Supplementary information

Transcriptome asymmetry within mouse zygotes but not between early embryonic sister blastomeres

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Supplementary materials and methods

Collection and culture of immature oocytes

For movies to monitor mitochondrial localization, fully-grown germinal vesicle (GV) oocyte-cumulus cell complexes were collected from 7-9-week-old transgenic females expressing mitochondrially-targeted Venus (mitoVenus) protein (Shoji *et al*, 2006) 44 to 48 hours after intraperitoneal injection of eCG. Following 1 hour of culture in Waymouth medium (Waymouth, 1959) supplemented with 10% (v/v) fetal calf serum (FCS), cumulus cells were displaced from GV oocytes by repeated pipetting in TaM medium (Miki *et al*, 2006) supplemented with 10% (v/v) FCS and 150 μM isobutylmethylxanthine (IBMX). The immature oocytes were held in TaM medium containing 150 μM IBMX until micromanipulation and without it afterwards. Oocyte and embryo culture was in humidified CO_2 (5% [v/v] in air) at 37°C.

Real-time fluorescence imaging

Mitochondrial tracking during in vitro maturation and following mII exit was in oocytes expressing mitoVenus (Shoji *et al*, 2006). Oocytes were placed in a KSOM droplet under mineral oil on a glass-bottomed dish on the stage of a TE2000 inverted microscope (Nikon, Japan) equipped with a CSU10 confocal scanning unit (Yokogawa, Japan) and a humidified chamber (5% [v/v] CO_2 in air) at 37°C. DIC images and fluorescent (488 nm) images (typically 13 focal planes, step size = 2 μm) were captured at 5 minute intervals by a C9100-13 ImagEM EM-CCD camera (Hamamatsu Photonics, Shizuoka, Japan) driven by MetaMorph (Molecular Devices, CA, USA) image analysis software.

Supplementary references

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Supplementary figure legends

Figure S1 Three models relating physiological pre-patterning to cell lineage specification in pre-implantation embryos. The models each represent the fates of two lineages arising from hypothetical blastomeres A and B of a 2-cell (2C) embryo. (**A**) Blastomeres A and B inherit distinct sets of maternal molecules (eg mRNAs) and are functionally non-equivalent. Daughters of one of them (in this case, lineage A) preferentially segregate to the morula interior to give rise to cells of the inner cell mass (ICM) and, in turn, epiblasts and the primitive endoderm (not shown) in 64-cell (64C) blastocysts (Plusa *et al*, 2005). (**B**) Blastomeres A and B inherit indistinguishable sets of maternal molecules and are functionally equivalent. The daughters of lineages A and B accordingly contribute randomly to pre-implantation embryonic specification (Hiiragi and Solter, 2004; Kurotaki *et al*, 2007). (**C**) Blastomeres A and B inherit distinct sets of maternal molecules and, is per (**A**) are functionally non-equivalent. Segregation is initially random, but internal morula cells of lineage A rapidly respond to the microenvironment of the morula interior and induce many of the neighboring (internal) cells of lineage B to do the same, so that many lineages at that location become ICM progenitors. The prompt and timely response of internal lineage A cells ensures coordinated development of the ICM and the embryo as a whole. The default pathway for all exterior cells is to trophectoderm (TE). Embryos split at the 2-cell stage are also able to develop fully, although note that the environment of each is non-physiological and the first mitotic cell cycle is effectively repeated. This model does not predict whether asymmetric inheritance at the 2-cell stage is transmitted along resultants lineages. Some maternal mRNAs are degraded in the mouse (Alizadeh *et al*, 2004), but even transient asymmetry at the 2-cell stage may be sufficient to affect lineage fate. The pre-patterning/induction model of (**C**) is compatible with reports that support random (stochastic) determination, such as the one of (**B**) (Kurotaki *et al*, 2007).

Figure S2 Sets of transcripts enriched in spindle and Pb_2 overlap. The observed overlap size between the top ranking mRNAs from lists of enriched mRNAs in the spindle (Supplementary Table S1) and Pb_2 (Supplementary Table S3) is significantly greater than the magnitudes of overlaps expected for randomly-selected mRNAs. Vertical orange bars indicate the spread of overlap sizes that occur if transcripts are chosen at random from both lists.

Figure S3 Pb_2-zygote segregation of mitochondria and RNAs associated with mouse oocyte polarity. (**A**) Paired DIC (upper) and fluorescence video captures of a representative mitoVenus-expressing oocyte, recorded at 5 minute intervals starting 30 minutes after sperm entry. The emergent Pb_2 in the final frame is marked with a red arrowhead. Bar = 50 μm. (**B**) Mitochondrial genomic DNA *mt-Co1* qPCR analysis in Pb_2-zygote couplets against an endogenous *Actb* genomic DNA standard, with the zygote value (open box) set at 1.0. (**C**) Comparative qPCR analysis respectively of spindle-oocyte (spin) and Pb_2-zygote (Pb_2) couplets shown as a -fold change in RQ values (spin/Pb_2 relative to oocyte/zygote) as per Figure 1B. RQ values were normalized against *H3f3a* (Supplementary Figure S5). A boxed inset gives p_{adj} values from microarray analysis (Supplementary Table S3) suggesting no significant Pb_2-zygote difference. RNAs correspond to the following with previously-reported non-uniform distributions in mouse oocytes: 16S mitochondrial RNA (*16S*), Stat3 protein (*Stat3*) and Leptin protein (*Lep*).

Figure S4 Relationships between experimental samples. Schematic summarizing relationships between the principal samples analyzed in this work.

Figure S5 PCR Standard Selection. Threshold cycle (C_t) values for candidate qPCR standards (CT) in the four principle sample types obtained microsurgically from mII oocytes in the present work (see Figures 1A and 1D).

