**Supplementary information**

**Table S1.** Primer sequences and amplicon sizes for human tissues. All primer sets worked under identical quantitative PCR cycling conditions with similar efficiencies to obtain simultaneous amplification in the same run. Sequences were taken from GeneBank, all accession numbers are denoted.

| Gene | Accession | | Sequences (5´ → 3´) | Length | Amplicon |
|---|---|---|---|---|---|
| CTGF | NM001901 | for | cct gca ggc tag aga agc aga | 21bp | 103bp |
| | | rev | ttt ggg agt acg gat gca ctt | 21bp | |
| CYR61 | NM001554 | for | aaa ggc agc tca ctg aag cg | 20bp | 110bp |
| | | rev | gca ctg gga cca tga agt tgt | 21bp | |
| HPRT1 | NM000194 | for | aag gac ccc acg aag tgt tg | 20bp | 157bp |
| | | rev | ggc ttt gta ttt tgc ttt tcc a | 22bp | |
| NOV | NM002514 | for | ccg tca atg tga gat gct gaa | 21bp | 107bp |
| | | rev | ttg gtg cgg aga cac ttt ttt | 21bp | |
| WISP1 | NM003882 | for | gta tgt gag gac gac gcc aag | 21bp | 104bp |
| | | rev | ggc tat gca gtt cct gtg cc | 20bp | |
| WISP2 | NM003881 | for | gac atg aga ggc aca ccg aag | 21bp | 94bp |
| | | rev | gta cat ggt gtc ggg cac ag | 20bp | |
| WISP3 | NM003880 | for | ctc cac tct tct gct tgc tgg | 21bp | 87bp |
| | | rev | agg cct tcc ttc agg tgt tgt | 21bp | |

**Table S2.** Primer sequences and amplicon sizes for mouse tissues. All primer sets worked under identical quantitative PCR cycling conditions with similar efficiencies to obtain simultaneous amplification in the same run. Sequences were taken from GeneBank, all accession numbers are denoted.

| Gene | Accession | | Sequences (5´ → 3´) | Length | Amplicon |
|---|---|---|---|---|---|
| β-catenin | NM007614 | for | tca aga gag caa gct cat cat tct | 24bp | 115bp |
| | | rev | cac ctt cag cac tct gct tgt g | 22bp | |
| Cdh16 | NM007663 | for | tgc aga aag cct gca cac a | 19bp | 130bp |
| | | rev | tgc cgt gtt tga gtc tcc tg | 20bp | |
| Col1a1 | NM007742 | for | cca aga aga cat ccc tga agt ca | 23bp | 128bp |
| | | rev | tgc acg tca tcg cac aca | 18bp | |
| Col1a2 | NM007743 | for | agc ttt gtg gat acg cgg act | 21bp | 86bp |
| | | rev | tcg tac tga tcc cga ttg ca | 20bp | |
| Ctgf | NM010217 | for | ctt ctg cga ttt cgg ctc c | 19p | 115bp |
| | | rev | tgc ttt gga agg act cac cg | 20bp | |
| Cyclin G1 | NM009831 | for | tgg ctg tca aga tga tag aag tac tga | 27bp | 94bp |
| | | rev | tgg ctg aca tct aga ctc ctg ttc | 24bp | |
| Cyclin B2 | NM007630 | for | gtc aac aag cag ccg aaa cc | 20bp | 75bp |
| | | rev | gag gac gat cct tgg gag cta | 2qbp | |
| Cyr61 | NM010516 | for | cca ccg ctc tga aag gga t | 19bp | 80bp |
| | | rev | ccc cgt ttt ggt aga ttc tgg | 21bp | |
| Fizz1 | NM020509 | for | tat gaa cag atg ggc ctc ctg | 21bp | 90bp |
| | | rev | tcc act ctg gat ctc cca aga | 21bp | |
| Fn | NM010233 | for | gtg tag cac aac ttc caa tta cga a | 25bp | 90bp |
| | | rev | gga att tcc gcc tcg agt ct | 20bp | |

| | | | | | |
|---|---|---|---|---|---|
| Fsp1 | NM011311 | for | agg agc tac tga cca ggg agc t | 22bp | 102bp |
| | | rev | tca ttg tcc ctg ttg ctg tcc | 21bp | |
| Fzd1 | NM021457 | for | aaa cag cac agg ttc tgc aaa a | 22bp | 58bp |
| | | rev | tgg gcc ctc tcg ttc ctt | 18bp | |
| Fzd2 | NM020510 | for | tcc atc tgg tgg gtg att ctg | 21bp | 66bp |
| | | rev | ctc gtg gcc cca ctt cat t | 19bp | |
| Fzd3 | NM021458 | for | gcc tat agc gag tgt tca aaa ctc a | 25bp | 78bp |
| | | rev | tgg aaa cct act gca ctc cat atc t | 25bp | |
| Fzd4 | NM008055 | for | gcc cca gaa cga cca caa | 18bp | 64bp |
| | | rev | ggg caa ggg aac ctc ttc at | 20bp | |
| Gsk3β | NM_019827 | for | ttt gag ctg gta ccc tag gat ga | 23bp | 75bp |
| | | rev | ttc ttc gct ttc cga tgc a | 19bp | |
| Hmbs | NM013551 | for | atg tcc ggt aac ggc ggc | 22bp | 135bp |
| | | rev | ggt aca agg ctt tca gca tcg c | 18bp | |
| Inha | NM008380 | for | gga ggg ccg aaa tga atg a | 19bp | 84bp |
| | | rev | tgc agt gtc ttc ctg gct gt | 20bp | |
| Kcne2 | NM134110 | for | ggt ctc ctg cat tgc tca cat | 21bp | 82bp |
| | | rev | cat cct cca gtg tct ggg tca | 21bp | |
| Ki67 | NM001081 117 | for | ttg acc gct cct tta ggt atg aa | 23bp | 138bp |
| | | rev | ggt atc ttg acc ttc ccc atc a | 22bp | |
| Lef1 | NM010703 | for | ggc ggc gtt gga cag at | 17bp | 67bp |
| | | rev | cac ccg tga tgg gat aaa cag | 21bp | |
| Lrp5 | NM008513 | for | caa cgt gga cgt gtt tta ttc ttc | 24bp | 138bp |
| | | rev | cag cga ctg gtg ctg tag tca | 21bp | |

