# Supplemental Material

*Table S1. List of primers, cycle numbers (# cycles) and temperatures (ºC)*

| rat qPCR | Gene | For | Rev | | |
|---|---|---|---|---|---|
| | *Ctgf* | ATCCCTGCGACCCACACA | ACGGACCCACCGAAGACA | | |
| | *Cyr61* | CCACCGCTCTGAAAGGGA | CCACAGCACCGTCAATACATG | | |
| | *Plk2* | CCGAGATCTCGCGGATTATAGT | CTGTCATTTCGTAACACTTTGCAA | | |
| | *Gata 4* | CCTGCGAGACACCCCAATC | TCCTGTCCCATCTCGCTC | | |
| | *Tnni3* | AGCCACATGCCAAGAAAAAGTC | TCACGCTCCATCTCCTGCTT | | |
| | *Myh7b* | GGTGAGCGTGGTTACCATGTCT | GTGGTGACCCCCTGACTGC | | |
| | *Vwf* | TGAGAACCAGCGGTGTAAACG | CCGACGCCGTCTTCAGTAAC | | |
| | *Pecam1* | AGCATTGTGACCAGTCTCCGA | GCAATGACCACTCCAATGACAA | | |
| | *Lats2* | GGAGTTGGTGAATGCAGGATGT | TTGCTCATTCCTGGGGTCC | | |
| | *r18S* | CCATTCGAACGTCTGCCCTAT | GTCACCCGTGGTCACCATG | | |
| **rat PCR** | **Gene** | **For** | **Rev** | # cycles | ºC |
| | *Abcg2* | ACCCTGCAGACTTCTTCCTTGAC | AGTAAAGGGCACCAATAATCAGTCC | 30 | 60 |
| | *Kit* | CGCCAGGAGACGCTGACTAT | TTAGGGTAGGCCTCGAACTCAAC | 30 | 60 |
| | *Pou5f1* | GCGCCGTGAAGTTGGAGA | TGATCCTCTTCTGTTTCAGCAGC | 30 | 60 |
| | *Nanog* | AGGTACCTCAGCCTCCAGCA | CTGCCACCTCTTGCACTTCA | 38 | 60 |
| | *Abcb1a* | AGCCCTGTTCTTGGACTGTCA | TTGCATAAGCCTGGAGTTCCTTA | 30 | 60 |
| | *Abcb1b* | AACCTGCTGTTGGCATATTCG | GTGGATGATAGCAGCGAGAGTTC | 30 | 60 |
| | *Nkx2.5* | ATTTTATCCGCGAGCCTACG | CAGGTACCGCTGTTGCTTGAA | 30 | 60 |
| | *Gata4* | CTGTGCCAACTGCCAGACTA | AGATTCTTGGGCTTCCGTTT | 25 | 57 |
| | *Myh7b* | GGTGAGCGTGGTTACCATGTCT | ACTCTGGCCCTTGGTCACATAC | 30 | 57 |
| | *Myh6* | TGATGACTCCGAGGAGCTTT | TGACACAGACCCTTGAGCAG | 39 | 60 |
| | *Tnni3* | ACGTGGAAGCAAAAGTCACC | CCTCCTTCTTCACCTGCTTG | 30 | 57 |
| | *Vwf* | TGAGAACCAGCGGTGTAAACG | CCGACGCCGTCTTCAGTAAC | 32 | 57 |
| | *Pecam1* | AGCATTGTGACCAGTCTCCGA | GCAATGACCACTCCAATGACAA | 32 | 57 |
| | *Gapdh* | CAGAACATCATCCCTGCATCC | AGGTCCACCACCCTGTTGC | 30 | 57 |
| | *Tbx18* | TGCCAAGGCTTCCGAGAC | AAGGTGAGAGTTCGTAGTGATGGC | 25 | 57 |
| | *Wt1* | CATCCTCTGTGGTGCCCAGT | CAGATGCTGACCGGACAAGAG | 30 | 57 |
| | *Aldh1a2* | TACATCGATTTGCAGGGAGTCA | TAGACCACAGTGTTACCACAGCA | 30 | 57 |
| | *Tcf21* | AAGGCCTTCTCCAGGCTCAA | CTCGCGGTCACCACTTCCT | 30 | 57 |

