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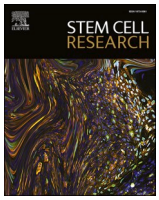

Lab Resource: Multiple Cell Lines

Generation of a set of induced pluripotent stem cell lines from two Alzheimer disease patients carrying APOE4 (MLUi007-J; MLUi008-A) and healthy old donors carrying APOE3 (MLUi009-A; MLUi010-B) to study APOE in aging and disease

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A B S T R A C T

Late-onset Alzheimer disease (LOAD) is the most frequent neurodegenerative disease, and the APOE ε4 allele is the most prominent risk factor for LOAD. Four human induced pluripotent stem cell (iPSC) lines MLUi007-J, MLUi008-B, MLUi009-A, and MLUi010-B were generated from LOAD patients and healthy matched donors by reprogramming of B-lymphoblastoid cells (B-LCLs) with episomal plasmids. The application of B-LCLs holds a great promise to model LOAD and other diseases because they can easily be generated from primary peripheral blood mononuclear cells (PBMCs) by infection with the Epstein-Barr virus (EBV).

Resource table

Unique stem cell lines identifier	1) MLUi007-J = APOE ε4 / ε4 2) MLUi008-B = APOE ε3 / ε4 3) MLUi009-A = APOE ε3 / ε3 4) MLUi010-B = APOE ε3 / ε3
Alternative name(s) of stem cell lines	N / A
Institution	*Medical Faculty of the Martin Luther University Halle-Wittenberg, Germany*
Contact information of distributor	Dr. Matthias Jung
Type of cell lines	iPSC
Origin	Human
Additional origin info required *for human ESC or iPSC*	Age: 64-79 Sex: female Ethnicity if known: Caucasian

(*continued on next column*)

Resource table (*continued*)

Cell Source	B-lymphoblastoid cells (B-LCLs; EBV-immortalized peripheral blood mononuclear cells)
Clonality	Different donors for each clone
Method of reprogramming	Episomal plasmids (Barrett et al., 2014)
Genetic Modification	No
Type of Genetic Modification	N / A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Real-time PCR confirmed absence of genes encoded on reprogramming plasmids
Associated disease	Late-onset Alzheimer disease (LOAD)
Gene / locus	APOE: 19q13.32; rs7412; rs429358
Date archived / stock date	June 2019
Cell line repository /bank	https://hpscreg.eu/cell-line/MLUi007-J
Ethical approval	This study was approved by the ethics committee of the Hospitals of the

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Resource table (*continued*)

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1. Resource utility

Late-onset Alzheimer disease (LOAD) is an age-related disease affecting increasing numbers of patients worldwide, but causal biological mechanisms are still poorly understood. To improve our understanding of aging and LOAD, we established iPSC lines using episomal plasmids from LOAD patients and healthy elderly donors with different APOE haplotypes.

2. Resource details

The APOE gene encoding for Apolipoprotein E (APOE) is the most important risk gene for LOAD (Harerimana et al., 2022). The APOE gene contains two SNPs (rs429358; rs7412) that generate three different isoforms, namely ε2 (rs7412-T, rs429358-T), ε3 (rs7412-C, rs429358-T), and ε4 (rs7412-C, rs429358-C). SNP rs429358 is part of the codon for amino acid 112 and SNP rs7412 is part of the codon for amino acid 158. For both SNPs, either a C or a T can be found on DNA level, leading to either an arginine or a cysteine in ε2 (cys112, cys158), ε3 (cys112, arg158), and ε4 (arg112, arg158). These different amino acids within the APOE protein determine its structure and function of the APOE protein. Together, both SNPs determine the APOE genotype. The APOE-ε4 allele was recently validated as the most strongest risk factor for LOAD in genome-wide association studies (Bellenguez et al., 2022).

We generated human induced pluripotent stem cell (iPSC) lines MLUi007-J and MLUi008-B from LOAD patients and MLUi009-A and MLUi010-B from healthy matched donors (CON), respectively. B-LCLs were reprogrammed with episomal plasmids based on an established protocol with minor modifications (Barrett et al., 2014). MLUi007-J and MLUi008-B were obtained from LOAD patients diagnosed using clinical parameters, the CERAD-plus neuropsychological battery, and the age of onset. MLUi009-A and MLUi010-B were obtained from healthy donors.

We observed that cells with epithelial colony-like morphology appeared approximately 20 days after transfection of B-LCLs with reprogramming plasmids and their seeding onto mouse embryonic feeder cell cultures (MEFs). Colonies were picked, propagated on MEF feeder cultures, and stained for alkaline phosphatase activity. We observed homogeneous blue staining of colonies, indicating abundant alkaline phosphatase activity (Fig. 1A). We adapted established iPSC lines after a several passages on feeder-free culture conditions using mTeSR™1 cell culture medium and Matrigel™ as matrix. The analyses presented here were performed in iPSCs in passages 10–15 adapted to feeder-free culture conditions. Bright field images of all lines displayed epithelial colony-like morphology of tightly packed cells with high nucleus to cytoplasm ratio representing hallmarks of human iPSCs (Fig. 1B). Using reverse transcriptase PCR, we checked the presence of pluripotency markers and the absence of B cell markers that may have retained resulting from somatic memory of the donor material (Supplementary Fig. 1A). We performed immunofluorescence (IF) staining for six reprogramming factors representing pluripotency marker proteins, namely: POU class 5 homeobox 1 (POU5F1, OCT4), SRY-box 2 (SOX2), Kruppel-like factor 4 (KLF4), MYC proto–oncogene, bHLH transcription factor (MYC), lin-28 homolog A (LIN28A), Nanog homeobox (NANOG) (Fig. 1C). All iPSC lines passed PluriTest™, a bioinformatic assay based on transcriptomic assessment, further confirming their pluripotency (Supplementary Fig. 1B). We analyzed all iPSC lines in comparison to HeLa cells (negative control; CVCL_0030), one previously established iPSC line (positive control; TMOi001-A), and H9 human embryonic stem cells (positive control; WAe009-A). Quantification of pluripotency markers by flow cytometry in all iPSC lines showed an abundant expression of OCT4, SOX2, NANOG and SSEA4. Stage-specific embryonic antigen 1 (SSEA1) was absent (Fig. 1D). B-LCLs represent immortalized cell lines that contain active extra chromosomal Epstein-Barr virus (EBV) episomes, which usually do not tend to integrate into the genome thereby enabling their application as genetic tools. During reprogramming, B-LCLs lost the EBV DNA, which was shown by quantitative real-time PCR for EBV-related genes, namely protein Tta (*Bzlf1*), Epstein-Barr nuclear antigens 1 and 2 (*Ebna1*, *Ebna2*), latent membrane protein 1 (*Lmp1*), and the origin of plasmid replication (*oriP*) (Fig. 1E). Because the plasmids also encode *Ebna1* and *oriP*, this result also showed the absence of these. A trilineage differentiation assay revealed that ecto-, *meso*-, and endodermal cell types could be generated from all the lines (Fig. 1F). A virology screening confirmed negativity for HIV, HBV, HCV and EBV. A screening for microbiological contamination with mycoplasma was negative (Supplementary Fig. 1C). Giemsa staining of metaphase chromosomes (G-banding) revealed a normal karyotype for MLUi009-A with no numerical or structural abnormalities. A multicolor fluorescence *in situ* hybridization (mFISH) karyotype was carried out by chromosome painting using 24 colors for MLUi007-J, MLUi008-B, and MLUi010-B. All lines showed a normal female karyotype of 46,XX (Fig. 1G). The APOE genotype was already known from previous screenings in B-LCL (not shown). We confirmed the presence of rs429358-T and rs7412-C haplotypes for the ε3 isoform and the presence of rs429358-C and rs7412-C haplotypes for the ε4 isoform by Sanger sequencing (Fig. 1H). For genotyping of iPSCs, we analyzed rs7412 and rs429358 using TaqMan™ SNP Genotyping Assays confirming homozygous and heterozygous presence of rs42958-C, which together with homozygous rs7412-T encode the ε4 isoform in MLUi007-J and MLUi008-B (Fig. 1H; Supplementary Fig. 1D), but not in the control iPSC lines. The identity of iPSCs was confirmed by comparing donor DNA from blood, B-LCLs, and iPSCs by the analysis of 16 short tandem repeats and Amelogenin loci (STR analysis).

