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Supplemental Information

Human embryonic genome activation initiates at the one-cell stage

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SUPPLEMENTAL INFORMATION

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This file includes:

- Legends for Figures S1 and S2
- Figures S1 and S2
- Tables S2 and S3

Other Supplemental Information for this manuscript includes:

- Table S1 (.xls)

Figure S1. Human one-cell embryo expression profiles and relationships

(A) Volcano plot showing genes exhibiting upregulated (up), downregulated (down) and non-changing (nc) expression in one-cell embryos from scRNA-seq. Top upregulated (blue) and down regulated (pink) genes are indicated.

(B) Upregulated gene number (FDR<0.1) from scRNA-seq of one-cell embryos at each log2FC (upper bound indicated for each bin).

(C) Ingenuity Pathway Analysis of down-regulated gene networks (FDR<0.10) in human one-cell embryos.

(D) Venn Diagrams showing upregulated gene overlap between previous datasets from human one-cell (1C) embryo RNA-seq and RNA-seq for two-cell (2C), four-cell (4C) and eight-cell (8C) embryos (L, Leng et al., 2019; X, Xue et al., 2013; W, Wu et al., 2018). Values in parentheses show percentages of the total in one-cell embryos. Applied parameters are provided in references or applied to datasets as follows: L-1C, L-2C, L-4C, L-8C (Leng et al., 2019); X-1C (p_{adj}<0.06, log2FC>2), X-2C (p_{adj}<0.6, log2FC>2), X-4C (p_{adj}<1, log2FC>2), X-8C (p_{adj}<0.06, log2FC>2); W-2C (log2FC>1), W-8C (log2FC>1).

(E) Venn Diagrams showing upregulated gene overlap between human one-cell embryo scRNA-seq obtained here (FDR<0.1, logFC>0.5) and data previously reported for one-cell (1C), two-cell (2C), four-cell (4C) and eight-cell (8C) embryos (L, Leng et al., 2019; X, Xue et al., 2013; W, Wu et al., 2018) or sperm intact RNA (sperm) (Sun et al., 2021) (FDR<0.1), showing (in parentheses) percentage of the total in one-cell embryos. Comparisons were of annotated genes using parameters of (D).

(F) Heatmap indicating expression trajectories of 153 of the top 200 upregulated DEGs from one-cell embryos determined by scRNA-seq in this work (FDR<0.1), relative to expression in single blastomeres from two-, four- and eight-cell embryos (Leng et al., 2019). Genes were sorted into three clusters (I, II, and III) as indicated.

See also Figure 1 and Table S1.

Figure S2. Differential gene expression networks in human one-cell embryos

(A) Histograms derived from scRNA-seq showing endogenous retrovirus (*hERV*) loci upregulated in human one-cell embryos. Locus coordinates are shown on the x-axis.

(B) Histograms derived from scRNA-seq showing endogenous retrovirus (*hERV*) loci down-regulated in human one-cell embryos. Locus coordinates are given on the x-axis.

(C) Pie chart showing functional classes of upregulated DEGs (FDR<0.10) related to molecular mechanisms of cancer (Ingenuity).

(D) Upstream transcription activators predicted by Ingenuity Pathway Analysis (IPA) of upregulated DEGs in human one-cell embryos (FDR<0.10).

(E-H) Upstream transcription regulators and associated networks inferred by IPA of upregulated gene networks (FDR<0.10) in human one-cell embryos for RABL6 (E), FOXM1 (F), YAP1 (G) and TP53 (H).

(I) Genes in the E2F4 cluster identified by IPA.

See also Figure 2 and Table S1.

