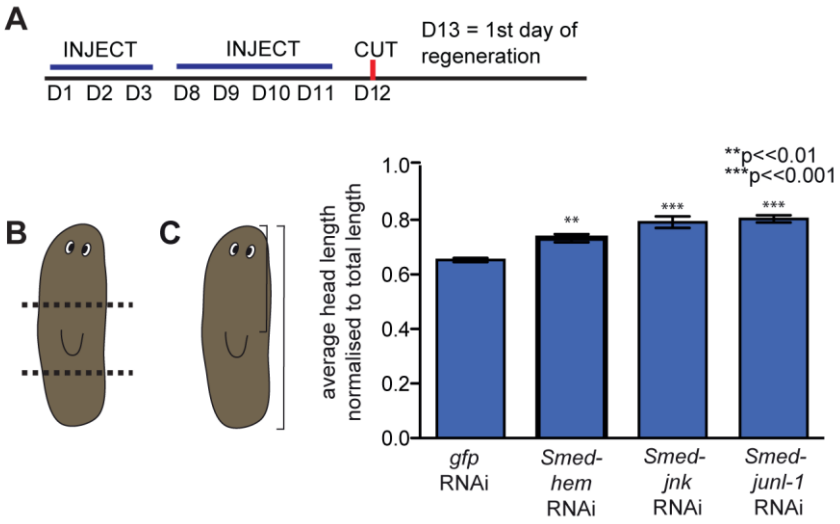
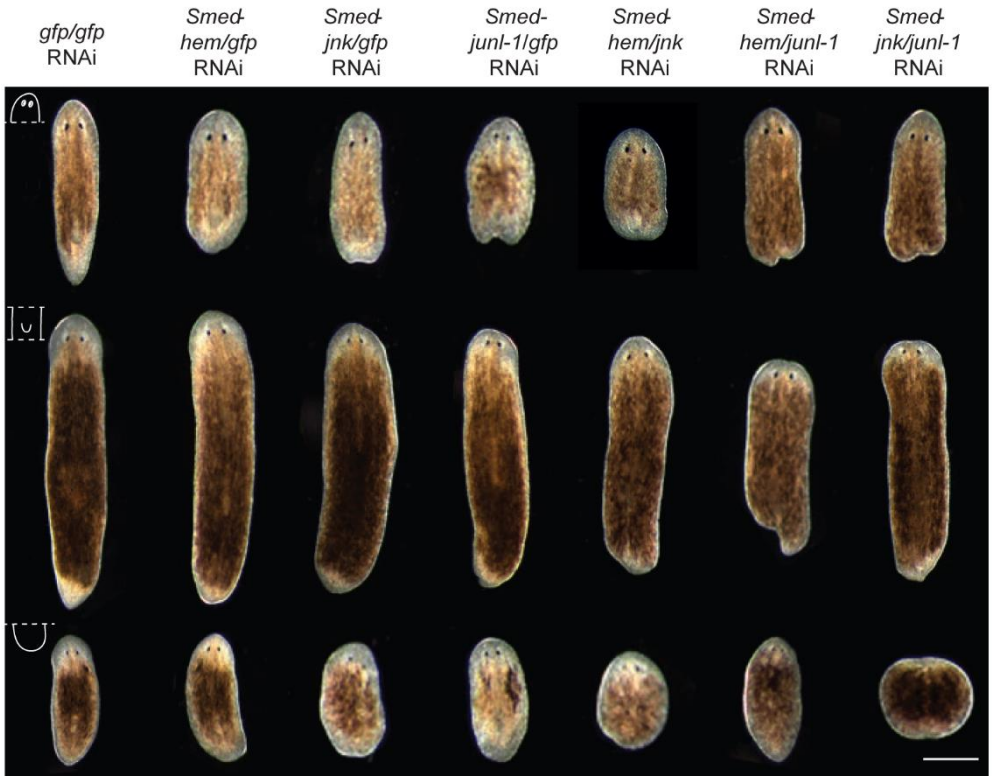
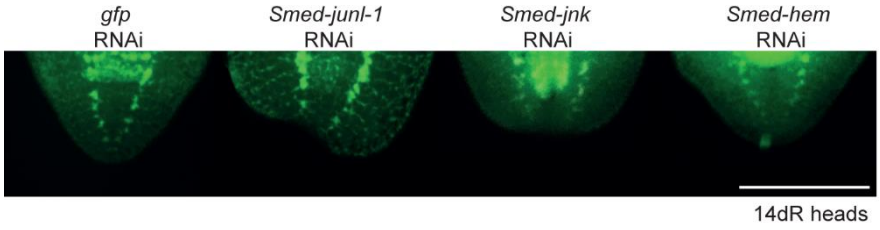
Supplementary figure 1: Phylogenetic analysis of *Smed-junl-1*. Phylogenetic tree of the b-zip domain of jun proteins across the Animal Kingdom, with the sequences identified in *Schmidtea mediterranea* boxed in red. The tree was generated using MrBayes with the following parameters: poisson fixed rate matrix, gamma rate variation model of evolution, chain length 1,100,000, subsampling frequency of 200 and unconstrained branch lengths. The b-zip domain from BACH1 was used as an outgroup.