3. Materials and methods

3.1. Reprogramming of B-LCLs

B-LCLs were maintained in RPMI1640 (Gibco, #31870082) supplemented with 10% FBS Superior (Sigma-Aldrich, #S0615), 2 mM L-glutamine (Gibco, #25030081), 1 mM sodium pyruvate (Gibco, #11360039), 0.1 mg / ml gentamicin (Gibco, #15750045), and 2.1 µg / ml Phytohämagglutinin-L (Millipore, #431784). B-LCLs were cultured at 37°C and 5% CO_2 in a humidified incubator. For reprogramming, we applied a recently published protocol (Barrett et al., 2014) with minor modifications. B-LCLs were reprogrammed into virus-free iPSC lines with the Neon™ Transfection System (Invitrogen, #MPK5000) using 1.5 µg of each episomal plasmid expressing seven factors, namely: OCT4, SOX2, KLF4, MYC, LIN28A, SV40 large T antigen (SV40LT), and cellular tumor antigen p53 (p53) shRNA. Episomal plasmids pEP4 E02S ET2K (#20927), pCXLE-hOCT3/4-shp53-F (#27077), pCXLE-hUL (#27080), and pCXLE-hSK (#27078) were obtained from Addgene. B-LCLs (0.5×10^6 cells) were collected, centrifuged at 200 × g for 5 min, resuspended in 100 µl R Buffer of the Neon™ Transfection System, and nucleofected using the B3 program (2000 V, 15 ms, 2 pulses). B-LCLs were plated on MEFs from CF-1™ mice (Charles River Laboratories; strain code: #023; 3×10^5 cells per well of a 6-well plate) pre-coated with 0.1% gelatin (Carl Roth, #4582). MEFs were cultured in DMEM with Glutamax™ and pyruvat (Gibco, #10569010) supplemented with FBS (Sigma-Aldrich, #S0615) and inactivated with a final concentration of 10 µg / ml mitomycin C (Applichem, #A2190) before use. Cells were maintained at hypoxic conditions (5% O_2, 5% CO_2, 90% N_2). Reprogramming media contained DMEM / F12 buffered

