Cell Stem Cell, *4*

Supplemental Data

Optimal Timing of Inner Cell Mass Isolation Increases the Efficiency of Human Embryonic Stem Cell Derivation and Allows Generation of Sibling Cell Lines

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Embryo Culture and ICM Isolation

Human zygotes and cleavage stage embryos were thawed using Quinn's Advantage Thaw Kit (Sage) and cultured in Global medium (LifeGlobal) supplemented with 15% Plasmanate (Talecris) until they reached the blastocyst stage. Embryos were graded as described (Gardner et al., 2000). No blastocoel (grade 2), blastocyst (grade 3), expanded blastoycst (grade 4), hatching blastocysts (grade 5), hatched blastoycst (grade 6). ICM of grade A: tightly packed, many cells; ICM of grade B: loosely grouped, several cells; ICM of grade C: no ICM visible. TE was graded A: many cells forming a cohesive epithelium, or B: few cells forming a loose epithelium, or C: very few large cells. If cultured beyond day6, embryos were switched to derivation medium. For poor quality embryos, we switched to conditioned ES cell media as early as day 5.

ICM isolation from these embryos was carried out by exposing the trophectoderm cells to 20-30 cell-lethal laser pulses from a Xyclone laser (Hamilton Thorne Biosciences) at an intensity of 500-700μs and subsequent removal of dead trophectoderm cells by using either piezo drill-assisted micromanipulation (Primetech), or by aspiration into a 50-75μm glass capillary pipette (**Figs. S1B**). A similar technique using the laser to isolate the ICM was recently reported by others (Turetsky et al., 2008), and isolation of the ICM by mechanical instead of enzymatic methods has also been reported elsewhere (Wang et al., 2005). A small number of lysed or living trophectoderm cells can be left attached to the ICM, as it does not interfere with ICM attachment to the fibroblast feeder layer, or with ES cell outgrowth. The isolated ICM (**Fig. S1C**) was then plated on a 95% confluent feeder layer of freshly plated gamma-irradiatiated mouse embryonic fibroblasts (MEFs), isolated from E12.5 embryos and passaged 3 times before irradiation, in hESC derivation media, a media conditioned for 24h by HUES9 cells grown on MEFs. This hESC derivation media consisted of 75% KO-DMEM (Invitrogen), 10% KO-SR replacement (Invitrogen), 10% Plasmanate (Talecris), 2.5% ES cell-tested Fetal Bovine Serum (FBS) (Hyclone), 2mM Glutamax-I, 1% non-essential amino acids, 50units/ml penicillin and 50μg/ml streptomycin (Invitrogen), 0.055mM β-mercaptoethanol (Invitrogen), 5ng/ml bFGF (Invitrogen), as described in (Akutsu et al., 2006; Sullivan et al., 2007) and was generated by exposure to a

growing culture of hESCs for 1 day, sterile filtering, and then a 1:1 dilution with non-conditioned medium (see also (Zhang et al., 2006)). When the hESC outgrowth reached a size of 0.5mm or more, usually 7 to 16 days after ICM plating, it was mechanically dispersed (Chen and Melton, 2007) and plated onto a new MEF feeder cell layer with conditioned derivation media (passage 1). The secondary colonies (**Fig. S1I**) that resulted were similarly dispersed until passage 2-5. The FBS content of the culture media was also eliminated during these initial passages to minimize ES cell differentiation. Cells were then further expanded using STEMPRO EZPassage (Invitrogen), or enzymatic passaging (**Fig.S1J**). Upon confirmation of embryonic stem cell identity by pluripotency and differentiation analyses (See Fig S2), all Harvard University lines are named according to their order of derivation as "HUES#".

To request stem cell lines, consult Table S2 for availability for distribution. More information can be found at (http://www.mcb.harvard.edu/melton/hues).

Immunohistochemistry of Human Preimplantation Stage Embryos

Human embryos were fixed in 4% paraformaldehyde at 4°C overnight. Washes were performed in PBS/0.1% Tween-20 (PBS/T). Permeabilization was performed in 0.5% PBS/T. Primary antibody incubations were diluted in 10% fetal bovine serum in 0.1% PBS/T overnight at 4°C. The following primary antibodies were used at the following dilutions: 1:500 of 0.6 mg immunoglobulin/ml monoclonal mouse anti-Cdx2 (CDX2-88, BioGenex, CA, USA); 1:500 of polyclonal rabbit anti-Oct4 (H134; Santa Cruz Biotechnology). Secondary antibodies (Cy3 or FITC donkey anti-rabbit or -mouse) were used at 1:300 (Molecular Probes, Invitrogen). Embryos were placed in 1:3 dilutions of Vectashield containing DAPI (Vector Labs): 0.1% PBS/T and placed onto coverslip dishes (MatTek) for imaging. (Alternatively, embryos were also permeabilized in 1% PBS/T). Imaging was performed on a Leica confocal microscope (LSM 510) using Meta software analysis. Cell numbers were counted using the IMARIS cell imaging software.

Microarray Analysis

Total RNA was isolated using Trizol (Invitrogen). RNA labeling and hybridization was done with the Illumina HumanRef-8 v3 beadchip and analyzed using BeadStudio and Bioconductor.

Genetic Analysis and Karyotyping

Genomic and mitochondrial DNA were isolated with the Qiagen DNAeasy tissue kit and the hypervariable region I (HVI) of the mitochondrial DNA (position 15983-16385) was amplified and sequenced using primers Mth1 5'-caccattagcacccaaagct-3' and Mth2 5'-tgatttcacggaggatggtg-3'. DNA alignment was done using Clustalw (http://www.ebi.ac.uk/), and Boxshade (http://www.ch.embnet.org/) using default parameters.

Karyotypes were done by the UMass Memorial Center, Cytogenetics Laboratory, Worcester, MA, USA. STR analysis was done by Global Stem Inc., Rockville MD, USA.

