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Supplemental Information

FGF-MAPK signaling regulates human deep-layer corticogenesis

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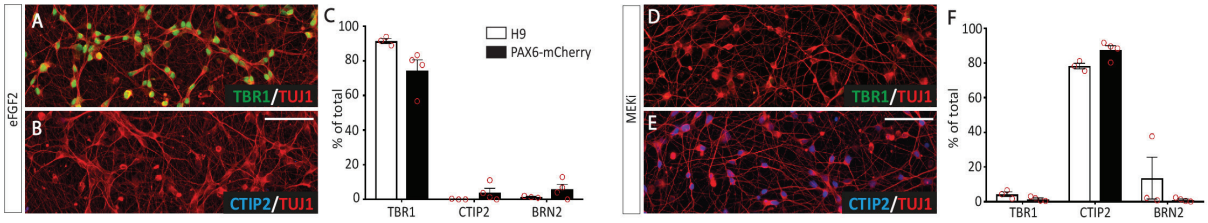
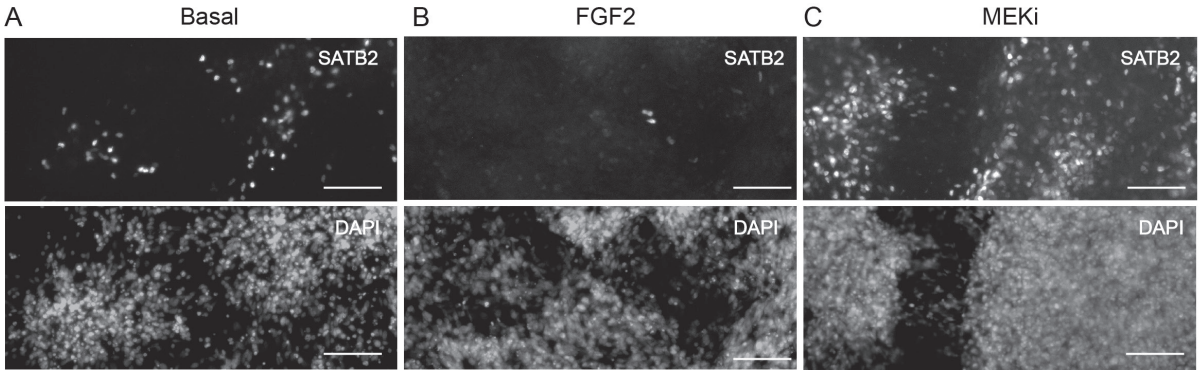
Supplementary Figure 1



Supplementary Figure S1 – Reproducibility of cortical laminar fate specification across human PSC lines following eFGF2 or MEKi treatment.

