Supporting Information

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SI Methods

Metabolic Labeling with [^{35}S]-Methionine. Primary hippocampal cells (10^6) from either *Prnp*$^{+/+}$ or *Prnp*$^{0/0}$, or 10^4 N2a or SN-56 cells, were plated for 24 h. For hippocampal cultures, half of the culture medium was substituted with DMEM lacking methionine (Invitrogen) and 10 μCi/mL of [^{35}S]-Met (Perkin-Elmer) was added 15 min before treatment. Hippocampal cells were treated with STI1, 2.4 μM Δ, 80 μM PepSTI1$_{230–245}$, or 80 μM PepSTI1$_{61–76}$ for 30 min. When inhibitors were used, neurons were preincubated with 5 μM Ly294002, 20 nM rapamycin, 50 μM PD98059, or 1.5 μM actinomycin D along with ^{35}S-Met for 15 min, followed by 2.4 μM STI1 for 30 min. For N2a and SN-56 cells, all medium was replaced with DMEM lacking methionine and 4 μCi/mL of [^{35}S]-Met (Perkin-Elmer) was added 15 min before stimulating with 2.4 μM STI1 for 30 min. A 300-μg quantity of synaptosomes was prewarmed in homogenization buffer for 10 min at 37 °C in the presence of 15 μCi [^{35}S]-Met, followed by treatment with 2.4 μM STI1 for 30 min. Cells or synaptosomes were rinsed with ice-cold PBS and lysed in RIPA buffer. Lysates were spotted onto 3MM filter paper (Whatmann) previously blocked with 100× glutamine solution (Invitrogen). Filter papers were allowed to air dry and were subsequently incubated in ice-cold 20% trichloroacetic acid for 20 min. Filter papers were boiled in 10% trichloroacetic acid for 15 min and air dried. The remaining radioactivity was measured by scintillation counting.

Synaptosome Preparations. Synaptosomes were prepared from 3-wk-old WT (*Prnp*$^{+/+}$) mice as described previously (1) with a few modifications. Briefly, dissected cortex was homogenized at 4 °C in 10 volumes of homogenization buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.53 mM KH_2PO_4, 212.7 mM glucose, and 0.2 mM DTT, pH 7.4), supplemented with Complete Protease Inhibitor Mixture (Roche) and 200 μg/mL chloramphenicol (Sigma). The homogenate was passed through two 100-μm nylon filters and one 5-μm PVDF membrane (Millipore), and centrifuged. Supernatants were discarded between filtration steps. The final filtrate was centrifuged at 1,000 × *g* for 10 min, and the pellet was washed, ressuspended in homogenization buffer, and used immediately for protein synthesis assays.

Polysome Profiles. Primary cultures of cortical neurons (1.5×10^8 cells for each condition, 5 d in vitro) were treated with 2.4 μM STI1 for 30 min, followed by treatment with 100 μg/mL cycloheximide for 3 min at 37 °C. Cells were washed with PBS containing 100 μg/mL cycloheximide, lysed in 300 μl lysis buffer (20 mM Tris · HCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 1% Triton X-100, 1 mM DTT, 100 μg/mL cycloheximide, and protease inhibitor mixture) and cleared by two centrifugations at 16,100 × *g* for 10 min at 4 °C. Ten A_{254nm} units of extract were applied to a linear 7–47% sucrose gradient prepared in 20 mM Tris · HCl (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, and 1 mM DTT and centrifuged for 2.5 h at 39,000 rpm in a SW41Ti rotor (Beckman Coulter) at 4 °C. The gradients were collected from the top, and absorbance at 254 nm was detected in a continuous flow. Quantification was performed in ImageJ software (National Institutes of Health) by measuring the area under the peak of 80S and the polysomes and calculating the polysomes/monosome ratio.

PK Digestion. Primary hippocampal neurons were lysed and a fraction was kept as control, nondigested extract. The remaining lysate was digested with 20 μg/mL of PK at 37 °C for 30 min. Proteins were separated by SDS/PAGE and immunoblotted with anti-PrP^C antibody 4H11.

Detection of PrP^{Sc} by Immunofluorescence Microscopy. Primary hippocampal neurons exposed to 22L-infected brain homogenate were fixed after 3 d and permeabilized by 0.1% Triton X-100, and proteins were denatured using 6 M guanidinium hydrochloride. Prion fluorescence was detected using antibody 4H11 and Cy3-conjugated secondary antibody (Dianova). Slides were mounted in Permafluor Aqueous Mounting Medium Liquid (Beckman Coulter), and confocal laser scanning microscopy was performed on an LSM 510 laser-scanning microscope (Zeiss). Mock-infected cells were imaged and the detector threshold was set, as no image could be seen in the red channel, and fluorescence from 22L-infected cells was detected.

