Expanded View Figures

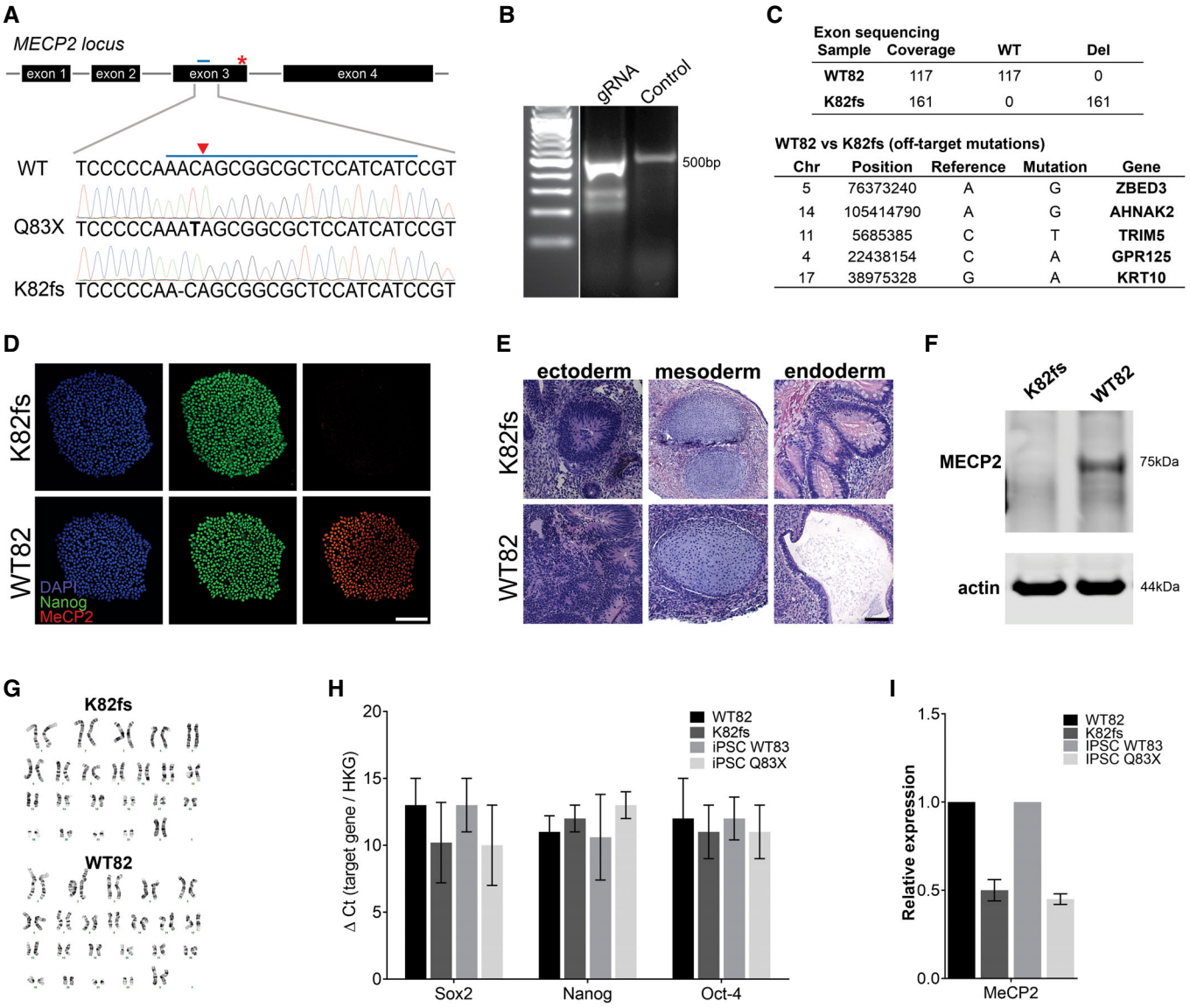

Figure EV1. Generation of *MECP2*-KO pluripotent stem cells.

A Mutations resulting in loss of functional MeCP2 protein shown by Sanger sequencing. CRISPR guide RNA is depicted as a blue bar over WT sequence and the cut site as a red triangle.

