Supporting Information

Deogracias et al. 10.1073/pnas.1206093109

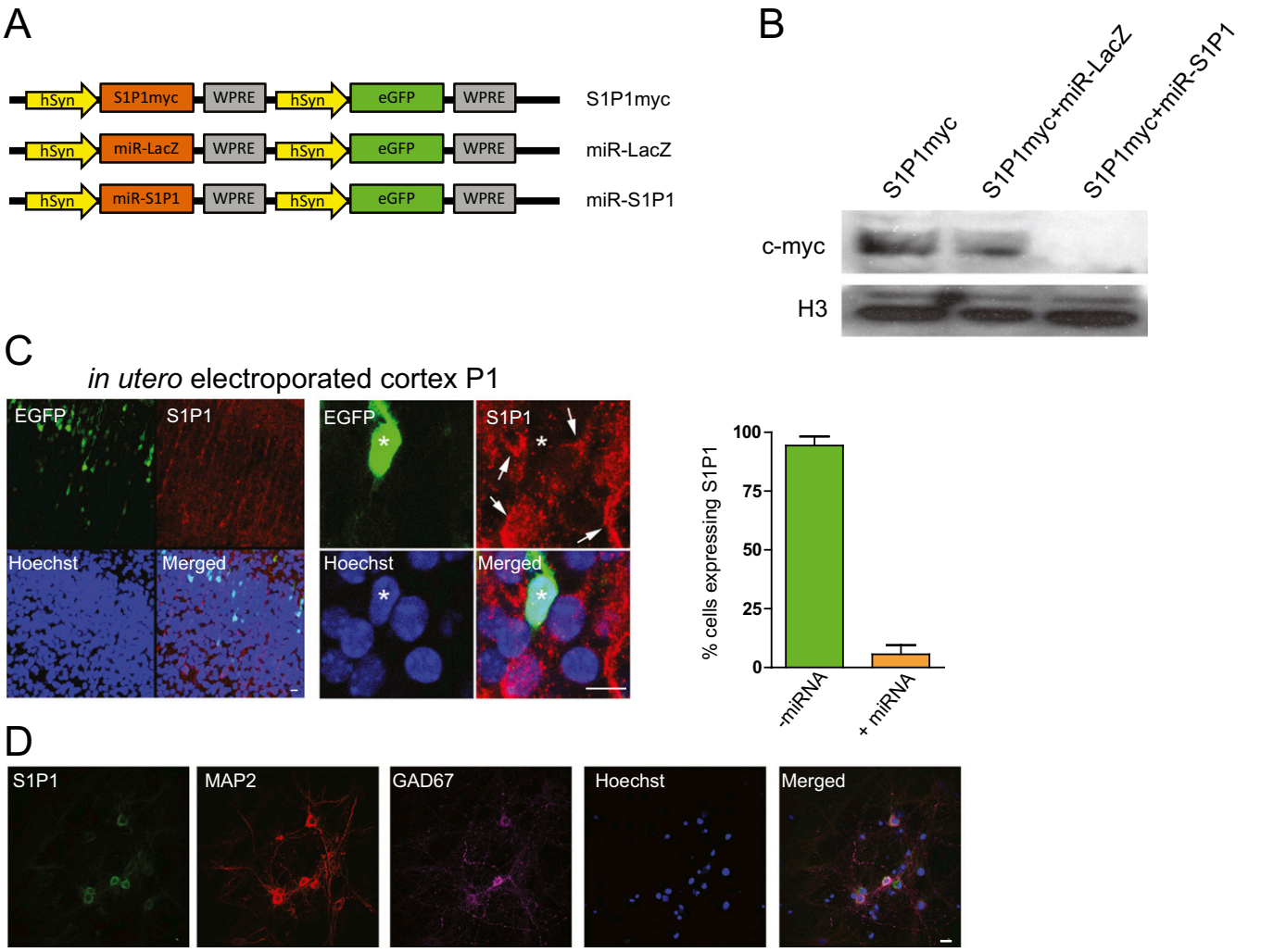

Fig. S1. Validation of anti-S1P1 antibodies. (*A*) Dual h-Synapsin-promoter (1) driving the expression of S1P1myc-tagged, miRNA against β-galactosidase (miR-LacZ), or miRNA against S1P1 from the first promoter, and eGFP from the second. (*B*) Validation of the miR-S1P1 expressing vector by Western blot on co-transfected HEK cells. (*C*) Immunohistochemistry using S1P1 antibodies on brain slices of P1 mice in utero electroporated at E14.5 with the miR-S1P1 expressing vector showed absence of S1P1 on transfected (eGFP positive) neurons. (*D*) Quantification of the number of neurons immunoreactive for S1P1 antibodies (n = 250). P1 mice transfected by in utero electroporation (E14.5) showed a reduction in the number of neurons expressing miRNA-S1P1 (eGFP positive) and S1P1. Cells stained with S1P1 receptor antibodies (green), MAP2 (red), GAD67 (magenta) and the Hoechst nuclear stain (blue). (Scale bar: 10 μm.) Note the lack of correlation between S1P1 receptor and neuronal subtype.

1. Gascón S, Paez-Gomez JA, Díaz-Guerra M, Scheiffele P, Scholl FG (2008) Dual-promoter lentiviral vectors for constitutive and regulated gene expression in neurons. *J Neurosci Methods* 168:104–112.

