Supplemental Material to:

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Smyd3 regulates cancer cell phenotypes and catalyzes histone H4 lysine 5 methylation
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Supplementary information

Materials and Methods

Plasmids

Full length Smyd3 was cloned into either pGEX-6P1 or pDESTT7 plasmid with a Flag/6His N-terminal tag which includes a TEV cleavage site. Single point mutations of Smyd3 were generated using the QuikChange site-directed mutagenesis kit (Stratagene), and clones were confirmed by DNA sequencing. The pGEX-derived plasmids generated by mutagenesis were: pGEX-Smyd3 F183A, pGEX-Smyd3 N205A and pGEX-Smyd3 Y239A. pDESTT7-Smyd3 N205A was also generated. Set8 was cloned in pGEX-6P1 using a similar method. The histone H4 mutants library were made by cloning histone H4 into pGEX-6P1 plasmid, lysines K5, K8, K12, K16, K20 and K31 were all mutated to arginine using the QuikChange site-directed mutagenesis kit (Stratagene) and each arginine was then individually mutated back to the original lysine one by one. Smyd3 shRNA was cloned in puromycine resistant pSICOR. Smyd3 reconstitution plasmids were created using the pGateway system according to manufacturer recommendations (Invitrogen) with either the WT or point mutant Smyd3 constructs, into hygromycin resistant pMSCV.

Cell culture, reagents and transfections.

Breast and liver carcinoma cell lines were maintained in RPMI-1640 medium containing 10% fetal bovine serum and glutamine. MCF7 cells were grown in advanced- Dulbecco's modified Eagle's medium (advanced-DMEM; GIBCO) supplemented with 10% fetal calf serum (FCS, GIBCO), 100 units/ml penicillin and glutamine. All cells were cultured at 37°C in a humidified incubator with 5% CO2.

Reverse siRNA transfections were performed using Lipofectamine RNAiMAX reagent (Life Technologies, Carlsbad, CA) and 25nM siRNA. Smyd3 siRNAs used were: siRNA A (CAGTATCTCTTTGCTCAATCA), siRNA B

(CAGGCAACTCGTAATGACATT) (Qiagen, Valencia, CA). For stable knockdown of Smyd3 in Hep3B cells, cells were transduced with lentiviral shRNA construct against Smyd3 3'UTR (TGCTGTTGACAGTGA GCGACACGATTATAATAAATTCAAATAGTGAAGCCACAGATGTATTTGAATTT ATTATAATCGTGCTGCCTACTGCCTCGGA, Open Biosystems), followed by puromycin selection for two weeks. For Bacmam infections with wild-type or catalytically inactive Smyd3 (N205A), cells were plated the day before the infection with bacmam virus. For catalytic activity rescue experiments the infection was repeated every 3 days to allow for continuous expression of Smyd3 throughout the study. For growth analysis, cells were plated in 96-well plates and the accumulation of cellular material was monitored using a CTG assay accordingly to manufacturer instructions (Promega). For growth analysis in anchorage-independent conditions, cells were plated in 0.65% methylcellulose (Sigma-Aldrich) and the colonies were counted using microscopy (Olympus IX71).

Retro- and lentiviral transductions of MCF7 cells were performed as previously described[1]. MCF7 cells were first transduced with pSICOR Smyd3 shRNAs (Smyd3 shRNA 3'UTR target sequence: TGCGTGTGTCTTTGTTGAATTTCAAGAGAAT TCAACAAAGACACACGCTTTTTTC) or empty pSICOR and selected with 2 μg/mL puromycin for 4 days. The puromycin-resistant cells were then transduced with pMSCV Smyd3 (wt or F183A) or with empty pMSCV, and then selected with hygromycin-B (250 μg/ml, Invitrogen) for 4 days.

For soft agar assay of MCF7 cells, 2×10^4 cells were plated in triplicate in 0.8% agarose on 1% agarose base agar medium as previously described[2]. Colony formation was quantified by counting the numbers of colonies per field after 21 days.

Smyd3 KO mice and culture of MEFs

Smyd3$^{tm1a(KOMP)Wtsi}$ mice were obtained from the KOMP Repository at UC Davis in a C57BL/6NTac genetic background. Briefly, in this allele, insertion of a *lacZ*

cassette in intron 2 of the *Smyd3* gene creates a hypomorphic allele; expression of the Cre recombinase in cells removes the *lacZ* cassette and further deletes several *Smyd3* exons, resulting in a null allele. Details on the targeted allele are available on the KOMP web site and upon request. Experiments with mice were approved by Stanford Institutional Animal Care and Use Committee.
MEFs were generated from embryos 13.5 days after fertilization. MEFs were cultured in media supplemented with 10% serum, penicillin-streptomycin, and L-glutamine. MEFs were used between passages 3 and 5. To delete *Smyd3*, 3×10^5 MEFs were infected with Adeno-GFP (control adenovirus) or Adeno-Cre (University of Iowa gene transfer core) as they were plated in DMEM supplemented with 10% serum. Cells were allowed to grow for 3 days before being replated for experiments.

Immunoblot analysis. Cells were lysed by sonication in buffer containing following components: 20 mM Tris-HCl (pH 7.5) , 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA , 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml leupeptin, containing fresh protease inhibitors (Roche) and phosphatase inhibitor cocktail (Sigma-Aldrich). Protein concentration of samples was determined by BCA assay (Pierce). MCF7 cell fractionation was performed by collecting supernatant after 5 min of 1300 g centrifugation following a 10 min incubation in hypotonic buffer (10 mM Hepes pH7.9, 10 mM KCL, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% glycerol, 1 mM DTT and protease inhibitors). The pellet was then lysed by sonication and incubated 15 min in LSDB500 buffer (Glycerol 20%, $MgCl_2$ 3 mM, Hepes pH 7.9 50 mM, KCL500 mM, DTT 0.5 mM, PMSF 0.5 mM, NP40 0.1%, protease inhibitors). Proteins were resolved by SDS-PAGE, transferred to nitrocellulose membrane and analyzed by immunoblot. Antibodies used were as follows: Smyd3 rabbit polyclonal antibodies (generated by Yenzym), histone H3 rabbit polyclonal (Abcam), histone H4 rabbit polyclonal (Millipore) and B-Tubulin (Millipore), H3K4me3 (Cell Signaling), H4K20me3 (Active Motif).

Expression and purification of recombinant proteins

Following baculovirus SF9 expression, cells were lysed and Smyd3 was purified on nickel NTA, and Flag peptide affinity columns. Following TEV cleavage, native, untagged Smyd3 was purified using size exclusion chromatography. The same strategy was used to purify N205A mutant Smyd3. For GST tagged recombinant proteins, transfected BL21 cells were induced with 0.1 mM IPTG O/N at 20°, and proteins were purified using Glutathione beads (Amersham) and eluted in 10 mM reduced glutathione (Sigma) as previously described[2].

