Supporting Information

Kickstein et al. 10.1073/pnas.0912793107

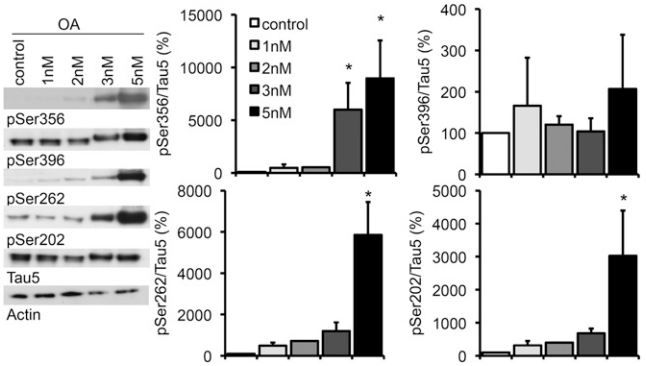

Fig. S1. Several phosphorylation sites of the tau protein are PP2A sensitive. Primary cortical neurons of wild-type mice were treated with the PP2A inhibitor okadaic acid (OA) in increasing concentrations. Cell lysates were analyzed by Western blot using antibodies detecting phosphorylation at specific tau sites (pSer356, pSer396, pSer262, pSer202). Band intensities were normalized to actin and compared with total tau (Tau 5). Representative Western blots and quantifications of three independent experiments are shown ($n = 3$). *$P < 0.05$.

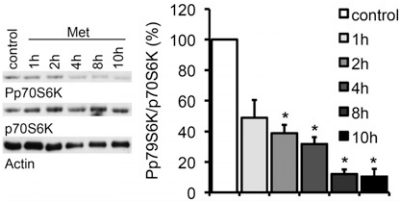

Fig. S2. Metformin treatment leads to a decrease of pS6 kinase phosphorylation. Primary cortical neurons of wild-type mice were treated with 2.5 mM metformin (Met) over increasing time intervals. Cell lysates were analyzed by Western blot using an anti-phospho S6 kinase (Pp70S6K) antibodies. Band intensities were normalized to actin and compared with total S6 kinase protein. Representative Western blots and quantifications of three independent experiments ($n = 3$) are shown. *$P < 0.05$.

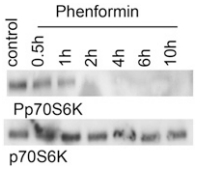

Fig. S3. Phenformin treatment leads to a decrease of pS6 kinase phosphorylation. Primary cortical neurons of wild-type mice were treated with 0.25 mM phenformin over increasing time intervals. Cell lysates were analyzed by Western blot using an anti-phospho S6 kinase (Pp70S6K) antibody. Band intensities were compared with total S6 kinase protein (p70S6K).

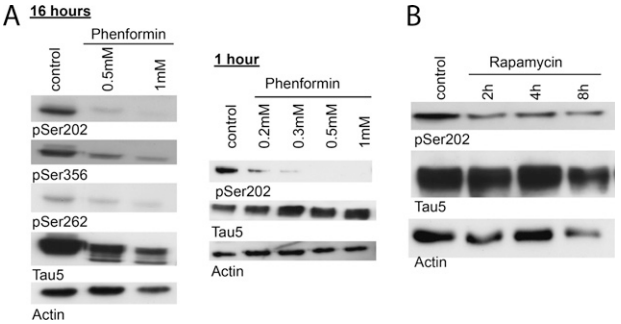

Fig. S4. (*A*) Phenformin induces dephosphorylation of tau at PP2A-sensitive sites. Primary cortical neurons of wild-type mice were treated with increasing concentration of phenformin over 16 h or 1 h, respectively. Cell lysates were analyzed by Western blot using antibodies detecting phosphorylation at specific PP2A-sensitive tau sites. Band intensities were compared with total tau (Tau 5) and actin. (*B*) Primary cortical neurons of human tau-expressing mice were treated with 10 μM of rapamycin over increasing time intervals. Cell lysates were analyzed by Western blot using antibodies detecting phosphorylation at a specific PP2A-sensitive tau site. Band intensities were compared with total tau (Tau 5) and actin.

