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Supplemental Information

Stroma-Derived Connective Tissue Growth Factor Maintains Cell Cycle Progression and Repopulation Activity of Hematopoietic Stem Cells In Vitro

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Supplemental experimental procedures

Construction of a Ctgf-signaling network.

Computational modeling allows the formulation of a systems-level hypothesis and proposes targeted experiments. However, for feasible experimental approaches, the size of a constructed network should be limited, as computationally and mathematically, it is more feasible to model and simulate a network with a small number of genes (Bauer-Mehren et al., 2009; Gonzalez et al., 2006; Singhania et al., 2011; Yener et al., 2008). The construction of models is best approached in a bottom-up directionality, where a small number of ”seed-genes“ are first extracted from within the experimental data and then used to define the network (Schlitt and Brazma, 2007).

For construction of the Ctgf signaling network, we first selected a list of “seed genes” from the CTGF interactome (Table S5) according to the following criteria: (i) hematopoiesis-associated genes (Table S4); (ii) genes involved in cell proliferation, GO:BP, GO:0008283-cell proliferation p: 2.9e-61, (106 PPIs, not shown). We obtained 12 genes which satisfied both criteria:

#	Gene Symbol	RT-qPCR
1	*Ccnd1*	⇓
2	*Cdkn1b*	⇑
3	*Foxo1*	⇑
4	*Foxo3*	
5	*Lef1*	⇓
6	*Stat1*	⇓
7	*Aqp*	
8	*Thbs1*	
9	*Serpine1*	
10	*Nfatc2*	
11	*Cebpe*	⇑
12	*Itgb3*	⇑

From these, we first identified Ctgf, and the Ctgf receptor sub-unit Itgb3 as our starting genes/proteins (ligands or transmembrane receptors) and as our terminal nodes, we defined two well known cell cycle regulators G0/G1-specific Cyclin D1 (*Ccnd1*) and the cyclin-dependent kinase inhibitor *Cdkn1b*, as well as two transcription factors (TFs) Forkhead box protein O1 (*Foxo1*) and Lymphoid enhancer-binding factor1 (*Lef1*).

Next, we performed EXCERBT literature search (Barnickel et al., 2009; Mewes et al., 2011) to identify the pathways and major molecular players relaying a signal from our start genes/proteins to the terminal nodes. This analysis extended the number of Ctgf receptors to *Lrp6*, *Igf2R*, *Egfr*, and *Tgfbr1*. Important to note, similarly as already described (Saez-Rodriguez et al., 2007), we also only considered local interactions (e.g., a kinase phosphorylates its substrate). At the same time, in order to keep the size of the network meaningful for experimental validation, parts of it were simplified: for example, the MAPK cascade, in which a series of nodes and edges impinge only on each other, was reduced to FAK → (activates)! ERK1/2. The complete network can be inferred from **Table S6**.

Supplementary Figures

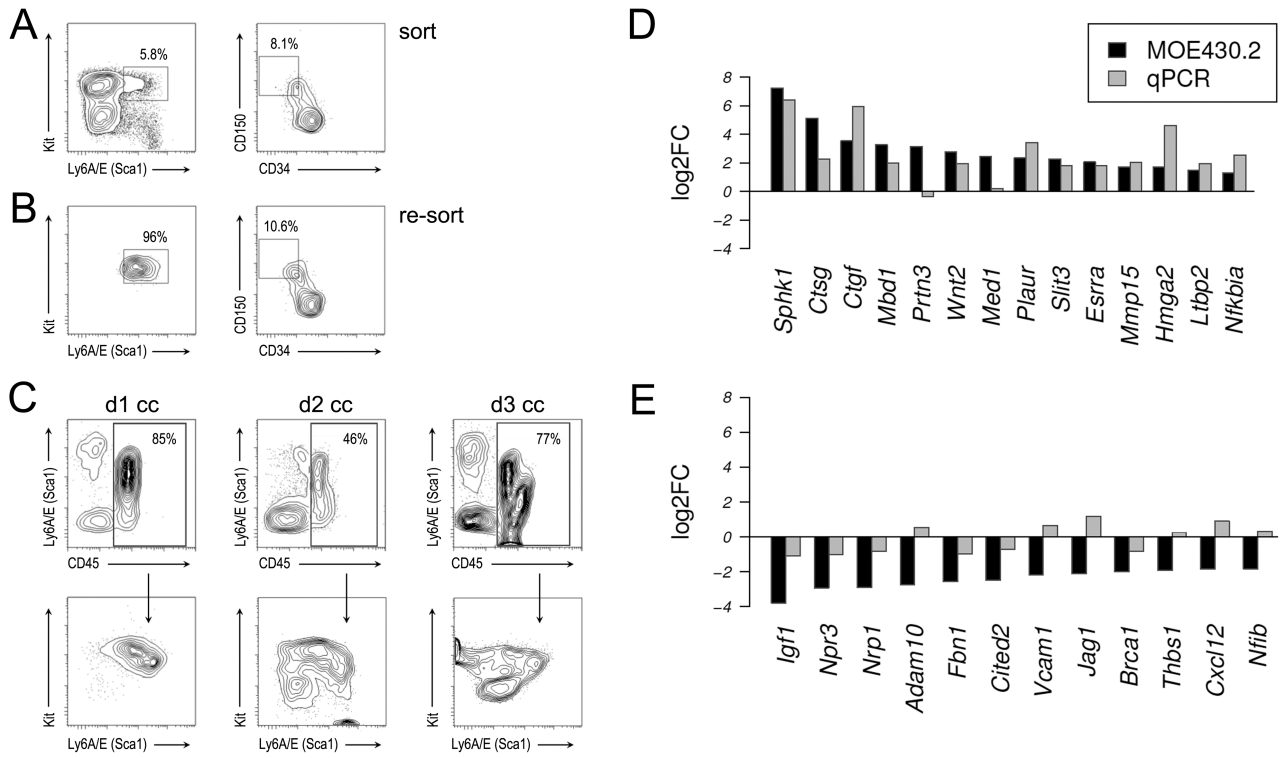


Figure S1 (related to Figure 1). *LSK cells before and after co-culture, including gene expression validation.* (A) LSK cells shown prior to sort and (B) after cell sorting. LSK cells were plated on UG26-1B6 stromal cells and co-cultured for (C) one day: d1 cc, two days: d2 cc, or three days: d3 cc. Relative gene expression of co-cultured (d1 cc) with mono-cultured (d0) UG26-1B6 cells ((D) up-regulated and (E) down-regulated genes)). The microarray (MOE430.2, black bars) and RT-qPCR (grey bars) results presented as fold change of mRNA expression levels on log2 scale (log2FC). Data presents results of 3 to 5 independent samples.

