Supplemental Information

HDAC inhibitor-induced mitotic arrest is mediated by Eg5/KIF11 acetylation

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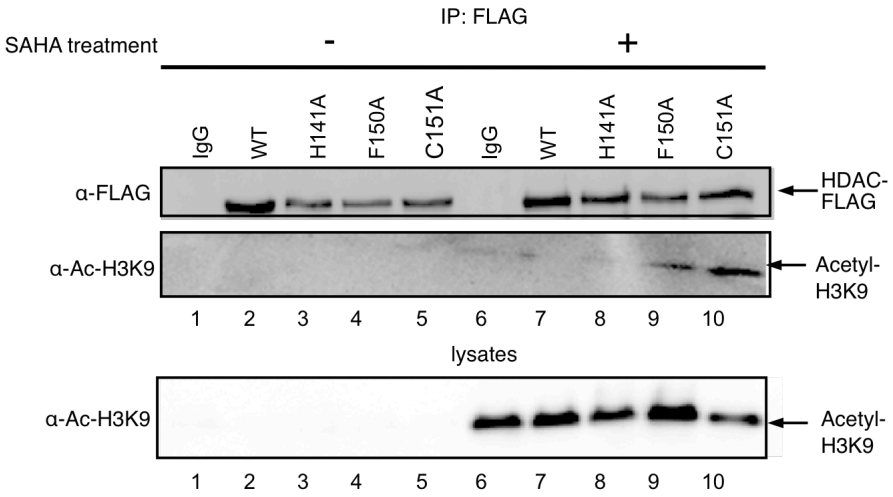
Trial 1



Trial 2

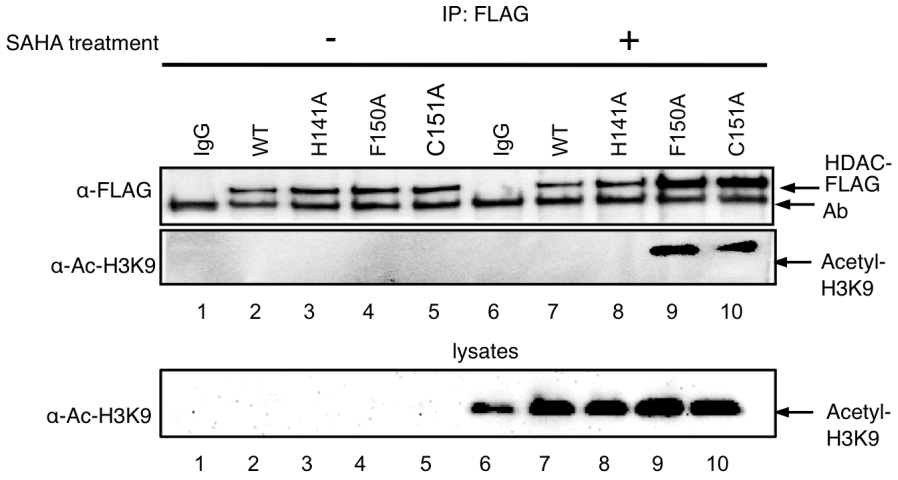


Figure S1. Independent trials used for histone binding assay, related to Figure 1. FLAG-tagged wild type or mutant HDAC1 were transfected into HEK293 cells, treated with or without SAHA (10 μM) for 24h, and then immunoprecipitated with a FLAG antibody, separated by SDS-PAGE, and immunoblotted with FLAG (top) or acetyl-H3K9 (bottom) antibodies. Lysates before immunoprecipitation (lysates) were probed with the acetyl-H3K9 as a control for equal acetylation in all cells. The third trial is shown in Figure 1D.

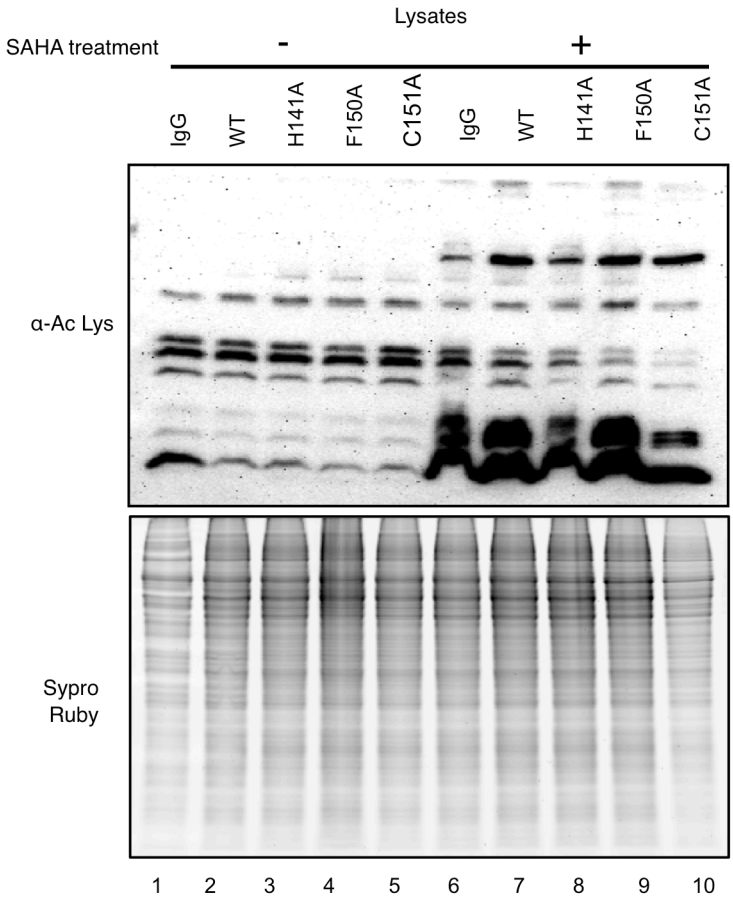


Figure S2. Acetylation levels of lysates expressing wild type and mutant HDAC1, related to Figure 1. FLAG-tagged wild type or mutant HDAC1 were transfected into HEK293 cells and treated with or without SAHA (10 uM) for 24h. After lysis, lysates were separated by SDS-PAGE and immunoblotted with acetyl lysine antibodies (top) or Sypro Ruby total protein stain (bottom). The lysates used here are the same lysates used in the Figures 1D. The results document that similar levels of acetylated proteins are observed with wild type and mutant expressing cells, showing that the mutants do not have a significant effect on acetylation levels.

Table S1- Proteins identified after trapping by mass spectrometry (MS), related to Figure 2.

Protein band	Protein name (Accession)	MW (kDa)	Sample	Unique peptide count[a]	Unique spectral Count[b]	% Coverage[c]
p130	Kinesin like protein 11(KIF11)/Eg5 (KIF11_HUMAN)	120	HDAC1 (WT)	ND[d]	ND[d]	ND[d]
			H141A	1	1	1
			F150A	2	2	3
			C151A	2	2	2
p45	Actin cytoplasmic 2 (ACTG_HUMAN)	42	HDAC1 (WT)	10	13	38
			H141A	11	15	49
			F150A	6	6	29
			C151A	4	8	24

[a] **Unique Peptide count -** Number of different amino acid sequences that are associated with a protein. [b] **Unique Spectral count –** Number of unique spectra that identified each unique peptide including modifications. [c]% Coverage – the percentage of the protein amino acid sequence was identified. [d] ND- Not Detected (No peptide ID observed by MS analysis after immunoprecipitation with the indicated HDAC1 protein).

