Developmental Cell, Volume *46*

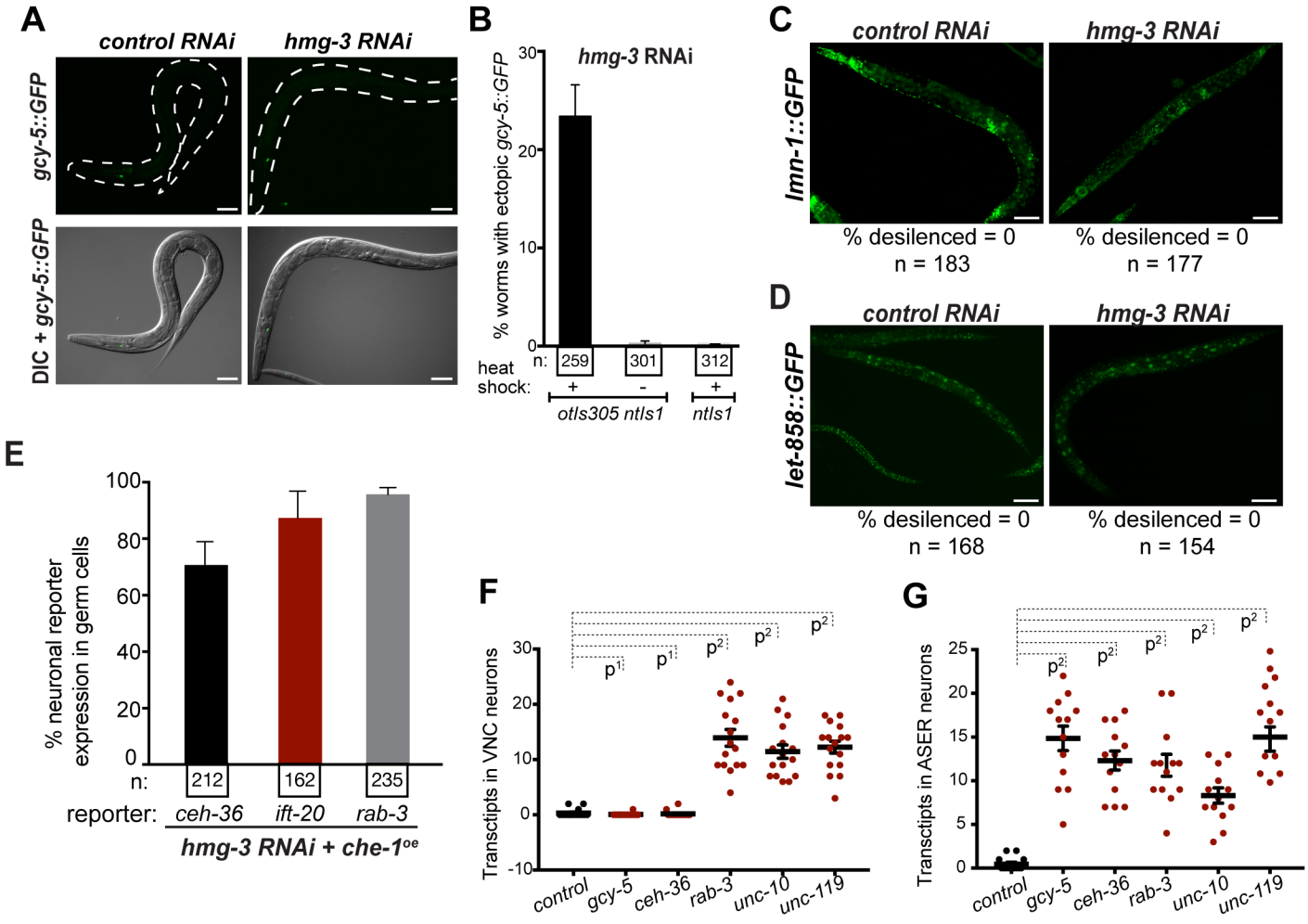
Supplemental Information

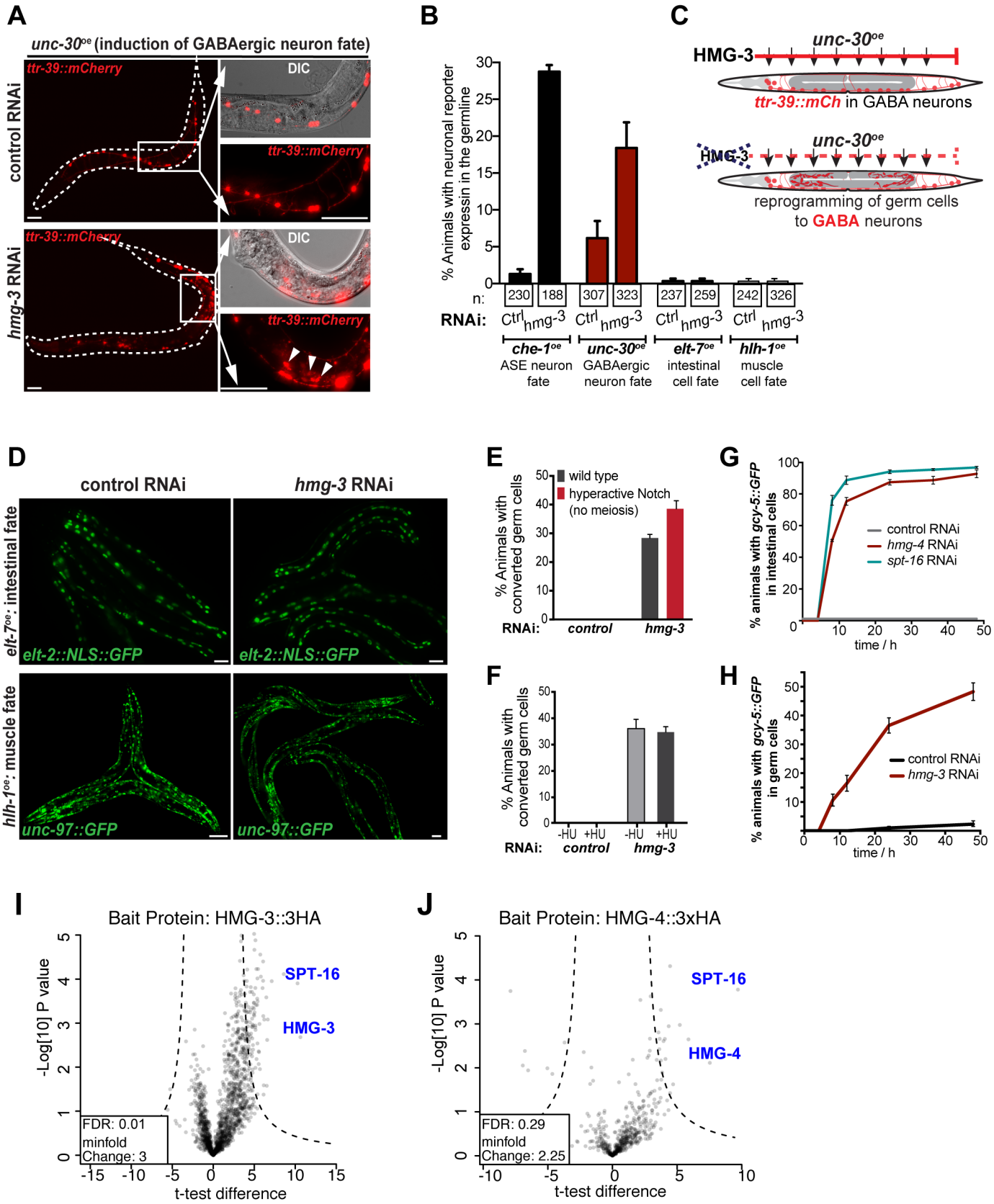
FACT Sets a Barrier for Cell Fate Reprogramming in *Caenorhabditis elegans* and Human Cells

