Supplementary Figure Legends

Figure S1. Schematic of myeloid lineage reporter mouse model used in this study.

(A) The Lyzs-Cre mouse was crossed with Gt(ROSA)26Sor$^{tm1(EYFP)Cos/}$J mice to label cells expressing Lysz. In order to monitor β-catenin/Tcf transcriptional activity, these mice were bred with the Tcf reporter mouse. Tcf transcriptional activity is identified by the production of ß-gal. These mice were used in lineage studies during wound repair. (B) Flow cytometry analysis of bone marrow derived macrophages indicates that 87% of EYFP-positive myeloid cells are F4/80-positive.

Figure S2. Bone marrow derived macrophages of Lysz-Cre;ROSA-EYFP mouse are EYFP+.

(A) Bone marrow derived cell culture from a ROSA-EYFP mouse expanded in macrophage specific medium. (B) Bone marrow cell culture from Lysz-Cre;ROSA-EYFP mouse showing EYFP-positive macrophage cells. (C) Flow cytometry analysis of bone marrow derived macrophages from ROSA-EYFP mouse, showing absence of EYFP positive cells. (D) Flow cytometry analysis of bone marrow derived macrophages from Lysz-Cre; ROSA-EYFP mouse, showing a big population of EYFP positive cells.

Figure S3. Schematic of myeloid lineage β-catenin deficient reporter mouse model used in this study.

(A) Lysz-Cre transgenic mice were crossed with Catnb$^{tm2Kem(fl/fl)}$. β-catenin is deleted when Cre-recombinase is expressed in mice expressing the Catnb$^{tm2Kem(fl/fl)}$ allele. These mice were crossed with an EYFP reporter mouse (Gt(ROSA)26Sor$^{tm1(EYFP)Cos/}$J). In order to monitor β-catenin/Tcf transcriptional activity, these mice were then bred with the Tcf reporter mouse. Tcf transcriptional activity is identified in these mice (Lysz-Cre;ROSA-EYFP;Tcf) by the production of ß-gal. (B) Quantitative RT-PCR analysis, showing decreased expression of the β-catenin/Tcf target *Axin2* in β-catenin-deficient bone marrow derived macrophages from Lysz-Cre;Catnb$^{tm2Kem(fl/fl)}$;ROSA.EYFP mice compared to control littermates. (C) Western blot analysis of β-catenin-deficient bone marrow derived macrophages show a substantial decrease in β-catenin at protein level in compare to control mice. (D) Relative wound bed quantification shows a significant increase in the wound area bed in Lysz.Cre;Catnb^{tm2Kem} compared to control mice. Data represent the mean± 95% confidence interval of 6 mice.

Figure S4. A subpopulation of myeloid lineage cells change their morphology during wound healing.

Double immunofluorescence staining of the healing dermis in a Lysz-Cre;ROSA-EYFP mouse, stained with EYFP and other antibodies. (A) co-staining with F4/80. Arrows show cells that are positive only for EYFP while arrowheads show cells that are positive for EYFP and F4/80. (B) Co-staining with an antibody to FAP. Arrows shows cells that are positive only for FAP while arrowheads show cells that are positive for EYFP and FAP. (C) Co-staining with an antibody to α-SMA. Arrows show cells that are positive only for α-SMA while arrowheads show cells that are positive for EYFP and α-SMA. Data represent the average frequency from 8 mice.

Figure S5. A subpopulation of EYFP-positive myeloid cells express FAP and α-SMA in healing dermis at the end of the healing process.

Double immunofluorescence staining of healing dermis in Lysz-Cre;ROSA-EYFP mouse with EYFP and FAP in (A) or α-SMA in (B). Arrowheads show cells which are positive only for FAP (A) or α-SMA (B) while arrows show cells that are positive for EYFP and the specified marker, indicating that a group of FAP or α-SMA positive cells are myeloid lineage progeny.

Figure S6. β-catenin mediates the development of fibroblastic phenotype of myeloid lineage cells.

(A) Bone marrow derived cells from a Lysz-Cre;ROSA-EYFP mouse grown in macrophage specific medium (MSM), showing cells expressing the macrophage marker Mac1. Arrowheads show round shaped macrophage cells which are EYFP-positive and Mac1-positive. (B) A subpopulation of bone marrow derived macrophages that are deprived of MSM, showing a change in their morphology to spindle shape fibroblast-like cells that do not express Mac1. Arrows show that fibroblast-like cells are EYFP-positive and Mac1-negative. Arrowhead points to rounded shape cells showing a macrophage phenotype that are EYFP-positive and Mac1-positive. (C) Quantitative RT-PCR showing down-regulation of genes characteristically expressed by macrophages, and up-regulation of genes characteristically expressed by fibroblasts in cultured macrophages when deprived of MSM. (D) Quantification of percentage of cells that are positive for each marker in macrophage medium compare to DMEM medium. (E) The phenotypes of bone marrow derived macrophage cells cultured in either MSM medium (top panel) or deprived of MSM medium (lower panel). Arrows show rounded cells characteristic of macrophages while arrowheads show spindle shape fibroblasts like cells. Macrophages which are deprived of MSM medium change their phenotype to a fibroblast-like shaped cell in cell from Lyzs.Cre; ROSA-

EYFP mice but not in cells from Lyzs.Cre;Catnb^{tm2Kem};ROSA-EYFP. (F) Quantitative RT-PCR showing down-regulation of genes characteristically expressed in fibroblast and up-regulation of genes characteristically expressed in macrophages. Data represent the mean± 95% confidence interval of 6 mice.

Figure S7. Peripheral fibroblasts of Lysz-Cre;ROSA-EYFP mouse do not produce EYFP in macrophage specific media.

Peripheral fibroblasts of Lysz-Cre;ROSA-EYFP mouse were cultured in macrophage specific media for 96 hours. Note that unlike bone marrow cells (A), established fibroblast do not produce EFYP protein (B), indicating that fibroblasts do not express *lysozyme* while exposed to MSM.

