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Generation of a set of isogenic, gene-edited iPSC lines homozygous for all main APOE variants and an APOE knock-out line

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Abstract

Alzheimer's disease (AD) is the most frequent neurodegenerative disease amongst the elderly. The SNPs rs429358 and rs7412 in the APOE gene are the most common risk factor for sporadic AD, and there are three different alleles commonly referred to as APOE-ε2, APOE-ε3 and APOE-ε4. Induced pluripotent stem cells (iPSCs) hold great promise to model AD as such cells can be differentiated *in vitro* to the required cell type. Here we report the use of CRISPR/Cas9 technology employed on iPSCs from a healthy individual with an APOE-ε3/ε4 genotype to obtain isogenic APOE-ε2/ε2, APOE-ε3/ε3, APOE-ε4/ε4 lines as well as an APOE-knock-out line.

Resource Table: *Please fill in right-hand column of the table below. All information requested in the table is MANDATORY, except where otherwise indicated. Manuscripts with incomplete or incorrect information will be sent back to author*

Unique stem cell lines identifier	1) EBiSC reference BIONi010-C-6 = *APOE-ε2/ε2* = Biosample ID: SAMEA4454009; ECACC 66540368 2) EBiSC reference BIONi010-C-2 = *APOE-ε3/ε3* = Biosample ID: SAMEA4342705; ECACC 66540268 3) EBiSC reference BIONi010-C-4 = *APOE-ε4/ε4* = Biosample ID: SAMEA4452060; ECACC 66540366 4) EBiSC reference BIONi010-C-3 = *APOE*-knock-out

	= Biosample ID: SAMEA4342740; ECACC 66540269 5) EBiSC reference BIONi010-C = *APOE-ε3/ε4* = Biosample ID: SAMEA3158050; ECACC 66540023
Alternative names of stem cell lines	*N/A*
Institution	*Bioneer A/S*
Contact information of distributor	*Benjamin Schmid, bsc@bioneer.dk*
Type of cell lines	*iPSC*
Origin	*Human*
Cell Source	*Fibroblasts*
Clonality	*clonal*
Method of reprogramming	*Episomal plasmids (Okita et al., 2011)*
Multiline rationale	*Isogenic clones*
Gene modification	*YES*
Type of modification	*Single base change*
Associated disease	*Alzheimer's disease*
Gene/locus	*APOE; 19q13.32; rs7412; rs429358*
Method of modification	*CRISPR/CAS9*
Name of transgene or resistance	*N/A*
Inducible/constitutive system	*Not inducible*
Date archived/stock date	*June 2016*
Cell line repository/bank	https://www.ebisc.org/
Ethical approval	https://www.ebisc.org/

Resource utility

The biological mechanisms, by which the APOE-ε4 allele increases the risk of developing AD, are still unknown. To better understand the role of APOE in the aetiology of AD, we have established a set of isogenic iPSC lines harbouring all different haplotypes in homozygosity (APOE-ε2/ε2, APOE-ε3/ε3, APOE-ε4/ε4) and an APOE knock-out (KO) line.

Resource Details

The APOE gene encoding for Apolipoprotein E is the most important risk gene for AD. The SNPs rs429358, which is part of the codon for amino acid 112, and rs7412, which is part of the codon for amino acid 158, determine the APOE genotype. For both SNPs, either a C or a T can be found on DNA level, leading to either an arginine or a cysteine on protein level, respectively. The three major combinations of the bases (rs429358-T/rs7412-T; rs429358-T/rs7412-C; rs429358-C/rs7412-C) result in three genotypes commonly referred to as APOE-ε2/ε2, APOE-ε3/ε3 and APOE-ε4/ε4. The APOE-ε4 allele increases the risk for late-onset AD comprising over 95% of AD cases (Chouraki and Seshadri, 2014). By contrast, the APOE-ε2 allele reduces the risk for AD and is considered to be protective (Conejero-Goldberg et al., 2014). Despite this genetic link, little is known about the role of APOE in the progression of the disease. We combined iPSC and CRISPR/Cas9 technology to generate a set of isogenic iPSC lines with either an APOE KO or an APOE-ε2/ε2, an APOE-ε3/ε3, or an APOE-ε4/ε4 genotype, which could help dissecting the contribution of each genetic *APOE* variant to the risk or protection to develop AD. Reprogramming of the iPSC line BIONi010-C, previously generated from human skin fibroblasts of an 18-year-old healthy individual (Rasmussen et al., 2014, original name of the iPS cell line: K3_shp53) carrying an *APOE-ε3/ε4* genotype, was performed by electroporation with three episomal plasmids encoding *hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC*, *hLIN28* and *shp53* (Okita et al., 2011; Takahashi et al., 2007). Sample meta-data is available in the Biosamples database (http://www.ebi.ac.uk/biosamples) under accession number SAMEA3158050. This cell line is part of the EBiSC repository (https://cells.ebisc.org/) and can be ordered via ECACC (https://www.phe-culturecollections.org.uk/collections/ecacc.aspx) under catalog number 66540368.