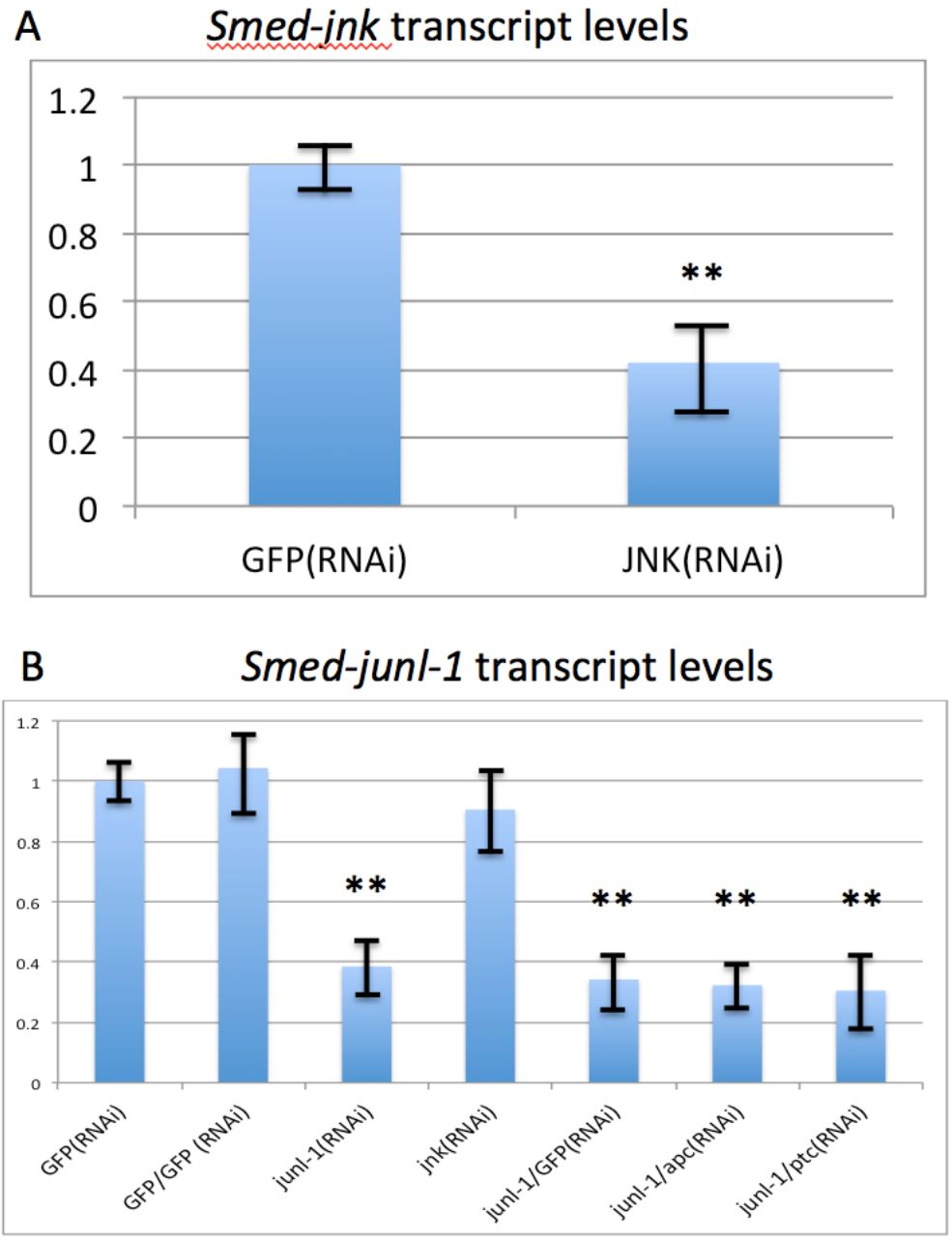
Supplementary figure 2: Injection schedule and anterior length monitoring. (A) Animals were injected six times over the course of two weeks, and amputated the day after the last injection. The first day of regeneration is the day after amputation. **(B)** Amputations were performed pre- and post-pharyngeally. (**C**) Anterior length increases in *Smed-hem*, *Smed-jnk*, and *Smed-junl-1(RNAi)* animals. Anterior length was measured from the anterior tip of the pharynx to the front of the animal and normalized to total length. A minimum of 60 animals were measured with the "measure" tool in Fiji, using brightfield pictures. Graph values represent the mean ± s.e.m.; ***P<0.001, two-tailed t-test compared to *gfp(RNAi)* animals.



