Liu et al., http://www.jcb.org/cgi/content/full/jcb.201509023/DC1

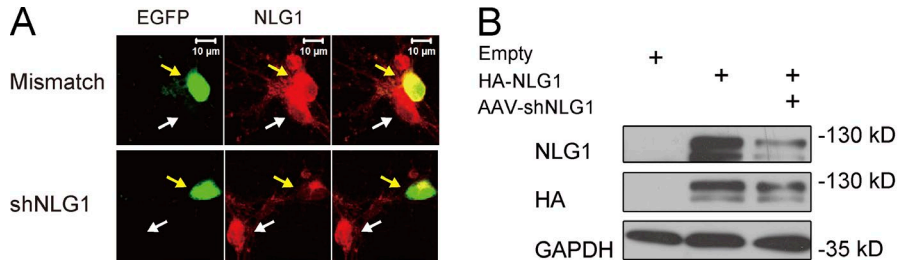

Figure S1. **Knockdown of NLG1.** Immunostaining of cultured hippocampal neurons (A) infected with AAV-NLG1shRNA or AAV-mismatch control shRNA virus (left) and Western blot analysis of HEK293 cells (B) transfected with HA-NLG1 and infected with AAV-NLG1shRNA virus (right) showing the knockdown of NLG1. Yellow arrows indicate infected cells and white arrows indicate noninfected cells.

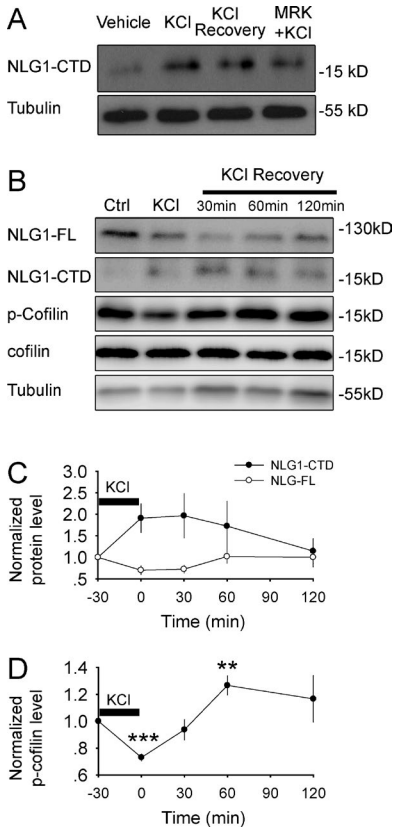

Figure S2. **Endogenous NLG1-CTD and its regulation by MRK and KCl.** (A) Western blots of protein lysate prepared from cultured neurons treated with KCl and/or MRK showing that KCl treatment increased the production of the CTD of NLG1 immediately (KCl) or 1 h (KCl recovery) after the treatment, and MRK blocked the effect of KCl (MRK + KCl). (B) Western blots of protein lysate of WT brain slices immediately or at various time points after KCl treatment showing that the level of the full-length NLG1 (NLG1-FL), NLG1-CTD, and p-cofilin was dynamically changed. (C and D) Summary graphs of B showing the time course of NLG1-FL, NLG1-CTD, and p-cofilin level during and after KCl treatment. **, $P < 0.01$; ***, $P < 0.001$.

