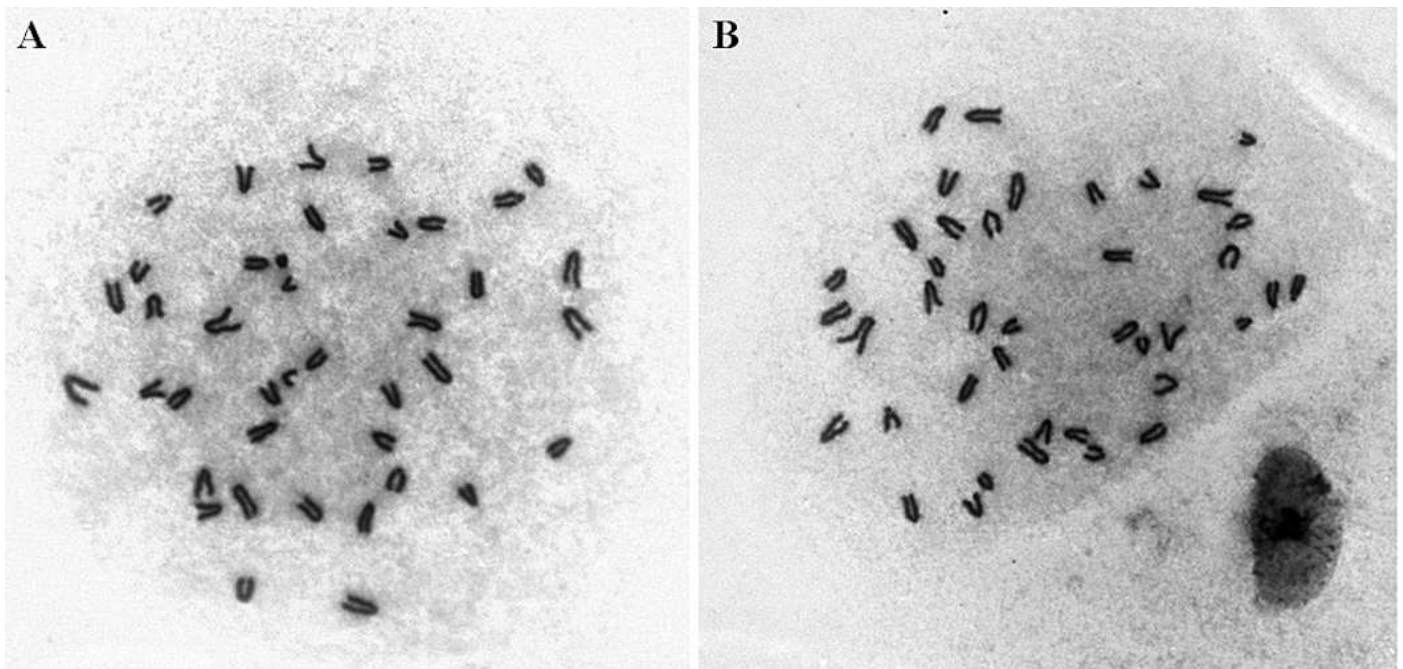


Mouse iPSC generated with porcine reprogramming factors as a model for studying the effects of non-silenced heterologous transgenes on pluripotency

## Supplementary Information

### Supplementary Figures



**Figure S1.** Karyotypes of miPSC produced with porcine reprogramming factors. A) Metaphase spread of a TetO-miPSC line 2 at passage 59. B) Metaphase spread of CAG-miPSC line 1 at passage 60.

