Supplementary Fig. 5. Brefeldin A treatment: EphB2 is trapped in the Golgi apparatus in GRIP1 siRNA expressing hippocampal neurons.

(a) Double immunostaining for surface and total EphB2-GFP in hippocampal neurons transfected at DIV13 with control pSuper vector or pSuper-GRIP1 siRNA#1. The projection of 6 single-plane confocal sections are shown. Most of EphB2-GFP is present at the surface (yellow); some EphB2-GFP signal can be found in intracellular punctate structures (green, indicated by arrows).

(b) Representative images of the cell body of hippocampal neurons triply transfected at DIV13 for 4 days with GFP, FLAG-EphB2 plus control pSuper vector or pSuper-GRIP1-siRNA#1. The neurons were incubated overnight with vehicle (Mock), 2 µM BFA or 2 µM BFA followed by a washout and 3h incubation in medium, and then stained with anti-FLAG antibody and anti-GM130 antibody to visualize EphB2 and the Golgi apparatus, respectively.

(c) Representative images of the cell body of hippocampal neurons cotransfected at DIV13 for 4 days with GFP and FLAG-EphB2Δ4C. Neurons were treated and stained as in (a).

Quantification of the ratio of immunostaining intensity in the Golgi / immunostaining intensity in dendrites for EphB2 is shown in Fig. 7c. BFA specifically interferes with ADP ribosylation factor (Arf)-dependent vesicle budding in the secretory pathway and blocks vesicle transport from the endoplasmic reticulum (ER) to the Golgi. Overnight treatment of control Flag-EphB2-transfected neurons with low concentration BFA (2 µM) blocks receptor trafficking out of the ER and results in a diffuse ER staining of Flag-EphB2 throughout the neuron and a reduced Golgi staining in the cell. After overnight treatment, washout of BFA allows the receptors to leave the ER and continue trafficking in the secretory pathway. Three hours after washout, EphB2 was found highly concentrated in the Golgi region. By contrast, neither BFA nor washout had any effect on the Golgi-pattern distribution of FLAG-EphB2 in neurons transfected with GRIP1-siRNA#1. The BFA data suggest that EphB2 is trapped in the Golgi region in GRIP1-knockdown neurons and cannot be distributed into the downstream compartments of the secretory pathway.