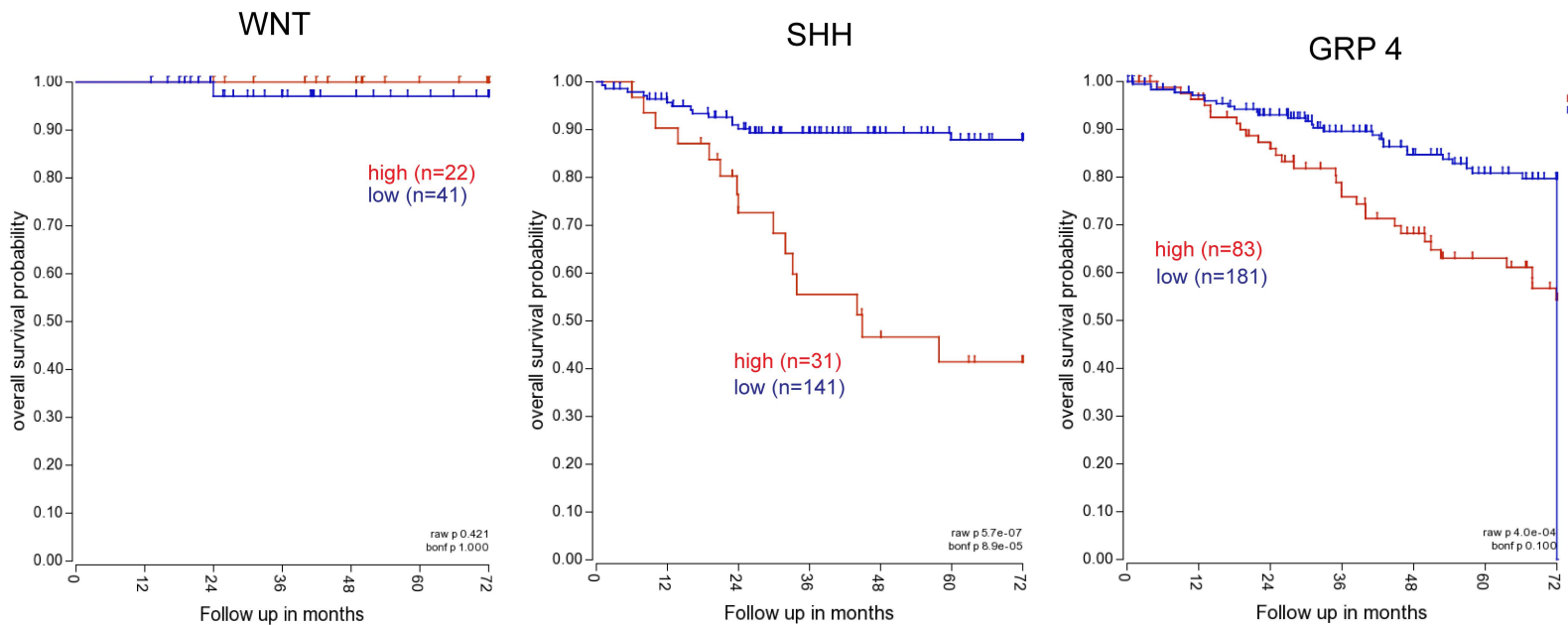


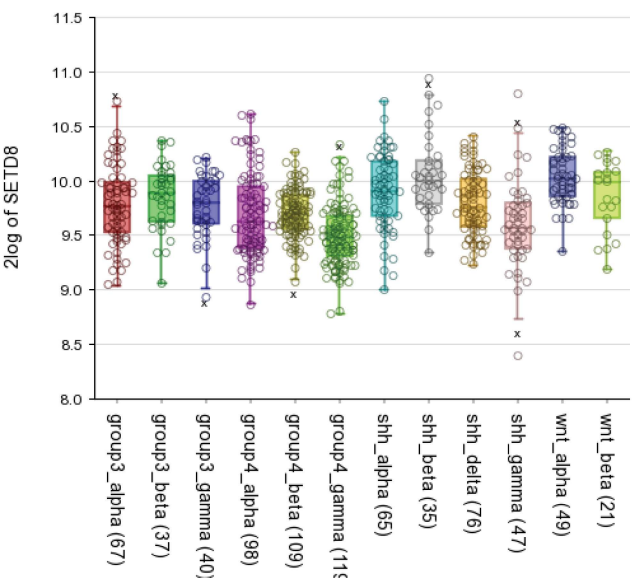
Supplemental Figures.

Supplementary Figure S1. (a) Kaplan-Meier plots indicating overall survival in relation to SETD8 expression in WNT ($p=0.421$), SHH ($p=5.7e-07$), and Group 4 ($p=4e-04$) subtypes. (b) SETD8 protein levels in patient Medulloblastoma subtypes.