Figure S8. F4/80 positive macrophages and β-catenin+ cells are enriched in human hypertrophic scar in compare with normal scar.

Accumulation of macrophages and β-catenin positive cells in the dermal component of hypertrophic scars compared with that observed in normal scars. This, shows a correlation between numbers of F4/80+ cells and β-catenin accumulation. Arrows show β-catenin positive cells in the upper panels and F4/80+ cells in the lower panels. Data in right panel represent the mean± 95% confidence interval of 10 hypertrophic scar samples and 3 normal scar samples.

Table S1. Tcf transcriptionally active cells express genes characteristically expressed by macrophages during skin healing.

LacZ-expressing cells were sorted from digested granulation tissue using fluorescein di-β-D-galactopyranoside subjected to microarray. The table summarizes the relative expression ratio of

genes characteristically expressed by macrophages between β-gal-positive cells with active β-catenin/Tcf signalling and β-gal-negative cells.

Table S2. Down-regulation of genes attributed to migration in macrophages lacking β-catenin.

Data from the microarray analysis (Geo accession number: GSE52163) shows down-regulation of genes attributed to macrophage migration in macrophages from Lyzs.Cre;Catnb^{tm2Kem};ROSA-EYFP in comparison with control macrophages.

Table S3. Modulation of Integrin gene family in macrophages lacking β-catenin.

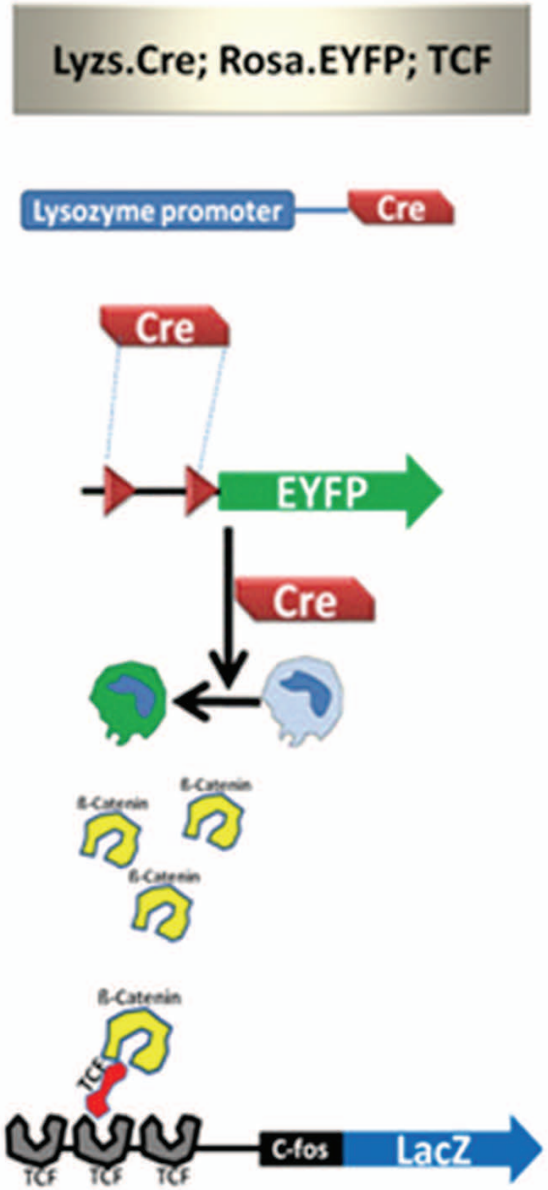
Integrin family member genes differentially expressed in the microarray analysis of expression in macrophages from Lyzs.Cre;Catnb^{tm2Kem};ROSA-EYFP mice compared with control macrophages.

Table S4. Modulation of Adam gene family in macrophages lacking β-catenin.

Summary of Adam gene family differential expression in cDNA microarray analysis of RNA from macrophages from Lyzs.Cre;Catnb^{tm2Kem};ROSA-EYFP mice compared with control macrophages.

Figure S1

A


Lyzs.Cre; Rosa.EYFP; TCF
Lysozyme promoter
Cre
Cre
EYFP
Cre
β-Catenin
β-Catenin
β-Catenin
β-Catenin
TCF
TCF
TCF
TCF
C-fos
LacZ

