Supplementary Table 1 - Epiprobe chemicals

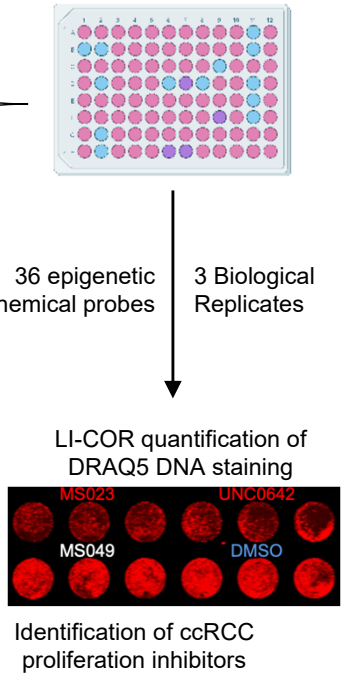
Protein Family	Target	Probe	PubMed ID
Methyltransferase	SUV20H1/H2	A-196	28114273
Methyltransferase	EHMT2, EHMT1	A-366	24900801
Methyltransferase	SMYD2	BAY-598	27075367
Bromodomain	BAZ2A/2B	BAZ2-ICR	25719566
Bromodomain	BRD9, BRD7	BI-9564	26914985
Bromodomain	BAZ2A/2B	GSK2801	25799074
Methyltransferase	EZH2	GSK343	24900432
PAD	PAD-4	GSK484	25622091
Methyltransferase	PRMT5	GSK591	26985292
KDM	KDM6B, KDM6A	GSKJ4	22842901
KDM	KDM1A	GSKLSD1	26175415
Bromodomain	BRD9	I-BRD9	25856009
Bromodomain	CREBBP P300	I-CBP112	26552700
PHD	pan-2-OG	IOX1	24504543
Bromodomain	BRD2-4	JQ-1	20871596
Bromodomain	BRD9, BRD7	LP99	25864491
Methyltransferase	Type I PRMT	MS023	26598975
Methyltransferase	PRMT4, PRMT6	MS049	27584694
Bromodomain	BRPF1-3	NI-57	28714688
Bromodomain	BRPF1-3	OF-1	21804994
Bromodomain	BRD2-4	PFI-1	23095041
Methyltransferase	SETD7	PFI-2	25136132
Bromodomain	SMARCA2,4	PFI-3	26139243
Bromodomain	BRPF1B	PFI-4	28849908
Methyltransferase	DOT1L	SGC0946	23250418
Methyltransferase	PRMT3	SGC707	25728001
Bromodomain	CREBBP P300	SGC-CBP30	24946055
Methyltransferase	EHMT2, EHMT1	UNC0638	21743462
Kme	L3MBTL3	UNC1215	23292653
Methyltransferase	EZH2	UNC1999	23614352
Bromodomain	Pan-Bromodomain	Bromosporine	27757418
Bromodomain	CECR2	NVS-CECR2-1	-
HDAC	HDAC	LAQ824	12816865
HDAC	HDAC	CI-994	17455259
Methyltransferase	EHMT2, EHMT1	UNC0642	24102134
WD40 repeats	WD40 (WDR5)	OICR-9429	26167872

Supplementary Table 2 - sgRNA's

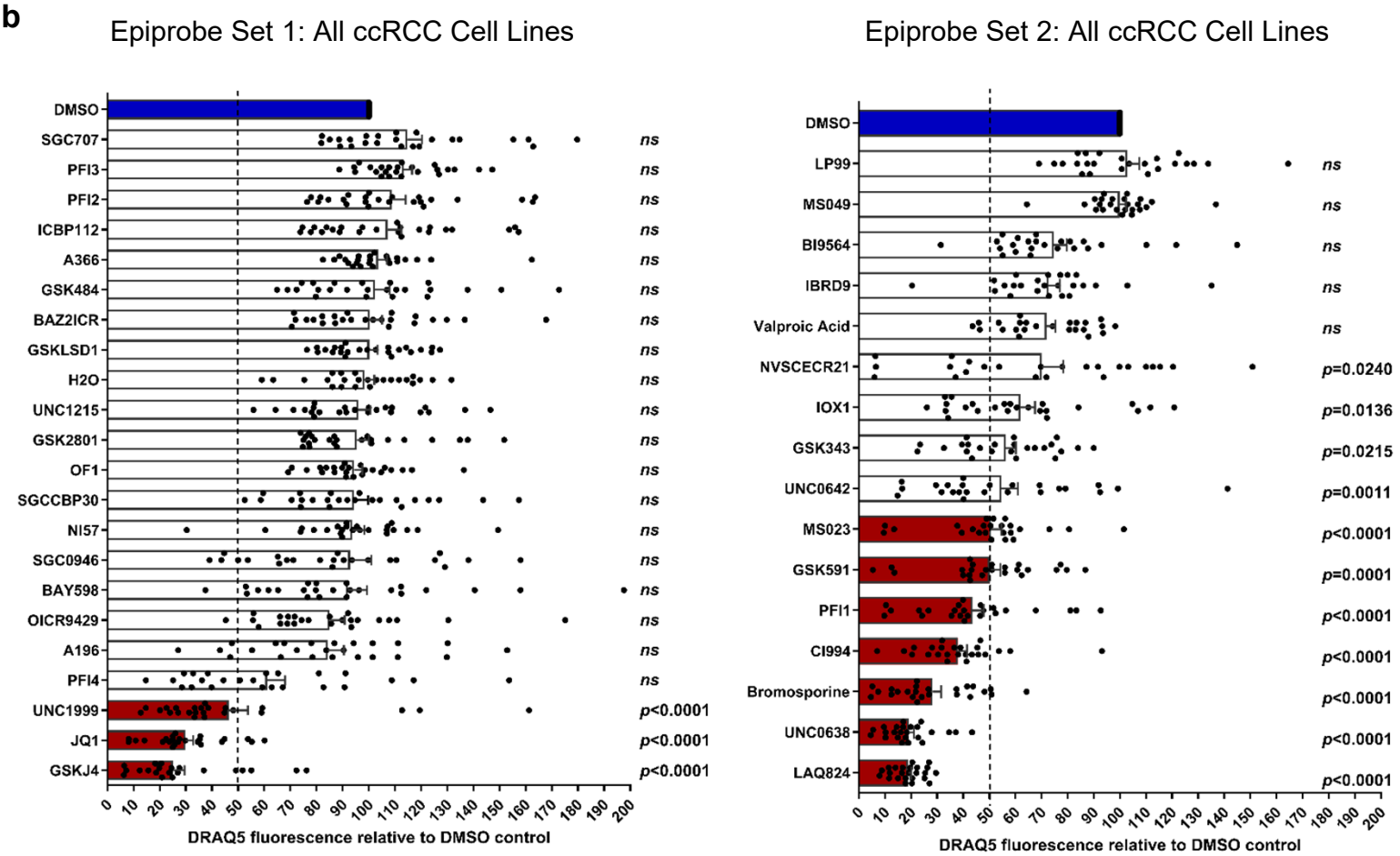
sgRNA name	sgRNA Target Sequence	PAM Sequence	Target Transcript	Exon Number (Relative to transcript)	Strand	On-Target Efficacy Score (Azimuth 2.0)	Position of Base After Cut (1-based)
PRMT1_e4	AAAGCCAACAAGTTAGACCA	CGG	NM_198318.4 (PRMT1v1)	4	sense	0.7305	49682255
PRMT1_e6	GATGGCCGTCACATACAGCG	TGG	NM_198318.4 (PRMT1v1)	6	antisense	0.7528	49684791
PRMT1_e7	GGGTCCACGACATCCACTAG	GGG	NM_198318.4 (PRMT1v1)	7	antisense	0.7112	49684987
PRMT1_e2	GTGGATGCCAAAGTGTGCGT	AGG	NM_198318.4 (PRMT1v1)	2	antisense	0.6442	49680569
PRMT3e8	CCAACATCCAAAACTACCTA	GGG	NM_001145167.1	8	antisense	0.6114	20407911
PRMT3e4	GAATTCATGTACTCAACTGT	AGG	NM_001145167.1	4	antisense	0.5647	20392906
PRMT3e6	GTCATCTACTAGTGTCATTG	CGG	NM_001145167.1	6	sense	0.6283	20397642
PRMT3e9	TAGATGTTATCATATCTGAG	TGG	NM_001145167.1	9	sense	0.7266	20426857
PRMT6e1.1	CGTTCCCGCTTAGTCCTCCG	GGG	NM_018137.2	1	antisense	0.7238	107056827
PRMT6e1.2	CTCGGACGTTTCGGTCCACG	AGG	NM_018137.2	1	sense	0.6903	107056885
PRMT6e1.3	GCCCATCCACTCGCTCACGA	TGG	NM_018137.2	1	antisense	0.6718	107057173
PRMT6e1.4	GTGGCCCATGAGACAGCGCG	TGG	NM_018137.2	1	antisense	0.6809	107057398
CARM1e7	TAGAGCTGTTCATCCGTGAA	GGG	NM_199141.1	7	antisense	0.6214	10916451
CARM1e1	TCGCGTCGCCGATGGTGAGG	AGG	NM_199141.1	1	antisense	0.6159	10871816
CARM1e5	TGGAGCACGGAAAATCTACG	CGG	NM_199141.1	5	sense	0.8313	10912257
CARM1e6	TTGAAGAGCATGTAGCCCAT	GGG	NM_199141.1	6	antisense	0.631	10913985
RPA3_e5.1	GATGAATTGAGCTAGCATGC	CGG	NM_002947.3	5	antisense	0.6306	7640373
RPA3_e7	GGTTGGAAGAGTAACCGCCA	AGG	NM_002947.3	7	sense	0.7045	7637924
RPA3_e6	TACGGGTTCCATCAACTCGA	TGG	NM_002947.3	6	antisense	0.6307	7639084
RPA3_e5.2	TGGACATGATGGACTTGCCC	AGG	NM_002947.3	5	sense	0.6221	7640398
Rosa26 g1 (THUMPD3-AS1)	ACCTCTCTGTGCTTACGCAA	NGG	NR_132780.1	4	antisense	0.6255	9390454
Rosa26 g2 (THUMPD3-AS1)	GTTGCTTTATCACTCAACAG	NGG	NR_132780.1	4	antisense	0.7707	9389195
Rosa26 g3 (THUMPD3-AS1)	GGAATGATCTTGAATAGTGT	NGG	NR_132780.1	4	sense	0.5791	9389474
Rosa26 g4 (THUMPD3-AS1)	AAGCAGTAGTCAAGATCACC	NGG	NR_132780.1	2	antisense	0.5735	9395543