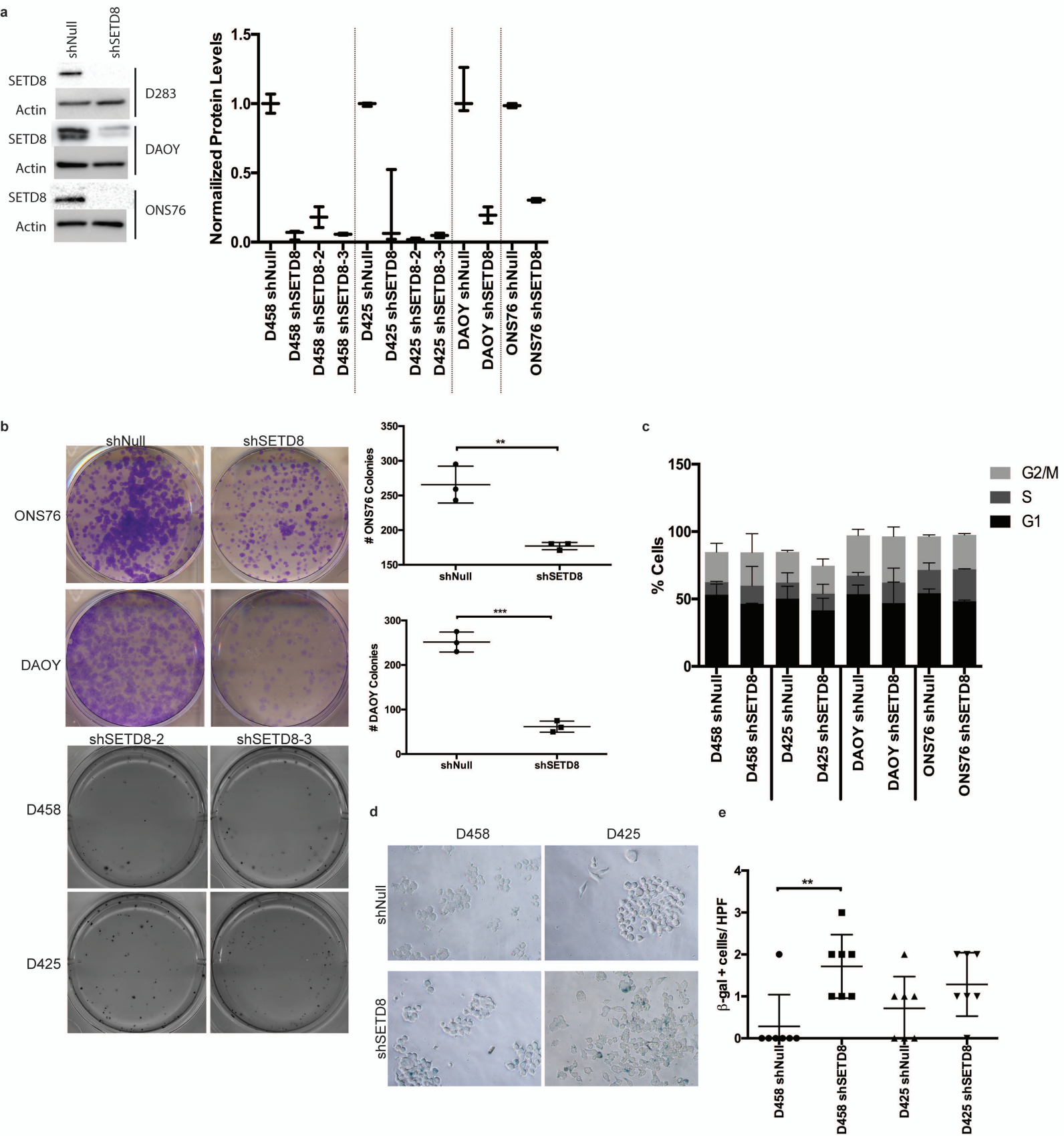
a



b

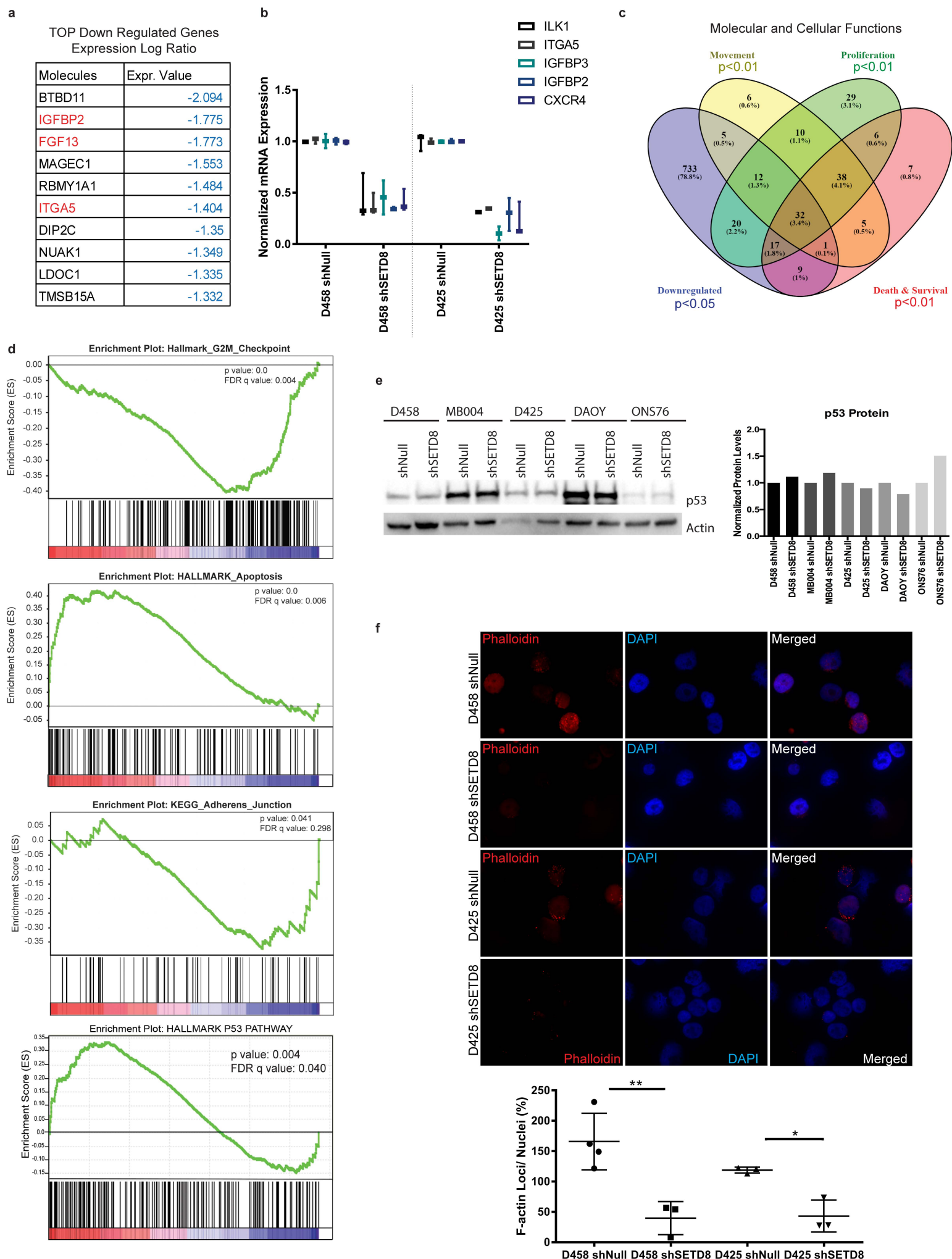


Supplementary Figure S2. (a) Representative images shown for D283, DAOY, and ONS76 western blot for SETD8 and Actin. Line and whisker plot represents quantification of three independent replicates. (b) Clonogenic assay of DAOY or ONS76 shnull and shSETD8 cells and methylcellulose assay with D458 and D425 shSETD8-2 and -3. Representative images are shown. Dot plots represent the mean \pm SD from three independent replicates. Asterisks indicate significant differences (unpaired t test; **,p-value<0.01; ***, p-value<0.001). (c) Cell Cycle kinetics determined by flow cytometry after staining with DRAQ5 in shNull and shSETD8 MB cells. (d) β -galactosidase staining in D458 and D425 shNull and shSETD8 cells. Representative images shown. (e) Dot plot represents mean β -gal+ cells per well \pm SD. Asterisks indicate significant differences (unpaired t test; **,p-value<0.01).