B

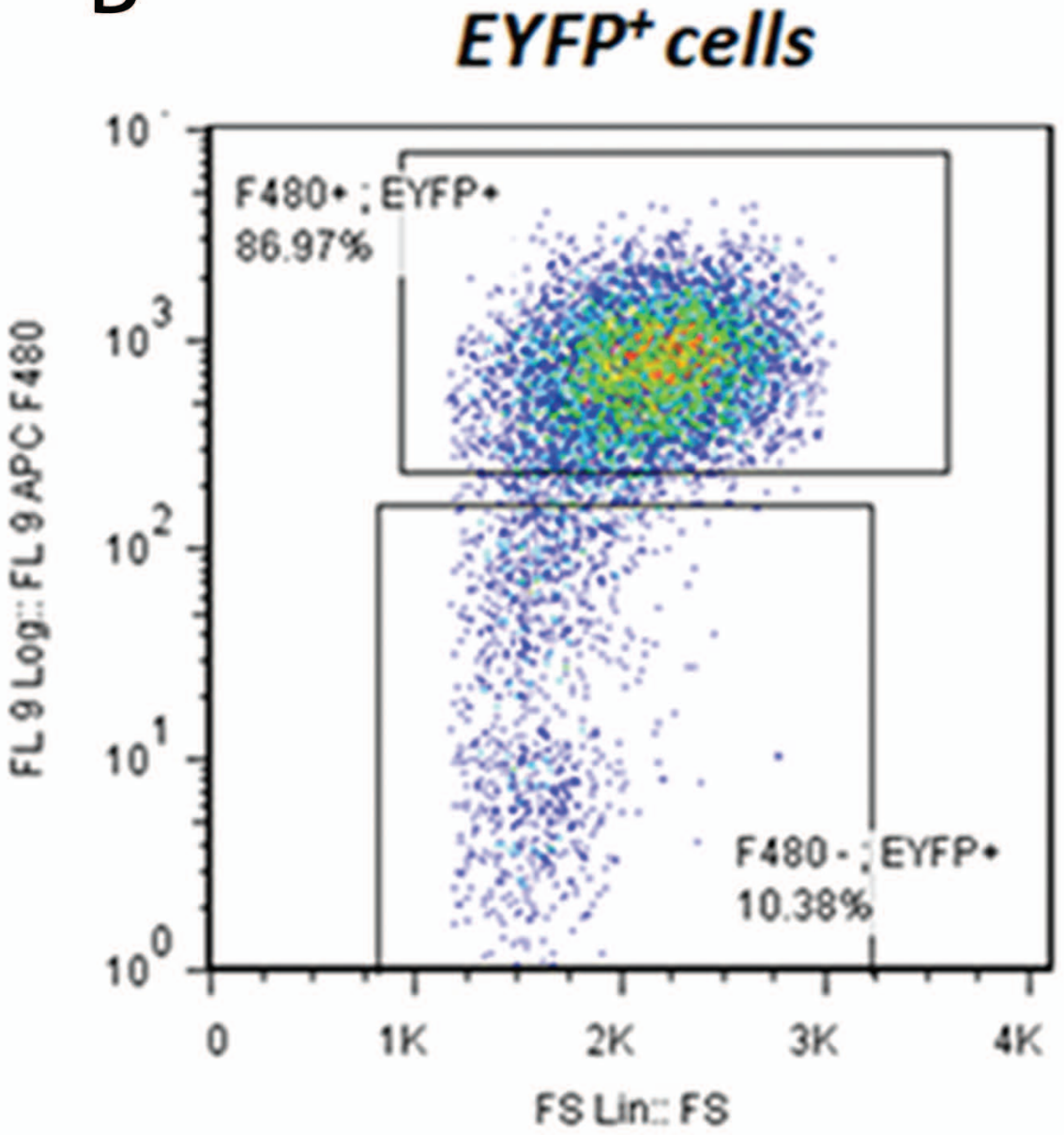

EYFP+ cells
F480+ ; EYFP+
86.97%
F480 - ; EYFP+
10.38%
FL 9 Log:: FL 9 APC F480
FS Lin:: FS
10^4
10^3
10^2
10^1
10^0
0
1K
2K
3K
4K

Figure S2

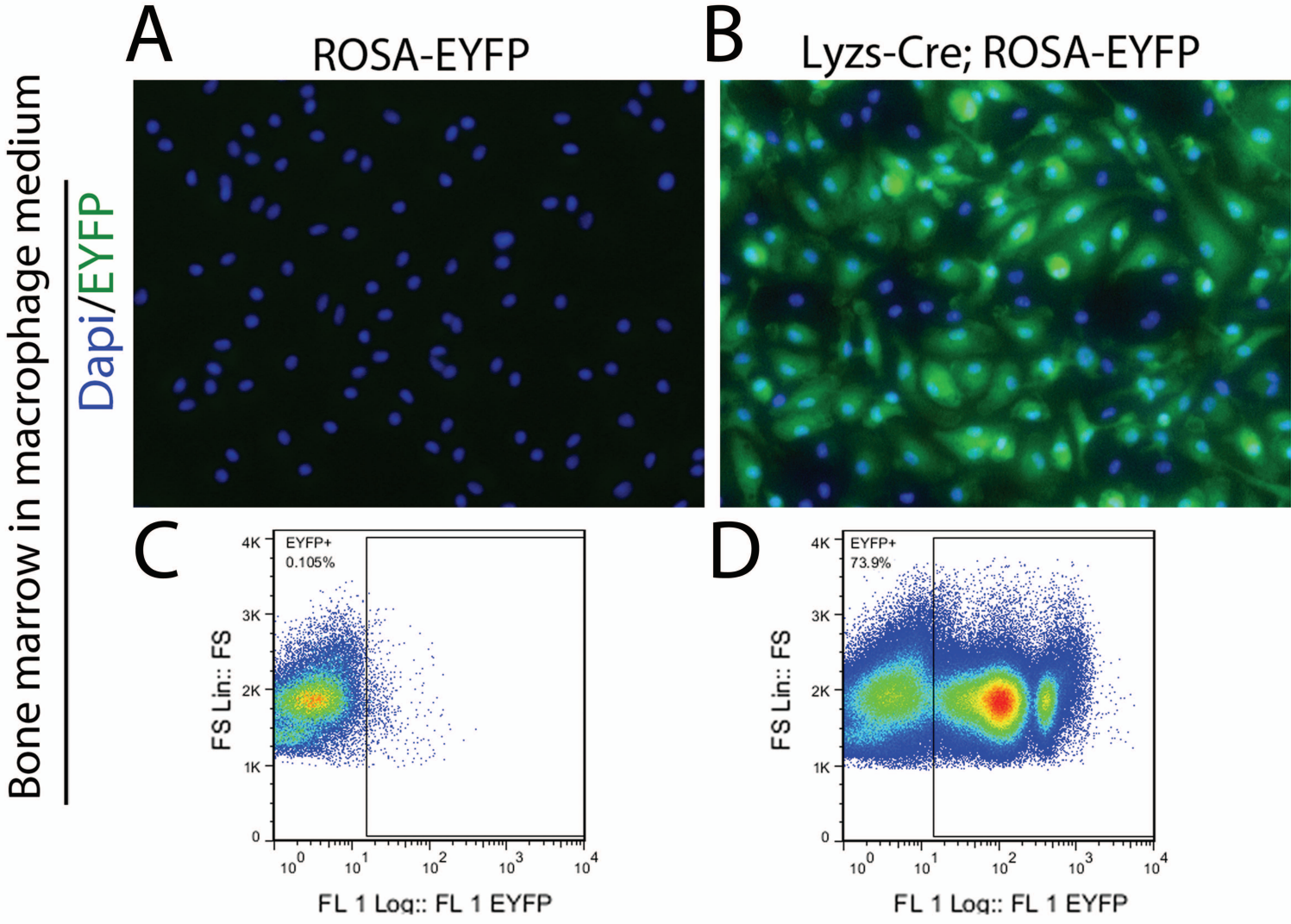

A
ROSA-EYFP
B
Lyzs-Cre; ROSA-EYFP
Bone marrow in macrophage medium
Dapi/EYFP
C
EYFP+
0.105%
FS Lin:: FS
FL 1 Log:: FL 1 EYFP
D
EYFP+
73.9%
FS Lin:: FS
FL 1 Log:: FL 1 EYFP

