SMARCA4 deficient tumours are vulnerable to KDM6A/UTX and KDM6B/JMJD3 blockade

Octavio A. Romero[1#*], Andrea Vilarrubi[1#], Juan J. Alburquerque-Bejar[1], Antonio Gomez[2], Alvaro Andrades[3,4], Deborah Trastulli[5], Eva Pros[1], Fernando Setien[1], Sara Verdura[5], Lourdes Farré[6], Juan F. Martín-Tejera[6], Paula Llabata[1], Ana Oaknin[7], Maria Saigi[1,8] Josep M. Piulats[8], Xavier Matias-Guiu[9], Pedro P. Medina[3,4], August Vidal[9,10], Alberto Villanueva[6,10], Montse Sanchez-Cespedes[1*]

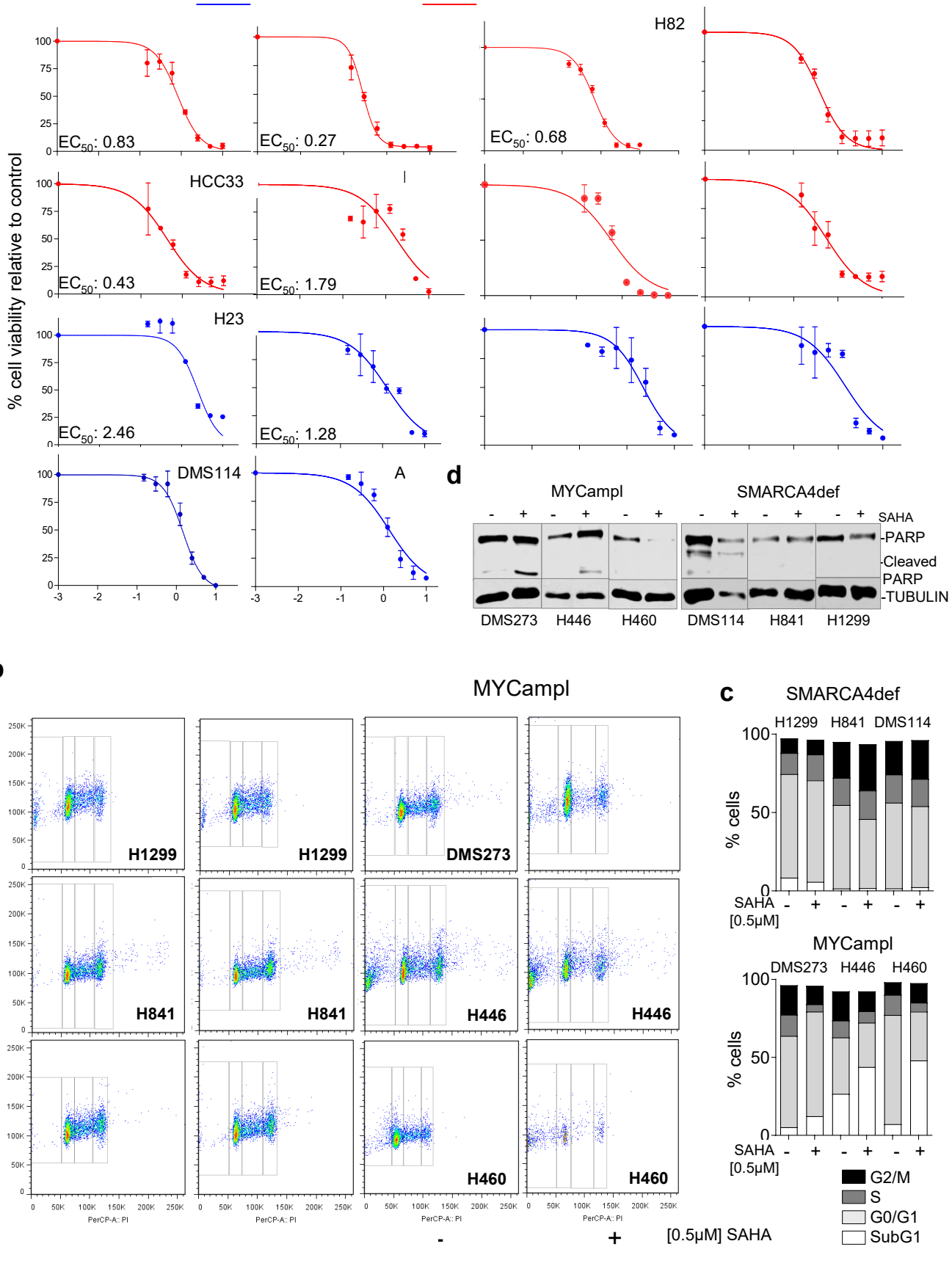
Author affiliations:

[1]Cancer Genetics Group, Josep Carreras Leukaemia Research Institute (IJC), Badalona, Barcelona, Spain. [2]Rheumatology Research Group, Vall d'Hebron Research Institute, Barcelona, Spain. [3]Centre for Genomics and Oncological Research (GENYO), Granada, Spain. [4]Department of Biochemistry and Molecular Biology III, University of Granada, Granada, Spain.[5]Genes and Cancer Group, Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute-IDIBELL Barcelona, Spain. [6]Chemoresistance and Predictive Factors Group, Program Against Cancer Therapeutic Resistance (ProCURE), Catalan Institute of Oncology (ICO), Oncobell Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet del Llobregat, Barcelona, Spain. [7]Department of Medical Oncology, Vall d'Hebrón Hospital, Barcelona, Spain. [8]Department of Medical Oncology, Catalan Institute of Oncology (ICO), Barcelona, Spain. [9]Department of Pathology, University Hospital of Bellvitge, IDIBELL, CIBERONC, L'Hospitalet del Llobregat, Barcelona, Spain. [10]Xenopat S.L., Parc Cientific de Barcelona (PCB), Barcelona, Spain.

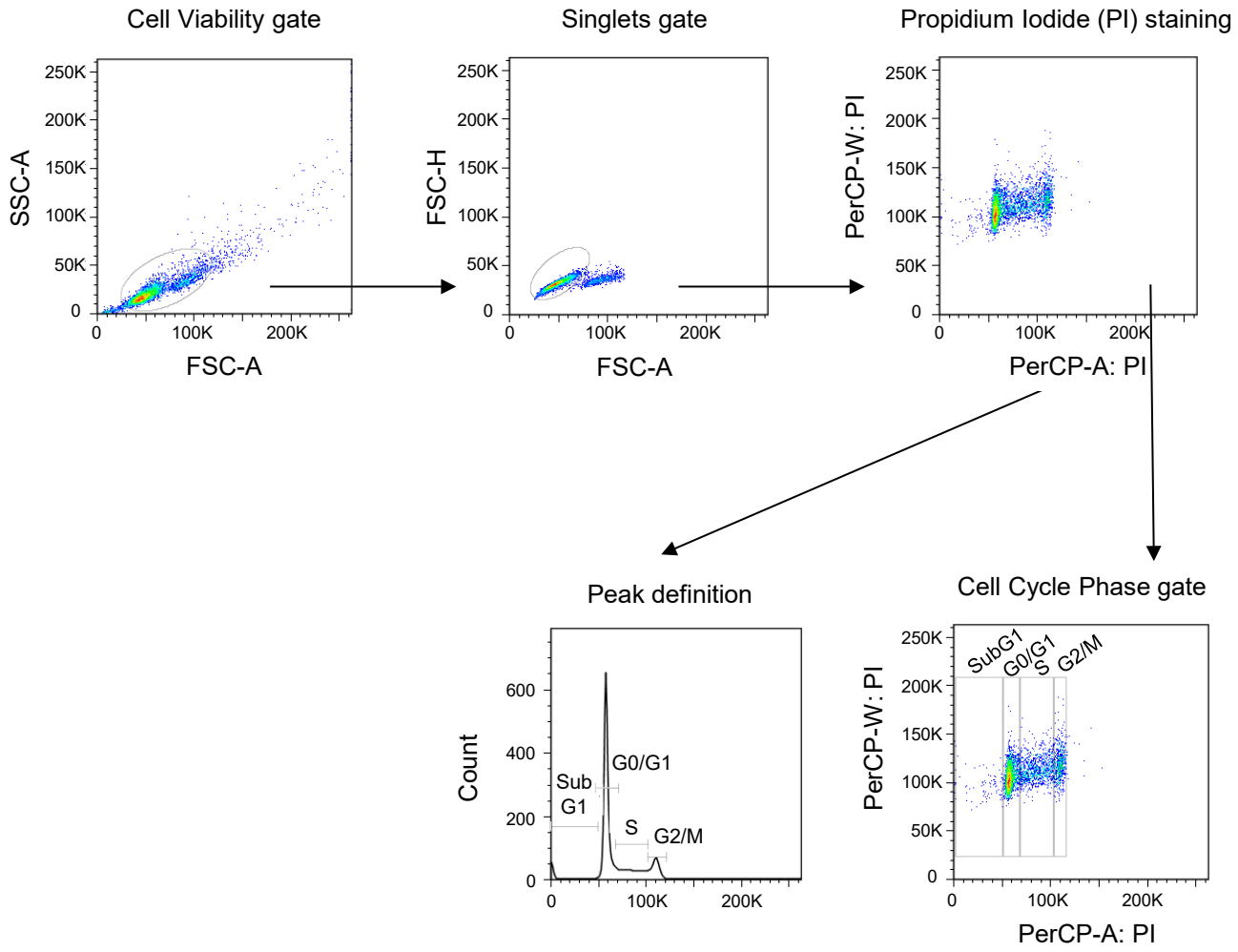
Corresponding authors mscespedes@carrerasresearch.org; oromero@carrerasresearch.org

