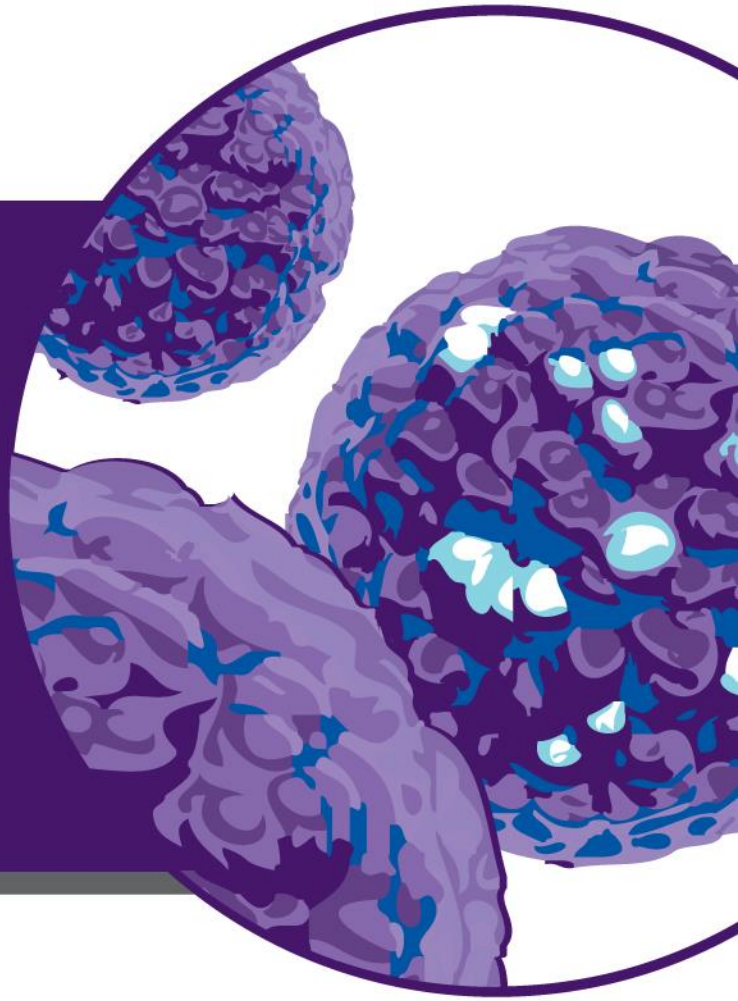
The use of validated chemical probes to understand responder populations to epigenetic drugs



Thomas Paul

Oncology Research Unit, Pfizer Worldwide Research and Development, La Jolla, CA

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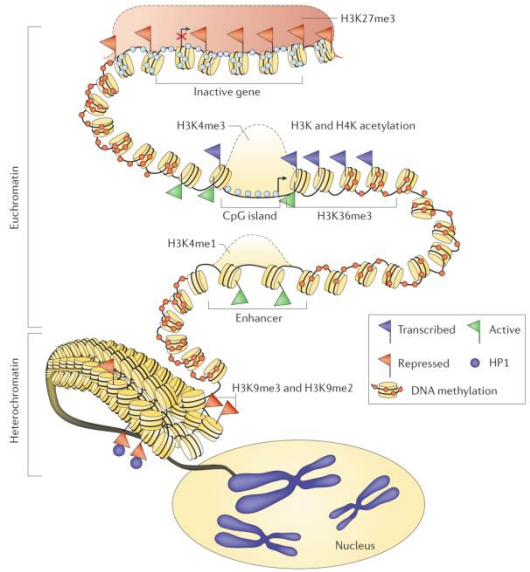
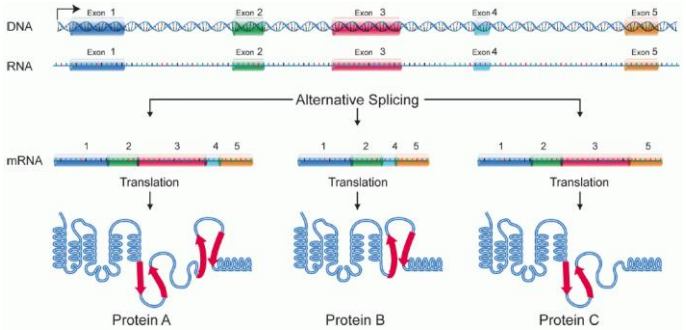
Epigenetic Therapies in Oncology

Goal: Reverse cell fate decisions that maintain cancer cells in proliferative/self-renewing, drug intolerant/resistant, or immunosuppressive state

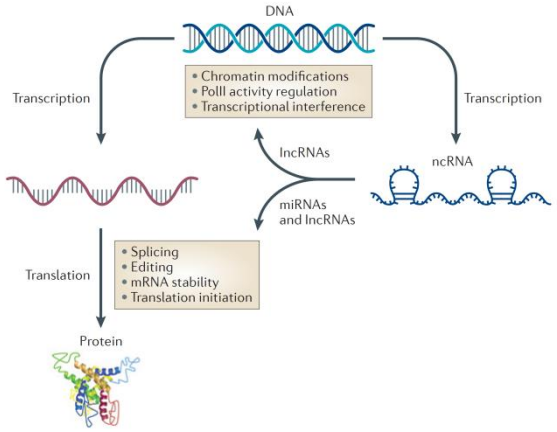
Chromatin states control transcriptional programs and DNA repair



Alternative splicing and mRNA stability alter repertoire of transcripts for translation



Non-coding RNA programs fine tune transcriptional outputs



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Responders to epigenetic therapy

Cell

Amplification

Mutation

Translocation

Overexpression

Aberrant recruitment of complexes

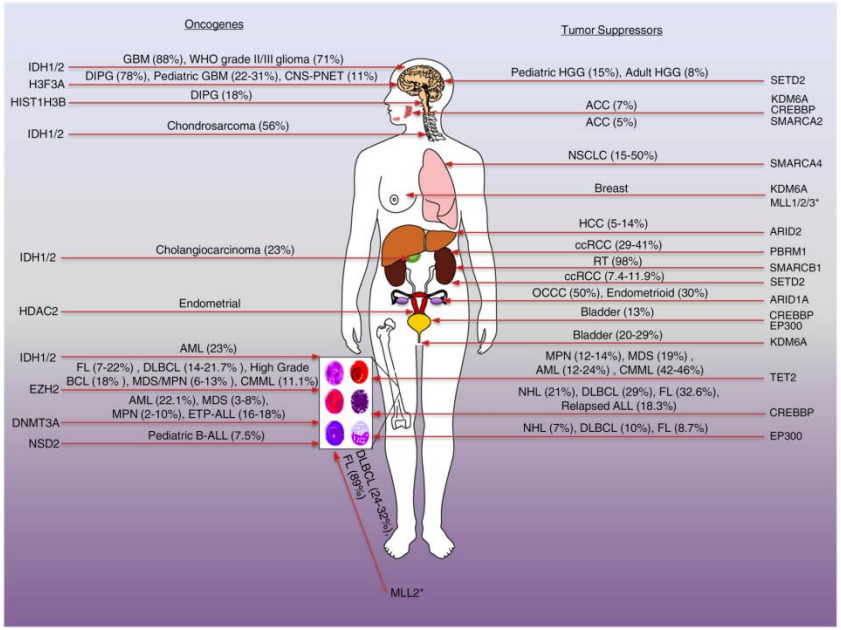
Epigenetic Silencing of tumor suppressor genes

Transcriptional Programs/ Differentiation State

Inhibitory immune checkpoints

Cancer

Mutations in epigenetic regulators



Roy et al., Protein Cell, 2014

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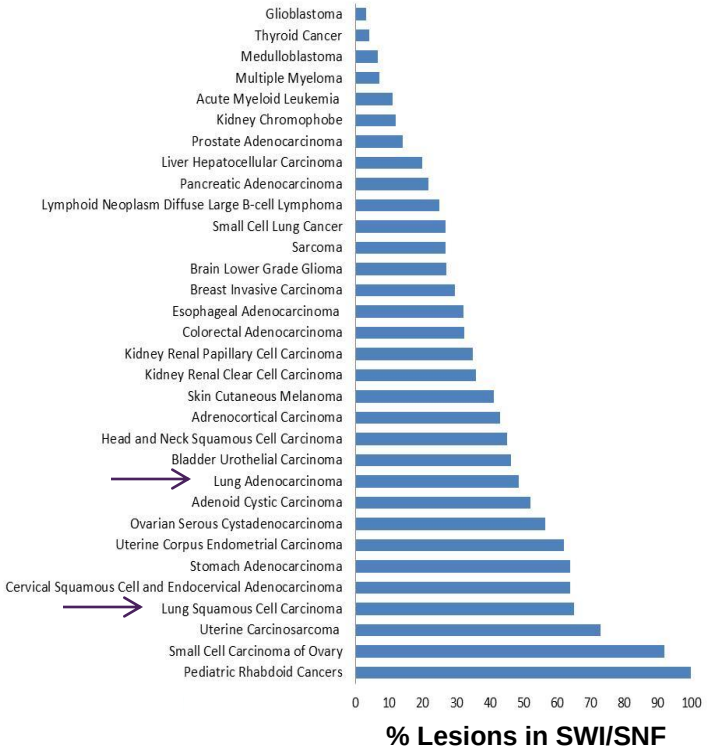
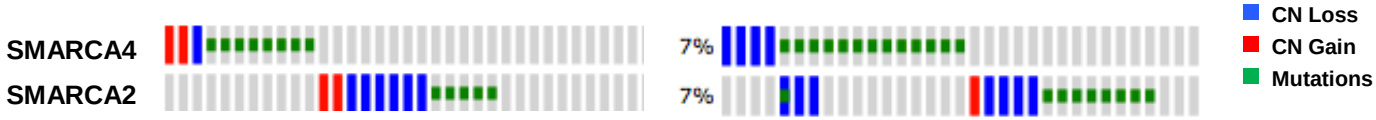
Multi-subunit SWI/SNF Complex is Frequently Mutated in Cancer

SWI/SNF mutations across tumor types (all complex members)



- SWI/SNF chromatin-remodeling complex performs fundamental roles in gene regulation, cell lineage specification, and DNA repair
- Mutated in nearly 20% of all cancers
- Overall frequency approaches that of p53
- Most mutations are inactivating (LOF)
- Mutations are most common in the enzymatic subunit SMARCA4 and the subunits that confer functional specificity (ARID1A, ARID1B, ARID2, PRBM1)

SMARCA2 and SMARCA4 mutations are mutually exclusive in NSCLC (TCGA)



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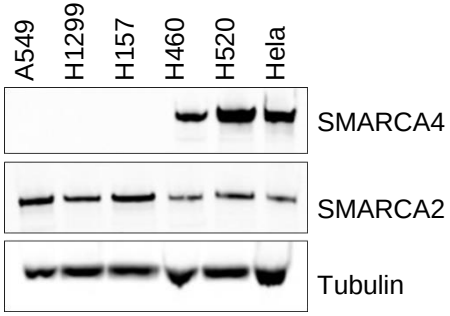
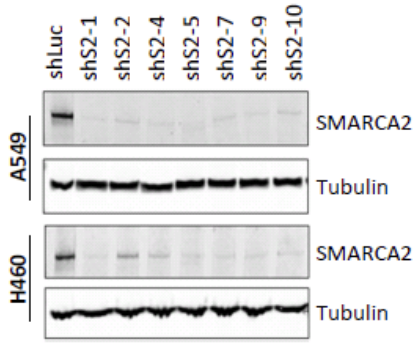
Pfizer

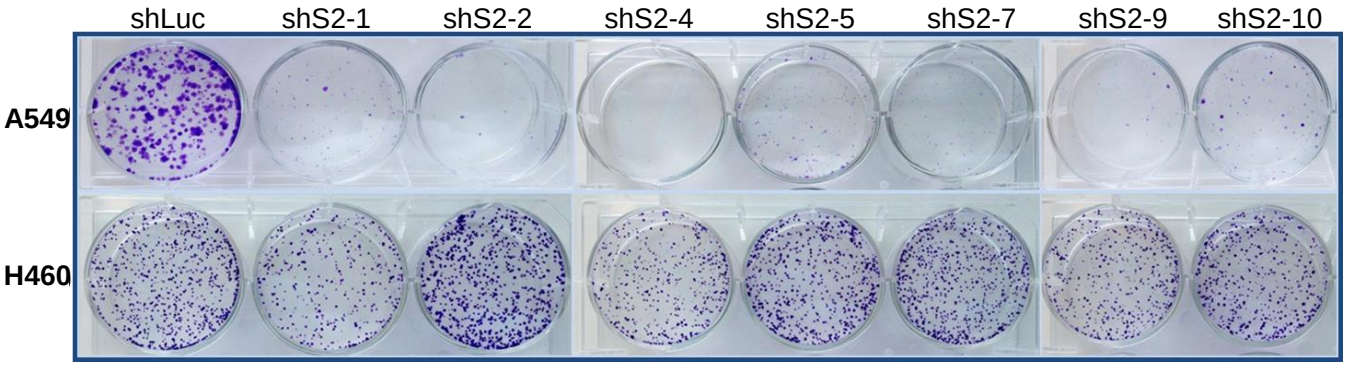
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Vangamudi et al. Cancer Res. 2015

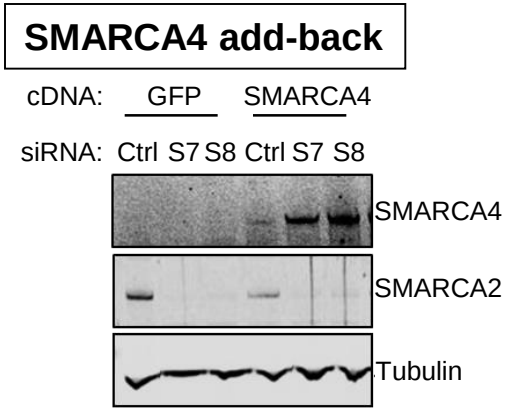
SMARCA4-deficient Lung Cancer Cells Are Selectively Dependent on SMARCA2

