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Supplemental information

The Calmodulin-interacting peptide Pcp4a regulates feeding state-dependent behavioral choice in zebrafish

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Supplemental Information

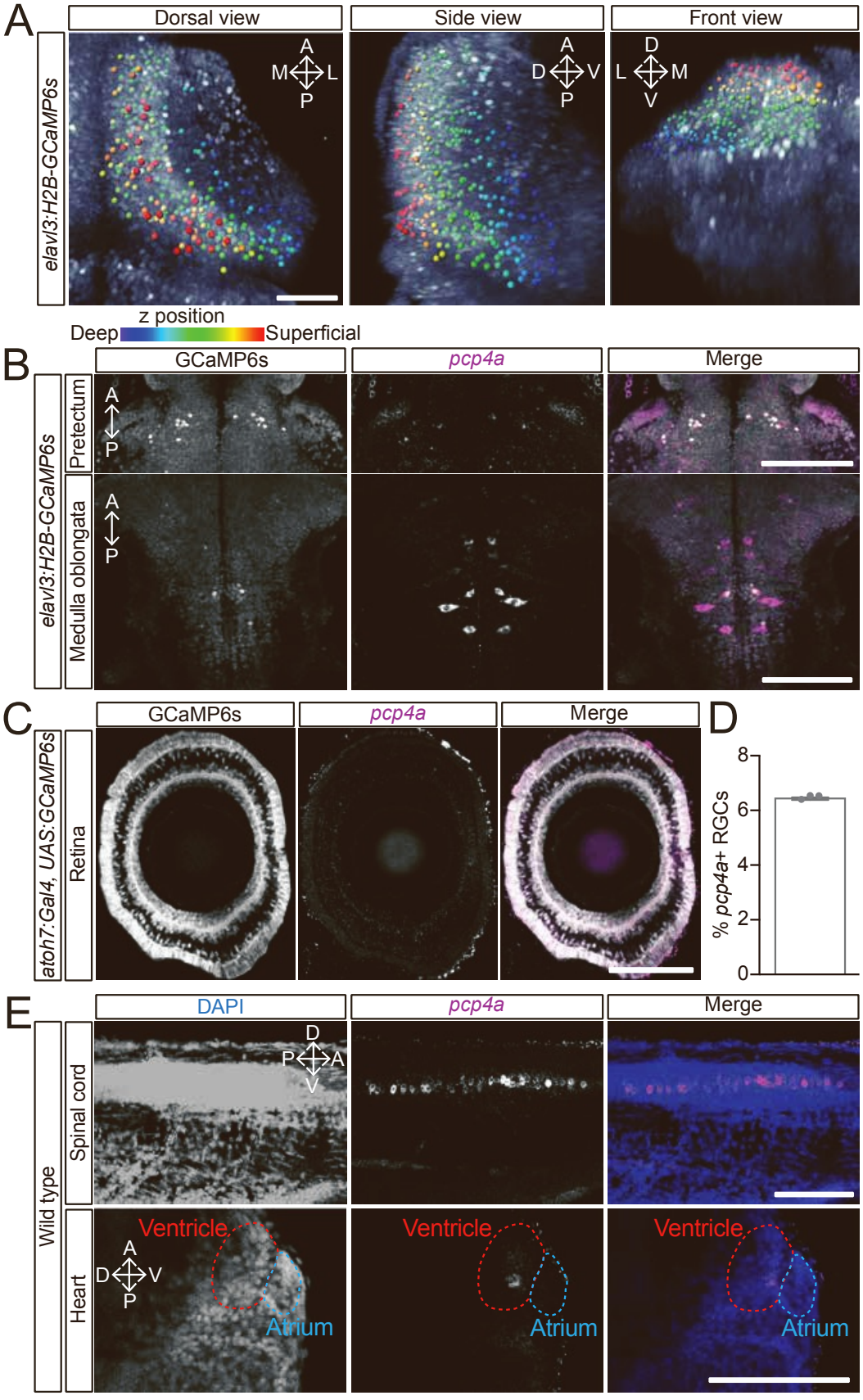

Figure S1. Localization of *pcp4a*+ cells inside and outside the central nervous system. Related to Figure 1. **A.** Top, side, and front views of a 3D-rendered volume of the tectum of a 7 dpf *elavl3:H2B-GCaMP6s* larva. Spheres indicate *pcp4a*+ PVNs identified by *in situ* HCR, and color-coded according to depth. **B, C.** Confocal images showing localization of the mRNA coding for Pcp4a in the pretectum and medulla oblongata of 7 dpf *elavl3:H2B-GCaMP6s* fish (B), and in the retina of a 7 dpf *atoh7:gal4, UAS:GCaMP6s* larva (C). **D.** Graph depicting average percentage of *pcp4a*+ RGCs in the retina. Data are shown as mean ± standard error of the mean. n = 3 larvae. **E.** Confocal images displaying expression of *pcp4a* in the ventral spinal cord and heart of 7 dpf wild-type fish. Outside the central nervous system, Pcp4a-coding mRNA was detectable only in the heart. Scale bars, 50 μm (A), 100 μm (B, C, E). Abbreviations: A, anterior; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral.

