Zeng et al., http://www.jcb.org/cgi/content/full/jcb.201207172/DC1

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Homologue	Phenotype	Protein complex	Function in ES cells	Known function
HP1-1	++++	N/A	N/A	Heterochromatin formation, Transcription regulation
SSRP1	++++	FACT	Viability	Transcription elongation
SPT16	++++	FACT	N/A	Transcription elongation
HDAC1	+++	NuRD	Pluripotency	Histone deacetylation
CHAF1A (P150)	+++	CAF1	Viability	Chromatin assembly
CHAF1B (P60)	+++	CAF1	N/A	Chromatin assembly
MCM3	+++	Mcm2-7 helicase	N/A	DNA replication
MCM4	++++	Mcm2-7 helicase	N/A	DNA replication
MNAT1	++	CAK	Self-renewal	Cell cycle control
BAF53A	++++	BAF	Self-renewal, Pluripotency	Chromatin remodeling
RBBP4 (P48)	+++	CAF1	N/A	Chromatin assembly
MBD3	++	NuRD	Pluripotency	Histone deacetylation

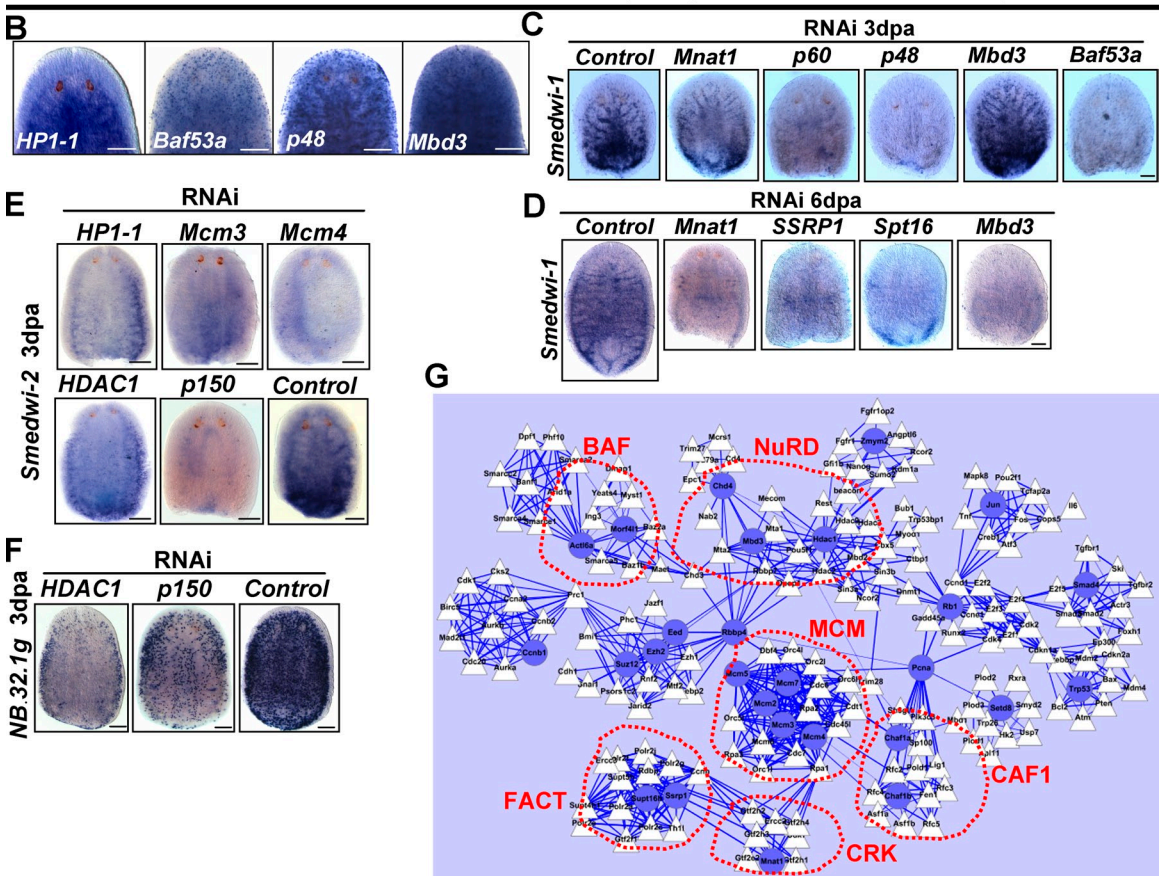

Figure S1. **RNAi screen identifies genes essential for planarian regeneration.** (A) List of genes from the RNAi screen showing phenotypes. The number of plus signs indicates the degree of regeneration defect. (B) WISH showing the expression of four identified genes in the anterior region of planarians. *p48* animal is the magnified view of *p48* in Fig. 1 G. (C–E) WISH analysis with *smedwi-1* (C and D) and *smedwi-2* (E) riboprobes after knockdown of the indicated genes. Shown are head pieces fixed at different time points after amputation (at least six worms showed similar results). dpa, days postamputation. (F) WISH for early progeny marker *NB.32.1g* after knockdown of the indicated genes (at least six worms showed similar results). (G) Network of chromatin regulators that result in regeneration defects upon knockdown. Genes are shown as nodes, and blue in dotted red circles denotes the genes identified. The remaining genes are sorted according to their known interaction with the blue notes in mice (STRING [Search Tool for the Retrieval of Interacting Genes/Proteins] database). N/A, not available. Bars, 0.1 mm.