| mouse qPCR | Gene | For | Rev | | |
|---|---|---|---|---|---|
| | *Ctgf* | CTTCTGCGATTTCGGCTCC | ACACCGACCCACCGAAGAC | | |
| | *Cyr61* | CCACCGCTCTGAAAGGGAT | CACGGCGCCATCAATACAT | | |
| | *Plk2* | ATGGAGCTGAAGGTGGGAGAC | GAGGACTTCGGGGGAGAGATA | | |
| | *Gata 4* | GCCAACCCTGGAAGACACC | GACATGGCCCCACAATTGAC | | |
| | *Tnni3* | CTGCCAACTACCGAGCCTATG | CGTTCCATCTCCTGCTTCG | | |
| | *Vwf* | GATGGAGGGGAGCTTGAACTG | CGACTCCACCACCTCAAAGTG | | |
| | *r18S* | CCATTCGAACGTCTGCCCTAT | GTCACCCGTGGTCACCATG | | |
| **mouse PCR** | **Gene** | **For** | **Rev** | # cycles | ºC |
| | *Abcg2* | CATGAAACCTGGCCTTAATGC | CTCCTCCAGAGATGCCACG | 25 | 60 |
| | *Kit* | CAGGACCTCGGCTAACAAAGG | TGGTCAGGCGAAGTTGGTTC | 25 | 60 |
| | *Pou5f1* | GGAGTCCCAGGACATGAAAGC | TGCTGTAGGGAGGGCTTCG | 25 | 60 |
| | *Nanog* | GGTGGCAGAAAAACCAGTGG | GCTTCCAGATGCGTTCACC | 38 | 60 |
| | *Abcb1a* | ATAATAGGATTTACCCGTGGCTGG | CCCATACCAGAATGCCAGAGC | 25 | 60 |
| | *Abcb1b* | TCAACTACCCATCGAGAAGCG | GGCATTGGCTTCCTTGACAG | 25 | 60 |
| | *Nkx2.5* | CCTGACCCAGCCAAAGACC | CACTTGTAGCGACGGTTCTGG | 38 | 60 |
| | *Gata4* | GCCGTATCATCACCAGAATCC | TCCAGCCTCTCGGTCATCTC | 25 | 60 |
| | *Myh7b* | CCGTTTTGGCAAGTTCATCC | AAGTTCCTCGCCGTCATCC | 30 | 57 |
| | *Myh6* | GCCAACCCTGGAAGACACC | TTGCAAGAGGCCTGGGAA | 38 | 60 |
| | *Tnni3* | CTGCCAACTACCGAGCCTATG | CCCTCAGGTCCAAGGATTCC | 30 | 60 |
| | *Vwf* | GATGGAGGGGAGCTTGAACTG | AGTTGACGGGGTCTTCCTCC | 38 | 60 |
| | *Pecam1* | CGTGAATGACACCCAAGCG | CACGGGTTTCTGTTTGGCC | 38 | 60 |
| | *Gapdh* | CAGAACATCATCCCTGCATCC | AGGTCCACCACCCTGTTGC | 25 | 60 |

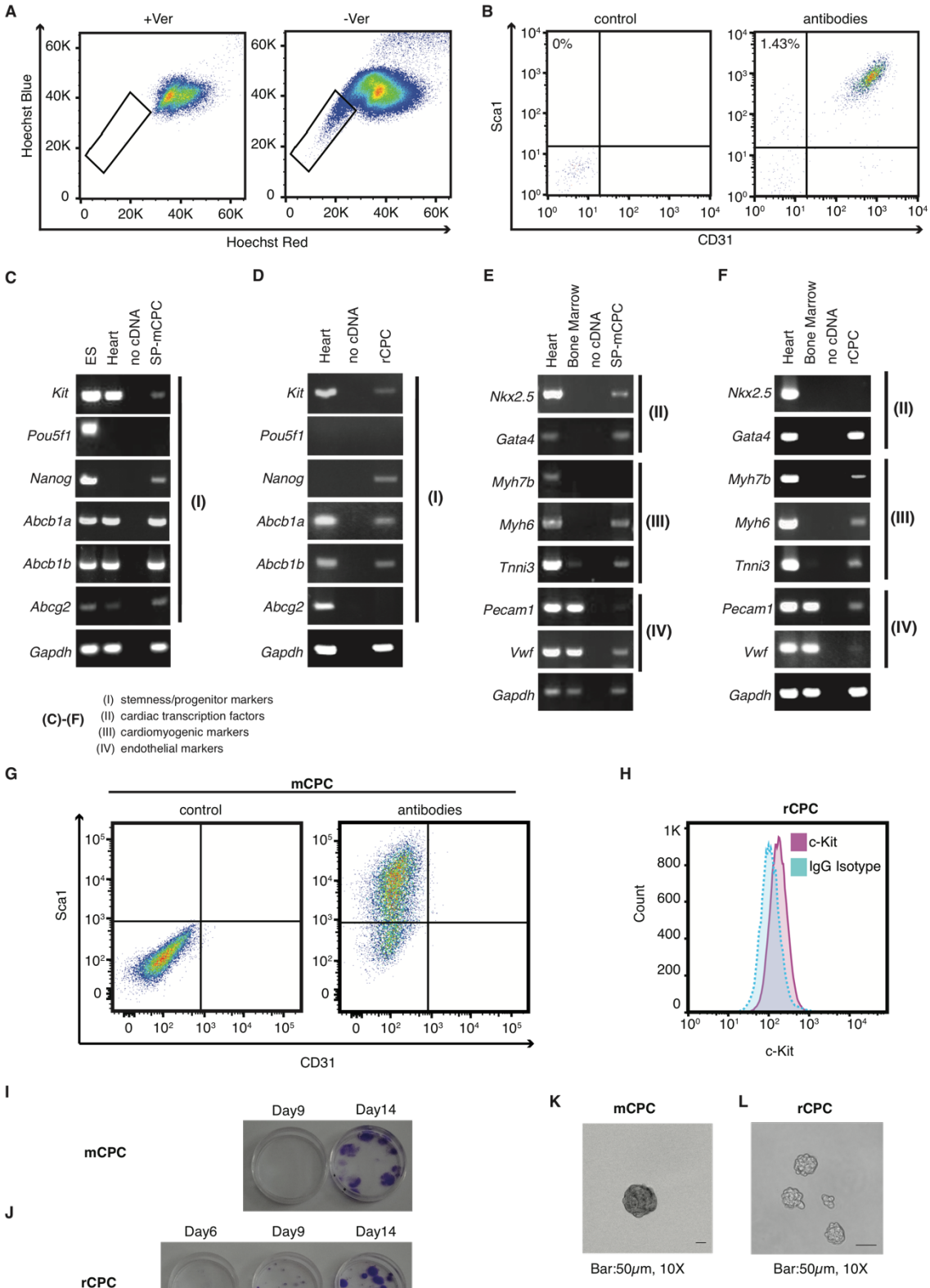
*Table S2. Complete list of genes regulated on FN versus LN*