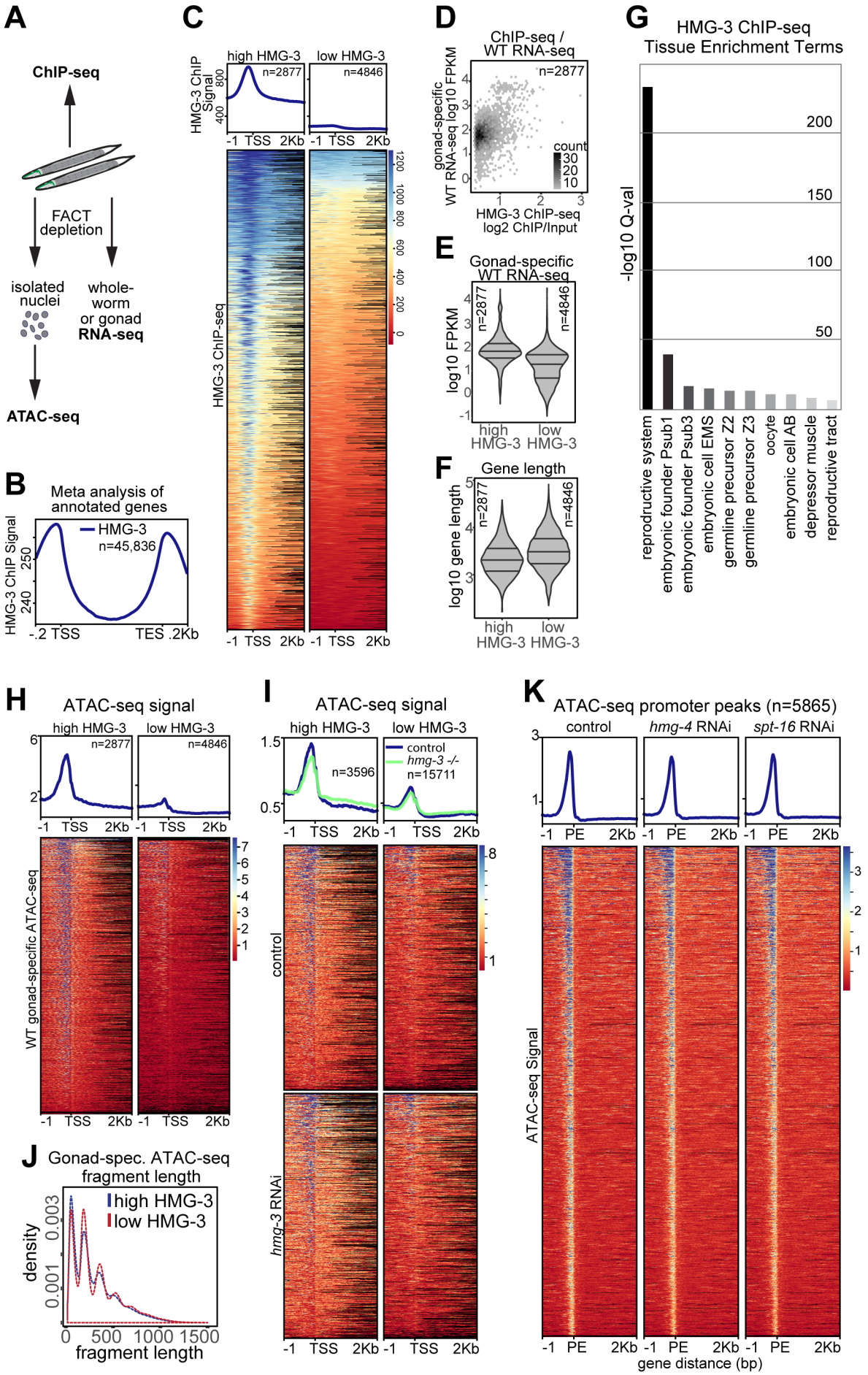
Ena Kolundzic, Andreas Ofenbauer, Selman I. Bulut, Bora Uyar, Gülkiz Baytek, Anne Sommermeier, Stefanie Seelk, Mei He, Antje Hirsekorn, Dubravka Vucicevic, Altuna Akalin, Sebastian Diecke, Scott A. Lacadie, and Baris Tursun

