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Lab Resource: Multiple Cell Lines

# Generation of human induced pluripotent stem cell lines (hiPSC) from one bipolar disorder patient carrier of a *DGKH* risk haplotype and one non-risk-variant-carrier bipolar disorder patient

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A B S T R A C T

Fibroblasts were isolated from skin biopsies from two patients with bipolar I disorder. One patient was a 26 year old female carrying a risk haplotype in the *DGKH* (diacylglycerol kinase eta) gene and the other was a non-carrier 27 year old male. Patient fibroblasts were reprogrammed into human induced pluripotent stem cells (hiPSCs) by using a Sendai virus vector. *DGKH*-risk haplotype and non-risk haplotype hiPSCs showed expression of pluripotency markers and were able to differentiate into cells of the three germ layers. These cell models are useful to investigate the role of risk gene variants in bipolar disorder.

Resource table.

| | |
|---|---|
| Unique stem cell lines identifier | KGUi001-A<br>KGUi002-A |
| Alternative names of stem cell lines | AR1023 hiPSC (KGUi001-A)<br>AR1034 hiPSC (KGUi002-A) |
| Institution | Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, University Hospital Frankfurt, Frankfurt Am Main, Germany |
| Contact information of distributor | Dr. Sarah Kittel-Schneider, sarah.kittel-schneider@kgu.de |
| Type of cell lines | iPSC |
| Origin | Human |
| Cell Source | Dermal fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Sendai virus |
| Multiline rationale | Same disease non-isogenic cell lines |
| Gene modification | No |
| Type of modification | N/A |
| Associated disease | Bipolar Disorder |
| Gene/locus | SNPs *DGKH* (rs994856/rs9525580/rs9525584 GAT haplotype; GG/AG/TT) and NON-GAT haplotype (AG/GG/CT); 13q14.11 |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | May 2018 |
| Cell line repository/bank | N/A |
| Ethical approval | Ethics committee University of Würzburg, 10.06.2011, Ethical approval number 96/11<br>Ethics committee University of Frankfurt, 04.3.2015, Ethical approval number 425/14 |

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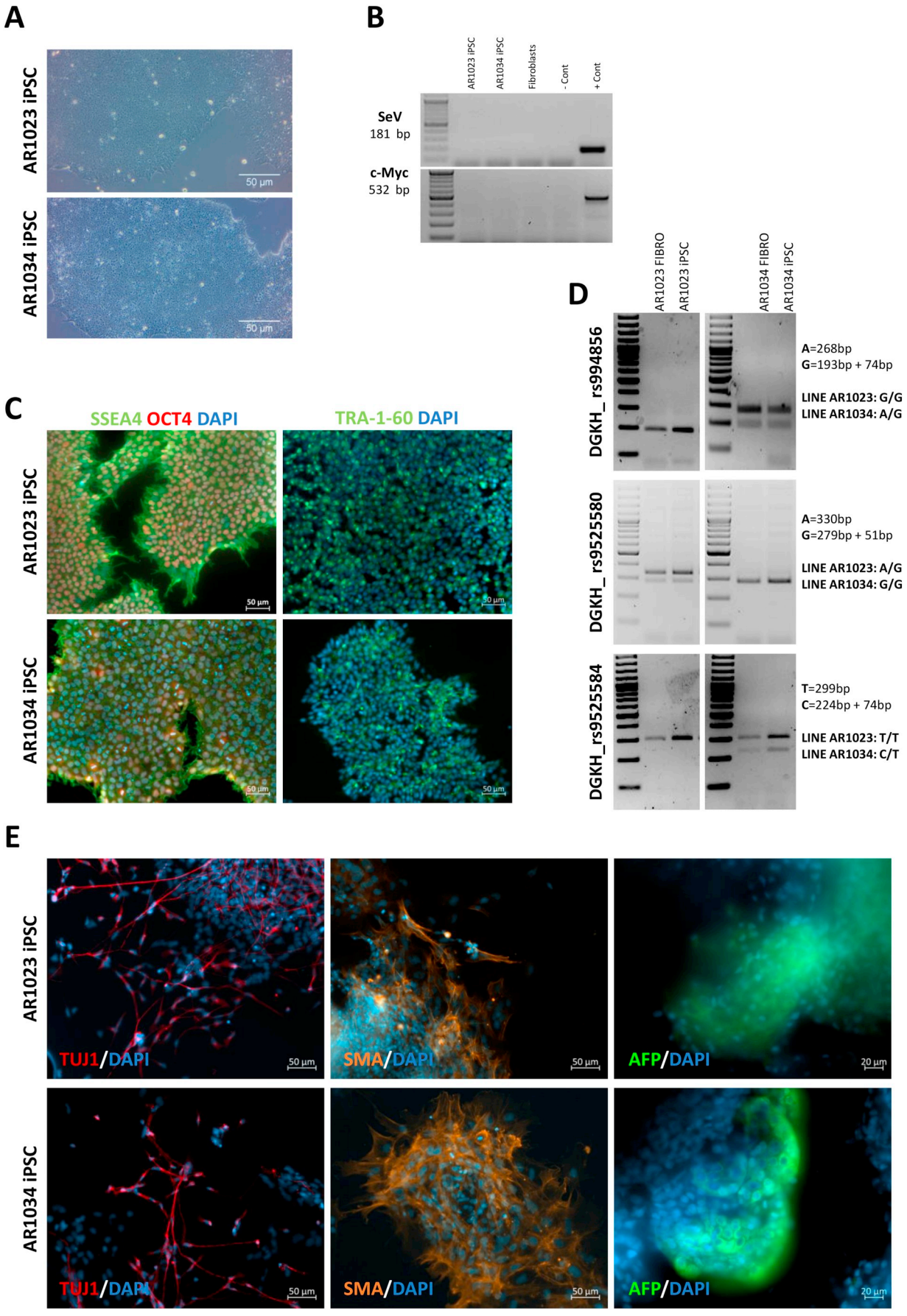


