Supporting Information

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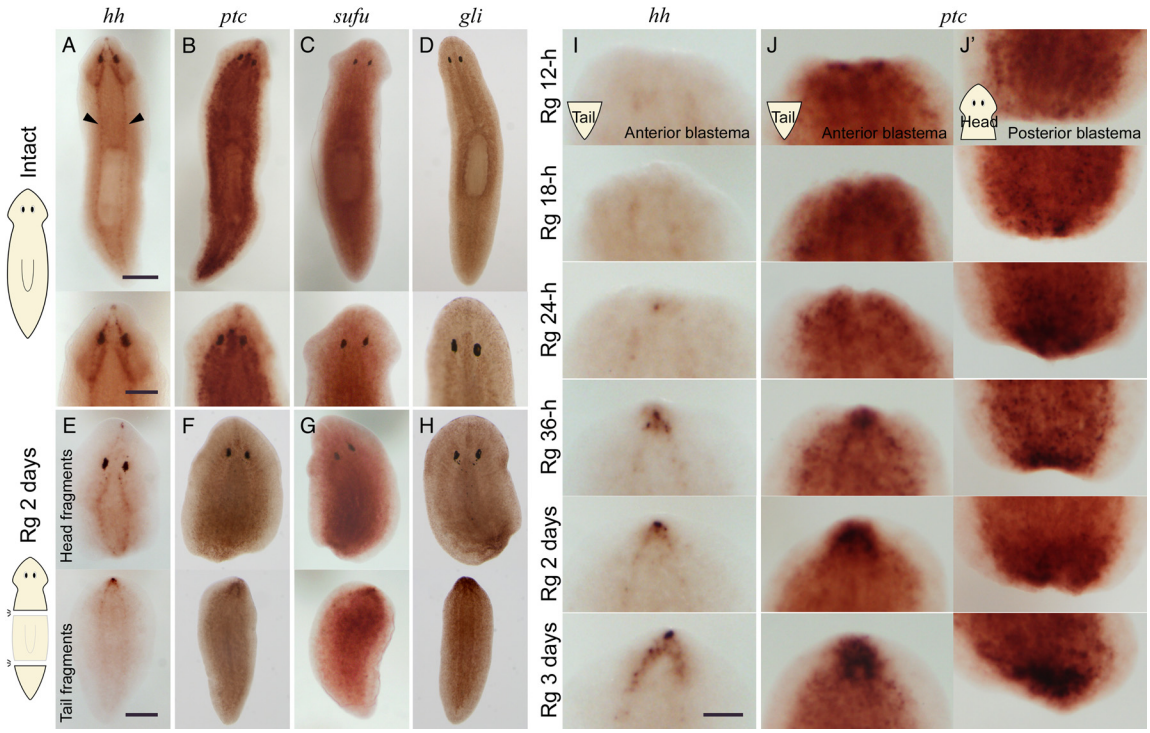

Fig. S1. Expression pattern of Hh signaling component genes in *D. japonica*. (*A–D*) Expression pattern in intact animals (*Upper*), and a magnified view of the same animals' head region (*Lower*). *Djhh* expression was specifically localized at the anterior tip of the head, VNCs (arrowheads), medial border of the brain and eye in intact planarians (*A*), whereas the other Hh signaling components, *Djptc* (*B*), *Djsufu* (*C*), and *Djgli* (*D*), were expressed ubiquitously. [Scale bars, 500 μm (whole) and 300 μm (magnified).] (*E–H*) Expression pattern in head and tail regenerants at day 2. *Djhh* expression was induced in the anterior blastema only (*E*), whereas the other genes were induced in both anterior and posterior blastemas (*F–H*). (Scale bar, 300 μm.) (*I–J'*) Induction of the expression of *Djhh* (*I*) and *Djptc* (*J* and *J'*) during the course of regeneration. *Djhh* expression was first detected in the anterior blastema of regenerating tail fragments at least 24 h after amputation and was strongest in the anterior tip of regenerating. The expression of *Djptc* was induced in both anterior blastema (of tail fragments) and posterior blastema (of head fragments) from the earliest stage examined during regeneration. (Scale bar, 200 μm.)

