Figures and figure supplements

Hedgehog signaling regulates gene expression in planarian glia

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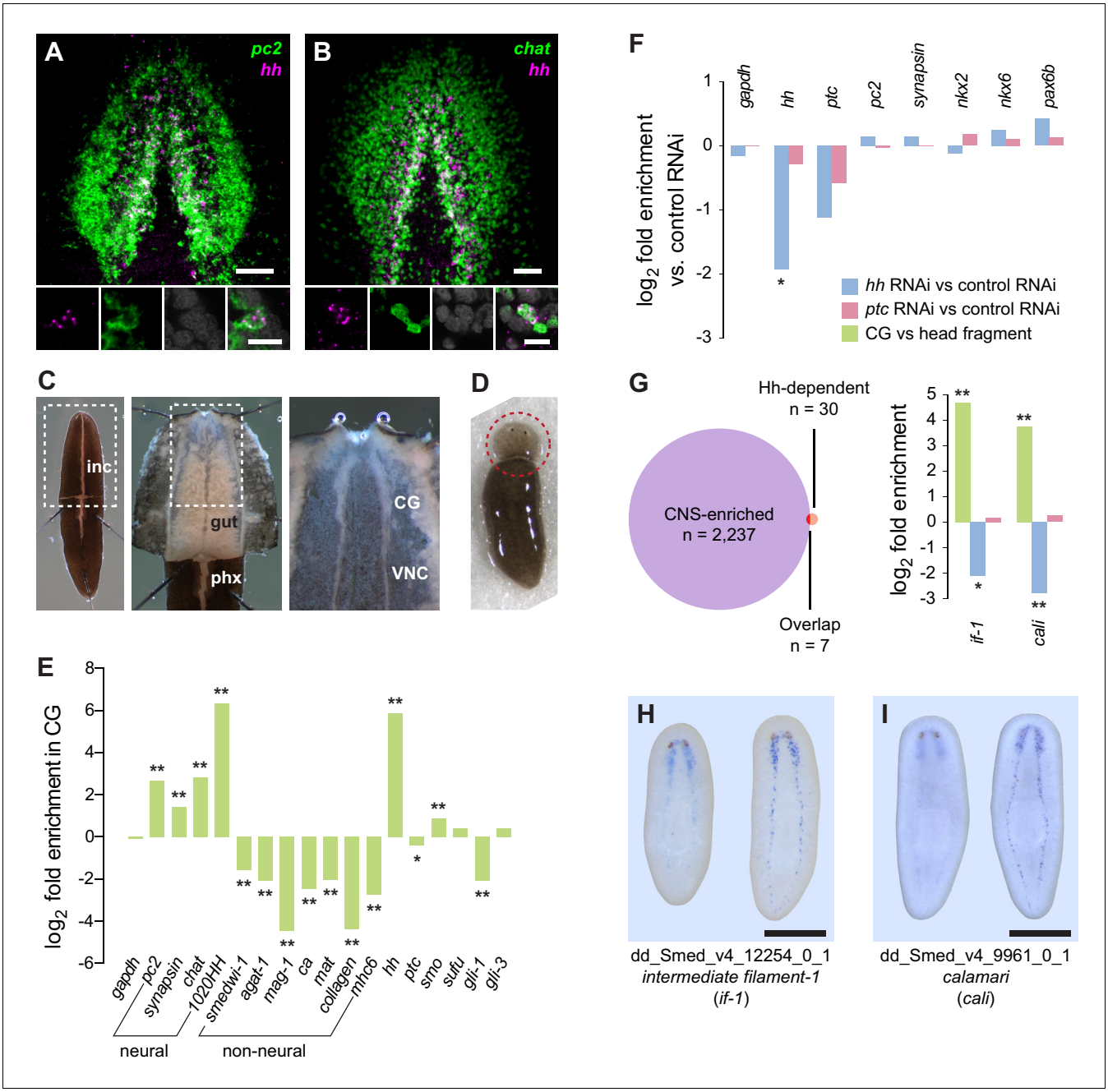