Starting from this control line, we generated the four different gene-edited clones (Supplementary 1A). To generate the homozygous APOE-ε4/ε4 genotype, the *APOE* alleles of the original BIONi010-C line were changed to a homozygous C at rs429358 using CRISPR2-recT, a CRISPR recognizing the T at rs429358, and the ssODN rs429358-C as homologous template with a C at rs429358 (Table 3, Supplement 1A). To generate the homozygous APOE-ε3/ε3 genotype, the APOE alleles of the original BIONi010-C line were changed to a homozygous T at rs429358 using CRISPR2-recC, a CRISPR recognizing the C at rs429358, and the ssODN rs429358-T as homologous template with a T at rs429358 (Table 3, Supplement 1A). To generate the homozygous APOE-ε2/ε2 genotype, the alleles of the BIONi010-C2 line with the APOE-ε3/ε3 genotype were changed to a homozygous T at rs7412 using CRISPR3 recC, a CRISPR recognizing the C at rs7412, and the ssODN rs7412-T as homologous template with a T at rs7412 (Table 3, Supplement 1A). Finally, the APOE-KO line was generated in the original BIONi010-C line using CRISPR 1 (Table 3, Supplement 1A) targeting exon 2 of the APOE gene without an ssODN in order to delete a part of the gene resulting in a gene KO. After gene-editing was completed, 50 vials of each line were frozen down. Subsequently, one vial of each line was thawed and analyzed. All the lines showed a normal iPSC morphology by light microscopy one or two days after thawing (Figure 1A). They all expressed the pluripotency markers Nanog, Oct4, SSEA3 and Tra-1-81 by ICC (Figure 1B) as well as Oct4 and Sox2 by flow cytometry, whereas the differentiation marker SSEA1 was negative (Figure 1C). A directed trilineage differentiation assay revealed that ecto-, meso- and endodermal celltypes were detectable in all the lines (Figure 1D). A karyotype was carried out by G-banding, and up to the banding quality of 400 – 500, no numerical or structural abnormalities were detectable. All the lines showed a normal male karyotype of 46, XY (Figure 1E). Resequencing of the relevant part of the APOE gene confirmed the correct genotypes of APOE-ε2/ε2, ε3/ε3, ε4/ε4 (Figure 1F) and a frame shift in exon 2 of the BIONi010-C-3, the APOE KO line (Supplement 1C). Western blot analysis of the APOE KO line BIONi010-C-3 showed that no ApoE protein was present (Figure 1F). In contrast to that, ApoE protein was still expressed in all the other gene-edited lines to a similar extent to that of the original BIONi010-C line (Supplement 1D). Microbiological contaminations were investigatd by three tests: First, a general microbiological test on all the lines was carried out by inoculating 1 mL of the supernatant of the iPSC medium in 10 mL LB growth medium (all lines negative, data not shown). Second, a PCR based virology screening at the Institute for medical virology (University of Tübingen) was carried out on the original line BIONi010-C (negative for HIV, HBV and HCV, data not shown). Third, a PCR based mycoplasma test on all the lines was done (all negative, Supplement 1B). No contaminations were found in either of the three tests. Finally, an STR analysis revealed that all the gene-edited lines including the original line BIONi010-C had the same identity (in archive with journal).

