MicroRNA-144-3p targets relaxin/insulin-like family peptide receptor 1 (RXFP1) expression in lung fibroblasts from patients with idiopathic pulmonary fibrosis

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Running title: *miR-144-3p targets RXFP1 expression in IPF*

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Table S1. Baseline Characteristics of IPF Patients and HV

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Characteristic	**IPF** N=126	**HV** N=18
Age -yr	71.6 ± 8.1	63.1 ± 8.8
Male sex -%	83	62
FVC percent predicted value -	69 ± 19	98 ± 18
Bronchoalveolar Lavage		
Alveolar macrophages -%	75 ± 16	78 ± 15
Lymphocytes -%	11 ± 10	18 ± 12
Neutrophils -%	10 ± 11	3 ± 4
Eosinophils -%	4 ± 6	0 ± 1

* Plus–minus values are means ±SD. FVC denotes for forced vital capacity. HV denotes for healthy volunteers. IPF denotes for idiopathic pulmonary fibrosis.

Supplemental Figure legends

FIGURE S1. Silencing of RXFP1 by siRNA.

Donor lung fibroblasts (N=4) were incubated with RXFP1 siRNA. RXFP1 protein was silenced significantly upon treatment with SiRXFP1 compared to scramble control SiRNA. RXFP1 protein was detected using mouse anti-human RXFP1 antibody purchased from Santa Cruz biotech (sc-293228) at 1:500. Goat anti-mouse-HRP secondary antibody was used for detection.

FIGURE S2. Original uncropped blot images for Fig2D and Fig3A.

(*C*) IPF (N=3) lung fibroblasts were processed for quantitative RT-PCR for miR-144-3p at several time points following incubation with TGFβ and Actinomycin D. Data represent the relative abundance of miR-144-3p (δδCt) remaining compared to 0 hr time point, and the best fit curves for miR-144-3p decay were plotted.

FIGURE S3. Original uncropped blot image for Fig7C.

FIGURE S4. Original uncropped blot image for Fig8A.

FIGURE S5. hsa-miR-144-3p targets human NFE2L2 (Nrf2)

(***A***) The seed region of hsa-miR-144-3p predicted to target the 3' UTR of human Nrf2. There are two potential targets for miR-144-3p in 3' UTR of human Nrf2. Donor lung fibroblasts (N=4) were treated with miR-144-3p mimic or scrambled control. RNA was isolated and processed for qPCR for **(*B*)** Nrf2. Transfection of mimic resulted in significantly lower basal levels of Nrf2 mRNA in Donor lung fibroblasts compared to scrambled negative control.

FIGURE S6. Effect of IL1b on miR-144-3p and RXFP1 in lung fibroblasts
Donor and IPF lung fibroblasts were treated with IL1b (10 ng/ml) or vehicle control for 24 hours. Total RNA was isolated and processed for qPCR for miR-144-3p (*A*) Donor or (*B*) IPF lung fibroblasts. There were no significant differences in miR-144-3p levels in IL1b treated cells compared to vehicle controls in both donor and IPF lung fibroblasts (*Student t-test*, N=3). Treatment of Donor and IPF lung fibroblasts with the IL1b has no significant effect on RXFP1 expression (*student t-test*, N=3).

FIGURE S7. Transfection efficiency of 5' FAM labeled miR-144-3p AntagomiR in lung fibroblasts.

Primary lung fibroblasts were transfected on days 0 and 1 as described in Experimental Procedures with (A) 5' FAM-labeled complexes or (B) unlabeled controls. Cells were counterstained with DAPI. Yellow arrows identify the punctate positive staining with the labeled complexes. FAM+ cells were counted as the percentage of total cells in the field (Magnification x600, N=3).