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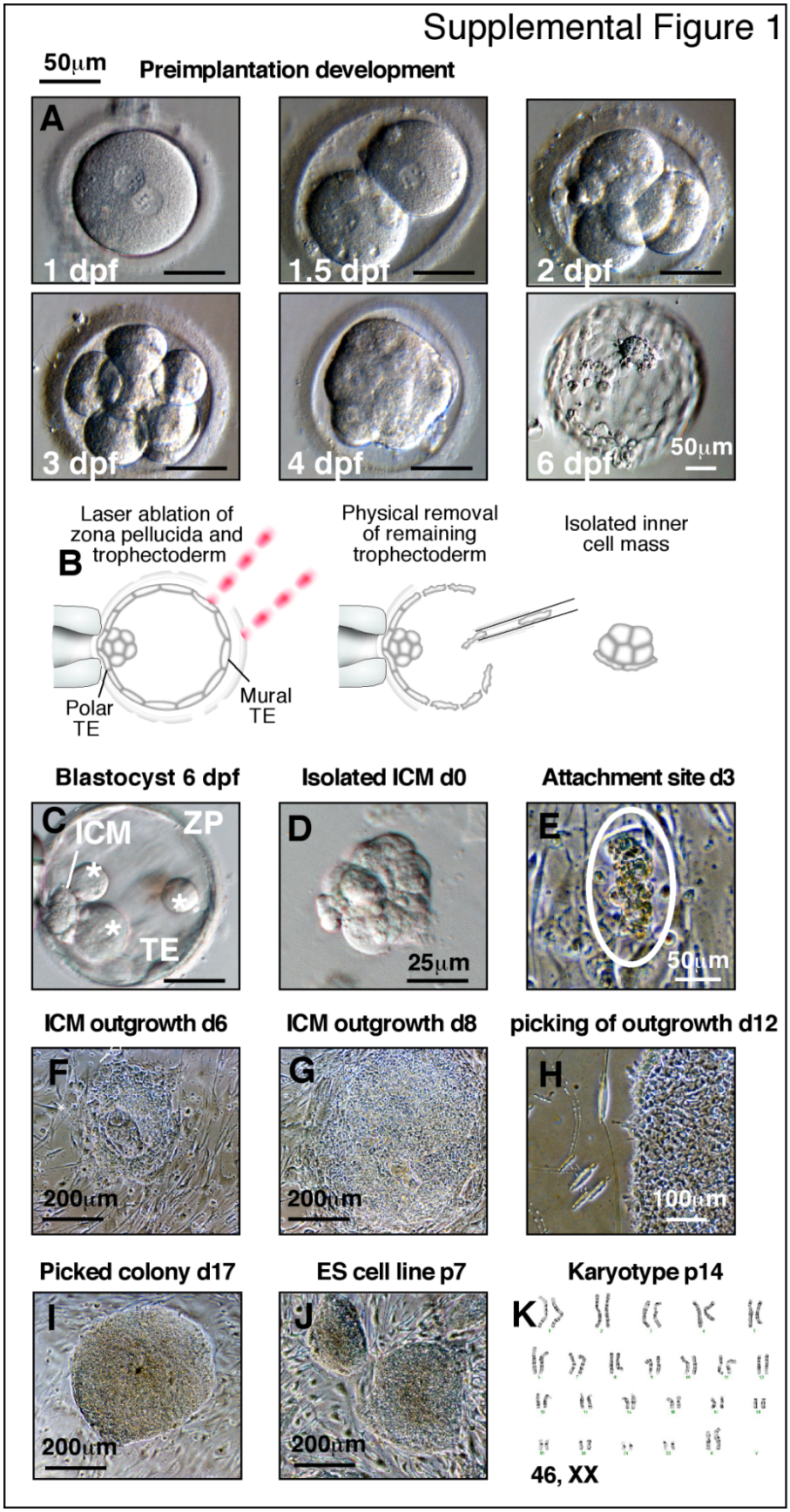
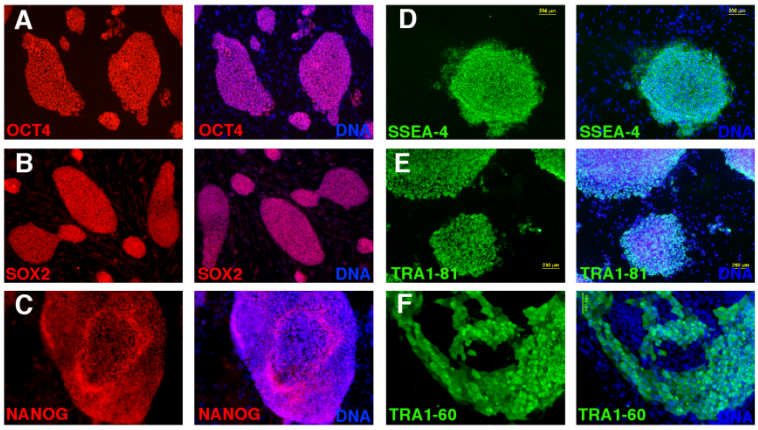

Supplemental Figure 1
50µm
Preimplantation development
A
1 dpf
1.5 dpf
2 dpf
3 dpf
4 dpf
6 dpf
50µm
B
Laser ablation of zona pellucida and trophectoderm
Physical removal of remaining trophectoderm
Isolated inner cell mass
Polar TE
Mural TE
Blastocyst 6 dpf
Isolated ICM d0
Attachment site d3
C
ICM
ZP
TE
D
25µm
E
50µm
ICM outgrowth d6
ICM outgrowth d8
picking of outgrowth d12
F
200µm
G
200µm
H
100µm
Picked colony d17
ES cell line p7
Karyotype p14
I
200µm
J
200µm
K
46, XX

Figure S1. Derivation of Pluripotent ES Cells by Laser Surgery of Human Embryos

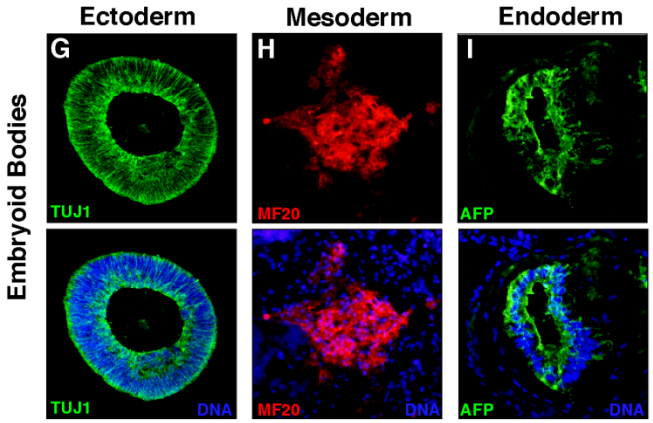
(A) Development of preimplantation embryos from the zygote stage to the expanded blastocysts stage. Dpf, days post-fertilization. (B) Schematic of inner cell mass (ICM) isolation. In multiple pulses, the zona pellucida (ZP) and most of the trophectoderm (TE) are targeted. Ablation of polar TE is avoided. Dead cells and remaining living TE cells are removed by a blunt glass pipette coupled to a piezo microaccentuator. (C) Human blastocyst at day 6 dpf before ICM isolation. Asterisk, blastomeres that were excluded from the developing blastocyst. (D) Isolated ICM on day 0 (d0), consisting of approximately 10 cells. (E-G) Attachment and outgrowth of the ICM into an embryonic stem cell colony (circled). Days of culture following isolation of the ICM are indicated. (H) Picking of a hESC outgrowth. Typical ES cell morphology is readily visible at high magnification. (I, J) Primary outgrowth is broken up into several colonies and passaged to give rise to a hESC line. The scale bar of 50mm is valid for panels A-J, except where otherwise indicated. (K) Chromosome analysis of cell line above shows a normal 46, XX female karyotype.

