Supplementary material figures

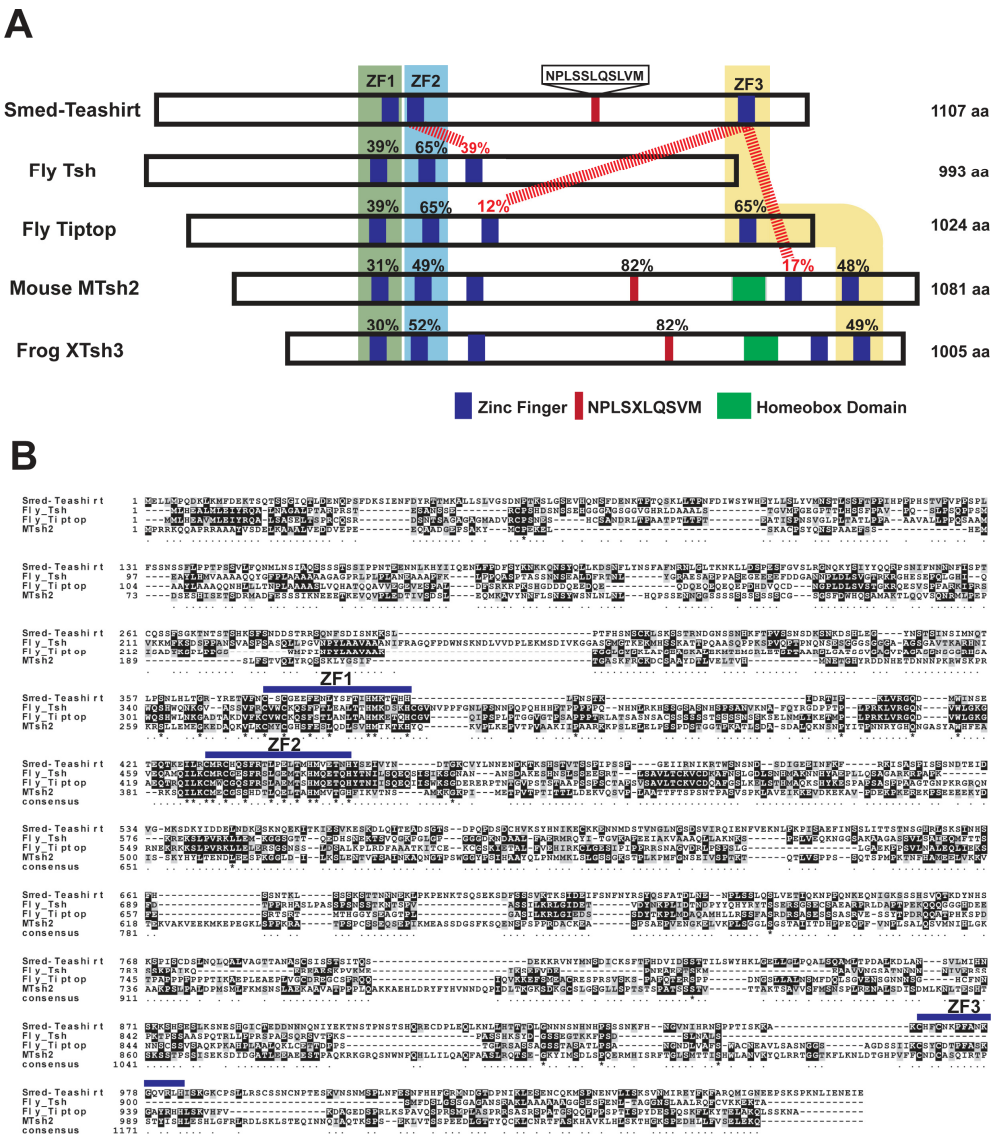

Fig. S1. Sequence analysis of the predicted Smed-Teashirt protein. (A) A comparison of the domain structure of planarian Teashirt to orthologs in several species. The percent amino acid identity shared between domains in Teashirt and its orthologs is listed with homologous domains in black and non-homologous domains in red. **(B)** Multiple sequence alignment shows two Cx2Cx12HMx4H type zinc