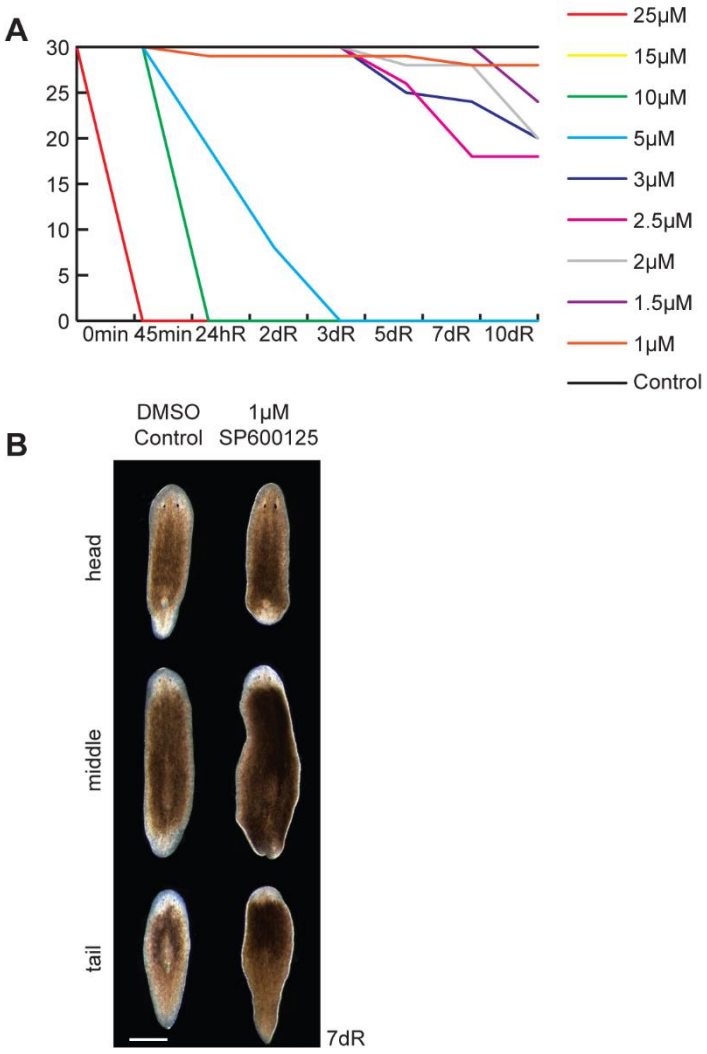
Supplementary figure 3: RNAi phenotype of combinations of members of the JNK signalling pathway. Double RNAi knockdown of combinations of the members of the JNK pathway resulted in tailless head and middle pieces, and normal regenerating tails. An exception was a reduced anterior with faint eyes in the *Smed-jnk/junl-1* double RNAi regenerating tails (*gfp/gfp* heads n=10/10, middles n=10/10, tails n=10/10; *hem/gfp* heads a=10/10, middles a=9/10, tails n=9/9; *jnk/gfp* heads a=9/10, middles a=7/9, tails n=10/10; *jun/gfp* heads a=10/10, middles a=9/11, tails n=7/9, *hem/jnk* heads a=8/8, middles a=8/9,1/9 eyeless), tails n=7/8, 1/8 eyeless; *hem/junl- 1* heads a=7/9, middles a=7/7, tails n=8/9; *jnk/junl-1* heads a=8/8, middles a=6/9, 3/9 with an eye phenotype, tails ae=7/7). Counts indicate normal (n) or abnormal (a, ae) animals as described per condition. Scale bars 500 μm. All pieces are at 14 days of regeneration.



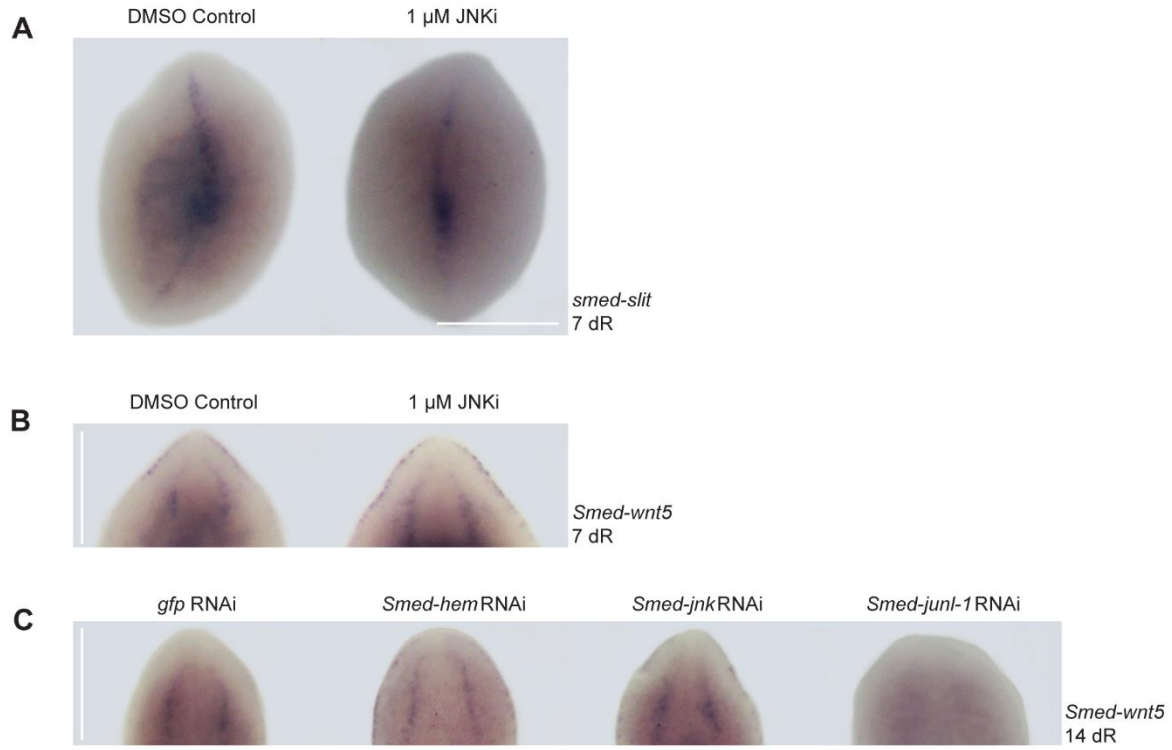