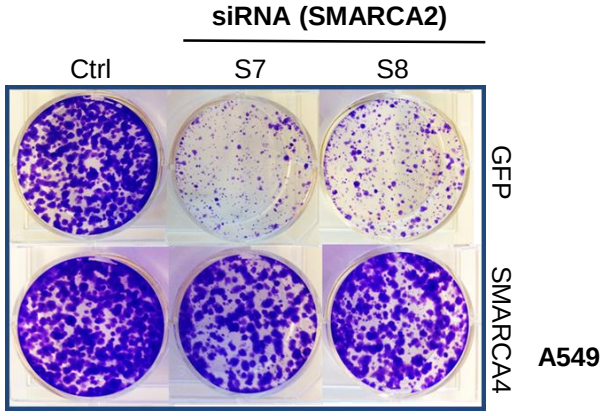






SMARCA4 add-back





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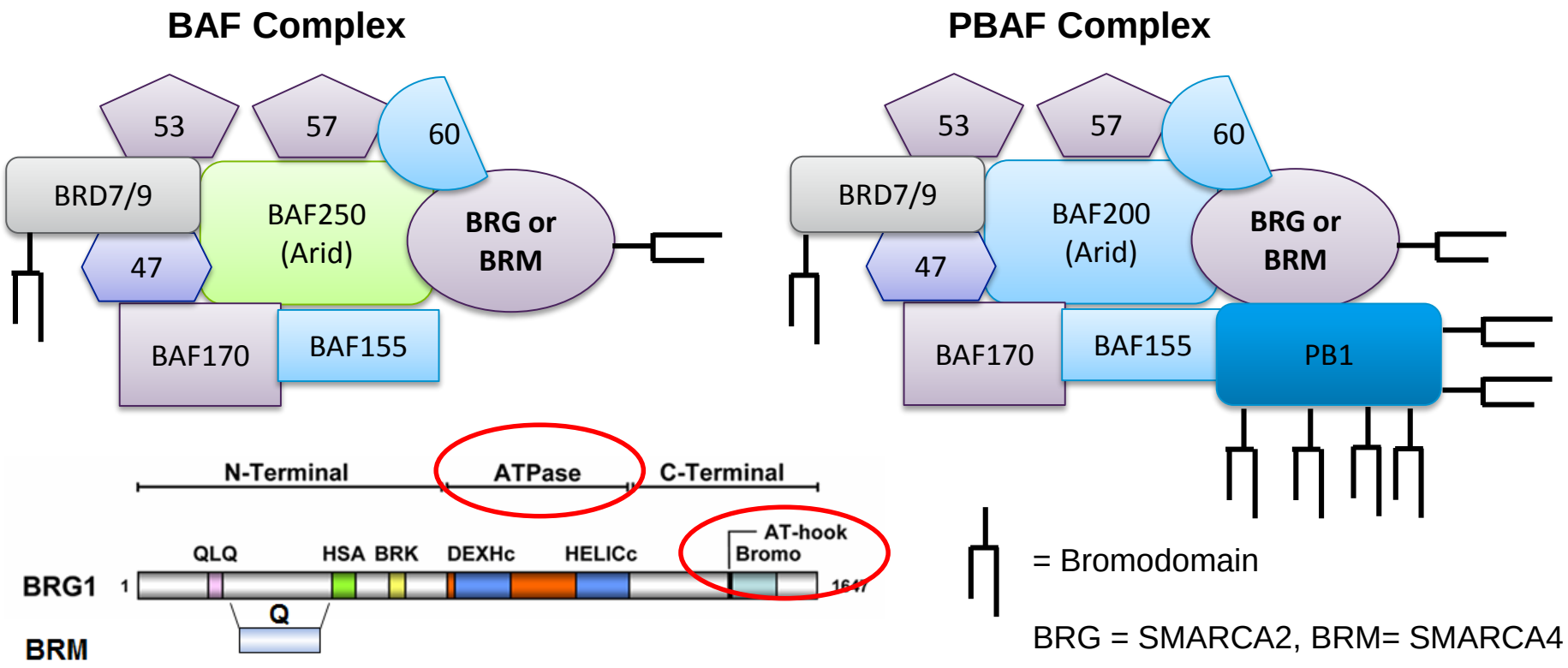
Targeting SWI/SNF Complexes



Hypothesis: SWI/SNF complex can be targeted by bromodomain or ATPase inhibition

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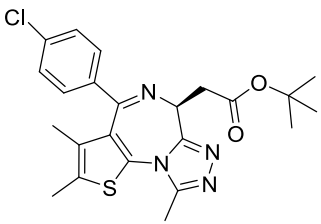
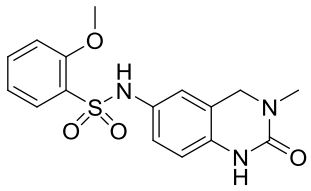
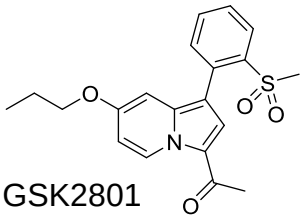
SGC Collaborative Bromodomain Probes so Far



JQ1

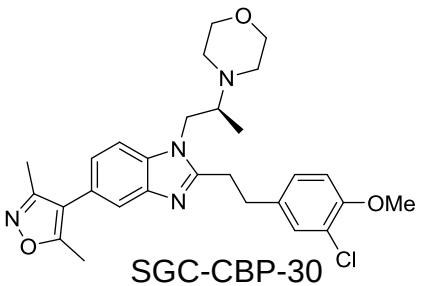


PFI-1

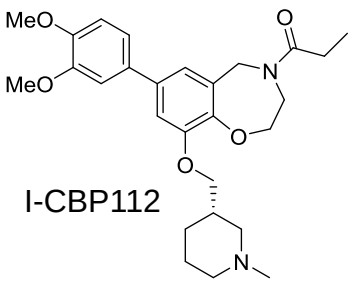


GSK2801

BAZ2-ICR

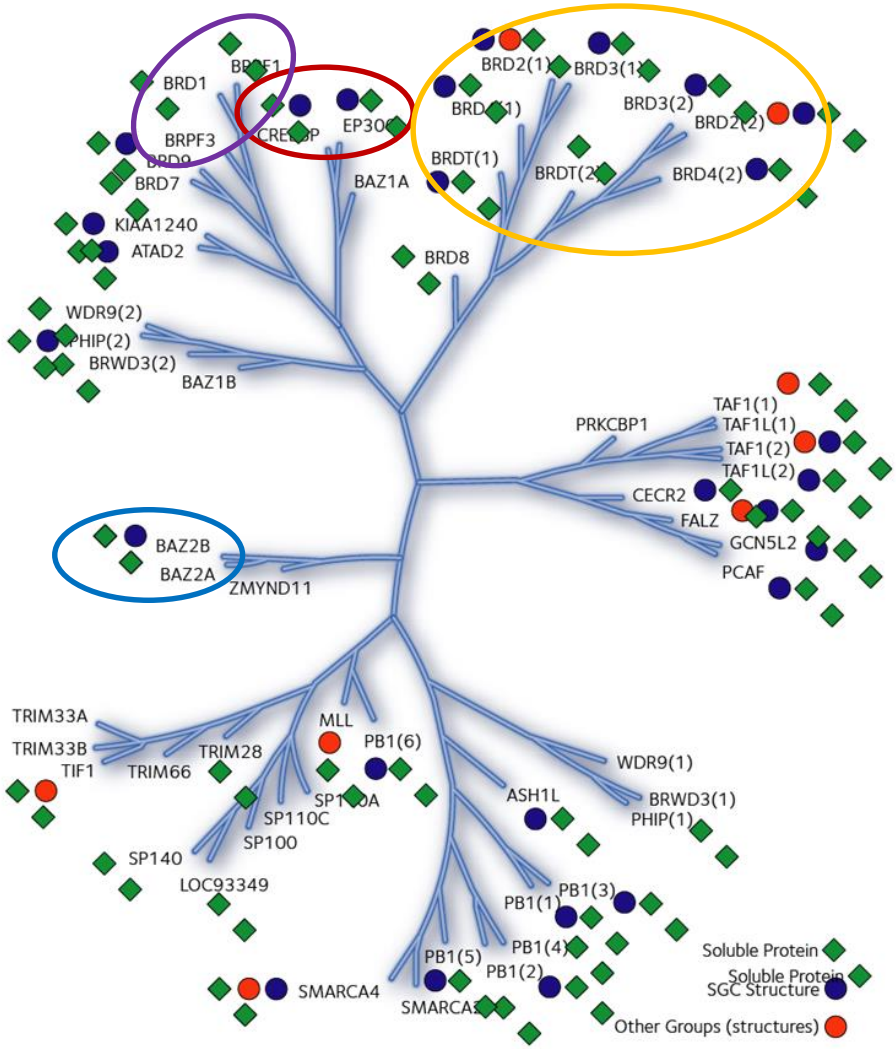


SGC-CBP-30



I-CBP112

OF-1



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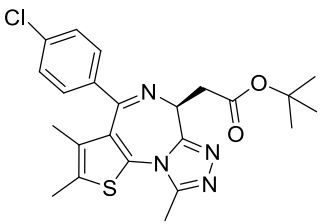
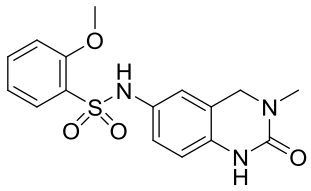
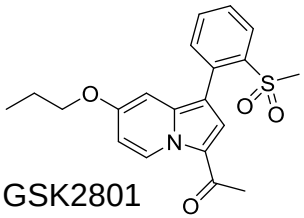
SGC Collaborative Bromodomain Probes so Far



JQ1



PFI-1



GSK2801

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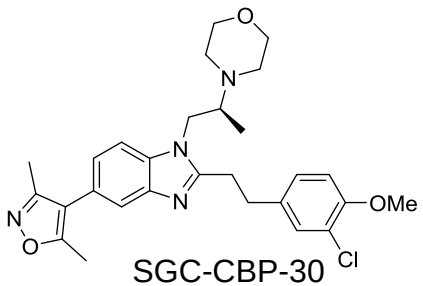
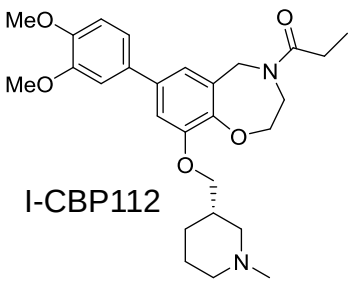
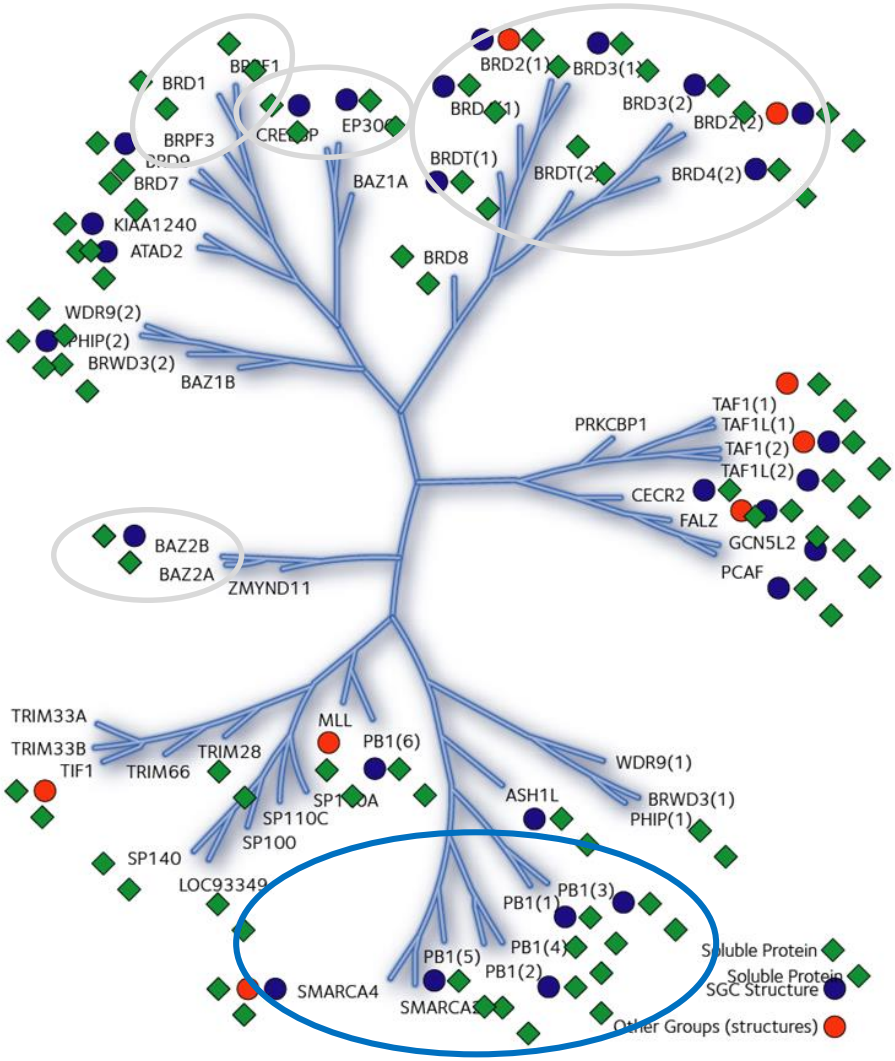