Supplementary table legends

Table S1 Differential gene expression analysis performed in R using the Limma package (Smyth and Speed, 2003) for matched spindle-oocyte samples. A positive logFC indicates relatively high levels in the spindle; a negative logFC indicates relatively low levels in the spindle.

Table S3 Differential gene expression analysis performed in R using the Limma package (Smyth and Speed, 2003) for matched Pb_2-zygote samples. A positive logFC indicates relatively high levels in the Pb_2; a negative logFC indicates relatively low levels in the Pb_2.

Table S5 Genes differentially expressed between the two comparisons (higher in Pb_2 and lower in spindle, or lower in Pb_2 and higher in spindle). A positive logFC indicates higher levels in the Pb_2 compared to zygote and lower in spindle compared to oocyte; a negative logFC indicates lower levels in the Pb_2 compared to zygote and higher levels in the spindle compared to the oocyte.

Array data are deposited at the Gene Expression omnibus (GEO) with the accession number, GSE27396:
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ljcljmkyuooawjo&acc=GSE27396

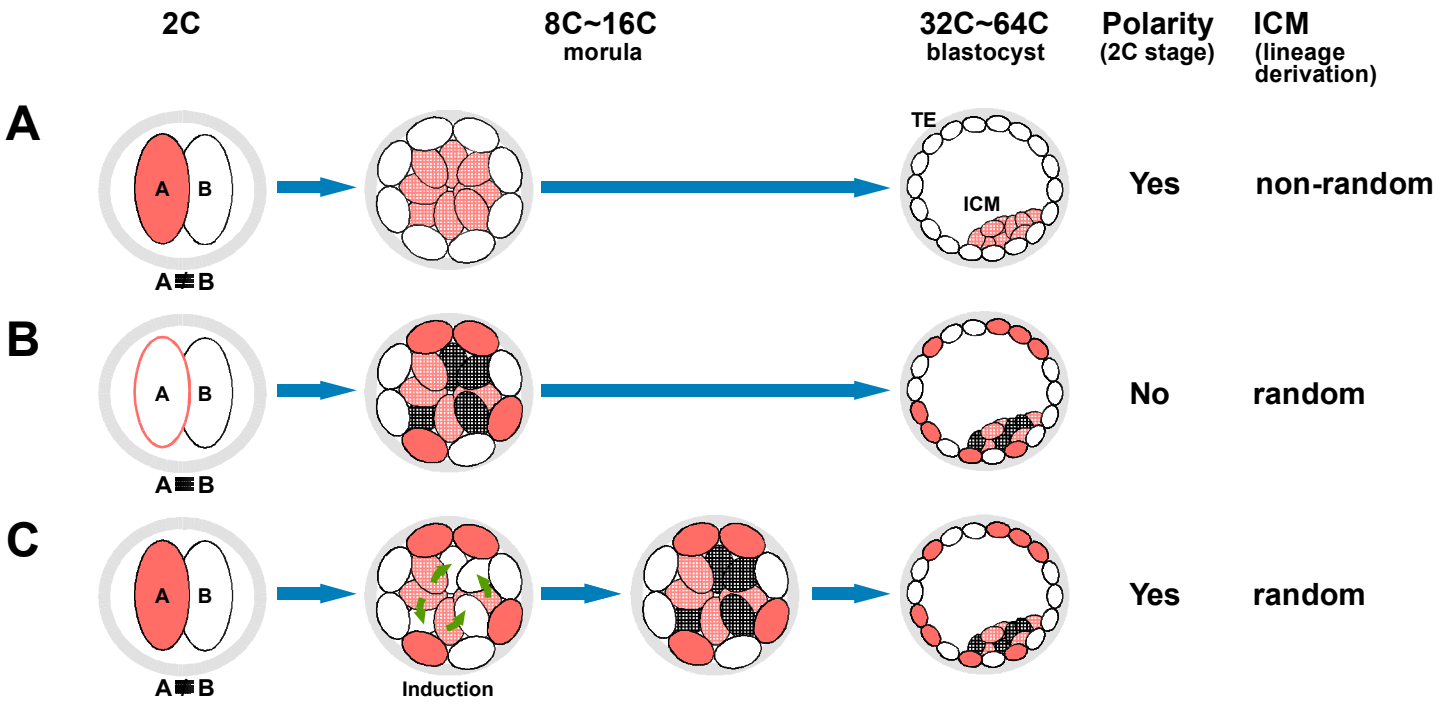
2C
8C~16C
morula
32C~64C
blastocyst
Polarity
(2C stage)
ICM
(lineage
derivation)
A
B
A
A
B
TE
ICM
Yes
non-random
B
A
B
A
B
No
random
C
A
B
A
B
Induction
Yes
random

Figure S1

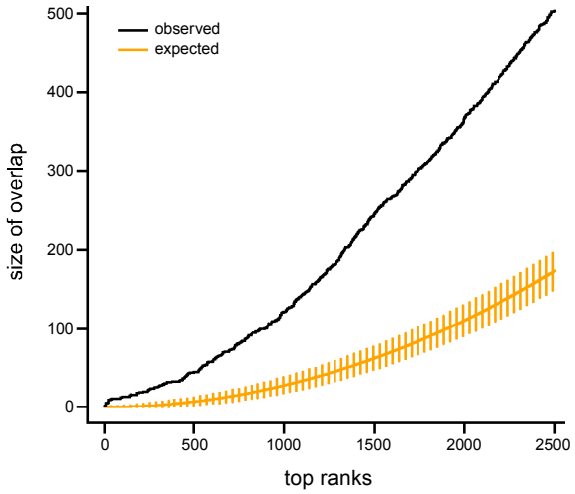
observed
expected
size of overlap
top ranks
0
100
200
300
400
500
0
500
1000
1500
2000
2500

