Supplemental Figure Legends

Figure S1. Generation of iPSCs derived from RTT patients' fibroblasts carrying distinct mutations in the MeCP2 gene; related to Figure 1. (A), Morphology of fibroblasts before retroviral infection. (B), Aspect of iPSCs colonies growing in the absence of feeder layer. Colonies are compact and have well-defined borders. Cells display high nucleus-to-cytoplasm ratio and are morphologically similar to hESCs. (C), Representative immunofluorescence analysis of RTT-iPSC clones. Expression of pluripotent markers such as Nanog and Tra-1-81 is observed. Bar = 100 μm. (D), Hierarchical clustering and correlation coefficients of microarray profiles of triplicate WT Fibroblasts, RTT Fibroblasts, WT-iPSC clone 1, WT-iPSC clone 2, RTT-iPSC clones 15 and 18 (1155del32), RTT-iPSC clones 1 and 2 (Q244X) and the hESC line HUES6. Color bar indicates the level of correlation (from 0 to 1), with color bar reporting log2 normalized expression values (green/red indicates high/low relative expression). (E), Reprogrammed iPSCs showed expressions similar to hESC-enriched genes (Lin28, CXADR, Nanog and PTRZ1; black bars) and showed distinct differences from fibroblast-enriched genes (GREM1, MMP1, DKK1 and PTX3; white bars). (F), RT-PCR from reprogrammed iPSCs showed endogenous expressions of hESC-enriched genes (Oct4, Sox2 and Nanog) but not from a fibroblast-enriched gene (Msx1).

Figure S2. Neuronal differentiation from individual WT and RTT-iPSC clones; related to Figure 2. Clones from WT and RTT-iPSCs were differentiated into neurons for approximately 1 month. (A), Neurons were stained with the Map2 neuronal marker. (B), Neurons were infected with a lentiviral vector expressing the DsRed reporter under the control of the Synapsin promoter region. (C), Inhibitory neurons were revealed in the cultures after staining with anti-GABA antibody. Each bar represents 3 independent experiments for each individual clone. Data shown as mean ± s.d.m.

Figure S3. Androgen receptor analysis; related to Figure 3. Example of X-inactivation analysis using the X-linked androgen receptor locus for the RTT-1155del32 C15 genomic DNA. After the PCR, 2 different-sized amplicons were detected (different peaks) and digested with a methylation-sensitive restriction enzyme (*Hpa*II). The PCR using undigested DNA shows if two distinct alleles are present and also allows a correction factor due to the advantage on the amplification of the smaller allele. When the template DNA is digested, amplification occurs if the restriction sites are methylated. If the site is unmethylated, digestion will occur between the flanking oligonucleotides and amplification will not be possible. The peak areas after *Hpa*II restriction digestion of genomic DNA are used to distinguish each parental X chromosome. (A), When random inactivation is present, the maternal and paternal alleles are represented at similar proportions. (B), In contrast, in a condition where non-random inactivation is present, the more commonly inactive allele will be preferentially amplified and this will be detected by a stronger peak. (C), A male control is displayed showing a single peak before *Hpa*II digestion. (D), A PCR was run without DNA template as a control. (E), Fibroblasts carrying the1155del32 MeCP2 mutant (GM11272) displayed random X-inactivation. (F), RTT-1155del32-derived neurons showed highly skewed X-inactivation.

Figure S4. Phenotypic analysis iPSC-derived neurons from several clones; related to Figure 4. (A), Representative images showing co-localization between VGLUT1 and Psd95 (arrows). Bar = 5 μm. (B), Experimental and clonal variation of VGLUT1 puncta quantification in different individuals. (C), Efficient expression and knockdown of both MeCP2 isoforms by a specific shRNA against MeCP2. Bar = 50 μm. Two alternatively spliced MeCP2 transcripts have been characterized, isoforms A and B, which differ only in their most 5′ regions. The MeCP2 isoform B is more prevalent in the brain and during neuronal differentiation (Mnatzakanian et al., 2004).

(D), Graph shows cell soma radius for several RTT and WT clones. (E), WT MeCP2 protein levels detected in control and RTT neurons (Q244X). Gentamicin treatment in RTT neurons increased protein levels after 2 weeks. Numbers of neurons analyzed (*n*) are shown within the bars in graphs (B) and (D). Data shown as mean ± s.e.m.

