Supplemental Materials

Molecular Biology of the Cell

Sabbattini et al.

A H3K9/S10 methyl-phospho switch modulates Polycomb and Pol II binding at repressed genes during differentiation

Pierangela Sabbattini[1,4], Marcela Sjoberg[1,4], Svetlana Nikic[1,4], Alberto Frangini[1], Per-Henrik Holmqvist[1], Natalia Kunowska[1], Tom Carroll[1], Emily Brookes[2], Simon J. Arthur[4], Ana Pombo[2] and Niall Dillon[1,*]

[1]Gene Regulation and Chromatin Group and [2]Genome Function Group, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN. [4]MRC Protein Phosphorylation Unit, Sir James Black Complex, University of Dundee, Dundee DD1 5EH, Scotland, U.K.

SUPPLEMENTAL MATERIALS

This supplement contains:

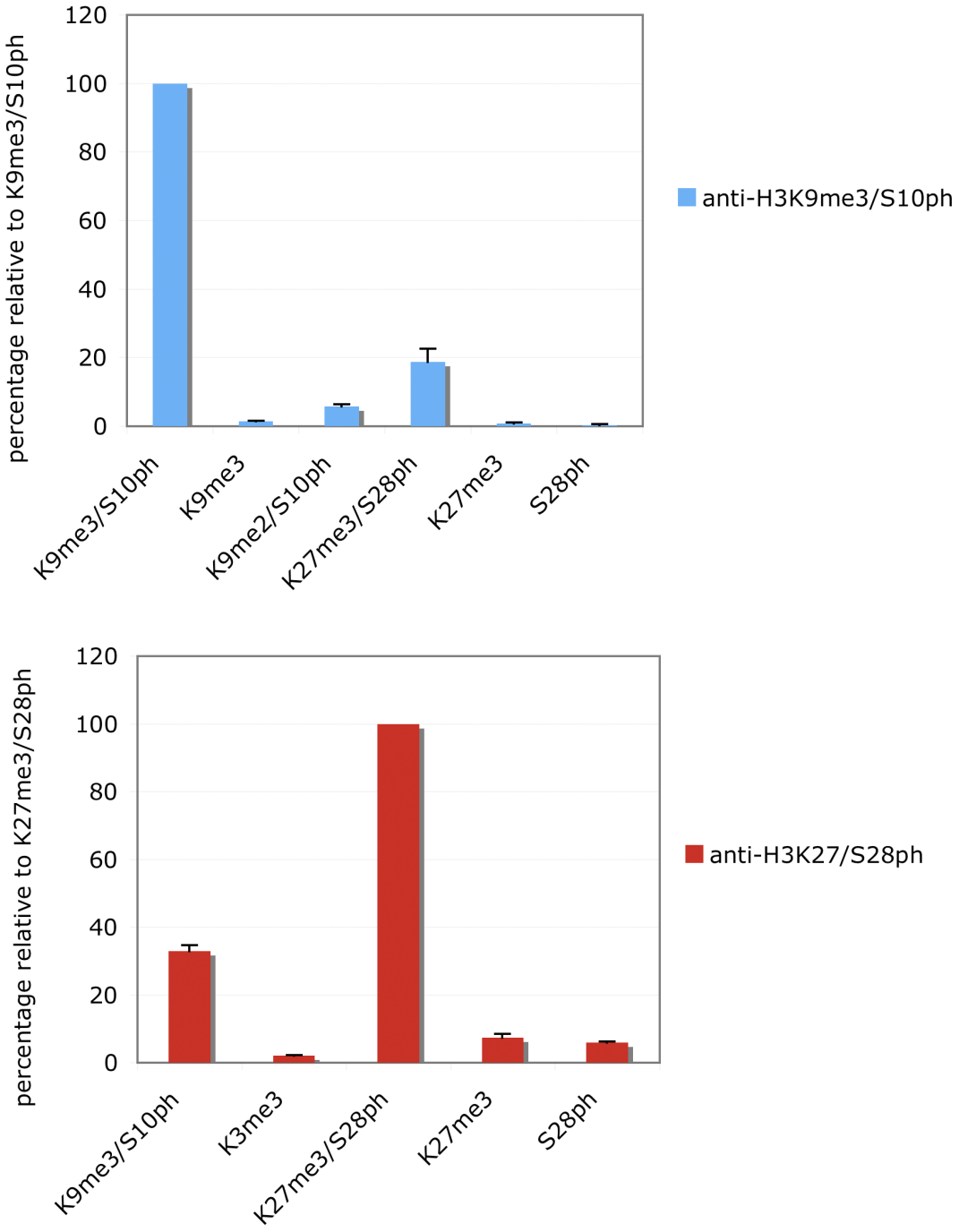

FIGURE S1: Validation of the specificities of the anti- H3K9me3/S10ph antibody (upper panel) and the anti- H3K27me3/S28ph antibody (lower panel). Antibody specificities were analysed by ELISA using the indicated peptides. The y-axes represent the percentage cross-reaction relative to the value for the cognate peptide sequence for each antibody (shown as 100%). Bars = mean ± SD; n = 2.

The similarity of the amino acid sequences surrounding H3K9/S10 and H3K27/S28 makes it important to assess whether there is cross-reaction between antibodies

recognising the H3K9me3/S10ph and H3K27me3/S28ph modifications. For the two antibodies used in this study, specificity was initially assessed using ELISA carried out with peptides containing single and double modifications. The two antibodies raised against the binary H3K9me3/S10ph and H3K27me3/S28ph modifications showed little or no cross-reaction with peptides containing any of the single H3 modifications (H3K9me3, H3K27me3, H3S10ph, H3S28ph). Each of the two antibodies also showed predominant binding to its cognate binary modification peptide. However there was a weak cross-reaction (~ 20%) between the anti-H3K9me3/S10ph antibody and the H3K27me3/S28ph peptide. Analysis of the anti-H3K27me3/S28ph antibody also showed approximately 30% cross-reaction with the H3K9me3/S10ph peptide in the ELISA assay, despite having been depleted with the H3K9me3 peptide.

The cross-reaction between the two antibodies that was observed in the ELISA could make it difficult to assess the levels of the two binary modifications in ChIP assays that make use of the individual antibodies if both modifications are present together on the same region of chromatin. However, comparison of the binding profiles obtained with the two antibodies has made it possible to measure the levels of both binary modifications. The results of the ChIP analysis show that co-existence of the two binary modifications is only observed in resting B cells and demonstrate that the H3K27me3/S28ph modification is absent from Polycomb regulated genes in G1 activated B cells. Similar results were obtained for differentiated C3H cells (Fig. 1C and 5A). Interestingly, the anti-H3K27me3/S28ph antibody does not show any evidence of cross-reaction with the H3K9me3/S10ph modification at silent genes in G1 activated B cells or in differentiated mesenchymal stem cells, despite the presence of high levels H3K9me3/S10ph at these genes. This suggests that the ELISA results might be overestimating the degree of cross reaction that is observed in native chromatin.

