

Supplemental Figures

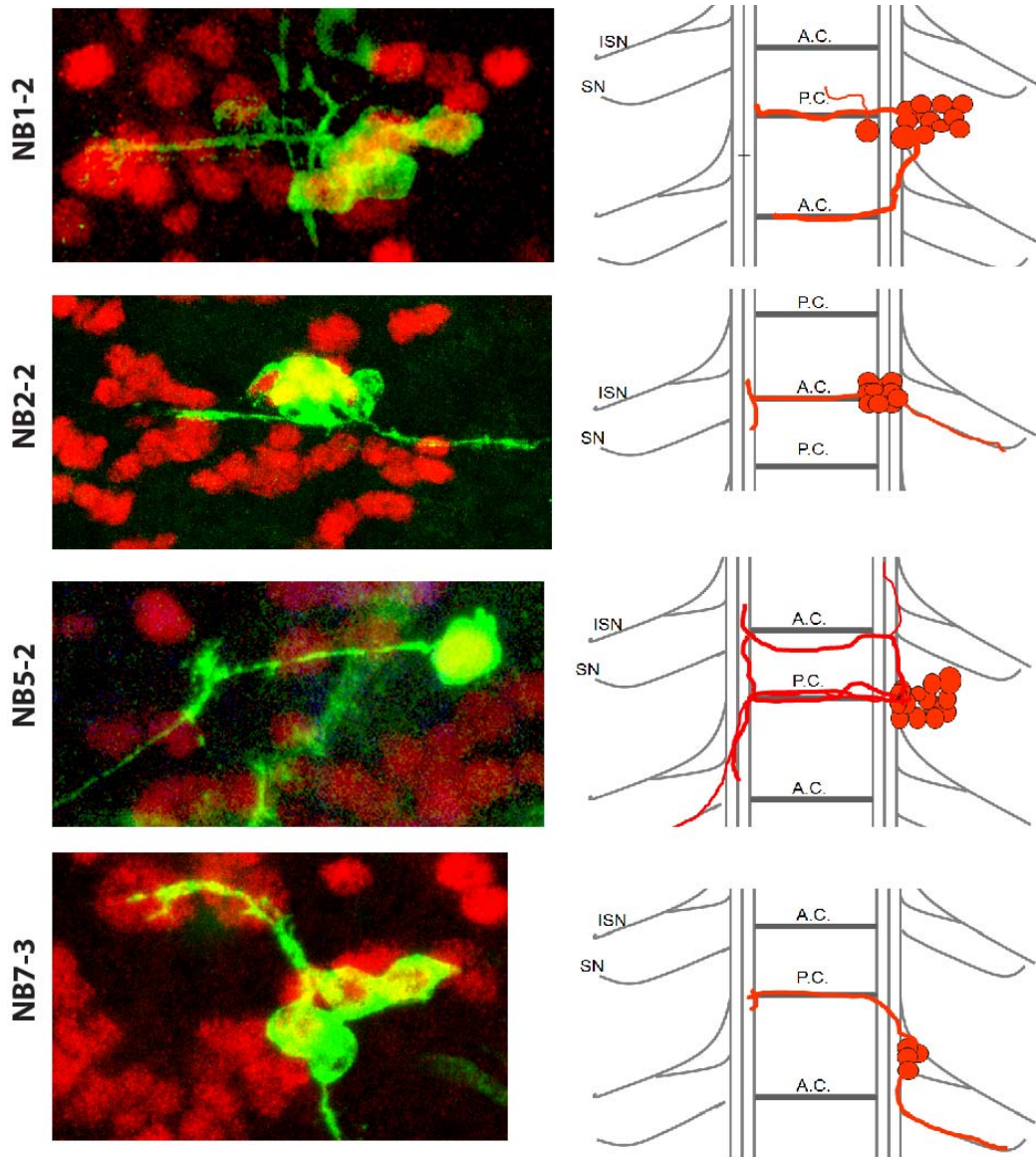


Figure S1) Lineage clones of Hb9-positive neurons. (Left) Abdominal segments of late-stage wild-type embryos containing lineage clones labeled for Hb9 (red) and GFP (green) for NB 1-2, NB 2-2, NB 5-2, and NB 7-3. (Right) Schematic representations of the cell body position and axonal projection patterns of the lineage produced by the indicated neuroblast; schematics models are based on the results from the Dil labeling studies of Bossing et al. (1996), Schmidt et al. (1997), and Schmid et al. (1999).

