Supplementary Materials

Supplementary Material and Methods

Hydroxyproline assay for collagen content. The frozen left lung lobe was weighed and dried overnight at 65°C in a heat-resistant glass tube (left lobe was always used for this technique). 1 mL of 6 N HCl /100 mg of lung tissue was added to the tube and hydrolyzed at 110°C for 18 h. One volume of 6N NaOH was added, solid particles removed by 22μm filtration and the samples incubated with 1.4% Chloramine T solution in 0.5 M sodium acetate/10% isopropanol for 10' at room temperature, Ehrlich reagent (Sigma) was added and the samples at 65°C incubated for 20'. Optical density at 550 nm was measured and hydroxyproline concentration was calculated by extrapolation from a hydroxyproline (Sigma) standard curve. Data are plotted as the total amount (μg) of hydroxyproline measured per mass of left lung lobe.

Histology and immunofluorescence. Tissue was fixed in 4% paraformaldehyde, embedded in paraffin and sections cut according to standard procedures. Lung right lobes were used for histology. For Sirius red staining, after deparaffination in decreasing alcohol concentrations (xylene, 100%-96%-70% ethanol), slides were placed in water and then stained with Direct Red 80 in Picrosirius acid (Sigma) for 1 hour at room temperature. After two washes in 0.5% Acetic Acid in dH_2O, samples were de-hydrated in 100% alcohol, incubated in xylene and mounted using permanent non-aqueous mounting media (Entellan® new. Germany).

For immunohistochemistry and immunofluorescence of paraffin-embedded mouse tissue sections, antigen retrieval was performed using citrate buffer, pH 6 in a pressure cooker. PBS supplemented with 0.1% TritonX, 0.05% Tween 0.05% and 5% BSA and 10% serum compatible with the secondary antibody was used for antibody dilution. The following antibodies were used for IHC and F-IHC: Fra-2 (CNIO mAb Unit), Fms (UBI: 06-174), Ym1 (R&D Systems: AF2446), αSMA

(Neomarker: MS-113-PO), vimentin (Cell Signaling: 5741), type VI collagen (Abcam: ab6588), S100A4 (Abcam) and SPC (Millipore: AB3786). Alexa Fluor conjugated secondary antibodies (Invitrogen) were used for fluorescence, and ABComplex kit (Vectastain) followed by peroxidase-based visualization system (3,3'-Diaminobenzidine, DAB) was used for Fra-2 immunohistochemistry. Nuclei were stained with DAPI and sections were mounted in ProlongGold anti-fade mounting media (Invitrogen: P36934). For confocal, TMA acquisition was performed with a TCS SP5 confocal microscope (Leica Microsystems) equipped with Leica HCS-A and the custom made iMSRC software (1). Final images were acquired with a 20x HCX PL APO 0.7 N.A. dry objective. Images of Fra-2 and Ym1 staining in Fra-2^{tg} mouse lungs were quantified by using Definiens Developer v2.5 (Definiens, Germany) with a custom made ruleset for Cell detection and intensity quantification. For ColVI quantification, pictures from 8 fields from alveolar areas of the lungs (to exclude big bronchi/arterial structures, rich in ColVI) were taken under a 10x objective, all at the same conditions. ImageJ software (2) was used to quantify DAPI nuclei number and ColVI area.

For immunohistochemistry in human tissues, an automated immunostaining platform was used (Autostainer Link, Dako and Ventana Discovery ULTRA, Roche). First round, antigen retrieval was performed first with low pH buffer; endogenous peroxidase was blocked (peroxide hydrogen at 3%) and slides were then incubated with the appropriate primary antibody as detailed: rat monoclonal anti-Fra2 (REY286; 1/400; Monoclonal Antibodies Core Unit, AM (286)). After the primary antibody, slides were incubated with the corresponding secondary antibodies when needed (rabbit anti rat, Abcam) and visualization systems (Novolink Polymer anti-Rabbit, Leica) conjugated with horseradish peroxidase. Immunohistochemical reaction was developed using 3, 30-diaminobenzidine tetrahydrochloride (DAB) (Dako).

Second round, antigen retrieval was performed with high pH buffer (CC1 mild); endogenous peroxidase was blocked (peroxide hydrogen at 3%) and slides were then incubated with the appropriate primary antibody as detailed: rabbit polyclonal anti-Collagen VI (1/300; Abcam, ab6588) and mouse monoclonal anti-CD68 (KP1; Ready to use, Dako, IR609). Slides were then incubated with the corresponding secondary antibodies (rabbit anti mouse, Abcam) and visualization systems (OmniMap anti-Rabbit, Ventana, Roche) conjugated with horseradish peroxidase. Immunohistochemical reaction was developed using purple chromogen (Discovery Purple Kit, Ventana, Roche) and blue chromogen (Discovery Teal Kit, Ventana, Roche), respectively. Nuclei were counterstained with Harrys's hematoxylin. Finally, the slides was dehydrated, cleared and mounted with a permanent mounting medium for microscopic evaluation. The three antibodies were performed consecutive; first Fra-2 in Autostainer Link and them, Collagen VI and CD68 in Discovery ULTRA. It's necessary to neutralize HRP using peroxide hydrogen between second and third antibody. Positive control sections known to be primary antibody positive, were included for each staining run.

Flow cytometry. Lung single-cell suspension was obtained by a two-step enzymatic digestion with Dispase (10 U/ml. Gibco) after perfusion of mouse lungs and filtered through a 70 μm cell strainer. After lysing red blood cells (Sigma), cells were incubated with FC-Block (BD) and the following antibodies against immune cell surface markers: CD206-PE, CD31-PerCPCy5.5, CD326-AF647, CD36-PE, CD86-PE, F4/80-AF647, Ly6G-PerCPCy5.5, MHCII / I-A/I-E-PE (BioLegend), B220/CD45R-PE, CD11b-PECy7, CD11b-PerCPCy5.5, CD11c-PE (BD Pharmingen), CD11b-BUV395 (BD Horizon), CD140a-PE (Thermofisher), CD29-PE (Miltenyi Bioscience), CD73-PECy7 (Thermo Scientific), siglecF-APCCy7 (BD Bioscience), CD45-PECy7 (EBioscience). Data were acquired on a BD LSRII Fortessa and analyzed using FlowJo 9.5.3. Cell

sorting was performed in MoFlow® Astrios (Beckman Coulter) and BD FACSAria™ III (BD). In all analysis, events were subsequently selected first on the time gate to avoid fluidic perturbations, then on the size to exclude doublets, and finally on the negative LIVE/DEAD™ Fixable Aqua Dead Cell Stain (Thermofisher) or DAPI (Sigma) uptake of living cells. Cell debris was excluded by FSC/SSC. Specific experiment gating strategies for analysis and sorting are defined in Fig. 4G and figs. S1H, S1E and S5I. Conventional macrophages were directly sorted into TrizolLS for RNA isolation (Life Technologies).