Methylation assays

Smyd3 methylation activity on a peptide library (obtained from Alta Bioscience or custom synthesized by 21st Century Biochemicals) was assessed by radiometric assays utilizing ^{3}H-SAM (PerkinElmer) with specific product capture of biotin-tagged peptides on streptavidin SPA beads (PerkinElmer). Untagged histone reagents were captured on arginine binding SPA beads. Alternatively, phosphocellulose 96 well multiscreen filter plates (MSPH, Millipore) were used to capture histones and peptides based on charge-charge interactions. Assay conditions were 25 mM Tris pH8, 4 mM DTT, 50 uM $ZnCl_2$, 0.01% Tween-20 final.

In vitro histone lysine methylation assays were performed as previously described[2]. Briefly, histone peptides, free recombinant histones (New England Biolabs and Millipore) or recombinant nucleosomes (kindly provided by S. Tan, Penn State) were incubated with recombinant full length protein (Smyd3 WT, Smyd3 mutants or Set8) and 0.1 mM S-adenosyl-methionine (AdoMet, Sigma) or 2 µCi ^{3}H-AdoMet (Amersham) in methylation buffer containing 50 mM Tris-HCl (pH 8.0), 10 % glycerol, 20 mM KCl, 5 mM $MgCl_2$, and 1 mM PMSF at 30°C over-night. The reaction mixture was resolved by SDS-PAGE, followed by either autoradiography, Coomassie stain (Pierce), or Mass Spectrometry analysis.

Analysis of H4 methylation by mass spectrometry. Total methyl incorporation was measured by LC-ESMS using an Agilent LC-TOF 6220

(Agilent). The Smyd3-H4 reaction was injected onto a 2.1 mm R2/10 Poros column (Applied Biosystems) and the mass of the intact H4 protein was determined from the individual charge states which were measured at a resolution of 15,000. To determine the specific sites of methylation, H4 was digested with trypsin and analyzed by LC-ESMS using data-dependent and targeted MS/MS. Prior to digestion with trypsin, H4 was derivatized with propionic anhydride to block trypsin cleavage at free and mono-methylated lysines[3]. H4 tryptic peptides were loaded onto a 100 um i.d. trap cartridge and separated on a 100 um i.d. x 5 cm RP-PSDVB monolithic column (Dionex) using an Agilent 1100 nanoHPLC system. The gradient program was 2% B (5 min), 2%-30% B (20 min), 30%-95% B (5 min with a 5 min hold). Mobile phases are; A: H_2O, 0.2% formic acid, B: CH_3CN, 0.2% formic acid and trap loading solution of 0.05% HFBA. The nanoHPLC was coupled to an LTQ-Orbitrap XL (ThermoFisher Scientific). Data-dependent LC-MS/MS experiments consisted of an Orbitrap MS spectrum, acquired from m/z 400-2000 at 30,000 resolution followed by collision induced dissociation (CID) MS/MS in the LTQ on the top six peptide ions based on their intensities in the prior MS scan and whether or not MSMS had already been done. Targeted LC-MS/MS methods utilized the same Orbitrap MS scan; however, a parent mass list was used to trigger high resolution CID in the Orbitrap (7,500 resolution).

Quantitative mass spectrometry

In vivo derived samples were analyzed as previously described [4]. In brief, histone samples from the Smyd3 knock out MEFs or knock down HeLa cells were modified by D5-propionylation and wild-type samples by D0-propionylation. Following trypsin digestion, samples were desalted before loading onto a C18 packed 75 µm fused silica capillary column with ESI tip by an autosampler (AS-2; Eksigent Technologies Inc.). Peptides were introduced into an Orbitrap mass spectrometer and separated by RP-HPLC gradient (1-100% buffer B in 110 mL, buffer A = 0.1 M acetic acid, Buffer B = 70% acetonitrile in 0.1 M acetic acid) by an HPLC pump (1200 series; Agilent). The Orbitrap mass spectrometer was

operated in data-dependent MS/MS mode, obtaining a full MS at 30000 resolution and 7 MS/MS spectra in the ion trap. All MS/MS spectra were manually inspected.

References:

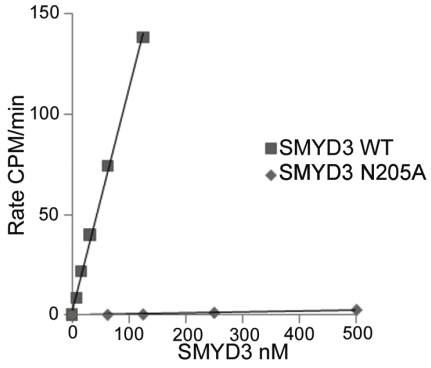
1 Michishita, E. *et al.* SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* **452**, 492-496, (2008).

2 Kuo, A. J. *et al.* NSD2 Links Dimethylation of Histone H3 at Lysine 36 to Oncogenic Programming. *Mol Cell* **44**, 609-620, (2011).

3 Garcia, B. A. *et al.* Chemical derivatization of histones for facilitated analysis by mass spectrometry. *Nat Protoc* **2**, 933-938, (2007).

4 Plazas-Mayorca, M. D. *et al.* Quantitative proteomics reveals direct and indirect alterations in the histone code following methyltransferase knockdown. *Mol Biosyst* **6**, 1719-1729, (2010).

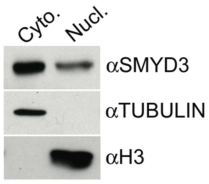
SUPPLEMENTARY FIGURE 1



Supplementary figure 1.

Quantitation of methylation assays with the indicated concentrations of recombinant WT and catalytically inactive N205A Smyd3 on recombinant histone H4.

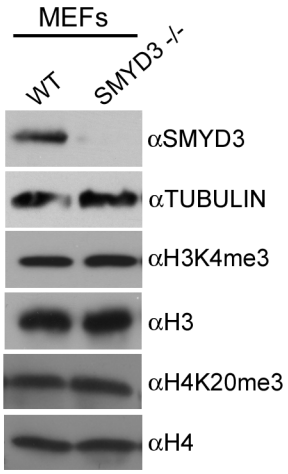
SUPPLEMENTARY FIGURE 2



Supplementary figure 2**.**

Western blot with the indicated antibodies of MCF7 cells biochemically separated into cytoplasmic and nuclear fractions. Tubulin and histone H3 are shown to control the integrity of the fractionation.

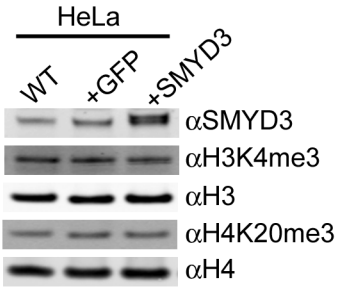
SUPPLEMENTARY FIGURE 3



Supplementary figure 3.