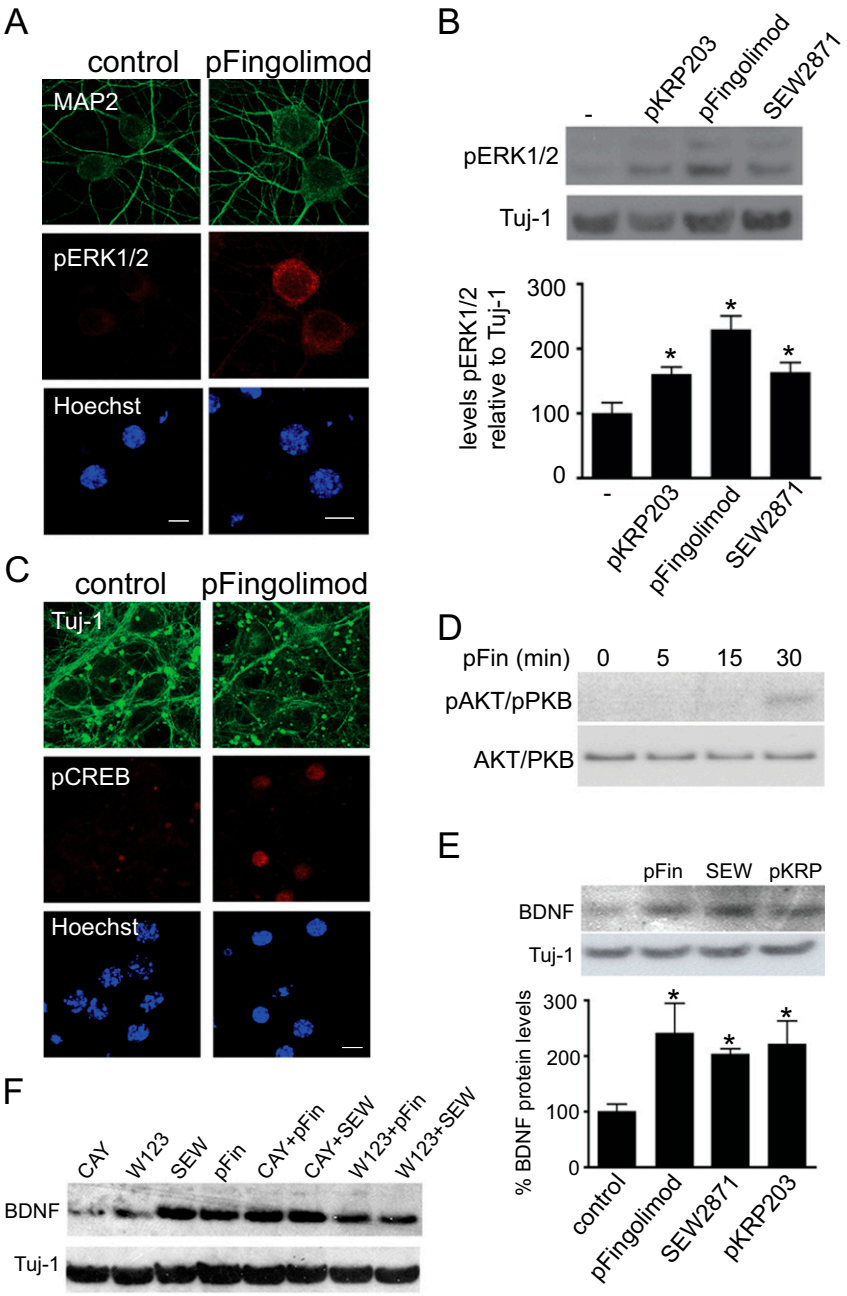

Fig. S2. Increased phosphorylation of ERK1/2, CREB, and AKT by modulation of S1P1 receptors. (*A*) Antibody staining showing neuro-specific phosphorylation of ERK1/2 after 30 min stimulation with phospho-fingolimod (10 nM). MAP2 was used to label neurons. Nuclei were stained with Hoechst. (*B*) Thirty minutes stimulation of cortical cultured with the specific S1P1 receptor agonists pKRP203 and SEW2871 lead to increased pERK1/2 (Mean ± SEM, $n = 3$, $^*P < 0.05$ Student's t test). (*C*) Rapid phosphorylation of AKT in cultured neurons after 30 min of stimulation with phospho-fingolimod (10 nM). (*D*) Cortical neurons stimulated with pFTY720 for 30min lead to increased pCREB-Ser133 immunoreactivity in the nucleus. Neurons were labeled with β-III-Tubulin (Tuj1) antibodies and nuclei with Hoechst. (*E*) BDNF protein levels were increased in cortical neurons treated with the S1P1 agonists SEW2871 and pKRP203 compared with nonstimulated cultures. Graph represents the mean ± SEM of three independent experiments. ($^*P < 0.05$ Student's t test). (*F*) The presence of the S1P1 antagonist W123, but not of the S1P3 antagonist CAY10444, abrogated the increased on BDNF levels after 24 h of treatment with phospho-fingolimod and SEW2871.

