Supporting Information

HDAC1 Substrate Profiling using Proteomics-Based Substrate Trapping

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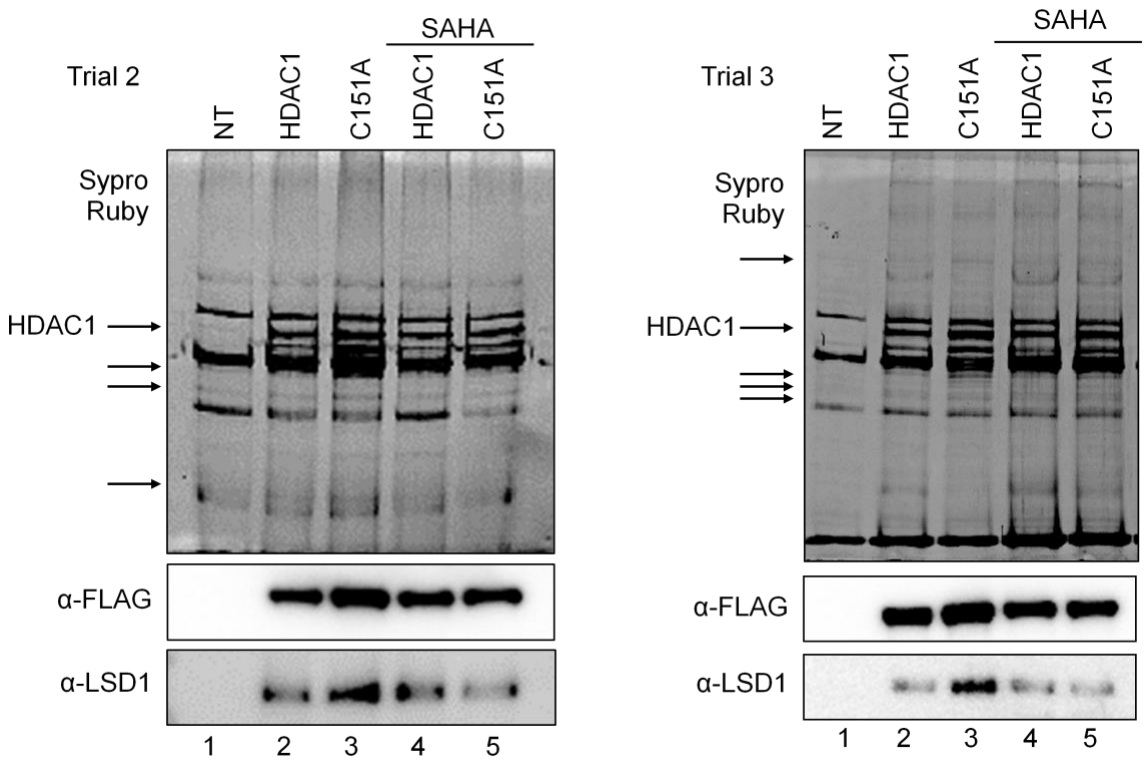


Figure S1- Repetitive trials of substrate trapping with high stringency wash condition. HEK293 cells were transiently tranfected with expression plasmids for FLAG-tagged wild type and C151A mutant HDAC1. After 48h of growth, cells were treated with SAHA for another 24h to induce robust acetylation. After cell lysis, wild type and C151A mutant HDAC1 were immunoprecipitated from the lysates in the absence and presence of SAHA. After high stringency washing, bound proteins were eluted, separated by SDS-PAGE, and visualized with Sypro Ruby staining or western blotting with antibodies to FLAG and LSD1 after transfer to a PVDF membrane. Arrows indicate the expressed HDAC1 wild type or C151A mutant protein band and protein bands enriched in the C151A mutant lane but not the wild type or SAHA-competed lanes. Two independent trials are shown here, with a third trial shown in Figure 2 of the manuscript.