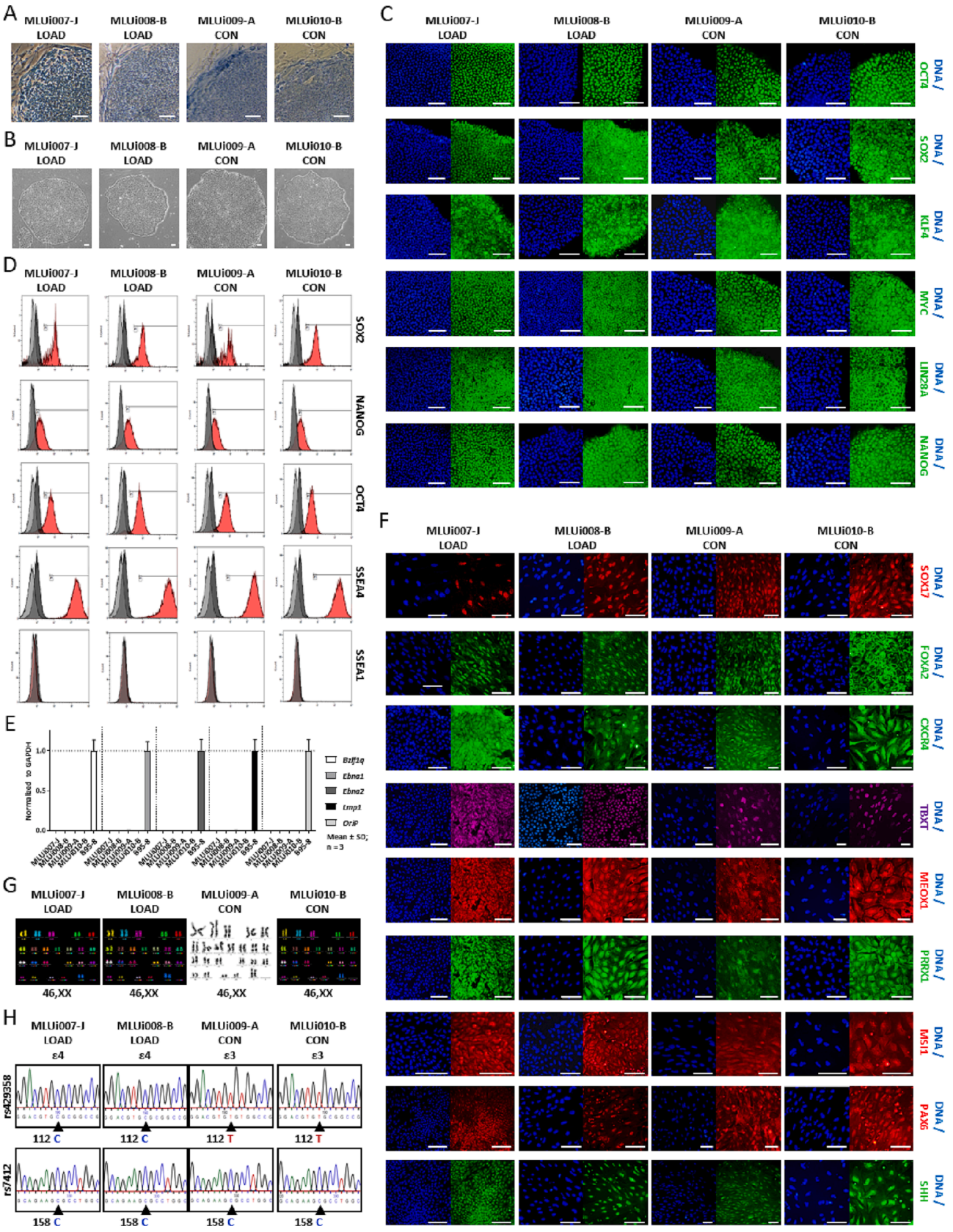
A
MLUi007-J LOAD
MLUi008-B LOAD
MLUi009-A CON
MLUi010-B CON
B
C
DNA / OCT4
DNA / SOX2
DNA / KLF4
DNA / MYC
DNA / LIN28A
DNA / NANOG
D
SOX2
NANOG
OCT4
SSEA4
SSEA1
E
Normalized to GAPDH
Bzlf1q
Ebna1
Ebna2
tmp1
OriP
Mean ± SD; n = 3
F
DNA / SOX17
DNA / FOXA2
DNA / CXCR4
DNA / TBXT
DNA / MEOX1
DNA / PRRX1
DNA / MSI1
DNA / PAX6
DNA / SHH
G
46,XX
H
ε4
ε3
rs429358
rs7412
112 C
112 T
158 C

Fig. 1.

with HEPES (Gibco, #11330057) supplemented with 1% GlutaMax™ (Gibco, #35050038), 1% non-essential amino acids (NEAA; Gibco, #11140050), 1% N-2 supplement (Gibco, #17502001), 2% B–27 supplement without vitamin A (Gibco, #12587001), 1% 10000 U / 10000 µg penicillin / streptomycin (Gibco, #10378016), 0.1 µM β-mercaptoethanol (BME; Sigma-Aldrich, #M3148), 100 ng / ml basic fibroblast growth factor (bFGF, FGF2; Peprotech, #100-18B), 0.5 ng / ml human leukemia inhibitory factor (hLIF; Gibco, #PHC9484), 0.5 µM MEK inhibitor PD0325901 (Stemcell Technologies, #72184), 3 µM GSK3 inhibitor CHIR99021 (Stemcell Technologies, #72054), 10 µM protein kinase inhibitor HA-100 (Santa Cruz, #sc-203072A), and 0.5 µM activin / NODAL/ TGF-β pathway specific ALK inhibitor A-83-01 (Santa Cruz, #sc-203791). Media was changed every other day. Colonies of iPSCs were first observed around 20 d. These were picked manually and cultured on MEFs for several passages. Cells were maintained in DMEM / F12 buffered with HEPES supplemented with 1% GlutaMax™, 20% KnockOut™ serum replacement (Gico, #10828010), 1% NEAA, 1% 10000 U / 10000 µg penicillin / streptomycin, 0.1 µM BME, and 10 ng / ml bFGF. Established iPSC lines were maintained without MEFs using 0.5 mg Matrigel™ (VWR, #7340268) in 6 ml DMEM (Gibco, #41965039) for coating of one 6-well plate. These iPSCs were cultured in mTeSR™1 (Stemcell Technologies, #85850) supplemented with 1% 10000 U / 10000 µg penicillin / streptomycin. For passaging, cells were treated with HA–100 and passaged as clumps with collagenase IV (Gibco, # 17104019) at a ratio of 1:6 every other week.

3.2. In vitro differentiation into germ layers

Human iPSCs were cultivated in a medium for spontaneous differentiation (trilineage differentiation) for ten days after reaching a confluence of about 70-80%. Spontaneous differentiation medium consists of DMEM / F12 buffered with HEPES supplemented with 1% GlutaMax™, 1% NEAA, 1% 10000 U / 10000 µg penicillin / streptomycin, 20% FBS Superior, and 0.1 µM BME. The medium was changed every other day. The differentiation into germ layer derivatives was analyzed at 3-10 d (Table 1).

3.3. Alkaline phosphatase staining and morphology

Alkaline phosphatase is a hydrolase responsible for dephosphorylating molecules such as nucleotides and proteins under alkaline conditions. Alkaline phosphatase staining is a universal pluripotent marker for all types of pluripotent stem cells including iPSCs and is not observed in MEFs. Cells were analyzed using the Blue–Color Alkaline Phophatase Staining Kit (SBI, #AP100B-1) according to manufacturer's instructions at 3 d after passaging. Alkaline phosphatase staining was investigated by light microcopy 3 d after passaging of cells onto MEFs. Morphology was investigated by light microcopy 3 d after passaging of cells onto Matrigel™.