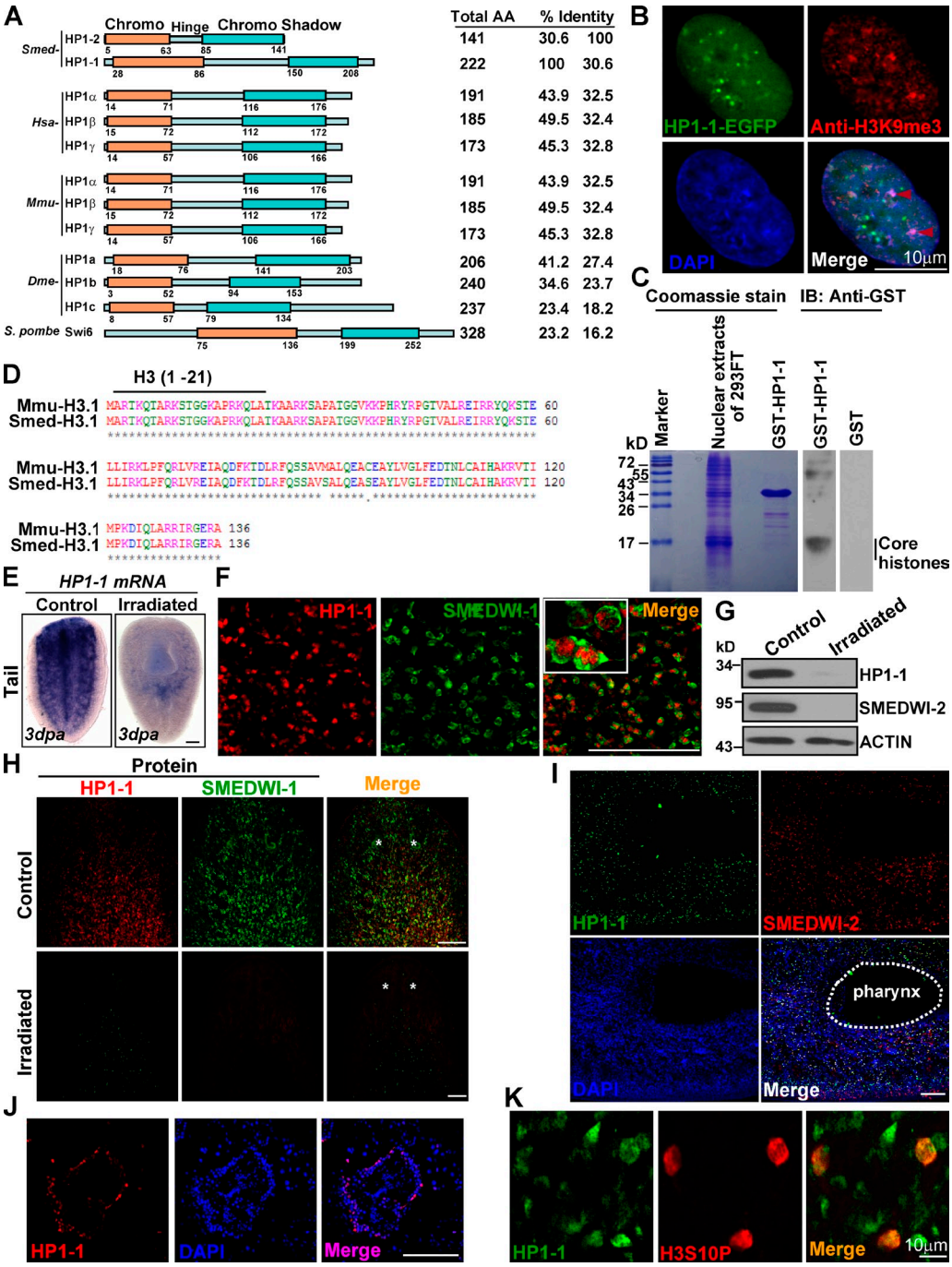

Figure S2. **HP1-1 is localized to ASCs.** (A) Schematic representation of HP1 family proteins from different species. Planarian *S. mediterranea* (*Smed-*), *H. sapiens* (*Hsa-*), *M. musculus* (*Mmu-*), *D. melanogaster* (*Dme-*), and *S. pombe* are shown. The three domains, the N-terminal chromo domain (Chromo), the central hinge region (Hinge), and the C-terminal chromo shadow domain (Chromo Shadow) are indicated. Total protein length is indicated (total AA) and drawn to relative scale. Percentage of identity when compared with SMED–HP1-1 (left) or –HP1-2 (right) over the full length was calculated by alignment (GeneStream II; Centre National de la Recherche Scientifique). (B) IF analysis of HeLa cells transfected with EGFP-tagged HP1-1. The nuclear region is shown. Colocalization is indicated (red triangles). (C) Far Western–type overlay assays. Nuclear extracts of 293FT cells were resolved by SDS-PAGE and either stained with Coomassie blue (left) or transferred to nitrocellulose (right). Purified GST–HP1-1 is shown on the Coomassie-stained gel. The membrane with fractionated nuclear extract was incubated with either GST–HP1-1 (first lane) or GST (second lane). Size markers are indicated by numbers (in kilodaltons). (D) Sequence alignment showing the high degree of sequence identity between planarian histone H3 protein and its murine homologue. The amino