SGC-CBP-30



I-CBP112

OF-1



Family VIII

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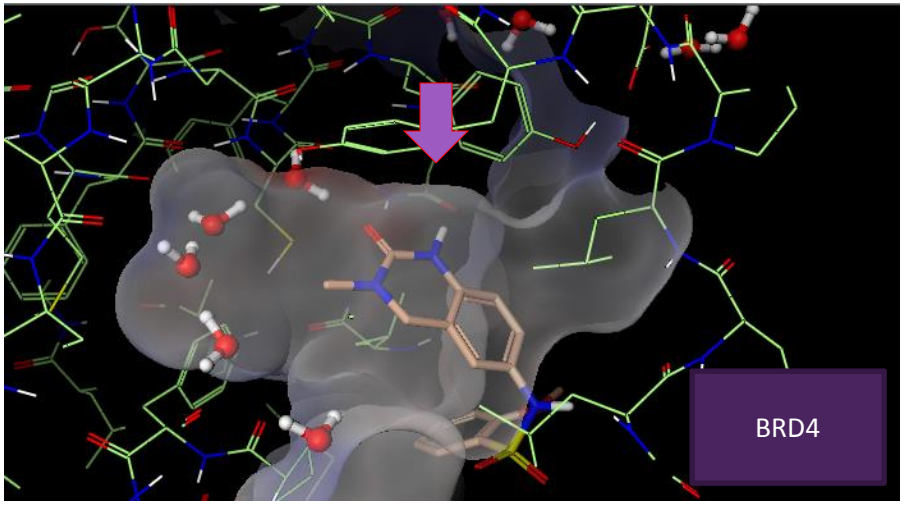
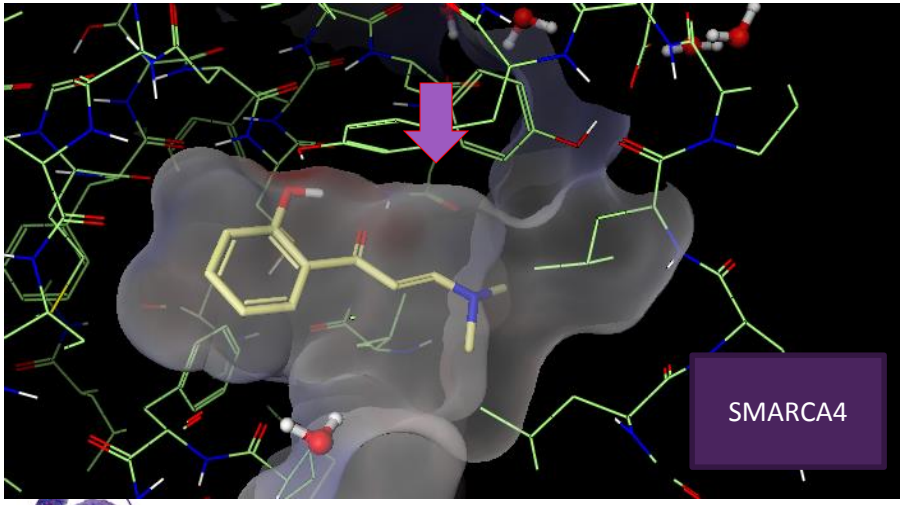
Fragment Screening Identifies A New Bromodomain Binding Mode in SMARCA4





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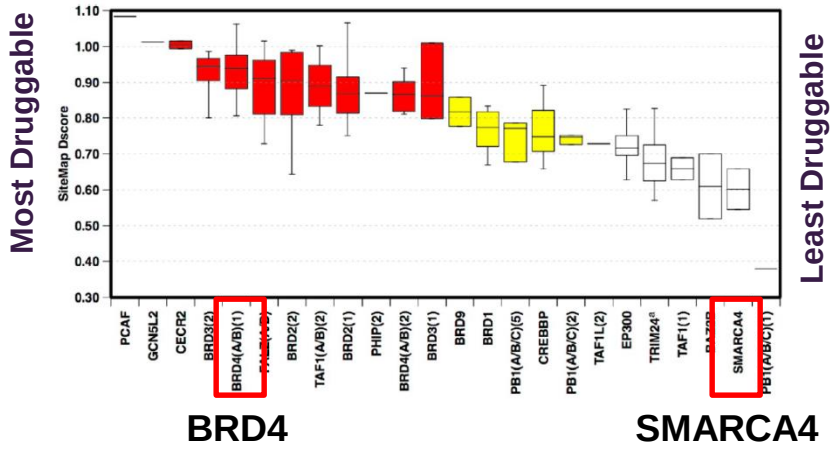
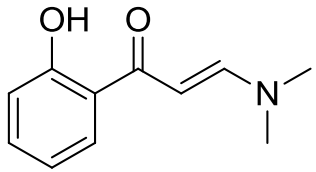
Journal of Medicinal Chemistry
Featured Article
pubs.acs.org/jmc

Druggability Analysis and Structural Classification of Bromodomain Acetyl-lysine Binding Sites

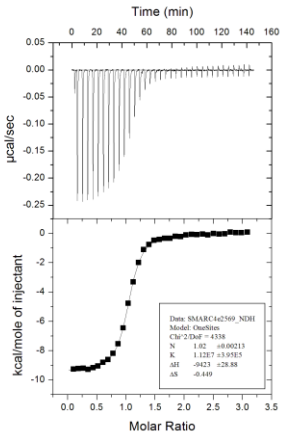
Lewis R. Vidler,† Nathan Brown,† Stefan Knapp,‡ and Swen Hoelder*,†

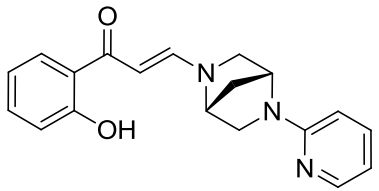




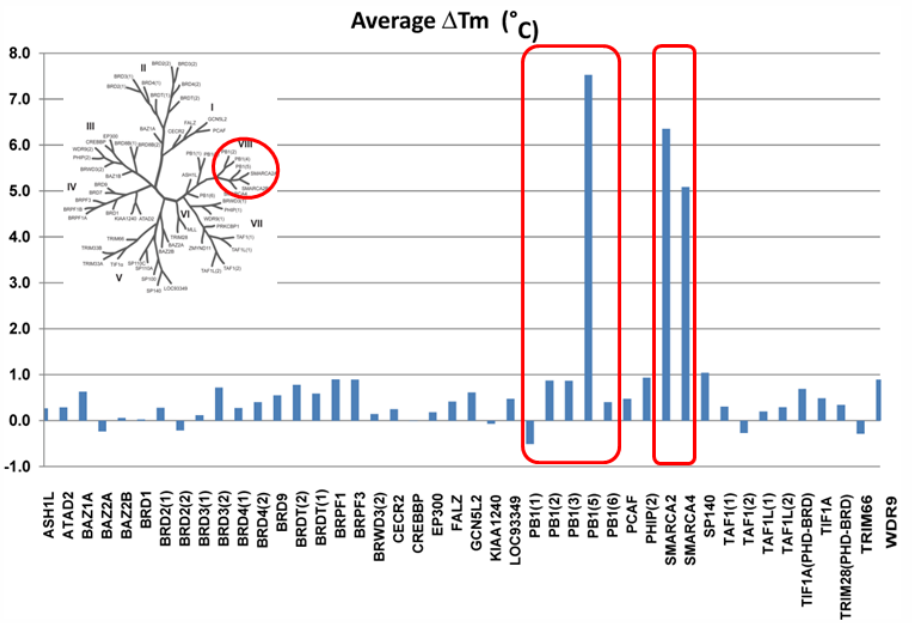
- **Unprecedented bromodomain binding mode**
- **Atypical ligand penetration deep into pocket**
- **Deep waters displaced by salicylic acid fragment**

PFI-3 Potency and Selectivity to Family VIII Bromodomains in SWI/SNF Complexes

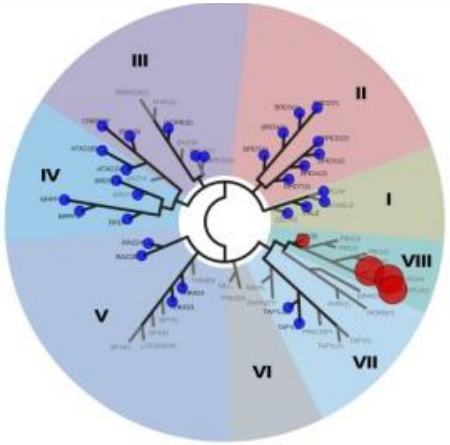




PFI-3
SMARCA4
K_D= 89 nM
PB1 (5) K_D= 48nM



- PFI-3 only shows a significant Tm shift with members of the PB1/SMARCA family
- No interaction with PB1(2)
- Tm confirmed by use of DiscoveRx Bromoscan
- $T_{1/2}$ >264 h in PBS and cell media @ 37 °C
- No cross-reactivity in kinase panel
- Cytotox >50µM



PFI-3 Bromoscan

DiscoveRx

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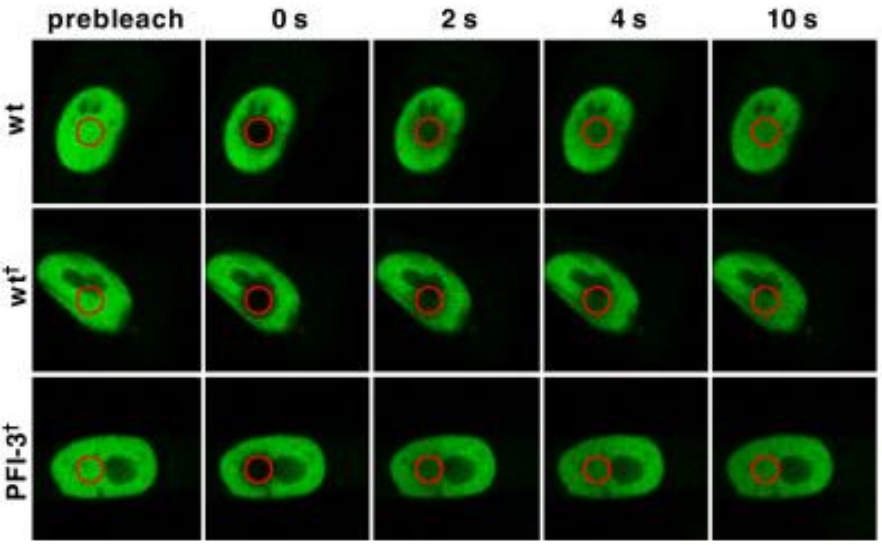
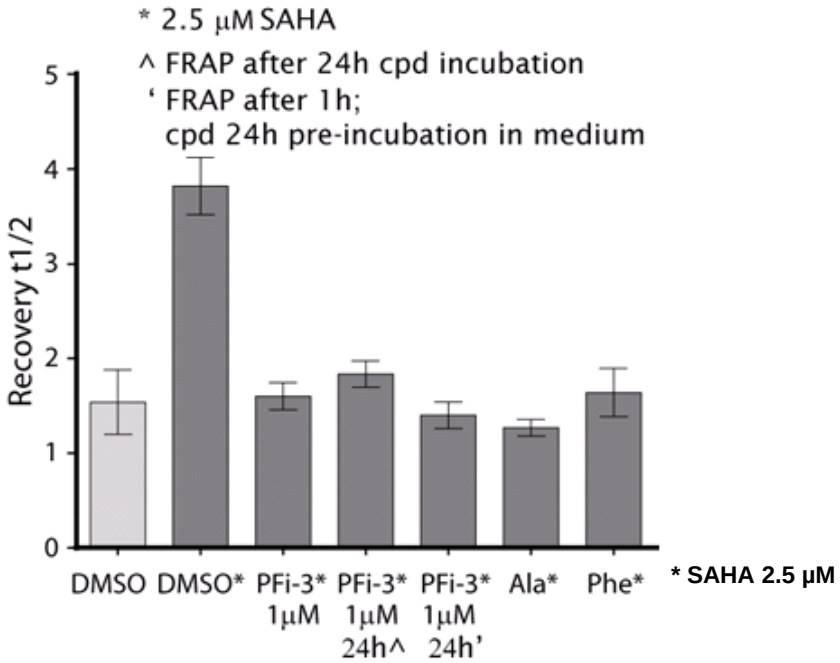
PFI-3 Gets Into Cells: SMARCA2 FRAP in U2OS cells





Transfect Full-length GFP-tagged SMARCA2

↓

Treat PFI-3/DMSO + SAHA

↓

Photobleach Nuceli

↓

Measure Recovery Time

- **Diffusion of unbleached protein back into the bleached region is retarded by protein binding to chromatin**
- **PFI-3 reduced t1/2 recovery of full-length GFP-tagged SMARCA2 from chromatin (less chromatin interaction)**
- **PFI-3 is stable in cells for at least 24h**

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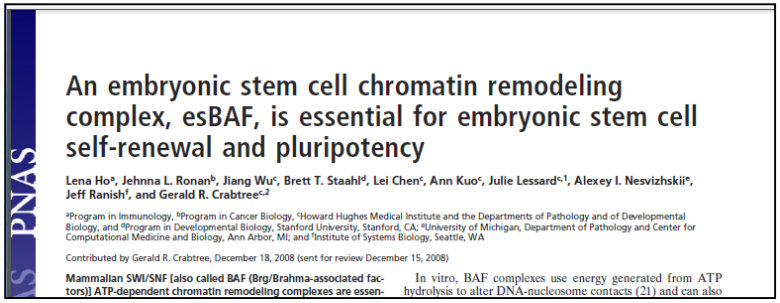
Philpott et al.; Epigenetics Chromatin. 2014