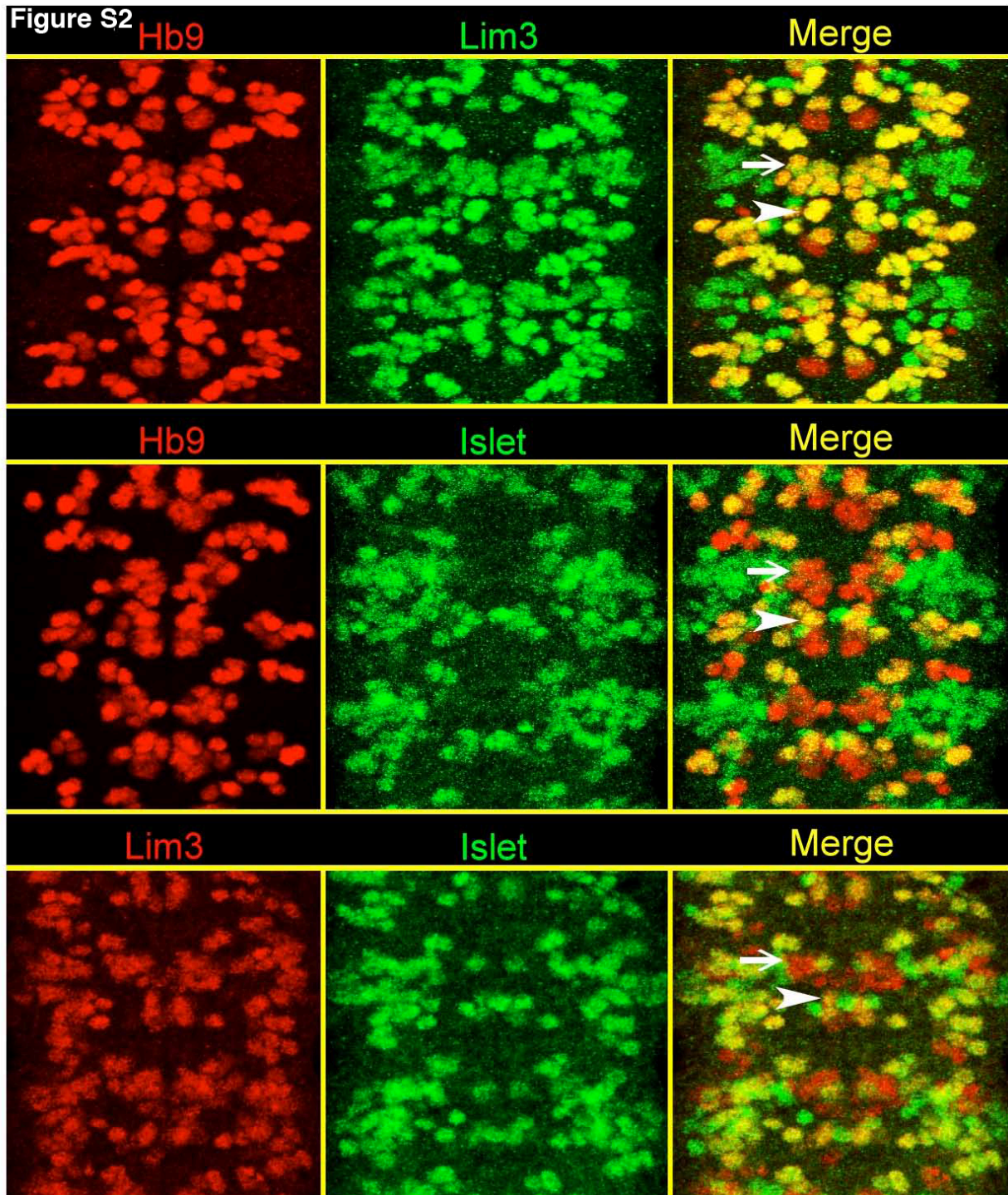


Figure S2) Relative expression patterns of Hb9, Lim3, and Islet in the Drosophila embryonic CNS: arrowheads point to the RP neurons; arrows point to the Hb9 and Lim3 expression neurons of the NB2-2 lineage.

