Supplementary material

HSP90 inhibition induces selective destabilization of HDAC6 in a subset of tumor cell types

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Table S1. List of antibodies used in the study

Antibody	Producer	Final concentration
anti-HDAC1 rabbit monoclonal antibody (D5C6U)	Cell Signaling 34589	4 ng/mL
anti-HDAC2 rabbit monoclonal antibody (D6S5P)	Cell Signaling 57156	50 ng/ml
anti-HDAC3 rabbit monoclonal antibody (D2O1K)	Cell Signaling 85057T	50 ng/ml
anti-HDAC4 rabbit monoclonal antibody (D8T3Q)	Cell Signaling 15164	160 ng/mL
anti-HDAC5 rabbit monoclonal antibody (D1J7V)	Cell Signaling 20458	230 ng/mL
anti-HDAC6 mouse monoclonal antibody (159)	inhouse, clone 159/17/45	250 ng/mL
anti-HDAC6 rabbit monoclonal antibody (D21B10)	Cell Signaling 7612	90 ng/mL
anti-HDAC7 rabbit monoclonal antibody (D4E1L)	Cell Signaling 33418	902 ng/mL
anti-HDAC8 rabbit monoclonal antibody (E7F5K)	Cell Signaling 66042	75 ng/mL
anti-HDAC9 rabbit monoclonal antibody (EPR5223)	Abcam ab109446	550 ng/mL
anti-HDAC10 rabbit polyclonal antibody	Abcam ab18971	100 ng/mL
anti-HDAC10 rabbit polyclonal antibody	Sigma H3413	500 ng/mL
anti-HDAC11 rabbit monoclonal antibody (D5I8E)	Cell Signaling 58442	40 ng/mL
anti-HDAC11 rabbit polyclonal antibody	GeneTex GTX116403	620 ng/mL
anti-HDAC11 rabbit polyclonal antibody	Abcam ab18973	100 ng/mL
anti-c-Raf rabbit polyclonal antibody	Cell Signaling 9422	10 ng/mL
anti-Akt pan rabbit monoclonal antibody (C67E7)	Cell Signaling 4691	20 ng/mL
anti-Akt1 rabbit monoclonal antibody (C73H10)	Cell Signaling 2937	20 ng/mL
anti-Akt2 mouse monoclonal natibody (L79B2)	Cell Signaling 5239	700 ng/mL
anti-Akt3 rabbit monoclonal antibody (62A8)	Cell Signaling 3788	50 ng/mL
anti-HSP90α rabbit monoclonal antibody (D1A7)	Cell Signaling 8165	25 ng/mL
anti-HSP90β rabbit monoclonal antibody (D3F2)	Cell Signaling 7411	*dilution 2000x
anti-acetyl-tubulin α mouse monoclonal antibody	Sigma T7451	200 ng/mL
anti-tubulin alpha rabbit polyclonal antibody	Abcam ab18251	100 ng/mL
anti-GAPDH mouse monoclonal antibody	HyTest 5G4-6C5	100 ng/mL
goat anti-rabbit IgG (H + L)-HRP conjugate	Bio-Rad 1706515	*dilution 10 000x
goat anti-mouse IgG (H + L)-HRP conjugate	Bio-Rad 1706516	*dilution 10 000x