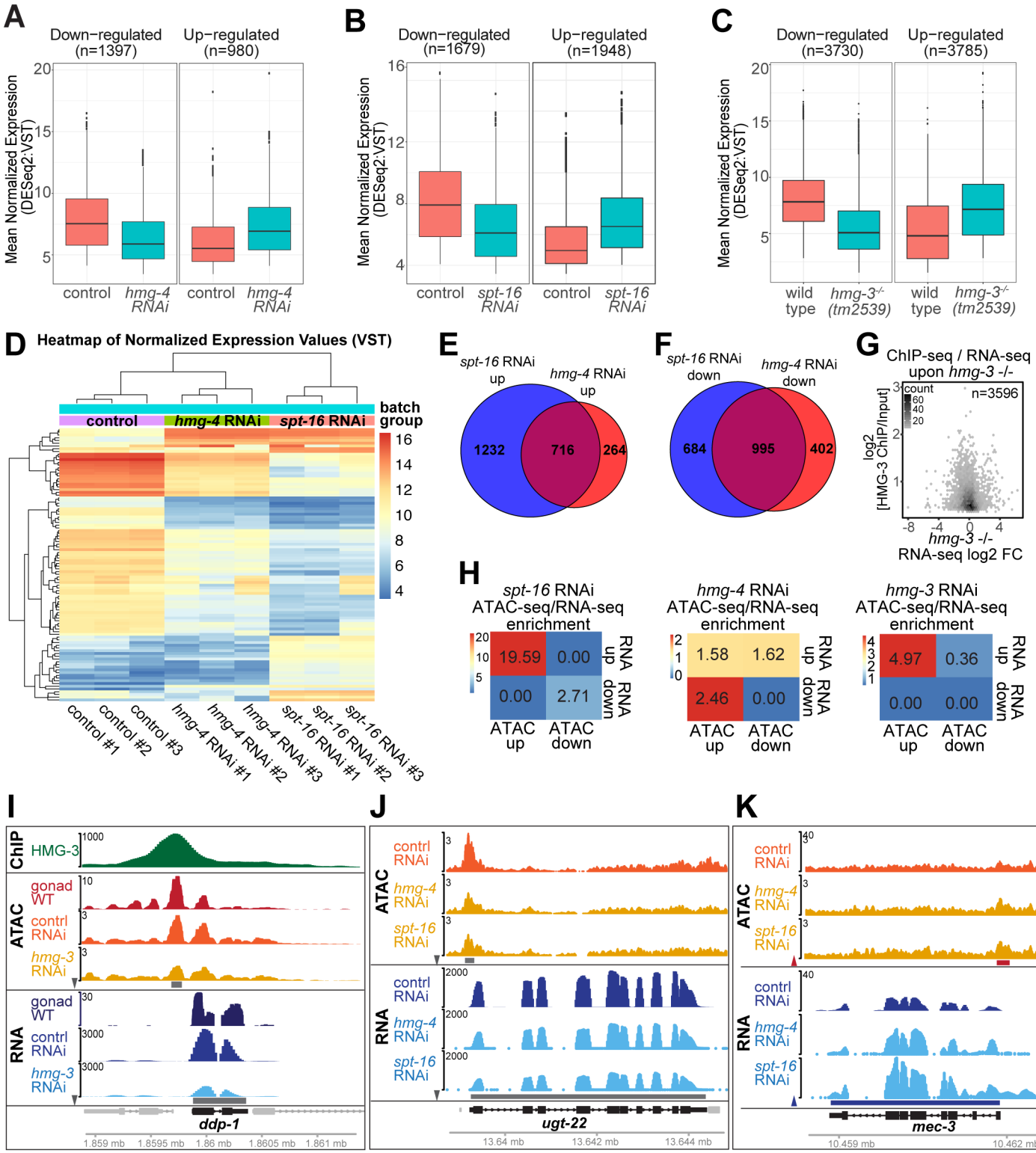
Supplemental Items

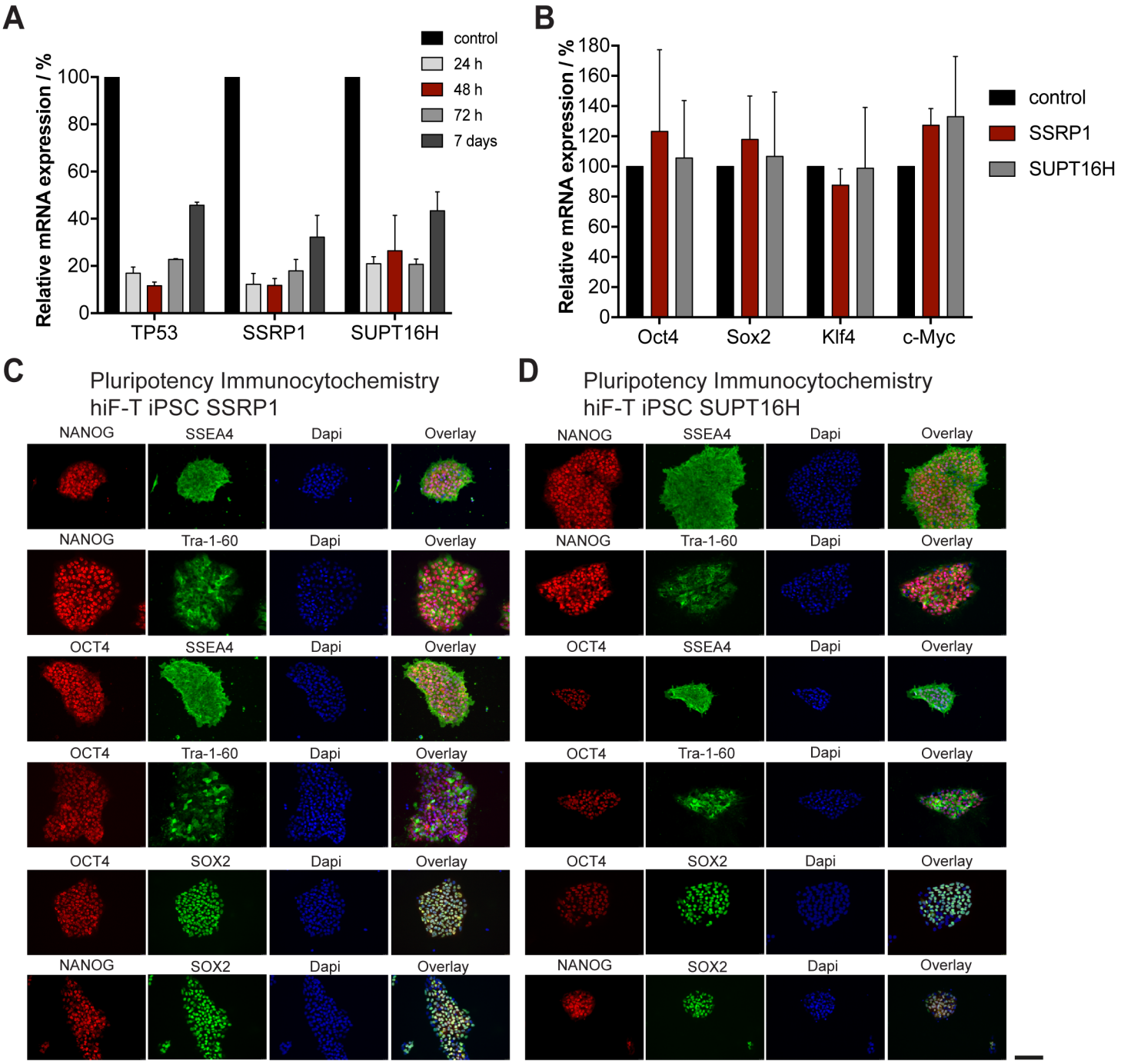
Supplemental Figures S1 – S6 and figure legends
Supplemental Tables S6 – S8 and description of all tables
Supplemental Tables S1 - S5 can be downloaded as Excel files

