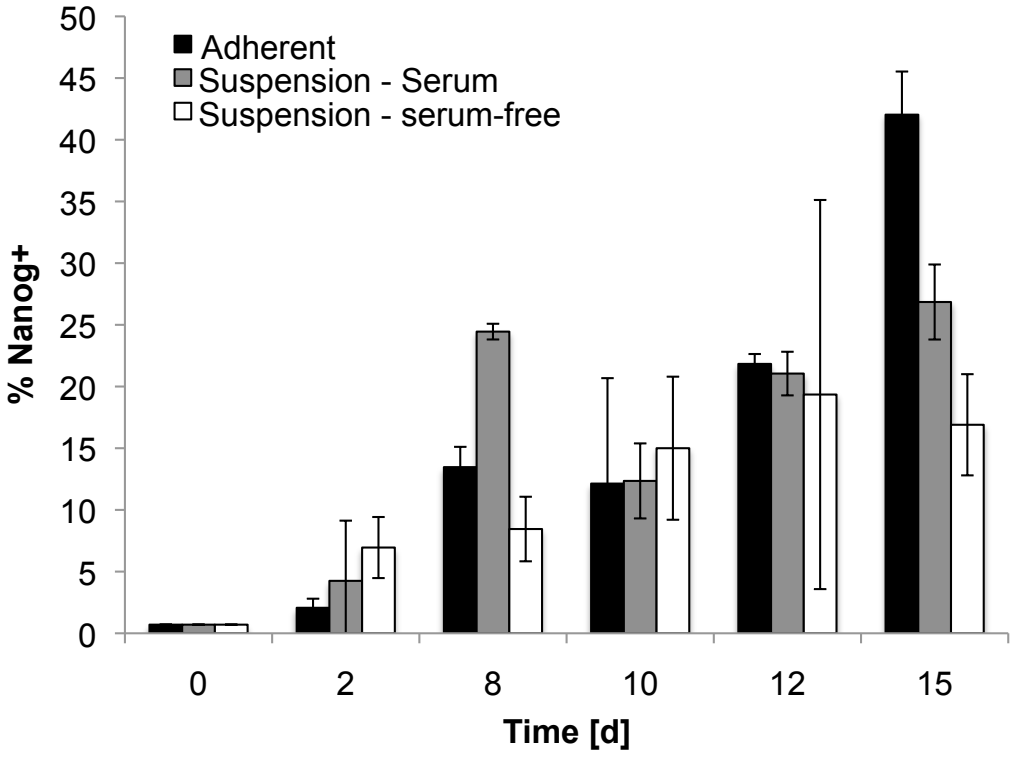
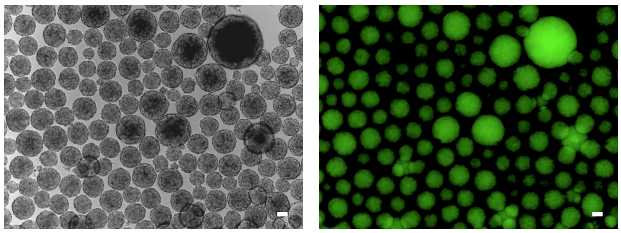
