

Figure S1

Identification of non-histone target proteins of HDAC11 during myoblast differentiation. C2C12 cells stably expressing CV, wild-type HDAC11 or catalytically inactive HDAC11 were differentiated, and the samples were collected at day 0, 1, 3 and 5. Protein samples from cells expressing CV, HDAC11 or HDAC11 mutant at each day were separated by 2-dimensional gel electrophoresis and western blot analysis was performed using an anti-acetyl-lysine antibody. (A) Table showing results of identification of four candidate proteins. (B) Western blot analysis reconfirming protein identification by measurement of the lysine acetylation level of annexin A1. (Changes in the lysine acetylation level of annexin A1 are represented by an asterisk (*)).

A

Nonhistone Target	Acetylation pattern	Mascot score	Coverage	Sequences (Underline)
1 AnnexinA1 (1, 5day)	CV HDAC11 HDAC11 mut Anti-Acetyl 2D-Gel	1171	71%	MAMVSEFLKQARFLENQEQEYVQAVKSYKGGPGS AVSPYP SFNVSSDVAALHKAIMVKGVDEATIIDILTK RTNAQRQKI K AAYLGENGKPLDEVLRKALTGHLE EVVLA MLKTPAGFDA DELRGAMKGLGDEEDTLIEI LTTR SNEQIREINRVYREELKRDLAKDIT S DT SGDFR KAL LALAKGDRCC DL SVNQDLADTDARALYEAG ERRKGTDVNV FTTLT SRSYPHLRVFGNY GKYSQ HDMNKALDLELKGDIKCLTTIVKCATSTPAFFAEK LYEAMKGG TRHKALIRIMVSR SEIDMNE IKVFYQK KYG ISLQQAILE TKGDYEKILV <u>ALCGGN</u>
2 Voltage-dependent anion-selective Channel protein 1 (3day only)	CV HDAC11 HDAC11 mut Anti-Acetyl 2D-Gel	739	45%	MAVPPTYADLGSARDVFTK GYGFGLIKLDLK TKSE <u>NGLE FTSSG SANEITTKVNGSLETKYRWTE YGLT</u> <u>FTEKWNTDNT LGTEITVEDQ LARGLKLTFDSSFSP</u> <u>NTGKKAIKITGYKREHINLGCDDV FDIAGPSIRGAL</u> <u>VLGYEGVWLAGYQMNFFETSKSRVYQSNFAVGYKTD</u> <u>FQL HTNVNDGTEFGGSIYQKVNK KLETAVNLAWT A</u> <u>GNSNTRFG IAAKYQVDPDACFSAKVNN S SLIGLGYT</u> <u>QTLKPGIKLTL SALLDGNKVNAGGHKLGLE FQA</u>
3 Enolase 1B (3day only)	CV HDAC11 HDAC11 mut Anti-Acetyl 2D-Gel	2712	70%	MSILRIHAREIFDSRGNPTVEVDLYTAKGLFRAAVPS <u>GA STGIYEALERDNDKTRFMKGVSGAVEHINKTI</u> <u>APALVSKKVNVEGEKIDKLMIEDGTENKSKFGA</u> <u>NAILGVSLAVCKAGAVEKGVPLYRHIADLAGNPEVIL</u> <u>PVPAFNVINGG SHAGNKLAMQEFMILPVGA S SFRE</u> <u>AMRIGAEVYHNLKNVKEIKYKGDATNVGDEGGFAP</u> <u>NILENKEALELLKTAAKAGYTDQVIGMDVAA SE</u> <u>FYRSGKYDLDFK SPDDP SRYITPDQLADLYK SFVQN</u> <u>YPVVSIEDPPFDQDDWGAWQKFTA SA GIQVVGDDL</u> <u>TYTNPKRIAKAASEK SCNCLLLKVNQIG SVTE SLQAC</u> <u>KLAQ SNGWGVVMV SHRSGETEDTFIADLVVGLCTGQI</u> <u>KTGAPCRSERLAKYNQILRIEELGSKAKFAGR SFRN</u> <u>PLAK</u>
4 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 (0, 1, 3 day)	CV HDAC11 HDAC11 mut Anti-Acetyl 2D-Gel	325	24%	MSELDQLRQE AEQLKNQIRD ARKACADATL SQIT <u>NMIDPV GRICMTRRRLRGLAKIYAMHWGTD SRLL</u> <u>VSA SGGDKLI IWD SYTTNKV HAIPLRSSWVM TCAY</u> <u>APSGN YVACGGLDNI CSIYNLKTRE GNVRVRSRELA</u> <u>GHTGYL SCCRFDDNQIVTS SGGTTCALWDIETGG</u> <u>QTTTF TGHTGVMSL SLAPDTRLFVSGACDA SAKL</u> <u>WDVREGMCRQ TFGHESDIN AICFFPNGNA FATG</u> <u>SDDATCRFLDRLADQE LMTYSHDNII CGITSVFSK</u> <u>SGRLLLAGYD DFNCONMWDALKADRAGVLAG HON</u> <u>RVSLGVTDDGMAVATG SWDSFLKIWN</u>

B

IP: Anti-acetyl Lysine

*Annexin A1

Input

*Annexin A1

M2-Flag

HSP90

C	H	M	C	H	M
0 day			1 day		

C: control vector
H: HDAC11
M: HDAC11 (H142/143A)