

Cell Stem Cell, Volume 1

Supplemental Data

***Oct4* Expression Is Not Required**

for Mouse Somatic Stem Cell Self-Renewal

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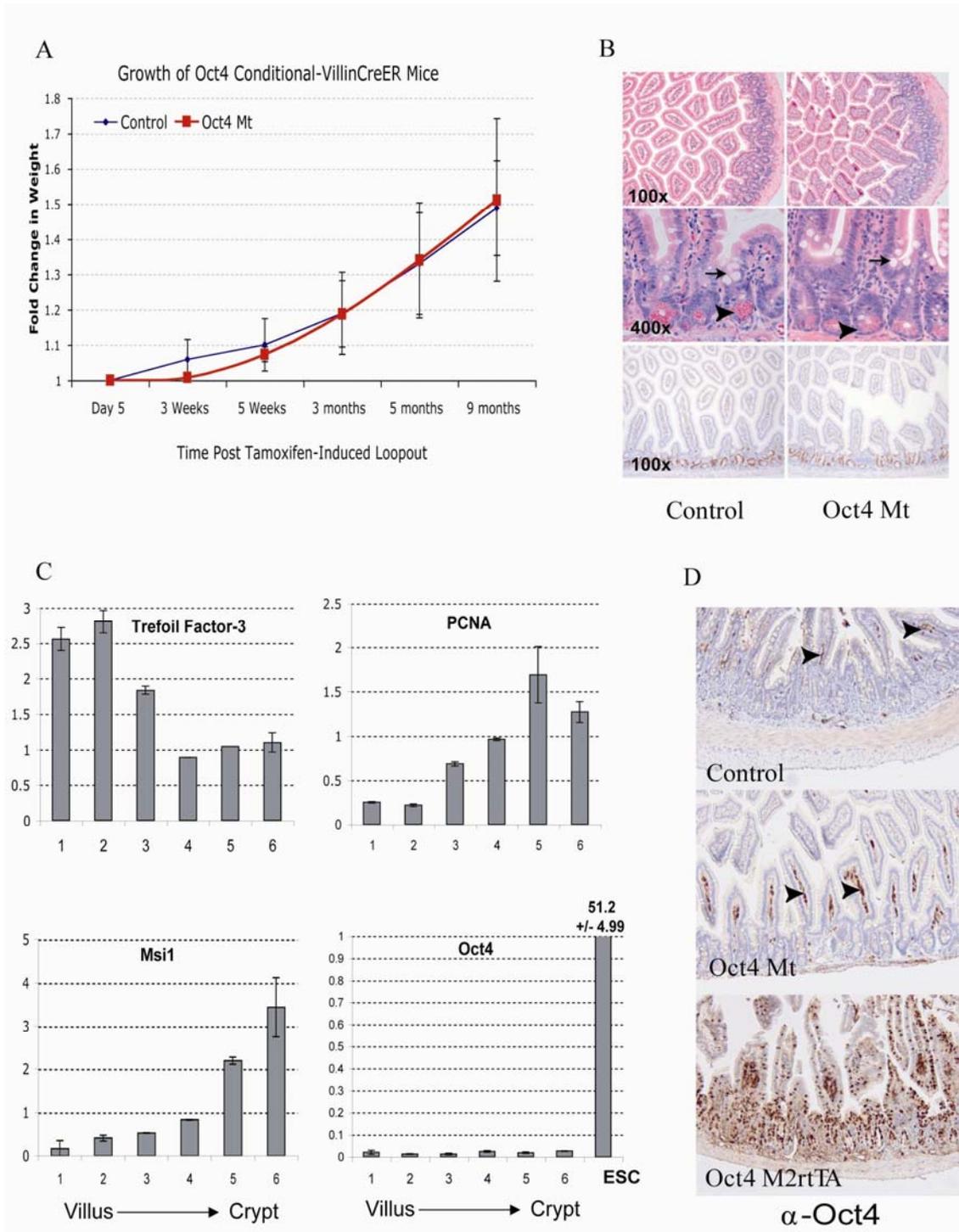


Figure S1. Long-Term Effects of *Oct4* Loss in the Intestinal Epithelium

(A) Growth of *Villin-CreER* mice was analyzed for a period of 9 months after inactivation of the *Oct4* conditional allele in 8-week old mice. Weight on the final day of Tamoxifen treatment (day5) was set equal to 1. Data are mean \pm SD, n=5.