finger motifs (ZF1 and ZF2) that are conserved across Teashirt homologs. Most Teashirt genes have a third Cx2Cx12HMx4H zinc finger except for the tsh1 family of vertebrate homologs (Onai et al., 2007). Planarian Teashirt has an additional Zinc finger (ZF3) near the N terminus that is not conserved in fly Teashirt but is present in other homologs. A domain with the sequence NPLSALQSVM is conserved between planarians and vertebrates; however, the function of this domain is unknown.

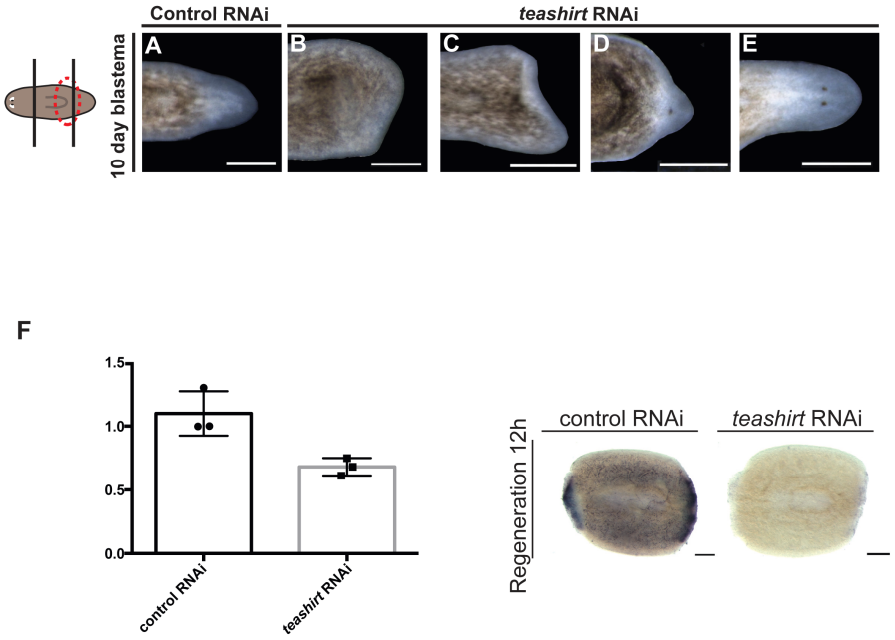

Fig. S2. A range of posterior defects caused by RNAi of *teashirt*. Less severe phenotypes include **(B)** a lack of posterior blastema or **(C)** misshapen posterior blastema. **(D)** *teashirt(RNAi)* animals regenerated both partial and **(E)** complete posterior heads. **(F)** RNAi of *teashirt* perturbs *teashirt* expression. Left, qRT-PCR on *teashirt(RNAi)* animals shows depletion of *teashirt* mRNA. Total RNA was isolated from dissociated cells from control, *teashirt*, and *β-catenin-1* RNAi irradiated head fragments. cDNA was prepared using an oligo-dT primer and quantitative PCR (qRT-PCR) was performed using EvaGreen (Bio-Rad). Data were normalized to the expression of *GAPDH* as previously described (Eisenhoffer et al., 2008), and Y-axis indicates relative mRNA abundance. Right, *in situ* hybridization following *teashirt* RNAi results in less detectable signal in trunk fragments, assessed two days after amputation. 4/4 animals showed less signal, most clearly assessed in the epidermis, than in control animals (n=4). Bar, 200 microns.