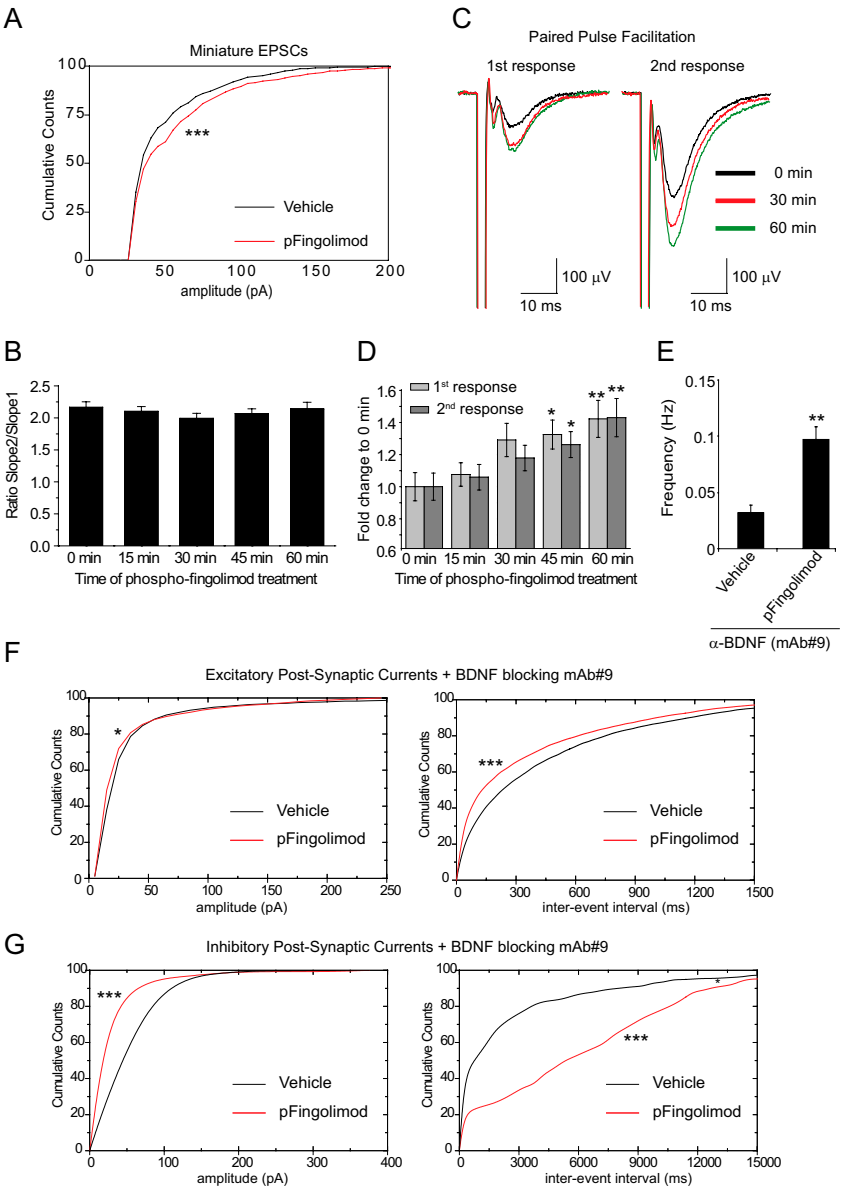

Fig. S3. Phospho-fingolimod increases postsynaptic excitability. (*A*) Phospho-fingolimod increases the average amplitude of miniature EPSCs (***$P < 0.001$; Kolmogorov–Smirnov test), whereas the frequency was not changed (5.8 vs. 4.3 Hz; $P = 0.31$; Student *t* test; 14 recorded cells per condition). (*B*) The ratio between the slopes did not alter ($P = 0.57$, Repeated measures one-way ANOVA, 8 recorded cells per condition). (*C*) Paired-pulse facilitation experiments on acute hippocampal slices confirmed an increase in excitability (each trace is an average of a 5-min recording). (*D*) The phospo-fingolimod induced increase in excitability was only observed at time points later than 30 min (*$P < 0.05$, **$P < 0.01$, Repeated measures one-way ANOVA, followed by Tukey's post hoc test, 8 recorded cells per condition). (*E*) In spontaneous activity recordings of DIV14-16 cortical cultures in the presence of the mAb#9 BDNF blocking antibody, the frequency of excitatory bursts was increased after phospho-fingolimod treatment (**$P < 0.01$, Student *t* test, 23 recorded cells per condition). (*F*) The mAb#9 BDNF blocking antibody reverses the phospho-fingolimod induced increase in EPSC amplitudes, while the interevent interval was still reduced (*$P < 0.05$, ***$P < 0.001$, one-sided Kolmogorov–Smirnov test, 23 recorded cells per condition). (*G*) The IPSC amplitude is reduced after phospho-fingolimod treatment in the presence of the mAb#9 BDNF blocking antibody, whereas the interevent interval is increased (***$P < 0.001$, one-sided Kolmogorov–Smirnov test, 23 recorded cells per condition).