[#]**These authors contributed equally:** Octavio A. Romero, Andrea Vilarrubi

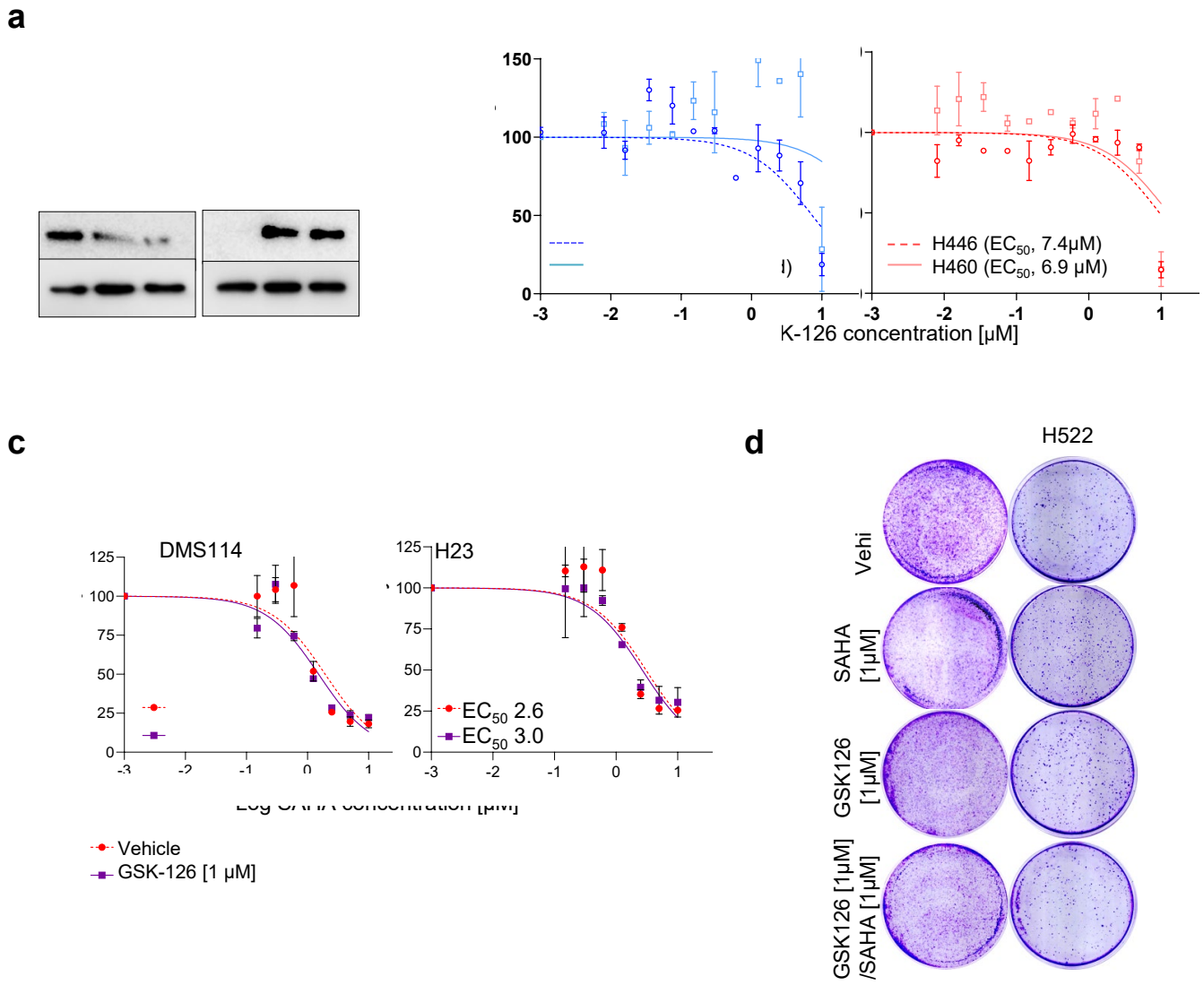
Supplementary Fig.1



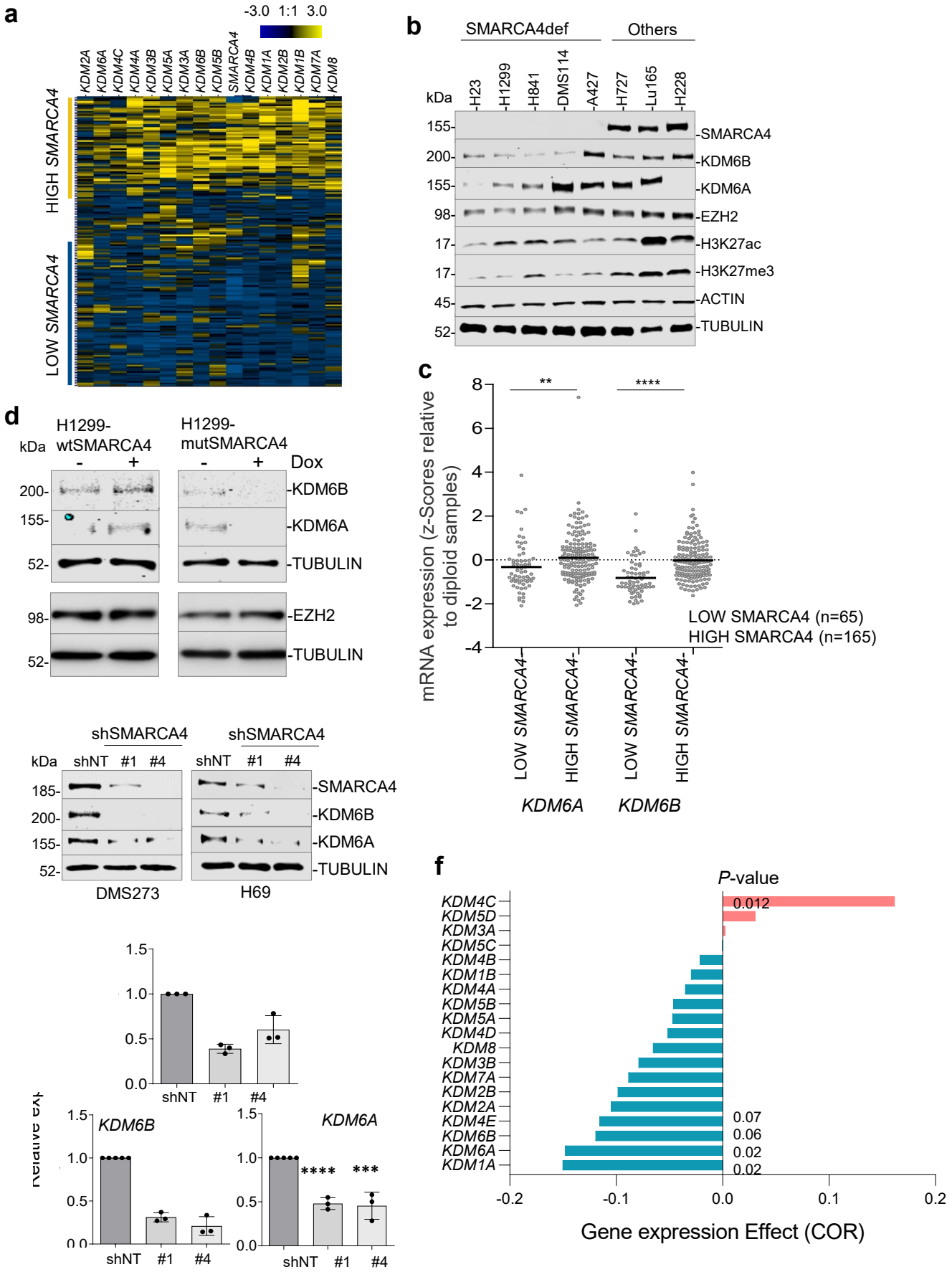
Supplementary Fig.1. SAHA reduces growth in MYCamp, but not in SMARCA4def cancer cells. a, Representative examples of effects on cell viability, measured using MTT assays, in the indicated cell lines after treatment with increasing concentrations of SAHA for 5 days**.** EC_{50}, half maximal effective concentration**.** Error bars, means ± SD of three replicates**. b,** SAHA induced G1 phase arrest in the cell cycle, in MYCamp but not in SMARCA4def cells. Cell cycle distribution was obtained by propidium iodide staining and flow cytometry analysis in cells following treatment with 0.5 μM of SAHA for 48hrs. **c**, Bar graphs depicting the percentage of the indicated cell in each of the cell cycle phases with and without SAHA treatment. **d,** Western blotting depicting the cleavage of PARP in the indicated cells, following the treatment with SAHA or vehicle. A Source Data file is available for this figure.



