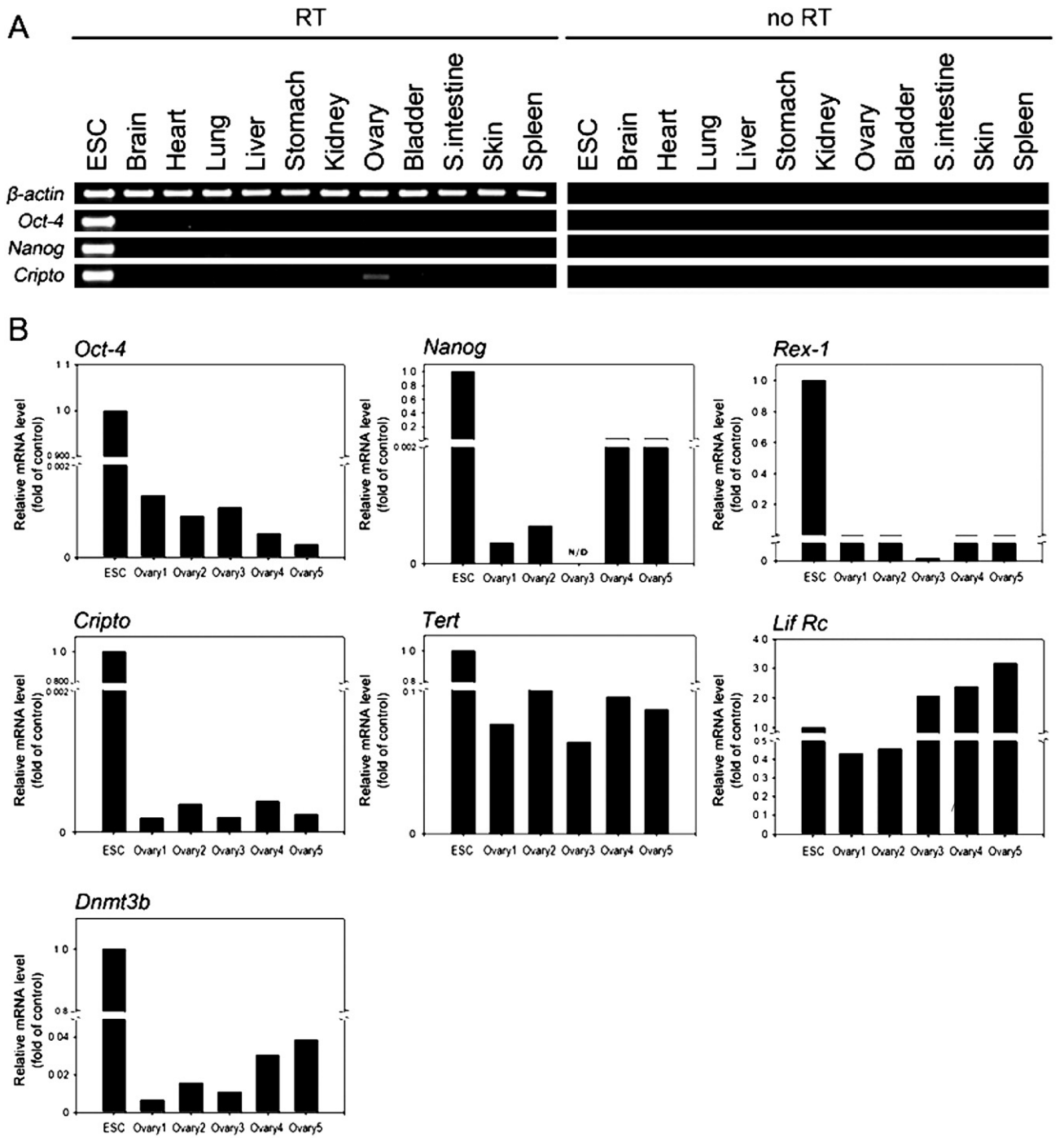
