

Supporting information

LSD1 substrate binding and gene expression are affected by HDAC1-mediated deacetylation

Dhanusha A. Nalawansa and Mary Kay. H. Pflum*

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202

Table of contents	Page
Figure S1- Substrate trapping in HEK293 cells	S2
Figure S2- HDAC inhibitors used in the study	S3
Table S1- Mass spectrometric analysis of new substrate bands	S4
Figure S3- Peptides of LSD1 observed in the wild type substrate trapping experiment	S5
Figure S4- Spectrum of LSD1 peptide in wild type sample identified by MS/MS analysis	S5
Figure S5- Peptides of LSD1 observed in the C151A substrate trapping experiment	S6
Figure S6- Spectrum of LSD1 peptide in C151A sample identified by MS/MS analysis	S6
Figure S7- Peptides of RuvB like 2 observed in the wild type substrate trapping experiment	S7
Figure S8- Spectrum of RuvB like 2 peptide in wild type sample identified by MS/MS analysis	S7
Figure S9- Peptides of RuvB like 2 observed in the C151A substrate trapping experiment	S8
Figure S10- Spectrum of RuvB like 2 peptide in C151A sample identified by MS/MS analysis	S8
Figure S11- Peptides of rRNA-2'-O-methyltransferase observed with wild type trapping	S9
Figure S12- Spectrum of rRNA-2'-O-methyltransferase peptide with wild type trapping	S9
Figure S13- Peptides of rRNA-2'-O-methyltransferase observed with C151A trapping	S10
Figure S14- Spectrum of rRNA-2'-O-methyltransferase peptide with C151A trapping	S10
Figure S15- Quantification of <i>in vitro</i> deacetylation assay	S11
Figure S16- Quantification of <i>in cellulo</i> deacetylation assay	S12
Figure S17- <i>In vitro</i> demethylation assay of LSD1	S13
Table S2 - MS analysis of LSD1 acetylation	S14
Figure S18- MS data identifying acetylated lysines on LSD1	S15-S17
Figure S19- Independent trials used for quantification of mRNA signal in RT-PCR	S18
References	S18

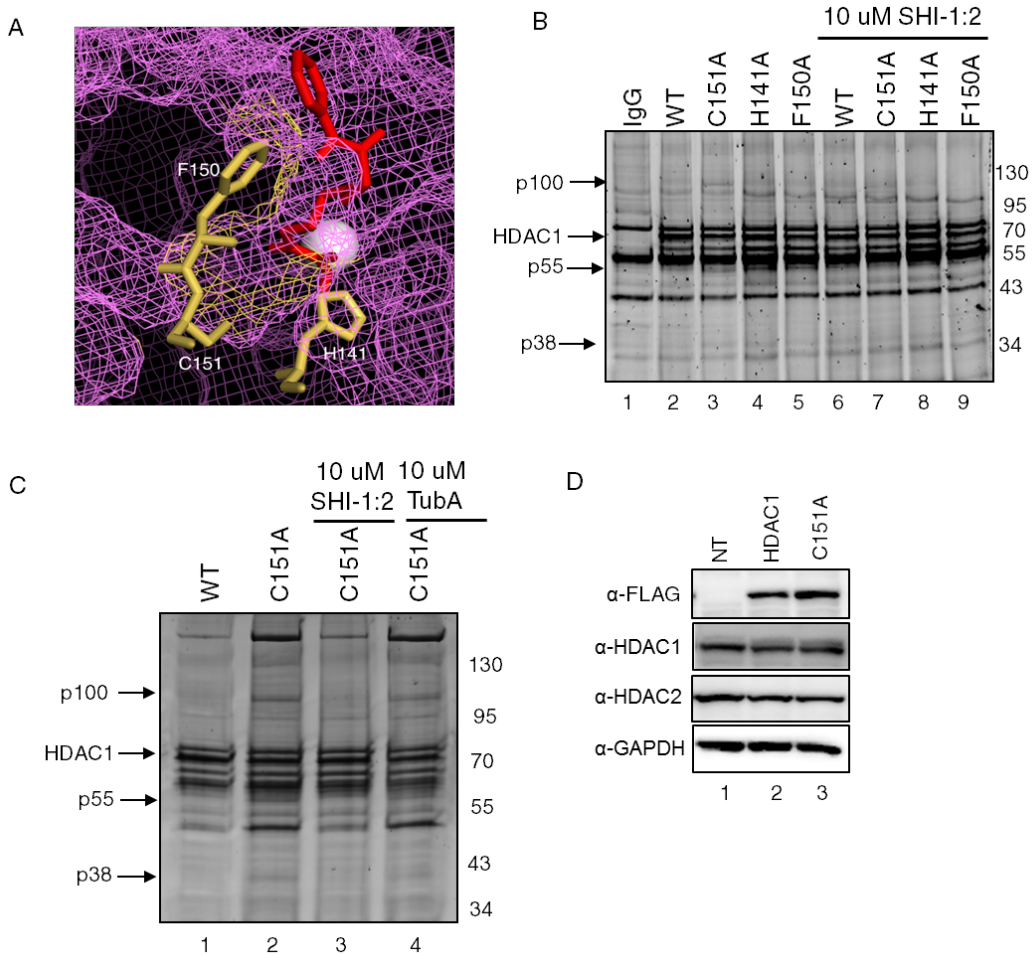
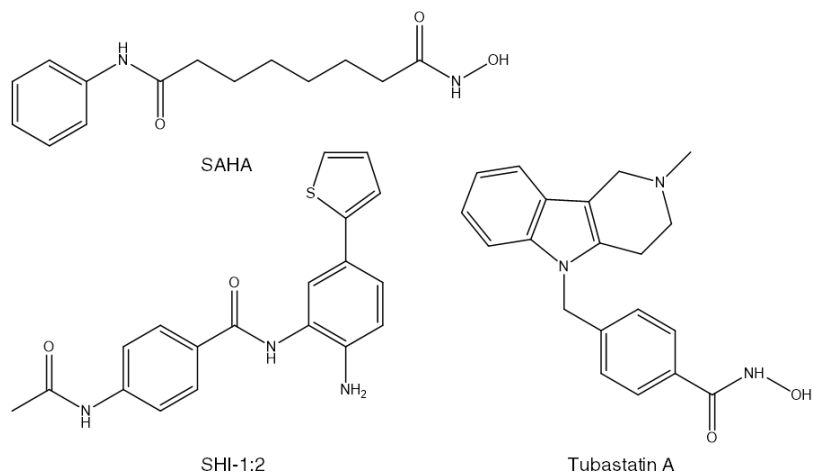


