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

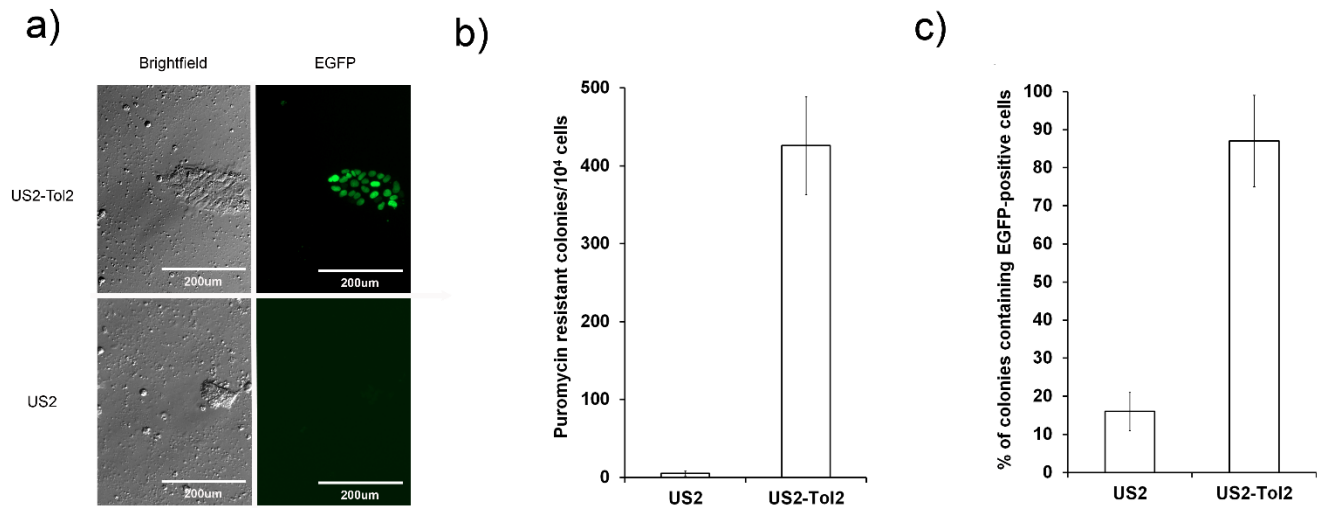
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Michael Uhler*‡

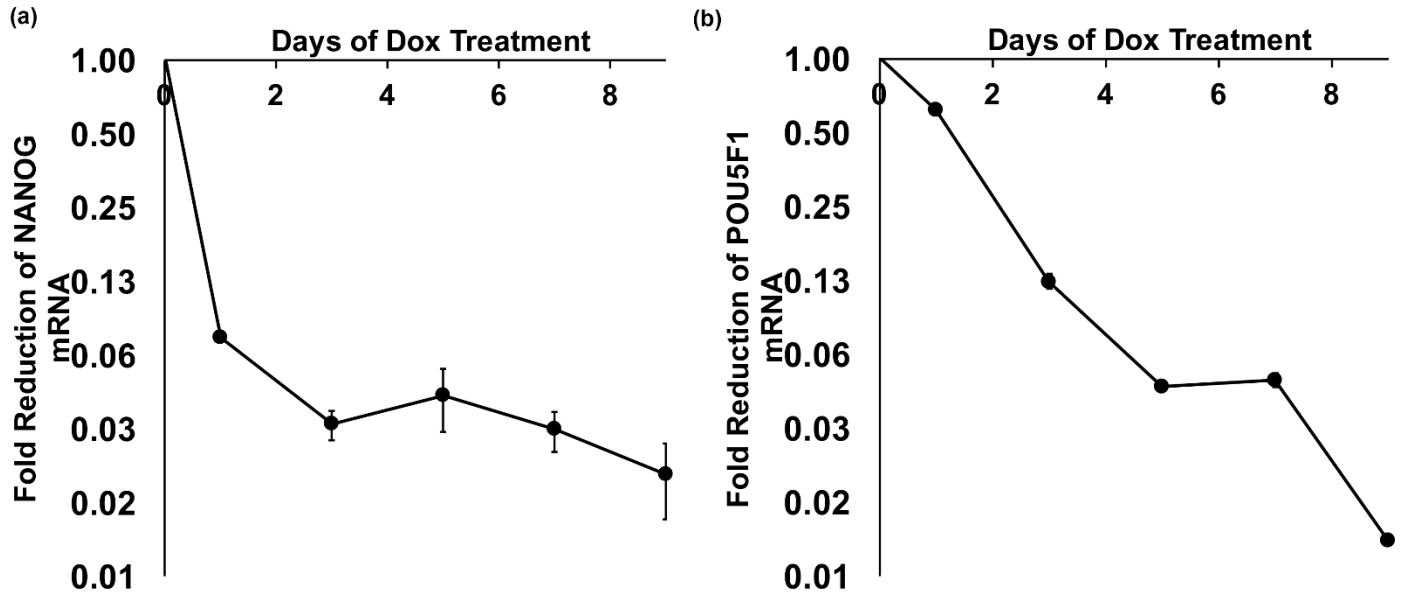
*Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, Michigan, USA

