

Highlighting fibroblast plasticity in lung fibrosis: the WI-38 cell line as a model for investigating the myofibroblast and lipofibroblast switch

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Supplementary figures

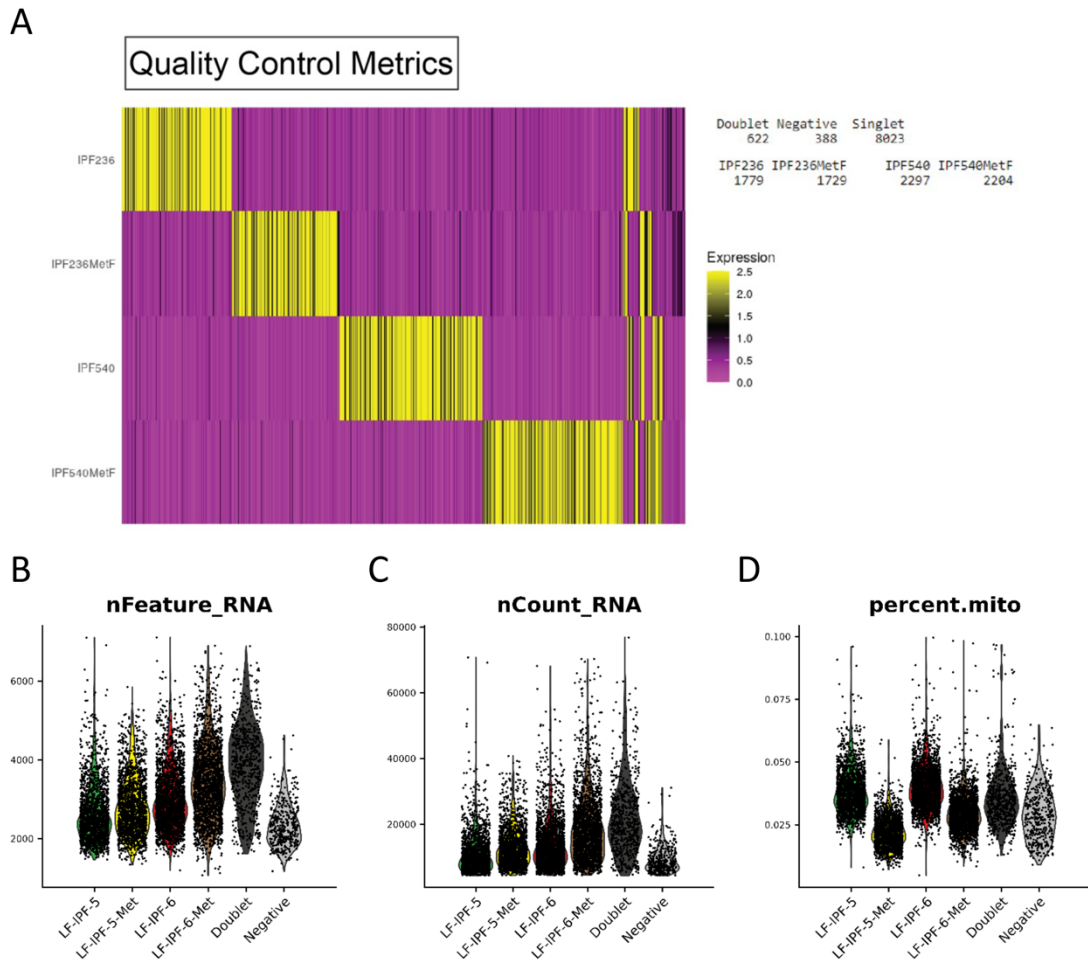


Figure S1: Quality control metrics of the scRNA-seq experiment. A) Heatmap of HTO counts in each cell, labelled according to assigned experimental condition after demultiplexing. Violin plots showing **B)** the number of genes detected in each cell, **C)** the number of UMI per cell and **D)** the percentage of cell reads originating from the mitochondrial genes.

