Supplementary Information

Resurrection of chromosomes from frozen animals by single chromosome transfer into mouse oocytes

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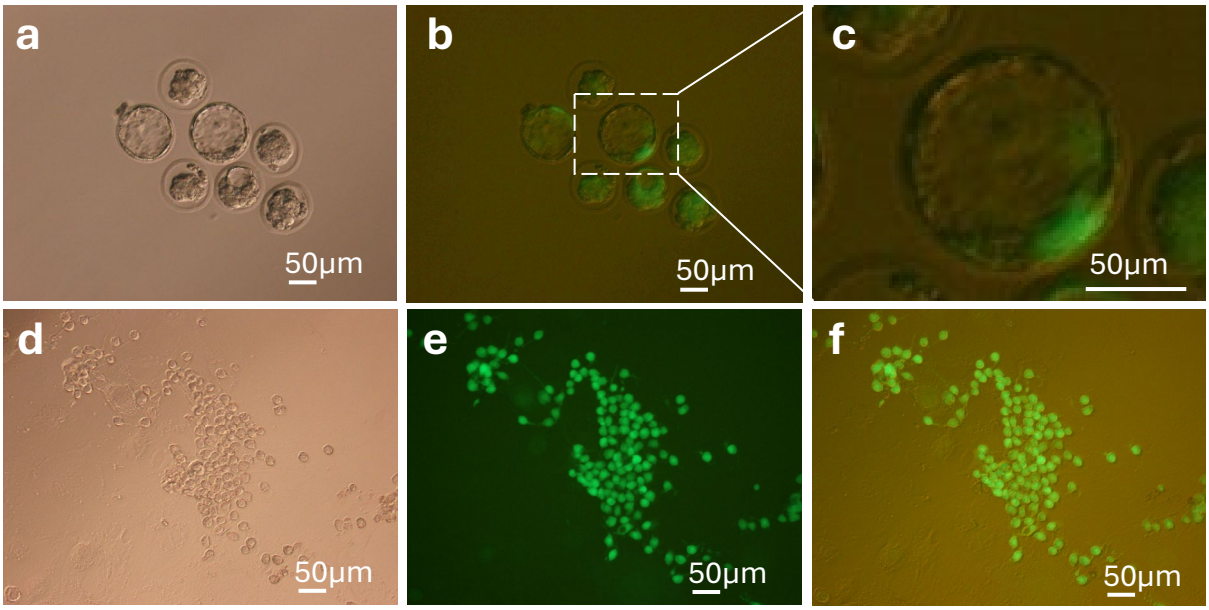


Figure S1. Development of hybrid embryos to the blastocyst stage and establishment of cell lines following the transfer of GFP-Tg rat somatic nuclei into mouse oocytes

(**a**) Blastocysts developed from artificially activated embryos generated by injecting GFP-Tg rat somatic nuclei into intact mouse oocytes in the presence of cytochalasin B.

(**b**) Fluorescence microscopy images of the same blastocysts, all of which expressed GFP.

(**c**) Magnified view of the same image.

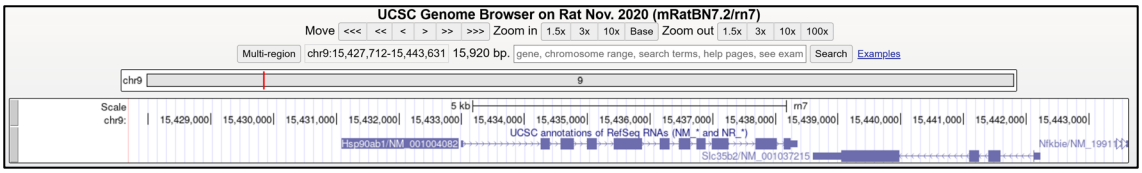
(**d**) Cells that proliferated after transferring the blastocysts into the embryonic stem cell derivation medium.

(**e**) Fluorescence microscopy images of the same cells, all of which expressed GFP.

(**f**) Merged bright-field and fluorescence microscopy images.

a

Rattus norvegicus heat shock protein 90 alpha family class B member 1 (Hsp90ab1), mRNA.



