New PCT application
Technische Universität München
Vossius Ref.: AD1813 PCT S3

Figure 1

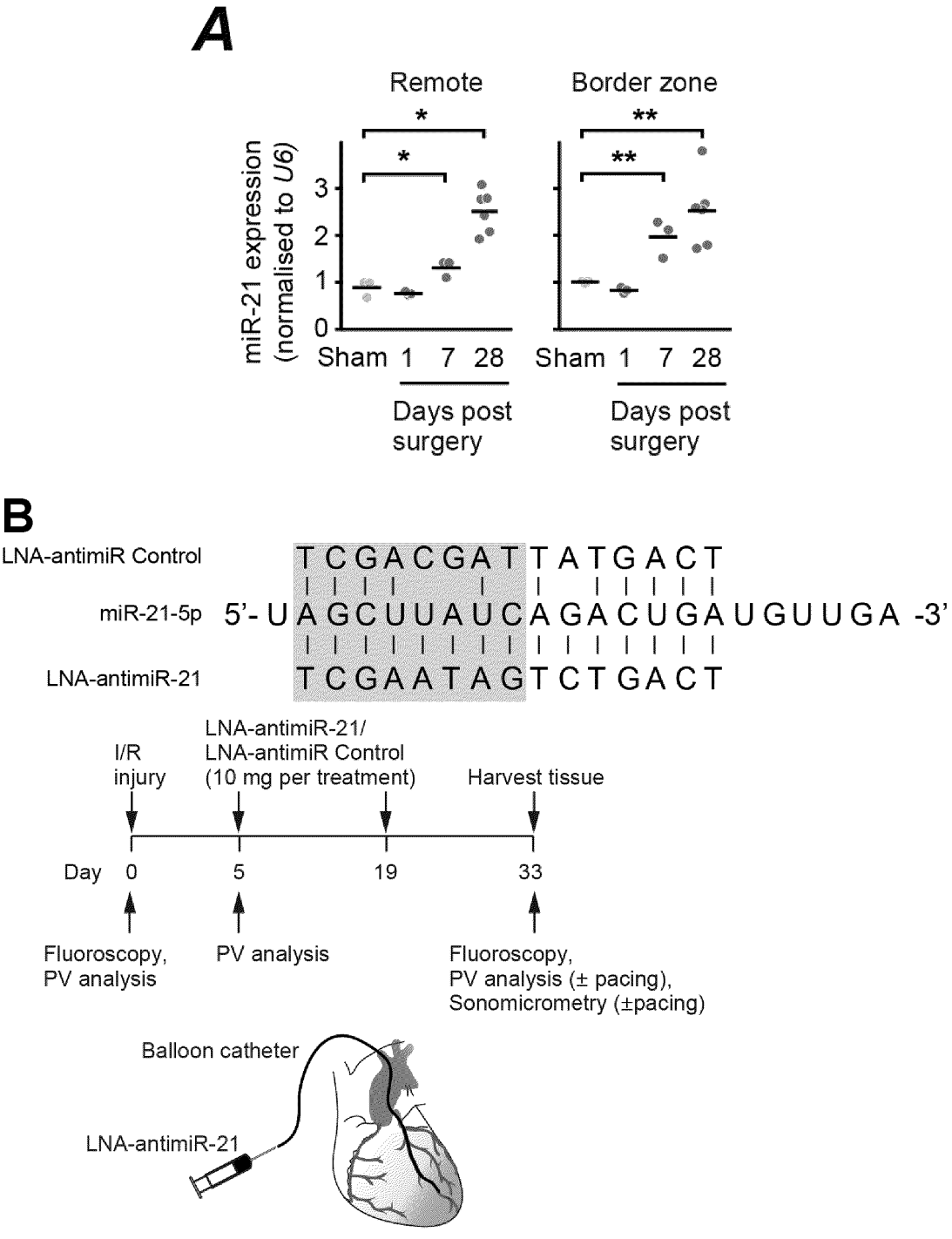


Figure 1 cont.

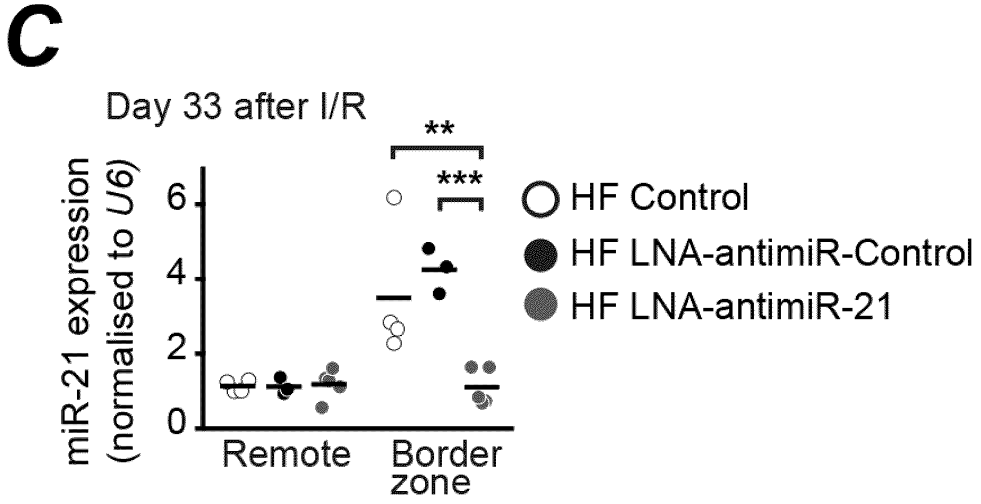


Figure 2

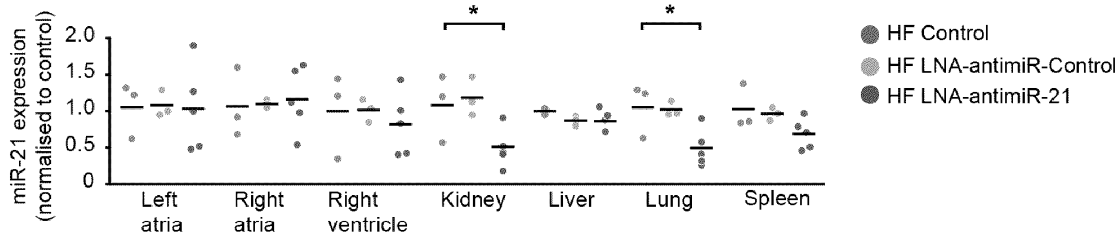


Figure 3
A

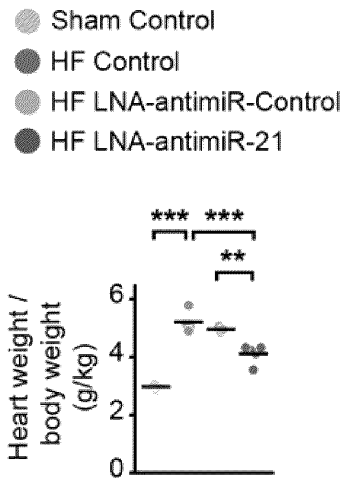


Figure 3 cont.

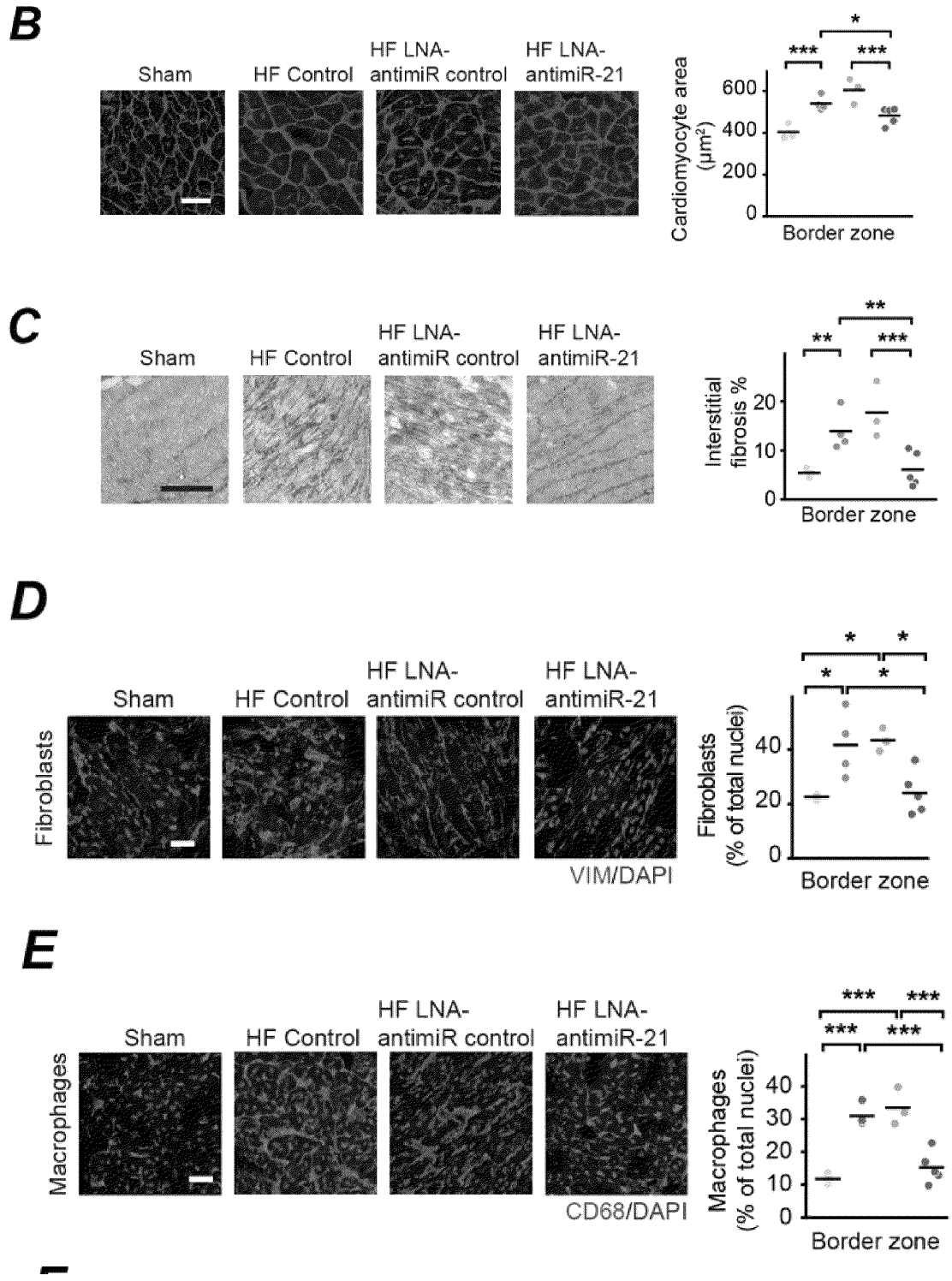


Figure 3 cont.

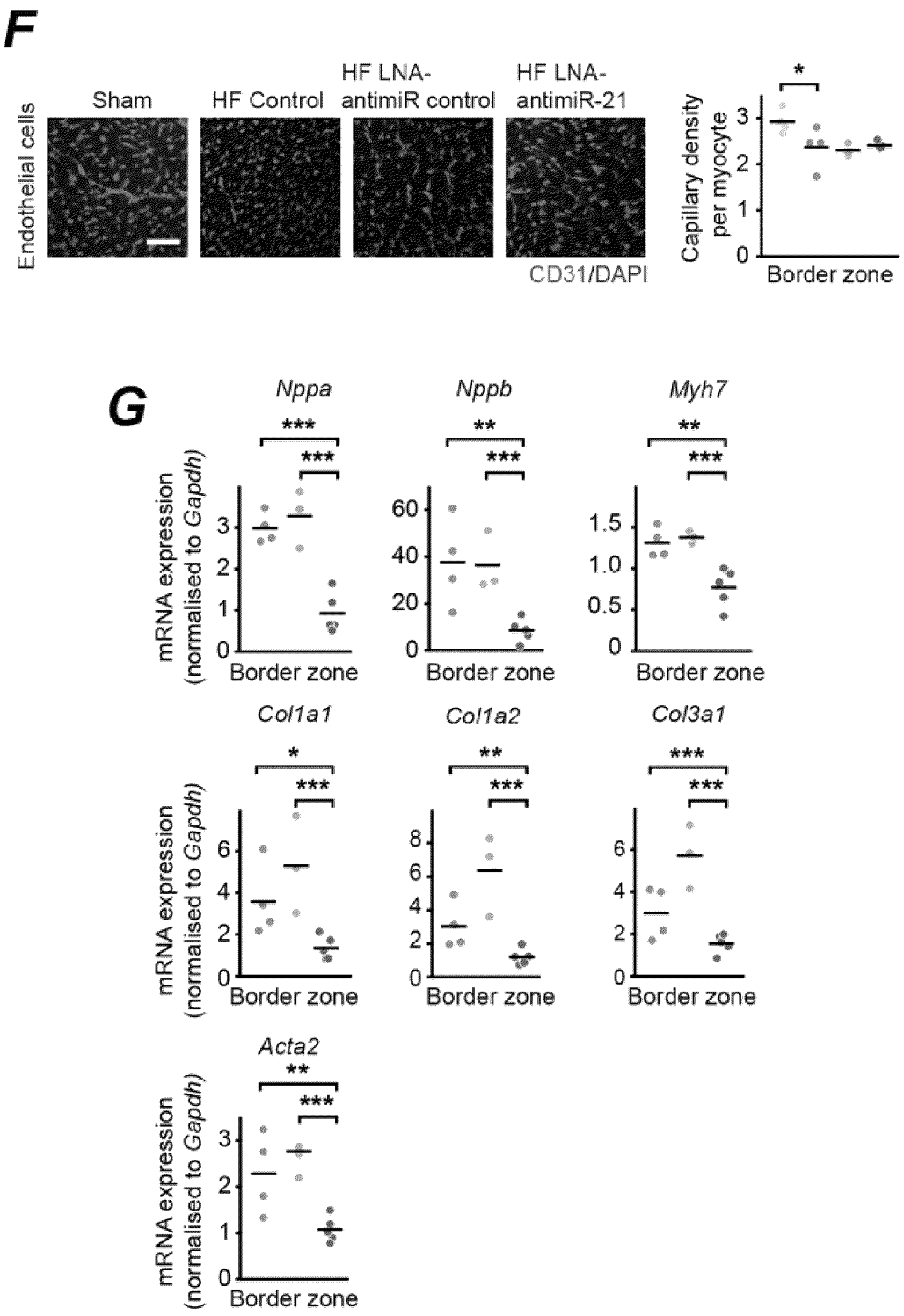


Figure 4

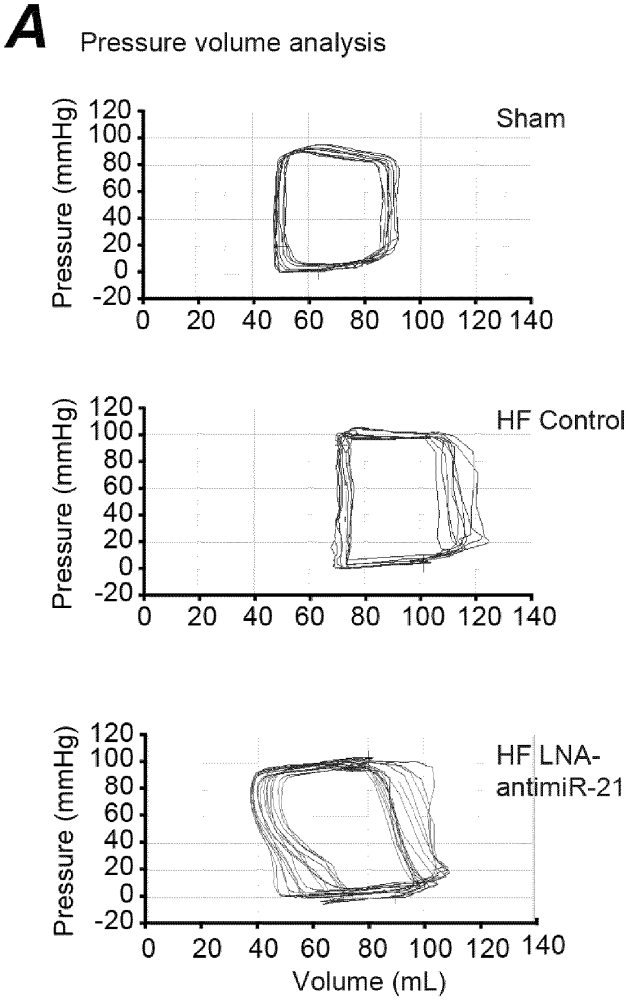

A
Pressure volume analysis
Sham
HF Control
HF LNA-antimiR-21
Pressure (mmHg)
120
100
80
60
40
20
0
-20
0 20 40 60 80 100 120 140
Volume (mL)

Figure 4 cont.

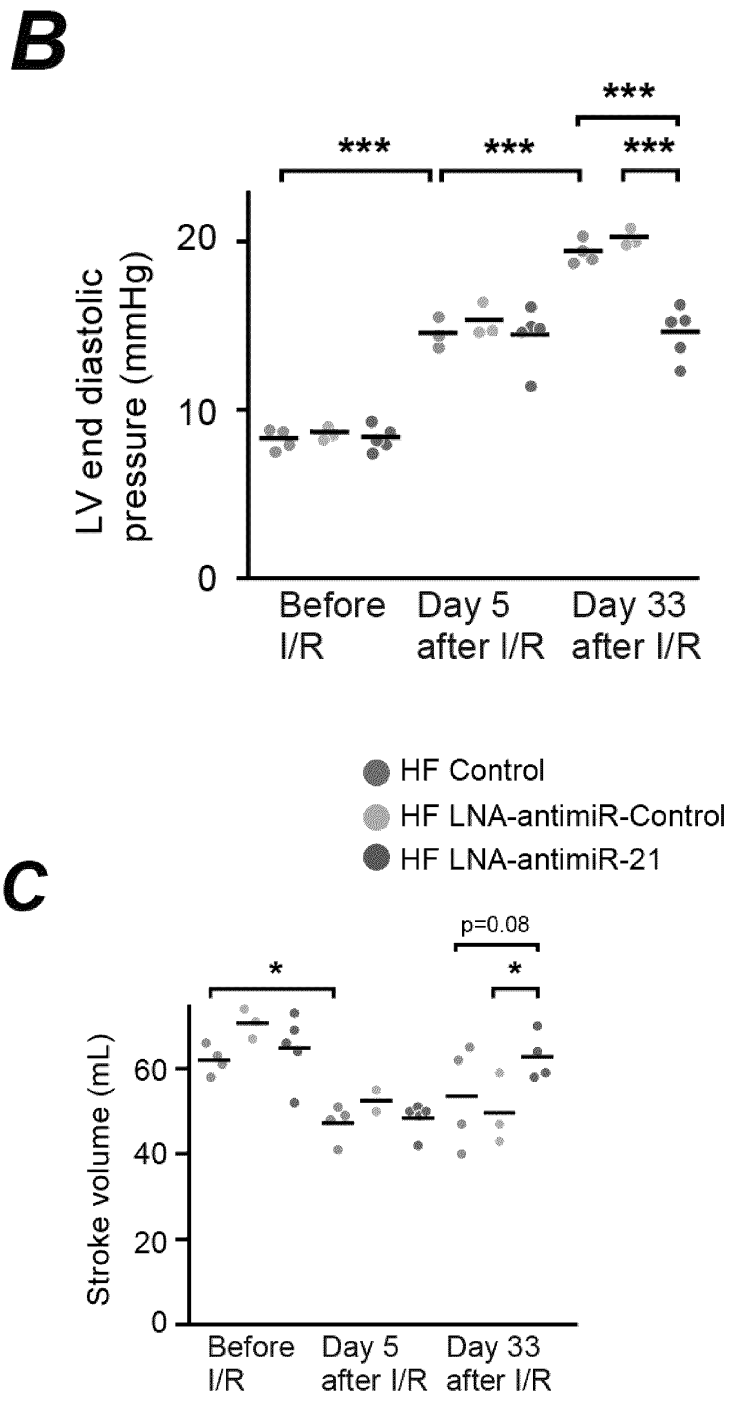


Figure 4 cont.

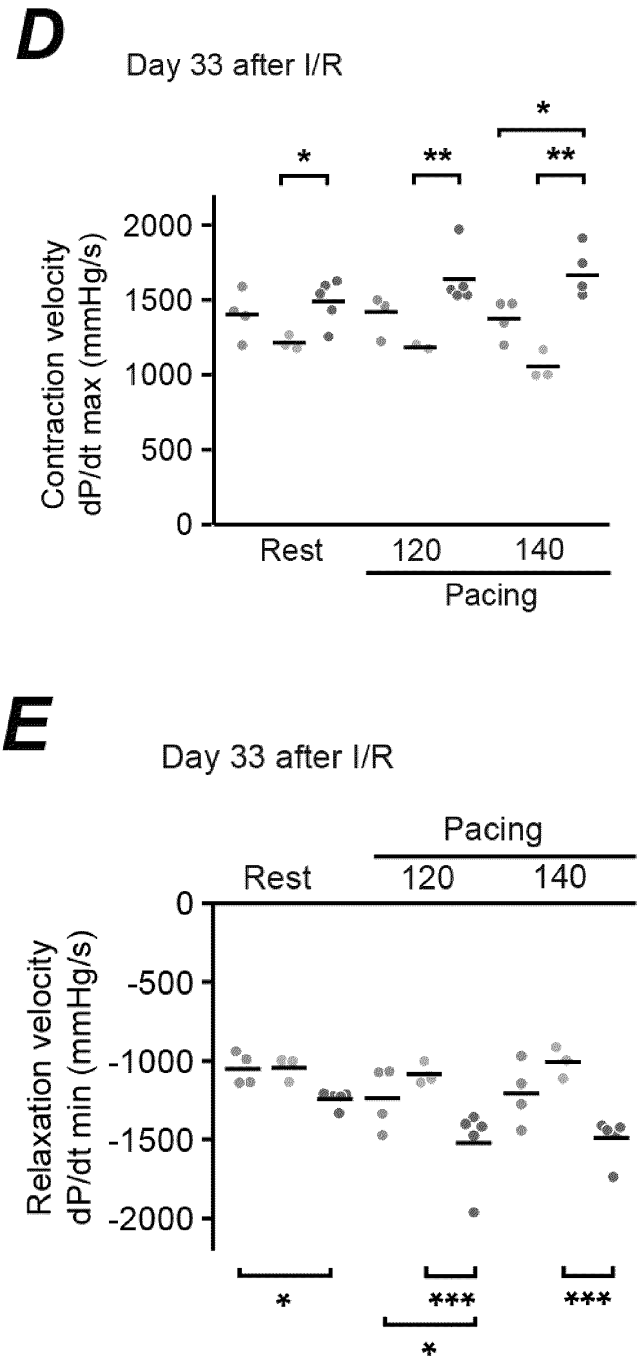
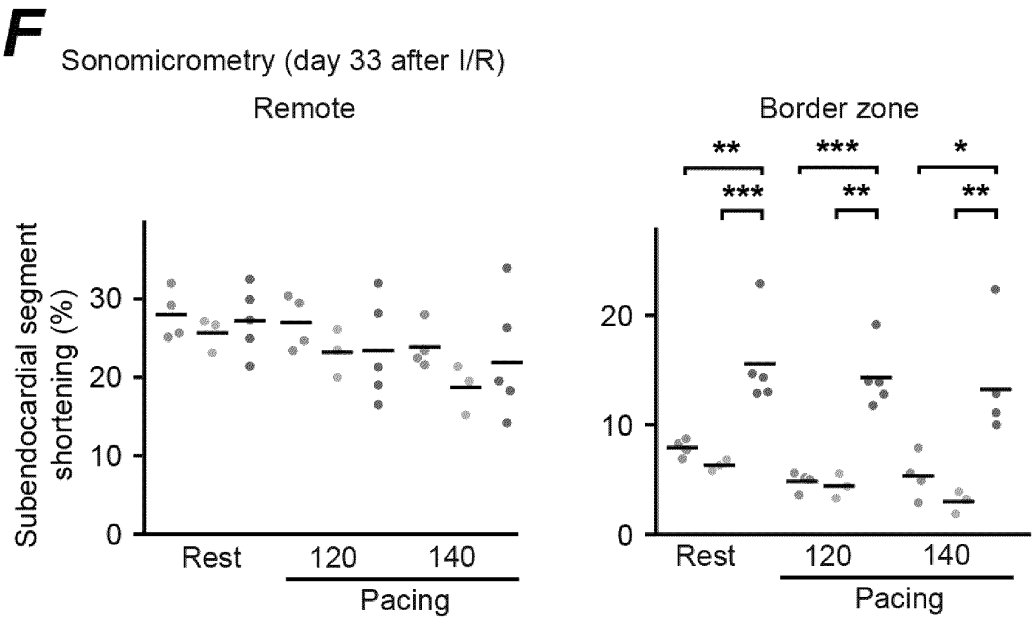


Figure 4 cont.