Figure S3

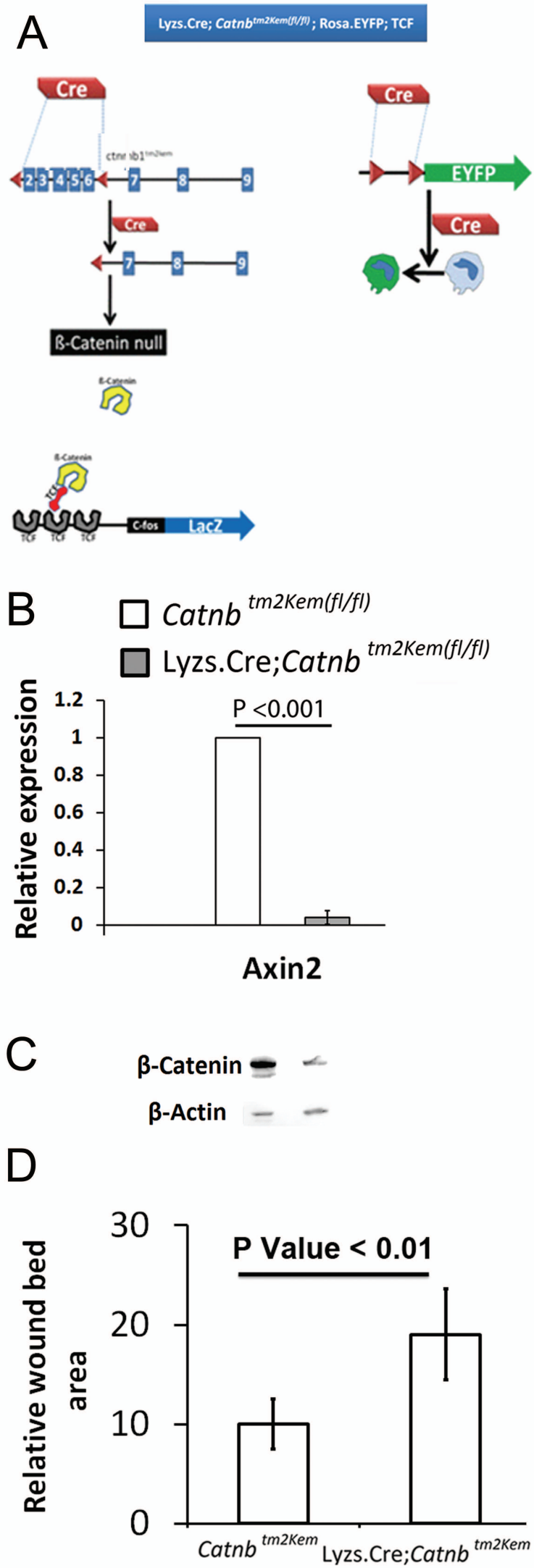

A
Lyzs.Cre; Catnb tm2Kem(fl/fl) ; Rosa.EYFP; TCF
Cre
2 3 4 5 6
7
8
9
Cre
7
8
9
ß-Catenin null
ß-Catenin
ß-Catenin
TCF
TCF
TCF
TCF
C-fos
LacZ
Cre
EYFP
Cre
B
Catnb tm2Kem(fl/fl)
Lyzs.Cre;Catnb tm2Kem(fl/fl)
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
P <0.001
Axin2
C
β-Catenin
β-Actin
D
Relative wound bed area
30
20
10
0
P Value < 0.01
Catnb tm2Kem
Lyzs.Cre;Catnb tm2Kem