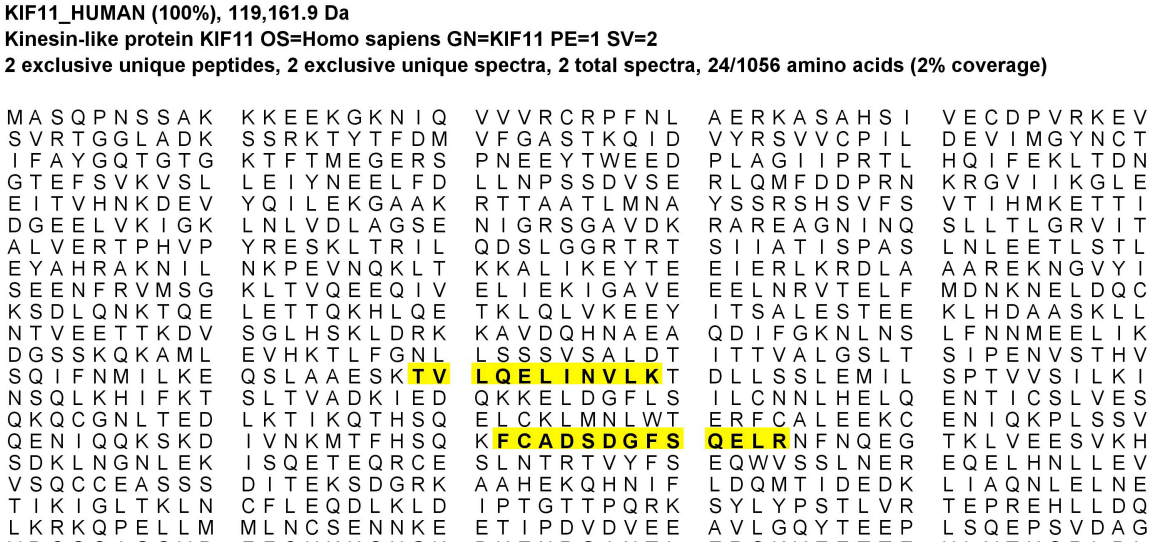
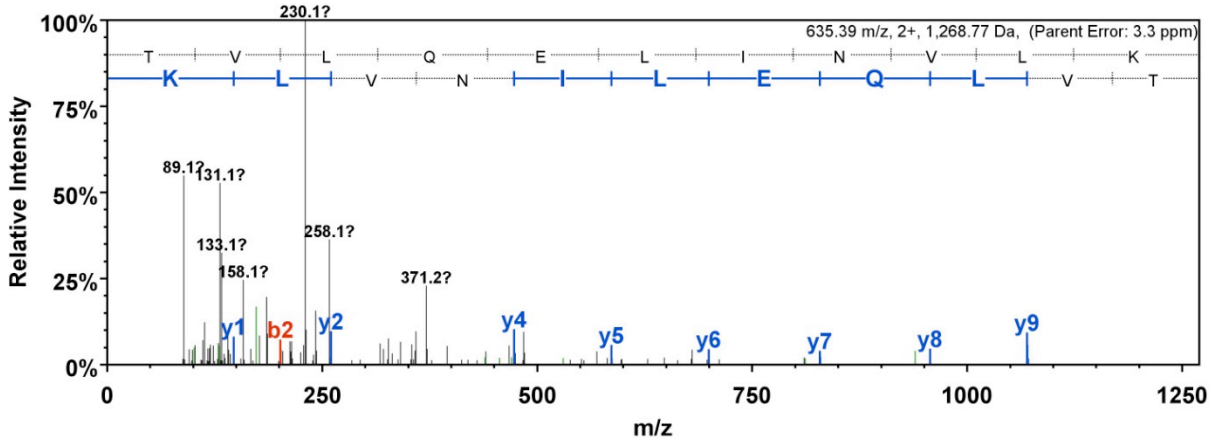


Figure S3 - Peptides of Eg5 (KIF11) observed in the substrate trapping experiment, related to Figure 2. Primary sequence of Eg5 (KIF11), which was identified in the substrate trapping experiment as p130. Peptides observed in the MS/MS analysis are highlighted in yellow. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2.

A) Peptide sequence- TVLQELINVLK (C151A-1, H141A- 1, F150A- 1)



B) Peptide sequence- FCADSDGFSQELR (C151A-2, H141A- 1, F150A- 3)

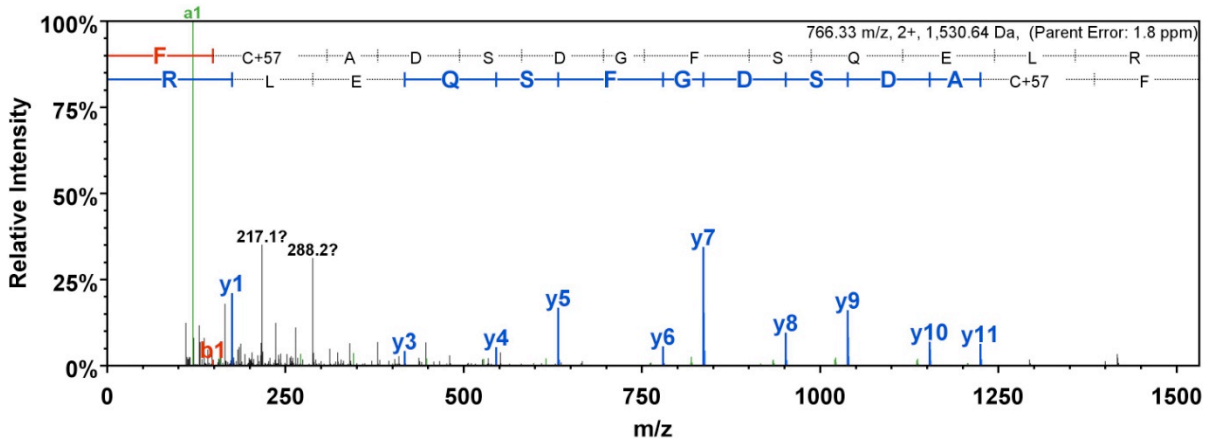


Figure S4 – Spectra of Eg5 (KIF11) peptides identified by MS/MS analysis, related to Figure 2. The annotated spectra of Eg5 (KIF11) peptides identified by MS/MS analysis (Figure S2). Each spectrum represents the different peptides identified from Eg5. The number of times each peptide was observed in each mutant sample is shown in parenthesis next to the peptide sequence.

ACTG_HUMAN (100%), 41,793.9 Da
Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1
8 exclusive unique peptides, 11 exclusive unique spectra, 15 total spectra, 120/375 amino acids (32% coverage)

MEEEIAALVI DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
TTTAERFIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
GNERFRCPEA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS
TFQQMWISKQ EYDESGPSIV HRKCF

Figure S5 - Peptides of actin observed in the substrate trapping experiment, related to Figure 2. Primary sequence of cytoplasmic actin identified in the substrate trapping experiment as p45. Peptide sequences observed in the MS/MS analysis are highlighted in yellow, while modified amino acids are in green. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2.

Peptide sequence- TTGIVmDSGDGVTHTVPIYEGYALPHAILR (C151A-2, WT HDAC1-1, H141A-1, F150A-2)

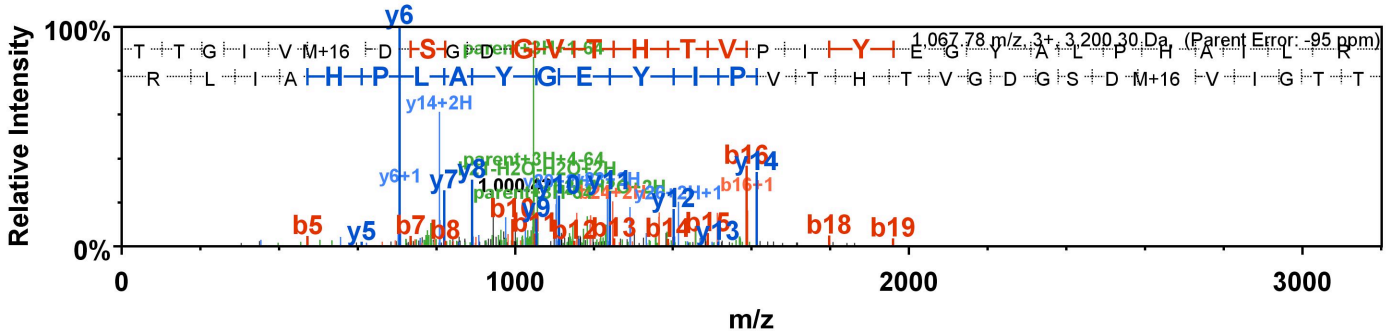


Figure S6– Spectra of actin peptides identified by MS/MS analysis, related to Figure 2. The annotated spectra of one of the cytoplasmic actin peptides identified by MS/MS analysis. The number of times each peptide was observed in each mutant sample is shown in parenthesis next to the peptide sequence.