FIGURE S8. Original uncropped blot images for Fig8A for fibroblast lines used in the densitometry analyses

FIGURE S1

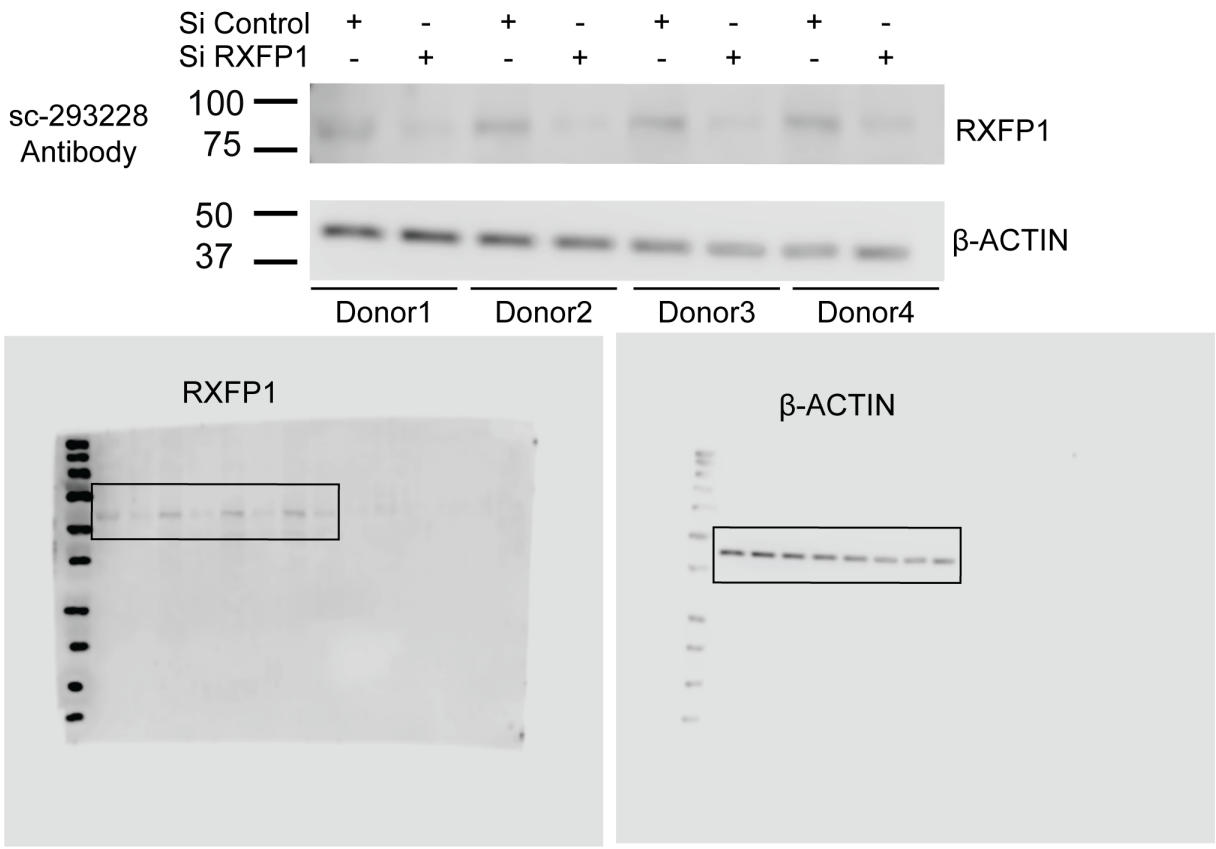

Si Control + - + - + - + -
Si RXFP1 - + - + - + - +
sc-293228 Antibody
100
75
50
37
RXFP1
β-ACTIN
Donor1
Donor2
Donor3
Donor4
RXFP1
β-ACTIN

