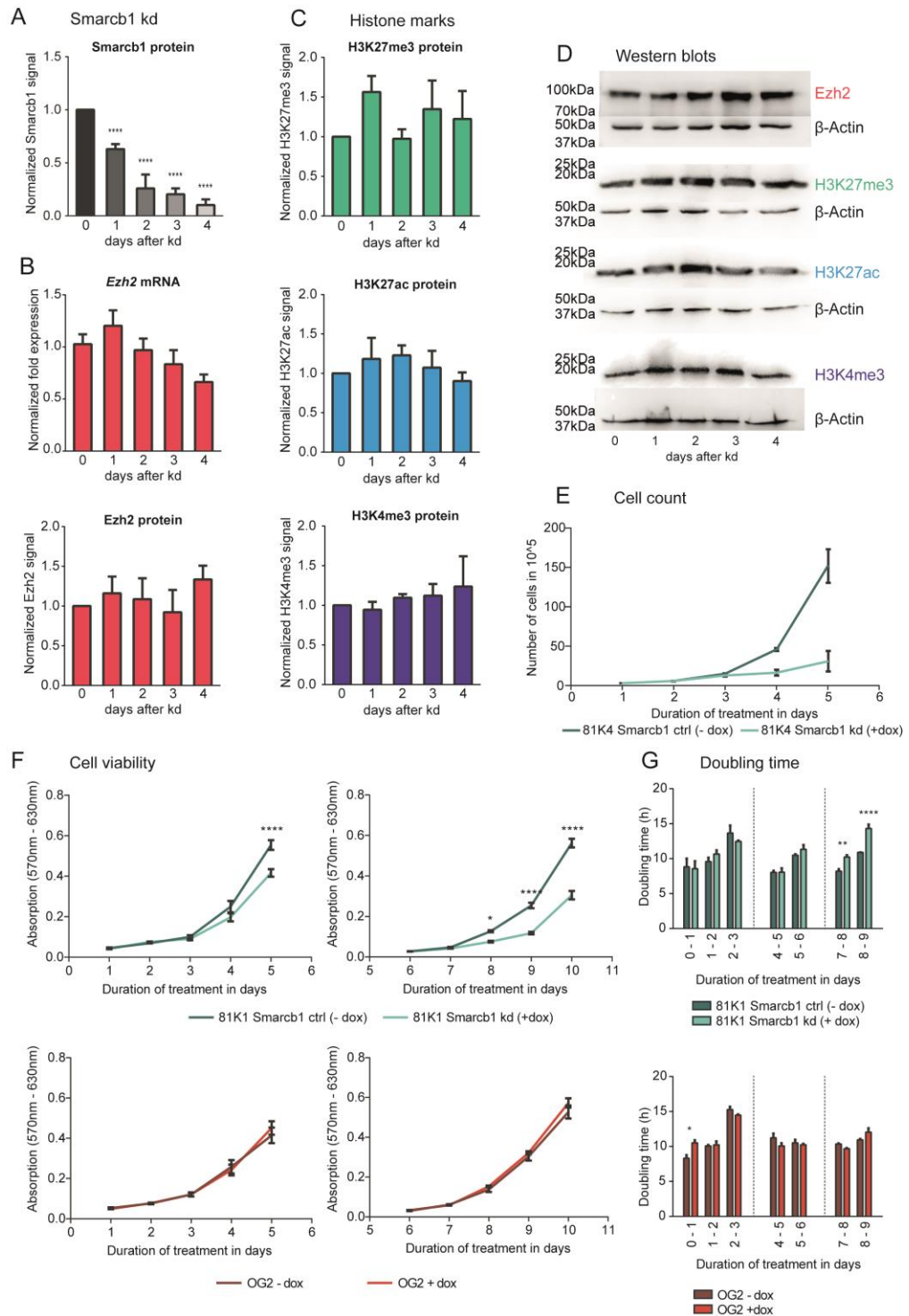