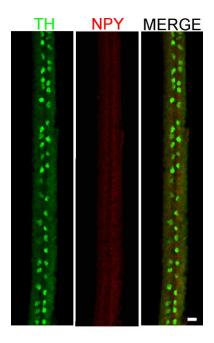
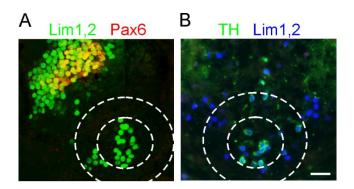


Supplementary Figure 1. Activity-dependent dopamine specification is absent from the PT at earlier stages of development. A. Percent change in TH-IR neurons in activity-manipulated embryos compared to control in the brain, adding OB, DLSC, VSC and PT together. B. Overexpression of Na<sub>v</sub> increases spike frequency to a similar level in the PT and VSC. Stage 35. C-D. Percent change in TH-IR neurons in activity-manipulated embryos compared to control in PT at stage 37 (C), or in stage 40 (D) (n>4 larvae per stage; values are mean  $\pm$  SEM. \*p>0.05; comparing control versus hKir2.1 and rNa<sub>v</sub>2a  $\alpha\beta$  by the Kruskal-Wallis test followed by Conover post-hoc analysis).



Supplementary Figure 2. NPY expression in the spinal cord. NPY is not expressed in dopaminergic cells of the spinal cord. Scale bar is 25  $\mu$ m. Wholemount from a stage 42 larva.



Supplementary Figure 3. Lim1,2 and Pax6 expression in the VSC. A. Transcription factors Lim1,2 and Pax6 are expressed in the diencephalon but Pax6 is not expressed in the VSC (dashed circles). **B**. TH neurons of the VSC core and annulus express Lim1,2, as previously shown (Dulcis and Spitzer, 2008). Scale bar is 40  $\mu$ m. Transverse sections are from stage 42 larvae.