* antibody concentration not specified by producer

Table S2. List of primers utilized in quantitative RT-PCR

Protein name	Gene name	Accession ID	forward primer	reverse primer
HDAC1	HDAC1	NM_004964.3	CGGGAGGCGAGCAAGATG	AGTCATGCGGATTCGGTGAGG
HDAC2	HDAC2	NM_001527.4	CATGGCGTACAGTCAAGGAGG	AGTTATGGGTCATGCGGATTCT
HDAC3	HDAC3	NM_001355039.2	GATTGGGCTGCTTTAACCTCA	ACAGTATAACCACCACCACCC
HDAC4	HDAC4	NM_006037.4	CACGACCTGACCGCCATTT	ACAGCGTTTGCATTGGGTCT
HDAC5	HDAC5	NM_005474.5	TCTGTAGCCATCACCGCAAA	GGGGTCATTGTAGAACGCCTG
HDAC6	HDAC6	NM_001321231.2	CACTGGCTTGGTGTTGGATG	GGCCTGAAAGGACACGCAG
HDAC7	HDAC7	NM_015401.5	CAGAAACCCAACCTCAATGCC	ACTTCTTCTTTGTCAGCCCCT
HDAC8	HDAC8	NM_018486.3	TGTCAGTATGTGTGACTCCCTG	GCATCAGTGTGGAAGGTGG
HDAC9	HDAC9	NM_001204146.2	TTGGAGAAGCAGAAGCAATACC	TCAAGGTGACTGCCTGGTTG
HDAC10	HDAC10	NM_001159286.2	TCTACTTCCACCCGAGTACCTT	ATTTTGCACAGCTCCAGTGA
HDAC11	HDAC11	NM_001330636.2	ACCGCCACATCTACCCAG	TGTTCCTCTCCACCTTATCCA
Akt1	AKT1	NM_001014431.2	CGTGGCTATTGTGAAGGAGGG	ATTCTTGAGGAGGAAGTAGCGT
c-Raf	RAF1	NM_001354695.3	GCAGACGGCTCAGGGAATG	CCACTCCAGCGTGACTTTACT
HSP90α	HSP90AA1	NM_005348.4	GAAACTGCGCTCCTGTCTTCT	GTCATCTTCATCAATACCCAGACC
HSP90β	HSP90AB1	NM_001271969.2	TCCCTTTGACCTTTTTGAGAACA	ACCACACCACGGATAAAATTGAG

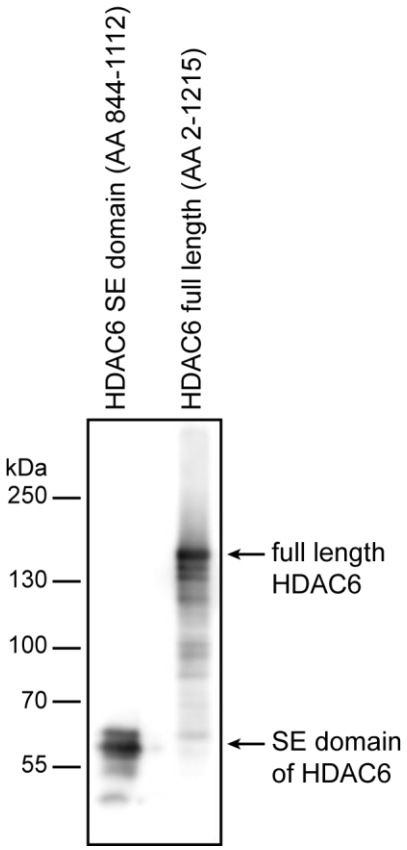

Figure S1. Specificity of anti-HDAC6 mouse monoclonal antibody Ab 159. β-mercaptoethanol-reduced samples of purified recombinant protein variants of HDAC6 were separated by SDS PAGE, and electroblotted on PVDF membrane that was further incubated in 5% milk/PBS/0.05% Tween-20 buffer for 45 min, and probed overnight with 1 µg/mL anti-HDAC6 Ab 159 diluted in 2% milk/PBS/0.05% Tween-20 buffer. Specific signal was detected using HRP-conjugated anti-mouse IgG secondary antibody. Antibody Ab 159 specifically recognizes epitope in SE domain of HDAC6 and detects full length HDAC6 construct.

