SUPPLEMENTARY INFORMATION:

The microtubule-associated tau protein has intrinsic acetyltransferase activity

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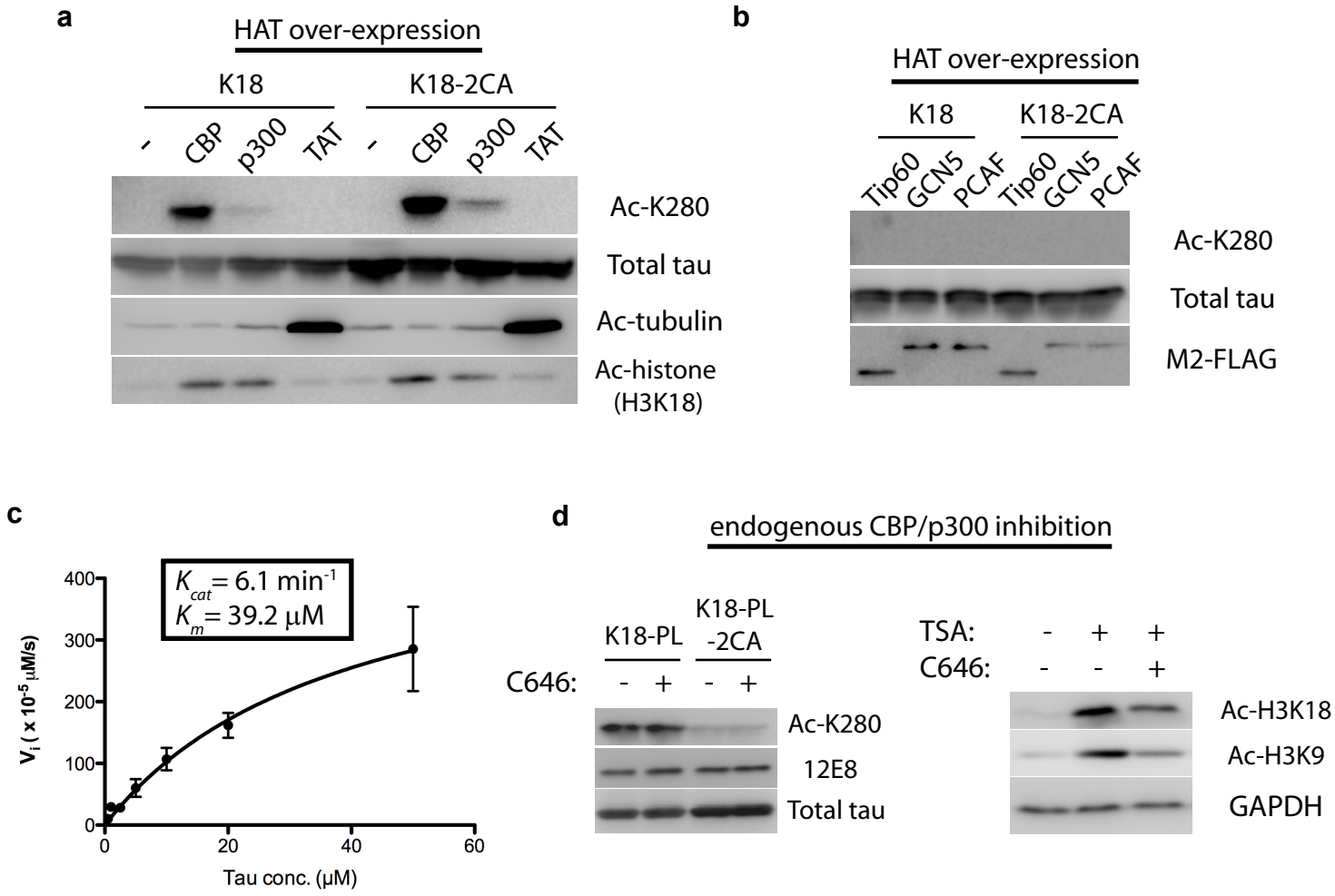
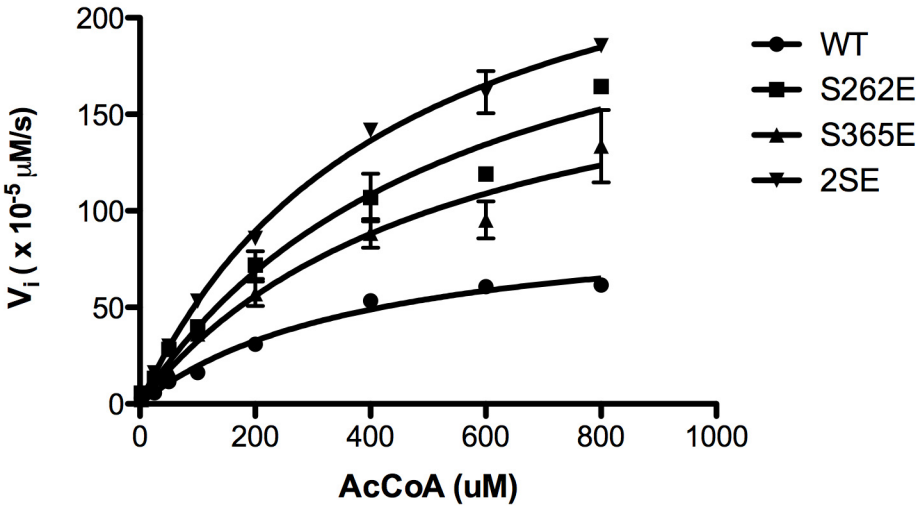
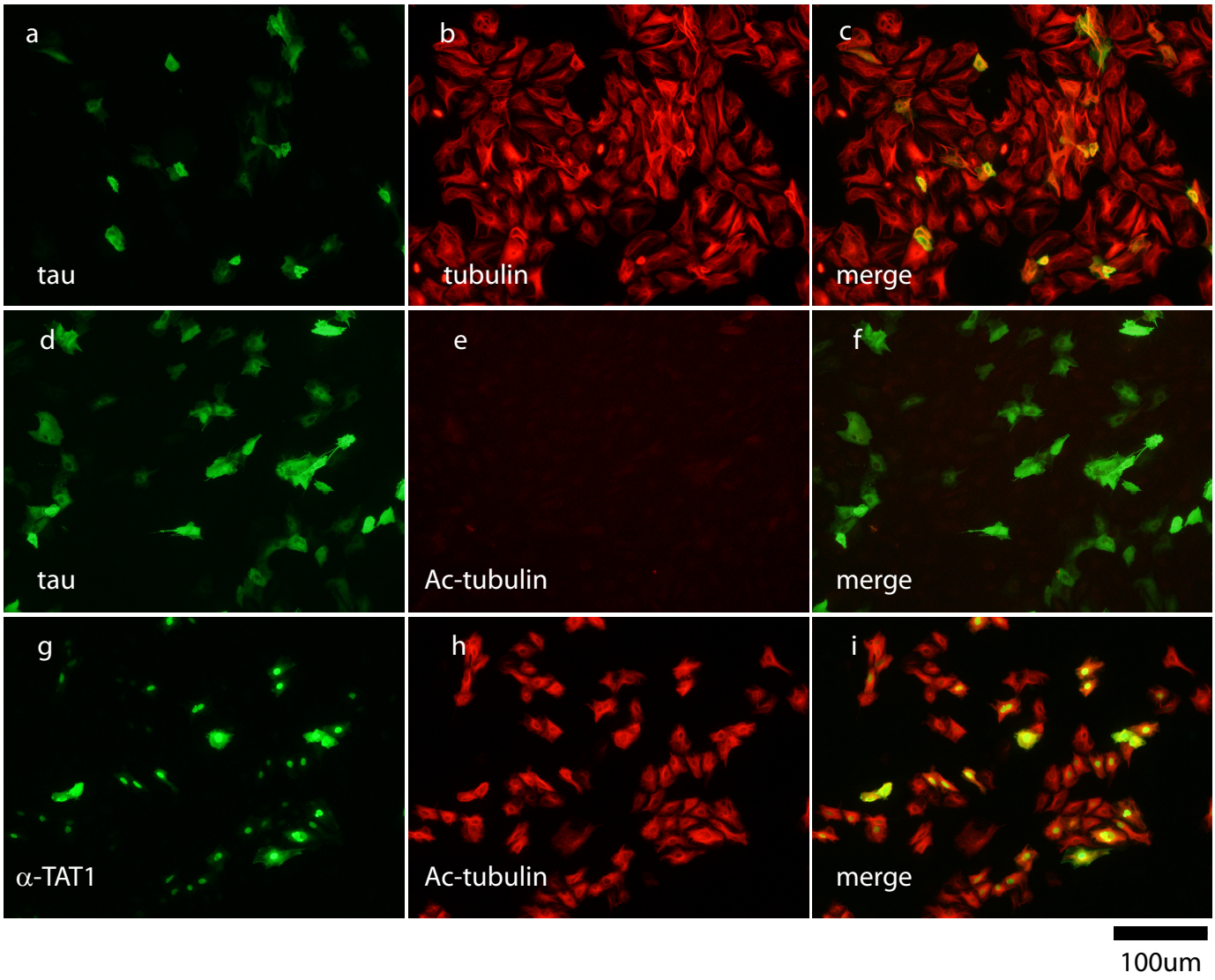


