## Supplemental Figures and Figure Legends

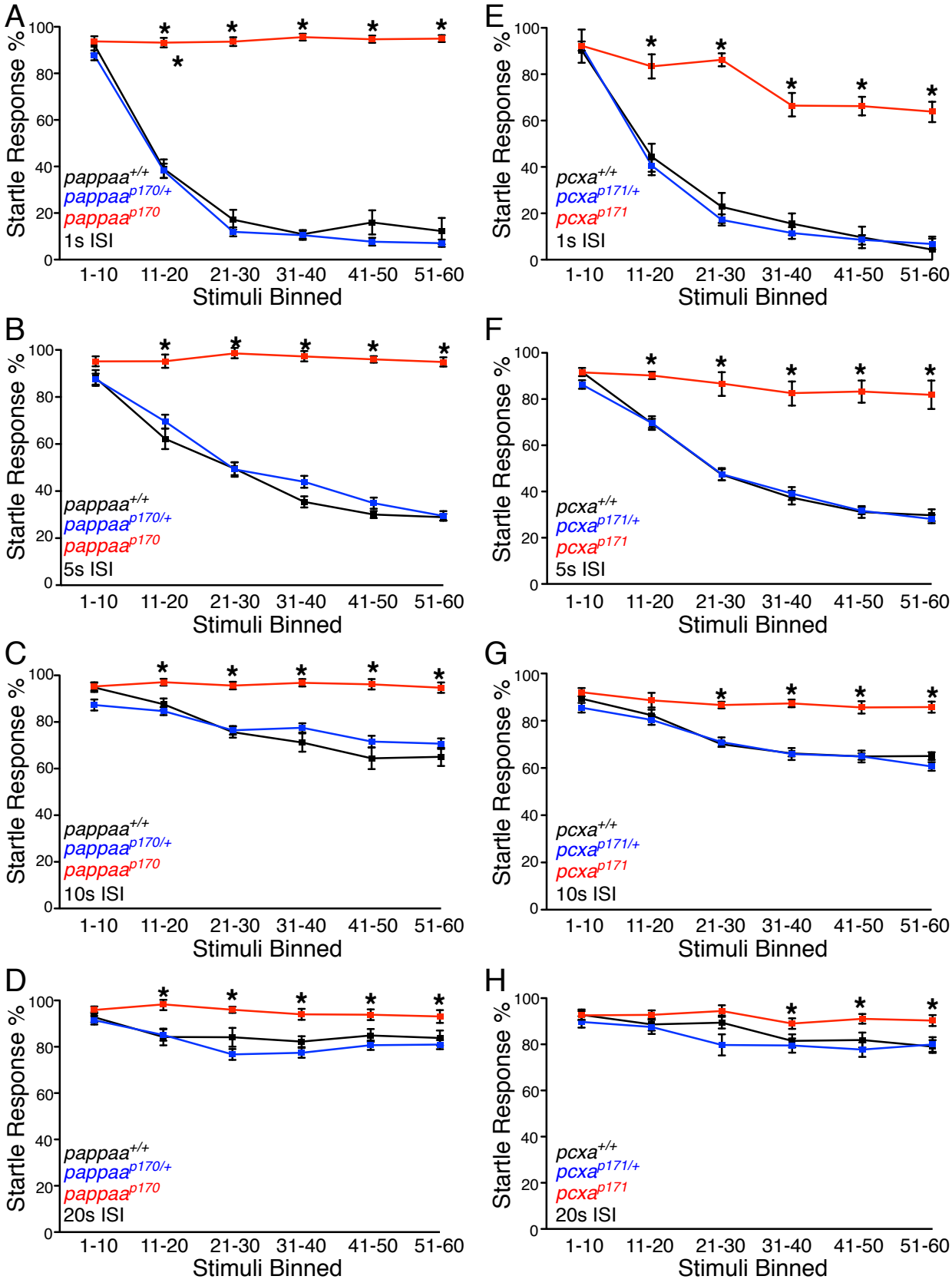

**Figure S1. Short term habituation profile of *pappaa*$^{p170}$ and *pcxa*$^{p171}$ mutants to repetitive acoustic stimuli presented at varying interstimulus intervals (related to Figure 1).**
Mean startle response trend of 5 dpf larvae to a modified version of the acoustic stimulation protocol described in Figure 1A. Stimuli 1-10 were delivered at 20s intervals and stimuli 11-60 were delivered at 1s (A,E), 5s (B,F), 10s (C,G), or 20s (D,H) intervals. Mean startle responses are binned by sets of 10 successive stimuli. *p<0.01 ANOVA vs. sibling homozygous wild type larvae at identical phase of assay. N larvae ranges from 17 to 51. Error bars indicate SEM.