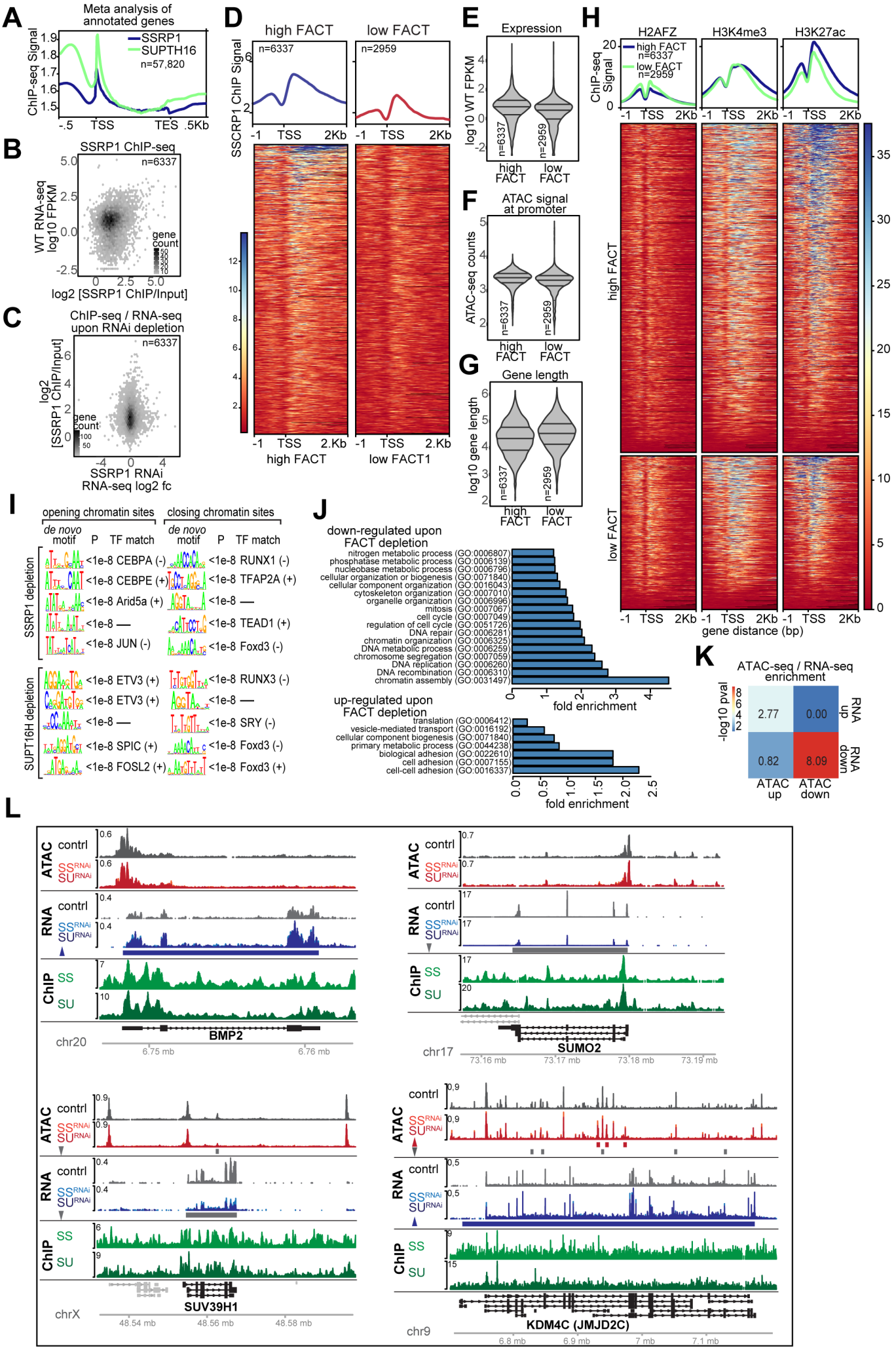

A
control RNAi
hmg-3 RNAi
gcy-5::GFP
DIC + gcy-5::GFP
B
hmg-3 RNAi
% worms with ectopic gcy-5::GFP
n: 259 301 312
heat shock: + - +
otls305 ntls1
ntls1
C
control RNAi
hmg-3 RNAi
lmn-1::GFP
% desilenced = 0
n = 183
% desilenced = 0
n = 177
D
control RNAi
hmg-3 RNAi
let-858::GFP
% desilenced = 0
n = 168
% desilenced = 0
n = 154
E
% neuronal reporter expression in germ cells
n: 212 162 235
reporter: ceh-36 ift-20 rab-3
hmg-3 RNAi + che-1oe
F
Transctipts in VNC neurons
control
gcy-5
ceh-36
rab-3
unc-10
unc-119
G
Transctipts in ASER neurons
control
gcy-5
ceh-36
rab-3
unc-10
unc-119
H
che-1oe
control RNAi
hmg-3 RNAi
gcy-5::GFP + pie-1::mCherry