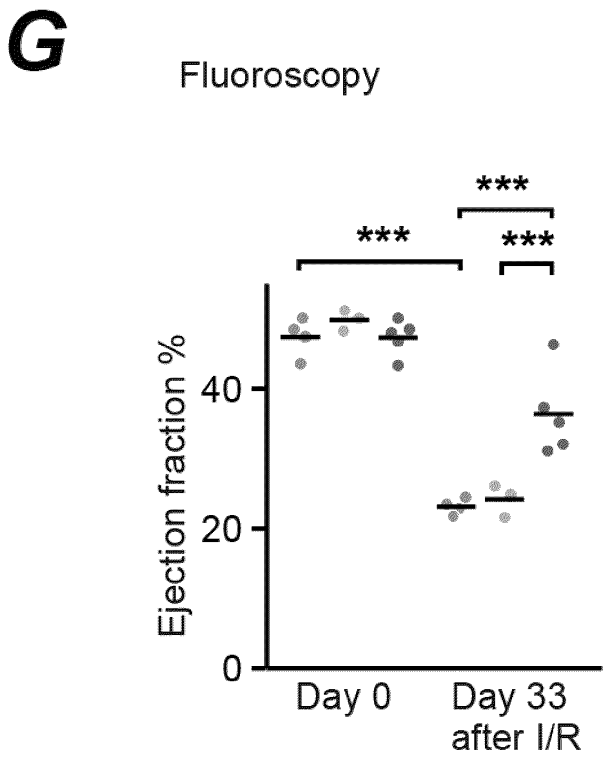


Figure 5

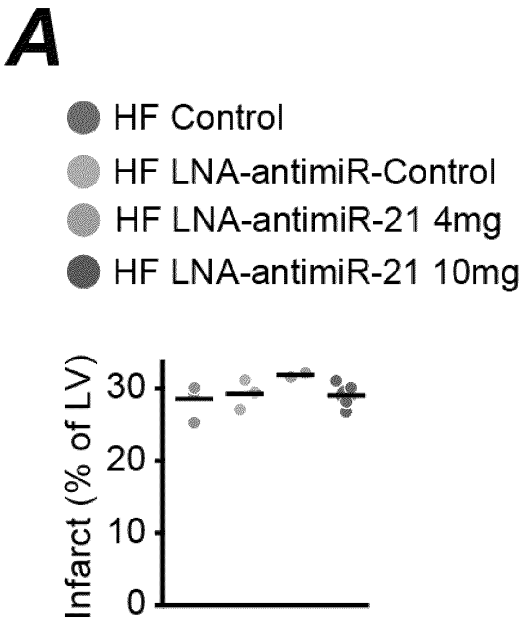
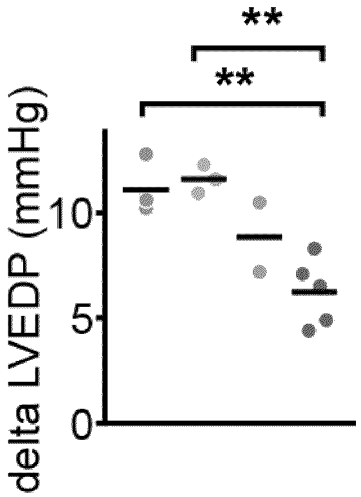
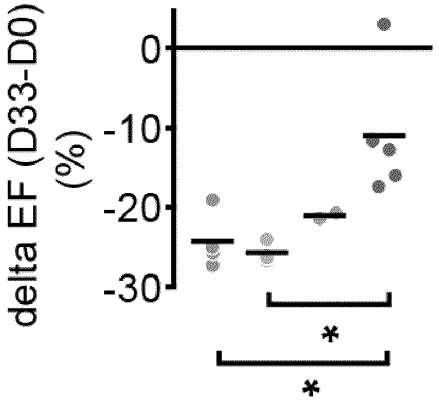

A
HF Control
HF LNA-antimiR-Control
HF LNA-antimiR-21 4mg
HF LNA-antimiR-21 10mg
Infarct (% of LV)
30
20
10
0
B
**
**
miR-21 expression (normalised to U6)
6
4
2
0

Figure 5 cont.

C



D



E

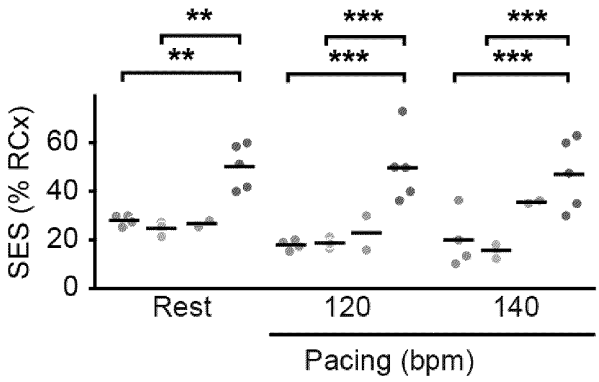


Figure 6

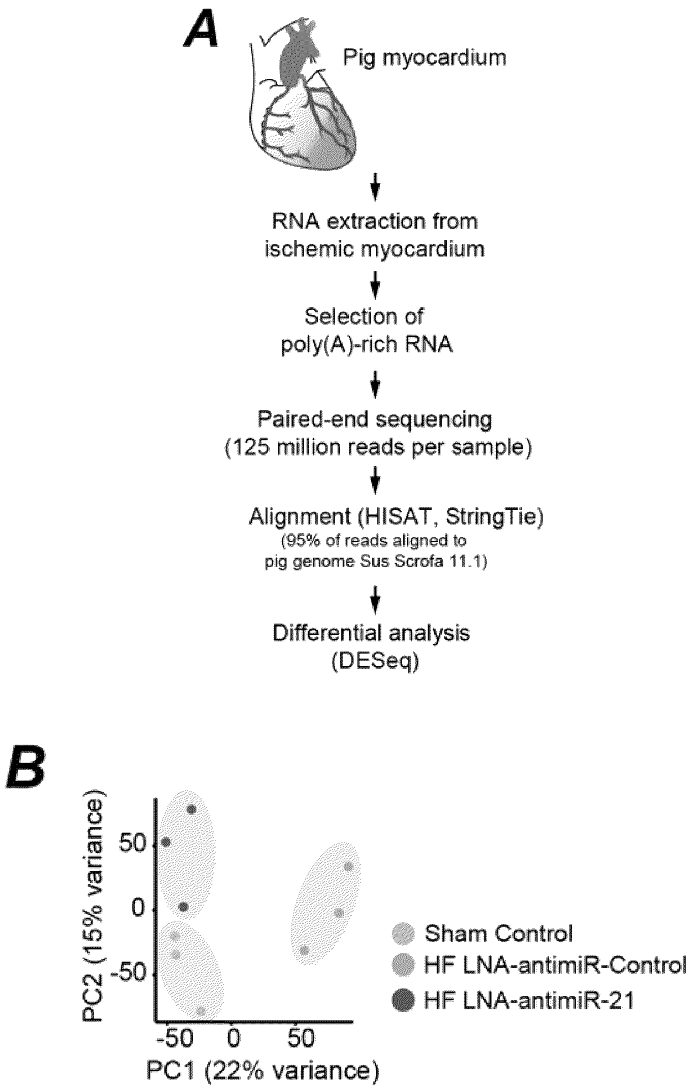

A
Pig myocardium
RNA extraction from ischemic myocardium
Selection of poly(A)-rich RNA
Paired-end sequencing (125 million reads per sample)
Alignment (HISAT, StringTie)
(95% of reads aligned to pig genome Sus Scrofa 11.1)
Differential analysis (DESeq)
B
PC2 (15% variance)
50
0
-50
-50
0
50
PC1 (22% variance)
Sham Control
HF LNA-antimiR-Control
HF LNA-antimiR-21

Figure 6 cont.

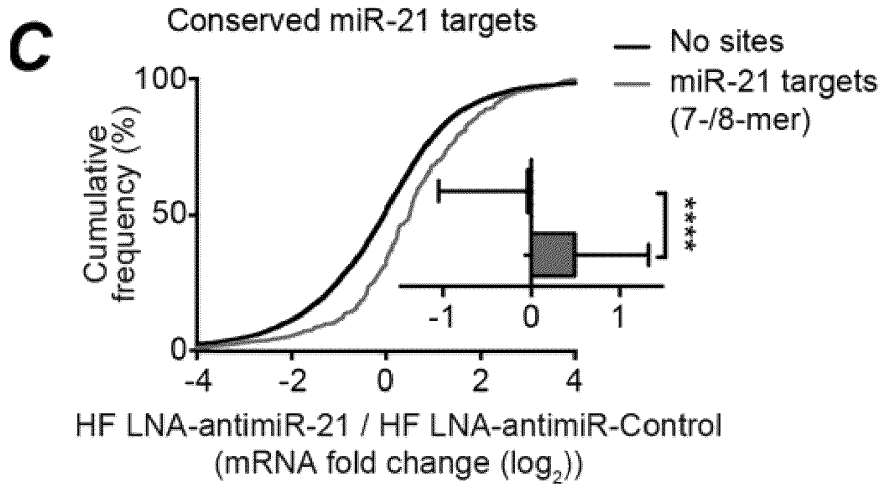


Figure 6 cont.

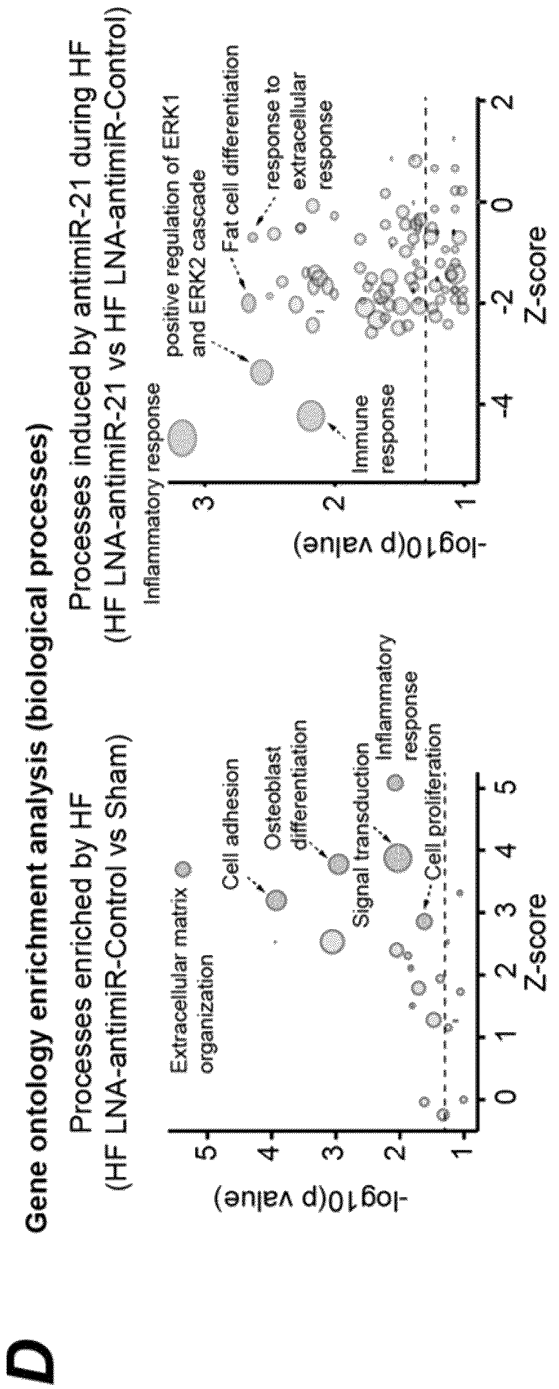
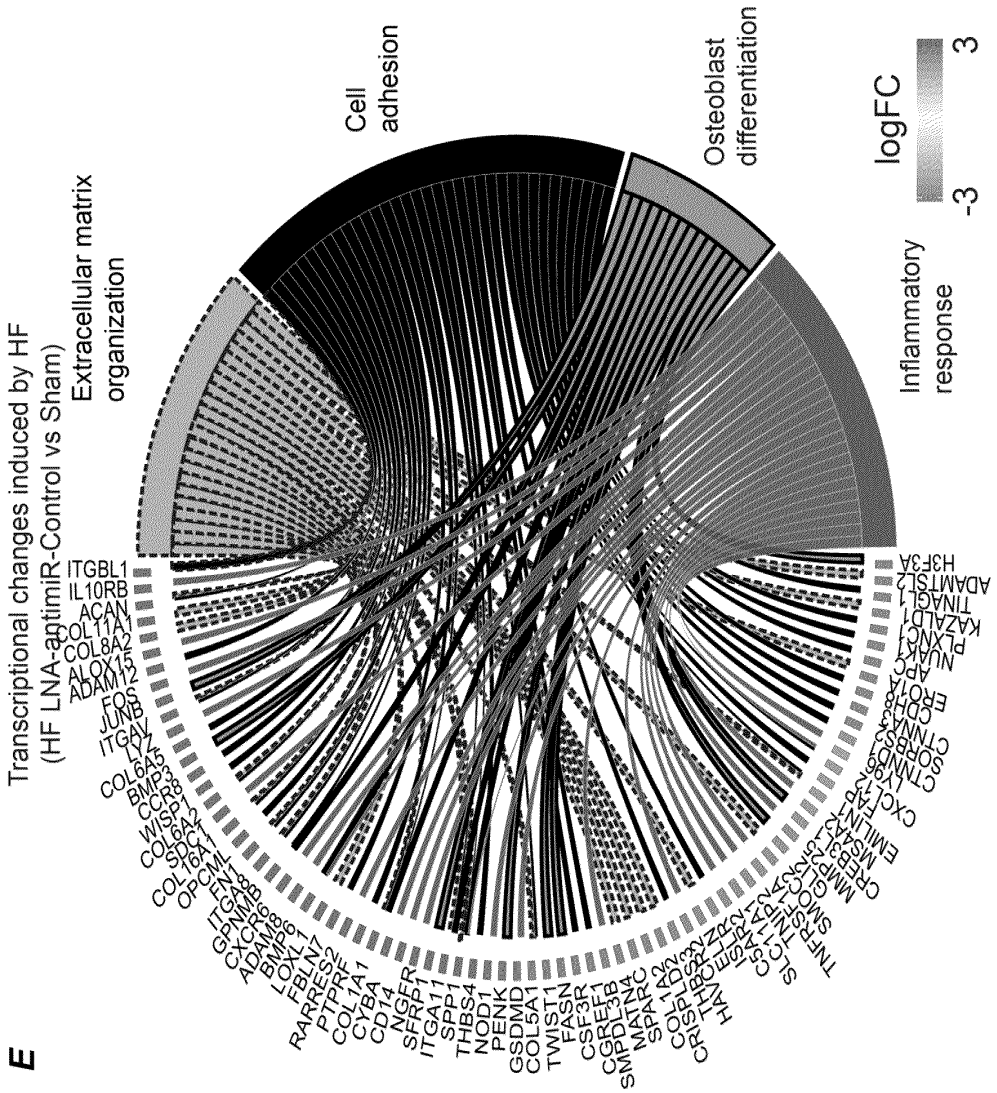


Figure 6 cont.



E

Figure 6E cont.

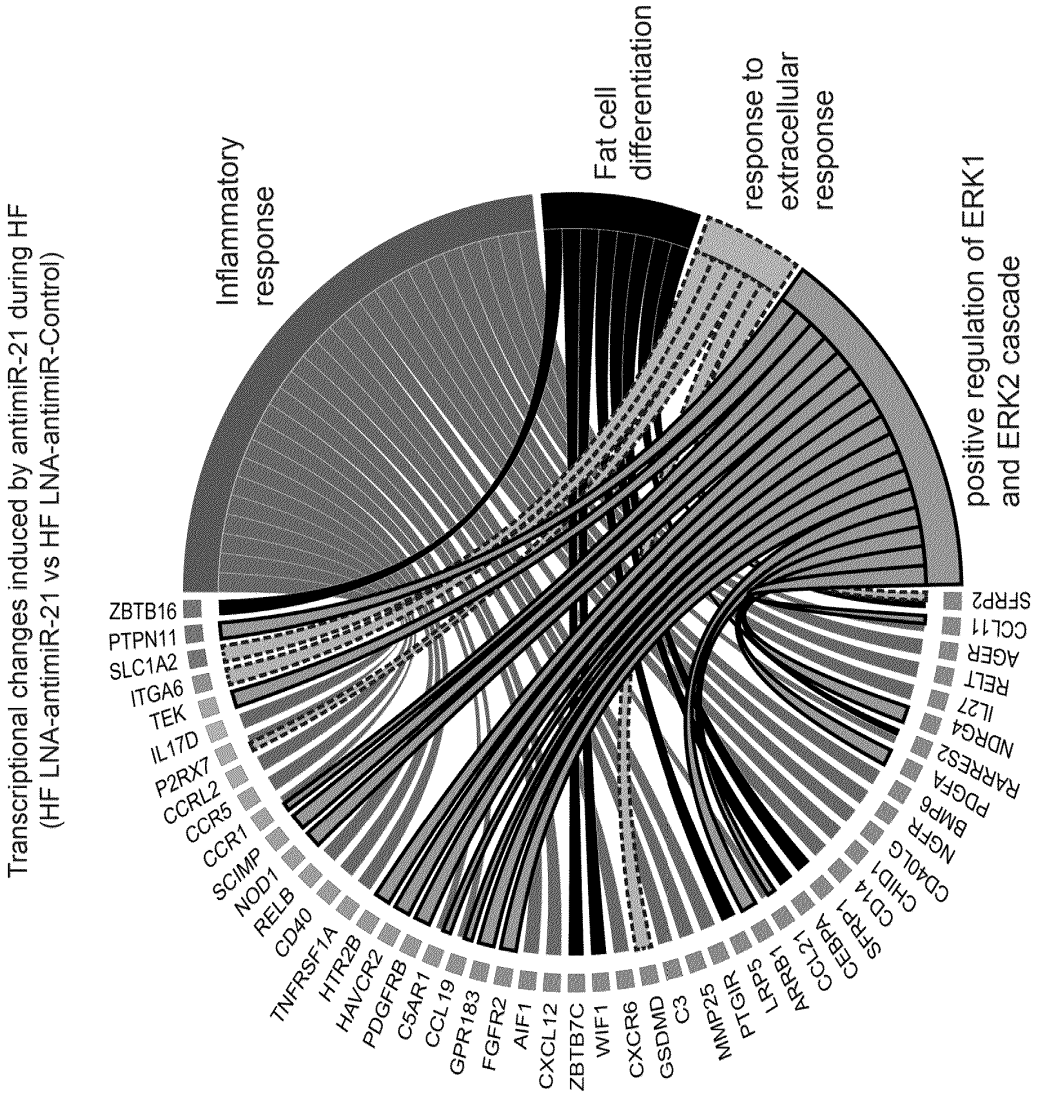
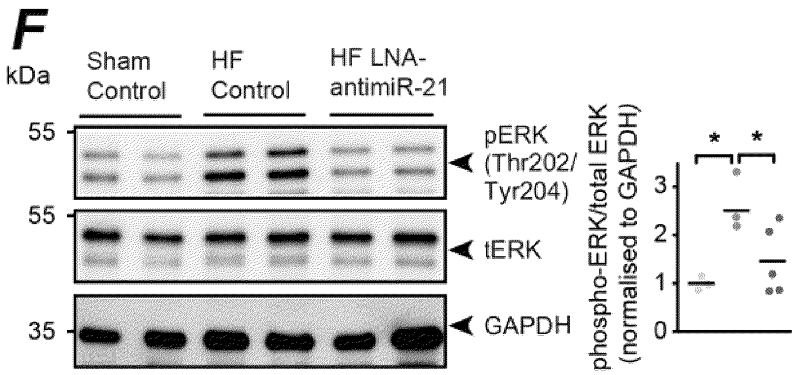


Figure 6 cont.



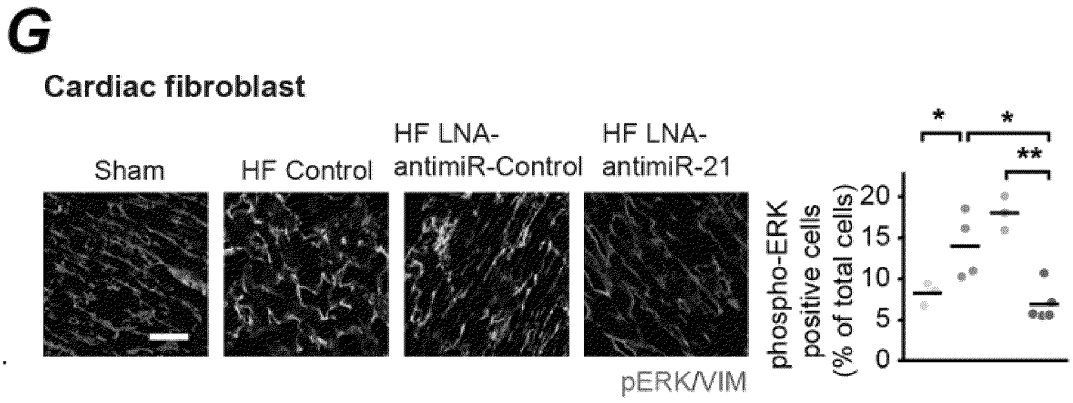


Figure 7

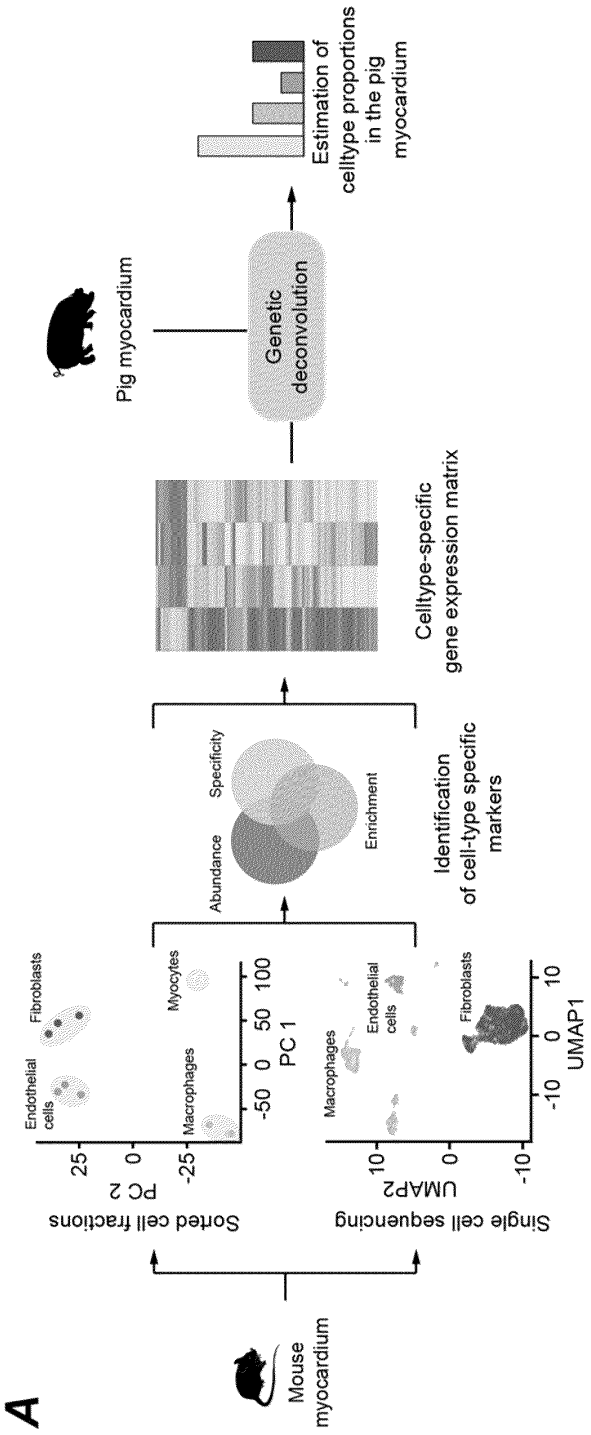

A
Mouse myocardium
Sorted cell fractions
PC 2
25
0
-25
Endothelial cells
Fibroblasts
Macrophages
Myocytes
-50 0 50 100
PC 1
Single cell sequencing
UMAP2
10
0
-10
Macrophages
Endothelial cells
Fibroblasts
-10 0 10
UMAP1
Abundance
Specificity
Enrichment
Identification of cell-type specific markers
Celltype-specific gene expression matrix
Pig myocardium
Genetic deconvolution
Estimation of celltype proportions in the pig myocardium

Figure 7 cont.