Figure S5. Calcium transient analysis in iPSC-derived neurons; related to Figure 5. Neurons were selected after the confirmation that calcium transients were blocked with 1 μM of TTX or the glutamate receptor antagonists CNQX/APV treatments. (A), Blocking glutamatergic signaling in the neuronal network using CNQX and APV resulted in significant reduction in intracellular calcium transients. (B), Blocking voltage-gated sodium channels using TTX prohibited the generation of action potentials and resulted in complete elimination of neuronal intracellular calcium transients. (C), Gabazine increased the number of calcium transients in the iPSC-derived neuronal networks. Red traces correspond to the calcium rise phase detected by the algorithm used. (D), Bar graph shows the normalized frequency of neurons with calcium transients after drug treatments. (E), Bar graph shows the event frequency decrease in RTT and shMeCP2-treated WT neurons compared to WT control neurons. (F), Bar graph shows the percentage of signaling neurons in RTT and shMeCP2-treated WT neurons compared to WT control neurons.

Supplemental Tables

Supplemental Table 1; related to Figure 1

Table S1. MeCP2 mutations, phenotype description and respective cell lines used in this study.

Cell line name	*iPSC code*	*Gender*	*MeCP2 Mutation*	*Nucleotide change*	*Individual phenotype*
GM11270*	R306C	Female	missense	916C>T	Clinically affected; classical symptoms; normal lysosomal enzymes; 46,XX in PBL; donor carries missense mutation, 916C>T, in the gene encoding methyl-CpG binding protein 2 (MECP2).
GM11272*	1155del32	Female	frameshift	1155del32	Clinically affected; classical symptoms; normal lysosomal enzymes; 46,XX in PBL; donor subject carries a frameshift mutation, 1155del32, in the gene encoding methyl-CpG binding protein 2 (MECP2).
GM16548*	Q244X	Female	nonsense	730C>T	Clinically affected; abnormal sleep patterns; ambulatory; breath holding; constipation; decelerating head circumference; loss of purposeful hand use; nonverbal; poor hand and feet circulation; repetitive hand motions; self injurious behavior; small feet; teeth grinding; tremors; donor subject is heterozygous for a 730C>T transition in the MECP2 gene resulting in a stop codon.
GM17880*	T158M	Female	missense	473C>T	Growth and developmental delay; can walk only with assistance; nonverbal; no hand use; constant repetitive hand motions; no seizures, but significantly abnormal EEG; teeth grinding; some sleep difficulties; eating problems with minor reflux; breath holding and hyperventilation; small feet; some tremor; this culture had a lifespan of 56 population doublings (PDLs); the donor subjects carried a 473C>T transition, resulting in the substitution of threonine 158 by methionine.
AG09319*	AG09319	Female	WT	-	Healthy individual. The karyotype is 46,XX; normal diploid female.
CRL2529**	CRL2529	Male	WT	-	Healthy individual. The karyotype is 46,XY; normal diploid male.
WT-126***	126	Male	WT	-	Healthy individual. The karyotype is 46,XY; normal diploid male.
WT-33***	33	Female	WT	-	Healthy individual. The karyotype is 46,XX; normal diploid female.
WT-ARDC40***	ARDC40	Male	WT	-	Healthy individual. The karyotype is 46,XY; normal diploid male.

*From Coriell. ** From ATCC ***From biopsies of healthy individuals.

Supplemental Table 2; related to Figures 1-6.

Table S2. Summary of the iPSC subjects and clones utilized for each experiment. Numbers represent experimental replications for each individual clone. The clones utilized in neuronal differentiation experiments were determined by availability at the end time-point.

	SUBJECTS														
	ADRC40	**AG09319**		**CRL2529**	**WT-33**		**WT-126**		**1155del32**			**Q244X**		**T158M**	**R306C**
Experiments/clones	*C1*	*C1*	*C2*	*C1*	*C1*	*C7*	*C5*	*C8*	*C13*	*C15*	*C18*	*C3*	*C4*	*C3*	*C1*
Pluripotency assays	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Gene expression		3	3							3	3	3	3		
NPC cell cycle		3	3	3					3	3	3				
Map2 labeling	3	3			3		3	3		3	3	3	3	3	
Syn::DsRed labeling		3			3			3		3	3	3			
GABA labeling		3			3			3		3		3		3	
H3K27me3 labeling		3			3					3	3	3			
Xist FISH					2					2	2	2			
Androgen receptor					2					2		2		2	
Cell cycle		3		3					3	3	3			3	
VGLUT puncta	3	3			3		3	3		3	3		3	3	
Soma size	2		2			2		2	2	2	2	2	2	2	
Spine density			3					3			3	3			
Calcium transients					3			3		3	3	3			
Electrophysiology					3			4		4		4			

Supplemental Experimental Procedures

Cell culture and retrovirus infection

Female RTT (1155del32, GM11272; Q244X, GM16548; T158M, GM17880 and R306C, GM11270; from Coriell Institute) and WT fibroblasts (AG09319; from Coriell Institute, CRL2529; from ATCC). WT-126, ADRC40 and WT-33 were cultured in Minimum Essential Medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone Laboratories).