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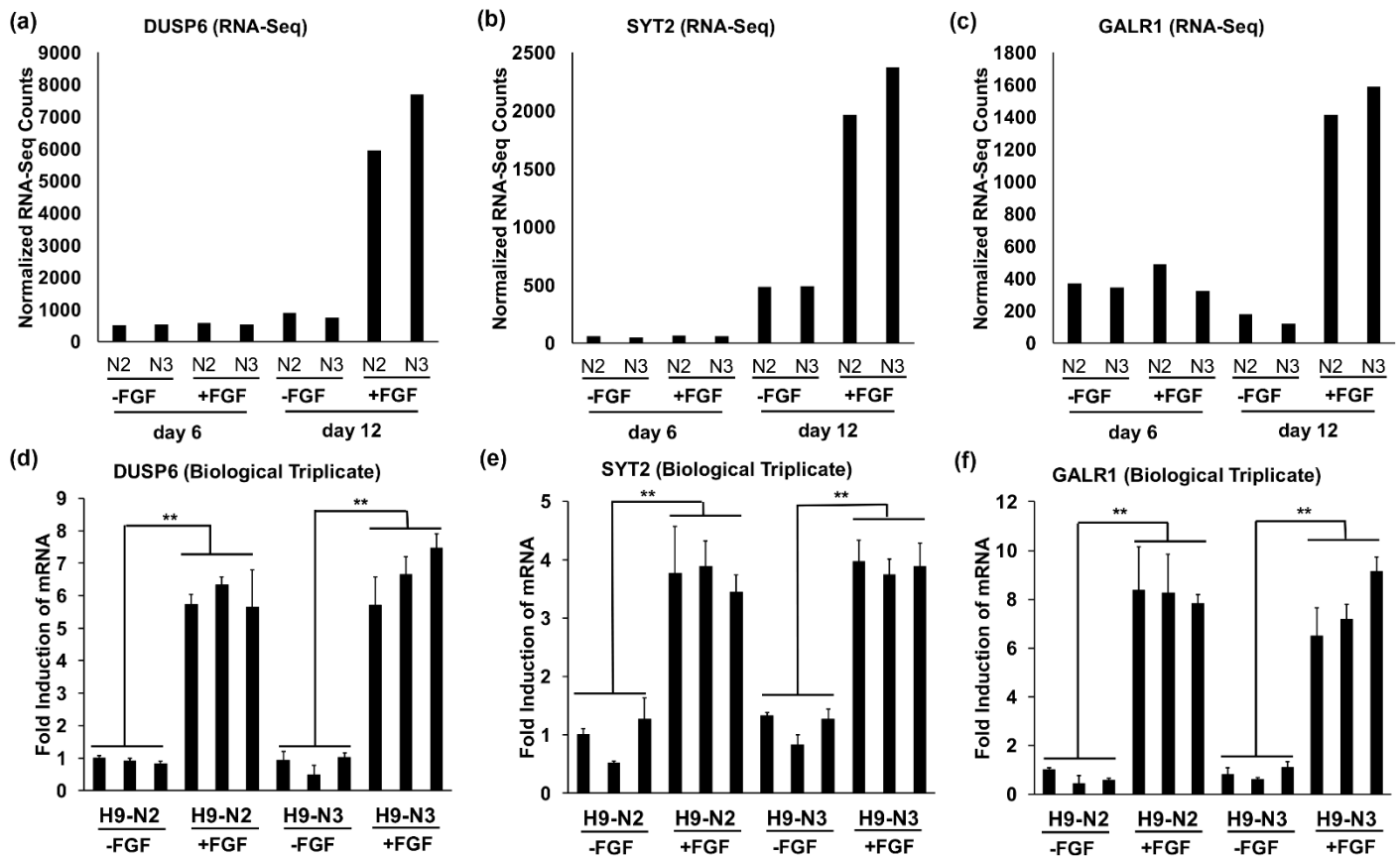
‡Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan, USA