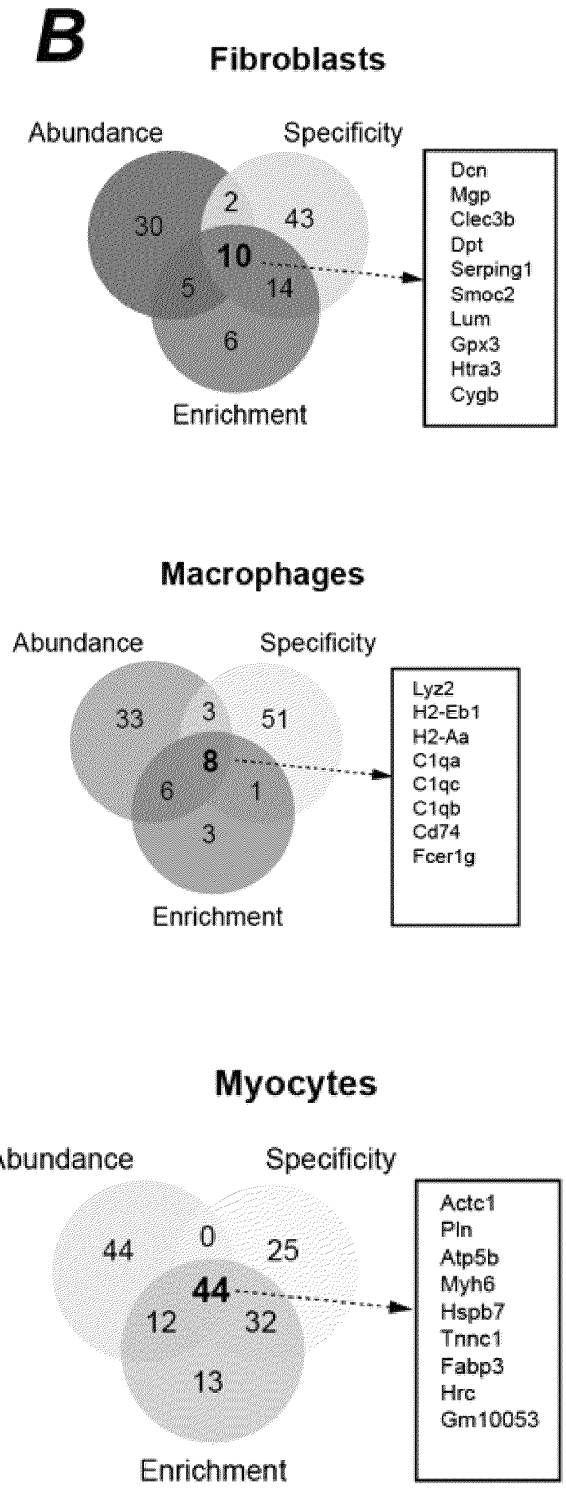


Figure 7 B cont.

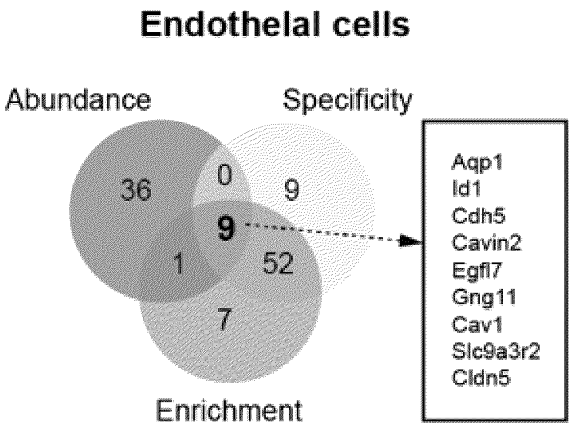


Figure 7

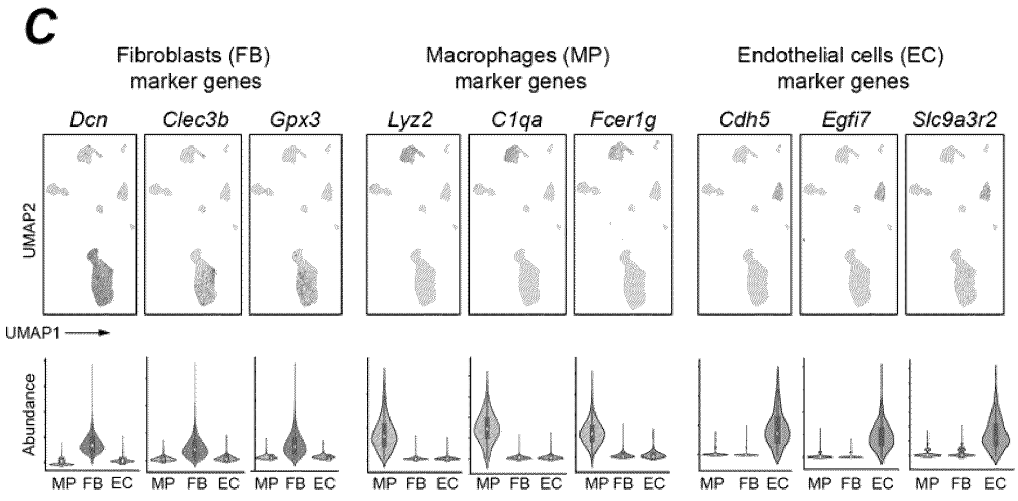


Figure 7 cont.

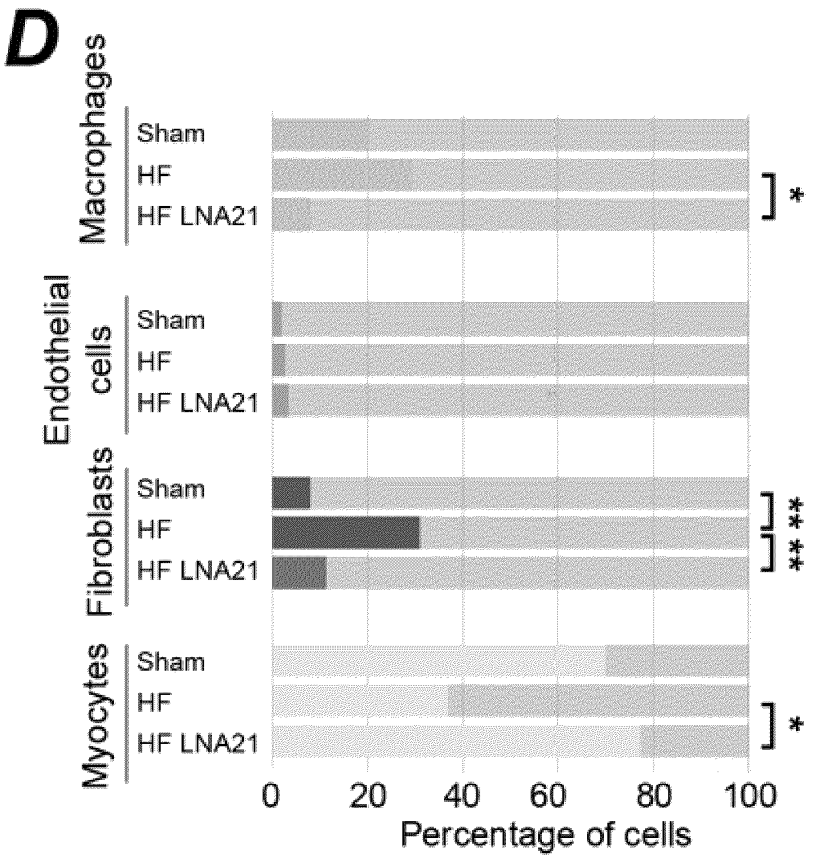


Figure 8

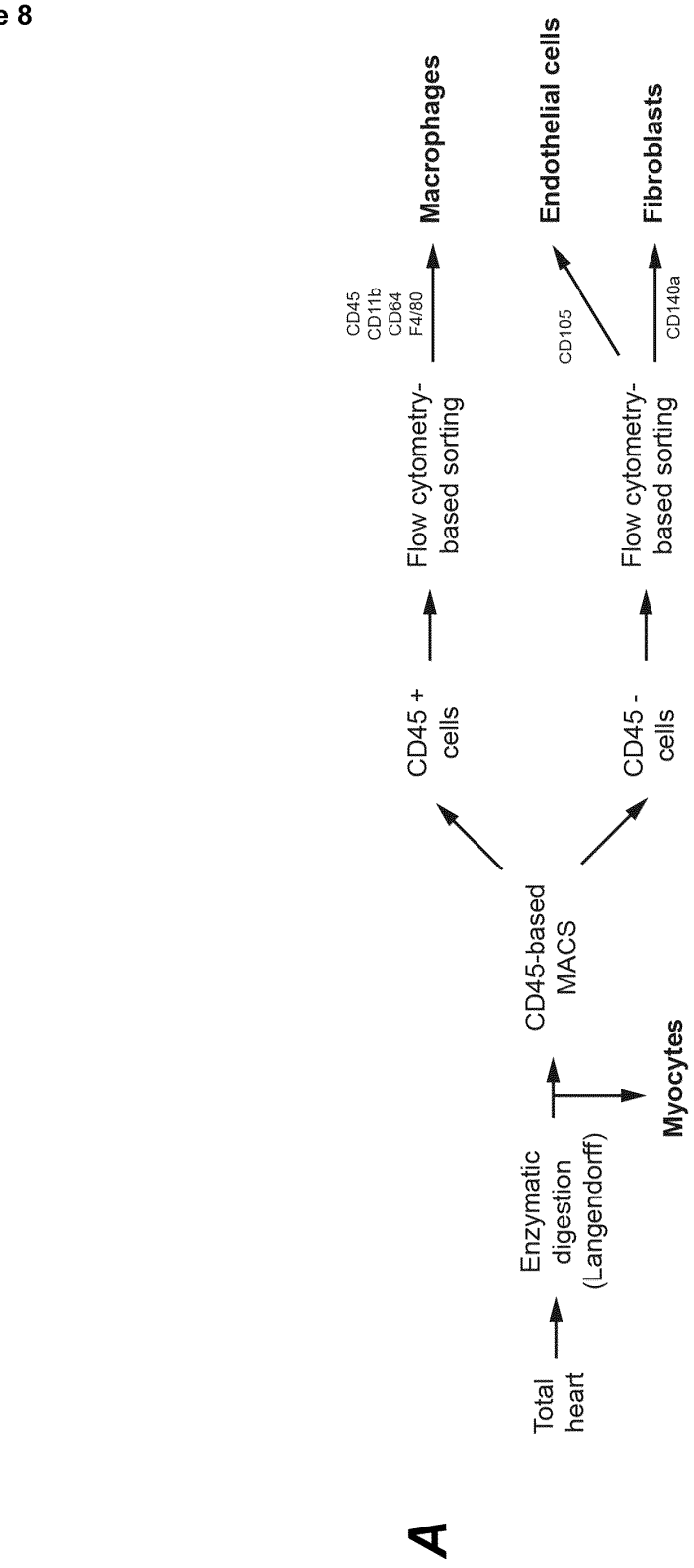

Total heart
Enzymatic digestion (Langendorff)
Myocytes
CD45-based MACS
CD45 + cells
CD45 - cells
Flow cytometry-based sorting
Flow cytometry-based sorting
CD45
CD11b
CD64
F4/80
CD105
CD140a
Macrophages
Endothelial cells
Fibroblasts
A

Figure 8 cont.

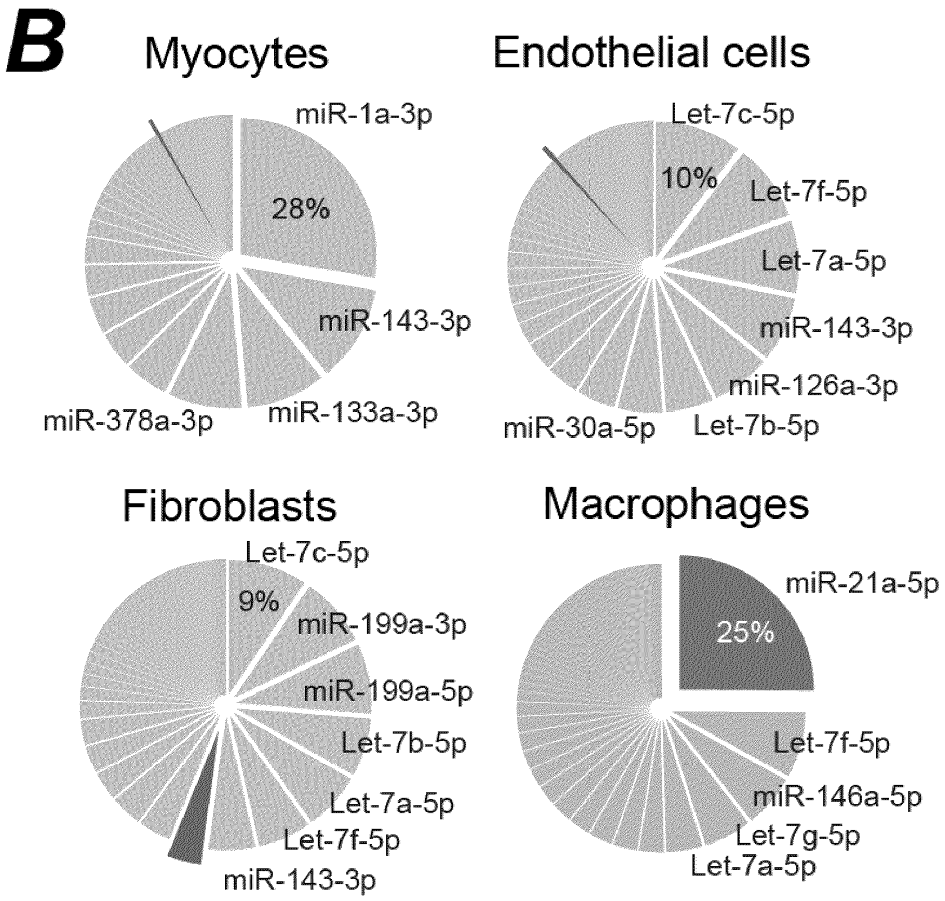
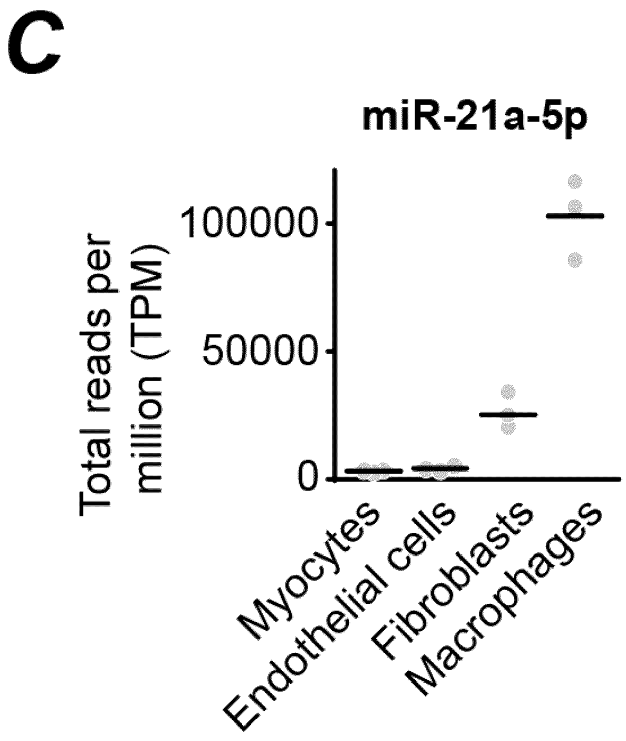


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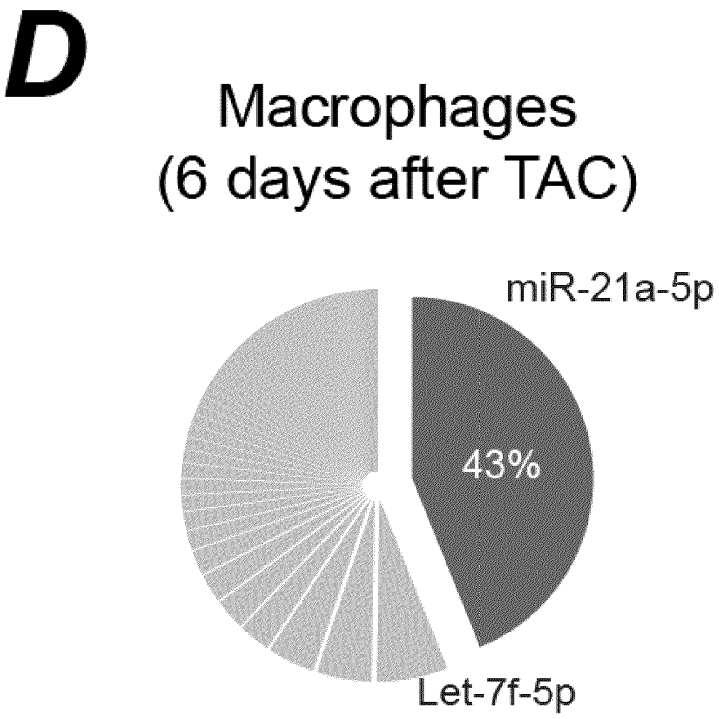


Figure 8 cont.

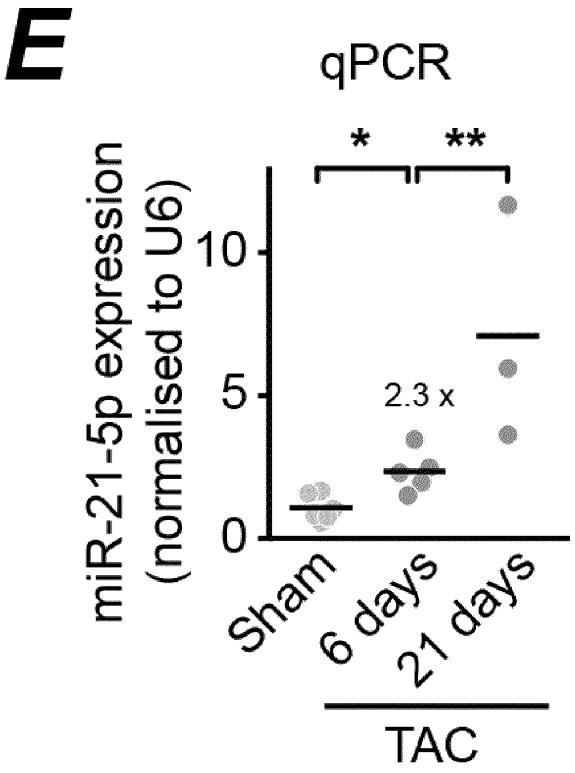


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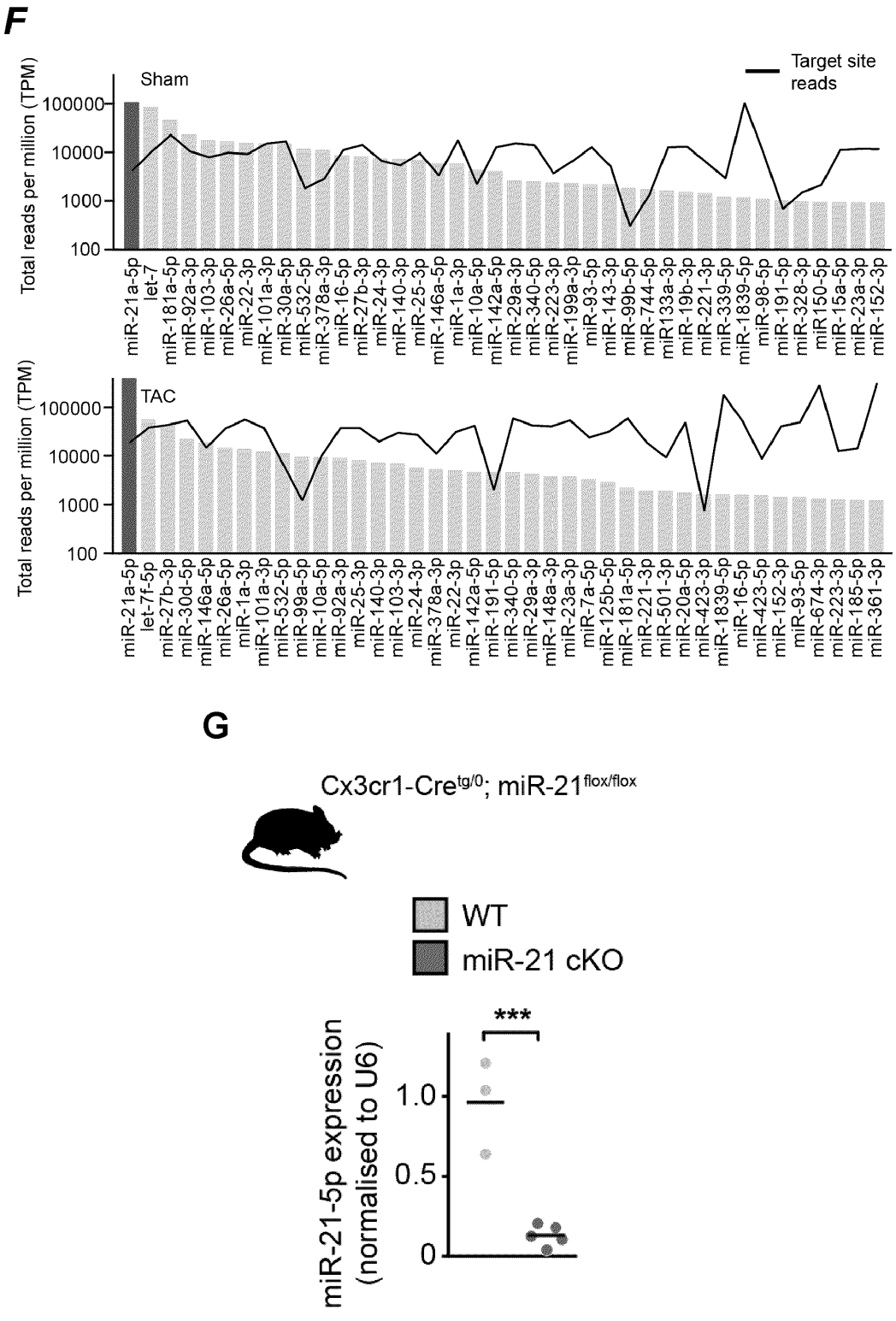


Figure 9

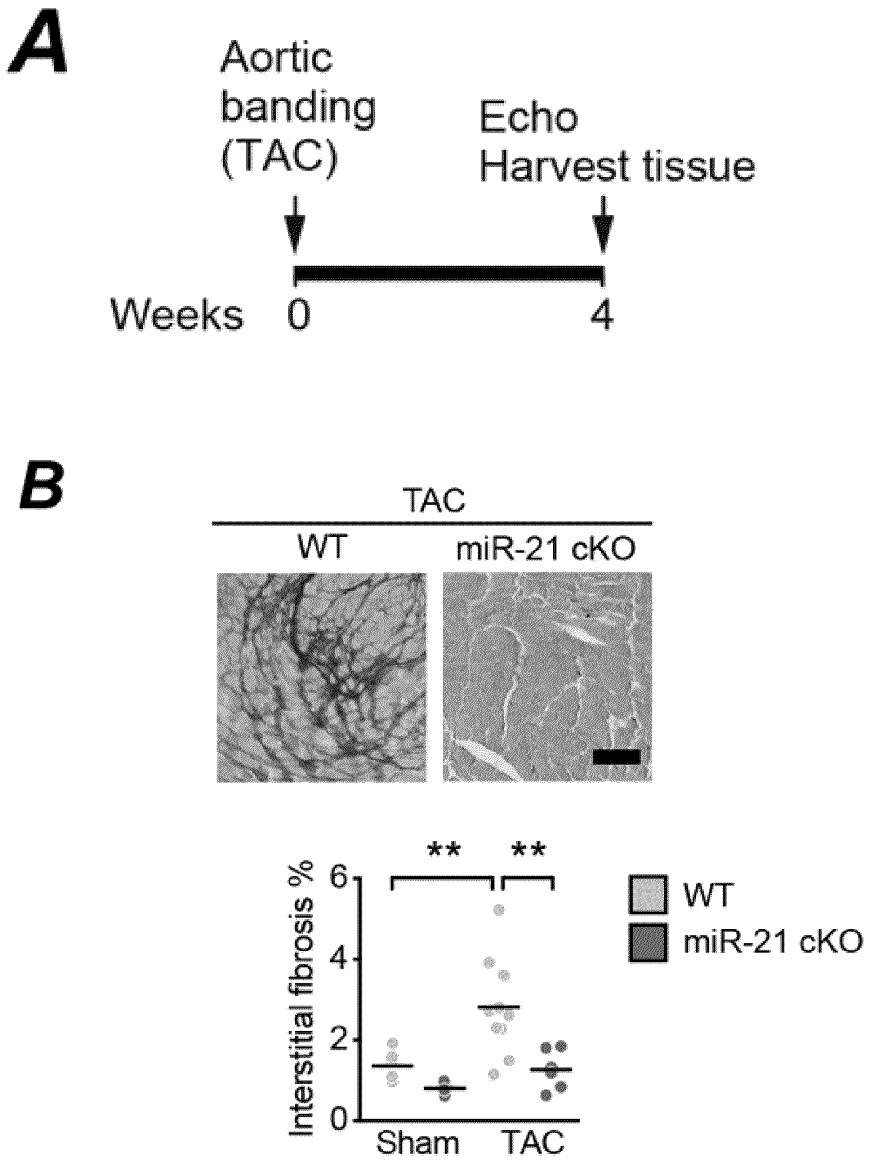

A
Aortic banding (TAC)
Echo
Harvest tissue
Weeks 0 4
B
TAC
WT
miR-21 cKO
Interstitial fibrosis %
0
2
4
6
**
**
Sham
TAC
WT
miR-21 cKO

Figure 9 cont.

