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Supplementary Materials for

Antibodies recognizing the C terminus of PP2A catalytic subunit are unsuitable for evaluating PP2A activity and holoenzyme composition

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Table S1. Antibodies used in this study.

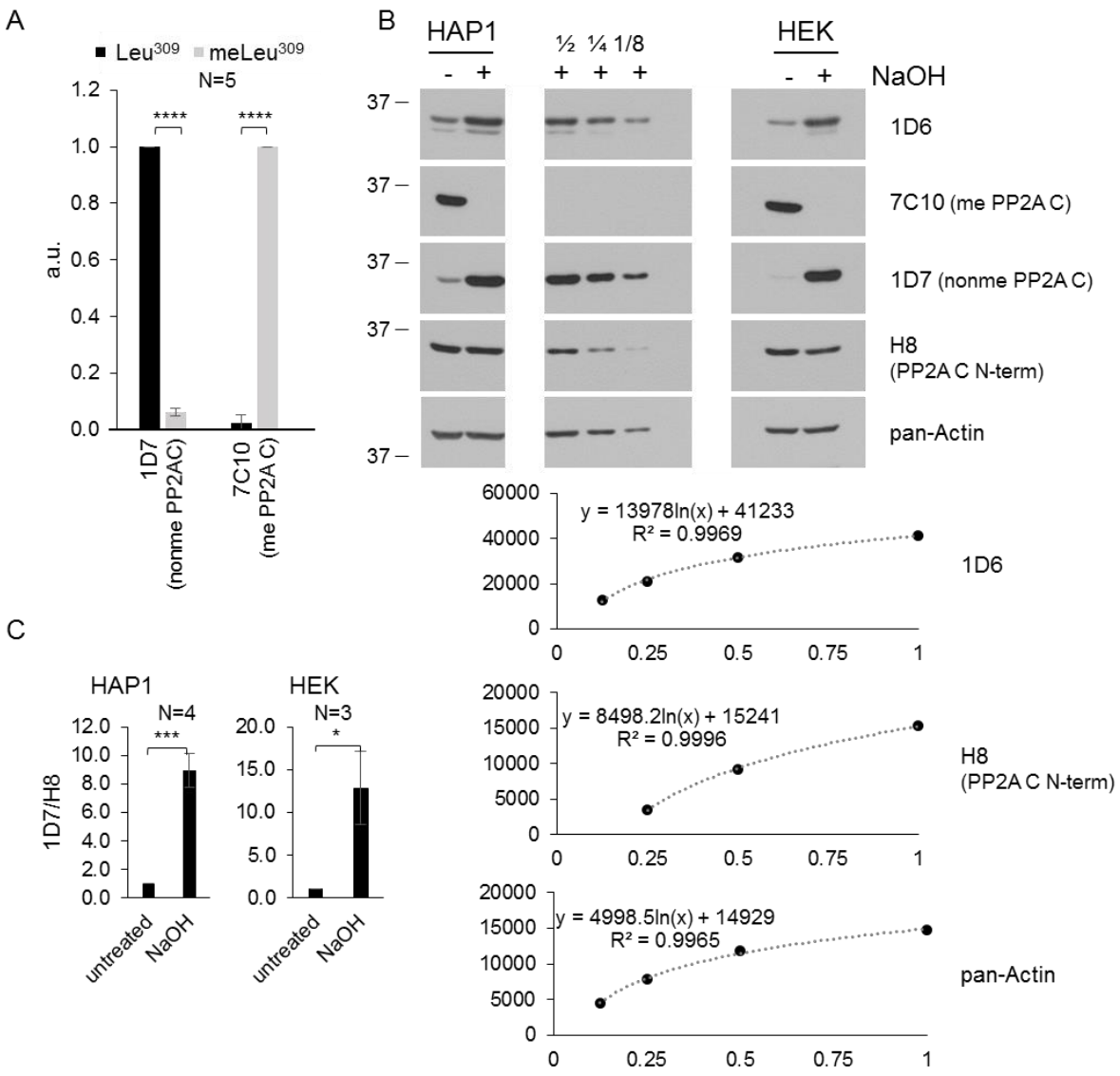

Fig. S1. 1D6 preferentially recognizes nonmethylated PP2A C subunit. (A) Quantification of monoclonal antibodies 1D7 and 7C10 binding to the peptides Leu^{309} (HVTRRTPDYFL) and meLeu^{309} (HVTRRTPDYFL-Me) by ELISA. Antibody binding data are shown as the average and standard deviation of *n*=5 independent ELISA experiments. The signals for 1D7 with the Leu^{309} peptide and 7C10 with the meLeu^{309} peptide were set arbitrarily to 1. (**B**) Immunoblotting of untreated or NaOH-treated lysates from HAP1 and HEK293Trex cells (same lysates as in Fig. 1A) using the indicated antibodies. The panel originates from 4 different blotting membranes for the C subunit antibodies, and the H8 blot was reincubated with a pan-actin antibody as loading control. The blots are representative of *n*=4 (HAP1) or *n*=3 (HEK) independent immunoblotting experiments. The blots were quantified by loading a dilution series of the HAP1 NaOH-treated sample. The equation of the logarithmic trendline was used to calculate the Western blot values of untreated and NaOH-treated samples, and the untreated samples were arbitrary set to 1. (**C**) Quantification of 1D7 blots in (B). Statistical significance was assessed using Student's t test. P values are indicated with *, ***, ****, which correspond to values of <0.05, <0.001, and <0.0001, respectively. a.u. arbitrary units.