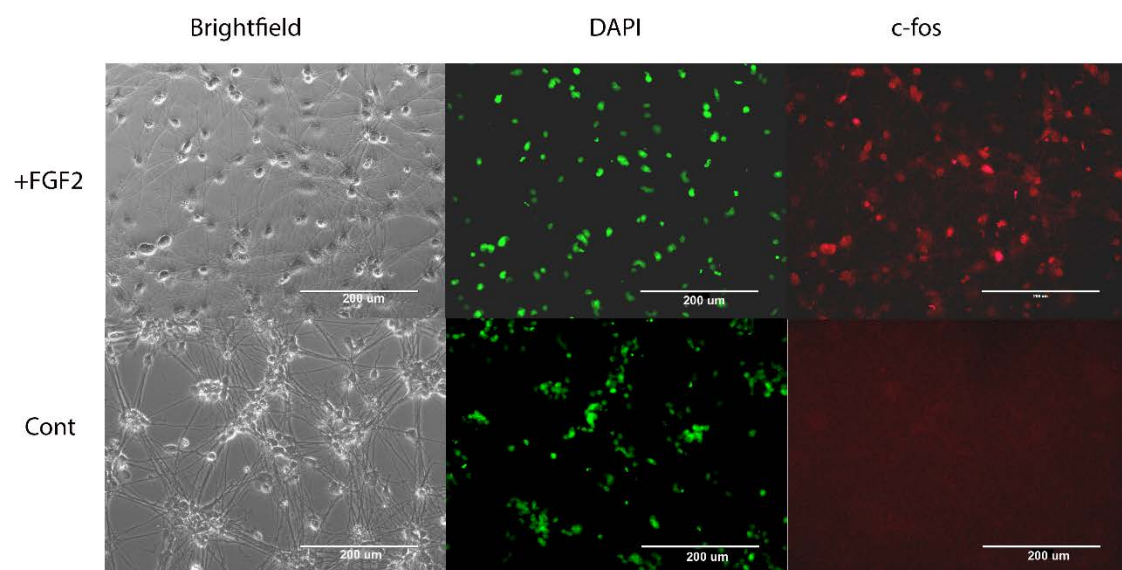
Supplementary Fig 1. Tol2 increases the efficiency of transfection. (A) Brightfield and epifluorescence images showing the increase in EGFP+ colonies in presence of Tol2 recombinase. (B) Number of puromycin resistant colonies increase 100-fold when the cells are transfected with Tol2 recombinase. (C). Quantification of EGFP+ colonies showing a 10-fold increase with Tol2 recombinase. Data is representative of three independent experiments (n=3).



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.