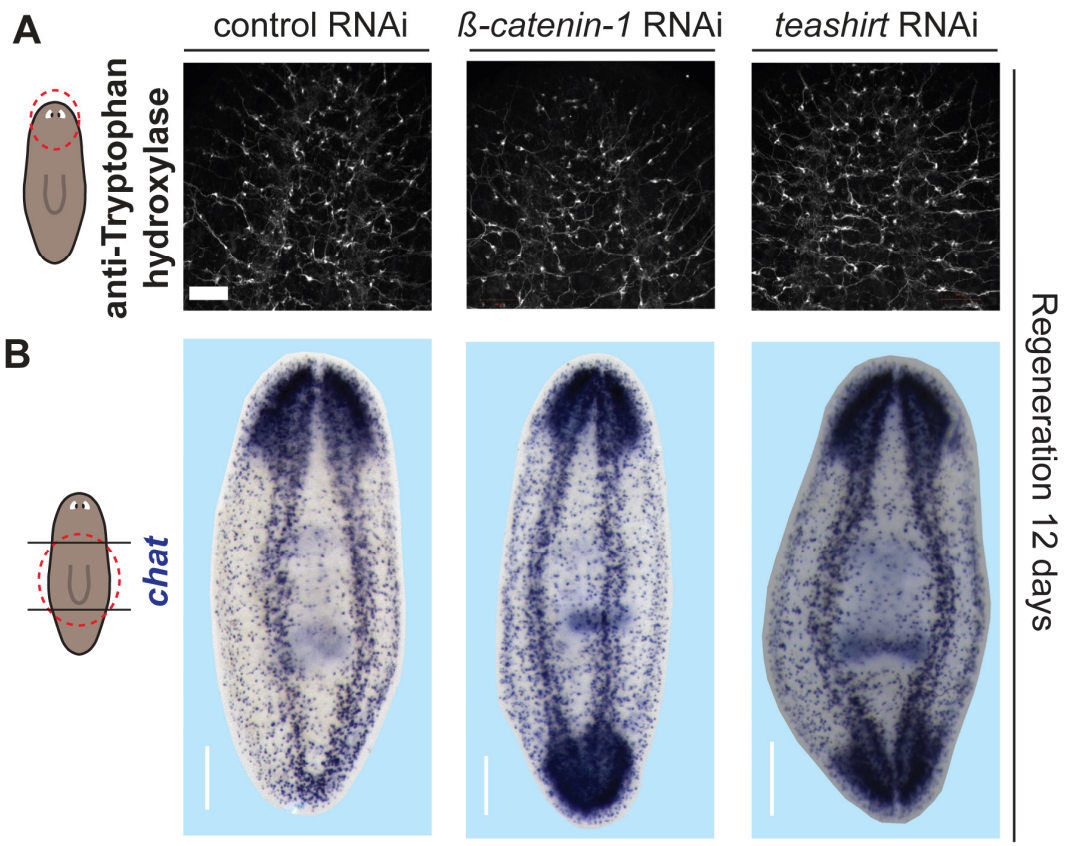

Fig. S3. *teashirt* and *β-catenin-1* RNAi animals regenerate similar nervous systems. (A) Neurons labeled with an anti-Tryptophan hydroxylase antibody (Chen et al., 2013) in the cephalic ganglia in anterior heads of *teashirt* and *β-catenin-1* RNAi trunk fragments are shown at 12 days of regeneration. No robust difference in presence or abundance of these neurons was apparent (n=8 control RNAi, *teashirt* RNAi, and *β-catenin-1* RNAi animals). Bar, 50 microns. Anterior, up. **(B)** RNAi trunk fragments were labeled with an RNA probe for *chat* mRNA, which is broadly expressed in the planarian nervous system. Anterior brains in *teashirt* and *β-catenin-1* RNAi are comprised of two well-formed cephalic ganglia and were morphologically similar to each other and the control (n=4 controls and *teashirt* RNAi animals produced by dsRNA injection each; n=7 control and *β-catenin-1* RNAi animals

produced by feeding of dsRNA-containing bacteria each). Posterior brains are known to be variable in morphology from animal-to-animal in two-headed *β-catenin-1(RNAi)* animals and are as well in *teashirt(RNAi)* animals with some animals having medial collapse of cephalic ganglia. Anterior, up.

