**SUPPLEMENTAL INFORMATION** (Rincon-Arano et al)

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1 (related to Figure 1). UpSET is a nuclear protein with an inactive, but conserved, SET domain.**
(A) Sequence alignment of the conserved UpSET SET domain in Drosophila species. Gray shading: conserved residues. Red shading: conserved Threonine suggested to be glycosylated.
(B) Schematic of Trx and MLL1 protein domain organization and their overall percent identity with UpSET.
(C) Loading controls for evaluating specificity of the nuclear versus cytoplasmic extracts. Pc: Polycomb, a nuclear transcription factor; SpireC: a cytoplasmic actin nucleation factor.
(D) Specificity of UpSET antibodies. Polyclonal and monoclonal antibodies generated against the N-terminus and C-terminus of UpSET were evaluated by western blot with *in vitro* translated proteins encoding the NH3- or COOH- terminal regions used as indicated.
(E) Production of SXC evaluated by radioactive *in vitro* translation.
(F) Expression of the murine MLL5 in ovaries. Western blot analysis of ovary extracts from wild type (1,2) or *upSET*$^{e00365}$ (3) mutants carrying the conditional UASp-mMLL5 transgene probed with anti-mMLL5 antibodies. Expression of the UASp-mMLL5 transgene was driven by the presence of the actin-Gal4 transgene as indicated. Full-length mMLL5 protein is denoted by the arrow. *:Non-specific bands provide internal loading controls.
(G) Murine MLL5 does not exhibit histone methyltransferase activity (HMT). Radioactive HMT assays performed with bacterially purified GST or GST-MLL5 (encoding the first 1-622aa of MLL5) were used in radioactive HMT assays. Recombinant proteins were incubated with mammalian nuclear extracts (NE). Controls are the same as in Figure 1K.

**Figure S2 (related to Figure 2). UpSET preferentially associates to active regions.**
(A) Histogram of UpSET bound regions. DamID based technology targets Dam methylation to +/-2.5 Kb around the binding site of the tagged protein. UpSET bound single peaks were considered to range between 3.5-6 Kb. UpSET bound regions were considered to be larger than 6 Kb.
(B) Box plots show DamID signal mapped to the 5 chromatin states described in Filion et al, 2010. UpSET based DamID signal mainly maps to the red and yellow chromatin states, which are considered to be transcriptionally active. Horizontal line represents the median.
(C) UpSET enrichment over active promoter regions is not dependent on pausing features. UpSET chromatin profiles were mapped to promoter regions with increasing RNA Polymerase II pausing indexes according to Gilchrist et al. (2010). Horizontal line represents the median. No pol = promoter without RNA pol II.

**Figure S3 (related to Figure 4). UpSET does not affect methylation of H3K4.**
(A) Tracks show binding of Rpd3 and Sin3, but not UpSET, over the C15 promoter region, which is regulated by PcG (Schwartz et al., 2006).
(B) Chromatin immunoprecipitation for histone acetylation marks (H3Ac and H4K16Ac) in knock down cells. Additional examples of histone acetylation levels of UpSET target gene including *viking* (*vkg*), *complexin* (*cpx*), *couch potato* (*cpo*), *sarcoplasmic calcium-*

*binding protein* (*cbp*) and *Cg25C*. P-values: *: p<0.05; **: p<0.01; non-significant p values are not marked. Bars represent mean +/- SEM
(C-D) UpSET knock down does not affect nucleosome abundance. H4 and H3 levels over UpSET bound promoters bound were evaluated by ChIP-qPCR. Tested Promoters are the same as in Figure 4B. Bars represent mean +/- SEM
(E-F) Levels of H3K4 methylation levels of histones from knock down and *upSET*$^{e00365}$ mutant ovaries were evaluated by western blot. H3K27m3 and H3 were used as controls.
(G-L) Staining of UpSET (green) and H3K4 methylation (red) on salivary gland polytene chromosomes from wildtype (G, I, K) and *upSET*$^{e00365}$ mutant (H, J, L) third instar larvae.

**Figure S4 (related to Figure 4). Murine MLL5 increases histone acetylation, but not chromatin accessibility, of the Ccna2 gene in C2C12 myoblasts.**
(A) Genomic region of the Ccna2 gene highlighting MLL5 binding region, as well as the 5 other regions amplified by ChIP-qPCR.
(B) Western blot showing MLL5 knock down in C2C12 myoblasts with two different siRNA concentration. GFP-specific siRNA was used as mock control. PARP1 was used a loading control.
(C) ChIP of histone modifications, including H3tetra-Acetylated, H3K9Ac, and H4K16Ac, on the 5 primer sets targeting the Ccna2 gene and neighboring regions. The 3rd exon of the hemaglobin β-major gene was used as a non-acetylated control. Normal IgG was used as a ChIP control. Bars represent mean +/- SEM. (*:p<0.05; **:p<0.01; non-significant values are not market).
(D) Chromatin accessibility evaluated by qPCR. MNase I-hypersensitive nucleosomes were isolated from mock- and MLL5 siRNA-treated nuclei. Mono- and di-nucleosomal DNA was recovered and evaluated by qPCR using the same set of primers as in C, as well as a primer set that targets the MyoD promoter region (as a positive control). Bars represent mean +/- SEM
(E) Accessibility ratios from D. Blue line indicates a value of 1 (no change). Bars represent mean +/- SEM.

**Figure S5 (related to Figure 4). UpSET modulates chromatin accessibility.**
(A) Low salt extraction (80mM) of hypersensitive nucleosomes from mock and UpSET knock down Kc cells. 600mM salt buffer was used to recover MNase insensitive chromatin. Hypersensitive DNA was labeled and hybridized on tiling arrays in order to compare knock down and mock-treated cells.
(B) Chromatin accessibility profiles of UpSET target genes in knock down Kc cells. Despite the different UpSET binding signals, target genes exhibit different degrees of increased accessibility. Gene examples are the same as in Figure 4B.
(C) 5-methyl cytosine immuno-Dot-Blot. Denatured genomic DNA from Drosophila ovaries treated or not with M.SssI (as indicated) was spotted and cross-linked to a membrane. Mouse genomic DNA was used as control.
(D-G) M.SssI titration for *in situ* chromatin accessibility in Drosophila ovaries. 5mC immunofluorescence of germariums incubated with increasing M.SssI units for 1 h at 25°C. The units of M.SssII used are indicated. Scale bars represent 10 μm.
(H-I) Extended (24h) M.SssI treatment shows a higher cytosine modification in both wildtype and *upSET*$^{e00365}$ mutant ovaries. Scale bars represent 10 μm.
(J) Chromatin accessibility quantification. M.SssI methylated genomic DNAs from wildtype and *upSET*$^{e00365}$ ovaries were analyzed by ELISA. P-values: **: p<0.01; non-significant p values are not marked. Bars represent mean of triplicates +/- SEM.

**Figure S6 (related to Figure 5). UpSET modulates off-target gene expression**
(A-B) UpSET knockdown does not affect transcription at non-coding. RT-qPCR from knock down Kc cells using primers in shown in Figure 4E, as well as a primer set for the CG13689 coding region (A). Bars represent mean +/- SEM
(C) UpSET up-regulates genes not targeted by RNA polymerase II. RT-qPCR from knock down Kc cells compared to mock cells. Primers amplify coding regions of the genes targeted by UpSET and RNA polymerase II or with low or no binding for those complexes. Genes analyzed were described previously in the manuscript, except for *CG1730*, *CG12200*, *CG6282*, *spn43Ab*, *CG4680*, *cypher* (*cyr*) and *prickle* (*pk*). P-values: *: $p<0.05$; **: $p<0.01$; non-significant p values are not marked. Bars represent mean +/- SEM

