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# Generation of two induced pluripotent stem cell lines (NHLBIi001-A and NHLBIi001-B) from a healthy Caucasian female volunteer with normal cardiac function

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## Abstract

Human-derived induced pluripotent stem cells (iPSCs) have proven to be indispensable in cardiovascular drug development, disease modeling, and developmental biology research. For this reason, it is particularly useful to develop wild-type iPSC lines to be used in experimental or control conditions. Here, we present two such cell lines generated from a sample of peripheral blood mononuclear cells (PBMCs) from a healthy patient with normal cardiac function.

## 1. Resource utility

These well-characterized human induced pluripotent stem cell (iPSC) lines derived from an apparently healthy Caucasian female volunteer are useful as wild-type control iPSC lines, especially for cardiovascular development research and disease modeling.

## 2. Resource details

Cardiovascular diseases are the number one cause of death globally. A recent scientific statement from the American Heart Association (AHA) highlighted the opportunities of using healthy donor and patient-derived iPSC lines for cardiovascular disease modeling and regenerative medicine. Well-characterized wild-type iPSC lines from racially diverse backgrounds are valuable controls in iPSC-based studies, especially when patients with specific mutations are unavailable and gene-editing technologies are applied (Ma et al., 2018; Musunuru et al., 2018). A Cardiovascular Discovery Protocol (10-H-0126, https://clinicalstudies.info.nih.gov/ProtocolDetails.aspx?A_2010-H-0126.html) at the National Heart, Lung, and Blood Institute (NHLBI) was established to study known or suspected metabolic or genetic factors that carry the risk of developing cardiovascular



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Supplementary materials

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disease. Patients and healthy volunteers from various racial backgrounds underwent tests including an echocardiography, a TC angiogram and screening laboratory studies to evaluate cardiac and metabolic health. Study subjects also signed a public stem cell database consent form, which allows NHLBI researchers to use donors' blood samples to generate iPSCs for research on tissue development, disease modeling, and drug development, and to submit genetic data or iPSC lines to a public database. In this study, two human iPSC lines were established from one such volunteer: an apparently healthy 51-year old Caucasian female who had a normal heart on echocardiography and CT angiography and no evidence of metabolic disease.

Following her signing consent, erythroblasts from the healthy volunteer's peripheral blood mononuclear cell (PBMC) sample were expanded and reprogrammed with the pluripotency factors OCT3/4, KLF4, SOX2 and C-MYC using Cytotune 2.0 Sendai Virus (SeV) to generate NHLBIi001-A and NHLBIi001-B iPSC lines, which maintained a human embryonic stem cell (ESC)-like morphology. Their undifferentiated state was characterized by immunofluorescent staining and flow cytometry analysis of several common human ESC/iPSC markers including SOX2, NANOG, OCT4, SSEA4, and TRA-1–60 (Fig. 1A and B). In addition, G-banding karyotyping indicated a normal karyotype (46, XX) (Supplemental Figure S1A) and short tandem repeat (STR) DNA profiling analysis at 15 loci showed the genotypes of these two iPSC lines did match that of the parental PBMC (submitted in archive with journal). Mycoplasma status was also confirmed to be negative by quantitative PCR (qPCR) (Supplemental Figure S1B). Clearance of the Sendai viral vector (SeV) was confirmed after passage 15 with quantitative reverse transcription polymerase chain reaction (qRT-PCR) using SeV-specific primers (Supplemental Figure S1C). Lastly, pluripotency was demonstrated by a teratoma formation assay in which the cells successfully differentiated into all three germ layers (ectoderm, neural tube; mesoderm, cartilage; endoderm, gut) *in vivo* (Fig. 1C).