Figure 1. Perturbation of Hh signaling affects gene expression in the cephalic ganglia. (**A–B**) Double fluorescent RNA in situ hybridization (FISH) for *hh* (magenta) and neuronal markers (**A**) *pc2* or (**B**) *chat* (green) in wild-type animals. Main panels show cephalic ganglia. Lower panels show high magnification images of, from left to right, *hh* (magenta), *pc2* or *chat* (green), DAPI (gray), and merged channels from a representative double-positive neuron. (**C**) Excision of cephalic ganglia tissue from acid-killed animals for RNA isolation. The left panel shows incision in the dorsal epidermis. Middle panel shows detail of the boxed region in the left panel after removal of dorsal epidermis. The right panel shows the detail of the boxed region in the middle panel after removal of gut tissue overlying the cephalic ganglia and ventral nerve cords. Abbreviations: inc, incision; gut, gut branches; phx, pharynx; CG, cephalic ganglia; VNC, ventral nerve cords. See methods for dissection protocol. (**D**) Representative image of amputation used to collect tissue for generating the head fragment Illumina libraries. Circle indicates the portion of the animal taken for RNA isolation. (**E**) Bar graph depicting $\log_2$ fold enrichment of selected markers in cephalic ganglia transcriptome over the head fragment transcriptome. Experimentally-verified neural markers and non-neural markers identified by brackets. Average $\log_2$ fold enrichment of all 7 CNS genes listed in ***Figure 1—source data 2*** in cephalic ganglia transcriptome is 2.57. Average $\log_2$ fold depletion of all 22 non-CNS genes listed in ***Figure 1—source data 2*** in cephalic ganglia transcriptome is 1.22. Statistically significant $\log_2$ fold change indicated by asterisks (*$p_{adj} \leq 0.05$, **$p_{adj} \leq 0.001$). For a list of all analyzed genes, see ***Figure 1—source data 1***. (**F**) Bar graph depicting $\log_2$ fold enrichment of transcript expression level in the cephalic ganglia tissue of *hh(RNAi)* animals (blue bars) or *ptc*

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(RNAi) animals (red bars) over cephalic ganglia tissue from *control(RNAi)* animals. (**G**) Intersection of CNS-enriched genes (n = 2237) and Hh-dependent genes (n = 30) reveals 7 CNS genes misregulated following Hh pathway perturbation. Bar graph shows CNS enrichment (green bar) and relative expression following RNAi of *hh* (blue bar) or *ptc* (red bar) for *if-1* and *cali* (*$p_{adj} \leq 0.05$, **$p_{adj} \leq 0.01$). (**H–I**) WISH for (**H**) *if-1* and (**I**) *cali*. Dorsal surface shown on left, ventral surface shown on the right. Anterior up, maximum intensity projection of the ventral domain shown for **A**, **B**. Anterior up for **H**, **I**. Scale bars: 50 um for overviews, 10 um for insets for **A**, **B**; 500 um for **H**, **I**.

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The following source data is available for figure 1:

Source data 1. Neuronal markers used in RNA-seq analysis and co-expression studies.

DOI: 10.7554/eLife.16996.003

Source data 2. Enrichment of neuronal markers and depletion of non-neuronal markers in cephalic ganglia tissue libraries.

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Source data 3. Genes with significant differential expression levels following inhibition of *hh* or *ptc*.

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Source data 4. Accession numbers of protein sequences used in phylogenetic analysis of intermediate filament proteins.

