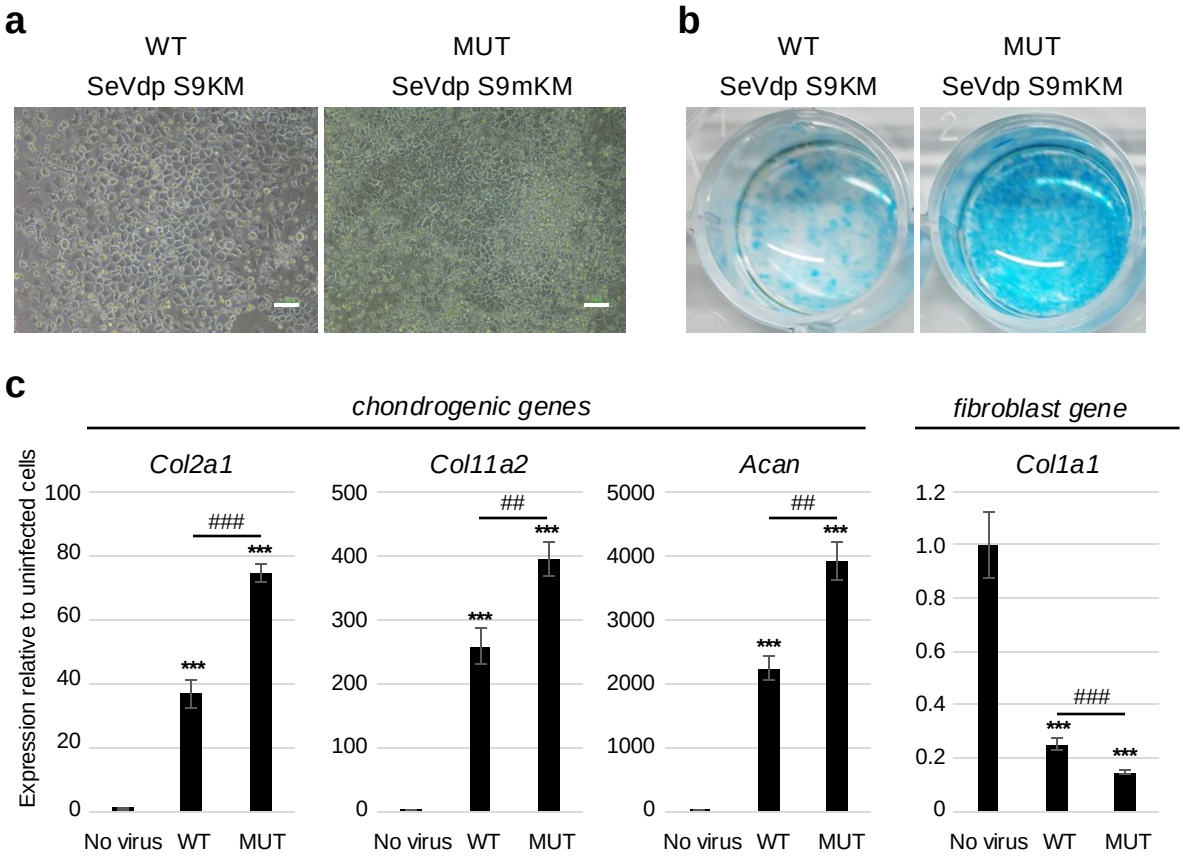
Supplementary information

A Sendai virus-based expression system directs efficient induction of chondrocytes by transcription factor-mediated reprogramming

Jingwen Zhou[1], Yuya Sekiguchi[1], Masayuki Sano[2], Ken Nishimura[1], Koji Hisatake[1*], Aya Fukuda[1*]