| | | | | | |
|---|---|---|---|---|---|
| Lrp6 | NM008514 | for | cca ttc ctc tca ctg gtg tca a | 22bp | 146bp |
| | | rev | gcc aaa ctc tac cac atg ttc ca | 23bp | |
| Mmp2 | NM008610 | for | atc gag acc atg cgg aag c | 19bp | 123bp |
| | | rev | atc cac ggt ttc agg gtc c | 19bp | |
| Mmp7 | NM010810 | for | cct agg cgg aga tgc tca ct | 20bp | 96bp |
| | | rev | gct gcc acc cat gaa ttt g | 19bp | |
| Mmp9 | NM01399 | for | cgc ctt ggt gta gca caa ca | 20bp | 106bp |
| | | rev | aca ggg ttt gcc ttc tcc gtt | 21bp | |
| Nov | NM010930 | for | aac aac cag act ggc att tgc | 21bp | 133bp |
| | | rev | cag cca atc tgc cca tct ct | 20bp | |
| Pai1 | NM008871 | for | gtc ttt ccg acc aag agc ag | 20bp | 104bp |
| | | rev | gac aaa ggc tgt gga gga ag | 20bp | |
| Sfrp1 | NM013834 | for | gta caa ccg tgt gtc ctc cat | 21bp | 89bp |
| | | rev | cat cct cag tgc aaa ctc gct | 21bp | |
| αSMA | NM007392 | for | gct ggt gat gat gct ccc a | 19bp | 80bp |
| | | rev | gcc cat tcc aac cat tac tcc | 21bp | |
| Spp1 | NM009263 | for | gtt tgg cat tgc ctc ctc c | 19bp | 83bp |
| | | rev | gga tct ggg tgc agg ctg ta | 20bp | |
| Tcf3 | NM009332 | for | tcc agc aca ctt gtc caa caa | 21bp | 61bp |
| | | rev | cag cgg gtg cat gtg atg | 18bp | |
| Tcf4 | NM009333 | for | gtg gga act gcc ccg ttt | 18bp | 59bp |
| | | rev | gtt cta aga gca cag ggc agt tg | 23bp | |
| Wisp1 | NM018865 | for | gtc ctg agg gtg ggc aac at | 20bp | 97bp |
| | | rev | ggg cgt gta gtc gtt tcc tct | 21bp | |

| | | | | | |
|---|---|---|---|---|---|
| Wisp2 | NM016873 | for | tac agg tgc cag gaa ggt gc | 20bp | 119bp |
| | | rev | cag atg cag gag tga caa ggg | 21bp | |
| Wisp3 | XM282903 | for | ggc gtg tgc gca tat ctt g | 19bp | 98bp |
| | | rev | agg cag ctg aac agt ggg tg | 20bp | |
| Wnt1 | NM021279 | for | caa atg gca att ccg aaa cc | 20bp | 112bp |
| | | rev | gat tgc gaa gat gaa cgc tg | 20bp | |
| Wnt2 | NM023653 | for | agc cct gat gaa cct tca caa c | 22bp | 78bp |
| | | rev | tga cac ttg cat tct tgt ttc aag | 24bp | |
| Wnt3a | NM009522 | for | gca cca ccg tca gca aca | 18bp | 57bp |
| | | rev | ggg tgg ctt tgt cca gaa ca | 20bp | |
| Wnt7b | NM009528 | for | tcg aaa gtg gat ctt tta cgt gtt t | 25bp | 67bp |
| | | rev | tga caa tgc tcc gag ctt ca | 20bp | |
| Wnt10b | NM011718 | for | tgg gac gcc agg tgg taa | 18bp | 60bp |
| | | rev | ctg acg ttc cat ggc att tg | 20bp | |

**Supplementary Figure Legends**

**Figure S1. (A)** The phenotype of ATII cell isolations from saline- or bleomycin-treated mice, 14 days after instillation, as indicated, was analyzed by immunofluorescent staining. ATII cells were fixed after 24h of attachment and subsequently stained with antibodies against occludin (OCCL) or pan-cytokeratin (panCK) (magnification 40×). Nuclei were visualized by DAPI staining (inlet). All stainings are representative of at least three independent experiments. **(B)** Control negative immunostainings (single or double, as indicated) for the antibodies used in the study. Cells were prepared as described, irrelevant IgG used in replacement of a specific primary antibody, and secondary antibodies used as indicated (magnification 10×).

**Figure S2.** ATII cell gene expression profiles were analyzed by whole genome expression analysis using RNA from freshly isolated ATII cells from saline- or bleomycin-treated mouse lungs 14 d after administration. Functional annotation of regulated gene clusters was performed according to Gene Ontology (GO) or the Kyoto Encyclopedia of Genes and Genomes (KEGG), as indicated.

**Figure S3.** Treatment scheme of TOPGAL mice. Recombinant mouse WNT3A or vehicle control was administered orotracheally (500 ng or 1000 ng in 80µl total volume) and mouse lungs were excised after 24h for the detection of β-galactosidase in the challenged TOPGAL mice. In a second arm, TOPGAL reporter mice were challenged with Bleomycin or saline as described in detail in *Material & Methods* and analysed on different time points, as indicated. At least four mice per time point were analysed.

**Figure S4.** Control immunohistochemical staining of sections from control (transplant donor) or IPF lung tissue specimen for the WISP1 antibody used in Figure 6D. The antibody was preincubated with recombinant human WISP1 protein before processing.