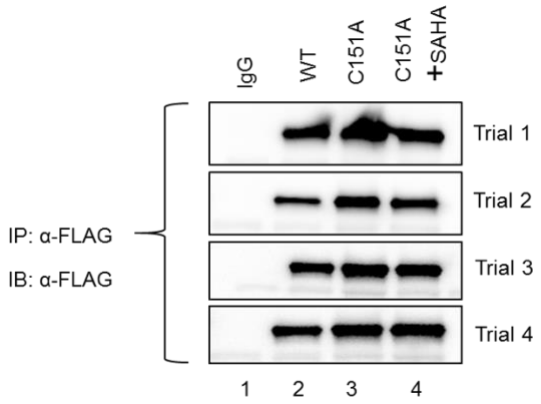


Figure S2- HDAC1 expression in samples for proteomics analysis. Wild type (WT) and C151A mutant HDAC1 were expressed as Flag-tagged proteins in HEK293 cells. After 48h of growth, cells were treated with SAHA for another 24h. Cells were lysed, and WT and C151A mutant HDAC1 were immunoprecipitated from the lysates in the presence or absence of SAHA. Bound Flag-tagged HDAC1 wild type or mutant proteins were eluted, separated by SDS-PAGE, and visualized by western blotting using a FLAG antibody to assure equal protein levels of HDAC1 proteins in the immunoprecipitates. These samples were subsequently analyzed by LC-MS/MS.