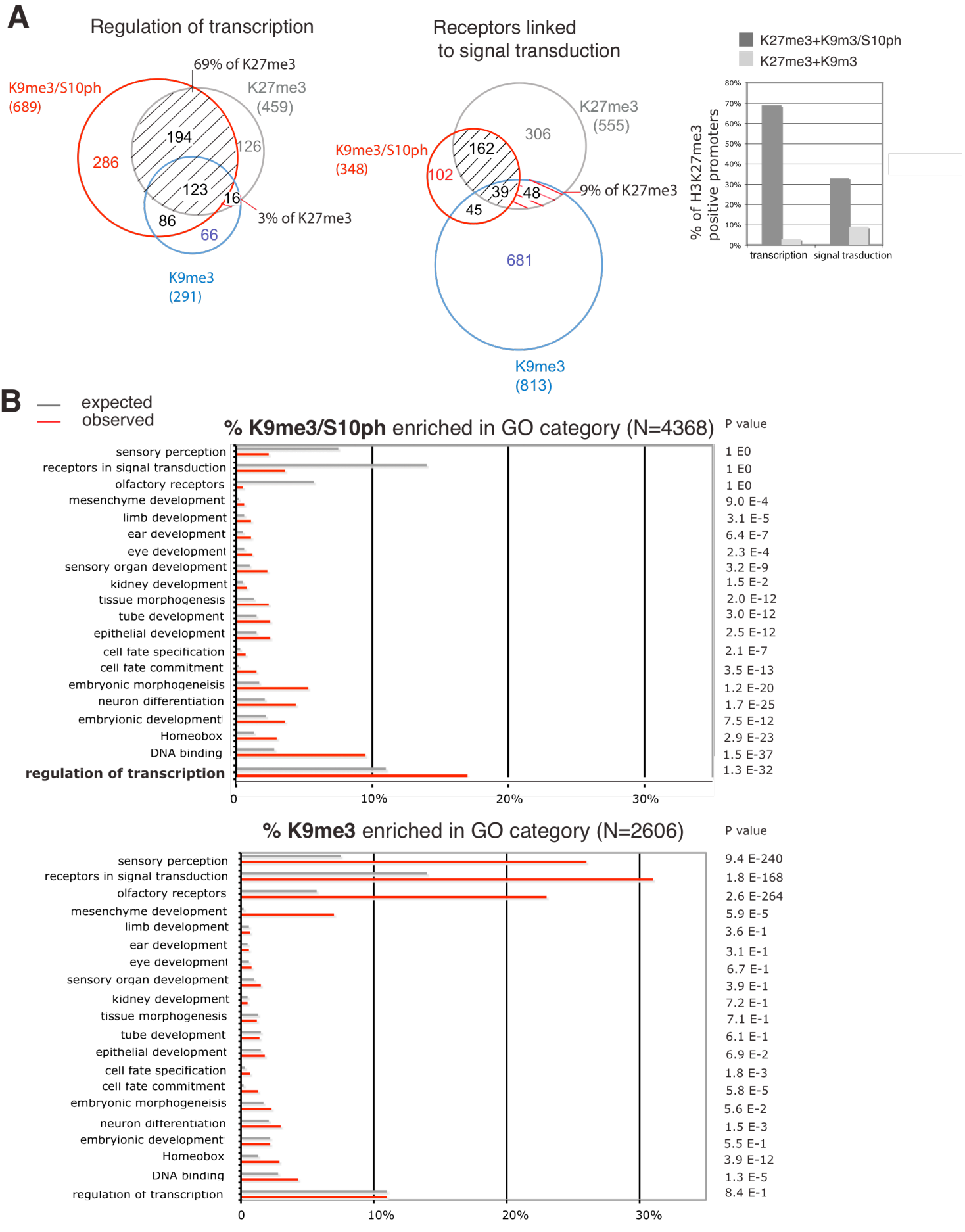
A
Regulation of transcription
69% of K27me3
K9me3/S10ph
(689)
K27me3
(459)
194
126
286
123
16
3% of K27me3
86
66
K9me3
(291)
Receptors linked
to signal transduction
K27me3
(555)
306
K9me3/S10ph
(348)
162
102
39
48
9% of K27me3
45
681
K9me3
(813)
K27me3+K9m3/S10ph
K27me3+K9m3
% of H3K27me3
positive promoters
80%
70%
60%
50%
40%
30%
20%
10%
0%
transcription
signal trasduction
B
expected
observed
% K9me3/S10ph enriched in GO category (N=4368)
P value
sensory perception
receptors in signal transduction
olfactory receptors
mesenchyme development
limb development
ear development
eye development
sensory organ development
kidney development
tissue morphogenesis
tube development
epithelial development
cell fate specification
cell fate commitment
embryonic morphogeneisis
neuron differentiation
embryionic development
Homeobox
DNA binding
regulation of transcription
1 E0
1 E0
1 E0
9.0 E-4
3.1 E-5
6.4 E-7
2.3 E-4
3.2 E-9
1.5 E-2
2.0 E-12
3.0 E-12
2.5 E-12
2.1 E-7
3.5 E-13
1.2 E-20
1.7 E-25
7.5 E-12
2.9 E-23
1.5 E-37
1.3 E-32
0
10%
20%
30%
% K9me3 enriched in GO category (N=2606)
P value
sensory perception
receptors in signal transduction
olfactory receptors
mesenchyme development
limb development
ear development
eye development
sensory organ development
kidney development
tissue morphogenesis
tube development
epithelial development
cell fate specification
cell fate commitment
embryonic morphogeneisis
neuron differentiation
embryionic development
Homeobox
DNA binding
regulation of transcription
9.4 E-240
1.8 E-168
2.6 E-264
5.9 E-5
3.6 E-1
3.1 E-1
6.7 E-1
3.9 E-1
7.2 E-1
7.1 E-1
6.1 E-1
6.9 E-2
1.8 E-3
5.8 E-5
5.6 E-2
1.5 E-3
5.5 E-1
3.9 E-12
1.3 E-5
8.4 E-1
0
10%
20%
30%

FIGURE S2: The H3K9me3/S10ph-H3K27me3 combination preferentially marks promoters of genes encoding transcriptional regulators in differentiated C3H cells. ChIP DNA was hybridised to the Nimblegen 24,000 promoter array. The top 20% of promoters enriched in two biological replicates for each of the indicated histone modifications were analysed for gene ontology clustering using DAVID http://david.abcc.ncifcrf.gov (Huang et al, 2009).
(A) Number of promoters with overlapping H3K27me3 and H3K9me3/S10ph and H3K9me3 modifications of genes that encode transcriptional regulators or receptors linked to signal transduction. Percentages refer to the number of promoters enriched for H3K27me3 that also show enrichment for either H3K9me3/S10ph or H3K9me3 only (summarised in the bar chart on the right).
(B) Gene ontology clustering indicates that H3K9me3/S10ph and H3K9me3 mark genes with different functions, with H3K9me3/S10ph preferentially found on transcription and developmental regulators and H3K9me3 found on genes encoding receptors involved in signal transduction. N corresponds to the number of annotated genes among the top 20% fraction of enriched promoters for the indicated histone modification.