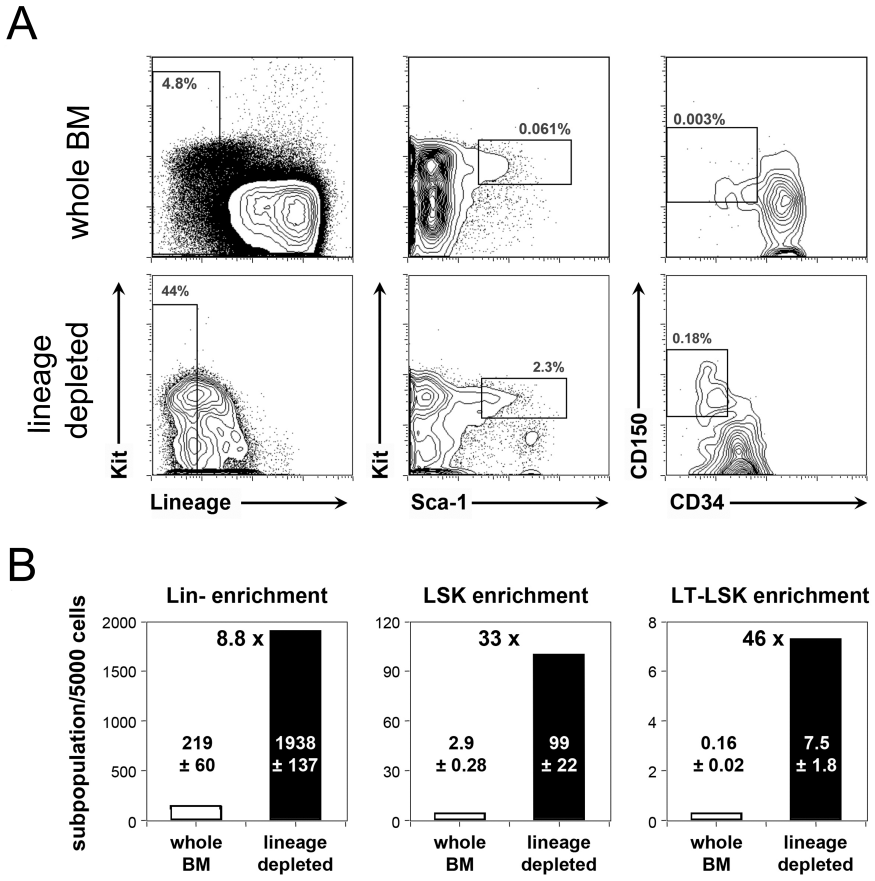


Figure S2 (related to Figure 2). The enrichment of LSKs and LT-LSKs cells after lineage depletion of whole bone marrow.

(A) Representative FACS plots of the whole BM and lineage depleted BM with gating strategy of LSKs and $CD34^{-}$ $CD150^{+}$ (LT)-LSKs. (B) Graphs representing level of enrichment of Lin^{-}, LSK and LT-LSK cells after lineage separation. Data shown are the results of two independent representative experiments (n=9).

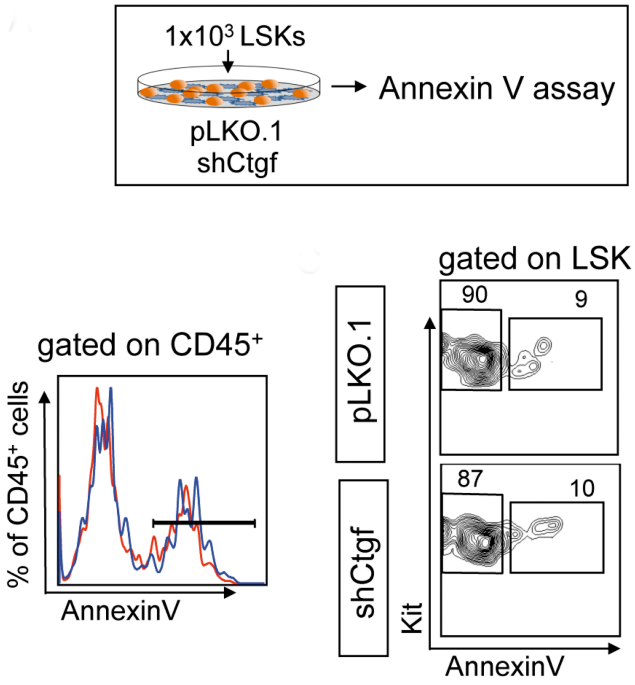


Figure S3 (related to Figure 3). Apoptosis in LSK cells cultured on pLKO.1 and shCtgf stroma. (A) Experimental design: $1x10^3$ LSK cells co-cultured for one day (d1 cc) on shCtgf, pLKO.1 stromal cells were harvested and stained for CD45, lineage markers, Kit, Sca-1, and AnnexinV. (B) Histogram of AnnexinV stain in $CD45^+$ cells (blue line – pLKO.1; red line – shCtgf). (C) The representative FACS plots of the AnnexinV stain in LSKs. Data represents results of two independent experiments.