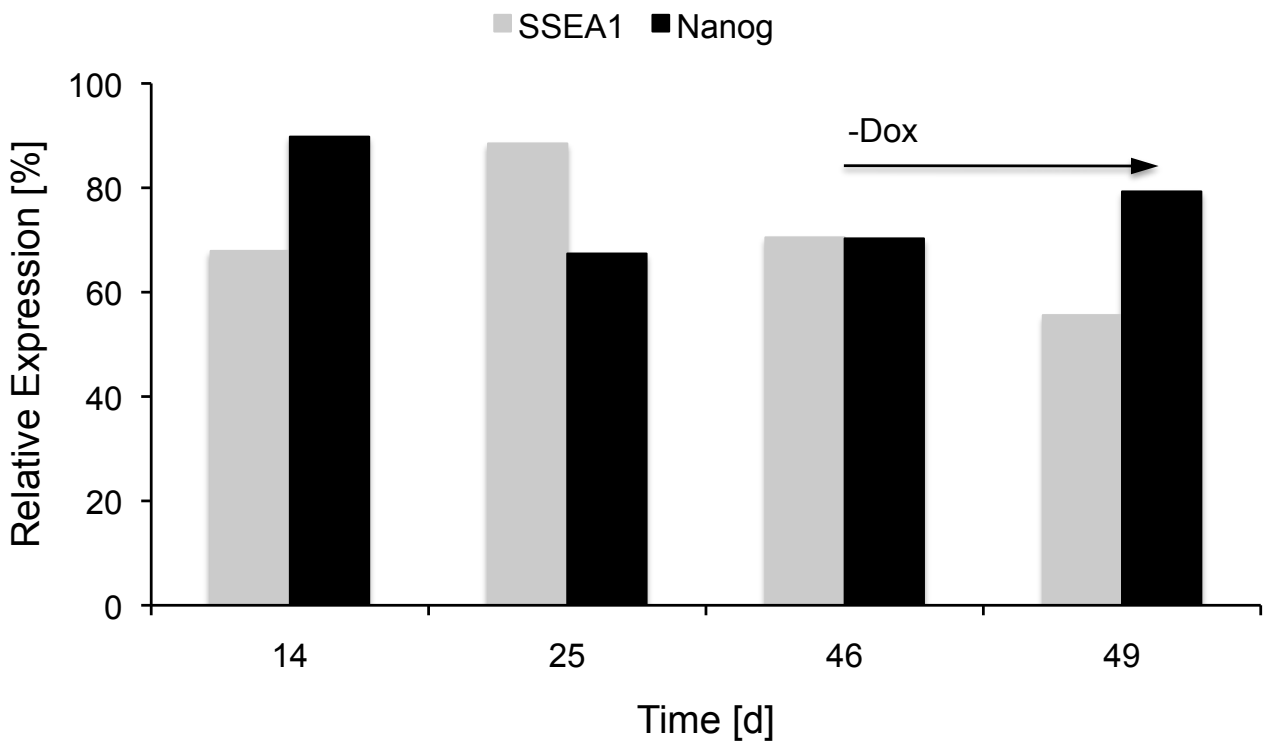