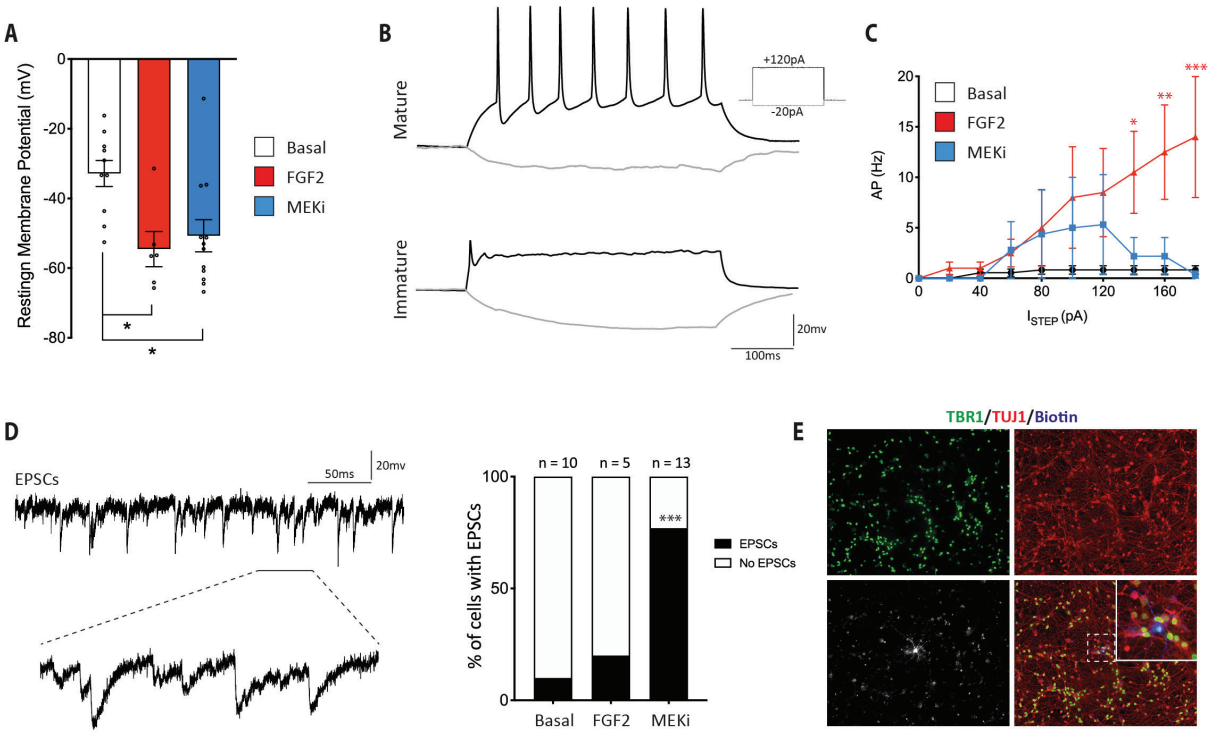
Cortical progenitors generated from H9 or HES3:PAX6^{mCherry} human ESCs were treated with eFGF2 (A-C) or MEKi (D-F) between D21-D35, DAPT-treated at D35 and analysed for their laminar phenotype at D55. Immunocytochemical staining for (A) TBR1+ and (B) CTIP2+ after eFGF2. (C) Quantification of layer subtype markers at D55 revealed that eFGF2 treatment generated TBR1+ neurons almost exclusively, regardless of cell line. Immunocytochemical staining for (D) TBR1+ and (E) CTIP2+ in MEKi treated cultures. (C) Cell counts revealed the majority of neurons were immunoreactive for CTIP2 for both PSC lines. (F). White bars - H9, black bars - HES3:PAX6^{mCherry}. Scale bar 50μm.

Supplementary Figure 2



Supplementary Figure S2 – eFGF2 treatment blocks SATB2+ neurogenesis.
SATB2 immunohistochemical labelling of D55 HES3:PAX6^{mCherry}-derived cortical cultures under Basal (A), extended FGF2 (eFGF2) (B) or MEKi (C) conditions, in the absence of DAPT. eFGF2-treated cultures lacked SATB2+ cells, which were notably increased following MEKi treatment. Scale bar 50μm.

