

## SUPPLEMENTARY MATERIALS

to the article by Mhd A. Arssan, A.I. Shevchenko, S.M. Zakian, I.S. Zakharova  
 “Naïve human iPSCs obtained by culturing the ICGi022-A cell line with primed pluripotency  
 in HENSM medium efficiently differentiate into endothelial derivatives”

### Supplementary Material 1. Antibodies used in the study

Markers	Antibody	Dilution	Manufacturer, catalogue number	RRID
Common markers of pluripotency	Mouse IgM anti-TRA-1-60	1:200	Abcam, Cat # ab16288	RRID: AB_778563
	Mouse IgG2b anti-OCT3/4	1:100	Santa Cruz Cat # sc-5279	RRID: AB_628051
	Rabbit IgG anti-NANOG	1:200	ReproCELL Cat # RCAB003P	RRID: AB_2714012
Markers of naïve pluripotency	Mouse IgM anti-CD75	1:40	eBioscience Cat # 50-0759-42	RRID: AB_2574175
	Rabbit IgG anti-KLF17	1:300	Sigma-Aldrich Cat # HPA024629	RRID: AB_1668927
	Rabbit IgG anti-TEF3	1:600	Sigma-Aldrich Cat # HPA023881	RRID: AB_1857931
Markers of differentiated derivatives of three germ layers	Mouse IgG2a anti-TUBB3	1:1,000	BioLegend Cat # 801201	RRID: AB_2313773
	Mouse IgG1 anti-human CD90	1:100	eBioscience Cat # 14-0909-82	RRID: AB_763535
	Mouse IgG2a anti-AFP	1:200	Sigma-Aldrich Cat # A8452	RRID: AB_258392
Trophoblast early placentation markers	Mouse IgG2a anti-TROP2 (TACSTD2)	1:100	R&D Cat # MAB650	RRID: AB_2205665
	Mouse IgG1 anti-CD249 (ENPEP)	1:100	BD Biosciences Cat # 564532	RRID: AB_2738837
Mature endothelial cells marker and FACS isotype control	Mouse IgG1 anti-human CD31	1:100	CellMarque Cat # 131M-96	RRID: AB_1516761
	CD31	1:20	eBioscience	RRID:
	Mouse IgG1 CD31-APC	5 µl /test	Cat # 17-0319-42	AB_10852842
	IgG1(kappa) Isotype control	1:20 5 µl /test	Invitrogen Cat # 17-4714-42	N/A
Secondary antibodies	Goat anti-Mouse IgM Heavy Chain Cross- Adsorbed Secondary Antibody, Alexa Fluor 568	1:400	Thermo Fisher Scientific Cat # A21043	RRID: AB_2535712
	Goat anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:400	Thermo Fisher Scientific Cat # A21134	RRID: AB_2535773
	Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:400	Thermo Fisher Scientific Cat # A21144	RRID: AB_2535780
	Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:400	Thermo Fisher Scientific Cat # A21124	RRID: AB_2535766
	Goat anti-Rabbit IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:400	Thermo Fisher Scientific Cat # A11008	RRID: AB_143165
	Goat anti-Rabbit IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:400	Thermo Fisher Scientific Cat # A11011	RRID: AB_143157