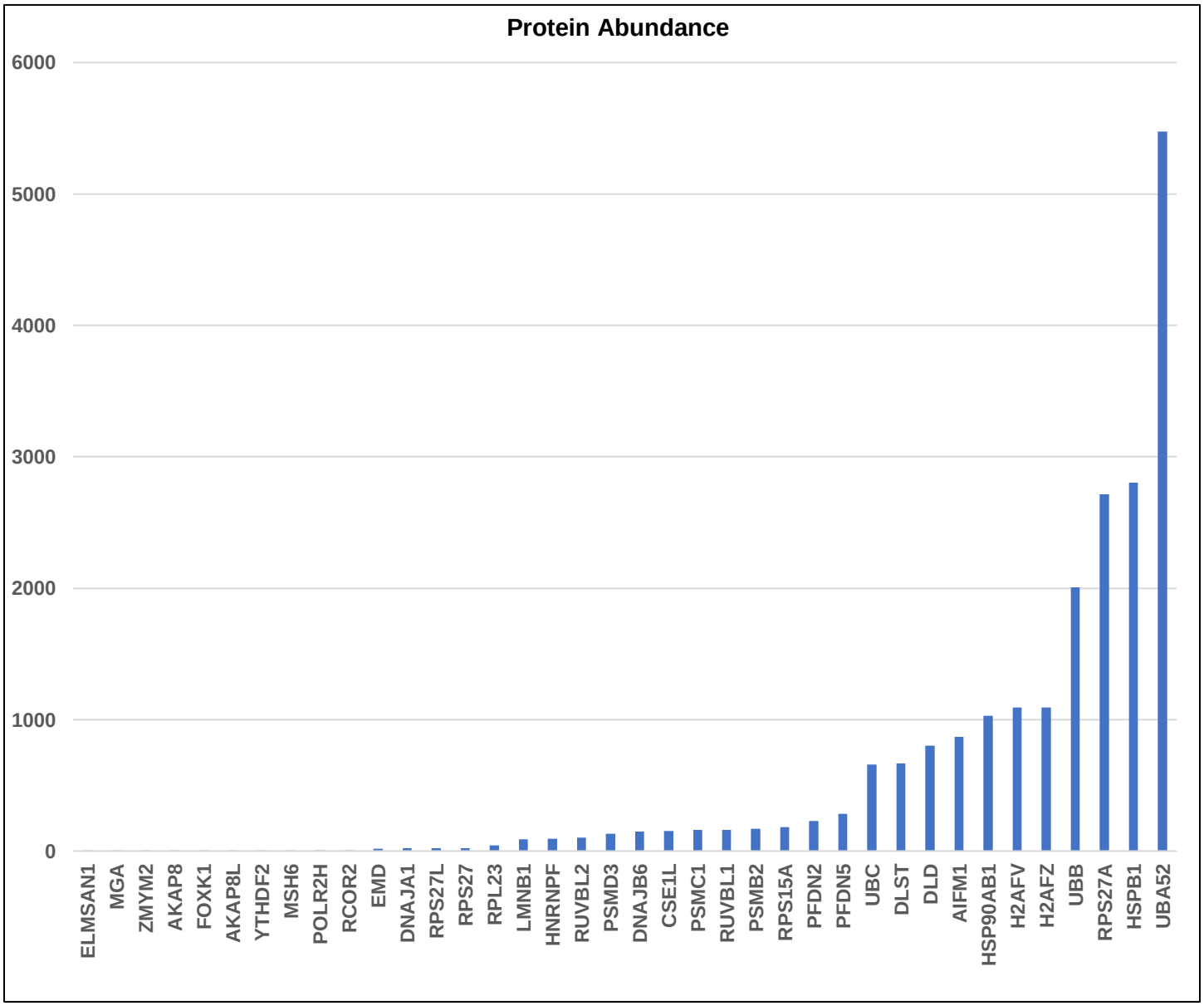


Figure S3- Abundance analysis of proteins identified by substrate trapping. The abundance of the 38 protein hits from Table S4 (all isoforms) were analyzed using PaxDb database (1). Protein abundance is shown ppm units in y-axis and names of hit proteins on x-axis. The range of abundances observed was 0.11 to 5474 ppm, which is similar to the abundance range for all proteins in HEK293 cells (0.01 to 10,000 ppm). C9orf41 (CARNMT1) is not included in the figure due to lack of data in PaxDb.