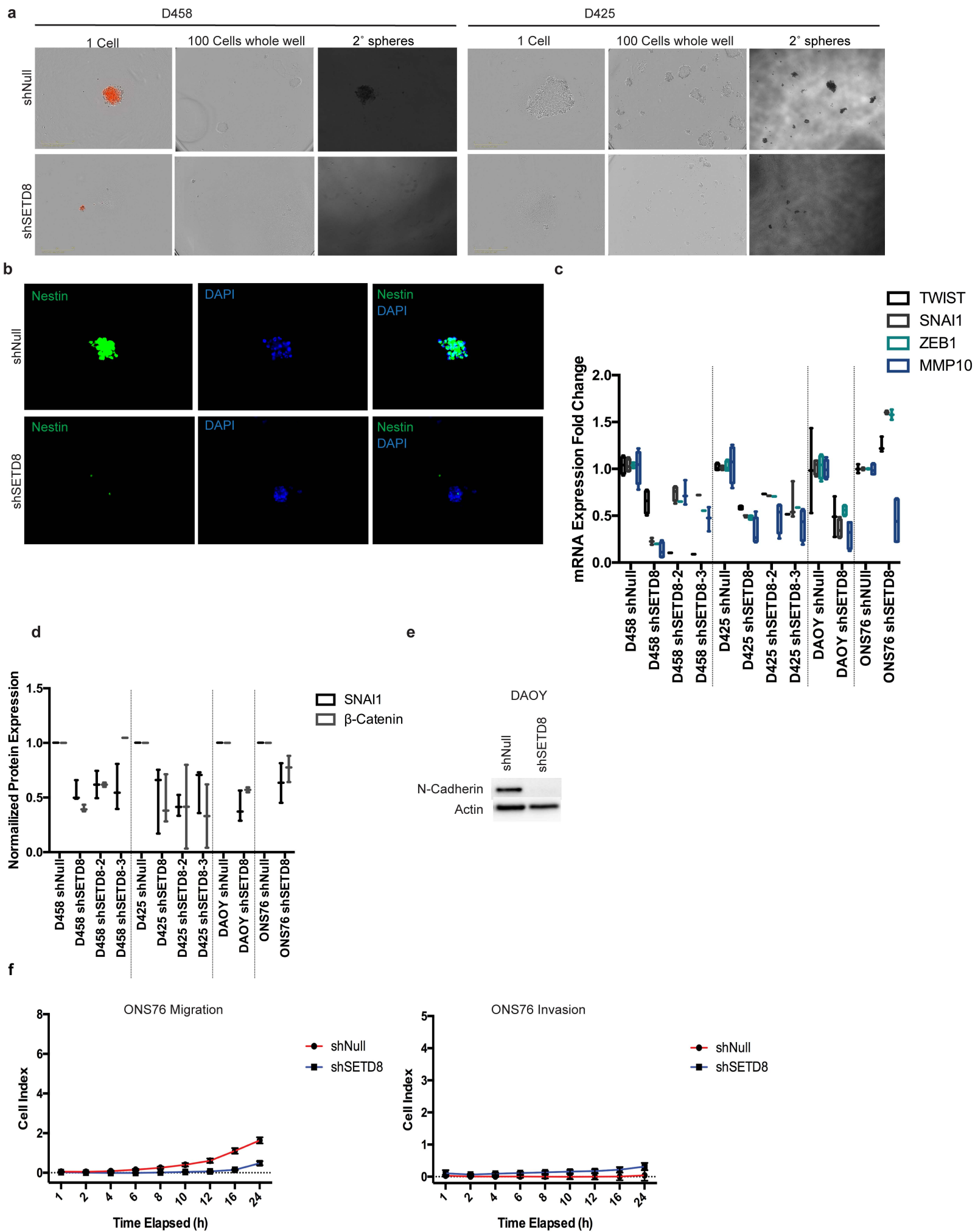
Supplementary Figure S3. (a) Top downregulated genes from Ingenuity analysis of the RNA-seq data and expression log ratio. Select genes in red. (b) Relative mRNA expression of *ILK1*, *ITGA5*, *IGFBP2*, *IGFBP3*, and *CXCR4* in D458 and D425 shSETD8 cells normalized to shNull in the respective cell line. Three independent replicates are shown as mean \pm SD. (c) Venn diagram of the top molecular and cellular functions represented in the downregulated gene set ($p < 0.05$). (d) GSEA of D458 shNull vs shSETD8 RNA-Seq. FDR q-value and p-value are shown for each plot. G2M, Apoptosis, Adherens Junction, and P53 pathway are shown. (e) Western blot of P53 protein in all cell lines with shNull or shSETD8. Quantification shown. (f) Immunofluorescence staining of phalloidin and dapi from D458 and D425 shNull and shSETD8. Cells were stained with Alexa Fluor[®] 555 phalloidin and DAPI. Imaged on Keyence BZ-X800 fluorescence microscope. Representative images are shown. Dot blots represent three independent replicates and show number of F-actin loci/nucleus \pm SD. Images quantified on ImageJ. Asterisks indicate significant differences (unpaired t test; *, p-value < 0.05; **, p-value < 0.01).

Supplementary Figure S3.



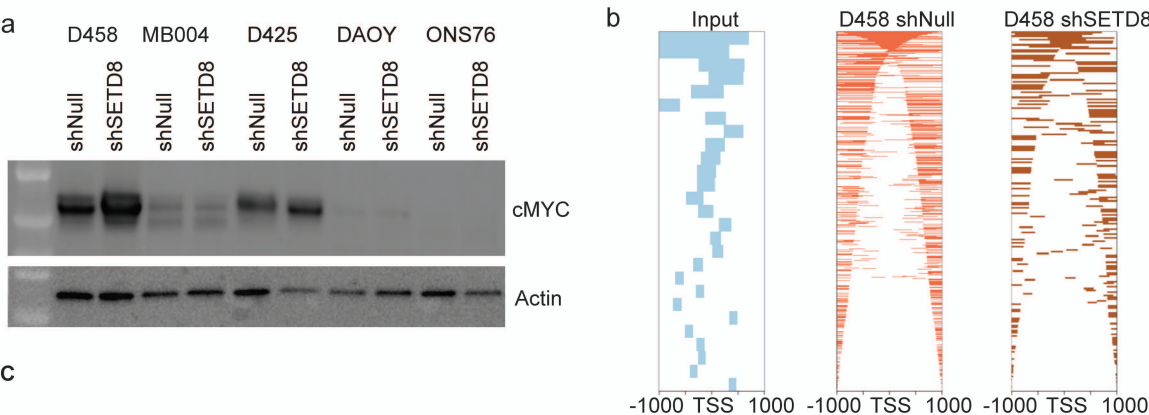
Supplementary Figure S4. (a) D458 and D425 shnull or shSETD8 neurosphere assay representative images for whole well 100 (4X), single-cells (10X), and secondary spheres (4X) as captured on Incucyte imaging system or Olympus U-CMAD microscope. (b) Immunofluorescence staining of 14 day old D458 shNull or shSETD8 neurospheres with α -nestin and DAPI. Representative images are shown. (c) Relative mRNA expression of TWIST1, SNAI1, ZEB1, and MMP10 in shSETD8 cells normalized to shNull in the respective cell line. Three independent replicates are shown as mean \pm SD. (d) Protein quantification of western in Figure 5B. Three independent replicates are shown as mean \pm SD in a line and whisker plot. (e) Western blot of N-cadherin and Actin in DAOY shNull and shSETD8 cell line. (f) ONS76 migration and invasion data. Line graphs represent the cell index mean vs time \pm SD in three independent replicates.

