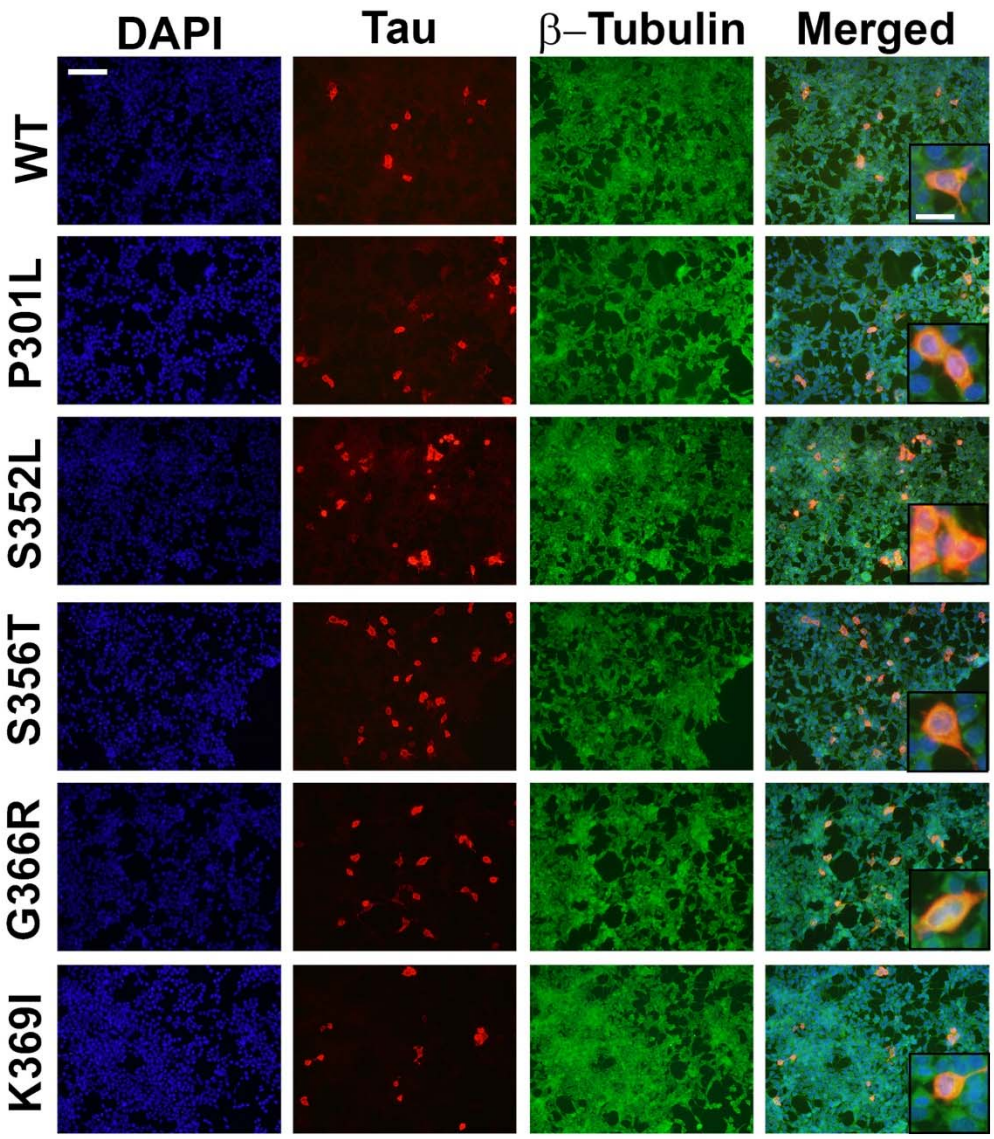
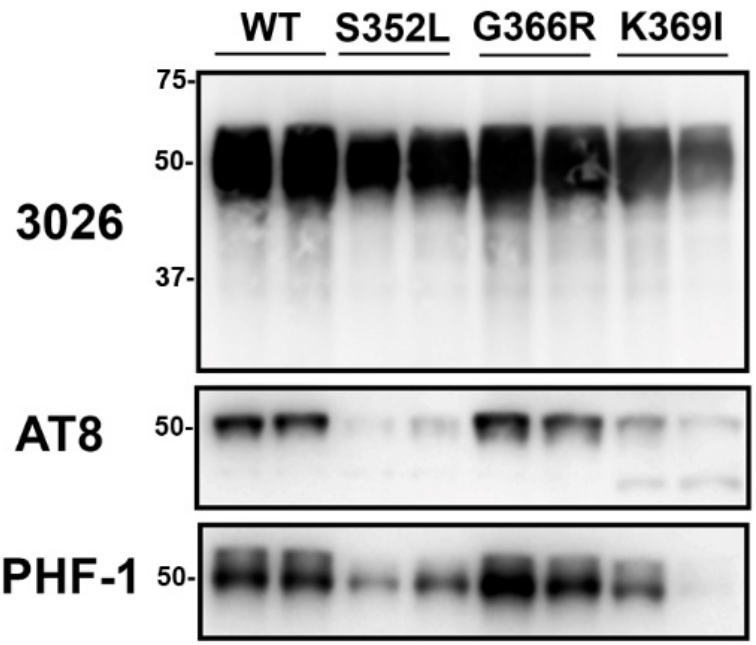
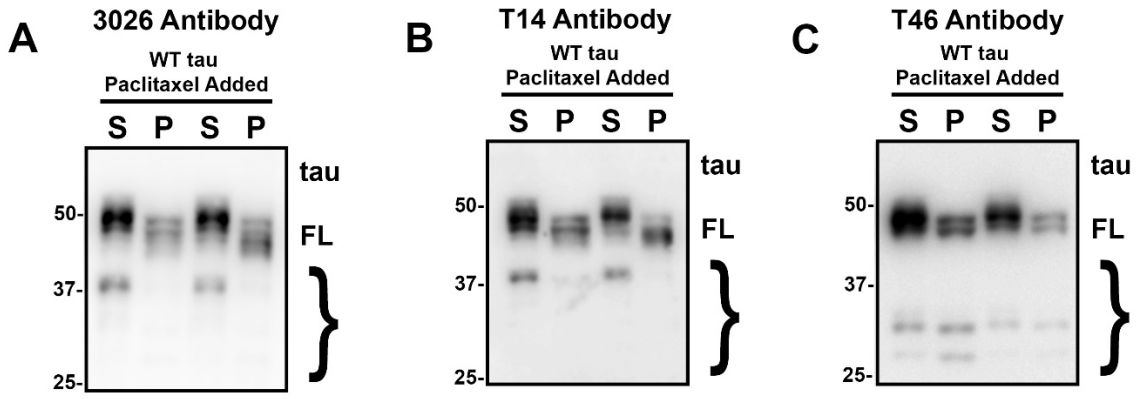