Supplementary figure 4: Staining with anti-Synapsin confirmed the tailless phenotype after knockdown of JNK-signaling family members. After *Smed-junl-1*, *-jnk* and *-hem (RNAi)* the tails appear shorter (a). In addition, the VNCs did not join at the tip of the animal (aa) after *junl-1* and *jnk(RNAi)* (*gfp* n=24/24, *junl-1* a=6/7 *jnk* aa=19/32 VNCs don't join, a=13/32 join but have short tails, *hem* a=17/20). Counts indicate normal (n) or abnormal (a, aa) animals as described per condition. Scale bars 500 μm.



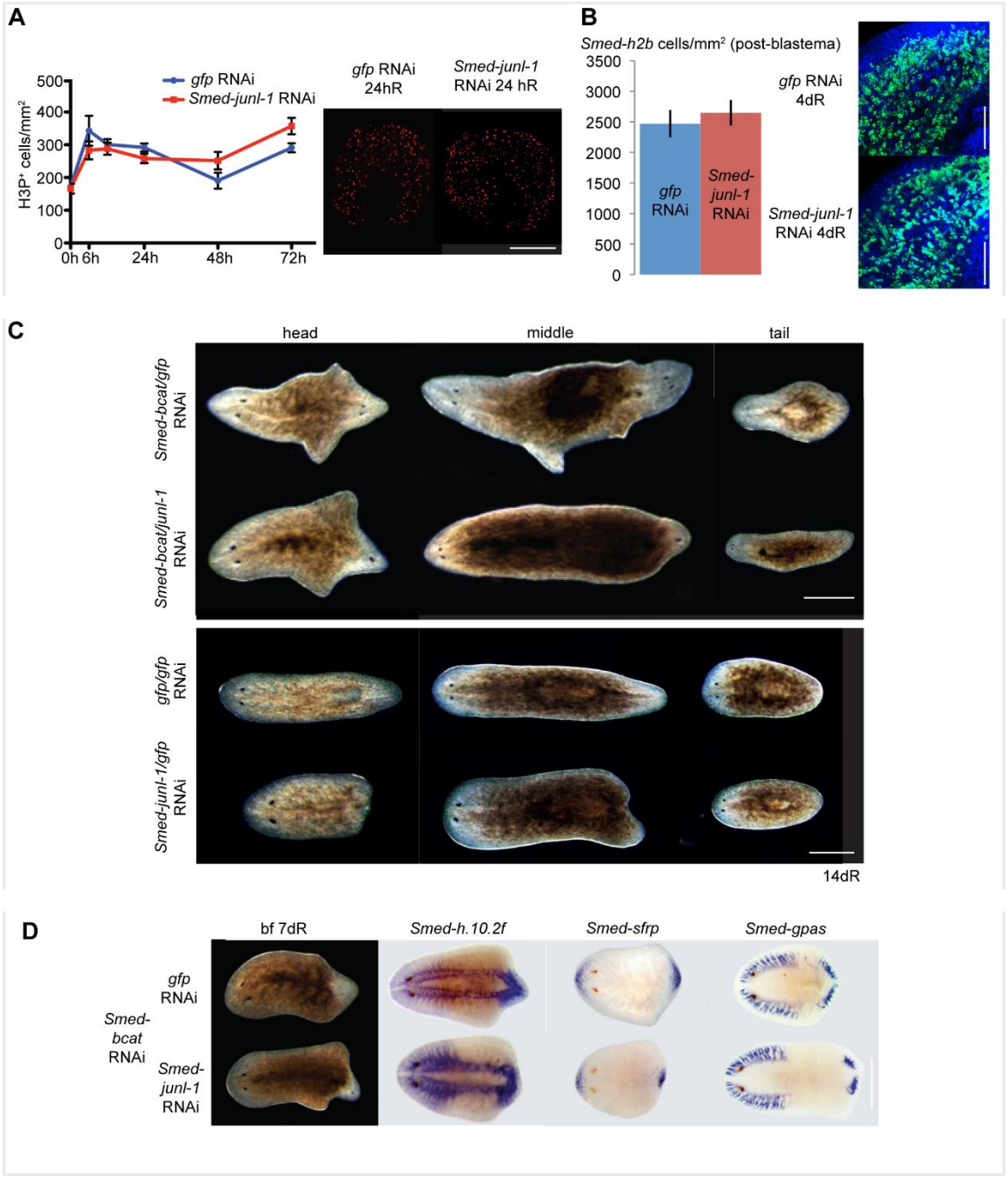
Supplementary figure 5: Quantitative RT-qPCR to confirm transcript knockdown by RNAi. (A) Quantitative RT-qPCR to confirm transcript knockdown of Smed-jnk. Levels of knockdown compared to GFP(RNAi) controls are comparable to those achieved in a previous study (Almeudo-Castillo,. et al. 2014). **(B)** Quantitative RT-qPCR to confirm transcript knockdown of *Smed-junl-1*. We observe consistent knockdown of *Smed-junl-1* across all RNAi experiments including double RNAi with *Smed-apc* and *Smed-ptc* compared to control *GFP(RNAi)* animals and *Smed-jnk(RNAi)* animals. **P<0.01, two tailed t-test compared to *GFP(RNAi)*. Error bars represent the standard deviation across all replicates.