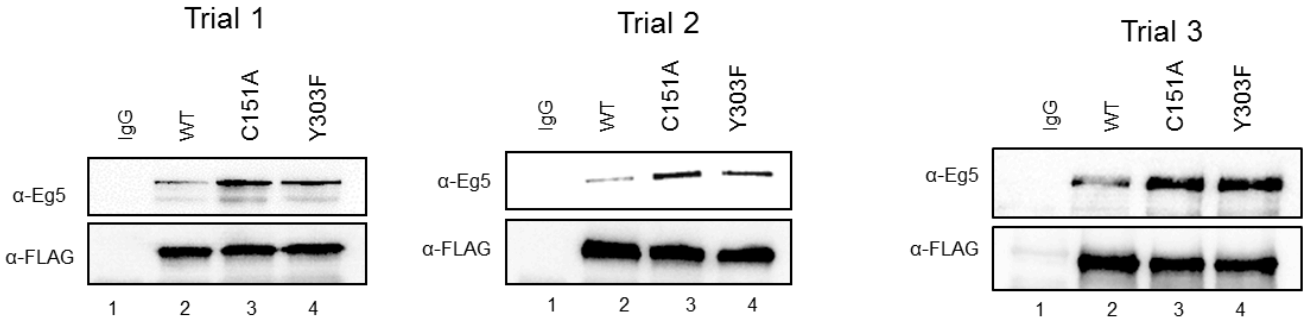
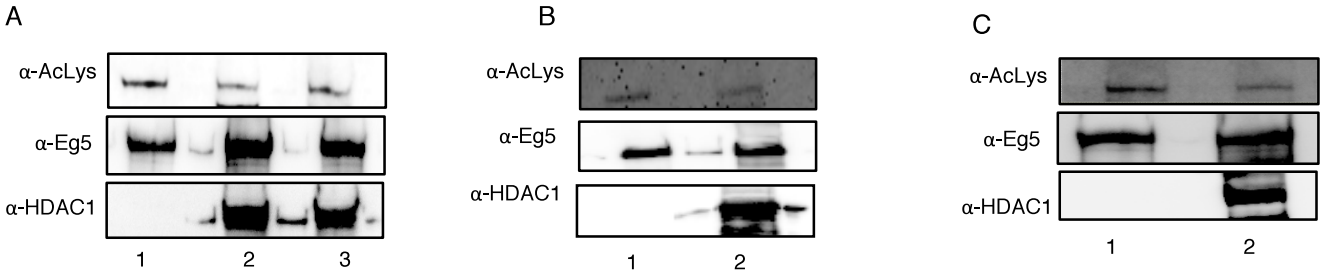


Figure S7- Substrate trapping with Y303F and C151A HDAC1 mutants, related to Figure 3. Wild type (WT) or mutant HDAC1 were expressed as FLAG-tagged proteins in HEK293 cells, immunoprecipitated (IP) with anti-FLAG agarose, separated by SDS-PAGE, and Western blotted with Eg5 (top) and FLAG (bottom) antibodies. The C151A and Y303F mutants trapped similar elevated levels of Eg5 compared to wild type HDAC1 (lanes 3 and 4 compared to lane 2, Eg5 blot), indicating that both mutants are effective for trapping of substrates.



D.

	Trial 1	Trial 2	Trial 3	Trial 4	Mean	Standard Error
rEg5 Ac-Lys	1	1	1	1	1	0.0
rEg5 Ac-Lys + rHDAC1	0.35	0.37	0.4	0.3	0.36	0.02
rEg5 Ac-Lys + rHDAC1 + SAHA	0.7	0.85			0.78	0.08

E.

	Trial 1	Trial 2	Trial 3	Trial 4	Mean	Standard Error
Eg5	1	1	1	1	1	0.0
rEg5 + rHDAC1	1	0.97	1.1	1.1	1	0.03
rEg5 + rHDAC1 + SAHA	0.98	0.95			0.97	0.02

F.

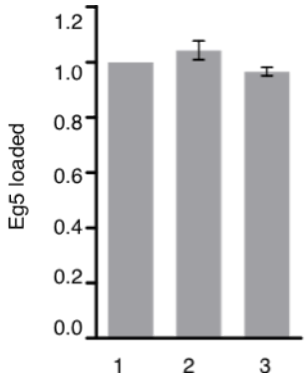
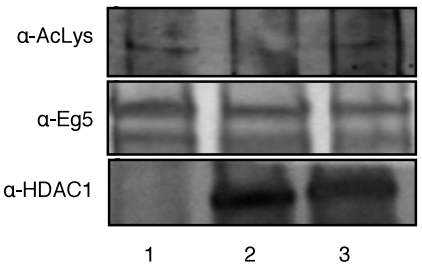
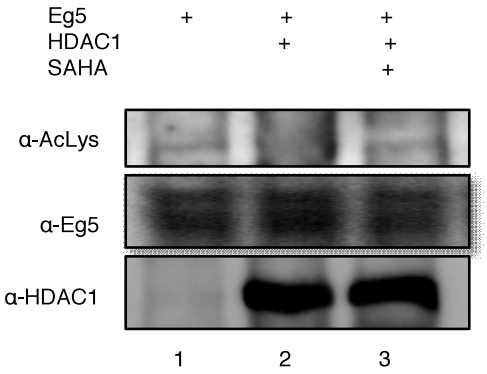


Figure S8 – Independent trials used for quantification of in vitro assay with recombinant HDAC1, related to Figure 4. A-C) Western blot analyses from four independent trials were quantified with the raw data shown as a table (D) or a histogram (Figure 4B in the manuscript). Three trials are shown here, with the fourth trial shown in Figure 4A. The components of each lane are shown in Figure 4A where lane 1 contains recombinant Eg5 alone, lane 2 contains recombinant Eg5 and recombinant HDAC1, and lane 3 contains recombinant Eg5, recombinant HDAC1, and SAHA. To assure equal protein loading, the Eg5 bands in the Eg5 western blot were quantified, with the data shown as a table (E) or histogram (F).

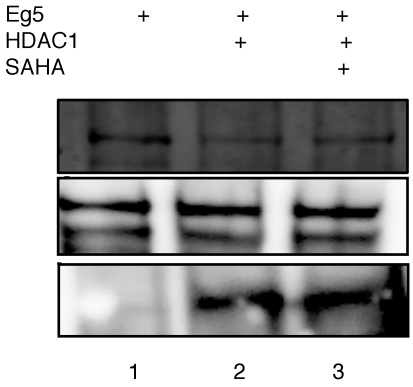
A. Trial 1



B. Trial 2



C. Trial 3



D.

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
Eg5 Ac-Lys	100	100	100	100	0.0
Eg5 Ac-Lys + HDAC1	12	11	16	13	2
Eg5 Ac-Lys + HDAC1 + SAHA	90	120	80	97	12

E.

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
Eg5	100	100	100	100	0.0
Eg5 + HDAC1	97	90	110	99	6
Eg5 + HDAC1 + SAHA	96	110	90	92	2

F.

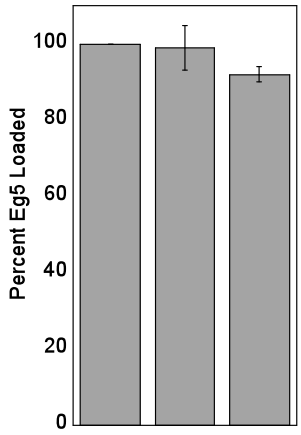


Figure S9– Independent trials used for quantification of deacetylation with cellular HDAC1, related to Figure 2. A-C) Western blots from three independent trials (Trial 1 in part A is shown in Figure 4C) were quantified with the raw data shown as a table (D) or histogram (Figure 4C in the manuscript). To assure equal protein loading, the Eg5 bands in the Eg5 western blot were quantified with the data shown as a table (E) or histogram (F).