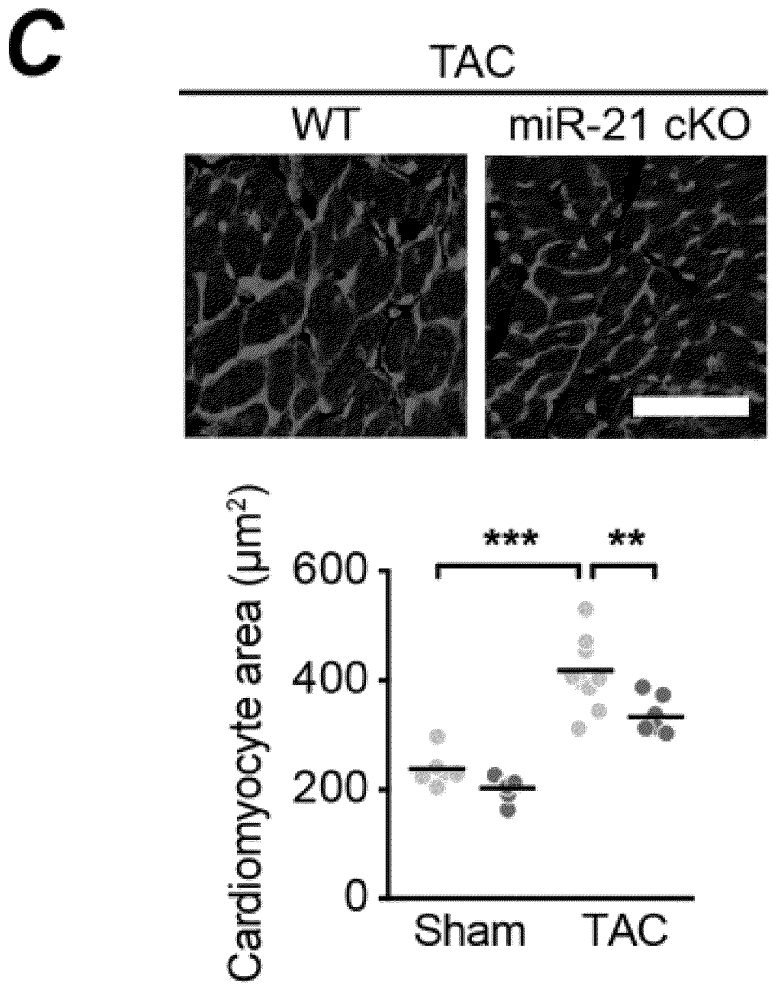


Figure 9 cont.

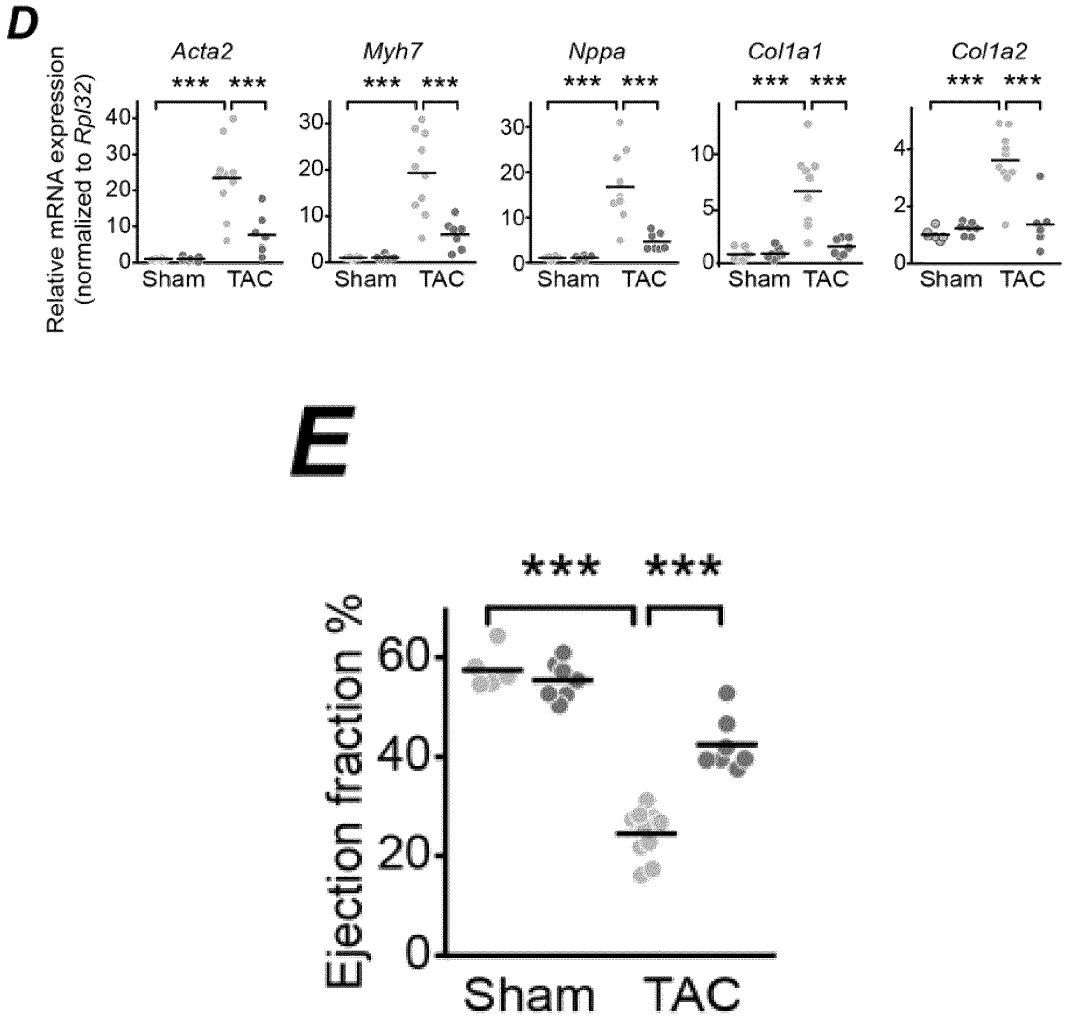


Figure 9 cont.

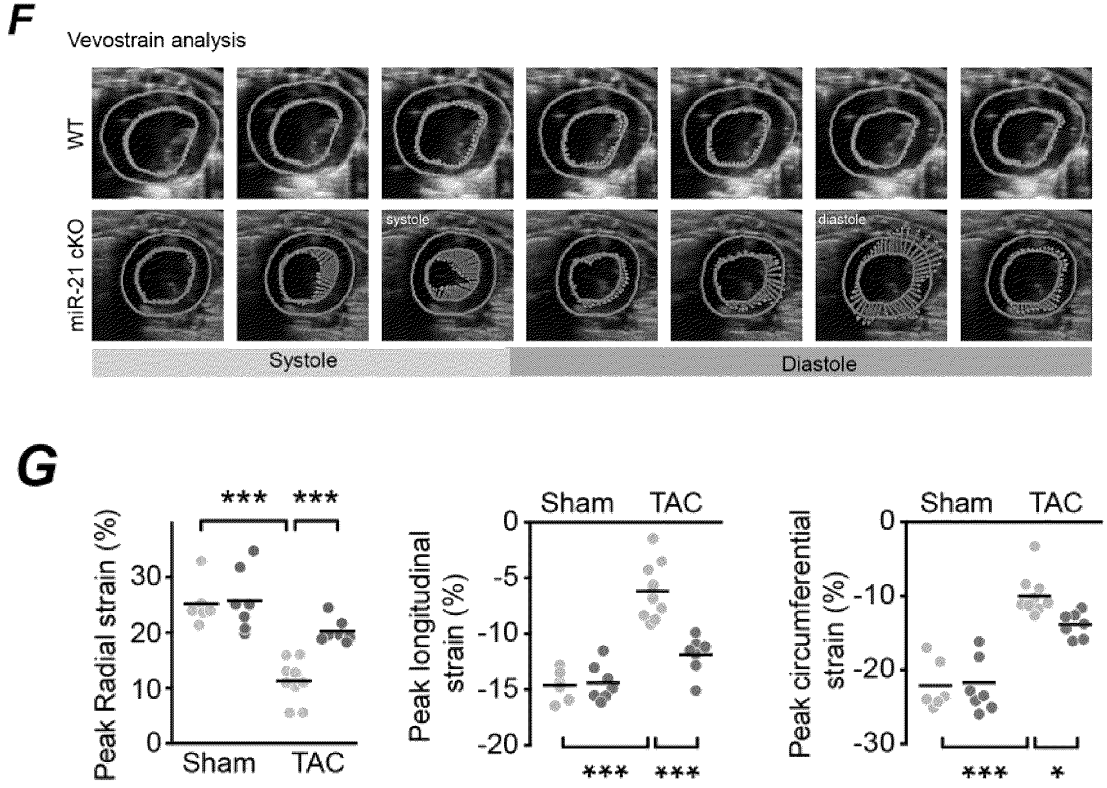


Figure 10

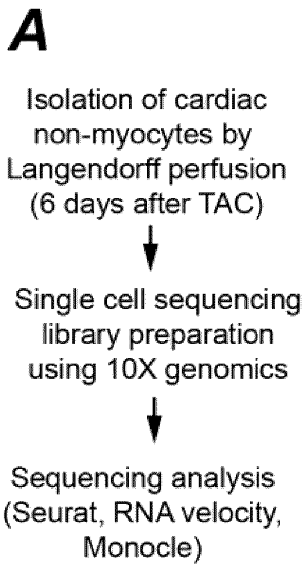


Figure 10 cont.

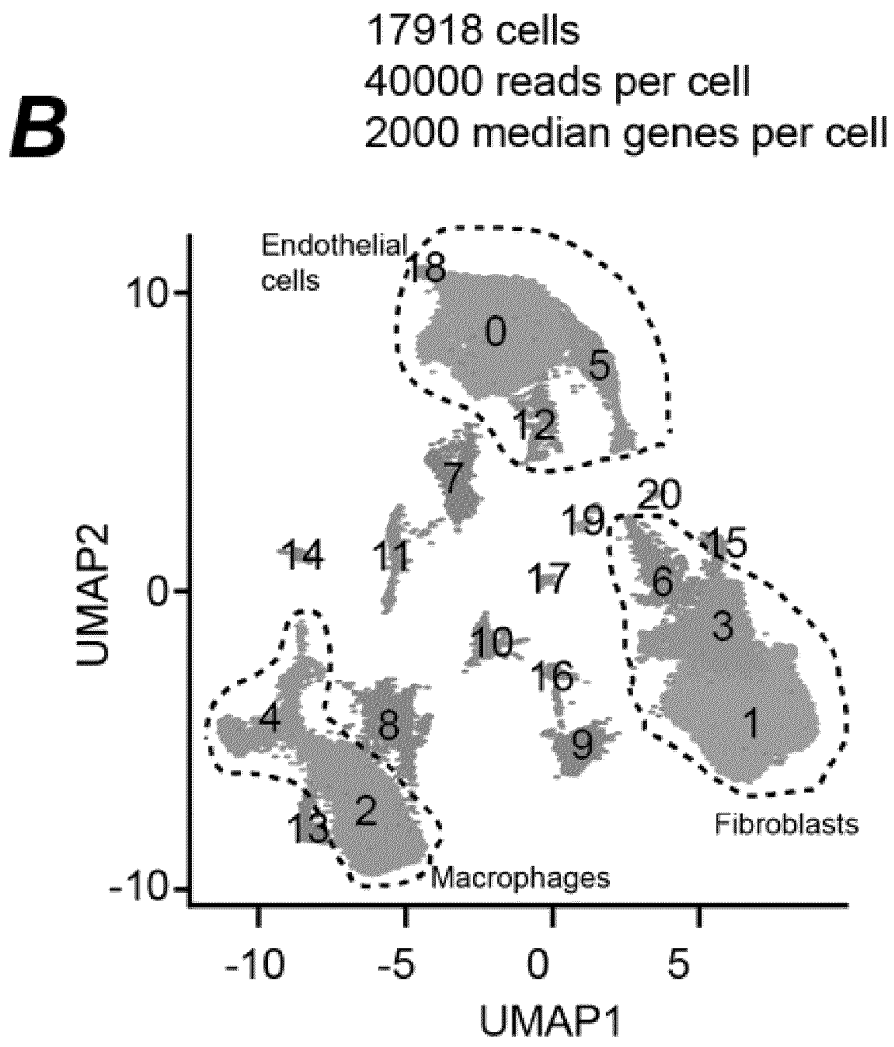


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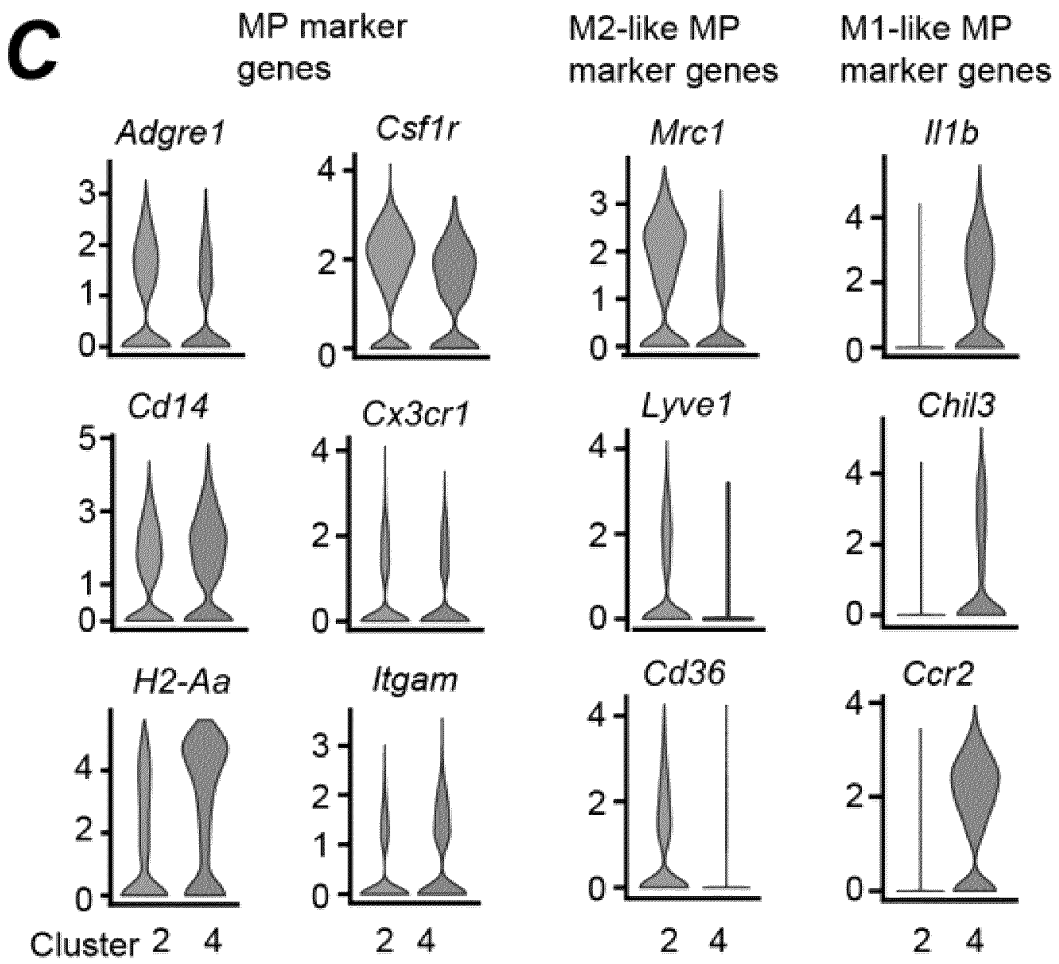


Figure 10 cont.

D

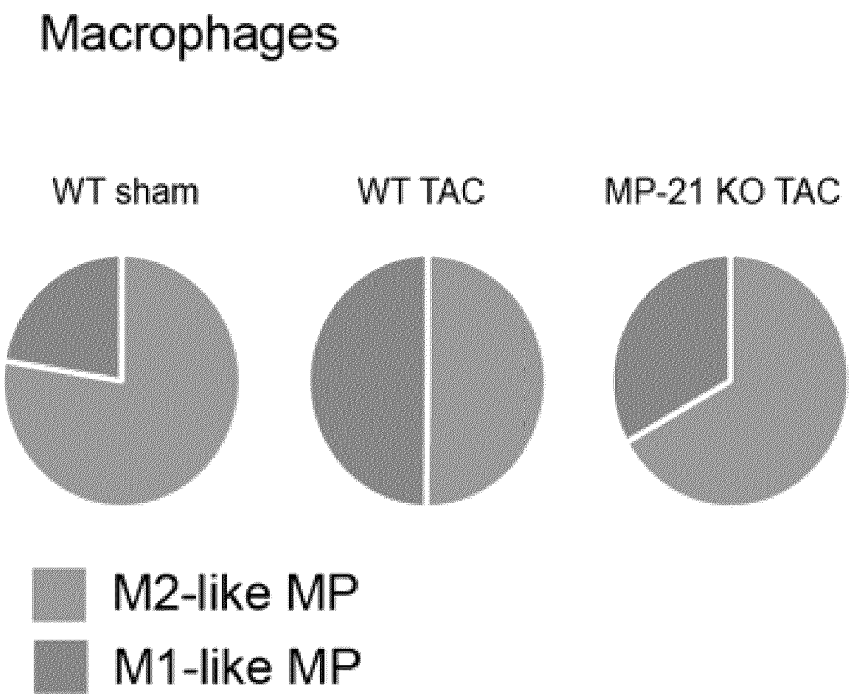


Figure 10 cont.

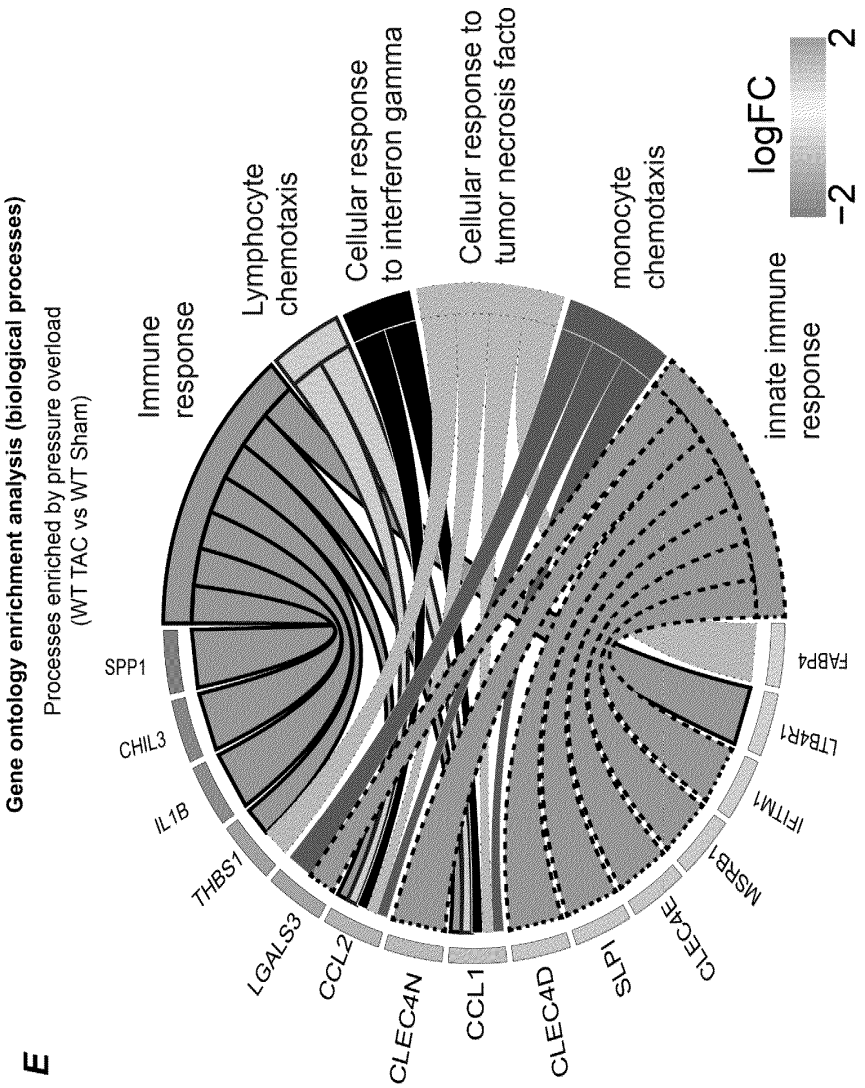


Figure 10E continued

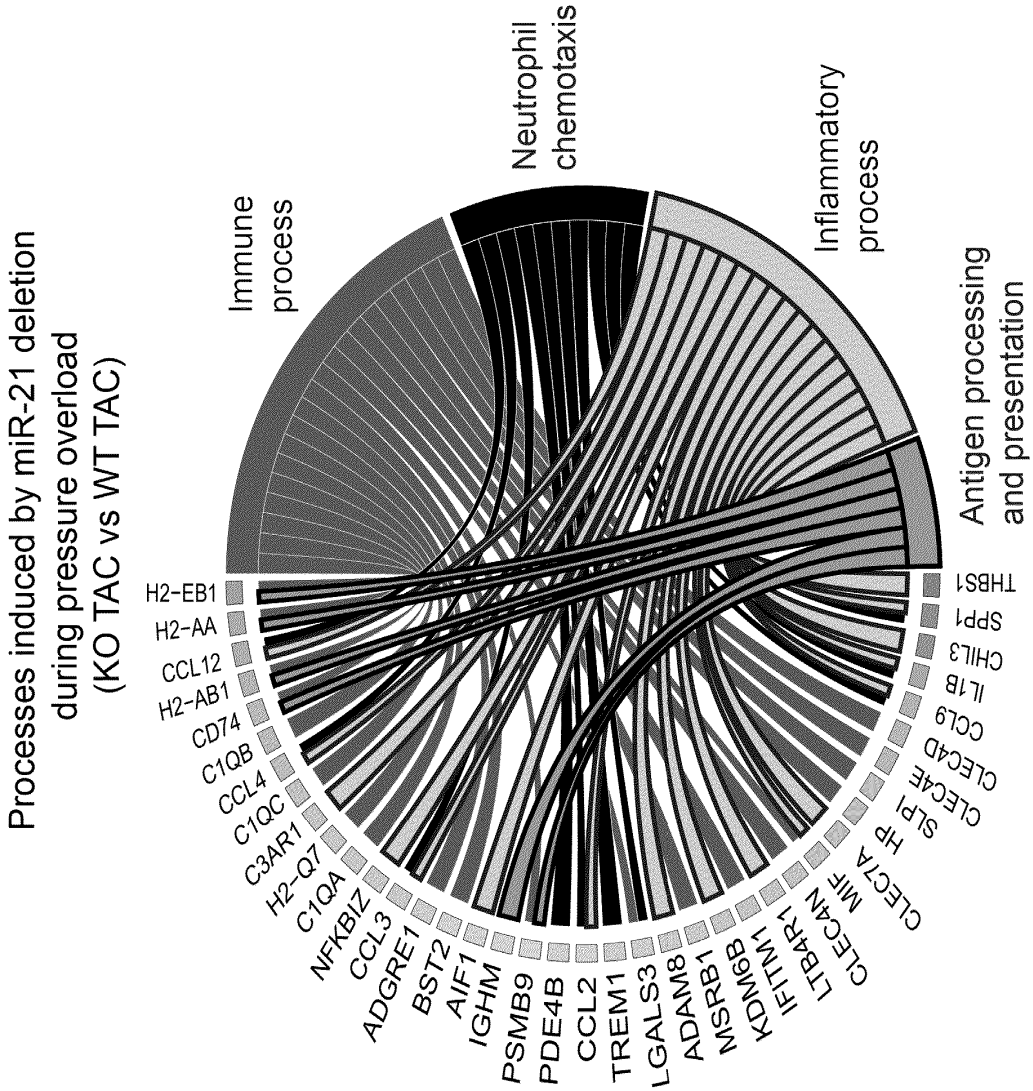


Figure 10 cont.