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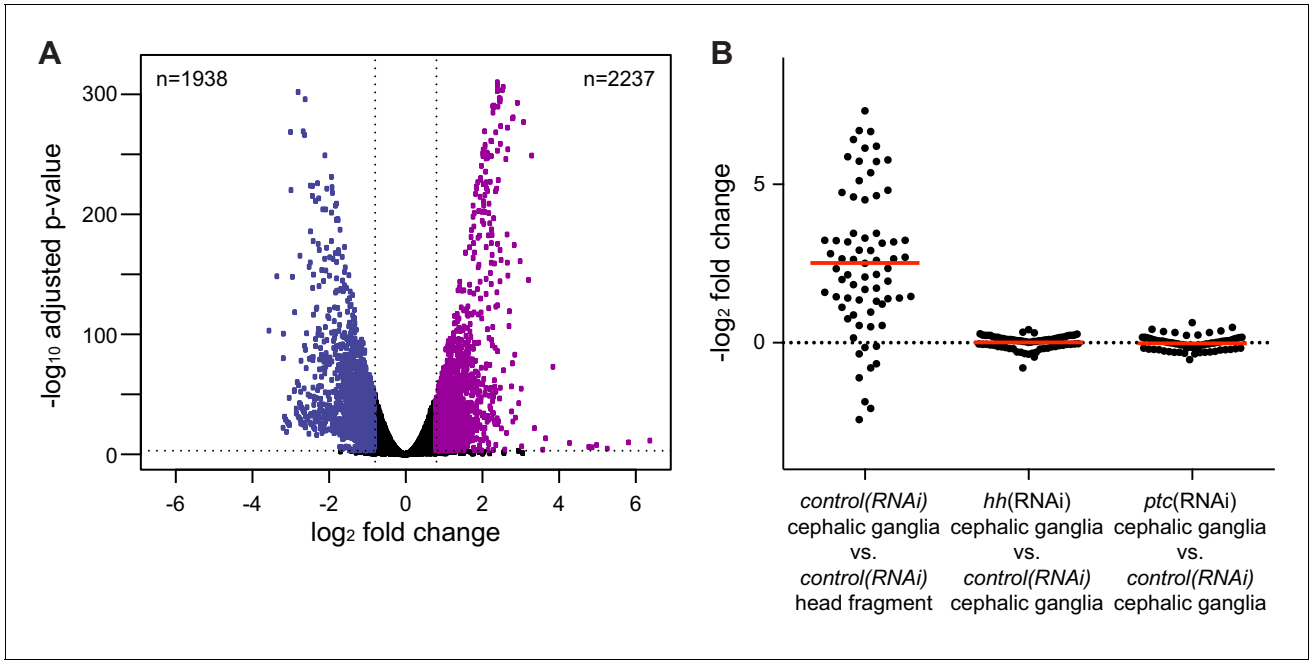

Figure 1—figure supplement 1. Analysis of RNA-seq libraries. (**A**) Volcano plot of differential expression between head fragment transcriptome and cephalic ganglia transcriptome. Dots represent the magnitude of differential expression versus the significance for each gene with an average RPKM over 100. A horizontal dotted line indicates significance cutoff and vertical lines indicate the differential expression magnitude cutoff. Number of genes significantly enriched (purple dots) or depleted (blue dots) in cephalic ganglia tissue listed in the upper right and left corners, respectively. (**B**) Column scatter plot of differential expression of neural markers between conditions. Each dot represents one neural marker. The solid red line indicates mean $\log_2$ fold change of all analyzed neural markers for each condition.