Materials and Methods

CRISPR design

Isogenic gene-corrected controls were obtained using the CRISPRs/Cas9 system in combination with an ssODNs serving as homologous template covering the site of the mutation. The CRISPRs were generated following the protocol from Ann Ran (Ran et al., 2013). Briefly, small guide RNAs (sgRNAs, Table 3) targeting either SNP rs429358, SNP rs7412 or exon2 of the APOE gene were designed at http://crispr.mit.edu/. The sgRNAs were cloned into a plasmid containing the sequence for Cas9 using BsaI (pSpCas9(BB)-2A-Puro (PX459); Addgene plasmid #62988). The sequences of the CRISPRs were confirmed using primer U6-FW and pSpCas9-(339)-RV1 (Table 3).

Nucleofection

iPSCs were cultured in 6 well plates coated with Matrigel (Corning Bioscience) in E8 medium and detached using Accutase. When they reached a density of 70 – 90%, a total of 1.5 x 10^6 cells were co-nucleofected with 10 µg of the CRISPR/Cas9 plasmid "pSpCas9(BB)-2A-Puro (PX459) V2.0" containing a puromycin resistance

cassette, and 1 µL of 100 µM ssODN using the P3 Primary Cell Kit (Lonza) using program CA167 following to the manufacturer's instructions (Lonza). iPSCs were subsequently transferred back to a Matrigel-coated 100 mm dish in E8 medium supplemented with 1:200 diluted Revita cell supplement (Gibco). 24 h post-nucleofection, cells were subjected to puromycin (Invitrogen) selection for 4 hours at a concentration of 10 µg/mL and allowed to recover for one week. Resistant colonies were then picked and expanded for genotyping.

Genotyping

DNA for genotyping was extracted using the prepgem kit from ZyGEM following the manufacturer's instructions. The DNA solution was diluted 1:5 with water. Genotyping was carried out using AmpliTaq Gold Polymerase (Thermo Fisher) according to the manufacturer's instructions at an annealing temperature of 60° C and the PCR primers APOE HhaI FW/RV (Table 3). The PCR products were digested using the restriction enzyme HhaI (NEB) for 1h to detect genetically modified clones. Positive candidates were then sequenced using sequencing primer APOE HhaI Seq FW (Table 3). Sequencing analysis of the KO line was carried out with the PCR primers SURV APOE KO FW/RV and the sequencing primer SURV APOE KO seq FW (Table 3). Clones with a frame shift were subjected to Western blot analysis. Briefly, iPSCs from one well of a 6-well plate were detached with a cell scraper and transferred to a 2 mL Eppendorf tube and spun down at 120 g for 5 minutes. The cell pellets were lysed in 50 µL of RIPA buffer (Invitrogen) containing Roche protease inhibitor. Lysates were centrifuged at 14.000 g for 10 minutes at 4°C. The protein concentration was determined using the Pierce BCA protein kit (Thermo Scientific) and 15 µg protein were loaded on an Invitrogen™ Novex™ pre-cast Tris-Glycine 12% gel with trysglycine running buffer at 126V for 90 minutes and blotted on an Invitrogen™ Novex™ Nitrocellulose membrane at 35 V for 70 minutes. The membrane was blocked in 5% skim milk diluted in TBS with 0.1% Tween 20 for 1 hour. Membrane was incubated with the ApoE antibody (NOVUS Biologicals, NB110-60531, WUE-4, mouse, 1:1000) over night at room temperature. After washing, the blot was incubated with goat anti-mouse IgG-HRP (sc-2005) from Santa Cruz Biotechnology (1:5000). Bands were visualized with Pierce™ ECL Western Blotting Substrate (ThermoFisher).

Cell banking

iPSCs were grown in three 15 cm plates on matrigel in E8 medium to a density of 80%. Cells were detached with 0.1% EDTA and centrifuged at 120g for 5 minutes. The cell pellet was resuspended in 50 mL of freezing medium (50% E8, 40% FCS, 10% DMSO), and 1 mL aliquots were distributed in cryo vials. The vials were transferred in isopropanol containers into a -80° C freezer over night. For long term storage, the cells were transferred into a nitrogen tank.

Morphology

The morphology was investigated by light microscopy 1 or 2 days after thawing one vial from the bank.

Karyotyping

For karyotyping, the cells were treated with colcimid (Gibco) when they were 60 – 80 % confluent. The cells were then incubabted with 0.075 M KCl for 30 minutes at 37° C and fixed with 1:3 acidic acid:methanol and sent for G-band karyotyping (University of Tübingen). At least 15 metaphases were counted and 6 of them were structurally evaluated by G-banding and a banding quality of 400-500.

