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Supplemental Information

Plasticity of Synaptic Transmission in Human Stem Cell-Derived Neural Networks

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Transparent Methods

Construction of donor plasmid.

To generate AAVS1-pur-CAG-ChR2 (H134R)-EYFP donor plasmid, we amplified ChR2 (H134R)-EYFP cDNA by PCR from pAAV-hSyn-hChR2 (H134R)-EYFP (Addgene #26973). The ChR2 (H134R)-EYFP fragment was inserted into the AAVS1-pur-CAG-EGFP donor plasmid (Chen et al., 2016) to replace EGFP, generating the AAVS1-pur-CAG-ChR2 (H134R)-EYFP donor plasmid. Human codon-optimized wild type Cas9 (Cas9-2A-EGFP) and sgRNA T2 plasmids were obtained from Addgene (#44719, #41818) (Ding et al., 2013; Mali et al., 2013).

Generation of ChR2 (H134R)-EYFP expressing hESC and iPSC lines.

H9 hESCs were obtained from WiCell (line WA09, passages 20–40). The Down Syndrome (DS) iPSCs (UWWC1-DS1, trisomy 21) and isogenic control iPSCs (UWWC1-DS2U, euploid) was generated from mosaic fibroblasts of a Down syndrome patient (Weick et al., 2013). hESCs or iPSCs were pretreated by Rho Kinase (ROCK)-inhibitor (0.5 μM, Calbiochem, H-1152P) overnight. After disaggregation of hESCs or iPSCs by TrypleLE^{TM} (Life Technology), cells were dispersed into single cells and resuspended in the Electroporation Buffer (KCl 5 mM, $MgCl_2$ 5 mM, HEPES 15 mM, Na_2HPO_4 102.94 mM, NaH_2PO_4 47.06 mM, PH = 7.2) with 15 μg Cas9 plasmid, 15 μg sgRNA T2 plasmid, and 30 μg AAVS1-pur-CAG-ChR2 (H134R)-EYFP donor plasmid. Electroporation was carried out using the Gene Pulser Xcell System (Bio-Rad). Cells were subsequently plated and selected by

puromycin (0.5 μg/ml, Invivogen, ant-pr-1) for two weeks on the mouse embryonic fibroblast (MEF) feeder. Individual colonies were picked up and identified by genomic PCR.

Generation of glutamate neurons and GABA neurons from hESCs.

The procedure for generating glutamate or GABA neurons from hESCs was described previously (Li et al., 2009; Liu et al., 2013). ChR2-EYFP cells or H9 (passages 25–35) ESCs as well as ChR2-EYFP DS or isogenic iPSCs were cultured on a feeder layer with a daily change of medium for 1 week. Then, ESC colonies were detached from the feeder layer and grown in the ESC medium for 4 days to help form cell aggregates. For neural induction, cell aggregates were cultured with the Neural Induction Medium (NIM) (DMED/F12, 1% N2, 1% MEM NEAA, Life Technologies) supplemented with 2uM SB431542 (Stemgent), 2 uM DMH1 (Torcris) for additional 3 days. The ESC aggregates were then adhered to 6-well plates in NIM (plus 5%FBS) for 20h followed by another 2 days with the NIM from day4-7. By 10 days after ESC differentiation, neuroepithelial cells appeared in the form of rosettes. For glutamate neuron differentiation, the neuroepithelial cells were cultured in the NIM till neural tube-like rosettes formed at around day 16, which were gently blown off by a 1-ml pipette and suspended in the same medium for another 2 weeks. To induce GABA progenitors, the neuroepithelial cells were treated with SAG (sonic hedgehog agonist, 1 μM, Calbiochem) from d 10 till d 26.