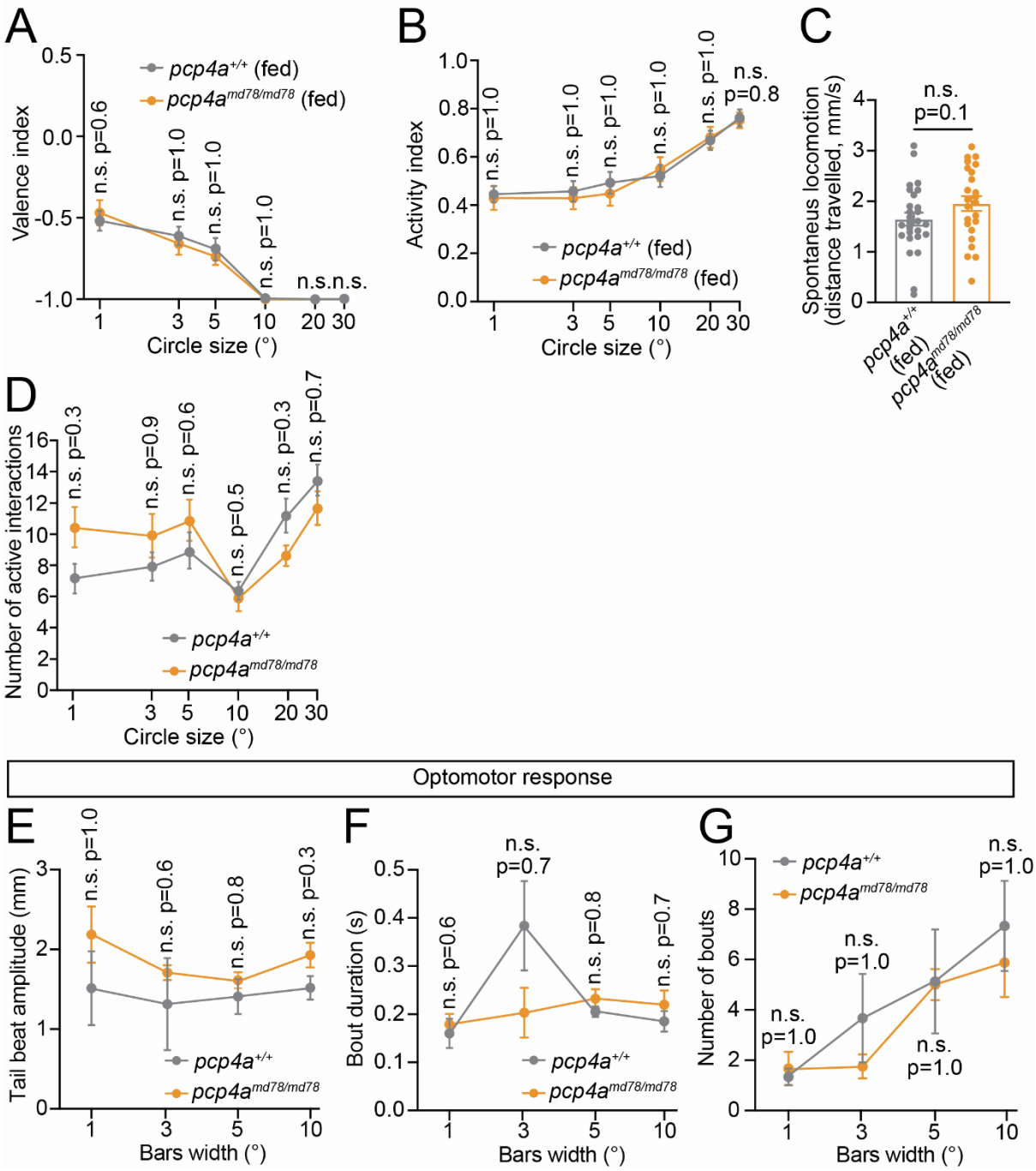

Figure S2. Behavioral performance of *pcp4a* mutants. Related to Figure 2. **A, B.** Graphs depicting average valence indexes (A) or activity indexes (B) for different sizes of visual stimuli of 7 dpf fed *pcp4a*$^{+/+}$ and *pcp4a*$^{md78/md78}$ larvae. $n_{pcp4a+/+}$ = 26 larvae, $n_{pcp4amd78/md78}$ = 22 larvae. **C.** Bar graph showing average spontaneous locomotion of 7 dpf fed *pcp4a*$^{+/+}$ and *pcp4a*$^{md78/md78}$ larvae. $n_{pcp4a+/+}$ = 27 larvae, $n_{pcp4amd78/md78}$ = 24 larvae. **D.** Graph displaying average number of active interactions (approaches + avoidances) in 7 dpf fed *pcp4a*$^{+/+}$ and *pcp4a*$^{md78/md78}$ fish. $n_{pcp4a+/+}$ = 22 larvae, $n_{pcp4amd78/md78}$ = 18 larvae. **E – G.** Graphs showing tail beat amplitude (E), bout duration (F), and number of bouts during optomotor responses performed by 5 dpf unfed *pcp4a*$^{+/+}$ and *pcp4a*$^{md78/md78}$ fish. $n_{pcp4a+/+}$ = 10 larvae, $n_{pcp4amd78/md78}$ =

9 larvae. Data are shown as mean ± standard error of the mean. n.s., not significant. Statistical significance was tested using two-tailed t test with Bonferroni-Holm correction, with the exception of the dataset shown in (C), in which correction for multiple comparisons was not applied.