PFI-3 Promotes Differentiation in Stem Cell Models



PNAS

An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency

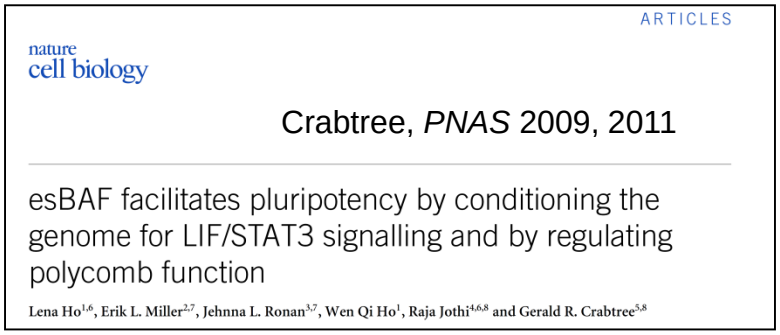
Lena Ho[a], Jehnna L. Ronan[b], Jiang Wu[c], Brett T. Staahl[d], Lei Chen[c], Ann Kuo[c], Julie Lessard[c,1], Alexey I. Nesvizhskii[e], Jeff Ranish[f], and Gerald R. Crabtree[d,2]

[a]Program in Immunology, [b]Program in Cancer Biology, [c]Howard Hughes Medical Institute and the Departments of Pathology and of Developmental Biology, and [d]Program in Developmental Biology, Stanford University, Stanford, CA; [e]University of Michigan, Department of Pathology and Center for Computational Medicine and Biology, Ann Arbor, MI; and [f]Institute of Systems Biology, Seattle, WA

Contributed by Gerald R. Crabtree, December 18, 2008 (sent for review December 15, 2008)

Mammalian SWI/SNF [also called BAF (Brg/Brahma-associated factors)] ATP-dependent chromatin remodeling complexes are essen-

In vitro, BAF complexes use energy generated from ATP hydrolysis to alter DNA-nucleosome contacts (21) and can also



ARTICLES

nature cell biology

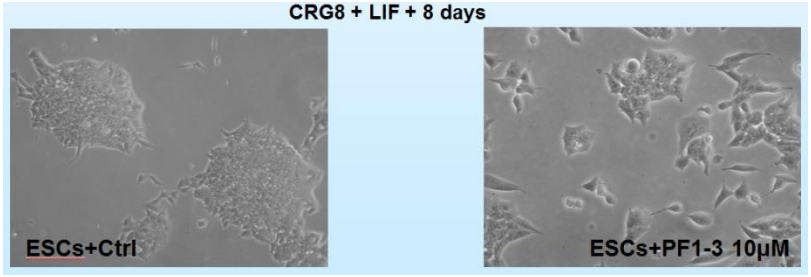
Crabtree, *PNAS* 2009, 2011

esBAF facilitates pluripotency by conditioning the genome for LIF/STAT3 signalling and by regulating polycomb function

Lena Ho[1,6], Erik L. Miller[2,7], Jehnna L. Ronan[3,7], Wen Qi Ho[1], Raja Jothi[4,6,8] and Gerald R. Crabtree[5,8]

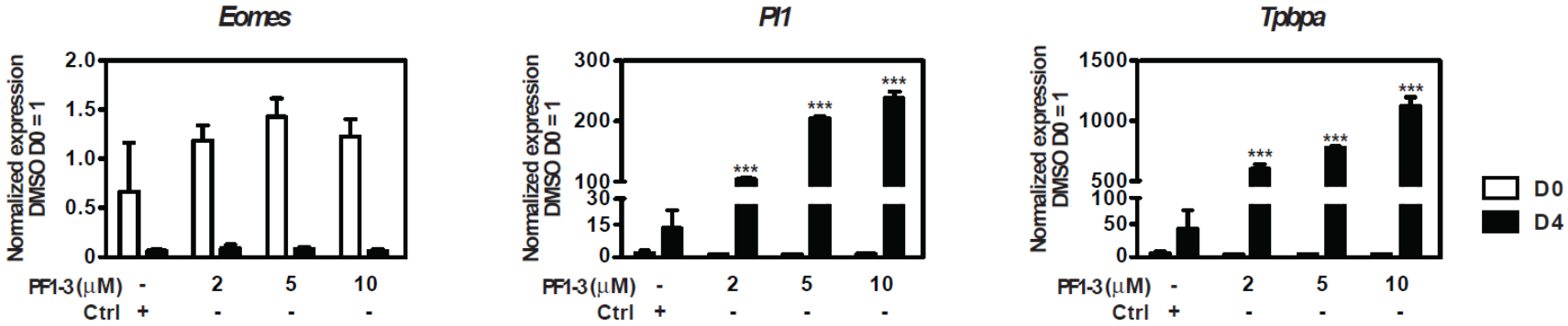
Embryonic Stem Cells

ES cells differentiate in presence PFI-3 despite presence of LIF which maintains stemness



PFI-3 down-regulates pluripotency markers Pou5f1 and Nanog

PFI-3 promotes differentiation of trophoblast stem cells and expression of differentiation-associated genes



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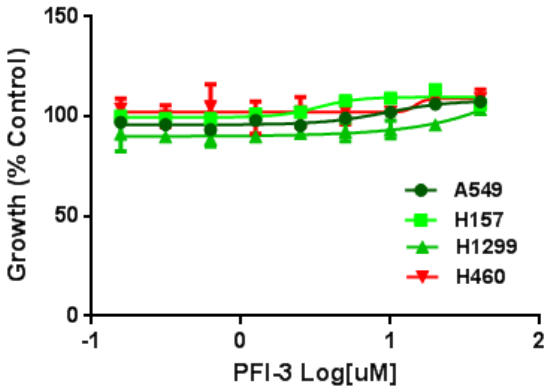
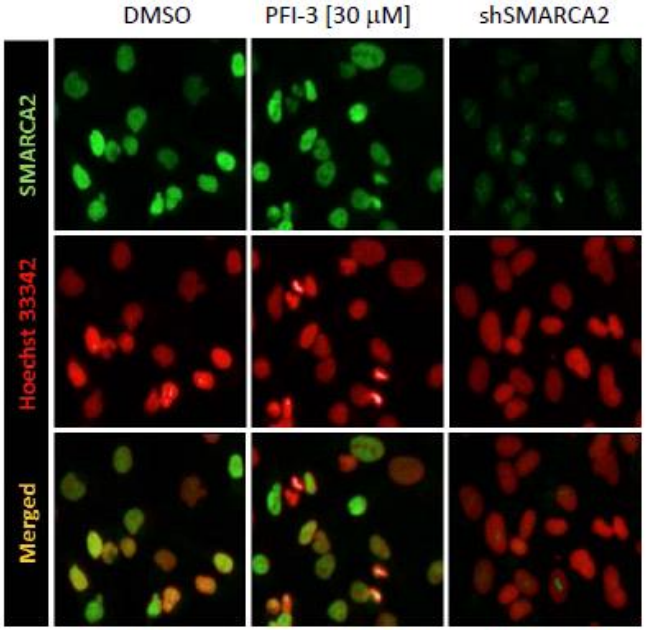
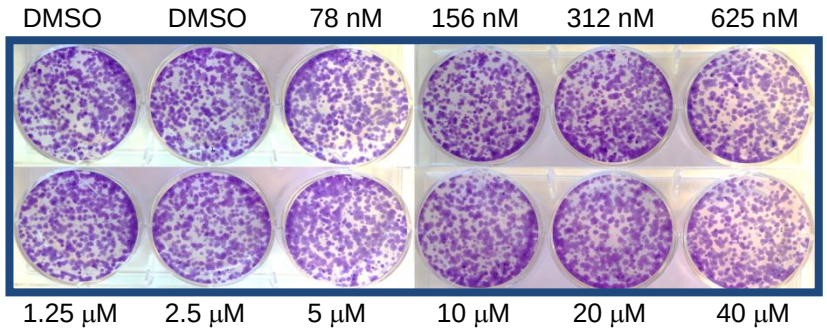
Fedorov et al.; Sci Adv. 2015

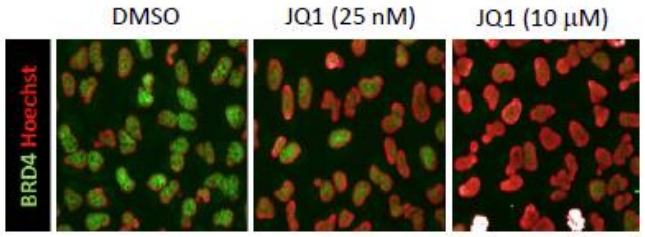
Pharmacological Inhibition of SMARCA2 Bromodomain Does Not Inhibit Growth of SMARCA4-deficient Lung Cancer Cells

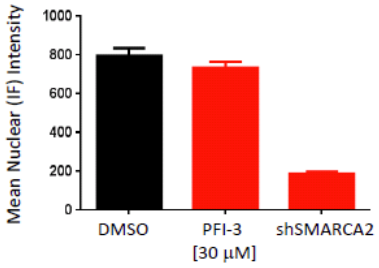
PFI-3 does not displace endogeneous SMARCA2 from chromatin

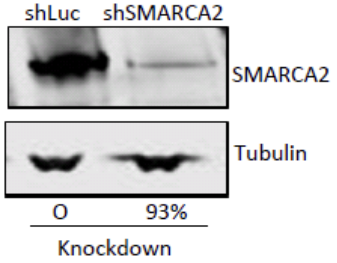












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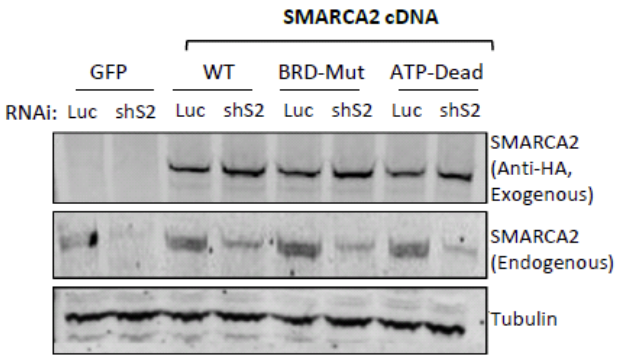
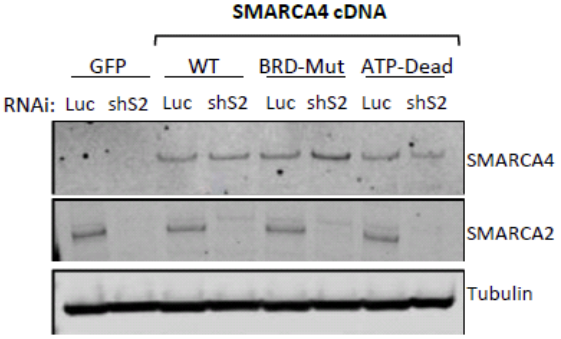
SMARCA2 ATPase, But Not the Bromodomain, Activity Is Essential for Its Tumorigenic Potential

Rescue with SMARCA2

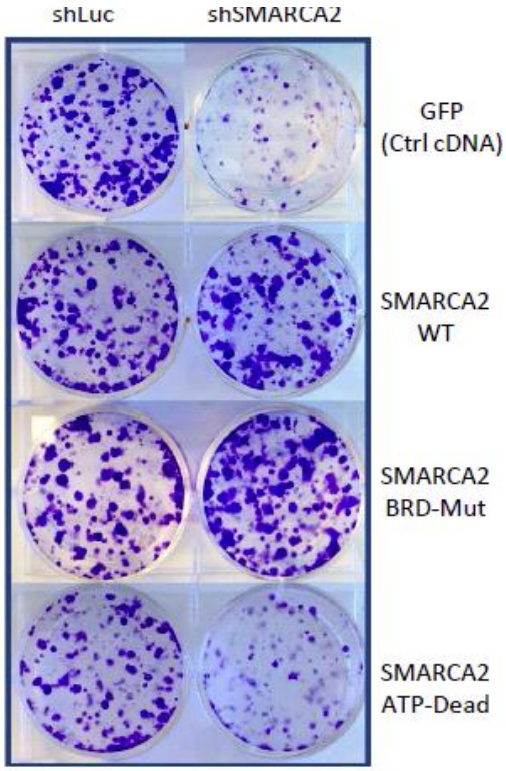


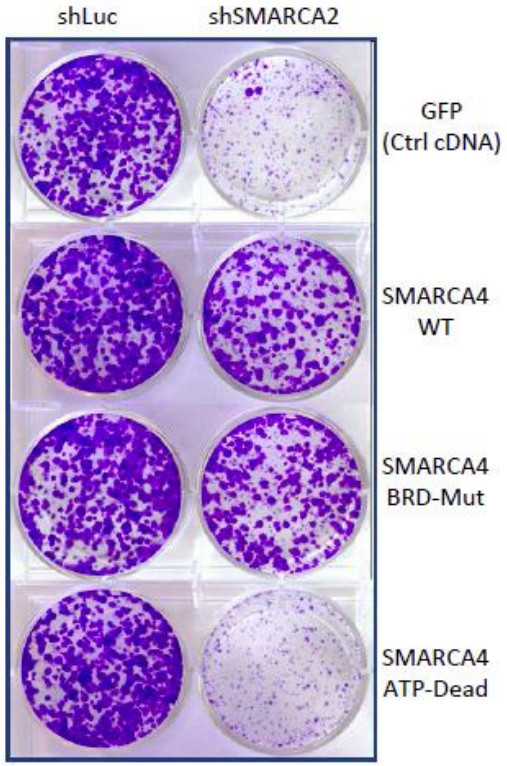
Rescue with SMARCA4



- **SMARCA 2 or 4 bromodomain wild-type or mutant rescues NSCLC cell growth in SMARCA4 mutant cells (A549, H1299)**

- **ATPase-dead mutant SMARCA2 or 4 fails to rescue shRNA phenotype**





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Summary

- PFI-3 is a viable chemical probe and a first in class chemotype for Family VIII bromodomains
- Not a drug-like structure, but fit for purpose (cell biology)
- Cell activity is confirmed by displacement of ectopically expressed GFP-tagged SMARCA2 or 4 from chromatin
- PFI-3 treatment fails to recapitulate shRNA phenotypes in multiple cancer models dependent on SWI/SNF complexes (SMARCA4 mut. NSCLC, synovial sarcoma, AML, malignant rhabdoid tumors)
  - Lack of activity can be explained by failure of bromodomain inhibition to displace endogenous SWI/SNF complexes from chromatin
  - Activity in certain developmental contexts? esBAF?