For co-culture, the ChR2-EYFP glutamate neuronal progenitors and non-ChR2 GABA progenitors (day-26) were mixed by 2:1 under the neuron maturation medium (neural basal medium containing 1% N2 (Life Technologies), 2% B27 (LifeTechnologies), brain-derived neurotrophic factor (10 ng/ml, PeproTech), glial cell line derived neurotrophic factor (10 ng/ml, PeproTech), ascorbic acid (200 μM,

Sigma-Aldrich), cAMP (1 μM, Sigma-Aldrich), insulin-like growth factor I (PeproTech,10 ng/ml), Compound E (1 μM, Calbiochem, for 1 week after attachment) and glutamax (1:1000, Life technologies) for up to 5 weeks before whole-cell patch-clamp recordings

Immunochemical staining.

Immunostaining of coverslip co-cultures was performed as previously described (Chen et al., 2016). Briefly, neurons on coverslips were fixed in 4% paraformaldehyde for 30min and rinsed with PBS 3 times. Coverslips were incubated in a blocking buffer for 1 hour followed by primary antibodies (Goat GFP, Abcam, 1:1000; Rb GABA, sigma, 1:5000; Mouse monoclonal GFAP, Millipore, 1:1000) overnight at 4℃. Coverslips were incubated for 1 h at room temperature with fluorescently conjugated secondary antibodies (Invitrogen). The nuclei were stained with Hoechst 33258. Images were collected with a Nikon A1R-Si laser-scanning confocal microscope (Nikon, Tokyo, Japan).

Electrophysiology and ChR2 stimulation.

The cultured cells were continuously perfused with artificial cerebrospinal fluid (ACSF) saturated with 95% O_2/5% CO_2. The composition of ACSF was (in mM) 124 NaCl, 3.5 KCl, 1.5 $CaCl_2$, 1.3 $MgSO_4$, 1.24 KH_2PO_4, 18 $NaHCO_3$, 20 glucose, PH 7.4. Electrodes (Sutter instrument) were pulled from glass capillaries using a Sutter instrument puller (model P-97). The electrodes were filled with a inner solution consisting of (in mM) 140 K-gluconate, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 ATP K_2, 0.1

GTP Na_3, and 10 HEPES, PH 7.25 (290 mOsm) and had a resistance of 4-6 MΩ. Whole-cell voltage-clamp and current-clamp recordings were carried out at 30 ˚C. Neurons were visualized using an Olympus Optical (Tokyo, Japan) BX51WI microscope with differential interference contrast optics at 40X. Voltage and current clamp recordings were obtained using a MultiClamp 700B amplifier (Axon instruments). Signals were filtered at 4 kHz using a Digidata 1322A analog-to-digital converter (Axon instruments). Access resistance was observed prior to and following recordings and neurons with resistances > 25 MΩ at either point were discarded from analyses. Series resistance was compensated > 60% except in post-synaptic current (PSC) recording where it was not compensated.

To observe paired pulse facilitation (PPF), two synaptic responses were evoked by a pair of stimuli (5 ms pulse) given at short intervals (50 ms) at 0.05 Hz, PPF ratio is the ratio of the second eEPSC amplitude to the first, or P2/P1. Miniature EPSCs (mEPSCs) were recorded in sweeps of 2 s at a holding potential of -70 mV under the voltage clamp mode in the presence of tetrodotoxin (TTX; 0.5 μM) and picrotoxin (50 μM) to block voltage-dependent sodium channels and $GABA_A$ receptors, respectively.

A light stimulation fiber was placed 5mm from the dish and picrotoxin (50 μM) was included in ACSF to block $GABA_A$ receptors. Light stimulation was achieved by a custom-made LED device (1 Watt, 470 nm; Cree lighting Inc.) coupled to a fiber optic cable. A National Instruments USB-6501 DAQ provided the trigger pulses, with timing controlled by pClamp (Axon). The light-evoked EPSCs (eEPSCs) were elicited by

0.05 Hz under the voltage-clamp mode. After at least a 5 min baseline eEPSC collection, LTP of unitary eEPSCs was induced by 200 ms light stimulation pairing with postsynaptic depolarization (voltage step to 0 mV, 200 ms) under the current-clamp mode. Pairing was repeated 20 times at 0.1 Hz. For LTD induction, we applied low frequency stimulation (LFS, 900 pulse, 3 Hz) by blue light.