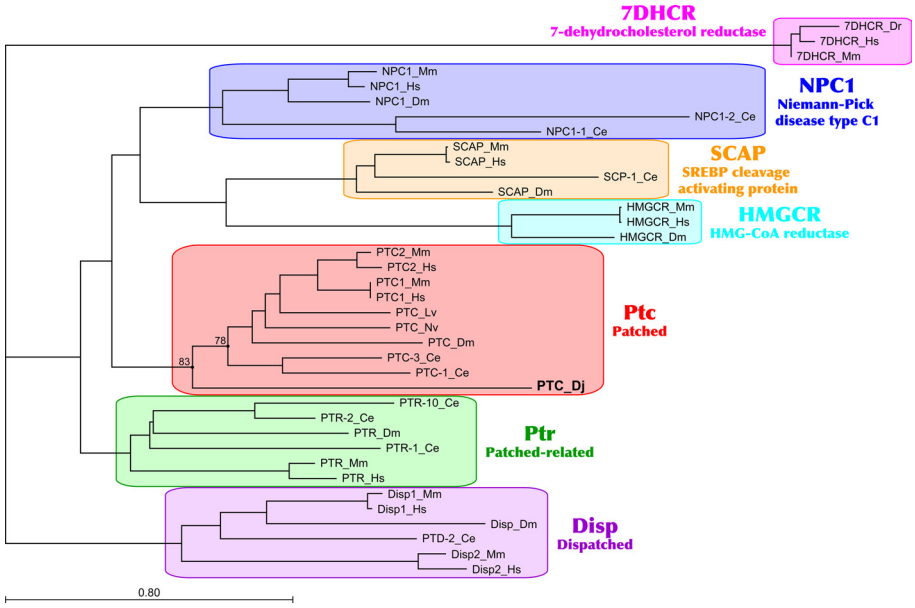

Fig. S2. Phylogenetic tree of SSD-containing proteins. Phylogenetic tree (Neighbor Joining, bootstrap replicas: 100) of conserved sterol-sensing domain (SSD) shows that DjPtc is more closely related to the Patched (Ptc) family (red) than any other proteins. Protein sequences were derived from the GenBank database. 7DHCR, 7-dehydrocholesterol reductase (pink); DISP, Dispatched (purple); HMGCR, 3-hydroxy-3-methylglutaryl CoA-reductase (light blue); NPC1, Niemann-Pick disease type C1 (blue); PTR, Patched-related (green); SCAP, sterol regulatory element-binding protein (SREBP)-cleavage activating protein (orange). Ce, *Caenorhabditis elegans*; Dj, *Dugesia japonica*; Dm, *Drosophila melanogaster*; Dr, *Danio rerio*; Hs, *Homo sapiens*; Lv, *Lytechinus variegatus*; Mm, *Mus musculus*; and Nv, *Nematostella vectensis*.