Protein isolation, Western blot and ELISA. Tissue was disrupted using a Precellys® device (Bertin Technologies) in RIPA buffer (50mM Tris ph7.4, 150mM NaCl, 1% NP40, 0.5% Na-deoxycholate,0.1% SDS). OCT-embedded human lung tissue was washed with cold HBSS and centrifuged at 10000g, three times. Then, pellet was lysed with SDS buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS and 10% glycerol). Protein lysates were quantified by using BCA protein assay reagent (Thermo Scientific). For Western blot analysis, 20-60μg protein per sample was loaded. Membranes were blocked with 5% BSA or non-fat dry milk in PBS-T (1% Tween 20). The following primary antibodies were used: Fra-2 (CNIO mAb Unit), fibronectin, type I pro-collagen (Santa Cruz: sc-8787), type I collagen (Santa Cruz: sc-28654), vinculin (Sigma: V 9131), alpha-smooth muscle actin (Neomarker: MS-113-PO), periostin (R&D systems: BAF2955) Osteopontin (R&D systems: AF808), COL6A1 (Santa Cruz: sc-377143), FRA-2 (Santa Cruz: sc-166102), ACTIN (Sigma: A4700), phospho-Smad2 (Cell signaling: 3101) and Smad2 (Cell signaling: 3103). Blots were incubated with the appropriate secondary horseradish peroxidase-coupled antibody (GE) and developed using the Luminata Western HRP Substrate (Millipore) and Amersham ECL Hyperfilms (GE Healthcare) or a ChemiDoc XRS+ imaging system with Image

Lab image acquisition and analysis software (Bio-Rad). Mouse IL-4 ELISA was performed using 50 µg of lung protein lysate and following the manufacturer's instruction (R&D: M4000B).

DNA/RNA isolation and PCR. Total RNA from lung tissue and cell cultures was isolated using Trizol (Sigma), complementary DNA was synthesized using Ready-To-Go-You-Prime-First-Strand Beads (GE Healthcare), RT-qPCR using GoTaq RT-qPCR Master Mix (Promega) and Eppendorf fluorescence thermocyclers (Eppendorf), all following manufacturers' instructions. The $2^{\Delta\Delta CT}$ method was used to quantify amplified fragments. Expression levels were normalized using at least one housekeeping gene (*Rps29* or *Rpl4*). DNA was isolated by digestion of bone marrow with proteinase K (400 µg/mL) and subsequent precipitation with high salt (6M NaCl) and isopropanol. BM DNA was subjected to PCR with ColVI$^{-/-}$ specific primers and the products were separated in a 2% agarose gel to identify knock-out bands. All primer sequences are listed in **Supp Table S1**.

RNA-seq and data analysis. 1 µg of total RNA was used. Sample RNA Integrity Numbers were between 7.3 and 9 (average 8.3) when assayed on an Agilent 2100 Bioanalyzer. PolyA+ fraction was purified and randomly fragmented, converted to double stranded cDNA and processed through subsequent enzymatic treatments of end-repair, dA-tailing, and ligation to adapters as in Illumina's "TruSeq Stranded mRNA Sample Preparation Part # 15031047 Rev. D" kit (this kit incorporates dUTP during 2nd strand cDNA synthesis, which implies that only the cDNA strand generated during 1st strand synthesis is eventually sequenced). Adapter-ligated library was completed by PCR with Illumina PE primers (8 cycles). The resulting purified cDNA library was applied to an Illumina flow cell for cluster generation and sequenced on an Illumina instrument by following manufacturer's protocols. RNA-seq reads quality was checked with FastQC (Andrews). Fastq files (3) were randomly down-sampled to generate datasets with similar number of reads in all the

samples. Differentially expressed genes were obtained using the Nextpresso pipeline. Sequencing quality was analyzed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/); reads were aligned to the mouse genome (GRCm38/mm10) using TopHat-2.0.10 (ref), Bowtie 1.0.0 (http://bowtie-bio.sourceforge.net/index.shtml) and Samtools 0.1.19.0 (http://samtools.sourceforge.net/); and transcripts assembly, abundances estimation and differential expression were calculated with Cufflinks 2.2.1 (http://cole-trapnelllab.github.io/cufflinks/). The estimated significance level (P value) was corrected to account for multiple hypotheses testing using Benjamini and Hochberg False Discovery Rate (FDR) adjustment. Genes with FDR less than or equal to 0.05 were selected as differentially expressed.

Gene set enrichment analysis (GSEA) of genes differentially expressed was performed using the GSEA software, Version 2.0.6, available at the Broad Institute (http://www.broad.mit.edu/gsea/). GSEA was performed to analyze the enrichment of the gene sets following the developer's protocol (4) and using pathway annotations from Reactome, Biocarta and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. All differentially expressed genes were ranked according to t-statistic and the ranked file was used as input for the enrichment analysis. All basic and advanced fields were set to default and only those gene sets significantly enriched at a False Discovery Rate (FDR) q-values < 0.25 where considered. Web tool DeconvoluteME from GlioVis (5) was used for deconvoluting gene expression profiles into cell-type-specific subprofiles .

Two public curated data bases on gene expression analysis from human lung tissues from lung fibrosis patients and controls were explored for *FOSL2*, *COL1A2*, *COL6A1*, *COL6A2* and *COL6A3* gene expression (Lung Genomics Research Consortium (LGRC) database: GSE47460; and GDS1252). Gene expression values were subjected to comparative analysis within data bases.

Proteomics analysis of Exosomes. Bronchioalveolar lavage fluid (BALF) was obtained from euthanized and subsequently tracheotomized mice by washing the lungs three times with 1 mL of cold and sterile PBS until recovering a total of 3 mL of BALF, which was then centrifuge for removing the cells. Individual mouse BALF were analyzed with a NanoSight NS500 (Malvern, UK) to characterize the number and size of the vesicles. Samples from individual mice were pooled in ultracentrifuge polycarbonate tubes (Beckman: 355645) and subjected to ultra-centrifugation at 100000 g for 1h. Pellet was resuspended with PBS to wash soluble factors and samples were subjected to a second ultra-centrifugation at 100000 g. Pellet was resuspended in PBS and one sample was taken for Nanosight analysis.

Proteins were solubilized using 8 M urea in 100 mM Tris-HCl pH 8.0. Samples (10 µg) were digested by means of the standard FASP protocol (6). Briefly, proteins were reduced (10 mM DTT, 60 min, RT), alkylated (55 mM IAA, 20 min in the dark, RT) and sequentially digested with Lys-C (Wako) (protein:enzyme ratio 1:50, o/n at RT) and trypsin (Promega) (protein:enzyme ratio 1:50, 6 h at 37 °C). Resulting peptides were desalted using C18 stage-tips.

LC-MS/MS was done by coupling a nanoLC-Ultra 1D+ system (Eksigent) to an Impact mass spectrometer (Bruker) via a Captivespray source (Bruker) supplemented with a nanoBooster operated at 0.2 bar/min with isopropanol as dopant. Peptides were loaded into a trap column (NS-MP-10 BioSphere C18 5 µm, 20 mm length, Nanoseparations) for 10 min at a flow rate of 2.5 µl/min in 0.1% FA. Then peptides were transferred to an analytical home-made column (ReproSil Pur C18-AQ 3 µm, 500 mm length and 0.075 mm ID, Dr. Maisch) and separated using a 128 min effective linear gradient (buffer A: 4% ACN, 0.1% FA; buffer B: 100% ACN, 0.1% FA) at a flow rate of 300 nL/min. The gradient used was: 0-2 min 2% B, 2-119 min 20% B, 119-129 min 34% B 130-140 min 98% B, 141-145 min 2% B. The peptides were electrosprayed (1.35 kV) into the

mass spectrometer with a heated capillary temperature of 180 °C. The mass spectrometer was operated in a data-dependent mode (130-1600 m/z), with an automatic switch between MS and MS/MS scans using a MS cycle time of 7 sec (threshold signal ≥ 500 counts, z ≥ 2 and m/z ≥ 350). An active exclusion of 30 sec was used. The precursor intensities were re-evaluated in the MS scan (n) regarding their values in the previous MS scan (n-1). Any m/z intensity exceeding 5 times the measured value in the preceding MS scan was reconsidered for MS/MS. Peptides were isolated using a 2 Th window and fragmented using collision induced dissociation (CID) with a collision energy of 23-56 eV as function of the m/z value.