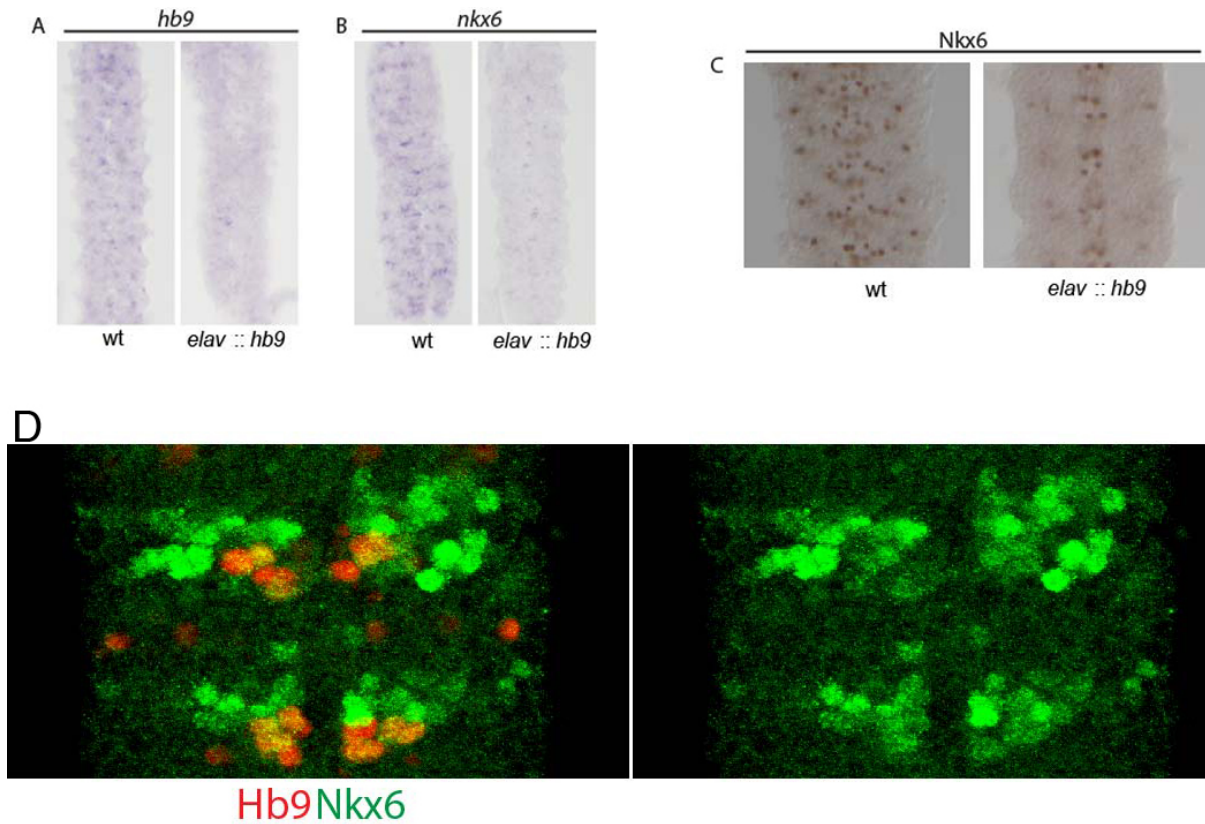


Figure S3) Hb9 overexpression represses both Hb9 and Nkx6 expression. A-C) Dissected nerve cords from stage 15-16 wild-type embryos and embryos that over-expressed Hb9 in all neurons (*elav::hb9*) stained for Hb9 mRNA (A), Nkx6 mRNA (B), or Nkx6 protein (C). (D) High magnification view of two segments from a stage 14 wild-type CNS labeled for Hb9 (red) and Nkx6 (green). Nkx6 expression is lower in cells that also co-express Hb9.

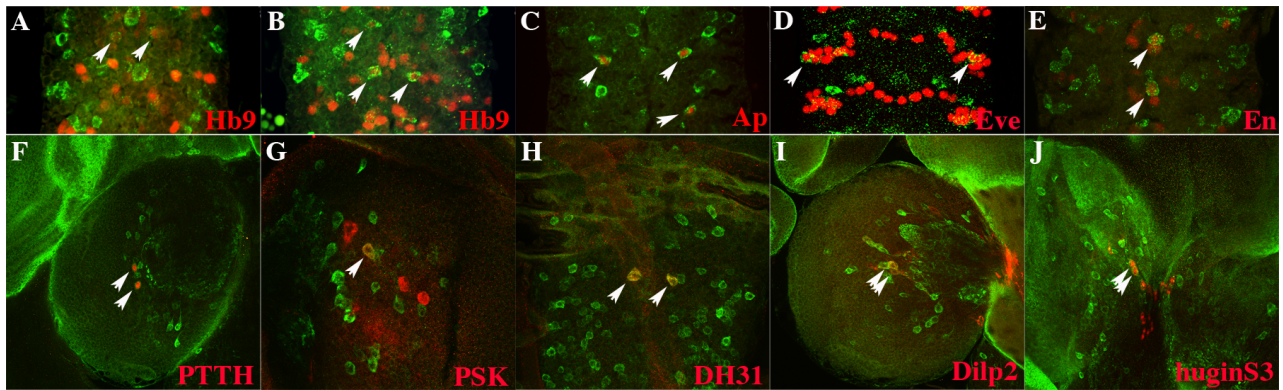


Figure S4) NOS expression in the embryonic and larval CNS.