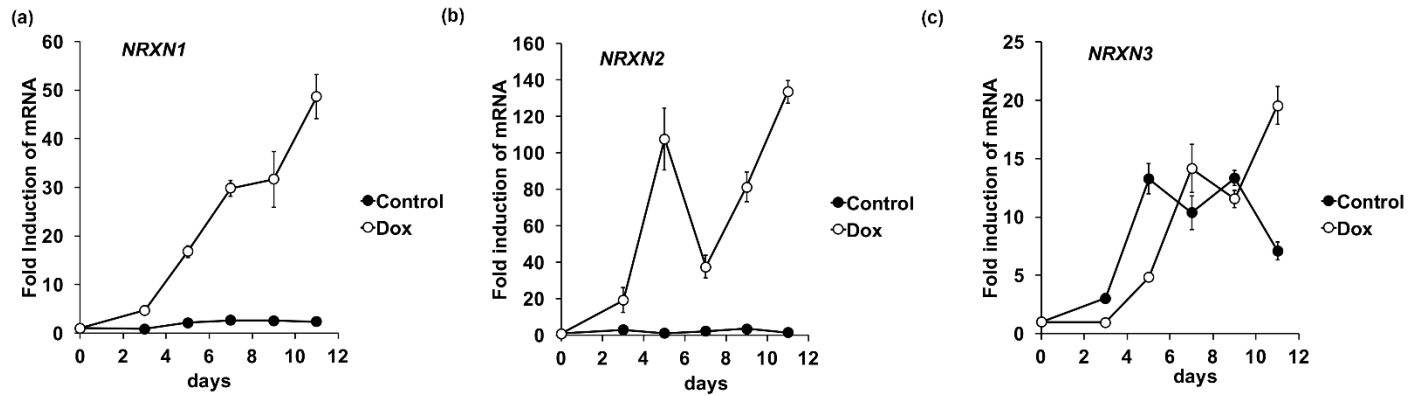


Supplementary Fig 3. FGF2 regulated genes in H9-N2 and H9-N3 siNeurons. (A-C) Normalized gene expression levels reported by RNA-Seq for the induced genes, DUSP6, SYT2, and GALR1 in two independent cell lines at day 6 and day 12. No significant difference was seen at day 6 but the expression level increased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

