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IDENTIFICATION AND CHARACTERIZATION OF DEAFNESS GENES IN *DROSOPHILA MELANOGASTER*

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I herewith declare that the Ph.D. thesis entitled “Identification and Characterization of Deafness Genes in *Drosophila melanogaster*” has been written independently and with no other sources and aids than quoted.

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Table of Contents

1 Summary

The antennal auditory organ of *Drosophila*, Johnston's organ (JO), provides a valuable system to study hearing. JO neurons and hair cells of mammals are developmentally specified via homologous *atonal* family genes and share equivalent transduction machineries. In this study, a novel reverse genetics approach is used to establish a catalogue of JO genes by comparing gene expression profiles between *atonal* mutants and controls. 274 candidate genes are identified to be expressed in JO.

Apart from four known auditory relevant genes, we identify novel genes that have not yet been associated with JO or hearing. The genes include three TRP channels, 7 axonemal dynein motors, and chemo- and phototransduction genes such as ionotropic receptors (IRs) and rhodopsins.

The microarray data is validated with quantitative real-time PCR (qPCR) and cluster analysis. Gene expression in JO is further confirmed by *in situ* hybridizations, antibody stainings, and Gal4 enhancer trap lines. 30 new genes are identified whose disruption impairs JO function, doubling the number of auditory relevant *Drosophila* genes. Photo- and chemotransducer such as ionotropic receptors (IRs) and Rhodopsins are confirmed to be expressed in JO and to contribute to JO function.

This extends the genetic parallels between sensory modalities from development to sensory signal processing.

2 Introduction

2.1 Hearing impairment

Hearing is a specialized form of mechanotransduction that mediates the detection of sound. Vibration of air particles produces sound waves that move with a speed of about 330m/sec through the air [Christopher, 2005]. Ears receive these acoustic signals and transduce them into nerve impulses. These impulses are then forwarded to the brain.

Hearing impairment is the most common sensory deficit in humans. In 2005 the World Health Organization estimated that 278 million people worldwide have moderate to profound hearing loss [WHO, 2010]. Hearing deficits can result from a variety of different causes. Two main types of hearing loss can be distinguished: conductive hearing loss, which can be caused by e.g. infections and affects the middle and the outer ear structures [Nadol, 1993], and sensory neural hearing loss, which mostly affects the inner ear and can be caused by loud or long term noise exposure as well as by infections or genetic disorders [Nadol, 1993].

Depending on the phenotype, hereditary hearing loss can be divided into nonsyndromic and syndromic hearing loss [Smith *et al.*, 1999]. Nonsyndromic hearing loss specifically affects only hearing, while syndromic hearing loss also affects functions of other organs. One example for syndromic hearing loss is the Usher syndrome. Besides hearing loss, people with Usher syndrome also suffer from retinal degeneration and blindness [Williams, 2008; Kremer *et al.*, 2006].

In general all genetic disorders affecting hearing can be inherited in autosomal (dominant or recessive) or in x-linked mode. There are also few exceptions that are inherited via the maternal mitochondria [Van Camp & Smith, 2000; Fischel-Ghodsian *et al.*, 1995]. Disruptions of some of these mitochondrial genes are nonsyndromic and only affect hearing [Jacobs, 1997, Fischel-Ghodsian, 1999].

2.2 Genes involved in human hearing impairment

Over the past years at least 96 genes involved in hereditary hearing loss have been identified [Van Camp & Smith, 2010]. Examples are OTOF, which is believed to be the Ca^{2+} sensor maintaining the transmitter release at the hair cell synapse [Adato *et al.*, 2000; Roux *et al.*, 2006], CDH23, which is a component of the hair cell tip-links [Kazmierczak *et al.*, 2007], and SLC26A5 (Prestin), a unconventional motor protein that promotes the electromotility of outer hair cells [Zheng *et al.*, 2000; Homma & Dallos, 2010]. While OTOF, CDH23, and Prestin have been well characterized, the function of most of the 96 auditory-relevant genes remains elusive and auditory key components such as the hair cell transduction channels have not yet been discovered.

Genetic model organisms that are endowed with ears thus and can be used to search for auditory relevant genes include *Drosophila* [Bermingham *et al.*, 1999; Eberl *et al.*, 1997], zebrafish [Ernest *et al.*, 2000; Whitfield, 2002], and mouse [Mikaelian, 1979; Steel & Bock, 1980].

2.3 *Drosophila* as a model organism for the study of hearing

The fruit fly *Drosophila melanogaster* provides a valuable model system to search for auditory relevant genes. When the whole genome of the fruit fly *Drosophila melanogaster* was sequenced [Adam *et al.*, 2000], Reiter *et al.*(2001) found that homologues of 548 fly genes are implicated in 714 human diseases, including 13 genes that are implicated in syndromic and non-syndromic deafness [Reiter *et al.*, 2001].

Drosophila is a well-studied genetic model organism that offers a variety of powerful tools for genetic analysis, including balancer chromosomes [Thompson, 1977] and the well-established UAS-Gal4 system [Fischer *et al.*, 1988; Brand & Perrimon, 1993]. The fly genome is sequenced [Adam *et al.*, 2000], most of its genes and their homologues are known, and many of them are already well-studied. In addition, many mutant and transgenic fly lines have been created and are available at public stock centers. Non-invasive techniques allow to measure the auditory performance in living flies and to access the consequences of genetic defects on hearing [Albert *et al.*, 2006; Albert *et al.*, 2007b].

2.4 *Drosophila* sound communication

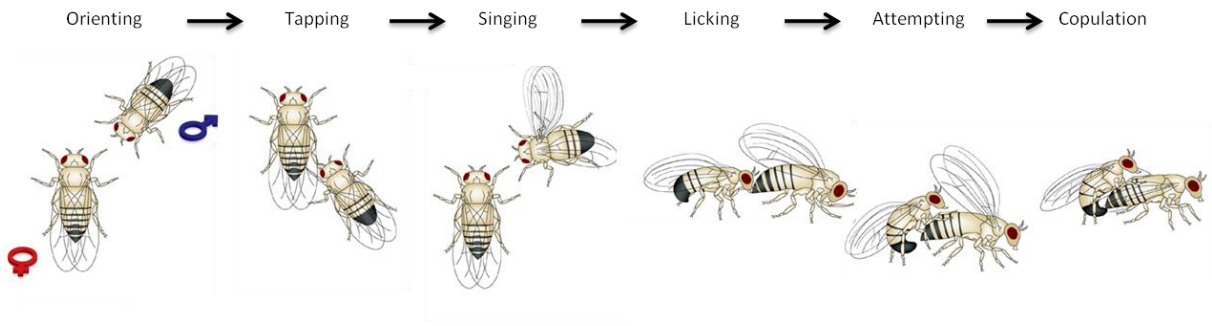


Figure 1 Courtship behavior of *Drosophila melanogaster*. Six steps of mating behavior: a) orienting, b) tapping, c) singing, d) licking, e) attempting copulation, and f) copulation (modified after Sokolowski, 2001)

In addition to visual, olfactory, and gustatory cues, auditory cues are important for *Drosophila* mating behavior [Hall, 1994] (Figure 1). During courtship, male flies produce songs by fanning one of their wings. These songs consist of short sound pulses that are dominated by frequencies around 150-200 Hz and are delivered at a rate of ca 30 pulses per second [Eberl *et al.*, 1997]. Apart from these 'pulse songs', the flies also produce 'sine songs' by continuously vibrating the wings [Ewing *et al.*, 1968; Eberl *et al.*, 1997]. Both song types are believed to have different effects on mating [Schilcher, 1976]. While the sine songs are suggested to prime the female prior to courtship, the pulse songs act as a final trigger and also increase the activity of nearby males [Ewing *et al.*, 1968; Alt *et al.*, 1998; Gleason, 2005].

2.5 The *Drosophila* hearing organ

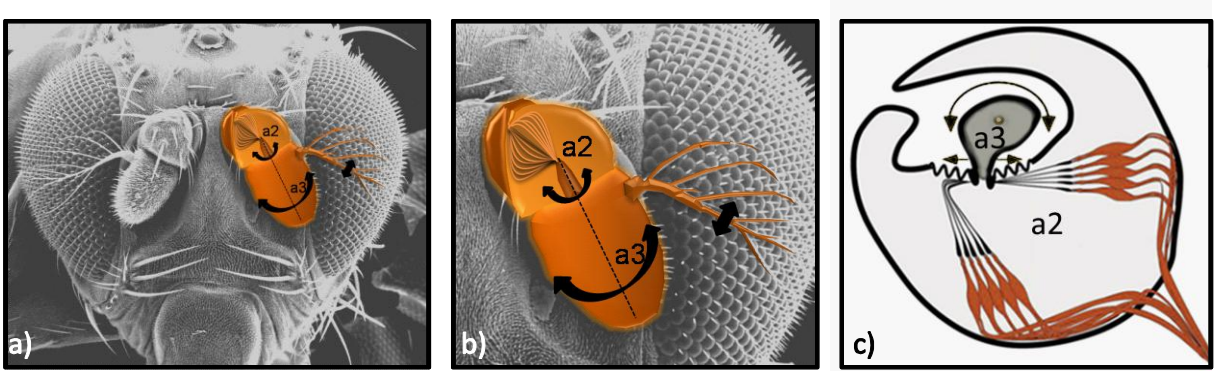


Figure 2 *Drosophila* antenna a) Frontal view of the *Drosophila* head; b) Morphology of the antenna: the third antennal segment (a3) with its feather-like arista is directly connected to the neurons in the second antennal segment (a2). Directions of the movement of the arista and the third antennal segment are indicated by black arrows; c) cross section through the second antennal segment showing the direct connection of the third segment (a3) to the neurons in the second segment (a2).

The *Drosophila* antenna consists of three main parts: the scape (first segment; a1), the pedicel (second segment; a2), and the funiculus (third segment; a3). The third segment and its arista form the sound receiver and vibrate in response to acoustic stimuli [Göpfert & Robert 2001; Göpfert & Robert 2002; Albert *et al.*, 2007a]. These movements are directly coupled to, and picked up by, the primary mechanosensory neurons of Johnston's organ (JO), fly's chordotonal organ (CHO) in the second antennal segment [Eberl & Boeckhoff-Falk, 2007] (Figure 2).

Chordotonal organs are mechanosensory organs found in insects and crustaceans [Field & Matheson, 1998, Yack 2004]. They are stretch receptors that serve proprioception [Smith & Shepherd, 1996; Cheng *et al.*, 2010], gravity and wind detection [Desroches *et al.*, 2010; Yorozu *et al.*, 2009], the detection of substrate vibration [Sauer & Stein, 1999], and hearing [Göpfert *et al.*, 2002; Eberl *et al.*, 2000]. Chordotonal organs are composed of multicellular units called scolopidia each of which consists of one to three neurons, a cap cell, a scolopale cell, and a ligament cell [Field & Matheson, 1998; Elliott *et al.*, 2005] (Figure 3). All these cells that make up one scolopidium are developmentally derived from a single sensory organ precursor cell by lineage [Witt *et al.*, 2010, zur Lage *et al.*, 2004].

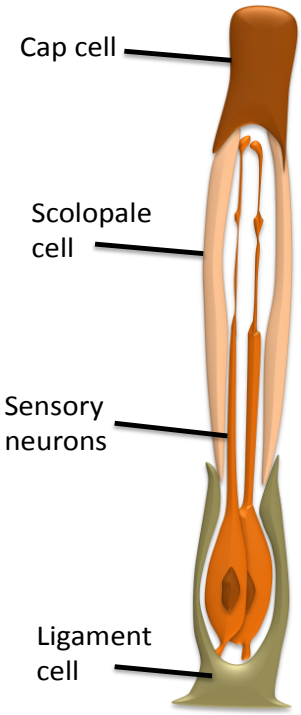


Figure 3 Structure of the scolopidium One to three neurons are encased in three different types of cells: cap cell, scolopale cell, and ligament cell, adapted from Bechstedt *et al.*, 2010.

Chordotonal sensory neurons are monodendritic and bipolar. They have a single distal ciliated dendrite and a proximal axon that conveys information to the CNS [Field & Matheson, 1998]. The scolopale cell connects, in case of JO, the sensory neurons to the cuticle at the third antennal segment [Caldwell & Eberl, 2002]. Proximally, the neurons are supported by the ligament cells [Field & Matheson, 1998]. The antennal chordotonal organ JO consists of about 200 scolopidia and about 480 sensory neurons [Kamikouchi *et al.*, 2006]. These neurons are mediating hearing and the detection of gravity and wind [Kamikouchi *et al.*, 2009; Yorozu *et al.*, 2009].

2.6 *atonal*

At the first glance, the *Drosophila* hearing organ and mammalian ears seem to have little in common. Accumulating evidence, however, suggests that the mechanosensory cells have evolved from a common ancestral cell [Fritsch & Beisel 2004]: The mechanosensory cells of *Drosophila* chordotonal organs are developmentally specified by the proneural gene *atonal* (*ato*) [Jarman *et al.*, 1993]. *atonal* is a member of the basic helix-loop-helix (bHLH) transcription factor family [Murre *et al.*, 1994; Simionato *et al.*, 2008] that is important for the formation of the sense organ precursors (SOPs). SOPs are produced from characteristic groups of ectodermal cells in the embryos and in the imaginal discs [Jarman *et al.*, 1993].

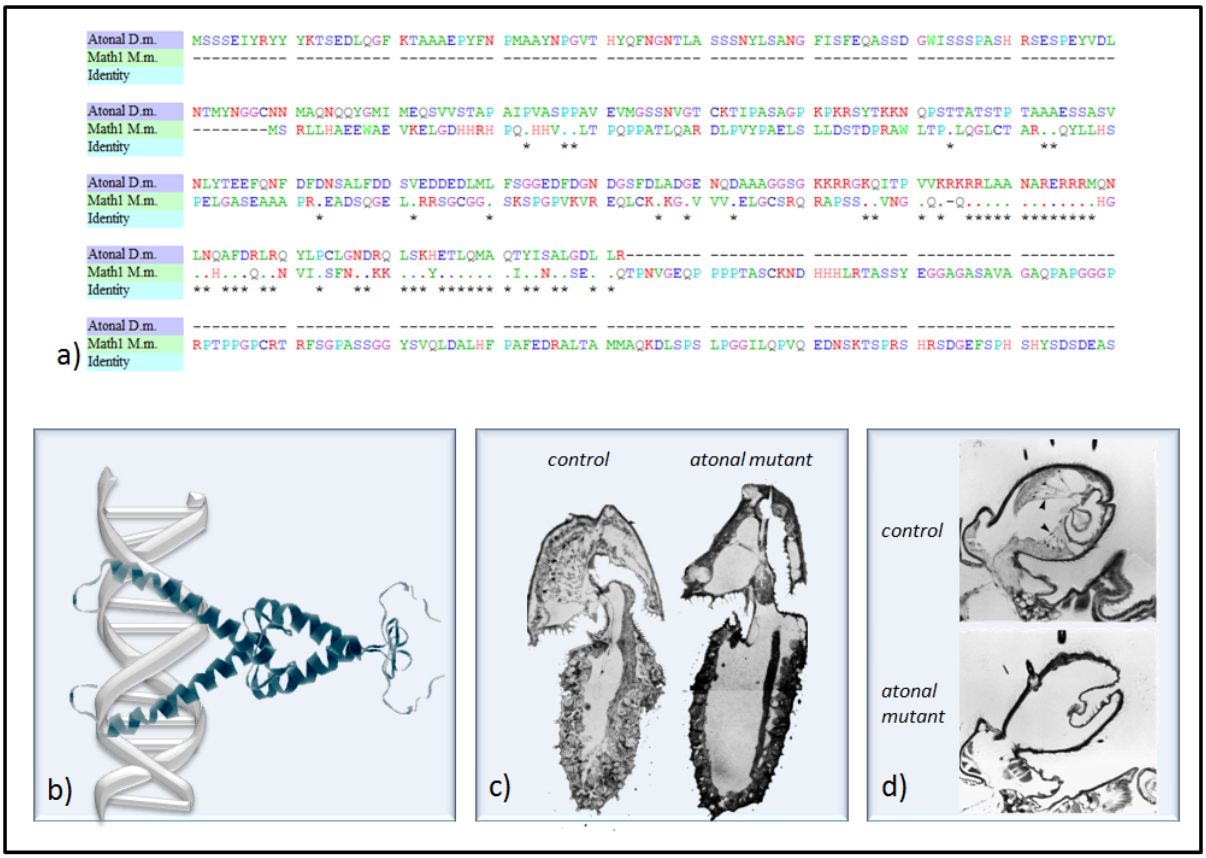


Figure 4 *atonal* a) Alignment of *Drosophila* Atonal and mouse Math1 (Atoh1) protein sequences, calculated with the Smith-Waterman local alignment algorithm using GENtle version 1.9.4., b) Schematic representation of the two Atonal proteins binding to the DNA double-helix with their bHLH-loops; bHLH-loop was modeled with the *Drosophila* Atonal protein sequence using 3Djigsaw and RasWin version 2.7.5; c) longitudinal sections of the antenna of controls and *atonal* mutants (*ato1/Df(3R)p13*) flies. *atonal* mutants lack the complete JO in the second segment and some olfactory neurons in the third segment (from Göpfert *et al.*, 2002); d) cross section of the second antennal segment of controls and *atonal* mutants, *atonal* mutant lack the entire organ and fail to form the connection between the second and third segment (from Jarman *et al.*, 1995).

atonal mutants fail to form JO in their antennae [Jarman *et al.*, 1993; Jarman *et al.*, 1995; Göpfert *et al.*, 2002]. *atonal* is also required to form all other chordotonal organs except the lch5 (lateral chordotonal organ 5) [zur Lage *et al.*, 1997], photoreceptor cells [Jarman *et al.*, 1994; Baker *et al.*, 1996], the sacculus, and some olfactory neurons [Gupta & Rodrigues 1997; Jhaveri *et al.*, 2000]. *atonal* mutants lack all *atonal*-dependent cells. There are two *atonal* homologues in mouse. *Mouse atonal homolog 5 (Math5; Atoh7)* is responsible for eye development [Brown *et al.*, 2001], while *Mouse atonal homolog 1 (Math1; Atoh1)* [Ben-Arie *et al.*, 1997] is essential for hair cell development in the cochlea [Bermingham *et al.*, 1999].

Mice that lack *Math1*, fail to develop hair cells in their ears [Bermingham *et al.*, 1999]. Interestingly, the fly gene can rescue the *Math1* phenotype in the cochlea and vice versa [Hassan *et al.*, 2000; Wang *et al.*, 2002] even though both proteins only share only a small conserved region that spans their bHLH domain (Figure 4).

2.7 Similar molecular machineries

In addition to the developmental similarities, hairs cells and fly JO neurons also share functionally equivalent molecular machineries, for mechanosensory transduction, adaptation, and amplification [Nadrowski *et al.*, 2008; Albert *et al.*, 2007a]. Transduction machineries seem to consist of serially arranged ion channels, and adaptation motors [Howard & Hudspeth, 1988; Nadrowski & Göpfert, 2009] and can be described with the gating-spring model [Albert *et al.*, 2007a; Howard & Hudspeth, 1988]. This functional equivalence of the modules suggests that some components of these machineries may be eventually conserved.

2.8 Genetic Screens

So far, only few genes have been implicated in fly hearing. The majority of these genes has emerged from two behavioral screens. In 1994, Kernan *et al.*, screened 5248 *Drosophila* larvae for mechanosensation defects [Kernan *et al.*, 1994]. They described 28 mutant lines, in which larval touch response was impaired. This screen led to the identification of six auditory-relevant genes including the gene coding for the bonafide mechanotransduction channel NompC [Walker *et al.*, 2000; Kang *et al.*, 2010], which is still the main candidate for the auditory transduction channel [Howard & Bechstedt, 2004; Göpfert *et al.*, 2006; Kamikouchi *et al.*, 2009]. Additional genes

identified in the screen are *nompA*, *nompB*, *tilB*, *unc* and *rempA*. *nompA* codes for a protein containing zona pellucida (ZP) domain, which is necessary for the connection of the mechanosensory neurons [Chung *et al.*, 2001]. *nompB and rempA* are involved in the intraflagellar transport [Han *et al.*, 2003; Lee *et al.*, 2008], while *tilB* [Kavile *et al.*, 2010; Göpfert *et al.*, 2005] and *unc* [Baker *et al.*, 2004] seem to be involved in genesis or maintenance of cilia.

In 1997, Eberl *et al.*screened for genes whose disruption impairs a behavioral response to courtship song. When male flies separated from females are triggered to court by exposing them to courtship song [Eberl *et al.*, 1997], they form characteristic courtship chains [Gailey & Hall, 1989]. Eberl *et al.*(1997) used this behavior to screen for auditory mutants. 15 out of nearly 400 mutant lines studied fail to show this behavior. One of the responsible genes turned out to be *beethoven* (*btv)* [Eberl *et al.*, 2000; Tauber & Eberl, 2001; Göpfert *et al.*, 2005], an additional auditory-relevant gene that encodes a cytoplasmic dynein heavy chain. The other mutations have not yet been linked to genes.

Forward genetic approaches in which mutants are first identified by their phenotypes and then traced down to genes are time consuming, and this is one of the reasons why only few auditory relevant genes have been described. Browsing the *Drosophila* genome for genes implicated in the sensory perception of sound (GO: 0007605) currently yields 30 entries of which only 23 refer to annotated genes [Carbon *et al.*, 2009].

2.9 Reverse genetics

An attractive alternative to forward genetics is reverse genetics, in which candidate genes are identified by expression profiling prior to testing for mutant effects [Cirelli & Tononi 1999; Ostrin *et al.*, 2006]. Such approaches have recently been used to identify genes that are expressed in campaniform mechanoreceptors in the *Drosophila* halteres [Bechstedt *et al.*, 2010] and in hair cells of zebrafish [McDermott *et al.*, 2007]. In both screens gene expressed in different tissues were compared. While Bechstedt *et al.*, compared gene expression between the second and third haltere segments of *Drosophila*, McDermott *et al.*, compared gene expression in zebrafish hair cells and in zebrafish liver cells.

In contrast to the strategies used in Bechstedt *et al.*(2010) and in McDermott *et al.*(2007), here we use a novel genetic approach with *atonal* mutants to establish a catalogue of candidate JO genes.

3 Material and Methods

3.1 Microarray Assay

3.1.1 Total RNA Preparation

To identify genes that are preferentially expressed in the fly's JO, we performed a microarray assay (Affymetrix®, Santa Clara, CA) of the second antennal segment of *atonal* mutants. Microarray analysis was performed using GeneChip® *Drosophila* Genome 2.0 Array (see 3.9.2.1). Flies carrying the ato^1 null allele were tested as $ato^1/Df(3R)p^{13}$ mutants [Jarman *et al.*, 1995] and heterozygous $ato^1/TM3$ and $Df(3R)p^{13}/TM3$ flies were used as control flies. Antennal second segments were isolated from 30-50 *atonal* mutant and control flies, including both males and females. Additionally, brains from 10 control flies ($ato^1/TM3$) were prepared. All tissues were directly homogenized and lysed in RLT buffer (Qiagen RNeasy® Micro Kit 3.9.2.1) containing 1µl freshly added β-Mercaptoethanol (3.9.1.19) per 100µl RLT buffer. Total RNA was extracted using the Qiagen RNeasy® Micro Kit (3.9.2.1). Total RNA was eluted with 14µl RNase free water supplied with the Kit. The quality of total RNA was assessed by comparing the ratios between 18S and 28S RNA with Agilent 2100 Bioanalyzer (3.9.7.1) using the Agilent RNA 6000 Nano Kit (3.9.2.1). The concentration of the total RNA was determined using NanoDrop (3.9.7.16). Three biological replicates with separately prepared tissues were run for each strain and tissue. Details for total RNA preparation are indicated in Table 1:

Table 1 Total RNA extraction

	Used flies	Dissected Tissue	Genotype	Obtained Concentration (ng/µl)	Used Volume (µl)	Used amount (ng)
M1	50	Mutant 2nd Antennal Segment	$ato^1/Df(3R)p^{13}$	22	3	66
M2	50	Mutant 2nd Antennal Segment	$ato^1/Df(3R)p^{13}$	7	3	21
M3	50	Mutant 2nd Antennal Segment	$ato^1/Df(3R)p^{13}$	20	3	60
A1	30	Control 2nd Antennal Segment	$ato^1/TM3$	23	3	69
A2	30	Control 2nd Antennal Segment	$ato^1/TM3$	29	3	87
A3	30	Control 2nd Antennal Segment	$ato^1/TM3$	39	2	78
D1	30	Control 2nd Antennal Segment	$Df(3R)p^{13}/TM3$	24	3	72
D2	30	Control 2nd Antennal Segment	$Df(3R)p^{13}/TM3$	40	2	80
D3	30	Control 2nd Antennal Segment	$Df(3R)p^{13}/TM3$	26	3	78
B1	10	Control Brain	$ato^1/TM3$	43	2	86
B2	10	Control Brain	$ato^1/TM3$	86	1	86
B3	10	Control Brain	$ato^1/TM3$	75	1	75

3.1.2 Two-cycle amplification and hybridization

For amplification and labeling of total RNA, we followed the GeneChip Expression Analysis Technical Manual, Eukaryotic Target Preparation, Two-Cycle protocols (Affymetrix® technical notes: http://media.affymetrix.com/support/downloads/manuals/expression_analysis_technical_manual.pdf). This protocol included mRNA amplification via two cycles of reverse transcription and probe labeling via *in vitro* transcription.

After labeling with biotin, cRNA was hybridized with Affymetrix® microarrays (3.9.2.1) for 16 hours at 45°C following the Affymetrix® protocol. Affymetrix® microarrays contained 14 25-mer oligonucleotides for 18500 probe sets representing different *Drosophila* transcripts. Arrays were scanned with the G2500A GeneArray scanner (3.9.7.10) and quantified following the standard Affymetrix ®protocol.

3.1.3 Data analysis

After scanning the arrays, we imported the raw data into the Gene Profile Analysis Suite (GEPAS, v. 4.0) [Herrero *et al.*, 2003] for statistical analysis. Probe level intensities were processed using background correction ('RMA'), log2-transformation, perfect match (PM) correction, median polish, and quantile normalization prior to differential expression analysis. Genes that are preferentially expressed in JO were identified by separately comparing the respective expression levels between *Df(3R)p*^{13}*/TM3* controls and mutants and *ato*^{1}*/TM3* controls and mutants. Differential expression was assessed using Student's two-sample t-tests. P-values were adjusted using the False Discovery Rate (FDR) control procedure to correct for multiple comparisons with an FDR below 10% [Benjamini & Hochberg 1995; Chuaqui *et al.*, 2002]. Differential expression falling within this cut-off (FDR<0.10) was considered significant.

The Online Tutorial GeneVenn (3.9.11) was used to obtain a consensus list of genes that were expressed in both controls, but not in mutants. The Cluster analysis was performed using the "Expression Profiler: Next Generation" tool v. 1.0. (3.9.11) [Kapushesky *et al.*, 2004].

3.2 Microarray Validation

3.2.1 RNA extraction and cDNA amplification

Total RNA was extracted from *atonal* mutants *($ato^1/Df(3R)p^{13}$)* and control flies *($ato^1/TM3$* and *$Df(3R)p^{13}/TM3$*), 30-50 flies each, transcribed, the second-strand was synthesized, and the cRNA was transcribed as described for the Microarray screen. The enzymes described in 3.9.2.2 were used for these procedures. The transcribed cRNAs were then purified by adding 50µl 8M LiCl (Lithium chloride 3.9.1.17) to the transcription reaction. Following the addition of 400µl 100% ethanol (3.9.1.9), the mixture was incubated overnight at -80°C (3.9.7.6). After pelleting via centrifugation, the cRNAs were washed with 70% ethanol (3.9.4.9). cRNA was then resuspended in 100µl Mol.bio.Water (3.9.3.11) and concentration was determined with NanoDrop (3.9.7.16). The second-cycle, first strand synthesis was performed with 1µg cRNA following the Affymetrix® protocol.

The cDNA mixture was then diluted to 1:100 with Mol.bio.Water prior to quantitative real-time PCR (qPCR). 1µl of the cDNA (~5pg) was used for each qPCR.

3.2.2 Quantitative real-time PCR (qPCR)

5pg of cDNA obtained in the second-cycle, first strand synthesis reaction was mixed with 2x Absolute QPCR SYBR Green ROX Mix (3.9.2.2) and 70nM (70fmol/µl) forward and reverse primers (3.9.9.1). The reaction was carried out in 96-well plates and the plates were vortexed and centrifuged for three minutes at 3000 rpm using the Eppendorf A-2-DWP rotor (3.9.7.2). Probes were amplified using the BIO-RAD MyiQ Single Color Real-Time PCR Detection System (3.9.7.13). Three biological samples have been used for each tissue. Each biological sample was replicated three times; in the end 9 replicates were run for each strain. The PCR protocol is described below.

	Step	Time (min:sec)	Temperature (°C)	
A	Initialization	15:00	95°C	
B.1	Denaturation	0:15	95°C	← 45 repeats
B.2	Annealing	0:30	60°C	
B.3	Elongation	0:30	72°C	
C.1	Denaturation	0:30	95°C	
C.2	Hybridization	0:30	55°C	
C.3	Stepwise Denaturation	0:10	C.2+0.5°C	— 81 repeats
D	Hold	Hold	12°C	

3.2.3 Comparing qPCR Data with Microarray Data

Log_2-fold changes in qPCR were estimated by comparing the threshold cycle C(t) [Pfaffl *et al.*, 2002] values of mutant and control templates. Because of the exponential amplification during the PCR, the difference between the two C(t) values (deltaC(t)) was considered as the log_2 fold change value. For both controls the deltaC(t) was calculated separately.

To calculate the microarray fold-change, fluorescence intensities of all three replicates were averaged first, and the obtained values from controls were divided with those of mutants. For further comparison of the qPCR data, these quotients were converted to log_2-scale.Like in qPCR deltaC(t) value, the microarray fold-changes were calculated separately for both controls.

All 9 Log_2-fold changes obtained through qPCR and Microarray were averaged and the standard deviation was calculated. For Microarray the standard deviations have been calculated using the boot strap method. Therefore fluorescence intensities of all three control replicates were paired with fluorescence intensities of all three mutant replicates, so that 9 quotients were obtained. From these 9 possible quotients the average and the standard deviation were calculated.

3.3 *In situ* hybridization

In situ hybridizations were used to visualize mRNA localization. DIG-labeled complementary (anti-sense) RNA probes were synthesized to bind the mRNA of interest *in situ*. Then, antibodies against DIG, coupled with alkaline phosphatase activity, were applied to the fixed tissue.

3.3.1 cDNA synthesis

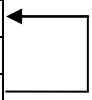
Total RNA was extracted using Qiagen RNeasy® Mini Kit (3.9.2.3). 10 adult flies were directly homogenized in a 1.5ml reaction tube filled with 350µl of RLT Buffer (containing 3.5µl β-Mercaptoethanol 3.9.1.19). Further steps for cDNA transcrition were done as suggested in Qiagen QuantiTect® Reverse Transcription Kit (3.9.2.3) protocol. Following transcription, the cDNA was diluted with 80µl Mol.bio.Water (3.9.3.11).