Figure S2

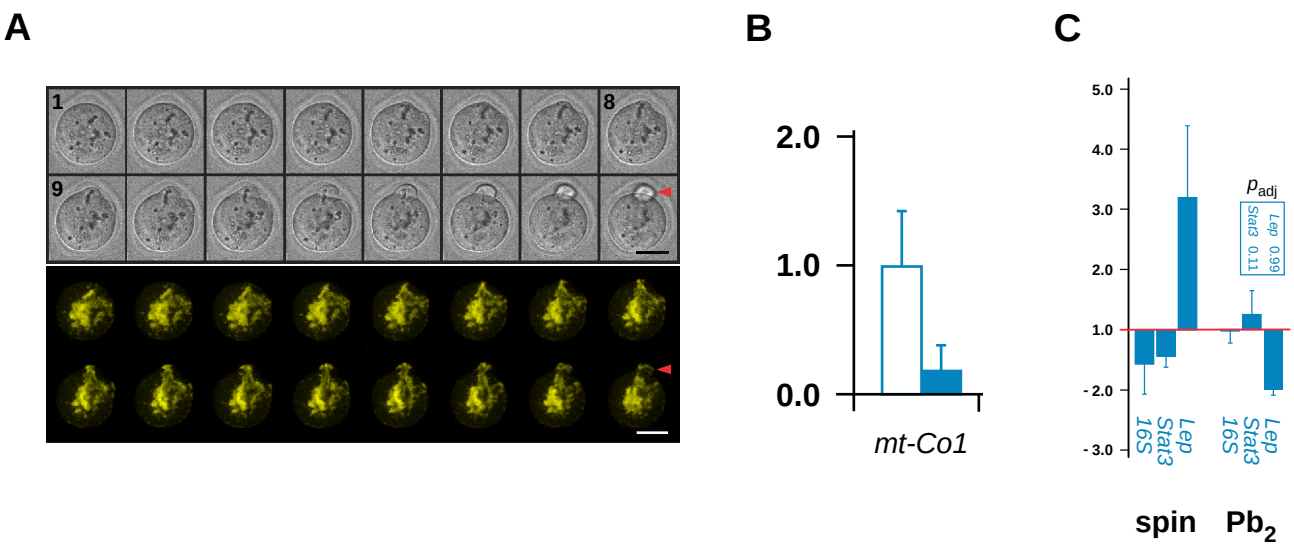
A
1
8
9
B
2.0
1.0
0.0
mt-Co1
C
5.0
4.0
3.0
2.0
1.0
- 2.0
- 3.0
p_{adj}
Lep 0.99
Stat3 0.11
16S
Lep
Stat3
16S
Lep
spin
Pb_2

Figure S3

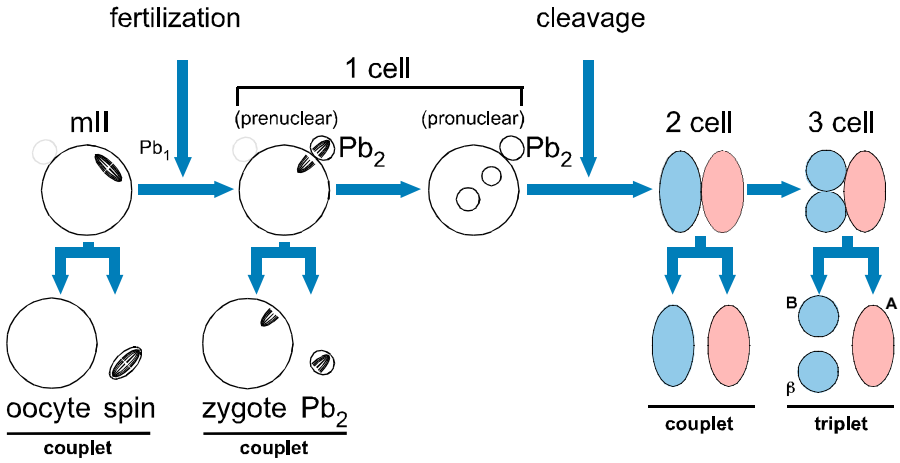
fertilization
cleavage
1 cell
mII
Pb1
(prenuclear)
Pb2
(pronuclear)
Pb2
2 cell
3 cell
B
A
β
oocyte spin
couplet
zygote Pb2
couplet
couplet
triplet

Figure S4

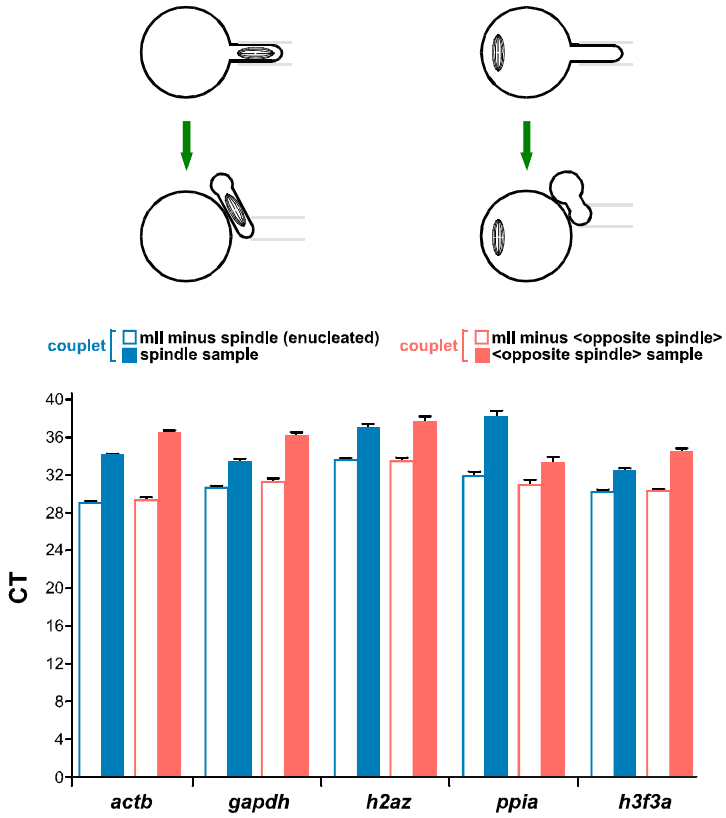
couplet
mII minus spindle (enucleated)
spindle sample
couplet
mII minus <opposite spindle>
<opposite spindle> sample
CT
40
36
32
28
24
20
16
12
8
4
0
actb
gapdh
h2az
ppia
h3f3a

Figure S5

Table S2. Spindle *vs* oocyte qPCR and corresponding microarray analyses
p_{adj}, adjusted *p* value. Note that position number, not row number, is shown (position no. = row no. -1) from Table S1. LogFC with a positive value (+) predicts spindle>mII oocyte. p_{adj} <0.05 shown in red.