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**Fig. 1.** A: hiPSCs clones show ES-like morphology; B:Absence of Sendai virus and c-myc after 12 passages, confirmed bei PCR; C:Expression of pluripotency markers OCT4, TRA-1-60, SSEA4 tested by immunofluorescence, D: Genotyping for the DGKH risk genotypes by PCR; E: Expression of pluripotency markers SMA, TUJI, AFP in the embryoid bodies by immunofluorescence; F: relatedness matrix of the molecular karyotyping; G:Pluripotency markers OCT4, NANOG, SOX2, DPPA5 of hIPSCs tested by PCR; H: Genotyping of the DGKH risk genotypes by KASP assay. I: Testing for mycoplasma and acholeplasma contamination by PCR; J: Pluripotency associated markers expression was quantified by colocalization analysis (colocalization nuclear stain/marker).

## Resource utility

*DGKH* rs994856/rs9525580/rs9525584 (GAT) risk and non-risk haplotype hiPSCs can be used as tools to investigate the role of diacylglycerol kinase eta (DGKη) as a risk gene for bipolar disorder and explore the pathophysiological role of pathways up- and downstream the inositol-1,4,5-triphosphate ($IP_3$) second messenger pathway in which DGKη is involved.

## Resource details

Bipolar disorder (BD) is a psychiatric disorder with a high heritability that affects 1–2% of the population worldwide. Common variants in the *DGKH* (diacylglycerol kinase eta) gene have been implicated in BD. In our own work we identified a risk haplotype (rs994856/rs9525580/rs9525584 GAT) as a shared risk variant for BD, major depression and adult attention-deficit/hyperactivity disorder (aADHD) (Weber et al., 2011). Even though *DGKH* did not emerge as a top bipolar risk gene in the most recent GWAS studies, current pathway analysis confirmed signalling pathways in which the *DGKH*-encoded protein diacylglycerol kinase η (DGKη) is involved as being associated with BD (Pandey et al., 2012). Additionally, a *Dkgh* knockout mouse model shows behavioural changes similar to manic symptoms in human BD patients that were normalized by lithium chloride treatment (Isozaki et al., 2016). Our previous work showed that the *DGKH* risk haplotype influenced amygdala volume specifically in BD patients (Kittel-Schneider et al., 2015). Moreover, increased *DGKH* expression was found in the peripheral blood of bipolar risk haplotype carriers compared to bipolar and healthy controls non-GAT carriers. In the fibroblast cells, *PRKCD* expression was significantly increased in bipolar *DGKH* GAT carriers (Kittel-Schneider et al., 2016). BD donors were recruited while being treated as in-patients in the Department of Psychiatry, Psychotherapy and Psychosomatic Medicine, University Hospital of Würzburg. The *DGKH* GAT haplotype carrier was a 26 year old female Caucasian bipolar I patient, lithium responder. The *DGKH* non-risk variant carrier was a 27 year old male Caucasian Bipolar I patient, lithium non-responder.