3.3.2 PCR using BioThermD-™ Taq DNA Polymerase

The obtained cDNA was used to amplify the transcript region of interest via PCR (Polymerase chain reaction). A master mix containing 1µl of the diluted cDNA (3.3.1), 1µl 10mM dNTPs, 2,5µl 10x Reaction buffer, 0.5µl Taq polymerase, and Mol.bio.Water (3.9.2.3) was prepared for all PCR reactions. 5 pmol forward and reverse primers were added separately (3.9.9.2). The total volume of the reaction was 25µl. PCR was performed using the Biometra professional Thermocycler (3.9.7.13).

Protocol for the Polymerase-chain-reaction

	Step	Time (min:sec)	Temperature (°C)
A	Initialization	3:00	95°C
B.1	Denaturation	0:30	95°C
B.2	Annealing	0:30	60°C
B.3	Elongation	0:30	72°C
C	Final elongation	7:00	72°C



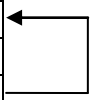
The steps B.1 to B.3 were repeated for 35 times.

3.3.3 PCR using illustra™ PuReTaq™ READY-TO-GO™ PCR beads

READY-TO-GO™ PCR beads (3.9.2.3) already have dNTPs, reaction buffer and Taq polymerases in their beads included. 1µl diluted cDNA (3.3.1), 1µl of each 5 pmol/µl forward and reverse primers (3.9.9.2), and 22µl Mol.bio.Water (3.9.3.11) were added to the ready-to-use beads. The reaction was mixed well until the beads were dissolved. PCR was then performed using the Biometra professional Thermocycler (3.9.7.13).

Protocol for the Polymerase-chain-reaction:

	Step	Time (min:sec)	Temperature (°C)
A	Initialization	5:00	95°C
B.1	Denaturation	0:30	95°C
B.2	Annealing	0:30	55°C
B.3	Elongation	1:00	72°C
C	Final elongation	5:00	72°C



3.3.4 Gel Electrophoresis

Gel electrophoresis with agarose-TBE gels were used to seperate DNA or RNA fragments by length and Ethidium bromide (EtBr) was used to visualize the nucleic acids with UV light. To verify the PCR efficiency, PCR products were loaded on a 1% agarose gel. The sizes of PCR fragments were determined with DNA ladders (3.9.6).To separate fragments bigger than 1kb, 0.5% agarose gels were used. PCR products shorter than 300 base pairs were loaded on 2% agarose gels.

3.3.5 TOPO cloning

The Invitrogen™ TOPO Cloning technique relies on the ability of adenine (A) and thymine (T) to hybridize and to become ligated together in the presence of topoisomerase I or ligase. Since Taq polymerases don't have proof-reading functions and have a nontemplate-dependent terminal transferase activity, they preferentially add an adenine (A) to the 3' end of the PCR product. TOPO vectors were supplied linearized with a single 3'-thimidine (T) overhangs. Depending on the TOPO kit they were supplied with or without Topoisomerase I, which was covalently bound to the vector.

When PCRII-TOPO 4.0 kb -vectors with Topoisomerase I (3.9.2.3) were used, the Invitrogen TA-cloning protocol was followed. When PCRII-TOPO 4.0 kb -vectors without Topoisomerase I (3.9.2.3) were used, the linearized TOPO vector was mixed with Fermentas Fast ligation enzymes (3.9.2.3) and a ligation reaction following Fermentas protocol was performed.

TOPO vectors carry the coding sequence for β -Galactosidase (lacZalpha) in their multiple cloning sites (MCS). When the PCR product was ligated into the vector, the reading frame of LacZalpha would be disrupted resulting in no expression of β-Galactosidase. Since bacterial colonies with intact β-Galactosidase turns blue and without β-Galactosidase stay white, we could perform a so called blue/white-screening to separate bacterial colonies with and without the PCR insert in the MCS.

3.3.6 Chemical Competent Cells

5ml LB Media (3.9.4.15) was inoculated with 1µl of TOP10 bacterial stock (delivered with the TOPO® TA Cloning Dual Promoter Kit, Cat. No. K4600-01; 3.9.2.3). Media was then incubated overnight at 37 °C without shaking. This pre-culture was inoculated into 100 ml of fresh LB Media and incubated at 37 °C with shaking (150 rpm) in the incubator shaker (3.9.7.9). The bacterial growth was monitored in

the Eppendorf photometer (3.9.7.16) using fresh LB Media as reference, until the culture reached an OD600 of 0.4.

The bacterial media was then centrifuged in the Eppendorf centrifuge (3.9.7.2) in pre-cooled 50ml falcon tubes (3.9.8.15) with 2000 g at 4 °C for 20 minutes. The supernatant was discarded and the pellet was briefly centrifuged to remove all residual medium. Pellet was then resuspended in 25 ml TFB1 (3.9.4.29) and incubated for 90min on ice. The solution was then centrifuged in pre-cooled 50ml falcon tubes with 2000 g at 4 °C for 15 min. Supernatant was removed, and a further centrifugation step was performed to remove the remaining media. Pellet was then resuspended in 5ml TFB2 (3.9.4.30). 50µl of the bacterial solution was aliquoted in pre-cooled 1.5ml reaction tubes (3.9.8.15) and stored at -80 °C (3.9.7.6) until usage.

3.3.7 Transformation

Aliquots of 50µl chemical competent cells (E. coli TOP10, Invitrogen, 3.3.6) were thawed on ice. 5µl of the ligation reaction (3.3.5) was added to the bacteria and incubated on ice for 20 minutes. The cells were heat-shocked at 42°C in the neoBlock II thermoblock (3.9.7.22) for 60 seconds. During this step, after ca. 30 sec., the cells were vortexed (3.9.7.26) for 1-2 seconds for effective cell wall disruption. After heat-shock, 200µl LB media (3.9.4.15) was added to the cells at room temperature. The bacterial cells were then incubated without shaking for one hour at 37°C in the Hereaus incubator (3.9.7.9). 20µl X-Gal-Solution (3.9.4.31) was added to the bacterial culture and the culture was immediately plated in LB+Amp agar plates (3.9.4.14) and incubated overnight at 37°C in the Hereaus incubator. Following successful transformation, blue and white bacterial colonies were observed on the plates next day.

3.3.8 Colony PCR

To test whether molecular cloning worked (3.3.5;3.3.7), 3 to 5 individual white bacterial colonies were picked (blue/white screening 3.3.5) with a freshly autoclaved pipette tip (3.9.8.12) and the whole pipette tip was put into 1ml LB+Amp Media (3.9.4.16). After one-hour incubation at 37°C on the incubator shaker (3.9.7.9), 5µl of each bacterial solution was separately collected in a 0.2ml reaction tube (3.9.8.15). To kill the bacteria and to disturb their cell wall, tubes were incubated for 5 minutes in an ultrasonic bath (3.9.7.24) and heated for three minutes at 95°C in the Biometra

professional Thermocycler (3.9.7.22). Taq polymerases (BioTherm™), dNTPs, 10x Reaction buffer (GeneCraft) and primer sets used for the initial PCR (3.3.2) were added to the lysed bacteria. The colony PCR was run with the same initial PCR conditions (3.3.2) in a total volume of 25µl. To detect whether the bacterial colonies have the correct insert, PCR products were loaded on a 1% Agarose gel (3.3.4). Since the same primers (3.9.9.2) were used as in the RT-PCR, PCR only produced bands, when the insert was correctly ligated into the TOPO vector. When the vector circularized itself or when a different product was ligated into the vector, PCR didn't produce any product.

3.3.9 Mini Preparation

When colony PCR itself didn't work, especially for the inserted fragments longer than 1kb, a Mini Preparation (3.3.9) was performed to purify the transformed vector before proceeding colony PCR or restriction digestion with EcoRI enzymes.

Like in Colony PCR, one white clone was picked and grown in 5ml LB+Amp Media (3.9.4.16) and incubated overnight in the incubator shaker (3.9.7.9). On the next day, 2ml of the bacterial overnight culture were taken into a 2ml reaction tube (3.9.8.15), and the culture was centrifuged in the Heraeus Fresco21 centrifuge (3.9.7.2) for two minutes at 14 800 rpm to pellet the bacteria. The supernatant was almost completely removed. The pellet was then resuspended in the small amount of that remained media. The Invitek Invisorb®Spin Plasmid Mini Two Kit (3.9.2.3) and its protocol were used for further steps with the exception, that the Plasmid DNA was eluted with 50µl Mol.bio.Water (3.9.3.11).

3.3.10 Midi Preparation

To amplify and purify large quantity of vector, Midi Preparation was performed. Any colony that showed a PCR product with the expected size (3.3.8) was chosen and amplified via overnight culture. Therefore 100µl of the bacterial culture was added to 100ml autoclaved Erlenmeyer flask containing 55ml LB+Amp Media and incubated overnight at 37°C in the incubator shaker (3.9.7.9) at 220rpm.

For the Midi preparation, the SIGMA-Aldrich Gen-Elute™ Plasmid Midiprep Kit (3.9.2.3) was used. The whole procedure was done following the Sigma Protocol with few modifications mentioned below. 50ml of bacterial overnight culture was used for the whole preparation. They were filled into a 50ml Falcon tube (3.9.8.15) and centrifuged in the Eppendorf centrifuge (3.9.7.2) with Eppendorf

fixed angle rotor (3.9.7.2) at 7000 rpm for 5 minutes. After removing most of the supernatant, the pellet was resuspended in the remaining media before adding the Resuspension solution of the SIGMA-Aldrich Gen-Elute™ Plasmid Midiprep Kit. The Neutralization solution was kept at 4°C prior to adding to the lysed bacterial cells. The cell debris was centrifuged in the fixed angle rotor (3.9.7.2) at 10000rpm for 10 minutes. All the following centrifugation steps were performed with the Eppendorf swing-bucket rotor (3.9.7.2) and the final elution was done with 1ml 0.05mM Tris pH 8.0 (3.9.4.23) 700µl icecold Isopropanol (3.9.1.16; stored in -20°C) and 100µl 3M sodium acetate pH 5.2 (3.9.4.25) were added to the eluate and incubated for 1 hour at -20°C (3.9.7.5). The samples were centrifuged at 14.800rpm in the Hereaus Fresco21 (3.9.7.2) for 30min at 4°C and the supernatant was removed. DNA pellet was washed with 70% ethanol (3.9.4.9) and centrifuged at 14.800rpm for 10 minutes at 4°C. The remaining supernatant was removed and the DNA pellets were dried at 37°C for 10 minutes until all ethanol was evaporated. The pellet was resuspended in 100µl Mol.bio.Water (3.9.3.11). The DNA concentration was measured with the NanoDrop (3.9.7.16).

3.3.11 Sequencing

The inserts were verified by sequencing for the fidelity and the direction of insertion. Since TOPO vectors are linear and have in both sides 3'T overhang, PCR product can be ligated in both directions. Sequencing was done by the MPI-Sequencing Facility in Hermann-Rein-Str. 3, 37075 Goettingen, Germany. For that 1.6 µg Plasmid DNA was needed in a total volume of 16µl. SP6-Sequencing Primer (3.9.9.3) was used for the sequencing reaction.

3.3.12 Restriction digestion

Since TOPO vector has no defined termination signal for transcription so that the reaction would proceed to the end of the DNA template reducing the total yield for the desired region, TOPO vectors (3.3.5) with the insert of interest were linearized by restriction digestion. Linearization of the vector ensured that RNA transcripts of a defined length and sequence were generated. The restriction site had to be unique and did not affect the insert and the promoter region.

The PCRII-TOPO 4.0 kb plasmid with the ligated PCR product is constructed in a way that the restriction enzymes BamHI, KpnI, XbaI and XhoI cut the construct only once. BamHI and KpnI are slightly downstream of the Sp6 promoter; XhoI and XbaI restriction sides are slightly downstream of

the T7 promoter. When using the Sp6 Polymerase, the vector was digested by XhoI or XbaI and in case of T7 polymerase vector was digested with BamHI or KpnI. Restriction digestions of ca. 10µg plasmid DNA were carried out using Fermentas FastDigest® Enzymes (3.9.2.3) in a total volume of 20µl for 30 minutes at 37°C in the Hereaus incubator (3.9.7.9). Regardless of the ligation direction, restriction reactions were carried out for T7 and SP6 polymerase IVT reactions (3.3.13), since both sense and antisense probes were needed for the *in situ* hybridization. The digested plasmid DNA was purified following the Invitek MSB® Spin PCRapace (3.9.2.3) protocol.

3.3.13 IVT (*in vitro* transcription)

In situ probes were generated by *in vitro* transcription. Fermentas SP6 and T7 RNA polymerases (3.9.2.3) were added to 1µg digested and purified Plasmid DNA from 3.3.12. Transcription buffer, RNase Inhibitors and Roche DIG RNA Labeling Mix containing DIG-labeled nucleotides (3.9.2.3) were added to the IVT reaction and incubated in the Hereaus Incubator (3.9.7.9) at 37°C for one hour. To purify the probe, 50µl 8M LiCl (Lithium chloride 3.9.1.17) and 400µl 100% ethanol were added to the transcription reaction, and incubated overnight at -80°C (3.9.7.6). On the following day ethanol precipitation was performed by pelleting the probe (as written in 3.2.1). The pellet was resuspended in 100µl Mol.bio.Water (3.9.3.11).

3.3.14 Fixation of Antennae

In situ hybridizations were performed in longitudinal section of frontal *Drosophila* antennae. w^{1118} flies were anesthetised in cold (-20°C, for three minutes 3.9.7.5). Flies' heads were removed from their bodies and treated with the fixation solution (3.9.4.11), which was prepared by mixing 900µl 1% PBT (3.9.4.21) with 100µl 37% formaldehyde (3.9.1.11) in a 1.5ml reaction tube (3.9.8.15). After one hour fixation on the neoLab rotator (3.9.7.18), the fixation solution was removed and the heads were washed with 1ml methanol (3.9.1.20) for 5 minutes. After removing methanol, 1ml PBS (3.9.4.20) was added to the samples. Complete removal of methanol was ensured by repeating this step 3-5 times.

3.3.15 Vibratome Sections

Albumin-gelatine (3.9.4.1) was heated in a water-bath (a 300ml beaker filled with water on the Medite Stretching Table 3.9.7.22) to 45°C. Silicon moulds (3.9.8.16) for the embedding were also preheated on the Medite Stretching Table. Albumin-gelatine was carefully poured into the moulds avoiding formation of air bubbles. 4 to 5 heads were put inside albumin gelatine, in a way that the antennae were placed frontally. The moulds were then chilled for 5 minutes at 4°C. Moulds were then post-fixed for two to three days in 6% paraformaldehyde (3.9.4.18) at 4°C, followed by 5 minutes in methanol (3.9.1.20) at room temperature. 30µm sections, made using Leica vibratome (3.9.7.25), were used for *in situ* hybridizations. Sections were stored in PBS at 4°C until use.

3.3.16 *In situ* hybridization in antennal sections

Sections were placed in a 24-well plate (3.9.8.1) containing suitable net-baskets filled with 0.3% PBT. Net-baskets were made of a polyamide fabric with a mesh size of 0.1mm (3.9.8.14). The fabric was attached to a 1.5 cm chopped piece of a 10ml syringe (3.9.8.17) at 200 °C on a hot plate covered with an aluminium foil (3.9.8.3). To remove fixation solution (3.9.4.11) and methanol (3.9.1.20), sections in the net-baskets were washed 5 times for 5 minutes with 0.3% PBT (3.9.4.21) on the shaker (3.9.7.20). Sections were then prehybridized with 500µl HybI (3.9.4.13) for one hour at 65°C in the Hereaus incubator (3.9.7.9). For each mRNA to be detected, two reactions, for both anti-sense and sense (negative control), were run. First, sense and anti-sense RNA-probes were diluted with HybI (3.9.4.13) to get a concentration of about 1ng/µl. Probes were then heated for 10 minutes at 80°C on the neoBlock II (3.9.7.22) and cooled for 1 minute at room temperature prior to adding them to the sections. Probes were hybridized overnight in the Hereaus incubator at 65°C with the tissue mRNA. To avoid evaporation overnight, the 24-well plate was closed with its lid and the edge was sealed with Parafilm (3.9.8.9). The whole plate was then covered with an aluminium foil (3.9.8.3).

On the next day the hybridization mixture was removed and the net baskets containing the sections were washed with 500µl HybI (3.9.4.13) for 20 minutes at 65°C in the Hereaus incubator. Later they were washed three times for 20 minutes with preheated 0.3% PBT at 65°C. Blocking solution was prepared by diluting 10x Blocking solution (3.9.4.5) in 1% PBT (1ml 10x Blocking solution in 9ml 1% PBT). Sections were incubated with Blocking solution for 1 hour at room temperature on the shaker (3.9.7.20). Roche anti-DIG-AP antibodies (3.9.5.1) were diluted to 1:500 with the remaining Blocking

solution and added to the sections in the net-basket. Sections were first incubated with antibodies on the shaker for 1 hour at room temperature. Later the incubation was continued overnight at 4°C.

On the next day antibodies were removed by washing 5 times with 0.3% PBT. Since alkaline phosphatase only works in alkaline buffers, the sections were washed twice with AP-buffer (3.9.4.3) and then removed from the net-baskets. 200µl BCIP/NBT solution (3.9.2.3) was added to the sections and the staining was monitored in the Olympus SZ61 microscope (3.9.7.11). The reaction was stopped by adding 0.3% PBT buffer. Both anti-sense and sense probe reactions were stopped simultaneously. To remove remaining BCIP and NBT, sections were washed twice with 70% Ethanol (3.9.4.9) for 20 minutes. Sections were incubated for one hour in a glycerol PBS mixture (1:1) and mounted on a glass slide (3.9.8.6).

3.3.17 Collection and Fixation of *Drosophila* embryos

Approximately 100 w^{1118} flies were anesthetised using CO_2, transferred into an apple juice-agar urinary cup (3.9.8.19) which was covered with a fabric net (3.9.8.14) and kept at 25°C for 6 hours. Then the flies were removed and embryos were kept at 25°C for another 16 hours to get 13-16 stage embryos. These are the stages when the anlagen of chordotonal organs are visible.

Embryos were carefully removed with a fine painting brush (3.9.8.8) wetted with deionised tab water and transferred into an egg basket. Egg baskets were made of polyamide fabric (3.9.8.14) that was attached to the chopped piece (3 cm length) from a 50ml falcon tube including its thread (3.9.8.15) and its falcon cap. The middle of the falcon cap was cut out, so that it had a hole of 2.4cm diameter, and could act as a sieve, so that the embryos didn't fall out. To remove the embryonic chorion, the egg basket was placed into a Petri dish with 100% Klorix (3.9.3.7) for two minutes. It was then rinsed thoroughly in deionised tab water for a minute. Embryos were then dried for about 5 minutes in room temperature. Using the painting brush, the dechorionated embryos were transferred to the Fixation solution (3.9.4.10) and incubated for 15 minutes on the rotator (3.9.7.18). The aqueous phase (lower phase) was removed with a Pasteur pipette (3.9.8.10). To devitellinize the embryos, 1ml methanol (3.9.1.20) was added to the mixture and vortexed for 30 seconds. Devitellinized embryos sunk to the bottom of the reaction tube. Methanol was then removed and embryos were then washed 4 times again with additional methanol to remove Fixation solution completely before storage at –20°C.

3.3.18 *In situ* hybridization in *Drosophila* embryos

The embryos obtained from the previous step (3.3.17) were washed 5 times for 5 minutes with 0.3% PBT (3.9.4.21), then once with equal amounts of 0.3% PBT and HybI (3.9.4.13) and finally once only with HybI. The embryos were then prehybridised with HybI for one hour at 65°C in order to prevent unspecific RNA binding of the probe. Afterwards, the embryos were equally distributed into two new 1.5ml reaction tubes (3.9.8.15). 400ng RNA probes (sense or antisense, 3.3.13) were diluted with 200µl HybI buffer and they were heated for 10 minutes at 80°C on the Thermoblock (3.9.7.22) to remove secondary structures. After cooling at room temperature, the probe solution was applied to the embryos overnight at 65°C in the Heraus Incubator (3.9.7.9) to hybridise with the embryonal mRNA.

Next day, the embryos were washed for 20 minutes in 500µl HybI, then for 20 minutes in a mixture of 250µl HybI and 250µl PBT (0.3%) and 5 times in 500µl PBT (0.3%), all at 65°C. The embryos were then blocked in Blocking solution (3.9.4.5) for one hour at room temperature on the neoLab rotator (3.9.7.18). Afterwards, the embryos were incubated with the primary antibodies (anti-DIG alkaline phosphatase, 3.9.5.1) in PBT with 10% Blocking Solution for two hours at room temperature on a rotator. Antibodies were then removed by washing 5 times 5 minutes with 0.3% PBT. Since alkaline phosphatases only work in alkaline buffers, the embryos were washed twice with AP-buffer (3.9.4.3) before adding 200µl BCIP/NBT solution (3.9.2.3). Staining by the BCIP/NBT reaction was monitored under the Leica SZ51 microscope (3.9.7.11). The reaction was stopped by adding and washing with 0.3% PBT buffer. Both anti-sense and sense probe reactions were stopped at the same time. To remove BCIP and NBT completely, sections were washed twice with 70% ethanol (3.9.4.9) for 20 minutes. Embryos were mounted on a microscopes slide (3.9.8.6) with glycerol/PBS (3.9.4.12).

3.3.19 Light Microscopy

In situ hybridizations of sections and embryos have been analysed with a Zeiss fluorescence microscope (3.9.7.11) equipped with a Spot CCD camera (Intas, Göttingen, Germany or Invisitron, Sterling Heights, USA). Figures have been arranged using Adobe Photoshop CS3.

3.4 GAL4-lines

3.4.1 Long Range PCR

To make Gal4 fusion constructs of the gene of interest, its 5' upstream region was amplified using its genomic region. Genomic DNA was extracted from 20 w^{1118} flies using the Qiagen DNeasy® Blood & Tissue Kit (3.9.2.4) following the Purification of total DNA from insects protocol (http://www.qiagen.com/literature/render.aspx?id=528). 1µl RNase A (from Sigma GenElute™ Plasmid Midiprep Kit 3.9.2.3) with 180µl ALT buffer was incubated for 20 minutes before adding 20µl Proteinase K (included in the kit). Flies were disrupted in the ALT buffer, RNase A, and Proteinase K mixture on the Eppendorf Thermoshaker (3.9.7.23) overnight at 56°C, 1400 rpm. All washing steps were performed as described in the DNA from insects protocol. In the end 100 µl Mol.bio.Water was used for the elution of the genomic DNA. Amplification of the region of interest was performed using the Fermentas Long PCR Enzymes (3.9.2.4) following the protocol as described below:

	Step	Time (min:sec)	Temperature (°C)	
A	Initialization	3:00	94°C	
B.1	Denaturation	0:20	95°C	
B.2	Annealing	0:30	60°C	10 repeats
B.3	Elongation	1 min / kb	68°C	
C.1	Denaturation	0:20	95°C	
C.2	Annealing	0:30	60°C	25 repeats
C.3	Elongation	(1min /kb)+10 sec	68°C	
D	Final Elongation	10:00	68°C	

3.4.2 Molecular Cloning

1µl of the PCR product was loaded on a 0.5% agarose gel (3.3.4) to prove the PCR efficiency prior to purification of the PCR products using the Invitek® MSB®Spin PCRapace Kit (3.9.2.4). 14µl of the purified PCR product was used for the restriction digestion. 2µl of each of the needed (see table 3.9.9.4) Fermentas FastDigest® enzymes (3.9.2.4) and 2µl of the 10X Reaction buffer was added to the PCR product. Restriction digestion was carried out for 1 hour at 37°C in the Hereaus Incubator (3.9.7.9).

In parallel 2µg of the pPTGAL vector [Sharma *et al.*, 2002] was also digested using the same restriction enzymes as for the PCR product. The linear vector was then purified with Invitek®

MSB®Spin PCRapace Kit and dephosphorylated using Roche Rapid DNA Dephos & Ligation Kit (3.9.2.4). The vector was then ligated with the PCR product following the Roche DNA Dephos & Ligation Protocol. Chemical transformation and plating the bacteria in LB+Amp agar plates (3.9.4.14) were done as described in 3.3.7. X-Gal was not added to the LB+Amp agar plates, since pPTGAL has no β -Galactosidase activity.

3.4.3 Mini Prep & Restriction Digestion

To check whether molecular cloning (3.4.2) worked we performed Mini Preparation of bacterial colonies using Invitek Invisorb®Spin Plasmid Mini Two Kit as described in 3.3.9. An additional restriction digestion using the same restriction enzymes as for the molecular cloning followed by an agarose gel electrophoresis, confirmed the ligation of the DNA fragment of interest into the pPTGAL vector. One colony showing a restriction product of the expected size was then amplified and Midi Prep was performed with the Sigma GenElute™ Plasmid Midiprep Kit (3.9.2.4) to extract the constructed vector.

3.4.4 Sequencing

Sequencing was performed as described in3.3.11. pPTGAL sequencing primers (3.9.9.3) and 5' gene specific primers used for the initial PCR (3.9.9.4) were used for the sequencing.

3.4.5 TheBestGene

About 50 µg of pPTGAL-construct (in a concentration of 1 µg/µl) was sent to TheBestGene (www.thebestgene.com) company for injection into w^{1118} fly embryos. We used the Plan C service, so called "P-element Premium". This service included injection of about 200 fly embryos, the generation of individual stable transformants, determining the inserted chromosome, and balancing with balancer chromosome.

3.4.6 Crossing

For each Gal4 contruct we received up to eight different insertion strains. All of them were crossed with flies carrying an UAS-reporter gene construct. Therefore, male flies carrying the Gal4 construct were crossed to virgin female flies carrying an UAS-2xEGFP (Bloomington: 6658) or UAS-2xEYFP (Bloomington: 6661) construct. When the first offspring hatched, their heads were removed and placed to a microscope slide with 1 hollow filled with Glycerol (3.9.1.13) in a way that the heads were frontal sided. Since heads were not fixed and no additional antibody staining was performed, monitoring the GFP or YFP expression under the confocal microscopy had to be done immediately.

3.4.7 Confocal Microscopy

GFP and YFP expressions and fluorescence antibody stainings have been monitored with the laser scanning confocal microscope (3.9.7.11). All figures were generated using ImageJ and arranged with Adobe Photoshop CS3 (3.9.11).

3.5 Fly pushing

Flies (3.9.10) were grown at 18°C in a 12h/12h light-dark rhythm. 20 to 50 flies per stock were grown in a 28ml cylindrical vial with a diameter of 26 mm that were covered with mites-free plugs (3.9.8.7). The mutant fly lines were kept in two different vials to ensure stock security as well to have access to different developmental stages. The vials were filled to its ¼th with fly food (3.5.1). When rapid breeding of stocks was needed, vials were kept at room temperature.

3.5.1 Fly Food

For 10 liter fly food 102 g of Agar was soaked overnight in 5 liters of tap water. On the following day, 100 g soy bean flour, 180 g yeast was mixed with one liter tap water, and 800 g cornmeal was dissolved in two liters of tap water. 220 g treacle was mixed with one liter tab water. All ingredients were mixed and boiled at 100 °C in the Varioklav® Steampot DT44580604. The temperature was then lowered to 55 °C and 800g Malzin dissolved in one liter tab water, 62ml propionic acid (anti-biotic), and 150g Nipagin (fungicide) diluted in 80ml ethanol were added to the mixture. With an Isomatic®

MCP pump the warm liquid food was filled into the vials. After cooling the fly food overnight at room temperature, the vials were closed with mite-free plugs and stored up to 4 weeks at 4°C.

3.6 Mechanical measurements

Mechanical measurements were done to verify the hearing ability of mutant flies by David Piepenbrock. The complete procedure is described in Albert *et al.*, 2006 and Albert *et al.*, 2007b and in his Diploma thesis ("Identifying and Characterizing Genetic Hearing Defects in *Drosophila melanogaster*", December 2009, University of Cologne).

3.7 Mutant qPCR

Since most of the mutants, used for mechanical measurements, carried a P-element insertion, we tested whether these insertions affect their gene expression. Therefore we compared mRNA transcript levels in mutant and in control flies (w^{1118}). W^{1118} was used as control strain, since most of the mutant strains have a w^{1118} background.

3.7.1 RNA extraction & cDNA synthesis

Second antennal segments of 5 flies per strain (mutants and w^{1118} controls) were dissected and lysed in Qiagen RLT buffer containing β-Mercaptoethanol (included in Qiagen RNeasy® Micro Kit). Qiagen RNeasy® Micro Kit (3.9.2.5) was used for total RNA extraction as describes in 3.1.1 RNA was eluted with 14µl RNase free water (Qiagen RNeasy® Micro Kit). Since the amount of extracted total RNA was smaller than 1ng, the concentration was not determined via NanoDrop**.** 12µl of the eluate was directly used for the cDNA transcription with Qiagen QuantiTect® Rev. Transcription Kit (as described in 3.3.1).

3.7.2 qPCR

cDNA from the former reaction was diluted with 80µl Mol.bio.Water (3.9.3.11) before proceeding to qPCR. qPCR was performed as described in 3.2.2. Since the cDNA amount was not determined prior to qPCR, 4 different control genes (3.9.9.6) that should be expressed in all cells in the same manner

were used to calculate the expression changes in mutant and control flies. The calculation was done with the delta-delta-Ct-method as described in [Schmittgen & Lilak, 2008]. 5 technical replicates were produced to estimate the average.

3.8 Antibody staining

3.8.1 Single staining

Antennal sections were fixed and prepared as described in the steps 3.3.14, 0. The blocks were post-fixed overnight and no more than 2-3 days as described in 3.3.14. Sections were washed three times for 10 minutes with 0.3% PBT (3.9.4.21) and blocked with 1% blocking solution (3.9.4.6) for two hours at room temperature. Primary antibodies were diluted in the blocking solution as described in 3.9.5 and added to the sections overnight at 4℃. Then the primary antibody solution was removed and the sections were washed 5 times with 0.3% PBT prior to adding secondary antibodies. Dilutions of antibodies are described in 3.9.5. Secondary antibodies were removed after two hours and the sections were washed 5 times with 0.3% PBT and incubated in glycerol/PBS (3.9.4.12) for at least one hour. The sections were then mounted on a microscope slide (3.9.8.6).

3.8.2 Double staining

When two different antibodies were used to perform a double-staining, both primary antibodies were added simultaneously and both secondary antibodies were added simultaneously.

3.8.3 Propium idodide to stain the nucleus

When Propium iodide staining was performed additionally to stain the nucleus, Propium iodide solution (3.9.4.22) was added to the sections for 5 to 10 minutes and removed by washing 5 times with 0.3% PBT (3.9.4.21).