Sequence	paired	p_{adj}	logFC
mrpl13	315	0.042	+
mrpl55	17	0.002	+
Cd52	639	0.073	+
Dach1	33,545	0.981	-
Twf1	1,723	0.168	+
Actb	6,760	0.446	+
Sat1	24,404	0.879	+
Ccdc72	9,955	0.571	+
Rsu1	745	0.085	-
H2afx	28,576	0.933	+
Tapbp	12,959	0.661	+
BC088983	741	0.085	-
Ddx59	1,324	0.130	-
Rbm22	4,027	0.313	-
Ripk2	2,391	0.213	-
Becn1	14,845	0.700	-
Atp1a1	4,927	0.363	-
Tnks	14,038	0.682	-
Cenpe	35,174	0.993	+
Ica1	14,553	0.692	-
Sf3b1	19,983	0.805	-
Cdc20	1,885	0.174	-
Ptov	3,177	0.259	+
2610110g12Rik	21,266	0.829	+
Prr7	34,703	0.990	-
Klk13	2,817	0.239	-
Daf2	15,039	0.704	+
Rshl2a	97	0.016	-
Mtpn	11,657	0.628	-
Ubl3	24,074	0.875	-

Table S4. Pb_2 *vs* zygote qPCR and corresponding microarray analyses
p_{adj}, adjusted *p* value. Note that position number, not row number, is shown (position no. = row no. -1) from Table S3. LogFC with a positive value (+) predicts Pb_2>zygote. p_{adj} <0.05 shown in red.

Sequence	position	p_{adj}	logFC
mrpl13	238	0.011	+
mrpl55	73	0.004	+
Cd52	779	0.035	+
Dach1	1,261	0.059	+
Twf1	167	0.008	+
Actb	5,289	0.235	+
Sat1	208	0.009	+
Ccdc72	280	0.028	+
Rsu1	2,604	0.118	+
H2afx	407	0.016	+
Tapbp	4,500	0.201	+
BC088983	10	0.001	-
Ddx59	32	0.002	-
Rbm22	2,680	0.122	-
Ripk2	13	0.001	-
Becn1	1,471	0.068	-
Atp1a1	107	0.005	-
Tnks	123	0.005	-
Cenpe	1,783	0.083	-
Ica1	4,140	0.184	-
Sf3b1	7,791	0.331	-
Cdc20	242	0.011	-
Ptov	14,641	0.559	+
2610110g12Rik	15,784	0.593	+
Prr7	2,990	0.136	-
Klk13	2,783	0.126	+
Daf2	4,307	0.191	+
Rshl2a	15,448	0.584	+
Mtpn	2,414	0.110	-
Ubl3	15,721	0.592	-

Table S6. No marked correspondence between enrichment of mRNAs in Pb_2/zygote and in *Xenopus* or human microtubules

p_{adj}, adjusted *p* values in paired setting. Values for spindle-mII oocyte couplets (spin-mII) are from Table S1, and those for second polar body-zygote couplets (Pb_2-zygote) are from Table S3. Each shows position no., not row no. (position no. = row no. -1). LogFC are given; a positive value (+) predicts spin>mII or Pb_2>zygote. All sequences were selected and searched where $p<0.05$ for *Xenopus* and/or human, taken from Blower et al., 2007.

p*<0.05, *Xenopus

Xl Gene name	Xl Log2 (MT/total)	Xl p-value	polysome enrichment	Hum gene name	Hum Log2 (Mt/total)	Hum p-value	spin-mII	Pb_2-zygote	Lowest p_{adj}
cenpe-A	2.291030504	0.014434491	14.35618364	CENPE	0.82699099	0.009340004	35,174	1,783	p_{adj} Pb_2-zygote=0.083 (-)
kif2c	0.707022245	0.045689399	13.4941008	KIF2C	-0.134948026	0.847522584	27,194	10,339	
TPX2	0.667149477	0.024565963	13.9809868	TPX2	-0.189446059	0.794693028	22,027	10,398	
Fzd7	0.65607925	0.005085621	12.65569241	FZD7	0.81224496	0.557071824	31,110	7,116	
ahsa1	0.617485507	0.019168603	12.08716718	AHSA1	0.177019199	0.830328847	33,667	8,395	
wdr23	0.456788541	0.00605576	9.893581433	WDR23	-0.696196529	0.189931244	22,717	15,048	
dbr1	0.440371158	0.037942005	10.43843054	DBR1	-0.139165918	0.892648221	33,975	31,815	
ppp1ca	0.411460017	0.006204685	13.2715399	PPP1CA	-0.027574463	0.953676683	5,275	27,565	p_{adj} spin-mII=0.378 (+)
Sfrs1	0.341660065	0.03650361	13.04739205	SFRS1	0.584064634	0.615844314	10,632	22,205	
ube2r2	0.316462818	0.045793425	11.29881947	UBE2R2	0.020019822	0.983931062	35,210	20,809	
gnai3	0.221482938	0.033674428	12.17328066	GNAI3	-0.003516342	0.997410992	20,165	14,790	