B CRISPR guide RNA to generate K82 frameshift mutation.

C Identification of off-target mutations by exome sequencing.

D–I *MECP2*-KO was successfully generated and did not affect pluripotency, as confirmed by immunochemistry (D), teratoma assay (E), Western blot (F), karyotype (G), and RT–qPCR (H, I). Experiments were performed in triplicate; data are presented as mean ± s.e.m. Scale bar = 200 μm.

Figure EV2. *MECP2*-KO neurons exhibit defects in spine-like morphology.

A Schematic showing neuronal differentiation from human PSCs. Scale bar = 200 μm.

B Neuronal markers immunostaining of 28-days *MECP2*-KO and control neurons. Scale bar = 50 μm.

C No difference in the resultant proportions of neurons or glia was observed between 28-days *MECP2*-KO and control neural differentiations. Similar results were observed for the proportions of glutamatergic and GABAergic neurons. Experiments were performed in triplicates.

D 28-days *MECP2*-KO neurons had decreased soma area and spine number (WT83/Q83X cell lines were used; $N = 13$ neurons per genotype, Student's t-test, $t_{24} = 5.03$, $^{***}P < 0.0001$). Left: representative images of Syn::GFP + neurons; Right: neuronal feature quantification. Scale bar = 20 μm.

E Observation over time showed decreased spine stability ($^{*}P < 0.01$), but spine motility ($P = 0.17$), spine formation ($P = 0.26$), and spine length ($P = 0.28$) were unchanged in *MECP2*-KO neurons. Scale bar = 5 μm. WT83/Q83X cell lines were used, $N = 10$-15 neurons/condition. Analyses were two-sided Student's t-tests. Top: representative images of neuronal spine dynamics at 5-min intervals; arrows denote representative spines. Bottom: quantification of features of neuronal spine dynamics.

Data information: Data are presented as mean ± s.e.m.

