SUPPLEMENTARY INFORMATION

BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation

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Figure S1

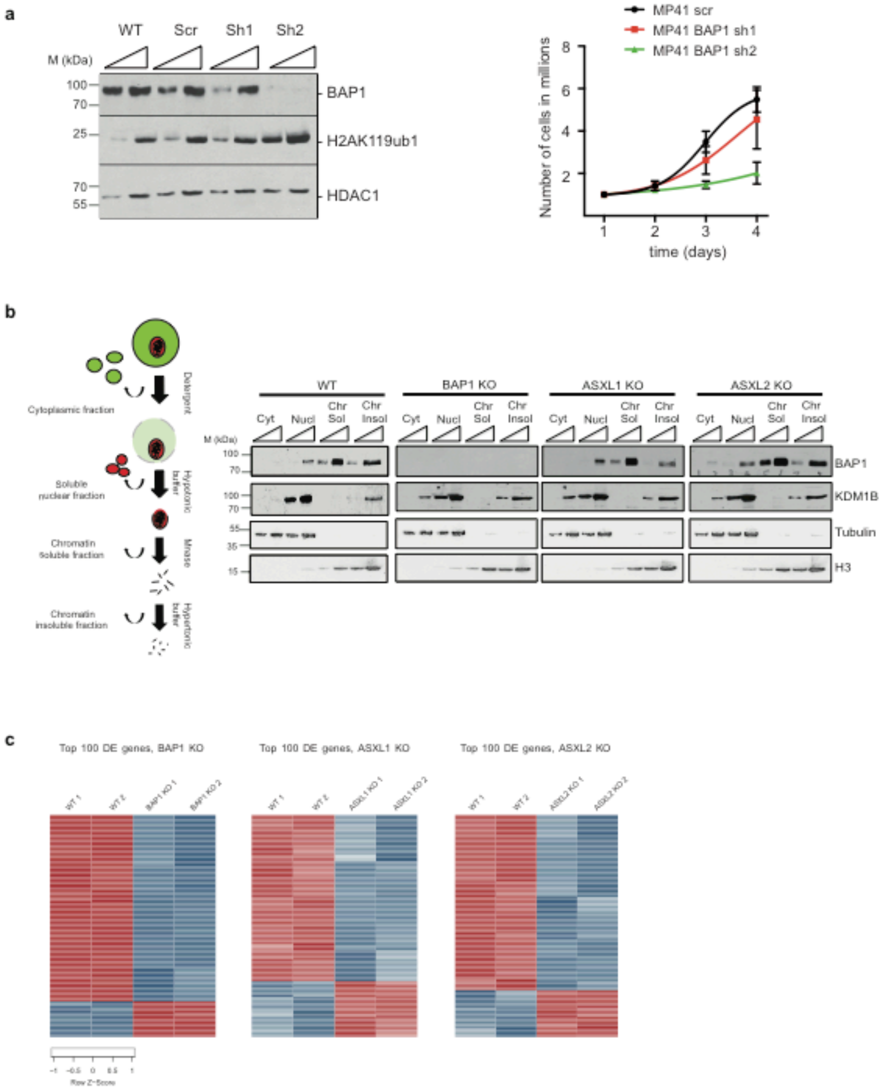
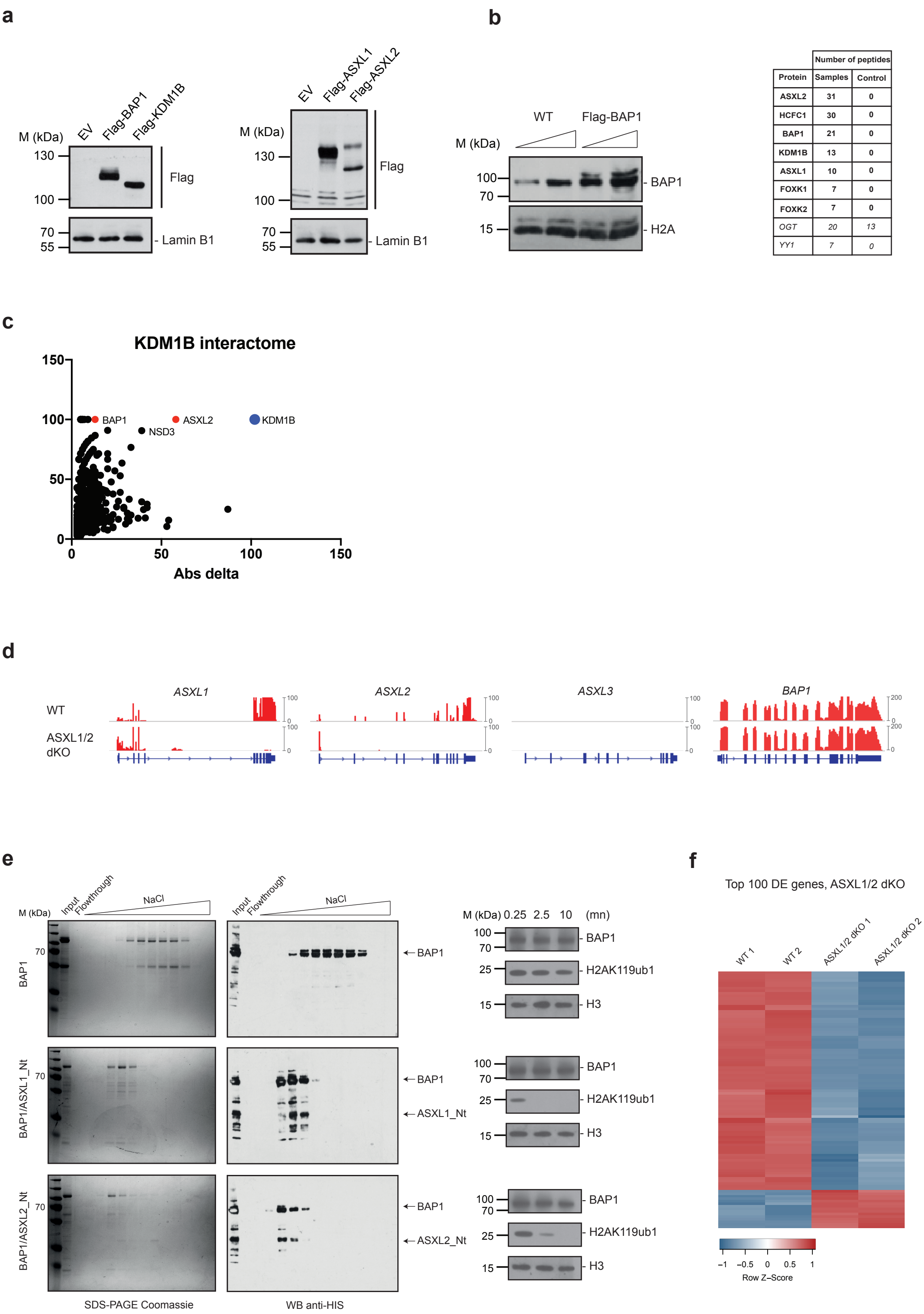