STR Analysis

For the STR analysis, DNA was extracted (Qiagen) and analyzed using the AmpFLSTR Identifiler PCR Amplification kit (Applied Biosystems).

Microbiology

General microbiology was investigated by growing 500 µL of the supernatant in LB medium for 2 days at 37° C. Virology was investigated on the medium supernatant of the parental line by PCR analysis (Rasmussen et al., 2014).

Integration of CRISPR plasmids

Analysis for the integration of CRISPR plasmid was carried out by PCR using primers U6-FW and pSpCas9-(339)-RV1 (Table 3).

Expression of pluripotency markers:

Expression of pluripotency markers were investigated by both ICC and trilineage differentiation followed by flow cytometry.

ICC:

iPSCs were grown on a glass coverslip coated with matrigel in E8 medium. When defined colonies were detectable, the cells were fixed with ice cold methanol at -20 C° C for 10 minutes. The cells were washed with PBS and blocked with blocking solution (2% BSA and 0.1% Triton-X-100 in PBS) for 15 minutes at room temperature. Primary antibodies were added in the respective dilution (Table 2) and incubated over night at 4° C. The cells were washed three times with blocking solution and incubated with the respective secondary antibody (Table 2) in blocking solution at room temperature for 1 hour. The cells were washed again three times with blocking solution and once in water. The coverslips were finally put on glass slides with mounting solution containing DAPI from Invitrogen and investigated by fluorescence microscopy.

Trilineage differentiation:

For trilineage differentiation, the iPSCs were split with accutase into a well of a 12-well plate with E8 medium on matrigel in different densities: 200,000 cells/cm^2 for ecto- and endoderm and 50,000 cells/cm^2 for mesoderm. For ectodermal differentiation, the medium was changed to neural induction medium (50% DMEM F12 and 50% Neurobasal medium, 1X B27 without retinoic acid, 1X N2 supplement, 1X glutamax, 1X Pen/Strep (all Gibco), 10 µM SB431542, 0.1 µM LDN193189 (both from Selleckchem)) on day one. The medium was changed every day until day six. For endodermal differentiation, the medium was changed to MCDB131-1 medium (MCDB131 medium, 1.5 g/L NaHCO3, 1X glutamax, 1X Pen/Strep (all Gibco), 10 mM glucose (Sigma), 0.5% BSA) on day one including 3 µM CHIR99021 (Selleckchem) and 100ng/mL Activin A (Cell Guidance Systems). On day two, CHIR99021 was withdrawn and MCDB131-1 medium with activing A was changed every day until day six. For mesodermal differentiation, the medium was replaced by mesodermal induction medium (APEL medium (Gibco), 25 µg/mL Activin A (Cell Guidance Systems), 30 ng/mL BMP4 (Peprotech), 50 ng/mL VEGF (peprotech), 1.5 µM CHIR99021 (Selleckchem)), which was left on the cells for two days. On day three, the medium was changed to vascular specification medium (APEL medium, 50 ng/mL VEGF, 10 µM SB431542 (Selleckchem)), which was then changed every day until day six.

Flow cytometry:

After 6 days, all the trilineage differentiations were split with 0.5 mL accutase (10 minutes incubation at 37° C). The cells were mixed with 1.5 mL 2% BSA solution and resuspended with a pipet to generate single cells. 200,000 cells were spun down at 120g. the pellet was resuspended in 0.5 mL of Foxp3 fixation/permeabilization working solution (diluted 1:3, Invitrogen) and incubated at RT for 30 minutes. Cells were washed in 1 mL 1X permeabilization buffer, centrifuged at 120g and resuspended in permeabilization

buffer containing the antibodies in the respective contrentration. After 45 minutes incubation at room temperature, the cells were centrifuged and resuspended in 200 µL permeabilization buffer. The cells were transferred to one well of a 96 well plate with round bottom shape and washed three times by repeating the centrifugation and resuspending step. The analysis was run at a calibrated flow cytometer (analyze 50.000 cells at high speed in 150 µl buffer).

Supplementary Fig. 1

Acknowledgments

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References:

Chouraki, V., and Seshadri, S. (2014). Genetics of Alzheimer's disease. Adv Genet *87*, 245-294.