The gene ontology analysis showed a strong enrichment of H3K9me3/S10ph on the promoters of genes that are involved in transcriptional and developmental regulation. Of the genes that are classified as transcriptional regulators and are marked by H3K27me3, 69% also have the H3K9me3/S10ph modification. In contrast, the single H3K9me3 modification without the adjacent H3S10ph is found at only 3% of the transcriptional regulator genes that are marked by H3K27me3 (A). The analysis also showed that the H3K9me3/S10ph modification is underrepresented on promoters of genes that encode signalling receptors (A and B). Instead, the promoters of these genes are marked by either H3K27me3 (23%), or H3K9me3 (51%), with only 9% having the two marks co-localised on the promoters. Together these results reveal striking differences in the combinations of H3 modifications that are found at promoters of different types of gene. They suggest that the double H3K9me3/S10ph modification forms part of a histone code that differentially repressed genes that have different functions during cell differentiation.

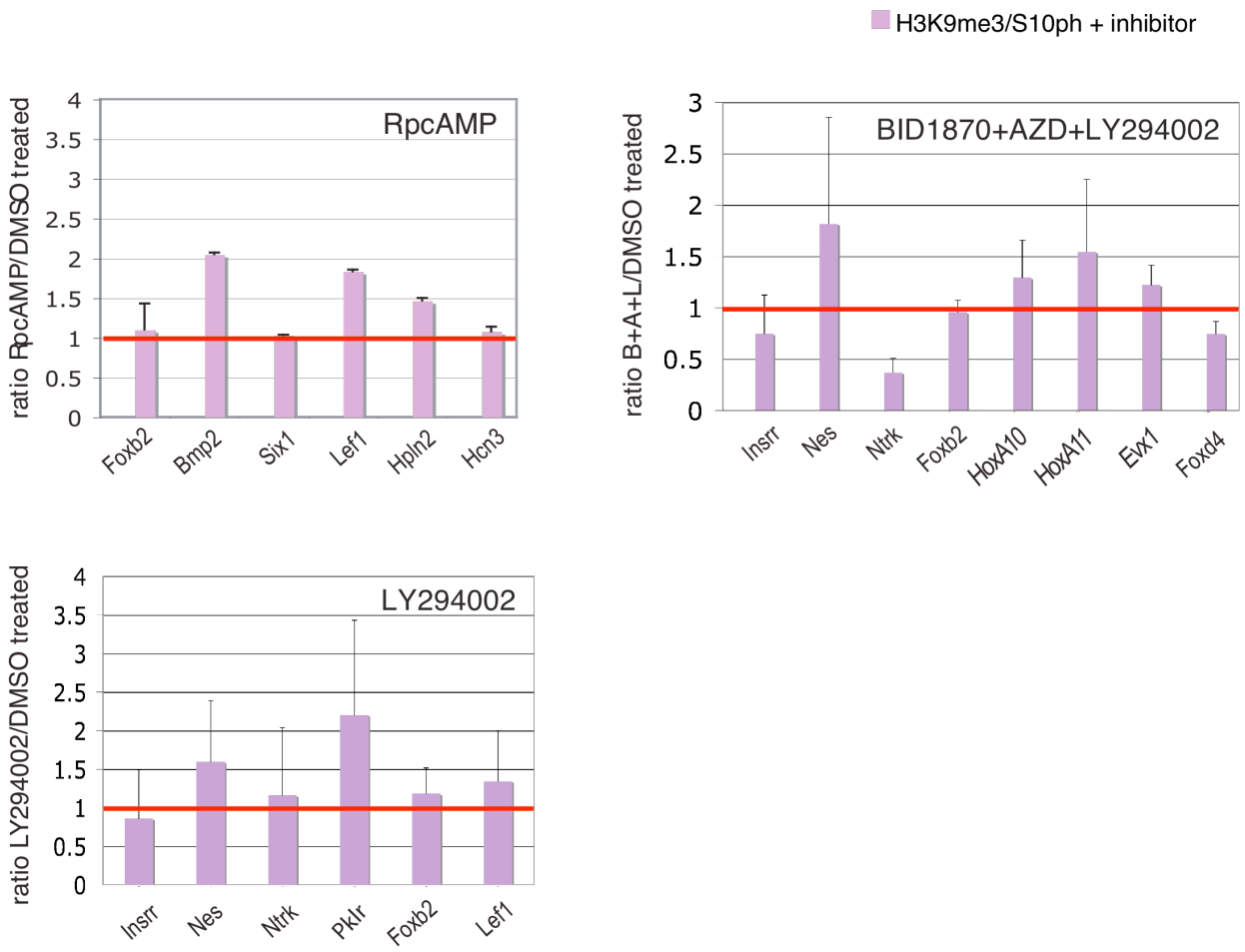

FIGURE S3: Effect of kinase inhibitors on H3K9me3/S10ph levels.
Chip analysis for H3K9me3/S10ph was carried out on chromatin from differentiated C3H10T1/2 cells treated with RpcAMP (10μM), LY294002 (30μM), BID1870 (10μM), AZD1151 (500nM) or vehicle (DMSO) for 14 hours. Levels are expressed as the ratio of the amount of double H3K9me3/S10ph modification detected in cells treated with inhibitors relative to cells treated with vehicle. The red line corresponds to a ratio of 1 and represents the value for the control cells. Bars = mean ± SD. n = 2 biological replicates for each inhibitor treatment.