Supplementary Figure 1. FACS analysis of NANOG expression during suspension reprogramming in the presence of dox. NANOG expression profiles for doxycycline (Dox) induced 6C secondary fibroblast cells cultured in adherent conditions and in suspension conditions in the presence or absence of serum (data is presented as mean ± s.d., *n* = 2).

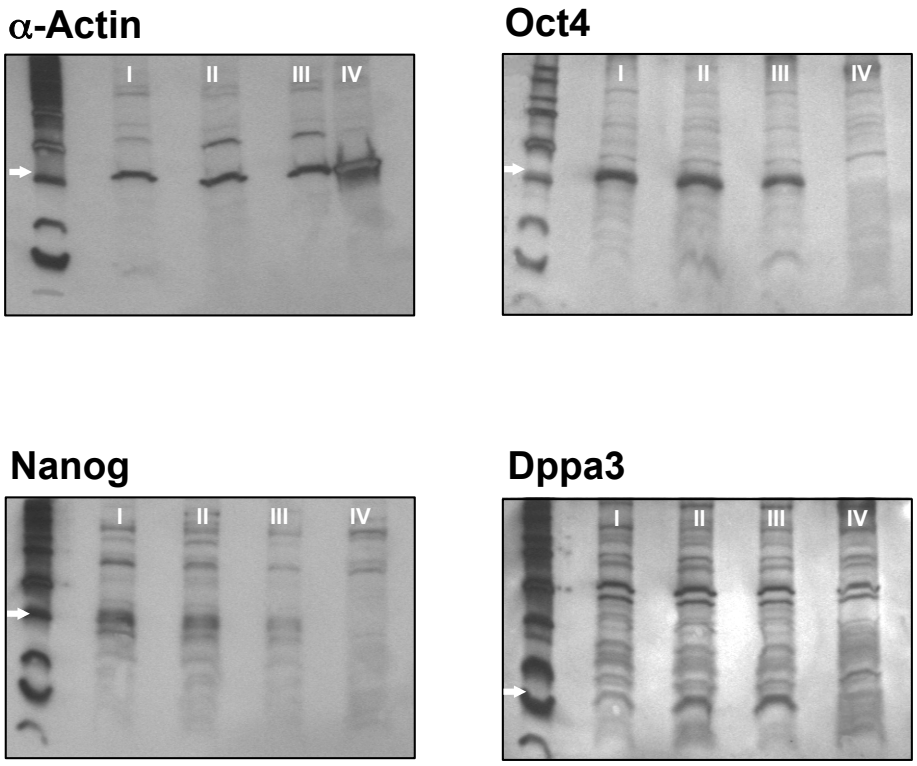


BF EGFP

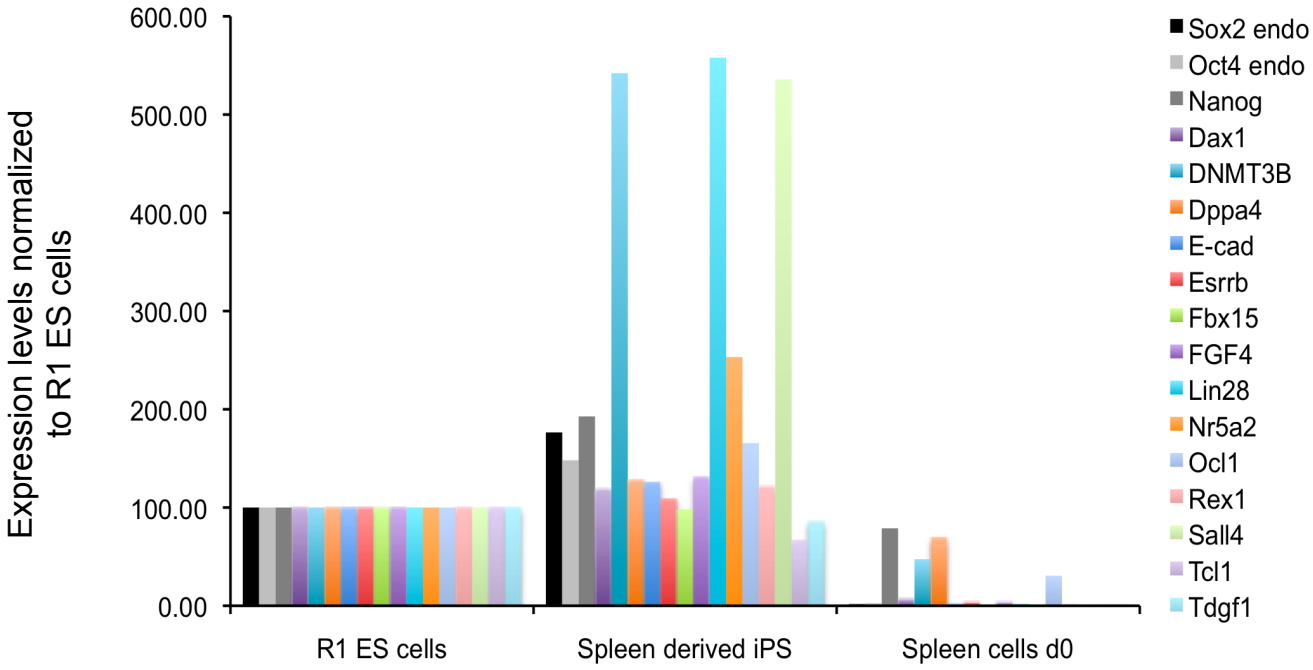
Supplementary Figure 2. Example micrographs of suspension reprogrammed iPSC aggregate cultures after dox removal. Withdrawal of dox from SiPSC cultures leads to formation and compaction of aggregates. SiPSC aggregates express EGFP from a *Rosa26-rtta-IRES-GFP* cassette expressed in secondary 6C cells. BF = bright field; EGFP = Enhanced Green Fluorescent Protein. Scale bars = 100μm