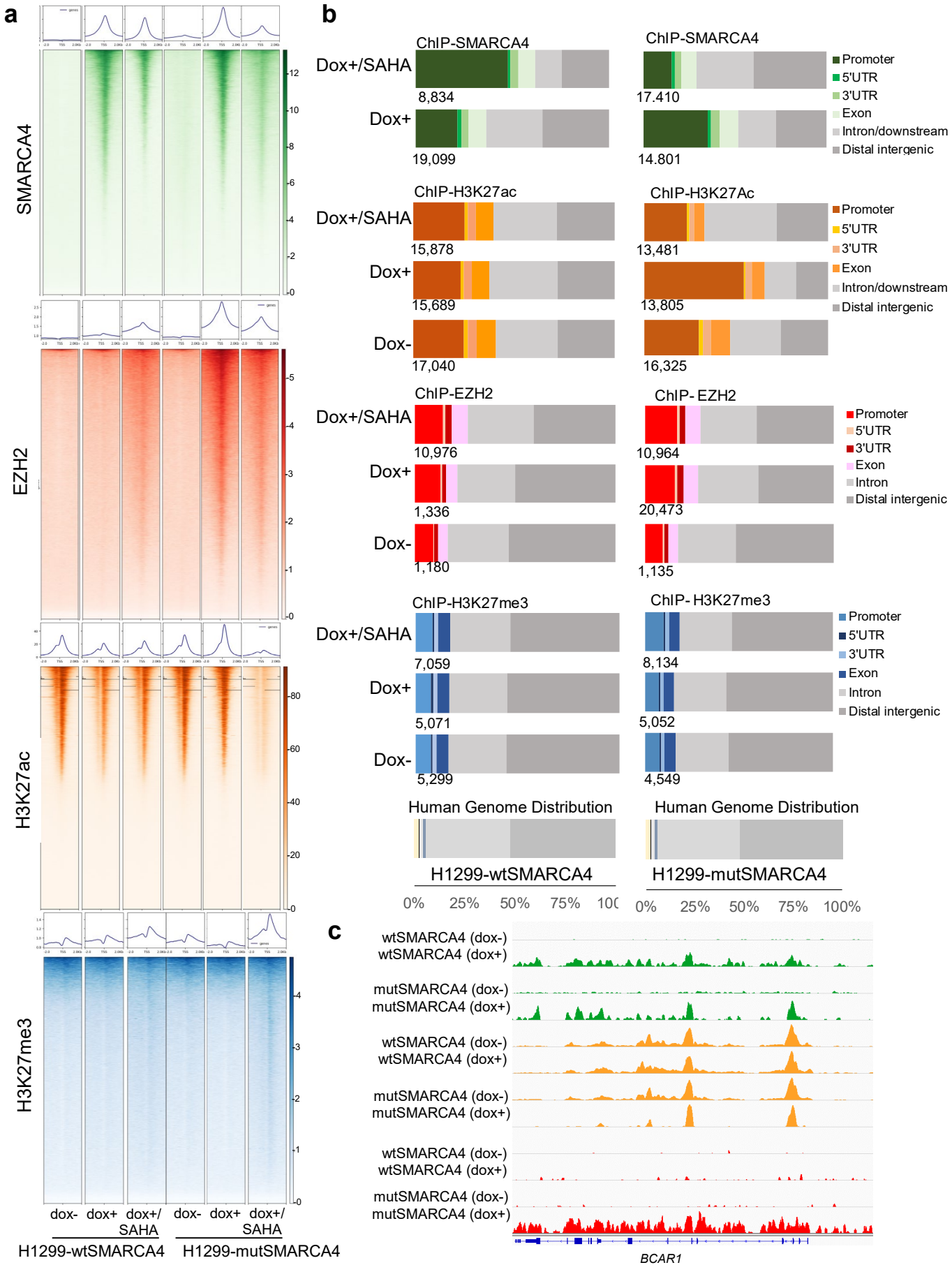
Supplementary Fig.2. Gating strategy for cell cycle analysis of stained MYCamp and SMARCA4def cells with Propidium iodide (PI) as per Supplementary Figure 1b. The gaiting strategy is based on first morphology of viable cells (excludes cell death) > singlets cells (excludes doublets) > PI staining (DNA content estimation). First FACS plot represent relative size (FSC-A) and complexity (SSC-A) of lung cancer cells. Second plot represent FSC-A and FSC-H parameters indicating singlets cells to exclude doublets. Third panel show PI-A and PI-W parameters to detect DNA content of stained cells with Propidium Iodide (PI). Next FACS plot and histogram showing the defined gates to identify SubG1, G0-G1, S and G2-M phase and the associated percentage.



Supplementary Fig.3. GSK126, alone or in combination with SAHA, do not affect proliferation or viability in SMARCA4def cells a, Western blot depicting the levels of EZH2 in the indicated cancer cells, following SAHA treatment. ACTIN, protein-loading control. **b, c**, Effects on cell viability, measured using MTT assays, in the indicated cell lines after treatment with increasing concentrations of GSK-126 (**b**) or of SAHA and 1 μM of GSK-126, for 5 days (**c**). EC_{50}, half maximal effective concentration**.** Values from two independent experiments per cell line are represented**.** Error bars, means ± SD of three replicates**.** **, $P < 0.01$; ****, $P < 0.0001$. **d**, Representative clonogenic assays for the indicated cells and treatments. A Source Data file is available for this figure.


a
-3.0 1:1 3.0
KDM2A KDM6A KDM4C KDM4A KDM3B KDM5A KDM3A KDM6B KDM5B SMARCA4 KDM4B KDM1A KDM2B KDM1B KDM7A KDM8
HIGH SMARCA4
LOW SMARCA4
b
SMARCA4def
Others
kDa
H23 H1299 H841 DMS114 A427 H727 Lu165 H228
155- SMARCA4
200- KDM6B
155- KDM6A
98- EZH2
17- H3K27ac
17- H3K27me3
45- ACTIN
52- TUBULIN
c
mRNA expression (z-Scores relative to diploid samples)
**
****
LOW SMARCA4 (n=65)
HIGH SMARCA4 (n=165)
LOW SMARCA4
HIGH SMARCA4
LOW SMARCA4
HIGH SMARCA4
KDM6A
KDM6B
d
H1299-wtSMARCA4
H1299-mutSMARCA4
kDa
- + - + Dox
200- KDM6B
155- KDM6A
52- TUBULIN
98- EZH2
52- TUBULIN
shSMARCA4
shSMARCA4
kDa shNT #1 #4 shNT #1 #4
185- SMARCA4
200- KDM6B
155- KDM6A
52- TUBULIN
DMS273
H69
Relative exp
KDM6B
KDM6A
shNT #1 #4
****
***
f
P-value
KDM4C 0.012
KDM5D
KDM3A
KDM5C
KDM4B
KDM1B
KDM4A
KDM5B
KDM5A
KDM4D
KDM8
KDM3B
KDM7A
KDM2B
KDM2A
KDM4E 0.07
KDM6B 0.06
KDM6A 0.02
KDM1A 0.02
Gene expression Effect (COR)

