Supplementary figures and methods

Supplementary methods:

Image Quantitation

We used confocal image stacks with three channels (nucleus marked with Hoechst 25 (Ch1), tissue markers and *pabpc2* marked with different fluorophores, Ch2 and Ch3 respectively. (Ch3). The three different channels are read as three separate arrays for independent analysis along 3D. To cover the complete brightness range of the acquired confocal images, the co-localization algorithm first enhances the contrast of the images. The next step is to convert all the images to their binary counterparts. For this, we employ Matlab's inbuilt thresholding technique using Otsu's method to choose the optimum threshold. Otsu's thresholding method chooses an optimal value of the threshold by minimizing the interclass variance of black and white pixels. The holes in the image are filled to provide continuity. The binary images are then filtered with an average filter to remove the noisy pixels. The next step is to filter the binary images of the tissue marker channel with an average filter and then to find the regions that have more than 200 bright connected pixels across 3D of the stack. This ensures elimination of noisy pixels and non-specific binding sites of the fluorophore. This is the mask for the tissue marker population. We next count the number of *pabpc2* positive cells in the 3D stack. The binary images of the *pabpc2* positive cells are processed in a manner similar to that of marker positive cells to obtain the mask for *pabpc2* positive cells. Co- localization of the tissue marker with *pabpc2* is determined by simply multiplying the masks of the tissue marker and pabpc2 cells. The resultant stack is finally scanned to count the co-localization in the cells. Also, once the cells with tissue markers are located, an edge detection algorithm using Canny's edge detector traces the boundary of the stained region. The edges are then overlaid on the *pabpc2* positive image. This provides an estimate of the co- localization of *pabpc2* with different tissue markers.

Transcriptome analysis

We obtained ~50 to 80 million reads from sequencing. These reads were adapter trimmed using Trimmomatic (Bolger et al., 2014). Reads that mapped to *Schmidtea mediterranea* mitochondrial genome, rRNA and other contamination databases were removed and remaining reads were taken for analysis. We used Tophat v2.0.9 (Trapnell et al., 2009) to align the reads back to Smed SxlV4 genome. We collated Smed Unigenes (Robb et al., 2015), Dresden v6 (Brandl et al., 2016) and Oxford (Blythe et al., 2010) transcriptome using CD-HIT (Li and Godzik, 2006) (with 90% protein sequence identity cutoff) and obtained ~47,000 unique clusters. We derived gene co-ordinates by mapping to SmedSxlV4 genome using Blat (Kent, 2002). For this collated gene model, we derived raw read count using HT-Seq (Anders et al., 2015). The data was normalized using DESeq (Anders and Huber, 2010). To remove noise in the data, we used minimum cut-off of at least 10 normalized reads in all the four samples. We performed two-tailed Fisher exact test to derive statistical significance using R. Adjusted P values are obtained by correcting *P values* from Fisher exact using *bonferroni* method.

From previous literatures we mined all known and reported progenitors in planaria. We also mined data from (Wurtzel et al., 2015) and (van Wolfswinkel et al., 2014). These datasets were blasted with the collated transcript model. Transcripts that showed at least 90% sequence identity & coverage were considered as best hit and further used for analysis.

References:

