Supporting Information

Robust Induced Presynapse on Artificial Substrate as a Neural Interfacing Method

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Figure S1. Vector design of eNLG1. HA, hemagglutinin affinity tag; H6, hexa-His tag; GS linker, glycine-serine linker; RFP, red fluorescent protein; Avi-tag, biotin acceptor peptide

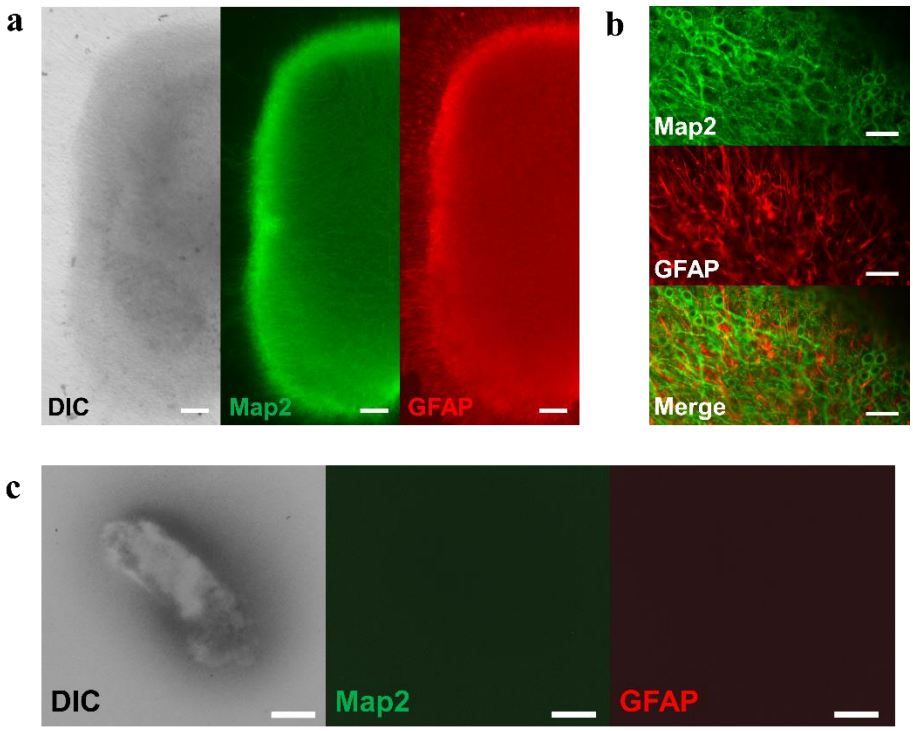

Figure S2. Organotypically cultured hippocampal slice. (a) DIC image and fluorescence image of organotypically cultured hippocampal slice in 13 DIV. Fluorescent labels are for Map2(green) and GFAP(red). Map2 is a neuronal marker protein and GFAP is glial marker protein especially for astrocyte. Both neurons and glia preserved themselves and grew for longer than 2 weeks period. Scale bar: 100 μm. (b) Confocal fluorescence images of the organotypically cultured hippocampal slice. Scale bar: 50 μm. (c) DIC and fluorescence images of cultured slice out of condition. Immunoscence labeling is for Map2(green) and GFAP(red). Neither Map2 nor GFAP signal is detected. Scale bar: 200 μm.

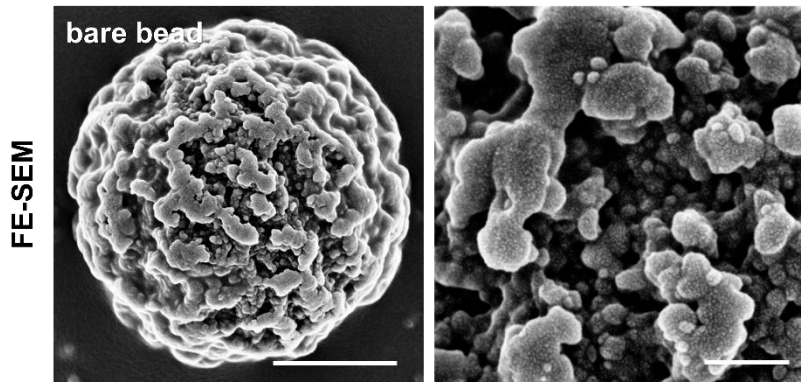

Figure S3. FE-SEM image of bare beads. Streptavidin coated beads have nano-grooves. Scale bar: 1 μm, 200 nm.