### Supplementary Material 2. Oligonucleotides used in the study

Type of test	Locus	Size, bp	Forward/reverse primer (5'–3')
Detection of episomal vectors	<i>EBNA-1</i>	196	TCCCGCAGATCTTCTGCTCCTGTTCCACCG / CTCAAAGGATCCGGGGTGATAACCATGGACGA
Mycoplasma detection	The ribosomal 16S RNA gene	280	GGGAGCAAACAGGATTAGATACCCT / TGCACCATCTGTCCTGTGTTAACCTC
Reference gene for quantitative RT-PCR	<i>TFRC</i>	80	GTCGCTGGTCAGTTCGTGATT / AGCAGTTGGCTGTTGTACCTCTC
	<i>B2M</i>	167	CACCCCCACTGAAAAAGATG / ATATTA AAAAGCAAGCAAGCAGAA
	<i>ACTB</i>	308	AGGCACCAGGGCGTGAT / GATAGCACAGCCTGGATAGCA
Target naïve related genes for quantitative RT-PCR	<i>NANOG</i>	116	TTTGTGGGCCTGAAGAAACT / AGGGCTGCTCTGAATAAGCAG
	<i>TFCP2L1</i>	129	TTTGTGGGACCTGCGAAG / TGCTTAAACGTGCAATCTGGA
	<i>KLF17</i>	200	AACATTGTTGGGCCCGACT / CGGGCTGCTCTGGTAGAAAT
	<i>TFE3</i>	135	AGCCATCACTGTCAGCAACT / TCTCTTCTTCTGCCGTTCT
	<i>DAZL</i>	118	ATGTTGTACCTCCGGCTTATTCA / CCATTTCCAGAGGGTGGAGTA
	<i>LEUTX</i>	105	TGGCTTCAAAGCTACAACCTTGAT / ATGGCCGTGCTGCATTT
	<i>LTR7Y</i>	190	GCCATTTTATAGGATTTGGGAAG / TAACTGATGACATTCCACCATTG

### Supplementary Material 3. Results of short tandem repeat analysis at 26 genomic loci

Locus	K7.4LF (N)	K7.4LF
<i>Amelogenin</i>	X,X	X,X
<i>D8S1179</i>	10,13	10,13
<i>D21S11</i>	30,32.2	30,32.2
<i>D7S820</i>	11,13	11,13
<i>CSF1PO</i>	10,12	10,12
<i>D3S1358</i>	14,17	14,17
<i>TH01</i>	9,9	9,9
<i>D13S317</i>	9,10	9,10
<i>D16S539</i>	12,13	12,13
<i>D2S1338</i>	23,25	23,25
<i>D5S818</i>	12,12	12,12
<i>FGA</i>	20.2,22	20.2,22
<i>D19S433</i>	13.2,15.2	13.2,15.2
<i>vWA</i>	15,16	15,16
<i>TPOX</i>	10,11	10,11
<i>D18S51</i>	17,17	17,17

**Supplementary Material 4. Cell line naïve ICGi022-A-1 passport**  
(preliminary, the line has not been registered because karyotype instability)

Unique stem cell line identifier	naïve ICGi022-A-1
Alternative name for the cell line	naïve K7.4LF (N)
Institution	Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
Contact information for inquiring about the cell line	Irina Zakharova, zakharova@bionet.nsc.ru
Cell type	Naïve iPSC
Species of organism	<i>Homo sapiens</i>
Additional information on the origin of the cell line	Age: 32 Gender: Female Ethnicity: unknown
Source of the original cells	Primed ICGi022-A-1 (primed K7.4LF)
Reprogramming method	Chemical resetting from primed to naïve pluripotency in HENSM medium
Genetic modification type	None
Disease	None
The gene/locus in which the genetic modification has occurred	N/A
Modification method/site-specific nuclease used	N/A
Delivery method of programmable nucleases	N/A
Genetic material introduced into cells	N/A
Method for analysing a modification made	Naïve status of iPSCs was confirmed by real time PCR, immunofluorescent staining and differentiation studies
Method for assessing off-target activity	N/A
Morphology	Compacted dome-shaped morphology, corresponding to human naïve pluripotent stem cells
Naïve pluripotency	Validated in the tests for naïve-specific gene expression, trophoblast cells and blastoids production, and the generation of derivatives of three germ layers resulting from spontaneous differentiation
Karyotype	46, XX at passages 12 and 15 46, XX, der(15) at passage 18
Contamination detection	No bacteria, fungi, mycoplasma detected
Application field	The cell line may potentially be used to study early human development, gastrulation, trophoblast development and diseases, drug screening and diseases modeling
Cultivation system	Based on mitotically inactivated mouse embryonic fibroblasts, and matrigel culture
Cultivation medium	HENSM medium
Temperature, °C	37
CO <sub>2</sub> concentration, %	5
O <sub>2</sub> concentration, %	Atmospheric (about 21 %)
Seeding method	Recombinant enzyme TrypLE™ Express (ThermoFisher Scientific)
Seeding rate	1:3
Cryopreservation	90 % FBS, 10 % DMSO
Storage conditions	Liquid nitrogen
Registry entry	
Date of passporting/depositing	