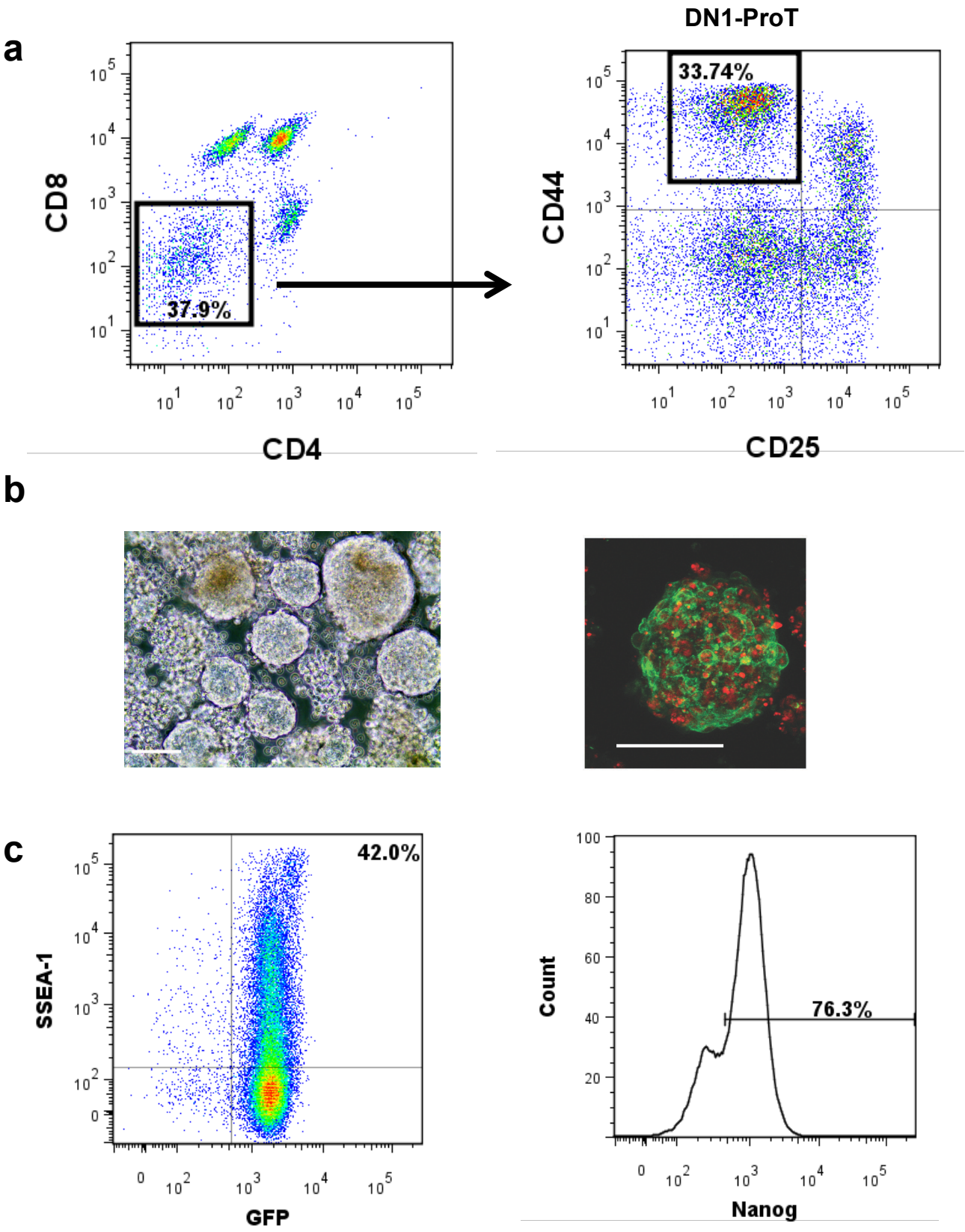
Supplementary Figure 3. Sustained SSEA-1 and NANOG expression in suspension reprogrammed and expanded secondary inducible MEF cells. Secondary 6C MEF cells were induced in suspension bioreactors and continously cultured for 46 days in the presence of dox. Cells were assayed for pluripotency marker expression at indicated timepoints. At day 46 dox was removed and cells were cultured in the absence of dox for another 13 days in suspension.



