



## **Supplementary Information**



Figure S1. Expression pattern of posterior wnts in intact and regenerating animals. (A) In situ hybridization of posterior wnts in intact animals. Smed-wnt1 is expressed as a stripe of cells in the posterior dorsal midline; Smed-wnt11-1 is expressed in the mouth and as a posterior gradient from the mouth to the tail; Smed-wnt11-2 is expressed as a posterior gradient, concentrated in the posterior midline; and Smed-wnt11-5 is expressed in the esophagus and as a gradient from the prepharynx to the tail; and (B) In situ hybridization of posterior wnts in regenerating trunks at 12 h, 1 day, 2 days and 3 days post-amputation. Anterior blastemas are shown on the left and posterior blastemas on the right. The first wnt to be expressed in the regenerating region is Smed-wnt1, at 12 h. At 1 day, Smedwnt1 decreases its expression in anterior blastemas and concentrates in the posterior. At 2 days, it recovers the expression pattern observed in intact animals (posterior dorsal midline) and disappears in anterior blastemas. Smed-wnt11-1 expression is only maintained in the mouth during early regeneration stages, and appears in the regenerating region at day 2, at the same time that Smedwnt11-2. Smed-wnt11-5 keeps the expression observed in intact animals. At 2 days of regeneration, it starts to re-scale from anterior to posterior to recover the gradient seen in intact animals. At 3 days of regeneration, the expression of all posterior wnts resembles the one observed in intact planarians. (The number of animals analyzed for each condition was at least n = 5.) Scale bar: 100 µm (A,B).

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Figure S2. Phenotypes of posterior wnts RNAi during regeneration. Stereomicroscope views of regenerating trunk pieces showing the different phenotypes. From left to right: control animals have a wild-type appearance; Smed-wnt1 RNAi planarians exhibit 2 different phenotypes, "tailless" (50% of animals) and "two-headed" (33% of animals); Smed-wnt11-1 RNAi generates "short tail" planarians (93% of animals); Smed-wnt11-2 RNAi generate "tailless" planarians (100% of animals); and Smedwnt11-5 RNAi planarians have a wild type tail. Images correspond to 20 days regenerating animals. (The number of animals analyzed for each condition was at least n = 14.) Scale bar: 500 µm.



different phenotypes following RNAi in intact planarians. From left to right: controls resemble wild type; Smed-wnt1 RNAi planarians also resemble wild type; Smed-wnt11-1 RNAi generates "short tail" planarians; Smed-wnt11-2 RNAi generates "tailless" planarians (100% of animals); and Smed-wnt11-5 RNAi planarians have a tail that resembles wild type. (The number of animals analyzed for each condition was at least n = 10); and (B)  $\alpha$ -Tubulin immunostaining showing the morphology of the ventral nerve cords in the posterior tip of planarians after silencing posterior wnts. All images correspond to 20 days regenerating animals. Scale bar: 500 µm (A) and 100 µm (B).