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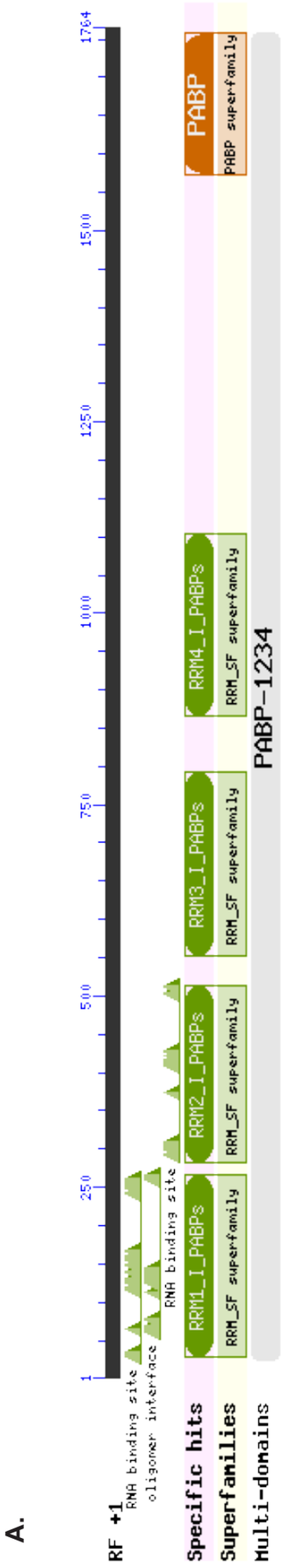
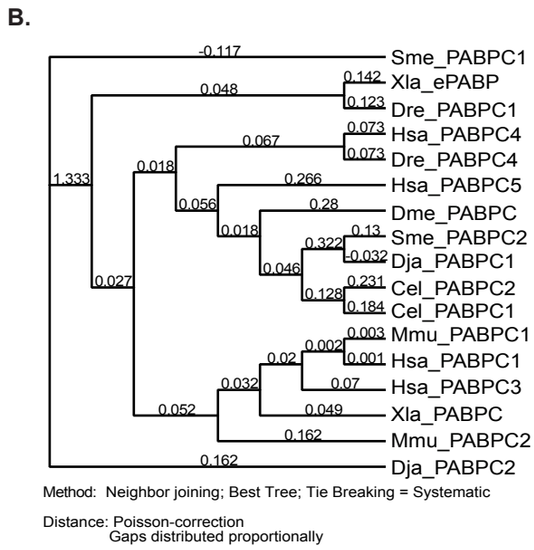
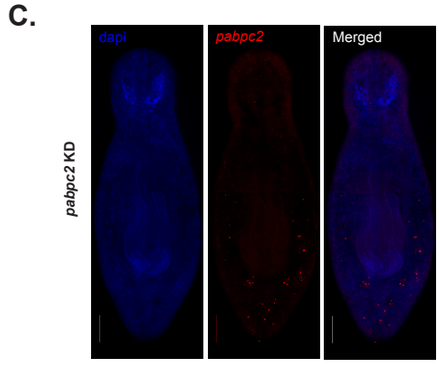
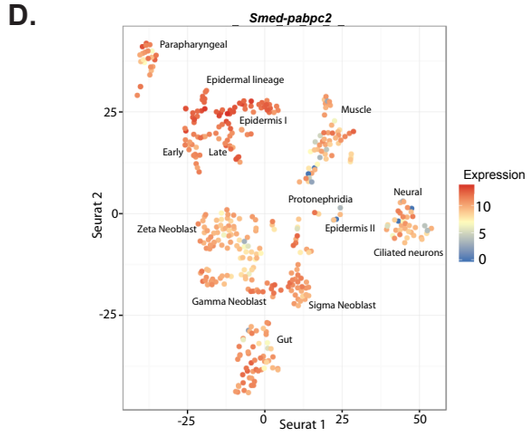
Figure S1