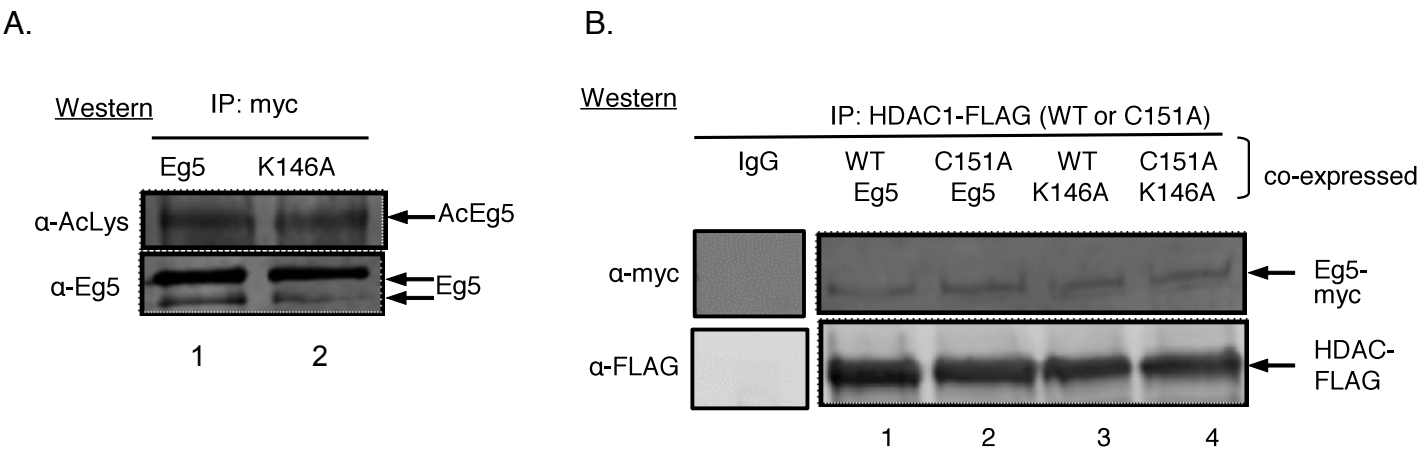


Figure S10 – K146 is not a predominant Eg5 acetylation site regulated by HDAC1, related to Figure 5. A) Myc-tagged wild type or K146A mutant Eg5 were transfected into HEK293 cells, treated with SAHA for 24h, and immunoprecipitated with a myc antibody. SDS-PAGE separation and immunoblotting was performed with acetyl lysine (top) and myc (bottom) antibodies. No change in the acetylation levels of the wild type and mutant proteins were observed (top gel), which indicated that K146 is not a predominant acetylation site on Eg5. B) FLAG-tagged wild type (WT) or C151A mutant HDAC1 were cotransfected with myc-tagged wild type or K146A mutant Eg5 into HEK293 cells. Wild type and C151A mutant HDAC1 were immunoprecipitated using anti-FLAG agarose beads, separated by SDS-PAGE, and immunoblotted with myc (top) and FLAG (bottom) antibodies. No changes in the levels of immunoprecipitated Eg5 were observed with either wild type or mutant proteins (top gel), which indicated that K146 is not a site bound and regulated by HDAC1.

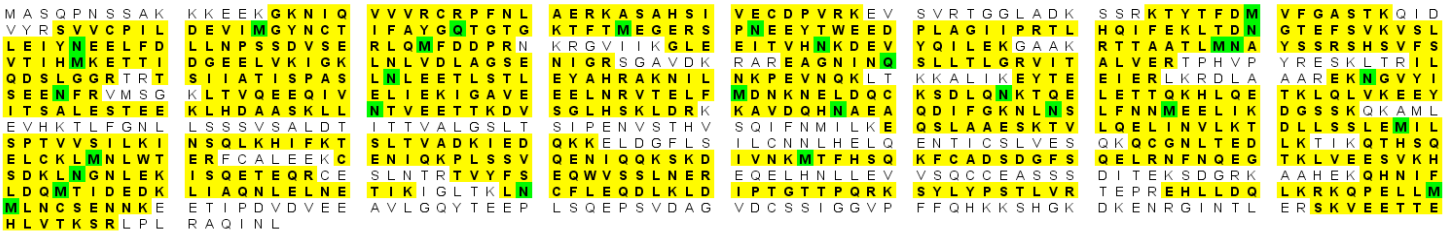
Table S2 – MS analysis of K890 in Eg5, related to Figure 5.

Protein name/ Accession number	Trial	Sample	% Coverage[a]	Unique # of acetylated peptides[b]
Kinesin like protein 11(KIF11)/Eg5 (KIF11_HUMAN)	1	Eg5+DMSO	71	0
		Eg5+SHI-1:2	72	1
		Eg5+SAHA	82	0
		Eg5+HDAC1	73	0
	2	Eg5+DMSO	80	0
		Eg5+SHI-1:2	69	1
		Eg5+SAHA	75	0
		Eg5+HDAC1	83	0

[a] **Unique # of acetylated peptides -** Number of unique peptide sequences containing an acetylated lysine. [b] **% Coverage –** The percentage of total amino acids in Eg5 that were represented in the peptides identified by LC-MS/MS.

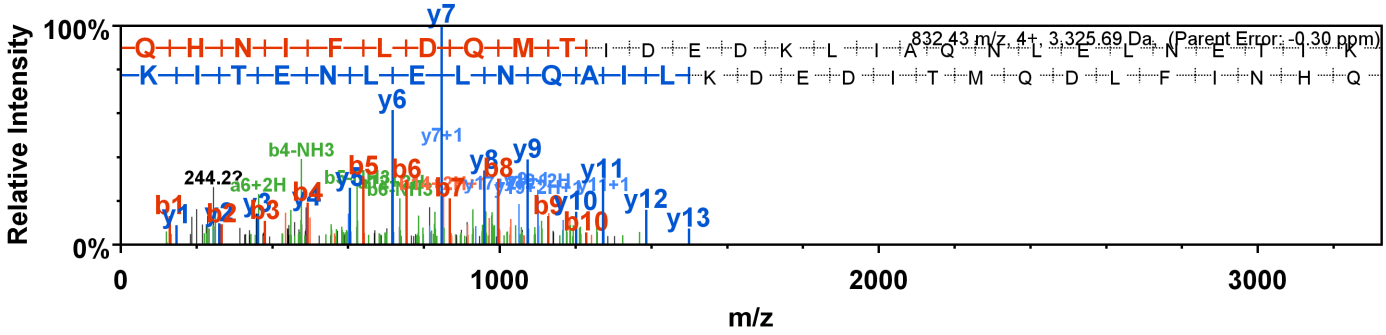
A. Eg5 + DMSO sample - sequence converage

KIF11_HUMAN (100%), 119,161.9 Da
Kinesin-like protein KIF11 OS=Homo sapiens GN=KIF11 PE=1 SV=2
89 exclusive unique peptides, 177 exclusive unique spectra, 438 total spectra, 745/1056 amino acids (71% coverage)



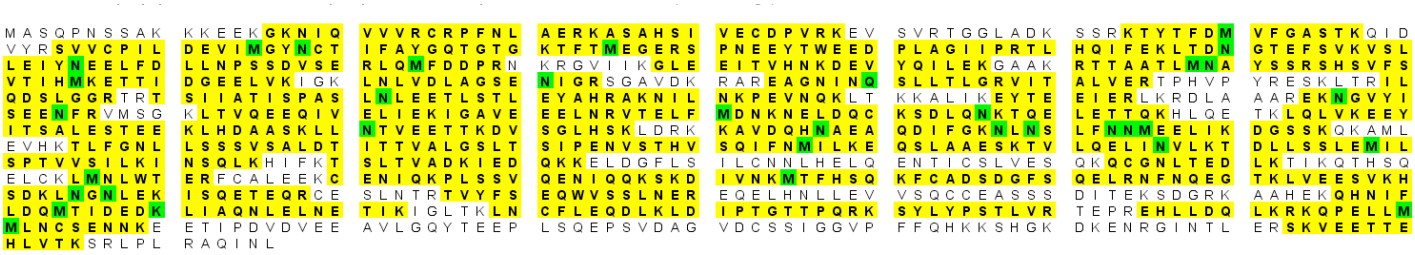
B. Eg5 + DMSO sample - peptide identified