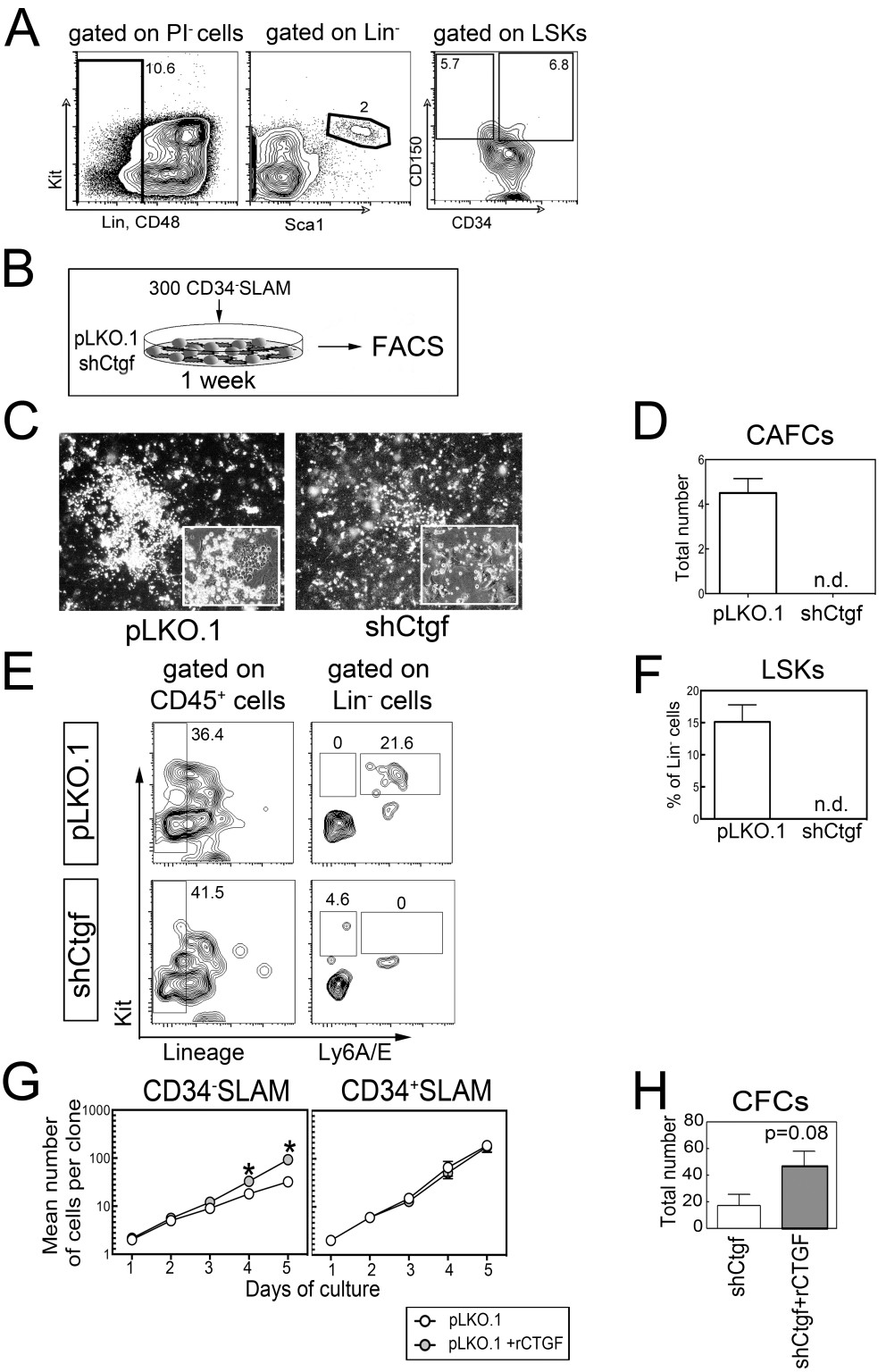
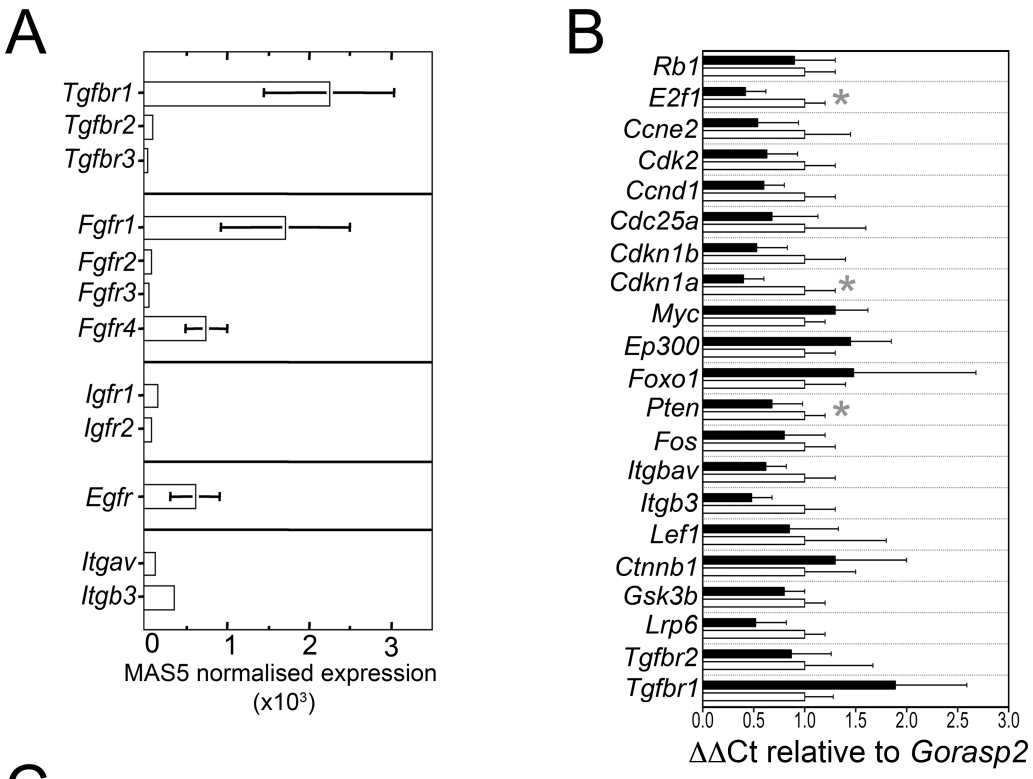
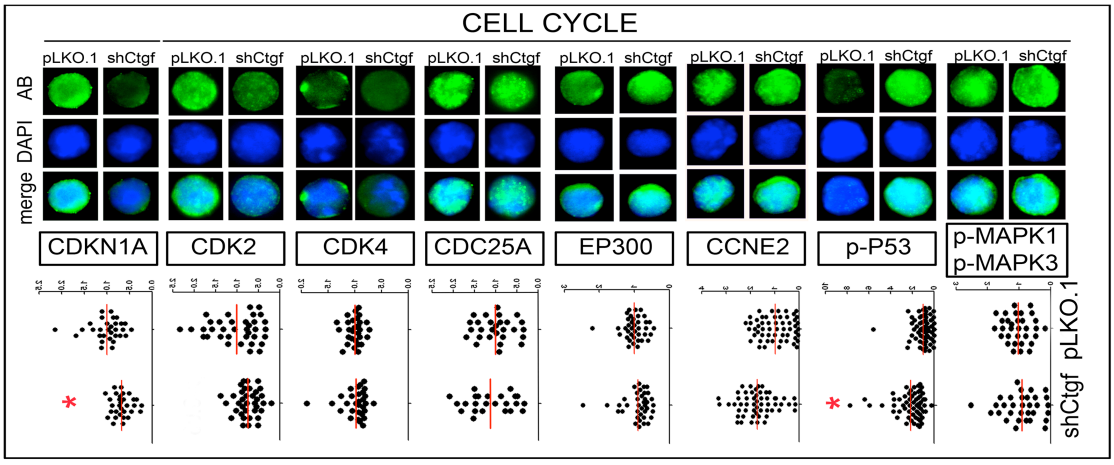


Figure S4. Sorting of CD34$^+$ and CD34$^-$ SLAM cells for co-cultures and single cell cultures (related to Figure 4). (A) Representative FACS plots of Lin$^-$ cells stained for SLAM marker (CD48, CD150) and sorted after CD34 expression as CD34$^+$ SLAM cells and CD34$^-$ SLAM cells. (B) Experimental design: 300 sorted CD34$^-$ SLAM cells were co-cultured on pLKO.1 and shCtgf stromal cells for one week in LT-medium and were further harvested and analysed by FACS. (C) Cobblestone area forming cells (CAFCs) observed under the light microscope (X5). (D) Total number of CAFCs in pLKO.1 and shCtgf co-cultures. (E) Representative FACS plots of co-cultures stained for LSKs. (F) Percentage of LSKs in one-week co-cultures. Data represents results of two (B-F) or three (G, H) independent experiments.