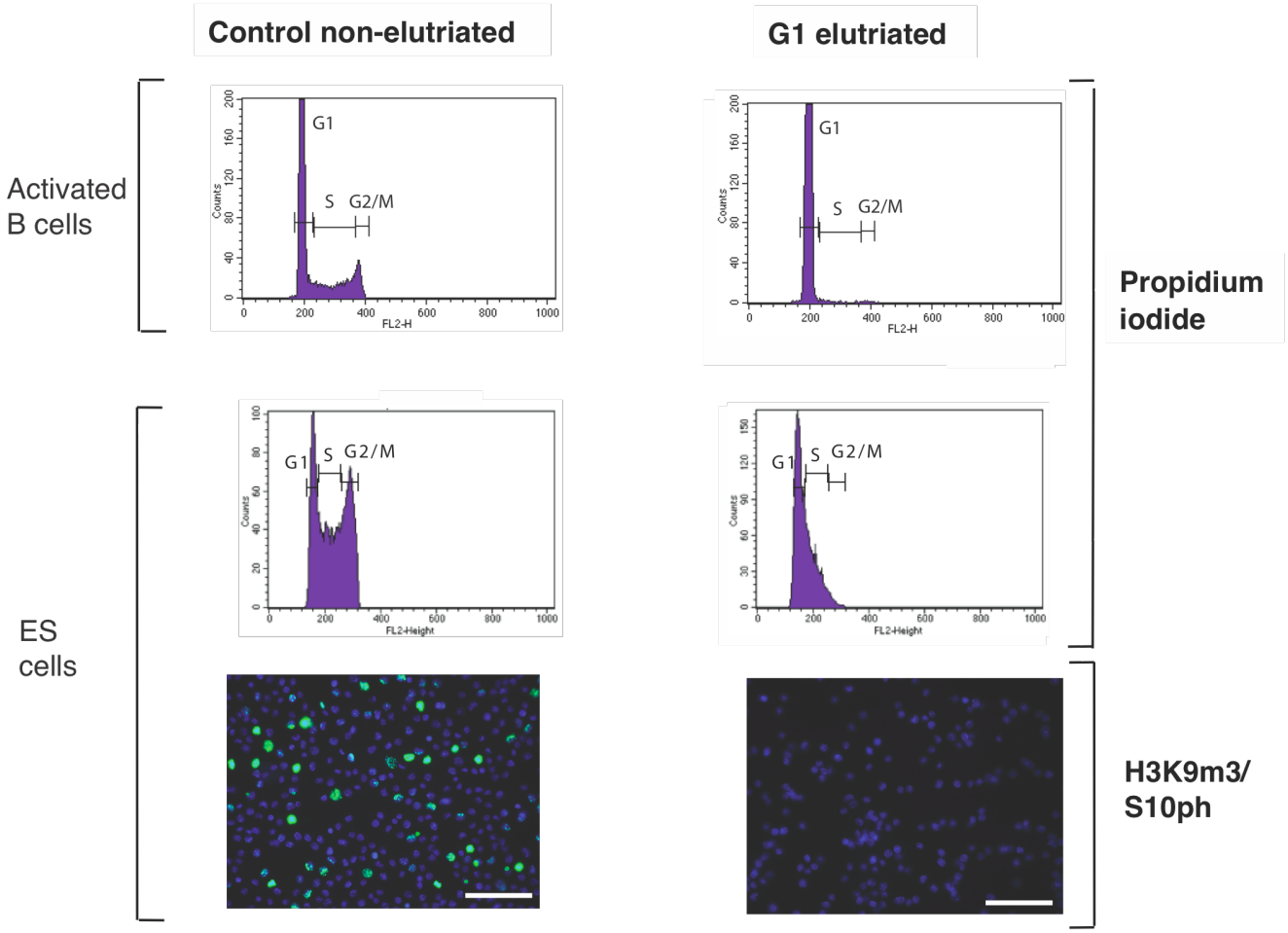

FIGURE S4: Examples of FACS analysis of ES cells and activated B cells stained with propidium iodide carried out on non-elutriated and elutriated cells in the G1 phase of the cell cycle.
Cell fractions isolated by centrifugal elutriation (see methods) were stained with propdium iodide and analysed for DNA content by FACS. ES cells have an unusual cell cycle profile with a high proportion of the cells showing S and G2/M staining profiles. The elutriated ES cell fraction used for the analysis was enriched for G1, but also contained some S-phase cells. Immunofluorescence staining with anti-H3K9me3/S10ph antibody (bottom panels) was used to confirm that the elutriated cell fraction was free of high-staining G2/M cells. Scale bars represent 50μm.