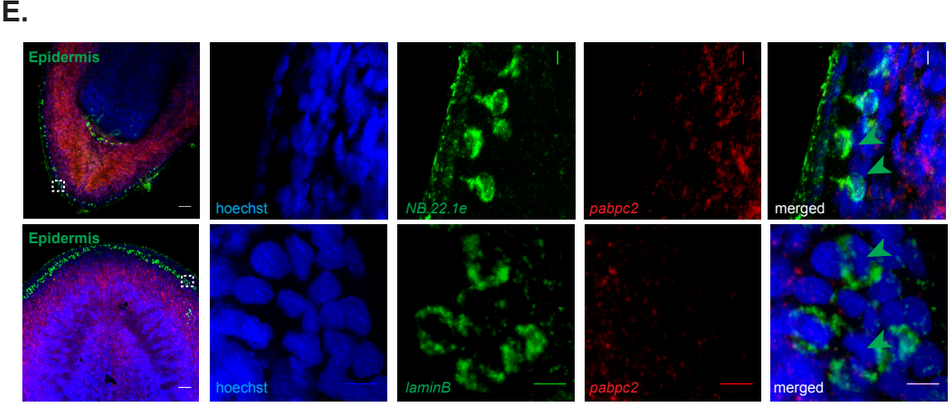


Figure S1. Related to Figure 1: A) PABPC2 protein with domain organization. The domain organization of PABPC2 predicted using Conserved Domain Database (CDD). The protein has four RNA Recognition Motif (RRM) domains and a poly (A) binding protein (PABP) domain. **B)** Phylogenetic tree depicting the relationship of PABPC2 with other PABPC across different species among metazoans. **C)** FISH showing lack of *pabpc2* staining in *pabpc2* KD animals depicting specificity of *pabpc2* probe used. Animals were fixed 2 days post 2nd feed. Bars, 500µm (n=5). **D)** Seurat plot showing expression of *Smed-pabpc2* across different cell types obtained from single cell transcriptome (Wurtzel et al., 2015) (Source: https://radiant.wi.mit.edu/app/). **E)** Double fluorescent *in situ* hybridization (dFISH) showing co-expression of *pabpc2* with *laminB* and *Nb.22.1e*. The first panel images were taken at 20X. Bars, 50µm. White box represents the zoomed in area. Probes are indicated; (Green arrows) epidermal cells; Bars, 5µm; n=6.