Supplemental Figure 2

Undifferentiated hESC culture



Differentiation into cell types of three germ layers *in vitro*



Differentiation into cell types of three germ layers *in vivo*

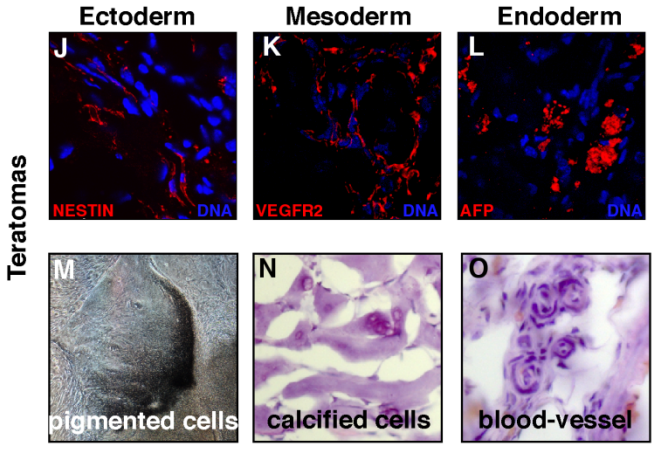


Figure S2. HESCs Isolated by Laser Surgery are Pluripotent

(A-F) In the undifferentiated state, cell lines derived by laser surgery express markers characteristic of pluripotent hESCs including OCT4, SOX2, NANOG, SSEA-4, TRA1-81, and TRA1-60. (G-I) hESCs differentiated *in vitro* via embryoid bodies express markers of the primary germ layers. Shown are immunohistochemical analyses using markers of the ectoderm (TUJ1), mesoderm (MF20), endoderm (AFP). HESCs differentiated *in vivo* via teratoma formation. (J-L) Immunohistochemical analyses using markers of the ectoderm (NESTIN), mesoderm (VEGFR2), and endoderm (AFP). (M) Pigmented cells formed in adherent culture (ectoderm). (N, O) Hematoxylin and Eosin staining reveal germ layer derivatives such as calcified cells (mesoderm) and blood vessels (mesoderm).

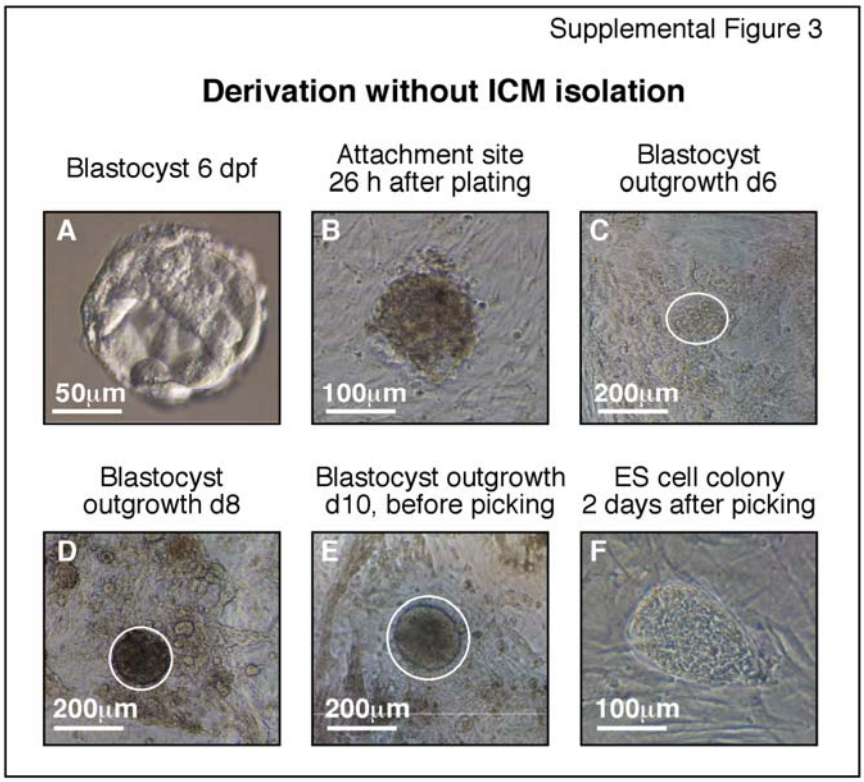


Figure S3. Isolation of Embryonic Stem cells after Plating of Whole Blastocysts

(**A**) Human blastocyst at day 6 post-fertilization (dpf), before plating. (B-D) whole blastocysts efficiently attach to the mouse embryonic fibroblast feeder layer (B) and give rise to a variety of differentiated cell types, surrounding the putative ICM cells (circled) (C, D). (E) Differentiated cells force the ICM to adopt an embryonic body like structure (circled) rather than a flat epithelial colony as observed after isolation of the ICM. (F) After picking of this structure, colonies with ES cell-like morphology form.