Raw files were processed with MaxQuant (v 1.5.1.2) using the standard settings against a mouse protein database (UniProtKB/Swiss-Prot and TrEMBL, August 2016, 43,965 sequences) supplemented with contaminants. Label-free quantification was done with match between runs (match window of 0.7 min and alignment window of 20 min). Carbamidomethylation of cysteines was set as a fixed modification whereas oxidation of methionines and protein N-term acetylation as variable modifications. Minimal peptide length was set to 7 amino acids and a maximum of two tryptic missed-cleavages were allowed. Results were filtered at 0.01 FDR (peptide and protein level). Afterwards, the "proteinGroup.txt" file was loaded in Excel for further analysis. GSEA analysis on cell type-specific subprofiles was perform based on published data sets (7).

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (8) partner repository with the dataset identifier PXD007194.

Vascular leakage assay. 200 µL of Evans blue (0.5% in PBS sterile-filtered. Sigma) was slowly injected in the mouse tail vein and mice observed for the following 30'. Mice were euthanized a after 2 hours and the thoracic cavity photographed. Lungs were next harvested, weighed and dried. 0.5 mL Formamide (Sigma) was added and tubes incubated at 55˚C for 24 hours. Optical density

was measured in the supernatant at 610 nm. Evans blue concentration was calculated by extrapolation from an Evans blue standard curve.

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) was performed using cultured bone-marrow derived macrophages fixed in 1% formaldehyde. Nuclear lysates were obtained by lysis of cell membrane in a hypotonic buffer (50 mM, HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% Glycerol, 0.5% NP-40, 0.25% Triton-X-100) and clearance of detergents (10 mM Tris-HCL pH 8.0, 200 mM NaCl, 1mM EDTA, 0.5 mM EGTA). Nuclei were resuspended in ChIP sonication buffer (100mM NaCl, 10mM TrisHCl pH 8, 1mM EDTA, 0.5mM EGTA, 0.1%Na-Deoxycholate, 0,5% N-lauroylsarcosine). Sonication was performed in a Covaris ultrasound device. Whole nuclear extract was quantified with BCA protein assay and 0.5-1mg of protein was used for preclearing with 10µl of protein G dynabeads. Immunoprecipitation was performed with 2-5µg of antibodies coupled to protein G dynabeads against Fra-2 (CNIO Monoclonal Antibody Unit), overnight at 4ºC. Rat IgG2a was used as a negative control (Millipore). After washing once with low salt (1% Triton X-100, 0.1% SDS, 2 mM EDTA pH 8.0, 150 mM NaCl, 20 mM Tris-HCl pH 8.0), once with high salt buffer (1% Triton X-100, 0.1% SDS, 2 mM EDTA pH 8.0, 500 mM NaCl, 20 mM Tris-HCl pH 8.0), once with LiCl buffer (1% NP-40, 1% Na-Deoxycholate, 1mM EDTA, 250mM LiCl, 20mM Tris-HCl pH8.0), and once with TE Buffer (10mM Tris, 8.0, 1 mM EDTA), Protein/DNA complexes were eluted from beads and DNA was decrosslinked at 65ºC in elution buffer (1%SDS, 10mM EDTA, 50mM Tris-HCl pH 8) overnight. Eluate was treated with RNase A (Sigma) for 2h at 37ºC and with Proteinase K (VWR) for 2h at 55ºC. DNA was recovered with Phenol/Chlorophorm extraction and amplified using GoTag PCR master mix (Promega) and primers specific for AP-1 binding sites using an Ep-Realplex light cycler (Eppendorf).

References for Supplementary Material and Methods

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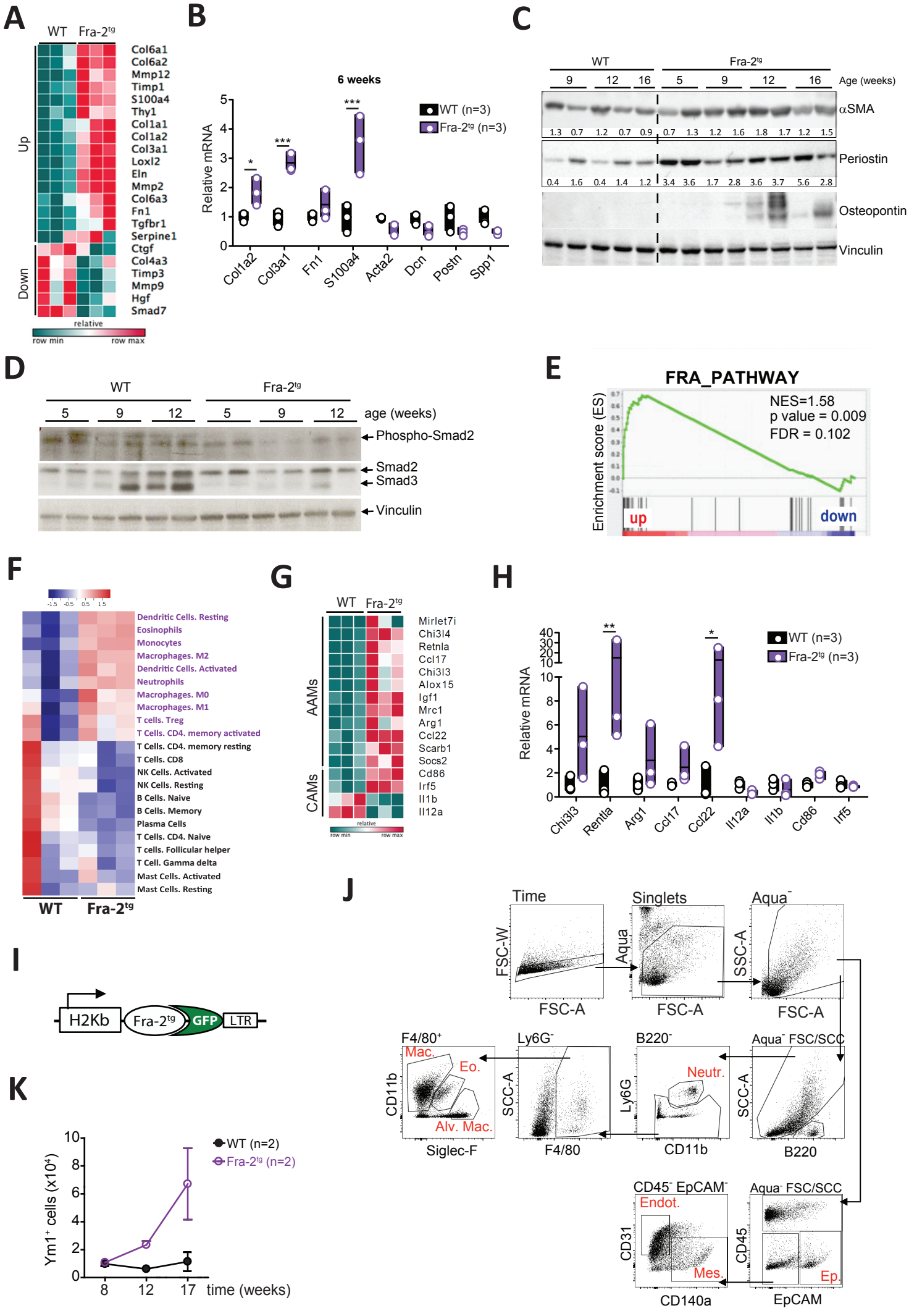
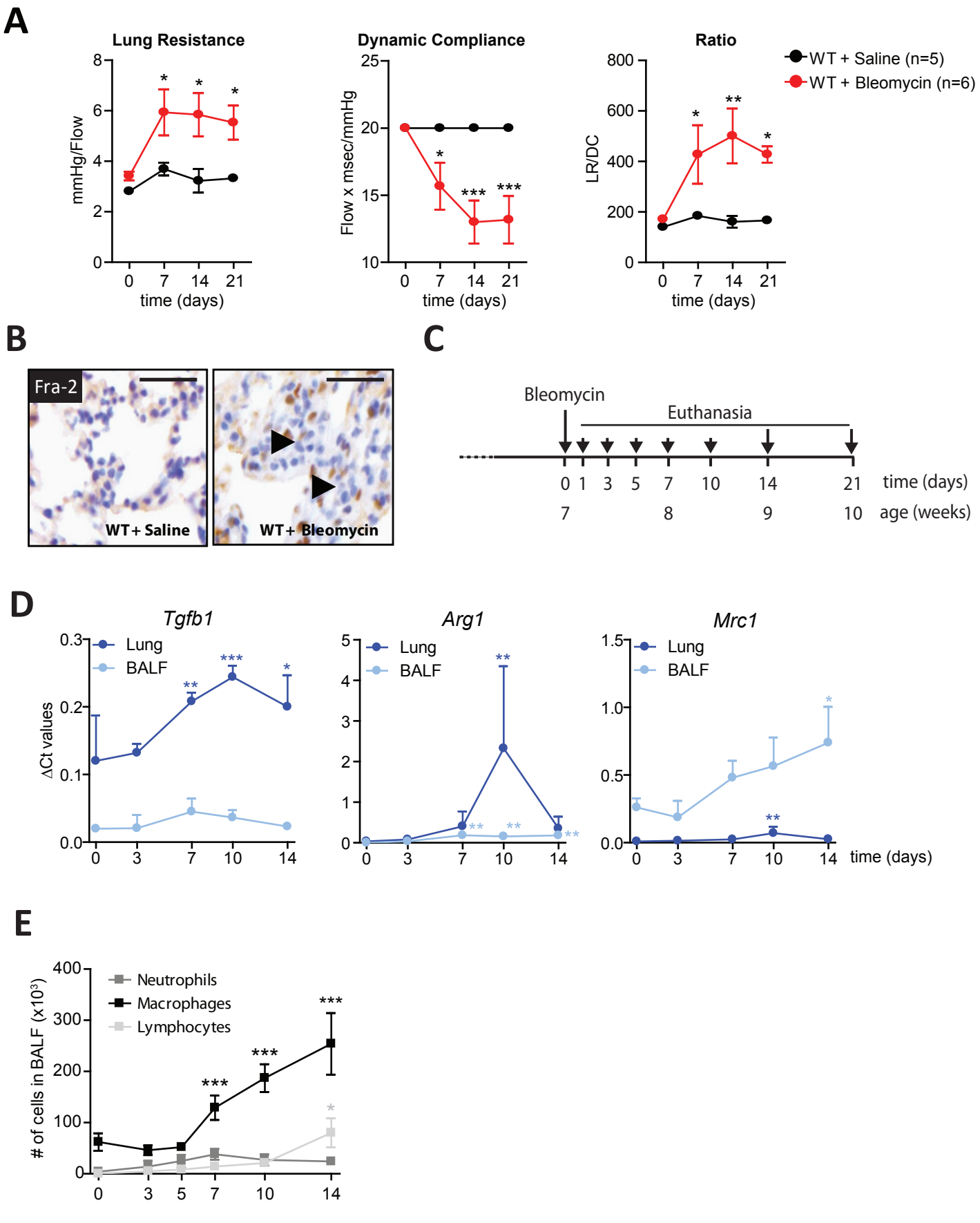