Figure S2

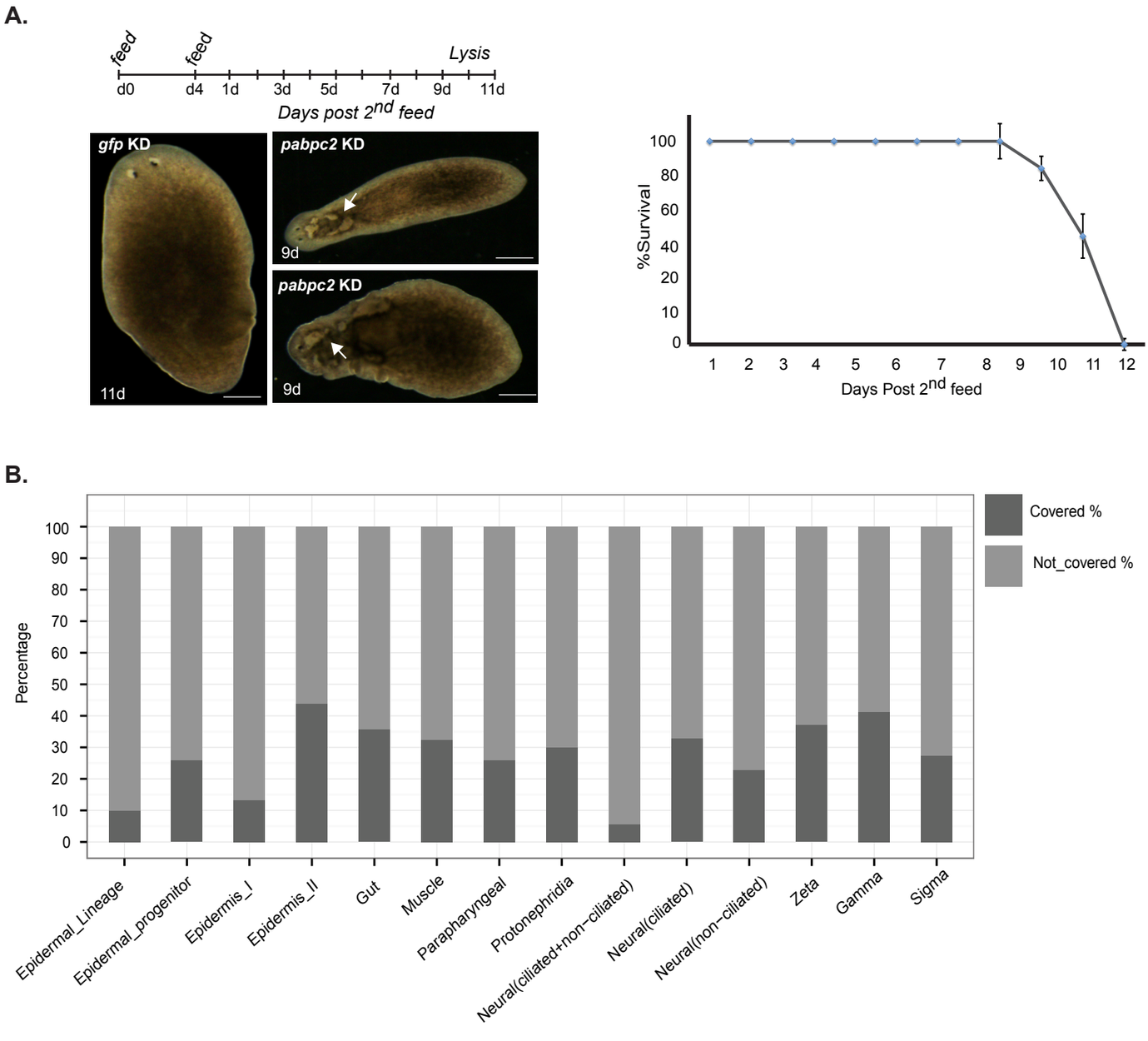


Figure S2. Related to Figure 2: A) Time line showing RNAi feed schedule. Images showing homeostatic defects in animals after *pabpc2* knockdown. The uncut animals treated with *pabpc2* dsRNA showed lesions on the body and subsequently lysed (100/100). *Gfp* dsRNA treated animals were used as negative control. All images were taken using Olympus BX53 wide- field microscope. Bars, 100μm. Graph showing the survival of uncut *pabpc2* KD animals. *Pabpc2* KD animals died 11 days post dsRNA treatment. *Gfp* dsRNA treated animals survived even after 21 days post treatment (not depicted in the graph). Error bars are drawn from three biological replicates. B) Stack bar showing percentage of cell type specific transcripts coverage in our transcriptome compared to the available single cell transcriptome data. C) qPCR analysis was done to check *zfp-1* transcript level in *gfp* and *pabpc2* KD animals 24 hpa. Histogram depicting no significant change in *zfp-1* transcript level. Error bars are drawn from three biological replicates. D) WISH showing decrease in expression of epidermal progenitors *prog1* and *agat1* as early as 6hpa. Bars, 200 μm; n=8/10.