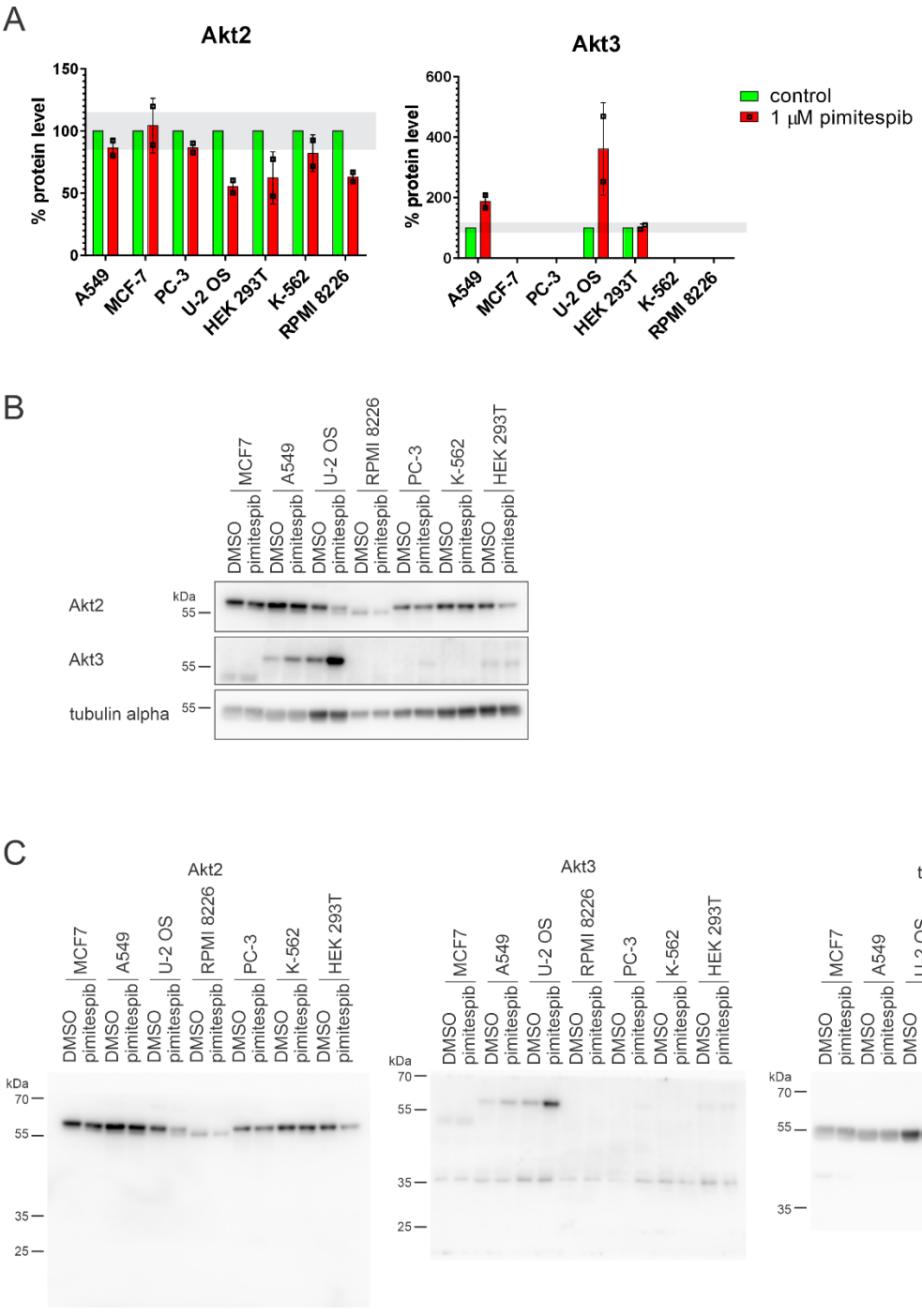

Figure S2. Protein level of Akt2 and Akt3 upon HSP90 inhibition. Cells were treated by 1 μM pimitespib or 0.1% DMSO (control) for 24 hours. Isoforms of Akt were immunodetected in cell lysates transferred on western blot membrane. The quantification error of the chemiluminescence detection equal 15% is marked by grey area. (A) Data were normalized by tubulin alpha level, then protein levels upon pimitespib treatment were related to control samples of individual cell lines. (B) Representative images of individual proteins immunodetected on western blot. (C) Uncropped unprocessed images of western blots.