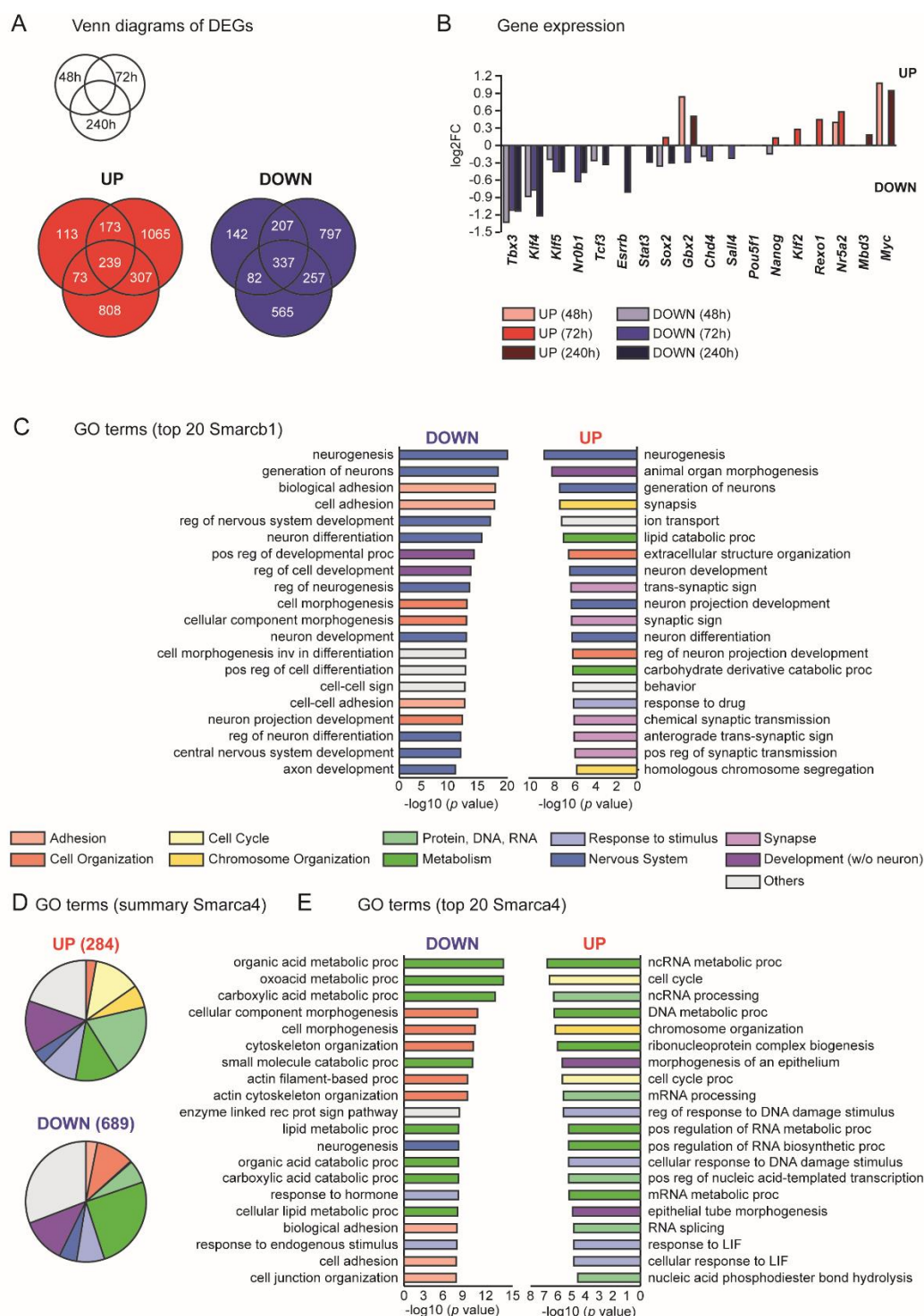## SUPPLEMENTARY FIGURES

