Supplemental Information

Identification of optimal substrate trapping mutants to discover substrates of HDAC1

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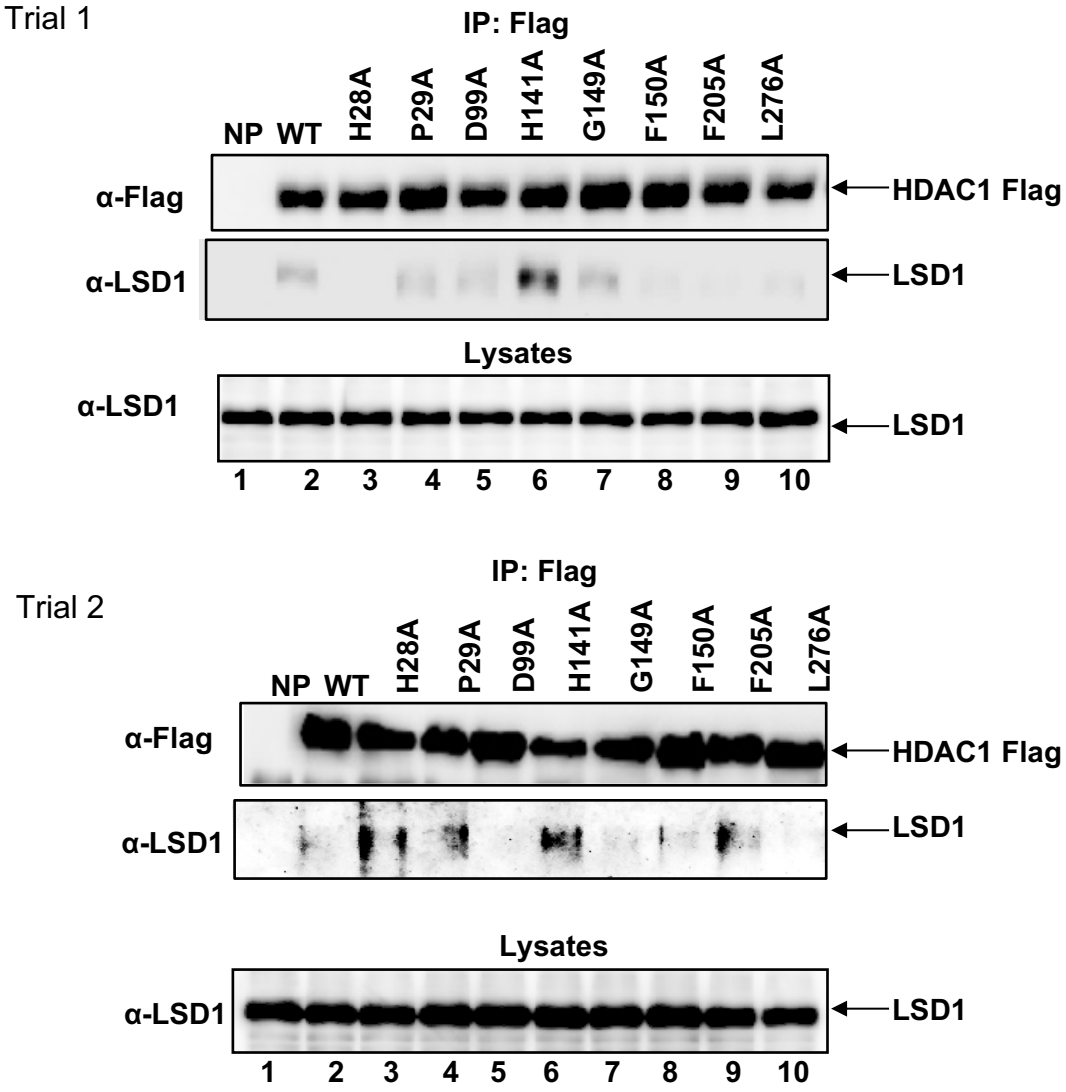
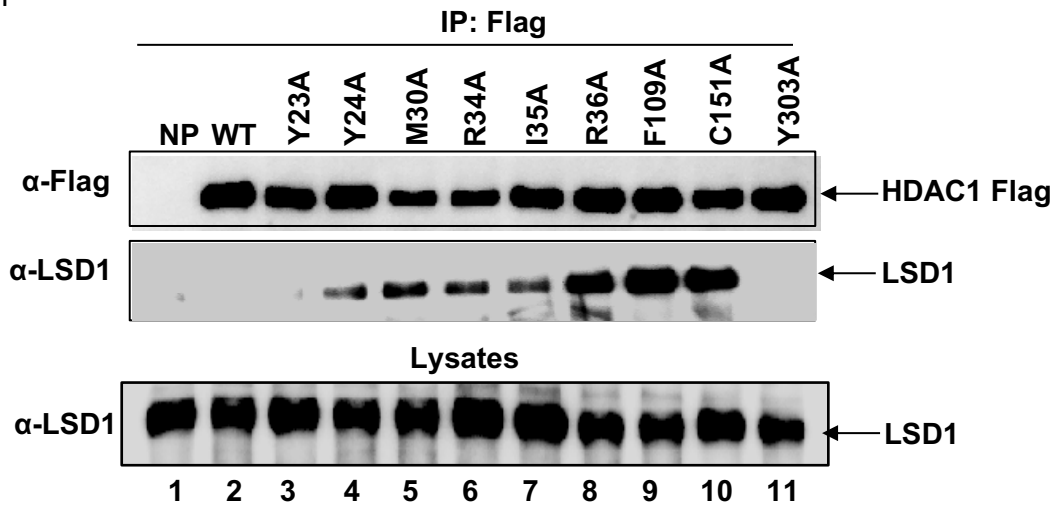


