A BRG1 peaks

B Genes associated BRG1

C

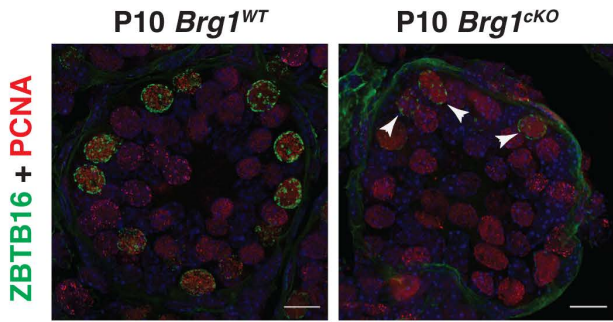**D** BRG1 occupancy at recombination hotspots

Figure S1. Features of BRG1 genomic associations. (A) Annotation of regions associated with P12 and P18 BRG1 peaks. Number (n) of P12 and P18 peaks are indicated within parentheses. (B) Distribution of temporal expression profile of genes associated with class 1-3 TSS's. PMei: pre-meiotic, Mei: Meiotic, Const: constant. (C) UCSC genome browser views depicting H3K4me3 and H3K27me3 peak associations with promoters of candidate BRG1 target genes. Thick black bars denote BRG1 Macs2 peak calls. (D) P12 (green line) and P18 (orange line) BRG1 enrichment across an 8 Kb window centered at PRDM9 peaks (purple line).
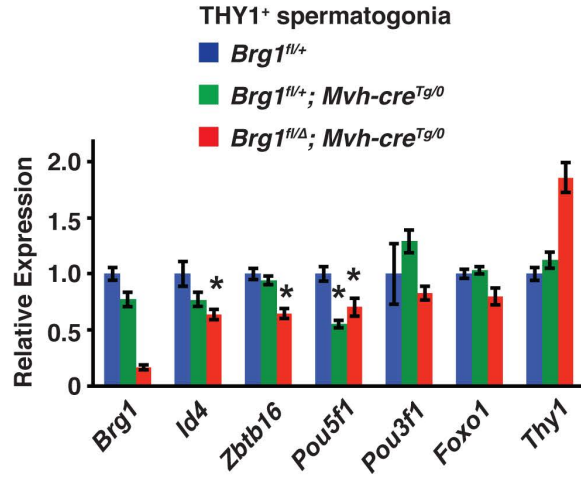
A

Staging spermatocytes

B

Expression of staging markers
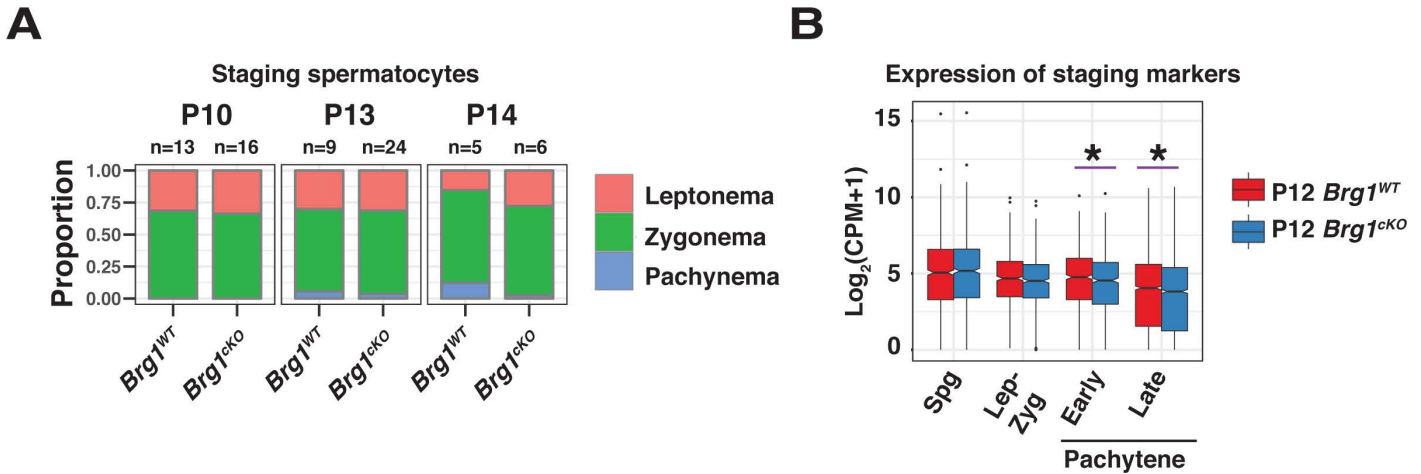
Figure S2. Comparison of meiotic cellular profiles between Brg^{WT} and $Brg1^{cKO}$. (A) Quantification of spermatocyte populations from P10, P13 and P14 Brg^{WT} and $Brg1^{cKO}$ testes cryosections. Meiotic prophase I substages were staged by γH2Ax immunostaining. Total number of tubules analyzed (n) are indicated. (B) Abundance of spermatogonial and meiotic sub stage specific protein-coding genes (x-axis) between P12 Brg^{WT} (red box) and $Brg1^{cKO}$ (blue boxes). Transcript abundance is expressed as the Log2 value of counts per million (CPM) added with a pseudo count (y–axis).
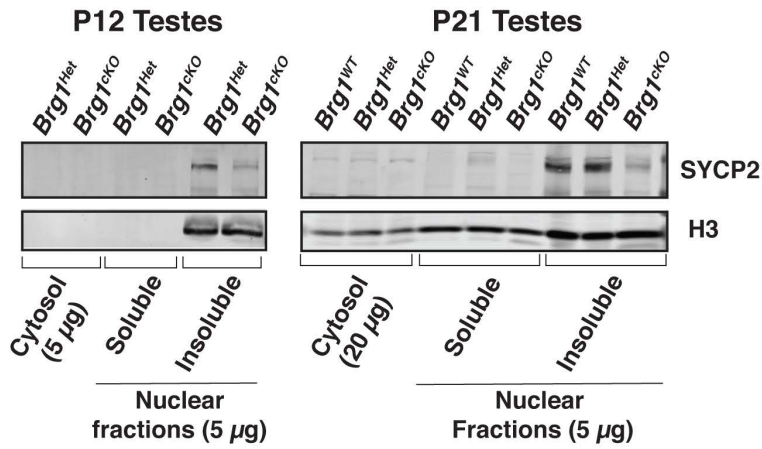
A

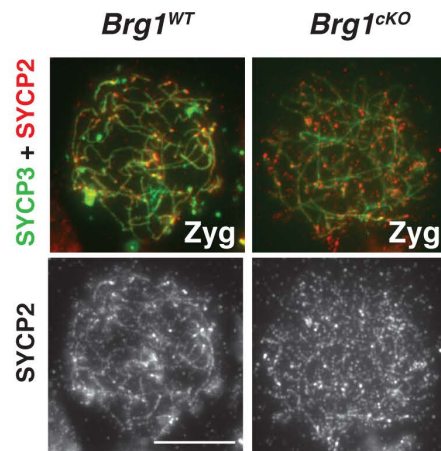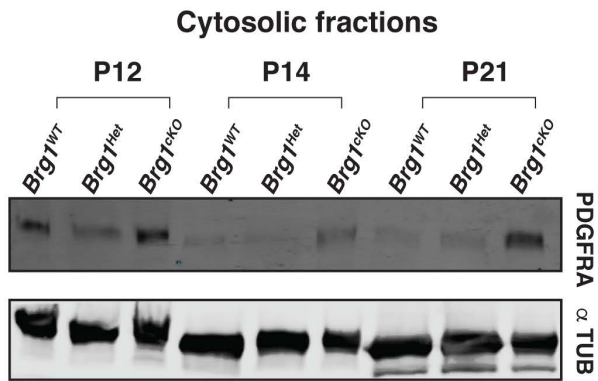P10 *Brg1^{WT}*   P10 *Brg1^{cKO}*

ZBTB16 + PCNA

B

THY1^{+} spermatogonia

- *Brg1^{fl/+}*
- *Brg1^{fl/+}; Mvh-cre^{Tg/0}*
- *Brg1^{fl/Δ}; Mvh-cre^{Tg/0}*

Relative Expression

Brg1 *Id4* *Zbtb16* *Pou5f1* *Pou3f1* *Foxo1* *Thy1*

C

P12 Testes   P21 Testes

SYCP2
H3

Cytosol (5 µg)   Soluble   Insoluble
Nuclear fractions (5 µg)

Cytosol (20 µg)   Soluble   Insoluble
Nuclear Fractions (5 µg)

