SUPPLEMENTARY INFORMATION

Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses

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Nature Neuroscience: doi:10.1038/nn.3041

Supplementary Movie (available online): Interkinetic nuclear migration, apical and basal mitoses in cortical rosettes

Time-lapse movie of the hESC-derived cortical rosette shown in Figure 2. In the initial frames the blue arrow indicates the apical progenitor and the yellow arrowhead indicates the basal progenitor shown in Figure 2. Frames were collected at 15 minute intervals.

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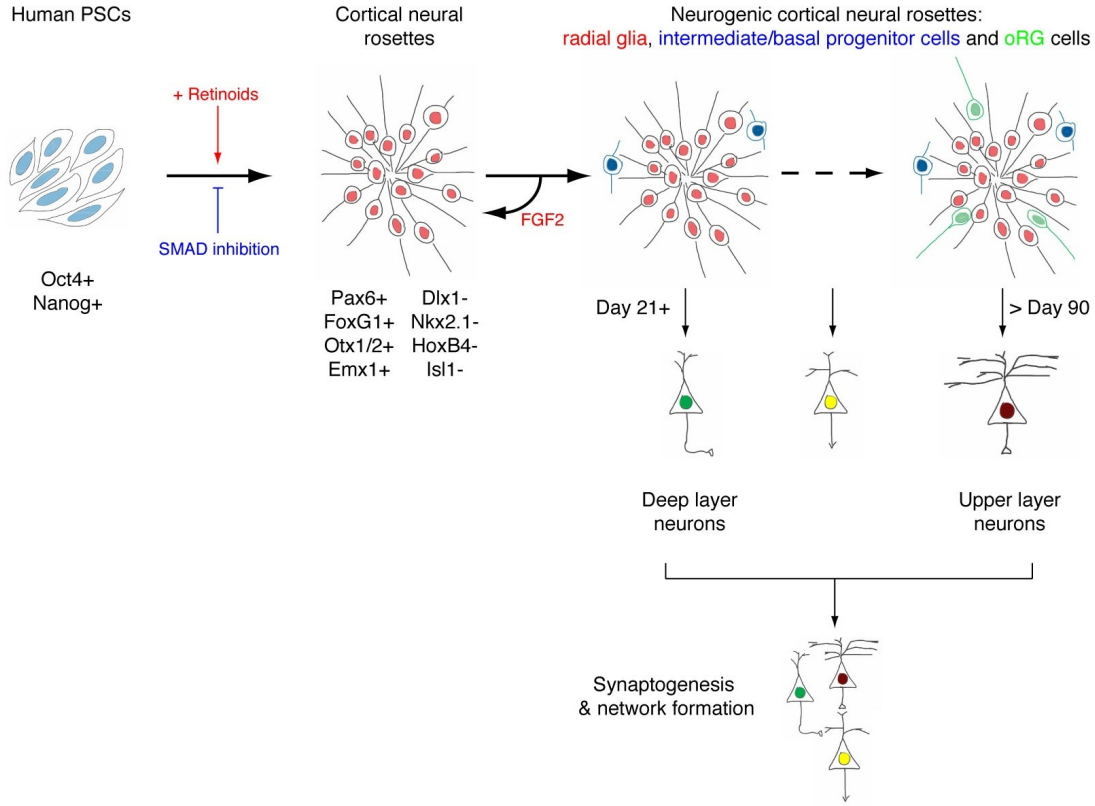
Supplementary Figure 1: Directed differentiation of human PSCs to cerebral cortex

Schematic of the differentiation procedure reported here: a combination of dual SMAD inhibition, combined with retinoids, differentiates PSCs to cortical stem and progenitor cells that can be expanded/maintained with FGF2. Within cortical rosettes three populations of stem/progenitor cells are generated: Pax6-expressing radial glia, Tbr2-expressing basal progenitor cells and Pax6+/Tbr2- outer radial glia (oRG) cells that have a basal process and lack an apical process that projects to the rosette lumen. Removal of FGF2 allows neurogenesis to take place. Cortical stem cells follow the same developmental progression in the genesis of cortical cell types as occurs in vivo, with deep layer neurons generated early, beginning around day 21 and upper layer neurons generated last, continuing beyond day 90. Cortical projections of all layers are generated from PSCs and form networks of functional excitatory, glutamatergic synapses.



