Modeling Apolipoprotein E ε3/4 allele-associated sporadic Alzheimer’s disease in an induced neuron

Supplementary Fig. 1-8.
Supplemental Figure 1. (A) Immunofluorescence staining of Tuj1+ cells in control and APP expressing iNs at 7 days after Ascl1, Brn2, and Myt1I infection. Scale bar = 100 µm. (B) The number of Tuj1 positive cells in the control iNs and APP expressing iNs on day 7 after initial factor infection. Data represent mean ± SEM. (C) Representative image of VGlut1 and Map2 positive cells in control iNs and APP expressing iNs on day 7 after initial factor infection. Scale bar = 100 µm. (D) mRNA expression levels of control iNs and APP expressing iNs for additional neuronal marker genes Tuj1, Map2, Gap43 and Synspain1. (E) Quantification of VGlut1/Tuj1 positive cells in control and APP expressing iNs. Data represent mean ± SEM. (F) Immunostaining of mouse iNs and APP expressing iNs for a neuronal stem cell marker Nestin, at 8 days after doxycycline induction. Scale bar = 100 µm. (G) Quantitative real time-polymerase chain reaction analysis of mouse iNs and APP expressing iNs for mature neuronal markers, Mapt, Map2, GAD67, Synapsin1, Tbr1 and Ctip2 at 8 days after doxycycline induction. Data represent mean ± SEM. ANOVA-test, *p<0.05, **p<0.01; (n=3 per each sample). (H) Fluorescence-activated cell sorting analysis of Tau-eGFP positive cells reprogrammed from Tau-eGFP knockin fibroblasts in control and APP expressing iNs.
Supplemental Figure 2. (A) Co-localization analysis of EEA positive puncta with total APP positive puncta in iNs and APP expressing iNs. Scale bar = 50 µm. (B) The percentage of APP(+) and EEA1(+) puncta per total EEA1 positive puncta. Data represent mean ± SEM. Student’s t-test, **p<0.01. (C) Quantitative real time-polymerase chain reaction analysis of BACE1 in controls and APP expressing iNs on 16 days after doxycycline induciton. (D) Immunostaining of Tuj1 and activated Caspase-3 positive cells in control and APP expressing iNs. Scale bar = 50 µm. (E) The number of Tuj1+ cells in the hydrogen peroxide treated control and APP expressing iNs. Data represent mean ± SEM. Student’s t-test, *p<0.05, **p<0.01. (F) Survival rate of hydrogen peroxide treated control and APP expressing iNs. Data represent mean ± SEM. Student’s t-test, **p<0.01; (n=3 per each sample). (G) The percentage of amyloid-β42/VGluT1 positive cells on 15 days after doxycycline induction with β-secretase inhibitor treatment. Data represent mean ± SEM. ANOVA test, *p<0.05, **p<0.01. (H) Measurement of neurite length in iNs, APP expressing iNs and following γ-secretase inhibitor treatment on day 20. Data represent mean ± SEM. ANOVA test, *p<0.05, **p<0.01; (n=10 per each sample). (I) Quantitative real time-polymerase chain reaction of neuronal markers (NeuroD1, Map2, Synapsin1 and Gad67) on control and APP expressing iNs treated with β-secretase inhibitor. Data represent mean ± SEM. ANOVA test, *p<0.05, **p<0.01. (J) Thioflavine-T staining for control and APP expressing iNs at 16 days after doxycycline induction. In the thioflavine-T solution treated sample (50 µM), amyloid beta deposits were detected in the APP expressing iNs. Scale bar = 100 µm. (K) The percentage of thioflavine-T positive cells per Tuj1 positive cells in the control and APP expressing iNs at 16 days after doxycycline induction. Data represent mean ± SEM. ANOVA test, *p<0.05, **p<0.01. (I) Intensity of Thioflavine-T staining in the amyloid beta deposits. Amyloid beta deposits were decreased in APP expressing iNs with β-secretase inhibitor treatment. Data represent mean ± SEM. ANOVA test, *p<0.05, **p<0.01; (n=5 per each sample).
Supplemental Figure 3. (A) Schematic of the direct lineage reprogramming in human fibroblasts into hiNs. FUW-Ascl1, Brn2, Mytl1, NeuroD and M2rtta, TetO-mutant APP were introduced to human fibroblasts and plated on nanopatterned substrates. (B) Representative images of nanopatterned substrates using a scanning electron microscope (spacing of 400 nm, ridge width of 300 nm). Scale bars = 1 µm. (C) Scannig electron microscope image of hiNs plated on nanopatterned substrates. Scale bars=1 µm. (D) Fluorescence-activated cell sorting analysis for RFP+ cells derived from hSynapsin-RFP infected hiNs and APP expressing hiNs on nanopatterned substrates. The left pannel was not infected by hSynapsin-RFP. (E) Measurement of neuron length and soma size on the nanopatterned substrates. Data represent mean ± SEM. Student’s t-test, **p<0.01. (F) Morphology of human fibroblasts and sporadic AD patient fibroblasts (AG11414) on control and nanopatterned substrates for the indicated time of direct reprogramming. Human fibroblasts from healthy control were purchased from Coriell institute. Scale bar = 100 µm. (G) The number of TUJ1, MAP2+ iNs between control and sporadic AD patient iNs. 10 independent fields for each group were counted in the three independent set of experiments. Data represent mean ± SEM. Student’s t-test, **p<0.05.