3.9 Materials

3.9.1 Chemicals

3.9.1.1 Agarose ($C_{12}H_{18}O_9$; M=290.27g/mol)
AppliChem: Agarose low EEO – Agarose Standard (Cat.No. A21114,0500)

3.9.1.2 Ampicillin (C16H18N3O4S; M= 349,41g/mol)
Roche: Ampicillin, Na-Salz (Cat.No. 835242)

3.9.1.3 Boric acid (BH_3O_3; M=61.83g/mol)
AppliChem: Boric Acid – Molecular biology grade (Cat.No. A2940,1000)

3.9.1.4 Calcium chloride dihydrate ($CaCl_2$*$2H_2O$; M=147.02g/mol)
Calcium chloride dihydrate – Molecular biology grade (Cat.No. A4689,1000)

3.9.1.5 DEPC ($C_6H_{10}O_5$; M=162.14g/mol)
AppliChem: DEPC – BioChemica (Cat.No. A0881,0050)

3.9.1.6 Dimethylformamide (DMF) (C_3H_7NO; M= 73.09 g/mol)
Sigma: N,N-Dimethylformamide, for molecular biology, minimum 99%
(Cat.No. D4551-250ML)

3.9.1.7 EDTA ($C_{10}H_{14}N_2Na_2O_8$*$2H_2O$; M=372.24g/mol)
AppliChem: EDTA disodium salt dihydrate – Molecular biology grade (Cat.No. A2937,1000)

3.9.1.8 Ethanol (C2H6O; M=46.07g/mol)
Sigma-Aldrich®: Ethanol absolute - puriss. p.a. (Cat.No. 32205)

3.9.1.9 Ethanol (C2H6O; M=46.07g/mol)
J.T.Baker: Ethanol absolute – 99.9%/vol (Cat.No. 8006)

3.9.1.10 Ethidium bromide ($C_{21}H_{20}BrN_3$; M= 394.29g/mol)
AppliChem: Ethidium bromide solution 1% - BioChemica (Cat.No. A1152,0100)

3.9.1.11 Formaldehyde (CH_2O; M=30.03 g/mol)
Merck: Formaldehyde solution min. 37% - free from acid (Cat.No. 1.03999.1000)

3.9.1.12 Formamide(CH_3NO; M=45.04g/mol)
Fluka® Analytical: Formamide – Ultra for molecular biology (Cat.No. 47671)

3.9.1.13 Glycerol ($C_3H_8O_3$; M=92.09g/mol)
Th.Geyer Chem$^{solute®}$: Glycerol anhydrous puriss. –Min. 99.0% (Cat.No. 2039.1000)

3.9.1.14 Heptane (C_7H_{16}; M= 100.21 g/mol)
Sigma®: Heptane, minimum 99%, capillary GC (Cat.No. H9629-1L)

3.9.1.15 Hydrochlorid acid (HCl; M= 36.46g/mol)
Applichem: Hydrochlorid acid (1M) – Molecular biology grade (Cat.No. A6578,0500)

3.9.1.16 Isopropanol (C_3H_8O; M=60.1g/mol)
AppliChem: 2-Propanol – Molecular biology grade (Cat.No. A3928,0500GL)

3.9.1.17 Lithium chloride (LiCl; M=42,39g/mol)
Sigma® Life Science: Lithium chloride solution, 8M – Molecular Biology
(Cat.No. L7026-100ML)

3.9.1.18 Magnesium chloride ($MgCl_2*6H_2O$; M=203.3g/mol)
AppliChem: Magnesium chloride hexahydrate – Molecular biology grade
(Cat. No: A4425,0250)

3.9.1.19 Mercaptoethanol (C_2H_6OS; M=78.13g/mol)
AppliChem: β-Mercaptoethanol – Molecular biology grade (Cat.No. A1108,025)

3.9.1.20 Methanol (CH_3OH; M=32.04g/mol)
J.T.Baker: Methanol – HPLC Gradient Grade (Cat.No. 8402)

3.9.1.21 MOPS ($C_7H_{15}NO_4S$; M=209.27g/mol)
AppliChem: MOPS – Molecular biology grade (Cat.No.: A2947,0100)

3.9.1.22 Nipagin ($C_8H_8O_3$; M=152.15g/mol)
Sigma-Aldrich®: Methyl 4-hydroxybenzoate - puriss., ≥99.0% (GC) (Cat.No:54750)

3.9.1.23 Paraformaldehyde ($(CH_2O)_n$; M= 30.03g/mol)
Merck: Paraformaldehyde (Cat.No.: 104051000)

3.9.1.24 Propium iodide ($C_{27}H_{34}I_2N_4$; M=668.39g/mol)
Sigma®Life Science: Propium iodide - >94% (HPLC) (Cat.No. P4170-10MG)

3.9.1.25 Propionic acid ($C_3H_6O_2$; M= 74.08g/mol)
Merck: Propionic acid (Cat.No.: 8006050100)

3.9.1.26 Rubidium chloride (RbCl; M=120,92g/mol)
ABCR: Rubidium chloride - 99% (Cat.No. AB121752)

3.9.1.27 Sodium acetate trihydrate ($C_2H_3NaO_2*3H_2O$; M=136.08g/mol)
AppliChem: Sodium acetate trihydrate – Molecular biology grade (Cat.No. A5268,0250)

3.9.1.28 di-Sodium hydrogen Phosphate dihydrate ($Na_2HPO_4*2H_2O$; M=177.99g/mol)
AppliChem: di-Sodium hydrogen phosphate dihydrate -BioChemica (Cat.No. A3905,0500)

3.9.1.29 Sodium chloride (NaCl; M=58.44g/mol)

AppliChem: Sodium chloride – Molecular biology grade (Catalog No: A2942,1000)

3.9.1.30 tri-Sodium citrate dehydrate ($C_6H_5Na_3O_7*2H_2O$; M=294.1g/mol)

AppliChem: tri-Sodium citrate dehydrate – p.A. BioChemica (Cat.No.: A3901,0500)

3.9.1.31 Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4*H_2O$; M=156.01g/mol)

AppliChem: Sodium dihydrogen phosphate monohydrate - BioChemica (Cat.No. A1047,0500)

3.9.1.32 Sodium hydroxide (NaOH; M=40g/mol)

AppliChem: Sodium hydroxide - Pellets –Molecular biology grade (Cat.No. A6829,0500)

3.9.1.33 Tris base ($C_4H_{11}NO_3$; M=121.14g/mol)

AppliChem: Tris – Molecular biology grade (Cat.No. A2264,1000)

3.9.1.34 Triton®X-100 ($C_{34}H_{62}O_{11}$; M=646.85g/mol)

AppliChem: Triton®X-100 (Cat.No.:A1388,0500)

3.9.2 Enzymes, Kits and Substrates

3.9.2.1 Microarray

- Qiagen: RNeasy® Micro Kit (Cat.No. 74004)
- Affymetrix®: Two-Cycle cDNA Synthesis Kit (Cat. No. 900432)
- Affymetrix®: GeneChip® Expression 3'-Amplification Reagents - For IVT Labeling (Cat.No. 900449)
- Affymetrix® GeneChip® Eukaryotic Poly-A RNA Control Kit (Cat.No. 900433)
- Affymetrix® GeneChip® Expression 3'-Amplification Reagents – Hybridization Controls (Cat.No. 900454)
- Ambion®: MEGAscriptTM T7 (Cat.No. 1334)
- Affymetrix®: GeneChip® Hybridization, Wash and Stain Kit – Hybridization Module (Cat.No. 900720)
- Affymetrix®: Wash Buffer A (Cat.No. 900721)
- Affymetrix®: Wash Buffer B (Cat.No. 900722)
- Affymetrix®: GeneChip® Sample Cleanup Module (Cat.No. 900371)
- Affymetrix®: GeneChip® *Drosophila* Genome 2.0 Array (Cat.No. 900532, lot number 4028865; part number 520087)
- Agilent Technologies: Agilent RNA 6000 Nano Kit (Cat.No. 5067-1511)

3.9.2.2 Quantitative real-time PCR (qPCR)

- Qiagen: RNeasy® Micro Kit (Cat.No. 74004)
- Ambion®: T7 Oligo(dT)24 Primer (Cat. No. AM5712)
- InvitrogenTM: 5x First Strand Buffer (YO2321)
- InvitrogenTM: 0.1M DTT (Y00147)
- Roche: RNase Inhibitor 20U/µl
- InvitrogenTM: 10mM dNTP Mix (18427-013)
- InvitrogenTM: SuperScript® II RNase H – Reverse Transcriptase (Cat.No. 18064-022)
- Roche: MgCl2 (Cat.No. 14271100)
- InvitrogenTM: 10mM dNTP Mix (18427-013)
- Roche: DNA Polymerase I (10642711001)
- Roche: RNase H (10786349001)
- Ambion®: MEGAscriptTM T7 (Cat.No. 1334)
- InvitrogenTM: Random Hexamers (58875)
- ThermoScientific: Absolute QPCR SYBR Green ROX Mix (Cat.No.

3.9.2.3 *In situ* hybridization

- Qiagen: RNeasy® Mini Kit (Cat.No. 74104)
- Qiagen: QuantiTect® Rev. Transcription Kit (Cat. No. 205311)
- GeneCraft: BioThermD-™ Taq DNA Polymerase (Cat.No. GC-064-0250)
- Invitrogen: 100mM dATP Solution (Cat.No. 55082)
- Invitrogen: 100mM dCTP Solution (Cat.No. 55083)
- Invitrogen: 100mM dGTP Solution (Cat.No. 55084)
- Invitrogen: 100mM dTTP Solution (Cat.No. 55085)
- GE Healthcare: illustraTM PuReTaqTM READY-TO-GOTM PCR beads (Cat.No. 27-9559-01)
- Invitrogen: TOPO® TA Cloning Dual Promoter Kit (Cat. No. K4600-01)
- Invitrogen: TOPO® TA Cloning Dual Promoter Kit without topoisomerase I (Cat.No. K2070-40)
- Fermentas: Rapid DNA Ligation Kit (Cat. No. K1422)
- Biomol: X-Gal (Cat.No. 02249)
- Invitek: Invisorb®Spin Plasmid Mini Two (Cat. No. 1010140400)
- Sigma: GenElute™ Plasmid Midiprep Kit (Cat. No.PLD35)
- Fermentas: FastDigest® BamHI (Cat.No. FD0054)
- Fermentas: FastDigest® KpnI (Cat.No. FD0524)
- Fermentas: FastDigest® XbaI (Cat.No. FD0684)
- Fermentas: FastDigest® XhoI (Cat.No. FD0694)
- Fermentas: SP6 RNA Polymerase (Cat.No. EP0131)
- Fermentas: T7 RNA Polymerase (Cat.No. EP0112)
- Roche: DIG RNA Labelling Mix (Cat. No. 11277073910)
- Fermentas: RiboLockTM RNase Inhibitor (Cat.No. EO0381)
- Sigma-Aldrich®: Albumin from Chicken, egg white, Grade II (Cat.No. A5253)
- Sigma® Life Science: Gelatin from porcine skin - Type A (Cat.No. G2500-100G)

- Sigma-Aldrich: Heparin soldium salt (Cat. No. H4784-250MG)
- Sigma: Deoxyribonucleic acid, low molecular weight from salmon sperm (Cat. No. 31149)
- Sigma: Ribonucleic acid, transfer from baker's yeast (S. cerevisiae) - Type X, lyophilized powder (Cat.No. R9001 2KU)
- Sigma-Aldrich®: BCIP®/NBT Liquid Substrate System – Ready-to-use (Cat.No. B1911-100L)

3.9.2.4 Gal4-Lines

- Qiagen: DNeasy® Blood & Tissue Kit (Cat. No. 69504)
- GeneCraft: BioThermPlus-™ Taq DNA Polymerase (Cat.No. GC-061-0250)
- Fermentas: Long PCR Enzyme Mix 100 units (Cat.No. K0181)
- Invitrogen: 100mM dATP Solution (Cat.No. 55082)
- Invitrogen: 100mM dCTP Solution (Cat.No. 55083)
- Invitrogen: 100mM dGTP Solution (Cat.No. 55084)
- Invitrogen: 100mM dTTP Solution (Cat.No. 55085)
- Invitek: MSB®Spin PCRapace (Cat.No. 1020220300)
- ZymoResearch: GelDNA RecoveryKit (Cat.No.
- Fermentas: FastDigest® BamHI (Cat.No. FD0054)
- Fermentas: FastDigest® EcoRI (Cat.No. FD0274)
- Fermentas: FastDigest® HindIII (Cat.No. FD0504)
- Fermentas: FastDigest® KpnI (Cat.No. FD0524)
- Fermentas: FastDigest® NotI (Cat.No. FD0593)
- Fermentas: FastDigest® SacI (Cat.No. FD1133)
- Fermentas: FastDigest® XbaI (Cat.No. FD0684)
- Fermentas: FastDigest® XhoI (Cat.No. FD0694)
- Fermentas: FastDigest® BglII (Cat.No. FD0083)
- Fermentas: FastDigest® PstI (Cat.No. FD0614)
- Fermentas: FastDigest® AvaI (Cat.No. FD0384)
- Fermentas: FastDigest® PvuI (Cat.No. FD0624)
- Roche: Rapid DNA Dephos & Ligation Kit (Cat. No. 04 898 117 001)
- Invitek: Invisorb®Spin Plasmid Mini Two (Cat. No. 1010140400)
- Sigma: GenElute™ Plasmid Midiprep Kit (Cat. No.PLD35)

3.9.2.5 Mutant qPCR

- Qiagen: RNeasy® Micro Kit (Cat.No. 74004)
- Qiagen: QuantiTect® Rev. Transcription Kit (Cat. No. 205311)
- ThermoScientific: Absolute QPCR SYBR Green ROX Mix (Cat.No. AB-1162/B)

3.9.3 Other Reagents

3.9.3.1 Agar AppliChem: Agar – Bacteriology grade (Cat.No. A0949,0250)

3.9.3.2 Albumin: Sigma® (Cat.No. A5253)

3.9.3.3 Apple Juice: from conventional supermarket

3.9.3.4 Blocking Reagent: Roche (Cat.No. 11096176001)

3.9.3.5 Cornmeal Maismehl: Kampffmeyer – Wesermühle Hameln 25kg

3.9.3.6 Gelatine: Sigma® (Cat.No. G2500)

3.9.3.7 Klorix DanKlorix: Dan Klorix- Hygiene Reiniger “Klassik”

3.9.3.8 Malzin Ulmer Spatz 15kg

3.9.3.9 LB-Agar Sigma®Life Science: LB Agar – Molecular Biology Tested (Cat. No. L2897-1KG)

3.9.3.10 LB-Media Sigma®Life Science: LB Broth – Molecular Biology Tested (Cat.No. L3022-1KG)

3.9.3.11 Mol. bio. Water Merck: LiChrosolv® Water for chromatography (Cat.No. 1.15333.2500)
Water was autoclaved, aliquoted, and stored at -30°C prior usage.

3.9.3.12 Treacle Hellmi.eu 13kg (Cat.No. 1905)

3.9.3.13 Ultrapure Water Deionised tab water purified with Sartorium arium®611UV

3.9.3.14 Yeast Probio GmbH Eggenstein 5kg (Cat.No. 03462)

3.9.4 Buffers and Solutions

3.9.4.1 Albumin Gelatine

24.2g Albumin was carefully dissolved in 66g ultrapure water. 5,7g Gelatine was dissolved in 25ml ultrapure water at 55°C. Gelatine solution was then carefully added to the Albumin solution. Albumin Gelatine was aliquoted prior to use.

3.9.4.2 Ampicillin Stock (1000x)

0.5 g Ampicillin was mixed with 5.0 ml ultrapure water. The solution was sterilized by filtration with a Sterile Syringe Filter 0.2µM (3.9.8.4). Filtrate was then aliquoted and stored at -20°C.

3.9.4.3 AP (alkaline phosphotase) buffer

24.2g Tris was mixed with 11.68g sodium chloride and dissolved in 2 liters ultra pure water. pH was adjusted with HCl to 9.5 and the solution was autoclaved. Then 2.04g magnesium chloride was added.

3.9.4.4 Apple juice-Agar

4g Agar was diluted with 200ml apple juice in an Erlenmeyer flask. The mixture was heated in the microwave until it boiled and then cooled at room temperature to 60°C. 10ml-15ml of the Apple juice-Agar solution was aliquoted into urinary cups. Cups were closed with their caps and stored at 4°C. Prior usage a drop of fresh baker's yeast was given to the agar-ground.

3.9.4.5 Blocking solution for *in situ* hybridization

10x Blocking solution (Cat. No. 1109617600) was prepared as described in Roche protocol. Prior to blocking it was diluted 1:10 with 1% PBT.

3.9.4.6 Blocking solution for Antibody staining

10x Blocking solution (Cat. No. 11 09617600) was prepared as described in Roche protocol. Prior to blocking it was diluted 1:100 with 1% PBT.

3.9.4.7 DEPC Water

1 ml DEPC (1.0 %) was added to 1000 ml ultrapure water and incubated for 1 hour at room temperature. It was then autoclaved for 20 min at 121°C for sterilization and stored at room temperature.

3.9.4.8 EDTA pH 8.0 (0.5M)

46.53 g EDTA disodium salt dehydrate ($C_{10}H_{14}N_2Na_2O_8*2H_2O$) was diluted in ca. 200 ml ultrapure water. 10N Sodium hydroxide (NaOH) was added to adjust the pH to 8.0. Ultrapure water was then added to have a final volume of 250ml.

3.9.4.9 Ethanol (70%)

15ml ultrapure water was added to 35ml ethanol.

3.9.4.10 Fixation Solution for Embryos

100µl 37% Formaldehyde was mixed with 400µl 1xPBS and 500µl Heptane was added and the mixture was vortexed for 1 minute.

3.9.4.11 Fixation Solution for Sections

100µl 37% Formaldehyde was diluted in 900µl PBS.

3.9.4.12 Glycerol/PBS (1:1)

5ml Glycerol was mixed with 5ml PBS and stored at room temperature.

3.9.4.13 HybI

25ml deionized Formamide, 12.5ml 20xSSC, 100µl tRNA (50µg/µl), 100µl ssDNA (50µg/µl), 50µl heparin (50µg/µl), and 50µl Triton were mixed in a 50ml Falcon Tube. DEPC water was filled up to a volume of 50ml.

3.9.4.14 LB+Amp agar plates

8.75g LB-Agar was dissolved in 250ml ultrapure water in a Erlenmeyer flask. Flask was covered with aluminium foil and autoclaved for 20 minutes at 121 °C, cooled to ca. 60°C. 250µl Ampicillin (0.1mg/µl) was added to the solution before pouring ca. 10ml of it in each Petri dish.

3.9.4.15 LB-Media

16g LB-broth was dissolved with 800ml ultrapure water in a 1L bottle. With half-closed cap the bottle was then autoclaved for 20 minutes at 121°C. After cooling at room temperature the cap was closed and the medium was stored in the fridge at 4°C.

3.9.4.16 LB+Amp Media

800µl Ampicillin (0.1mg/µl) was added to 800ml LB-medium and stored in fridge at 4°C.

3.9.4.17 Loading Buffer (10x)

40g Sucrose, 0.2 g Orange G were dissolved and mixed in 100ml TE buffer. The mixture was aliquoted and stored at -20°C.

3.9.4.18 Paraformaldehyde solution

6g Paraformaldehyde was diluted in 70ml ultrapure water and heated up to 55°C. When the solution was cleared, ultrapure water was added till 100ml.

3.9.4.19 PBS (10x)

85g sodium chloride, 15g di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4*2H_2O$), 2.1g sodium dihydrogen phosphate monohydrate ($NaH_2PO_4*H_2O$) were dissolved in 1000ml ultrapure water. This solution was then sterilized by autoclaving for 20 minutes at 121°C.

3.9.4.20 PBS (1x)

100ml 10x PBS was diluted in 900ml ultrapure water. The pH of the buffer should be at 7.4; this was controlled with pH-metry.

3.9.4.21 PBT

For 0.3% PBT, 3ml Triton-X-100 was added to 1L 1xPBS. For 1% PBT 10ml Triton-X-100 was added to 1L 1xPBS.

3.9.4.22 Propium iodide solution

1mg Propium iodide was solved in 1ml 1x PBS. Propium iodide was diluted 1:2000 prior usage.

3.9.4.23 Tris (0.05mM) pH 8.0

50mM Tris/HCl pH 8.0 was diluted 1:1000 with Mol.bio.water. 50mM Tris/HCl was prepared by dissolving 30.3g Tris in 500ml ultrapure water and adjusting the pH to 8.0 with HCl.

3.9.4.24 Sodium hydroxide solution (10N)

100g Sodium hydroxide (NaOH) was dissolved in 250ml ultrapure water and sterilized by filtration.

3.9.4.25 Sodium acetate (3M, pH 5.2)

4.1g Sodium acetate was dissolved in 50ml ultrapure water and the pH was adjusted to 5.2 with HCl.

3.9.4.26 SSC (20x)

175.3g of sodium chloride and 88.2g sodium citrate were dissolved in 950ml ultrapure water. The pH was adjusted to 7.0 with 1M HCl. Additional ultrapure water was added to adjust the final volume to 1L. SSC buffer was sterilized by autoclaving.

3.9.4.27 TBE (10x)

106g of Tris base, 55g boric acid, and 40ml 0.5M EDTA pH 8.0 were dissolved in 1l of ultrapure water. For gel electrophoresis 10x TBE buffer was diluted with ultrapure water and 0.5x TBE was used.

3.9.4.28 TE

0.606 g Tris (10 mM), 1ml 0.5 M EDTA (1 mM) were dissolved in 500 ml ultrapure water. pH was adjusted to 8.0 with 5N HCl. TE buffer was then autoclaved and stored at room temperature.

3.9.4.29 TFB1

3.02 g Rubidium chloride, 2.47 g magnesium chloride, 1.10 g calcium chloride, and 37.5 g Glycerol were dissolved in 200ml Mol.bio. Water. Mol.bio. water was then added to make a final volume of 250ml. TFB1 was sterilized by sterile filtration.

3.9.4.30 TFB2

0.52 g MOPS , 0.30 g rubidium chloride, 2.76 g calcium chloride, and 37.5 g glycerol were dissolved in 200ml Mol.bio.Water. pH was adjusted to 6.8 with KOH and Mol.Bio.Water was added to make the final volume of 250ml. TFB2 was sterilized by sterile filtration.

3.9.4.31 X-Gal-Solution

30g X-gal was diluted in 1ml dimethylformamide (DMF), aliquoted, and frozen in -20°C.

3.9.5 Antibodies

3.9.5.1 anti-DIG (1:500)
Roche: Anti-Digoxigenin- AP Fab fragments (Cat.No. 11 093 274 910)

3.9.5.2 anti-Glass (1:300)
DSHB: 9.B2.1 anti-glass-s 1ea 11/12/09 6µg/mlIg

3.9.5.3 anti-Rhodopsin (1:300)
DSHB: 4C5-s 1es 2/18/10 35g/mlIg

3.9.5.4 anti-GFP (1:1000)
GeneTex; Inc.: GFP antibody Lot.No.23817 10mg/ml (Cat.No. GTX13970)

3.9.5.5 Alexa Fluor® 546 anti-mouse (1:200)
Invitrogen™: Alexa Fluor 546 goat anti - mouse IgG (Cat.No. A11003)

3.9.5.6 Cy5 anti-mouse (1:200)
Millipore: Goat anti-Mouse IgG, Cy5 conjugate (Cat.No: AP124S)

3.9.5.7 Alexa Fluor® 488 anti-chicken (1:200)
Invitrogen™: Alexa Fluor 488 goat anti - mouse IgG (Cat.No. A21070)

3.9.6 DNA Ladder

Fermentas: GeneRuler™ DNA Ladder Mix (Cat.No. SM0331)

Fermentas: GeneRuler™ 100bp DNA Ladder (Cat.No. SM0241)

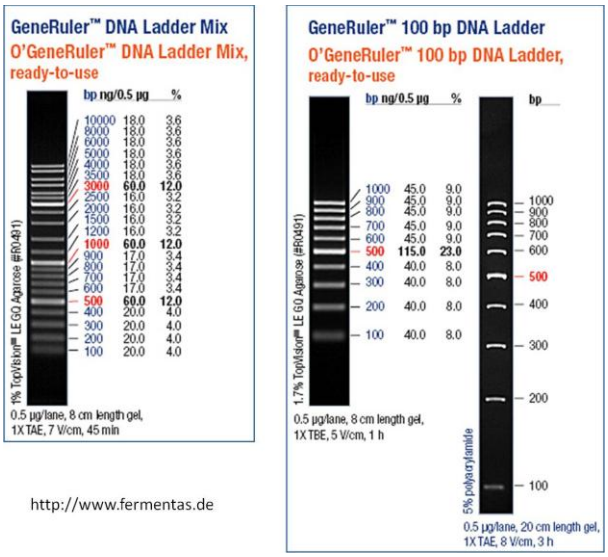


Figure 5 DNA ladder

3.9.7 Equipments

3.9.7.1 Bioanalyzer

Agilent Technologies: Agilent Bioanalyzer 2100

3.9.7.2 Centrifuges

Eppendorf: Centrifuge 5804 R

Eppendorf fixed-angle rotor F-34-6-38

Eppendorf swing-bucket rotor A-4-44

Eppendorf swing-bucket rotor A-2-DWP (for 96 well plates)

Thermo: Heraeus Fresco21 Centrifuge

Heraeus: BIOFUGE pico

A.Hartenstein: Combi-Spin

3.9.7.3 Electronic Magnetic Stirrer

VARIOMAG®: Monotherm Electronicrührer

3.9.7.4 Fridges (4°C)

Comfort NoFrost

Liebherr Economy

3.9.7.5 Freezer (-30°C)

Sanyo: Biomedical Freezer MDF-U537

3.9.7.6 Freezer (-80°C)

New Brunswick Scientific: Ultra low Temperature Freezer

3.9.7.7 Gel Electrophoresis

BIO-RAD: WIDE MINI SUB® CELL GT

BIO-RAD: MINI SUB® CELL GT

3.9.7.8 Imaging System

PerkinElmer precisely: Geliance 200 Imaging System

3.9.7.9 Incubator

Hereaus: Thermo Scientific T6 Incubator, Function line

New Brunswick Scientific: Incubator Shaker Series Innova® 40

3.9.7.10 Microarray Scanner

Agilent: G2500A Gene Array Scanner

3.9.7.11 Microscopes

Olympus: SZ51

Olympus: SZ61

Olympus: SZX16 with Olympus DP70 Camera

Zeiss: Fluorescence microscope Axioskop combined with Intas Spot CCD camera

Leica: laser scanning confocal microscope TCS-4D

3.9.7.12 Microwave

SIEMENS: - Elektrogeräte GmbH Typ MM817ASM

3.9.7.13 PCR Machines

Biometra: professional Thermocycler

BIO-RAD: My**iQ**™ Single Color Real-Time PCR Detection System

3.9.7.14 Pipettes

Gilson: Pipetman®neo 0.2µl-2µl

STARLAB: ErgoOne 0.5µl-10µl

STARLAB: ErgoOne 2µl-20µl

STARLAB: ErgoOne 10µl-100µl

STARLAB: ErgoOne 20µl-200µl

STARLAB: ErgoOne 100µl-1000µl

LLG: Micropipette 1ml-10ml

Brand: accu-jet® *pro*

3.9.7.15 pH-meter

METTLER TOLEDO: **Seven**Easy

3.9.7.16 Photometers

Thermo Fischer Scientific Inc./Peqlab: NanoDrop 1000 Spectrophotometer

Eppendorf: BioPhotometer

3.9.7.17 Power Supply

BIO-RAD: PowerPac™ Basic

3.9.7.18 Rotator

neoLab: Stuart rotator SB2

3.9.7.19 Scale

AND: GX-400

3.9.7.20 Shaker

Heidolph Vibramax 100

3.9.7.21 Electronic Magnetic Stirrer

VARIOMAG®: Monotherm Electronicrührer

3.9.7.22 Thermoblock

neoLab: neoBlock II

Medite: Stretching Table OTS 40

3.9.7.23 Thermomixer

Eppendorf: Thermomixer compact

3.9.7.24 Ultrasonic Bath

QUIGG: Digital Ultrasonic Cleaner

3.9.7.25 Vibratome

Leica: VT 1000 S combined with Leica MS5 microscope

3.9.7.26 Vortexer

Scientific Industries: Vortex-Genie2

3.9.7.27 Water system

Sartorius arium®611UV Ultrapure Watersystem

3.9.8 Consumables

3.9.8.1 24-well-plates
Nunclon™DELTA Surface Disposables for cell culture (Cat.No. 142475)

3.9.8.2 96-well-plates
ABgene®PCR plates: Thermo-Fast(r)96, semi-skirted (Cat.No. AB-0900)

3.9.8.3 Aluminium Foil
neoLab (Cat.No. 16595)

3.9.8.4 Filter
VWR: Sterile Syringe Filter 0.2µM Cellulose Acetate (Cat.No 28145-477)

MILLEX®HV: Filter Unit 0.45µm Durapors®PVDF Membrane

NALGENE®: 50mm Filter Unit – 250ml (Cat.No. 157-0020)

3.9.8.5 Fly vials
K-TK: 28cm 40300

3.9.8.6 Microscope Slides and Cover Slips
MARIENFELD: Microscope Slides ~76x26x1mm (Catalog No: 0810000)

MENZEL-GLÄSER: 100 Deckgläser (cover slips) 24x50mm #1

Wiegang: neoLab-slides Microscope slide with 1 hollow (16293)

3.9.8.7 Mites-free plugs
K-TK: Mites-free plugs

3.9.8.8 Painting brush
Da Vinci: Nr. 3

3.9.8.9 Parafilm
Hartenstein (Cat.No.PF10)

3.9.8.10 Pasteur pipette
Hartenstein (Cat.No. PP004)

3.9.8.11 PCR-Sealer™
Bio-Rad: Microseal® 'B' Film (Cat. No. MSB 1001)

3.9.8.12 Pipette tips

STARLAB: TipOne 0.1µl-10µl Extended Length, Natural Tips (Catalog No. S1110-3000)

STARLAB: TibOne 1-200µl Yellow Pipette Tips (Catalog No. S1111-0006)

STARLAB: TipOne 101-1000µl Blue, Graduated Pipette Tips (Catalog No. S1111-2021)

Greiner bio-one: Cellstar® Serological Pipette 10ml in 1/10ml (Cat.No. 607180)

Corning: COSTAR®Stripette® Serological Pipet 25ml in 2/10mL (Cat.No. 4489)

3.9.8.13 Plastic Scale Pan

Hartenstein: Cat. No. WAE1

3.9.8.14 Polyamide fabric

Polyamid fabric, mesh size 0.1mm

3.9.8.15 Reaction Tubes

STARLAB: 0.2ml 8-Strip PCR Tube, Individually Attached Flat Caps (Catalog No: A1402-3700)

STARLAB: 0.2ml 8-Strip PCR Tubes, Natural (Catalog No: I1402-3500)

SARSTEDT: Multiply(r)-Pro cup 0.5ml, PP (Catolog No: 72.735.0022)

STARLAB: TubeOne 1.5ml Natural Flat Cap Microcentrifuge Tubes (Catalog No: S1615-5550)

STARLAB: TubeOne 2.0ml Natural Flat Cap Microcentrifuge Tubes (Catalog No: S1620-2700)

FALCON®: BLUE MAX™ 50ml Polypropylene Conical Tube (Catalog No: 352017)

FALCON®: 14ml Polypropylene Round-Bottom Tube (Catalog No: 352059)

greiner: Tubes, 15ml, PS, graduated, conical bottom, blue screw cap, sterile (Cat.No. 188261)