Figure S1: BAP1.com activity

a) RNA interference against *BAP1* in MP41 cells. Left panel: MP41 were infected with lentivirus containing shRNAs and nuclear extracts were analyzed by western blot. Scr = sh-scramble. A two-point titration (1:3 ratio) is shown for each condition. Right panel: proliferation assay in wild type or BAP1-silenced MP41 cells. Figures represent the mean of 3 independent biological replicates. Error bars represent the standard deviation between the replicates. **b)** Left panel: scheme summarizing the fractionation strategy for the isolation of chromatin-interacting proteins. Right panel: Western blots assessing the distribution of BAP1 and KDM1B in the different cellular compartments and chromatin fractions in HAP1 wild type and knockout cells. Tubulin and Histone H3 serve as controls for the cytoplasmic and chromatin associated fractions respectively. Two quantities were loaded for each condition, respecting a 1:3 ratio. **c)** Heatmap of 100 top differentially expressed (DE) genes in *BAP1*, *ASXL1*, and *ASXL2* KO cells. Individual replicates are shown side by side.

Figure S2



	Number of peptides	
Protein	**Samples**	**Control**
ASXL2	**31**	**0**
HCFC1	**30**	**0**
BAP1	**21**	**0**
KDM1B	**13**	**0**
ASXL1	**10**	**0**
FOXK1	**7**	**0**
FOXK2	**7**	**0**
OGT	*20*	*13*
YY1	*7*	*0*

Figure S2: BAP1.com composition.

a) Western blot analysis of nuclear extracts from HeLa cells expressing Flag-tagged constructs or empty vector as control. Lamin B1 was used as a loading control. **b)** Left panel: western blot showing overexpression of Flag-BAP1 in MP41 nuclear extracts. H2A was used as a loading control. Right panel: table recapitulating mass spectrometry result of Flag-BAP1 IP as control to control in MP41 cells. **c)** Mass spectrometry analysis of HeLa cells overexpressing FLAG-tagged KDM1B. Graphs represent proteins relative to their absolute and relative delta compared to mass spectrometry analysis of empty vector expressing cells. **d**) Screenshots of RNA-seq on wild-type and ASXL1/2 dKO cells showing expression of ASXL1, ASXL2, ASXL3 (not expressed), and BAP1. **e)** Left panel: coomassie and corresponding western blot analyses showing the anion exchange purification of recombinant BAP1, BAP1+ASXL1_Nt and BAP1+ASXL2_Nt (Mono Q PC 0.1ml). The input is issued from a first Flag IP on whole cell extracts of SF9 expressing the different combinations of recombinant proteins. The asterisk represents a non-specific protein co-IPed with Flag-BAP1. Right panel: representative result of deubiquitinase assay following H2AK119 ubiquitination, western blot for BAP1 serves as a control. **f**) Heatmap of 100 top differentially expressed (DE) genes in *ASXL1/2* dKO cells. Individual replicates are shown side by side.