Supplemental Figure 4

Maturation of blastocysts after prolonged *in vitro* culture

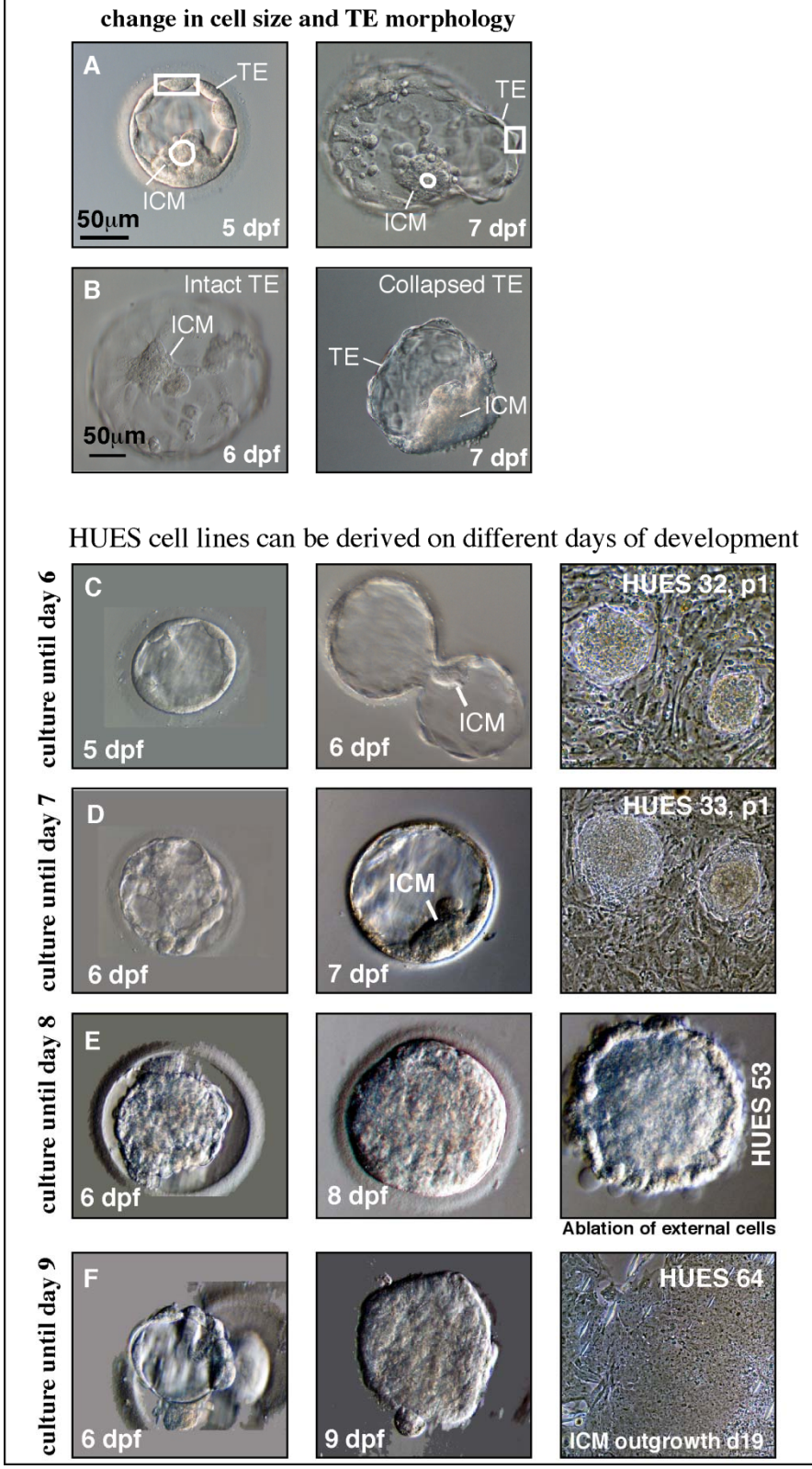


Figure S4. Blastocysts Yield Stem Cells on Different Days of Development

Extended *in vitro* culture allows for maturation of healthy blastocysts and improved development of poor quality blastocysts. (A) A healthy blastocyst on day 5 post-fertilization (dpf) (3AA) and on day 7 (5AA). Note that individual cells of the inner cell mass (ICM; circled), and the trophectoderm (TE; boxed), decrease in size while their number increases after prolonged culture. (B) A healthy blastocyst on day 6 (5AA) and day 7. Note the expanded TE on day 6 and subsequent collapse and deterioration of TE on day 7. Despite deterioration of TE morphology, the ICM expands. (C) A blastocyst on day 5 post-fertilization without a discernible ICM (3CA) and on day 6 with a distinct ICM (5AA). (D) A poor quality blastocyst on day 6 (3CC) without an apparent ICM and on day 7 with a distinct ICM (3BB). (E) A poor quality blastocyst on day 6 (3CC) and after 2 days of culture on day 8. Following ablation of external cells with the laser, a hESC line was derived. (F) Poor quality blastocyst on day 6 (3CC) and on day 9. Embryos were cultured in hESC-conditioned derivation medium from days 5 or 6 post-fertilization.

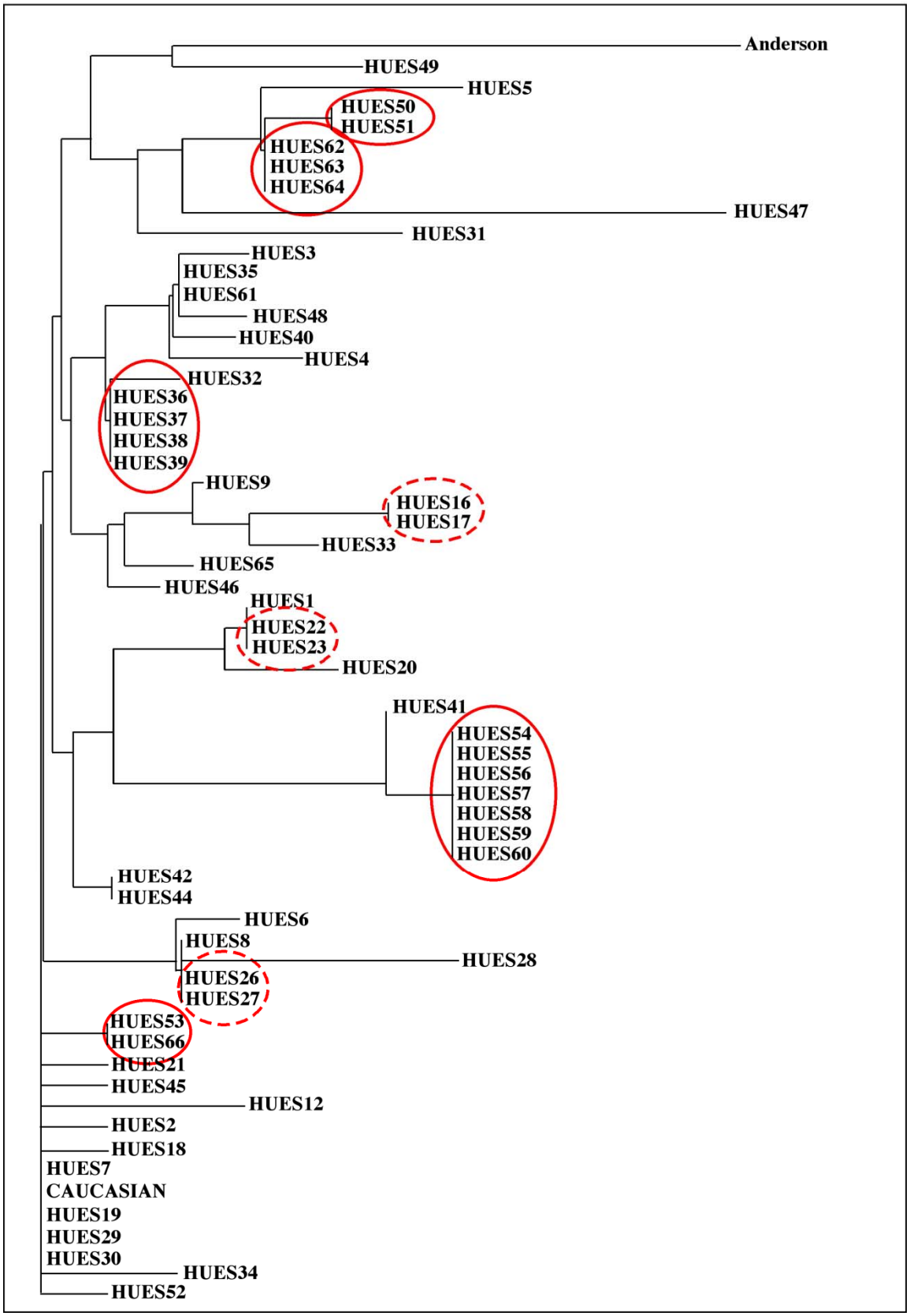

Anderson
HUES49
HUES5
HUES50
HUES51
HUES62
HUES63
HUES64
HUES47
HUES31
HUES3
HUES35
HUES61
HUES48
HUES40
HUES4
HUES32
HUES36
HUES37
HUES38
HUES39
HUES9
HUES16
HUES17
HUES33
HUES65
HUES46
HUES1
HUES22
HUES23
HUES20
HUES41
HUES54
HUES55
HUES56
HUES57
HUES58
HUES59
HUES60
HUES42
HUES44
HUES6
HUES8
HUES28
HUES26
HUES27
HUES53
HUES66
HUES21
HUES45
HUES12
HUES2
HUES18
HUES7
CAUCASIAN
HUES19
HUES29
HUES30
HUES34
HUES52

