

**Developmental Cell, Volume 46**

**Supplemental Information**

**FACT Sets a Barrier for Cell Fate Reprogramming  
in *Caenorhabditis elegans* and Human Cells**

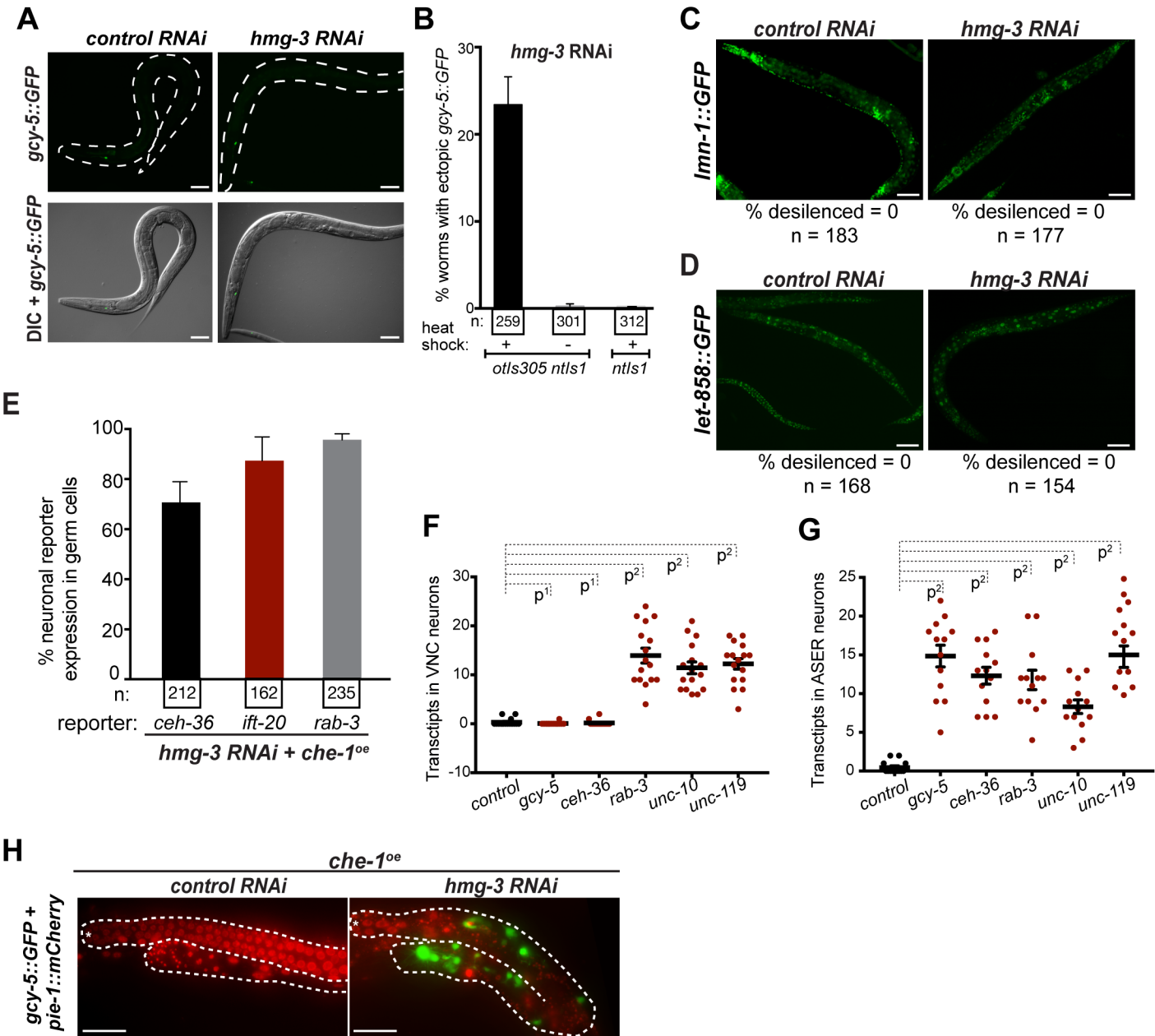
**Ena Kolundzic, Andreas Ofenbauer, Selman I. Bulut, Bora Uyar, Gülkiz Baytek, Anne Sommermeier, Stefanie Seelk, Mei He, Antje Hirsekorn, Dubravka Vucicevic, Altuna Akalin, Sebastian Diecke, Scott A. Lacadie, and Baris Tursun**