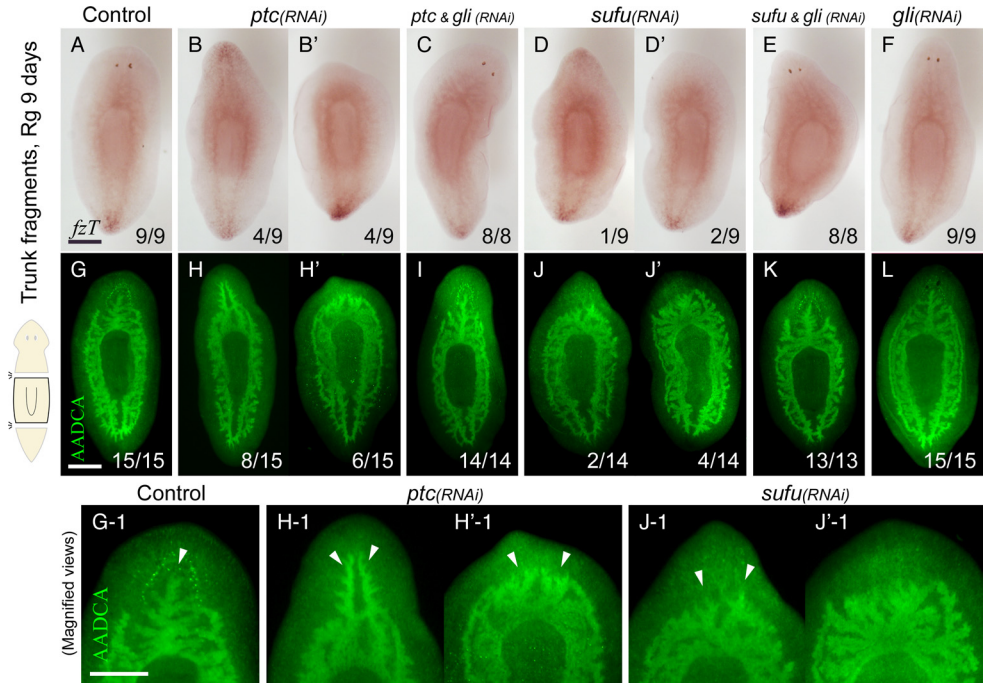

Fig. S3. Phenotypes induced by RNAi of Hh signaling component genes were characterized with respect to head-less morphologies in RNAi trunk regenerants. RNAi of *Djptc* and *Djsufu* caused various phenotypes in the regeneration of the anterior region, ranging from Janus-tails formation, to posteriorization of the anterior region, and loss of the head identity. RNAi trunk fragment regenerants at day 9 were examined for *DjfzT* expression (*A*–*F*), followed by immunostaining using anti-DjAADCA antibody to analyze gut-branching morphology (*G*–*L*). A portion of the anterior region of specimens after immunostaining is magnified in the lower panel (*G*–*1*, *H*–*1*, *H*′–*1*, *J*–*1*, and *J*′–*1*). *DjfzT* expression was ectopically induced in the anterior blastema of trunk regenerants after *Djptc* (*B*) or *Djsufu* (*D*) RNAi, indicating Janus-tails formation. Actually, these regenerants showed bifurcated branching of the main tract of the gut in the anterior region (*H* and *H*′–*1*). However, *DjfzT* expression was sometimes detected only in the posterior end (without ectopic expression in the anterior blastema) of RNAi regenerants (*B*′ and *D*′). They showed a rounded anterior end, and did not generate eyes or induce *Djndk* expression in the anterior region (Fig. 3*N*). Regarding gut morphology, regenerants did show bifurcated branching of the main tract of the gut in the anterior region after *Djptc* (*H*′ and *H*′–*1*) or *Djsufu* (*J* and *J*′–*1*) RNAi, indicating the posteriorization of the anterior region (but not transformation to a tail fate). Furthermore, *Djsufu*(*RNAi*) regenerants sometimes showed abnormal anterior gut-branching (*J* and *J*′–*1*), suggesting an abnormal anterior end, which possessed neither a head (anterior fate) nor a tail (posterior fate), that is, loss of the head identity. These phenotypes would correspond to those of Janus-heads formation and tail-less regeneration in the reported RNAi for *Djhh* and *Djgli* (Fig. 3 *A*–*D*). Moreover, all of these phenotypes were suppressed by simultaneous *Djgli* RNAi (*C*, *E*, *I*, and *K*), as indicated by the single main tract of the gut and also the detection of DjAADCA-positive neurons in the cephalic brain. (Scale bars, 300 μm.)

