Genome-wide RNAi screening reveals glial phosphoethanolamine-ceramide is critical for axonal ensheathment

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submitted by

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I, hereby declare that my doctoral thesis entitled **"Genome-wide RNAi screening reveals glial phosphoethanolamine-ceramide is critical for axonal ensheathment"** has been written independently with no other sources and aids than quoted.

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Abbreviations

Brn	Brainiac
C1P	Ceramide-1-phosphate
Cdase	Ceramidase
cDNA	Complementary DNA
CDP	Cytidine 5'-diphosphate
Cerk	Ceramide Kinase
CerS	Ceramide Synthase
CK	Ceramide Kinase
CNS	Central nervous system
cVA	Vaccenyl acetate
EGFR	Epidermal growth factor receptor
Egh	Egghead
ELOVL	Elongation of very long chain protein
ER	Endoplasmic reticulum
FA	Fatty acid
Gal-ceramide	Galactosyl ceramide
GFP	Green fluoroscent protein
Glc-ceramide	Glucosyl ceramide
GMR	Glass multimer reporter
GSL	Glycosphingoslipid
HRP	Horseradish peroxidase
JNK	c-Jun N-terminal kinase
Lac-ceramide	Lactosyl ceramide
LCB	Long chain base
MAG	Myelin- associated-glycoprotein
MAPK	Mitogen activated protein kinase

mRNA	messenger RNA
NL	Neural lamina
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PE	Phosphatidylethanolamine
PE-ceramide	Phosphoethanolamine-ceramide
PECT	Phosphoethanolamine cytidylyltransferase
PECS	Phosphoethanolamine ceramide synthase
PG	Perineurial glia
PI3K	Phosphoinositide 3-kinase
PLP	Proteolipid protein
PNS	Peripheral nervous system
pSJ	Pleated septate junction
qPCR	Real-time polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
SAT	Sialyl transferase
shRNA	Short hairpin RNA
SK	Sphingosine kinase
SM	Sphingomyelin
SMase	Sphingomyelinase
SMS	Sphingomyelin synthase
SPG	Subperineurial glia
Sply	S1P lyase
SPT	Serine palmitoyl transferase
UAS	Upstream activation sequence
VLCFA	Very long chain fatty acids
WG	Wrapping glia

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Abstract

Glia play a major role in many processes during the development of the nervous system both in vertebrates and in invertebrates. One of the crucial functions of glia is the insulation of axons to provide them with trophic support. This insulation renders electrical isolation to allow faster conduction of nerve impulses. This project aimed to identify novel glia-specific functions that alter axonal morphology.

In this study, we first characterized a *Drosophila* model to study glial specific functions in the mature nervous system. At first, we investigated the consequence of acute loss of glia on neuronal survival and for that UAS-GAL4 system in combination with $GAL80^{ts}$, a temperature-sensitive suppressor of GAL4, was used to eliminate glia exclusively in the adult nervous system. Ablation of glia in the mature nervous system of *Drosophila* induced neuronal cell death and had a dramatic impact on survival and motor performance of the flies. This result underscores the pivotal role of glia in maintaining the normal physiological milieu in the nervous system. This model was further exploited to identify genes contributing glia-specific function in the mature nervous system. Hence, we performed a genome-wide RNAi screen with a sublibrary of human homologs in the adult *Drosophila* model that we characterized. Each shRNA was specifically expressed in the mature glial cells and lethality or reduced locomotor activity of adult flies were scored. Interestingly, metabolic pathways are predominantly represented in our primary hit list. Next, a systematic bioinformatic analysis followed by secondary assays unveils that glial sphingolipids are critical for axonal ensheathment. Furthermore, we have determined that a specific sphingolipid phosphoethanolamine-ceramide (PE-ceramide) is required for a subtype of glia namely wrapping glia in order to maintain axonal enwrapping to preserve axonal integrity and electrical insulation. The loss of PE-ceramide in wrapping glia is likely to interfere with glial differentiation or axon-glia interaction and therefore, errors in ensheathment processes occurs. Rearrangement of cytoskeleton in glia, that are necessary for insulation

process might also be affected upon loss of PE-ceramide.

In parallel, we report that the loss of two very long chain fatty acid elongases *CG 18609* and *baldspot* in glia shows reduced viability and they are expressed in the brain. Morphological assay revealed that the loss of *baldspot* in glia altered glial morphology and subsequent axonal wrapping. *baldspot* is a homolog of mammalian Elovl6 that synthesizes short monounsaturated and saturated fatty acyl chain. Therefore, it is likely that PE-ceramide with short fatty acyl chain is critical for axonal ensheathment by glia. Moreover, these sphingolipids may influence the integrity or permeability of different physiological barrier present in the skin or nervous system.

Chapter 1

Introduction

1.1 Biology of glia

All complex nervous systems consist of two main cell types: neuron and glia. Neurons establish the basic architecture of the nervous system and they relay information from one neuron to the next or to the muscle whereas glia provide trophic support and electrical insulation to the neurons. Glial cells are pivotal in neuronal remodeling, proliferation, migration and synaptogenesis during development, underscoring the necessity of neuron-glia communication [1–4]. In *Drosophila* nervous system, glia also possess an intricate anatomical relationship with neurons throughout their lifespan. Glial elimination triggers neuronal cell death during the embryonic development and in adulthood [5, 6]. Moreover, the entire nervous system is encapsulated by a layer of glial cells forming the so called blood-brain-barrier in flies that restricts entry of solute and ions to the nervous system [7, 8]. Furthermore, glia delimit axonal outgrowth, fasciculation [9–11] and secrete factors necessary for the maintenance and ensheathment of synapses [12–14]. Recent studies have also shown a phagocytic role of glia to clear dying cells and degenerating axons of pupae and adult [15–19].

In contrast to mammalian nervous system where glia cells outnumber the neuron by far, in *Drosophila* there are only 10 % glia cells [20]. This makes *Drosophila* an excellent model system to study neuron-glia biology because of less compensatory pathways. Moreover, glial organization in flies is structurally very much similar to that of mammals and can be genetically manipulated with tools like mutagenesis, RNAi knockdown etc.

1.1.1 Mammalian glia biology

In the mammalian nervous system, there are four distinct types glia: oligodendrocytes, Schwann cell, astrocytes and microglia. Oligodendrocytes and Schwann cells ensheathe axons and form myelin sheath in the central nervous system (CNS) and in the peripheral nervous system (PNS), respectively. This insulation provides the basis for faster neuronal conduction and helps in maintaining axonal integrity (for review [21]). Astrocytes are critical for the maintenance of neural homeostasis. They participate in tripartite synapse formation, secretion of gliotransmitters and recycling of neurotransmitters [22–24]. Microglia are the macrophages present in the CNS and provide the first line of active immune defence. They produce an inflammatory response against pathogen invasion into the CNS [25, 26].

1.1.2 *Drosophila* glia biology

Based upon characteristic positioning during embryogenesis, glial cells are broadly classified into three categories: midline glia, longitudinal glia and peripheral glia. Midline glia are located in the midline of embryo enwrapping the neurons from each hemisegments of the CNS. Longitudinal glia are located along the longitudinal tracts of the embryonic ventral nerve cord and peripheral glia are positioned along sensory and motor axons [27–29]. During the embryonic development of *Drosophila*, the glial cells originate from progenitor cells called neuroblasts. For instance, midline glia and longitudinal glia are derived from the midline neuroblast of mesectoderm and the longitudinal glioblast in neuroectoderm respectively. Not only in origin, midline glia and longitudinal glia also differ distinctly in their gene expression profiles as well as in their functions [30–32]. Peripheral glia, as the name suggests are generated from sensory neuroblasts and sensory organ precursors [33].

Midline glia play a crucial role in axon guidance in the CNS of flies as demonstrated by the targeted elimination of midline glia or mutants blocking their differentiation. They provide both positive and negative cues that attract or repel axonal growth cones around the midline and thereby establish proper formation of axonal commissures in the CNS [28, 34, 35]. For instance, midline glia secrete Netrins (UNC-6 homolg) which navigate the CNS commissural axons and the motor axons in the periphery [36, 37]. Longitudinal glia also play a major role in proper formation of axonal commissures that run anterior

to posterior along the longitudinal tracts. These glial cells guide the axonal pathfinding and maintain proper fasciculation of the longitudinal tracts [2, 38]. One of the most remarkable features of peripheral glia is their ability to migrate throughout the embryonic development. Glial cells that are developed at lateral border of the CNS, migrate to the periphery along the axonal tracts of motor neurons. Actin-rich fillapodia-like structure of glia cell at the leading edge of migratory glia cells directs the migration of all glial cell. Sensory axons use glial cues to find their target in the CNS, but its role in motor axon guidance is very limited [3, 4, 39, 40].

A transcription factor that is instrumental for glial development, differentiation and function is *glial cell missing* or *gcm*. Except the midline glia which require EGFR signalling for development, all glial cells express *gcm* [41, 42]. *gcm* is sufficient for glial fate specification and it can switch neuronal cell fate to a glial cell fate [43]. One of the most well-studied direct transcriptional downstream target of *gcm* is *reversed polarity* or *repo* which is also expressed in all glia except midline glia of the embryo. *repo* retains its glia-specific expression pattern throughout the adult life-span of flies [44–46] and plays a dual role in the glial differentiation. On one hand it promotes terminal glial differentiation together with *pointed P1* [47–50], while on the other hand, it impedes neuronal differentiation in combination with *tramtrack* [50, 51].

1.1.3 Glia subtypes and their function in *Drosophila*

In the *Drosophila* nervous system, based upon their morphology, glial cells are broadly classified into four major categories : cortex, surface, neuropil and peripheral glia. Cortex glia are closely associated with neurons and they make honeycomb like processes that fill the gap between neuronal cell bodies. Notably, this type of glia exists in close proximity with oxygen supplying trachea and blood-brain barrier, the major entry sites for oxygen and nutrients to the fly brain. This suggests a possible regulatory role of cortex glia in the influx of gas, ions and food [52, 53]. Surface glia form the blood-brain barrier to protect the CNS from hemolymph and favors the faster propagation of the nerve impulses. Surface glial membrane form the pleated septate junctions (pSJs) with membranes of cortex glia. Neurexin IV, gliotactin and coracle are crucial proteins in septate junction formation and functional assembly. This pSJs restrain ionic influx and preserve the ionic balance in neural tissues [54–57]. Neuropil glia extend sheath like structures around the bundle

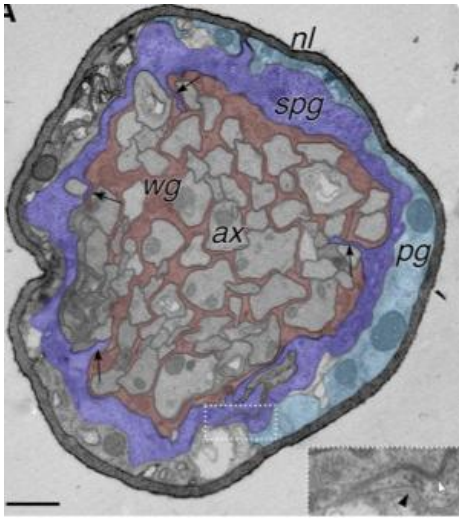


Figure 1.1: Electron Microscopic view of cross-section of a third instar larval peripheral nerve. Three types of glia that present in peripheral nerve are designated with colors. Wrapping glia (wg) is shown in red. The surrounding subperineurial glia (spg) is shown in blue. The glia also form pleated septate junction shown in higher magnification as inset. Perineurial glia (pg) (one cell is shown in light blue) sends small projections to spg. A basal lamina that consists extracellular matrix encapsulates the whole nerve called neural lamina (nl). Ax denotes axons in the nerve. Adapted from Stork *et al*, 2008 [7]. Reproduced with permission from Society for Neuroscience.

of axons and provide the electrical insulation to nerves. This insulation acts as a barrier between the nerves and the surrounding environment and thus it is beneficial for the neuronal function and activity. Neuropil glia is also necessary to provide trophic support to the neurons [5]. Peripheral glia ensheathe peripheral nerves in the embryonic and larval nervous system. Unlike the CNS, the peripheral nerves are enwrapped with several layers of glial cells which have distinct function and molecular markers [58]. The innermost layer of wrapping glia (wg) ensheathe individual axons or a group of axons called fascicles. wg layer is enwrapped by another glial cell layer called subperineurial glia (spg) which form septate junctions and establish the blood-nerve-barrier [7, 8]. spg glial layer is surrounded by the perineurial glia which consist of monolayer of squamous-like cells [59, 60]. Finally all peripheral nerves are encapsulated by a dense basal lamina called neural lamina (nl) [61].

1.2 Axonal ensheathment by glia

In the vertebrates, oligodendrocyte and Schwann cells produce a highly specialized multilayered membrane called myelin that ensheathe axons in the CNS and in the PNS, respectively. Myelin, composed of lipid and proteins, insulates the axons to form a low capacitance and high resistance barrier. This electrical insulation in turn allows fast conduction of the nerve impulse, called saltatory conduction [62–64]. A similar relationship between glia and axons also exist in *Drosophila*. In flies, inner glial cells or wrapping glia ensheathe multiple peripheral axons at the same time similar to Schwann cells in vertebrate [49, 65]. One axon or multiple axons called fascicles are insulated by cellular processes of inner glial cells. Subperineurial and perineurial glia which are together called outer glial cell, encapsulate the whole peripheral nerve to protect it from hemolymph ionic environment. This insulation of nerves by glial processes ensures the high-speed conduction of the nerve impulse and decreases the reaction time in response to stimuli. It is interesting to note that, glia in *Drosophila* lacks orthologs of most of myelin genes and do not form myelin but it still can produce myelin-like multilayered glial sheaths around axons at least observed in thoracic ganglia of adult flies [66–68]. This indicates that a conserved molecular and cellular pathway is possibly involved in the formation of multilayered glial membrane structure around the axons.

1.2.1 Glial migration and axonal ensheathment

Glial cells of *Drosophila* that ensheathe axons in the periphery are first generated in the CNS and then migrate to the periphery to ensheathe the peripheral nerves during the embryonic development. This migration of glia is very important for the proper development of the *Drosophila* nervous system [29, 69–71]. Since glial population in *Drosophila* is relatively low, glial membrane undergoes ramification to achieve a tortuous morphology in order to accommodate and enwrap a large number of axons. By stage 17 (17 hours after egg laying), inner glial cells enwrap axons and outer glial cells encapsulate the nerve by forming glial septate junctions [68, 72]. Axonal ensheathment begins at the embryonic stage but continues until third instar larval stage when all peripheral nerves are wrapped completely by glial cellular processes. Segmental nerves are insulated gradually from embryonic stage to larval stages whereas intersegmental nerves are wrapped during larval stages only. It is interesting to note that segmental and intersegmental axon tracts fuse

to form the peripheral nerve trunk that grows until larval peripheral nerve is completely developed [73, 74]. The ensheathment of axons by inner glial cells or outer glial cells accommodate the growing peripheral nerves by different means: outer glial cells undergo post-embryonic proliferation and inner glial cells stretch their cellular processes [3, 66, 68].

Glial migration is therefore, a critical function to attain proper axonal ensheathment in the *Drosophila* peripheral nerves. Several studies have shown that the actin cytoskeleton plays a crucial role to instruct glial migration to the periphery as well as guide to the ensheathment process. For instance, the Rho family of GTPases (Rac, Rho) has been identified to mediate cytoskeleton changes, which affect the motility of glial cells. Both gain and loss of function of Rac1 and RhoA by expressing constitutively active and dominant negative forms respectively, shows changes of actin dynamics in glial cytoskeleton resulting in the stalling of peripheral glia at the CNS-PNS transition zone. Additionally, as a secondary effect impaired nerve ensheathment was also observed. A balance of these GTPases is also crucial for glial migration and wrapping as evident from the distinct phenotype of their mutants; Rac1 mutant shows ball-shaped collapsed glia whereas RhoA mutant shows very long, spike-shaped actin processes [3, 4, 39]. On the contrary, overexpression of dominant negative and constitutively active Rho and Rac1 in neurons did not show any glial migration defect suggesting a glial specific role of actin cytoskeleton and these GTPases [68]. Interestingly, a downstream target of Rho, ROCK or Rho kinase has also been shown to be involved in myosin regulation and actomyosin assembly and thereby orchestrate glial cytoskeleton changes during Schwann cell dependent myelination in vertebrate [75] Therefore, it can be speculated that actin cytoskeleton dynamics is essential for glial insulation of axons and it is most likely conserved across the species.

Another study from Edenfeld *et al* shows that a splicing factor, crooked neck or *Crn* regulates axonal ensheathment in flies. Crn mutant shows impaired formation of cellular processes around axonal fascicles as a result of defects in glial migration and differentiation [76]. Biochemical studies indicate that Crn is a component of splicing machinery but it lacks the RNA binding motif. Therefore, it interacts with How to exert its effect [77, 78]. The vertebrate ortholog of How is Quaking which is known to be involved in glial differentiation and myelination [79]. These striking similarities potentiate the fact that basic molecular mechanism coordinating neuronal ensheathment in insects and mammals

are possibly the same.

1.2.2 L3 larva as a model to study axonal ensheathment

L3 larva is the third instar larval stage of *Drosophila* life cycle before entering the puparium formation. L3 larval PNS has been the most established model system to study axonal ensheathment. L3 larvae have eight pairs of peripheral nerves innervating the muscle from each abdominal hemisegments of larva (Figure 1.2. Each nerve originates from the ventral lateral edge of a ventral ganglion and innervate ventral oblique muscle fibers [66, 80]. A1 nerves send their projection to the muscles most adjacent to the ventral ganglion and A8 nerves target the muscles most distal to the ventral ganglion. Upon reaching the bodywall, each nerve divides into five branches that run ventral to dorsal. These branches resemble the embryonic segmental and intersegmental nerves [81, 82]. Another innervation of the bodywall is contributed by transverse nerves that emerge from dorsal midline of the ganglion and run ventral to dorsal along the segment border to project bilaterally at the alary muscles [83]. Each peripheral nerve consists of three components: sensory and motor axons, inner glia or wrapping glia that ensheathe axons and outer glia or perineurial glia that encapsulate the whole nerve [84]. There are approximately 85-90 afferent and efferent axons present in each nerve [85].

Studies on *Drosophila* mutants *fray, neurexin IV (nrx IV), gliotactin (gli)* reveal several aspects of peripheral nerve ensheathment. Analysis of *fray* mutant shows that glial ionic homeostasis is crucial for the encapsulation of peripheral nerve. *fray* encodes a 552 amino acid protein which is homologous to mammalian serine/threonine kinase. Mutation in *fray* gene results in swelling of L3 larval peripheral nerve. Ultrastructure analysis shows that inner glial processes fail to enwrap the axons completely. As a result, a severe defasciculation and splitting of axons are observed [66]. Later studies indicate that *fray* regulates a conserved Na-K-Cl cotransporter Ncc69 (a homolog of mammalian NKCC1) and thereby maintain the ionic balance in glial cells [57]. Neurexin IV, transmembrane protein, is critical for proper septate junctions assembly. *nrx IV* mutant lack glial septate junctions in peripheral nerve and consequently the blood-nerve-barrier integrity and nerve ensheathment is affected [72, 74, 86, 87]. *gli* is also a transmembrane protein that is expressed in ensheathing glia. *gli* mutant shows improper glial ensheathment of axons. It is spec-

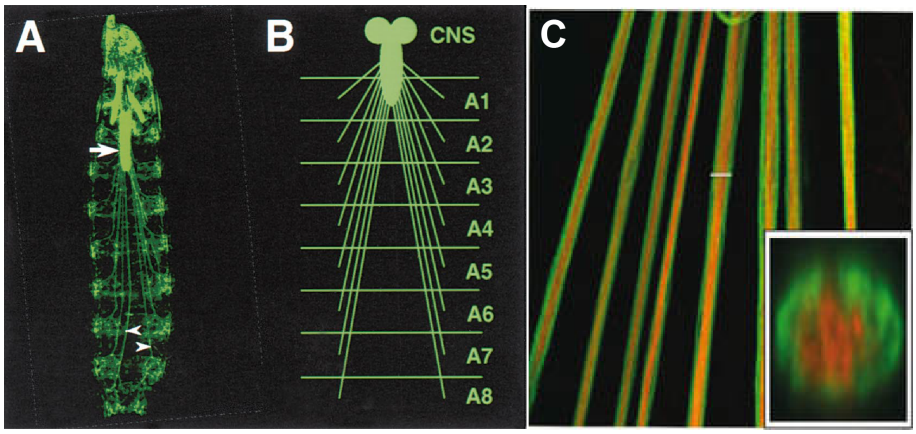


Figure 1.2: L3 larval abdominal nerves of *Drosophila*.(A) The whole larval nervous system is labelled with GFP. **(B)** Diagram of projection and nomenclature of peripheral nerves innervating different segments. They are numbered according to the segments they innervate. Adapted from Leiserson *et. al*, 2000 [66]. Reproduced with permission from Elsevier. **(C)** *Drosophila* glial membrane morphology (green) of peripheral nerves of L3 stage larva is visualized with mCD8-GFP expression under repo-GAL4. Neuronal membrane (red) is observed with HRP staining. Inset shows glial encapsulation of neuron of an orthogonal section from the region of the nerve marked with white line.

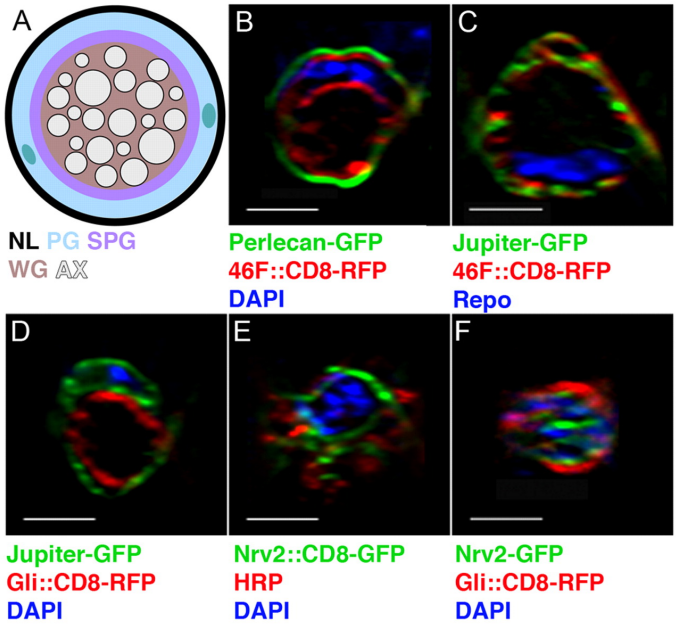


Figure 1.3: Axons and glial layers in larval peripheral nerve. Orthogonal section of a L3 larval peripheral nerve. **(A)** Scheme of a transverse section of a peripheral nerve showing its components: neural lamella (NL), perineurial glia (PG), subperineurial glia (SPG), wrapping glia (WG) and axons (AX). **(B-F)** Different subtypes of glia are visualized using a combination of either GFP-trap lines or subtype-restricted expression UAS-mCD8-GFP/RFP. Adapted from Xie *et. al*, 2011 [58]. Reproduced with permission from Development.

ulated that gliotactin acts as signaling molecule that mediate cell recognition effect [66, 73].

Thus L3 larval PNS is an excellent tool to study cellular communications or interactions between neuron and glia, especially, the function of glia during axonal ensheathment. Visualization of axonal enwrapping by glial processes is hindered due to the unavailability of any glia-specific marker protein localized to cell surface. But this is circumvented by driving UAS-mCD8-GFP under pan glial driver repo-GAL4 [4]. UAS-mCD8-GFP encodes a protein that is targeted to glial membrane and thus visualization of glial membrane morphology is possible [68, 88]. Membrane morphology of the subtypes of glia is also possible by combining UAS-mCD8-GFP with subtype-specific glia driver line, for instance, nervana2-GAL4 for wrapping glia, gliotactin-GAL4 for subperineurial glia. A list of different glia subtype-specific drivers are presented in Figure 1.4 [58]. By recombining glial-subtypes specific drivers with GFP, membrane morphology of glial subtypes can

	Glia subtypes			
	NL	PG	SPG	WG
GAL4 drivers				
repo-GAL4		X	X	X
46F-GAL4		X		
SPG-GAL4			X	
Gli-GAL4			X	
Nrv2-GAL4				X
GFP trap lines				
perlecan-GFP	X			
viking-GFP	X			
Jupiter-GFP		X		
nrv2-GFP				X

NL, neural lamella; PG, perineurial glia; SPG, subperineurial glia; WG, wrapping glia; Gli, Gliotactin; Nrv2, Nervana 2.

Figure 1.4: Summary of markers for glia and its subtype and neural lamella in the peripheral nerve. Adapted from Xie *et. al*, 2011 [58]. Reproduced with permission from Development.

be examined easily with the fluorescent imaging techniques. A recent study represents a comprehensive overview of available subtype-specific driver lines and GFP-trap lines (Figure 1.3). These fly lines can be further exploited to address the functional significance of glia and its different subtypes in axonal conformity in larval PNS.

1.3 Biosynthesis of sphingolipid

Sphingolipids are vital components of cellular membranes. Different metabolites generated by sphingolipid metabolism (Figure 1.5), act as second messengers and regulate several cellular signaling pathways involved in cell growth, differentiation, survival and apoptosis [89–94]. Sphingolipid biosynthesis starts in the endoplasmic reticulum (ER) when ceramide is generated. Ceramide is then modified both in the ER and Golgi to yield complex sphingolipids such as the different glycosphinlgolipids (for review [95]).

1.3.1 Ceramide biosynthetic pathway

Ceramide biosynthesis begins with the condensation of palmitoyl CoA with serine. In the first step, serine-palmitoyl transferase (SPT) catalyzes this condensation process to generate 3-ketosphinganine, a sphingoid base product. SPT is the rate-limiting enzyme in

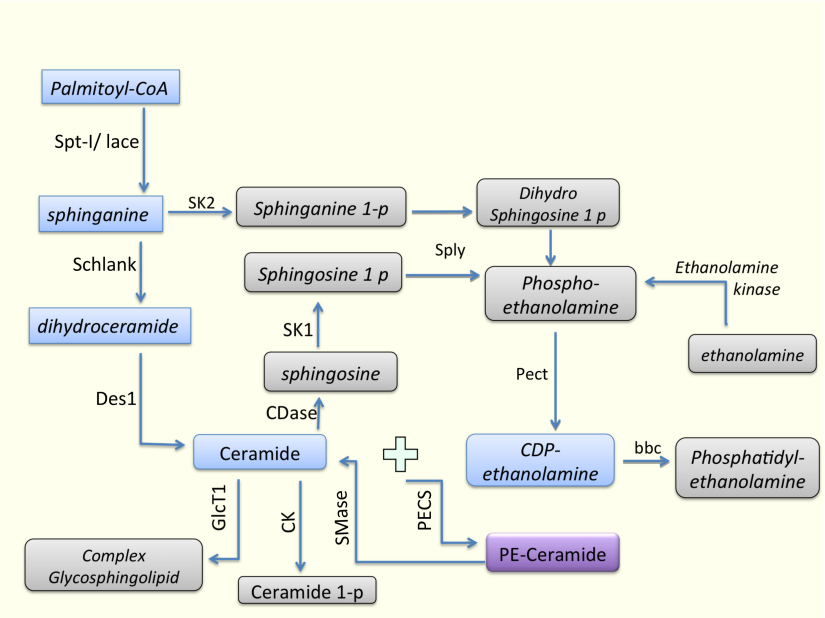


Figure 1.5: Sphingolipid metabolic pathway

mammals and also in yeast. It is a hetero-oligomer of two transmembrane proteins LCB1 and LCB2. The biological significance of this enzyme in this catalysis was first described in yeast [96, 97]. In *Drosophila*, LCB1 is encoded by *Spt-I* gene which encodes a protein containing 485 amino acids. This gene is annotated as CG4016 and is located in the second chromosome. Mutation of LCB1 in human is associated with human hereditary sensory neuropathy type 1, a disease that affects autonomic and sensory nervous system of lower limbs [98]. *lace* encodes LCB2 subunit and is annotated as CG4162 in flybase. It is located in second chromosome and encodes a 597 amino acid containing protein. This gene is critical for proper development of *Drosophila* as demonstrated by *lace* mutant flies namely $lace^{k05305}$ and $lace^{2}$. However, the hypomorphic combination of $lace^{k05305}$ and $lace^{2}$ mutant flies grow until adulthood but they show several defects in wings, eyes and bristles [99]. It is important to note that *spt* in *Drosophila* recognizes laurate (C12) instead of palmitate as in the mammals and generates a sphingoid base, 3-ketosphinganine with alkyl chain of C16 in place of C18 [100, 101].

In the next step, a NADPH dependent 3-ketodihydrosphingosine reductase converts 3-ketosphinganine to dihydrosphingosine or sphinganine. A fly homolog of this enzyme is yet to be identified. The following step is catalyzed by an acyl-CoA dependent dihydroceramide synthase that transfers an acyl group to sphinganine to generate dihydroceramide. This acylation step is carried out by a family of six enzymes called ceramide synthase (CerS) in mammals. There are six CerS which differ in their substrate specificity and expression pattern. CerS1 and CerS2 are widely expressed in brain and specifically synthesize C18 and C20-26 acyl chains respectively [102, 103]. CerS1 modulates the growth of squamous cell carcinoma in head and neck [104] and might also play role in a genetic metabolic disorder named ceroid lipofuscinoses as indicated by its enriched expression in most neurons of patients [105, 106]. CerS2 is predominantly expressed in oligodendrocytes and Schwann cells. Studies from CerS2 knockout mice have shown defects in myelin sheath formation [107] and an increase in membrane fluidity [108]. This implies a role of long chain fatty acyl CoA in membrane organization and axonal ensheathment. Recently, in *Drosophila*, a gene called *schlank* has been identified and it belongs to the ceramide synthase family. It controls growth and body fat metabolism of flies, but no biochemical study has been done so far to confirm its ceramide synthase activity [109]. The final step of *de novo* ceramide biosynthesis is catalyzed by a desaturase which converts dihydroceramide to ceramide. A *Drosophila* gene called *Des-1* has been proposed by Basu and Li [110]. Loss of this gene shows degenerative spermatocyte phenotype, because of the defects in spindle assembly during meiosis of spermatocytes. Its colocalization with microtubules suggests a possible role of ceramide in anchoring of cytoskeletal elements during cell division.

1.3.2 Sphingomyelin metabolism

Ceramide serves as the backbone of several complex sphingolipids. Ceramide undergoes a series of species-specific modifications in the plasma membrane and in the *trans*-Golgi network to generate sphingomyelin and glycosphingolipids. In humans, two sphingomyelin synthase (SMS) genes are known, namely SMS1 (found in the *trans*-Golgi) and SMS2 (found in the plasma membrane) [111, 112]. SMS transfers the phosphorylcholine head group from phosphatidylcholine to ceramide to yield phosphocholine-ceramide or sphingomyelin (SM). In *Drosophila*, the amount of SM is very negligible, but phosphoethanolamine-ceramide (PE-ceramide) which is the most abundant sphingolipid

in flies, probably acts as a sphingomyelin analog [113, 114]. Even though several ortholog of SMSs in fly have been identified, a cognate SMS in fly is yet to be identified. A recent study from Varcau *et. al* determined SMSr (sphingomyelin synthase 1-related), annotated CG32380 in flybase that can synthesize PE-ceramide *in vitro* but not *in vivo*. This study has shown that SMSr rather acts as a sensor for ceramide homeostasis both in fly and mammalian cells [115]. They proposed that a PE-ceramide generating enzyme is likely to be one of three other homologs of SMS, namely *CSS1a* (CG11438), *CSS1b* (CG11426), and *CSS2* (CG31717) [101].

Sphingomyelin is catabolized by sphingomyelinase (SMase) to produce ceramide. In mammals, there are two types of SMases that have been reported based on their pH optima, namely acidic SMase and neutral SMase [116, 117]. In *Drosophila*, CG3376, CG3376 and CG15533 have been identified as potential acidic SMases for flies whereas CG12034 has been found as the only homolog of mammalian neutral SMase 1 and 2 [101]. Since, PE-ceramide acts as sphingomyelin analog in flies, it is most likely that these SMases cleave PE-ceramide to produce ceramide. However, further studies are required to corroborate this notion.

1.3.3 Glycosphingolipid metabolism

In mammals, glycosphingolipids (GSL) are synthesized by a series of addition of sugar moieties to ceramide base. At first, glucosyl-transferase adds a glucose molecule to ceramide in the Golgi network to produce glucosyl-ceramide (Glc-ceramide) [118]. Glc-ceramide in turn is converted to lactosyl-ceramide (Lac-ceramide) by lactosyl-ceramide synthase [119, 120]. This Lac-ceramide is the starting point for the generation of different series of gangliosides and other series of complex GSL (Figure 1.6). A series of sialosylation catalyzed by different sialyl-transferases generate precursor for Ganglio-series while precursors for Globo-series and Lacto-series are generated by β3GnT5 (a N-acetyl-glucosamine transferase) and α4GalT (a galactosyl transferase), respectively. These series of complex GSLs are produced by subsequent addition of specific sugar moieties to the respective precursors [121, 122].

In vertebrates, ceramide acts also as a precursor of another GSL called galactosyl-ceramide (Gal-ceramide). In the ER, Gal-ceramide is generated by ceramide galactosyl-transferase

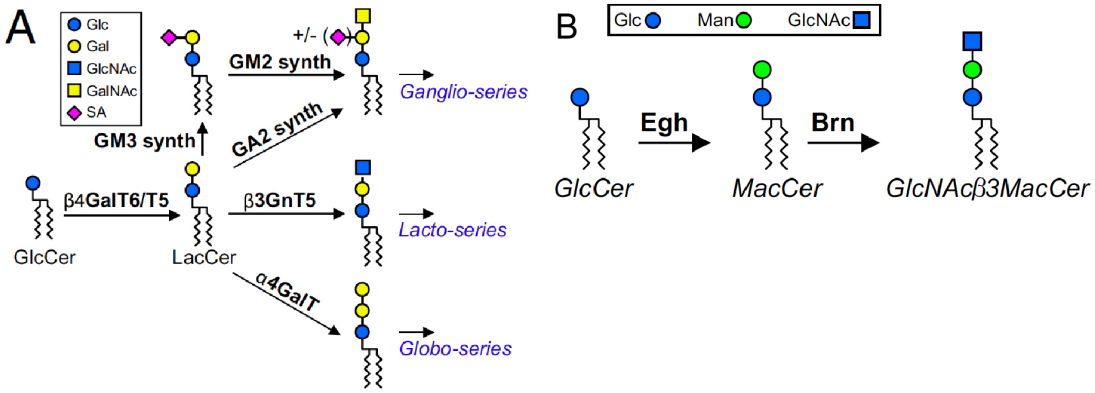


Figure 1.6: Biosynthesis of Glc-ceramide related glycosphingolipids both in vertebrate (A) and in *Drosophila* (B). Adapted from Dahlgaard *et. al*, 2012 [186]. Reproduced with permission from Proceedings of the National Academy of Sciences.

[123, 124] and the product is transferred to the Golgi stack where it has two fates. On one hand, Gal-ceramide undergoes sulphation by a sulphate transferase involving PAPS (3'-phosphoadenosine, 5'-phosphosulphate) [125]. On the other hand, Gal-ceramide is sialosylated to gangliosides by the action of a sialyl-transferase (SAT II) [122].

Biosynthesis of Glc-ceramide related GSLs in flies is very much similar to that in mammals. A *Drosophila* glucosyltransferase (Glc-T1) transfers a glucose moiety to ceramide core in order to yield glucosyl-ceramide. The next steps that generate complex GSL are very different as compared to mammals. The major GSLs in flies are not sialylated as in mammals. They contain $Man^{\beta 1-4}Glc^{\beta 1}-Cer$ as core disaccharide in place of a $Gal^{\beta 1-4}Glc^{\beta 1}-Cer$ as in mammals [126]. *egghead (Egh) and brainiac (Brn)* are two enzyme which encode β4-manosyl transferase and β3-N-Acetylglucosaminyl transferase respectively acting one by one to generate β3-N-Acetylglucosaminyl-ceramide. Mutations of these two genes result in a widespread phenotype during embryonic development involving neuronal hypertrophy, EGFR signaling and oogenesis [126–129]. There are two more enzymes which produce more complex glycosphinlgolipids; β-4-N-Acetylga-lactosaminyl transferase A/B (βGalNacTA/B) and α4 N-Acetylgalactosaminyl transferase 1/2 (αGT1/2) which catalyze a series of further additions to N-Acetylgalactosamines.

All complex GSLs bear a long chain of sugar residues containing GalNAc and GlcNac. One or two of GlcNAc of sugar chain is/are substituted by PE. Loss of βGalNacTA/B causes defects in the neuromuscular junction and also in the coordination of the movements of the flies [130, 131]. In spite of some differences, *egghead* mutant flies which block the synthesis of mactosyl-ceramide shows an accumulation defect of truncated GSLs. This defect is rescued by the introduction of human galactosyltransferase, which makes lactosyl-ceramide [132]. This indicates that the GSL of flies can normally function with human lactosyl-core instead of its own mactosyl-core. Hence, these properties can further be used to study structure-function relationship of GSL in both vertebrates and invertebrates.

1.3.4 Catabolism of ceramide

This pathway involves generation of several metabolic intermediates which have a role in cell signaling, growth and survival (see Figure 1.7). On one hand, ceramide is converted to ceramide phosphate by the action of ceramide kinase. This conversion step is very necessary to stall the apoptotic signal of ceramide to promote cell survival. The enzyme was first identified in human and later its homolog has been studied in *Drosophila*, plants and others. *Drosophila* ceramide kinase or *Cerk* is annotated as CG16708 in flybase [133, 134]. Ceramidase (CDase) converts ceramide to sphingosine. In mammals, acidic CDase catalyzes the conversion of ceramide to sphingosine [135]. However, no acidic CDase has been reported in flies, rather a neutral CDase (CG1471) and an alkaline CDase (CG13969) have been identified in *Drosophila* [136]. Mutation in neutral-CDase(*slab*) result in defects in synaptic transmission as a result of failure of synaptic vesicle fusion during exocytosis of neurotransmitters [137]. Targeted expression of this gene rescues retinal degeneration in arrestin and PLC mutant flies [138]. Likewise, the alkaline CDase, known as *brainwashing*(*bwa*) has a role in mushroom and ellipsoid body development in *Drosophila* CNS [139]. A recent study,however, indicates that *bwa* does not have any ceramidase activity instead it acts as a regulator of sphingolipid flux. It is suggested that the apparent CDase activity of *bwa* was observed due to strong genetic interactions with other sphingolipid metabolites [140].

In the following step, sphingosine kinase catalyzes sphingosine to sphingosine-1-phosphate (S1P). Two *Drosophila* genes *SK1, SK2* have been identified as sphingosine kinases and they are closely related to mouse SphK1 and 2. Mutation of SK2 showed a reduction in

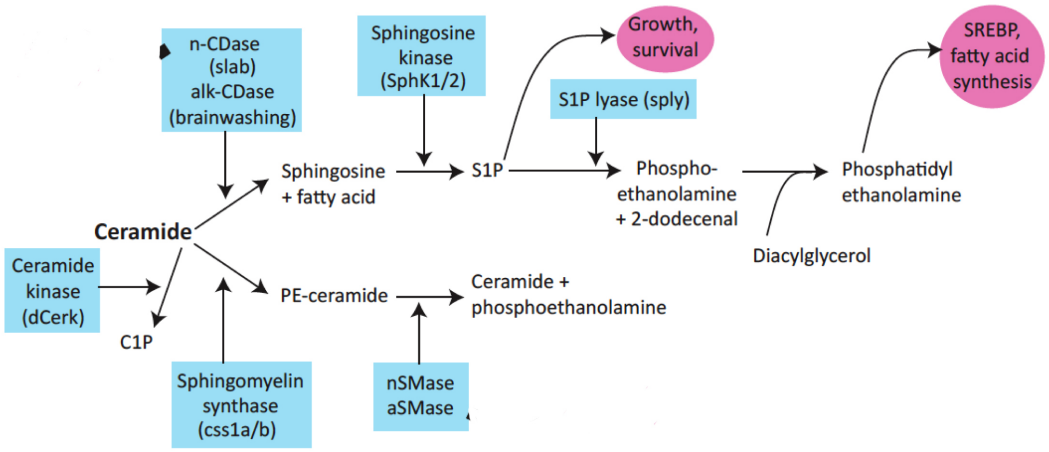


Figure 1.7: Catabolism of ceramide in flies. Several ceramide metabolites namely sphingosine-1-phosphate (S1P), Phosphoethanolamine (PE)-ceramide, ceramide-1-phosphate (C1P) are produced. Adapted from Kraut *et. al*, 2011 [141]. Reproduced with permission from John Wiley and Sons.

fight performance and fecundity. Additionally, the egg laying was delayed in female flies but they were viable. This viability of homozygous mutant of SK2 flies indicates a possible compensation from SK1 activity. Evidently, this compensatory function is not complete as these flies show an increased level of long chain bases [142]. Both SK1 and SK2 have the ability to phosphorylate sphingosine, dihydrosphingosine or sphinganine to yield S1P which acts as a second messenger and is involved in many signaling pathways viz. platelet-derived growth factor (PDGF) dependent cell-proliferation, apoptosis [143, 144]. S1P also acts as an extracellular ligand for a family of G-protein coupled S1P receptors (S1P-GPCR) and regulates cardiac development and angiogenesis [145]. S1P is irreversibly broken down to phosphoethanolamine and 2-dodecanol by an enzyme called S1P lyase (Sply). *Sply* is annotated as CG8946 in flybase. Mutation of this gene shows hypertrophy of flight muscle and reduction in the number of muscle fibers along with degeneration of testes and ovaries. Interestingly, the loss of both copies of *Sply* can rescue the lethality of *lace*, suggesting that the loss of sphingosine or S1P is responsible for the *lace* mutant phenotype [141, 146].

In the next step of ceramide catabolism, cytidine 5′-diphosphate (CDP) is transferred to phosphoethanolamine, catalyzed by phosphoethanolamine cytidylyltransferase (PECT) to produce CDP-ethanolamine. In flybase, *pect* is annotated as CG5547. CDP-ethanolamine can also be generated by another pathway bypassing the sphingolipid intermediates. When ethanolamine is available, ethanolamine kinase directly converts it to phosphoethanolamine which in turn produces CDP-ethanolamine by *Pect* [147]. CDP-ethanolamine has two fates in flies (see Figure 1.5). On one hand, it donates the head group to diacylglycerol to synthesize phosphatidylethanolamine (PE) by the action of CDP-ethanolamine phosphotransferase called *bb in a boxcar* or *bbc* in flies. On the other hand, PE-ceramide synthase (PECS) uses its head group to synthesize PE-ceramide [115]. This step is contrary to the mammalian PE-ceramide synthesis where PE donates the head group to yield the phosphatidylethanolamineceramide [148, 149]. A cognate PECS enzyme is yet to be identified. Therefore, the characterization of fly homologs of PECS as discussed before could provide clues to identify this enzyme in *Drosophila*.

1.4 Role of sphingolipids in nervous system

1.4.1 Sphingolipids in vertebrate nervous system

Sphingolipids are the essential components of eukaryotic membranes where they constitute 10-20 % of total membrane lipids [150]. They regulate the geometrical and structural properties and lateral order of biological membranes. Moreover, sphingolipids metabolism is tightly regulated both temporally and spatially during the development of nervous system to support its functional integrity. Importantly, sphingolipids participate in the regulation of a number of biological processes such as neuronal survival, migration, differentiation, neuron-glia interaction [151–155]. Ceramide, the central metabolite of sphingolipid metabolism controls cell survival and death through various signaling pathways. High concentration of ceramide is critical for neuronal development while low concentration of the same abrogate Purkinje cell differentiation and reduces axonal branching in cultured neurons [94, 156]. Furthermore, ceramide gives rise to all complex sphingolipids such as GSL and SM.

GSLs are pivotal in the development and maintenance of the nervous system as demonstrated by the ceramide glucosyltransferase knockout mice. These mice are embryonically

lethal and shows defect in cellular differentiation [157]. GSLs undergo complex modification during development of nervous system. First simple gangliosides (GM3, GD3) are produced which later give rise more complex gangliosides (e.g GM1, GD1a, GD1b). In humans, the increase of ganglioside level begins at six months of gestation, reaches its peak at five years after birth and declines with aging [158, 159]. Notably, GSLs regulate multiple biological functions either by direct lipid-protein interaction or indirectly via lipid rafts [160–163]. Inhibition of GlcCer synthase by pharmacological inhibitors affects axonal branching, neurite outgrowth, synaptic formation and activity in neuronal cultures. Conversely, pharmacological stimulation of GSL synthesis promotes neurite outgrowth, synapse formation, synaptic activity [164–167]. Another subclass of GSL, the galactolipids (GalCer) and sulfatide have been shown to be involved in myelin biogenesis. These lipids are found in compactly wrapped myelin around axons and stabilize the paranodal loops as evident form the studies on knockout mice [168–170]. Interaction of GalCer and sulfatide in oligodendrocytes governs the clustering and proper transport of myelin proteins, vital for myelin biogenesis and function [171].

SM is the most abundant sphingolipids of the nervous system incorporating 25 % of total myelin lipids [172]. SM primarily serves as a source for bioreactive second messengers (e.g ceramide, S1P)[173, 174]. SM is found in the micro-domain structures called lipid-raft, present in the membrane of many cell types. Lipid-rafts help specific protein-protein interactions and thereby activate the downstream signaling cascades to regulate various cellular functions [175].

