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Lab Resource: Multiple Cell Lines

# Generation of 20 human induced pluripotent stem cell lines from patients with focal segmental glomerulosclerosis (FSGS)

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A B S T R A C T

Focal segmental glomerulosclerosis (FSGS) is a major cause of familial nephrotic syndrome. We generated 20 induced pluripotent stem cell lines from patients diagnosed with FSGS. The iPSC lines include 8 female and 12 male lines and cover a donor age range from 31 to 78. The lines were generated from peripheral blood mononuclear cells by integration-free reprogramming using Sendai virus vectors. Cell lines were fully characterized regarding their pluripotency and differentiation potential, and quality controlled for karyotypic integrity, identity and clearance of reprogramming vectors. The generated cell lines represent a valuable tool for disease modelling and drug development for FSGS.

## 1. Resource table

| | |
|---|---|
| Unique stem cell lines identifier | BIHi006-D, BIHi007-A, BIHi008-A, BIHi009-A, BIHi010-A, BIHi011-A, BIHi012-A, BIHi015-A, BIHi016-A, BIHi017-A, BIHi018-A, BIHi019-A, BIHi024-A, BIHi025-A, BIHi028-B, BIHi029-A, BIHi030-C, BIHi031-B, BIHi032-A, BIHi038-B |
| Alternative names of stem cell lines | N/A |
| Institution | Charité – Universitätsmedizin Berlin, Berlin Institute of Health (BIH) Berlin, Germany |
| Contact information of distributor | BIH Stem Cell Core FacilityHarald Stachelscheid, Sebastian Diecke (stemcellcore@bihealth.de) |
| Type of cell lines | hiPSC |
| Origin | human |
| Cell Source | peripheral blood mononuclear cells |
| Clonality | clonal |
| Method of reprogramming | sendai virus (Oct3/4, Sox2, c-Myc, Klf4) |
| Multiline rationale | same disease non-isogenic cell lines |
| Gene modification | no |
| Type of modification | N/A |
| Associated disease | Focal Segmental Glomerulosclerosis (FSGS) |
| Gene/locus | N/A |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | 2017 |
| Cell line repository/bank | Berlin Institute of Health, Stem Cell Core Facility *https://www.bihealth.org/en/research/core-facilities/stem-cells/* European Bank for induced pluripotent Stem Cells (EBiSC) *https://cells.ebisc.org/* |
| Ethical approval | approved by the Ethics Committee of Charité – Universitätsmedizin Berlin (EA2/047/14) |

## 2. Resource utility

FSGS is a severe chronic disease leading to renal failure. It is a cause of one sixth of all cases of nephrotic syndrome in children and adolescents, and a leading cause of kidney failure in adults. The term FSGS

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**Table 1**
Summary of lines.

| iPSC line names | Gender | Age | Ethnicity | Genotype of locus | Disease |
|---|---|---|---|---|---|
| BIHi012-A | female | 31 | N/A | N/A | FSGS |
| BIHi024-A | female | 31 | N/A | N/A | FSGS |
| BIHi031-B | female | 37 | N/A | N/A | FSGS |
| BIHi011-A | female | 44 | N/A | N/A | FSGS |
| BIHi016-A | female | 47 | N/A | N/A | FSGS |
| BIHi038-B | female | 51 | N/A | N/A | FSGS |
| BIHi008-A | female | 61 | N/A | N/A | FSGS |
| BIHi028-B | female | 78 | N/A | N/A | FSGS |
| BIHi029-A | male | 33 | N/A | N/A | FSGS |
| BIHi006-D | male | 36 | N/A | N/A | FSGS |
| BIHi018-A | male | 36 | N/A | N/A | FSGS |
| BIHi017-A | male | 37 | N/A | N/A | FSGS |
| BIHi015-A | male | 44 | N/A | N/A | FSGS |
| BIHi032-A | male | 45 | N/A | N/A | FSGS |
| BIHi010-A | male | 46 | N/A | N/A | FSGS |
| BIHi019-A | male | 49 | N/A | N/A | FSGS |
| BIHi007-A | male | 54 | N/A | N/A | FSGS |
| BIHi030-C | male | 61 | N/A | N/A | FSGS |
| BIHi025-A | male | 63 | N/A | N/A | FSGS |
| BIHi009-A | male | 67 | N/A | N/A | FSGS |