Peptide sequence- (K)QHNIFLDQMTIDEDKLIAQNLELNETIK(I)



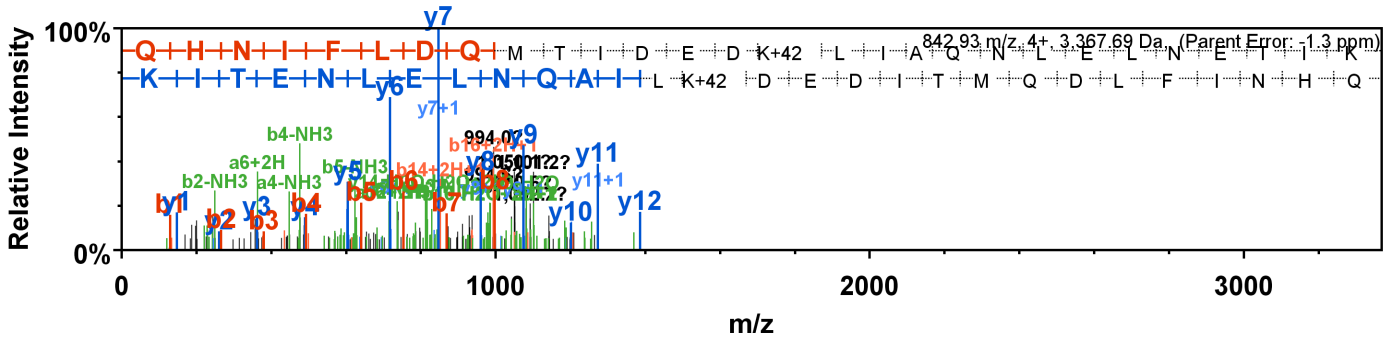
C. Eg5 + SHI-1:2 sample - sequence coverage

KIF11_HUMAN (100%), 119,161.9 Da
Kinesin-like protein KIF11 OS=Homo sapiens GN=KIF11 PE=1 SV=2
80 exclusive unique peptides, 178 exclusive unique spectra, 584 total spectra, 763/1056 amino acids (72% coverage)



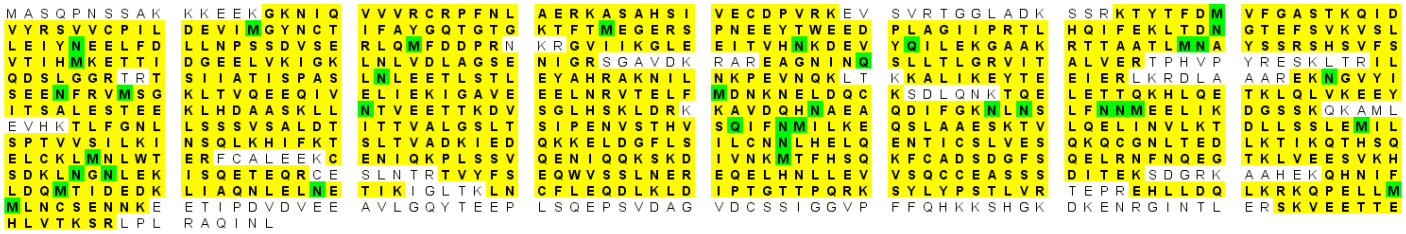
D. Eg5 + SHI-1:2 sample - peptide identified

Peptide sequence- (K)QHNIFLDQMTIDEDKAcLIAQNLELNETIK(I)



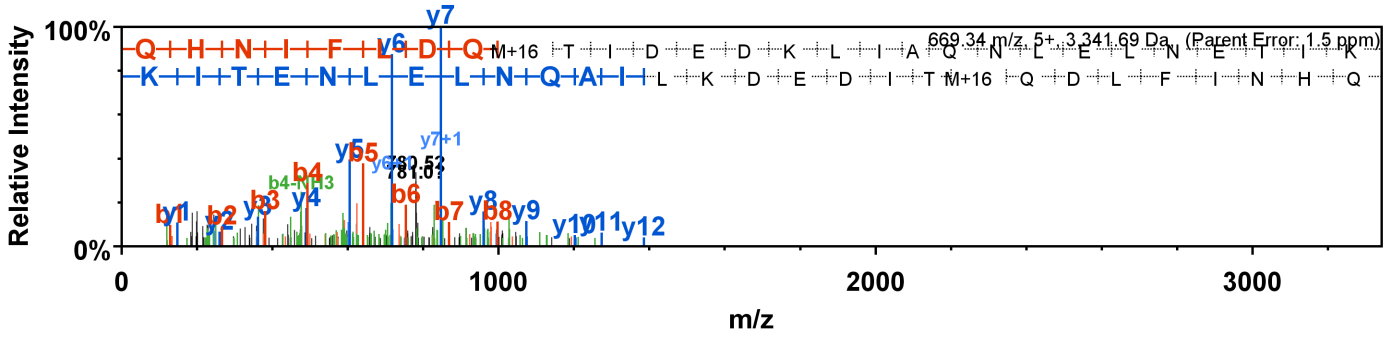
E. Eg5 + SAHA sample - sequence coverage

KIF11_HUMAN (100%), 119,161.9 Da
Kinesin-like protein KIF11 OS=Homo sapiens GN=KIF11 PE=1 SV=2
100 exclusive unique peptides, 211 exclusive unique spectra, 794 total spectra, 866/1056 amino acids (82% coverage)



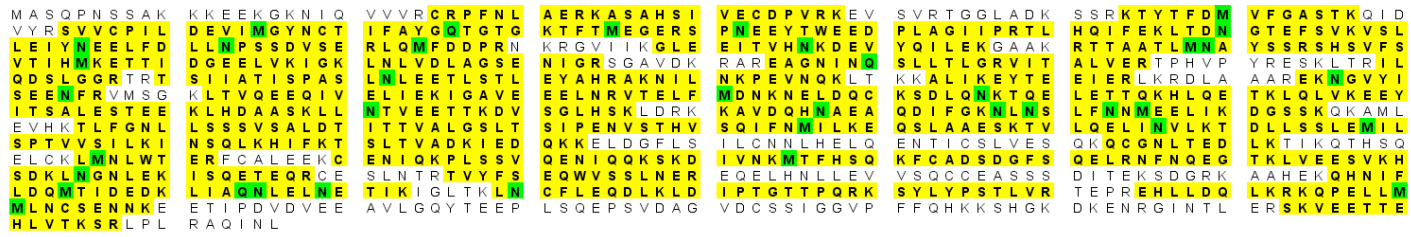
F. Eg5 + SAHA sample - peptide identified

Peptide sequence- (K)QHNIFLDQMTIDEDKLIAQNLELNETIK(I)



G. Eg5 + overexpressed HDAC1 sample - sequence coverage

KIF11_HUMAN (100%), 119,161.9 Da
Kinesin-like protein KIF11 OS=Homo sapiens GN=KIF11 PE=1 SV=2
91 exclusive unique peptides, 206 exclusive unique spectra, 723 total spectra, 773/1056 amino acids (73% coverage)



H. Eg5 + overexpressed HDAC1 sample - peptide identified

Peptide sequence- (K)QHNIFLDQMTIDEDKLIAQNLELNETIK(I)