Western blot analysis with the indicated antibodies of whole cell extracts (WCE) from wild-type and Smyd3-/- MEFs

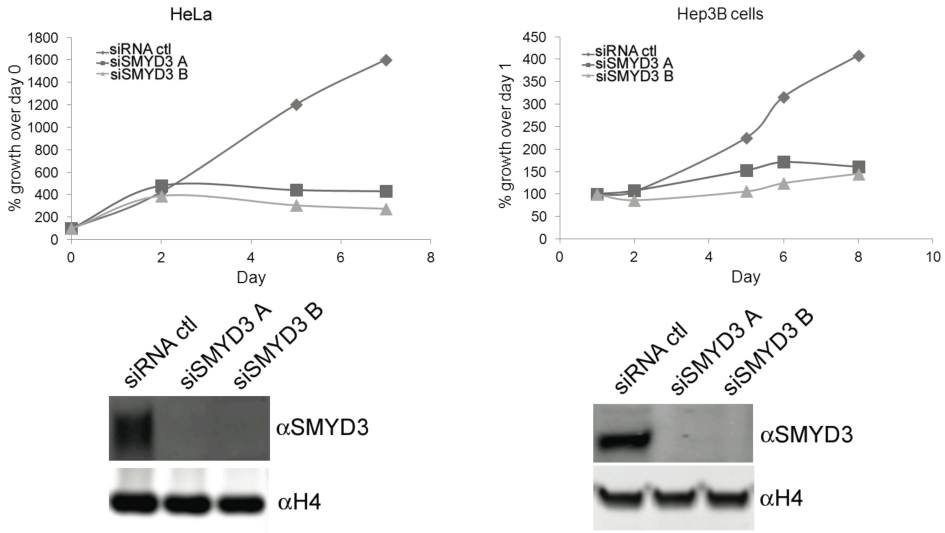
SUPPLEMENTARY FIGURE 4



Supplementary figure 4.

Western blot analysis of HeLa whole cell extract.

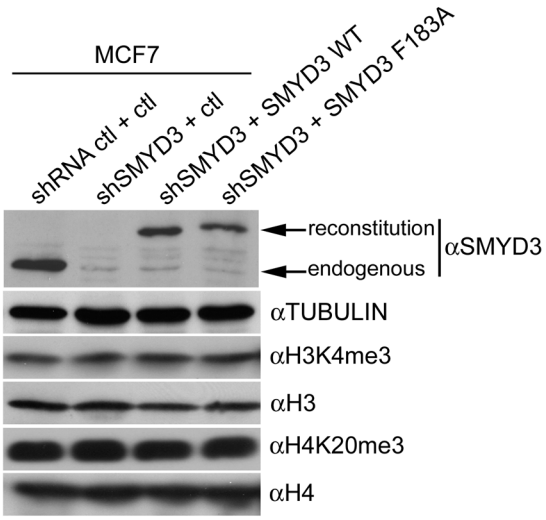
SUPPLEMENTARY FIGURE 5



Supplementary figure 5.

Depletion of Smyd3 leads to diminished cell proliferation of carcinoma cell lines.
Top panel: Growth analysis of HeLa (left panel) and liver Hep3b (right panel) carcinoma cell lines after Smyd3 knockdown with two independent siRNAs targeting Smyd3.
Bottom panel: Western blot analysis with the indicated antibodies of WCE from the cell lines described in the top panel.

SUPPLEMENTARY FIGURE 6



Supplementary figure 6.

Western blot analysis of WCEs from the indicated MCF7 cell lines.

SUPPLEMENTARY TABLE 1

Histone	% of control	% of control	Sequence																								N-term	C-term
H2a (1-21)	102	94	S	G	R	G	K	Q	G	G	K	A	R	A	K	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	136	118	Σ	G	R	G	K	Q	G	G	K	A	R	A	K	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	99	83	Σ	G	R	G	Δ	Q	G	G	K	A	R	A	K	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	78	84	S	G	R	G	Δ	Q	G	G	K	A	R	A	K	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	91	81	S	G	R	G	Δ	Q	G	G	Δ	A	R	A	K	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	98	86	S	G	R	G	K	Q	G	G	Δ	A	R	A	K	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	98	112	S	G	R	G	K	Q	G	G	Δ	A	R	A	Δ	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	97	98	S	G	R	G	K	Q	G	G	K	A	R	A	Δ	A	K	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	86	91	S	G	R	G	K	Q	G	G	K	A	R	A	Δ	A	Δ	T	R	S	S	R	A					biotin	CONH
H2a (1-21)	98	96	S	G	R	G	K	Q	G	G	Δ	A	R	A	Δ	A	Δ	T	R	S	S	R	A					biotin	CONH
H2a (21-41)	105	107	A	G	L	Q	F	P	V	G	R	V	H	R	L	L	R	K	G	N	Y	A	E					biotin	CONH
H2a (21-41)	95	95	A	G	L	Q	F	P	V	G	R	V	H	R	L	L	R	Δ	G	N	Y	A	E					biotin	CONH
H2a (86-106)	107	97	L	A	I	R	N	D	E	E	L	N	K	L	L	G	K	V	T	I	A	Q	G					biotin	CONH
H2a (86-106)	96	92	L	A	I	R	N	D	E	E	L	N	Δ	L	L	G	K	V	T	I	A	Q	G					biotin	CONH
H2a (86-106)	116	110	L	A	I	R	N	D	E	E	L	N	K	L	L	G	Δ	V	T	I	A	Q	G					biotin	CONH
H2a (86-106)	97	94	L	A	I	R	N	D	E	E	L	N	Δ	L	L	G	Δ	V	T	I	A	Q	G					biotin	CONH
H2a (112-130)	83	81	I	Q	A	V	L	L	P	K	K	T	E	S	H	H	K	A	K	G	K							biotin	COOH

H2a (112-130)	83	94	I	Q	A	V	L	L	P	K	Δ	T	E	S	H	H	K	A	K	G	K							biotin	COOH
H2a (112-130)	96	92	I	Q	A	V	L	L	P	K	Δ	Ω	E	S	H	H	K	A	K	G	K							biotin	COOH
H2a (112-130)	99	76	I	Q	A	V	L	L	P	K	K	Ω	E	S	H	H	K	A	K	G	K							biotin	COOH
H2b (1-21)	95	91	P	E	P	A	K	S	A	P	A	P	K	K	G	S	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	90	90	P	E	P	A	Δ	S	A	P	A	P	K	K	G	S	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	87	92	P	E	P	A	Φ	S	A	P	A	P	K	K	G	S	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	83	73	P	E	P	A	Π	S	A	P	A	P	K	K	G	S	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	95	91	P	E	P	A	Θ	S	A	P	A	P	K	K	G	S	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	85	78	P	E	P	A	K	S	A	P	A	P	K	Δ	G	S	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	86	91	P	E	P	A	K	S	A	P	A	P	K	K	G	Σ	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	88	85	P	E	P	A	K	S	A	P	A	P	K	Δ	G	Σ	K	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	97	93	P	E	P	A	K	S	A	P	A	P	K	K	G	Σ	Δ	K	A	V	T	K	A					biotin	CONH
H2b (1-21)	86	90	P	E	P	A	K	S	A	P	A	P	K	Δ	G	Σ	Δ	K	A	V	T	K	A					biotin	CONH
H2b (15-35)	104	98	K	K	A	V	T	K	A	Q	K	K	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	87	100	K	K	A	V	T	Δ	A	Q	K	K	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	84	97	K	K	A	V	T	Δ	A	Q	Φ	K	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	100	88	K	K	A	V	T	Δ	A	Q	Θ	K	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	110	99	K	K	A	V	T	K	A	Q	Φ	K	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	109	115	K	K	A	V	T	K	A	Q	Π	K	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	112	96	K	K	A	V	T	K	A	Q	Θ	K	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH

H2b (15-35)	96	94	K	K	A	V	T	K	A	Q	Φ	Δ	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	94	86	K	K	A	V	T	K	A	Q	Θ	Δ	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (15-35)	92	103	K	K	A	V	T	K	A	Q	K	Δ	D	G	K	K	R	K	R	S	R	K	E					biotin	CONH
H2b (27-47)	186	202	K	K	R	K	R	S	R	K	E	S	Y	S	V	Y	V	Y	K	V	L	K	Q					biotin	CONH
H2b (27-47)	152	184	K	K	R	K	R	Σ	R	K	E	S	Y	S	V	Y	V	Y	K	V	L	K	Q					biotin	CONH
H2b (27-47)	123	140	K	K	R	K	R	Σ	R	K	E	Σ	Y	S	V	Y	V	Y	K	V	L	K	Q					biotin	CONH
H2b (27-47)	176	175	K	K	R	K	R	S	R	K	E	Σ	Y	S	V	Y	V	Y	K	V	L	K	Q					biotin	CONH
H2b (27-47)	110	107	K	K	R	K	R	S	R	K	E	Σ	Y	S	V	Y	V	Y	Δ	V	L	K	Q					biotin	CONH
H2b (27-47)	104	86	K	K	R	K	R	S	R	K	E	S	Y	S	V	Y	V	Y	Δ	V	L	K	Q					biotin	CONH
H2b (37-74)	105	101	Y	S	V	Y	V	Y	K	V	L	K	Q	V	H	P	D	T	G	I	S	S	K					biotin	CONH
H2b (37-74)	89	97	Y	S	V	Y	V	Y	Φ	V	L	K	Q	V	H	P	D	T	G	I	S	S	K					biotin	CONH
H2b (37-74)	104	94	Y	S	V	Y	V	Y	Π	V	L	K	Q	V	H	P	D	T	G	I	S	S	K					biotin	CONH
H2b (37-74)	91	83	Y	S	V	Y	V	Y	Θ	V	L	K	Q	V	H	P	D	T	G	I	S	S	K					biotin	CONH
H3 (1-21)	99	115	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	96	108	A	Ξ	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	104	102	A	Ψ	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	95	83	A	Ψ	Ω	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	90	81	A	Ψ	Ω	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	90	103	A	Ψ	T	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	94	83	A	R	Ω	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH

H3 (1-21)	98	91	A	R	Ω	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	99	88	A	R	T	Φ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	92	89	A	R	T	Π	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	107	86	A	R	T	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	105	94	A	R	T	Θ	Q	T	A	R	Δ	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	97	97	A	R	T	Θ	Q	T	A	R	Θ	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	96	97	A	R	T	K	Q	T	A	R	Δ	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	98	81	A	R	T	K	Q	T	A	R	Δ	Σ	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	92	107	A	R	T	K	Q	T	A	R	Δ	S	Ω	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	92	75	A	R	T	K	Q	T	A	R	Δ	Σ	Ω	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	94	78	A	R	T	K	Q	T	A	R	Φ	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	95	88	A	R	T	K	Q	T	A	R	Π	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	95	89	A	R	T	K	Q	T	A	R	Θ	S	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	83	85	A	R	T	K	Q	T	A	R	Θ	S	Ω	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	107	98	A	R	T	K	Q	T	A	R	Θ	Σ	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	83	87	A	R	T	K	Q	T	A	R	Θ	Σ	Ω	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	99	94	A	R	T	K	Q	T	A	R	K	Σ	T	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	88	94	A	R	T	K	Q	T	A	R	K	S	Ω	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	81	93	A	R	T	K	Q	T	A	R	K	Σ	Ω	G	G	K	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	91	75	A	R	T	K	Q	T	A	R	K	Σ	Ω	G	G	Δ	A	P	R	K	Q	L	A					biotin	CONH

H3 (1-21)	93	62	A	R	T	K	Q	T	A	R	K	Σ	Ω	G	G	Θ	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	101	91	A	R	T	K	Q	T	A	R	K	S	Ω	G	G	Δ	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	83	71	A	R	T	K	Q	T	A	R	K	S	Ω	G	G	Θ	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	91	102	A	R	T	K	Q	T	A	R	K	S	T	G	G	Δ	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	107	86	A	R	T	K	Q	T	A	R	K	S	T	G	G	Δ	A	P	Ψ	K	Q	L	A					biotin	CONH
H3 (1-21)	87	78	A	R	T	K	Q	T	A	R	K	S	T	G	G	Φ	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	91	92	A	R	T	K	Q	T	A	R	K	S	T	G	G	Π	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	92	91	A	R	T	K	Q	T	A	R	K	S	T	G	G	Θ	A	P	R	K	Q	L	A					biotin	CONH
H3 (1-21)	104	86	A	R	T	K	Q	T	A	R	K	S	T	G	G	Θ	A	P	Ψ	K	Q	L	A					biotin	CONH
H3 (1-21)	102	87	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	Ξ	K	Q	L	A					biotin	CONH
H3 (1-21)	92	87	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	Ψ	K	Q	L	A					biotin	CONH
H3 (14-34)	87	95	K	A	P	R	K	Q	L	A	T	K	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	96	79	K	A	P	Ψ	Δ	Q	L	A	T	K	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	105	80	K	A	P	R	Δ	Q	L	A	T	K	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	93	90	K	A	P	R	Δ	Q	L	A	T	Θ	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	89	75	K	A	P	R	Δ	Q	L	A	T	Δ	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	92	89	K	A	P	R	Δ	Q	L	A	T	Δ	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	96	81	K	A	P	R	K	Q	L	A	T	Δ	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	97	91	K	A	P	R	K	Q	L	A	T	Δ	A	A	Ψ	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	98	83	K	A	P	R	K	Q	L	A	T	Δ	A	A	Ψ	Δ	S	A	P	A	T	G	G					biotin	CONH