FIGURE S2

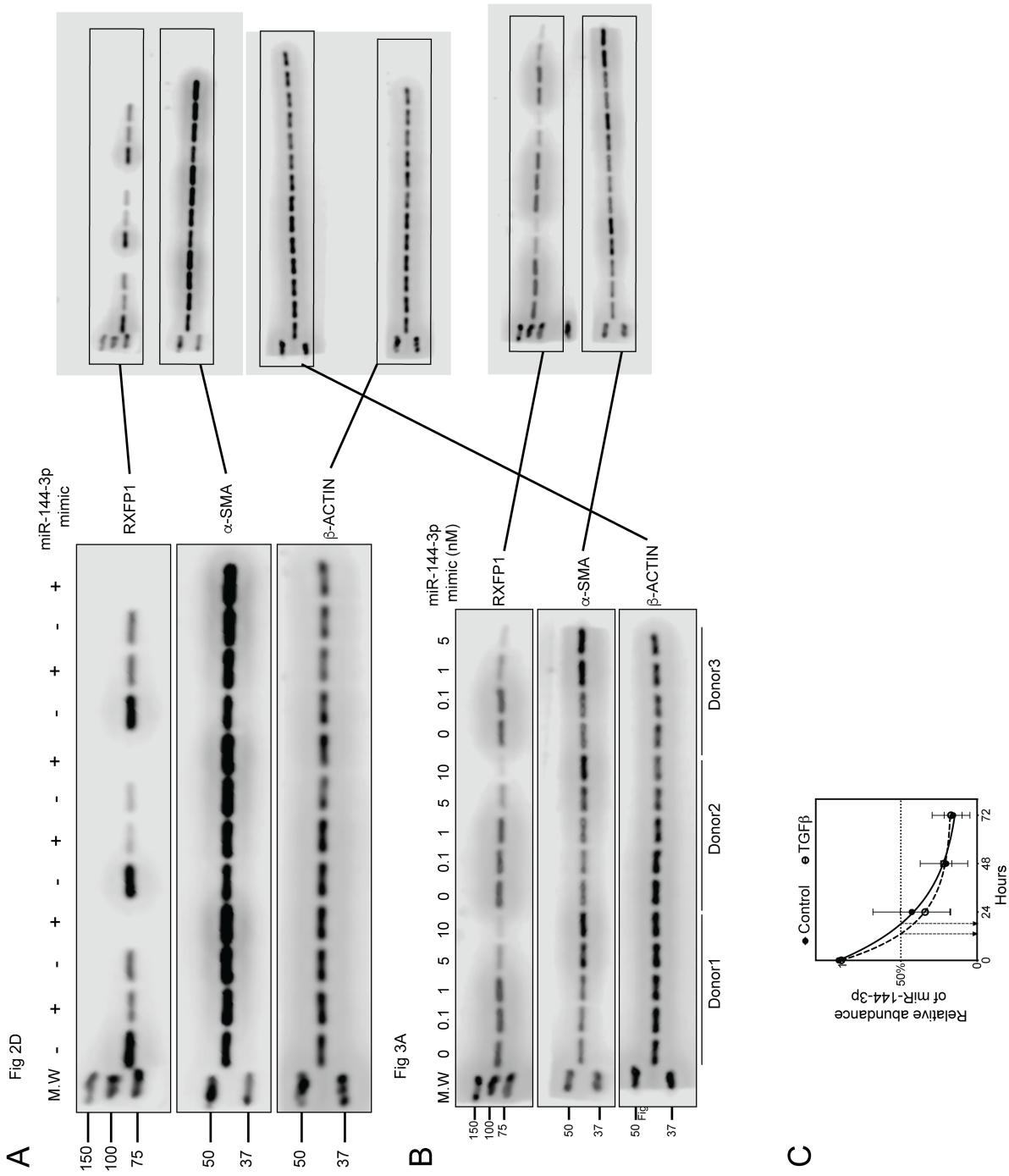

A
Fig 2D
M.W
miR-144-3p mimic
RXFP1
α-SMA
β-ACTIN
150
100
75
50
37
B
Fig 3A
miR-144-3p mimic (nM)
Donor1
Donor2
Donor3
C
Control
TGFβ
Relative abundance of miR-144-3p
50%
Hours
0
24
48
72