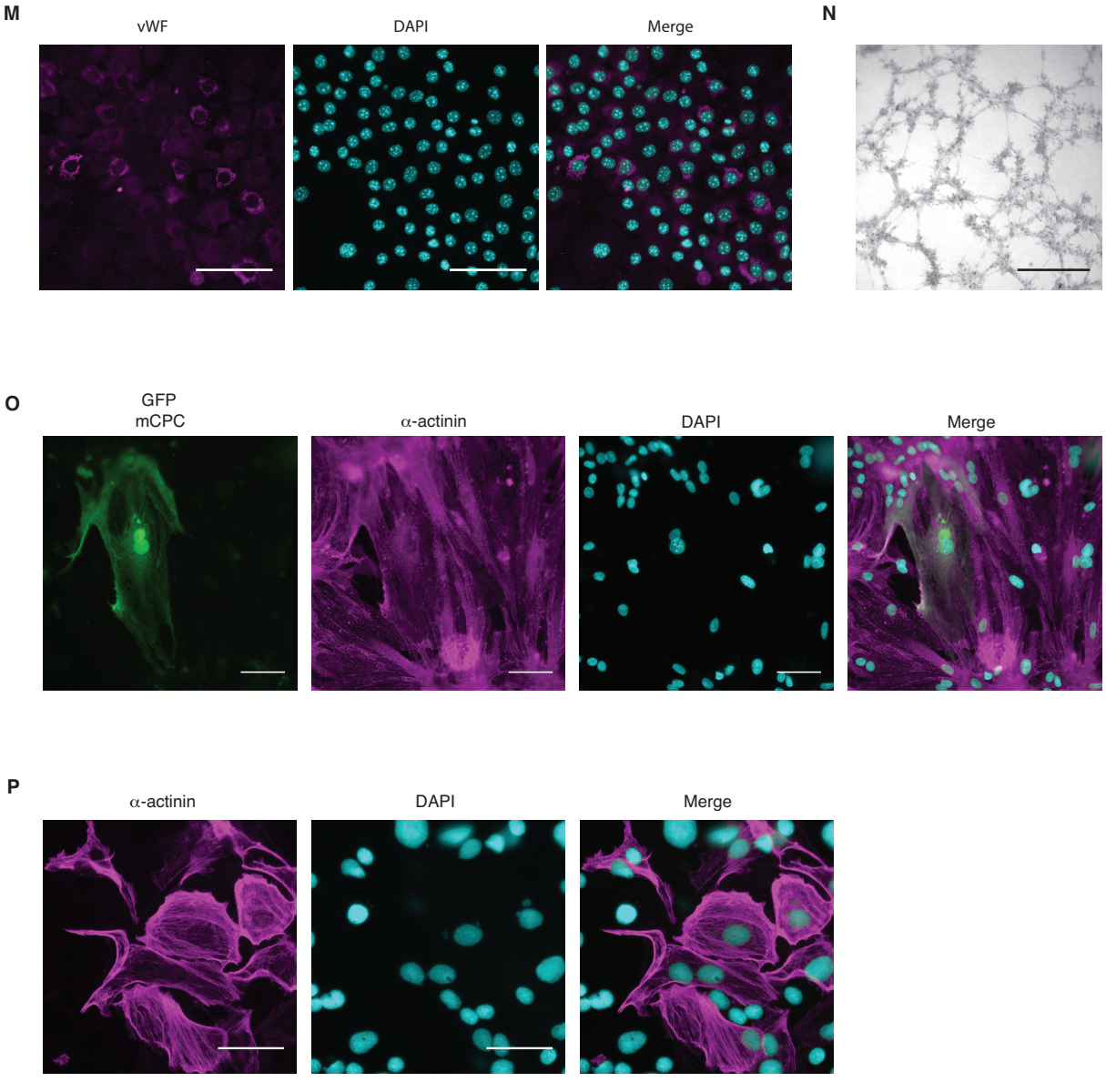
| | EntrezID | Symbol | log2FC | adj.P.Val |
|---|---|---|---|---|
| **1** | 83476 | Cyr61 | 3.39 | 2.5E-40 |
| **2** | 64032 | Ctgf | 2.04 | 2.7E-30 |
| **3** | 83722 | Plk2 | 1.71 | 4.2E-28 |
| **4** | 287362 | Nlrp3 | 1.69 | 1.4E-14 |
| **5** | 100861535 | Rn28s | 1.51 | 1.1E-03 |
| **6** | 24723 | Rn45s | 1.47 | 2.4E-06 |
| **7** | 65157 | Amotl2 | 1.43 | 1.3E-27 |
| **8** | 85265 | Ajuba | 1.41 | 3.5E-29 |
| **9** | 289419 | Nuak2 | 1.34 | 3.8E-20 |
| **10** | 259227 | Vof16 | 1.31 | 9.2E-10 |
| **11** | 27064 | Ankrd1 | 1.23 | 2.4E-06 |
| **12** | 299626 | Gadd45b | 1.20 | 2.2E-13 |
| **13** | 85471 | Gata3 | 1.16 | 9.0E-11 |
| **14** | 362993 | Rnd1 | 1.09 | 1.3E-10 |
| **15** | 25433 | Hbegf | 1.07 | 1.1E-03 |
| **16** | 29637 | Hmgcs1 | 1.06 | 1.7E-06 |
| **17** | 366492 | Epha2 | 1.03 | 1.1E-06 |
| **18** | 361679 | Dusp8 | 0.96 | 1.6E-05 |
| **19** | 64534 | Pim3 | 0.93 | 3.6E-12 |
| **20** | 306330 | Klf2 | 0.91 | 1.4E-07 |
| **21** | 64194 | Insig1 | 0.88 | 2.4E-06 |