Supp. Figure 1. Ucero et al.



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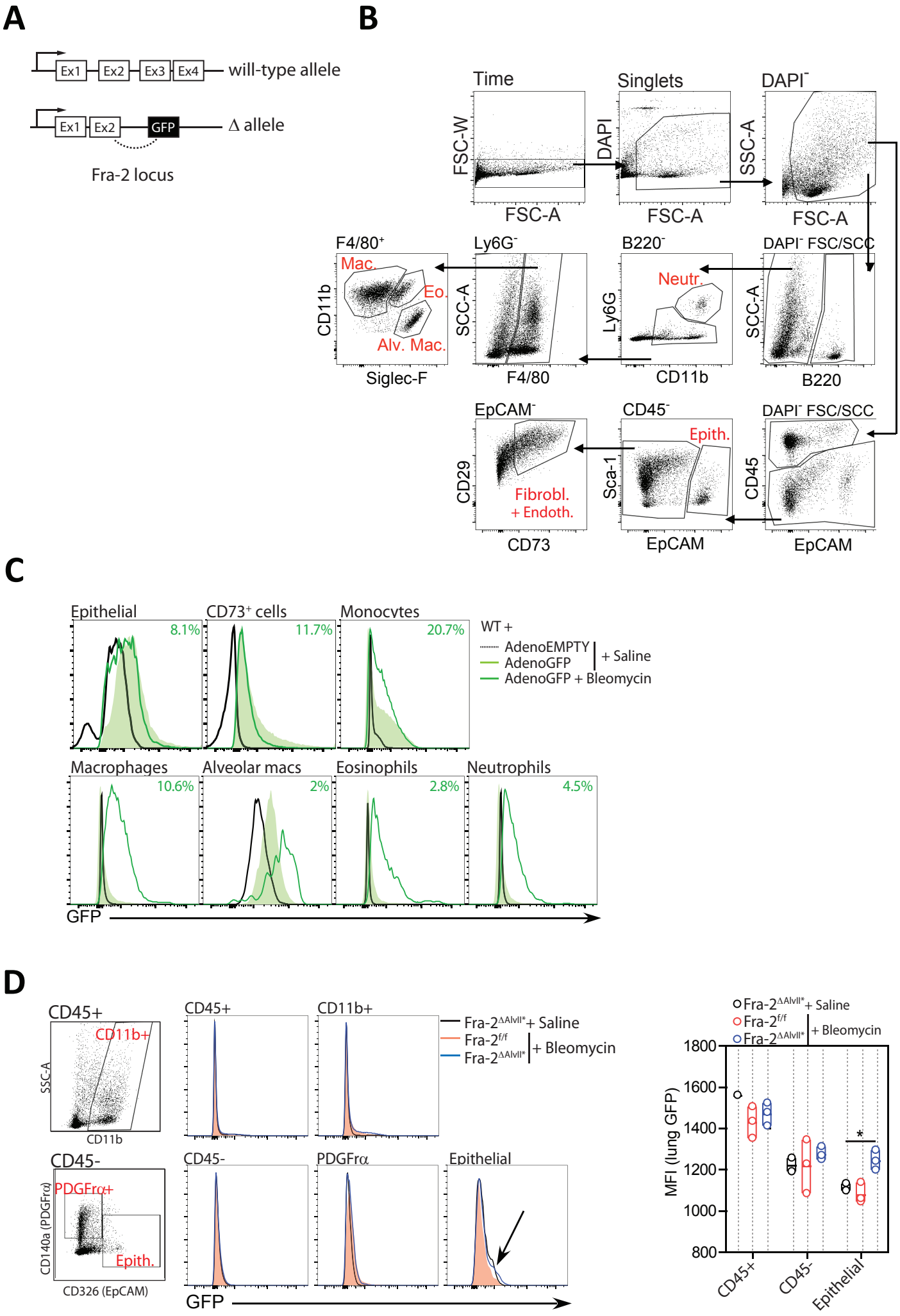