A
Tgfbr1
Tgfbr2
Tgfbr3
Fgfr1
Fgfr2
Fgfr3
Fgfr4
Igfr1
Igfr2
Egfr
Itgav
Itgb3
0
1
2
3
MAS5 normalised expression
(x10³)
B
Rb1
E2f1
Ccne2
Cdk2
Ccnd1
Cdc25a
Cdkn1b
Cdkn1a
Myc
Ep300
Foxo1
Pten
Fos
Itgbav
Itgb3
Lef1
Ctnnb1
Gsk3b
Lrp6
Tgfbr2
Tgfbr1
0.0
0.5
1.0
1.5
2.0
2.5
3.0
ΔΔCt relative to Gorasp2

C


CELL CYCLE
pLKO.1 shCtgf
AB
DAPI
merge
CDKN1A
CDK2
CDK4
CDC25A
EP300
CCNE2
p-P53
p-MAPK1
p-MAPK3
pLKO.1
shCtgf

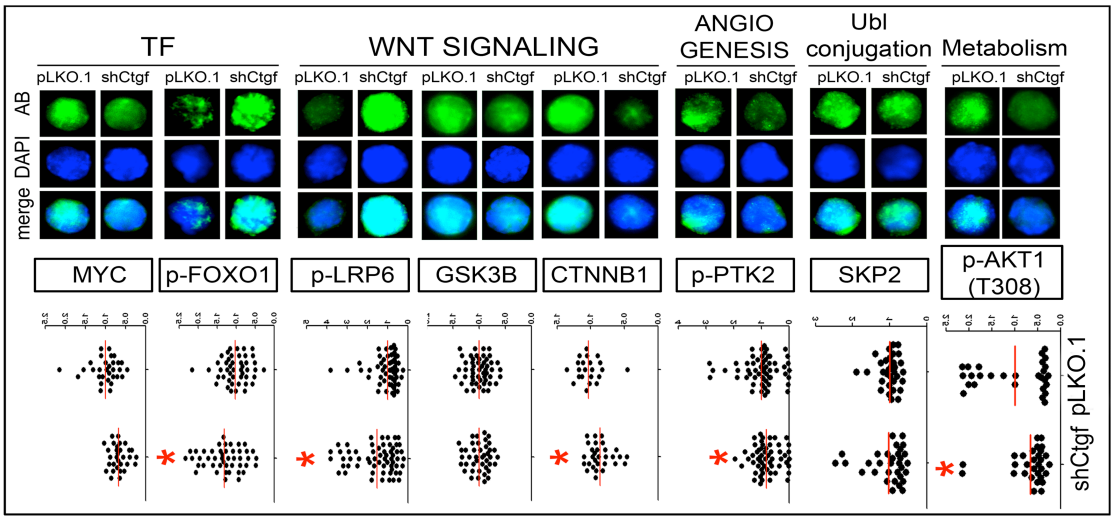

TF
WNT SIGNALING
ANGIO GENESIS
Ubl conjugation
Metabolism
pLKO.1 shCtgf
AB
DAPI
merge
MYC
p-FOXO1
p-LRP6
GSK3B
CTNNB1
p-PTK2
SKP2
p-AKT1 (T308)
pLKO.1
shCtgf

Figure S5 (related to Figure 5). Experimental validation of the Ctgf interaction model. (A) Shown are MAS5-normalized values for the TGFBR, FGFR, IGFR, EGF and ITGAV/ITGB3 receptor families in sorted in three independent analyses of 15.000 sorted LSK cells. (B) Relative expression of selected genes was detected by RT-qPCR from LSK co-cultured for one day (d1 cc) on shCtgf and pLKO.1 stromal cells in three or four independent experiments. (C) Shown are the representative pictures of LSK cells co-cultured on shCtgf and pLKO.1 stromal cells for one day (1d cc) and stained with respective antibodies. DAPI was used as a counter stain. Scale bars represent 5 μm. Each dot represents relative pixel number in an individual cell counted with ImageJ software from cells analysed on a fluorescent microscope. The total number of dots represent all cells measured in three independent experiments.

