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Lab Resource: Single Cell Line

Establishment of *STUB1*/CHIP mutant induced pluripotent stem cells (iPSCs) from a patient with Gordon Holmes syndrome/SCAR16

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ABSTRACT

STUB1/CHIP is a central component of cellular protein homeostasis and interacts with key proteins involved in the pathogenesis of many neurodegenerative diseases. Here, we reprogrammed human skin fibroblasts from a 12-year-old male patient with recessive spinocerebellar ataxia type 16 (OMIM #615768), carrying compound heterozygous mutations (c.355C>T, c.880A>T) in *STUB1*. Genomic integrity of the iPSC line HIHCNi001-A without transgene integration and genomic aberration but with maintained disease-relevant mutations was proven by SNP array analysis and Sanger sequencing while pluripotency was verified by the expression of important pluripotency markers and the capacity to differentiate into cells of all three germ layers.

Resource table.

Unique stem cell line identifier	HIHCNi001-A
Alternative name(s) of stem cell line	iPSC-STUB1
Institution	Hertie Institute for Clinical Brain Research and German Center for Neurodegenerative Diseases (DZNE), University of Tübingen, Germany
Contact information of distributor	Stefan Hauser, Stefan.hauser@dzne.de
Type of cell line	Induced pluripotent stem cell (iPSC)
Origin	Human
Additional origin info	12 years, male
Cell Source	Fibroblasts
Clonality	clonal
Method of reprogramming	Non-integrating episomal plasmids
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Spinocerebellar ataxia, autosomal recessive 16 (SCAR16), OMIM #615768
Gene/locus	*STUB1*, c.[355C>T]; c.[880A>T]
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	December 2016
Cell line repository/bank	N/A
Ethical approval	Institutional Review Board of the Medical Faculty, University of Tübingen Approval Number: 598/2011BO1

Resource utility

CHIP is a central component of cellular protein homeostasis and interacts with several key proteins associated with neurodegenerative diseases, with mutations in *STUB1* leading to SCAR16 (Hayer et al., 2017; Synofzik et al., 2014). iPSC-derived neurons will help to further decipher the exact role of this neurodegenerative key protein, including central steps in the pathogenesis of SCAR16.

Resource details

HIHCNi001-A was generated by reprogramming fibroblasts cultured from a skin biopsy of a 12 year old boy suffering from autosomal recessive spinocerebellar ataxia type 16 (SCAR16). SCAR16 patients develop a severe early-onset multi-systemic neurodegenerative disorder resulting in a broad phenotypic spectrum, including cerebellar ataxia, spasticity, epilepsy and hypogonadism. While it is of high interest to determine the pathophysiological role of CHIP, the protein encoded by *STUB1*, it is also a promising possible key player of neurodegeneration as it interacts with *inter alia* α-Synuclein, LRRK2, Huntingtin, Ataxin-3 and Tau. Our patient carried two heterozygous mutations c.355C >T and c.880A >T in the *STUB1* gene leading to a premature stop p.Arg119* and an amino acid exchange p.Ile294Phe on the protein level. Compound heterozygosity of the mutations was shown by segregation analysis (Hayer et al., 2017). Fibroblasts were reprogrammed by the delivery of episomal plasmids encoding human OCT4, SOX2, KLF4, L-MYC (OSKM) and LIN28 (Okita et al., 2011). iPSCs exhibited a morphology