Supplementary Figure S1

Supplementary Figure S1. (related to Figure 1): Smarcb1 loss has little impact on *Ezh2* expression and histone marks but impairs mESC proliferation (A) Levels of Smarcb1 protein in Western blot semi-quantitatively analysed using Image J including n = 3 biological replicates. (B) *Ezh2* expression in RT-qPCR (n = 3 biological replicates in technical triplicates) and Ezh2 signal in Western blot semi-quantitatively analysed using Image J (n = 3 biological replicates). (C)

Western blots of histone marks H3K27me3, H3K27ac and H3K4me3 semi-quantitatively analysed using Image J. Per antibody, experiments were carried out in n = 3 biological replicates. (D) Representative Western blots for all antibodies presented in (B) and (C). (E) Manual counting experiment of 81K4 ESC clone in n = 3 biological replicates. (F) MTT assays performed in n = 3 biological replicates per condition with 81K1 ESC with and without kd of *Smarcb1*. As control cell line, OG2 ESC were tested under equal conditions. Cells treated for 1 to 5 days and cells treated for 6 to 10 days (pre-treated before seeding for MTT assay) were examined in two different experiments to ensure optimal growth conditions. (G) Doubling time determined by manual counting in n = 3 independent experiments. In (A) – (C) and (E) – (G), error bars indicate SEM, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.
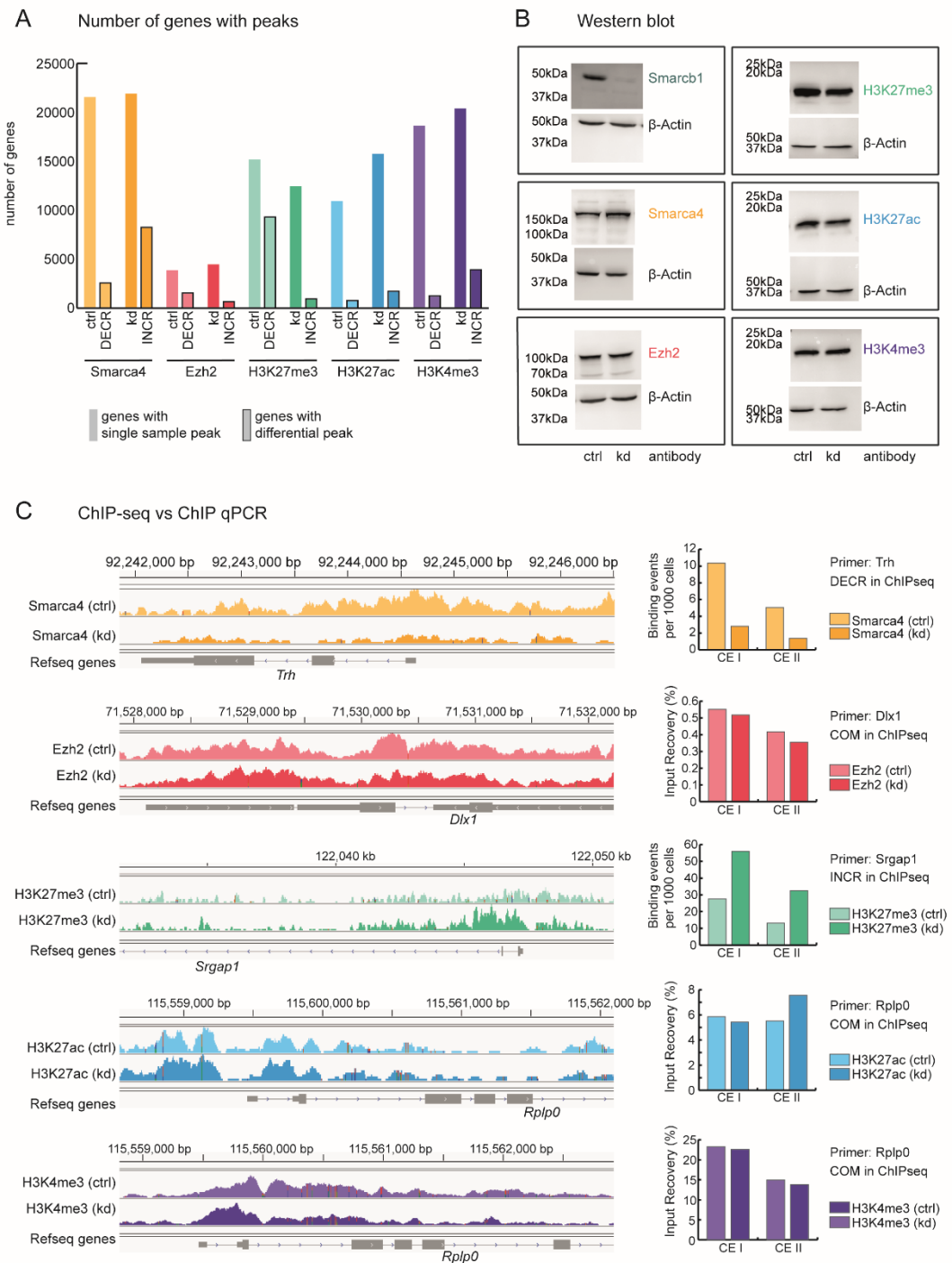
Supplementary Figure S2