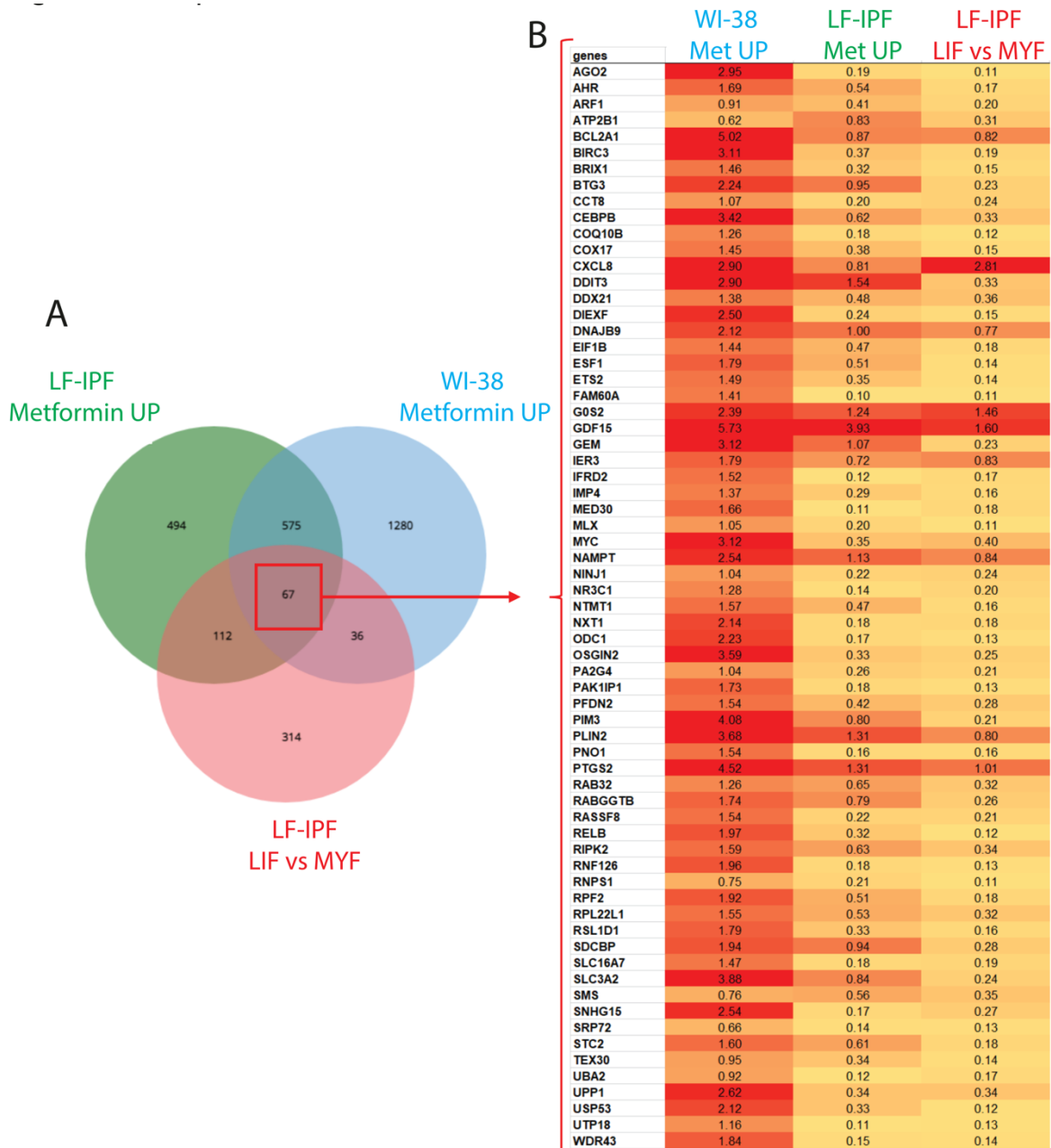
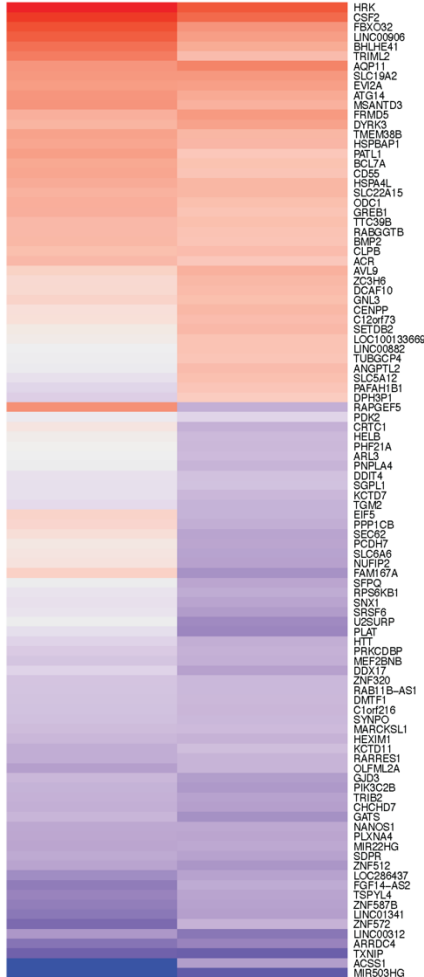