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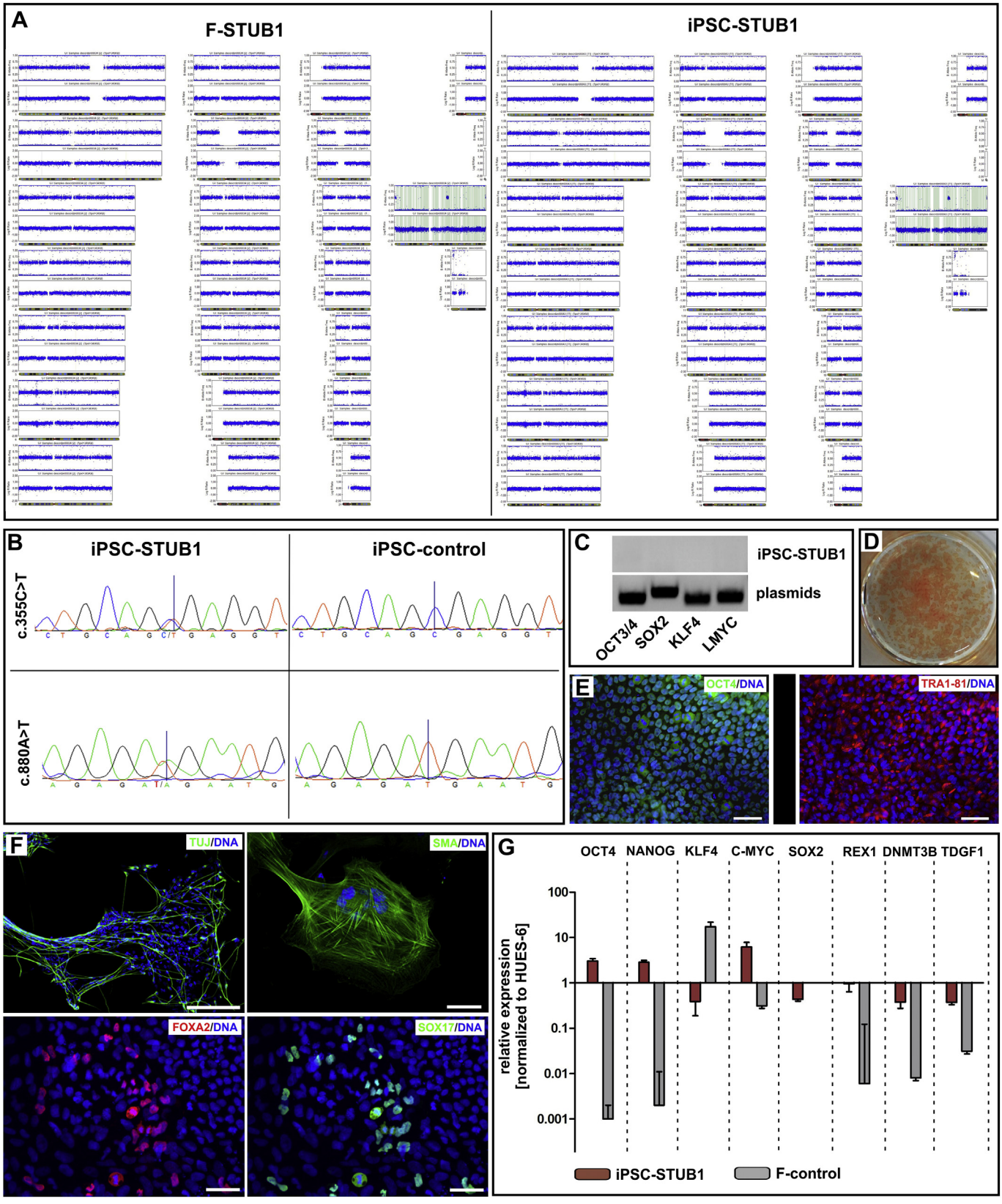

Fig. 1. Characterization and validation of HIHCNi001-A.

similar to those of human embryonic stem cells and were assessed after manual picking and expansion for several passages. Cells expressed pluripotency-associated surface markers such as alkaline phosphatase (Fig. 1D) and did not express exogenous reprogramming factors after passage 5 (Fig. 1C). Endogenous expression of pluripotency genes OCT4 and TRA1-81 on protein level was verified by immunocytochemistry (Fig. 1E). OCT4, NANOG, KLF4, c-MYC, SOX2, REX1, DNMT3B and TDGF1 were further verified on transcript level *via* qRT-PCR to have a