Figure S1



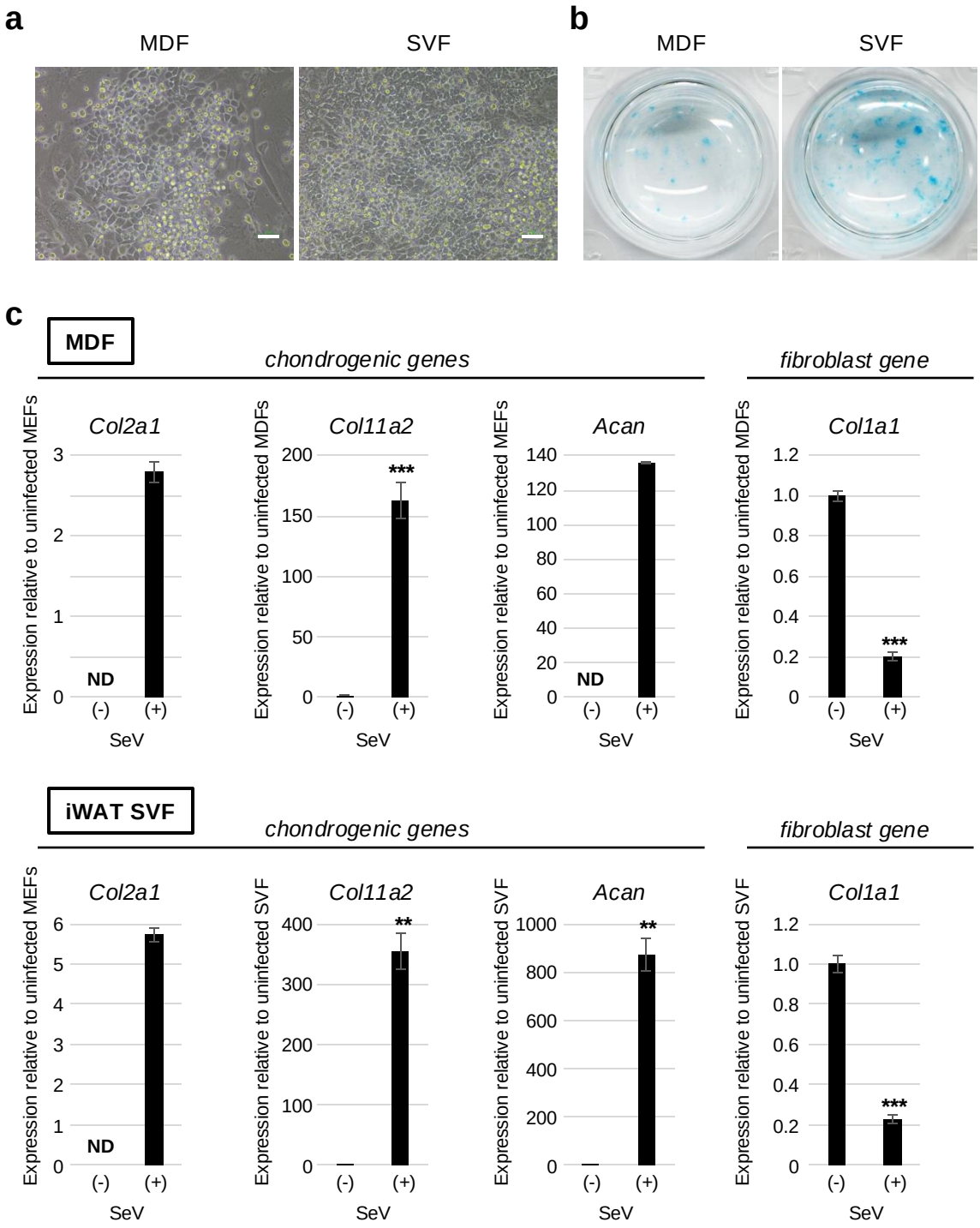
Induction of chondrocytes by wild-type SOX9- and mutant SOX9$_{H131A/K398A}$ (S9m)-expressing SeV vectors

a Morphology of chondrocytes induced by wild-type SOX9- or mutant SOX9-expressing SeV vector. Scale bar: 100 μm

b Alcian blue staining of the differentiated chondrocytes.

c Expressin of chondrogenic genes and a fibroblast gene in the induced chondrocytes. n = 5. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. * represents a significant difference versus uninfected cells (No virus) $^{***}p < 0.001$.