D

Brg1^{WT}   *Brg1^{cKO}*

SYCP3 + SYCP2

Zyg   Zyg

SYCP2

E

Cytosolic fractions

P12   P14   P21
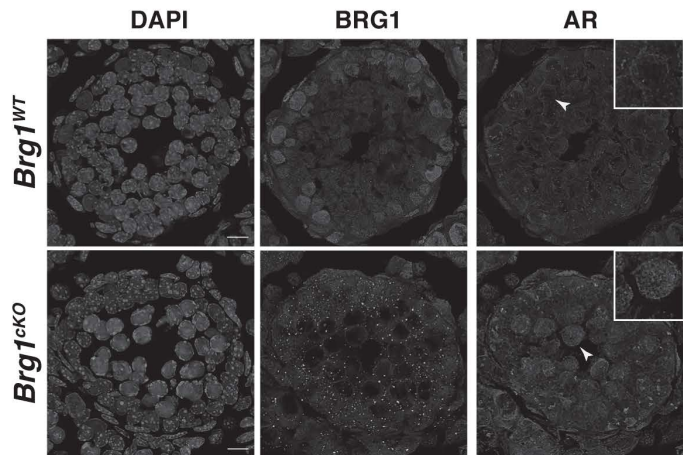
PDGFRA
α TUB

F

DAPI   BRG1   AR

Brg1^{WT}

Brg1^{cKO}

Figure S3. Transcriptional response to the loss of BRG1 in the male germ line. (A) P10 Brg^{WT} and $Brg1^{cKO}$ testes cryosections (63x objective Scale bar: 10 µm), immuno-labeled for ZBTB16 (green), PCNA (red) and counter stained with DAPI (blue). (B) Quantitative RT-PCR analysis to determine the transcript abundance (y-axis) of candidate stem cell factors (x-axis) in Brg^{Het} and $Brg1^{cKO}$ relative to Brg^{WT} spermatogonia (THY1$^+$). The transcript abundance of candidate factors was normalized to genes constantly expressed (*Sdha* and *Ywhaz*). * denotes a p-value < 0.05, calculated using an unpaired students t-test. (C) Western blot showing the abundance of SYCP2 in sub-cellular fractions obtained from $Brg1^{WT}$, Brg^{Het} and $Brg1^{cKO}$ spermatogenic cells. Nuclear loading control: Histone- H3. (D) Brg^{WT} and $Brg1^{cKO}$ zygotene spermatocytes immunofluorescently labeled for SYCP2 (red) and SYCP3 (green). Images were captured using a 100x objective Scale bar: 10 µm. (E) Western blot showing the abundance of PDGFRA in cytosolic fractions obtained from P12, P14, P21 Brg^{WT}, Brg^{Het} and $Brg1^{cKO}$ spermatogenic cells. Loading control: α TUBULIN. (F) P13 Brg^{WT} and $Brg1^{cKO}$ testes cryosections (63x objective Scale bar: 10 µm), immuno-labeled for BRG1, AR and counter stained with DAPI (blue).Panel insets highlight Brg^{WT} and $Brg1^{cKO}$ germ cells.

A
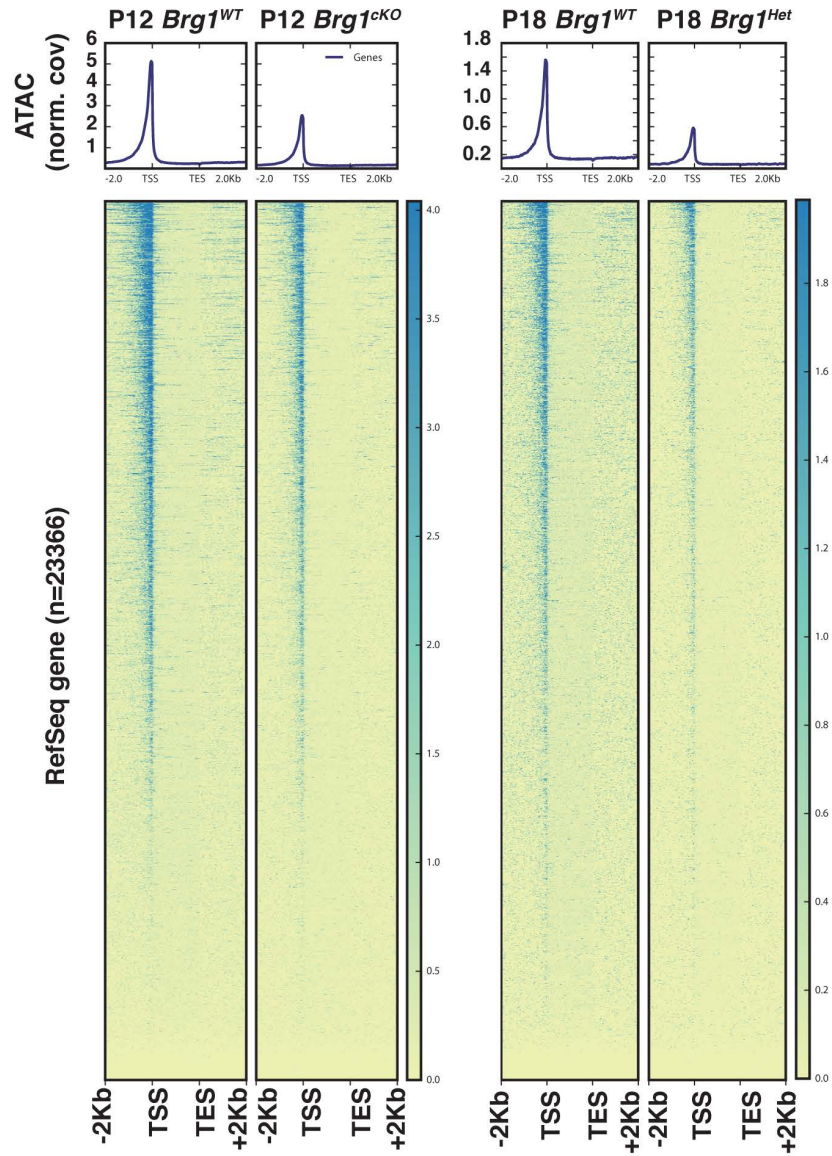
B
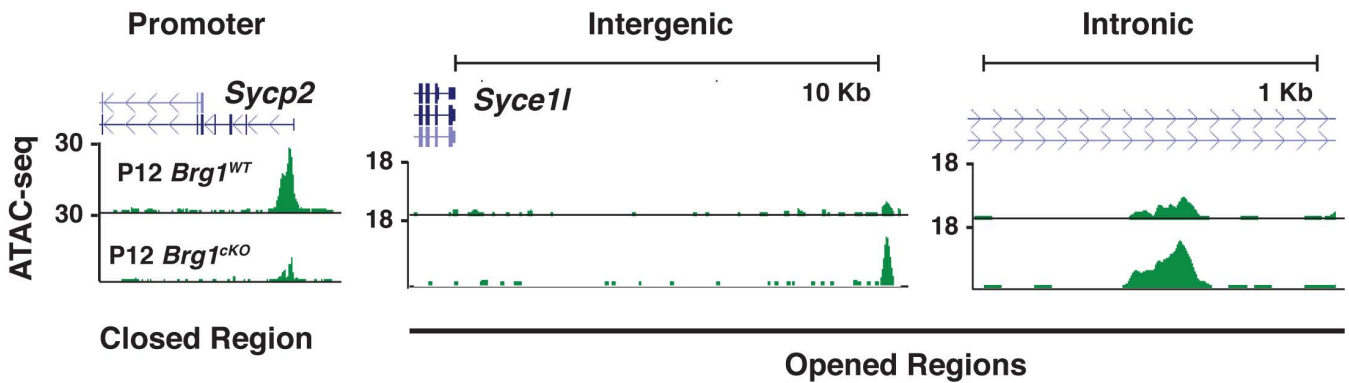
Figure S4. BRG1 directed changes in chromatin accessibility (A) Metaplots (top) and corresponding heatmaps (bottom) depicting the pairwise comparisons of normalized ATAC-seq signal at RefSeq genes ± 2Kb, between P12 $Brg1^{WT}$ and $Brg1^{cKO}$ spermatogenic cells, P18 $Brg1^{WT}$ and $Brg1^{Het}$ ($Brg1^{fl/\Delta}$) spermatogenic cells. TSS: Transcription start site, TES: Transcription end site. (B) UCSC browser view of candidate closed and opened regions.