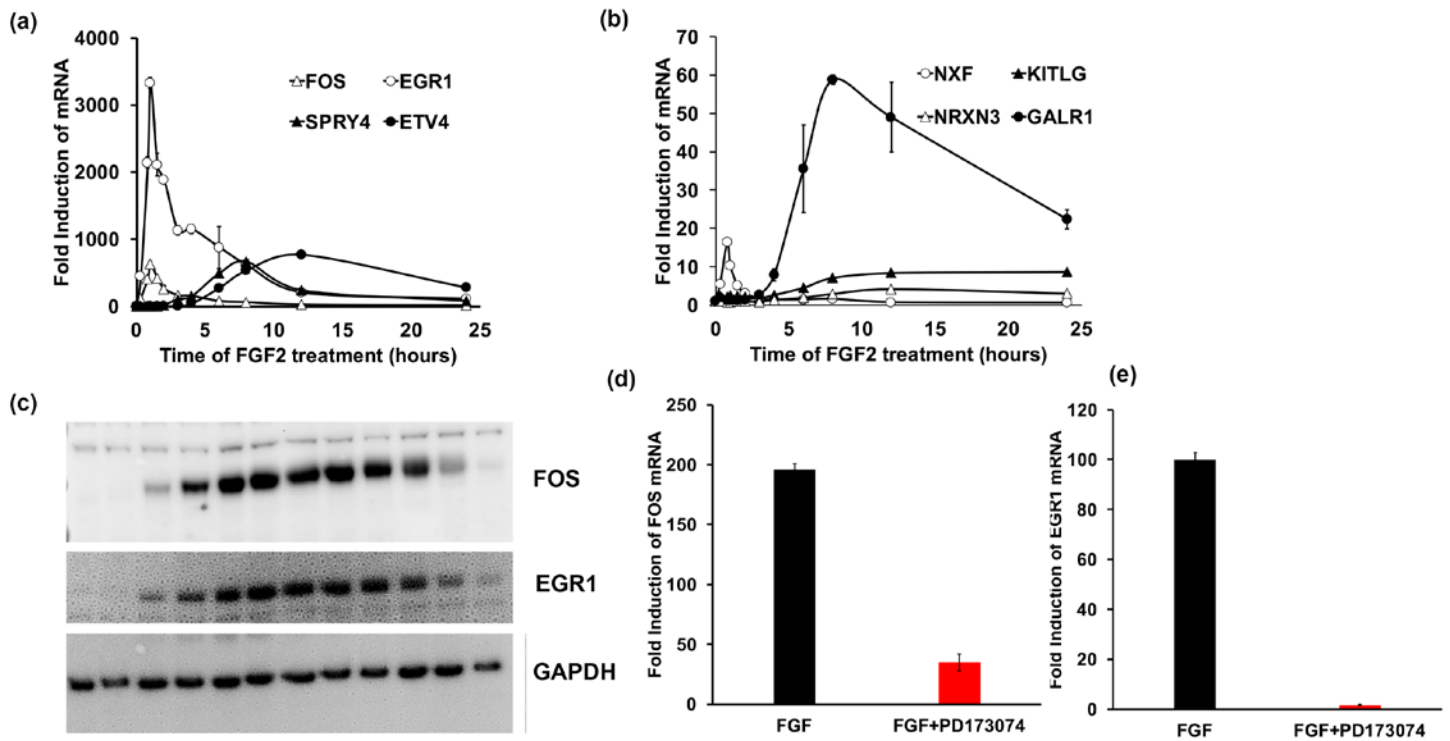


38 **Supplementary Fig 4. Immunostaining of cFOS upon treatment with FGF2.** c-FOS immunolabelling of H9-N2
39 siNeurons in presence and absence of FGF2 shows c-FOS induction upon FGF2 treatment.

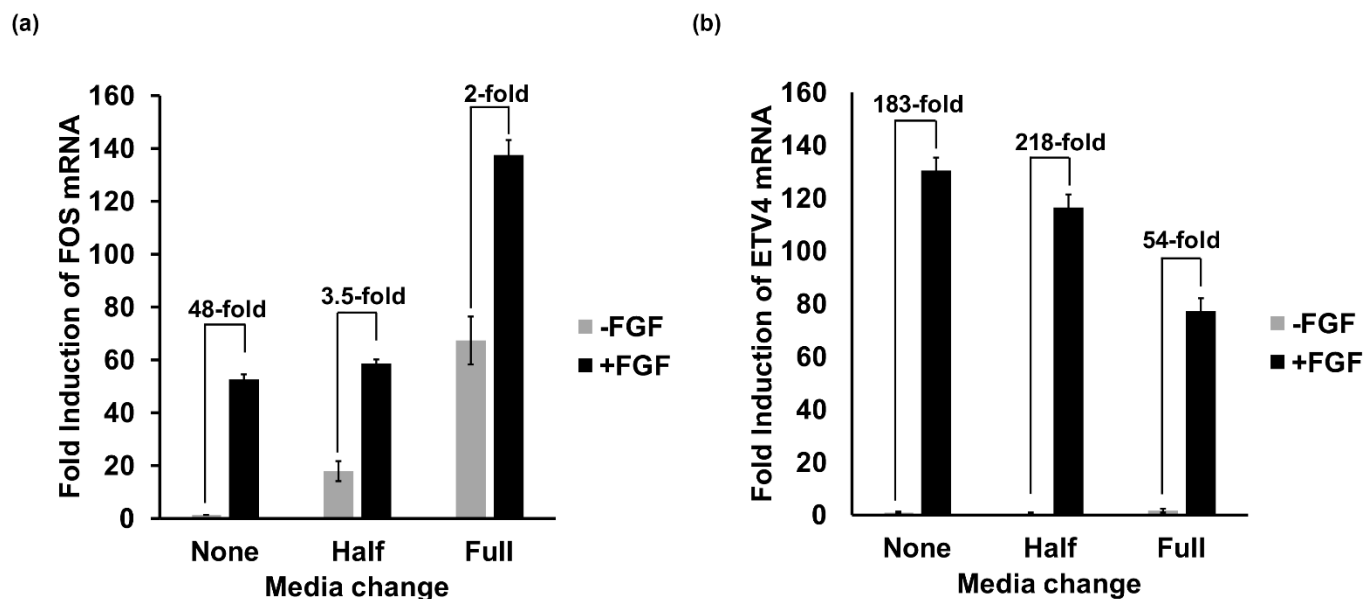
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Supplementary Fig 5. Regulation of Neurexin genes during siNeuron differentiation. (A-C) Quantification of NRXN1, NRXN2, and NRXN3 mRNA levels in H9 hESC-derived siNeurons over a 11-day time course shows a 50-fold and 100-fold increase in NRXN1 and 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.