(A-E) High magnification ventral views of two consecutive segments of stage 15 embryonic nerve cords labeled for NOS (green) and the indicated proteins. Arrows in B likely identify Hb9+ neuron in NB 2-2 lineage. Anterior is at the top. (F-J) High magnification views of late-third instar larval brains (F, G, I, J) and nerve cord (H) labeled for NOS and the indicated neuropeptides.

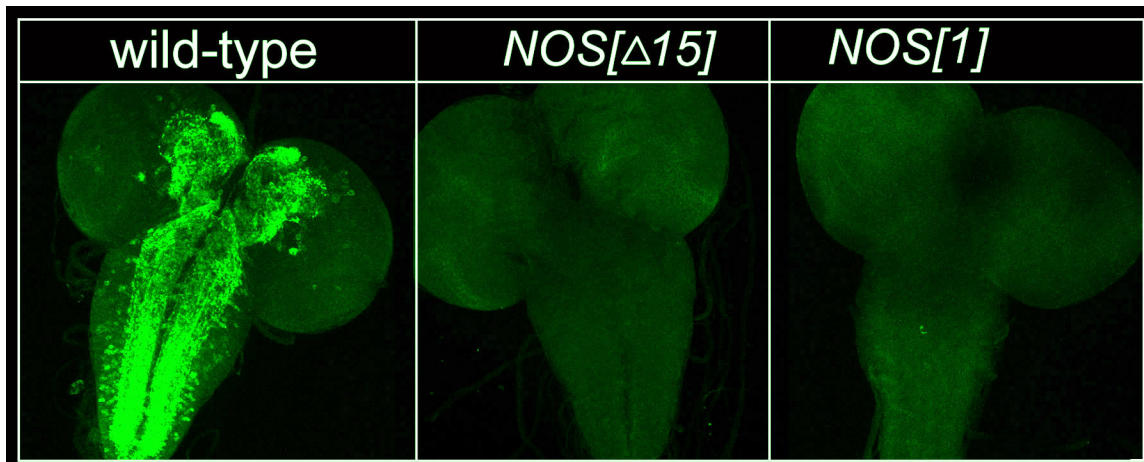


Figure S5) NOS protein expression in the late-third instar larval CNS of wild-type, *Nos* mutant, and *Nos-RNAi*-treated larvae.

NOS expression in a wild-type CNS and the CNS from larvae homozygous mutant for *Nos*^{*A15*} or *Nos*^{*F107*}.

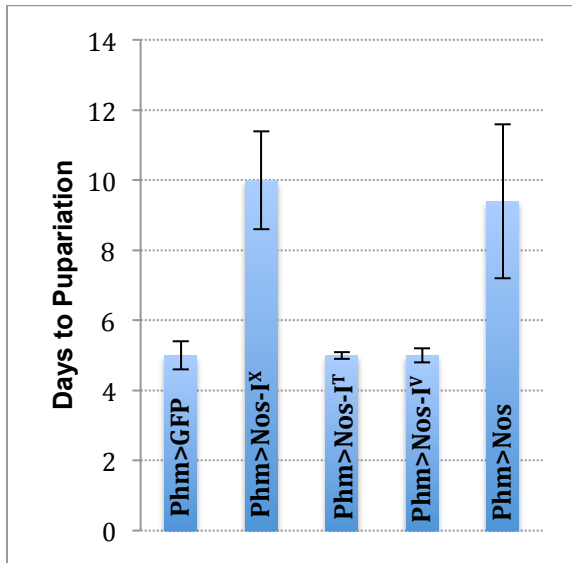


Figure S6) Time to pupariation (from larval hatching) is shown for indicated genotypes. Phm: Phantom-GAL4. *Nos-i^x*: *UAS-Nos-RNAi^{IR-X}* from Caceres et al. 2011; *Nos-i^T*: line JF03220 from TRiP RNAi collection (BSC28792); *Nos-i^V* (line P{GD12013}v27722 from VDRC collection). NOS: UAS-NOS (wild-type UAS-NOS transgene; see methods).