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Not shown, available with author
Phenotype	Qualitative analysis	Immunocytochemistry of pluripotency markers: OCT4, TRA1-81	Fig. 1 panel E
	Quantitative analysis	qRT-PCR for OCT4, NANOG, KLF4, c-MYC, SOX2, REX1, DNMT3B and TDGF1	Fig. 1 panel G
Genotype/identity	Whole genome SNP genotyping with Infinium OmniExpressExome-8 BeadChip (Illumina) Spacing (kb): Mean: 3,03, Median: 1,36	No larger chromosomal aberrations or copy number variations; Genotype 46, XY	Fig. 1 panel A
Mutation analysis	Sequencing Southern Blot OR WGS	Compound heterozygous, c.355C>T and c.880A>T N/A	Fig. 1 panel B
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR, negative	Supplementary file 1
Differentiation potential	Embryoid body formation	Smooth muscle actin (SMA), β-tubulin (TUJ) and FOXA2, SOX17	Fig. 1 panel F
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

similar expression compared to the human embryonic stem cell line HUES 6 and a discriminative expression pattern compared to fibroblasts (Fig. 1G). Pluripotency was further demonstrated by embryoid-body-based differentiation to endodermal, mesodermal and ectodermal cell lineage (Fig. 1F). Genomic integrity was confirmed by SNP genotyping (Fig. 1A) and Sanger sequencing of the mutation site (Fig. 1B) of both original fibroblasts and generated iPSCs. The cell line was confirmed to be mycoplasma-free (Suppl. Fig. 1).

Materials and methods

Culturing and reprogramming fibroblasts

Human dermal fibroblasts were cultured in fibroblast culture medium [DMEM high glucose (Life Technologies) + 10% FBS (Life Technologies)] for 10 days at 37 °C, 5% CO_2. Reprogramming was achieved by nucleofection with the episomal plasmids pCXLE-hUL, pCXLE-hSK and pCXLE-hOCT4 as described by Okita et al. Briefly, 10^5 cells were nucleofected with 1 μg of each plasmid. After electroporation, fibroblasts were cultivated in fibroblast medium before adding FGF2 (2 ng/ml (Peprotech)) on day 2. The following day, medium was changed to Essential 8 (E8) medium with 100 μM NaB (Sigma-Aldrich). After 3–4 weeks with medium change every other day, colonies were picked and expanded on matrigel-coated plates in E8 medium. After ≥5 passages, iPSCs were genomically and functionally analysed, passaged or frozen in E8 medium with 40% KO-SR (Life Technologies), 10% DMSO (Sigma-Aldrich) and 1 μM Y-27632 (Abcam Biochemicals) (Table 1).

Genomic integrity analysis

To verify genomic integrity, DNA of iPSCs and fibroblasts was isolated with DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. Whole-genome SNP genotyping was conducted

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Goat anti-OCT4	1:100	Santa Cruz, AB_653551
	Mouse anti-TRA1-81	1:500	Millipore, AB_177638
In vitro differentiation	Mouse anti-SMA	1:100	Dako, AB_2223500
	goat anti-SOX17	1:250	R&D Systems, AB_355060
	rabbit anti-FOX-A2	1:300	Millipore, AB_390153
	mouse anti-TUJ	1:1000	Sigma Aldrich, AB_477590
Secondary antibodies	Alexa Fluor 488 Donkey anti-Goat IgG	1:300	Life Technologies
	Alexa Fluor 488 Goat anti-Rabbit IgG	1:300	Life Technologies
	Alexa Fluor 488 Goat anti-Mouse IgG	1:300	Life Technologies
	Alexa Fluor 568 Goat anti-Mouse IgG	1:300	Life Technologies
	Alexa Fluor 568 Goat anti-Rabbit igG	1:300	Life Technologies