| **22** | 24883 | Wt1 | 0.84 | 1.0E-02 |
| **23** | 316842 | Metrnl | 0.84 | 1.3E-10 |
| **24** | 306636 | Efnb2 | 0.81 | 1.3E-06 |
| **25** | 300866 | Lca5 | 0.81 | 2.5E-03 |
| **26** | 295588 | Rnd3 | 0.79 | 2.6E-02 |
| **27** | 29517 | Sgk1 | 0.79 | 1.4E-07 |
| **28** | 362598 | Sh3d21 | 0.79 | 2.2E-02 |
| **29** | 363243 | Klf7 | 0.77 | 8.5E-08 |
| **30** | 24323 | Edn1 | 0.74 | 2.2E-02 |
| **31** | 498159 | Spry3 | 0.74 | 4.6E-02 |
| **32** | 64373 | Rhob | 0.74 | 1.4E-07 |
| **33** | 310533 | Rapgef2 | 0.72 | 6.1E-06 |
| **34** | 25675 | Hmgcr | 0.70 | 1.3E-06 |
| **35** | 81503 | Cxcl1 | 0.69 | 4.9E-04 |
| **36** | 287925 | Pkp2 | 0.69 | 2.1E-03 |
| **37** | 500400 | Fam110b | 0.69 | 8.1E-04 |
| **38** | 292844 | Siglec10 | 0.67 | 2.2E-02 |
| **39** | 499528 | Ccno | 0.63 | 2.2E-02 |
| **40** | 25556 | Il1rl1 | 0.62 | 2.2E-02 |
| **41** | 364754 | Fzd8 | 0.60 | 3.0E-04 |
| **42** | 24577 | Myc | 0.60 | 4.9E-04 |
| **43** | 24530 | Lcat | 0.60 | 2.9E-02 |
| **44** | 500300 | LOC500300 | 0.60 | 5.5E-03 |

