Supporting Information for:

Fibroblast Growth Factor 2 Regulates Activity and Gene Expression of Human Postmitotic Excitatory Neurons

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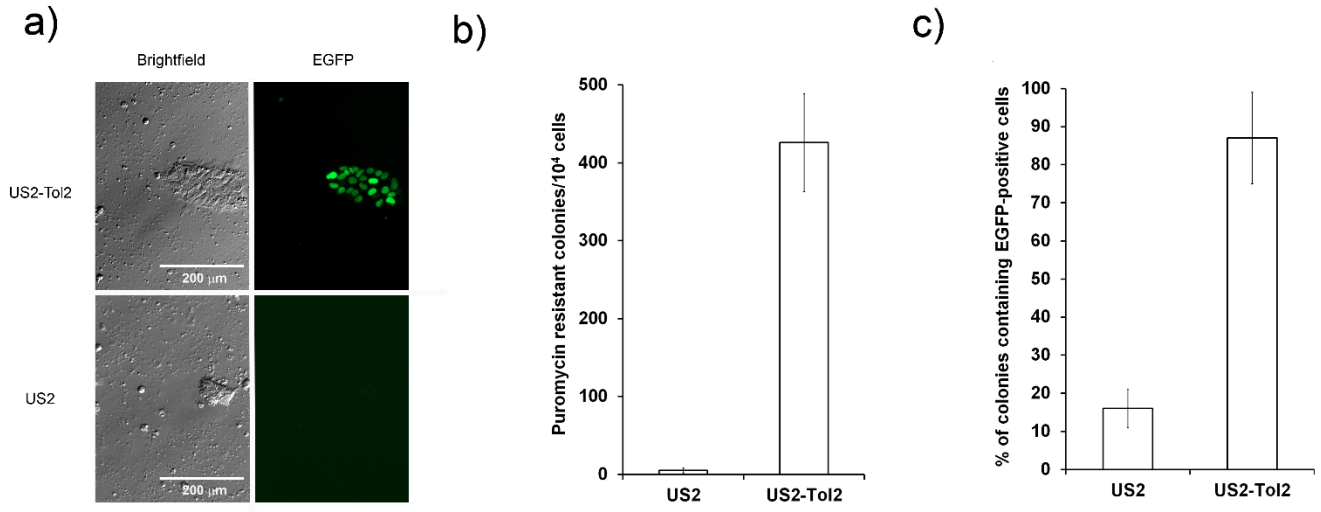
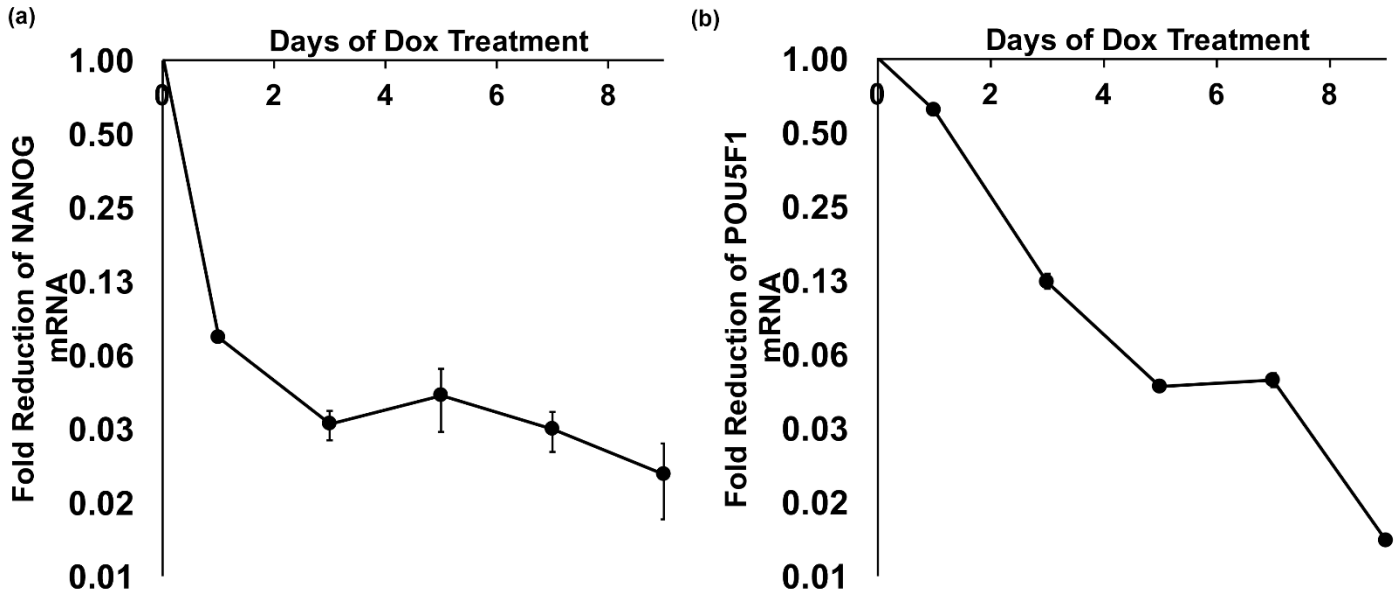
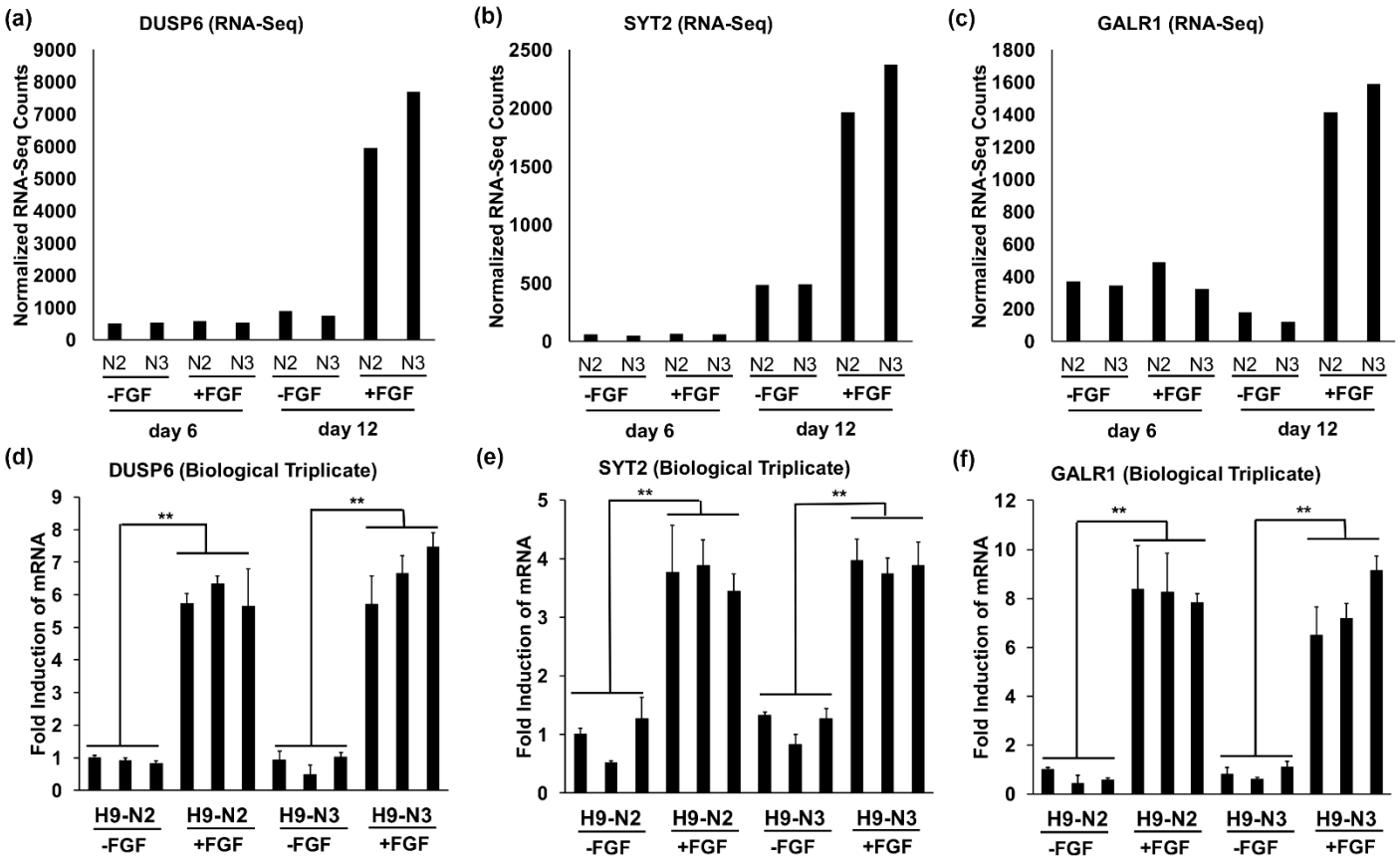