Figure S1—Substrate trapping in HEK293 cells. A) SAHA (red line structure) is docked into the crystal structure of HDAC1 (purple mesh; PDB ID: 4BKX). Amino acid residues mutated in the trapping strategy are shown as yellow line structures and are labeled. The Zn^{2+} metal is shown as a gray sphere. B) and C) Wild type (WT) or mutant HDAC1 (indicated above each lane) were expressed as Flag-tagged proteins in HEK293 cells and then cells were treated with 10 μ M SAHA for 24 hr to induce robust protein acetylation. Cells were lysed and proteins were immunoprecipitated with anti-Flag agarose in the presence or absence of A) 10 μ M SHI-1:2, or B) 10 μ M SHI-1:2 or 10 μ M Tubastatin. Bound proteins were separated by SDS-PAGE, and stained with Sypro Ruby total protein stain. Arrows point to HDAC1 or possible substrates (p100, p55, p38) present in mutant but not WT or inhibitor treated samples. D) Wild type (WT) or mutant HDAC1 (indicated above each lane) were expressed as Flag-tagged proteins along with a non-transfected control (NT) in HEK293 cells and then cells were treated with 10 μ M SAHA for 24 hr to induce robust protein acetylation. Cells were lysed and lysates were separated by SDS-PAGE and immunoblotted with FLAG, HDAC1, HDAC2 and GAPDH antibodies. The levels of overexpressed HDAC1 and mutant were similar to endogenous levels of HDAC1 and HDAC2.

A

B IC₅₀ values (μM) of HDAC inhibitors SAHA, SHI-1:2 and Tubastatin

	SAHA	SHI-1:2	Tubastatin A
HDAC1	0.03	0.007	16.4
HDAC2	0.17	0.049	>30
HDAC3	0.1	10	>30
HDAC4	>50	>10	>30
HDAC5	>30	>10	>30
HDAC6	0.038	>10	0.015
HDAC7	>30	>10	>30
HDAC8	0.41	>10	0.854

Figure S2 – HDAC inhibitors used in the study. A) Chemical structures of SAHA, SHI-1:2 and Tubastatin used in this study. B) IC₅₀ values of SAHA,¹ SHI-1:2,² and Tubastatin A,³ for different HDAC isoforms (HDAC1-8).

Table S1- Mass spectrometric analysis of new substrate bands

Protein band	Protein name (Accession)	MW (kDa)	Sample	Unique peptide count*	Unique spectral count[#]	% Coverage[€]
p100	Lysine specific histone demethylase 1A (LSD1) (KDM1A_HUMAN)	93	HDAC1 (WT)	6	6	11
			C151A	26	31	43
p55	RuvB- like 2 (RUVB2_HUMAN)	51	HDAC1 (WT)	3	3	8
			C151A	10	13	25
p38	rRNA 2'-O-methyltransferase fibrillarin (FBRL_HUMAN)	34	HDAC1 (WT)	1	1	3
			C151A	6	7	26

*Unique Peptide count - Number of different amino acid sequences that are associated with a protein. [#]Unique Spectral count – Number of unique spectra that identified each unique peptide including modifications. [€]% Coverage – the percentage of the protein amino acid sequence was identified.

KDM1A_HUMAN (100%), 92,903.4 Da
 Lysine-specific histone demethylase 1A OS=Homo sapiens GN=KDM1A PE=1 SV=2
 7 exclusive unique peptides, 7 exclusive unique spectra, 7 total spectra, 102,852 amino acids (12% coverage)

```

MLSGKKA AAAA AAAAAAATG TEAGPGTAGG SENGSEVAAQ PAGLSGPAEV GPGAVGERTP RKKEPPRAS P PGLAEPPGS
AGPQAAGPTVV PGSATPMETG IAETPEGRRT SRRKRAKVEY REMDESLANL SEDEY YSEEE RNAAKAEK K LPPPPQAPP
EEENESEPEE PSQVEGAAFO SRLPHDRMTS QEAACFPDII SGQQQTQKVF LFIRNRTLQL WLDNPKIQLT FEATLQQLEA
PYN S D T V L V H RVH SYLERHG L I N F G I Y K R I K E L P T K K T G K V I I I G S G V S G L A A A R Q L O S F G M D V T L L E A R D R V G G R V A T F
R K G N Y V A D L G A M V V T G L G G N P M A V V S K Q V N M E L A K I K Q K C P L Y E A N G Q A V P K E K D E M V E Q E F N R L L E A T S Y L S H O L D F N V
L N N K P V S L G Q A L E V V I Q L Q E K H V K D E Q I E H W K K I V K T Q E E L K E L L N K M V N L K E K I K E L H Q Q Y K E A S E V K P P R D I T A E F L V
K S K H R D L T A L C K E Y D E L A E T Q G K L E E K L Q E L E A N P P S D V Y L S S R D R Q I L D W H F A N L E F A N A T P L S T L S L K H W D Q D D D F E V
T G S H L T V R N G Y S C V P V A L A E G L D I K L N T A V R Q V R Y T A S G C E V I A V N T R S T S Q T F I Y K C D A V L C T L L P L S L V L K Q Q P P A V Q F V
P P L P E W K T S A V Q R M G F G N L N K V V L C F D R V F W D P S V N L F G H V G S T T A S R G E L F L F W N L Y K A P I L L A L V A G E A A G I M E N I S D
D V I V G R C L A I L K G I F G S S A V P Q P K E T V V S R W R A D P W A R G S Y S Y V A A G S S G N D Y D L M A Q P I T P G P S I P G A P Q P I P R L F F A G
E H T I R N Y P A T V H G A L L S G L R E A G R I A D Q F L G A M Y T L P R Q A T P G V P A Q Q S P S M
  
```

Figure S3 - Peptides of LSD1 observed in the wild type substrate trapping experiment. Primary sequence of LSD1, which was identified in the substrate trapping experiment as p100. Peptides observed in the MS/MS analysis are highlighted in yellow. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2. Oxidation of methionine (M) is shown in green.

Peptide sequence - (K)APILLALVAGEAAGImENISDDVIVGR(C)

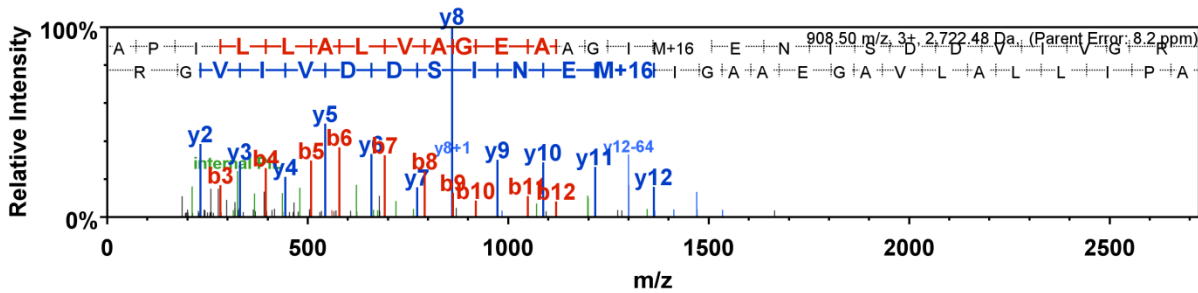


Figure S4 – Spectrum of LSD1 peptide observed in the wild type LC-MS/MS analysis. The annotated spectrum of one representative peptide from LSD1 identified by LC-MS/MS analysis (Figure S3).