Figure S3

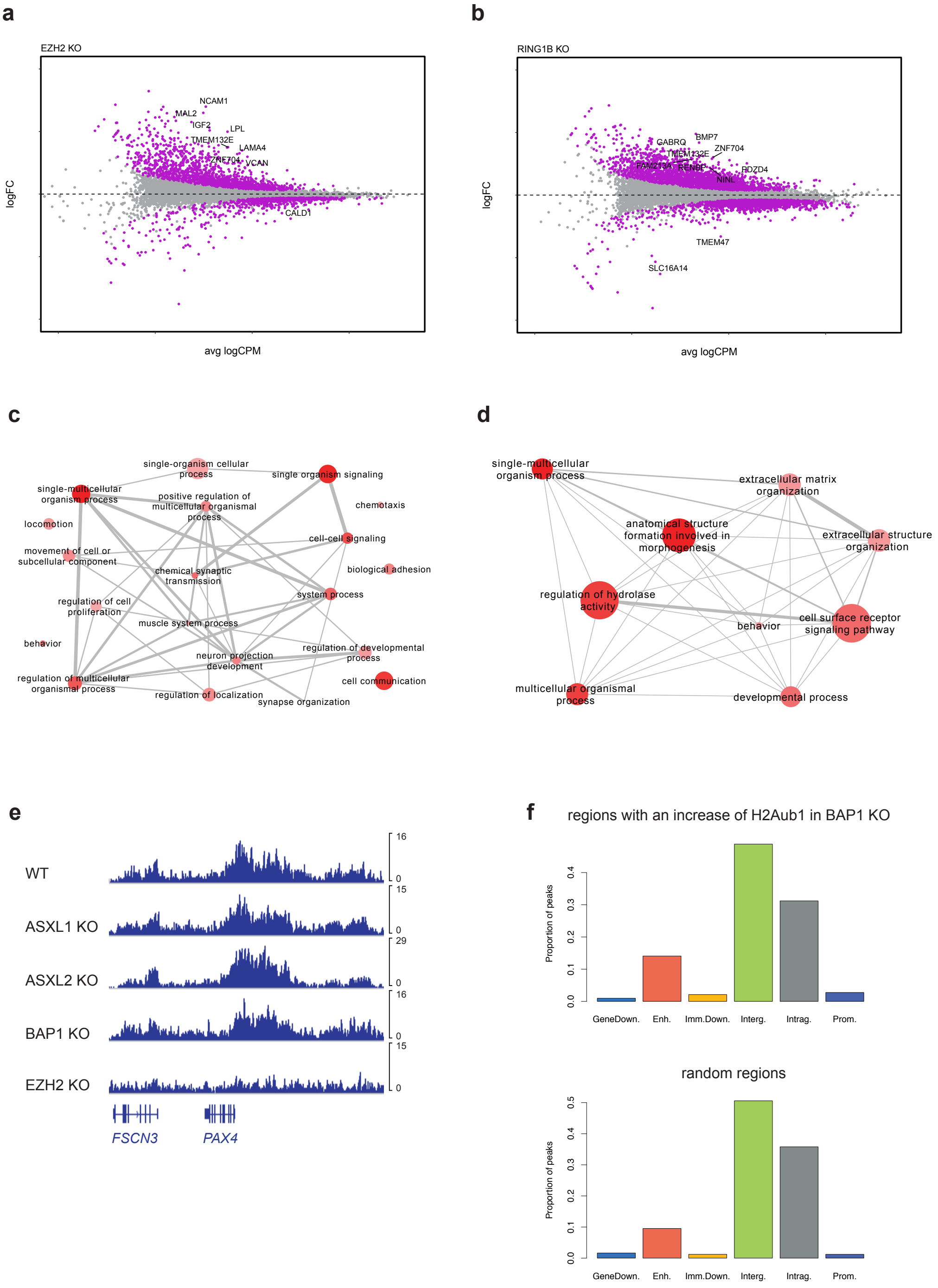


Figure S3: BAP1 versus the Polycomb machinery

a, b) Scatterplots showing log2 fold-change (logFC) expression between wild-type and *EZH2* KO (a) or *RING1B* KO (b) cells as a function of average log2 counts per million (logCPM). Differentially expressed genes (DEGs) are highlighted in magenta. **c, d**) Gene ontology analysis of DEGs in *EZH2* KO (c) and *RING1B* KO (d) cells. **e**) Screenshot showing H3K27me3 enrichment at the *PAX4* locus in the different conditions indicated on the left. **f**) Annotation of regions that gain H2AK119ub1 upon *BAP1* KO (top panel) versus a random permutation of the same regions over the human genome (bottom panel).

Figure S4

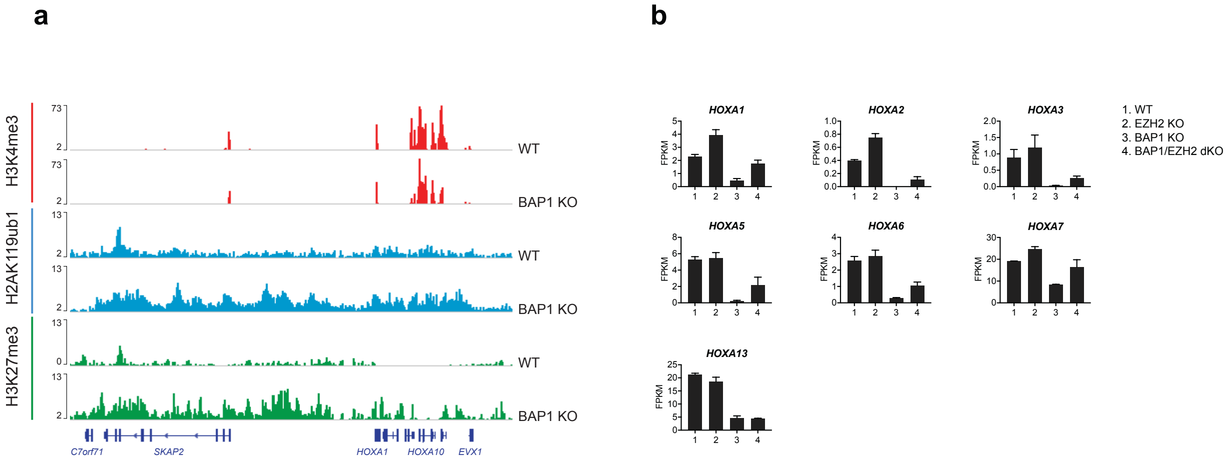


Figure S4: BAP1 versus PRC2

a) Screenshot showing enrichment for H3K4me3, H2AK119ub1 and H3K27me3 at the HOXA cluster in wild-type and *BAP1* KO cells. **b**) Expression of the individual HOXA genes represented as column bar graphs across wild-type, *EZH2*, *BAP1* and *BAP1/EZH2* KO conditions detected from corresponding RNA-seq data. Mean + SD, n=2.