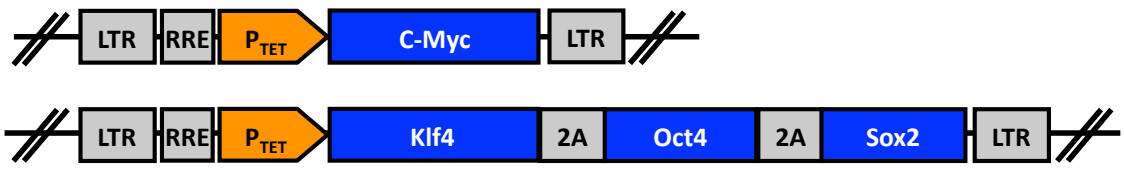
Supplementary Figure 4. Western blot analysis of alpha-actin, OCT4, NANOG and DPPA3 in R1 ES control (I), suspension-derived primary B6 iPSC (II), suspension-derived secondary 6C iPSC (III) and primary fibroblasts (IV). Protein standards of 10kD, 15kD, 20kD, 25kD, 37kD, 50kD, 75kD, 100kD and 150kD were used. Arrow indicates Mr(alpha-actin)~42kD, Mr(OCT4)~40kD, Mr(NANOG)~40kD and Mr(DPPA3)~17kD, respectively.



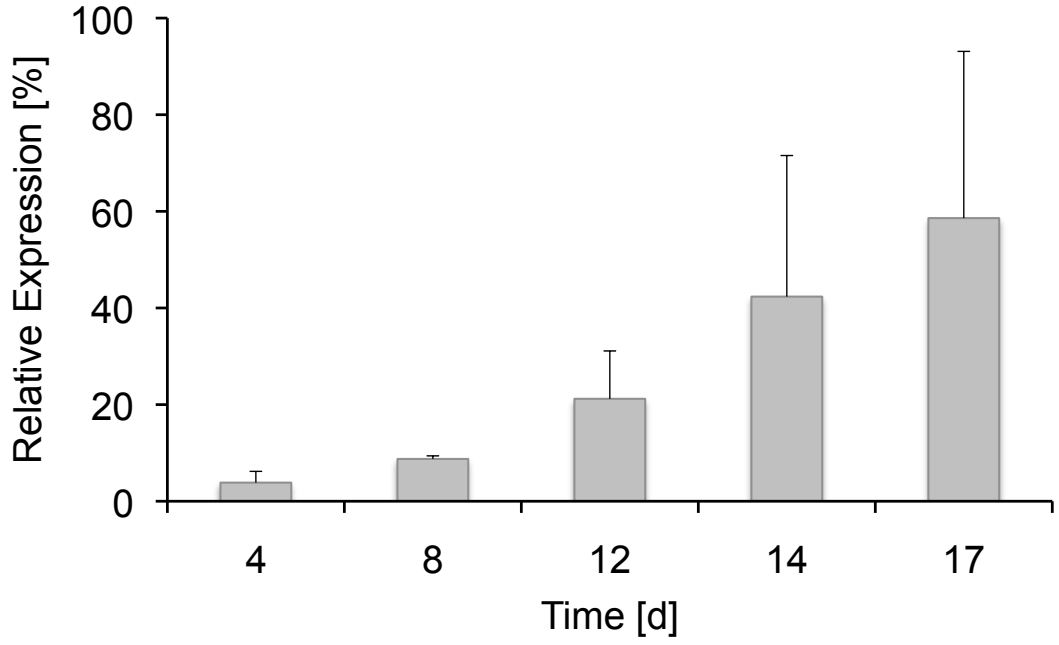
Supplementary Figure 5. Q-PCR analysis of pluripotency factor expression in spleen derived SiPSCs and non induced parental spleen cells from the same chimera. Quantitative PCR was performed for a panel of pluripotency associated genes. Expression levels of the factors in spleen derived suspension iPS cells and spleen cells at day 0 before dox induction were normalized to R1 ES control cells and compared.



Supplementary Figure 6. Suspension reprogrammed iPSCs from purified DN1-progenitor T cells from the thymus. (a) Gating strategy for isolation of immature DN1 progenitor T cells from secondary chimeric mice. Cells were isolated from the thymus, stained as described in materials and methods and sorted for $CD4^{-}CD8^{-}CD25^{-}CD44^{+}$ surface marker expression. (b) Morphology of DN1-derived SiPSCs reprogrammed with doxycycline in suspension after 16 days (left). Confocal immunofluorescence image of SSEA-1 expression (green) and nuclear counterstain (red) of DN-1-derived iPSCs (scale bar is 100 µm). (c) SSEA-1 surface marker and intracellular NANOG expression on suspension reprogrammed DN1-iPSCs after 25 days.