Predicted length of the PCR product: 333 base (CG-AT)

MspI

ccggcgagctcccttgacctctttgagaacaagaagaagaagaacaacatcaaattgtatgtccgtcgtgtgttcatcatggaca

competitive primer 1

gctgtgatgagctgatacctgagtacctcaacttcatccgtggtgtggttgattccgaggacctgcctctgaacatctcccgagaa

competitive primer 2

atgctccagcagagcaagatcctgaaagtcatccgcaaaaacattgtgaagaagtgccttgagctcttctctgagttggctgaaga

caaagagaactacaagaaattttatgaggcattctctaagaattaa

MseI

b

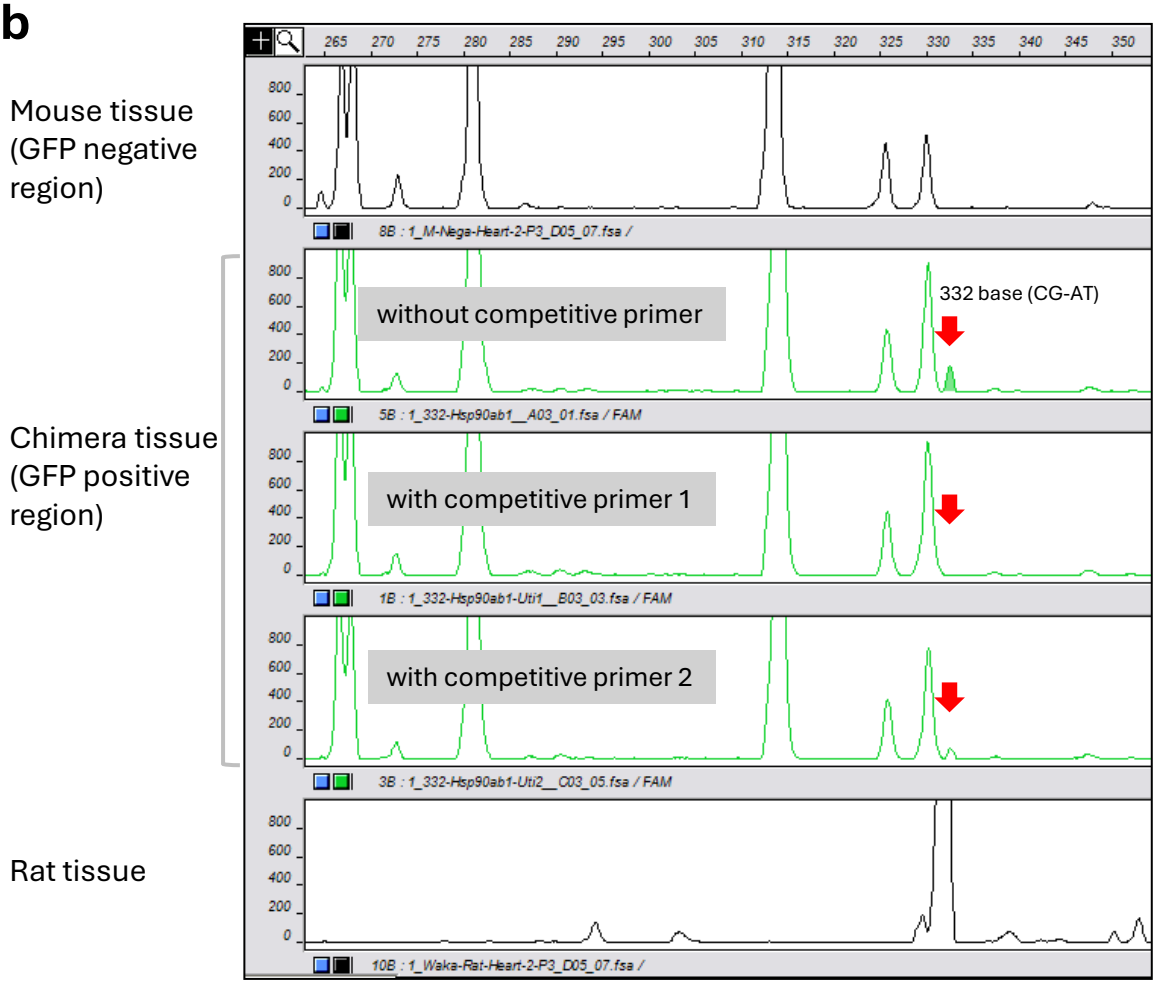


Figure S2. Confirmation of the detection of the rat transcript

(**a**) NM_00100408/Hsp90ab1 rat transcript. The sequence of the *Msp*I-*Mse*I fragment (332 bases) was predicted from the Rat RefSeq database, and the competitive primers that we designed were indicated.