Supplementary Figure S4.



Supplementary Figure S5. (a) Western of cMYC and actin in shNull and shSETD8 MB cell lines. (b) TSS mapped H4K20me1 enriched regions (c) Kegg pathway hepatocellular carcinoma associated gene list.

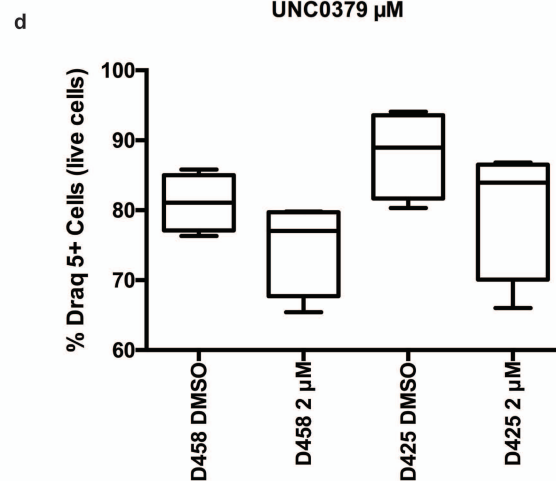
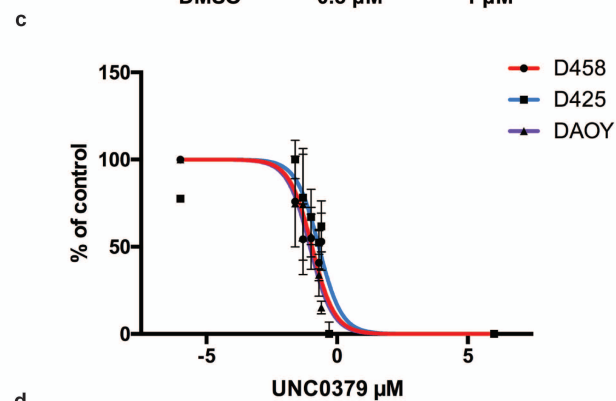
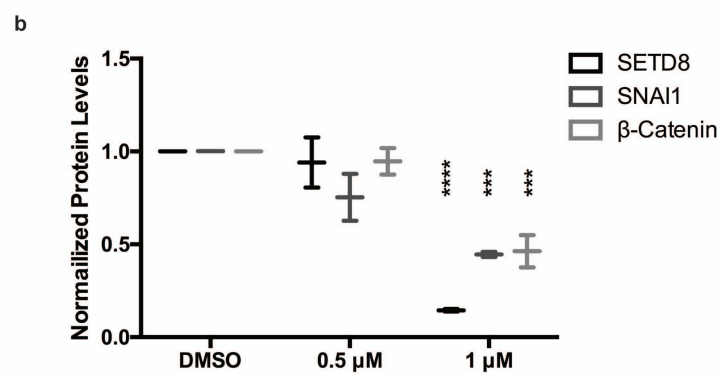
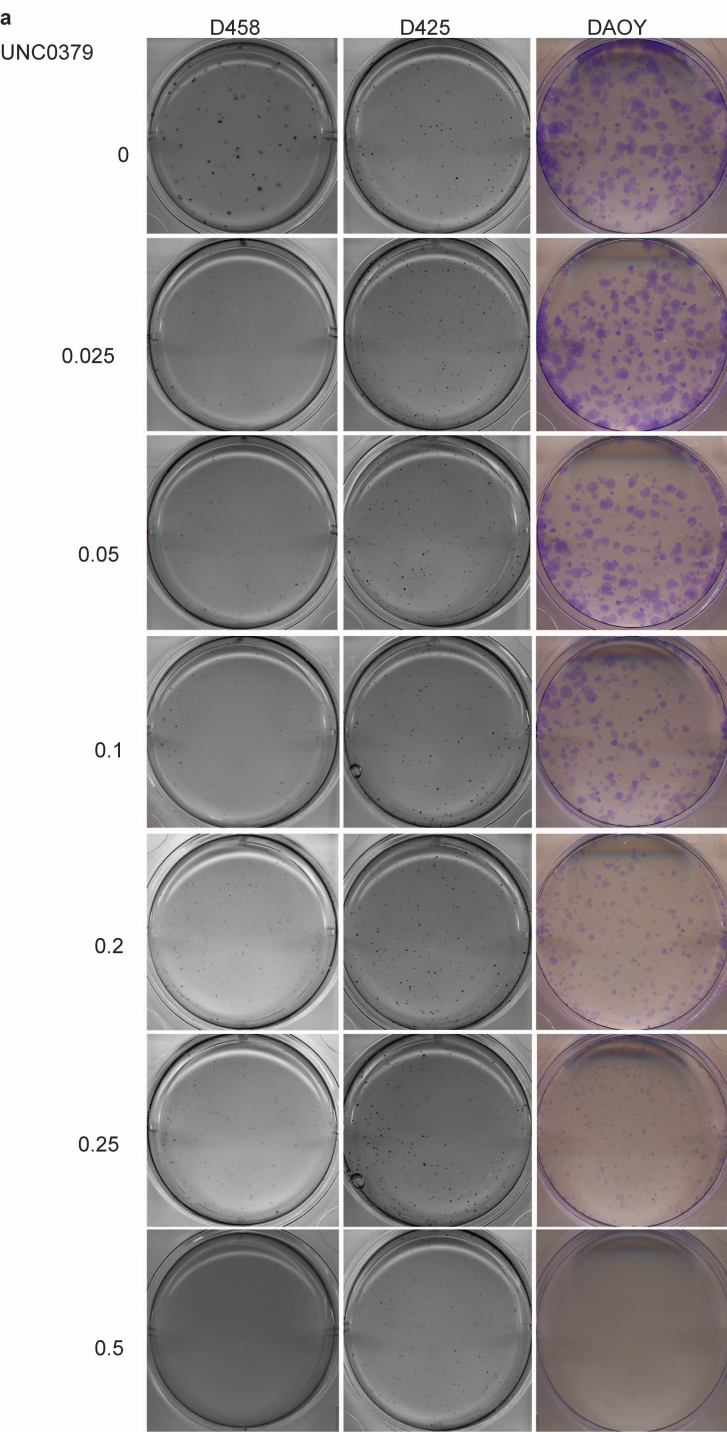
Supplementary Figure S5.