3.4. Immunofluorescence analysis

Cells were cultured on Matrigel™-coated glass cover slips. For staining, cells were washed thrice with PBS, fixed in 4% paraformaldehyde (Applichem, #211511) for 15 min, and washed thrice with PBS. Fixed cells were incubated with PBS containing 0.1% Triton™ X-100 (Sigma-Aldrich, #X100) and 1% donkey / goat / rabbit serum (Sigma-Aldrich, #D9963 / #G9023 / #R9133) for 30 min followed by incubation with 3.5% serum for 30 min. Afterwards, cells were incubated with primary antibodies (Table 2) at 4°C for overnight, washed thrice in PBS, and incubated with secondary antibodies (Table 2) for 2 h at room temperature. Then, cells were washed thrice in PBS and stained with 5 µg / ml Hoechst 33342™ (Invitrogen, #H1399) for 5 min at room temperature. Finally coverslips were mounted using the fluorescence mounting medium (Dako, #S302380-2) and sealed with transparent nail polish.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography bright field	Visual record of the lines: normal iPSC morphology	Fig. 1B
Phenotype	Qualitative analysis by alkaline phophatase staining on MEFs, IF analysis on Matrigel™, transcript analysis on Matrigel™	All lines stain positive for alkaline phosphatase activity. All lines express mRNA for *CDH1*, *DPPA2*, *REST*, *OCT4*, *SOX2*, *KLF4*, *MYC*, *LIN28A*, and *NANOG*. *GPR183*, *FCLRA*, and *BTLA* are not expressed. All lines are positive for protein stem cell markers OCT4, SOX2, KLF4, MYC, LIN28A, and NANOG.	Fig. 1A Fig. 1C Supplementary Fig. 1A
	Quantitative analysis by flow cytometry and quantitative real-time PCR	MLUi007-J: SOX2: 86.4%; NANOG: 70.1%; OCT4: 93.5%; SSEA4: 99.9%; SSEA1: 0.0% MLUi008-B: SOX2: 77.1%; NANOG: 78.8%; OCT4: 88.2%; SSEA4: 99.8%; SSEA1: 0.0% MLUi009-A: SOX2: 90.3%; NANOG: 66.5%; OCT4: 89.7%; SSEA4: 99.9%; SSEA1: 0.0% MLUi010-B: SOX2: 72.9%; NANOG: 73.3%; OCT4: 91.7%; SSEA4: 99.9%; SSEA1: 0.0% None of the lines express *Bzlf1*, *Ebna1*, *Ebna2*, *Lmp1* and *oriP*.	Fig. 1D Fig. 1E
Genotype	Karyotype (G-banding) and mFISH	All lines are XX,46. Giemsa staining: Metaphases produced between 300 and 400 bands distributed among the 23 pairs of human chromosomes with a resolution of ~ 10 Mb. mFISH staining: Stained metaphases showed a resolution of ~ 1 Mb	Fig. 1G
Identity	Microsatellite PCR (mPCR) OR STR analysis	Not performed 16 sites were tested and identity was verified	N / A Submitted to journal archive

(*continued on next page*)

Table 1 (*continued*)

Classification	Test	Result	Data
Mutation analysis	Sequencing	Confirmed genotype: MLUi007-J: APOE ε4 / ε4 MLUi008-B: APOE ε3 / ε4 MLUi009-A: APOE ε3 / ε3 MLUi010-B: APOE ε3 / ε3	Fig. 1H Supplementary Fig. 1D
	Southern Blot OR WGS	N / A	N / A
Microbiology and virology	Mycoplasma	All lines are negative for mycoplasma as confirmed by PCR.	Supplementary Fig. 1C
Differentiation potential	Trilineage differentiation, PluriTest™	The following markers were positive in all lines: Endoderm: SOX17, FOXA2, CXCR4 Mesoderm: TBXT, MEOX1, PRRX1 Ektoderm: MSI1, PAX6, SHH All lines passed Pluripotency and Novelty Score treshholds.	Fig. 1F Supplementary Fig. 1B
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Supplementary Fig. 1C
Genotype additional info	Blood group genotyping	N / A	N / A
	HLA tissue typing	N / A	N / A

3.5. Flow cytometry

Cells were washed in PBS and detached using 1 ml TrypLE™ (Gibco, #12563029) for 5 min at 37°C. The reaction was stopped by adding 1 ml iPSC medium containing 20% FCS. Then, cells were centrifuged with 300 g for 10 min and suspended in 1 ml FC buffer consisting of PBS supplemented with 0.5% BSA and 2 mM EDTA. Cells were centrifuged again and suspended in 1 ml FC buffer. For detection of SOX2, 10^6 cells were fixed and permeabilized with a FoxP3 staining buffer set (Miltenyi Biotec, #130–093-142). Cells were first centrifuged, resuspended in 1 ml of fixation / permeabilization solution, and placed on ice for 30 min. Then, the cells were centrifuged and resuspended in 1 ml of FC buffer. Cells were then centrifuged, washed with 1 ml permeabilization buffer, and resuspended in 50 µl permeabilization buffer. The cell suspension was incubated with fluorescence-labeled primary antibody (Table 2) on ice for 30 min. Finally cells were washed in permeabilization buffer and resuspended in FC buffer for analysis. For detection of OCT4, SSEA1, and SSEA4, we applied the Human and Mouse Pluripotent Stem Cell Analysis Kit (BD Biosciences, #560477). The included reagents were also applied for staining NANOG. 2×10^6 cells were first centrifuged, resuspended in 1 ml of BD Cytofix™ fixation buffer, and incubated for 20 min. Then, the cells were centrifuged, washed twice in BD Perm / Wash™ buffer, resuspended in 1 ml of that buffer, and incubated for 10 min. Afterwards, 50 µl (10^5 cells) of the cell suspension were stained with fluorescence-labeled primary antibody (Table 2). Isotype controls were used to test for non-specific staining in all cell lines. A BD FACScanto™ II flow cytometer from BD Biosciences was used and all data were analyzed using the BD FACSDiva™ software.

3.6. Transcript analysis

RNA isolation and desoxyribonuclease treatment were performed according to the manufacturer's protocol using the RNeasy™ Mini Kit (Qiagen, #74104) in comination with the RNase-Free DNase Set (Qiagen, #79254). Reverse transcription of RNA into complementary DNA was performed using reverse transcription reaction mix (20 µl) containing 0.5 µl RiboLock RNase inhibitor, 1 µl 200 U / µl Revert Aid™ M-MuLV Reverse Transcriptase, 4 µl 5x buffer, 2 µl 10 mM deoxyribonucleotides (dNTPs), 1 µl 20 pmol / µl Oligo(dt)$_{18}$ primer, and 11.5 µl ribonuclease-free water including 1 µg RNA (all from Thermo Scientific, #EO0381, #EP0441, #R0182, #SO131, #R0581). Semi-quantitative PCR reactions were performed in 25 µl containing 2.5 µl 10x buffer-BD, 2.5 µl 25 mM $MgCl_2$, 0.25 µl 5 U / µl Firepol™ DNA polymerase (Solis Biodyne, #01-01, #), 2 µl 2.5 mM dNTPs, 1 µl 10 pmol / µl forward and reverse primer (Biomers) (Table 2), and RNase-free water including template DNA. Glycerinaldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as expression control.