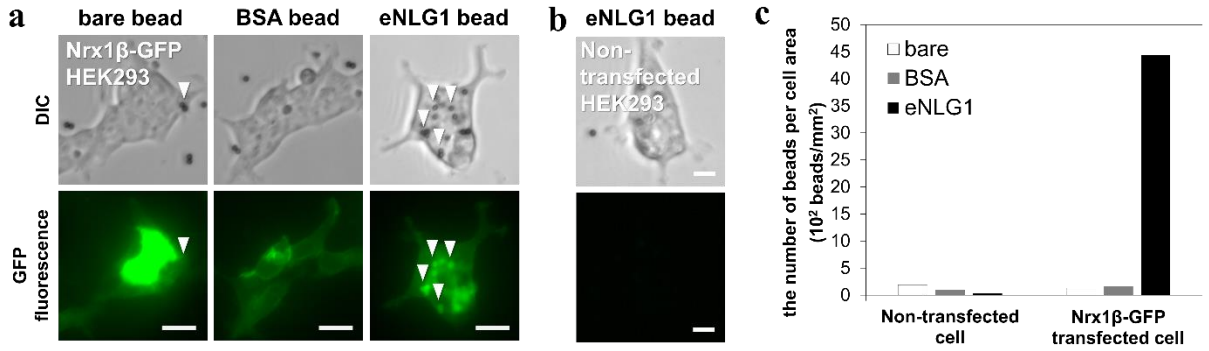

Figure S4. eNLG1 modified beads gather Nrx1β expressed on the plasma membrane of the HEK 293 cell. (a) DIC (upper row) and GFP fluorescent (lower row) images of HEK 293 cells transfected with NRx1β-GFP, incubated with bare, BSA-modified, and eNLG1-modified beads. White arrows indicate beads contacted with HEK 293 cell. Scale bar: 10 μm. (b) DIC (upper) and GFP fluorescent (lower) images of non-transfected HEK 293 cells, incubated with eNLG1-modified beads. Scale bar: 10 μm. (c) Number of beads bound per cell area in each condition (total cell area analyzed is over 50,000 um^2). We incubated Nrx1β-GFP transfected HEK293 cells with eNLG1-modified beads for 1 hour. The culture medium was replaced several times to wash out unbound beads in the medium. Bare beads and BSA coated microbeads were used as control groups. As a result, the eNlg1 bead made stronger adhesion to Nrx1β-GFP transfected cells than the control (p<0.01). Bare and BSA beads, or non-transfected cell surfaces showed poor binding affinity. Moreover, Nrx1β-GFP fluorescent intensity increased when the eNLG1 beads made contact with the membrane surface. Moreover, no change in fluorescent intensity occurred at the contact spot between non-transfected cell and eNLG1. Taken together, we deduced that the eNLG1-immobilized bead binds to the Nrx1β anchored in the cell membrane and causes an increase in local Nrx1β density.

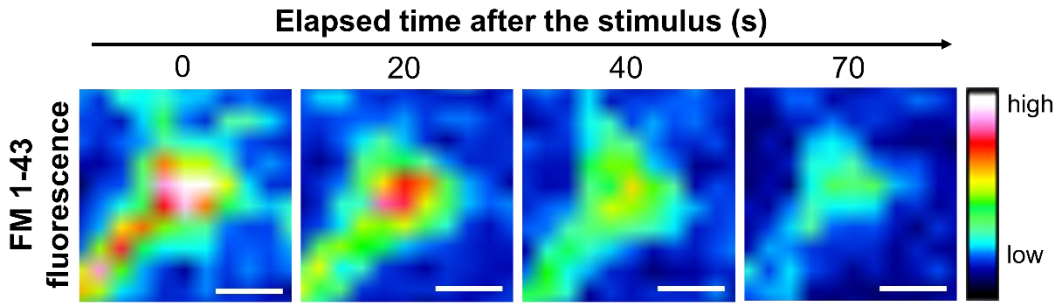

Figure S5. FM 1-43 release in the endogenous synapse. Time-lapse image of the fluorescence from FM 1-43 after the stimulating an endogenous synapse. Scale bar: 5 μm.

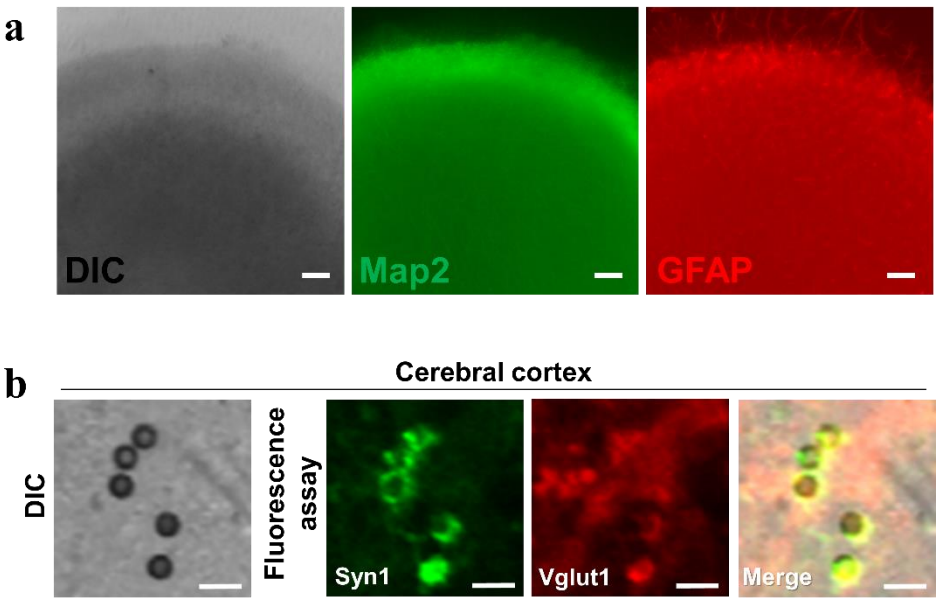

Figure S6. eNLG1 bead induces synapse formation in cultured cortex brain slice. (a) DIC and fluorescence image of cultured cortex brain slice. Fluorescent labels are for Map2 (green) and GFAP (red). Scale bar: 50 μm. (b) Representative image of immunostaining on synapsin1 (green) and Vglut1 (red) in hippocampal silce contact with eNLG1. 3 independent experiments were conducted. Scale bar: 5 μm.