Supplemental Figure S1. Time course of phosphorylation of tau. (A) Phosphorylation of WT tau, P301L or R406W by Cdk5. WT tau (circle), P301L (square) or R406W (triangle) at 0.1 mg/ml was incubated with Cdk5-p25 at 35°C for indicated times in the presence of [γ-^{32}P]ATP. Phosphorylation was measured by ^{32}P incorporated into tau after SDS-PAGE and expressed as mol phosphate per mol tau (mean ± SE, n=3). (B) Phosphorylation of WT tau by GSK3β or PKA. WT tau at 0.25 mg/ml was incubated with GSK3β (square) or PKA (circle) at 35°C for indicated times in the presence of [γ-^{32}P]ATP. Phosphorylation was measured as described above and expressed as mol phosphate per mol tau (mean ± SE, n=3).

Supplemental Figure S2. Dephosphorylation of tau phosphorylated by Cdk5. (A) Effect of the protein phosphatase inhibitor microcystin LR on dephosphorylation of tau phosphorylated by Cdk5-p35 in rat brain extracts. Tau phosphorylated by Cdk5-p35 using [γ-^{32}P]ATP was incubated with rat brain extract in the presence (circles) or absence (triangles) of 100 nM microcystin LR at 37°C for the indicated times. The dephosphorylation reaction was stopped by boiling in SDS-PAGE sample buffer. After SDS-PAGE, radioactivity remaining in tau was measured by a scintillation counter. (B) WT tau phosphorylated by Cdk5-p25 using [γ-^{32}P]ATP was incubated with wild type (black) or Pin1-KO (white) mouse brain extract at 35°C for 10 or 30 min. Radiolabels remaining in tau were measured by a scintillation counter after SDS-PAGE. A significant difference in dephosphorylation between wild type and Pin1-KO brain extracts was detected at 10 min incubation (*$P < 0.05$).

Supplemental Figure S3. Four control experiments addressing other possibilities associated with experimental conditions of dephosphorylation of P-tau bound to microtubules. (A) Effect of microtubules on dephosphorylation of P-WT tau. P-WT tau was incubated with microtubules, and the extent of phosphorylation was measured after SDS-PAGE (triangles). Dephosphorylation of P-WT tau in the absence (closed circles) or presence (open circles) of rat brain extract is also shown. (B) Effect of GTP used for tubulin polymerization on dephosphorylation of P-WT tau in rat brain extract. P-WT tau was dephosphorylated in the absence (closed circles) or presence (open circles) of 0.5 mM GTP. (C) Effect of microtubules on dephosphorylation of NF-L phosphorylated by Cdk5-p25. NF-L was dephosphorylated, but a little slowly, in the presence of microtubules (closed circles) compared to that in the absence (open circles), suggesting that microtubules marginally inhibit phosphatase activity in brain extract. (D) Effect of the order of the addition of tau and microtubules on dephosphorylation. P-WT tau was incubated with tubulin before (closed circles) or after (triangles) polymerization, and dephosphorylated. Dephosphorylation of P-WT tau in the absence of microtubules is also shown (open circles). These data show that entrapment of tau inside microtubules, if it occurs, is not a major means by which dephosphorylation is suppressed.

Supplemental Figure S4. Phosphorylation of ΔMTB by Cdk5-p25. (A) Schematic representation of human tau (1N4R) and ΔMTB used in this study. One N-terminal insertion and four C-terminal microtubule-binding repeats are indicated by light and dark gray, respectively. Numbers indicate residues in sequence, with the longest human tau isoform containing 441 residues. Mutation sites P301L and R406W used in this study are indicated above the WT tau molecule. Cdk5-p25 phosphorylation site serine residues are indicated below the WT tau molecule. ΔMTB is a mutant tau lacking four microtubule-binding repeats, from residues 255-368. (B) Coomassie brilliant blue staining of an SDS-PAGE gel indicating that ΔMTB does bind microtubules. WT tau and ΔMTB were incubated with microtubules polymerized in the presence of 20 μM taxol, and then centrifuged at 100,000 × *g* for 30 min. The supernatant (S) and pellet (P) were then analyzed by SDS-PAGE. (C) 2D phosphopeptide map of WT tau or ΔMTB phosphorylated by Cdk5-p25. Both proteins exhibited a similar phosphorylation pattern.

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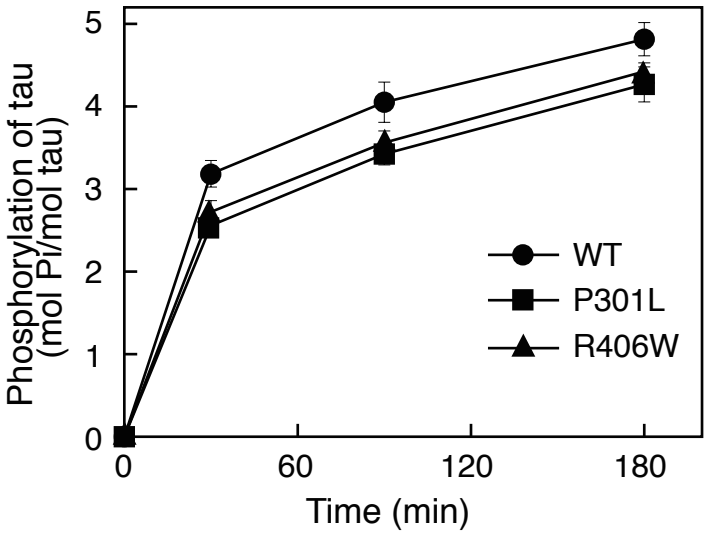
A