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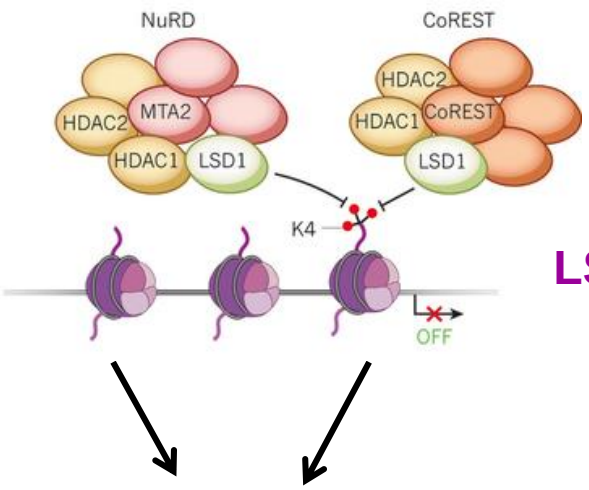
LSD1 (Lysine Specific Demethylase), KDM1A



Histone H3 Lysine 4 Demethylase

Gene Repression

LSD1 - Enzyme Function

- Histone Lysine Demethylase targeting Histone H3 Lysine 4 mono and dimethylation
  - Leads to gene repression

LSD1 - Role in Cancer

- LSD1 is over-expressed or mis-expressed in tumors leading to aberrant activation or repression of genes involved in oncogenic programming.
- LSD1 regulates cellular differentiation/self-renewal pathways critical for tumor maintenance
- LSD1 inhibitors shown to be active in Breast Cancer, Colon Cancer, Prostate cancer, Lung cancer, Neuroblastoma, Leukemias (APL, AML)

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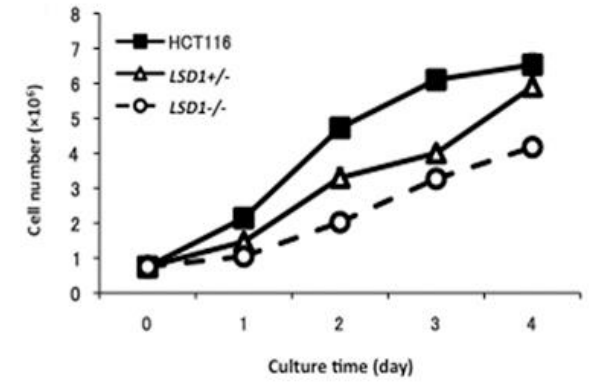
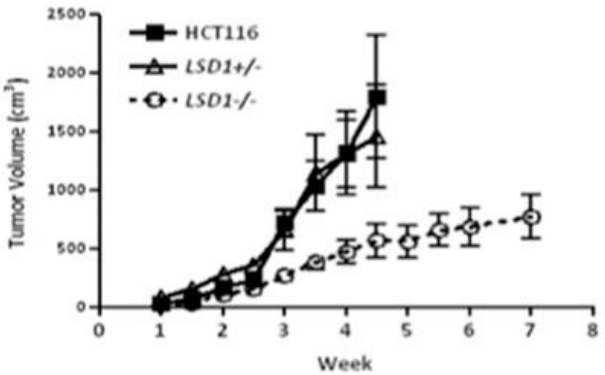
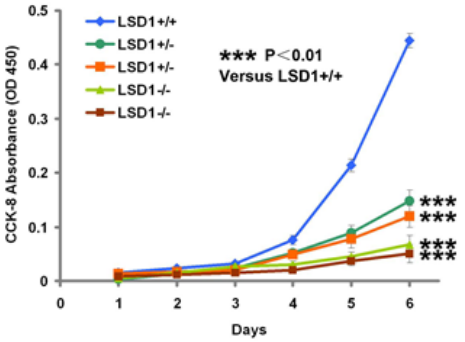
Early literature suggests LSD1 inhibition maybe effective in many solid tumor models

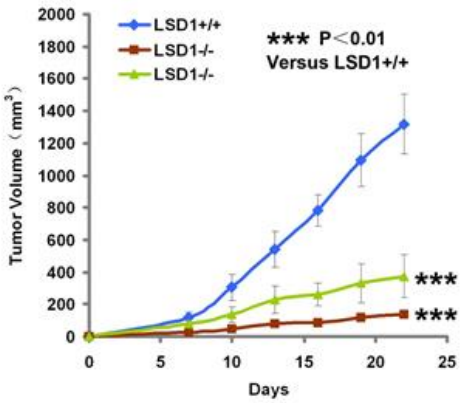
HCT-116 LSD1 -/- isogenic cell models show reduced tumor growth in vitro and in vivo





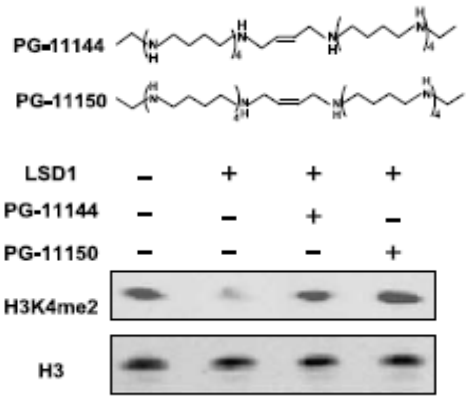
Casero, R. et al
Biochem. J. 2013. 449, 459-468

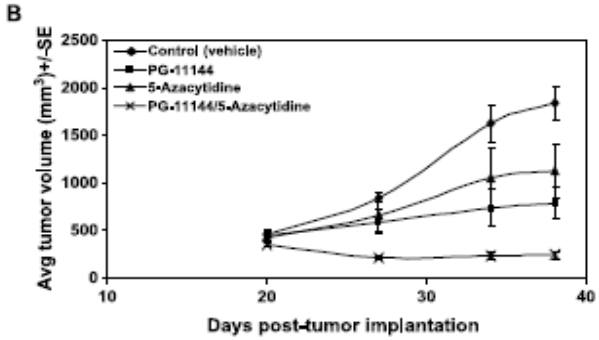




Huang, et al PlosOne 2013

LSD1 inhibitors show anti-tumor activity in HCT-116 xenograft models





Huang Y, Casero et al., Clin Cancer Res., 2009
Lin J. et al. Biochem J. 2012

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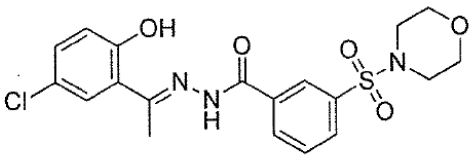
New Generation of Selective LSD1 Tool Compounds

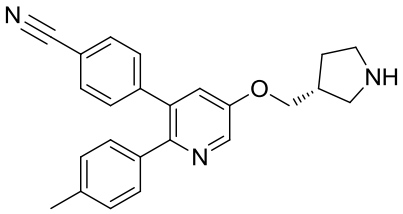
University of Utah



SP2509
LSD1/CoREST Ki = 13 nM
Cell Growth IC50

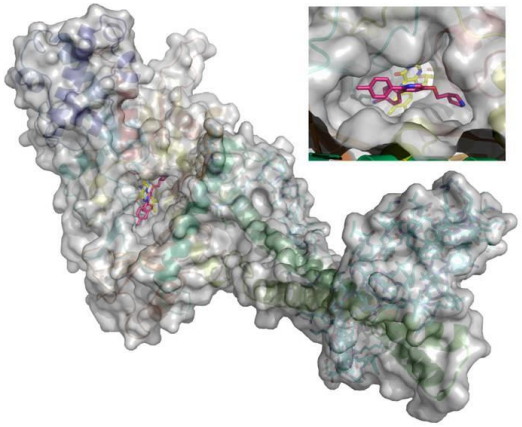
Cell-line	Cell Growth, IC50 (μM)
AN3 Ca	0.356
BT-20	0.489
BT-549	1.010
HCT 116	0.614
HER218	0.612
Hs-578-T	1.700
HT29	0.429
MCF-7	0.637
MDA-MB-231	1.040
MDA-MB-235	0.728
MDA-MB-435	1.440
MDA-MB-468	2.730
MIA PaCa-2	0.468
PANC-1	1.104
PC-3	2.160
SK-N-MC	0.329
T-47D	0.649
U87	1.160

GSK



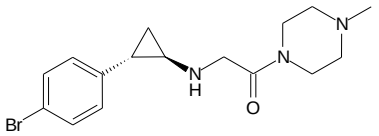
GSK690
LSD1/CoREST Ki = 4 nM
Cell/Gene Exp IC50 = 308 nM

Selective vs. LSD2, MAO-A, MAO-B
Competitive w/ H3K4Me2 peptide



Binding GSK690 (purple) at the opening of the FAD pocket

Oryzon



OG-86
LSD1 IC50 (uM) - HRP Assay = 0.047
IC50 (uM) - TR-FRET Assay = 0.004

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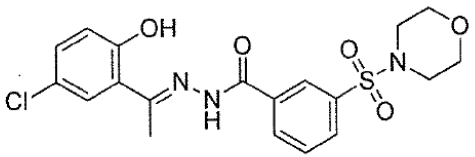
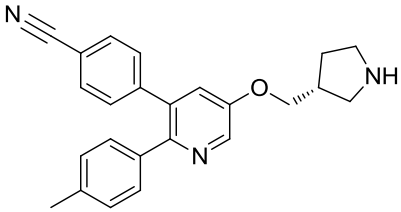
Off-target activities of LSD1 inhibitors in LSD1-/- isogenic cell lines