**Figure S5. (A)** The effect of WISP1 (1 µg/ml) on proliferation of A549 cells was assessed by cell counting 24 h after treatment, as indicated. **(B, C)** The *Wisp1* knockdown was analysed by Western Blot and qRT-PCR. Two different siRNA sequences were tested in different concentrations, as indicated. Cells were harvested and lysed 24 h after transfection. For all further experiments si #1 (150 nm) was used. **(B)** Recombinant mouse WISP1 protein served as a positive control, β-actin served as a loading control. Data are representative of at least three independent experiments. **(C)** *Wisp2* mRNA levels served as a control. **(D)** ATII cells were attached in 48-well plates, transfected with 150 nM of *Wisp1* (W1, si #1) or non-specific scrambled (scr) siRNA, and cultured for another 24h. Cells were then labelled with [$^3$H]-thymidine as described in *Material and Methods* and results are presented as relative proliferation (n = 3), * $p < 0.05$, ** $p < 0.02$.

**Figure S6.** Single layers of all double immunofluorescent stainings presented in **Figure 8 (B)** of this manuscript are depicted. Immunofluorescent detection was performed for DAPI (blue), TJP1 (red), or αSMA (green), as indicated.

**Figure S7. (A)** The effect of WISP1 on primary lung fibroblast proliferation under different serum conditions was assessed by [$^3$H]-thymidine incorporation. Data are presented as relative proliferation of WISP1-stimulated fibroblasts compared with vehicle controls (n =5).

**Figure S8.** Mice were subjected to saline or bleomycin instillation, and treated either with neutralizing αWISP1 antibodies or pre-immune serum (IgG control) by orotracheal

application as described in detail in *Material & Methods*. Lungs were processed 14 d after bleomycin application for immunohistochemical analysis and stained for tenascin C. Pictures are representative of at least two independent experiments using at least three different lung tissues for each condition. Arrows point to positive smooth muscle cells, arrowheads depict weak staining of the interstitium.

**Figure S9.** The mRNA levels of the profibrotic marker genes *Col1a1*, *Spp1*, *Mmp7*, *Pai1*, and *Ctgf* **(A)** and the EMT markers *eCad*, *Tjp1*, *Occl*, *Fsp1*, *Vim* and *αSMA* **(B)** were analyzed by qRT-PCR in lung homogenates of mice instilled with saline, bleomycin, bleomycin plus IgG control, or bleomycin plus αWISP1 antibody, 14 days after initial bleomycin exposure (n = 5 each). * $p < 0.05$, ** $p < 0.02$. Results are plotted as relative mRNA levels (ΔCt) and presented as mean ± s.e.m., log fold-changes in mRNA expression for Bleo + αWISP1 compared to Bleo + IgG is presented in **Figure 11 (B, C)**.

**Figure S1**

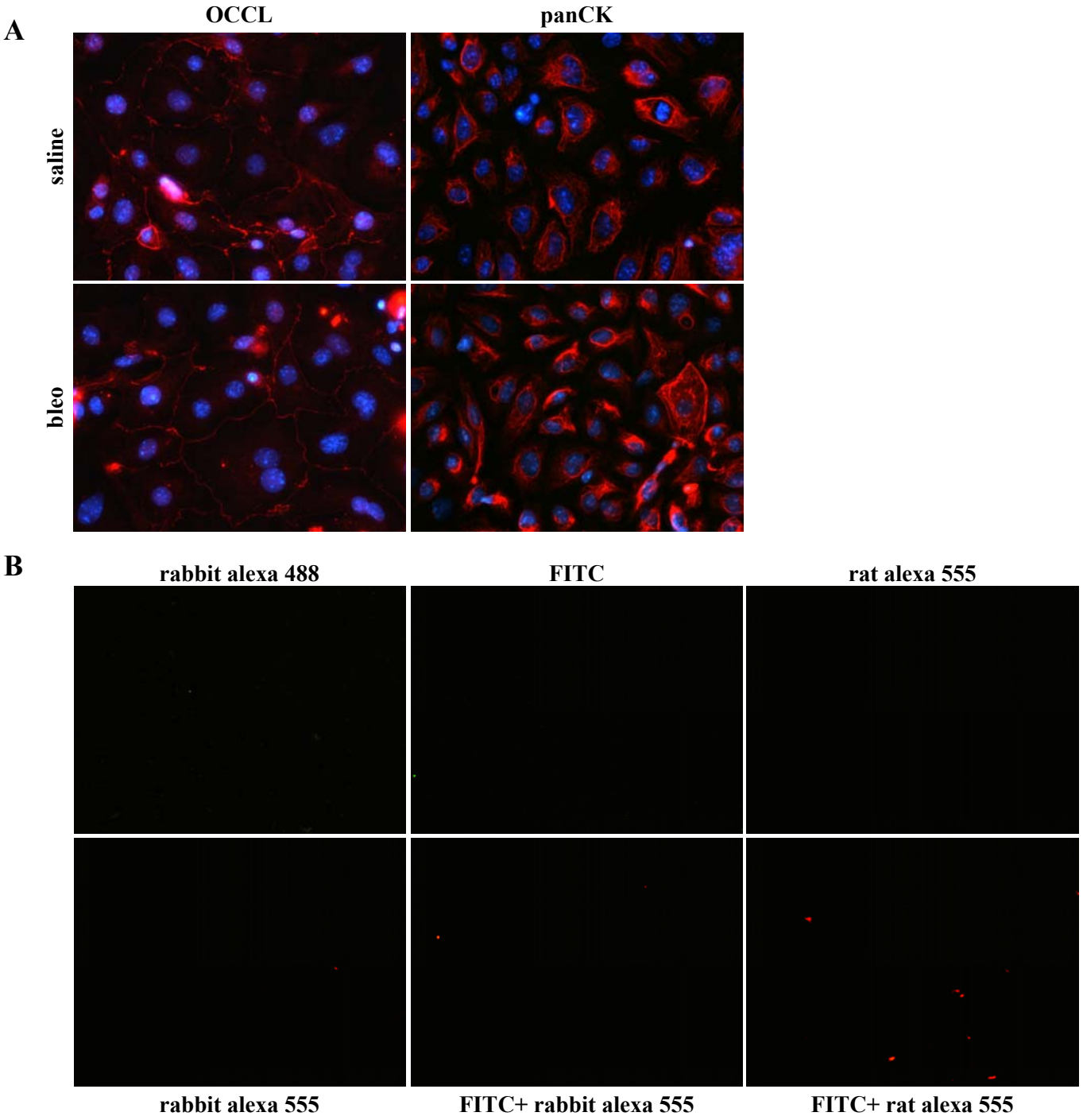
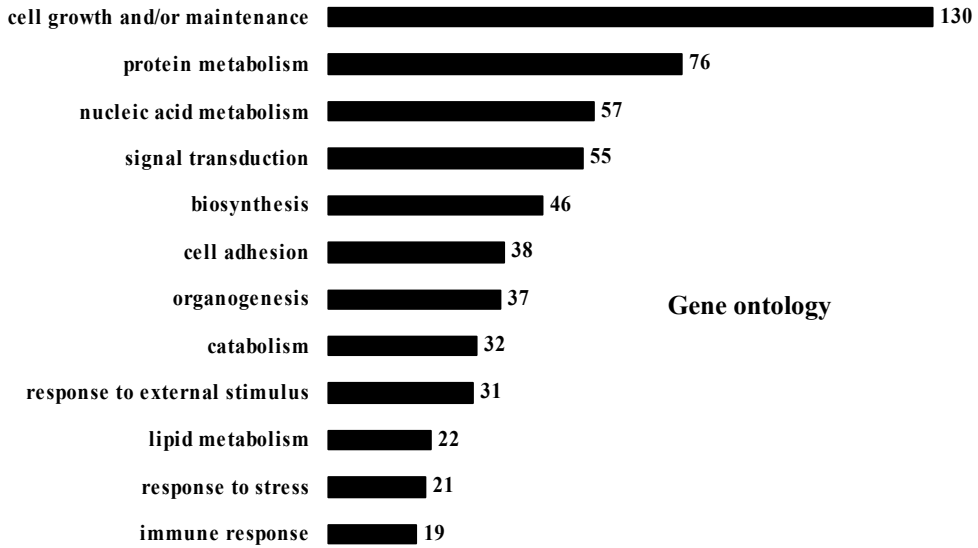