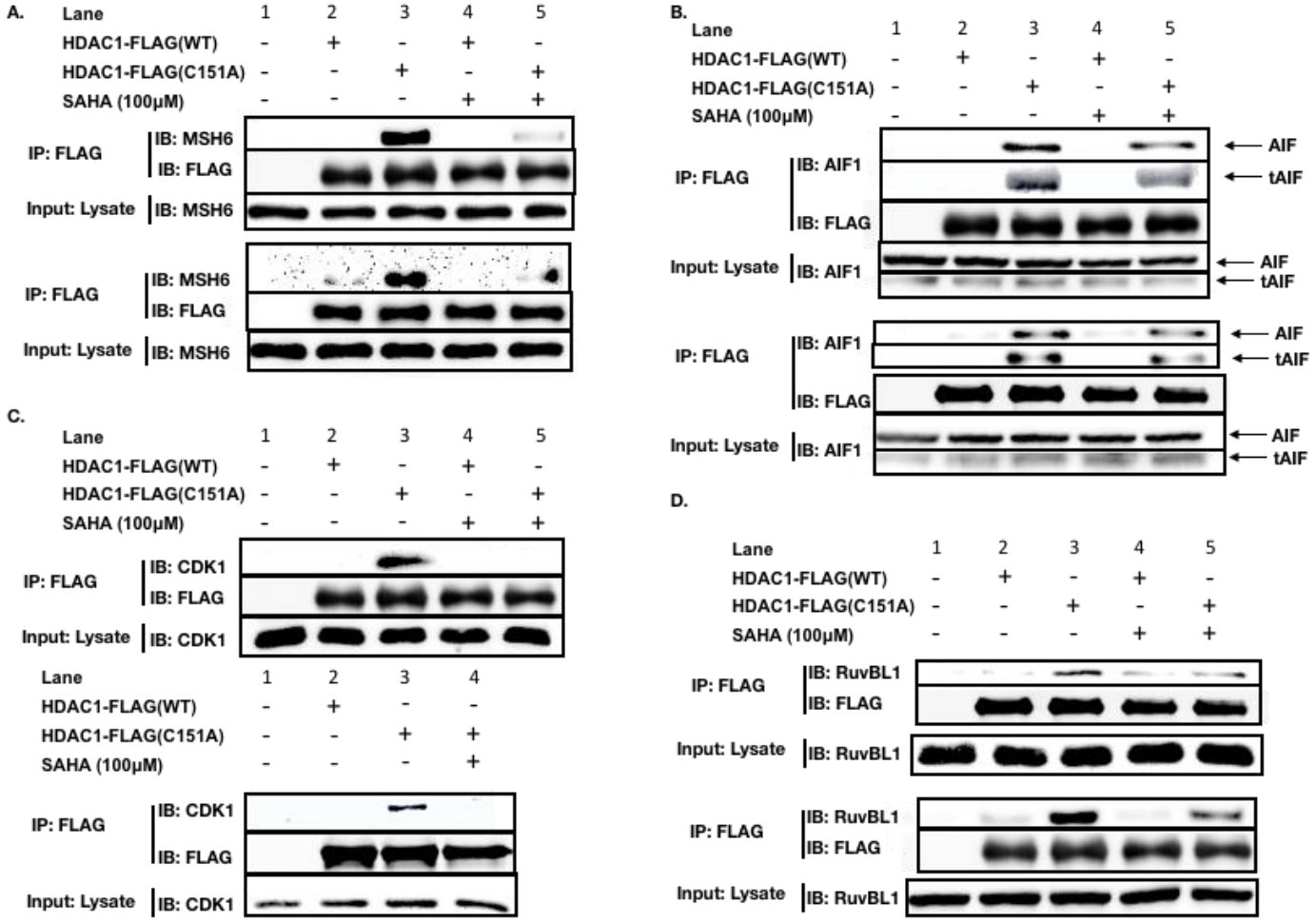


Figure S4 - Confirmation of four potential substrates identified by substrate trapping. HEK293 cells were transfected with HDAC1-Flag or C151A HDAC1 mutant-Flag. After 48h, cells were treated with SAHA for another 24h to induce robust acetylation and then subjected to immunoprecipitation (IP) using anti-FLAG agarose beads, followed by immunoblot (IB) analysis of bound MSH6 (A), AIF1 (B), CDK1 (C) and RuvBL1 (D) proteins. As controls, anti-FLAG beads were incubated with lysates without overexpression of wild type of mutant HDAC1-Flag (IgG, lane 1). As an input control, lysates from each condition were probed with individual antibodies to demonstrate equal level of protein expressed and loaded. Two independent assays for each substrate are shown here, with the third shown in Figure 4 of the manuscript.

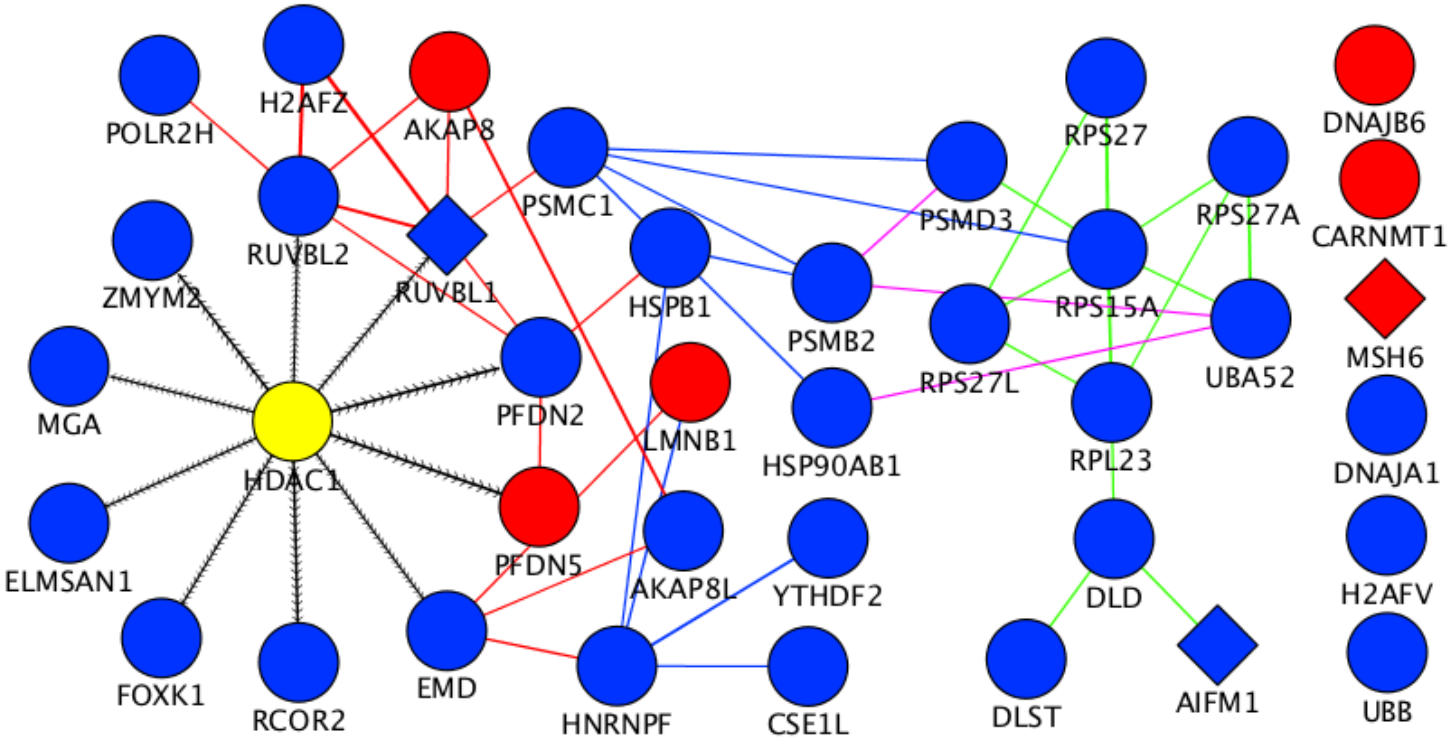
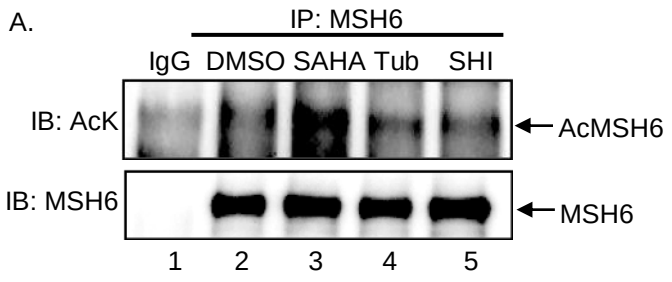
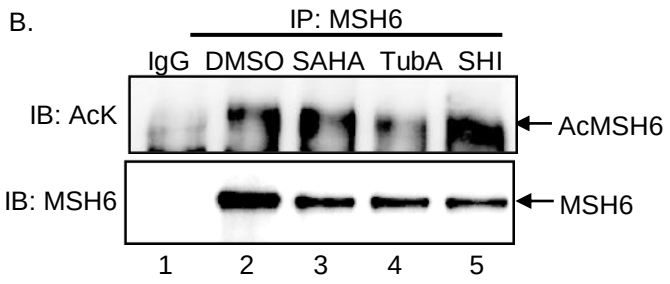


