

Fig. S1. *pbx* is expressed in cells colocalizing with neoblasts and is expressed in the regenerating central nervous system and the regenerating pharynx. (A) Adjacent sagittal sections of whole worms showed that *pbx* is expressed in a pattern resembling that of the neoblast marker *H2B*. Images were false colored and overlaid to illustrate the colocalization of *pbx*- and *H2B*-expressing cells within the parenchyma ($n=5$ section pairs from three animals). (B) *pbx* expression is broadly observed in both anterior and posterior blastemas of regenerating pieces. Expression in the CG of head pieces was also clearly visible. By 10 days, expression resembles that seen in intact animals. Expression in the regenerating pharynx is also observed in head and tail pieces from 3 dpa. (C) Regenerating fragments were γ -irradiated with a dose of 100 Gy 1 day prior to fixation to aid visualization of *pbx* in cells other than neoblasts, and fixed at the time points indicated. As in B, expression is observed in anterior and posterior blastemas and in the CG of head pieces. Expression in blastemas is bilateral, suggesting expression in the regenerating CNS. Expression in the regenerating pharynx is also clear from 3 dpa. Scale bars: 200 μ m. CG, cephalic ganglion; dpa, days post-amputation.

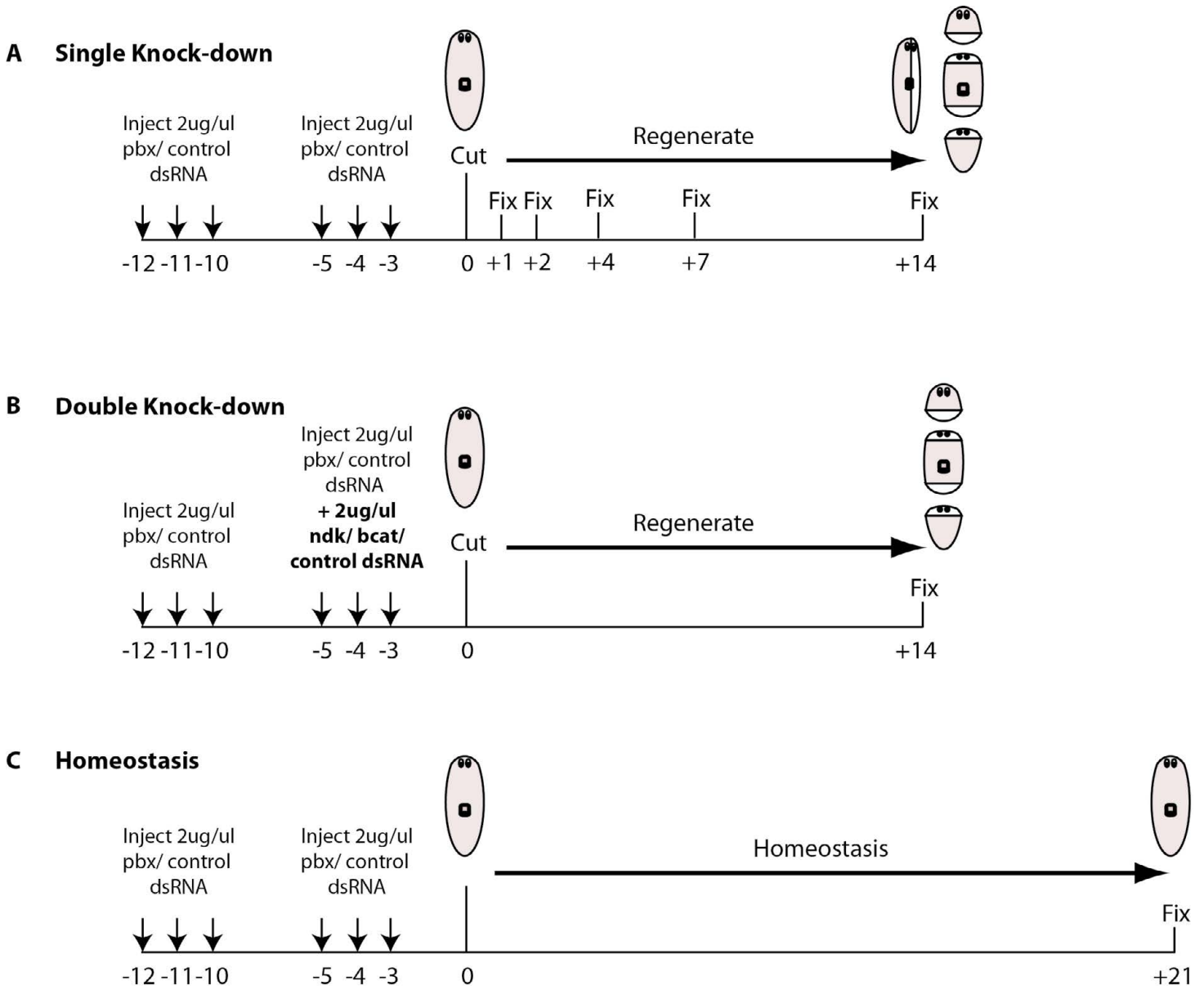


Fig. S2. Experimental procedures for RNAi experiments. (A) For single gene RNAi experiments, animals were injected with 2 $\mu\text{g}/\mu\text{l}$ dsRNA with 33 nl injections for each day as shown. Animals were either cut laterally or into head, trunk and tail pieces, and fixed at the appropriate time-point for analysis. (B) For double RNAi experiments, animals were injected as for single gene injections, except on the second set of 3 days when dsRNA for the second gene was added so that both genes were at a final concentration of 2 $\mu\text{g}/\mu\text{l}$ dsRNA. (C) For homeostasis experiments, the injection protocol was the same as for regeneration experiments as outlined in A. In this case animals were not amputated.