A

	Protein	# Unique Peptide	% Coverage
Shared SWI/SNF sub units	BAF60A	37	82
	BAF60B	26	60
	BAF60C	19	45
	BAF45A	14	36
	BAF45B	10	38
	BAF45C	8	33
	BAF45D	15	49
	BAF57	20	51
	BAF53A	22	55

B
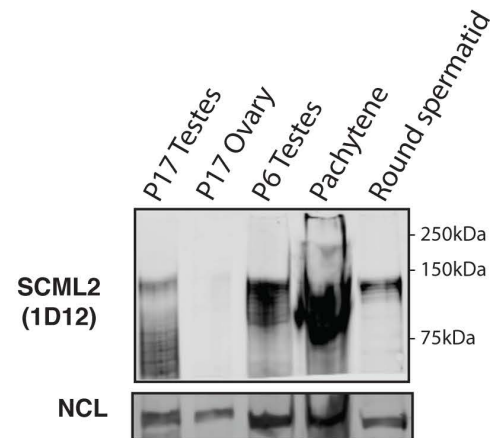
C
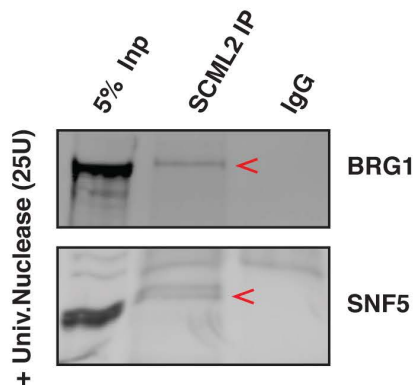
D
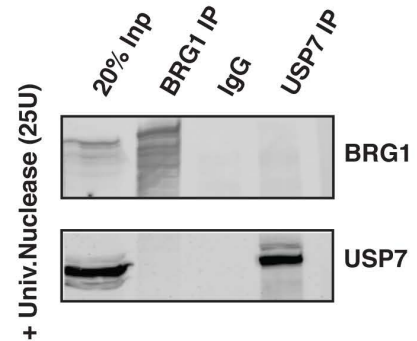
Figure S5. Summary and validation of IP-MS. (A) List of known SWI/SNF subunits identified by IP-MS that are common to BAF and PBAF subcomplexes. (B) Validation of SCML2 antibody (1D12) specificity on nuclear extracts prepared from testes, ovaries and purified spermatogenic cells. Nucleolin (NCL) was used as a nuclear marker. (C) SCML2 Co-immunoprecipitation of BRG1 and SNF5. (D) BRG1 and USP7 co-immunoprecipitations. Negative control: IgG. All lysates were treated with 25 U of universal nuclease (benzonase) prior to IP

A

BRG1 Enrichment

pach *Scml2* Act   pach *Scml2* Rep

$\log_2$ (BRG1/Input)

— P12 BRG1   — P18 BRG1

B

Promoter accessibility

pach *Scml2* Act   pach *Scml2* Rep

ATAC Read Coverage

— P12 *Brg1*^{WT} — P12 *Brg1*^{cKO}
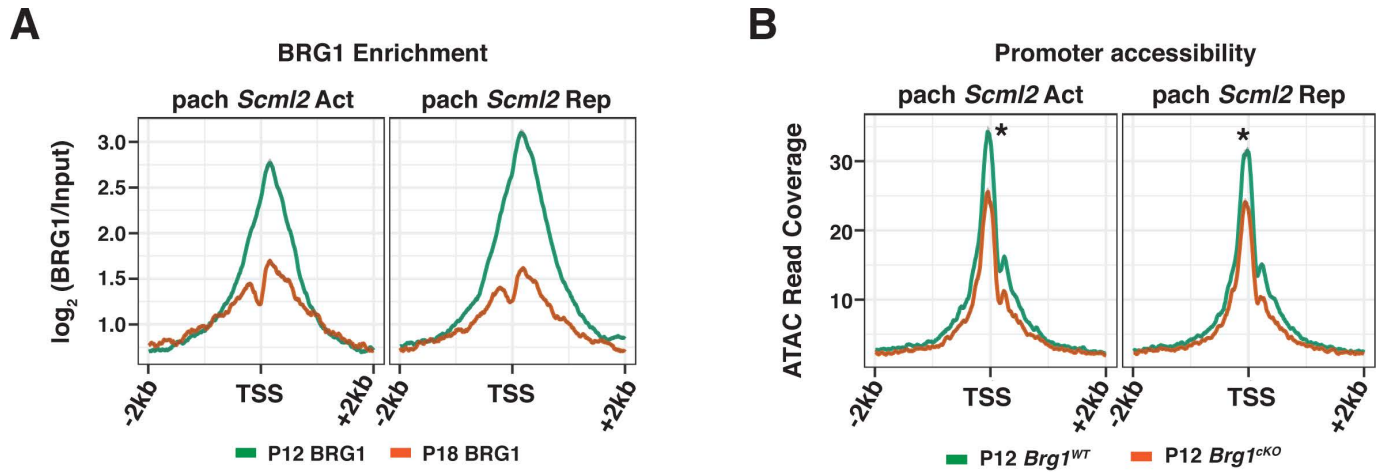
C

Enriched GO terms - Concordant genes

spindle checkpoint
positive regulation of peptidyl–threonine phosphorylation
mitotic spindle assembly checkpoint
mitotic sister chromatid segregation
spindle assembly checkpoint
regulation of hormone levels
response to wounding
wound healing
cellular response to oxidative stress
negative regulation of transport

Act   Rep

p.adjust
0.03
0.02
0.01

GeneRatio
0.08
0.10
0.12

Figure S6. BRG1 association with SCML2 regulated genes. (A) BRG1 enrichment (P12: green, P18: orange) at TSS ± 2 Kb, of genes differentially regulated by SCML2 in pachytene spermatocytes. (B) ATAC seq coverage from P12 *Brg1^{WT}* (green) and *Brg1^{cKO}* (orange) at TSS ± 4 Kb, associated with genes regulated by SCML2 in pachytene spermatocytes. pach *Scml2* Act: Activated, pach *Scml2* Rep: Repressed. (C) Enriched gene ontology terms associated with genes concordantly activated or repressed by BRG1 (P12) and SCML2 (pachynema).

