1 Supplementary Figure Legends

**Supplementary Figure S1.** (A-B) Double fluorescence in situ hybridization showing co-expression of prpf19 (magenta) with marker genes (green) for stem cells (piwi-1, h2b), early epidermal progeny marker (prog-1), and late epidermal progeny marker (agat-1). (C) Western blot analysis using an anti-ubiquitin antibody, which detects all forms of ubiquitin and ubiquitylated proteins. Whole worm homogenate protein extracts were obtained from control(RNAi) or prpf19(RNAi) animals at days indicated. Scale bars in A-B = 20 µm. (D) RT-qPCR experiments measuring the relative expression of epidermal lineage markers, zfp-1, prog-1, agat-1, or vim-1 in control(RNAi) or prpf19(RNAi) planarians following 14 days of RNAi treatment. Graph shows mean ± SD expression levels relative to the controls. *p-value < 0.05, ***p-value < 0.0001, Student’s t-test with Holm-Sidak correction for multiple comparisons.

**Supplementary Figure S2.** WISH to NTC core elements cdc5l, plrg1, spf27, and spliceosomal RNP members prpf3 and prpf8. Scale bars = 200 µm.

**Supplementary Figure S3.** (A) Western blot analysis shows no reduction in H2Aub1 levels following disruption of canonical PRC1 genes ring1, cbx, and phc after 21 days of RNAi treatment. (B) WISH analysis showing expression patterns for PRC1 genes phc and cbx. (C) WISH marker gene analysis for dorsoventral marker bmp4 and anterior-posterior markerndl-3 showed no change in expression domains following phc RNAi. Scale bars = 200 µm.

**Supplementary Figure S4.** (A) Feeding and sampling schedule for RNA-seq experiments. Worms were fed twice a week and sampled for RNA extraction at the days indicated by the red arrowheads. (B) Volcano plot of differentially expressed genes after 14 days of rnf2 RNAi treatment. (C) Venn diagram showing the overlap between day 28 and day 14 sample sets for rnf2(RNAi). (D) GO analysis of down-regulated genes in rnf2(RNAi) worms after 28 days of treatment. (E) GO analysis of up-regulated genes in rnf2(RNAi) worms after 28 days of treatment.
Supplementary Figure S1

A

piwi-1
prpf19
merge

h2b
prpf19
merge

B

prog-1
prpf19
merge

agat-1
prpf19
merge

C

RNAi: control control prpf19 prpf19 control prpf19

day 11 day 14 day 18

D

Relative Gene Expression

control(RNAi) prpf19(RNAi)

control prog-1 agat-1 vim-1

* ***
Supplementary Figure S2
Supplementary Figure S3

A  
day 21

RNAi: control m2 ring1 cbx phc
ub-H2A

B  
phc  cbx

C  
bmp-4  ndl-3

control

phc(RNAi) d28

d28

Supplementary Figure S3
Supplementary Figure S4

A. No. of feeds: 1 2 3 4 5 6 7 8
   Days after first feed: 1 7 14 21 28 28
   Sampling days and genes:
   ![Gene expression graph]

B. ![Scatter plot graph]

C. ![Circle graph]

D. Down-regulated genes GO term
   - cellular catabolic process
   - primary metabolic process
   - organic substance catabolic process
   - organic substance metabolic process
   ![Bar chart]

E. Up-regulated genes GO term
   - response to hypoxia
   - alpha-amino acid catabolic process
   - aromatic amino acid family catabolic process
   - proteasomal ubiquitin-independent protein catabolic process
   - cellular amino acid catabolic process
   - neutrophil degranulation
   - regulation of cellular amino acid metabolic process
   - regulation of transcription from RNA polymerase II promoter in response to stress
   - ATF6-mediated unfolded protein response
   - chaperone cofactor-dependent protein refolding
   - protein-containing complex assembly
   - protein folding in endoplasmic reticulum
   - antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent
   - cellular response to decreased oxygen levels
   - glycosyl compound catabolic process
   - protein refolding
   - cellular biosynthetic process
   - regulation of stem cell differentiation
   ![Bar chart]