Supplementary figure 6: Incubation with SP600125 results in tailless animals. (A) Kaplan-Meyer survival curve of animals incubated in different concentrations of SP600125 (JNKi) after amputation. Lower concentrations of JNKi (0.25 μM, 0.5 μM and 0.75 μM) did not affect viability but did not result in tailless animals (0.25 μM and 0.5 μM) or resulted in only 40% of tailless animals (0.75 μM). We chose 1 μM as the optimal concentration, to use in our experiments, as the highest concentration that did not affect viability. **(B)** Animals that are incubated in JNKi for 7 days of regeneration cannot regenerate the tail (a) but can regenerate the anterior correctly (n). Control heads n=40/40, middles n=39/39, tails n=39/40; JNKi heads a=40/40, middles a=38/39, tails n=35/39, 4/39 have faint eyes. Counts indicate normal (n) or abnormal (a) animals as described per condition. Scale bars 500 μm.

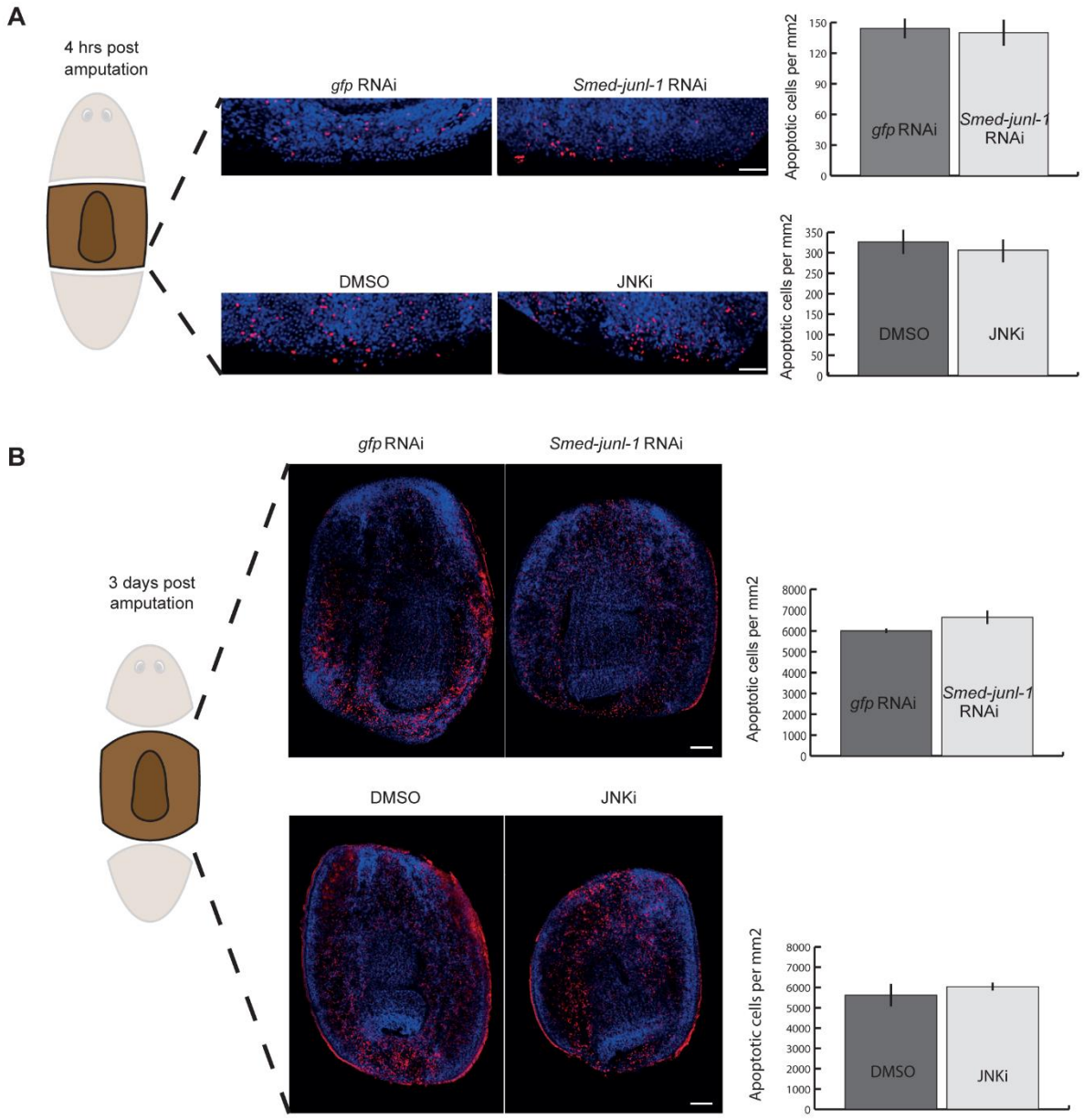


Supplementary figure 7: *Smed-jnk* is not required for correct anterior midline specification. (A) The expression of *Smed-slit* (DMSO n=8/8, JNKi n=7/7) and (**B**) *Smed-wnt5* (DMSO n=8/8 JNKi n=8/8) appeared normal (n) in JNKi regenerating middle pieces at 7dR. (**C**) The expression of *Smed-wnt5* is only reduced (a) in *junl-1(RNAi)* (*gfp* n=8/9 *hem* n=10/11 *jnk* n=11/11 *junl-1* a=6/6) 14dR animals. Counts indicate normal (n) or abnormal (a) animals as described per condition. Scale bars 500 µm.

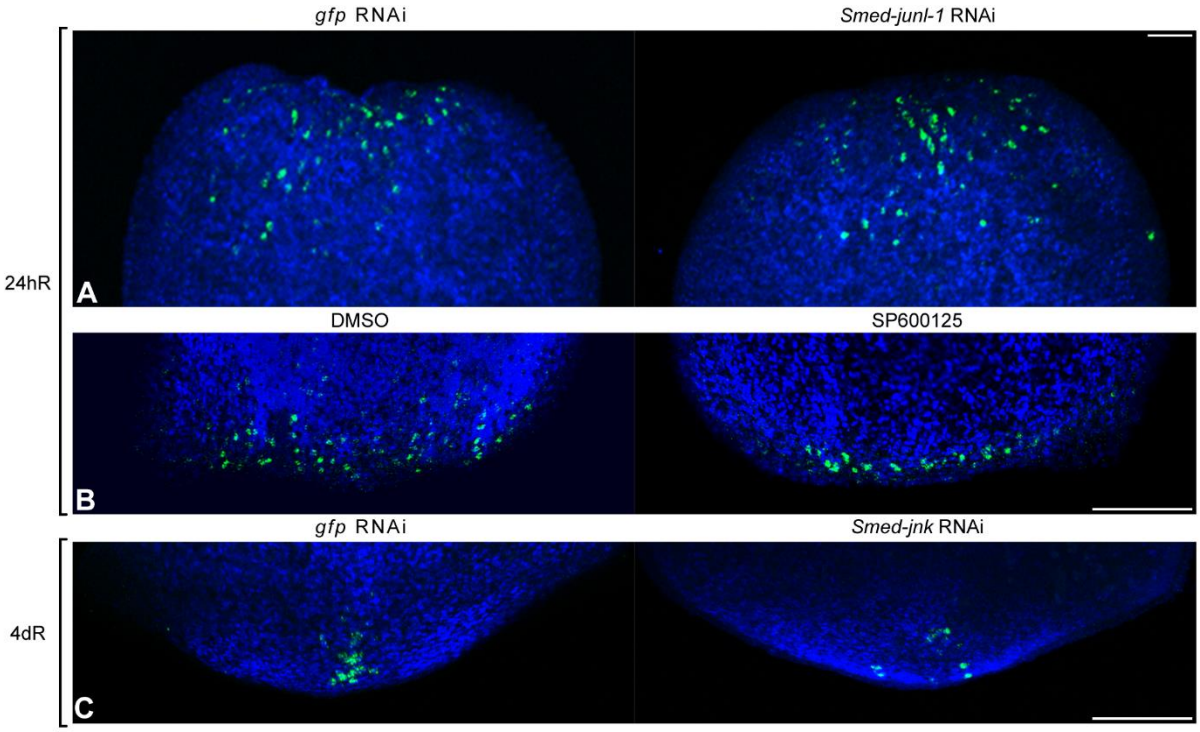


Supplementary figure 8: The tailless phenotype is not due to general differentiation or proliferation defects. (A) Proliferation after wounding was not negatively affected after *Smed-junl-1(RNAi)*. H3P positive cells were counted from a minimum of 15 regenerating pieces 6, 12, 24, 48 and 74 hours after amputation. H3P counts are presented as the average number of mitosis per mm^2, the error bars represent the s.e.m.. Representative images of middle pieces stained with anti-H3P antibody at 24 hours of regeneration are shown. (**B**) Neoblast numbers after wounding were not significantly affected by *Smed-junl-1(RNAi)* at

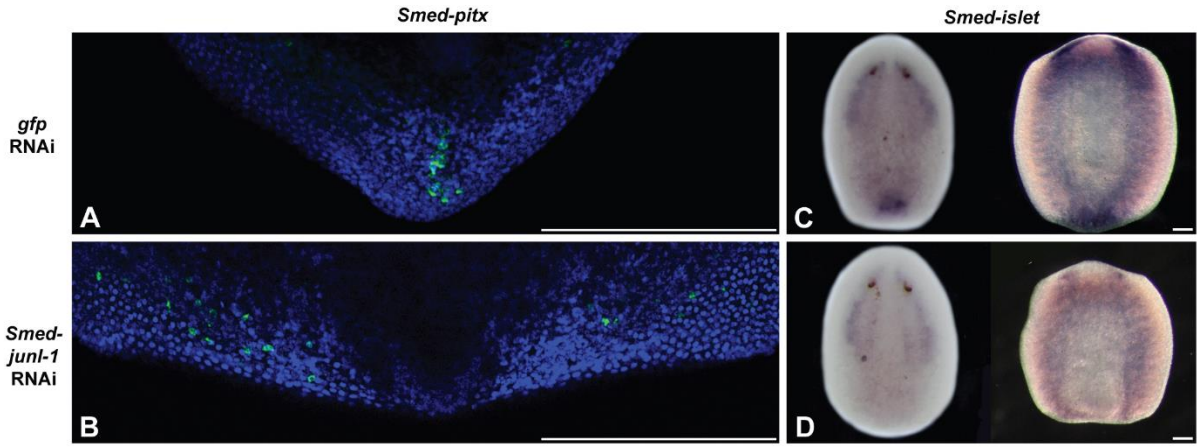