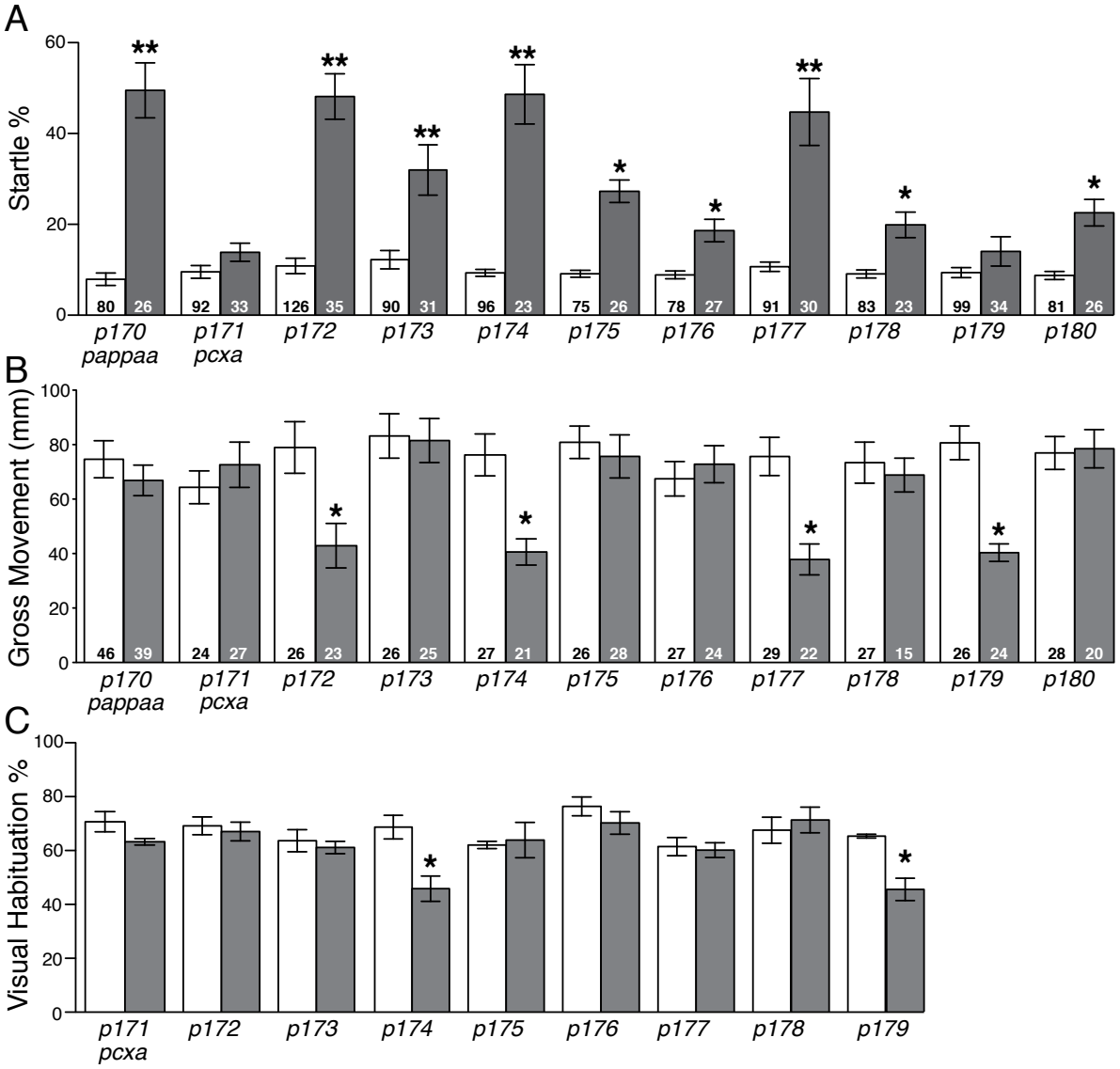


**Figure S2. Behavioral characterization of startle habituation mutants (related to Figure 1, Table 1).**
(A) Mean acoustic startle responsiveness to 10 non-habituating, low-level acoustic stimuli. (B) Mean total movement over 160s. (C) Mean degree of habituation to repetitive visual dark flash stimuli. *p<0.01 and **p<0.001, Student's t-test between mutants (gray bars) and wild type siblings (white bars) for each family. N larvae shown within each bar. For C, n= 3 sets of 15-20 larvae per set. Error bars indicate SEM.