Figure S1- Independent trials screening the 11Å channel mutants for LSD1 trapping. Wild type (WT) and mutant HDAC1 were expressed as Flag-tagged proteins in HEK293 cells. After transfection and 48 hr recovery, cells were incubated with SAHA (10 μM) for 24 hr to induce robust acetylation. After lysis, proteins in the lysates were immunoprecipitated with anti-FLAG agarose beads, separated by SDS-PAGE, and analyzed by western blot analysis with flag and LSD1 antibodies. Two trials are shown here with the third in Figure 3A.

Trial 1



Trial 2

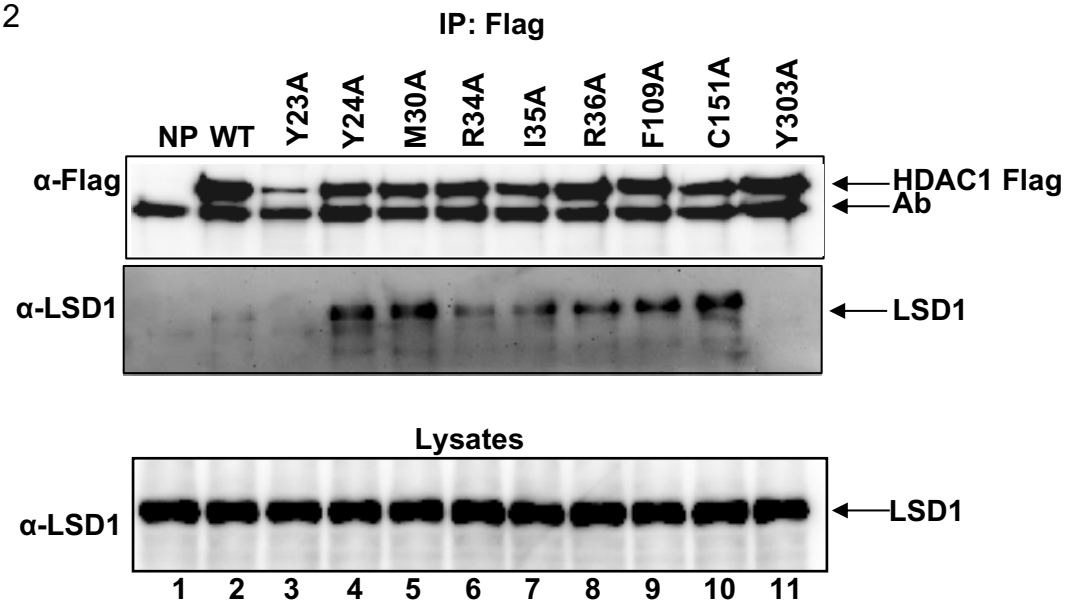


