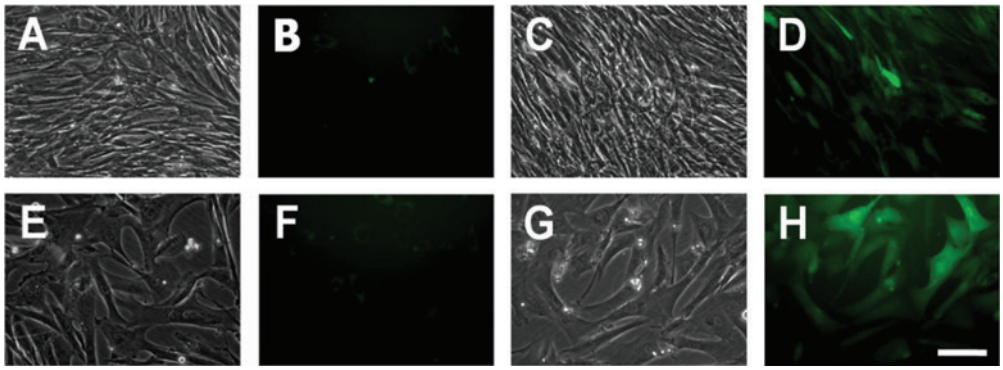
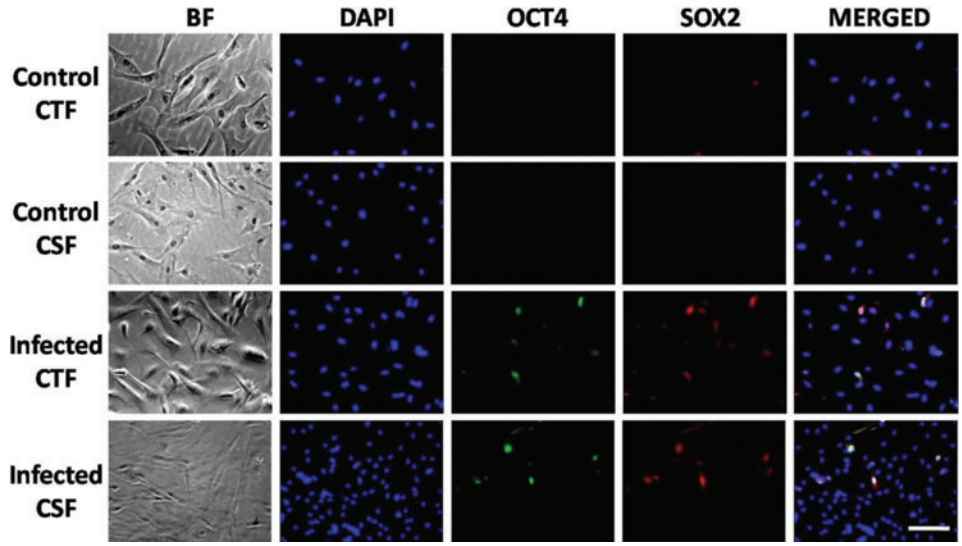
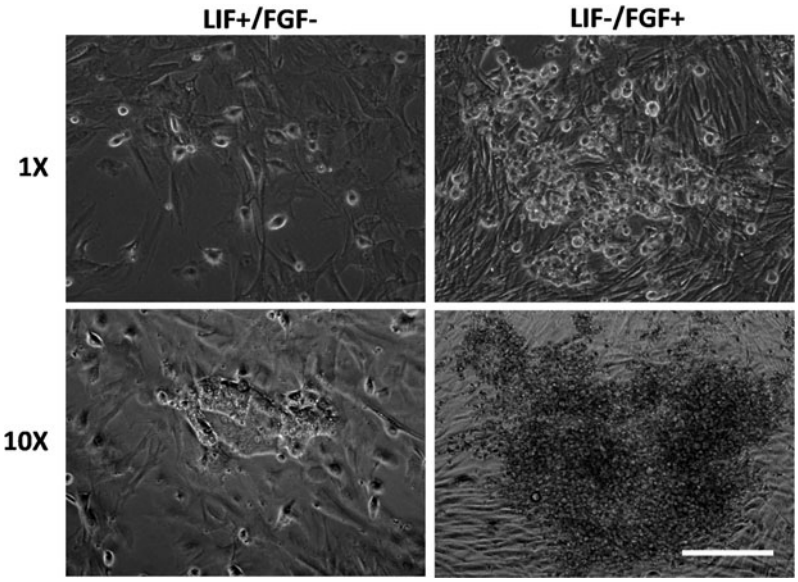
Supplementary Data