H3 (14-34)	85	81	K	A	P	R	K	Q	L	A	T	Φ	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	96	97	K	A	P	R	K	Q	L	A	T	Π	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	99	95	K	A	P	R	K	Q	L	A	T	Θ	A	A	R	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	90	85	K	A	P	R	K	Q	L	A	T	Θ	A	A	Ψ	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	87	90	K	A	P	R	K	Q	L	A	T	Θ	A	A	Ψ	Δ	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	86	91	K	A	P	R	K	Q	L	A	T	K	A	A	Ξ	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	89	91	K	A	P	R	K	Q	L	A	T	K	A	A	Ψ	K	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	90	78	K	A	P	R	K	Q	L	A	T	K	A	A	Ψ	Δ	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	96	90	K	A	P	R	K	Q	L	A	T	K	A	A	Ψ	Δ	Σ	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	99	82	K	A	P	R	K	Q	L	A	T	K	A	A	Ψ	K	Σ	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	86	89	K	A	P	R	K	Q	L	A	T	K	A	A	R	Δ	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	102	86	K	A	P	R	K	Q	L	A	T	K	A	A	R	Δ	Σ	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	88	72	K	A	P	R	K	Q	L	A	T	K	A	A	R	Φ	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	82	97	K	A	P	R	K	Q	L	A	T	K	A	A	R	Π	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	90	78	K	A	P	R	K	Q	L	A	T	K	A	A	R	Θ	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	85	76	K	A	P	R	K	Q	L	A	T	K	A	A	Ψ	Θ	S	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	90	81	K	A	P	R	K	Q	L	A	T	K	A	A	R	Θ	Σ	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	93	94	K	A	P	R	K	Q	L	A	T	K	A	A	Ψ	Θ	Σ	A	P	A	T	G	G					biotin	CONH
H3 (14-34)	91	83	K	A	P	R	K	Q	L	A	T	K	A	A	R	K	Σ	A	P	A	T	G	G					biotin	CONH
H3 (18-37)	99	94	K	Q	L	A	T	K	A	A	R	K	Σ	A	P	A	Ω	G	G	V	K	K	P					biotin	CONH

H3 (29-48)	88	84	S	A	P	A	T	G	G	V	K	K	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	88	84	S	A	P	A	Ω	G	G	V	K	K	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	95	69	S	A	P	A	T	G	G	V	Φ	K	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	88	79	S	A	P	A	T	G	G	V	Π	K	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	93	99	S	A	P	A	T	G	G	V	Θ	K	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	97	88	S	A	P	A	Ω	G	G	V	Θ	K	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	89	94	S	A	P	A	T	G	G	V	K	Φ	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	84	85	S	A	P	A	T	G	G	V	K	Π	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	95	89	S	A	P	A	T	G	G	V	K	Θ	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	96	86	S	A	P	A	Ω	G	G	V	K	Θ	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	86	86	S	A	P	A	T	G	G	V	Θ	Θ	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (29-48)	101	91	S	A	P	A	Ω	G	G	V	Θ	Θ	P	H	R	Y	R	P	G	T	V	A	L					biotin	CONH
H3 (72-92)	104	99	V	R	E	I	A	Q	D	F	K	T	D	L	R	F	Q	S	S	A	V	M	A					biotin	CONH
H3 (72-92)	96	84	V	R	E	I	A	Q	D	F	Φ	T	D	L	R	F	Q	S	S	A	V	M	A					biotin	CONH
H3 (72-92)	99	89	V	R	E	I	A	Q	D	F	Π	T	D	L	R	F	Q	S	S	A	V	M	A					biotin	CONH
H3 (72-92)	95	97	V	R	E	I	A	Q	D	F	Θ	T	D	L	R	F	Q	S	S	A	V	M	A					biotin	CONH
H3 (111-131)	83	79	C	A	I	H	A	K	R	V	T	I	M	P	K	D	I	Q	L	A	R	R	I					biotin	CONH
H3 (111-131)	86	99	C	A	I	H	A	Δ	R	V	T	I	M	P	K	D	I	Q	L	A	R	R	I					biotin	CONH
H3 (111-131)	99	89	C	A	I	H	A	Δ	R	V	Ω	I	M	P	K	D	I	Q	L	A	R	R	I					biotin	CONH
H3 (111-131)	81	97	C	A	I	H	A	K	R	V	Ω	I	M	P	K	D	I	Q	L	A	R	R	I					biotin	CONH

H3 (111-131)	81	92	C	A	I	H	A	K	R	V	T	I	M	P	Δ	D	I	Q	L	A	R	R	I					biotin	CONH
H3 (111-131)	86	84	C	A	I	H	A	K	R	V	Ω	I	M	P	Δ	D	I	Q	L	A	R	R	I					biotin	CONH
H3 (111-131)	93	94	C	A	I	H	A	Δ	R	V	Ω	I	M	P	Δ	D	I	Q	L	A	R	R	I					biotin	CONH
H3 (116-136)	104	92	K	R	V	T	I	M	P	K	D	I	Q	L	A	R	R	I	R	G	E	R	A					biotin	COOH
H3 (116-136)	106	97	K	R	V	T	I	M	P	K	D	I	Q	L	A	Ξ	R	I	R	G	E	R	A					biotin	COOH
H3 (116-136)	103	99	K	R	V	T	I	M	P	K	D	I	Q	L	A	Ψ	R	I	R	G	E	R	A					biotin	COOH
H3 (116-136)	101	105	K	R	V	T	I	M	P	Δ	D	I	Q	L	A	Ψ	R	I	R	G	E	R	A					biotin	COOH
H3 (1-21)	98	112	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	94	101	A	Ξ	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	101	109	A	Ψ	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	93	102	A	Ψ	Ω	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	93	81	A	Ψ	Ω	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	91	109	A	Ψ	T	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	98	97	A	R	Ω	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	99	116	A	R	Ω	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	99	91	A	R	T	Φ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	103	84	A	R	T	Π	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	98	94	A	R	T	Θ	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	87	96	A	R	T	Θ	Q	T	A	R	Δ	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	101	99	A	R	T	Θ	Q	T	A	R	Θ	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin

H3 (1-21)	98	84	A	R	T	K	Q	T	A	R	Δ	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	106	87	A	R	T	K	Q	T	A	R	Δ	Σ	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	81	94	A	R	T	K	Q	T	A	R	Δ	S	Ω	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	74	70	A	R	T	K	Q	T	A	R	Δ	Σ	Ω	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	88	88	A	R	T	K	Q	T	A	R	Φ	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	89	96	A	R	T	K	Q	T	A	R	Π	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	96	89	A	R	T	K	Q	T	A	R	Θ	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	100	94	A	R	T	K	Q	T	A	R	Θ	S	Ω	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	97	90	A	R	T	K	Q	T	A	R	Θ	Σ	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	96	101	A	R	T	K	Q	T	A	R	Θ	Σ	Ω	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	91	88	A	R	T	K	Q	T	A	R	K	Σ	T	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	94	89	A	R	T	K	Q	T	A	R	K	S	Ω	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (1-21)	98	80	A	R	T	K	Q	T	A	R	K	Σ	Ω	G	G	K	A	P	R	K	Q	L	A					NH2	biotin
H3 (4-24)	103	97	K	Q	T	A	R	K	Σ	Ω	G	G	Δ	A	P	R	K	Q	L	A	T	K	A					Ac	biotin
H3 (4-24)	90	99	K	Q	T	A	R	K	Σ	Ω	G	G	Θ	A	P	R	K	Q	L	A	T	K	A					Ac	biotin
H3 (4-24)	98	86	K	Q	T	A	R	K	S	Ω	G	G	Δ	A	P	R	K	Q	L	A	T	K	A					Ac	biotin
H3 (4-24)	85	80	K	Q	T	A	R	K	S	Ω	G	G	Θ	A	P	R	K	Q	L	A	T	K	A					Ac	biotin
H3 (4-24)	91	84	K	Q	T	A	R	Δ	S	T	G	G	Δ	A	P	R	K	Q	L	A	T	K	A					Ac	biotin
H3 (8-28)	91	102	R	K	S	T	G	G	Δ	A	P	R	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (8-28)	90	108	R	K	S	T	G	G	Δ	A	P	Ψ	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin