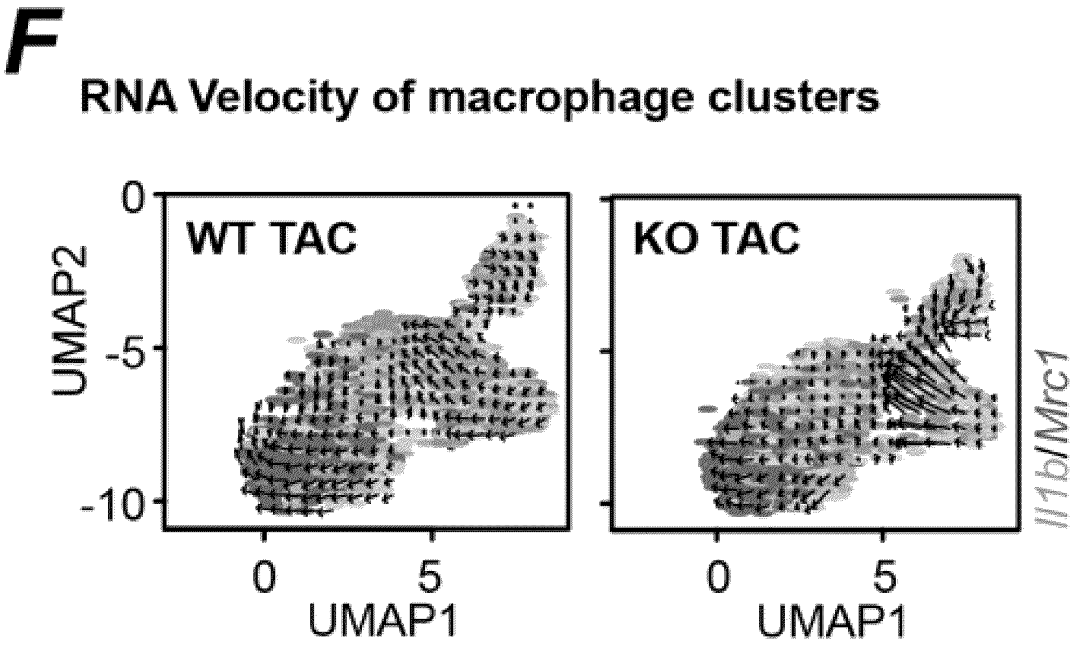


Figure 10 cont.

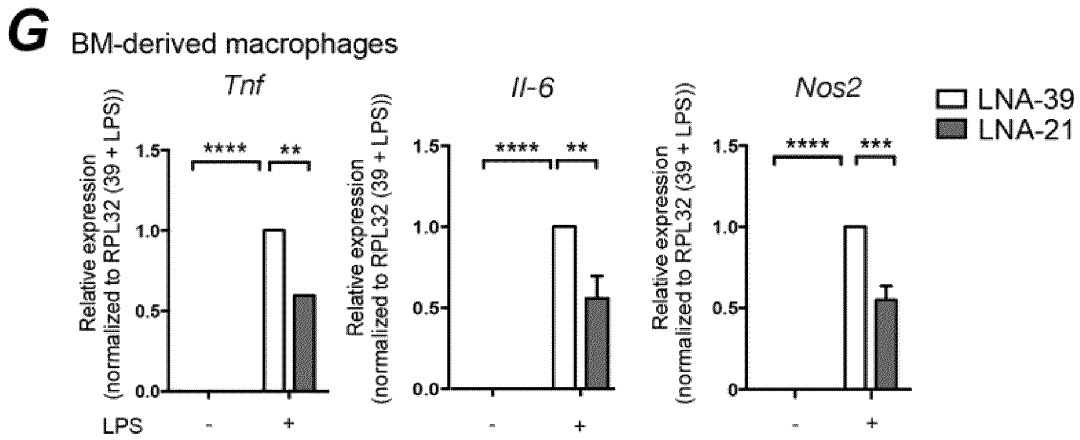


Figure 11

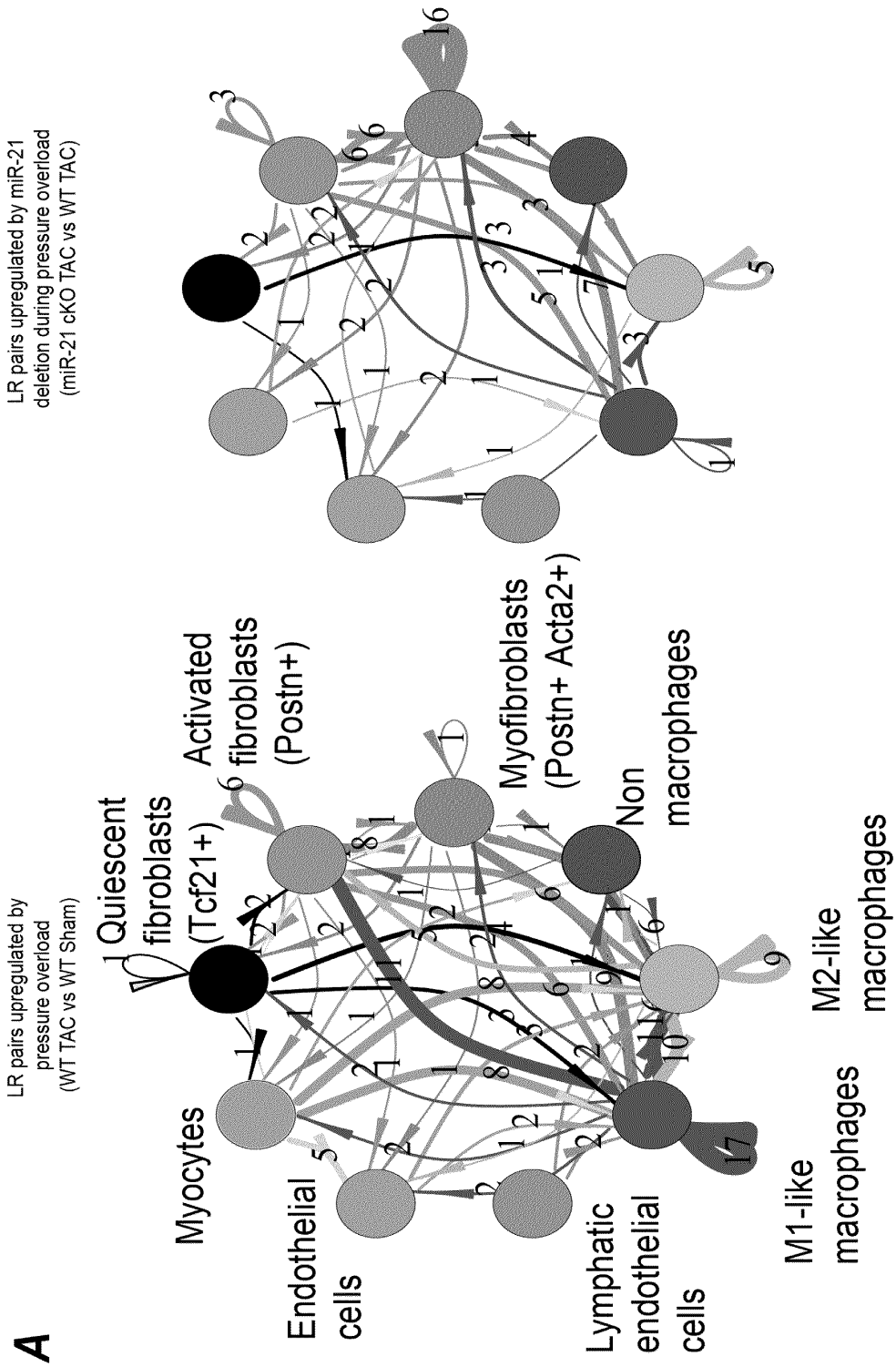
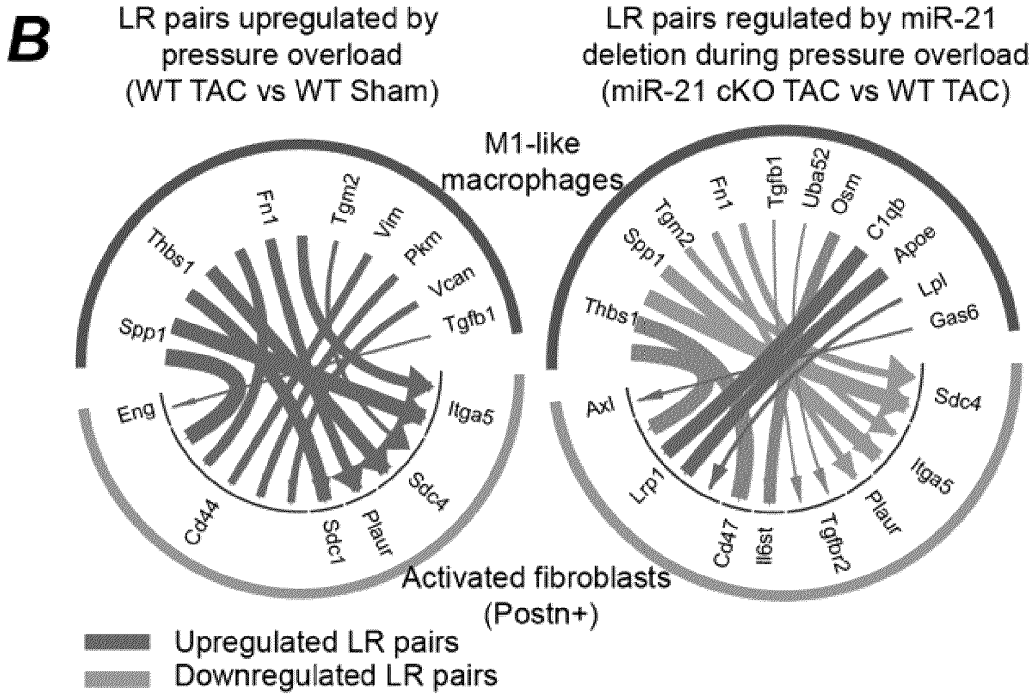

A
LR pairs upregulated by pressure overload (WT TAC vs WT Sham)
LR pairs upregulated by miR-21 deletion during pressure overload (miR-21 cKO TAC vs WT TAC)
Quiescent fibroblasts (Tcf21+)
Activated fibroblasts (Postn+)
Myofibroblasts (Postn+ Acta2+)
Myocytes
Endothelial cells
Lymphatic endothelial cells
Non macrophages
M1-like macrophages
M2-like macrophages

Figure 11 cont.



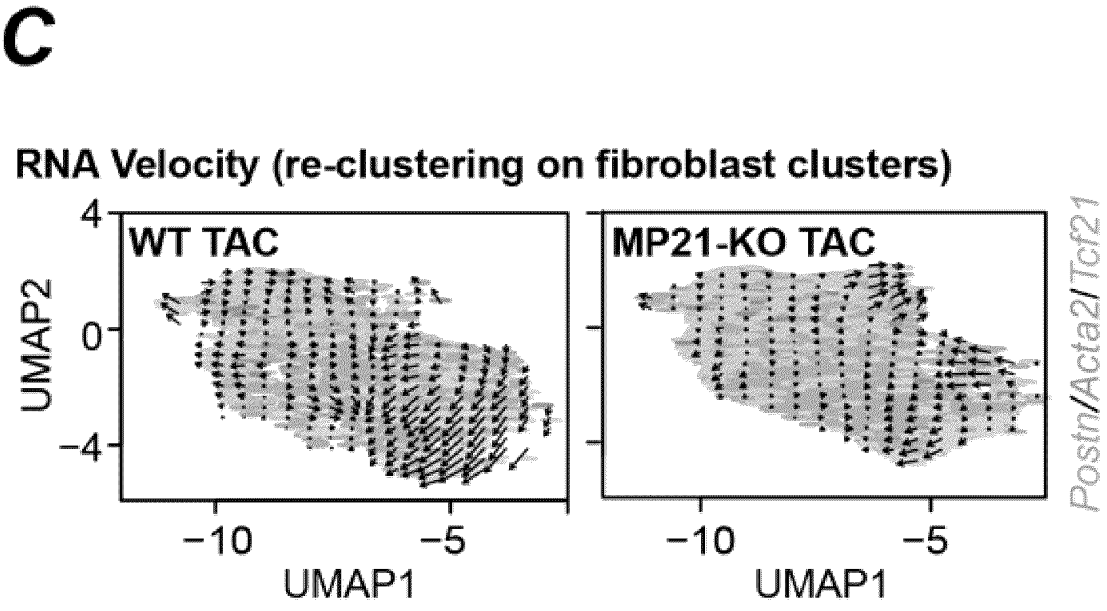
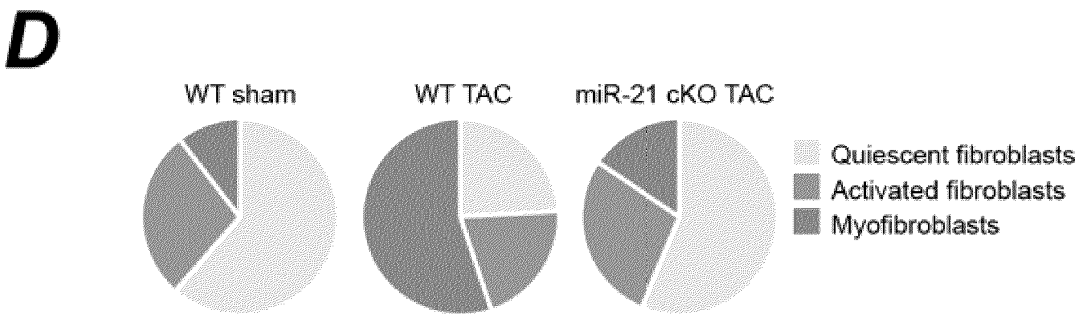


Figure 11 cont.



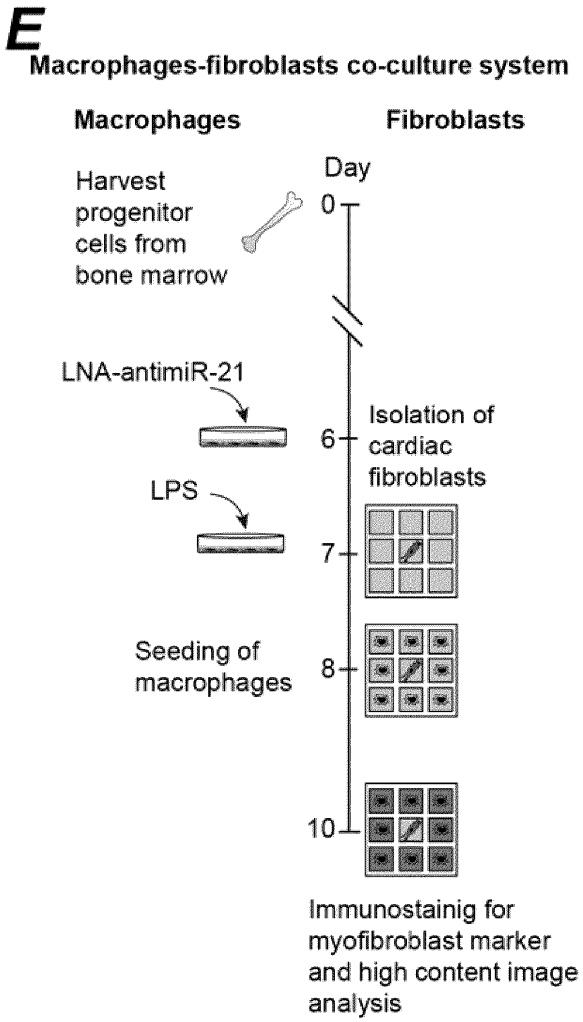


Figure 11 cont.

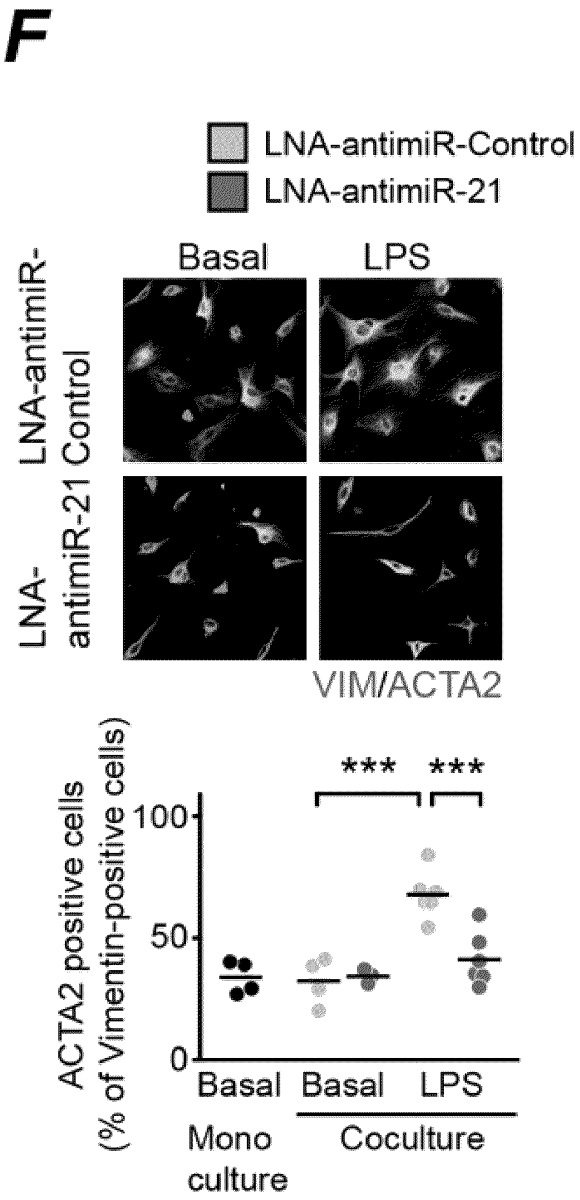


Figure 12

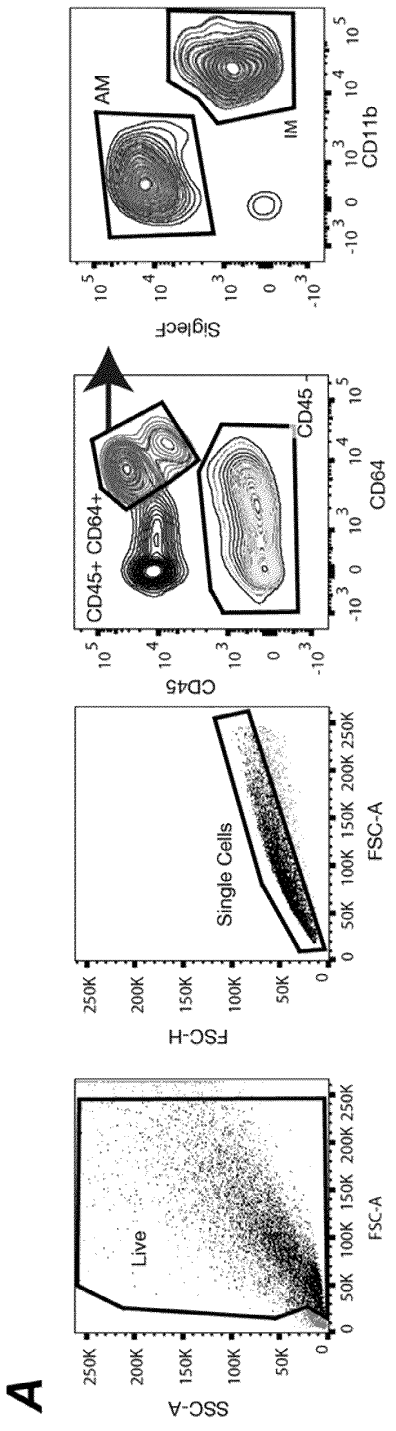
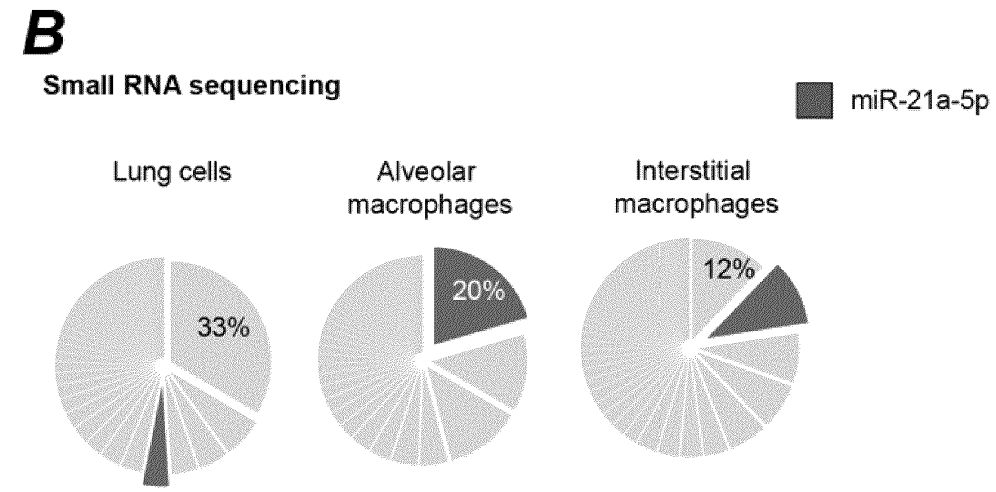

A
SSC-A
FSC-A
Live
FSC-H
FSC-A
Single Cells
CD45
CD64
CD45+ CD64+
CD45
SiglecF
CD11b
AM
IM

Figure 12 cont.



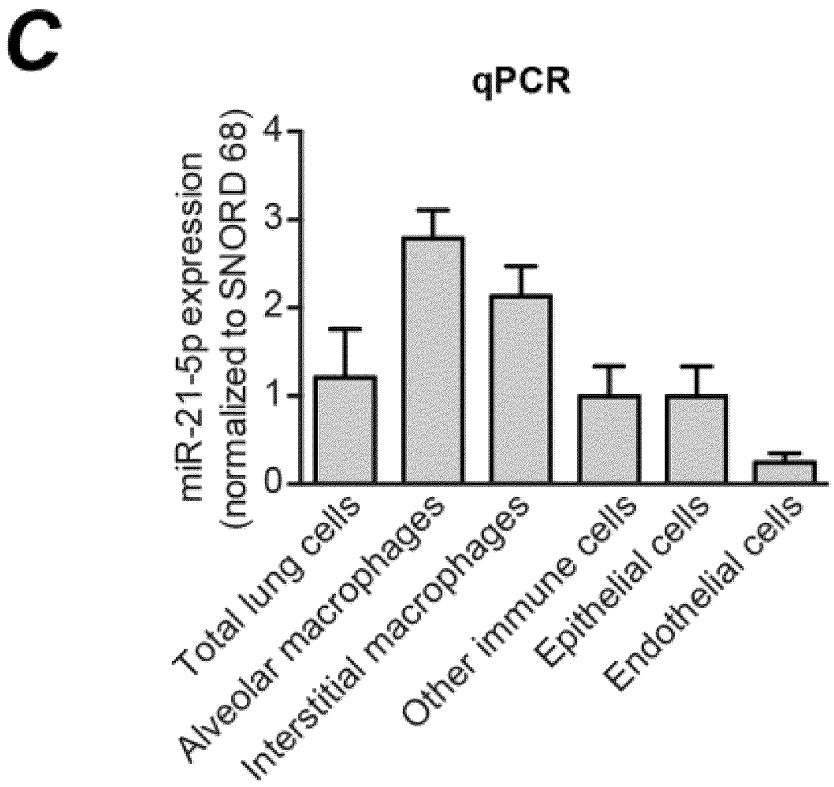


Figure 13

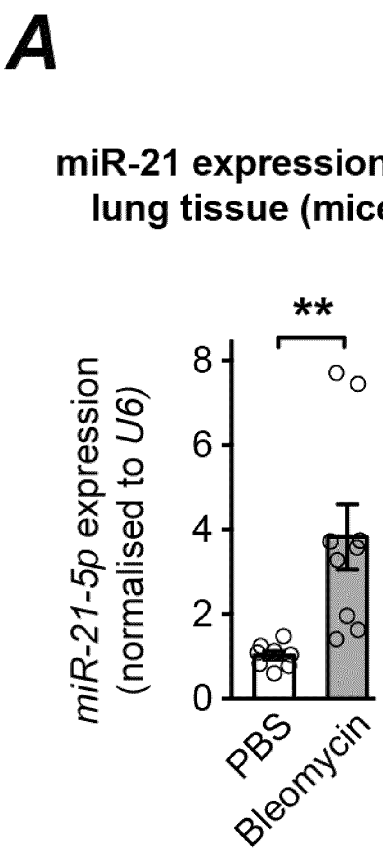

A
miR-21 expression in lung tissue (mice)
**
miR-21-5p expression (normalised to U6)
8
6
4
2
0
PBS
Bleomycin

Figure 13 cont.