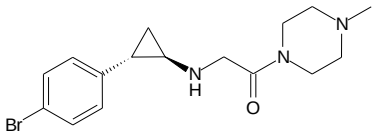
University of Utah SP2509

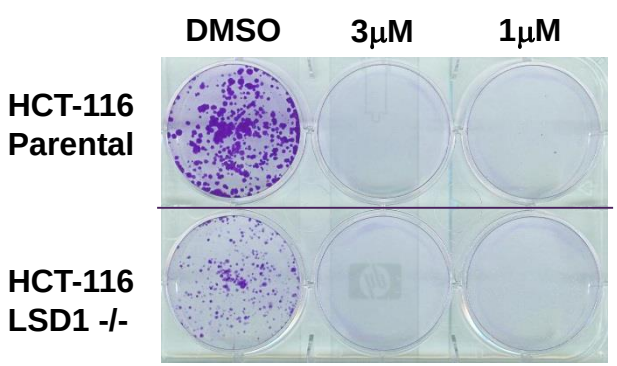


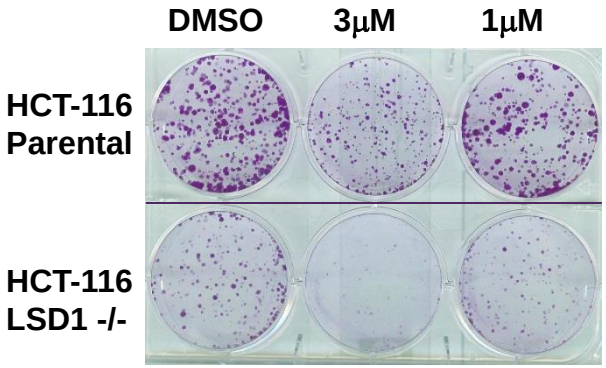
GSK690

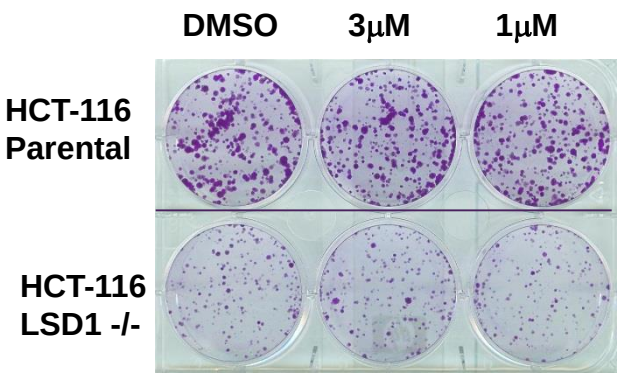


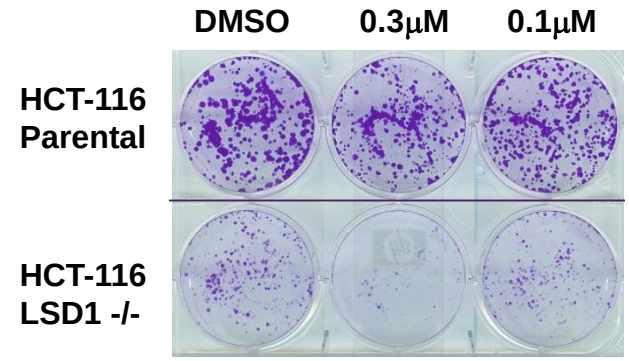
Oryzon OG-86

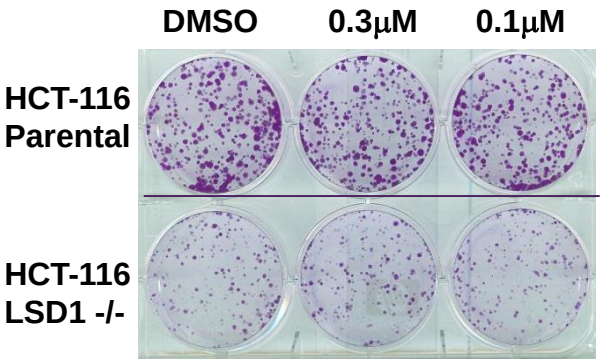


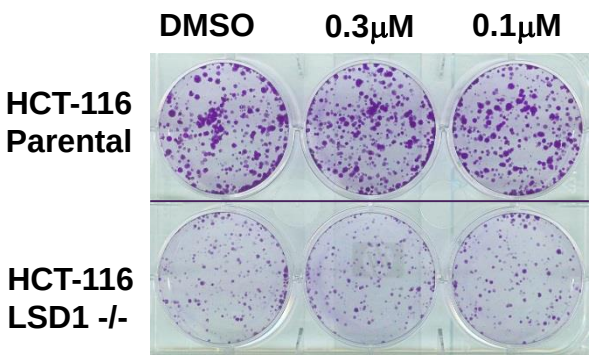






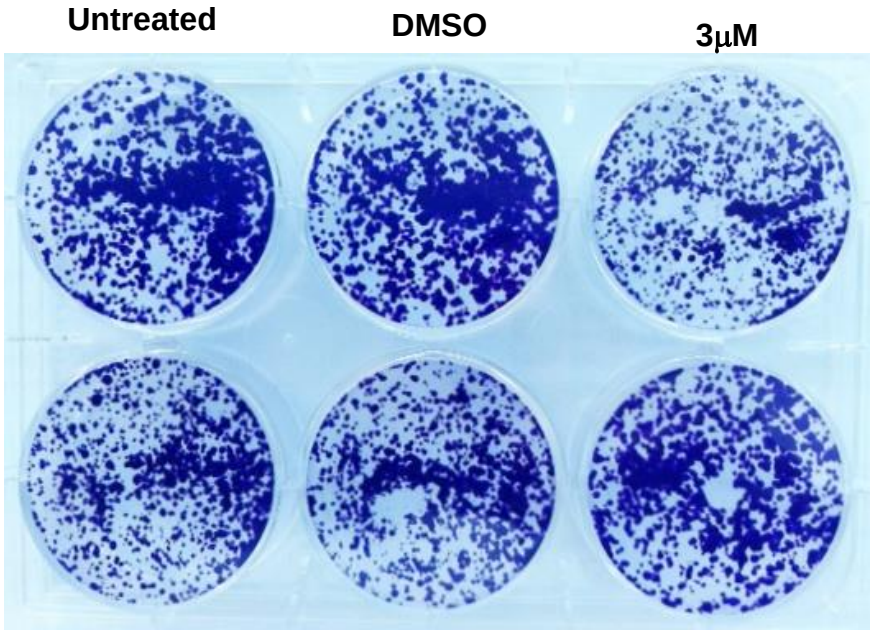




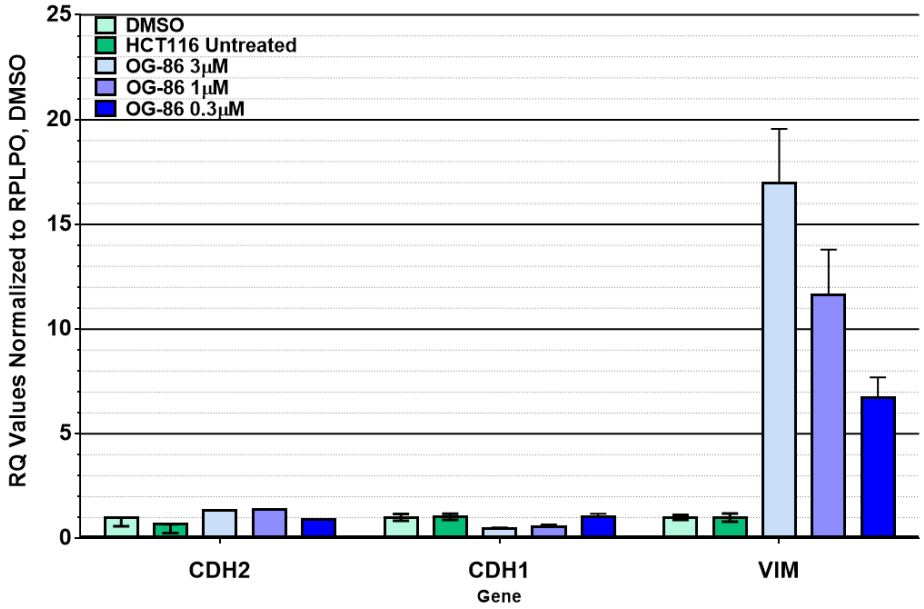


LSD1 inhibitors do not alter cell growth however induce mesenchymal markers in HCT-116 cells

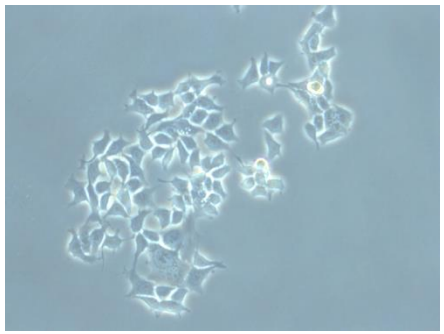
HCT-116 Parental Cells are not sensitive to LSD1 inhibition (OG-86)



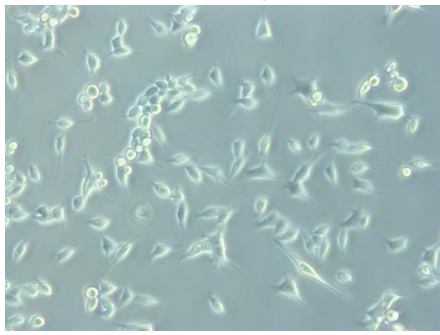
LSD1 inhibitors induce mesenchymal markers and morphological changes in HCT-116 cells



DMSO



OG-86 1μM



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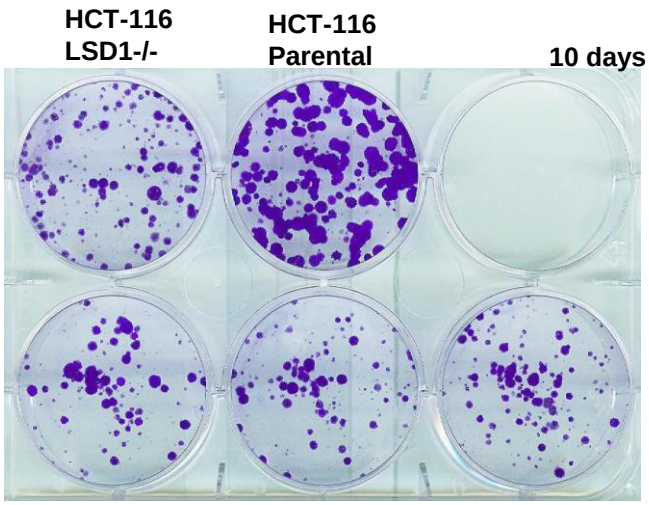
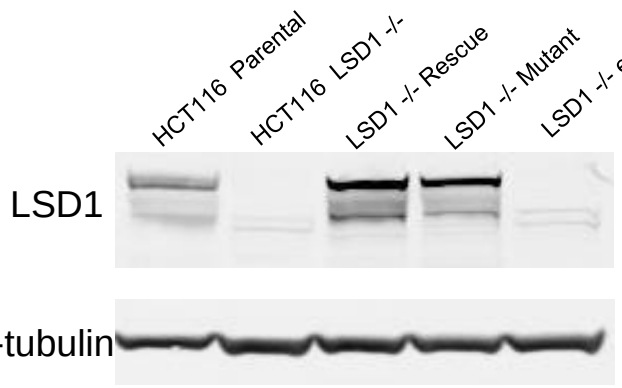
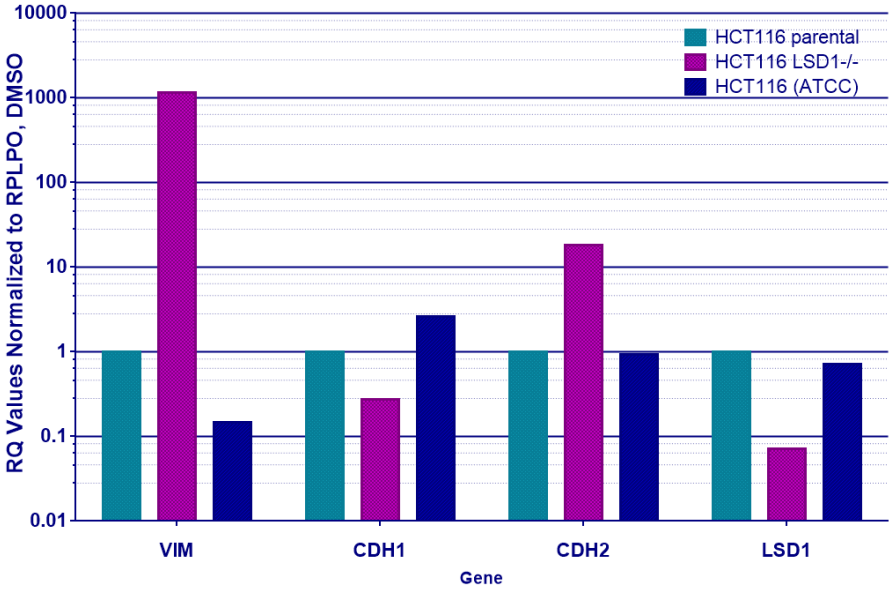
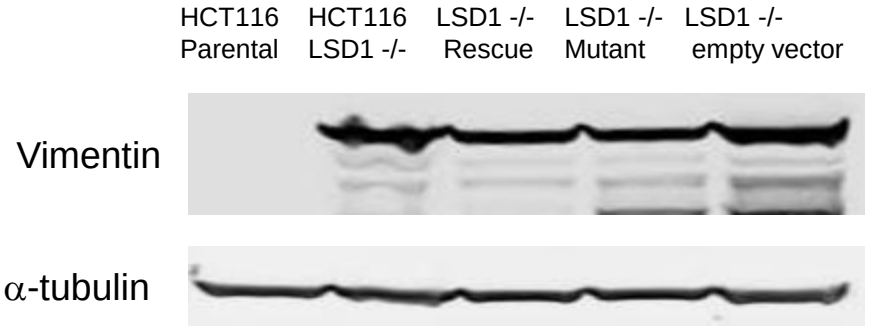
Isogenic LSD1 -/- display mesenchymal differentiation and can not be rescued by LSD1 re-expression





LSD1-/- isogenic cells show high EMT gene markers





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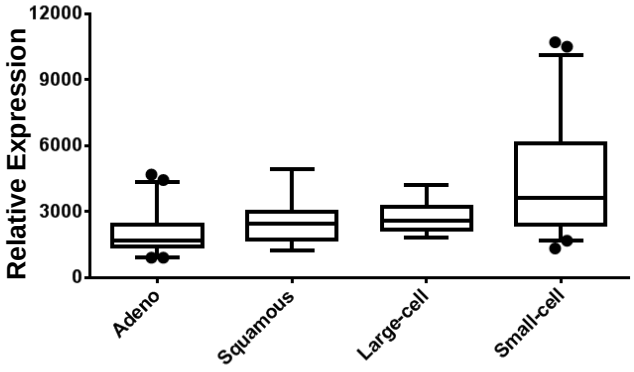
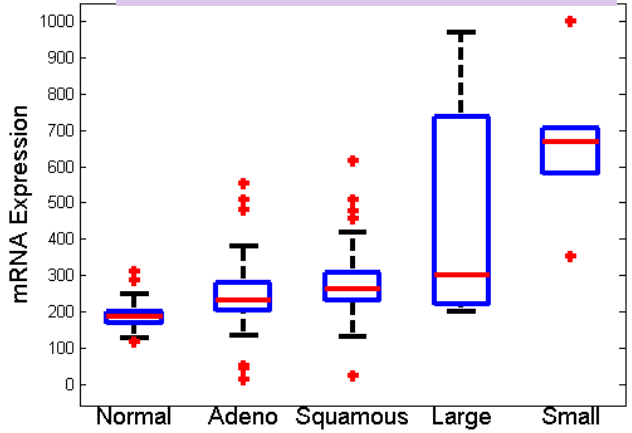
LSD1 is over-expressed in SCLC

Lung Cancer Cell Lines



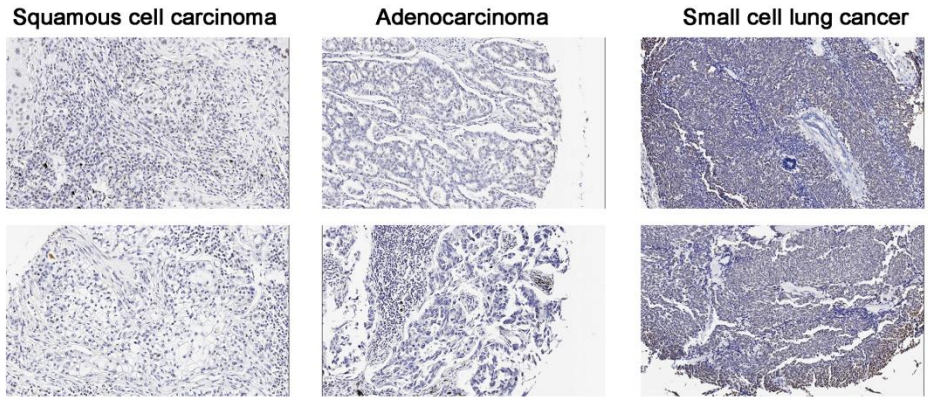
Patient tumor expression



LSD1 in Small Cell Lung Cancer

- LSD1 is over-expressed in SCLC
- LSD1 and corepressor of REST (CoREST) form complex shown to regulate neurological differentiation
  - SCLC is less-differentiated neuroendocrine cell (neural crest) origin
  - LSD1 inhibitors entered clinic for SCLC in 2014