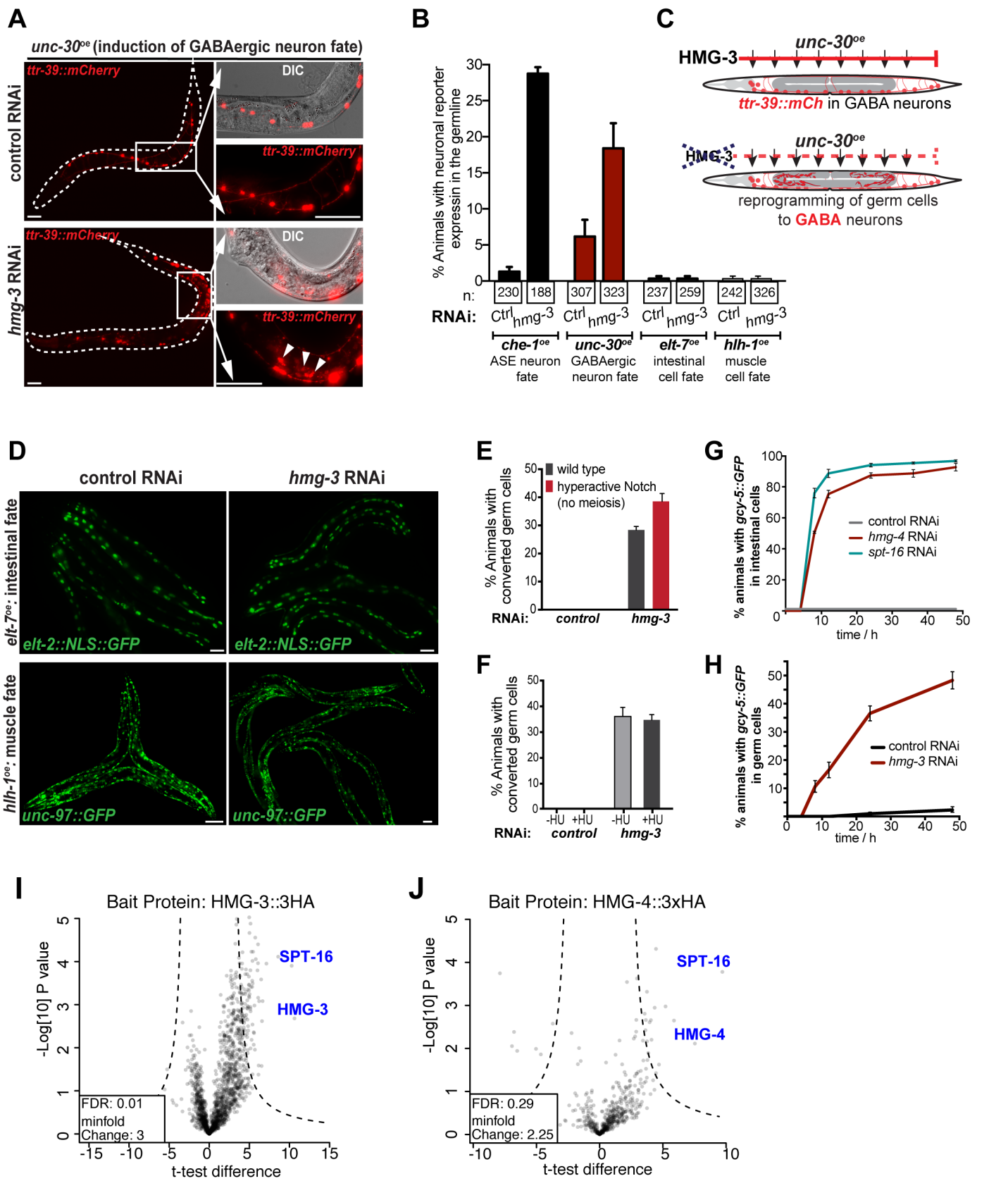
## **Supplemental Items**

Supplemental Figures S1 – S6 and figure legends

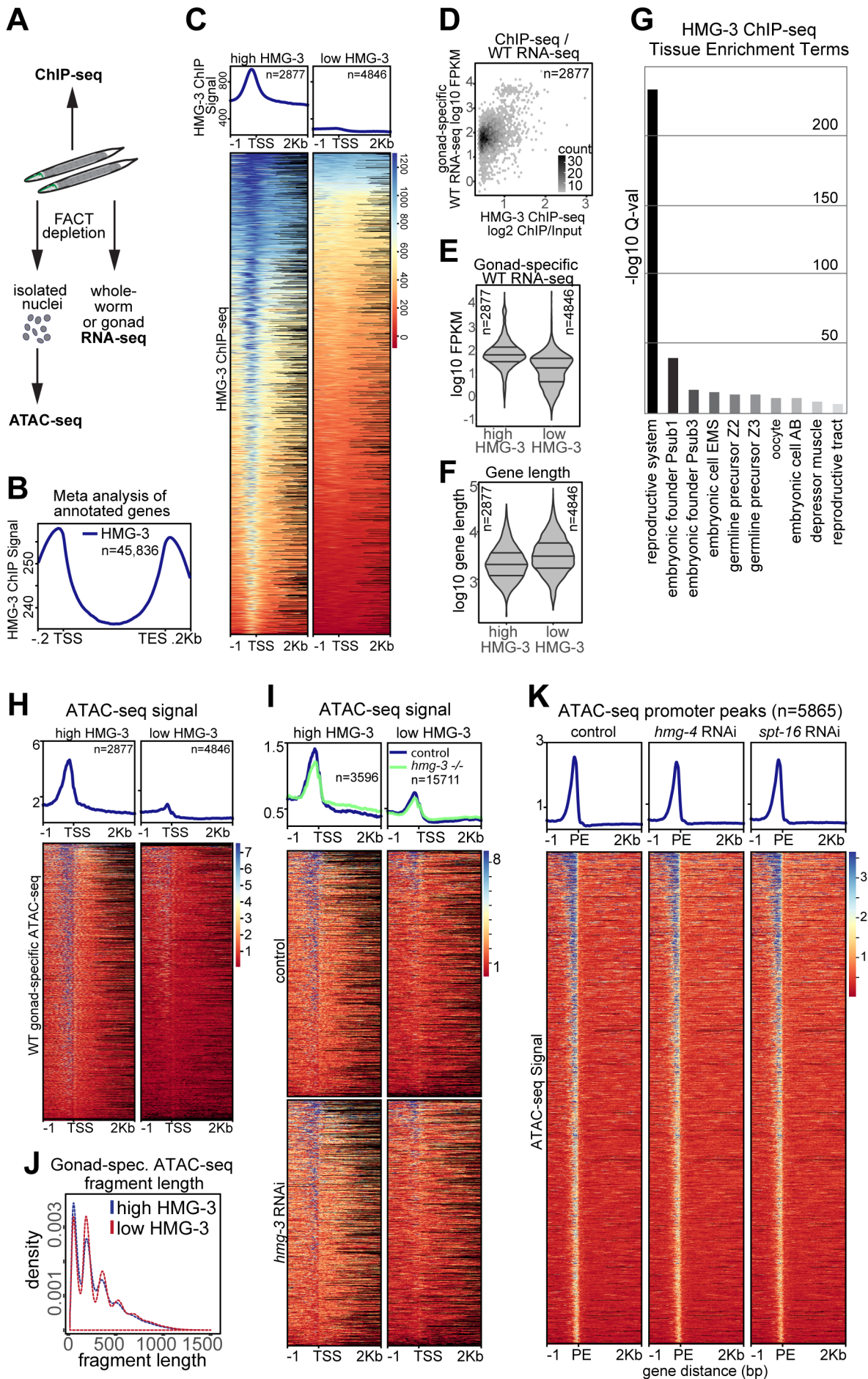
Supplemental Tables S6 – S8 and description of all tables