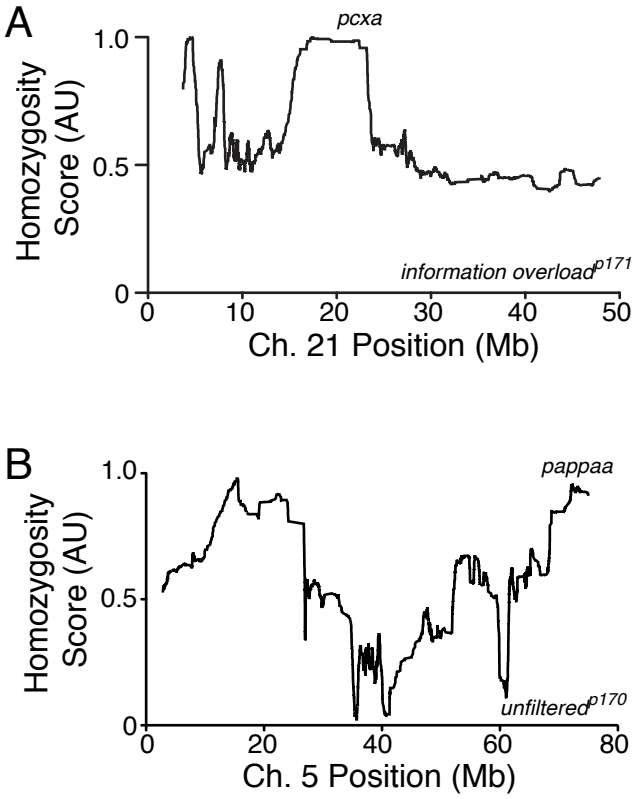


**Figure S3. Homozygosity mapping of *information overload*$^{p171}$ and *unfiltered*$^{p170}$ mutant locus (related to Figure 1)**

Homozygosity plots of *information overload*$^{p171}$ mutant larvae showing linkage to Chromosome 21(A) and *unfiltered*$^{p170}$ mutant larvae showing linkage to Chromosome 5 (B). Arbitrary values were assigned to SNPs unique to TLF background (AU = 1) and WIK-L11 background (AU=0) and a homozygosity score was calculated by taking average homozygosity value across a sliding 100-SNP window (see Experimental Procedures). Chromosomal locus of *pcxa* and *pappaa* shown above plots.