## 3. Materials and methods

### 3.1. Cell culture

The NHLBIi001-A and NHLBIi001-B iPSC cell lines were derived from peripheral blood mononuclear cells (PBMCs) from a healthy 51 years old female Caucasian volunteer with a normal cardiovascular and metabolic profile. The PBMCs were isolated from 5 to 10 ml whole blood by the standard Ficoll gradient centrifugation method. They were cultured in 1 well of a 12-well tissue culture plate with 1 ml StemSpan™SFEM II medium with an added Erythroid expansion supplement (100ng/ml SCF, 10ng/ml IL-3, 2U/ml EPO, 40ng/ml IGF-1, 1 μM Dexamethasone, and 100 μg/ml holo-transferrin) for 8–10 days. Half of the medium was changed every 2 days to promote erythroblast expansion. Reprogramming of PBMC-derived erythroblasts with the CytoTune 2.0 SeV kit (A16517, Thermo Fisher) is similar to fibroblast reprogramming as previously described (Beers et al., 2015) except for a few modifications: (1) 200,000 PBMCs were infected by 20ul of premixed SeVs (1:1:1 ratio of three SeVs) using centrifugation at 2250 rpm for 1 h, (2) SeVs were removed by centrifugation one day after infection, (3) erythroblasts were plated onto Matrigel (Corning, 354277)-coated 48-well plates on day 2–3 post-infection by a serial dilution of 20–15,000

cells/well. Established NHLBIi001-A and NHLBIi001-B iPSCs were maintained with Essential8 (E8) medium (A1517001, Thermo Fisher) using the EDTA dissociation method (Beers et al., 2012) and were expanded for >15 passages prior to further characterization and use.

**3.1.1. Immunocytochemistry—**NHLBIi001-A and NHLBIi001-B iPSCs were fixed and stained as previously described, though we blocked the cells and diluted the primary antibodies with a 10mg/ml Bovine Serum Albumin (BSA) in DPBS solution (Hong et al., 2019). Cell nuclei were stained with DAPI and the cells were imaged with an EVOS® FL Cell Imaging System (Thermo Fisher) and a 10 or 20 × objective lens with Texas Red, FITC, and DAPI filters.

**3.1.2. Flow cytometry analysis—**iPS cells were dissociated from the plate with TrypLE (12563029, Thermo Fisher) and were prepared for flow cytometry as previously described (Beers et al., 2015), except that a different permeabilization buffer (2%FBS and 0.2% Tween 20 in DPBS) was used. We used fluorophore conjugated antibodies as listed inTable 2. The cells were analyzed with an AccuriC6 Flow Cytometry system (BD Biosciences).

**3.1.3. G-banding karyotyping—**G-banding karyotyping was performed by WiCell Cytogenetics lab (Madison, WI) using twenty randomly selected metaphases.

**3.1.4. Short tandem repeat (STR) analysis—**STR analysis was performed by WiCell Cytogenetics lab using a Powerplex® 16 System (Promega) and genomic DNA extracted from the iPSCs with DNeasy Blood and Tissue Kit (Qiagen).

**3.1.5. Mycoplasma detection—**2 ml of medium from the iPSC culture was spun down at >20,000 g for 20 min to collect a small pellet of cells. After removing all medium, the pellet was lysed by 0.5x Phusion HF Buffer (NEB, #B0518S) with 8U/ml Proteinase K (NEB, #P8107S) at 55 °C for 1–3 h followed by heat-inactivation at 95 °C for 10 min. Quantitative PCR (qPCR) detection of mycoplasma was carried out using the primer pair GPO-1_MGSO with the SsoAdvanced™Universal SYBR Green Supermix (Bio-Rad Laboratories) for 40 cycles. The RFU values at the end of the PCR were used to compare samples with positive (a known contaminated sample) and negative (sterile water) controls to evaluate the presence of mycoplasma contamination. A pair of GAPDH primers (GAPDH-3) that amplify in human samples was used to ensure cell material was present.