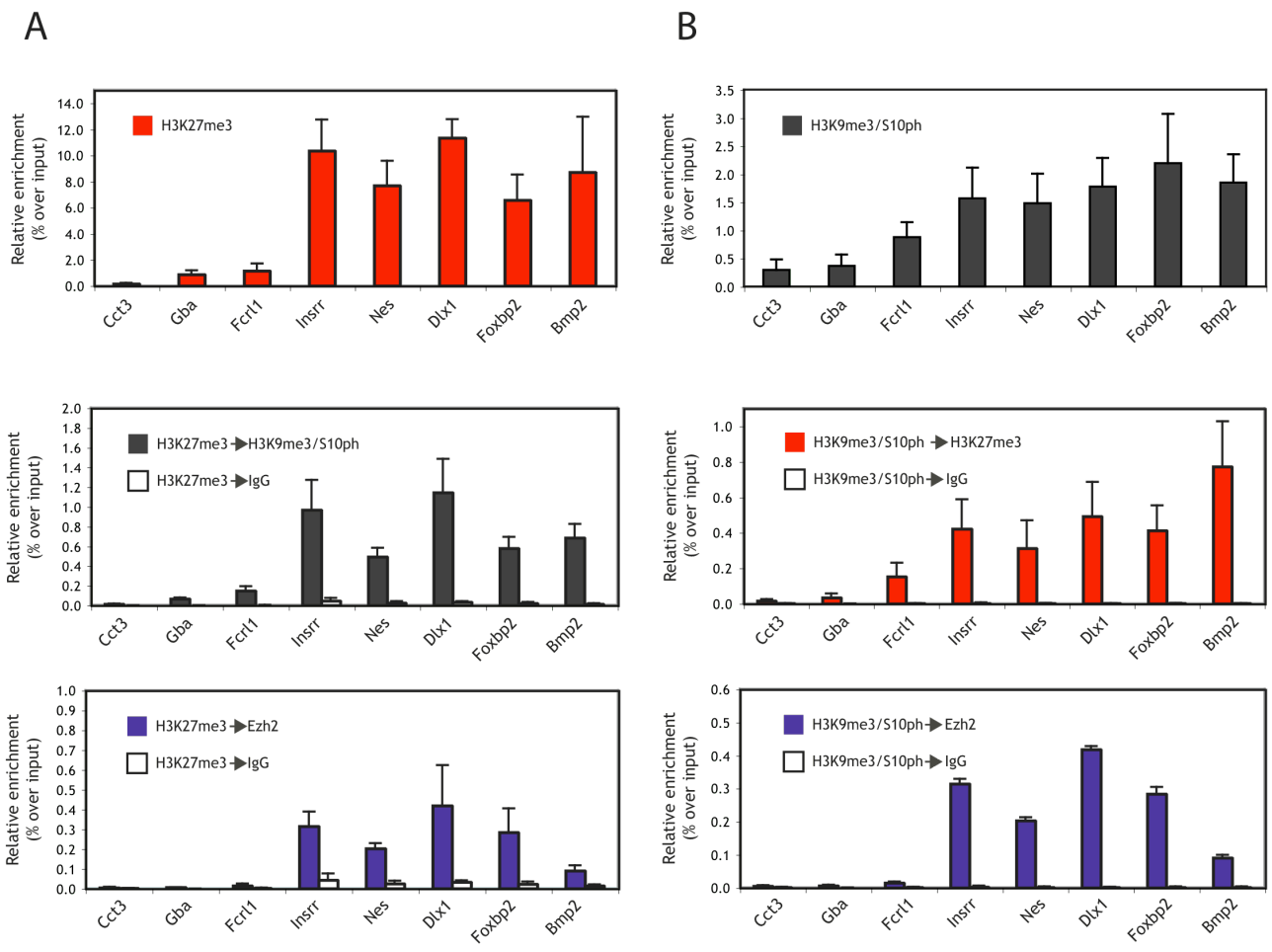

FIGURE S5: Sequential ChIP confirms co-localisation of H3K27me3 and H3K9me3/S10ph on chromatin in G1 activated B cells.
The first ChIP was performed with anti-H3K27me3 (A) and anti-H3K9me3/S10ph (B). Re-ChIP was then carried out on the recovered chromatin using the antibody indicated after the arrow in each graph. The results show that H3K9me3/S10ph co-immunoprecipitates with H3K27me3 and with the PRC2 protein Ezh2 on silent genes in G1 activated B cells. Bars = mean ± SD.

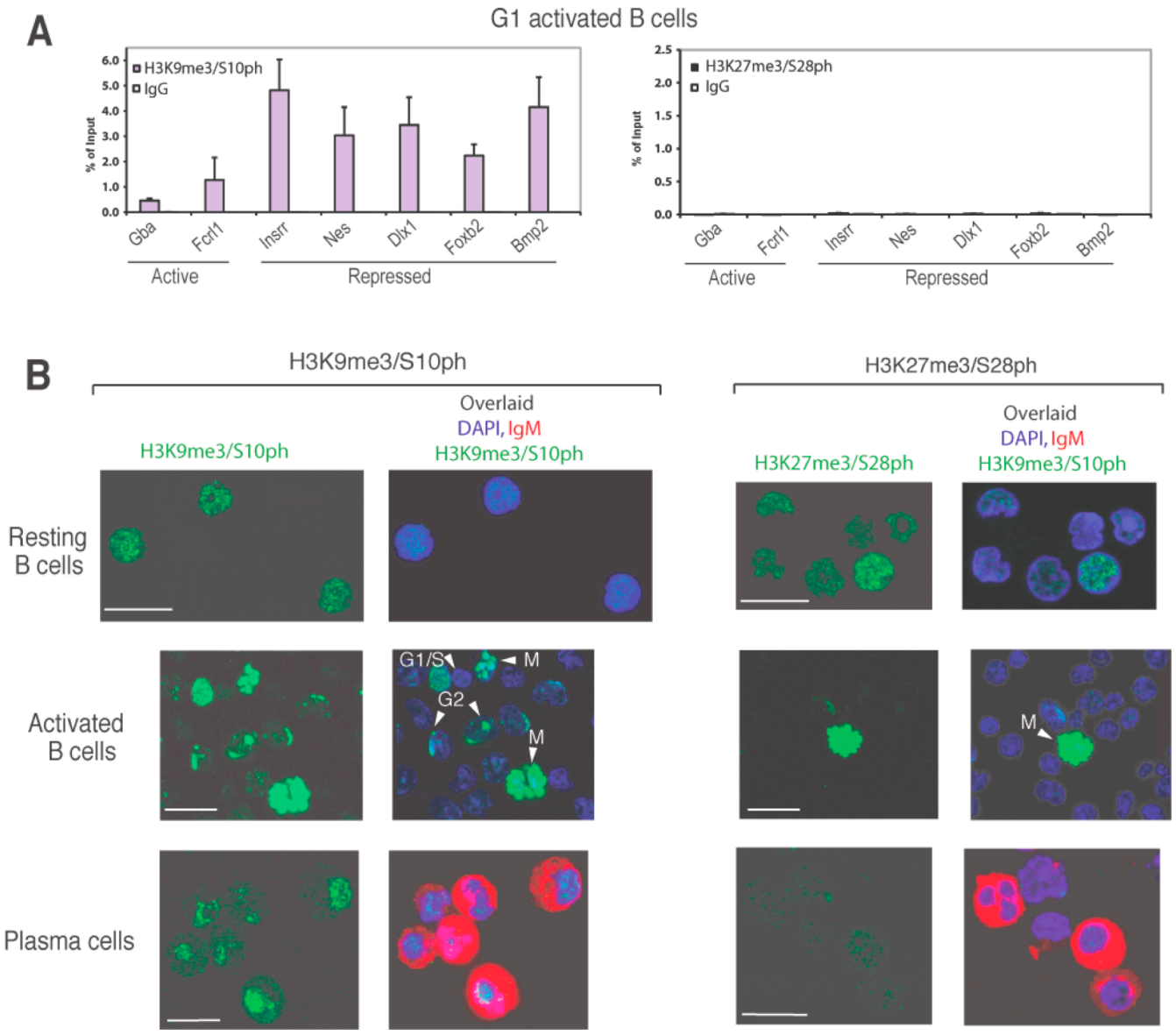

FIGURE S6: Different distributions of the H3K9m3/S10ph and H3K27me3/S28ph double modifications on B cell chromatin. (A) ChIP analysis was carried out on chromatin from LPS-activated B cells using anti-H3K27me3/S28ph (black histograms) and anti-H3K9m3/S10ph (purple histograms). Immunoprecipitated chromatin was analysed with primers for the promoters of a panel of silent and active genes. Bars = mean ± SD. (B) Immunofluorescence analysis of the distribution of H3K9me3/S10ph (left hand panels) and H3K27me3/S28ph (right hand panels) in cells at different stages of B cell development and of the cell cycle. Top panels: isolated resting B cells. Middle panels: In vitro differentiated plasma cells. Bottom panels: Splenic B cells activated for 3 days with LPS. Arrows indicate nuclei of cells in G1/S, G2, or mitosis(M). Scale bars represent 10μm.