A
OCCL
panCK
saline
bleo
B
rabbit alexa 488
FITC
rat alexa 555
rabbit alexa 555
FITC+ rabbit alexa 555
FITC+ rat alexa 555

**Figure S2**


cell growth and/or maintenance
130
protein metabolism
76
nucleic acid metabolism
57
signal transduction
55
biosynthesis
46
cell adhesion
38
organogenesis
37
catabolism
32
response to external stimulus
31
lipid metabolism
22
response to stress
21
immune response
19
Gene ontology

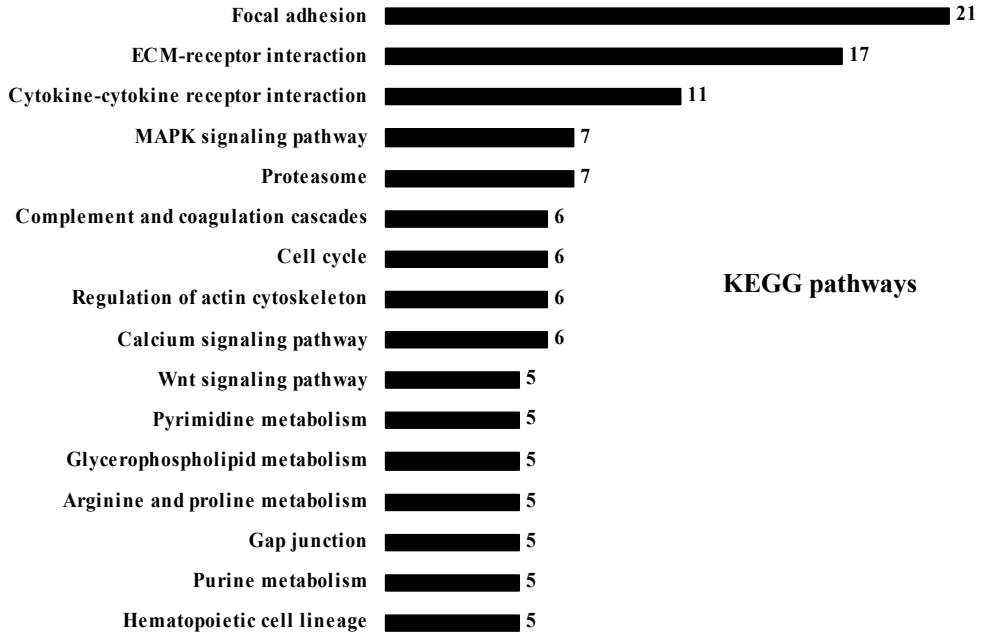

Focal adhesion
21
ECM-receptor interaction
17
Cytokine-cytokine receptor interaction
11
MAPK signaling pathway
7
Proteasome
7
Complement and coagulation cascades
6
Cell cycle
6
Regulation of actin cytoskeleton
6
Calcium signaling pathway
6
Wnt signaling pathway
5
Pyrimidine metabolism
5
Glycerophospholipid metabolism
5
Arginine and proline metabolism
5
Gap junction
5
Purine metabolism
5
Hematopoietic cell lineage
5
KEGG pathways