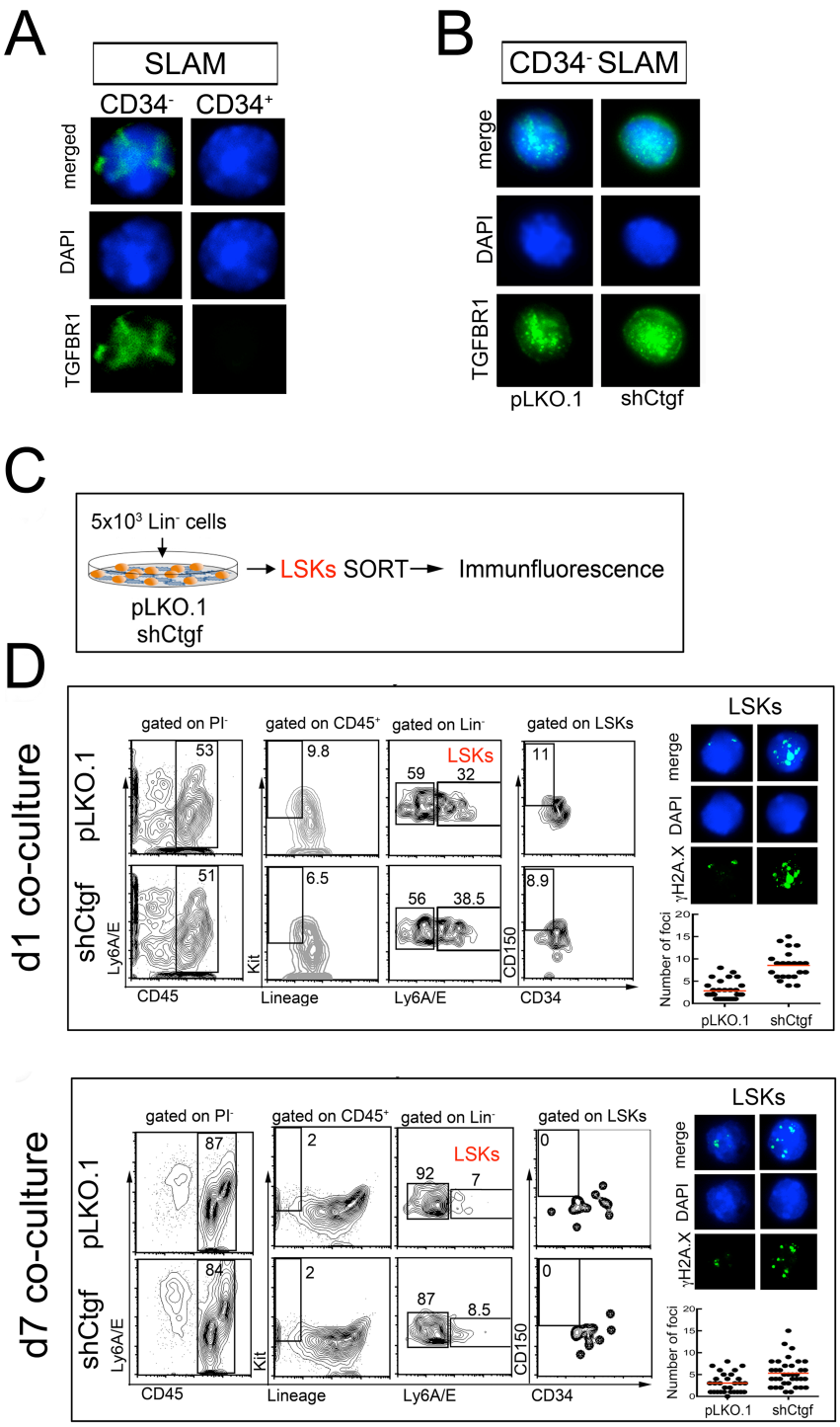


Figure S6 (related to Figure6). Expression of TGFBR in SLAM cells and distribution of senescence-associated γH2A.X foci in LSKs from d1 and d7 co-cultures (A) Expression of TGFR1 in $CD34^-$ and $CD34^+$ SLAM cells. (B) TGFBR1 expression in $CD34^-$ SLAM cells after d1 co-culturing on pLKO.1 and shCtgf stromal cell. (C) Experimental design: $5x10^3$ lineage depleted cells were cultured on pLKO.1 and shCtgf stroma for one (d1) and seven (d7) days. After co-culture, LSKs were sorted from trypsin-detached cultures and analysed for γH2A.X expression. (D) Representative FACS plots of sorted d1 and d7 co-cultures. The immunofluorescence pictures represents expression of γH2A.X in sorted LSKs. DAPI was used as a counter stain. One experiment was performed with three technical replicates. Each dot represents the number of foci counted in individual cells analysed on fluorescent microscope. Scale bars represent 5 μm.

Table S7

Target	Sequence 5'-3'	Tm	GC%	PCR product size
Adam10-F	CCTGGGAAGCAGTGCAGTCCGA	60.75	63.64	140
Adam10-R	GCTGGGCAAAGGGCTGTGAAGC	61.06	63.64	140
Atf4-F	TTCTCCAGCGACAAGGCGGGC	67.58	66.67	100
Atf4-R	CTGTCCCGGAAAAGGCATCCTCCTT	66.58	56.00	100
Axin2-F	TGGGGAGCAGTTTTGTGGCAGCA	61.35	56.52	116
Axin2-R	CCCCCGCTGCACTGGACATCC	61.77	71.43	116
Brca1-F	ATGCTGCAGCTGTGTGGGGCT	67.37	61.90	100
Brca1-R	TCCAGGCGCTTGGCTGCACG	68.45	70.00	100
Ccnd1-F	GCGTACCCTGACACCAATCT	59.75	55.00	329
Ccnd1-R	CACAACTTCTCGGCAGTCAA	58.42	50.00	329
Ccne1-F	GCAGCGAGCAGGAGACAGA	62.00	63.16	66
Ccne1-R	GCTGCTTCCACACCACTGTCTT	63.28	54.55	66
Ccne2-F	CGCAGCCGTTTACAAGCTAAG	60.20	52.38	65
Ccne2-R	TGGGTTTCTTGCGGAGAGTCT	61.38	52.38	65
Cdc25a-F	AAACCTTGCCGATCGTTGCGGG	60.55	59.09	146
Cdc25a-R	CCTTCACAGGGCTGGGCACAC	59.91	66.67	146
Cdk2-F	GCCTTTGGAGTCCCTGTCCGAACT	60.41	58.33	152
Cdk2-R	GCCCTGCGGGTCACCATTTCAG	60.30	63.64	152
Cdkn1a-F	CGGCAGGAGGCATATCTAGG	59.47	60.00	145
Cdkn1a-R	GACCCACCCTAGACCCACAA	60.84	60.00	145
Cdkn1b-F	GGCCCGGTCAATCATGAA	57.34	55.56	77
Cdkn1b-R	TTGCGCTGACTCGCTTCTTC	61.28	55.00	77
Cebpb-F	TCGGGACTTGATGCAATCCGGATCA	66.13	52.00	101
Cebpb-R	AGTGATTACTCAGGGCCCGGCTG	66.34	60.87	101
Cited2-F	TGGGCCAAACCGTTCTGGATCAGG	67.02	58.33	112
Cited2-R	TGCCACTGACGACATTCCACACCC	67.17	58.33	112
Ctgf-F4	GCGAGAGCTGAGCATGTGTCCTCC	61.38	62.50	148
Ctgf-R4	ACTTGCCACAAGCTGTCCAGTCT	58.44	52.17	148
Ctsg-F	GACCAAATGTGCGCCAATCGC	57.93	57.14	104
Ctsg-R	CCACCAGAATCACCCCTGAAGGCA	60.41	58.33	104
Cxcl12-F	GCCCTTCAGATTGTTGCACGGC	59.24	59.09	139
Cxcl12-R	TCGGGCGTCTGACTCACACCT	59.58	61.90	139
Cxcr4-F	GAGGCGTTTGGTGCTCCGGT	59.63	65.00	147
Cxcr4-R	CCCGGAAGCAGGGTTCCTTGT	58.69	61.90	147
Ddit3-F	GTACCCAGCACCATCGCGCCA	67.63	66.67	104
Ddit3-R	TGTGCAAGCCGAGCCCTCTCCT	68.19	63.64	104
Dnmt3a-F	AGGAAAACGCCGGAGGGCTTGG	67.57	63.64	124
Dnmt3a-R	AGGACCGGAGGGGAAGAAGGGGA	68.34	65.22	124
E2f1-F	GGGGAGGGTACGTGAGGGCCT	67.53	71.43	103
E2f1-R	ACTGGAGGGTGGGGAGGACAGC	67.72	68.18	103
Eed-F	TGGCAAAAGATGCTTGCATTGGGCA	66.70	48.00	133
Eed-R	TGGTTTGTCGAATAGCCGCGCCA	67.48	56.52	133
Ep300-F	GCCGAGAATGTGGTGGAACCCG	65.91	63.64	103
Ep300-R	GTGAACCAAAATCTGTGCCATCGCT	64.49	48.00	103
Esrra-F	AGGACCCAGGAAGACAGCCCCAG	67.68	65.22	128
Esrra-R	AGAGAGTGGCCACAGCGGGGA	67.70	66.67	128
Ezh2-F	CTGTGAGCTCATTGCGCGGGACT	67.51	60.87	107
Ezh2-R	AGGCACCGAGGCGACTGCATTC	67.58	63.64	107
Fbn1-F	AGGCCCCCTGCAGTTACGGT	59.82	65.00	118
Fbn1-R	CCTCGGCCCATGCCCATTCC	59.83	70.00	118
Fos-F	GGCAGCCGGCATCCAGACGT	61.84	70.00	132
Fos-R	TCCTTGAGGCCCACAGCCTGGT	60.99	66.67	132
Foxo1-F	GGCCATCGAGAGCTCAGCCG	65.38	70.00	125
Foxo1-R	TTGAATTCTTCCAGCCCGCCGA	64.80	54.55	125