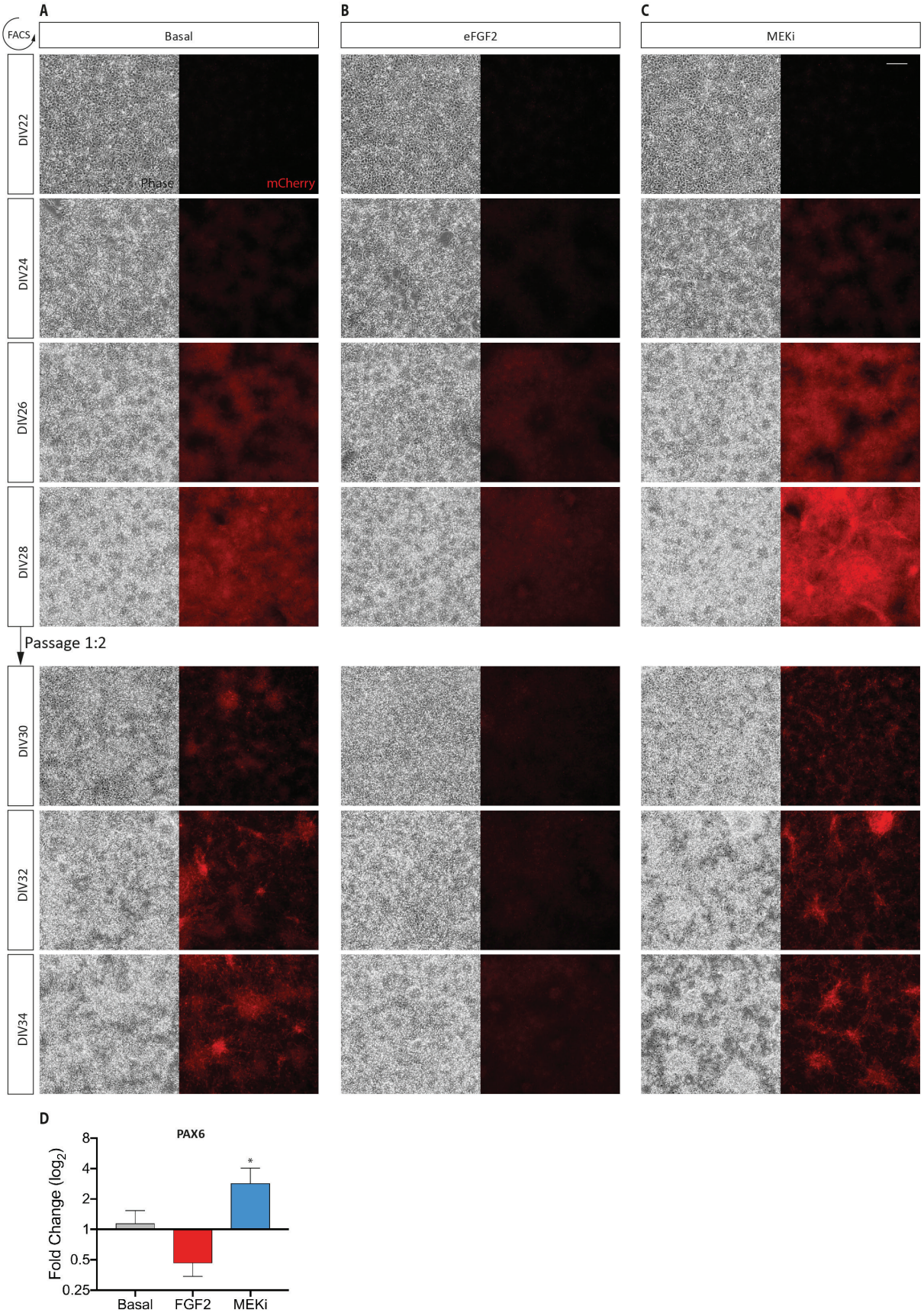
Supplementary Figure 3



Supplementary Figure S3 - Electrophysiological characterization of basal, FGF2 or MEKi treated cultures at D80.

(A) eFGF2 and MEKi treated cultures, which underwent DAPT-induced cell cycle exit, displayed lower resting membrane potentials compared to Basal cells (Basal n = 10, eFGF2 n = 5, MEKi n = 13; from two separate experiments (One-Way ANOVA with Tukey post hoc). (B) Neurons from all groups were capable of firing action potentials (AP) with large current injections and could be grouped into Mature or Immature types. (C) eFGF2 treated neurons maintained and increased AP initiation in response to increasing current injections compared to MEKi treated and basal cells (Two-Way ANOVA with Tukey post hoc). Combined these data indicate cells existed along a gradient of maturation and displayed varying channel expression and density. (D) Example traces of EPSC events within a representative MEKi treated neuron. The majority of neurons derived from MEKi treated progenitors exhibited excitatory postsynaptic currents (EPSCs), indicating they received greater synaptic input. (E) Representative biocytin filled, TBR1+ cortical neuron recovered after whole cell recording.