Supplementary Figure S2. (related to Figure 1): In-detail analysis of gene expression changes after *Smarcb1* kd and *Smarca4* kd (A) Venn diagrams of DEG after *Smarcb1* kd at three different time points (48 h, 72 h, 240 h). Only genes with *p* value < 0.05 and abs. log2FC > 0.58 were considered. (B) Expression changes of genes connected to naïve pluripotency in ESC that were described by Dunn et al. [4] 48 h, 72 h and 240 h after *Smarcb1* kd. Only expression of genes with *p* < 0.05 is displayed. (C) Top 20 GO terms (ranked by *p* value) assigned to up- and downregulated genes after 72 h of *Smarcb1* kd. Colour code is based on categories as explained in Fig. 1E. (D and E) Biological process related GO terms that were assigned to genes up- or downregulated after
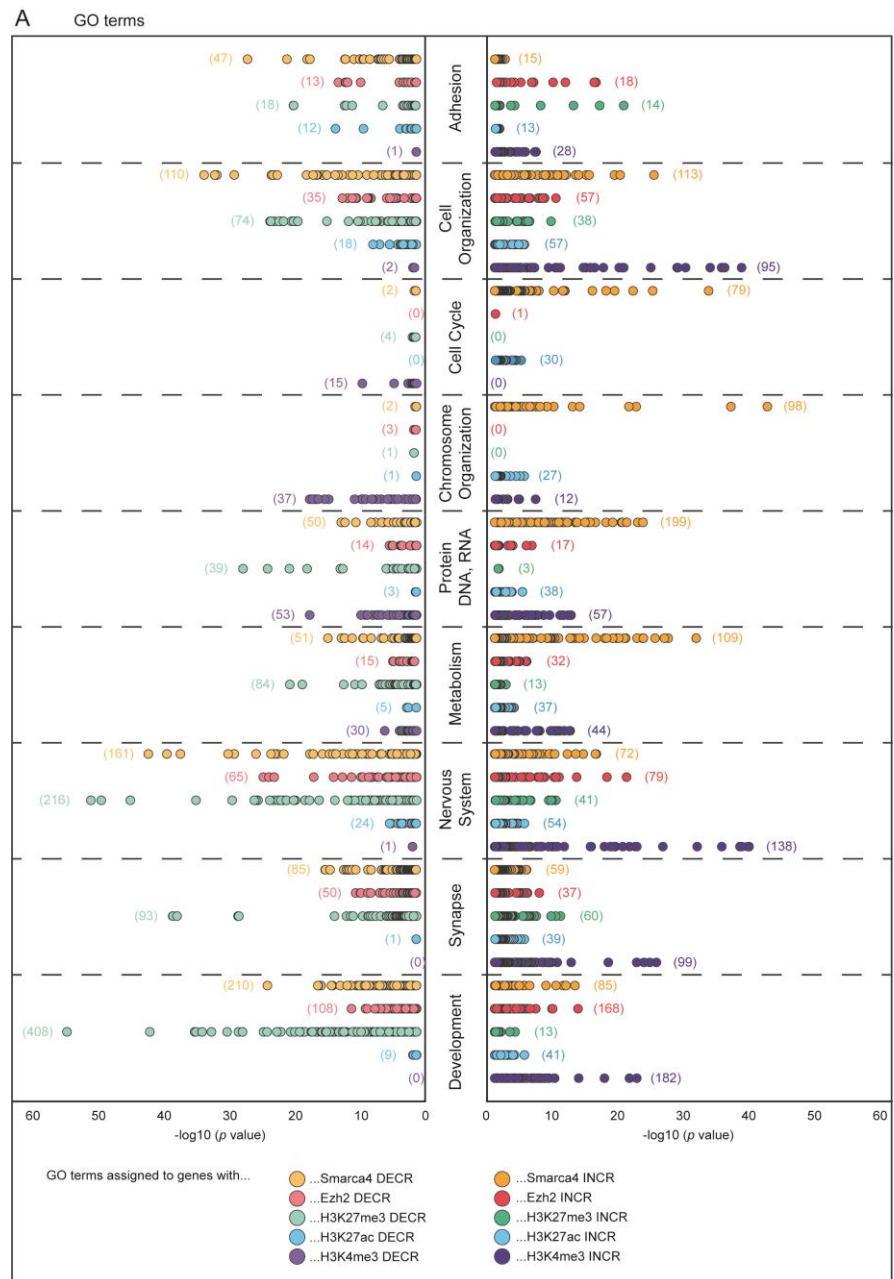
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Smarca4 knockdown (data obtained from [34]). Genes were analysed separately using ToppGene and terms were separated into categories already used for *Smarcb1* knockdown related GO terms. Pie charts give a summary of these categories, bar charts only include GO terms within the top 20 (ranked by *p* value) In parenthesis, total numbers of GO terms are given.
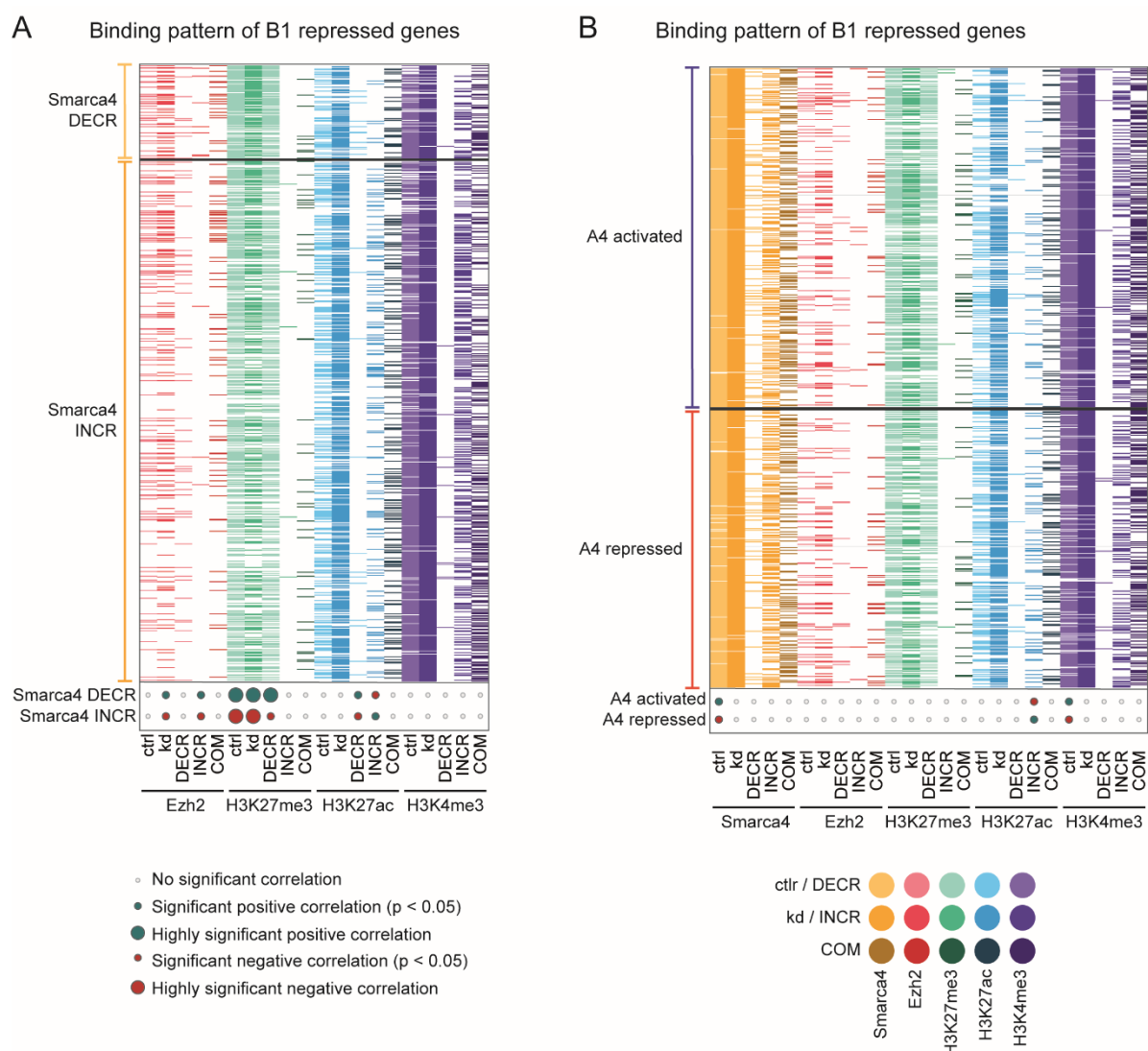
Supplementary Figure S3