**Figure S7 (related to Table 1 and Figure 6). *upSET*$^{e00365}$ does not affect Polycomb chromatin recruitment nor activity and affects cell specification.**
(A) Schematic showing the Minos insertion site in the *upSET*$^{MB8950}$ allele used for genetic interactions. The Minos insertion truncates the UpSET coding sequence. Gray bars represent the protein fragments used for antibody production.
(B-C') Immuno-detection of UpSET (green) on salivary gland polytene chromosomes isolated from *upSET*$^{MB8950}$ mutant larvae. Antibodies recognizing the NH3- (B-B') or COOH- (C-C') terminus of UpSET were incubated independently. Staining for RNA Pol II (red) was used as control. DNA is visualized with DAPI (grayscale).
(D) UpSET does not bind Polycomb associated PREs. Co-staining of Pc (red) and UpSET (green) on salivary gland polytene chromosomes from wildtype third instar larvae. UpSET was detected with a mix of antibodies targeting the NH3- and COOH-termini.
(E-F) Lack of UpSET does not affect chromatin recruitment of the class I PcG protein Posterior sex comb (Psc). Polytene chromosomes from wildtype (E) and *upSET*$^{e00365}$ mutant (F) third larval instar salivary glands were stained with a mouse antibody against Psc (green).
(G-H) UpSET does not affect class II PcG-dependent H3K27 methylation. Immuno-detection of H3K27m3 on polytene chromosomes from wildtype (G) and *upSET*$^{e00365}$ third larval instar salivary glands (H). UpSET antibodies used as in D.
(I-J') Co-staining of SOCS36C (green) and Eya (red) in wildtype (I-I') and *upSET*$^{e00365}$ (J-J') S9 egg chambers. (I', J') Higher magnification views of areas boxed in I-J.
(K) *upSET*$^{e00365}$ mutant ovaries exhibit high Notch and Socs36E levels. Western blot analysis of ovary whole cell extracts with antibodies against Notch and Socs36E, as well as Histone H3 as a loading control. Quantification is shown below each blot as a comparison to wildtype ovary extracts (WT).
(L-M) Lack of UpSET impairs the Jak/Stat pathway. To evaluate JAK/STAT activation, we used the reporter STAT92E-GFP, which expresses GFP at high levels in stalk cells and to a lesser extent in follicle cells (L) (Bach et al., 2007). GFP staining (green) of wildtype (L) and *upSET*$^{e00365}$ mutant (M) ovarioles containing the STAT92-GFP reporter. EYA staining (red) was used to label somatic cells. Strikingly, *upSET*$^{e00365}$ ovarioles show lower levels of GFP and complete absence of activation in stalk cells (arrowheads), suggesting that, in the absence of UpSET, the JAK/STAT pathway is impaired.
(N-O) UpSET is required for proper specification of polar cells. The lack of JAK/STAT signaling observed could be a consequence of improper specification of polar cells that are required for ligand secretion. To examine this possibility, we used two polar cell specific markers, Fasciclin III (FasIII) and the reporter line NeurA101-Lacz, to evaluate the establishment of these cells. Wildtype (N) and *upSET*$^{e00365}$ mutant (O) ovarioles

containing a NeurA101-Lacz reporter (arrowheads) were stained with an antibody against LacZ (green) and FASIII (red). DAPI was used to visualize DNA. In *UpSET* mutant ovaries, FasIII expression is properly established and maintained; however, no activation of the NeurA101-Lacz reporter line is observed, suggesting an incomplete specification of polar cells (O). Confocal projections of 5 serial 1 μm optical sections are shown. Scale bars represent 50 μm.

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Plasmids and sequence analysis

UpSET cDNA was cloned from 0-6 hr embryonic mRNA by amplifying four fragments. The four fragments were then connected by standard cloning procedures and sequenced. The resulting full-length cDNA was cloned into pCRII-TOPO (Invitrogen Life technologies, Carlsbad, CA, USA). UpSET cDNA was amplified by PCR using the Long template amplification kit (Roche Diagnostics Inc., Indianapolis, IN, USA) and cloned into pNDAM (van Steensel and Henikoff, 2000). For in vitro translation, the first and last 1.5 Kb of the *upSET* cDNA were cloned into pCite-4(+). The murine MLL5 cDNA was cloned from mouse erythroblasts. A DNA fragment corresponding to the 994-1319aa region of UpSET, as well as the 1-622aa region of mMLL5, were cloned *in frame* into pGEX-5X. For the rescue construct with the UAS-MLL5, the murine MLL5 cDNA was cloned into pUASp. Insect conservation data was retrieved from the UCSC genome browser. Sequence homology analyses were performed with BLAST using the default settings.

### Supplemental fly stocks and genetics

Genetic interactions were performed with the following alleles from the Bloomington Drosophila Stock Center: $st^{1}$ $trx^{1}$/TM1, $brm^{2}$ $trx^{E2}$ $ca^{1}$/*TM6B*, $Pc^{1}$ *Abd-*$B^{Mc}$/TM3, Sb, and *In(3R)P(*$Pc^{3}$*),* $Pc^{3}$/TM1. To rescue *upSET* female sterility, a BAC containing the *upSET* locus (CH322.187J08) was inserted on chromosome 2 using PACMAN technology (Venken et al., 2009). Additionally, a UAS-MLL5 rescue construct was inserted on the second chromosome. Germline transformations were performed by BestGene Inc (Chino Hills, CA, USA) using standard procedures. All transgenic lines used in this study were mapped to a single chromosome and shown to have non-lethal insertions. For testing phenotypic rescue with the upSET-containing BAC, *BACCH322.187J08/CyO; MKRS/TM3,Ser* males were crossed to $Kr^{If-1}$*/CyO;* $upSET^{e00365}$*/TM3,Ser* using standard methods. For testing phenotypic rescue with the murine MLL5 gene, *mMLL5/CyO;* $upSET^{e00365}$*/ TM3,Ser* males and *Act-GAL4/CyO;* $upSET^{e00365}$*/ TM3,Ser* females were mated in order to generate *mMLL5/Act-GAL4;* $upSET^{e00365}$ flies. The 10XSTATGFP reporter line was kindly provided by Erika Bach (Bach et al., 2007). The percentage of sterility was evaluated by mating 50 female flies of the proper phenotypes with wild type males and the tubes were screened for laid eggs. The number of eggs produced was determined by mating 25 females of the proper genotype with 8 males in individual tubes for 24 h at 25°C. The flies were then transferred to collection chambers with grape agar plates. Plates were exchanged every 12 h for 1 week and the number of laid eggs was scored.

### RNA extraction, cDNA preparation, and qPCR analyses

Total RNA was extracted with Trizol (Invitrogen Life technologies, Carlsbad, CA, USA) from Kc cells or fly ovaries according to the manufacturer's protocol. mRNA was treated with DNase I for 30min at RT and the integrity of the mRNA was assessed by electrophoresis. A Qiagen clean up protocol was used to purify mRNA. For RT-qPCR, 500ng of mRNA was reverse transcribed using random primers and the MLV-RT as reported (Rodriguez et al., 2008). RNA was removed by treating the samples with RNase H for 30min at 37°C and the samples were diluted 1:4 with RNAase-free water.

qPCR analysis was performed using an ABI 7900HT Real Time PCR machine and analyzed with SDS 2.3 software (Applied Biosystems/Life Technology Corporation, Carlsbad, CA, USA) using standard SYBR Green incorporation to detect PCR products. qPCR reactions (in triplicates) were performed at least two times and analyzed by the ΔΔCt method +/- SE. For gene expression, control signals were adjusted to 1 to allow for comparison with the knock down and mutant ovary expression. The expression primers used in this study are listed below. For RNA tiling arrays, the same reaction was scaled up to 3μg of RNA. cDNA was treated with RNase H for 30min at 37°C. 3μg of cDNA was labeled and hybridized according to the NimbleGen protocol (Roche NimbleGen Inc., Madison, WI, USA).

**Immunoprecipitations**

500μg of nuclear extract (NE) was incubated with 8μl polyclonal antibodies for Sin3 (Pile and Wassarman, 2000) or Rpd3 (Winkler et al., 2010) for 4h at 4°C. BSA-blocked protein A/G sepharose was added then incubated for 2h at 4°C. Complexes were washed with RIPA buffer three times for 5min each and the immunoprecipitated proteins were resolved on a 4-12% acrylamide gel. Unfortunately, the UpSET monoclonal antibodies we generated do not work for immunoprecipitation.