A
unc-30oe (induction of GABAergic neuron fate)
control RNAi
hmg-3 RNAi
ttr-39::mCherry
DIC
B
% Animals with neuronal reporter expressin in the germline
n:
RNAi:
Ctrl
hmg-3
che-1oe ASE neuron fate
unc-30oe GABAergic neuron fate
elt-7oe intestinal cell fate
hlh-1oe muscle cell fate
C
HMG-3
unc-30oe
ttr-39::mCh in GABA neurons
reprogramming of germ cells to GABA neurons
D
control RNAi
hmg-3 RNAi
elt-7oe: intestinal fate
hlh-1oe: muscle fate
elt-2::NLS::GFP
unc-97::GFP
E
% Animals with converted germ cells
wild type
hyperactive Notch (no meiosis)
RNAi:
control
hmg-3
F
-HU
+HU
G
% animals with gcy-5::GFP in intestinal cells
control RNAi
hmg-4 RNAi
spt-16 RNAi
time / h
H
% animals with gcy-5::GFP in germ cells
hmg-3 RNAi
I
Bait Protein: HMG-3::3HA
-Log[10] P value
SPT-16
HMG-3
FDR: 0.01
minfold Change: 3
t-test difference
J
Bait Protein: HMG-4::3xHA
HMG-4
FDR: 0.29
minfold Change: 2.25


A
ChIP-seq
FACT depletion
isolated nuclei
whole-worm or gonad RNA-seq
ATAC-seq
B
Meta analysis of annotated genes
HMG-3
n=45,836
HMG-3 ChIP Signal
-.2 TSS
TES .2Kb
C
high HMG-3
low HMG-3
n=2877
n=4846
HMG-3 ChIP Signal
HMG-3 ChIP-seq
-1 TSS 2Kb
D
ChIP-seq / WT RNA-seq
n=2877
gonad-specific WT RNA-seq log10 FPKM
count
HMG-3 ChIP-seq log2 ChIP/Input
E
Gonad-specific WT RNA-seq
log10 FPKM
high HMG-3
low HMG-3
F
Gene length
log10 gene length
G
HMG-3 ChIP-seq Tissue Enrichment Terms
-log10 Q-val
reprodructive system
embryonic founder Psub1
embryonic founder Psub3
embryonic cell EMS
germline precursor Z2
germline precursor Z3
oocyte
embryonic cell AB
depressor muscle
reprodructive tract
H
ATAC-seq signal
WT gonad-specific ATAC-seq
I
ATAC-seq signal
control
hmg-3 -/-
n=3596
n=15711
hmg-3 RNAi
J
Gonad-spec. ATAC-seq fragment length
density
fragment length
K
ATAC-seq promoter peaks (n=5865)
control
hmg-4 RNAi
spt-16 RNAi
-1 PE 2Kb
ATAC-seq Signal
gene distance (bp)


A
Down−regulated (n=1397)
Up−regulated (n=980)
Mean Normalized Expression (DESeq2:VST)
control
hmg-4 RNAi
B
Down−regulated (n=1679)
Up−regulated (n=1948)
control
spt-16 RNAi
C
Down−regulated (n=3730)
Up−regulated (n=3785)
wild type
hmg-3-/- (tm2539)
D
Heatmap of Normalized Expression Values (VST)
batch group
control #1
control #2
control #3
hmg-4 RNAi #1
hmg-4 RNAi #2
hmg-4 RNAi #3
spt-16 RNAi #1
spt-16 RNAi #2
spt-16 RNAi #3
E
spt-16 RNAi up
hmg-4 RNAi up
1232
716
264
F
spt-16 RNAi down
hmg-4 RNAi down
684
995
402
G
ChIP-seq / RNA-seq upon hmg-3 -/-
log2 [HMG-3 ChIP/Input]
n=3596
hmg-3 -/- RNA-seq log2 FC
H
spt-16 RNAi ATAC-seq/RNA-seq enrichment
19.59
0.00
0.00
2.71
hmg-4 RNAi ATAC-seq/RNA-seq enrichment
1.58
1.62
2.46
0.00
hmg-3 RNAi ATAC-seq/RNA-seq enrichment
4.97
0.36
0.00
0.00
RNA up
RNA down
ATAC up
ATAC down
I
ChIP
HMG-3
ATAC
gonad WT
contrl RNAi
hmg-3 RNAi
RNA
ddp-1
J
hmg-4 RNAi
spt-16 RNAi
ugt-22
K
mec-3
L
hmg-3 RNAi: ATAC down
hmg-4 RNAi: ATAC down
spt-16 RNAi: ATAC down
de novo motif
P-value
best TF match
<1e-8 cnd-1 (-)
<1e-8 unc-55 (+)
<1e-8 lin-32 (-)
<1e-8 lin-39 (+)
<1e-8 sptf-3 (-)
<1e-8 —
<1e-8 —
<1e-8 —
<1e-8 lin-32 (-)
<1e-8 ztf-14 (+)
0.19 ceh-40 (+) ceh-20 (+)
0.2 skn-1 (+)
0.31 —
0.50 pha-4 (-) fkh-6 (-)
0.53 elt-2/3/7 (+)
0.85 unc-62 (-)
0.95 —
0.95 ceh-40 (-) ceh-20 (-)
0.98 tbp-1 (-)
0.98 —
<1e-8 elt-2/7 (+)
<1e-8 —
<1e-8 —
<1e-8 ceh-40 (-) ceh-20 (-)
8.86e-6 unc-62 (+)
1.79e-5 ceh-40 (-) ceh-20 (-)
5.87e-4 —
0.0063 ceh-40 (-) ceh-20 (-)
0.25 unc-62 (+)
0.34 php-3 (+)
M
hmg-3 RNAi: ATAC up
hmg-4 RNAi: ATAC up
spt-16 RNAi: ATAC up
<1e-8 elt-6 (+)
<1e-8 elt-7 (+)
<1e-8 —
<1e-8 —
<1e-8 —
<1e-8 —
<1e-8 ztf-16 (+)
<1e-8 —
<1e-8 jun-1 (-)
<1e-8 jun-1 (-)
<1e-8 crh-1 (+)
2e-8 jun-1 (-)
1.2e-7 atf-8 (+)
1.2e-7 ztf-28 (+)
1.6e-7 lin-1 (-) ets-5 (-) ast-1 (-)
1.06e-6 —
3.33e-6 —
6.72e-6 —
<1e-8 jun-1 (+)
<1e-8 —
<1e-8 B0310.2 (+)
<1e-8 jun-1 (-)
<1e-8 tag-97 (-)
<1e-8 gei-11 (-)
<1e-8 zip-11 (+)
<1e-8 ztf-6 (-)
<1e-8 zip-3 (+)
<1e-8 —