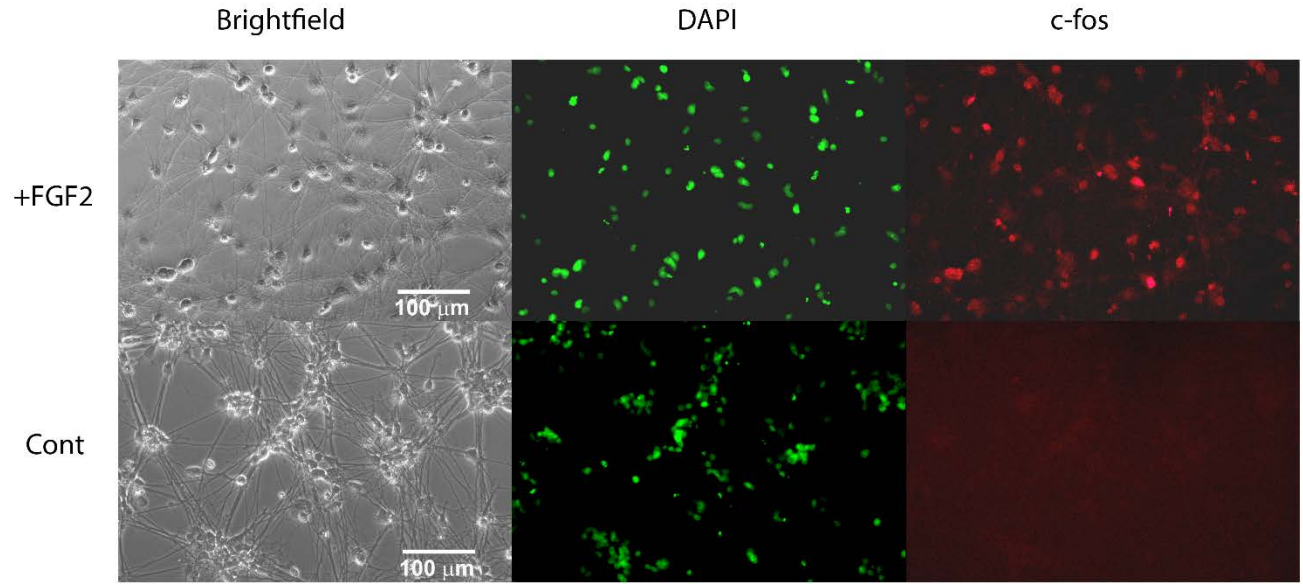
Supplementary Fig 1. Tol2 increases the efficiency of transfection. (A) Brightfield and epifluoroscence images showing the increase in EGFP+ colonies in presence of Tol2 recombinase. (B) Number of puromycin resistant colonies increase 100-fold when the cells are transfected with Tol2 recombinase. (C). Quantification of EGFP+ colonies showing a 10-fold increase with Tol2 recombinase.



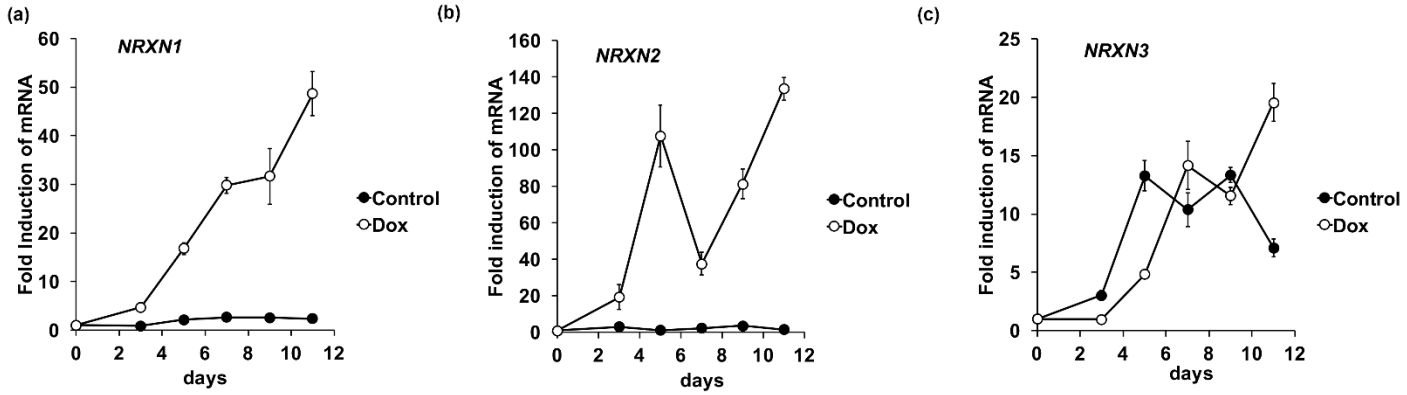
Supplementary Fig 2. Regulation of pluripotency genes during siNeuron differentiation. (A) Quantification of NANOG mRNA levels in H9 hESC-derived siNeurons over a 11-day time course show the rapid decrease in NANOG upon dox induction. (B) qRT-PCR of POU5F1 mRNA level showing the decline in expression over 11 days upon dox treatment. Data are represented as means and bars indicate the standard deviation between the triplicate samples.



