c186/+ x fh252/+

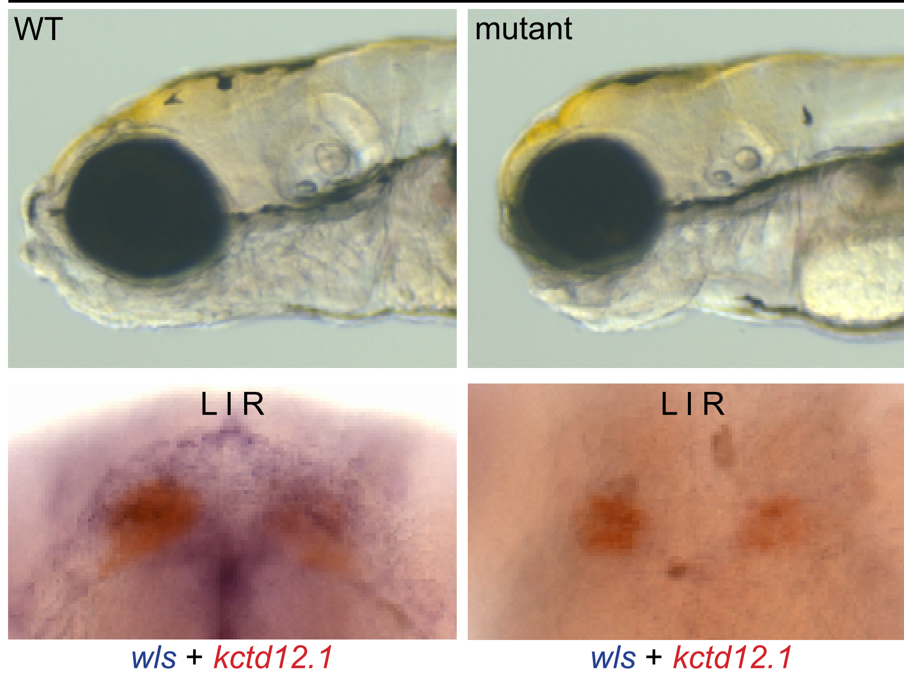
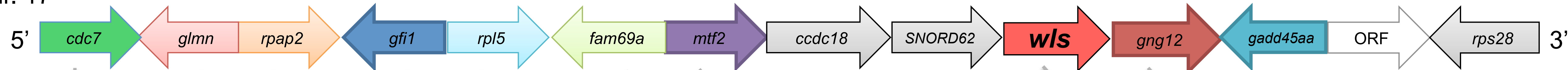
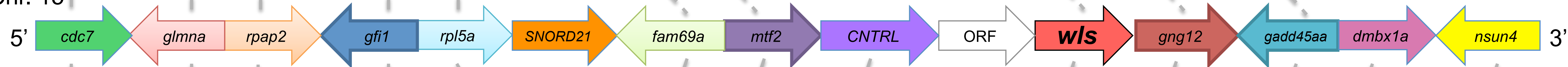


Fig. S1: c186 and fh252 alleles fail to complement
Transheterozygous (c186/fh252) <i>wls</i> mutants can be readily distinguished from WT larvae at 4 dpf and have similar morphological and dorsal habenular phenotypes to mutants homozygous for each allele.

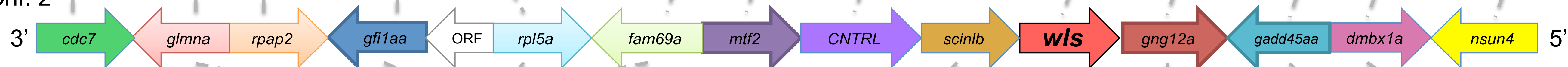
Medaka Chr. 17



Pufferfish Chr. 15



Zebrafish Chr. 2



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Zebrafish Chr. 6

8 Mb

25 Mb

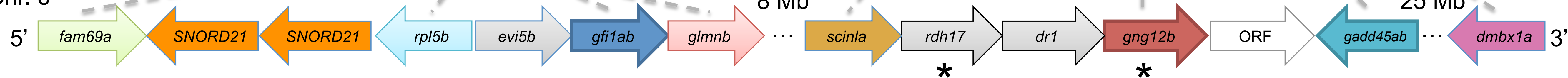


Fig. S2: Syntenic analysis supports a single zebrafish <i>wls</i> gene
The regions surrounding the zebrafish <i>wls</i> gene on chromosome (Chr.) 2 are conserved with other teleost species, such as medaka (<i>Oryzias latipes</i>) and pufferfish (<i>Tetradon nigroviridis</i>). Zebrafish chromosome 6 also shows partial synteny, but contains multiple insertions, deletions and inversions. DNA sequence homologous to the <i>wls</i> gene is not found within the expected region between <i>scinla</i> and <i>gng12a</i>. Asterisks signify previously unannotated genes and ORF indicate open reading frames of unknown genetic identity.