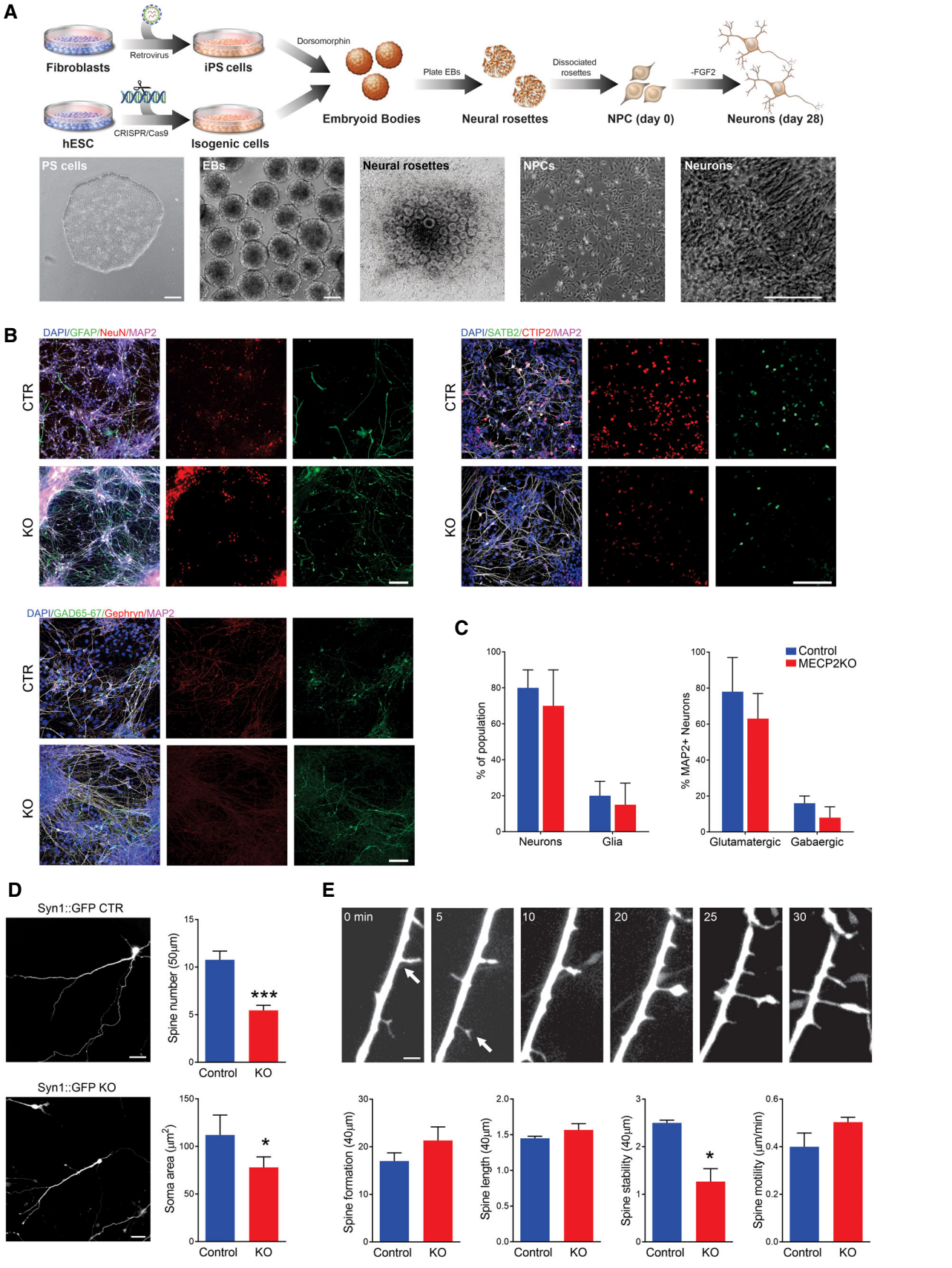

Figure EV2.

Figure EV3. RT–PCR array and single-cell RT–qPCR analyses.

A, B RT–PCR array revealed that differences in genetic expression showed variation between control and *MECP2*-KO NPCs and neurons, as depicted on volcano plots (A) and heatmap (B). Each heatmap column is one gene.

C–K Single-cell RT–qPCR analysis likewise showed differences in gene expression throughout neuronal differentiation due to *MECP2*-KO that appear to involve synaptic function. 28-days WT83/Q83X and WT82/K82fs neurons were used. Principal component analysis (C) of control and *MECP2*-KO NPCs (day 0 of same cell lines) and comparative expression of select genes between genotypes (D). Principal component analysis shows differences in expression between NPCs and neurons within each genotype (E) and differences in neuronal gene expression between genotypes (F). Heatmap shows differences in expression of particular genes (columns; G), and schematic denotes single-cell qPCR of individual markers (H). Number (H) and graphical depiction (J) of differentially expressed genes between genotypes. Table presents differentially expressed genes (K).

