Electronic Supplementary Material (ESI) for Molecular Omics.

Supporting Information

Differential profiles of HDAC1 substrates and associated proteins in breast cancer cells revealed by trapping

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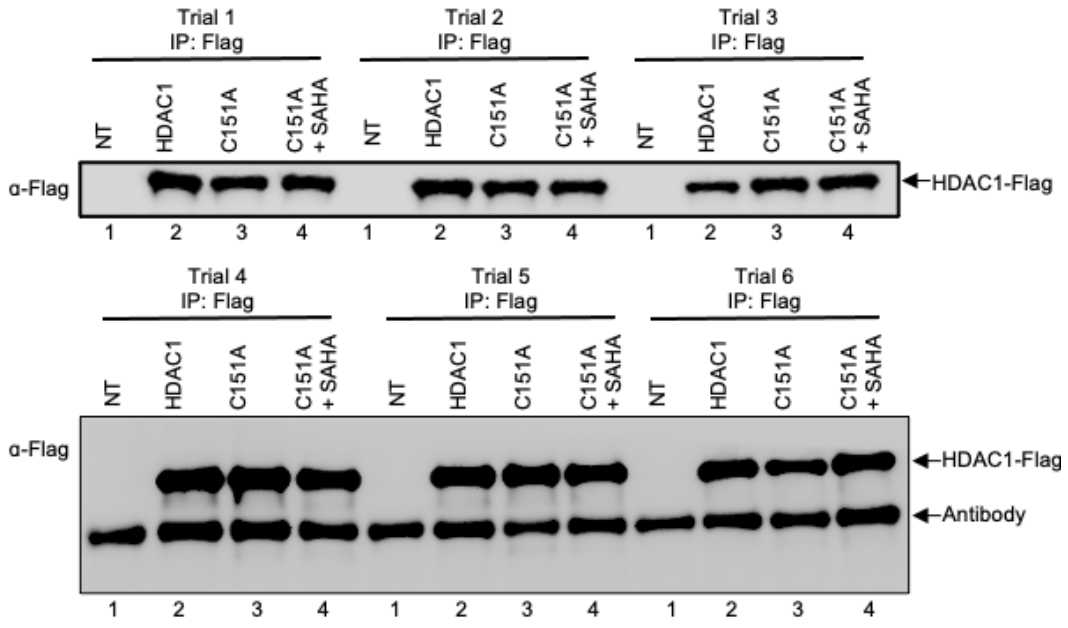

Figure S1- HDAC1 immunoprecipitation levels in MCF10A cells. Wild type (WT) and C151A mutant HDAC1 were expressed as Flag-tagged proteins in MCF10A cells. As a negative control, untransfected cells (NT, lane 1) were also generated. After 48h, cells were treated with SAHA (5 μM) for another 24h to induce robust acetylation. Proteins were immunoprecipitated from each lysate using Flag-bound agarose beads. SAHA was included as a binding competitor in a separate HDAC1 C151A mutant sample (lane 4). Bound proteins were eluted, separated by SDS-PAGE, and analyzed by Western blotting with FLAG antibody (α-Flag), with the arrows indicating HDAC1-Flag or the Flag antibody bands. The six trials used for LC-MS/MS analysis are shown here, with trial 1 shown in Figure 2A of the manuscript. We note that trial 1 showed unequal intensities of HDAC1 in the LC-MS/MS data and was removed from further analysis (see red highlighted columns in Table S1A). Full gel images are shown for the last three trials.