Supplemental Figure 4. (A) Co-localization analysis of EEA1/APP positive puncta in APOE ε3/4 hiNs and APP expressing APOE ε3/4 hiNs. Scale bar = 50 µm. (B) The percentage of EEA1/APP+ puncta per total EEA1 positive puncta on a nanogrooved pattern. Data represent mean ± SEM. (C) Quantitative real time-polymerase chain reaction analysis of BACE1 in APOE ε3/4 hiNs and APP expressing APOE ε3/4 hiNs on day 15. Data represent mean ± SEM. (D) Immunostaining showing the expression of TUJ1 and amyloid-β42 on a nanogrooved pattern with β-secretase inhibitor and γ-secretase inhibitor treatment. Scale bar = 100 µm. (E) Quantitative real time-polymerase chain reaction of neuronal markers (Map2, Synapsin1, TBR1 and CTIP2) on APOE ε3/4 iNs and APP expressing APOE ε3/4 iNs treated with β-secretase inhibitor. Data represent mean ± SEM. Student’s t-test, **p<0.05, ***p<0.01.
Supplemental Figure 5. (A) Boxplots showing microarray expression levels of differentially expressed genes. The color of boxplots indicates specific group color in Figure 4A and average median of boxplots are showed by yellow dotted line. (B) Protein-protein interaction network showing full network of differentially expressed genes (|fold change| ≥ 1.5). (C) Subnetwork of (B) directly interacts with DSG2. (D) Nucleotide sequencing data of APOE ε3 and APOE ε4 genotyping in sporadic AD patient cell lines (AG05810 and AG11414). APOE ε3 allele has cysteine residues at the amino acid residues 112 and arginine at position 158, while APOE ε4 allele has arginine residues at both sites. (E) We confirmed upregulation of DSG2 genes in sporadic AD patient fibroblasts (APOE ε3/4 genotypes) using both DSG2 primers.
Supplemental Figure 6. (A) Quantitative real-time polymerase chain reaction analysis of neuronal markers (ChAT, MAPT and Synapsin1) in APP expressing APOE ε3/4 patient iNs and APP expressing APOE ε3/4 patient iNs treated with DSG2-shRNA at 10 days after doxycycline induction. (B and C) Knockdown of DSG2 did not significantly reduce amyloid-β (6E10) oligomer levels in APP expressing familial AD patient (APOE ε3/3 genotype) iNs. (D) Analysis of neurite length (left) and soma size (right) in patient iNs and APP expressing patient iNs treated with DSG2-shRNA about control human cell line and 5 patient cell lines.
Supplemental Figure 7. (A and B) β-secretase inhibitors treatment (0.1μM) for 3 days decreased the number of amyloid puncta in APP-/DSG2-expressing control (APOE ε3/3 genotype) iNs and sporadic AD patient (APOE ε3/4 genotype) iNs. Scale bar = 50 µm. Data represent mean ± SEM. Student’s t-test, *p<0.05 **p<0.01; n=6 per each sample. (C) Quantitative real time-polymerase chain reaction analysis of DSG2 expression in APP-/DSG2-expressing control (APOE ε3/3 genotype) iNs and sporadic AD patient (APOE ε3/4 genotype) iNs. Data represent mean ± SEM. Student’s t-test, *p<0.05 **p<0.01; n=6 per each sample. (D and E) Overexpression of DSG2 significantly increase amyloid-β (6E10) oligomer levels in APP expressing sporadic AD patient (APOE ε3/4 genotype) iNs. Western blot intensity shows reduction of β-amyloid oligomers in APP-/DSG2-expressing control (APOE ε3/3 genotype) iNs and sporadic AD patient (APOE ε3/4 genotype) iNs by β-secretase inhibitors treatment.
Supplemental Figure 8. (A) Immunofluorescence analysis of amyloid-β42 was performed in APP expressing human APOE ε4 mouse iNs. In shDSG2-GFP treated APP expressing APOE ε4 mouse iNs, amyloid-β42 levels were decreased. Scale bar = 50 µm. (B) Relative intensity of amyloid-β42 staining in APP expressing APOE ε4 mouse iNs. Expression of amyloid-β42 shows a significantly lower level of amyloid-β in DSG2-shRNA treated iNs as compared to untreated iNs. Data represent mean ± SEM. ANOVA test, *p<0.05, **p<0.01; (n=6 per each sample).