H3 (8-28)	98	78	R	K	S	T	G	G	Φ	A	P	R	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (8-28)	106	99	R	K	S	T	G	G	Π	A	P	R	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (8-28)	80	98	R	K	S	T	G	G	Θ	A	P	R	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (8-28)	92	102	R	K	S	T	G	G	Θ	A	P	Ψ	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (8-28)	101	86	R	K	S	T	G	G	K	A	P	Ξ	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (8-28)	90	91	R	K	S	T	G	G	K	A	P	Ψ	K	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (8-28)	103	92	R	K	S	T	G	G	Δ	A	P	R	Δ	Q	L	A	T	K	A	A	R	K	S					Ac	biotin
H3 (12-32)	84	100	G	G	K	A	P	Ψ	Δ	Q	L	A	T	K	A	A	R	K	S	A	P	A	T					Ac	biotin
H3 (12-32)	91	89	G	G	K	A	P	R	Δ	Q	L	A	T	K	A	A	R	K	S	A	P	A	T					Ac	biotin
H3 (12-32)	92	98	G	G	K	A	P	R	Δ	Q	L	A	T	Θ	A	A	R	K	S	A	P	A	T					Ac	biotin
H3 (12-32)	93	94	G	G	K	A	P	R	Δ	Q	L	A	T	Δ	A	A	R	K	S	A	P	A	T					Ac	biotin
H3 (18-38)	106	85	K	Q	L	A	T	Δ	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (18-38)	92	91	K	Q	L	A	T	Δ	A	A	Ψ	K	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (18-38)	92	103	K	Q	L	A	T	Δ	A	A	Ψ	Δ	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (18-38)	88	102	K	Q	L	A	T	Φ	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (18-38)	89	100	K	Q	L	A	T	Π	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (18-38)	91	97	K	Q	L	A	T	Θ	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (18-38)	104	95	K	Q	L	A	T	Θ	A	A	Ψ	K	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (18-38)	82	99	K	Q	L	A	T	Θ	A	A	Ψ	Δ	S	A	P	A	T	G	G	V	K	K	P					Ac	biotin
H3 (20-40)	96	89	L	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin

H3 (20-40)	101	114	L	A	T	K	A	A	Ξ	K	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	104	117	L	A	T	K	A	A	Ψ	K	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	104	97	L	A	T	K	A	A	Ψ	Δ	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	97	95	L	A	T	K	A	A	Ψ	Δ	Σ	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	92	93	L	A	T	K	A	A	Ψ	K	Σ	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	88	77	L	A	T	K	A	A	R	Δ	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	102	88	L	A	T	K	A	A	R	Δ	Σ	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	106	97	L	A	T	K	A	A	R	Φ	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	95	89	L	A	T	K	A	A	R	Π	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	100	88	L	A	T	K	A	A	R	Θ	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	95	96	L	A	T	K	A	A	Ψ	Θ	S	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	86	94	L	A	T	K	A	A	R	Θ	Σ	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	93	94	L	A	T	K	A	A	Ψ	Θ	Σ	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (20-40)	96	108	L	A	T	K	A	A	R	K	Σ	A	P	A	T	G	G	V	K	K	P	H	R					Ac	biotin
H3 (1-8)	100	90	A	R	T	K	Q	T	A	R																		NH2	K-Ahx-biotin
H3 (1-13)	104	97	A	R	T	A	Q	T	A	R	K	S	T	G	G													NH2	K-Ahx-biotin
H3 (1-20)	91	86	A	R	T	A	Q	T	A	R	R	S	T	G	G	K	A	P	R	A	Q	L						NH2	K-Ahx-biotin
H3 (1-20)	102	89	A	R	T	A	Q	T	A	R	R	S	T	G	G	A	A	P	R	K	Q	L						NH2	K-Ahx-biotin
H3 (1-20)	85	83	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L						NH2	K-Ahx-biotin
H3 (1-20)	88	102	A	R	T	A	Q	T	A	R	A	S	T	G	G	K	A	P	R	K	Q	L						NH2	K-Ahx-biotin

H3 (1-20)	99	91	A	R	T	A	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L						NH2	K-Ahx-biotin
H3 (1-20)	87	82	A	R	T	K	Q	T	A	R	A	S	T	G	G	K	A	P	R	K	Q	L						NH2	K-Ahx-biotin
H3 (1-20)	100	90	A	R	T	R	Q	T	A	R	R	S	T	G	G	K	A	P	R	K	Q	L						NH2	K-Ahx-biotin
H3 (1-20)	97	80	A	R	T	R	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L						NH2	K-Ahx-biotin
H3 (1-20)	93	85	A	R	T	K	Q	T	A	R	R	S	T	G	G	K	A	P	R	K	Q	L						NH2	K-Ahx-biotin
H3 (1-21)	98	104	A	R	T	K	Q	T	A	R	A	S	T	G	G	A	A	P	R	K	Q	L	A					NH2	GGK-Ahx-biotin
H3 (1-21)	100	94	A	R	T	A	Q	T	A	R	K	S	T	G	G	A	A	P	R	K	Q	L	A					NH2	GGK-Ahx-biotin
H3 (1-21)	100	84	A	ß	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	GGK-biotin
H3 (21-44)	90	99	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	104	102	A	T	A	A	A	R	Θ	S	A	P	A	T	G	G	V	A	A	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	94	95	A	T	K	A	A	R	Θ	S	A	P	A	T	G	G	V	K	K	P	H	R	Y	R	P	G		NH2	GK-biotin
H3 (21-44)	95	103	A	T	A	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	97	88	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	A	K	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	93	102	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	K	A	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	85	102	A	T	A	A	A	R	K	S	A	P	A	T	G	G	V	A	K	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	98	93	A	T	A	A	A	R	K	S	A	P	A	T	G	G	V	K	A	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	93	104	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	A	A	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	88	101	A	T	A	A	A	R	A	S	A	P	A	T	G	G	V	A	A	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	98	97	A	T	K	A	A	R	A	S	A	P	A	T	G	G	V	K	K	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	99	96	A	T	A	A	A	R	A	S	A	P	A	T	G	G	V	A	K	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin

H3 (21-44)	90	104	A	T	A	A	A	R	A	S	A	P	A	T	G	G	V	K	A	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (21-44)	94	97	A	T	K	A	A	R	A	S	A	P	A	T	G	G	V	A	A	P	H	R	Y	R	P	G		NH2	GK-Ahx-biotin
H3 (1-21)	93	73	A	R	T	K	Q	T	A	Φ	K	S	T	G	G	K	A	P	R	K	Q	L	A					NH2	K-biotin
H3 (1-21)	91	86	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	ß	K	Q	L	A					NH2	GGK-biotin
H3 (1-21)	99	97	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	Φ	Q	L	A					NH2	GGK-biotin
H3 (1-21)	94	93	A	R	T	K	Q	T	A	R	K	S	T	G	G	K	A	P	R	Π	Q	L	A					NH2	GGK-biotin
H3 (1-21)	87	80	A	R	T	K	Q	T	A	R	K	Σ	T	G	G	Δ	A	P	R	K	Q	L	A					NH2	GGK-biotin
H3 (21-44)	89	87	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	Φ	K	P	H	R	Y	R	P	G		NH2	GK-biotin
H3 (21-44)	94	90	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	Π	K	P	H	R	Y	R	P	G		NH2	GK-biotin
H3 (21-44)	90	80	A	T	K	A	A	R	K	S	A	P	A	T	G	G	V	Θ	K	P	H	R	Y	R	P	G		NH2	GK-biotin
H3 (21-44)	93	89	A	T	Θ	A	A	R	K	S	A	P	A	T	G	G	V	K	K	P	H	R	Y	R	P	G		NH2	GK-biotin
H3 (44-63)	91	87	G	T	V	A	L	R	E	I	R	R	Y	Q	K	S	T	E	L	L	I	R						NH2	GGK-biotin
H4 (1-16)	85	81	S	G	ß	G	K	G	G	K	G	L	G	K	G	G	A	K										NH2	Ahx-C-biotin
H4 (1-21)	104	90	S	G	ß	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	K-Ahx-Biotin
H4 (1-23)	93	99	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	Θ	V	L	R			NH2	GGK-biotin
H4 (1-23)	97	105	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	Φ	V	L	R			NH2	GGK-biotin
H4 (1-25)	144	168	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V	L	R	D	N	NH2	GK-biotin-Ahx
H4 (11-30)	103	86	G	K	G	G	A	K	R	H	R	K	V	L	R	D	N	I	Q	G	I	T						NH2	GK-biotin-Ahx
H4 (1-23)	95	75	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	Π	V	L	R			NH2	GGK-biotin
H4 (1-21)	134	154	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin

H4 (1-21)	149	176	Σ	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	122	126	S	G	Ξ	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	116	143	S	G	Ψ	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	101	97	S	G	Ψ	G	Δ	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	97	106	Σ	G	Ψ	G	Δ	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	99	97	S	G	R	G	Δ	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	92	99	S	G	R	G	Δ	G	G	Δ	G	L	G	Δ	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	92	103	S	G	R	G	Δ	G	G	Δ	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	104	99	S	G	R	G	K	G	G	Δ	G	L	G	K	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	99	103	S	G	R	G	K	G	G	Δ	G	L	G	Δ	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	123	139	S	G	R	G	K	G	G	K	G	L	G	Φ	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	126	140	S	G	R	G	K	G	G	K	G	L	G	Π	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	111	120	S	G	R	G	K	G	G	K	G	L	G	Θ	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	93	106	S	G	R	G	K	G	G	Δ	G	L	G	Φ	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (1-21)	96	96	S	G	R	G	K	G	G	Δ	G	L	G	Θ	G	G	A	K	R	H	R	K	V					NH2	biotin
H4 (6-26)	102	91	G	G	K	G	L	G	Φ	G	G	A	Δ	R	H	R	K	V	L	R	D	N	I					Ac	biotin
H4 (6-26)	100	96	G	G	K	G	L	G	Θ	G	G	A	Δ	R	H	R	K	V	L	R	D	N	I					Ac	biotin
H4 (6-26)	85	80	G	G	K	G	L	G	K	G	G	A	Δ	R	H	R	K	V	L	R	D	N	I					Ac	biotin
H4 (11-31)	97	95	G	K	G	G	A	K	R	H	R	K	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin
H4 (11-31)	108	89	G	K	G	G	A	K	R	H	R	Δ	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin

H4 (11-31)	95	88	G	K	G	G	A	Δ	R	H	R	Δ	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin
H4 (11-31)	102	112	G	K	G	G	A	Δ	R	H	R	Φ	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin
H4 (11-31)	106	97	G	K	G	G	A	Δ	R	H	R	Θ	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin
H4 (11-31)	97	107	G	K	G	G	A	K	R	H	R	Φ	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin
H4 (11-31)	94	97	G	K	G	G	A	K	R	H	R	Π	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin
H4 (11-31)	97	123	G	K	G	G	A	K	R	H	R	Θ	V	L	R	D	N	I	Q	G	I	T	K					Ac	biotin
H4 (1-21)	228	322	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	573	950	Σ	G	R	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	240	368	S	G	Ξ	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	179	227	S	G	Ψ	G	K	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	108	94	S	G	Ψ	G	Δ	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	99	105	Σ	G	Ψ	G	Δ	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	106	110	S	G	R	G	Δ	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	100	86	S	G	R	G	Δ	G	G	K	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	111	83	S	G	R	G	Δ	G	G	Δ	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	120	116	S	G	R	G	K	G	G	Δ	G	L	G	K	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	104	93	S	G	R	G	K	G	G	Δ	G	L	G	Δ	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	181	290	S	G	R	G	K	G	G	K	G	L	G	Φ	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	229	297	S	G	R	G	K	G	G	K	G	L	G	Π	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	107	146	S	G	R	G	K	G	G	K	G	L	G	Θ	G	G	A	K	R	H	R	K	V					biotin	CONH

H4 (1-21)	104	103	S	G	R	G	K	G	G	Δ	G	L	G	Φ	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	105	81	S	G	R	G	K	G	G	Δ	G	L	G	Θ	G	G	A	K	R	H	R	K	V					biotin	CONH
H4 (1-21)	123	124	S	G	R	G	K	G	G	K	G	L	G	Φ	G	G	A	Δ	R	H	R	K	V					biotin	CONH
H4 (1-21)	111	89	S	G	R	G	K	G	G	K	G	L	G	Θ	G	G	A	Δ	R	H	R	K	V					biotin	CONH
H4 (1-21)	118	111	S	G	R	G	K	G	G	K	G	L	G	K	G	G	A	Δ	R	H	R	K	V					biotin	CONH
H4 (13-33)	99	80	G	G	A	K	R	H	R	K	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (13-33)	97	81	G	G	A	K	R	H	R	Δ	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (13-33)	88	86	G	G	A	Δ	R	H	R	Δ	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (13-33)	102	85	G	G	A	Δ	R	H	R	Φ	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (13-33)	93	99	G	G	A	Δ	R	H	R	Θ	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (13-33)	95	102	G	G	A	K	R	H	R	Φ	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (13-33)	91	100	G	G	A	K	R	H	R	Π	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (13-33)	88	98	G	G	A	K	R	H	R	Θ	V	L	R	D	N	I	Q	G	I	T	K	P	A					biotin	CONH
H4 (43-63)	95	99	V	K	R	I	S	G	L	I	Y	E	E	T	R	G	V	L	K	V	F	L	E					biotin	CONH
H4 (43-63)	96	67	V	K	R	I	Σ	G	L	I	Y	E	E	T	R	G	V	L	K	V	F	L	E					biotin	CONH
H4 (43-63)	95	93	V	K	R	I	S	G	L	I	Y	E	E	T	R	G	V	L	Δ	V	F	L	E					biotin	CONH
H4 (51-71)	107	91	Y	E	E	T	R	G	V	L	K	V	F	L	E	N	V	I	R	D	A	V	T					biotin	CONH
H4 (51-71)	105	91	Y	E	E	T	R	G	V	L	Δ	V	F	L	E	N	V	I	R	D	A	V	T					biotin	CONH
H4 (68-88)	101	83	D	A	V	T	Y	T	E	H	A	K	R	K	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (68-88)	103	97	D	A	V	T	Y	T	E	H	A	Δ	R	K	T	V	T	A	M	D	V	V	Y					biotin	CONH