KDM1A_HUMAN (100%), 92,903.4 Da
 Lysine-specific histone demethylase 1A OS=Homo sapiens GN=KDM1A PE=1 SV=2
 27 exclusive unique peptides, 32 exclusive unique spectra, 35 total spectra, 373,852 amino acids (44% coverage)

```

MLSGKKA AAAA AAAAAAATG TEAGPGTAGG SENGSEVAAQ PAGLSGPAEV GPGAVGERTP RKKEPPR ASP PGGLAEP PGS
AGPOACPTVV PGSATP METG IAETPEGRRT SRRKRAKVEY REMDESLANL SEDEYYSEEE RNAKAKEKKK LPPPPPQAPP
EEENESEPEE PSGVEGAAAFQ SRLPHDRMRTS QEAACFPDII SGPOQTOKVF LFI RNRTLOL WLDNPKIQLT FEATLQQLEA
PYNSDTVLVH RVHSYLERHG LINFGIYKRRI KPLPTKRTGK VI IIGSGVSG LAAROLOSF GMDVTLLEAR DRVGGRVATF
FKGNYVADLG AMVVTGLGSH PMAVVSQGVN MELAKIKQKQ PLYEANGQAV PKEKDE MVEQ EFNRLLLEATS YLSHQLDENV
LNKKNPVS LGQ ALEVVIQLOE KHVKDEQIEH WKKIVKTQEE LKELLNKMVN LKEKIKELHQ QYKEASEVKK PRDITAEFLV
KSKHRDLTAL CK EYDELAET QGKLEEKLOE LEANPPSDVY LSSRDROI LD WHFANLEFAN ATPLSTLSLK HWDQDDDFEF
TGSHLT VTRNG YSCVPVALAE GLDIKLN TAV RQVRYTASGC EVIAVNT RST SQTFIYK CDA VLCTLP LSLK KQPPAVQFV
PPLPEWK TSA VQR MGFGNLN KVVLCFDRVF WDP SVNLFGH VGS TTASRGE LFLFWNL YKA PILLALVAGE AAGIMENISD
DVI VGRCLAI LKGI FGSSAV PQPK ETV VSR WRADPWARGS YSYVAA GSSG NDYDLMAQPI TPGPS I PGAP QPI PRLFFAG
EHTIRNYPAT VHGALLSGLR EAGR IADQFL GAMYTLPRQA TPGVPAQQSP SM
  
```

Figure S5 - Peptides of LSD1 observed in the C151A substrate trapping experiment. Primary sequence of LSD1, which was identified in the substrate trapping experiment as p100. Peptides observed in the MS/MS analysis are highlighted in yellow. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2. Oxidation of methionine (M) is shown in green.

Peptide sequence - (K)EKDEmVEQEFNR(L)

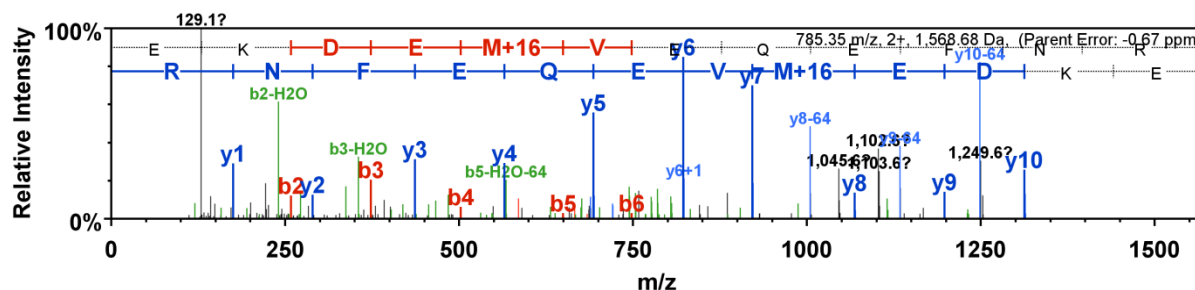


Figure S6 – Spectrum of LSD1 peptide observed in the C151A LC-MS/MS analysis. The annotated spectrum of one representative peptide from LSD1 identified by LC-MS/MS analysis (Figure S5).

RUVB2_HUMAN (100%), 51,158.1 Da
 RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3
 3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 36/463 amino acids (8% coverage)

```

MATVTATTKV PEIRDVTRIE RIGAHSHIRG LGLDDALEPR QASQGmVGO L AARRAAGYVL EMIREGKIAG RAVLIAGQPP TGTKTAIANGM
AQUALGPDTPF TAIAGSEIFS LEMSKTEALT QAFRRSIGVR IKEETEIIIEG EVVEIQIDRP ATGTGSKVVK LTLKTTMET IYDLGTMIE
SLTKDKVQAG DVITIDKATG KISKLGSRFT RARDYDAMGS OTKFWQCPDG ELQKRKEVVH TVSLHEIDVI NSRTQGF LAL FSGDTGEIKS
EVREQINAKV AEWREEGKAE IIPGVLFIDE VHMLDIESFS FLNRALES DM APVLI MATNR GITRIRGTSY QSPHGIPIDL LDRLLIVSTT
PYSEKDTKQI LRIRCEEEDV EMSDAYTVL TRIGLETSLR YAIQLITAA S LVORRKR GTE WQVDDIKR VY SLFLDES RST QYMKEYQDAF
LFNELKGETM DTS
  
```

Figure S7 - Peptides of RuvB like 2 observed in the wild type substrate trapping experiment. Primary sequence of RuvB like 2, which was identified in the substrate trapping experiment as p55. Peptides observed in the MS/MS analysis are highlighted in yellow. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2. Oxidation of methionine (M) is shown in green.

Peptide sequence - (R)QASQGMVQLAAR(R)

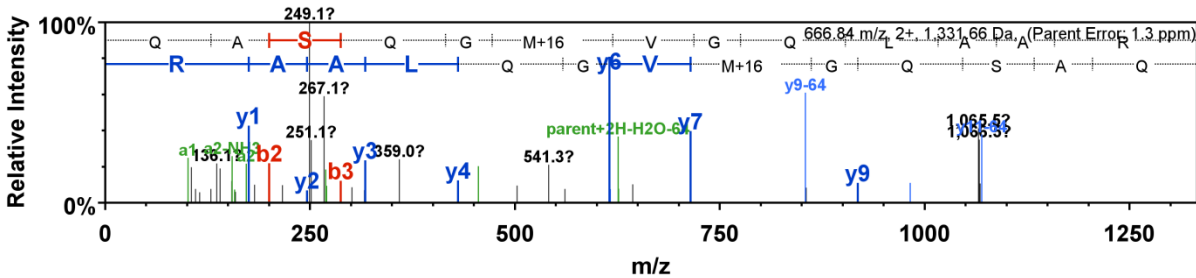


Figure S8 – Spectrum of RuvB like 2 peptide observed in the wild type LC-MS/MS analysis. The annotated spectrum of one representative RuvB like 2 peptides identified by LC-MS/MS analysis (Figure S7).