Figure S5. Dendrogram of Mitochondrial DNA Sequence of the Hypervariable Region I

Note the identical sequence of sibling cell lines HUES 54-60, HUES 66 and 53, HUES 36-39, HUES 62-64, and HUES 50-51, circled, and HUES 16 and 17, HUES 22 and 23, and HUES 26 and 27. Most other cell lines are different, demonstrating independent origin. The reference sequences are a Caucasian (helvetic) sequence, and the published Anderson sequence (Anderson et al., 1981). For cell lines with dotted circles, documentation of IVF history was not available but mitochondrial sequencing suggests sibling relations (see also **Figure S6 and Table S4**). The IVF history of fully circled lines is known and agrees with the genetic data of identical parenthood.

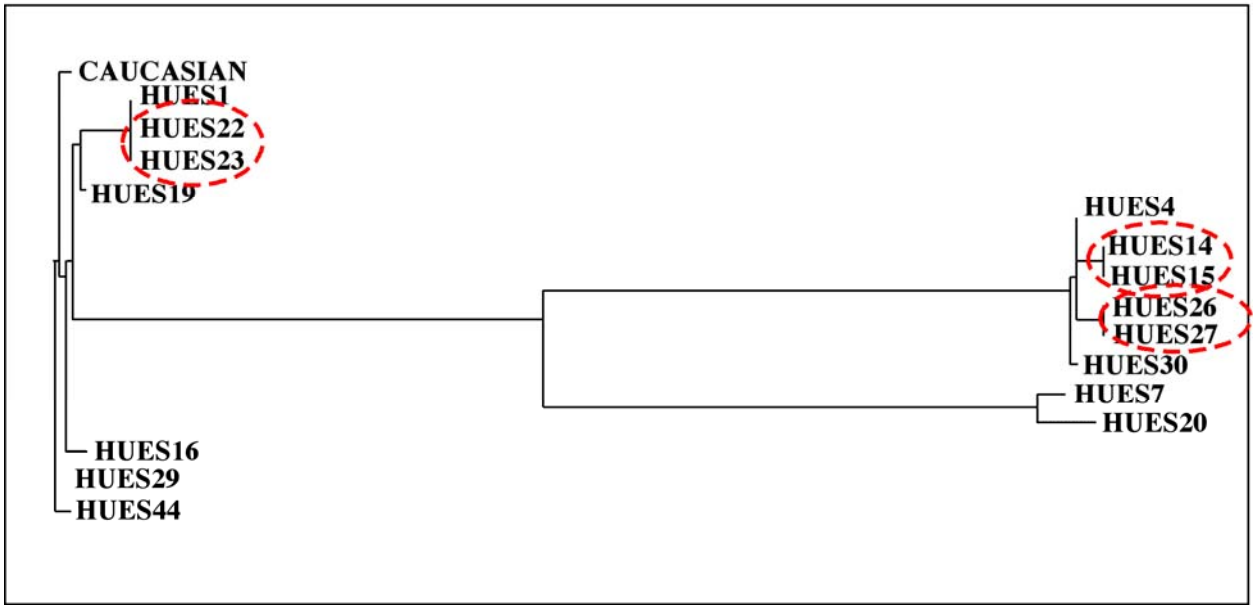


Figure S6. Dendrogram of Mitochondrial DNA Sequence of the Hypervariable Region II

Select hESC lines were analyzed at hypervariable region II. Most of them differed in sequence, sibling stem cell lines are circled. HUES 14 and 15 (Cowan et al. 2004), HUES 22 and 23, and HUES 26 and 27 are sibling stem cell lines, but their IVF history was not available (see also **Table S4**).

Table S1. Variations in Derivation Efficiency Using the Published Protocol (Cowan et al., 2004).

Authors	Method	# ICM isolated	# cell lines	% derivation efficiency[ẑ]
Cowan *et al.* 2004	Immunosurgery	97	17	18%
Experiment A	Immunosurgery	8	2	25%
Experiment B	Immunosurgery	14	7	50%
Experiment C	Immunosurgery	5	2	40%
Experiment D	Immunosurgery	8 (day 5-6)	1	13%
Total Experiments A to D	Immunosurgery	35	12	34%

Derivation efficiency varied between different experiments using identical methods. The embryonic day of development was not taken into consideration in experiments A-C. [ẑ]expressed as % according to the number of cell lines obtained per total number of ICMs isolated for derivation.

Table S2. Characterization of Human Embryonic Stem Cell Lines 18-66

Cell lines with limited availability for distribution can only be used for studies related to diabetes and endoderm due to consent restrictions. # Within the population of karyotypically normal cells, a few karyotypically abnormal cells were also detected (e.g. 3 of 20). * Pluripotency markers that were tested are indicated. In all cases, the tested markers were present. All cell lines showed low levels of spontaneous differentiation in culture. Consistent with previous reports, all hESC lines derived thus far express the same set of pluripotency markers and have the ability to differentiate into various tissues (The International Stem Cell Initiative, 2007). EB: embryoid body, dpf: days post-fertilization, ND: not determined. ** this line was produced by aggregation of blastomeres from two poor quality embryos to form a single blastocysts and may therefore be mosaic.