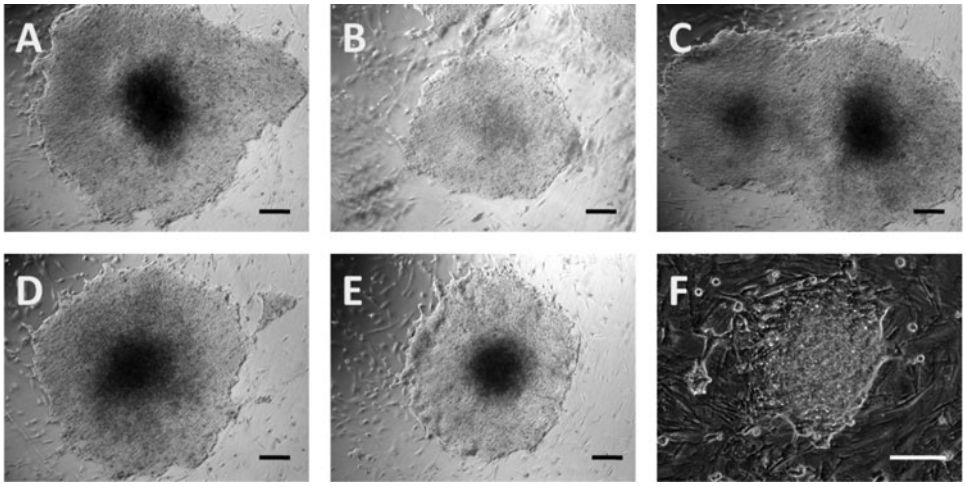
SUPPLEMENTARY FIG. S1. Lentivirus-infected canine fibroblasts show YFP expression. **(A, B)** Uninfected canine skin fibroblasts (CSFs); **(C, D)** infected CSFs; **(E, F)** uninfected CTFs; **(G, H)** infected CTFs. Scale bar: 100 μm. CTF, canine testicular fibroblast.



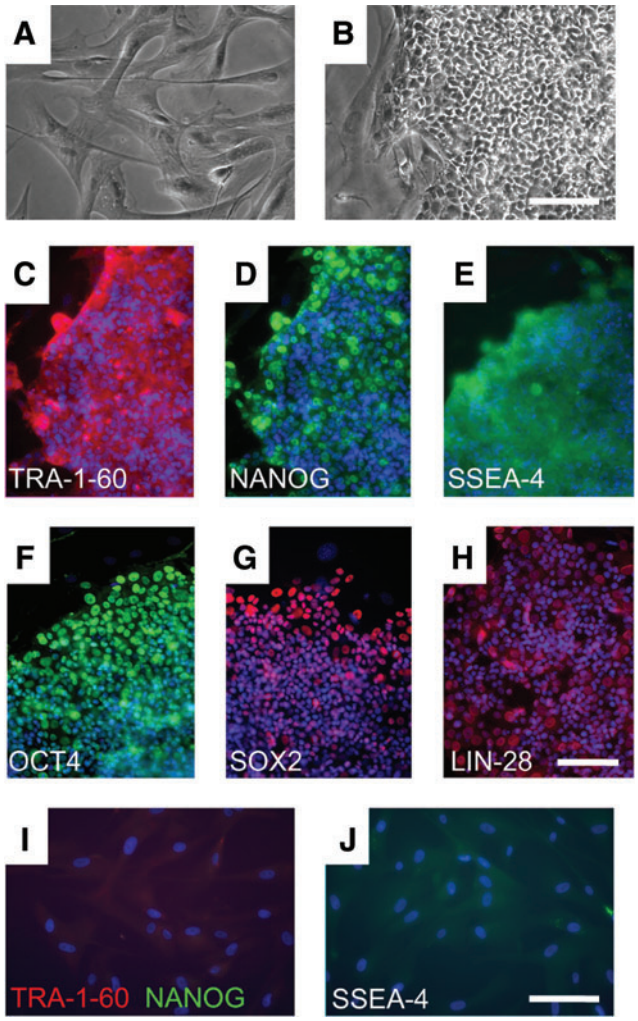
SUPPLEMENTARY FIG. S2. Immunocytochemistry of human OCT4 and SOX2 after transduction. CTFs and CSFs on day 3 after viral transduction partially express introduced genes (human OCT4 and SOX2), whereas the uninfected CTFs and CSFs remain negative after immunostaining. The DNA was labeled by DAPI staining. Scale bar: 250 μm.