acids were colored by physicochemical properties: red, small (small + hydrophobic [include aromatic − Y]); blue, acidic; magenta, basic − H; green, hydroxyl + sulfhydryl + amine + G. (E, left) WISH analysis shows that HP1-1 is highly expressed in the postblastema region of wild-type regenerating worms. (right) This expression was eliminated by irradiation (12/12 animals displayed similar results). Anterior is to the top. (F) Double IF for the SMEDWI-1 and HP1-1 antibodies showing that HP1-1–positive nuclei were mostly surrounded by cytoplasmic SMEDWI-1 signal. Insets show magnified views of dividing neoblasts. (G) Representative Western blot analysis of HP1-1 protein levels in intact worms at day 3 after irradiation. β-Actin was used as a loading control. (H) IF analyses for comparing the expression of HP1-1 before and after exposure to irradiation. SMEDWI-1 was used as a positive control. Worms were harvested at day 1 after irradiation. Asterisks indicate photoreceptors. (I) Double IF showing coexpression of HP1-1 and SMEDWI-2 in intact worms. (J) IF analysis showing expression of HP1-1 in testes lobules. (K) Double IF showing the colocalization of HP1-1 and H3S10P in intact worms. The colocalization of the HP1-1 and H3S10P signal indicates that a small fraction of HP1-1–positive cells is progressing through mitosis. A single confocal optical section is shown. Bars, 0.1 mm, unless otherwise indicated.