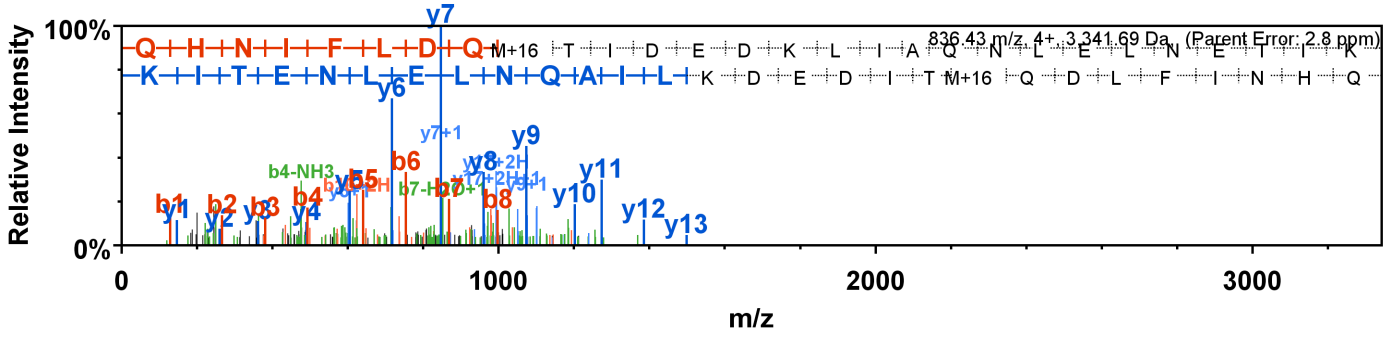
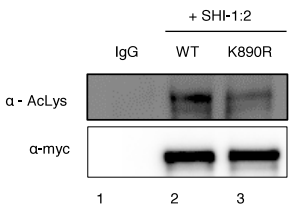
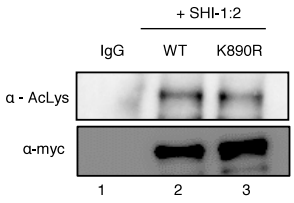


Figure S11- MS analysis of K890, related to Figure 5. A) Primary sequence of Eg5 (KIF11) identified in the control DMSO-treated sample. Peptides observed in the LC-MS/MS analysis are highlighted in yellow, with modified amino acids in green. The parameters were set to protein threshold 95%, peptide threshold 99% with minimum number of peptides set to 2 for all samples discussed here. B) Spectra of the Eg5 peptide containing K890 from control DMSO-treated sample identified by LC-MS/MS analysis (unacetylated K890 shown in red). C) Primary sequence of Eg5 (KIF11) identified in the SHI-1:2-treated sample. Peptides observed in the MS/MS analysis are highlighted in yellow, with modified amino acids in green. D) Spectra of Eg5 peptide containing acetylated K890 from the SHI-1:2-treated sample identified by MS/MS analysis (acetylated K890 shown in red). E) Primary sequence of Eg5 (KIF11) identified in the SAHA-treated sample. Peptides observed in the LC-MS/MS analysis are highlighted in yellow. F) Spectra of Eg5 peptide containing K890 from SAHA treated sample identified by MS/MS analysis (unacetylated K890 shown in red). G) Primary sequence of Eg5 (KIF11) identified in the Eg5 sample where HDAC1 was overexpressed. Peptides observed in the MS/MS analysis are highlighted in yellow. D) Spectra of Eg5 peptide containing K890 from Eg5 where HDAC1 was overexpressed identified by LC-MS/MS analysis (unacetylated K890 shown in red).

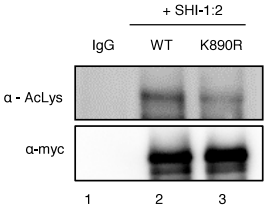
A. Trial 1



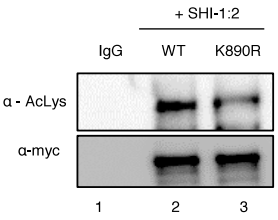
B. Trial 2



C. Trial 3



D. Trial 4



E.

	Trial 1	Trial 2	Trial 3	Trial 4	Mean	Standard Error
Eg5 AcLys	100	100	100	100	100	0.0
K890R AcLys	31	28	29	32	30	1

F.

	Trial 1	Trial 2	Trial 3	Trial 4	Mean	Standard Error
Eg5-myc	100	100	100	100	100	0.0
K890R myc	100	104	102	104	103	1

G.

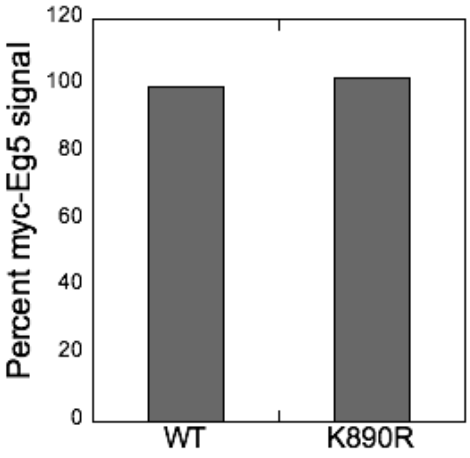


Figure S12 – **Independent trials used for quantification of K890R acetylation, related to Figure 5**. A-D) Western blots from four independent trials (Trial 1 in part A is shown in Figure 5A) were quantified with the raw data shown as a table (E) or histogram (Figure 5B in the manuscript). To assure equal protein loading, the Eg5-myc bands in the myc western blot were quantified with the data shown as a table (F) or histogram (G).

Table S3 – ATPase activity of Eg5*, related to Figure 5.

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
NT	-0.13	0.04	-0.16	-0.08	0.06
WT SHI-1:2	1	1	1	1	0.0
K890R SHI-1:2	3.6	3.6	3.9	3.7	0.1

*Data is depicted in Figure 5E. Negative values in the nontreated (NT) control were due to background correction.

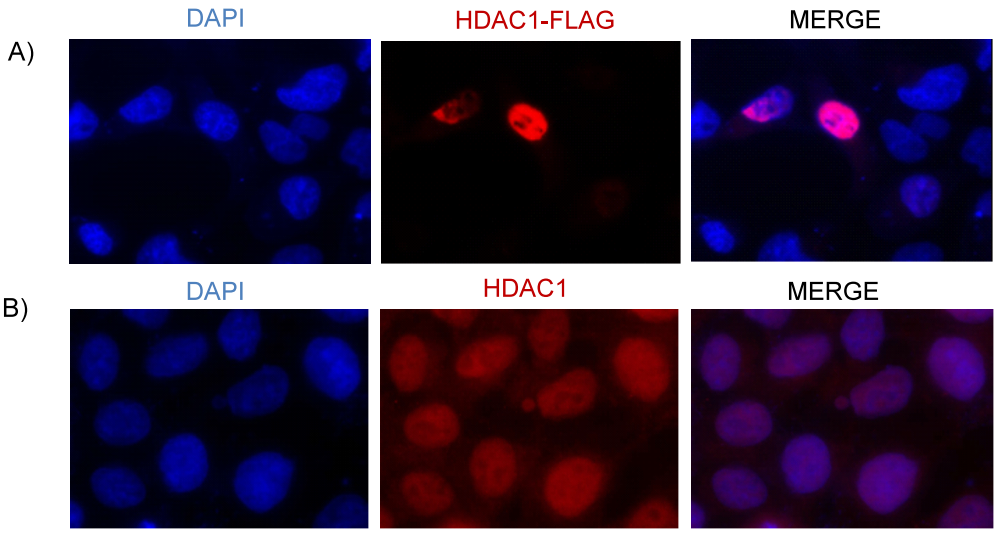


Figure S13 – Endogenous and overexpressed HDAC1 localize to the nucleus, related to Figure 6. HEK293 cells expressing FLAG tagged HDAC1 (A) or HEK293 cells alone (B) were fixed and stained with FLAG (A) or HDAC1 (B) antibodies (red). Cells were counterstained with DAPI (blue) and visualized using fluorescence microscopy. Both endogenous and overexpressed HDAC1 were predominantly nuclear. The data suggests that the FLAG tag does not affect the localization of HDAC1-FLAG.