Figure S5

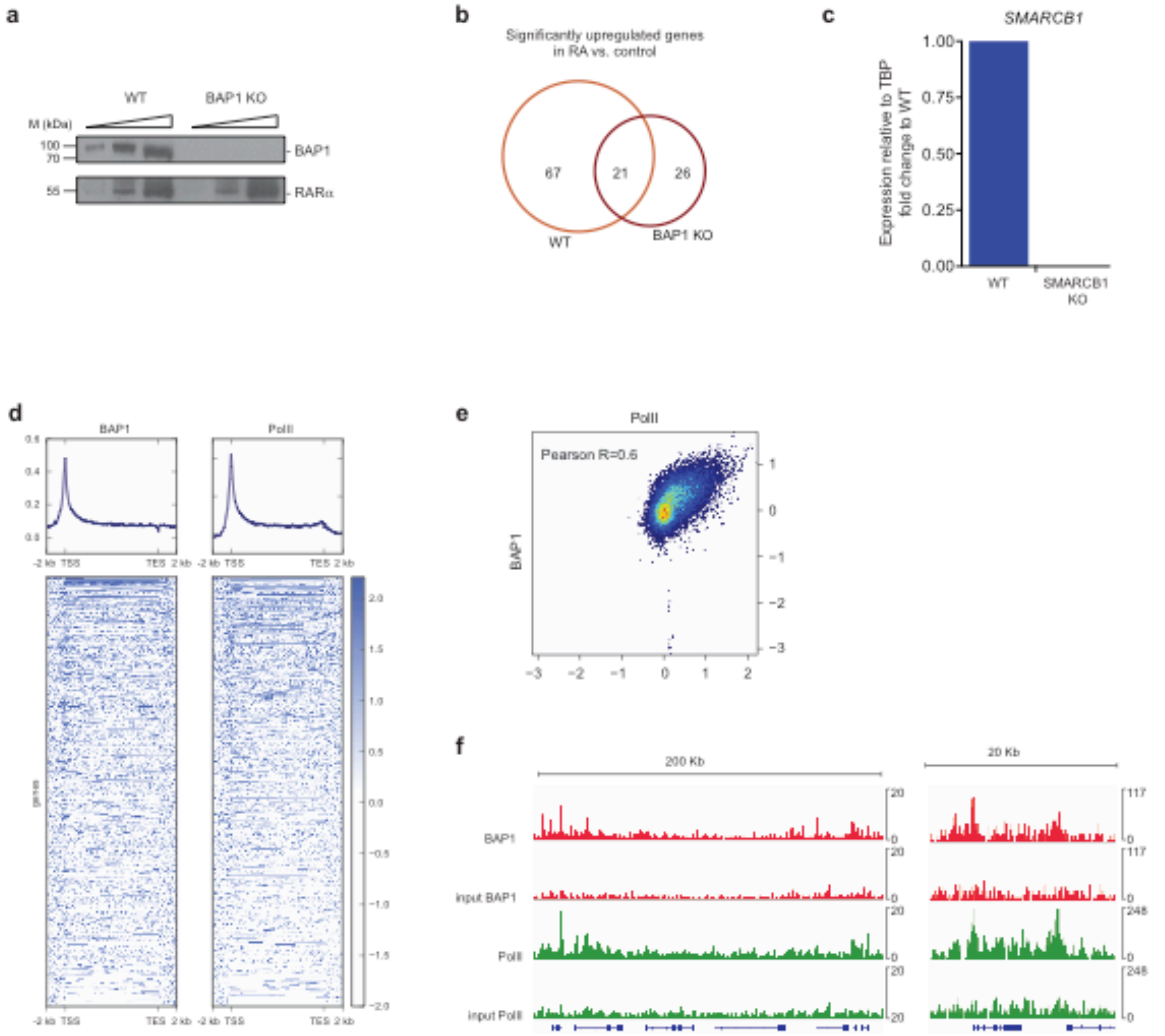


Figure S5: BAP1 and transcription

a) Titration of nuclear extracts from wild-type or *BAP1* KO HAP1 cells analyzed by western blot and probed with antibodies recognizing either BAP1 or RARα. **b**) Venn diagram showing the overlap between RA-regulated genes in wild-type and BAP1 KO cells. **c)** RT-qPCR analysis SMARCB1 expression in the different in wild-type and SMARCB1 KO HAP1 cells. Mean ± SD. n = 3. **d)** Heatmaps showing BAP1 and RNA PolII along gene bodies of all annotated genes from TSS to termination end site (TES) scaled to an equivalent 10 kb window and including 2 kb upstream and downstream of TSS and TES respectively. Corresponding average profiles are plotted on top of each heatmap. **e**) Scatterplot showing PolII versus BAP1 enrichment around the TSS (-/+ 2 kb) at all annotated genes in mouse Bone Marrow-Derived Macrophages. Pearson correlation coefficient is displayed. **f**) Screenshots of BAP1 and RNA PolII enrichment at representative regions. The input displayed below each corresponding ChIP-seq experiment.

Figure S6

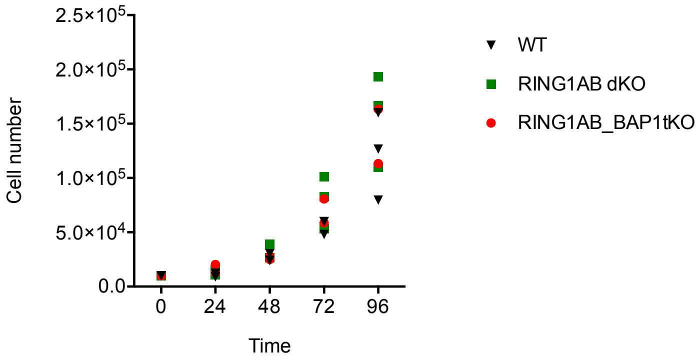


Figure S6: BAP1 versus PRC1.

Proliferation curve of wild-type, *RING1A/B* dKO and *RING1A/B*;*BAP1* KO HAP1 cells. n = 3.