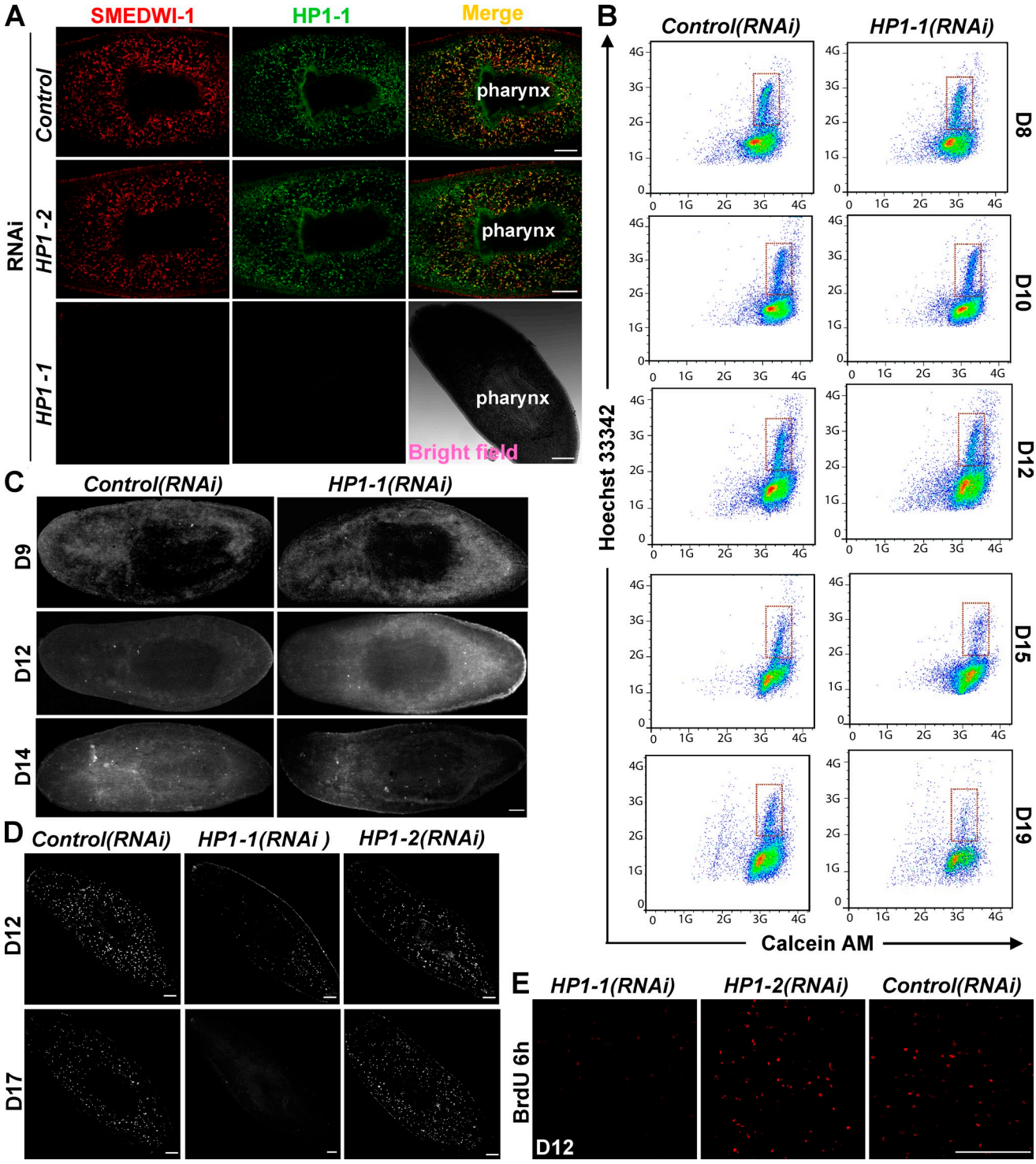

Figure S3. **HP1-1 is required for ASC self-renewal.** (A) The SMEDWI-1–positive cell population was dramatically decreased after HP1-1 depletion. Worms were harvested at D15. 8/8 worms per condition showed similar results. (B) The X1 population (depicted by red boxes) was analyzed by FACS assay. Quantification of the results is shown in Fig. 4 D. Shown is a representative experiment of three independent biological replicates with similar results. 10–15 thousand cells were analyzed for each graph. (C) TUNEL staining for intact worms upon knockdown of the indicated genes. 5/5 worms per condition showed similar results. Quantification of the results is shown in Fig. 4 E. (D) Representative immunostaining results for the H3S10P antibody. More than 10 worms per condition showed similar results. Maximum projections of confocal scanning are shown. Quantification of the results is shown in Fig. 5 C. Anterior is to the top left. (E) *HP1-1(RNAi)* worms display substantially reduced BrdU incorporation relative to control worms at D12. Shown are representative images from whole-mount planarians. 5/5 worms per condition showed similar results. Bars, 0.1 mm.