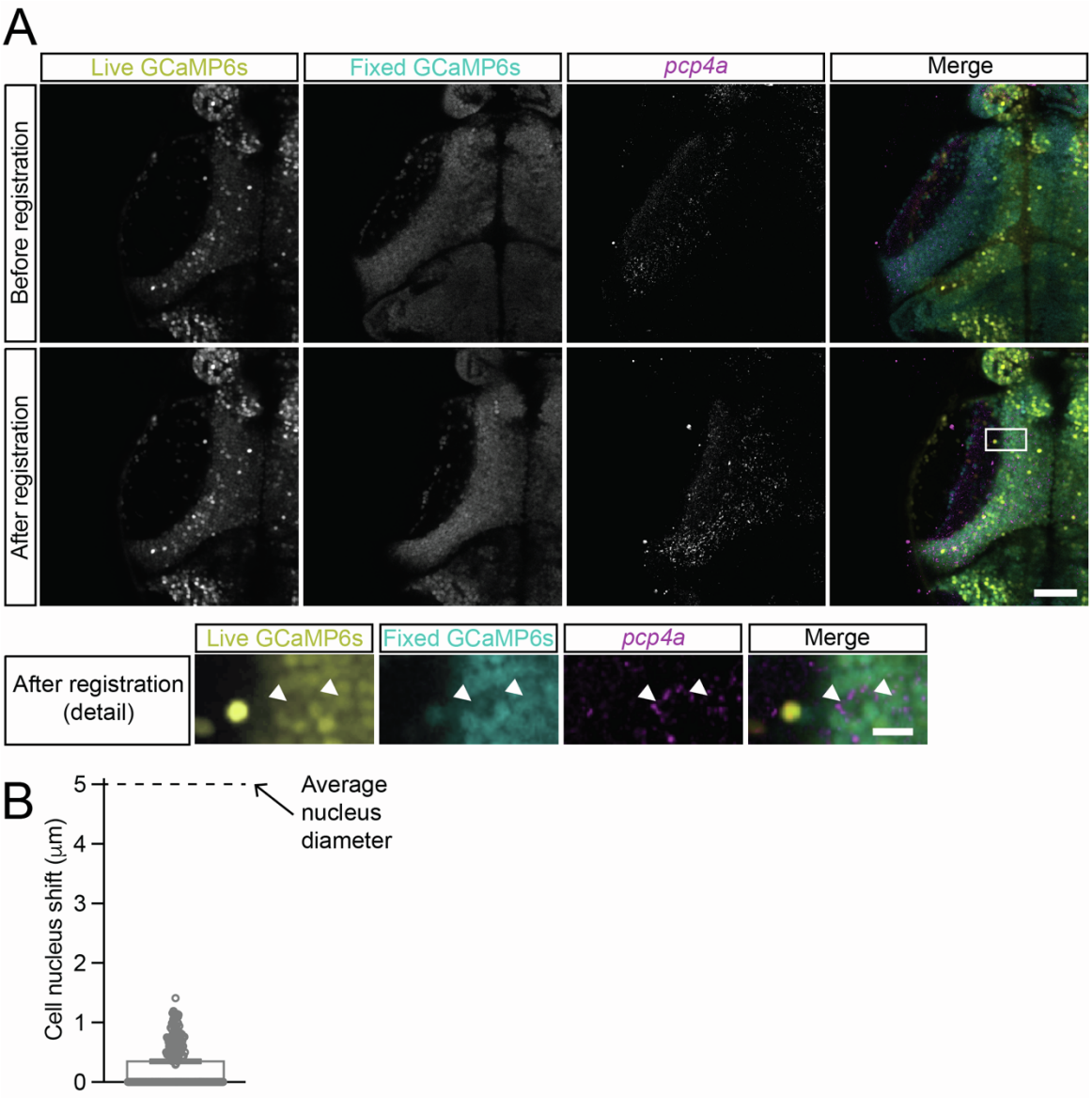

Figure S3. Registration of *in vivo* and *post mortem* confocal images following Ca^{2+} imaging and *in situ* HCR. Related to Figure 3. **A.** Images showing GCaMP6s signal before (live GCaMP6s, average projections of a Ca^{2+} imaging timelapse of a single z-plane) and after fixation (fixed GCaMP6s, corresponding z-plane of the fixed and stained sample), and *pcp4a*'s mRNA localization (detected by *in situ* HCR) in a 7 dpf *elavl3:H2B-GCaMP6s* larva, before and after registration of the *in vivo* and fixed confocal images. At the bottom, high magnification images of the area marked by the white rectangle in the top panel are shown. Scale bars, 50 μm (top panel) or 10 μm (bottom panel). Note that registration of the periventricular layer is very efficient, while the neuropil region of the two images does not align well. **B.** Graph depicting quantification of shift of cell nuclei between the *in vivo* and *post mortem* images after registration. The average shift was minimal, compared to the average cell nucleus diameter. n = 168 randomly selected neurons from 3 fish. Data are shown as mean ± standard error of the mean.