RUVB2_HUMAN (100%), 51,158.1 Da

RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3

10 exclusive unique peptides, 13 exclusive unique spectra, 14 total spectra, 117/463 amino acids (25% coverage)

MATVTATTKV PEIRDVTRIE RIGAHSHIRG LGLDDALEPR **QASQGMVQQL** AARPAAGVVL EMIREGKIAG RAVLLIAGOPG TGKTAIAMGM
 AALGPDTPF TAIAGSEIFS LEMSKTEALT QAFRRSIGVR IKEETEIEEG EVVEIQIDRP ATGTGSKVVK LTLK**TTEMET** **LYDLGTMIE**
SLTKDKVQAG **DVITIDK**ATG KISKLGSRFT RARDYDAMGS QTKFVQCPDG ELQKRK**EVVH** **TVSLHEIDVI** **NSRTQGF LAL** FSGDTGEIKS
 EVREQINAKV AEWREEGKAE IIPGVLFIDE VHMLDIESFS FLNRALES DM APVLI MATNR GITRIRGTSY QSPHGIPIDL LDRLLIVSTT
PYSEKDTKQI LRIRCEEEDV EMESEDAYTVL TR**IGLETSLR** YAIQLITAA S LVCRKRK**GTE** **VOVDDIKRVY** **SLFLDES**RST QYMK EYQDAF
 LFNELKGETM DTS

Figure S9 - Peptides of RuvB like 2 observed in the C151A substrate trapping experiment. Primary sequence of RuvB like 2, which was identified in the substrate trapping experiment as p55. Peptides observed in the MS/MS analysis are highlighted in yellow. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2. Oxidation of methionine (M) is shown in green.

Peptide sequence - (K)GTEVQVDDIKR(V)

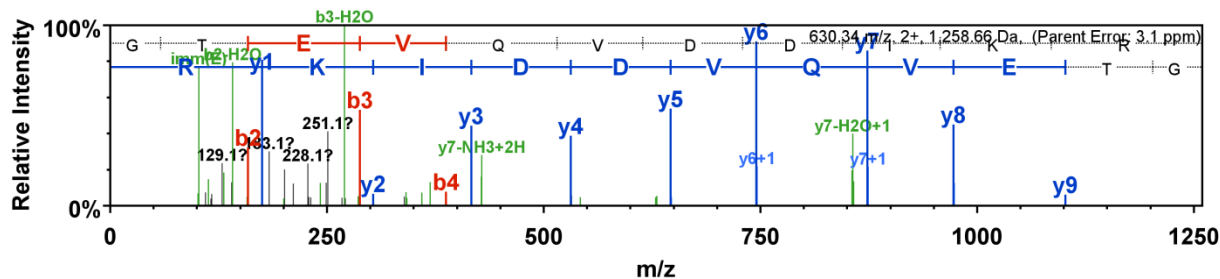


Figure S10 – Spectrum of RuvB like 2 peptide observed in the C151A LC-MS/MS analysis. The annotated spectrum of one representative RuvB like 2 peptides identified by LC-MS/MS analysis (Figure S9).

FBRL_HUMAN (43%), 33,784.1 Da

rRNA 2'-O-methyltransferase fibrillarins OS=Homo sapiens GN=FBRL PE=1 SV=2

1 exclusive unique peptides, 1 exclusive unique spectra, 1 total spectra, 9/321 amino acids (3% coverage)

```
MKPGFSPRGG GFGGRGGFGD RGGRRGGGGF GGGRRGGGGF RRRRRGGGGG GGGGGGGGGG GGGFHSGGNR GRRRGGKRGH
OSGKNVMVEP HRHEGVFTCR GKEDALVTKN LVPQESVYGE KRVSISEGDD KIEYRAWNPF RSKLAAAILG GVDQIHKPG
AKVLYLGAAS GTTVSHVSDI VGPDGLVYAV EFSHRSGRDL INLAKKRTNI IPVIEDARHP HKYRMLIAMV DVI FADVAQP
DQTRIVALNA HTFLRNGGHF VISIKANCID STASAEAVFA SEVKKMQQEN MKPQEQLTLE PYERDHA VVV GYRPPPKVK
N
```

Figure S11 - Peptides of rRNA-2'-O-methyltransferases fibrillarins (FBRL) observed in the wild type substrate trapping experiment. Primary sequence of FBRL, which was identified in the substrate trapping experiment as p38. Peptides observed in the MS/MS analysis are highlighted in yellow. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2.

Peptide sequence - (R)GKEDALVTK(N)

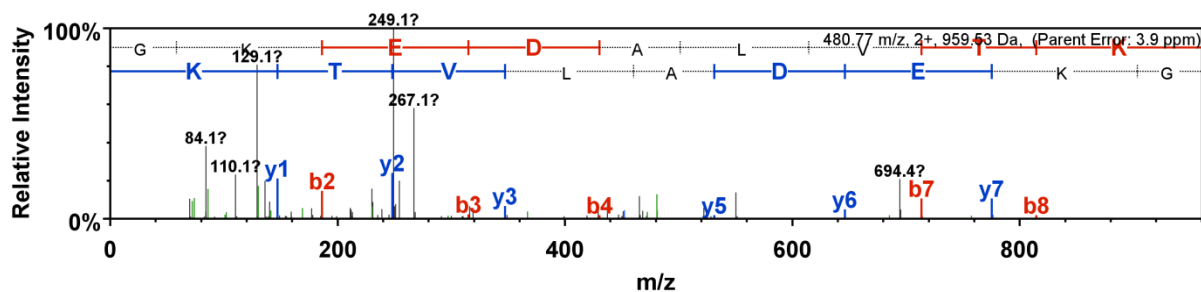


Figure S12 – Spectrum of rRNA-2'-O-methyltransferases fibrillarins (FBRL) peptide observed in the wild type LC-MS/MS analysis. The annotated spectrum of one representative FBRL peptides identified by LC-MS/MS analysis (Figure S11).