refers to a disease of primary podocyte injury, or a lesion caused by secondary scarring processes in any type of chronic kidney disease (Fogo, 2015). FSGS is characterized by glomerular injury, associated with podocyte damage and proteinuria. Causes include monogenetic mutations causing alterations in structural genes of the podocyte, many of which result in early onset of disease. These genes include nephrin (NPHS1), podocin (NPHS2), alpha-actinin-4 (ACTN-4), CD2-associated protein (CD2AP), Wilm's tumor gene (WT1), and transient receptor potential cation 6 (TRPC6) (Woroniecki and Kopp, 2007). The recurrence of FSGS in many patients that received a healthy kidney graft points also to systemic factors in disease pathogenesis. Primary FSGS leads to end-stage renal disease (ESRD) with the current only available treatment options dialysis and kidney transplantation. Research to elucidate the diverse disease mechanism is urgently needed to enable the identification of new therapeutic targets and treatment strategies. We generated iPSC lines from 20 FSGS patients to build up a cohort to be utilized in research projects.

## 3. Resource details

PBMCs from 20 FSGS patients (8 female, 12 male, ages between 31 and 78) (Table 1) were reprogrammed with the Sendai virus reprogramming kit Cytotune 2.0 which includes vectors for the genes Oct3/4, Sox2, c-Myc and Klf4. For each iPSC line a single colony was picked after reprogramming, checked for the absence of Sendai viral vectors by PCR (data not shown) and expanded into a master cell bank. The master cell banks were subsequently characterized and quality controlled. All cell lines show the typical morphology of undifferentiated iPSC when maintained in E8 medium (Fig. 1A and Table 2). Immunofluorescence staining of adherent cells confirmed expression of the markers for undifferentiated iPSCs octamer-binding transcription factor 3/4 (Oct3/4), Nanog, tumour rejection antigen (Tra-1–60) and stage specific embryonic antigen 4 (SSEA4) (Fig. 1B, Table 2). The quantification of these markers by flow cytometry confirmed their undifferentiated state. Tra-1–60 is expressed by more than 82%, Oct3/4 is expressed by more than 97%, Nanog by more than 94% and SSEA4 by more than 95% of all cells in all cell lines. Unstained cells (grey histograms) served as negative gating control (Fig. 1C, Table 2, Suppl.Table 1). Furthermore, the PluriTest analysis was applied to additionally confirm the stem cell identity. All cell lines reached high pluripotency and low novelty scores, comparable to a fully characterized iPSC line, which validates their iPSC identity (Fig. 1E, Table 2, Suppl.Table 2). The differentiation into all three germ-layers was tested for all iPSC lines and confirmed their pluripotent differentiation potential. To this end, the cells were tested either by teratoma formation in immunodeficient mice or by directed differentiation *in vitro*. All cell lines differentiated into endodermal, mesodermal and ectodermal cells, as shown by histological analysis of the teratomas or by quantification using flow cytometric analysis of the *in vitro* differentiated cells. Here, at least 68% co-expressed the ectodermal markers Sox2+/Pax6+, 65% stained positive for the mesodermal markers CD140b+ or CD144+ and over 49% co-expressed the endodermal markers CD184+/Sox17+ in the ectodermal, mesodermal and endodermal differentiation assays, respectively. Unstained cells (grey) served as negative and gating control (Fig. 1D, Table 2, Suppl. Fig. 3, Suppl.Table 4). The karyotypes of the cell lines were tested by KaryoLite™ BoBs™ and by SNP analysis. The tests confirmed normal karyotypes for all cell lines but BIHi019-A. In BIHi019-A a deletion on the p-arm and a amplification on the q-arm of chromosome 20 were identified by both test methods (Fig. 1F, Fig. 1G, Table 2, Suppl.Table 3, Suppl.Fig. 2). The identity of all cell lines was confirmed by short tandem repeat (STR) analysis of 10 genomic loci. Identical DNA profiles between patient's PBMCs and the generated hiPSC line were observed (data available with journal). Moreover, tests for mycoplasma contamination and donor screenings for HIV 1 + 2 Hepatitis B and Hepatitis C were all negative (Table 2, Suppl.Table 6).