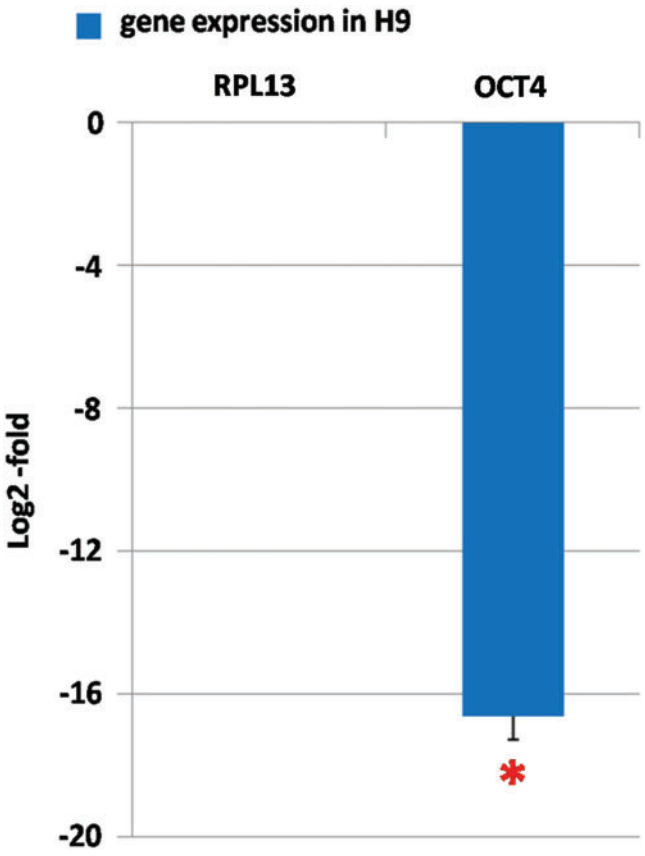
SUPPLEMENTARY FIG. S3. Leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) dependency of canine induced pluripotent stem cells (ciPSCs). Morphology of the canine donor cells on day 10 after viral infection based on different treatments of growth factors. The cells were cultured, respectively, with human LIF (LIF+) or bFGF (FGF+) in concentrations of 1 × (10 ng/mL for human LIF and 4 ng/mL for bFGF) or 10 × (100 ng/mL for human LIF and 40 ng/mL for bFGF). Scale bar: 250 μm.



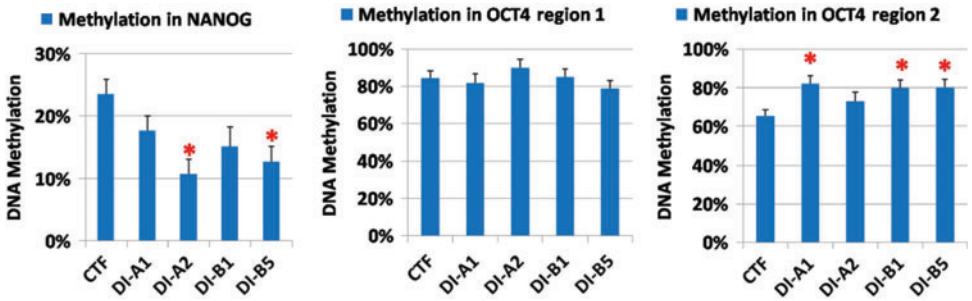
SUPPLEMENTARY FIG. S4. The ciPSCs derived from the second batch of donor fibroblasts. **(A–E)** The typical ciPSC colonies from cell line DI-B1 to DI-B5 at P1; **(F)** the first colony of cell line DI-B2 on day 9 postviral transduction. Scale bar: 100 μm.