Figure S4

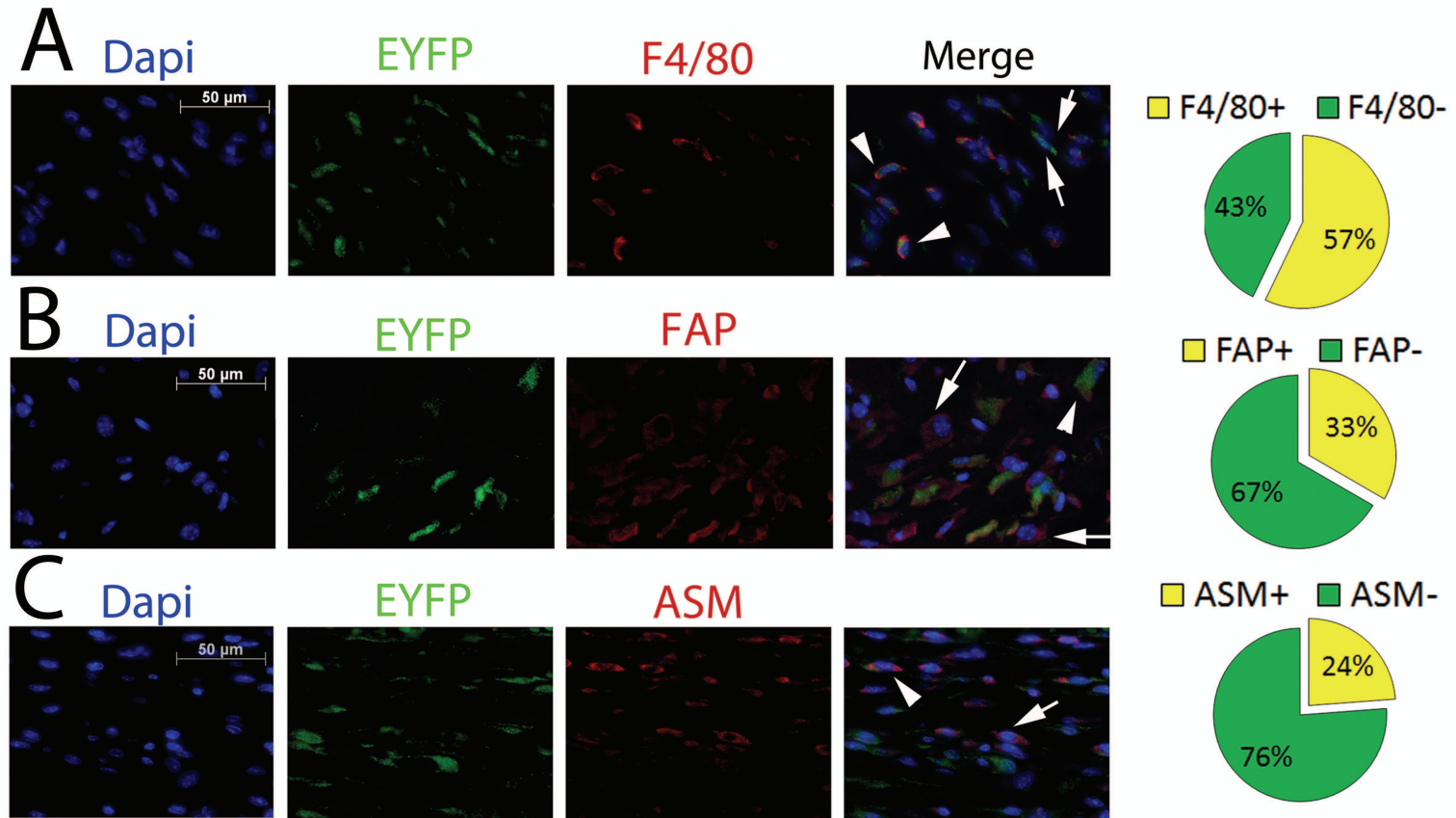

A
Dapi
EYFP
F4/80
Merge
50 µm
F4/80+
F4/80-
43%
57%
B
Dapi
EYFP
FAP
50 µm
FAP+
FAP-
33%
67%
C
Dapi
EYFP
ASM
50 µm
ASM+
ASM-
24%
76%

