Supplementary Data for

Differential HDAC1/2 network analysis reveals a role for prefoldin/CCT in HDAC1/2 complex assembly

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Supplementary Table S3: HDAC1 complexes in HeLa cells

Supplementary Tables S4: HDAC2 complexes in HeLa cells

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Supplementary Data – DNA Sequences

1. **Primers used to construct vectors expressing Halo tagged HDAC subunits**

For cloning Halo-HDAC1 in pFN21A:

HDAC1 SgfI F 5'- CAGGCGATCGCCATGGCGCAGACGCAGG - 3'

HDAC1 PmeI R (with stop) 5'- CAGGTTTAAACTCAGGCCAACTTGACCTCCTC - 3'

For cloning HDAC1-Halo in pFC14A:

HDAC1 SgfI F (2) 5'- AAAAGCGATCGCCATGGCGCAGACG - 3'

HDAC1 PmeI R (no stop) 5'- AAAAGTTTAAACGGCCAACTTGACCTCC - 3'

For cloning HDAC2-Halo in pFC14A

HDAC2 SgfI F 5'- AAAAGCGATCGCCATGGCATATAGC - 3'

HDAC2 PmeI R (no stop) 5'- AAAAGTTTAAACCGGATTAGAGAGCTGCTCAG - 3'

2. Synthetic sequence used to express HDAC2 (subcloned into pFN21A)

5’ - GCGATCGCCATGGCATATAGCCAAGGTGGGGGCAAAAAAAAAGTATGTTATTATTATGA
TGGAGATATCGGTAACTATTACTATGGTCAGGGCCACCCAATGAAGCCACATAGAATCAGG
ATGACTCACAACCTGCTGTTGAATTATGGGTTGTATCGAAAGATGGAGATCTATAGACCACA
TAAGGCCACTGCTGAAGAGATGACCAAGTATCATTCTGATGAGTATATTAAATTTCTGAGGT
CCATCAGGCCAGACAACATGAGTGAGTACAGTAAACAAATGCAGCGCTTTAATGTGGGTGA
GGATTGCCCAGTGTTCGACGGTCTGTTTGAGTTCTGTCAGCTCAGCACTGGCGGCTCTGTTGC
AGGGGCGGTAAAACTTAACCGCCAGCAAACGGATATGGCTGTTAATTGGGCAGGTGGCTTG
CATCATGCGAAAAAGTCCGAGGCTAGTGGGTTTTGTTACGTCAACGATATTGTCTTGGCCAT
CCTTGAGTTGCTCAAATACCATCAGCGCGTTCTCTACATAGATATCGATATCCATCACGGTG
ACGGCGTGGAAGAGGCCTTTTACACAACAGACAGAGTGATGACGGTTTCCTTCCACAAGTAT
GGCGAGTATTTCCCTGGCACTGGCGATCTCCGCGACATCGGTGCGGGTAAAGGAAAGTACTA
CGCGGTTAACTTCCCCATGCGAGACGGGATAGACGACGAGTCCTATGGCCAAATATTTAAGC
CCATAATATCTAAAGTGATGGAAATGTATCAACCGAGCGCCGTGGTTTTGCAGTGCGGGGCA
GATAGTTTGTCTGGTGATCGCCTTGGGTGCTTTAATCTTACCGTCAAAGGTCACGCCAAGTGC
GTAGAAGTAGTCAAGACATTCAACTTGCCTCTCCTCATGTTGGGGGGCGGCGGGTACACGAT
CAGAAATGTGGCTAGATGTTGGACGTATGAAACCGCAGTTGCGCTGGATTGCGAGATTCCAA
ATGAGTTGCCTTACAACGATTATTTTGAATATTTTGGCCCGGATTTCAAACTTCATATTTCCC
CTAGTAATATGACTAACCAAAATACCCCGGAATATATGGAAAAAATAAAACAGCGACTCTT
CGAGAATCTTCGGATGTTGCCCCATGCTCCCGGCGTGCAGATGCAGGCGATACCAGAGGATG
CGGTCCACGAGGACAGCGGAGATGAAGATGGTGAGGACCCGGATAAGCGAATATCCATTCG
CGCCAGTGATAAACGGATAGCCTGTGACGAAGAATTTTCTGACAGTGAAGATGAGGGAGAG
GGAGGCCGCAGGAATGTGGCTGACCATAAAAAGGGCGCGAAGAAAGCAAGAATTGAAGAG
GACAAAAAGGAAACAGAGGACAAGAAGACTGATGTTAAGGAGGAGGACAAGTCAAAAGAC
AACAGTGGTGAGAAAACAGATACTAAAGGAACTAAATCTGAGCAGCTCTCTAATCCGTGAG
TTTAAAC - 3’