3.9.8.16 Silicon moulds for embedding

Plano: G3690

3.9.8.17 Syringe

HENKE SASS WOLF: 10ml (12ml) NORM-JECT®

3.9.8.18 Petri dishes

Hartenstein: PP60

Hartenstein: PP90

3.9.8.19 Urinary Cup

SARSTEDT: Urinary Cup (100ml)

3.9.9 Primers

3.9.9.1 Primers used for Microarray Validation qPCR

Table 2 Primers for Microarray Validation

Intern No.	Primer Name	CG-No.	Sequence (5‘-3‘)	MPI No.
V 1b	CG5948 1637030_at-3'	CG5948	TTCTCCTCGTTCTGATAGGCCAG	18005
V 1b	CG5948 1637030_at-5'	CG5948	ATCAATGGGATCGTTGGGCGTTC	18004
V 2a	run 1634413_at-3'	CG1849	CGAGGGCCTGTGATTCAAACTC	18001
V 2a	run 1634413_at-5'	CG1849	GCATCAACTAGCTGAGCAAAAGT	18000
V 3b	3b 1638001_a_at-3'	CG8889	GAGCAATTCTTGAGAATCTTGGGC	18117
V 3b	3b 1638001_a_at-5'	CG8889	GGGACTATTCGTCGTCTGTGGT	18116
V 4	DAG 1628730_AT-3'	CG42667	GCTCCCTACTCCTTTCCCTTTCC	16851
V 4	DAG 1628730_AT-5'	CG42667	GATTGAGATCGAGAGCGACGAG	16850
V 5	Caps 1628675_AT-3'	CG33653	CGCGCAGAAGGCGAATGAATAGTG	16853
V 5	Caps 1628675_AT-5'	CG33653	GGAGTATAGGCTCAACGACCCATATG	16852
V 6	mRpL1 1628481_AT -3'	CG7494	CACATCTCTGGAACGTAGACAAAGGG	16855
V 6	mRpL1 1628481_AT -5'	CG7494	CAGCGGCATTAGTTACAGTGCAG	16854
V 7	AnnX 1627472_AT-3'	CG9579	GCGTTTATTTATTGCCAACAGTGTGC	16857
V 7	AnnX 1627472_AT-5'	CG9579	GAGACCTCTGGTGACTACAAGCG	16856
V 8	beta4GalN 1637032_AT-3'	CG14517	CAGCCGTAATACAGATTGGACATG	16859
V 8	beta4GalN 1637032_AT-5'	CG14517	TCATCCTTCATGACGTGGACCTCC	16858
V 9a	CG31955 1633946_at-3'	CG31955	GTTTTCTGGGCTCACTGTATTCAT	17813
V 9a	CG31955 1633946_at-5'	CG31955	GCACATGCCGGATTCCAAATTAAG	17812
V 10	mthl5 1639045_AT-3'	CG6965	GTGGCTCATTGTAGCAGCAGGTC	16863
V 10	mthl5 1639045_AT-5'	CG6965	TCATGTTCTCGCTGATGCTGTTAG	16862
V 11a	CG9733 1640085_at-5'	CG9734	CTCGGACAACATTCAGCCCAT	17815
V 11a	CG9733 1640085_at-3'	CG9734	AGAATCTCTGGCGGCATTTGG	17814
V 13	a5 1628647_AT-3'	CG5430	CCTTCATAATATCCAATCCGGGAAC	16836
V 13	a5 1628647_AT-5'	CG5430	AGGGAGTTACTTAGGATCAAGTACG	16837
V 14	CG11041 1635777_AT-3'	CG11041	AGATAGCCCTTGTTCTCCGGATCC	16838
V 14	CG11041 1635777_AT-5'	CG11041	CATCGACGTTAGAGAAGTGGGCAC	16839
V 15	CG17279 1638756_AT-3'	CG17279	TAGCTTGCCATCGTCTCGGAGATC	16840
V 15	CG17279 1638756_AT-5'	CG17279	GAGGCAAAGGGATTCGATGCGGA	16841
V 16	Os-C 1636865_AT-3'	CG3250	CGGTCCAGTTCCTTCAATGGTAGTC	16842
V 16	Os-C 1636865_AT-5'	CG3250	ATACGGACAACACGCCATCGGCTC	16843
V20a	CG13133 1636818_at-3'	CG13133	GTGATGCACAACTGACCCTTCTCC	17808
V20a	CG13133 1636818_at-5'	CG13133	TGGTTGGGCGAGTGGTCATAGA	17809

3.9.9.2 Primers used for *in situ* hybridization

Table 3 Primers for *in situ* hybridization

Primer Name	CG- No.	Sequence (5‘-3‘)	MPI/Sigma No.
Dhc93AB-5'	CG3723	TTGGATCAAGTGCCGCCCATTTGG	MPI 19622
Dhc93AB-3'	CG3723	AGGTTTGCAAGAGCAGCGCTAC	MPI 19623
Bmcp-RA-5' ISH	CG7314	CGACCTATGGCACCATCAAGTTTGG	Sigma B8219-007
Bmcp-RA-3' ISH	CG7314	CCTTCGTTCCTGATAGTCTGTACAGC	Sigma B8223-001
CG8086 affy probe 5' ISH	CG8086	CTTTGGCCTGAAGACAGTGCCCAC	Sigma B8219-017
CG8086 affy probe-3' ISH	CG8086	TCGCCTCCACAGCGTACTTGAC	Sigma B8219-016
10-CG13133 -RA-5'	CG13133	ACTACTATCTCTCCCACGACCTGGAC	Sigma B2553-066
10-CG13133 -RA-3'	CG13133	GGACACGCTGCCAAAGTAATTGCC	Sigma B2553-065
CG14921 Dys Affymetrix-5'	CG14921	CATGCAGAAGCGCCTAGAGATCG	Sigma B8606-001
CG14921 Dys Affymetrix-3'	CG14921	TATTAGCACAGTCTCGCACGCACGC	Sigma B8612-004
Arr2_affy-5'	CG5962	TATCGCCCTGGATGGTCACTTGAAG	MPI 19613
Arr2_affy-3'	CG5962	AGCGACACGAGGTCTGTTCAACTC	MPI 19614
trpl-RB-5'	CG18345	ATTCGAGAACAGCGGAATGGATG	MPI 17668
trpl-RB-3'	CG18345	GTGCAGCGACCTATTTGGGAATCC	MPI 17669
CG14585-affy-5'	CG14585	AGTCGTTTCATCTCTACTAAGCTCGC	MPI 19757
CG14585-affy-3'	CG14585	AGGCCAGTTCCACCAGCAGGATTAC	MPI 19758
CG32373 ISH-5'	CG32373	CTCCAAATTACCGCCATCGAAAGACG	MPI 19992
CG32373 ISH-3'	CG32373	ACGGCTTTGTGGAATCCGGTTAAGG	MPI 19993
CG18516 ISH-5'	CG18516	GTTGATCAATCTGATAGCCTCGGATCAC	MPI 19990
CG18516 ISH-3'	CG18516	GGCATTTAGTAGCAGAGTCTCTGGAG	MPI 19991
trpA3 CG31284-RC wtrw-5'	CG31284	GGTTGTCTAAGCTCGTGCGTCA	MPI 17680
trpA3 CG31284-RC wtrw -3'	CG31284	TGAGGAACTCGATCCGATCAGC	MPI 17681
CG14076-RA 5' ISH	CG14076	CCGTCGGATGCTATCCAGTCATTG	Sigma B8219-011
CG14076-RA 3' ISH	CG14076	TGCCAATGGAGACAAATCCGCCAAC	Sigma B8219-010

3.9.9.3 Primers used for sequencing

For sequencing of TOPO-constructs

MPI-SP6 5’-AACAGCTATGACCATGATTACG-3’

For sequencing of pPTGAL-constructs:

MPI-17924 5’-CCTGGTACTTCAAATACCCTTGG-3’

3.9.9.4 Primers used for Gal4-Lines

Table 4 Primers for Gal4-lines

Intern Name	Primer Name	CG- No.	Sequence (5‘-3‘)	MPI No.
G 0	CG9313 ext. gene reg-BamHI-3'	CG9313	CAGGATCCGAGCCAACTGAGCCCTTACTTTAC	17696
G 0	CG9313 ext. gene reg-EcoRI-5'	CG9313	GGATGAATTCACATGACCACCGCTACTTC	17697
G 1b	dyslexia CG14921 EcoRI-3' Primer2	CG14921	CCCGAATTCCAGAAATGCTTTAATCCTC	18486
G 1b	dyslexia CG14921-NotI 5' P 2	CG14921	CAAGCGGCCGCGGAAAGTGGCTAAAG	18485
G 3b	CG13636 BamHI-3' P 2	CG13636	CGGATCCGCTCCGCCAGAAAGTCCGAAG	18483
G 3b	CG13636 NotI-5' Primer2	CG13636	CAAGCGGCCGCAAGGCGATTAAATTCAGGC	18484
G 5	CG11253 EcoRI-5'	CG11253	ACAGGAATTCCATGGACATTTGTGACAG	18081
G 5	CG11253 BamHI-3'	CG11253	GAGGATCCTCTCGTGCACCTCCAG	18082
G 6a	6a CG4329 BamHI-3'	CG4329	GGAGGATCCGAAATTCGATCAAACAACC	19206
G 6a	6a CG4329 EcoRI-5'	CG4329	TCCCAAGAATTCCCCAGCTGAAGAGG	19207
G 7	PNC1 CG31216-NotI-5' Primer1	CG31216	GCGGCCGCACGAGGGATTAATCGCATTC	18473
G 7	PNC1 EcoRI-3' Primer1	CG31216	ATACCACTTAGAATTCGTTGATGTAGTC	18474
G 12	Dhc93AB BamHI-3' Primer1	CG3723	GCGCGGATCCGGGCCACCAGCTG	18487
G 12	Dhc93AB EcoRI-5' Primer1	CG3723	GGAATTCAGCGCAACAAATGGCCATTGAC	18488
G 13	13 Klp68D-KpnI-3'	CG7293	GAGGTACCCGTTTGGCGTCTGGCTGCTG	19214
G 13	13-Klp68D-BglII-5'	CG7293	AAGATCTACTAAACTGGGTGGTAAGTTAG	19215
G 14	14 Ir94b CG31424 BamHI-3'	CG31424	GGGATCCCAGTTTTGAAGGCTACAATTG	19216
G 14	14 Ir94b CG31424 EcoRI-5'	CG31424	CATGAATTCGGATGTATACTAAACACTTTG	19217

3.9.9.5 Primer used for qPCR controls

Table 5 Primers for qPCR controls

Intern Name	Sequence 5' - 3'
corto Contrl Genes Affy-3'	TTACACAGAGCGGTTGCTGTTGTTTC
corto Contrl Genes Affy-5'	CGGTGAGAAAAGAGGAAATGAGCGA
cpa Contrl Gene Affy-3'	AGTCGGCTGGTTGGTCTTCAAAGAC
cpa Contrl Gene Affy-5'	TCTACGTCACGATCGCCACCAATTG
Moesin Contrl Gene Affy-3'	TTGTCACGTCCCTGGCGAACGTTC
Moesin Contrl Gene Affy-5'	GAGCATATCAAGGACCCCATCGAGGAC
spinster Contrl Gene Affy-3'	GCTCGCAGTCTTTATGTCGCTTTC
spinster Contrl Gene Affy-5'	CGAATTGGAGAAATGTGTGCACCCTC

3.9.9.6 Primers used for Mutant qPCR

Table 6 Primers for Mutant PCR

Affected gene	5' Primer (5'-3')	3'Primer (5'-3')
Naam	AAGCTTTCACAGCTGACGGACG	AATGTGGTAGCAATGTCAAATTCGTG
Dhc36c	GACTACGTGTTCGCCATGAAGAAG	GTCTTCATCAGGACATTGCGGTTC
CG14693	GAGCACGGTTCAATGCCTGATAC	GGGCTTCAAGGTCTCGGAAATTAG
Ank2	TCCGAAGGCACTGATAGCACTAC	CCTCTGTGAGCTCAAAACTCCTC
CG11253	ATCTCAAGATGGGCGAAGCAAAG	TTTGTATACTAGACGCTGGGTCAC
CG8086	GCGCTATTAGCACCGCAATAAAGTG	CCACGTTGCCGCCCATGTTAATTG
CG9492	GCACTTGGATATCAACAGATAGACCAAAGG	TGATGTCACAGAGCAGGGCCACTC
CG13636	ATGTCCAGCAAGACCAAGCTGTGTC	TTCGACGATCGCCTTCTAGAAATGC
norpA	TCAGTTTATGAACGACAAACAGCGCG	GTTTTCGTCGGACATTAGATAGCGC
Bw	CACATGAAGACCGGCGACCTCATC	TTCACGTTGATCTCGAACTGCGGC
ninaC	AGTTCCATGCCAAGTATCGCTTCAAG	AAGTAGCACAGATGCTTGATTAGCAGC
Bmcp	CTAATTCGTTGCGCAACAGAGCG	TGCCAAATTCCGCCGTGATTGAG
CG4329	GGTCTCAATCAGAACAACGTGCAGTTG	GGCGTGTTTGCGGAACAGTTCTTTC
CG14921	GGCCTGAGATGTTCCAGAAGCTGG	CGCCGCTTGTTATCGTAACGTTCC
CG14636	CATCGACCTGTGCTCTCCTCTTC	CTACAGAGTGTGGCAAGTAGGTCACG
Ir94b	CGCCTAAATCCTTTCCCATCTCCCC	CCCATTTGGATAGTCCAGTTGTAATCGTG
Dhc93AB	CCTCGTCCATATTCCACAGCGATATC	TGAAAAGTCCATAAAATCGGCGGCCG
CG10633	CAGTTGCATACATGGTTTCGGACCAC	GAAGCGGTTCATCGTGTCGATGTG
Klp68D	AAAGAAGCCAGCTTCGGCGTATC	AAAGAAGCCAGCTTCGGCGTATC
Gl	AATTAGTCGCCGTCTTATGCTCCTC	CAAATGGCATGATACCAAGATCACCAATAG
Gol	ATATTGCCGTGCAAGCACGAGTTC	TTGGCGCTGTATGCTGTGGACTC
CG8419	CAGTGAGCCCACTAGCAGCTTG	TGAGTAGCCTCTTGGGATGCAGTTG
Stops	GGAATCCTGCCAATAGGGCCAGTC	AGCCCTTGATGATGGAGCTCAGG
Lin29	CGGTCGAGCCTTTATACAGCTTTC	TGCTGCTGCAAGACACCAAGATG
Sulf1	GCGCTACTCAAAAACTGTCTACCTGG	GACTACTCCGTCGGGAGTGAAACAG
CG9313	CAAATGCTGGCAGATACTCGAGCG	TGTCGGTGACGTTCATCTTCTTGGTC
Arr2	ATCCACTAATCCACAATGGTTGTATCCG	CAGTTGGCCAAAGACTTTCCTGTTC
Trpl	CTGGCCATCATCCTGAACTACGATCC	GATGACTTTGTCCACAATGCTCTTGCG
inaD	GCAATCGATGCAGAAGCGGAATAC	TCGCGATCCTTTTCCTGCTTGTTG
Futsch	CATGCAACATGCCCCATGCAACAG	GAGCGCTAATGCGGATCATGATCC
Yuri	TTGAAGGATCAGTGCATAGCACAGC	GCATATCGCTTGTAATCGCGTCCG
kek4	TTCAGTCACCTGGCAGTGAAGAAG	GTGTGCATATACTCGCTGCCATTCG
CG6053	TCACCGGATGGCGGCATCAAAATG	ATCCTTCTGGTTGTACTCGAGACACACG
Sei	CCTATTTCGACAACCGCACTGGTG	AGCCTTTGAATAATGGCAGATACGTTTCC
ninaA	AGTTCTACCCGGACGACTTCAACATC	ATTCGGAGTGTATGGGGGCTTCTTAG
Trp	ATCGTCCATCGTGATCTCTGGTTCCG	GTCGATTATCATGCGACCCAGTGAAAC
CG11388	CTTGCATTGATTTTTACGCCGTGCTC	CTATTGCAACAGTACGTCGGCGTC
Rh5	CCTACTACAAGCTCTTCACCCACGTC	TGTCCAGGCCAGAATGAAGAGCATG
Rh6	TATCGCTGTGGTTCTTCGCCTGG	AGCACCTGCTTGTACTTCGGGTG

3.9.10 Fly Stocks

Table 7 BL: Bloomington Stock Center, S.B.:Susanne Bechstedt Zuker Tilling Collection (Dresden, Germany), R.H.: gifted from Roger Hardie (Cambridge, U.K), C.H-F: gifted from Charlotte Helfrich-Förster (Würzburg, Germany)

Genotype	Donor	Symbol	CG-No	Chrom	Described in
y[1]; ry[506] P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG31216[KG10548]	BL: 15278	Naam	CG31216	3R	
y[1] w[*]; P{y[+m8]=Mae-UAS.6.11}Dhc36C[LA00085]	BL: 22169	Dhc36c	CG5526	2L	
w[1118]; PBac{w[+mC]=WH}CG14693[f03110]	BL: 18620	CG14693	CG14693	3R	
w[1118]; PBac{w[+mC]=WH}Ank2[f02001] CG32373[f02001]/TM6B, Tb[1]	BL: 18502	Ank2	CG42734	3L	
y[1] w[67c23]; P{w[+mC] y[+mDint2]=EPgy2}CG11253[EY10866]	BL: 20637	CG11253	CG11253	3L	
w[1118]; PBac{w[+mC]=WH}CG8086[f03214]	BL: 18629	CG8086	CG8086	2L	
y[1] w[67c23]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG9492[KG02504] ry[506]	BL: 13745	CG9492	CG9492	3R	
w[1118]; Mi{ET1}CG13636[MB03846]	BL: 24534	CG13636	CG13636	3R	
norpA[7]	BL: 5685	norpA	CG3620	X	
bw[1]	BL: 245	bw	CG17632	2R	
w[1118]; Mi{ET1}ninaC[MB02664]	BL: 23459	ninaC	CG5125	2L	
w[1118]; P{w[+mGT]=GT1}Bmcp[BG02446]	BL: 12688	Bmcp	CG7314	3L	
y[1] w[67c23]; Mi{ET1}CG4329[MB01614]	BL: 23200	CG4329	CG4329	2R	
y[1] w[1118]; PBac{y[+mDint]=3HPy[+]} CG14921[C247]/CyO	BL: 16317	CG14921	CG14921	2L	
w[1118]; Mi{ET1}CG14636[MB03866]	BL: 24538	CG14636	CG14636	3R	
w[1118]; Mi{ET1}CG31424[MB02190]	BL: 23424	Ir94b	CG31424	3R	
w[1118]; Mi{ET1}Dhc93AB[MB05444]	BL: 25298	Dhc93AB	CG3723	3R	
w[1118]; Mi{ET1}CG10633[MB05283]	BL: 24610	CG10633	CG10633	3L	
y[1] w[67c23]; P{w[+mC] y[+mDint2]=EPgy2} Klp68D[EY00199]	BL: 20090	Klp68D	CG7293	3L	
gl[3]	BL: 508	gl	CG7672	3R	Moses *et al.*, 1989
w[1118]; Mi{ET1}gol[MB03006]	BL: 23834	gol	CG2679	2R	
w[1118]; Mi{ET1}CG8419[MB06410]	BL: 25321	CG8419	CG8419	2L	
y[1] w[*]; stops[1]	BL: 24893	stops	CG31006	3R	
y[1]; Mi{ET1}CG2052[MB00729]	BL: 23728	Lin29	CG2052	4	
w[1118]; Mi{ET1}Sulf1[MB11661]	BL: 29237	Sulf1	CG6725	3R	
CG9313[Z2-5863]	S.B.	CG9313	CG9313	2R	
arr2[5]	R.H.	Arr2	CG5962	3L	Alloway & Dolph, 1999

Genotype	**Donor**	**Symbol**	**CG-No**	**Chrom**	**Described in**
trp[343]	R.H.	trpl	CG18345	2R	Scott *et al.*, 1997
inaD[1]	R.H.	inaD	CG3504	2R	Tsunoda *et al.*, 1997
y[1] futsch[K68]	BL: 8794	futsch	CG34387	X	
w; PBac{GAL4D,EYFP}yuriPL00114 cn1 bw1; P{FRT(whs)}2A P{neoFRT}82B*	BL: 19394	yuri	CG31732	2L	
w[1118]; Mi{ET1}kek4[MB11415]	BL: 29204	kek4	CG9431	2L	
w[1118]; Mi{ET1}CG6053[MB06262]	BL: 25491	CG6053	CG6053	3L	
w[1118]; P{w[+mC]=EPg}sei[HP21840]	BL: 21935	sei	CG3182	2R	
w[*]; ninaA[1]	BL: 3530	ninaA	CG3966	2L	
trp[343]	R.H.	trp	CG7875	3R	
w[1118]; PBac{RB}CG11388e03063	BL: 18113	CG11388	CG11388	2R	
rh5[2]	C.H-F	Rh5	CG5279	2L	Yamaguchi *et al.*, 2008
rh6[1]	C.H-F	Rh6	CG5192	3R	Yamaguchi *et al.*, 2008
w[1118]		Control			
CantonS		Control			
OregonR		Control			
y[*] w[*]; P{w[+mC]=UAS-2xEGFP}AH3	BL: 6658	UAS-2xEGFP		3L	Halfon *et al.*, 2002
y[*] w[*] P{w[+mC]=UAS-2xEYFP}AX	BL: 6661	UAS-2xEYFP		X	Halfon *et al.*, 2002
w[1118]; ;Dhc93AB-Gal4[6254-4-4M]/TM3(sb)	own	Dhc93AB-Gal4		3	
w[1118]; Klp68D-Gal4[6623-4-2M]/CyO	own	Klp68D-Gal4		2	
w[1118]; CG9313-Gal4[6135-1-4M]/TM3(sb)	own	CG9313-Gal4		3	
w[1118];CG4329-Gal4[6623-3-3M]/TM3(sb)	own	CG4329-Gal4		3	
w[1118]; Naam-Gal4[6254-4-3M]/CyO	own	Naam-Gal4		2	
w[1118]; Ir94b-Gal4[6623-5-2M]/TM3(sb)	own	Ir94b-Gal4		3	
w[1118]; CG11253-Gal4[6135-3-6M]/TM3	own	CG11253-Gal4		3	
w[1118]; CG14921-Gal4[6623-1-2M]/TM3(sb)	own	CG14921-Gal4		3	
w[1118];CG13636-Gal4[6254-1-2M]/TM3(sb)	own	CG13636-Gal4		3	

3.9.11 Online Resources and Software

AmiGo: AmiGo v.1.7 http://amigo.geneontology.org/cgi-bin/amigo/term_enrichment [Carbon *et al.*, 2009]

BIO-RAD IQ:Bio-Rad iQ5 Version 2.0

BLAST:Refseq protein, BlastP, Sep/2011 http://blast.ncbi.nlm.nih.gov/Blast.cgi

Excel: Microsoft Office, Excel 2007

Expression Profiler: Next Generation tool v. 1.0 http://www.ebi.ac.uk/microarray-srv/EP/cgi-bin/ep_ui.pl [Kapushesky *et al.*, 2004]

FlyAtlas: http://flyatlas.org/ [Chintapalli *et al.*, 2007]

FlyExpress: http://www.flyexpress.net/ [Konikoff *et al.*, in prep.]

FlyBase: http://FlyBase.org/ [Tweedie *et al.*, 2009]

FlyMine: http://www.flymine.org/ [Lyne *et al.*, 2007]

Gel-Documentation: Perkin Elmer GeneSnap Version 7.04

Gene Ontology: Dec/2010 http://www.Gene Ontology.org/ [Ashburner *et al.*, 2000]

GeneVenn: http://www.bioinformatics.org/gvenn/

GENtle: GENtle V1.9.4 Magnus Manske, University of Cologne, GER http://gentle.magnusmanske.de/

GEPAS: GEPAS, v.4.0 http://www.gepas.org/ [Herrero *et al.*, 2003]

Hereditaryhearingloss: http://hereditaryhearingloss.org [Van Camp & Smith, 2010]

HOMOPHILA: http://superfly.ucsd.edu/homophila/ [Chien *et al.*, 2002]

IHOP: http://www.ihop-net.org [Hoffmann & Valencia, 2004]

ImageJ: ImageJ 1.43u Wayne Rasband, National Institutes of Health, USA, http://rsb.info.nih.gov/ij

NetAffx™:http://www.affymetrix.com/estore/analysis/index.affx?category=34005&categoryIdClicked=34005&rootCategoryId=34005&navMode=34005&aId=netAffxNav [Liu *et al.*, 2003]

Photoshop: Adobe Photoshop CS3

Phylogeny.fr: http://www.phylogeny.fr/version2_cgi/simple_phylogeny.cgi [Dereeper *et al.*, 2008]

Pubmed: http://www.ncbi.nlm.nih.gov/pubmed

SMART: http://smart.embl-heidelberg.de/smart/show_motifs.pl [Leutunic *et al.*, 2009]

String: http://string-db.org/ [Jensen *et al.*, 2009]

4 Results

4.1 Experiments

To identify genes that are expressed in JO, gene expression profiles were compared between *atonal* mutants and controls. Flies carrying the *ato*^{1} null allele [Jarman el al. 1995] were tested as hemizygous *ato*^{1}*/Df(3R)p*^{13} mutants. Balanced *Df(3R)p*^{13}*/TM3* and *ato*^{1}*/TM3* flies were used as controls (see Figure 6). RNA from the second antennal segments of mutants and controls was extracted. Brains of *ato*^{1}*/TM3* controls served as neuronal control tissue.

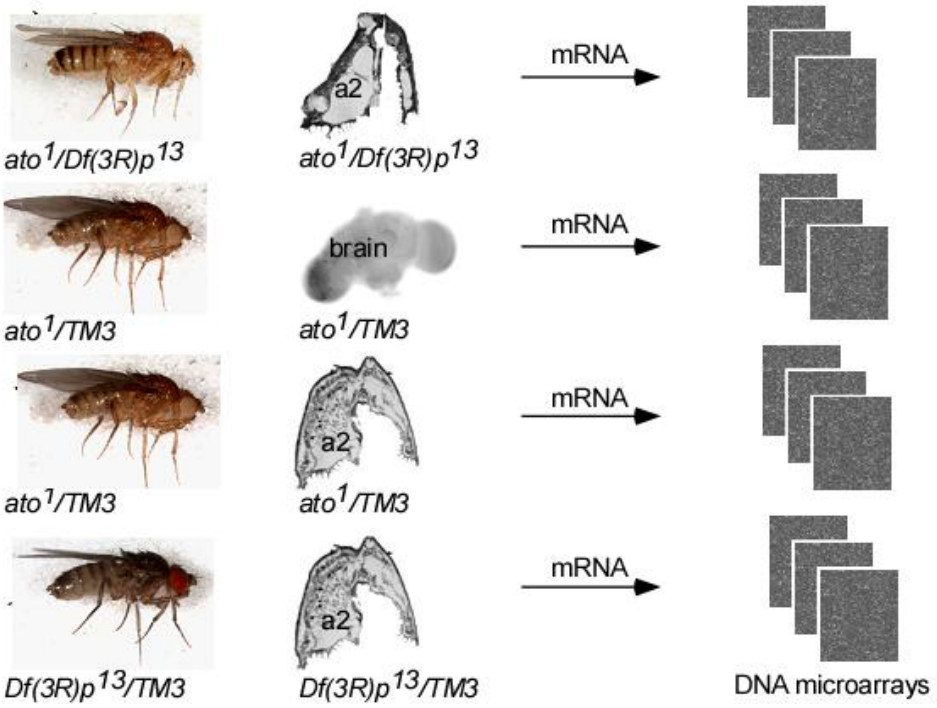


Figure 6 The microarray approach. RNA from the second antennal segments of *atonal* mutants (*ato*^{1}*/Df(3R)p*^{13} and controls (*ato*^{1}*/TM3* and *Df(3R)p*^{13}*/TM3*) have been extracted for the microarray assay. Brains of *ato*^{1}*/TM3* controls served as neuronal control tissue.

Biotin-labeled cRNA probes were hybridized to Affymetrix® GeneChip® *Drosophila* Genome 2.0 arrays containing 25-mer-oligonucleotides probes for 18952 probe sets (including 183 Affymetrix® internal controls). Arrays were scanned with the Agilent G2500A Gene Array Scanner, and three chips were run for each strain. The raw scanning data was corrected using the Gene Profile Analysis Suite GEPAS, v. 4.0 [Herrero *et al.*, 2003].

To assess the consistency of the microarray data, Log_2-scale fluorescence intensities of all Affymetrix® probe sets were compared between *Df(3R)p^{13}/TM3* and *ato^{1}/TM3* controls. Scatter plots (Figure 7) document similar gene expression profiles for both controls. Comparing the fluorescence intensities of controls with those of *atonal* mutants identified a small proportion of probe sets that is significantly downregulated in *atonal* mutants. The Student's two-sample t-test was used to assess this differential expression [Benjamini & Hochberg, 1995; Chuaqui *et al.*, 2002]. P-values were adjusted with the False Discovery Rate (FDR) control procedure to correct for multiple comparisons. Differential expression of genes that falls within a cut-off of 10% (FDR<0.10) was considered as significant [Xie *et al.*, 2007, Benjamini & Hochberg, 1995; Chuaqui *et al.*, 2002].

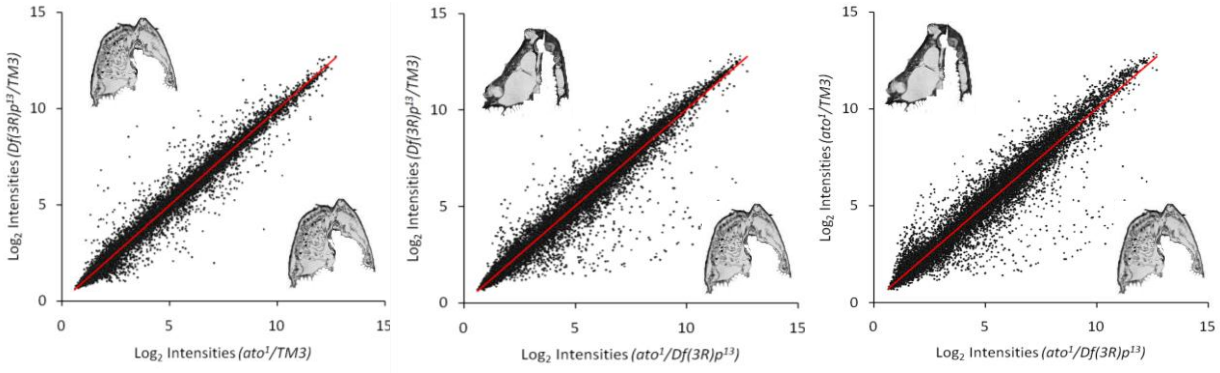


Figure 7 Fluorescence intensities Scatter plots comparing microarray intensity profiles in the 2nd antennal segment of *ato* mutants and controls (mean intensities of three arrays/ strain). Left: *Df(3R)p^{13}/TM3* control vs. *ato^{1}/TM3* control; middle: *ato^{1}/Df(3R)p^{13}* mutant vs. *Df(3R)p13/TM3* control; right: *ato^{1}/Df(3R)p^{13}* mutant vs. *Df(3R)p^{13}/TM3* control.