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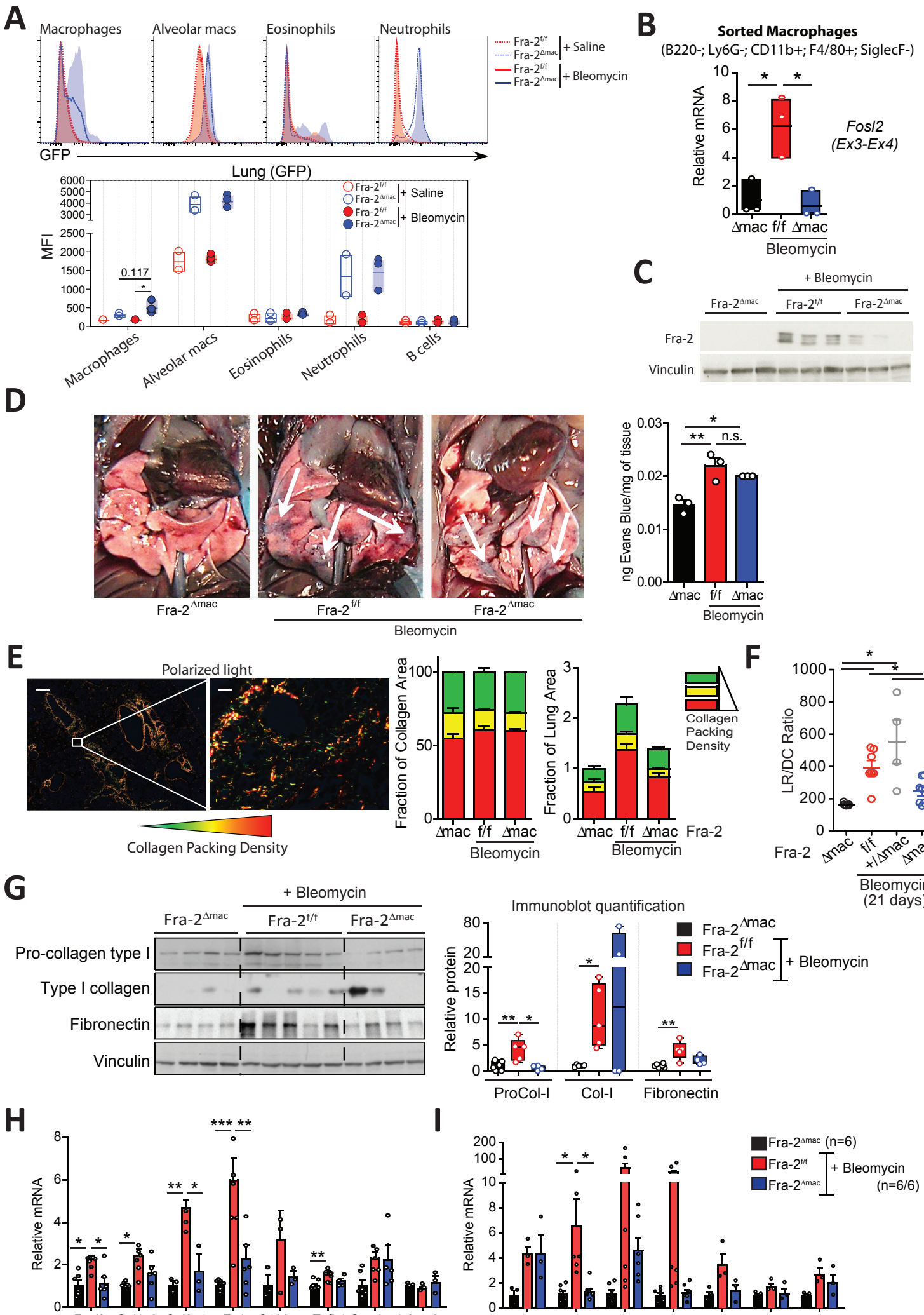

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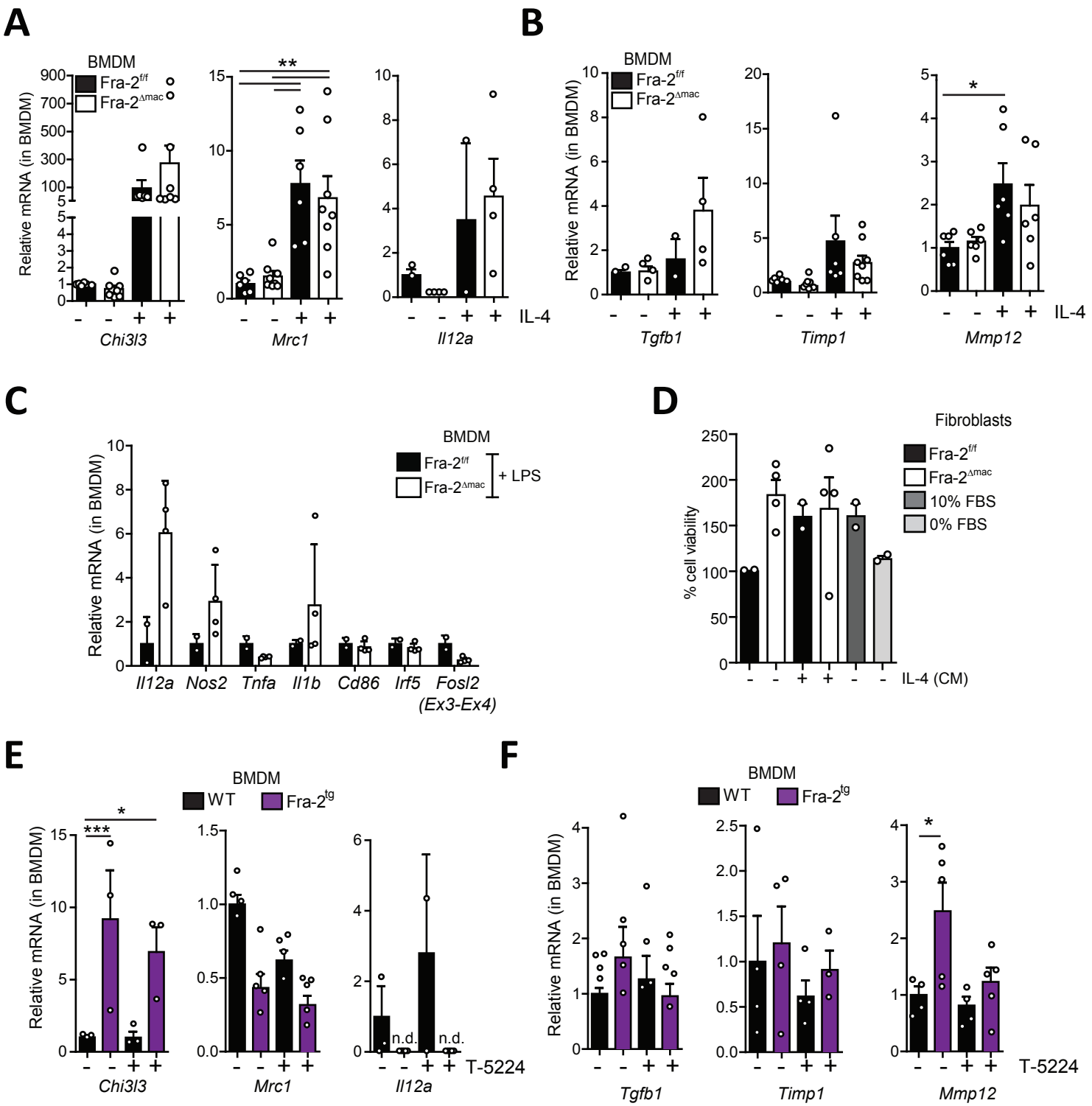