a

Cell Line	*VHL*	*PBRM1*	*SETD2*	*BAP1*
22	Nonsense: p.Y175*	Nonsense: p.E1416*	In frame deletion: p.Q1292 Y1293delinsH	
162	Nonsense[#]: p.R161*	Missense: p.D727Y		
222	Frame shift deletion: p.T157fs	Frame shift deletion: p.E1201fs Missense: p.P1188L		
243	Nonsense: p.R113*			Missense: p.H141Y
323	Frame shift insertion: p.N174fs			
364	Nonsense: p.Y185*			
407	Frame shift deletion: p.E188fs Frame shift deletion: p.R200fs	Nonsense: p.R512*		
786-0	Frame shift deletion: p.G104Afs			

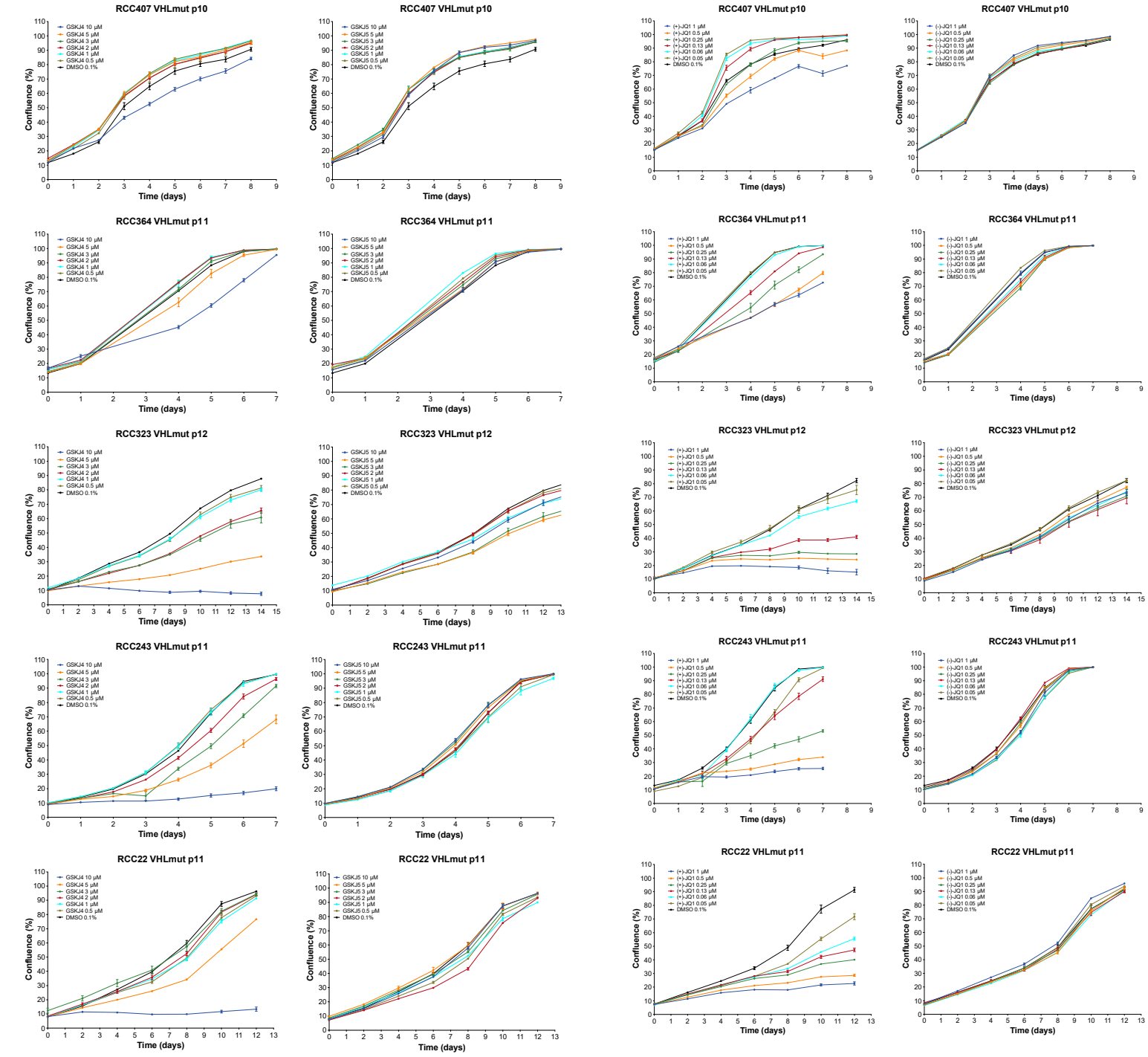


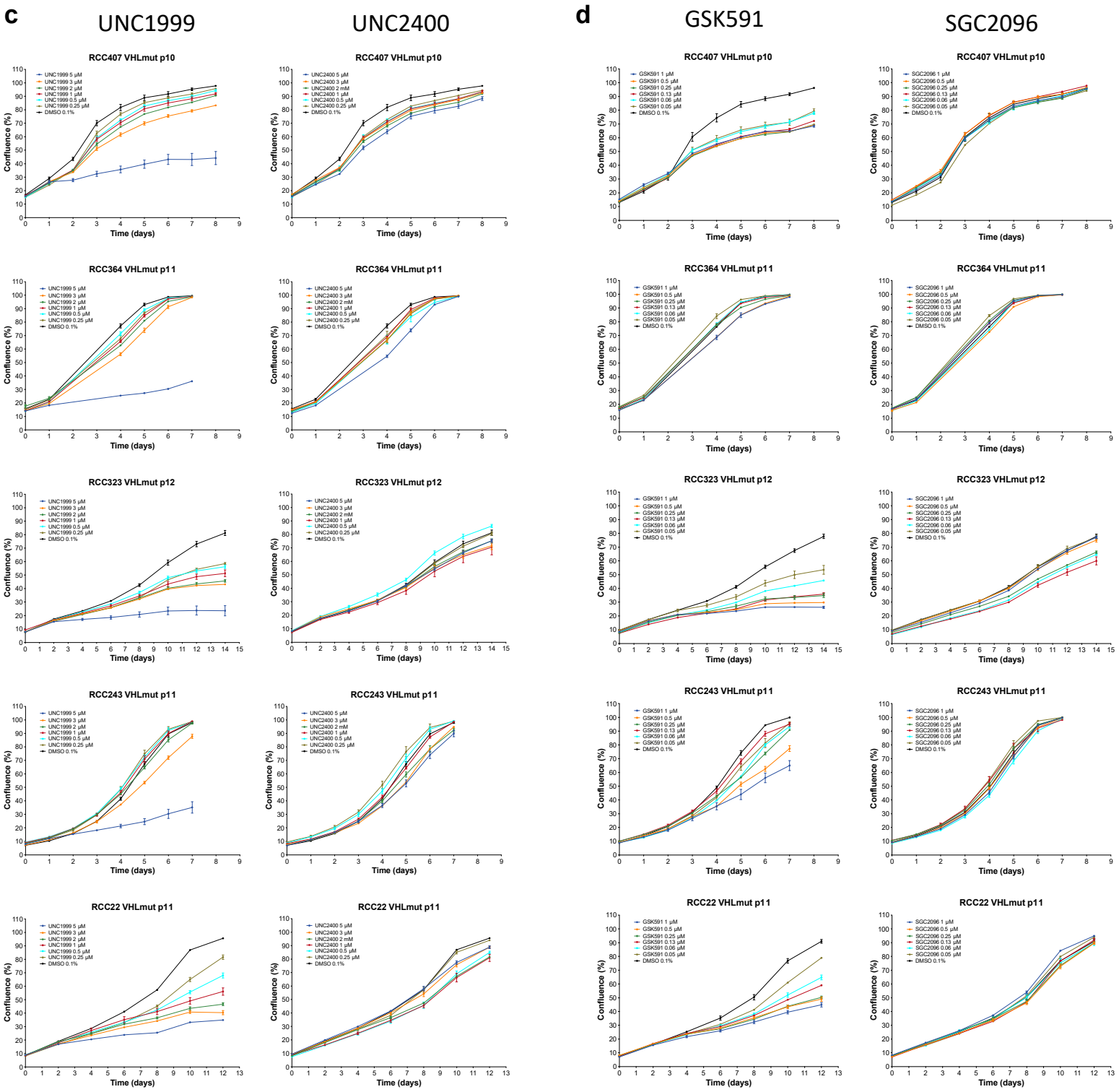
b



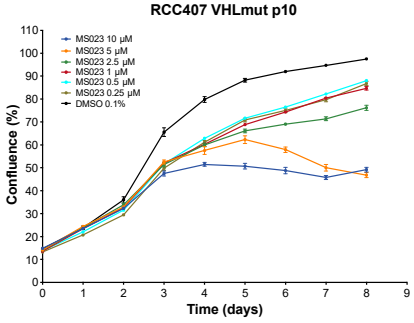
Supplementary Figure 1. Epiprobe Screen

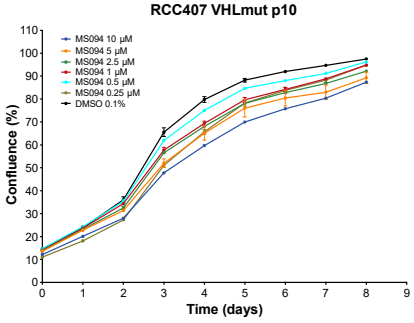
a, Mutation profiles for select genes in eight ccRCC cell lines and the schematic workflow of epigenetic chemical probe screen. **b,** Epiprobe chemical screen results across all cell lines. Data are presented as the mean fluorescence normalized to DMSO of 8 biological replicates with 3 technical replicates each ± SD and *p* values are calculated by 1-way ANOVA with Dunnett's multiple comparison relative to DMSO control group. Red bars indicate p-values <0.05 and a mean fluorescence intensity value <50% compared to DMSO control (dotted line). Data corresponds to heat map shown in Figure 1. ns=nonsignificant. Source data are provided as a Source Data file. Panel (a) 96-well plate schematic created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license.

