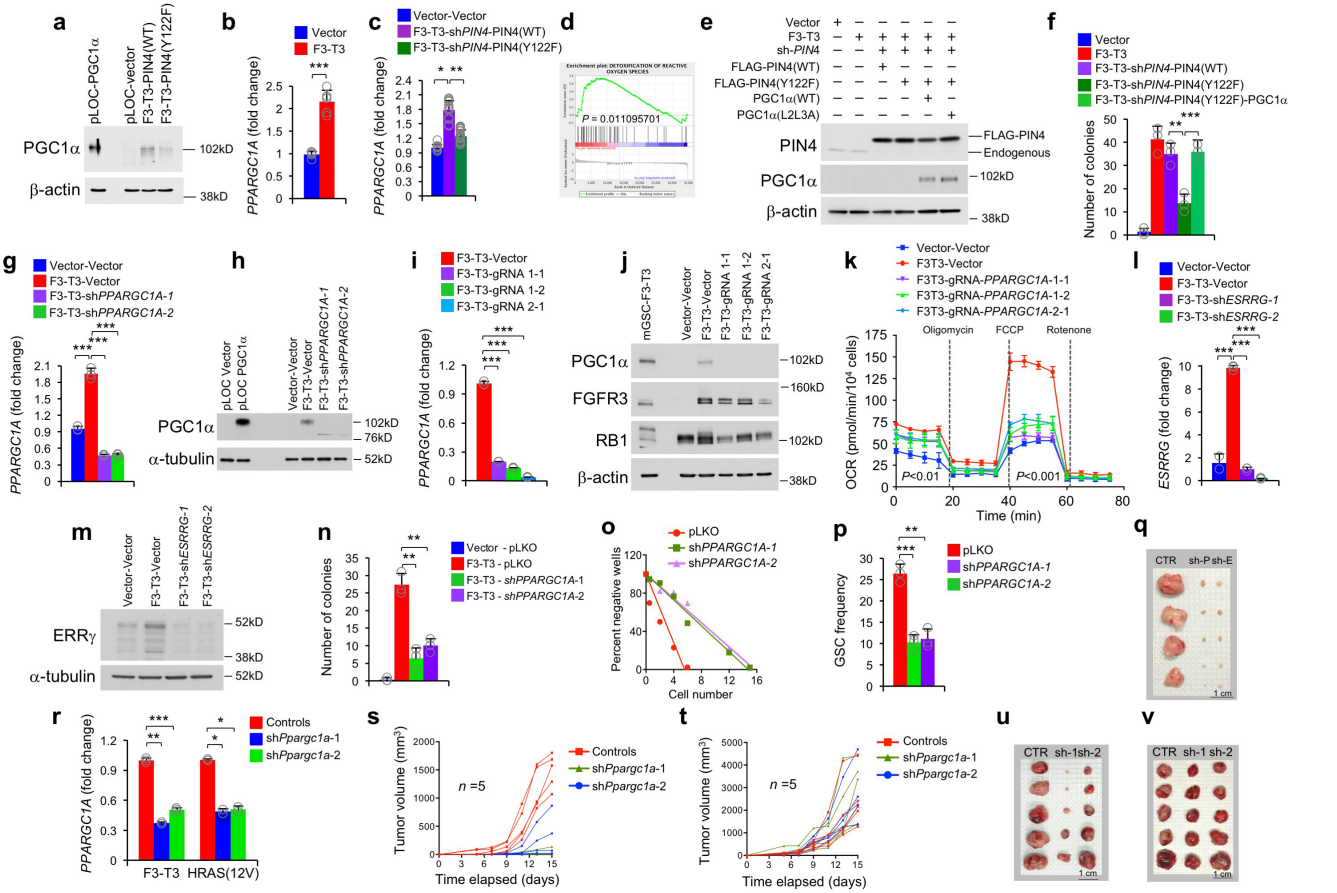

Extended Data Figure 7 | PGC1α and ERRγ are required for mitochondrial metabolism and tumorigenesis of cells transformed by F3–T3. a, Immunoblot of endogenous PGC1α in human astrocytes expressing F3–T3 following silencing of *PIN4* and reconstitution with wild-type or PIN4(Y122F). Exogenous expression of PGC1α in human astrocytes is included as positive control. Experiment was independently repeated three times with similar results. **b**, RT–qPCR of *PPARGC1A* in human astrocytes expressing F3–T3 or vector. Data are mean ± s.d. ($n = 6$ replicates) from two independent experiments each performed in triplicate. **c**, RT–qPCR of *PPARGC1A* in human astrocytes expressing F3–T3 treated as in **a**. Data are mean ± s.d. ($n = 4$ biological replicates) from four independent experiments. **d**, GSEA shows upregulation of ROS detoxification genes in human astrocytes expressing F3–T3 ($n = 5$ biological replicates) compared with vector ($n = 3$ biological replicates). Nominal *P* value is indicated. **e**, Immunoblot of Flag-PIN4 (wild-type and Y122F) and PGC1α (wild-type and L2L3A) in human astrocytes expressing F3–T3 after silencing of *PIN4*. Experiment was repeated twice independently with similar results. **f**, Soft agar colony-forming assay of human astrocytesF3–T3 following silencing of *PIN4* and reconstitution with wild-type or Y122F Flag-PIN4 in the presence or the absence of PGC1α. Data are mean ± s.d. ($n = 3$ technical replicates) from one representative experiment out of two independent experiments. **g**, RT–qPCR of *PPARGC1A* in human astrocytes expressing vector or F3–T3 transduced with *PPARGC1A* shRNA1 or *PPARGC1A* shRNA2 lentivirus. Data are mean ± s.d. ($n = 3$ technical replicates) from one representative experiment. **h**, Immunoblot analysis of PGC1α in HA-F3–T3 treated as in **g**. Experiment was repeated two times independently with similar results. Exogenous expression of PGC1α is included as positive control. Experiment was repeated twice independently with similar results. **i**, RT–qPCR of *PPARGC1A* in F3–T3 human astrocytes expressing two independent gRNAs against *PPARGC1A* (*PPARGC1A* gRNA1, two clones; *PPARGC1A* gRNA2, 1 clone) or the empty vector. Data are mean ± s.d. ($n = 3$ technical replicates) from one representative experiment. **j**, Western blot of cells treated as in (**i**) using the indicated antibodies. Experiment was repeated twice independently with similar results. **k**, OCR of human astrocytes expressing vector or F3–T3 transduced with *PPARGC1A* gRNA1 or gRNA2. Data are mean ± s.d. ($n = 5$ technical replicates) from one representative experiment out of