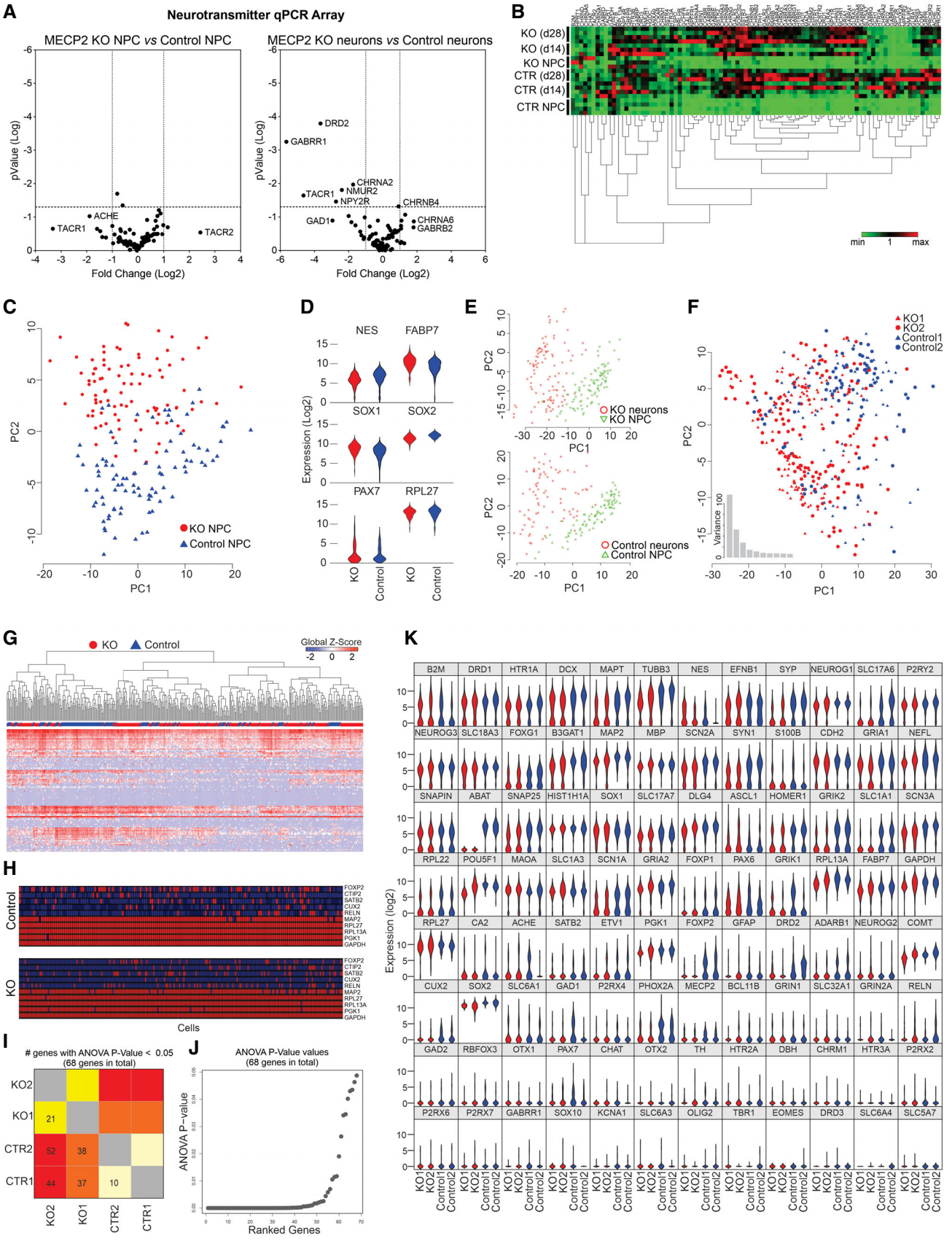
A
Neurotransmitter qPCR Array
MECP2 KO NPC vs Control NPC
MECP2 KO neurons vs Control neurons
pValue (Log)
Fold Change (Log2)
ACHE
TACR1
TACR2
DRD2
GABRR1
CHRNA2
NMUR2
TACR1
NPY2R
CHRNB4
GAD1
CHRNA6
GABRB2
B
KO (d28)
KO (d14)
KO NPC
CTR (d28)
CTR (d14)
CTR NPC
min 1 max
C
PC2
PC1
KO NPC
Control NPC
D
NES
FABP7
SOX1
SOX2
PAX7
RPL27
Expression (Log2)
KO
Control
E
PC2
PC1
KO neurons
KO NPC
Control neurons
Control NPC
F
KO1
KO2
Control1
Control2
Variance
G
KO
Control
Global Z-Score
H
Control
KO
FOXP2
CTIP2
SATB2
CUX2
RELN
MAP2
RPL27
RPL13A
PGK1
GAPDH
Cells
I
genes with ANOVA P-Value < 0.05
(68 genes in total)
KO2
KO1
CTR2
CTR1
J
ANOVA P-Value values
(68 genes in total)
ANOVA P-value
Ranked Genes
K
B2M
DRD1
HTR1A
DCX
MAPT
TUBB3
NES
EFNB1
SYP
NEUROG1
SLC17A6
P2RY2
NEUROG3
SLC18A3
FOXG1
B3GAT1
MAP2
MBP
SCN2A
SYN1
S100B
CDH2
GRIA1
NEFL
SNAPIN
ABAT
SNAP25
HIST1H1A
SOX1
SLC17A7
DLG4
ASCL1
HOMER1
GRIK2
SLC1A1
SCN3A
RPL22
POU5F1
MAOA
SLC1A3
SCN1A
GRIA2
FOXP1
PAX6
GRIK1
RPL13A
FABP7
GAPDH
RPL27
CA2
ACHE
SATB2
ETV1
PGK1
FOXP2
GFAP
DRD2
ADARB1
NEUROG2
COMT
CUX2
SOX2
SLC6A1
GAD1
P2RX4
PHOX2A
MECP2
BCL11B
GRIN1
SLC32A1
GRIN2A
RELN
GAD2
RBFOX3
OTX1
PAX7
CHAT
OTX2
TH
HTR2A
DBH
CHRM1
HTR3A
P2RX2
P2RX6
P2RX7
GABRR1
SOX10
KCNA1
SLC6A3
OLIG2
TBR1
EOMES
DRD3
SLC6A4
SLC5A7
Expression (log2)
KO1
KO2
Control1
Control2