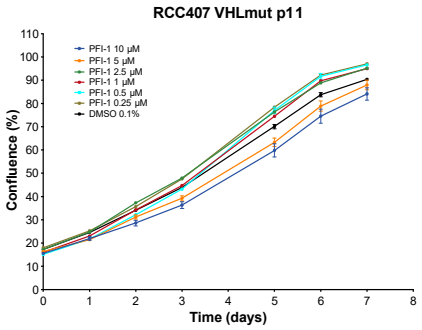

a
GSKJ4
GSKJ5
b
(+)-JQ1
(-)-JQ1
RCC407 VHLmut p10
RCC364 VHLmut p11
RCC323 VHLmut p12
RCC243 VHLmut p11
RCC22 VHLmut p11
Confluence (%)
Time (days)
GSKJ4 10 μM
GSKJ4 5 μM
GSKJ4 3 μM
GSKJ4 2 μM
GSKJ4 1 μM
GSKJ4 0.5 μM
DMSO 0.1%
GSKJ5 10 μM
GSKJ5 5 μM
GSKJ5 3 μM
GSKJ5 2 μM
GSKJ5 1 μM
GSKJ5 0.5 μM
DMSO 0.1%
(+)-JQ1 1 μM
(+)-JQ1 0.5 μM
(+)-JQ1 0.25 μM
(+)-JQ1 0.13 μM
(+)-JQ1 0.06 μM
(+)-JQ1 0.05 μM
DMSO 0.1%
(-)-JQ1 1 μM
(-)-JQ1 0.5 μM
(-)-JQ1 0.25 μM
(-)-JQ1 0.13 μM
(-)-JQ1 0.06 μM
(-)-JQ1 0.05 μM
DMSO 0.1%

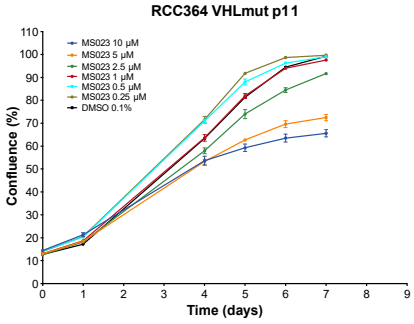

c
UNC1999
UNC2400
d
GSK591
SGC2096
RCC407 VHLmut p10
RCC364 VHLmut p11
RCC323 VHLmut p12
RCC243 VHLmut p11
RCC22 VHLmut p11
Confluence (%)
Time (days)
UNC1999 5 µM
UNC1999 3 µM
UNC1999 2 µM
UNC1999 1 µM
UNC1999 0.5 µM
UNC1999 0.25 µM
DMSO 0.1%
UNC2400 5 µM
UNC2400 3 µM
UNC2400 2 mM
UNC2400 1 µM
UNC2400 0.5 µM
UNC2400 0.25 µM
GSK591 1 µM
GSK591 0.5 µM
GSK591 0.25 µM
GSK591 0.13 µM
GSK591 0.06 µM
GSK591 0.05 µM
SGC2096 1 µM
SGC2096 0.5 µM
SGC2096 0.25 µM
SGC2096 0.13 µM
SGC2096 0.06 µM
SGC2096 0.05 µM