**Nuclear extracts, histone preparation and western blot analyses**

Cells were washed with PBS once, resuspended in NP-40/sucrose buffer (0.32M Sucrose, 3mM $CaCl_2$, 2mM $MgCl_2$, 0.1mM EDTA and 1.5% NP-40) and incubated on ice for 5min. Nuclei were pelleted at 1500xg and washed once with sucrose buffer without NP-40. For western blots, the nuclei were resuspended in RIPA buffer, sonicated (avoiding overheating) and incubated on ice for 10min. The samples were spun at 14,000xg for 15min at 4°C and the supernatants were recovered. For immunoprecipitation experiments, nuclei were resuspended in Buffer B (20mM Hepes pH8, 0.5mM EDTA, 100mM KCl, 10% glycerol, 2mM DTT, 3mM $CaCl_2$, 1.5mM $MgCl_2$) and MNase I was added at 1U/10μg DNA. Samples were incubated at 37°C for 5min and cooled on ice. Samples were nutated for 1h at 4°C and 3mM of EDTA was added to stop the MNase I reaction. Samples were spun at 14,000xg for 15min at 4°C and the supernatants were recovered. Protease and phosphatase inhibitors were added to all the buffers. For histone preparation, the nuclei were treated as previously described (Brand et al., 2008).

For larvae nuclear extracts, 50 larvae were washed three times with PBS and resuspended in NP-40/sucrose buffer. Larvae were homogenized with a tight fit dounce homogenizer with twenty strokes on ice. The suspension was passed through a mesh to remove the cuticles. Nuclei were spun at 1500xg for 5min at 4°C and resuspended in RIPA buffer with protease and phosphatase inhibitors. Nuclei were sonicated 5 times using a Sonic Dismembrator (Model 60; Fisher Scientific, Hampton, NH, USA) at setting 4 with 30 seconds per pulse. Nuclei were spun at 16,000xg for 10min at 4°C and the supernatant recovered.

For ovary whole cell extracts, ovaries were resuspended in RIPA buffer plus protease inhibitors, sonicated briefly and spun for 10min at 14,000xg. Supernatant was collected.

**Cell culture and RNAi lines**

Kc cells were grown in CCM3 media supplemented with L-glutamine at 25°C. For stable transfections, $1x10^6$ Kc cells were transfected with Fugene for 24h according to the manufacturer specifications using a 5:1 Fugene/DNA ratio. A 0.4 Kb (corresponding to 351-489aa) UpSET fragment was cloned into a modified pMT vector and co-transfected with the pCoBlast plasmid at a 20:1 ratio. Pools of stable clones were expanded after a 3-week selection period. RNAi was induced by adding $CuSO_4$ to 500nM and incubating for 7 days at 25°C, with one change of media plus $CuSO_4$ on day 3. Knock down efficiency was addressed by western blot. dsCheck was used to determine any off-target genes in the Drosophila genome and no off-target hits were identified (Naito et al., 2005). C2C12 cells were grown in DMEM supplemented with 10% FBS, penicillin and streptomycin as reported in Sebastian et al. (2009). Cells were transfected with stealth siRNA against murine MLL5 or GFP (see sequences below; Invitrogen Life technologies, Carlsbad, CA, USA) using the Amaxa system according to the manufacturer protocol and grown for 48 h. Knock down efficiency was evaluated by western blot.

**Histone methyltransferase assays**

Recombinant proteins bound to GST-sepharose beads were incubated with nuclear extracts (200µg) or *in vitro* translated SXC in the presence of 50 mM Tris pH 8, 150 mM NaCl, 0.5% Nonidet P-40, 12.5 mM MgCl2, 1 mM β-ME, and 700 nM UDP- GlcNAc for 2 h at 30°C. Beads were washed twice with RIPA buffer supplemented with protease inhibitors and twice with TE. Histone methyltransferase assays were performed in 50 mM Tris-HCl pH 8, 5 mM MgCl2, 4 mM DTT supplemented with 2 µg of chicken histones and 1 µCi of S-[methyl-3H]-adenosyl-L-methionine (Perkin Elmer) for 2 hr at 37°C. Samples were resolved on a 4-20% acrylamide gel, dried and exposed to a film.

**UpSET antibody characterization**

Western blotting was used to test antibody specificity against endogenous protein in nuclear and cytoplasmic Kc cell extracts using a 1:100 dilution (Figure 1E). The monoclonal lines were generated in the FHCRC Antibody Development Shared Resource Facility as described (Wayner et al., 1989; Harmon et al., 1995), obtaining 2 and 3 clones for the NH3- and COOH-terminal ends, respectively. Western blotting was used to test antibody specificity against endogenous protein in nuclear and cytoplasmic Kc cell extracts using a 1:12 dilution (Figure S1C).

**Ovary Immunostaining**

Immunofluorescence and confocal microscopy were performed as described previously (Rosales-Nieves et al., 2006; Liu et al., 2009). 2 day old well-fed females were dissected in CCM3 media, washed in PBS and fixed in 6% EM-grade formaldehyde (Polysciences, Warrington, PA) diluted in PBS, with three times volume of heptane. After washing, tissues were blocked in 1.5% BSA, hand dissected, then incubated with primary antibodies at 4°C overnight. The following antibodies, obtained from the Developmental Studies Hybridoma Bank, were used: mouse anti-bam and anti-FasIII (1:100 dilution); anti-Orb, anti-lacZ, and anti-EYA (1:50 dilution). Rabbit polyclonal anti-GFP (Roche Diagnostics Inc., Indianapolis, IN, USA) and rabbit anti-SOCS36E (kindly provided by Steven Hou, NIH) were used at 1:1000. Alexa-488 and -568 (1:1000 dilution; Invitrogen

Life technologies, Carlsbad, CA, USA) were used for fluorescence visualization. 0.1µg/ml of DAPI (Invitrogen Life technologies, Carlsbad, CA, USA) and 0.5µg/ml of Phalloidin-Alexa630 were used for DNA and actin cytoskeleton staining, respectively. Ovaries were further hand dissected and mounted in SlowFade Gold with DAPI (Invitrogen Life technologies, Carlsbad, CA, USA).

**Polytene Chromosome staining**

Salivary gland polytene chromosomes from WT and *UpSET* third instar larvae were fixed and prepared as previously described (Bianchi-Frias et al., 2004). Mouse monoclonal antibodies for UpSET were used in a 1:6 dilution. Other antibodies were used at 1:500 dilution: rabbit anti-H3K4m3 (Active Motif, Carlsbad, CA); rabbit anti-H3K4m2, H3K4m1, H3K27m3 (Upstate-Millipore, Billerica, MA); rabbit anti-H3 pan-acetylated, anti-H3K9Ac, anti-H3K27Ac, anti-H4K16Ac (Abcam, Cambridge, MA); rabbit anti-Sin3a (Pile and Wassarman, 2000); rabbit Rpd3 (Winkler et al., 2010); mouse anti-CTD Pol II (Abcam, Cambridge, MA); and rabbit anti-Polycomb (Santa Cruz Biotechnology). Polytene chromosomes were imaged on a DeltaVision microscope. Average projections of 0.2mm stacks are shown with adjustments in brightness and contrast only.

**Western blot**

Western blots were performed as described previously (Rincón-Arano et al., 2003). Anti-histone antibodies were used at 1:2000 dilution. Antibodies against RNA polymerase II, Sin3 and Rpd3 were used at 1:2000. Polyclonal anti-SpireC antibodies were used 1:20 (Liu et al., 2009). Rabbit anti-murine MLL5 antibody was used 1:2000 (Rincon-Arano et al., in preparation) Blots were developed by chemi-luminescence using HRP-conjugated antibodies (Cell Signaling, Beverly, MA) or by infrared fluorescence detection using an Odyssey LiCor detection system according to the manufacturer recommendations. In both cases, secondary antibodies were used at 1:15000 dilution.