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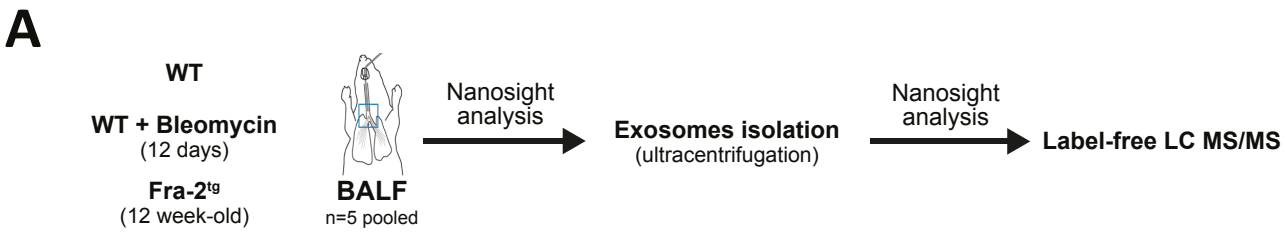

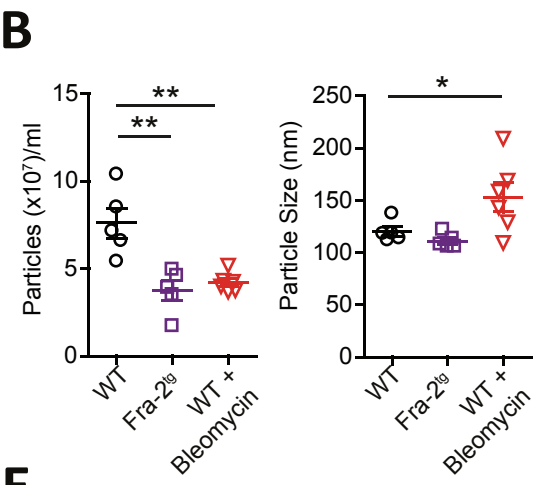

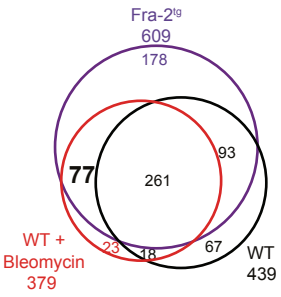

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#pathway ID	pathway description	observed gene count	FDR
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GO.0044421	extracellular region part	126	2.48E-59
GO.0031988	membrane-bounded vesicle	121	1.10E-57
GO.0005576	extracellular region	129	3.77E-55
GO.0031982	vesicle	120	4.75E-55
GO.0005615	extracellular space	59	3.39E-30
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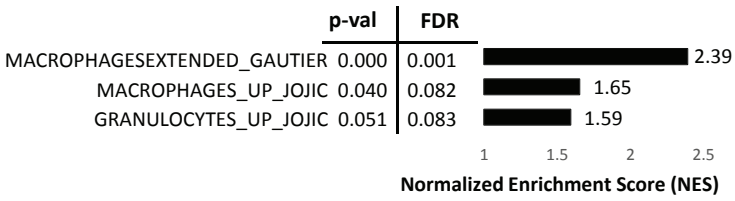
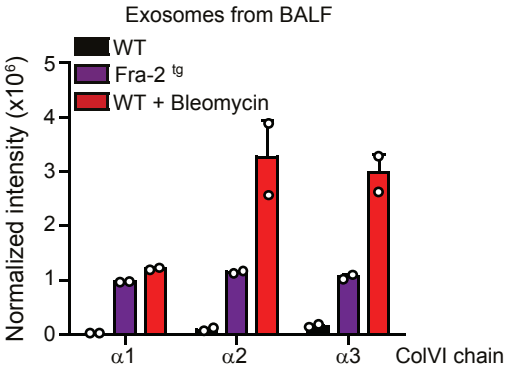
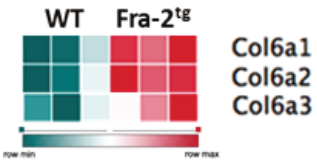
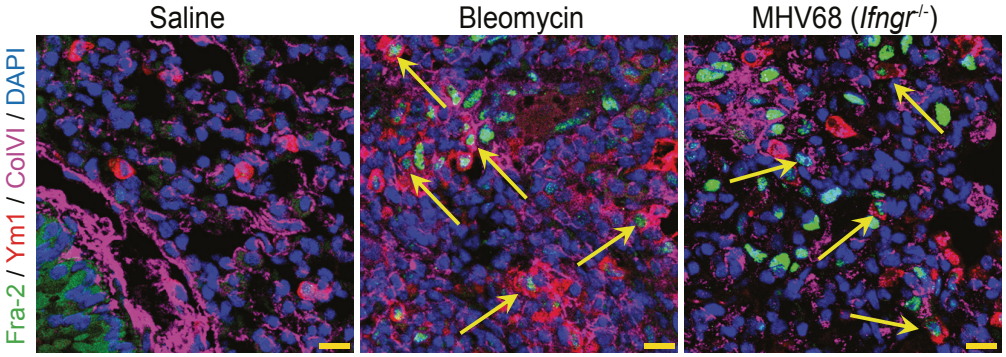
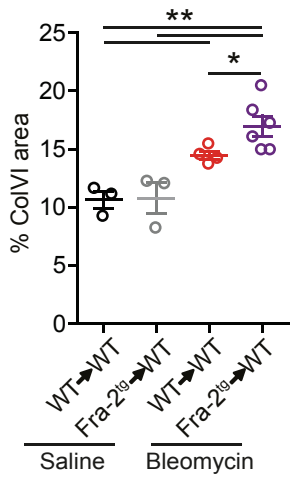