A
Relative mRNA expression / %
control
24 h
48 h
72 h
7 days
TP53
SSRP1
SUPT16H
B
Relative mRNA expression / %
control
SSRP1
SUPT16H
Oct4
Sox2
Klf4
c-Myc
C
Pluripotency Immunocytochemistry
hiF-T iPSC SSRP1
NANOG SSEA4 Dapi Overlay
NANOG Tra-1-60 Dapi Overlay
OCT4 SSEA4 Dapi Overlay
OCT4 Tra-1-60 Dapi Overlay
OCT4 SOX2 Dapi Overlay
NANOG SOX2 Dapi Overlay
D
Pluripotency Immunocytochemistry
hiF-T iPSC SUPT16H
NANOG SSEA4 Dapi Overlay
NANOG Tra-1-60 Dapi Overlay
OCT4 SSEA4 Dapi Overlay
OCT4 Tra-1-60 Dapi Overlay
OCT4 SOX2 Dapi Overlay
NANOG SOX2 Dapi Overlay


A
Meta analysis of annotated genes
ChIP-seq Signal
SSRP1
SUPTH16
n=57,820
-.5 TSS TES .5Kb
B
SSRP1 ChIP-seq
n=6337
WT RNA-seq log10 FPKM
gene count
log2 [SSRP1 ChIP/Input]
C
ChIP-seq / RNA-seq upon RNAi depletion
n=6337
log2 [SSRP1 ChIP/Input]
gene count
SSRP1 RNAi RNA-seq log2 fc
D
high FACT
low FACT
n=6337
n=2959
SSCRP1 ChIP Signal
-1 TSS 2Kb
-1 TSS 2.Kb
high FACT
low FACT1
E
Expression
log10 WT FPKM
n=6337
n=2959
high FACT
low FACT
F
ATAC signal at promoter
ATAC-seq counts
G
Gene length
log10 gene length
H
H2AFZ
H3K4me3
H3K27ac
high FACT n=6337
low FACT n=2959
ChIP-seq Signal
-1 TSS 2Kb
high FACT
low FACT
gene distance (bp)
I
opening chromatin sites
closing chromatin sites
de novo motif
P
TF match
SSRP1 depletion
<1e-8 CEBPA (-)
<1e-8 CEBPE (+)
<1e-8 Arid5a (+)
<1e-8 —
<1e-8 JUN (-)
<1e-8 RUNX1 (-)
<1e-8 TFAP2A (+)
<1e-8 —
<1e-8 TEAD1 (+)
<1e-8 Foxd3 (-)
SUPT16H depletion
<1e-8 ETV3 (+)
<1e-8 ETV3 (+)
<1e-8 —
<1e-8 SPIC (+)
<1e-8 FOSL2 (+)
<1e-8 RUNX3 (-)
<1e-8 —
<1e-8 SRY (-)
<1e-8 Foxd3 (-)
<1e-8 Foxd3 (+)
J
down-regulated upon FACT depletion
nitrogen metabolic process (GO:0006807)
phosphatase metabolic process (GO:0006139)
nucleobase metabolic process (GO:0006796)
cellular organization or biogenesis (GO:0071840)
cellular component organization (GO:0016043)
cytoskeleton organization (GO:0007010)
organelle organization (GO:0006996)
mitosis (GO:0007067)
cell cycle (GO:0007049)
regulation of cell cycle (GO:0051726)
DNA repair (GO:0006281)
chromatin organization (GO:0006325)
DNA metabolic process (GO:0006259)
chromosome segregation (GO:0007059)
DNA replication (GO:0006260)
DNA recombination (GO:0006310)
chromatin assembly (GO:0031497)
fold enrichment
up-regulated upon FACT depletion
translation (GO:0006412)
vesicle-mediated transport (GO:0016192)
cellular component biogenesis (GO:0071840)
primary metabolic process (GO:0044238)
biological adhesion (GO:0022610)
cell adhesion (GO:0007155)
cell-cell adhesion (GO:0016337)
fold enrichment
K
ATAC-seq / RNA-seq enrichment
-log10 pval
2.77
0.00
0.82
8.09
RNA up
RNA down
ATAC up
ATAC down
L
ATAC
RNA
ChIP
contrl
SSRNAi
SURNAi
SS
SU
BMP2
chr20
6.75 mb
6.76 mb
SUMO2
chr17
73.16 mb
73.17 mb
73.18 mb
73.19 mb
SUV39H1
chrX
48.54 mb
48.56 mb
48.58 mb
KDM4C (JMJD2C)
chr9
6.8 mb
6.9 mb
7 mb
7.1 mb

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*^{RNAi} animals with and without *che-1*^{oe} (no *hs::che-1* construct in the background), or with only *gcy-5::GFP* (*ntIs1*) construct in the background. *gcy-5::GFP* is not induced upon *hmg-3* RNAi without *che-1*^{oe}. Scale bars, 20 μm.
(B) Quantification of *gcy-5::GFP* induction in *hmg-3*^{RNAi} with and without *che-1*^{oe} (no *hs::che-1* construct in the background) or with only *gcy-5::GFP* (*ntIs1*) 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 μm.
(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*^{RNAi} 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*^{RNAi} animals upon overexpression of GABA neuron-fate inducing TF UNC-30 (*unc-30*^{oe}). 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*^{oe} (ASE neuron) or *unc-30*^{oe} (GABA neuron), but no intestinal or muscle fate induction by over-expressed ELT-7 (*elt-7*^{oe}) or HLH-1 (*hlh-1*^{oe}), 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*^{oe}. *unc-30*^{oe} 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*^{RNAi} animals. Scale bars, 20 μm.
(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*^{RNAi} and *spt-16*^{RNAi} animals. Time in h after induction of *che-1*^{oe}. 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*^{RNAi} animals. Time in h after induction of *che-1*^{oe}. 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*^{RNAi} and *spt-16*^{RNAi} 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*^{RNAi} and *spt-16*^{RNAi} animals.
(G) Log2 fold-changes in expression levels from whole worm RNA-seq after *hmg-3*^{RNAi} 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*^{RNAi}, *hmg-4*^{RNAi} and *spt-16*^{RNAi}. 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 μm.