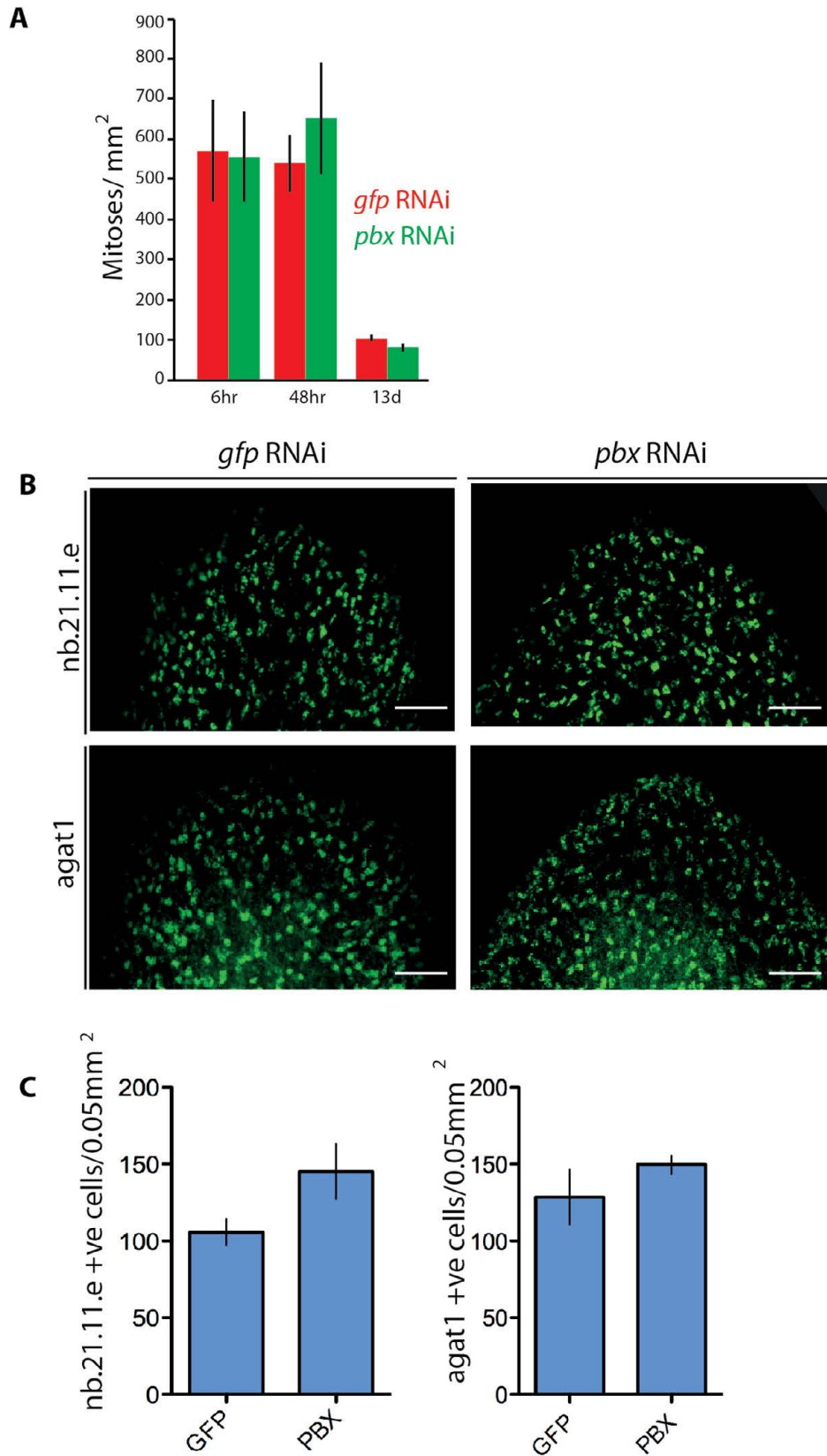


Fig. S3. *pbx* is not required for the neoblast proliferative response or for neoblast differentiation to early and late progeny. (A) Neoblast proliferation at 6 hours and 48 hours after amputation is not significantly different from controls in *pbx*(RNAi) animals, and neoblast proliferation after regeneration is also not affected at 13 days after amputation ($P > 0.2$, two tailed students *t*-test, $n > 7$ in each of three separate experiments). (B) Staining with markers of early (nb.21.11.e) and late (agat1) progeny in anterior blastemas reveal that both these cell populations are reconstituted correctly. Scale bars: 100 μ m. (C) Cell counts of both nb.21.11.e- and agat1-positive cells confirm that *pbx*(RNAi) animals can reconstitute these cell types normally ($P > 0.15$, two-tailed test, $n = 4$ animals, three different regions).