Cell line	Embryo Quality (dpf)	Isolation method, day of isolation, (quality)	Karyotype	Pluripotency marker expression tested *	Differentiation	Available for distribution
HUES 18	ND	Immunosurgery, ND	46, XY # (p13)	ND	Spontaneous	yes
HUES 19	ND	Immunosurgery, ND	46, XY # (p21)	ND	Spontaneous	yes
HUES 20	ND	Immunosurgery, ND	46,XY (p15)	ND	Spontaneous	yes
HUES 21	ND	Immunosurgery, ND	46,XX (p13)	ND	Spontaneous	yes
HUES 22	ND	Immunosurgery, ND	46,XY	OCT4, SSEA4, TRA1-81, TRA1-60	Spontaneous/Teratoma	yes
HUES 23	ND	Immunosurgery, ND	46,XX,der(2)t(2;17)(q37;q11.2) (p14)	OCT4, SSEA4, TRA1-81, TRA1-60	Spontaneous/Teratoma	yes
HUES 24	ND	Immunosurgery, ND	47,XY # (p14)	OCT4, SSEA4, TRA1-81, TRA1-60	Spontaneous/Teratoma	yes
HUES 25	ND	Immunosurgery, ND	ND	ND	Spontaneous	yes
HUES 26	ND	Immunosurgery, ND	46,XY (p13)	ND	Spontaneous	yes
HUES 27	ND	Immunosurgery, ND	46, XY (p13)	ND	Spontaneous	yes
HUES 28	ND	Immunosurgery, ND	46, XX (p14)	OCT4, SSEA4, TRA1-81, TRA1-60	Spontaneous/Teratoma	yes
HUES 29	3BB (day 6)	Immunosurgery, day 6 (3BB)	45, XX,-21	OCT4, SSEA4, TRA1-81, AP	Spontaneous	no
HUES 30	3AB (day 6)	Laser, day 6 (3AB)	46,XX (p14)	AP, OCT4, SSEA4, SOX2, TRA1-81, NANOG	Spontaneous/EB/Teratoma	no
HUES 31	4AA (day 5)	Laser, day 5 (4AA)	46,XX (p11)	AP, OCT4, NANOG, TRA1-81	Spontaneous	no
HUES 32	3CC (day 6)	Immunosurgery, day 7 (5AA)	46, XY (p6)	ND	Spontaneous	no
HUES 33	2CC (day 6)	Immunosurgery, day 7 (4AB)	46, XX (p7)	OCT4	Spontaneous	no
HUES 34	3CB (day 6)	Laser, day 7 (4AA)	46, XX (p15)	AP, OCT4, SSEA4, SOX2, TRA1-81, NANOG, TRA1-60	Spontaneous/EB	no
HUES 35	3CB (day 6)	Laser, day 8 (filled cavity)	Male	AP, OCT4, SOX2, TRA1-81, NANOG, TRA1-60	Spontaneous/EB/Teratoma	no
HUES 36	4CA (day 5)	Immunosurgery, day 6 (4AA)	46, XX, (p11)	AP, OCT4, SSEA4, SOX2, TRA1-81, NANOG, TRA1-60	Spontaneous/EB	no
HUES 37	3BB (day 5)	Immunosurgery, day 6 (5AA)	46, XX (p8)	OCT4, SSEA4, TRA1-81, TRA1-60	Spontaneous	no
HUES 38	3AA (day 6)	Laser, day 7 (5AA)	Male	ND	Spontaneous	no
HUES 39	3BC (day 5)	Laser, day 6 (4BA)	Female	OCT4, TRA1-81, TRA1-60	Spontaneous/EB	no

HUES 40	5AA (day 6)	Laser, day 7 (6AA)	46, XX (p12)	OCT4, SSEA4, SOX2, NANOG, TRA1-60	Spontaneous/EB	no
HUES 41	3BB (day 5)	Laser, day 7 (6BC)	Female	AP, OCT4, SSEA4, SOX2, TRA1-81	Spontaneous/EB/Teratoma	no
HUES 42	4AA (day 6)	Laser, day 7 (5AA)	46, XY (p16)	OCT4, TRA1-81	Spontaneous/EB/Teratoma	yes
HUES 43	2CC (day 6)	Laser, day 8 (clump of cells)	ND	ND	Spontaneous	no
HUES 46	3BB (day 6)	No isolation	46, XX (p5)	ND	Spontaneous	no
HUES 48	4BB (day6)	Laser, day 9 (filled cavity)	46, XX (p9)	OCT4, NANOG, TRA 1-81	Spontaneous	yes**
HUES 49	5AA (day 6)	Laser, day 9 (filled cavity)	46, XX (p6)	OCT4, NANOG	Spontaneous	yes
HUES 50	5AA (day 6)	Laser, day 6 (5AA)	47, XX, +14 (p9)	OCT4, NANOG	Spontaneous	no
HUES 51	3BB (day 6)	Laser, day 7 (4AB)	Female	OCT4, NANOG	Spontaneous	no
HUES 52	6AA (day 6)	Laser, day 6 (6AA)	Female	OCT4, NANOG, TRA1-60	Spontaneous	no
HUES 53	3BB (day 6)	Laser, day 8 (filled cavity)	46, XY (p9)	OCT4, NANOG, TRA1-60, TRA1-81	Spontaneous	yes
HUES 54	Clump of cells (day 6)	Laser, day 7 (clump of cells)	Female	OCT4, NANOG	Spontaneous	no
HUES 55	3BB (day 6)	Laser, day 7 (3AB)	Female	OCT4, NANOG	Spontaneous	no
HUES 56	4AA (day 6)	Laser, day 6 (4AA)	Male	OCT4, NANOG, TRA1-60	Spontaneous	no
HUES 57	2CC (day6)	Laser, day 8 (clump of cells)	46,XX (p9)	ND	Spontaneous	no
HUES 58	5AA (day 6)	Laser, day 6 (5AA)	Female	OCT4, NANOG	Spontaneous	no
HUES 59	3CC (day 6)	Laser, day 7 (filled cavity)	Nd	OCT4, NANOG	Spontaneous	no
HUES 60	4AA (day 6)	Laser, day 6 (4AA)	Male	OCT4, NANOG	Spontaneous	no
HUES 61	4AA (day 6)	Laser, day 6 (4AA)	46, XX (p9)	OCT4, NANOG, TRA1-60	Spontaneous	no
HUES 62	4AB (day 6)	Laser, day 6 (4AB)	46, XX (p6)	OCT4, NANOG	Spontaneous	yes
HUES 63	5AA (day 6)	Laser, day 6 (5AA)	46, XY (p12)	OCT4, NANOG	Spontaneous	yes
HUES 64	3CC (day 6)	Laser, day 9 (filled cavity)	46, XY (p9)	OCT4, NANOG	Spontaneous	yes
HUES 65	4AA (day 6)	Laser, day 9 (filled cavity)	46, XY (p10)	OCT4, NANOG	Spontaneous	yes

HUES 66	3AB (day 6)	Laser, day 8 (filled cavity)	Female	OCT4, NANOG, TRA1-60	Spontaneous, EB	yes

Table S3. Derivation of Sibling Cell Lines

Donor couple #	# of cleavage stage embryos donated	# of blastocysts	# of cell lines
217	10	7	4 (HUES 36-39)
324	19	5	7 (HUES 54-60)
398	14	5	3 (HUES 62-64)
230	15	7	2 (HUES 50, 51)
291	3	3	2 (HUES 53, 66)
Total	61	27	18

In addition to these 18 lines, HUES 22 and HUES 23, as well as HUES 26 and HUES 27 are sibling cell lines (Fig. S5-S6, Table S4). IVF history was not available for the sibling cell lines, HUES 22 and 23, HUES 26 and 27, HUES 14 and 15 (Cowan et al. 2004), and HUES 16 and 17 (Cowan et al. 2004).

Table S4. Short Tandem Repeat (STR) Profiles of Human Embryonic Stem Cell Lines 1-66

Length of STR fragments at each locus is listed. * Lower levels than other co-alleles, indicating mosaicism. Colored cell lines are siblings. Sibling cell lines share a high number of identical alleles. E.g. HUES 22 and HUES 23 share 21 of 30 alleles, while two unrelated cell lines, HUES 47 and HUES 48 share 7 alleles. HUES 14 and HUES 15, as well as HUES 16 and HUES 17 (see Cowan et al. 2004), are most likely sibling cell lines. Together with the lines reported here, there are a total number of 26 sibling cell lines, 13 of which are available for distribution.