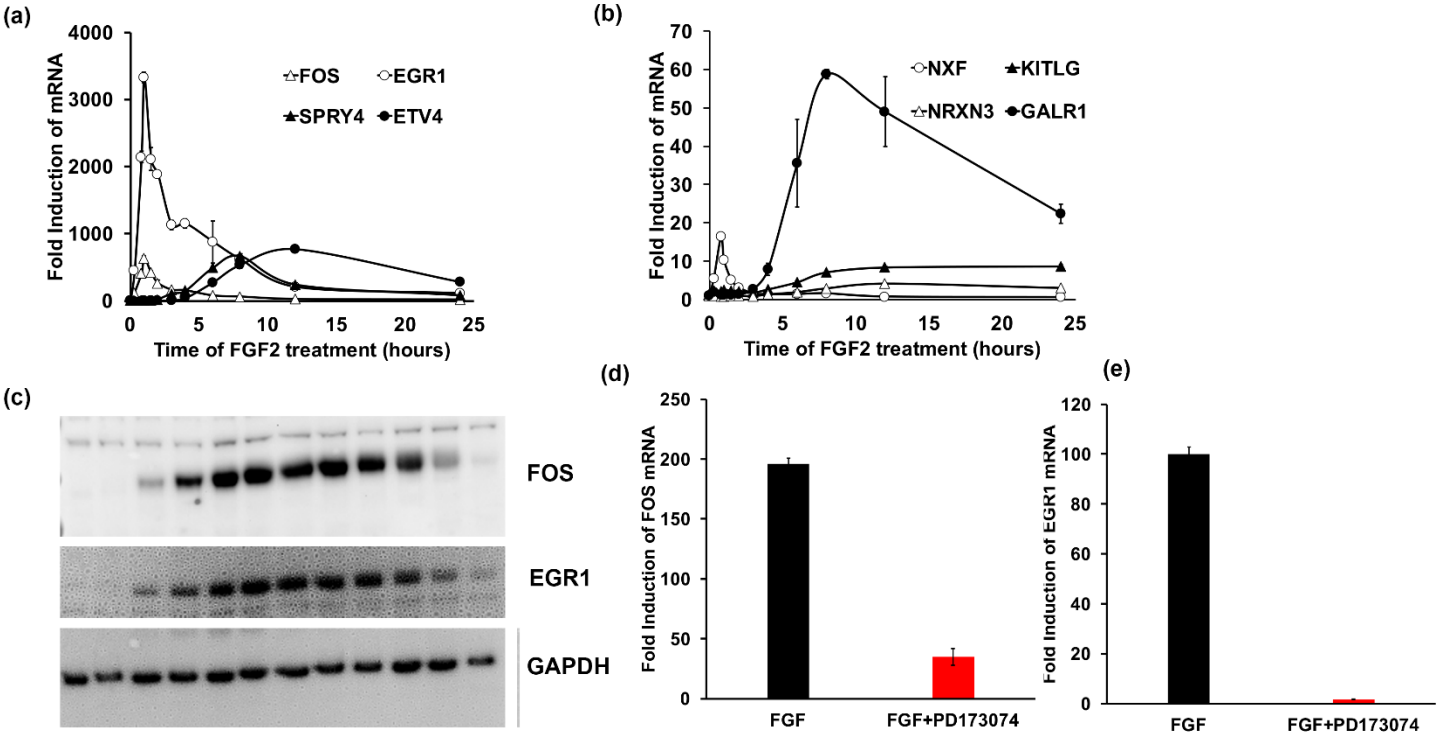
Supplementary Fig 3. FGF2 regulated genes in H9-N2 and H9-N3 siNeurons. (A-C) Normalized gene expression levels reported by RNA-Seq for the induced genes, DUSP6, SYT2, and GALR1 in two independent cell lines at day 6 and day 12. No significant difference was seen at day 6 but the expression level increased significantly in the presence of FGF at day 12. **(D-F)** Quantification of mRNA levels for the genes DUSP6, SYT2, and GALR1 in biological triplicate of two independent cell lines showing a significant increase in expression in the presence of FGF. **, $p<0.01$.