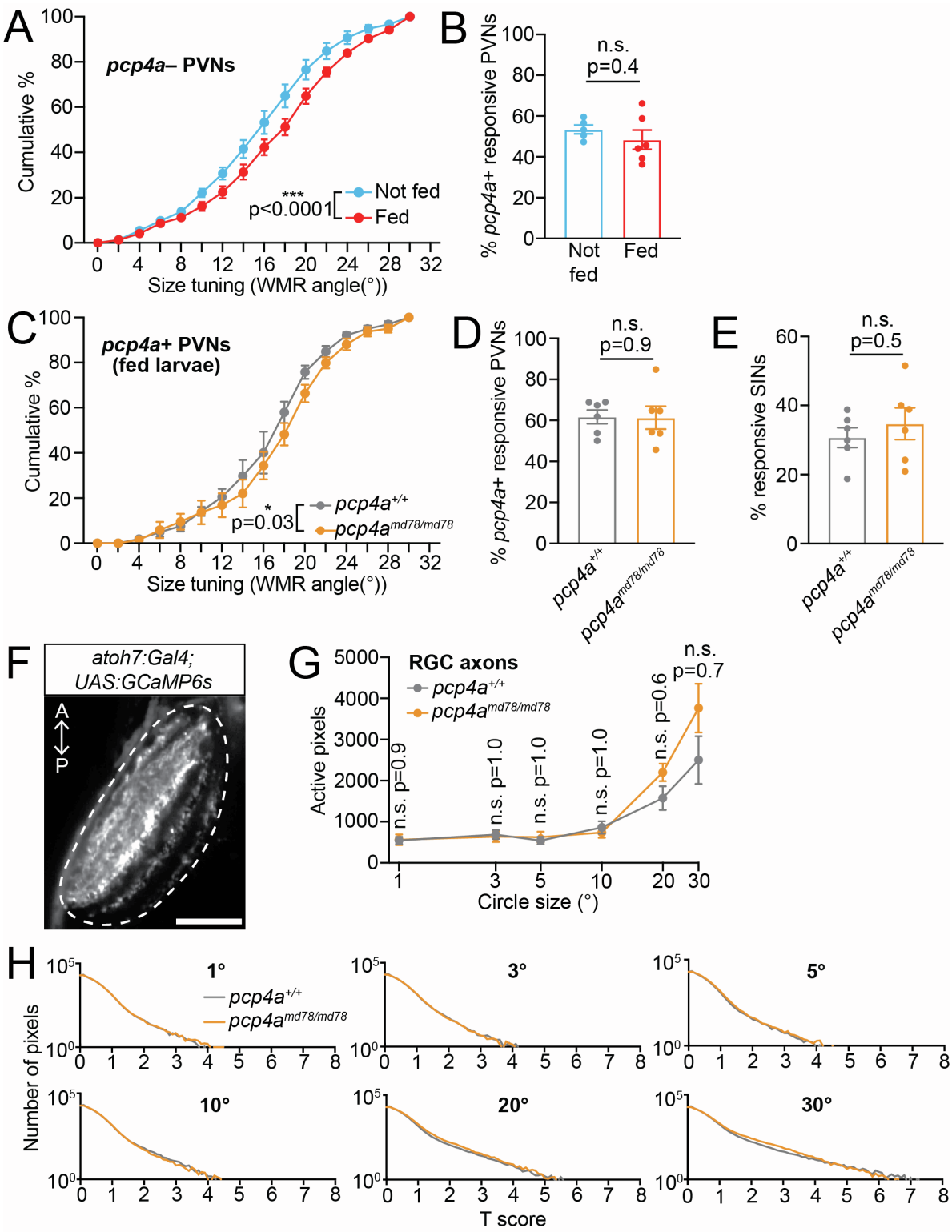

Figure S4. Food intake modifies response properties of *pcp4a*– PVNS but not number of responsive PVNs. The *pcp4a*^{md78} mutation has only a small effect on size tuning of PVNs in fed larvae, and does not alter number of responsive tectal neurons or responses of RGC axons to visual stimuli. Related to Figure 3. **A.** Graph depicting cumulative percentages of WMR angles of *pcp4a*– PVNs in food-deprived and fed 7 dpf *elavl3:H2B-GCaMP6s* larvae. n_{fed} = 6 fish, $n_{not\text{-}fed}$ = 5 fish. *** $p < 0.0001$, two-way ANOVA. **B.** Bar graph depicting average numbers

of *pcp4a*+ PVNs responsive to visual stimuli in 7 dpf fed and not-fed *elavl3:H2B-GCaMP6s*. $n_{not\text{-}fed}$ = 5 larvae, n_{fed} = 6 larvae. n.s., not significant, two-tailed t test. **C.** Graph depicting cumulative percentages of WMR angles of *pcp4a*+ PVNs in fed 7 dpf *elavl3:H2B-GCaMP6s; pcp4a*$^{+/+}$ and *elavl3:H2B-GCaMP6s; pcp4a*$^{md78/md78}$ larvae. n = 5 fish per group. * p = 0.03, two-way ANOVA. **D.** Bar graph showing average numbers of *pcp4a*+ PVNs responsive to visual stimuli in unfed 7 dpf *elavl3:H2B-GCaMP6s; pcp4a*$^{+/+}$ and *elavl3:H2B-GCaMP6s; pcp4a*$^{md78/md78}$ larvae. $n_{pcp4a+/+}$ = 6 larvae, $n_{pcp4amd78/md78}$ = 6 larvae. n.s., not significant, two-tailed t test. **E.