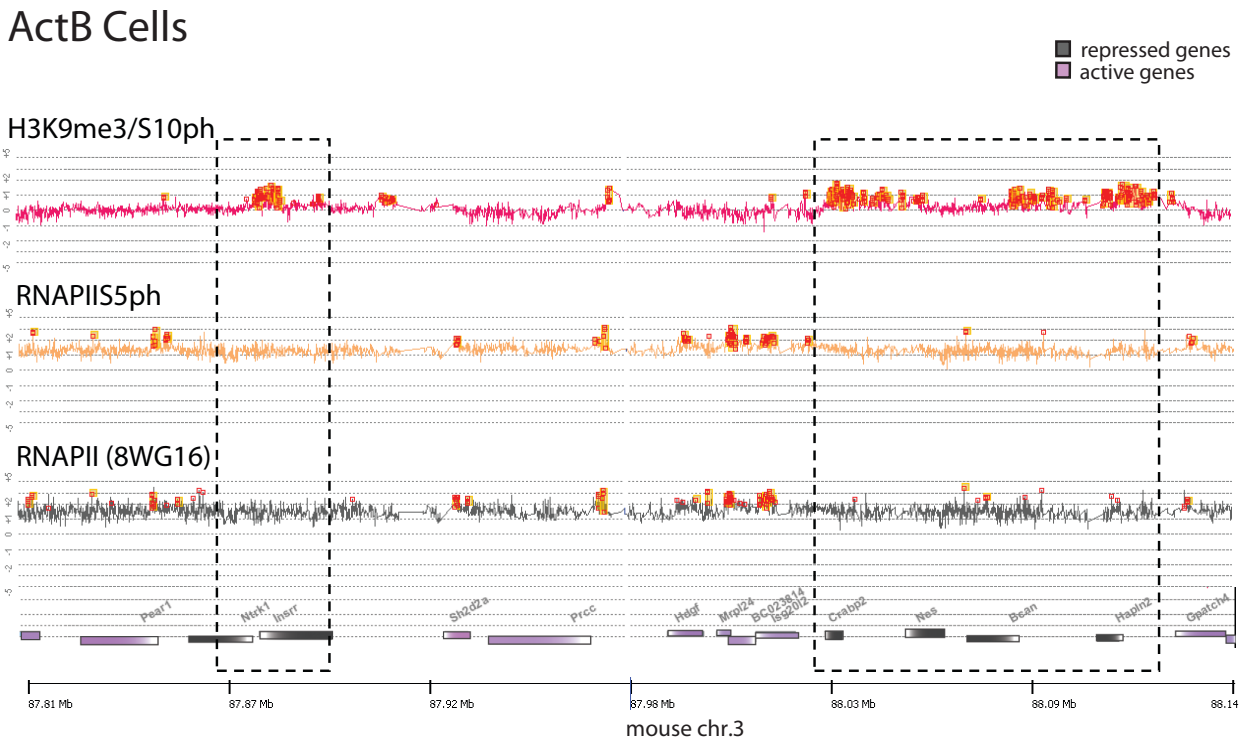

FIGURE S7: Unphosphorylated RNAPII and RNAPIIS5ph do not bind to regions that are marked by H3K9me3/S10ph in activated B cells.
ChIP analysis was carried out using anti-RNAPIIS5ph and the 8WG16 antibody, which recognises hypo-phosphorylated RNAPII. Calculation of peaks of enrichment (orange squares) is described in the legend to Figure 4. Dashed boxes indicate regions containing repressed genes (black rectangles). Purple rectangles = active genes.

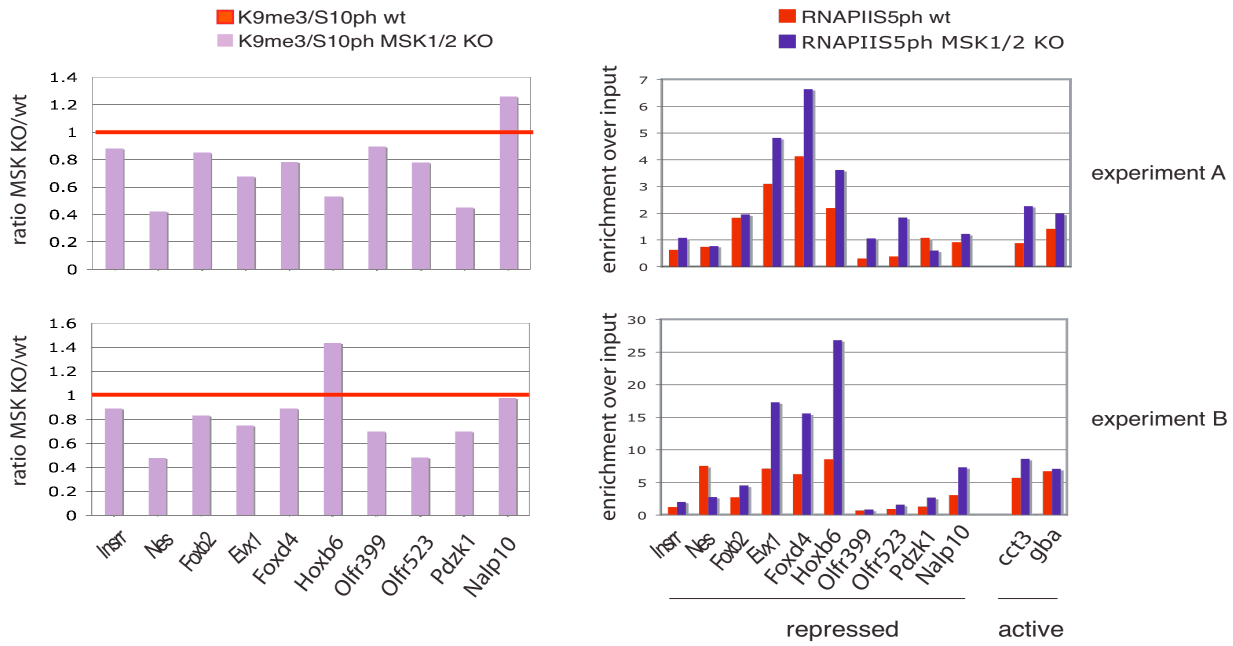

FIGURE S8: Differentiated MSK1/2 KO MEFs show increased binding of RNAPIIS5ph on repressed genes.
ChIP analysis of H3K9me3/S10ph and RNAPIIS5ph at repressed genes in differentiated MSK1/2 dko MEFs. Values are expressed as the levels of enrichment relative to the levels in wt cells. The red line correspond to ratio of 1, which represents the value for the control cells.