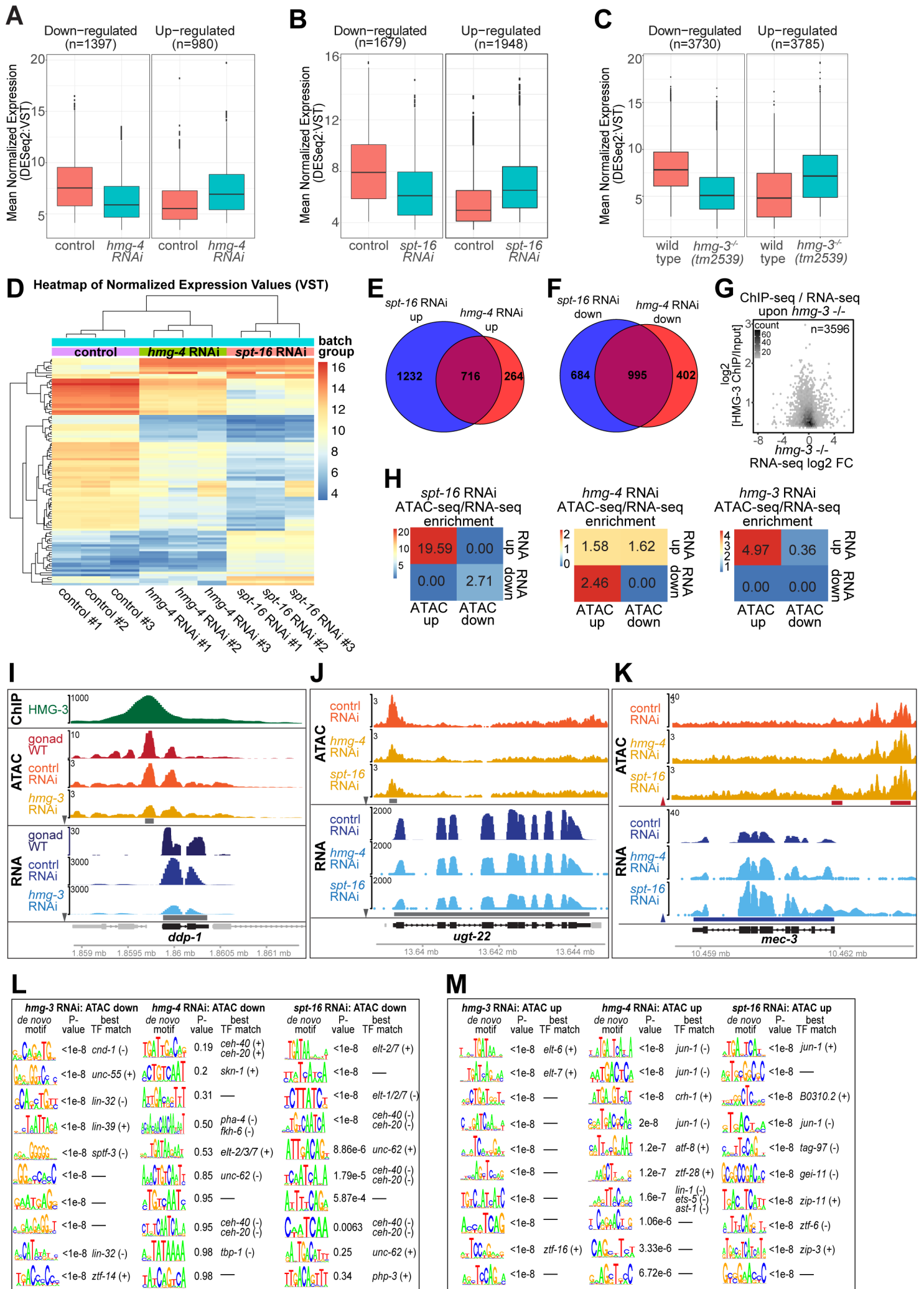
Supplemental Tables S1 - S5 can be downloaded as Excel files