A
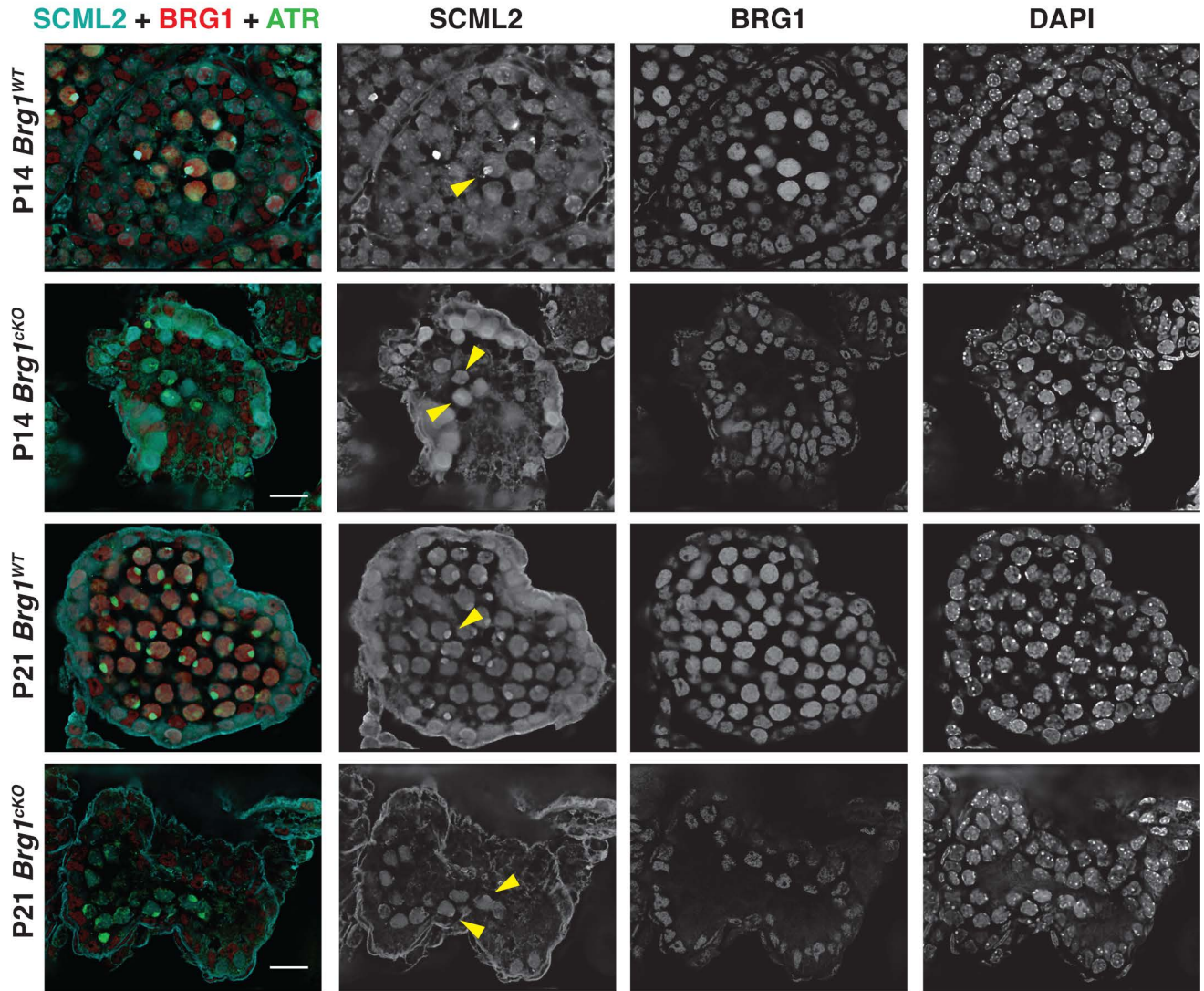
B
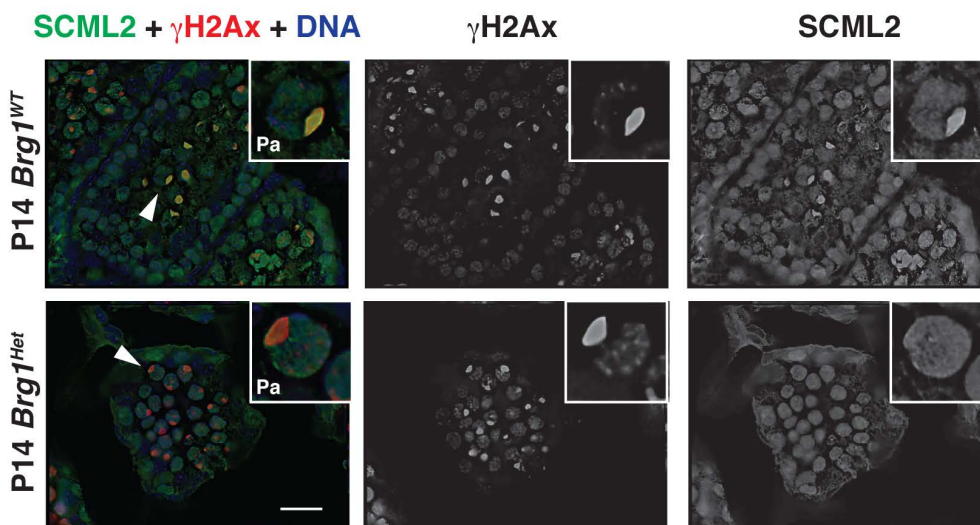
Figure S7. Validating the role of BRG1 in the recruitment of SCML2 to the sex body. Cryosections prepared from (A) P14 and P21 *Brg1^WT^* and *Brg1^cKO^* testes, immunofluorescently labeled for SCML2 (cyan), BRG1(red) and ATR (green), (B) P14 *Brg1^WT^* and *Brg1^Het^* testes, immunofluorescently labeled for SCML2 (green) and γH2Ax (red), DNA was stained with DAPI. Arrowheads label pachytene spermatocytes with completely formed sex body. Images were captured using a 63x objective. Scale bar: 20 µm.
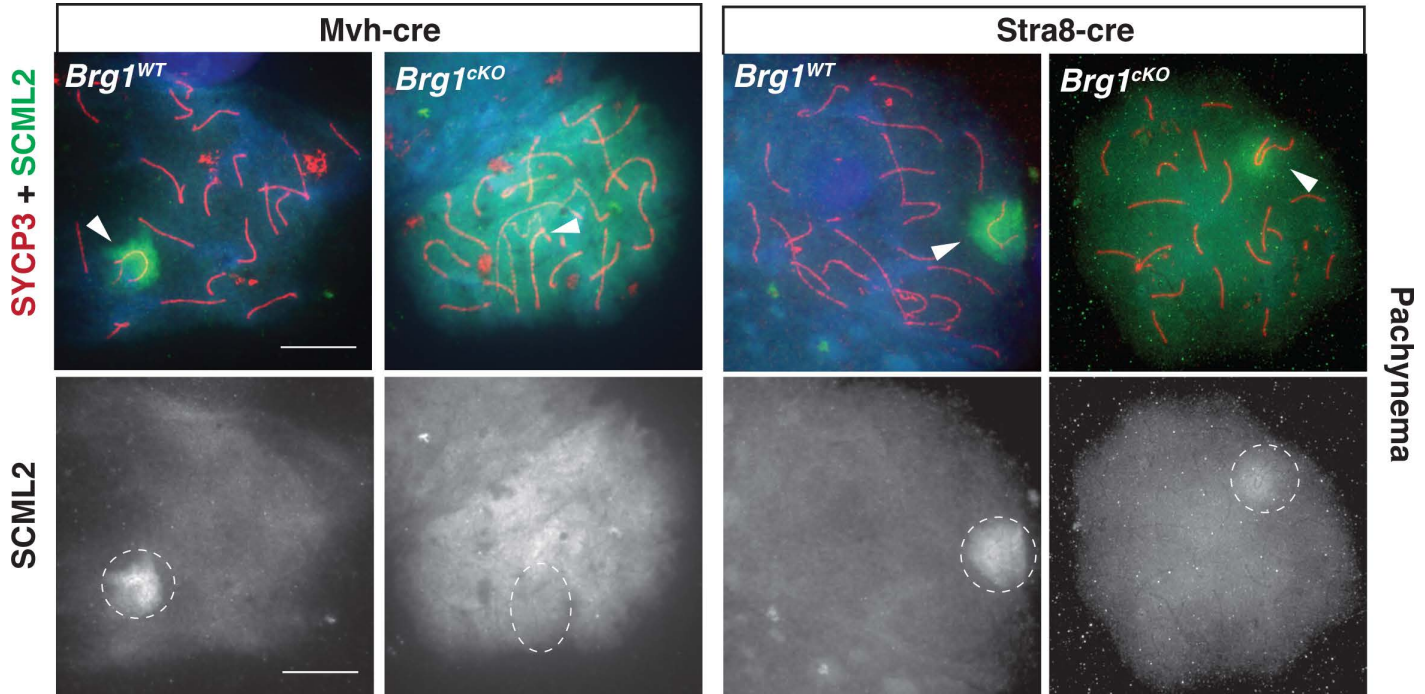
Figure S8. Analysis of SCML2 localization in meiotic spreads. Pachytene spermatocytes from *Mvh-Cre* (left panel) and *Stra8-Cre* (right panel) induced $Brg1^{cKO}$ and $Brg1^{WT}$ testes, immunofluorescently labeled for SCML2 (green) and SYCP3 (red). Arrowheads denote the sex chromosomes and dotted circle outlines the SCML2 signal around the sex body. Images were captured using a 100x objective. Scale bar: 20 µm.

A

C

B

D
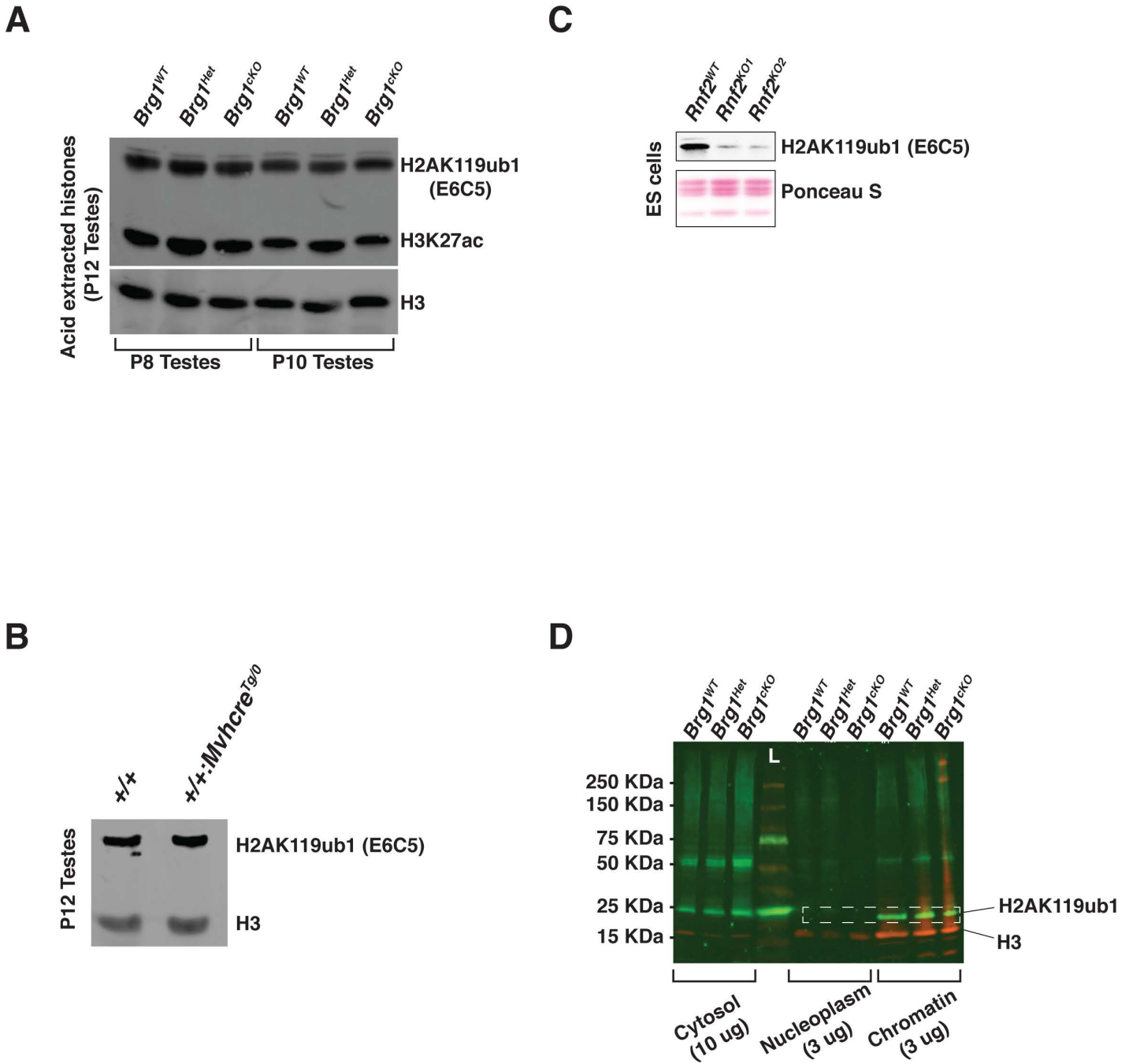
Figure S9. Temporal regulation of H2AK119ub1 in testes and validation of anti- H2AK119ub1 (clone E6C5) . (A) Western blot depicting H2AK119ub1, H3K27ac abundance in acid extracted histones obtained from P8,P10 *Brg^WT^*, *Brg^Het^* and *Brg1^cKO^* testes. H3 was used as a loading control (B) Western blot showing H2AK119ub1 and H3 abundance in acid extracted histones