3.7. Detection of episomal plasmids and EBV

The vector backbone of all episomal plasmids is pCEP4 encoding *oriP* and *Ebna1*, genes that have been cloned from EBV. Therefore, absence of *oriP* and *Ebna1* DNA indicates absence of episomal plasmids. To monitor absence of EBV DNA, we also monitored the genes *Bzlf1*, *Ebna2*, and *Lmp1*. DNA Isolation was performed using the DNeasy Blood & Tissue Kit (Qiagen, #69504) according to manufacturer's instructions. Quantitative real-time genomic PCR reactions were performed in 20 µl containing 4 µl Hot Firepol™ EvaGreen qPCR Mix Plus (Solis Biodyne, #08-24), 1 µl 10 pmol / µl forward and reverse primer (Table 2), and RNase-free water including template DNA. Ct values were measured in triplicate and their mean was used with a standard curve to calculate relative expression. Normalization of expression was performed with genomic *GAPDH*. B95–8 (CVCL_1953) cells containing EBV served as a positive control.

3.8. APOE genotyping

Although the genotype of LOAD patients and healthy individuals was known, we verified iPSCs by genotyping the single nucleotide polymorphisms rs7412 and rs429358 and Sanger sequencing of the respective loci in the APOE gene. DNA isolation was performed using the DNeasy Blood & Tissue Kit according to manufacturer's instructions. Sanger sequencing of rs7412 and rs429358 required the amplification of the respective loci by genomic PCR. Therefore, genomic PCR reactions were performed in 25 µl containing 2.5 µl 10x PCR buffer B, 2.5 µl 25 mM $MgCl_2$, 5 µl 10x solution S, 2 µl 2.5 mM dNTPs, 0.25 µl 5 U / µL Firepol™ DNA polymerase, 0.5 µl 10 pmol / µl forward and reverse primer (Table 2), and nuclease-free water including template DNA. The PCR product of 514 bp was separated in a 2% agarose gel, excised using a scalpel under ultraviolet light, and extracted using the QIAquick™ Gel Extraction Kit (Qiagen, #28704) according to manufacturer's instructions. PCR products were cloned into *E.coli* (strain JM109) using the pGEM™-T Easy Vector System (Promega, #A1380) according to manufacturer's instructions followed by vector DNA extraction using the Monarch™ Plasmid DNA Miniprep Kit (New England Biolabs, #T1010) according to manufacturer's instructions and sequenced by Eurofins Genomics. TaqMan™ SNP Genotyping Assays for rs7412 (Applied Biosystems, Assay ID 904973_10) and rs429,358 (Applied Biosystems, Assay ID 3084793_20) were performed in 25 µl containing 12.5 µl 2x TaqMan™ Genotyping Master Mix (Applied Biosystems, #4371353), 1.25 µl 20x Genotyping Assay, and 11.25 µl nuclease-free water including 10 ng template DNA. Measurements were performed using a CFX Connect Real-Time PCR Detection System from Biorad.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency marker / immunofluorescence	Monoclonal mouse IgG anti human OCT4 (C-10)	1 : 100	Santa Cruz Biotechnology Cat# sc-5279	AB_628051
	Monoclonal rabbit IgG anti human SOX2 (*N*-term)	1 : 100	Acris Cat# AM09112PU-S	AB_10556441
	Polyclonal goat IgG anti human KLF4	1 : 400	R and D Systems Cat# AF3640	AB_2130224
	Monoclonal mouse IgG anti human MYC (9E10)	1 : 100	Santa Cruz Biotechnology Cat# sc-40	AB_627268
	Monoclonal mouse IgG anti human LIN28A (6D1F9)	1 : 100	Santa Cruz Biotechnology Cat# sc-293120	AB_10920408
	Polyclonal goat IgG anti human NANOG (N-17)	1 : 100	Santa Cruz Biotechnology Cat# sc-30331	AB_649884
Differentiation marker / immunofluorescence	Monoclonal mouse IgG anti human SOX17 (3.5CH)	1 : 100	Santa Cruz Biotechnology Cat# sc-130295	AB_2286667
	Polyclonal rabbit IgG anti human FOXA2	1 : 100	Millipore Cat# AB4125	AB_2104889
	Monoclonal rat IgG anti human CXCR4	1 : 100	R and D Systems Cat# MAB21651	AB_2261636
	Polyclonal rabbit IgG anti human TBXT	1 : 1000	Abcam Cat# ab20680	AB_727024
	Monoclonal mouse IgG anti human MOX1 (1A10)	1 : 500	Novus Cat# H00004222-M01	AB_608872
	Polyclonal goat IgG anti human PRRX1	1 : 200	Novus Cat# NBP1-06067	AB_1556179
	Polyclonal rabbit IgG anti human MSI1	1 : 200	Millipore Cat# AB5977	AB_92184
	Monoclonal mouse IgG anti human PAX6 (AD2.35)	1 : 100	Santa Cruz Biotechnology Cat# sc-53108	AB_630089
	Monoclonal rat IgG anti human SHH	1 : 250	Abcam Cat# ab50515	AB_882647
Secondary antibodies / immunofluorescence	Cy™ 3 AffiniPure donkey anti mouse IgG (H + L)	1 : 400	Jackson ImmunoResearch Labs Cat# 715-165-151	AB_2315777
	Cy™ 3 AffiniPure donkey anti rabbit IgG (H + L)	1 : 400	Jackson ImmunoResearch Labs Cat# 711-165-152	AB_2307443
	Cy™ 5 AffiniPure goat anti rabbit IgG (H + L)	1 : 400	Jackson ImmunoResearch Labs Cat# 111-175-144	AB_2338013
	Goat anti rabbit IgG (H + L) Alexa Fluor™ 488	1 : 400	Thermo Fisher Scientific Cat# A-11034	AB_2576217
	Rabbit anti rat IgG (H + L) Alexa Fluor™ 488	1 : 400	Thermo Fisher Scientific Cat# A-21210	AB_2535796
	Donkey anti goat IgG (H + L) Alexa Fluor™ 488	1 : 400	Thermo Fisher Scientific Cat# A-11055	AB_2534102
	Goat anti mouse IgG (H + L) Alexa Fluor™ 488	1 : 400	Thermo Fisher Scientific Cat# A-11001	AB_2534069
Pluripotency marker / flow cytometry	FITC REAfinity™ anti human SOX2	1 : 10	Miltenyi Biotec Cat# 130-104-993	AB_2653499
	Alexa Fluor™ 488 mouse anti human NANOG	1 : 25	BD Biosciences Cat# 560,791	AB_1937305
	Alexa Fluor™ 647 mouse anti human SSEA4	1 : 6	BD Biosciences Cat# 560,477	AB_2869350
	PE mouse anti human SSEA1	1 : 6	BD Biosciences Cat# 560,477	AB_2869350
	PerCP-Cy™ 5.5 mouse anti human OCT4	1 : 6	BD Biosciences Cat# 560,477	AB_2869350