Figure EV3.

Figure EV4. Drug treatment of *MECP2*-KO and control neurons.

A Representative Western blot images after *MECP2*-KO drug treatment. Samples run at the same time in different gels.
B Analysis of treatment effect on quantities of the astrocytic marker GFAP and neuronal marker MAP2. Experiments were performed in triplicate.
C Full depiction of synaptic puncta following drug treatment. Scale bar = 5 μm.
D Synaptic puncta co-localization following drug treatment of 6-week control neurons (WT83/Q83X cell lines were used; $N = 8$ neurons per condition; one-way ANOVA, $F_{6,49} = 10.94$, $^{*}P < 0.05$). $^{**}P < 0.01$, $^{***}P < 0.001$. *Z* scores of control untreated vs. compound: Nefiracetam = 1.010; PHA 543613 = 0.282; Acamprosate = 0.175; GR73632 = 1.010; IGF-1 = 3.231; Hyperforin = 2.639).
E, F Calcium imaging showed increasing spike frequency with time (E) that was tractable to manipulators of synaptic function (F). Experiments were performed in triplicate.
G Representative image of MEA activity of control and *MECP2*-KO neurons.

Data information: Data are presented as mean ± s.e.m.

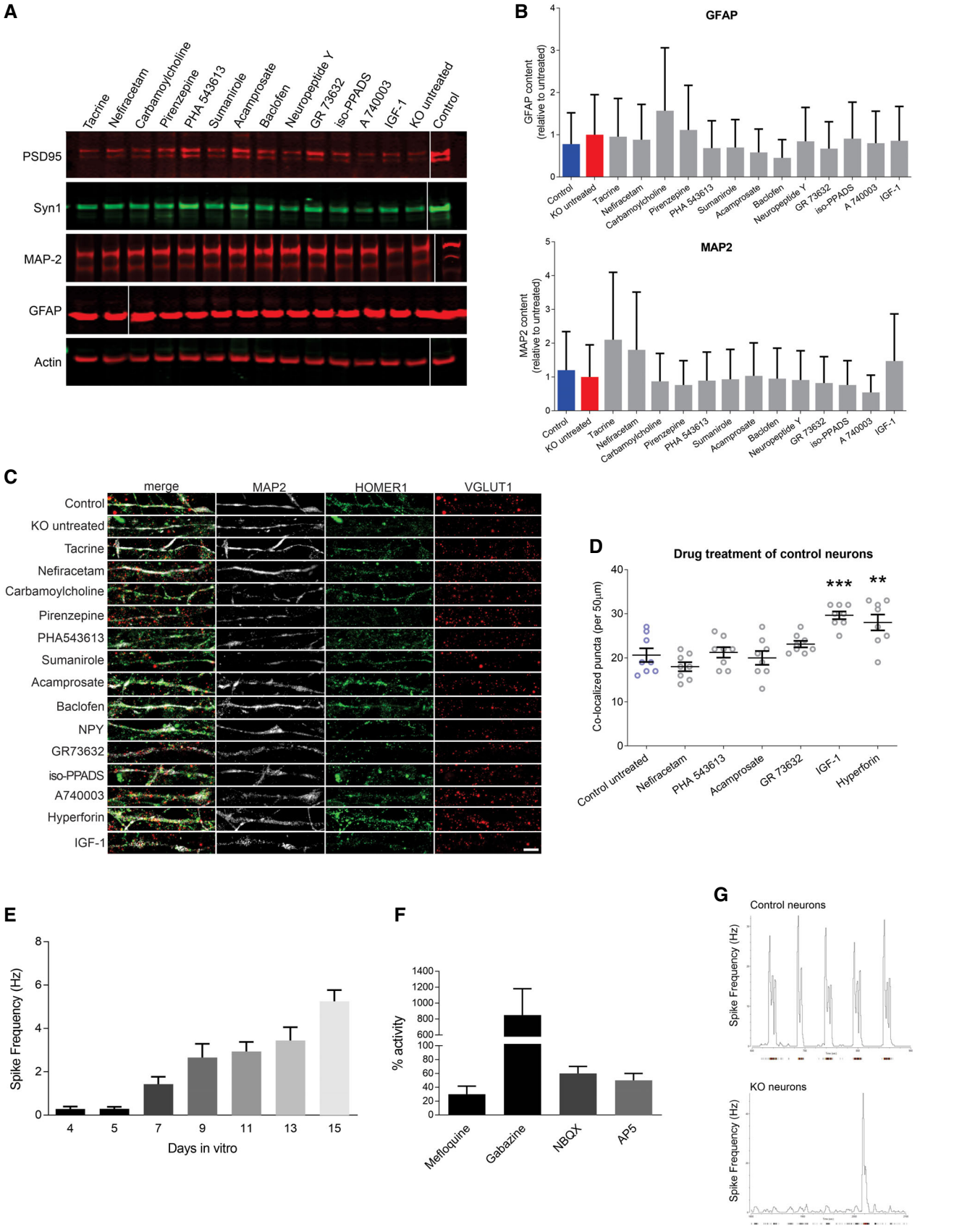
A
Tacrine
Nefiracetam
Carbamoylcholine
Pirenzepine
PHA 543613
Sumanirole
Acamprosate
Baclofen
Neuropeptide Y
GR 73632
iso-PPADS
A 740003
IGF-1
KO untreated
Control
PSD95
Syn1
MAP-2
GFAP
Actin
B
GFAP
GFAP content (relative to untreated)