Conejero-Goldberg, C., Gomar, J.J., Bobes-Bascaran, T., Hyde, T.M., Kleinman, J.E., Herman, M.M., Chen, S., Davies, P., and Goldberg, T.E. (2014). APOE2 enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. Mol Psychiatry *19*, 1243-1250.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K.*, et al.* (2011). A more efficient method to generate integration-free human iPS cells. Nat Methods *8*, 409-412.

Rasmussen, M.A., Holst, B., Tumer, Z., Johnsen, M.G., Zhou, S., Stummann, T.C., Hyttel, P., and Clausen, C. (2014). Transient p53 suppression increases reprogramming of human fibroblasts without affecting apoptosis and DNA damage. Stem Cell Reports *3*, 404-413.

Table 1: Summary of lines

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
BIONi010-C-6	*APOE-ε2/ε2*	*Male*	*18*	*African*	*APOE-ε2/ε2*	*N/A*
BIONi010-C-2	*APOE-ε3/ε3*	*Male*	*18*	*African*	*APOE-ε3/ε3*	*N/A*
BIONi010-C-4	*APOE-ε4/ε4*	*Male*	*18*	*African*	*APOE-ε4/ε4*	*N/A*
BIONi010-C-3	*APOE-KO*	*Male*	*18*	*African*	*APOE-knock-out*	*N/A*

Table 2: Characterization and validation

Classification	Test	Result	Data
Morphology	Photography	*Visual record of the lines: normal iPSC morphology*	*Figure 1A*
Phenotype	Qualitative analysis by ICC	*All lines express the stem cell markers Nanog, Oct4, SSEA3 and Tra-1-81*	*Figure 1B*
	Quantitative analysis by flow cytometry	*Oct4, Sox2: more than 96 % positive for all lines (negative control: same line without antibody) SSEA1: less than 0.11% positive for all lines (negative control: same line without antibody)*	*Figure 1C*
Genotype	Karyotype (G-banding) and resolution	*All lines show 46XY, Resolution 450-500*	*Figure 1E*
Identity	Microsatellite PCR (mPCR) OR STR analysis	*N/A*	*N/A*
		15 sites tested, identity verified	*in archive with journal*
Mutation analysis	Sequencing	*Confirmed genotypes: BIONi010-C-6: APOE ε2/ε2 Bioni010-C-2: APOE ε3/ε3 BIONi010-C-4: APOE ε4/ε4 BIONi010-C-3: APOE KO*	*Figure 1F*
	Southern Blot OR WGS	*N/A*	*N/A*
Microbiology and virology	Growth in LB medium, Mycoplasma test	*all negative*	*not shown but available with author*
Differentiation potential	directed trilineage differentiation	*The following markers were positive in all lines: Ectoderm: Sox1/Pax6 (all >25.5%); Mesoderm: CD34/CD56 (all >11.2%); Endoderm: CD184/Sox17 (all >48.5%)*	*Figure 1D*
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	*Negative*	*Data not shown but available with author*
Genotype additional info (OPTIONAL)	Blood group genotyping	*N/A*	N/A
	HLA tissue typing	*N/A*	N/A