Supplementary Figure 1. Western blot analysis of Halo-HDAC1/HDAC1-Halo expressed in HeLa cells. Lysates from HeLa cells transiently transfected with plasmids expressing either Halo tag alone, Halo-HDAC1 or HDAC1-Halo were prepared as described in Materials and Methods. HDAC complexes were purified from lysates using Halo affinity purification as described in Materials and Methods. Samples of the HeLa cell lysates (left panel) or Halo purified protein complexes (right panel) were fractionated by SDS-PAGE and analysed by Western blotting using rabbit anti-HDAC1 primary antibodies and IRDye® 800CW labeled goat anti-Rabbit secondary antibodies. Proteins were visualised using an Odyssey CLx infra-red imaging system (LI-COR Biosciences).

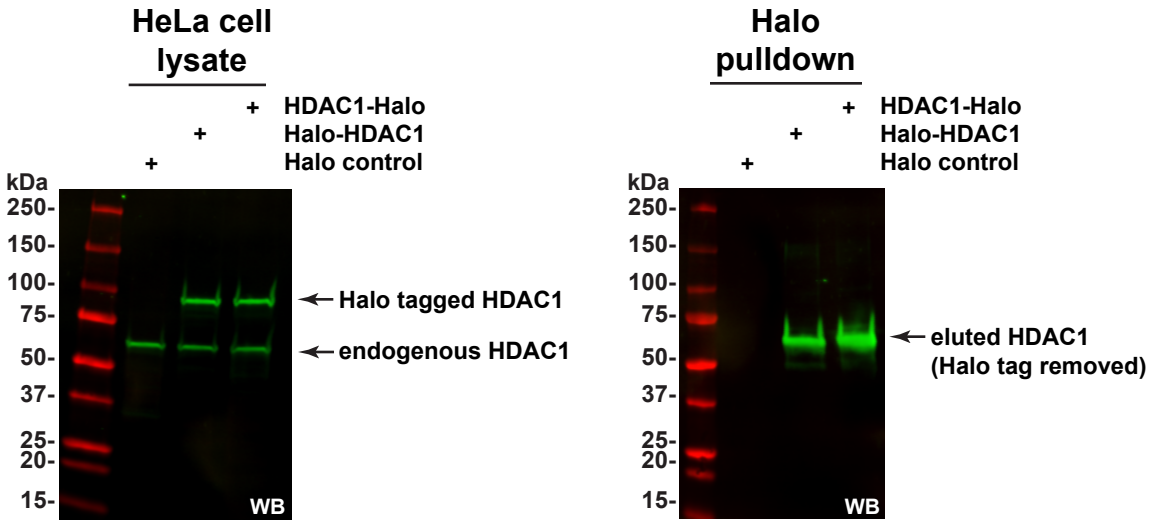
Supplementary Figure 2. Full length gel images corresponding to figure 3D. Images are of Western blots probed with the indicated primary antibodies and fluorescently labeled secondary antibodies. Blots were scanned using a Li-Cor Odyssey infra-red scanner and visualised using the corresponding Li-Cor software Image Studio (version 2.1). Images were exported as .tiff files at 600 dpi.