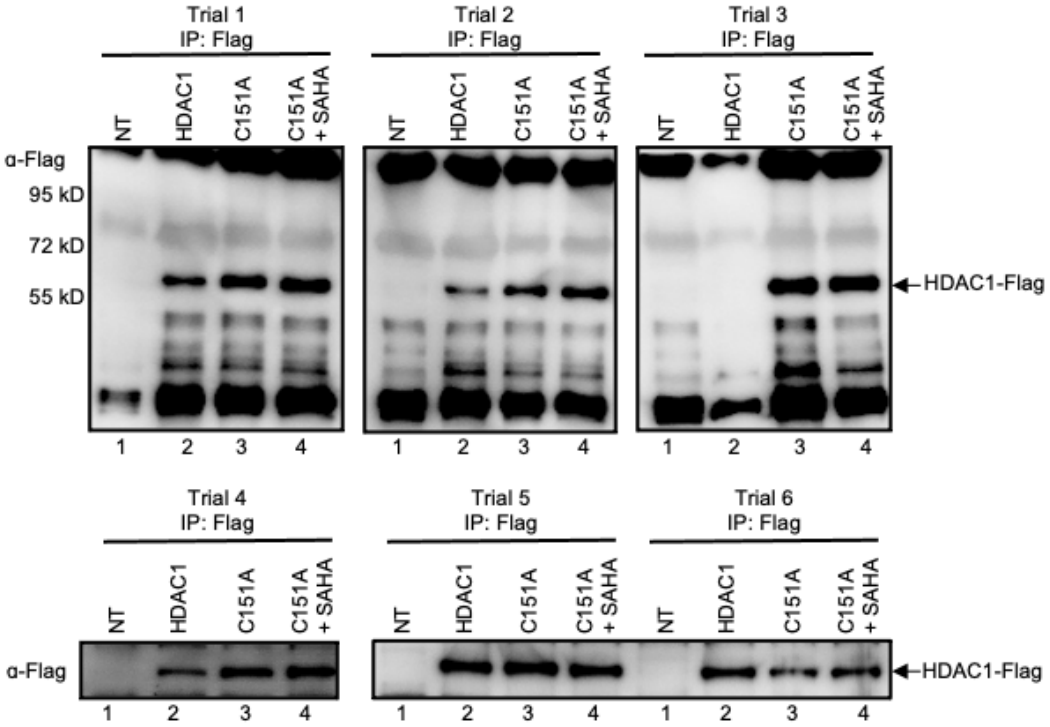

Figure S2- HDAC1 immunoprecipitation levels in MDA-MB-468 cells. Wild type (WT) and C151A mutant HDAC1 were expressed as Flag-tagged proteins in MDA-MB-468 cells. As a negative control, untransfected cells (NT, lane 1) were also generated. After 48h, cells were treated with SAHA (5 μM) for another 24h to induce robust acetylation. Proteins were immunoprecipitated from each lysate using Flag antibody-bound agarose beads. SAHA was included as a binding competitor in a separate HDAC1 C151A mutant sample (lane 4). Bound proteins were eluted, separated by SDS-PAGE, and analyzed by Western blotting with FLAG antibody (α-Flag). The six trials used for LC-MS/MS analysis are shown here, with trial 1 shown in Figure 2B of the manuscript. We note that trial 3 showed unequal intensities of HDAC1 in the LC-MS/MS data and was removed from further analysis (see red highlighted columns in Table S2A). Full gel images and molecular weight markers are shown for the first three trials, with the arrow indicating HDAC1-Flag**.** All other bands present in all lanes are antibody bands derived from the Flag antibody-bound agarose beads. Full gel images are shown for the first three trials.