MAP2
MAP2 content (relative to untreated)
C
merge
MAP2
HOMER1
VGLUT1
Control
KO untreated
Tacrine
Nefiracetam
Carbamoylcholine
Pirenzepine
PHA543613
Sumanirole
Acamprosate
Baclofen
NPY
GR73632
iso-PPADS
A740003
Hyperforin
IGF-1
D
Drug treatment of control neurons
Co-localized puncta (per 50µm)
***
**
Control untreated
Nefiracetam
PHA 543613
Acamprosate
GR 73632
IGF-1
Hyperforin
E
Spike Frequency (Hz)
Days in vitro
F
% activity
Mefloquine
Gabazine
NBQX
AP5
G
Control neurons
KO neurons
Spike Frequency (Hz)

Figure EV4.

Figure EV5. Cellular and molecular characterization of human cortical organoids.

A Schematic of the protocol used to generate cortical organoids. Scale bar = 200 μm.

B Reproducibility of organoid size at 3 weeks of maturation (*N = 20* independent experiment, 7 different cell lines).

C, D Gene expression of specific neurotransmission markers of treated vs. untreated cortical organoids ($N = 8$ samples of treated organoids and 8 samples of control organoids, ~10–15 organoids pooled per sample). Boxplot: center band of the box is median $\log_2$ normalized expression and edges are 25^{th} and 75^{th} percentiles; whiskers are minimum and maximum $\log_2$ normalized expression values.

E, F Representative images of quantification of Synapsin1 puncta. Scale bar = 50 μm. SYN1 puncta mask was made using ImageJ to access the number and area of the puncta.