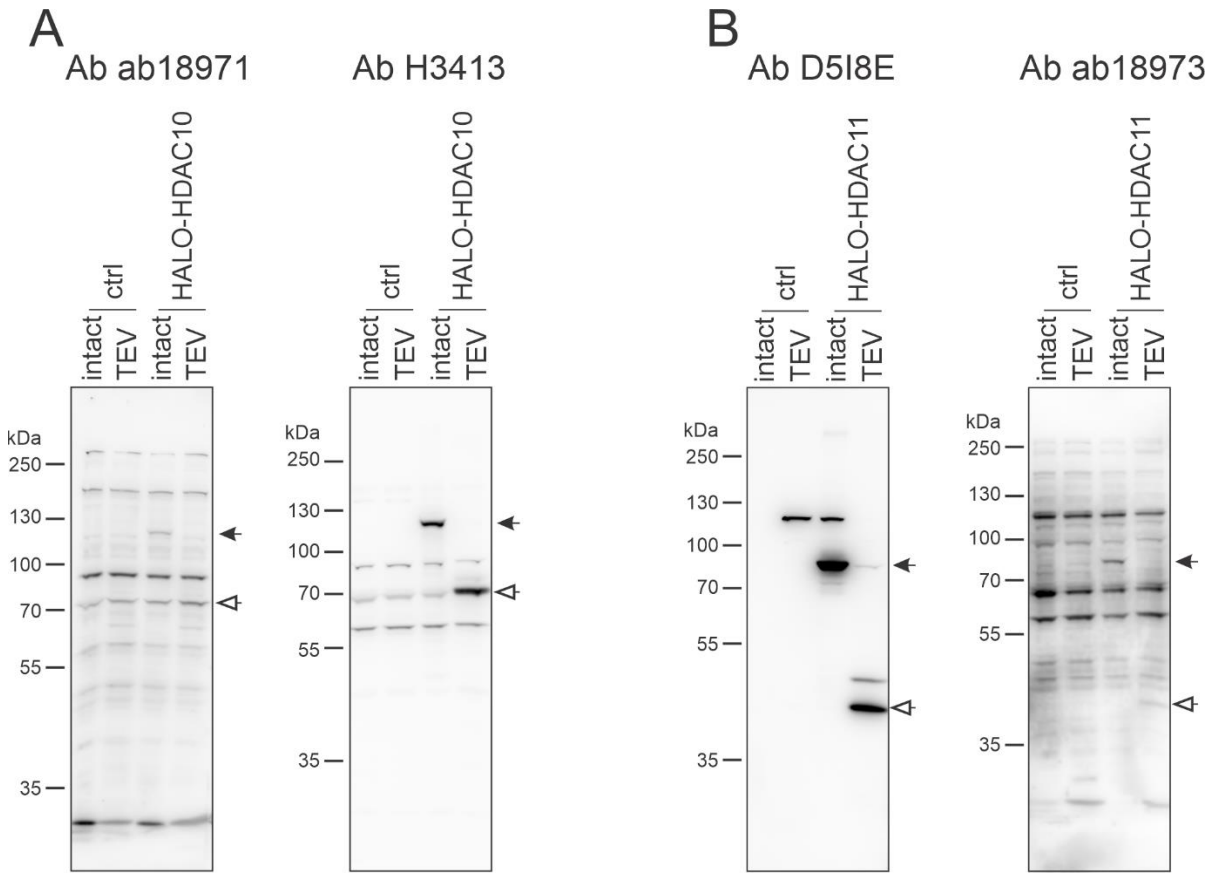

Figure S3. Evaluation of antibody specificity. Cells were transfected by constructs of HDAC10 and HDAC11 fused with HALO tag cleavable by TEV protease. Cell lysates were incubated with TEV, separated by SDS PAGE, and electroblotted onto membrane. HDACs were detected on membrane using antibodies from different sources. Samples without TEV treatment (intact) show signal of fusion construct (black arrow) while TEV-treated samples reveal signal of HDAC cleaved from fusion (white arrow). (A) Molecular weight of HALO-HDAC10 construct ~ 116 kDa, molecular weight of TEV-cleaved HDAC10 ~ 71 kDa. Both antibodies recognize human recombinant HDAC10. (B) Molecular weight of HALO-HDAC11 construct ~ 84 kDa, molecular weight of TEV-cleaved HDAC11 ~ 39 kDa. Both antibodies recognize human recombinant HDAC11.

