

High specificity of widely used phospho-tau antibodies validated using a quantitative whole-cell based assay

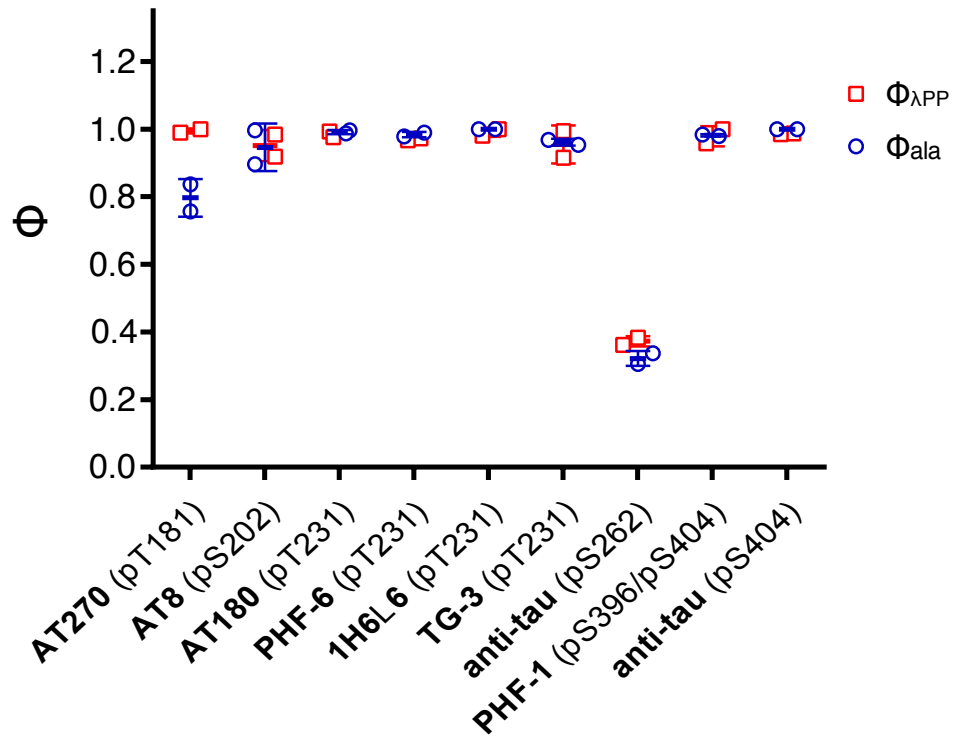
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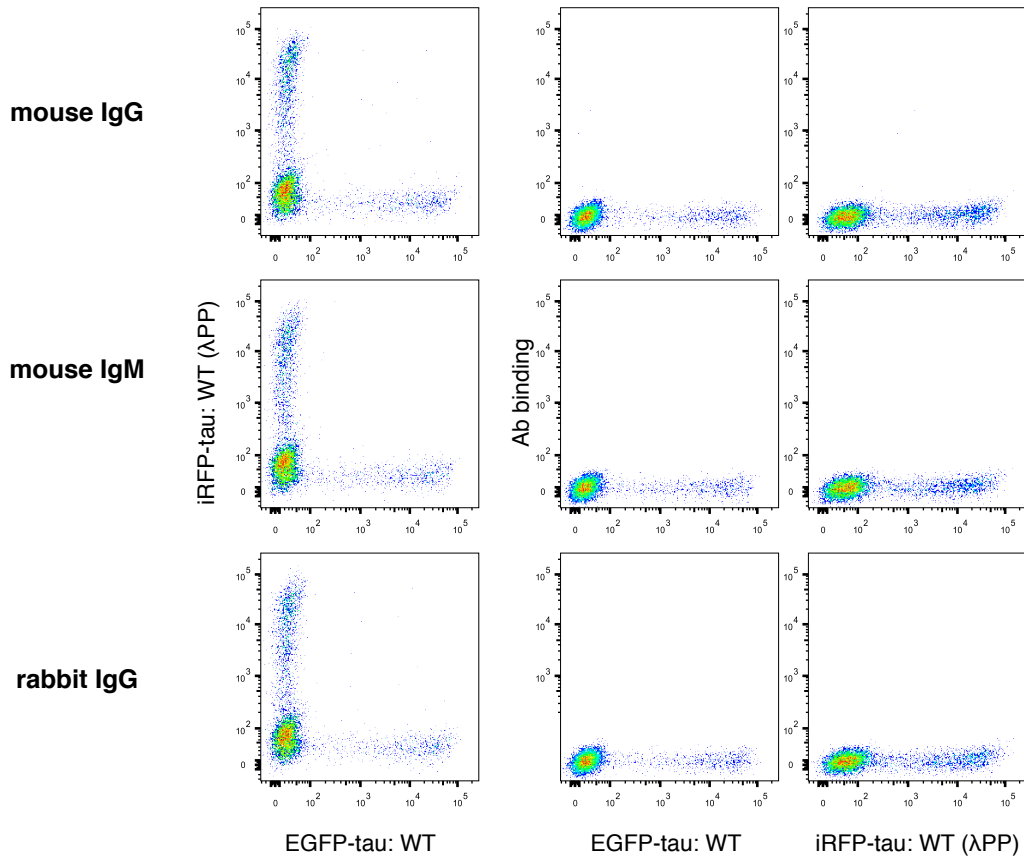
Supplementary Figures and Tables