(**b**) Competitive inhibition assay. The HiCEP products from the green fluorescent protein-positive regions in the heart (PCR ID: CG-AT) were diluted and used as the template for the competitive polymerase chain reaction (PCR) assay. Competitive PCR was conducted using competitive primers 1 or 2, in addition to the fluorescent HiCEP primer for CG-AT.

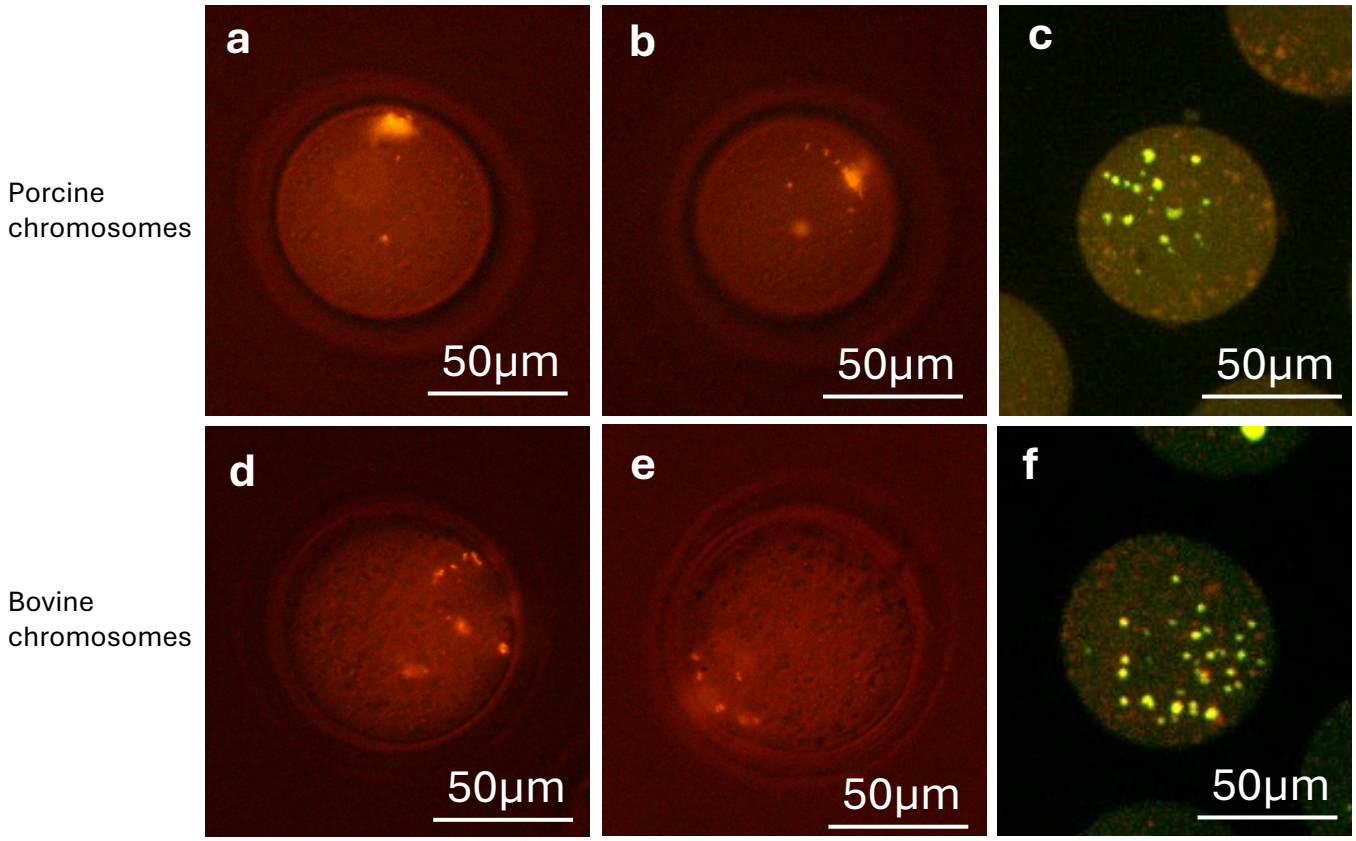


Figure S3. Dispersion of chromosomes derived from porcine and bovine somatic cells within mouse oocytes.

(a–c). Mouse oocytes were enucleated and injected with a somatic cell nucleus isolated from porcine skeletal muscle. Each panel represents a different oocyte.