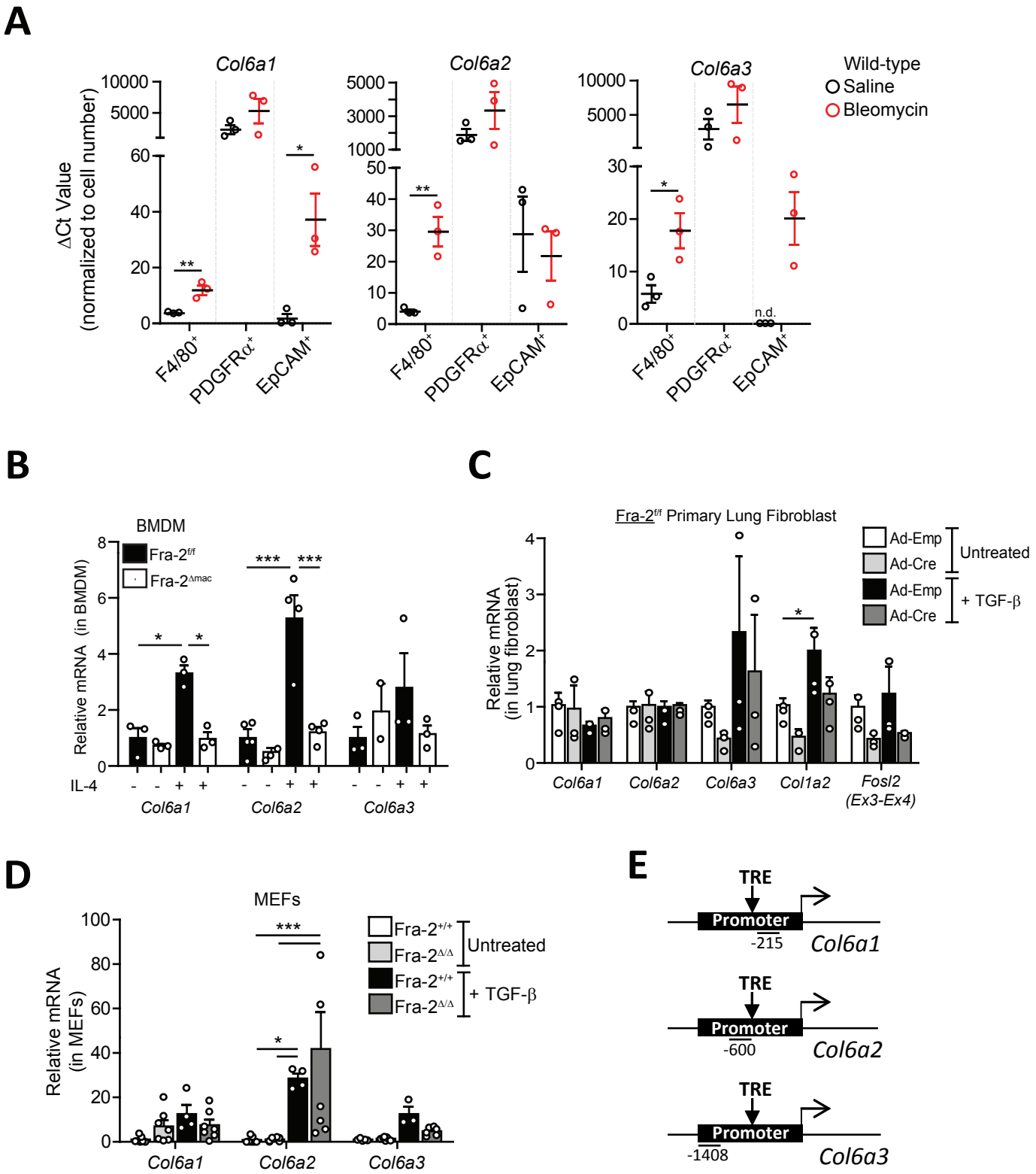








Supp. Figure 6. Ucero et al.



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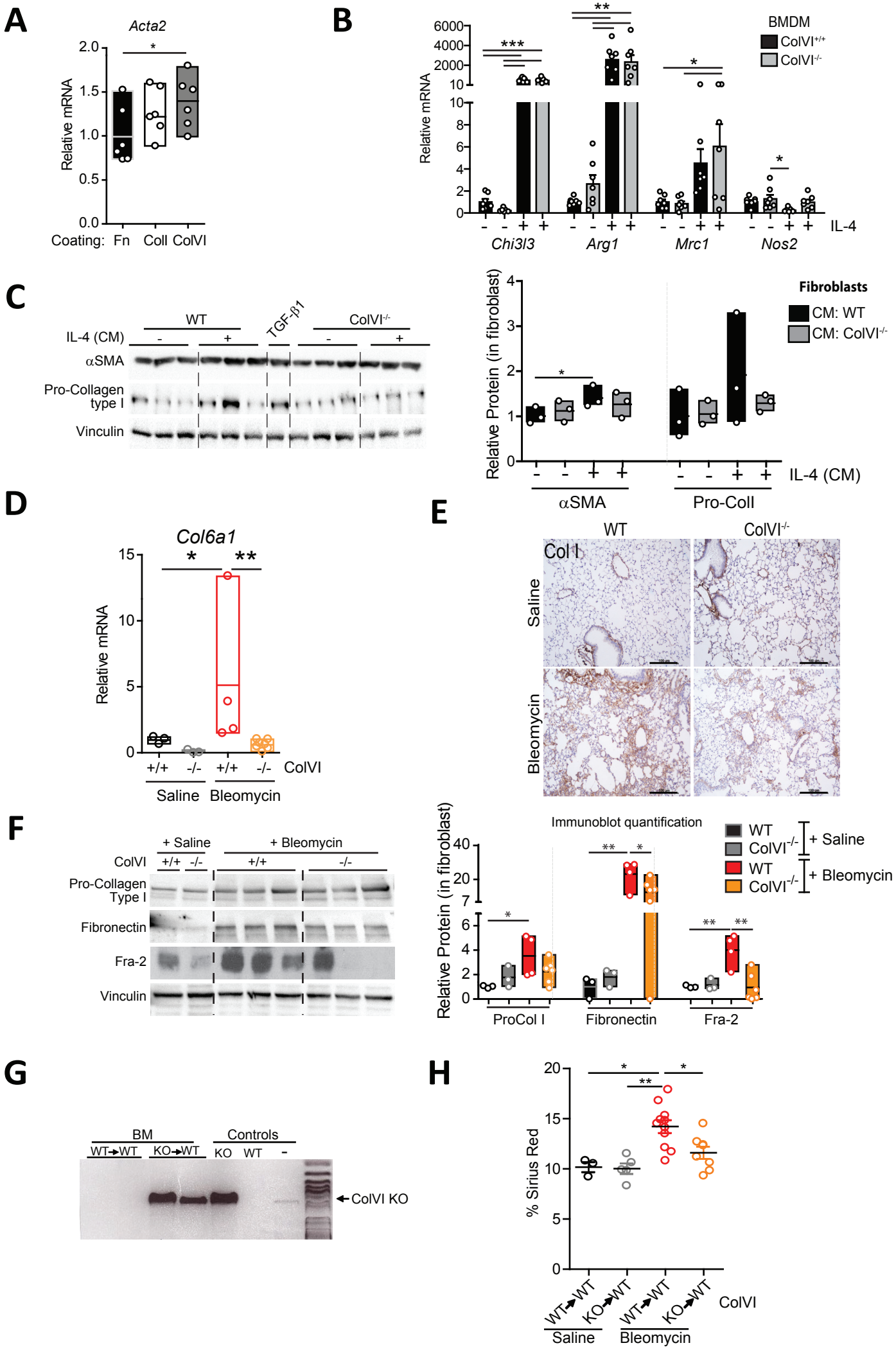