| | | | | |
|---|---|---|---|---|
| **45** | 360580 | Dusp14 | 0.59 | 5.1E-04 |
| **46** | 498796 | Fam107b | 0.59 | 5.6E-06 |
| **47** | 365864 | Tuft1 | 0.59 | 3.9E-05 |
| **48** | 89830 | Ptch1 | 0.58 | 1.1E-03 |
| **49** | 58834 | Dlc1 | 0.57 | 4.2E-04 |
| **50** | 299694 | Nuak1 | 0.57 | 7.9E-04 |
| **51** | 246760 | Mafk | 0.56 | 3.5E-04 |
| **52** | 360202 | Ston1 | 0.55 | 3.5E-04 |
| **53** | 501584 | Amer1 | 0.55 | 3.9E-05 |
| **54** | 24373 | Fst | 0.55 | 2.3E-03 |
| **55** | 300438 | Ldlr | 0.54 | 1.7E-03 |
| **56** | 315259 | Prickle1 | 0.54 | 2.4E-03 |
| **57** | 688993 | Kctd7 | 0.53 | 2.7E-02 |
| **58** | 65154 | Wisp1 | 0.53 | 4.0E-02 |
| **59** | 63839 | Fhl2 | 0.52 | 6.0E-04 |
| **60** | 64562 | Prkab2 | 0.51 | 1.1E-02 |
| **61** | 498963 | Spata2L | 0.51 | 4.2E-02 |
| **62** | 81809 | Tgfb2 | 0.51 | 5.4E-04 |
| **63** | 305922 | Lats2 | 0.50 | 1.3E-03 |
| **64** | 686117 | Meis1 | 0.50 | 2.7E-04 |
| **65** | 25690 | Ahr | 0.50 | 4.2E-02 |
| **66** | 140910 | Msmo1 | 0.50 | 9.3E-04 |
| **67** | 300803 | Lactb | 0.50 | 3.3E-03 |
| **68** | 299139 | Slc38a6 | 0.50 | 4.9E-02 |
| **69** | 25105 | Nppb | 0.50 | 1.5E-02 |
| **70** | 29376 | Irs2 | 0.48 | 9.5E-03 |
| **71** | 310341 | Fat4 | 0.47 | 3.3E-02 |
| **72** | 29619 | Btg2 | 0.46 | 1.5E-02 |
| **73** | 291699 | Stard4 | 0.46 | 2.9E-02 |
| **74** | 83514 | Tsc22d3 | 0.46 | 8.9E-03 |
| **75** | 309728 | Arid5b | 0.44 | 2.6E-03 |
| **76** | 501099 | Srf | 0.43 | 1.8E-02 |
| **77** | 296583 | Nacc2 | 0.43 | 2.6E-02 |
| **78** | 500000 | Cldn12 | 0.43 | 3.9E-02 |
| **79** | 691922 | Nt5dc3 | 0.42 | 3.4E-02 |
| **80** | 499891 | RGD1565616 | 0.41 | 1.4E-02 |
| **81** | 29230 | Sqle | 0.40 | 4.2E-02 |
| **82** | 311630 | Zswim3 | 0.39 | 2.5E-02 |
| **83** | 360899 | Sertad4 | 0.38 | 4.6E-02 |
| **84** | 303185 | Flcn | -0.38 | 4.2E-02 |
| **85** | 94268 | Efna1 | -0.42 | 6.3E-03 |
| **86** | 314480 | Gpr132 | -0.48 | 1.3E-02 |
| **87** | 246142 | Bmf | -0.51 | 2.9E-02 |
| **88** | 501925 | Slc2a9 | -0.51 | 3.2E-02 |
| **89** | 295934 | Chst1 | -0.52 | 2.6E-02 |
| **90** | 100526644 | Mir3593 | -0.60 | 6.7E-03 |
| **91** | 289399 | RGD1311892 | -0.69 | 1.5E-02 |