Figure S5

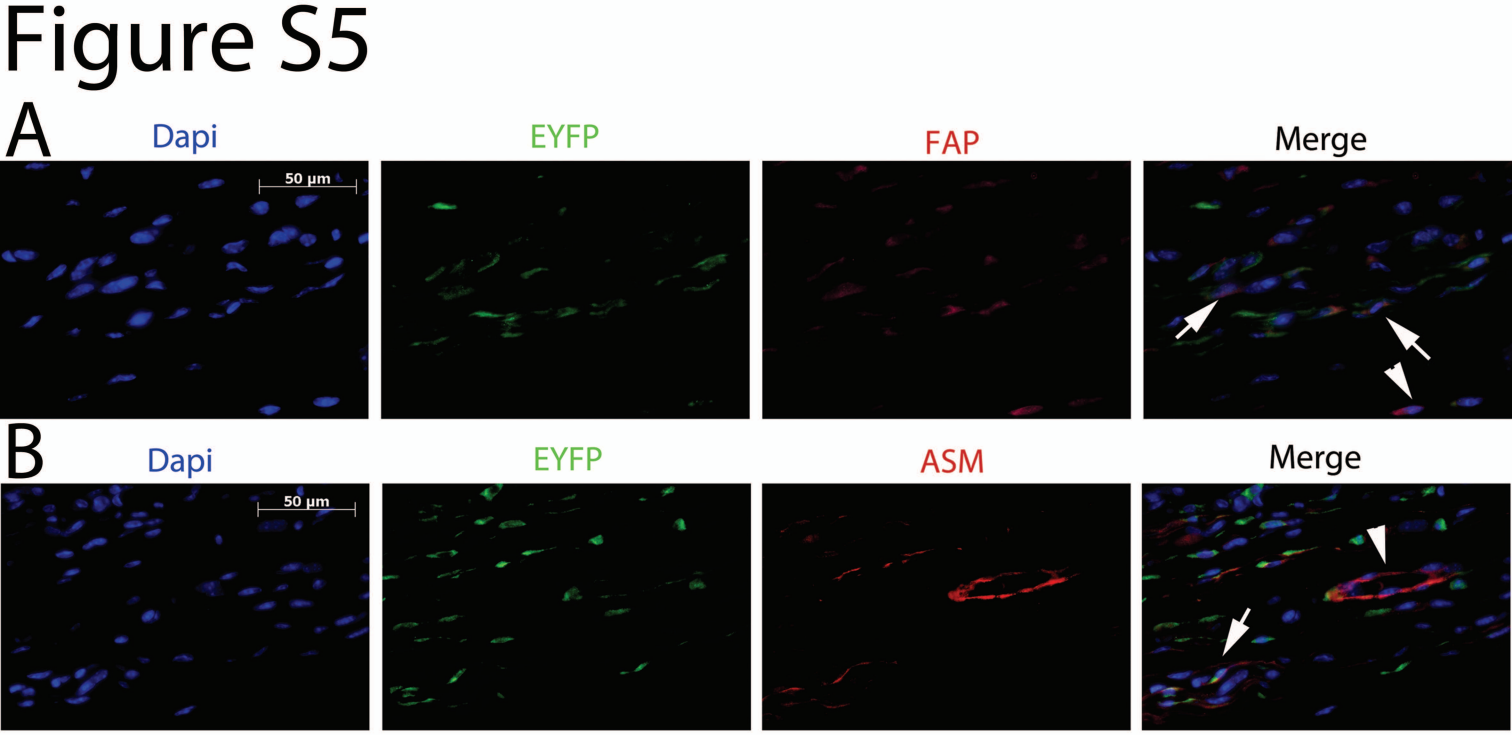


Figure S6

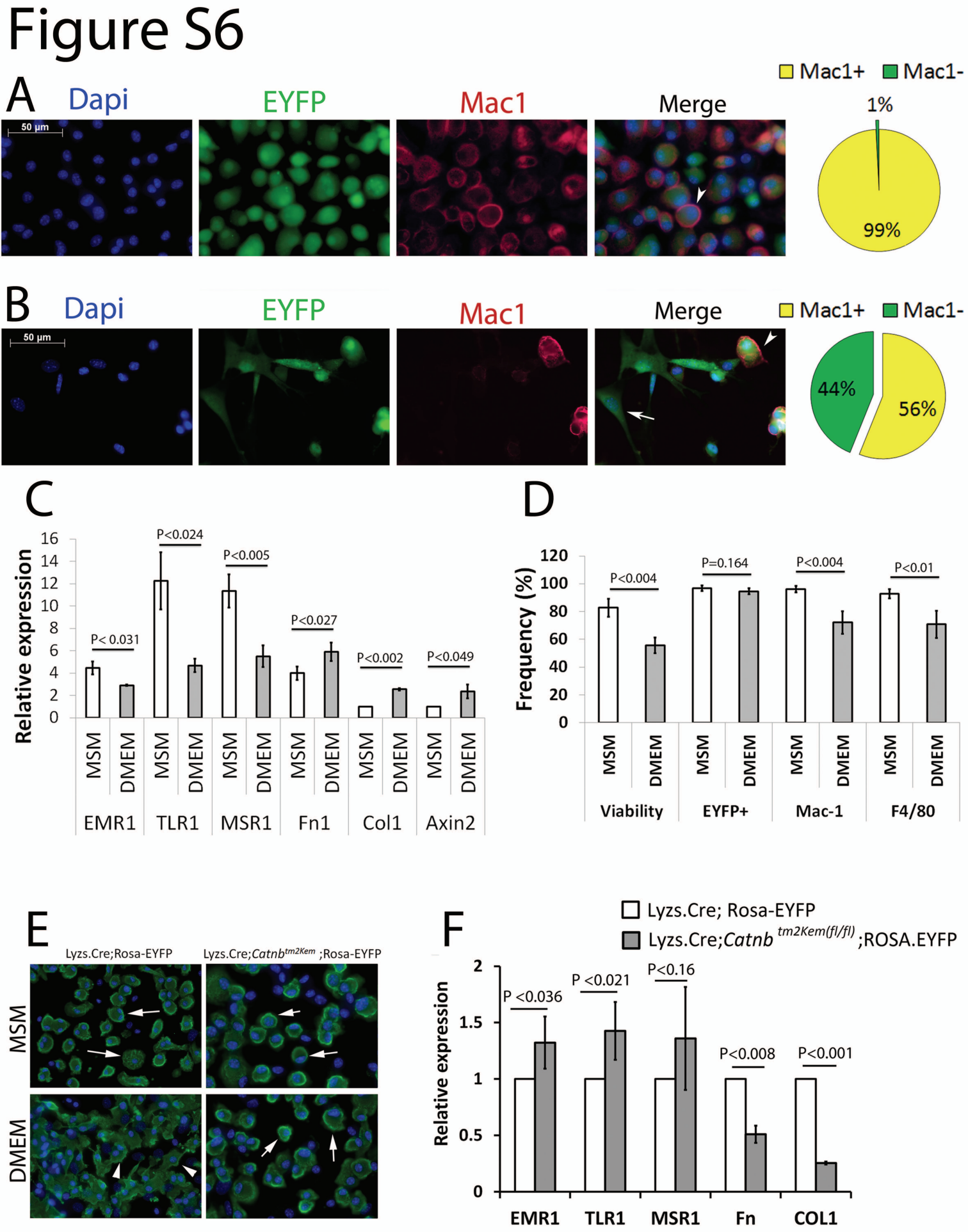

A
Dapi
EYFP
Mac1
Merge
50 µm
Mac1+
Mac1-
1%
99%
B
Dapi
EYFP
Mac1
Merge
50 µm
Mac1+
Mac1-
44%
56%
C
Relative expression
16
14
12
10
8
6
4
2
0
P< 0.031
P<0.024
P<0.005
P<0.027
P<0.002
P<0.049
MSM
DMEM
EMR1
TLR1
MSR1
Fn1
Col1
Axin2
D
Frequency (%)
120
100
80
60
40
20
0
P<0.004
P=0.164
P<0.004
P<0.01
MSM
DMEM
Viability
EYFP+
Mac-1
F4/80
E
Lyzs.Cre;Rosa-EYFP
Lyzs.Cre;Catnb tm2Kem ;Rosa-EYFP
MSM
DMEM
F
Lyzs.Cre; Rosa-EYFP
Lyzs.Cre;Catnb tm2Kem(fl/fl) ;ROSA.EYFP
Relative expression
2
1.5
1
0.5
0
P <0.036
P <0.021
P<0.16
P<0.008
P<0.001
EMR1
TLR1
MSR1
Fn
COL1