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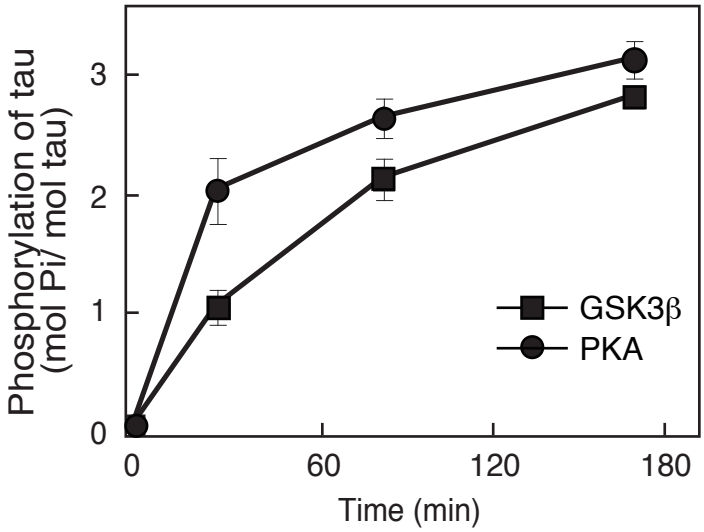

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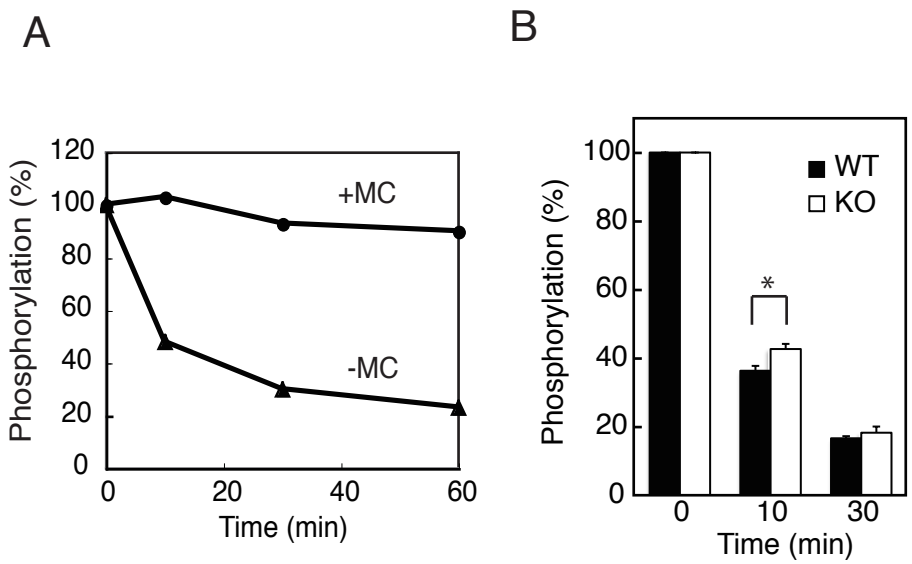

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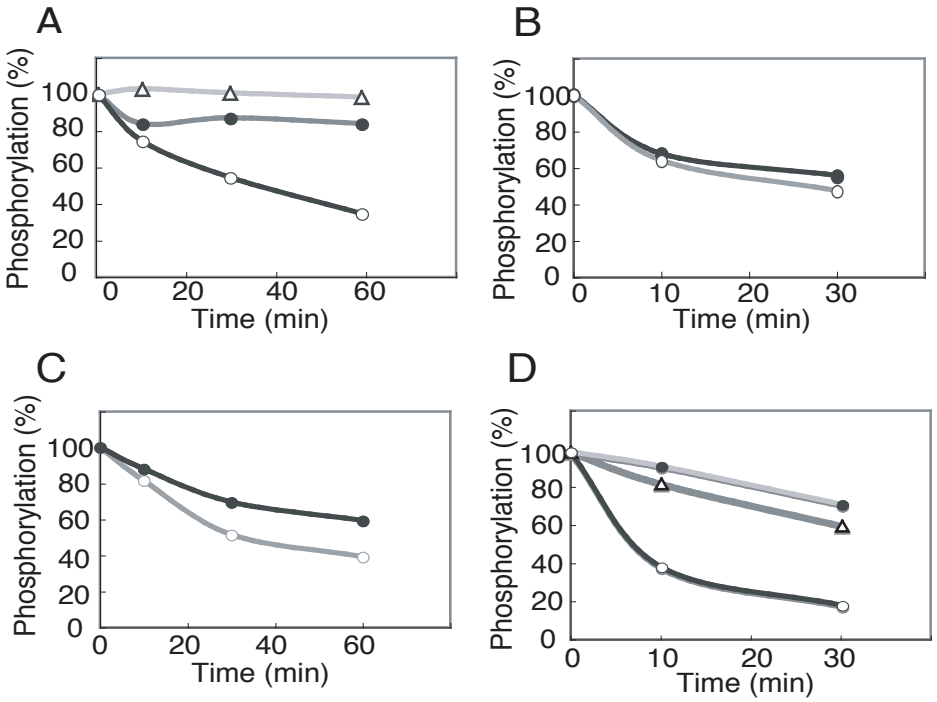

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