1. Villasana, et al. (2006) Rapid isolation of synaptoneurosomes and postsynaptic densities from adult mouse hippocampus. *J Neurosci Methods* 158:30–36.

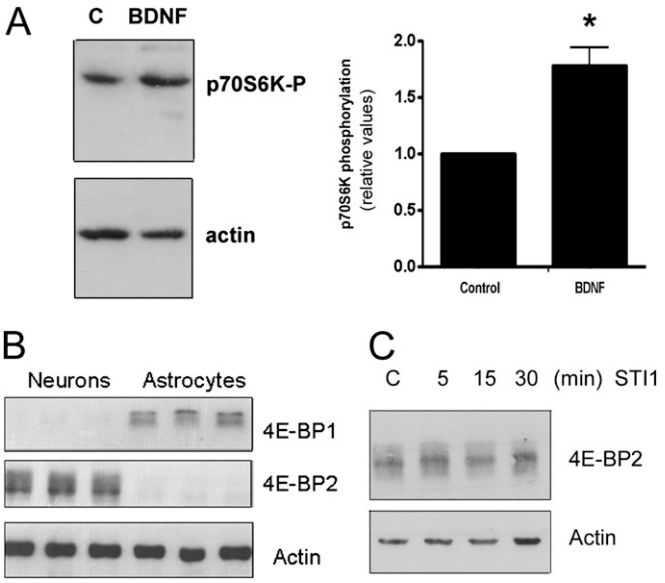

Fig. S1. 4E-BP2 is predominantly expressed in neurons and its expression is unaltered by STI1. The mTOR pathway can still be stimulated in $Prnp^{0/0}$ cells. (*A*) $Prnp^{0/0}$ primary cultured hippocampal neurons were treated with 100 ng/mL BDNF for 15 min. Cell extracts were resolved in SDS/PAGE followed by immunobloting with antibodies against phospho-p70S6K and actin. The phospho-p70S6K/actin ratio was calculated, and phosphorylation relative to control was plotted in the graph. *Statistically different from control. Student *t* test, $P < 0.01$. (*B*) $Prnp^{+/+}$ primary cultured hippocampal neuron and astrocyte lysates were resolved in SDS/PAGE followed by immunobloting with antibodies against 4E-BP1, 4E-BP2, and actin. (*C*) Immunobloting from lysates of primary cultured hippocampal $Prnp^{+/+}$ neurons treated with 2.4 μM STI1 for the indicated time.

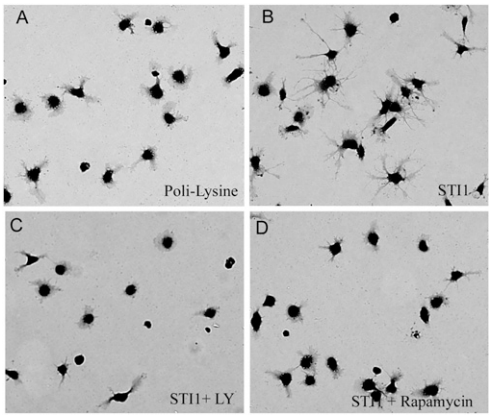

Fig. S2. PrP^{C}–STI1–induced neuritogenesis is dependent on PI3K and mTOR signaling. Hippocampal $Prnp^{+/+}$ neurons were cultured in the absence (*A*) or presence of 0.6 μM STI1 (*B*), STI1 plus Ly294002 (*C*), or STI1 plus rapamycin (*D*) for 24 h. Cells were fixed and stained, and representative images were taken.