Hepatocellular Carcinoma KEGG Pathway

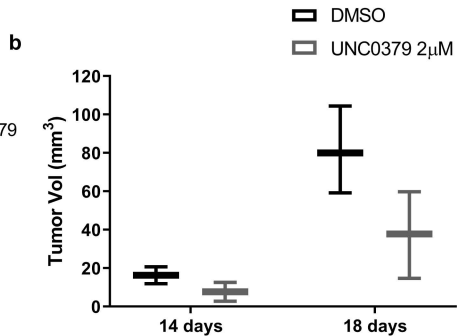
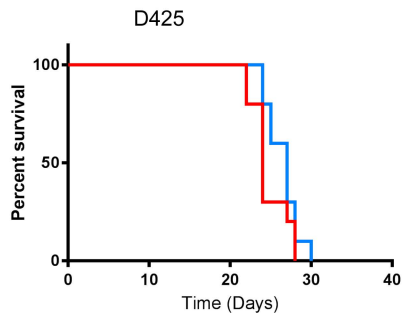
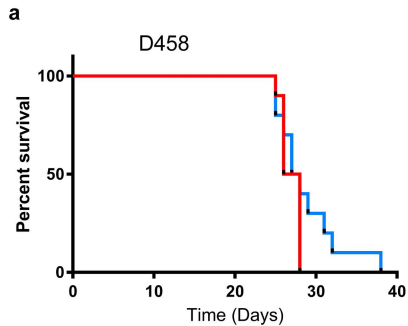
ENTREZ_GENE_ID	Name
71	actin gamma 1(ACTG1)
4609	v-myc avian myelocytomatosis viral oncogene homolog(MYC)
1647	growth arrest and DNA damage inducible alpha(GADD45A)
1026	cyclin dependent kinase inhibitor 1A(CDKN1A)
4257	microsomal glutathione S-transferase 1(MGST1)
8324	frizzled class receptor 7(FZD7)
6934	transcription factor 7 like 2(TCF7L2)
6932	transcription factor 7 (T-cell specific, HMG-box)(TCF7)
6654	SOS Ras/Rac guanine nucleotide exchange factor 1(SOS1)
5296	phosphoinositide-3-kinase regulatory subunit 2(PIK3R2)
3265	HRas proto-oncogene, GTPase(HRAS)
2932	glycogen synthase kinase 3 beta(GSK3B)
3480	insulin like growth factor 1 receptor(IGF1R)
10912	growth arrest and DNA damage inducible gamma(GADD45G)
7477	Wnt family member 7B(WNT7B)
7015	telomerase reverse transcriptase(TERT)
81029	Wnt family member 5B(WNT5B)
2885	growth factor receptor bound protein 2(GRB2)
6598	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1(SMARCB1)
2950	glutathione S-transferase pi 1(GSTP1)
6599	SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily c member 1(SMARCC1)
4041	LDL receptor related protein 5(LRP5)
4040	LDL receptor related protein 6(LRP6)
3845	KRAS proto-oncogene, GTPase(KRAS)
23401	frequently rearranged in advanced T-cell lymphomas 2(FRAT2)
7296	thioredoxin reductase 1(TXNRD1)
9817	kelch like ECH associated protein 1(KEAP1)
55274	PHD finger protein 10(PHF10)
1869	E2F transcription factor 1(E2F1)
2549	GRB2 associated binding protein 1(GAB1)
1857	dishevelled segment polarity protein 3(DVL3)
8313	axin 2(AXIN2)
60	actin beta(ACTB)

Supplementary Figure S6. (a) Methylcellulose assay with D458, D425, and clonogenic assay with DAOY cells treated with 0.2 μ M UNC0379. Representative images are shown. (b) Protein quantification of western in Figure 8C. Three independent replicates are shown as mean \pm SD in a line and whisker plot. Asterisks indicate significant differences (unpaired t test; ***, p-value<0.001; ****, p-value<0.0001). (c) Total number of colonies formed in (8D) of all three cell lines. Represented as the percentage of the control wells (DMSO) vs concentration of UNC0379 \pm SD. Three independent replicates were done. (d) Box and whisker plot of % DRAQ5 + cells per cell line treatment. Three independent replicates are represented.



Supplementary Figure S7. (a) Kaplan-Meier survival plot from intracranial orthotopic mouse model with drug treatment. D458 DMSO (n=10) and UNC0379 (n=10) and D425 DMSO (n=10) and UNC0379 (n=10). (b) Tumor volume analysis of D458 DMSO and UNC0379 treated tumors at 14 and 18 days post injection.

Supplemental Figure S7.



Supplementary Table S1. Complete chemical inhibitor list that met the 70% cell viability cut off.

Drug target and RNAi gene list shown.

Supplementary Table S1.

Drug	Target	RNAi Gene List	
MI-nc (hydrochloride)	Menin-ml1	BRD3	ATAD2B
IOX1	2OG-oxygenase	WDR5	SATB1
UNC0646	G9a/GLP	SP100	NCOA3
UNC0631	G9a/GLP	ASH1L	SCML2
S-(5'-Adenosyl)-L-methionine (tosylate)	SAM	USP27X	PAXIP1
PFI-1	BRD2, BRD4	SSRP1	RNF2
5-Azacytidine	analog cytidine	EHMT2	SETD6
CPTH2 (hydrochloride)	GCN5	ASH2L	PCGF1
(R)-PFI-2 (hydrochloride)	SETD7/9	HCFC1	SUZ12
Etoposide	TOPO II	RING1	UBE2A
2,4-DPD	HIF1 α -PH	BAZ2B	BAZ2A
(-)-Nepanocin A	SAH	EP400	HDAC6
Decitabine	analog cytidine	CHAF1A	SMARCA5
DMOG	HIF1 α -PH	SIN3A	UHRF1
6-Thioguanine	analog thiopurine	MTA2	ING2
2,4-Pyridinedicarboxylic Acid	α -ketoglutarate	ORC1	EPC2
SGI-1027	DNMT1, DNMT3a/3b	KAT6A	HLTF
S-Adenosylhomocysteine	SAM	MLL5	SMARCA2
UNC0638	G9a/GLP	ARID4B	PBRM1
C646	P300	BRD4	PADI4
UNC0379	SETD8	INO80	PRMT3
Mirin	MIRN	BMI1	PRDM2
Isoliqurritigenin	quinone reductase-1	MLL2	MBD4
JIB-04	Jumonji	BRD1	DNMT3L
GSK126	EZH2	SETD8	SIRT3
3-Deazaneplanocin A (hydrochloride)	EZH2	RBBP4	DNMT1
Sinefungin	SET domain	PHF19	HDAC5
GSK-J5 (hydrochloride)	JMJD3	SUV420H2	UBE2I
4-pentynoyl-Coenzyme A (trifluoroacetate sa	acetyl-coA reporter	KANSL1	RBBP7
RVX-208	BRD4	NCOR1	HAT1
3-amino Benzamide	PARP	RNF20	PRMT8
α -Hydroxyglutaric Acid (sodium salt)	2OG-oxygenase	PRKAA1	CARM1
5-Methyl-2'-deoxycytidine	analog pyrimidine	PHC2	EZH2
SGC-CBP30	CREB/P300	SETD2	SFMBT2
BIX01294 (hydrochloride hydrate)	G9a/GLP	TRIM33	JMJD1C
Anacardic Acid	P300, PCAF	SETDB2	BRD7
PFI-3	SMARCA2/4	ERCC5	HDAC3
Rucaparib (phosphate)	PARP	NAP1L2	BPTF
Garcinol	P300, PCAF, GCN5		
MI-2 (hydrochloride)	Menin-ml1		
3-Deazaneplanocin A	EZH2		
GSK-J4 (hydrochloride)	JMJD3		
GSK2801	BAZ2A/B		
UNC0642	G9a/GLP		
UNC1215	L3MBTL3		
2-PCPA (hydrochloride)	LSD1		
Chaetocin	BRD4		