two independent experiments. $P < 0.001$ for rate 1–4 and 9–12; two-tailed *t*-test with unequal variance. **l**, RT–qPCR of *ESRRG* in human astrocytes expressing vector or F3–T3 infected with *ESRRG* shRNA1 or *ESRRG* shRNA2 lentiviruses. Data are mean ± s.d. ($n = 3$ technical replicates) from one representative experiment. **m**, Immunoblot analysis of ERRγ in human astrocytes expressing F3–T3 treated as in **l**. Experiment was repeated twice independently with similar results. **n**, Soft agar colony-forming assay of human astrocytes treated as in Fig. 3g. Data are mean ± s.d. ($n = 3$ technical replicates) of one representative experiment out of two independent experiments performed in triplicate. **o**, GSC1123 cells were transduced with *PPARGC1A* shRNA lentiviruses or the empty vector. Cells were analysed by *in vitro* LDA. Representative regression plot used to calculate the frequency of gliomaspheres in 96-well cultures from three independent infections. **p**, Bar graph shows the frequency of gliomaspheres from three independent infections analysed by LDA as shown in **o**. Data are mean ± s.d. ($n = 3$ biological replicates). **q**, The photograph shows tumours generated by human astrocytes F3–T3 transduced with *PPARGC1A* shRNA1, *ESRRG* shRNA1 or vector lentivirus in Fig. 3i at the time of mouse euthanasia. sh-P, *PPARGC1A* shRNA1; sh-E, *ESRRG* shRNA1. **r**, RT–qPCR of *Ppargc1a* in F3–T3-shTrp53 and HRAS(12V) shTrp53 mGSCs transduced with *Ppargc1a* shRNA1 or *Ppargc1a* shRNA2 lentivirus. Data are mean ± s.d. ($n = 3$ technical replicates) of one representative experiment. **s**, Tumour volume of F3–T3-shTrp53 mGSCs expressing a pLKO-vector ($n = 5$), *Ppargc1a* shRNA1 ($n = 5$) or *Ppargc1a* shRNA2 ($n = 5$). Data are the tumour growth curve of individual mice. **t**, Tumour volume of mice injected subcutaneously with HRAS(12V);shTrp53 mGSCs expressing pLKO-vector ($n = 5$) or *Ppargc1a* shRNA1 ($n = 5$) or *Ppargc1a* shRNA2 ($n = 5$). Data are tumour growth curve of individual mice; NS, not significant, two-tailed *t*-test with unequal variance (time points 1–7). **u**, Photograph shows tumours generated from F3–T3;shTrp53 mGSCs transduced with *Ppargc1a* shRNA1 or *Ppargc1a* shRNA2 or vector lentivirus in **s** at the time of mouse euthanasia. **v**, Photograph shows tumours generated by HRAS(12V) shTrp53 mGSCs transduced with *Ppargc1a* shRNA1 or *Ppargc1a* shRNA2 or vector lentivirus in **t** at the time of mouse euthanasia. Molecular weights are indicated and β-actin or α-tubulin is shown as a loading control in all immunoblots. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; two-tailed *t*-test with unequal variance.