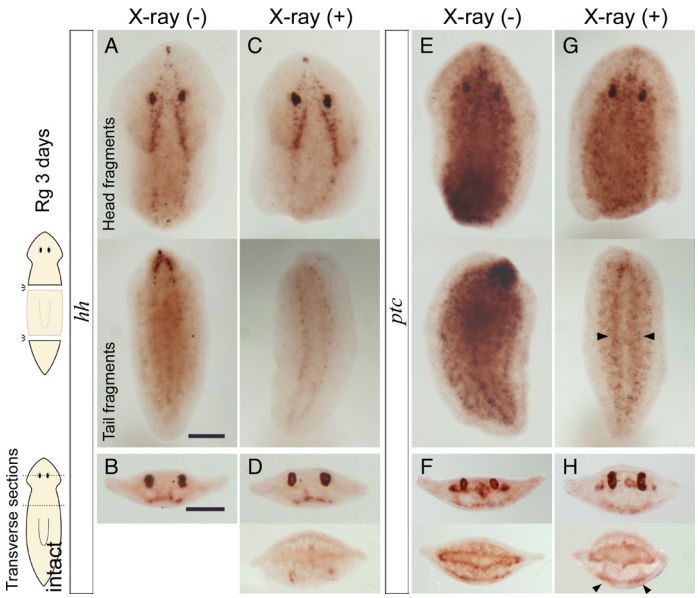

Fig. S4. Gene expression pattern of *Djhh* and *Djptc* in X-ray-irradiated planarians and regenerants. Regenerants from head (*Upper*) and tail (*Lower*) fragments of X-ray-irradiated animals were analyzed for the expression of *Djhh* (*A* and *C*) and *Djptc* (*E* and *G*) 3 days after amputation. Also, intact animals (no amputation) after X-ray irradiation were examined by in situ hybridization, followed by transverse sectioning at the head (*Upper*) and prepharyngeal (*Lower*) regions as indicated by dotted lines in the diagram (*B*, *D*, *F*, and *H*). *Djhh* expression in the head region and VNCs was not affected by X-ray irradiation (*C* and *D*), indicating its localization in differentiated cells. In contrast, the expression in the anterior blastema of regenerants from tail fragments (tip and brain precursor) was eliminated by X-ray irradiation (*C*), suggesting that these expressing cells were derived from the stem cell population. In contrast, X-ray irradiation not only abolished the up-regulation of *Djptc* in both the anterior and posterior blastemas of regenerants from body fragments after amputation, but also markedly reduced the ubiquitous expression of *Djptc* (*E* and *G*). In X-ray-resistant cells, *Djptc* was predominantly expressed in cells neighboring the VNCs (arrowheads) and gut epithelial cells (*F* and *H*). Taken together, these results indicate that Hh signaling mediated by *Djptc* occurs in both differentiated and undifferentiated cell populations, and suggest that AP polarity is defined by Hh signaling in differentiated cells of the VNCs. (Scale bars, 300 μm.)

Table S1. PCR primers used in RT-qPCR analysis

Gene	Sequence		Accession No.*
	Forward	Reverse	
DjG3PDH	accaccaactgtttagctcccttag	gatggtccatcaacagtcttttgc	AB504746
Djndk	tcacaaactccaccgcagtacttt	ggtatggattagcattattgaattgtg	AB071948
DjZicB	gtcgtttctaacgtctaatcaacattctg	cgttctttcattttacattgagattcg	AB231881
DjAbd-Ba	gattatcacgcattgtcagcttatacttc	agctgggaatagaattgtgaggataata	AB049972
DjotxA	ggaacaatttgtccgttctacattagac	tcgtttcaatcttcttatagtcgccata	AB008834
DjwntP-1	agtgacatagtttatttagaagacgttcca	aaatgcgtcctgtagttcccaa	AB504744
Djwnt11–2	gcggaaagattaccatacattggtaaag	caaataacaatgatgcactactaaaggca	AB504745
DjfzT	cgcacaatctaaacagaatgaaactc	tcattgactggaaatgaagaattcg	AB504743
Djptc	gagtaatggctctttggtcaggc	tttaccggtcgctggaatatcc	AB504738
Djsufu	gatgctggcgaaattatgtattcagt	tacagccatatcagaccgacaatga	AB504740
Djgli	tgtcaaaacaaagtcactttcagcaaa	gtaatcccatgcagtatcatatcaacact	AB504741

*Accession number refers to the GenBank database.