The hESC Cyth25 (CyThera Inc., San Diego) and HUES6 (Harvard) cell lines were cultured as previously described (Muotri et al., 2005). Recombinant viruses were produced by transient transfection in 293T cells, as previously described (Muotri et al., 2005).

To obtain NPCs, EBs were formed by mechanical dissociation of cell clusters and plating onto low-adherence dishes in hESC medium without FGF2 for 5-7 days. After that, EBs were plated onto poly-ornithine/laminin (Sigma)-coated dishes in DMEM/F12 (Invitrogen) plus N2. Rosettes were visible to collect after 7 days. Rosettes were then dissociated with accutase (Chemicon) and plated again onto coated dishes with NPC media (DMEM/F12; 0.5X N2; 0.5X B27 and FGF2). Homogeneous populations of NPCs were achieved after 1-2 passages with accutase in the same condition. To obtain mature neurons, floating EBs were treated with 1 µM of retinoic acid for 3 more weeks (total time of differentiation 4 weeks). Mature EBs were then dissociated with Papain and DNAse (Worthington) for 1 hour at 37^{o}C and plated in poly-ornithine/laminin-coated dishes in NPC media without FGF2.

Primary antibodies used for immunofluorescence in this study

Primary antibodies used in this study were TRA-1-60, TRA-1-81 (1:100, Chemicon); Nanog and Lin28 (1:500, R&D Systems); human Nestin (1:100, Chemicon); Tuj-1 (1:500, Covance); Map2 (1:100; Sigma); MeCP2 (1:1000, Sigma); VGLUT1 (1:200, Synaptic Systems); Psd95 (1:500,

Synaptic Systems), GFP (1:200, Molecular Probes-Invitrogen); Sox1 (1:250, BD Biosciences), Musashi1 (1:200, Abcam) and me3H3K27 (1:500, Millipore).

Oligonucleotide sequences used in this study

1-ShRNA against the human MeCP2 gene (5'-GGAGTCTTCTATCCGATCTGT-3') was cloned in the LentiLox3.7 lentivirus vector, which forms a part of hairpin loop 5'-GGAGTCTTCTATCCGATCTGTTCAAGAGACAGATCGGATAGAAGACCTCC-3'.

2- The primer sequences were: hOct4-f: gggaggggaggagctagg and hOCt4-R: tccaaccagttgccccaaac; hSox2-F: tgggaggggtgcaaaagagg and hSox2-R: gagtgtggatgggattggtg; hNanog-F: cctatgcctgtgatttgtgg and hNanog-R: ctgggaccttgtcttccttt; hMSX1-F: 5' aggaccccgtggatgcagag and hMSX1-R: 5' ggccatcttcagcttctccag; hGAPDH-Fw: 5' accacagtccatgccatcac 3', hGAPDH-Rv: 5' tccaccaccctgttgctgta 3'. PCR products were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide and visualized by UV illumination.

Microarray analysis. Gene-level signal estimates were derived from the CEL files by RMA-sketch normalization as a method in the apt-probeset-summarize program. Hierarchical clustering of the full dataset by probeset values was performed by complete linkage using Euclidean distance as a similarity metric in Matlab.

Quantification of calcium transients.

The recipe for Krebs HEPES Buffer (KHB) used for calcium Imaging was: 10 mM HEPES, 4.2 mM $NaHCO_3$, 10 mM dextrose, 1.18 mM $MgSO_4 \cdot 2H_2O$, 1.18 mM KH_2PO_4, 4.69 mM KCl, 118 mM NaCl, 1.29 mM $CaCl_2$; pH 7.3). Neurons were selected after the confirmation that calcium transients were blocked with 1 μM of tetrodotoxin (TTX) or the glutamate receptor antagonists CNQX/APV (6-cyano-7-nitroquinoxaline-2,3-dione at 10 μM / (2R)-amino-5-phosphonovaleric