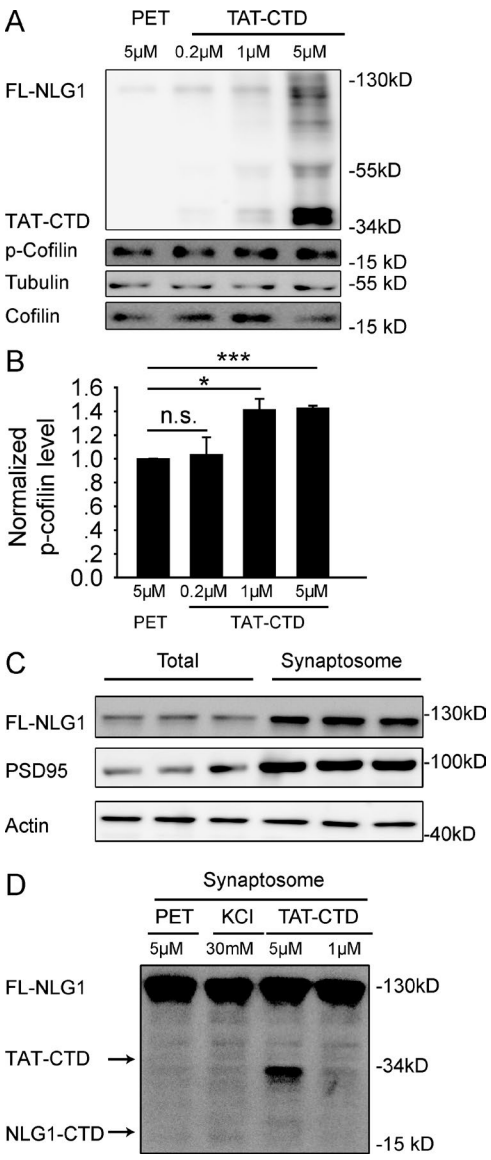

Figure S3. **Effect of recombinant proteins at various concentrations.** Western blot analysis (A) and summary graph (B) of total protein lysate of cultured neurons treated with PET and TAT-CTD recombinant proteins at various concentrations (0.2, 1, and 5 μM), showing that TAT-CTD increased p-cofilin in a dosage dependent manner, with 1 or 5 μM being sufficient to induce this increase. n.s., not significant. *, $P < 0.05$; ***, $P < 0.001$. (C) Western blots of total and synaptosomal protein lysate prepared from mouse brain slices showing that synaptic proteins NLG1 and PSD95 were enriched in the synaptosomal fraction compared with the total protein lysate. (D) Western blots of synaptosomal protein lysate of mouse brain slices treated with PET, KCl, or TAT-CTD showing that 1 μM TAT-CTD treatment produced a physiologic level of TAT-CTD, which was sufficient to induce an increase in p-cofilin shown in A.

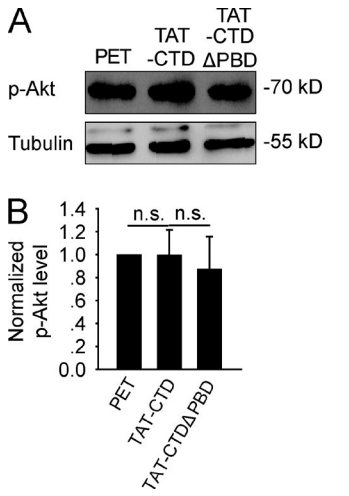

Figure S4. **Lack of effect of recombinant proteins on p-Akt.** Western blots (A) and summary graph (B) of protein lysate of cultured cortical neurons treated with various recombinant proteins for 1 h showing that neither TAT-CTD nor TAT- NLG1ΔPBD affected the p-Akt level compared with the PET group. n.s., not significant.

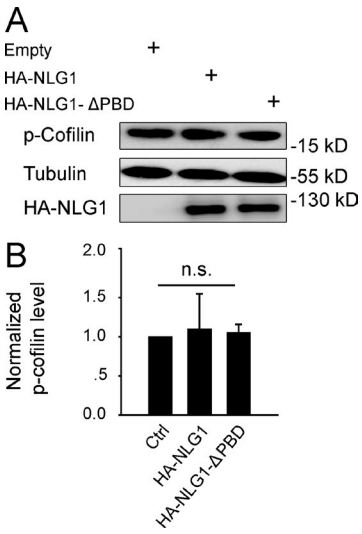

Figure S5. **HA-NLG1 alone has no effect on p-cofilin.** Western blots (A) and summary graph (B) of protein lysate of transfected HEK 293 cells showing that neither HA-NLG1 nor HA-NLG1ΔPBD transfection alone affected p-cofilin levels compared with the empty vector control group. n.s., not significant.