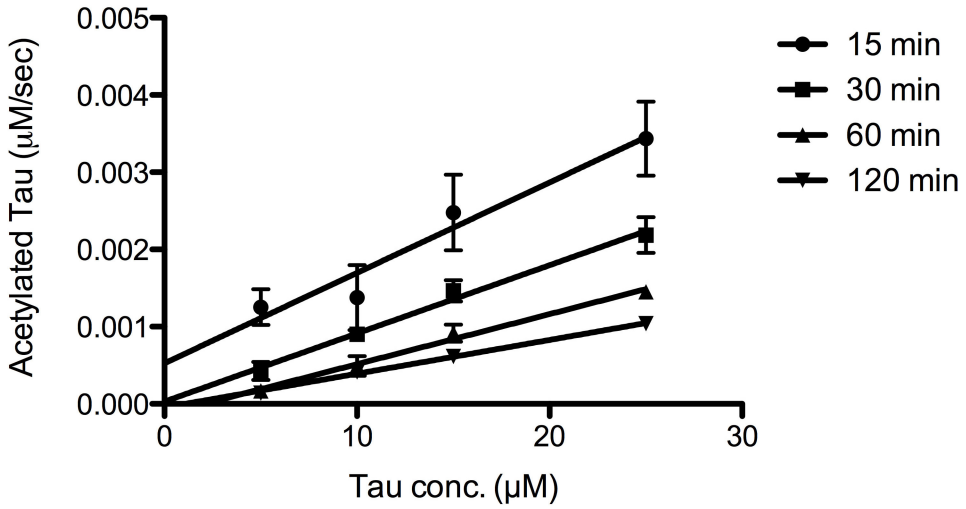
Supplementary Figure 1 Analysis of CBP-mediated acetylation of tau.
(**a-b**) QBI-293 cells were co-transfected with plasmids expressing wild-type tau-K18 or tau-2CA mutant as well as a panel of the indicated HATs (histone acetyltransferases). Cell lysates were analyzed by immunoblotting using antibodies detecting acetylated tau (ac-K280), total tau (K9JA), acetylated tubulin, and acetylated histone H3 (ac-H3K18). Tip60, GCN5, and PCAF proteins are FLAG-tagged and their expression was confirmed using anti-FLAG antibody (M2, Sigma). Note that only CBP and p300 are capable of acetylating wild-type tau-K18 or tau-2CA. The decreased efficiency of p300 compared to CBP is likely due to low level full-length p300 over-expression observed in these cells. (**c**) Semi-synthetic p300 (ss-p300) catalytic domain was analyzed for its ability to acetylate tau-K18 using filter-binding acetyltransferase assays in the presence of 25 µM [^{3}H]-acetyl-CoA. A saturation plot analysis generated K*cat* and K*m* values for ss-p300 acetyltransferase activity towards tau-K18 substrate. Error bars represent standard error of the mean (SEM). (**d**) Cells were transfected with K18-PL or K18-PL-2CA plasmids followed by overnight treatment with 10 µM 3-methyladenine (3MA) to induce tau acetylation and 20 µM C646 to block CBP/p300 activity, where indicated. Cell lysates were analyzed by immunoblotting as described in **a** above. To confirm C646 inhibition of CBP/p300, cells were treated overnight with C646 followed by 5 hr incubation with 200 nM Trichostatin-A (TSA), where indicated. Acetylation of the CBP/p300 substrate, histone H3, was analyzed by immunoblotting using the acetylated histone antibodies ac-H3K9 and ac-H3K18.