**Cross-linked and native Chromatin Immunoprecipitation (ChIP)**

Cross-linked ChIP was performed according to the protocols established by the modENCODE Consortium (Schwartz et al., 2006). Native ChIP for histone modifications was performed by extracting nuclei with a 5%NP-40/Sucrose buffer (0.32M Sucrose, 3mM $CaCl_2$, 2mM $MgCl_2$, 0.1mM EDTA). Chromatin was shredded with Micrococcal nuclease for 5min at 37°C in order to generate mono-, di- and tri-nucleosomes. 200µg of chromatin was incubated in IP buffer (10mM Tris-HCl pH8, 0.1% Deoxycholate, 1% Triton X-100, 2mM EDTA, 90mM NaCl) with 4µg of Ab overnight at 4°C. Immuno-complexes were precipitated with Protein A/G mixture for 2h at 4°C. Washes were performed 2 times with each of the following buffers: ChIP wash buffer (20mM Tris-HCl pH8, 0.1% SDS,1% Triton X-100, 2mM EDTA, 150mM NaCl) and Final Wash buffer (20mM Tris-HCl pH8, 0.1% SDS, 1% Triton X-100, 2mM EDTA, 500mM NaCl). DNA was eluted in 0.1M $Na_2HCO_3$, 1% SDS, 0.1mg/ml Proteinase K at 65°C for at least 6h. DNA was cleaned with a Qiagen PCR purification kit. ChIP experiments were performed at least two times. qPCR reactions were performed in triplicate and analyzed by the ΔΔCt method +/- SE. ChIP primers used in this study are listed below.

### *In situ* chromatin accessibility

2 day old well-fed females were dissected in CCM3 media, washed in PBS and fixed in 6% EM-grade formaldehyde (Polysciences, Warrington, PA) diluted in PBS, with three times volume of heptane. After washing, tissues were permeabilized in PBS with 1% Triton X-100, then washed three times with PBS. Ovaries were blocked in PAT (1% BSA, 0.1% Triton X-100 in PBS) overnight. 300 ovarioles were hand dissected and washed twice with 250ul M.SssI reaction buffer supplemented with 16 μM S-Adenosyl-L-methionine. Ovarioles were resuspended in 100ul of M.SssI reaction buffer supplemented with 25U of M.SssI (NEB, Ipswich, MA) and incubated for 1h at 25°C on an orbital shaker. These conditions were established by titrating different enzyme concentrations without reaching saturating conditions. The accessibility reaction was stopped and DNA denatured by adding 1ml of 2N HCl for 30min at room temperature. Ovarioles were neutralized in 100mM Borax for 5min and washed twice with PBS. The tissue was blocked with PAT for 30min and the 5-methylcytosine antibody (monoclonal anti-5-mC, clone 33D3, Active Motif, Carlsbad, CA) was added at 1ug/ml overnight at 4°C. Ovarioles were washed three time with PBT (0.1% BSA, 0.1% Triton X-100 in PBS). Alexa-488 (1:1000 dilution; Invitrogen Life technologies, Carlsbad, CA, USA) was used for fluorescence visualization. 0.1μg/ml of DAPI (Invitrogen Life technologies, Carlsbad, CA, USA)) was used for DNA staining. Ovaries were further mounted in SlowFade Gold with DAPI (Invitrogen Life technologies, Carlsbad, CA, USA). The ovarioles were imaged as described above (Ovary Immunostaining).

For 5mC quantification, genomic DNA was extracted from M.SssI-treated and control ovarioles using standard protocols. Briefly, ovarioles were resuspended in TENS+PK (10mM Tris, 1mM EDTA, 100mM NaCl, 0.5% SDS and 200μg/ml Proteinase K) and incubated overnight at 65°C. The samples were extracted twice with Phenol:chloroform:isoamyl alcohol (25:24:1). The genomic DNA was precipitated with 1/10 vol of 3M sodium acetate pH 5.5 and 3 vol 100% ethanol. The pellet was washed with 70% ethanol, resuspended in 10mM Tris and quantified. CpG content was analyzed by ELISA (Cell Biolabs, San Diego, CA) according to manufacturer instructions.

### Chromatin accessibility-qPCR

C2C12 cells were washed with PBS once, resuspended in NP-40/sucrose buffer (0.32M Sucrose, 3mM $CaCl_2$, 2mM $MgCl_2$, 0.1mM EDTA and 1.5% NP-40) and incubated on ice for 5min. Nuclei were pelleted at 1500xg and washed once with sucrose buffer without NP-40. $3x10^6$ nuclei were treated with 0.1 U MNase I/ml for 5 min at 37°C. EDTA was added to a final concentration of 10 mM to stop the reaction. Nuclei were further incubated in 80 mM salt extraction buffer for 2 h at 4°C. Nuclei were spun at 2000 rpm for 10 min and the supernatant was recovered. DNA was extracted from the supernatant and resolved in a 1.5% agarose gel. Mono- and di-nucleosomal DNA was excised and cleaned with a Qiagen Gel extraction and PCR clean up systems. DNA concentration was adjusted at 0.1 ng/ul and used for qPCR. Relative accessibility was calculated by normalizing the signal from test primers to the accessibility of the 3$^{rd}$ exon of the Hbb gene (Not accessible). MyoD was used as a positive control.

**Immuno dot blot**

Genomic DNA from Drosophila ovaries and the mouse erythroid G1E cell line were extracted using standard protocols. Genomic DNA was fragmented in a bioruptor for 30min in 30" pulses on and off, heat denatured and chilled on ice for 5min. Overnight M.SssI-treated Drosophila genomic DNA was used as a control to assess all available CpG. 1:5 serial dilutions were prepared from a 200μg/ml starting stock and spotted on Hybond N+ membrane (GE Healthcare Life Sciences, Pittsburg, PA). The membrane was air-dried and UV cross-linked. Membrane was incubated with LiCor blocking solution (LiCor Biosciences, Lincoln, NE) overnight at 4°C. Anti-5mC antibody was used at 1μg/ml and incubated for 2h at 4°C. The membrane was washed three times with PBS+0.1% Tween 20 and incubated with goat anti-mouse IgG IRDye 680. After three washes, the membrane was analyzed with a LiCor Odyssey scanner (LiCor Biosciences, Lincoln, NE).

**DamID-based chromatin profiling**

Chromatin profiling was performed using the full-length cDNA of UpSET, which was cloned in frame C-terminal to Dam methylase. Kc cells were transfected and methylated DNA was recovered as reported before (Orian et al., 2009). Briefly, Kc cells were transfected with Dam-UpSET or Dam alone by electroporation. 48h post-transfection, genomic DNA was recovered and digested with DpnI. 200-2500bp fragments were recovered by ultracentrifugation in a 5-30% sucrose gradient. Dam-UpSET and Dam DNA were labeled by random priming with Cy3- and Cy5-hexamers and hybridized to a Nimblegen high-density genome-wide tiling array according to the manufacturer's protocol (Roche NimbleGen Inc., Madison, WI, USA).

**DamID Data analysis**

Data analysis was performed using R/Bioconductor (www.R-project.org; www.bioconductor.org). Raw signals of corresponding experimental replicates were loess normalized within arrays and quantile normalized between arrays. Enrichment statistics (test versus control signals) were computed using the 'sam' algorithm within Bioconductor (Tusher et al., 2001). FDR values of the sam statistic were determined using the package 'locfdr'. Region summarization was performed using the HMM algorithm of TileMap (Ji and Wong, 2005). We applied this HMM model to reliably establish the regions in the genome bound by UpSET (Straub et al., 2008). Probes were considered to be bound significantly if the posterior probability of the HMM was greater than 0.9 and larger than 4Kb. Statistical tests and presentations were performed using R defaults. Visualization was carried out using the UCSC browser. p values were obtained with Welch Two Sample t-test to evaluate significance to Random sequences. 6000 random sequences ranging between 1-5Kb were generated in XLSTAT considering a similar number of binding site for UpSET. Data used the *Drosophila melanogaster* built DM5. End analyses were performed as described (Deal et al., 2010). End analysis for RNA polymerase and H3K4m3 was performed with data generated in S2 cells, which share a similar transcriptional profile with Kc cells (Cherbas et al., 2011).

*Additional Data Source:* Chromatin states and Ovary RNA-seq data from the modENCODE Consortium, available at: www.modencode.org/publications/integrative_fly_2010; (modENCODE Consortium et

al., 2010); GSE20472 (Gilchrist et al., 2010), (Deal et al., 2010)GSE19788; GSE22069 (Filion et al., 2010). For dividing the ovary transcriptome into quintiles, RNA-seq reads were associated with genes using the default "union" overlap mode of HTSeq-counts (http://www-huber.embl.de/users/anders/HTSeq/doc/count.html). Reads with zero counts across the three available RNA-seq experiments or mapping multiple locations or across genes were discarded. The filtered reads were used to break the genes up into quintiles, based on total counts.