(B) Histological analysis of intestinal epithelium with H&E staining 9 months after inactivation of

the *Oct4* conditional allele. Top panels show normal intestinal architecture. Center panels reveal goblet cells (arrows) and Paneth cells (arrowheads) in the presence or absence of a functional *Oct4* gene. Ki67 staining (lower panels) shows a normal distribution of proliferating cells near the base of the villi.

(C) qRT-PCR analysis after fractionation of the intestinal crypt villus structure with early fractions (1-3) corresponding to the tip of the villi (as evidenced by *Trefoil Factor-3* expression) and later fractions (4-6) corresponding to the transit amplifying compartment near the base of the crypt (marked by *PCNA* expression), with intestinal stem cells most represented in fraction 6 (marked by expression of the putative stem cell marker *Msi1*). *Oct4* expression is negligible in all of these fractions when compared to ESCs. All data are mean \pm SD, relative to GAPDH, n=3.

(D) *Oct4* immunostaining in the control and mutant intestine reveals no *Oct4*⁺ epithelial nuclei in comparison to *Oct4* *M2rtTA* intestinal epithelium in which *Oct4* expression is ectopically induced through doxycycline administration. Non-specific staining is seen in the mesenchyme of control and mutant tissue (arrowheads).

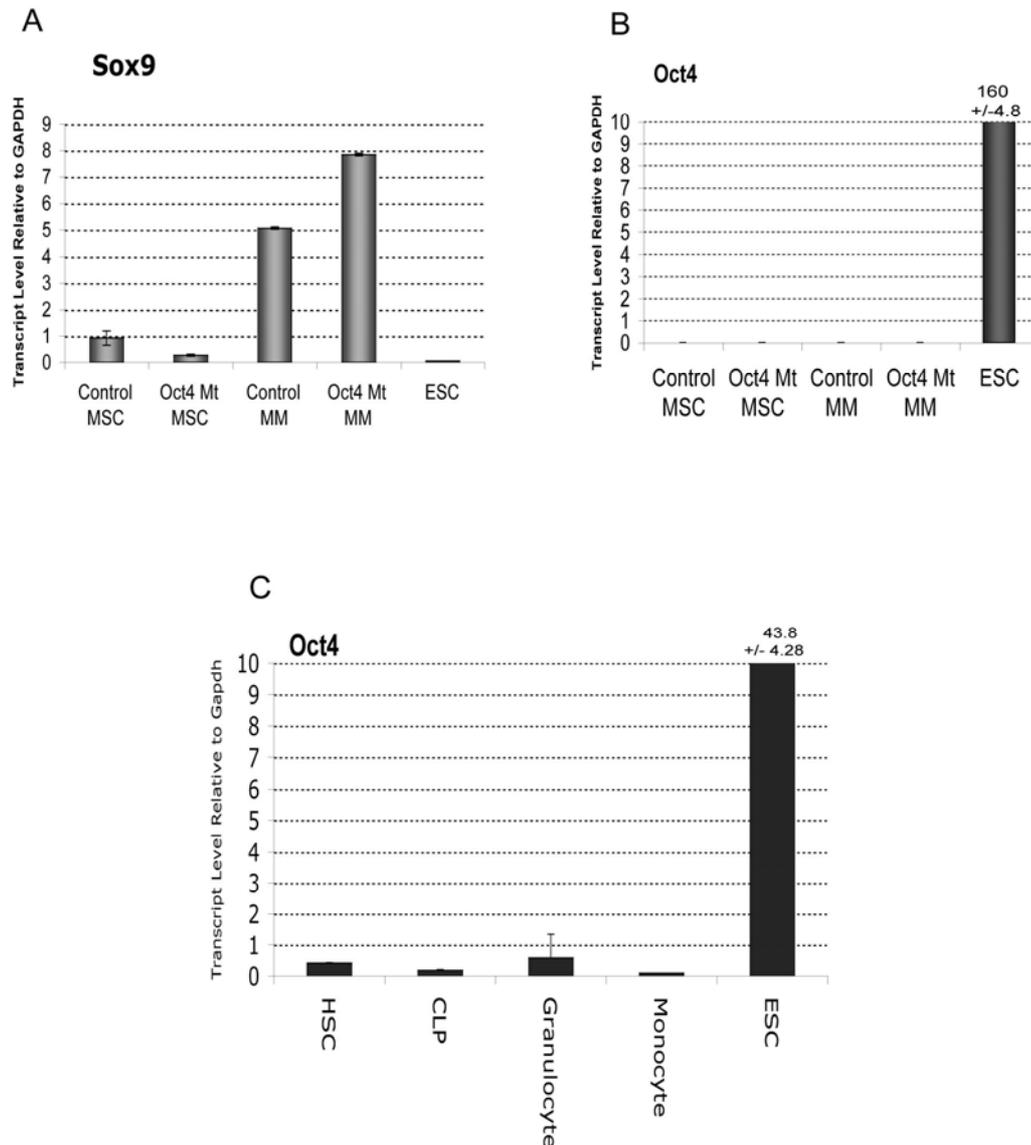


Figure S2. *Oct4* Gene Expression in Progenitor Cells of the Bone Marrow

(A) Quantitative RT-PCR analysis of high-density micromass (MM) cultures of marrow-derived MSCs under chondrogenic culture conditions shows activation of the chondrogenic transcriptional regulator Sox9.

(B) *Oct4* gene expression is undetectable in chondrogenic MSC cultures in comparison to ESCs.

(C) *Oct4* expression in hematopoietic cell populations purified by flow sorting. All data are mean \pm SD, relative to GAPDH, n=3.