Calcium imaging.

Coverslip cultures were loaded with the calcium-sensitive fluorescent dye Quest Rhod-4 AM (10 μM) for 15 min at 37 °C (Lock et al., 2015). The fluorescence intensity was measured at 549 nm in response to 578 nm excitation and the fluorescence increase upon binding Ca^{2+} with little shift in wavelength. The data use the time points just before and after the light stimulation. The Ca^{2+} concentration was analyzed using intergrated optical density (IOD), which reflects a relative, not an absolute measurement of the free Ca^{2+} concentration. Images were collected with a Nikon A1R-Si laser-scanning confocal microscope (Nikon, Tokyo, Japan).

Single cell harvesting and in-tube reverse transcription.

Cell harvesting was performed according to the previous published paper (Fuzik et al., 2016). At the end of induction of LTP or LTD, the pipette was clamped to −5 mV (Vhold) and then a continuous series of depolarizing rectangular voltage pulses (5 ms at 5 ms intervals) were applied for 6 min with amplitudes of 25 mV from Vhold. One hour after that, we aspirated cell body with all the intracellular

components into the pipette tips with a minimal negative pressure. All the contents (about 0.5 µl) in the tip was ejected into freshly prepared 4.5 µl lysis buffer (0.25% NP40, 2.3 mM DTT, 0.4 U/µl RNAse inhibitor, and 1 µM OL(dT)30 Primer) in a 200 µl EP tube and stored at -80°C until batch processing. After all the samples were collected, the in-tube reverse transcription and amplification were performed as previously described with minor modification (Picelli et al., 2014). The samples were thawed and incubated at 70 °C for 3 min, then cooled to 4 °C. 5 µl reverse transcription buffer containing 0.7 mM dNTP, 1 µM TSO primer, 0.8 U/ µl RNAse inhibitor, and 1 U/ µl SMartase was added and incubated at 42 °C for 90 min followed by 70 °C for 10 min. The products of the reverse transcription were mixed with PCR buffer (1X EX Taq buffer, 0.4 mM dNTP, 0.4 µM IS PCR primer, and 0.05 U/µl TaKaRa EX Taq hot start) and amplified using thermal cycling 95 °C for 1 min (1 cycles), 95 °C 30 s, 65 °C 30 s, 72 °C 8 min (12 cycles). All the samples (cDNA) then underwent purification by AMPure XP beads (Beckman coulter). The Ct of GAPDH of all the samples was between 17-23.

Quantitative real time PCR.

Quantitative real time PCR was performed on CFX RT-PCR detection system (BioRad) using the iTaq SYBR green supermix (BioRad) according to the manufacturer's instructions. To determine the relative expression levels of different genes, ΔCt (Ct GAPDH-Ct gene) was calculated and normalized to the control cells (without laser, group LTP and LTD). Primers are listed in Table S1.

Statistical analysis.

All data were obtained from independent coverslip cultures. Offline data analysis was performed using MiniAnalysis software (Synaptosoft), Clampfit 9.0 (Axon) and origin (Origin). All data were presented as the mean ± SEM and significance was determined using the paired Student's t-test, one-way ANOVA, or two-way ANOVA.

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Figure S1. Related to Figure 1