In summary, we generated integration free hiPSC lines from PBMCs of 20 FSGS patients which can be used to investigate the different disease mechanisms of FSGS and to identify possible drugs and therapies for FSGS.

## 4. Materials and methods

A detailed description of the methods for PBMCs isolation and erythroblast expansion, reprogramming, test for absence of the reprogramming vector, culture of hiPSC, immunofluorescence staining for pluripotency markers, FACS staining and analysis for pluripotency and differentiation markers, PluriTest, *in vitro* directed differentiation into the three germ layers, karyotyping with KaryoLite™ BoBs™ and short tandem repeat analysis can be found in our earlier publication of the cell line BIHi002-A by Hennig et al. (2019). Information on antibodies and primers used can be found in Table 3.

## 5. SNP analysis for karyotyping

PBMCs and iPSCs where karyotyped using the Illumina platform and the OMNI-EXPRESS-8v1.4 Chip. The analysis was done in Karyostudio 1.4.

## 6. Teratoma formation

The hiPSC were harvested as single cells using accutase treatment. A cell pellet of $1x10^6$ was resuspended in 50 µl PBS w/o CaMg and 50 µl of Matrigel was added. The yielded 100 µl hiPSC suspensions were transplanted s.c. into the left flank of immunodeficient mouse. After about six to eight weeks when the tumour reached a size of more than 1 $cm^3$ the animals were sacrificed, and the tumour was histologically evaluated.

## 7. Mycoplasma screening

The cell cultures and master banks were tested for Mycoplasma contaminations by the MycoAlert™ Mycoplasma Detection Kit according to the manufacturer's instructions.

## 8. Screening for HIV 1 + 2 Hepatitis B and Hepatitis C

Testing of the donor PBMCs for viruses was conducted by external diagnostic laboratories (Central lab of Charite – Universitätsmedizin Berlin or IDEXX BioResearch, Ludwigsburg, Germany).