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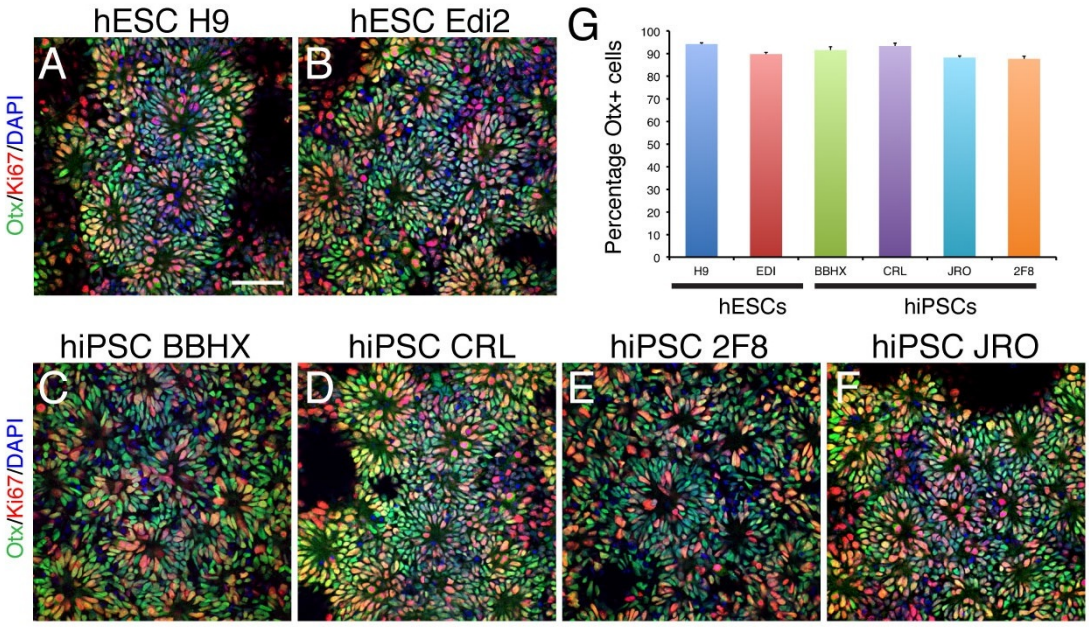
Supplementary Figure 2: Robust cortical stem cell differentiation from two independent hESC and four independent hiPSC lines.

A-F. Representative images of Otx immunofluorescence at day 15 of differentiation in human ESC- (A, B) and iPSC- (C-F) derived neural stem cell cultures.
G. Percentage of Otx+ cells per culture on day 15 are shown for H9 and Edi2 hESCs, and for BBHX, CRL, 2F8 and JRO hiPSCs.