Figure S2- Independent trials screening the 14Å cavity mutants for LSD1 trapping. Wild type (WT) and mutant HDAC1 were expressed as Flag-tagged proteins in HEK293 cells. After transfection and 48 hr recovery, cells were incubated with SAHA (10 μM) for 24 hr to induce robust acetylation. After lysis, proteins in the lysates were immunoprecipitated with anti-FLAG agarose beads, separated by SDS-PAGE, and analyzed by western blot analysis with flag and LSD1 antibodies. Two trials are shown here with the third in Figure 3B. In trial 2, the co-migrating HDAC1-Flag and contaminating IgG antibody (Ab) bands are indicated by arrows.

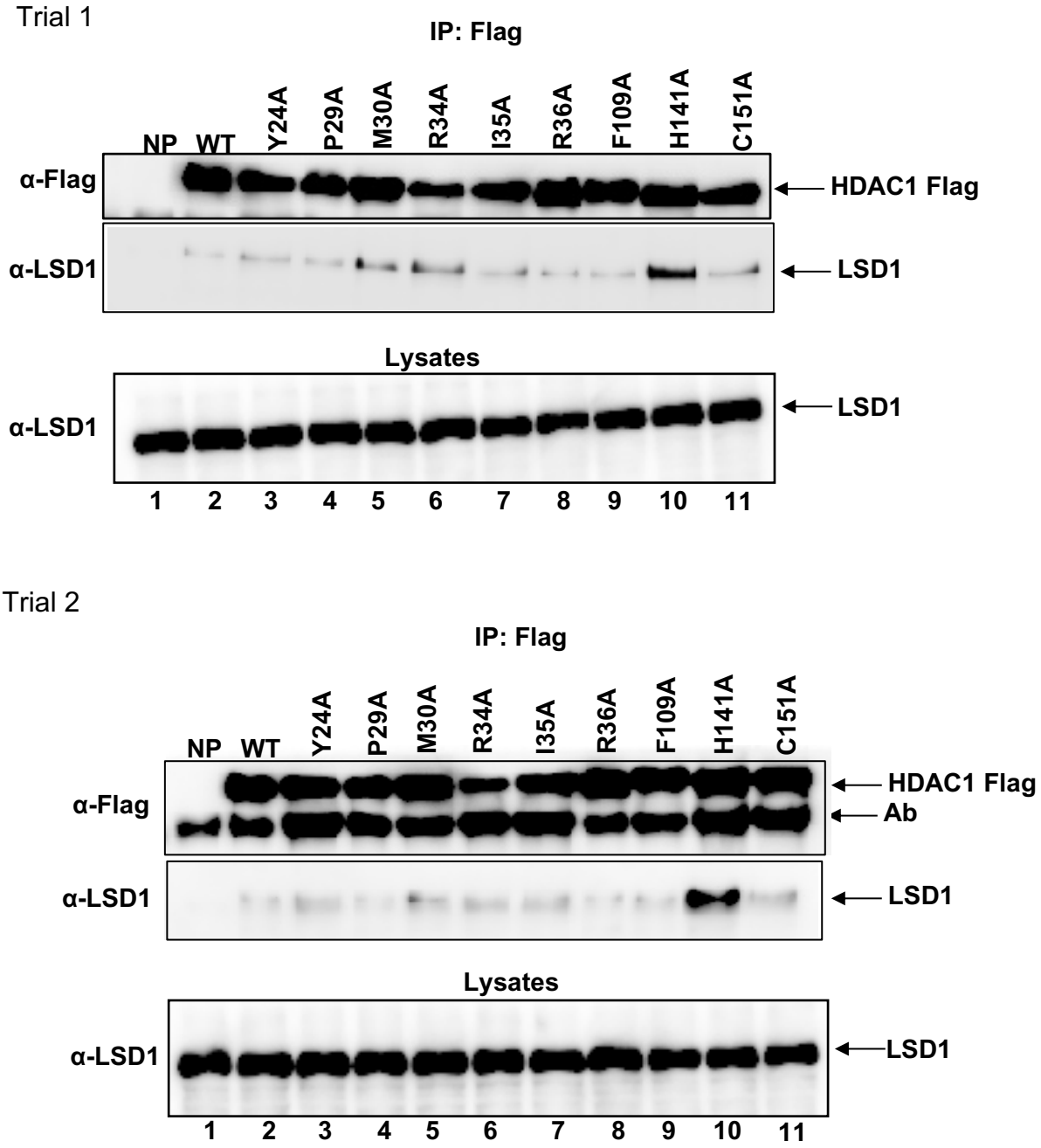
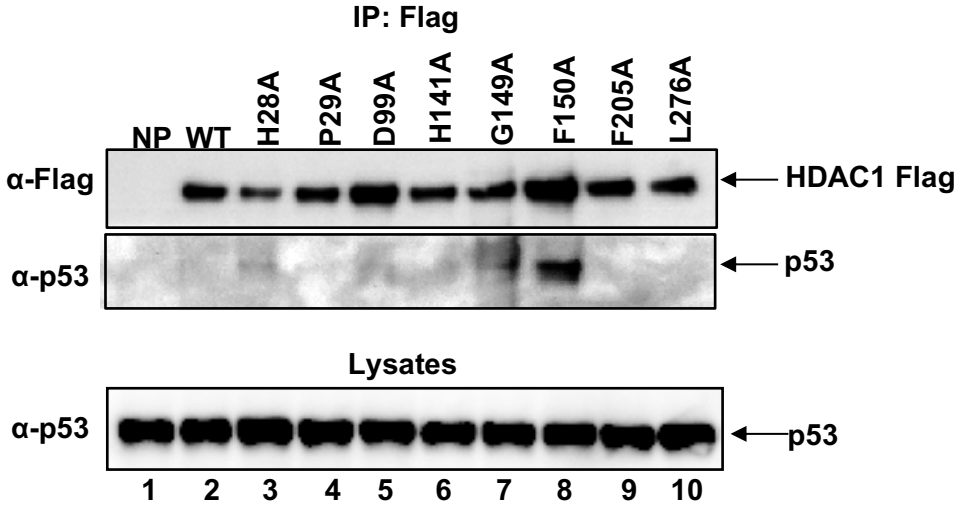