Differentiated C3H
repressed genes
active genes
H3K9me3/S10ph
H3K27me3
Ezh1
Ezh2
RNAPIIS5ph
Pear1
Ntrk1
Inarr
Shroom2a
Shroom2a
Prcc
Hdgf
Mrpl24
BC023814
Crabp2
Isg20l2
Nea
Bcan
Bcan
Hapln2
Opalin4
Apoa1bp
Tfc24
Iqgap3
Mef2d
87.80 Mb
87.89 Mb
87.97 Mb
88.05 Mb
88.13 Mb
88.21 Mb
mouse chr. 3
H3K9me3/S10ph
H3K27me3
Ezh1
Ezh2
RNAPIIS5ph
Fdps
Pkir
Pkir
Hyi3
Clk2
Scamp3
1110013L07Rik
Gba
Mtx1
Thbs3
Muc1
Trim46
Dpm3
Ragiap1
Efna1
Efna3
Efna4
Adam15
Dcst1
Zbtb7b
Lenep
Flad1
Cks1b
Pygo2
Pbxip1
89.18 Mb
89.26 Mb
89.34 Mb
89.42 Mb
89.50 Mb
mouse chr. 3

FIGURE S9: Ezh1 co-localizes with H3K9me3/S10ph-H3K27me3 at repressed genes in differentiated C3H10T1/2 cells

Upper and lower panels show a microarray analysis of different sections of the 2 Mb region of mouse chromosome 3. Chromatin from differentiated C3H10T1/2 cells was precipitated with the indicated antibodies and hybridised to an Agilent tiling oligonucleotide microarray as described in figure 4. Peaks of enrichment are indicated by orange squares. Dashed boxes indicate regions containing repressed genes.

Supplementary Table 2. List of antibodies used in the ChIP assays.

Antibody	Host	Supplier	Cat nr.	Amount used per IP
Anti-EZH2	rabbit	Active Motif	AR-0163-200 (39103)	5μg for 100μg of chromatin
Anti-EZH2	rabbit	Active Motif	39639	10μl for 400μg of chromatin
Anti-EZH2	rabbit	Active Motif	39901	10μl for 400μg of chromatin
Anti-H3K4me3	rabbit	Abcam	ab8580	2μg for 25μg of chromatin
Anti-H3K27me3	mouse	Abcam	ab6002	5-10μg for 100μg of chromatin
Anti-H3K9me3/S10ph	rabbit	Abcam	ab5819	5μg for 200μg of chromatin
Anti-Ezh1	goat	Santa-Cruz	sc-20347	8 μg for 400μg of chromatin
Anti-Ezh1	rabbit	Abcam	ab64850	10μg for 400μg of chromatin
Anti-RBP1 CTD phosphorylated on Ser5	mouse	Covance	MMS-128P	25μg for 400μg of chromatin
Anti-non phosphorylated RBP CTD (8WG16)	mouse	Covance	MMS-126R	25μg for 400μg of chromatin
IgG	goat	Santa Cruz	sc-2018	5μg for 100μg of chromatin
Anti-H3K9me3	rabbit	Millipore	07-442	5μg for 250μg of chromatin

SUPPLEMENTARY METHODS

Generation and purification of the anti-H3K27me3/S28 antibody

The rabbit polyclonal antibody used to detect the H3K27me3/S28ph double modification was generated using the following peptide: KAARK(me3)S(ph)APATGG-C**.** To minimise cross-reactivity with the homologous K9me3/S10ph region, the antibody was immunodepleted by incubation with NeutrAvidin agarose beads (Thermo Scientific) conjugated to a peptide containing the H3K9me3/S10ph epitope (biotin-ARTKQTARK(me3)S(ph)TGGKAP-RKQL). Assessment of the specificity of the antibody is described in Figure S1.

Osteogenic differentiation of MEFs

MEFs isolated from wt and MSK1/2 double KO littermates were grown in α-MEM/10% FCS/ 100U penicillin/ 100 μg/ml streptomycin to 50% confluence. For osteogenic differentiation the cells were transferred into osteogenic medium (α-MEM/10% FCS/ 100U penicillin/ 100 μg/ml streptomycin/ 50μg/ml ascorbic acid/ 10mM β-glycerophosphate/50 ng/ml BMP-2. The medium was replaced every 72 h for up to 14 days.

Centrifugal elutriation

Conditions to obtain G1 LPS activated mouse B cells were as follows: Elutriation was carried out on 4 X 10^8 cells after 72 hours of activation with LPS. The cells were suspended in 10 ml of RPMI media supplemented with 1% FBS and loaded into the system at 2300 r.p.m and 4°C, using a starting flow rate of 6 ml/min. G1 mouse B cells were flushed out of the chamber at a flow rate of 10 ml/min. Conditions to obtain G1 mouse ES cells were as follows: 2.5 X 10^8 cells were suspended in 10 ml of PBS supplemented with 1% FBS and loaded into the system at 1800 r.p.m and 4°C, using a starting flow rate of 7 ml/min. G1 mouse ES cells were flushed out of the chamber at a flow rate of 9ml/min. For both cell types, cell fractions of 200 ml were collected at different flow rates (from 7 to 13 ml/min). Cell cycle stage was assessed by measuring the DNA content using propidium iodide (PI) staining on a Becton Dickinson FACSCalibur Flow Cytometer, using BD CellQuest Pro software.

Chromatin Immunoprecipitation

ChIP analysis was carried out as described by (Szutorisz et al., 2005) with the following modifications. Cells were fixed in medium + 1% formaldeheide for 10 min at 37^{o}C. Formaldeheide-fixed chromatin from 2.5 - 3 × 10^7 cells/ml was sonicated in sonication buffer (50 mM Hepes pH 7.9/ 140 mM NaCl/ 1mM EDTA/ 1% triton X/ 0.1% Na-deoxycholate/ 0.1% SDS/ Complete-mini Roche protease inhibitors/ SIGMA phosphatase inhibitors cocktail 3) using a Diagenode Bioruptor to obtain DNA fragments of around 500 bp. Aliquots of 100-400 μg of sonicated chromatin were subjected to immunoprecipitation in sonication buffer with 5 mg of antibody.

For ChIP analysis of resting B cells with the antibodies against H3K9me3/S10ph and H3K27me3/S28ph the protocol described above was used with the following modifications. 10^8 cells were lysed in 10 ml of lysis buffer (10 mM HEPES pH 7.5, 10

mM EDTA, 0.5 mM EGTA and 0.5% Triton) and incubated at 4ºC on a rotating platform for 10 minutes. After centrifugation at 1700g, the nuclei were resuspended in 10 ml of nuclei wash buffer (10mM HEPES pH 7.5, 1mM EDTA, 0.5 mM EGTA and 200 mM NaCl) and incubated at 4ºC on a rotating platform for 10 minutes. The nuclei were then resuspended in 1ml of lysis/sonication buffer (25 mM Tris pH7.5, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.2% sodium deoxycholate), lysed on ice for 30 minutes and sonicated. The material was centrifuged at 14,000rpm (4ºC, 15 min) and the chromatin concentration was measured. For the immunoprecipitation, 70 µl of purified anti-H3K27me3S28ph antibody was coupled to 20 µl of protein G-dynabeads (Invitrogene 100-03D) in wash buffer 1 (50 mM Tris pH8.0, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP40 and 1mM EDTA) at 4ºC for 4hr on a rotating wheel. The beads-antibody conjugates were washed twice with buffer 1 and subsequently incubated with 200 µg of chromatin at 4ºC overnight. After washes and DNA elution, the eluted DNA fragments were treated with 200 µg/ml of proteinase K and purified using the QIAquick PCR purification Kit. Aliquots of 2 µl of 100 µl of immunoprecipitated and input DNA were analyzed by quantitative real-time PCR (qPCR). Amplification (40 cycles) was performed using SYBR Green Jump Start (Sigma). All buffers were supplemented with protease inhibitors cocktail (Roche, Burgess Hill, UK) and phosphatase inhibitors cocktail (Sigma).