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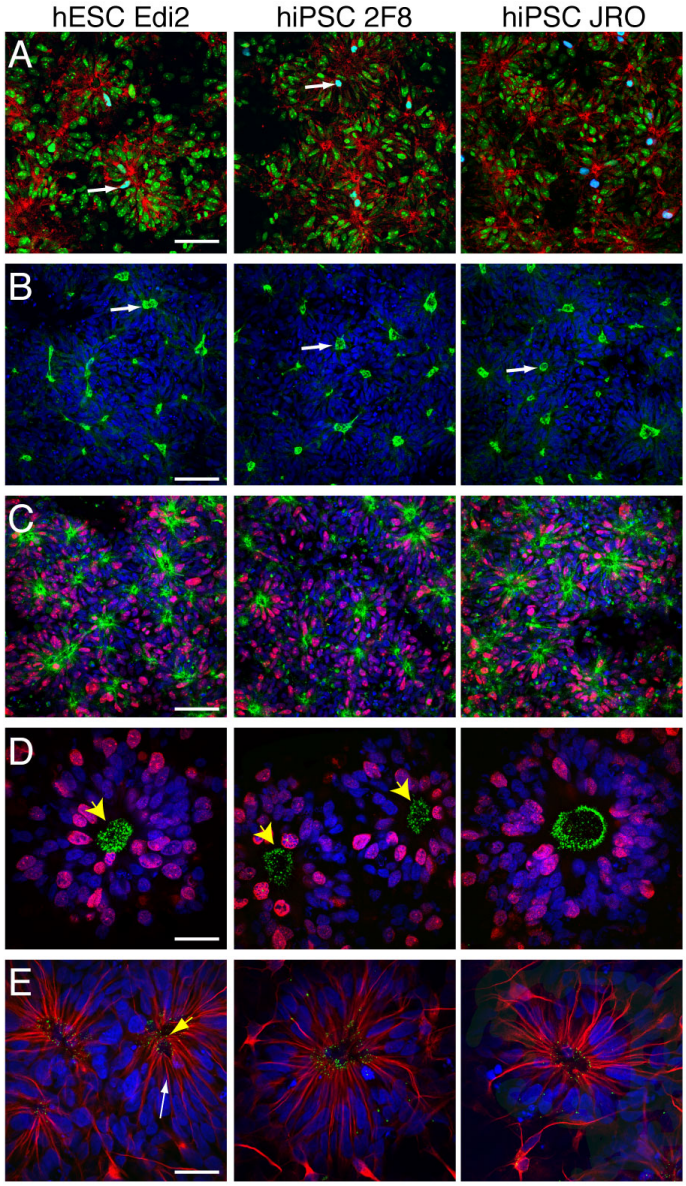
Supplementary Figure 3: Human ESC and iPSC-derived cortical stem/progenitor cells form a polarised neuroepithelium.

All hESC and hiPSC lines reported here differentiate efficiently to polarized neuroepithelial rosettes.

A.hESC (Edi2) and hiPSC (2F8 and JRO)-derived cortical stem and progenitor cells form polarized neuroepithelial rosettes of proliferating cells (Ki67) in which many mitoses (phospho-histone H3) take place near a central lumen (white arrows) formed from the apical surfaces of the neuroepithelial cells (CD133/Prominin1, red in all panels).

B-D. In addition to CD133/Prominin1, cortical rosettes localize aPKC (B), Transferrin receptor (TfR, C) and ASPM (D) to the apical, luminal pole of the neuroepithelial cells. Scale bars A-C, 100 µm; D, 25 µm.

E. Centrosomes (detected by CEP135 immunostaining) are located apically in hiPSC-derived cortical rosettes, as they are in the neuroepithelium *in vivo.* Acetylated tubulin (white arrows) extends throughout the cortical stem/progenitor cells. Scale bars in all panels, 25 µm.



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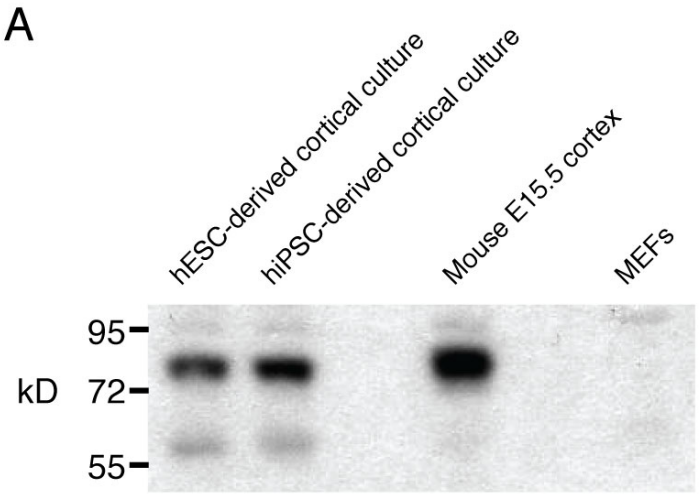
Supplementary Figure 4: Western blot to demonstrate the specificity of the anti-Tbr1 polyclonal antibody

A, B. The predicted molecular weight of human Tbr1 is 74 kD (B), and a single major band of that size is detected in cortical cultures derived from human ES and iPS cells, and in developing mouse cerebral cortex (embryonic day 15.5), but not in mouse embryonic fibroblasts (MEF), demonstrating the specificity of the antibody.

