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Supplementary Materials for

Cadherin-11–mediated adhesion of macrophages to myofibroblasts establishes a profibrotic niche of active TGF-β

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The PDF file includes:

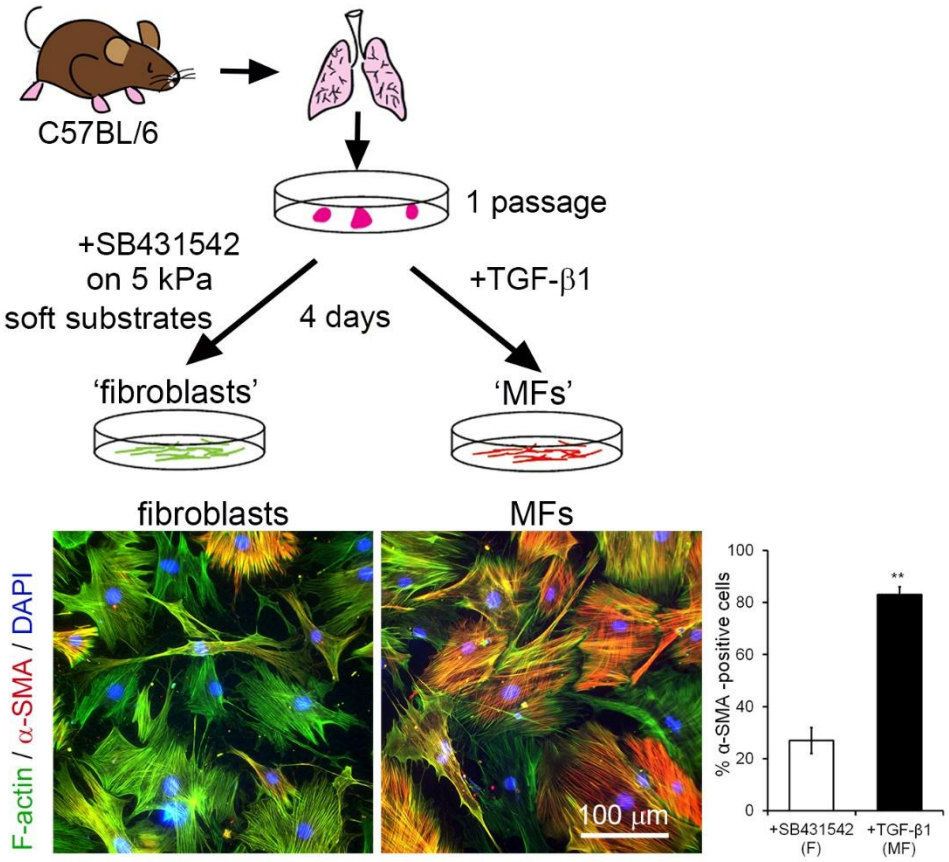
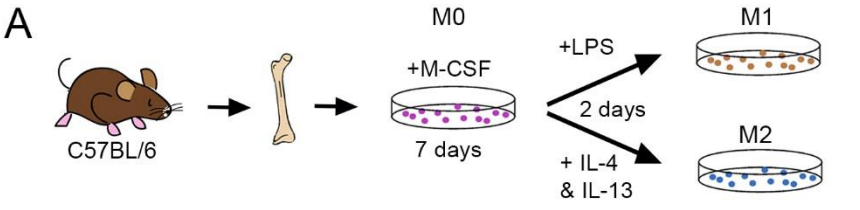
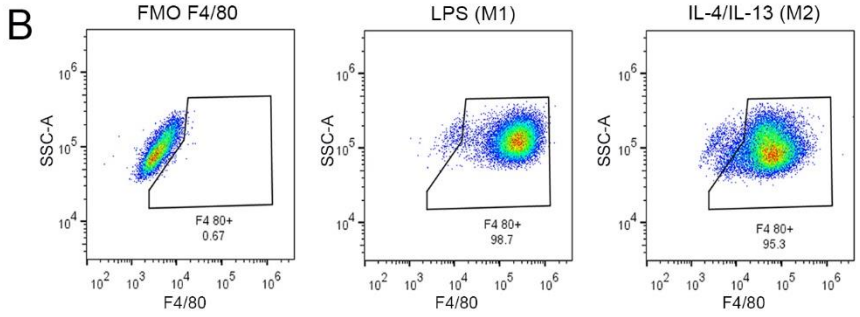
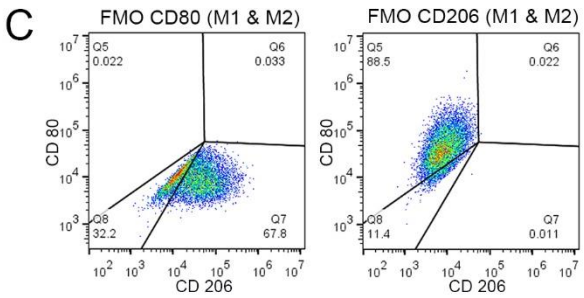
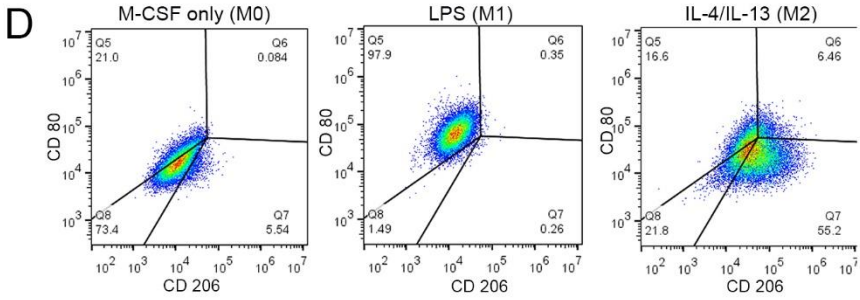