Supplementary Figure S3. (related to Figure 3): Validation of ChIP-seq data (A) Number of genes with peaks / DECREASE / INCREASE of examined factors and histone marks. For merging ChIP-seq data with genomic information, a gene region was defined to start 1 kbp upstream of TSS (Transcription start site) until TES (Transcription end site). (B) Representative Western blots performed with protein extracts of cells that were also used for ChIP-seq. (C) Validation of ChIP-seq experiments via ChIP-qPCR. Gene regions with antibody binding were chosen randomly and peak calling after ChIP-seq (left-hand) were compared with ChIP-qPCR results (right-hand). For a more detailed review on analysis of ChIP-qPCR, see materials and methods. ChIP-qPCR experiments were performed in n = 2 biological replicates.

Supplementary Figure S4. (related to Figure 3): Detailed analysis of GO terms connected to genes with changed antibody binding after *Smarcb1* kd (A) GO terms being assigned to the indicated gene-sets (genes with changed antibody binding after *Smarcb1* knockdown). On the left-hand site, terms associated with genes showing a DECREASE of antibody binding (lighter colours) are represented, on the right-hand site, those with an INCREASE (darker colours.) Each dot represents one individual GO term, numbers in parentheses report the overall number of GO terms that have been assigned to the gene set and category of interest. Terms fitting into more than one category are depicted in all of these categories to prevent loss of information. This graph focuses on the most frequently occurring topics, therefore not including all GO terms that are presented in Figure 3C.

Supplementary Figure S5

Supplementary Figure S5. (related to Figure 4): **Genes upregulated after *Smarcb1* knockdown share their antibody binding pattern** (A) Heatmap showing antibody binding patterns of genes fulfilling the following criteria: (I) upregulated after *Smarcb1* kd ($p < 0.05$, log2FC > 0.58) and (II) Smarca4 DECREASE or INCREASE. Depending on Smarca4 binding, two groups were formed whose antibody binding patterns were compared using permutation tests (results below the heatmap). Aquamarine circles represent a positive correlation (genes are more likely to be bound by the antibody of interest if they are in the tested group) while red circles represent a negative correlation (genes are less likely to be bound by the antibody of interest if they are in the tested group). For a more detailed explanation of significance levels, see materials and methods. (B) Heatmap displaying antibody binding patterns of genes (I) upregulated after *Smarcb1* kd ($p < 0.05$, abs. log2FC > 0.58) and (II) changed expression after *Smarca4* kd (data obtained from Ho et al. [34]). Depending on expression after *Smarca4* kd, two groups were formed whose antibody binding patterns were compared using permutation tests (results below the heatmap).