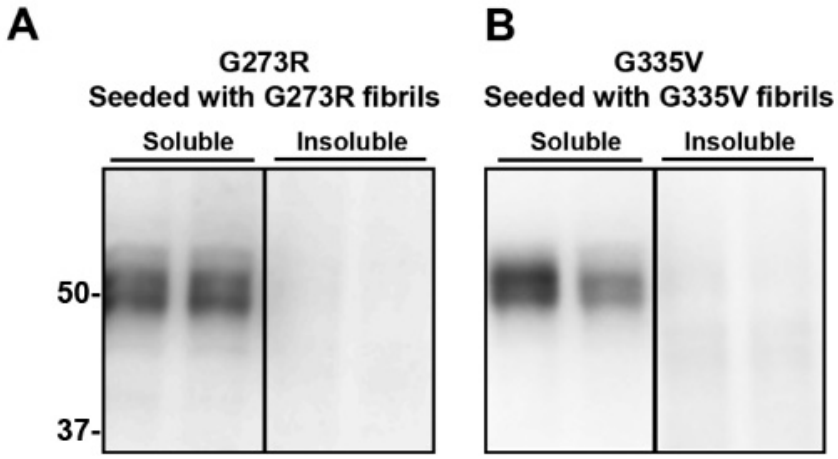
Supplemental Figure 1: Double immunofluorescent labeling of HEK293T cells transfected with WT human tau or different human tau mutants showing colocalization between tau and MTs. HEK293T cells were transfected with WT human tau or the different tau mutants P301L, S352L, S356T, G366R, and K369I. Double immunofluorescence was performed on fixed cells as described in "Experimental Procedures". Cells were labeled with DAPI for nuclei (blue), 3026 antibody for tau (red), and an antibody against β-tubulin (green). Overlay shown on the right. Tau and tubulin colocalize within the cytoplasm in WT tau and all different tau mutations at similar levels. Scale bar = 250 μm for panels and 50 μm for inserts.



Supplemental Figure 2. Whole cell lysate of WT human tau and tau mutants are expressed at similar levels and are phosphorylated at the AT8 and PHF-1 phospho-epitopes. HEK293T cells were transfected with WT human 0N4R tau or the tau mutants S352L, G366R, or K369I. Whole cell lysates were extracted in SDS lysis buffer and equally loaded, showing similar total tau expression as detected by tau antibody 3026. The AT8 antibody reacts with tau phosphorylated at S202 and T205 and the PHF-1 antibody reacts with tau phosphorylated at S396 and S404. Both WT tau and different tau mutants are phosphorylated at multiple sites similar to or less than that of WT tau.



Supplemental Figure 3: All the main tau protein bands are detected by N- or C-terminal specific tau antibodies. A cell-based MT binding assay performed with HEK293T cells transfected to express WT tau with paclitaxel as described in "Experimental Procedures". (A) 3026 is a polyclonal antibody against total tau, (B) T14 is an antibody against N-terminal region of tau from 83-120 residues, and (C) T46 is an antibody against C-terminal region of tau from 404-441 residues. All the main protein bands around 50 kDa are reactive with all 3 antibodies. Some minor lower molecular mass protein bands react only with T14 or T46 showing that these are either N- or C-terminal truncated. S = supernatants; P = pellet fractions. The relative molecular masses of protein markers are indicated on the left. On the right, FL is for full length tau and the bracket indicates degradative tau bands.



Supplemental Figure 4: Homotypic seeding of tau mutants G273R and G335V do not result in aggregation. HEK293T cells were transfected with human tau mutants G273R or G335V and assessed for aggregation with exogenous K18 fibrillar seeds with their respective mutations. Both G273R (A) and G335V (B) did not aggregate even when homotypically seeded with K18 fibrils comprised of G273R and G335V K18, respectively. Immunoblots were probed with total tau antibody 3026. The relative molecular masses of protein markers are indicated on the left.