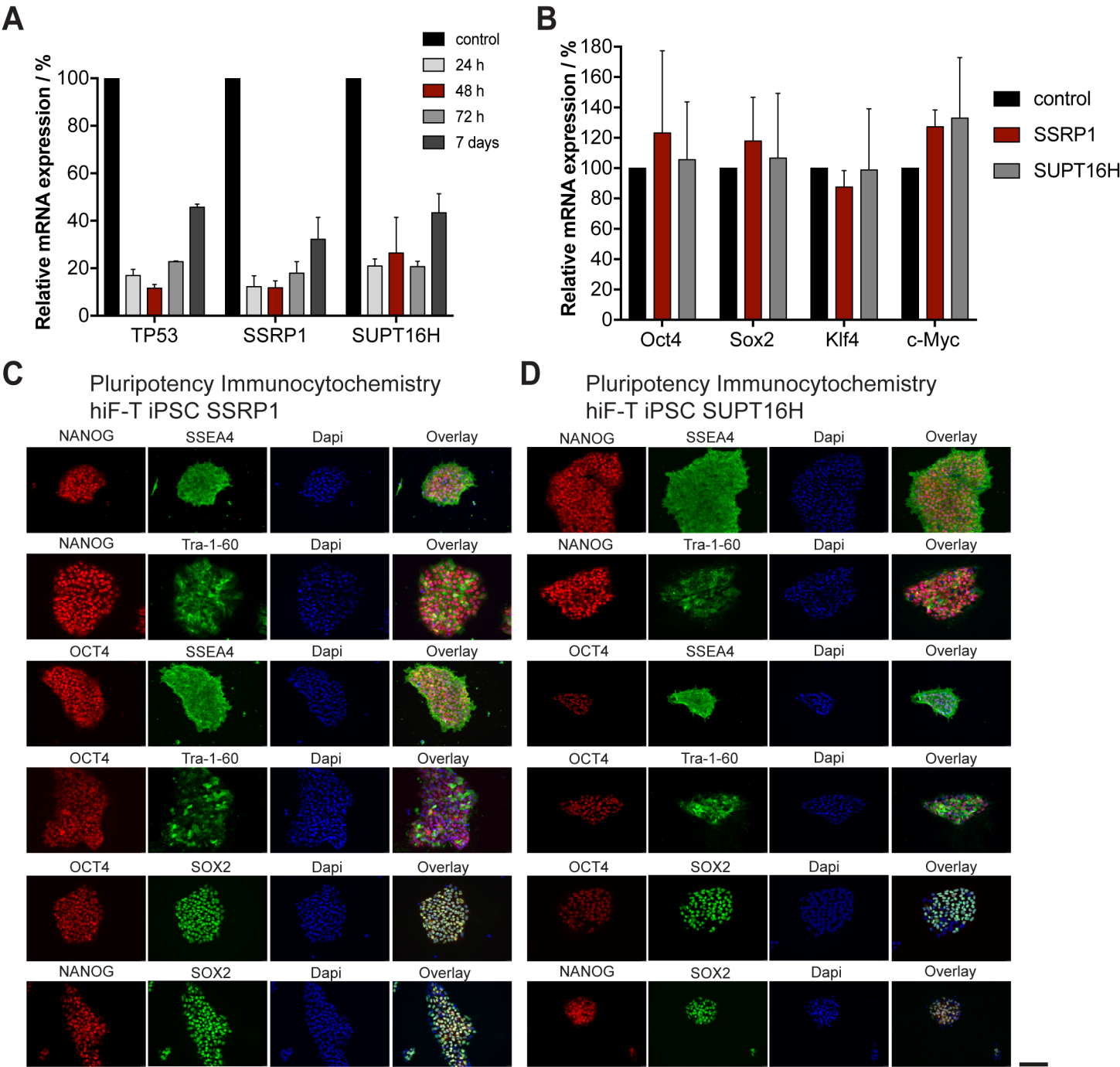




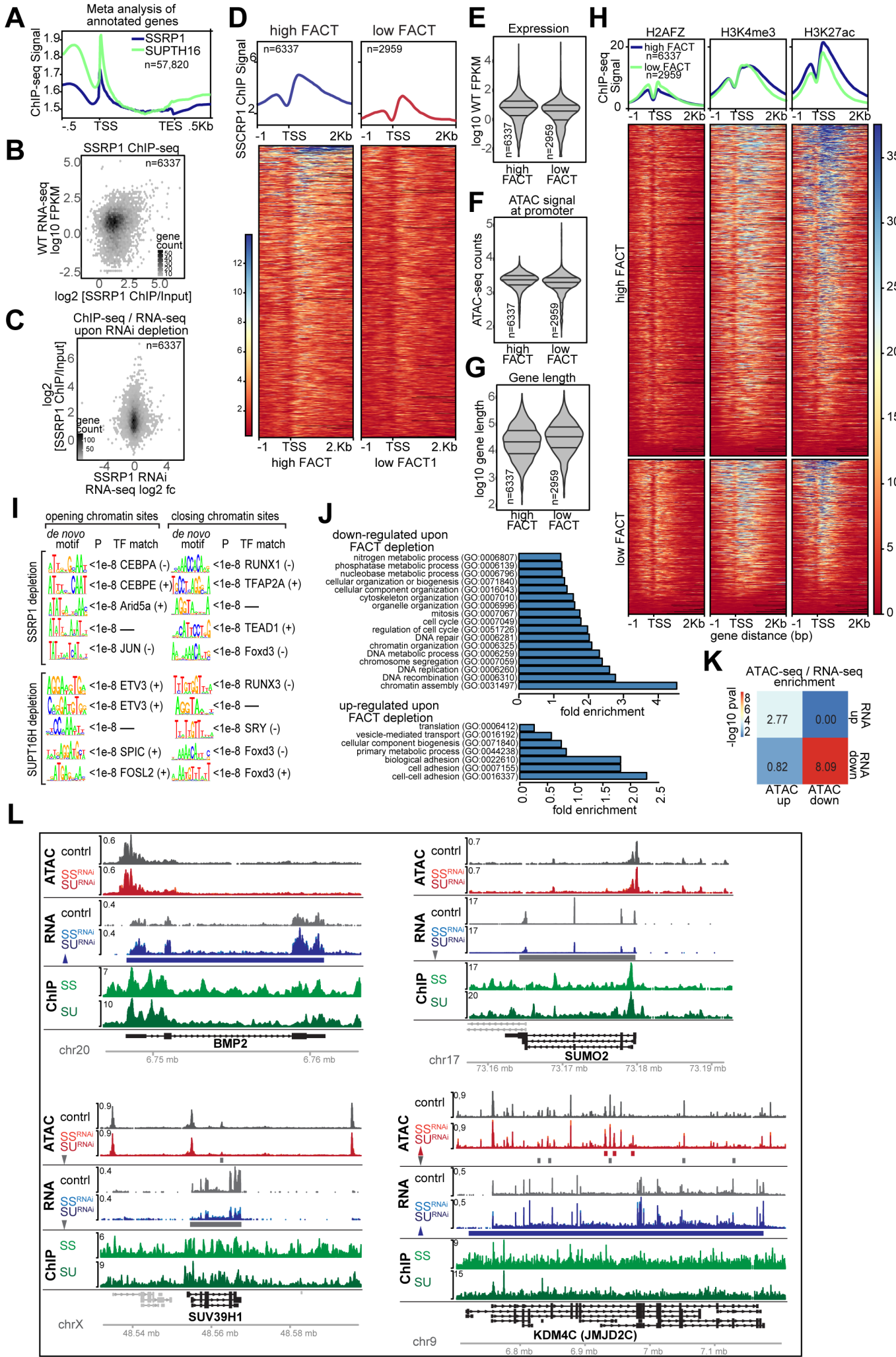












## Supplemental Figure Legends:

### Figure S1. Assessment of Ectopic *gcy-5::GFP* Induction in Germ Cells Upon RNAi Against *hmg-3*, Related to Figure 2

(A) Representative images of *hmg-3<sup>RNAi</sup>* animals with and without *che-1<sup>oe</sup>* (no *hs::che-1* construct in the background), or with only *gcy-5::GFP* (*ntl-1*) construct in the background. *gcy-5::GFP* is not induced upon *hmg-3* RNAi without *che-1<sup>oe</sup>*. Scale bars, 20  $\mu$ m.

(B) Quantification of *gcy-5::GFP* induction in *hmg-3<sup>RNAi</sup>* with and without *che-1<sup>oe</sup>* (no *hs::che-1* construct in the background) or with only *gcy-5::GFP* (*ntl-1*) construct in the background. Error bars represent SEM.