Target	Sequence 5'-3'	Tm	GC%	PCR product size
Fzd7-F	TCAGCCATATCACGGCGAGA	61.11	55.00	141
Fzd7-R	GCGTCCTCTTGGTTCGTGT	60.30	57.89	141
Gorasp2-F	CACTGGGTTCCCTGTACCAC	59.96	60.00	173
Gorasp2-R	GATGCGACTCACAGAGACCA	59.47	55.00	173
Hdac1-F	CACGGGAGGCTCTGTCGCAAGTG	67.70	65.22	149
Hdac1-R	GTTCCAGGATGGCCAGGACGATGT	66.59	58.33	149
Hdac2-F	TGGTGCTGCAGTGTGGCGCA	68.07	65.00	133
Hdac2-R	CCTCCACCGAGCATCAGCAATGGC	68.10	62.50	133
Hmga2-F	AGGCAGGATGAGCGCACGCG	68.22	70.00	138
Hmga2-R	GAGGGCTCACAGGTTGGCTCTTGC	67.74	62.50	138
Hoxa9-F	ATCGATCCCAATAACCCGGCTGCCA	68.21	56.00	146
Hoxa9-R	ACCTCGTACCTGCGGTCCCGT	67.63	66.67	146
Igf1-F	TGGCGCTCTGCTTGCTCACCT	67.00	61.90	138
Igf1-R	AGCCATAGCCTGTGGGCTTGTTGAA	66.78	52.00	138
Itgav-F	ACTGGTGAACAGATGGCTGCGT	58.77	54.55	147
Itgav-R	TGAGACCTGGCCAACCTCCTGG	59.85	63.64	147
Itgb3-F	GGGACACAGCAAACAACCCGC	59.07	61.90	109
Itgb3-R	TCCCACGGTCCTGGCGTCAT	59.90	65.00	109
Jag1-F	ATCTGTCCACCTGGCTATGC	59.53	55.00	155
Jag1-R	TCCAGCTGACAGAGGTTTCC	59.31	55.00	155
Kdm5d-F	GCCATTGGTTGGCAAGGCCGT	66.71	61.90	149
Kdm5d-R	TCAAAGGCAAAGCCTGAAGGCAAGG	66.17	52.00	149
Kdm6a-F	TGACCCTACAGCCGAGCCGTC	66.20	66.67	130
Kdm6a-R	TTATTTCTGCCTCCTCCTCTGCCGC	66.62	56.00	130
Kdm6b-F	GGTCCCTGGCAGCCGAACGC	68.51	75.00	145
Kdm6b-R	ACCATGCCGGTCGCAGAAGGC	67.91	66.67	145
Lef1-F	CACCCATTGGCTGGCAAGGTCAG	60.24	60.87	145
Lef1-R	CCAGTTGTGTGGGGGCCAGGG	61.60	71.43	145
Lgals3-F	CCTCCGGGAAATCAGCCAACTGGG	67.35	62.50	106
Lgals3-R	CACAGGGCCGGTTTCGGTGC	66.64	70.00	106
Ltbp2-F	GGGCGATGCAGCAACACGGA	60.32	65.00	147
Ltbp2-R	GGAGCCAGGGGAGTTGACGC	59.42	70.00	147
Mbd1-F	TGGAGAAGAGCCGAGGGTGTGGC	68.38	65.22	106
Mbd1-R	TGGCGCTTGAGACCAGGGCG	67.62	70.00	106
Med1-F	GCTAGCAGCCCAGGATCAAA	60.11	55.00	121
Med1-R	CGGCTCCCTGTTAAGCAAGT	60.32	55.00	121
Meis1-F	GTGCAGCCCATGATAGACCA	59.82	55.00	142
Meis1-R	CTGGCATACTTTGCAGCCCT	60.68	55.00	142
Mll1-F	CCGAGACACCGACCCCGCAC	67.57	75.00	119
Mll1-R	CTGCCGGCTGCCCACACTCC	68.25	75.00	119
Mmp15-F	AGCCCAGCCGCCACATGTCC	67.74	70.00	135
Mmp15-R	GGGGCCGCTTCATCCACGTTTT	66.16	59.09	135
Nfib-F	ACCCTGGGACGAGGTACCCCC	67.23	71.43	139
Nfib-R	ACCCTGGTGTGTGGCTAGCAAGC	67.25	60.87	139
Nfkbia-F	CCGTCCTGCAGGCCACCAACT	67.29	66.67	130
Nfkbia-R	CCATTGCAGGGCTCCTGAGCG	65.97	66.67	130
Npr3-F	ACTCAGTGCCTGTGTCTGAACGTGT	66.30	52.00	100
Npr3-R	TGCCCAGGGAAGAAGGCTCCGA	67.69	63.64	100
Nrp1-F	GGGCTGTGAAGTGGAAGCACCT	59.02	59.09	144
Nrp1-R	GTGGCCAGGACAGTGGTGCC	59.90	70.00	144
Pak1-F	GGGCAGGAGGTGGCCATTAAACA	65.19	56.52	149
Pak1-R	ACCCACAGCTCATCTCCCACAAGGT	67.55	56.00	149
Pbrm1-F	TGGCTCCCCCACCAAAGACCCA	61.71	63.64	130
Pbrm1-R	ACATCCCGTCTTCGAGCTGCCA	60.24	59.09	130
Pbx1-F	AAGCGCAGGCCAGAAAACATGCT	66.15	52.17	135
Pbx1-R	GCTGGGGGTCTGTGGGCTCCT	68.37	71.43	135