1.4.2 Sphingolipids in *Drosophila* nervous system

Although there are considerable differences among *Drosophila* and vertebrate sphingolipid species, several common biological functions have been implicated. Previously, ceramide was only considered as proapoptotic as an increase in its level showed deleterious effects including reduction of lifespan [176, 177]. However, recent studies on CDase mutant flies (known as *bwa*) have elucidated its diverse functions. For instance, CDase converts ceramide to sphingosine and as a result, mutant CDase shows an increase of ceramide level. Even though the ceramide level is high in these flies, the larval developmental time is prolonged and an increase in stress resistance is also apparent [178]. Therefore it is now speculated that ceramide acts as stress-response coordinator which promotes sur-

vival through mitogen-activated protein kinase (MAPK) pathway and induces cell death via c-Jun N-terminal kinases (JNK) pathway [141, 179]. Eye-specific overexpression of CDase lowers the level of ceramide which in turn rescues the phenotype of degenerating photoreceptors of arrestin and phospholipase C β mutant flies (*norpA*). This strongly indicates a neuroprotective role of low-levels of ceramide [99, 180]. Another study with CDase mutant flies has shown that the high level of ceramide decreases light response of photoreceptor-neurons and induces apoptosis in a non-autonomous fashion. CDase function is indispensable for peri-synaptic neuronal transmission and synaptic vesicular fusion as evident from *Slab (Slugabed)* mutant flies where a mutation is present in the CDase gene [138]. In addition, ceramide promotes autophagy which is necessary for the expansion of motor neurons during larval development and is mediated by two kinase signaling pathways namely *basket* (fly JNK) and *wallenda* (fly MAPKKK) [181, 182]. Another metabolite of ceramide is ceramide-1-phosphate (C1P) yielded by the action of Ceramide kinase (*Cerk*). C1P orchestrates the distribution of phosphatidylinositol-4,5-bisphosphate at the photoreceptor membrane by localization of PLC and consequently, induces neurodegeneration [183].

The importance of GSL in *Drosophila* nervous system was first demonstrated by studies with *egghead* and *brainiac* mutants. These two genes add sugar residues to generate more complex GSL from Glc-ceramide. Mutations in these genes perturb germ line-follicle cell interaction which leads to hypertrophy of neurons in the embryo. This phenotype is similar to that of Notch-EGFR signaling pathway defect [184, 185]. A recent study reveals that the loss of *egghead* creates neurofibromatosis-like pathophysiology. Larval peripheral nerves become swollen and are attacked by immune cells like plasmatocytes. An increase of subperineurial glia growth and proliferation caused by activation of phosphatidylinositol 3-kinase (PI3K) signaling is also observed indicating a role of GSL in terminal glia differentiation [186]. *GalNAc-T-A* catalyze further elongation of GSL preceded by *brainiac* and *egghead*. Mutation in this enzyme results in defects in the neuromuscular junction and consequently, in locomotion and in coordination. Moreover, a reduction in size, branching, number of synaptic boutons and consequent muscular hypercontractility are also observed. Targeted expression GalNAc-T-A cDNA in the neurons or in the muscles in the mutant background, only partially rescues the phenotype. Hence, it suggests the distinct role of GSL when having a specific combination of sugar residues [130, 131].

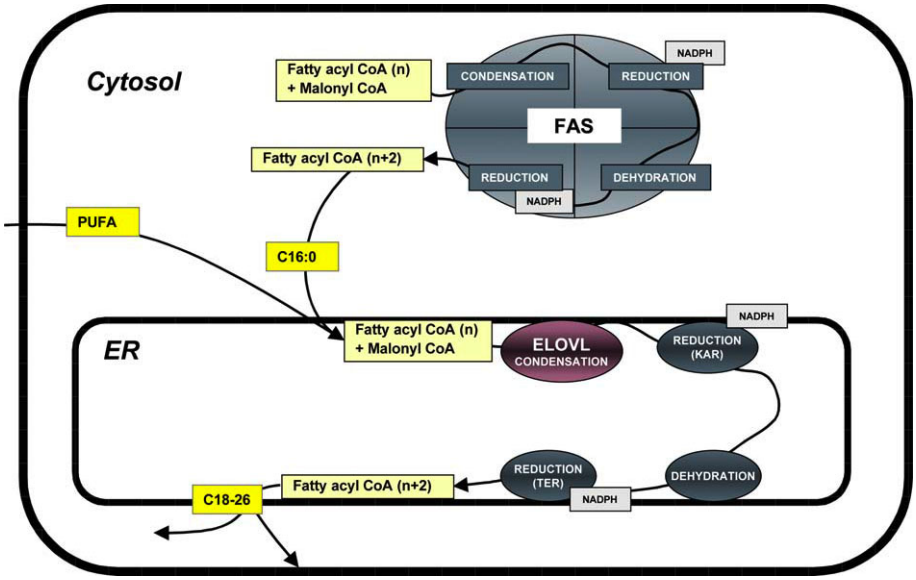


Figure 1.8: Elongation of fatty acids in mammals. Fatty acids are synthesized by the fatty acid synthase (FAS) in the cytosol and then the FA is transported to the ER for further elongation. ELOVL, elongation of very long chain fatty acid, KAR, 3-ketoacyl-CoA reductase, TER, trans 2,3 enoyl CoA reductase. Adapted from Jakobsson *et al*, 2006 [196]. Reprinted with permission from Elsevier.

1.5 Very Long Chain Fatty acid Elongation protein

1.5.1 In vertebrate

Very Long Chain Fatty acid Elongation proteins (Elovl) are fatty acid elongases that catalyze the elongation of fatty acids (FA) [187]. In mammals, fatty acids with a chain length of 16 carbons (C16) are synthesized by fatty acid synthase complex in the mitochondria [188–190]. Next, the C16 acyl chain containing FA (palmitic acid) is transported to the ER for further elongation. Elovls are ER-resident protein, where they elongate different length of fatty acyl chains in a four step reaction involving condensation, reduction, dehydration and reduction (see Figure 1.8). Different enzymes catalyze each of these steps but it is the elongases that determine the substrate-specificity, and in addition, it is the rate-limiting enzyme in this process [187, 191, 192].

Studies on fatty acyl chain length of the different lipids show that the saturated and unsaturated fatty acyl chains are present in different mammalian tissues. The chain length

and the degree of saturation or unsaturation of fatty acids is also determined by the elovl proteins [193–195]. There are seven Elovl proteins present in vertebrates and they are broadly classified into two categories: i) Elovl1,3,6 and 7 that elongate saturated and monounsaturated FA and ii) Elovl2,4 and 5 that elongate polyunsaturated FA. Intriguingly, the expression of all elongases is spatially and temporally restricted indicating a tissue-specific functional significance [196, 197]. These long chains FA are transferred to sphinganine to generate sphingolipids. FA chain of sphingolipids regulates the compactness and the order of membrane lipids [198, 199]. Elovl1 protein is ubiquitously expressed and therefore, is probably related to basic maintenance of membrane architecture. However, the relative abundance of Elovl1 mRNA in oligodendrocytes [200], corpus callosum and spinal cord strongly indicates its role in myelination [196]. Interestingly, *Quaking* and *Jimpy*, two known mutant mice harboring mutation in two oligodendrocyte proteins myelin-associated protein (Mag) and proteolipid protein (Plp) respectively, not only show defects in myelination, but also show a dramatic reduction of very long chain fatty acids (VLCFA) level in the brain and mRNA level of Elovl1 [201–203]. But there is a discrepancy in the severity of defects. Intriguingly, the degree of severity seems to be associated with the degree of reduced expression of Elovl1 mRNA level [196]. Another very important function of Elovl protein has been identified by the generation of Elovl3 knockout mice. These mice show a significant reduction of VLCFA and the skin-barrier is compromised [204]. Since most of the elongases identified are known to be expressed in the skin, it suggests that they are the structural components, required for the preservation of skin-barrier integrity [205–207].

Although an abnormal VLCFA level has been associated with many disorders of the nervous system, metabolism, skin permeability etc [208–210], the precise role of the of the FA chain length is not fully understood. A tissue specific expression pattern of certain Elovl protein underscores the fact that FA chain length serves different purposes in different tissues. Whether Elovl influences the membrane fluidity, lateral order or basic membrane architecture in order to exert its effect needs further investigations.

1.5.2 In *Drosophila*

In *Drosophila*, there are 20 putative elongases present in its genome and they are closely related to known Elovl proteins of yeast and mice (see Figure 1.9) [211]. These elongases

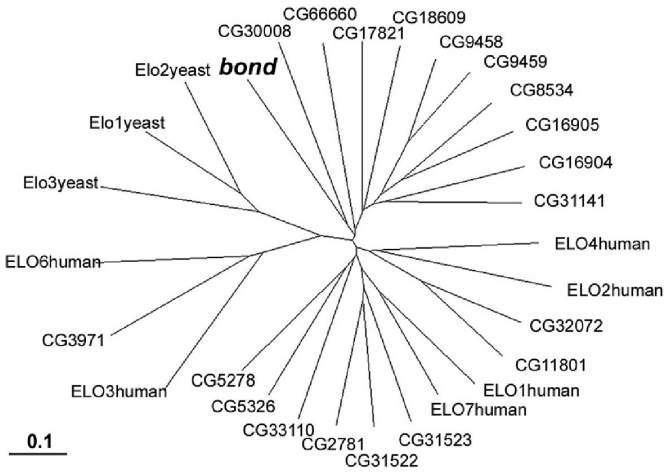


Figure 1.9: Phylogenetic tree of elongases from human, *S. cerevisiae, D. melaogaster*. Adapted from Szafer-Glusman *et al*, 2008 [211]. Reproduced with permission from Elsevier.

show substrate recognition similar to mammalian elongases [212]. The first elongase that is characterized in flies is *Elo68alpha*, an elongase that is expressed almost exclusively in the male reproductive system. It has the potential to elongate myristoleic and palmitoleic acids and likely to influence biosynthesis of vaccenyl acetate (cVA). cVA is a potent male pheromone that control male courtship and aggregation behavior [212]. Another elongase identified called *bond* which is homologous to mammalian Elovl6. It regulates the cytokinesis during spermatogenesis by participating in spindle assembly. It, probably, maintains acyl chain length of certain lipid components that are required to bring two plasma membranes close together during cell division [211]. *baldspot* or *noa* is another *Drosophila* elongase that is also involved in spermatogenesis. It has a somatic function and modulates male germline development. Additionally, *baldspot* mutants shows lethality and some viable mutants show motor deficits, suggesting that this elongase is critical for the cell vitality functions that cannot be compensated by other elongases [213].

Although several studies in mammals show various functions of Elovl proteins in the nervous system, no elongase has been identified in the nervous system of *Drosophila*. Further experimentation is required to check whether these elongases perform similar functions. A detailed study of all putative *Drosophila* elongases is therefore the key to shed light on the role of the elongases in the nervous system of flies.

1.6 Aim of the project

This study aimed to identify the genes with glial specific functions. So far, glial functions have been implicated in supporting axonal guidance, pathfinding and migration during development. How glia function in the mature nervous system is still elusive. Therefore, at first, an adult *Drosophila* model to study neuron-glia communication was established. By using GAL80^{ts}, GAL4 system, this model can drive expression of UAS-transgene specifically in the mature glial cells. A sublibray (containing genes predicted to have human homolog) of Vienna Drosophila RNAi Centre (VDRC) library was used to conduct the screen. All fly lines harbor a shRNA directed against a specific gene under a GAL4-UAS promoter. RNAi expression was specifically triggered in mature glia and the survival or motor defects of adult flies were scored. Candidates from the screening might have a potential glia-specific function. Notably, the knowledge from these results could be translated to the mammalian nervous system because of two reasons: first, all candidates have human homolog and second, glial functions are likely to be conserved across the species.

Chapter 2

Materials and Methods

2.1 Materials

2.1.1 Chemicals and consumables

All chemicals used in this study were purchased from AppliChem GmbH (Darmstadt, Germany) or Sigma-Aldrich Chemie GmbH (Munich, Germany) unless mentioned elsewhere. Consumables used were obtained from Falcon (Becton Dickinson Labware Europe, Le Pont De Claix, France), Eppendorf AG (Hamburg, Germany).

2.1.2 Buffers and solutions

2.1.2.1 Phosphate buffered saline (PBS)

PBS was prepared according to the following protocol.

10× PBS (1 L)

80.0 g	NaCl
2.0 g	KCl
14.4 g	Na_2HPO_4 (or 18.05 g $Na_2HPO_4 \times 2H_2O$)
2.4 g	KH_2PO_4

To obtain 1× PBS, 10× PBS was diluted 10 times with milliQ H_2O. The pH value was adjusted to 7.4.

2.1.3 *Drosophila* stocks and genetics

All fly stocks were maintained in normal cornmeal fly food at 18°C with 12:12 hour light and dark cyle with constant humidity. All the fly crossings were performed in 25°C or 18°C incubator. For temperature shift experiment to 29°C, another incubator was used. The following table (see Table 2.1) lists the genotype of all the fly lines used in the study.

Table 2.1: *Drosophila* stocks used in the study

Fly line	Genotype	Origin
repo-GAL4	w[1118]; P{w[+m*]=GAL4}repo/TM3, Sb	Auld *et al*, 2000
elav^{c155}-GAL4	P{w[+mW. hs]=GawB}elav[c155]	Bloomington
tub-GAL80	w[*]; P{w[+mC]=tubP-GAL80[ts]}20; TM2/TM6B,Tb	Bloomington
nrv2-GAL4	w[*]; P{w[+mC]=nrv2-GAL4.S}3	Bloomington
gliotactin-GAL4	w[*]; P{rl82-GAL4}/CyO	Sepp *et al*, 1999
NP6293-GAL4	y[*] w[*]; P{w[+mW.hs]=GawB}Bsg[NP6293] / CyO, P{w[-]=UAS-lacZ.UW14}UW14	DGRC,Japan
UAS-mCD8-GFP	PUAS-mCD8::GFP.L	Bloomington
UAS-reaper	w[1118]; P{w[+mC]=UAS-rpr.C}14	Bloomington
OregonR	wildtype	Bloomington

All RNAi fly lines were obtained from Vienna *Drosophila* RNAi Centre (VDRC). Each RNAi line was generated by the random insertion of shRNA in *Drosophila* genome and shRNAs are under the control of UAS-GAL4. In this study, a sub-library of 7881 genes with known or predicted human homologs were selected by VDRC. Complete list of genes of the library are given in the appendix. To validate RNAi knock down of the candidates, a second RNAi line was also ordered from VDRC. Additionally, RNAi against all genes involved in sphingolipid biosynthetic pathway were obtained from VDRC(see Table 2.2).

Table 2.2: Sphingolipid RNAi lines

Gene	CG	Transformant ID	RNAi Library	Off target
lace	4162	11081	KK	0
lace	4162	21805	GD	0
Spt-I	4016	108833	KK	0
Spt-I	4016	10020	GD	0
CDase	1417	110671	KK	0

CDase	1417	30189	GD	0
Ugt86Da	18578	105923	KK	1
Ugt86Da	18578	105923	GD	0
GlcT1	6437	108064	KK	1
GlcT1	6437	45275	GD	1
Sply	8946	103485	KK	1
Sply	8946	37974	GD	0
SK1	1747	32932	KK	0
SK1	1747	32930	GD	0
SK2	32484	101018	KK	0
SK2	32484	41905	GD	0
Cerk	16708	101550	KK	0
Cerk	16708	43413	GD	0
SMase	32052	105825	KK	0
SMase	32052	121437	GD	0
bbc	6016	110371	KK	0
bbc	6016	7989	GD	0
Pect	5547	27459	KK	1
Pect	5547	10982	GD	0
Schlank	3576	109418	KK	0
Schlank	3576	4114	GD	0
Des-1	9078	16665	KK	1
Css1beta	11426	42599	GD	0
Css2	31717	105379	KK	0
CSss2	31717	7662	GD	0

2.1.4 Equipments

Following equipments were used for the study.

Table 2.3: Equipments

Equipments	**Company**
LSM 510 Confocal Laser Scanning Microscope	Carl Zeiss
TCS SP2 Confocal Laser Scanning Microscope	Leica
SZ51 Zoom Stereo Microscope	Olympus
SteREO Discovery.V8 Microscope	Carl Zeiss
Real Time LightCycler 480	Roche

2.1.5 Softwares

The following softwares and online resources were used for the study.

Table 2.4: Software

Name of the Software	Source
Adobe Illustrator CS5	http://www.adobe.com/products/illustrator.html
BibTeX	http://www.bibtex.org/
Bingo bioinformatics	http://www.psb.ugent.be/cbd/papers/BiNGO/Home.html
Bloomington	http://flystocks.bio.indiana.edu/
Cytoscape	http://www.cytoscape.org/
DGRC, Japan	http://kyotofly.kit.jp/cgi-bin/stocks/index.cgi
Flybase	http://flybase.org/
GraphPad Prism	http://www.graphpad.com/prism/Prism.htm
ImageJ	http://rsbweb.nih.gov/ij/
LaTeX	http://www.latex-project.org/
qPCR primers	https://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp?id=UP030000
STRING	http://string-db.org/
Uniprot	http://www.uniprot.org/
VDRC	http://stockcenter.vdrc.at/control/main
Zeiss LSM image browser	http://www.zeiss.de/ImageBrowser

2.2 Methods

2.2.1 Fly lines generated for the study

To perform the primary screening, a fly line was generated by recombining repo-GAL4 with temperature sensitive tubulin-GAL80. Next for morphological analysis of the candidates, pan-glial driver repo-GAL4, wrapping glia driver nervana2-GAL4, subperineurial glia driver gliotactin-GAL4 and perineurial glia driver NP6293-GAL4 was recombined with UAS-mCD8-GFP. This enabled us to study glial membrane morphology upon knockdown of genes specifically in a subset of glia. The genotypes of the flies are mentioned before (see Table 2.1).

2.2.2 The primary screening

tub-GAL80^{ts}; repo-GAL4 fly line was used to induce the expression of shRNA under GAL4-UAS promoter specifically in the adult stage. 5-7 virgin females of this line was crossed with 3-4 males from each RNAi line. All the crossings were set at 18^{o}C to inhibit the expression of GAL4 by GAL80^{ts}. After raising the offsprings until adulthood at 18^{o}C, the adult males were shifted to 29^{o}C to check the lethality or climbing defect after 10 days. At this restrictive temperature, GAL80^{ts} was inhibited and thereby allowed the expression of GAL4 that was under the control of glial specific repo promoter. Thus, by temperature-switching all shRNA were expressed in the mature glial cells. This fly line was also used to ablate glia in adult flies in order to study the neuron-glia communication in the mature nervous system of *Drosophila*. UAS-reaper was crossed with this fly line as mentioned above. Adult males were analyzed for longevity and climbing assay.

2.2.3 Longevity of flies upon adult glia ablation

To deplete the mature glial cells tub-GAL80^{ts};repo-GAL4 flies were crossed with UAS-reaper, and OregonR (negative control). The flies were raised in permissive temperature 18^{o}C. Then 3-4 days post-hatching, adult male flies with the respective combination of GAL4-driver, UAS-transgene and GAL80^{ts} were shifted to restrictive 29^{o}C (10-15 flies per vial). The number of dead flies were counted everyday and fresh fly food was provided every 2-3 days. At least 50 flies per genotype were used for the assay. For statistical significance Log Rank Test (Mantel-Cox) was performed using GraphPad prism software.

2.2.4 Climbing assay

3-4 days post-hatching, 30-40 adult male flies per genotype were shifted to 29^{o}C and raised for required days. Fresh food was provided every 2-3 days after shifting to 29^{o}C. In order to asses locomotion, the negative geotaxis assay or climbing assay was performed. In this experiment, flies were partitioned up into six tubes by giving them the choice five times to stay or to climb up the side of the tube. After the assay, flies were distributed into six tubes depending on how many times (between 0 and 5 times) they climbed up. To represent the distribution of the flies, the number of the flies in the tubes 1st and 2nd (group 1), 3rd and 4th (group 2), and 5th and 6th (group 3) tubes were summed up and

plotted graphically. For every time point, flies in group 1 and group 3 were compared to respective controls and t-test was performed for statistical significance. Only one time point of 10 days was considered for the experiment [214].

2.2.5 *Drosophila* dissection procedure

2.2.5.1 Adult brain

At first, adult flies were anesthetized with CO_2 and the flies were soaked with 100 % ethanol to break the surface tension. Next, the flies were placed on a silicon plate with PBT solution. The fly was fixed with insect pins at the thorax with ventral side up and the proboscis was removed with the help of a pair of fine forceps. Once proboscis was removed, an opening at the mouth was created. Now, slowly the head capsule was removed by one forceps, grabbing the mouth opening part with another forceps. After completely removing the head capsule, remaining air sacs, tracheal tissues were cleaned up as far as possible. The whole brain dissection was performed as quickly as possible in order to avoid apoptosis due to hypoxia [215].

2.2.5.2 L3-larval peripheral nervous system

Drosophila L3 wandering larva was collected from the crossing vials and placed on a silicon plate. After several washing with ice-cold PBS, each larva was fixed with insect pins. Larva with dorsal side up was fixed by placing two insect pins, one at the mouth part and the other at the edge of the abdomen. Next, a fine scissor was used to make incision at midline and the larva was opened up along the midline. After removing the trachea completely, the larval PNS was exposed by clearing off the gut. By placing four insect pins at the larval fillet, the PNS architecture was properly aligned. Ice-cold PBS was used to wash from time to time during the dissection to minimize the movements of the larva.

2.2.6 TUNEL assay

To detect apoptotic cells, *in situ* cell death detection kit from Roche was used and performed according to the manufacturer's protocol. 5 μl enzyme solution and 225 μl label

solution were mixed to prepare terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) reaction mixture. To the TUNEL reaction mixture, quickly dissected adult brain fixed with 4% PFA were added and incubated for 1 hour at 37°C in dark. Next, the saline-sodium citrate buffer (Promega) was used to stop TUNEL reaction to avoid unspecific staining.

2.2.7 Immunohistochemistry

Adult *Drosophila* brains were dissected in 1× PBS +0.1% Triton X-100 (PBT) and fixed with 4% PFA for 30 min at room temperature. After fixation 1× PBT was used 3 times for washing. 10% horse serum was used as blocking solution for 30 min. Primary antibodies (both obtained from Developmental Studies Hybridoma Bank, University of Iowa, USA) anti-Repo (1:100) and anti-Elav (1:200) were diluted in PBT with 2% horse serum and incubated at 4°C overnight. 1× PBT was used thrice for washing after primary antibody incubation. For primary antibody detection, Cy3-coupled antibodies anti-mouse or anti-rat (Dianova) were used in a 1:200 dilution. After extensive washing by 1× PBT, brains were mounted with Vectashield + DAPI (Vectorlab).

Drosophila L3 stage larva was dissected in 1× PBS and PNS was fixed with bouins fixative solution for 3 min. Then the tissue was permeabilized with 1× PBT solution for 15 min. For blocking (1 hour) and antibody dilutions 10% goat serum was used. Primary antibodies GFP (invitrogen, Germany), anti-HRP-Cy3 (Dianova, Germany), antiHRP-aexa647 (Dianova, Germany), anti-repo (DSHB, University of Iowa, USA) were used with 1:1000, 1:200, 1:200, 1:20 dilutions, receptively. Primary antibodies were incubated overnight whereas secondary antibodies anti-rabbit alexa-488, mouse alexa-647 (both form Invitrogen) mouse-Cy3 (Dianova, Germany) were used with 1:200 dilutions for 2 hours. After washing with PBT 3 times, larva fillet was mounted in Vectashield and mouth part was removed.

2.2.8 Quantification of lace phenotype

Approximately 200 μm nerve segments were imaged randomly from A3 or A4 body wall segment. Five nerve width were measured approximately after every 40 μm along the length of the nerve. Every five measurements of each nerve were considered as an ordered

quintuplet $(d_1, d_2, d_3, d_4, d_5)$. This five values were used to estimate average cross-sectional area of the nerve with the following equation:

$$A = (\pi/48)(d_1^2 + d_1 d_2 + 2d_2^2 + d_2 d_3 + 2d_3^2 + d_3 d_4 + 2d_4^2 + d_4 d_5 + d_5^2)$$

This estimated cross-sectional area of the nerve was calculated by considering the volume of the nerve same as that of the cylinder. At least 5-7 nerves per animals were used to measure this A-value. A-values form each animal were averaged and mean of these average values were compared among control and lace knockdown groups. Unpaired t-test was performed for the statistical significance analysis [57].

2.2.9 Quantification of wrapping glia phenotype

For the analysis of wrapping glia defects, Nrv-GAL4 was crossed with different UAS-shRNA lines. Images of L3 larva stage PNS were taken for both control and treated groups with exactly same settings of the confocal microscope. Quantification of the intensity was performed using ImageJ software (NIH, USA). The signal density was given as the mean grey value per square micrometers.

2.2.10 Microscopy

2.2.10.1 Confocal microscopy

To visualize TUNEL positive nuclei, images of the central region of the adult drosophila brain were acquired with a Leica confocal (LSM/SP2) with a 63× oil-immersion objective. Z-stacks images were obtained with Leica TCS SP2 AOBS confocal laser scanning setup. Image processing and colocalization analysis was done with ImageJ software.

L3 PNS was imaged with Zeiss confocal microscope (LSM 510) having 40× water-immersion objective. Images with z-stacks were taken and digital projections of the stack and optical orthogonal section was analyzed using Zeiss LSM image browser software. ImageJ was used for the image processing.

2.2.10.2 Electron microscopy

Larval fillets were fixed with a mixture of 4% PFA and 2.5% glutaraldehyde in 0.1M PBS for 4 hours at room temperature. The fillets were washed with PBS and then were subjected to perform a post-fixation with 1% osmium tetroxide for 1 hour at 4°C. Next, the post-fixed fillets were dehydrated and stained with a mixture of freshly prepared 1.5% uranyl acetate and 1.5% tungstophosphoric acid. After completion of dehydration process, the fillets were embedded in Epon. Then the silver sections were cut and contrasted with 4% uranyl acetate followed by 0.3% lead citrate. Multiple sections were cut, contrasted and imaged for every genotype. The sections were imaged with a LEO EM912 Omega electron microscope (Carl Zeiss, Germany) and the digital micrographs were obtained with an on-axis 2048 × 2048 CCD camera (Proscan GmbH, Germany).

(Electron microscopic imaging was performed by Nicolas Snaidero and Tina Kling.)

2.2.11 Quantification of mRNA expression

2.2.11.1 RNA isolation

30 fly heads were flash frozen in liquid nitrogen and they were lysed in 1 ml Trizol using a pestle. After keeping it for 10 min in 37°C, the homogenate was centrifuged at 12000g for 10 min at 4°C. The supernatant was collected and proceeded further for chloroform: isopropanol based RNA extraction. 200 μl chloroform was added to the supernatant and vortexed. Following the centrifugation at 12000 g at 4°C for 15 min, the aqueous phase was transferred to 500 μl isopropanol and centrifuged for 15 min at 4°C. Isopropanol was discarded form from the pellet and the pellet was resuspended with 200 μl H_2O, 500 μl 96% ethanol and 70 μl ammonium acetate (5 M). The mixture was precipitated at -80°C overnight. Following day, it was centrifuged at 12000g at 4°C for 30 mins and the supernatant was discarded. The pellet was washed twice with 70% ethanol and resuspended in 25 μl sterile RNAse free water. RNA isolation procedure did not yield pure RNA. Therefore, DNA free kit from Ambion was used to clear genomic DNA contamination from the RNA according to the manufacturer's protocol. Finally pure RNA was dissolved in RNAse free water provide in the kit.

2.2.11.2 cDNA synthesis

2 μg of RNA was used for cDNA synthesis using SuperScript III® First-Strand synthesis kit. 2 μg RNA, 1 μl of 50 μM oligo(dT)$_{20}$, 1 μl of 10 mM dNTP mix and sterile water were mixed to make up volume to 10 μl. The mixture was incubated at 65°C for 5 min and then cooled down to 4°C. A Reverse Transcriptase mix (RT mix) was prepared by mixing 2 μl 10X RT buffer, 4 μl 25mM $MgCl_2$, 2 μl 0.1M DTT, 1 μl RNAseOUT and 1 μl Superscript III RT. All of the reagents were provided in kit. RT mix was added to pre-cooled RNA-mix and incubated for 50 mins at 50°C. The reaction was stopped by increasing the temperature to 85°C for 5 min. 1 μl RNAse H was added and incubated for 20 min at 37°C to cleave remaining RNA. The mixture was cooled to 4°C and cDNA samples were stored at -20°C.

2.2.11.3 Semi-quantative RT-PCR

RT-PCR was performed to analyze the expression of the genes in different tissues. cDNA samples were used as template to do a normal semi-quantitative PCR. 2 μl cDNA (1:10 dilution), 0.3 μl of each primer, 2.5 μl of 25 mM $MgCl_2$, 1 μl 10 mM dNTP, 10 μl 5X GoTaq® flexi reaction buffer, 0.2 μl of Go-Taq® flexi DNA polymerase were mixed and sterile water was added to make final volume 50 μl. The primers used are mentioned below. General protocol used for the RT-PCR was as the following:

98°C for 1 min
25 cycles of
98°C for 30 sec
56°C for 15sec
72°C for 1 min

72°C for 10 min
4°C pause

Table 2.5: Primers for RT-PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Baldspot	GACTCTTCCACTCCGTCTGC	AGCAGGGTGATGTGGTGATA

CG18609	CAGGTGTTCATGTCCTTT GG	GATGGCATAACTGAGCAGCA
Elo68alpha	TTATATAGGTTTCTTGCC	ATGGCATCACTTGGCATCTCATTTC
Elo68beta	ATGACGTCGTCGATGGGTAATGA	TTACTTGGCTTTCTTTACAACTGCCG
elav	CGCACAAACCTTATTGTCAACTAC	AATTTTACCACTATGGGGTCTGTG
GAL80	CCGTGCCTAATGCAGCTCC	ATAAACGCTCTCGATTAACC

2.2.11.4 Quantitative real-time PCR

In order to quantify the relative abundance of mRNA level, quantitative real-time PCR (qPCR) was performed using SYBR green based detection method in a real-time cycler. Primers were generated by the primer design tool from Roche Applied Science: Universal probe library. 200 ng cDNA was used for the amplification using appropriate primers and Power SYBR Green qPCR mix (Roche, Germany) following manufacturer's instructions. Relative mRNA expression was calculated using the ΔCt method and normalized to housekeeping gene actin. General protocol and the primers (Table 2.6) used for the quantitative real-time PCR were as follows.

98^{o}C for 1 min
25 cycles of
98^{o}C for 30 sec
56^{o}C for 15sec
72^{o}C for 1 min

72^{o}C for 10 min
4^{o}C pause

Table 2.6: Primers for qPCR

Gene	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
actin5C	CACACCGTGCCCATCTACGAGG	CTTCTGCATACGGTCGGCGATGC
Elo68alpha	TGGATATATCTGCCTGGAACTCT	CCAAAATGCCTTTGTAAGACG
Elo68beta	TGTTCTTGTTTTGCTGGACTTATG	CAAAAGAGTTTATCATGCTTGGAA

2.2.12 Statistical analysis

Statistical analysis of the data was performed with GraphPad Prism software (USA) (seeTable 2.4). To compare two independent groups with sample sets showing normal distribution and equal variance, the parametric t-test was used. For the analysis of *Drosophila* survival curves, Log-Rank test (Mantel-Cox) was performed using Prism. p-values were corrected using the Bonferroni correction. p-value less than 0.05 was considered as significantly different.

Chapter 3

Results

Some parts of the results have been published in:

> Targeted Ablation of Oligodendrocytes Triggers Axonal Damage
> Aniket Ghosh*, Natalia Manrique-Hoyos*, Aaron Voigt, Jörg B. Schulz, Mario Kreutzfeldt, Doron Merkler, Mikael Simons
> PLoS ONE 6(7): e22735. (2011)

3.1 Glial ablation triggers neuronal damage in adult *Drosophila*

3.1.1 Glial ablation causes neuronal apoptosis

We used *Drosophila melanogaster* as a model system to analyze the impact of glial damage on neurons in the mature nervous system. To ablate all the glial cells in adult fly UAS-GAL4 system was used in combination with temperature sensitive $GAL80^{ts}$. A proapoptotic gene *reaper* was expressed in adult glia to deplete the mature glial cells using pan-glial driver repo-GAL4 in combination with tubulin-$GAL80^{ts}$. All crossings were set at 18°C and therefore, glial specific GAL4 expression was suppressed during the development by the action of $GAL80^{ts}$ which is under control of tubulin promoter. All the male offsprings were then switched to 29°C, subsequently inhibiting the action of $GAL80^{ts}$. Thus *reaper* was expressed specifically in the mature glial cells to trigger apoptosis.

Next, we performed TUNEL staining and immunofluorescence using antibodies against neuronal and glial markers to detect apoptotic cells (Figure 3.1A). Optical sections of adult brain were examined 0, 3 and 10 days after the induction of glial apoptosis to quantify the extent of neuronal and glial damage. We observed a significant increase in the

number of TUNEL-positive neurons (Elav-positive cells) with time (Figure 3.1C) whereas apoptotic neurons were almost absent from control brains (Figure 3.1B).

3.1.2 Glial damage causes reduction of lifespan and locomotion defect

In order to analyze the consequences of glial cell death, we performed two behavioral experiments. First, longevity analysis was done to asses the lifespan and second, negative geotaxis was scored to measure the locomotion defect. There was a dramatic reduction of longevity of adult flies upon induction of glial apoptosis. Flies died within 20 days after switching the temperature form 18°C to 29°C. However, control flies were alive beyond 50 days (Figure 3.2A). Median life span was also significantly reduced for flies expressing *reaper* in glia (Figure 3.2B). Survival curve analysis showed a dramatic reduction of lifespan and statistical analysis of survival curves was presented in (Figure 3.2C).

Additionally, to measure the locomotion defect we used negative geotaxis assay. Normally all flies climb away from gravity and thus the negative geotaxis is directly correlated with their motor performance or locomotion. Hence, this assay was used to asses motor deficit of the flies upon inducing apoptosis in glia. Our result indicated that there was a significant reduction of climbing ability of the flies after 10 days of temperature-shift (Figure 3.3).

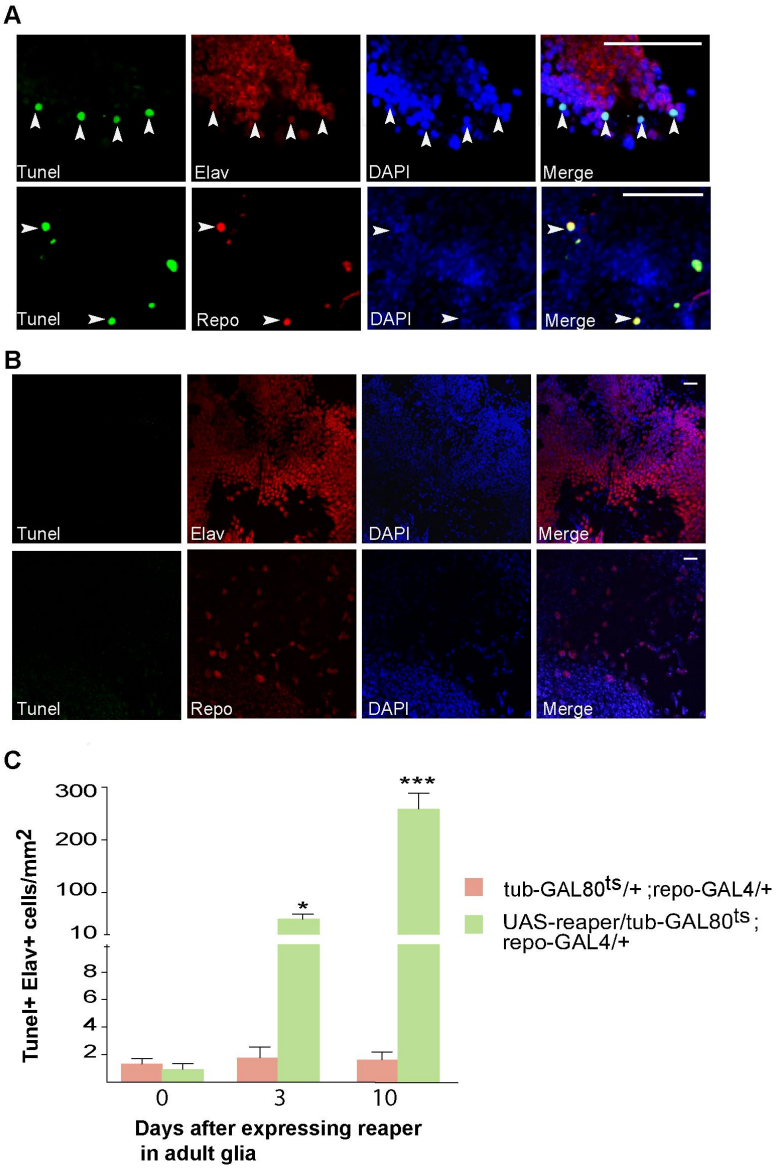
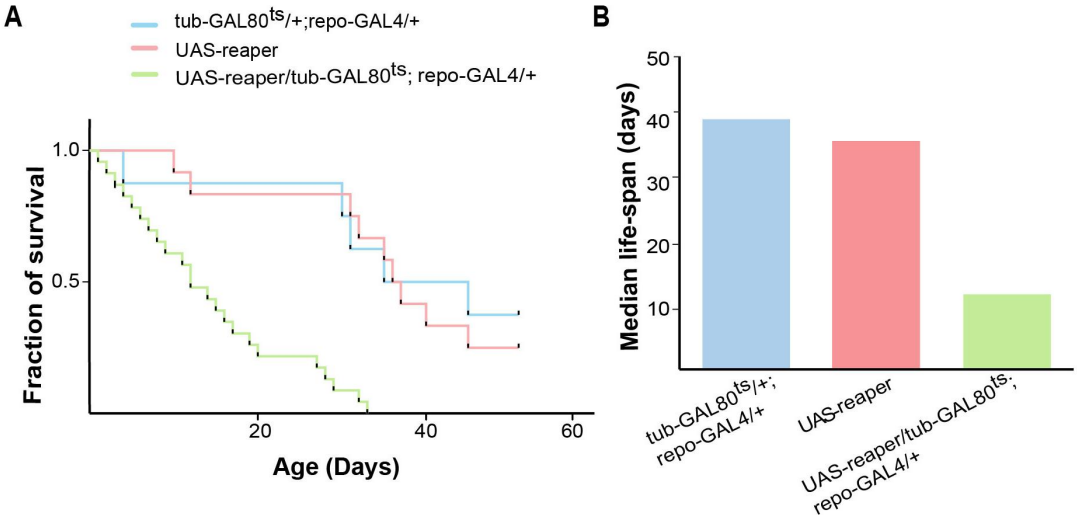


Figure 3.1: Glial ablation causes neuronal apoptosis. **(A)** Glial cell death was induced in transgenic flies by expressing *reaper* for 10 days in adult flies (UAS-reaper/tub-GAL80^{ts}; repo-GAL4/+). Double-label immunofluorescence and TUNEL staining on adult fly brains. Colocalization of glia-specific protein repo and neuronal-specific protein elav with TUNEL positive nuclei (green) reveals the presence of apoptotic neuronal and glial nuclei (arrows). **(B)** Double-label immunofluorescence and TUNEL on the brain of control flies (tub-GAL80^{ts}/+; repo-GAL4/+). Scale bar: 20 μm. DAPI serves as nuclear staining (blue). **(C)** Quantitative analysis of TUNEL positive neurons. Unpaired t-test was performed for statistical analysis. Values presented as mean+ SEM. $*$ p$<$ 0.05, $***$ p$<$ 0.001.



C

Comparison -of survival curves			
tub-GAL80^{ts}/+; repo-GAL4/+	vs	UAS-reaper/tub-GAL80^{ts}; repo-GAL4/+	***
UAS-reaper	vs	UAS-reaper/tub-GAL80^{ts}; repo-GAL4/+	***
tub-GAL80^{ts}/+; repo-GAL4/+	vs	UAS-reaper	ns

Figure 3.2: Glial ablation in adult *Drosophila* reduces lifespan. (A) Glial cell death was induced in transgenic flies by expressing *reaper* for 10 days in adult flies (UAS-reaper/tub-GAL80^{ts}; repo-GAL4/+). tub-GAL80^{ts}/+; repo-GAL4/+) and UAS-reaper were used as negative control. **(B)** Median survival of respective survival curve.**(C)** Summary of statistical significance (Log-rank-Mantel-Cox Test) by cross-comparison of the survival curves. $*p < 0.05$, $**$ $p < 0.001$, ns not significant.

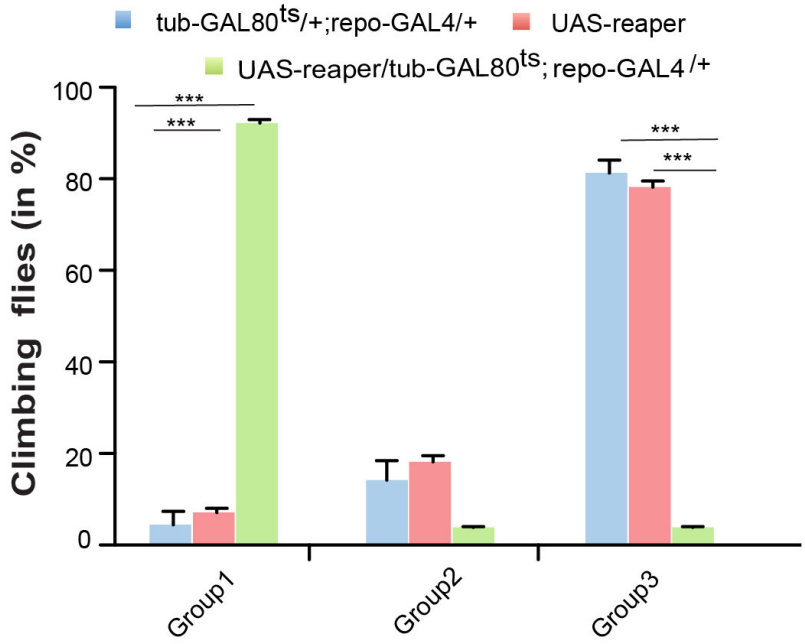


Figure 3.3: Glial ablation in adult *Drosophila* impairs locomotion. Locomotion defect of flies was analyzed with negative geotaxis and was quantified 10 days after shifting the flies from 18°C to 29°C in a countercurrent apparatus. Experimental flies (UAS-reaper/tub-GAL80^{ts}; repo-GAL4/+) were compared to control flies (tub-GAL80^{ts}/+; repo-GAL4/+) in group1 and group3 to have better assessment of motor defect. One-way ANOVA followed by Bonferroni *post hoc* test was used for statistical significance ($***p<0.0001$).

3.2 The screen

All VDRC-derived RNAi lines were entered into a database and an internal number was annotated with every RNAi line. This provides the opportunity for blinded experiments and reduces the bias of the outcome. A sub-library of 7881 RNAi lines (complete list in the appendix) known to have human homolog were obtained from Vienna *Drosophila* RNAi Center (VDRC). Each RNAi line encodes a shRNA against a specific gene of *Drosophila* as they have minimal or no known off-target effect [216].

The scheme of the screening (Figure 3.4) is presented below. Expression of shRNA was restricted to glial cells and in the adult stage by using pan-glial driver line repo-GAL4 in combination with temperature sensitive (ts) GAL80 under the control of ubiquitous tubulin promoter (tub-GAL80^{ts}). Crossing of virgin females (tub-GAL80^{ts}; repo-GAL4) with 2-3 males from UAS-shRNA fly lines were set at 18^{o}C. After 3 weeks male adult flies from F1 generation were shifted to 29^{o}C to induce shRNA expression. After 10 days, RNAi lines showing lethality or motor defect in at least in 50% flies were counted as primary hits.

Before, the genome-wide screening was performed, a pre-screening was performed by crossing males of UAS-nejire RNAi with tub-GAL80^{ts}; repo-GAL4 virgin females flies at 18^{o}C. Adult males from F1 generation were then shifted to 29^{o}C to induce RNAi. We found that glial loss of cell-vitality protein *nejire* showed a drastic reduction longevity and all flies died within 10 days, whereas controls (tub-GAL80^{ts}/+; repo-GAL4/+) lived more than 50 days (Figure 3.5). This is an important result for our screening that suggests the importance of glia in the mature nervous system and indicated the utility of our GAL4-GAL80^{ts} based fly model system to reveal genes with glial specific functions.

3.2.1 Primary screening

Primary screening data indicated that 11% of the total RNAi lines used in the screening, showed lethality (861 lines) and 0.45% was scored for motor defect (30). 82% RNAi lines of the library did not show any phenotype (6360 lines) while 630 lines were not analyzed. The results are summarized in the figure below (Figure 3.6).

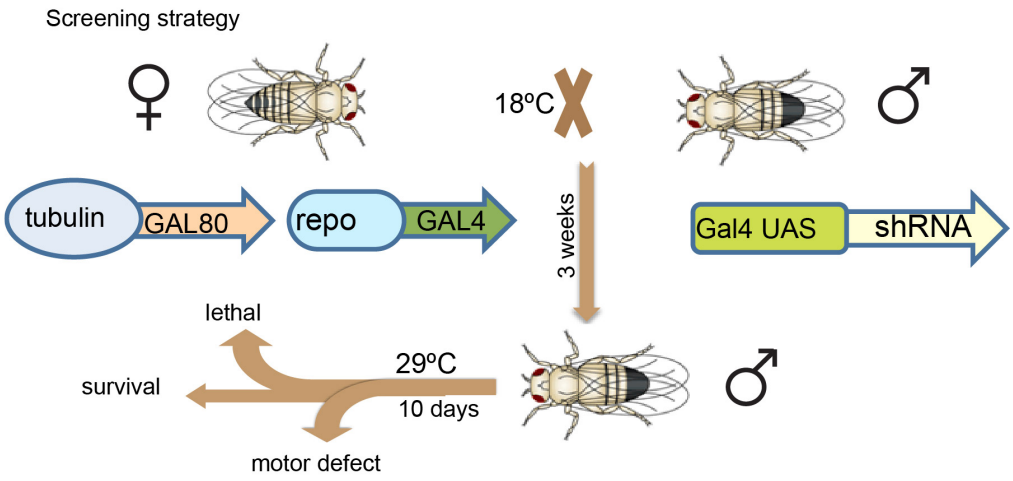


Figure 3.4: Scheme for genome-wide RNAi screening strategy. *Drosophila* shRNA was expressed specifically in adult by using GAL80-GAL4 system and temperature shift from 18ºC to 29º. Adult flies were switched to 29ºC to induce shRNA expression. Data analyzed after 10 days for motor defect and lethality.