***p*<0.05, Human**

Xl Gene name	Xl Log2 (MT/total)	Xl p-value	polysome enrichment	Hum gene name	Hum Log2 (Mt/total)	Hum p-value	spin-mII	Pb_2-zygote	Lowest p_{adj}
cenpe-A	2.291030504	0.014434491	14.35618364	CENPE	0.82699099	0.009340004	35,174	1,783	p_{adj} Pb_2-zygote=0.083 (-)
mcm7	0.80316826	0.141637931	14.04891801	MCM7	1.039992656	0.01648752	5,303	19,591	p_{adj} spin-mII=0.379 (-)
Xe-Wee1A	0.596412137	0.109621888	13.91992331	WEE1	1.102939937	0.008097551	23,205	31,566	
tpr	0.530048162	0.18158509	14.00722159	TPR	1.13175161	0.045389788	31,811	4,129	p_{adj} Pb_2-zygote=0.183 (-)
ddx39	0.327671262	0.120172918	14.10299638	DDX39	0.764752439	0.049275213	15,268	31,937	
ppp1cc	0.316420473	0.185544551	13.00501458	PPP1CC	-1.498378956	0.034601549	9,116	3,759	p_{adj} Pb_2-zygote=0.171 (+)
Ptk2	0.184324621	0.050856873	10.20242115	PTK2	-3.513329867	0.020000133	34,143	27,614	

Table S7. Polar mRNAs localized to the *Xenopus* animal hemisphere and Pb_2-zygote segregation in mouse counterparts
Position numbers are shown (position no. = row no. -1) from Table S3. LogFC with a positive value (+) predicts Pb_2>zygote. p_{adj} <0.05 shown in red.

Name	*Xenopus* prot (aa)	*Mus* prot (aa)	*Mus* mRNA	position	p_{adj}	Notes *(UniGene expression)*
βTrCP	Q91854 (518)	NP_033901 (569)	NM_009771	30,613	0.918 (-)	Similar. *Btrc mRNA: zyg, 473; brain, 225; testis, 76. mII, zyg = 245, 458.* NF: NM_009771, Btrc, TrCP.
An2	NP_001081246 (553)	NP_031531 (553)	NM_007505	15,418	0.583 (+)	*Mus* ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1 identities = 515/552 (93%). Similar. *Atp5a1 mRNA: zyg, 72; brain, 175; testis, 42. mII, zyg = 196, 70.* NF: NM_007505, An2, Atp5a1.
An3	P24346 (697)	NP_034158 (662)	NM_010028	27,245	0.858 (-)	*Mus* DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked identities = 558/708 (78%). Similar. *Ddx3x mRNA: zyg, 0; brain, 0; testis, 0. mII, zyg = 49, 0.* NF: NM_010028, An3, Ddx3x.
xlan4	AAA73357 (315)	EDL00149 (436)	NM_198127	18,273	0.656 (-)	*Xenopus* aka animal 4. *Mus* abl-interactor 2 identities = 294/317 (92%). Similar. *Abi2 mRNA: zyg, 0; brain, 164; testis, 50. mII, zyg = 0, 0.* NF: NM_198127, xlan4, Abi2, abl-interactor 2.
FN	NP_001081270 (2481)	NP_034363 (2477)	NM_010233	23,733	0.793 (-)	Fibronectin. Identities = 1750/2456 (71%). Similar. *Fn1 mRNA: zyg, 0; brain, 111; testis, 177. mII, zyg = 0, 0.* NF: NM_010233, FN, Fibronectin.
xlPou60	NP_001081583 (426)	NP_032926 (497)	NM_008900	6,388	0.278 (-)	POU domain, class 3, transcription factor 3 (Brain-specific homeobox/POU domain protein 1) (Brn-1 protein). Like Oct4. Identities = 91/155 (58%). Related. *Pou3f3 mRNA: zyg, 0; brain, 33; testis, 0. mII, zyg = 0, 0.* NF: NM_008900, xlPou60, Brn-1, Brn 1, Brn1, Pou3f3.
Tcf-1	NP_001082691 (464)	NP_034833 (397)	NM_010703	104	0.005 (-)	*Mus* lymphoid enhancer binding factor 1. Identities = 224/382 (58%). Related. *Lef1 mRNA: zyg, 182; brain, 18; testis, 76. mII, zyg = 0, 176.* NF: NM_010703, Tcf-1, lymphoid enhancer binding factor, Lef1.

Table S8. Polar mRNAs localized to the *Xenopus* vegetal hemisphere and Pb_2-zygote segregation in mouse counterparts
Position numbers are shown (position no. = row no. -1) from Table S3. LogFC with a positive value (+) predicts Pb_2>zygote.

