



Extended Data Figure 3 | Phosphorylation of Y122 of PIN4 by F3-T3.

a, Amino acid sequence flanking Y122 of PIN4 (in red) is evolutionarily conserved. **b**, Immunoprecipitation-western blot analysis of human astrocytes expressing F3-T3 or F3-T3(K508M) with or without silencing of endogenous PIN4. β-Actin is shown as a loading control. WCL, whole-cell lysate. **c**, Immunoblot analysis of phosphotyrosine immunoprecipitates (left) or whole-cell lysates (right from U87 glioma cells expressing empty vector, FGFR3, F3-T3 or F3-T3(K508M) using the indicated antibodies. The asterisk indicates a non-specific band. **d**, Immunoblot analysis of phosphotyrosine immunoprecipitates (left) or whole-cell lysates (right) from human astrocytes expressing empty vector, F3-T3 or F3-T3(K508M) using the indicated antibodies. FAK is shown as a loading control. **e**, Immunoblot analysis of phosphotyrosine immunoprecipitates (left) or whole-cell lysates (right) from GSC1123 cells expressing endogenous F3-T3 shows decreased phosphorylation of F3-T3 substrates following treatment with AZD4547 for the indicated times. Paxillin is shown as a

loading control. **f**, Immunoblot analysis of canonical FGFR signalling proteins in GSC1123 cells treated with AZD4547 for the indicated time. β-Actin is shown as a loading control. **g**, Immunoblot analysis of phosphotyrosine immunoprecipitates from human astrocytes expressing F3-T3 or vector transduced with wild-type or the unphosphorylatable Y to A F3-T3 kinase substrate mutants. Paxillin is shown as a loading control. The asterisk indicates a non-specific band. **h**, Confocal images of immunofluorescence staining using the phospho-PIN4(Y122)-specific antibody (red) in human astrocytes transduced with vector or F3-T3 without or with silencing of endogenous PIN4. Nuclei were stained with DAPI (blue). **i**, Immunoblot analysis of phospho-PIN4(Y122), total PIN4 and FGFR3 in SF126 cells transduced with FGFR3, F3-T3, F3-T3(K508M) or the empty vector. β-Actin is shown as a loading control. Molecular weights are indicated in all panels. Experiments in **b-g, i** were repeated independently three times with similar results. Experiment in **h** was repeated independently four times with similar results.