33 **Supplementary Fig 7. Gene expression changes after media change.** (A) Quantification of c-FOS mRNA in the
34 presence or absence of FGF2 shows that to obtain maximal induction of FOS mRNA, the cells need to be starved for 24 h.
35 (B) Quantification of ETV4 mRNA in the presence or absence of FGF2 shows that ETV4 is induced only in presence of
36 FGF2 and that greater responses were seen when the cells were starved of fresh media or had half media changes. Data is
37 representative of three independent experiments (n=3).
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Supplementary Table 1. List of primers used in this study: qRT-PCR

Target	Forward	Reverse
NEUROG2	ATCCGAGCAGCACTAACACG	GCTGAGGCACAGTTAGAGCC
VGLUT2	AGATTCCGGGAGGCTACATC	ACTCTGGCTGCTGATGGAAT
SYN	ACTTCTGTGACAAGCCCTGG	ACCACGGGGTACGTTGTA
MAPT	CACACTTGGACTGGACGTTG	AAGATCGGCTCCACTGAGAA
CAMKIIA	TGATCTTGGCAGCATACTCCT	CGCTTCACGGAAGAGTACCA
DCX	TCAGGACCACAGGCAATAAA	AGACCGGGGTTGTCAAAAA
MAP2	TTCGTTGTGTCGTGTTCTCA	AACCGAGGAAGCATTGATTG
CAMKIIIB	CACCGACGAGTACCAGCTCT	GTTGATGATCTTGGCTGCAT
ETV4	GCCCCTCGACTCTGAAGAT	TGGAAATCAGGAACAACTGC
SPRY4	GAGTACAGCGGCGGCTAA	CAAACCGCTCAATACAGGCT
NRXN3	ATTTGCCGTCATTTACAGGG	TGACTTCCTCCAGCTTACACA
c-FOS	GTGACCGTGGGAATGAAGTT	CCGGGGATAGCCTCTCTTAC
EGR1	GGAAAAGCGGCCAGTATAGG	AGCCCTACGAGCACCTGAC
NXF	CTGCATCTACACTCGCAAGG	GTAGTGCCGCTACGATGTCC
KITLG	AGCGCTGCCTTTCCCTTATG	TCCTGCAGATCCCTTCAGTT
GALR1	CTTCTGCTATGCCAAGGTCC	CAACCACCACCAGAAGTGTCT
DUSP6	CAGTGACTGAGCGGCTAATG	TGTCCAGTTTTTCCCTGAG
GAPDH	AAGGTGAAGGTCGGAGTCAA	AATGAAGGGGTCATTGATGG

Peer Review

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Supplementary Table 2. Gene Ontology Analysis of Genes Induced by Doxycycline in H9-N1 cells