acid; (2R)-amino-5-phosphonopentanoate at 20 μM, respectively) treatments. Calcium transients increased after 30 μM Gabazine treatment. For quantification of calcium transients, ImageJ, an NIH-funded open source, JAVA-based morphometric application, was used to allow manual selection of individual neurons on the Syn::DsRed image that correspond to each calcium movie using circular regions of interest (ROI) of 4 pixels (~5μm) in diameter. Each cell was considered as an individual ROI and the average fluorescence intensity was calculated for each ROI through the entire acquired image sequence. Quantitative signal analysis and processing were done with custom-written Matlab routines. Individual temporal fluorescence intensity signals indicative of intracellular calcium fluctuations were filtered using power spectrum calculated from Fourier transforms to reduce noise. Amplitude of signals was presented as relative fluorescence changes (ΔF/F) after background subtraction. A first-derivative filter was used to identify regions of increase in calcium signal and a calcium event was identified by a positive derivative value of 2 SD or more above background with a rise phase that persisted a minimum of 5 consecutive frames (~70ms).

Electrophysiology

Recipe for HEPES-buffered saline: 115 mM NaCl, 2 mM KCl, 10 mM HEPES, 3 mM CaCl2, 10 mM glucose and 1.5 mM MgCl2 (pH 7.4).

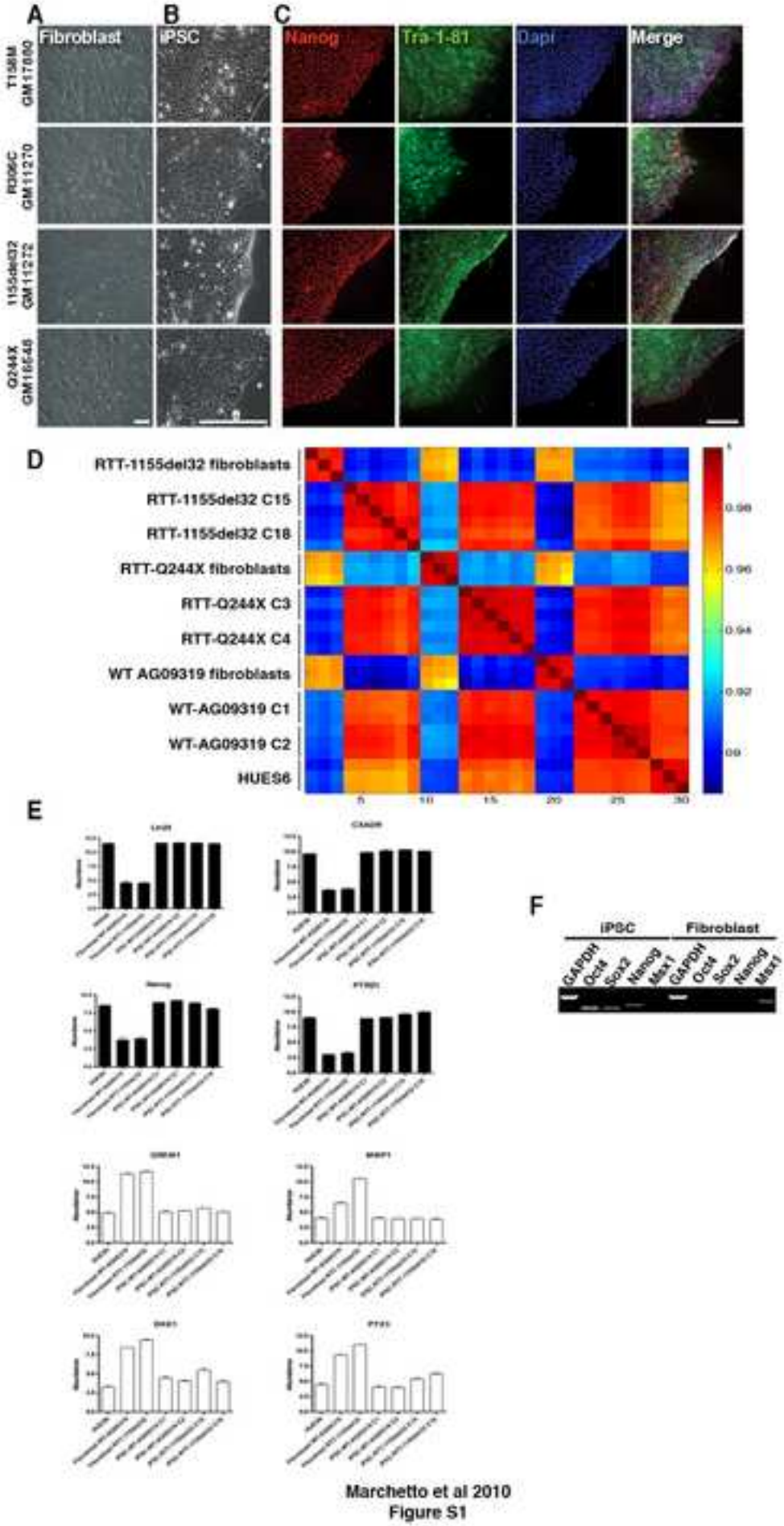
Recipe for solution inside the recording micropipettes (tip resistance 3–6 MΩ): 140 mM K-gluconate, 5 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES and 0.2 mM EGTA, 2.5 mM Na-ATP, 0.5 mM Na-GTP, 10 mM Na_2-phosphocreatine (pH 7.4).

