Briz et al., http://www.jcb.org/cgi/content/full/jcb.201504092/DC1

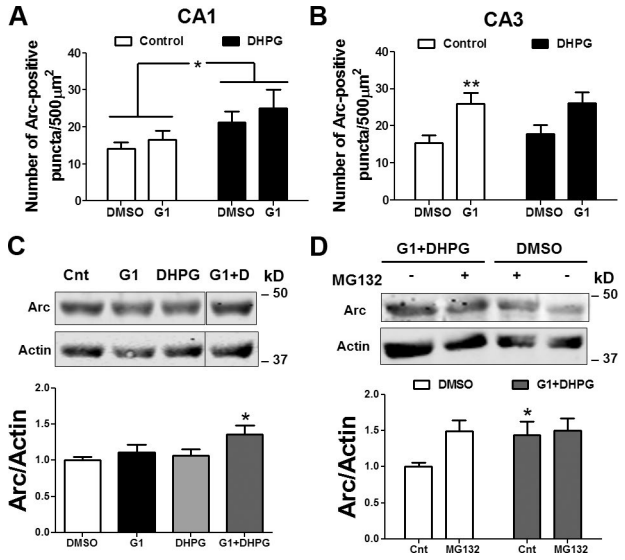

Figure S1. **Region-specific effects of G1 and DHPG on Arc immunostaining.** Hippocampal slices were incubated with DMSO or G1 for 1 h and then treated with DHPG for 15 min. Treatment with MG132 was performed 30 min before DHPG application. (A and B) Quantification of density of Arc-positive punctas in CA1 (A) and CA3 (B). *, P < 0.05; **, P < 0.01 versus control (n = 6–23, two-way ANOVA). (C and D) Representative immunoblots (top) and quantitative analysis (bottom) for changes in Arc levels in whole hippocampal slices. Data are presented as the ratio (fold of control) of Arc over actin (n = 5–16). *, P < 0.05 as compared with control (one- or two-way ANOVA). Black lines indicate that intervening lanes have been spliced out. Error bars indicate means ± SEM.

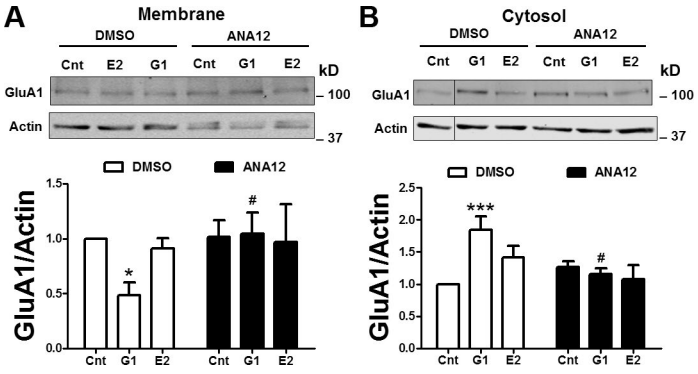

Figure S2. **G1-induced GluA1 internalization requires TrkB receptor activation.** Hippocampal slices were preincubated with DMSO or ANA-12 (50 µM) for 30 min and then treated with G1 or E_2 for an additional 60 min. At the end of treatments, samples were homogenized and processed for Western blotting after membrane fractionation (A, membrane; B, cytosol). Data are presented as the ratio (fold of control) of GluA1 over actin (n = 3–9). *, P < 0.05; ***, P < 0.001, as compared with control. #, P < 0.05, as compared with G1 alone (one- or two-way ANOVA). Black lines indicate that intervening lanes have been spliced out. Error bars indicate means ± SEM.

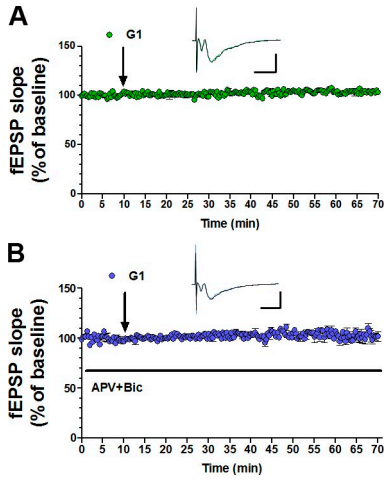

Figure S3. **G1 does not modify fEPSPs in the CA3 area of the hippocampus.** Hippocampal slices were incubated with G1 (100 nM) in the absence (A) or presence (B) of APV (100 µM) and bicuculline (10 µM). Slopes of fEPSPs are expressed as the percentage of the mean values recorded during the 10-min baseline (n = 3). (B, top) Traces of fEPSPs measured in field CA3 50 min after G1 application. Bar, 1 mV/5 ms.