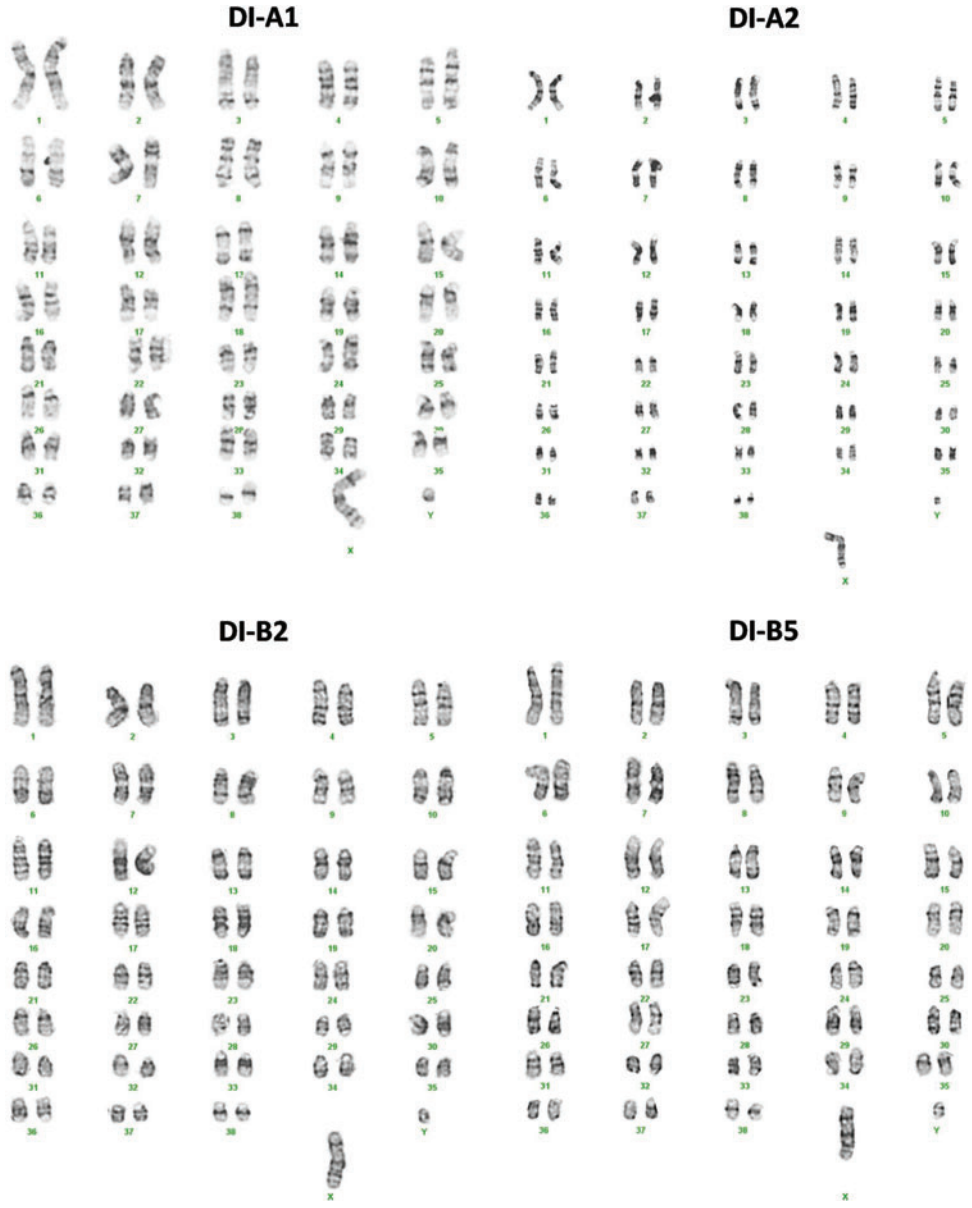
SUPPLEMENTARY FIG. S5. Immunocytochemistry of DI-A1, DI-A2, and CTFs. **(A)** Phase-contrast image of canine fibroblasts used for ciPSC generation at P3. **(B)** Phase-contrast image of ciPSCs at P7. **(C–H)** Immunocytochemistry of pluripotency markers in ciPSCs as labeled. The pluripotency markers include **(C)** TRA-1-60, **(D)** NANOG, **(E)** SSEA-4, **(F)** OCT4, **(G)** SOX2, and **(H)** LIN-28. **(I, J)** Examples showing that pluripotency markers are not expressed in input canine fibroblasts. DNA was labeled by DAPI staining and shown in blue. Scale bar: 250 μm.