Fibroblast primary cultures were derived from skin biopsies and reprogrammed into hiPSCs by transfection with the Yamanaka factors with a Sendai virus (SeV)-based vector. The presence or absence of the GAT risk haplotype was confirmed by PCR in fibroblast and HiPSC samples (Fig. 1D) and by KASP assay (Fig. 1H). hiPSCs clones from both the cell lines displayed a specific ES-like morphology (compact and round-shaped colonies characterized by distinct borders, individual tightly packed cells, high nuclear to cytoplasm ratio) (Fig. 1A). We confirmed the absence of the reprogramming viral vector specific transcripts by Reverse transcription polymerase chain reaction (RT)-PCR after 12 passages (Fig. 1B). The expression of key pluripotency markers was assessed by immunofluorescence (OCT4, TRA-1-60, SSEA4) (Fig. 1C) and by (RT)-PCR (OCT4, NANOG, SOX2, DPPA5) (Fig. 1G). The 3-germ layer differentiation capacity was confirmed *in vitro* by promoting hiPSCs growth as spherical three-dimensional aggregates (embryoid bodies -EBs) and evaluated by immunofluorescence. Marker for the mesodermal (smooth muscle actin SMA), ectodermal (β-III-tubulin TUJ1) and endodermal (α-fetoprotein AFP) lineage were expressed by both the cell lines (Fig. 1E). Molecular karyotyping (by Illumina Infinium Omni2.5–8 bead array) and short tandem repeat (STR) analysis did not show any significant increase in DNA aberration or anomalies when comparing fibroblasts and hiPSCs clones from the same patient and confirmed a high rate of genetic similarity among hiPSCs, fibroblast and blood samples from the same cell line, as described in the Relatedness Matrix (Fig. 1F). The two different hiPSC lines generated in our facility have thus been confirmed to be *Bona Fide* hiPSCs and can be used for further applications.

## Materials and methods

### *Skin biopsies and fibroblast primary cultures*

Skin biopsies were performed under local anaesthesia. After incubation for 16 h at 4 °C in dispase solution (2.4 U/ml-PAN Biotech) the epidermis layer was removed and the sample incubated with collagenase (Serva) for 45 min at 37 °C. Fibroblasts were detached by Trypsin/EDTA (PANBiotech) incubation and fed with DMEM, 10%FBS (Life Technologies) twice weekly.

### *Reprogramming and hiPSCs culture*

Fibroblasts (passage 5) were reprogrammed in hiPSCs using CytoTune-Ips 2.0 Sendai Reprogramming Kit (Invitrogen). Fibroblast were incubated with the viral vectors for 24 h in DMEM, 10% FBS, 1% MEM Non-Essential Amino Acids 100×, 55 μM 2-Mercaptoethanol (Life Technologies). At day 7 cells were seeded on irradiated mouse embryonic fibroblasts (Amsbio) and feed daily with KnockOut DMEM/F-12, 20%KnockOut Serum Replacement, 1%MEM Non-Essential Amino Acids 100×, 55 μM 2-Mercaptoethanol (Life Technologies), 1% Penicillin/streptomycin (Sigma).

Emerging colonies were manually picked for single clone expansion.

HiPSCs were cultured on Matrigel matrix (Corning) and feed daily with mTeSR1 (StemCell Technologies). Cells were split 1:2 ratio one day before reaching confluence using ReLeSR (StemCell Technologies) For a summary of the generated cell lines see (Tables 1 and 2).