4dR. *Smed-H2B* positive cells are presented as the average number of cells per mm^2 (error bars indicate s.e.m) counted over 7 samples. Scale bars 100 µm. **(C)** Double *Smed-bcatenin/junl-1(RNAi*) animals can regenerate anterior tissue at the posterior site (xh). A proportion of *Smed-bcat/junl-1* animals show mispatterning of the eyes. Shown are animals at 14 days of regeneration. *Smed-bcat/gfp* heads xh=59/59, middles xh=60/60, tails n=60/60; *Smed-bcat/junl-1* heads xh=60/60, middles xh=53/53 (double headed) where 2/53 have 2 eyes in both blastemas, 12/53 have one eye in each blastema, 14/53 have two eyes in the anterior blastema and one eye in the posterior blastema and 9/53 have two eyes in the anterior blastema but produce unpigmented tissue with no eyes in the posterior blastema; tails 43/56 are cyclopic, 11/56 have two eyes and 2/56 have very faint eyes (scored as eyeless). **(D)** Anterior fate (xa) was confirmed by the use of neural marker *Smed-h.10.2f* (*bcat/gfp* xa=14/14, *bcat/junl-1* xa=10/10), anterior polarity marker *Smed-sFRP* (*bcat/gfp* xa=25/25, *bcat/junl-1* xa=13/19) and brain marker *Smed-GPAS* (*bcat/gfp* xa=9/9, *bcat/junl-1* xa=13/13). Counts indicate posterior head (xh, xa) phenotypes as described per condition. Scale bars 500 µm.