obtained from testes of P12 wild type mice carrying a *Mvh-Cre* transgene and their littermate controls (+/+). (C) Validation of anti-H2AK119ub1 (clone E6C5) specificity by immunoblotting for total H2AK119ub1 in acid extracted histones obtained from $Rnf2^{WT}$ and two different $Rnf2^{KO}$ ES cell lines. Blots were stained with Ponceau S to show total histone levels. (D) Western blot using anti-H2AK119ub1 (clone E6C5), to show H2AK119ub1 (green) levels in sub-cellular fractions of spermatogenic cells obtained from P12 $Brg1^{WT}$, $Brg1^{Het}$ and $Brg1^{cKO}$ testes. H3 (red) was used as nuclear loading control. Dotted rectangle labels the nuclear levels of H2AK119ub1 levels. L: ladder.
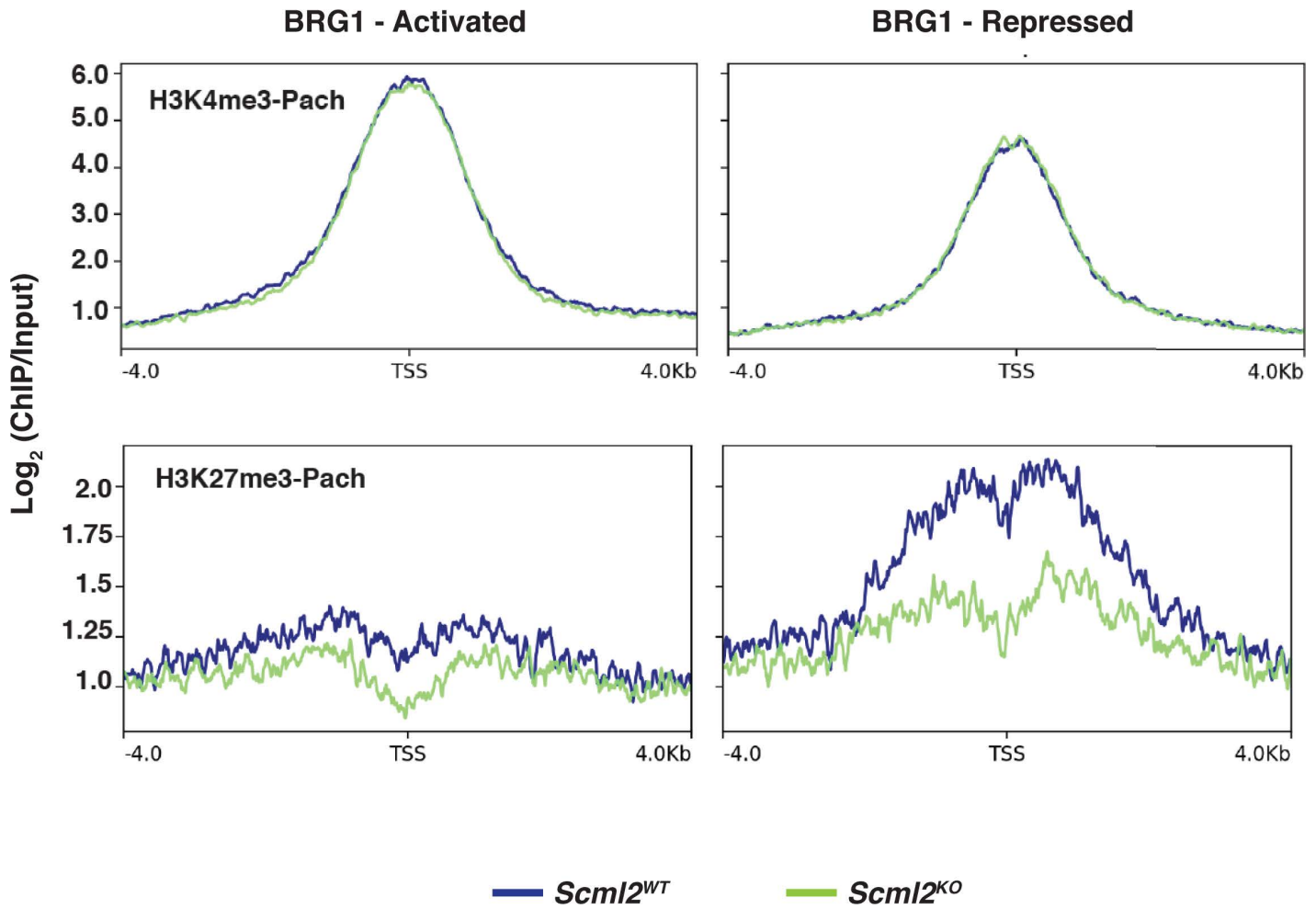
Figure S10. H3K4me3 and H3K27me3 enrichment from *Scml2^{WT}* and *Scml2^{KO}* pachytene spermatocytes. Enrichment is expressed as Log2 ratio of ChIP/Input (Y-axis) in *Scml2^{WT}* (blue) and *Scml2^{KO}* (green) pachytene spermatocytes at TSS ± 4 Kb of genes transcriptionally regulated by BRG1 (FDR<0.05).

A
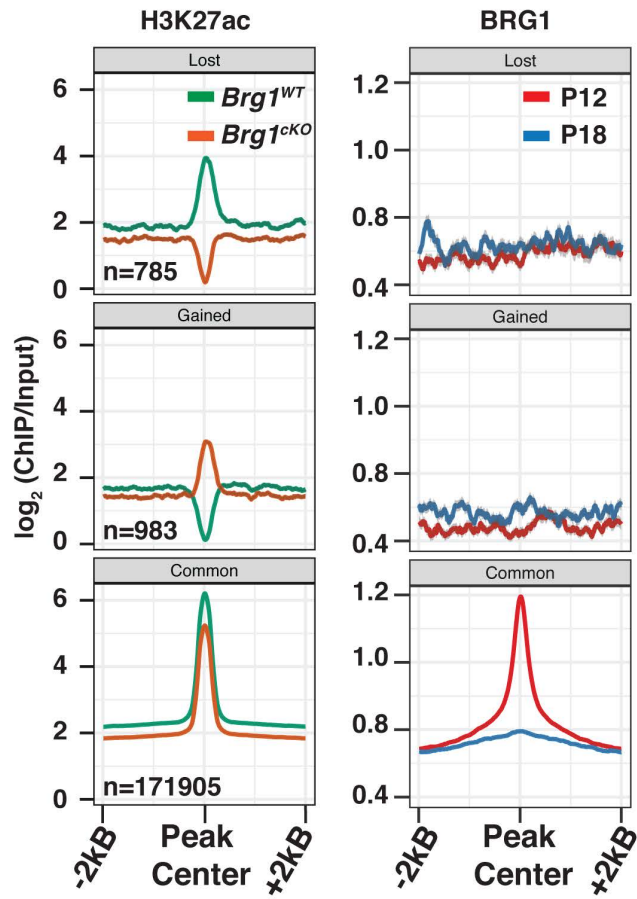
B
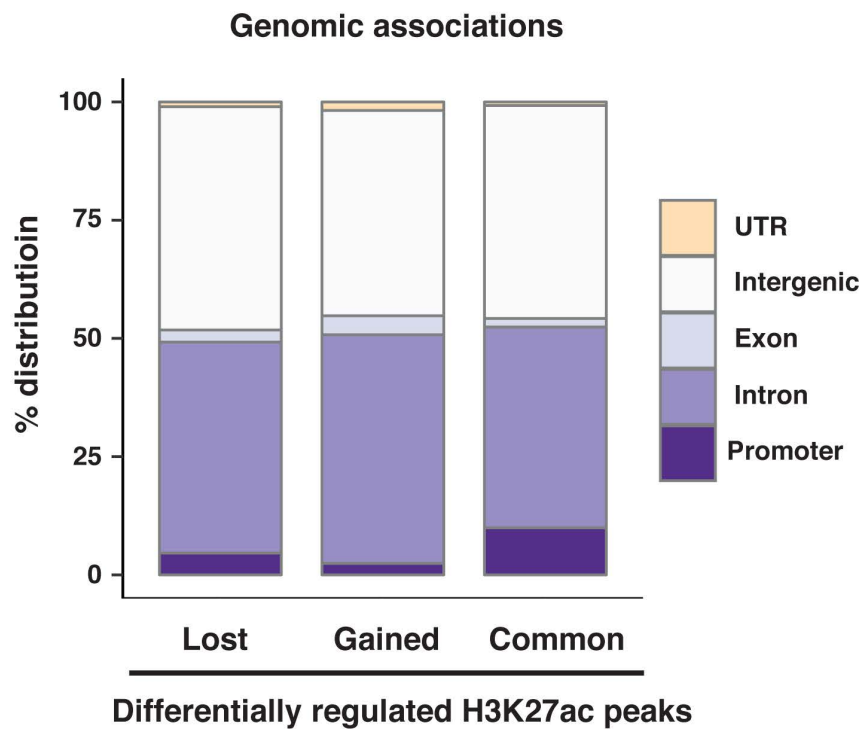
Figure S11. Differential analysis of H3K27ac peaks. (A) H3K27ac (left) and BRG1 (right) enrichment at lost, gained and common H3K27ac peaks. (B) Genomic associations of lost, gained and common H3K27ac peaks.