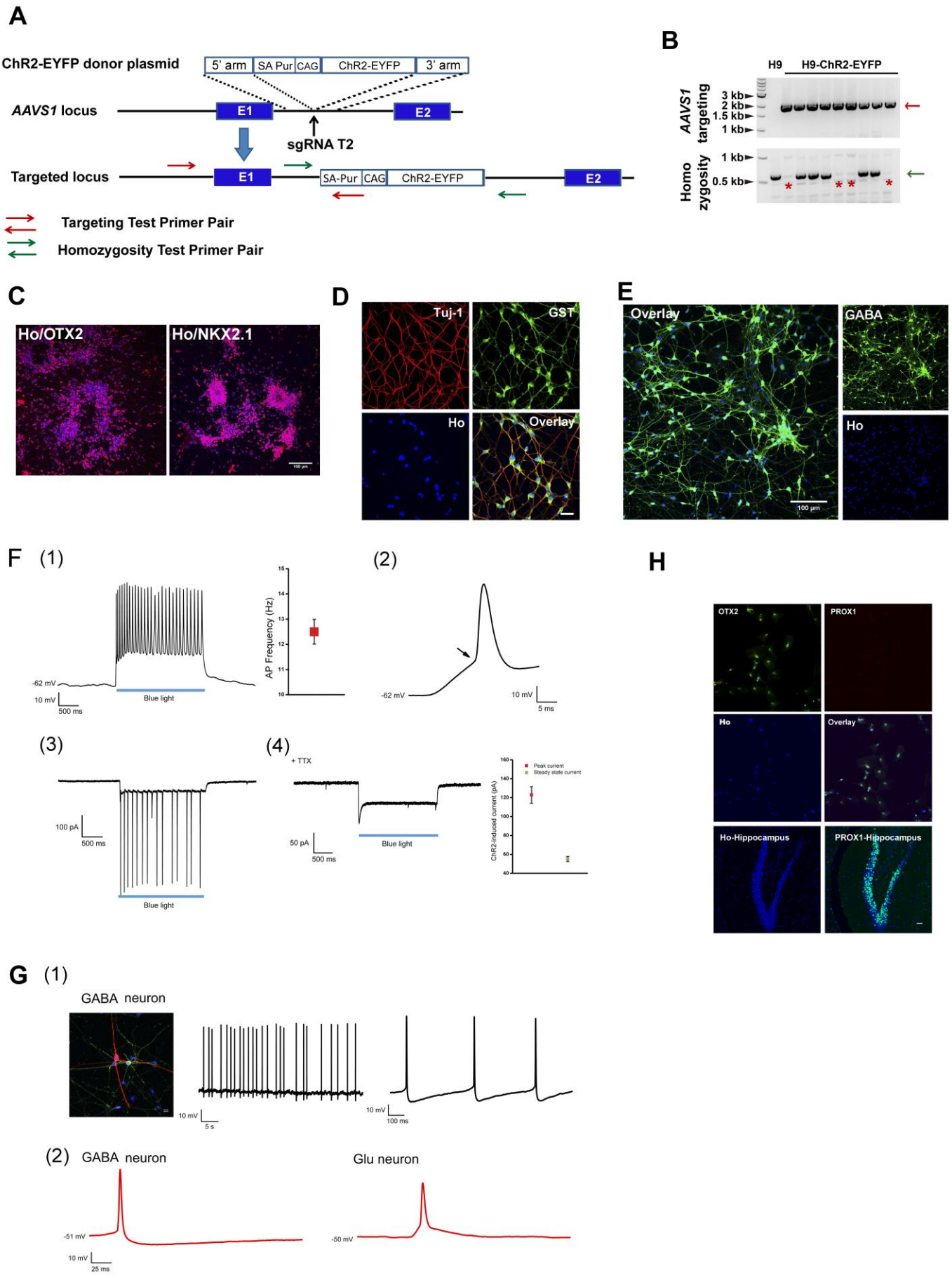

Figure S1. Establishment of ChR2-expressing hESCs and neural differentiation. A. Schematic diagram showing the strategy for knock-in of ChR2-EYFP-expressing cassette into the AAVS1 locus. Exons are shown as blue boxes. The vertical arrows indicate targeting site by sgRNA T2 in AAVS1 locus. SA-Pur, splice acceptor sequence followed by a T2A self-cleaving peptide sequence and the puromycin resistance gene. CAG, synthetic CAGGS promoter. EYFP is fused to the C terminus of ChR2. PCR primers for AAVS1 locus insertion or homozygosity are indicated as red arrows and green arrows, respectively. B. PCR genotyping of ChR2-EYFP-expressing hESC clones. Red arrow indicates the expected PCR products (~2000bp) for correctly targeted AAVS1 locus. Green arrow indicates the PCR products (~650bp) of heterozygous clones. Red asterisks indicate the homozygous clones without PCR products. C. Immunostaining of the co-cultured progenitors. Almost all cells were stained by OTX2 and NKX2.1, indicating ventral forebrain identity. D. Immunocytochemistry analysis of hESC-derived ChR2-expressing glutamate neurons. The efficiency for directing ChR2-expressing hESCs into glutamatergic neurons is 92.9 ± 3.3 % based on expression of glutamine synthase (GST) (n = 5 different differentiation cultures). Ho, Hoechst. Scale bar, 25 μm. E. Immunocytochemistry analysis of hESC-derived GABAergic neurons. The efficiency for directing hESCs into GABAergic neurons is 