| | | | | |
|---|---|---|---|---|
| **92** | 66015 | Adamts4 | -0.70 | 2.7E-06 |
| **93** | 117274 | Nr0b2 | -0.70 | 8.5E-03 |
| **94** | 297902 | Gem | -0.81 | 8.2E-09 |

***Figure S1. Characterization of mouse and rat CPCs.***

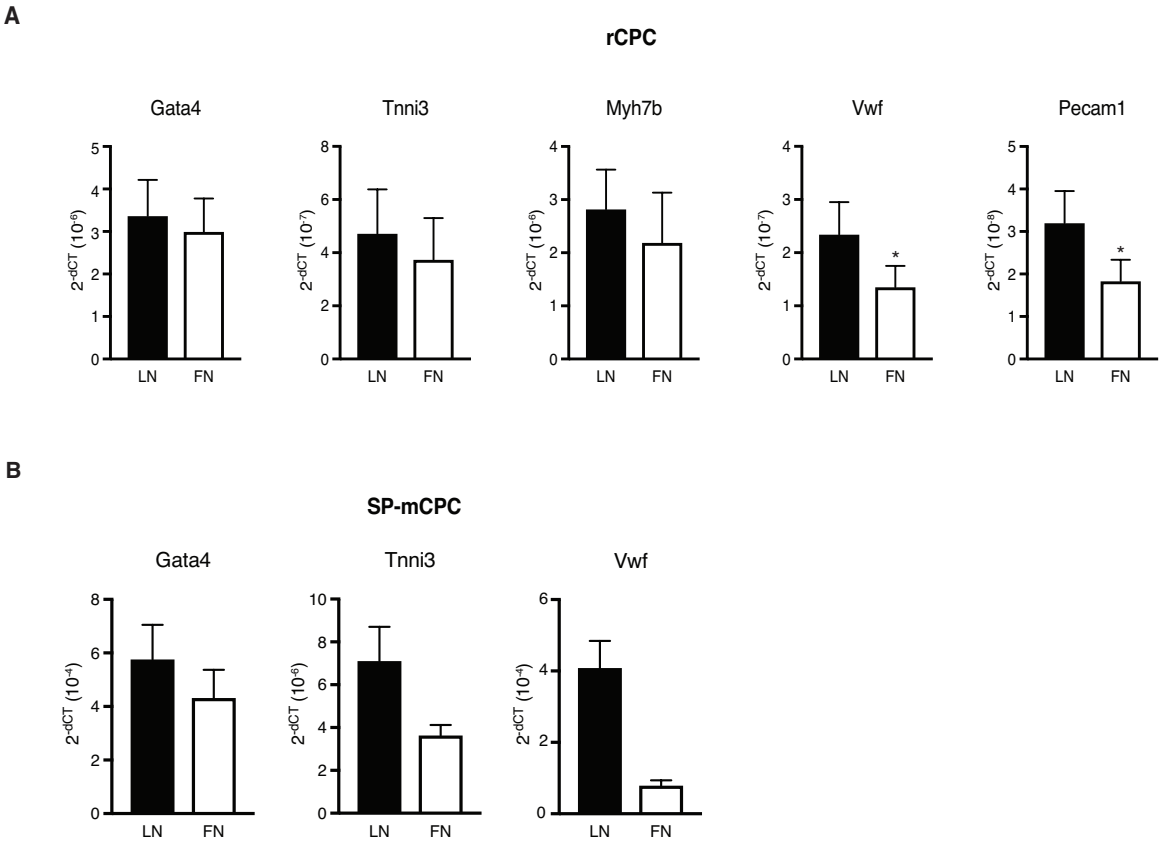



M
vWF
DAPI
Merge
N
O
GFP
mCPC
α-actinin
DAPI
Merge
P
α-actinin
DAPI
Merge
Q
Bacteriological dishes
Culture dishes
3 Days
2-3 Days
7-12 Days
CardioStem sphere generation
Culture Medium (-supp. +FCS)
Cardiomyogenic Medium
100nM Oxytocin

**(A), (B)** *Isolation of Sca1$^+$CD31$^-$ side population (SP) mouse CPCs (SP-mCPCs).* (A) Sorting of freshly isolated cardiac SP-mCPCs by Hoechst 33324 staining with/without Verapamil (Ver; ABC transporter inhibitor) using flow cytometry. (B) Staining of freshly isolated SP-mCPCs with anti-CD31 and Sca1 antibodies (control: non-stained freshly isolated SP-mCPCs; percentage given for Sca1$^+$/ CD31$^-$ fraction). **(C), (D)** *Gene expression of stemness and progenitor markers of SP-mCPCs and of rat CPCs isolated based on c-kit positivity (rCPCs).* Gene expression was assessed by PCR. Mouse embryonic stem cells (ES) and mouse or rat whole heart homogenate were used as positive and negative controls, respectively. **(F), (E)** *Gene expression of cardiac transcription factors and cardiomyogenic and endothelial lineage markers.* Whole heart homogenate was used as positive control for all markers, and bone marrow as positive control for endothelial and negative control for cardiomyogenic markers. **(G), (H)** *Assessment of surface marker expression in expanded mouse and rat CPCs.* (G) Flowcytometry of expanded SP-mCPCs stained with anti-CD31 and Sca1 antibodies (control: non-stained expanded SP-mCPCs). (H) Flowcytometry of expanded rCPCs stained with anti-c-Kit antibody or IgG isotype. **(I), (J**) *Clonogenicity of mouse and rat CPCs.* Representative images of clone formation after the days indicated for mCPCs (I) and rCPCs (J). **(K), (L**) *CardioStem Sphere (CSS) formation* of mCPC (K) and rCPC (L). Bar: 50µm. **(M), (N)** *Expanded SP-mCPCs can differentiate into endothelial cells.* (M) vWF protein expression after 3 weeks in endothelial differentiation medium. (N) Tube formation of SP-mCPCs primed and differentiated on LN for three weeks after 24 hours in matrigel. **(O)-(Q)** *Cardomyogenic differentiation of SP-mCPCs and rCPCs.* (O) Cardiomyogenic differentiation of freshly isolated GFP-SP-mCPCs co-cultured with neonatal rat cardiomyocytes for 3 weeks. Bar: 50µm. (P) Cardiomyogenic differentiation of CSS-derived rCPC according to the protocol depicted in (Q) (for detailed description please see manuscript Methods section) [1]. Bar: 50µm.

