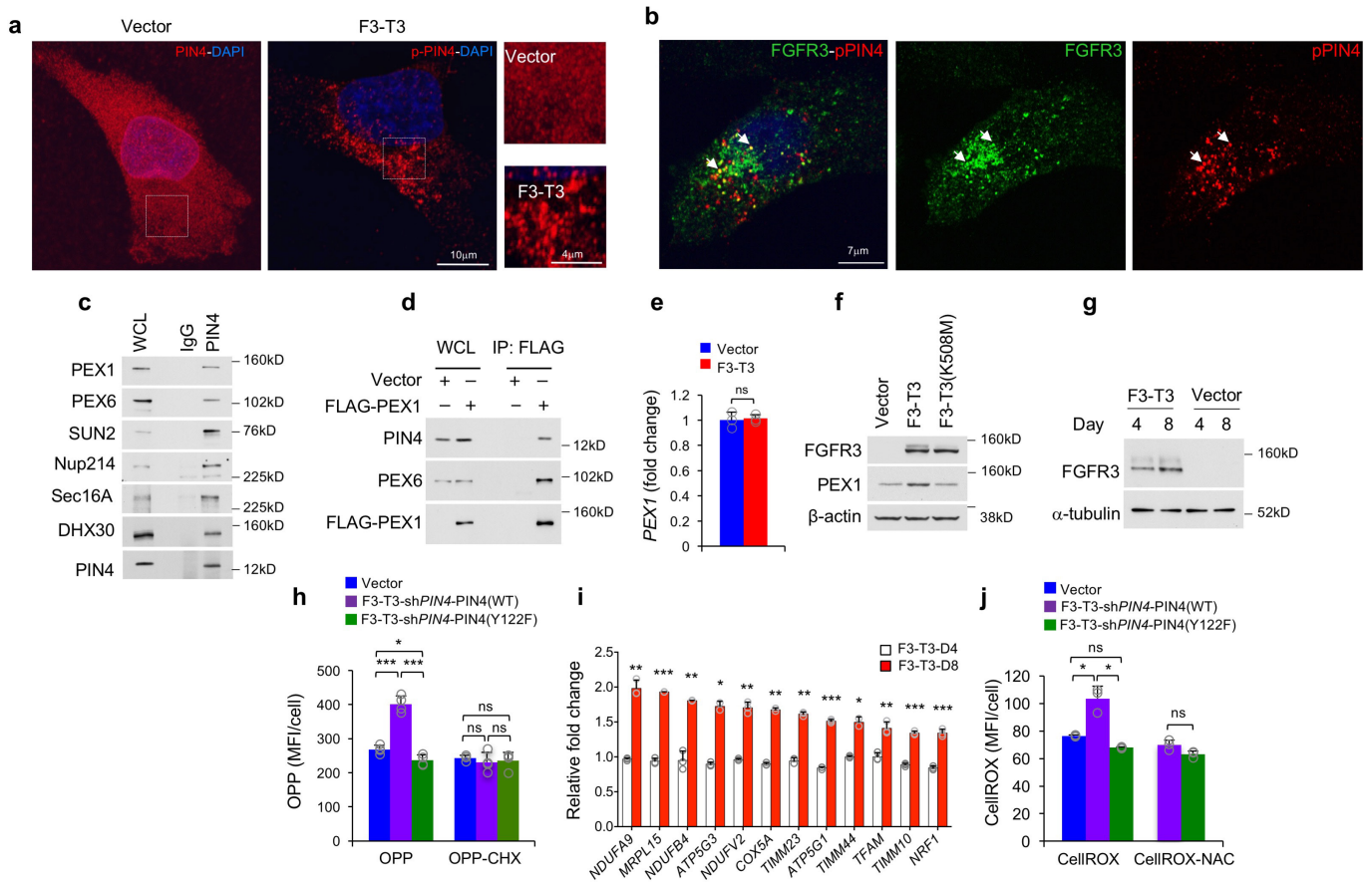

Extended Data Figure 10 | Acute expression of F3–T3 fusion induces peroxisome biogenesis through phosphorylation of PIN4(Y122). a, Representative confocal images (maximum intensity) of immunofluorescence staining for total PIN4 (PIN4, red, left) and phospho-PIN4(Y122) (p-PIN4, red, middle panel) in human astrocytes expressing the empty vector and F3–T3. Right, higher magnification of dotted boxes. Nuclei were counterstained with DAPI (blue). Experiment was repeated independently twice with similar results. **b**, Maximum intensity of confocal images of double immunofluorescence staining for FGFR3 (green, middle) and phospho-PIN4(Y122) (red, right) in human astrocytes expressing F3–T3. Arrows indicate protein co-localization. Experiment was repeated independently twice with similar results. **c**, Co-immunoprecipitation from H1299 cells using the PIN4 antibody. Endogenous PIN4 immunocomplexes and input (WCL) were analysed by western blot using the indicated antibodies. Input is 10% for PEX1, PEX6, SUN2 and NUP214; 5% for SEC16A and DHX30; 2% for PIN4. **d**, Western blot analysis of co-immunoprecipitation of exogenous Flag-PEX1 in human astrocytes expressing F3–T3. WCL: 1% for PIN4 and 10% for PEX1 and PEX6. Experiment was repeated independently four times with similar results. **e**, RT–qPCR of *PEX1* in human astrocytes expressing F3–T3 or vector. Data are mean ± s.d. ($n = 3$ technical replicates) of one representative experiment out of three independent experiments performed in triplicate. **f**, Western blot analysis of PEX1 expression in human astrocytes transduced with F3–T3, F3–T3(K508M) or the empty vector. β-Actin is shown as a loading control. Experiment was repeated independently three times with similar results. **g**, Time-course analysis of F3–T3 expression in human astrocytes by western blot. α-Tubulin is shown as a loading control. Experiment was repeated independently twice with similar results. **h**, Quantification of protein biosynthesis by OPP incorporation measured by high-content fluorescent microscopy in human astrocytes reconstituted with PIN4(WT) or PIN4(Y122F) after silencing of the endogenous *PIN4* and acutely transduced with F3–T3 or vector. Representative bar plots ($n = 4$ technical replicates) from one out of three independent experiments. $^*P < 0.05$, $^{***}P < 0.001$; two-tailed *t*-test with unequal variance. CHX-treated cultures were used as negative controls. **i**, Time-course expression analysis by RT–qPCR of the indicated mitochondrial genes in human astrocytes expressing F3–T3 or empty vector. Data are mean ± s.d. ($n = 3$ technical replicates) of one representative experiment out of two independent experiments performed in triplicate. Values were normalized to vector (dotted line). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; two-tailed *t*-test with unequal variance. **j**, Quantification of cellular ROS (measured by high-content microscopy) in human astrocytes reconstituted with PIN4(WT) or PIN4(Y122F) after silencing of the endogenous *PIN4* and acutely transduced with F3–T3 or vector. Representative bar plots from one out of three independent experiments. Data are mean ± s.d. ($n = 3$ technical replicates). $^*P < 0.05$; two-tailed *t*-test with unequal variance. *N*-acetyl-L-cysteine-treated cultures were used as negative controls. Molecular weights are indicated in all immunoblots.

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