Figure S3- Independent trials screening all trapping mutants for LSD1 binding. Wild type (WT) and mutant HDAC1 were expressed as Flag-tagged proteins in HEK293 cells. After transfection and 48 hr recovery, cells were incubated with SAHA (10 μM) for 24 hr to induce robust acetylation. After lysis, proteins in the lysates were immunoprecipitated with anti-FLAG agarose beads, separated by SDS-PAGE, and analyzed by western blot analysis with flag and LSD1 antibodies. Two trials are shown here with the third in Figure 3C. In trial 2, the co-migrating HDAC1-Flag and contaminating IgG antibody (Ab) bands are indicated by arrows.

Trial 1



Trial 2

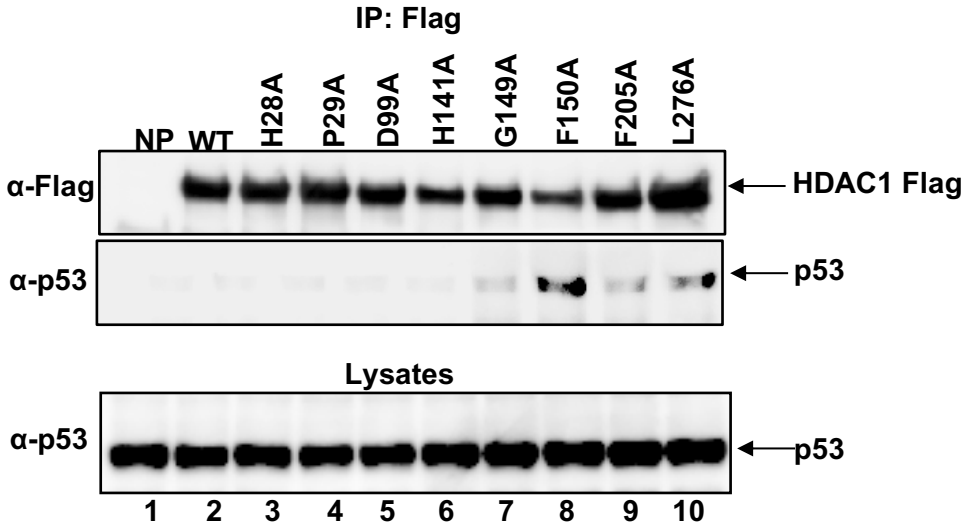
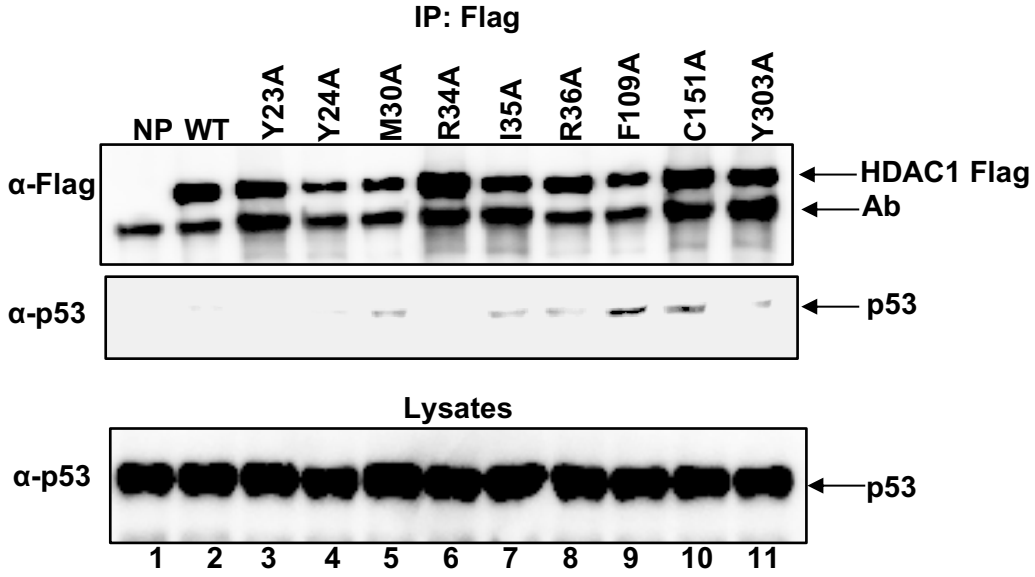


Figure S4- Independent trials screening the 11Å channel mutants for p53 trapping. Wild type (WT) and mutant HDAC1 were expressed as Flag-tagged proteins in HEK293 cells. After transfection and 48 hr recovery, cells were incubated with SAHA (10 μM) for 24 hr to induce robust acetylation. After lysis, proteins in the lysates were immunoprecipitated with anti-FLAG agarose beads, separated by SDS-PAGE, and analyzed by western blot analysis with flag and p53 antibodies. Two trials are shown here with the third in Figure 4A.