Supplementary Figure 7. **Schematic representation of vector constructs used to reprogram primary mouse fibroblasts**. IRES, internal ribosome entry site; LTR, long terminal repeat; PTET, doxycycline responsive Promoter; RRE, rev responsive element; 2A, self-cleaving peptide sequence;



Supplementary Figure 8. **FACS analysis of SSEA-1 expression in adult tail tip derived fibroblasts reprogramming in suspension**. Tail tip fibroblast cells from adult mice were transduced with MKOS and reprogrammed in suspension for a time period of 17 days in the presence of dox. Data is presented as mean ± s.d. (*n* = 2).

ID	Gene Name
Adam19	a disintegrin and metallopeptidase domain 19 (meltrin beta); similar to metalloprotease-disintegrin meltrin beta
Adamts4	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 4
Adamts5	similar to a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2); a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2)
Ahsg	alpha-2-HS-glycoprotein
Angptl4	angiopoietin-like 4
Aspn	asporin
Bmp4	bone morphogenetic protein 4
Ccdc80	coiled-coil domain containing 80
Chl1	cell adhesion molecule with homology to L1CAM
Col10a1	collagen, type X, alpha 1
Col12a1	collagen, type XII, alpha 1
Col18a1	collagen, type XVIII, alpha 1
Col5a2	collagen, type V, alpha 2
Col6a1	collagen, type VI, alpha 1
Col6a2	collagen, type VI, alpha 2
Col6a3	collagen, type VI, alpha 3
Col8a1	collagen, type VIII, alpha 1
Comp	cartilage oligomeric matrix protein
Ctgf	connective tissue growth factor
Dpt	dermatopontin
Fbln1	fibulin 1
Hapln1	hyaluronan and proteoglycan link protein 1
Kazald1	Kazal-type serine peptidase inhibitor domain 1
Lgals3bp	lectin, galactoside-binding, soluble, 3 binding protein
Lox	lysyl oxidase
Lum	lumican
Mamdc2	MAM domain containing 2
Matn2	matrilin 2
Mfap2	microfibrillar-associated protein 2
Mfap5	microfibrillar associated protein 5
Mmp11	matrix metallopeptidase 11
Mmp13	matrix metallopeptidase 13
Mmp19	matrix metallopeptidase 19
Mmp2	matrix metallopeptidase 2
Mmp3	matrix metallopeptidase 3
Nid1	similar to Nidogen precursor (Entactin); nidogen 1; similar to Nid1 protein
Olfml2a	olfactomedin-like 2A
Podn	podocan
Postn	periostin, osteoblast specific factor
Ptn	pleiotrophin
Sparcl1	SPARC-like 1
Spon2	spondin 2, extracellular matrix protein
Tgfb3	transforming growth factor, beta 3
Tgfbi	transforming growth factor, beta induced
Timp4	tissue inhibitor of metalloproteinase 4
Tnfrsf11b	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)
Tnxb	tenascin XB
Wnt16	wingless-related MMTV integration site 16
Wnt7a	wingless-related MMTV integration site 7A

Supplementary Table 1A. Gene list of transcripts grouped under the GO term “extracellular matrix” exhibiting > two-fold expression differences between adherent and suspension conditions at day two after induction.