Name	*Xenopus* prot (aa)	*Mus* prot (aa)	*Mus* mRNA	position	p_{adj}	Notes *(UniGene expression)*
βTrCP	Q91854 (518)	NP_033901 (569)	NM_009771	30,613	0.918 (-)	Similar. *Btrc mRNA: zyg, 473; brain, 225; testis, 76. mII, zyg = 245, 458.* NF: NM_009771, Btrc, TrCP.
VegT	AAB93301 (455)	AAC53110 (540)	NM_011538	30,341	0.913 (-)	Mouse seq is ortholog of Tbx6; Tbx6 also exists in *Xenopus*, but in Blast of *Xenopus* VegT, the first *Mus* hit is with Tbx6. Related. *Tbx6 mRNA: zyg, 0; brain, 0; testis, 0. Restricted to juvenile. mII, zyg = 0, 0.* NF: AY654733, VegT, Tbx6.
Vg1	AAH90232 (361)	NP_032134 (366)	NM_008108	22,739	0.771 (-)	Mouse seq is growth differentiation factor 3 (Gdf3). *Xenopus* also has a Gdf3-like protein called Derriere. Related. *Gdf3 mRNA: zyg, 0; brain, 0; testis, 0. Restricted to blastocyst. mII, zyg = 0, 0.* NF: NM_008108, Vg1, Gdf3.
Xcat-2	CAA51067 (128)	NP_918953 (136)	NM_194064	20,407	0.711 (-)	Mouse seq is Nanos homologue 2; 41% identities over 111. Similar. Nanos1 also similar (69% over 56). *Nanos2 mRNA: zyg, 0; brain, 0; testis, 0. Restricted to ovary. mII, zyg = 0, 0.* NF: NM_194064, Xcat-2, Nanos.
Xcat-3	Q9DGP9 (483)	NP_031942 (478)	NM_007916	1,870	0.087 (-)	*Xenopus* protein is ATP-dependent RNA helicase DDX25 (Dead South RAN helicase; Zn-responsive). *Mus*, Ddx19-like protein, is very like Xcat3 (292/423 identity = 69%). Similar. *Ddx19a mRNA: zyg, 145; brain, 86; testis, 110. mII, zyg = 0, 140.* NF: NM_007916, Xcat-3, ddx25, ddx19.
Xdazl	O57437 (286)	NP_034151 (298)	NM_010021	1,741	0.082 (-)	*Xenopus* is deleted in azoospermia-like-A (DAZ-like protein A). *Mus* deleted in azoospermia-like. Identities = 161/270 (59%). Similar. *Dazl mRNA: zyg, 2477; brain, 10; testis, 896. Restricted to ovary and unfertilized ovum. mII, zyg = 18,953, 2,395.* NF: NM_010021, Xdazl, DAZ.
xpat	CAA05358 (293)	none	none			No clearly-related protein sequences; xpat not found.
Xwnt-11	NP_001084327 (353)	NP_033545 (354)	NM_009519	11,778	0.470 (+)	Mouse wingless-related MMTV integration site 11 (Wnt11) identities = 221/337 (65%). Similar. *Wnt11 mRNA: zyg, 0; brain, 0; testis, 16. Expressed in oviduct (274). mII, zyg = 0, 0.* NF: NM_009519, Xwnt, Wnt11,

						Wnt-11, Wnt 11.
fatvg	NP_001081960 (428)	P43883 (425)	NM_007408	15,130	0.574 (+)	*Mus* is perilipin 2 (Plin2) identities = 237/407 (58%). Related. *Plin2 mRNA: zyg, 109; brain, 21; testis, 33. mII, zyg = 98, 105.* NF: NM_007408, fatvg, Adipophilin, ADRP, Adfp. Listed as Adfp.
xvelo1	AAP43960 (779)	none	none			No clearly related protein sequences; xvelo1 not found.
XNIF	AAP43959 (276)	NP_598471 (276)	NM_133710	34,595	0.983 (-)	*Mus* CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like, identities = 224/277 (80%). Similar. *Ctdspl mRNA: zyg, 36; brain, 23; testis, 33. mII, zyg = 49, 35.* NF: NM_133710, XNIF, Ctdspl.
Xlerk	NP_001080948 (329)	NP_034240 (345)	NM_010110	21,856	0.745 (+)	*Xen* and *Mus* is ephrin-B1 (EPH family ligand). *Mus* (aka LERK-2) identities = 242/329 (73%). Similar. *Efnb1 mRNA: zyg, 0; brain, 23; testis, 8. Expressed in oviduct (548). mII, zyg = 0, 0.* NF: NM_010110, Xlerk, ephrin-B1, LERK-2, LERK 2, LERK2, Efnb1.
Germes	NP_001082512 (587)	none	none			No clearly related protein sequences; Germes not found.
Hermes	NP_001081864 (196)	NP_082306 (206)	NM_028030	5,933	0.260 (-)	RRM-type RNA-binding protein hermes. Identities = 164/206 (79%). Similar. *Rbpms2 mRNA: zyg, 72; brain, 14; testis, 16. mII, zyg = 343, 70.* Not found: NM_028030, Hermes, Rbpms2.
C3H-3	NP_001081886 (364)	NP_031590 (338)	NM_007564	10,547	0.432 (-)	Related to *Mus* zinc finger protein 36, C3H type-like 1, identities = 203/378 (53%). Related. *Zfp36l1 mRNA: zyg, 0; brain, 37; testis, 42. mII, zyg = 0, 0.* NF: NM_007564, C3H-3, Zfp36l1.
xARHα	Q801G1 (309)	NP_663529 (308)	NM_145554	7,496	0.319 (+)	Low density lipoprotein receptor adapter protein 1-A (Autosomal recessive hypercholesterolemia protein homolog alpha) (ARH alpha) (xARH) (Xcat4). *Mus* identities = 219/311 (70%). Similar. *Ldlrap1 mRNA: zyg, 0; brain, 14; testis, 0. mII, zyg = 0, 0.* NF: NM_145554, xARH, Xcat4, ARH.

Table S9. Pb_2-zygote differential transcripts that are abundant during oocyte maturation

Sequences are taken from Wang et al. (2004). Values in paired column show position no., not row no. (position no. = row no. -1) from Table S3. LogFC with a positive value (+) predicts Pb_2>zygote. p_{adj} <0.05 shown in red. Where multiple matches exist (eg for Hmgcr) the highest is shown.