(C and D) Representative images of (D) *lmn-1::GFP* and (E) *let-858::GFP* animals after *hmg-3* RNAi. Neither expression of *lmn-1::GFP* nor expression of *let-858::GFP* changes upon FACT depletion. Scale bars, 20  $\mu$ m.

(E) Quantification of neuronal markers *ceh-36::RFP*, *ift-20::NLS::RFP* and *rab-3::NLS::RFP* in *gcy-5::GFP* positive germline of *hmg-3<sup>RNAi</sup>* animals. Error bars represent SEM.

(F and G) Control quantification of smFISH based on counts of hybridization signals (red dots) in (F) ventral nerve cord (VNC) and (G) ASER neurons. For each condition, more than 15 cells were counted for smFISH-derived transcript detection based on fluorescence signals. Ordinary one-way ANOVA was used for statistical analysis.  $p^1$  not significant,  $p^2 < 0.0001$ .

(H) The germ cell fate marker *pie-1::mCherry::his-58* is lost in reprogrammed germ cells. Dashed lines indicated the outline of the gonad, Asterisk labels distal tip of the germline. Scale bars, 5 mm.

### Figure S2. Depletion of HMG-3 Allows Germ Cell Reprogramming to GABA Neuron-Fate and Mass Spectrometry Analysis of HMG-3 / HMG4 Immunoprecipitations, Related to Figures 2 and 3

(A) Representative images for induction of the GABA fate reporter *ttr-39::mCherry* in the germ line of *hmg-3<sup>RNAi</sup>* animals upon overexpression of GABA neuron-fate inducing TF UNC-30 (*unc-30<sup>oe</sup>*). Dashed lines indicated the outline of the worm, boxes indicate the magnification area and arrows in the zoom indicate reprogrammed germ cells. Scale bars, 20 mm, and 5 mm in magnifications.

(B) Quantification of germ cell conversion upon induction of different fate-inducing TFs. Neuronal fate induction can be detected in 20-30% animals after *che-1<sup>oe</sup>* (ASE neuron) or *unc-30<sup>oe</sup>* (GABA neuron), but no intestinal or muscle fate induction by over-expressed ELT-7 (*elt-7<sup>oe</sup>*) or HLH-1 (*hlh-1<sup>oe</sup>*), respectively. Number of animals counted for each condition are indicated as n. Error bars represent SEM.

(C) Schematic representation of transgenic animals for GABA neuron fate induced by *unc-30<sup>oe</sup>*. *unc-30<sup>oe</sup>* in adults after *hmg-3* RNAi induces reprogramming of germ cell to GABA neurons.

(D) Representative pictures showing that overexpression of intestinal (ELT-7) or muscle (HLH-1) fate-inducing TFs do not convert germ cells into gut or muscle cells in *hmg-3<sup>RNAi</sup>* animals. Scale bars, 20  $\mu$ m.

(E) Germ cell conversion is not lost in the *glp-1(gf)* mutant background (hyperactive Notch) which lacks meiotic germ cells, but retains mitotic germ cells. More than 150 animals were counted for each condition. Error bars represent SEM.

(F) Reprogramming is independent of cell cycle. Phenotype penetrance remained unchanged after HU-mediated cell cycle arrest (-HU, no treatment, +HU, 6 hr HU treatment). More than 150 animals were counted. Error bars represent SEM.

(G) Time course experiment *gcy-5::GFP* induction in *hmg-4<sup>RNAi</sup>* and *spt-16<sup>RNAi</sup>* animals. Time in h after induction of *che-1<sup>oe</sup>*. More than 150 animals were counted for each time point. Error bars represent SEM.

(H) Time course experiment of *gcy-5::GFP* induction in *hmg-3<sup>RNAi</sup>* animals. Time in h after induction of *che-1<sup>oe</sup>*. More than 150 animals were counted for each time point.

(I and J) In order to assess HMG-3 and HMG-4 protein interactions, coimmunoprecipitations with subsequent mass spectrometry (IP-MS) were performed using protein lysates of HMG-3::HA and HMG-4::HA CRISPR tagged animals. Volcano plots show statistically significant enrichment of co-precipitated proteins by (I) HMG-3::HA IP and (J) HMG-4::HA IP. SPT-16 is the most significant co-precipitated protein for both HMG-3 and HMG-4, indicating that both proteins associate predominantly with SPT-16. All IP-MS measurements and statistical values are provided in Table S2.

**Figure S3. Analysis of HMG-3 ChIP-seq, ATAC-seq, and RNA-seq, Related to Figure 4.**

(A) Schematic illustration of ChIP-seq, RNA-seq, and ATAC-seq using isolated nuclei of animals treated with RNAi against FACT subunits without induction.

(B) Meta-analysis of library-normalized, input-subtracted HMG-3 ChIP-seq signal at annotated genes where gene bodies have been scaled to a relative length.

(C) Average positional profiles and heat maps of HMG-3 ChIP-seq signal for gene TSS windows classified as HMG-3 high or HMG-3 low (see Methods) and detected in isolated gonad RNA-seq. Signal is library normalized and input subtracted (see Methods).

(D) Log2 ratio of HMG-3 ChIP/Input plotted against FPKM expression values from isolated gonad RNA-seq for genes classified as HMG-3 high. Density scatter plot scale shows number of genes plotted per hexbin.

(E) Distribution violin plots of detected gene FPKM values from isolated gonad RNA-seq for genes with high or low HMG-3.

(F) Log10 gene length for genes determined as HMG-3 high or HMG-3 low and detected in gonad-isolated RNA-seq.

(G) Tissue enrichment analysis of genes identified as HMG-3 ChIP-seq positive using wormbase.org provided analysis (Angeles-Albores et al., 2016).

(H and I) Average positional profiles and heat maps for library-normalized ATAC-seq signal from (H) isolated gonads and (I) whole worms following FACT knockdown for genes classified as HMG-3 high or HMG-3 low and detected in RNA-seq.

(J) Density distributions of isolated gonad ATAC-seq fragment lengths intersecting TSS windows for genes classified as HMG-3 high or HMG-3 low and detected in RNA-seq.

(K) Average positional profiles and heat maps of library-normalized whole worm ATAC-seq signal with and without FACT knockdown. Plots are anchored on downstream edges of promoter-annotating ATAC-seq peaks (see Methods).