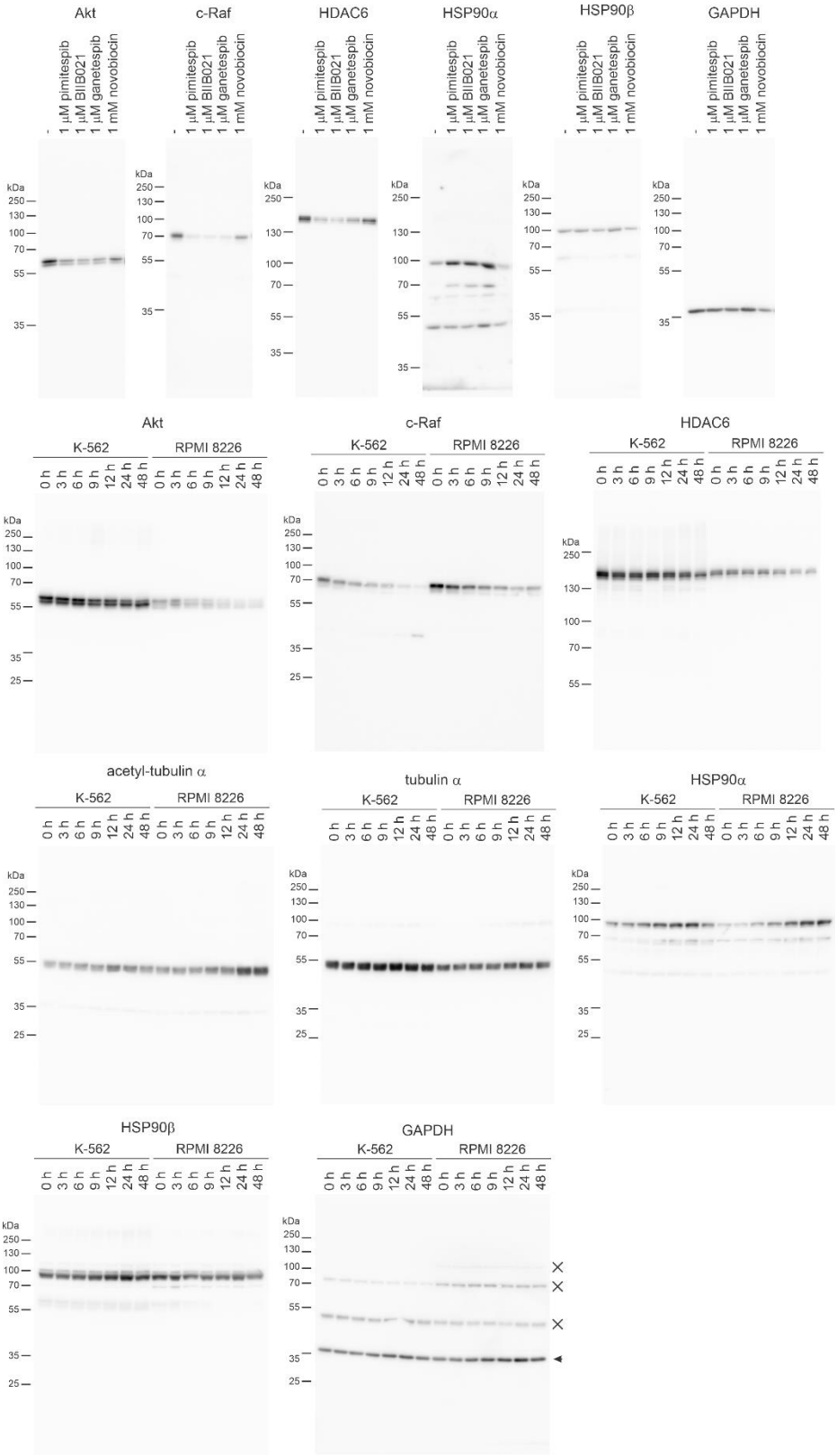

Figure S4. Uncropped unprocessed western blots shown in Figure 1. Position of detected antigen is marked by arrow while signals originated from the former detection of different antigen on the same membrane are marked by cross.