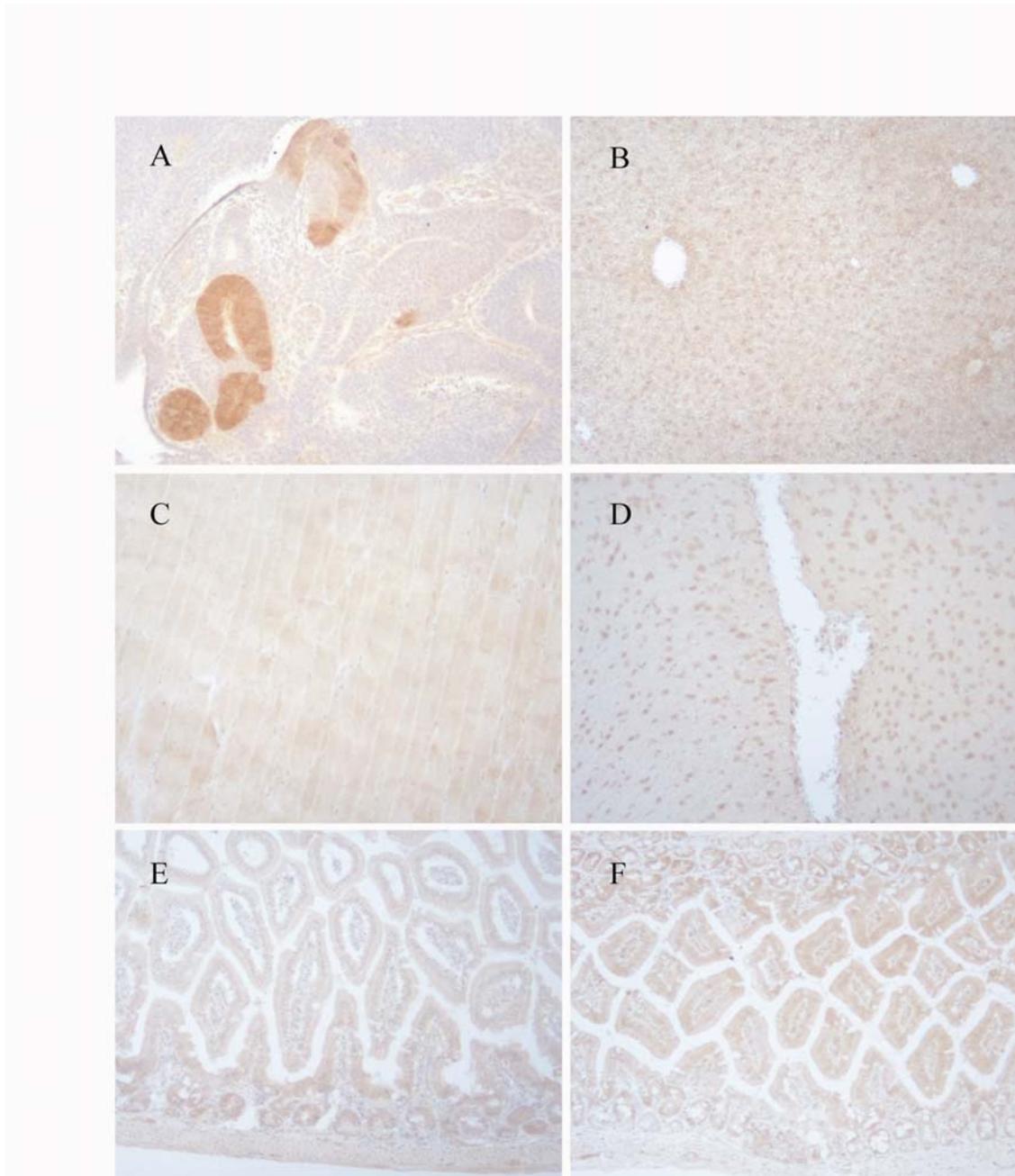


Figure S3. *Oct4-EGFP* in Somatic Tissues

(A) Teratoma derived from *Oct4-EGFP* ES cells stained with an anti-GFP antibody reveals pockets of undifferentiated *Oct4-EGFP* expressing cells.
(B–E) Sections of liver, skeletal muscle, the lateral ventricle of the brain, and intestinal epithelium, respectively, from *Oct4-EGFP* mice stained with an anti-GFP antibody revealing no positive cells.
(F) Intestinal epithelium from wild-type mouse stained with an anti-GFP antibody acts as a negative control.