Only genes that were significantly enriched in the second antennal segments of both controls (*ato^{1}/TM3* and *Df(3R)p^{13}*) were taken into consideration. Analyzing the data in terms of Venn diagrams yielded a consensus list of 282 transcripts representing 274 *Drosophila* genes (Figure 8). All these genes were downregulated in *atonal* mutants; 101 genes showed higher expression in the JO than in the brain, while 173 genes were equally or higher expressed in the brain.

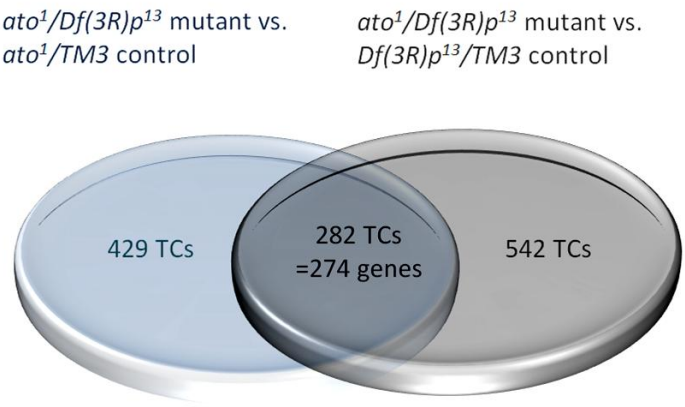
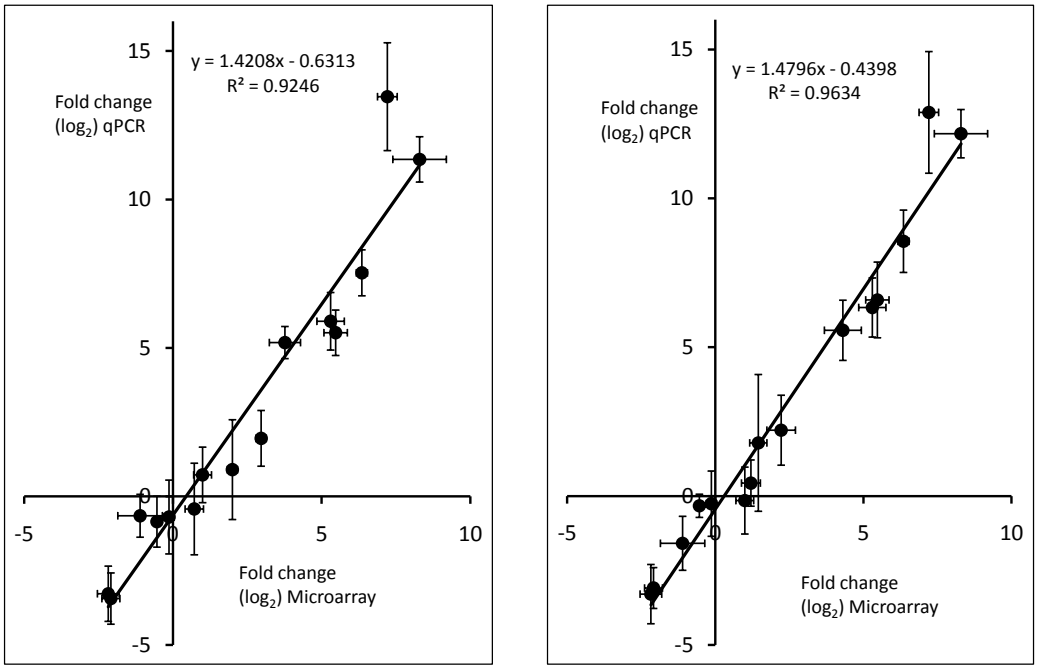


Figure 8 Venn diagram In *atonal* mutants, 429 transcripts (TCs) are downregulated compared to *ato*^{1}/TM3 controls, and 542 transcripts are downregulated when compared to *Df(3R)p*^{13}*/TM3* controls. A consensus list of 282 transcripts representing 274 *Drosophila* genes is obtained.

4.2 Global validity of the microarray data



ato^{1}*/TM3* controls vs. *ato*^{1}*/Df(3R)p*^{13} mutants

Df(3R)p^{13}*/TM3* controls vs. *ato*^{1}*/Df(3R)p*^{13} mutants

Figure 9 Comparison of microarray and qPCR results 15 genes spanning the entire microarray fluorescence range were chosen and qPCR was performed. QPCR was performed separately for each control (*ato*^{1}*/TM3* and *Df(3R)p*^{13}*/TM3*) and Log_2 fold changes were calculated as described in 3.2.3.

To exclude technical pitfalls with microarrays that may lead to the under- or overestimation of expression levels, quantitative real-time PCR (qPCR) was performed for 15 selected genes to assess the validity of the microarray data. Genes spanning the entire range of gene expression covered by the microarray were selected by a random-stratified sampling strategy following the global validation scheme described by Miron *et al.*(2006).

As in the microarray experiments RNA was extracted from *ato*^{1}*/Df(3R)p*^{13} mutants and *ato*^{1}*/TM3* and *Df(3R)p*^{13}*/TM3* controls. For each strain three biological replicates were run and each replicate was tested three times. Log_2 ratios were calculated as described in 3.2.3.

Microarray and qPCR results correlated with each other, supporting the global validity of the microarray screen (R^2=0.9246 for *ato*^{1}*/TM3* controls, and an R^2=0.9634 for *Df(3R)p*^{13}*/TM3* controls) (Figure 9).

4.3 Consistency of the microarray data

Fluorescence intensities of the 282 transcripts representing the 274 candidate genes were subjected to cluster analysis to assess the consistency of the microarray data. All biological replicates of each sample tissue clustered together. Also antennal transcription pattern of the two controls (*ato^{1}/TM3* and *Df(3R)p^{13}/TM3*) grouped together, whereas that of mutants (*ato^{1}/Df(3R)p^{13}*) was distinct. Antennal transcription patters of controls turned out to be more similar to those of brains than to the antennal transcription patterns of the mutants, illustrating that JO expresses many common neuronal genes (Figure 10).

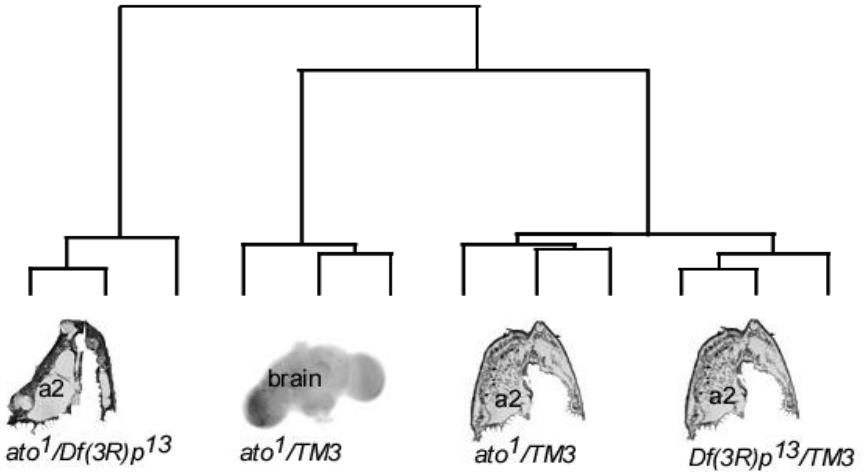


Figure 10 Cluster analysis. Cluster analysis was performed using Expression Profiler: Next Generation tool v. 1.0 [Kapushesky *et al.*, 2004]

4.4 The auditory organ gene sets

Signal transmission GO:0023060

Rh3	Rhodopsin 3
Rh4	Rhodopsin 4
Rh5	Rhodopsin 5
Rh6	Rhodopsin 6
Gbeta76C	G protein beta subunit 76C
Ggamma30A	G protein gamma30A
Arr2	Arrestin 2
norpA	no receptor potential A
rdgA	retinal degeneration A
rdgC	retinal degeneration C
inaC	inactivation no afterpotential C
inaD	inactivation no afterpotential D
ninaA	neither inactivation nor afterpotential A
ninaC	neither inactivation nor afterpotential C
trp	transient receptor potential
trpl	trp-like
Cam	Calmodulin
boss	bride of sevenless
laza	laza
qvr	quiver
cpx	complexin
cac	cacophony
Pask	PAS kinase
stops	slow termination of phototransduction
pum	pumilio
sif	still life
CdsA	CDP diglyceride synthetase
dlg1	discs large 1
Calx	Na/Ca-exchange protein
Slob	Slowpoke binding protein
Ank2	Ankyrin2
n-syb	n-synaptobrevin
Syn	Synapsin
Sh	Shaker
stj	straightjacket
flw	flapwing
bsk	basket
dtr	defective transmitter release
Syt1	Synaptotagmin 1
CG13830	(calcium ion binding)
CG42674	(Rho guanyl-nucleotide exchange factor activity)

Ion channel activity GO:0005216

trp	transient receptor potential
trpl	trp-like
iav	inactive
nan	nanchung
wtrw	waterwitch
Ir8a	Ionotropic receptor 8a
Ir64a	Ionotropic receptor 64a
Ir75a	Ionotropic receptor 75a
Ir75d	Ionotropic receptor 75d
Ir76a	Ionotropic receptor 76a
HisCl1	Histamine-gated chloride channel subunit 1
Sh	Shaker
cac	cacophony
tipE	temperature-induced paralytic E
stj	straightjacket
sei	seizure
CG9935	(Glutamate receptor)

Motor activity GO:0003774

ninaC	neither inactivation nor afterpotential C
Klp68D	Kinesin-like protein at 68D
Dhc16F	Dynein heavy chain at 16F
CG6053	(Dynein intermediate chain)
Dhc36C	Dynein heavy chain at 36C
CG9313	(Dynein intermediate chain)
CG3339	(Dynein heavy chain)
Dhc93AB	Dynein heavy chain at 93AB
CG9492	(Dynein heavy chain)

Figure 11 Significantly enriched Gene Ontology categories Enrichment were identified using AmiGO (Version: 1.7)

To annotate the list of candidate JO genes, we tested for significantly enriched Gene Ontology categories [Carbon *et al.*, 2009; Ashburner *et al.*, 2000]. We found that significant proportion of the 274 genes serve signal transmission (20.7%, p=1.54•E-09) and encode ion channels (9.1%, p=1.49•E-06) or motors (4.5%, p=8.99•E-03). Several phototransducer components were also identified, including four rhodopsins [Earl & Britt, 2006], the G protein subunits Gbeta76C [Running Deer *et al.*, 1995] and Ggamma30A [Schulz *et al.*, 1999], Arrestin2 [Smith *et al.*, 1990], the phospholipase C NorpA [Paj *et al.*, 1976], the scaffolding matrix protein InaD [Shieh & Niemeyer, 1995], and the protein kinase C InaC [Ranganathan *et al.*, 1991]. Furthermore the transcription factor Glass [O'Neill *et al.*, 1995; Moses *et al.*, 1989; Treisman & Rubin, 1996] and the two phototransduction channels Trp [Minke 1977; Minke, 1982] and Trp-like [Phillips *et al.*, 1992; Niemeyer *et al.*, 1996] were included. Three additional TRP channels Iav, Nan, and Wtrw [Liu *et al.*, 2007] were identified, as well as six ionotropic glutamate receptors (IRs) [Benton *et al.*, 2009]. The TRP channels Iav and Nan are necessary for *Drosophila* hearing [Gong *et al.*, 2004; Kim *et al.*, 2003]. Apart from *nan* and *iav*, the

only known auditory relevant *Drosophila* genes that are included in the catalogue are *tilB* and *eys*. TilB [Göpfert *et al.*, 2005] is involved in cilia maintenance [Kavile *et al.*, 2010] and Eys protects JO neurons from environmental stress [Cook *et al.*, 2008]. Several motor proteins were identified, including the Myo3A homologue NinaC [Walsh *et al.*, 2002; Matsumoto *et al.*, 1987], the kinesin Klp68D [Pesavento *et al.*, 1994], and 7 axonemal dyneins [Goldstein & Gunawardena, 2000]. Ca. 25% of the candidate genes have not been associated with any Gene Ontology terms [Ashburner *et al.*, 2000].

To test whether the catalogue includes relatives of human auditory genes, we browsed the Homophila [Chien *et al.*, 2002] database that links fly genes to their related human genes and known human diseases. We found that 51 of the 275 genes have human relatives that are implicated in syndromic or in non-syndromic deafness, including Myo3A [Schneider *et al.*, 2006], EYA4 [Wayne *et al.*, 2001; Makishima *et al.*, 2007], PTPN11 [Porciello *et al.*, 2008; Gelb & Tataglia *et al.*, 2007], RAF1 [Gelb & Tataglia *et al.*, 2007], and PAX3 [Karaman & Aliagaoglu, 2006] (supplement Table 7.2).

4.5 Auditory organ gene expression

To determine whether candidate genes identified in the microarray are indeed expressed in the JO, we selected 15 genes and determined their expression pattern using *in situ* hybridization [Lehmann & Tautz, 1994]. Sense probes, which had the same reading frame and sequence as the mRNA of interest, were used as controls for each staining. *In situ* probes had a length between 200 and 500 base pairs and were produced via *in vitro* transcription (IVT) using RNA from the whole w^{1118} flies. Primers used for RT PCR are listed in 3.9.9.2. RT-PCR products separated in a 1.5% Agarose gel are shown in the supplement Figure 17. *In situ* hybridizations have been performed using antennal sections (thickness: 30µm).

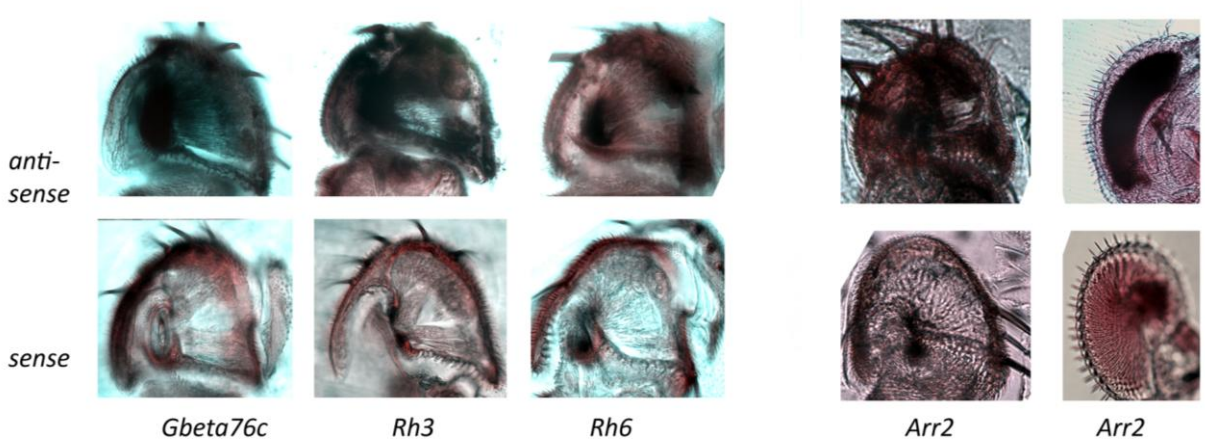


Figure 12 Auditory organ gene expression (phototransducer components). *In situ* hybridization are shown for the second antennal segments. For Arr2 hybridizations are also shown for the eye (right).

Anti-sense probes but not sense probes produced clear stainings in JO for all 16 selected genes. Expression was confirmed for four phototransduction genes: the G-protein *Gbeta76c* [Running Deer *et al.*, 1995], the rhodopsins *Rh3* [Zuker *et al.*, 1987] and *Rh6* [Huber *et al.*, 1997], and Arrestin *Arr2* [Smith *et al.*, 1990]. For *Arr2*, *in situ* hybridizations have also been performed in 30µm head and eye sections. Anti-sense probes, but not sense probes, stained cells in fly's eyes (Figure 12).

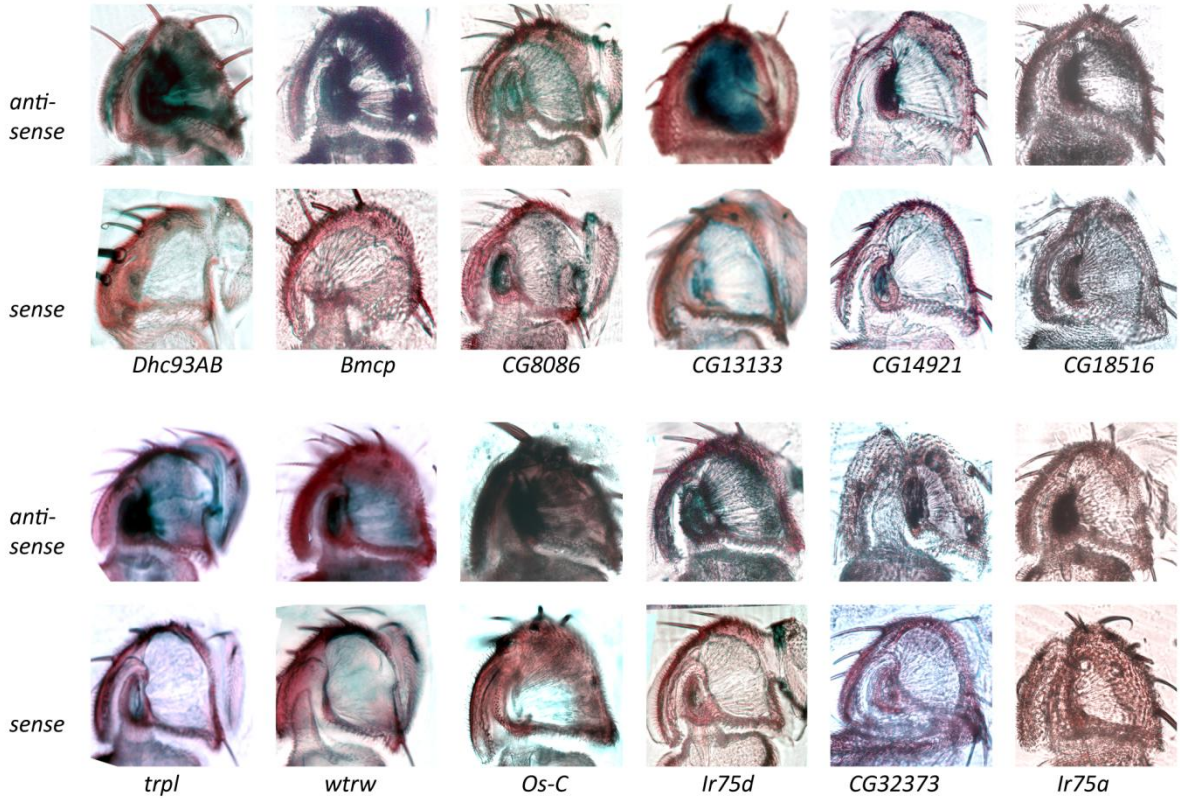


Figure 13 Auditory organ gene expression. *In situ* hybridization in the second antennal segment for *Dhc93AB* (*dynein heavy chain 93AB*), *Bmcp*, *CG8086*, *CG13133*, *CG14921*, and *CG18516*. *trpl* (trp-*like*), *wtrw* (*water witch*), Os-C, *CG14076*, *CG32373*, and *CG14585* are shown.

JO expression was also confirmed for the axonemal dynein heavy chain Dhc93AB [Goldstein & Gunawardena, 2000], for *Bmcp*, which belongs to the mitochondrial carrier super family [Fernández-Ayala *et al.*, 2010; Sánchez-Blanco *et al.*, 2006], for two TRP channels, *trp-like* [Phillips *et al.*, 1992; Niemeyer *et al.*, 1996] and *wtrw* [Liu *et al.*, 2007], and for two ionotropic receptors [Benton *et al.*, 2009], *Ir75a*, and *Ir75d*. *CG8086 is* a gene of unknown function [Lebo *et al.*, 2009]. *CG13133* encode a protein with HSP20-like chaperone domain [Tweedie *et al.*, 2009]. *CG14921* encode the homologue of the dyslexia susceptibility 1 candidate 1, and *CG18516* encodes a protein with electron carrier activity [Tweedie *et al.*, 2009]. *Os-C*, which encode a protein of unknown function is highly expressed in JO. Same is true for *CG32373* which encode a protein which is predicted to bind calcium [Tweedie *et al.*, 2009] (Figure 13). Together these results document JO expression for representative of diverse families of genes.

4.6 Cell type-specific gene expression

To determine cellular expression pattern, Gal4 promoter fusion constructs [Fischer *et al.*, 1988; Brand & Perrimon, 1993] were generated for 9 genes. Promoter fusion constructs were generated by ligating the extended promoter sequence into the pPTGAL vector [Sharma *et al.*, 2002], upstream of the gene sequence that codes for the Gal4 protein of yeast. Transgenic flies carrying these Gal4 constructs were crossed to flies carrying the reporter genes 2xEYFP or 2XEGFP under the control upstream activating sequence (UAS) [Halfon *et al.*, 2002] using the Gal4/UAS-system [Fischer *et al.*, 1988; Brand & Perrimon, 1993]. Expression of reporter genes in the adult antenna was examined via confocal microscopy (Figure 14).

Gal4 lines have been generated for three genes encoding motor proteins [Goldstein & Gunawardena, 2000]: *Klp68D*, which encodes a kinesin motor [Pesavento *et al.*, 1994], the *Dhc93AB*, which encodes a dynein heavy chain, and *CG9313*, which encodes a dynein intermediate chain. All three genes are widely expressed in JO sensory neurons. *CG4329* [Baeg *et al.*, 2005], a WD40-containing protein [Tweedie *et al.*, 2009; Leutunic *et al.*, 2009], is also widely expressed in the neurons.

Subpopulations of JO neurons are labeled by *CG11253-Gal4*, *CG14921-Gal4*, and *CG13636-Gal4*. *CG11253* encodes a putative interaction partner of TilB [Giot *et al.*, 2003], *CG14921* encodes the dyslexia susceptibility 1 candidate 1 homologue and *CG13636* encodes two small protein isoforms of unknown function that are insect-specific.

Two genes were found to be expressed in support cells of JO. *Naam* (*Nicotinamide amidase*), which is expressed in JO scolopale cells, encodes a calcium binding protein that is involved in negative regulation of neuronal apoptosis [Balan *et al.*, 2008]. *Ir94b*, an ionotropic glutamate receptor [Benton *et al.*, 2009] is expressed in JO ligament cells. In addition, *Ir94b* is expressed in some cells of the third antennal segment and in the ocelli (supplement Figure 18). Accordingly, the catalogue includes genes that are expressed in different cell types of JO.

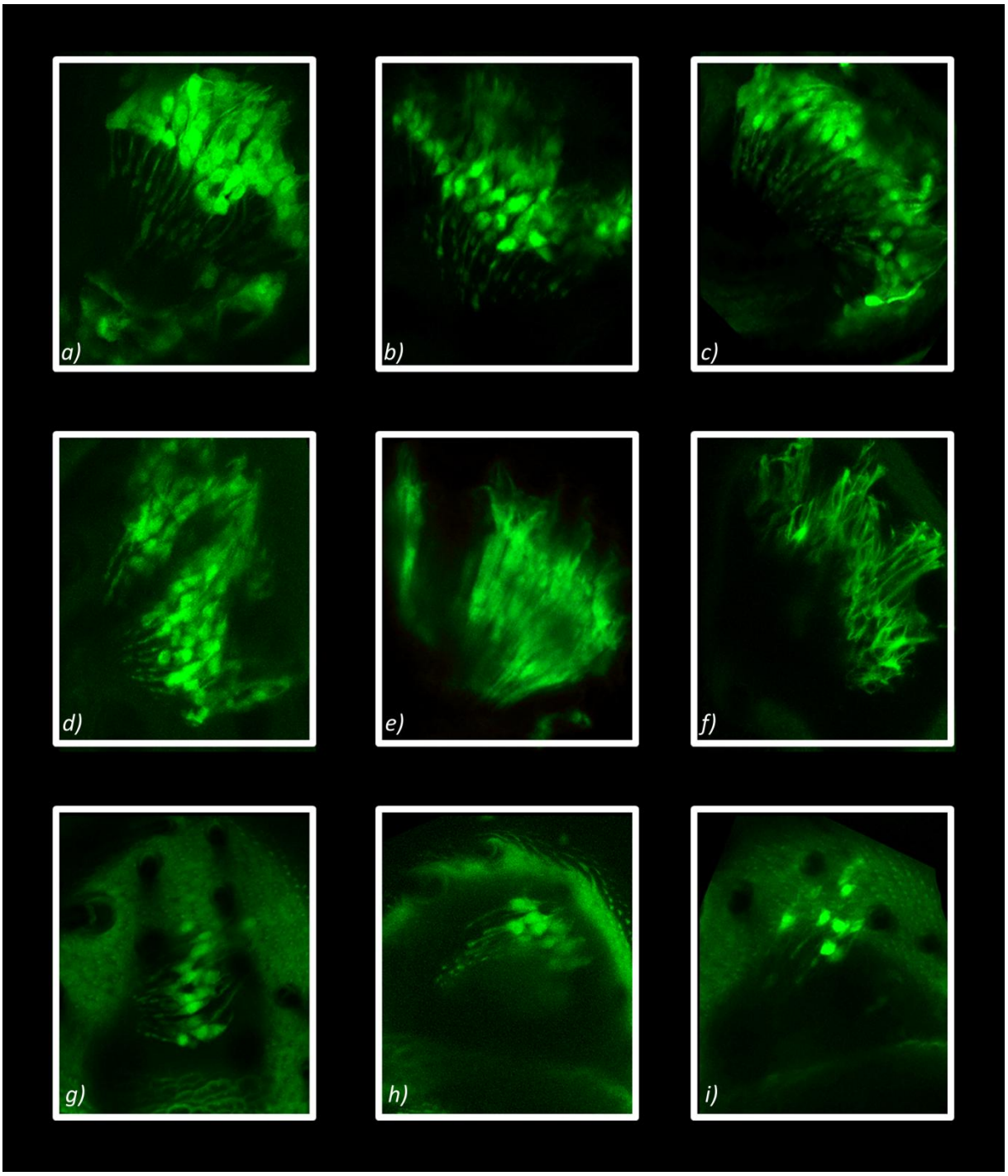


Figure 14 Cell type-specific gene expression. Flies carrying Gal4 constructs were crossed with flies carrying *UAS-2xEGFP* or *UAS-2xEYFP* reporters. Confocal images of the second antennal segments are shown for the following Gal4-constructs: a) *Klp68D-Gal4*, b) *Dhc93AB-Gal4*, c) *CG9313-Gal4*, d) *CG4329-Gal4*, e) *Naam-Gal4*, f) *Ir94b-Gal4*, g) *CG11253-Gal4*, h) *CG14921-Gal4*, and i) *CG13636-Gal4*.

4.7 Gene requirements for JO function

To evaluate whether the catalogue includes genes that contribute to JO function, we disrupted 39 selected genes and tested for alterations in sound-evoked mechanical and electrical responses of JO. Genes were selected according to the availability of classic mutants or mutants carrying intronic transposon insertions. The efficacy of all mutations was assessed using qPCR (supplement Table 11). JO responses were evoked by stimulating the flies with pure tones near the best frequency of their antennal sound receiver, which was determined from the power spectrum of its free fluctuations (Göpfert *et al.*, 2005). The gain in mechanical sensitivity provided by the motility of JO neurons was deduced from displacement responses of the antenna (Göpfert *et al.*, 2006), and their electrical activity was assessed by recording compound action potentials from the antennal nerve. All measurements were performed by David Piepenbrock. Five flies were tested for each mutant line, and w^{1118}, CantonS, and OregonR flies were used as controls. Significant changes in JO performance were identified using two-tailed Mann-Whitney U-tests.

Mutations in three genes caused deafness, entirely abolishing sound-evoked mechanical and electrical responses of JO. The respective genes are the dyslexia susceptibility 1 candidate 1 homologue gene *CG14921* as well as *Dhc93AB* and *CG9492*, which both encode axonemal dynein heavy chains [Goldstein & Gunawardena, 2000]. Mutations in 25 genes caused hearing impairments, reducing the mechanical activity of JO neurons and the sensitivity of their electrical responses to sound. The respective genes include *CG11253*, *which* encodes the putative TilB binding protein [Giot *et al.*, 2003], the insect specific *CG13636*, *trpl* [Phillips *et al.*, 1992; Niemeyer *et al.*, 1996], *Arr2* [Smith *et al.*, 1990] *Rh5* [Papatsenko *et al.*, 1997]. Mutations in 2 genes increased the motility of JO neurons and their sensitivity to sound. The respective genes are *bw*, which encodes an ABC-transporter [Waaler, 1921; Dreesen *et al.*, 1988], and *norpA* that encodes phospholipaseC [Paj *et al.*, 1976]. Taken together, mutations in 30 of the 39 genes cause alterations in JO sound responses, which means that ca. ¾ of the gene from the catalogue seem to be functionally relevant JO genes.