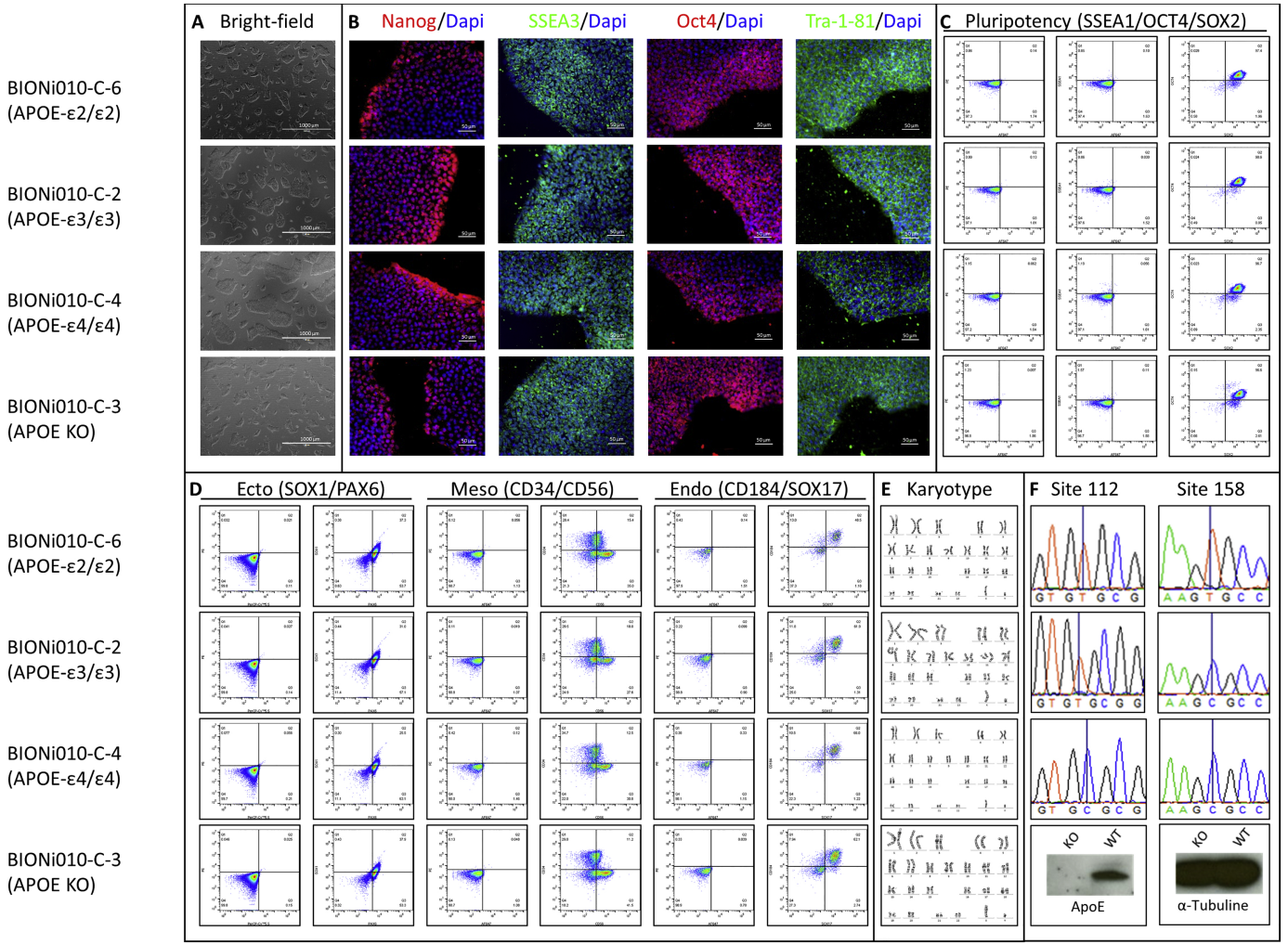
Table 3: Reagents details

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Marker	Mouse anti-OCT4	1:200	Stem cell Technologies (cl. 40) 01550
Pluripotency Marker	Rabbit anti-NANOG	1:200	Millipore AB5731
Pluripotency Marker	Rat anti-SSEA3	1:100	Biolegend Cat: 330302 RRID: AB_1236554
Pluripotency Marker	Mouse anti-TRA-1-81	1:200	Biolegend Cat: 330702 RRID: AB_1089240
Pluripotency Marker	OCT4 PE	1:200	BD Pharmigen Cat: 560186
Pluripotency Marker	SOX2 AF647	1:200	BD Pharmigen Cat: 560294
Differentiation Markers	SSEA1 PE	1:200	BD Pharmigen Cat: 560142
Differentiation Markers	SOX1 PE	1:50	BD Pharmigen Cat: 561592
Differentiation markers	PAX6 AF647	1:50	BD Pharmigen Cat: 562249
Differentiation Markers	CD34 PE	1:25	BD Pharmigen Cat: 555822
Differentiation Markers	CD56 APC	1:25	BD Pharmigen Cat: 555518
Differentiation Markers	CD184 PE	1:25	BD Pharmigen Cat: 555974
Differentiation Markers	SOX17 AF647	1:50	BD Pharmigen Cat: 562594
Secondary antibodies	Donkey Anti-Mouse IgG Alexa fluor 488	1:1000	Life technologies Cat#A21202 RRID: AB_141607
Secondary antibodies	Donkey Anti-Mouse IgG Alexa fluor 647	1:1000	Invitrogen Cat# A31571 RRID: AB_162542
Secondary antibodies	Donkey Anti-Rat IgG Alexa fluor 594	1:1000	Invitrogen Cat# A21209 RRID: AB_2535795
Secondary antibodies	Goat anti-Rabbit IgG Alexa fluor 647	1:1000	Life technologies Cat# A-21245 PRID: AB_2535813
APOE antibody	Apolipoprotein E/APOE Antibody	1:1000	NOVUS Biologicals; NB110-60531