FBRL_HUMAN (100%), 33,784.1 Da
 rRNA 2'-O-methyltransferase fibrillarin OS=Homo sapiens GN=FBL PE=1 SV=2
 6 exclusive unique peptides, 7 exclusive unique spectra, 7 total spectra, 91/321 amino acids (28% coverage)

```

M K P G F S P R G G G F G G R G G F G D R G G R G G R G G F G G R G R G G G G G G G G G G G G G G R G G G F H S G G N R G R G R G G K R G N
Q S G K N V M V E P H R H E G V F I C R G K E D A L V T K N L V P G E S V Y G E K R V S I S E G D D K I E Y R A W N P F R S K L A A A I L G G V D Q I H I K P G
A K V L Y L G A A S G T T V S H V S D I V G P D G L V Y A V E F S H R S G R D L I N L A K K R T N I I P V I E D A R H P H K Y R M L I A M V D V I F A D V A Q P
D Q T R I V A L N A H T F L R N G G H F V I S I K A N C I D S T A S A E A V F A S E V K K M Q Q E N M K P Q E Q L T L E P Y E R D H A V V V G V Y R P P P K V K
N
  
```

Figure S13 - Peptides of rRNA-2'-O-methyltransferases fibrillarin (FBRL) observed in the C151A substrate trapping experiment. Primary sequence of FBRL, which was identified in the substrate trapping experiment as p38. Peptides observed in the MS/MS analysis are highlighted in yellow. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2. Oxidation of methionine (M) is shown in green.

Peptide sequence - (R)DHAVVVG VYRPPPK(V)

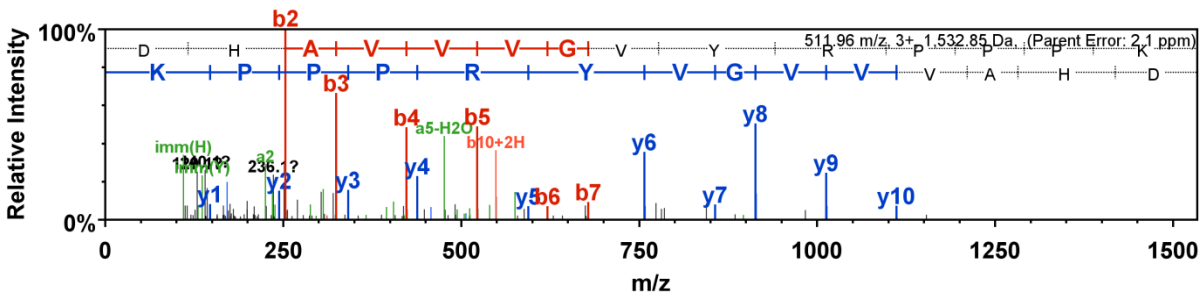


Figure S14 – Spectrum of rRNA-2'-O-methyltransferases fibrillarin (FBRL) peptide observed in the C151A MS/MS annotated spectrum of one representative FBRL peptides identified by MS/MS analysis (Figure S13).

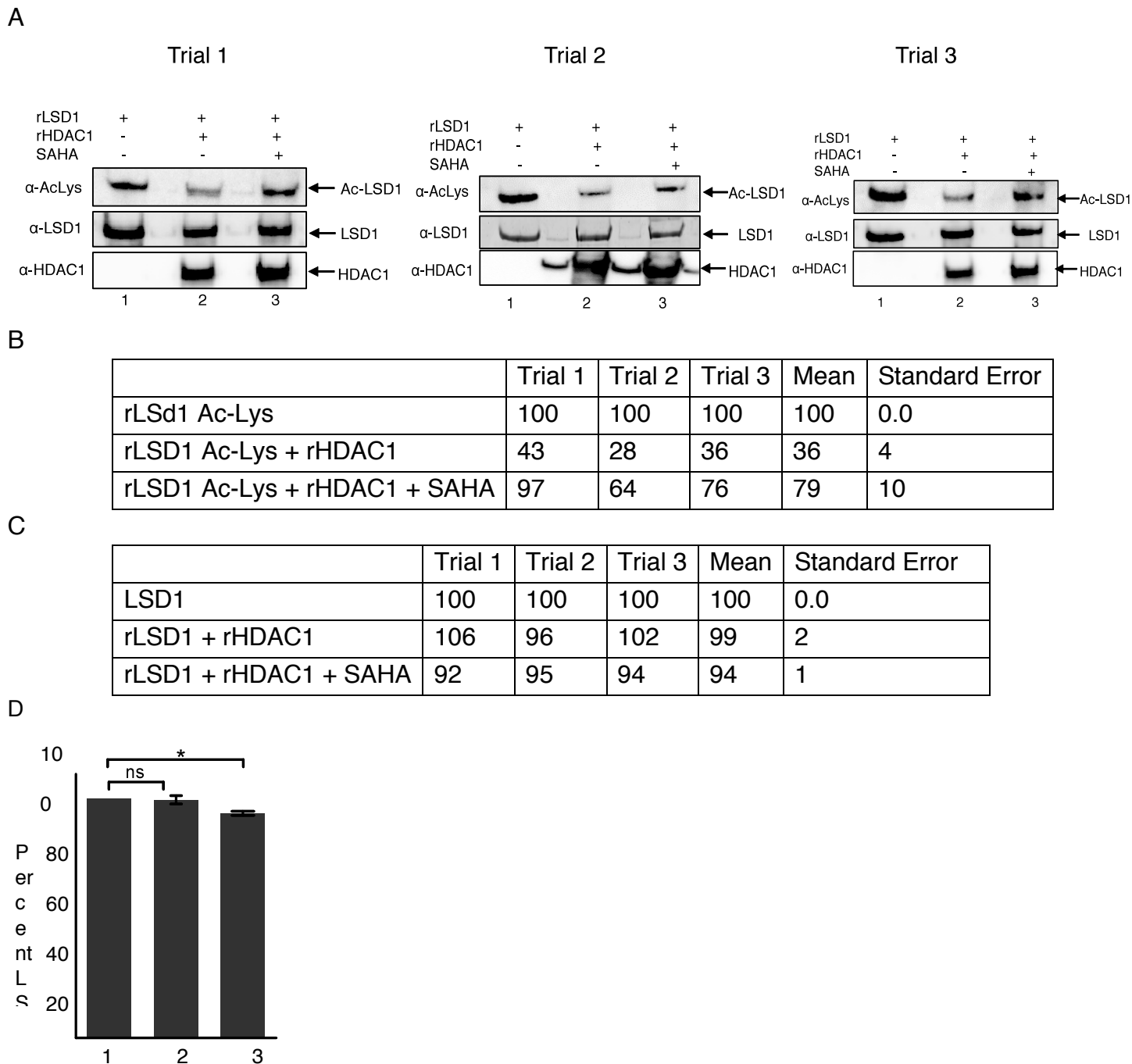
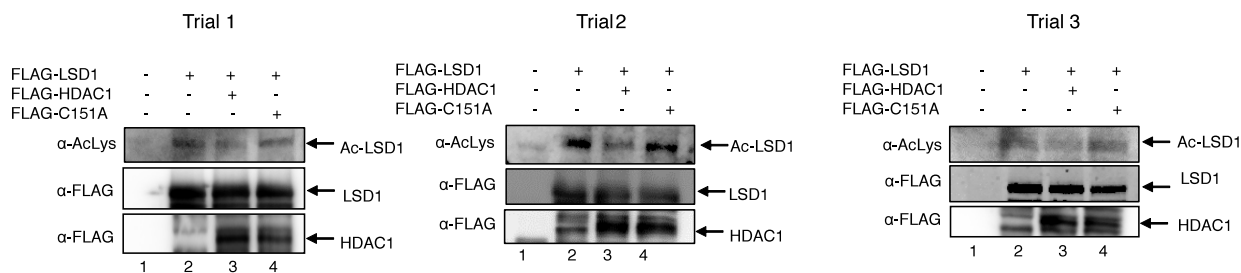