Supplementary Figure 1.



Supplementary Figure 1. Measurement of Φ_{ala} and $\Phi_{\lambda PP}$ of phospho-tau antibodies. Error bars indicate standard deviation from two independent cell culture preparations. Red, $\Phi_{\lambda PP}$. Blue, Φ_{ala} .

Supplementary Figure 2.



Supplementary Figure 2. Flow cytometry plots showing binding of isotype-matching control antibodies under the specificity quantification assay conditions.

Supplementary Table 1. Primers for Golden Gate assembly of plasmids

DNA template	Primers
pRK5-EGFP-Tau	5'- TCCGCTGAGCCCCG -3' 5'- CCATGGTGGCGACCATC -3'
piRFP670-N1	5'- ATGGTAGCAGGTCATGCC -3' 5'- CGGATCCGCTCTCAAGCGCGG -3'

Supplementary Table 2. Primers used for site-directed mutagenesis

Mutant	Primers
T181A	5'- GAGCTGGGTGGTG C CTTTGGAGCGGGC -3' 5'- GCCCGCTCCAAAG G CACCACCCAGCTC -3'
S202A*	5'- AGTGCCTGGGG C GCCGGGGCTGC -3' 5'- GCAGCCCCGGC G CCCCAGGCACT -3'
T212A/S214A	5'- GTTGAAGGG C CGGGG C GCGGGAGCGG -3' 5'- CCGCTCCCGC G CCCC G CCCTTCCAAC -3'
T231A	5'- GACTTGGGTGGAG C ACGGACCACTGCCACCTTCT -3' 5'- AGAAGGTGGCAGTGGTCCGT G CTCCACCCAAGTC -3'
S262A	5'- TTCAGTTTCTCAGTGG C GCCGATCTTGGACTTG -3' 5'- CAAGTCCAAGATCGGC G CCTACTGAGAACCTGAA -3'
S396A/S404A [§]	5'- CAGACCACTGGCG C CTTGTACACGATCTC -3' 5'- GAGATCGTGTACAAG G CGCCAGTGGTGTCTG -3'
S404A	5'- AGATGCCGTGGAG C CGTGTCCCCAGAC -3' 5'- GTCTGGGGACACG G CTCCACGGCATCT -3'

Note: *Previous study has shown that AT8 recognition requires only Ser202 to be phosphorylated in tau (Goedert *et al.* 1993).

[§]S396A/S404A were obtained using the S404A mutant as a template to generate double mutations.

References

Goedert, M., Jakes, R., Crowther, R. A., Six, J., Lubke, U., Vandermeeren, M., Cras, P., Trojanowski, J. Q. and Lee, V. M. (1993) The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. *Proc Natl Acad Sci U S A* **90**, 5066-5070.