**Figure S3**

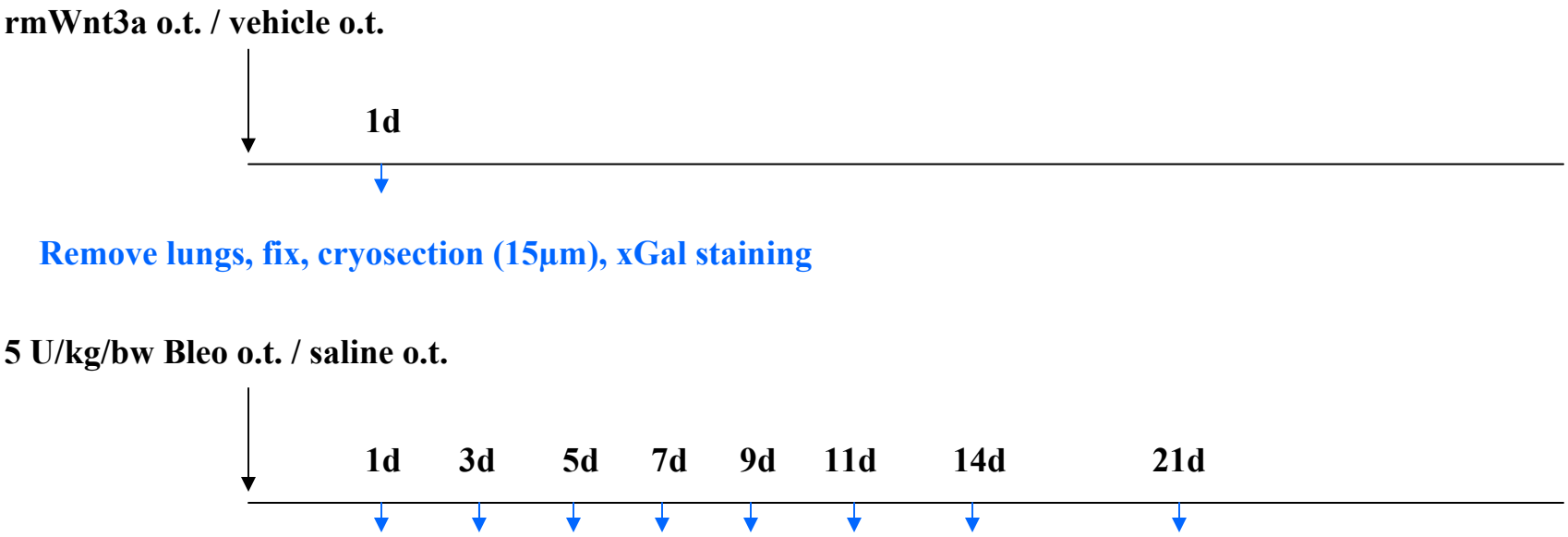

rmWnt3a o.t. / vehicle o.t.
1d
Remove lungs, fix, cryosection (15µm), xGal staining
5 U/kg/bw Bleo o.t. / saline o.t.
1d
3d
5d
7d
9d
11d
14d
21d
Remove lungs, fix, cryosection (15µm), xGal staining