**3.1.6. Testing for Sendai reprogramming vector clearance—**RNA from NHLBIi001-A and NHLBIi001-B iPSCs was extracted using 1 ml TRIzol Reagent Solution (15596026, Thermo Fisher) following the manufacturer's protocol, however we used 100 µl 1-bromo-3-cholopropane (BCP) in place of chloroform. RNA from another recently generated passage 2 iPSC line was used as a positive control for SeV detection. Following RNA extraction, 2 ug of RNA was reverse transcribed into cDNA with Maxima H Minus Reverse Transcriptase (Thermo Fisher) primed with PolyN(15-mer) (Eurofins) in accordance with the manufacturer's protocol. Leftover RNA template was removed by incubating the sample with 1 µl Ribonuclease H (Life Technologies) from *Escherichia coli* for 20 min at

37 °C. The Real-time PCR reaction was then prepared with the SsoAdvanced™Universal SYBR Green Supermix and run on a BIO-RAD CFX96 (Bio-Rad Laboratories) machine.

**3.1.7. Teratoma assay—**NHLBIi001-A and NHLBIi001-B iPSCs were removed from 6-well plates when ~90% confluent using the EDTA dissociation method. $1 \times 10^7$ cells per clone were resuspended in E8 medium and kept on ice. The suspension was mixed with a 50% volume of cold Matrigel (Corning, 354277) and 150 μl of the resulting mixture was injected subcutaneously into NSG mice (JAX No. 005557) at two sites. Tumors were visible after 6–8 weeks at which point they were removed and fixed in 10% Neutral Buffer Formalin. They were then embedded in paraffin and stained with hematoxylin and eosin.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgement

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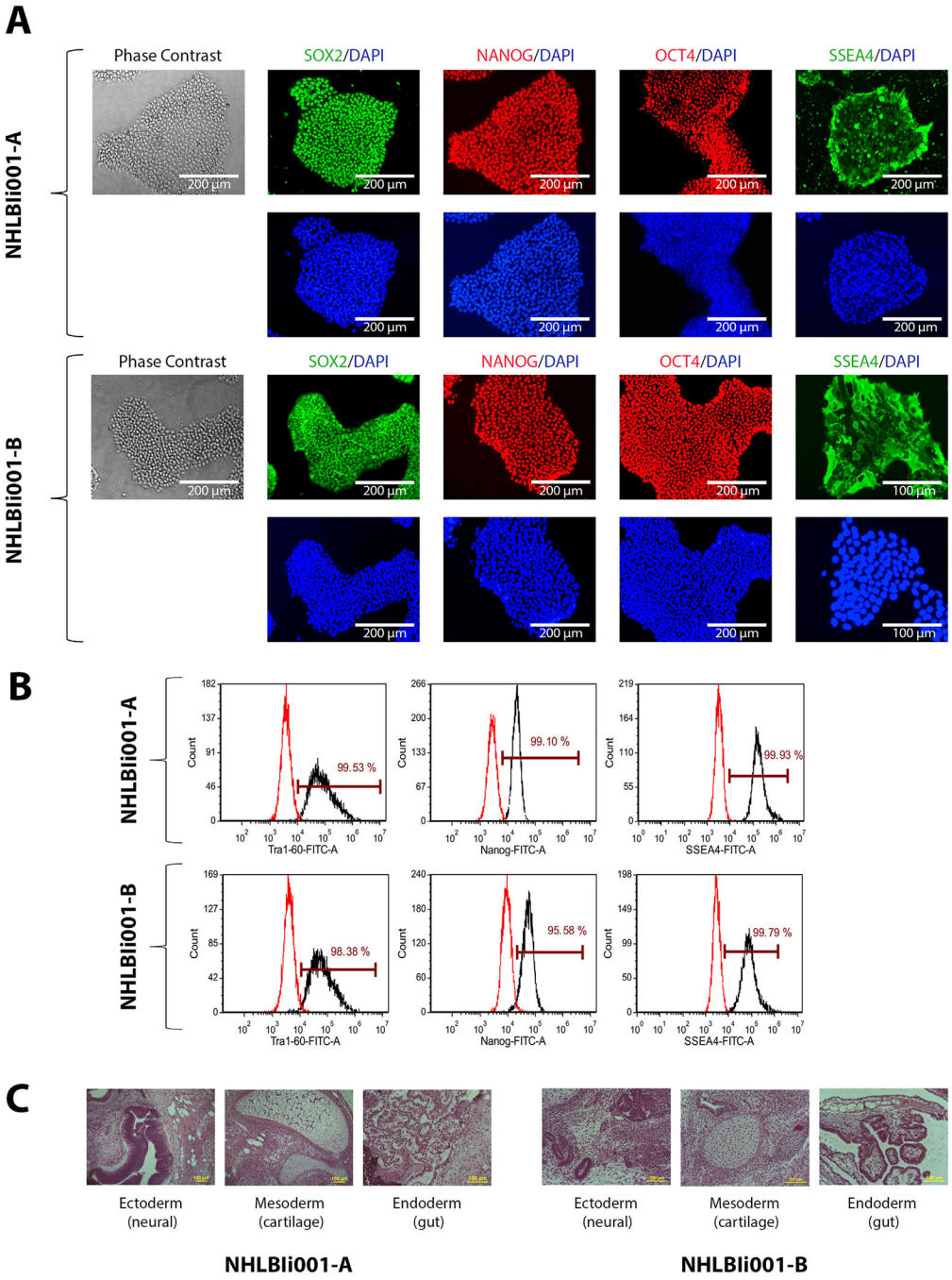