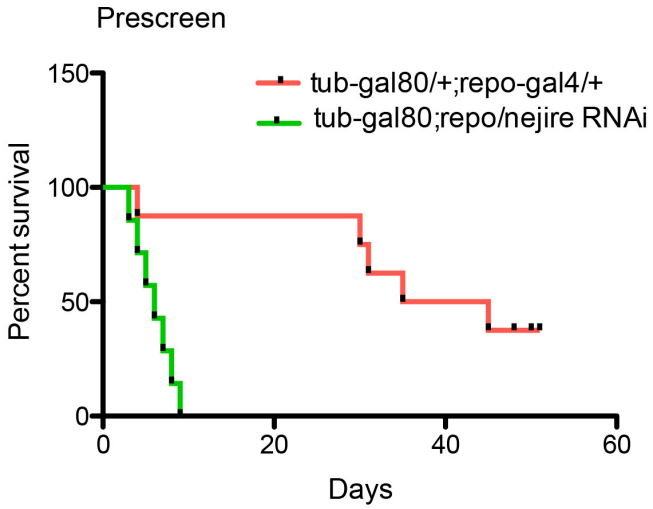


Figure 3.5: Pre-screening with nejire RNAi. nejire RNAi was expressed specifically in the mature glia and survival of the flies was assessed. Flies expressing nejire RNAi (tub-GAL80; repo/UAS-nejire RNAi) died within 10 days of temperature-shift, but control flies(tub-GAL80/+; repo-GAL4/+) survived more than 50 days. Survival curves were analyzed with Log-Rank Mantel Cox test. $p < 0.0001$.

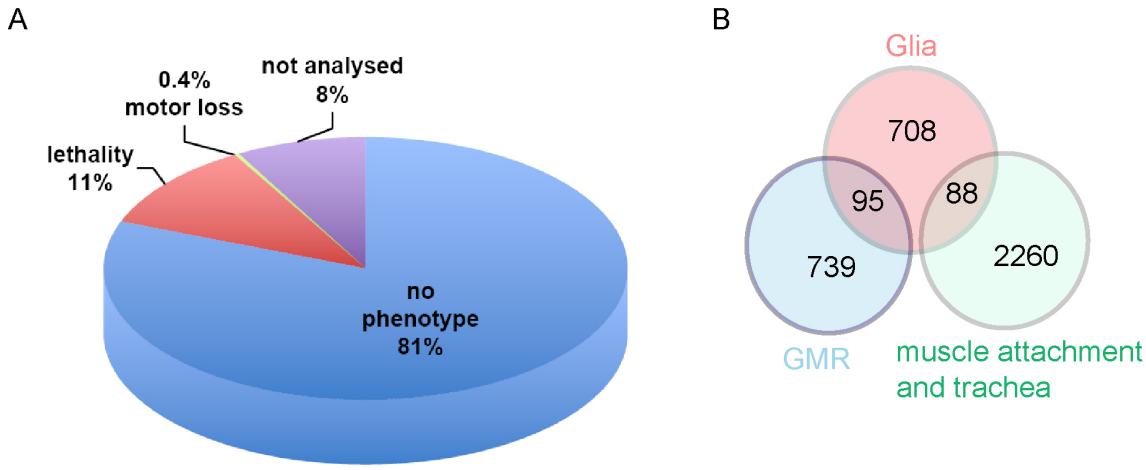


Figure 3.6: Results from primary screening. (A) Out of 7881 lines, 7251 lines were analyzed. 861 lines showed lethality, 30 lines showed impaired climbing performance and 6360 lines showed no phenotype. 630 lines were not analyzed. **(B)** By comparing glial screening data with three other RNAi screening results (GMR, Muscle attachment, trachea) that used same RNAi library, 708 candidates having glia specific function were identified.

To exclude RNAi lines having unspecific effects, our screening data was compared with three other screening data where same RNAi sub-library was used but shRNA was expressed specifically in the eye (GMR), trachea and muscle attachment site. 95 lines from glial screening, showed lethality when they were expressed by using eye (GMR). By comparing the screening results using muscle attachment and trachea specific driver line, 88 RNAi lines were excluded.

The data for GMR screen was kindly provided by Aaron Voigt; muscle attachment and tracheal screen data was provided by Reinhard Kühmlein.

3.2.2 Functional categories of candidates with glial specific functions

After exclusion of RNAi lines that showed a phenotype in other screenings, a list of 708 RNAi lines were identified as genes having glial specific functions. All glial screening-specific candidates were then subjected to categorization. Categorization was done based upon GO annotated biological processes of all the candidates and their predicted human homologs (Figure 3.7). For this purpose, we used the BiNGO plug-in of Cytoscape (see Table 2.4). The hypergeometric test was performed and overrepresented categories were

displayed after Benjamini and Hochberg False Discovery Rate correction. The database used for the analysis was GOSlim-generic and the significance level was set at 0.05.

Functional categorization reveals that the screened candidates are significantly overrepresented in certain categories such as metabolism, cell proliferation and differentiation, protein transport, cellular component organization, multicellular organismal development and anatomical structure morphogenesis. Interestingly, our list for glia-specific candidates were significantly enriched with carbohydrate and lipid metabolic process. This result suggests that glia might support neurons by providing sugars, fatty acids and other metabolites to maintain its integrity. Since, we found two general metabolic pathways appeared to converge to perform a common function in relation to neuron-glia communication, the proteins involved in these pathways might have close interactions with each other. In order to check this, we generated an interactome map (Figure 3.8) with carbohydrate and lipid metabolic candidates by STRING, a software for known and predicted protein-protein interactions (see Table 2.4). The interactome map was based upon experiments, text mining, database search and had a confidence level 0.15. This map clearly unveils that most of the lipid and sugar metabolic candidate genes have three or more interacting partners pointing out a common biological function in neuron-glia communication.

3.3 Secondary screening

Based upon interactome data, a secondary screening was performed using the candidates that have at least three binding partners. In order to identify the glia specific functions, these genes were knockdown using pan-glial driver line repo-GAL4. A membrane-tagged GFP (mCD8-GFP) was recombined with repo-GAL4 to visualize the glial membrane morphology. The PNS of *Drosophila* L3 larva was dissected and immunostained for the morphological investigation. Immunolabeling with GFP and HRP showed glia and neuronal membrane morphology, respectively. Different phenotypes viz. swelling, wrapping defect, neuronal splitting, glial organization defects were observed (Figure 3.9). Strikingly, *lace, baldspot, CG4095* that alter integrity of neurons and glia are components of sphingolipid metabolism. Hence, this data indicates a crucial role of glial sphingolipid metabolism in

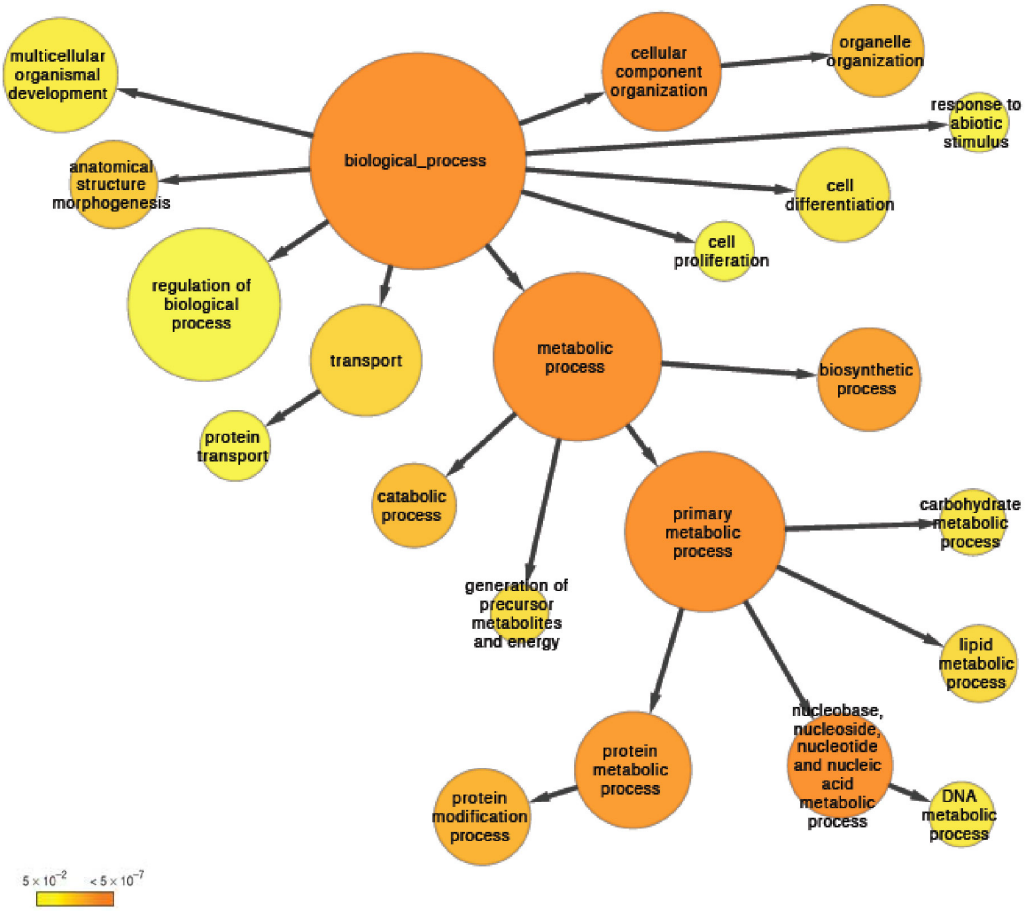


Figure 3.7: Categories of candidates based on GO biological process. Overrepresented categories from primary screening candidates are shown. The color shade of the circles indicates significance level (yellow, false discovery rate ¡ 0.05), and the size of each circle denotes the number of genes in each category.

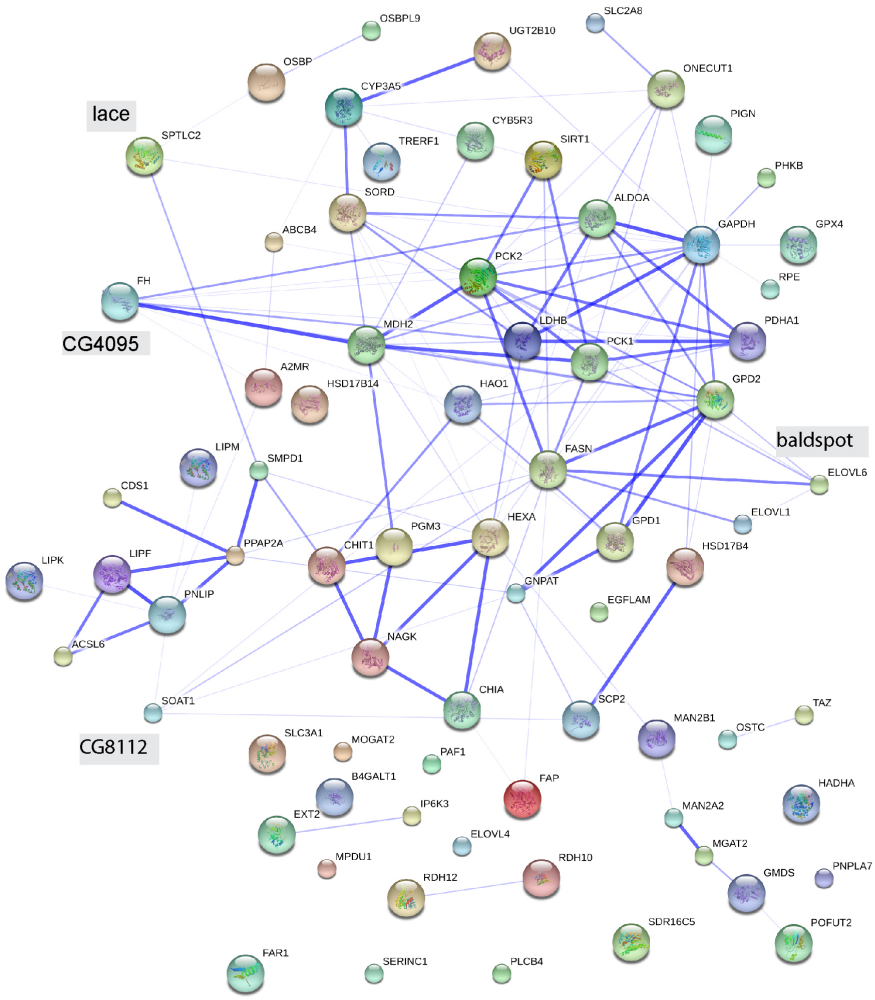


Figure 3.8: Interactome of metabolic candidates. Confidence view is presented in the map. Thicker lines represent the stronger association. *Drosophila* genes that show morphological defects in later analysis are highlighted.

maintenance of neuron-glia morphology.

3.4 *lace* is critical for glial wrapping around axons

3.4.1 Specificity of the candidate: *lace*

From secondary screening, it appeared that glial loss of *lace* affects axonal insulation by glia in the PNS. Knockdown of *lace* by repo-GAL4 with two different RNAi lines showed glial swelling and defects in axonal ensheathment (Figure 3.10A). Additionally, orthogonal section of the peripheral nerves showed wrapping defects of glial processes (Figure 3.10B). By using two different RNAi lines, we excluded the possibility of the nonspecific effects of RNAi knockdown. From the quantification of lace phenotype upon knockdown by two different RNAi, it turned out that glial loss of *lace* significantly affects glial membrane wrapping (Figure 3.10C). *lace* phenotype was 100% penetrant as we observed the bulging in all eight pairs of abdominal nerves in all larval fillet preparations examined (n=>15). The bulging of glia was localized to one region but their appearance was random in nature along the peripheral nerves. The diameter of the nerve at bulging regions ranged from 10 μm to 30 μm. In contrast, repo-GAL4/+ control flies have nerves with uniform diameter of 5-7 μm and axons were straight and packed in bundles.

3.4.2 *lace* is required for wrapping glia

Next, we wanted to check which of the glial subtypes requires *lace* specifically. Therefore, lace RNAi was expressed in combination with different glial subtype-specific driver lines. nervana-GAL4, gliotactin-GAL4 and NP6293-GAL4 lines were used to asses the role of wrapping, subperineurial, perineurial glia respectively. By using glial subtype specific drivers, we observed that *lace* was crucial for wrapping glia to mediate axonal ensheathment. Quantification of GFP signal from nervana-positive cells was significantly reduced in flies having genotype Nrv2>mCD8GFP/lace RNAi compared to control (Nrv2>mCD8GFP/+) (Figure 3.11). This indicates that sphingolipids are necessary for normal membrane morphology of the wrapping glia. In contrast, neuronal loss of *lace* by using pan-neuronal elav-GAL4 did not alter neuronal membrane morphology suggesting that sphingolipids are preferentially required for glial processes to insulate axons (Fig-

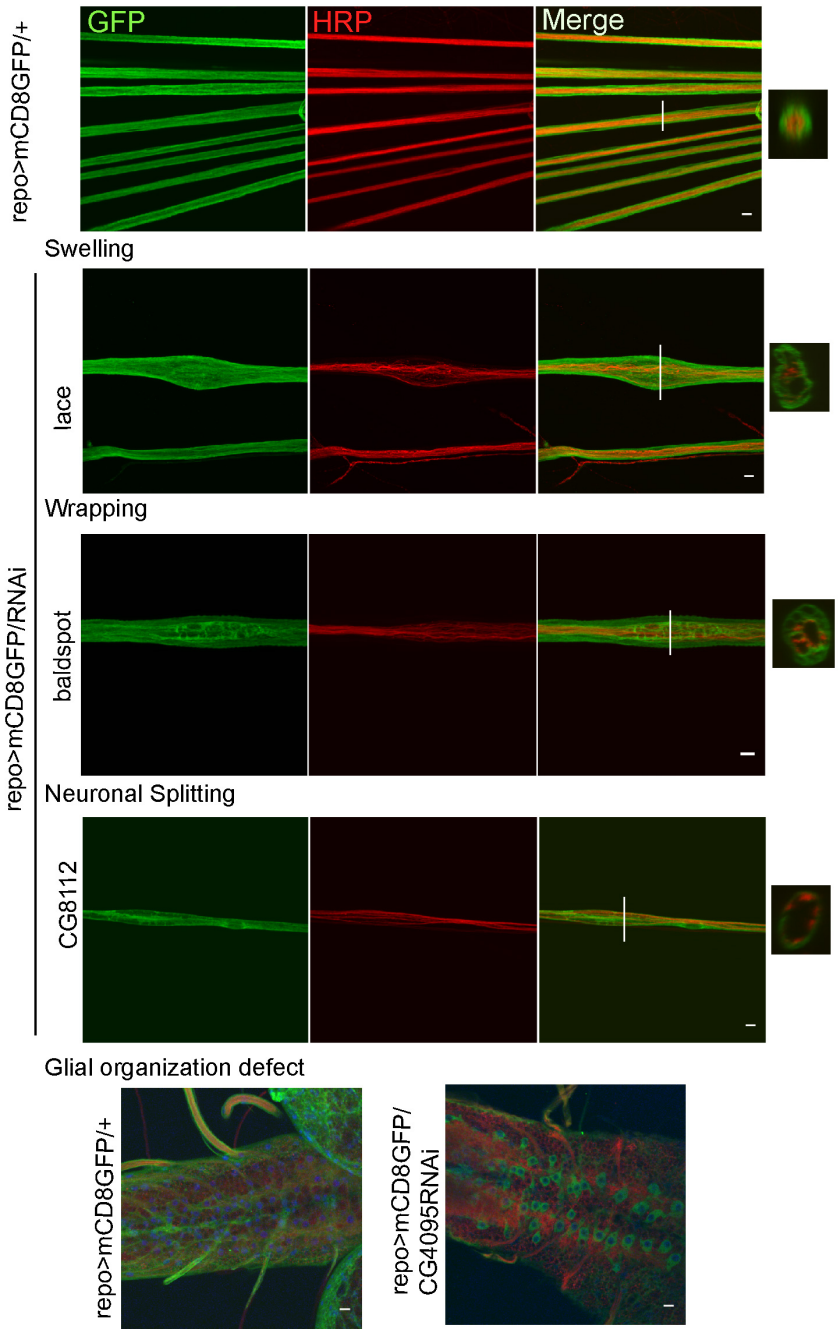


Figure 3.9: Secondary screening with metabolic candidates. UAS-RNAi was expressed using pan-glial driver *repo*-GAL4 and the effects were visualized in L3 PNS. Different phenotypes observed, were shown in the figure. Glial membrane was imaged by expressing UAS-mCD8-GFP(Green). HRP (red) stained neuronal membrane. Projection of all z-stacks are presented in the panel, orthogonal sections are presented as inset. The position of orthogonal section is indicated in the respective panel by a white line. Scale bar 10 μm.

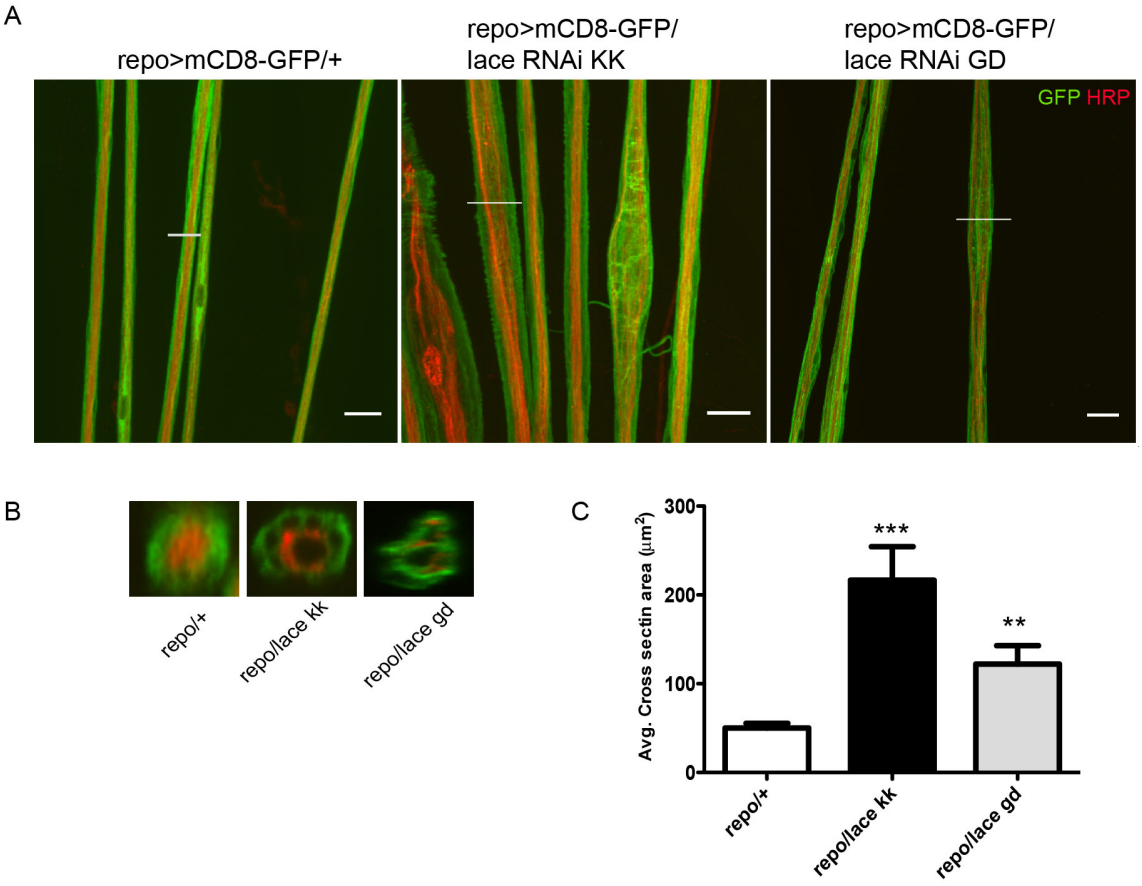


Figure 3.10: Knockdown of *lace* shows glial wrapping defect. (A) In the PNS, glial membrane (green) swelling and wrapping defect around axons (red) were observed upon knockdown of *lace* with two different RNAi lines (kk and gd). repo>mCD8-GFP/+ served as control. Merged projection of all confocal z-stacks is presented. **(B)** Orthogonal section of the nerve region is marked by a white line in respective genotype. **(C)** Quantification of average cross-section area upon *lace* knockdown specifically in glia. Scale bar 20 μm.

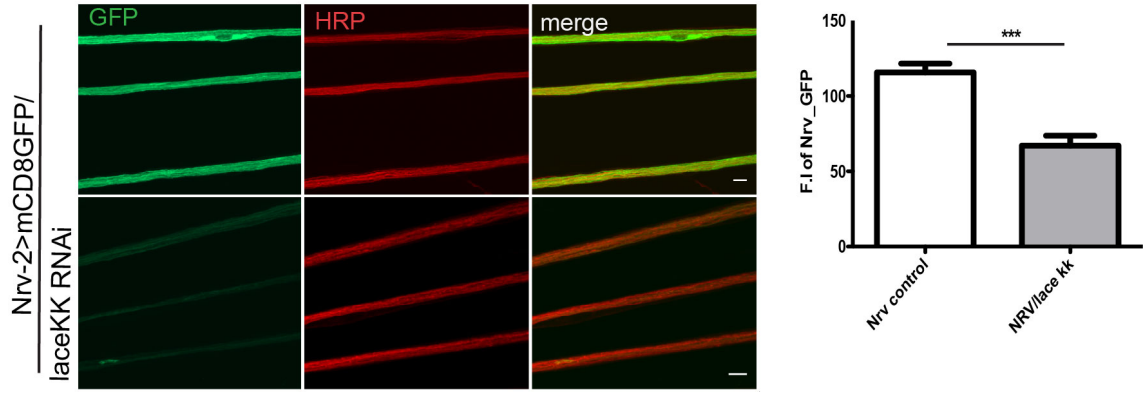


Figure 3.11: Wrapping glia requires *lace* for axonal ensheathment. *lace* RNAi kk was expressed by wrapping glial specific driver line Nrv-GAL4. mCD8-GFP (green) marks the membrane of wrapping glia and HRP (red) stains the neuronal membrane. Quantification of GFP signal was performed with merged projection of all z-stacks. A significant reduction of GFP signal density in Nrv>mCD8-GFP/lace RNAi kk was observed compared to control (Nrv>mCD8-GFP/+). Scale bar 10 μm. p< 0.0001.

ure 3.11).

3.4.3 Ultrastructural analysis reveals the defects in wrapping glial organization

Transmission electron microscopy (TEM) was performed to elucidate the cellular ultrastructure of glia and axons upon loss of *lace* both in all glial cell or exclusively in wrapping glial cells. In wild type flies, normally glial processes usually encircle on axon or a group of axons. TEM sections identifies loss of *lace* in all glia cells specifically affects wrapping glia and both swelling and non-swelling regions of nerve shows extensive errors in glial insulation of axons. Intriguingly, non-swelling region of nerve that appear normal in confocal microscopy also shows defects in axonal ensheathment. This is consistent with the fact that the loss of *lace* in wrapping glia results in the failure of wrapping glial processes to encircle axons (Figure 3.13).

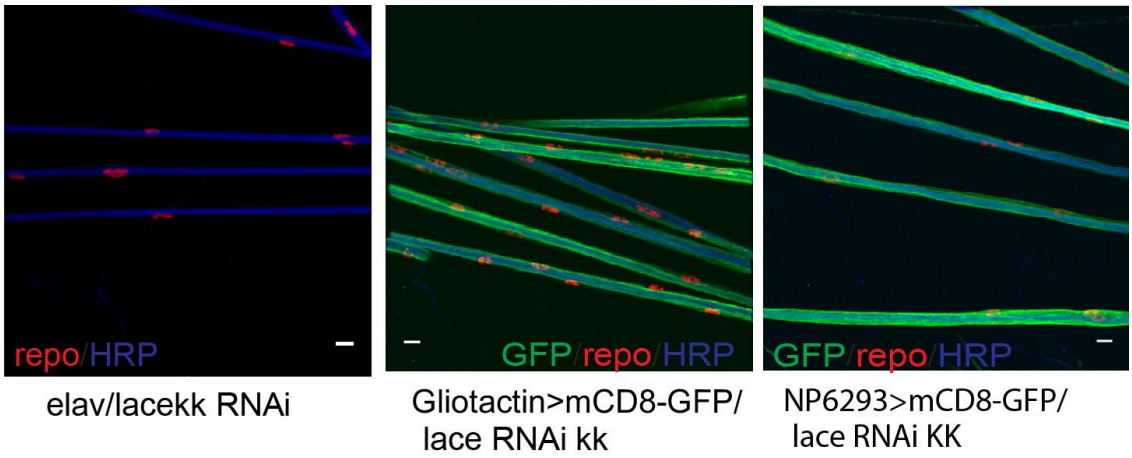


Figure 3.12: Effect of *lace* knockdown on glial subtype and neuron. Merged projection of all confocal z-stacks from larval peripheral nerves. Knockdown of lace in neuron (elav/lace RNAi kk), subperineurial glia (gliotactin>mCD8-GFP/lace RNAi kk) perineurial glia (NP6293>mCD8-GFP/lace RNAi kk) shows normal glial (green) and neuronal (blue) membrane morphology. repo (red) stains glial nuclei. Scale bar 10 μm.

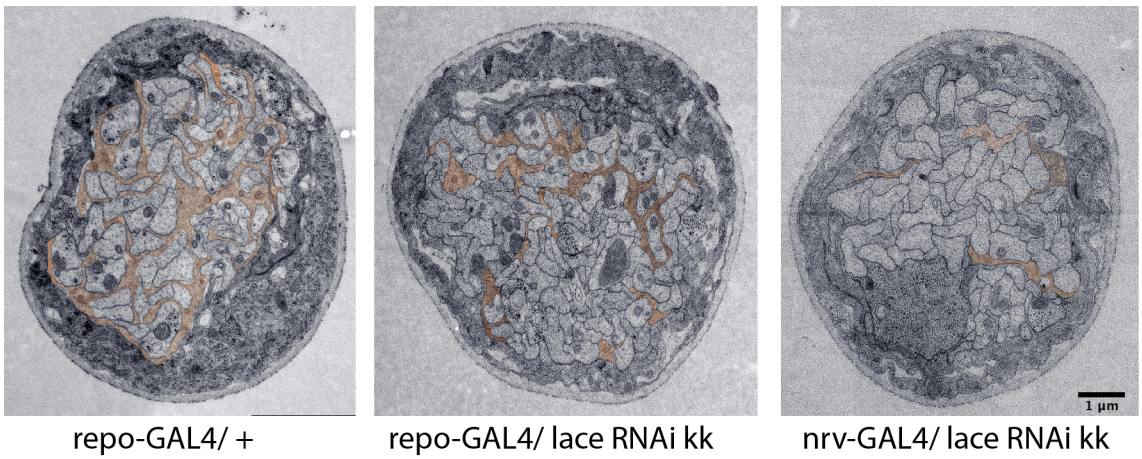


Figure 3.13: Ultrastructural analysis of *lace* phenotype. TEM micrograph of a cross-section of L3 larval peripheral nerve. Images were obtained using MIA. Wrapping glia is color coded with red. Axonal ensheathment is incomplete upon loss of *lace* both in all glia (middle) and in wrapping glia (right). Proper ensheathment of axons is observed in driver line control (left). Scale bar 1 μm.

3.5 Glial PE-ceramide is critical for axonal ensheathment

Glial loss of *lace* triggers axonal ensheathment defect. Since *lace* is the rate limiting enzyme for sphingolipid biosynthesis, glial sphingolipids might be necessary for the glial wrapping process around axons. In order to test this hypothesis and to determine the sphingolipid metabolic intermediates critical for axonal ensheathment, a genetic dissection study using RNAi against all known sphingolipid metabolic enzymes was performed. This study revealed that the loss of *Spt-I, schlank, Des1, pect* in glia caused axonal wrapping defect similar to *lace* phenotype (Figure 3.14). Phenotype of all the candidates were reproduced with two independent RNAi lines (Figure 3.15) to eliminate the possible off-target effects. Thus, glial PE-ceramide appears to be critical for axonal insulation.

Spt-I, lace, schlank, des1 are the enzymes of ceramide biosynthetic pathway in flies. This suggests the necessity of ceramide in the maintenance of glial membrane morphology and consequent axonal ensheathment. Furthermore, we wanted to confirm whether PE-ceramide is crucial for this axonal ensheathment by glia. Therefore, the role of other metabolites generated from ceramide such as GSL and C1P were examined. Hence, two different RNAi lines were used to knockdown the enzymes (*GlcT1, CGT, CK*) involved in biosynthesis of these metabolites. As shown in Figure 3.16 no change in glia or neuronal membrane morphology was observed. Hence, we concluded that glycosphinlgolipids and ceramide phosphate are not critical for axonal insulation by glial cellular processes.

Moreover, in order to check role of PE in glial membrane morphology and axonal ensheathment, two different bbc-RNAi was expressed using repo-GAL4. Notably, *bbc* converts CDP-ethanolamine to PE. But no wrapping defect glial processes could be observed in L3 larval PNS (Figure 3.17).

Now by combing the results from genetic dissection of sphingolipid biosynthesis pathway, it appears that PE-ceramide is critical for glial wrapping and consequently, axonal insulation (Figure 3.18).

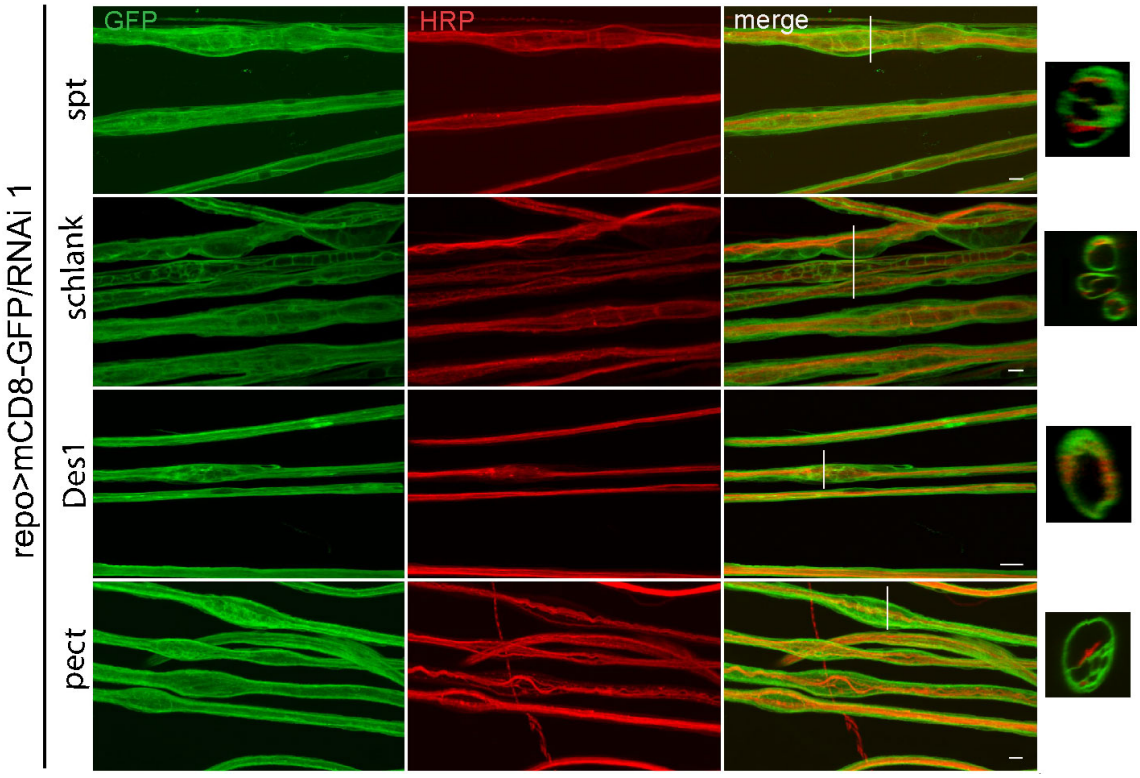


Figure 3.14: Genetic dissection of *lace* phenotype. Knockdown of all sphingolipid synthesizing enzymes by repo-GAL4 driven RNAi. *spt*, *schlank*, *Des1*, *pect* knockdown shows glial swelling and wrapping defect same as that of *lace*. Merged projection of all confocal stacks are presented. As inset, orthogonal section of the nerve region marked white in respective panel. Scale bar 10 μm.

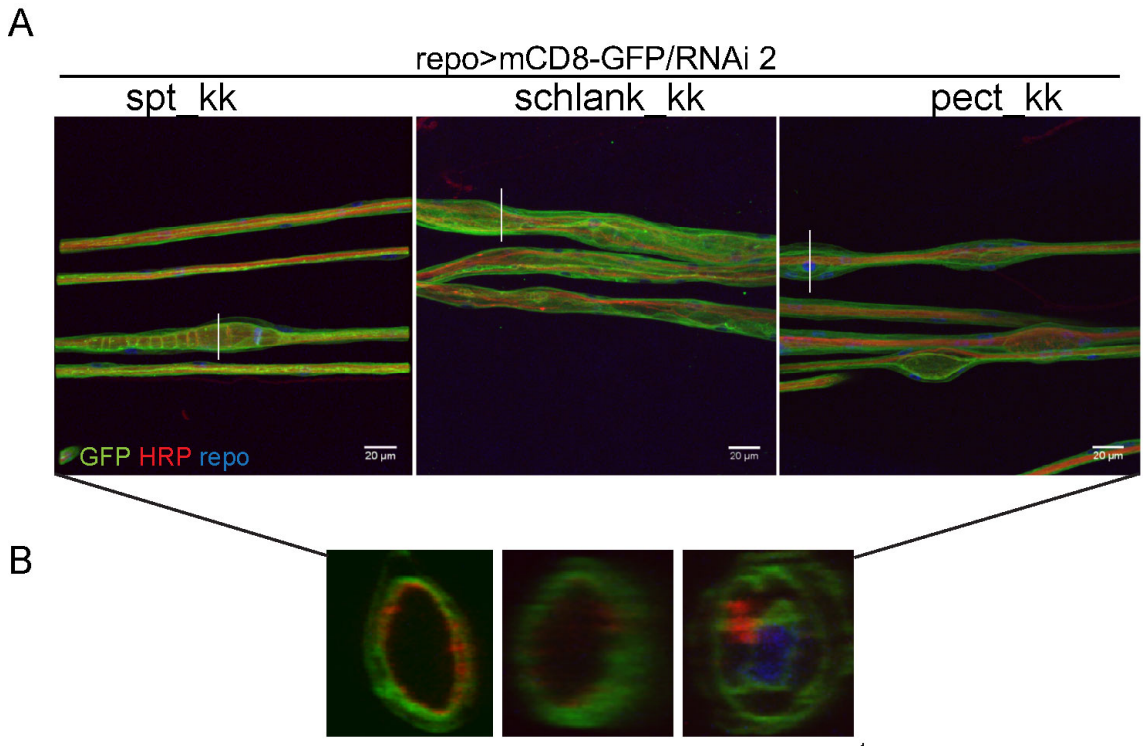


Figure 3.15: Confirmation of sphingolipids essential for axonal ensheathment. Knockdown of *spt*, *schlank*, *pect* with a second RNAi (kk) by repo-GAL4. **(A)** Projection of all confocal stacks after immuno-labeling with GFP and HRP shows glial swelling. **(B)** Orthogonal section of the nerve region marked white in A, shows axonal defect (red) by glial membrane (green). repo labels glial nuclei (blue). Scale bar 20 μm.

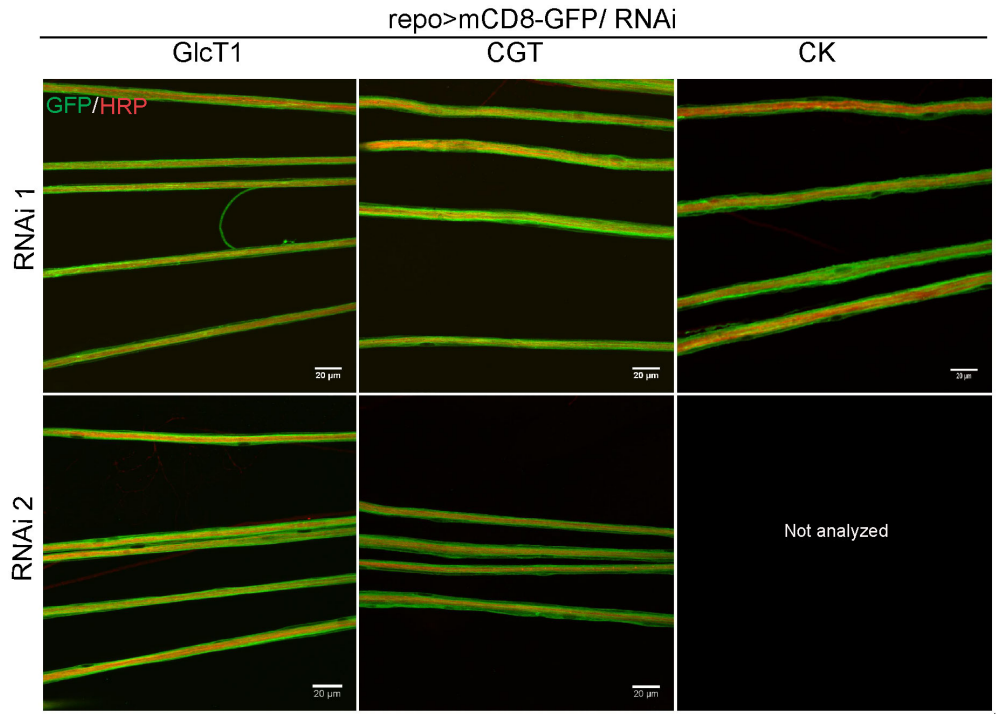


Figure 3.16: Glycosphingolipids are not essential for axonal ensheathment by glia. Glia-specific knockdown of GlcT1 and CGT with two different RNAi driven by repo-GAL4 shows no effect in the morphology of glial membrane (green) and neuron (red). Ceramide Kinase (CK) knockdown by repo-GAL4 also shows no effect. Scale bar 20 μm.

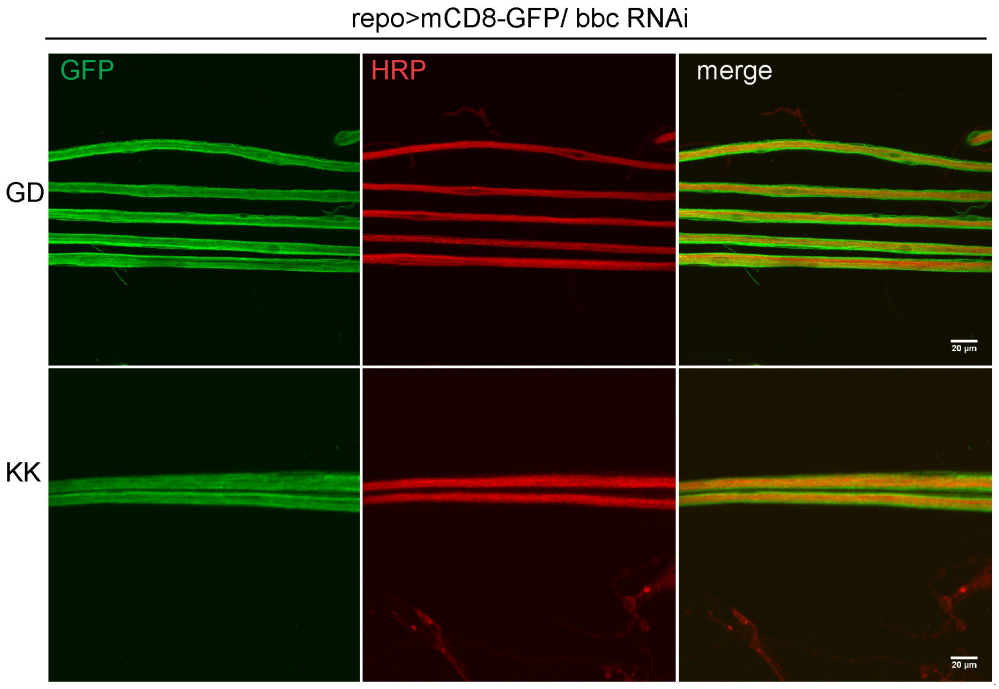


Figure 3.17: Phosphatidylethanolamine is not crucial for glial wrapping. Glial specific (repo-GAL4) knockdown of *bbc* with two different RNAi (gd and kk) shows that PE has no effect on neuron (red) and glia (green) morphology. Projection of confocal z-stacks is presented in the panel. Scale bar 20 μm.

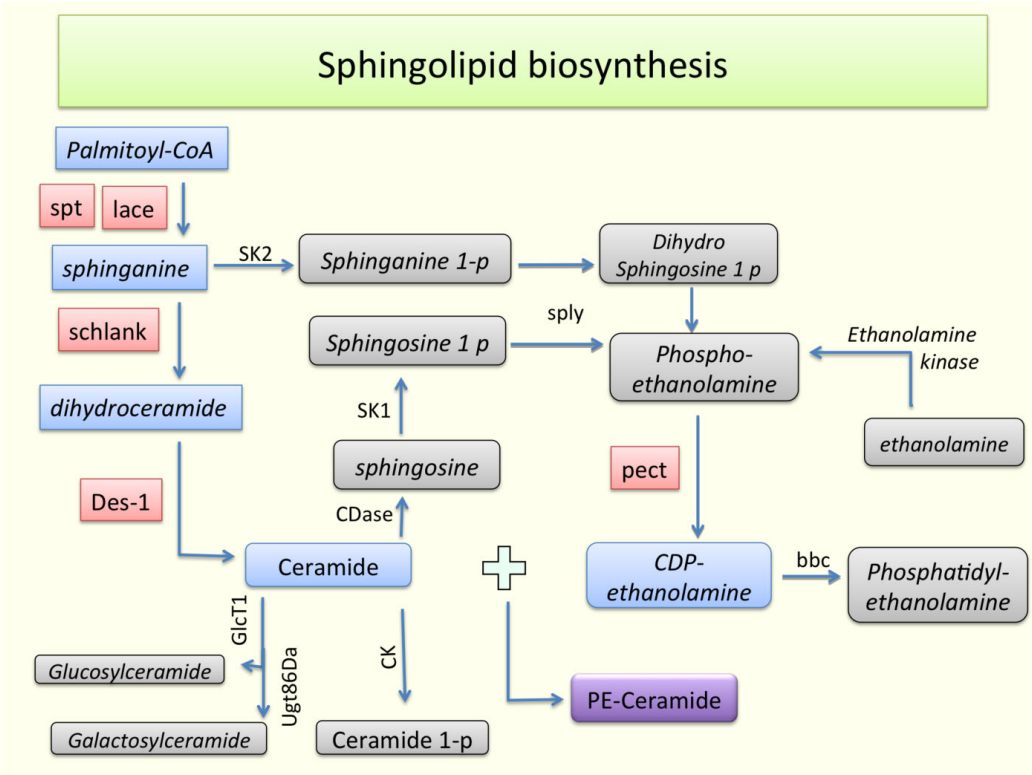


Figure 3.18: KEGG pathway for sphingolipid biosynthesis. Glial loss of certain enzymes (red) shows axonal ensheathment defect. Genetic dissection study shows that PE-Ceramide is critical for glial wrapping around the axons. Lipid species marked in blue are crucial for the maintenance of glial membrane architecture. Lipid species marked in grey color are not involved in the maintenance of glial morphology.

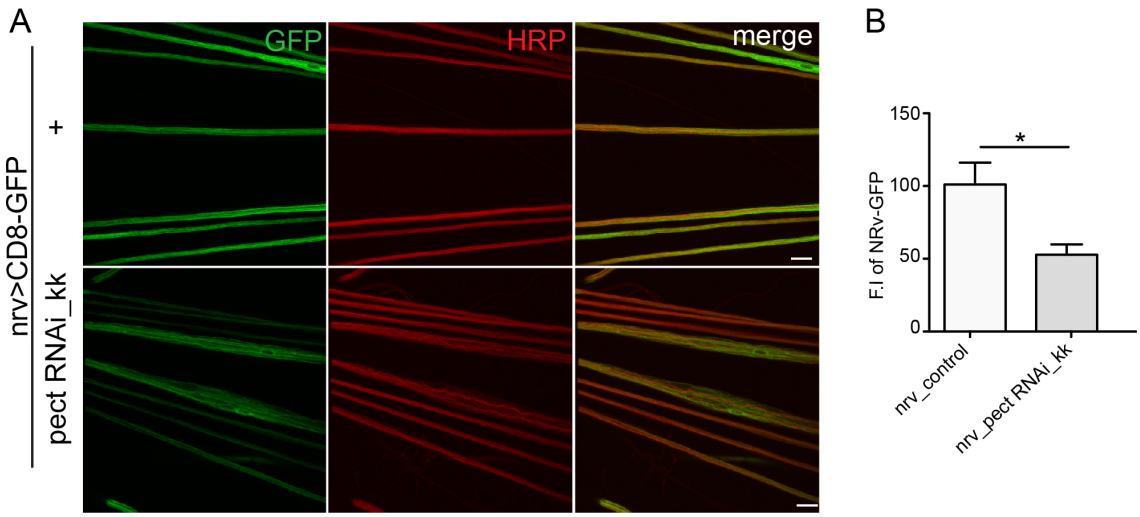


Figure 3.19: Wrapping glia requires PE-ceramide for axonal ensheathment. (A) pect RNAi kk was expressed in wrapping glia using nrv-GAL4. GFP marks the wrapping glial membrane (green) and HRP stains the neuronal membrane (red). **(B)** Quantification of GFP signal density was performed with merged projection of all peripheral nerves examined.