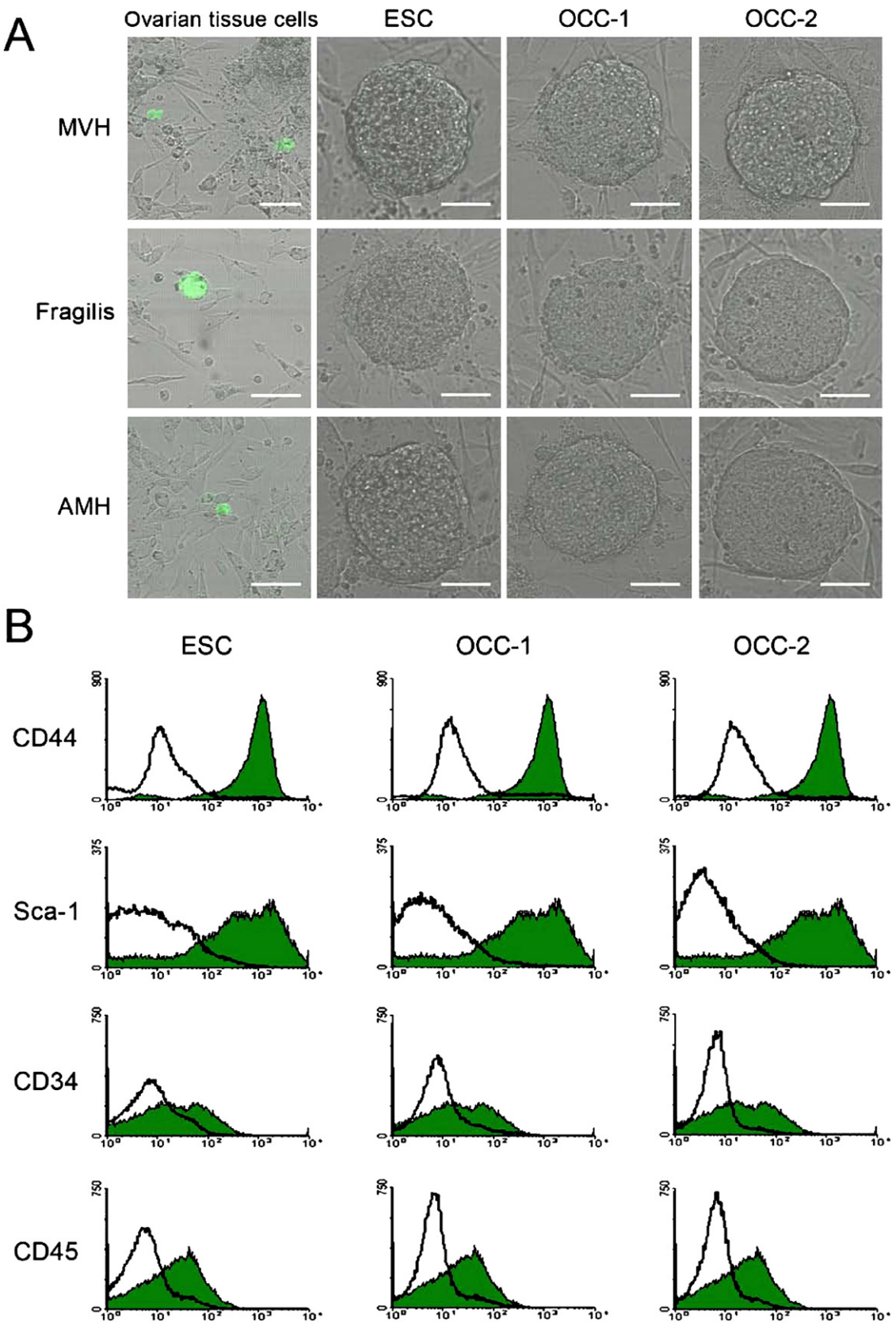
SUPPLEMENTARY FIGURE 1