**Figure S4. RNA-seq Analysis and *de novo* Motif Generation from Worm ATAC-seq, Related to Figure 4.**

(A, B, and C) Expression changes in *C. elegans* with RNAi against (A) *hmg-4*, (B) *spt-16*, or (C) containing a mutation in *hmg-3*. Up/down regulated genes are detected based on the differential expression criteria of adjusted p-value of at least 0.1 and at least two-fold increase or decrease in expression levels in relation to the control samples. Transcription levels of these up- and down regulated genes are represented as boxplots.

(D) A heatmap of unsupervised hierarchical clustering of the top 100 genes with most variant gene expression across control, *hmg-4* and *spt-16* RNAi samples shows that *hmg-4*<sup>RNAi</sup> and *spt-16*<sup>RNAi</sup> are more similar to each other than the control. Independently generated biological replicates clustered together (individual samples and batches are indicated).

(E and F) Venn-diagram showing overlap of (E) up-regulated and (F) down-regulated genes (numbers given) in *hmg-4*<sup>RNAi</sup> and *spt-16*<sup>RNAi</sup> animals.

(G) Log2 fold-changes in expression levels from whole worm RNA-seq after *hmg-3*<sup>RNAi</sup> plotted against log2 ratio of HMG-3 ChIP/Input for genes classified as HMG-3 high and detected in the RNA-seq. Density scatter plot scale shows number of genes plotted per hexbin.

(H) –Log10 enrichment pvalues of genes assigned only up-regulated or only down-regulated ATAC-seq peaks that intersected genes detected as up- or down-regulated in differential RNA-seq analysis for *spt-16* (left), *hmg-4* (middle), and *hmg-3* (right) depletion experiments.

(I-K) Browser shots of library-normalized, input-subtracted HMG-3 ChIP-seq signal, library-normalized ATAC-seq from isolated gonads, library-normalized ATAC-seq signal from whole worms with and without RNAi against *hmg-3*, *hmg-4*, or *spt-16*, and library-normalized RNA-seq signal with and without RNAi against *hmg-3*, *hmg-4*, or *spt-16*. Genes and ATAC-seq peaks called as differentially regulated upon FACT knockdown are shown below the respective signal tracks.

(L and M) *De novo* motif generation (see Methods) in closing (L) or opening (M) regions upon *hmg-3*<sup>RNAi</sup>, *hmg-4*<sup>RNAi</sup> and *spt-16*<sup>RNAi</sup>. Top 10 enriched motifs for each indicated set together with the p value and the best TF match are given. The orientation of the generated motif relative to the best TF match in the database is indicated in parentheses.

**Figure S5. FACT siRNA Knockdown efficiency and Pluripotency Marker Expression in FACT-Depletion Derived iPSCs, Related to Figure 5**

(A) Quantitative RT-PCR (qRT-PCR) analysis to confirm knock-down of SSRP1, SUPT16H and TP53 at 24 h, 48 h, 72 h and 7 days after transfection with siRNAs.

(B) qRT-PCR analysis for expression of levels of Oct4, Sox2, Klf4, and c-Myc 48 hours after SSRP1 or SUPT16H depletion. Gene expression levels were normalized to GAPDH expression levels and compared to control siRNA. Error bars represent SD.

(C and D) Representative images of antibody staining for NANOG, OCT4, SOX2 SSEA-4 and Tra-1-60 in iPSC colonies derived from SSRP1 (C) or SUPT16H (D) depleted hiF-T cells. Scale bars, 25  $\mu$ m.

**Figure S6. FACT ChIP-seq, RNA-seq, and ATAC-seq Analysis, Related to Figure 6.**

(A) Meta-analysis of library- and input-normalized SSRP1 and SUPT16H ChIP-seq signal at annotated genes. Signal in gene bodies is scaled to relative lengths.

(B) Log2 ratio of SSRP1 ChIP/Input plotted against FPKM expression values from control RNA-seq for genes classified as FACT high and detected in the RNA-seq. Density scatter plot scale shows number of genes plotted per hexbin.

(C) Log2 fold-changes in expression levels from RNA-seq after SSRP1 knockdown plotted against log2 ratio of SSRP1 ChIP/Input for genes classified as FACT high and detected in the RNA-seq. Density scatter plot scale shows number of genes plotted per hexbin.

(D) Average positional profiles and heat maps of library- and input-normalized SSRP1 ChIP-seq signal for genes whose TSS windows classified as FACT high or FACT low (see Methods).

(E-G) Violin distribution plots of wild-type RNA-seq FPKM expression values, E, ATAC-seq counts in promoter windows, F, and log10 gene lengths, G, for genes classified as FACT high or FACT low and detected in the RNA-seq.

(H) Average positional profiles and heat maps of H2AFZ, H3K4me3, and H3K27ac ChIP-seq signal from ENCODE human lung fibroblasts datasets for genes classified as FACT high or FACT low and detected in the RNA-seq.

(I) *De novo* motif generation results (Methods) in closing and opening regions upon SSRP1<sup>RNAi</sup> and SUPT16H<sup>RNAi</sup>. Top 5 enriched motifs for each indicated set together with the p value and the best TF match are given. The orientation of the generated motif relative to the best TF match in the database is indicated in parentheses.

(J) Observed/Expected Go analysis enrichments using PANTHER on differentially expressed genes called as up-regulated or down-regulated in either SSRP1 or SUPT16H knockdown compared to all genes detected in the RNA-seq analysis.

(K) -Log10 enrichment pvalues of genes assigned only up-regulated or only down-regulated ATAC-seq peaks following SSRP1 or SUPT16H knockdown that intersect genes called as differentially expressed in RNA-seq after SSRP1 or SUPT16H knockdown.

(L) Browser shots as described in Figure 7M for BMP2, SUMO2, SUV39H1, and KDM4C genes.