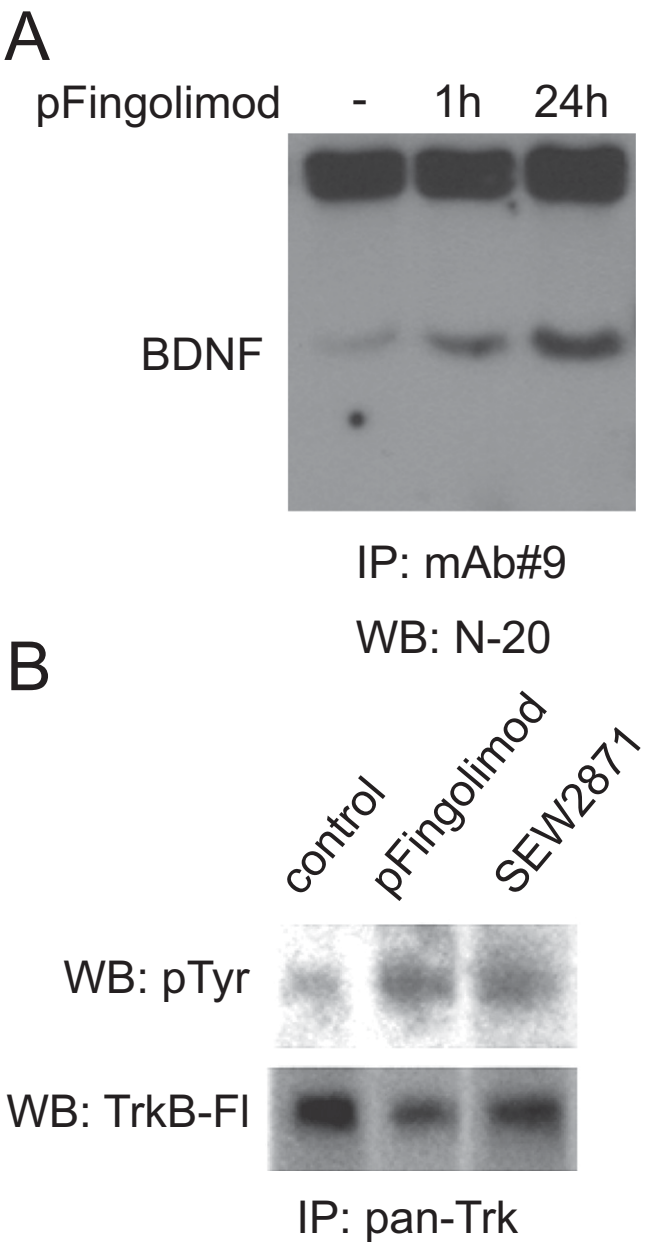

Fig. S4. Increased BDNF secretion and TrkB phosphorylation by phospho-fingolimod. (*A*) Cortical neurons (DIV14) were treated with phospho-fingolimod (10 nM) for 1 or 24 h with the BDNF antibody mAb#9 (12 μg/mL) added to the medium 1h before treatment. The medium was collected, immunoprecipitated and analyzed by Western blot using the BDNF antibody N-20. (*B*) Immunoprecipitation with pan-Trk antibodies, followed by detection with phospho-tyrosine and TrkB-Fl antibodies. Note the increase in phospho-TrkB levels after phospho-fingolimod and SEW2871 treatment for 1 h.