**A** Alignment of Oct4 mouse (NP\_038661.2) and porcine (NP\_001106531) sequences

Score	Identities	Positives	Gaps
545 Bits (1404)	297/360 (83%)	317/360 (88%)	(8/360 (2%))
Mouse 1	MAGHLASDFAFSPFFGGGDSAGLEPG-WDFPRTWLSFGPPGGGIGPG---SEVLGI	55	
Porcine 1	MAGHLASDFAFSPFFGGG G WDFPRTWLSFGPPGG GIGPG +EV G+	60	
Mouse 56	SPCPAYEFCGGMAYCGPQGLGIVPQVGVETLQPEQAGARVESNSSESTSEPCADRP	115	
Pig 61	PACPPFYDFCGGMAYCAPQVGVIVPQGGLETQPEGEAGAVESNSSEGA.SPEPCAA	120	
Mouse 116	AVKL--EKVEPTPEESQDMKALQLELQFAKLLKQKRITLGYTQADVGLTIGVLFKQVFS	173	
Pig 121	A.KL EK+EP PEE SQ+HAIQK+LEQFAKLLKQKRITLGYTQADVGLTIGVLFKQVFS	180	
Mouse 174	QTTICRFEALQLSKNCKLRFLLKQVEADNNEMLQEI CKSETLVQARKRRTSIENR	232	
Pig 181	QTTICRFEALQLSKNCKLRFLLKQVEADNNEMLQEI CKSETLVQARKRRTSIENR	240	
Mouse 234	VKWSLEIMFLKCPKSLQQTITHANQLGLEKDVVWVFNCRKQKGRSSIEYSQREEYEA	293	
Pig 241	VR +LE+MFL+CPK+LQI+HIA QLGLEKDVVWVFNCRKQKGRSS +YSQE+EA	300	
Mouse 294	TGTFPFGGAVSFPFPFGHEFTGPGYSPHTTLYL-SVPFPEGEAFPSVLTGSPMHSN	352	
Pig 301	AGSPFPFGGAVSFPFPFGHEFTGPGYSPHTTLYL-SVPFPEGEAFPSVLTGSPMHSN	360	

**B** Alignment of Sox2 mouse (AAH57574.1) and porcine (NP\_001116669.1) sequences

Score	Identities	Positives	Gaps
638 bits (1645)	310/319 (97%)	314/319 (98%)	0/319 (0%)
Mouse 1	MYTMMETELKPPGCGQA.SGGGGGGNATAAATGGQKNSFDVVKRPMIAFMVW.SRGGRRK	60	
Pig 1	MYTMMETELKPPGCGQT.SGGGGGGNATAAATGGQKNSFDVVKRPMIAFMVW.SRGGRRK	60	
Mouse 61	MAQENPKMHNSEISIRLGAENKLLSETEKRPFTDEAKRLRALHMKHEFDYKYPERRKTK	120	
Pig 61	MAQENPKMHNSEISIRLGAENKLLSETEKRPFTDEAKRLRALHMKHEFDYKYPERRKTK	120	
Mouse 121	IMKKDKYTLFGGILLAPGGNSMAGSVGVGAGL.GAGVQRMDSYAHMNGW.SNGSYTSMQCFQL	180	
Pig 121	IMKKDKYTLFGGILLAPGGNSMAGSVGVGAGL.GAGVQRMDSYAHMNGW.SNGSYTSMQCFQL	180	
Mouse 181	GYFQHPGLNANHAQAQMFMHRVYDVSALQYNSMT.SQTYMNGSPTYSMSYSQQGTFGMLG	240	
Pig 181	GYFQHPGLNANHAQAQMFMHRVYDVSALQYNSMT.SQTYMNGSPTYSMSYSQQGTFGMLG	240	
Mouse 241	SMGSVVKSEASSSEPVVTS.SSHSRAPCQAGLDLDMISMLYLGAEVPEPAA.PSRLLMHAQHY	300	
Pig 241	SMGSVVKSEASSSEPVVTS.SSHSRAPCQAGLDLDMISMLYLGAEVPEPAA.PSRLLMHAQHY	300	
Mouse 301	QSGFVPGTAINGLPLSHM 319		
Pig 301	QSGFVPGTAINGLPLSHM 319		