A



B

>gi|5730081|ref|NP_006584.1| T-box brain protein 1 [Homo sapiens]

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        10         20         30         40         50         60
MQLEHCLSPS IMLSKKFLNV SSSYPHSGGS ELVLHDHPII STTDNLERSS PLKKITRGMT

        70         80         90        100        110        120
NQSDTDNFPD SKDSPGDVQR SKLSPVLDGV SELRHSFDGS AADRYLLSQS SQPQSAATAP

       130        140        150        160        170        180
SAMFPYPGQH GPAHPAFSIG SPSRYMAHHP VITNGAYNSL LSNSSPQGYP TAGYPYPQQY

       190        200        210        220        230        240
GHSYQGAPFY QFSSTQPGLV PGKAQVYLCN RPLWLKFHRH QTEMIITKQG RRMFPFLSFN

       250        260        270        280        290        300
ISGLDPTAHY NIFVDVILAD PNHWRFQGGK WVPCGKADTN VQGNRVYMHP DSPNTGAHWM

       310        320        330        340        350        360
RQEISFGKLK LTNNKGASNN NGQMVVLQSL HKYQPRLHVV EVNEDGTEDT SQPGRVQTFT

       370        380        390        400        410        420
FPETQFIAVT AYQNTDITQL KIDHNPFAKG FRDNYDTIYT GCDMDRLTPS PNDSPRSQIV

       430        440        450        460        470        480
PGARYAMAGS FLQDQFVSNY AKARFHPGAG AGPGPGTDRS VPHTNGLLSP QQAEDPGAPS

       490        500        510        520        530        540
PQRWFVTPAN NRLDFAASAY DTATDFAGNA ATLLSYAAAG VKALPLQAAG CTGRPLGYYA

       550        560        570        580        590        600
DPSGWGARSP PQYCGTKSGS VLPCWPNSAA AAARMAGANP YLGEEAEGLA AERSPLPPGA

       610        620        630        640        650        660
AEDAKPKDLS DSSWIETPSS IKSIDSSDSG IYEQAKRRRI SPADTPVSES SSPLKSEVLA

       670        680
QRDCEKNCAK DISGYYGFYS HS

Theoretical pI/Mw: 6.89 / 74053.26
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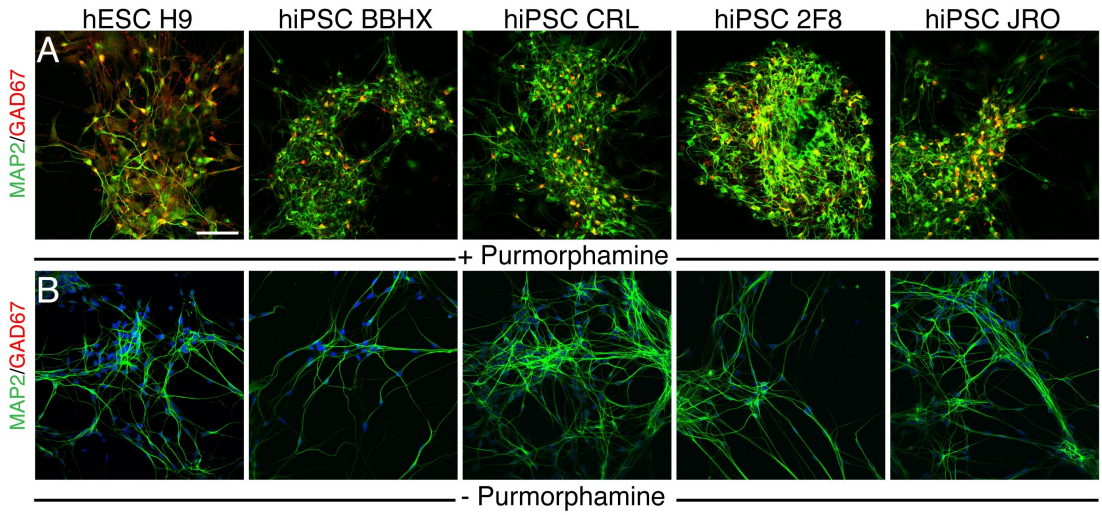
Supplementary Figure 5: Cortical rosettes can be ventralised to generate GABAergic interneurons.