sgRNAs, ssODNs and primers	**Target**	**Forward/Reverse primer (5′-3′)**
CRISPR1	Exon2	GGTTCTGTGGGCTGCGTTGCTGG
CRISPR2_recT	rs429358	GCGGACATGGAGGACGTGTGCGG
CRISPR2_recC	rs429358	GCGGACATGGAGGACGTGCGCGG
CRISPR3_recC	rs7412	ACACTGCCAGGCGCTTCTGCAGG
ssODN rs429358-T	rs429358	AGGAGCTGCAGGCGGCGCAGGCCCGG-CTGGGCGCGGACATGGAGGACGTGTG-CGGCCGCCTGGTGCAGTACCGCGGCG-AGGTGCAGGCCATGCTCGGCCAG
ssODN rs429358-C	rs429358	AGGAGCTGCAGGCGGCGCAGGCCCGG-CTGGGCGCGGACATGGAGGACGTGCG-CGGCCGCCTGGTGCAGTACCGCGGCG-AGGTGCAGGCCATGCTCGGCCAG
ssODN rs7412-T	rs7412	TGCGCAAGCTGCGTAAGCGGCTCCTCC-GCGATGCCGATGACCTGCAGAAGTGCC-TGGCAGTGTACCAGGCCGGGGCCCGCG-AGGGCGCCGAGCGCGGCCTC
APOE HhaI	rs 429358; rs7412	GCACGGCTGTCCAAGGAG/ GCCCCGGCCTGGTACAC
APOE HhaI Seq	rs 429358;	TGTCCAAGGAGCTGCAGG

	rs7412	
SURV APOE KO	APOE exon2	GAACACGGCGCTTAACTGTG/ CAGAGAGCGTCAAATCGCTGT
SURV APOE KO Seq	APOE exon2	GCGGCTTGGTAAATGTGCTG
U6-FW	pSpCas9n(BB)-2A-Puro plasmid	GAGGGCCTATTTCCCATGATTCC
pSpCas9-(339)-RV1	pSpCas9n(BB)-2A-Puro plasmid	CGACTCGGTGCCACTTTTTC