Gene Ontology Term Number	Term Description	Gene Count	% of total genes	PValue	Genes	Fold Enrichment	Bonferroni	Benjamini	FDR
GO:0007399	Nervous System Development	66	5.94	2.66E-22	MEF2C, GRIK1, FGF14, SCN3B, FGF17, ZEB2, L1CAM, TLL7, SRRM4, LGI1, NRG1, CHRNA3, DSCAM, RBFOX1, STMN3, ENCL1, MYT1L, HES4, LSAMP, CNTN4, NRSN1, SMARCA2, NEURL1, KALRN, DPF3, ACHE, CPLX2, CNTFR, ST8SIA2, NRN1, MYT1, EPHB2, DPF1, APLP1, PCSK2, LHX1, CRMP1, LY6H, AVIL, GPSM1, APBA2, DCX, SIM1, DCLK1, APBA1, GLRB, CYP46A1, MAP1B, DPYSL5, DPYSL4, EVL, DPYSL2, ELAVL3, SHOX2, KIAA2022, SLC4A10, TENM1, ST8SIA4, SMPD1, TMOD2, NEUROD2, GFRA1, MPPED2, MAB21L2, GFRA2, FEZ1	4.01	7.88E-19	7.88E-19	4.79E-19
GO:0007411	Axon Guidance	42	3.78	1.19E-16	DCC, CDK5R1, NRP1, L1CAM, DRGX, EPHB2, CRMP1, TNFR, POU4F2, SEMA3C, ROBO2, UNC5C, SPON2, LGI1, TUBB3, FLRT3, FLRT2, KLF7, KIF5A, DRAXIN, KIF5C, SPTBN4, DPYSL5, NFASC, EVL, DPYSL2, NR4A3, NRXN1, PTPRO, SLIT1, SLIT3, NCAM1, EPHAS, TENM2, NTRK1, CNTN2, SPTBN2, CNTN4, RELN, CHL1, KIF26B, FEZ1	4.61	3.29E-13	1.64E-13	2.00E-13
GO:0007269	Neurotransmitter Secretion	22	1.98	1.46E-13	PPFIA2, SYT1, RAB3A, STX1A, CPLX1, NRXN2, PTPRN2, LIN7B, STXB1, DGKI, NRXN1, BRSK1, RIMS1, SYN1, SNPH, SLC18A3, SLC5A7, VAMP2, SNAP25, APBA1, CHAT, CACNA1B	7.53	4.31E-10	1.44E-10	2.62E-10
GO:0001764	Neuron Migration	28	2.52	2.39E-11	DCC, MEF2C, CDK5R1, NRP1, CDK5R2, TUBB2B, ASTN1, DRGX, NRCAM, GPM6A, MAPT, DNER, PAFAH1B1, DCX, TLX3, DCLK1, PITX2, KIRREL3, PHOX2B, MDGA1, NKX6-1, NTRK2, CNTN2, RELN, NEUROD4, KIAA0319, ADGRL3, CHL1	4.65	7.09E-08	1.77E-08	4.30E-08
GO:0007268	Chemical Synaptic Transmission	41	3.69	9.37E-10	MYO5A, SNGC, SYT1, CPLX1, SNAP91, GRIK1, GRIK2, SYTS, TACR1, GLRA3, CACNB1, TAC1, CACNB3, GABBR2, KCNIP2, AMPH, SLC1A2, KCNQ3, SYN1, CHRNA4, APBA2, PAFAH1B1, LRFN5, SNAP25, NOVA1, APBA1, GLRB, NRXN2, OPR1, KIF5A, BSN, PCDH8, GRIA3, NRXN1, KCNK3, GABARAP, GRM2, GRIA2, SLC18A3, CACNA1E, CACNA1B	2.98	2.77E-06	5.55E-07	1.68E-06
GO:0014047	Glutamate Secretion	14	1.26	1.09E-09	PPFIA2, RAB3A, SYT1, STX1A, CPLX1, STXB1, RIMS1, SLC17A7, SLC1A2, GRM2, NTRK2, VAMP2, SNAP25, APBA1	8.73	3.24E-06	5.39E-07	1.97E-06
GO:0007416	Synapse Assembly	19	1.71	4.68E-09	FLRT3, DNM3, ACHE, NRXN2, CLSTN3, SPOCK2, DRD2, BSN, NRXN1, PCLO, NRCAM, GPM6A, DNER, SPTBN2, POU4F1, NRG1, ADGRL3, DSCAM, KIRREL3	5.44	1.38E-05	1.98E-06	8.41E-06
GO:0007156	Homophilic Cell Adhesion	31	2.79	5.38E-09	CADM3, ME3, CLSTN2, CADM1, CLSTN3, CADM2, DSCAML1, PCDHGC4, CDH2, DCHS1, CDH6, CDH22, CDH7, CDH20, TRO, ROBO2, DSCAM, KIRREL3, RET, CDHR1, CDHR2, PCDH9, PCDHGB6, PCDH8, PCDH17, PCDH19, PCDHGB4, CDH12, CDH13, CDH18, CDH10	3.42	1.59E-05	1.99E-06	9.67E-06
GO:0043524	Negative Regulation of Neuron Apoptotic Process	26	2.34	1.05E-07	MEF2C, NRP1, GRIK2, CPEB4, SNCA, TGFB3, CNTFR, CITED1, POU4F1, AGAP2, NEFL, DRAXIN, STXB1, PARK2, NR4A3, ISL1, PPARGC1A, FAM134B, CORO1A, NTRK1, JUN, SIX1, VSTM2L, NTRK2, FAIM2, CHL1	3.44	3.12E-04	3.47E-05	1.89E-04
GO:0010976	Positive Regulation of Neuron Projection Development	21	1.89	1.07E-07	RET, PPP2R5B, STMN2, ENCL1, DPYSL3, KIDINS220, ADCYAP1, EPHA3, TRIM67, NDRG4, NTRK1, CNR1, NTRK2, AVIL, ATP8A2, CNTN1, RELN, CAMK2B, APBB1, NEGR1, FEZ1	4.12	3.16E-04	3.16E-05	1.92E-04