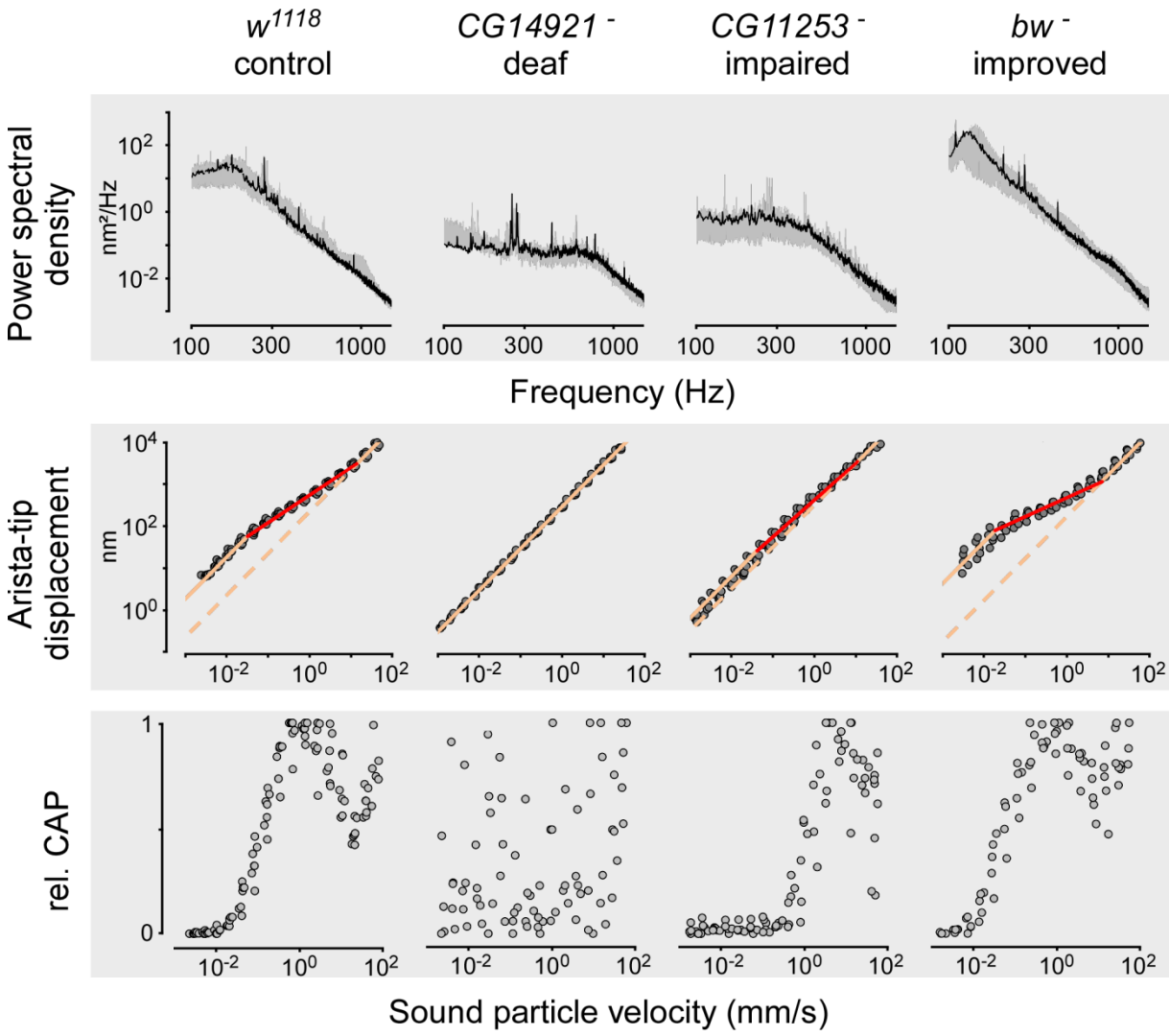


Figure 15 JO function. Power spectra of receiver fluctuations (upper panels), receiver displacement as a function of the sound particle velocity (middle panels), and relative amplitude of compound action potentials as a function of the sound particle velocity (lover panels) in w^{1118} controls and *CG14921*, *CG11253*, and *bw* mutants. One of the mutants is deaf (*CG14921*), one hearing impaired (*CG11253*), and one shows improved hearing (*bw*).

Table 8 Mutant genotypes and respective auditory phenotypes and mRNA expression levels. Expression levels are compared to those of w^{1118} controls.

Tested line	**Affected gene**	**Hearing ability**
y[1]; ry[506] P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG31216[KG10548]	*Naam*	impaired
y[1] w[*]; P{y[+m8]=Mae-UAS.6.11}Dhc36C[LA00085]	*Dhc36c*	impaired
w[1118]; PBac{w[+mC]=WH}CG14693[f03110]	*CG14693*	impaired
w[1118]; PBac{w[+mC]=WH}Ank2[f02001] CG32373[f02001]/TM6B, Tb[1]	*Ank2*	impaired
y[1] w[67c23]; P{w[+mC] y[+mDint2]=EPgy2}CG11253[EY10866]	*CG11253*	impaired
w[1118]; PBac{w[+mC]=WH}CG8086[f03214]	*CG8086*	impaired
y[1] w[67c23]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG9492[KG02504] ry[506]	*CG9492*	deaf
w[1118]; Mi{ET1}CG13636[MB03846]	*CG13636*	impaired
norpA[7]	*norpA*	improved
bw[1]	*Bw*	improved
w[1118]; Mi{ET1}ninaC[MB02664]	*ninaC*	normal
w[1118]; P{w[+mGT]=GT1}Bmcp[BG02446]	*Bmcp*	impaired
y[1] w[67c23]; Mi{ET1}CG4329[MB01614]	*CG4329*	impaired
y[1] w[1118];PBac{y[+mDint]=3HPy[+]} CG14921[C247]/CyO	*CG14921*	deaf
w[1118]; Mi{ET1}CG14636[MB03866]	*CG14636*	impaired
w[1118]; Mi{ET1}CG31424[MB02190]	*Ir94b*	normal
w[1118]; Mi{ET1}Dhc93AB[MB05444]	*Dhc93AB*	deaf
w[1118]; Mi{ET1}CG10633[MB05283]	*CG10633*	normal
y[1] w[67c23]; P{w[+mC] y[+mDint2]=EPgy2}Klp68D[EY00199]	*Klp68D*	impaired
gl[3]	*gl*	impaired
w[1118]; Mi{ET1}gol[MB03006]	*gol*	impaired
w[1118]; Mi{ET1}CG8419[MB06410]	*CG8419*	normal
y[1] w[*]; stops[1]	*stops*	impaired
y[1]; Mi{ET1}CG2052[MB00729]	*Lin29*	impaired
w[1118]; Mi{ET1}Sulf1[MB11661]	*Sulf1*	normal
CG9313[Z2-5863]	*CG9313*	impaired
arr2[5]	*Arr2*	impaired
trp[343]	*trpl*	impaired
inaD[1]	*inaD*	impaired
y[1] futsch[K68]	*futsch*	normal
w; PBac{GAL4D,EYFP}yuriPL00114 cn1 bw1; P{FRT(whs)}2A P{neoFRT}82B*	*yuri*	normal
w[1118]; Mi{ET1}kek4[MB11415]	*kek4*	impaired
w[1118]; Mi{ET1}CG6053[MB06262]	*CG6053*	impaired
w[1118]; P{w[+mC]=EPg}sei[HP21840]	*sei*	impaired
w[*]; ninaA[1]	*ninaA*	normal
trp[343]	*trp*	normal
w1118; PBac{RB}CG11388e03063	*CG11388*	impaired
rh5[2]	*Rh5*	impaired
rh6[1]	*Rh6*	impaired

4.8 Rhodopsin and Glass expression

Antibody stainings have been performed against proteins that are involved in phototransduction (Figure 16): the transcription factor Glass [O'Neill *et al.*, 1995; Moses *et al.*, 1989; Treisman & Rubin, 1996] and Rhodopsins [Earl & Britt, 2006; Johnson & Pak, 1986]. The antibody staining against Glass labels cells of unknown identity in JO. Rhodopsin antibody, which is directed against all *Drosophila* Rhodopsins, stained some cells in the second and third antennal segments, including cells surrounding the sacculus [Gupta & Rodrigues, 1997; Jhaveri *et al.*, 2000]. When the antibody staining against Rhodopsin was performed in flies expressing GFP in JO sensory neurons, no co-localization was observed.

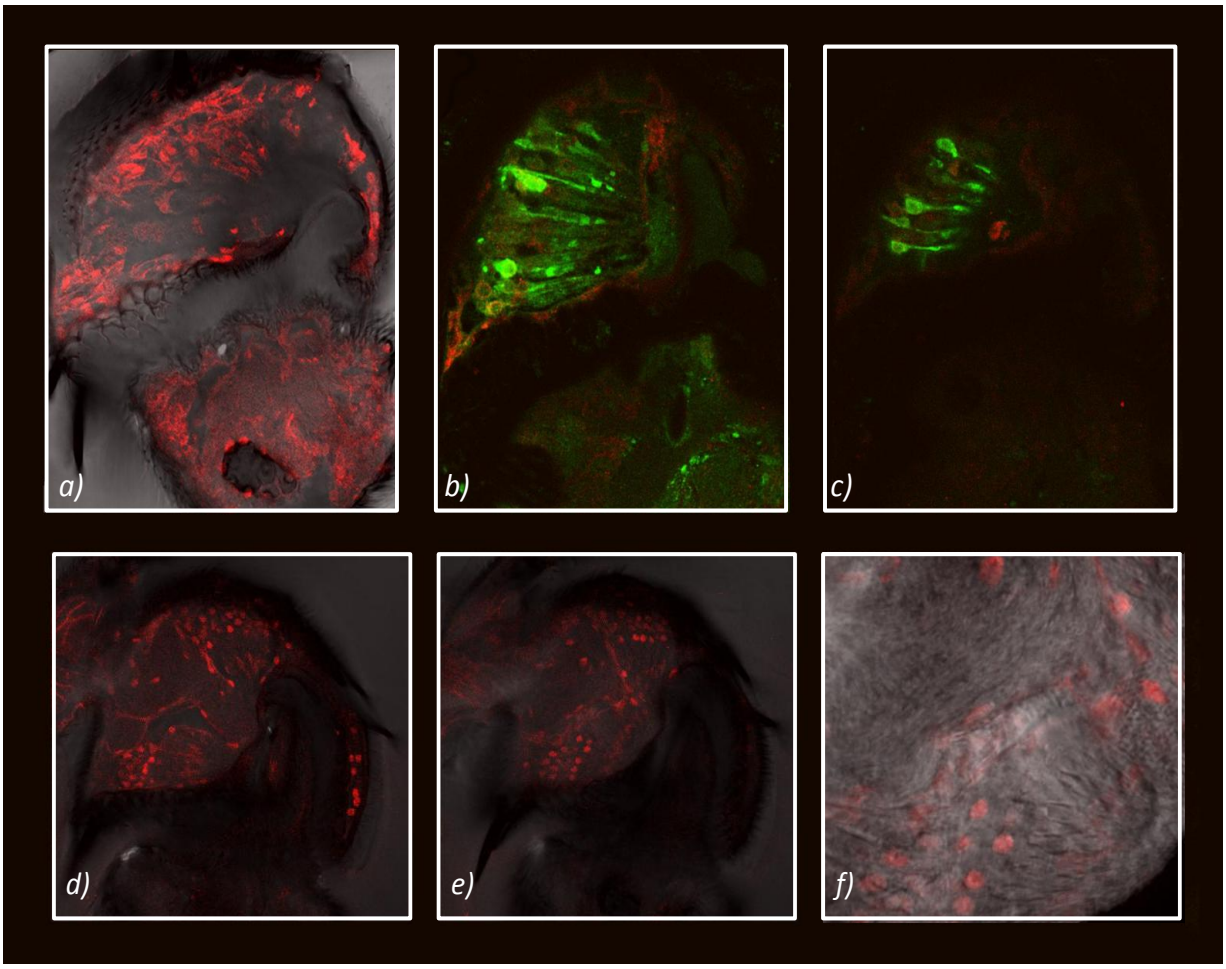


Figure 16 Rhodopsin and Glass expression in JO. Antibody staining have been performed on 30µm antennal sections a) Rhodopsin antibody staining in w^{1118} background (primary: 4C5-s, secondary: anti-mouse Alexa Fluor® 546), b-c) Rhodopsin antibody staining in *Dhc93AB-Gal4* x *UAS-2xEGFP* background red: Rhodopsin antibody; green: JO neurons (primary: 4C5-s & anti-GFP, secondary: Cy5 anti-mouse & anti-chicken Alexa Fluor® 488), d-f) Glass antibody staining in *w1118* background (primary: anti-glass, secondary: anti-mouse Alexa Fluor® 546)

5 Discussion

Mechano-, chemo-, and photoreceptors are developmentally specified by transcription factors of the *atonal* and *achaete-scute* families across taxa [Jarman & Ahmed, 1998; Fritsch & Beisel, 2001; Simionato *et al.*, 2008]. Here, we show that these transcription factors can be used to define the genetic repertoire of sensory cells. The approach is illustrated using the *Drosophila* auditory organ as an example, and should in principle be applicable to a wide range of sensory organs and cells.

By comparing gene expression profiles between *atonal* mutants and controls, we have identified 274 genes that are downregulated in the absence of –and thus seem to be expressed in– the *Drosophila* auditory organ. Gene expression in the auditory organ is confirmed by *in situ* hybridization and promoter fusions, and many of the genes are shown to contribute to auditory organ function. Our analysis doubles the number of the auditory-relevant *Drosophila* genes and documents auditory roles for representatives of diverse gene families. For example, novel ion channels and motors for hearing are identified, some of which are shown to be required for auditory transduction and may serve as auditory transducer components. The newly defined genes for hearing also include ciliary compartment genes that, judged from our analysis, are required for mechanosensory cilium function. Our analysis also documents auditory roles for chemo- and phototransduction genes such as ionotropic receptors and rhodopsins, adding new levels of complexity to auditory organ function and extending the genetic parallels between mechano-, chemo-, and photoreceptor cells from development to sensory signal processing.

5.1 Screen identifies novel auditory organ genes

According to gene ontology, 23 annotated *Drosophila* genes have so far been associated with the sensory perception of sound (GO:0007605). Three of these genes were identified in our screen: *nan* and *iav*, which encode TRPV (Transient Receptor Potential Vanilloid) channels that are expressed in the sensory cilia of JO neurons [Gong *et al.*, 2004; Kim *et al.*, 2003], and *tilB*, which encodes a leucine rich protein that is involved in cilium formation or maintenance [Kavile *et al.*, 2010]. Orthologues of *nan* and *iav* are expressed in vertebrate hair cells [Liedke *et al.*, 2000], and the human homologue of *tilB*, *Lrr6* (*leucine rich repeat containing 6; LRTP*), is expressed in spermatocytes and is assumed to be a cell cycle regulator [Xue & Goldberg, 2000]. We also identified *Eyes shut*, which reportedly protects JO neurons from environmental stress [Cook *et al.*, 2008], and *yuri*, which is expressed in JO and involved in gravity sensing [Armstrong *et al.*, 2006; Baker *et al.*, 2007]. Known auditory-relevant

Drosophila genes that have not been identified in our study such as *crinkled* [Todi *et al.*, 2008], *cp309* [Martinez-Campos *et al.*, 2004], *cut* [Ebacher *et al.*, 2007], *diaphanous* [Cosetti *et al.*, 2008], *forked* [Cosetti *et al.*, 2008], and *atonal* [Jarman *et al.*, 1993] are mostly implicated in JO morphgenesis and may not be transcribed in adults. Hence, most of the 274 genes identified by our screen have not previously been associated with JO, and all the 30 genes whose disruption is shown to affect JO function are novel auditory relevant genes.

5.2 Auditory organ genes include conserved genes of cilia and mechanosensory cells

JO neurons use cilia for mechanotransduction. 66 ciliary genes that are only found in the genomes of organisms that are endowed with cilia were identified by Avidor-Reiss (2004). According to our screen, at least 9 of these 66 genes are expressed in the *Drosophila* JO (hypergeometric probability P = 4.6 $\bullet 10^{-6}$). Six of the 9 genes encode axonemal dynein motor components, and disrupting four of these dyneins (*CG6053*, *CG9492*, *CG9313*, and *Dhc36c*) is shown to affect JO function. Apparently, the mechanosensory function of JO relies at least partly on conserved cilium genes.

To assess whether the JO genes identified in our screen are expressed in other types of mechanosensory cells, we tested for genes that are reportedly enriched in campaniform mechanoreceptors of the *Drosophila* haltere [Bechstedt *et al.*, 2010]. Campaniform receptors monitor cuticular deformations and are developmentally specified by *achaete-scute* transcription factors [Jennings *et al.*, 1995]. Of the 625 campaniform receptor genes identified by Bechstedt *et al.*(2010), we identified 62 identified in JO (P = 3.6 $\bullet$ 10^{-27}). Examples include *Ank2*, which encodes a cytoskeletal protein [Bouley *et al.*, 2000; Koch *et al.*, 2008], rdga, which encodes diacylglycerol kinase [Harris & Stark, 1977], *wtrw*, which encodes a TRP channel [Liu *et al.*, 2007], and the Shaker potassium channel encoded by *Sh* [Kaplan, 1969]. Accordingly, at least one quarter of the JO genes identified in our screen seem to be expressed in other *Drosophila* mechanoreceptors as well.

At least 13 of the ca. 1000 genes that are enriched in zebrafish hair cells according to McDermott *et al.*, (2007) have orthologs that are expressed in JO. Examples include the gluthathione S-transferase Sepia [Kim *et al.*, 2006], the dynein heavy chain Dhc16F [Rasmusson *et al.*, 1994], the NDRG3 homolog MESK2 [Zhao *et al.*, 2001], and Calmodulin [Yamanaka *et al.*, 1987; Doyle *et al.*, 1990]. Additionally TilB and its putative binding partner CG11253 seem to be expressed in zebrafish hair cells and in JO. Whereas *tilB* is widely expressed in JO neurons and essentially required for JO

function [Kavile *et al.*, 2010], *CG11253* is expressed in a subset of JO neurons and specifically required for sensitive hearing. *CG11253* may thus a gene that distinguishes JO sound-receptors from JO gravity/wind-receptor cells [Kamikouchi *et al.*, 2009; Yoruzu *et al.*, 2009].

To determine further highly conserved candidate genes, a systematic BLAST analysis (3.9.11) was performed. We found that 190 of the 274 (69%) JO genes have related human cognates (E-Value $\leq 10^{-10}$), and 105 (38%) of these cognates are highly conserved (E-Value$\leq 10^{-50}$). The conserved genes encode dyneins [Goldstein & Gunawardena, 2000], the cytoskeletal protein Ank2 [Koch *et al.*, 2008], the calcium/sodium antiporter Calx [Schwarz & Benzer, 1997], the ubiquitin-specific protease faf [Wu *et al.*, 1999; Fischer-Vize *et al.*, 1992], the anion exchanger Ndae1 [Romero *et al.*, 2000], and the voltage-gated calcium channels cac and stj [Chan *et al.*, 2002; Ly *et al.*, 2008]. In addition, screening Homophila database revealed that 51 JO genes have human relatives, which are involved in syndromic and nonsyndromic deafness. Overall, theese indicate that many JO genes are conserved throughout taxa.

5.3 Auditory organ genes include candidate mechanotransducer components

Analogous to the transduction modules of vertebrate hair cells, those of the fly's JO neurons reportedly consist of serially arranged force-gated ion channels and adaptation motors [Albert *et al.*, 2007b; Nadrowski *et al.*, 2008]. The TRP channel NompC has been surmised to serve as transduction channel in JO sound-receptors [Göpfert *et al.*, 2006; Kamikouchi *et al.*, 2009; Nadrowski *et al.*, 2010], but neither the transduction channels nor the adaptation motors have molecularly been identified yet.

Apart from NompC, TRP channels Nan and Iav as well as Pyrexia and Pain are reported to be expressed in JO [Sun *et al.*, 2009]. We show that also Trp, Trpl, and Wtrw are also expressed in this organ, which means that JO expresses at least 8 of the totally 13 *Drosophila* TRPs. The newly added TRPs are implicated in hygrosensation (Wtrw) [Liu *et al.*, 2007] as well as in thermosensation and vision (TRP and TRPL) [Minke, 1982; Philips *et al.*, 1992; Niemeyer *et al.*, 1996; Rosenzweig *et al.*, 2008; Montell, 2005]. Judged from our functional analyses, mutations in *trpl* phenocopy the auditory defects caused by loss-of-function alleles of *nompC.* Besides NompC, TRPL thus is a candidate for the fly's auditory transduction channel.

Motor proteins that are reportedly expressed in JO are the dynein heavy chain Btv [Eberl *et al.*, 2000] and the kinesin associated protein Kap3 [Sarpal *et al.*, 2003], both of which are implicated in intraflagellar transport. Electron microscopy indicates that the proximal regions of the sensory cilia of JO neurons are endowed with axonemal dynein arms, but whether axonemal dyneins are expressed in -and required for the function of- JO had hitherto remained unclear. Our analysis shows that at least 9 dynein axonemal dyneins are expressed in JO. Based on our functional analyses, at least 4 of them are required for mechanosensory cilium function and might serve as adaptation motors.

5.4 *atonal*: Connecting mechano-, chemo-, phototransduction

atonal is the proneural gene of *Drosophila* chordotonal organs [Jarman *et al.*, 1993], photoreceptors [Jarman *et al.*, 1994], and chemosensory coeloconic sensilla [Gupta & Rodrigues, 1997], indicating that these organs share a common evolutionary origin. Because of genetic parallels in the formation of these organs, it has been proposed that these organs have evolved from an ato-dependent protosensory organ [Niwa *et al.*, 2004]. Our results extend the genetic parallels between these organs from development to function by demonstrating that key signaling molecules of photoreceptors and coeloconic sensilla contribute to chordotonal organ function.

Coeloconic sensilla are functionally distinguished from other *Drosophila* chemoreceptors in that they use ionotropic receptors (IRs) as chemosensory receptors [Benton *et al.*, 2009]. IRs are reportedly also expressed in receptors of the *Drosophila* sacculus, a sensory organ that is located in the third segment of the antenna and that presumably serve hygro- and thermosensation [Stocker & Gendre, 1988; Shanbhag *et al.*, 1995; Benton *et al.*, 2009; Ai *et al.*, 2010]. We found that at least 6 ionotropic receptors are expressed in JO, and that one of them (I*R94b*) is expressed in the ligament cells of JO chordotonal sensilla. We further show that IRs contribute to JO function, possibly by influencing ion homeostasis in JO chordotonal sensilla. Hence, the chemosensory receptors of coeloconic sensilla are present in chordotonal organs and contribute to chordotonal organ function.

Apart from IRs, we show that almost the entire *Drosophila* phototransduction cascade is present in JO, ranging from rhodopsins (Rh3, Rh4, Rh5 and Rh6) [Chou *et al.*, 1999] to phototransduction channels (Trp, Trpl) [Minke, 1977; Philips *et al.*, 1992]. Disrupting phototransducer components also affects JO function, whereby the phenotypes observed e.g. in *arr2 arr2*^{5} [Alloway & Dolph, 1999], *rh5*^{2} [Halfon *et al.*, 2002], *rh6*^{1} [Halfon *et al.*, 2002] *mutants* closely resemble those of *nompC* nulls. Like IRs, Rhodopsins seem not to be expressed in JO sensory neurons; this suggests that they serve regulatory roles than being integral mechanotransducer components.

The mere presence of phototransduction molecules in JO suggests that the function of this organ may be influenced by light. In *Drosophila*, many sensory organs are endowed with peripheral circadian clocks [Krishnan *et al.*, 1999]. The presence of such clock has been recently documented for the *Drosophila* femoral organ [Sehadova *et al.*, 2009], suggesting that a clock also might be present in JO. Circadian clocks are entrained by two main zeitgebers, i.e. light and temperature [Ben-Shlomo & Kyriacou, 2002]. Recent studies suggest that the temperature-entrainment of central circadian clocks involves chordotonal organs [Sehadova *et al.*, 2009], and several of the phototransduction genes we identified are also implicated in temperature-transduction, including for example the TRP channels TRP and TRPL [Rosenzweig *et al.*, 2008]. It will be interesting to see whether JO is also implicated in light-dependent clock entrainment, and particularly why this organ expresses not just one as seen in the ocelli [Pollock & Benzer, 1988], but four distinct rhodopsins.

6 References

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7 Supplement

7.1 JO genes and their annotated functions

Table 9 JO genes

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1622920_at	trp	calcium ion transport /// visual perception /// phototransduction /// sensory perception of smell /// protein localization /// olfactory learning /// light-induced release of internally sequestered calcium ion ///calcium-mediated signaling /// inhibition of phospholipase C activity involved in G-protein coupled receptor signaling pathway /// detection of light stimulus involved in visual perception /// transmembrane transport	ion channel activity /// intracellular ligand-gated calcium channel activity /// protein binding /// calmodulin binding /// light-activated voltage-gated calcium channel activity /// store-operated calcium channel activity /// protein homodimerization activity
1622932_s_at	sn	actin filament organization /// establishment or maintenance of cell polarity /// multicellular organismal development /// bristle morphogenesis /// epidermal cell differentiation /// microvillar actin bundle assembly /// actin cytoskeleton organization /// cuticle pattern formation /// hemocyte migration /// imaginal disc-derived wing hair organization /// wound healing /// oogenesis /// antennal morphogenesis /// neuron projection morphogenesis /// actin filament bundle assembly	actin binding /// protein binding, bridging /// actin filament binding
1623017_at	CG7149	phagocytosis, engulfment /// phospholipid biosynthetic process	diacylglycerol cholinephosphotransferase activity
1623069_s_at	CG17544	fatty acid beta-oxidation	acyl-CoA dehydrogenase activity /// acyl-CoA oxidase activity /// pristanoyl-CoA oxidase activity /// oxidoreductase activity, acting on the CH-CH group of donors /// FAD binding
1623128_at	CG13455	---	---
1623206_a_at	eya	optic lobe placode formation /// Bolwig's organ morphogenesis /// protein amino acid dephosphorylation /// pole cell migration /// spermatogenesis /// salivary gland morphogenesis /// eye-antennal disc morphogenesis /// mesoderm development /// metabolic process /// male gonad development /// response to light stimulus /// negative regulation of cell fate specification /// ovarian follicle cell development /// compound eye photoreceptor development /// regulation of transcription	phosphoprotein phosphatase activity /// protein serine/threonine phosphatase activity /// protein tyrosine phosphatase activity /// protein binding /// hydrolase activity /// nucleotide phosphatase activity /// lipid phosphatase activity /// metal ion binding
1623234_s_at	Calx	calcium ion transport /// cell communication /// phototransduction /// transmembrane transport	calcium:sodium antiporter activity
1623294_at	CG17669	---	---
1623342_at	CG8369	wing disc dorsal/ventral pattern formation	---
1623466_at	bab1	transcription /// eye-antennal disc morphogenesis /// leg disc morphogenesis /// imaginal disc-derived leg morphogenesis /// sex differentiation /// behavior /// female gonad development /// regulation of transcription /// regulation of developmental pigmentation /// sex-specific pigmentation /// negative regulation of developmental pigmentation /// negative regulation of male pigmentation	DNA binding /// transcription factor activity /// protein binding
1623682_a_at	Cam	detection of calcium ion /// protein amino acid phosphorylation /// mitotic spindle organization /// centriole replication /// deactivation of rhodopsin mediated signaling /// metarhodopsin inactivation /// regulation of light-activated channel activity /// adaptation of rhodopsin mediated signaling /// kinetochore organization /// positive regulation of NFAT protein import into nucleus	calcium ion binding /// protein binding /// calmodulin binding /// myosin heavy chain binding /// myosin VI head/neck binding
1623763_at	CG14274	---	protein binding
1623838_s_at	CG4629	protein amino acid phosphorylation /// cell adhesion /// regulation of cell shape	nucleotide binding /// protein serine/threonine kinase activity /// ATP binding /// transferase activity
1623862_at	ninaA	protein folding /// 'de novo' protein folding /// transport /// visual perception /// phototransduction, visible light /// rhodopsin biosynthetic process /// response to stimulus /// apoptosis in response to endoplasmic reticulum stress	peptidyl-prolyl cis-trans isomerase activity /// protein binding /// cyclosporin A binding /// isomerase activity
1623874_at	CG14215	---	---
1623894_a_at	CG8086	---	protein binding
1623917_a_at	Sh	regulation of action potential /// potassium ion transport /// potassium ion transport /// learning or memory /// courtship behavior /// flight behavior /// proboscis extension reflex /// larval locomotory behavior /// detection of visible light /// regulation of circadian sleep/wake cycle, sleep /// behavioral response to ether /// axon extension /// sensory perception of taste /// transmembrane transport /// regulation of synaptic activity	voltage-gated potassium channel activity /// protein binding
1623923_a_at	gl	compound eye photoreceptor fate commitment /// regulation of transcription, DNA-dependent /// visual perception /// entrainment of circadian clock /// response to red light /// ring gland development /// compound eye photoreceptor development /// entrainment of circadian clock by photoperiod	DNA binding /// specific RNA polymerase II transcription factor activity /// zinc ion binding

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1623966_s_at	Nrt	cell adhesion /// axonogenesis /// axon guidance /// axonal fasciculation /// central nervous system development	---
1623971_at	CG9150	metabolic process /// oxidation reduction	catalytic activity /// binding /// oxidoreductase activity /// oxidoreductase activity, acting on CH-OH group of donors
1624002_a_at	CG31291	---	protein binding
1624143_a_at	CG12071	phagocytosis, engulfment	nucleic acid binding /// zinc ion binding
1624265_at	l(1)G0196	inositol metabolic process	nucleotide binding /// inositol 1,3,4,5,6-pentakisphosphate kinase activity /// inositol hexakisphosphate 5-kinase activity /// acid phosphatase activity /// ATP binding /// kinase activity /// transferase activity /// diphosphoinositol-pentakisphosphate kinase activity
1624383_at		---	---
1624519_at	Gbeta76C	G-protein coupled receptor protein signaling pathway /// activation of phospholipase C activity /// visual perception /// phototransduction /// deactivation of rhodopsin mediated signaling	GTPase activity /// signal transducer activity /// protein binding
1624655_at	CG6472	lipid metabolic process	catalytic activity /// lipase activity /// hydrolase activity
1624755_a_at	flw	instar larval development /// protein amino acid dephosphorylation /// chromosome segregation /// cell adhesion /// ovarian nurse cell to oocyte transport /// female germline ring canal formation /// imaginal disc-derived wing morphogenesis /// mesoderm development /// striated muscle tissue development /// muscle attachment /// negative regulation of JNK cascade	protein serine/threonine phosphatase activity /// protein binding /// hydrolase activity /// myosin phosphatase activity /// metal ion binding
1624820_at	CG8800	---	protein binding /// GTP binding
1624834_at	CG14015	metabolic process	mannan endo-1,6-alpha-mannosidase activity /// hydrolase activity, acting on glycosyl bonds
1624877_at	CG1561	---	---
1625011_at	CG11206	---	---
1625262_at	CG17786	---	---
1625424_at	CG17360	---	protein binding
1625428_at	CG13707	---	protein binding
1625799_a_at	CG7196	---	---
1625874_at	CG6761	---	---
1625881_at	iav	calcium ion transport /// sensory perception of sound /// sensory perception of smell /// flight behavior /// response to heat /// behavioral response to cocaine /// transmembrane transport	calcium channel activity
1625954_at	CG2681	ubiquitin-dependent protein catabolic process /// multicellular organismal development	ubiquitin-protein ligase activity /// protein binding /// zinc ion binding
1625965_a_at	Osi2	---	---
1626200_s_at	tipE	sodium ion transport /// cellular response to heat /// male courtship behavior, veined wing generated song production /// positive regulation of sodium ion transport via voltage-gated sodium channel activity	voltage-gated sodium channel activity /// protein binding /// sodium channel regulator activity
1626251_at	Rh4	signal transduction /// G-protein coupled receptor protein signaling pathway /// visual perception /// phototransduction, UV /// protein-chromophore linkage	signal transducer activity /// G-protein coupled photoreceptor activity
1626358_at	Fer1	regulation of transcription	transcription factor activity
1626404_a_at	bsk	MAPKKK cascade /// establishment of planar polarity /// compound eye photoreceptor fate commitment /// glycogen biosynthetic process /// protein amino acid phosphorylation /// defense response /// response to oxidative stress /// JUN phosphorylation /// border follicle cell migration ///initiation of dorsal closure /// response to heat /// Wnt receptor signaling pathway /// antibacterial humoral response /// ovarian follicle cell development /// wound healing, spreading of epidermal cells /// negative regulation of JUN kinase activity /// imaginal disc fusion, thorax closure /// dorsal appendage formation /// micropyle formation /// embryonic morphogenesis /// axon extension	nucleotide binding /// protein serine/threonine kinase activity /// phosphorylase kinase activity /// JUN kinase activity /// MAP kinase activity /// protein binding /// calmodulin binding /// ATP binding /// transferase activity
1626537_at	CG17321	---	---
1626540_at	CG14077 CG14076	ion transport /// oxidation reduction	cytochrome-c oxidase activity /// receptor activity /// ionotropic glutamate receptor activity /// extracellular-glutamate-gated ion channel activity /// oxidoreductase activity
1626574_at	CG9935	transport /// ion transport	ionotropic glutamate receptor activity /// extracellular-glutamate-gated ion channel activity /// kainate selective glutamate receptor activity
1626593_at	CG14693	---	---
1626722_at	CG14087	---	---
1626740_at	Cpr72Ec	---	structural constituent of chitin-based cuticle
1626753_at	CG12947	---	---
1626897_at	HisCl1	ion transport	extracellular-glycine-gated ion channel activity /// histamine-gated chloride channel activity