Fig. 1


A Bright-field
B Nanog/Dapi
SSEA3/Dapi
Oct4/Dapi
Tra-1-81/Dapi
C Pluripotency (SSEA1/OCT4/SOX2)
BIONi010-C-6 (APOE-ε2/ε2)
BIONi010-C-2 (APOE-ε3/ε3)
BIONi010-C-4 (APOE-ε4/ε4)
BIONi010-C-3 (APOE KO)
D Ecto (SOX1/PAX6)
Meso (CD34/CD56)
Endo (CD184/SOX17)
E Karyotype
F Site 112
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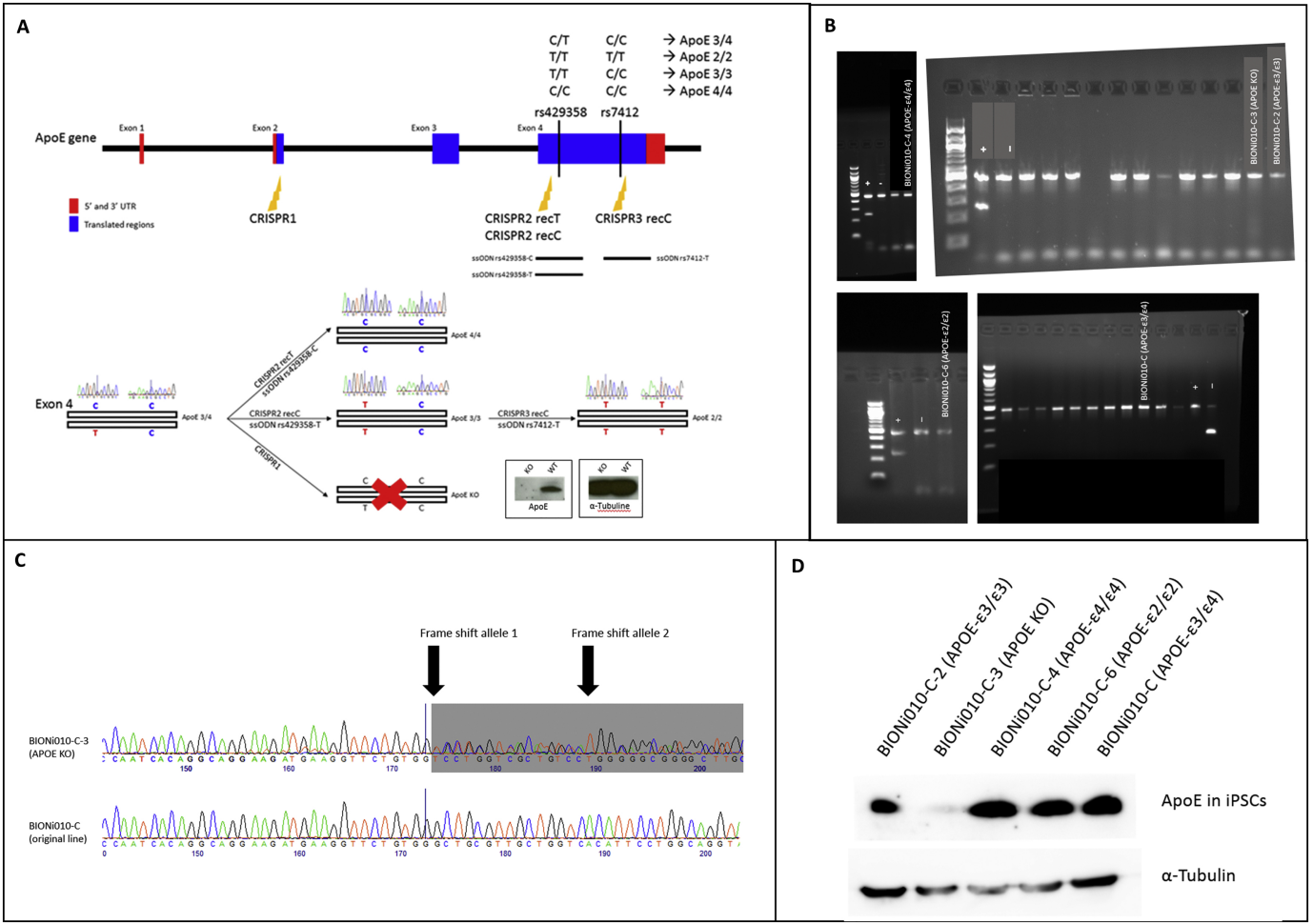
AAGCGCC
KO
WT
ApoE
α-Tubuline

Figure 1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C/C T/T C/C C/C → ApoE 2/2
→ ApoE 3/3
→ ApoE 4/4
rs429358 rs7412
ApoE gene
Exon 1
Exon 2
Exon 3
Exon 4
5' and 3' UTR
Translated regions
CRISPR1
CRISPR2 recT
CRISPR2 recC
CRISPR3 recC
ssODN rs429358-C
ssODN rs429358-T
ssODN rs7412-T
Exon 4
ApoE 3/4
ApoE 4/4
ApoE 3/3
ApoE 2/2
ApoE KO
CRISPR2 recT ssODN rs429358-C
CRISPR2 recC ssODN rs429358-T
CRISPR3 recC ssODN rs7412-T
CRISPR1
KO WT
ApoE
α-Tubuline
B
BIONi010-C-4 (APOE-ε4/ε4)
BIONi010-C-3 (APOE KO)
BIONi010-C-2 (APOE-ε3/ε3)
BIONi010-C-6 (APOE-ε2/ε2)
BIONi010-C (APOE-ε3/ε4)
C
Frame shift allele 1
Frame shift allele 2
BIONi010-C-3 (APOE KO)
BIONi010-C (original line)
D
BIONi010-C-2 (APOE-ε3/ε3)
BIONi010-C-3 (APOE KO)
BIONi010-C-4 (APOE-ε4/ε4)
BIONi010-C-6 (APOE-ε2/ε2)
BIONi010-C (APOE-ε3/ε4)
ApoE in iPSCs
α-Tubulin

Figure 2