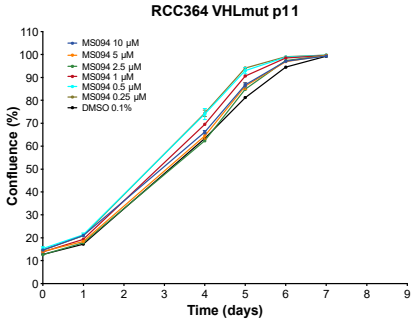
e MS023 MS094 **f** PFI-1

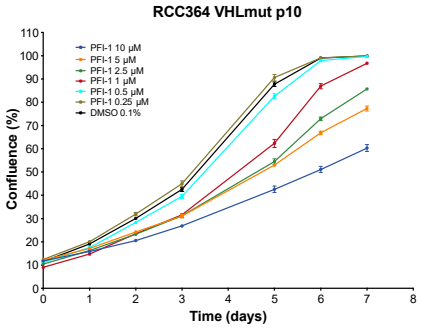


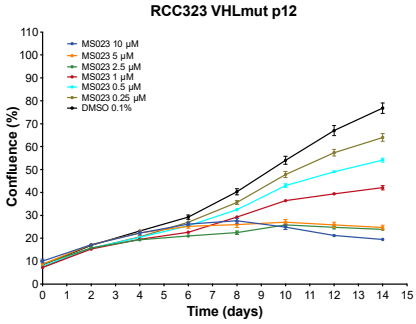


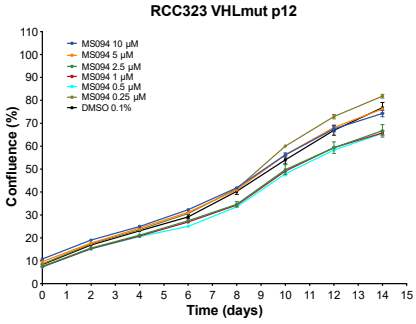


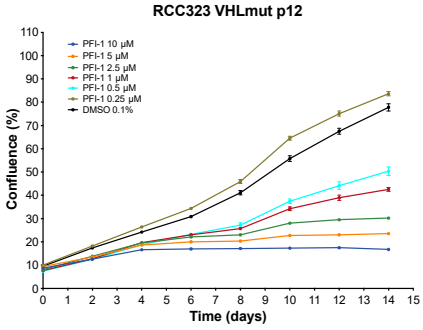


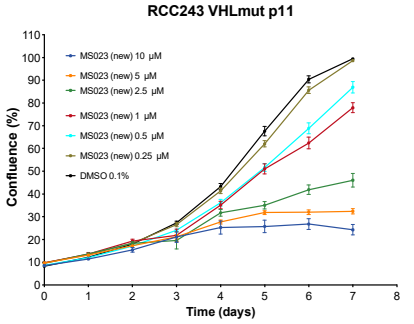


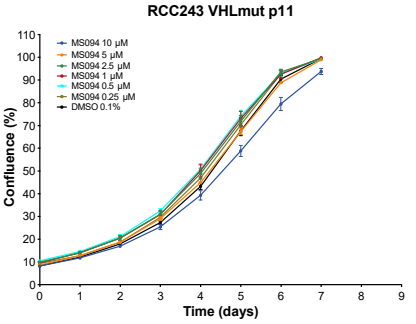


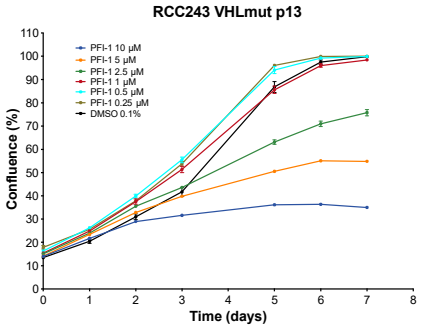


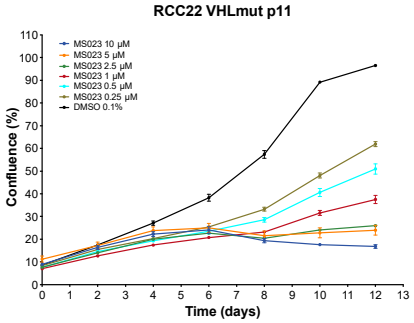


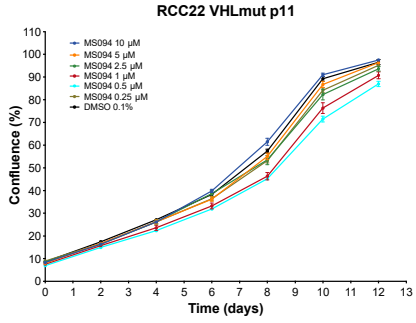


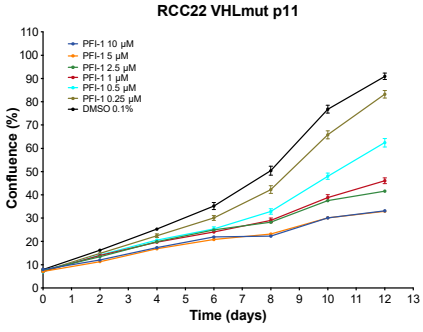


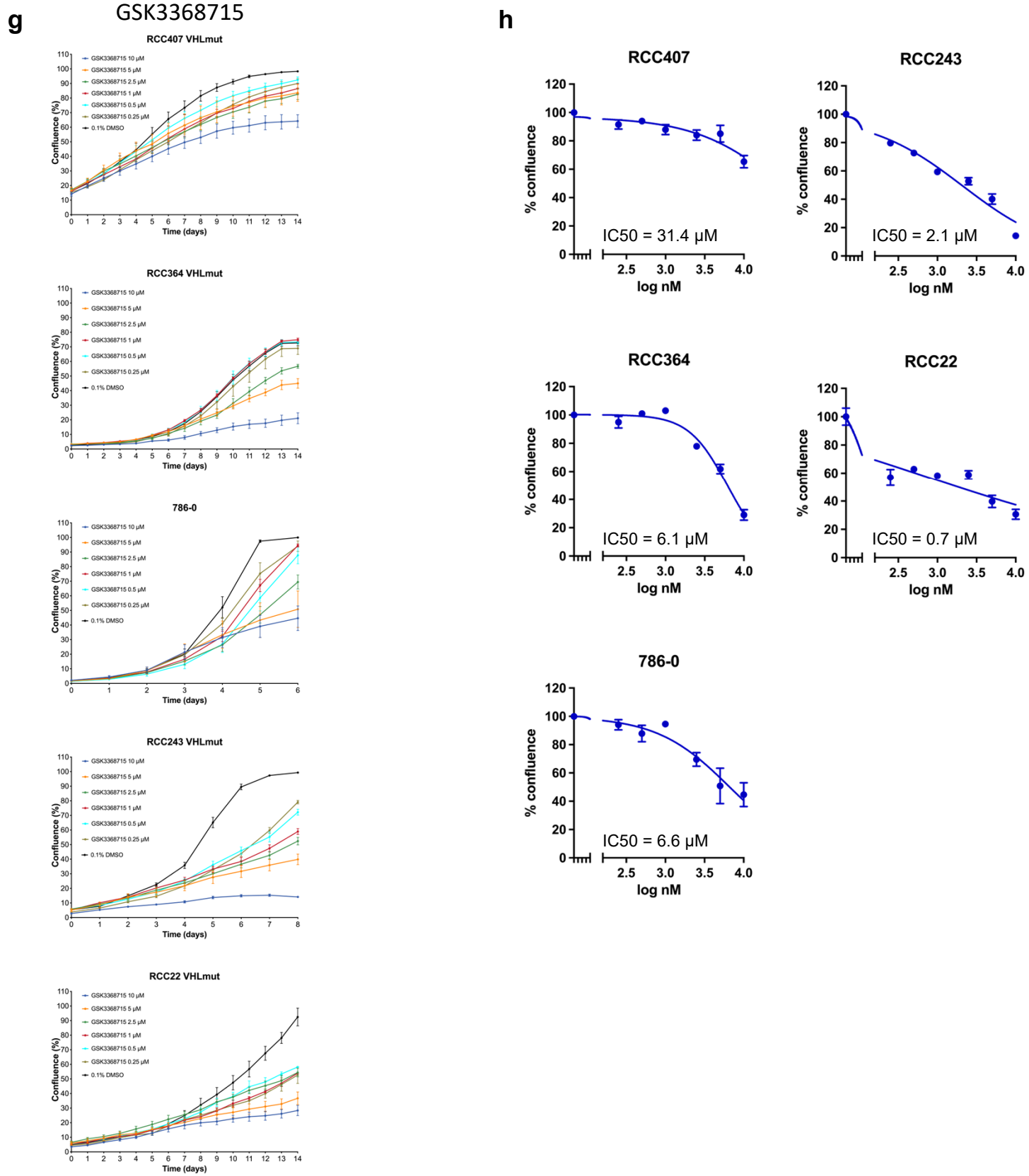






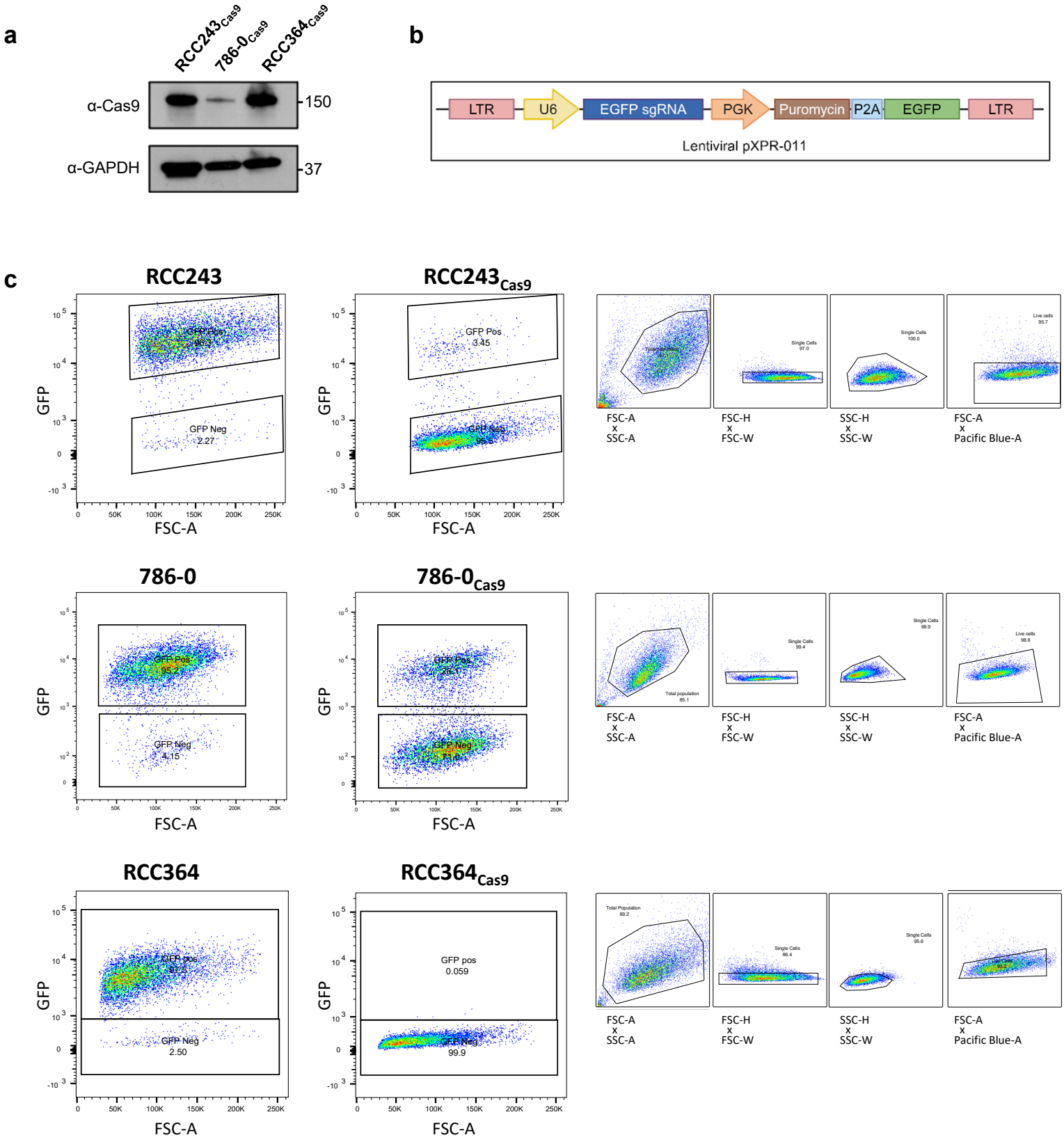






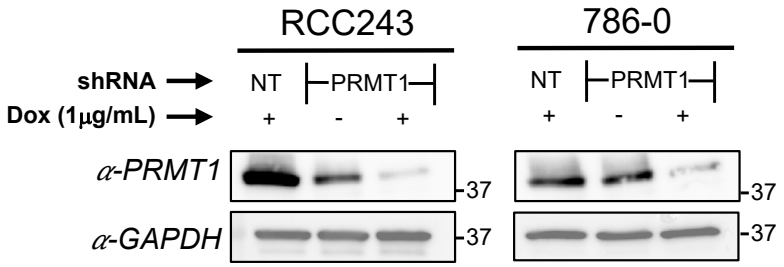
Supplementary Figure 2. Dose Response Curves for Epiprobe Hits

Dose response curves across cell lines RCC407, RCC364, RCC323, RCC243 and RCC22, for **a,** GSKJ4 *vs* inactive control GSKJ5. **b,** (+)JQ1 *vs* inactive