	Primers		
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Episomal plasmids and EBV (real-time genomic PCR)	*oriP*	101 bp	GTGAGATGGACATCCAGTCTTTACG / GGGCAATAAATACTAGTGTAGGAATGAAACATT
Episomal plasmids and EBV (real-time genomic PCR)	*Ebna1*	61 bp	ATCAGGGCCAAGACATAGAGA / GCCAATGCAACTTGGACGTT
EBV (real-time genomic PCR)	*Ebna1*	190 bp	GGTTGCTGGAGAGGGCAAGG / GCCCAGAGGCTCCCATTCTC
EBV (real-time genomic PCR)	*Lmp1*	109 bp	CAGTCAGGCAAGCCTATGA / CTGGTTCCGGTGGAGATGA
EBV (real-time genomic PCR)	*Bzlf1*	92 bp	AAATTTAAGAGATCCTCGTGTAAAACATC / CGCCTCCTGTTGAAGCAGAT
House-keeping gene (real-time genomic PCR)	*GAPDH*	143 bp	CAGCCTCAAGATCATCAGCA / GTCTTCTGGGTGGCAGTGAT
House-keeping gene (semi-quantitative PCR)	*GAPDH*	497 bp	CAAGGTCATCCATGACAACTTTG / GTCCACCACCCTGTTGCTGTAG
Epithelial marker (semi-quantitative PCR)	*CDH1*	825 bp	GGAGAAGAGGACCAGGACTTTG / AGGGACACACCAGTGTAGTAA
Pluripotency marker (semi-quantitative PCR)	*DPPA2*	606 bp	CCGTCCCCGCAATCTCCTTCCATC / ATGATGCCAACATGGCTCCCGGTG
Pluripotency marker (semi-quantitative PCR)	*KLF4*	397 bp	TGATTGTAGTGCTTTCTGGCTGGGCTCC / ACGATCGTGGCCCCGGAAAAGGACC
Pluripotency marker (semi-quantitative PCR)	*LIN28A*	313 bp	GGTTCGGCTTCCTGTCCATGA / GGTGGCAGCTTGCATTCCTTG
Pluripotency marker (semi-quantitative PCR)	*MYC*	490 bp	TTCGGGTAGTGGAAAACCAG / TAGGAGGCCAGCTTCTCTGA

(*continued on next page*)

Table 2 (*continued*)

	Antibodies used for immunocytochemistry/flow cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency marker (semi-quantitative PCR)	*NANOG*	483 bp	TTCCTTCCTCCATGGATCTG / ACTGGATGTTCTGGGTCTGG	
Pluripotency marker (semi-quantitative PCR)	*OCT4*	467 bp	CAAGCCCTCATTTCACCAG / TTGATGTCCTGGGACTCCTC	
Pluripotency marker (semi-quantitative PCR)	*REST*	333 bp	CCAGCACCCAACTTTACCAC / ACCGACCAGGTAATCACAGC	
Pluripotency marker (semi-quantitative PCR)	*SOX2*	410 bp	AGTCTCCAAGCGACGAAAAA / GGAAAGTTGGGATCGAACAA	
B-LCL marker (semi-quantitative PCR)	*GPR183*	352 bp	GACCCGAACGAGTCACTGAT / ACACAAGGCATCTCCGATTC	
B-LCL marker (semi-quantitative PCR)	*FCRLA*	295 bp	AACCCTAGGTGTTGGGCTCT/ TGGTGGAAGGGTGAGTTAGC	
B-LCL marker (semi-quantitative PCR)	*BTLA*	331 bp	GACACAGCAGGAAGGGAAAT / ATGGTCCCTGTTGGAGTCAG	
Genotyping	*APOE*	514 bp	ACTGACCCCGGTGGCGGAGGA / CAGGCGTATCTGCTGGGCCTGCTC	

3.9. Pluritest™ analysis

Sample processing and array analysis were performed at the Center for Integrative Psychiatry (ZIP) in Kiel as previously published (Tandon et al., 2018). PluriTest™ analysis required RNA isolation as described for transcript analysis followed by microarray analysis using 200-500 ng RNA. TotalPrep™ RNA Amplification Kit (Illumina, #IL1791) was used for preamplification of RNA and TargetAmp™ Nano-g Biotin-aRNA Labeling Kit (Illumina, #TAN07924-142) was used to generate Biotin-aRNA, which was diluted to a concentration of 150 ng / μL. Hybridization of 750 ng Biotin-aRNA was performed using the Human HT-12v4.0 Expression BeadChip (Illumina, #BD-901-1001) at 58°C for 16-20 h followed by a washing step and staining corresponding to the manufacturer's protocol. BeadChips were scanned using the iScan system from Illumina. Finally, raw data (*.idat files) were submitted to PluriTest™ analysis at https://www.pluritest.org.

3.10. Virology screening and mycoplasma testing

Cells were first washed in PBS and then detached with a cell scraper in 1 ml PBS. The obtained cell suspension was then treated in an ultrasonic bath for 1 min. The sample was then centrifuged at RT for 5 min at 3000 rpm and the supernatant was used for further analysis. Virology screening for hepatitis B and C (HBV, HCV) and human immunodeficiency virus (HIV) was performed by analyzing the hepatitis B surface antigen (HBsAg), hepatitis C virus core antigen (HCV Ag), and HIV p24 antigen. HBs Ag levels were detected using the Architect HBsAg Qualitative II assay and the HCV Ag levels were detected using the Architect HCV Ag assay. The Architect HIV Ag / Ab Combo assay was used for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV-1 and HIV-2. All assays used are chemiluminescent microparticle immunoassays (CMIA) and were performed according to the manufacturer's instructions on the Architect i1000 from Abbott Diagnostics. For EBV DNA detection, the artus EBV RG PCR Kit (Qiagen, # 4501263) was used in a manual setup on the Rotor–Gene Q instrument from Qiagen according to the manufacturer's protocol. To demonstrate the absence of mycoplasma, the Venor™ *GeM* Classic (Minerva Biolabs, #11-1025) was performed for cell culture supernatants according to the manufacturer's instructions.