Methyltransferase inhibitor
Histone Acetylase inhibitor
Oxygen sensing
Demethylase inhibitor
DNA Damage
Bromodomain Anagtonist

Supplementary Table S2. ChIP primer sequences.

Supplemental Methods.

Clonogenic assay. Cells were plated at 1000 cells/well in a 6-well dish in 2 mL complete media and allowed to grow for ten days. Cells were then stained with crystal violet and counted.

Immunofluorescence. D458 neurospheres were spun down onto slides using Shandon Cytospin 4 (Thermo Fisher). Neurospheres were fixed in 4% paraformaldehyde in PBS. Cells were then washed 0.2% Triton X-100 in PBS and blocked in 5% milk in 0.05% Triton X-100 in PBS for 30min. Cells were stained with α -nestin Cat#ab105389 (AbCam) overnight. Cells were washed then exposed to 2° Alexa fluor 488 Cat#A11001 (ThermoFisher) 1hr RT. Cells were washed again then Prolong® Gold antifade with DAPI Cat#P36935 (Thermo Fisher) was applied and coverslipped. Alexa Fluor 555 Phalloidin Cat#8953 (Cell Signaling Technology) was used for phalloidin staining. Cells were plated in 8-well chamber slide overnight. Fixed and blocked as previously described, then stained for 15 mins, washed, DAPI applied, and coverslipped. Images were obtained on the Keyence BZ-X800 fluorescence microscope, and analyzed with ImageJ.

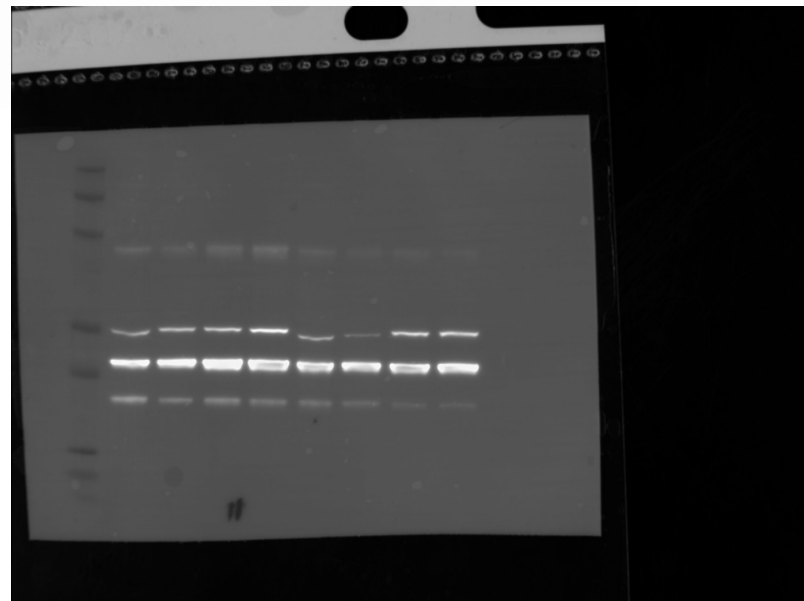
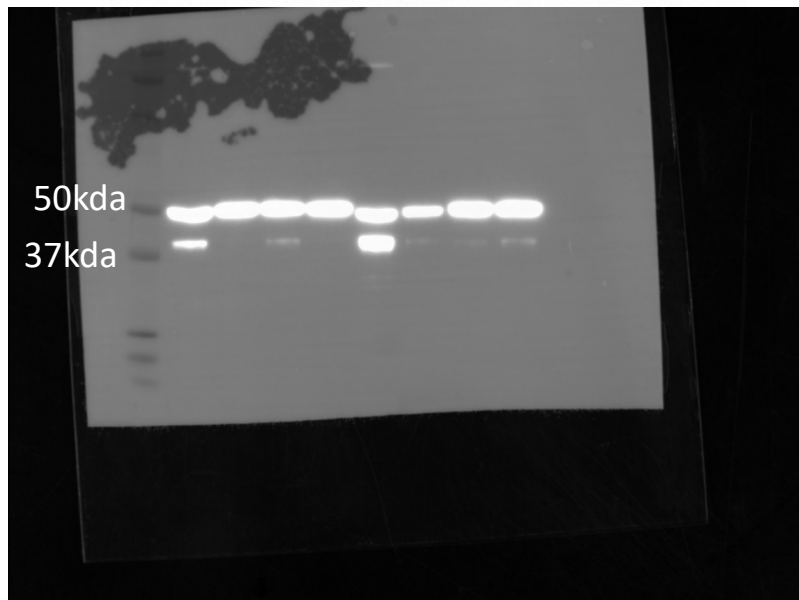
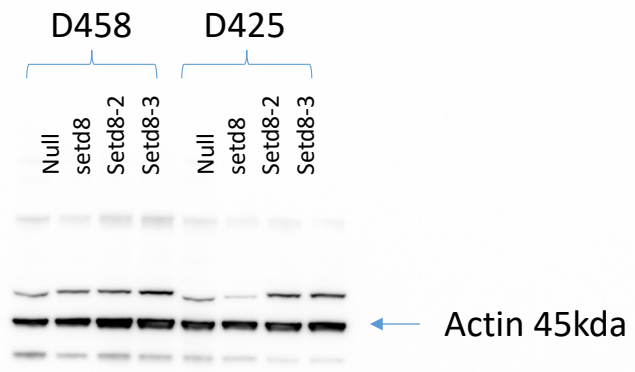
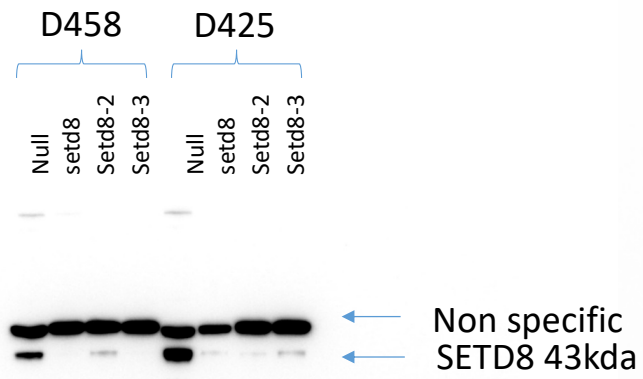
Cell cycle analysis. Cells in growth phase were collected and fixed in 70% ethanol overnight. Fixed cells were washed with PBS and suspended in 100 μ L 2% FBS/PBS, stained with 1:1000 dilution of DRAQ5™ fluorescent probe (ThermoFisher) for 30 min in the dark. Cells were studied on Amnis Flowsight® Imaging Flow Cytometer (Millipore Sigma) and analyzed by Amnis IDEAs software.