Supplemental References

Mnatzakanian, G.N., Lohi, H., Munteanu, I., Alfred, S.E., Yamada, T., MacLeod, P.J., Jones, J.R., Scherer, S.W., Schanen, N.C., Friez, M.J.*, et al.* (2004). A previously unidentified MECP2 open reading frame defines a new protein isoform relevant to Rett syndrome. Nat Genet *36*, 339-341.

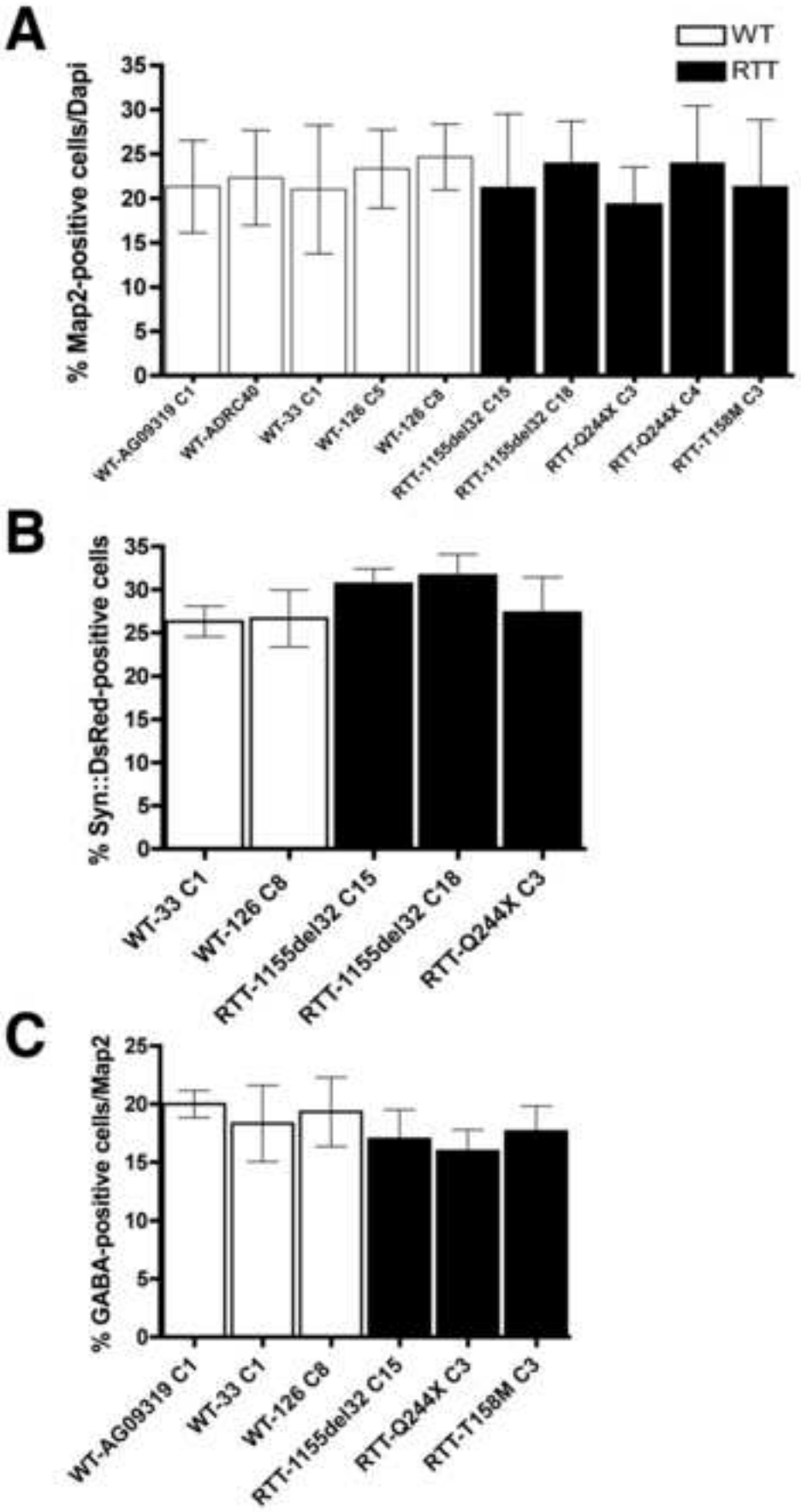
Muotri, A.R., Nakashima, K., Toni, N., Sandler, V.M., and Gage, F.H. (2005). Development of functional human embryonic stem cell-derived neurons in mouse brain. Proc Natl Acad Sci U S A *102*, 18644-18648.

