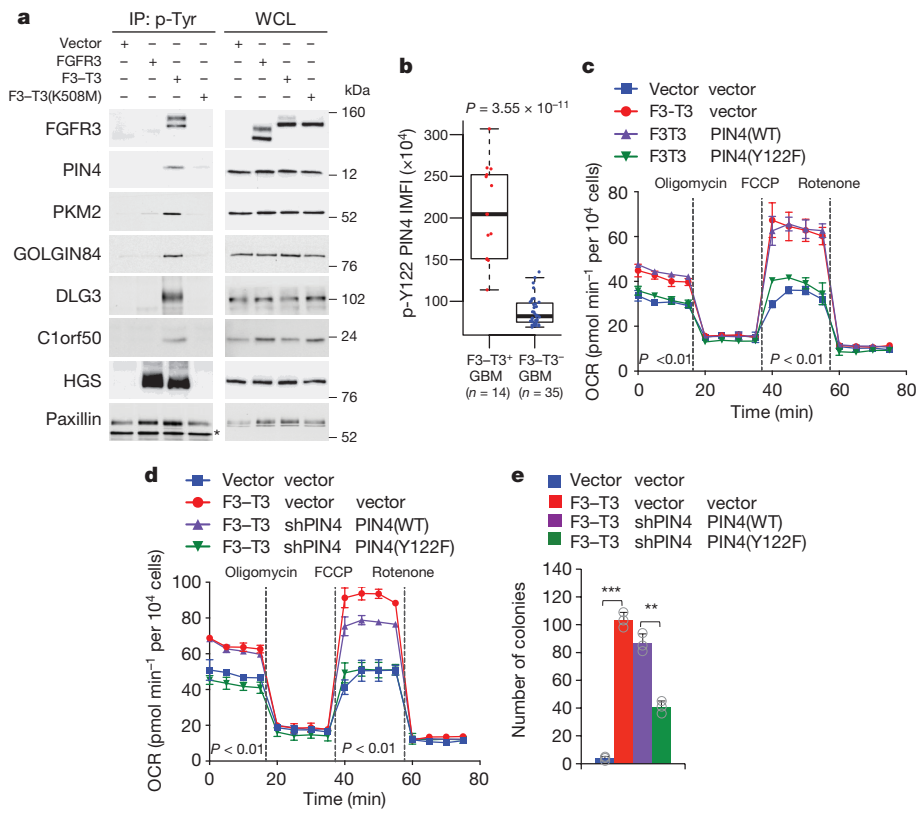

Figure 2 | Phosphorylation of PIN4 at Y122 affects mitochondrial metabolism. a, Immunoblot of phosphotyrosine immunoprecipitates from SF126 glioma cells (left) or whole-cell lysate (WCL, right). Paxillin is a loading control. IP, immunoprecipitation. **b**, Quantification of phospho-PIN4(Y122) integrated mean fluorescence intensity (IMFI) from F3–T3-positive and F3–T3-negative GBM. Box plot spans the first to third quartiles and whiskers show the 1.5× interquartile range. $P \leq 0.0001$, two-sided MWW test. **c**, OCR of F3–T3 human astrocytes transduced with PIN4(WT), PIN4(Y122F) or vector. **d**, OCR of F3–T3 human astrocytes following silencing of *PIN4* and reconstitution with PIN4(WT) or PIN4(Y122F). **e**, Soft agar colony-forming assay of human astrocytes treated as in **d**. Data in **c**–**e** are mean ± s.d. of one representative experiment with $n = 3$ technical replicates. Experiments were repeated three times with similar results. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, two-tailed *t*-test with unequal variance.

GBM transcriptome from The Cancer Genome Atlas (TCGA)[13]. The combination of the easy ensemble (ee) undersampling technique and Mann–Whitney–Wilcoxon (MWW) test statistics (ee-MWW) exhibited the best performance for correct identification of imbalanced samples and reproducible clustering (Supplementary Information and Supplementary Table 3). We used ee-MWW to generate a ranked list of genes discriminating F3–T3-positive samples in the GBM dataset of the TCGA and built a hierarchical cluster (confirmed by consensus clustering), including a small cluster of nine F3–T3-positive samples and nine fusion-like GBM (Fig. 3a, Extended Data Fig. 5a and Supplementary Table 4). The most significant biological processes enriched in F3–T3-positive GBM were mitochondrial categories (Fig. 3b and Extended Data Fig. 5b). Mitochondrial functions were also increased in fusion-like GBM, which were enriched for amplification and high expression of mitochondrial RNA polymerase (*POLRMT*, Extended Data Fig. 5c–e). Immunostaining of oxidative phosphorylation biomarkers in an independent GBM cohort revealed that F3–T3-positive tumours expressed higher levels of mitochondrial proteins (Fig. 3c and Extended Data Fig. 5f). The ee-MWW method clustered tumours with other rare oncogenes (oncogenic *RAS* in GBM and invasive breast carcinoma, *EGFR-SEPT14* gene fusion in GBM[12]) and identified their associated biological functions (Extended Data Fig. 5g–i and Supplementary Table 5). Using ee-MWW, we detected small and homogeneous clusters of F3–T3-positive tumours enriched for mitochondrial categories in each tumour containing recurrent F3–T3 fusions in the TCGA dataset (pan-glioma, lung squamous cell carcinoma, head and neck squamous cell carcinoma, oesophageal carcinoma, urothelial bladder carcinoma and cervical squamous cell carcinoma and endocervical adenocarcinoma; Extended Data Fig. 6a–k and Supplementary Table 6). The transcriptional similarity of F3–T3-positive glioma was confirmed by Topological Data Analysis[14,15] (Extended Data Fig. 6l). Finally, expression of the *F3–T3* fusion gene correlated with mitochondrial activities in the analysis of multiple cancer types (Extended Data Fig. 6m).

To identify the transcription factors that are causally related to the gene expression signature that is activated in F3–T3-positive glioma (master regulators)[16], we assembled transcriptional networks from the GBM and pan-glioma datasets using the regularized gradient boosting machine algorithm that was developed for the inference of gene regulatory networks[17]. In both datasets, the two most active master regulators of F3–T3-positive tumours were *PPARGC1A* and *ESRRG* (encoding the PGC1α transcriptional coactivator and the nuclear receptor ERRγ, respectively; Fig. 3d, Extended Data Fig. 6n and Supplementary Table 7). Expression of *PPARGC1A* and *ESRRG* mRNA was higher in F3–T3-positive than F3–T3-negative GBM (Extended Data Fig. 6o). Because PGC1α is a coactivator of the oestrogen-related receptor (ERR) subfamily of nuclear receptors and acts as a master regulator of mitochondrial biogenesis and metabolism[18,19], we investigated whether PGC1α and ERRγ enable the mitochondrial functions induced by F3–T3. Introduction of F3–T3 in human astrocytes expressing PIN4(WT) increased *PPARGC1A* mRNA and PGC1α protein and the expression of genes involved in reactive oxygen species (ROS) detoxification[20] (Extended Data Fig. 7a–d and Supplementary Table 7). Accordingly, PGC1α accumulated at higher levels in F3–T3-positive GSC1123 cells and F3–T3;shTrp53 mGSCs compared to F3–T3-negative GSC308 cells and HRAS(12V);shTrp53 mGSCs, respectively (Fig. 3e). However, replacement of PIN4 with the un-phosphorylatable Y122F mutant PIN4(Y122F) blunted