Supplementary Figure 3. Constructing a differential protein interaction network using Diffany. To illustrate the rewiring of the HDAC1/2 interaction network after affinity tag relocation, we first created two separate interaction networks and then subtracted one network from the other using Diffany software. The initial networks mapped interactions of prey proteins with either N terminally or C terminally tagged versions of HDAC1/2 (network N or network C). Each interaction in these networks has an associated dBNSAF value. We next subtracted network C from network N to generate the differential interaction network. Edge widths (unbundled regions in Fiigure 4) represent differences in dBNSAF values with positive values in red and negative values in green.

Supplementary Figure 4. HDAC1 copurifies with SNAP-FLAG-VBP1 captured from HEK293T cell lysates. HEK293T cells were transiently transfected with plasmids expressing either Halo-HDAC1 or HDAC1-Halo with or without SNAP-FLAG-VBP1 as indicated. Lysates were prepared as described in Materials and Methods. SNAP-FLAG-VPB1 associated proteins were purified using SNAP affinity purification. Samples were fractionated by SDS-PAGE and analysed by Western blotting using rabbit anti-Halo primary antibodies and IRDye® 800CW labeled goat anti-Rabbit secondary antibodies (green bands),or using mouse anti-FLAG primary antibodies and IRDye® 680RD labeled goat anti-Mouse secondary antibodies (red bands). Proteins were visualised using an Odyssey CLx infra-red imaging system (LI-COR Biosciences). Full length images of Western blots are presented in Supplementary Figure 5.

Supplementary Figure 5. Full length gel images corresponding to figures 5B, Supplementary Figure 4, and Figure 6C. Western blots were probed with the indicated primary antibodies and fluorescently labeled secondary antibodies. Western blots and the Coomassie stained gel were scanned using a Li-Cor Odyssey infra-red scanner and visualised using the corresponding Li-Cor software Image Studio (version 2.1). Images were exported as .tiff files at 600 dpi.

Supplementary Figure 1: Western blot analysis of Halo-HDAC1/HDAC1-Halo expressed in HeLa cells



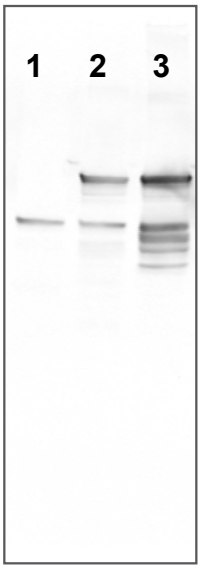
Lysates from HeLa cells transiently transfected with plasmids expressing either Halo tag alone, Halo-HDAC1 or HDAC1-Halo were prepared as described in Materials and Methods. HDAC complexes were purified from lysates using Halo affinity purification as described in Materials and Methods. Samples of the HeLa cell lysates (left panel) or Halo purified protein complexes (right panel) were fractionated by SDS-PAGE and analysed by Western blotting using rabbit anti-HDAC1 primary antibodies and IRDye® 800CW labeled goat anti-Rabbit secondary antibodies. Proteins were visualised using an Odyssey CLx infra-red imaging system (LI-COR Biosciences).