Figure S2: Identification of a cluster of 67 genes commonly upregulated in LIF vs MYF subclusters in LF-IPF (scRNAseq Figure 3) and by Metformin (vs. vehicle) in LF-IPF (scRNAseq Figure 2) and in WI-38 (Figure 4). A) Venn diagram showing the indicated comparison (left) and B) the heatmap of the identified 67 genes common to the 3 comparisons. The log₂ ratio for each condition is indicated in the table. The intensity of the red is directly proportional to the expression level.

C
MET vs. Vehicle
WI-38 LF-IPF
 Kheirollahi et al. 2019



D
TGF-β1 vs. Vehicle
WI-38 LF-IPF
 Jones et al. 2019

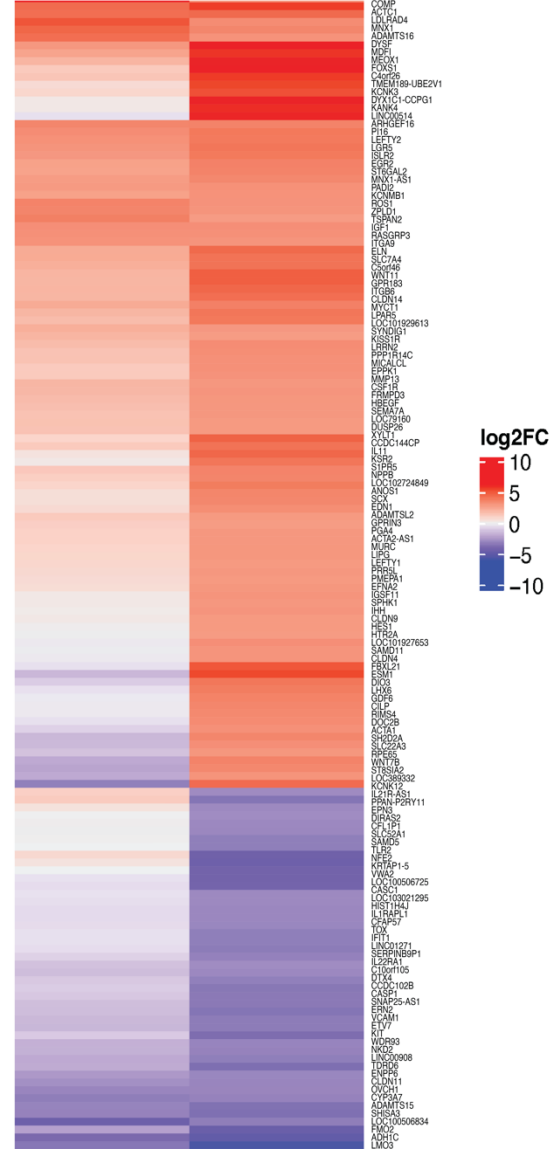
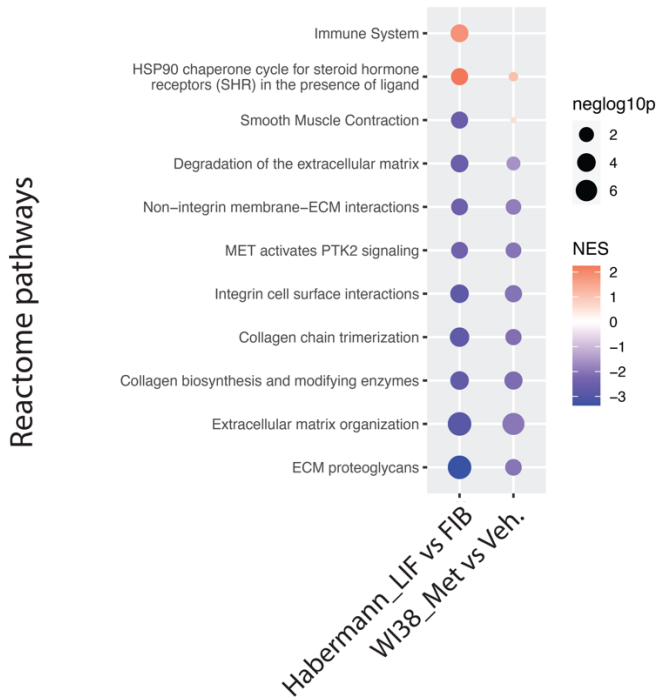