Fig. S1. Primary mLF cultures. Primary lung fibroblasts were explanted from the lungs of C57BL/6 mice. Cells were subcultured in control medium for 1 passage (7 d), then transferred into medium containing the TGF-β receptor inhibitor SB431542 to inhibit MF activation, or to medium containing active TGF-β1 to activate MFs. Cells from each culture condition were immunostained for α-SMA, F-actin (Phalloidin), and nuclei (DAPI) and the percentage of α-SMA–positive activated MFs was quantified by image analysis. Graph shows averages ±SD from N=3 independent experiments performed with cells from at least 3 different animals (**$p \leq 0.01$ using ANOVA followed by a post-hoc Tukey's multiple comparison test).


A
C57BL/6
M0
+M-CSF
7 days
+LPS
2 days
+ IL-4
& IL-13
M1
M2


B
FMO F4/80
LPS (M1)
IL-4/IL-13 (M2)
SSC-A
F4/80
F4 80+
0.67
F4 80+
98.7
F4 80+
95.3


C
FMO CD80 (M1 & M2)
FMO CD206 (M1 & M2)
CD 80
CD 206
Q5
0.022
Q6
0.033
Q8
32.2
Q7
67.8
Q5
88.5
Q6
0.022
Q8
11.4
Q7
0.011


D
M-CSF only (M0)
LPS (M1)
IL-4/IL-13 (M2)
CD 80
CD 206
Q5
21.0
Q6
0.084
Q8
73.4
Q7
5.54
Q5
97.9
Q6
0.35
Q8
1.49
Q7
0.26
Q5
16.6
Q6
6.46
Q8
21.8
Q7
55.2