3.5.1 Wrapping glia requires PE-ceramide to ensheathe axons

lace is required for wrapping glia specifically in order to maintain proper axonal insulation. Therefore, we wanted to check whether wrapping glia requires PE-ceramide specifically. Upon wrapping glial specific knockdown of *pect* using nrv-GAL4, the morphology of wrapping glial processes were altered significantly (Figure 3.19). Quantification of the GFP signal density showed a significant reduction upon loss of PE-ceramide in wrapping glia. This suggests that the processes of wrapping glia in absence of PE-ceramide fail to encircle axons completely.

3.6 Role of elongases in *Drosophila* glia

3.6.1 Glial elongases are required for the long-term survival

Primary screening data indicated that glial elongases are necessary for long-term survival of adult flies. Five elongases were identified in the primary screening and therefore, all the elongases were validated by repeating the crossing and were analyzed with more flies. Glia-specific knockdown of four elongases that were confirmed after secondary screening reduced the lifespan of adult *Drosophila*. *baldspot, CG18609, Elo68alpha, Elo68beta* were identified as the candidate genes necessary for the long-term survival of adult flies. Cross-comparison of survival curves shows significant difference as compared to controls. p-values obtained by comparing the survival curves of *CG18609, baldspot, Elo68alpha, Elo68beta* with control were <0.05, <0.001, <0.01, <0.001, respectively. These long chain fatty acids (LCFA) are known to be involved in sphingolipid formation. Therefore, our data suggests that LCFA containing sphingolipids required for glial cells are essential for normal lifespan.

3.6.2 HXXHH motif is present in all elongases

Next, the pairwise alignment of all *Drosophila* elongase with known*Drosophila* elongase *bond*, mouse elovl1 and human Elovl7 show sequence identity. A conserved elongase motif HXXHH (in the box) is also present in all putative elongases (Figure 3.21).

3.6.3 Expression of elongases in the fly brain

RT-PCR analysis of total mRNA isolated from the fly brain show that *baldspot, CG18609* are expressed both male and female brains (Figure 3.22A). However, *Elo68a* is selectively expressed in male flies (Figure 3.22B) and *Elo68beta* expression is not detected either in male or female (Figure 3.22C). Both *Elo68alpha* and *Elo68beta* are not detectable in the brain of either sex. Further confirmation with qPCR, showed that *Elo68alpha* is preferentially expressed in male fly but not in the brain (Figure 3.22D).

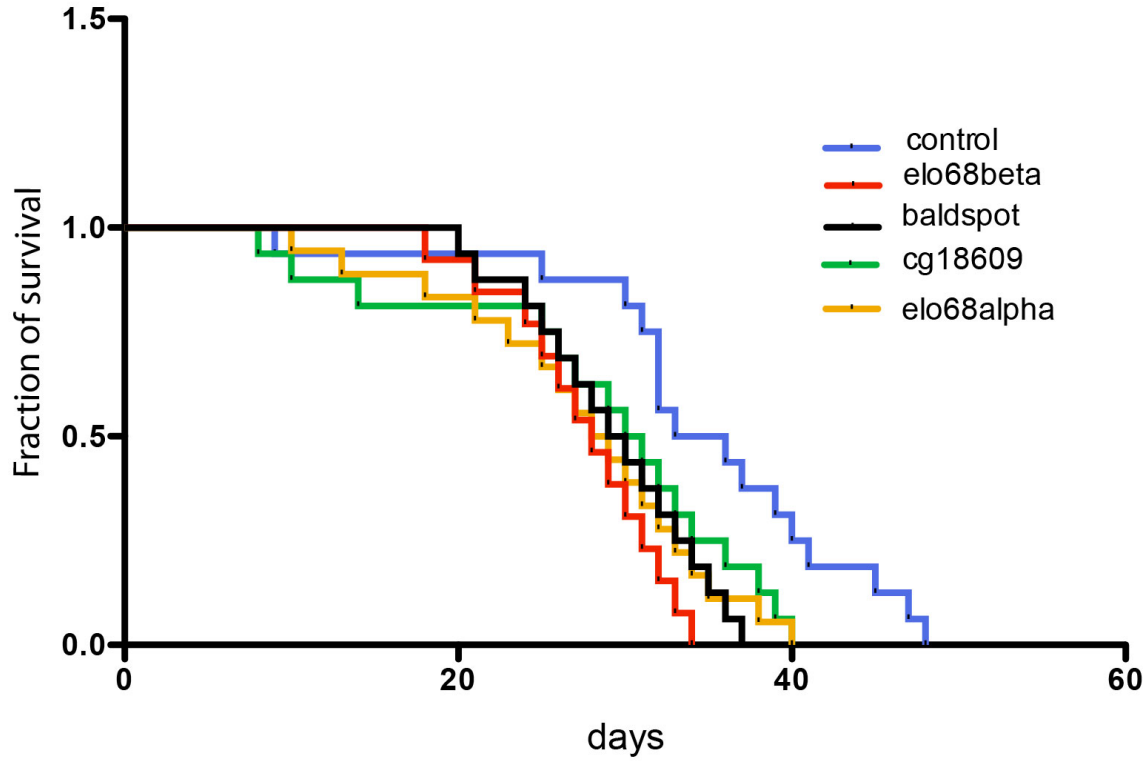


Figure 3.20: Glial elongases are required for long-term survival. Knockdown of four elongases in the mature glia driven by tub-GAL80^{ts}; repo-GAL4 showed reduction of lifespan. GAL80^{ts}/+; repo-GAL4/+ was used as control. The survival curves were analyzed by Log-Rank test.

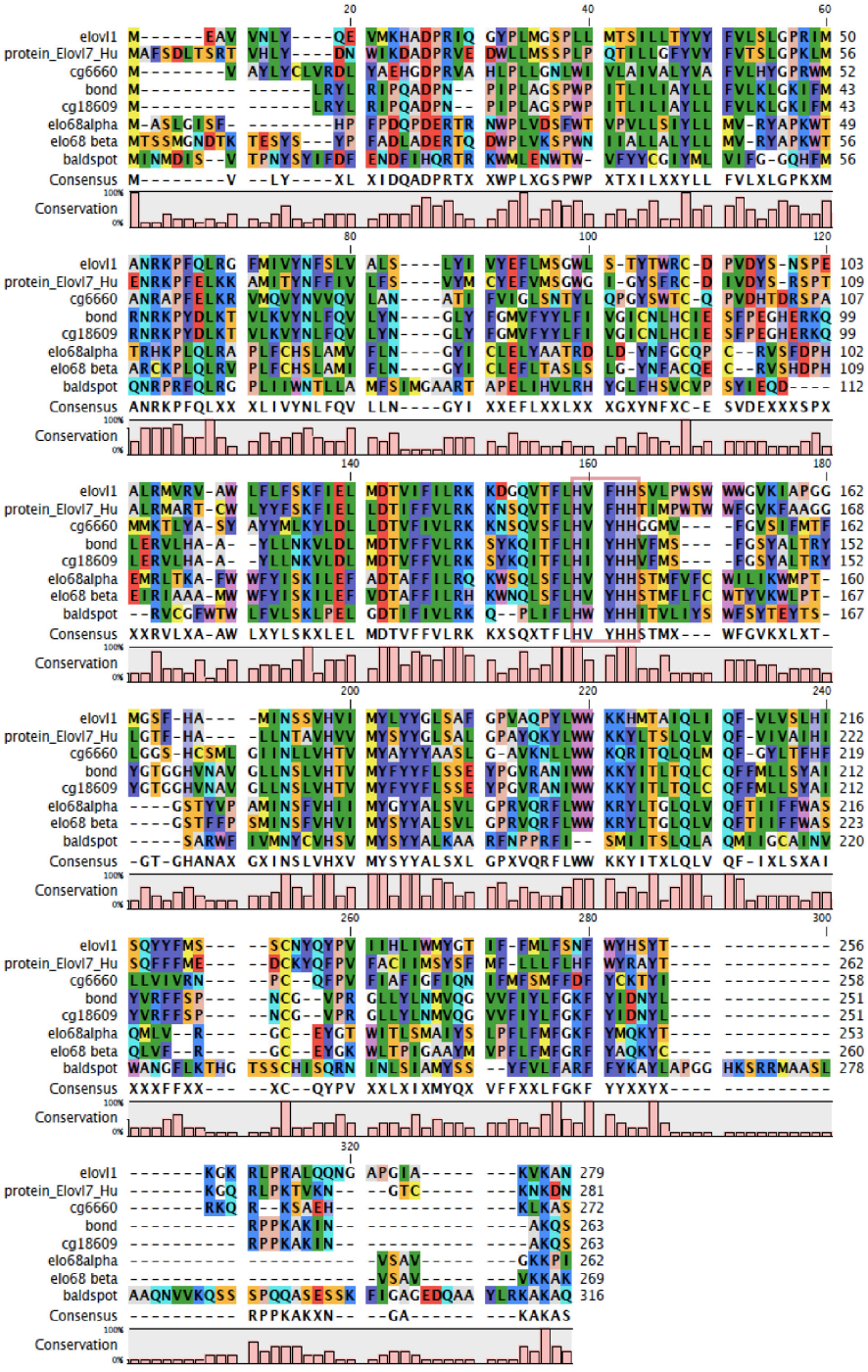


Figure 3.21: HXXHH motif is present in all putative elongases found in the screening. Alignment of all five elongases to known *Drosophila* elongase *bond* and known human elovl7 and mice elov1. Conserved HXXHH motif is shown in a box.

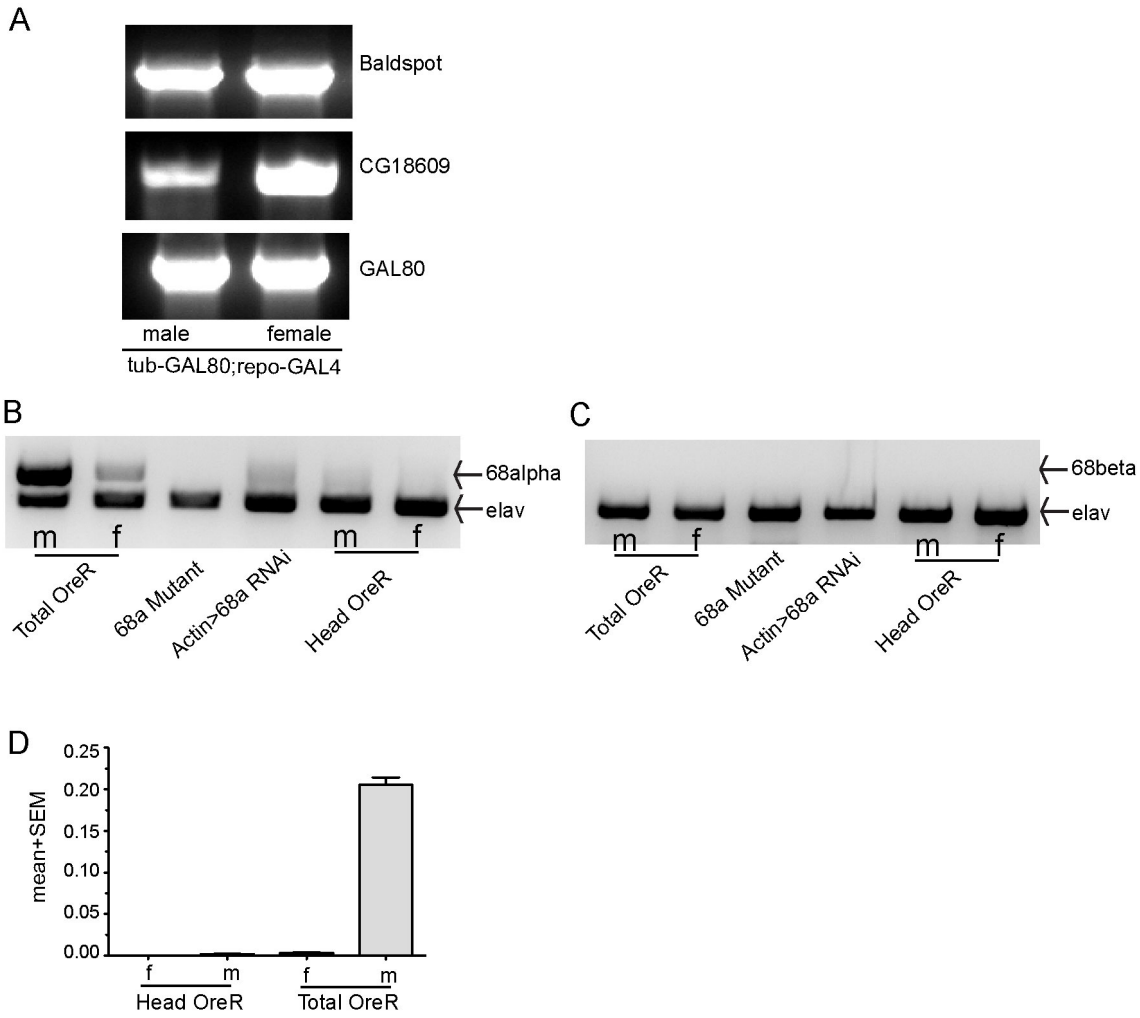


Figure 3.22: Expression pattern of elongases. **(A)** RT-PCR analysis shows that *baldspot, CG18609* are expressed in the adult fly brain, but *Elo68alpha, Elo68beta* cannot be detected in the brain. **(B,C)** RT-PCR shows that *Elo68alpha* is strongly expressed in male. Elo68alpha mutant (68a mutant) and the loss of Elo68alpha in fly (Actin>68a RNAi) are used as negative control. *elav* is used as loading control. *Elo68beta* cannot be detected even in total *Drosophila* body. **(D)** Quantitative-PCR also confirms that *Elo68alpha* is not expressed in the brain but strongly expressed in wild type OregonR males.

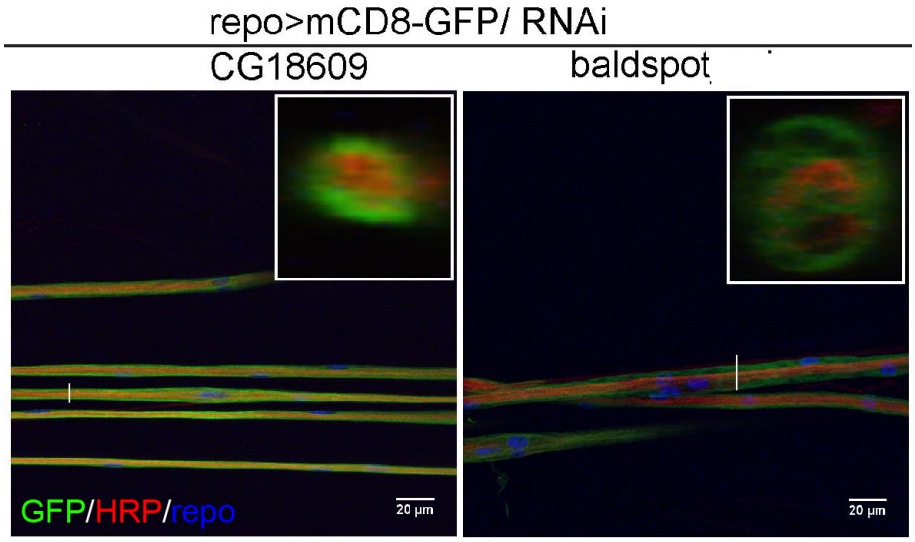


Figure 3.23: *baldspot* alters the morphology of glial membrane. Peripheral nerve of L3 larva was immunostained and projection of all confocal z-stacks are displayed. Membrane morphology of glia (green) and neuron (red) is affected upon glial loss of *baldspot*. Orthogonal section of the nerve-region marked by the white line is presented as inset. Scale bar 20 μm.

3.6.4 Loss of *baldspot* affects glial morphology

We have shown that the loss of PE-ceramide in glia severely damages axonal insulation of peripheral nerves produced by glia. Fatty acyl chain length is an intricate part of PE-ceramide. Therefore, we investigated whether fatty acyl CoA produced by two elongases *baldspot, CG18609* had any effect. We have observed that loss *baldspot* in glia impede axonal wrapping processes whereas loss CG18609 does not show any phenotype. This could be inferred that glial morphology is determined by very specific elongases and this cannot be compensated by other elongases even if they are expressed in the brain. The loss of *baldspot* in glia shows focal detachment of glial membrane from axonal membrane (Figure 3.23). This focal detachment is observed only in two to three regions per nerve examined and this phenotype is more evident in the orthogonal section of the nerve.

Chapter 4

Discussion

Glia was first discovered in retina, renamed later as Muller glia, in 1851 by Heinrich Muller. However, the term "glia" was coined by Rudolph Virchow in 1885. He proposed that it is the *nervenkitt* or nerve glue that surrounds neurons. Although glial biology research lagged behind as compared to neurons, towards end of last century it drew attention of many neuroscientists. Subsequently, some remarkable functions of glia during development of the nervous system came out establishing the fact that it is more than just a glue. Complexities of nervous system are evolved followed by a steady increase of glial population in the nervous system. For e.g, in invertebrates as in flies the ratio of neuron to glia is 10:1 while in vertebrates the ratio is changed to 1:1 [20, 217]. Although glia contributes significantly in the nervous system population, glial function is very much under-appreciated. Therefore, this study aimed to identify novel glia-specific functions and genes involved in it. *Drosophila* is used as a model system for two reasons: first, it is only *Drosophila* which allows conditional gene-inactivation *in vivo* in high-throughput and second, basic glial functions are likely to be conserved across the species.

4.1 Adult *Drosophila* as a model for neuron-glia communication

4.1.1 Characterization of a *Drosophila* model

The role of glia has been well demonstrated in axonal guidance, electrical insulation, neurotransmitter recycling, trophic support during the development (for review [218]). In contrast, only few glial genes like *swiss cheese*, *drop dead* have been shown to impair neuronal functions in adult [219, 220]. These functional relationships between neuron and glia raise the questions if acute loss of glia impairs neuronal integrity. During development,

loss of glia by mutation of *glial cell missing* or exclusive elimination of glia by apoptosis impairs neuronal survival [5]. These studies clearly shows critical role of glia for neuronal survival and function during development, but so far no study has shown whether acute loss of glia would affect neuronal integrity in the mature nervous system.

Hence, the characterization of *Drosophila* as a model for neuron-glia communication in adult is necessary. Targeted genetic ablation of glia in mature nervous system was performed by using GAL4-UAS system in combination with temperature sensitive $GAL80^{ts}$. *repo*, a glia-specific homeodomain transcription factor that is expressed in all glial cells in mature nervous system, is used to drive GAL4 expression in all glial cells. Upon exclusive elimination of mature glia, neuronal survival was severely affected. The motor performance and lifespan of adult flies was also compromised significantly within few days after triggering apoptosis in glia [6]. This is an important finding that underlies the fact that even though glia population is only 10% in flies, it is indispensable in the mature nervous system. The loss of glia compromises the physiological milieu of the nervous system to such an extent that their functional role cannot be compensated by neurons. Such changes of physiological environment in the CNS as a consequence of glial dysfunction resemble several neurological diseases. For instance, amyotrophic lateral sclerosis (ALS), Huntington Disease (HD) provide evidences of non-cell-autonomous mechanism of neurodegeneration primarily caused by glial dysfunction [221–224].

This model to study neuron-glia communication can further be exploited to identify novel glial function and factors that are crucial for neuron-glia interaction. Additionally, this can also be used to address the long-standing question of how glial trophic support for neurons is mediated and genes involved in it, are determined by performing a genetic screening. It is important to mention here that to eliminate glial cells, apoptosis was triggered instead of necrosis by GAL4/UAS system. Expression of necrosis inducing factors such as tetanus toxins can be leaky and has the ability to affect the neurons and physiological surroundings directly or indirectly via released cytokines. Spatial restriction of apoptosis to glia provides the opportunity to study the effect on neuronal integrity as direct consequence of glial impairment.

4.2 Screening of glial factors affecting neuronal integrity

4.2.1 Glial factors that affect survival of *Drosophila*

With a genome-wide RNAi screening, our study sought to reveal glia specific functions and genes modulating neuronal function or morphology. The screening was performed in the mature nervous system because of various reasons: i) RNAi expression was very efficiently induced in all glia cells of adult flies with the help of pan-glial driver repo-GAL4, ii) since, *repo* was not expressed in midline glia of embryo, RNAi could not reduce the expression of gene of interest in all glia cells during development, and iii) genes that were primarily required for glial specification, differentiation and and migration could be excluded. As a read-out of screening, lethality and motor defects are considered as these two behavioral defects are direct manifestation of neuronal dysfunction or loss of conformity.

With the primary screening, 891 candidates have been identified as glial factors modulating neuronal survival or function. Since many of these could also be cell-viability factors, therefore, a comparative analysis was performed with other screening that used same RNAi library but were expressed in different tissues such as eye (GMR), muscle and trachea (personal communication Dr. Aaron Voigt, Dr. Reinhard Schuh and Dr. Ronald Kühnlein). This comparative analysis filtered out the common genes that appeared in other screenings, as these genes were most likely to have role in normal viability of cell. Thus, a list of genes likely to have glial specific functions was obtained. Like any other genome-wide screening study, our primary screening brought a large data-set. In order to make the meaningful and precise understanding, a systematic approach was necessary. Therefore, a networking analysis was performed by Bingo online resource to reveal gene ontology annotated biological processes. Interestingly, it appears that a vast majority of candidates are involved in metabolic processes suggesting role of glial metabolism in long-term survival of flies. These results suggest a glial specific function of the metabolic genes in preserving the neuronal architecture. Many of the candidates from our screening have not been annotated in flybase, but strikingly their human homologs are known to be expressed in mammalian astrocytes and oligodendrocytes [200]. A staggering overlap between astrocyte, oligodendrocyte enriched genes with our candidates indicates two things: first, the mechanism of neuron-glia communication is highly conserved across the species and second, *Drosophila* as a model system with powerful genetic tools can be useful to dissect this basic mechanism which would also have an impact on the mammalian nervous

system.

4.2.2 Metabolic factors perturb neuron-glia morphology

Systematic networking analysis of the candidates reveals that carbohydrate and lipid metabolic genes are necessary for the long-term survival of adult flies. Together these two metabolic processes and its regulators account for 79 genes. An interactome analysis uncovers that these hits are functionally close to each other. Since, bioinformatic analysis is based upon database search, secondary screenings are necessary to pinpoint the possible functions of these hits. In order to conduct the secondary screening, L3 PNS was chosen and the hits that have three or more binding partners are selected, as these hits are likely to function collectively in certain biological processes. The L3 larval PNS system was used because of its accessibility and the possibility of visualization by different molecular markers [225]. At L3 stage, glial migration is complete and they are terminally differentiated to promote axonal ensheathment [3, 34, 68]. Ensheathment of all afferent and efferent axons is accomplished at wandering L3 larva stage, the final stage of larva before entering puparium. Therefore, this wandering L3 stage is the best stage to study neuron-glia communication.

Another advantage of the secondary screening is that it helps comprehensive interpretation of large data set, which often genome-wide screening suffers from. The rational of this screening is to find out the morphological changes of axons and glia upon glia-specific loss of selected metabolic hits based on the interactome results. Various phenotypes such as glial swelling, wrapping defect, axonal splitting and glial organization defect were observed. This suggests that the metabolic genes affect the anatomical structures of neuron-glia in diverse ways. Evidently, these genes maintain the cohesiveness of glial insulation around axons. Defective glial membrane organization at L3 stage is also described in different mutants namely *gliotactin, fray, neurexin IV*. They interfere with glial intracellular signaling processes and subsequently cause glial wrapping defect [66, 73, 74].

The role of glial metabolic factors in neuron-glial morphology is not clear. Our study sheds light into the role of glial metabolic support for axonal integrity. Two hits CG8812 and CG4095 that affect the architecture of axonal membrane are yet to be annotated in flybase. Their functions in flies are unknown but their potential human homologs are

sterol-o-acetyl transferase 1 (Soat1) and fumarate hydrase (FH), respectively. Whether they function in the same way as they do in vertebrates, further investigation is required. Glial loss of *lace* shows glial bulging and the compactness of neuronal membrane is altered suggesting a possible role of glial sphingolipids in order to preserve axonal ensheathment. *lace*, being a rate-limiting enzyme for sphingolipid biosynthesis, it is very tempting to speculate that the intermediates of sphingolipid metabolism or a specific sphingolipid is necessary for the maintenance of axon-glia membrane morphology.

4.2.3 Specificity of the selected candidate: lace

RNAi screening experiments and the phenotypes are quite robust but often suffer from an inherent problem of sequence dependent or independent off-target effects. Consequently, they are associated with generation of false-positive results. To avoid this, careful validation of *lace* as candidate was performed with two different RNAi lines. Both RNAi lines (gd and kk) show similar swelling and wrapping defect, suggesting a strong correlation of observed phenotype with lace.

Loss of *lace* in glia causes incomplete or defective wrapping by glial cellular processes around axons or axonal fascicles resulting in defasciculation or errors in ensheathment. *Drosophila* serine-palmitoyl transferase (SPT) consists of two subunits encoding two genes: LCB1 encoding Spt-I and LCB2 encoding lace. SPT is the first enzyme catalyzing biosynthesis of sphingolipids in *Drosophila*. It has been shown that sphingolipid is essential for cell survival in mammalian cells as well as in yeast. Treatment with ISP-1, an inhibitor of SPT, can elicit apoptosis which can be further rescued by the addition of sphingosine in diet [226, 227]. *lcb2* mutant yeast strain also shows lethality which can also be rescued by addition of long chain bases to media [228, 229]. Mutation in *Drosophila* lace or LCB2 homolog causes developmental lethality and they cannot grow beyond embryonic stage but some hypomorphic allelic combination grows until adulthood but shows severe defects in the wing, legs and antenna discs. Moreover, *lace* mutant shows apoptosis in all tissues, although the degree of severity is not the same. JNK has been identified as a mediator of lace mutant phenotype although which of the specific sphingolipid modulates this signaling pathway is not known. It is generally believed that ceramide orchestrates cell death and survival by JNK and MAP kinase pathways, respectively [141].

Our study demonstrates a novel function of *lace* in the maintenance of glial membrane morphology. Glia-specific knockdown of *lace* alters the structure of glial processes involved in axonal encapsulation but does not elicit apoptosis in glia or reduced viability during development. The absence of glial apoptosis is apparent, as previous studies have shown that apoptosis in glia rendered developmental lethality in flies. Functional role of *lace* in ensheathment is further established by using three different glial subtype-specific GAL4 lines (nervana2-GAL4, gliotactin-GAL4 and NP6293-GAL4) lines and a pan-neuronal GAL4 (elav-GAL4) line [230–233]. The loss of *lace* only in wrapping glia (nerava-GAL4) resembles the phenotype of impaired axonal ensheathment which is in the line with our hypothesis that sphingolipids are required for wrapping glia to mediate axonal insulation. Again, this result is consistent with almost exclusive function of wrapping glia in axonal encapsulation.

4.3 Glia requires specific sphingolipids

4.3.1 Role of PE-ceramide in the PNS

Mutation in *lace* clearly demonstrates the necessity of sphingolipid in glial membrane organization in *Drosophila* PNS. Furthermore, we performed a genetic dissection study to determine the specific sphingolipid required for glia to insulate axons. PE-ceramide is identified as the critical sphingolipid in this cellular process. Interestingly, the *lace*-phenotype is observed upon glia-specific loss of the other subunit of SPT, *Spt-I* or LCB1 and also with downstream genes of *lace* namely *schlank, Des-1, pect.* This bolsters the significance of sphingolipids in glial morphology. All genes upstream of ceramide biosynthesis shows identical errors in glial membrane organization and the phenotype can be further reproduced by two different RNAi. This data unequivocally underlies the functional significance of ceramide in axonal enwrapping by glial processes.

The role of ceramide in axonal ensheathment has recently been described in mammalian system as well. Knockout mice of ceramide synthase 2 (Css2) shows myelination defect both in the CNS and PNS, clearly going along with our data. Myelination is a process of axonal-wrapping by oligodendrocyte and Schwann cells in mammalian system. Css2 knockout mice shows focal separation of inner lamella of myelinating glial processes from axons [107]. Moreover, conditional knockout of Sptlc2 in Purkinje neurons shows partial

loss of these neurons [95] while mice mutant of Sptlc1 also shows myelin thinning and loss of large myelinated axons [234]. Taken together, these evidences suggest a possible role of sphingolipids in glial wrapping in vertebrates. Our results from invertebrates, also hints towards this possibility.

In flies, ceramide biosynthetic enzymes *Spt-I, schlank, Des-1* have so far not been implicated in development of the nervous system. The expression of Spt-I is detected both in the CNS and in the PNS [235], but no function has been described so far. *Schlank* is recently described as a potential ceramide synthase in *Drosophila* that regulates growth and body fat and control fatty acid biosynthesis. It also induces the expression of sterol-responsive element binding protein (SREBP) in *Drosophila* [236]. Additionally, the mutation in *Des-1* causes the failure of spindle assembly during spermatogenesis resulting in defective cytokinesis and male sterility [110]. Our study reveals novel functions of these genes in the maintenance of axon-glial morphology that has not been explored so far. Since, the vertebrate counter-part of these enzymes already hinted towards possible role in axonal ensheathment, our study shed a light in glia membrane biology of *Drosophila* which can phenocopy mutant mice and thus provides an excellent tool to study basic molecular and cellular mechanisms of axonal ensheathment process by glia.

4.3.2 Role of PE-ceramide in membrane wrapping

Our study reveals that the loss of *Pect* in wrapping glia impairs axonal ensheathment. Lim *et al*, reported that *Pect* ensures the homeostasis of PE level mediated by the SREBP pathway. The loss of *Pect* causes accumulation of triglycerides resulting cardiac steatosis and cardiomyopathy. Strikingly, the integrity of the longitudinal muscle fibers is compromised and disorganized inner transverse myofibrils are observed. This suggests a possible role of *Pect* in the preservation of membrane morphology [237], in congruent with our observation in this study.

Pect catalyzes the conversion of CDP-ethanolamine from phosphoethanolamine (PEth). Thereafter, CDP-ethanolamine is converted to yield either PE or PE-ceramide. We confirm with two different RNAi that loss of PE-synthesizing enzyme or *bbc* has no effect on glial morphology. Hence, we conclude that the errors in axonal encapsulation are caused by the loss of PE-ceramide, which is generated from CDP-ethanolamine by the action of

PECS. Moreover, complex sphingolipids such as Glc-ceramide, Gal-ceramide, C1P that can also be produced from ceramide by the action of different enzymes. Loss of these enzymes in glia did not alter the architecture of glial membrane. Taken together, our data posits a novel role of PE-ceramide in organization of glial membrane that encapsulates axons in order to preserve its integrity.

We could not detect any alteration of glial morphology upon glia-specific loss of *Sply* that catalyzes the production of PEth, a source of CDP-ethanolamine. We reason that PEth is generated by another parallel pathway where ethanolamine is phosphorylated by ethanolamine kinase (*eas*) to yield PEth. It is important to mention here, that no PECS that are used in the study shows glial wrapping defect. Since there is no established PECS exists in flies, we consider the predicted homologs CG 31717, CG11438, CG11426 for our study. Of these putative enzymes, no RNAi is available against CG 11438. There can be four reasons not to find a functional PECS: i) since PE-ceramide is most abundant phospholipid in *Drosophila* there may be compensatory pathways that stringently maintain the PE-ceramide level, ii) RNAi against the CG 31717 and CG 11426 may be nonfunctional, iii) CG 11438 may be the potential PECS in the nervous system, iv) oher PECS might exist and therefore, careful genome-wide study is therefore necessary. However, the first problem of compensatory enzymatic activity of PECS can be dealt with the generation of double mutant fly line or by using two RNAi lines at the same time against two proteins (combinatorial knockdown). These approaches can potentially explore the role of these putative enzymes and can provide fundamental insights into axonal insulation.

4.3.3 PE-ceramide and membrane structure

PE-ceramide is most enriched phospholipid in flies whereas PC-ceramide or sphingomyelin (SM) is most abundant in mammals. This lipid species is also found in fresh water invertebrates and some species of protozoa[238, 239]. The reason why *Drosophila* possesses more PE-ceramide instead of SM is unclear. SM essentially serves as a reservoir of ceramide that regulate several intracellular signaling pathways and it forms so called "lipid rafts" with cholesterol to facilitate these signaling pathways. Whether PE-ceramide performs similar functions in *Drosophila* needs further investigation. One of the main reasons for having excess PE in flies is to give tolerance to flies against ethanol, which is an environmental stress factor for rotting food inhabitants like flies. *Drosophila* sequesters ethanol in the

membrane by converting it to PE. Moreover, ectotherms like *Drosophila* have to adjust their membrane fluidity in response to changes of environmental temperature. PE plays a critical role in order to maintain the biophysical properties of membrane and its fluidity to counter stress, related to ethanol and temperature-shift [240].

Biophysical properties PE-ceramide is also a bit different as compared to SM. The melting temperature of PE-ceramide lipid bilayer is 64°C whereas that of SM is 41°C indicating very strong intermolecular interaction in PE-cermaide. This strong interaction is achieved because of small cross-section area of phosphoethanolamine head group in PE-ceramide that allows closer contact of molecules in hydrophobic membrane leading to the tight packing of acyl chains [241, 242]. Therefore, organisms like *Drosophila* which has to adapt to a wide range of temperature, PE-ceramide is beneficial. However, its interaction with cholesterol is relatively poor compared to SM [241]. Seemingly, SM is evolved and replaces PE-ceramide in higher vertebrates where cholesterol content is very high unlike insects.

In mammals, a tightly packed membranous structure or myelin provides axonal ensheathment. Flies do not produce myelin and the axonal ensheathment is provided by wrapping glia. Due to strong intermolecular interaction, PE-ceramide might contribute to the tight packing wrapping glial membranous structure which is essential for axonal insulation.

4.4 Elongases in glia

4.4.1 Glia-specific function of elongases

Fatty acid chain length and its degree of unsaturation is very essential for determining the biophysical properties of sphingolipids and thereby membrane morphology; for instance, ceramide with different chain length differentially regulate phospholipase A2 and subsequently membrane permeability [243–245]. Our study puts forward a novel role of glial elongases in long-term survival. Out of 20 *Drosophila* elongases only three have been described before but two of them viz. *Elo68alpha, bond* seem to have gender-specific effect. Our screening identified *Elo68alpha, Elo68beta, baldspot, CG18609* as a glial elongase that regulate lifespan of flies. Identification of Elo68alpha and beta was confusing as it was described that Elo68 was expressed in the testes of adult male flies and Elo68beta was not detectable in fly cDNA pool. Our qPCR results also confirm this finding. Since, RNAi

against Elo68alpha is functional as observed in the mRNA level, the phenotype related to it is likely to be an off-target effect. However, Elo68beta may be expressed in extremely low level and that is why it is not detected in total mRNA isolated from head. The isolation of mRNA directly from brain, avoiding the cuticle and other structures of the head region might be able to detect this very low level of expression.

Here, we present that the expression and function of two elongases that are present in both sexes and also in the brain of flies. Previous studies have shown that *baldspot* determines viability and also has a role during spermatogenesis. It is a homolog of mammalian ELOVL6 that elongates fatty acid chain with 12 carbon atom (C12) to 16 carbon atom (C16) [246]. The reason behind the lethality and motor defect observed with P-element mutant of *baldspot* is unknown, but our results provide an explanation. *baldspot* sustain the integrity of glial membrane that protects axons from the surroundings and thereby controls the long-term survival and functions of neurons. In addition, the biophysical property of short chain fatty acid provides an advantage of not getting solidified at 25°C, the preferred environmental temperature of experimental flies [150]. Consistent with the fact that C14-16 unsaturated and saturated fatty acids are expressed in the *Drosophila* brain, our results propose a novel function of *baldspot* in the synthesis of C14-16 acyl chain that in turn gets attached to PE-ceramide and modulates the glial enwrapping of axons and determines the long-term survival.

Chapter 5

Summary and Conclusions

Glia share very intimate relationship with neuron. This relationship, at first is established during the development and continued throughout adult life. Several studies indicate that neuron-glia communication is very similar both in vertebrate and in *Drosophila*. But glial specific functions that preserve this strong association with neuron are still unclear.

In our study, at first we aimed to establish a model to study neuron-glia communication in the mature nervous system. Therefore, we used UAS-GAL4 in combination with the temperature sensitive suppressor of GAL4, $GAL80^{ts}$ to selectively expressed any transgene under UAS promoter in spatially and temporally restricted manner. By using this model, we report that acute loss of mature glia triggers neuronal cell death, motor paralysis and reduces viability. Hence, we employ this characterized *Drosophila* model to identify genes with glial specific functions by a genome-wide RNAi mediated gene-silencing approach. Screening uncovers a list of genes that indicates several modes of neuron-glia communication such cell adhesion, transcription factors, kinases, DNA binding proteins and metabolism. Thus, these results open up future direction for wide variety of research related to neuron-glia communication. Interestingly, our study reveals that the genes involved in metabolic pathways are considerably overrepresented in our primary hit list. A systematic bioinformatics analysis together with secondary assays reveals that a specific sphingolipid, PE-ceramide in wrapping glia is critical for axonal ensheathment. PE-ceramide is predominantly expressed in the brain of adult flies but its function is unknown in the nervous system. Our study indicates its possible function in glial wrapping around axons. Furthermore, we show that PE-ceramide in wrapping glia together with short-chain fatty acyl chain, synthesized by glial elongases conform structure of glial membrane that covers axon and axonal fascicles. Loss of PE-ceramide in glia may cause mislocalization of

certain proteins that are crucial for axon-glia interaction. Perturbation of this interaction affects the stability of axonal ensheathment process. Loss of this lipid might also interfere with glia-mediated signaling cascade required for the enwrapping processes and therefore most of axons remain naked or unwrapped by the wrapping glial processes. Moreover, differentiation of wrapping glia during ensheathment process might also be affected by the loss of PE-ceramide. A change in the cytoskeletal structure during the differentiation of wrapping glia might be involved in this erroneous insulation processes. Elvol or elongases have been shown to be involved in the different physiological barrier present in the skin and gut. Glial sphingolipids with specific fatty acid chain synthesized by the elongases might be crucial for the integrity of the physiological barrier of the nervous system such as blood-brain or blood-nerve barrier.

Axonal insulation by glia in *Drosophila* is poorly understood. Our study signifies the role of different sphingolipid species that regulate the architecture of glial membrane. Given that, these sphingolipids are also present in vertebrate nervous system, it would be intriguing to know if they perform similar functions. One of major difficulty to study effect of these sphingolipid biosynthetic enzymes in the nervous system is their ubiquitous expression and functional significance during growth and development. As a result, all conventional knockout mice of sphingolipid biosynthetic enzymes show embryonic lethality and therefore conditional knockout is the only solution. *Drosophila* as a model system can be an alternative to this. With the tools like mutagenesis and RNAi, fly as a model system can provide better understanding of this basic neuron-glia relationship mediated by sphingolipids.

In conclusion, our experimental approach concerted with strong bioinformatics offers comprehensive dissection of biological processes of glia in order to maintain axonal integrity. Besides identification of many known glial processes, it uncovers several genes with possible glia-specific functions. Given, all these genes are conserved in human, results from this large screen in *Drosophila* most likely is translatable to the vertebrate nervous system.

Part I

Appendix

M= motor defect, L= 50% or more lethality after 10 days.