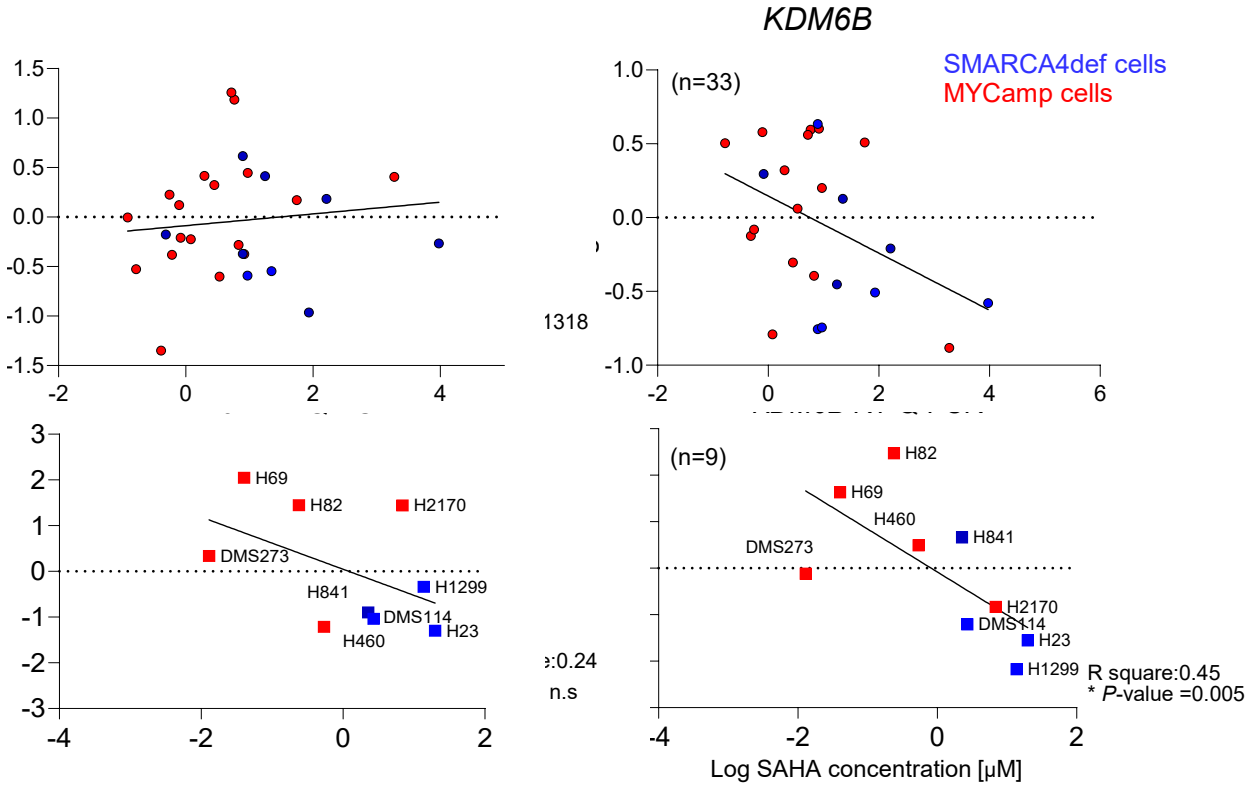
Supplementary Fig. 4. SMARCA4def cells carry lower levels of KDM6A and KDM6B, as compared to the MYCamp cells. a, Heatmap depicting mRNA levels (RPKMs, reads/kb/ million) of the indicated *KDMs* and of *SMARCA4* from a panel of 179 lung cancer cell lines (from Cancer Cell Line Encyclopedia-CCLE at cBioportal; https://www.cbioportal.org/). The heatmap was generated using the Software Genesis. **b,** Western blot depicting endogenous levels of the indicated proteins in lung cancer cell lines. ACTIN and TUBULIN, protein-loading controls. The H228 cells do not have KDM6A protein due to a genetic inactivating alteration at the *KDM6A* gene. **c,** Plot comparing the mRNA levels of the indicated *KDMs* of the SMARCA4-low expressing and SMARCA4-high expressing tumors (lung primary adenocarcinomas from TCGA, Nature 2014 (https://www.cbioportal.org/). Each dot represents a single tumor. Bars show mean ± SD. Two-sided unpaired Student's t-test. **, P =0.01; ****, $P < 0.0001$.**d,** Determination of protein levels of KDM6A, KDM6B and EZH2 by western blot in the H1299-wtSMARCA4 and H1299-mutSMARCA4 cell models (dox, doxycycline, 1 µg/mL; 72 h). Bars show mean ± SD. Two-sided unpaired Student's t-test. **e,** Upper panel, western blots depicting levels of indicated proteins in the indicated cells infected with the shNT and with different shSMARCA4 (#1, #4) (see Ref. 7 for more information). Lower panel, real-time quantitative PCR of *SMARCA4*, *KDM6A* and *KDM6B* (relative to ACTB) for comparing mRNA levels in the H69 cells infected with the shNT and with two different shSMARCA4 (#1, #4). n=3, for each gene and shRNA. Error bars, means ± SD of replicates**.** Two-sided unpaired Student's t-test. *, P =0.011; ***, P =0.002; ****, $P < 0.0001$. **f,** Analysis of the changes in the expression levels of the indicated transcripts from a panel of 242 genomically characterized human cancer cell lines after knockout of SMARCA4 using CRISPR/CAS9 (Sanger CRISPR). Data and statistics are available at the Depmap portal (https://depmap.org/portal/depmap/). A Source Data file is available for this figure.

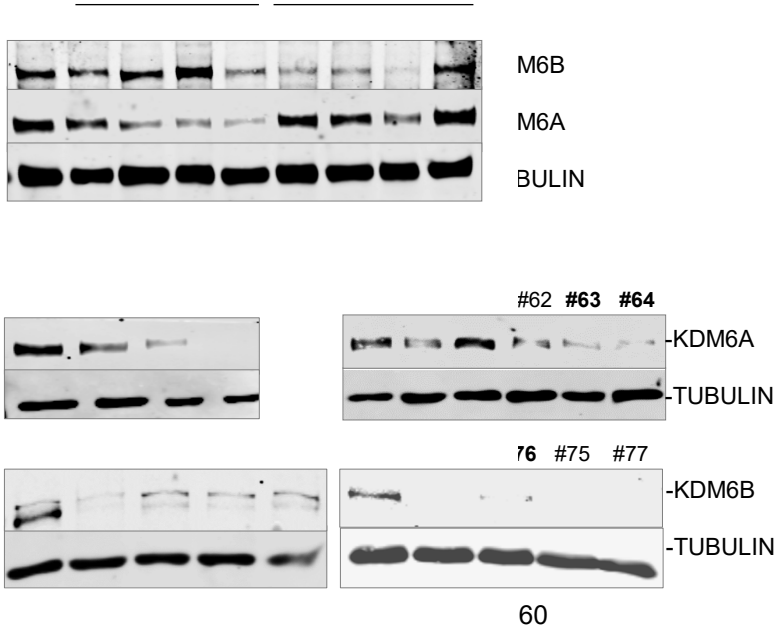

a
SMARCA4
EZH2
H3K27ac
H3K27me3
dox-
dox+
dox+/ SAHA
dox-
dox+
dox+/ SAHA
H1299-wtSMARCA4
H1299-mutSMARCA4
b
ChIP-SMARCA4
Dox+/SAHA
8,834
Dox+
19,099
ChIP-SMARCA4
17.410
14.801
Promoter
5'UTR
3'UTR
Exon
Intron/downstream
Distal intergenic
ChIP-H3K27ac
Dox+/SAHA
15,878
Dox+
15,689
Dox-
17,040
ChIP-H3K27Ac
13,481
13,805
16,325
ChIP-EZH2
Dox+/SAHA
10,976
Dox+
1,336
Dox-
1,180
ChIP- EZH2
10,964
20,473
1,135
Promoter
5'UTR
3'UTR
Exon
Intron
Distal intergenic
ChIP-H3K27me3
Dox+/SAHA
7,059
Dox+
5,071
Dox-
5,299
ChIP- H3K27me3
8,134
5,052
4,549
Promoter
5'UTR
3'UTR
Exon
Intron
Distal intergenic
Human Genome Distribution
H1299-wtSMARCA4
0%
25%
50%
75%
100
Human Genome Distribution
H1299-mutSMARCA4
0%
25%
50%
75%
100%
c
wtSMARCA4 (dox-)
wtSMARCA4 (dox+)
mutSMARCA4 (dox-)
mutSMARCA4 (dox+)
wtSMARCA4 (dox-)
wtSMARCA4 (dox+)
mutSMARCA4 (dox-)
mutSMARCA4 (dox+)
wtSMARCA4 (dox-)
wtSMARCA4 (dox+)
mutSMARCA4 (dox-)
mutSMARCA4 (dox+)
BCAR1