Primers			
	Target	Forward primer	Reverse primer (5′–3′)
Episomal plasmids	KLF4	CCACCTCGCCTTACACATGAAG	TAGCGTAAAAGGAGCAACATAG
	L-MYC	GGCTGAGAAGAGGATGGCTAC T	TTTGTTTGACAGGAGCGACAA
	OCT3/4	CATTCAAACTGAGGTAAGGG	TAGCGTAAAAGGAGCAACATAG
	SOX2	TTCACATGTCCCAGCACTACCAG	TTTGTTTGACAGGAGCGACAAT
Pluripotency markers (qPCR)	c-MYC	ATTCTCTGCTCTCCTCGACG	CTGTGAGGAGGTTTGCTGTG
	DNMT3B	ACGACACAGAGGACACACAT	AAGCCCTTGATCTTTCCCCA
	KLF4	CCATCTTTCTCCACGTTCGC	CGTTGAACTCCTCGGTCTCT
	NANOG	CAAAGGCAAACAACCCACTT	TGCGTCACACCATTGCTATT
	OCT4	GGAAGGTATTCAGCCAAACG	CTCCAGGTTGCCTCTCACTC
	SOX2	TGATGGAGACGGAGCTGAAG	GCTTGCTGATCTCCGAGTTG
	TDGF1	GGTCTGTGCCCCATGACA	AGTTCTGGAGTCCTGGAAGC
Housekeeping gene (qPCR)	GAPDH	AGGTCGGAGTCAACGGATTT	ATCTCGCTCCTGGAAGATGG
Targeted sequencing	Mutation_1	GCTACCTGAAGATGCAGCAG	TGAGCCTGGAGAGGTAAGAG
	Mutation_2	GTGCAGTGCCCCTTTTCAG	GTCCAACAGCAGAACTTGGG

using Infinium OmniExpressExome-8-BeadChip (Illumina) and GenomeStudio V2.0.3 (Illumina) for evaluation. Copy number analysis was performed using CNVPartition plugin (Illumina). Early mosaicism states were evaluated by manual review on B allele frequency plots on chromosomal level. DNA was also sequenced for both mutations in *STUB1* using mutation-specific primers (Table 2) according to standard procedures, using 3130xl Genetic Analyzer (Applied Biosystems) and Staden 2.0.0b10 (Staden Sourceforge) for visualisation. To verify non-integration of plasmids, RT-PCR was performed with plasmid-specific primers (Table 2).

Pluripotency assessment

iPSCs were fixed with 4% paraformaldehyde (PFA) and either assessed for alkaline phosphatase expression or permeabilized with 0.1% Triton X-100, blocked with 5% FBS and stained overnight at 4 °C with primary antibodies for immunocytochemical analysis (Table 2). Samples were visualized after staining with Alexa Fluor 488- or 568-conjugated secondary antibodies (Table 2) for 1 h at room temperature. Nuclei were counterstained with Hoechst 33,342 (1:10.000, Invitrogen). Samples were embedded in ProLong Gold Antifade Reagent (Life Technologies) and observed with AxioImager Z1 (Zeiss). On transcript level, qRT-PCR with primers specific for pluripotency genes (Table 2) was performed: RNA was extracted with High Pure RNA Isolation Kit (Roche) according to manufacturer's instructions and reverse-transcribed to cDNA, using Transcriptor High Fidelity cDNA Synthesis Kit (Roche). qRT-PCR was performed as triplicates with LightCycler 480 SYBR Green I Master (Roche). Normalization of C_T values for GAPDH and the reference hESC line HUES6 was achieved by using the $2^{-\Delta\Delta Ct}$ method. Pluripotency was further confirmed by embryoid-body based differentiation by cultivating iPSCs in EB medium (80% DMEM/F12 (Life Technologies), 20% KO-SR, 1× NEAA (Sigma-Aldrich), 1× Penicillin-Streptomycin (Merck Millipore), 2 mM L-Glutamine (Gibco), 0.1 mM β-Mercaptoethanol (Merck)) on AggreWell 800 Plates (StemCell Technologies) with medium change on day 2. Embroid bodies were collected on day 4 and plated onto 0.1% gelatine- or matrigel-coated plates, for *endo*- and mesodermal or ectodermal differentiation, respectively. Cells were cultivated for 2–3 weeks with medium change every other day and immunocytochemically stained for SMA, TUJ, FOXA2 and SOX17 (Table 2).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.04.001.

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