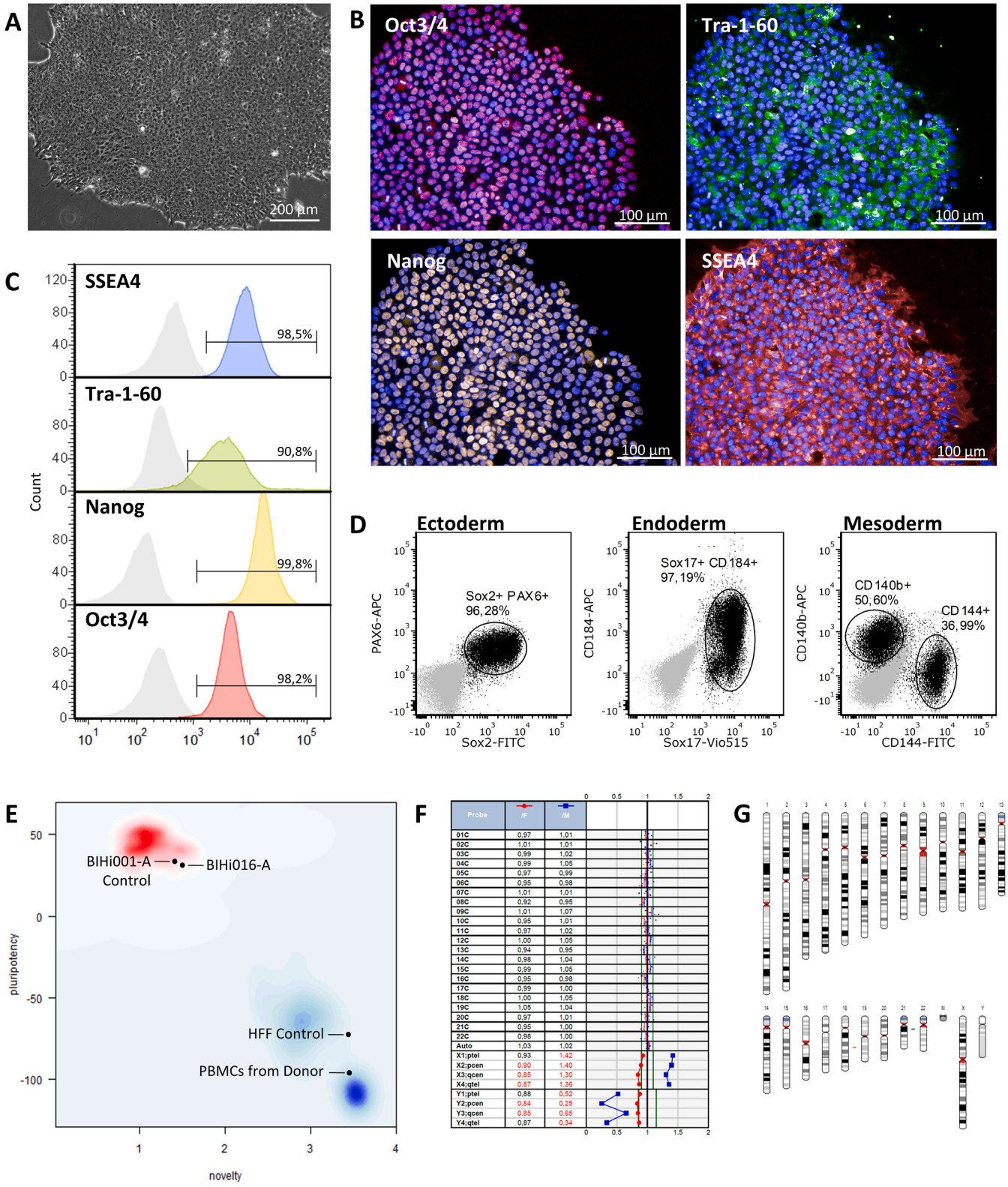


**Fig. 1.** Morphology, pluripotency, differentiation and karyotype of BIHi016-A, exemplary for all 20 iPSC lines. Data for the other lines can be found in the supplementary materials.