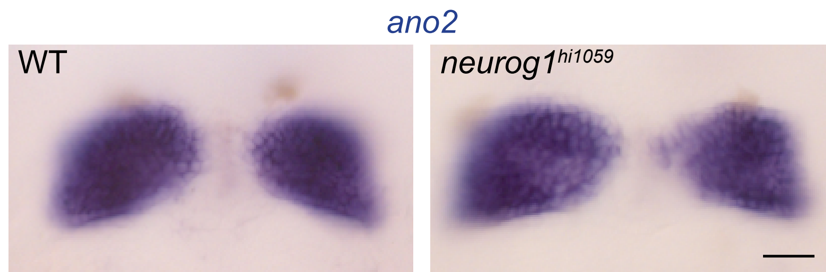
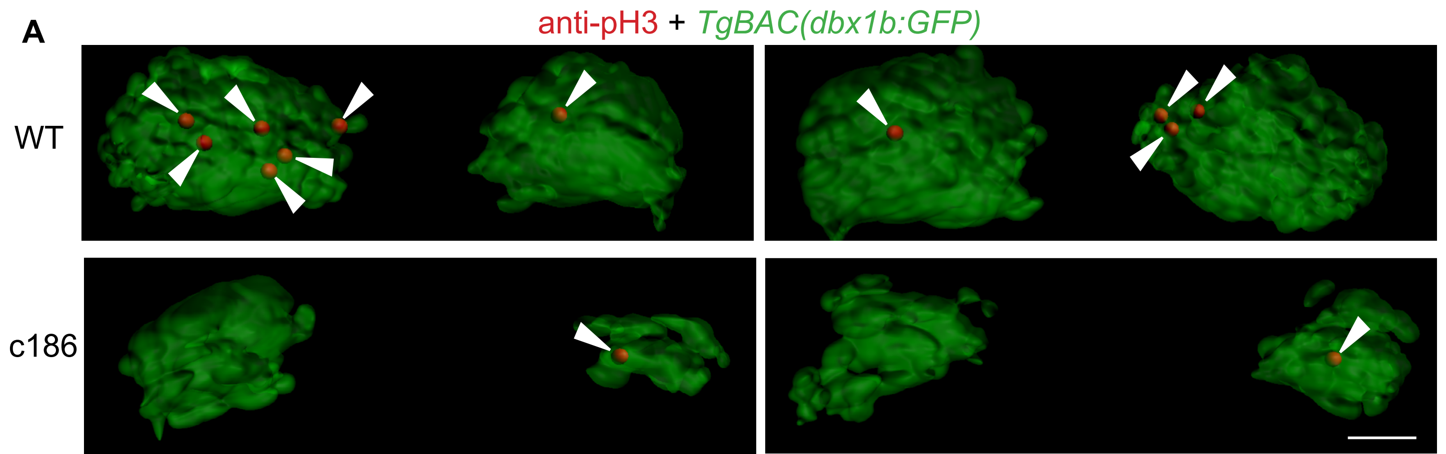


Fig. S3: Dorsal habenular nuclei develop normally in <i>neurogenin-1</i> mutants
WT and homozygous <i>neurog1<super>hi1059</super></i> mutants show similar expression of <i>ano2</i> in the dorsal habenular nuclei. The <i>neurog1<super>hi1059</super></i> allele is due to a retroviral insertion (Golling, G., Amsterdam, A., Sun, Z., Antonelli, M., Maldonado, E., Chen, W., Burgess, S., Haldi, M., Artzt, K., Farrington, S., et al. (2002). Insertional mutagenesis in zebrafish rapidly identifies genes essential for early vertebrate development. Nat Genet 31, 135-140) and, although not confirmed to be a null mutation, when homozygous, it disrupts formation of sensory neurons (McGraw, H.F., Nechiporuk, A., Raible, D.W. (2008). Zebrafish dorsal root ganglia neural precursor cells adopt a glial fate in the absence of <i>*neurogenin-1</i>*. J Neurosci 28, 12558-12569).