**C** Alignment of c-myc mouse (AAB59728.1) and porcine (CAA65753.1) sequences

Score	Identities	Positives	Gaps
719 bits (1857)	400/438 (91%)	410/438 (94%)	2/438 (0%)
Mouse 1	MPLNVFTRNRYLDYDVSQYFYFCEEEENFYHQQQQSELQFPAPSEDIWKKFELLTPTFP	60	
Pig 1	MPLNVFTRNRYLDYDVSQYFYFCEEEENFYHQQQQSELQFPAPSEDIWKKFELLTPTFP	60	
Mouse 61	LSPSRRSGLCSPSYVAVATSFSPREDDGGGGFSTADQLQMMTELLGDMVWQSFICDP	120	
Pig 61	LSPSRRSGLCSPSYVAVA-SFSPREDDGGGGFSTADQLQMMTELLGDMVWQSFICDP	119	
Mouse 121	DDETFHKNI IQDCMWSGFSAAKLVSEKIASYQAARKDSTSLSPARGHVCSTSSLYLQ	180	
Pig 120	DDETFHKNI IQDCMWSGFSAAKLVSEKIASYQAARKDSTSLSPARGHVCSTSSLYLQ	179	
Mouse 181	DLTAAASECIDP.SVVFYPLNDSSEPKCSTS.SDSTAFSPSSDLSLSESSP-RASPEPLV	239	
Pig 180	DLTAAASECIDP.SVVFYPLNDSSEPKCSTS.SDSTAFSPSSDLSLSESSP-RASPEPLV	239	
Mouse 240	LHEETPFTTSSDSEEEQEDEEEDVWVVEKRTQFAKRSSESSPFRGHSKPPHSPVLVLR	299	
Pig 240	LHEETPFTTSSDSEEEQEDEEEDVWVVEKRTQFAKRSSESSPFRGHSKPPHSPVLVLR	299	
Mouse 300	CHVSTHQHNYAAPSTRKDYPAKRAKIDSGVVLQKISNNRKC.SPSSDTEENDKRRTH	359	
Pig 300	CHVSTHQHNYAAPSTRKDYPAKRAKIDSGVVLQKISNNRKC.SPSSDTEENDKRRTH	359	
Mouse 360	NVLERQRNLEKRSFFALRDQIPELENNKAKKVVILKATAYLLSQADEHKITSEKDL	419	
Pig 360	NVLERQRNLEKRSFFARRDQIPELENNKAKKVVILKATAYLLSQADEHKITSEKDL	419	
Mouse 420	LRKRRQLKLEQLRNS 437		
Pig 420	LRKRRQLKLEQLRNS 437		

**D** Alignment of Klf4 mouse (NP\_034767.2) and porcine (ACF93739.1) sequences (the mouse sequence contains 9 aa fragment in the beginning which is not found in the pig)

Score	Identities	Positives	Gaps
827 bits (2135)	438/477 (92%)	449/477 (94%)	3/477 (0%)
Mouse 10	MAVSDALLPSTFTFASGPAGREKTLR.PAGAPTRWRREELSHMK-RLFP-L.PGRPYDLAA-	66	
Pig 1	MAVSDALLPSTFTFASGPAGREKTLR.PAGAPTRWRREELSHMKRLFP.LPGRPYDLAA	60	
Mouse 67	TVATDLESGG GAAC S+NFALL RRETEFNWLLDLDLDFILSMISLTHQESVAATVTSAS	126	
Pig 61	TVATDLESGGVAACSGSNPALLPARETEFNWLLDLDLDFILSMISLTHQESVAATVTSAS	120	
Mouse 127	ASSSSPASSGPASPSTCF.SFYPTRAGDGPVAASNTGGGLLYSRASAPPTAPFNLAD	186	
Pig 121	ASSSSPSSSGPASPSTCF.SFYPTRAGDGPVAGSTGGGLLYSRASAPPTAPFNLAD	180	
Mouse 187	DIWVSPSGGFVAELRLPELDPVYI.PPQQPQP.PGGGLMKGKFKLAKSLITPGSEYSSPVLIS	246	
Pig 181	DIWVSPSGGFVAELRLPELDPVYI.PPQQPQP.PGGGLMKGKFKLAKSLITPGSEYSSPVLIS	240	
Mouse 247	VSKGSDGSHFVVVAPYSGGPPRMCPKIKQEA.VPACTVSRSELAHL.SAGPQLSNGHRENT	306	
Pig 241	VSKGSDGSHFVVVAPYSGGPPRMCPKIKQEA.VPACTVSRSELAHL.SAGPQLSNGHRENT	300	
Mouse 307	HDFFLGRQLPRTTPTLSPPELLNSRDCHPGLPFPFGFHPGPNYFPLFDQMCSQVRS	366	
Pig 301	HDFFLGRQLPRTTPTL EELL+SRDCHP LPLPFGFHPGPNYFPLFDQ+Q QVF	360	
Mouse 367	LHYQELMPPGSCLEPEPKPKRGRSSP.RKRTATHTCDYAGCGKTYTFS.SHLKAHLRTHTG	426	
Pig 361	LHYQELMPPGSCMPEEPKPKRGRSSP.RKRTATHTCDYAGCGKTYTFS.SHLKAHLRTHTG	420	
Mouse 427	EKPYRCDWDGCGWKFAR.SDELT.RHYRKHGHRP.FQCCKCDRAF.SSDHLL.HMKRHF	483	
Pig 421	EKPYRCDWDGCGWKFAR.SDELT.RHYRKHGHRP.FQCCKCDRAF.SSDHLL.HMKRHF	477	