Trial 1



Trial 2

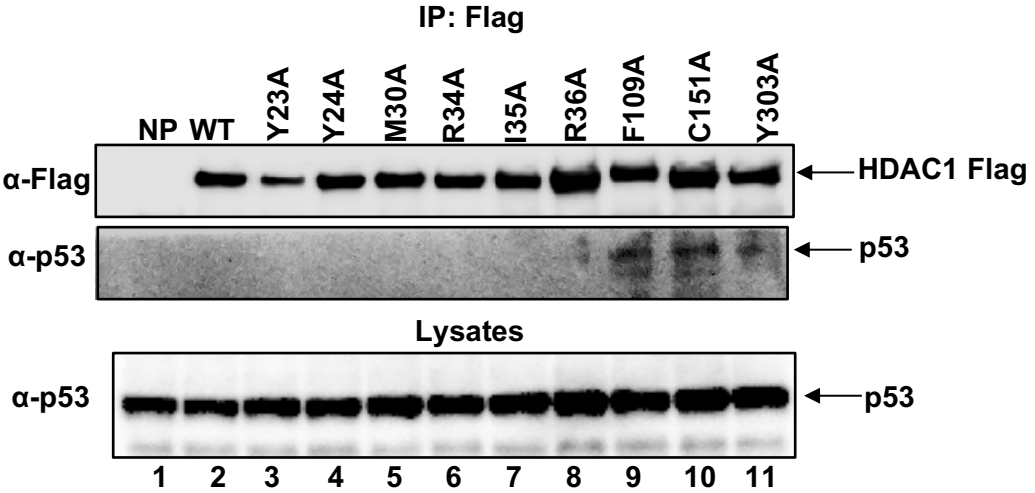


Figure S5- Independent trials screening the 14Å cavity mutants for p53 trapping. Wild type (WT) and mutant HDAC1 were expressed as Flag-tagged proteins in HEK293 cells. After transfection and 48 hr recovery, cells were incubated with SAHA (10 μM) for 24 hr to induce robust acetylation. After lysis, proteins in the lysates were immunoprecipitated with anti-FLAG agarose beads, separated by SDS-PAGE, and analyzed by western blot analysis with flag and p53 antibodies. Two trials are shown here with the third in Figure 4B. In trial 1, the co-migrating HDAC1-Flag and contaminating IgG antibody (Ab) bands are indicated by arrows.