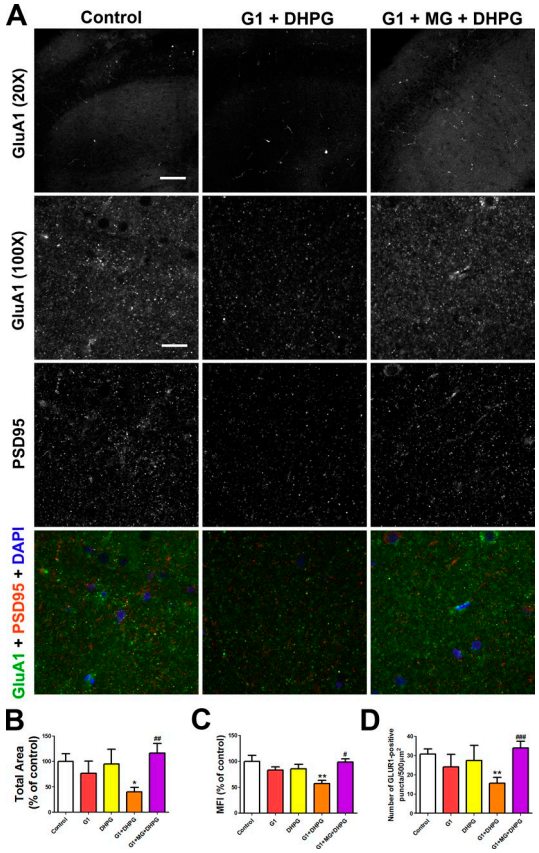

Figure S4. **Proteasome inhibition blocks down-regulation of GluA1 immunostaining by G1+DHPG.** Hippocampal slices were incubated with DMSO or G1 for 1 h and then treated with DHPG (100 µM) for 10–15 min. (A) Representative images of double immunostaining for GluA1 (green) and PSD95 (red) in CA3. Bars: (20×) 100 µm; (100×) 20 µm. (B–D) Quantification of GluA1 immunostaining in CA3 by means of: total area (B), mean fluorescence intensity (MFI; C) and density of GluA1-positive punctas (D). *, P < 0.05; **, P < 0.01 versus control. #, P < 0.05; ##, P < 0.01; ###, P < 0.001, as compared with G1+DHPG (n = 7–14, one-way ANOVA). Error bars indicate means ± SEM.

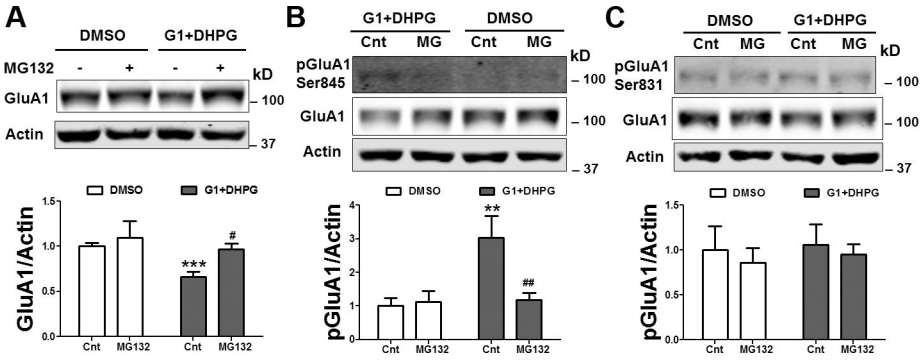

Figure S5. **Effects of MG132 and G1+DHPG on GluA1 phosphorylation.** Hippocampal slices were incubated with DMSO or G1 for 1 h and then treated with DHPG for 15 min. Treatment with MG132 was performed 30 min before DHPG application. Representative immunoblots (top) and quantitative analysis (bottom) for changes in GluA1 (A), phospho-GluA1 (pGluA1) at Ser845 (B), and pGluA1 at Ser831 (C) in whole hippocampal slices are shown. Data are presented as the ratio (fold of control) of GluA1 over actin (n = 5–16) and pGluA1 over actin (n = 4–6). ***, $P < 0.001$ as compared with control. #, $P < 0.05$, as compared with G1+DHPG (two-way ANOVA). Error bars indicate means ± SEM.