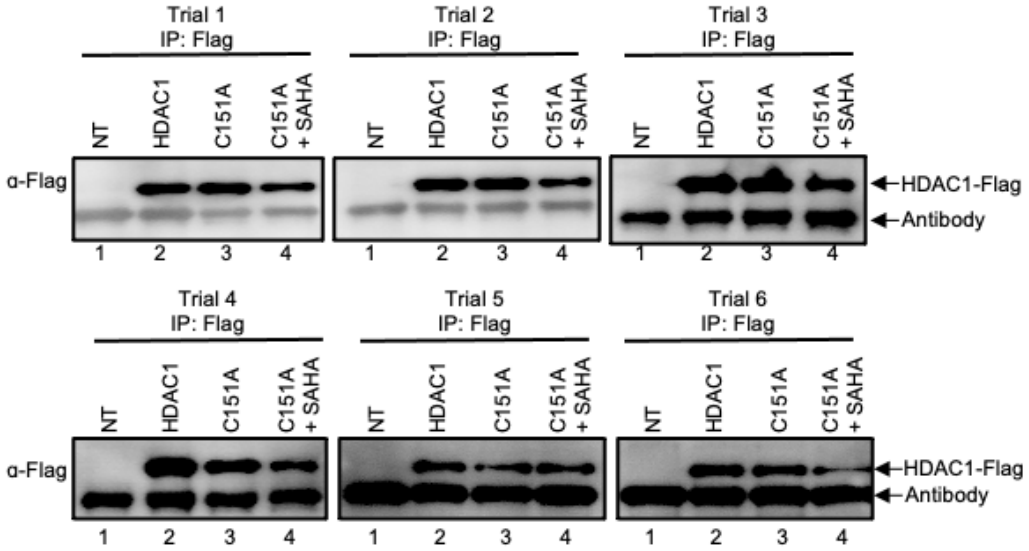

Figure S3- HDAC1 immunoprecipitation levels in MDA-MB-231 cells. Wild type (WT) and C151A mutant HDAC1 were expressed as Flag-tagged proteins in MDA-MB-231 cells. As a negative control, untransfected cells (NT, lane 1) were also generated. After 48h, cells were treated with SAHA (5 μM) for another 24h to induce robust acetylation. Proteins were immunoprecipitated from each lysate using Flag-bound agarose beads. SAHA was included as a binding competitor in a separate HDAC1 C151A mutant sample (lane 4). Bound proteins were eluted, separated by SDS-PAGE, and analyzed by Western blotting with FLAG antibody (α-Flag), with the arrows indicating HDAC1-Flag or the Flag antibody bands. The six trials used for LC-MS/MS analysis are shown here, with trial 1 shown in Figure 2C of the manuscript. We note that trial 1 showed unequal intensities of HDAC1 in the LC-MS/MS data and was removed from further analysis (see red highlighted columns in Table S1A).

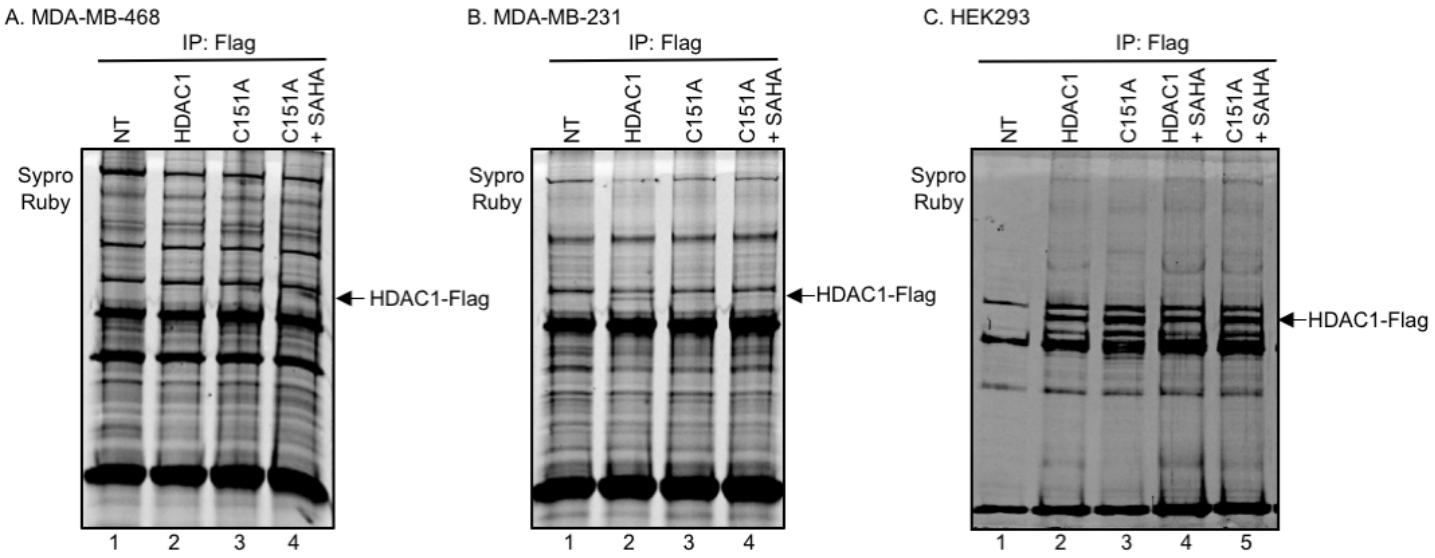

Figure S4- Substrate trapping with MDA-MB-468, MDA-MB-231, and HEK293 cells. Wild type (WT) and C151A mutant HDAC1 were expressed as Flag-tagged proteins in (A) MDA-MB-468, (B) MDA-MB-231, and (C) HEK293 cells. As a negative control, untransfected cells (NT, lane 1) were also generated. After 48h, cells were treated with SAHA (5 μM) for another 24h to induce robust acetylation. Proteins were immunoprecipitated from each lysate using Flag-bound agarose beads. SAHA was included as a binding competitor in separate samples (lanes 4 and 5). Bound proteins were eluted, separated by SDS-PAGE, and analyzed by SyproRuby total protein stain, with the arrow indicating HDAC1-Flag. The full gel image of one representative trial with each cell line is shown here. A truncated version of the trial with MDA-MB-231 (B) is shown in Figure 2D of the manuscript.