A
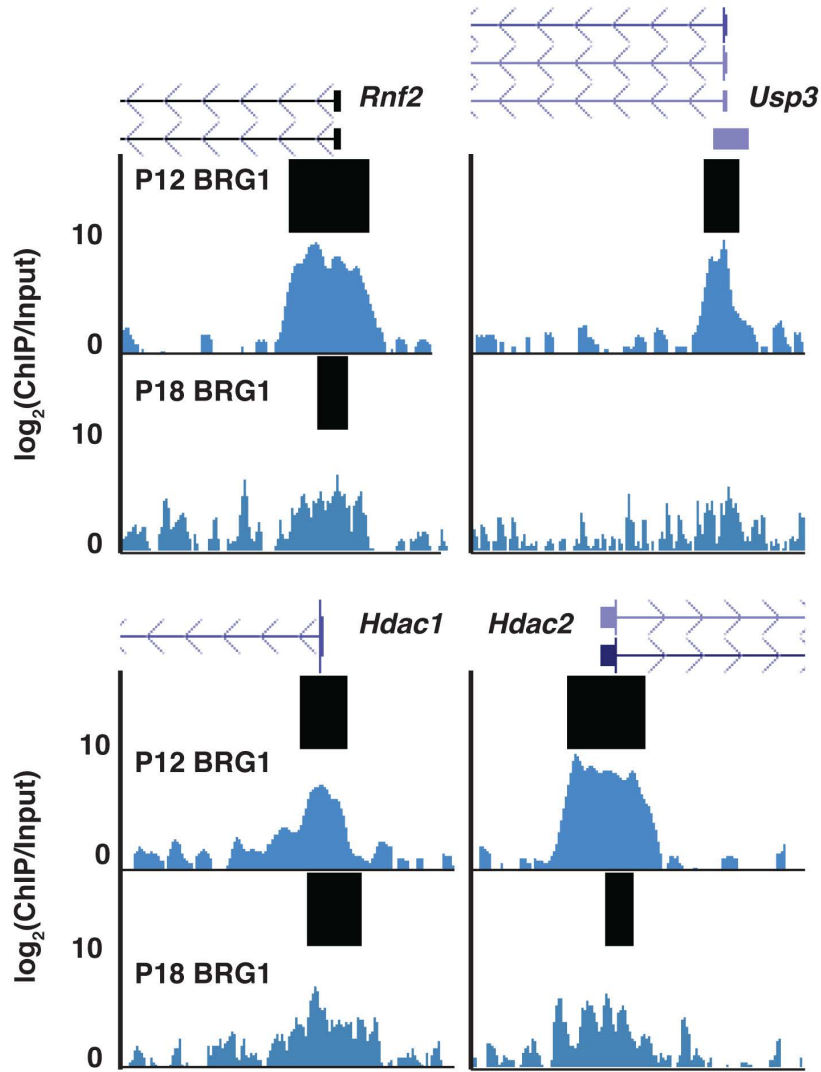
B
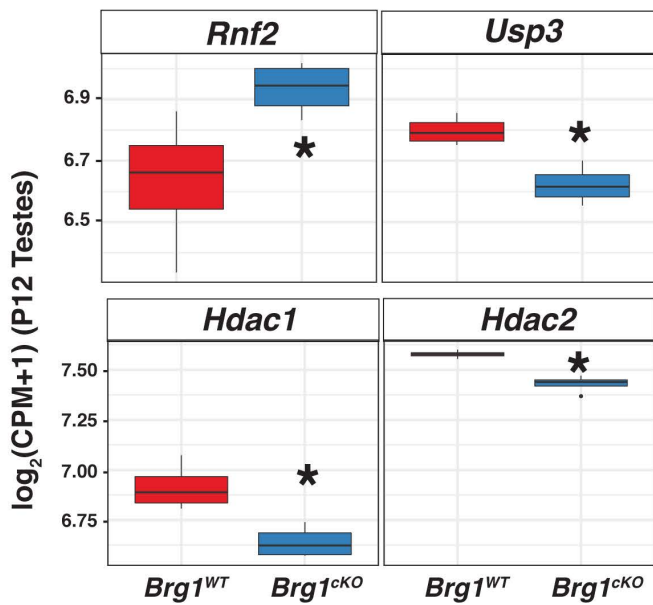
C
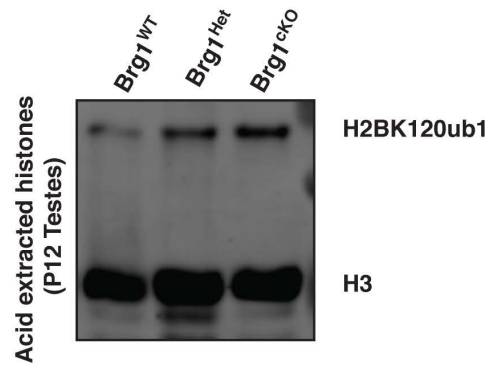
Figure S12. BRG1 regulates the expression of epigenetic modifiers of H2AK119ub1, H2BK120ub1 and H3K27ac. (A) UCSC browser view depicting BRG1 occupancy at *Rnf2*, *Usp3*, *Hdac1* and *Hdac2* promoters. Thick black bars label BRG1 peaks. (B) Transcript abundance (y-axis) of *Rnf2*, *Usp3*, *Hdac1* and *Hdac2* in P12 *Brg1^{WT}* (red box) and *Brg1^{cKO}* (blue box). Transcript abundance is expressed as the Log2 value of normalized abundance in counts per million (CPM) added with a pseudo count. (C) Western blot depicting H2BK120ub1 levels in acid extracted histones obtained from P12 *Brg^{WT}* , *Brg^{Het}* and *Brg1^{cKO}* testes. H3 was used as a loading control.

Table S1. List of genes differentially regulated by BRG1 at P12.

Click here to Download Table S1

Table S2. List of regions displaying significant differences in chromatin accessibility in the *Brg1^cKO^* relative to *Brg1^WT^*.

Click here to Download Table S2

Table S3. List of BRG1 ChIP-seq peak calls and the genomic location of sites differentially bound by BRG1 between P12 and P18 testes.