G Nefiracetam and PHA 543613 treatment did not rescue *MECP2*-KO neuronal morphology, for either the number of neurites (Kruskal–Wallis test, $P = 0.011$; Dunn's multiple comparisons test vs. KO untreated: control, $^{**}P = 0.01$ and $Z = 3.13$; Nefiracetam, $P = 0.49$ and $Z = 1.74$; PHA 543613, $P > 0.99$ and $Z = 0.87$; WT83/Q83X and WT82/K82fs cell lines; $N = 5$–10 neurons per condition) or the total neurite length (one-way ANOVA, $F_{3,21} = 9.62$, $P = 0.0003$; Dunnett's multiple comparisons test vs. KO untreated: control, $^{**}P = 0.002$ and $Z = 3.08$; Nefiracetam, $P = 0.89$ and $Z = 0.64$; PHA 543613, $P = 0.99$ and $Z = 0.17$; WT83/Q83X and WT82/K82fs cell lines; $N = 5$–10 neurons per condition) and, nearly uniformly, did not rescue nuclei size in neurons (one-way ANOVA, $F_{3,221} = 26.04$, $P < 0.0001$; Dunnett's multiple comparisons test vs. KO untreated: control, $^{***}P < 0.0001$ and $Z = 6.37$; Nefiracetam, $P = 0.91$ and $Z = 0.39$; PHA 543613, $P = 0.19$ and $Z = 1.74$; WT83/Q83X and WT82/K82fs cell lines; $N = 45$ nuclei per condition), neurospheres (one-way ANOVA, $F_{5,264} = 5.579$, $P < 0.0001$; Dunnett's multiple comparisons test vs. CTR/KO untreated: control, $^{***}P < 0.0001$ and $Z = 3.782$; KO untreated, $P = 0.51$ and $Z = 1.066$; Nefiracetam, $P = 0.23$ and $Z = 1.35$; PHA 543613, $P = 0.99$ and $Z = 0.05$; Nefi + PHA, $P = 0.24$ and $Z = 1.012$; WT83/Q83X cell lines; $N = 45$ nuclei per condition), or cortical organoids (one-way ANOVA, $F_{3,281} = 6.594$, $P = 0.0003$; Dunnett's multiple comparisons test vs. KO untreated: control, $^{**}P = 0.009$ and $Z = 2.38$; Nefiracetam, $^{**}P = 0.01$ and $Z = 3.05$; PHA 543613, $P = 0.98$ and $Z = 0.30$; WT83/Q83X and WT82/K82fs cell lines; $N = 45$-90 nuclei per condition). Spine density was rescued or partially rescued by the compounds (one-way ANOVA, $F_{3,63} = 8.449$, $P < 0.0001$; Dunnett's multiple comparisons test vs. KO untreated: control, $^{***}P < 0.0001$ and $Z = 4.35$; Nefiracetam, $P = 0.16$ and $Z = 2.01$; PHA 543613, $^{***}P = 0.001$ and $Z = 3.24$; WT83/Q83X and WT82/K82fs cell lines; $N = 11$–28 neurons per condition). Note that statistical comparisons are presented relative to KO untreated or, for mosaic neurospheres, CTR/KO untreated.

Data information: Data are presented as mean ± s.e.m.