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 $SSRP1^{RNAi}$ and $SUPT16H^{RNAi}$. 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	*barIs12[elt-2prom::gfp; myo-3p::NmBirAo]*
BAT026	*otIs284 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.; hdIs30 [glr-1::dsRED]*
BAT028/OH9846	*otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.*
BAT032	*glp-1(ar202) III.; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.*
BAT044	*juIs244 [ttr-39prom::mCherry, ttx-3prom::gfp]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.*
BAT046	*otIs133 [ttx-3prom::mCherry]; otIs284 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.; hdIs30 [glr-1::dsRED]*
BAT068	*otEX4945 [hs:hlh-1, rol-6(su1006)]; mgIs25 [unc-97prom::gfp]*
BAT109	*otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)] V.*
BAT139	*stIs10086 [ges-1::H1-Wcherry + unc-119(+)]*
BAT160	*itIs37 [pie-1p::mCherry::his-58(pAA64), unc-119(+)]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)] ntIs1 [gcy-5p::GFP, lin-15(+)] V.*
BAT282	*barIs40 [vit-5::2xNLS::TagRFP]*
BAT284	*stIs10131 [elt-7::H1-wCherry + unc-119(+)]*
BAT287	*ntIs1 [gcy-5::gfp; lin-15b(+)] V.*
BAT326	*otIs263 [ceh-36prom::tagRFP]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.*
BAT453	*barEx147 [hsp-16.4prom::unc-30; hsp-16.2prom::unc-30; rol-6(su1006)]; juIs244 [ttr-39prom::mCherry, ttx-3prom::gfp]*
BAT522	*otis393 [ift-20prom::NLS::tagRFP]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.*
BAT525	*hmg-3 (tm2539) / dpy-5(e61) unc-13(e1091) I.*
BAT527	*otIs355 [rab-3prom::NLS::TagRFP]; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)]; ntIs1 [gcy-5::gfp; lin-15b(+)] V.*
BAT606	*edIs6 [unc-119::gfp + pRF4[rol-6(su1006)]] IV.; otIs305 [hsp-16.48prom::che-1::3XHA::BLRP; rol-6(su1006)] V.*
BAT1560	*hmg-3(bar24[hmg-3::3xHA]) I. protein tag CRISPR engineered*
BAT1753	*hmg-3(bar24[hmg-3::3xHA]) I. 2x outcrossed*
BAT1945	*jun-1(gk557) II; otIs305[hsp::che-1::3xHA, rol-6] ntIs1[gcy-5::GFP]*
BAT1967	*hmg-4(bar32[hmg-4::3xHA]) III*
JR3373	*wIs125[hsp-16-2::elt-7 hsp-16-41::elt-7]; rrIs1 [elt-2::GFP + unc-119(+)]*
LW697	*ccIs4810[pJKL380.4; lmn-1p::lmn-1::GFP::lmn-1 3'utr + pMH86; dpy-20(+)] I.*
NL2507	*pkIs1582[let-858::GFP + rol-6(su1006)]*
SS104	*glp-4(bn2) I.*
OD56	*ltIs 37[pie-1p::mCherry::his-58 unc-119(+)];unc-119(ed3) III.*

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

7A. smFISH probes		
Probe set	**Sequences**	**Fluorophore**
gcy-5_set1	cattcggatgctccaagaac	Fluor Red 610
	caattccaactcgaagcgtc	
	caattggaagagttccacca	
	tatcgcattcggtatattcc	
	tcccactacaacatctacat	
	tattggtatcagccaactgg	
	tgccactcgatcaaattgga	
	tttacagtagtcttggtcgt	
	cttaaggttgcctcaacatc	
	atccgcactggatatagatc	
	cgatcttgttaatgcctcat	
	tacgagctcgactctttaca	
	ggaccactaattgcgcataa	
	ccaatactcctcattgtcaa	
	tctttcccaaacttgtttgt	
	tggagttagtccatttgcaa	
	ctactgtgaatgactcccaa	
	atttctaacagcatccgcaa	
	tgccatcccgtataagtaaa	
	tagtaaccatttgcggcata	
	gcggtagagatttttgaccaa	
	tcatgttaactagtgccact	
	ccgtgacaattgcgaagacg	
	cgtttttctttttgtggcat	
	gtgatcttcgactatttggc	
	actttctccggttatagttg	
	gctatgatgtttggtggtta	
	atttctccttctcttcttta	
	ggtccatcgatagataatcc	
	gatatcctgaagtgatcctc	
	aaagttcataccctctgcaa	
	ggcaagtagctgaacgtaga	
	ctcccaatccaaaatctgtt	
	tacgatttcctcctttttc	
	aagtattaactccggtcgga	
	acttgcaaatttgctcagct	
	gttctgcaacttgttttgga	
	tctccaattgattccacttt	
	tgtcggtaacccagaaacac	
	ggaaccttgaagctcttaca	
	gcccactattaattccaatt	
	atggatagaccaacgacacc	
	gtatccccaaataggcaata	
	tttccattactttccattct	
	tgtgcagcttctgacatatg	
	tctcctcttgaacttgtttc	
	tgtttccattacacctttttc	
	gattttgtgtcactgtcagt	
ceh-36_set1	gtgtagaagttggtggtcat	Fluor Red 610
	ggataagcagtgtagccgag	
	tgcggcagcaaatgcaaatt	
	atgtaagactgggtgccgtg	
	cattgtcgttgagcttgtgg	
	ttcactgtttggagccattg	
	ctctgttgaacgaggtacgt	
	ttttccagctgatcgagttg	
	gatactgtgtttcgcggaaa	
	gcttctcttctgtgcacatc	
	caaattgattgccttcgcca	
	ttacttgtaccttccatca	
	cgatttttgaaccaaaccgt	
	gttgtttctatccttggctc	
	gatggactccatccatttttt	
	gatcttgatgaagtgcttcc	
	cgttgtgtggagaaccattg	
	gtgatttagtatcaggcttt	
	tgtgcctggtatgtgaattc	
	cactgtgtgcattgaattcc	
	gagtttgcctcatatttggc	
	ttgcagttgactcaagactg	
	agtcctccagttcacttttt	
	atttggtatctgcaagtggt	
	cttgagcctgaggaagaagt	
	agttgcgtaggatgcatatg	
	tagttgtacgggtaaggagc	
	gtttgatgggaagtagctgt	
	tgcttccatattgttggtag	
	aggcagtaatatttggggtg	