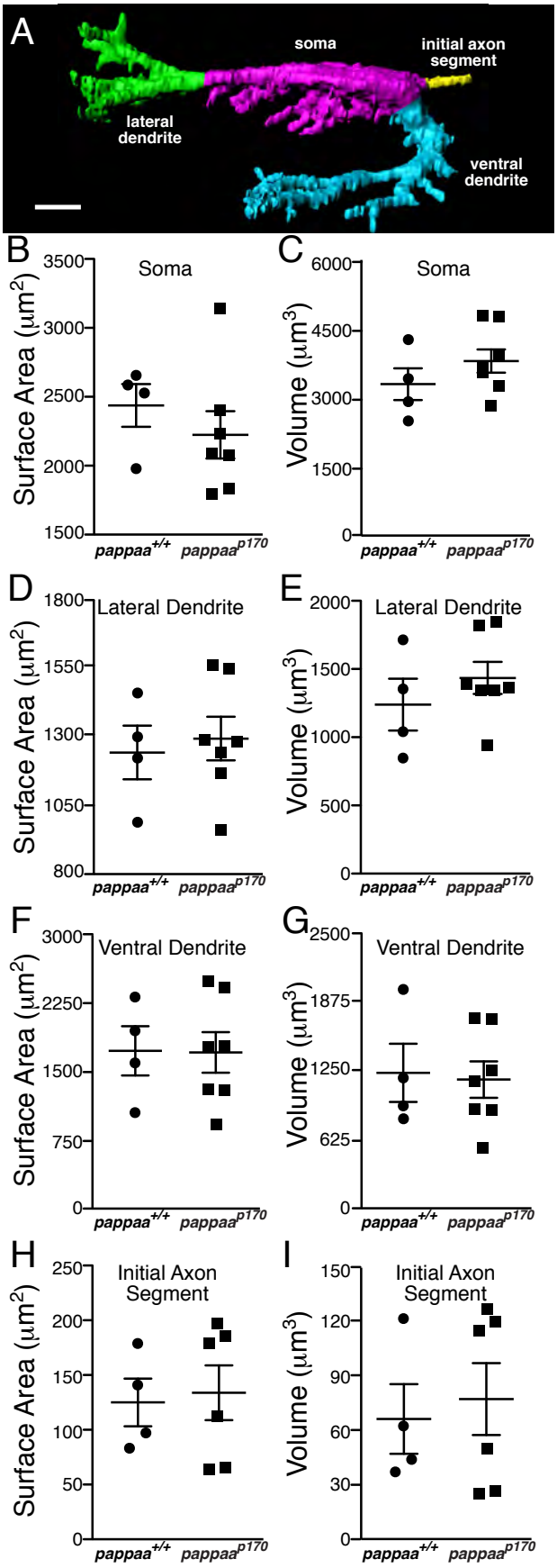
A
soma
initial axon segment
lateral dendrite
ventral dendrite
B
Soma
Surface Area (μm2)
3500
3000
2500
2000
1500
pappaa+/+
pappaap170
C
Soma
Volume (μm3)
6000
4500
3000
1500
0
pappaa+/+
pappaap170
D
Lateral Dendrite
Surface Area (μm2)
1800
1550
1300
1050
800
pappaa+/+
pappaap170
E
Lateral Dendrite
Volume (μm3)
2000
1500
1000
500
0
pappaa+/+
pappaap170
F
Ventral Dendrite
Surface Area (μm2)
3000
2250
1500
750
0
pappaa+/+
pappaap170
G
Ventral Dendrite
Volume (μm3)
2500
1875
1250
625
0
pappaa+/+
pappaap170
H
Initial Axon Segment
Surface Area (μm2)
250
200
150
100
50
0
pappaa+/+
pappaap170
I
Initial Axon Segment
Volume (μm3)
150
120
90
60
30
0
pappaa+/+
pappaap170

**Figure S4. Mauthner neuron morphology (related to Figure 3).**

(A) 3D reconstruction of Mauthner neuron of *pappaa*$^{+/+}$ larva showing soma, lateral and ventral dendrites, and initial axon segment. Scale bar = 10μm. Quantification of Mauthner neuron morphology includes: mean surface area of soma (B), lateral dendrite (D), ventral dendrite (F), and initial axon segment (H) and mean volume of soma (C), lateral dendrite (E), ventral dendrite (G), and initial axon segment (I). Each mark indicates a Mauthner neuron from a single larva. Error bars denote SEM. No significant differences between *pappaa*$^{+/+}$ and *pappaa*$^{p170}$ *l*arvae.

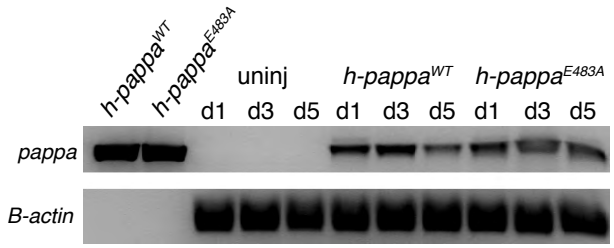


**Figure S5. RT-PCR of *h-pappa* injected in zebrafish (related to Figure 5)**

RT-PCR of 1, 3, or 5 dpf larvae that were injected with human wild type *pappa* or proteolytically inactive *pappa*$^{E483A}$ *mRNA* at the 1 cell stage. Leftmost two lanes show amplification of DNA constructs as proof of principle. *B-actin* expression used as positive control.