** Bar graph showing average number of SINs responsive to visual stimuli in 7 dpf *elavl3:H2B-GCaMP6s; pcp4a*$^{+/+}$ and *elavl3:H2B-GCaMP6s; pcp4a*$^{md78/md78}$ larvae. $n_{pcp4a+/+}$ = 6 larvae, $n_{pcp4amd78/md78}$ = 6 larvae. n.s., not significant, two-tailed t test. **F.** Confocal image showing the tectal neuropil, labeled by retinal ganglion cell axons, of a 7 dpf *atoh7:gal4; UAS:GCaMP6s* larva. The dashed white line marks the borders of the neuropil region. Scale bar, 50 μm. A and P indicate anterior and posterior directions, respectively. **G.** Graph showing average numbers of active pixels in RGC axons of food-deprived 7 dpf *atoh7:gal4; UAS:GCaMP6s; pcp4a*$^{+/+}$ and *atoh7:gal4; UAS:GCaMP6s; pcp4a*$^{md78/md78}$ larvae in response to visual stimuli of different sizes. $n_{pcp4a+/+}$ = 14 larvae, $n_{pcp4amd78/md78}$ = 9 larvae. n.s., not significant, two-tailed t test with Bonferroni-Holm correction. **H.** Graphs depicting distribution of T scores obtained with the pixel-wise analysis based on a regression model to analyze responses of RGC axons to visual stimuli of different sizes in 7 dpf *atoh7:gal4; UAS:GCaMP6s; pcp4a*$^{+/+}$ and *atoh7:gal4; UAS:GCaMP6s; pcp4a*$^{md78/md78}$ larvae (see Materials and Methods for details). $n_{pcp4a+/+}$ = 14 larvae, $n_{pcp4amd78/md78}$ = 9 larvae. Data in (A – E, G) are presented as mean ± standard error of the mean.