**Figure S4**

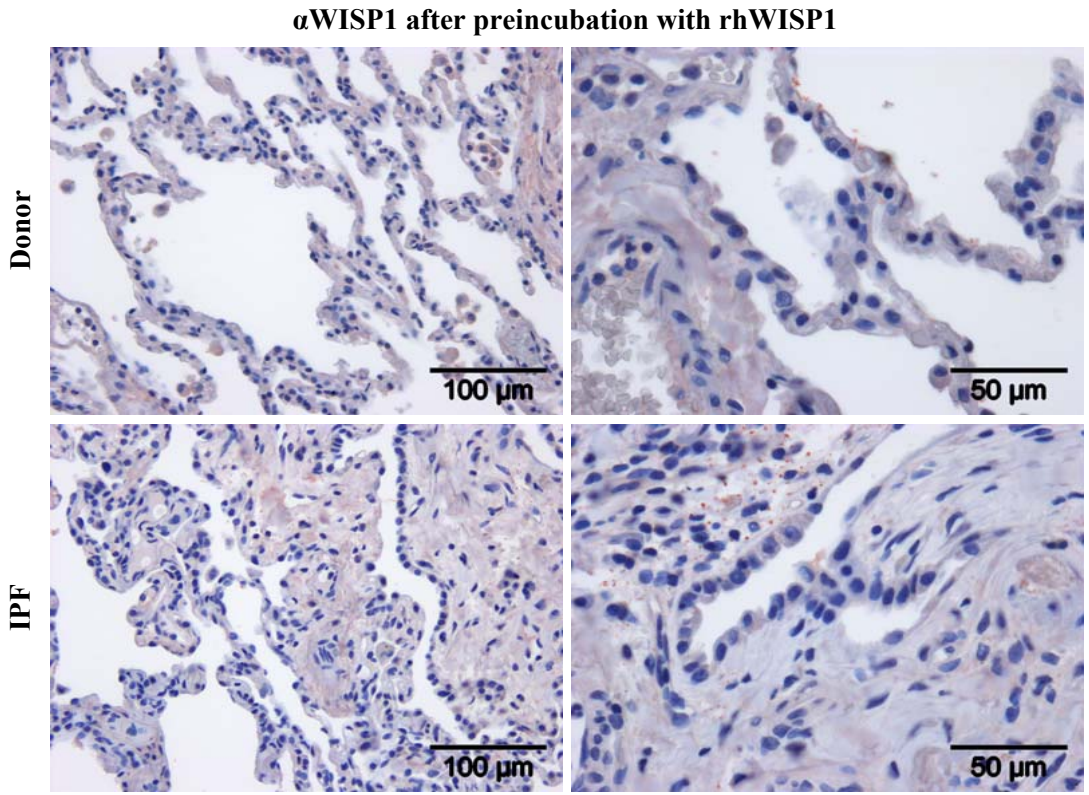

αWISP1 after preincubation with rhWISP1
Donor
IPF
100 µm
50 µm
100 µm
50 µm

**Figure S5**

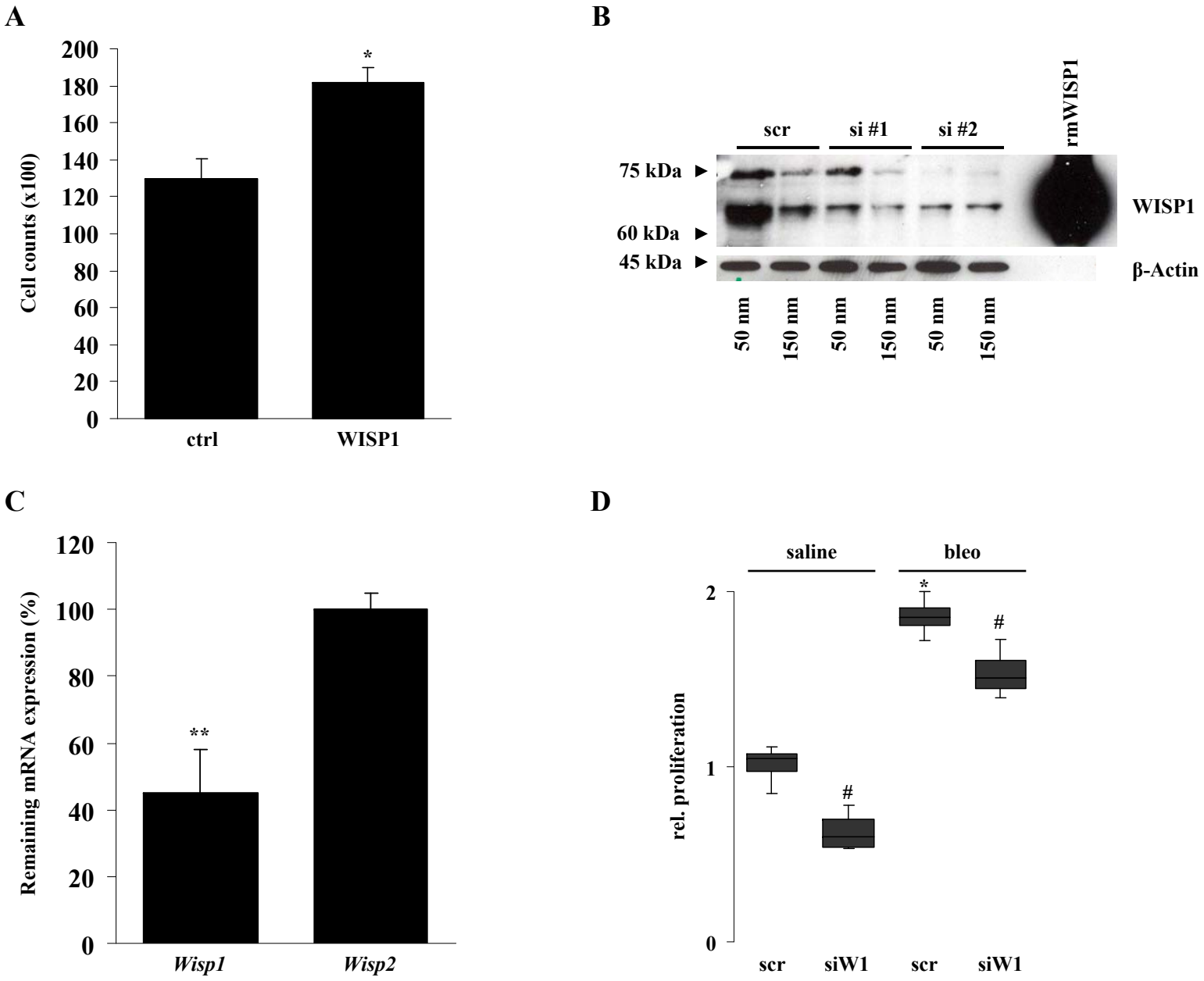

A
Cell counts (x100)
200
180
160
140
120
100
80
60
40
20
0
*
ctrl
WISP1
B
scr
si #1
si #2
rmWISP1
75 kDa
60 kDa
45 kDa
WISP1
β-Actin
50 nm
150 nm
50 nm
150 nm
50 nm
150 nm
C
Remaining mRNA expression (%)
120
100
80
60
40
20
0
**
Wisp1
Wisp2
D
saline
bleo
rel. proliferation
2
1
0
*
#
#
scr
siW1
scr
siW1