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1626946_at	oc	embryonic development via the syncytial blastoderm /// compound eye morphogenesis /// regulation of transcription/// anterior region determination /// pattern specification process /// central nervous system development /// adult walking behavior /// ocellus development /// brain segmentation /// anterior head segmentation /// compound eye photoreceptor development /// rhabdomere development /// regulation of transcription /// photoreceptor cell fate commitment /// ocellus morphogenesis	DNA binding /// transcription regulator activity /// sequence-specific DNA binding
1626959_at	Obp58b	transport /// sensory perception of chemical stimulus	odorant binding /// hydrolase activity
1627024_s_at	CG9164	---	sulfotransferase activity
1627074_at	CG10185	---	---
1627130_at	CG7568	---	---
1627167_a_at	CG40485	metabolic process /// oxidation reduction	catalytic activity /// oxidoreductase activity
1627288_a_at	Cpn	visual perception /// rhabdomere development /// response to stimulus	calcium ion binding /// protein binding
1627297_at	CG15270	---	---
1627406_at	CG3009	phospholipid metabolic process /// lipid catabolic process	phospholipase A2 activity /// calcium ion binding /// hydrolase activity
1627533_at	stj	neuromuscular synaptic transmission /// synaptic vesicle fusion to presynaptic membrane /// negative regulation of synaptic growth at neuromuscular junction	voltage-gated calcium channel activity
1627636_at	CG14636	---	---
1627662_at	Ugt35a	metabolic process	UDP-glycosyltransferase activity
1627711_at	CdsA	terminal branching, open tracheal system /// visual perception /// phototransduction /// phospholipid biosynthetic process /// CDP-diacylglycerol biosynthetic process /// rhodopsin mediated signaling pathway /// thermotaxis /// response to stimulus	phosphatidate cytidylyltransferase activity /// protein binding /// transferase activity, transferring phosphorus-containing groups /// nucleotidyltransferase activity
1627736_at	Actbeta	multicellular organismal development /// cell growth /// dendrite morphogenesis	transforming growth factor beta receptor binding /// growth factor activity
1627742_at	Bmcp	transport /// regulation of metabolic process /// transmembrane transport	transporter activity /// binding /// transmembrane transporter activity
1627755_at	CG14185	---	protein binding
1627825_at	CG13305	---	---
1627829_at	Rh3	signal transduction /// G-protein coupled receptor protein signaling pathway /// visual perception /// phototransduction, UV /// detection of UV /// absorption of UV light /// protein-chromophore linkage	signal transducer activity /// receptor activity /// G-protein coupled photoreceptor activity
1627935_a_at	Obp59a	transport /// sensory perception of chemical stimulus	odorant binding
1628005_at	chinmo	mushroom body development /// dendrite morphogenesis	transcription factor activity /// protein binding /// zinc ion binding
1628238_at	Tektin-C	microtubule cytoskeleton organization /// microtubule-based process	microtubule binding
1628245_at	sens-2	---	nucleic acid binding /// zinc ion binding
1628254_at	CG31847	---	---
1628275_at	Trl	nuclear division /// tRNA-type intron splice site recognition and cleavage /// chromatin organization /// regulation of transcription from RNA polymerase II promoter /// tRNA splicing, via endonucleolytic cleavage and ligation /// mitosis /// imaginal disc-derived wing morphogenesis /// dosage compensation /// chromatin modification /// syncytial blastoderm mitotic cell cycle /// oogenesis /// protein oligomerization	tRNA-intron endonuclease activity /// DNA binding /// specific RNA polymerase II transcription factor activity /// protein binding /// zinc ion binding /// transcription activator activity /// protein heterodimerization activity
1628369_at	boss	signal transduction /// G-protein coupled receptor protein signaling pathway /// R7 cell fate commitment /// visual perception /// response to glucose stimulus /// glucose homeostasis /// R7 cell differentiation /// R7 cell development /// R8 cell-mediated photoreceptor organization /// sevenless signaling pathway /// compound eye development /// response to stimulus /// lipid homeostasis	signal transducer activity /// transmembrane receptor activity /// G-protein coupled receptor activity /// receptor binding /// sevenless binding
1628572_s_at	stops	deactivation of rhodopsin mediated signaling /// intracellular signaling pathway	---
1628604_at	D	instar larval development /// multicellular organismal development /// blastoderm segmentation /// central nervous system development /// brain development /// hindgut morphogenesis /// regulation of gene-specific transcription from RNA polymerase II promoter /// negative regulation of gene-specific transcription from RNA polymerase II promoter /// post-embryonic appendage morphogenesis positive regulation of transcription, DNA-dependent	transcription factor binding /// DNA bending activity /// transcription activator activity /// sequence-specific DNA binding
1628616_at	CG8419	---	protein binding /// zinc ion binding
1628647_at	a5	---	phosphatidylethanolamine binding
1628725_at	CG14127	---	---
1628730_at	rdgA	activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway /// visual perception /// response to stimulus	nucleotide binding /// diacylglycerol kinase activity /// ATP binding /// transferase activity /// metal ion binding
1628754_s_at	Ggamma30A	signal transduction /// G-protein coupled receptor protein signaling pathway /// visual perception /// phototransduction /// response to stimulus	GTPase activity /// signal transducer activity
1628763_at	Ptpmeg	protein amino acid dephosphorylation /// mushroom body development /// salivary gland cell autophagic cell death /// central complex development /// axon extension involved in axon guidance	phosphoprotein phosphatase activity /// protein tyrosine phosphatase activity /// cytoskeletal protein binding /// hydrolase activity

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1628806_at	Rh5	G-protein coupled receptor protein signaling pathway /// visual perception /// phototransduction /// UV-A, blue light absorption of visible light /// protein-chromophore linkage	signal transducer activity /// G-protein coupled photoreceptor activity
1629129_at	Tie	protein amino acid phosphorylation	nucleotide binding /// receptor activity /// protein binding /// ATP binding /// kinase activity /// transferase activity
1629147_at	mRpL37	translation	structural constituent of ribosome
Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1629225_s_at	CG31386	---	---
1629325_at		---	---
1629417_s_at	CG14853	---	protein binding
1629418_s_at	sei	potassium ion transport	voltage-gated potassium channel activity
1629421_at	CG16789	---	ATP binding
1629540_a_at	bw	eye pigment biosynthetic process /// ommochrome biosynthetic process /// pteridine biosynthetic process /// eye pigment precursor transport	nucleotide binding /// eye pigment precursor transporter activity /// ATP binding /// pigment binding /// ATPase activity, coupled to transmembrane movement of substances
1629593_at	CG10866	---	---
1629729_at	CG31019	proteolysis	metallocarboxypeptidase activity /// zinc ion binding /// hydrolase activity
1629747_at	Cpr49Ag	---	structural constituent of chitin-based cuticle
1629778_s_at	CG5130	cation transport	zinc ion transmembrane transporter activity
1629817_at	CG3339	microtubule-based movement	microtubule motor activity /// ATP binding /// hydrolase activity /// ATPase activity, coupled /// ATPase activity, uncoupled
1629887_at	CG8180	---	---
1629897_a_at	CG6044	---	---
1629976_at	Ir94b	---	---
1630018_at	spn-B	DNA double-strand break repair /// regulation of translation /// meiosis /// reciprocal meiotic recombination /// germarium-derived oocyte fate determination /// intracellular mRNA localization /// polarity specification of anterior/posterior axis /// polarity specification of dorsal/ventral axis /// oocyte differentiation /// chromosome condensation /// karyosome formation /// oogenesis	recombinase activity /// DNA binding /// protein binding /// ATP binding /// DNA-dependent ATPase activity /// hydrolase activity /// ATPase activity, uncoupled
1630065_at	CG6912	---	protein binding
1630106_at	CG10362	intracellular signaling pathway	protein binding /// diacylglycerol binding /// metal ion binding
1630118_s_at	Dyb	---	calcium ion binding /// cytoskeletal protein binding /// zinc ion binding /// structural constituent of muscle
1630119_s_at	norpA	mucosal immune response /// phospholipid metabolic process /// diacylglycerol biosynthetic process /// sensory perception of smell /// adult locomotory behavior /// light-induced release of internally sequestered calcium ion /// rhodopsin mediated phototransduction /// response to abiotic stimulus /// entrainment of circadian clock /// deactivation of rhodopsin mediated signaling /// calcium-mediated signaling /// phosphatidylinositol metabolic process /// negative regulation of compound eye retinal cell programmed cell death /// elevation of cytosolic calcium ion concentration involved in G-protein signaling coupled to IP3 second messenger	phosphoinositide phospholipase C activity /// GTPase activator activity /// calcium ion binding
1630163_at	CG32373	---	calcium ion binding
1630257_s_at	pncr015:3L-RA	---	---
1630304_at	Dhc93AB	microtubule-based movement	nucleotide binding /// microtubule motor activity /// ATP binding /// hydrolase activity ATPase activity, coupled /// ATPase activity, uncoupled
1630337_at	CG15927	---	---
1630376_at	onecut	regulation of transcription, DNA-dependent	DNA binding /// RNA polymerase II transcription factor activity /// sequence-specific DNA binding
1630437_s_at	CG15118	---	---
1630504_at	CG13830	---	calcium ion binding /// protein binding
1630577_at	CG17378	---	---
1630602_at	CG13842	---	---
1630766_at	CG13800	---	---
1630829_at	PIP5K59B	phosphorylation /// phosphatidylinositol metabolic process	phosphatidylinositol phosphate kinase activity /// 1-phosphatidylinositol-4-phosphate 5-kinase activity /// transferase activity
1630963_at	Pbprp4	transport /// sensory perception of chemical stimulus	phenylalkylamine binding
1631110_at	CG10257	negative regulation of apoptosis	---
1631168_at	CG5687	sodium ion transport	transporter activity /// sodium:iodide symporter activity
1631175_a_at	CG10283	---	protein binding
1631203_at	CG6362	---	---
1631403_at	CG14445	---	protein binding
1631491_at	CG9335	---	---
1631526_s_at	wtrw	calcium ion transport /// response to humidity	calcium channel activity

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1631552_at	pyr	hindgut morphogenesis /// heart development /// mesoderm migration /// pericardial cell differentiation /// larval somatic muscle development /// glial cell migration /// fibroblast growth factor receptor signaling pathway /// cardioblast differentiation /// positive regulation of glial cell proliferation	fibroblast growth factor receptor binding
1631555_at	CG10062	proteolysis	peptidase activity /// hydrolase activity
1631579_a_at	fry	imaginal disc-derived wing morphogenesis /// bristle morphogenesis /// Wnt receptor signaling pathway /// non-sensory hair organization /// imaginal disc-derived wing hair organization /// rhabdomere development /// regulation of transcription /// positive regulation of protein kinase activity /// antennal morphogenesis /// regulation of dendrite morphogenesis	protein binding /// transcription activator activity
1631651_at	CG14905	---	protein binding
1631659_at	Ir8a	transport /// ion transport	extracellular-glutamate-gated ion channel activity /// kainate selective glutamate receptor activity
1631662_a_at	Ank2	cytoskeletal anchoring at plasma membrane /// signal transduction /// axon extension	structural constituent of cytoskeleton /// cytoskeletal protein binding
1632111_at	Arr2	visual perception /// phototransduction /// deactivation of rhodopsin mediated signaling /// metarhodopsin inactivation	metarhodopsin binding
1632183_at	ru	compound eye photoreceptor fate commitment /// signal transduction /// epidermal growth factor receptor signaling pathway /// epithelial cell migration, open tracheal system /// leg disc proximal/distal pattern formation /// compound eye cone cell fate commitment	serine-type endopeptidase activity /// hydrolase activity
1632319_at	CG18598	---	---
1632392_s_at	Slob	regulation of synaptic transmission	protein kinase activity /// protein binding
1632418_s_at	pum	nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay /// cell fate determination /// regulation of translation /// mitosis /// pole cell migration /// germ cell development /// long-term memory /// head involution /// regulation of synaptic growth at neuromuscular junction /// anterior/posterior axis specification, embryo /// cell migration /// negative regulation of translation /// germ-line stem cell division /// positive regulation of translation /// negative regulation of cell cycle /// negative regulation of transcription, DNA-dependent /// oogenesis /// dendrite morphogenesis /// regulation of synaptic transmission	translation repressor activity, nucleic acid binding /// mRNA 3'-UTR binding /// protein binding /// SUMO binding
1632478_a_at	Pkc53E	protein amino acid phosphorylation /// intracellular signaling pathway	nucleotide binding /// protein serine/threonine kinase activity /// calcium-dependent protein kinase C activity /// ATP binding /// zinc ion binding /// transferase activity /// diacylglycerol binding /// metal ion binding
1632570_at	CG2052	---	nucleic acid binding /// protein binding /// zinc ion binding
1632701_at	Pal	cellular metabolic process	peptidylamidoglycolate lyase activity /// lyase activity /// metal ion binding
1632753_a_at	Cep97	centriole replication	protein binding /// protein binding /// protein phosphatase type 1 regulator activity
1632968_at	CG6053	microtubule-based movement	motor activity /// hydrolase activity /// ATPase activity, coupled /// ATPase activity, uncoupled
1633198_a_at	5-HT2	G-protein coupled receptor protein signaling pathway /// serotonin receptor signaling pathway /// germ-band extension	G-protein coupled serotonin receptor activity
1633207_at	Ndae1	sodium ion transport /// chloride transport /// bicarbonate transport	transporter activity /// inorganic anion exchanger activity /// protein binding /// sodium:bicarbonate symporter activity
1633313_at	Ank2	cytoskeletal anchoring at plasma membrane /// signal transduction /// axon extension	structural constituent of cytoskeleton /// protein binding /// cytoskeletal protein binding
1633353_s_at	CAP	---	vinculin binding
1633393_at	CG6499	transmembrane transport	ammonium transmembrane transporter activity
1633499_at	retinin	---	---
1633636_at	Ir64a	ion transport	extracellular-glutamate-gated ion channel activity
1633646_at	inaF	response to light stimulus /// rhodopsin mediated signaling pathway /// regulation of membrane potential in photoreceptor cell /// regulation of membrane potential	calcium channel regulator activity /// protein binding
1633649_s_at	trpl	calcium ion transport /// body fluid secretion /// visual perception /// response to light stimulus /// calcium-mediated signaling /// detection of light stimulus involved in visual perception /// transmembrane transport	protein binding /// calmodulin binding /// light-activated voltage-gated calcium channel activity /// ion transmembrane transporter activity /// store-operated calcium channel activity /// protein heterodimerization activity
1633714_at	eys	temperature compensation of the circadian clock /// rhabdomere development	calcium ion binding /// protein binding
1633727_s_at	wnd	protein amino acid phosphorylation	protein tyrosine kinase activity /// ATP binding /// transferase activity
1633782_at	HDC06237	---	---
1633821_at	B52	regulation of alternative nuclear mRNA splicing, via spliceosome /// mRNA splice site selection /// mRNA processing	nucleotide binding /// nucleic acid binding /// RNA binding /// mRNA binding /// protein binding
1633825_at	CG14221	cell redox homeostasis	---
1633880_s_at	Ir76a	ion transport	ionotropic glutamate receptor activity
1633913_at	a10	sensory perception of chemical stimulus	pheromone binding

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1633998_s_at		---	---
1634050_at	CG17122	---	protein binding
1634054_at	ninaC	protein amino acid phosphorylation /// intracellular protein transport /// cytoskeleton organization /// visual perception /// phototransduction, visible light /// phototransduction, UV /// protein localization /// deactivation of rhodopsin mediated signaling /// dsRNA transport	nucleotide binding /// motor activity /// actin binding /// receptor signaling protein serine/threonine kinase activity /// calmodulin binding /// ATP binding /// kinase activity /// transferase activity /// phosphoinositide binding /// ATPase activity, coupled
1634061_a_at	rdgC	protein amino acid dephosphorylation /// visual perception /// phototransduction /// deactivation of rhodopsin mediated signaling /// calcium-mediated signaling /// thermotaxis /// photoreceptor cell maintenance /// response to stimulus /// detection of stimulus involved in sensory perception	calcium-dependent protein serine/threonine phosphatase activity /// iron ion binding /// calcium ion binding /// calmodulin binding /// hydrolase activity /// manganese ion binding
1634264_at	CG13202	---	---
1634272_at	CG18516	oxidation reduction	catalytic activity /// iron ion binding /// electron carrier activity /// oxidoreductase activity /// FAD binding /// iron-sulfur cluster binding
1634295_at	hoe2	citrate transport /// transmembrane transport	L-tyrosine transmembrane transporter activity /// citrate transmembrane transporter activity
1634325_a_at	MESK2	---	---
1634413_at	run	DNA-dependent positive regulation of transcription from RNA polymerase II promoter /// multicellular organismal development /// periodic partitioning by pair rule gene /// segment polarity determination /// germ-band extension /// neuroblast fate determination /// axon guidance /// central nervous system development /// ventral cord development /// sex determination, establishment of X:A ratio /// eye morphogenesis /// dendrite morphogenesis	specific RNA polymerase II transcription factor activity /// protein binding /// ATP binding /// transcription activator activity /// transcription regulator activity /// sequence-specific DNA binding
1634438_at	Pph13	regulation of transcription, DNA-dependent /// rhabdomere development	RNA polymerase II transcription factor activity /// sequence-specific DNA binding
1634570_at	CG5375	---	protein binding
1634631_at	CG3105	protein amino acid phosphorylation /// signal transduction	nucleotide binding /// protein serine/threonine kinase activity /// signal transducer activity /// protein binding /// ATP binding /// transferase activity
1634692_at	CG18336	---	---
1634752_a_at	CG14085	---	---
1634836_a_at	CG9317	transmembrane transport	secondary active organic cation transmembrane transporter activity
1634855_s_at	CG9813	ATP synthesis coupled proton transport	protein binding /// hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances
1634856_at	Hdc	cellular amino acid and derivative metabolic process /// carboxylic acid metabolic process /// compound eye photoreceptor development /// catecholamine biosynthetic process	catalytic activity /// histidine decarboxylase activity /// protein binding /// lyase activity /// carboxy-lyase activity /// pyridoxal phosphate binding
1634988_a_at	CG17352	---	---
1635007_at	Sulf1	pattern specification process /// metabolic process	catalytic activity /// N-acetylglucosamine-6-sulfatase activity /// sulfuric ester hydrolase activity /// alkyl sulfatase activity /// metal ion binding
1635158_at	CG13924	---	---
1635300_at	CG1268	ATP hydrolysis coupled proton transport	hydrogen-exporting ATPase activity, phosphorylative mechanism /// hydrolase activity
1635338_a_at	retinophilin	engulfment of apoptotic cell	protein binding
1635348_at	CG8086	---	protein binding
1635382_at	dlg1	morphogenesis of a polarized epithelium /// cytoskeleton organization /// cell adhesion /// signal transduction /// synaptic transmission /// multicellular organismal development /// dorsal closure /// central nervous system development /// peripheral nervous system development /// male courtship behavior /// asymmetric protein localization /// negative regulation of cell proliferation /// establishment or maintenance of polarity of embryonic epithelium /// establishment or maintenance of polarity of follicular epithelium /// morphogenesis of larval imaginal disc epithelium /// establishment or maintenance of polarity of larval imaginal disc epithelium /// septate junction assembly /// cell differentiation /// neuron differentiation /// regulation of border follicle cell delamination /// regulation of cell proliferation /// asymmetric protein localization involved in cell fate determination /// basal protein localization /// zonula adherens assembly /// establishment or maintenance of neuroblast polarity /// establishment or maintenance of epithelial cell apical/basal polarity /// locomotor rhythm /// positive phototaxis /// establishment of spindle orientation /// regulation of cell cycle	guanylate kinase activity /// signal transducer activity /// epidermal growth factor receptor binding /// structural molecule activity /// protein binding /// transferase activity
1635396_at	CG30203	---	serine-type endopeptidase inhibitor activity
1635400_at	dtr	synaptic transmission /// cilium morphogenesis	dynein binding
1635463_at	CG8407	microtubule-based movement	microtubule motor activity /// hydrolase activity /// ATPase activity, coupled /// ATPase activity, uncoupled
1635468_a_at	CG7730	---	

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1635519_at	CG13950	---	protein binding /// sugar binding
1635541_s_at	MESK2	---	---
1635644_at	CG33203	---	protein binding
1635757_at	laza	phototransduction /// response to light intensity /// dephosphorylation /// thermotaxis	catalytic activity /// phosphatidate phosphatase activity /// hydrolase activity
1635777_at	CG11041	---	calcium ion binding
1635802_s_at	CG9279	microtubule-based movement	---
1635961_at	qvr	positive regulation of circadian sleep/wake cycle, sleep /// rhythmic process	GPI anchor binding
1635963_a_at	CG7990	---	---
1636288_at	yuri	sperm individualization /// microtubule basal body organization /// gravitaxis	nucleotide binding /// protein binding
1636345_s_at	Syt1	transport /// regulation of pole plasm oskar mRNA localization /// larval locomotory behavior /// synaptic vesicle exocytosis /// vesicle-mediated transport /// synaptic vesicle endocytosis /// calcium ion-dependent exocytosis of neurotransmitter /// regulation of synapse structure and activity	transporter activity /// calcium ion binding /// protein binding /// calcium-dependent phospholipid binding
1636376_at	CG14947	---	---
1636378_a_at	Syn	neurotransmitter secretion /// behavior /// synaptic vesicle exocytosis	catalytic activity /// protein binding /// ATP binding
1636405_a_at	CG4329	---	---
1636407_at	gol	mesoderm formation /// regulation of transcription, DNA-dependent /// multicellular organismal development /// mesoderm development	DNA binding /// protein binding /// zinc ion binding /// transcription regulator activity
1636488_at	CG4468	---	protein binding
1636576_s_at	norpA	mucosal immune response /// lipid metabolic process /// phospholipid metabolic process /// diacylglycerol biosynthetic process /// signal transduction /// visual perception /// phototransduction /// sensory perception of smell /// adult locomotory behavior /// light-induced release of internally sequestered calcium ion /// rhodopsin mediated phototransduction /// response to abiotic stimulus /// entrainment of circadian clock /// lipid catabolic process /// deactivation of rhodopsin mediated signaling /// calcium-mediated signaling /// phosphatidylinositol metabolic process /// negative regulation of compound eye retinal cell programmed cell death /// elevation of cytosolic calcium ion concentration involved in G-protein signaling coupled to IP3 second messenger	phosphoinositide phospholipase C activity /// signal transducer activity /// GTPase activator activity /// calcium ion binding /// protein binding /// phosphoric diester hydrolase activity
1636602_at	CG11253	---	protein binding /// zinc ion binding
1636643_at	CG6983	---	---
1636745_at	Art4	chromatin modification /// histone methylation /// peptidyl-arginine methylation, to asymmetrical-dimethyl arginine /// induction of programmed cell death by ecdysone /// regulation of transcription	histone-arginine N-methyltransferase activity
1636818_at	CG13133	---	protein binding
1636838_a_at	n-syb	neurotransmitter secretion /// synaptic vesicle docking during exocytosis	SNAP receptor activity
1636865_at	Os-C	---	pheromone binding
1636880_s_at	cpx	neurotransmitter transport /// synaptic vesicle exocytosis /// synaptic growth at neuromuscular junction	neurotransmitter transporter activity /// syntaxin binding
1637030_at	CG5948	superoxide metabolic process /// oxidation reduction	superoxide dismutase activity /// oxidoreductase activity /// metal ion binding
1637110_at	CG3618	actin filament organization /// cell adhesion /// regulation of cell shape	guanylate cyclase activity /// lyase activity
1637157_at	CG34297	---	binding
1637389_at	Ir100a	---	---
1637435_at	kek4	---	---
1637514_at	CG14142	---	---
1637526_s_at	Ggamma30A	G-protein coupled receptor protein signaling pathway /// visual perception /// phototransduction	GTPase activity /// signal transducer activity
1637771_s_at	CG7220	proteolysis /// post-translational protein modification /// regulation of protein metabolic process	ubiquitin-protein ligase activity
1637801_at	se	pteridine biosynthetic process /// oxidation reduction	glutathione transferase activity /// pyrimidodiazepine synthase activity /// oxidoreductase activity /// protein homodimerization activity /// glutathione dehydrogenase (ascorbate) activity
1637807_at	inaC	protein amino acid phosphorylation /// phagocytosis, engulfment /// cellular component movement /// visual perception /// phototransduction /// female gonad development /// response to light stimulus /// deactivation of rhodopsin mediated signaling /// calcium-mediated signaling /// inhibition of phospholipase C activity involved in G-protein coupled receptor signaling pathway /// detection of light stimulus involved in sensory perception	protein serine/threonine kinase activity /// calcium-dependent protein kinase C activity /// protein binding /// ATP binding /// zinc ion binding /// transferase activity /// diacylglycerol binding /// metal ion binding
1637828_a_at	sif	synaptic transmission /// multicellular organismal development /// actin cytoskeleton organization /// regulation of Rho protein signal transduction /// regulation of axonogenesis /// regulation of synapse structure and activity	receptor signaling protein activity /// Rho guanyl-nucleotide exchange factor activity /// protein binding
1637870_a_at	dpr5	---	---
1637894_at	CG14509	olfactory behavior	---

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1637895_at	Dhc16F	regulation of transcription, DNA-dependent /// microtubule-based movement	microtubule motor activity /// ATP binding /// transcription factor binding /// hydrolase activity ///ATPase activity, coupled /// ATPase activity, uncoupled
1637975_at	Dhc36C	glycerol-3-phosphate metabolic process /// microtubule-based movement /// oxidation reduction	microtubule motor activity /// glycerol-3-phosphate dehydrogenase activity /// ATP binding /// oxidoreductase activity /// hydrolase activity /// ATPase activity, coupled /// ATPase activity, uncoupled
1638001_a_at	CG8889	---	hydrolase activity
1638022_at	CG7971	nuclear mRNA splicing, via spliceosome /// RNA splicing	protein binding
1638032_at	CG14921	---	---
1638049_at	CG30494	---	---
1638078_at	Side	negative regulation of transcription from RNA polymerase II promoter	DNA binding /// transcription repressor activity /// transcription regulator activity
1638091_at	CG15143	---	protein binding
1638110_at	BcDNA:GH10292	transmembrane transport	---
1638205_s_at	futsch	microtubule cytoskeleton organization /// nervous system development /// axonogenesis /// neuromuscular junction development /// axon cargo transport /// olfactory learning /// regulation of synaptic growth at neuromuscular junction /// negative regulation of neuron apoptosis /// compound eye development /// dendrite morphogenesis /// neurofilament cytoskeleton organization	microtubule binding
1638299_at	CG42674	regulation of Rho protein signal transduction	Rho guanyl-nucleotide exchange factor activity
1638319_at	inaD	visual perception /// phototransduction /// protein localization /// deactivation of rhodopsin mediated signaling /// detection of light stimulus involved in sensory perception	structural molecule activity /// calmodulin binding /// receptor signaling complex scaffold activity /// myosin III binding
1638397_at	CG11388	---	transferase activity, transferring glycosyl groups
1638464_a_at	qtc	male courtship behavior	protein binding
1638505_at	t	histamine biosynthetic process /// visual perception /// flight behavior /// penicillin biosynthetic process /// dopamine biosynthetic process /// cuticle pigmentation	beta-alanyl-dopamine hydrolase activity /// hydrolase activity /// beta-alanyl-histamine hydrolase activity
w1638728_at	CG17994	---	---
1638756_at	CG17279	---	---
1638785_at	Ir75a	---	extracellular-glutamate-gated ion channel activity
1638828_a_at	CG17378	---	---
1638844_s_at	CG3714	response to oxidative stress /// pyridine nucleotide biosynthetic process	nicotinate phosphoribosyltransferase activity /// transferase activity, transferring glycosyl groups
1638851_at	CG5280	---	protein binding
1639022_at	CG12836	---	
1639269_a_at	Pdh	phagocytosis, engulfment /// metabolic process /// oxidation reduction	catalytic activity /// alcohol dehydrogenase (NAD) activity /// receptor activity /// protein binding /// oxidoreductase activity
1639333_at	al	negative regulation of transcription, DNA-dependent /// proximal/distal pattern formation, imaginal disc /// imaginal disc-derived leg morphogenesis /// bristle development /// elongation of arista core /// leg disc development /// positive regulation of Notch signaling pathway /// antennal morphogenesis	specific RNA polymerase II transcription factor activity /// sequence-specific DNA binding
1639525_at	king-tubby	sensory perception of smell	protein binding
1639576_at	Klp68D	microtubule-based movement /// anterograde axon cargo transport	motor activity/// ATP binding
1639641_at	CG8560	proteolysis	metallocarboxypeptidase activity /// zinc ion binding /// hydrolase activity
1639782_at	CG6931	---	---
1639789_at	CG18130	cell redox homeostasis	---
1639862_at	Eaat2	dicarboxylic acid transport /// taurine transport	taurine:sodium symporter activity /// L-aspartate transmembrane transporter activity /// glutamate:sodium symporter activity /// sodium:dicarboxylate symporter activity
1639936_at	Sas	carbohydrate biosynthetic process	N-acylneuraminate-9-phosphate synthase activity
1640030_at	CG4168	---	G-protein coupled receptor activity /// protein binding
1640119_a_at	dlg1	cytoskeleton organization /// cell adhesion /// signal transduction /// synaptic transmission /// dorsal closure /// central & peripheral nervous system development /// male courtship behavior /// negative regulation of cell proliferation /// morphogenesis, establishment or maintenance of polarity of follicular epithelium /// morphogenesis, establishment or maintenance of polarity of larval imaginal disc epithelium /// septate junction assembly /// cell differentiation /// neuron differentiation /// regulation of border follicle cell delamination /// regulation of cell proliferation /// asymmetric protein localization involved in cell fate determination /// basal protein localization /// zonula adherens assembly /// establishment or maintenance of neuroblast polarity /// establishment or maintenance of epithelial cell apical/basal polarity /// locomotor rhythm /// positive phototaxis /// establishment of spindle orientation /// regulation of cell cycle	guanylate kinase activity /// signal transducer activity /// epidermal growth factor receptor binding /// structural molecule activity /// transferase activity