Click here to Download Table S3

Table. S4. List of antibodies used in this study

Antibody	Vendor (catalog #)	Application
Rabbit anti-BRG1	Abcam (ab110641)	ChIP-seq (10 µg) IF (1:500)
Rabbit anti- YH2Ax	Cell signaling (9718)	IF (1:1000)
Mouse anti- YH2Ax	Millipore (05-636)	IF (1:1000)
Mouse anti-SYCP3	Abcam (ab97672)	IF (1:500)
Rabbit anti-SYCP3	Abcam (ab154255)	IF (1:500) WB (1:1000)
Rabbit anti-SYCP2	Millipore (ABE2622)	IF (1:500) WB (1:1000)
Goat anti- ATR (N-19)	Santa Cruz Biotech (sc-1887)	IF (1:50)
Mouse anti-SCML2 (1D12)	Developmental Studies Hybridoma Bank (PCRP-SCML2-1D12)	IF (1:10) IP (10 µg)
Recombinant anti-SCML2	Recombinant Antibody Network (anti-SCML2-RAB-C218)	CUT&RUN (1:100)
Rat anti- RNAPII subunit B1 (phosphor CTD Ser-2)	Millipore (04-1571)	IF (1:200)
Mouse anti- H2AK119ub1 (E6C5)	Millipore (05-678)	ChIP-seq (10 µg) WB (1:1000)
Rabbit anti- H3K27ac	Active Motif (39133)	ChIP-seq (5 µg) WB (1:1000)
Rabbit anti- H2BK120ub1	Cell signaling (5546)	WB (1:1000)
Mouse anti- H3K27me3	Abcam (ab6002)	WB (1:1000)
Rabbit anti– H3K4me3	Abcam (ab1012)	WB (1:1000)
Rabbit anti- H3	Abcam (ab1791)	WB (1:5000)
Mouse anti- RNF2	Santa Cruz Biotech (sc-1887)	WB (1:1000)
Mouse anti- USP3	GeneTex (GTX128238)	WB (1:1000)
Mouse anti- USP7	Santa Cruz Biotech (sc-1887)	IF (1:100) IP (2 µg) WB (1:1000)
Mouse anti- HDAC1	Abcam (ab7028)	WB (1:1000)
Rabbit anti- PDGFRA	Cell signaling (3164)	WB (1:1000)
Rabbit anti- AR	Abcam (ab74278)	IF (1:200)
Rabbit anti- PCNA	Santa Cruz Biotech (sc 7907)	IF (1:100)
Rabbit anti- SNF5	Abcam (ab192864)	WB (1:1000)
Mouse anti α TUBULIN	Developmental Studies Hybridoma Bank (12G10)	WB (1:1000)
Rabbit anti NCL (Nucleolin)	Bethyl (A300-711A)	WB (1:5000)

Table. S5. Sequences of qRT-PCR primer used in this study

Gene Symbol	Forward Primer (5′ - 3′)	Reverse Primer (5′ - 3′)
Zbtb16	TATCTCGAAGCATTCCAGCGAGGA	ACTCATGGCTGAGAGACCGAAAGA
Id4	CCCGCGCCACCTCTCCAC	CAGAGAATGCTGTCA CCCTG
Pou3f1	TGGGGGCTGTCACTTTATTC	GGAGTTAGAAGGACCCCAGG
Pou5f1	AGCTGCTGAAGCAGAAGAGG	GTGGTCTGGCTGAACACCTT
Foxo1	AAGAGCGTGCCCTACTTCAA	TGCTGTGAAGGGACAGATTG
Sdha	TGGACCTTGTAGTCTTTGGCA	AACCGATTCTTCTCCAGCATT
Ywhaz	GAGAAAAAGCAGCAGATGGC	CTTTCTGGTTGCGAAGCATT
Brg1	GAAGACCATCCAGACCATCG	TTCATACGCCCAGTTTGACA

Supplemental Materials

ChIP from low input chromatin:

Spermatogenic cells obtained from 12-day-old *Brg1*[WT] and *Brg1*[cKO] mice were fixed as described (Raab et al., 2015). Frozen pellets (10^6 cells each) were thawed on ice and then resuspended in 50 µl of nuclear isolation buffer (Sigma NUC-101). For the H3K27ac ChIPs, 5mM sodium butyrate was added to the nuclear suspensions. Samples were mixed by pipetting the cell suspension 15-20 times. 50 µl of nuclear preparations were mixed with 10 µl of MNase digestion buffer [6x MNase buffer (NEB), 8.8 mM DTT, 12 gel units MNase (NEB)] and incubated at 37 C for 15 minutes. The digestion was stopped by adding 1/10th volume of 100 µM EDTA (Ethylenediaminetetraacetic acid). 6.6 µl of a 1% Triton X-100/1% sodium deoxycholate solution was added to the digested chromatin and vortexed gently for 30 seconds after which the samples were held on ice until the next step. The DNA content of each chromatin preparation was quantified from 5 ul of chromatin. Briefly, chromatin was mixed with 95 µl H_2O and 100 µl 10% chelex-100 (Bio-Rad) and incubated at 95 C for 10 min. Following RNaseA digestion for 15 min at 37 C, ProteinaseK digestion for 1 hr at 56 C, the DNA was purified using ChIP-DNA clean and concentrator kit (Zymo) and quantified on a qubit. This way we ensured that for each pairwise comparison (wild type versus mutant ChIP) we began with an equal amount of input. We started with 145 ng and 285 ng of input DNA for each H3K27ac and H2AK119ub1 ChIP respectively. Digested chromatin was mixed with complete immnuoprecipitation (IP) buffer (20mM Tris-HCl pH 8.0, 2mM EDTA, 150 mM NaCl, 0.1% Triton X-100, 1x Protease inhibitor cocktail -PIC, 1mM Phenylmethanesulfonyl fluoride -PMSF) such that it comprised less than 25 % of the total volume. Chromatin was pre-cleared with 10 ul of magnetic Protein A (Bio-Rad) or G (Invitrogen/Dynalbeads) beads for 1 hour on a rotator at 4 C. After pre-clearing, 10% chromatin was set aside as input. Pre-cleared lysates were then mixed with antibodies to perform the ChIP. 5µg of rabbit anti-H3K27ac (Active Motif – 39133) conjugated to Protein A beads (Bio-Rad) and 10 µg of mouse anti-H2AK119ub1 IgM antibodies (Millipore E6C5, 05-678) were added to the lysates and left to bind chromatin by rotating the tubes overnight at 4 C. The following day anti-mouse IgM (Millipore, 12-488) conjugated to proteinA/G beads were added to the H2AK119ub1 ChIP samples and rotated at 4 C for 3 hours to capture anti-H2AK119ub1 bound chromatin. Chromatin bound to bead-antibody conjugates from all ChIP samples were isolated using a magnetic separator. Beads were then washed twice in low salt buffer followed by two washes in high salt buffer and eventually re-suspended in 100 µl of elution buffer (1%SDS/100mM $NaHCO_3$) in a 1.5 ml eppendorf tube. DNA was

eluted at 65 C on a shaking incubator (Eppendorf) at 800 rpm for 30 min. Eluate was separated from beads on a magnetic separator. 5 μl of 5M NaCl was added to eluate and incubated in 65 C water bath overnight to reverse crosslinks. ChIP DNA was digested with RNAseA for 30 min at 37 C followed by ProteinaseK digestion for 1 hr at 56 C and then purified using a ChIP-DNA clean and concentrator kit (Zymo) and quantified with a qubit. Libraries were prepared from ChIP and Input samples using the Kapa Hyperprep kit.

ChIP-seq data analysis:

Reads were aligned to mm9 using bowtie/bowtie2 (Langmead and Salzberg, 2012; Langmead et al., 2009). The resulting sam output files were converted to the BAM format using Samtools, version 1.6.0 (Li et al., 2009). The BAM files were filtered to remove PCR duplicates using Picard tools, MarkDuplicates (**http://broadinstitute.github.io/picard**). The BAM files were converted to bigwig files for visualization on the UCSC browser (Kent 2002). The bigwig files were generated using DeepTools (Ramírez et al., 2016), bamCompare with a bin size of 10 bp ,extending fragments to 150bp (nucleosome size), filtered for mm9 blacklisted regions and normalized to 1X depth of coverage. Each bigwig file represents the $\log_2$ ratio of ChIP to the corresponding Input sample. For comparison across samples the bigwig files were normalized to effective library size. Replicates were merged into a single bigwig using UCSC tools, bigWigMerge (http://hgdownload.soe.ucsc.edu/admin/exe/). Read coverage over regions of interest were generated from matrix files generated using DeepTools, computeMatrix following which metagene plots were made in R using ggplo2 (Wilkinson, 2011). Peaks from each sample were called using Macs2, version2.1.0, (Zhang et al., 2008) in broadpeak mode with --broad-cutoff 0.05 (FDR ≤ 0.05). Overlapping peaks between replicates were identified using bedtools, intersectBed and were used for subsequent analysis. A list of all BRG1 peak calls are provided (Table S3) Peaks were annotated using HOMER, peakannotate.pl (Heinz et al., 2010). Differential peak analysis was performed on bedgraph files using Macs2, bdgdiff run with default parameters.

CUT&RUN data analysis:

Reads were trimmed to remove n's at either end using TrimGalore (version 0.4.3, https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) keeping the --trim-n option. Following trimming, reads were aligned to mm9 (mouse) and sacCer3 (yeast, spike in control) reference genomes using bowtie2. Replicate BAM files were then merged using samtools merge followed by their conversion to the bigWig format using DeepTools, bamCoverage. The resulting bigWig files were filtered for mm9 black listed regions and were generated with the following options -bs 1 (bin size), --normalizeUsing RPKM, --extendReads=140 and --ignoreDuplicates. Scaling factors calculated from spike-in normalization were the same across samples thereby obviating the need for scaling.

ATAC-seq data analysis:

Reads were aligned to the mm9 genome using bowtie, with following paramters: -S –q –m 1 –p 2 –best –strata –chunkmbs 256 (Langmead et al., 2009). The outputed sam files were converted to BAM files using Samtools version 1.3.1. Next Bedtools version 2.25.0 (Quinlan and Hall, 2010), bamtobed was used to convert BAM output files to bed file format to do the subsequent steps . BAM files were converted to bigWig files for visualization on UCSC browser and to generate metagene plots as described above (see supplementary materials on ChIP-seq data analysis).