**Figure S6**

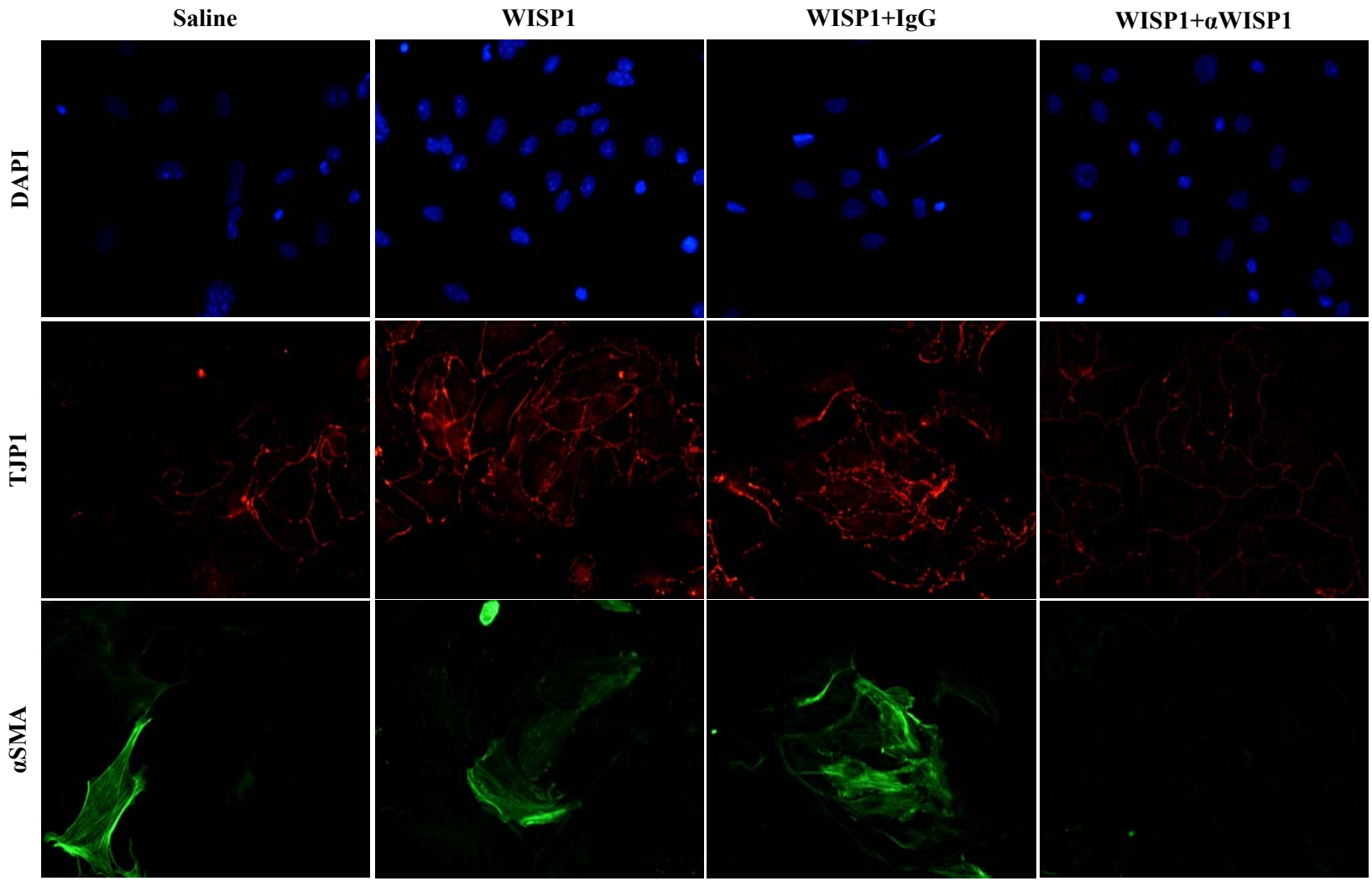


**Figure S7**

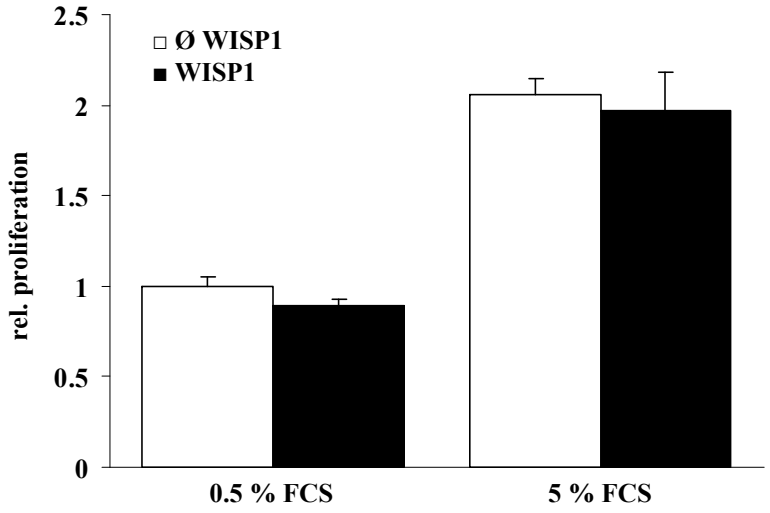


**Figure S8**

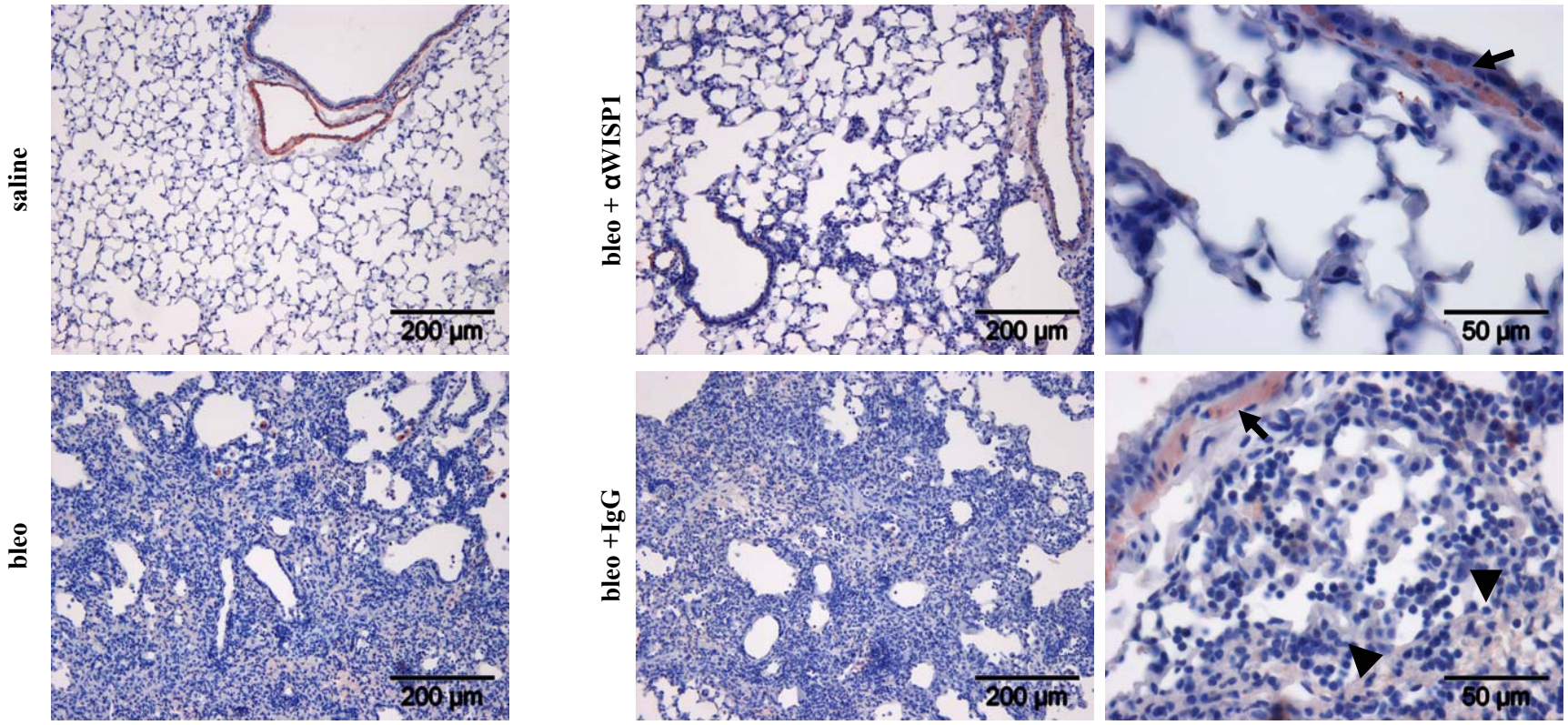
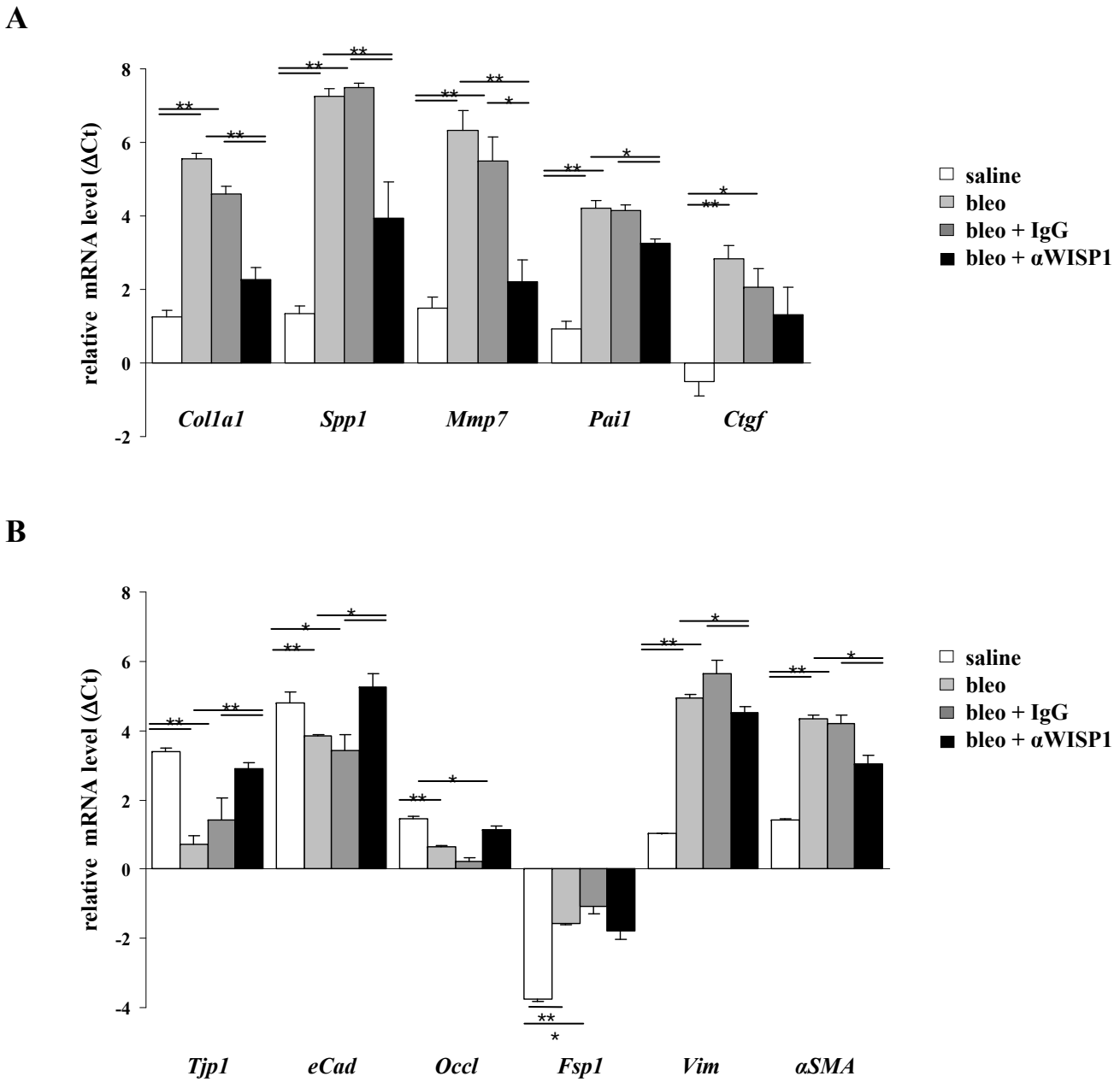

saline
bleo
bleo + αWISP1
bleo +IgG
200 μm
200 μm
200 μm
50 μm
200 μm
50 μm

**Figure S9**


A
relative mRNA level (ΔCt)
8
6
4
2
0
-2
Col1a1
Spp1
Mmp7
Pai1
Ctgf
saline
bleo
bleo + IgG
bleo + αWISP1
B
relative mRNA level (ΔCt)
8
6
4
2
0
-2
-4
Tjp1
eCad
Occl
Fsp1
Vim
αSMA
saline
bleo
bleo + IgG
bleo + αWISP1