Table 1: List of Candidates

CG	Gene synbol	Human homolog	Phenotype
		CELL ADHESION	
8079		Angiogenic factor with G patch and FHA domains 1	L
5803	Fas3	cell adhesion molecule 4	L
6120	Tsp96F	CD81 antigen (tetraspanin)	L
32796	boi	Cdon /Cell adhesion molecule-related	L
15211		CKLF-like MARVEL transmembrane domain-containing pr	L
11326	Tsp	COMP/Thrombospondin 5	L
32183		connective tissue growth factor	L
33531	Ddr	discoidin domain receptor family, member 1	L
8390	vlc, vulcan	Disks large-associated protein 1	L
8403	SP2353	EGF-like, fibronectin type III and laminin G domains iso 1	L
7749	fat2	Protocadherin Fat 1	L
32593	Flo-2	flotilin 2	L
3171	Tre1	G protein-coupled receptor 84	L
3322	LanB2	Laminin subunit gamma-1 Precursor	L
2198	Ama	limbic system-associated membrane protein	L
3665	Fas2	NCAM2	L
8581	fra	neogenin homolog 1	L
6449	NijA	ninjurin 1	L
7050	Nrx-1	Neurexin-3-beta Precursor	L
7050	Nrx-1	Neurexin-3-beta Precursor	L
31009	Cad99C	Protocadherin-15	L
6378	BM-40-SPARC	SPARC like 1(cell adhesion ECM related Ca binding	L
17739		Spondin-F	L
3359	mfas	Transforming growth factor-beta-induced protein ig-h3	L
5550		tenascin C/R	L
3299	Vinc	Vinculin (Metavinculin)	L
		KINASE/PHOSPHATASE/SIGNALING	
9451		lysosomal acid phosphatase 2 precursor	L
10564	Ac78C	adenylate cyclase 8/5	L
17146	Adk1	Adenylate kinase isoenzyme 1	M
8243		Arf GTPase Activator	L
3365	drongo	ARF GTPase activator	L
6477	RhoGAP54D	Rho GTPase activating protein 19	L
10188		Rho guanine nucleotide exchange factor 18	L
10637	Nak	BMP-2-inducible protein kinase	L
13597		Bromodomain-containing protein 2	L
8203		Cdk5	L
30291		CDK5 regulatory subunit-associated protein 3	L
2048	dco	casein kinase 1 epsilon	L
33242		casein kinase 2, beta polypeptide	L
33246		casein kinase 2	L
13197		RNA/RNP complex-1-interacting phosphatase	L
7378		Dual specificity protein phosphatase 13	L
15528		Dual specificity protein phosphatase 14	L
16932	Eps-15	EGFR pathway substrate 15 like 1	L
8433	Ext2	Exostosin-2	L

17269	Fancd2	Fanconi anemia group D2 protein (pr. kinase)	L
2872	AlstR	Galanin /Allatostatin receptor type 1	L
34372		GPCR 158	L
34357		guanylate cyclase 2F	L
1410	waw	GUF1 GTPase homolog	L
10535		IkappaB kinase complex-associated protein	L
10082		Inositol hexakisphosphate kinase 3	L
2899	ksr	kinase suppressor of ras 2	L
2061		LanC-like protein 2(GPCR)	L
5680	bsk	MAP kinase 10/ JNK3	L
32703		MAP kinase 15	L
4720	Pk92B	MAPK/ERK kinase kinase 15	L
7717	Mekk1	MAP kinase kinase kinase	L
13778	Mnn1	menin isoform (MAPKK cascade)	L
4946	Mob3	MOB1, Mps One Binder kinase activator-like 2B	L
32717	sdt	membrane associated guanyl kinase 5	L
10951	niki	Serine/threonine-protein kinase Nek8	L
1669	κB-Ras	kappa B-ras 1	L
4550	ninaE	opsin 4 isoform 1	L
9662		Oligosaccharyltransferase complex subunit/DC2	L
18582	mbt	p21 activated kinase	L
11444		PDGFA associated protein 1	L
10797	dnc	cAMP-specific 3',5'-cyclic phosphodiesterase 4B	L
8475		Phosphorylase b kinase regulatory subunit beta	L
18662		phosphohistidine phosphatase 1 isoform 3	L
7933	janA	phosphohistidine phosphatase iso 3	L
6167	PICK1	Protein interacting with C kinase 1	L
10260		1-phosphatidylinositol 4-kinase activity	L
33275		Pleckstrin homology domain-containing family G member 4B	L
33275		Pleckstrin homology domain-containing family G member 4B	L
32418	Myt1	Membrane-tyrosine- and threonine-specific cdc2-inhibitory kinase	L
7186	SAK	Serine/threonine-protein kinase PLK4	L
11426		phosphatidic acid phosphatase type 2A	L
6571	rdgC	Serine/threonine-protein phosphatase EF-hands 2	L
2104		protein phosphatase 2A regulatory subunit	M,L
7913	PP2A-B	protein phosphatase type 2A regulator	L
12091		T-cell activation protein phosphatase 2C	L
15862	Pka-R2	cAMP-dependent protein kinase type II-alpha regulatory	L
42349	Pkc?	protein kinase C, delta	L
1954	Pkc98E	Protein kinase C epsilon	L
6453		protein kinase C substrate 80K-H isoform 1	L
10776	wit	Serine/threonine kinase-D	L
12788		phosphoseryl-tRNA kinase	L
12788		phosphoseryl-tRNA kinase	L
3101	l(1)G0232	tyr phosphatase non receptor 9	L
11516	Ptp99A	protein tyrosine phosphatase, receptor	L
10443	Lar	Receptor-type tyrosine-protein phosphatase delta	L
6772	Slob	PX domain containing serine/threonine kinase	L
8865	Rgl	Ral guanine nucleotide dissociation stimulator	L
13875		Ras association (RalGDS/AF-6) domain family	L
8331		Receptor expression-enhancing protein 5	L
4926	Ror	receptor tyrosine kinase-like orphan receptor 1	L
18085	sev	Proto-oncogene tyrosine-protein kinase ROS	L
17596	S6kII	Ribosomal-S6-kinase	L
17559	dnt	Tyrosine-protein kinase RYK	L

1695		Small G protein signaling modulator 2	L
8209		SAPK substrate protein 1	M,L
1921	sty	sprouty 2	L
2224		STAM-binding protein	L
11228	hpo	serine/ threonine kinase 3	L
14217	Tao-1	Serine/threonine-protein kinase TAO1	L
4063	ebi	Transducin beta-like protein 1	L
9222		Testis-specific serine/threonine-protein kinase 2	L
32019	bt	titin isoform novex-3	L
3172	twf	Twinfilin, tyrosine kinase 9	L
6386	ball	vaccinia related kinase 1	L
		TRANSCRIPTION REGULATION/ RNA BINDING	
8817	lilli	AF4/FMR2 family, member 1	L
3935	al	aristaless homeobox	L
5205		Activating signal cointegrator 1 complex subunit 3	L
13379	Sgf11	ataxin 7-like 3 isoform b	L
3905	Su(z)2	BMI1 polycomb ring finger oncogene	L
2922	exba	basic leucine zipper and W2 domains 2	L
12357	Cbp20	Nuclear cap-binding protein subunit 2	L
6059		coiled-coil domain containing 147	L
10750		Coiled-coil domain-containing protein 42B	L
3696	kis	chromodomain helicase DNA binding protein 7	L
31762	aret	CUG triplet repeat, RNA binding protein	L
1762	aret	CUG triplet repeat, RNA binding protein 2	L
9680	Dbp73D	ATP-dependent RNA helicase	L
32533		ATP-dependent RNA helicase	L
11837		dimethyladenosine transferase	L
11166	Eaf	ELL-associated factor	L
6907		Elongator complex protein 4 (hELP4)	L
15191	e(y)2	Enhancer of yellow 2 transcription factor	L
6249	Csl4	exosome component 1(3'-5' exoribonuclease)	M
11001	FK506-bp2	FK506 binding protein 1A	L
10002	fkh	forkhead box A2/Hepatocyte nuclear factor 3-beta	L
4029	jumu	forkhead box N1	L
16899		forkhead box P1	L
5041	Tfb4	general transcription factor IIH	L
14036		gametocyte specific factor 1	L
18144	Hand	basic helix-loop-helix transcription factor	L
11900		HD domain-containing protein 3	L
8333	HLHmγ	hairy and enhancer of split 1	L
42458		heterogeneous nuclear ribonucleoprotein C isoform b	L
11648	Abd-B	Homeobox protein Hox-C10	L
7379		Inhibitor of growth protein 2	L
7832	l(3)L1231	INO80 complex subunit D	L
15329	hdm	lysine (K)-specific demethylase 1	L
10384		KH domain-containing, RNA-binding, signal transduction p.r 2	L
8912	Psi	KH type-splicing regulatory protein	L
10699	Lim3	LIM homeobox protein 3	L
6061	mip120	lin-54 homolog	L
7662	veli	lin-7 homolog C	L
32105		LIM homeobox transcription factor 1-alpha	L
13624		luman-recruiting factor	L
3711		leucine-zipper-like transcription regulator	L
9648	max	MAX protein isoform a	L

7162	MED1	Mediator of RNA polymerase II transcription subunit 1	L
1057	MED31	Mediator of RNA polymerase II transcription subunit 31	L
4913	ear	Protein ENL (YEATS domain-containing protein 1	L
15001	nab	NGFI-A binding protein 1	L
3891	Nf-YA	nuclear transcription factor Y, alpha isoform 2	L
1922	onecut	Hepatocyte nuclear factor 6	L
12498		Paf1/RNA polymerase II complex	L
14956		piggyBac transposable element derived 4	L
15772		Polyhomeotic 1 like isoform 3	L
12238	e(y)3	PHD finger protein 10 isoform a	L
8068	Su(var)2-10	E3 SUMO-protein ligase PIAS1	L
1796	Tango4	Pleiotropic regulator 1	L
11820		Polyglutamine-binding protein 1	L
10348		PR domain containing 16 isoform	L
33206	Gmap	26S protease regulatory subunit 8	L
33323	Fer1	Pancreas specific Tf	L
1507	Pur-α	Transcriptional activator protein Pur-beta	L
1433	Atu	RNA polymerase-associated protein	L
1347		Rb1-inducible coiled coil protein 1 isoform 1	L
11982		ring finger protein 126	L
8998	Roc2	Ring finger 7	L
33183	Hr46	RAR-related orphan receptor B	L
3312	Rnp4F	Squamous cell carcinoma antigen recognized by T-cells 3	L
17181		Transcriptional repressor scratch 1	L
13893		SEC14-like protein 2 (TAP)	L
6987	SF2	splicing factor arg/ser rich isoform	L
31550		splicing factor 4	L
6695		splicing factor, arginine/serine-rich 16	L
17136	Rbp1	splicing factor, arginine/serine-rich 3	L
7129	l(3)05822	SH3 domain containing 19/ADAM binding protein Eve-1	L
3871	Six4	Homeobox protein SIX4	L
4152	l(2)35Df	Superkiller viralicidic activity 2-like 2	L
1775	Med	mothers against decapentaplegic homolog 4	L
3949	hoip	U4/U6.U5 tri-snRNP	L
8404	Sox15	SRY-box 18	L
11491	br	serine/arginine repetitive matrix 2	L
33520	Rpb4	Transcriptional adapter 2-alpha	L
2962		Transcription initiation factor TFIID subunit 4	L
10327	TBPH	TAR DNA-binding protein 43/ TDP-43	L
11490		TBC1 domain family, member 15	L
31367		Transcription elongation regulator 1	L
9973		CXXC finger6/ Methylcytosine dioxygenase	L
7238	sip1	tuftelin interacting protein 11 (spliceosome)	L
2980	thoc5	THO complex 5	L
8384	gro	Transducin-like enhancer protein 4	L
15440		tRNA selenocysteine associated protein 1	L
42281	bun	TSC22 domain family, member 1	L
31531		titin isoform N2-A	L
7246		hepatocellular carcinoma antigen 66	L
15897	wuho	tRNA (guanine-N(7)-)-methyltransferase	L
5247	Irbp	ATP-dependent DNA helicase 2 subunit 1	L
12647		YLP motif containing 1	L
3446		YjeF N-terminal domain-containing protein 3	L
18381	lola	Zinc finger and BTB domain-containing protein 20	L
6222	su(s)	Zinc finger CCCH domain-containing protein 4	L

15602		Zinc finger FYVE domain-containing protein 19	L
17440		zinc finger protein 853	L
31852	Tap42	zinc finger, HIT domain containing 2	L
		METABOLISM	
3425	T3dh	Alcohol dehydrogenase iron-containing protein 1	L
6058		aldolase A, fructose-bisphosphate	L
11058		AMP deaminase 2	L
18104	arg	arginase, type II	L
8536	β4GalNAcTA	Beta-1,4-galactosyltransferase 2	L
12539		choline dehydrogenase	L
9150		dehydrogenase/reductase (SDR family) member 11	L
34420		dipeptidase 1 (renal)	L
3744		Dipeptidyl peptidase 9	L
6660		elongation of very long chain fatty acids-like 1	L
32072	Elo68α	elongation of long chain fatty acids-like 4	L
11801	Elo68β	elongation of long chain fatty acids-like 4	L
18609		elongation of very long chain fatty acids-like 4	L
3971	Baldspot	elongation of very long chain fatty acids-like 6	L
8433	Ext2	exostosin 2 isoform 2 (glycosyltransferases)	L
4770		Fatty acyl-CoA reductase 1	L
3524	v(2)k05816	fatty acid synthase	L
4095		fumarate hydratase	L
8890	Gmd	GDP mannose dehydratase	L
4625		glyceronephosphate O-acyltransferase	L
3215		glycerol-3-phosphate dehydrogenase 1 (soluble)	L
2137		glycerol-3-phosphate dehydrogenase 2	L
1787	Hexo2	Beta-hexosaminidase subunit alpha/beta	L
4779	hgo	homogentisate dioxygenase	L
12171		17-beta-hydroxysteroid dehydrogenase 14	L
11151		17-beta hydroxysteroid dehydrogenase 4	L
3961		Long-chain-fatty-acid–CoA ligase 5	L
13334		L-lactate dehydrogenase	L
31091		lipase, gastric	L
11600		Lipase member K	L
8093		lipase, family member M	L
7921	Mgat2	mannosyl (alpha-1,6-)-glycoprotein	L
1942		monoacylglycerol O-acyltransferase 2	L
6218		N-acetyl-D-glucosamine kinase	L
31730		N-acetyltransferase 5 isoform a	L
31851		N-acetyltransferase 5 isoform a	L
7291	Npc2a	Epididymal secretory protein E1 Precursor	L
10924		phosphoenolpyruvate carboxykinase 1 (soluble)	L
17725	Pepck	phosphoenolpyruvate carboxykinase 2	L
7024		pyruvate dehydrogenase (lipoamide) alpha 1	L
10627		Phosphoacetylglucosamine mutase 3	L
13978		phosphatidylinositol glycan anchor biosynthesis, class N	L
4907		phosphatidylinositol glycan anchor biosynthesis, class N	L
3620	norpA	phospholipase C, beta 4	L
18258		pancreatic lipase	L
2212	sws	Patatin-like phospholipase domain-containing protein 7	L
14789	O-fut2	GDP-fucose protein O-fucosyltransferase 2	L
3073	l(1)G0144	protein prenyltransferase alpha subunit repeat containing 1	L
17121		retinol dehydrogenase 10	L
30499		Ribulose-phosphate 3-epimerase	L

7066	Sbp2	SECIS binding protein 2	L
4672	TMS1	serine incorporator 1	L
3307	pr-set7	Histone-lysine N-methyltransferase SETD8	L
8112		sterol O-acyltransferase 1	L
4162	lace	Serine palmitoyltransferase 2	L
3376		Sphingomyelin phosphodiesterase	L
32052		sphingomyelin phosphodiesterase	L
5103		transketolase	L
6649	Ugt35b	UDP-glucuronosyltransferase 2B10	L
		OTHERS	
3264		alkaline phosphatase, placental	L
9198	shtd	Anaphase-promoting complex subunit 1	L
14965		ankyrin repeat domain 12 isoform 2	L
10984		Ankyrin repeat domain-containing protein 12	L
15118		ankyrin repeat domain 13B	L
10011		Ankyrin repeat domain-containing protein 50	L
17149	Su(var)3-3	Ankyrin repeat and KH domain-containing protein 1	L
9968	Anxb11	annexin 7	L
9968	Anxb11	annexin VII isoform 2	L
4019		aquaporin 4 isoform b	L
32191		N-acetylgalactosamine-4-sulfatase	L
6763		astacin-like metalloendopeptidase	L
10814		gamma butyrobetaine dioxygenase	L
9908	disco	basonuclin 2	L
2252	fs(1)h	Bromodomain-containing protein 4 (HUNK1 pr)	L
9904		seipin	L
6906	CAH2	Carbonic anhydrase 2	L
6702	Cbp53E	Calbindin	L
17769	And	calmodulin	L
15373		cancer susceptibility candidate 1 isoform	L
14210		Coiled-coil domain-containing protein 86	L
14939		cyclin Y isoform	L
8258		T-complex protein 1 subunit theta	L
8360		cytidine deaminase	L
6392	cmet	Centromere-associated protein E	L
3986	Cht4	acidic chitinase	L
9357	Cht8	Acidic mammalian chitinase	L
2989	Cht6	chitotriosidase	L
1019	Mlp84B	Cysteine and glycine-rich protein 1	L
42309	Mlp60A	cysteine and glycine-rich protein 3	L
12163		cathepsin F	L
10246	Cyp6a9	cytochrome P450, family 3, subfamily A, polypeptide 5	L
10242	Cyp6a23	cytochrome P450, family 3, subfamily A, polypeptide 5	L
3506	vas	ATP-dependent RNA helicase DDX4	L
3735		digestive-organ expansion factor homolog	L
9099		density-regulated protein (translation initiation factor)	L
8915		DEAH (Asp-Glu-Ala-His) box polypeptide 36	L
7020	DIP2	DIP2 disco-interacting protein 2 homolog C	M
2239	jdp	DnaJ (Hsp40) homolog, subfamily C	L
10379	mbc	Dedicator of cytokinesis protein 1	L
2245	l(3)s1921	deoxyhypusine hydroxylase/monooxygenase	L
13190	cuff	DOM-3 homolog Z	L
8340	128up	developmentally regulated GTP binding protein 1	L
30460		dentin sialophosphoprotein	L

14853		dentin sialophosphoprotein	L
6148	Past1	EH domain containing 1	L
7439	AGO2	Argonaute 2	L
8335		eukaryotic translation initiation factor 3, subunit 5 epsilon	L
8846	Thor	eukaryotic initiation factor 4E binding	L
32859	eIF4E-7	eukaryotic translation initiation factor 4E isoform 3	L
15102	Jheh2	Microsomal epoxide hydrolase	L
1333	ERO1-L	Endoplasmic reticulum oxidoreductin-1-like	L
3631		Protein FAM20B Precursor	L
31232	koko	Cyclin-related protein FAM58A (Cyclin-M)	L
10158		FGFR1 oncogene partner 2	L
3006	Fmo-1	dimethylaniline monooxygenase [N-oxide-forming] 6	L
10703		GRIP and coiled-coil domain-containing protein 1	L
33214		golgi apparatus protein 1 isoform 3	L
30496		glomulin/FKBP associated protein	L
4840	cbs	golgin 97	L
11061	GM130	Golgi autoantigen, golgin subfamily a2	L
30000		glutathione S-transferase theta 1	L
17523	GstE2	lutathione S-transferase theta 2	L
18003		hydroxyacid oxidase 1	L
16989		HEAT repeat-containing protein 6	L
33714		heterogeneous nuclear ribonucleoprotein AB	L
16901	sqd	heterogeneous nuclear ribonucleopro..	L
33147	Hs3st-A	heparan sulfate (glucosamine) 3-O-sulfotransferase 5	L
2525	Hus1-like	HUS1 checkpoint homolog	L
5414		Isoleucyl-tRNA synthetase	L
9333	Oseg5	Intraflagellar transport protein 80 homolog	L
5859		integrator complex subunit 8	L
13855		IQ and ubiquitin-like domain-containing protein	L
10793		katanin p60 subunit A-like 2	L
4799	Pen	Importin subunit alpha-1	L
3793		Leucine carboxyl methyltransferase 2	L
12818		leukocyte receptor cluster member 1	L
15735		LSM 12 homolog	M,L
9111	LysC	Lysozyme C	L
9116	LysP	Lysozyme C-1 Precursor	L
9116	LysP	Lysozyme 1/2	L
1179	LysB	Lysozyme 1/2	L
2072	TXBP181-like	Mitotic spindle assembly checkpoint protein MAD1	L
18802	α-Man-II	mannosidase, alpha, class 2A, member 2	L
8031		mediator of cell motility 1	L
10238	Mocs2	Molybdenum cofactor synthesis protein 2B	L
13090		Molybdopterin synthase sulfurylase	L
1919	Cpr62Bc	mucin-2 precursor	L
13722		nascent polypeptide-associated complex	L
13667		NADPH dependent diflavin oxidoreductase 1	L
15669	MESK2	N-myc downstream-regulated gene 3 isoform a	L
1009	Psa	aminopeptidase puromycin sensitive	L
9019	dsf	photoreceptor-specific nuclear receptor isoform b	L
10581		nucleoside-triphosphatase	M,L
17904		Nucleotide-binding protein 1	L
10347		NudC domain-containing protein 1	L
8128		Nucleoside diphosphate-linked moiety X motif 6	L
7360	Nup58	nucleoporin like 1 isoform c	L
12752	Nxt1	NTF2-related export protein 2	L

1513		oxysterol binding protein	L
11486		PAN3 poly(A) specific ribonuclease subunit homolog	L
8363	Papss	Papss-2	L
7228	pes	pescadillo homolog 1	L
7266	Eip71CD	Peptide methionine sulfoxide reductase	L
11858		peptidyl propyl cis/trans isomerase	L
17266		peptidylprolyl isomerase H	L
17268	Pros28.1A	proteasome alpha 8 subunit	L
9588		26S proteasome non-ATPase regulatory subunit 9	L
31000	heph	Polypyrimidine tract-binding protein 1	L
6168		glutaminyl-peptide cyclotransferase-like	M,L
34422		retinoblastoma binding pr.	L
9088	lid	retinoblastoma binding protein 2	L
6434		retinoblastoma binding protein 5	L
30495		retinol dehydrogenase 12	L
7694		E3 ubiquitin-protein ligase RNF181	L
13344		E3 ubiquitin-protein ligase RNF25	L
10343		RWD domain-containing protein 4A	L
4170	vig	SERPINE1 mRNA binding protein 1	L
9456	Spn1	Serine (or cysteine) proteinase inhibitor	L
9334	sp3 ,Spn3	Serpin B4	L
9455		serine (or cysteine) proteinase inhibitor, clade B, member 9	L
5094	Sgt	small glutamine rich tetratricopeptide	L
4909	POSH	SH3 domain containing ring finger 3	L
1311		CDW92 antigen/Choline transporter-like protein 1	L
8595	Toll-7	SLIT and NTRK-like protein 5	L
8032		Spermine oxidase	L
4649	Sodh-2	sorbitol dehydrogenase	L
2720	Hop	stress-induced-phosphoprotein 1 (Hsp70/Hsp90	L
5241	Taspase1	Threonine aspartase 1	L
13472		Tudor domain-containing protein 3	L
10118	ple	tyr hydroxylase	L
1102	MP1	transmembrane protease, serine 4 isoform 3	L
7398	Trn	transportin 1 isoform 2	L
4843	Tm2	Tropomyosin alpha-3 chain	L
17556		tetratricopeptide repeat domain 35	L
11323	TTLL3A	Tubulin–tyrosine ligase-like protein 3	L
8993		thioredoxin	L
3315	TrxT	thioredoxin	L
11588		thioredoxin domain containing 1	L
5495	Txl	Thioredoxin-like	L
3589		Peroxisomal leader peptide-processing protease	L
17030		ubiquitin-conjugating enzyme E2L 3 isoform 1	M,L
10254		ubiquitin-conjugating enzyme E2O	L
15817		ubiquitin specific peptidase 1	L
4165		Ubiquitin thioesterase 16	L
4202	Sas10	small subunit processome component	L
1520	WASp	Wiskott-Aldrich syndrome-like	L
6724		Ribosome biogenesis protein WDR12	L
2812		WD repeat domain 47	L
17766	Rbcn-3B	WD repeat-containing protein 7	L
17293		WD repeat domain 8	L
9900	mit(1)15	ZW10, kinetochore associated, homolog	L

ION TRANSPORTER

12602		ATPase, H+ transporting, lysosomal V0	L
4624		Lysosomal ATPase H+ transporting, V0 sub unit	L
2934	VhaAC39	V-type proton ATPase subunit d 1	L
5075		ATPase, H+ transporting, lysosomal V1	L
17369	Vha55	V-type proton ATPase subunit B	L
7779	Cng	cyclic nucleotide gated channel alpha	L
4587		voltage-gated calcium channel alpha(2)delta-4 subunit	M
5284		H(+)/Cl(-) exchange transporter 3	L
10997	Clic	intracellular Cl- channel 5	L
32688	Hk	potassium voltage-gated channel, shaker-related subfamily, beta member 2 isoform 2	L
5890		Kv channel interacting protein 1	L
6504	Pkd2	polycystin-2	L
9903		solute carrier family 10 (sodium/bile cotransporter family), member 2	L
10413		K/Cl symporter	L
11665		monocarboxylate transporter 13	L
14694		solute carrier family 19, member 2	L
6293		L-Ascorbate Na+ symporter	L
8323		solute carrier family 25, member 34	L
6484		solute carrier family 2 member 8	L
8234		solute carrier protein family 2 member 8	L
7623	sll	solute carrier family 35, member B2	L
8695	LvpL	solute carrier family 3, member 1	L
15890		solute carrier family 46, member 3	L
9657		solute carrier family 5 (I- transporter) member 8	L
15444	ine	solute carrier family 6 (betaine/GABA) member 12	L
4545	SerT	Serotonin transporter	L
12531		Solute carrier family 7 member 14	L
10806	Nha1	Na+/H+ hydrogen antiporter	L
		INTRACELLULAR TRANSPORT	
9388	AP-47	adaptor-related protein complex 1, mu 1 subunit	L
9463		mannosidase, alpha, class 2B, member 1	L
7435	Arf84F	ARF factor 2	L
8156	Arf51F	ARF III	L
5429	Atg6	beclin 1, autophagy related	L
9308		coated vesicle membrane protein	L
4848		component oligomeric golgi complex 1	L
2038	CSN7	COP9 signalosome complex subunit 7b	L
17604	c(3)G	early endosome antigen 1	L
12855	HPS	Hermansky-Pudlak syndrome 1 protein	L
3792		mannose-P-dolichol utilization defect 1	L
8683		MON2–Arf family exchange factor (golgi trafficking)	L
1513		Oxysterol-binding protein-related protein 9	L
6760	l(3)70Da	Peroxin1	L
7864		Peroxisome biogenesis factor 10	L
7062	Rab-RP3	Rab 3	L
12156	Rab39	Rab39	M,L
8287	Rab8	Rab8	L
34397	Rgk3	GTP-binding protein REM 1	L
1167	Ras64B	Ras-like protein TC21	L
6678		RCC1 domain containing 1	L
11857		Retention in ER1	L
10043	rtGEF	Rho guanine nucleotide exchange factor 7	L
34418	sif	T-lymphoma invasion and metastasis-inducing protein 2	L
3988	γSnap	Gamma-soluble NSF attachment protein	L

9958	snapin	SNAP-associated protein	L
9474	Snap24	SNAP25	L
32758		sorting nexin family member 27	L
6410		Sorting nexin-16	L
17320	ScpX	Sterol carrier potein 2	L
17248	n-syb	Synaptobrevin-2	L
11278	Syx13	Syntaxin 13	L
1467		Syntaxin 16	L
7736	Syx6	Syntaxin 6	L
4758	Trp1	Translocation protein SEC62	L
7919	fan	vesicle-associated membrane protein-associated protein A	L
		DNA/CHROMATIN BINDING	
1795	Ogg1	8-oxoguanine DNA glycosylase isoform 1a	L
10385	msl-1	Male-specific lethal 1 homolog	L
4036		alkylation repair homolog 4	L
14130		alkylation repair homolog 7	L
5316		aprataxin isoform c	L
3675	Art2	arginine methyltransferase 8	L
7602	DNApolι	polymerase (DNA directed) iota	L
11301	Mes4	DNA polymerase epsilon subunit 4	L
1925	mus205	DNA polymerase zeta	L
9148	scf	Calumenin	L
33650	DNApol-?35	DNA-directed DNA polymerase gamma 2	L
13418	RpI12	DNA-directed RNA polymerase I subunit	L
31679		endonuclease G	L
10387	tosca	exonuclease 1	L
8862	EndoG	EndoDNAase	L
10215	Ercc1	excision repair cross-complementing 1 isofrom 2	L
2128	Hdac3	HDAC3	L
6990	HP1c	heterochromatin protein 1-beta	L
12223	Dsp1	High mobility group protein B1	L
17949	His2B	Histone H2B	L
4976	Mes-4	Wolf-Hirschhorn syndrome candidate 1	L
4565		Histone-lysine N-methyltransferase	L
5017		nucleosome assembly protein 1-like 1	L
9601		Polynucleotide 3' Kinase	M,L
4299	Set	SET nuclear oncogene	L
10336		TIMELESS interacting protein	L
3458	Top3β	topoisomerase-3	M
15104	Topors	topoisomerase I binding, arginine/serine-rich	L
		STRUCTURAL PROTEINS	
32531	mRpS14	mitochondrial ribosome S14	L
5012	mRpL12	mRpL12	L
8849	mRpL24	mRpL24	L
15442	mRpL27	mRpL27	L
1577		mRpL52	L
2033	RpS15A	Ribosomal protein S15	L
4882		Rp S27	L
9873	RpL37b	RpL37b	L
9378	Rlc1	RpL47	L
12324	RpS15Ab	RpS15Ab	L
7215		ubiquitin and ribosomal protein S27a	L

APOPTOSIS REGULATORS

4199		Apoptosis-inducing factor 3	L
13887		B-cell receptor-associated protein 31	L
7945		BCL2-associated athanogene 2	L
10992		cathepsinB	L
8357	Drep-1	DNA fragmentation factor subunit alpha	L
14715		FK506-binding protein 2 Precursor	L
4201	ird5	inhibitor of nuclear factor kappa B kinase	L
7896		insulin-like growth factor binding protein	L
8853		Intraflagellar transport protein 57 homolog	L
13510		LPS induced TNF factor 1	L
5216	Sir2	NAD-dependent deacetylase sirtuin-1	L
5073		programmed cell death 10	L
3939		thioredoxin domain containing 17	L
5140	nopo	TRAF-interacting protein	L

TRANSMEMBRANE PROTEINS

1718		ATP-binding cassette, sub-family A member 3	L
10181	Mdr65	BBOX1 gamma butyrobetaine dioxygenase 10814 3429	L
9270		ATP-binding cassette, sub-family C (CFTR/MRP), member 4	L
7627		ATP binding cassette superfamily C, member 4	L
4562		ATP-binding cassette, sub-family C, member 4	L
4140		Ankyrin repeat domain-containing protein 49	L
9703	Axs	transmembrane protein 16K/Anoctamin-10	M,L
11951		membrane alanine aminopeptidase	L
32513	bves	blood vessel epicardial substance	L
9496	Tsp29Fb	CD63	L
7962	CdsA	CDP-diacylglycerol synthase 1	L
15358		C-type lectin domain family 4, member M	L
6383	crb	crumbs homolog 1 precursor	L
13095	Bace	cathepsin E isoform/ b- APP-cleaving enzyme	L
3061		DNAJB12	L
42400		dipeptidase 3	L
4662		EF-hand domain family, member A2	L
3059	NTPase	Ectonucleoside triphosphate diphosphohydrolase 5	L
13160		endoplasmic reticulum metallopeptidase 1	L
10081		endoplasmic reticulum metallopeptidase 1	L
42280	ome	fibroblast activation protein, alpha subunit	L
5907	Frq2	Neuronal calcium sensor 1	L
4114	ex	FREM domain containing 1 isoform	L
4521	mthl1	G protein-coupled receptor 64	L
16857		immunoglobulin superfamily 9B	L
3653	kirre	kin of IRRE like 3	L
11136		leucine-rich repeats and Ig-like domains 1	L
11136		leucine-rich repeats and Ig-like domains 1	L
33087		LRP1/ alpha 2 macroglobulin/APOER	L
15254		meprin A beta	L
12021	Patj	Multiple PDZ domain protein	L
10588		nardilysin isoform b	L
17610	grk	neuregulin 3	L
14969		osteopetrosis associated transmembrane protein 1	L
8297		disulfide isomerase	L
8297		disulfide isomerase	L
9046	Vm26Ab	protocadherin 15 isoform CD1-7 precursor	L
9084		phospholipid scramblase 1	L

4679		Pentatricopeptide repeat domain 3	L
5423	robo3	roundabout 1 isoform b	L
8895	Rtnl1	Reticulon-1 (Neuroendocrine-specific protein)	L
10497	Sdc	Syndecan-3	L
15629		Epidermal retinal dehydrogenase 2	L
3326		spastin	L
8766	Taz	Tafazzin	L
1021		transmembrane and coiled-coil domain family 2	L
9536		PL6 protein	L
12341		Transmembrane protein 170A	L
13603		transmembrane protein 179	L
7071		transmembrane protein 199	L
14238		Transmembrane protein 26	L
13920		Transmembrane Protein 35	L
6982		Brain cell membrane protein 1	L
4613		transmembrane protease, serine 3	L
4050		Transmembrane and TPR repeat-containing protein 3	L
12846	Tsp42Ed	tetraspanin 9	L
3078		unc-93 homolog A isoform 1	L
8624	melt	ventricular zone expressed PH domain homolog 1	L
14001	bchs	WD repeat and FYVE domain containing 3	L

MITOCHONDRIAL PROTEINS

16986		Acyl-coenzyme A thioesterase 13	L
42252	mmd	ADAM metallopeptidase domain 11	L
6030	ATPsyn-d	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	M
10575	Ppat-Dpck	coenzyme A synthase	L
4942		Mitochondrial inner membrane protein COX18 Precursor	L
2140	Cyt-b5	Cytochrome b5	L
6816		cytochrome P450, family 2, subfamily U, polypeptide 1	L
5599		dihydrolipoamide branched chain transacylase precursor	L
10361		glycine C-acetyltransferase	L
14407		glutaredoxin 5	M
15116		glutathione peroxidase 4	L
4389		Trifunctional enzyme subunit alpha,	L
7235	Hsp60C	chaperonin	L
9836		Iron-sulfur cluster assembly enzyme ISCU	L
10749		mitochondrial malate dehydrogenase precursor	L
7791		mitochondrial intermediate peptidase	L
10757		mitochondrial ribosomal protein S18B	L
4610		mitochondrial translation optimization 1	L
7598		NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 1	L
8844	Pdsw	NADH dehydrogenase	L
8132		nitrilase family, member 2	L
3107		pitrilysin metallopeptidase 1	L
6888		peroxiredoxin 2	M
14757		succinate dehydrogenase complex assembly factor 2	L
18418		solute carrier family 25 member 11 (mitochondrial carrier)	L
3057	colt	solute carrier family 25 member 20	L
4994		Mitochondrial phosphate carrier protein	L
9090		Phosphate carrier protein	L
1065	Scsα	succinate-CoA ligase, alpha subunit	L
11611	Tim13	Tim13	L
15257	Tim17b2	Tim-17	L
3021		Mitochondrial tRNA-specific 2-thiouridylase 1	L

4335		trimethyl lysine hydroxylase epsilon	L
11401	Trxr-2	thioredoxin reductase 2	L
		CYTOSKELETON PROTEINS	
8604	Amph	bridging integrator 1	L
18631		coiled-coil and C2 domain containing 2A	L
17150		dynein, axonemal, heavy chain 3	L
9764	yrt	Band 4.1-like protein 5	L
3399	capu	formin 2	L
5022		FERM domain-containing protein 3	L
33556	form3	Inverted formin 2 isoform 1	M
10229	katanin-60	katanin p60 subunit A-like 1	L
9910	kat80	Katanin p80 WD40-containing subunit B1	L
12298	sub	Kinesin family member 20A	L
5300	Klp31E	Kinesin-like protein KIF21A	L
8649	Fim	lymphocyte cytosolic protein 1	L
13221	Vhl	LIM domain binding 3 isoform 1	L
17927	Mhc	myosin, heavy chain 7, cardiac musc...	L
5596	Mlc1	fast skeletal myosin alkali light chain 1	L
3849	Lasp	nebulette	L
13458		piccolo isoform 1	L
11739		sideroflexin 1	L
34379	shroom	shroom family member 4	L
12117	Sptr	sepiapterin reductase	M
14168		synaptopodin 2-like isoform a	L
17566		gamma-tubulin	L
		UNKNOWN	
17068		BTB/POZ domain containing protein 3 isoform	L
4593		coiled-coil domain-containing protein 25	L
10383		serine active site containing 1	L
15439		PHD finger pr. 14 isoform	L
6424		Protein FAM13C	L
30338		RWD domain containing 2B	L
32544		hypothetical	L
10075		hypothetical	L
10566		hypothetical	L
11388		hypothetical	L
7289		hypothetical	L
12118		hypothetical	L
5903		hypothetical	L
5793		hypothetical	L
4186		hypothetical	L
12608		hypothetical	L
11454		hypothetical	L
13018		hypothetical	L
9867		hypothetical	L
31050		hypothetical	L
9879		hypothetical	L
15133		hypothetical	L
5745		hypothetical pr	L
8675		hypothetical pr	L
7044		hypothetical pr	L
12929		hypothetical pr	L
3309		hypothetical pr	L

14777		hypothetical pr	L
14299		hypothetical pr	L
15456		hypothetical pr	L
10581		hypothetical pr	L
6443		hypothetical protein	L
13567		hypothetical protein	L
31800		hypothetical protein	L
32813		hypothetical protein	L
2818		hypothetical protein	L
9752		hypothetical protein	L
9166	312	hypothetical protein LOC115416	L
5435		hypothetical protein LOC127003	L
10517		hypothetical protein LOC221443	L
11178		hypothetical protein LOC23080	L
10674		hypothetical protein LOC51398	L
15706		hypothetical protein LOC54842	L
9986		hypothetical protein LOC57102	L
30100		hypothtical protein	L
17726		chromosome 2 open reading frame 56	L
7974		chromosome 9 open reading frame 78	L
31076		chromosome 10 open reading frame 11	L
13926		Uncharacterized protein C11orf73	L
12279		similar to KAT protein	L
14903		Uncharacterized protein C2orf79	L

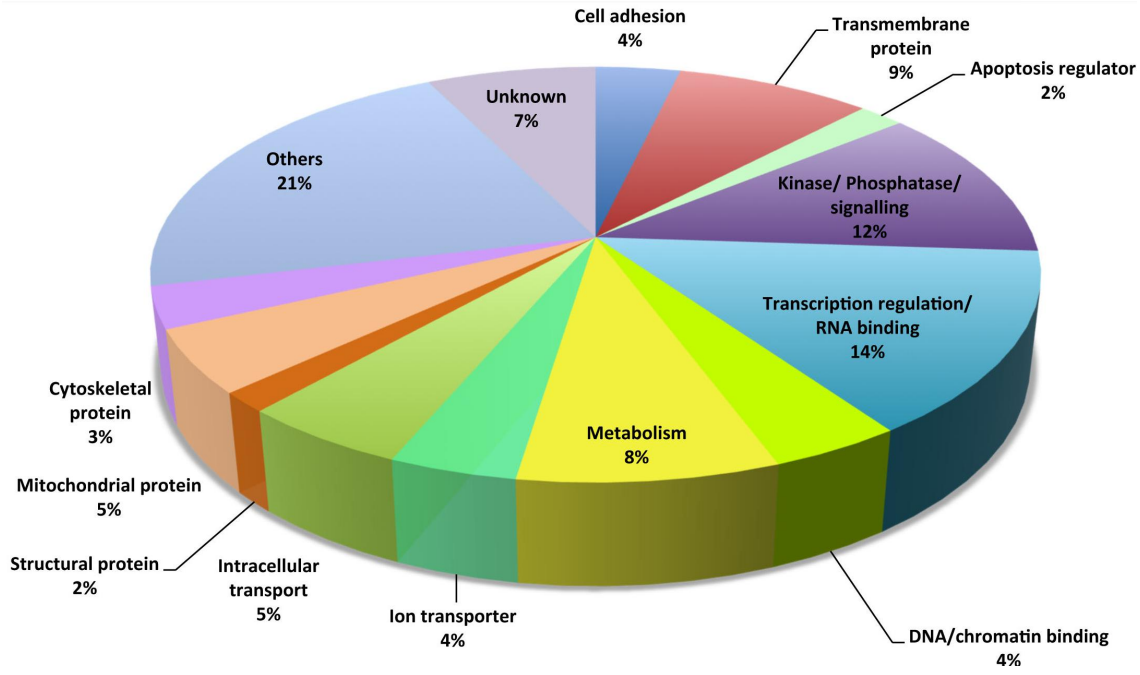


Figure 1: Sorted candidates. The distribution of GO-annotated biological processes of predicted human homologs of the candidates obtained after comparing with GMR, muscle and trachea screening. The sorting was performed manually using Uniprot database.

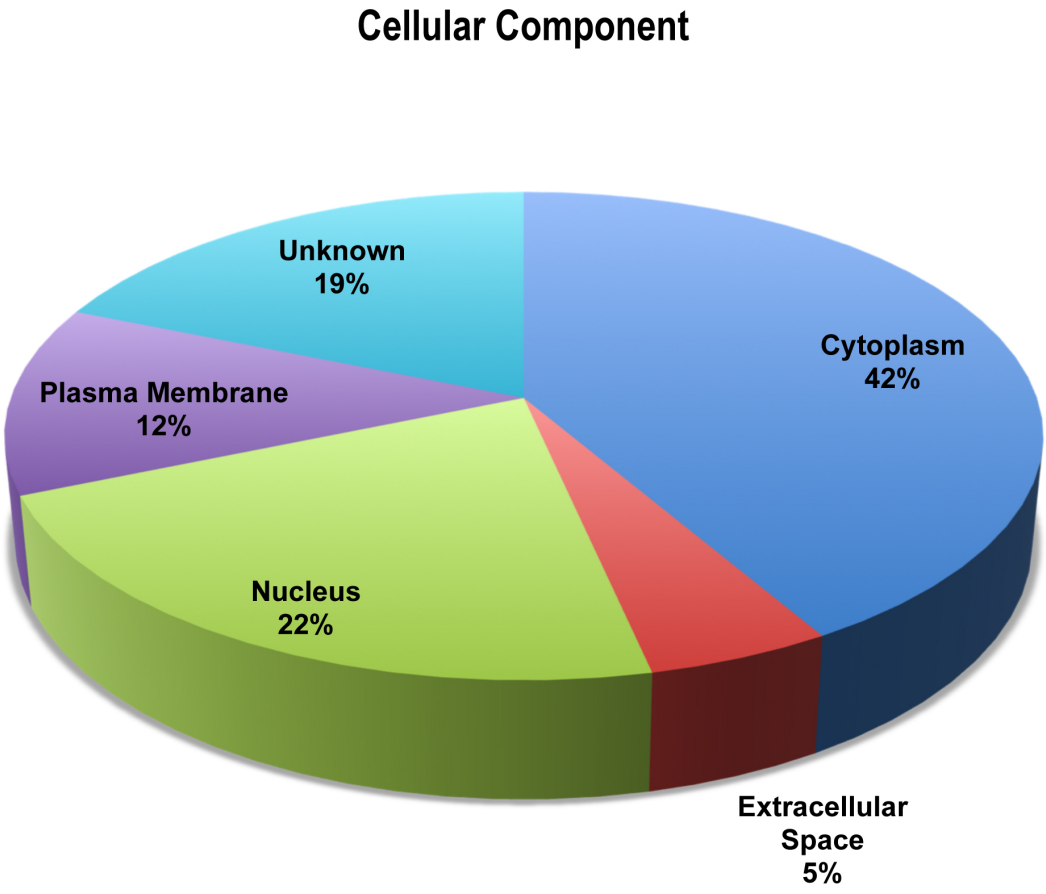


Figure 2: Sorted candidates. Distribution of GO annotated cellular components of the glial screening specific candidates. The data was extracted using Ingenuity Pathway Analysis. Candidates that belong to plasma membrane category are potential candidates involved in neuron-glia interaction.

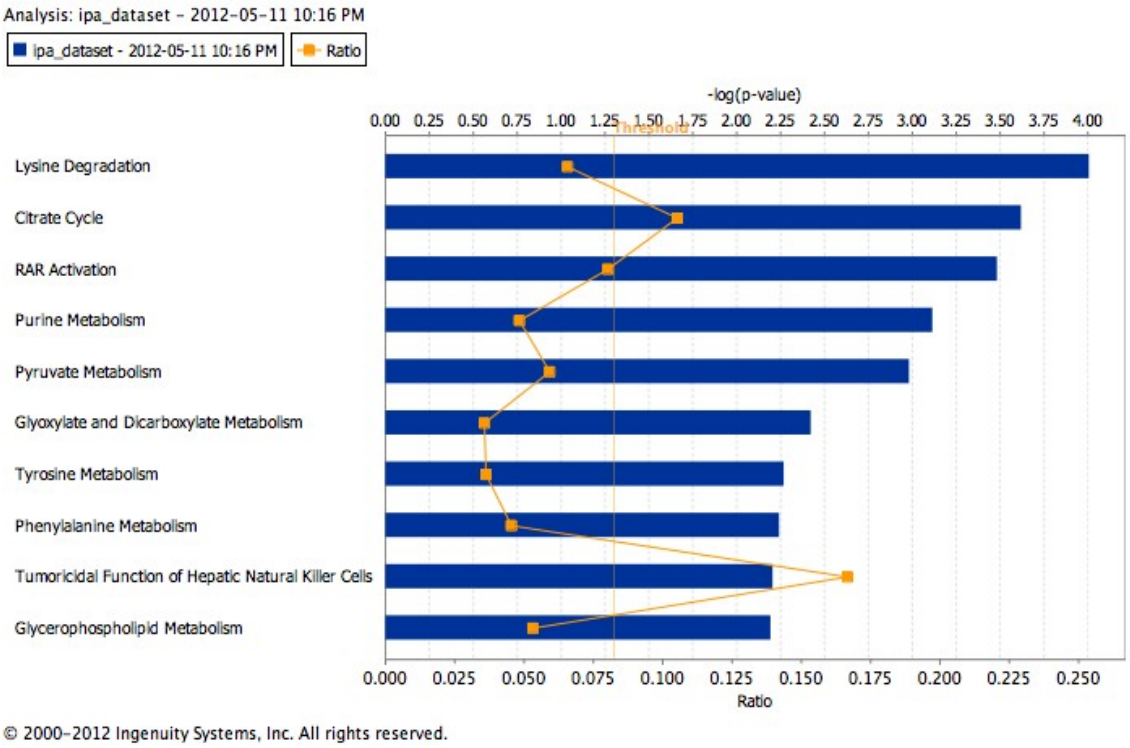


Figure 3: Canonical pathways. Top 10 canonical pathways that are significantly enriched in our list of candidates. Ingenuity Pathway Analysis shows that glial metabolism is crucial for neuronal survival.

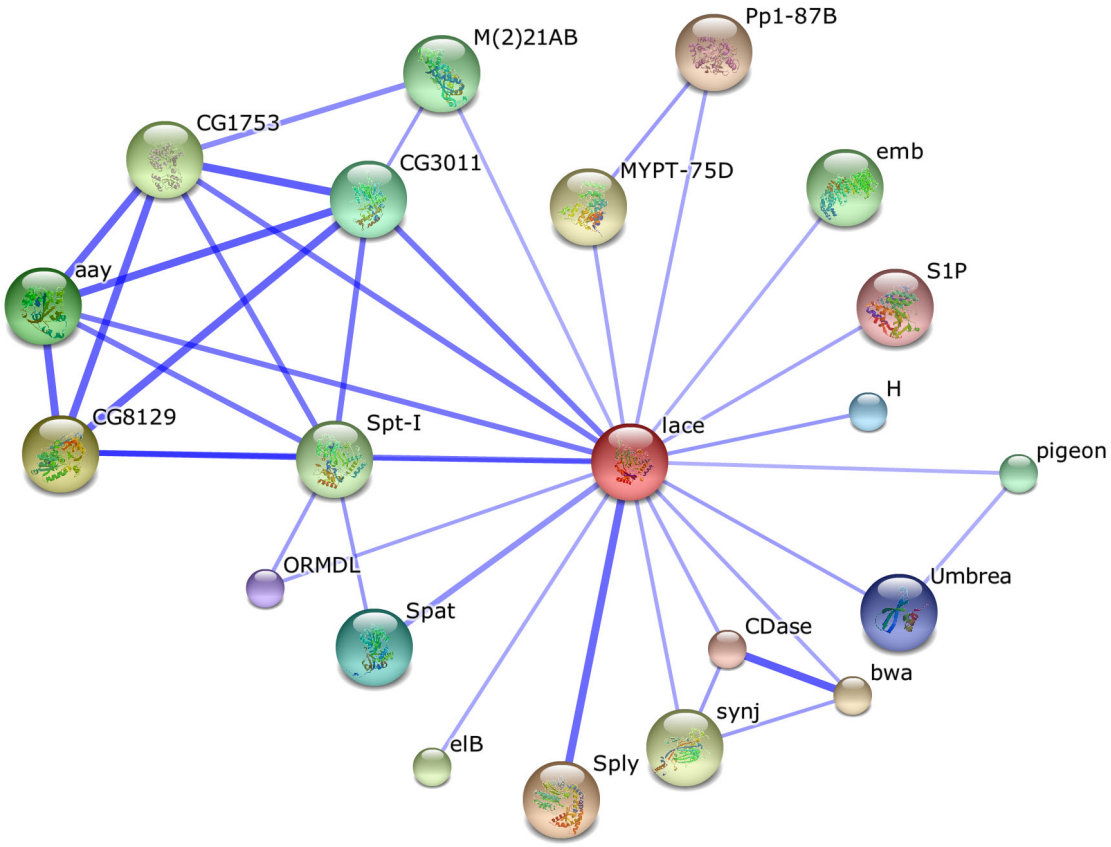


Figure 4: Interactome map of *lace*. The STRING protein interactome map of *lace*. Thicker lines indicates a stronger association. This map identifies lace as a central regulator of sphingolipid biosynthesis and it also has a potential role in the regulation of other metabolic pathways.