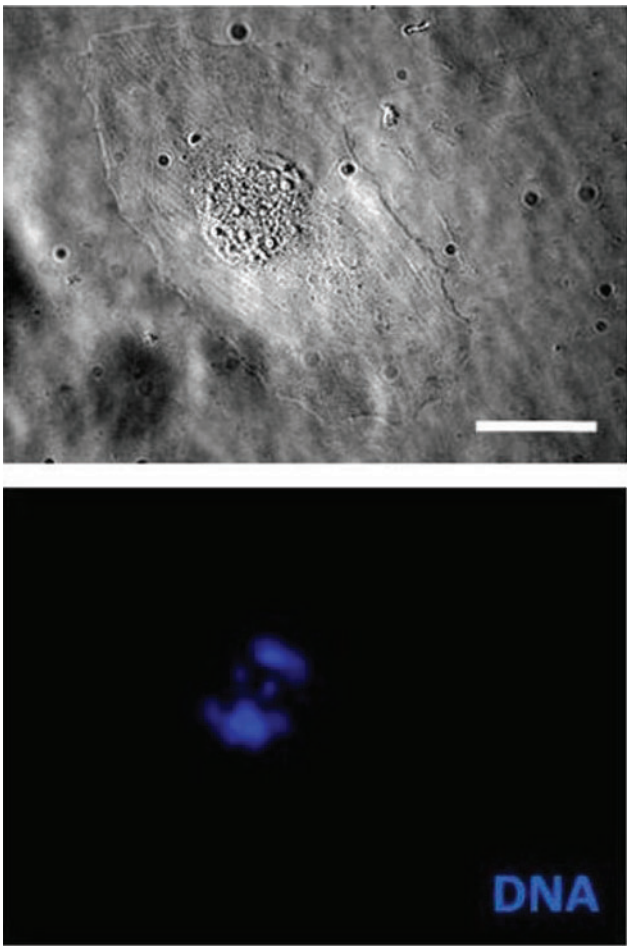
SUPPLEMENTARY FIG. S6. Validation of specificity of canine OCT4 primers for ciPSCs. Quantitative reverse transcription–polymerase chain reaction analysis of relative transcript amount of canine RPL13 and OCT4 in human ESC H9. The *y*-axis represents the fold change (Log_2) relative to RPL13. *$P < 0.05$.



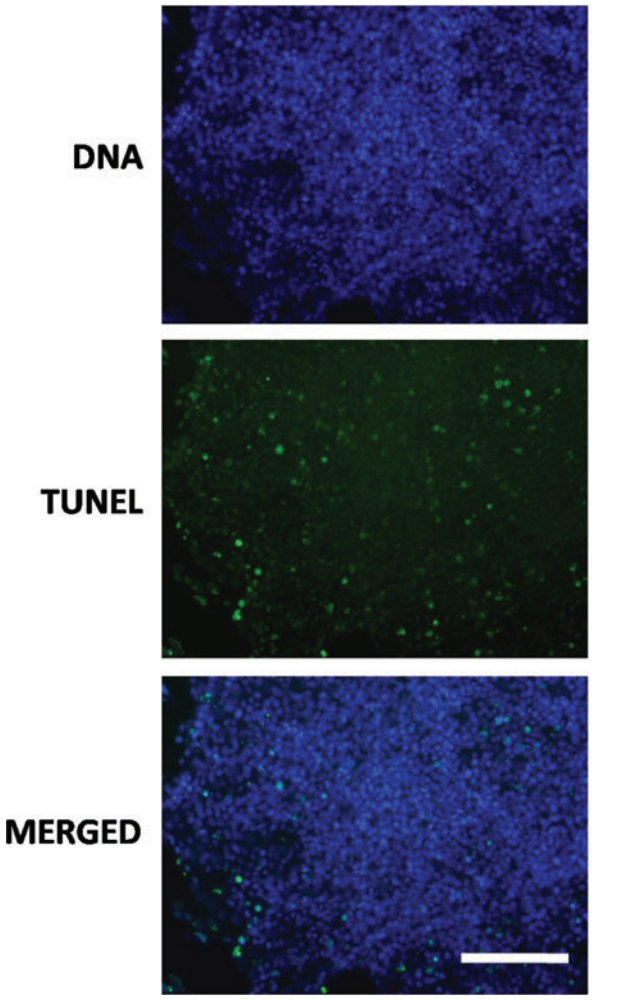
SUPPLEMENTARY FIG. S7. Epigenetics analysis of ciPSCs. Bisulfate genomic sequencing for DNA methylation in the promoter regions of canine NANOG and OCT4 within CTFs and ciPSC lines DI-A1, DI-A2, DI-B1, and DI-B5. The percentages of methylation in 4 ciPSC lines are compared with that in CTF by PROC GLM from SAS. Error bars stand for the standard errors of each column. $*P < 0.05$.