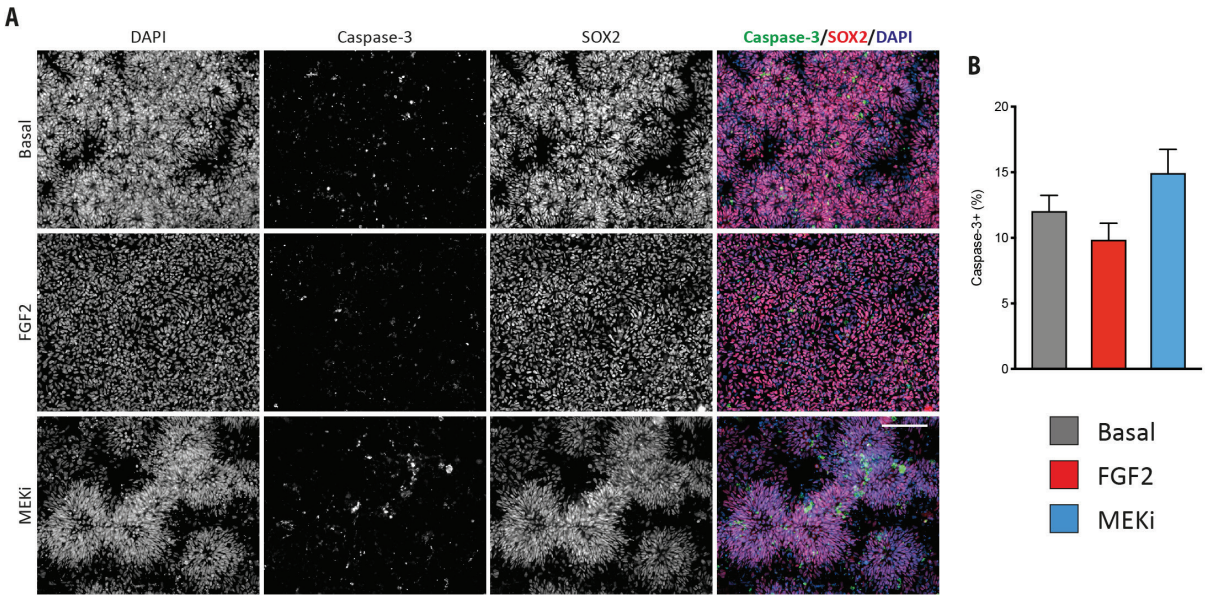
Supplementary Figure 4



Supplementary Figure S4 – PAX6^{mCherry} expression in cortical progenitors after FGF2 or MEKi addition.

(A) After FACS, PAX6+ progenitors decrease mCherry expression due to dissociation-induced stress. After replating, mCherry expression gradually increases as rosettes reform. Ongoing neurogenesis results in heterogenous cultures of PAX6- cells with neuronal morphology and PAX6+ progenitors. (B) PAX6^{mCherry} expression is notably reduced after eFGF2 treatment but remains detectable by FACS. (C) In contrast, MEKi treated cortical progenitors increase PAX6^{mCherry} reporter expression. Following a further passage, cultures contain PAX6- neurons and progenitors as well as PAX6+ cortical progenitors. (D) qPCR analysis of PAX6 expression confirms mCherry observations.