β -Galactosidase senescence Assay. Cells were plated at 5x10⁴ in a 6 well dish and grown overnight. Senescence β -Galactosidase staining kit Cat#9860 (Cell signaling technology) was used to stain overnight with 1mL of β -galactosidase solution with 20mg/ml of X-gal at 37°C in dry incubator. Cells were then imaged on Nikon ECLIPSE TS100 with a Nikon DSFi1.

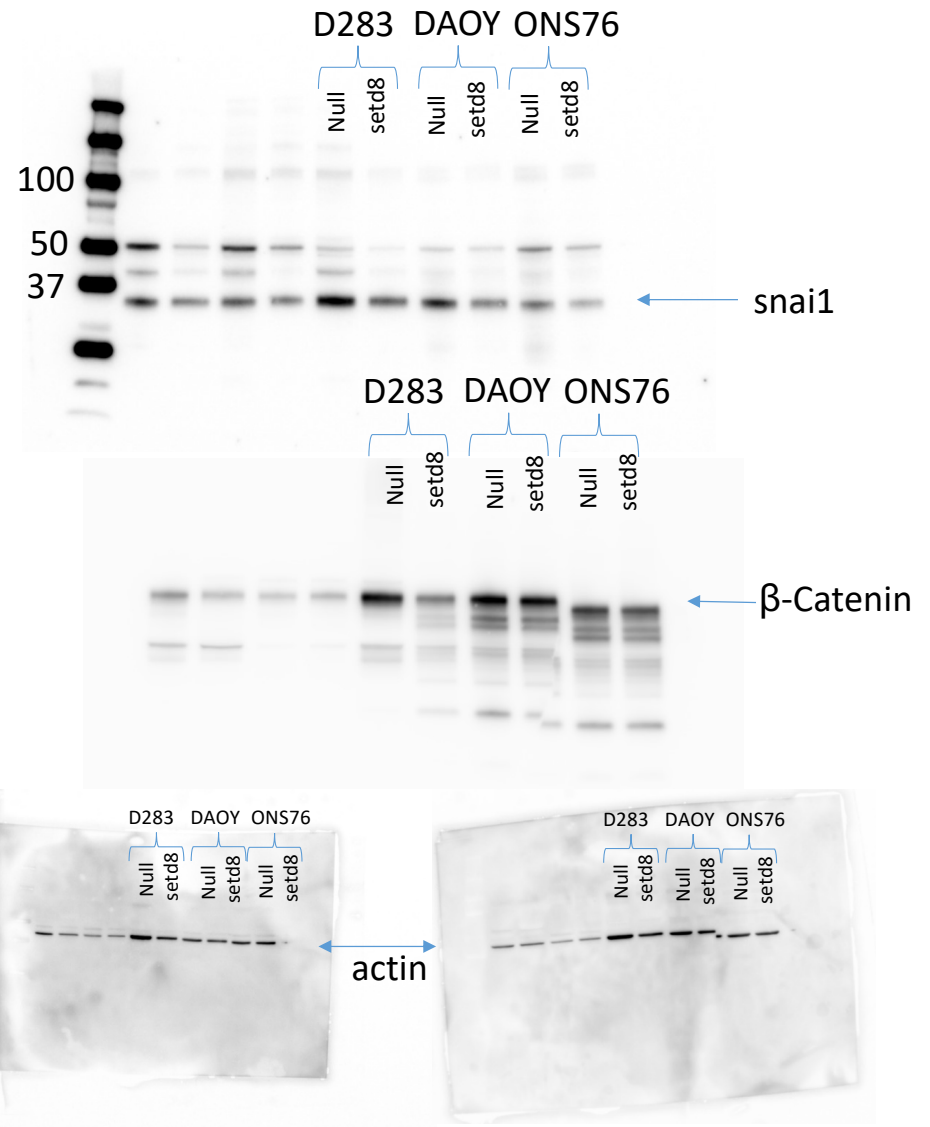
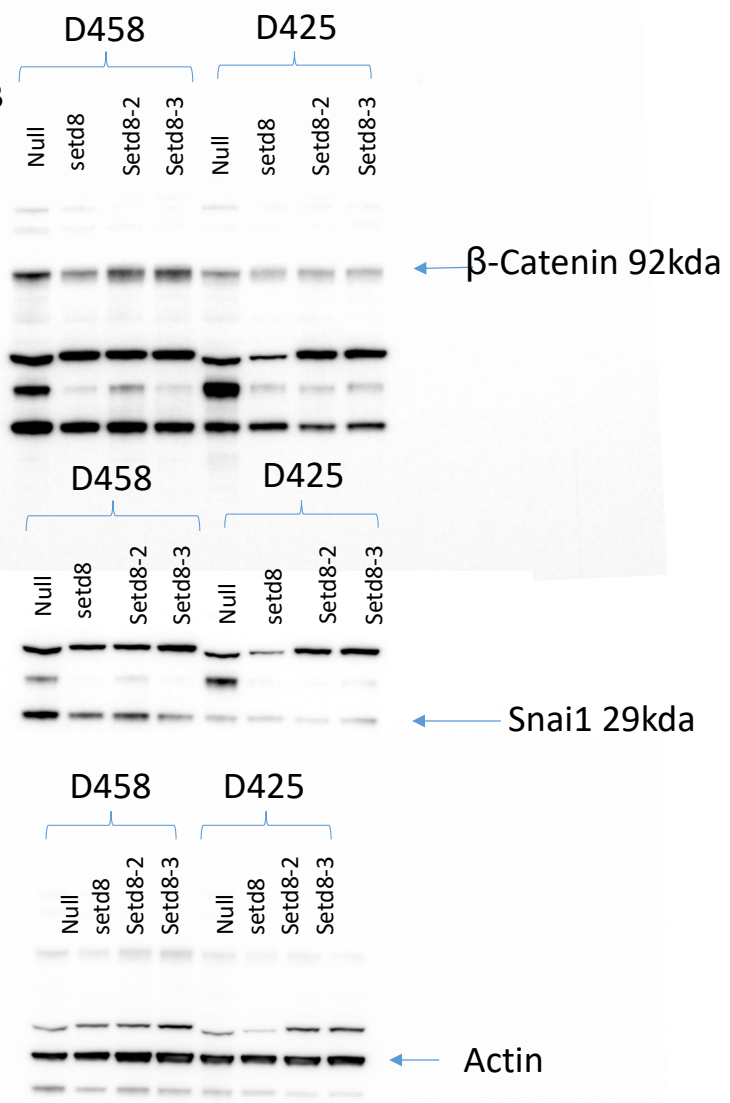
Supplementary Table S2.