Figure S7

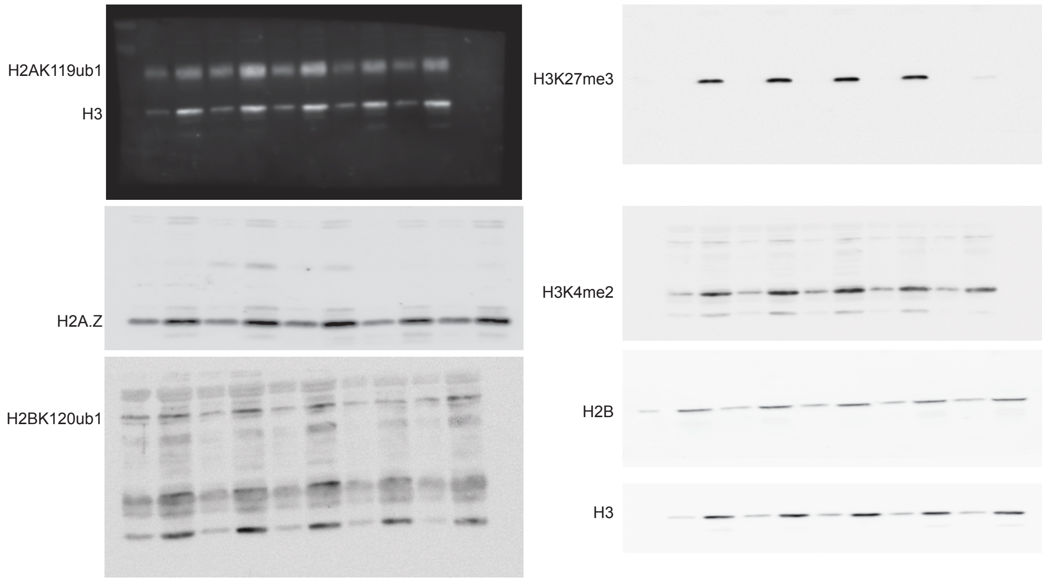


Figure S7: raw data for Figure 1C

Figure S8

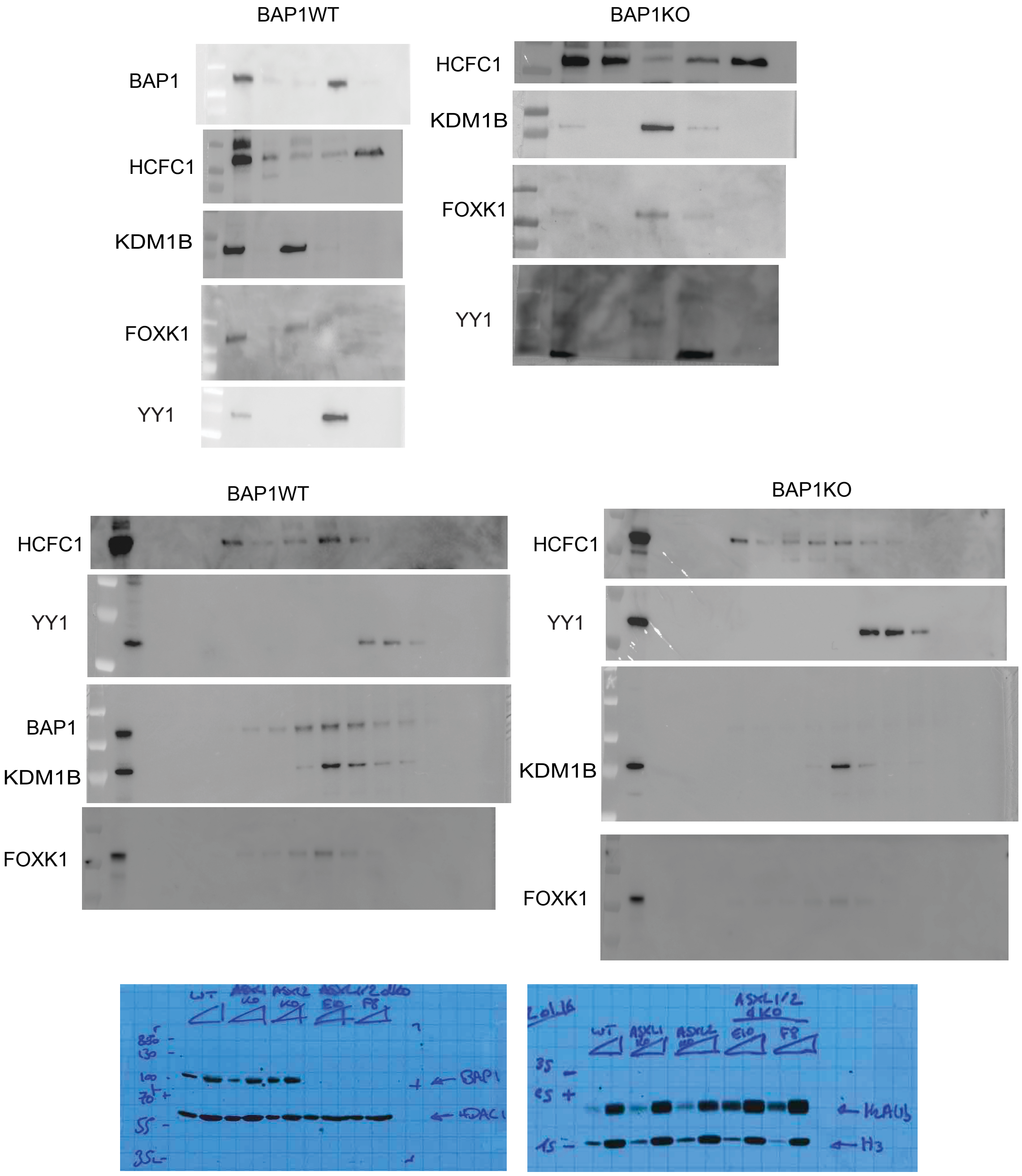


Figure S8: raw data for Figure 2B and 2D

Figure S9

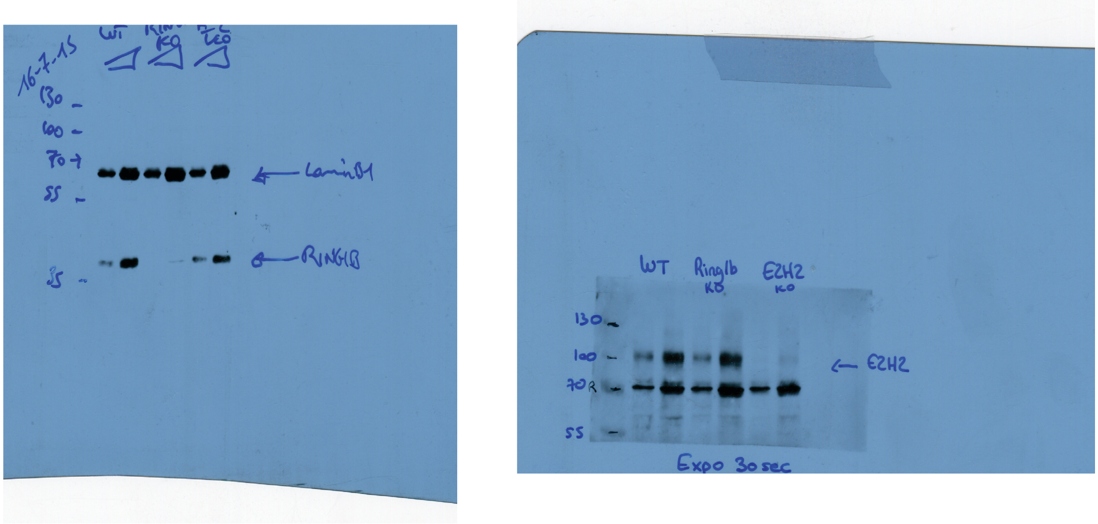


Figure S9: raw data for Figure 3A

Figure S10

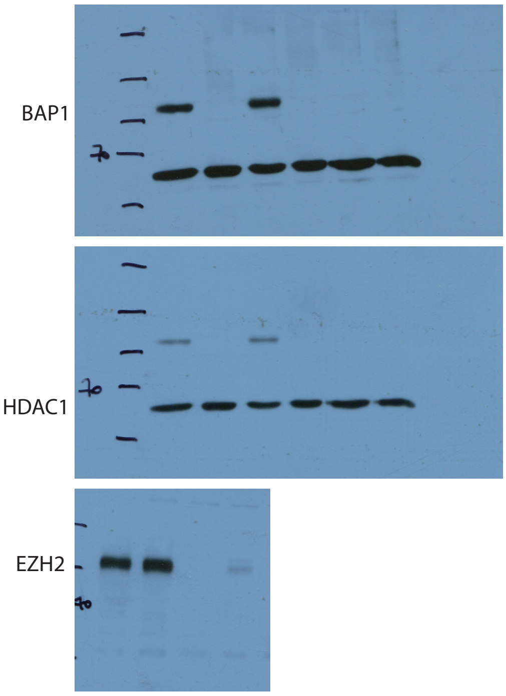


Figure S10: raw data for Figure 4B

Figure S11

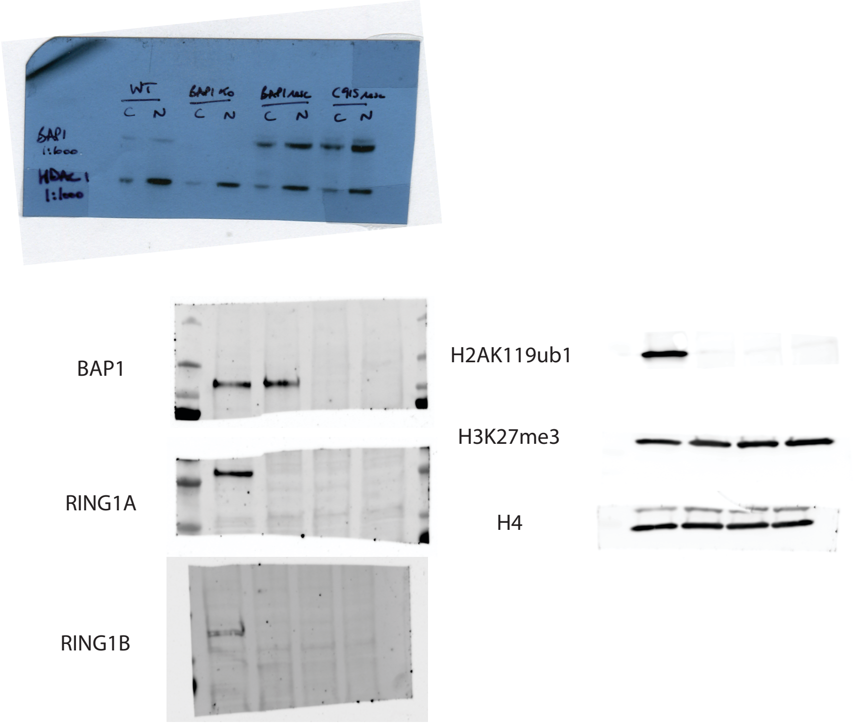


Figure S11: raw data for Figure 6A and 6B

Figure S12

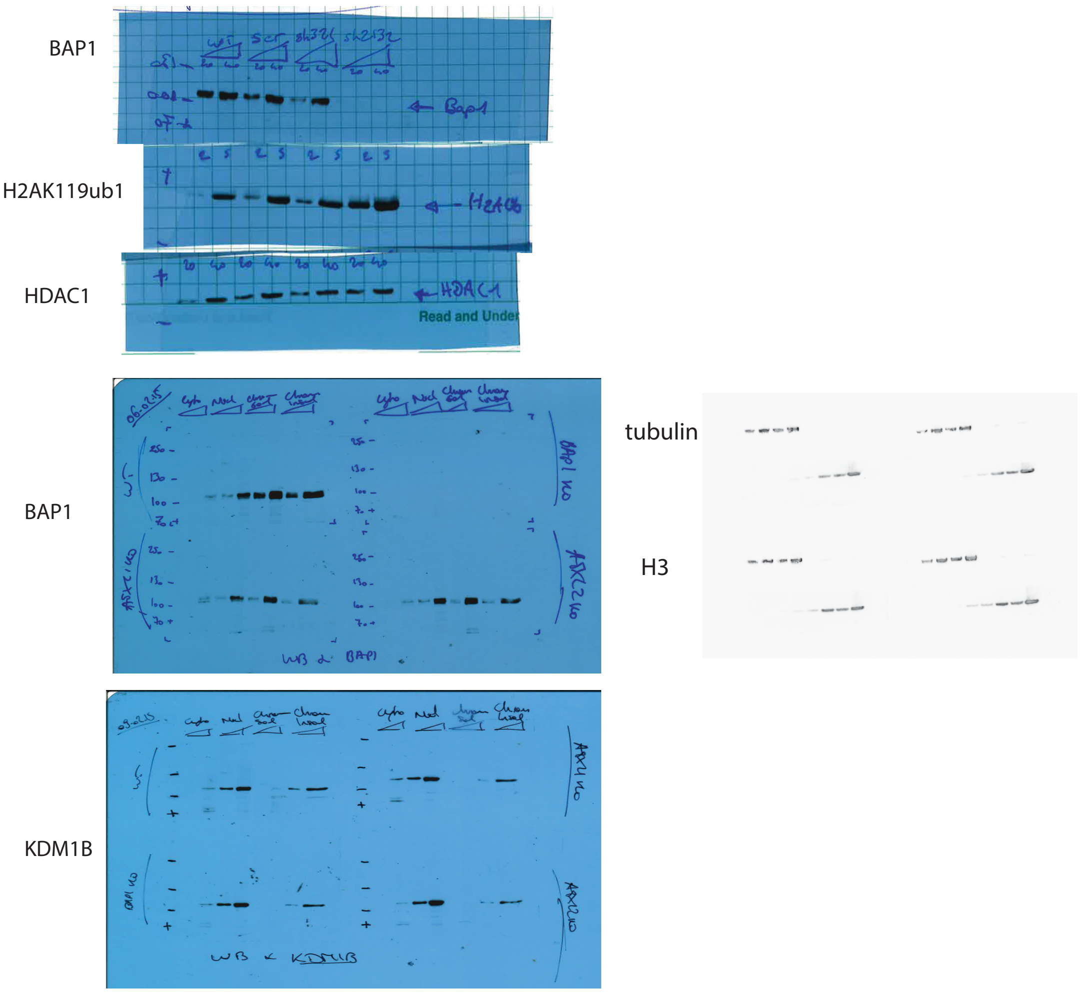