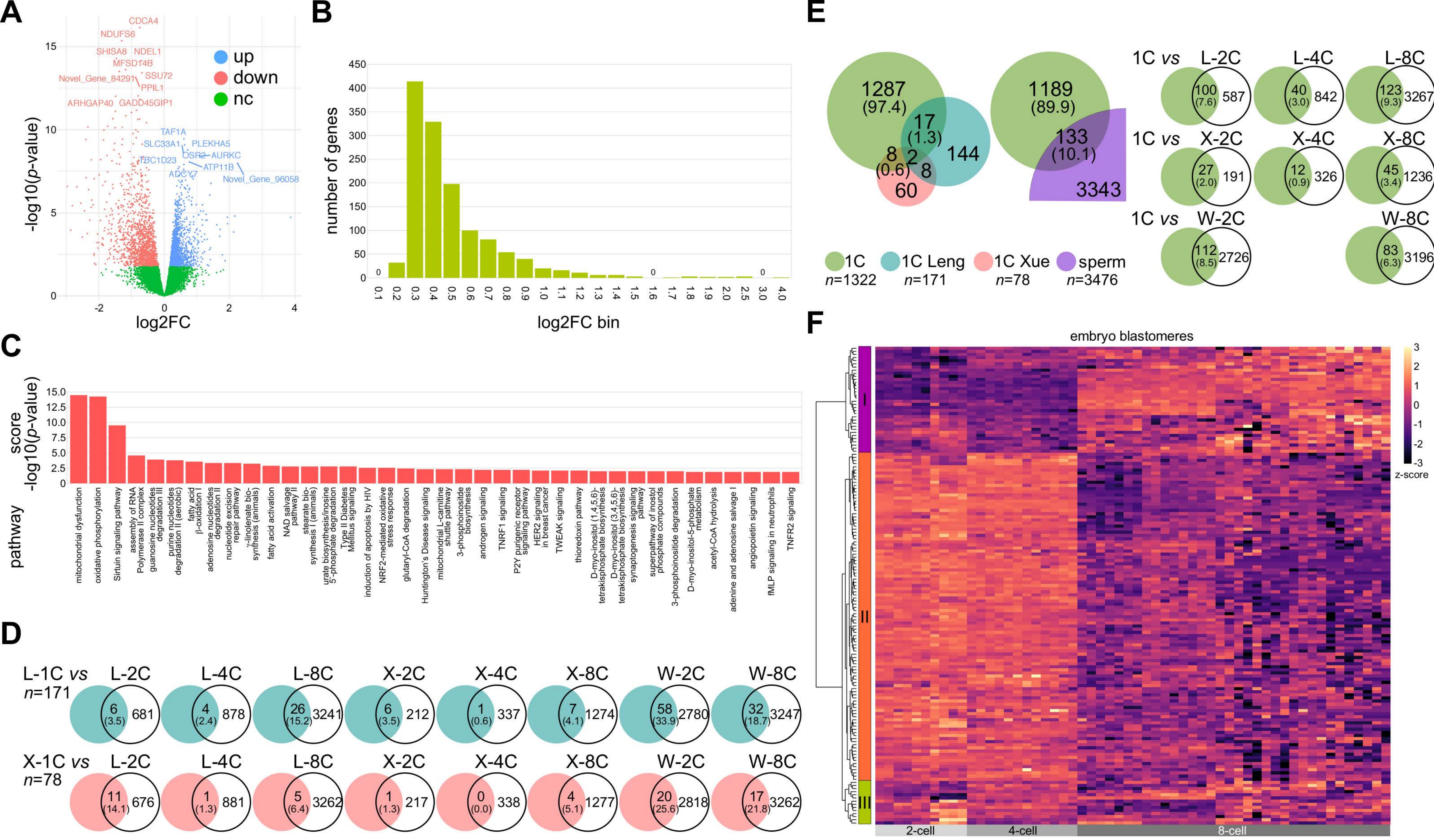
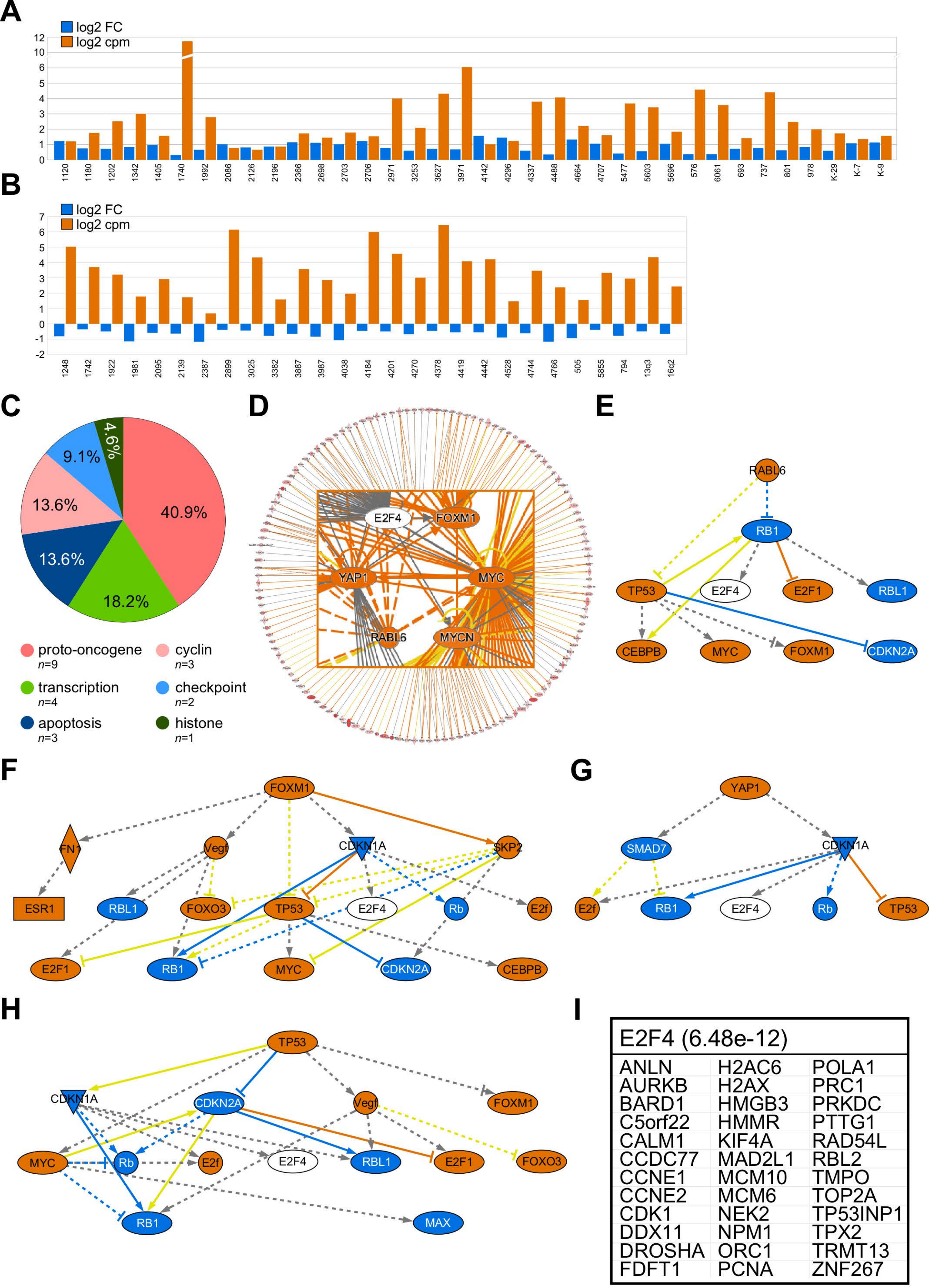