Figure S3

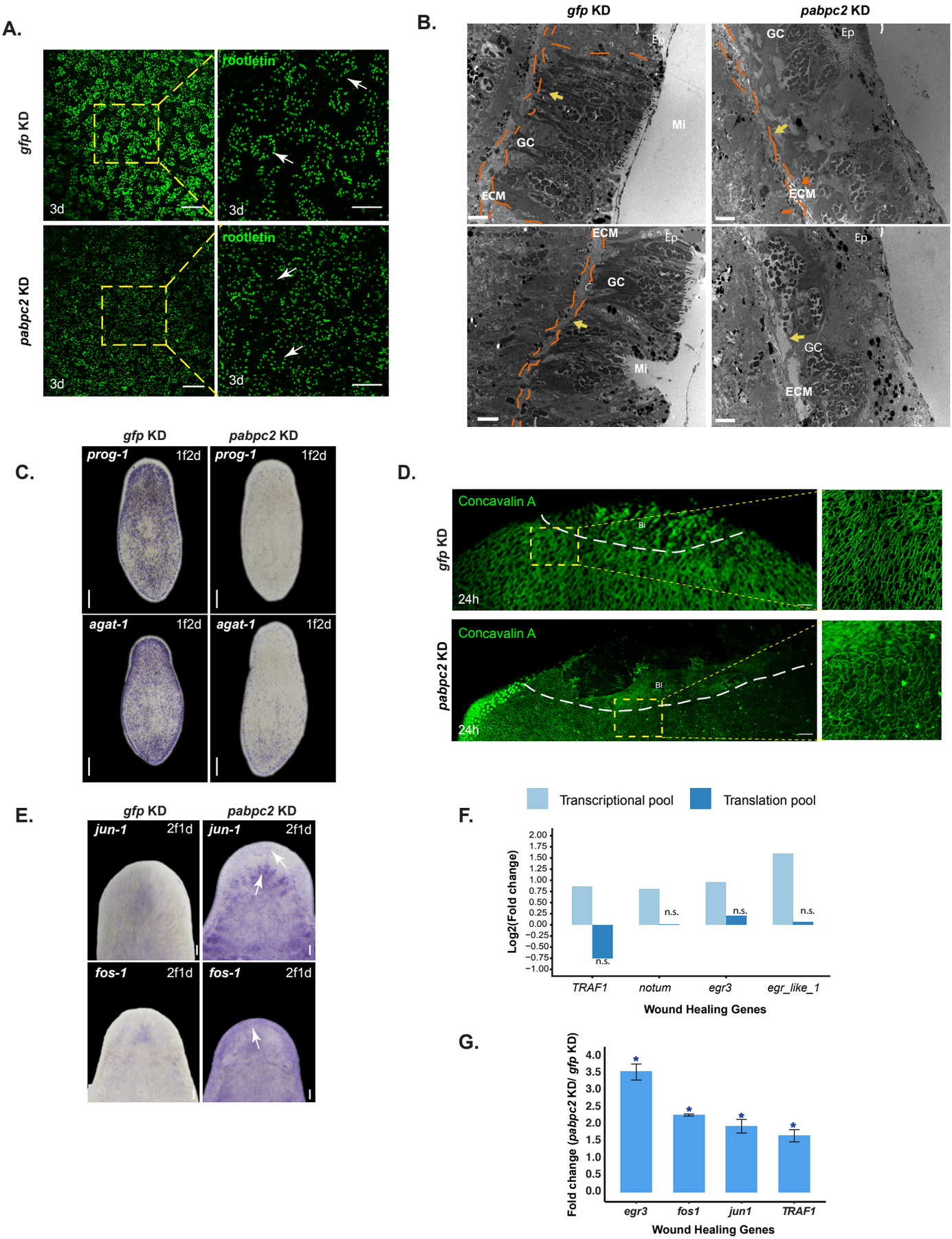


Figure S3. Related to Figure 3. A) Confocal images of *gfp* and *pabpc2* KD animals 3d post amputation stained with anti-rootletin antibody to visualize ciliated epidermal cells. Images were taken on ventral side below the eye region at 40X at 1.1 and 2.3 magnifications respectively. *Pabpc2* KD animals show disorganized pattern of ciliated cells. *Gfp* KD was used as a control. Bars, 20µm and 10µm for 1.1 and 2.3 magnification respectively; n=10. B) EM studies showing epidermal tissue organization in homeostasis worms 10 days post RNAi induction in *gfp* and *pabpc2* KD animals. Yellow arrows indicate disorganized gland cells and reduced basal lamina in *pabpc2* KD animals as compared to the control animals. GC- gland cells, MI- Microvilli, ECM- Extracellular matrix, Ep- Epidermis. Bars, 2µm; n=5. C) WISH indicating loss of expression of epidermal progenitors *prog1* and *agat1* in *pabpc2* KD animals 2 days post 1st feed (1f2d). Bars, 200µm; n=5. D) Confocal images of *gfp* and *pabpc2* KD animals stained with concavalinA-FITC showing the organization of epidermal tissue near the amputated region at 24hpa. Bl- Blastema. Bars, 50µm; n=12. E) WISH showing up regulation of wound healing genes like *jun-1* and *fos-1* in *pabpc2* KD animals 2f1d post induction of RNAi compared to the control animals. Bars, 100µm; n=5. Arrows indicate expression of genes in KD animals. F) Bar graph showing fold change in transcriptional pool of wound healing genes in *pabpc2* KD animals 24hpa. n.s.- non-significant. G) Quantification of level of expression of wound healing genes by qRT-PCR. Histogram showing fold change of wound healing genes in *pabpc2* KD animals 2f1d post induction of RNAi compared to the control animals. The error bars are drawn from biological triplicates and asterisk represent statistical significance. Error bars represent standard error of mean (SEM).