Supplementary Figure 2: Full length gel images corresponding to figures 3D

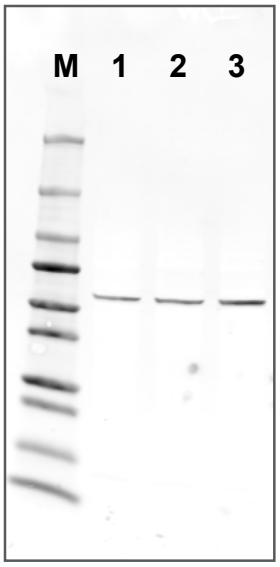
Figure 3D

Key: **M Size markers**
1 Halo control
2 Halo-HDAC1
3 HDAC1-Halo

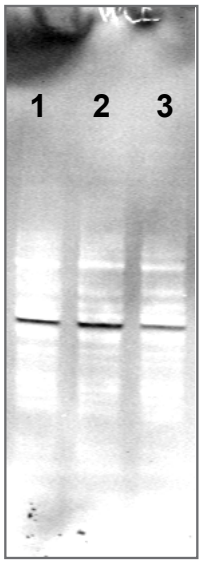
293 cell lysate



HDAC1



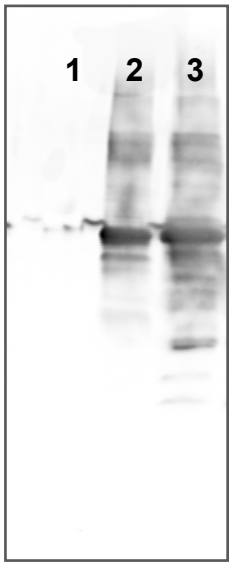
TCP1



SUDS3

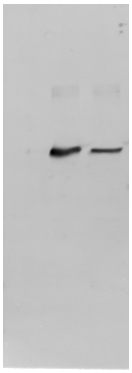
SAP30

Halo Pulldown

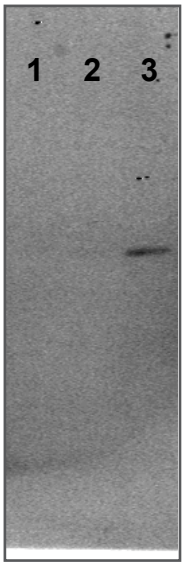


HDAC1

TCP1



TCP1 Western blot with background signal increased abouve default settings using Image Studio (version 2.1)