Figure S3: High magnification of the heatmap shown in Figure 4C and D

Pathway analysis LIF

A



B

Pathway analysis MYF

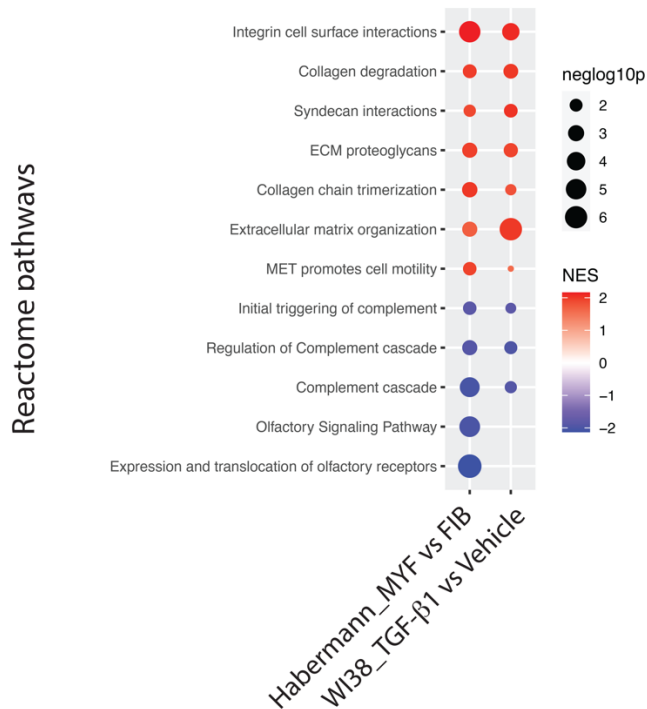
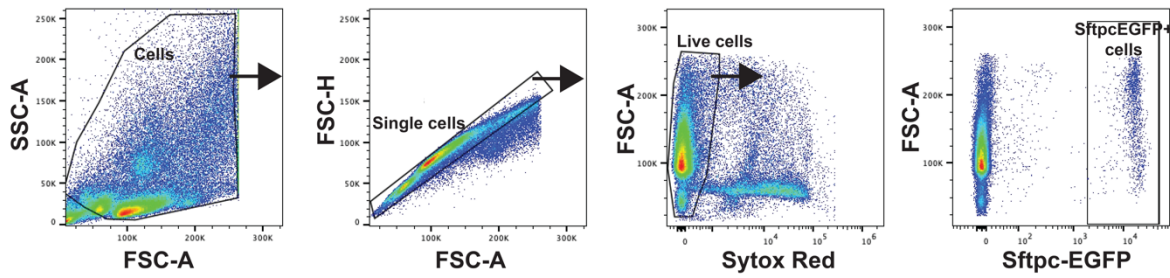


Figure S4: Pathway analysis for LIF and MYF using the Habermann dataset and WI-38 cells. A) Pathway analysis of the LIF using the Habermann *PLIN2*+ FIB vs FIB and WI-38 Met vs. Vehicle. **B)** Pathway analysis of the MYF using the Habermann MYF vs FIB and WI-38 TGF-β1 vs. Vehicle.