Figure S15 - Quantification of *in vitro* deacetylation assay. A) Three independent trials used for quantification. B) Percent AcLys signal of rLSD1 was quantified from western blots using three independent trials and raw data is shown in the Table here or the histogram in Figure 2D. C-D) As a loading control, total rLSD1 was quantified from western blots, with raw data shown in the table (C) and the histogram (D). ns - not significant. * $p < 0.05$.

A



B

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
LSD1 Ac-Lys	100	100	100	100	0.0
LSD1 Ac-Lys + WT HDAC1	31	43	34	36	4
LSD1 Ac-Lys + C151A HDAC1	86	88	96	90	3

C

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
LSD1	100	100	100	100	0.0
LSD1 + WT HDAC1	100	104	91	98	4
LSD1 + C151A HDAC1	93	101	92	95	3

D

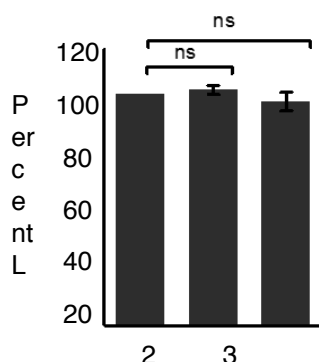
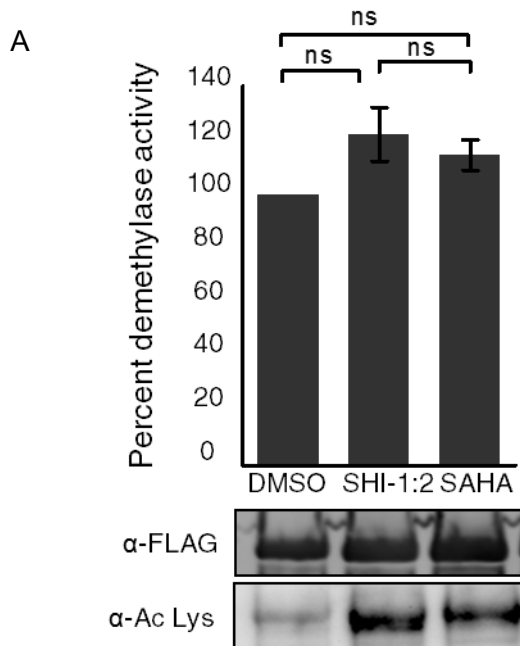


Figure S16 - Quantification of *in cellulo* deacetylation assay. A) Three independent trials used for quantification. B) Percent AcLys signal of LSD1-FLAG was quantified from the western blots of three independent trials and raw data is shown in the table here or in the histogram in Figure 2F. C-D) As a loading control, total LSD1-FLAG was quantified from western blots, with raw data shown in the table (C) and the histogram (D). ns - not significant.



B

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
LSD1+DMSO	100	100	100	100	0.0
LSD1+SHI-1:2	104	138	125	122	10
LSD1+SAHA	104	117	123	115	6

Figure S17 - *In-vitro* demethylation assay of LSD1. A) HEK293 cells were transfected with the LSD1-FLAG expression construct and grown for 48 hr. Then, 10 μ M SHI-1:2 or 10 μ M SAHA was added for another 24 hr. Cells were harvested and lysed before proteins were immunoprecipitated with anti-FLAG agarose beads. Half of the immunoprecipitate was subjected to a fluorescence-based LSD1 activity assay (Enzo life) and other half was analyzed by western blotting with FLAG and acetyl lysine antibodies to assure equal protein content and acetylation level in each reaction. The fluorescence signal due to demethylase activity was background corrected using a negative control reaction using cell lysates where no LSD1 was overexpressed. Then the signal of each sample was normalized to DMSO untreated LSD1 enzyme (set to 100%). The mean and the standard error from at least three independent trials are shown. ns – not significant B) Percent demethylation activity mean and standard error for the three independent trials used to plot the data in part A are shown as a table.

Table S2- MS analysis of LSD1 acetylation

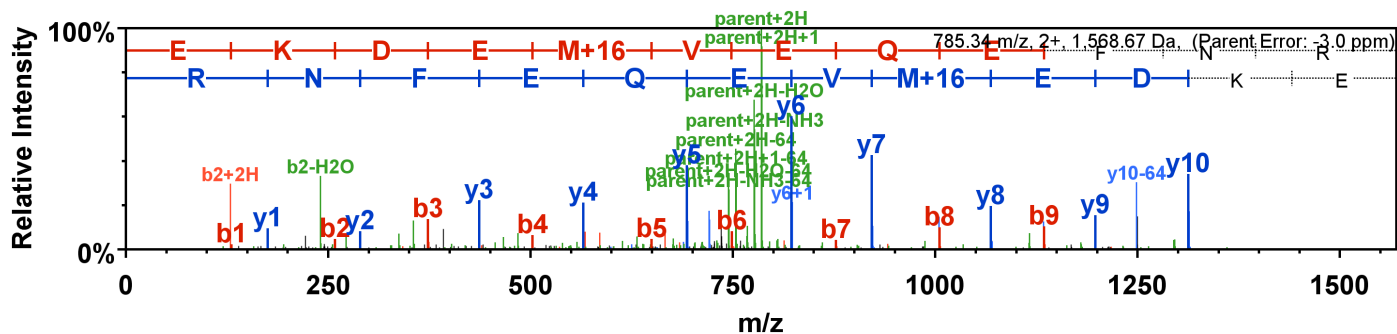
Protein name/ Accession	Trials	Sample	% Coverage ^a	Unique # of acetylated peptides ^b
Lysine Specific histone demethylase 1 (LSD1) (KDM1A_HUMAN)	1	LSD1+DMSO	90	3 (K6, K268, K280) (K374 covered, but no acetylation)
		LSD1+SHI-1:2	94	6 (K6, K268, K280, K374 , K404, K503, K507)
		LSD1+SAHA	59	0 (K374 not covered)
	2	LSD1+DMSO	71	0 (K374 covered, but no acetylation)
		LSD1+SHI-1:2	84	3 (K268, K374 , K404)
		LSD1+SAHA	75	0 (K374 covered, but no acetylation)
	3	LSD1+DMSO	75	0 (K374 covered, but no acetylation)
		LSD1+SAHA	79	1 (K374)

^a % Coverage – the percentage of the protein amino acid sequences identified. ^b Unique acetylated peptides - Number of different peptides containing acetylated lysines identified. K374 indicated in red highlights the presence of peptides containing acetylated K374 in that trial. The presence of peptides containing unacetylated K374 is indicated by the statement in parenthesis.