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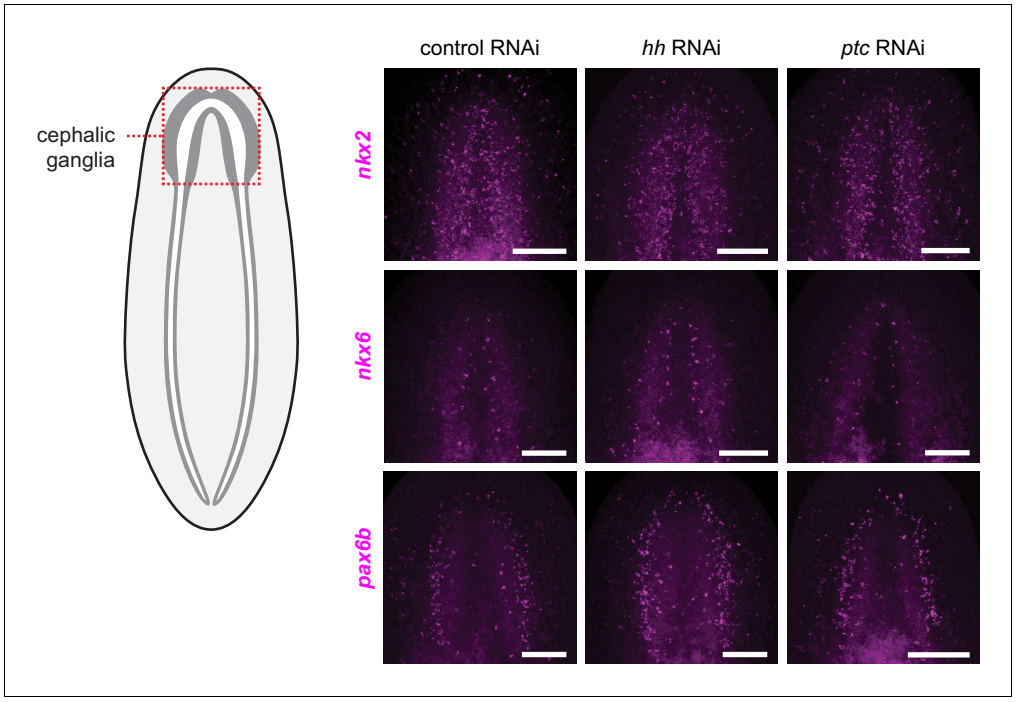

Figure 1—figure supplement 2. Hh signaling pathway perturbation does not affect regional expression of transcription factors in the central nervous system. FISH of orthologs of vertebrate CNS development transcription factors following perturbation of Hh signaling pathway components. Schematic indicates a region of