3.11. Chromosome analysis

Metaphase arrest was induced by incubating 60-80% confluent cells with 0.1 μg / ml colcemid (Sigma-Aldrich, #234109) for 3 h at 37°C. Cells were washed in PBS, detached with 400-600 Units / ml Accutase™ (Gibco, #A1110501), and centrifuged at 200 × g for 5 min. The supernatant was discarded. For chromosomes release, the cell suspension was incubated with 10 ml pre-warmed 75 mM KCl hypotonic solution (Carl Roth, #P017) at 37°C for 20 min. Cell suspension was centrifuged at 200 × g for 10 min. The supernatant was discarded. For fixing the metaphases, the cells treated with 10 ml ice cold fresh fixative (methanol:acetic acid; 3:1; Carl Roth, #T909, #6755) and centrifuged at 300 × g for 10 min. The supernatant was discarded. Cells were resuspended in fresh fixative and incubated for 30 min at 4°C and centrifuged again. The supernatant was removed and cells were suspended in 1-2 ml fresh fixative depending on cell density. Finally, cell suspension was dropped onto coverslips. Coverslips dried at least 1 d prior G-banding or mFISH. For G-banding, coverslips were stained with 5% Giemsa solution (azure, eosin, and methylene blue in PBS; pH: 6.8; Sigma-Aldrich, #109204) for 2 min followed by a 30 s trypsin (Gibco, # 15090046) treatment. A total of 15 metaphases spreads were analyzed for chromosomal aberrations by Giemsa banding (G-banding). We performed mFISH analysis as recently described (Luft et al., 2017). In brief, chromosome spreads were stained with the 24XCyte human multicolor FISH probe (MetaSystems, #D-0125-060-DI) according to manufacturer's instructions. Stained chromosomes were recorded using an Axio Imager Z1 microscope from Zeiss and analyzed using ISIS software from MetaSystems. We analyzed 100-150 karyotypes. All types of structural chromosome aberrations were recorded and classified according to the mPAINT system.

3.12. STR analysis

For short tandem repeat (STR) analysis, DNA was isolated as described for APOE genotyping. Then, DNA was amplified with the PowerPlex™ ESX 17 Fast System (Promega, #DC1711) for 30 cycles. This system allows co-amplification of 16 autosomal STR loci and Amelogenin (gender determination). Amplification products were separated using an Applied Biosystems™ 3500 Genetic Analyzer.

3.13. STR analysis

Summary table.

3.14. Cell lines identity testing

Done.

3.15. Abnormal karyotype

Done.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Barrett, R., Ornelas, L., Yeager, N., Mandefro, B., Sahabian, A., Lenaeus, L., Targan, S.R., Svendsen, C.N., Sareen, D., 2014. Reliable generation of induced pluripotent stem cells from human lymphoblastoid cell lines. Stem Cells Translat. Med. 3, 1429–1434. https://doi.org/10.5966/sctm.2014-0121.