FIGURE S3

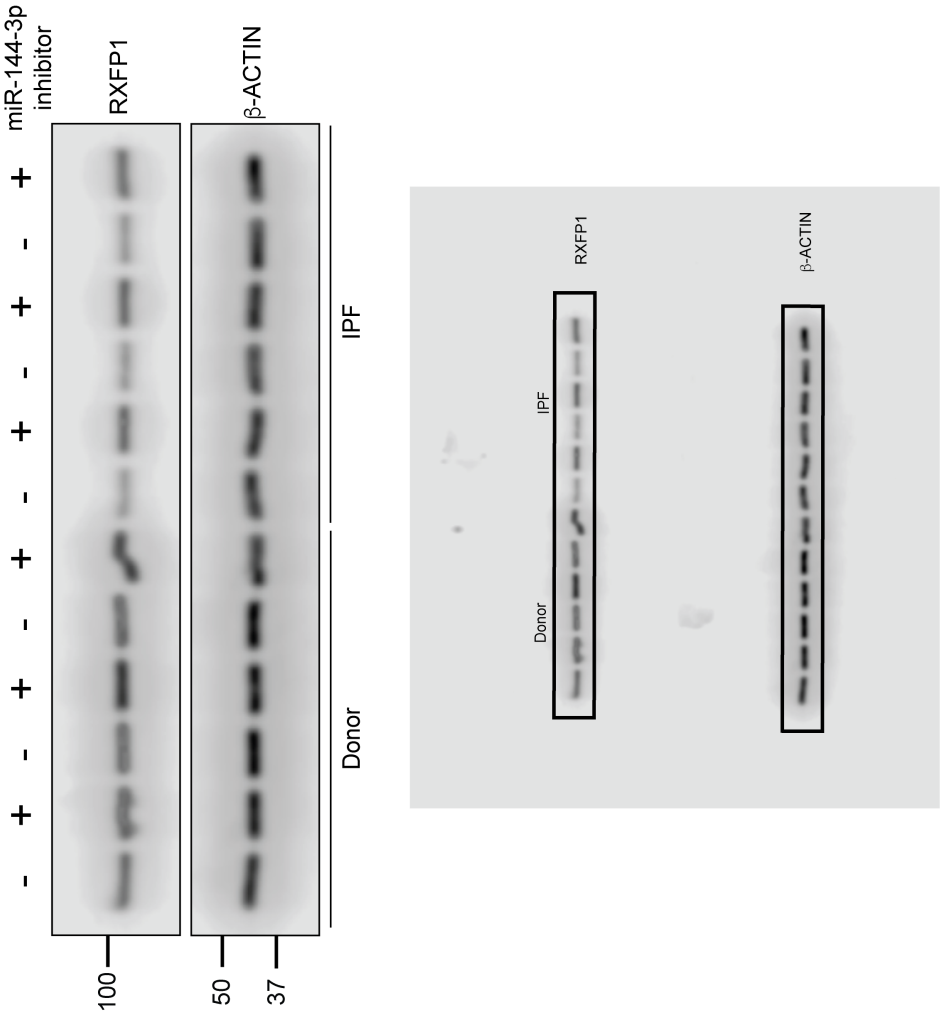


Fig 7C

FIGURE S4

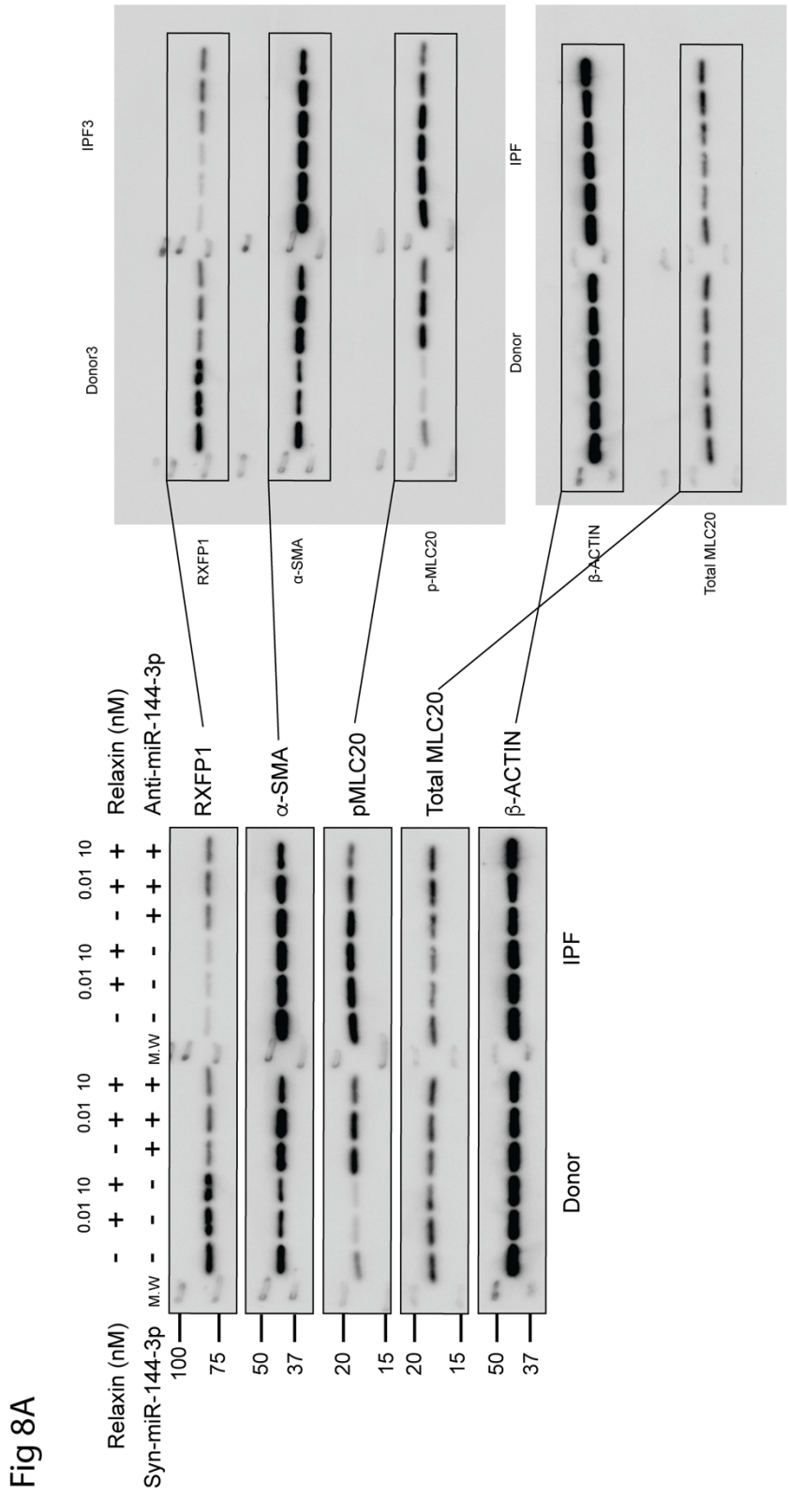
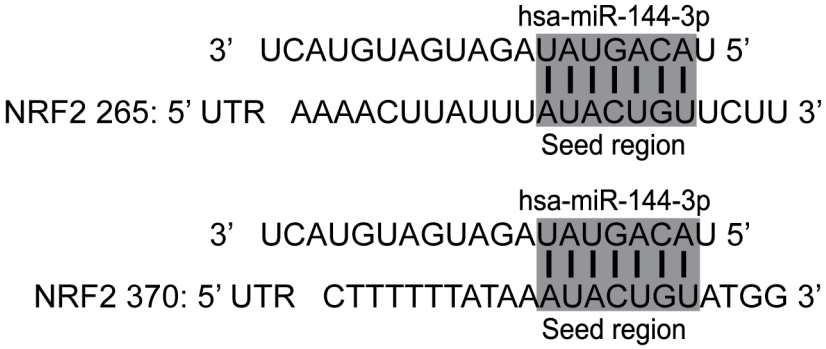


Fig 8A
Relaxin (nM)
Syn-miR-144-3p
M.W
0.01 10
0.01 10
0.01 10
0.01 10
Relaxin (nM)
Anti-miR-144-3p
100
75
50
37
20
15
20
15
50
37
RXFP1
α-SMA
pMLC20
Total MLC20
β-ACTIN
Donor
IPF
Donor3
IPF3
Donor
IPF
RXFP1
α-SMA
p-MLC20
β-ACTIN
Total MLC20

FIGURE S5

A


hsa-miR-144-3p
3’ UCAUGUAGUAGAUAUGACAU 5’
NRF2 265: 5’ UTR AAAACUUAUUUAUACUGUUCUU 3’
Seed region
hsa-miR-144-3p
3’ UCAUGUAGUAGAUAUGACAU 5’
NRF2 370: 5’ UTR CTTTTTTATAAAUACUGUATGG 3’
Seed region