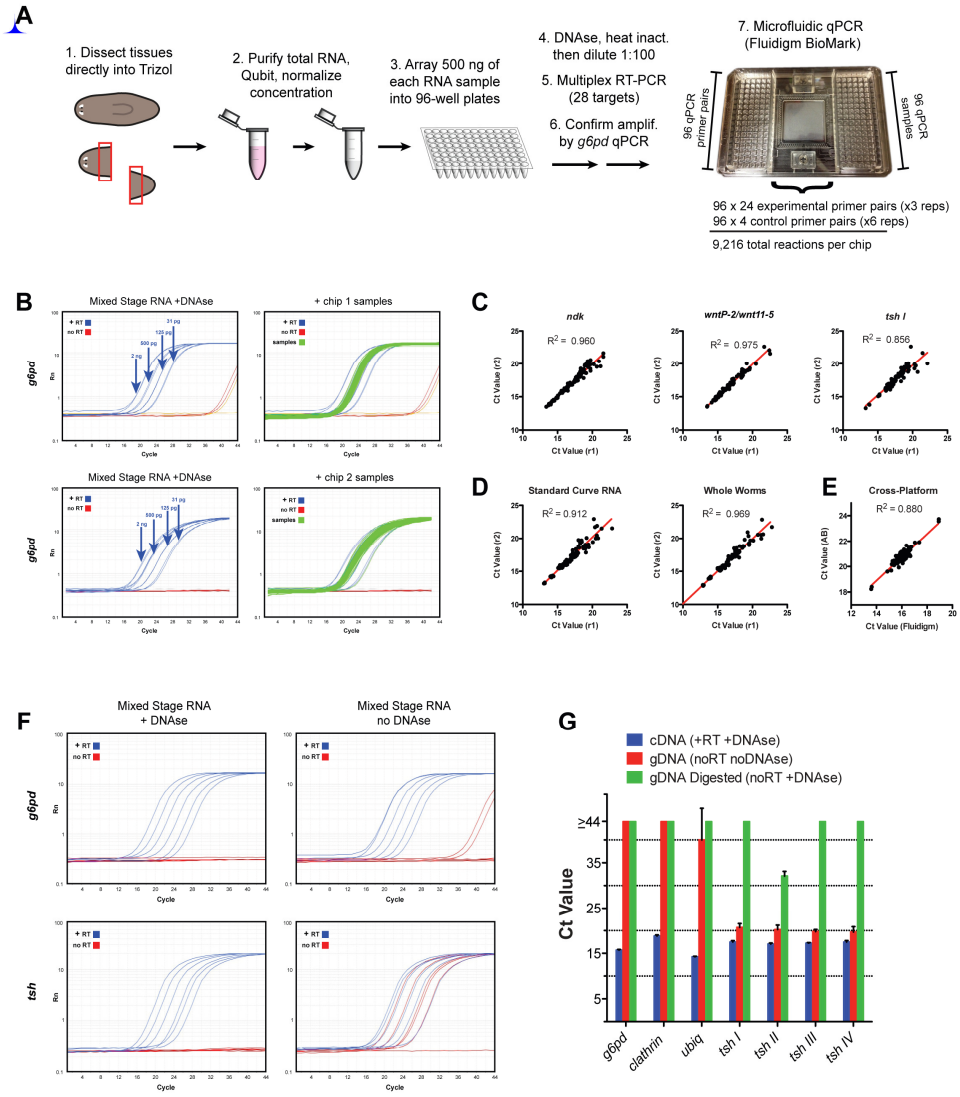

Figure S4. Development of the multiplexed qRT-PCR procedure with planarian fragments.