Figure S2

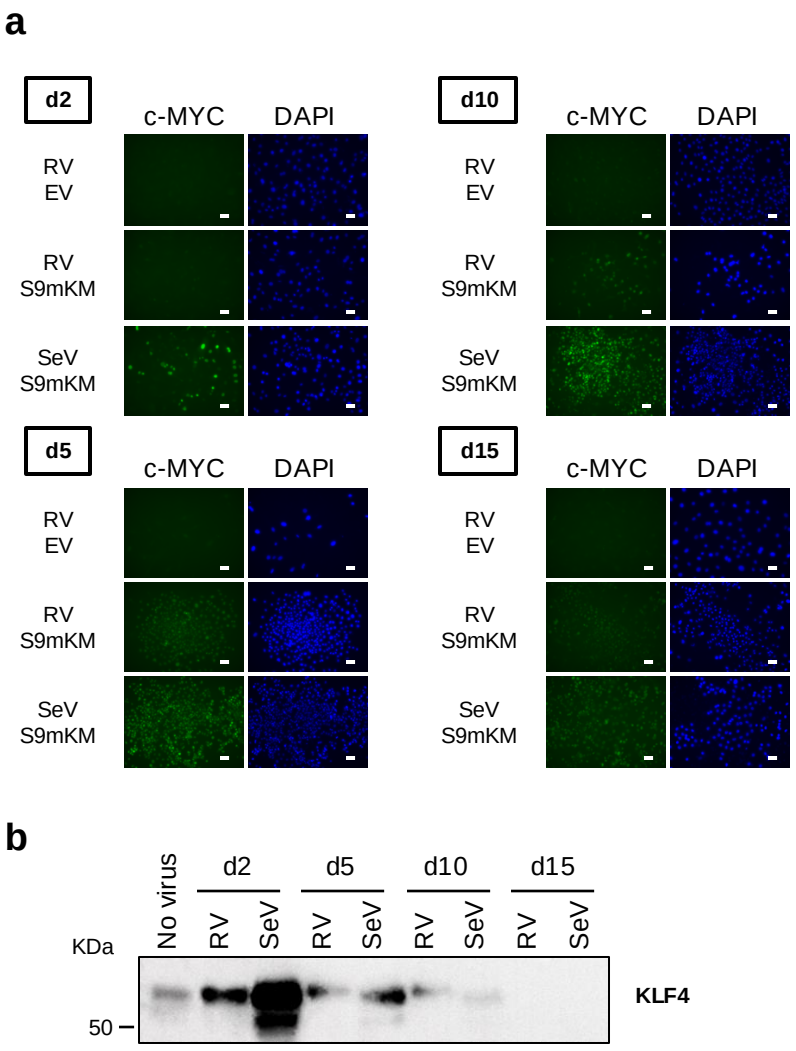


Induction of chondrocytes from MDFs and iWAT SVF cells by SeVdp S9mKM

a Morphology of chondrocytes induced by the SeV vector (S9mKM). Scale bar: 50 μm

b Alcian blue staining of the differentiated chondrocytes.

c Expressin of chondrogenic genes and a fibroblast gene in the induced chondrocytes. n = 3. * represents a significant difference versus uninfected cells (-). **p < 0.01, ***p < 0.001. ND: not detected.