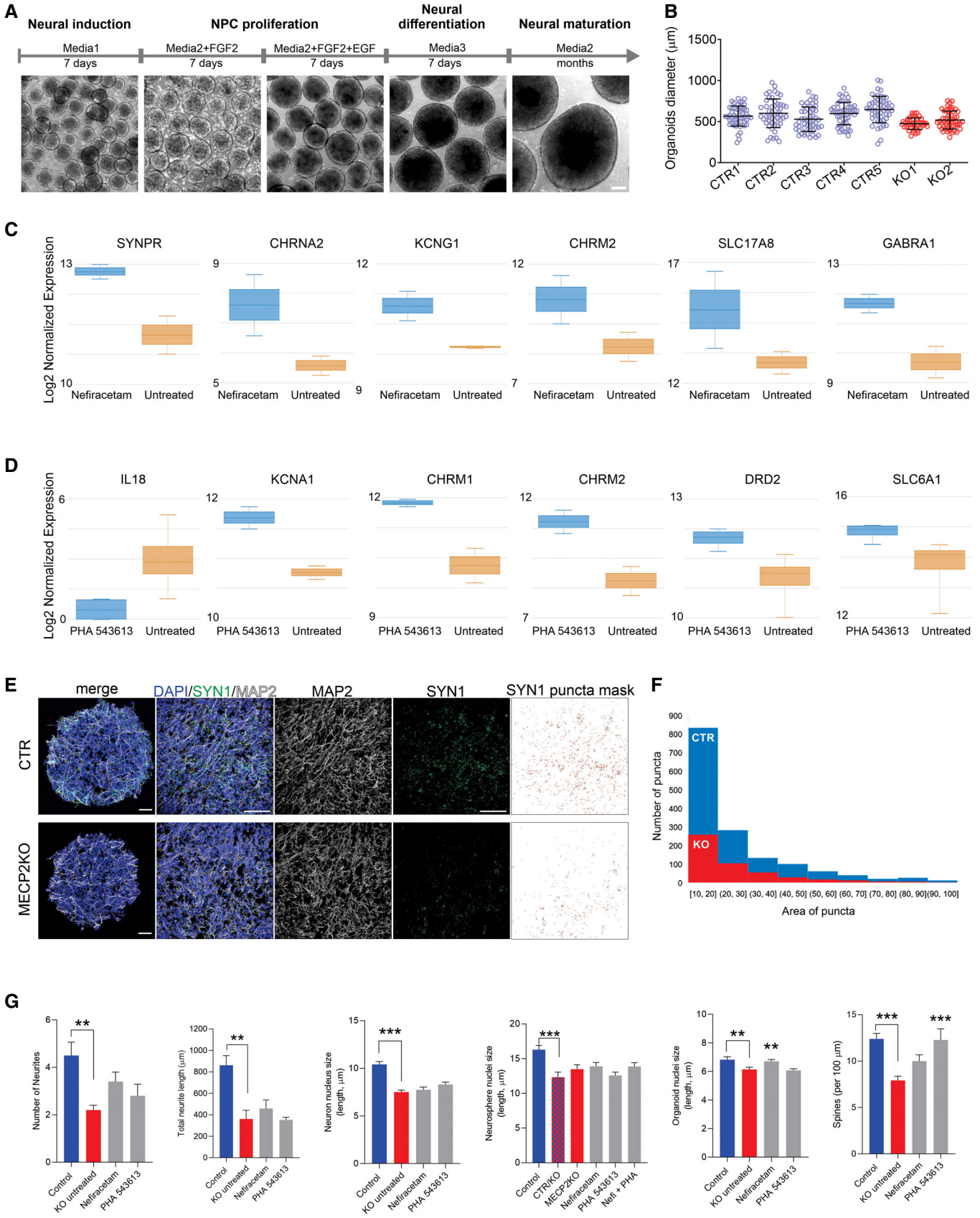
A
Neural induction
NPC proliferation
Neural differentiation
Neural maturation
Media1
7 days
Media2+FGF2
7 days
Media2+FGF2+EGF
7 days
Media3
7 days
Media2
months
B
Organoids diameter (µm)
1500
1000
500
0
CTR1
CTR2
CTR3
CTR4
CTR5
KO1
KO2
C
Log2 Normalized Expression
SYNPR
CHRNA2
KCNG1
CHRM2
SLC17A8
GABRA1
Nefiracetam
Untreated
D
Log2 Normalized Expression
IL18
KCNA1
CHRM1
CHRM2
DRD2
SLC6A1
PHA 543613
Untreated
E
merge
DAPI/SYN1/MAP2
MAP2
SYN1
SYN1 puncta mask
CTR
MECP2KO
F
Number of puncta
CTR
KO
[10, 20] (20, 30] (30, 40] (40, 50] (50, 60] (60, 70] (70, 80] (80, 90](90, 100]
Area of puncta
G
Number of Neurites
Total neurite length (µm)
Neuron nucleus size (length, µm)
Neurosphere nuclei size (length, µm)
Organoid nuclei size (length, µm)
Spines (per 100 µm)
Control
KO untreated
Nefiracetam
PHA 543613
CTR/KO
MECP2KO
Nefi + PHA

Figure EV5.