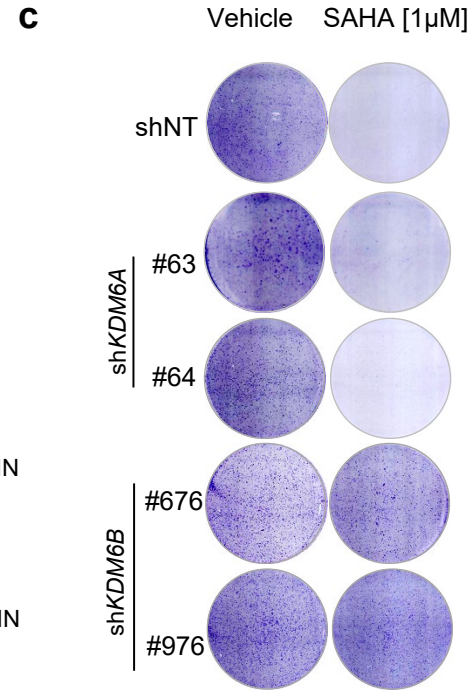
Supplementary Fig. 5. Genome-wide effects of wild type and mutant SMARCA4 and of SAHA on the dynamics of H3K27 modification and on EZH2 occupancy. a, Heatmaps of normalised ChIP-seq intensities, centred ± 2 kb around the transcriptional start sites (TSS), of SMARCA4, H3K27ac, H3K27me3 and EZH2, in the H1299 cell model. **b,** Genome-wide functional annotations for peaks generated by ChIP-seq analyses. Promoters are defined as the regions ± 2 kb around the annotated TSS. Numbers below each bar indicate the absolute number of peaks at promoters called for the ChIP-seq of each indicated protein and condition. **c,** Representative snapshots from IGV of ChIP-seq profiles at selected target loci, performed in the H1299 cell model. H1299-wtSMARCA4 or H1299-mutSMARCA4 cell models after induction of SMARCA4 (dox, doxycycline, 1 μg/mL; 72 h) with or without SAHA treatment (1 μM, 72 h).


a
H1299-wtSMARCA4
H1299-wtSMARCA4/ H1299-mutSMARCA4
H1299-mutSMARCA4
SMARCA4
H3K27ac
EZH2
dox+
SAHA
dox-
7546 7221 696
3712 11055 988
4452 7591 2207
1842 12749 845
2460 11134 1592
2423 11767 959
762 12832 972
2615 11189 985
2152 10574 1600
70 148 252
4 1 447 8432
14 204 8675
74 414 4210
1497 3127 3994
3898 4981 2140
H1299-wtSMARCA4 (dox +)
H1299-mutSMARCA4 (dox +)
3290 11447 2117
1907 10136 2590
14307 460 28
5035 7008 1871
103 386 13280
2160 6719 6007
1661 6152 7652
3776 6022 6152
5204 2609 2015
4919 4879 2242
856 3768 10036
2124 4997 7177
b
wtSMARCA4
mutSMARCA4
[0-25]
[0-7]
[0-300]
[0-5]
dox-
dox+
dox+/SAHA
SMARCA4
EZH2
H3K27ac
H3K27me3
KDM4B
KDM2B

Supplementary Fig. 6. Venn diagrams showing shared occupancies and examples of snapshots for the different ChIP-sequencing experiments. **a,** Venn diagrams of the overlap of SMARCA4, H3K27ac and EZH2 peaks in the H1299 cell model. **b,** Representative snapshots from IGV of ChIP-seq profiles at selected target loci, performed in the H1299 cell model. H1299-wtSMARCA4 or H1299-mutSMARCA4 cell models after induction of SMARCA4 (dox, doxycycline, 1 μg/mL; 72 h) with or without SAHA treatment (1 μM, 72 h).

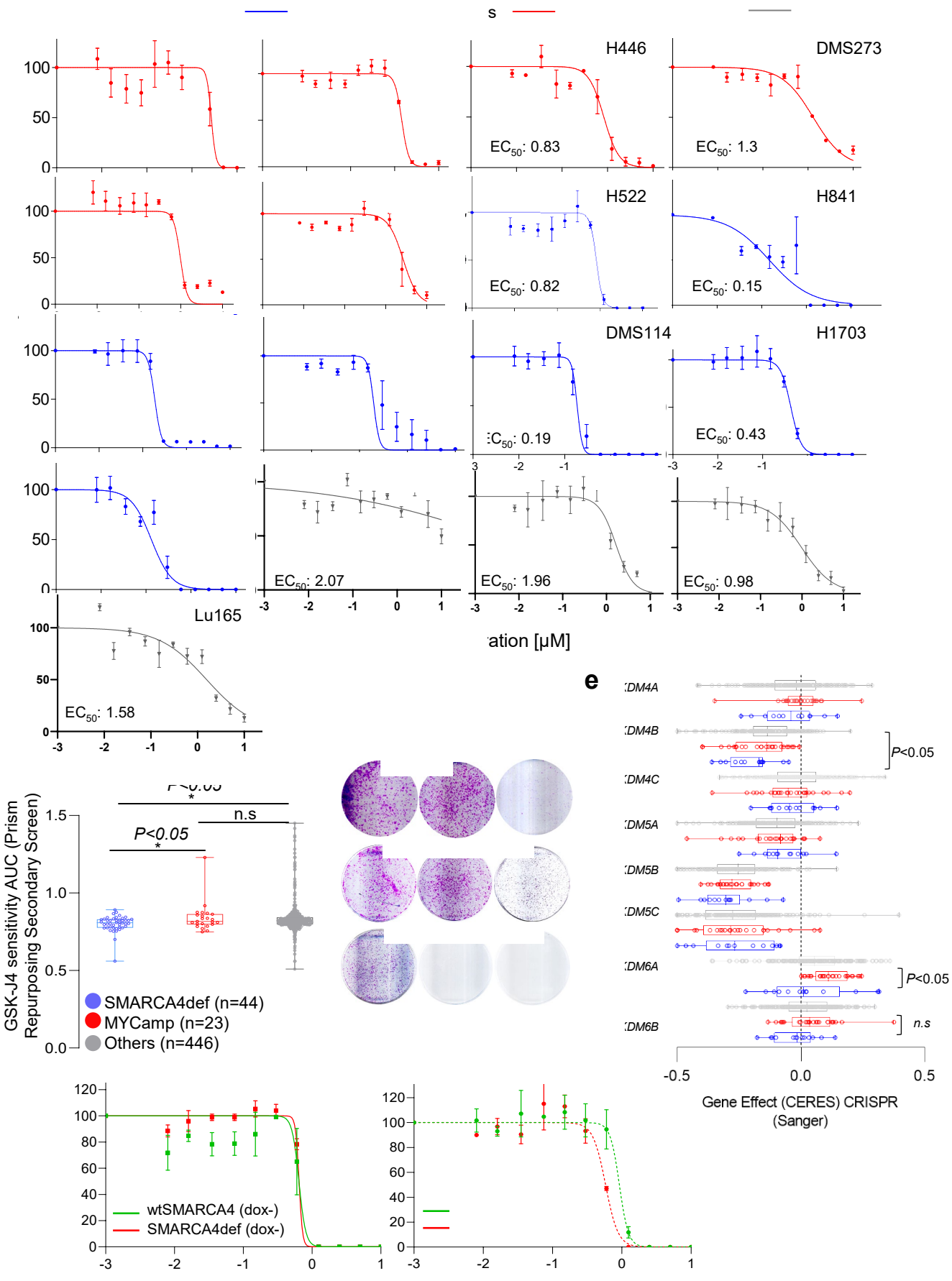

KDM6B
SMARCA4def cells
MYCamp cells
(n=33)
1318
(n=9)
H69
H82
H2170
DMS273
H841
H1299
DMS114
H460
H23
:0.24
n.s
R square:0.45
* P-value =0.005
Log SAHA concentration [μM]