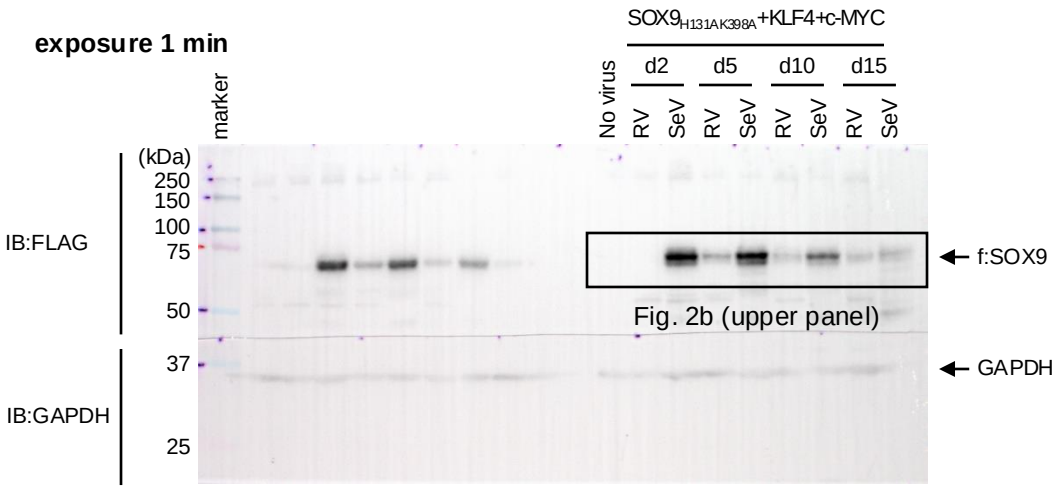


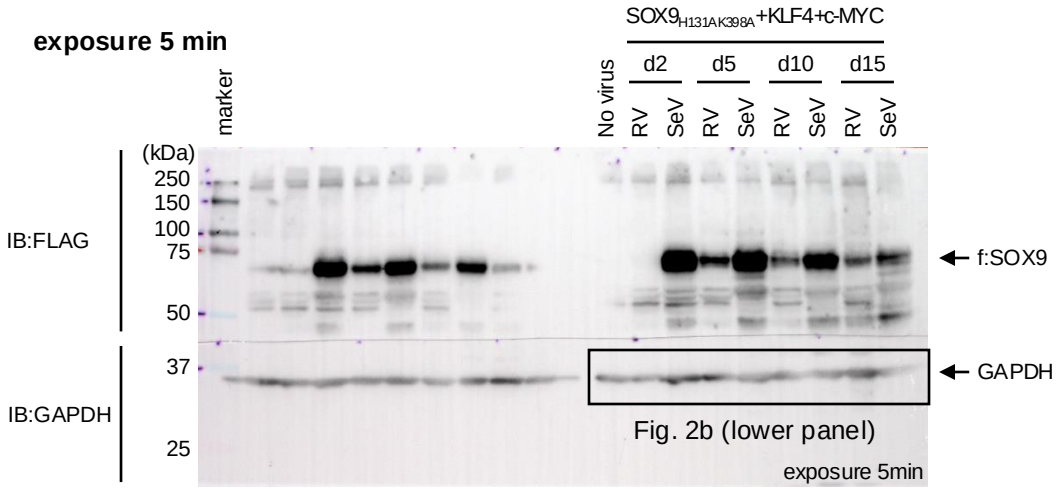
Rapid and strong expression of the reprogramming factors by the Sendai virus vector

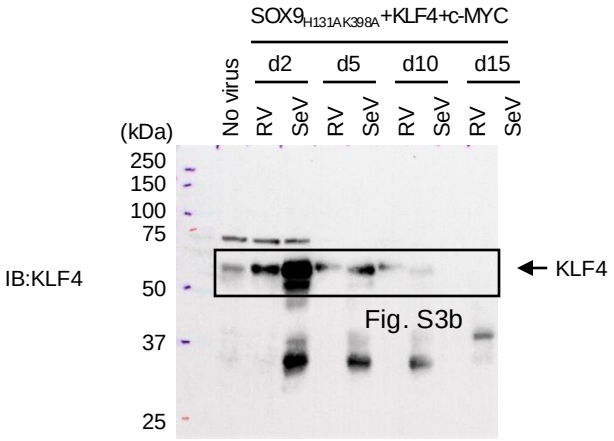
a Immunofluorescence staining of the retrovirus (RV)- or Sendai virus (SeV)-infected cells using an anti-c-MYC antibody.

b Western blotting analysis of KLF4 expression in RV- or SeV-infected cells.