81.9 ± 2.3 % based on expression of GABA (n = 5 different differentiation cultures). Ho, Hoechst. Scale bar, 25 μm. F. Light-induced action potentials and currents in ChR2-expressing hESC-derived glutamatergic neurons. (1) Left: Representative traces from current-clamp recordings of a ChR2+ cell during a 2 s, 470nm light stimulus. Right: The averaged frequency of APs (n = 7). (2) Expanded timescale of current-clamp recording demonstrates the presence of action potentials by a clear threshold deflection (arrow). (3) Representative traces from voltage-clamp recordings of a ChR2+ cell during a 2 s, 470nm light stimulus. (4) Left: ChR2-induced inward currents in the presence of 1 μM Tetrodotoxin (TTX). Right: the averaged amplitude of the two components of ChR2-induced currents (n = 7). G. Difference of action potentials between glutamatergic neurons and GABA neurons. (1) Left, neurobiotin staining of recorded GABAergic neurons (Red: GABA neuron;

Green: ChR2-expressing neuron). Middle, spontaneous action potentials in an hESC-derived GABA neuron. Right, expanded timescale of the spontaneous action potentials in an hESC-derived GABA neuron. (2) Left, action potential in an hESC-derived GABA neuron. Right, action potential in an hESC-derived glutamatergic neuron. H. Immunofluorescence images of co-cultured neurons immunostained for OTX2, PROX1, Ho as well as immunofluorescence images of hippocampus immunostained for PROX1, Ho (Bottom panel). Ho, Hoechst. Scale bar, 100 μm.