Bellenguez, C., Küçükali, F., Jansen, I.E., Kleineidam, L., Moreno-Grau, S., Amin, N., Naj, A.C., Campos-Martin, R., Grenier-Boley, B., Andrade, V., Holmans, P.A., Boland, A., Damotte, V., van der Lee, S.J., Costa, M.R., Kuulasmaa, T., Yang, Q., Rojas, I. de, Bis, J.C., Yaqub, A., Prokic, I., Chapuis, J., Ahmad, S., Giedraitis, V., Aarsland, D., Garcia-Gonzalez, P., Abdelnour, C., Alarcón-Martín, E., Alcolea, D., Alegret, M., Alvarez, I., Álvarez, V., Armstrong, N.J., Tsolaki, A., Antúnez, C., Appollonio, I., Arcaro, M., Archetti, S., Pastor, A.A., Arosio, B., Athanasiu, L., Bailly, H., Banaj, N., Baquero, M., Barral, S., Beiser, A., Pastor, A.B., Below, J.E., Benchek, P., Benussi, L., Berr, C., Besse, C., Bessi, V., Binetti, G., Bizarro, A., Blesa, R., Boada, M., Boerwinkle, E., Borroni, B., Boschi, S., Bossù, P., Bråthen, G., Bressler, J., Bresner, C., Brodaty, H., Brookes, K.J., Brusco, L.I., Buiza-Rueda, D., Bûrger, K., Burholt, V., Bush, W.S., Calero, M., Cantwell, L.B., Chene, G., Chung, J., Cuccaro, M.L., Carracedo, Á., Cecchetti, R., Cervera-Carles, L., Charbonnier, C., Chen, H.-H., Chillotti, C., Ciccone, S., Claassen, J.A.H.R., Clark, C., Conti, E., Corma-Gómez, A., Costantini, E., Custodero, C., Daian, D., Dalmasso, M.C., Daniele, A., Dardiotis, E., Dartigues, J.-F., Deyn, P.P. de, Paiva Lopes, K. de, Witte, L.D. de, Debette, S., Deckert, J., Del Ser, T., Denning, N., DeStefano, A., Dichgans, M., Diehl-Schmid, J., Diez-Fairen, M., Rossi, P. D., Djurovic, S., Duron, E., Düzel, E., Dufouil, C., Eiriksdottir, G., Engelborghs, S., Escott-Price, V., Espinosa, A., Ewers, M., Faber, K.M., Fabrizio, T., Nielsen, S.F., Fardo, D.W., Farotti, L., Fenoglio, C., Fernández-Fuertes, M., Ferrari, R., Ferreira, C. B., Ferri, E., Fin, B., Fischer, P., Fladby, T., Fließbach, K., Fongang, B., Fornage, M., Fortea, J., Foroud, T.M., Fostinelli, S., Fox, N.C., Franco-Macías, E., Bullido, M.J., Frank-García, A., Froelich, L., Fulton-Howard, B., Galimberti, D., García-Alberca, J. M., García-González, P., Garcia-Madrona, S., Garcia-Ribas, G., Ghidoni, R., Giegling, I., Giorgio, G., Goate, A.M., Goldhardt, O., Gomez-Fonseca, D., González-Pérez, A., Graff, C., Grande, G., Green, E., Grimmer, T., Grünblatt, E., Grunin, M., Gudnason, V., Guetta-Baranes, T., Haapasalo, A., Hadjigeorgiou, G., Haines, J.L., Hamilton-Nelson, K.L., Hampel, H., Hanon, O., Hardy, J., Hartmann, A.M., Hausner, L., Harwood, J., Heilmann-Heimbach, S., Helisalmi, S., Heneka, M.T., Hernández, I., Herrmann, M.J., Hoffmann, P., Holmes, C., Holstege, H., Vilas, R.H., Hulsman, M., Humphrey, J., Biessels, G.J., Jian, X., Johansson, C., Jun, G.R., Kastumata, Y., Kauwe, J., Kehoe, P.G., Kilander, L., Ståhlbom, A.K., Kivipelto, M., Koivisto, A., Kornhuber, J., Kosmidis, M.H., Kukull, W.A., Kuksa, P.P., Kunkle, B.W., Kuzma, A.B., Lage, C., Laukka, E.J., Launer, L., Lauria, A., Lee, C.-Y., Lehtisalo, J., Lerch, O., Lleó, A., Longstreth, W., Lopez, O., Munain, A.L. de, Love, S., Löwemark, M., Luckcuck, L., Lunetta, K.L., Ma, Y., Macías, J., MacLeod, C.A., Maier, W., Mangialasche, F., Spallazzi, M., Marquié, M., Marshall, R., Martin, E.R., Montes, A.M., Rodríguez, C. M., Masullo, C., Mayeux, R., Mead, S., Mecocci, P., Medina, M., Meggy, A., Mehrabian, S., Mendoza, S., Menéndez-González, M., Mir, P., Moebus, S., Mol, M., Molina-Porcel, L., Montrreal, L., Morelli, L., Moreno, F., Morgan, K., Mosley, T., Nöthen, M.M., Muchnik, C., Mukherjee, S., Nacmias, B., Ngandu, T., Nicolas, G., Nordestgaard, B.G., Olaso, R., Orellana, A., Orsini, M., Ortega, G., Padovani, A., Paolo, C., Papenberg, G., Parnetti, L., Pasquier, F., Pastor, P., Peloso, G., Pérez-Cordón, A., Pérez-Tur, J., Pericard, P., Peters, O., Pijnenburg, Y.A.L., Pineda, J.A., Piñol-Ripoll, G., Pisanu, C., Polak, T., Popp, J., Posthuma, D., Priller, J., Puerta, R., Quenez, O., Quintela, I., Thomassen, J.Q., Rábano, A., Rainero, I., Rajabli, F., Ramakers, I., Real, L.M., Reinders, M.J.T., Reitz, C., Reyes-Dumeyer, D., Ridge, P., Riedel-Heller, S., Riederer, P., Roberto, N., Rodriguez-Rodriguez, E., Rongve, A., Allende, I.R., Rosende-Roca, M., Royo, J.L., Rubino, E., Rujescu, D., Sáez, M.E., Sakka, P., Saltvedt, I., Sanabria, Á., Sánchez-Arjona, M.B., Sanchez-Garcia, F., Juan, P.S., Sánchez-Valle, R., Sando, S.B., Sarnowski, C., Satizabal, C.L., Scamosci, M., Scarmeas, N., Scarpini, E., Scheltens, P., Scherbaum, N., Scherer, M., Schmid, M., Schneider, A., Schott, J.M., Selbæk, G., Seripa, D., Serrano, M., Sha, J., Shadrin, A.A., Skrobot, O., Slifer, S., Snijders, G.J.L., Soininen, H., Solfrizzi, V., Solomon, A., Song, Y., Sorbi, S., Sotolongo-Grau, O., Spalletta, G., Spottke, A., Squassina, A., Stordal, E., Tartan, J.P., Tárraga, L., Tesí, N., Thalamuthu, A., Thomas, T., Tosto, G., Traykov, L., Tremolizzo, L., Tybjærg-Hansen, A., Uitterlinden, A., Ullgren, A., Ulstein, I., Valero, S., Valladares, O., van Broeckhoven, C., Vance, J., Vardarajan, B.N., van der Lugt, A., van Dongen, J., van Rooij, J., van Swieten, J., Vandenberghe, R., Verhey, F., Vidal, J.-S., Vogelgsang, J., Vyhnalek, M., Wagner, M., Wallon, D., Wang, L.-S., Wang, R., Weinhold, L., Wiltfang, J., Windle, G., Woods, B., Yannakoulia, M., Zare, H., Zhao, Y., Zhang, X., Zhu, C., Zulaica, M., Farrer, L.A., Psaty, B.M., Ghanbari, M., Raj, T., Sachdev, P., Mather, K., Jessen, F., Ikram, M.A., Mendonça, A. de, Hort, J., Tsolaki, M., Pericak-Vance, M.A., Amouyel, P., Williams, J., Frikke-Schmidt, R., Clarimon, J., Deleuze, J.-F., Rossi, G., Seshadri, S., Andreassen, O.A., Ingelsson, M., Hiltunen, M., Sleegers, K., Schellenberg, G.D., van Duijn, C.M., Sims, R., van der Flier, W.M., Ruiz, A., Ramirez, A., Lambert, J.-C., 2022. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nature genetics 54, 412–436. https://doi.org/10.1038/s41588-022-01024-z.

Harerimana, N.V., Goate, A.M., Bowles, K.R., 2022. The influence of 17q21.31 and APOE genetic ancestry on neurodegenerative disease risk. Front. Aging Neurosci. 14, 1021918. https://doi.org/10.3389/fnagi.2022.1021918.

Luft, S., Arrizabalaga, O., Kulish, I., Nasonova, E., Durante, M., Ritter, S., Schroeder, I.S., 2017. Ionizing radiation alters human embryonic stem cell properties and differentiation capacity by diminishing the expression of activin receptors. Stem Cells Develop. 26, 341–352. https://doi.org/10.1089/scd.2016.0277.

Tandon, R., Brändl, B., Baryshnikova, N., Landshammer, A., Steenpaß, L., Keminer, O., Pless, O., Müller, F.-J., 2018. Generation of two human isogenic iPSC lines from fetal dermal fibroblasts. Stem Cell Res. 33, 120–124. https://doi.org/10.1016/j.scr.2018.10.004.