**Fig. 1.**

(A) Images of phase contrast microscopy and immunofluorescence staining of pluripotency markers of NHLBIi001 iPSCs. (B) Flow cytometry analysis of pluripotency markers of NHLBIi001 iPSCs. (C) Teratoma formation assay shows NHLBI001 iPSCs can generate three germ layers in vivo.

**Table 1**

Summary of lines.

| iPSC line names | Abbreviation in figures | Gender | Age | Ethnicity | Genotype of locus | Disease |
|---|---|---|---|---|---|---|
| NHLBIi001-A | NHLBIi001-A | Female | 51 | Caucasian | N/A | N/A |
| NHLBIi001-B | NHLBIi001-B | Female | 51 | Caucasian | N/A | N/A |

**Table 2**

Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography | Normal | Fig. 1A |
| Phenotype | Immunocytochemistry | SOX2, OCT4, NANOG, SSEA-4 | Fig. 1A |
| | Flow Cytometry | TRA–1–60; NANOG; SSEA-4 | Fig. 1B |
| Genotype | Karyotype (G-banding) and resolution | 46XX; Resolution 425–500 | Supplementary Fig. S1A |
| Identity | Microsatellite PCR (mPCR) OR STR analysis | Not performed | N/A |
| | | 15 loci plus amelogenin (Promega PowerPlex 16) tested, all matched | Submitted in archive with journal |
| Mutation analysis (IF APPLICABLE) | Sequencing | N/A | N/A |
| | Southern Blot OR WGS | N/A | N/A |
| Microbiology and virology | Mycoplasma | Mycoplasma testing by qPCR; Negative | Supplementary Fig. S1B |
| Differentiation potential | e.g. Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation | Teratoma formed with three germ layers: Ectoderm, Mesoderm, and Endoderm | Fig. 1C |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | N/A | N/A |
| Genotype additional info (OPTIONAL) | Blood group genotyping | N/A | N/A |
| | HLA tissue typing | N/A | N/A |

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**Table 3**

Reagents details.