Supplementary Figure 5

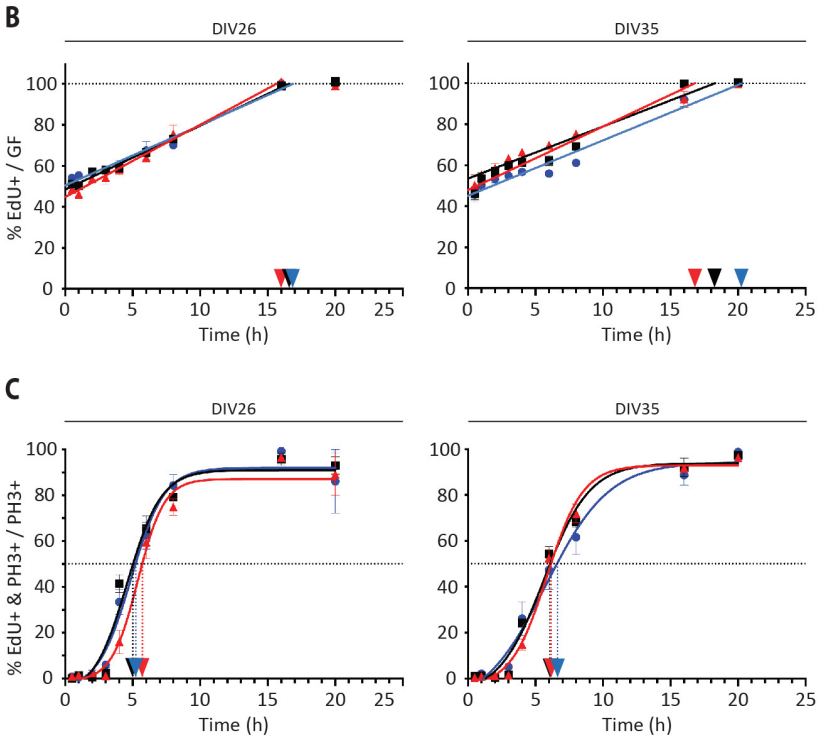


Supplementary Figure S5 – eFGF2 or MEKi treatments do not induce apoptosis.
(A) D26 cultures treated with eFGF2 or MEKi displayed no changes in toxicity relative to basal conditions as assessed by staining for cleaved caspase-3. A basal level of apoptosis (10-15%) was present within all conditions. (B) Quantification of caspase-3+ cells within cortical progenitor cultures. Scale bar is 100μm.

Supplementary Figure 6

Cell Cycle Parameters of hPSC-derived Cortical Progenitors (h)

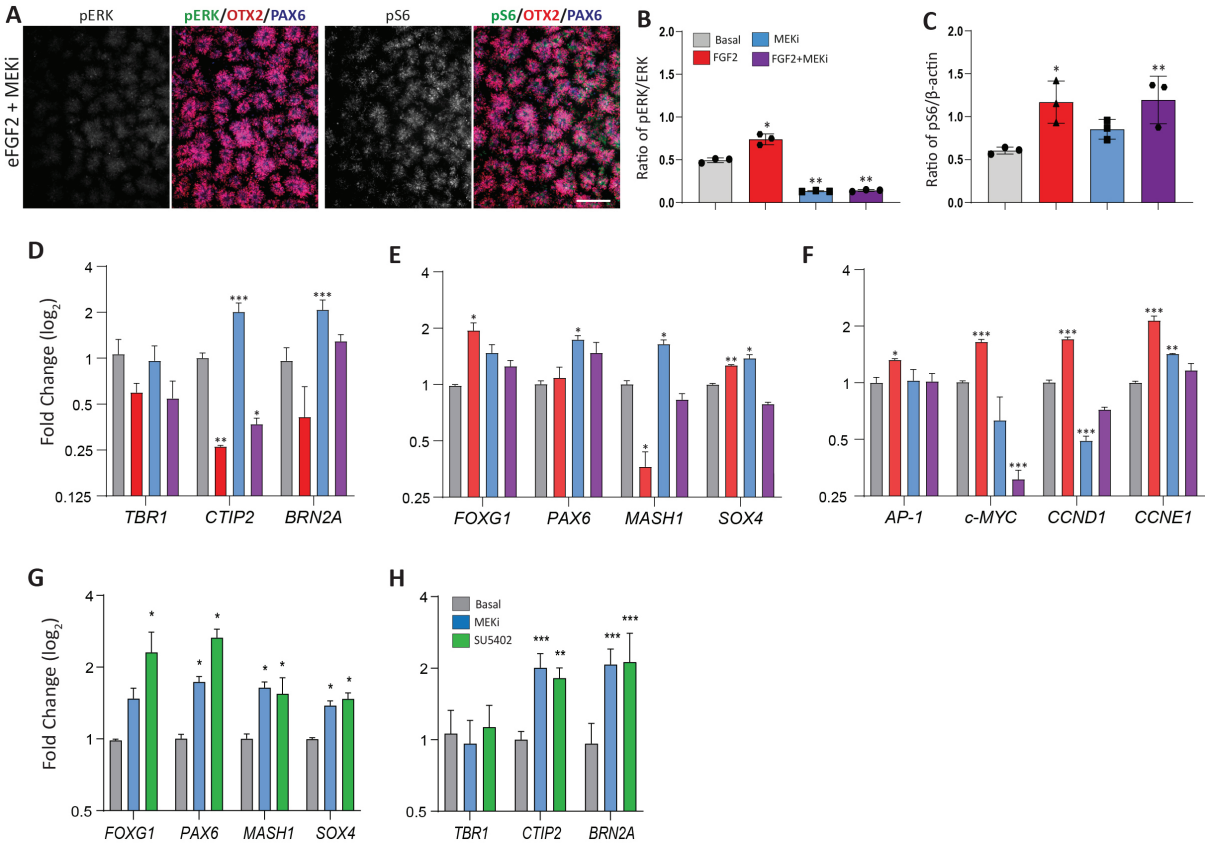
DIV	Group	T_C-T_S	T_S	T_S (% of T_C)	T_C	T_{G2}	T_M	T_{G1}	T_{G1} (% of T_C)
26	Basal	16.5	15.5	(48.4)	32.1	4.7	0.7	11.1	(34.7)
	eFGF2	15.6	12.7	(44.7)	28.3	5.4	0.4	9.9	(34.9)
	MEKi	16.8	17.1	(50.3)	33.9	4.9	0.8	11.1	(32.9)
35	Basal	18.3	21.1	(53.5)	39.4	5.8	0.4	12.1	(30.7)
	eFGF2	16.8	15.5	(47.9)	32.3	5.9	0.2	10.8	(33.4)
	MEKi	20.3	16.6	(44.9)	36.9	6.4	0.7	13.2	(35.9)