Sequence	paired	p_{adj}	logFC
Atp1a1	107	0.005	-
Il1r1	7,324		
Por	24,787		
Evx1	19,703		
Dag1	125	0.006	-
Pparg	31,192		
Gas6	14,104		
Daxx	15,575		
Rnaseh1	4,065		
Hmgcr	3,604		
Tpra1	not found		
Lepr	30,441		
Col4a1	29,259		
Psmb3	24,799		
Klra3	249		+
Gspt2	10,611		
nuclear protein 95	not found		
Nfkbia	333	0.014	-
Plxna2	20,998		
Prep	3,458		
Gorasp2	772	0.034	-
Dtymk	18,046		
Slc12a7	22,843		
Plat	5	0.000	-
Pcbp2	1,347		
Ube2h	766	0.034	-
Efna4	3,195		
Lama2	22,929		
Txnip	14,255		
Lamb1-1	2,676		
Psma2	3,027		
Itpr2	32,320		
Orc5l	20,285		
Aup1	11,487		
Wars	6,001		
Myd88	2,386		
BANP homolog	not found		
Eef1b2	31,632		
Rdh11	4,522		
Mum1l1	24,548		
UBE-1c3	not found		
Rdx	1,488		
Usp2	28,166		
Ppib	13,829		
Magea7	23,287		

Xtrp3s1	5,403		
Mbl2	34,796		
Cd160	802	0.036	-
Celsr1	6,201		
Ext2	3,709		
Slc19a1	30,926		
Mos	35,437		
Hdh	9,291		
Cnih2	9,374		
Pdcd7	24,906		
Psma5	27,453		
Iigp2 (now Irgm2)	1,715		

Table S10. Pb_2-zygote segregation of transcription factor mRNAs that are potentially discriminatory in 16-cell embryos

Sequences are taken from Guo et al. (2010). p_{adj}, adjusted *p* value. Values in paired column show position no., not row no. (position no. = row no. -1) from Table S3. LogFC with a positive value (+) predicts Pb_2>zygote. p_{adj} <0.05 shown in red. The highest table position is given where there are multiple matches (eg Sall4 and Tcf23).

Sequence	position	p_{adj}	logFC
Actb	5,289	0.235	+
Ahcy	16,428	0.611	+
Aqp3	24,524	0.807	+
Atp12a	3,304	0.151	+
Bmp4	5,955	0.260	+
Cdx2	9,221	0.385	-
Cebpa	16,045	0.600	+
Creb3l2	26,489	0.845	-
Dab2 (D630005B22Rik)	5,452	0.241	-
Dppa1	30,902	0.924	-
Eomes	30,198	0.910	+
Esrrb	28,726	0.885	+
Fgf4	10,131	0.419	+
Fgfr2	24,348	0.803	-
Fn1	23,733	0.793	-
Gapdh	6,954	0.299	+
Gata3	30,913	0.924	-
Gata4	30,574	0.917	-
Gata6	7,067	0.305	+
Grhl1	25,906	0.834	-
Grhl2	14,113	0.543	-
Hand1	13,632	0.529	-
Hnf4a	5,762	0.254	+
Id2	10,855	0.443	+
Klf2	4,205	0.187	-
Klf4	31,963	0.941	-
Klf5	25,354	0.825	-
Krt8	16,011	0.600	+
Lcp1	6,801	0.294	+
Mbnl3	24,077	0.799	-
Msc	15,674	0.590	+
Msx2	20,592	0.715	+
Nanog	14,416	0.552	-
Pdgfa	8,669	0.363	+
Pdgfra	29,333	0.898	-
Pecam1	11,275	0.455	-
Pou5f1 (NM_013633)	31,382	0.931	-
Runx1	9,585	0.396	-
Sall4	71	0.004	-
Snai1	30,736	0.920	+
Sox13	34,711	0.985	+

Sox17	24,191	0.800	+
Sox2 (NM_011443)	19,297	0.680	-
Tcf23	10,830	0.442	+
Tcfap2a	3,939	0.177	+
Tcfap2c	1,356	0.062	+
Tspan8	25,470	0.827	+
Utf1	15,515	0.587	-