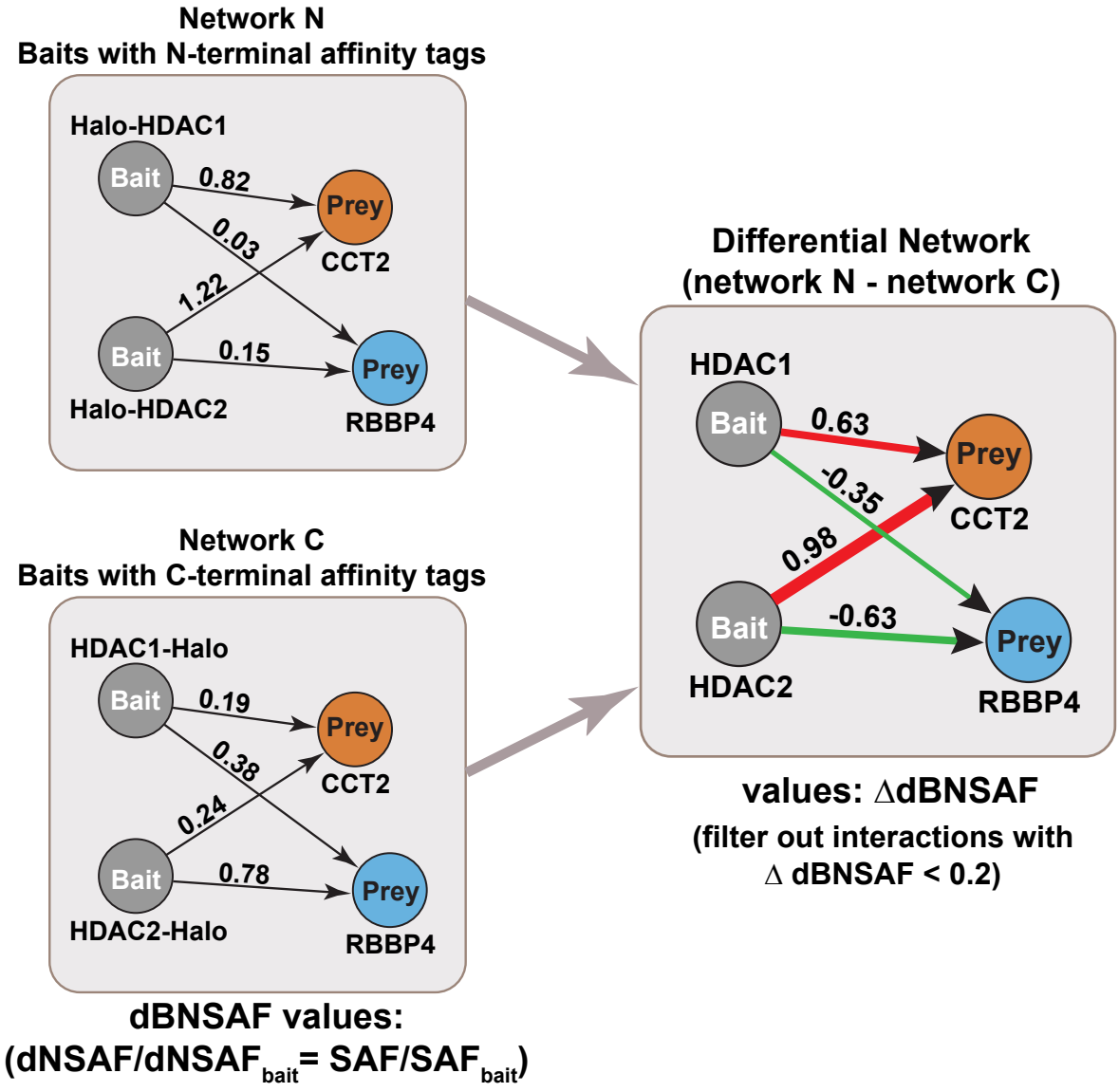
SUDS3

SAP30

Images are of Western blots probed with the indicated primary antibodies and fluorescently labeled secondary antibodies. Blots were scanned using a Li-Cor Odyssey infra-red scanner and visualised using the corresponding Li-Cor software Image Studio (version 2.1). Images were exported as .tiff files at 600 dpi.

Supplementary Figure 3

Differential Interaction Network Analysis using Diffany[1]

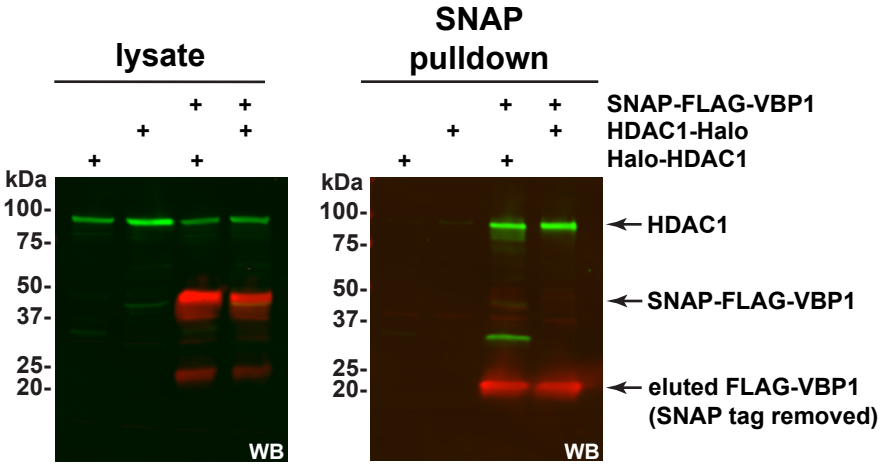


Contructing a differential protein interaction network using Diffany[1]–To illustrate the rewiring of the HDAC1/2 interaction network after affinity tag relocation, we first created two separate interaction networks and then subtracted one network from the other using Diffany software[1]. The initial networks mapped interactions of prey proteins with either N terminally or C terminally tagged versions of HDAC1/2 (network N or network C). Each interaction in these networks has an associated dBNSAF value.
We next subtracted network C from network N to generate the differential interaction network. Edge widths (unbundled regions in Fiigure 4) represent differences in dBNSAF values with positive values in red and negative values in green.