Supplementary Figure S6 – Cell cycle analysis of developing cortical progenitors

(A) Cell cycle values of cortical progenitors under basal, eFGF2 or MEKi conditions. (B) Plots of EdU+ cycling progenitors normalised to the growth fraction (GF) at D26 and D35. GF is defined as the maximal percentage of EdU+/Ki67+ of total Ki67+ proliferating cells and ranged between (85.5-96.1%). Arrow heads represent the average time taken to reach EdU saturation and represent the T_C-T_S interval. The x-intercept is T_S. Data is from 3 independent experiments in duplicate. (C) Plots of EdU+/PH3+ of total PH3+ and fitted sigmoidal curves at D26 and D35. The time taken to 50% is the average time from S-phase incorporation of EdU to mitosis and hence represents average G2. Data from 3 independent experiments in duplicate.

Supplementary Figure 7



Supplementary Figure S7 – Western blot and gene expression analyses of cortical progenitors after FGF/MAPK modulation.

(A) Immunofluorescence staining for pERK and pS6 in cortical progenitors treated with both FGF2 and MEKi. See Figure 3A for comparison to Basal and/or single-treatment conditions. (B-C) Western blot analysis following acute (24 hours) exposure to mediators of FGF/MAPK signalling pathway. ERK activity (B) was blocked upon MEK inhibition and could not be rescued by co-incubation with FGF2, while analysis of pS6 activity (C) revealed an FGF-mediated, and MEK-independent increase in pS6 signalling. Transcriptional changes (D-F) were assessed using qPCR hours after 5 days of eFGF2 or MEKi treatment. Gene families were stratified into groups investigating the influence of FGF/MAPK regulation on cortical layer (D), neurogenic (E) and cell cycle (F) genes. (G-H) Treatment of cortical progenitors with FGFR inhibitor SU5402 at D20 (and assessed at D26) phenocopied MEK inhibitor treatment, with comparable increases in neurogenic and laminar fate gene expression, showing a maturation to layer V (and UL) neurogenesis. Data represented as mean ± SEM, Two-way *ANOVA* with Dunnett's correction, *p<0.05, **p<0.01, ***p<0.001 and was obtained from at least three independent experiments. Scale bar is 100μm.