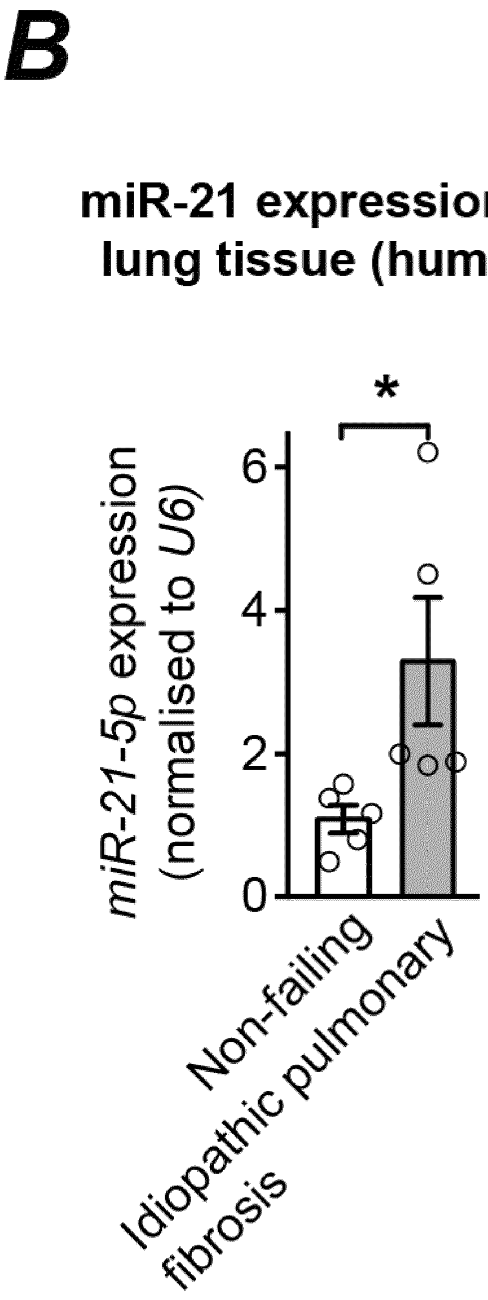
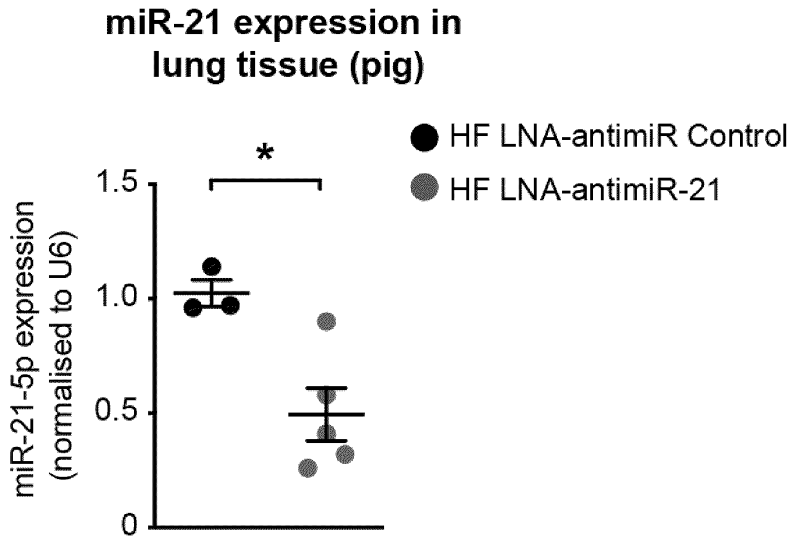


Figure 13 cont.

C



D

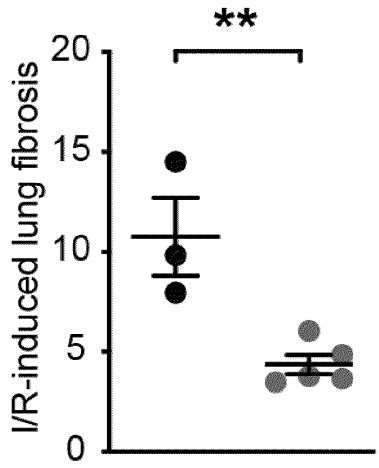


Figure 14

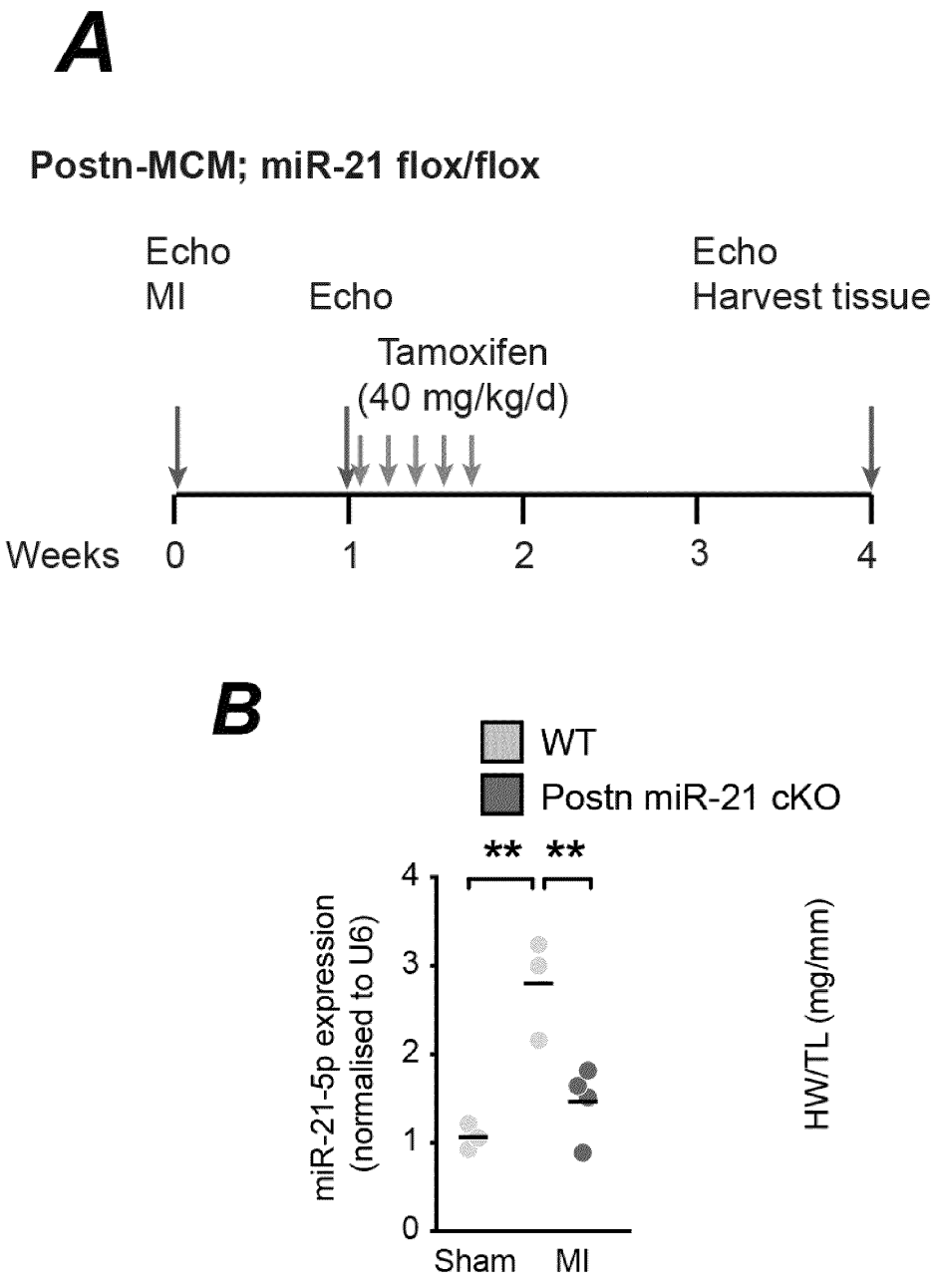
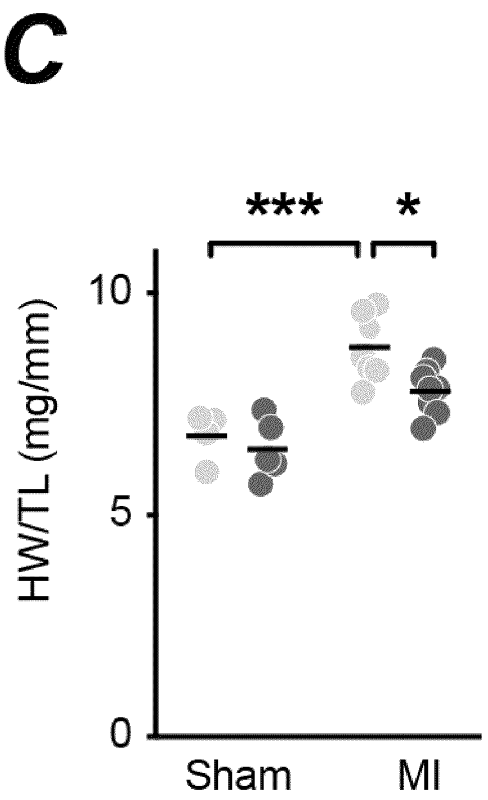

A
Postn-MCM; miR-21 flox/flox
Echo
MI
Echo
Echo
Harvest tissue
Tamoxifen
(40 mg/kg/d)
Weeks
0
1
2
3
4
B
WT
Postn miR-21 cKO
**
**
miR-21-5p expression
(normalised to U6)
HW/TL (mg/mm)
Sham
MI

Figure 14 cont.



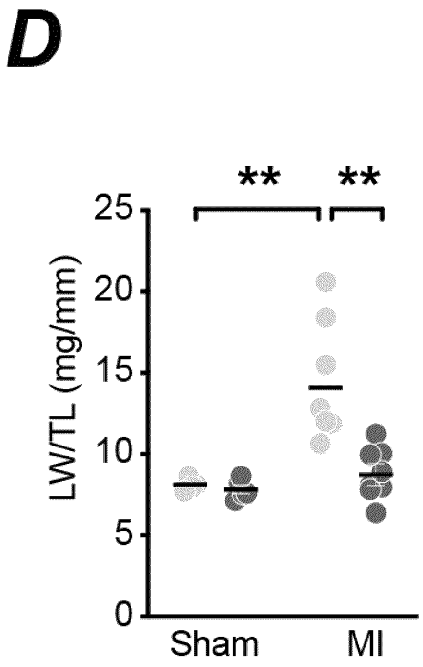


Figure 14 cont.

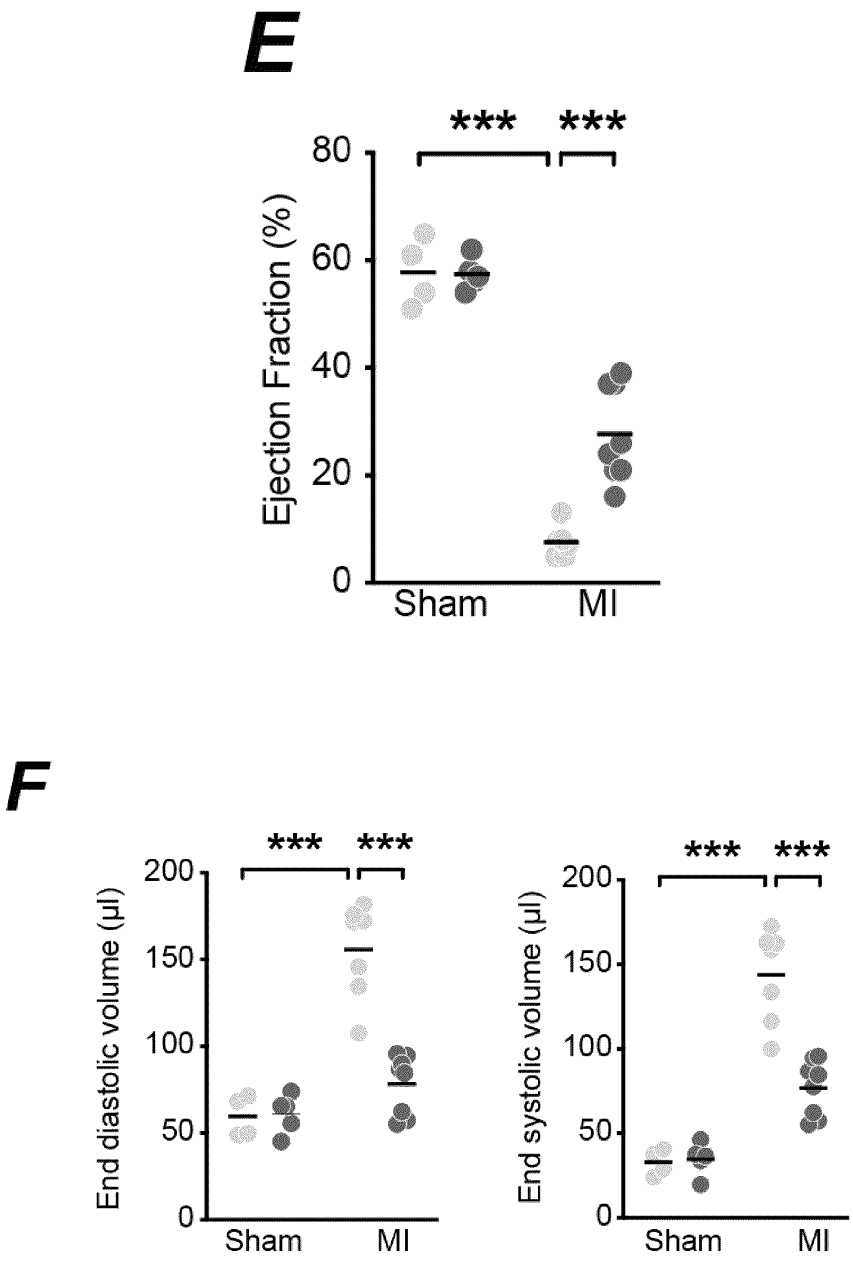


Figure 14 cont.

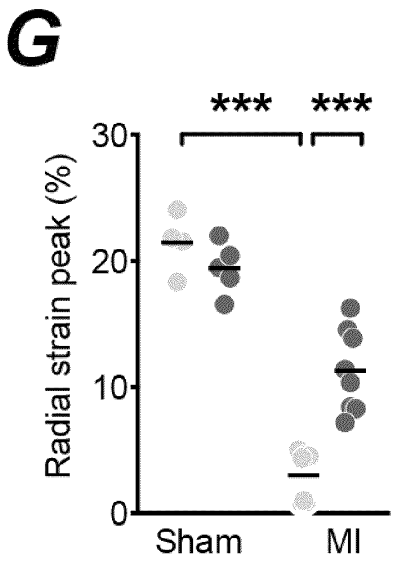


Figure 15

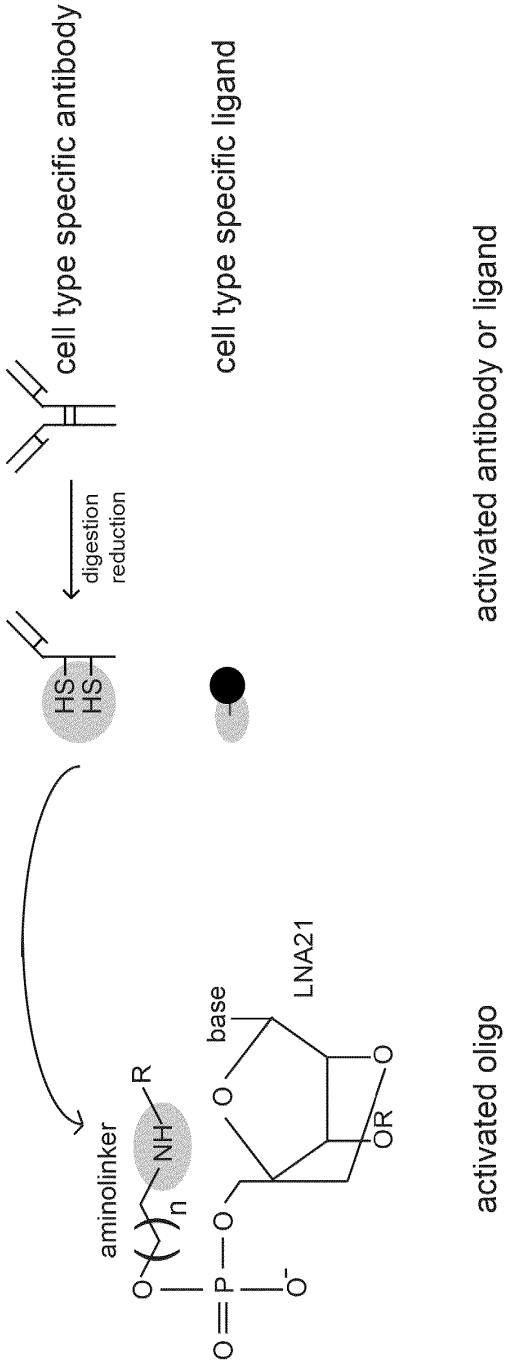

cell type specific antibody
digestion
reduction
HS
HS
cell type specific ligand
activated antibody or ligand
aminolinker
NH
R
n
O
P
O
O-
base
LNA21
O
OR
activated oligo

Figure 16
Table 1
Serological analysis of selected biochemical parameters of liver and kidney function

	HF Control	HF LNA-antimiR-Control	HF LNA-antimiR-21	p-value
n	3	3	5	
Day 0				
AST (GOT) (U/l)	32 ± 3.8	23 ± 3.1	29 ± 3.8	n.s.
ALT (GPT) (U/l)	42 ± 5.0	36 ± 3.7	46 ± 8.8	n.s.
γ-GT (U/l)	33 ± 10.7	28 ± 8.4	30 ± 7.5	n.s.
Creatinine (µmol/l)	87 ± 5.9	72 ± 5.4	88 ± 2.4	n.s.
Urea (mmol/µl)	2.5 ± 0.6	3.6 ± 0.7	3.1 ± 0.9	n.s.
Day 5				
AST (GOT) (U/l)	33 ± 7.1	32 ± 7.3	30 ± 1.3	n.s.
ALT (GPT) (U/l)	47 ± 7.4	55 ± 1.2	63 ± 10.6	n.s.
γ-GT (U/l)	48 ± 22.8	23 ± 3.5	32 ± 7	n.s.
Creatinine (µmol/l)	93 ± 10.5	58 ± 5.2	99 ± 5.4	n.s.
Urea (mmol/µl)	2.7 ± 0.6	3.9 ± 0.3	3.2 ± 0.6	n.s.
Day 33				
AST (GOT) (U/l)	31 ± 3.3	22 ± 7.3	27 ± 4.9	n.s.
ALT (GPT) (U/l)	44 ± 12.6	22 ± 3.7	53 ± 9.5	n.s.
γ-GT (U/l)	49 ± 11.7	25 ± 4.7	33 ± 4.8	n.s.
Creatinine (µmol/l)	120 ± 20.7	97 ± 0.8	111 ± 2.3	n.s.
Urea (mmol/µl)	3.3 ± 0.8	3.4 ± 0.2	4.3 ± 0.7	n.s.

(MEAN±SEM, p-value HF Control vs. HF LNA-antimiR-21)

Reference values: AST (GOT) (<59 U/l); ALT (GPT) (<68 U/l); γ-GT (<54 U/l); Creatinine (<160 µmol/l); Urea (2.5-6.7 mmol/µl).

Figure 17
Table 2
Cardiovascular events

	Sham	HF Control	HF LNA-antimiR-21
	Day0		
Ventricular extrasystoly	0%	67%	85%
Reanimation (Defibrillation)	0%	33%	14%
Cardiac death	0%	16%	14%
	Day 5		
Ventricular extrasystoly		20%	16%
Reanimation (Defibrillation)		20%	16%
Cardiac death		20%	16%
	Day 19		
Ventricular extrasystoly		0%	0%
Reanimation (Defibrillation)n		0%	0%
Cardiac death		0%	0%
	Day 33		
Ventricular extrasystoly	0%	0%	0%
Reanimation (Defibrillation)	0%	0%	0%
Cardiac death	0%	0%	0%

(MEAN±SEM, p-value HF Control vs. HF LNA-antimiR-21)

Figure 18.

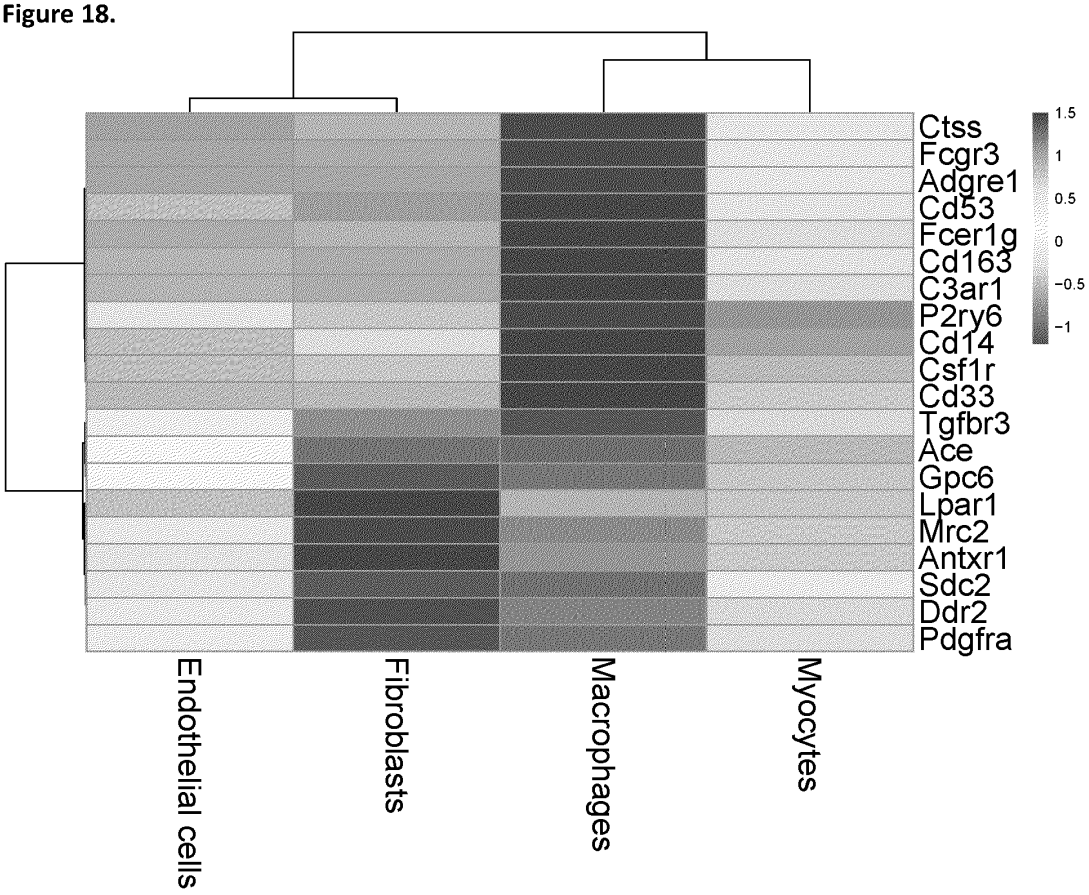

Ctss
Fcgr3
Adgre1
Cd53
Fcer1g
Cd163
C3ar1
P2ry6
Cd14
Csf1r
Cd33
Tgfbr3
Ace
Gpc6
Lpar1
Mrc2
Antxr1
Sdc2
Ddr2
Pdgfra
1.5
1
0.5
0
−0.5
−1
Endothelial cells
Fibroblasts
Macrophages
Myocytes

Figure 19

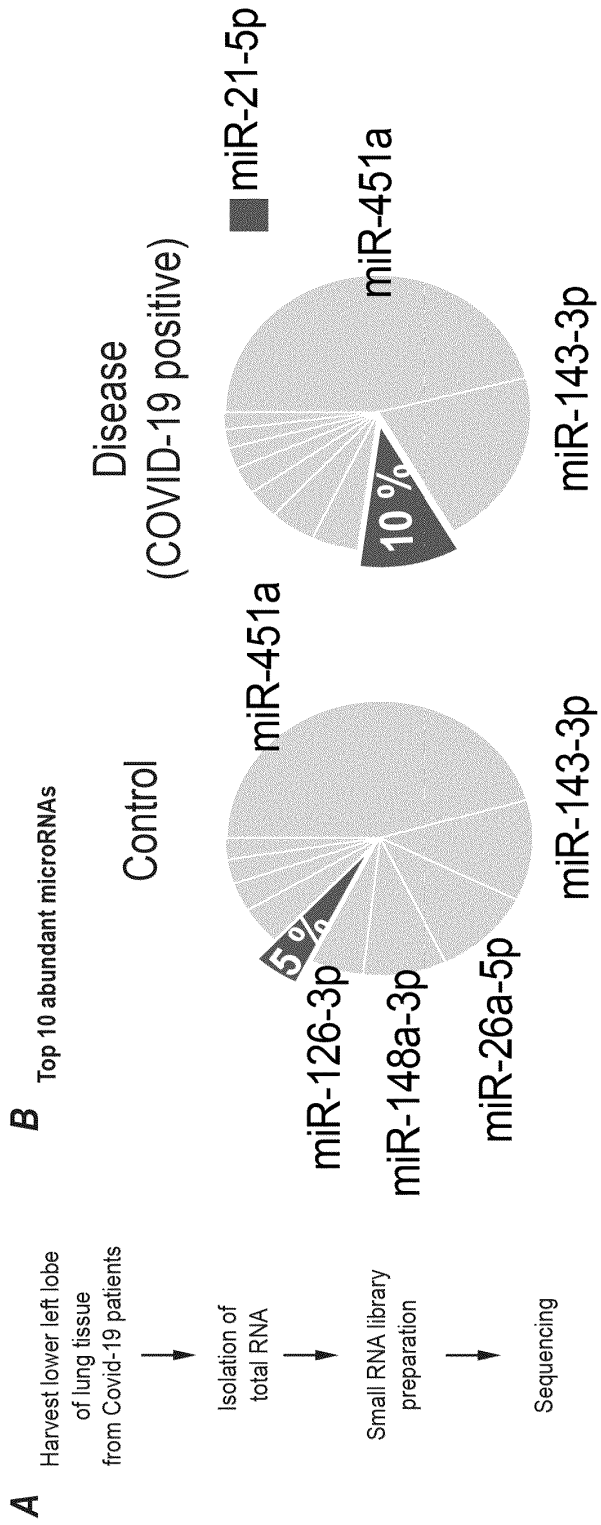

A
Harvest lower left lobe of lung tissue from Covid-19 patients
Isolation of total RNA
Small RNA library preparation
Sequencing
B
Top 10 abundant microRNAs
Control
miR-451a
5 %
miR-126-3p
miR-148a-3p
miR-26a-5p
miR-143-3p
Disease (COVID-19 positive)
miR-21-5p
miR-451a
10 %
miR-143-3p