control (-)JQ1. **c,** UNC1999 *vs* inactive control UNC2400. **d,** GSK591 *vs* inactive control SGC2096. **e,** MS023 *vs* inactive control MS094. **f,** PFI-1. **g,** GSK3368715, a pan-type I PRMT inhibitor (786-0 evaluated in place of RCC323). Data in **a-g** presented as the mean ± SD calculated from 3 technical replicates for each cell line **h,** GSK3368715 IC50 values across indicated ccRCC models. Data are presented as the mean ± SD calculated from 4 technical replicates for each cell line. Data presented at Day 6 for 786-0, Day 8 for RCC243 and Day 14 for RCC22, RCC364 and RCC407 (determined based on the time at which control-treated cells reached confluence). Source data are provided as a Source Data file.

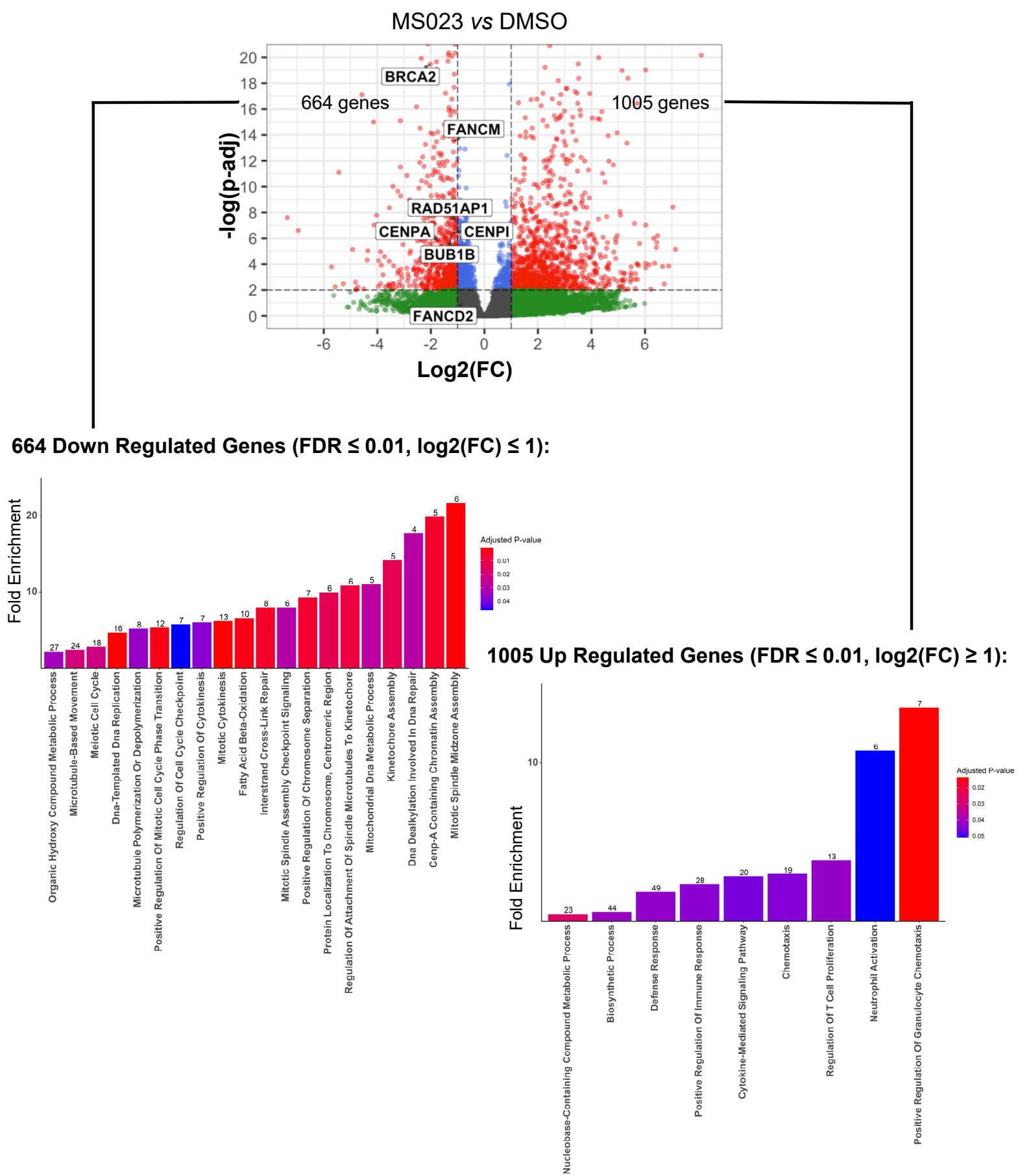


Supplementary Figure 3. Validation of Functional Gene Editing in ccRCC Cell Lines