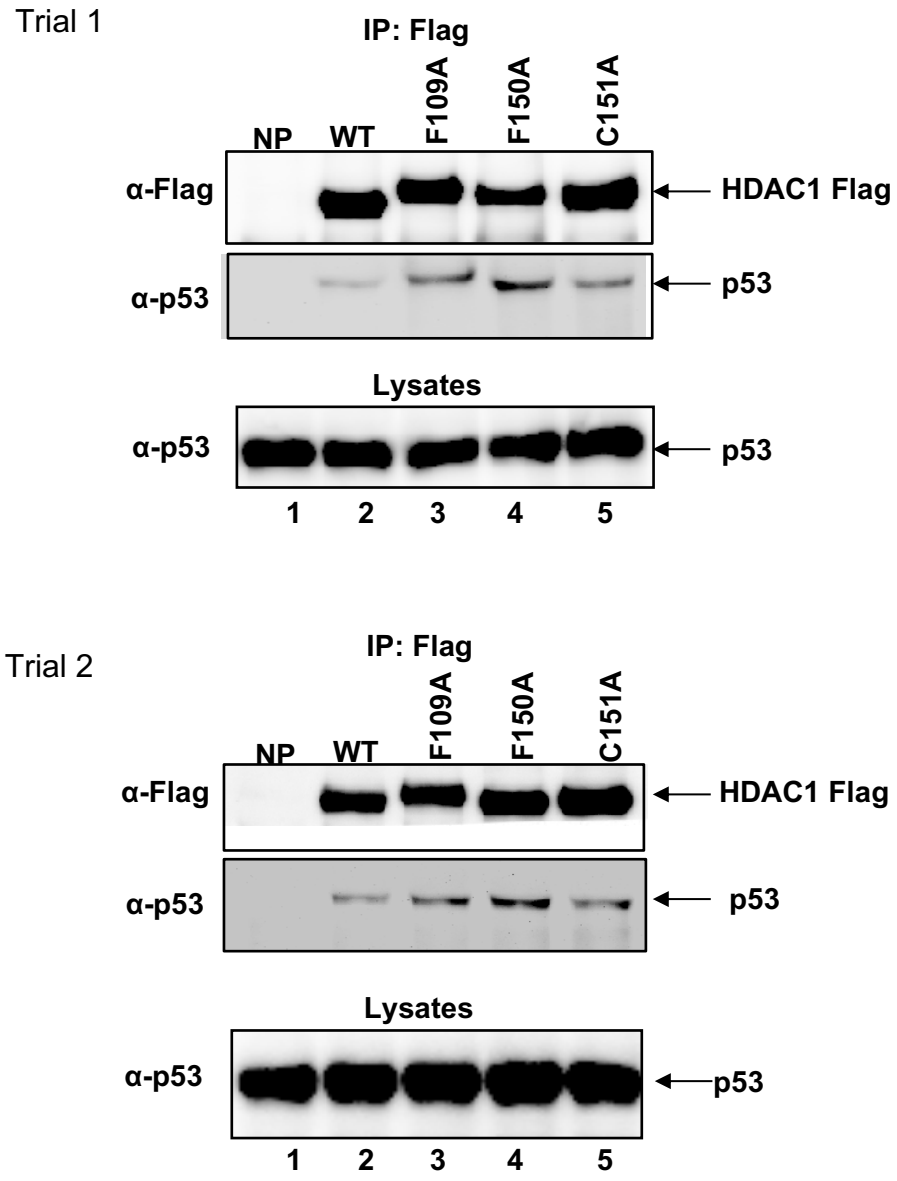


Figure S6- Independent trials screening all trapping mutants for p53 binding. Wild type (WT) and mutant HDAC1 were expressed as Flag-tagged proteins in HEK293 cells. After transfection and 48 hr recovery, cells were incubated with SAHA (10 μM) for 24 hr to induce robust acetylation. After lysis, proteins in the lysates were immunoprecipitated with anti-FLAG agarose beads, separated by SDS-PAGE, and analyzed by western blot analysis with flag and p53 antibodies. Two trials are shown here with the third in Figure 4C.

A)

	Y23	Y24	M30	R34	I35	R36	F109	C151	Y303
HDAC1	Y	Y	M	R	I	R	F	C	Y
HDAC2	Y	Y	M	R	I	R	F	C	Y
HDAC3	H	Y	M	R	L	A	F	C	Y
HDAC4	T	C	E	R	I	Q	A	C	H
HDAC5	M	C	E	R	I	Q	A	C	H
HDAC6a	L	W	E	R	L	H	C	C	Y
HDAC6b	L	W	E	R	I	L	C	C	Y
HDAC7	S	C	E	R	I	Q	A	C	H