A



B

WI-38	differentiated cell name	first treatment	second treatment	endpoint
FIB	MYF1	TGF-b1	-	4 days
FIB	LIF1	Metformin	-	4 days
FIB	LIF2	TGF-b1	Metformin	4 days+4days
FIB	MYF2	TGF-b1	vehicle	4 days+4days
FIB	LIF-1 like	vehicle	Metformin	4 days+4days
FIB	MYF3	Metformin	TGF-b1	4 days+4days
FIB	LIF3	Metformin	vehicle	4 days+4days
FIB	MYF1-like	vehicle	TGF-b1	4 days+4days

Figure S5: A) Gating strategy for the isolation of GFP positive cells from adult Sftpc^{GFP+} mouse lungs. **B)** Abbreviation used for the different culture conditions

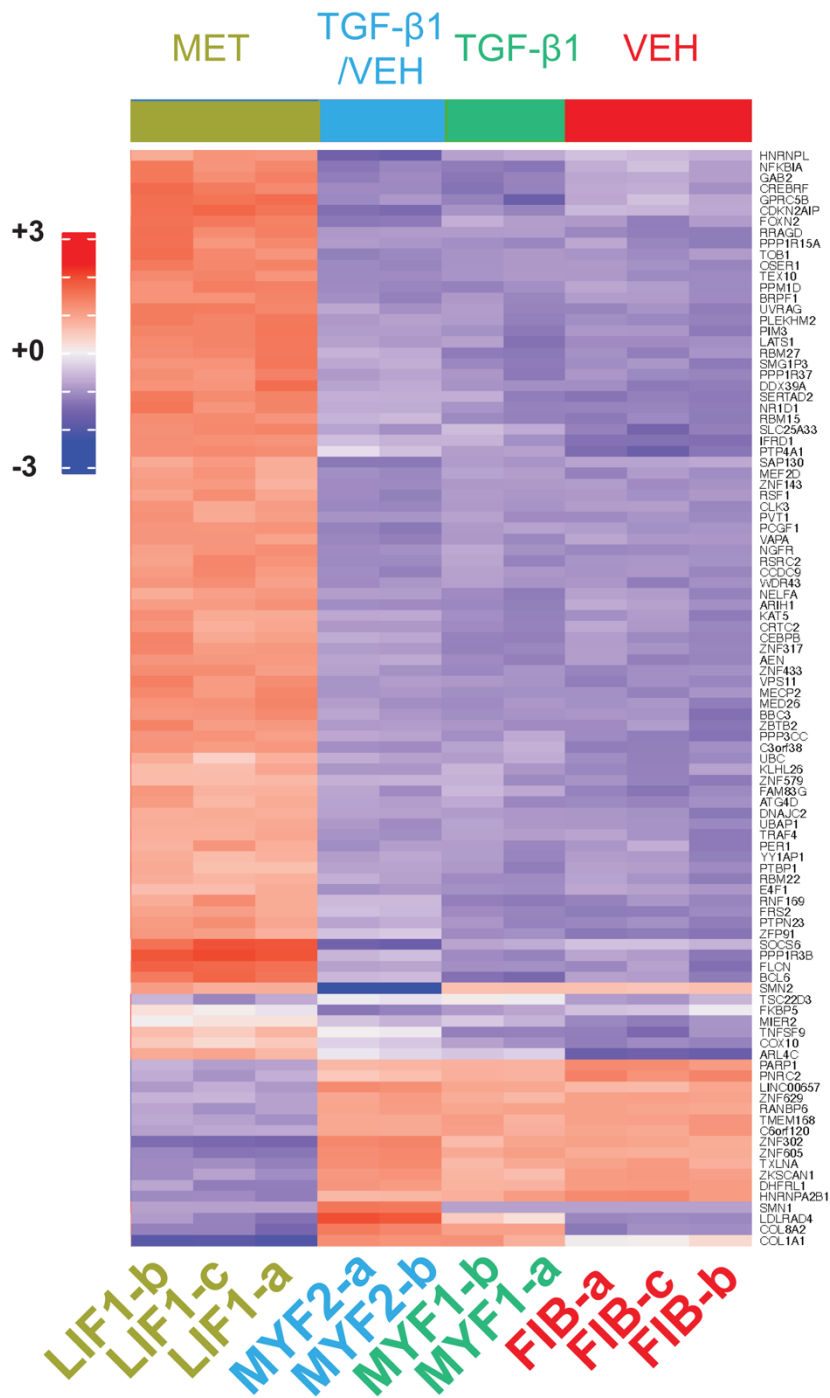


Figure S6: High magnification of the heatmap in Figure 7F
 For LIF1 n = 3, MYF1 n = 2, MYF2 n = 2, FIB n = 3.