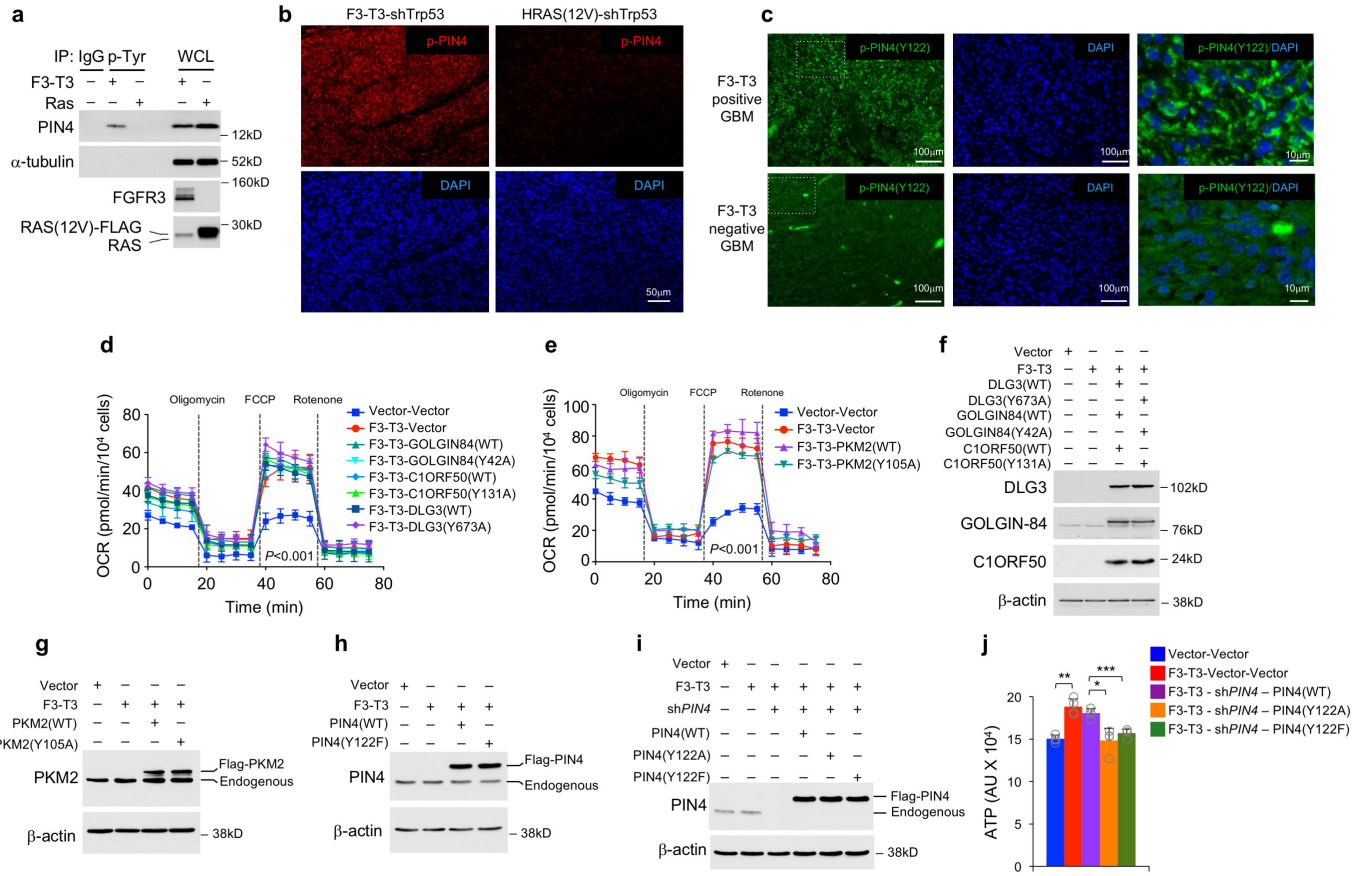

Extended Data Figure 4 | Functional analysis of tyrosine phosphorylation of F3–T3 kinase substrates. a, Western blot analysis of phosphotyrosine immunoprecipitation of F3–T3;shTrp53 and HRAS(12V);shTrp53 mGSCs using the PIN4 antibody. F3–T3 and HRAS(12V) expression are shown. α-Tubulin is shown as a loading control. **b**, Immunofluorescence images using the phospho-PIN4(Y122)-specific antibody (red, top) in tumours from F3–T3;shTrp53 and HRAS(12V)shTrp53 mGSCs. Nuclei were counterstained with DAPI (blue, bottom). Experiment was repeated independently twice with similar results. **c**, Left, representative images of phospho-PIN4(Y122) immunofluorescence in F3–T3-positive (top) and F3–T3-negative (bottom) GBM (green). Right, higher magnification images of phospho-PIN4(Y122)–DAPI co-staining depicting cytoplasmic localization of phospho-PIN4(Y122). Middle, DAPI staining of nuclei is shown as an indication of cellular density. **d**, Analysis of OCR in human astrocytes F3–T3 transduced with wild-type or the unphosphorylable Y to A mutant of GOLGIN84, C1orf50 and DLG3. Human astrocytes expressing the empty vector are included as a control. Data are mean ± s.d. ($n = 5$ technical replicates) of one representative experiment out of two independent experiments performed in triplicate with similar results. $P < 0.001$, rate 9–12 for vector versus each F3–T3 combination, two-tailed *t*-test with unequal variance. **e**, Analysis of OCR of human astrocytes expressing F3–T3 transduced with PKM2(WT), PKM2(Y105A) or the empty vector. Human astrocytes expressing the empty vector are included as control. Data are mean ± s.d. ($n = 3$ technical replicates) of one representative experiment out of three independent experiments; $P < 0.001$, rate 9–12 for vector versus each F3–T3 combination, two-tailed *t*-test with unequal variance. **f**, Immunoblot analysis of GOLGIN84, C1orf50 and DLG3 wild-type or Y to A mutants in human astrocytes expressing F3–T3 or vector. **g**, Immunoblot analysis of human astrocytes transduced with empty vector or F3–T3 expressing PKM2(WT) or PKM2(Y105A). **h**, Immunoblot analysis of human astrocytes transduced with F3–T3 or the empty vector for the expression of PIN4(WT) or PIN4(Y122F). **i**, Immunoblot analysis of PIN4 proteins in human astrocytes expressing F3–T3 following silencing of endogenous *PIN4* and reconstitution with PIN4(WT), PIN4(Y122A) or PIN4(Y122F). In **f**–**i**, β-actin is shown as a loading control. Molecular weights are indicated on all immunoblots. **j**, Quantification of ATP levels in human astrocytes treated as in **i**. Data are mean ± s.d. ($n = 4$ technical replicates) of one out of two independent experiments. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; two-tailed *t*-test with unequal variance. Experiments in **a**, **f**–**i** were repeated independently three times with similar results.