(A) Overview of sample processing for qRT-PCR. (see also Experimental Procedures). **(B)** Prior to microfluidic qRT-PCR, each sample is assessed for levels of a broadly expressed gene (*g6pd*) alongside standard curve dilutions of planarian total RNA (representing uninjured and mixed regenerative stages). *g6pd* levels indicate that the vast majority of samples ranged between 100-500pg following DNAse treatment. **(C)** Assessment of technical replicates. Ct values for two technical

replicates of the same preamplified samples run in parallel on a single Fluidigm Biomark chip. Ct values for 3 genes are plotted separately. Highly correlated values indicate robustness. **(D)** Assessment of enzymatic and biological replicates. Left, Ct values for two samples of 500 pg planarian total RNA, processed in parallel. Right, Ct values for biological replicates (each consisting of 5-10 whole animals), processed in parallel. **(E)** Comparison of *g6pd* Ct values between the AB 7500 and Fluidigm platforms showed strong linear correlation. **(F)** Assessment of DNAse-I treatment. Shown are qRT-PCR amplification plots for a target transcript in which primers span introns (*g6pd)*, and a target transcript lacking introns (*teashirt*). Samples are standard curve dilutions of planarian total RNA. Low levels of *g6pd* amplification in the noRT-noDNAse control indicate that genomic DNA does not significantly contribute to qRT-PCR measurements (Top panels). By contrast, significant *teashirt* amplification is detected in the noRT-noDNAse control, indicating that DNAse-treatment is critical for elimination of contaminating genomic DNA. **(G)** Confirmation of successful DNAse-I treatment in microfluidic qRT-PCR samples. Means of Ct values are plotted for three control genes, and four sets of *teashirt* primers (I-IV). Error bars indicate standard deviation. Lower Ct values reflect higher expression, and undetected Ct measurements are indicated as >44 (the number of cycles performed). cDNA-specific amplification levels (+RT +DNAse) are indicated by blue bars. As expected, high amounts of genomic DNA amplification are observed for four sets of *teashirt* primers (red bars). However, addition of DNAse significantly reduces RT-independent amplification of the *teashirt* target ("*tsh*", green bars).