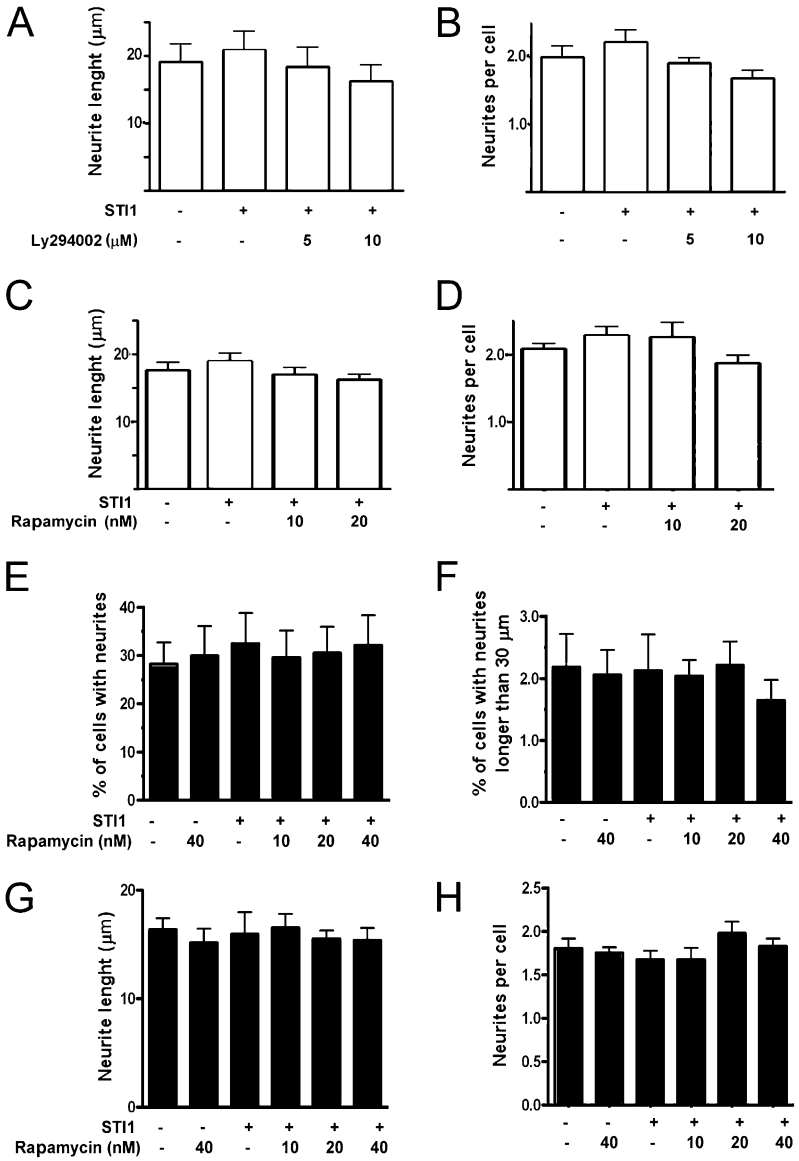

Fig. S3. STI1 does not elicit neuritogenesis in PrP^{C}-null neurons and mTOR inhibition does not affect basal neuritogenesis. Primary hippocampal neurons from WT mice (*Prnp*$^{+/+}$, open bars) were cultured in the presence of 0.6 μM STI1 and Ly294002 (*A* and *B*) or rapamycin (*C* and *D*) at the indicated concentrations for 24 h. Cells were fixed and stained for morphometric quantification. Neurite length (*A* and *C*) and number of neurites per cell (*B* and *D*) were quantified. Primary hippocampal neurons from PrP^{C}-null mice (*Prnp*$^{0/0}$, filled bars) were cultured in the presence of 0.6 μM STI1 and rapamycin at the indicated concentrations for 24 h. Cells were fixed and stained for morphometric quantification. The following parameters were quantified: (*E*) percentage of cells presenting neurites, (*F*) percentage of cells presenting neurites longer than 30 μm, (*G*) neurite length, and (*H*) number of neurites per cell.

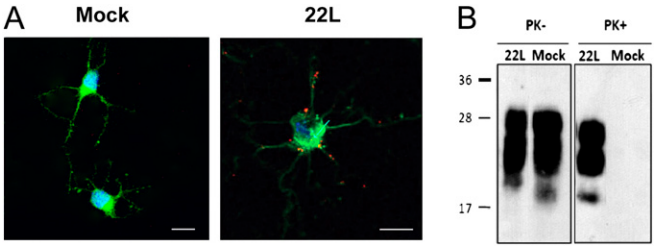

Fig. S4. PrP^{Sc} exposure in primary hippocampal neurons. (*A*) Primary neurons were exposed to mock-infected or 22L-infected brain extracts, fixed, treated with guanidinium hydrochloride, and labeled by immunofluorescence using antibodies against PrP (red), and neurofilament (green). (Scale bar, 10 μm.) (*B*) Primary neurons were exposed to mock-infected or 22L-infected brain extracts for 3 d. Cell lysates were subjected to PK treatment followed by Western blotting using antibodies against PrP.