**Microarray Expression Profiling**

Expression profiles were generated for mutant and wildtype ovary samples using NimbleGen *D. melanogaster* Gene Expression 4x72k Arrays (Roche NimbleGen Inc., Madison, WI, USA). Samples were processed in triplicate, each replicate from an independent collection of flies. Total RNA was assessed for quality using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Inc, Wilmington, DE, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). For each array, 1μg of total RNA was labeled with Cy3 and hybridized to the array following the manufacturer's recommendations. Arrays were scanned on a GenePix 4000B Microarray Scanner (Molecular Devices, Sunnyvale, CA, USA) and data was extracted using NimbleScan v2.4 software (Roche NimbleGen Inc., Madison, WI, USA).

**Microarray Expression Analysis**

Microarray data was processed in NimbleScan using the RMA (Robust Multi-array Analysis) protocol (Bolstad et al., 2003). To ensure all signal intensity values were positive, a small adjustment was uniformly applied to the overall dataset. The dataset was filtered by applying a lower-bound signal intensity cutoff, followed by the application of a variance filter using the 'shorth' function in the Bioconductor package *genefilter*. Pair-wise significance testing (mutant ovaries vs. WT ovaries) was performed using the bioconductor package *limma* (Smyth, 2005) and p-values were corrected for multiple testing using the false discovery rate (FDR) method of Benjamini and Hochberg (Benjamini et al., 1995). For this comparison, differential expression was defined as $|\log_2(\text{ratio})| \geq 1$ with the FDR set to 5%. Gene ontology was performed with The Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov/home.jsp) using default settings (Huang et al., 2009b; 2009a).

**Primers used for chromatin immunoprecipitation**

| Name | SEQUENCE |
|---|---|
| Chip_OrbF | AGCCAGGCGAAAAAGGTTAT |
| Chip_OrbR | TGGCACCACATTAACTCGAA |
| chip_DlF | GAGCGAGTACGTTTCGTGGT |
| chip_DlR | CGCGGTAATCTGCAATTCTT |
| chip_NeurF | CTTATGCGAGGGCTGGTCTA |
| chip_NeurR | TTACGCGACATCCCATGTTA |
| chip_RELF | AGCAGTGGCGCACTAAAGTT |
| chip_RELR | CGAGATGACTCACGGGTTTT |
| chip_NothcF | ACCGCTATGACGGCACTAAA |
| chip_NothcR | TCTGTTTCAAATCGGCAGTG |

| | |
|---|---|
| chip_Su(H)F | CCTGCGGCCACATATCTATT |
| chip_Su(H)R | TTCTTCCATTCGGAACCAGT |
| C15_Fp | AATCCGTGAATCCAAAGCAG |
| C15_Rp | CTCATGGTCATCCTCCTCGT |
| c_Up13689F | CCTGCGGCCACATATCTATT |
| c_Up13689F | TTCTTCCATTCGGAACCAGT |
| c_13689F | TTTCTGGCCACATTTTGGAT |
| c_13689R | TCGAGATGTATGTGGCTTCG |
| c_Dw13689F | ATCGCATTGCTCGATAGTCC |
| c_Dw13689R | CCAACTGTGATTCCCCAGAT |
| c_Up4380F | CTCAGTCCATCCAGGAGAGC |
| c_Up4380R | TAGCATGGATCCTCCGTTTC |
| c_4380F | CCTGCGCCACCTAGTTAAAA |
| c_4380R | ACTTGTTGCACATCCCCAGT |
| c_Dw4380F | TTAACAGATCCCCCATTGCT |
| c_Dw4380R | AGTTCTTCCGCAGCATTCTC |
| ch_VGK_F | GAGCCCGGAGTCACATCTAC |
| ch_VGK_R | CTCGGCTCCACTCGTAAAAG |
| ch_CG25C_F | TTAGTCAGCGCTGTGGAGTG |
| ch_CG25C_R | GCACAACGACTTGCTGGATA |
| ch_CBP_F | GACCGCTCTGCTTTTACAGG |
| ch_CBP_R | TTTTCGAAGATGCACCACAA |
| ch_cpo_F | TATTGGCCTATCTCGCAACC |
| ch_cpo_R | TTACGCCGTTGGGTCTTTAG |
| ch_cyrF | TCCATTGGCAATCTTTCTCC |
| ch_cyrR | GGTAGCGGAAGTCTTGATCG |
| bmaj +15558 | TAATTTGTCAGTAGTTTAAGGTTGCA |
| bmaj -115805 | CATTGTTCACAGGCAAGAGCAGG |
| Ccna2F-1000 | GGGGTGGGGTAGTTTACTGG |
| Ccna2R-1000 | GAGGCAGGGTCTCATAATGC |
| Ccna2F CCRE | AATAGTCGCGGGCTACTTGA |
| Ccna2R CCRE | CTCCGGTCCGGTTCTGTC |
| C-1500Exosc9F | ATGTGTGCACCGTATCCTCA |
| c-1500Exosc9R | TTGGTTTATGGGCTGGAGAC |
| c-3ExosC9F | CTGACAGGAGACATGGCAGA |
| c-3ExosC9R | AGGCCTCTCATGCTGACCTA |
| c-3Bbs7F | GAGCTCCGGGCTTTCTAAGT |
| c-3Bbs7R | AGATGTGCAGGCTGCTTTTT |
| myodP2F | CAGGCTTGCTCACCTAGACC |
| myodP2R | AGACCAGGCAAAGCATAGGA |

**Primers used for RT-qPCR**

| | |
|---|---|
| Copia_F | GGAGGTTGTGCCTCCACTTA |
| Copia_R | CTCTTGGAGACGCTTTACGG |
| Burdock_F | CTCCTCGCCGAAAATGATAG |
| Burdock_R | ATGGTCCTCGAATCGTTCTG |
| Tirant_F | GAAAAACTGCACCTCGAAGC |
| Tirant_R | TGTGGACGATGTGTTTCGTT |
| LTR_Retro_F | TGGGATCCGCTGAAACTATC |
| LTR_Retro_R | TTGCTTGCATTCTTTGTTGC |
| Gypsy_F | CGTATTTGGGTGGTCCAAAC |
| Gypsy_R | TAGCGTTGAAAGAGGGCAGT |
| Line_F | TCCAACTACAACCGCATTCA |
| Line_R | AGGCCTAGCAGGGAGTAAGC |

| | |
|---|---|
| Roo_F | TAAGGCGGAGGAGTTACCAA |
| Roo_R | TCCAGAGCCGAGCAGTAAAT |
| DM297_F | TGGACGGACAAATTACACGA |
| DM297_R | TCCGATTGGTTACCTTCCAG |
| Jockey_F | CGCTGTGCCAGCTGTAAATA |
| Jockey_R | TGTTTTGAGGGCTGAGCTTT |
| Actin_F | TGCCCATCTACGAGGGTTAC |
| Actin_R | GCCATCTCCTGCTCAAAGTC |
| GAPDH_F | TCAAGGCTAAGGTCGAGGAG |
| GAPDH_R | ACCGAACTCGTTGTCGTACC |
| rt_CG25C_F | GTTGTACGTCGATGGCAATG |
| rt_CG25C_R | GTACTGGCGGATCTCGATGT |
| rt_CBP_F | CGGCAACAATAACACCAATG |
| rt_CBP_R | GGACAAGTGTTGCCGAAAAT |
| rt_cpo_F | CTGACCTTATGCGACAACGA |
| rt_cpo_R | AGGCCGCTATGTGACTGACT |
| rt_VGK_F | TGGACGCTGCAACTACTACG |
| rt_VGK_R | ACCATCTGTGAAACCGGAAG |
| rt_cyr_F | TGGGTCTGGGAGTAGGACTG |
| rt_cyr_R | CGGATAGTAGGGCATCAGGA |
| rt_pk_F | TCCAGCTCGGAGGACTACAT |
| rt_pk_R | TGCAGTTCTTGTCCTTGTCG |
| rt_CG6282F | GCGAGACGACCCCAATATAA |
| rt_CG6282R | AGTCGCCTTCAAAGTGCTGT |
| rt_17350F | GCAAACGAAAGAGTCCAAGG |
| rt_17350R | CCCTTGAAAATTGGCACACT |
| rt_spn43AbF | AATCGCAAGGATGGTCTGAG |
| rt_spn43AbR | ACATGTTGCCAAAGTCACCA |
| rt_CG4680F | TTCAGCTACAATGGCACCAG |
| rt_CG4680R | TCCAAGGGATTAGGTCATGC |
| rt_C3GF | ACCGATCTCAAAGAGCTCCA |
| rt_C3GR | CTGCTCAATGCGATCTTCAA |
| rt_Cp1F | GAGGCATCGATACCGAGAAG |
| rt_Cp1R | TAGACGCCCTCCGAGTAGAA |
| rt_Fur1F | CCCTGCAACTGACGGTTAAT |
| rt_Fur1R | CCATTGATTGAATCCGGAAC |
| rt_OrdF | GCAATATGTGACGGCCTTTT |
| rt_ordR | AGGTTCTGGAGGAGTCAGCA |
| rt_HerF | GAATTGGCAGTTCACCACCT |
| rt_HerR | GTGTTGCAGAAATTGGGACA |
| rt_alyF | AGAGGATGGCAATTGGTACG |
| rt_alyR | ACACGCTTGGGTATCTGGTC |
| rt_wF | CTAATATCCTGCGCCAGCTC |
| rt_wR | ACGGAACCATGAGAGGTACG |
| rt_CG12200F | ATCGCTTACCACGAGAATGC |
| rt_CG12200R | TTAGTGTCGCTGCCAAAAGA |
| rt_spn47CF | CGGATTTGAGCGATCTTTTC |
| rt_spn47CR | AATGGCCAACGAAGTAAACG |
| rt_rbp4F | CACAAACATGCCCAGACATC |
| rt_rbp4R | TCCGTTGGTCGGAGATTTAG |