1. Landeghem, S. Van, Parys, T. Van, Dubois, M., Inzé, D. & Van De Peer, Y. Diffany: an ontology-driven framework to infer, visualise and analyse differential molecular networks. BMC Bioinformatics 17, 18 (2016).

Supplementary Figure 4:

HDAC1 copurifies with SNAP-FLAG-VBP1 captured from HEK293T cell lysates

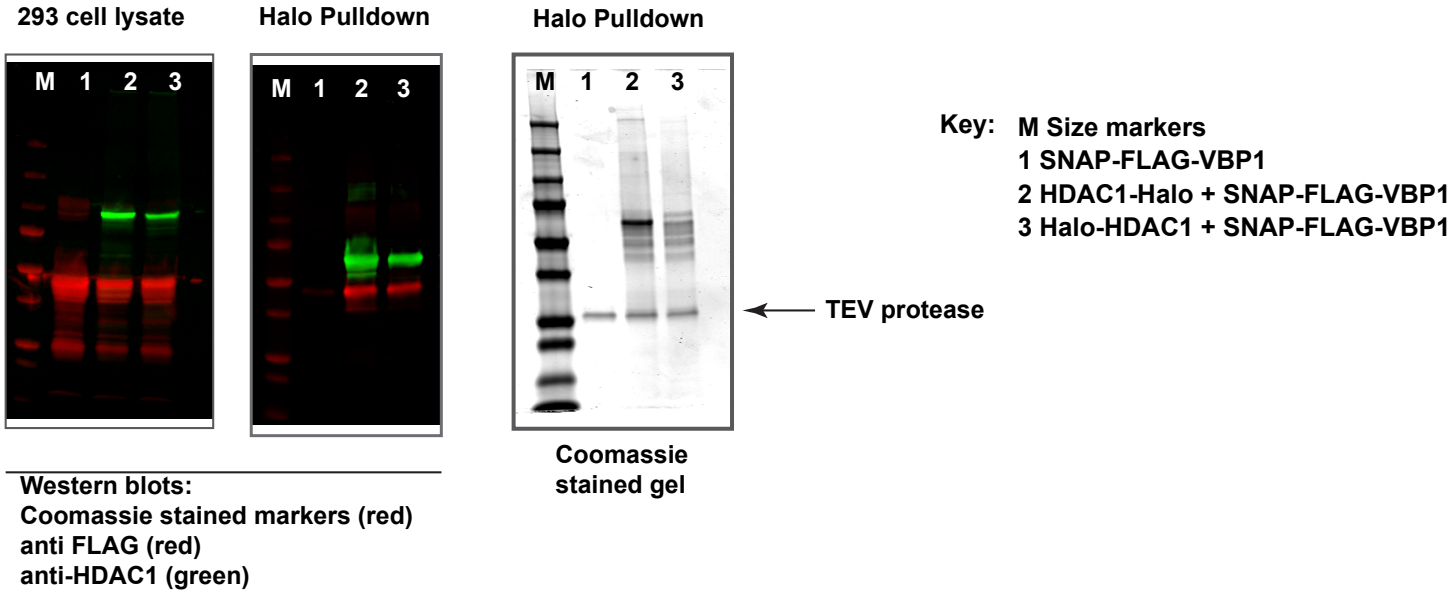


HEK293T cells were transiently transfected with plasmids expressing either Halo-HDAC1 or HDAC1-Halo with or without SNAP-FLAG-VBP1 as indicated. Lysates were prepared as described in Materials and Methods. SNAP-FLAG-VPB1 associated proteins were purified using SNAP affinity purification as described previously[1]. Samples were fractionated by SDS-PAGE and analysed by Western blotting using rabbit anti-Halo primary antibodies and IRDye® 800CW labeled goat anti-Rabbit secondary antibodies (green bands), or using mouse anti-FLAG primary antibodies and IRDye® 680RD labeled goat anti-Mouse secondary antibodies (red bands). Proteins were visualised using an Odyssey CLx infra-red imaging system (LI-COR Biosciences). Full length images of Western blots are presented in Supplementary Figure 5.