SUPPLEMENTARY TABLES

Supplementary Table S1: Log2FC thresholds for comparison of Smarca4 versus Smarcb1 kd

CATEGORY	DEFINITION

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B1 / A4 repressed	Upregulated after Smarcb1 knockdown → $p < 0.05$, log2FC > 0 AND Upregulated after Smarca4 knockdown → $p < 0.05$, log2FC > 0
B1 / A4 activated	Downregulated after Smarcb1 knockdown → $p < 0.05$, log2FC < 0 AND Downregulated after Smarca4 knockdown → $p < 0.05$, log2FC < 0
B1 repressed / not A4 repressed	Upregulated after Smarcb1 knockdown → $p < 0.05$, log2FC > 0.58 AND Not Upregulated after Smarca4 knockdown → either $p > 0.05$ or $p < 0.05$ and log2FC < 0
B1 activated / not A4 activated	Downregulated after Smarcb1 knockdown → $p<0.05$, log2FC< -0.58 AND Not Downregulated after Smarca4 knockdown → either $p >0.05$ or $p < 0.05$ and log2FC > 0
A4 repressed / not B1 repressed	Upregulated after Smarca4 knockdown → $p < 0.05$, log2FC > 0.58 AND Not Upregulated after Smarcb1 knockdown → either $p > 0.05$ or $p < 0.05$ and log2FC < 0
A4 activated / not B1 activated	Downregulated after Smarca4 knockdown → $p < 0.05$, log2FC < -0.58 AND Not Downregulated after Smarcb1 knockdown → either > 0.05 or $p < 0.05$ and log2FC > 0

Supplementary Table S2: Lysis buffers used for chromatin extraction

INGREDIENTS	LB1	LB2	LB3
HEPES KOH 1 M, pH 7.4	50 mM	-	-

Tris HCl 1 M, pH 8.0	-	10 mM	10 mM
NaCl 5 M	140 mM	200 mM	140 mM
EDTA 0. 5 M, pH8.0	1 mM	1 mM	1 mM
EGTA 0.5 M, pH 8.0	0.5 mM	0.5 mM	0.5 mM
Glycerol Anhydrous	10 %	-	-
IGEPAL CA-630, 10 %	0.5 %	-	-
Triton X-100, 10 %	0.25 %	-	-
N-lauroyl-sarcosine, 10 %	-	-	1 %
Sodium deoxycholate	-	-	0.2 %

Supplementary Table S3: Oligonucleotides' sequences

OLIGONUCLEOTIDES	FORWARD	REVERSE
mSmarcb1	GAGGTGGGAAACTACCTGCG	CGCCAGAGTGAGGGGTATC
mRpl3	GGAAAGTGAAGAGCTTCCCTAAG	CTGTCAACTTCCCGGACGA
mEzh2	AATCAGAGTACATGCGACTGAGA	GCTGTATCCTTCGCTGTTTC
mDlx1	ATGTCTCCTTCTCCCATGTCC	ACTGCACGGAACTGATGTAGG

mRplp0	GAATAAAATCTCTGCCCTGT GG	TACTCTCCCTTACTCTCCCA CCT

Supplementary Table S4: shRNA sequences

shRNA	Anti-*Smarcb1*
Upper	TCCCGAAGCTAATGACTCCTGAGATTTCAACAGAATCTCAGGAGTCATTAGCTTCTTTTT
Lower	CGCGTAAAAAGAAGCTAATGACTCCTGAGATTCTCTTGAAATCTCAGGAGTCATTAGCT

Supplementary Table S5: Antibodies uses in this study

ANTIBODIES	SOURCE	IDENTIFIER
beta-actin mouse, monoclonal	Santa Cruz Biotechnology	SC-47778
α-Tubulin (B-7)	Santa Cruz Biotechnology	sc-23948
Smarca4 Rabbit, monoclonal	Abcam	ab110641
Brg1 (H-88)	Santa Cruz Biotechnology	sc-10768
Brg1 (N-15)	Santa Cruz Biotechnology	sc-8749
anti-Baf155	Santa Cruz Biotechnology	sc-9746
Baf155 (D7F8S)	Cell Signaling Technology	9053
EZH2 (D2C9) Rabbit, monoclonal	Cell signalling	5246S
H3K27ac	Abcam	ab4729