SUPPLEMENTARY FIG. S8. Karyotype analysis of ciPSCs. G-banding chromosomes of DI-A1 (P4), DI-A2 (P3), DI-B2 (P4), and DI-B5 (P5) demonstrate the normal male karyotypes.



SUPPLEMENTARY FIG. S9. Morphology and DNA staining of ciPSC-differentiated trophoblast cell-like cell. Scale bar: 100 μm.



SUPPLEMENTARY FIG. S10. Terminal deoxynucleotidyl transferase dUTP nick-end labeling assay for the DNase-treated ciPSCs as the positive control. Green cells represent the apoptotic cells. Scale bar: 250 μm.

Supplementary Table S1. Antibodies Used for Immunocytochemistry Assay

Antigen	*Catalog no.*	*Isotype*	*Manufacturer*	*Concentration*
OCT4	SC8628	Goat IgG	Santa Cruz Biotechnology	1:300
SOX2	AB5603	Rabbit IgG	Abcam	1:500
NANOG	SC33759	Rabbit IgG	Santa Cruz Biotechnology	1:500
LIN-28	67266	Rabbit IgG	Santa Cruz Biotechnology	1:250
SSEA-4	MC813-70	Mouse IgG	Abcam	1:500
TRA-1-60	09-0009	Mouse IgG	Stemgent	1:250
Fibronectin	HFN7.1	Mouse IgG	Abcam	1:500
TUJ1	14545	Mouse IgG	Abcam	1:7,000
Vimentin	AMF17B	Mouse IgG	Developmental Studies Hybridoma Bank	1:250
AFP	SC8108	Goat IgG	Santa Cruz Biotechnology	1:500
Cy-3 goat anti-mouse IgG + IgM	09-0038	N/A	Stemgent	1:1,000
Alexa 488 donkey anti-mouse IgG	A21207	N/A	Invitrogen	1:1,000
Alexa 488 donkey anti-goat IgG	A11055	N/A	Invitrogen	1:1,000

AFP, alpha-fetoprotein; IgG, immunoglobulin G; TUJ1, β-III neuron-specific tubulin.

Supplementary Table S2. Primers Designed for Quantitative Reverse Transcription–Polymerase Chain Reaction

Primers		*Sequence*
RPL13	Forward	GGAGAAGGCCAGAGTCATCACA
	Reverse	TTTGCCCTGATGCCAAAAAG
OCT	Forward	ACGATCAAGCAGTGACTATTCG
	Reverse	GAGGGACTGAGGAGTAGAGCGT
NANOG	Forward	CTAGGGACCCTTCTCCAATGC
	Reverse	CATTGGCAAGGATGCAGGAT
TERT	Forward	TTACAGAGCATAGGAATCAGACAACTC
	Reverse	GGTGTCTCCTGACCTCTGCTTCT
SOX2	Forward	AACCCCAAGATGCACAACTC
	Reverse	CGGGGCCGGTATTTATAATC
c-MYC	Forward	GGACGCGCAGAGGCTCTACC
	Reverse	GGTTTCCACTCTCCGGAGGAG
LIN-28	Forward	CCACCCCAGCCCAAGAA
	Reverse	CAGTGGACACGAGGCTACCA
SOCS3	Forward	CGAGAAGATCCCTCTGGTGTTG
	Reverse	TTTCTCGTAGGAGTCCAGGTG
STAT3	Forward	ACTGGCGTCCAGTTCACTACCA
	Reverse	TGCGACATCCCCAGAGTCTTT
GBX2	Forward	AAGGCTGGCAATGCCAATT
	Reverse	TGACTTCTGATAGCGAACCTGC
FOXD3	Forward	GCAGAGCCCGCAGAAGAAG
	Reverse	GGGAAGCGGTTGCTAATGAA
O-K	Forward	TCTCCCATGCATTCAAACG
	Reverse	GTGGAGAAAGATGGGAGCAG
NESTIN	Forward	AGCCCTACTTCCCTCTCCTT
	Reverse	CTGAAGTGTGGGCGGGATGGGG
NEFL	Forward	GGAAACTCTTGGAAGGTGAGGA
	Reverse	TAACCCACCATAGGCAGATCG
CD34	Forward	CCTACAACAGCACCAGCCTTGT
	Reverse	CCGGAACATTTGATTTCTCCCT
GATA2	Forward	GCCACTGACCATGAAGAAGGAA
	Reverse	ACAGCTCCTCAAAGCACTCTGC
CXCR4	Forward	ACTCCATGAAGGAACCCTGCTT
	Reverse	TGCCCACTATGCCAGTCAAGA
AFP	Forward	CTGAAAACCCTCTTGAATGCCA
	Reverse	TTTCTGGAAGAGGCCACAGCT
CDX2	Forward	CCAAGTGAAAACCAGGACGAA
	Reverse	CGGATGGTGATGTAACGACTGT