**Stealth siRNA for knock down (Forward strand only)**

| | |
|---|---|
| stGFP_232 | CACAUGAAGCAGCACGACUUCUUCA |
| stGFP_415 | GGGCACAAGCUGGAGUACAACUACA |
| stGFP_696 | CGGCAUGGACGAGCUGUACAAGUAA |

| | |
|---|---|
| siMLL5A_833 | CAUGAUGACGGAUACAUGAUCUGUU |
| siMLL5B_861 | ACAAAUGCAGUGUUUGGCAACAUAU |
| siMLL5C_908 | CAGCAUAUUCCUGAUACAUACCUAU |

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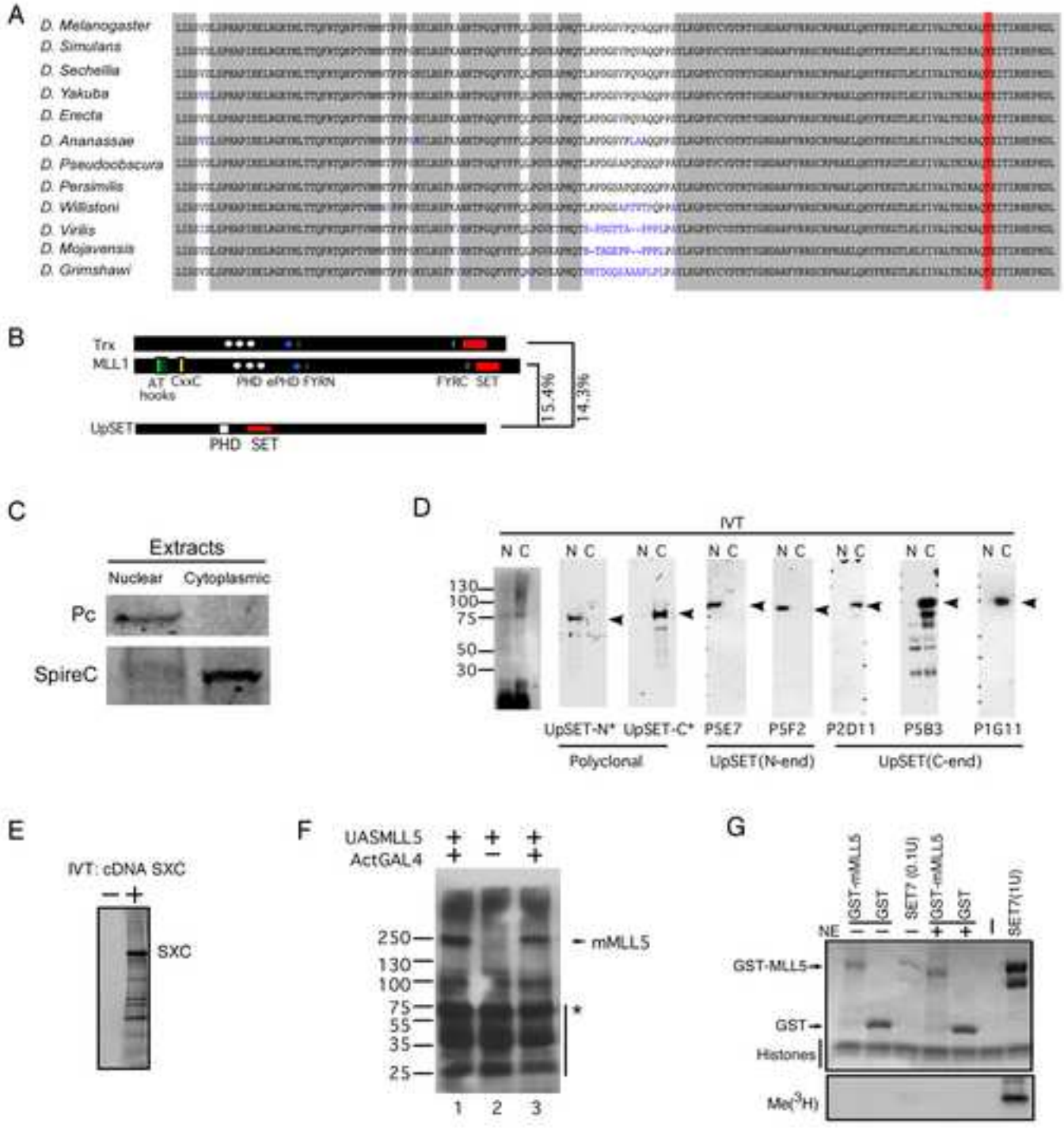
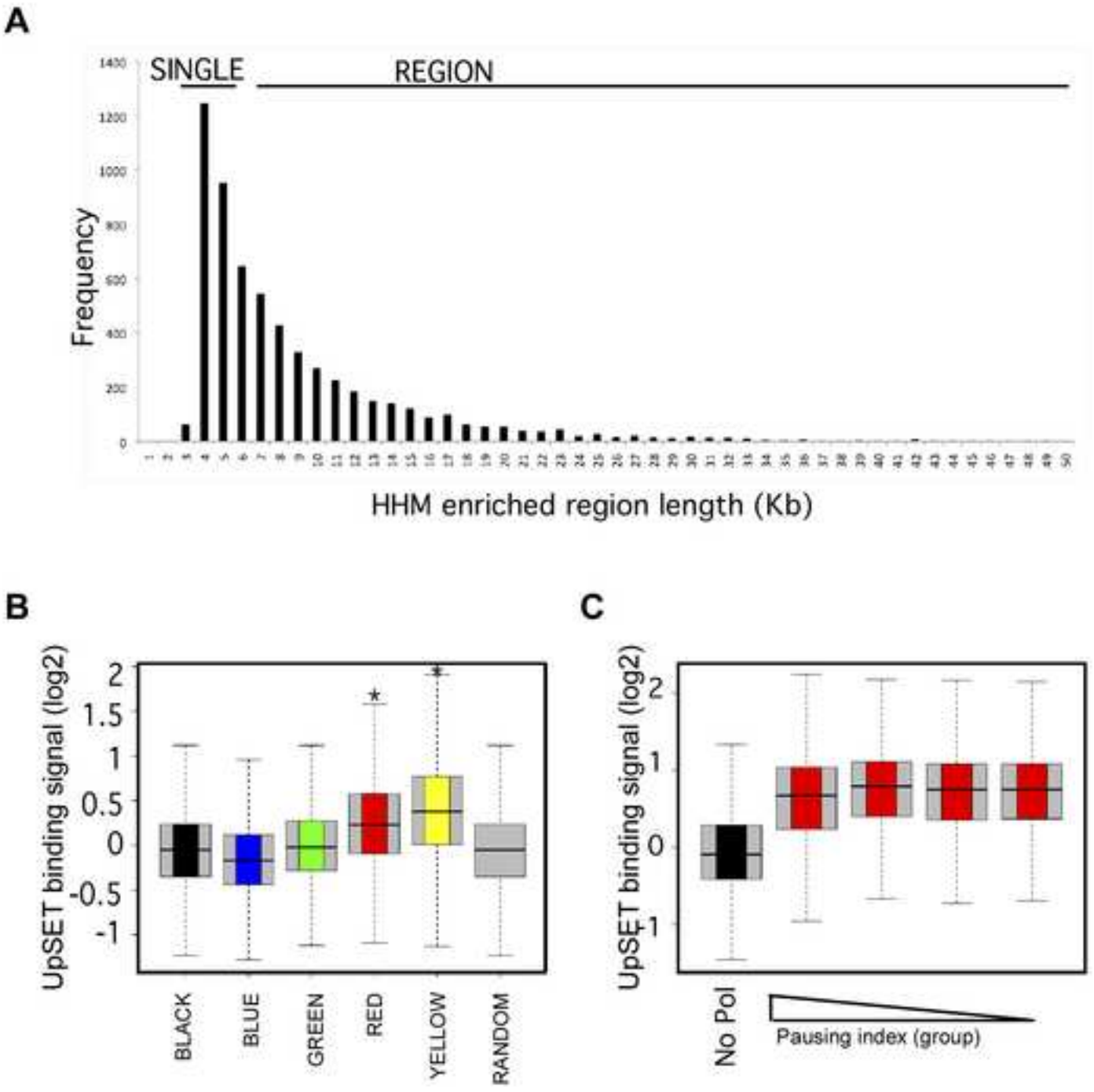
**Supplemental Figure 1**