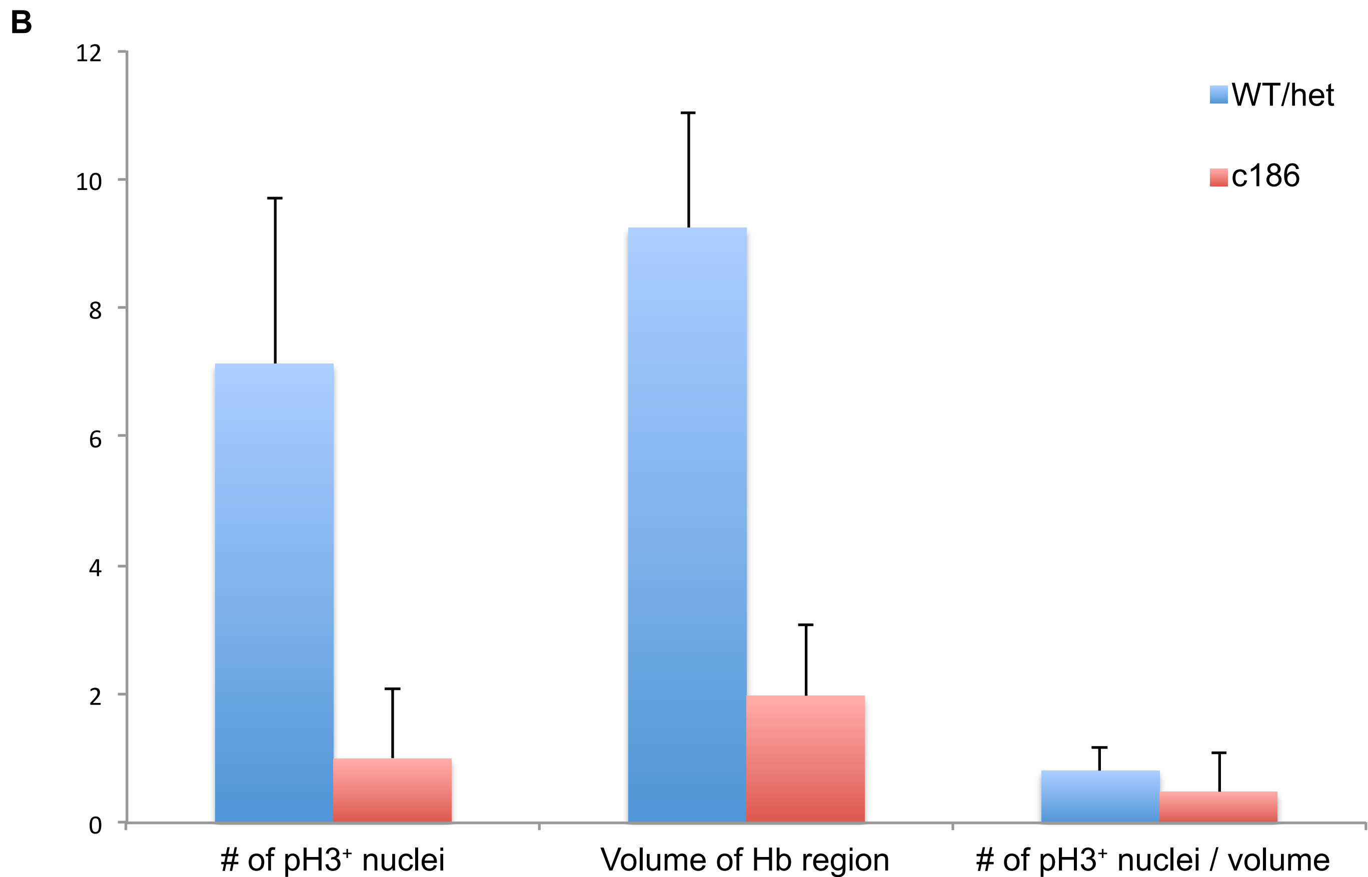


Fig. S4: Cells proliferate within the developing habenular region of <i>wls</i> mutants (A) Immunolabeling was performed at 48 hpf on <i>*TgBAC(dbx1b:GFP)*</i> embryos to visualize pH3<super>+</super> mitotic nuclei within the developing habenulae. Habenular volume was calculated by generating a 3D virtual representation of the <i>*dbx1b:GFP*</i> labeled domain (green) using the surfaces function of Imaris image processing software (Bitplane). pH3<super>+</super> cells unambiguously located within this domain (red) were identified computationally using the spots function of Imaris. Two representative examples of <i>*wls*</i><super>c18<i>6</i></super> homozygous mutants and WT siblings are shown postprocessing. Scale bar = 20 µm. (B) Although the number of pH3<super>+</super> cells and volume of the developing habenulae (value of y axis X 10<super>4</super> µm<super>3</super>) differed between mutant (n = 11) and WT (n = 15) embryos (two-tailed t-test, p=6.29 x 10<super>-8</super> and p=4.21 x 10<super>-12</super>, respectively), when normalized for volume, the number of mitotic cells within the <i>*dbx1b:GFP*</i> habenular domain was not significantly different between the two groups (p=0.12).