M6B
M6A
BULIN
#62 #63 #64
-KDM6A
-TUBULIN
-KDM6B
-TUBULIN
60

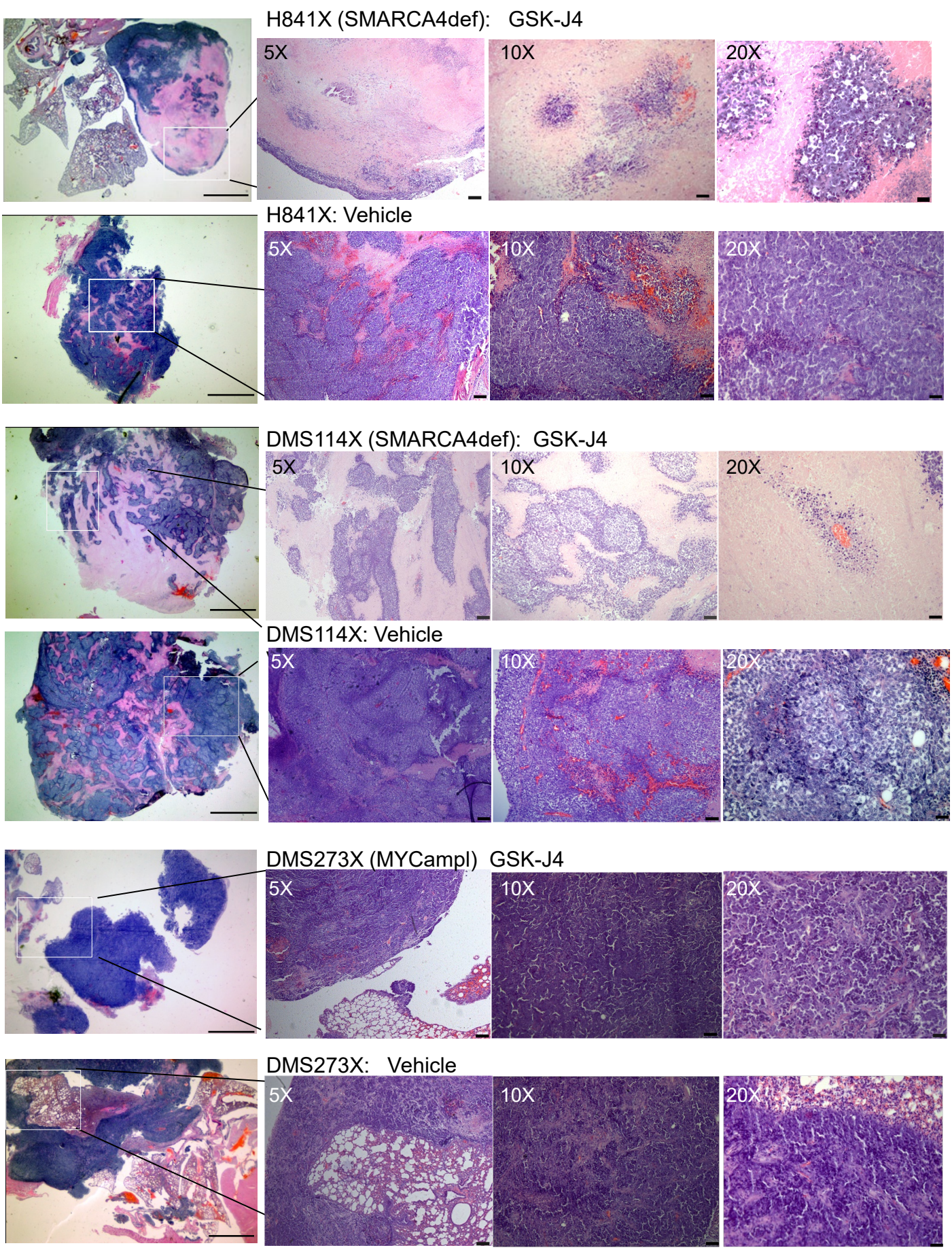

c
Vehicle
SAHA [1μM]
shNT
shKDM6A
#63
#64
shKDM6B
#676
#976

Supplementary Fig. 7. Lower levels of KDM6A and KDM6B in the SMARCA4def cells and shRNAs, to downregulate KDM6A and KDM6B expression in different MYCamp cells. a, Significant correlation between mRNA levels of *KDM6B* (RNA-sequencing from the Sanger database or RT-QPCR data from our current study) and EC_{50} (half maximal effective concentration) for SAHA (upper panels, from www.cancerrxgene.org or lower panels, from our own data). Each dot represents a single cancer cell line**.** Simple linear regression analysis. *, $P < 0.05$; n.s, not significant. **b,** Western blots depicting levels of the indicated proteins in the indicated cells infected with the shNT and with different shKDM6A (#60, #61, #62, #63 and #64) and shKDM6B (#676, #976, #75 and #77). **c,** Clonogenic assays for the H460 cells infected with shNT, shKDM6A (#63, #64) or shKDM6B (#676, #976) treated with SAHA for 5 days. A Source Data file is available for this figure.

Supplementary Fig.8

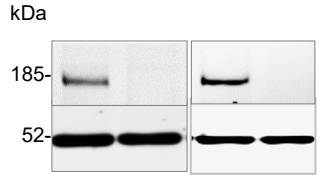


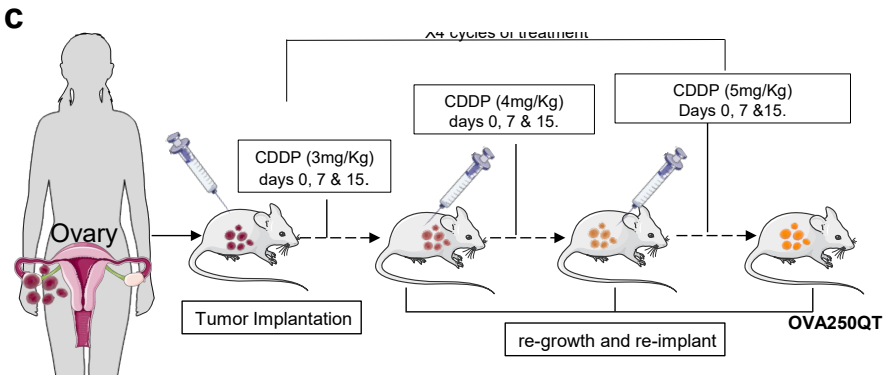
Supplementary Fig. 8. GSK-J4 induces cell growth inhibition of SMARCA4def lung cancer cells. a, Examples of the effects on cell viability, measured using MTT assays, after treatment with increasing concentrations of GSK-J4 in the indicated lung cancer cell lines. Lines, percentage of viable cells relative to the total number at 0 h. Error bars, means ± SD of three replicates. **b,** Analysis of the sensitivity to the GSK-J4 drug in a panel of 750 genomically characterized human cancer cell lines using the PRISM (Profiling Relative Inhibition Simultaneously in Mixtures) technology. Each dot represents a single cancer cell line. In box-whisker plots, the horizontal band inside box indicates the median, the bottom and top edges of the box 25th–75th percentiles and the whiskers indicate the min to max. Two-sided unpaired Student's t-test.*, P= 0.032 in both cases; n.s, not significant. Data available at the Achilles project at the depmap portal (https://depmap.org/portal/achilles/). **c-d,** clonogenic (**c**) and cell viability (MTT) (**d**) assays in the H1299-wtSMARCA4 or H1299-mutSMARCA4 cell models before and after induction of SMARCA4 (doxycycline, 1 µg/mL; 5 days) treated with GSK-J4. Lines, percentage of viable cells relative to the total number at 0 h. Error bars, mean ± SD of three replicates. **e,** Dependency of different cancer cell lines for each of the indicated KDM from the Achilles project at the depmap portal (https://depmap.org/portal/achilles/). Blue, SMARCA4def cell lines (n=13); Red, MYCamp cell lines (n=25) and grey (n=204), cancer cell lines that are wild type for both SMARCA4 and MYC. Two-sided unpaired Student's t-test. *, $P < 0.05$; n.s, not significant. In box-whisker plots, the horizontal band inside box indicates the median, the bottom and top edges of the box 25th–75th percentiles and the whiskers indicate the min to max. A Source Data file is available for this figure.