LSD1 protein is over-expressed by IHC



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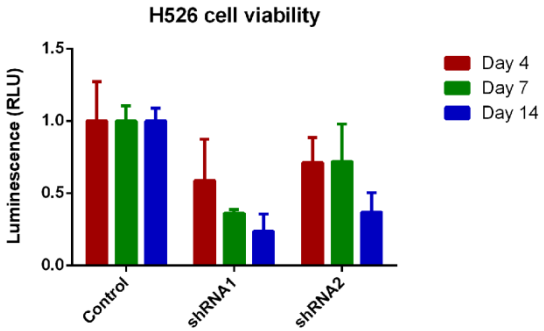
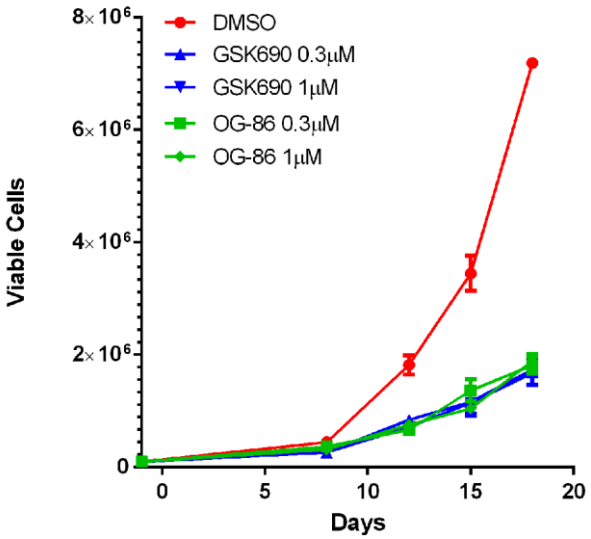
SCLC cell lines are sensitive to LSD1 inhibition

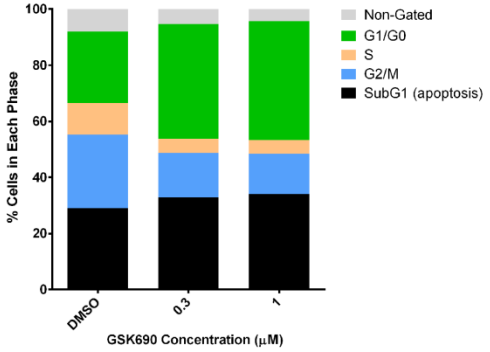
SCLC cell lines are sensitive to LSD1 shRNA inhibition



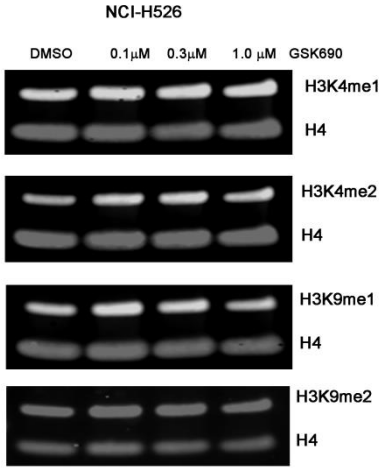
LSD1 inhibitors show delayed activity in SCLC cell lines consistent with epigenetic mechanism



LSD1 inhibitors show cytostatic effects with G1 arrest at day 14



No changes observed in global histone modification levels in H3K4me1/2 or H3K9me1/2



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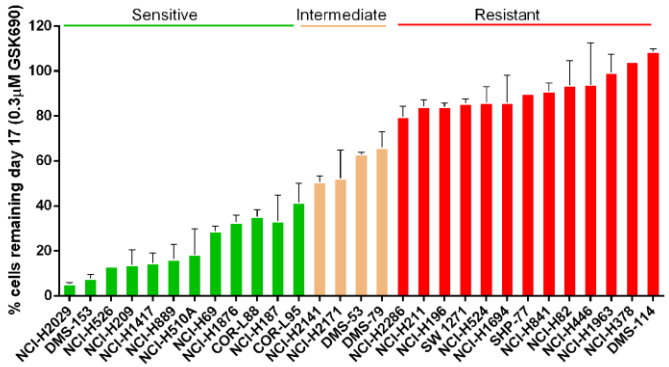
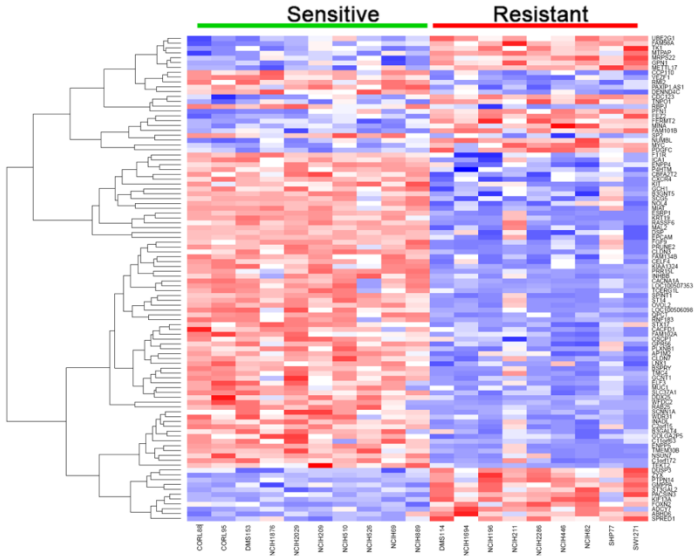
Differential sensitivity to LSD1 inhibitors in predicted by a neuroendocrine/mesenchymal gene expression signature

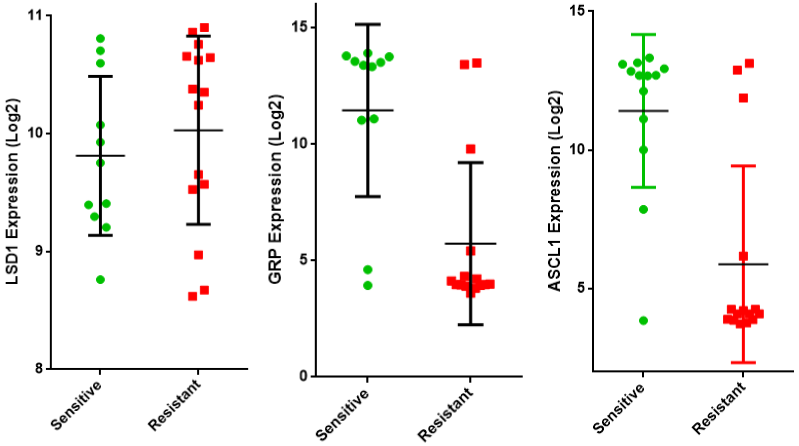
LSD1 inhibitors are active over a broad panel of SCLC cell models

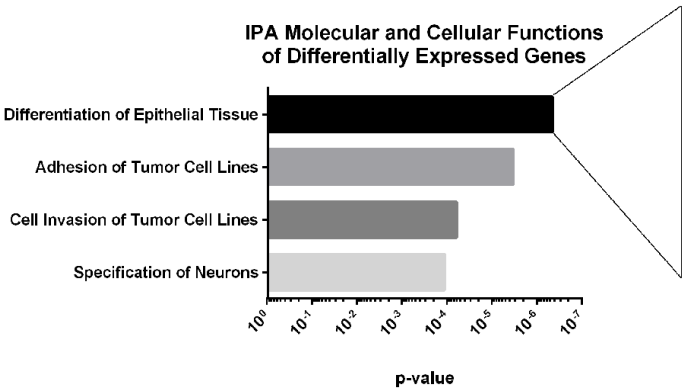


A differential gene signature enriched in EMT and neuroendocrine markers predicts LSD1 inhibitor sensitivity



LSD1 inhibitor sensitive models are enriched for neuroendocrine markers





Genes	Expression Level in Resistant vs. Sensitive
MYC	4.048
RND3	2.165
ZEB1	2.029
SPRED1	1.424
CLIC4	1.027
CSF1	0.239
IHH	0.226
RBPJ	-0.715
KDF1	-1.644
TXNIP	-1.731
CBFA2T2	-1.747
BARX1	-1.855
GRHL2	-2.175
F11R	-2.463
CXCR4	-2.812
CDH1	-2.977
SOX2	-3.455
EFL3	-3.493
FOXA1	-3.493
FGF9	-3.581
FOXA2	-3.706
KIT	-3.817
OVOL2	-3.967
ST14	-4.029
DSP	-4.747
ASCL1	-5.554

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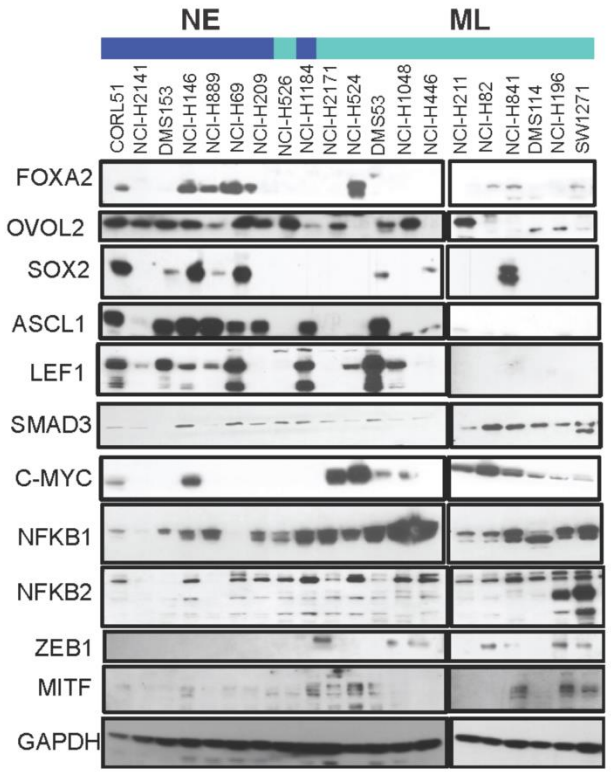
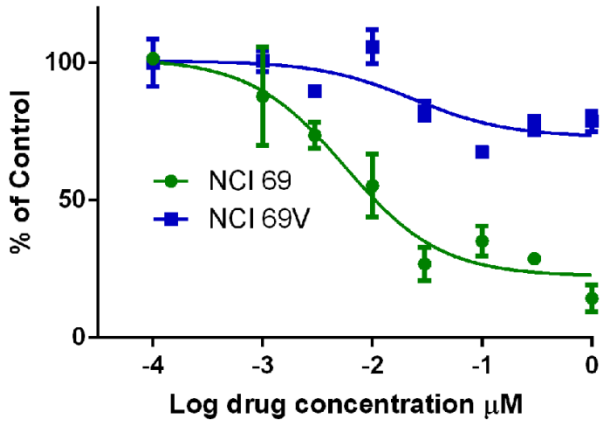
Mesenchymal shifted SCLC cells are insensitive to LSD1 inhibitors

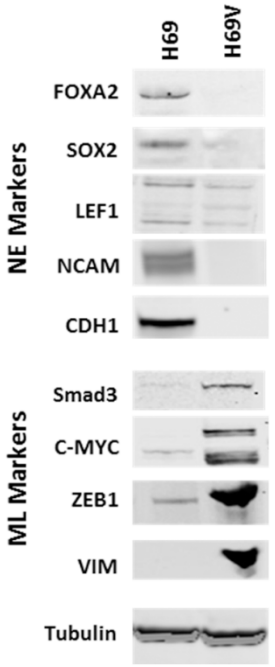
Transcription factor signatures define differentiation state of small cell lung cancer models



A. Udyavar et al. Cancer Research 2016

H69V mesenchymal variant cells are insensitive to LSD1 inhibition





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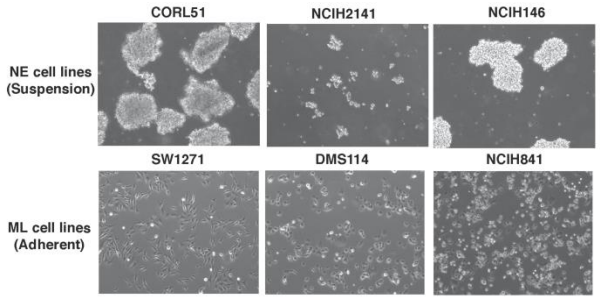
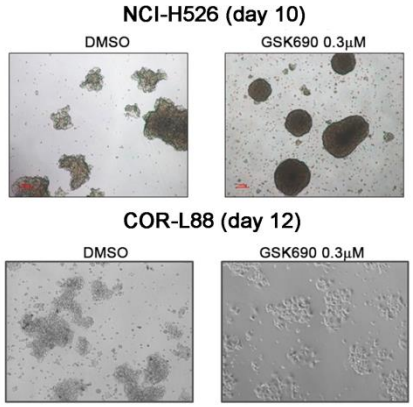
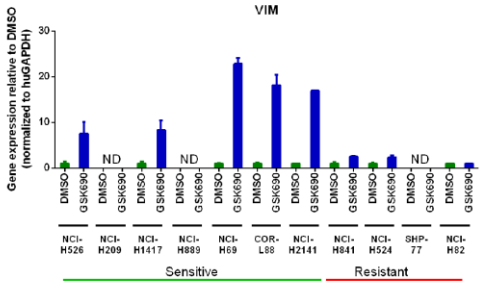
LSD1 inhibitor treatment alters neuroendocrine and mesenchymal genes in SCLC

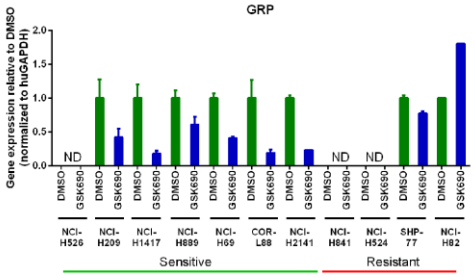
LSD1 inhibitor treatment induces morphological changes characteristic of mesenchymal-like cell lines