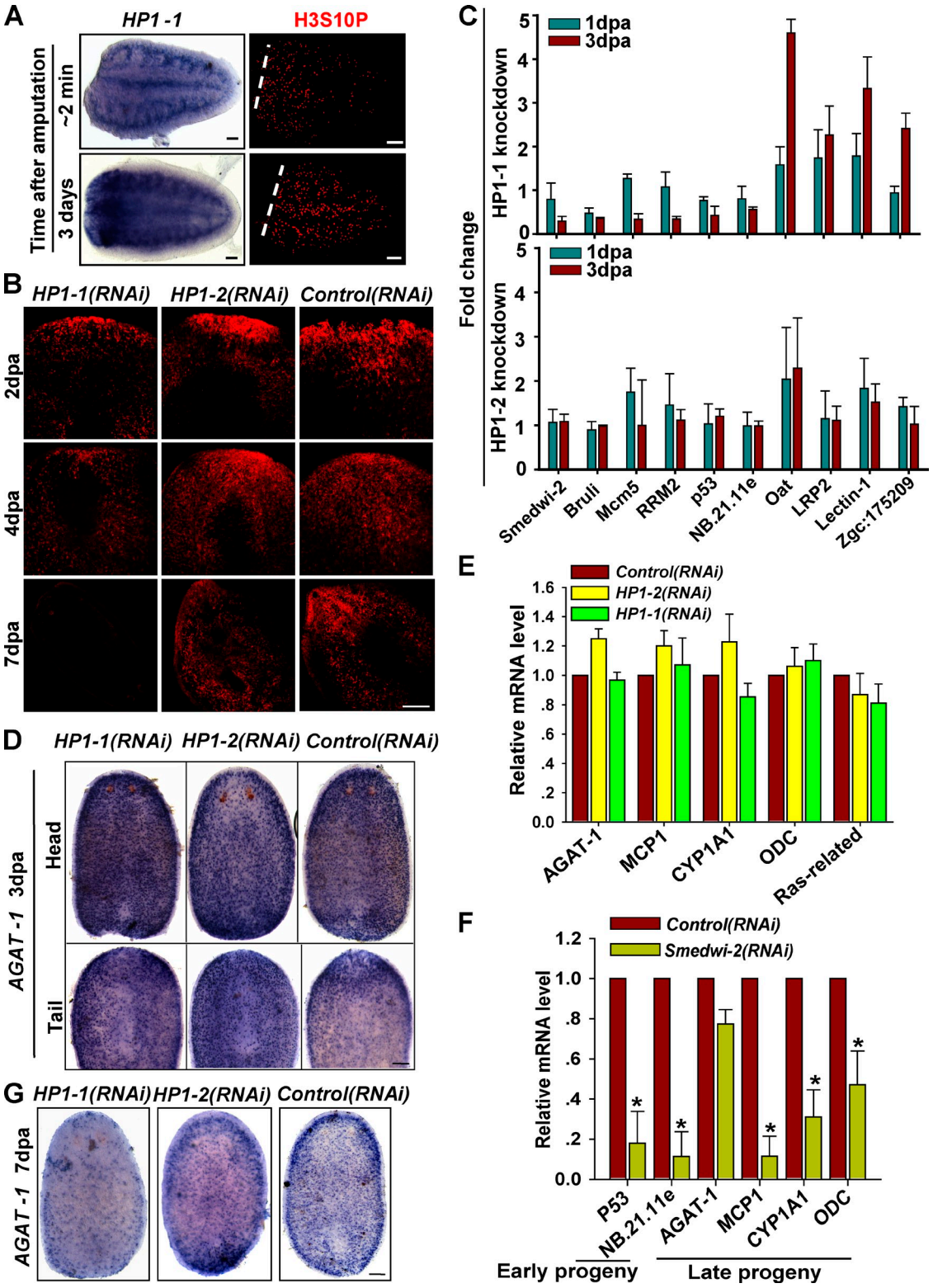

Figure S4. **HP1-1 is essential for the ASC-directed proliferative response.** (A, left) WISH analysis of HP1-1 expression after amputation. *HP1-1* increased and accumulated beneath the blastema during regeneration. (right) Proliferation of wild-type regenerating worms was analyzed with H3S10P immunostaining. Shown are tail fragments. White dotted line indicates amputation site. (B) Representative IF with SMEDWI-1 antibody in regenerating animals. Head fragments are shown; posterior is to the top (top and middle rows) or top left (bottom row), $n = 15$. (C) qRT-PCR shows that expression of genes specific for stem cells progressively decreased in *HP1-1(RNAi)* (top) but not in *HP1-2(RNAi)* (bottom) worms. Relative mRNA levels were obtained by comparing the data from *HP1-1* or *HP1-2(RNAi)* worms with *Control(RNAi)* worms at the corresponding time point. $n = 3$. (D) WISH analysis with the late-progeny marker *AGAT-1* at 3 dpa shows that HP1-1 knockdown did not affect expression of the late-progeny marker (>10 worms per riboprobe were similar). Head and tail pieces of regenerating worms are shown, and anterior is to the top. (E) qRT-PCR showing expression levels of neoblast progeny markers at 3 dpa after knockdown of the indicated genes. $n = 3$. ODC, ornithine decarboxylase. (F) qRT-PCR demonstrates that *smedwi-2* knockdown leads to significant reduction of both early and late-progeny marker genes. $n = 3$. *, $P < 0.01$. (G) WISH analysis of the late-progeny marker *AGAT-1* at 7 dpa. Shown are head pieces of regenerating worms. 5/5 worms per condition showed similar results. Bars, 0.1 mm. Error bars show SDs.