Supplementary Table S3. Primers Designed for Canine NANOG and OCT4 Promoter Regions in Bisulfite Genomic Sequencing

dNANOG1outF	GTATTTTGATTTTAAAGGATGGA
dNANOG1outR	AAAACCTCCACATATAAAAAATAAA
dNANOG1in F	TAGAAATATTTAATTGTGGGGTT
dNANOG1in R	CATATAAAAAATAAAAAAAAAACAAAAT
dOCT41out F	ATATAGGGAGGAGTGTTTAGGTTA
dOCT41in F	GAGGAGTGTTTAGGTTATTTTAT
dOCT41in R	CTCAACACCTCTCTCCCTCC
dOCT41out R	AAAAACTCTCCTAAAAACTACTCAA
dOCT42out F	AGGTTAGTGGGTGGGATTGG
dOCT42in F	AGGTGTTGAGTAGTTTTTAGGAGA
dOCT42in R	ACTCCCACCTAAAATCCACAATA
dOCT42out R	CCTTAAAACAACAACCCCACTC

"Out" or "in" stands for that the primers were designed for the amplification of the outer or inner region in nested genomic polymerase chain reaction. "F" or "R" stands for the forward or reverse primers.

Supplementary Table S4. Genotypes for the Induced Pluripotent Stem Cells Using Five Canine Tetranucleotide Repeat Microsatellites

Samples markers	*CSF*	*CTF*	*DI-A1*	*DI-A2*	*DI-B1*	*DI-B2*	*DI-B3*	*DI-B4*	*DI-B5*
FH2054	157, 162	150, 166[ab]	150, 166	150, 166	150, 166	150, 165	N/A	150, 165	150, 166
FH2165	386, 394	453, 470	453, 470	453, 470	453, 470	453, 470	453, 470	453, 470	453, 470
FH2233[c]	359, 367	281, 351, 409	281, 351, 409	281, 351, 409	281, 351, 409	281, 351, 408	281, 351, 409	281, 351, 409	N/A
FH2313	269, 272	293, 307	293, 307	293, 307	292, 306	292, 306	292, 306	292, 306	292, 306
FH2324	253, 262	253, 257	253, 257	253, 257	253, 257	253, 257	253, 257	253, 257	253, 257

[a]Allele sizes are shown without the M13 tail used to label the amplicons so that direct comparisons can be made with the allele frequency data of Irion et al. [1].

[b]Sizes are rounded to the nearest whole number. Single base differences among allele sizes are deemed to represent the same allele.

[c]This marker showed 3 alleles in all cell lines except CSF. The 3 alleles are caused by a duplication ("copy number polymorphism" or CNP) that contains the FH2233 marker [2].

CSF, canine skin fibroblast; CTF, canine testicular fibroblast.

Supplementary References

1. Irion DN, AL Schaffer, TR Famula, ML Eggleston, SS Hughes and NC Pedersen. (2003). Analysis of genetic variation in 28 dog breed populations with 100 microsatellite markers. [Translated from Eng.] J Hered 94:81–87. [In Eng.]
2. Onogi A, M Nurimoto, Y Sato Y and M Morita. (2008). A chromosomal duplication that includes the canine microsatellite INRA21 in Labrador Retrievers. [Translated from Eng.] Anim Genet 39:241–248. [In Eng.]