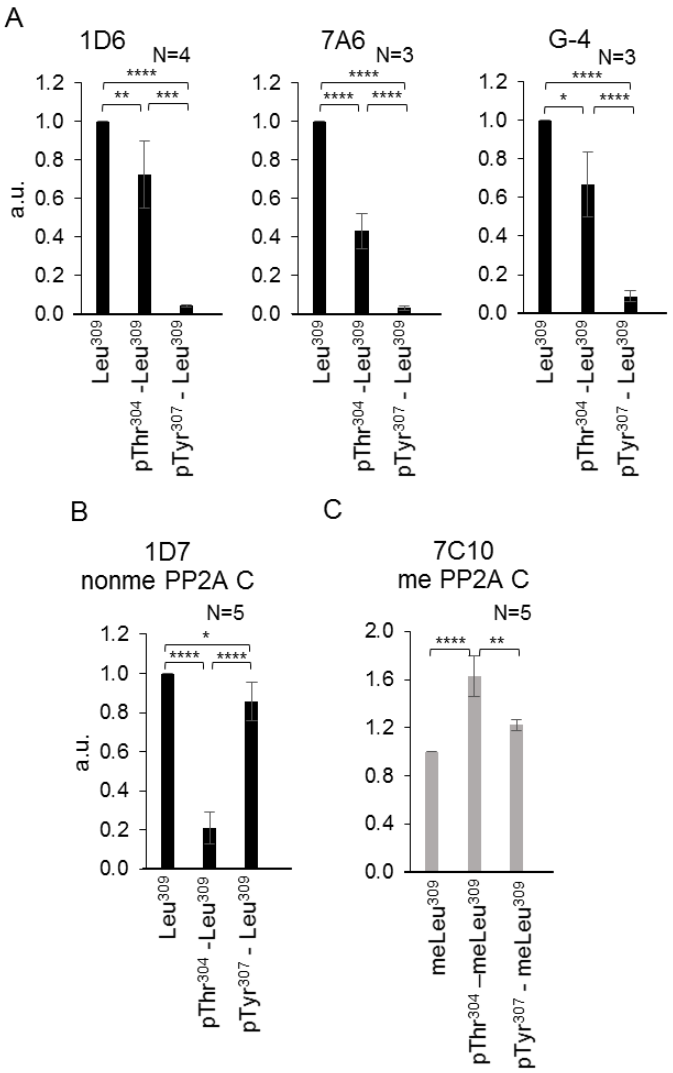

Fig. S2. Phosphorylation of Thr^{304} or Tyr^{307} influences epitope recognition by PP2A C-terminal antibodies. (**A**) Quantification of monoclonal antibodies 1D6, 7A6 and G4, binding to peptides Leu^{309} (HVTRRTPDYFL) and pThr^{304}-Leu^{309} (HVTRRpTPDYFL) or pTyr^{307}-Leu^{309} (HVTRRpTPDYFL) by ELISA. *n*=4 (1D6) or 3 (7A6 and G-4) independent ELISA experiments. Signals were normalized to Leu309 peptide, which was arbitrarily set to 1. (**B**) Quantification of monoclonal antibody 1D7 binding to peptides Leu^{309} (HVTRRTPDYFL) and pThr^{304}-Leu^{309} (HVTRRpTPDYFL) or pTyr^{307}-Leu^{309} (HVTRRpTPDYFL) by ELISA. N=5 independent ELISA experiments. Signals were normalized to Leu^{309} peptide, which was arbitrarily set to 1. (**C**) Quantification of monoclonal antibody 7C10 binding to peptides meLeu^{309} (HVTRRTPDYFL-Me) and