**Table 2**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Phase contrast microscopy | Normal PSC morphology | Fig. 1A<br>Suppl.Fig. 1 |
| Phenotype | Qualitative analysis (immunocytochemistry) | Positive stainings for Oct3/4, Nanog, Tra 1–60, SSEA-4 confirm the undifferentiated state of the iPSC lines | Fig. 1B<br>Suppl.Fig. 1 |
| | Quantitative analysis (flow cytometry) | High percentages of positively stained cells for Oct3/4, Nanog, Tra 1–60, SSEA-4 confirm the undifferentiated state of the iPSC lines | Fig. 1C<br>Suppl.Table 1 |
| | PluriTest | Pluripotency and novelty scores confirm the iPSC identity | Fig. 1E<br>Suppl.Table 2 |
| Genotype | Karyotype (KaryoLite™ BoBs™) | Normal karyotype in all lines, except for BIHi019-A<br>Resolution: 4 probes per chromosome | Fig. 1F<br>Suppl.Table 3 |
| | Karyotype (SNP array) | Normal karyotype in all lines, except for BIHi019-A<br>Resolution: ≈960000 markers | Fig. 1G<br>Suppl.Fig. 2 |
| Identity | STR analysis | 10 STR sites analysed, all matching between donor PBMCs and hiPSC lines | Submitted in archive with journal |
| Microbiology and virology | Mycoplasma testing (luminescence) | Negative | Suppl.Table 6 |
| Clearance of reprogramming vectors | PCR for SeV, SeV-cMyc, SeV-Klf4, SeV-KOS | No detectable Sendai virus genes, vectors and transgenes | Suppl.Table 5 |
| Differentiation potential | Directed differentiation and quantitative analysis (flow cytometry) | Germ layer-specific marker expression:<br>Endoderm: $CD184^{+}/Sox17^{+}$<br>Mesoderm: $CD140b^{+}$, $CD144^{+}$<br>Ectoderm: $Pax6^{+}/Sox2^{+}$ | Fig. 1D<br>Suppl.Table 4 |
| | Teratoma formation and qualitative analysis (histology) | Teratomas containing derivatives of all three germ layers were formed | Suppl.Fig. 3<br>Suppl.Table 4 |
| Donor screening | HIV 1 + 2 Hepatitis B, Hepatitis C | Negative | Not shown but available with the author |

**Table 3**
Reagents details.

| Antibodies used for immunocytochemistry/flow cytometry | | | |
|---|---|---|---|
| | Antibody | Dilution | Company Cat # and RRID |
| Pluripotency Markers Immunocytochemistry | Anti-Nanog-PE | 1:50 | Cell Signaling Cat# 14955, RRID: N/A |
| | Anti-Oct3/4-APC | 1:10 | Miltenyi Biotec Cat# 130–105-607, RRID:AB_2653086 |
| | Anti-SSEA4-PerCP-Vio700 | 1:10 | Miltenyi Biotec Cat# 130–105-083, RRID:AB_2653526 |
| | Anti-Tra1-60-Vio488 | 1:100 | Miltenyi Biotec Cat# 130–106-872, RRID:AB_2654228 |
| Pluripotency Markers Flow Cytometry | Anti-Nanog-PE | 1:100 | Cell Signaling Cat# 14955, RRID: N/A |
| | Anti-Oct3/4-APC | 1:20 | Miltenyi Biotec Cat# 130–105-555, RRID:AB_2653087 |
| | Anti-Tra1-60-Vio488 | 1:700 | Miltenyi Biotec Cat# 130–106-872, RRID:AB_2654228 |
| Differentiation Markers Flow Cytometry | CD140b-APC | 1:11 | Miltenyi Biotec Cat# 130–105-280, RRID:AB_2655085 |
| | CD144 (VE-Cadherin)-FITC | 1:11 | Miltenyi Biotec Cat# 130–100-742, RRID:AB_2655151 |
| | CD184 (CXCR4)-APC | 1:11 | Miltenyi Biotec Cat# 130–109-844, RRID:AB_2655771 |
| | Anti-PAX-6-APC | 1:11 | Miltenyi Biotec Cat# 130–107-776, RRID:AB_2653169 |
| | Anti-Sox17-Vio515 | 1:50 | Miltenyi Biotec Cat# 130–111-031, RRID:AB_2653497 |
| | Anti-Sox2-PE | 1:11 | Miltenyi Biotec Cat# 130–104-994, RRID:AB_2653501 |
| Primers | | | |
| | Target | Forward/Reverse primer (5′-3′) | |
| Sendai virus vectors (PCR) | SeV | GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC | |
| | SeV-Klf4 | TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA | |
| | SeV-cMyc | TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG | |
| | SeV-KOS | ATGCACCGCTACGAGTGAGCGC/ | |
| House-Keeping Genes (PCR) | Hu18SRNA | GTAACCCGTTGAACCCCATT/ CCATCCAATCGGTAGTAGCG | |

## 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102406.

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