Figure 20

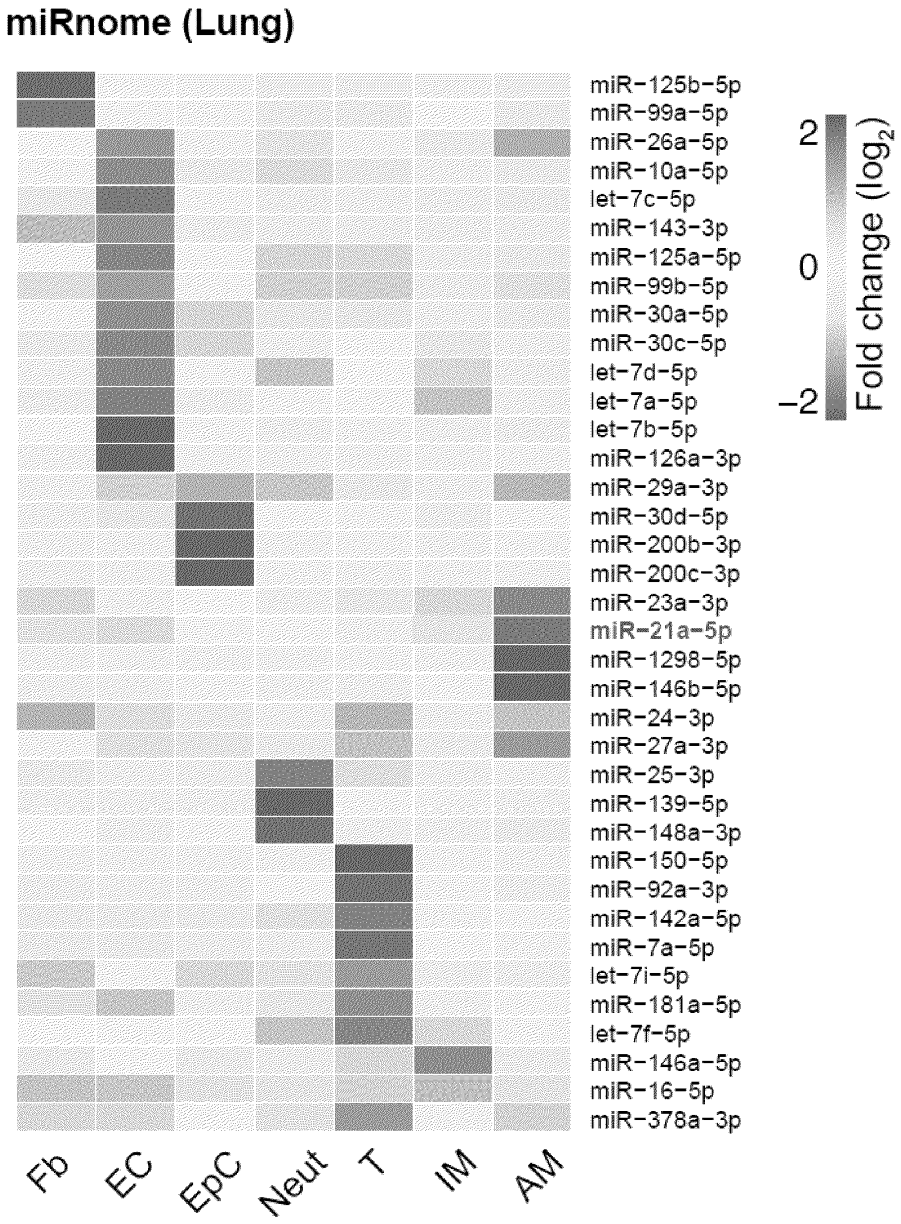

miRnome (Lung)
miR-125b-5p
miR-99a-5p
miR-26a-5p
miR-10a-5p
let-7c-5p
miR-143-3p
miR-125a-5p
miR-99b-5p
miR-30a-5p
miR-30c-5p
let-7d-5p
let-7a-5p
let-7b-5p
miR-126a-3p
miR-29a-3p
miR-30d-5p
miR-200b-3p
miR-200c-3p
miR-23a-3p
miR-21a-5p
miR-1298-5p
miR-146b-5p
miR-24-3p
miR-27a-3p
miR-25-3p
miR-139-5p
miR-148a-3p
miR-150-5p
miR-92a-3p
miR-142a-5p
miR-7a-5p
let-7i-5p
miR-181a-5p
let-7f-5p
miR-146a-5p
miR-16-5p
miR-378a-3p
Fb
EC
EpC
Neut
T
IM
AM
2
0
−2
Fold change (log2)

Figure 21

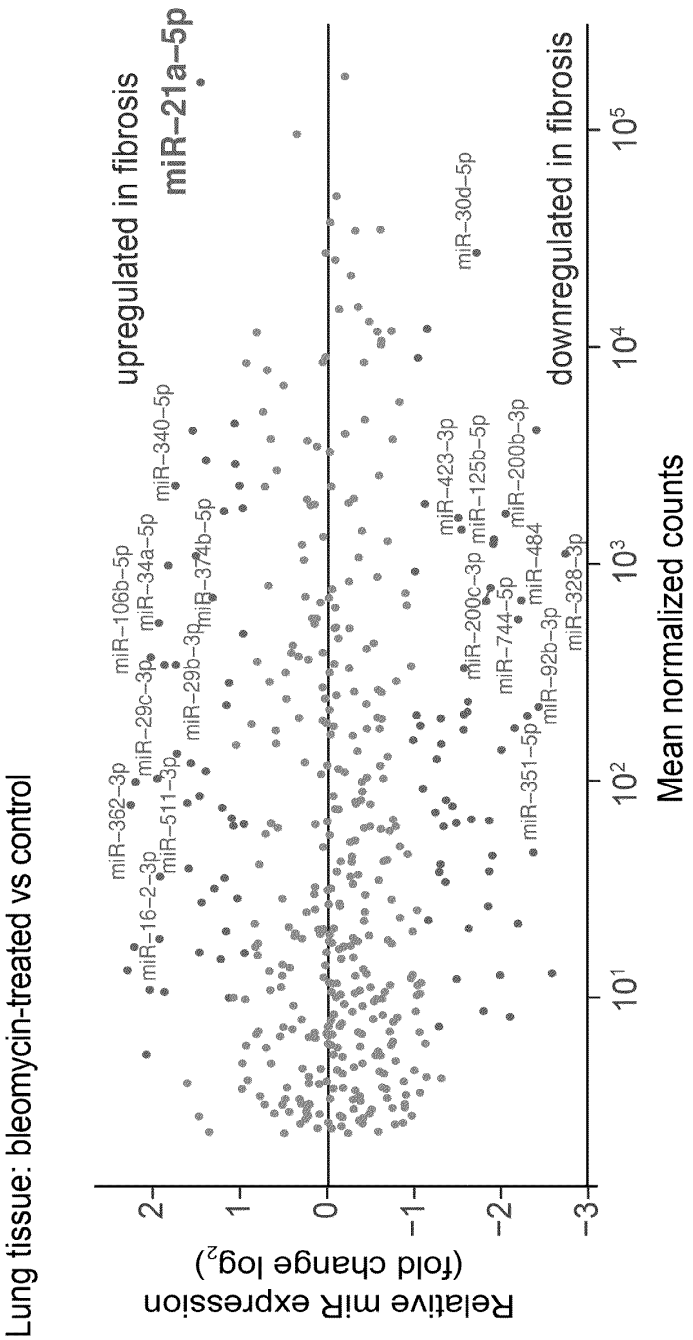

Lung tissue: bleomycin-treated vs control
upregulated in fibrosis
downregulated in fibrosis
miR−21a−5p
miR−362−3p
miR−16−2−3p
miR−511−3p
miR−29c−3p
miR−106b−5p
miR−29b−3p
miR−34a−5p
miR−374b−5p
miR−340−5p
miR−30d−5p
miR−423−3p
miR−125b−5p
miR−200c−3p
miR−744−5p
miR−200b−3p
miR−484
miR−92b−3p
miR−351−5p
miR−328−3p
Relative miR expression
(fold change log2)
Mean normalized counts
2
1
0
−1
−2
−3
10^1
10^2
10^3
10^4
10^5

Figure 22

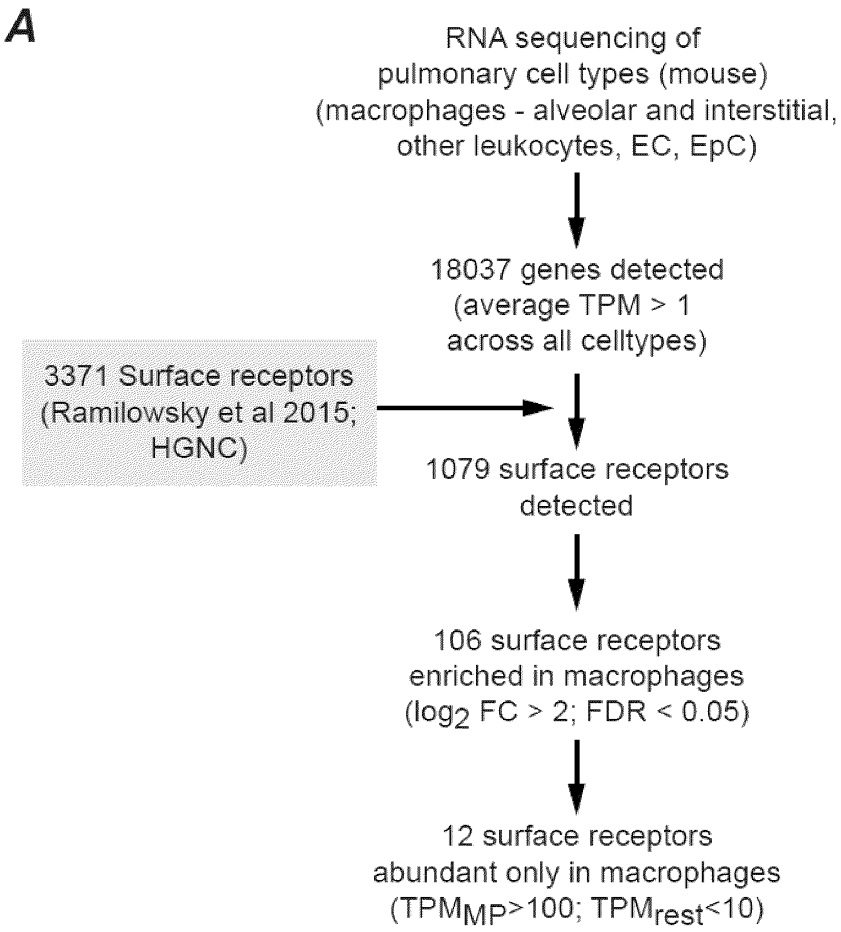
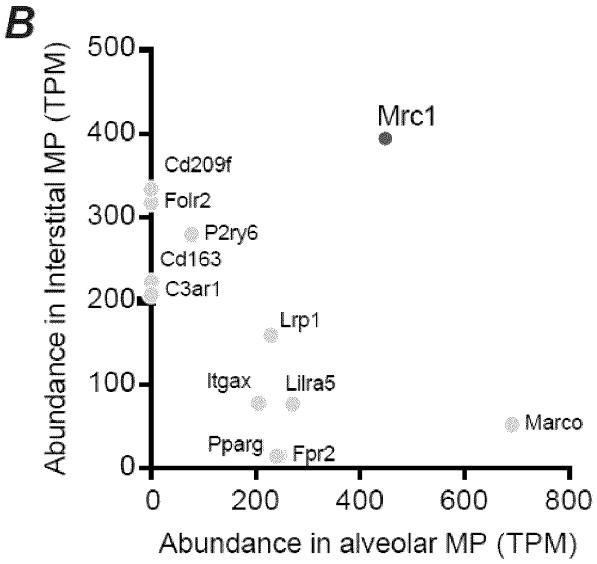

A
RNA sequencing of pulmonary cell types (mouse) (macrophages - alveolar and interstitial, other leukocytes, EC, EpC)
18037 genes detected (average TPM > 1 across all celltypes)
3371 Surface receptors (Ramilowsky et al 2015; HGNC)
1079 surface receptors detected
106 surface receptors enriched in macrophages (log_2 FC > 2; FDR < 0.05)
12 surface receptors abundant only in macrophages (TPM_{MP}>100; TPM_{rest}<10)

Figure 22 cont.



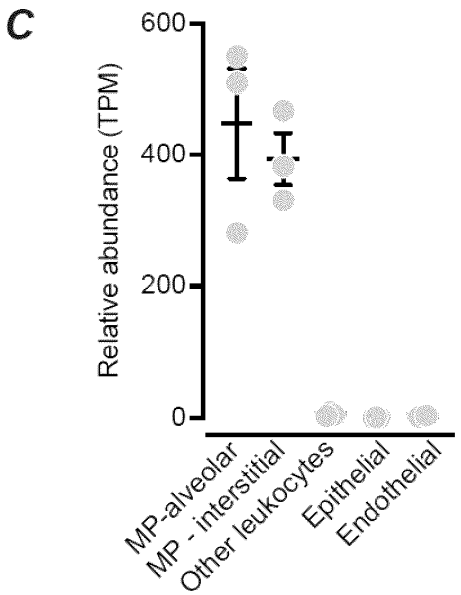
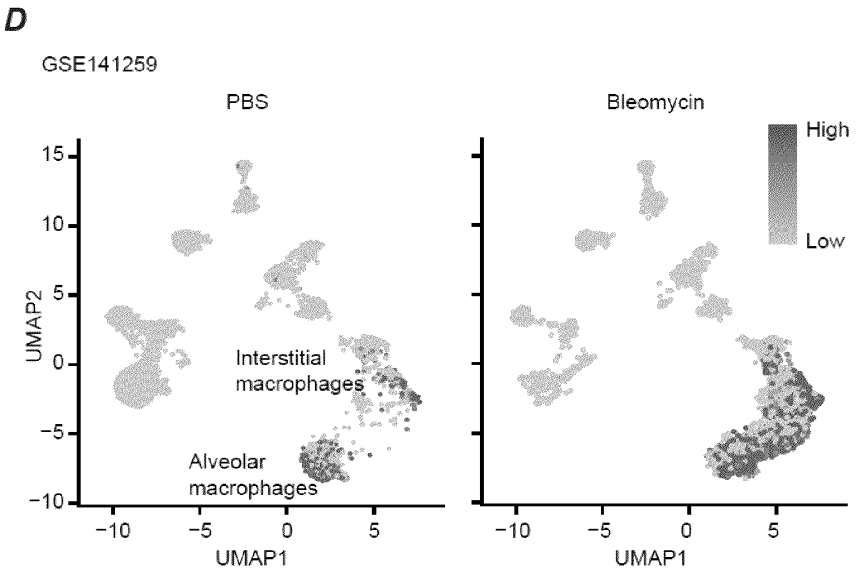


Figure 22 cont.



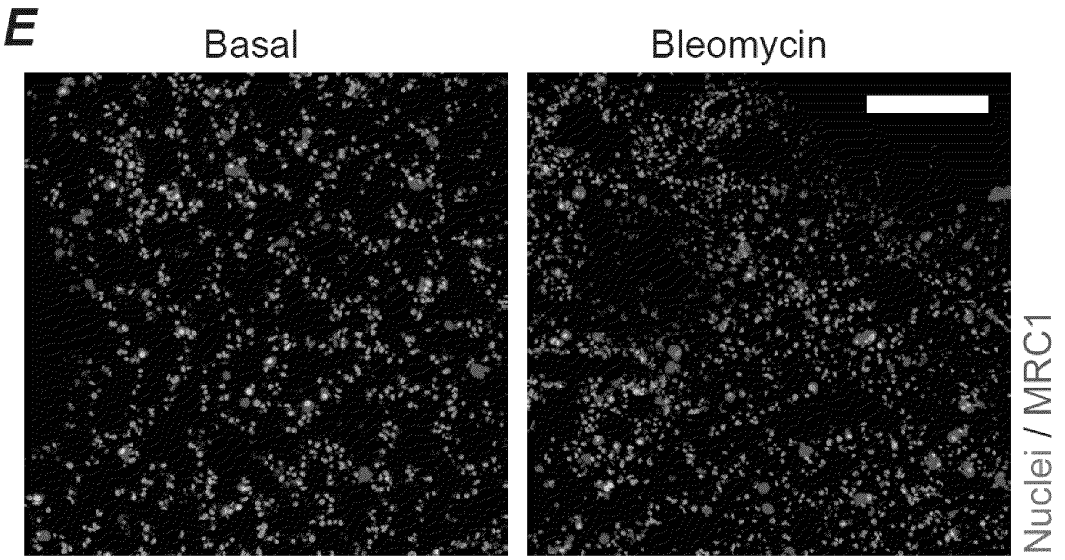


Figure 22 cont.

F

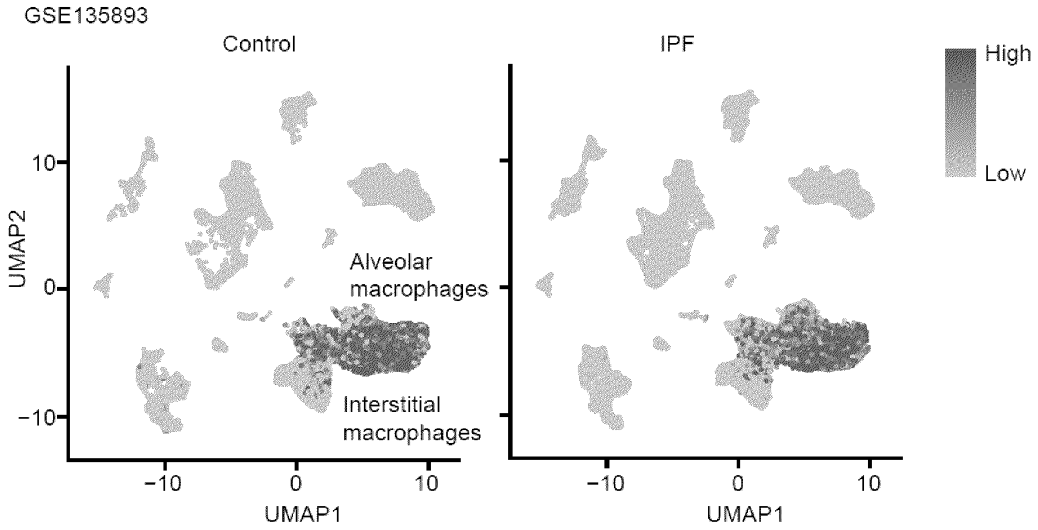
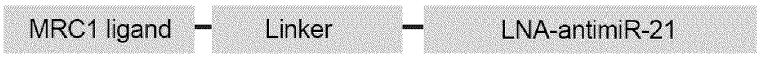


Figure 23

A


MRC1 ligand
Linker
LNA-antimiR-21

B

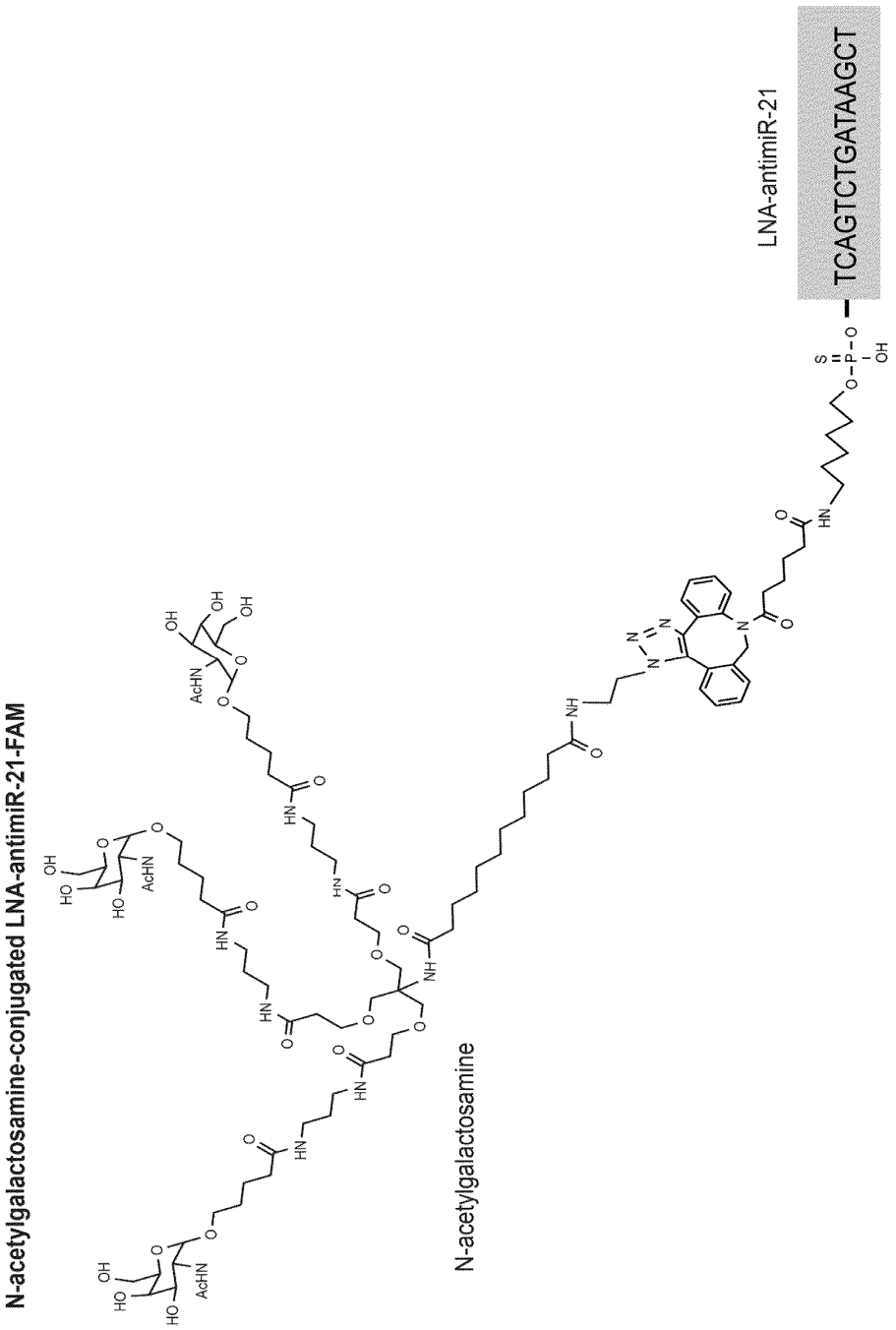

N-acetylgalactosamine-conjugated LNA-antimiR-21-FAM
LNA-antimiR-21
TCAGTCTGATAAGCT
N-acetylgalactosamine