### *Reverse transcription polymerase chain reaction (RT-PCR) and genotyping*

For all RT-PCR applications, total RNA was isolated using RNeasy-Plus Mini Kit (Qiagen) and 500 ng converted into cDNA by RT-iScript cDNA Synthesis Kit (Bio Rad). PCR reagents were from Biozym and primers from Eurofins Medigenomix (Table 3). Genotyping of *DGKH* haplotype was performed as described in (Kittel-Schneider et al., 2016). Briefly, DNA was extracted from fibroblasts and hiPSCs, diluted to a working solution of 50–100 ng/μl and used for PCR. PCR products were digested with restriction enzymes (New England Biolab), separated in a 3% agarose gel and imaged with myECL Imager (Thermo Fisher Scientific). rs994856 was digested with *Hpy*188I (10,000 U/ml) leading to the following products: A = 268 bp G = 193 bp + 74 bp; rs9525580 with *Bsr*DI (5000 U/ml): G = 279 bp + 51 bp A = 330 bp and rs9525584 with *Msp*I (20,000 U/ml): C = 224 bp + 74 bp *T* = 299 bp. The genotype was also confirmed by KASP Assay as suggested by the manufacturer by wet DNA method (LGC Genomics). Fluorescent signal from the two FRET cassettes was detected with LightCycler 480 (Roche).

### *3-germ layer in vitro differentiation*

Ten million hiPSCs were seeded on AggreWell400Ex plates (Stem Cell Technologies) as single cell suspension in AggreWell Formation Medium, 10 μM Y-27632 (Stem Cell Technologies) and harvested after 24 h. EBs were maintained in suspension culture and fed every third day. From day 11 EBs were fed with DMEM/F-12, 10%KnockOut Serum