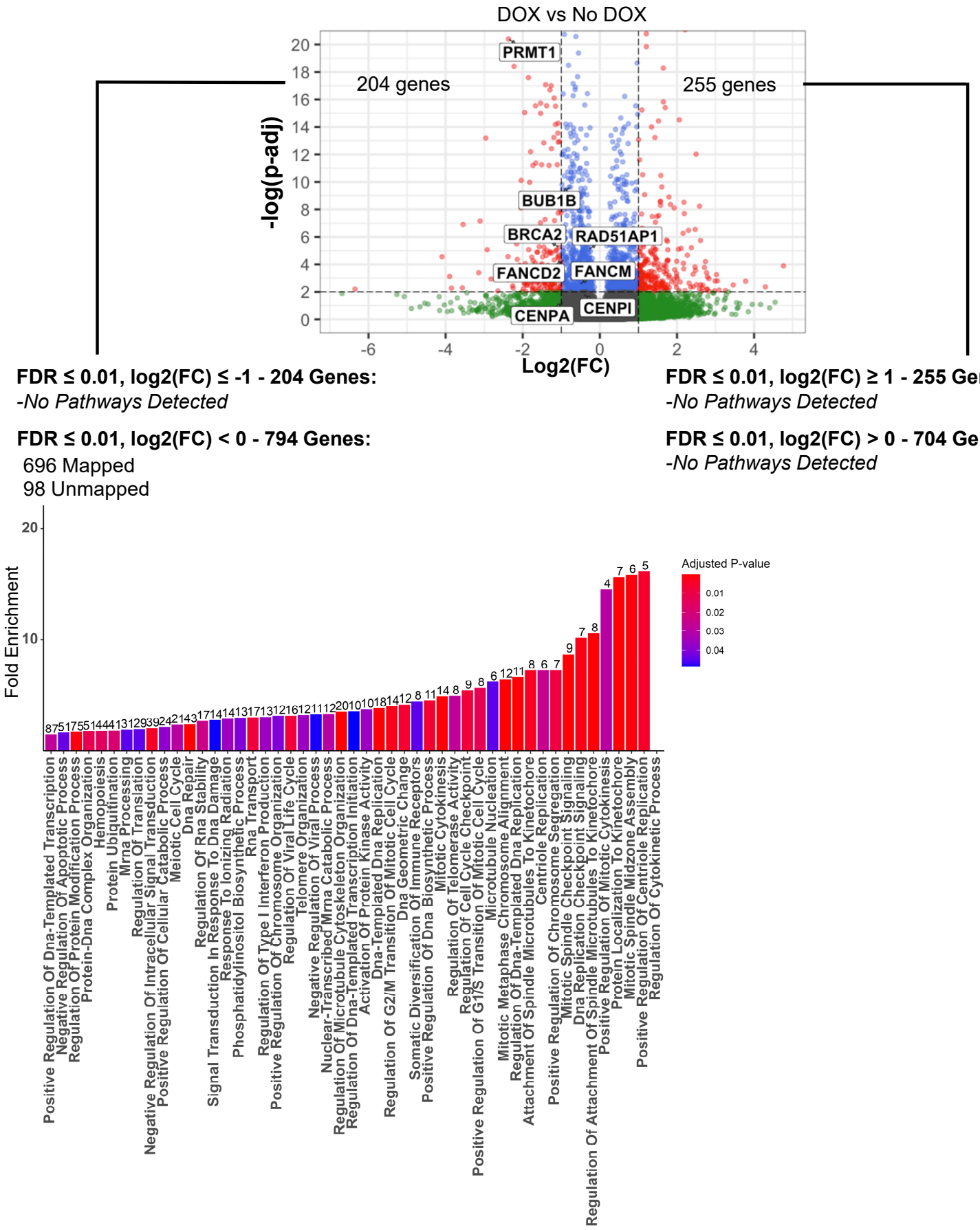
a, Western blot analysis of Cas9 expression in RCC243Cas9, 786-0Cas9 and RCC364Cas9 following transduction with lentivirally delivered humanized Cas9 gene and serial dilutions to establish monoclonal lines. Experiment performed once. **b,** Functional testing lentiviral genetic construct that expresses both the green fluorescence protein (GFP) and the guide (g-)RNA targeting GFP. **c,** Analytical flow cytometry analysis of GFP expression in parental and Cas9-expressing lines 5 days after genetic cassette in (b) introduced *via* lentiviral transduction. The gating strategy to select for viable, non-doublet cells is shown to the right. Source data are provided as a Source Data file. Panel (b) created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license.



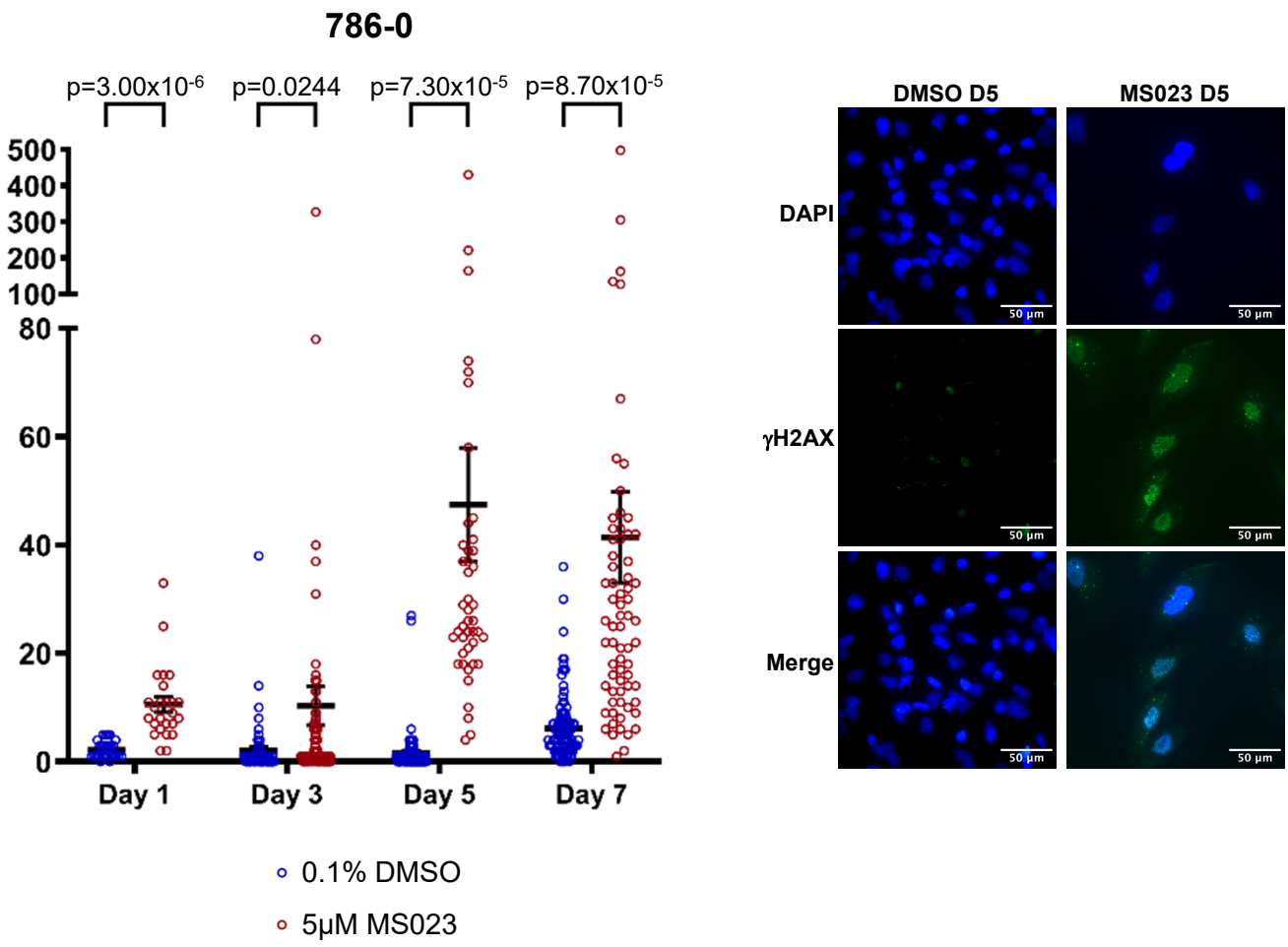
Supplementary Figure 4. Western blot analysis of PRMT1 expression in RCC 243 and 786-0 cells
Cell lines were engineered to express doxycycline (Dox)-inducible PRMT1 targeting or non-targeting (NT) shRNAs and treated with or without 1.0 μg/mL Dox for 4 days prior to RNA-seq analysis. Source data are provided as a Source Data file.