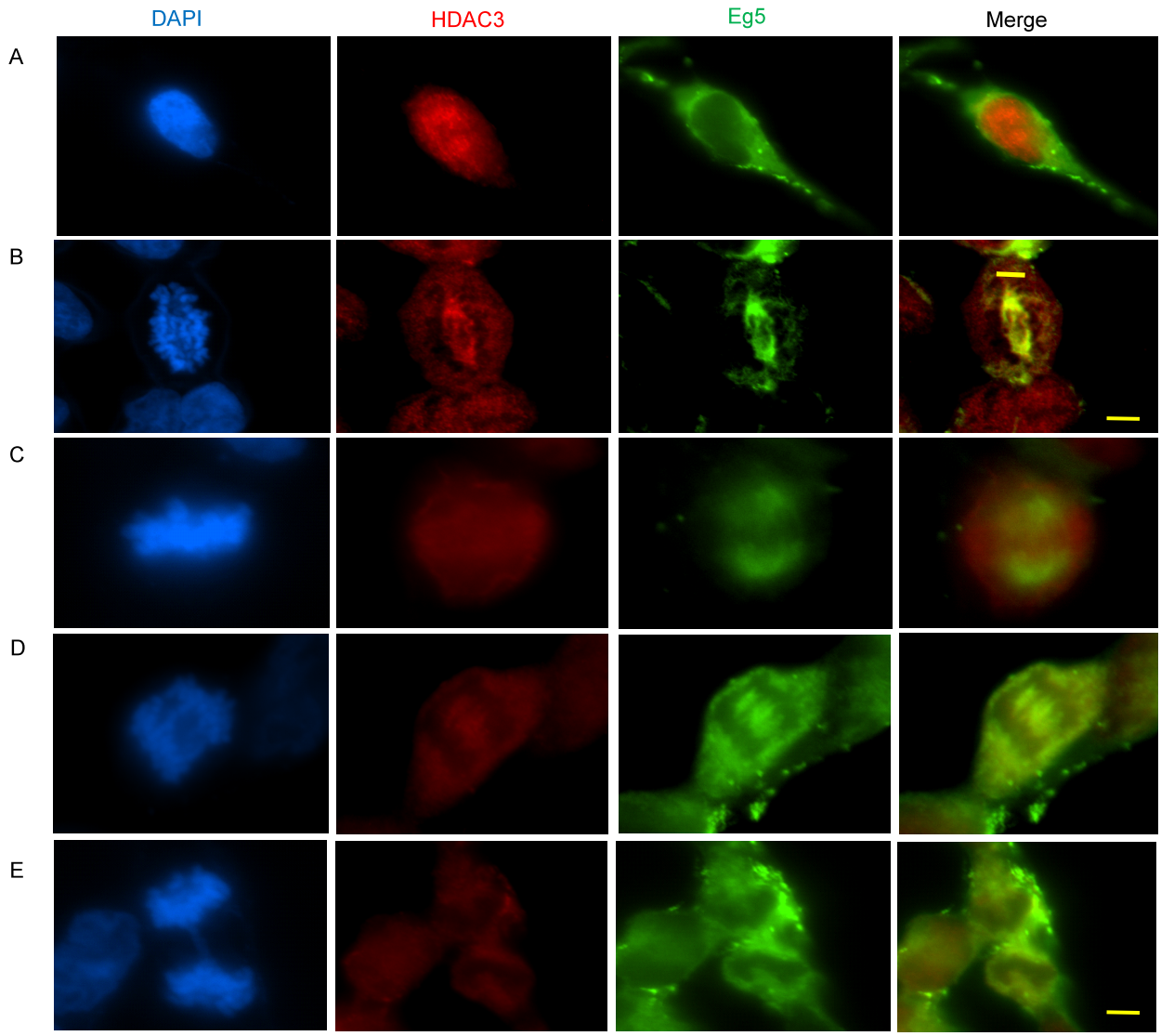


Figure S14. Eg5 partially colocalized with HDAC3 during prophase, related to Figure 6. HEK293 cells were fixed and stained with HDAC3 (red) and Eg5 (green) antibodies. Cells were counterstained with DAPI (blue). Fluorescence microscopy was used to visualize HDAC3 and Eg5 in each cell. Cells in interphase (A), prophase (B), metaphase (C), anaphase (D), and telophase (E) are shown. HDAC3 (red) and Eg5 (green) images were used to generate merged images (yellow). Scale bar 10 μm.

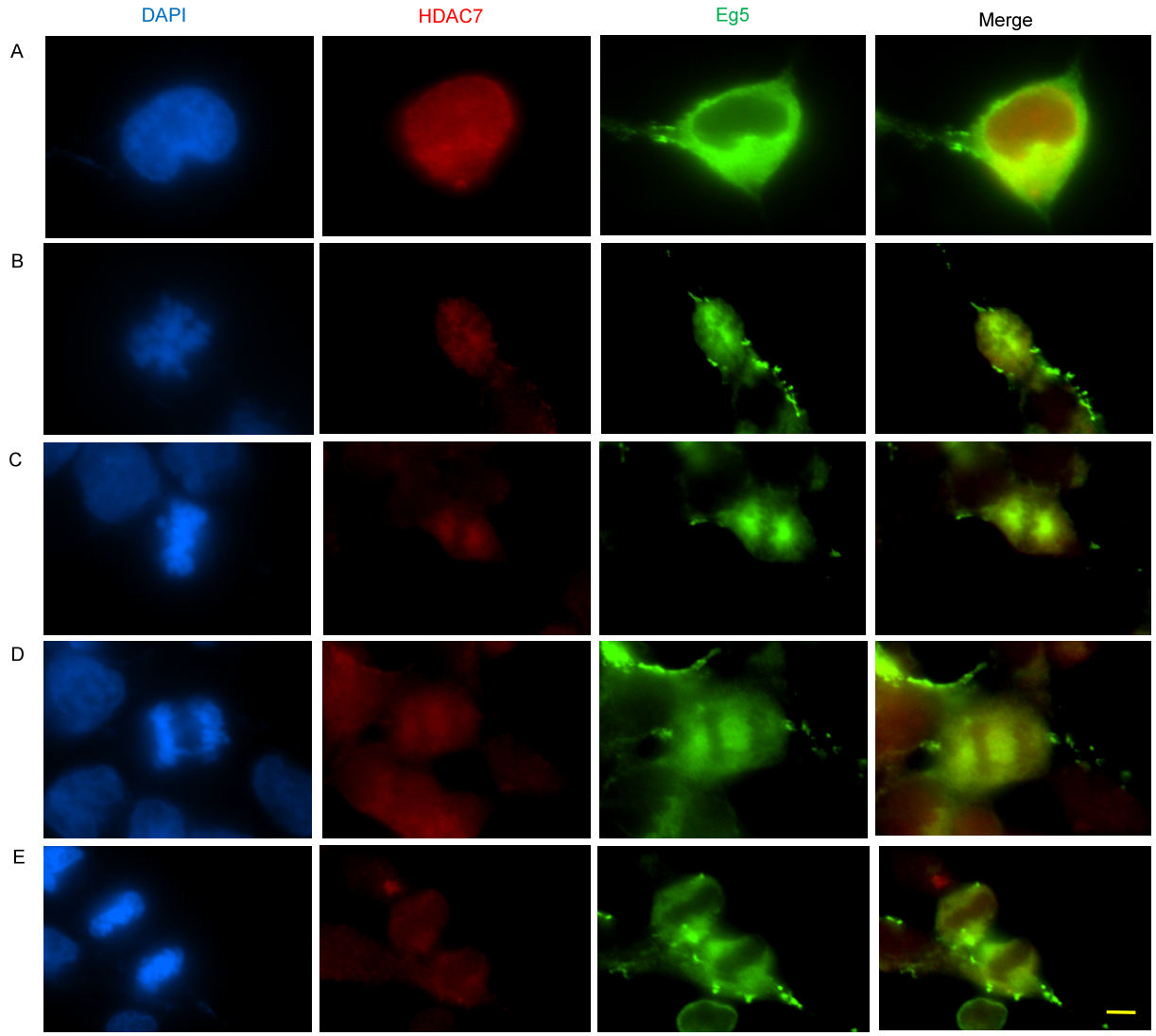


Figure S15. Eg5 does not colocalize with HDAC7 during prophase, related to Figure 6. HEK293 cells were fixed and stained with HDAC7 (red) and Eg5 (green) antibodies. Cells were counterstained with DAPI (blue). Fluorescence microscopy was used to visualize HDAC7 and Eg5 in each cell. Cells in interphase (A), prophase (B), metaphase (C), anaphase (D), and telophase (E) are shown. HDAC7 (red) and Eg5 (green) images were used to generate merged images (yellow). Scale bar 10 μm.