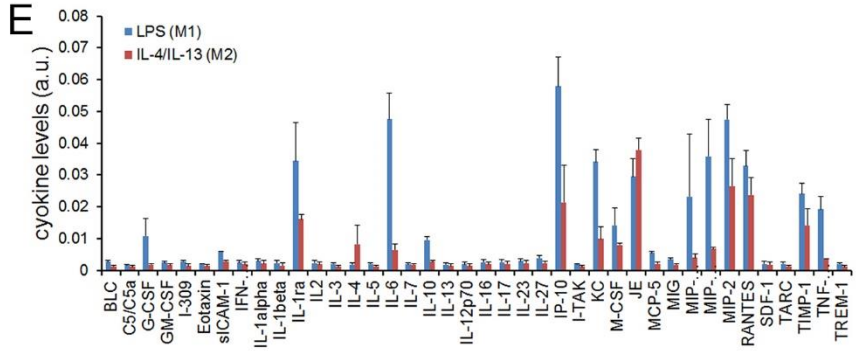

E
LPS (M1)
IL-4/IL-13 (M2)
cyokine levels (a.u.)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
BLC
C5/C5a
G-CSF
GM-CSF
I-309
Eotaxin
sICAM-1
IFN-
IL-1alpha
IL-1beta
IL-1ra
IL2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-10
IL-13
IL-12p70
IL-16
IL-17
IL-23
IL-27
IP-10
I-TAK
KC
M-CSF
JE
MCP-5
MIG
MIP-..
MIP-..
MIP-2
RANTES
SDF-1
TARC
TIMP-1
TNF-..
TREM-1

Fig. S2. Primary mouse macrophage cultures. (**A**) To obtain primary macrophages, femurs and tibiae of C57BL/6 mice were dissected, and the bone marrow was flushed out with macrophage base medium plus M-CSF, followed by 7 d of culture in the same medium. These cells are designated as mature (M0) macrophages. Mature macrophages were polarized by adding LPS (to generate M1 macrophages) or 10 ng/ml IL-4 plus 10 ng/ml IL-13 (to generate M2 macrophages) for an additional 4 d. (**B-D**) For flow cytometry, macrophages were detached from culture dishes using Accutase, Fc receptors were blocked using CD16/CD32 antibody, and cells were stained for viability with using fixable viability dye eFluor506. Cells were further live-labeled with fluorochrome-conjugated primary antibody mix containing: F4/80-BV, CD80-AF488, CD206-PE. (**B**) Polarized macrophages were first assessed for F4/80 expression, which was used for gating in all subsequent analyses. (**C**) FMO (fluorescence minus one) conditions contained all antibodies except the control and were performed with equimolar mixes of all macrophage polarization types. (**D**) Different in vitro polarized macrophages were assessed by flow cytometry for the presence of of CD80 and CD206. (**E**) To quantify cytokine production, macrophages were polarized as described and subsequently cultured in base medium for 2 d. Conditioned culture supernatants were analyzed using a mouse cytokine array comprising BLC, C5/C5a, G-CSF, GM-CSF, I-309, Eotaxin, sICAM-1,INF-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-12p70, IL-16, IL-17, IL-23, IL-27, IP-10, I-TAC, KC, M-CSF, JE, MCP-5, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, SDF-1, TARC, TIMP-1, TNF-α, and TREM-1. Graph shows averages ±SD from N=3 independent experiments performed with macrophages extracted from at least 3 different animals.