Figure S1
Asami et al.



E2F4 (6.48e-12)		
ANLN	H2AC6	POLA1
AURKB	H2AX	PRC1
BARD1	HMGB3	PRKDC
C5orf22	HMMR	PTTG1
CALM1	KIF4A	RAD54L
CCDC77	MAD2L1	RBL2
CCNE1	MCM10	TMPO
CCNE2	MCM6	TOP2A
CDK1	NEK2	TP53INP1
DDX11	NPM1	TPX2
DROSHA	ORC1	TRMT13
FDFT1	PCNA	ZNF267

Figure S2
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Table S2 PCR Primers used in this work, related to Figures 1 and 2

Sequence	Forward (5'→3')	Reverse (5'→3')
H3F3A	ACAAAAGCCGCTCGCAAGAGTG	TTTCTCGCACCAGACGCTGGAA
CDH15	AGCGTATCCGAGAACCACAAGC	CTGGATGCTGTAGATGACGCTG
TAF1A	TCTGTGCTCAGTGGTGCAGGAA	TGCCATTGGTGCTTCAGCAGAG
ZCCHC8	TTTCCAGCAGCGATACCACGCA	GGAAGACTCTTGTCTGTCACACC
AIF1L	CCTTCCAGAAAAGCTCACAGCC	CTTCATCTCCAGGTGGGTCTTG
TXNIP	CAGCAGTGCAAACAGACTTCGG	CTGAGGAAGCTCAAAGCCGAAC
TIGD5	GTGCTGTTCCTGTCCAAAGGCA	AGGACACAGCCAGTCGCAGCA
CMTR2	CTGACCACTCTTGGAAACGGTG	GACTTCGGAGTTTCCTGCCTTG
RIPK4	CTGAAACCGAGGACCTGTGTGA	GCTGTAGTCGTTATCGAAGGTGG
OPRM1	CGTCAGAACACTAGAGACCACC	CTTGGTGAAGGTCGGAATGGCA
RAP2C	GAGCAGTTTGCCTCCATGAGAG	CCTACTAGGATTAGTGGGACTTTT
PIAS3	TGGTGATGAGTTTCCGGGT	GGAAAGCGTCGTCGGTAA
UGDH	TGTGATGGTGCCCATGCTGTTG	GTCCATCGAAGATAAAGGCTGGC
NFKBIA	TCCACTCCATCCTGAAGGCTAC	CAAGGACACCAAAAGCTCCACG
CCNE1	TGTGTCCTGGATGTTGACTGCC	CTCTATGTCGCACCACTGATACC