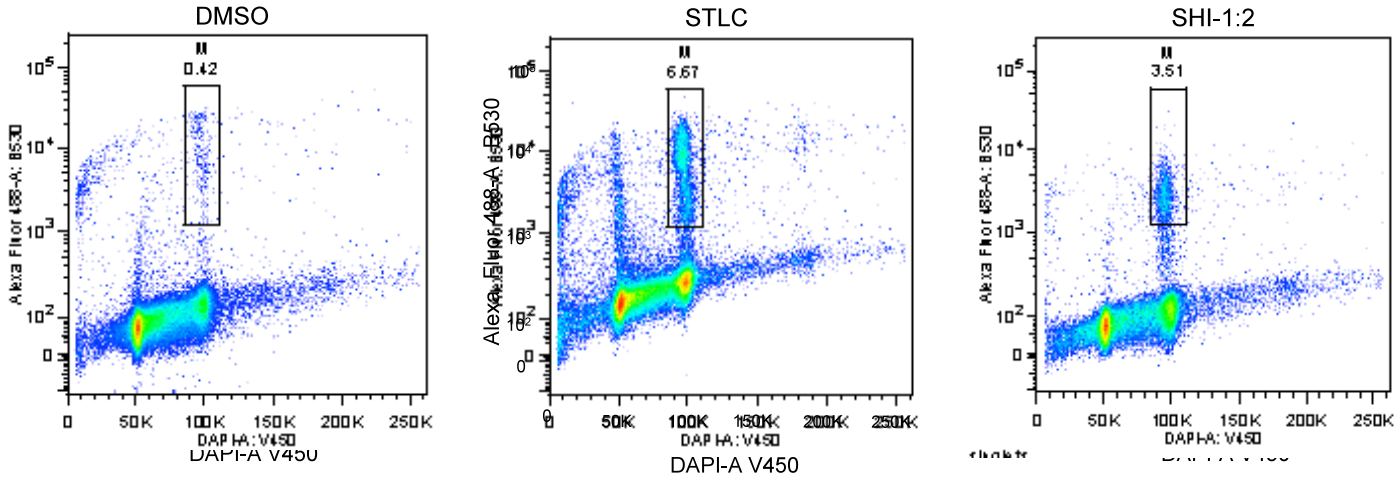


Figure S16. Percentage mitotic cells after inhibitor treatment analyzed by flow cytometry, **related to Figure 7.** HEK293 cells were treated with DMSO, STLC (10 µM) or SHI-1:2 (10 µM) for 48h followed by fixing and staining with Alexafluoro®488 conjugated phospho-histone H3 Ser10 antibody and DAPI. Samples were analyzed by flow cytometry. A representative trial is shown here with the percentage of cells in mitosis (M) shown as the number at the top. Quantification of three independent trials shown in Table S4 and Figure 7A.

Table S4 – Percentage mitotic cells after inhibitor treatment analyzed by flow cytometry*, related to Figure 7.

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
DMSO	0.46	0.4	0.42	0.43	0.02
STLC	6.2	5.4	6.7	6.1	0.4
SHI-1:2	3.4	4.2	3.5	3.7	0.3

*Data depicted in Figure 7A of the manuscript.

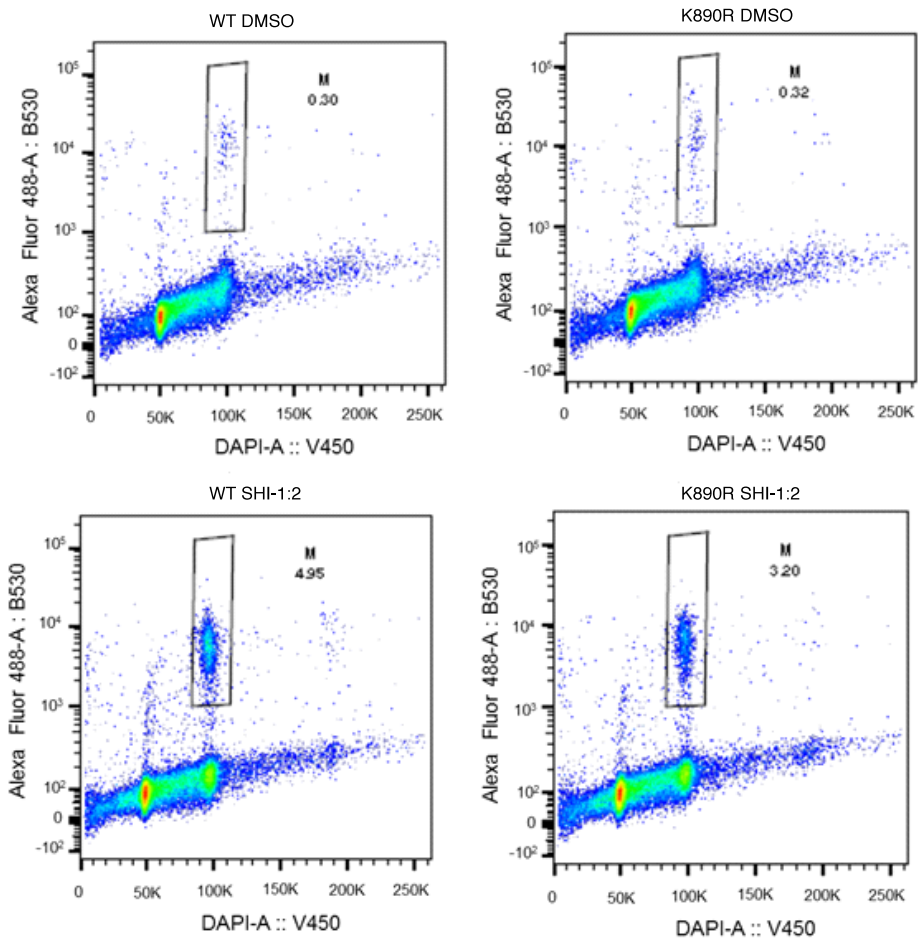


Figure S17. Flow cytometry analysis of SHI-1:2-induced mitotic cells with WT and K890R Eg5, related to Figure 7. Myc-tagged wild type or K890R mutant Eg5 were transfected into HEK293 cells, treated with SHI-1:2 for 48h and subjected to staining with Alexa fluoro®488 conjugated phospho-histone H3 (Ser10) antibody and DAPI prior to flow cytometric analysis. A representative trial is shown here with the percentage of cells in mitosis shown as the number in the top right. Quantification of three independent trials shown in Table S5 and Figure 7B.

Table S5 – Flow cytometry analysis of SHI-1:2-induced mitotic cells with WT and K890R Eg5*, related to Figure 7.

	Trial 1	Trial 2	Trial 3	Mean	Standard Error
WT DMSO	0.27	0.42	0.30	0.30	0.05
K890R DMSO	0.3	0.26	0.32	0.30	0.02
WT SHI-1:2	3.8	4.9	5.0	4.6	0.4
K890R SHI-1:2	3.0	3.8	3.2	3.3	0.2

*Data depicted in Figure 7B of the manuscript.

Table S6 - Percentage of monopolar spindles observed by fluorescence microscopy after inhibition*, related to Figure 7.

	Trial 1	Trial 2	Trial 3	Trial 4	Mean	Standard Error
STLC	100	100	100	100	100	0.0
SHI-1:2	53	48	57	55	53	2.0

*Data depicted in Figure 7D of the manuscript.