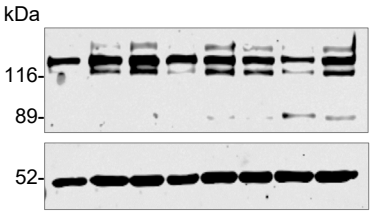

H841X (SMARCA4def): GSK-J4
5X
10X
20X
H841X: Vehicle
5X
10X
20X
DMS114X (SMARCA4def): GSK-J4
5X
10X
20X
DMS114X: Vehicle
5X
10X
20X
DMS273X (MYCampl) GSK-J4
5X
10X
20X
DMS273X: Vehicle
5X
10X
20X

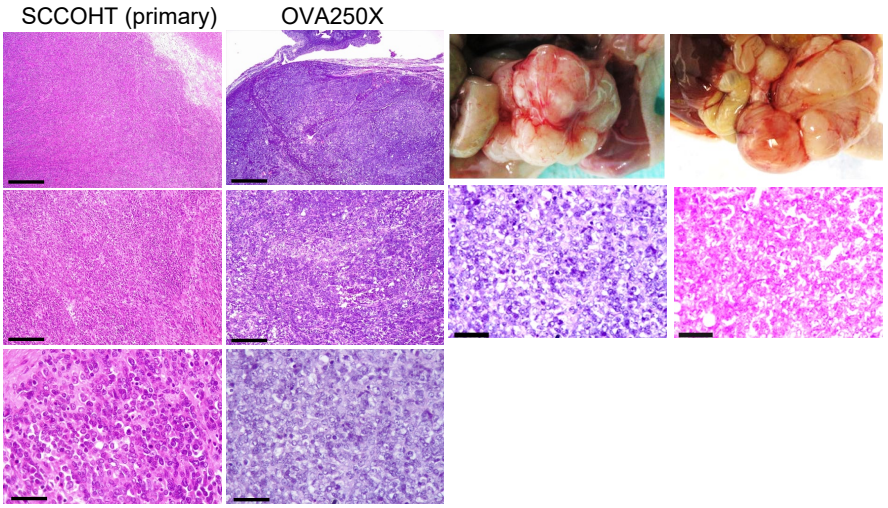
Supplementary Fig. 9. GSK-J4 induces tumour regression of SMARCA4def lung tumours *in vivo*

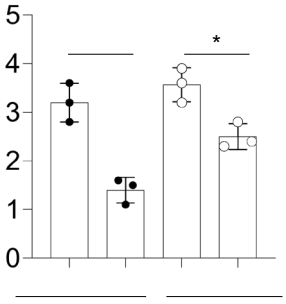
Representative sections of H&E staining of the indicated tumours developed from the indicated lung cancer cells grown in nude mice (lung orthotopic implantation of small solid fragments of engrafted cell lines previously grown subcutaneously), treated with GSK-J4 or vehicle. Left panels, scale bars, 2.5 mm. Magnification as indicated (5x, 10x and 20x panels; scale bars, 100 μm, 50 μm and 25 μm, respectively).

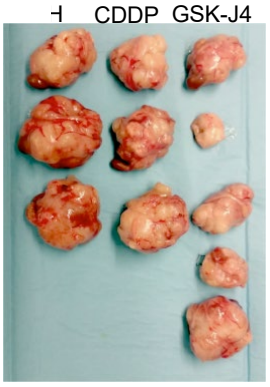

kDa
185-
52-

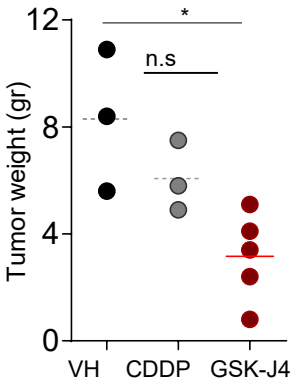

c
X4 cycles of treatment
CDDP (3mg/Kg)
days 0, 7 & 15.
CDDP (4mg/Kg)
days 0, 7 & 15.
CDDP (5mg/Kg)
Days 0, 7 &15.
Ovary
Tumor Implantation
re-growth and re-implant
OVA250QT

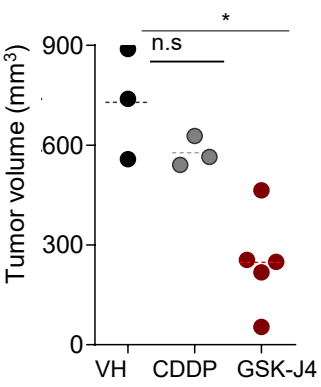

kDa
116-
89-
52-

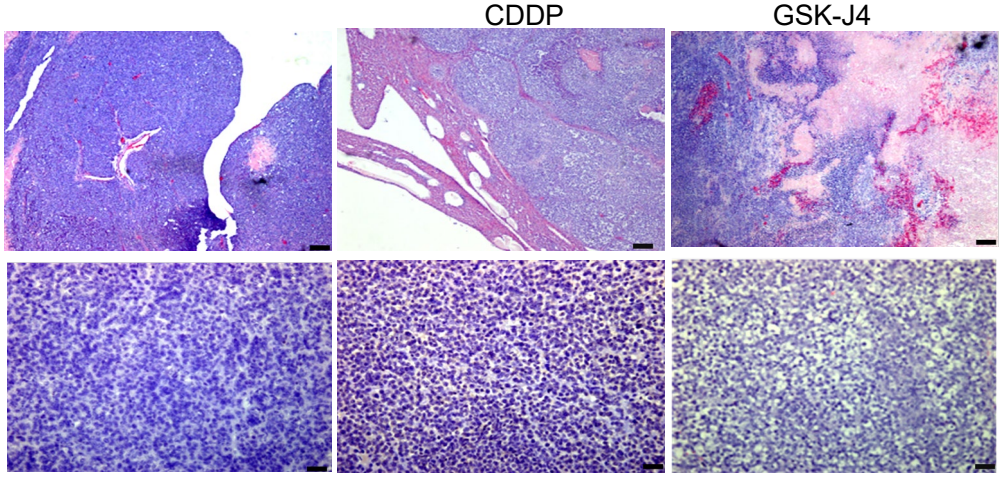

SCCOHT (primary)
OVA250X

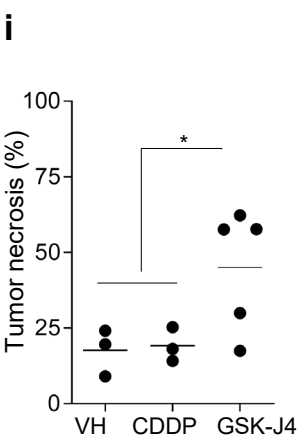

5
4
3
2
1
0
*


CDDP
GSK-J4


12
8
4
0
*
n.s
Tumor weight (gr)
VH
CDDP
GSK-J4


900
600
300
0
*
n.s
Tumor volume (mm3)
VH
CDDP
GSK-J4


CDDP
GSK-J4


i
100
75
50
25
0
*
Tumor necrosis (%)
VH
CDDP
GSK-J4

Supplementary Fig. 10. *In vivo* establishment of paired cisplatin-resistant SCCOHT xenografted tumour and effect of cisplatin and GSK-J4 treatments. a, Western blot to show presence or absence of SMARCA4 in the indicated ovarian cancer cells. **b**, Western blot depicting the cleavage of PARP in the indicated cells, following the different treatments or vehicle. TUBULIN, protein-loading control. **c**, The experimental approach used for cisplatin (CDDP)-resistant tumour generation combines: (i) iterative cycles of cisplatin treatment (3 doses of cisplatin administered by i.v. injection on days 0, 7 and 15); (ii) successive increase of administered doses through four cycles applied to different mice. Lateral laparotomy was conducted in isofluorane-anaesthetised mice. The ovary was mobilised and small tumour pieces of primary tumour were anchored on the mouse ovarian surface with prolene 7.0 sutures. Engrafted OVA250X tumours grew as large solid masses, usually to 1,000–1,500 mm^3 in diameter, which determined the time of sacrifice. Figure schematics were generated using https://smart.servier.com/ (https://creativecommons.org/licenses/by/3.0/). **d,** Representative H&E staining, from at least five different tumours of each group, reveals a similar morphology between primary SCCOHT and paired engrafted OVA250X. Top panels, scale bar 500 µm, middle panels scale bar, 200 µm. Bottom panels scale bar, 50 µm. **e,** Gross pathological photographs at necropsy (top panels) and representative H&E staining (bottom panels) of the OVA250X and OVA250XR tumours, from at least five different tumours of each group. Scale bar, 50 µm. **f,** Measure of the weight of each of the tumours from mice treated with vehicle (VH) (n=3) or cisplatin (CDDP) (n=3). *, P =0.046 (OVA250X) and *, P =0.018 (OVA250XR); Error bars, means ± SD of replicates**. g,** Left panel, gross pathological photographs of all ovarian tumours that developed in the mice treated with vehicle (VH) (n=3), cisplatin (CDP) (n=3) or GSK-J4 (n=5). Right panels, volume and weight of each tumour. *, P =0.036 in both cases; n.s, not significant. **h,** Representative H&E staining of OVA250XR tumours, from at least five different tumours of each group, treated as indicated. Scale bar, 100 µm (upper panels) and 25 µm (lower panels). **i**, Quantification of necrotic areas from the tumours from mice treated with vehicle (VH) (n=3) or cisplatin (CDDP) (n=3) or GSK-J4 (n=5). *, P =0.044; All tests are two-sided unpaired Student's t-testn.s, not significant. A Source Data file is available for this figure.