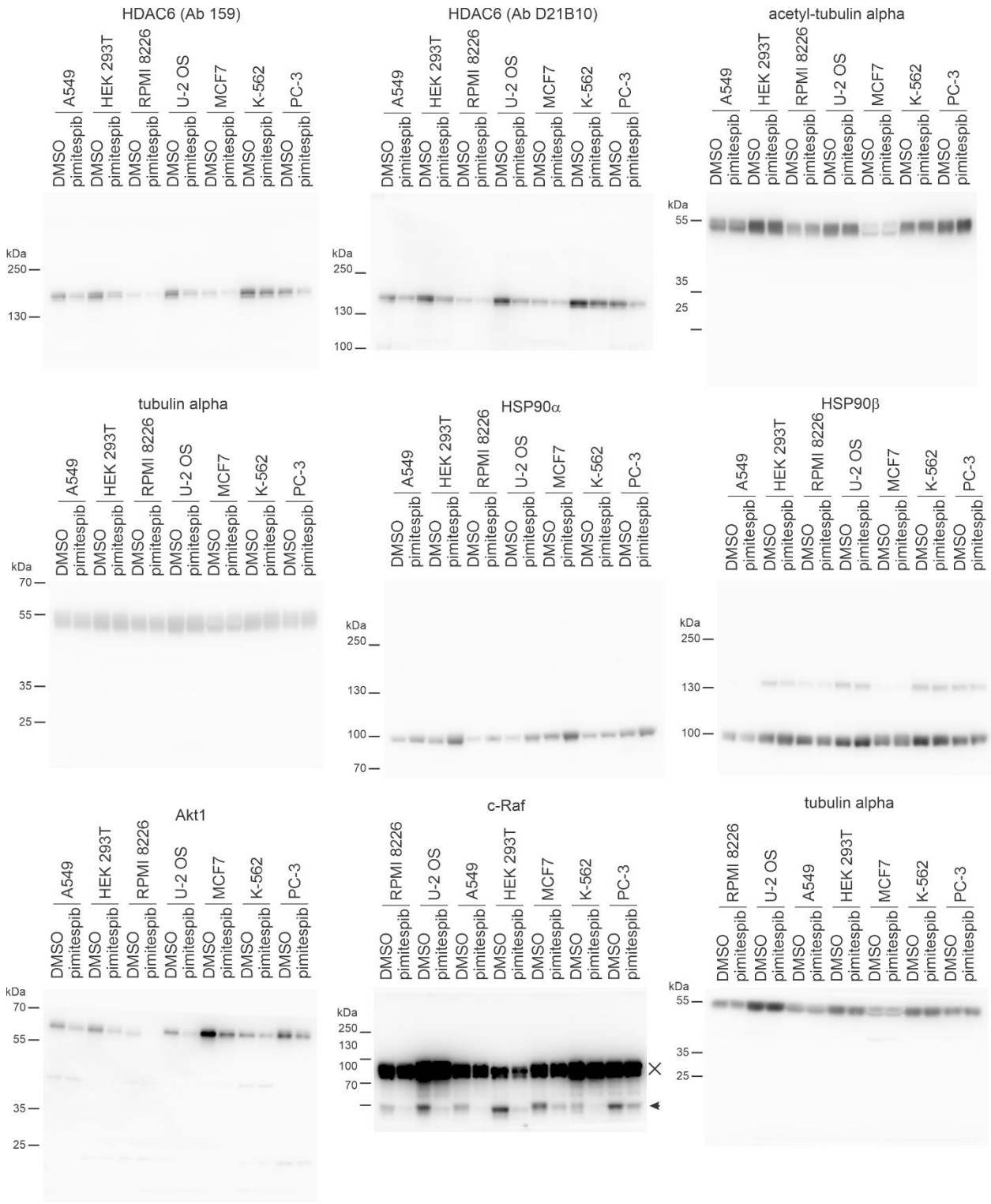

Figure S5. Uncropped unprocessed western blots shown in Figure 2. Position of detected antigen is marked by arrow while signals originated from the former detection of different antigen on the same membrane are marked by cross.