Figure S12: raw data for Figure S1A and S1B

Figure S13

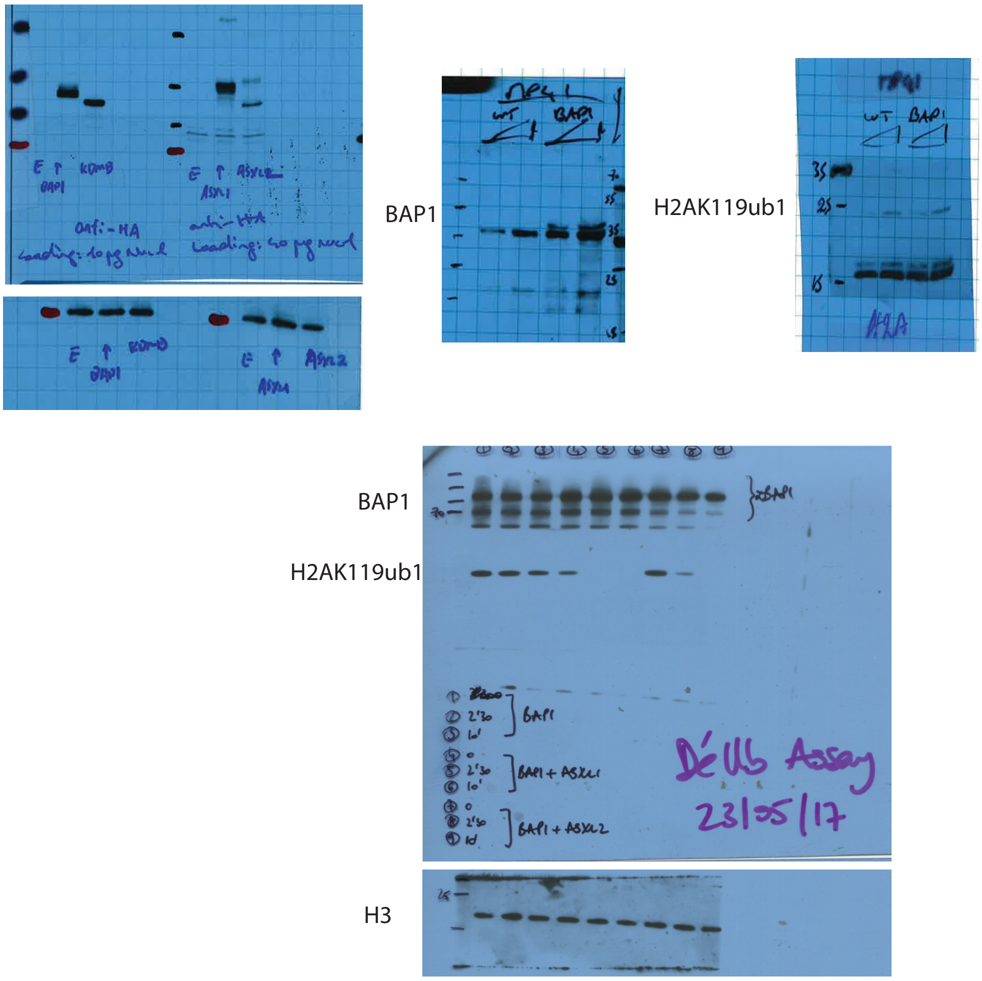


Figure S13: raw data for Figure S2A, S2B and S2E

Figure S14

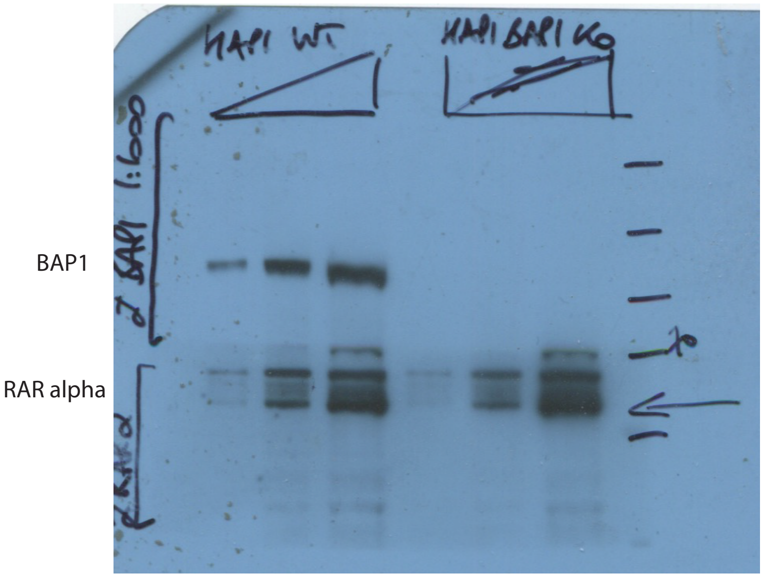


Figure S14: raw data for Figure S5A

primer	**sequence**
TBP_FW	GTTCTGGGAAAATGGTGTGC
TBP_REV	GCTGGAAAACCCAACTTCTG
SMARCB1_FW	AGACCAGCGCGTCATCATCA
SMARCB1_REV	CAGCTCCGAGCACAGCTTCA
EZH2_FW	TTTTTGCCAAGAGAGCCATC
EZH2_REV	TCGATGCCGACATACTTCAG
ASXL1_FW	AAAGCCACAGCCCACTAAAG
ASXL1_REV	AGTGGGCTGACCTTTAACCAC
ASXL2_FW	GACAGAATCCAGGTGCGAAAAG
ASXL2_REV	TTCTGGCTCCTGTTCTGTTAGG
BAP1_FW	CCACAAGTCTCAAGAGTCACAG
BAP1 _REV	CTGCACCATCTGTGTGGTT
RARB_FW	AGCAAGCCTCACATGTTTCC
RARB_REV	CTCTGCACcTTTAGCACTGATG
CYP26A1_FW	AACATTCGCGCCAAGATCTG
CYP26A1_REV	TGCTTTAGTGCcTGCATGTC
S100A11_FW	TCGCTCAGCTCCAACATGGCAA
S100A11_REV	ACAGCAATCAGGGACTCGATGCAC
TNFRSF1A_FW	TGCCAGGAGAAACAGAACACCGT
TNFRSF1A_REV	AGGCACAACTTCGTGCACTCCA
TMSB4X_FW	TCGCTTCGCTTTTCCTCCGCAA
TMSB4X_REV	GCCTGCTTGCTTCTCCTGTTCAATC
DHRS3_FW	CTACTGCACATCCAAAGCGTC
DHRS3_REV	GGGAAACCTGACTCTCATGCC
FAM46A_FW	CTGGACTGCCTGTTGGACTTC
FAM46A_REV	TCCATCGGTCAGAGTCATTGC

