Supplemental Figure 1. Apparatus for testing the VOR response in zebrafish larvae shown from above. The larvae are stably positioned in a drop of methyl cellulose on the sample platform situated perpendicular to the red circle. The platform also supports the light source, lens and camera for recording the movements of the eye relative to the platform position.

Supplemental Figure 2. *vglut1* expression levels are comparable between *asteroid/vglut3* mutant and sibling larvae at 96 hpf. *In situ* hybridization of *vglut1* mRNA in wild-type siblings (*A,B*) and *asteroid/vglut3* mutant hair cells (*C,D*). Images are of whole mounts (*A,C*) or 14 μm sections of whole-mount *in situs* (*B,D*). Scale bar: 100 μm in *A,C*, and 3 μm in *B,D*. *E*, Neither *vglut1* nor *vglut3* expression levels (fold expression compared to WT) were significantly changed in *asteroid/vglut3* mutant larvae. Semi-quantitative PCR was performed with *gapdh* serving as an internal standard. Expression levels are: *vglut1* ("VG1N1"): 1.0 ± 0.4, *vglut3* ("VG3N8"): 1.0 ± 0.1, *vglut3* exon2 ("VG3E2"): 0.0 ± 0.0. Error bars denote standard deviation. Data from 3 biological replicates. *F*, Morpholino knockdown of Vglut1 causes deafness and a balance defect in 96 hpf WT larvae. VG1_GT morpholino targets the exon2 donor splice site (GT) of *vglut1* and causes early truncation of the resulting protein. Percentage larvae with auditory/vestibular defects: 0 ± 0% uninjected; 4 ± 6% control MO; 34 ± 6% GT MO; ($p << 0.001$, Student's t-test). Error bars denote standard deviation. Number of larvae examined in parentheses. *G-I*, Vglut1 is localized to the basal half of hair. *G*, Vglut1-GFP fluorescence in neuromast hair cells driven by the *myo6b* promoter (n=5). *H*, Same cells expressing *myo6b:gap43-td tomato*, outlining the cell bodies. *I*, Overlay of *G* and *I*. Scale bar: 3 μm.

Supplemental Figure 3. Setup for recording posterior lateral line ganglion (pLLg) activity in response to mechanical stimulation of lateral line hair cells. ***A,*** Cartoon depicting the location of stimulus and recording pipettes as well as the approximate locations of pLLg (green) and a typical lateral line neuromast (brown). ***B,*** DIC image of pLLg (dashed line) with recording pipette in position. Each soma is 10-15 μm in diameter. ***C,*** Day 5 *neuroD:gfp* transgenic larva. *Left panel:* GFP fluorescence of pLLg with incoming afferent fibers. *Right panel:* lateral line afferent fibers contacting three neuromasts.