Table S1. Published Literature Citing *Oct4* Expression in Somatic Tissue

Somatic Stem Cells

Tissue origin	Sample Type	Method of detection	Species	Reference
bone marrow	MAPC (multi potent adult progenitor cell)	RT-PCR	Mouse	Jiang et al. <i>Nature</i> 2002
bone marrow	CD34+ HSCs	IHC	Mouse	Goolsby et al. <i>Proc. Nat. Acad. Sci.</i> 2003
bone marrow	MIAMI cells (Marrow-isolated adult multilineage inducible)	RT-PCR	Human	D'ippolito et al. <i>J Cell Sci.</i> 2004
bone marrow	stromal cell subpopulation	RT-PCR	Human	Pochampally et al. <i>Blood</i> 2004
bone marrow	whole marrow	RT-PCR	Mouse	Johnson J et al. <i>Cell</i> 2005
bone marrow	hMSCs (human mesenchymal stem cells)	RT-PCR	Human	Morisot C et al. <i>Stem Cells</i> 2005
bone marrow	MMSC (multipotent marrow mesenchymal cells)	RT-PCR	Human	Zhang et al. <i>Cell Transplant</i> 2005
bone marrow	MIAMI cells (Marrow-isolated adult multilineage inducible)	RT-PCR	Human	D'ippolito et al. <i>Bone</i> 2006
bone marrow	MIAMI cells (Marrow-isolated adult multilineage inducible)	RT-PCR	Human	D'ippolito et al. <i>Rejuven. Res.</i> 2006
bone marrow	MSC (mesenchymal stem cells)	IHC	Mouse	Lamoury FJ et al. <i>Cytotherapy</i> 2006
bone marrow	bone marrow-derived germ cells	RT-PCR	Mouse	Nayernia K et al. <i>Lab. Invest.</i> 2006
bone marrow	MSCs (marrow stromal cells)	RT-PCR, IHC	Mouse	Ren H et al. <i>Biochem. Biophys. Res. Comm.</i> 2006
bone marrow/adipose	rASCs (rhesus adipose stem cells) hBMCs (human BMSCs)	WB, RT-PCR	Human, NH Primate	Izadpanah R et al. <i>J. Cell. Biochem.</i> 2006
bone marrow	cardiomyogenic progenitor cells	IHC, RT-PCR	Mouse	Pallante BA et al. <i>Circulation Research</i> 2007
bone marrow	MAPC (multi potent adult progenitor cell)	IHC, RT-PCR	Mouse	Serafini M et al. <i>J. Exp. Medicine</i> 2007
bone marrow	MSC (mesenchymal stem cells)	RT-PCR	Human	Roche S et al. <i>J. Biol. Chem.</i> 2007
bone marrow	MSC (mesenchymal stem cells)	RT-PCR	Human	Grayson WL et al. <i>BBRC</i> 2007
bone marrow	CD 45+, Lin- cells	RT-PCR, IHC, FACS	Human	Rogers I et al. <i>Exp. Cell Res.</i> 2007
peripheral blood	CD14+CD34low EPCs (endothelial progenitor cells)	RT-PCR	Human	Romagnani et al. <i>Circulation Research</i> 2005
peripheral blood	MSCs (mesenchymal stem cells)	RT-PCR	Human	Tondreau T et al. <i>Stem Cells</i> 2005