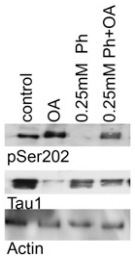

Fig. S5. Inhibition of PP2A blocks the phenformin effect on the phosphorylation of tau. Primary cortical neurons of wild-type mice were treated with either okadaic acid (OA; 10 nM), only phenformin (Ph; 0.25 mM), or with OA (10 nM) before phenformin (0.25 mM) over 4 h. Cell lysates were analyzed by Western blot using antibodies detecting phosphorylated (pSer202) or dephosphorylated tau (Tau-1) at a specific PP2A-sensitive tau site.

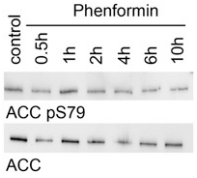

Fig. S6. Phenformin effects on the phosphorylation of tau are AMPK independent. Primary cortical neurons of wild-type mice were treated with 0.25 mM of phenformin over increasing time intervals. Cell lysates were analyzed by Western blot using an antibody detecting phosphorylation of the AMPK target ACC. Band intensities were compared with total ACC.

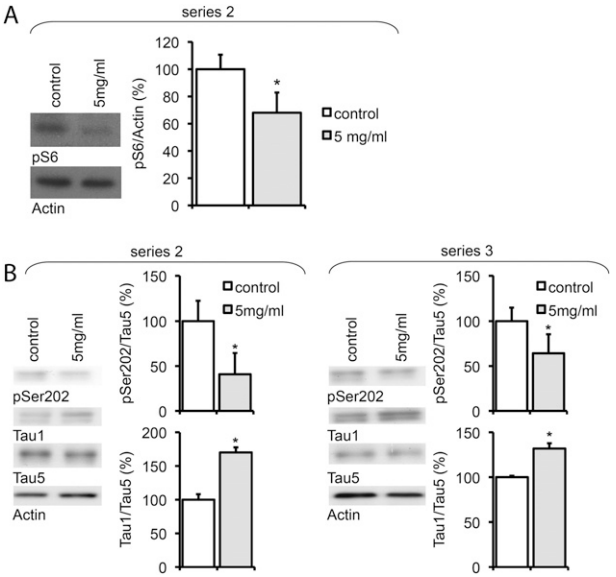

Fig. S7. Metformin decreases tau phosphorylation in vivo. Pairs of wild-type mice were fed with or without 5 mg/mL of metformin in the drinking water for 16–24 d. (*A*) Brains were lysed and analyzed via Western blots for phosphorylation of S6. Band intensities were compared with actin. Representative Western blots and quantification of three technical replicates are shown. (*B*) Brains were lysed and analyzed via Western blots for phosphorylation of Tau. pSer202 was detected using a phospho-specific pSer202 antibody and a tau1 antibody, which detects dephosphorylated Ser202. Band intensities were normalized to actin and compared with total tau (Tau5). Representative Western blots and quantification of three technical replicates are shown. $*P < 0.05$.

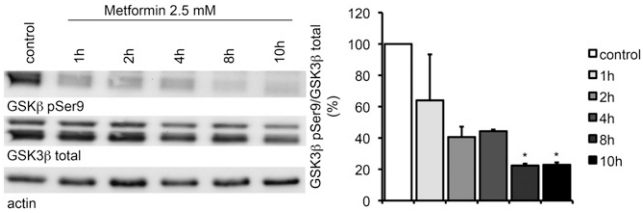

Fig. S8. Metformin increases the activity of GSK3β. Primary cortical neurons of wild-type mice were treated with 2.5 mM metformin (Met) over increasing time intervals. Cell lysates were analyzed by Western blot using an anti-phospho GSK3β antibody (pSer9). Dephosphorylation at this site activates the enzyme. Band intensities were compared with total GSK3β and actin. $*P < 0.05$.