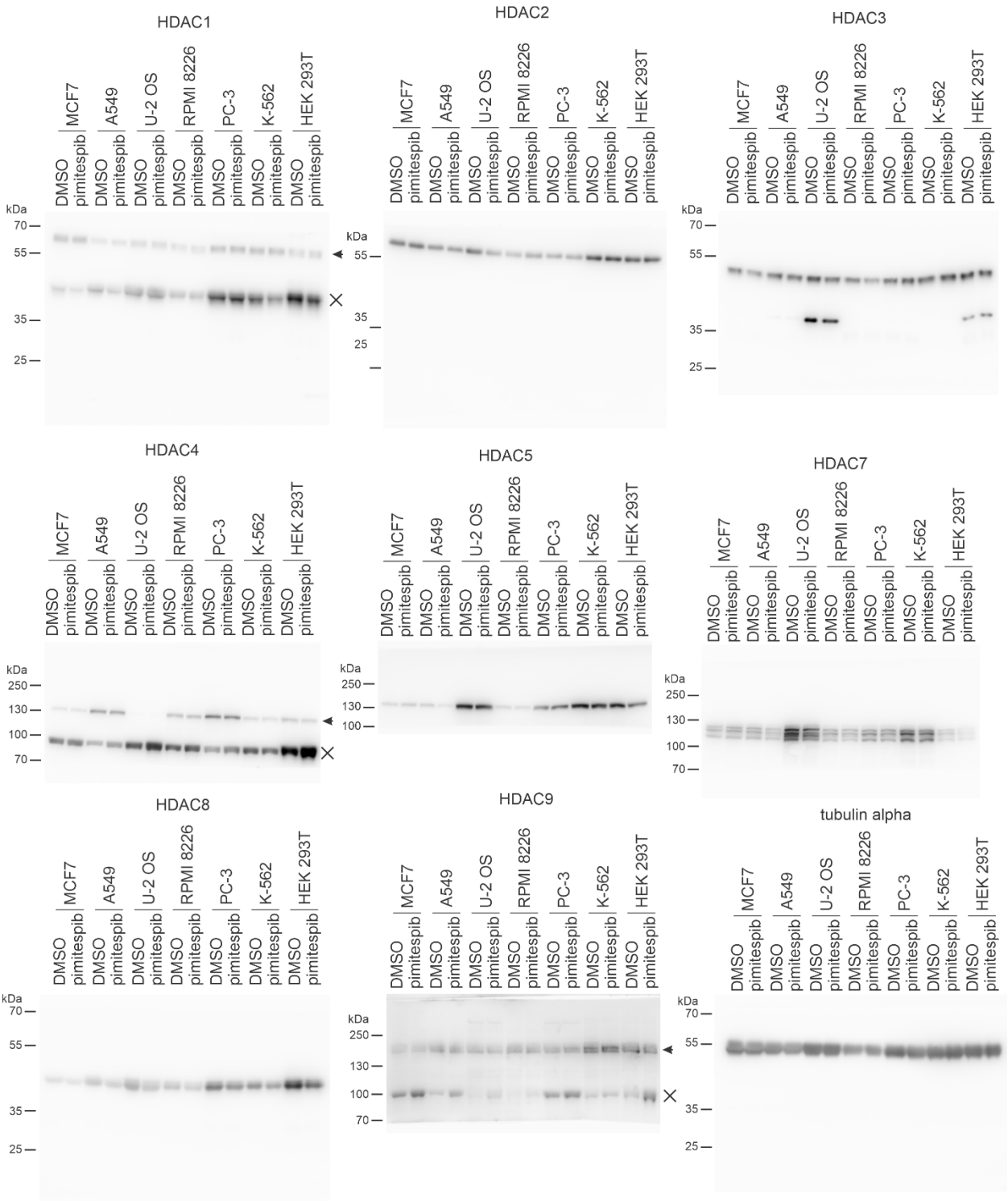

Figure S6. Uncropped unprocessed western blots shown in Figure 3. Position of detected antigen is marked by arrow while signals originated from the former detection of different antigen on the same membrane are marked by cross.