A. Trial 1

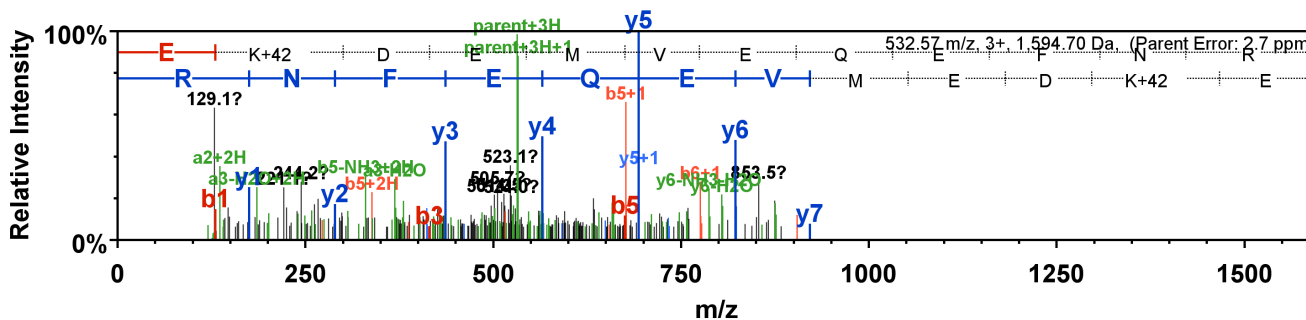
LSD1+DMSO

Peptide sequence- (K)EKDEmVEQEFNR(L)



LSD1+SHI1:2

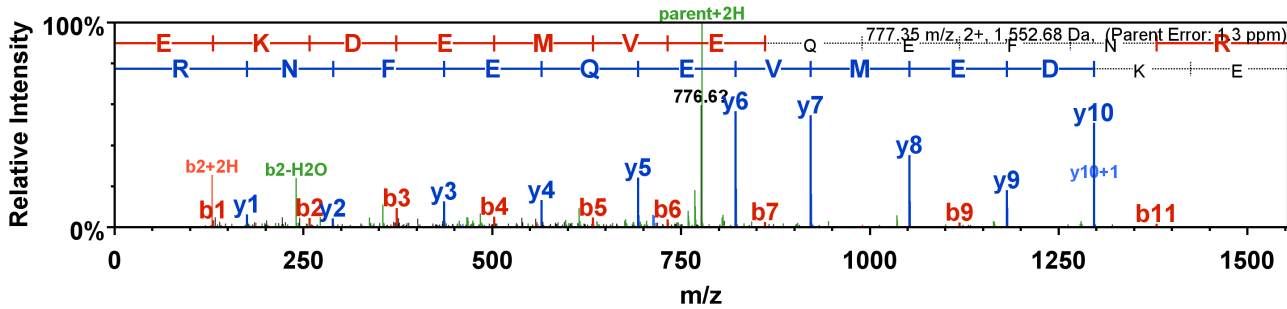
Peptide sequence- (K)EKacDEMVEQEFNR(L)



B. Trial 2

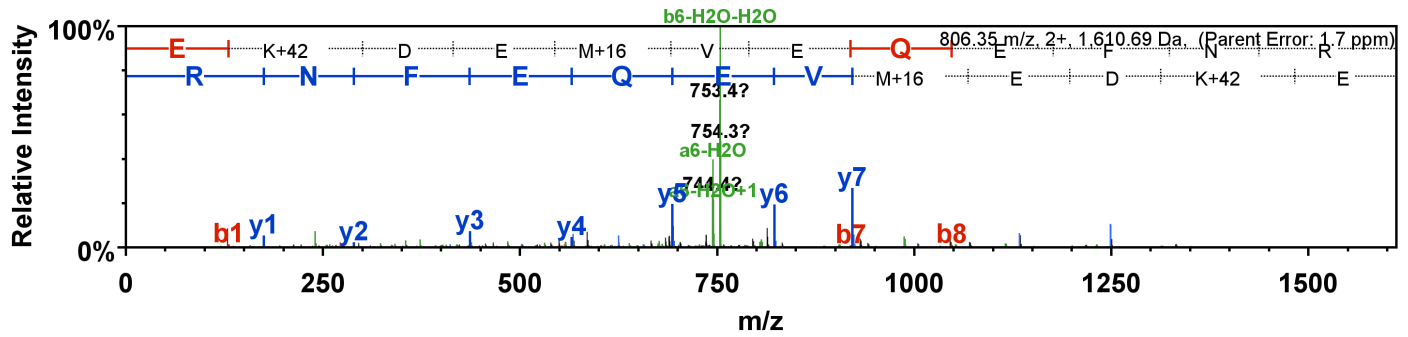
LSD1+DMSO

Peptide sequence- (K)EKDEMVEQEFNR(L)



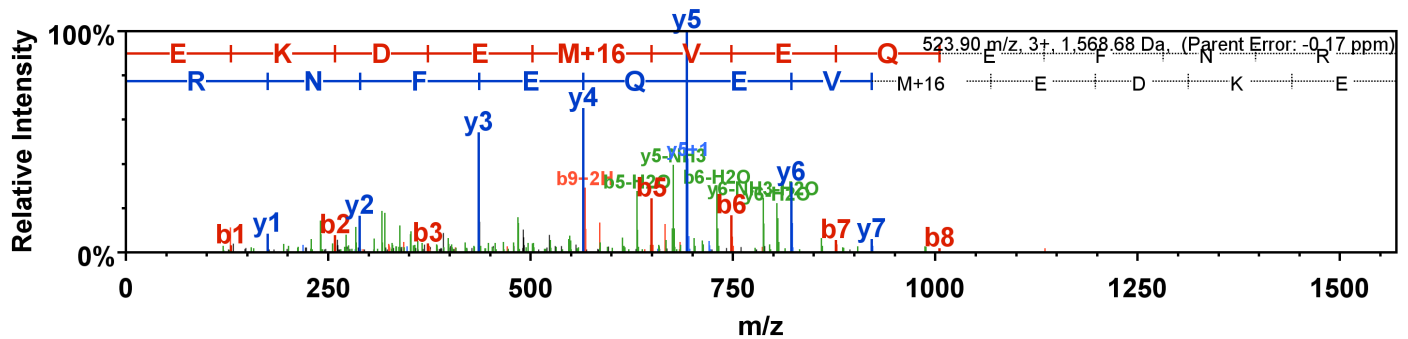
LSD1+SHI-1:2

Peptide sequence- (K)EKacDEmVEQEFNR(L)