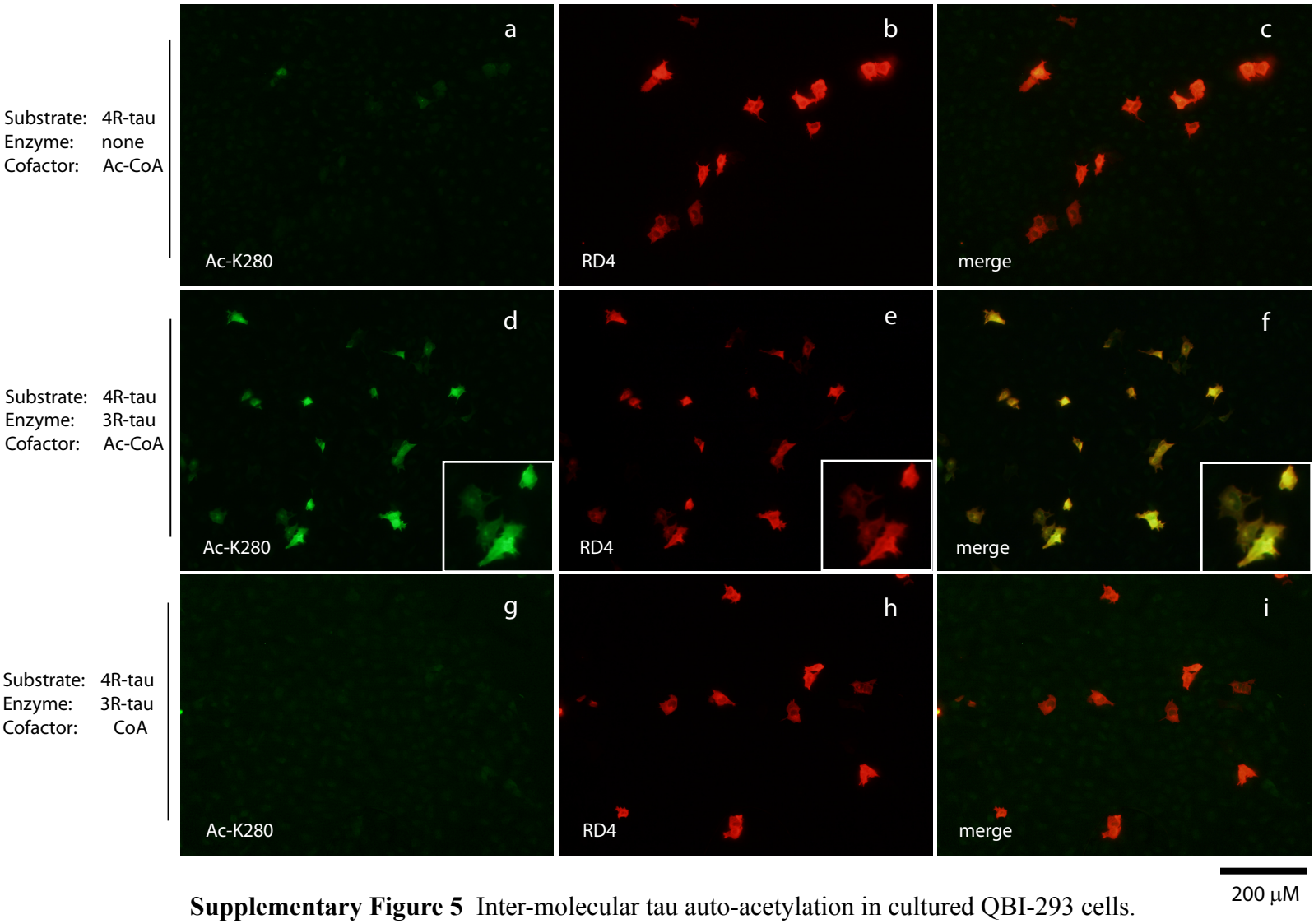
Supplementary Figure 2 Kinetic analysis of phospho-mimic tau-K18 mutants.
Phospho-mimic serine to glutamic acid substitutions at residues 262, 356, or both (2SE) were assayed for acetyltransferase activity *in vitro*. An Ac-CoA titration was performed ranging from 2.5 to 800 uM to calculate catalytic parameters. Experiments were performed in triplicate and error bars represent standard error of the mean (SEM).