H4 (68-88)	103	90	D	A	V	T	Y	T	E	H	A	Δ	R	Δ	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (68-88)	101	88	D	A	V	T	Y	T	E	H	A	K	R	Δ	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (68-88)	96	76	D	A	V	T	Y	T	E	H	A	Δ	R	Φ	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (68-88)	97	81	D	A	V	T	Y	T	E	H	A	Δ	R	Θ	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (68-88)	93	80	D	A	V	T	Y	T	E	H	A	K	R	Φ	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (68-88)	95	91	D	A	V	T	Y	T	E	H	A	K	R	Π	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (68-88)	98	97	D	A	V	T	Y	T	E	H	A	K	R	Θ	T	V	T	A	M	D	V	V	Y					biotin	CONH
H4 (82-102)	94	85	T	A	M	D	V	V	Y	A	L	K	R	Q	G	R	T	L	Y	G	F	G	G					biotin	COOH
H4 (82-102)	95	91	T	A	M	D	V	V	Y	A	L	K	Ξ	Q	G	R	T	L	Y	G	F	G	G					biotin	COOH
H4 (82-102)	100	91	T	A	M	D	V	V	Y	A	L	K	Ψ	Q	G	R	T	L	Y	G	F	G	G					biotin	COOH

Δ	=	acetyl-lysine
Φ	=	monomethyl-lysine
Π	=	dimethyl-lysine
Θ	=	trimethyl-lysine
Σ	=	phospho-serine
Ω	=	phospho-threonine
Ξ	=	monomethyl-arginine
ß	=	symmetric dimethyl-arginine
Ψ	=	asymmetric dimethyl-arginine
A	=	alanine mutations
R	=	arginine mutations

Supplementary table 1.

Peptide library screening. A library of 327 peptides was tested as Smyd3 substrates. Peptides were derived from histones H2a, H2b, H3, and H4 and possessed various post-translational modifications, including acetylation, methylation and phosphorylation, as noted in the key. Results are from two independent measurements and expressed as percent of control.

SUPPLEMENTARY TABLE 2

HeLa cells

Histone H3	Control	siRNA SMYD3	Control/siRNA SMYD3
TKQTAR			
K4un	93.35	91.78	1.02
K4me1	6.59	8.14	0.81
K4me2	0.04	0.05	0.82
K4me3	0.03	0.04	0.76
KSTGGKAPR			
K9unK14un	13.31	11.03	1.21
K9me1K14un	10.86	10.44	1.04
K9me2K14un	26.01	27.50	0.95
K9me3K14un	18.83	21.85	0.86
K9unK14ac	3.67	3.84	0.96
KSAPATGGVKKPHR			
K27unK36un	3.69	3.08	1.20
K27unK36me1	1.52	1.74	0.88
K27me1K36un	9.67	11.87	0.81
K27me2K36un	22.26	21.38	1.04
K27unK36me2	4.78	3.42	1.40
K27me3K36un	12.99	11.12	1.17
K27me1K36me1	5.49	5.07	1.08
K27me2K36me1	14.18	13.53	1.05
K27me1K36me2	11.44	13.71	0.83
K27me2K36me2	2.54	3.48	0.73
K27me3K36me1	6.06	5.64	1.08
K27me1K36me3	4.83	5.01	0.97
EIAQDFKTDLR			
K79un	84.76	84.84	1.00
K79me1	12.32	12.15	1.01
K79me2	2.92	3.01	0.97
Histone H4	**Control**	**siRNA SMYD3**	**Control/siRNA SMYD3**
KVLR			
K20un	10.68	11.89	0.90
K20me1	33.45	37.40	0.89
K20me2	55.12	49.66	1.11
K20me3	0.75	1.05	0.72
GKGGKGLGKGGAKR			
unmodified	65.16	70.09	0.93
1 Ac (mostly K16)	27.86	24.36	1.14
2 Ac (mostly K12 and K16)	5.71	4.68	1.22
3 Ac (mostly K5, K12 and K16)	1.06	0.72	1.48
4 Ac (K5, K8, K12 and K16)	0.09	0.11	0.79
K5 me1	0.12	0.03	**3.56**

MEF cells

Histone H3	WT MEF	SMYD3 KO MEF	WT/SMYD3 KO
TKQTAR			
K4un	91.26	90.69	1.01
K4me1	8.68	9.25	0.94
K4me2	0.04	0.05	0.76
K4me3	0.02	0.02	1.14
KSTGGKAPR			
K9unK14un	7.31	10.01	0.73
K9me1K14un	7.49	7.37	1.02
K9me2K14un	24.89	24.36	1.02
K9me3K14un	20.64	17.35	1.19
K9unK14ac	3.67	3.22	1.14
KSAPATGGVKKPHR			
K27unK36un	16.98	11.09	1.53
K27unK36me1	4.63	4.95	0.93
K27me1K36un	17.53	16.80	1.04
K27me2K36un	9.78	13.38	0.73
K27unK36me2	4.35	4.31	1.01
K27me3K36un	3.99	2.57	1.55
K27me1K36me1	8.55	11.58	0.74
K27me2K36me1	13.50	14.19	0.95
K27me1K36me2	7.11	10.78	0.66
K27me2K36me2	3.15	1.90	1.66
K27me3K36me1	7.09	5.40	1.31
K27me1K36me3	3.34	3.05	1.09
EIAQDFKTDLR			
K79un	87.85	90.75	0.97
K79me1	10.53	8.02	1.31
K79me2	1.63	1.23	1.33
Histone H4	**WT MEF**	**SMYD3 KO MEF**	**WT/SMYD3 KO**
KVLR			
K20un	14.10	13.62	1.04
K20me1	30.79	40.71	0.76
K20me2	54.68	45.12	1.21
K20me3	0.42	0.55	0.77
GKGGKGLGKGGAKR			
unmodified	62.01	63.54	0.98
1 Ac (mostly K16)	32.30	31.73	1.02
2 Ac (mostly K12 and K16)	4.81	3.82	1.26
3 Ac (mostly K5, K12 and K16)	0.69	0.72	0.96
4 Ac (K5, K8, K12 and K16)	0.12	0.17	0.74
K5 me1	0.06	0.02	2.80

Supplementary table 2.
The indicated post-translational modifications were quantified on Histones H3 and H4 in both HeLa and MEF cells with and without Smyd3 knockdown or knockout respectively