A. GABAergic (GAD67+) neurons (MAP2+) generated by hESC and hiPSC-derived neural stem cells following treatment with 10 µm purmorphamine to ventralise the rosettes.
B. No GAD67+ GABAergic neurons were found in control, parallel cultures from which purmorphamine was omitted.



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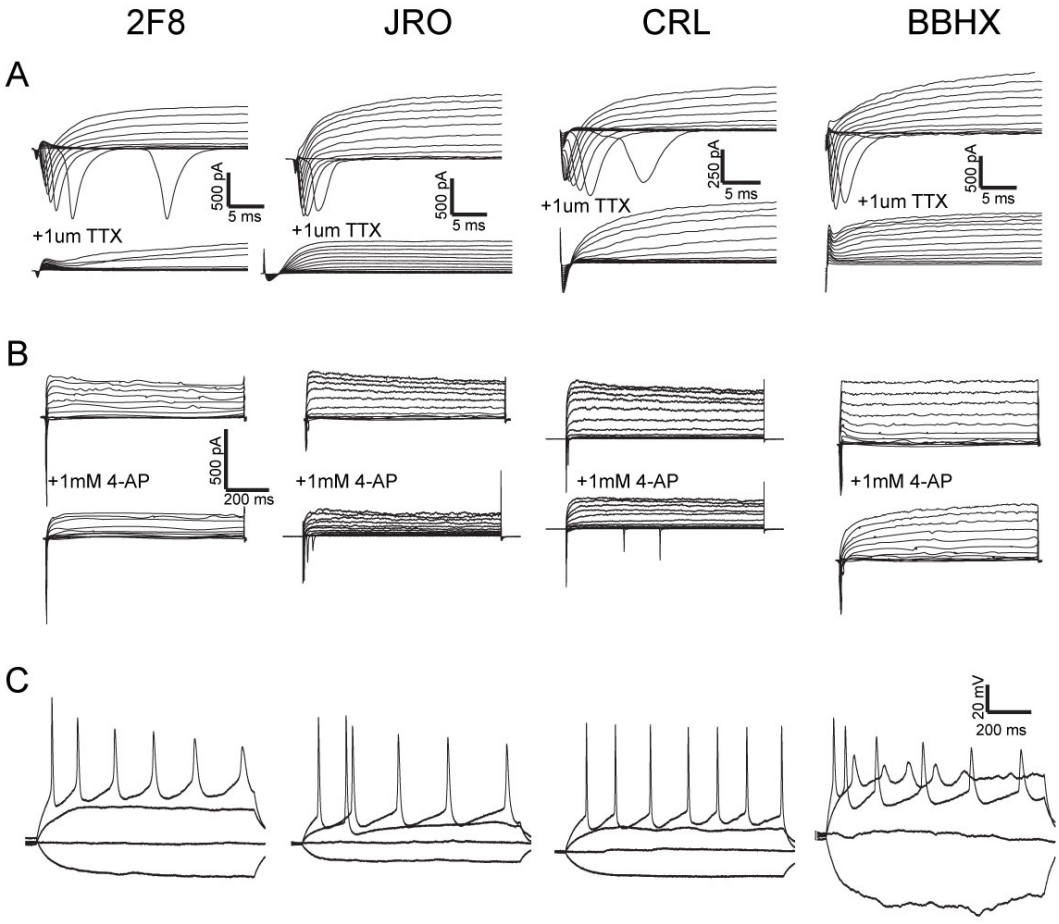
Supplementary Figure 6: Single neuron electrophysiological properties of iPSC-derived cortical neurons.