Supplemental Figure1



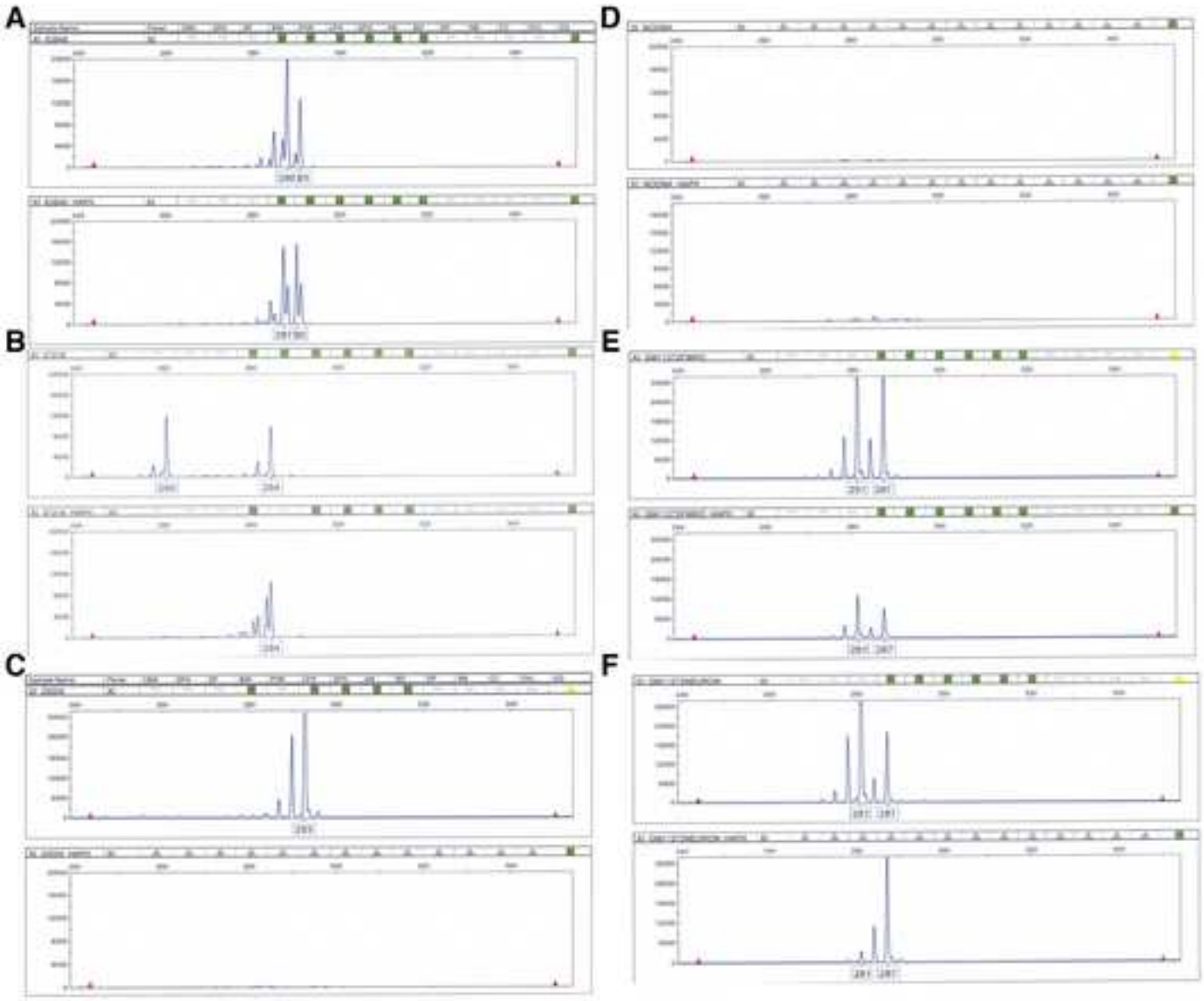
Marchetto et al 2010
Figure S1

Supplemental Figure2



Marchetto et al 2010
Figure S2

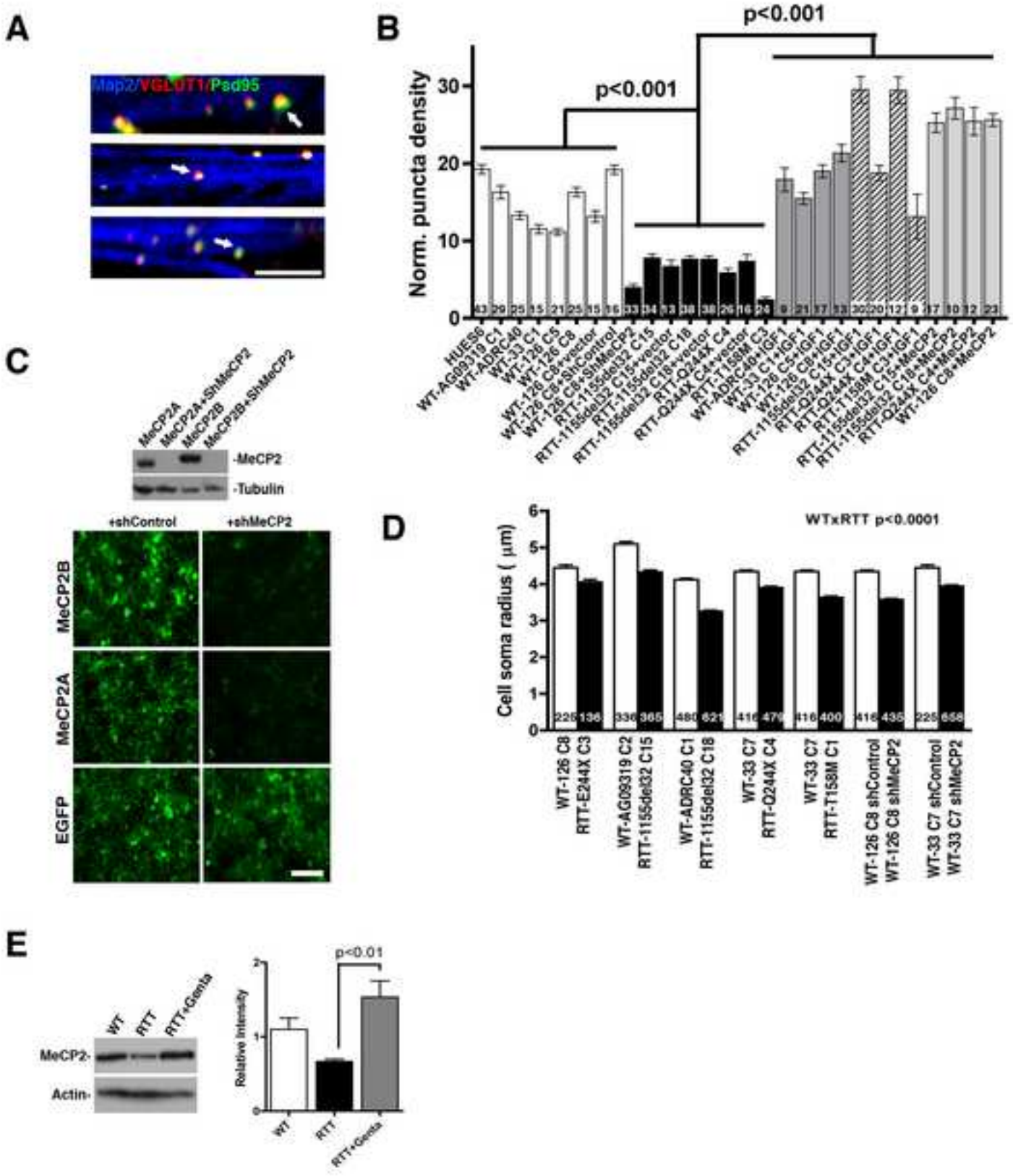
Supplemental Figure3



Sample Name	Peak Area1	Peak Area2	Corr. Factor	Corr. Peak2 (Hpa)	Corr. Total	Peak1 %	Peak2 %	Xl:Xa
Xl_63846	156508	87390	1.7909143					
Xl_63846_HapII	100309	103847		185961.0765	286290.0765	35	65	65:35 (Random Control)
Xl_57216	87250	73588	1.1856553					
Xl_57216_HapII	79106			0	79106	100	0	100 (Highly Control)
Xl_GM11272Fibro	273822	245617	1.1148333					
Xl_GM11272Fibro_HapII	85193	61995		69114.08775	154307.0877	55.2	44.8	55:45 (Random)
Xl_GM11272Neuron	330180	150314	2.1966018					
Xl_GM11272Neuron_HapII	19851	229017		503059.1499	522910.1499	3.8	96.2	96:4 (Highly)
Xl_20000	325981							
Xl_20000_HapII								Male Control
Xl_NoDNA								
Xl_NoDNA_HpaII								No DNA