Target	Sequence 5'-3'	Tm	GC%	PCR product size
Pcbd1-F	GGCTGGCCCTTGCTCCCTGAC	67.70	71.43	116
Pcbd1-R	AGCCCCAGTGAGGAGAGTGGCAC	68.15	65.22	116
Plaur-F	CACTGCAATGGTGGCCCAGTTCT	59.62	56.52	126
Plaur-R	CCGGCAGTTGATGAGAGACGCC	59.87	63.64	126
Prtn3-F	ATGCTTCGGAGACTCGGGCGG	60.97	66.67	122
Prtn3-R	ACATGGACACCCGGGCGAAGA	60.18	61.90	122
Rad51-F	TGCGTCAACCACCAGGCTGTACCT	68.22	58.33	132
Rad51-R	TTGGCATCGCCCACTCCATCTGC	67.63	60.87	132
Rad54l-F	CGTGGGGAGGAGCGTCTGCG	67.61	75.00	131
Rad54l-R	AGGGGTGTCAGCCTACAACAAACCA	66.17	52.00	131
Rpl13a-F	CCCTCCACCCTATGACAAGA	58.12	55.00	153
Rpl13a-R	TTCTCCTCCAGAGTGGCTGT	60.18	55.00	153
Rpl23-F	CCCGTTCATATCCCAGTGTCCCCTG	66.42	60.00	135
Rpl23-R	CAGCTCCGACCGGAAGACCCA	66.18	66.67	135
Rpl39-F	ATTCCTCCGCCATCGTGCGCG	68.20	66.67	130
Rpl39-R	TCCGGATCCACTGAGGAATAGGGCG	67.47	60.00	130
Rplp0-F	TCCTATAAAAGGCACACGCGGGCA	66.78	54.17	106
Rplp0-R	ACGGCGGTGCGTCAGGGATTG	67.85	66.67	106
Slit3-F	TGCGGGAGGGTGCCTTCGAT	60.25	65.00	132
Slit3-R	GGTTGCTCCGCAACATCAGCG	59.28	61.90	132
Smad4-F	ATGCAGCAACAGGCGGCCACT	67.89	61.90	128
Smad4-R	CCAGCAGCAGCAGACAGACTGATGG	67.48	60.00	128
Smarca4-F	GTACAAAGACAGCAGCAGTGGACG	64.10	54.17	149
Smarca4-R	TGCGGTACTTGTGGTTTCGGATGC	65.90	54.17	149
Sphk1-F	CCACTATGCTGGGTACGAGCAGGT	60.06	58.33	126
Sphk1-R	AGCCGCAGCCCAGAAGCAGTG	61.96	66.67	126
Stat1-F	CGCGTGGTGGTCCCAGCTCTCA	68.89	68.18	120
Stat1-R	CCAGCATTAGGGCCCAGCAGCTT	67.43	60.87	120
Stat6-F	ACCCCCAGGGTCTGCTGCAGT	68.01	66.67	134
Stat6-R	GGTGCCTTGGGGGAAACCTCCC	67.39	68.18	134
Suz12-F	AAGGAGACGCTGACTACAGAGCTGC	66.71	66.67	147
Suz12-R	CGGGCAGTGCAGGTCGTCTCT	66.25	56.00	147
Tgfb1-F	ACCCCCATTGCTGTCCCGTGC	61.66	66.67	131
Tgfb1-R	TGGGGGTCAGCAGCCGGTTAC	60.78	66.67	131
Tgfb2-F	GCAGGAGAAGGCAAGCCGGAG	65.64	66.67	124
Tgfb2-R	CGGGATGGCATTTTCGGAGGGG	65.80	63.64	124
Tgfb3-F	TGCTTCCGCAACCTGGAGGAGA	65.90	59.09	145
Tgfb3-R	CTGCGCTGCGGAGGTATGGG	65.68	70.00	145
Tgfbr1-F	GGGTCTGGATCAGGTTTACCACTGC	65.07	56.00	118
Tgfbr1-R	CTCCCCGCCATTTGCCTCGC	66.52	70.00	118
Tgfbr2-F	CGCACGTTCCCAAGTCGGATGT	65.56	59.09	141
Tgfbr2-R	GAAGCTTGACCGCACCGCCA	66.03	65.00	141
Thbs1-F	AATGCCAACCAGGCCGACCA	58.90	60.00	150
Thbs1-R	GTCACCTCGGCCATCACCATCA	58.64	59.09	150
Vcam1-F	TGTCAACGTTGCCCCCAAGGA	58.55	57.14	124
Vcam1-R	GCTCCACAGGATTTTGGGAGCTGG	59.76	58.33	124
Wnt2-F	AGCGGGCCGTGTGTGCAACTT	62.10	61.90	149
Wnt2-R	AGTCCTGACAGCGCACGGCA	61.08	65.00	149

Table S7 (related to Figures 1, S2, S5, and Experimental Procedures). Gene-specific primers used for RT-qPCR analysis used in this study. The primers were designed using the NCBI primer design tool Primer-BLAST http://www.ncbi.nlm.nih.gov/tools/primerblast/ using the default parameters, except that the PCR product size was restricted to 100-150 bp and the primers were required to span an exon-exon junction in order to eliminate genomic DNA amplification.

Table S8

Antibody	Manufacturer	Catalog Nr.	Dilution	Antibody species
anti-CDC25A	Cell Sign. Techn., US	3652	1:50	rabbit
anti-CDK2	Cell Sign. Techn., US	2546	1:50	rabbit
anti-CDK4	Cell Sign. Techn., US	2906	1:50	mouse
anti-CTGF	Santa Cruz Biotec., US	sc-25440	1:50	rabbit
anti-Cyclin D1	Cell Sign. Techn., US	2978	1:25	rabbit
anti-Cyclin E2	Cell Sign. Techn., US	4132	1:100	rabbit
anti-p21Cip1 (CDKN1A)	Santa Cruz Biotec., US	sc-271532	1:50	mouse
anti-p27Kip1 (CDKN1B)	BD Transduct. Laborat., US	610242	1:100	mouse
anti-p300	Upstate/Millipore, US	05-2576	1:100	mouse
anti-phospho- Ser473 AKT	Cell Sign. Techn., US	9271	1:25	rabbit
anti-phospho- Thr308 AKT	Cell Sign. Techn., US	2965	1:100	rabbit
anti-phospho-Ser 33/ Ser 37/ Thr 41 beta-catenin	Cell Sign. Techn., US	9561	1:100	rabbit
anti-phospho- Tyr925 FAK	Cell Sign. Techn., US	3284	1:50	rabbit
anti-phospho-FoxO1 Ser256	Cell Sign. Techn., US	9461	1:50	rabbit
anti-phospho-GSK3-beta Ser9	Cell Sign. Techn., US	5558	1:400	rabbit
anti-phospho-Lrp6 Ser1490	Cell Sign. Techn., US	2568	1:200	rabbit
anti-phospho-Thr202/ Tyr204 p44/42 MAPK (Erk1/2)	Cell Sign. Techn., US	4377	1:200	rabbit
anti-phospho-Ser15 TP53	Cell Sign. Techn., US	9284	1:50	rabbit
anti-phospho-Ser780 RB	Cell Sign. Techn., US	8180	1:200	rabbit
anti-phospho-Ser465/467 SMAD2 - Ser423/425 SMAD3	Cell Sign. Techn., US	9510	1:200	rabbit
PTEN	Cell Sign. Techn., US	9552	1:100	rabbit
SKP2	Cell Sign. Techn., US	4358	1:50	rabbit

Table S8 (related to Figures 1, 6, S5, S6, and Experimental Procedures). Primary antibodies used for the Immunofluorescence (IF) staining in this study. Antibody manufacturer with catalog-number and species, as well as the dilution at which the antibody was used are represented.

Legends to additional supplementary tables (Excel supplement)

Table S1. Downregulated genes in Cluster C1 (related to Figure 1B and 1C). ToppFun analysis of functional categories significantly associated with genes down-regulated after performing two-way comparison of 24 h co-culture-derived (Day1; d1) vs. separately cultured UG26-1B6 (Day0; d0) cells (Supplementary Table1) and unified in STEM ((Ernst and Bar-Joseph, 2006); http://www.cs.cmu.edu/jernst/stem) cluster #1 (C1; Figure 1B, C). ToppFun is part of the ToppGene Suite http://toppgene.cchmc.org (Chen et al., 2009). Detects enriched terms of the gene annotations and sequence features, namely, GO: Molecular Function, GO: Biological Process, Mouse Phenotype, Pathways, Protein Interactions, Protein Domains, transcription factor binding sites, miRNA-target genes, disease-gene associations, drug-gene interactions and Gene Expression, compiled from various data sources. Hypergeometric distribution with Bonferroni correction (p-Value cutoff <= 0.05, default parameters) was used for determining statistical significance.