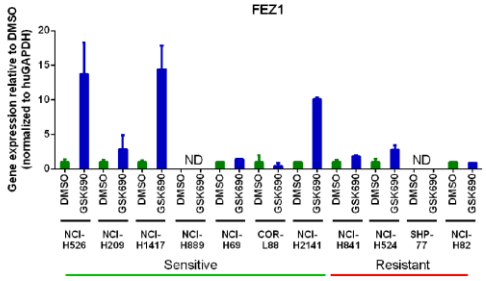


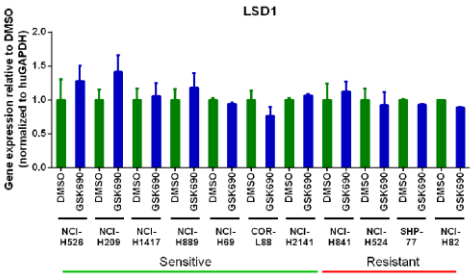


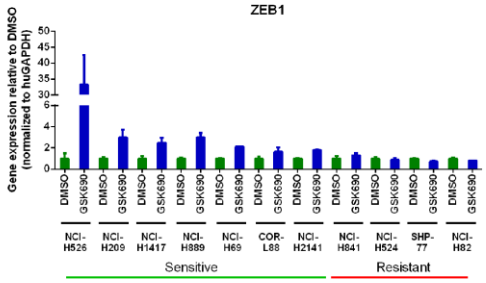
LSD1 inhibition alters mesenchymal and neuroendocrine gene expression markers in sensitive models











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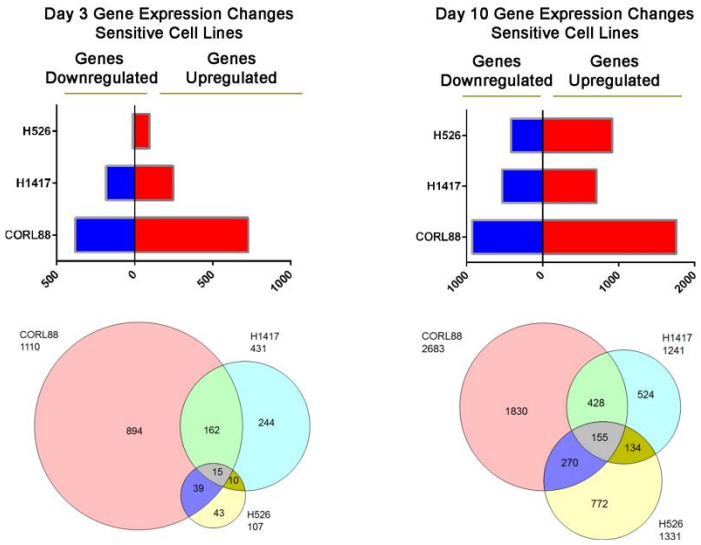
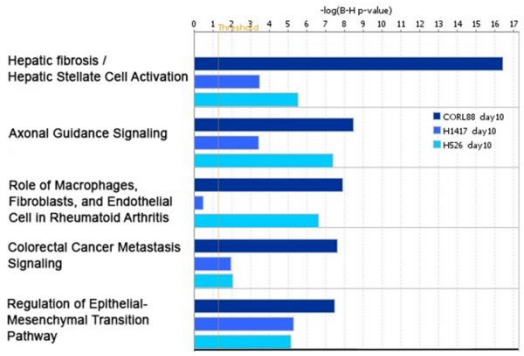
RNA-seq analysis shows different gene expression responses in LSD1 inhibitor treated sensitive and resistant models

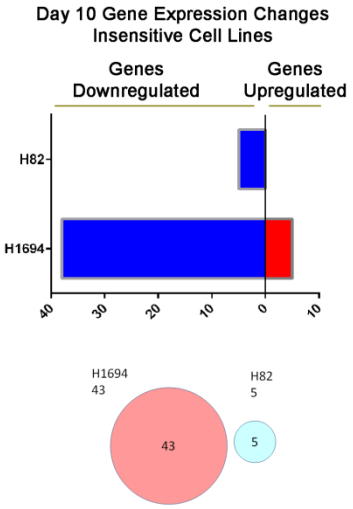
Neuronal and EMT pathways are enriched LSD1 inhibitor treated sensitive SCLC cell models



IPA pathways analysis of genes altered by LSD1 inhibitor treatment at day 10



Minimal gene expression changes are observed in LSD1 inhibitor treated resistant SCLC cell models



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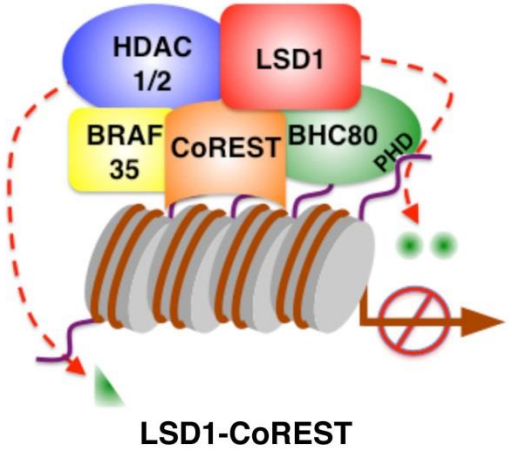
LSD1-containing complexes in SCLC resemble BHC complexes previously shown to be important for neurological gene repression

Sensitive			Resistant	
COR-L88	NCI-H526	NCI-H69	NCI-H82	NCI-H1694
KDM1A (LSD1)	KDM1A (LSD1)	KDM1A (LSD1)	KDM1A (LSD1)	KDM1A (LSD1)
RCOR1 (co-REST)	RCOR1 (co-REST)	RCOR1 (co-REST)	RCOR1 (co-REST)	RCOR1 (co-REST)
RCOR2			RCOR2	RCOR2
				RCOR3
HDAC1				
HDAC2	HDAC2	HDAC2		HDAC2
ZMYM2 (ZNF198)	ZMYM2 (ZNF198)		ZMYM2 (ZNF198)	ZMYM2 (ZNF198)
ZMYM3				ZMYM3
GSE1	GSE1	GSE1	GSE1	GSE1
				PHF21A (BHC80)
				HMG20A (BRAF35)
				HMG20B



Mosammaparast and Shi, *Annual Rev. Biochem.*, 2010

A core–BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes

Mohamed-Ali Hakimi*†, Daniel A. Bochar*†, Josh Chenoweth‡, William S. Lane§, Gail Mandel‡, and Ramin Shiekhattar*¶

*The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104; ‡Howard Hughes Medical Institute, Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794; and §Harvard Microchemistry and Proteomics Analysis Facility, Harvard University, Cambridge, MA 02138

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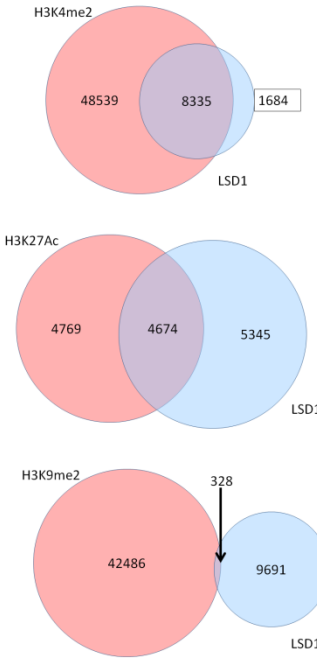
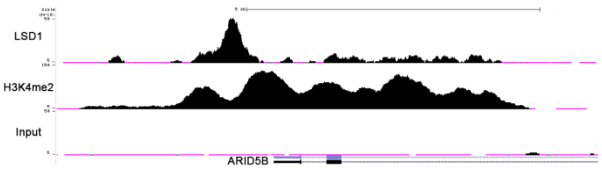
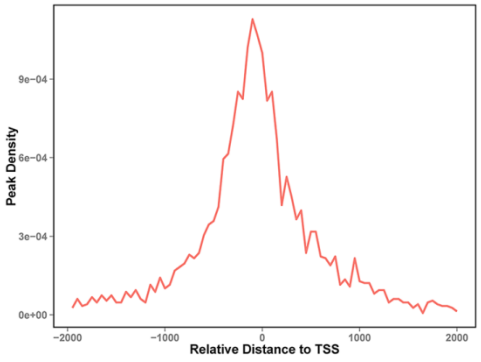
CHIP-seq analysis demonstrates LSD1 regulation of H3K4me2 with enrichment for transcription factors involved in neurological and mesenchymal differentiation

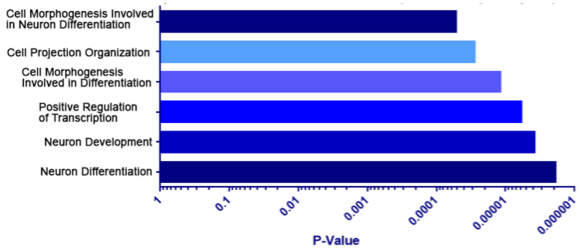
LSD1 binding in SCLC overlaps regions of H3K4me2 with an enrichment at gene promoters







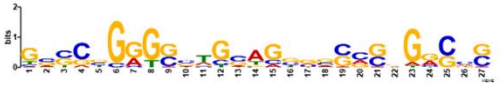
LSD1 direct target genes in overlap of RNA-seq and ChIP-seq datasets suggests LSD1 key transcription factors in neurological and mesenchymal differentiation



ABLIM2	KDM3A	RORA
BARX2	KIT	RORB
CD40	KITLG	RUNX1
EGR1	LEF1	SHH
ESRRG	MAML2	SKAP1
EYA1	MAML3	SOX4
FOXO1	MYC	SOX6
FZD4	NEUROG2	TESC
FZD7	NR4A3	WT1
FZD10	PAX6	WWTR1
GRIP1	PBX1	YAP1
HES1	PRKCG	ZEB1

MEME analysis shows LSD1 binding is enriched at octamer (POU-family) DNA binding sites

E-value= 1.3e-140
POU2F, Pou2f2_3748.1, Pou2f3_3986.2, Pou5f1::Sox2



E-value= 1.8e-008
ZNF263, Zfp281, E2F1, SP2, E2F6, Zfp740, Hic1, E2F3, EGR1, E2F4

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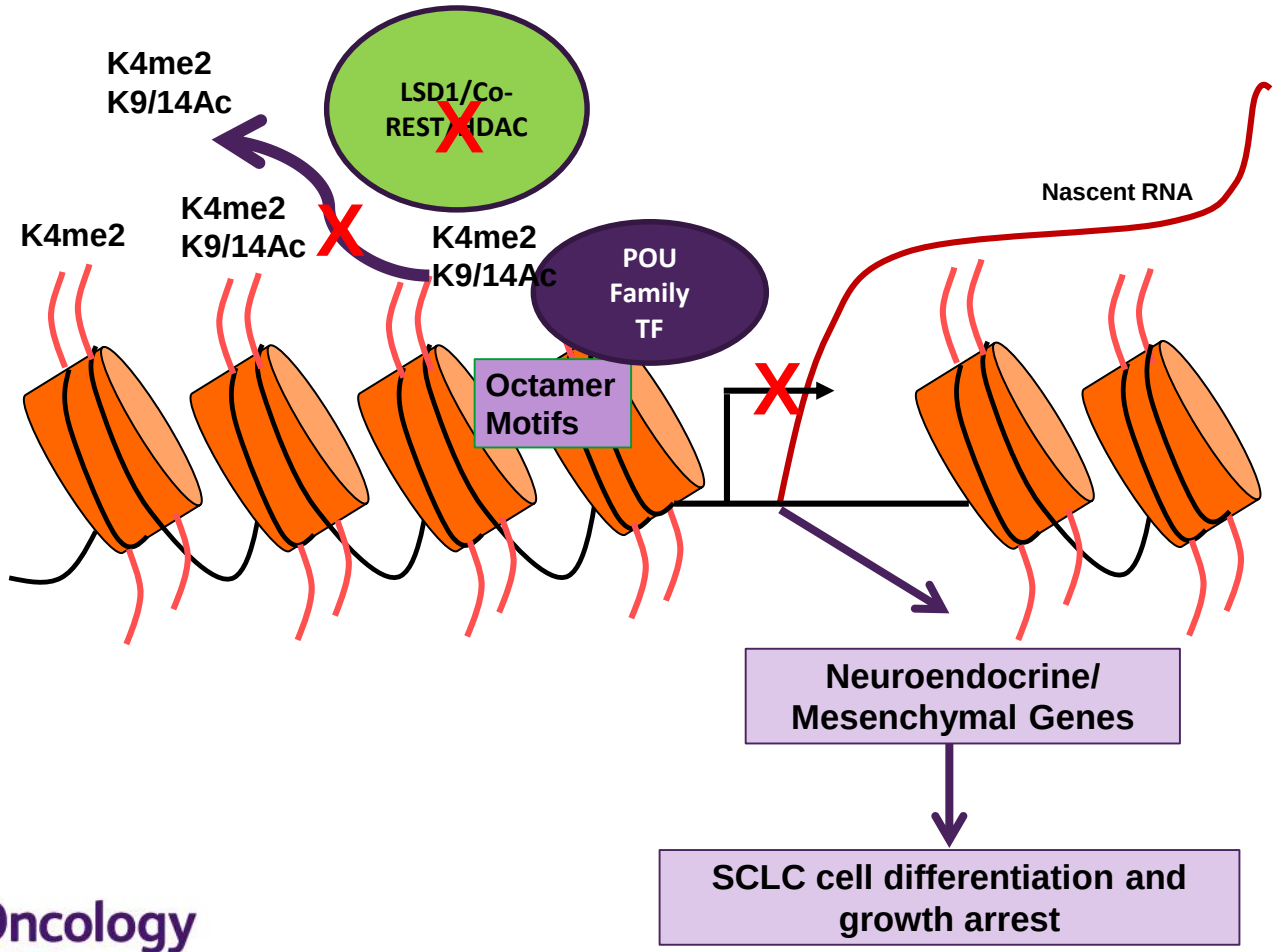
Model for LSD1 Regulation in SCLC



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