List of screened RNAi lines Provided by VDRC as human homolog sub-library

Gene	TID
CG10001	1326
CG10002	37063
CG10002	49961
CG10006	44539
CG10009	17954
CG10011	45096
CG10018	37591
CG10021	3774
CG10023	17957
CG10029	16583
CG10030	44480
CG10032	51688
CG10033	38319
CG10034	30525
CG10036	15425
CG10037	10756
CG10037	47182
CG1004	51953
CG10043	17966
CG10047	33317
CG10050	30020
CG10052	44717
CG10053	50811
CG10053	17972
CG10055	17973
CG10060	28150
CG10061	17975
CG10062	4697
CG10064	38322
CG10066	23303
CG10067	17979
CG10068	15948
CG10069	6591
CG10072	29434
CG10073	11133
CG10075	50067
CG10075	15399
CG10076	44092
CG10078	48823
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CG10079	43268
CG10080	46133
CG10081	10066
CG10082	38326
CG10083	38330
CG10084	38336
CG10089	17991
CG1009	35354
CG10090	37346
CG10096	50765
CG10097	6090
CG10098	50126
CG10103	31174
CG10104	13969
CG10105	18002
CG10106	7934
CG10107	18004
CG10110	18009
CG10117	4871
CG10118	3308
CG10122	12688
CG10123	10635
CG10126	44104
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CG10130	8785
CG10133	18014
CG10137	38342
CG10142	15246
CG10143	51564
CG10144	18019
CG10145	15194
CG10149	18022
CG10153	31186
CG10155	18024
CG10157	14004
CG10158	47388
CG10160	31192
CG10162	23362
CG10165	3909
CG10166	46385
CG10166	13731
CG10168	1151
CG1017	15610
CG10170	1141
CG10171	43837
CG10174	31195
CG10175	1140
CG10178	8064
CG10181	9019
CG10184	3311
CG10185	18135
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CG10188	18029
CG10189	51692
CG1019	18593
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CG10193	45109
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CG10203	31202
CG10206	31204
CG1021	37336
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CG10211	12352
CG10212	10711
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CG10320	8837
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CG10326	5894
CG10326	47194
CG10327	38377
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CG10336	16019
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CG10354	27254
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CG10379	16044
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CG10399	18617
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CG10420	1753
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CG10425	6734
CG10426	16048
CG10435	38384
CG10438	23296
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CG10459	41451
CG10463	31248
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CG10473	16052
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CG10546	31258
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CG10584	28681
CG10585	31273
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CG10616	36605
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TID= transformant ID, VDRC

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CG10623	31294	CG10723	30304	CG10862	31373	CG10984	15627	CG11096	38444
CG10624	44928	CG10724	22850	CG10863	48619	CG10986	31390	CG11098	7625
CG10626	22845	CG10726	26759	CG10866	3234	CG10988	2983	CG11099	9272
CG10627	31298	CG10728	50141	CG10868	45009	CG1099	16158	CG11102	13478
CG10628	47392	CG10732	18664	CG10869	27326	CG10990	16162	CG11103	5562
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CG10635	35481	CG10742	10140	CG10877	45750	CG10996	16168	CG11109	17528
CG10635	46220	CG10743	16850	CG1088	45374	CG10997	28303	CG11110	3801
CG10637	35482	CG10747	38403	CG10881	35495	CG10999	16171	CG11111	6226
CG10638	31306	CG10748	14072	CG10882	37543	CG1100	18676	CG11115	49942
CG10639	30737	CG10749	27311	CG10887	16082	CG11001	45015	CG1112	42942
CG1064	12645	CG10750	27313	CG10889	27329	CG11006	31394	CG11121	8950
CG10640	30890	CG10751	22761	CG1089	27332	CG11007	40833	CG11123	18142
CG10641	31307	CG10753	31343	CG10890	50554	CG11009	16173	CG11124	13911
CG10642	45372	CG10754	31347	CG10895	44981	CG1101	12031	CG11125	18143
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CG10646	38396	CG10757	45494	CG10898	13643	CG11015	30892	CG1113	31426
CG10648	51699	CG1076	40807	CG10899	16084	CG1102	18970	CG11130	18145
CG1065	27298	CG10760	13996	CG1090	26783	CG11024	46489	CG11133	18150
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CG10657	3326	CG10776	42244	CG10908	44211	CG11029	44228	CG11137	48241
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CG10685	17847	CG10805	17000	CG10931	52107	CG11058	31409	CG11156	31432
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CG10687	31320	CG10808	8784	CG10938	16104	CG11059	37291	CG11162	8326
CG10688	39715	CG10809	38408	CG10939	16958	CG1106	37867	CG11163	13311
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CG10695	27307	CG10824	16588	CG10954	45013	CG1107	16182	CG11172	30566
CG10697	3329	CG10825	31360	CG10955	27341	CG11070	31413	CG11173	18172
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CG10701	37917	CG10833	7870	CG10961	16125	CG1108	16184	CG11178	18178
CG10702	3691	CG10837	31364	CG10962	30850	CG11081	27238	CG11180	31438
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CG1072	46755	CG10850	18847	CG10977	37238	CG11092	16188	CG11190	14821
CG1072	9830	CG10859	27322	CG10979	16144	CG11093	15478	CG11194	30561
CG10721	33098	CG1086	13326	CG1098	27346	CG11094	11096	CG11196	36497

TID= transformant ID, VDRC

CG11197	41368	CG1132	30553	CG11449	31503	CG11601	42737	CG11783	10958
CG11198	8105	CG11320	18054	CG11450	41069	CG11601	50814	CG11788	47245
CG11199	51707	CG11321	18055	CG11454	45116	CG11606	44093	CG1179	14123
CG1120	18184	CG11323	18057	CG11455	12838	CG11607	15876	CG1179	49832
CG11200	4725	CG11324	18061	CG11459	16573	CG11608	19932	CG11793	31551
CG11201	31456	CG11325	9546	CG11459	48053	CG1161	44964	CG11796	31563
CG11202	37656	CG11326	7535	CG11465	28457	CG11611	39157	CG11799	45697
CG11206	42943	CG11329	18065	CG11466	38045	CG11614	3004	CG1180	13842
CG11207	7833	CG1133	51292	CG1147	9605	CG11617	30533	CG11801	30042
CG11208	4720	CG11333	44110	CG11474	31507	CG11621	16240	CG11802	31570
CG11210	7363	CG11334	38485	CG11475	22864	CG11622	16244	CG11804	16313
CG11217	28762	CG11335	17259	CG11482	12685	CG11639	23285	CG11807	38564
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CG11236	38460	CG11342	38488	CG11490	20040	CG1165	13886	CG11820	39724
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CG11251	38467	CG11357	5027	CG11511	38526	CG11659	16260	CG11825	33917
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CG11257	31475	CG11374	15561	CG1152	38041	CG11665	7314	CG11837	38574
CG11258	23376	CG11376	40673	CG11522	51715	CG11669	15794	CG11838	31575
CG11259	17537	CG11386	38493	CG11523	46794	CG1167	6225	CG11839	31576
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TID= transformant ID, VDRC

CG11922	13721	CG12058	31635	CG12163	15366	CG12275	44589	CG12376	22879
CG11926	38600	CG12068	16388	CG12165	17044	CG12276	18528	CG12384	36388
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CG11935	15472	CG12070	51129	CG12170	31672	CG12278	29484	CG12390	38682
CG11936	37927	CG12071	49512	CG12171	47411	CG12279	44123	CG12395	17101
CG11940	16369	CG12072	9928	CG12173	31674	CG1228	38652	CG12396	31927
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CG11949	9787	CG12077	4125	CG12175	32503	CG12283	4761	CG12398	11540
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CG11951	48791	CG12079	13856	CG12178	7245	CG12286	37348	CG1240	45758
CG11956	3335	CG12081	31642	CG1218	31685	CG12287	52272	CG12400	37462
CG11958	1335	CG12082	17568	CG1218	46777	CG12287	30708	CG12403	46397
CG11963	20097	CG12083	7434	CG12186	17068	CG12289	31724	CG12403	17102
CG11963	50300	CG12084	31646	CG12189	13617	CG12297	7926	CG12404	1463
CG11964	35511	CG12085	20144	CG12190	52138	CG12298	45402	CG12405	24522
CG11968	20130	CG12090	16390	CG12192	41484	CG12301	51733	CG12405	50772
CG11975	26799	CG12091	13985	CG12194	7846	CG12304	38654	CG12410	9727
CG11980	38616	CG12092	35514	CG12194	46796	CG12306	20177	CG1242	7716
CG11981	31608	CG12093	41293	CG12200	31688	CG1231	17076	CG12424	25222
CG11982	38623	CG12099	18734	CG12200	50229	CG12311	5583	CG1245	13697
CG11984	31615	CG1210	18736	CG12201	42648	CG12313	4075	CG12455	3849
CG11985	22773	CG12101	18739	CG12202	17571	CG12314	48764	CG12467	46883
CG11986	20136	CG12104	16398	CG12203	42983	CG12317	45191	CG12467	41377
CG11987	10735	CG12106	16400	CG12207	18747	CG12318	12375	CG12477	31942
CG11988	10662	CG12107	8731	CG12208	20157	CG12320	17781	CG12478	35526
CG11989	31619	CG12108	5499	CG1221	15396	CG12321	17077	CG12489	20239
CG11990	28318	CG12109	20151	CG12210	30605	CG12323	38658	CG1249	31947
CG11992	49413	CG12110	38626	CG12211	38634	CG12324	49105	CG12497	24549
CG11994	18719	CG12111	16513	CG12211	38634	CG12324	17785	CG12498	17793
CG11999	16423	CG12113	18742	CG12212	3788	CG12325	31726	CG12499	24550
CG1200	50007	CG12116	6498	CG12214	31689	CG12327	8372	CG1250	24552
CG12000	16381	CG12117	17018	CG12215	8754	CG12333	23091	CG12505	31122
CG12001	16383	CG12118	13340	CG12217	31690	CG12334	17079	CG12505	48131
CG12002	15277	CG1212	41479	CG12218	31693	CG12338	35521	CG12506	19879
CG12004	10287	CG12125	5492	CG12220	23040	CG12339	17081	CG12506	50327
CG12005	11205	CG12127	3295	CG12223	41029	CG1234	17084	CG12511	12451
CG12006	39481	CG12128	42979	CG12225	31701	CG12340	22878	CG1252	4566
CG12007	20137	CG12129	17065	CG12230	4548	CG12341	7391	CG12524	28745
CG12008	37075	CG1213	6487	CG12231	31705	CG12342	31736	CG12529	3337
CG12010	6463	CG12130	7388	CG12233	50829	CG12343	31737	CG12531	6203
CG12012	33097	CG12131	45744	CG12233	41191	CG12345	20183	CG12532	7720
CG12014	13990	CG12132	31651	CG12234	31706	CG12345	20183	CG12533	11670
CG12016	22871	CG12134	31654	CG12235	31711	CG12345	40918	CG12533	11670
CG12018	13621	CG12135	17032	CG12238	38637	CG12346	48112	CG12539	38689
CG12019	47776	CG12136	5520	CG12240	6655	CG12346	20185	CG12542	51748
CG12020	18727	CG12139	1110	CG12244	20166	CG12352	31741	CG12544	31961
CG12021	31620	CG12139	27242	CG12245	2961	CG12355	47109	CG12548	31964
CG12024	20143	CG12139	5094	CG12245	2961	CG12357	50433	CG12560	29725
CG12025	4098	CG1214	4080	CG1225	31716	CG12358	46299	CG1257	31974
CG12026	43385	CG12140	15508	CG12252	38640	CG12359	31744	CG1258	46137
CG12026	42480	CG12149	17035	CG12253	31719	CG1236	17789	CG12582	15028
CG12029	26980	CG12151	31661	CG12254	47922	CG12360	41487	CG12598	7763
CG12030	16385	CG12152	17037	CG12259	38646	CG12361	10513	CG12600	31993
CG12031	15877	CG12153	13690	CG12261	17060	CG12362	31747	CG12602	6121
CG12038	51178	CG12154	51480	CG12262	15053	CG12363	31749	CG12605	49556
CG12042	31623	CG12156	31666	CG12263	10060	CG12366	44045	CG12605	20250
CG12048	1346	CG12157	13177	CG12265	43112	CG12367	31920	CG12608	24629
CG12050	42976	CG1216	17043	CG12267	43528	CG12369	35524	CG12608	49840
CG12051	12456	CG12161	31669	CG12268	1166	CG12370	43314	CG1263	17262
CG12052	12574	CG12162	20156	CG1227	38648	CG12373	24521	CG12630	48656
CG12054	45986	CG12163	46406	CG12272	18749	CG12374	13919	CG12630	18762
CG12055	31631	CG12163	12787	CG12273	42919	CG12375	45309	CG1264	2990

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CG12646	17267	CG12848	31900	CG13095	15541	CG13344	17141	CG1354	17235
CG12647	22781	CG12855	5639	CG13096	41499	CG13345	17145	CG13551	31786
CG12648	23518	CG12858	1688	CG13097	38675	CG13348	36555	CG13559	6644
CG1265	44951	CG12859	8787	CG13098	38015	CG13349	23873	CG13567	43344
CG12653	12668	CG1287	12127	CG13108	43559	CG13350	44474	CG13570	23895
CG12657	29843	CG12876	32047	CG13109	15709	CG13364	28464	CG1358	13313
CG12659	31757	CG12877	20265	CG1311	39695	CG13364	48265	CG13585	13323
CG12661	47422	CG12878	38722	CG13124	29648	CG13366	29606	CG13585	50392
CG1268	9174	CG12879	4407	CG13125	17123	CG13367	17157	CG13588	45755
CG12690	15743	CG12891	4047	CG13137	41500	CG13369	17161	CG13597	32225
CG12698	18256	CG12892	20270	CG13139	32146	CG13375	2944	CG13598	32230
CG12701	38706	CG12895	49607	CG13142	24511	CG13379	17167	CG13601	19953
CG12702	24588	CG12895	18093	CG1315	47096	CG13383	23886	CG13602	32235
CG1271	13044	CG12896	32111	CG13151	39782	CG13383	50566	CG13603	6778
CG12713	17296	CG12904	10268	CG1316	23851	CG13384	44246	CG13604	32239
CG12723	41389	CG12909	32114	CG13160	4629	CG13387	3347	CG13605	1170
CG12727	17302	CG1291	32116	CG13162	32148	CG13388	5647	CG13608	22713
CG12728	24592	CG12913	44296	CG13163	39785	CG13389	17168	CG13609	17326
CG12728	52287	CG12915	38737	CG13167	18770	CG13390	17169	CG13610	48870
CG12733	24601	CG12918	38082	CG13168	18776	CG13391	17171	CG13610	1176
CG12734	32008	CG12919	45252	CG1317	4106	CG13393	33164	CG13611	45793
CG12737	24604	CG12921	20271	CG13175	50775	CG13396	30760	CG13617	32247
CG1274	43254	CG12924	20280	CG13176	24643	CG13398	38669	CG1362	32249
CG12740	49123	CG12929	4180	CG13178	44816	CG13399	31781	CG13623	17334
CG12740	18090	CG12935	47569	CG13185	18782	CG13399	50778	CG13624	32250
CG12743	47431	CG12936	38750	CG1319	39792	CG1340	32192	CG13626	1209
CG12746	33313	CG12938	50331	CG13190	43001	CG13400	29954	CG13628	29253
CG12749	51759	CG12938	38754	CG13192	32157	CG13401	43572	CG13633	14398
CG1275	4103	CG12948	41492	CG13197	36422	CG13402	30051	CG13645	32255
CG12750	17304	CG12951	46921	CG1320	18101	CG13404	30255	CG13646	1421
CG12752	52631	CG12951	16569	CG1320	52257	CG13409	47577	CG13651	11515
CG12752	44146	CG12954	18765	CG13201	2976	CG1341	17176	CG13654	13550
CG12753	1617	CG12956	13014	CG13202	28290	CG13410	13443	CG13661	14730
CG12756	31761	CG12959	17946	CG13213	43004	CG13415	49138	CG13663	6731
CG12759	17306	CG12975	18098	CG1322	42856	CG13415	17178	CG13667	36578
CG1276	17917	CG12990	6160	CG13221	32164	CG13418	39049	CG13671	52026
CG12765	17307	CG12993	20304	CG13223	1684	CG13423	45404	CG13686	31867
CG12766	50403	CG12994	11864	CG13232	28772	CG13424	10514	CG13688	43824
CG12770	31894	CG12995	13346	CG13251	17127	CG13425	2912	CG13690	24648
CG12772	46140	CG12997	33914	CG13252	33301	CG13426	8821	CG13691	32058
CG12773	9899	CG13001	42347	CG13263	17129	CG1343	12606	CG13693	46527
CG12775	32016	CG13016	37428	CG1327	31086	CG13430	49662	CG13702	50000
CG12782	31857	CG13018	33859	CG13277	23862	CG13431	40834	CG1371	13276
CG12785	31830	CG13019	31769	CG13281	12648	CG13442	4736	CG1372	36346
CG12787	7854	CG13020	17116	CG13287	36601	CG1345	17187	CG1372	4309
CG12788	49610	CG13029	8502	CG13293	39797	CG13458	17193	CG13722	46470
CG12788	23044	CG1303	41494	CG13295	32171	CG13466	3152	CG13722	30939
CG12789	33153	CG1303	50742	CG13296	17133	CG13467	17196	CG1374	16892
CG12792	41489	CG13030	17117	CG13298	31777	CG1347	24515	CG13743	40974
CG12795	32019	CG13032	29454	CG13306	44960	CG13470	17198	CG13744	44796
CG12797	32021	CG13035	14247	CG13308	13862	CG13472	32193	CG13745	24655
CG12799	20260	CG13037	32138	CG13309	14133	CG13473	32195	CG13746	41403
CG12812	32025	CG13072	49484	CG13310	43960	CG13475	12608	CG13748	43985
CG12813	31094	CG13072	18099	CG13311	51574	CG1349	17214	CG1375	39808
CG12816	17314	CG13076	45906	CG13312	14060	CG13502	32203	CG13752	877
CG12817	40655	CG13077	51496	CG1332	32175	CG13510	28433	CG13752	3070
CG12818	31898	CG13078	3917	CG13320	23866	CG13521	42241	CG13756	29592
CG12818	46248	CG1308	44503	CG13322	32178	CG13521	4329	CG13758	42724
CG12822	32031	CG13089	2810	CG13326	18784	CG13521	42578	CG13760	17698
CG12833	32038	CG1309	37227	CG1333	51169	CG13526	32206	CG13771	31868
CG12836	24614	CG13090	43558	CG13334	15005	CG13531	17230	CG13772	44236
CG12846	52561	CG13091	11729	CG13343	17137	CG13533	6633	CG13773	43011

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CG13775	44814	CG13972	47444	CG1414	12630	CG14305	17477	CG1455	49987
CG13777	41507	CG13977	1294	CG14142	17410	CG14322	30026	CG14550	50317
CG13778	17701	CG13978	47488	CG14149	24851	CG14325	17485	CG14550	4369
CG13779	49153	CG13985	51447	CG1416	41548	CG1433	17490	CG14551	24625
CG13779	31789	CG1399	24826	CG14163	5201	CG14339	17493	CG14562	32286
CG1378	6236	CG13993	18210	CG14164	48825	CG1434	30996	CG14575	43325
CG13780	7629	CG13994	48622	CG14164	17863	CG14353	24860	CG14575	13384
CG13784	8042	CG13994	31791	CG14168	17414	CG14366	17505	CG14577	9117
CG1379	41030	CG13996	46532	CG1417	18561	CG1438	8631	CG1458	4439
CG1380	44934	CG13999	7889	CG14174	17416	CG14394	39307	CG1458	33925
CG13801	45925	CG14001	45028	CG1418	37728	CG14396	12292	CG14591	9945
CG13807	47439	CG1401	52176	CG14181	42548	CG14396	29907	CG14593	1658
CG13809	24793	CG14013	17343	CG14182	5370	CG14396	842	CG14611	31811
CG1381	24797	CG14015	45891	CG14183	17423	CG1440	24622	CG14615	24872
CG13822	38068	CG14016	28329	CG14184	35613	CG14407	43020	CG14617	24874
CG13827	24481	CG14016	49613	CG14185	32097	CG1441	4034	CG14618	50597
CG13829	28321	CG1402	39251	CG14192	35684	CG14411	17579	CG14619	37930
CG13830	44583	CG14020	44221	CG14194	6181	CG14413	22740	CG1462	6862
CG13832	24802	CG14022	31796	CG14198	51785	CG14414	48176	CG14620	24881
CG13833	6845	CG14022	48332	CG14199	40884	CG14414	18217	CG14621	8661
CG13842	17714	CG14023	36480	CG1420	32100	CG1442	17580	CG14622	24885
CG13844	29689	CG14024	13402	CG14201	37047	CG14428	9730	CG1463	31877
CG13849	52165	CG14025	13998	CG14206	49844	CG14428	39362	CG14630	24894
CG13850	15233	CG14026	862	CG14208	50572	CG14429	11683	CG1464	42845
CG13852	17716	CG14026	3059	CG14208	37420	CG1443	1333	CG14641	38790
CG13855	17717	CG14028	13403	CG14209	49642	CG14435	17600	CG14647	18297
CG13859	48179	CG14028	33841	CG14210	31803	CG14437	17602	CG14648	23918
CG13859	17718	CG14029	5650	CG14210	49168	CG1444	40949	CG14650	45458
CG1386	13188	CG1403	17344	CG14211	3146	CG14440	23916	CG1467	8644
CG13867	24806	CG14030	24833	CG14212	17429	CG14440	48156	CG14670	32301
CG13875	44819	CG14031	48920	CG14213	32104	CG14444	17621	CG14671	51803
CG13876	39817	CG14033	23052	CG14214	11989	CG14446	10274	CG14680	32304
CG13880	24809	CG14034	32085	CG14214	46474	CG14463	44552	CG14683	52664
CG13886	17730	CG14036	28475	CG14216	18212	CG1447	19831	CG14685	24905
CG13887	23207	CG1404	24835	CG14217	17432	CG14472	17648	CG14688	39840
CG13889	17732	CG14040	13399	CG1422	44510	CG14476	48375	CG14689	24907
CG1389	1016	CG14041	39821	CG14221	32106	CG14477	28731	CG14690	5985
CG1389	36280	CG14043	17351	CG14222	18213	CG14480	32270	CG14693	18332
CG1389	4298	CG14048	39648	CG14224	47449	CG14480	46974	CG14694	5994
CG13890	24813	CG1405	31908	CG14228	7161	CG14482	33849	CG14701	28517
CG13892	44823	CG14057	48963	CG14230	46903	CG1449	13305	CG14704	13159
CG13893	32077	CG14057	22718	CG14232	47897	CG14490	17660	CG14709	6053
CG13898	23905	CG1406	17358	CG14234	30309	CG14505	17683	CG1471	30189
CG13900	18955	CG14066	24841	CG14235	26848	CG14507	35712	CG14712	18347
CG13906	12584	CG1407	50031	CG14238	2673	CG1451	51468	CG14715	12829
CG1391	17740	CG14073	39823	CG14255	39397	CG14511	4426	CG14717	38813
CG13916	51569	CG14077	7563	CG14256	35615	CG14512	47661	CG14718	32311
CG13917	32082	CG14079	14337	CG14267	24615	CG14512	41527	CG1472	44460
CG13919	4086	CG14080	45415	CG14268	14535	CG14513	26806	CG14721	18354
CG13920	46993	CG14084	8420	CG1427	17457	CG14514	41530	CG14721	50259
CG13922	41516	CG14085	45757	CG14270	17459	CG14514	49989	CG14722	23919
CG13926	15261	CG1409	30988	CG14271	31807	CG14515	51637	CG14724	44490
CG13927	4084	CG1410	24845	CG14283	37802	CG14517	42693	CG14739	18358
CG13929	17752	CG1410	50568	CG14283	50292	CG14522	32275	CG1474	42996
CG13930	17753	CG14100	46739	CG14286	17463	CG1453	41534	CG14740	32318
CG13937	3375	CG14105	17388	CG1429	15549	CG14536	11725	CG14743	4142
CG13941	3464	CG1411	15320	CG14290	15858	CG14542	24869	CG14744	40829
CG13946	17858	CG1412	43955	CG14291	16897	CG14543	4563	CG14745	52289
CG1395	17760	CG14122	51782	CG14296	24617	CG14544	32279	CG14745	6444
CG13956	17766	CG14127	32914	CG14299	17469	CG14547	14767	CG14746	50792
CG13957	31840	CG14130	31911	CG1430	17473	CG14549	43588	CG14746	43201
CG13969	8070	CG14133	23322	CG14303	17474	CG1455	32283	CG14749	24917

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CG1475	24921	CG14933	33920	CG15100	25193	CG15257	3837	CG15440	19494
CG14750	38821	CG14933	7683	CG15101	13749	CG15261	24964	CG15441	29123
CG14757	38293	CG14934	15833	CG15102	30909	CG15262	25100	CG15442	49184
CG14763	8737	CG14936	6123	CG15104	10642	CG15266	51827	CG15442	49184
CG14767	50355	CG14938	7779	CG15105	19290	CG15270	3829	CG15444	8880
CG14771	45498	CG14939	32334	CG15106	5008	CG1528	25101	CG15445	29158
CG14777	9737	CG14940	31038	CG1511	4771	CG15283	28550	CG15448	30894
CG14778	44970	CG14941	5690	CG1511	6545	CG15287	19379	CG15450	8645
CG14783	18226	CG14945	13459	CG1511	27236	CG15288	3141	CG15455	30539
CG14786	42899	CG14946	38306	CG15110	37185	CG15297	29623	CG15456	23106
CG14788	18379	CG14956	3254	CG15111	37189	CG15298	38884	CG15467	41584
CG14789	41361	CG14962	39855	CG15112	43056	CG15299	43501	CG15468	25116
CG14792	38823	CG14965	31848	CG15113	46485	CG1530	45166	CG15471	19504
CG14792	50129	CG14966	43612	CG15113	9558	CG15300	25140	CG1548	31012
CG14793	3195	CG14966	47189	CG15116	30877	CG15304	11317	CG15481	32612
CG14801	38826	CG14967	44431	CG15117	47527	CG15304	47846	CG15483	30763
CG14802	28264	CG14969	8913	CG15118	16854	CG15305	29622	CG15498	19508
CG14802	48491	CG14971	39479	CG1512	19298	CG15309	19388	CG15507	19512
CG14803	18395	CG14977	18233	CG15120	32374	CG1531	22901	CG15509	6212
CG14804	18396	CG14980	18479	CG15120	46222	CG15318	32382	CG15523	47481
CG14805	32325	CG14983	32341	CG1513	48600	CG15319	3787	CG15524	25072
CG14806	45107	CG14985	32342	CG1513	39148	CG1532	19398	CG15525	48660
CG14808	8305	CG14991	18484	CG15130	43491	CG15329	32564	CG15525	39993
CG14812	31815	CG14991	46494	CG15133	8059	CG15331	39285	CG15526	19518
CG14813	41549	CG14992	39857	CG15134	19305	CG1534	43097	CG15527	39323
CG14814	41553	CG14993	43032	CG1514	5467	CG15340	39973	CG15528	14865
CG14815	43473	CG14994	32344	CG15141	19307	CG15341	43660	CG15529	14867
CG14816	51655	CG14995	12725	CG15142	19309	CG15342	19428	CG15529	50227
CG14817	26894	CG14996	5654	CG15143	25195	CG15343	29569	CG1553	52179
CG14818	40700	CG14997	47016	CG15144	19315	CG15358	13372	CG15532	39877
CG14820	15456	CG14998	18491	CG15145	19316	CG1536	32579	CG15533	42520
CG14824	45594	CG14999	37756	CG15147	19320	CG15362	38282	CG15534	30186
CG14825	41554	CG1500	39575	CG15148	19323	CG15365	47648	CG15535	26825
CG1484	39528	CG15000	6273	CG1515	19329	CG15368	32583	CG15547	32621
CG14853	39734	CG15000	6273	CG15150	19332	CG15372	13184	CG1555	11322
CG14861	47458	CG15001	24943	CG15154	51821	CG15373	19439	CG15551	10263
CG14865	28532	CG15011	51517	CG1516	41576	CG15378	22908	CG15552	45961
CG14866	18419	CG15012	9176	CG15160	44601	CG15385	51859	CG15556	1791
CG14868	51810	CG15014	43615	CG15161	25198	CG15387	35759	CG15561	41582
CG14869	33347	CG15015	18492	CG15168	12248	CG15388	32599	CG1560	29619
CG1487	41559	CG15016	38842	CG15168	48614	CG15388	32599	CG15602	40001
CG14870	46307	CG15019	28438	CG1517	3306	CG15389	52199	CG15609	43406
CG14881	31821	CG1502	14894	CG1518	12250	CG15389	52199	CG15610	25074
CG14882	38838	CG15021	52038	CG1518	48653	CG1539	32601	CG15618	40006
CG14883	8028	CG15027	24627	CG15187	30628	CG15390	25112	CG15619	40007
CG14885	46916	CG1503	32349	CG1519	46544	CG15395	39929	CG15624	7858
CG14887	48256	CG15035	32352	CG15191	48720	CG15398	8873	CG15625	29368
CG1489	24936	CG15037	37789	CG15191	16751	CG15399	19450	CG15626	7844
CG1489	46803	CG1506	33217	CG1520	13757	CG15400	7261	CG15629	49077
CG14893	43598	CG15069	44611	CG15208	19368	CG15403	29945	CG15629	7839
CG14895	39843	CG1507	12765	CG15211	35704	CG15405	52652	CG15635	46490
CG14898	18229	CG1507	48249	CG15216	32541	CG1542	39976	CG15636	13072
CG14898	49177	CG15072	39866	CG15218	36216	CG15427	27046	CG15637	44029
CG1490	18231	CG15077	18524	CG1522	5551	CG15427	3064	CG15644	41587
CG14902	43028	CG15078	10061	CG15220	15380	CG15429	19462	CG15645	52291
CG14903	40658	CG15081	32361	CG15224	32378	CG1543	51667	CG15645	20206
CG14903	49107	CG15087	32527	CG1523	25199	CG15432	19466	CG15651	37206
CG14905	39848	CG15093	5655	CG15237	47255	CG15432	49216	CG15653	8827
CG14906	31851	CG15094	5002	CG1524	39870	CG15433	19470	CG15655	6586
CG14909	9082	CG15096	39463	CG15253	30729	CG15434	29236	CG15661	16904
CG1492	8320	CG15097	25188	CG15254	15604	CG15438	7303	CG15663	15445
CG14921	32331	CG15099	20126	CG15255	12412	CG15439	19490	CG15666	40013

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CG15667	19150	CG15879	38899	CG16708	43413	CG16885	48049	CG17033	32830
CG15669	19536	CG15881	32390	CG1671	32697	CG16886	38026	CG17034	8136
CG15671	2938	CG15890	11538	CG16716	40042	CG16889	32766	CG17035	44443
CG15676	20121	CG15897	41618	CG16717	29199	CG16890	40044	CG17036	2869
CG1569	19152	CG15898	41114	CG16718	37472	CG16892	23844	CG1704	32451
CG15693	32383	CG15898	33897	CG16719	39934	CG16894	10067	CG17043	8777
CG15694	40018	CG15902	8577	CG16721	25201	CG16896	20316	CG17048	8780
CG15696	16979	CG15907	40762	CG16725	45447	CG16899	15732	CG17051	25209
CG15697	32511	CG15908	19623	CG1673	25204	CG16901	32395	CG17054	40047
CG15701	19541	CG1591	38908	CG16732	1148	CG16902	37067	CG17059	23535
CG15706	4907	CG15910	38910	CG16733	47020	CG16903	37572	CG17060	46791
CG1571	51846	CG15912	32654	CG16738	15749	CG16905	12130	CG17060	28758
CG15715	28563	CG15914	41571	CG16740	7210	CG16905	48663	CG17064	32841
CG15723	28566	CG15916	19627	CG16742	23832	CG16908	32767	CG17065	32845
CG1573	22915	CG15923	37373	CG16749	46390	CG1691	20321	CG17068	32850
CG15730	19582	CG15925	8982	CG16749	7555	CG16910	7723	CG1707	26832
CG15735	33813	CG15929	26830	CG1675	32712	CG16912	20327	CG17075	2487
CG15736	23926	CG1594	40037	CG16751	19069	CG1692	20329	CG17077	7170
CG15737	19584	CG1597	37383	CG16756	45655	CG16928	30476	CG17081	14194
CG15738	47929	CG1598	32391	CG16757	19658	CG1693	43841	CG17083	39937
CG15739	14890	CG1599	13317	CG16758	32714	CG16932	19165	CG1709	22925
CG15743	42685	CG1607	13793	CG1676	32719	CG16933	35558	CG17090	32855
CG15744	1095	CG1609	32664	CG16764	26976	CG16938	20334	CG17097	41244
CG15744	4801	CG1615	40953	CG16766	51387	CG16940	51853	CG17098	46309
CG15749	16744	CG1616	44484	CG1677	50195	CG16941	20338	CG1710	46998
CG1575	19153	CG1617	23822	CG16771	6386	CG16944	48582	CG17100	42821
CG15751	19594	CG1618	46483	CG16779	40824	CG16944	11968	CG17109	32858
CG15752	43686	CG1620	12682	CG16781	7018	CG1695	48062	CG17109	50820
CG15759	41597	CG1622	38915	CG16783	32724	CG1695	20340	CG17117	12764
CG15762	29871	CG1624	41623	CG16784	48045	CG16952	19166	CG17118	37624
CG1577	23356	CG1625	32668	CG16785	30214	CG16954	19167	CG17119	51127
CG15771	43689	CG1627	14900	CG16787	19661	CG1696	12939	CG17121	49341
CG15772	46284	CG1628	47475	CG16787	48685	CG16965	32782	CG17122	25212
CG15775	39358	CG1630	19159	CG16788	51851	CG1697	44414	CG17124	19078
CG15775	28570	CG1634	6688	CG16789	32729	CG16975	52247	CG17134	18110
CG15776	47248	CG1634	27202	CG16790	19160	CG16979	51594	CG17136	21083
CG15776	19611	CG1635	32672	CG16799	35709	CG16982	32399	CG17137	39107
CG1578	41599	CG1637	12932	CG16801	37617	CG16983	46605	CG17141	50241
CG15792	7819	CG1638	32676	CG16804	16806	CG16983	32790	CG17143	46676
CG15793	40025	CG1638	46275	CG16807	23843	CG16985	32401	CG17146	25214
CG15797	41603	CG1639	23825	CG1681	43041	CG16986	39129	CG17149	25218
CG15803	43635	CG1640	32680	CG16812	14972	CG16987	13418	CG1715	52253
CG15804	48544	CG1646	32682	CG16817	15309	CG16988	32798	CG17150	35624
CG15804	48153	CG1650	12568	CG16827	37172	CG16989	32802	CG17158	45668
CG15811	19696	CG1652	38104	CG1683	45174	CG16995	23078	CG1716	30707
CG15814	30429	CG1656	35555	CG16833	32741	CG17002	39883	CG17161	12680
CG15816	19616	CG1657	19649	CG16837	32749	CG17003	32804	CG17166	20388
CG15817	41605	CG1658	19066	CG16838	32754	CG17004	11471	CG17168	41625
CG15818	15537	CG1658	19066	CG16840	20305	CG17018	32810	CG17170	32868
CG15819	32385	CG1658	19066	CG16857	24479	CG17019	48749	CG17173	8370
CG1582	19617	CG1659	19653	CG16857	4806	CG1702	48635	CG1718	44449
CG15820	43874	CG1660	28801	CG16857	36224	CG17024	50187	CG17180	29141
CG1583	8928	CG1662	50409	CG16858	16986	CG17026	49199	CG17181	20396
CG1583	50353	CG1662	12197	CG16863	20312	CG17026	32813	CG17183	32459
CG15835	32652	CG1664	32690	CG16865	19162	CG17027	50076	CG17184	20401
CG1584	10677	CG1665	9958	CG16868	8209	CG17027	32817	CG17187	40051
CG15862	39437	CG1666	32693	CG16873	15758	CG17028	32819	CG17195	1435
CG15865	19157	CG1666	50144	CG16874	13247	CG17029	49565	CG17196	6743
CG1587	19061	CG1667	4031	CG16879	32759	CG17029	32822	CG17197	1433
CG15871	43691	CG1669	52246	CG1688	30269	CG1703	32826	CG17198	1432
CG15877	40031	CG16700	6145	CG16882	52426	CG17030	32827	CG17200	6024
CG15879	46754	CG16705	30972	CG16884	51363	CG17031	32829	CG17202	41628

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CG17203	45360	CG17369	46553	CG17599	14682	CG17809	39572	CG18048	19184
CG17204	32872	CG17370	1438	CG1760	32961	CG17818	19089	CG18065	15361
CG17204	32872	CG17378	47367	CG17603	41099	CG17821	4997	CG18065	49677
CG17207	46640	CG1738	32464	CG17604	32962	CG17828	39887	CG18069	47280
CG17209	30512	CG17386	5701	CG17608	51162	CG17829	41659	CG18085	49924
CG1721	52336	CG17387	32902	CG17610	4331	CG1783	33007	CG1809	43231
CG17219	41635	CG1739	32904	CG17610	3121	CG17835	49950	CG18096	30873
CG17221	25229	CG17396	10833	CG17610	36251	CG17838	33012	CG18102	3798
CG17223	2608	CG1740	49848	CG17617	32965	CG17840	45037	CG18104	47186
CG17224	41177	CG1740	24968	CG17618	32403	CG17841	5530	CG18104	14933
CG17227	13330	CG17419	40076	CG1762	42234	CG1785	52467	CG18110	1349
CG17230	20409	CG1742	44445	CG1762	893	CG17856	33016	CG18112	43415
CG17233	25231	CG1743	32929	CG1762	40895	CG1787	5503	CG1812	15491
CG1724	30315	CG17437	38925	CG17633	47634	CG17870	48724	CG18128	40127
CG17245	8383	CG17440	20445	CG17636	8675	CG17888	37769	CG18130	20599
CG17245	46687	CG17441	13444	CG1764	20507	CG1789	20567	CG18136	13128
CG17245	27220	CG17446	47221	CG17642	51611	CG17894	37673	CG1814	19096
CG17248	44011	CG17446	20453	CG17642	30719	CG17896	5580	CG18144	23306
CG17248	49202	CG17450	50795	CG17645	41658	CG17903	33019	CG18146	36261
CG17249	19170	CG17461	43639	CG1765	37059	CG17904	41660	CG18146	3705
CG1725	41136	CG17462	19801	CG17657	40088	CG17907	3968	CG18146	3120
CG17251	48024	CG1747	32932	CG17660	2684	CG17912	40107	CG1815	40132
CG17252	20410	CG1748	32933	CG17664	49979	CG17919	38204	CG18155	42446
CG17255	48633	CG1749	32937	CG17665	46744	CG17919	46473	CG1817	1101
CG17256	40052	CG17492	40078	CG17669	36538	CG17921	12773	CG1817	8010
CG17257	2611	CG17494	8199	CG1768	20518	CG17922	6599	CG18171	25299
CG17258	44372	CG17498	47918	CG17680	45211	CG17923	3976	CG18173	4092
CG17262	2614	CG17498	25264	CG17681	40972	CG17927	7164	CG18174	19272
CG17265	36479	CG17508	1487	CG17683	19180	CG1793	51476	CG18176	20604
CG17266	25243	CG17509	36527	CG17691	40686	CG17934	19093	CG18177	40844
CG17268	46876	CG1751	51149	CG17697	43077	CG17935	23556	CG18182	40134
CG17268	40056	CG17514	47269	CG1770	20522	CG17941	36219	CG18208	10215
CG17272	32874	CG17521	19084	CG1771	5671	CG17941	4312	CG1821	24977
CG1728	28805	CG17523	32945	CG17712	32987	CG17945	42407	CG18214	40138
CG17280	12965	CG17525	20472	CG17716	6561	CG17945	23558	CG18217	41665
CG17284	2558	CG1753	32946	CG17716	42237	CG17946	23560	CG1824	5554
CG17286	36623	CG1754	8651	CG17723	7461	CG17947	19182	CG18241	47967
CG17287	49415	CG17540	32948	CG17725	20529	CG17949	33025	CG18247	25304
CG17291	49672	CG17544	47934	CG17725	50252	CG17949	50797	CG18251	40143
CG17293	25246	CG17556	49518	CG17726	32991	CG1795	37738	CG18258	20621
CG17294	39105	CG17556	20483	CG17734	13791	CG17950	19026	CG18259	50094
CG17301	25252	CG17559	27057	CG17734	49497	CG17952	39468	CG1826	33049
CG17302	32875	CG17559	8002	CG17735	50189	CG1796	44862	CG1827	46039
CG17309	32877	CG17559	6547	CG17737	29215	CG17960	33029	CG18278	22936
CG1732	13359	CG1756	37517	CG17739	36542	CG17964	3014	CG18278	50084
CG17320	49013	CG17560	52002	CG1774	16622	CG17970	16988	CG1828	10916
CG17324	6379	CG17562	37365	CG17743	39529	CG17973	20571	CG18281	50086
CG17326	32878	CG17564	47273	CG17746	40102	CG17985	7355	CG18281	8438
CG17327	41654	CG17565	32951	CG1775	19689	CG1799	40116	CG18284	49221
CG17329	19171	CG17566	25271	CG17753	20536	CG17991	1277	CG18284	31030
CG17331	19079	CG17569	41657	CG17754	47274	CG17998	1835	CG18287	36660
CG17332	19173	CG17574	45729	CG17757	35772	CG1800	40118	CG18296	18866
CG17335	46117	CG17577	4664	CG17759	51116	CG18000	48333	CG1830	33054
CG17336	37408	CG1759	49563	CG17759	19088	CG18005	25290	CG18301	31023
CG17342	32885	CG1759	3361	CG17765	32405	CG18009	10443	CG18313	49211
CG17348	3047	CG17592	30452	CG17766	14531	CG18012	20580	CG18313	44735
CG17348	27053	CG17593	13029	CG17768	49674	CG18013	33041	CG18315	19101
CG17349	32887	CG17595	29228	CG17769	20544	CG18024	41080	CG18315	48613
CG17358	12654	CG17596	5702	CG17779	45670	CG18026	25291	CG18317	44203
CG1736	32889	CG17597	49204	CG17785	1181	CG1803	39945	CG18319	9413
CG17360	40070	CG17597	25276	CG17800	36233	CG18039	40929	CG18324	33234
CG17367	32892	CG17598	32956	CG17800	3115	CG18041	46031	CG18331	48500

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List of screened RNAi lines Provided by VDRC as human homolog sub-library

CG18332	12821	CG18558	33366	CG18799	5528	CG1937	6870	CG2061	44186
CG18335	52470	CG1856	10855	CG18801	37110	CG1938	41686	CG2062	4018
CG18339	25317	CG18572	33375	CG18802	5838	CG1939	44765	CG2063	33462
CG18340	33128	CG18578	8573	CG18803	43082	CG1941	3998	CG2064	8728
CG18341	52475	CG18582	46044	CG18809	20702	CG1942	7942	CG2065	19119
CG18345	35571	CG18585	19917	CG18810	40825	CG1942	48583	CG2069	43382
CG18347	6076	CG18591	23569	CG18811	14011	CG1945	2955	CG2070	20793
CG1836	30498	CG18594	20650	CG18812	39224	CG1945	30679	CG2072	43714
CG18362	52606	CG18596	41675	CG18814	5283	CG1946	33428	CG2075	13673
CG1837	15660	CG18599	25363	CG18815	33414	CG1946	48259	CG2076	50221
CG18371	19193	CG18600	32470	CG18818	20705	CG1951	33430	CG2076	5537
CG18374	52478	CG18605	33377	CG1882	41406	CG1954	33434	CG2078	25402
CG18375	25332	CG18609	4994	CG18820	20706	CG1956	33437	CG2079	20796
CG18378	25333	CG1861	14872	CG18826	13336	CG1957	29575	CG2083	20798
CG1838	33132	CG18619	19110	CG18827	9894	CG1960	28342	CG2086	27084
CG18380	20634	CG18620	40811	CG18828	23572	CG1961	40161	CG2086	4830
CG18381	25335	CG18624	29883	CG18829	33415	CG1962	33444	CG2087	16427
CG18389	13075	CG18624	33893	CG1883	20711	CG1963	48872	CG2091	20799
CG1839	15476	CG18627	33386	CG18831	13007	CG1963	19117	CG2092	33465
CG18398	43647	CG18630	25366	CG18833	39189	CG1964	28347	CG2093	29971
CG18402	991	CG18631	47215	CG18833	39352	CG1965	43944	CG2095	45032
CG18405	36147	CG18635	8993	CG18834	14590	CG1966	33446	CG2096	2964
CG18405	4743	CG1864	2971	CG18838	12187	CG1967	12196	CG2097	33469
CG18408	19054	CG18642	7266	CG1884	12571	CG1968	20767	CG2098	20804
CG18412	50027	CG18647	9409	CG18842	6932	CG1970	38224	CG2099	39895
CG18412	50024	CG18654	38239	CG18843	24340	CG1972	33450	CG2100	38037
CG18414	10679	CG18656	14038	CG18844	39335	CG1973	19275	CG2101	33473
CG18418	9008	CG1866	20670	CG18844	28816	CG1975	20771	CG2102	2929
CG18419	42776	CG18660	51459	CG18845	14934	CG1977	25387	CG2103	33264
CG18428	35638	CG18662	26833	CG18846	29892	CG1981	13657	CG2104	30016
CG18432	25336	CG18667	25371	CG18848	45684	CG1982	47191	CG2105	39535
CG18436	16523	CG18668	20673	CG18849	42391	CG1982	3761	CG2108	13145
CG18437	1310	CG18671	33394	CG18851	20721	CG1983	24980	CG2109	45000
CG1844	23268	CG18675	33397	CG18853	50099	CG1986	51669	CG2112	25405
CG18445	4039	CG18678	23225	CG18858	50800	CG1989	46977	CG2118	25406
CG1845	30436	CG18679	3128	CG1886	8315	CG1989	39898	CG2121	3386
CG1846	41672	CG18679	36153	CG1890	39894	CG1994	13479	CG2124	14869
CG18466	5705	CG1868	25379	CG1891	46350	CG1998	37395	CG2125	51479
CG18467	47282	CG1869	42877	CG1891	42456	CG2005	27208	CG2126	47598
CG1847	43701	CG1871	39287	CG1893	20729	CG2005	6705	CG2128	20814
CG18472	40148	CG1873	52343	CG1894	41574	CG2005	8009	CG2128	50213
CG18473	25341	CG1873	40156	CG1897	7791	CG2009	48037	CG2135	16625
CG1848	25343	CG18730	50098	CG1898	33420	CG2013	46927	CG2136	33486
CG18480	1072	CG18730	30745	CG1900	29259	CG2013	23229	CG2137	41235
CG18480	3821	CG18732	35641	CG1903	28341	CG2014	50731	CG2140	43065
CG1849	3025	CG18734	1020	CG1905	48768	CG2014	40165	CG2143	20819
CG18493	18930	CG18735	30914	CG1906	32476	CG2017	20780	CG2144	3996
CG18495	49680	CG1874	41680	CG1907	1341	CG2019	10004	CG2145	14874
CG18495	43705	CG18740	6969	CG1909	20745	CG2022	9049	CG2146	44292
CG18497	48846	CG18741	3391	CG1911	33423	CG2025	25392	CG2151	47307
CG18497	49543	CG18745	20686	CG1912	43711	CG2028	13664	CG2152	19121
CG18497	20637	CG18746	33399	CG1913	52346	CG2031	20783	CG2155	3349
CG18505	28285	CG18747	41252	CG1913	33427	CG2033	50635	CG2158	20824
CG18508	52071	CG18766	20694	CG1915	47301	CG2033	19198	CG2160	33490
CG1851	33133	CG18767	38251	CG1916	38079	CG2034	20786	CG2161	20826
CG18516	46403	CG18769	9501	CG1919	15265	CG2038	40690	CG2162	33493
CG1852	8309	CG1877	42445	CG1921	6948	CG2048	9241	CG2163	33499
CG18528	40149	CG18780	52484	CG1922	10663	CG2051	33459	CG2165	30203
CG18530	6109	CG18787	20697	CG1924	52348	CG2052	20790	CG2168	50311
CG18530	47205	CG18787	49686	CG1924	42397	CG2053	13546	CG2168	43627
CG18549	6098	CG18789	49689	CG1925	24472	CG2060	49044	CG2173	36516
CG1855	40150	CG18789	20699	CG1935	52670	CG2060	9953	CG2174	37530