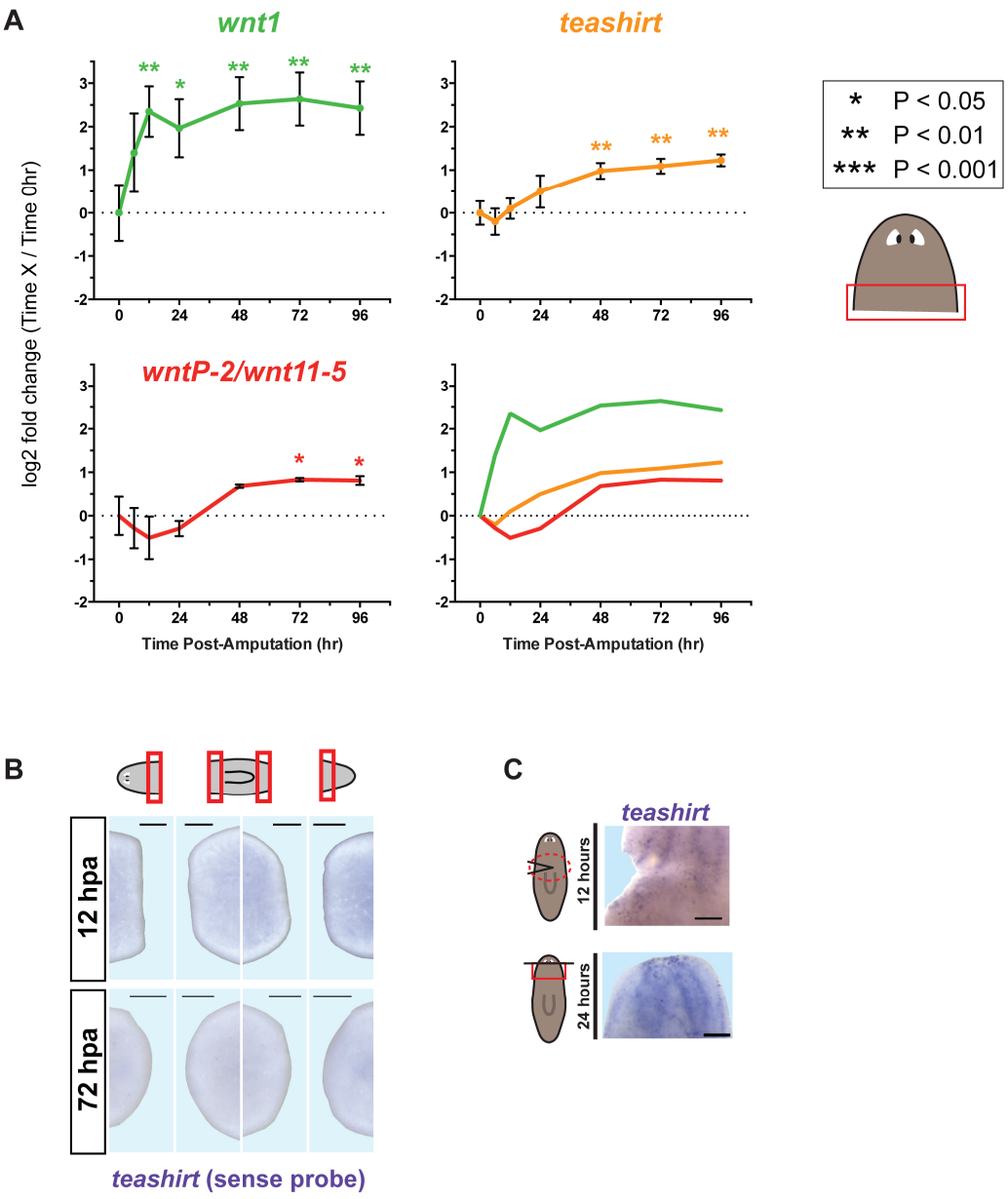

Figure S5. *teashirt* expression timing and controls

(A) Plots of log2 fold changes in *teashirt, wntP-2*, and *wnt1* levels at posterior-facing wounds. Error bars, standard deviation. Asterisks denote significance determined by t-tests using untransformed delta-Ct values. **(B)** Whole-mount *in situ* hybridization showing controls for wound-site expression of *teashirt* at 12 and 72hr post-amputation (hpa). Background staining for the method is shown by a "sense" probe

for *teashirt* transcript, with no wound site expression detected. Anterior, left. Bars, 200 μm. **(C)** Additional examples of *teashirt* expression at wound sites. Anterior, up. Bar, bottom: 200 μm.