Preparation of nuclear lysates for immunoprecipitation:

Spermatogenic cells isolated from 2- to 3-week-old males were washed once in PBS and centrifuged at 600g for 5 min at 4 C. The resulting cell pellet was re-suspended in 20 PCV (packed cell volumes) of buffer A (10mM HEPES-KOH pH7.9, 1.5mM MgCl2, 10mM KCl, 0.1% NP-40, 0.5mM DTT, 0.5mM PMSF, 1x PIC, 0.5) and left on ice to swell for 10 min. Cells were centrifuged at 600g for 5 min at 4 C, resuspended in 2 PCV of buffer A and then homogenized using a dounce (type B). The homogenate containing nuclei were centrifuged at 700g for 10 min at 4 C. Nuclear preparations were washed once again in 10 PCV of buffer A and pelleted at 5000 rpm for 10 min at 4 C. Lysates were extracted from pelleted nuclei at least twice for 1 hour each with an equal volume of high salt buffer C (20mM HEPES-KOH pH7.9, 1.5mM MgCl2, 420mM NaCl, 10mM KCl, 25 % glycerol, 0.2mM EDTA, 0.5mM DTT, 0.5mM PMSF, 1x PIC) on a nutator at 4 C. The resulting nuclear lysates were mixed with 2.8x volume of buffer D (20mM HEPES-KOH pH7.9, 20 % glycerol, 0.2mM EDTA ,0.5mM DTT, 0.5mM PMSF, 1x PIC) following which they were clarified by spinning at 14,000 rpm for 10 min at 4 C, pooled together and snap-frozen in liquid nitrogen and stored at -80 C until further use.

Co-Immunoprecipitation (Co-IP):

100 µl of magnetic protein A beads were initially washed twice in PBS for 5 min each at 4 C followed by one wash in PBS + 0.5% BSA for 10 min at 4 C. The antibodies (tableS3) were then mixed with beads resuspended in 1x volume of PBS + 0.5% BSA and allowed to conjugate for at least 2 hr at 4 C. After this, antibody-bead conjugates were washed once with 500 µl of PBS + 0.5% BSA , followed by two washes in IP buffer (20mM HEPES-KOH pH7.9, 0.15mM KCl, 10 % glycerol, 0.2mM EDTA, 0.5mM DTT, 0.5mM PMSF, 1x PIC) for 5 min each at 4 C. The antibody-coupled beads were stored in IP buffer until the nuclear lysates were processed for IP. Briefly, the lysates were thawed on ice and centrifuged at 14,000 rpm at 4 C to remove any precipitates. 500 µg of nuclear lysate was diluted in IP buffer to make up the volume to 1.3 ml and then incubated with unconjugated protein A beads to pre-clear the lysate. A fraction of lysate was set aside as input and the remaining was transferred to a tube containing the antibody coupled beads and left on a rotator overnight at 4 C. The following day the protein bound antibody-bead conjugates were separated using a magnetic separator and washed on a rotator with a series of buffers in the following order at 4 C for 5 min each: twice in IP buffer, twice in high salt wash buffer (20mM HEPES-KOH pH7.9, 300mM KCl, 10 % glycerol, 0.2mM

EDTA, 0.1 % Tween-20, 0.5mM DTT, 0.5mM PMSF, 1x PIC), twice in a low salt wash buffer (20mM HEPES-KOH pH7.9, <u>100mM KCl</u>, 10 % glycerol, 0.2mM EDTA, 0.1 % Tween-20, 0.5mM DTT, 0.5mM PMSF, 1x PIC), once in final wash buffer (20mM HEPES-KOH pH7.9, 60mM KCl, 10 % glycerol, 0.5mM DTT, 0.5mM PMSF, 1x PIC). The proteins were eluted by resuspending the beads in 2X Laemmli buffer and incubating them at 70 C for 10 min followed by boiling the samples at 95 C for 5 min.

BRG1-IP and mass spectrometry:

Prior to mass spectrometry the IPs were performed with anti-BRG1 antibody and non-specific IgG (table S4) as described above with a few modifications. After conjugation each antibody was crosslinked to protein A beads with BS[3] (bis[sulfosuccinimidyl] suberate) crosslinking agent, prepared as per the manufacturer's instructions (Thermo scientific, 21585). Crosslinking with BS[3] significantly reduces IgG elution and improves signal to noise ratio in samples obtained by boiling beads in Laemlli buffer (Sousa et al., 2011). Briefly, antibody coupled beads were washed once in PBS for 5 min at 4 C, followed by two washes in conjugation buffer pH 7.9 (20 mM Sodium Phosphate, 0.15M NaCl). The antibody-coupled beads were resuspended in 250 ul of conjugation buffer containing 5mM BS[3] in a tube and incubated on a rotator for 40 min at room temperature. Antibody crosslinked beads were separated and the crosslinker was quenched with an equal volume of 1M glycine (1x volume of beads). The crosslinked beads were then washed once in PBS at 4 C for 5 min, followed by incubation in 100 ul of 0.11M glycine (pH2.5) for 10 min at 4 C to remove any uncrosslinked antibody. The crosslinked beads were then washed thrice in PBS tween-20 (0.1%) + 0.5 % BSA, followed by another three washes in IP buffer before transferring them to a 15 ml falcon tube containing 4 mg of nuclear lysate made up in IP buffer up to a final volume of 10ml and left on rotator overnight at 4 C. The following day the beads were washed, and proteins were eluted as described above. Samples were run on a short gel from which the regions containing the proteins were cut out and processed for mass spectrometry and peptide identification.

In-gel Digestion

Gel slices were cut into 1x1 mm pieces and placed in 1.5 mL eppendorf tubes with 1 mL of water for 30 min. The water was then removed and 50 µL of 250 mM ammonium bicarbonate followed by10 µL of 45 mM 1, 4 dithiothreitol (DTT) were added prior to incubation at 50 °C for 30 min. The samples were cooled to room temperature and then alkylated with 10 µL of 100 mM iodoacetamide for 30 min. The gel pieces were washed 2x with 1 mL of water, removed and

added 1 mL of 50 mM ammonium bicarbonate:acetonitrile (1:1) and allowed to incubate at room temperature for 1 hr. The solution was then removed and 200 µL of acetonitrile was added, removed, and the gel pieces dried by SpeedVac. Gel pieces were rehydrated in 70 µL of 2 ng/µL trypsin (Sigma) and 0.01% ProteaseMAX surfactant (Promega) in 50mM ammonium bicarbonate and incubated at 37 °C for 21hrs. Supernatant was removed, gel pieces added 100 µL of 80:20 (1% (v/v) formic acid in acetonitrile), combined with the former supernatant, and dried on a SpeedVac. Samples were reconstituted in 25 µL of 5% acetonitrile (0.1% (v/v) trifluroacetic acid) for LC-MS/MS analysis.

LC-MS/MS

Tryptic peptides were dissolved in 0.1% trifluoroacetic acid and directly loaded at 4 µL/min for 7 minutes onto a custom-made trap column (100 µm I.D. fused silica with Kasil frit) containing 2 cm of 200Å, 5 µm Magic C18AQ particles (Michrom Bioresources). Peptides were then eluted onto a custom-made analytical column (75 µm I.D. fused silica) with gravity-pulled tip and packed with 25 cm 100Å, 5 µm Magic C18AQ particles (Michrom). Peptides were eluted with a linear gradient from 100% solvent A (0.1% (v/v) formic acid in water:0.1% formic acid in acetonitrile (95:05)) to 35% solvent B (0.1% (v/v) formic acid in acetonitrile) in 90 minutes at 300 nanoliters per minute using a Waters NanoAcquity UPLC system directly coupled to a Thermo Scientific Q Exactive hybrid mass spectrometer. Data were acquired using a data-dependent acquisition routine of acquiring one mass spectrum (m/z 300 -1750) in the Orbitrap (resolution 70,000, 1e6 charges, 30 ms maximum fill time) followed by 10 tandem mass spectrometry scans (resolution 17,500, 1e5 charges, 110 ms maximum fill time, HCD collision energy 27 eV NCE). Dynamic exclusion was employed to maximize the number of peptide identifications and minimize data redundancy.

Data Analysis

Raw data files were processed into peak lists using Proteome Discoverer (version 1.4; Thermo Scientific) and then searched against the Uniprot mouse database with Mascot (version 2.5; Matrix Science) using precursor mass tolerances of 10 ppm and fragment mass tolerances of

0.5 Da. Full tryptic specificity was specified considering up to 2 missed cleavages; variable modifications of acetylation (protein N-term), pyro-glutamination (N-term glutamine), and oxidation (methionine) were considered and fixed modifications of carbamidomethylation (cysteine) were considered. Mascot search results were loaded into Scaffold (Proteome Software) with threshold values of 80% for peptides (1.0% false-discovery rate) and 90% for proteins (2 peptide minimum) for final annotation.

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