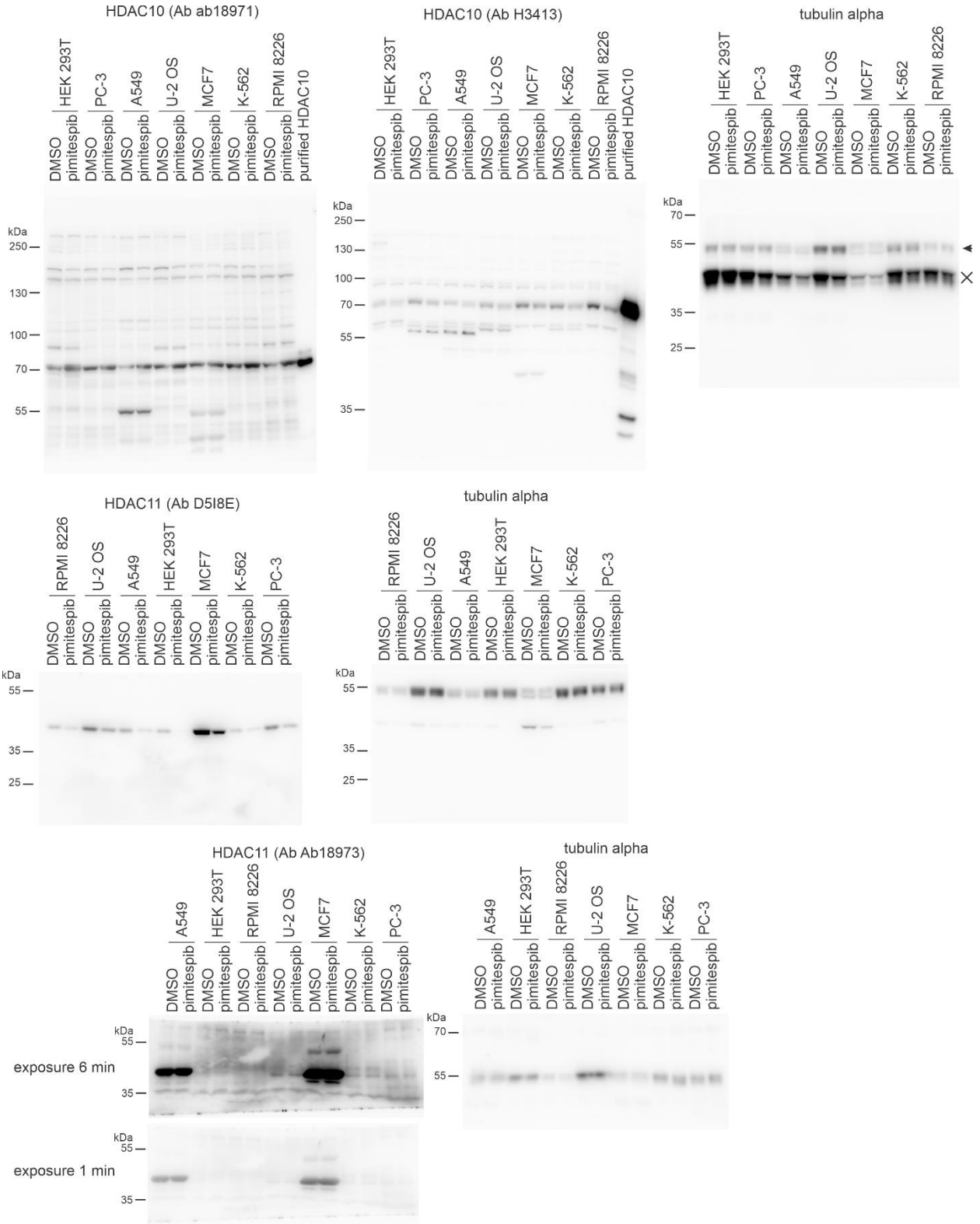

Figure S7. Uncropped unprocessed western blots shown in Figure 4. Position of detected antigen is marked by arrow while signals originated from the former detection of different antigen on the same membrane are marked by cross.