Figure S4

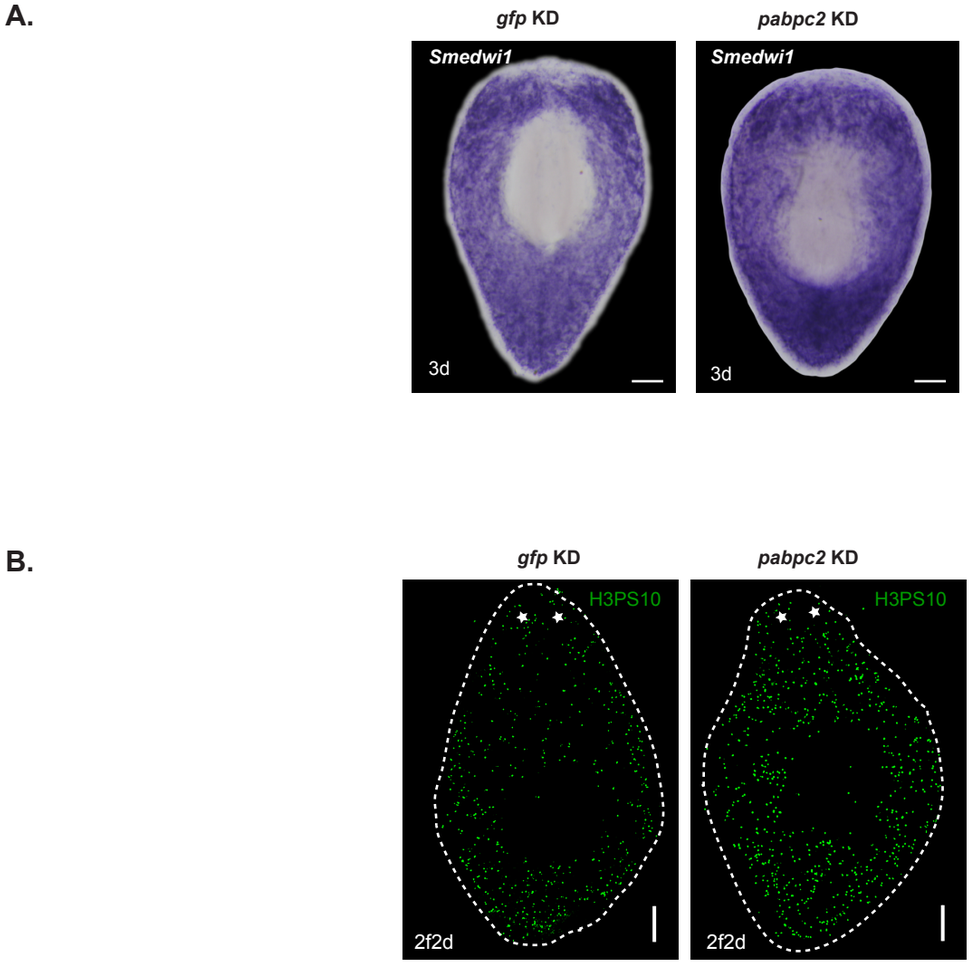


Figure S4. Related to Figure 4. *A*) WISH using neoblast marker, *smedwi1* showed no observable change in the expression at 3dpa in *gfp* and *pabpc2* KD animals. Bars, 200µm; n=10. C) Max intensity projections of confocal images showing H3PS10^{+} cells in homeostasis animals in *gfp* and *pabpc2* KD animals 2 days post 2nd feed (2f2d). KD animals show 1.5-fold increase ($p<0.01$) in neoblast proliferation. Bars, 200µm; n=8.