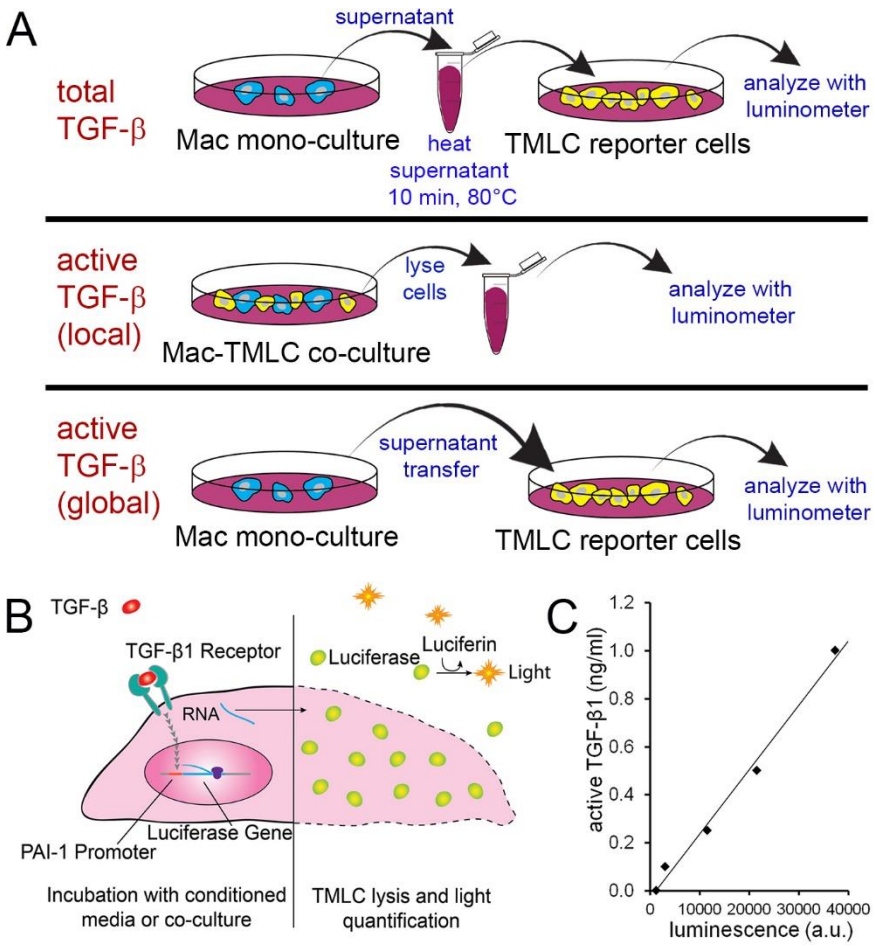


Fig. S3. Active TGF-β1 measurement using TMLC cultures. Active TGF-β was quantified using TMLCs, which produce luciferase under the control of the PAI-1 promoter in response to TGF-β. (**A**) To measure total TGF-β production in macrophage cultures, supernatants and macrophage lysates were collected after 24 h, heated to 80°C for 10 min to activate all latent TGF-β, and then transferred to TMLC cultures for another 24 h. To determine the amounts of locally active TGF-β, TMLCs were directly co-cultured with macrophages for 48 h. To determine amounts of active TGF-β in macrophage culture supernatants, 24 h conditioned medium was transferred to TMLC cultures for another 24 h. (**B**) TMLCs were lysed, and luciferase activity was measured by luminescence. The measured readings were corrected for TMLC baseline luciferase production in control medium. (**C**) Luminescence measurements were converted into ng/ml of TGF-β by establishing standard curves performed with known concentrations of active TGF-β. Graph shows one representative example of a typical standard curve.

Table S1. Primer list.

Target	**Forward 5' → 3'**	**Reverse 5' → 3'**	**Anneal at**
Cdh11	GCCTGGCTCAACATCTCTGT	CTGATGGCCACTGGGACTTT	59°C
Cdh2	AGCCCGGTTTCACTTGAGAG	CCTGGTGCAGAAACTCAGGT	59°C
Itgαv	TTGGTGTGGATCGAGCTGTC	CAGGCAACGGGCAGATCTTA	59°C
Itgβ1	GCGTGGTTGCTGGAATTGTT	GGATTTTCACCCGTGTCCCA	59°C
Itgβ3	GCTTGCCCATGTTTGGCTAC	TACGGGACACGCTCTGTTTC	59°C
Itgβ5	CCCAATGCCATGACCATCCT	CGCTCACTTTGGAACTTGGC	59°C
Itgβ8	CAGTGCCTTCACCCTCACAA	GCAGATAGCTTGGGCCAGAT	59°C
Acta2	CATCACCAACTGGGACGACA	GTTCAGTGGTGCCTCTGTCA	59°C
Cd64	GCAAGTTAGAAGCGATGGCG	CACAGTCACCCACTGAGCTT	59°C
Siglec-F	CCCTGGTGTCTGGCAATTCT	AGGGAAGTGAGGGCAGATGA	59°C
Cd206	GTCAGAACAGACTGCGTGGA	CAGCAGCAGTCTCGATGGAA	59°C
Nos2	GAGCCACAGTCCTCTTTGCT	CTGGTCCATGCAGACAACCT	59°C
Cd11c	TTCATCTCCACGTCAAGCCC	AGGACCTTGGTGGCATCTTG	59°C
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA	59°C
Hmbs	AAGGGCTTTTCTGAGGCACC	AGTTGCCCATCTTTCATCACTG	59°C
G6pd	CACAGTGGACGACATCCGAAA	AGCTACATAGGAATTACGGGCAA	59°C