1. Banks, C. A. S. et al. A Structured Workflow for Mapping Human Sin3 Histone Deacetylase Complex Interactions Using Halo-MudPIT Affinity-Purification Mass Spectrometry. Mol. Cell. Proteomics 17, 1432–1447 (2018).

Supplementary Figure 5:
Full length gel images corresponding to Figure 5B, Supplementary Figure 4, and Figure 6C

Figure 5B



Supplementary Figure 4

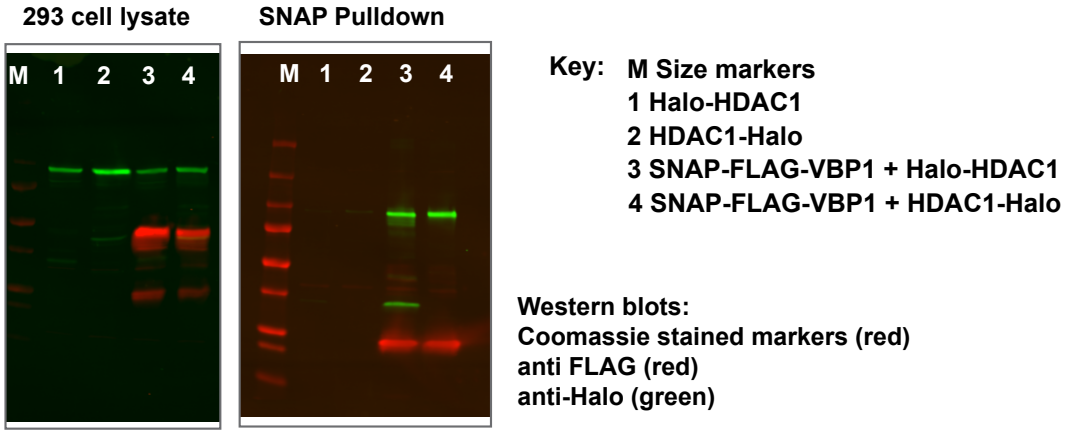
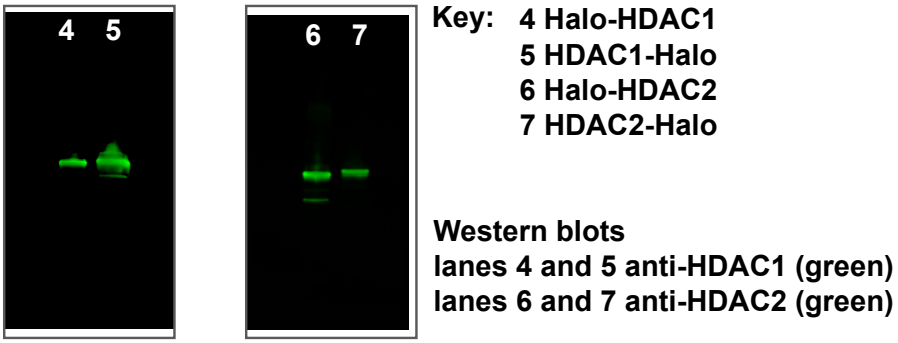


Figure 6C



Western blots were probed with the indicated primary antibodies and fluorescently labeled secondary antibodies. Western blots and the Coomassie stained gel were scanned using a Li-Cor Odyssey infra-red scanner and visualised using the corresponding Li-Cor software Image Studio (version 2.1). Images were exported as .tiff files at 600 dpi.