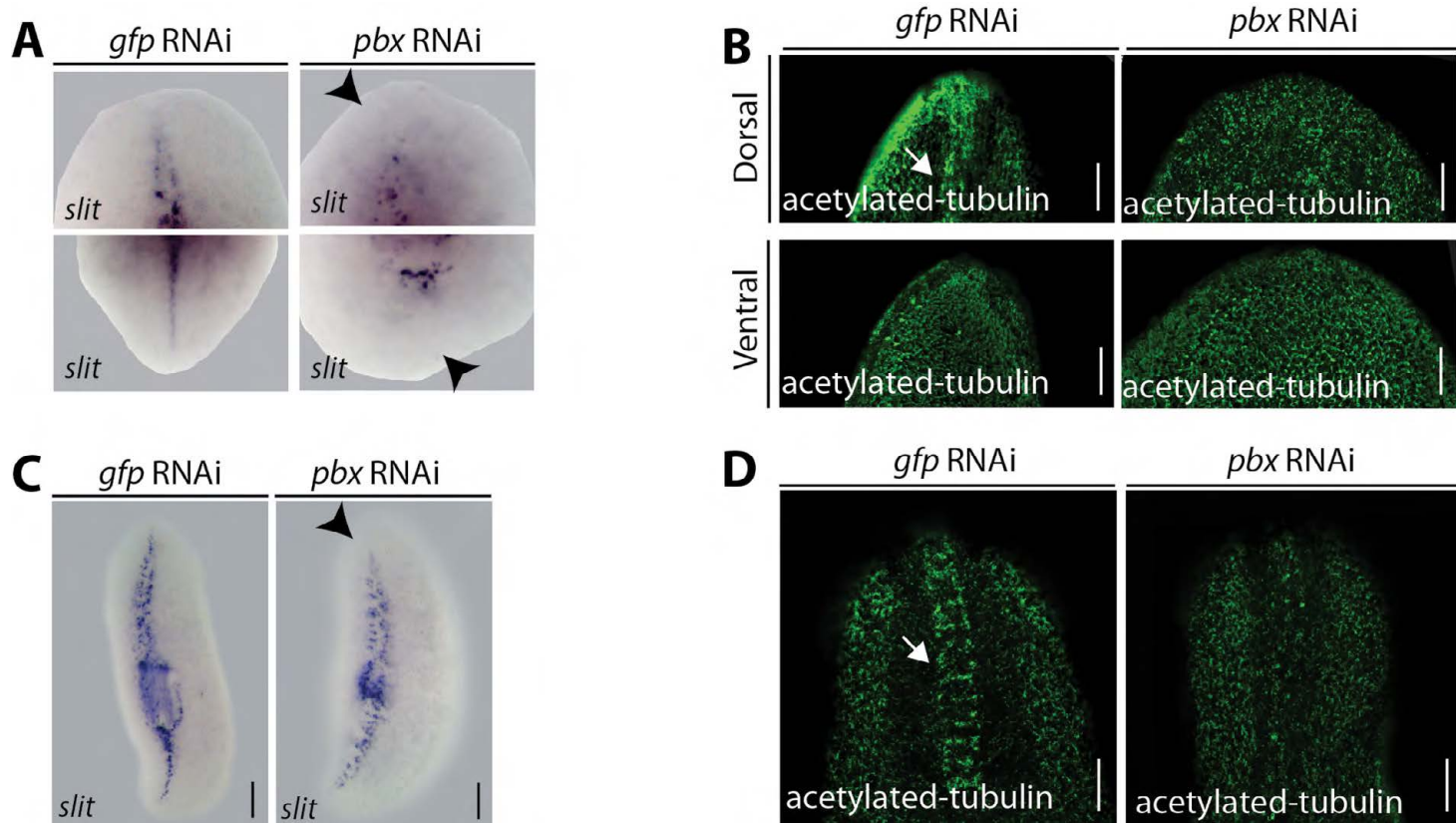


Fig. S4. *pbx* is required for patterning of the midline. (A) *slit*-expressing cells are absent from the anterior and posterior compartments of *pbx (RNAi)* regenerates at 12 dpa, whereas the characteristic stripe of cells is observed along the midline of control *gfp(RNAi)* regenerates. (B) Anti-acetylated tubulin immunostaining reveals that the dorsal stripe of cilia indicated by a white arrow in the anterior compartment of controls (7/7), but is absent from *pbx(RNAi)* regenerates (14/14). Ventral differentiation of cilia is not affected. (C) *slit*-expressing cells are observed along the midline of *pbx(RNAi)* and control *gfp(RNAi)* lateral regenerates; however, are absent from the anterior compartment of *pbx(RNAi)* regenerates. (D) The dorsal stripe of cilia observed in the anterior compartment of controls (7/7) was lost within 3 weeks of homeostasis following *pbx(RNAi)* (17/17).