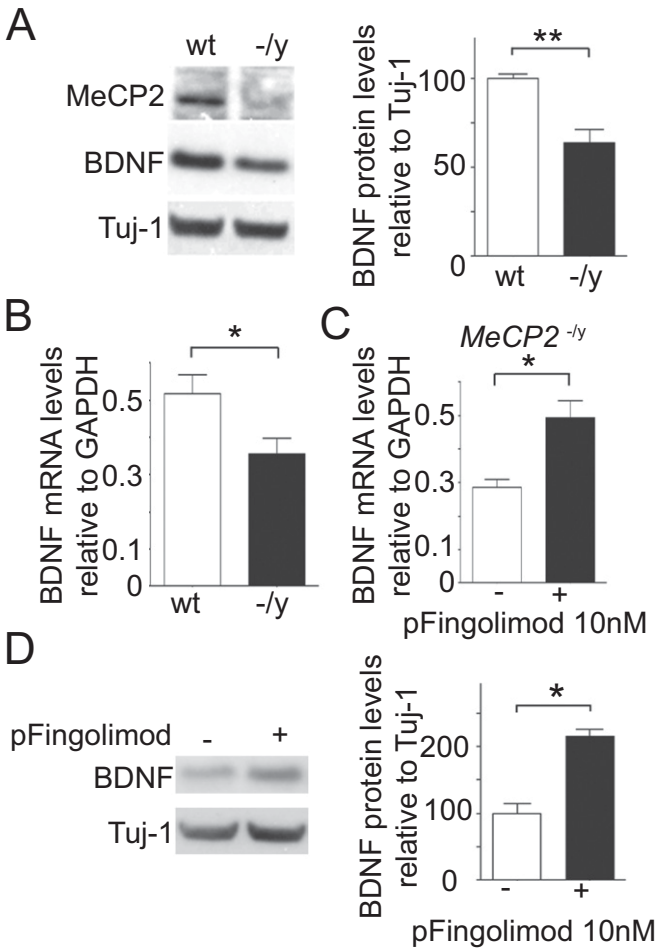

Fig. S5. Phospho-fingolimod increases BDNF levels in neurons derived from *Mecp2*$^{-/y}$ ES cells. (*A* and *B*) *Mecp2*$^{-/y}$ ES cell-derived neurons (14 DIV) show reduced BDNF levels as observed by Western blot (*A*) and qPCR (*B*) assays. (*C* and *D*) Stimulation of *Mecp2*$^{-/y}$ ESCDN with phospho-fingolimod (10 nM, 4 h) increases BDNF mRNA levels (*C*) and protein levels (*D*) after 16 h (means ± SEM, $n = 3$, $^{*}P < 0.05$ Student's *t* test).