Expression of genes related to pluripotency in various tissues of 8-week-old adult mice. (**A**) Expression of the pluripotency-related genes in various tissues of adult mice, detected by the 30-cycle RT-PCR. Weak expression of *Oct-4*, *Nanog*, and *Cripto* was detected in the ovaries. (**B**) Quantification of pluripotency-specific gene expression in mouse ovaries retrieved from different animals. The expression levels of *Oct-4*, *Nanog*, *Rex-1*, *Cripto*, *Tert*, *Lif Rc*, and *Dnmt3b* in the ovaries retrieved from different mice were measured by real-time PCR. Embryonic stem cells were used as the positive control cells. N/D, not detected.



Gong. Stem cells from ovarian tissue. Fertil Steril 2010.

SUPPLEMENTARY FIGURE 2



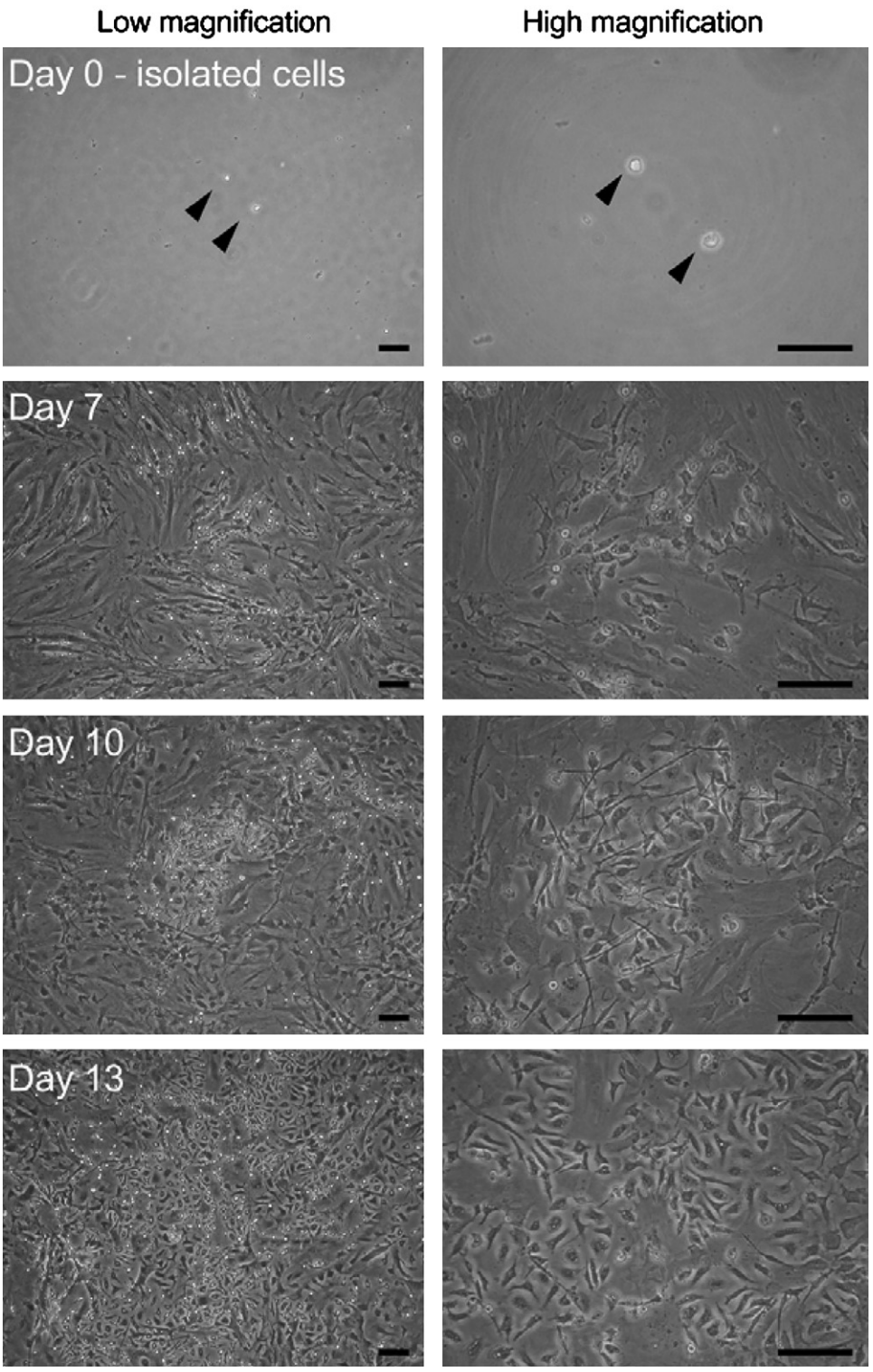
Gong. Stem cells from ovarian tissue. Fertil Steril 2010.