pThr^{304}-meLeu^{309} (HVTRRpTPDYFL-Me) or pTyr^{307}-meLeu^{309} (HVTRRpTPDYFL-Me), by ELISA. *n*=5 independent ELISA experiments. Signals were normalized to meLeu^{309} peptide, which was arbitrarily set to 1. Statistical significance was assessed using and ANOVA and Tukey's HSD as post-hoc test. P values are indicated with *, **, ***, ****, which correspond to values of <0.05, <0.01, <0.005, and <0.001, respectively. a.u. arbitrary units.

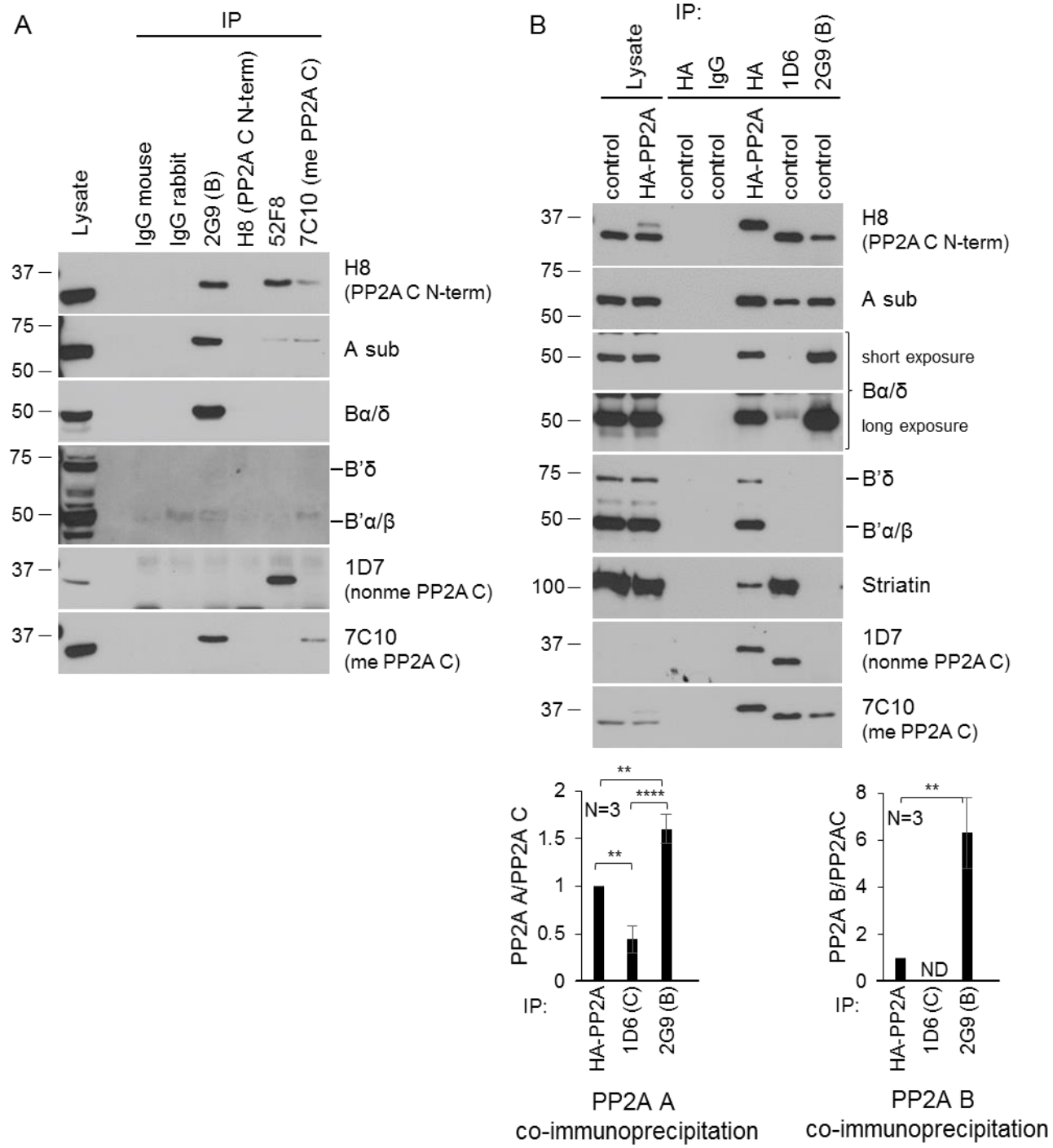
A
IP
Lysate
IgG mouse
IgG rabbit
2G9 (B)
H8 (PP2A C N-term)
52F8
7C10 (me PP2A C)
37
75
50
H8
(PP2A C N-term)
A sub
Bα/δ
B'δ
B'α/β
1D7
(nonme PP2A C)
7C10
(me PP2A C)
B
IP:
Lysate
HA
IgG
HA
1D6
2G9 (B)
control
HA-PP2A
control
control
HA-PP2A
control
control
short exposure
long exposure
100
Striatin
PP2A A/PP2A C
N=3
**
****
IP:
HA-PP2A
1D6 (C)
2G9 (B)
PP2A A
co-immunoprecipitation
PP2A B/PP2AC
ND
PP2A B
co-immunoprecipitation