**Movie S1. *unfiltered*$^{p170}$ larvae show reduced acoustic startle habituation (related to Figure 1).**

Acoustic startle responses recorded at 5 dpf for *unfiltered*$^{p170}$ larva and wild type sibling. Pre-habituation stimulus is stimulus 1 and post-habituation stimuli are stimuli 38-40, as schematized in Figure 1A. Stimulus delivered at bump (0) with 30 msec of video shown before and after delivery.

**Movie S2. 3D, rotational view of Mauthner neuron in *pappaa*$^{+/+}$ larva (related to Figure 3).**

Confocal projection of anti-GFP labeled Mauthner neuron in *pappaa*$^{+/+}$*; hspGFF130DMCA-UAS:GAP43-citrine* 5 dpf larva.

**Movie S3. 3D, rotational view of Mauthner neuron in *pappaa*$^{p170}$ larva (related to Figure 3).** Confocal projection of anti-GFP labeled Mauthner neuron in *pappaa*$^{p170}$*; hspGFF130DMCA- UAS:GAP43-citrine* 5 dpf larva.

**Supplemental Table 1: PCR primers for bulk segregant analysis, molecular cloning, and genotyping of *pappaa*$^{p170}$, *pcxa*$^{p171}$**

**(related to Experimental Procedures and Supplemental Experimental Procedures)**

| Primer | Forward | Reverse |
|---|---|---|
| ***gDNA amplification*** | | |
| z1366 | TACTTCAGCTTCATCCAGACCA | CTGAACTCTGAGTCCAGACCG |
| z1525 | CTTCAGCGCATGTGATCG | GACCTTCTCGCTGGACTGAC |
| z4951 | TTCCTTCATGGTGGGCGAAT | CCTCTCGTTTGGAGCCCAGA |
| z7104 | CCTCCCTTTAGAGCCACCTG | GCCCATCACAAACCAGAACT |
| z22250 | CTCATCCCCTTTTCTCCACA | GCGTTAAGTGAGGATGGCAT |
| z14591 | TCCCTCTCCGAGAGCTAACA | GGCATCGTGTCATTTTCTGA |
| z1454 | TTGGAGACTAGAAGCGGGATGA | TTTACTGTCATCACGGCAAAGA |
| CA5722 | CTGAGCTCCACTGCACTTCC | TTTCCCTTGTGCAGAAATAAGC |
| CA5731 | CATCATGCTGTTCCAAACTGG | CCGCACTACTTATGACCACACG |
| z7958 | TGTCCCTCTGGAGAGATGCT | TCATTCCCAACTCAGAACCC |
| z4396 | GGGATTGTGGTTCTCCACGC | AGGCAGCCCTTTCCTAAAGGC |
| z9189 | TCCAGGTTTGCGTGTGATAG | CCAGTGTGAAACCCGAGAAT |
| z13822 | CAAACTGCTACACTCGAGCG | GCGGAGCTGAGTGACATACA |
| *pappaa*$^{p170}$ genotyping | CACTCTGGAGCCTCCAGCTTGCGGT | ATTGCTGACGTTGTGTACG |
| *pcxa*$^{p170}$ genotyping | AGTTTCTGAAGGTCGAATGC | TTATGTTCCCTGCTGTTGG |
| ***cDNA amplification*** | | |
| *z-pappaa-FL* | AGCAGAAAACAAGTGGAAGG | GGAAAATGAATTGCCTAACC |
| *z-pappaa* for RT-PCR | AATCACTTCTGGAGCTTTCG | TATGAGGACTCCAAGTGTGG |
| *h-pappa* | TTGAACCCATCTTTCTATGG | ACTCCAGTGTCACAGAGTCC |
| *pcxa* SNP | TTGTAAATGAACCGGACACC | TGTGTTGTTCTTTGAGATCC |

## Supplemental Experimental Procedures

*PCR genotyping pappaa*$^{p170}$ *and pcxa*$^{p170}$

To genotype *pappaa*$^{p170}$ larvae, we developed a dCAPS assay using the dCAPS program (http://helix.wustl.edu/dcaps/dcaps.html) to design appropriate primers (Supplemental Table 1) (Neff et al., 2002). After gDNA isolation, PCR was performed as described above and the product was digested with Mse1 (New England Biolabs), cleaving the mutant allele and producing a 245 bp fragment distinguishable from the 271 bp wild type allele on a 3% agarose gel containing 1.5% Metaphor agarose (Lonza). To genotype *pcxa*$^{p171}$, we used the primers listed in Supplemental Table 1 and digested the product with Dde1, producing a banding pattern of 209 and 153bp for the wild type

allele and producing 209, 91, and 62bp fragments for the mutant allele. All genotyping was performed after behavioral or immunolabeling experiments.