Probe Set ID	Gene Symbol	GO biological process term	GO molecular function term
1640146_at	CG33116	phospholipid biosynthetic process	CDP-alcohol phosphotransferase activity
1640192_at	nan	calcium ion transport /// sensory perception of sound /// response to humidity /// transmembrane transport	calcium channel activity
1640206_at	CG4660	---	---
1640214_at	Naam	response to oxidative stress /// metabolic process /// determination of adult lifespan /// negative regulation of neuron apoptosis	catalytic activity /// calcium ion binding /// nicotinamidase activity
1640270_at	CG9492	microtubule-based movement	microtubule motor activity /// ATP binding /// hydrolase activity /// ATPase activity, coupled /// ATPase activity, uncoupled
1640291_at	Bili	negative regulation of Wnt receptor signaling pathway	binding
1640397_at	CG34360	carbohydrate transport	zinc ion binding
1640477_at	tilB	sensory perception of sound /// temperature compensation of the circadian clock /// male courtship behavior, veined wing generated song production /// detection of mechanical stimulus involved in sensory perception of sound	protein binding
1640541_at	faf	ubiquitin-dependent protein catabolic process /// endocytosis /// nuclear migration /// cellularization /// visual perception /// germ cell migration /// mystery cell fate differentiation /// protein deubiquitination /// negative regulation of proteolysis /// oogenesis /// compound eye development /// response to stimulus	ubiquitin thiolesterase activity /// ubiquitin-specific protease activity /// cysteine-type peptidase activity /// hydrolase activity
1640605_at	CG13855	---	---
1640614_at	cac	regulation of heart rate /// calcium ion transport /// exocytosis /// synaptic transmission /// multicellular organismal development /// phototransduction /// visual behavior /// adult locomotory behavior /// epithelial fluid transport /// male courtship behavior, veined wing generated song production /// positive regulation of synaptic growth at neuromuscular junction /// regulation of neurotransmitter secretion /// detection of light stimulus involved in visual perception /// transmembrane transport	voltage-gated calcium channel activity
1640642_at	Rh6	signal transduction /// G-protein coupled receptor protein signaling pathway /// visual perception /// phototransduction /// protein-chromophore linkage /// response to stimulus	signal transducer activity /// G-protein coupled photoreceptor activity
1640649_at	CG15878	---	protein binding
1640656_a_at	Pep	regulation of nuclear mRNA splicing, via spliceosome	single-stranded DNA binding /// single-stranded RNA binding /// protein binding /// zinc ion binding
1640767_s_at	CG14591	---	---
1640774_a_at	Mob2	cell morphogenesis /// olfactory learning	protein binding /// metal ion binding
1640962_s_at	CG4091	negative regulation of anti-apoptosis /// salivary gland cell autophagic cell death	caspase inhibitor activity
1640965_at	CG10050	---	---
1640970_at	CG9313	microtubule-based movement	motor activity /// protein binding /// hydrolase activity /// ATPase activity, coupled /// ATPase activity, uncoupled
1641002_at	CG13889	olfactory behavior /// oxidation reduction	peroxidase activity /// protein binding /// oxidoreductase activity
1641030_at	Obp58d	sensory perception of chemical stimulus	odorant binding
1641299_s_at	CG11353	---	acyltransferase activity /// transferase activity, transferring acyl groups other than amino-acyl groups
1641385_at	CG14342	---	---
1641390_at	dpr19	---	---
1641427_at	foi	zinc ion transport /// pole cell migration /// central nervous system development /// gonadal mesoderm development /// germ cell migration /// cell migration /// metal ion transport /// cell differentiation /// branch fusion, open tracheal system /// gonad morphogenesis /// transmembrane transport	zinc ion transmembrane transporter activity /// protein binding /// metal ion transmembrane transporter activity
1641501_a_at	Sh	regulation of action potential /// potassium ion transport /// learning or memory /// courtship behavior /// flight behavior /// proboscis extension reflex /// larval locomotory behavior /// detection of visible light /// sleep /// regulation of circadian sleep/wake cycle, sleep /// behavioral response to ether /// axon extension /// sensory perception of taste /// transmembrane transport /// regulation of synaptic activity	voltage-gated potassium channel activity /// protein binding
1641605_at	PIP82	---	---
1641738_a_at	CG13636	---	protein binding

7.2 Human relatives involved in Deafness

Table 10 Human homologues

Gene	CG-ID	E-Value	Human Gene	Disease
ninaC	CG5125-PA	3.00E-180	DFNB30; MYO3A	Deafness, autosomal recessive 30, 607101 (3)
eya	CG9554-PA	1.00E-121	CMD1J;DFNA10;EYA4	Deafness, autosomal dominant 10, 601316 (3)
Ptpmeg	CG1228-PA	9.00E-35	NS1; PTP2C; PTPN11; SHP2	Leopard syndrome, 151100 (3)
wnd	CG8789-PA	2.00E-33	CRAF; NS5;RAF1	LEOPARD syndrome 2, 611554 (3)
eys	CG33955-PB	2.00E-31	AGS;AHD;JAG1	Deafness, congenital heart defects, and posterior embryotoxon (3)
al	CG3935-PA	2.00E-27	CDHS; HUP2; PAX3; WS1	Craniofacial-deafness-hand syndrome, 122880 (3)
Pph13	CG2819-PA	6.00E-25	CDHS; HUP2; PAX3; WS1	Craniofacial-deafness-hand syndrome, 122880 (3)
inaC	CG6518-PA	3.00E-21	DFNB30; MYO3A	Deafness, autosomal recessive 30, 607101 (3)
CG4629	CG4629-PA	4.00E-18	DFNB30; MYO3A	Deafness, autosomal recessive 30, 607101 (3)
Pkc53E	CG6622-PA	1.00E-17	CRAF; NS5;RAF1	LEOPARD syndrome 2, 611554 (3)
CG7568	CG7568-PA	1.00E-17	PEX7; RCDP1	Refsum disease, 266500 (3)
oc	CG12154-PA	2.00E-15	CDHS; HUP2;PAX3;WS1	Craniofacial-deafness-hand syndrome, 122880 (3)
Sh	CG12348-PB	2.00E-15	DFNA2A; KCNQ4	Deafness, autosomal dominant 2A, 600101 (3)
gl	CG7672-PA	1.00E-14	HSAL1; SALL1; TBS	Townes-Brocks branchiootorenal-like syndrome, 107480 (3)
wtrw	CG31284-PA	6.00E-14	ESPN	Deafness, autosomal recessive 36, 609006 (3)
sens-2	CG31632-PA	3.00E-13	HSAL1;SALL1;TBS	Townes-Brocks branchiootorenal-like syndrome, 107480 (3)
Sulf1	CG6725-PA	2.00E-10	SGSH;MPS3A;SFMD	Sanfilippo syndrome, type A, 252900 (3)
CG2052	CG2052-PA	3.00E-10	HSAL1;SALL1;TBS	Townes-Brocks branchiootorenal-like syndrome, 107480 (3)
CG12071	CG12071-PB	4.00E-09	HSAL1;SALL1;TBS	Townes-Brocks branchiootorenal-like syndrome, 107480 (3)
Gene	CG-ID	E-Value	Human Gene	Disease
CG32373	CG32373-PA	8.00E-07	DBS;LRP2	Donnai-Barrow syndrome, 222448 (3)
inaD	CG3504-PA	5.00E-06	CIP98; DFNB31; USH2D	Usher syndrome, type IID, 611383 (3)
CG10283	CG10283-PA	7.00E-06	C3orf6; CCDC50; DFNA44	Deafness, autosomal dominant 44, 607453 (3)
Gβ76C	CG8770-PA	8.00E-06	PEX7; RCDP1	Refsum disease, 266500 (3)
dlg1	CG1725-PB	9.00E-06	DFNB18;USH1C	Usher syndrome, type 1C, 276904 (3)
Syt1	CG3139-PB	2.00E-05	AUNB1; DFNB9; OTOF	Auditory neuropathy, autosomal recessive, 1, 601071 (3)
HisCl1	CG14723-PA	4.00E-05	ACHRG; CHRNG	Escobar syndrome, 265000 (3)
nan	CG5842-PA	4.00E-05	SANS; USH1G	Usher syndrome, type 1G, 606943 (3)
tilB	CG14620-PA	1.00E-04	LRTOMT; LRTOMT1	Deafness, autosomal recessive 63, 611451 (3)
Rh6	CG5192-PB	3.00E-04	ABCDS; EDNRB; HSCR2	ABCD syndrome, 600501 (3)
sif	CG5406-PA	6.00E-04	DFNB18; USH1C	Usher syndrome, type 1C, 276904 (3)
CG8800	CG8800-PA	9.00E-04	LRTOMT; LRTOMT1	Deafness, autosomal recessive 63, 611451 (3)
Rh4	CG9668-PA	2.00E-03	ABCDS; EDNRB; HSCR2	ABCD syndrome, 600501 (3)
dtr	CG31623-PA	3.00E-03	LRTOMT; LRTOMT1	Deafness, autosomal recessive 63, 611451 (3)
CG14185	CG14185-PA	4.00E-03	LRTOMT; LRTOMT1	Deafness, autosomal recessive 63, 611451 (3)
iav	CG4536-PA	1.00E-02	SANS; USH1G	Usher syndrome, type 1G, 606943 (3)
CG8086	CG8086-PD	1.30E-02	TRIOBP;KIAA1662	Deafness, autosomal recessive 28, 609823 (3)
Rh3	CG10888-PA	1.60E-02	ABCDS;EDNRB;HSCR2	ABCD syndrome, 600501 (3)
5-HT2	CG1056-PA	2.10E-02	ABCDS; EDNRB; HSCR2	ABCD syndrome, 600501 (3)
onecut	CG1922-PA	2.80E-02	BRN3C;POU4F3	Deafness, autosomal dominant 15, 602459 (3)
CG9313	CG9313-PA	3.30E-02	PEX7; RCDP1	Refsum disease, 266500 (3)
CG4329	CG4329-PA	4.00E-02	CKN1; CSA; ERCC8	Cockayne syndrome, type A, 216400 (3)
CG10185	CG10185-PA	5.40E-02	PEX7; RCDP1	Refsum disease, 266500 (3)
CG4168	CG4168-PA	9.40E-02	LRTOMT; LRTOMT1	Deafness, autosomal recessive 63, 611451 (3)
CG15118	CG15118-PA	1.00E-01	SANS; USH1G	Usher syndrome, type 1G, 606943 (3)
CG16789	CG16789-PA	1.70E-01	PEX1; ZWS1	Refsum disease, infantile, 266510 (3);
CG8419	CG8419-PA	1.90E+00	PAF1; PEX2; PMP35	Refsum disease, infantile form, 266510 (3)
CG3980	CG3980-PB	2.40E+00	LRTOMT; LRTOMT1	Deafness, autosomal recessive 63, 611451 (3)
t	CG12120-PA	4.80E+00	DFNB49; MARVD2	Deafness, autosomal recessive 49, 610153 (3)
trp	CG7875-PA	7.90E+00	DFNA2A;KCNQ4	Deafness, autosomal dominant 2A, 600101 (3)
CG9164	CG9164-PA	9.70E+00	NPC;NPC1	Niemann-Pick disease, type C1, 257220 (3)
CG13889	CG13889-PA	9.90E+00	DFNA5	Deafness, autosomal dominant 5, 600994 (3)

7.3 RT-PCR for *In situ* hybridizations

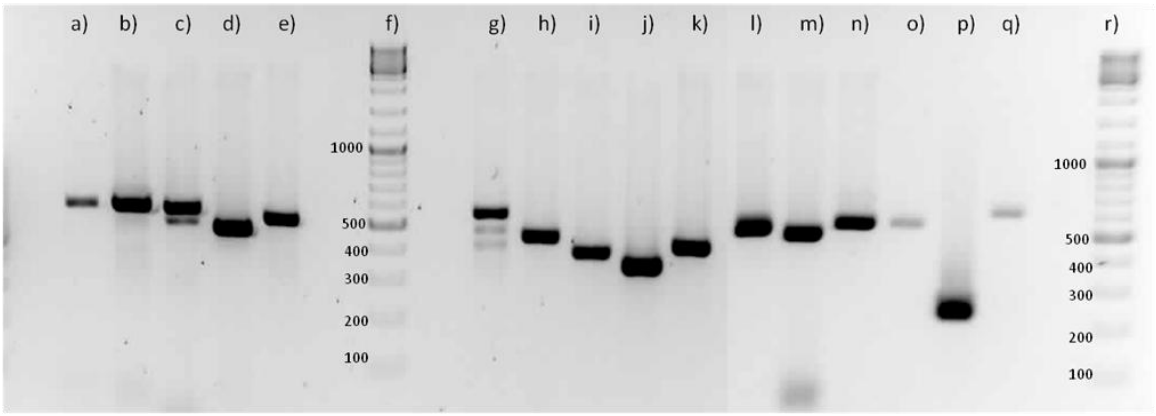

Figure 17 RT-PCR for *In situ* hybridization a) *Dhc93AB*, b) *Bmcp*, c) *CG8086*, d) *CG13133*, e) *CG14921*, f) GeneRuler™ DNA Ladder Mix, g) *Gbeta76c*, h) *Rh3*, i) *Rh6*, j) *trpl*, k) *wtrw*, l) *Os-C*, m) *CG14076*, n) *CG32373*, o) *CG18516*, p) *Arr2* q) *CG14585*, r) GeneRuler™ DNA Ladder Mix.

7.4 Cellular expression of *Ir94b-Gal4*

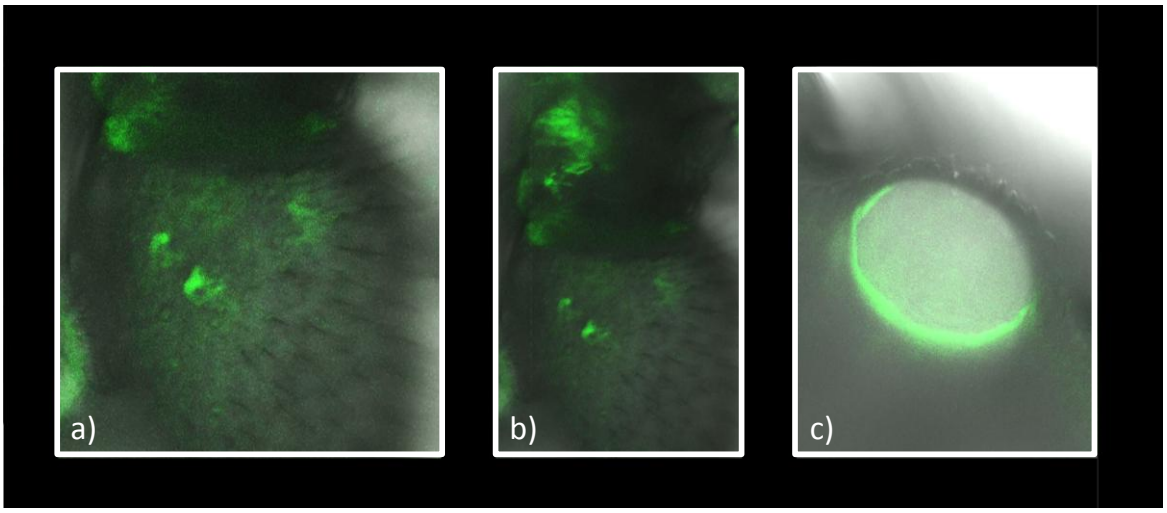

Figure 18 Cellular expression of *Ir94b-Gal4*. Tranzgenic flies carrying *Ir94b-Gal4* contructs were crossed with flies carrying *UAS-2xEYFP*. Confocal images show the EYFP expression in the following organs: a) third antennal segment, b) second and third antennal segment, c) ocelli

7.5 Gene expression in mutant lines

Table 11 Candidate gene expression in mutant lines. To determine whether candidate gene is affected in mutant lines, qPCR have been performed. mRNA expression in percentage indicate the mRNA level in mutant when compared to w^{1118} controls.

Tested line	**mRNA expression (%)**
y[1]; ry[506] P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG31216[KG10548]	6.61 ± 1.29
y[1] w[*]; P{y[+m8]=Mae-UAS.6.11}Dhc36C[LA00085]	41.27 ± 2.36
w[1118]; PBac{w[+mC]=WH}CG14693[f03110]	52.32±13.65
w[1118]; PBac{w[+mC]=WH}Ank2[f02001] CG32373[f02001]/TM6B, Tb[1]	59.38 ± 3.16
y[1] w[67c23]; P{w[+mC] y[+mDint2]=EPgy2}CG11253[EY10866]	0.40 ± 0.20
w[1118]; PBac{w[+mC]=WH}CG8086[f03214]	3.86 ± 0.90
y[1] w[67c23]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG9492[KG02504] ry[506]	0.04 ± 0.06
w[1118]; Mi{ET1}CG13636[MB03846]	3.30 ± 0.28
norpA[7]	248.39 ± 110.36
bw[1]	90.07 ± 23.97
w[1118]; Mi{ET1}ninaC[MB02664]	4.72 ± 0.92
w[1118]; P{w[+mGT]=GT1}Bmcp[BG02446]	10.66 ± 0.96
y[1] w[1118];PBac{y[+mDint]=3HPy[+]} CG14921[C247]/CyO	0.01 ± 0.01
w[1118]; Mi{ET1}CG14636[MB03866]	89.68 ± 6.91
w[1118]; Mi{ET1}CG31424[MB02190]	192 ± 14.23
w[1118]; Mi{ET1}Dhc93AB[MB05444]	18.94 ± 4.62
w[1118]; Mi{ET1}CG10633[MB05283]	8.56 ± 3.01
gl[3]	11.51 ± 6.34
w[1118]; Mi{ET1}gol[MB03006]	141.71 ± 34.83
w[1118]; Mi{ET1}CG8419[MB06410]	0.03 ± 0.00
y[1] w[*]; stops[1]	4.15 ± 0.09
y[1]; Mi{ET1}CG2052[MB00729]	42.58 ± 19.05
w[1118]; Mi{ET1}Sulf1[MB11661]	0.00 ± 0.00
CG9313[Z2-5863]	185.51 ± 16.61
arr2[5]	93.99 ± 15.74
trp[343]	56.64 ± 22.89
inaD[1]	22.82 ± 2.37
y[1] futsch[K68]	50.05 ± 25.38
w[1118]; Mi{ET1}kek4[MB11415]	0.01 ± 0.01
w[1118]; Mi{ET1}CG6053[MB06262]	0.06 ± 0.02
w[1118]; P{w[+mC]=EPg}sei[HP21840]	4.88 ± 2.26
w[*]; ninaA[1]	0.00 ± 0.00
trp[343]	0.00 ±0.00
w1118; PBac{RB}CG11388e03063	177.54 ± 20.35
rh5[2]	362.61 ± 46.47
rh6[1]	218.95 ± 7.59
w; PBac{GAL4D,EYFP}yuriPL00114 cn1 bw1; P{FRT(whs)}2A P{neoFRT}82B*	56.28 ± 14.71

7.1 Summary of gene functions, analyses, and homologues

Table 12 Summary: a) has a human relative involved in deafness (Homophila); b) is a ciliary gene (Avidor-Reiss et al., 2004); c) zebrafish homologue expressed in hair cells (McDermott et al., 2007); d) is also expressed in the campaniform mechanoreceptors (Bechstedt et al., 2010); e) in situ hybridizations have been performed, f) Gal4-Lines have been constructed; g) JO function was assessed; h) human homologues (BLAST); i) E-value when compared with human homologue (BLAST)

Gene Symbol	a	b	c	d	e	f	g	h	i
Ank2				x			impaired	ANK2	0
cac								CACNA1C	0
Calx								SLC8A3	0
CG3339								DNAH17	0
CG9492		x					deaf	DNAH5	0
CG9935								GRIK2	0
Dhc16F			x					DNAH6	0
Dhc36C		x					impaired	DNAH7	0
Dhc93AB					x	x	deaf	DNAH17	0
faf								USP9X	0
fry								FRY	0
Hdc								HDC	0
inaC	x							PRKCB	0
l(1)G0196				x				PPIP5K2	0
Ndae1								SLC4A8	0
ninaC	x		x				normal	MYO3B	0
norpA							improved	PLCB4	0
Pkc53E	x							PRKCA	0
rdgA								DGKI	0
sei				x			impaired	KCNH2	0
Sh	x			x				KCNA2	0
sif	x							TIAM2	0
trp	x						normal	TRPC5	0
trpl					x		impaired	TRPC5	0
bsk				x				MAPK8	2.00E-179
Ptpmeg	x							PTPN4	1.00E-175
flw								PPP1CB	3.00E-172
pum								PUM2	3.00E-171
Klp68D		x		x		x	impaired	KIF3B	1.00E-168
stj								CACNA2D3	3.00E-164
Art4								CARM1	1.00E-162
CG9313	x	x		x		x	impaired	DNAI1	7.00E-159
CG17544								ACOX3	7.00E-157
PIP5K59B				x				PIP5K1A	6.00E-156
CG6053		x					impaired	DNAI2	6.00E-153
CdsA								CDS2	1.00E-151
Sulf1	x						normal	SULF2	9.00E-149
Dyb								DTNB	5.00E-148
wnd	x							MAP3K12	1.00E-147
CG16789	x	x						IQCA1	4.00E-145
rdgC				x				PPEF1	5.00E-138
CG15118	x							ANKRD13D	1.00E-134
CG18516					x			XDH	2.00E-132
CG10185	x							KIAA1239	4.00E-130
CG31019								AGBL4	6.00E-123
Eaat2								SLC1A2	2.00E-119
Syt1	x							SYT1	2.00E-118
CG10062				x				Ermp1	2.00E-117
hoe2								OCA2	4.00E-110
eya	x		x					EYA1	6.00E-106
CG15270								ANO8	3.00E-105
eys	x							EYS	8.00E-105
king-tubby				x				TUB	1.00E-102
sn				x				FSCN1	3.00E-101
Syn				x				SYN1	2.00E-100
CG5687				x				SLC5A8	2.00E-98
Bmcp					x		impaired	SLC25A30	2.00E-94
Sas								NANS	2.00E-92
Arr2					x		impaired	ARRB1	5.00E-91
Gbeta76C	x				x			GNB4	2.00E-89
futsch							normal	MAP1B	1.00E-88
CG9279				x				DCTN1	1.00E-86
CG3105				x				PASK	2.00E-86
CG7568	x		x					WDR69	5.00E-86
dlg1	x							DLG1	1.00E-84
sens-2	x							GFI1	2.00E-82
CG33116				x				EPT1	9.00E-82
CG4629	x							MGC42105	1.00E-81
Cam			x	x				CALM2	3.00E-80

Gene Symbol	a	b	c	d	e	f	g	h	i
CG11206								KAZ	4.00E-80
tilB	x		x					LRRC6	5.00E-80
CG7149								EPT1	2.00E-79
CG3714								NAPRT1	4.00E-77
foi								SLC39A10	2.00E-75
dtr	x							LRRC50	9.00E-74
Cep97	x							CEP97	1.00E-73
CG42674								PLEKHG5	1.00E-72
CG9317								SLC22A5	2.00E-72
HisCl1	x							GLRA2	3.00E-70
CG30203								SPON1	2.00E-68
B52								SRSF4	3.00E-68
MESK2			x	x				NDRG3	4.00E-67
CG6761				x				KIAA1841	1.00E-65
Ir8a								GRIK3	2.00E-65
CG7220								UBE2W	7.00E-65
CG8419	x						normal	TRIM45	1.00E-64
CG11388				x			impaired	C3orf21	7.00E-64
CG11253			x			x	impaired	ZMYND10	2.00E-63
CG8560								CPA2	9.00E-62
CAP								SORBS1	3.00E-60
CG4329	x					x	impaired	WDR65	8.00E-60
Ugt35a				x				UGT2B4	8.00E-59
gol							impaired	RNF150	1.00E-58
CG13855								IQUB	2.00E-58
nan	x							TRPV5	3.00E-57
Pal								PAM	3.00E-57
CG8800	x	x						DNAL1	1.00E-56
CG17669								C19orf51	8.00E-55
iav	x							TRPV6 |	4.00E-54
Bili								FRMD8	1.00E-53
Mob2								MOB2	3.00E-53
run								RUNX1	2.00E-52
CG9150								DHRS11	5.00E-50
CG10362				x				PDZD8	9.00E-50
Rh6	x				x		impaired	OPN4	2.00E-49
CG14591				x				TMEM164	3.00E-49
inaD	x						impaired	MPDZ	3.00E-49
Tektin-C			x					TEKT1	3.00E-49
Tie				x				TEK	4.00E-48
ninaA							normal	PPIB	4.00E-47
Rh4	x							OPN4	3.00E-46
CG10050								DTWD2	9.00E-46
CG40485								DHRS11	3.00E-45
CG14215								AHCTF1	5.00E-45
CG33203								PAQR4	2.00E-44
Rh3	x				x			OPN4	3.00E-44
Rh5							impaired	OPN4	1.00E-43
gl	x						impaired	ZSCAN22	9.00E-43
onecut	x							ONECUT1	1.00E-42
se			x					GSTO1	2.00E-42
wtrw	x				x			TRPA1	2.00E-41
CG6472				x				PNLIPRP1	1.00E-40
CG4091				x				TNFAIP8	3.00E-40
laza								PPAP2A	9.00E-40
D								SOX21	3.00E-39
5-HT2	x							HTR2C	3.00E-38
CG4168	x							IGFALS	1.00E-37
ru				x				RHBDL3	2.00E-37
CG11041								EFCAB2	2.00E-36
retinophilin								MORN4	4.00E-36
CG14015				x				MANEAL	5.00E-36
CG9164	x							WSCD2	2.00E-35
BcDNA:GH10292								SLC45A1	3.00E-35
Slob				x				PXK	4.00E-35
CG10257				x				FAIM	5.00E-35
Actbeta								INHBB	8.00E-35
CG2052	x			x			impaired	ZNF384	1.00E-34
a5								PEBP1	4.00E-34
CG8407		x						DNAL4	4.00E-34
spn-B								XRCC3	4.00E-34
Pdh								HPGD	5.00E-34
CG7990								PGAP2	7.00E-34
CG14142			x					FAM188B2	1.00E-33
oc	x							OTX1	1.00E-33
al	x							ARX	2.00E-33
Pph13	x							ARX	2.00E-31
CG5130								SLC30A1	4.00E-31
n-syb				x				VAMP2	6.00E-31
CG13830				x				SPOCK2	1.00E-30

Gene Symbol	a	b	c	d	e	f	g	h	i
CG14085								C5orf36	2.00E-28
bw							improved	ABCG1	3.00E-28
Nrt				x				CES2	3.00E-26
Fer1								PTF1A	2.00E-25
CG6931								C18orf10	9.00E-24
CG7971								SRRM2	4.00E-23
CG14905		x						CCDC63	1.00E-22
CG17378			x					KIAA0513	1.00E-22
CG32373	x				x			SCUBE2	2.00E-22
kek4							impaired	LRRC24	2.00E-22
CG10866				x				C5orf28	4.00E-22
Side				x				HES1	4.00E-22
CG17122								C9orf117	3.00E-21
CG14185	x							LRRC56	4.00E-21
CG14127								CCDC151	1.00E-19
CG17360				x				PLEKHM2	2.00E-19
CG3009								PLA2G3	3.00E-19
mRpL37								MRPL37	2.00E-17
CG4660								C8orf55	2.00E-16
CG8086	x			x	x		impaired	ODF3L2	6.00E-16
CG18130								TXNDC3	1.00E-15
CG2681								SIAH1	2.00E-15
CG34297								TTC12	4.00E-15
CG1268				x				ATP6V0E2	1.00E-14
CG31291				x				SDCCAG8	2.00E-14
CG5280								C22orf23	2.00E-14
CG6983								C20orf27	2.00E-14
CG12071	x			x				ZNF484	4.00E-14
CG14077					x			GRID1	2.00E-13
CG34360								ZNF395	2.00E-13
CG8889								MPPE1	2.00E-13
CG5948								SOD1	9.00E-13
chinmo								ZBTB24	2.00E-12
cpx								CPLX1	2.00E-10
CG13133				x	x			HSPB1	3.00E-10
Ggamma30A								GNG13	4.00E-10
CG14853								LOC285141	7.00E-10
CG5375				x				SCHIP1	8.00E-10
Ir75a					x			GRIA1	8.00E-10
bab1								ZBTB17	9.00E-10
Trl								KLHL18	4.00E-09
CG13842				x				CCDC142	2.00E-08
CG15143								C3orf15	2.00E-08
CG17352								NETO2	2.00E-08
CG14921					x	x	deaf	DYX1C1	3.00E-08
dpr5								OBSCN	4.00E-08
HDC06237								RFWD2	0.0000003
Ir76a								GRID1	0.0000003
Pep								CIZ1	0.0000005
boss				x				GPRC5B	0.0000009
dpr19								LSAMP	0.0000009
CG12836								TCTEX1D2	0.000002
CG10283	x							CCDC50	0.00003
inaF								PRR24	0.00003
Ir64a							normal	GRIK2	0.00004
CG14693							impaired	PNPLA6	0.00006
CG13202								CCDC103	0.0002
CG13889	x			x				CEP290	0.0003
CG3618				x				KIAA1467	0.0003
CG6499								RHCG	0.0007
stops							impaired	ASB17	0.001
Naam				x		x	impaired	EFCAB1	0.002
Ir100a								GRIN1	0.004
a10								SLC9A9	0.005
CG13950				x				LGALS9	0.005
CG11353								no	no
CG12947								no	no
CG13305								no	no
CG13455								no	no
CG13636						x	impaired	no	no
CG13707								no	no
CG13800								no	no
CG14087								no	no
CG14221								no	no
CG14274								no	no
CG14342								no	no
CG14445				x				no	no

Gene Symbol	a	b	c	d	e	f	g	h	i
CG1561								no	no
CG15878								no	no
CG15927								no	no
CG17279								no	no
CG17321								no	no
CG17786								no	no
CG17994				x				no	no
CG18336								no	no
CG18598								no	no
CG30494								no	no
CG31386								no	no
CG31847								no	no
CG4468								no	no
CG6044				x				no	no
CG6362				x				no	no
CG6912								no	no
CG7196								no	no
CG7730				x				no	no
CG8180								no	no
CG8369				x				no	no
CG9335								no	no
CG9813				x				no	no
Cpn								no	no
Cpr49Ag								no	no
Cpr72Ec								no	no
Ir94b						x	normal	no	no
Obp58b								no	no
Obp58d								no	no
Obp59a								no	no
Os-C					x			no	no
Osi2								no	no
Pbprp4								no	no
PIP82								no	no
pncr015:3L-RA								no	no
pyr								no	no
qtc				x				no	no
qvr								no	no
retinin								no	no
t	x							no	no
tipE				x				no	no
yuri							normal	no	no
CG13924								no	no
CG14509				x				no	no
CG14636							impaired	no	no
CG14947								no	no

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Curiculum vitae

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Education

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2005-2006 **Diploma thesis** Dimerization: a tool for EGF receptor activation – Comparison of monomer and dimer kinases, Supervisor: Prof. H.W.Klein, Institute of Biochemistry, University of Cologne, Germany

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Publications

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Björn Nadrowski, Thomas Effertz, Pingkalai R. Senthilan, Martin C. Göpfert
Antennal hearing in insects - New findings, new questions (Hearing Research, 2010)

Qianhao Lu, Pingkalai R. Senthilan, Thomas Effertz, Björn Nadrowski and Martin C. Göpfert
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Pingkalai R. Senthilan, Qianhao Lu, Martin C. Göpfert
Grundlagen des Hör- und Gleichgewichtssystems (Plinkert, Hören und Gleichgewicht, 2009)