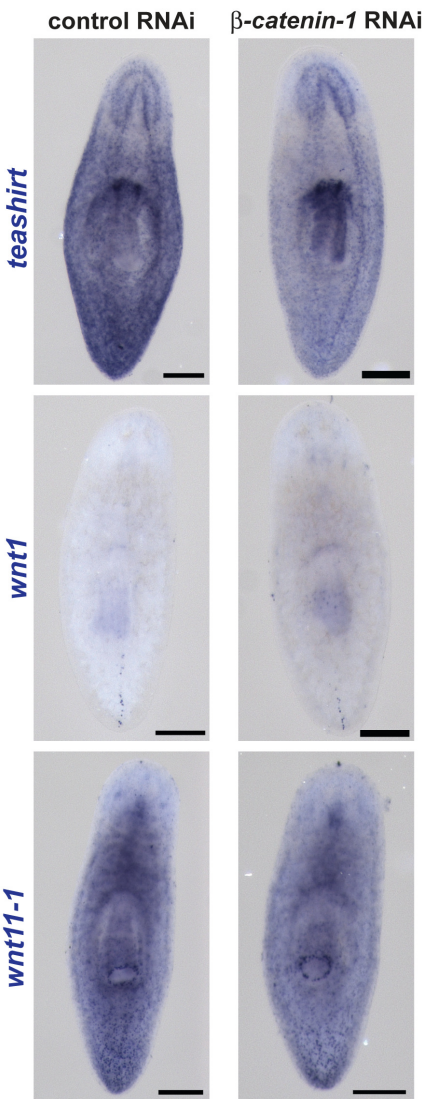

Figure S6. *β-catenin-1* is required for *teashirt* expression in intact animals

Uninjured animals were examined four days after control (*C. elegans unc-22*) or *β-catenin-1* RNAi for expression of *teashirt* and other posteriorly expressed position control genes. Posterior *teashirt* expression was decreased following *β-catenin-1* RNAi, whereas *wnt1* and *wnt11-1* expression was still present. Anterior, up. Bar, 200 μm.

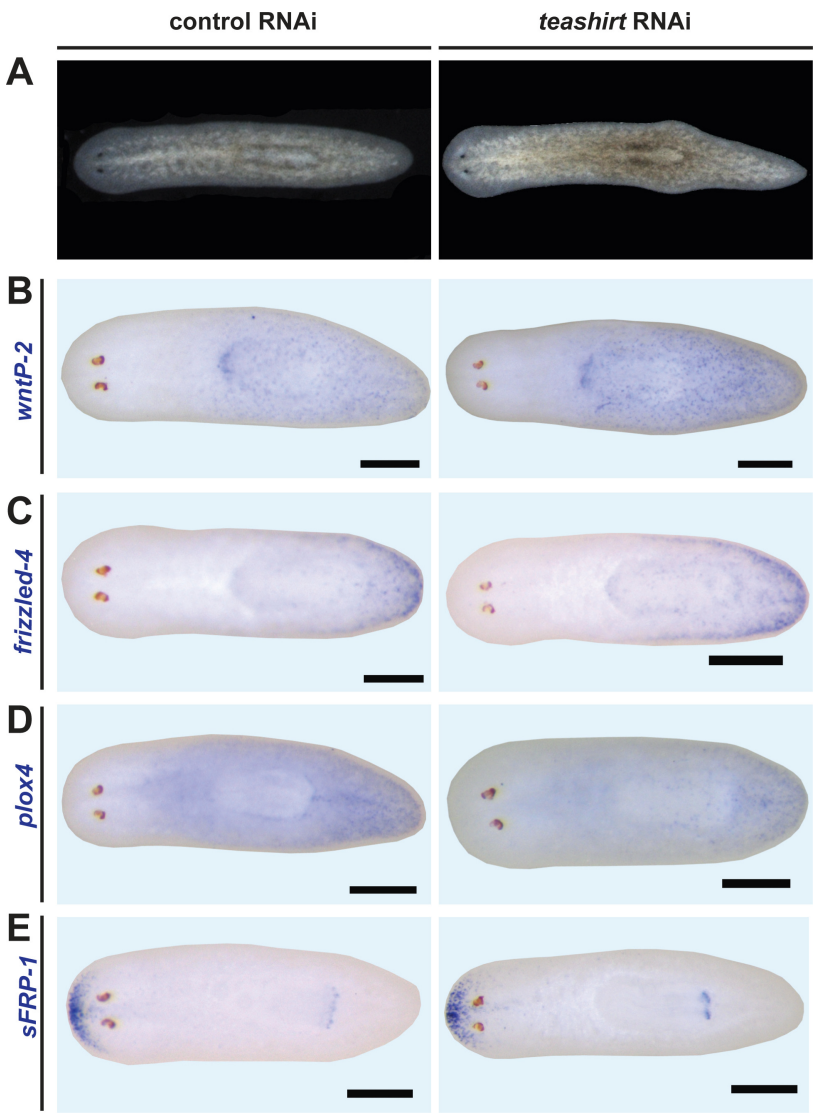

Figure S7. Long-term RNAi of *teashirt* in intact animals. **(A)** After eight weeks of continuous RNAi, a small number of *teashirt(RNAi)* animals show an enlarged pharynx causing the body to bulge outwards around the pharynx cavity (n=4/54) *in situ hybridization* on long term *teashirt(RNAi)* animals showed no detectable change in the expression levels of posterior genes such as **(B)** *wntP-2*, **(C)** *frizzled-4*, and **(D)** *plox4*. **(E)** They also do not express the anterior marker *sFRP-1* in the posterior.