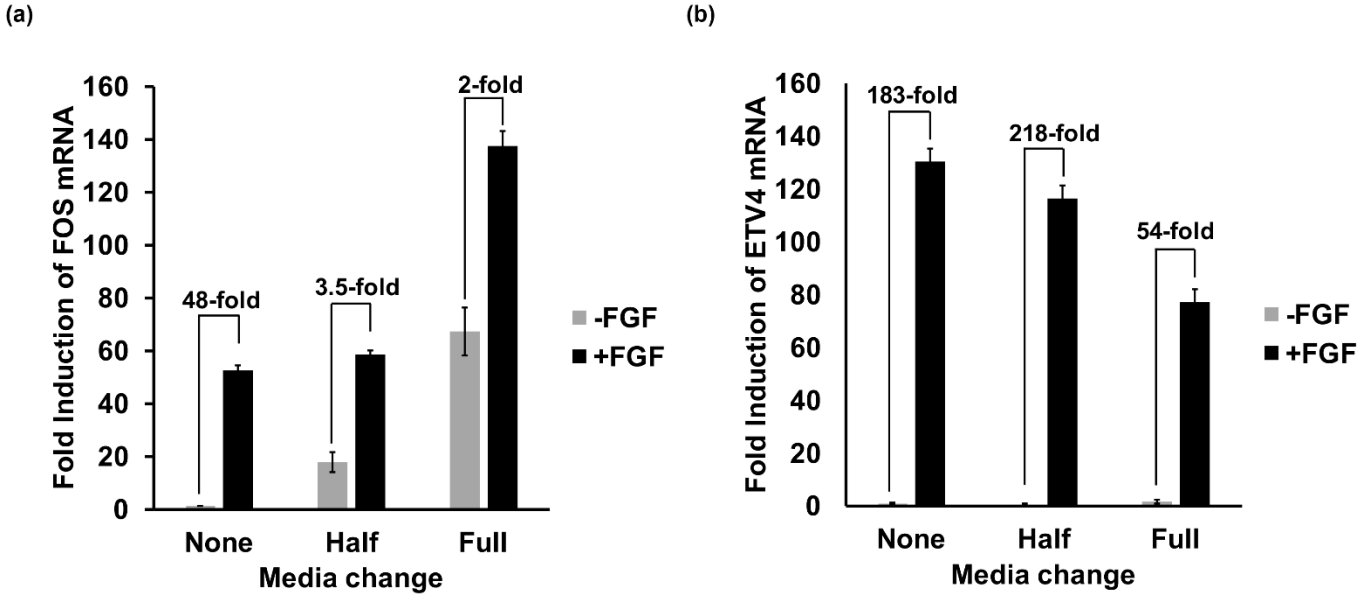
Supplementary Fig 4. Immunostaining of cFOS upon treatment with FGF2. c-FOS immunolabelling of H9-N2 siNeurons in presence and absence of FGF2 shows c-FOS induction upon FGF2 treatment.



Supplementary Fig 5. Regulation of Neurexin genes during siNeuron differentiation. (A-C) Quantification of NRXN1, NRXN2, and NRXN3 mRNA levels in H9 hESC-derived siNeurons over a 11-day time course shows a 50-fold and 100-fold increase in NRXN1 and NRXN2, respectively, upon induction with dox. siNeurons do not show dox induction of NRXN3 until day 11.



Supplementary Fig 6. FGF2 regulation in control iPSC generated siNeurons affect the downstream MAPK signaling pathway (A-B). Time- course induction of genes responsive to 2 ng/ml FGF2 over 24h. IEGs FOS, EGR1 and NXF are induced within 60 min, SPRY4 by 8h and ETV4, NRXN3, GALR1 within 12 h. **(C)** Immunoblot analyses of FOS and EGR1 proteins over 24 h treatment with 2nM FGF2. GAPDH is used as a loading control. **(D-E)** Inhibition of FOS and EGR1 gene expression upon treatment with PD173074, a FGFR1 inhibitor.



Supplementary Fig 7. Gene expression changes after media change. (A) Quantification of c-FOS mRNA in the presence or absence of FGF2 shows that to obtain maximal induction of FOS mRNA, the cells need to be starved for 24 h. (B) Quantification of ETV4 mRNA in the presence or absence of FGF2 shows that ETV4 is induced only in presence of FGF2 and that greater responses were seen when the cells were starved of fresh media or had half media changes.