Rabbit, polyclonal		
H3K27me3 Rabbit, polyclonal	Merck KGaA	07-449
H3K4me3 Rabbit, polyclonal	Diagenode	pAb-003-050
IgG Rabbit, monoclonal	Novusbio	NBP2-24891
Peroxidase-conjugated anti-Mouse Goat, polyclonal	Jackson Immuno Research	115-035-044
Peroxidase-conjugated anti-rabbit Goat, polyclonal	Jackson Immuno Research	111-035-045
Smarcb1 mouse, monoclonal	BD transduction laboratories	612110
Smarcb1 (Y-7)	Santa Cruz Biotechnology	sc-101161

Supplementary Table 6: Chemicals used in this study

CHEMICALS	SOURCE	IDENTIFIER
Absolute pure ethanol (75 %)	AppliChem	# A4230-1000PE
Acrylamide (30 %) / Bis Solution	Bio-Rad Life Science	1610156
A/G PLUS agarose beads	Santa Cruz Biotechnology	sc-2003

BSA (bovine serum albumin, molecular biology grade, 20 mg/ml)	New England BioLabs	B9000S
BSA (bovine serum albumin, fraction V, 7.5 %)	Fisher Scientific	15260037
Chloroform	Sigma-Aldrich Chemie GmbH	288306
cOmplete™	Sigma-Aldrich Chemie GmbH	11697498001
Coomassie Brilliant Blue G-250	Bio-Rad Life Science	# 1610406
DEPC water	Invitrogen	AM9915G
DMEM Glutamax	Invitrogen	31966021
Doxycycline	Sigma-Aldrich Chemie GmbH	D9891-1G
EDTA	Carl Roth GmbH & CoKG	8043.2
EGTA	Sigma-Aldrich Chemie GmbH	E4378
FBS superior	Biochrom	S0615
Formaldehyde	Sigma-Aldrich Chemie GmbH	F8775

Glycine	Carl Roth GmbH & CoKG	3790.2
Gylcerol	AppliChem GmbH	A1123,2500
HCl	Honeywell Fluka™	71763
HEPES KOH	Sigma-Aldrich Chemie GmbH	H3375
IGEPAL CA-630	Sigma-Aldrich Chemie GmbH	18896
IgG Dynabeads™	Novex by Life Technologies	10004D
Isopropanol	SAV Liquid Production GmbH	ISOP-5000-100-1
Laemmli Sample Buffer (4 X)	Bio-Rad Life Science	1610747
LiCl	Sigma-Aldrich Chemie GmbH	62476
MEM Non-essential amino acids	Sigma-Aldrich Chemie GmbH	M7145
Methanol	Carl Roth GmbH & CoKG	T145.2
MTT reagent	Merck KGaA	CT01
Murine embryonic stem cell leukaemia inhibitory factor (ESLIF)	PolyGene Transgenetics	PG-A1140-0100

NaCl	Carl Roth GmbH & CoKG	3957.2
NaDOC	Sigma-Aldrich Chemie GmbH	D6750
N-lauroyl-sarcosine	Sigma-Aldrich Chemie GmbH	L9150
Power SYBR Green PCR Master Mix	Life Technologies	4367659
Proteinase K	Sigma-Aldrich Chemie GmbH	3115828001
PVDF membranes	BioRad Life Science	1620177
RNase A	Invitrogen	AM2272
SDS	Sigma-Aldrich Chemie GmbH	74255
Triton X-100	AppliChem GmbH	A4975,0500
TRIzol®	LifeTechnologies	15596018
Trypsin 0.05 % EDTA	Gibco by life technologies	25300054
Western Blot ECL Pro Solution	Perkin Elmer	NEL120001EA
b-Mercaptoethanol	Gibco by life technologies	31350-010

Supplementary Table S7: critical commercial kits

CRITICAL COMMERCIAL KITS	SOURCE	IDENTIFIER
PrimeScript RT Reagent Kit with gDNA Eraser	TaKaRa	RR047B
RNeasy Mini Kit	Qiagen	74104
QIAquick PCR Purification Kit	Qiagen	28104