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CG2177	51084	CG2471	39140	CG2781	48139	CG2938	7035	CG30051	49256
CG2179	20829	CG2478	12482	CG2789	2507	CG2939	37658	CG30059	13010
CG2182	20834	CG2488	10461	CG2790	20903	CG2943	8477	CG30059	50732
CG2183	13762	CG2493	3929	CG2791	42622	CG2944	8688	CG3006	38148
CG2184	51201	CG2503	20876	CG2803	25160	CG2945	19280	CG30062	15433
CG2185	29477	CG2505	20879	CG2807	25161	CG2947	43725	CG30063	48780
CG2187	7575	CG2508	52280	CG2812	52485	CG2948	33593	CG30075	25563
CG2189	7782	CG2520	12732	CG2816	29700	CG2956	37091	CG30077	25568
CG2189	50110	CG2522	14877	CG2818	25441	CG2957	25462	CG30078	30610
CG2194	19279	CG2525	15880	CG2826	46588	CG2958	45294	CG3008	43731
CG2198	22944	CG2528	33532	CG2827	25444	CG2960	25466	CG30084	36564
CG2201	33502	CG2534	7769	CG2829	20905	CG2962	50422	CG30085	33672
CG2204	19124	CG2540	33536	CG2830	29339	CG2962	5526	CG3009	12216
CG2205	43630	CG2543	25417	CG2835	24959	CG2964	42293	CG30093	17893
CG2210	33198	CG2551	29635	CG2839	17952	CG2969	42751	CG30097	8977
CG2212	5469	CG2574	40173	CG2843	52326	CG2970	48124	CG30100	43236
CG2216	49537	CG2578	32482	CG2843	38933	CG2970	43497	CG30100	48945
CG2216	12925	CG2595	43717	CG2845	20909	CG2971	14637	CG30101	15514
CG2218	32517	CG2608	20886	CG2848	33571	CG2972	48771	CG30102	33677
CG2219	41690	CG2614	32407	CG2849	43623	CG2974	32484	CG30103	4949
CG2221	1054	CG2615	49365	CG2852	15069	CG2975	2601	CG30104	10050
CG2221	27247	CG2616	11052	CG2854	41703	CG2976	20962	CG30105	19204
CG2221	39505	CG2617	3931	CG2855	16820	CG2980	25470	CG30106	1678
CG2222	20849	CG2621	7005	CG2856	10970	CG2984	33599	CG30109	50925
CG2224	20852	CG2637	22348	CG2857	7417	CG2986	28822	CG3011	19208
CG2239	43044	CG2641	25422	CG2859	15334	CG2987	7182	CG30112	42444
CG2241	49245	CG2647	5709	CG2861	20918	CG2988	9419	CG30113	41562
CG2241	25412	CG2655	30564	CG2863	33573	CG2988	48649	CG30115	39952
CG2244	20855	CG2656	25423	CG2864	23962	CG2989	46286	CG30123	25571
CG2245	25415	CG2662	33544	CG2867	20926	CG2990	30507	CG30124	4998
CG2246	48877	CG2666	42611	CG2872	39221	CG2991	2604	CG30131	52556
CG2246	24987	CG2669	47309	CG2872	3399	CG2993	33605	CG30144	39920
CG2248	49247	CG2670	11499	CG2872	48496	CG2994	8176	CG30147	25580
CG2249	40977	CG2671	51249	CG2875	33575	CG2995	25473	CG30149	21109
CG2252	51305	CG2674	7167	CG2881	33577	CG2996	14613	CG30152	38848
CG2253	33507	CG2675	10149	CG2883	14646	CG2998	42419	CG3016	7090
CG2254	5470	CG2677	25427	CG2887	33581	CG2998	35783	CG30163	39687
CG2256	40170	CG2679	37435	CG2890	25446	CG2999	33609	CG30164	3633
CG2257	33510	CG2681	33549	CG2892	14868	CG3000	25553	CG30165	19709
CG2259	33512	CG2682	44783	CG2899	45041	CG30000	41965	CG3017	21110
CG2260	25154	CG2684	13308	CG2901	12209	CG30005	25555	CG30170	25590
CG2262	14609	CG2685	20887	CG2902	37333	CG3001	21054	CG30171	29412
CG2263	33514	CG2694	33551	CG2903	20933	CG30010	48606	CG30173	35794
CG2272	33516	CG2699	33556	CG2905	52486	CG30010	33660	CG30174	45060
CG2275	10835	CG2701	25433	CG2906	25449	CG30011	43070	CG30175	24378
CG2277	20869	CG2702	33557	CG2910	20942	CG30012	21057	CG30176	25593
CG2286	52049	CG2708	33561	CG2911	25452	CG30013	41415	CG30178	21111
CG2292	51933	CG2713	5587	CG2913	7028	CG30014	25557	CG30179	26836
CG2304	4448	CG2714	6308	CG2914	51225	CG30016	40488	CG3018	33684
CG2309	33522	CG2715	32413	CG2916	25456	CG30019	32429	CG30183	25594
CG2316	12170	CG2716	8307	CG2917	13613	CG3002	3269	CG3019	25597
CG2321	33523	CG2718	40174	CG2918	18440	CG30021	29965	CG30193	39474
CG2328	9285	CG2720	41696	CG2919	25457	CG30022	30880	CG30194	50234
CG2330	46555	CG2727	12232	CG2921	33585	CG30035	52360	CG30194	3527
CG2331	24354	CG2747	33566	CG2922	25166	CG30035	8126	CG30197	9586
CG2358	9055	CG2759	30033	CG2925	20943	CG30038	21068	CG30203	33913
CG2371	15483	CG2762	5712	CG2926	33589	CG3004	46557	CG3021	25603
CG2380	14918	CG2765	43721	CG2929	25458	CG30043	11708	CG3022	50176
CG2381	24989	CG2766	33888	CG2930	7031	CG30046	13947	CG3024	30985
CG2397	4019	CG2766	28819	CG2931	20946	CG30047	4623	CG30259	19211
CG2412	1494	CG2772	15577	CG2934	20950	CG30048	4634	CG3026	33688
CG2453	20872	CG2779	24992	CG2937	30087	CG30051	43066	CG30268	42703

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CG3027	25610	CG30481	13320	CG3105	25662	CG31212	40214	CG31408	41420
CG30271	32436	CG30483	21216	CG31050	48824	CG31223	43426	CG3141	7074
CG30271	50158	CG30489	49271	CG31050	23934	CG31229	9660	CG31414	21336
CG30275	25612	CG30489	21235	CG31053	33750	CG31232	13090	CG31421	25760
CG3028	25615	CG30491	12817	CG31057	25023	CG31234	30389	CG31426	21340
CG30280	34743	CG30493	43131	CG31063	45246	CG31237	23308	CG3143	30556
CG30284	21118	CG30495	21241	CG31064	33756	CG31240	10424	CG31445	51642
CG3029	25014	CG30496	21244	CG31069	25026	CG31241	29503	CG31447	25761
CG30290	49962	CG30497	41745	CG3107	40196	CG31243	14385	CG31447	50997
CG30290	41564	CG30498	13457	CG31072	1251	CG31249	43740	CG31450	26319
CG30291	21120	CG30499	25016	CG31075	25676	CG31251	25739	CG31452	27194
CG30295	47790	CG30502	21253	CG31076	28776	CG31256	40218	CG31452	3744
CG30327	33705	CG30503	9036	CG3108	25680	CG31259	46362	CG31453	21350
CG3033	7086	CG3051	1827	CG31089	12449	CG3126	21306	CG31454	5811
CG30336	21134	CG3052	13724	CG3109	14529	CG3127	33797	CG31460	33323
CG30338	29188	CG3054	13555	CG31091	33085	CG31274	40222	CG31467	52637
CG30342	21136	CG3057	47965	CG31092	25684	CG31278	38938	CG31467	40268
CG30345	42868	CG3058	21258	CG31095	14756	CG31285	33799	CG31469	23396
CG30346	45429	CG3059	7265	CG31106	3415	CG31289	39091	CG31473	14059
CG30349	39959	CG3060	2993	CG31110	25030	CG3129	40228	CG31477	42108
CG3035	21139	CG3061	5868	CG31111	40198	CG31290	38860	CG31478	21358
CG30354	23575	CG3064	6972	CG31115	25693	CG31291	21308	CG31482	45931
CG30359	43151	CG3069	15872	CG31120	3402	CG31293	21312	CG3149	47560
CG3036	42755	CG3071	29589	CG31122	40207	CG31299	45442	CG31501	25773
CG30360	47951	CG3073	25627	CG31123	25034	CG3130	40229	CG3151	33939
CG30368	13913	CG3074	6617	CG31126	23395	CG31301	40232	CG31522	37329
CG30372	19287	CG3075	41034	CG31126	49277	CG31302	40235	CG31523	9807
CG30373	4013	CG3077	25631	CG31132	40209	CG31304	45035	CG31527	43255
CG30378	21153	CG3078	51390	CG31133	33774	CG31306	40236	CG31528	30811
CG30382	21156	CG3083	25020	CG31136	33112	CG3131	2593	CG3153	16652
CG30387	3468	CG3085	43421	CG31137	13365	CG31311	23079	CG31531	40276
CG30387	48313	CG3086	3170	CG31138	29541	CG31314	44406	CG31534	21366
CG30388	41735	CG3090	10856	CG3114	4559	CG31317	21317	CG31536	25049
CG30389	19002	CG3093	33733	CG31140	38936	CG31318	21985	CG31546	13140
CG30390	41740	CG3095	14524	CG31141	29537	CG31319	40238	CG31547	8551
CG30392	12862	CG3099	25636	CG31145	25036	CG3132	16779	CG31548	13144
CG30394	3470	CG3100	14526	CG31146	42616	CG31320	25742	CG31550	25779
CG30398	21166	CG31000	33735	CG31148	14698	CG31321	44942	CG31551	40278
CG3040	19219	CG31002	22960	CG31151	51341	CG31325	45139	CG3156	12182
CG30401	2725	CG31002	48225	CG31152	14480	CG31332	49701	CG3157	47163
CG30404	29385	CG31004	40940	CG31155	39134	CG31332	16813	CG3157	19130
CG3041	47602	CG31007	33739	CG31156	44478	CG31342	51330	CG3158	21374
CG30410	40176	CG31008	19227	CG31158	42321	CG31345	24656	CG3160	7070
CG30418	38152	CG31009	27211	CG31159	46144	CG31346	45943	CG31601	21378
CG3042	25620	CG31009	27216	CG31163	25701	CG31349	38863	CG31605	2789
CG30420	7414	CG31009	3739	CG31168	43222	CG3135	14803	CG31605	43306
CG30421	33726	CG31009	3733	CG31169	25706	CG31352	49112	CG3161	44487
CG30423	10600	CG3101	21275	CG31170	25707	CG31352	25750	CG3161	49291
CG30426	21173	CG31012	38854	CG31175	19230	CG31357	40248	CG3161	49291
CG30427	7423	CG31014	30975	CG31183	4773	CG3136	36504	CG3161	44487
CG30429	21175	CG31015	41351	CG31183	861	CG31363	25044	CG31613	33972
CG30438	37106	CG3102	33744	CG31183	36323	CG31367	37625	CG31617	33975
CG30440	21178	CG31022	2464	CG31187	43739	CG31373	21726	CG31618	25782
CG30441	50583	CG31027	25644	CG31188	15364	CG31381	14720	CG31619	33102
CG3045	25621	CG31033	25651	CG31189	22962	CG31385	3704	CG3162	21380
CG30460	40624	CG31038	25655	CG31192	42318	CG31385	8394	CG31623	21383
CG30462	10046	CG3104	21289	CG31194	45433	CG31385	27178	CG31624	33976
CG30463	4923	CG31040	39926	CG31196	15884	CG3139	8875	CG31624	50803
CG30467	21203	CG31042	9704	CG31201	49547	CG31390	11504	CG31628	46295
CG3047	21206	CG31046	9701	CG31202	43522	CG31391	33801	CG31629	37935
CG30476	23495	CG31048	21293	CG3121	25712	CG31391	49286	CG31632	21386
CG3048	21214	CG31049	21294	CG31211	33787	CG3140	25046	CG31634	2650

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CG31634	2650	CG31739	21410	CG3189	34017	CG32064	34051	CG32179	6119
CG31635	33980	CG3174	42829	CG31908	51924	CG32066	44825	CG32179	27110
CG31637	7614	CG31740	33850	CG31911	7618	CG32067	13066	CG32180	45900
CG31638	33981	CG31741	7930	CG31913	3376	CG32068	23412	CG32183	38208
CG31639	48790	CG31743	7538	CG31915	37944	CG32069	9202	CG3219	48576
CG31639	32443	CG31746	35727	CG31916	25906	CG32072	9206	CG32190	5319
CG31640	3498	CG31751	21414	CG31917	25909	CG32075	19255	CG32191	14294
CG31641	32370	CG31756	25833	CG31919	52494	CG32076	52402	CG3220	23511
CG31648	44225	CG31758	23522	CG3192	30413	CG3208	41779	CG32202	26325
CG31649	50192	CG31759	47321	CG31922	36386	CG32082	50019	CG32210	41865
CG3165	25785	CG31760	7686	CG31924	36469	CG32082	21438	CG32211	34452
CG31650	30690	CG31761	46014	CG31928	12276	CG32085	34053	CG32217	34458
CG31651	2629	CG31762	41568	CG3193	25919	CG32086	19261	CG3222	34459
CG31652	33993	CG31762	48237	CG31935	25922	CG32087	47238	CG32220	23280
CG31653	25786	CG3178	19819	CG31937	3449	CG32087	8345	CG32225	5383
CG31654	15565	CG31783	3875	CG31938	40306	CG32089	8340	CG32226	34464
CG31655	45883	CG31784	39192	CG3194	7333	CG3209	10281	CG32227	43928
CG31657	25787	CG31787	6372	CG3195	32450	CG32090	7495	CG3223	30003
CG31659	23310	CG31790	46057	CG31950	34751	CG32092	41785	CG32230	9804
CG31660	7852	CG31792	50152	CG31953	49301	CG32094	21442	CG32238	29424
CG31663	2533	CG31793	8069	CG31954	13028	CG32095	13833	CG3224	29561
CG31665	8021	CG31794	25853	CG31956	46910	CG32099	43650	CG32245	14017
CG3167	11149	CG31795	7560	CG31956	7284	CG3210	44156	CG3225	24725
CG31671	25793	CG3180	45959	CG31957	25926	CG32101	41786	CG32250	7491
CG31673	25797	CG31800	28162	CG31960	50102	CG32103	29439	CG32251	34477
CG31678	1473	CG31802	18562	CG31961	29359	CG32104	41790	CG32253	34479
CG31679	21392	CG31803	45277	CG31964	25929	CG32105	51269	CG3226	47034
CG3168	48010	CG31807	25863	CG31965	44154	CG32108	21454	CG32262	44923
CG31682	7257	CG31809	50643	CG31967	25933	CG32110	34062	CG32263	33272
CG31683	6400	CG31809	32521	CG31968	25934	CG32113	41792	CG32276	49568
CG31684	29130	CG3181	29354	CG31988	50935	CG32120	11690	CG32276	15911
CG31687	21393	CG31810	49296	CG31988	25939	CG32130	34408	CG3228	37079
CG31688	50139	CG31810	6757	CG31989	9402	CG32131	23279	CG32280	15908
CG31689	2584	CG31811	37521	CG31990	34752	CG32133	13061	CG32281	41873
CG3169	10818	CG31812	23403	CG31990	34752	CG32134	949	CG3229	34498
CG31690	43813	CG31812	50487	CG31991	6367	CG32134	27108	CG32296	34502
CG31692	21396	CG3182	3606	CG31992	45773	CG32135	21665	CG32297	34506
CG31693	37131	CG31821	15496	CG31999	29155	CG32137	37872	CG32300	7442
CG31694	21398	CG31823	43145	CG3200	14125	CG32138	34412	CG3231	34515
CG31702	25807	CG31832	47633	CG32000	29174	CG32139	41098	CG32315	45459
CG31703	32493	CG31837	23407	CG32008	25945	CG3214	34079	CG32316	34087
CG31704	39450	CG3184	25880	CG3201	31160	CG32145	5263	CG32319	24728
CG31704	8900	CG31842	21429	CG32016	34755	CG32146	10299	CG32333	26118
CG31708	38261	CG31843	43198	CG32018	21610	CG32147	34419	CG32335	44172
CG3171	7219	CG31849	2876	CG32019	46253	CG32149	41861	CG3234	2886
CG31711	25814	CG31851	3754	CG32022	10105	CG3215	12723	CG32343	15741
CG31712	21402	CG31852	25179	CG32024	52031	CG32155	5279	CG32344	21675
CG31713	39150	CG31855	34295	CG32026	34032	CG32158	45641	CG32346	46645
CG31715	21505	CG31858	3367	CG3203	41778	CG3216	29915	CG32350	24732
CG31716	10850	CG3186	25129	CG32030	34034	CG3216	6115	CG32351	46784
CG31717	7662	CG31860	7688	CG32031	34036	CG32163	14259	CG32351	41881
CG31719	41758	CG31864	28079	CG32039	38867	CG32164	34423	CG32369	45129
CG3172	25817	CG31864	50051	CG3204	45230	CG32164	49304	CG32371	24735
CG31721	21405	CG31865	25897	CG32041	43632	CG32165	49307	CG32374	14107
CG31725	7836	CG31865	50589	CG32045	40308	CG32165	26112	CG32376	41885
CG31726	25823	CG31866	25899	CG32048	50237	CG32168	34429	CG3238	34533
CG31729	7697	CG3187	40295	CG32048	19251	CG32169	29799	CG32380	39482
CG3173	25825	CG31871	51241	CG32049	40312	CG32171	34081	CG32381	41835
CG31730	21407	CG31872	31028	CG32051	21435	CG32174	21468	CG32384	42302
CG31731	11082	CG31873	36375	CG32052	21437	CG32176	30015	CG32386	26126
CG31738	998	CG31874	41766	CG32062	34046	CG32178	8413	CG32387	1003
CG31738	29906	CG31884	36297	CG32063	15207	CG32179	1038	CG32387	36287

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List of screened RNAi lines Provided by VDRC as human homolog sub-library

CG32392	34537	CG32679	47158	CG33193	46445	CG33672	49774	CG3474	43956
CG32394	24736	CG3268	3988	CG33198	46214	CG33694	49776	CG3476	2734
CG3240	12677	CG32688	47804	CG3321	46764	CG33695	49777	CG3479	3010
CG32407	26131	CG3269	34766	CG33217	47818	CG33713	49611	CG3480	26205
CG32409	34543	CG32698	50316	CG3322	42559	CG33714	51020	CG3483	41939
CG3241	29356	CG3270	41196	CG33230	48803	CG33718	49781	CG3488	7534
CG32412	38277	CG32708	49325	CG33237	49758	CG33722	49646	CG3491	34631
CG32413	5043	CG3271	3983	CG3324	34595	CG3373	1278	CG3493	40450
CG32417	34547	CG32727	48271	CG33242	49760	CG3376	12226	CG3494	24760
CG32418	34549	CG3274	34581	CG33246	49762	CG33785	50678	CG3495	26208
CG32423	37863	CG32750	52403	CG3325	30490	CG33786	50682	CG3496	43768
CG32425	41142	CG32754	46509	CG33250	46450	CG3380	39469	CG3497	9925
CG32427	27252	CG32754	50591	CG3326	24746	CG3385	3618	CG3499	33256
CG32434	36625	CG32756	49853	CG33260	47820	CG3388	9277	CG3500	19703
CG32435	26051	CG32775	46421	CG3327	2620	CG3389	36164	CG3501	26025
CG32438	38969	CG32778	48869	CG33276	48363	CG3389	8408	CG3504	26211
CG32441	13134	CG32782	46425	CG33277	47828	CG33931	50510	CG3506	9271
CG32442	6481	CG32789	49744	CG33288	51189	CG33932	50690	CG3508	34632
CG32444	41899	CG3279	45726	CG33289	48289	CG33933	50694	CG3510	43771
CG32446	23058	CG32791	48926	CG3329	24749	CG3394	3621	CG3511	21733
CG32447	5417	CG32792	47047	CG33300	46768	CG3397	34604	CG3515	34636
CG32448	9120	CG3280	42556	CG3331	45689	CG3399	34278	CG3520	40455
CG32451	22975	CG3282	47599	CG3332	3451	CG3400	25959	CG3522	4053
CG32454	26141	CG32823	50763	CG3333	46279	CG3401	34607	CG3523	29349
CG32463	7254	CG32832	47810	CG3333	34597	CG3402	21483	CG3524	4290
CG32464	12781	CG3284	11219	CG33331	50209	CG3403	40442	CG3525	34286
CG32465	5783	CG32847	48422	CG3337	29169	CG3408	36306	CG3526	26214
CG32473	8281	CG32854	50338	CG3337	29170	CG3409	37141	CG3527	24762
CG32478	24740	CG3289	41913	CG3338	21678	CG3410	37836	CG3530	26216
CG32479	37859	CG3290	52378	CG3339	41917	CG3412	34274	CG3532	26221
CG3248	26055	CG3290	6622	CG3340	40873	CG3412	34273	CG3534	41276
CG32483	22977	CG3291	21677	CG3342	40324	CG3415	34613	CG3536	11816
CG32484	41905	CG3292	19989	CG3344	15212	CG3416	26183	CG3539	26223
CG32485	34565	CG3294	26110	CG33466	46260	CG3419	3626	CG3542	26227
CG32486	41908	CG3295	24709	CG33466	46260	CG3420	39052	CG3544	21684
CG32487	26154	CG3297	42485	CG33474	51188	CG3421	41934	CG3552	30952
CG32488	46571	CG32971	49456	CG33479	48461	CG3422	26187	CG3560	34090
CG3249	48005	CG3298	43751	CG33489	48465	CG3422	48735	CG3561	43773
CG32490	21477	CG3299	34585	CG3350	11259	CG3424	44536	CG3564	7039
CG32495	49719	CG33002	46440	CG33503	50506	CG3425	26190	CG3570	48219
CG32500	49724	CG3301	43225	CG33505	49525	CG3425	48882	CG3571	43778
CG3251	34574	CG33012	48408	CG3351	21480	CG3427	43445	CG3572	21686
CG32516	48377	CG3303	9916	CG3352	9396	CG3427	50372	CG3573	34649
CG3253	26159	CG3304	7280	CG3353	41920	CG3428	41938	CG3587	37902
CG32531	47915	CG3305	7308	CG3353	41921	CG3430	40444	CG3589	38154
CG3254	26162	CG33051	48793	CG33533	51100	CG3431	34618	CG3590	6343
CG32579	48384	CG33052	48410	CG33544	48807	CG3433	19045	CG3593	21688
CG3258	29041	CG33054	48413	CG3355	13037	CG3434	45130	CG3595	7916
CG32581	49727	CG33057	49458	CG33558	46456	CG3437	34221	CG3597	21693
CG32584	48387	CG3306	34588	CG3356	34601	CG3443	10133	CG3599	16665
CG3259	46163	CG3307	34589	CG3358	41819	CG3445	26196	CG3603	51663
CG3260	11521	CG3308	43756	CG3359	37889	CG3446	42696	CG3604	9394
CG32602	50435	CG3309	15415	CG3360	8287	CG3450	26871	CG3604	48507
CG32616	49729	CG33104	49750	CG3362	26171	CG3454	34620	CG3605	26252
CG3263	26328	CG3312	24742	CG3363	26176	CG3455	49574	CG3609	52600
CG3264	43250	CG3313	43758	CG33635	48813	CG3455	34624	CG3612	34663
CG3265	24451	CG3314	43760	CG33649	49765	CG3458	30627	CG3613	26332
CG32656	46776	CG3315	42858	CG3365	43763	CG3460	46165	CG3615	10045
CG32668	46666	CG33172	49462	CG33650	49769	CG3461	6975	CG3618	6977
CG32669	47916	CG33178	49464	CG33665	51606	CG3466	30218	CG3619	27187
CG3267	13259	CG33182	46444	CG33670	51016	CG3469	24759	CG3619	3720
CG32675	46667	CG3319	10442	CG33671	49772	CG3473	26201	CG3619	37288

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CG3620	21490	CG3723	41947	CG3837	44576	CG3959	34770	CG4058	16668
CG3625	40855	CG3725	4474	CG3839	12704	CG3960	34098	CG4059	2959
CG3626	34667	CG3727	37525	CG3839	47122	CG3961	37305	CG4061	40498
CG3629	11529	CG3730	34291	CG3842	7117	CG3964	38981	CG4062	21782
CG3630	21697	CG3731	40467	CG3843	40477	CG3967	26338	CG4063	40862
CG3631	13770	CG3733	26277	CG3845	34719	CG3969	26065	CG4064	26382
CG3632	26254	CG3734	12899	CG3848	10749	CG3971	47519	CG4065	3614
CG3633	34670	CG3735	26278	CG3849	47126	CG3973	34772	CG4067	26385
CG3634	16720	CG3736	30470	CG3849	21500	CG3977	46757	CG4068	34786
CG3638	6914	CG3737	45133	CG3850	21719	CG3978	6224	CG4069	21783
CG3639	34671	CG3739	29508	CG3857	44734	CG3979	9981	CG4070	12259
CG3640	15241	CG3740	13525	CG3858	51221	CG3980	34773	CG4071	26388
CG3641	40314	CG3743	30455	CG3861	26301	CG3981	40495	CG4071	47653
CG3642	26261	CG3744	34695	CG3862	34721	CG3983	26066	CG4074	26070
CG3644	15453	CG3751	34699	CG3869	40478	CG3985	35813	CG4076	21784
CG3647	47973	CG3752	21707	CG3871	30456	CG3985	46482	CG4078	37607
CG3648	10156	CG3753	34701	CG3871	48598	CG3986	13875	CG4079	30442
CG3652	35574	CG3756	15675	CG3874	47543	CG3988	26346	CG4080	9026
CG3653	27227	CG3758	9794	CG3876	44201	CG3989	2901	CG4082	13639
CG3653	3111	CG3759	15602	CG3878	34179	CG3991	26348	CG4083	34789
CG3653	6695	CG3760	43437	CG3879	42514	CG3994	3836	CG4086	14268
CG3654	21700	CG3760	46862	CG3880	34725	CG3996	34778	CG4087	12944
CG3656	12190	CG3761	40468	CG3881	42779	CG3997	23578	CG4088	33197
CG3658	41084	CG3762	34389	CG3885	35806	CG3999	26354	CG4090	34792
CG3661	23416	CG3763	33173	CG3886	30586	CG4001	3016	CG4091	34102
CG3663	12879	CG3764	34707	CG3889	34727	CG4005	40497	CG4094	34797
CG3664	34096	CG3766	34709	CG3891	11270	CG4006	2902	CG4095	47685
CG3665	8392	CG3769	40469	CG3893	40479	CG4007	36282	CG4095	26074
CG3665	36351	CG3770	4064	CG3894	21721	CG4007	841	CG4097	34801
CG3668	37637	CG3772	7238	CG3896	4913	CG4007	9653	CG4098	14265
CG3671	44000	CG3773	26281	CG3903	37115	CG4008	21772	CG4101	9089
CG3675	26057	CG3774	30238	CG3905	50368	CG4009	19942	CG4103	26390
CG3678	26267	CG3776	38269	CG3905	43869	CG4012	28367	CG4105	47499
CG3678	49792	CG3780	40471	CG3909	12758	CG4013	14915	CG4107	21786
CG3680	34675	CG3781	7113	CG3911	21738	CG4015	26356	CG4108	21788
CG3682	47027	CG3782	41949	CG3915	40484	CG4016	10020	CG4109	42561
CG3683	46799	CG3788	21715	CG3917	34731	CG4017	13282	CG4111	38950
CG3683	25961	CG3790	4667	CG3918	47144	CG4019	46880	CG4114	22994
CG3688	26269	CG3791	41954	CG3918	34734	CG4019	6650	CG4119	26395
CG3689	45280	CG3792	48233	CG3921	52608	CG4020	3290	CG4120	34806
CG3692	45939	CG3792	7862	CG3922	25964	CG4023	43781	CG4123	8493
CG3694	26872	CG3793	40475	CG3923	34737	CG4025	6924	CG4124	35580
CG3695	28361	CG3794	20117	CG3924	30454	CG4027	7139	CG4125	27225
CG3696	46685	CG3796	7756	CG3925	40486	CG4029	12610	CG4125	951
CG3696	10762	CG3798	28365	CG3926	34738	CG4030	26368	CG4128	8890
CG3697	12670	CG3799	41960	CG3929	7795	CG4032	2897	CG4129	21789
CG3699	5667	CG3803	3596	CG3931	26309	CG4033	37581	CG4132	21792
CG3702	7296	CG3806	34711	CG3935	4542	CG4035	7800	CG4140	26396
CG3703	34679	CG3808	34713	CG3936	1112	CG4036	26370	CG4141	38986
CG3704	34684	CG3809	43780	CG3936	27228	CG4038	21775	CG4143	12751
CG3705	23179	CG3810	6922	CG3938	47941	CG4039	13661	CG4145	28369
CG3707	34686	CG3811	22984	CG3939	21513	CG4040	26372	CG4147	14882
CG3708	26272	CG3812	44418	CG3940	13806	CG4040	48694	CG4152	21793
CG3709	34687	CG3814	4671	CG3943	21745	CG4041	34780	CG4153	9416
CG3710	22979	CG3817	21716	CG3944	21749	CG4042	26377	CG4153	48911
CG3711	11166	CG3820	41964	CG3947	40886	CG4043	21776	CG4154	21797
CG3712	42412	CG3821	7750	CG3948	34768	CG4045	34784	CG4157	21799
CG3712	36406	CG3822	40985	CG3949	21755	CG4046	21516	CG4158	6248
CG3714	26275	CG3825	15238	CG3953	16416	CG4049	6981	CG4159	26397
CG3715	40464	CG3830	16896	CG3954	21756	CG4050	33248	CG4161	26400
CG3717	45284	CG3832	52052	CG3956	50003	CG4051	21779	CG4162	21805
CG3719	21495	CG3835	30979	CG3956	6232	CG4057	21780	CG4163	51493

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CG4164	22996	CG4274	44834	CG4422	26537	CG4557	51263	CG4666	36293
CG4165	41977	CG4276	26482	CG4426	7143	CG4560	26548	CG4670	7984
CG4166	45775	CG4278	26487	CG4427	15555	CG4561	40541	CG4672	8380
CG4167	21806	CG4279	28793	CG4428	34843	CG4562	6770	CG4673	21917
CG4169	26404	CG4279	50653	CG4429	34301	CG4565	5665	CG4675	3664
CG4170	26408	CG4288	8620	CG4429	48119	CG4567	34874	CG4676	4689
CG4173	26412	CG4289	42591	CG4433	29468	CG4568	1405	CG4677	28376
CG4179	7054	CG4290	26496	CG4434	21521	CG4569	21871	CG4678	28379
CG4180	41980	CG4291	21819	CG4435	40519	CG4572	16428	CG4679	38111
CG4183	6983	CG4293	6885	CG4438	34845	CG4573	21874	CG4681	40552
CG4184	21809	CG4299	21827	CG4438	50033	CG4574	26557	CG4683	22173
CG4185	3161	CG4300	26500	CG4439	41232	CG4579	21878	CG4684	44282
CG4186	35814	CG4301	6125	CG4443	34109	CG4583	39562	CG4685	14751
CG4187	7226	CG4302	37207	CG4445	42864	CG4584	21883	CG4686	46370
CG4192	6354	CG4303	12673	CG4447	38195	CG4586	21886	CG4690	45740
CG4193	21811	CG4307	12794	CG4448	34847	CG4587	3840	CG4692	13324
CG4195	43120	CG4311	26504	CG4450	42770	CG4589	6662	CG4696	40554
CG4196	11926	CG4314	40875	CG4451	42658	CG4591	42643	CG4696	46854
CG4199	26424	CG4316	1613	CG4452	40525	CG4592	6255	CG4697	34308
CG4200	7173	CG4317	14163	CG4453	47155	CG4593	21888	CG4698	38011
CG4201	26427	CG4320	13112	CG4466	40530	CG4594	13284	CG4700	15810
CG4202	26432	CG4321	21615	CG4481	42891	CG4596	35590	CG4701	9763
CG4202	49946	CG4322	1800	CG4482	33186	CG4598	34879	CG4703	26601
CG4204	12952	CG4324	37211	CG4483	5176	CG4599	26075	CG4704	21921
CG4205	24497	CG4328	30516	CG4484	5174	CG4600	26562	CG4706	34888
CG4206	51217	CG4330	11078	CG4485	12498	CG4602	51088	CG4709	21923
CG4207	46688	CG4332	37392	CG4488	26543	CG4603	21893	CG4713	21928
CG4207	26439	CG4334	49330	CG4494	34113	CG4604	15389	CG4719	21932
CG4208	30505	CG4335	26514	CG4495	49350	CG4606	42652	CG4720	34892
CG4209	21611	CG4337	23299	CG4497	21859	CG4608	5732	CG4722	46675
CG4210	49494	CG4341	29341	CG4498	10330	CG4609	21895	CG4722	8892
CG4211	26441	CG4342	21519	CG4498	39411	CG4610	34881	CG4729	48592
CG4212	45774	CG4346	37389	CG4500	34852	CG4611	21898	CG4729	5284
CG4214	3857	CG4347	21832	CG4501	34853	CG4613	14089	CG4733	34893
CG4215	10374	CG4349	40505	CG4502	34858	CG4617	26568	CG4735	26615
CG4217	37819	CG4350	38988	CG4510	26544	CG4618	41993	CG4738	21937
CG4220	42813	CG4351	26517	CG4511	34861	CG4619	2755	CG4739	6019
CG4221	34810	CG4353	47507	CG4520	34862	CG4621	21903	CG4742	21534
CG4222	48009	CG4354	10709	CG4521	33135	CG4622	21904	CG4743	9487
CG4225	37356	CG4356	33123	CG4523	21860	CG4623	21624	CG4746	21629
CG4226	51438	CG4357	30000	CG4525	34864	CG4624	34303	CG4749	21941
CG4233	26452	CG4364	27607	CG4527	43784	CG4625	1429	CG4750	41998
CG4236	26455	CG4365	6261	CG4532	21861	CG4627	4681	CG4751	45530
CG4237	26459	CG4370	4341	CG4533	40531	CG4629	26573	CG4752	40557
CG4238	41982	CG4372	36431	CG4535	21862	CG4630	4687	CG4753	1730
CG4239	11857	CG4376	7760	CG4536	7128	CG4633	21905	CG4753	46850
CG4241	50706	CG4379	6993	CG4537	48669	CG4634	3200	CG4755	34895
CG4244	21813	CG4380	16893	CG4537	28832	CG4636	21908	CG4756	13683
CG4247	25967	CG4386	41291	CG4538	16432	CG4637	1402	CG4757	38052
CG4252	11251	CG4389	21845	CG4539	21865	CG4638	34885	CG4758	8895
CG4257	43867	CG4393	21616	CG4542	7132	CG4643	26578	CG4759	21943
CG4258	34821	CG4394	34835	CG4545	11346	CG4645	10164	CG4760	21536
CG4260	4267	CG4395	7223	CG4546	34868	CG4646	21461	CG4764	26625
CG4261	30495	CG4396	48891	CG4547	21870	CG4648	21914	CG4766	21944
CG4262	37915	CG4400	21618	CG4548	10618	CG4649	46701	CG4767	21946
CG4264	26465	CG4402	33252	CG4550	44179	CG4649	26585	CG4768	21541
CG4264	50222	CG4405	48531	CG4551	40534	CG4654	12722	CG4769	9180
CG4265	26469	CG4407	34228	CG4552	40537	CG4656	21916	CG4770	6783
CG4266	26475	CG4410	26536	CG4553	14743	CG4659	42852	CG4774	6742
CG4268	45127	CG4412	35385	CG4554	21620	CG4660	50362	CG4775	42499
CG4270	34105	CG4415	34839	CG4555	41990	CG4662	41260	CG4779	21544
CG4272	26479	CG4420	40512	CG4556	40540	CG4663	39544	CG4780	44535

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CG4785	21948	CG4901	34905	CG5012	26684	CG5119	22007	CG5241	34976
CG4787	21949	CG4904	26653	CG5012	50149	CG5121	34316	CG5242	17830
CG4789	26626	CG4907	6832	CG5013	38992	CG5122	27358	CG5242	48166
CG4792	24667	CG4908	44845	CG5013	48108	CG5124	22010	CG5245	27381
CG4795	13086	CG4909	26657	CG5014	30404	CG5125	27359	CG5247	16758
CG4798	26627	CG4911	45141	CG5017	21582	CG5127	47030	CG5248	9248
CG4799	34265	CG4912	26658	CG5018	42018	CG5131	42025	CG5249	34978
CG4800	26632	CG4913	15671	CG5021	36440	CG5133	16747	CG5249	50171
CG4802	42001	CG4916	49379	CG5022	8262	CG5140	22013	CG5252	27383
CG4803	34898	CG4917	42665	CG5023	34914	CG5142	22015	CG5254	6887
CG4805	44412	CG4918	31005	CG5025	26685	CG5144	30946	CG5258	35729
CG4806	26633	CG4920	37001	CG5026	34915	CG5147	37114	CG5261	48939
CG4807	41005	CG4921	24672	CG5027	12106	CG5149	34944	CG5263	22044
CG4810	26637	CG4924	43786	CG5029	39418	CG5150	15298	CG5264	30465
CG4813	21950	CG4924	50123	CG5029	29733	CG5155	27364	CG5264	50658
CG4816	21951	CG4925	45142	CG5029	33803	CG5157	34947	CG5265	34323
CG4817	44343	CG4926	935	CG5030	31092	CG5160	22019	CG5266	29686
CG4821	45232	CG4926	29930	CG5032	46172	CG5161	34951	CG5268	13796
CG4822	42730	CG4928	6143	CG5033	30551	CG5162	14661	CG5270	27390
CG4824	42004	CG4931	34907	CG5037	42786	CG5163	12746	CG5271	34325
CG4825	5391	CG4933	24674	CG5038	52421	CG5164	40582	CG5274	22046
CG4827	49359	CG4934	45457	CG5041	12559	CG5165	34953	CG5275	47621
CG4832	44526	CG4935	12563	CG5044	40570	CG5166	34955	CG5276	47995
CG4836	41431	CG4937	24679	CG5045	26687	CG5167	6092	CG5277	34979
CG4840	21959	CG4938	23004	CG5047	26692	CG5168	22021	CG5280	22050
CG4841	26645	CG4942	42888	CG5048	34120	CG5169	22024	CG5281	6049
CG4842	50516	CG4943	24680	CG5053	42019	CG5170	37583	CG5282	5387
CG4842	37490	CG4944	21549	CG5055	2914	CG5174	29752	CG5284	6465
CG4843	42010	CG4945	24683	CG5057	12755	CG5178	9780	CG5285	10376
CG4845	21960	CG4946	21550	CG5059	9847	CG5179	30448	CG5287	51882
CG4847	3230	CG4947	41644	CG5063	49383	CG5183	9235	CG5288	24440
CG4848	40559	CG4952	2942	CG5064	27351	CG5184	23249	CG5289	22052
CG4849	21962	CG4953	41214	CG5065	4921	CG5186	15185	CG5290	22057
CG4851	1459	CG4954	26664	CG5067	40867	CG5187	37634	CG5295	37880
CG4852	9014	CG4956	24487	CG5068	27352	CG5188	34320	CG5300	34983
CG4853	19877	CG4957	40565	CG5069	43858	CG5189	40318	CG5310	39402
CG4858	26649	CG4960	2732	CG5070	6150	CG5190	28282	CG5313	37762
CG4860	34899	CG4960	52392	CG5072	40576	CG5192	1836	CG5315	40936
CG4863	23420	CG4963	12342	CG5073	46549	CG5195	31044	CG5316	25953
CG4863	46265	CG4965	46064	CG5075	34927	CG5196	6096	CG5317	46572
CG4866	34116	CG4966	24687	CG5076	45198	CG5197	22028	CG5319	34986
CG4867	30360	CG4968	21978	CG5077	6766	CG5198	27370	CG5320	22059
CG4871	47955	CG4969	27610	CG5078	8085	CG5201	42840	CG5321	22061
CG4875	1830	CG4972	2777	CG5081	5413	CG5202	27373	CG5322	34989
CG4875	47226	CG4973	42015	CG5085	21999	CG5202	49982	CG5323	21563
CG4877	21966	CG4974	14136	CG5091	2782	CG5203	34125	CG5325	22064
CG4878	26651	CG4975	26673	CG5093	30550	CG5205	34128	CG5327	25972
CG4879	13363	CG4976	10836	CG5094	22002	CG5206	44284	CG5330	27392
CG4881	28386	CG4980	26676	CG5098	27356	CG5208	27378	CG5333	22066
CG4882	15718	CG4984	10055	CG5099	11784	CG5214	34966	CG5335	22068
CG4884	23421	CG4991	30264	CG5102	51300	CG5215	34969	CG5336	10455
CG4887	21970	CG4993	45863	CG5103	46604	CG5216	23201	CG5337	22069
CG4887	49022	CG4994	8366	CG5103	34931	CG5219	45542	CG5338	22074
CG4889	13351	CG4996	34913	CG5104	13787	CG5220	34972	CG5339	22075
CG4893	22358	CG4998	16453	CG5105	22003	CG5222	27380	CG5341	22077
CG4894	52644	CG4999	37252	CG5108	34934	CG5226	37181	CG5342	44404
CG4896	26652	CG5000	21982	CG5109	22004	CG5227	9437	CG5342	50058
CG4896	48197	CG5002	10058	CG5110	34935	CG5229	5684	CG5343	10468
CG4897	21973	CG5003	26680	CG5111	28642	CG5231	22039	CG5344	22082
CG4898	34119	CG5004	26683	CG5112	14747	CG5232	22040	CG5347	22088
CG4899	46766	CG5005	13725	CG5114	36618	CG5235	16705	CG5348	1698
CG4899	6470	CG5009	21991	CG5116	16476	CG5237	45780	CG5352	40587

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CG5353	7752	CG5455	35011	CG5554	20101	CG5661	9428	CG5790	45044
CG5354	22095	CG5458	27420	CG5555	35013	CG5662	35030	CG5792	34143
CG5355	40588	CG5461	19679	CG5558	39560	CG5663	27475	CG5793	35044
CG5358	15645	CG5462	27424	CG5558	46814	CG5669	45300	CG5793	47883
CG5359	21565	CG5462	27424	CG5560	14814	CG5670	12330	CG5794	27517
CG5362	27399	CG5462	27424	CG5561	47055	CG5671	35731	CG5796	40607
CG5363	41838	CG5463	22135	CG5561	27461	CG5675	27479	CG5798	8931
CG5364	2781	CG5465	14916	CG5565	27463	CG5675	28652	CG5798	8931
CG5365	18988	CG5467	45556	CG5567	44319	CG5676	47731	CG5799	3780
CG5366	12067	CG5469	39000	CG5569	21568	CG5677	1414	CG5800	27519
CG5367	12392	CG5473	21567	CG5571	22160	CG5680	34139	CG5802	6801
CG5370	34328	CG5474	12101	CG5577	22163	CG5682	7696	CG5803	941
CG5370	46616	CG5475	52277	CG5580	41845	CG5684	28396	CG5803	3091
CG5371	15683	CG5475	34238	CG5582	5322	CG5685	42660	CG5803	42229
CG5372	6646	CG5479	22138	CG5583	10932	CG5686	7777	CG5804	23587
CG5374	34070	CG5481	11823	CG5585	22166	CG5687	33262	CG5805	35592
CG5375	22099	CG5482	4991	CG5586	22169	CG5688	27482	CG5807	6726
CG5377	34392	CG5483	22139	CG5589	44322	CG5690	28651	CG5808	22199
CG5378	22104	CG5484	2679	CG5590	42039	CG5692	27486	CG5809	43148
CG5379	14461	CG5485	5341	CG5591	22170	CG5695	37535	CG5810	44988
CG5380	11228	CG5486	26027	CG5594	10278	CG5703	22194	CG5811	1258
CG5383	22108	CG5488	11570	CG5595	27465	CG5704	42044	CG5813	28401
CG5384	27405	CG5489	45558	CG5596	34331	CG5705	19854	CG5814	22201
CG5387	34990	CG5491	27433	CG5599	16506	CG5706	42046	CG5815	22203
CG5389	22112	CG5492	11901	CG5602	51315	CG5707	22195	CG5818	40608
CG5392	52427	CG5493	50637	CG5603	15340	CG5708	23425	CG5821	37850
CG5394	34995	CG5493	26082	CG5604	27467	CG5714	28398	CG5823	8301
CG5395	34999	CG5495	27436	CG5605	45027	CG5715	14710	CG5826	27521
CG5403	51314	CG5497	41800	CG5608	45569	CG5718	34239	CG5827	23590
CG5404	40907	CG5498	27440	CG5610	1189	CG5720	27487	CG5828	27522
CG5405	43790	CG5499	12768	CG5610	48159	CG5721	27488	CG5830	40611
CG5406	27406	CG5500	15376	CG5611	46344	CG5722	42782	CG5832	16755
CG5407	27411	CG5502	49443	CG5613	24694	CG5723	51173	CG5836	13426
CG5408	22113	CG5505	11152	CG5621	47549	CG5725	44157	CG5837	22207
CG5411	25976	CG5508	1316	CG5625	45570	CG5728	24696	CG5838	22210
CG5412	27413	CG5510	49025	CG5626	27469	CG5729	27490	CG5840	22214
CG5412	50266	CG5510	28393	CG5627	35019	CG5730	27493	CG5841	27526
CG5413	14356	CG5514	27445	CG5629	40601	CG5731	15543	CG5842	5261
CG5414	35001	CG5515	27447	CG5632	39087	CG5733	27495	CG5844	27528
CG5417	23422	CG5516	51402	CG5634	1106	CG5734	43798	CG5846	21645
CG5422	28649	CG5517	15957	CG5634	27199	CG5735	27498	CG5847	3162
CG5423	44702	CG5519	22146	CG5634	8016	CG5737	41048	CG5850	1456
CG5424	33200	CG5521	22150	CG5637	22693	CG5741	5158	CG5851	42051
CG5428	43796	CG5522	40595	CG5638	1748	CG5742	36428	CG5854	15649
CG5429	22123	CG5523	5038	CG5639	1305	CG5744	23428	CG5855	37722
CG5430	44712	CG5524	13650	CG5640	37663	CG5745	35034	CG5857	3408
CG5432	27417	CG5525	22155	CG5641	48200	CG5748	37699	CG5859	45677
CG5433	22125	CG5526	27450	CG5641	42043	CG5748	48692	CG5861	6360
CG5434	21641	CG5528	924	CG5642	46592	CG5751	37250	CG5861	47665
CG5435	35005	CG5528	36308	CG5643	27470	CG5753	27503	CG5862	45155
CG5439	27418	CG5529	19834	CG5645	9289	CG5755	3879	CG5863	14366
CG5440	49030	CG5532	30346	CG5648	35023	CG5757	28654	CG5864	12913
CG5442	40590	CG5535	42584	CG5650	35025	CG5760	44002	CG5869	34145
CG5443	46573	CG5537	22157	CG5651	44325	CG5771	22198	CG5870	42053
CG5443	47331	CG5543	27454	CG5653	14064	CG5772	6750	CG5871	41822
CG5444	12600	CG5545	13056	CG5654	27472	CG5776	27507	CG5874	43211
CG5446	41797	CG5546	27457	CG5656	18119	CG5783	23429	CG5876	3855
CG5447	21566	CG5547	27459	CG5657	51526	CG5784	49386	CG5877	39004
CG5450	42114	CG5548	24714	CG5658	40605	CG5786	39001	CG5880	1264
CG5451	42035	CG5549	8222	CG5659	35029	CG5788	48146	CG5882	27532
CG5452	39137	CG5550	31000	CG5660	29445	CG5788	27515	CG5884	19730
CG5454	22132	CG5553	30623	CG5661	1052	CG5789	1204	CG5884	19731