ID	Gene Name
Adam8	a disintegrin and metallopeptidase domain 8
Agt	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)
Anxa9	annexin A9
Cadm3	cell adhesion molecule 3
Cd97	CD97 antigen
Cdh2	cadherin 2; similar to N-cadherin
Cdh3	cadherin 3
Chl1	cell adhesion molecule with homology to L1CAM
Cldn1	claudin 1
Cldn11	claudin 11
Col12a1	collagen, type XII, alpha 1
Col18a1	collagen, type XVIII, alpha 1
Col6a1	collagen, type VI, alpha 1
Col6a2	collagen, type VI, alpha 2
Col8a1	collagen, type VIII, alpha 1
Comp	cartilage oligomeric matrix protein
Cpxm1	carboxypeptidase X 1 (M14 family)
Ctgf	connective tissue growth factor
Ctnnd2	catenin (cadherin associated protein), delta 2
Cx3cl1	chemokine (C-X3-C motif) ligand 1
Cyr61	cysteine rich protein 61
Dcbld2	discoidin, CUB and LCCL domain containing 2
Dpt	dermatopontin
Dsg1b	desmoglein 1 beta
Dsg3	desmoglein 3
Dsg4	desmoglein 4
Edil3	EGF-like repeats and discoidin I-like domains 3
Fblim1	filamin binding LIM protein 1
Fndc3a	fibronectin type III domain containing 3A
Gpnmb	glycoprotein (transmembrane) nmb
Hapln1	hyaluronan and proteoglycan link protein 1
Itga10	integrin, alpha 10
Itga11	integrin alpha 11
Itgb3	integrin beta 3
Itgb8	integrin beta 8
Jub	ajuba
Lgals3bp	lectin, galactoside-binding, soluble, 3 binding protein
Lpp	LIM domain containing preferred translocation partner in lipoma
Lypd3	Ly6/Plaur domain containing 3
Mcam	melanoma cell adhesion molecule
Megf10	multiple EGF-like-domains 10
Mpzl2	myelin protein zero-like 2
Msln	mesothelin
Nell2	NEL-like 2 (chicken)
Nid1	similar to Nidogen precursor (Entactin); nidogen 1; similar to Nid1 protein
Pcdh1	protocadherin 1
Pcdhb16	protocadherin beta 16
Pcdhb17	protocadherin beta 17
Pcdhb18	protocadherin beta 18
Pcdhb19	protocadherin beta 19
Postn	periostin, osteoblast specific factor
Pvr	poliovirus receptor
Sdk2	sidekick homolog 2 (chicken)
Sned1	sushi, nidogen and EGF-like domains 1
Sox9	SRY-box containing gene 9
Spon2	spondin 2, extracellular matrix protein
Spp1	secreted phosphoprotein 1
Svep1	sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1
Tgfbi	transforming growth factor, beta induced

Supplementary Table 1B. Gene list of transcripts grouped under the GO term “cell adhesion” exhibiting > two-fold expression differences between adherent and suspension conditions at day two after induction.

