

Supplementary Materials:

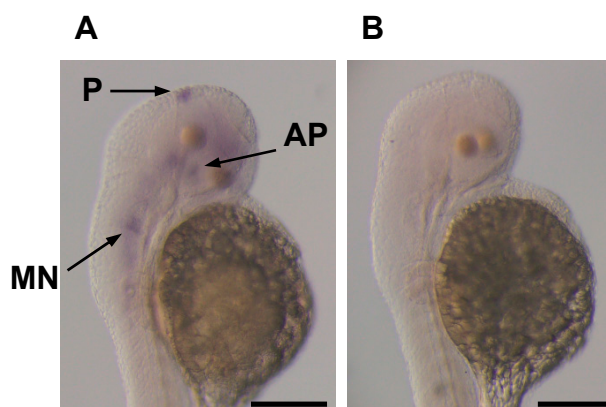


Figure S1. Spatiotemporal expression of *lhx4* in 48 hpf WT zebrafish. Whole-mount ISH staining of *lhx4* in 48 hpf WT zebrafish. **A)** *Lhx4* is expressed in motor-neurons (MN) in the spinal cord, pineal gland (P) and anterior pituitary (AP) (denoted by arrows). **B)** Negative control of whole-mount ISH staining with the sense probe of *lhx4*. Scale bar = 200 μm .

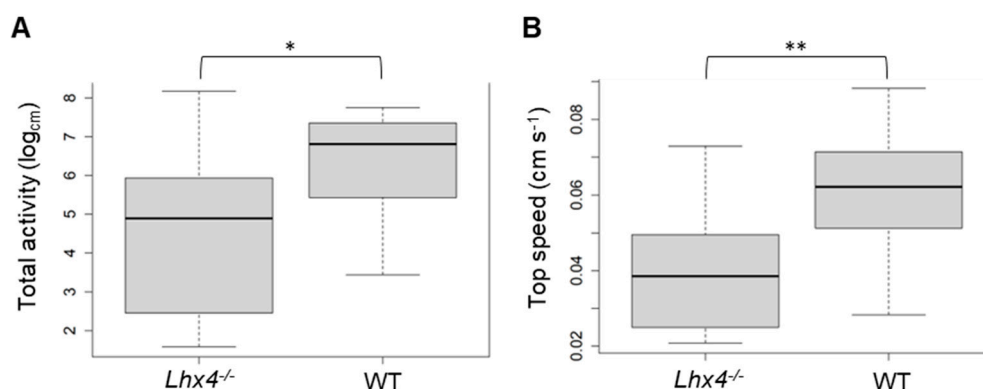


Figure S2. Reduced locomotor activity of *lhx4* mutant larvae. Locomotor activity of homozygous *lhx4* mutant larvae (N = 9) and their WT siblings (N = 13) was continuously monitored for 4 hours at 9 dpf. Boxplots present medians (solid lines) and interquartile ranges (gray boxes) within each group. **A)** Total activity (log_{cm}) of homozygous *lhx4* mutant larvae is significantly reduced in comparison with their WT siblings, **p* < 0.05, t-test with Benjamini-Hochberg correction (false discovery rate = 0.05). **B)** The maximal speed (cm sec⁻¹) of homozygous *lhx4* mutant larvae is significantly reduced compared with their WT siblings, ***p* < 0.01, t-test with Benjamini-Hochberg correction (false discovery rate = 0.05).

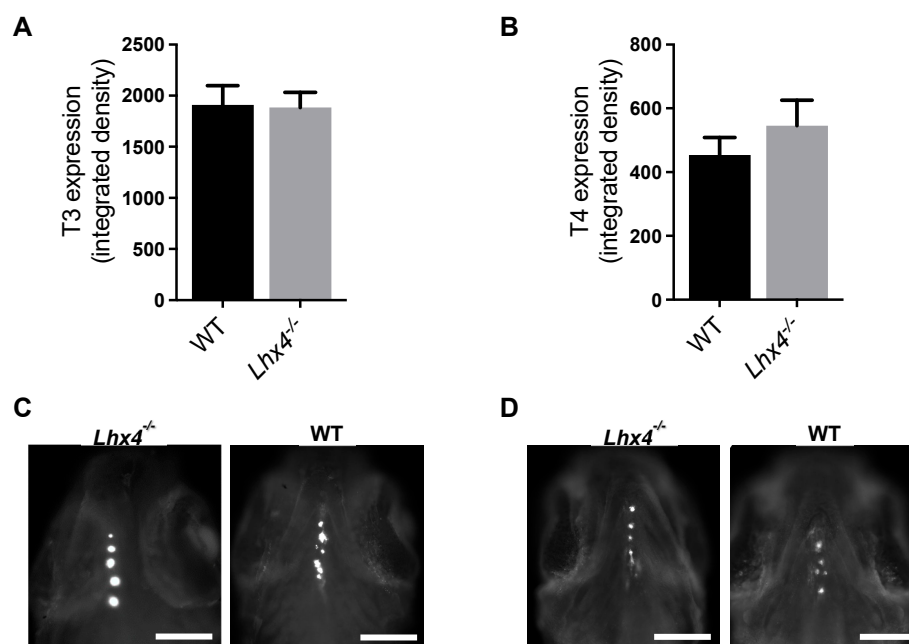


Figure S3. Unaltered levels of thyroid hormones in the thyroid of 7 dpf *lh4* KO larvae. Levels of T3 (A) and T4 (B) in the thyroid of 7 dpf larvae, analyzed by whole-mount immunohistochemistry (integrated density of the fluorescent signal). **A)** No differences were found in T3 levels between homozygous *lh4* mutants (N = 13) and their WT siblings (N = 12). **B)** No differences were detected in T4 levels between homozygous *lh4* mutants (N = 13) and their WT siblings (N = 12). Error bars indicate s.e.m. **C)** Representative samples (heads of 7 dpf larvae, ventral view) of homozygous *lh4* mutant and WT sibling immunostained with an antibody against T3. **D)** Representative samples (heads of 7 dpf larvae, ventral view) of homozygous *lh4* mutant and WT sibling immunostained with an antibody against T4. Scale bar = 200 μm.