**Figure S1.**
(Rincon-Arano et al.)



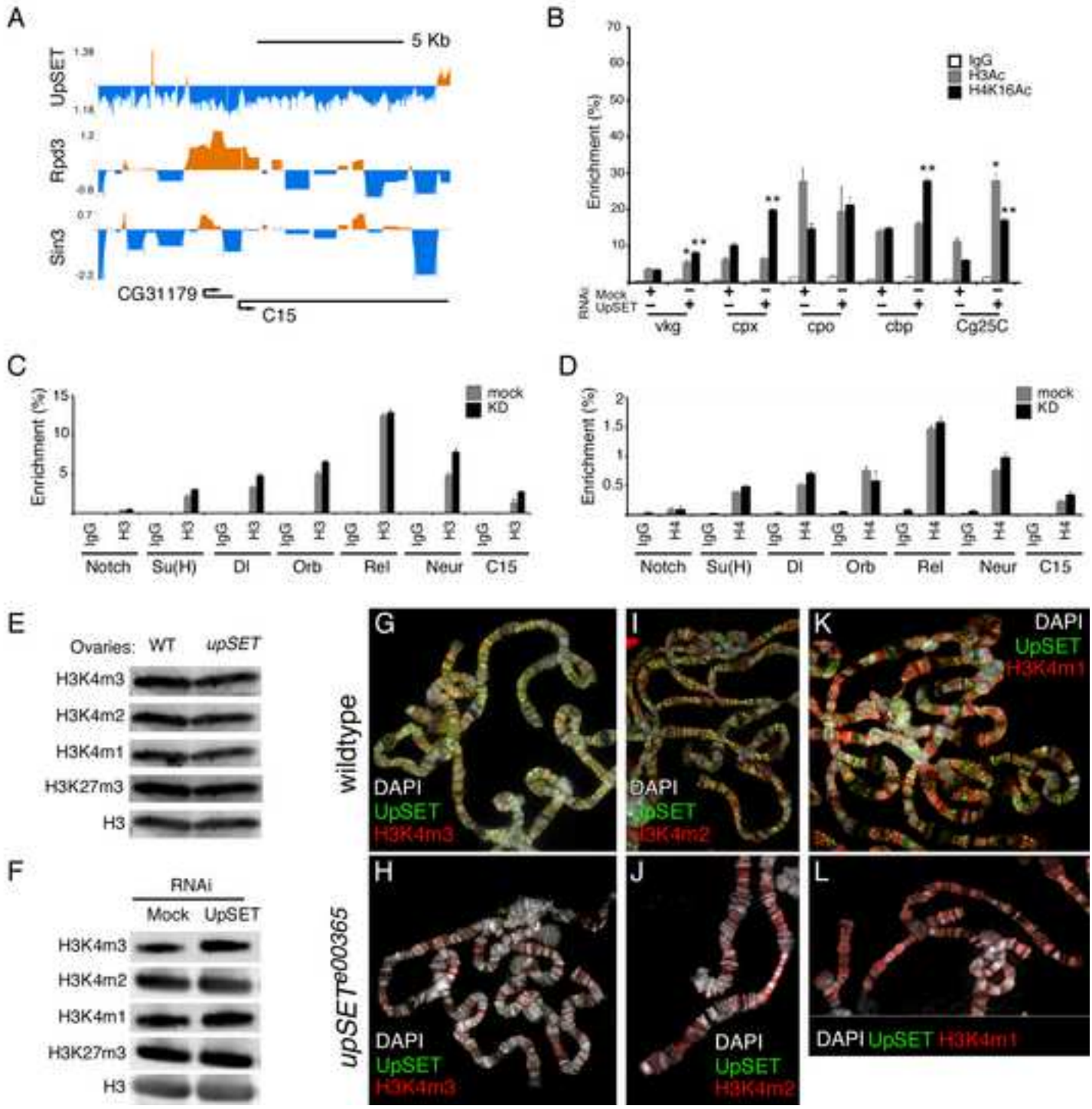
**Supplemental Figure 2**

**Figure S2.**
(Rincon-Arano et al.)



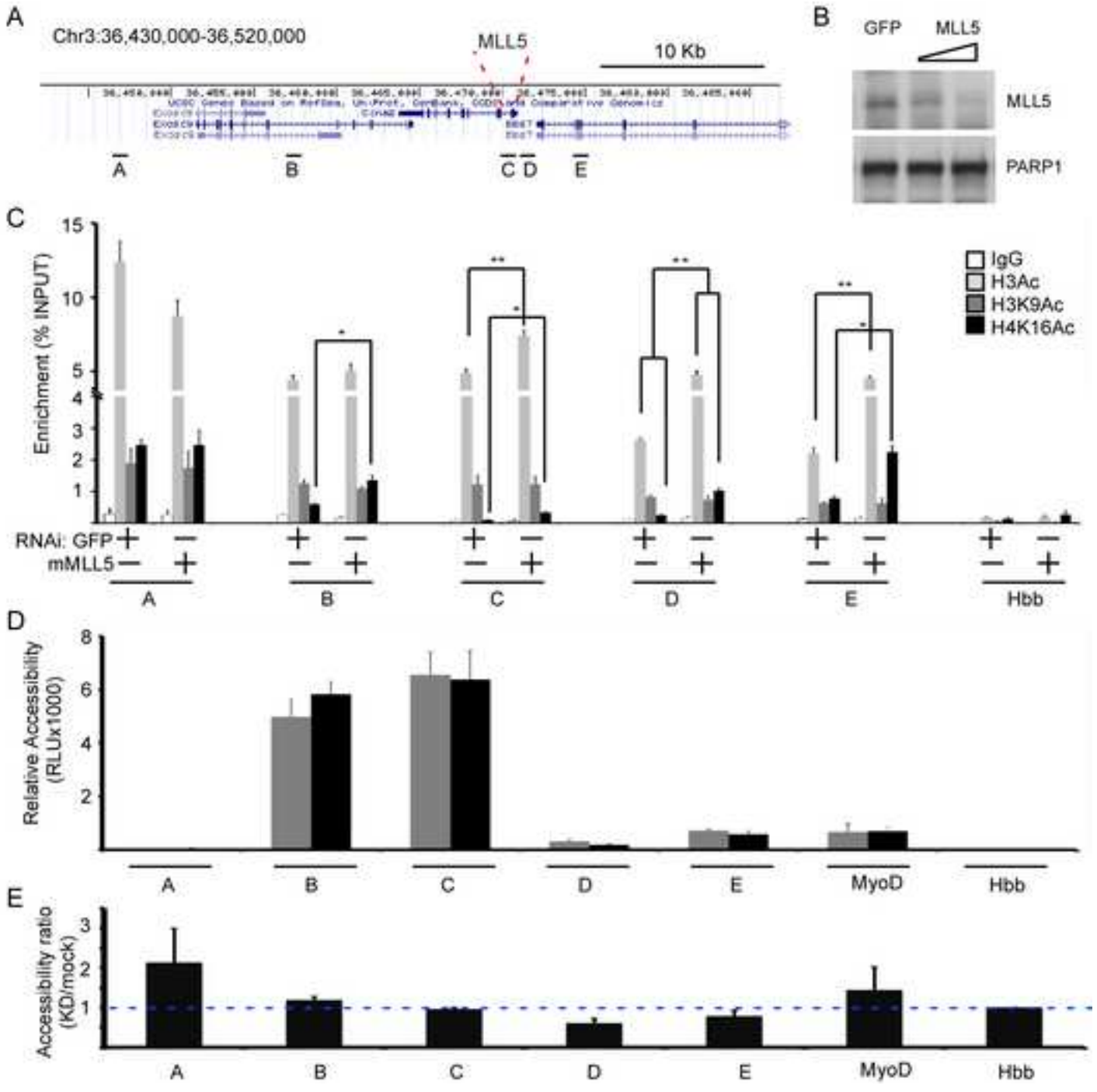
**Supplemental Figure 3**

## Figure S3.
(Rincon-Arano et al.)

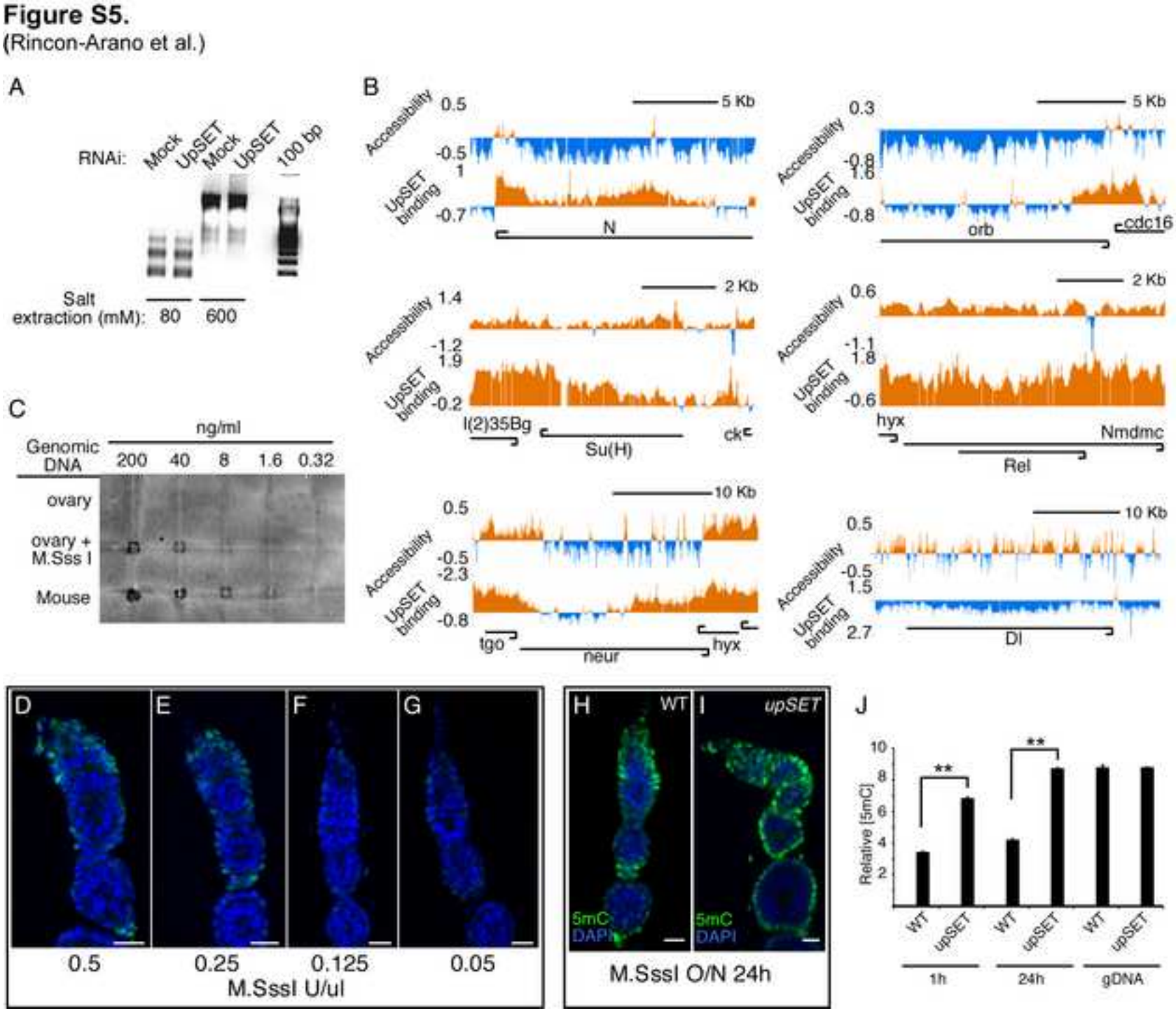