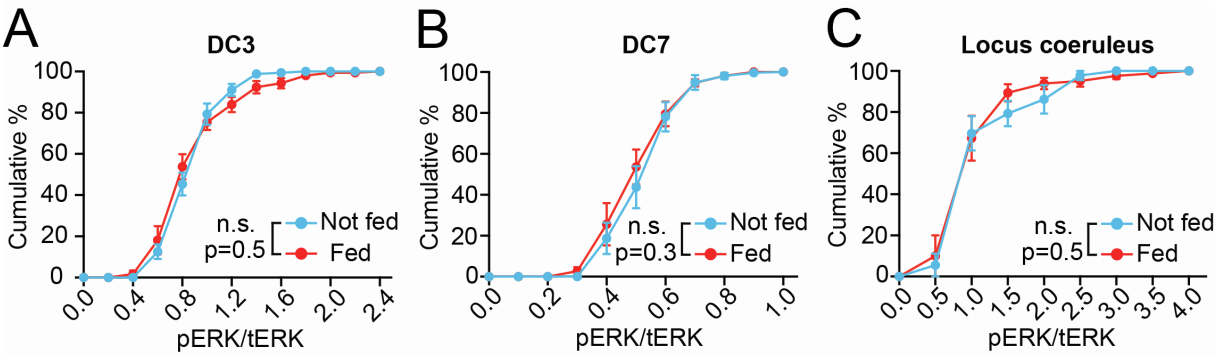

Figure S5. Food intake does not alter activity of DC3 and DC7 dopaminergic neurons, and noradrenergic neurons of the locus coeruleus. Related to Figure 4. **A – C.** Graphs showing cumulative percentages of pERK/tERK values in dopaminergic neurons in the DC3 (A) and DC7 (B) clusters, and in noradrenergic neurons in the locus coeruleus (C) in fed or food-deprived 7 dpf *th:gal4-VP16; UAS:EGFP-CAAX* larvae. $n_{not\text{-}fed} = 9$ larvae, $n_{fed} = 10$ larvae. n.s., not significant, two-way ANOVA. Data are shown as mean ± standard error of the mean.