SUPPLEMENTARY FIGURE 2 CONTINUED

Expression of the markers on germline, follicular, and adult stem cells. (**A**) Immunostaining of ovary-derived colony-forming cells (OCC) with antibodies against the germline markers mouse Vasa homolog (MVH) and Fragilis, and the follicular cell-specific marker antimüllerian hormone (AMH). Both lines of OCC were analyzed along with crude-dispersed ovarian cells before seeding, and embryonic stem cells (ESC) were used as control cells. Cells staining positive for MVH, Fragilis, or AMH were present in freshly dispersed adult ovarian cells; however, OCC retrieved at the 20th subpassage and ESC were not positive for those markers. Scale bar = 50 μm. (**B**) Characterization of OCC by fluorescence-activated cell sorting (FACS) using mesenchymal cell-specific markers CD44 and Sca-1, and the hematopoietic stem cell-specific marker CD34 and CD45. The green color represents peaks of mesenchymal stem cells used as the control. Both ESC and OCC were negative for reactivity with anti-CD44, anti-Sca-1, anti-CD34, and anti-CD45 antibodies.

SUPPLEMENTARY FIGURE 3

Culture of whole blood-derived mononuclear cells. Whole blood was retrieved from adult female mice by heart puncture, and only mononuclear cells were purified by density gradient. Isolated mononuclear cells (*arrowheads*) were cultured on an ICR feeder layer for 13 days. They proliferated as the monolayer starting on day 7, and no colony-forming cells were detected by the end of culture. Scale bar = 100 μm.



Gong. Stem cells from ovarian tissue. Fertil Steril 2010.

SUPPLEMENTARY TABLE 1

Outcome of ovarian stromal cell coculture with feeder.

Repl	Ovarian cell[a]	Feeder cell[b]	Culture outcome	Alternative supplements[c]
1	B6D2F1	ICR	Colony-like cell establishment (OCC-1)	None
2	B6D2F1	ICR	Layer formation	None
3	B6D2F1	ICR	Layer formation	None
4	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	None
5	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	None
6	B6D2F1	ICR	Cell aggregate at P0	None
7	B6D2F1	ICR	Cell aggregate at P0	None
8	B6D2F1	ICR	Layer formation	None
9	B6D2F1	ICR	Layer formation	None
10	B6D2F1	ICR	Cell aggregate at P0	None
11	B6D2F1	ICR	Layer formation	None
12	B6D2F1	ICR	Cell aggregate at P0	None
13	B6D2F1	ICR	Cell aggregate at P0	None
14	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	None
15	B6D2F1	ICR	Cell aggregate at P0	None
16	B6D2F1	ICR	Layer formation	None
17	B6D2F1	ICR	Layer formation	None
18	B6D2F1	ICR	Layer formation	None
19	B6D2F1	ICR	Cell aggregate at P0	None
20	B6D2F1	ICR	Cell aggregate at P0	LIF (1,000)
	B6D2F1	ICR	Cell aggregate at P0	LIF (5,000)
21	B6D2F1	ICR	Layer formation	LIF (1,000)
	B6D2F1	ICR	Layer formation	LIF (5,000)
22	B6D2F1	ICR	Layer formation	LIF (2,000)
	B6D2F1	ICR	Layer formation	LIF (3,000)
23	B6CBAF1	ICR	Cell aggregate at P0	LIF (2,000)
	B6CBAF1	ICR	Cell aggregate at P0	LIF (3,000)
24	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	LIF (3,000)+FSH (100)
25	B6CBAF1	ICR	Cell aggregate at P0	LIF (3,000)+FSH (100)
26	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	None
	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	FSH (100)
	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	LH (10)
	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	FSH (100)+LH (10)
27	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	cell strainer-/preculture-
28	B6D2F1	ICR	Cell aggregate at P0 then disappear at P1	preculture-
29	B6D2F1	ICR	Cell aggregate at P0	None
	B6D2F1	ICR	Cell aggregate at P0	FSH (100)
	B6D2F1	ICR	Cell aggregate at P0	LH (10)
	B6D2F1	ICR	Cell aggregate at P0	FSH (100)+LH (10)
30	B6D2F1	ICR	Cell aggregate at P0	cell strainer-/preculture-
31	B6D2F1	ICR	Layer formation	None
32	B6D2F1	ICR	Cell aggregate at P0	None
33	B6D2F1	ICR	Layer formation	None
34	B6D2F1	ICR	Layer formation	None
35	B6D2F1	ICR	Cell aggregate at P0	None
36	B6D2F1	ICR	Colony-like cell dump until day 12 then disappear at P1	None
37	B6D2F1	ICR	Cell aggregate at P0	None
38	B6D2F1	ICR	Colony-like cell establishment (OCC-2)	None
39	B6D2F1	ICR	Cell aggregate at P0	preculture-
40	B6D2F1	ICR	Layer formation	None
41	C57BL/6	ICR	Layer formation	15 month-old mouse
42	B6CBAF1	ICR	Cell aggregate at P0	4 week-old mouse
43	B6CBAF1	ICR	Colony-like cell dump until day 18 then disappear at P1	2 week-old/preculture-
44	B6D2F1	STO	Cell aggregate at P0	LIF (1,000)
45	Balb/c	STO	Layer formation	LIF (1,000)
46	B6CBAF1	STO	Layer formation	LIF (1,000)
47	B6CBAF1	ICR	No vital cell	A23187 (10 μM)
48	B6D2F1	ICR	Cell aggregate at P0	A23187 (1 μM)