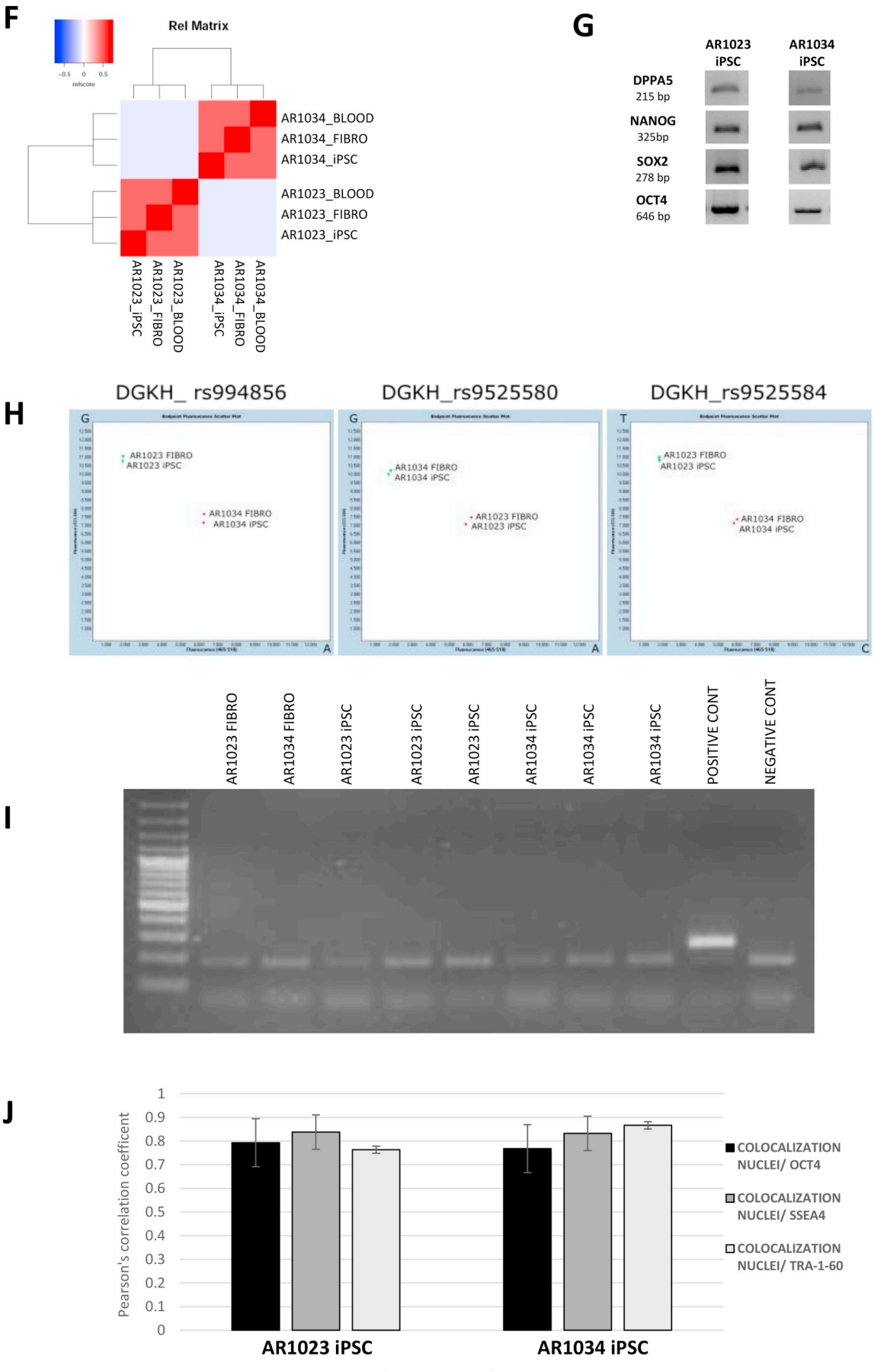


**Fig. 1.** (*continued*)

**Table 1**
Summary of lines.

| iPSC line names | Abbreviation in figures | Gender | Age | Ethnicity | Genotype of locus | Disease |
|---|---|---|---|---|---|---|
| KGUi001-A | AR1023 HiPSC | Female | 26 | Polish (Caucasian) | *DGKH* rs994856 G/G<br>*DGKH* rs9525580 A/G<br>*DGKH* rs9525584 T/T | Bipolar disorder |
| KGUi002-A | AR1034 HiPSC | Male | 27 | German (Caucasian) | *DGKH* rs994856 A/G<br>*DGKH* rs9525580 G/G<br>*DGKH* rs9525584 C/T | Bipolar disorder |

**Table 2**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Light microscopy | hESC-like morphology | Fig. 1 panel A |
| Phenotype | Qualitative analysis: immunofluorescence (OCT4, TRA-1-60, SSEA4) and (RT)-PCR (OCT4, NANOG, SOX2, DPPA5) | Expression of pluripotency markers | Fig. 1 panel C<br>Fig. 1G and Supplementary Figure panel G |
| | Quantitative analysis: immunofluorescence colocalization | Colocalization nuclear stain/marker (Pearson's correlation coefficient):<br>OCT4: r = 0,78<br>TRA-1-60: $r$ = 0,835<br>SSEA4: r = 0,815 | Fig. 1J and Supplementary Figure panel J |
| Genotype | DNA BeadChip array | *Infinium* Omni 2.5 Exom-8 V1.3 | Fig 1F and Supplementary Figure panel F |
| Identity | STR analysis | STR profile consists of 21 specific markers | Submitted in archive with journal |
| Mutation analysis | Human Single Nucleotide Polymorphisms determined by PCR and KASP Assay | AR1023 HiPSC: GAT haplotype<br>AR1034 HiPSC: non GAT haplotype | Fig. 1 panel D and H and Supplementary Figure panel H |
| Microbiology and virology | Mycoplasma | Venor GeM Mycoplasma Detection Kit: negative | Fig. 1I and Supplementary Figure panel I |
| Differentiation potential | Embryoid body formation undirected differentiation | Expression of smooth muscle actin, β-tubulin and α-feto protein. | Fig. 1 panel E |
| Donor screening | HIV 1 + 2 Hepatitis B, Hepatitis C | Negative | Not shown but available with author |
| Genotype additional info | Blood group genotyping | N/A | N/A |
| | HLA tissue typing | N/A | N/A |