(d–f). Mouse oocytes were enucleated and injected with a somatic cell nucleus isolated from bovine skeletal muscle. Each panel represents a different oocyte. Images a, b, d, and e were captured via fluorescence microscopy, whereas c and f were captured via confocal microscopy.

Table S1: Rat somatic cell nuclear transfer into enucleated or intact mouse oocytes

Condition of oocyte	No. of used oocytes	No. of enucleated oocytes	No. of survived after nuclear injection	No. of survived after Sr treatment	No. of pseud-pronuclei formation (%)	No. 2-cell at 24 h (%)*	No. Moru/ Blast at 72 h (%)*	No. established cell lines (%)*	No. GFP positive cell lines
Enucleated	244	244	195	181	106 (58.6)	99 (93.4)	0	-	-
Intact	442	-	351	296	218 (73.6)	211 (96.8)	67 (30.7)	9 (4.1)**	0
								2 (0.9)**	2

Moru, Morula: Blast, Blastocyst

*, Calculate against the number of pseud-pronuclei formation.

** Nine cell lines were morphologically identified as ES cells, but none of them expressed GFP. Two cell lines expressed GFP but displayed a morphology distinct from ES cells and ceased proliferation after several passages.

Table S2: Development of a method to disassemble the MII spindle of mouse oocytes

Type of inhibitor		No. examined oocytes	Separated	Partially separated	Aggregated	Activated
Nocodazole	Demecolcine					
+	-	59	37 (62.7) [a]	7 (11.9) [c]	3 (5.1) [e]	12 (20.3) [g]
-	+	40	0 [b]	18 (45.0) [d]	21 (52.5) [f]	1 (2.5) [h]
+	+	30	11 (36.7) [a]	11 (36.7) [d]	6 (20.0)	2 (6.7)

Reconstructed oocytes were observed at 5 h after inhibitor treatment.
a vs. b, c vs. d, e vs. f, g vs. h: $p<0.01$

Table S3. Development of a method to disassemble chromosomes of the mouse cumulus cell nucleus within enucleated mouse oocytes

Type of inhibitor		No. examined oocytes	Separated	Partially separated	Aggregated	Activated	No nucleus
Noco.	Deme.						
+	-	99	26 (26.3) [a]	9 (9.1)	30 (30.3)	13 (13.1)	21 (21.2) [e]
-	+	120	26 (21.7) [a]	24 (20.0) [c]	33 (27.5)	18 (15.0)	6 (5.0) [f]
+	+	57	27 (47.7) [b]	1 (1.8) [d]	18 (31.6)	5 (8.8)	6 (10.5)

Reconstructed oocytes were observed at 2-3 h after inhibitor treatment.
Noco, Nocodazole; Deme, Democoltine
a vs. b, c vs. d, e vs. f, g vs. h: $p<0.01$

Table S4. Development of oocytes injected with a single chromosome derived from mouse cumulus cells and establishment of ES cells

No. of survived oocytes after single chromosome injection	No. of survived oocytes after ICSI	No. of embryos developed to (%)				No. plated embryos	No. established cell lines
		Frag/1-cell	2-cell	4-8-cell	Mor/Bla		
15	15	1	1	7	6 (40)	6	2

Frag, Fragment: Moru, Morula: Blast, Blastocyst

Table S5: Generation of chimera mice using rat chromosome-containing rct-mES cell lines

Line no.	No. used embryos	No. offspring	No. Chimera		
			Total	High coat color	Low coat color
SCT-R4	80	46	8	2	6
SCT-R5	60	35	6	0	6

Table S6: Chimerism of mice derived from the SCT-R4 and R5 cell lines

		Animal ID		
		#R4-1	#R4-2	#R5-1
Origin	Organ	Male	Female	Female
Ectoderm	Brain	++	+	+
Endoderm	Lung	+	-	-
	Liver	-	-	-
	Pancreas	-	-	-
	Intestine	+	+	-
Mesoderm	Thymus	-	-	++
	Heart	+	+	+
	Spleen	-	-	-
	Kidney	-	-	-
	Uterus		-	-
	Testis	++		
	Muscle	+	++	+
	Born	-	++	-

Table S7. Chromosome dispersion of somatic cell nuclei from frozen porcine and bovine tissues

Animal species	No. examined oocytes	Separated	Partially separated	Aggregated	No nucleus
Porcine	40	6 (15.0)	11 (27.5)	16 (40.0)	6 (15.0)
Bovine	37	3 (8.1)	16 (43.2)	13 (35.1)	3 (8.1)

There is no significant difference