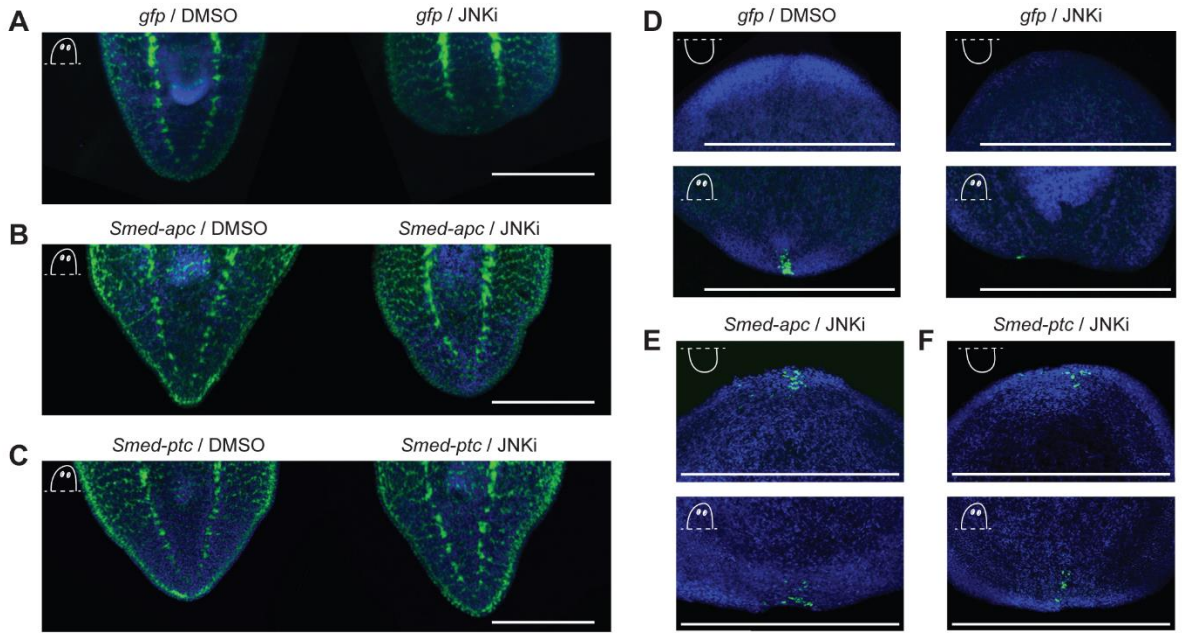
Supplementary figure 9: Cell death is unaffected after *Smed-junl-1*(RNAi) or SP600125 treatment. TUNEL analysis of **(A)** the early (4hR) apoptotic peak near the site of amputation (posterior) and (**B**) the late (3dR) apoptotic peak spread across the animal tissue (whole trunks shown) indicates no significant change in apoptosis after *Smed-junl-1*(RNAi) or JNKi treatment relative to controls. Ten animals per condition were analysed. Average counts are presented as cells per mm^2 ± s.e.m.. Scale bars as indicated.



Supplementary figure 10: *Wnt1* expression after *Smed-junl-1(RNAi),* SP600125 treatment and *Smed-jnk*(RNAi). Wound-induced *wnt1* expression is unaffected (n) in (**A**) tail pieces after *Smed-junl-1*(RNAi) (*gfp* n=26/30 *junl-1* n=32/35, over 3 different experiments) and (**B**) head pieces treated with JNK inhibitor at 24 hours of regeneration (*gfp* n=8/10 JNKi n=10/11). (**C**) Stem-cell dependent *wnt1* expression is disrupted (a) in head pieces at 4 days of regeneration after *Smed-jnk*(RNAi) (*gfp* n=10/10 *jnk* a=7/8). Counts indicate normal (n) and abnormal (a) animals as described per condition. Scale bars 100 μm.



Supplementary figure 11: JNK-signaling is required for the expression of *pitx* and *islet*. **(A)** Normal pole cell *pitx* expression (n) is disrupted (a) by (**B**) *Smed-junl-1*(RNAi) in heads at 4 days after regeneration however peripheral *pitx* expression is maintained (*gfp* n=9/10 *junl-1* a=9/11). (**C**) *Islet* expression is detected in the posterior blastema in both head and middle pieces but also laterally in the anterior blastema of middle pieces at 4 days after regeneration (*gfp* n=9/9). (**D**) *Smed-junl-1*(RNAi) abolishes posterior *islet* expression (a) and attenuates anterior expression (*junl-1* a=6/6). Counts indicate normal (n) and abnormal (a) animals as described per condition. Scale bars 200 µm.



Supplementary figure 12: Over activation of Wnt signalling rescues the tailless phenotype and pole cell *Wnt1* expression at every wound site. (A) Treatment with 1 μM of SP600125 coupled with *gfp(RNAi)* also results in tailless animals after 10 days of regeneration, compared to 0.05% DMSO controls. Immunohistochemistry with anti-Synapsin shows that the VNCs do not extend or join (a) at the tip (control n=26/27, JNKi a=20/23). **(B)** *Smed-apc(RNAi)* rescues the tailless phenotype including VNC joining (n) at 10dR (*Smed-apc/* DMSO n=15/15, *Smed-apc/*JNKi n=20/20) and **(C)** Smed-ptc(RNAi) also rescues the tailless phenotype with visible VNC joining at 10dR (Smed-ptc/DMSO n=13/13, Smed-ptc/JNKi n=20/20). **(D)** Treatment with 1 μM of SP600125 coupled with gfp(RNAi) also results in the loss of posterior pole expression of wnt1, compared to controls (*gfp/DMSO* n=5/5, *gfp/JNKi* a=4/5). **(E)** Posterior pole *wnt1* expression is rescued in both anterior and posterior blastemas at 4dR in *Smed-apc/JNKi* animals (n=11/12). and *Smed-ptc/JNKi* animals (n=12/13). Counts indicate normal (n) and abnormal (a) phenotypes as described per condition. Scale bars 500 μm.