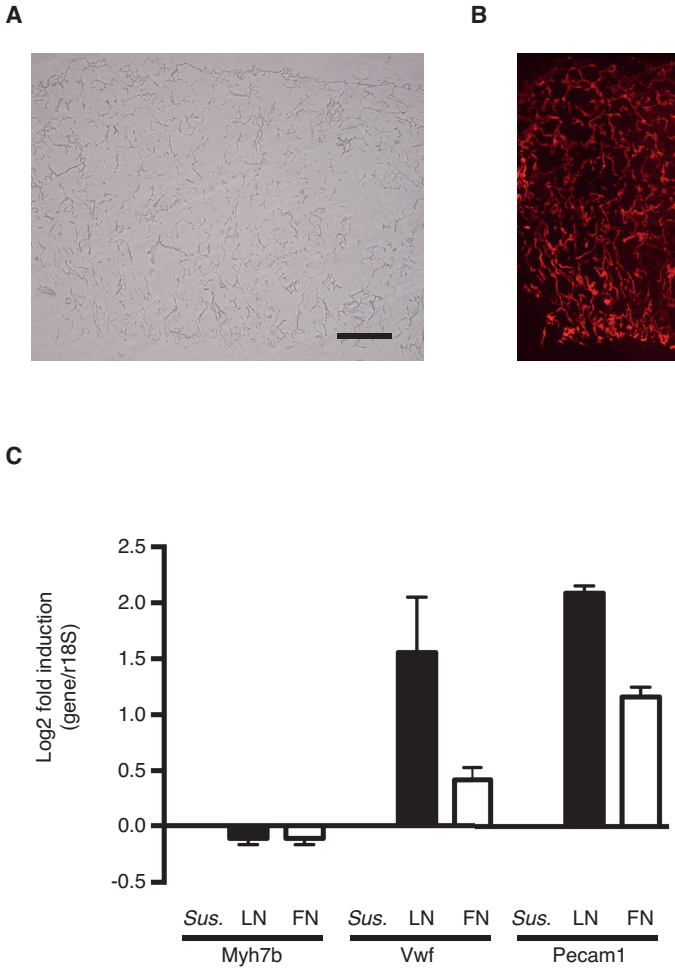
Abcb1a: ATP-binding cassette, sub-family B (MDR/TAP), member 1A (Mdr1a); Abcb1b: ATP-binding cassette, subfamily B (MDR/TAP), member 1B (Mdrab); Abcg2: ATP-binding cassette, subfamily G (WHITE), member 2 (BCRP1); Gapdh: glyceraldehyde-3-phosphate dehydrogenase; Gata4: GATA binding protein 4; Kit: v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; Myh6: myosin, heavy chain 6, cardiac muscle, alpha; Myh7b: myosin, heavy chain 7B, cardiac muscle, beta; Nanog: Nanog homeobox; Nkx2.5: NK2 homeobox 5; Pecam1: platelet/endothelial cell adhesion molecule 1 (CD31); Pou5f1: POU domain, class 5, transcription factor 1 (Oct-4); Tnni3: troponin I, cardiac 3; Vwf: Von Willebrand factor

***Figure S2. Non-normalized data of gene expression as per Figure 1.***



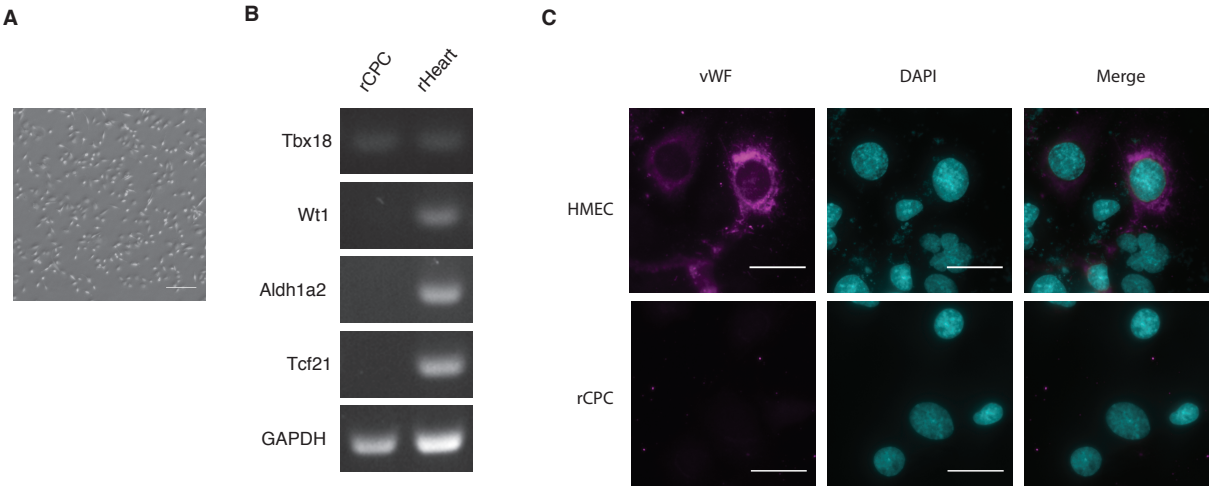

**(A)** Lineage gene expression of rCPCs on LN and FN as per Figure 1C. *p<0.05 for LN vs. FN (Wilcoxon signed rank test). **(B)** Lineage gene expression of SP-mCPCs on LN and FN as per Figure 1D. Data are given as mean±SEM. LN: laminin; FN: fibronectin; rCPCs: rat cardiac progenitor cells; SP-mCPCs: side population mouse cardiac progenitor cells.