Supplementary Figure 3 Tau is insufficient to promote microtubule acetylation in PTK2 cells. PTK2 epithelial cells were transfected with plasmids expressing wild-type tau-2N4R (**a-c** and **d-f**) or HA-tagged human tubulin acetyltransferase α-TAT-1 (**g-i**) followed by immunostaining analysis using anti-HA, anti-tubulin or anti-acetylated tubulin antibodies. Merged panels indicate extensive α-TAT-1 mediated acetylation of tubulin, as expected, while tau is unable to promote tubulin acetylation in PTK2 cells.



Supplementary Figure 4 Tau-K18 displays first-order reaction kinetics.
Filter-binding assays were performed with tau-K18 in the presence of of [^{3}H]-acetyl-CoA at the indicated timepoints. A plot of acetylated tau vs. tau concentration at all timepoints was generated to evaluate intra- or inter-molecular acetylation mechanism, as detailed in the methods section. Error bars indicate standard error of the mean (SEM) from N=3 independent experiments.



Supplementary Figure 5 Inter-molecular tau auto-acetylation in cultured QBI-293 cells. QBI-293 cells adhered to cover slips were transfected with a 4R-tau (2N4R) expression plasmid and fixed/permeabilized followed by control incubation (**a-c**) or exposure to purified 3R-tau (0N3R) (**d-i**) in the presence of either CoA (**g-i**) or acetyl-CoA (**d-f**). Cells were subsequently analyzed by immunostaining using Ac-K280, which detects acetylated cellular 4R-tau, and RD4, which detects total ectopically expressed 4R-tau, but not the exogenously added recombinant 3R-tau. Note, only 3R-tau enzyme in presence of acetyl-CoA is capable of acetylating intracellular 4R-tau substrate.

	Substrate	Kcat	Km (protein)	Km (AcCoA)	Reference
p300	H4-21	27.6 /min	1.2 ± 0.54 uM	1.24 ± 0.19 uM	J Biol Chem. 2001 Sep 7;276(36):33721-9
ESA1	H3p19	7.32 / min	0.922 ± 0.1543 uM	9.1136 ± 1.48 uM	Nat Struct Biol. 2002 Nov;9(11):862-9
Rtt109+Vps75	H3	22.5 / min	8.5 ± 0.7 uM	8.0 ± 2.0 uM	Nat Struct Mol Biol. 2008 Sep;15(9):998
Tau	ND	ND	ND	ND	
TAT	microtubules	0.037 / min	1.6 uM ± 0.36	2.2 ± 0.2 uM	Proc Natl Acad Sci U S A. 2010 Dec 14;107(50):21517-22

	Substrate	Kcat (auto)	Km AcCoA (auto)	Reference
p300	auto	ND	3.3 ± 0.8 uM (AcCoA)	J Biol Chem. 2000 Jul 21;275(29):21953-9
ESA1	auto	ND	9.4 uM (AcCoA)	EMBO J. 2011 Oct 21;31(1):58-70
Rtt109+Vps75	auto	.12 / min	9 ± 2 uM	J Biol Chem. 2011 Jul 15;286(28):24694-701
Tau	auto	.005 / min	146.8 uM	this study
TAT	ND	ND	ND	

Supplementary Table 1 Comparison of enzyme kinetics among acetyltransferases.
Kinetics parameters for nuclear and cytoplasmic acetyltransferases are listed. Kinetic values for known substrates including histones and microtubules are shown in the top panel, while auto-acetylation kinetics are shown in the bottom panel. Auto indicates auto-acetylated substrate, and ND indicates the value was not determined.