A, B. Voltage-gated sodium (A) and potassium (B) channels in cortical neurons generated from four different hiPSC lines. Current responses to families of step depolarizations from a holding potential of -80 mV to +4- mV are superimposed. In A, fast-activating and inactivating inward sodium currents are completely blocked by applying TTX. In B, 4-AP blocks a fast-activating transient fraction of outward K current.
C. hiPSC-derived cortical neurons develop robust regular-spiking behaviour in response to current injection.



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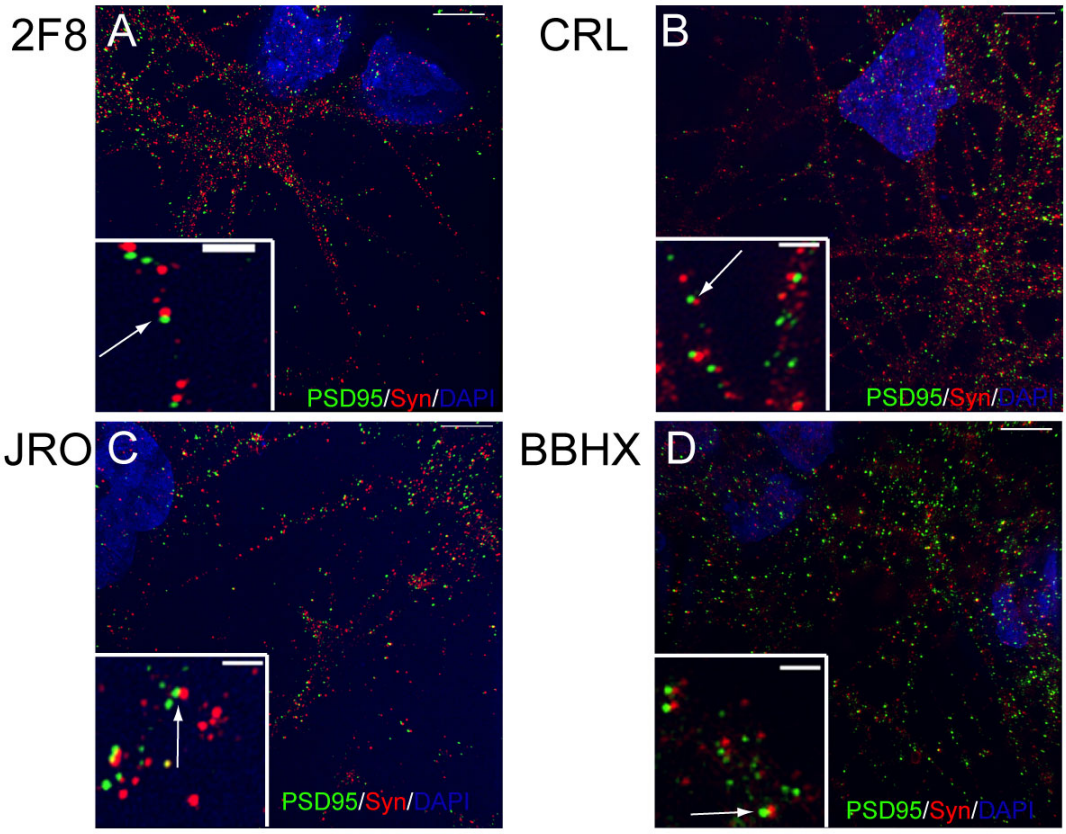
Supplementary Figure 7: Super-resolution microscopy to visualize synapses in iPSC-derived cortical neuron cultures.

A-D Super-resolution microscope images of synapses formed from cortical neurons derived from four different hiPSC lines. Physical synapses were identified by juxtaposition of pre- and post-synaptic protein complexes, in this case synaptophysin and PSD95.