B

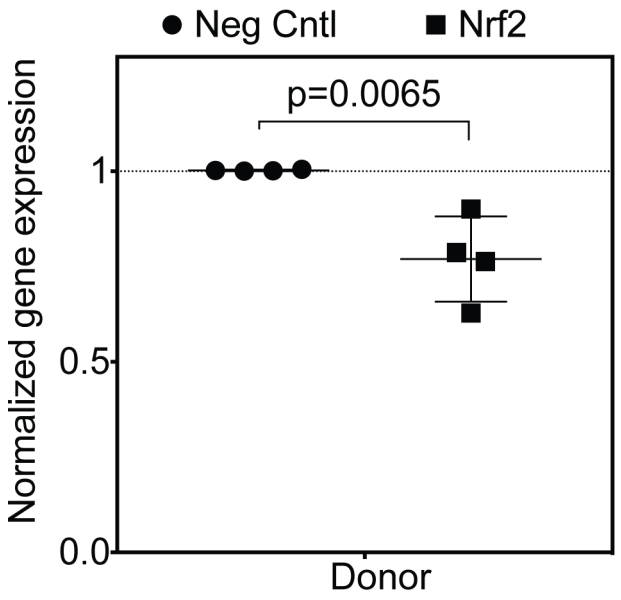

Neg Cntl
Nrf2
p=0.0065
Normalized gene expression
1
0.5
0.0
Donor

FIGURE S6

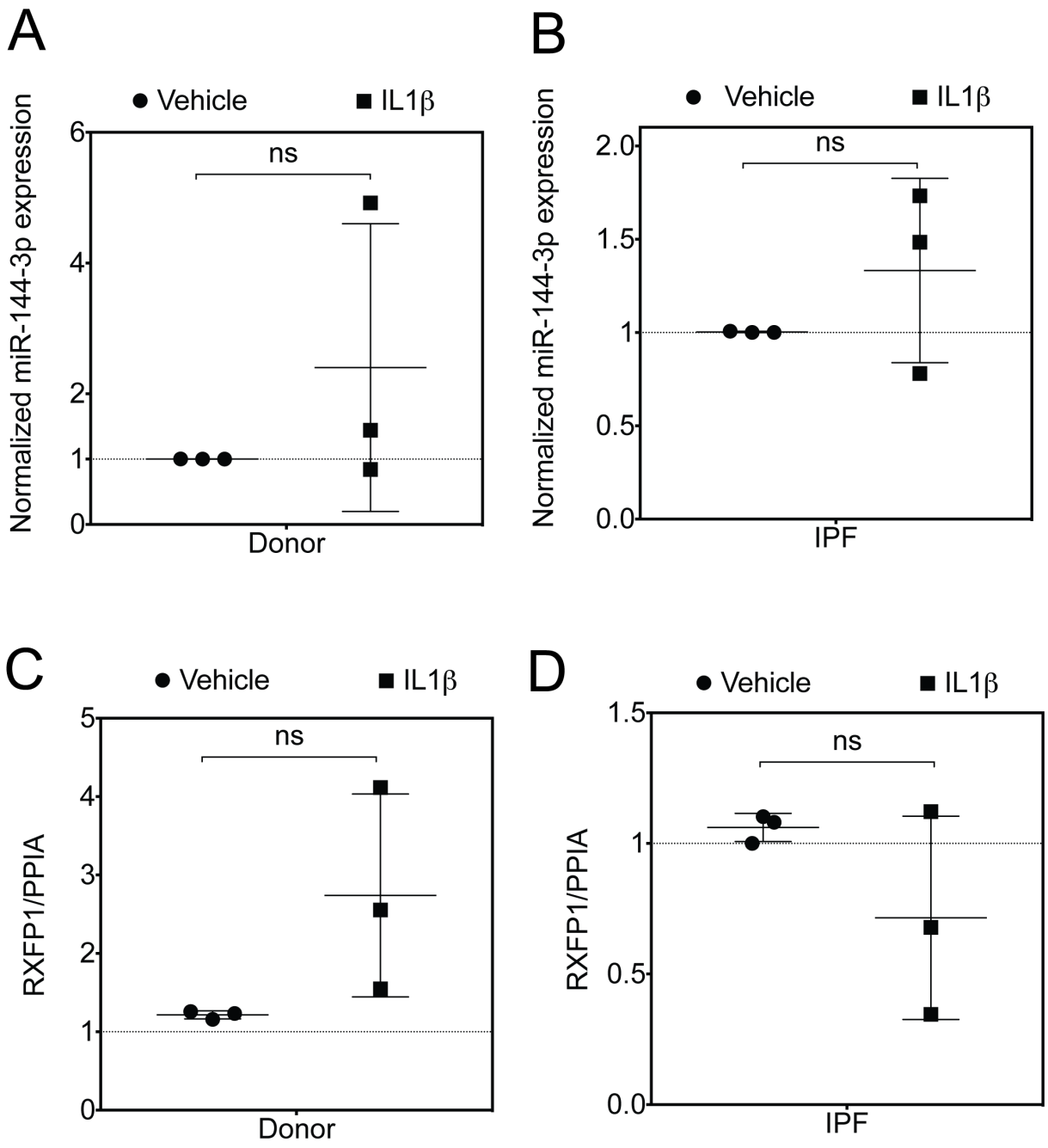

A
Vehicle
IL1β
Normalized miR-144-3p expression
6
4
2
1
0
ns
Donor
B
Vehicle
IL1β
Normalized miR-144-3p expression
2.0
1.5
1
0.5
0.0
ns
IPF
C
Vehicle
IL1β
RXFP1/PPIA
5
4
3
2
1
0
ns
Donor
D
Vehicle
IL1β
RXFP1/PPIA
1.5
1
0.5
0.0
ns
IPF