Figure S4

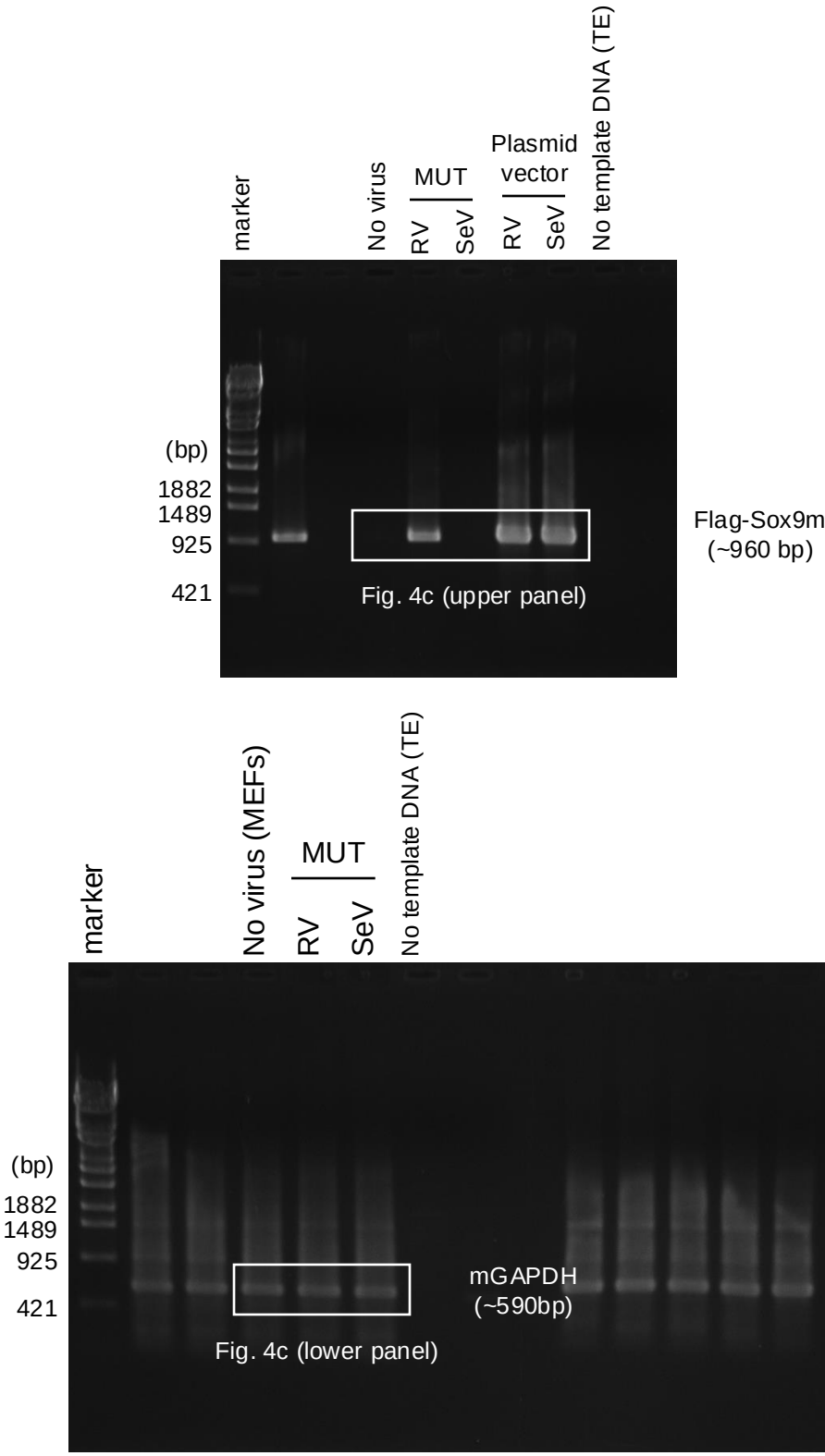






Full length immunoblots shown in Fig. 2b and Fig. S3b.