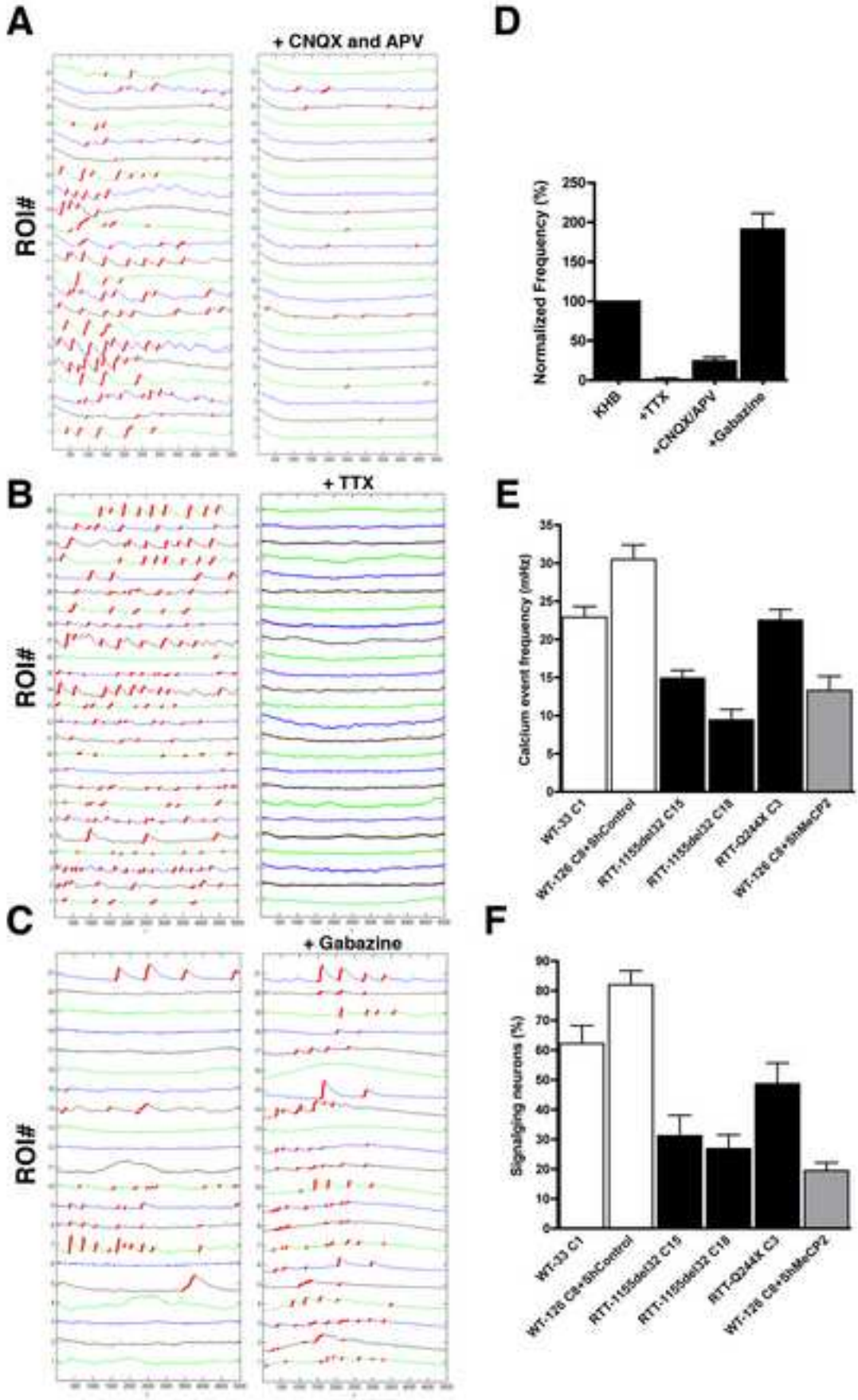
Marchetto et al 2010
Figure S3

Supplemental Figure4



Marchetto et al 2010
Figure S4

Supplemental Figure5



Marchetto et al 2010
Figure S5