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CG5886	22216	CG5987	21005	CG6094	48721	CG6194	22294	CG6321	47682
CG5887	47142	CG5989	5149	CG6094	27564	CG6196	47140	CG6321	27584
CG5887	33338	CG5991	25483	CG6095	30111	CG6196	22297	CG6322	34242
CG5889	27535	CG5992	16456	CG6096	37691	CG6197	46312	CG6323	4391
CG5890	23431	CG5992	50426	CG6096	47124	CG6198	22300	CG6325	35072
CG5892	6807	CG5994	21010	CG6098	27566	CG6199	45484	CG6327	43988
CG5893	49549	CG5996	9337	CG6106	30120	CG6201	13245	CG6330	44326
CG5893	2940	CG5998	25484	CG6110	22221	CG6202	5883	CG6331	6782
CG5894	27538	CG6000	23436	CG6113	31021	CG6203	8933	CG6331	47132
CG5898	23432	CG6004	25489	CG6114	22225	CG6204	30135	CG6332	43799
CG5899	15679	CG6005	25492	CG6115	29711	CG6205	47864	CG6335	43998
CG5902	6274	CG6007	21012	CG6120	3422	CG6205	9149	CG6338	12632
CG5903	31098	CG6008	5717	CG6121	22233	CG6206	49352	CG6339	15879
CG5904	35048	CG6009	33623	CG6122	22235	CG6208	40348	CG6340	34160
CG5905	7108	CG6009	46891	CG6125	12141	CG6210	5215	CG6341	22488
CG5906	5227	CG6011	13760	CG6126	7326	CG6213	25986	CG6342	30153
CG5907	49870	CG6013	33625	CG6127	27172	CG6218	35069	CG6343	14444
CG5907	23433	CG6014	31067	CG6128	50641	CG6220	40350	CG6345	27588
CG5911	42716	CG6015	41708	CG6129	22237	CG6222	10854	CG6347	16837
CG5912	6707	CG6016	7988	CG6133	37601	CG6223	15419	CG6349	11227
CG5912	4819	CG6017	8487	CG6136	22239	CG6224	22476	CG6350	50009
CG5912	36286	CG6018	33629	CG6137	30125	CG6225	8276	CG6352	51289
CG5913	40336	CG6019	47606	CG6139	4856	CG6226	22480	CG6353	40363
CG5915	40338	CG6020	13130	CG6140	50305	CG6227	40351	CG6355	27592
CG5917	8347	CG6022	45020	CG6141	22244	CG6230	8897	CG6358	40368
CG5919	28402	CG6025	17826	CG6142	19930	CG6232	31020	CG6359	34165
CG5920	20963	CG6027	43633	CG6143	22245	CG6233	24700	CG6363	43802
CG5921	37875	CG6028	52314	CG6144	41847	CG6235	34340	CG6364	11693
CG5926	24996	CG6030	21018	CG6145	48698	CG6238	30136	CG6369	27598
CG5927	17853	CG6034	33631	CG6146	10639	CG6246	6217	CG6372	52508
CG5930	41011	CG6036	21023	CG6147	22252	CG6249	30140	CG6375	27600
CG5931	43962	CG6042	49491	CG6148	22253	CG6251	44808	CG6376	15887
CG5933	20968	CG6042	25168	CG6149	40847	CG6253	44629	CG6378	16677
CG5934	20970	CG6045	48191	CG6151	39596	CG6255	40355	CG6379	29611
CG5935	25477	CG6045	21032	CG6152	41825	CG6258	12618	CG6380	29950
CG5937	33613	CG6046	15710	CG6153	22257	CG6259	25990	CG6383	39177
CG5938	32418	CG6049	25497	CG6154	23008	CG6262	25993	CG6385	27601
CG5939	33615	CG6050	48982	CG6155	34151	CG6264	5963	CG6386	48980
CG5940	32421	CG6051	25500	CG6156	22258	CG6267	30079	CG6390	4385
CG5941	48773	CG6052	5311	CG6167	22268	CG6267	42260	CG6391	25995
CG5942	37721	CG6053	35052	CG6168	28405	CG6269	10825	CG6392	35081
CG5946	5226	CG6054	35055	CG6169	22272	CG6271	50744	CG6393	40376
CG5949	41028	CG6056	34148	CG6171	30128	CG6272	34156	CG6395	47208
CG5950	5150	CG6057	6532	CG6171	49472	CG6275	3053	CG6395	34168
CG5952	17849	CG6058	27542	CG6172	13062	CG6275	845	CG6396	35082
CG5954	13994	CG6058	47667	CG6173	14703	CG6278	23598	CG6396	35082
CG5955	15836	CG6059	35056	CG6176	30131	CG6279	37267	CG6396	35082
CG5958	20982	CG6061	35061	CG6177	22280	CG6281	15372	CG6401	39552
CG5960	20983	CG6064	27545	CG6178	1172	CG6284	22483	CG6405	35087
CG5961	20988	CG6066	35065	CG6179	40341	CG6287	40358	CG6407	32257
CG5962	20989	CG6070	1262	CG6180	47677	CG6292	37562	CG6410	24701
CG5964	33617	CG6072	27546	CG6181	37945	CG6293	33220	CG6412	44327
CG5965	20994	CG6073	17256	CG6182	14705	CG6299	46105	CG6413	35090
CG5966	13164	CG6074	31148	CG6184	40920	CG6302	28794	CG6414	44328
CG5969	47116	CG6081	7868	CG6186	14666	CG6303	48309	CG6415	51541
CG5970	20998	CG6083	27549	CG6187	34152	CG6304	48212	CG6418	40379
CG5973	24998	CG6084	27551	CG6188	25983	CG6308	30273	CG6420	42060
CG5974	2889	CG6087	9699	CG6189	22287	CG6311	30149	CG6422	27614
CG5977	33110	CG6089	27554	CG6190	45876	CG6312	10416	CG6424	27619
CG5978	21000	CG6090	22218	CG6191	27133	CG6315	27577	CG6428	27622
CG5980	21003	CG6091	27558	CG6192	24719	CG6318	10759	CG6432	43451
CG5986	25479	CG6092	44165	CG6193	22290	CG6320	27581	CG6434	36424

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CG6437	45275	CG6549	27661	CG6678	26719	CG6778	44603	CG6891	50055
CG6438	37023	CG6550	4947	CG6682	26721	CG6779	37742	CG6892	30552
CG6439	14443	CG6551	27662	CG6684	52603	CG6781	34227	CG6894	9154
CG6443	15736	CG6554	40388	CG6686	21573	CG6782	50714	CG6897	35134
CG6444	27626	CG6562	46070	CG6690	14439	CG6784	39312	CG6898	37358
CG6445	36320	CG6565	39006	CG6691	3951	CG6788	48054	CG6899	1012
CG6449	5208	CG6567	27667	CG6692	13959	CG6788	42912	CG6899	4297
CG6450	40382	CG6570	52323	CG6693	27717	CG6789	27747	CG6899	27232
CG6451	27630	CG6570	30461	CG6695	36656	CG6790	12134	CG6900	49629
CG6453	37991	CG6571	35105	CG6696	28426	CG6792	35118	CG6900	23602
CG6454	36655	CG6574	40902	CG6696	49114	CG6794	30578	CG6903	3285
CG6455	11938	CG6576	26695	CG6697	27720	CG6796	6494	CG6904	35136
CG6459	15520	CG6577	12588	CG6699	42071	CG6798	1199	CG6905	13492
CG6461	18545	CG6578	6170	CG6700	39007	CG6800	40397	CG6906	8357
CG6463	34171	CG6582	51472	CG6701	36557	CG6811	34250	CG6907	22460
CG6464	3029	CG6584	25999	CG6702	41812	CG6812	8534	CG6910	22464
CG6464	3029	CG6589	46072	CG6703	34185	CG6814	22442	CG6913	46690
CG6465	27632	CG6593	27673	CG6704	46856	CG6815	39675	CG6914	35139
CG6472	22495	CG6597	28237	CG6706	1784	CG6816	5601	CG6915	35141
CG6475	40932	CG6603	27680	CG6707	44557	CG6817	10102	CG6919	47896
CG6476	33834	CG6604	28416	CG6711	37548	CG6818	49479	CG6920	13310
CG6476	39377	CG6604	50849	CG6712	39012	CG6818	26723	CG6921	30179
CG6477	27639	CG6605	27683	CG6713	27722	CG6819	47693	CG6923	26096
CG6479	45648	CG6607	27688	CG6716	6221	CG6822	5142	CG6928	5231
CG6480	23449	CG6608	6005	CG6717	18961	CG6824	12663	CG6930	35147
CG6484	4954	CG6612	42064	CG6719	27727	CG6827	9039	CG6931	22468
CG6485	14281	CG6613	42065	CG6721	23016	CG6831	40399	CG6931	49058
CG6486	43804	CG6614	26700	CG6723	13769	CG6835	49800	CG6932	22307
CG6492	6162	CG6615	27691	CG6724	27730	CG6838	35123	CG6937	22315
CG6493	25090	CG6618	8052	CG6725	37361	CG6840	23290	CG6938	37271
CG6495	42796	CG6619	15622	CG6726	34247	CG6841	34253	CG6939	22317
CG6498	35100	CG6620	35107	CG6733	50172	CG6842	35126	CG6944	45635
CG6500	2916	CG6621	27695	CG6736	12890	CG6844	1194	CG6946	27752
CG6502	27645	CG6622	27699	CG6737	46197	CG6846	40402	CG6948	22318
CG6504	6940	CG6623	40390	CG6738	48918	CG6847	22451	CG6949	40405
CG6506	34349	CG6625	22379	CG6738	27736	CG6850	16467	CG6950	22321
CG6508	28413	CG6627	42798	CG6741	16826	CG6851	44306	CG6951	27756
CG6509	46234	CG6630	26701	CG6742	27738	CG6852	26001	CG6953	1530
CG6509	22496	CG6632	52510	CG6743	22407	CG6854	12762	CG6954	27759
CG6512	8515	CG6633	6016	CG6744	48329	CG6856	34354	CG6961	35150
CG6513	34173	CG6634	30519	CG6745	46746	CG6857	7231	CG6963	26003
CG6514	27649	CG6637	21658	CG6746	46513	CG6859	12426	CG6964	13687
CG6515	13392	CG6643	28418	CG6747	28431	CG6860	7306	CG6966	22326
CG6516	27654	CG6649	7320	CG6750	9408	CG6863	2656	CG6967	27769
CG6517	39622	CG6650	8363	CG6751	12577	CG6866	22453	CG6971	35153
CG6518	2894	CG6652	26706	CG6752	43605	CG6867	37416	CG6971	48986
CG6519	13860	CG6653	8574	CG6753	16600	CG6868	1216	CG6972	27772
CG6521	22497	CG6656	1630	CG6754	28215	CG6869	5271	CG6975	6314
CG6522	22500	CG6657	8144	CG6755	6282	CG6870	52570	CG6976	37532
CG6523	34174	CG6658	8569	CG6756	18112	CG6871	6283	CG6978	7041
CG6524	33286	CG6659	8038	CG6757	22412	CG6873	22454	CG6980	29148
CG6533	19870	CG6660	6835	CG6758	43606	CG6875	2910	CG6983	35158
CG6534	30462	CG6662	26713	CG6759	52667	CG6876	35131	CG6984	21649
CG6535	22502	CG6664	12780	CG6760	27743	CG6877	22455	CG6987	27775
CG6538	12602	CG6665	8202	CG6762	35734	CG6878	9224	CG6988	23358
CG6539	49505	CG6666	6031	CG6763	18940	CG6881	1815	CG6990	50520
CG6539	7154	CG6667	45998	CG6766	38035	CG6883	22703	CG6990	27782
CG6543	27658	CG6668	6719	CG6767	35112	CG6884	27750	CG6993	10715
CG6544	34066	CG6672	12132	CG6768	13645	CG6888	26094	CG6998	43116
CG6545	12662	CG6673	41806	CG6770	35825	CG6890	9430	CG6999	41829
CG6546	24703	CG6674	26716	CG6772	30673	CG6890	13549	CG7000	42496
CG6547	46065	CG6677	7141	CG6775	26090	CG6890	27099	CG7002	37005

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CG7004	27785	CG7077	42713	CG7195	39303	CG7285	13560	CG7400	9406
CG7005	9797	CG7081	5136	CG7195	28160	CG7288	47663	CG7402	37302
CG7006	29512	CG7082	2554	CG7197	19736	CG7289	34281	CG7405	10398
CG7007	33342	CG7083	22342	CG7199	48979	CG7291	30725	CG7408	8415
CG7007	47187	CG7085	46150	CG7199	37072	CG7292	35200	CG7413	10696
CG7008	19013	CG7090	14610	CG7200	27913	CG7293	27943	CG7414	18804
CG7009	27790	CG7092	27837	CG7206	22530	CG7301	39384	CG7415	35242
CG7009	27789	CG7097	35166	CG7207	27914	CG7305	48072	CG7417	37555
CG7010	40410	CG7098	46320	CG7211	35386	CG7307	18553	CG7420	46316
CG7011	46860	CG7099	27848	CG7212	40436	CG7311	41813	CG7421	27995
CG7013	12834	CG7100	1092	CG7215	49802	CG7317	28694	CG7423	28003
CG7014	49879	CG7103	6175	CG7215	27916	CG7319	27949	CG7424	44630
CG7014	27792	CG7103	46875	CG7217	49806	CG7322	9258	CG7425	26011
CG7015	49498	CG7106	45634	CG7217	35196	CG7323	15631	CG7427	28006
CG7015	41858	CG7107	27853	CG7218	5908	CG7324	31064	CG7429	28008
CG7018	15355	CG7108	43870	CG7220	34198	CG7328	27951	CG7430	28011
CG7020	27796	CG7109	41924	CG7221	22536	CG7329	48340	CG7431	2857
CG7023	27799	CG7111	27858	CG7222	34377	CG7331	27955	CG7432	31091
CG7024	27803	CG7112	35174	CG7223	40627	CG7332	27959	CG7433	28014
CG7025	43187	CG7113	37083	CG7223	6692	CG7334	13375	CG7435	48430
CG7026	8254	CG7115	9404	CG7224	36437	CG7335	27962	CG7436	28019
CG7026	48830	CG7121	44705	CG7225	13863	CG7337	35204	CG7437	28023
CG7028	27808	CG7121	17903	CG7228	33155	CG7338	27963	CG7438	12558
CG7033	41190	CG7121	839	CG7230	15900	CG7339	13629	CG7439	49473
CG7034	35162	CG7123	23121	CG7231	2783	CG7340	3174	CG7441	28027
CG7035	22331	CG7125	22344	CG7233	27919	CG7343	35206	CG7446	5329
CG7036	13070	CG7127	27867	CG7234	7878	CG7343	35206	CG7447	30934
CG7037	22335	CG7128	27870	CG7235	22539	CG7343	35206	CG7449	40898
CG7038	47159	CG7129	52571	CG7238	34382	CG7345	10813	CG7449	27065
CG7038	40413	CG7131	52520	CG7241	5851	CG7347	46011	CG7449	9471
CG7039	26007	CG7134	27881	CG7245	22541	CG7349	51481	CG7452	36595
CG7041	26097	CG7137	27884	CG7245	22541	CG7351	27966	CG7457	46671
CG7042	23452	CG7139	35177	CG7246	34256	CG7352	40636	CG7457	26740
CG7044	27811	CG7140	27888	CG7250	27103	CG7354	14305	CG7459	5805
CG7047	15203	CG7143	44721	CG7250	927	CG7356	26100	CG7460	13115
CG7048	29811	CG7144	51346	CG7250	7995	CG7359	9888	CG7461	28028
CG7049	15846	CG7145	40422	CG7254	27928	CG7360	40773	CG7462	40638
CG7050	4306	CG7146	40427	CG7255	8373	CG7362	7556	CG7464	10558
CG7050	36328	CG7149	43821	CG7257	22548	CG7364	7706	CG7466	42462
CG7051	22340	CG7152	27893	CG7259	5273	CG7365	14318	CG7467	7810
CG7052	35611	CG7154	37669	CG7260	43909	CG7367	43822	CG7469	28033
CG7053	27815	CG7156	26036	CG7261	27931	CG7368	27978	CG7470	38955
CG7054	40416	CG7158	35179	CG7262	22552	CG7371	27984	CG7471	46930
CG7055	37684	CG7161	35186	CG7263	2544	CG7371	48711	CG7471	30599
CG7056	15719	CG7162	13054	CG7264	22554	CG7375	35220	CG7473	39151
CG7057	27820	CG7163	38247	CG7265	27932	CG7376	35222	CG7478	9776
CG7059	21651	CG7168	27894	CG7266	26009	CG7378	35226	CG7479	45048
CG7060	42883	CG7169	44999	CG7266	48992	CG7378	47855	CG7480	44263
CG7061	27823	CG7172	27898	CG7268	24453	CG7379	27988	CG7484	13358
CG7062	34190	CG7176	42915	CG7269	22556	CG7382	47494	CG7485	26876
CG7066	36572	CG7177	35194	CG7272	8374	CG7387	27993	CG7486	28041
CG7067	27831	CG7178	34196	CG7274	40440	CG7390	35229	CG7487	10614
CG7068	44241	CG7180	34368	CG7275	22561	CG7391	42834	CG7490	28618
CG7069	27834	CG7183	40429	CG7277	30691	CG7392	35232	CG7494	48961
CG7070	49533	CG7184	34373	CG7279	18107	CG7394	9210	CG7494	28042
CG7070	35165	CG7186	27904	CG7280	18550	CG7395	9379	CG7497	9374
CG7071	46823	CG7187	28610	CG7281	27937	CG7397	13429	CG7499	9179
CG7073	34191	CG7188	37108	CG7281	48835	CG7398	30066	CG7504	28050
CG7074	12721	CG7190	31070	CG7282	50406	CG7398	4769	CG7507	28053
CG7075	3666	CG7192	52526	CG7282	27941	CG7398	6543	CG7508	48674
CG7076	28740	CG7193	40434	CG7283	52411	CG7399	35240	CG7508	2924
CG7077	33835	CG7194	24723	CG7283	23458	CG7400	48719	CG7509	51585

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CG7510	8532	CG7635	9160	CG7760	37611	CG7865	15782	CG7962	5121
CG7511	52654	CG7636	13828	CG7761	8692	CG7867	28069	CG7964	50645
CG7512	13829	CG7637	42402	CG7762	25549	CG7870	2802	CG7966	22639
CG7513	35250	CG7637	23669	CG7764	12596	CG7872	9134	CG7970	8857
CG7514	37233	CG7638	8235	CG7765	44337	CG7873	26019	CG7971	34262
CG7515	35251	CG7639	33641	CG7766	52573	CG7875	1365	CG7972	28072
CG7516	28058	CG7640	23671	CG7768	35266	CG7878	35288	CG7974	28074
CG7518	26745	CG7642	25172	CG7769	44976	CG7879	15260	CG7975	40645
CG7519	21653	CG7646	35742	CG7770	34203	CG7882	8103	CG7978	51974
CG7520	15927	CG7650	41714	CG7771	26888	CG7883	40321	CG7979	5126
CG7524	35252	CG7654	47988	CG7772	30431	CG7885	14000	CG7980	28169
CG7530	5872	CG7655	12429	CG7773	35267	CG7886	22610	CG7985	8257
CG7532	12405	CG7656	26881	CG7776	35268	CG7887	1374	CG7986	22646
CG7536	11576	CG7659	12639	CG7777	8124	CG7887	43329	CG7988	46277
CG7538	10967	CG7659	46805	CG7779	29046	CG7888	37264	CG7988	22651
CG7542	47336	CG7662	46962	CG7785	36650	CG7891	26085	CG7989	28172
CG7546	35253	CG7662	43094	CG7787	45715	CG7892	3002	CG7990	46157
CG7550	35254	CG7664	26885	CG7788	28065	CG7893	6241	CG7993	35314
CG7555	13121	CG7665	13566	CG7791	35272	CG7894	3060	CG7995	22652
CG7556	28621	CG7669	29200	CG7793	42848	CG7894	869	CG7997	16840
CG7558	35258	CG7670	44595	CG7804	49886	CG7894	42251	CG7998	22654
CG7560	28063	CG7671	33645	CG7804	28067	CG7895	12656	CG7999	15878
CG7562	30441	CG7678	11649	CG7806	2804	CG7896	907	CG8001	35317
CG7563	35261	CG7685	5919	CG7807	41130	CG7896	36340	CG8003	22659
CG7564	29462	CG7686	33650	CG7808	35278	CG7896	3813	CG8005	22664
CG7565	36291	CG7693	41719	CG7809	22564	CG7899	3579	CG8007	11462
CG7565	8396	CG7694	25520	CG7810	22565	CG7900	43157	CG8008	4158
CG7565	3707	CG7697	21045	CG7811	2890	CG7904	37279	CG8009	41105
CG7568	45783	CG7698	39557	CG7813	22566	CG7904	848	CG8009	48955
CG7571	37295	CG7700	12152	CG7814	35279	CG7908	2733	CG8013	42423
CG7573	8337	CG7704	45957	CG7815	22567	CG7910	51546	CG8014	22671
CG7577	36659	CG7706	25524	CG7816	1362	CG7911	23075	CG8019	41022
CG7578	33634	CG7708	30301	CG7818	13541	CG7912	1377	CG8020	44076
CG7580	28839	CG7709	9865	CG7818	48559	CG7913	22614	CG8021	23675
CG7581	21037	CG7712	50214	CG7820	26015	CG7914	22619	CG8023	34210
CG7582	1353	CG7716	25526	CG7821	40641	CG7915	22620	CG8025	26039
CG7583	37609	CG7717	25528	CG7823	46154	CG7917	22623	CG8026	4163
CG7590	25506	CG7718	25532	CG7825	44723	CG7919	5117	CG8029	48016
CG7595	9265	CG7719	21046	CG7826	28628	CG7921	1381	CG8031	35326
CG7597	25510	CG7722	25534	CG7828	7728	CG7923	7492	CG8032	28175
CG7598	14861	CG7724	43927	CG7830	4253	CG7925	52533	CG8036	35330
CG7600	47473	CG7725	33654	CG7831	22570	CG7926	7748	CG8038	28179
CG7601	4456	CG7726	45427	CG7832	49339	CG7927	22627	CG8039	29473
CG7602	37594	CG7727	42673	CG7833	44030	CG7929	12920	CG8042	35331
CG7605	43730	CG7728	21048	CG7834	36661	CG7931	21578	CG8043	28181
CG7609	21041	CG7729	37010	CG7837	22573	CG7931	50524	CG8046	7380
CG7610	16539	CG7734	3226	CG7838	26109	CG7935	38963	CG8048	46563
CG7611	25511	CG7735	43508	CG7839	12691	CG7940	22633	CG8049	22675
CG7614	12575	CG7736	1501	CG7840	47495	CG7943	28632	CG8053	26022
CG7615	47312	CG7737	49437	CG7842	46334	CG7945	35296	CG8058	13314
CG7616	41408	CG7739	51521	CG7842	14279	CG7946	35298	CG8060	46991
CG7620	30391	CG7740	51956	CG7843	22574	CG7948	14021	CG8060	22684
CG7621	48032	CG7741	33655	CG7845	22578	CG7949	21657	CG8064	28182
CG7622	39914	CG7742	25535	CG7847	9921	CG7950	22635	CG8064	49076
CG7623	12149	CG7744	33247	CG7849	22588	CG7951	46696	CG8067	47871
CG7625	30382	CG7747	44854	CG7850	3018	CG7954	52538	CG8067	22687
CG7626	19793	CG7749	5098	CG7851	33157	CG7955	40838	CG8068	30709
CG7627	2807	CG7749	3749	CG7852	7178	CG7956	22638	CG8069	28189
CG7628	49973	CG7749	27113	CG7855	22590	CG7957	44027	CG8070	35333
CG7628	19713	CG7757	25547	CG7860	34395	CG7958	28070	CG8073	23020
CG7632	45675	CG7758	12823	CG7861	34388	CG7960	13679	CG8075	7376
CG7633	6178	CG7759	21052	CG7864	46613	CG7961	35306	CG8079	23023

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CG8083	37162	CG8202	35410	CG8297	8972	CG8402	24308	CG8506	24114
CG8085	28192	CG8203	35855	CG8297	46760	CG8403	42478	CG8507	42640
CG8086	23028	CG8207	24236	CG8298	43541	CG8404	45482	CG8509	28915
CG8090	30341	CG8208	9261	CG8300	24291	CG8405	11127	CG8517	28093
CG8091	23033	CG8209	35858	CG8302	51943	CG8407	23504	CG8520	36546
CG8092	28196	CG8211	24239	CG8303	4917	CG8408	12432	CG8522	37640
CG8093	19561	CG8211	24237	CG8306	23134	CG8409	31995	CG8523	51165
CG8094	35337	CG8212	44731	CG8308	24297	CG8411	28897	CG8524	30460
CG8095	4891	CG8213	7372	CG8311	8985	CG8412	5934	CG8525	28916
CG8097	28199	CG8214	24240	CG8314	1691	CG8415	50956	CG8527	39580
CG8098	6455	CG8219	24244	CG8315	28892	CG8415	35421	CG8529	44360
CG8102	16898	CG8222	976	CG8318	35877	CG8416	12734	CG8531	24122
CG8103	10766	CG8222	13503	CG8320	8797	CG8417	49508	CG8532	35949
CG8104	29788	CG8222	43459	CG8321	8765	CG8418	35929	CG8534	49893
CG8105	8037	CG8223	35861	CG8322	30280	CG8419	24097	CG8536	4867
CG8107	46241	CG8224	3825	CG8323	4861	CG8421	52589	CG8538	35952
CG8107	23037	CG8224	853	CG8325	35881	CG8425	44049	CG8542	24125
CG8108	35343	CG8226	8747	CG8326	23760	CG8426	37545	CG8544	37792
CG8109	11329	CG8230	37160	CG8327	35883	CG8427	35934	CG8545	35954
CG8110	35345	CG8231	23751	CG8330	23763	CG8428	3229	CG8546	5110
CG8111	29391	CG8233	24248	CG8331	35377	CG8431	26959	CG8548	28920
CG8112	37345	CG8234	49889	CG8332	35415	CG8432	28866	CG8549	28924
CG8114	35349	CG8234	40980	CG8333	10950	CG8433	49808	CG8552	35957
CG8116	45735	CG8237	9324	CG8334	18982	CG8433	4902	CG8553	35959
CG8117	23254	CG8239	24253	CG8335	15506	CG8434	43898	CG8556	28926
CG8117	47174	CG8240	28877	CG8336	23729	CG8434	42570	CG8556	50349
CG8127	44851	CG8241	47782	CG8338	28240	CG8434	4319	CG8557	28927
CG8128	47740	CG8243	26952	CG8339	5070	CG8435	28900	CG8561	44361
CG8129	46959	CG8244	33842	CG8340	35890	CG8439	47742	CG8566	23464
CG8129	24201	CG8244	30642	CG8343	7735	CG8440	6216	CG8566	47171
CG8132	17254	CG8245	28878	CG8344	15692	CG8442	44439	CG8567	39592
CG8134	24204	CG8250	11446	CG8349	48682	CG8443	42136	CG8568	18534
CG8135	42635	CG8251	24257	CG8351	28895	CG8444	5830	CG8569	35962
CG8138	24208	CG8253	35411	CG8353	35896	CG8445	47743	CG8571	35967
CG8142	10881	CG8254	13716	CG8354	28848	CG8446	23141	CG8577	51237
CG8144	24214	CG8256	19565	CG8355	20210	CG8448	39126	CG8578	35969
CG8146	48210	CG8257	35865	CG8356	7317	CG8449	24102	CG8580	24130
CG8146	23126	CG8257	50371	CG8357	30485	CG8453	4615	CG8581	16923
CG8147	2892	CG8258	45789	CG8360	41643	CG8454	23769	CG8581	24475
CG8149	24215	CG8261	28844	CG8361	16753	CG8455	47953	CG8581	29909
CG8151	12581	CG8266	35867	CG8362	35901	CG8461	24103	CG8582	35970
CG8152	13978	CG8267	35870	CG8363	35904	CG8464	24104	CG8583	33282
CG8153	15695	CG8268	23678	CG8364	52541	CG8465	24107	CG8584	26988
CG8155	24218	CG8269	23728	CG8365	37685	CG8468	6452	CG8587	26989
CG8156	24224	CG8270	23755	CG8368	45259	CG8470	40712	CG8589	24180
CG8161	35843	CG8271	4607	CG8370	42509	CG8472	28243	CG8590	35975
CG8166	8137	CG8272	24262	CG8372	8043	CG8474	51285	CG8591	30713
CG8167	44761	CG8273	28887	CG8376	37791	CG8475	2800	CG8593	47001
CG8169	39193	CG8274	24265	CG8378	40705	CG8481	28906	CG8594	4642
CG8171	23131	CG8276	28888	CG8379	35911	CG8481	49470	CG8595	6541
CG8173	35845	CG8277	24267	CG8380	12082	CG8483	41263	CG8595	39176
CG8174	26933	CG8280	49890	CG8383	35915	CG8485	35940	CG8596	5089
CG8177	39492	CG8280	24270	CG8384	6315	CG8486	2796	CG8598	35982
CG8184	26935	CG8282	24275	CG8385	23082	CG8487	42140	CG8601	28932
CG8186	49888	CG8284	35872	CG8386	35919	CG8491	23142	CG8603	47148
CG8187	24230	CG8285	4365	CG8390	46229	CG8492	14929	CG8603	26992
CG8189	14210	CG8286	14154	CG8392	35923	CG8493	24109	CG8604	9264
CG8190	43917	CG8287	28092	CG8394	45917	CG8494	42609	CG8605	29435
CG8194	13018	CG8288	24278	CG8395	24305	CG8495	28849	CG8606	48214
CG8197	42125	CG8289	24279	CG8396	47383	CG8497	24111	CG8609	14032
CG8199	24231	CG8290	12739	CG8400	35419	CG8498	35388	CG8610	35986
CG8200	42130	CG8293	2972	CG8401	45237	CG8500	28795	CG8611	28936

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CG8612	15199	CG8719	39121	CG8821	37660	CG8933	7802	CG9031	42189
CG8614	42146	CG8721	30038	CG8823	30821	CG8936	47207	CG9032	23685
CG8615	42152	CG8722	40717	CG8824	4637	CG8937	45596	CG9032	50958
CG8616	38249	CG8725	28942	CG8825	46268	CG8938	50140	CG9033	44287
CG8624	26995	CG8726	40719	CG8825	28958	CG8938	23084	CG9035	8759
CG8625	6208	CG8727	11765	CG8827	41219	CG8939	40726	CG9038	29012
CG8627	23680	CG8728	48677	CG8830	28960	CG8942	1031	CG9041	28163
CG8628	35392	CG8728	23617	CG8831	42153	CG8942	9976	CG9042	29013
CG8629	39155	CG8729	15533	CG8833	36408	CG8942	40747	CG9044	42193
CG8630	33340	CG8730	23772	CG8839	4620	CG8946	37974	CG9045	37711
CG8630	50290	CG8732	3222	CG8841	48253	CG8947	14218	CG9046	13230
CG8631	2998	CG8733	51921	CG8841	23625	CG8948	42165	CG9047	23153
CG8632	4654	CG8734	7949	CG8843	28873	CG8949	48307	CG9049	36085
CG8635	24131	CG8735	4025	CG8844	35437	CG8950	36069	CG9053	10168
CG8636	28937	CG8739	47750	CG8846	35439	CG8954	23659	CG9054	29019
CG8637	35988	CG8743	45989	CG8849	36050	CG8956	3343	CG9056	29021
CG8639	29968	CG8749	23150	CG8853	51322	CG8958	42169	CG9057	40734
CG8641	35993	CG8757	13110	CG8855	19050	CG8959	14837	CG9060	12665
CG8641	52260	CG8759	36017	CG8857	23475	CG8962	29003	CG9062	3810
CG8642	35997	CG8760	28945	CG8858	23634	CG8963	42110	CG9063	40738
CG8645	35431	CG8764	35829	CG8860	8768	CG8967	30834	CG9064	2647
CG8646	38092	CG8766	4658	CG8862	38085	CG8967	42565	CG9065	33879
CG8647	38189	CG8767	36533	CG8863	23637	CG8967	878	CG9065	29838
CG8648	15698	CG8768	36022	CG8865	23639	CG8968	48894	CG9066	45185
CG8649	47514	CG8771	36023	CG8873	45618	CG8968	26923	CG9067	35744
CG8649	6276	CG8772	7192	CG8874	36053	CG8969	30483	CG9071	4062
CG8651	37715	CG8773	10203	CG8877	18567	CG8972	45845	CG9075	42201
CG8652	46514	CG8774	5862	CG8881	28975	CG8974	5572	CG9081	38218
CG8654	4715	CG8776	7909	CG8882	28976	CG8975	7965	CG9084	45609
CG8655	40715	CG8776	40803	CG8884	35445	CG8976	7394	CG9086	28961
CG8656	24184	CG8778	23621	CG8885	7860	CG8977	36071	CG9088	42203
CG8657	4659	CG8779	979	CG8886	46702	CG8978	42171	CG9089	40966
CG8660	35432	CG8779	30073	CG8887	28982	CG8979	28860	CG9090	44297
CG8663	44486	CG8779	37282	CG8888	30336	CG8980	42175	CG9092	51445
CG8664	47568	CG8781	36025	CG8890	24148	CG8981	28098	CG9093	9696
CG8665	35999	CG8782	28950	CG8891	36055	CG8983	51675	CG9095	23159
CG8667	44470	CG8783	40721	CG8892	28985	CG8987	3133	CG9096	29023
CG8668	33156	CG8784	15989	CG8893	50351	CG8988	4601	CG9098	27001
CG8669	2935	CG8785	4650	CG8893	23645	CG8989	12771	CG9099	28106
CG8675	26997	CG8786	36028	CG8895	7866	CG8993	41126	CG9099	49895
CG8676	37694	CG8789	26910	CG8895	33919	CG8995	23665	CG9100	27002
CG8677	23608	CG8790	5863	CG8896	965	CG8996	44378	CG9102	8943
CG8678	36002	CG8793	36033	CG8896	36305	CG8998	28102	CG9102	49042
CG8679	30778	CG8795	1768	CG8896	44386	CG9000	37179	CG9104	10472
CG8680	23467	CG8798	36035	CG8900	23083	CG9001	4931	CG9108	30030
CG8681	1479	CG8799	39539	CG8902	23650	CG9002	49812	CG9109	16982
CG8690	15798	CG8800	42117	CG8905	42162	CG9003	23481	CG9111	39183
CG8693	7947	CG8803	4174	CG8907	28987	CG9004	40727	CG9113	3275
CG8694	28292	CG8804	51091	CG8909	29900	CG9005	36079	CG9115	29032
CG8695	15791	CG8804	6446	CG8912	28989	CG9009	12016	CG9116	50537
CG8696	15789	CG8805	4176	CG8914	26915	CG9010	40728	CG9116	38139
CG8705	11791	CG8806	40723	CG8915	28857	CG9012	23666	CG9117	52545
CG8706	8397	CG8808	37966	CG8916	9138	CG9013	4964	CG9118	49813
CG8706	39215	CG8809	39076	CG8918	28994	CG9014	36084	CG9118	14931
CG8706	3710	CG8811	29774	CG8919	28996	CG9015	35697	CG9119	46326
CG8707	36003	CG8814	2606	CG8920	28998	CG9018	40732	CG9120	49896
CG8708	45194	CG8815	10808	CG8922	36060	CG9019	33909	CG9124	36086
CG8709	36007	CG8816	50135	CG8923	36063	CG9020	42185	CG9126	47073
CG8711	44829	CG8816	36045	CG8928	23480	CG9022	45173	CG9126	47074
CG8714	9951	CG8817	13081	CG8930	29931	CG9023	51936	CG9127	47972
CG8717	8739	CG8819	49637	CG8930	905	CG9025	44428	CG9128	37216
CG8719	50531	CG8821	49640	CG8930	4753	CG9027	37794	CG9131	44362

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CG9134	48756	CG9242	24152	CG9359	24144	CG9463	15587	CG9582	2845
CG9135	36091	CG9243	37422	CG9360	13189	CG9465	52269	CG9586	28250
CG9138	1047	CG9244	12455	CG9361	8564	CG9466	46288	CG9588	47763
CG9139	46329	CG9245	11852	CG9362	24012	CG9466	13040	CG9590	29482
CG9140	43184	CG9246	24136	CG9363	37012	CG9467	45806	CG9591	44696
CG9143	46330	CG9247	52612	CG9364	30730	CG9468	15590	CG9593	24165
CG9144	48207	CG9248	41226	CG9373	44658	CG9469	15469	CG9594	13636
CG9144	27006	CG9249	47643	CG9375	28129	CG9471	24042	CG9595	24068
CG9147	29050	CG9250	48548	CG9376	9457	CG9472	8424	CG9596	27025
CG9148	45224	CG9250	36095	CG9377	42837	CG9473	15339	CG9597	36117
CG9150	16877	CG9257	6406	CG9378	28130	CG9474	48033	CG9598	15975
CG9151	47881	CG9258	46542	CG9379	22824	CG9480	35452	CG9601	24070
CG9151	9827	CG9261	2660	CG9381	44662	CG9484	44676	CG9602	29498
CG9153	37221	CG9265	46577	CG9383	23737	CG9485	45809	CG9603	37496
CG9154	29054	CG9267	2879	CG9384	14169	CG9488	29720	CG9606	44699
CG9154	49534	CG9270	29961	CG9386	47755	CG9490	23316	CG9609	30469
CG9155	49345	CG9271	15547	CG9388	24017	CG9491	27015	CG9610	48121
CG9156	29057	CG9272	41018	CG9389	44663	CG9493	40743	CG9611	36120
CG9159	7893	CG9273	30572	CG9391	23723	CG9494	44877	CG9613	5801
CG9160	43503	CG9277	24138	CG9393	44400	CG9495	3795	CG9615	24072
CG9160	39232	CG9279	45052	CG9394	13879	CG9496	2824	CG9619	36121
CG9163	1025	CG9283	15223	CG9398	29110	CG9499	7900	CG9620	42623
CG9163	45927	CG9286	23735	CG9399	13788	CG9501	7903	CG9621	16641
CG9163	30075	CG9288	46191	CG9400	43296	CG9508	24052	CG9623	5600
CG9166	28109	CG9290	14349	CG9401	28132	CG9510	44683	CG9629	44700
CG9169	27008	CG9291	15302	CG9406	48893	CG9512	14809	CG9630	31081
CG9170	29066	CG9294	29092	CG9406	29765	CG9514	37403	CG9633	11210
CG9171	13451	CG9296	29096	CG9410	44669	CG9517	24162	CG9636	28133
CG9171	50541	CG9300	24139	CG9412	29113	CG9518	8328	CG9637	9073
CG9172	23255	CG9302	15544	CG9413	45180	CG9519	16501	CG9638	24076
CG9176	40964	CG9304	11142	CG9414	30479	CG9519	47195	CG9643	24081
CG9177	29070	CG9305	30523	CG9415	15347	CG9520	2826	CG9646	14982
CG9181	37437	CG9306	23088	CG9416	10064	CG9521	16497	CG9648	15351
CG9184	10283	CG9307	23163	CG9418	37665	CG9521	47136	CG9650	23170
CG9187	44366	CG9308	6606	CG9422	30171	CG9522	19861	CG9655	37309
CG9191	52549	CG9310	12692	CG9423	36103	CG9523	1451	CG9657	43922
CG9195	9130	CG9311	14173	CG9426	10843	CG9526	51451	CG9660	24083
CG9198	29072	CG9313	29099	CG9427	15375	CG9527	24054	CG9662	7278
CG9200	36092	CG9314	44647	CG9428	3986	CG9528	44687	CG9662	7278
CG9201	29073	CG9320	24141	CG9429	51272	CG9533	5569	CG9666	45658
CG9203	29075	CG9322	29100	CG9430	7339	CG9536	7907	CG9667	36127
CG9204	28111	CG9323	44984	CG9433	41021	CG9537	29374	CG9668	46919
CG9206	3785	CG9325	29101	CG9436	24026	CG9539	42763	CG9670	24086
CG9207	12616	CG9326	24157	CG9438	37148	CG9540	14807	CG9674	24089
CG9209	44638	CG9328	28125	CG9441	33923	CG9542	45620	CG9677	27032
CG9210	11547	CG9330	44651	CG9443	5843	CG9543	24059	CG9678	23342
CG9211	1001	CG9331	44653	CG9444	43275	CG9548	35453	CG9680	36131
CG9211	29898	CG9333	52551	CG9446	44671	CG9550	43996	CG9682	16549
CG9211	42577	CG9334	49899	CG9448	24030	CG9554	43911	CG9683	22830
CG9212	28116	CG9334	30774	CG9450	24031	CG9556	48044	CG9688	43887
CG9214	42209	CG9339	44655	CG9451	14344	CG9564	45717	CG9695	13005
CG9218	28119	CG9342	15775	CG9452	51202	CG9565	37803	CG9696	7787
CG9219	35750	CG9343	41095	CG9453	47262	CG9569	1820	CG9699	7742
CG9220	29085	CG9344	23689	CG9454	24032	CG9569	1820	CG9701	3358
CG9222	27010	CG9345	16643	CG9455	13263	CG9571	10480	CG9702	6859
CG9224	37407	CG9346	27013	CG9456	37955	CG9573	49820	CG9703	6137
CG9227	40858	CG9347	29108	CG9458	48700	CG9576	47261	CG9705	40665
CG9231	9101	CG9350	30619	CG9459	48905	CG9577	24064	CG9706	49347
CG9232	29087	CG9351	24143	CG9459	5948	CG9578	45082	CG9709	29119
CG9236	39161	CG9353	35447	CG9460	24036	CG9580	50546	CG9712	23944
CG9238	24149	CG9354	49902	CG9461	24039	CG9581	48220	CG9715	36138
CG9240	14833	CG9357	44656	CG9463	48063	CG9581	39208	CG9717	42669

TID= transformant ID, VDRC

CG9722	6679	CG9879	29311	CG9987	36198
CG9723	37412	CG9881	28141	CG9987	36494
CG9725	23945	CG9882	29312	CG9994	36201
CG9726	41347	CG9884	38258	CG9994	43486
CG9727	30575	CG9886	48079	CG9995	29531
CG9728	42895	CG9886	29320	CG9995	36205
CG9730	36139	CG9887	2574	CG9996	36207
CG9732	27035	CG9890	23062	CG9998	24177
CG9734	23483	CG9895	41035	CG9999	30568
CG9735	23951	CG9899	46584		
CG9738	26928	CG9900	24171		
CG9739	44390	CG9901	29944		
CG9741	51061	CG9903	42690		
CG9742	39256	CG9904	45478		
CG9747	1394	CG9906	5597		
CG9748	6299	CG9907	6131		
CG9749	36142	CG9908	7001		
CG9750	19021	CG9910	24175		
CG9752	50282	CG9910	24175		
CG9752	28138	CG9911	46585		
CG9753	1385	CG9913	36459		
CG9755	45815	CG9914	29322		
CG9761	23171	CG9916	41015		
CG9762	11381	CG9920	29326		
CG9764	28674	CG9921	14921		
CG9770	43462	CG9922	35465		
CG9772	15636	CG9924	28798		
CG9774	3793	CG9925	29328		
CG9776	29266	CG9927	29332		
CG9778	11037	CG9930	47793		
CG9779	29275	CG9931	7743		
CG9783	29276	CG9934	36464		
CG9784	30098	CG9936	13777		
CG9786	11775	CG9938	29337		
CG9790	23702	CG9940	40756		
CG9796	36452	CG9941	29902		
CG9799	29280	CG9941	29596		
CG9802	39207	CG9943	48887		
CG9804	46579	CG9943	5081		
CG9805	28140	CG9945	23742		
CG9811	30103	CG9946	7799		
CG9818	29285	CG9949	50178		
CG9819	30105	CG9951	36172		
CG9828	29289	CG9951	29457		
CG9834	29290	CG9952	29903		
CG9836	29295	CG9953	9024		
CG9839	36455	CG9954	12712		
CG9842	46873	CG9958	49822		
CG9847	12863	CG9958	28145		
CG9849	12850	CG9961	36175		
CG9852	48717	CG9968	36185		
CG9854	42283	CG9968	29693		
CG9855	33309	CG9973	36187		
CG9862	29302	CG9973	36584		
CG9865	40701	CG9976	38002		
CG9867	44570	CG9977	49573		
CG9868	45506	CG9977	36193		
CG9870	44215	CG9981	11566		
CG9873	29760	CG9983	29523		
CG9876	10481	CG9984	42217		
CG9878	23705	CG9985	6229		
CG9878	50446	CG9986	46113		

TID= transformant ID, VDRC

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