**Table 3**
Reagents details.

Antibodies used for immunocytochemistry

| | Antibody | Dilution | Company Cat # and RRID |
|---|---|---|---|
| Pluripotency markers | Rabbit anti-OCT4 | 1:500 | Thermo Fisher Scientific Cat# 710788, RRID:AB_2633097 |
| | Mouse anti-SSEA4 | 1:200 | Thermo Fisher Scientific Cat# MA1-021, AB_2536687 |
| | Mouse anti-TRA-1-60 | 1:100 | Novus Cat# NB100–730, RRID:AB_10001809 |
| Differentiation markers | Rabbit anti-TUJ1 | 1:700 | Thermo Fisher Scientific Cat# A25532, RRID:AB_2651003 |
| | Mouse anti-AFP | 1:700 | Thermo Fisher Scientific Cat# A25530, RRID:AB_2651004 |
| | Mouse anti-SMA | 1:200 | Thermo Fisher Scientific Cat# A25531, RRID:AB_2651005 |
| Secondary antibodies | Alexa Fluor 594 donkey anti-rabbit | 1:250 | Thermo Fisher Scientific Cat# R37119, RRID:AB_2556547 |
| | Alexa Fluor 488 goat anti-mouse IgG3 | 1:250 | Thermo Fisher Scientific Cat# A-21151, RRID:AB_2535784 |
| | Alexa Fluor 488 goat anti-mouse IgM | 1:250 | Thermo Fisher Scientific Cat# A-21042, RRID:AB_2535711 |
| | Alexa Fluor 488 goat anti-mouse IgG1 | 1:250 | Thermo Fisher Scientific Cat# A25536, RRID:AB_2651011 |
| | Alexa Fluor 555 goat anti-mouse IgG2a | 1:250 | Thermo Fisher Scientific Cat# A25533, RRID:AB_2651012 |
| | Alexa Fluor 647 donkey anti-rabbit | 1:250 | Thermo Fisher Scientific Cat# A25535, RRID:AB_2651010 |

Primers

| | Target | Forward/Reverse primer (5′-3′) |
|---|---|---|
| Pluripotency markers, (RT)-PCR | hSOX2 | AACCAGCGCATGGACAGTTA/GACTTGACCACCGAACCCAT |
| | hNANOG | ACCAGTCCCAAAGGCAAACA/AAAGGCTGGGGTAGGTAGGT |
| | hOCT4 | GTTGATCCTCGGACCTGGCTA/GGTTGCCTCTCACTCGGTTCT |
| | hDPPA5 | CGGCTGCTGAAAGCCATTTT/AGTTTGAGCATCCCTCGCTC |
| SNP genotyping | DGKH2_rs9525584 | GGTGAACAGCACATGCAGCCG/TTTGAGGCTGGCTTCCAAATTTCA |
| | DGKH2_rs994856 | TGCTTTGCCTGAAAACCCAGTGC/TGGGAGTGAGAGACTGCACAAGA |
| | DGKH2_rs9525580 | TGCACAAGTTAGAGACCCGTTGGA/CCCTCCTAAGCCTGCCCCCA |
| Sendai virus (SeV) detection, (RT)-PCR | SeV | GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC |
| | c-Myc | TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG |

Replacement, 1%MEM Non-Essential Amino Acids 100×, 0.1 mM 2-Mercaptoethanol, L-glutamine (Life Technologies). After two weeks EB were seeded onto matrigel-coated coverslips and cultured for 6 days.

*Immunofluorescence*

Cells were seeded on Matrigel-coated coverslips and fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.2%Triton-X (hIPSCs) or 1%Saponin (EBs) for 15 min at RT and blocked with 3%BSA (Sigma Aldrich) for 30 min at room temperature (RT). Primary antibodies were incubated overnight at 4 °C, secondary antibodies were incubated for 1 h at RT. Coverslip were mounted on glass slide with ProLong Diamond Antifade Mountant with DAPI (Life Technologies). Slides were imaged with a fluorescence microscope with ApoTome function (Zeiss). Pluripotency associated markers expression was quantified by colocalization analysis (colocalization nuclear stain/marker) and Pearson's correlation coefficient calculated by the Fiji/ImageJ software plug-in Coloc2 (Fig. J).

*Mycoplasma testing*

Both fibroblast and hiPSCs lines were routinely tested for the absence of Mycoplasma and Acholeplasma contamination by Venor GeM Mycoplasma Detection Kit (Merk Millipore) (Fig. 1I).

*Molecular karyotyping and STR analysis*

Genomic DNA was extracted with DNeasy kit (Quiagen) and samples were analysed by Illumina Infinium Omni2.5–8 bead array in the Institute of Human Genetics, LIFE&BRAIN GmbH, University Bonn. SNP calling was performed using GenomeStudio software and GenTrain Algorithmus 2.0 with a GenCall Threshold of 0.2. All samples had call rates above 98%. Samples were tested for discordance between genetically inferred gender and annotated gender, relatedness was defined *via* identity by state (IBS) and doublets were defined by IBS $\leq 2$. The relatedness matrix is based on the PI HAT score, all calculation were done in Plink v1.9. STR analysis was conducted by CLS Cell Lines Service GmbH.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.09.008.

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