the animal displayed in images. Inhibition of *hh* (center column) or *ptc* (right column) shows no change in the expression pattern of *nkx2* (top row), *nkx6* (middle row), or *pax6b* (bottom row) from controls (left column). Anterior up, maximum intensity projection of ventral side shown. Scale bars: 100 um for all.
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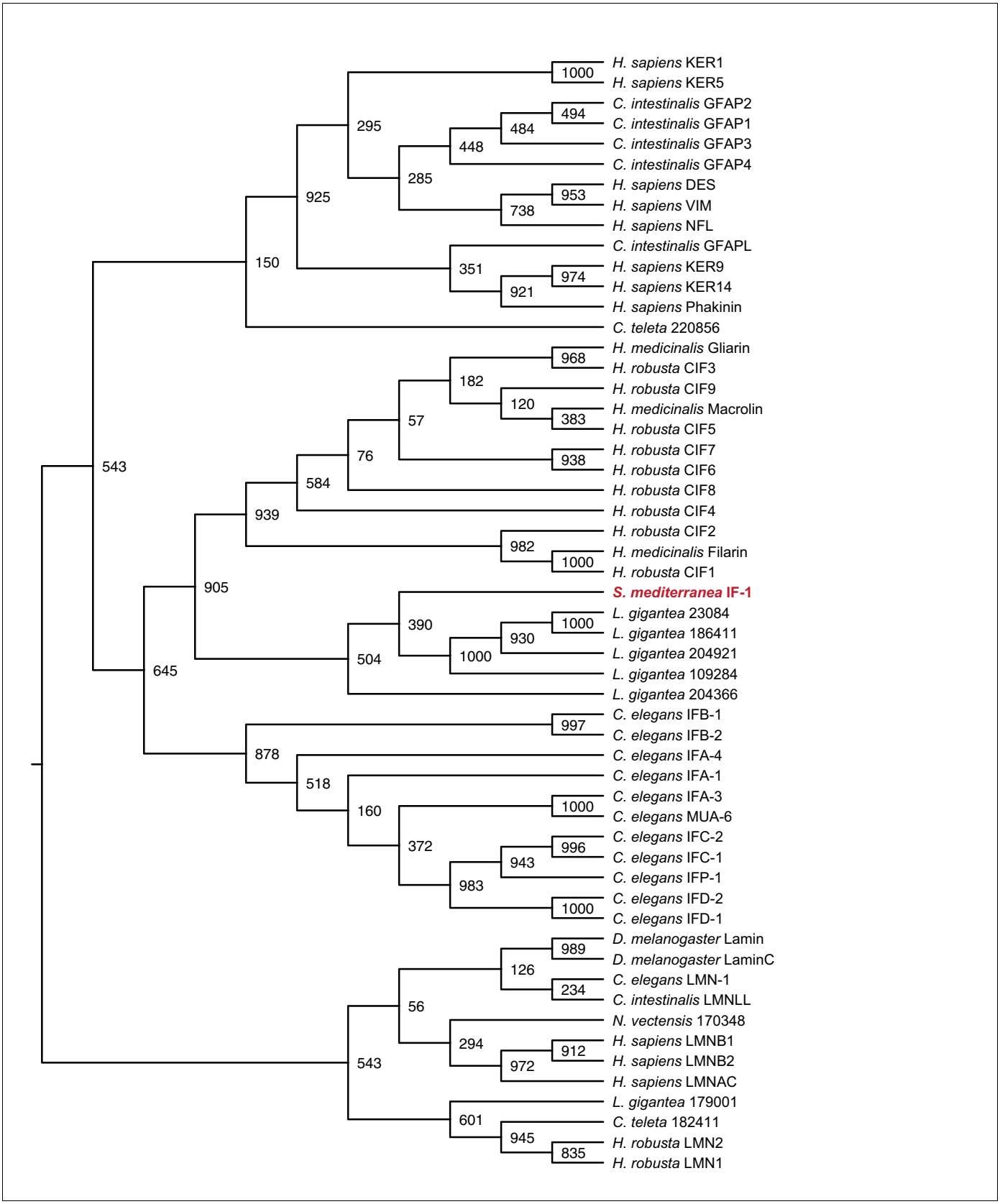