Figure 23 B continued

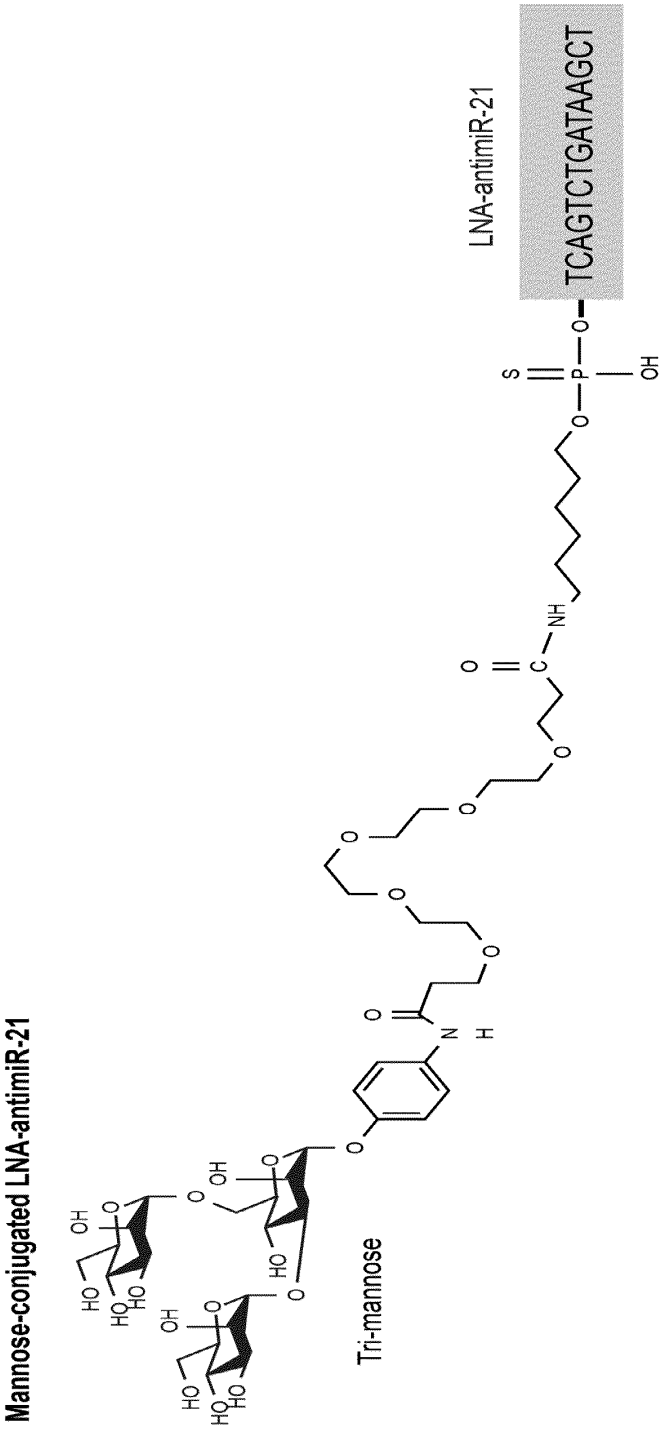


Figure 24

A

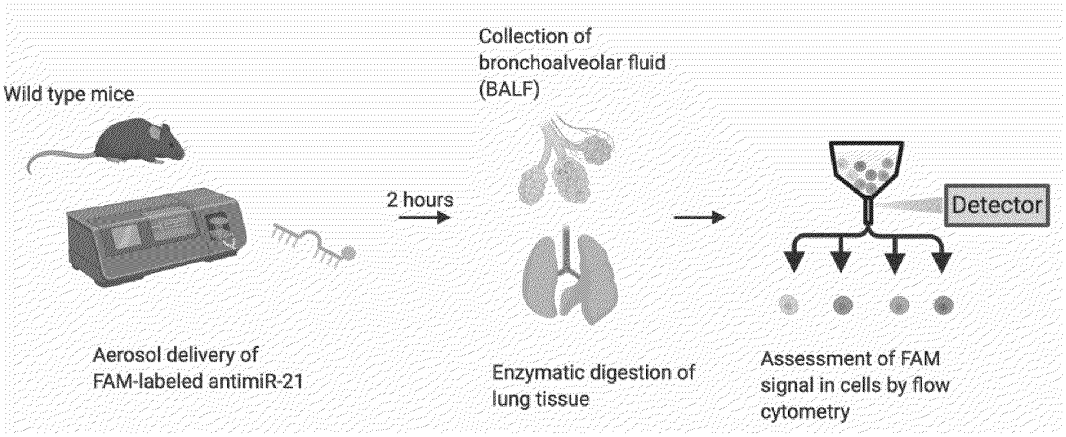
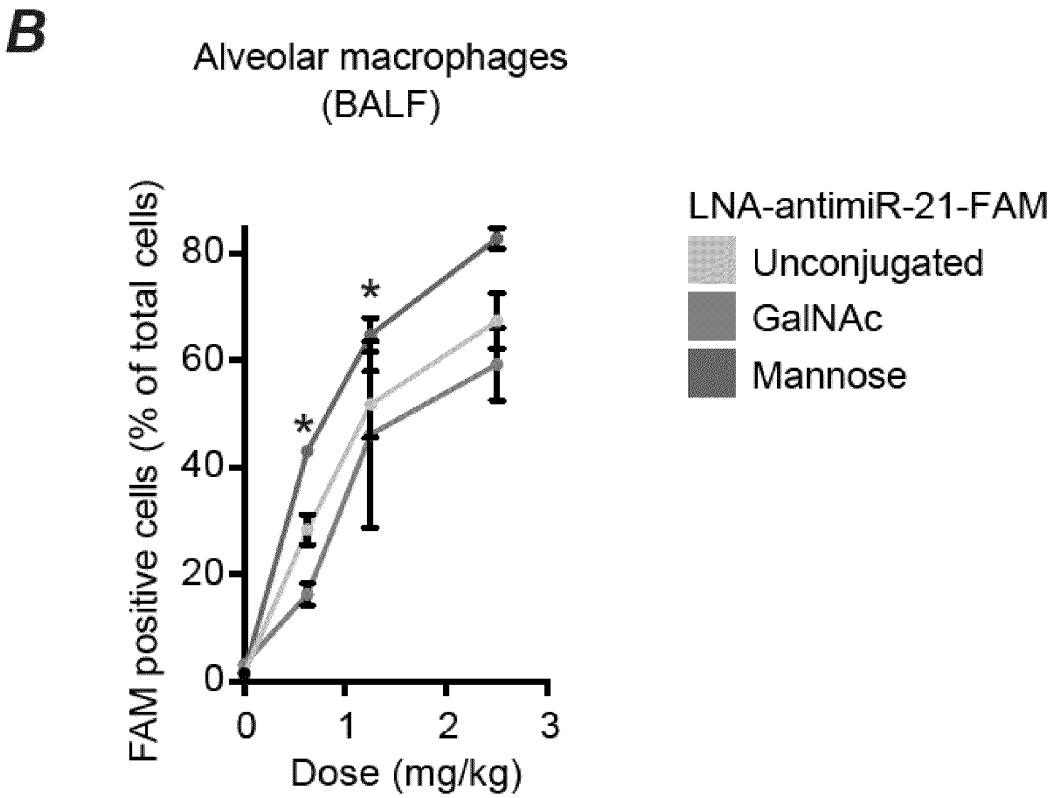

Wild type mice
Aerosol delivery of FAM-labeled antimiR-21
2 hours
Collection of bronchoalveolar fluid (BALF)
Enzymatic digestion of lung tissue
Detector
Assessment of FAM signal in cells by flow cytometry

Figure 24 cont.



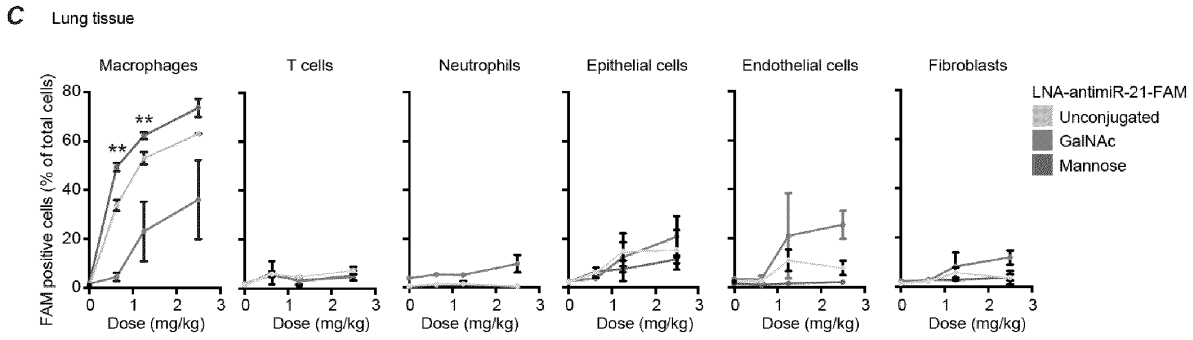


Figure 25

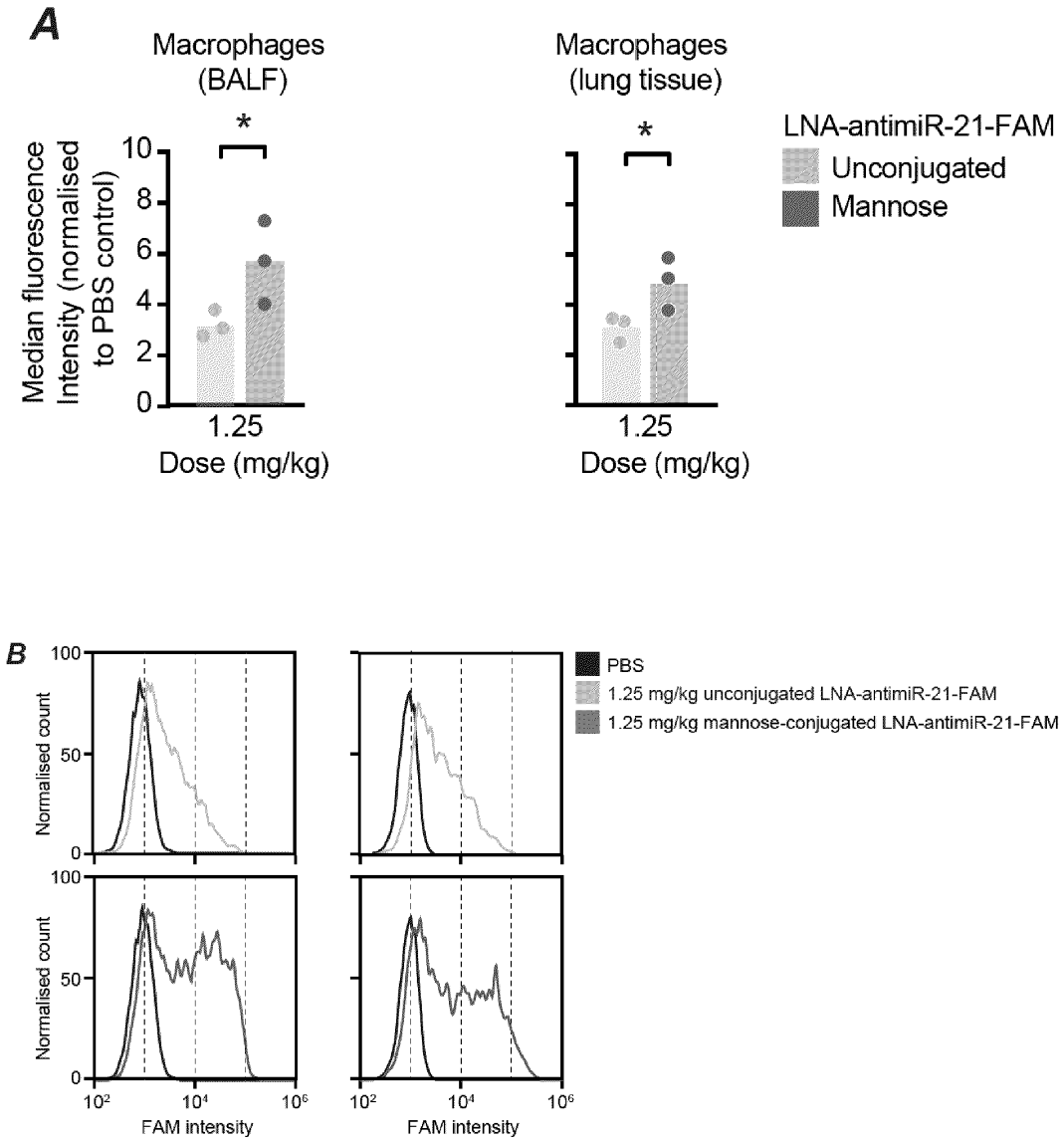

A
Macrophages (BALF)
Macrophages (lung tissue)
Median fluorescence Intensity (normalised to PBS control)
10
8
6
4
2
0
*
*
1.25
Dose (mg/kg)
1.25
Dose (mg/kg)
LNA-antimiR-21-FAM
Unconjugated
Mannose
B
100
50
0
Normalised count
100
50
0
Normalised count
10^2
10^4
10^6
FAM intensity
10^2
10^4
10^6
FAM intensity
PBS
1.25 mg/kg unconjugated LNA-antimiR-21-FAM
1.25 mg/kg mannose-conjugated LNA-antimiR-21-FAM

Figure 26

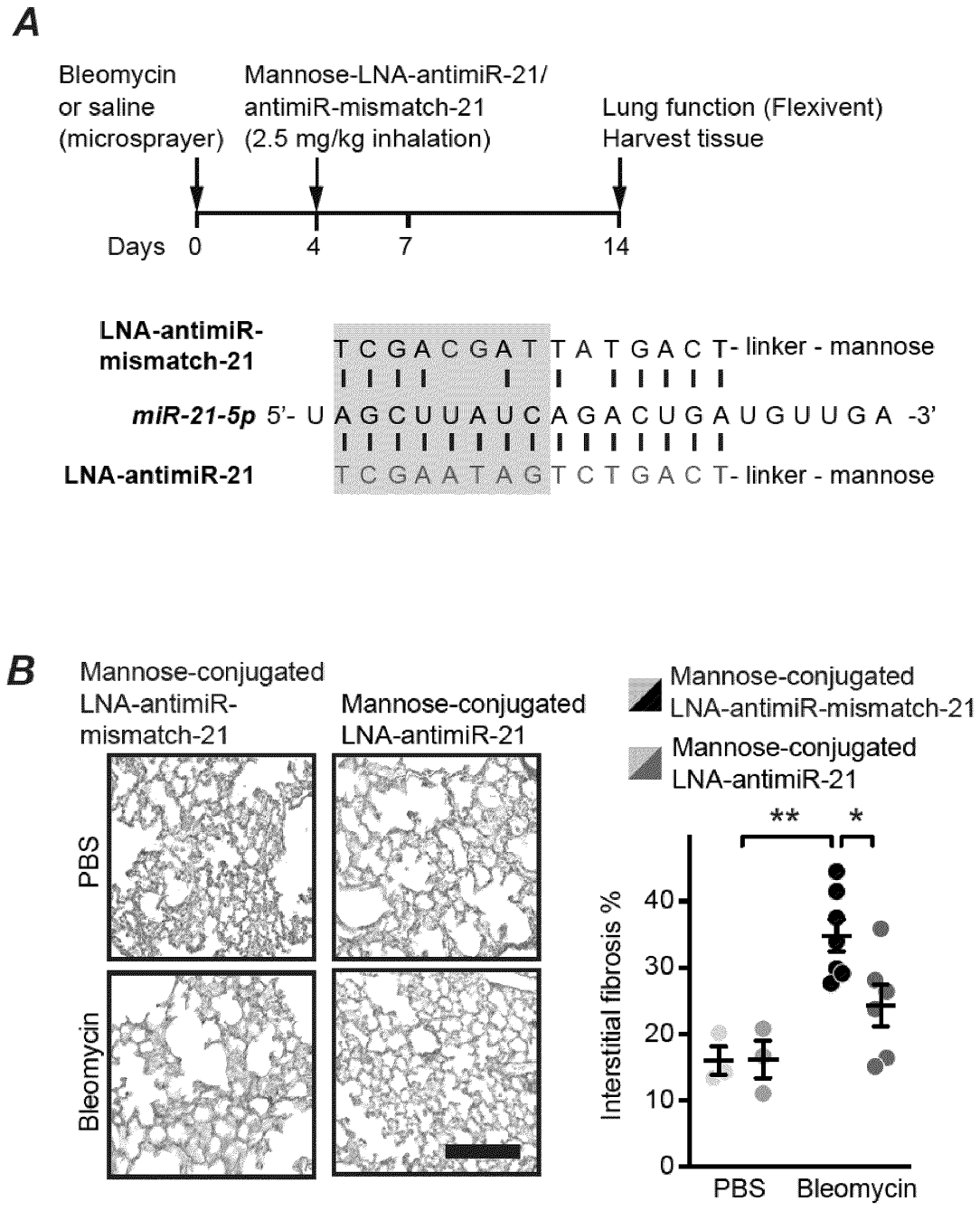

A
Bleomycin
or saline
(microsprayer)
Mannose-LNA-antimiR-21/
antimiR-mismatch-21
(2.5 mg/kg inhalation)
Lung function (Flexivent)
Harvest tissue
Days 0 4 7 14
LNA-antimiR-mismatch-21
T C G A C G A T T A T G A C T- linker - mannose
miR-21-5p 5'- U A G C U U A U C A G A C U G A U G U U G A -3'
LNA-antimiR-21
T C G A A T A G T C T G A C T- linker - mannose
B
Mannose-conjugated LNA-antimiR-mismatch-21
Mannose-conjugated LNA-antimiR-21
PBS
Bleomycin
Mannose-conjugated LNA-antimiR-mismatch-21
Mannose-conjugated LNA-antimiR-21
**
*
Interstitial fibrosis %
0
10
20
30
40
PBS
Bleomycin

Figure 26 cont.

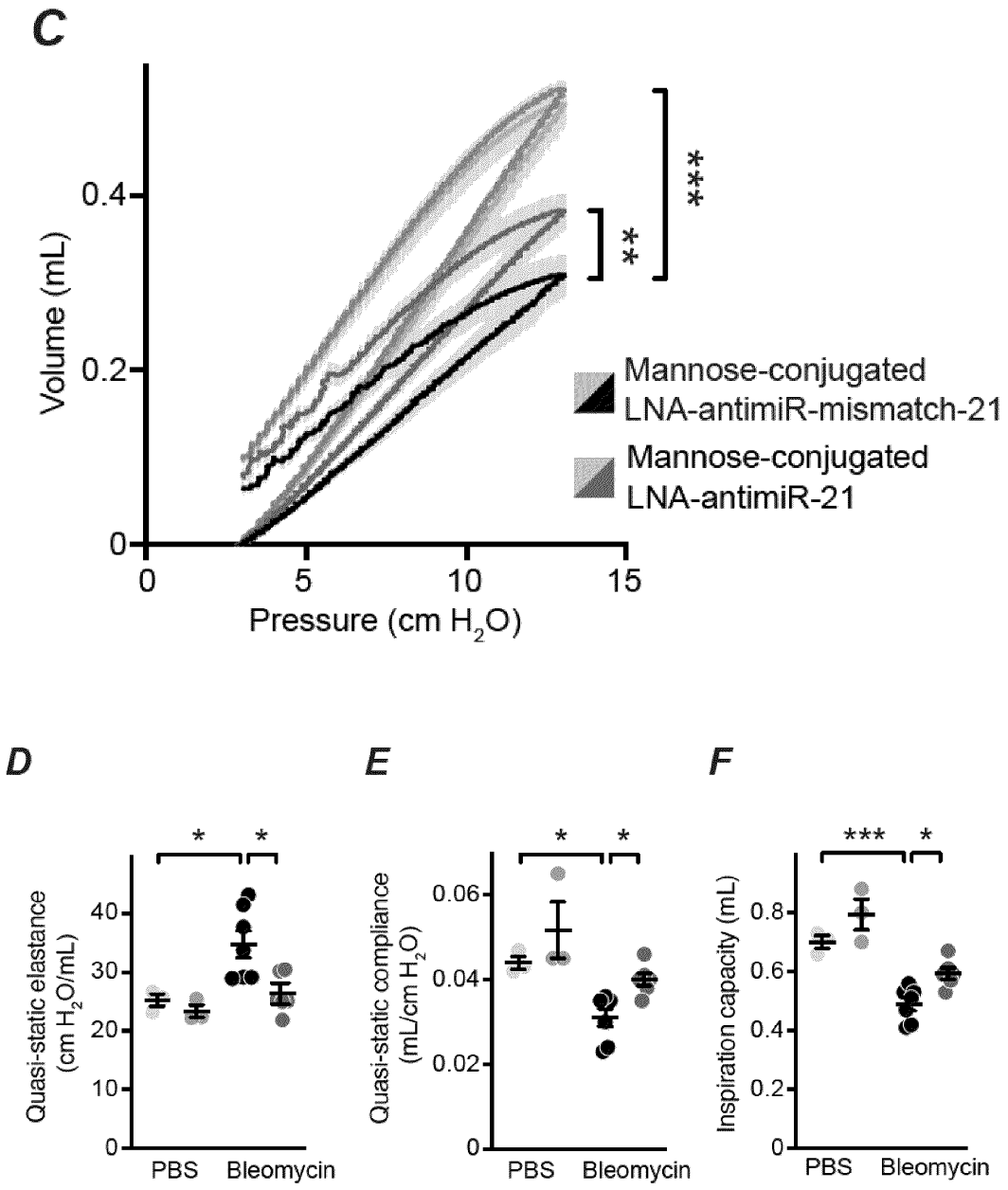


Figure 27

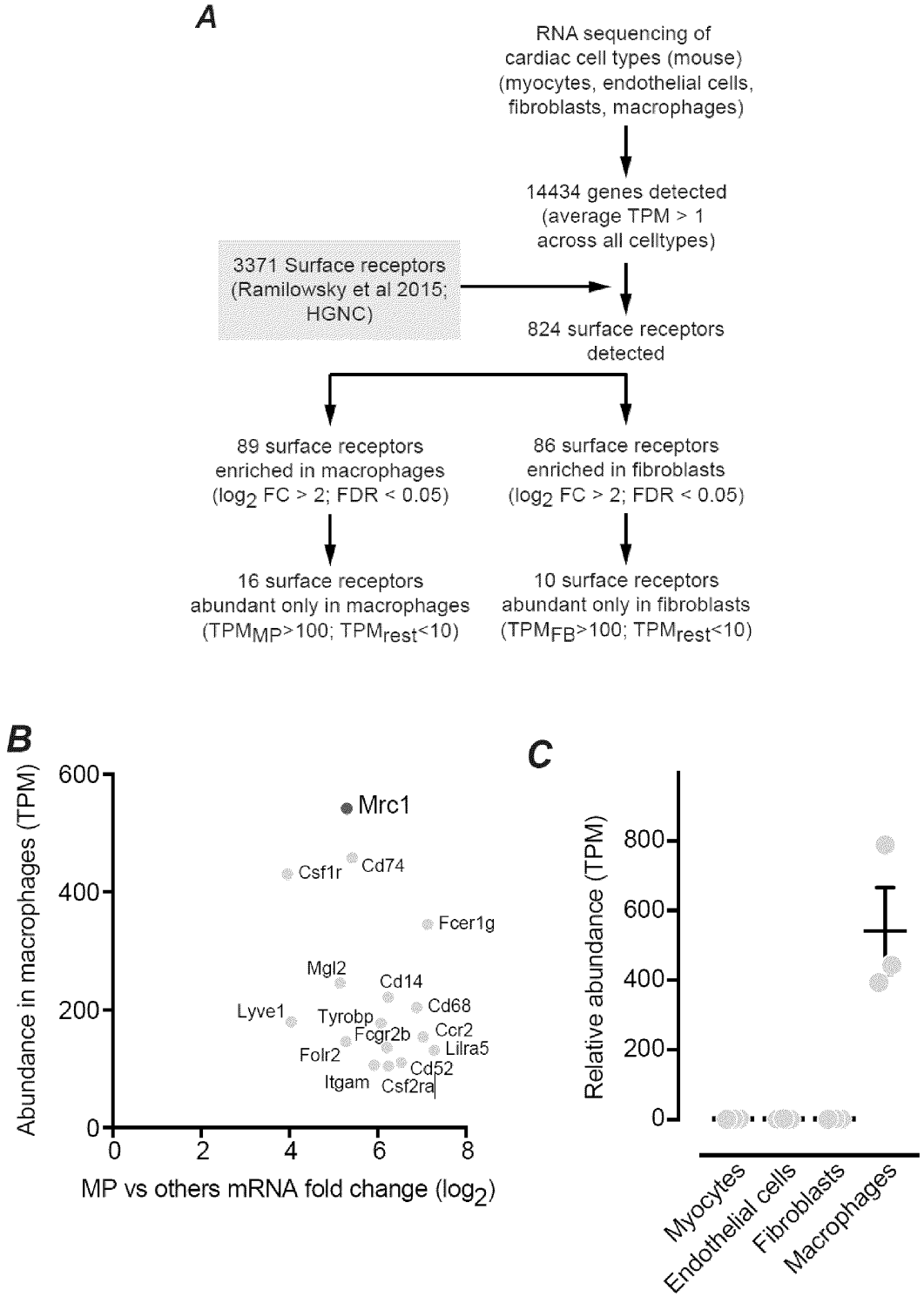

A
RNA sequencing of cardiac cell types (mouse) (myocytes, endothelial cells, fibroblasts, macrophages)
14434 genes detected (average TPM > 1 across all celltypes)
3371 Surface receptors (Ramilowsky et al 2015; HGNC)
824 surface receptors detected
89 surface receptors enriched in macrophages (log_2 FC > 2; FDR < 0.05)
86 surface receptors enriched in fibroblasts (log_2 FC > 2; FDR < 0.05)
16 surface receptors abundant only in macrophages (TPM_{MP}>100; TPM_{rest}<10)
10 surface receptors abundant only in fibroblasts (TPM_{FB}>100; TPM_{rest}<10)
B
Abundance in macrophages (TPM)
MP vs others mRNA fold change (log_2)
Mrc1
Csf1r
Cd74
Fcer1g
Mgl2
Cd14
Cd68
Lyve1
Tyrobp
Fcgr2b
Ccr2
Folr2
Lilra5
Cd52
Itgam
Csf2ra
C
Relative abundance (TPM)
Myocytes
Endothelial cells
Fibroblasts
Macrophages

Figure 27 cont.