All procedures were conducted according to standard protocol (green background) unless otherwise stated.
The basic medium was DMEM supplemented with 0.1 mM β-mercaptoethanol, 1% nonessential amino acids, 2 mM L-glutamine, 15% fetal bovine serum, and 5,000 units/ml LIF unless otherwise stated.
[a]The ovary dissociated cells were prepared from the ovaries retrieved from 8 to 12 week-old mice unless otherwise stated.
[b]All somatic cells used as feeder cell layer were treated with mitomycin C.
[c]FSH=mIU/ml; LIF=units/ml; A23187= μM; strainer-=Without passing through cell strainer in cell isolation; preculture-=no preculture was undertaken for preparing buoyant cell fraction

Notes: All procedures were conducted according to standard protocol (shaded background) unless otherwise stated. The basic medium was DMEM supplemented with 0.1 mM β-mercaptoethanol, 1% nonessential amino acids, 2 mM L-glutamine, 15% fetal bovine serum, and 5000 units/mL LIF unless otherwise stated.

[a] The ovary dissociated cells were prepared from the ovaries retrieved from 8- to 12-week-old mice unless otherwise stated.

[b] All somatic cells used as feeder cell layer were treated with mitomycin C.

[c] Follicle-stimulating hormone (FSH) = mIU/mL; leukemia inhibitory factor (LIF) = units/mL; A23187 = μM; strainer = without passing through cell strainer in cell isolation; preculture = no preculture was undertaken for preparing buoyant cell fraction.

Gong. Stem cells from ovarian tissue. Fertil Steril 2010.

SUPPLEMENTARY TABLE 2

Short-tandem repeat microsatellite analysis of established ovary-derived colony-forming cells (OCC) derived from coculturing ovarian cells and embryonic fibroblast feeder cells, and the strains of the ovary (B6D2F1) and feeder cell (ICR) donor.

	Samples			
Markers	**Ovary donor**	**Feeder cell**	**OCC-1**	**OCC-2**
D3Mit200[a]				
Size 1	101.09	124.24	100.96	100.95
Size 2	124.22	126.21	124.11	124.19
D11Mit4[a]				
Size 1	248.92	242.8	248.97	249.07
Size 2	285.39	248.98	285.44	285.53
D19Mit33[a]				
Size 1	251.6	223.0	251.6	251.5
Size 2	253.7	—	253.5	253.5
D4Mit251[a]				
Size 1	107.3	120.7	107.2	107.2
Size 2	120.7	—	120.7	120.6

[a] Microsatellite markers used were selected from MIT database for discerning mouse strains employed as ovary donor (B6D2F1) and feeder cells (ICR).

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SUPPLEMENTARY TABLE 3

Oligonucleotide primers and PCR cycling conditions.