Figure S7

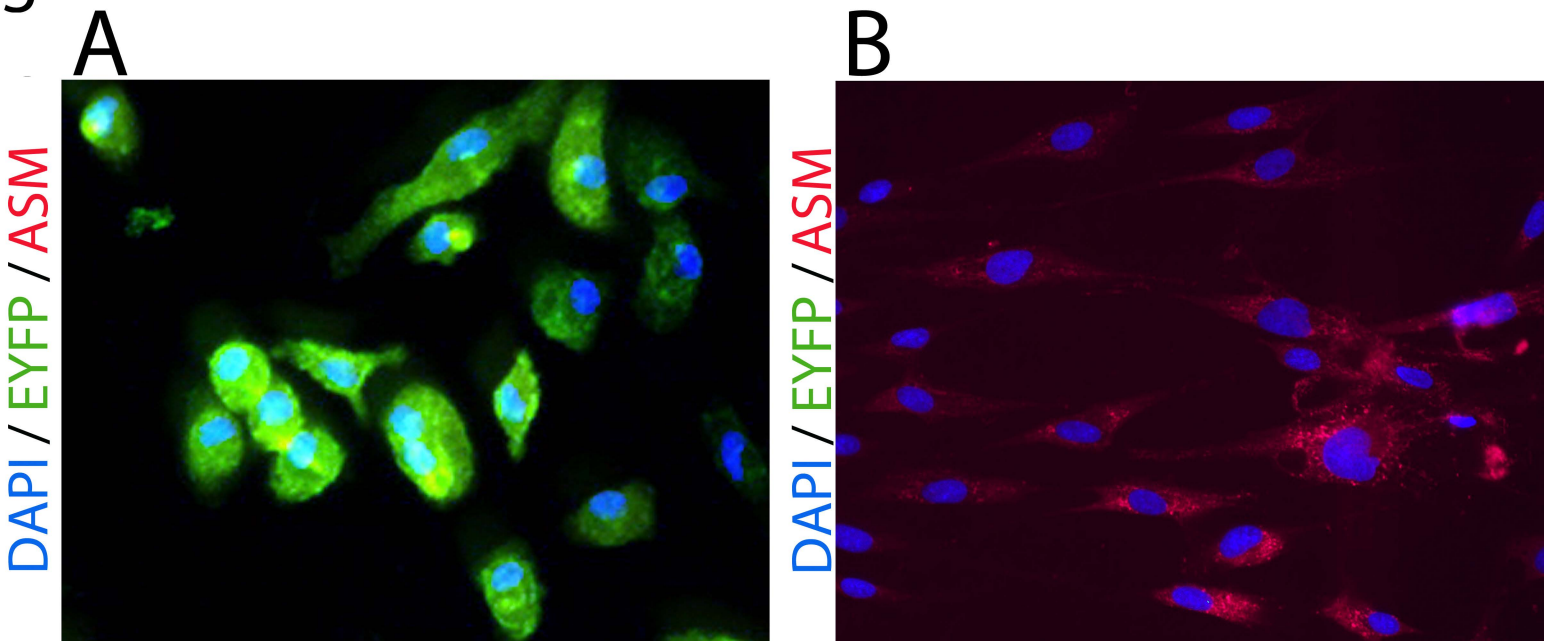


Figure S8

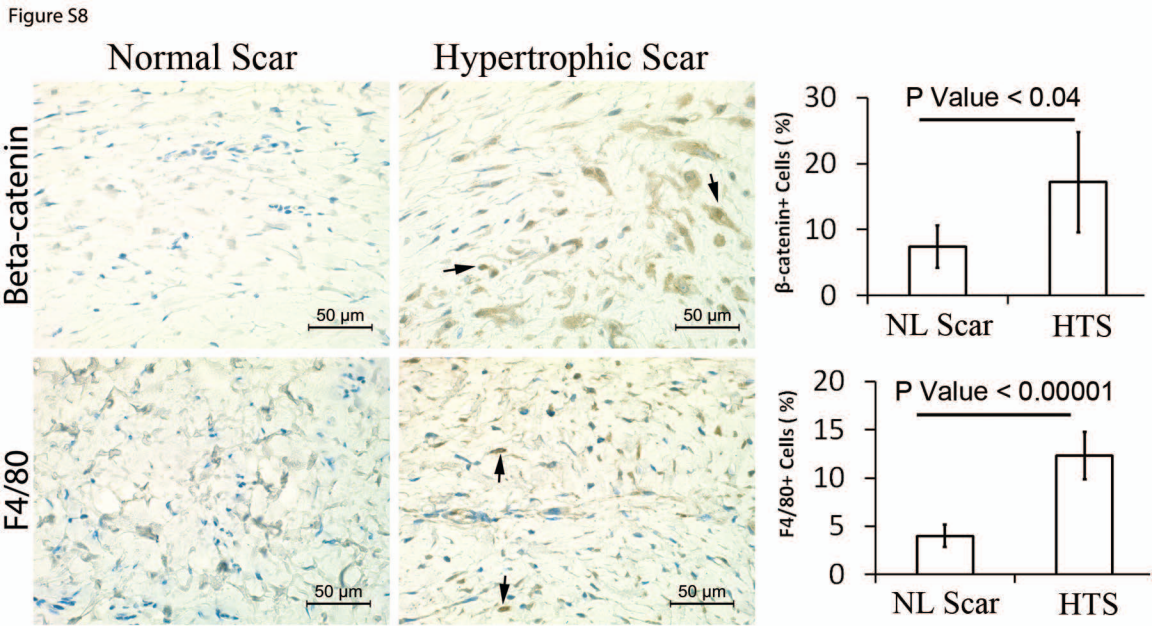

Normal Scar
Hypertrophic Scar
Beta-catenin
F4/80
50 µm
50 µm
50 µm
50 µm
P Value < 0.04
β-catenin+ Cells (%)
30
20
10
0
NL Scar
HTS
P Value < 0.00001
F4/80+ Cells (%)
20
15
10
5
0
NL Scar
HTS

Table S1. Tcf transcriptionally active cells express genes characteristically expressed by macrophages during skin healing.