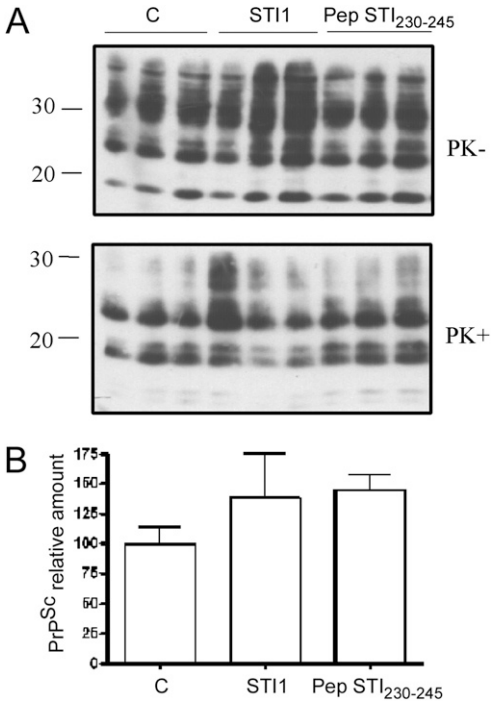

Fig. S5. STI1 does not modify PrP^{Sc} levels in prion-infected cells. (*A*) N2a cells persistently infected with prions were treated for 4 d with (350 nM) STI1 or with the peptide containing the STI binding site to PrP^{C} (16 μM $PepSTI1_{230–245}$). Cells were then lysed and subjected to PK digestion for detection of PrP^{Sc}. Total PrP (lysates without PK treatment, *Upper*) and PrP^{Sc} (PK-treated, *Lower*) were visualized by immunoblot. Experiments were performed in triplicate. (*B*) Relative levels of PrP^{Sc} in STI1 or $PepSTI_{230–245}$-treated N2a cells persistently infected with 22L compared with untreated cells. Data were compared by Student *t* test.

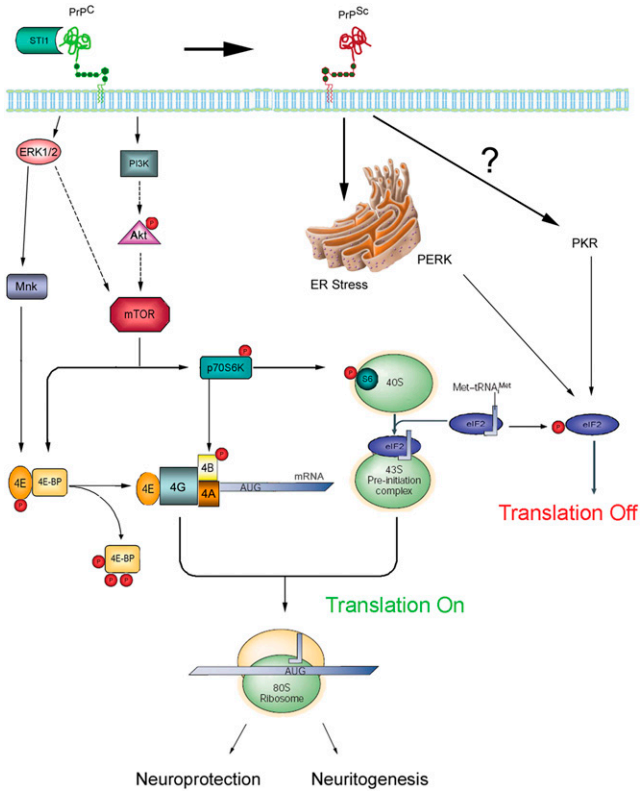

Fig. S6. Overview for PrP^C–STI1 signaling pathways controlling protein synthesis and translational blockage by PrP^{Sc} infection. STI1 binds to PrP^C at the cell surface, inducing the activation of both ERK1/2 and PI3K. PI3K activation leads to Akt phosphorylation, which in turn triggers mTOR activation. Downstream targets of mTOR include p70S6K and 4E-BPs. Activated p70S6K phosphorylates S6 and eIF4B. Phosphorylated 4E-BP releases eIF4E to form the 5′ cap-binding complex. ERK1/2 activates Mnk, which in turn phosphorylates eIF4E. The impact of S6 and eIF4E phosphorylation on translation is controversial, but it has been observed upon treatments that stimulate protein synthesis in neurons. ERK1/2 indirectly promotes the activation of mTOR, through RSK1 inactivation of tuberous sclerosis 2. Activation of these pathways leads to enhanced protein synthesis, which is essential to PrP^C–STI1–induced neuritogenesis and neuroprotection. PrP^{Sc} infection leads to ER stress, which could trigger eIF2α phosphorylation through PERK or PKR. eIF2α phosphorylation results in general inhibition of translation. PrP^C dysfunction by conversion to PrP^{Sc} may also alter the response to STI1.