Supplementary Information

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Comprehensive Analysis of GnRH2 Neuronal Projections in Zebrafish

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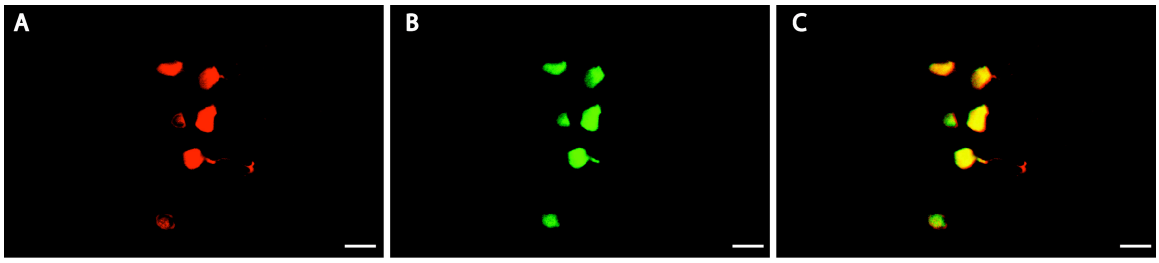

Figure S1| Double fluorescent staining of *gnrh2* mRNA and eGFP protein on a 25 dpf *GnRH2:eGFP Tg* juvenile brain section (7 µm cross section with dorsal at the top).

(A) *In situ* hybridization using *gnrh2* mRNA probe and TSA cy3 solution. (B) ICC using mouse anti-eGFP antibody and secondary fluorescein-conjugated antibody. (C) Overlay of Figures D & E. Scale bars = 20 µm (D-F).

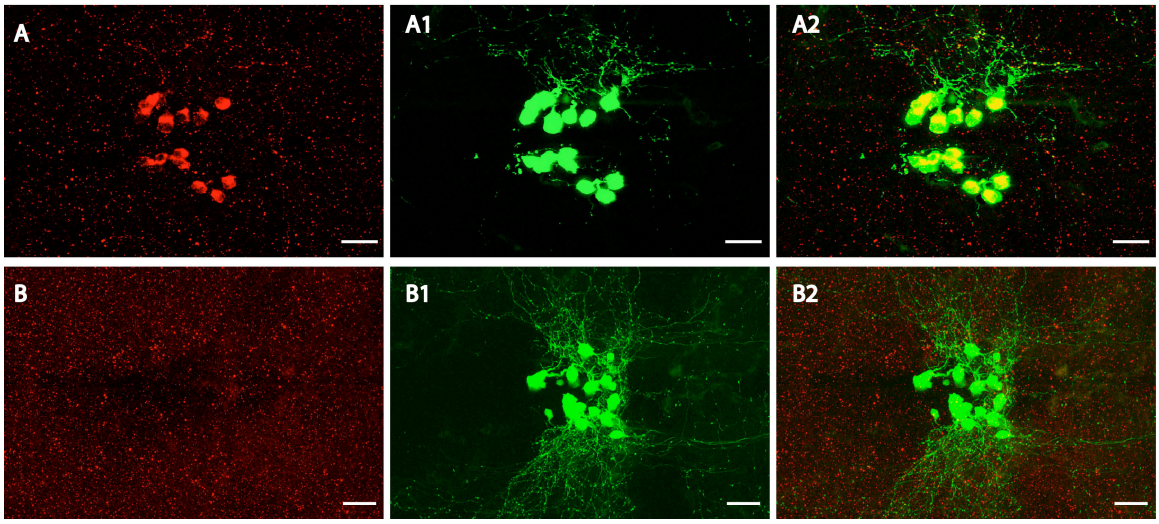

Figure S2| Immunocytochemistry detection of GnRH2 antibody specificity.

Consecutive 100 µm (thickness) horizontal sections (dorsal views with anterior to the left) were, respectively, immunostained by GnRH2 antibody (A) and the pre-absorbed GnRH2 antibody with excess recombinant GnRH2-GAP protein (B). The green fluorescent signals represent endogenously expressed eGFP proteins (A1, B1). Merged views (A2, B2) of GnRH2 antibody stained (A & A1) and pre-absorbed GnRH2 antibody stained (E & E1) *GnRH2:eGFP Tg* brain sections, showing the antibody signal was completely abolished by the pre-absorption in the eGFP-expressing neurons, despite the longer exposure causing more non-specific background (B) compared with non-pre-absorbed antibody (A) . Scale bars = 20 µm.

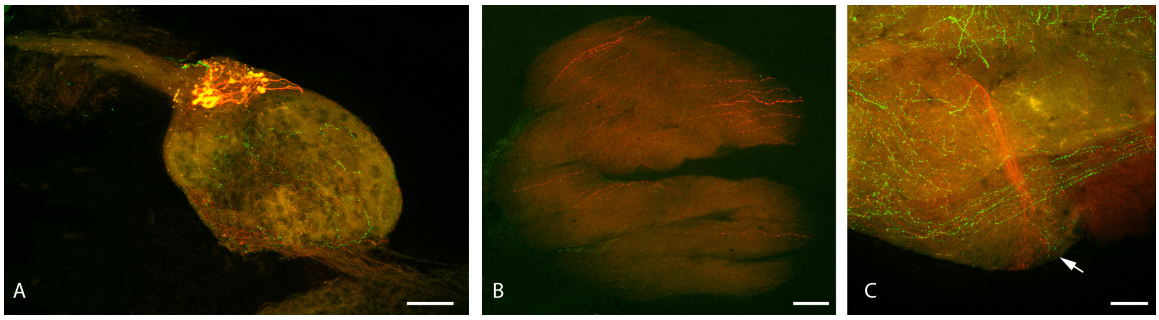

Figure S3| Sagittal sections of a double *GnRH2:eGFP* and *GnRH3:tdTomato Tg* adult fish, showing the olfactory and mediobasal hypothalamus areas.
Unlike GnRH3, GnRH2 fibers end in the olfactory bulbs (A) and are not detected in the olfactory epithelium (B). In the mediobasal hypothalamus (C), both GnRH2 and GnRH3 fibers can be observed projecting to the pituitary stalk (arrow). Scale bars = 100 µm.

Movie S1| Fibers projecting from tegmentum GnRH2 cells in the midbrain and hindbrain areas of a live 9 dpf larva.
Corresponding to Figure 3B & C. Anterior (head) to the right.

Movie S2| Observation of both GnRH projections in the midbrain and forebrain of a 4 dpf larva.
Corresponding to Figure 6A & C. Anterior (head) to the right.