Fig. S3. 1D6 and other C-terminal antibodies do not immunoprecipitate two major PP2A holoenzyme families. (**A**) Immunoblotting of 2G9, H8, 52F5, 7C10, and mouse or rabbit IgG (control) immunoprecipitates and lysates of HEK293 cells using the indicated antibodies. The panel originates from 5 different blotting membranes. The blots are representative of N=3 independent immunoprecipitation experiments. (**B**) Immunoblotting of HA, 1D6, 2G9, and IgG immunoprecipitates and lysates of HEK293Trex cells transfected with either a plasmid encoding HA-PP2A C subunit or empty vector using the indicated antibodies. The panel originates from 3 different blotting membranes. The blots are representative of *n*=3 independent immunoprecipitation experiments. The co-immunoprecipitated PP2A A and B subunits were quantified relative to the amount of immunoprecipitated PP2A C subunit. The amounts of PP2A A and B subunits co-precipitating with HA-PP2A C were arbitrarily set to 1. *n*=3 independent immunoprecipitation experiments. Statistical significance was assessed using and ANOVA and Tukey's HSD as post-hoc test. P values are indicated with **, ***, ****, which correspond to values of <0.01, <0.005, and <0.001, respectively. ND, not determined.

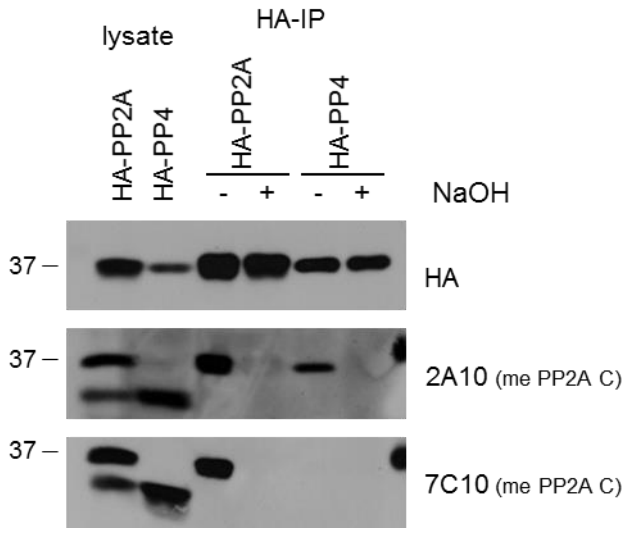

Fig. S4. 7C10 specifically recognizes methylated PP2A C, and 2A10 also recognizes methylated PP4 C. Immunoblotting of lysates and HA immunoprecipitates from NIH3T3 cells infected with retrovirus encoding HA-PP2A C or PP4 C or empty virus (-) using the indicated antibodies. The panel originates from separate blotting membranes for each antibody indicated in the panel. The blots are representative of *n*=3 independent immunoprecipitation experiments.

Table S1. Antibodies used in this study.