Table S3 Human embryos used in this work

#	Patient	Sample	Analysis	Ethnicity	Age (yr)	Yield (nM)	Useable reads	Normalization factor (TMM)	Genes detected (≥1cpm)
1	1	2PN	RNAseq	Asian	nd	61.7	68635512	0.9692079	15709
2	1	2PN	RNAseq	Asian	nd	60.8	65181145	0.9699501	15601
3	1	2PN	RNAseq	Asian	nd	48.8	88623885	0.9031541	15732
4	2	2PN	RNAseq	Caucasian	nd	31.2	46084528	1.001886	15726
5	2	2PN	RNAseq	Caucasian	nd	3.1	Amplification failure	nd	nd
6	3	2PN	RNAseq	Caucasian	nd	54.8	67065772	0.981549	15763
7	4	2PN	RNAseq	Caucasian	nd	52.9	76729549	0.957278	15630
8	4	2PN	RNAseq	Caucasian	nd	46.2	76189001	0.944969	15762
9	4	2PN	RNAseq	Caucasian	nd	51.6	73264323	0.9851035	15918
10	5	2PN	RNAseq	Caucasian	nd	43.7	75180066	0.9368282	15767
11	5	2PN	RNAseq	Caucasian	nd	46.6	73222174	0.9957537	15831
12	6	2PN	RNAseq	Caucasian	nd	67.1	66404656	0.9754091	15753
13	6	2PN	RNAseq	Caucasian	nd	43.5	58715420	0.9919022	15602
14	7	mII	RNAseq	Caucasian	nd	46.7	72657324	0.9876494	15585
15	7	mII	RNAseq	Caucasian	nd	15.4	Excluded outlier	nd	nd
16	8	mII	RNAseq	Caucasian	nd	66.1	65324304	1.0415315	15825
17	8	mII	RNAseq	Caucasian	nd	52.2	72466449	1.0608596	15885
18	9	mII	RNAseq	Caucasian	<30	72.5	Excluded outlier	nd	nd
19	9	mII	RNAseq	Caucasian	<30	25.2	54705074	1.0171751	15455
20	10	mII	RNAseq	Caucasian	24.5	68.2	51126979	1.0306027	15860
21	10	mII	RNAseq	Caucasian	24.5	57.8	55244789	1.026353	15818
22	11	mII	RNAseq	African American/Hispanic	31	94.2	67438079	1.0240237	15812
23	11	mII	RNAseq	African American/Hispanic	31	97.4	61061212	1.0471581	15985
24	12	mII	RNAseq	Caucasian	22.5	46.3	69363653	1.0382324	15837

25	12	mII	RNAseq	Caucasian	22.5	70.1	65550194	1.0569761	15920
26	13	mII	RNAseq	Caucasian	25	21.1	67377209	1.0439114	15942
27	13	mII	RNAseq	Caucasian	25	35.8	54760799	1.0324617	15977
28	7	mII	qPCR	Caucasian	nd				
29	7	mII	qPCR	Caucasian	nd				
30	11	mII	qPCR	African American/Hispanic	31				
31	8	mII	qPCR	Caucasian	nd				
32	10	mII	qPCR	Caucasian	24.5				
33	1	2PN	qPCR	Asian	nd				
34	1	2PN	qPCR	Asian	nd				
35	1	2PN	qPCR	Asian	nd				
36	1	2PN	qPCR	Asian	nd				
37	1	2PN	qPCR	Asian	nd				
38	5	2PN	qPCR	Caucasian	nd				
39	14	2PN	qPCR	Unknown	nd				
40	15	3PN	qPCR	Unknown	nd				
41	16	3PN	qPCR	Unknown	nd				
42	3	3PN	qPCR	Caucasian	nd				
43	17	1PN	qPCR	Unknown	nd				
44	15	1PN	qPCR	Unknown	nd				
45	18	1PN	qPCR	Unknown	nd				

Abbreviations: 1PN, 2PN, 3PN, respectively monopronuclear, bipronuclear and tripronuclear human one-cell embryos; nd, no data; TMM, trim-mean of M-values; cpm, counts per million.