Genes	GenBank number	Primer sequence		Size (bp)
		Sense (5' > 3')	Antisense (5' > 3')	
β-actin (RT)	X03672	ACCGTGAAAAGATGACCCAG	TCTCAGCTGTGGTGGTGAAG	254
β-actin (R-T)	X03672	TACCACAGGCATTGTGATGG	TCTTTGATGTCACGCACGATT	200
Oct-4 (RT, R-T)	M34381	GAAGCCCTCCCTACAGCAGA	CAGAGCAGTGACGGGAACAG	297
Nanog (RT, R-T)	AY455282	CCCCACAAGCCTTGGAATTA	CTCAAATCCCAGCAACCACA	255
Rex-1 (RT)	M28382	ACATCCTAACCCACGCAAAG	TGATTTTCTGCCGTATGCAA	294
Rex-1 (R-T)	M28382	TCCCCGTGTAACATACACCA	CTTCGTCCCCTTTGTCATGT	247
Cripto (RT)	M87321	CTTTAAGCAGGGAGGTGGTG	TAAAGCCATCTGCCACAATG	195
Cripto (R-T)	M87321	CGGAGATCTTGGCTGCTAAC	CTTCGACGGCTCGTAAAAAC	200
Dnmt3b (RT)	BC105922	AGTCCATCGCTGTGGGAACT	GGGCGGGTATAATTCAGCAA	226
Dnmt3b (R-T)	BC105922	GTCCGGAAAATCACCAAGAA	CCAGAAGAATGGACGGTTGT	201
Tert (RT)	AF051911	GGATCCTGGCTACGTTCCTG	TGCCTGACCTCCTCTTGTGA	208
Tert (R-T)	AF051911	GCAGTGGTCCGGAGAGATAG	ACACTGTGACGCAGGAAGTG	224
Lif Rc (RT, R-T)	BC031929	GCTGAGTGGTAAAGATACCG	TTCGTTGGACTCATACAACA	261
Stat3 (RT)	AY299489	TTTGGAATGAAGGGTACATC	CAAATGACATGTTGTTCAGC	228
Bmp4 (RT)	BC013459	TGAGAGACCCCAGCCTAAGA	AAACTTGCTGGAAAGGCTCA	259
Fgf4 (RT)	BC104312	CAGTCTTCTGGAGCTCTCTC	AGGAAGTGGGTTACCTTCAT	282
Foxd3 (RT)	AF067421	CAAGAACAGCCTGGTGAAG	GTCCAGGGTCCAGTAGTTG	262
Sox2 (RT)	AB108673	ACGCTCATGAAGAAGGATAA	GTAGGACATGCTGTAGGTGG	345
CD9 (RT)	U60473	ATGCTACCACTGTTTCCAAC	ACAAGTTAAACTGGCAGCAT	212
Gdf3 (RT)	BC101963	CGAGTTTCAAGACTCTGACC	TAGAGGACCTTCTGGAGACA	276
Zfy1 (gDNA)	AC163622	GTTACTCATTTTCAGGTGTTCTGGG	GTGTCAGCTGTTATAGGATCAGTGA	572
Xist (gDNA)	AJ421479.1	GAGATACATTTATTTGCTCA	GACTTAGTTTGGTTTCTTTA	540
Nanog (gDNA)	NC000072	GATTTTGTAGGTGGGATTAATT GTGAATTT	ACCAAAAAAACCCACACTCATATCAATATA	358
Oct-4 (gDNA)	NC000083	GGTTTTTTAGAGGATGGTTGAGTGG	CCATCACCCCCACCTAATAAAAATAA	458
H19 (gDNA)	AF049091	GGTGGTAAGATGTGTGTATTTTTGG	CTAACTAACTTAAAAAATCCCAAGACAAA AAAAAAC	508
Gtl2 (gDNA)	NT_166318	ATATTATGTTAGTGTTAGGAAGGATTGT	GATGGTTAAAATATTTTTATAGATTGGGAATG	465
Snrpn (gDNA)	AF081460	AATTTGTGTGATGTTTGTAATTATTTGG	ATAAAATACACTTTCACTACTAAAATCC	420
Peg3 (gDNA)	AF105262	TTTTGTAGAGGATTTTGATAAGGAGG	CCCCAAACACCATCTAAACTCTAC	288

Notes: gDNA, genomic DNA PCR; RT, reverse-transcriptase PCR; R-T, real-time PCR.

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