hair follicle	hair follicle progenitor cells and primary follicular bulge	IHC	Human	Yu H et al. <i>Am. J. Pathology</i> 2006
skin	PSOS cells (porcine skin-originated sphere)	RT-PCR	Pig	Dyce et al. <i>Nature Cell Biol.</i> 2006, <i>BBRC</i> 2004
epidermis	keratinocytes- adult and neonatal	RT-PCR	Human	Mongan NP et al. <i>Mol. Carcin.</i> 2006
epidermis	keratinocyte side population cells	RT-PCR	Mouse	Redvers RP et al. <i>Proc. Nat. Acad. Sci.</i> 2006
subdermal ear tissue	FSCCs (fetal somatic stem cells)	RT-PCR	Pig	Kues WA et al. <i>Biol. Reprod.</i> 2005
brain	NSPCs (neural stem and progenitor cells)	N/A	Rhesus	Davis SF et al. <i>Stem Cells Dev.</i> 2006
brain	neurospheres	WB, RT-PCR	Mouse	Okuda T et al. <i>Mol. Brain Res.</i> 2004
uterus-endometrium	endometrial label retaining cells	IHC	Mouse	Cerello I et al. <i>Human Reprod.</i> 2007
uterus-endometrium	primary tissue	IHC, RT-PCR	Human	Matthai C et al. <i>Mol. Hum. Reprod.</i> 2006
breast	HBEc (human breast epithelial cells)	RT-PCR, IHC	Human	Tai MH et al. <i>Carcinogenesis</i> 2005
muscle	PPSCs (pluripotent stem cells)	RT-PCR	Rat	Romero-Ramos M et al. <i>J. Neurosci. Res.</i> 2002
pancreatic islets	epithelial cells	RT-PCR	Rat	Wang et al. <i>J. Endocrinology</i> 2004
pancreas	pancreatic stem cells	N/A	Rat	Kruse C et al. <i>Ann. Anat.</i> 2006
lung	MRC-5 fetal lung-derived fibroblasts	IHC, RT-PCR, WB	Human	Rieske P et al. <i>Differentiation</i> 2005
lung	pulmonary cells	IHC, RT-PCR	Mouse	Ling TY et al. <i>Proc. Nat. Acad. Sci.</i> 2006
kidney	MRPC (multipotent renal progenitor cell)	RT-PCR	Rat	Gupta S et al. <i>J. Am. Soc. Neph.</i> 2005
kidney	parietal epithelial stem cells from Bowman's capsule	RT-PCR, Oct4-GFP Tg	Rat	Sagrinati C et al. <i>J. Am. Soc. Neph.</i> 2006
liver, heart, bone marrow	hMASCs (multipotent adult stem cells)	IHC, RT-PCR	Human	Beltrami AP et al. <i>Blood</i> 2007
liver	hepatic epithelial colonies	RT-PCR	Human	Selden C et al. <i>Stem Cells</i> 2003
whole embryo somatic tissue	FSCCs (fetal somatic stem cells)	RT-PCR, Oct4-GFP Tg	Mouse	Kues WA et al. <i>Biol. Reprod.</i> 2005
embryo	hFOB (human fetal osteoblastic cell)	RT-PCR	Human	Yen M et al. <i>Stem Cells</i> 2007
thyroid	thyroid cells and thyroid cancer-derived cells	IHC, RT-PCR	Human	Thomas T et al. <i>Thyroid</i> 2006