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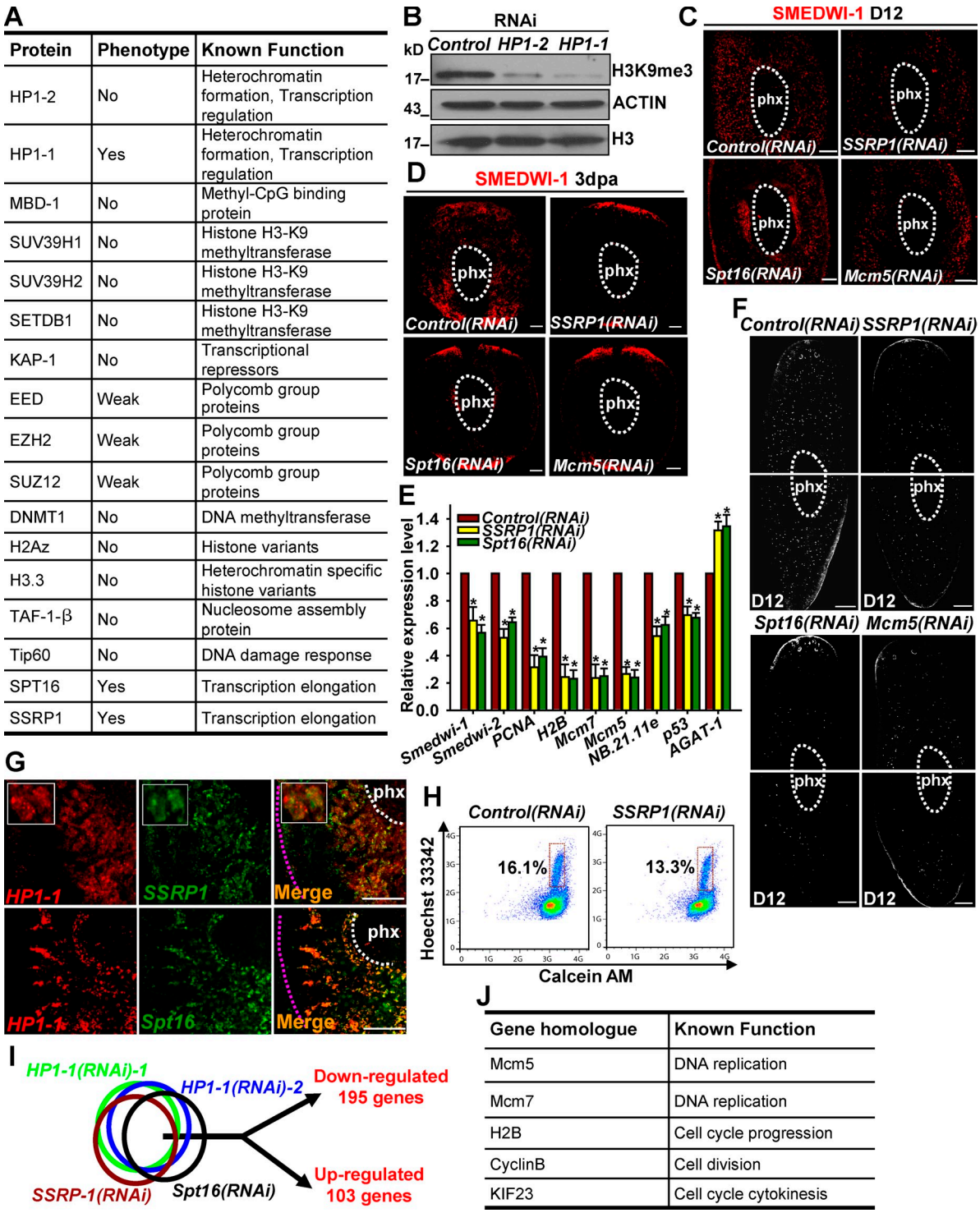
Protein	Phenotype	Known Function
HP1-2	No	Heterochromatin formation, Transcription regulation
HP1-1	Yes	Heterochromatin formation, Transcription regulation
MBD-1	No	Methyl-CpG binding protein
SUV39H1	No	Histone H3-K9 methyltransferase
SUV39H2	No	Histone H3-K9 methyltransferase
SETDB1	No	Histone H3-K9 methyltransferase
KAP-1	No	Transcriptional repressors
EED	Weak	Polycomb group proteins
EZH2	Weak	Polycomb group proteins
SUZ12	Weak	Polycomb group proteins
DNMT1	No	DNA methyltransferase
H2Az	No	Histone variants
H3.3	No	Heterochromatin specific histone variants
TAF-1-β	No	Nucleosome assembly protein
Tip60	No	DNA damage response
SPT16	Yes	Transcription elongation
SSRP1	Yes	Transcription elongation



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Gene homologue	Known Function
Mcm5	DNA replication
Mcm7	DNA replication
H2B	Cell cycle progression
CyclinB	Cell division
KIF23	Cell cycle cytokinesis

Figure S5. **Knockdown of FACT complex genes and *Mcm5* leads to a phenotype resembling that of HP1-1 depletion.** (A) Summary of the RNAi results of the potential HP1-1 binding proteins. Phenotype shows the regeneration defect. RNAi knockdown of components of the FACT complex, *SSRP1* and *Spt16*, abrogates regeneration capacity. (B) Western blotting showing