ChIP Primers	Sequence
C-MYC sites	
myc1SETD8F_qRT	ATCAAAGCTGCTGGAGAGGTCG
myc1SETD8R_qRT	GATCTGGGACTTCCGGTTCTC
myc2SETD8F_qRT	GAGGGCATAGGCAGAGTACC
myc2SETD8R_qRT	TAGCATCCTTCAAGGCAGGG
myc3SETD8F_qRT	ATGGTGTCTACCTTTGCAGGG
myc3SETD8R_qRT	AGAAGGGCTGTACTTGACCG
snail.6H4K20F_qRT	AAGCAGACACCTTCCCAATC
snail.6H4K20R_qRT	CACCTTTCCCATATCCAGCTC
bcatenin.3H4K20F.qRT	GTTCCCCTGAGGTGCTTG
bcatenin.3H4K20R.qRT	TGCTACGAAGTTTGGCTCC
twist.3H4K20F.qRT	AGCTTGCCATCTTGGAGTC
twist.3H4K20R.qRT	AGCAAGATTCAGACCCTCAAG
MYC3.H4K20F_qRT	TCCACGAACTTTGCCCATAG
MYC3.H4K20R_qRT	GAGAGTCGCGTCCTTGC

Full unedited gel for Figure 2A

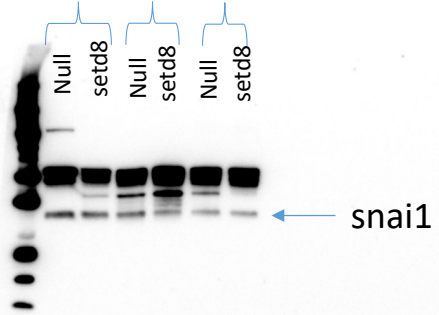


Full unedited gel for Figure 5B

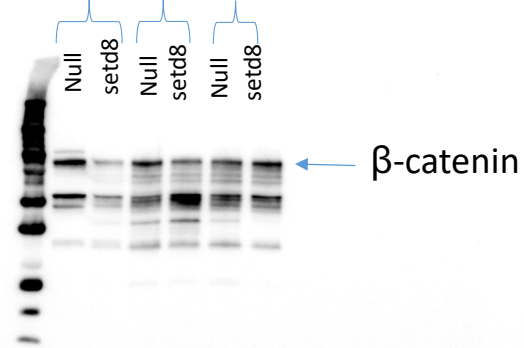


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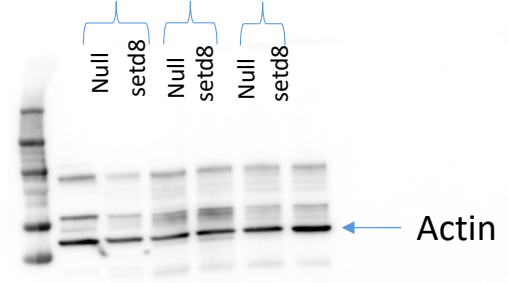
D283 DA0Y ONS76



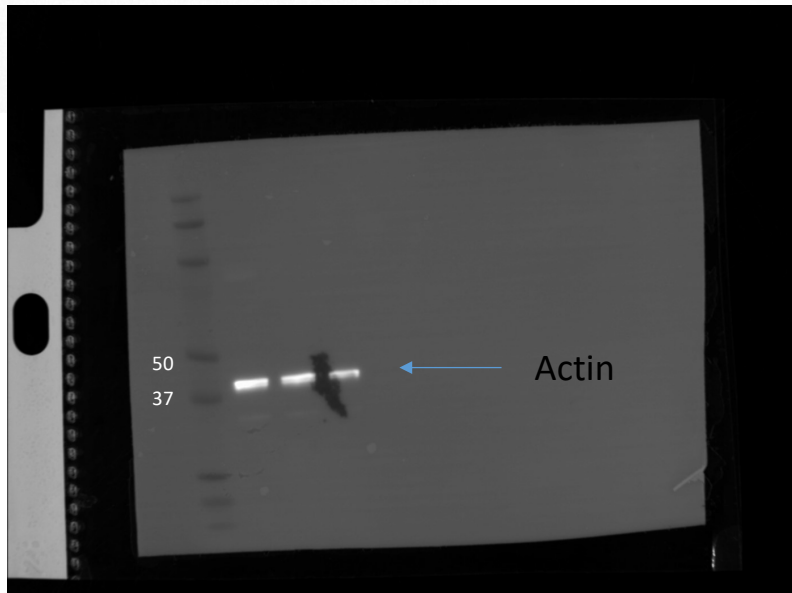
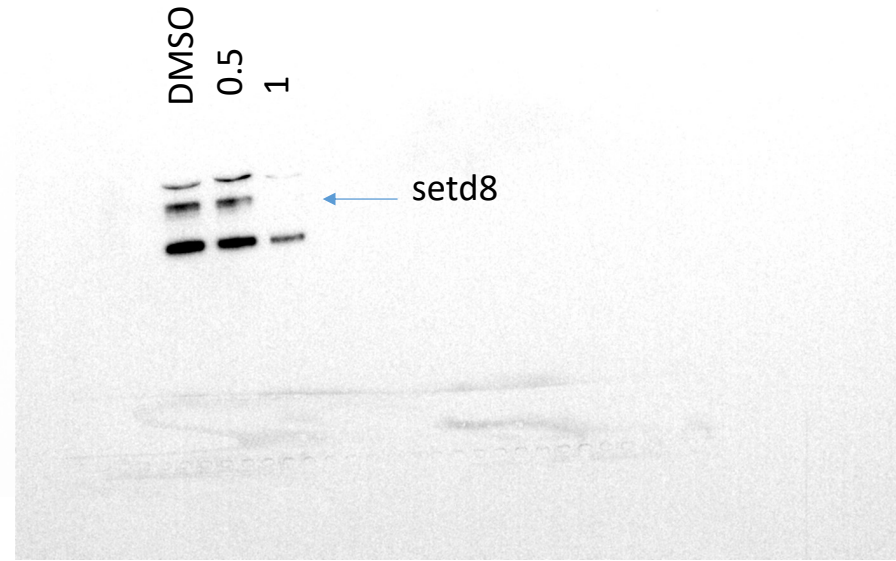
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D283 DA0Y ONS76



Full unedited gel for Figure 8C



Full unedited gel for Supplemental S3 and S5