Supplementary table 1: Primer sequences used for RT-qPCR analysis

RNA-seq

ID	NAME	TOTAL	MAPPED	DUPLICATION RATE
B140T5	A1_1	70241530	69450520	0.15
B140T6	A1_2	64937844	64203469	0.14
B140T7	A2_1	58673448	57958778	0.15
B140T8	A2_2	53059964	52345028	0.13
B178T11	ASXL1_2_KO_1	67091976	65946970	0.14
B178T12	ASXL1_2_KO_2	54790946	54118638	0.13
B140T3	B_1	63357286	62600276	0.15
B140T4	B_2	57635620	56811726	0.13
A733T146	B_EC2dKOcontrol_1	24281347	23941732	0.63
A733T151	B_EC2dKOcontrol_2	28806113	28311712	0.65
A733T143	B_EC2dKORA24h_1	31408623	31045728	0.65
A733T152	B_EC2dKORA24h_2	26898827	26484207	0.64
B178T7	BAP1_EZH2_KO_1	65846202	65017975	0.16
B178T8	BAP1_EZH2_KO_2	58402232	57109340	0.15
B178T3	BAP1_KO_1	54022002	53175371	0.13
B178T4	BAP1_KO_2	55476234	54567352	0.17
B178T13	BAP1_KO_3	59298136	58449034	0.14
B178T14	BAP1_KO_4	59040088	57956440	0.14
B178T15	BAP1_rescue_2	56640210	55663882	0.15
B178T16	BAP1_rescue_3	59806914	58635322	0.16
A733T142	BAP1KOcontrol_1	29799276	29478507	0.61
A733T149	BAP1KOcontrol_2	24683840	24423284	0.61
A733T141	BAP1KORA24h_1	26447921	26155065	0.59
A733T150	BAP1KORA24h_2	23339424	23041865	0.61
B140T9	E_1	74148166	72896586	0.16
B140T10	E_2	60561476	59849531	0.13
B178T9	EZH2_BAP1_KO_1	62641432	61827211	0.14
B178T10	EZH2_BAP1_KO_2	51817994	51068947	0.13
B178T5	EZH2_KO_1	59706336	58712569	0.13
B178T6	EZH2_KO_2	62309352	61359293	0.14
B140T11	R_1	66889238	65806493	0.13
B140T12	R_2	63433256	62060800	0.13
B140T1	W_1	66790126	65956987	0.15
B140T2	W_2	56378590	55652212	0.14
B178T1	WT_1	48609558	47885617	0.13
B178T2	WT_2	54067260	53298331	0.15
A733T144	WTcontrol_1	24508360	24275817	0.62
A733T147	WTcontrol_2	26780729	26551175	0.63
A733T145	WTRA24h_1	24723651	24443565	0.61
A733T148	WTRA24h_2	27751900	27449448	0.63

ChIP-seq

ID	NAME	TOTAL	MAPPED	DUPLICATION RATE
B139C13	A1_K27_1	44631872	43672079	0.05
B139C14	A1_K27_2	43962771	42998869	0.05
B139C15	A1_K4_1	43736044	42624486	0.08
B139C16	A1_K4_2	58785798	57249240	0.07
B139C17	A1_Ub_1	61794710	60499768	0.05
B139C18	A1_Ub_2	61923203	60577687	0.05
B139C19	A2_K27_1	41963427	41224761	0.06
B139C20	A2_K27_2	43886633	43283990	0.06
B139C21	A2_K4_1	37705765	37064784	0.3
B139C22	A2_Ub_1	42531701	41783297	0.04
B139C23	A2_Ub_2	47786002	46990575	0.05
B139C7	B1_K27_1	37654429	36784099	0.06
B139C8	B1_K27_2	43611916	42610006	0.06
B139C9	B1_K4_1	46233393	45066997	0.09
B139C10	B1_K4_2	43836218	42622688	0.07
B139C11	B1_Ub_1	58753665	57660167	0.05
B139C12	B1_Ub_2	49537001	48526449	0.04
B139C24	E2_K27_1	38119713	37044115	0.07
B139C25	E2_K27_2	43338116	42150861	0.09
B139C26	E2_K4_1	43621734	42502791	0.09
B139C27	E2_K4_2	52617910	51236451	0.07
B139C28	E2_Ub_1	47589844	46541422	0.05
B139C29	E2_Ub_2	42187354	41171030	0.05
B139C30	RB_K27_1	39666722	38921385	0.08
B139C31	RB_K27_2	36743282	36222362	0.06
B139C32	RB_K4_1	32513561	31984090	0.3
B139C33	RB_Ub_1	34491103	33826182	0.04
B139C34	RB_Ub_2	34774245	34164733	0.05
B139C1	WT_K27_1	37461281	36604007	0.05
B139C2	WT_K27_2	36936404	36074797	0.05
B139C3	WT_K4_1	38014632	37022611	0.08
B139C4	WT_K4_2	38243693	37203001	0.07
B139C5	WT_Ub_1	41263065	40368888	0.05
B139C6	WT_Ub_2	39021850	38084567	0.05

Supplementary table 2: Mapping statistics for RNA-seq and ChIP-seq samples

Supplementary methods

Cell lines

MP41 cells were kindly provided by Dr S. Roman-Roman and cultured in RPMI media supplemented with 10% FBS and 1% L-Glutamine (Invitrogen).

BAP1 knockdown

shRNA sequences targeting BAP1 were retrieved from the following publication [1] and cloned into pLKO.1 vector from addgene.
The target sequences are:

shBAP1.321	CGUCCGUGAUUGAUGAUGA
shBAP1.2132	GAGUUCAUCUGCACCUUUA

Supplementary bibliography

1. Machida, Y.J., Machida, Y., Vashisht, A.A., Wohlschlegel, J.A. & Dutta, A. The deubiquitinating enzyme BAP1 regulates cell growth via interaction with HCF-1. *J Biol Chem* **284**, 34179-88 (2009).