Figure 1—figure supplement 3. Maximum likelihood cladogram for cytoplasmic intermediate filaments. *S. mediterranea* IF-1 clusters with Protostome cytoplasmic intermediate filaments, which diverged prior to the vertebrate radiation of multiple intermediate filament types. Nuclear intermediate

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filament proteins were used as an outgroup to root the tree. Bootstrap values listed at branch junctions. Accession numbers of protein sequences used in the analysis listed in ***Figure 1—source data 4***.

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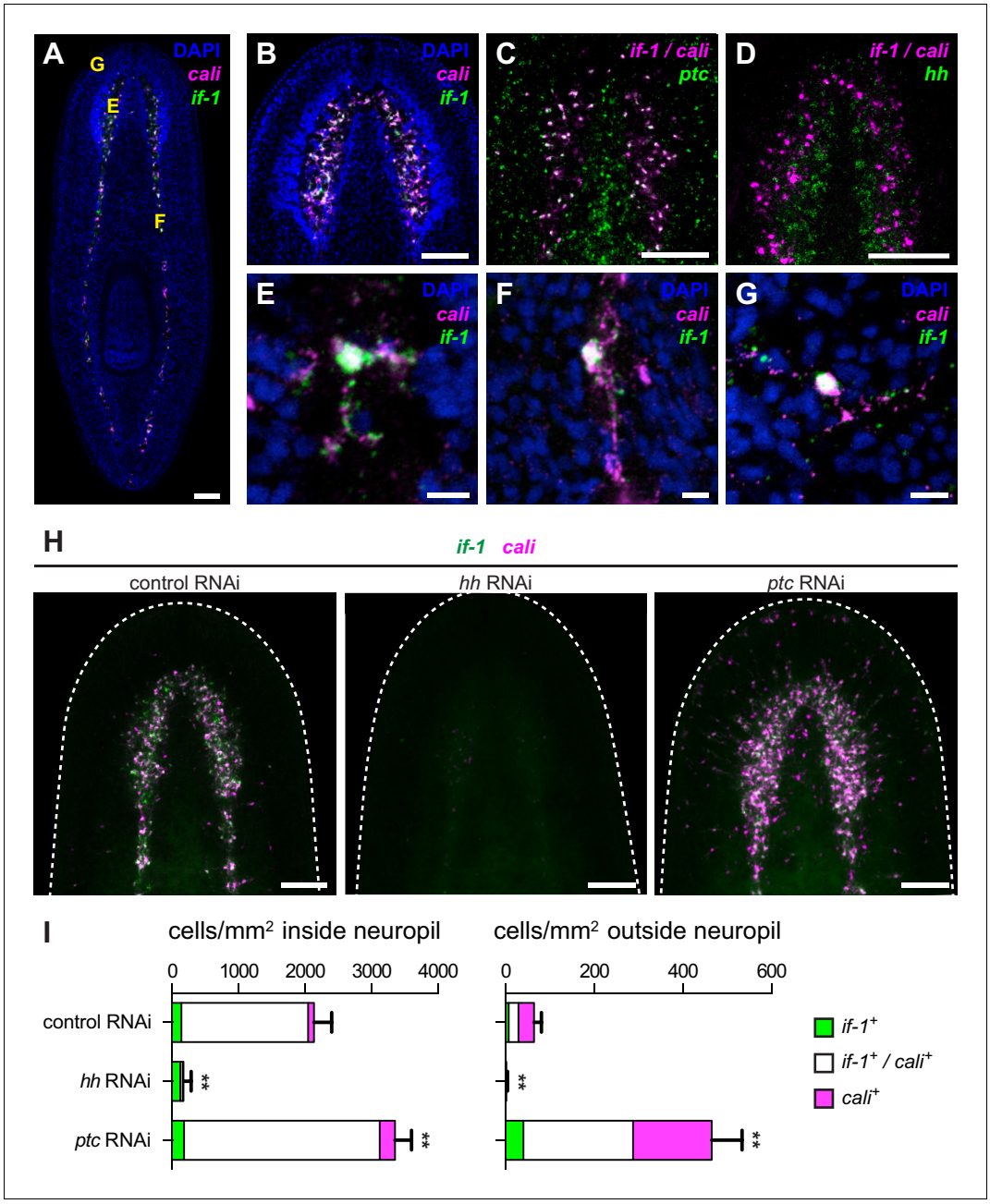

Figure 2. Expression of *if-1* and *cali* in neuropil cells is dependent on Hh signaling. (**A**) Double FISH for *if-1* (green) and *cali* (magenta) in wild-type animals. Cells co-expressing both markers are located in the cell body-sparse neuropil of the cephalic ganglia and ventral nerve cords. The cell body-rich cortical region is labeled by DAPI (blue). Yellow letters indicate regions detailed in **E–G**. (**B**) Double FISH for *if-1* and *cali* in cephalic ganglia neuropil. (**C**) Double FISH for *if-1/cali* (magenta) and *ptc* (green) indicates co-expression of the genes. Probes for *if-1* and *cali* were combined into a single channel (denoted *if-1/cali*) to improve coverage and signal intensity. 97.8 ± 2.1% of *if-1*$^+$/*cali*$^+$ cells in the neuropil and 100% of *if-1*$^+$/*cali*$^+$ cells outside the neuropil expressed *ptc*. (**D**) Double FISH for *if-1/cali* (magenta) and *hh* (green) indicates lack of co-expression. (**E–G**) Single *if-1*$^+$/*cali*$^+$ cells in the (**E**) cephalic ganglion neuropil, (**F**) ventral nerve cord, and (**G**) head rim. (**H**) Double FISH for *if-1* (green) and *cali* (magenta) in animals following inhibition of a control gene, *hh*, or *ptc*. White dotted line delineates the edge of animal. (**I**) Quantification of the results from (**H**), with distribution of *if-1*$^+$ only cells (green), *cali*$^+$ only cells (magenta), and *if-1*$^+$/*cali*$^+$ cells (white). Within the neuropil, cells expressing one or both markers are present at 2135.6 ± 265.8 cells/mm^2 in *control(RNAi)* conditions (n = 5 animals), 169.3 ± 118.6 cells/mm^2 in *hh(RNAi)* conditions (n = 4 animals), and 3354.0 ± 249.5 cells/mm^2 in *ptc(RNAi)* conditions (n = 5 animals). Differences were

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