Somatic Tumors and Transformed Cells

Tissue origin	Sample Type	Method of detection	Species	Reference
colon/breast/pancreas	primary tumor	RT-PCR	Human	Monk et al. <i>Cancer Research</i> 2001
breast	primary carcinoma, MCF7 cell line	RT-PCR	Human	Ezeh UI et al. <i>Cancer</i> 2005
bone/cartilage	primary osteo/chondrosarcoma	IHC	Human	Gibbs CP et al. <i>Neoplasia</i> 2005
breast	breast cancer derived stem cells	WB	Human	Ponti D et al. <i>Cancer Research</i> 2005
thyroid gland	primary insular carcinoma	IHC	Human	Ruangpratheep C et al. <i>J Med. Assoc. Thai.</i> 2005
pancreas	islets, primary tumors	IHC	Hamster	Iki K et al. <i>Pancreatolgy</i> 2006
prostate	prostate cancer cell lines, CD45+ fraction	RT-PCR	Human	Patrawala L et al. <i>Oncogene</i> 2006
bladder	primary carcinoma samples	RT-PCR, IHC, WB	Human	Atlasi Y et al. <i>Int. J. Cancer</i> 2007

Amniotic and Umbilical Cord-Derived Cells

Tissue origin	Sample Type	Method of detection	Species	Reference
amniotic fluid	amniocytes	RT-PCR, WB, IHC	Human	Prusa AR et al. <i>Hum. Reprod.</i> 2003
amniotic fluid	AFMSCs (amniotic fluid mesenchymal stem cells)	RT-PCR, IHC	Human	Tsai MS et al. <i>Biol. Reprod.</i> 2006
amniotic fluid	amniocytes	N/A	Human	Woodbury D et al. <i>Mol. Reprod. Dev.</i> 2006
amniotic fluid	AFCs (amniotic fluid cell)	RT-PCR	Human	Bossolasco P et al. <i>Cell Research</i> 2006
amniotic fluid	amniocytes	RT-PCR	Human	Kim J et al. <i>Cell Prolif.</i> 2007
amniotic fluid	AFS (amniotic fluid stem cells)	Flow cytometry	Mouse, Human	De Coppi P et al. <i>Nature Biotech.</i> 2007
umbilical cord	PUC (porcine umbilical cord) matrix cells	RT-PCR	Pig	Carlin R et al. <i>Reprod. Biol. Endocrinol.</i> 2006
umbilical cord	CB-SCs (cord blood stem cells)	IHC	Human	Zhao Y et al. <i>Exp. Cell Research</i> 2006
umbilical cord	RUCM (rat umbilical cord matrix) cells	RT-PCR, IHC	Rat	Jomura S et al. <i>Stem Cells</i> 2007
cord blood	HSPC (hematopoietic stem/progenitor cells)	RT-PCR, IHC	Human	Baal N et al. <i>Thromb Haemost.</i> 2004
cord blood	MSCs (mesenchymal stem cells)	RT-PCR	Human	Tondreau T et al. <i>Stem Cells</i> 2005
cord blood	cord-blood derived stem cells	IHC, WB	Human	Sun B et al. <i>BBRC</i> 2007
placenta	amniotic epithelial cells	RT-PCR	Human	Miki T et al. <i>Stem Cells</i> 2005
placenta	PDMSCs (placenta-derived multipotent stem cells)	RT-PCR	Human	Chang CM et al. <i>BBRC</i> 2007