Figure S2: BLAST alignments of amino acid sequences of mouse and porcine reprogramming factors (<http://blast.ncbi.nlm.nih.gov>). A) Alignment of *Mus Musculus* (NP\_038661.2) and *Sus Scrofa* (NP\_001106531) Oct4 amino acid sequences. B) Alignment of *Mus Musculus* (AAH57574.1) and *Sus Scrofa* (NP\_001116669.1) Sox2 amino acid sequences. C) Alignment of *Mus Musculus* (AAB59728.1) and *Sus Scrofa* (CAA65753.1) c-myc amino acid sequences. D) Alignment of *Mus Musculus* (NP\_034767.2) and *Sus Scrofa* (ACF93739.1) Klf4 amino acid sequences.

## Supplementary Table

**Table S1.** Primers used for RT-PCR and real-time PCR

ENDOGENOUS PLURIPOTENCY GENES	PRIMER SEQUENCE	SOURCE REFERENCE
Oct4	5'-ATGAAAGCCCTGCAGAAGGAGCTAGAAC-3' 5'-TCTCTAGCCCAAGCTGATTGGCGATGTG-3'	Mizuno and Kosaka, 2008
Sox2	5'-TAGAGCTAGACTCCGGGCGATGA-3' 5'-TTGCCTTAAACAAGACCACGAAA-3'	Takahashi and Yamanaka, 2006
Nanog	5'-AGGGTCTGCTACTGAGATGCTCTG-3' 5'-CAACCACTGGTTTTTCTGCCACCG-3'	Takahashi <i>et al.</i> , 2007
Klf4	5'-GCGAACTCACACAGGCGAGAAACC-3' 5'-TCGCTTCCTCTCTCCGACACA-3'	Takahashi and Yamanaka, 2006
c-myc	5'-TGACCTAACTCGAGGAGGAGCTGGAATC-3' 5'-AAGTTTGAGGCAGTTAAAATTATGGCTGAAGC-3'	Takahashi and Yamanaka, 2006
Rex1	5'-ACGAGTGGCAGTTTCTTCTTGGGA-3' 5'-TATGACTCACTCCAGGGGGCACT-3'	Takahashi <i>et al.</i> , 2007
Utf1	5'-GGATGTCCCGGTGACTACGTCTG-3' 5'-GGCGGATCTGGTTATCGAAGGGT-3'	Takahashi <i>et al.</i> , 2007
 TRANSGENES		
pOSMK	5'-GTCGGACCACCTTGCCTTACACATG-3' 5'- CGGCTTCGGCCAGTAACGTTAG-3'	Petkov <i>et al.</i> , 2013
 DIFFERENTIATION GENES		
Afp	5'-AGTGC GTGACGGAGAAGAAT-3' 5'-TGTCTGGAAGCACTCCTCCT-3'	Choi <i>et al.</i> , 2005
Gata4	5'-CTCCTACTCCAGCCCCTACC-3' 5'-GTGGCATTGCTGGAGTTACC-3'	Choi <i>et al.</i> , 2005
Gata6	5'-GCAATGCATGCGGTCTCTAC-3' 5'-CTCTTGGTAGCACCAGCTCA-3'	Hay <i>et al.</i> , 2004
Nestin	5'-GGACAGGACCAAGAGGAACA-3' 5'-TCCCACCTCTGTTGACTTCC-3'	Choi <i>et al.</i> , 2005
Pax6	5'-AGACTTTAACCAAGGGCGGT-3' 5'-TAGCCAGGTGCGAAGAAGACT-3'	Choi <i>et al.</i> , 2005
Sox17	5'-CTTTATGGTGTGGCCAAAG-3' 5'-GGTCAACGCCTTCCAAGACT-3'	Kim <i>et al.</i> , 2010