Supplementary Figure 5. Differentially expressed genes in MS023 *vs* DMSO-treated cells.
Volcano plot of log_2fold-change for genes significantly downregulated (red, left) or upregulated (red, right) genes following 3 days of 5 μM MS023 treatment in cell lines 786-0 and RCC243. Specific mitotic and DNA damage genes of interest explicitly labelled in plot. Overrepresentation analysis for gene ontology (GO) biological processes on indicated downregulated (505/664 mapped) and upregulated (376/1005 mapped) gene lists. Number of genes in down regulated list per GO biological process listed above each respective bar. Go terms filtered to most specific subclass.

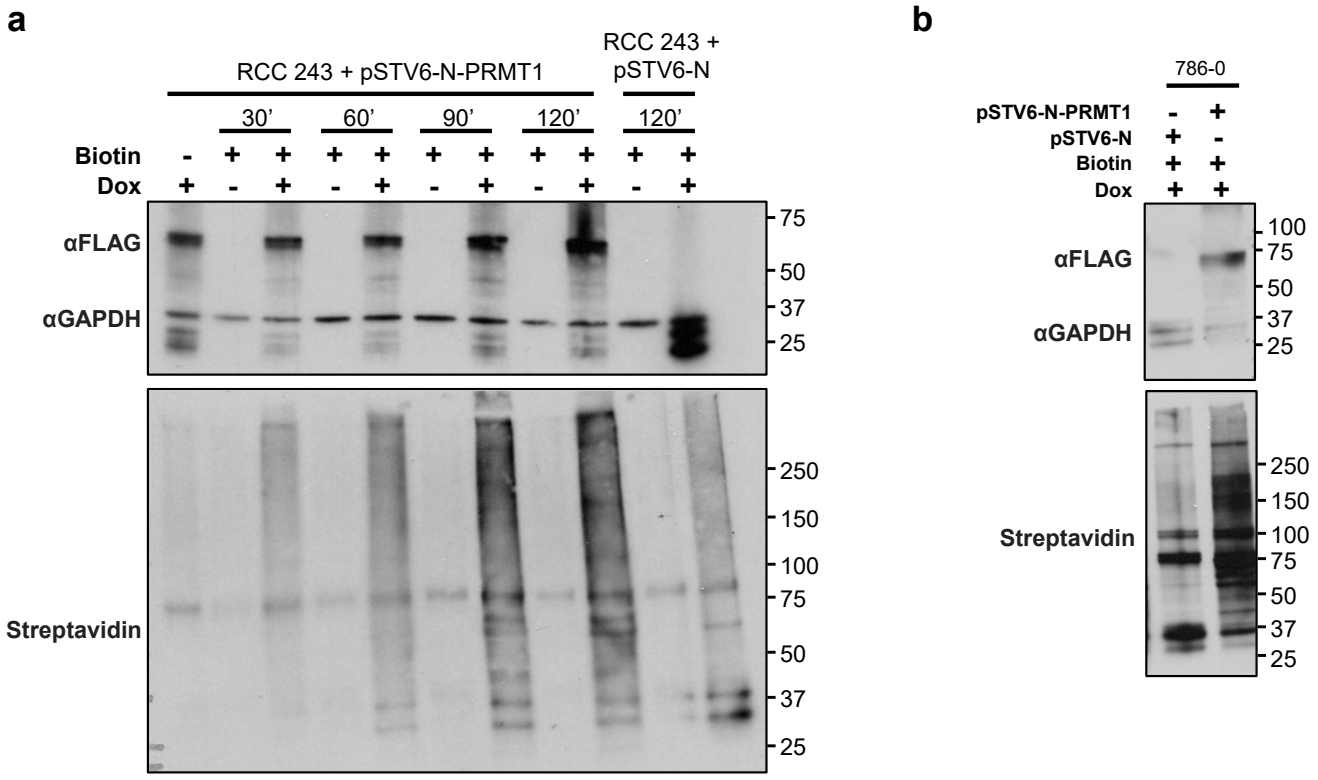


Supplementary Figure 6. Differentially expressed genes in PRMT1 knockdown *vs* control cells.
Volcano plot of log_2fold-change for genes significantly downregulated (red, left) or upregulated (red, right) genes in cell lines 786-0 and RCC243 expressing PRMT1 targeting shRNAs treated with or without 1.0 µg/mL Dox for 4 days. Specific mitotic and DNA damage genes of interest explicitly labelled in plot. Overrepresentation analysis for gene ontology (GO) biological processes on indicated downregulated and upregulated gene lists. Number of genes in down regulated list per GO biological process listed above each respective bar. GO terms filtered to most specific subclass.



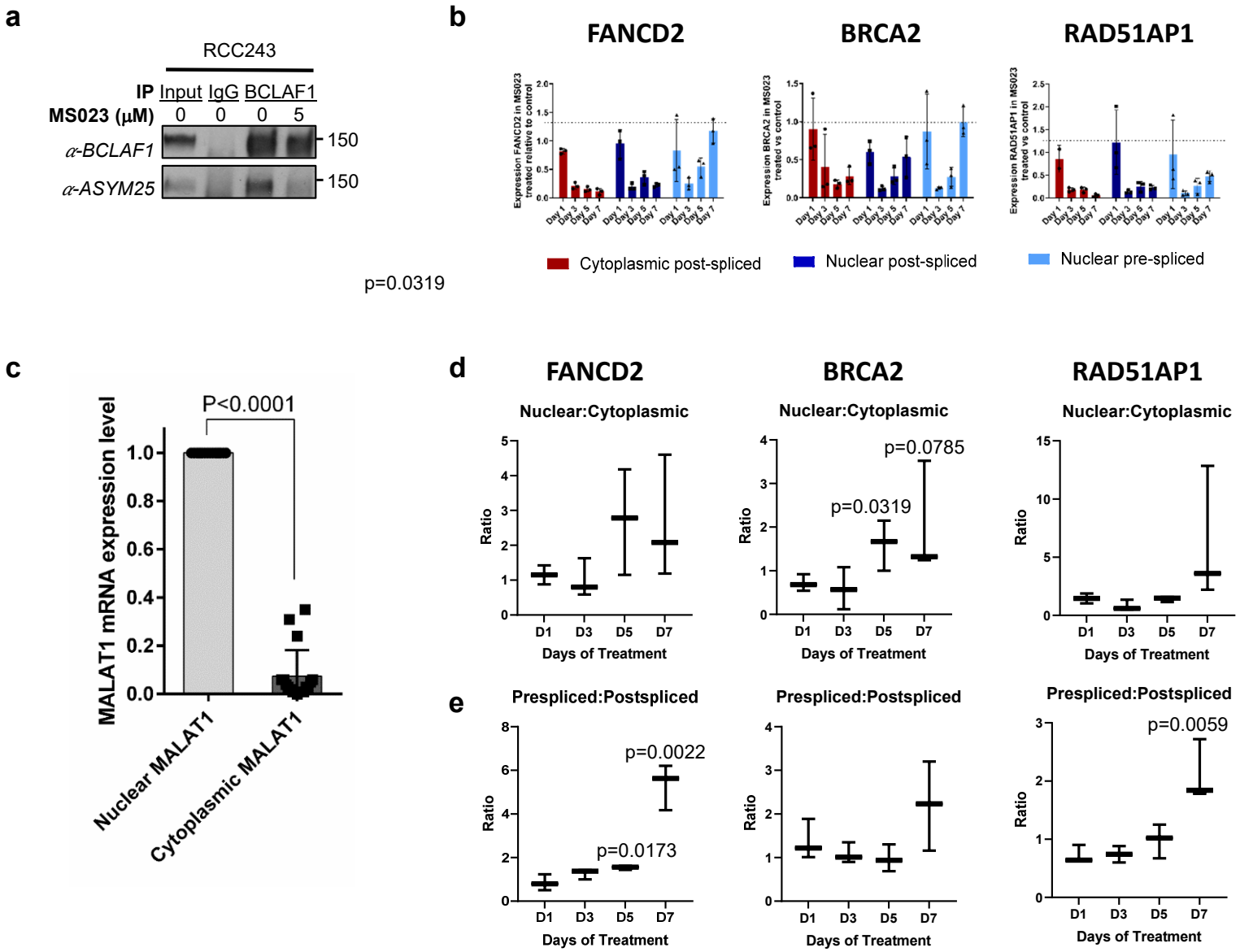
Supplementary Figure 7. Analysis of γH2AX foci in 786-0 cells