Figure S5 - Enlarged version of the Interactome Analysis from Figure 5 of the manuscript. Known physical protein-protein interactions among the 38 trapped hits in Table S3 were analyzed using GeneMANIA in Cytoscape. UBC was not included due to interaction with almost all proteins, which created a more complex interactome. Colored shapes represent proteins in the lower (black, 2.5-fold enrichment, Table S4) and higher (red, 5-fold enrichment, Table S5) stringency hit lists (Tables S3-S5). Proteins with a diamond shape were tested in confirmation or validation studies. Lines between proteins indicate a known physical interaction, with the thickness indicating the confidence in that interaction. Proteins encircling HDAC1 (black lines) are known direct interactors, whereas the other proteins bind HDAC1 indirectly through one (red lines), two (blue lines), three (purple lines), or four or more (green lines) associated proteins. The remaining proteins (right) show no known interactions. This figure is an enlarged version of the image in Figure 5 of the manuscript.





C.

Sample	Trial 1	Trial 2	Trial 3	Mean	Standard Error
DMSO	1	1	1	1	
SAHA	1.4	1.5	1.2	1.3	0.1
Tub A	0.83	0.85	0.55	0.74	0.10
SHI-1:2	0.83	1.3	1.2	1.1	0.2

Figure S6 - Validation of HDAC1-dependent MSH6 deacetylation. A and B) HEK293 cells were treated with DMSO, SAHA (10 µM), tubastatin A (TubA, 10 µM), or SHI-1:2 (10 µM in A; 20 µM in B) for 24 hr. After cell harvesting and lysis, endogenous MSH6 was immunoprecipitated (IP) with an MSH6 antibody and Protein A/G beads. Immunoprecipitates were analyzed by immunoblotting (IB) with acetyl lysine (AcK) and MSH6 antibodies. Two independent trials are shown here, with a third shown in Figure 6 of the manuscript. C) Band intensities were quantified using ImageJ (version 1.47, https://imagej.net/ImageJ)(2). Quantified band intensities were normalized to the untreated (DMSO) sample by dividing the intensity value of the SAHA, TubA, and SHI-1:2 samples by the intensity value of the untreated sample (untreated is set to 1.0 as a result). The mean and standard error of the three trials are shown, which were used to create the histogram in Figure 6B of the manuscript.

References

1. Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D., and von Mering, C. (2015) Version 4.0 of PaxDb: Protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163-3168
2. Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9**, 671-675