Re-ChIP analysis of H3K27me3 and H3K9me3/S10ph

For sequential re-ChIP, cross-linked chromatin was immunoprecipitated with the indicated first antibody. Immunocomplexes were eluted with 100 µl of ChIP elution buffer (50 mM Tris-HCl pH 7.5, 10 mM EDTA, 1% SDS) for 10 min at 68ºC on a rotating platform (Geisberg and Struhl, 2004). A 10 ml aliquot of eluted chromatin was used for qPCR to evaluate the first ChIP and the residual fraction was diluted 10-fold in dilution buffer (16.7 mM Tris-HCl pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton, 0.01% SDS). The diluted eluate was divided into equal fractions and the second ChIP was performed under the same conditions as the first, using anti-H3K27me3 or anti-H3K9me3S10ph antibodies. Anti-EZH2 antibody was used as a positive control and anti-IgG as a negative control.

ChIP-on-chip using Agilent tiling microarrays

Whole genome amplified DNA (3 mg) was labelled with Cy3 (input) and Cy5 (ChIP DNA) by random priming and hybridized to Agilent microarrays tiled across selected regions of the mouse genome (http://www.chem.agilent.com/cag/prod/dn/G4410-90020_CGH_Protocol_FFPE1_0.pdf). High frequency repeats were excluded from the array by repeat masking. Agilent scanner and Feature Extraction software were used to obtain feature intensity data. ChIP interactive analysis of the extracted features were performed with the CGH module of the DNA Analytics® software 4.0.85 (Agilent Technologies). Dye bias (Intra-array) and Inter-array median normalization methods were applied. The Whitehead Error Model was applied using the observed spread of negative controls source. The ChIP application used the Whitehead Per-Array Neighbourhood model for peak detection. p-values of $p<0.05$ for the central probe and its left and right neighbours were used to identify protein-DNA binding interactions (peaks). Two biological replicate ChIP/microarrays were performed for each antibody.

Peptide pulldown assay

NIH3T3 cells were harvested, washed once with ice-cold PBS and resuspended in 1ml sucrose buffer (10 mM Tris pH 8.0, 0.32 M sucrose, 3 mM CaCl2, 2 mM magnesium acetate, 1 mM DTT, 0.1 mM EDTA, 0.2% NP-40 and freshly added protease inhibitors (Roche)) per 10^8 cells to isolate the nuclei. After 5 min of incubation on ice, the nuclei were pelleted by centrifugation (500g, 5 min., 4°C) and washed once with sucrose buffer without the detergent. The quality of the nuclear preparations was confirmed visually by trypan blue staining. The nuclei were then resuspended in 1ml lysis buffer (20 mM Hepes, pH 7.9, 25% v/v glycerol, 420 mM KCl, 1.5 mM MgCl2, 0.2 mM EDTA, 1 mM DTT and freshly added protease inhibitors) per 3 x 10^8cells and subjected to 3 cycles of freezing and thawing, followed by centrifugation (10 min, max, 4 deg. C). The supernatant was collected as the nuclear fraction, while chromatin pellets were solubilized by digestion with MNase I (New England BioLabs). The nuclear and chromatin fractions were pooled together, adjusted to 150 mM NaCl and pre-cleared, and used for the peptide capture assay with biotinylated peptides corresponding to aa 1-20 of histone H3 (GL BIochem). The peptides contained the following defined post-translational modifications: trimethylation of lysine K9 alone or in combination with phosphorylation of serine S10. The unmodified peptide was used as a control.
The peptide pulldown assay was performed as described in (Wysocka, 2006) with the following modifications. $5x10^7$ cells were used per assay. Extracts were incubated at 4°C with 20 µl of NeutrAvidin beads (Pierce) coupled to biotinylated histone peptides. Beads were then washed 5 times with ice-cold wash buffer (20 mM Hepes, pH 7.9, 20% v/v glycerol, 0.2 mM EDTA, 0.2% Triton X-100, 150 mM KCl and freshly added protease inhibitors). Bound proteins were eluted by boiling in Laemmli buffer. Eluted proteins were subjected to SDS-PAGE followed by Western blotting and detection with the antibodies against Ezh1 and Ezh2 (Table II).

In vitro binding analysis by surface plasmon resonance (SPR)

Surface Plasmon Resonance was carried out as described in Sabbattini et al., 2007. The assays were performed on a Biacore X instrument (Biacore, Milton Keynes, UK) with Biacore HBS-EP buffer (10mM Hepes pH 7.4, 0.15M NaCl, 3mM EDTA, 0.005% Tween 20) at a flow rate of 10 ml/min. 6500 response units (RU) of anti-6X-His monoclonal antibody (Abcam) and 6700 RU of a control anti-RNA pol II monoclonal antibody (kind gift of Laszlo Tora) were immobilised using the Amine Coupling Kit (Biacore) on flow cell 1 and 2 respectively of a CM5 sensor chip. 300 nM 6Xhis-Cbx protein was injected simultaneously over flow channels 1 and 2 followed by injection over both flow channels of 50 mM solutions of peptides for 2 minutes. After the injection, the dissociation of the complex was followed for 180 seconds. Non-phosphorylated peptides were also injected at lower concentrations for comparability to phosphorylated peptide binding. The difference between the increase in RU between flow channel 1 and flow channel 2 was used to measure the specific binding of the peptides to His-Cbx. At least two injections were carried out for each peptide. Data were analysed using Bioevaluation 3.2 software (Biacore).

SUPPLEMENTARY REFERENCES

Geisberg, J.V., and Struhl, K. (2004). Quantitative sequential chromatin immunoprecipitation, a method for analyzing co-occupancy of proteins at genomic regions in vivo. Nucleic Acids Res *32*, e151.

Szutorisz, H., Canzonetta, C., Georgiou, A., Chow, C.-M., Tora, L., and Dillon, N. (2005). Formation of an active tissue-specific chromatin domain initiated by epigenetic marking at the embryonic stem cell stage. Mol Cell Biol *25*, 1804-1820.

Wysocka, J. (2006). Identifying novel proteins recognizing histone modifications using peptide pull-down assay. Methods *40*, 339-343.