Hues ID	passage number	Amelogenin	vWa	D8S1179	TPOX	FGA	D3S1358	THO1	D21S11	D18S51	PentaE	D5S818	D13S317	D7S820	D16S539	CSF1PO	PentaD	D2S1338	D19S433
HUES 1	25	X,X	17,18	10,12	8,9	21,23	15,18	7,9	28,30	13,13	7,10	11,12	11,12	10,10	8,12	12,12	9,12	ND	ND
HUES 2	24	X,Y	14,16	8,12	9,11	18,23	14,18	5,6	31,32	12,16	7,15	11,12	11,12	9,13	11,13	12,12	11,13	ND	ND
HUES 3	36	X,Y	16,17	12,13	8,11	22,24	15,16	7,8	28,29	13,17	11,12	12,12	8,14	8,11	12,14	11,12	9,12	ND	ND
HUES 4	25	X,Y	15,17	13,15	8,8	23,23	17,17	6,6	30,31	12,19	16,16	12,13	9,11	8,10	12,12	11,12	9,10	ND	ND
HUES 5	18	X,X	15,17	12,14	11,11	20,20	15,18	7,9	27,28	13,16	10,11	11,12	11,13	11,12	12,13	12,12	10,12	ND	ND
HUES 6	24	X,X	15,16	10,13	9,11	23,24	16,18	8,9.3	30,30	12,14	12,12	12,12	11,11	8,11	10,11	11,12	9,10	ND	ND
HUES 7	19	X,Y	16,18	10,11	8,8	25,25	15,18	6,9.3	27,32.2	14,15	7,13	11,13	11,12	8,11	11,12	12,12	9,11	ND	ND
HUES 8	28	X,Y	15,19	13,13	8,11	20,25	15,19	7,9	28,29	12,16	7,10	11,11	12,14	11,12	9,12	10,12	10,11	ND	ND
HUES 9	27	X,X	17,19	9,12	8,9	19,21	17,18	8,9	29,30	12,13	10,12	12,12	11,12	8,12	12,13	10,10	9,13	ND	ND
HUES 10	16	X,Y	16,17	12,13	8,8	19,19	14,18	9.3,9.3	29,30	12,13	ND	11,11	11,12	12,13	12,13	10,13	ND	29,30	15,16.2
HUES 11	30	X,Y	14,16	12,15	8,8	23,24	15,17	6,7	27,28	16,17	5,14	11,12	11,12	12,12	12,12	12,13	13,14	ND	ND
HUES 12	21	X,X	16,18	13,13	8,11	23,24	15,17	6,7	27,28	14,17	7,16	11,12	11,13	9,13	12,13	10,12	13,16	ND	ND
HUES 13																			
HUES 14	21	X,X	16,18	10,11	9,11	20,20	15,17	7,7	28,30	15,19	9,18	10,12	9,11	7,10	9,11	10,12	10,13	ND	ND
HUES 15	17	X,X	16,18	11,13	8,11	20,21	17,18	7,7	28,30	13,15	16,18	11,12	9,9	10,10	9,10	10,12	10,12	ND	ND
HUES 16	19	X,Y	16,17	10,13	8,8	21,21	16,17	7,9.3	30.2,32.2	12,15	7,15	11,12	11,12	8,10	12,13	11,12	11,11	ND	ND
HUES 17	23	X,Y	16,18	10,13	8,9	21,22	16,17	7,9,3	29,30.2	12,15	7,15	11,12	11,12	8,10	12,13	11,12	11,13	ND	ND
HUES 18	13	X,Y	15,17	12,14	8,11	23,26	15,15	6,9	30,31.2	14,21	7,12	11,11	11,11	10,10	9,11	10,11	9,13	ND	ND
HUES 19	23	X,Y	18,20	13,15	8,11	20,24	15,16	7,8	28,30	14,19	11,12	11,11	12,13	10,13	11,11	10,11	10,11	ND	ND
HUES 20	15	X,Y	15,19	13,14	11,11	21,25	15,18	8,9.3	29,30	12,13	12,12	12,12	9,11	10,10	11,12	12,12	9,13	ND	ND
HUES 21	13	X,X	18,19	13,14	9,11	20,22	15,16	6,8	30,30	13,13	8,12	12,12	11,11	10,11	11,13	10,11	9,12	ND	ND
HUES 22	15	X,Y	16,18	14,14	8,11	21,24	16,16	6,6	30,30.2	13,15	5,12	11,12	11,11	8,10	9,12	10,11	13,14	ND	ND

HUES 23	14	X,X	17,17	14,15	11,12	21,24	14,16	6,6	30,30.2	13,16	5,12	11,12	11,11	8,11	11,12	10,11	13,13	ND	ND
HUES 24	8	X,Y	17,17	10,13	8,9	21,24	11,17	6,7	30,30	13,18	ND	10,12	12,14	9,9	9,12	10,12	ND	19,23	14,15
HUES 25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HUES 26	15	X,Y	16,17	11,14	8,11	25,26	13,18	6,9	28,31.2	13,20	12,13	11,11	8,9	10,10	11,12	10,12	9,14	ND	ND
HUES 27	13	X,Y	15,17	11,14	8,11	21,23	13,18	6,9	28,28	14,20	12,17	12,13	8,9	8,10	9,12	10,12	9,14	ND	ND
HUES 28	14	X,X	15,17	14,15	8,8	21,22	15,18	9.3,9.3	28,30	12,14	12,16	11,11	9,10	11,12	12,12	11,12	9,12	ND	ND
HUES 29	13	X,X	16,17	11,13	8,8	21,22	14,15	6,6	31.2,31.2	14,16	16,18	11,12	11,12	8,9	11,12	10,12	12,12	ND	ND
HUES 30	14	X,X	15,19	11,14	8,11	22,25	14,19	6,9.3	30,31.2	13,13	11,21	11,13	12,12	10,11	12,13	10,12	12,14	ND	ND
HUES 31	11	X,X	16,18	13,13	8,8	20,21	16,17	6,9.3	28,30	14,17	13,20	11,12	10,12	8,13	9,11	11,12	9,13	ND	ND
HUES 32		X,Y	15,17	10,14	8,8	22,23	14,17	7,8	29,31	15,18	ND	13,13	9,11	10,11	9,11	10,11	ND	16,17	14,16
HUES 33		X,X	16,17	11,11	9,11	23,25	14,15	6,9.3	28,29	13,20	ND	12,13	8,11	9,11	11,12	10,13	ND	17,24	14,14
HUES 34		X,X	16,18	8,8	8,11	21,24	15,16,20*	6,9.3	28,30	14,17,11.2*	ND	11,13	11,12	9,11	12,12	11,13	ND	17,18	14,15
HUES 35		X,Y	16,18	10,13	8,11	20,21	15,20	9,9.3	28,32.2	15,18	ND	11,13	8,13	8,10	10,11	10,11	ND	17,17	14,14.2
HUES 36		X,X	15,18	13,14	8,8	20,22	15,17	9,9.3	27,31.2	16,16	ND	12,12	8,12	8,11	12,13	11,12	ND	17,20	13,15
HUES 37		X,X	14,18	14,15	8,11	20,22	15,17	7,9	31.2,32.2	14,15	ND	12,13	8,12	8,10	12,13	9,21	ND	17,20	15,17
HUES 38		X,Y	15,18	14,15	8,8	21,24	17,19	9,9.3	30,32.2	14,16	ND	11,12	8,12	8,8	11,12,13	11,12	ND	17,24	13,14
HUES 39		X,X	14,18	10,13	8,8	21,22	15,17	6,9.3	31.2,32.2	15,16	ND	12,12	8,12	8,11	12,13	9,12	ND	17,24	15,17
HUES 40		X,X	14,18	13,15	8,8	22,22	16,17	6,9.3	27,31	12,17	ND	12,13	10,11	9,11	9,11	10,11	ND	24,24	13,14
HUES 41		X,X	16,18	12,14	8,8	19,20	15,17	6,7	33.2,33.2	16,17	ND	13,14	8,10	9,11	12,14	12,12	ND	21,25	13,16
HUES 42		X,Y	18*,19	10,15	8,8	20,24	18,18	6,9.3	28,31.2	17,20	ND	11,12	11,11	9,10	11,12	11,11	ND	17,24	14,15
HUES 43	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HUES 44		X,X	16,16	13,15	8,11	20,23	16,17	6,7	27,30	15,16	ND	12,13	8,12	10,13	10,13	10,12	ND	19,21	14,15.2
HUES 45		X,X	16,19	10,13	8,9	20,24	16,18	7,7	30,32.2	13,17	ND	11,11	11,14	9,12	11,13	10,11	ND	17,17	12,15.2