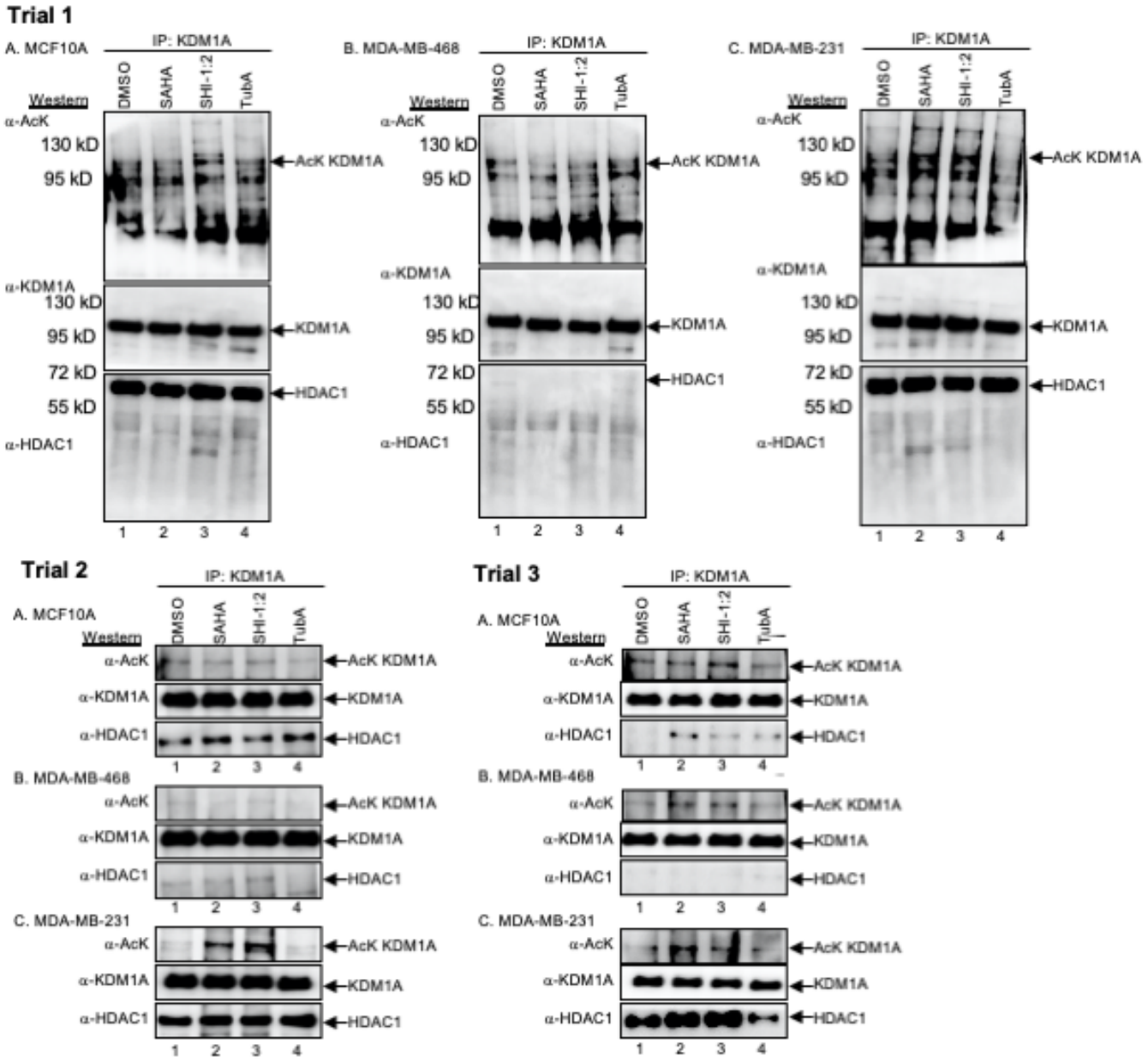

Figure S5- Repetitive trials of KDM1A deacetylase assays. MCF10A (A), MDA-MB-468 (B), and MDA-MB-231 (C) were untreated (DMSO) or treated with broad-spectrum SAHA, HDAC1/HDAC2-selective SHI-1:2, or HDAC6-selective tubastatin A (10 μM) for 24 h to inhibit deacetylation. KDM1A was immunoprecipitated from each lysate, and bound proteins were eluted, separated by SDS-PAGE, and analyzed by Western blot with acetyllysine (AcK), KDM1A, and HDAC1 antibodies. Three trials are shown here, with the full raw gel images from Figure 4 of the manuscript shown as Trial 1.