FIGURE S7

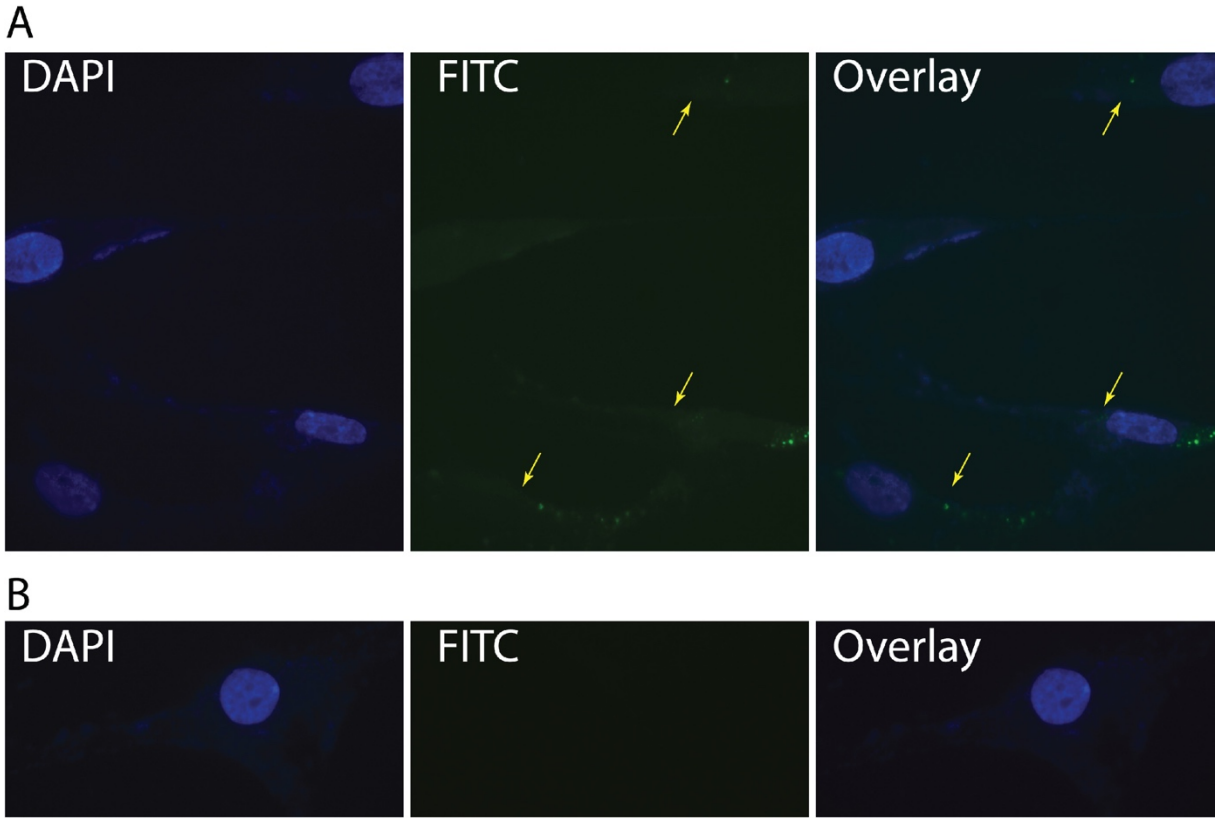