Figure S2. Related to Figure 2 and Figure 3

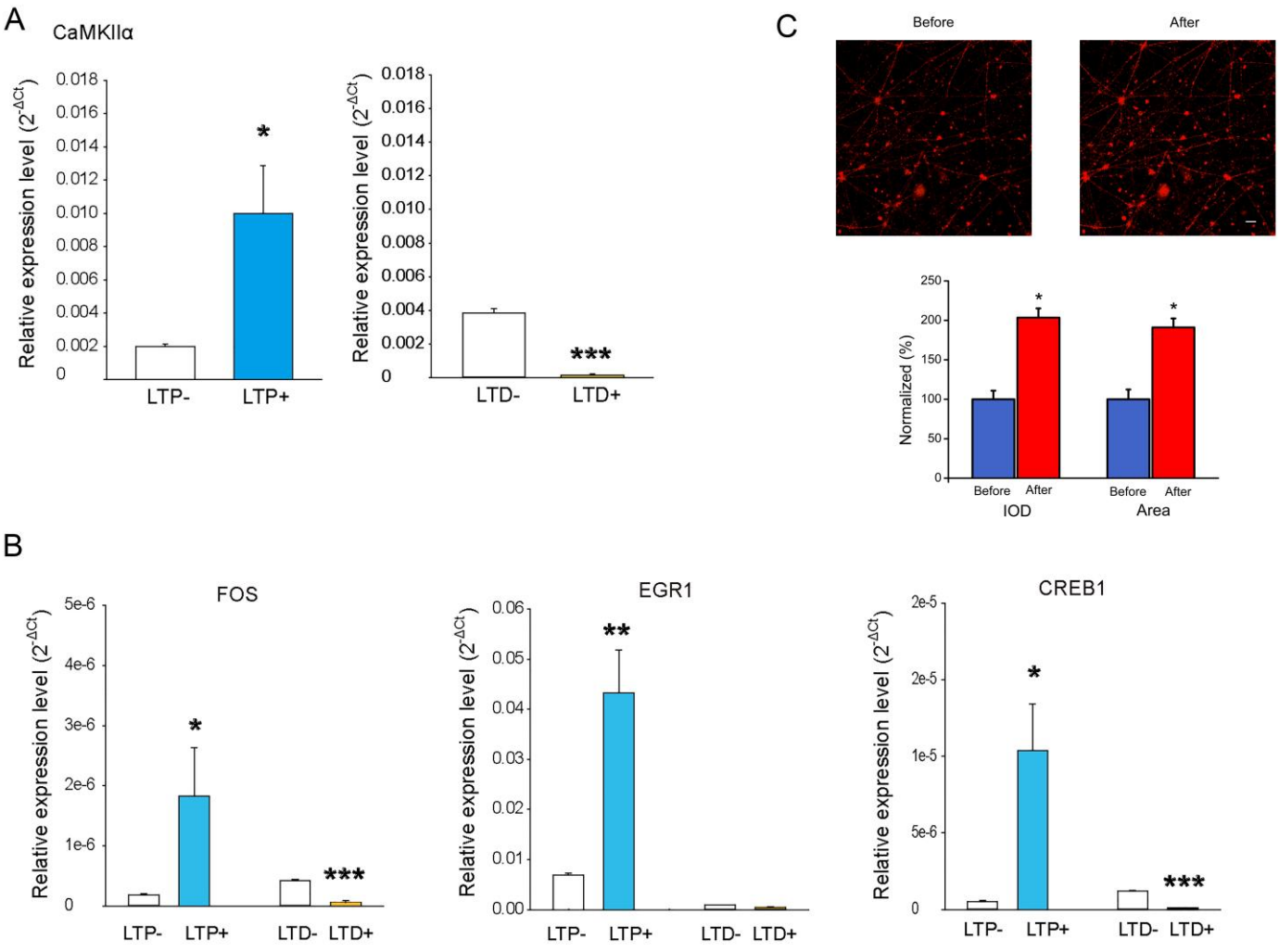

Figure S2. The molecular changes in postsynaptic cells after LTP or LTD induction. A. The expression changes of CAMKIIA in the postsynaptic neurons after LTP or LTD induction. The relative expression levels of CAMKIIA after LTP (Left) and LTD (Right) induction were shown in $2^{-\Delta Ct}$. $\Delta Ct = Ct_{GAPDH} - Ct_{gene}$. 4 cells in each group. * P < 0.05, ** P < 0.01, ***P<0.001; t-test. B. The expression changes of IEGs in the postsynaptic neurons after LTP or LTD induction. The relative expression levels of IEGs FOS (Left), EGR1 (Middle), and CREB1 (Right) after LTP and LTD induction were shown in $2^{-\Delta Ct}$. $\Delta Ct = Ct_{GAPDH} - Ct_{gene}$. 4 cells in each group. * P < 0.05, ** P<0.01, ***P < 0.001; t-test. C. Effect of light stimulation on calcium influx in co-cultured neurons. Scale bar, 100 μm. n= 4, P < 0.001, t-test.

Table S1 Related to Figure 2 and Figure 3. Primers for qPCR

Gene	5’ primer	3’ primer
GAPDH	GCACCGTCAAGGCTGAGAAC	AGGGATCTCGCTCCTGGAA
CAMK2A	ACCACTACCTGATCTTCGACC	CCGCCTCACTGTAATACTCCC
FOS	CCGGGGATAGCCTCTCTTACT	CCAGGTCCGTGCAGAAGTC
EGR1	GGTCAGTGGCCTAGTGAGC	GTGCCGCTGAGTAAATGGGA
CREB1	ATTCACAGGAGTCAGTGGATAGT	CACCGTTACAGTGGTGATGG