Figure S5

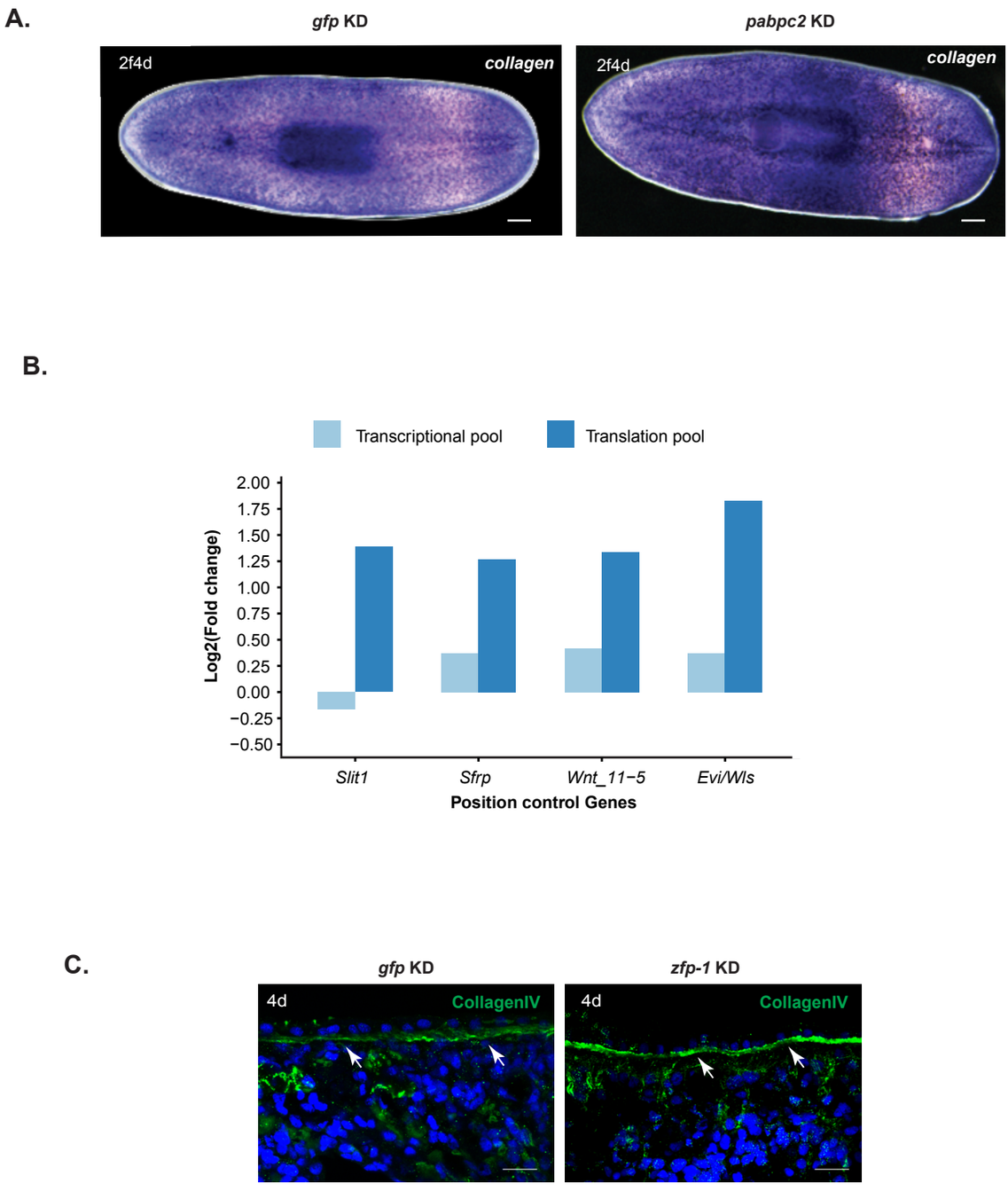


Figure S5 related to Figure 5. A) WISH with *collagen* probe in *gfp* and *pabpc2* KD uncut animals 4 days post 2nd feed (2f4d). Bars, 100µm; n=6. B) Bar graph showing fold change in transcriptional and translational pool of some of the position control genes (PCGs) in *pabpc2* KD animals 24hpa. C) Maximum intensity projections of Z-Stacks of *gfp* and *zfp-1* KD animals sagittal sections stained with Collagen IV antibody at 4d post amputation. Bars, 20µm; n=6.