the changes of H3K9me3 levels after depletion of either *HP1-1* or *HP1-2* at 3 dpa. Shown is a representative experiment of three independent biological replicates with similar results. (C and D) IF analysis of SMEDWI-1 in intact (C) and regenerating (D) animals upon knockdown of the indicated genes. 6/6 worms per condition showed similar results. (E) qRT-PCR analysis for expression of lineage markers after knockdown of *SSRP1* or *Spt16*. Error bars show SDs, obtained from three independent RNAi experiments. *, $P < 0.01$. (F) IF analysis of mitotic marker H3S10P in intact animals (D12) upon knockdown of the indicated genes. 8/8 worms per condition showed similar results. (G) Double FISH for *HP1-1* (left) and *SSRP1* or *Spt16* (right) in intact worms. Purple dotted lines represent the margin of the worm body. Insets in the top row show magnified views of cells coexpressing *HP1-1* and *SSRP1*. White dotted lines indicate pharynx (phx) regions. 5/5 worms per condition showed similar results. (H) Representative FACS analyses for assessing the X1 population. Shown is a representative experiment of three independent biological replicates with similar results. Animals were harvested at 3 dpa. (I) Venn diagram representation of differentially expressed genes (≥1.5-fold) in the four RNAi knockdown groups. (J) Five genes exhibiting phenotypes identical to that of HP1-1 knockdown were identified. List of genes that are selected from the array (I) and exhibit regeneration defects upon knockdown. Bars, 0.1 mm.