	β-Gal+ / β-Gal-
Tlr4	2.67
Tlr6	2.83
CD22	3.9
CD33	4.29
CD93	5.99
CD11b	6.5
CD68	9.8
CD204	11.67
MSR1	11.67
CD84	13.2
Tlr1	13.24
F4/80	26.9

Table S2. Genes Attributed to Macrophage Migration

RefSeq	Gene.Symbol	Gene name	KO/WT
NM_008118	Gif	Glycosylation-inhibiting factor	0.562284
NM_011223	Pxn	Paxillin	0.738619
NM_001162365	Ptk2b	Protein tyrosine kinase 2 beta	0.773482
NM_010576	Itga4	VLA-4	0.788286
NM_020505	Vav3	Vav3 oncogene	0.810314
NM_001111316	Ptprc	CD 45, role in migration	0.894839
NM_010736	Ltbr	Lymphotoxin B receptor	0.947183
NM_001082960	Itgam	Mac-1	0.971535

Table S3. Adam Gene Family

RefSeq	Gene.Symbol	KO/WT	RefSeq	Gene.Symbol	KO/WT
NM_010084	Adam18	0.537267	NM_177872	Adamts3	0.905477
NM_021475	Adamdec1	0.566615	NM_177872	Adamts3	0.913203
NM_177872	Adamts3	0.590272	NM_177872	Adamts3	0.916544
NM_001033877	Adamts17	0.617274	NM_029967	Adamtsl1	0.923991
NM_007402	Adam7	0.620423	NM_172845	Adamts4	0.943594
NM_175506	Adamts19	0.622105	NM_013906	Adamts8	0.968468
NM_175939	Adam29	0.629918	NM_001003911	Adamts7	0.968973
NM_145745	Adam34	0.665905	NM_153397	Adam32	0.971559
NM_177872	Adamts3	0.680757	NM_007400	Adam12	0.976602
NM_172125	Adam1b	0.702741	NM_001081401	Adamts3	0.976721
NM_177872	Adamts3	0.708564	NM_177872	Adamts3	0.978318
NM_001001322	Adamts13	0.70957	NM_033615	Adam33	1.012868
NM_011781	Adam25	0.7223	NM_001009547	Adam26b	1.014347
NM_010086	Adam24	0.726665	NM_007401	Adam5	1.01455
NM_145745	Adam34	0.743746	NM_001081127	Adamts14	1.037296
NM_009616	Adam19	0.750183	NM_009618	Adam2	1.039012
NM_172466	Adamts18	0.750865	NM_177872	Adamts3	1.049208
NM_177872	Adamts3	0.763838	NM_007404	Adam9	1.054751
NM_001037722	Adam15	0.767754	NM_001033877	Adamts17	1.070653
NM_010082	Adam28	0.7764	NM_177872	Adamts3	1.079829
NM_174885	Adam6a	0.778271	NM_013906	Adamts8	1.097105
NM_001037722	Adam15	0.787446	NM_175501	Adamts12	1.110269
NM_029967	Adamtsl1	0.788609	NM_009615	Adam17	1.110969
NM_177431	Adamts20	0.789541	NM_001081020	Adamts6	1.117838
NM_007399	Adam10	0.793108	NM_172126	Adam1a	1.155448
NM_001007220	Adam22	0.795107	NM_001113548	Adamtsl5	1.182471
NM_027665	Adam30	0.803075	NM_177872	Adamts3	1.191522
NM_001110778	Adam11	0.805177	NM_144899	Adamtsl4	1.230599
NM_001025380	Adam39	0.811513	NM_177872	Adamts3	1.230684
NM_009619	Adam3	0.821438	NM_177872	Adamts3	1.291045
NM_009620	Adam4	0.835456	NM_172619	Adamts10	1.306561
ENSMUST00000094237	Adamtsl3	0.836585	NM_175314	Adamts9	1.464681
NM_001033877	Adamts17	0.846534	NM_175314	Adamts9	1.57999
NM_001009545	Adam6b	0.846981	NM_177872	Adamts3	1.612718
NM_029981	Adamtsl2	0.84956	ENSMUST00000049189	Adamts9	1.691706
NM_001024139	Adamts15	0.852374	NM_175314	Adamts9	1.730321
NM_001033877	Adamts17	0.853589	NM_175314	Adamts9	1.85765
NM_001033877	Adamts17	0.856495	NM_011780	Adam23	2.06231
NM_177872	Adamts3	0.872939	NM_175314	Adamts9	2.147783
NM_010085	Adam26a	0.882167	NM_175314	Adamts9	2.409637
NM_007403	Adam8	0.891508	NM_175643	Adamts2	2.512744
NM_172053	Adamts16	0.899952	NM_009621	Adamts1	3.00974
NM_020330	Adam21	0.903909	NM_011782	Adamts5	3.04486

Table S4. Integrins

RefSeq	Gene.Symbol	KO/WT
NM_008400	Itgal	0.408643
NM_001001309	Itga8	0.470144
NM_133721	Itga9	0.583581
NM_001005608	Itgb4	0.655604
NM_001029872	Itgad	0.717612
NM_010577	Itga5	0.761503
NM_010576	Itga4	0.788286
NM_008402	Itgav	0.801325
NM_008399	Itgae	0.844142
NM_010578	Itgb1	0.85869
NM_027120	Itgb1bp3	0.86017
NM_008405	Itgb2l	0.882582
NM_001159564	Itgb6	0.898635
NM_008403	Itgb1bp1	0.93463
NM_001081053	Itga10	0.952872
NM_001082960	Itgam	0.971535
NM_145467	Itgbl1	0.974079
NM_010578	Itgb1	1.006318
NM_016780	Itgb3	1.019998
NM_008397	Itga6	1.040697
NM_001005608	Itgb4	1.047808
NM_026348	Itgb3bp	1.052541
NM_176922	Itga11	1.073923
NM_013565	Itga3	1.115873
NM_013712	Itgb1bp2	1.121421
NM_177290	Itgb8	1.138774
NM_013566	Itgb7	1.172588
NM_008404	Itgb2	1.200715
NM_008398	Itga7	1.211262
NM_001145884	Itgb5	1.323797
NM_008396	Itga2	1.34384
NM_001033228	Itga1	1.634017
NM_010575	Itga2b	1.774187
ENSMUST00000106237	Itgad	1.893578
NM_021334	Itgax	2.889488