| Antibodies used for immunocytochemistry/flow-cytometry | | | |
|---|---|---|---|
| | Antibody | Dilution | Company Cat # and RRID |
| Pluripotency markers | Mouse anti-SOX2 | 1:250 | BioLegend, Cat# 656102, RRID: AB_2562246 |
| Pluripotency markers | Rabbit anti-NANOG | 1:400 | Cell Signaling Technology, Cat# 4903, RRID: AB_10559205 |
| Pluripotency markers | Rabbit anti-OCT4 | 1:400 | Thermo Fisher, Cat# 701756, RRID: AB_2633031 |
| Pluripotency markers | Mouse anti-SSEA4 | 1:1000 | Cell Signaling Technology, Cat# 4755, RRID: AB_1264259 |
| Secondary antibodies | Donkey anti-Mouse IgG (Alexa Fluor 488) | 1:400 | Thermo Fischer, Cat# A21202, RRID: AB_141607 |
| Secondary antibodies | Donkey anti-Rabbit IgG (Alexa Fluor 594) | 1:400 | Thermo Fischer, Cat# A21207, RRID: AB_141637 |
| Flow cytometry antibodies | Anti-Tra-1–60-DyLight 488 | 1:50 | Thermo Fischer, Cat# MA1–023-D488X, RRID: AB_2536700 |
| Flow cytometry antibodies | Anti-Nanog-Alexa Fluor 488 | 1:50 | Millipore, Cat# FCABS352A4, RRID: AB_10807973 |
| Flow cytometry antibodies | Anti-SSEA-4-Alexa Fluor 488 | 1:50 | Thermo Fischer, Cat# 53–8843–41, RRID: AB_10597752 |
| Flow cytometry antibodies | Mouse-IgM-DyLight 488 | 1:50 | Thermo Fischer, Cat# MA1–194-D488, RRID: AB_2536969 |
| Flow Cytometry Antibodies | Rabbit IgG-Alexa Fluor 488 | 1:50 | Cell Signaling Technology, Cat# 4340S, RRID: AB_10694568 |
| Flow cytometry antibodies | Mouse IgG3-FITC | 1:50 | Thermo Fischer, Cat# 11–4742–42, RRID: AB_2043894 |

| Primers | | |
|---|---|---|
| | Target | Forward/Reverse primer (5′–3′) |
| SeV specific primers (qRT-PCR) | SeV/181 bp | 5′-GGATCACTAGGTGATATCGAGC<br>5′-ACCAGACAAGAGTTTAAGAGATATGTATC |
| SeV specific primers (qRT-PCR) | KOS/528 bp | 5′-ATGCACCGCTACGACGTGAGCGC/5′-ACCTTGACAATCCTGATGTGG |
| SeV specific primers (qRT-PCR) | Klf4/ 410 bp | 5′-TTCCTGCATGCCAGAGGAGCCC<br>5′-AATGTATCGAAGGTGCTCAA |

| | | |
|---|---|---|
| SeV specific primers (qRT-PCR) | C-Myc/523 bp | 5′-TAACTGACTAGCAGGCTTGTCG<br>5′-TCCACATACAGTCCTGGATGATGATG |
| House-keeping gene primers (qRT-PCR) | GAPDH/168 bp | 5′-AATGGGCAGCCGTTAGGAAA<br>5-GCGCCCAATACGACCAAATC |
| Mycoplasma detection primers (qPCR) | GPO-1_MGSO/724bp | 5′-ACGGCCCAGACTCCTACGGGAGGCAGCAGTA<br>5′-CCATGCACCATCTGTCACTCTGTTAACCTC |
| House-keeping gene primers (qPCR) | GAPDH-3/488 bp | 5′-GGGAGCCAAAAGGGTCATCA<br>5′-TGATGGCATGGACTGTGGTC |

RRID Requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

## Resource Table:

| | |
|---|---|
| Unique stem cell lines identifier | NHLBIi001-A |
| | NHLBIi001-B |
| Alternative names of stem cell lines | MS19-ES-D (NHLBIi001-A) |
| | MS19-ES-H (NHLBIi001-B) |
| Institution | National Heart, Lung, and Blood Institute (NHLBI) |
| Contact information of distributor | Dr. Jizhong Zou jizhong.zou@nih.gov |
| Type of cell lines | iPSC |
| Origin | Human |
| Cell Source | Blood |
| Clonality | Clonal |
| Method of reprogramming | Cytotune 2.0 Sendai viruses |
| Multiline rationale | Wild-type control iPSC lines from a healthy donor with normal cardiac function |
| Gene modification | No |
| Type of modification | N.A. |
| Associated disease | N.A. |
| 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 | September 2019 |
| Cell line repository/bank | N.A. |
| Ethical approval | The Cardiovascular Disease Discovery Protocol (#10-H-0126) has been approved by NHLBI Institutional Review Board (IRB) to collect blood samples for iPSC generation. |