LSD1+SAHA

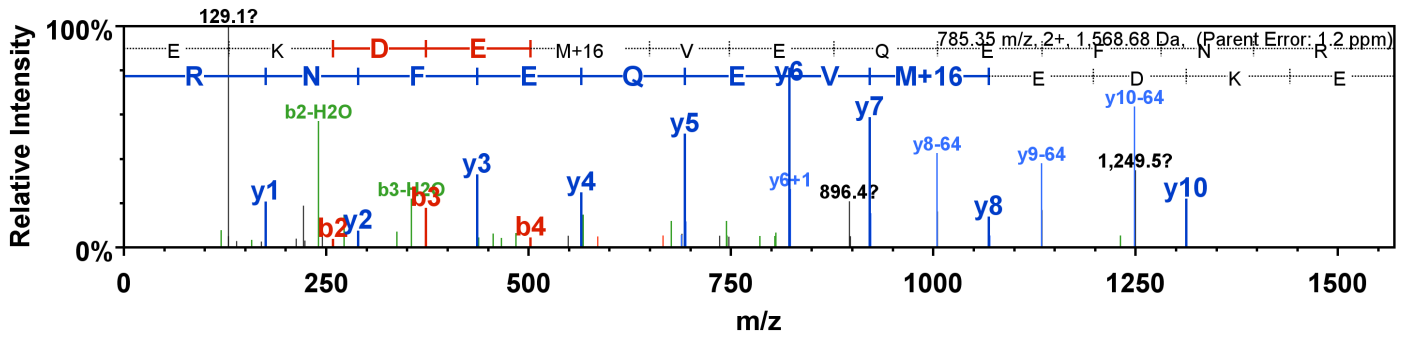
Peptide sequence- (K)EKDEmVEQEFNR(L)



C. Trial 3

LSD1+DMSO

Peptide sequence- (K)EKDEmVEQEFNR(L)



LSD1+SAHA

Peptide sequence- (K)EKacDEmVEQEFNR(L)

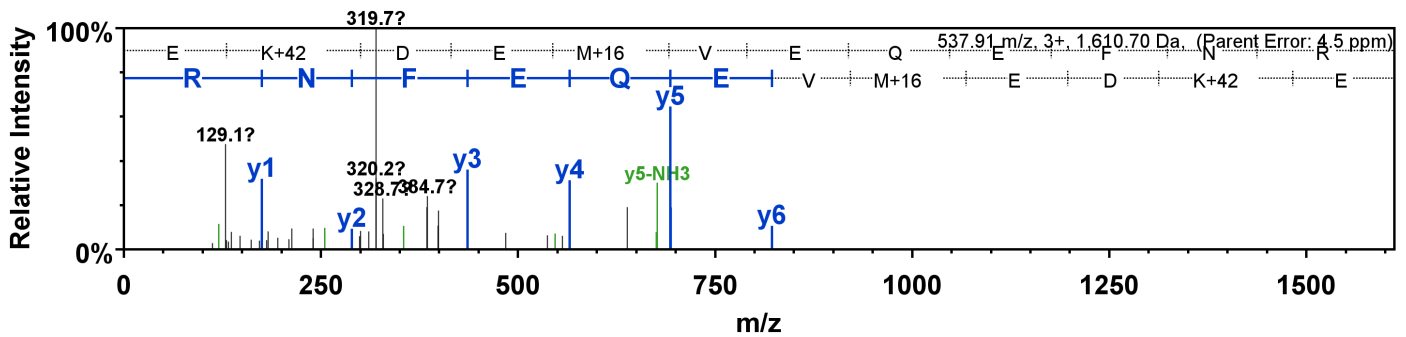


Figure S18- MS data identifying acetylated lysines on LSD1. Representative spectra for acetylated and unacetylated peptide of LSD1 identified from three independent trials (A, B, and C). An overview of the three independent trials are shown in Table S2.

A

SCN2A	Trial 1	Trial 2	Trial 3	Trial 4	Mean	Standard Error
NT	1.4	3.9	1.7	2.6	2.4	0.6
WT LSD1+DMSO	1.0	1.0	1.0	1.0	1.0	0.0
WT LSD1+SHI-1:2	7.8	5.9	2.7	5.0	5.4	1.0
K374R LSD1+SHI-1:2	2.7	1.7	1.0	0.70	1.5	0.4

B

SCN3A	Trial 1	Trial 2	Trial 3	Trial 4	Mean	Standard Error
NT	1.4	1.2	1.7	-	1.4	0.1
WT LSD1+DMSO	1.0	1.0	1.0	1.0	1.0	0.0
WT LSD1+SHI-1:2	2.3	2.3	2.6	2.5	2.4	0.1
K374R LSD1+SHI-1:2	0.40	0.80	1.2	1.2	0.90	0.2

Figure S19 – Real time PCR analysis of SCN2A and SCN3A genes. HEK293 cells were transfected with WT or K374R mutant LSD1-FLAG, treated with SHI-1:2, and lysed, before RNA was isolated, converted to cDNA, and then used as the template in PCR reactions using gene specific primers. Fold change calculated from four independent trials, with mean and standard error shown for SCN2A (A) and SCN3A (B). Data is presented in Figure 6 of the manuscript.

References

1. Witter, D. J., Harrington, P., Wilson, K. J., Chenard, M., Fleming, J. C., Haines, B., Kral, A. M., Secrist, J. P., and Miller, T. A. (2008) Optimization of biaryl Selective HDAC1&2 Inhibitors (SHI-1:2), *Bioorganic & medicinal chemistry letters* 18, 726-731.
2. Methot, J. L., Chakravarty, P. K., Chenard, M., Close, J., Cruz, J. C., Dahlberg, W. K., Fleming, J., Hamblett, C. L., Hamill, J. E., Harrington, P., Harsch, A., Heidebrecht, R., Hughes, B., Jung, J., Kenific, C. M., Kral, A. M., Meinke, P. T., Middleton, R. E., Ozerova, N., Sloman, D. L., Stanton, M. G., Szewczak, A. A., Tyagarajan, S., Witter, D. J., Secrist, J. P., and Miller, T. A. (2008) Exploration of the internal cavity of histone deacetylase (HDAC) with selective HDAC1/HDAC2 inhibitors (SHI-1:2), *Bioorganic & medicinal chemistry letters* 18, 973-978.
3. Butler, K. V., Kalin, J., Brochier, C., Vistoli, G., Langley, B., and Kozikowski, A. P. (2010) Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin A, *Journal of the American Chemical Society* 132, 10842-10846.