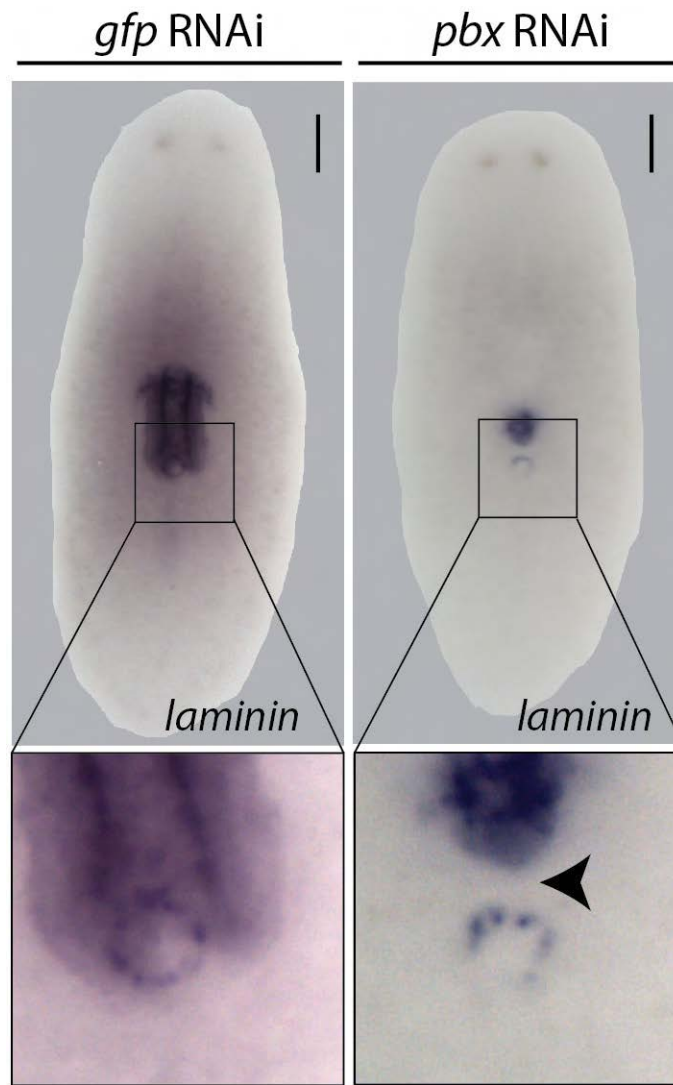


Fig. S5. Pharynx regeneration and patterning requires *pbx*. The pharynx was removed from control *gfp(RNAi)* and *pbx(RNAi)* worms. The complete pharynx was regenerated within 14 days in controls, as shown by *Smed-laminin in situ* hybridization; however, the pharynx was not regenerated properly following *pbx(RNAi)*. The mouth of the pharynx does not connect with the aborted pharynx rudiment following *pbx(RNAi)*.