Table S11. PCR primers used in this work

Target	Code	FORWARD	REVERSE	Product size (bp)	Tm (°C)
Actb	NM_007393	TGACAGGATGCAGAAGGAGA	GCTGGAAGGTGGACAGTGAG	131	60
H2az	NM_016750	GCGTATCACCCCTCGTCACTTG	TCTTCTGTTGTCCTTTCTTCCCG	146	58 or 60
ppia (cyclophilin A)	NM_008907	AGGGTGGTGACTTTACACGC	AAAACTGGGAACCGTTTGTG	150	58
H3f3a	NM_008210	CCATGCCAAACGTGTAACAA	TACCTTTGACCCCATGGAAA	182	58
Gapdh	NM_008084	TGCGACTTCAACAGCAACTC	ATGTAGGCCATGAGGTCCAC	143	58
Nanog	MGI:1919200	GCAAGCGGTGGCAGAAAAAC	GCAATGGATGCTGGGATACTCC	122	58
Ptov1	NM_133949	GGATCATGGACAATGGCTTC	TGACAAAGTTGCTCTGGTCG	141	58
2610110g12Rik	NM_028476	CCCTGTACTCCCAACCTGTG	GTGGTCTGGAGTCTGACCGT	133	58
Prr7	NM_001030296	GCCTCCGAGTTACAAGCCTC	CTGCTGAGCTCTGAGTCGGT	159	58
Klk13	NM_001039042	CACGTCCGTACCCTGAAACT	ATGTTGGCAGTGATCTTCCC	188	58
Daf2	NM_007827	AGGACAATGGAGCAGTCCAC	TGAGGAGTTGGTTGGTCTCC	156	58
Rshl2a	NM_025789	AGAGTCTCCTCACAGCCAGC	TTGTAAAGCCTGGATGGGTC	151	58
Mtpn	NM_008098	GCAATTGCAGTGTTTTGCAT	AAACCCAACTGTGTCAAGGG	174	58
Ubl3	NM_011908	AGTGGCTGTCCTCACAGGAT	ACCAGTTCTGCACAGTGACG	178	58
Mrpl13	NM_026759	TGCGGTTATAGCATCCAACA	GCCTGGGTAGCCAGTATGTG	163	58
Mrpl55	NM_026035	CTATGCTCGCCTCTACCCTG	TTCTTCCCTCTTCTGCTGGA	178	58
Cd52	NM_013706	AAAGCTGCTACAGAGCCCAG	GGTGGAGGTGCTGTTTTTGT	167	58
Dach1	NM_007826	ACAGAGGGTGAATTGTGGCT	TTAACTTCATCACCTGGGGC	153	58
Actb	NM_007393	TGACAGGATGCAGAAGGAGA	GCTGGAAGGTGGACAGTGAG	153	58
Twf1	NM_008971	CATGTTCGAAAGCCAAGGAT	CCCAAGCAACAGGACAATTT	165	58
Sat1	NM_009121	GACCCCTGAAGGACATAGCA	CATGGCAACCTGGCTTAGAT	163	58
Ccdc72	NM_183250	CGAAGAGCTAAAAGCCAAGG	AGGTGGCAAAGATGCTATGG	165	58
Rsu1	NM_009105	GCAGATACTCAGCCTCAGGG	GGTAACCCAGGGGTTGTTCT	184	58
H2afx	NM_010436	GGGCTTGAAGGTTAGTCCCT	GCGACAGGGTCAACTGGTAT	180	58
Tapbp	NM_009318	CCGCTGGACTCGAACTAAAC	GTAACCCCGACTGACCTGAA	157	58
BC088983	NM_001009951	GCGGAAGCTAGAACAAGTGC	CCATTAGGAGTTCGGCTACG	149	58
Ddx59	NM_026500	CCCTGGACGACTTCTGGATA	ATGCTATCTGGAATGGTGGC	186	58
Rbm22	NM_025776	AGGGGCTCTGAGGAAGAAAG	GAAGACATGTGGGGAAGCAT	147	58
Ripk2	NM_138952	CTGCAGCCTTACCCAGAAGT	TGACTTCCTCCCAAAGAAGG	100	58
Becn1	NM_019584	CGGCTCCTATTCCATCAAAA	CCTTAAAGGCAAACATCCCC	155	58
Atp1a1	NM_144900	TGGAGTTCACCTGCCATACA	AGCAAGGGCTGTCTCTTCAA	155	58
Tnks	NM_175091	GGCCCTTACCTTGAGGAGTC	CAGCTCGTTCATGTGCATTT	154	58
Cenpe	NM_173762	GTGCATCTCCATCCTACCGT	TTCACCAGCTGCATTTTGAG	155	58
Ica1	NM_010492	CATGACACATGCTCAGGACC	AACACTGCAGCCCACTCTCT	188	58
Sf3b1	NM_031179	TCAGGAGTGCTAGGCAGCTT	AAAGAACAAGAATCCGTGCG	154	58
Cdc20	NM_023223	ATGGAGCAGCCTGGAGACTA	TGTTCCAACTGAGGGAGCTT	169	58
H3f3a	NM_008210	CCATGCCAAACGTGTAACAA	TACCTTTGACCCCATGGAAA	182	58
16S ribosomal	Jeong et.al.2005; 71	AGATGATCGAGCCGCGC	GCTACCAGGGCCTTTGAGATGG/	163	62
Leptin	NM_008493	GGATCAGGTTTTGTGGTGCT	CTTCCTGTCACTCTTTCCCG	161	58
Stat3	NM_213659	CAAGCCTTTCCTGACAGAGG	ACTGTCTCGAAGCAGAAGGC	182	58
Leptin receptor	NM_146146	GGTCTCAGAGCACCCAGGTA	GCCTCAGTGGGGAATGATTA	168	58
D-loop	Dai/Wei et.al	ATAAACATTACTCTGGTCTTGTAA	ATTAATAAGGCCAGGACCAAACC	1100	58

D-Loop 5' fragment	Dai et.al.2005; 208	CCACCACCAGCACCCAAAG	CGGGTTGTTGGTTTCACGG	342	58
mtDNA	Gois/Bogue et.al	GGATCCTACTCTCTACAAACAC	CTATTGTCCTAGAAATAAGAGGG	500	58&60
mt-Co1	Larsson/Clayton et.al	CCCAATTCTCTACCAGCATC	GGCTCATAGTATAGCTGGAG	250	58&60
XP VegT	NM_001088196	GGCAAAATTTGGTGTTCCAT	TGCAGAGTTAGGCAGCCTTT	110	58
XP Vg1	NM_001095591	AGGGAGGTAGATGCCATTGA	CACAGACAAATGCAAGACCG	120	58
XP Xcat-3	NM_001088548	TATGTATGCCATCCCCCAGT	TGCAAGATGTCCCTGCAATA	136	58
XP Xdazl	NM_001088303	TGGCATTGTGTAGCTTGAGG	ACAAAGTGAACGGGGCTATG	110	58
XP Fatvg	NM_001088491	CCATTCCCTGGTGATGAGTG	TCTTTGAGGGAAGAGGCAGA	120	58
XP XNIF	NM_001090817	TGTGAAAAAGCTGCGAATTG	TGATGGATTTCTGTTTGGCA	119	58
XP Xlerk	NM_001087479	CCTGTCCCTAAGCACACTGG	TAGACAGGGTGGCCGTAGTC	147	58
XP xARH	NM_001089091	TGCAAATTGTCCACCACATT	GGCCTGGGTGTATTGAGCTA	135	58
XP An3	NM_001101775	GGTGCAGATGCACTTGAAGA	TTTTTCCTGATCGGAATTGG	118	58
XP xlan4	NM_001087591	GTTATTTCCAGATCTGCGGC	CTTTGGTGGCTGATTCAGGT	145	58
XP Tcf-1	NM_001089222	GACTTGGGAAAACGTTGCAT	GCAGGGGTTGCAAACAGTAT	123	58
XP xlPou60	NM_001088114	GGAGTTAATCAGCTGCCTGC	GCGCATCTACAGCATGAGAA	149	58
XP Gapdh	NM_001087098	CAGAGGTGCAGGTCAGAACA	AGATCCACGACGGACACATT	138	58
XP eef1a-o1	NM_001086133	TGAGCCTCCTTTTAGCCAGA	CATGTTGTCTCCATGCCATC	130	58