Table S2. Upregulated genes in Cluster C2 (related to Figure 1B and 1D). ToppFun analysis of functional categories significantly associated with genes up-regulated after performing two-way comparison of 24 h co-culture-derived (Day1; d1 cc) vs. separately cultured UG26-1B6 (Day0; d0) cells (Supplementary Table1) and unified in STEM ((Ernst and Bar-Joseph, 2006); http://www.cs.cmu.edu/jernst/stem) cluster #2 (C2; Figure 1B, D). ToppFun is part of the ToppGene Suite http://toppgene.cchmc.org (Chen et al., 2009). Detects enriched terms of the gene annotations and sequence features, namely, GO: Molecular Function, GO: Biological Process, Mouse Phenotype, Pathways, Protein Interactions, Protein Domains, transcription factor binding sites, miRNA-target genes, disease-gene associations, drug-gene interactions and Gene Expression, compiled from various data sources. Hypergeometric distribution with Bonferroni correction (p-Value cutoff <= 0.05, default parameters) was used for determining statistical significance.

Table S3. Total list of differentially expressed stromal genes upon contact with LSK cells. Genes differentially expressed (DEGs) after performing two-way comparison of 24 h co-culture-derived (Day1; d1 cc) vs. separately cultured UG26-1B6 (Day0; d0) cells. GcRMA-normalized gene expression data were first filtered using an additional control 24 h after changing the culture medium (d1 mc). Co-culture-derived transcripts that did not show significant positive (p-Value <= 0.05) associations with medium-control-derived transcripts in terms of Pearson's correlation coefficient, as well as transcripts that were part of our microarray validation set were further subjected to empirical Bayes test statistics as implemented in LIMMA (Smyth et al., 2005). Genes where considered differentially expressed (DEGs), if their expression level difference was -1 <= log2FC >= 1 and p-Value <= 0.05 across the two time points being compared.

Table S4. Seed list of hematopoiesis-associated genes for network modeling (related to Figure 5). Hematopoiesis-associated genes retrieved by performing extensive biomedical literature search using the text-mining tool EXCERBT (Extraction of Classified Entities and Relations from Biomedical Texts) (Barnickel et al., 2009; Mewes et al., 2011). Co-occurrence search was employed in order to retrieve all the genes associated with the phenotype 'hematopoiesis'. Thereafter, false positives were discarded by manual

curation. By this, a list of 374 genes shown to modulate hematopoietic stem cells (HSCs) or hematopoiesis in general was obtained. This seed list was further supplemented with ToppGene mouse phenotypic data associated with phenotypes 'leukemia' (HP:0001909), 'acute leukemia' (HP:0002488), 'hematological neoplasia' (HP:0004377), 'abnormal hematopoiesis' (MP:0002123), 'abnormal hematopoietic cell number' (MP:0011180) and 'abnormal hematopoietic stem cell morphology' (MP:0004808), yielding an extended list of 1737 genes.

Table S5. CTGF interaction partners for network modeling (related to Figure 5). CTGF interaction partners retrieved by performing extensive biomedical literature search using the textmining tool EXCERBT (Extraction of Classified Entities and Relations from Biomedical Texts) (Barnickel et al., 2009; Mewes et al., 2011). Co-occurrence search was employed in order to retrieve all the molecular species and phenotypes associated with Ctgf. Thereafter, false positives were discarded by manual curation. By this, a list of 274 unique interactions was obtained (since in some cases controverse results were reported and/or more than one source yealded the association, the total number of interactions was 548).

Table S6. CTGF signaling network model of cell cycle regulation (related to Figure 5). Construction of the literature-based signaling network model of CTGF-regulated HSC cell cycle progression. Literature mining using EXCERBT (Extraction of Classified Entities and Relations from Biomedical Texts) (Barnickel et al., 2009; Mewes et al., 2011) and manual curation was performed to identify the pathways and major molecular players relaying a signal from CTGF to the terminal nodes associated with the cell cycle regulation: *Ctgf*, Cyclin D1 (*Ccdn1*), p21Cip1 (*Cdkn1a*), FoxO1 (*Foxo1*) and LEF (*Lef1*). The network was split into two sub-networks associated with functional outcomes (i) G0/G1 defined as the activation of Cyclin D:Cdk4/6 and (ii) G1/S block, where the induction of p21Cip1 and/or p27Kip1 serves as the readout. In order to keep the size of the network meaningful, parts of it were simplified, for example, the MAPK cascade, in which a series of nodes and edges impinge only on each other (see KEGG map04510: Focal adhesion), was reduced to FAK → Erk1/2.

4. Supplemental References

Barnickel, T., Weston, J., Collobert, R., Mewes, H. W., and Stumpflen, V. (2009). Large scale application of neural network based semantic role labeling for automated relation extraction from biomedical texts. PloS one *4*, e6393.

Bauer-Mehren, A., Furlong, L. I., and Sanz, F. (2009). Pathway databases and tools for their exploitation: benefits, current limitations and challenges. Molecular systems biology *5*, 290.

Chen, J., Bardes, E. E., Aronow, B. J., and Jegga, A. G. (2009). ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic acids research *37*, W305-311.

Ernst, J., and Bar-Joseph, Z. (2006). STEM: a tool for the analysis of short time series gene expression data. BMC bioinformatics *7*, 191.

Gonzalez, A. G., Naldi, A., Sanchez, L., Thieffry, D., and Chaouiya, C. (2006). GINsim: a software suite for the qualitative modelling, simulation and analysis of regulatory networks. Bio Systems *84*, 91-100.

Mewes, H. W., Ruepp, A., Theis, F., Rattei, T., Walter, M., Frishman, D., Suhre, K., Spannagl, M., Mayer, K. F., Stumpflen, V., and Antonov, A. (2011). MIPS: curated databases and comprehensive secondary data resources in 2010. Nucleic acids research *39*, D220-224.

Saez-Rodriguez, J., Simeoni, L., Lindquist, J. A., Hemenway, R., Bommhardt, U., Arndt, B., Haus, U. U., Weismantel, R., Gilles, E. D., Klamt, S., and Schraven, B. (2007). A logical model provides insights into T cell receptor signaling. PLoS computational biology *3*, e163.

Schlitt, T., and Brazma, A. (2007). Current approaches to gene regulatory network modelling. BMC bioinformatics *8 Suppl 6*, S9.

Singhania, R., Sramkoski, R. M., Jacobberger, J. W., and Tyson, J. J. (2011). A hybrid model of mammalian cell cycle regulation. PLoS computational biology *7*, e1001077.

Smyth, G. K., Michaud, J., and Scott, H. S. (2005). Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics *21*, 2067-2075.

Yener, B., Acar, E., Aguis, P., Bennett, K., Vandenberg, S. L., and Plopper, G. E. (2008). Multiway modeling and analysis in stem cell systems biology. BMC systems biology *2*, 63.