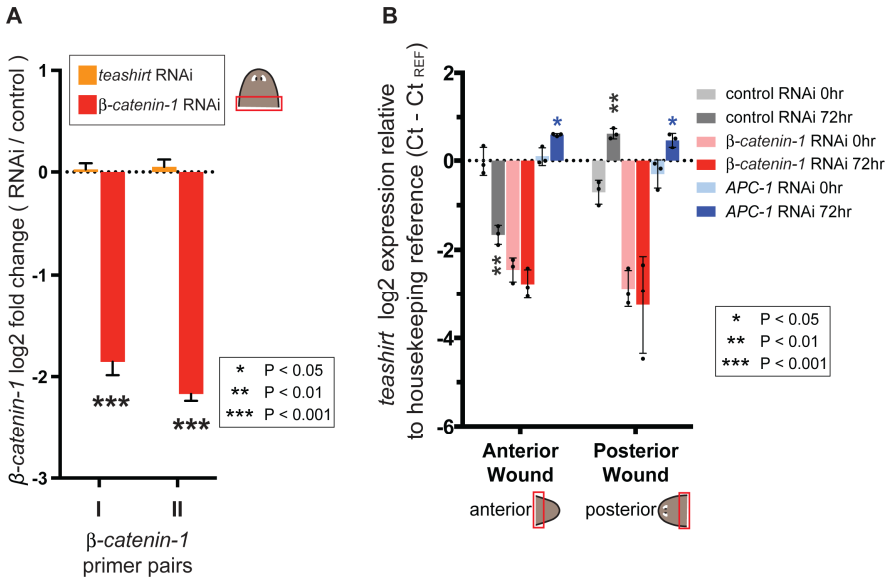

Figure S8. qRT-PCR controls for Figure 6. (A) qRT-PCR analysis of *β-catenin-1* transcript levels (using two independent primer sets) following *teashirt* or *β-catenin-1* RNAi. RNA was harvested from dissected posterior-facing wound sites of dsRNA-injected head fragments 0hr post-amputation. Biological triplicates consisted of pooled wound sites from 6-9 individual animals. Bars depict mean log2-fold changes in *β-catenin-1* levels between *teashirt* or *β-catenin-1* RNAi specimens and control RNAi specimens. Error bars, standard deviation. Asterisks denote significance determined by t-tests between sets of biological triplicate delta-Ct values. **(B)** qRT-PCR analysis of *teashirt* levels in *β-catenin-1* and *APC* RNAi animals (See also Figure 6C). Bars depict delta-Ct values (raw Ct measurements normalized to the average of Ct measurements for four housekeeping transcripts (*g6pd, clathrin, luc7,* and *ubiquilin*). Error bars, standard deviation. Individual replicates are indicated by dots. Asterisks denote significance determined by t-tests between timepoints for each wound type using sets of biological triplicate delta-Ct values.

SUPPLEMENTARY TABLE

Table S1

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References

Chen, C. C., Wang, I. E. and Reddien, P. W. (2013) '*pbx* is required for pole and eye regeneration in planarians', *Development* 140(4): 719-29.

Eisenhoffer, G. T., Kang, H. and Sánchez Alvarado, A. (2008) 'Molecular analysis of stem cells and their descendants during cell turnover and regeneration in the planarian *Schmidtea mediterranea*', *Cell Stem Cell* 3(3): 327-39.

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