Supplementary Table 1. List of primers used in this study for qPCR.

Gene	Forward	Reverse
AP-1	AACAGGTGGCACAGCTTAAAC	CAACTGCTGCGTTAGCATGAG
ASCL1	CGCGGCCAACAAGAAGATG	CGACGAGTAGGATGAGACCG
BRN2A	CGGCGGTTTGCTCTATTC	ATGGTGTGGCTCATCGTG
CCNE1	GCCAGCCTTGGGACAATAATG	CTTGCACGTTGAGTTTGGGT
CCND1	GCCAGCCTTGGGACAATAATG	CTTGCACGTTGAGTTTGGGT
c-MYC	GTCAAGAGGCGAACACACAAC	TTGGACGGACAGGATGTATGC
CTIP2 (BCL11B)	TCACCCACGAAAGGCATCTGT	TGAAGGGCTGCTTGCATGTTG
CUX1	ACCATCGGCTTCTTCTACAC	TGGTCAGCGAACTTCTTGG
FOXG1	AGAAGAACGGCAAGTACGAGA	TGTTGAGGGACAGATTGTGGC
HES5	AGGCTGGAGAGGCGGCTAAG	TGGAAGGTGACACTGCGTTGG
HPRT1	CATTATGCTGAGGATTTGGAAAGG	CTTGAGCACACAGAGGGCTACA
NESTIN	AGCGTTGGAACAGAGGTTG	AGGAGGGTCCTGTACGTG
NOTCH1	ATCCTGATCCGGAACCGAG	CGTCGTGCCATCATGCAT
PAX6	CTGAGGAATCAGAGAAGACAGGC	ATGGAGCCAGATGTGAAGGAGG
SATB2	CCAAACACACCATCATCAAGTTC	GCAGCTCCTCGTCCTTATATTC
SOX4	GACCTGCTCGACCTGAACC	CCGGGCTCGAAGTTAAAATCC
TBR1	TCCCAGTGCCATGTTCCC	AACCCATTTGCCTCCTTGA

Supplementary Table 2. List of antibodies used in this study.

Antibody	Species	Source	Identifier
BRN2	Goat	Santa Cruz	Cat# sc-6029, RRID: AB_2167385
CTIP2	Rat	Abcam	Cat# AB18465, RRID: AB_2064130
GFAP	Rabbit	DAKO	Cat# Z0334, RRID: AB_10013382
Ki67	Rabbit	Thermo Fisher	Cat# RM-9106, RRID: AB_2341197
MAP2	Rabbit	Millipore	Cat# AB5622, RRID: AB_91939
OTX2	Goat	R&D Systems	Cat# AF1979, RRID: AB_2157172
PAX6	Mouse	DSHB	Cat# pax6-s, RRID: AB_528427
p44/42 MAPK	Rabbit	Cell Signaling Technology	Cat# 4370, RRID: AB_2315112
pS6	Rabbit	Cell Signaling Technology	Cat# 2211, RRID: AB_331679
PH3	Rat	Abcam	Cat# AB10543, RRID: AB_2295065
RFP	Rabbit	Rockland Labs	Cat# 600-401-379, RRID: AB_2209751
SATB2	Mouse	Abcam	Cat# ab51502, RRID: AB_882455
SOX2	Goat	R&D Systems	Cat# AF2018, RRID: AB_355110
TBR1	Rabbit	Abcam	Cat# AB31940, RRID: AB_2200219
TBR2	Rabbit	Abcam	Cat# AB23345, RRID: AB_778267
TUJ1	Mouse	Promega	Cat# G7121, RRID: AB_430874
VIMENTIN	Mouse	Millipore	Cat# MAB3400, RRID: AB_94843
ZO-1	Mouse	Thermo Fisher	Cat# 339100, RRID: AB_2533147