Name	Forward	Reverse
Q-PCR primer		
B-actin	GAAATCGTGCGTGACATCAAAG	TGTAGTTTCATGGATGCCACAG
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Hprt1	CACAGGACTAGAACACCTGC	GCTGGTGAAAAGGACCTCT
C-myc (TOTAL)	TCCACCGCCGATCAGCTGGA	TGGCAGCGGCTGAGAAACCG
Cdh1 (1)	TCTACCAAAGTGACGCTGAAGTCC	GGTACACGCTGGGAAACATGAG
Cdh1 (2)	CAGGTCTCCTCATGGCTTTGC	CTTCCGAAAAGAAGGCTGTCC
Cdh2	AGCGCAGTCTTACCGAAGG	TCGCTGCTTTCATACTGAACTTT
Cripto (Tdgf1)	CAGTGCGTTTGAATTTGGACCCGT	AGTCCCTCCATTCAGACAGCAAGT
Dax1	CCTGCACTTCGAGATGATGGA	GCCTGGTGGTAAGCATTTCC
DNMT3B	AGCGGGTATGAGGAGTGCAT	CTGCGTGTAATTCAGAAGGCT
Dppa4	AGCATCTTGGGCCAGAATTGCATC	TTCTTATCGGGTACCCAGGCTTGT
Esrrb	AACCGAATGTCGTCCGAAGAC	GTGGCTGAGGGCATCAATG
Fbx15	TCGTGGGACTGAGCACAACTA	TGACAGATGAGCCTCTAACAAAC
Fgf4	GTGTGCCTTTCTTTACCGACG	CTGAGGGCCATGAACATACCG
Klf2	CTCAGCGAGCCTATCTTGCC	CACGTTGTTTAGGTCCTCATCC
Lin28	TGTTCTGTATTGGGAGTGAGC	GCTTGCATTCCTTGGCATG
Nanog (1)	AACCAAAGGATGAAGTGCAAGCGG	TCCAAGTTGGGTTGGTCCAAGTCT
Nanog (2)	TTGCTTACAAGGGTCTGCTACT	CCACAAACAGATCCGGCTT
Nr5a2	GTGGCGATAAAGTGTCTGGGT	GCGTTTTGTCAATTTGGCAATTC
Ocln	CCTCCAATGGCAAAGTGAATGGCA	TGTTTCATAGTGGTCAGGGTCCGT
PeCam1	GCTGGCAACAAGTTGCTCTCTGAA	CGTTGCACTGCTTGGCTTTGAAGA
Pou5f1 (endo)	CCATGCATTCAAACTGAGGCACCA	AGCTATCTACTGTGTGTCCCAGTC
Rex1	CCCTCGACAGACTGACCCTAA	TCGGGGCTAATCTCACTTTCAT
SALL4	TGGTCCAGCCAATGACTCTTCCTT	TCGGATAAATGTTGGAGGGAGGCT
Sox2 endo	ACTAGGGCTGGGAGAAAGAAGAGG	TTAAGCCTCGGGCTCCAAACTTC
Tcl1	TGGGAGAAGCACGTGTACTTGGAT	GTTGCCACATTAAAGGCAGCTCGT
Thy1	TTACCCTAGCCAACTTCACCACCA	AAATGAAGTCCAGGGCTTGGAGGA
Zfp42 (1)	CCCTCGACAGACTGACCCTAA	TCGGGGCTAATCTCACTTTCAT
Zfp42 (2)	GAAAGTGAGATTAGCCCCGAG	GTCCCCTTTGTCATGTACTCC
Cloning Primer		
cMyc	ACCCTGAACTTCGACCTGCTGAAGCTGGCCG GCGACGTGGAGAGCAACCCCGGCCCTGCTAG CATGCCCCTCAACGTGAACTTC	AGTCGATATCAGATCTTTATGCACCAGAGTTTCGAA
eGFP	GGAATTCCACCATGGGTAGCAACAAGAGCAAG CCCAAGGATGCCAGCCAGCGGCGCGAGGCC GCTAGCATGGTGAGCAAGGGCGAG	CGGGGTACCGTTTAAACTCTAGATCTTACTTGTACAG CTCGTCCA
IRES	GCGAATTCACGCGTAGTCGATAAGCTAATTCCG CC	GCTCTAGAGCTAGCGTTGTGGCCATATTATCATCGT
Klf4	ACCCTGAACTTCGACCTGCTGAAGCTGGCCG GCGACGTGGAGAGCAACCCCGGCCCTGCTAG CATGGCTGTCAGCGACGCTCTGC	
PCAG	CCATCGATACGCGTCTCGAGTCGACATTGATTA TTGACTAGT	
Ptet	GTTGGCGCGCCTGCTCTCGTTAATTAACTCG	CGATGCGCTAGCTTGTGATGGCCGCCACCGC
rTTA		GCTCTAGAGCTAGCACGCGTTACCCGGGGAGCATGT CAAGG
Sox2	ACCCTGAACTTCGACCTGCTGAAGCTGGCCG GCGACGTGGAGAGCAACCC	CGGCCCTGCTAGCATGTACAACATGATGGAGAC

Supplementary Table 2. **Sequences of oligonucleotides used in this study**.

Name	Company	Catalogue number
Actin	BD Biosciences	BD 612656
Actn1	Sigma	A7811
AnnexinV Alexa 647	Invitrogen	A23204
Tubb3	Sigma	T8660
cTnT	Thermo	MS-295-P1
CD4	BD Biosciences	552051
CD8a	BD Biosciences	553035
CD25	BD Biosciences	552880
CD44	BD Biosciences	553134
Flk-1-APC	BD Biosciences	560070
Foxa2	Abcam	ab40874
GFP	Invitrogen	G10362
Klf4	R&D	AF3158
Nanog	eBiosciences	14-5761-80
Nanog (western blot)	Milipore	AB5731
Oct3/4	BD Biosciences	611203
Pdgfrα	eBiosciences	13-1401-82
Sox2	R&D	MAB2018
SSEA-1	eBiosciences	14-8813-82
Dppa3	Santa-Cruz	Sc-67249
Tbx3	Santa-Cruz	Sc-17871

Supplementary Table 3. **Antibodies used in this study.**