**Supplemental Table S6. Summary of FACT depletion effects based on RNA-seq and ATAC-seq in *C. elegans* and Human Cells, Related to Figures 4, S3, S4, 6, and S6**

WORM:

RNA-seq

299/3596 genes bound strongly by hmg-3 ('HMG-3 high genes') are UP in hmg-3 RNAi  
540/3596 genes bound strongly by hmg-3 ('HMG-3 high genes') are DOWN in hmg-3 RNAi  
3593/15711 genes bound moderately by hmg-3 ('HMG-3 low genes') are UP in hmg-3 RNAi  
3136/15711 genes bound moderately by hmg-3 ('HMG-3 low genes') are DOWN in hmg-3 RNAi

ATAC

592/3596 genes bound strongly by hmg-3 ('HMG-3 high') have assigned UP peaks in hmg-3 RNAi  
369/3596 genes bound strongly by hmg-3 ('HMG-3 high') have assigned DOWN peaks in hmg-3 RNAi  
2021/15711 genes bound moderately by hmg-3 ('HMG-3 low') have assigned UP peaks in hmg-3 RNAi  
2006/15711 genes bound moderately by hmg-3 ('HMG-3 low') have assigned DOWN peaks in hmg-3 RNAi

HUMAN:

RNA

758/6337 genes bound strongly by FACT ('FACT high') are UP in either SSRP1 or SUPT16H RNAi  
884/6337 genes bound strongly by FACT ('FACT high') are DOWN in either SSRP1 or SUPT16H RNAi  
453/2959 genes bound moderately by FACT ('low') are DOWN in either SSRP1 or SUPT16H RNAi  
324/2959 genes bound moderately by FACT ('low') are UP in either SSRP1 or SUPT16H RNAi

ATAC

1072/6337 'FACT high' genes have assigned UP peaks in either SSRP1 or SUPT16H RNAi  
1089/6337 FACT high genes have assigned DOWN peaks in either SSRP1 or SUPT16H RNAi  
539/2959 FACT low genes have assigned UP peaks in either SSRP1 or SUPT16H RNAi  
544/2959 FACT low genes have assigned DOWN peaks in either SSRP1 or SUPT16H RNAi

**Table S7. *C. elegans* strains used in the study, Related to STAR Methods**

Name	Genotype
BAT012	<i>barIs12[elt-2prom::gfp; myo-3p::NmBirAo]</i>
BAT026	<i>otIs284 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.; hdlIs30 [glr-1::dsRED]</i>
BAT028/OH9846	<i>otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.</i>
BAT032	<i>glp-1(ar202) III.; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.</i>
BAT044	<i>julS244 [ttr-39prom::mCherry; ttx-3prom::gfp]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.</i>
BAT046	<i>otIs133 [ttx-3prom::mCherry]; otIs284 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.; hdlIs30 [glr-1::dsRED]</i>
BAT068	<i>otEX4945 [hs:hlh-1, rol-6(su1006)]; mglS25 [unc-97prom::gfp]</i>
BAT109	<i>otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)] V.</i>
BAT139	<i>stIs10086 [ges-1::H1-Wcherry + unc-119(+)]</i>
BAT160	<i>itIs37 [pie-1p::mCherry::his-58(pAA64), unc-119(+)]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)] ntlIs1 [gcy-5p::GFP, lin-15(+)] V.</i>
BAT282	<i>barIs40 [vit-5::2xNLS::TagRFP]</i>
BAT284	<i>stIs10131 [elt-7::H1-wCherry + unc-119(+)]</i>
BAT287	<i>ntlIs1 [gcy-5::gfp; lin-15b(+)] V.</i>
BAT326	<i>otIs263 [ceh-36prom::tagRFP]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.</i>
BAT453	<i>barEX147 [hsp-16.4prom::unc-30; hsp-16.2prom::unc-30; rol-6(su1006)]; julS244 [ttr-39prom::mCherry, ttx-3prom::gfp]</i>
BAT522	<i>otIs393 [ift-20prom::NLS::tagRFP]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.</i>
BAT525	<i>hmg-3 (tm2539) / dpy-5(e61) unc-13(e1091) I.</i>
BAT527	<i>otIs355 [rab-3prom::NLS::TagRFP]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntlIs1 [gcy-5::gfp; lin-15b(+)] V.</i>
BAT606	<i>edIs6 [unc-119::gfp + pRF4[rol-6(su1006)]] IV.; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)] V.</i>
BAT1560	<i>hmg-3(bar24[hmg-3::3xHA]) I. protein tag CRISPR engineered</i>
BAT1753	<i>hmg-3(bar24[hmg-3::3xHA]) I. 2x outcrossed</i>
BAT1945	<i>jun-1(gk557) II; otIs305[hsp::che-1::3xHA, rol-6] ntlIs1[gcy-5::GFP]</i>
BAT1967	<i>hmg-4(bar32[hmg-4::3xHA]) III</i>
JR3373	<i>wIs125[hsp-16-2::elt-7 hsp-16-41::elt-7]; rrls1 [elt-2::GFP + unc-119(+)]</i>
LW697	<i>cclIs4810/pJKL380.4; lmn-1p::lmn-1::GFP::lmn-1 3'utr + pMH86; dpy-20(+)] I.</i>
NL2507	<i>pkIs1582[let-858::GFP + rol-6(su1006)]</i>
SS104	<i>glp-4(bn2) I.</i>
OD56	<i>itIs 37[pie-1p::mCherry::his-58 unc-119(+)];unc-119(ed3) III.</i>

Supplemental Table S8. Sequences of smFISH Oligos, PCR primers, and siRNAs, Related to Star Methods