Supplementary Table 1. List of antibodies used. WB, western-blot; IHC, immunohistochemistry; ChIP, chromatin immunoprecipitation

Protein	Reference	Supplier	Technique	Dilution (WB)	Total amount (ChIP)	Dilution (IHC)
KDM6B/JMJD3	#3457	Cell signaling	WB	1:1000		
	ab38113	Abcam	WB & IHC	1:1000		1:50
KDM6A/UTX	#33510	Cell signaling	WB & IHC	1:1000		1:100
H3K27me3	07-449	Sigma	WB & ChIP-seq & IHC	1:1000	2µg	1:100
H3K27ac	ab4729	Abcam	WB & ChIP-seq & IHC	1:1000	2µg	1:100
EZH2	#5246	Cell signaling	WB & ChIP-seq	1:1000	2µg	
SMARCA4	#49360	Cell signaling	WB & ChIP-seq	1:1000	2µg	
ACTIN	A2228	Sigma	WB	1:10000		
TUBULIN	200-301-880S	Rockland	WB	1:10000		
PARP	#9542	Cell signaling	WB	1:1000		
CASPASE-3	#9668	Cell signaling	IHC	1:1000		1:100
Donkey anti-rabbit IgG IRDye 800CW fluorescent secondary antibody	926-32213	Li-Cor	WB	1:10000		
Donkey anti-mouse IgG IRDye 680LT fluorescent secondary antibody	926-68022	Li-Cor	WB	1:10000		

Supplementary Table 2. List of primers used for the quantitative RT-PCRs.

Gene	Forward (5'-3')	Reverse (5'-3')	Scale (µmols)	Supplier
ACTB	GGCATCCTCACCCTGAGGTA	AGGTGTGGTCGGAGATTTTC	0.025	Sigma
GUSB	CTGTACACGACACCCACCAC	GATGAGGAACTGGCTCTTGA	0.025	Sigma
KDM1A/LSD1	GCCATGGTGGTACAGGTCTA	TGGCCAGTTCCATATTTACA	0.025	Sigma
KDM2A	CGGATAGTTGAGAAAGCCAT	CTCTTTGGTGGGCCTCTGTA	0.025	Sigma
KDM3A	ACCTGCAGTTATTCTTCAGC	TAATGCCAGTCCTATGCCAT	0.025	Sigma
KDM4A/JMJD2A	CCTCACTGCGCTGTCTGTAT	CCAGTCGAAGTGAAGCACAT	0.025	Sigma
KDM4B/JMJD2B	GATCTCCATGGACGTGTTCG	CGTTTCCGGTGAGACCTGCC	0.025	Sigma
KDM4C/JMJD2C	GGCATAGGTGACAGGGTGTG	CGGGGACCAAACTCTGGAAA	0.025	Sigma
KDM4D/JMJD2D	CGGGATCTGCACAGATTATC	AGTTTCTGAGGAGGGCGACC	0.025	Sigma
KDM5A	GGTTTCTTAAGGTGGCAAGT	TTCTTTTGTACTGTTCCCTA	0.025	Sigma
KDM5B	AGCTTTTCTCAGAATGTTGG	GCAGAGTCTGGGAATTCACA	0.025	Sigma
KDM5C	GGGTTTCTAAAGTGTAGATC	CCCACACATCTGAGCTTTAG	0.025	Sigma
KDM6A/UTX	GTCCGAGTGTCAACCAACTG	TGAGAGTCCTGGAGTAGGAG	0.025	Sigma
KDM6B/JMJD3	CTCAACTTGGGCCTCTTCTC	GCCTGTCAGATCCCAGTTCT	0.025	Sigma

Supplementary Table 3. References for the shRNAs

Name	Clone ID	Vector	Target gene	Supplier
#60	TRCN0000107760	pLKO.1	*KDM6A*	Sigma
#61	TRCN0000107761	pLKO.1	*KDM6A*	Sigma
#63	TRCN0000107763	pLKO.1	*KDM6A*	Sigma
#64	TRCN0000107764	pLKO.1	*KDM6A*	Sigma
#75	TRCN0000236675	pLKO.1	*KDM6B*	Sigma
#77	TRCN0000236677	pLKO.1	*KDM6B*	Sigma
#676	TRCN0000236676	pLKO.1	*KDM6B*	Sigma
#976	TRCN0000359976	pLKO.1	*KDM6B*	Sigma

Supplementary Table 4. List of primers used for the cloning of KDM6A and KDM6B.

Gene	Forward (5'-3')	Reverse (5'-3')	Scale (µmols)	Supplier
KDM6A	aaaaaaaaCTCGAGCCGCCACCATGAAATCCTGCGGAGTGTCGCTCGCTAC	aaaaaaaaGCGGCCGCTCAAGATGAGGCGGATGGTAATGGAGGAGCTAATG	0.025	Sigma
KDM6B	tttttttGGTACCGCCGCCACCATGCATCGGGCAGTGGACCCTCCAG	tttttttCTCGAGTCATCGCGACGTGCTGGCTGGGG	0.025	Sigma

Supplementary Table 5. List of primers used for the sequencing of KDM6A and KDM6B.

Gene	Forward (5'-3')	Scale (µmols)	Supplier
KDM6A	ATGAGTCTAGTTTAAAGCATTTTCAG	0.025	Sigma
KDM6A	ATTAAATGCTACTTAAATGCAACTAG	0.025	Sigma
KDM6A	GGAGTTGCACAGGTAC	0.025	Sigma
KDM6A	GGTGGACAACAAGGC	0.025	Sigma
KDM6A	TAAAAATGGCTTATCTAACAGTAGC	0.025	Sigma
KDM6A	TGACTAAACTTCCTGCTTTTG	0.025	Sigma
KDM6A	GCACGAAATATCAAGGTCTCAGATCCAAAGC	0.025	Sigma
KDM6B	AACGGAACTATGGAGCC	0.025	Sigma
KDM6B	CTCCAGCGTTTCACC	0.025	Sigma
KDM6B	AGGATTCTCACACCCC	0.025	Sigma
KDM6B	CCGCTGAAGGAGCCC	0.025	Sigma
KDM6B	CGAAGAGCCAGTCCC	0.025	Sigma
KDM6B	TGCCCAGCCCACACC	0.025	Sigma
KDM6B	GTGGAAGCGAGTGGC	0.025	Sigma
KDM6B	ACGAGCACTACTGGG	0.025	Sigma
KDM6B	AAGAAAATCGCTTACCAGGGC	0.025	Sigma

Supplementary Table 6. Distribution of H3K27me3 staining among tumours (three replicates per cell line and condition) from the DMS273X, H841X, DMS114X and OVA250X tumours treated with vehicle or GSK-J4. Low (intensity values 1 & 2); high (values 3 & 4).

		Eval 1		Eval 2		Eval 3		Mean		Categories	
		H3K27me3		H3K27me3		H3K27me3		H3K27me3		H3K27me3	
	Replicate	Vehicle	GSK-J4	Vehicle	GSK-J4	Vehicle	GSK-J4	Mean-Vehicle	Mean-GSK-J4	Categories Vehicles	Categories GSK-J4
DMS114X	R1	1	3	1	2	1	1	1	2	Low	Low
	R2	2	4	2	3	2	3	2	3.3	Low	High
	R3	2	4	2	4	2	4	2	4	Low	High
H841X	R1	1	3	1	3	1	3	1	3	Low	High
	R2	3	4	3	3	3	3	3	3.3	High	High
	R3	2	4	2	4	2	3	2	3.6	Low	High
DMS273X	R1	2	3	1	2	1	2	1.3	2.3	Low	Low
	R2	1	3	2	3	2	3	1.6	3	Low	High
	R3	2	4	2	3	2	4	2	3.6	Low	High
OVA250X	R1	2	3	1	3	2	3	1.6	3	Low	High
	R2	2	4	1	4	2	4	1.6	4	Low	High
	R3	2	3	2	4	3	4	2.3	3.6	Low	High