*pappaa$^{p170}$ morphology, body length, and survival*
Gross morphological, size measurements, and survival curves were determined on a clutch of larvae from an incross of *pappaa$^{p170/+}$* adults. Assessment of morphology, body length, and survival was made under a stereoscope and larvae were genotyped post hoc.

*DASPEI labeling, immunohistochemistry, and in situ hybridization*
To label neuromasts, we performed DASPEI staining at 5 dpf as previously described (Raible and Kruse, 2000). For immunostaining, larvae were fixed in 4% paraformaldehyde containing 4% sucrose for 1-3 hours at room temperature. After peeling the skin, larvae were blocked for 1 hour with 5% normal goat serum, 1% DMSO, 0.3% triton-X, and 2mg/mL bovine serum albumin in phosphate buffered saline. Larvae were then incubated in the primary antibodies anti-GFP (1:500, A11122, Life Technologies), anti-HuC (1:500, 16A11, Santa Cruz), anti-Cx35/36 (1:200, MAB3045, Millipore), anti-neurofilament (1:200, 3A10), and/or anti-GlyR (1:200, 146011, Synaptic Systems) overnight at 4°C in blocking solution, washed out, and then detected by addition of AlexaFluor488, AlexaFluor594, or AlexaFluor633 conjugated secondary antibodies (1:500, Life Technologies). After staining, samples were mounted in Vectashield (Vector Labs). Images were acquired with a Zeiss 710 confocal laser scanning microscope (LSM 710) using ZEN2010 software.

For *in situ* hybridization, digoxygenin-UTP labeled antisense riboprobes for *pappaa* were synthesized and hydrolyzed from the full length *pappaa* cDNA construct in pBS-SK+ (Cox et al., 1984). Whole mount *in situ* hybridization was performed as described previously (Chalasani et al., 2007; Halloran et al., 1999). Images of colorimetric developed embryos were acquired using a Zeiss Axioskop compound microscope and images of FISH labeled larvae were acquired with a Zeiss LSM 710. For RT-PCR based expression analysis, *pappaa* and *B-actin* primers (Supplemental Experimental Procedures) were run against cDNA prepped from total mRNA extracted from 25 embryos/larva at each stage.

*Pharmacology*
Larvae were treated with the compounds: BMS-754807 (Selleckchem.com), SC-79 (Tocris), and 740 Y-P (Tocris). BMS-754807 and SC-79 were dissolved in 100% DMSO and administered in a final concentration of 1% DMSO. 740 Y-P was dissolved and administered in E3 media. For acute treatments larvae were bathed in the compound for 30 minutes prior to and during habituation testing. For chronic treatments with SC-79, compound bath was replaced daily and larvae were tested in compound. Doses of each compound were prescreened for potential effects on embryonic and larval health, gross behavior, baseline startle responsiveness and the highly stereotyped kinematic parameters of startle behavior (Burgess and Granato, 2007).

*Mauthner morphology analysis*
Confocal stacks were processed into maximum intensity projections using ImageJ. 3D reconstructions and surface area and volume quantifications were generated using Imaris software (Bitplane). To define the initial axon segment, we located the ventral inflection point between the axon and soma and set a cut plane from this point to the closest dorsal surface of the axon. To segment the ventral dendrite from the soma, we used the same inflection point used for the initial axon segment, and set a cut plane to the closest lateral surface of the Mauthner neuron. To segment the lateral dendrite from the soma, we measured from the most distal point of the lateral dendrite inward by 30μm and set a perpendicular

vertical cut plane. The soma was defined as the remaining central portion of the Mauthner neuron.

*Statistics*
Statistical analyses, including calculation of means, standard error, ANOVA, and Kaplan-Meier curve, were performed using the Graphpad prism software (www.graphpad.com).

**Supplemental References**
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Raible, D.W., and Kruse, G.J. (2000). Organization of the lateral line system in embryonic zebrafish. The Journal of comparative neurology *421*, 189-198.