HUES 46		X,X	17,17	11,13	8,11	21,26	17,17	9,9.3	31.2,32.2	13,16	ND	11,12	12,13	10,12	11,12	10,11	ND	20,24	15,16
HUES 47		X,X	16,16	13,14	8,11	21.2,22	14,16	7,7	27,29	15,18	ND	12,13	12,12	8,12	10,10	7,12	ND	23,26	12,17.2
HUES 48		X,X	14,17	10,13	8,8	21*,24	15,16	9.3,9.3	30.2,31.2	12,13	ND	12,13	12,13	9,9	11,12	11,12	ND	20,21	14,15
HUES 49		X,X	14,18	12*,13	8,11	21,22	16,18,20	6,6	32.2,33.2	11.2*,14,16	ND	9,12	10,12	10,11	12,12	10,12	ND	17,17	14,14
HUES 50		X,X	14,20	12,14	9,11	23,23	16,19	6,9	29,30	15,15	ND	11,13	12,14	8,9	9,11	9,11	ND	17,25	13,14
HUES 51		X,X	17,17	11,14	9,12	23,23	16,19	7,7	29,32.2	15,16,23	ND	11,13	8,12	8,10	12,13	9,10	ND	29,32.2	13,14
HUES 52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HUES 53		X,Y	17,18	12,14	8,8	21,23	15,16	6,7	30,31.2	15,20***	ND	11,12	11,12	11,12	11,12	12,13	ND	17,23	14,14
HUES 54		X,X	14,16	14,15	9,11	21,23	15,16	7,7	29,32	ND	ND	11,12	9,10	11,11	9,10,13	7,11	ND	21,22	13,14
HUES 55		X,X	14,17	14,15	9,11	22,24	14,17	7,7	32.2,32.2	15,16	ND	11,11	nd	9,11	9,10	11,12	ND	18,22	13,13.2
HUES 56		X,Y	14,17	14,15	10,11	21,23	14,17	7,7	32,32.2	14,16	ND	11,12	9,10	10,11	9,9	7,11	ND	18,22	13,13.2
HUES 57		X,X (Y*)	14,15,16	14,15	9,11	23,24	14,15	7,7	29,32.2	15,16	ND	11,12	11,14	9,10	9,10,13	7,11	ND	18,21,22	13.2,14
HUES 58		X,X	14,16	14,15	10,11	21,23	15,16	7,7	32.2,32.2	14,19	ND	11,11	11,14	9,10	9,13	11,12	ND	18,22	13,14
HUES 59		X,Y	14,16	14,15	9,11	23,24	16,17	6.3,7	29,32	14,19	ND	11,12	10,11	10,11	9,13	7,11	ND	18,22	13.2,14
HUES 60		X,Y	16,17	14,15	10,11	21,23	14,15	6.3,7	29,32	ND	ND	11,12	11,14	11,11	10,13	7,11	ND	18,22	13,14
HUES 61		X,X	14,17	13,16	9,9	20,21	16,17,20	9.3,9.3	29,30	14,14	ND	11,12	10,11	8,11	8,14	9,11	ND	17,24	13,15
HUES 62		X,X	16,17	13,13	8,11	23,24	15,20	9,9.3	29,31.2	15,16	ND	11,11	9,12	10,12	12,12	9,12	ND	20,22	13,14
HUES 63		X,Y	16,16	13,13	8,11	22,24	15,16,20	6,9	31,31.2	ND	ND	11,11	ND	10,12	12,13	9,12	ND	18,20	13,13
HUES 64		X,Y	16,16	12,14	8,10	ND	15,15	6,9	31,31.2	ND	ND	11,13	ND	11,12	11,13	12,12	ND	18,20	13,14
HUES 65		X,Y	16,19	13,14,15	8,8	21,25	16,18,20	8,9.3	30,30	ND	ND	12,13	9,9	9,12	12,13	11,12	ND	19,20	14,14
HUES 66		X,X	14,17	11,14	8,8	21,23	17,17	6,7	31.2,32.2	15,15	ND	9,11	12,12	11,12	11,12	11,12	ND	20,23	14,15

Table S5. Comparison of Derivation Efficiencies to Previous Reports

Authors	Method	# embryos	# blastocysts	# of blastocysts used for derivation	day of derivation	# cell lines	% derivation efficiency[~]
Thomson *et al.* 1998	Immunosurgery	36 (fresh and frozen)	20	14		5	35%
Cowan *et al.* 2004	Immunosurgery	344 (frozen)	N/A	97	5-7	17	18%
Lerou *et al., 2008*	No Isolation	242 (fresh, low quality)	94	94	5	8	8.5%
Stojkovic *et al.* 2004	Immunosurgery	11	7	7	8	1	14%
This study	No isolation, Immunosurgery, or Laser-mediated ICM isolation	584 (frozen)	156*	140	5-9	45	32%
This Study	Laser-mediated ICM isolation, (day6)			19	6	11	58%

[~]expressed as % according to the number of cell lines obtained per total number of blastocysts used for derivation. The developmental time of derivation is indicated in parenthesis. N/A: data not available. * 16 blastocysts were used for antibody staining, leaving 140 blastocysts of high and low quality (Table S2) for derivation.