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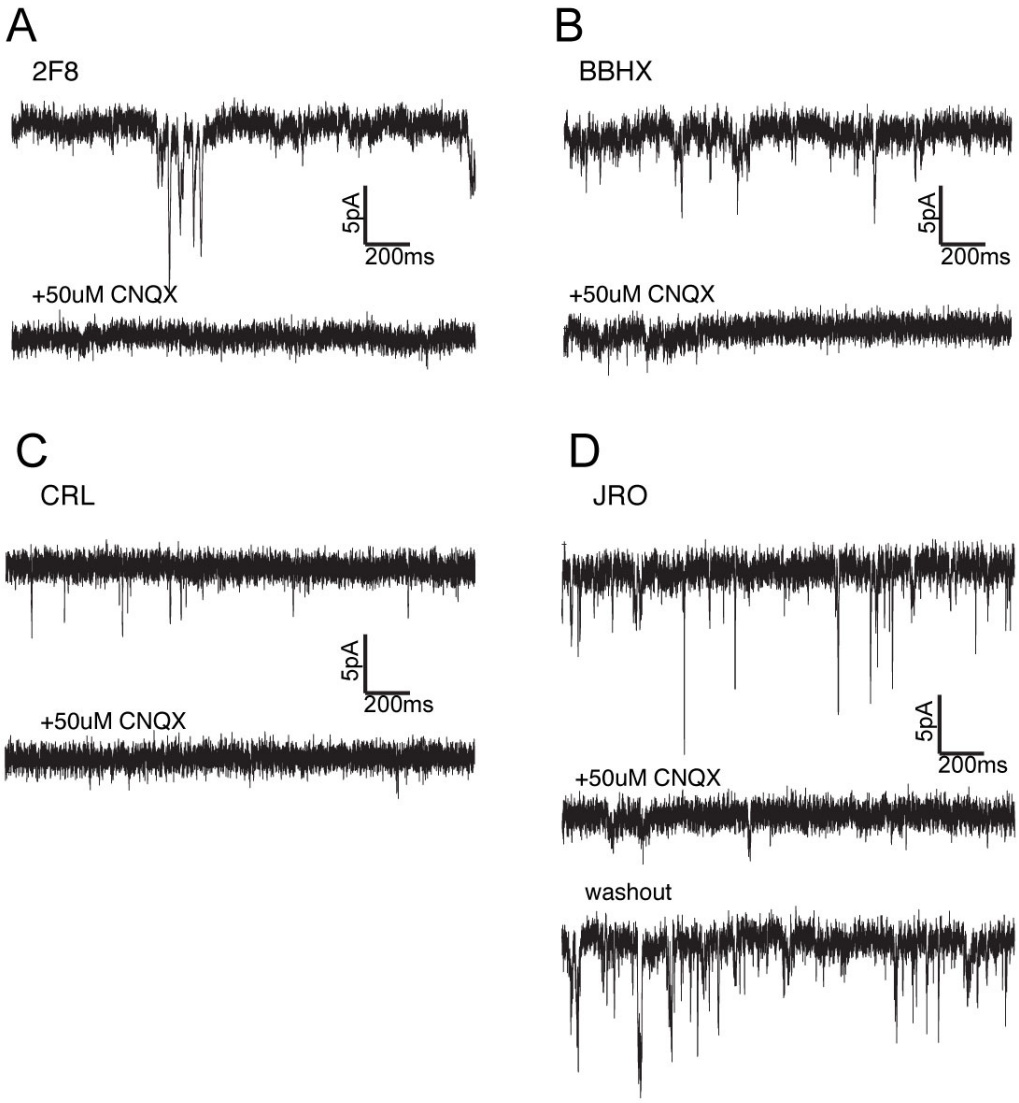
Supplementary Figure 8: Functional excitatory synapses in iPSC-derived cortical neuron cultures.

A-D. Detection of mEPSCs in whole cell recordings of hiPSC-derived cortical neurons, from four different iPSC lines. In each case, the AMPA receptor antagonist CNQX blocked the appearance of mEPSCs.



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