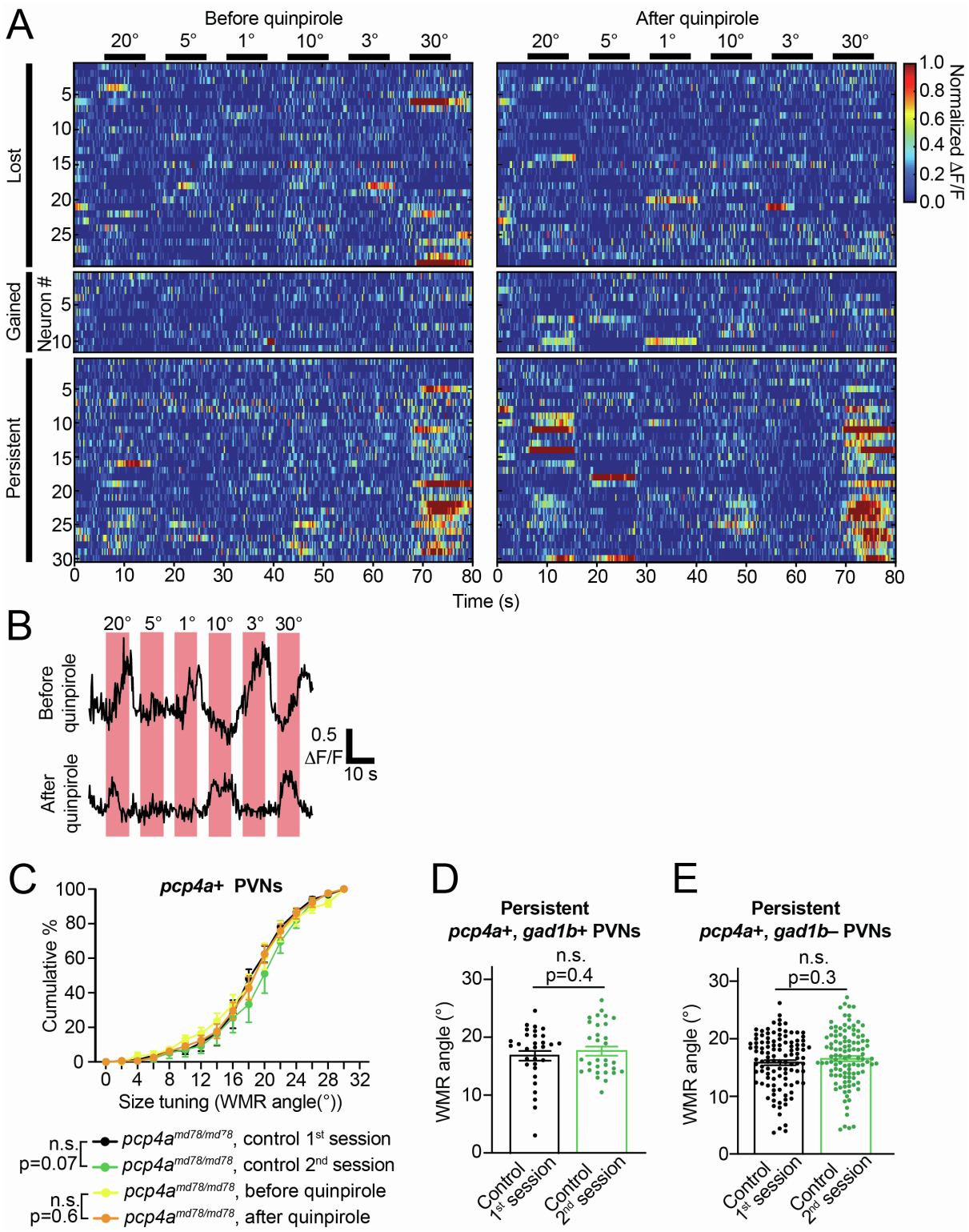

Figure S6. Responses to visual stimuli of *pcp4a*+ PVNs before and after treatment with quinpirole. Related to Figure 5 and 6. **A.** Heatmap showing activity of lost, gained and persistent *pcp4a*+ PVNs in response to visual stimuli of different sizes in a 7 dpf *elavl3:H2B-GCaMP6s* larvae before and after treatment with quinpirole. **B.** Examples of traces of a persistent *pcp4a*+ PVNs which responded to small and large visual stimuli before treatment with quinpirole, and only to large ones after. **C.** Graph showing cumulative percentages of

WMR angles of *pcp4a*+ PVNs in unfed 7 dpf *pcp4a*$^{md78/md78}$; *elavl3:H2B-GCaMP6s* larvae before and after three hours treatment with quinpirole and in untreated controls kept in agarose for the same duration of the drug treatment. $n_{control}$ = 3 fish, $n_{quinpirole}$ = 4 fish. n.s., not significant, two-way ANOVA. **D, E.** Graphs depicting WMR angles of *pcp4a*+/*gad1b*+ (D) or *pcp4a*+/*gad1b*– (E) PVNs in the tecta of 7 dpf *elavl3:H2B-GCaMP6s* larvae before (1st session) or after three hours of permanence in agarose (2nd session) without drug treatment. *pcp4a*+/*gad1b*+: 31 neurons from 4 fish, *pcp4a+/gad1b–:* 110 neurons from 4 fish. n.s., not significant, paired t test. Data are shown as mean ± standard error of the mean. Individual data points are also shown.