Scatter plots of γH2AX foci in 786-0 cells treated with and without 5 μM MS023 for indicated times. P-values are calculated by unpaired t-test with Welch's correction. Representative images from Day 5 of treatment are shown to the right. *n* = 33 *vs* 25 cells (Day 1 DMSO *vs* MS023), n = 69 *vs* 99 cells (Day 3 DMSO *vs* MS023), n = 122 *vs* 45 cells (Day 5 DMS0 *vs* MS023) and n = 110 *vs* 70 cells (Day 7 DMSO *vs* MS023). Source data are provided as a Source Data file.



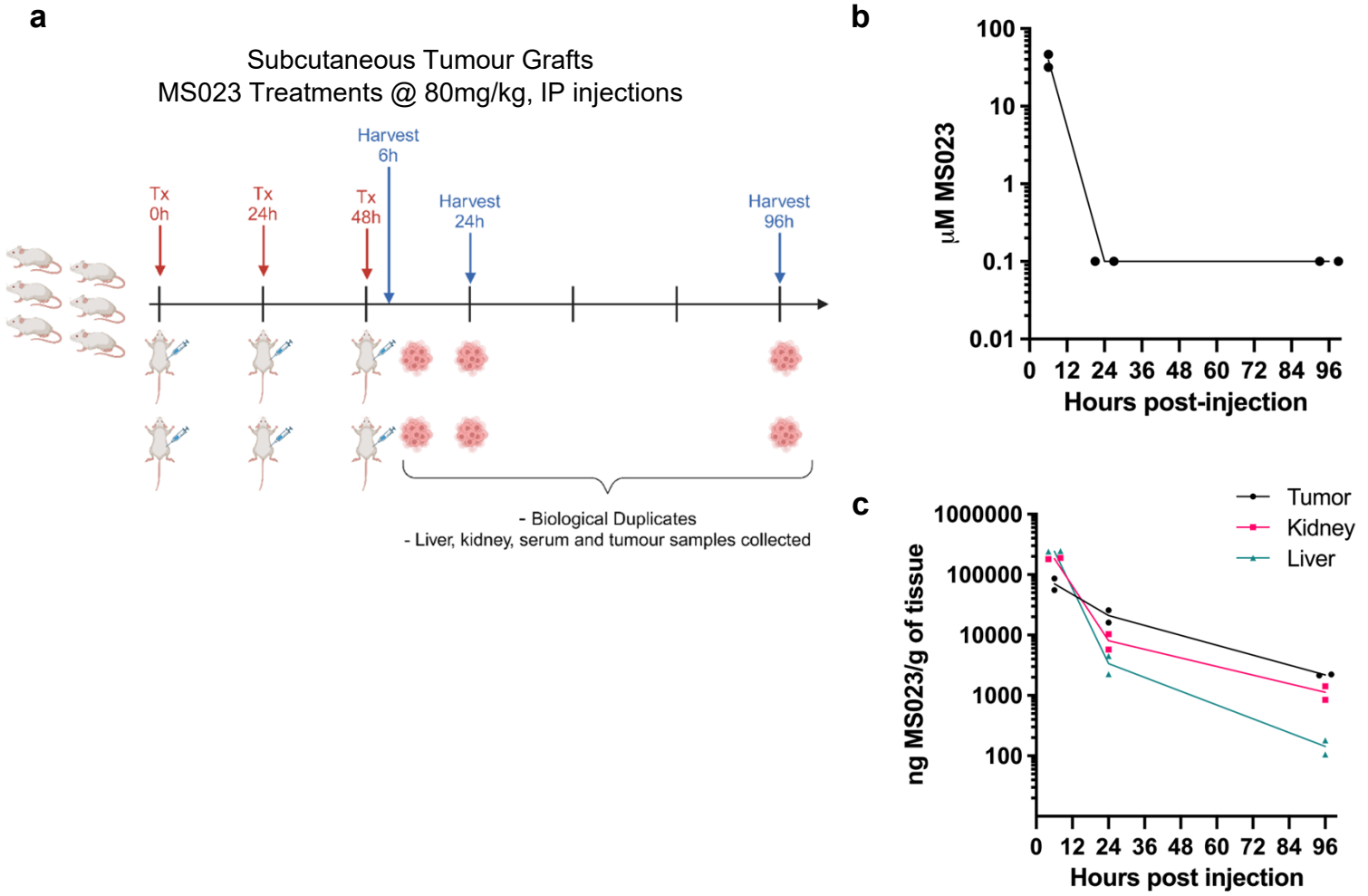
Supplementary Figure 8. Western blots for induction of PRMT1v1 expression and global biotinylation levels in cells used for Bio-ID

a, RCC243 cells were transduced with PRMT1-miniTurbo fusion (pSTV6-Nterm-PRMT1) or miniTurbo alone (pSTV6-N) and 1 μg/mL doxycycline was added for 24 hours to induce protein expression. 50 μM biotin was then added for the indicated times. Western blot analysis of protein expression *via* FLAG-tag detection and biotinylation levels *via* streptavidin detection are shown. 90 minutes was chosen as the appropriate time point for analysis. **b,** 786-0 cells were transduced with PRMT1-miniTurbo fusion (pSTV6-Nterm-PRMT1) or miniTurbo alone (pSTV6-N) and 1 μg/mL doxycycline was added for 24 hours to induce protein expression. 50 μM biotin was added for 90 min. Western blot analysis of protein expression *via* FLAG-tag detection and biotinylation levels *via* streptavidin detection are shown. Source data are provided as a Source Data file.



Supplementary Figure 9. Validation of BCLAF1 as a PRMT1 substrate and effect of MS023 on expression and nucleocytoplasmic transport of selected DDR genes

a, RCC243 cells were incubated with 5 μM of MS023 or 0.1% DMSO for 3 days, lysed and anti-BCLAF1 immunoprecipitations were performed. The bound proteins were separated by SDS-PAGE and immunoblotted with the indicated antibodies. **b,** mRNA levels for indicated genes in denoted cellular fraction after exposure to 1, 3, 5 and 7 days treatment with 5 μM MS023. Data presented as the mean of *n* = 3 biological replicates per gene per day (3 technical replicates per biological replicate used to calculate ΔΔCt values). **c,** MALAT1 lncRNA levels in the nuclear *vs* cytoplasmic fractions for samples in (b) as assessed by qRT-PCR. MALAT1 is largely absent from cytoplasmic fractions relative to nuclear compartments. Data presented as mean of n=18 biological replicates per gene (3 technical replicates per biological replicate used to calculate ΔΔCt values) **d, e,** Ratio of nuclear/cytoplasmic post-spliced transcripts and ratio of nuclear pre-spliced to nuclear post-spliced transcripts. mRNA expression levels assessed *via* qRT-PCR on cDNA generated from DNAse treated RNA samples and normalized to Actin. Graphs represent the mean of three independent experiments ±SD. Significant differences in the ratios were assessed using Student's two-tailed t-test in comparison to DMSO-treated cells. Only significant or near significant p-values are shown. Source data are provided as a Source Data file.



Supplemental Figure 10. Pharmacokinetics of MS023 in tumor-bearing NSG mice

a, Dosing and tissue collection schedule for PK study. **b,** Mass spectrometry serum measurements of MS023 post Day 3 injection. *n* = 2 mice/time point. **c,** Mass spectrometry tissue measurements of MS023 in tumor tissue, kidney and liver post Day 3. *n* = 2 mice/time point. Source data are provided as a Source Data file. Panel (a) created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license