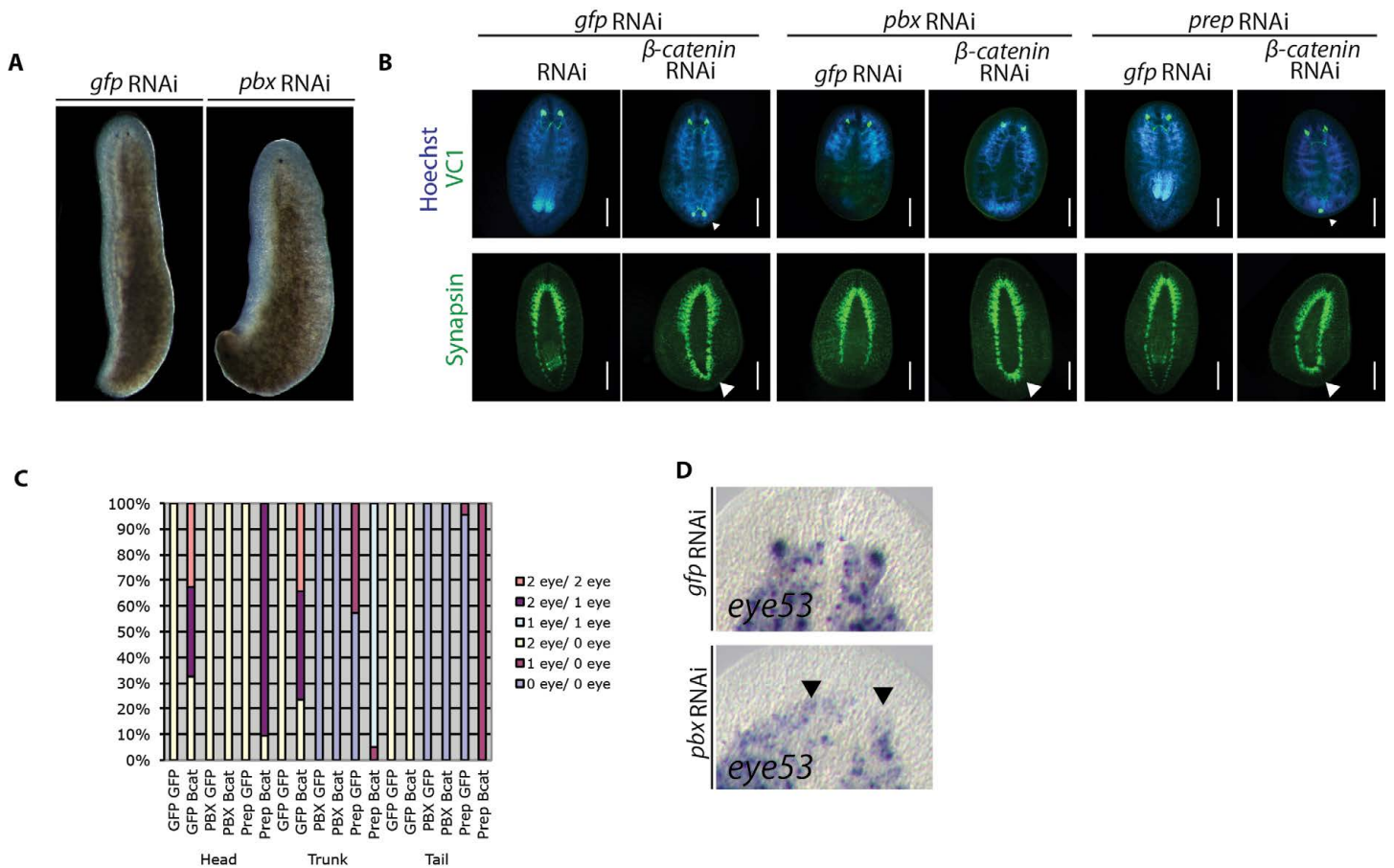


Fig. S6. *pbx* is required for eye regeneration independently of its role during CG regeneration. (A) By 14 dpa, regenerated eyes were observed in control *gfp*(RNAi) lateral blastemas, whereas they are absent following *pbx*(RNAi). (B) VC1 anti-arrestin immunolabeling of eye structures in 14 dpa head pieces. Ectopic posterior eye regeneration was observed following combined *gfp*/ β -catenin RNAi and *prep*/ β -catenin RNAi (small arrowheads), whereas it was not observed following *pbx*/ β -catenin RNAi, despite regeneration of a comparable degree of ectopic posterior CG in each case, revealed by 3C11 anti-synapsin immunolabeling (shown by large arrowheads). VC1 labeled the existing eye structures of *pbx*(RNAi) heads. Hoechst staining reveals the outline of the regenerating head. (C) Eye scoring of 14 dpa regenerates following combined *pbx* / β -catenin and *prep*/ β -catenin RNAi. Each pool consists of more than 30 regenerated pieces. Eyes were not regenerated in any of the conditions following *pbx*(RNAi). (D) By 3 weeks of homeostasis, *eye53* expression is lost from the eyes following *pbx*(RNAi). Scale bars: 200 μ m.