FIGURE S8

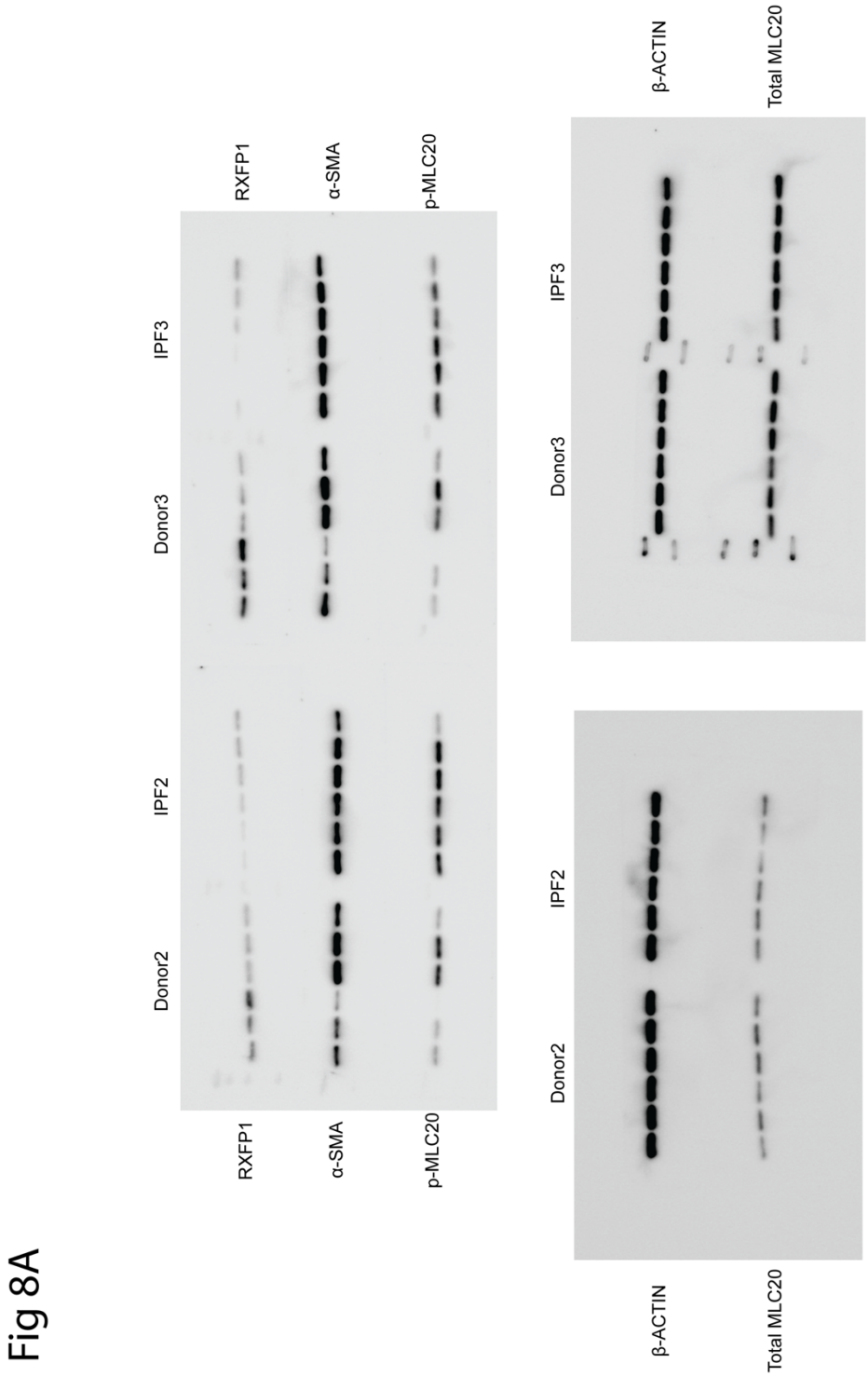


Fig 8A