***Figure S3. Lineage marker expression in CPCs in 3D-culture on LN- and FN-coated scaffolds using a perfusion bioreactor.***



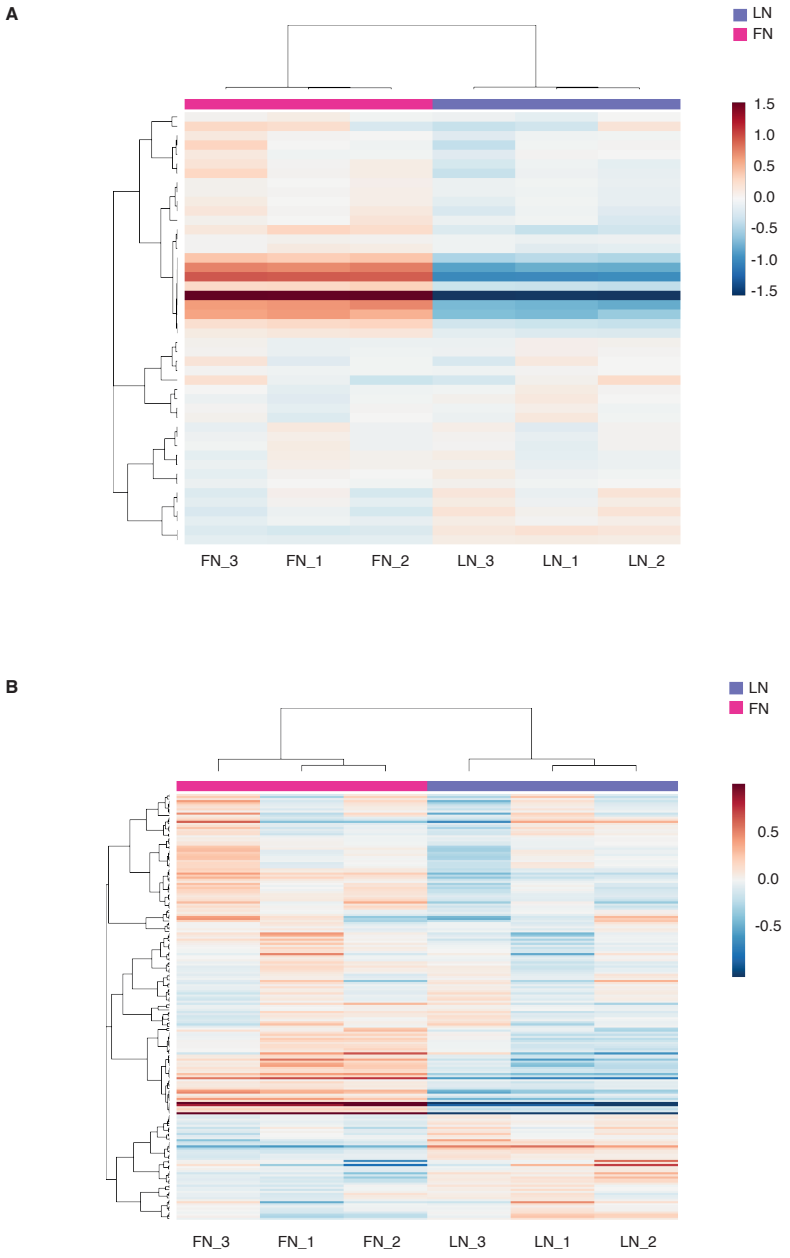

*Collagen scaffold coated with Laminin.* **(A)** *Bright filter;* **(B)** *Alexa546 shows LN*. Scaffolds were incubated with 10µg/mL LN; Bar: 500µm. **(C)** *LN also enhances commitment towards the endothelial lineage compared to FN in perfusion-based bioreactor 3D-culture.* Gene expression by qRT-PCR (n=3). rCPCs were plated on LN- or FN-coated scaffolds with 1% FBS. Data are given as mean ±SEM. Sus.: suspension; LN: laminin; FN: fibronectin; rCPCs: rat cardiac progenitor cells.

***Figure S4.***




**(A)** ***Morphology of rat CPCs under growth conditions.*** rCPCs are an inhomogeneous cell population encompassing cells with different shapes. **(B)** ***Gene expression of epicardial markers in rat CPCs.*** Tbx18 is the only marker expressed in rCPCs. Wt1: Wilms tumor protein 1; Aldh1a2: aldehyde dehydrogenase 1 family member a2; Tcf21: transcription factor 21. rHeart: RNA from neonatal rat heart serving as positive control. **(C)** ***Rat CPCs do not express von Willebrand factor protein in culture medium (F12).*** Human microvascular endothelial cells (HMEC) were used as positive control. vWF: von Willebrand Factor, rCPC: rat CPCs. Bar: 25µm.

***Figure S5***




***Heat maps of gene expression from enriched gene sets as identified by Gene Set Enrichment Analysis (GSEA) in rat CPCs on LN and FN.***
**(A)** *CORDENONSI_YAP_CONSERVED_SIGNATURE.* **(B)** *WGGAATGY_V$TEF1_Q6.*

**Supplemental References:**

1. Smith AJ, Lewis FC, Aquila I, Waring CD, Nocera A, Agosti V, Nadal-Ginard B, Torella D, Ellison GM. Isolation and characterization of resident endogenous c-kit+ cardiac stem cells from the adult mouse and rat heart. *Nat Protoc*. 2014;9:1662-1681.