7A. smFISH probes		
Probe set	Sequences	Fluorophore
<i>gcy-5_set1</i>	caticggatgctccaagaac	Fluor Red 610
	caattccaactcgaagcgc	
	caattggaagattccacca	
	tatcgcatcggatatatcc	
	tcccactacaacatctacat	
	tattggatcatcgccaactgg	
	tgcactcgatcaaatggga	
	tttacagtatcttggtcgt	
	cttaaggttgctccaacatc	
	atccgactggatatagatc	
	cgatcttgaatgectcat	
	tacgagctcgactctttaca	
	ggaccactaatggcataa	
	ccaatactcctcattgcaa	
	tcttcccaactgtttgt	
	tggagttagtcatttgcaa	
	ctactgtgaatgactccaa	
	atttctaaacgactccgaa	
	tggcatccgataaagtaa	
	tagtaaccatttgcggcata	
	gcgctagagatttgaccaa	
	tcatgttaactagtgcacat	
	ccgtgacaattgcgaagacg	
	cgttttctttttgtggcat	
	gtgatctcgactatttggc	
	actttcccggttatagttg	
	gctatgatgtttggtgtta	
	atttctcctctctcttta	
	ggtcacatgataataacc	
	gatactcgaatgatcttc	
	aaagttcataccctgcga	
	ggcaagtagctgaacgtaga	
	ctcccaatccaatctgtt	
	tacgatttctcctttttc	
	aagtattactccggtcgga	
	acttgcaaatgtctcagct	
	gttctgcaactgttttga	
	tctccaattgattccactt	
	tgtcggtaaccgaaacac	
	ggaaccttgaaagctcttaca	
	gcccactattaattccaatt	
	atggatagaccaacgacacc	
	gtatccccaataggcaata	
	tttccattcttccattct	
	tgtcagctctcgacatag	
	tctcctcttgaactgtttc	
	tgtttccattacacctttc	
	gattttgtgctactgacgt	
	gtgtagaagttgtgtgcat	
	ggataagcaggtatagccgag	
	tgcggcgcaaatgcaaat	
	atgtaagactgggtgccgtg	
	cattgtcgttgacctgtgg	
	ttcactgtttggagccattg	
	ctctgttgaacgaggtacgt	
	tttccagctgatcgagtg	
	gatactgtgtttcgcgaaaa	
	gctctcttctgtgcacatc	
	caaatgattgcttgcgca	
	ttactgttacccttccatca	
	cgatttttgaaccaaacgt	
	gtttgttctatccttggctc	
	gatggactccatcattttt	
	gatcttgatgaagtgcttcc	
	cgttgtgtggagaaccattg	
	gtgatttagtatcaggcttt	
	tgtgcctggtatgtgaattc	
	cactgtgtgcatgaattcc	
	gagtttgctcatatttggc	
	ttgcagttgactcaagactg	
	agtccctccagttcacitttt	
	atttgatctgcaagtgtgt	
	cttgagcctgaggaagaagt	
	agttgcgtagatgcataag	
	tagttgtacgggtaaggagc	
	gtttgatgggaagttagctgt	
	tgttccataatgttgtag	
	agcgagtaataattgggggtg	

7B. Oligonucleotides used for qRT-PCR	
Name	Sequence
SSRP1 fwd	TTTGCCCGAATGTGTGTGTC
SSRP1 rev	AGTCAAAGGCTTGCCATGC
SUPT16H fwd	GCAGAAAGAGAAGCGAAGAGC
SUPT16H rev	TTTCCCGAATAIGTGGTTCC
TP53 fwd	TCAACAAGATGTTTGCCAACTG
TP53 rev	ATGTGCTGTGACTGCTTGTAGA TG
GAPDH fwd	TGCACCACCAACTGCTTAGC
GAPDH rev	GGCATGGACTGTGGTCATGAG
POU5F1 fwd	GAGAACCGAGTGAGAGGCAAC C
POU5F1 rev	CATAGTCGCTGCTTGATCGCTT G
SOX2 fwd	GCCGAGTGGAACTTTTGTGCG
SOX2 rev	GCAGCGTGTAATTATCCTTCTT
KLF4 fwd	ACCAGGCACTACCGTAAACAC A
KLF4 rev	GGTCCGACCTGGAAAATGCT
MYC fwd	CGTCTCCACATCAGCACAA
MYC rev	CACTGTCCAACCTGACCCTCTT G

7C. List of siRNAs and their sequences			
Targeted gene	siRNA pool ID	siRNA Name	Sequence
mock	D-001210-01-05	siGENOME N	UAGCGACUAAACACAUCAA
RLuc	P-002070-01-20	RLuc Duplex	AAACAUGCAGAAAUGCUG
TP53	MU-003329-03-0002	D-003329-05	GAGGUUGGUCUCUGACUGUA
		D-003329-07	GCACAGAGGAAGAGAAUCU
		D-003329-08	GAAGAAACCACUGGAUGGA
		D-003329-26	GCUUCGAGAUUGUCCGAGA
SSRP1	MU-011783-01-0002	D-011783-01	GAUGAGAUCCUUUGUCA
		D-011783-03	GACUUAACUGCUUACAAA
		D-011783-04	GCAAGACCUUUGACUACAA
		D-011783-17	GAGGGAGGAGUACGGGAAA
SUPT16H	MU-009517-00-0002	D-009517-01	GAAGAUUUGUGACGUGUAU
		D-009517-02	GAACAAAGUCGAAGUAUGA
		D-009517-03	AGACAUGGUCUUUGGUUA
		D-009517-04	GAAGAACUUUGUAUUGGUA

*ceh-36\_set1*

Fluor Red 610

rab-3_set1	caaagttctgatcggttgt	Fluor Red 610
	atcaggagcttgaacatgta	
	tccaactgatgaattccga	
	catcacagtaacggaagagg	
	gttagagacgaaggcagaagt	
	cactttgaaatcgattccga	
	tttgtctccacggaacacag	
	ggfatcccagatttgaagt	
	gatatagaggcgggtgtag	
	cagaatgaatcccatgtctc	
	actctcattatgtatgta	
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## **Supplemental Tables:**

**Table S1.** Whole-Genome RNAi screening results, Related to Figure 1  
(provided as Excel file)

**Table S2.** Results of HMG-3 and HMG-4 Co-Immunoprecipitation with subsequent Mass Spectrometry analysis, Related to Figure S2 and Mass Spectrometry Analysis as described in Star Methods  
(provided as Excel file)

**Table S3.** ChIP-seq, ATAC-seq and RNA-seq analysis of *hmg-3*, *hmg-4*, and *spt-16* , Related to Figures 4, S3 and S4  
(provided as Excel file)

**Table S4:** ChIP-seq for SSRP1 and SUPT16H with RNA-seq analysis of SSRP1 and SUPT16H knockdown, Related to Figures 6 and S6  
(provided as Excel file)

**Table S5.** ATAC-seq analysis of SSRP1 and SUPT16H knockdown, Related to Figures 6 and S6  
(provided as Excel file)

**Table S6.** Summary of FACT depletion effects based on RNA-seq and ATAC-seq in *C. elegans* and Human Cells, Related to Figures 4, S3, S4, 6, and S6

**Table S7.** *C. elegans* strains used in the study, Related to STAR Methods

**Table S8.** Sequences of smFISH Oligos, PCR primers, and siRNAs, Related to Star Methods