**Supplemental Figure 4**

**Figure S4.**
(Rincon-Arano et al.)

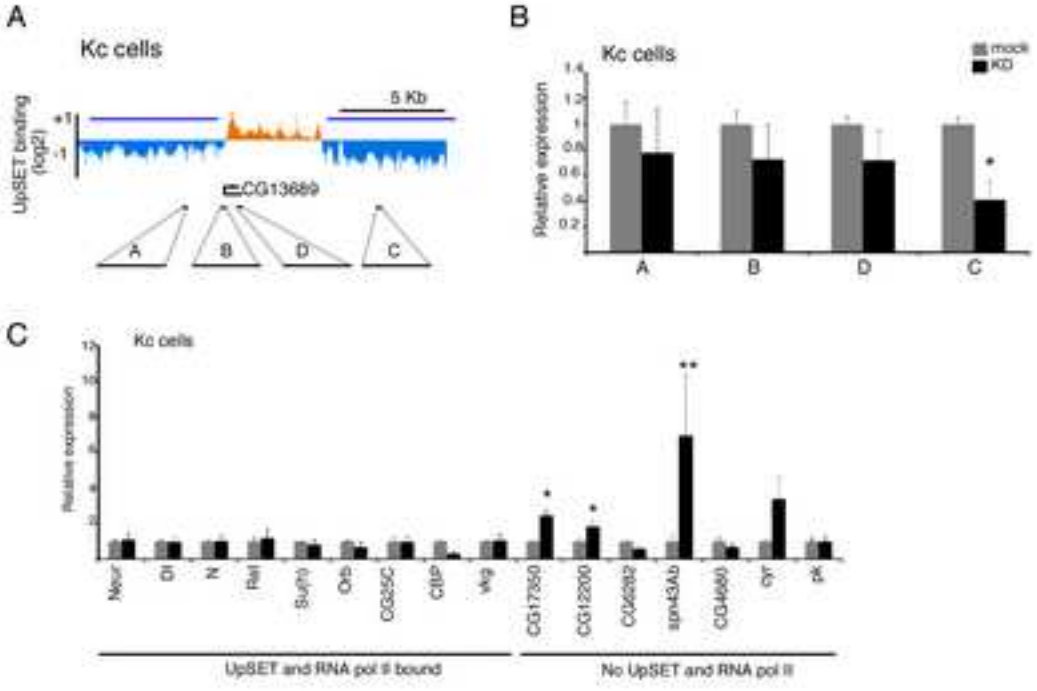


**Supplemental Figure 5**



**Supplemental Figure 6**

**Figure S6.**
(Rincon-Arano et al.)



**Supplemental Figure 7**

**Figure S7.**
(Rincon-Arano et al.)