7B. Oligonucleotides used for qRT-PCR	
Name	**Sequence**
SSRP1 fwd	TTTGCCCAGAATGTGTTGTC
SSRP1 rev	AGTCAAAGGTCTTGCCATGC
SUPT16H fwd	GCAGAAGAGAAGCGAAGAGC
SUPT16H rev	TTTCCCGAATATGTGGTTCC
TP53 fwd	TCAACAAGATGTTTTGCCAACTG
TP53 rev	ATGTGCTGTGACTGCTTGTAGATG
GAPDH fwd	TGCACCACCAACTGCTTAGC
GAPDH rev	GGCATGGACTGTGGTCATGAG
POU5F1 fwd	GAGAACCGAGTGAGAGGCAACC
POU5F1 rev	CATAGTCGCTGCTTGATCGCTTG
SOX2 fwd	GCCGAGTGGAAACTTTTGTCG
SOX2 rev	GCAGCGTGTACTTATCCTTCTT
KLF4 fwd	ACCAGGCACTACCGTAAACACA
KLF4 rev	GGTCCGACCTGGAAAATGCT
MYC fwd	CGTCTCCACACATCAGCACAA
MYC rev	CACTGTCCAACTTGACCCTCTTG

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	AAACAUGCAGAAAAUGCUG
TP53	MU-003329-03-0002	D-003329-05	GAGGUUGGCUCUGACUGUA
		D-003329-07	GCACAGAGGAAGAGAAUCU
		D-003329-08	GAAGAAACCACUGGAUGGA
		D-003329-26	GCUUCGAGAUGUUCCGAGA
SSRP1	MU-011783-01-0002	D-011783-01	GAUGAGAUCUCCUUUGUCA
		D-011783-03	GACUUAAACUGCUUACAAA
		D-011783-04	GCAAGACCUUUGACUACAA
		D-011783-17	GAGGGAGGAGUACGGGAAA
SUPT16H	MU-009517-00-0002	D-009517-01	GAAGAUAUGUGACGUGUAU
		D-009517-02	GAACAAAGUCGAAGUAUGA
		D-009517-03	AGACAUGGCUCUUUGGUUA
		D-009517-04	GAAGAACUUUGAUAUGGUA

rab-3_set1	caaagttctgatcgggttgt	Fluor Red 610
	atcaggagcttgaacatgta	
	tccaactgatgaatttccga	
	catcacagtaacggaagagg	
	gtagagacgaaggcagaagt	
	cactttgaaatcgattccga	
	tttgtctccacggaacacag	
	ggtatcccagatttgaagtt	
	gatagtaggcggtggtgatg	
	cagaatgaatcccattgctc	
	actcttcattagtgatgtca	
	gcaccaatcctgaacactat	
	tttcccatgagtatgtcttg	
	ccaaccaaaacaacttgagc	
	ttcagagtccatatcacatt	
	ccctatccatagatacaact	
	aagttgatcagcaagttggc	
	ggctgatgtttcgaagaatt	
	ccttttacattaatgttctcc	
	tctccaccaacttctcaaaa	
	tctgccatcttatcacaaat	
	ctgtgggtccttatccaaac	
	ttcgagcttctgtccttttg	
	aattgcattgctgttgagca	
	attgcgtttggaatttggga	
	agagctacgcgcttttagaa	
	cctagatgttgagagaggga	
	tttacgatccatatatctgg	
	taattaaaccaactacgccc	
	ggggaatatgattgaacgtt	
	gctctgggaattgtttggaa	
	ggcgactatgattagttaga	
	tgggaactgggaagtcacta	
	aatcaatctttcagcgggtg	
	cctcgaaaataatttcctcc	
unc-10_set1	taaatccggcatcatcgacg	Fluor Red 610
	ttcacgttcttctgcagata	
	ccgtgatctgtttgtctaac	
	agatttgacagatcgcgtca	
	caattccgtccgcaaatttg	
	cagattgccttatttttgct	
	gattttgactcattggctgt	
	catattctgattgtggctct	
	tttgtccttgttggttttg	
	aggcgtttgtttcatagttc	
	tcttgttgtccatgttgatt	
	attctctctcattctgttgt	
	tctcggaatttccagtgtag	
	ggttgtttttggttctgattc	
	ggttcaaatggtcgtcagta	
	tcgaagttgcctatgcaatc	
	cgagtttttatgatcgccat	
	atggtgacaaggacagcgat	
	ccctgttccaaaatgatcat	
	tggctgcagaattttcagtt	
	gtaatgaatgcaccgagctt	
	atgtggcattttgcagagac	
	ttgcagcgatgctatcatat	
	gaatacgccgaggatgacat	
	gatggatatgcagatggcac	
	ggctgattgtgaatgtggta	
	gatgtcgaacgattgcgtga	
	gagcaactgagagttgtcga	
	aaactggcatgagtgtctct	
	ggttcagtaaggccattata	
	tcgtaatcccagacagttag	
	gtcatttggggcaagatgat	
	tcgtcgtcgtcaatgtattc	
	atgatcagatgtgtagcctg	
	tgttggatcgtacatatcct	
	tccatcactataatatccct	
	cattgtagttggcatgctat	
	aaacttttctttcgctcctt	
	cctcagatctagcaaaaccg	
	gtgagccgatctgaagacaa	
	cttgcttcagaaagggagga	
	gcaaacttgtctgagtgagc	
	aagcacttgacgaccgacaa	
	cttttacatagggagctgga	
	tttggcaatgcattgtttgc	
	ttccatacgaccgtaatcac	
	tttgcgaaatccccatgaat	
	cagtttataccaccctatta	
	cgatcgattgttgttgttgc	
	catctgagacgggaaggttg	
	gttatagcctgttcggttac	

unc-119_set1	tgatttttcgcgagaagctc	Fluor Red 610
	agagctagcacatcatttgg	
	gcataggaatccttgagtga	
	ttatagacgtttgccgatgg	
	cgaggtcacggatttggaat	
	gcaatttcgaagagcacgtg	
	attctcttccgtctcatttt	
	gatatcggacatatcttgcc	
	aatgtgtgatcggcacatcg	
	aagtgccgttcaatcattcg	
	gcatttcaataaacgatcct	
	ggcatacagaatccaaattc	
	tgttcacagttgtttctcga	
	gttgttgtgaaagttgtgga	
	attattgatcatgtcgtcca	
	aatagaagctatcggagcgg	
	gtgcattacgagcttattct	
	tgcatcatacgagtagtcgg	

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