HDAC8	S	M	K	R	A	S	Y	C	Y
HDAC9	V	C	E	R	I	Q	A	C	H
HDAC10	L	W	E	R	L	T	C	C	Y
HDAC11	F	M	F	K	W	G	L	C	Y

B)

	H28	P29	D99	H141	G149	F150	F205	L276
HDAC1	H	P	D	H	G	F	F	L
HDAC2	H	P	D	H	G	F	F	L
HDAC3	H	P	D	H	G	F	F	L
HDAC4	H	P	D	H	G	F	F	L
HDAC5	H	P	D	H	G	F	F	V
HDAC6a	F	P	D	H	G	Y	W	A
HDAC6b	H	P	D	H	G	F	F	V
HDAC7	H	P	D	H	G	F	F	V
HDAC8	L	A	D	H	G	F	F	M
HDAC9	H	P	D	H	G	F	F	V
HDAC10	E	I	D	H	G	F	W	A
HDAC11	H	P	N	H	G	F	Y	I

Figure S7 – Sequence alignment for all metal-dependent HDAC isoforms. The catalytic domains of the class I, II, and IV human HDAC proteins were aligned (Clustal W) and the residues located in the 14 Å internal cavity (A) and 11 Å active site channel (B) are shown with the numbering at the top is for HDAC1. The three conserved optimal substrate trapping mutants are highlighted in red.