Figure S5



Full length agarose gel images shown in Fig. 4c.

Supplementary Table S1

Antibodies used in this study

Anti-DYKDDDDK (Flag) tag antibody	Wako, 012-22384, mouse monoclonal	1:1000 (IF) 1:1500 (IB)
Anti- GKLF (KLF4) antibody (H-180)	Santa Cruz, sc-20691, rabbit polyclonal	1:500 (IF)
Anti-KLF4 antibody	prepared in our lab, rabbit polyclonal	1:500 (IB)
Anti- c-MYC antibody (Y-69)	Abcam, ab32072, rabbit monoclonal	1:400 (IF)
Alexa Fluor™ 488 donkey anti-mouse IgG (H+L)	Thermo Fisher Scientific, A21202	1:1000 (IF)
Alexa Fluor® 555 goat anti-rabbit IgG (H+L)	Thermo Fisher Scientific, A21428	1:1000 (IF)
Anti- COL2A1 antibody (M2139)	Santa Cruz, sc-52658, mouse monoclonal	1:50 (IF)
Anti- COL1A1 antibody (3G3)	Santa Cruz, sc-293182, mouse monoclonal	1:50 (IF)
Anti-GAPDH antibody (6C5)	Santa Cruz, sc-32233, mouse monoclonal	1:1000 (IB)
Anti-Mouse IgG, HRP-Linked Whole Ab Sheep	Cytiva, NA931V	1:3000 (IB)

IF : immunofluorescent staining

IB : immunoblotting

Supplementary Table S2

Primers used for qPCR

m*Col2a1* Forward	TGGCCTTAGTGCAGGAAACTTC
m*Col2a1* Reverse	ACCACCAGCCTTCTCGTCA
m*Col11a2* Forward	AGACCAGCCCTTATGTCAAGGA
m*Col11a2* Reverse	ACCGTCCGGCCTTGCT
m*Acan* Forward	TCTCCAGGTGCAGCTGAAGTC
m*Acan* Reverse	TGGGCGATAGTGGAATACAACTC
m*Sox5* Forward	TGATGGATTTCAATATGAGTGGAGAT
m*Sox5* Reverse	TCCCTGTAAATTCTTGACTCTGAGACT
m*Sox6* Forward	GGACCAGCCCTGTAACTCAAGT
m*Sox6* Reverse	GGCCGGGATGAGAGATTCA
m*Sox9 (endo)* Forward	CCCCGGTTTCGTTCTCTGTT
m*Sox9 (endo)* Reverse	TCAGCTGCCGGCTCTAAAC
m*Col1a1* Forward	CCCTGGCCTGGAGGAA
m*Col1a1* Reverse	CAGCTGATTTTTCATCATAGCCA
m*Col1a2* Forward	CACAGTGGTATGGATGGATTAAAGG
m*Col1a2* Reverse	GCTTGACCTGGAGTTCCATTCT
m*Nono* Forward	GCTCTGGACAGATGCAGTGAAG
m*Nono* Reverse	CAGTCACAGGCCGAGGAAA
m*Col10a1* Forward	GCTGCCCCACGCATCTC
m*Col10a1* Reverse	GGTATTTGAGGCAGCATATTTTCA
m*Mmp13* Forward	CCACTCCCTAGGTCTGGATCA
m*Mmp13* Reverse	TCAAGGGATAGGGCTGGGTC
SeV genome RNA Forward	TCAGTCTCTTACGTCTCTCACAG
SeV genome RNA Reverse	CAGAAGGGTTTTGGGAGGA

Supplementary Table S3

Primers used for genome PCR

Flag Forward	GACTACAAAGACGACGATGACAAA
m*Sox9* Reverse	GCCGTAGCTGCCCGTGTAGGT
m*Gapdh intron 1* Forward	TGTACGGGTCTAGGGATGCT
m*Gapdh intron 2* Reverse	TATGCACCTCACAACGCCAT