ID#	Species	Antigen	Dilution	Source	Reference /Cat Nr/Lot
1D6	Mouse monoclonal	Peptide (RGEPHVTRRTPDYFL) corresponding to amino acids 295-309 of human PP2A C subunit	E 1:5000 WB: 1:5000	Millipore	05-421, lot 2834654
7A6	Mouse monoclonal	Peptide (C-bA-RRTPDYFL) corresponding to amino acids 302-309 of human PP2A C subunit	E 1:50 WB 1:100	Ogris Lab, various companies	Lab stock
G-4	Mouse monoclonal	specific for an epitope mapping between amino acids 275-309 at the C-terminus of PP2A-Cα/β of human origin	E 1:500 WB 1:1000	SCBT	sc-166034, lot K2910
52F8	Rabbit monoclonal	Peptide surrounding P293 of human PP2A C subunit	WB: 1:2000	Cell signaling	#2259, lot 2
2038	Rabbit polyclonal	Peptide corresponding to amino acids at the C-terminus of human PP2A C subunit	WB 1:2000	Cell signaling	#2038, lot 2
1D7	Mouse monoclonal	Peptide (TPDYFL) corresponding to amino acids 304 to 309 of human PP2A C subunit	E: 1:500 WB 1:200	Ogris Lab, various companies	Lab stock
7C10	Mouse monoclonal	Peptide (TPDYFL-OMe) corresponding to amino acids 304-309 of human PP2A C subunit	E: 1:200 WB 1:100	Ogris Lab	Lab stock
2A10	Mouse monoclonal	Peptide (RRTPDYFL-OMe) corresponding to amino acids 302-309 of human PP2A C subunit	WB 1:50	Ogris Lab, various companies	Lab stock
H8	Mouse monoclonal	46-90aa of human PP2A catalytic subunit, α isoform	WB: 1:100	Ogris Lab	Lab stock, This study
11H12	Mouse monoclonal	Peptide (DTLKYSFLQFDPAPR) corresponding to amino acids 280-294 of human PP2A catalytic subunit, α isoform	WB 1:100	Ogris Lab	Lab stock, This study
2G9	Mouse monoclonal	Peptide (CASGKRKKDEISVD) corresponding to amino acids 398-411 of rat PP2A B55 alpha subunit, it may crossreact with the β, γ or δ isoforms of the B subunit family, but expression of B β, and B γ is restricted to testis and/or brain, (for review see Sontag, 2001)	WB: 1:200	Ogris Lab, various companies	Lab stock (*26*)
B’	Rabbit polyclonal	1-278 of human B’ B56 alpha subunit and purified. It primarily recognized B56 alpha and slightly cross-reacted with B56 gamma, B56 δ, and B56 ε, but not B56 beta.	WB 1:5000	Ogris Lab	Lab stock (*36*)
Asub	Rabbit polyclonal	Peptide (MAAADGDDSLY) corresponding to amino acid 1-11 of human PP2A A alpha	WB 1:5000	Ogris Lab, Millipore	Lab stock
PP4 Clone 7B5-G4	Mouse monoclonal	Peptide (EHLQKDFIIFEAAPQET) corresponding to amino acids 277-293 of mouse PP4 catalytic subunit	WB 1:100	Ogris Lab	Lab stock, This study

PP6 Clone 7G7-A7	Mouse monoclonal	Peptide (DVNTREPKLFRAVPDSE) corresponding to amino acids 276-292 of mouse PP6 catalytic subunit	WB 1:100	Ogris Lab	Lab stock, This study
LCMT-1	Rabbit polyclonal	Full length human LCMT1		Ogris Lab	Lab stock, This study
Pan-actin	Mouse monoclonal	Peptide (SKQEYDESGPSIVHRKC) corresponding to amino acid 358-374 of human actin	WB 1:500	Ogris Lab	Lab stock, This study
16B12	Mouse monoclonal	peptide CYPYDVPDYASL (Covance MMS-101R, lot B211583)	WB 1:20000	Covance	# MMS-101R, lot B211583
12CA5	Mouse monoclonal	36aa from the human influenza virus hemagglutinin protein			Lab stock
mouse heavy chain	Goat polyclonal	Reacts with Fc portion of mouse IgG heavy chain but not with Fab	WB and E 1:10000	Jackson ImmunoResearch	115-035-008, lot 124594
mouse light chain	Goat polyclonal	Reacts with light chain of mouse IgG	WB 1:5000	Jackson ImmunoResearch	115-035-174, lot 126230
rabbit heavy chain	Goat polyclonal	Reacts with Fc portion of rabbit IgG heavy chain but not with Fab portion	WB 1:10000	Jackson ImmunoResearch	111-035-008, lot 129411
IgG	Mouse	normal mouse IgG		SCBT	# sc-2025