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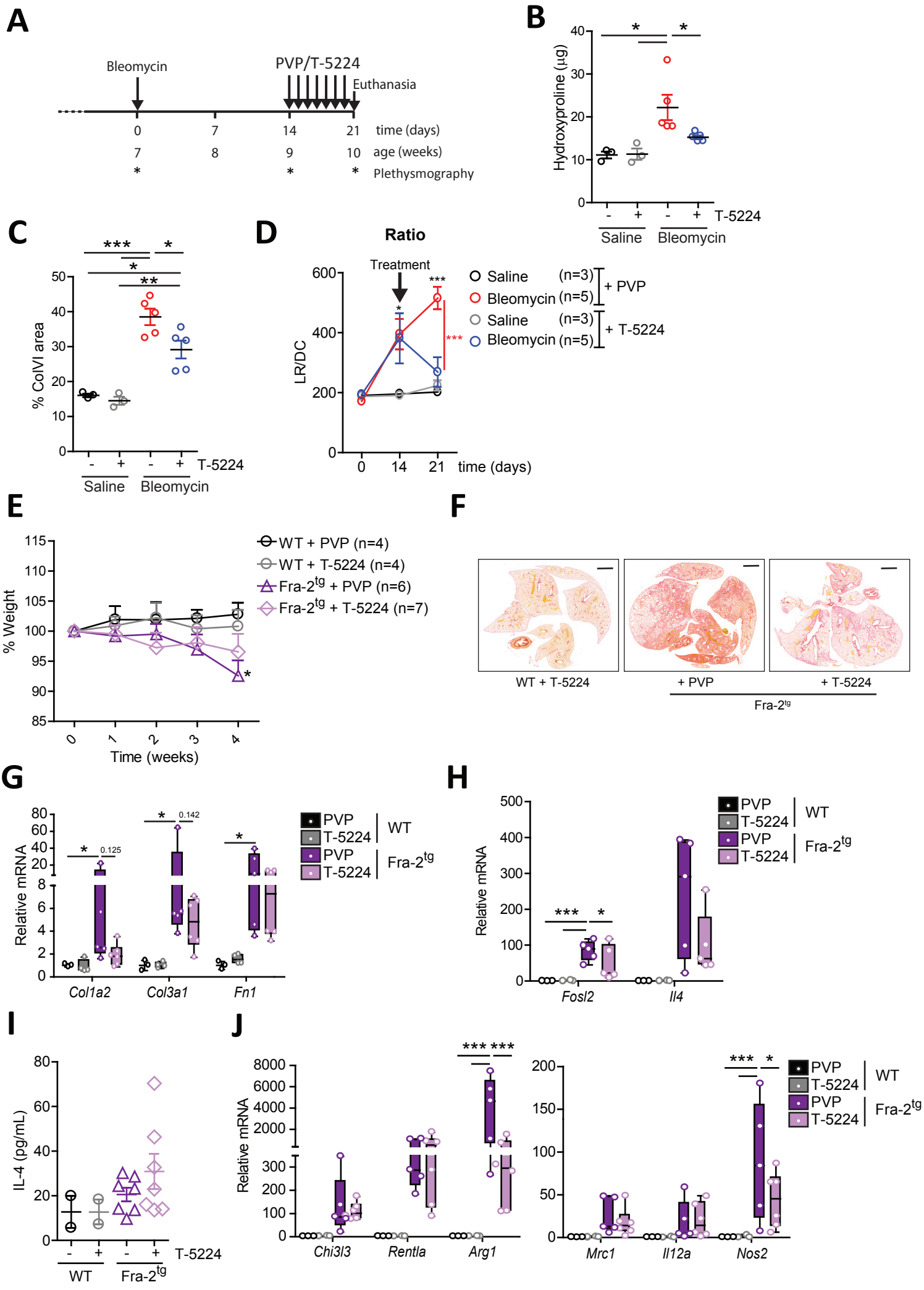

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Supplementary Figure Legends

Supplementary Figure 1. A role for alternatively activated macrophages in the Fra-2^{tg} lung fibrosis model (Related to Figure 1).

A) Heat-maps for fibrosis-related differentially expressed genes in wild-type (wt) and Fra-2^{tg} lungs (6 weeks, RNA-seq, n=3/3).

B) qRT-PCR analysis of fibrosis marker genes in the lungs of wt and Fra-2^{tg} mice. Mean, maximum and minimum are plotted with individual values as biological replicates. Relative expression in wt is set to 1. * $p < 0.05$, *** $p < 0.001$ (Unpaired t-test; two-tailed).

C) Immunoblot analysis of αSMA, Periostin and Osteopontin in lung lysates from 5, 9, 12 and 16 week-old Fra-2^{tg} and wild-type (wt) control littermates. Not all mice across the time points are littermates. Vinculin is used to control loading. Numbers show the vinculin-normalized arbitrary density unit fold-change relative to wt average at 9 weeks of age for αSMA and Periostin.

D) Immunoblot analysis of phospho-Smad2, Smad2 and vinculin in lung lysates from 5, 9 and 12 week-old Fra-2^{tg} and wild-type (wt) control littermates. Not all mice across the time points are littermates. Vinculin is used to control loading.

E) GSEA plot of Fra-1 and Fra-2 transcriptional targets (PID_FRA_PATHWAY) in pre-fibrotic Fra-2^{tg} lungs compared to wt littermates (6 weeks, RNA-seq, n=3/3). NES, FDR and p value are indicated.

F) Normalized enrichment heat-maps and hierarchical clustering of immune cell-type-specific sub-profiles in wt and Fra-2^{tg} lungs (6 weeks, RNA-seq, n=3/3).

G) Heat-maps for macrophage polarization marker differentially expressed genes in wt and Fra-2^{tg} lungs (6 weeks, RNA-seq, n=3/3).

H) qRT-PCR analysis of macrophage marker genes in the lungs of Fra-2^{tg} mice. Mean, maximum and minimum are plotted with individual values as biological replicates. Relative expression in wt is set to 1. * $p < 0.05$, ** $p < 0.01$ (Unpaired t-test; two-tailed).

I) Simplified scheme of the Fra-2 transgene: Expression of the complete murine Fra-2 mRNA fused with an IRES-EGFP and a LTR-polyA sequence is controlled by an H2Kb promoter fragment.

J) Gating strategy for Flow cytometry analyses in wt and Fra-2^{tg} mice. In all flow cytometry analyses, first we discard acquisition perturbations over time and select single events by size. Then we gate on live cells, negative for DAPI or Aqua staining. Finally, cell debris is discarded by FSC/SCC. In the flow cytometry experiments with wt and Fra-2^{tg} mouse lungs we performed two different antibody panels for myeloid subpopulations, and epithelial, endothelial and mesenchymal cell lineages as shown in the dot plots.

K) Absolute number of Ym1-positive cells. Not all mice across the time points are littermates (n=2; biological replicates from one experiment).

Supplementary Figure 2. Fra-2 expression is relevant to fibrogenesis (Related to Figure 2).

A) Longitudinal analyses of respiratory function of wt controls after Saline or Bleomycin treatment. Lung resistance (LR: mmHg/mL x s^{-1}) and Dynamic Compliance (DC: mL/mmHg) were measured by plethysmography in the same mice over time and mean±sem plotted. Lung Resistance and Dynamic Compliance ratios are also shown as a more sensitive analysis to identify fibrosis-specific dysfunction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Two-way ANOVA; Bonferroni post-test).

B) Fra-2 immune-histochemistry (IHC) of lungs from wt mice 10 days after Saline or Bleomycin treatment. Nuclei are counterstained with hematoxylin. Arrows point to positive nuclei. Scale bar= 25µm.

C) Experimental timeline for the Bleomycin lung fibrosis model. i.t.: intra-tracheal delivery.

D) qRT-PCR analysis in lungs and BALF from Bleomycin-treated wt mice. In order to compare RNA expression on samples from different origin, ΔCt values are plotted. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to initial time point (0 days; untreated). (One-way ANOVA; Dunnet post-test).

E) Quantification of immune cell populations in bronchioalveolar lavage fluid (BALF) from Bleomcyin-treated wt mice over time. *** $p < 0.001$ compared to initial time point (0 days; untreated). (One-way ANOVA; Dunnet post-test).

Supplementary Figure 3. Fra-2 expression during lung fibrosis (Related to Figure 3).

A) Scheme of the wild-type and knock-out (Δ) Fra-2 alleles depicting the knocked in GFP reporter expressed under the control of the endogenous Fra-2 regulatory elements.

B) Gating strategy for Flow cytometry analyses in Fra-$2^{+/+}$ and Fra-$2^{+/-}$ mice after either Saline or Bleomycin treatment. In all flow cytometry analyses, first we discard acquisition perturbations over time and select single events by size. Then we gate on live cells, negative for DAPI or Aqua staining. Finally, cell debris is discarded by FSC/SCC. In these flow cytometry experiments we performed two different antibody panels for myeloid subpopulations and, epithelial and $CD73^{+}$ cells (include lung endothelial and mesenchymal cells), as shown in the dot plots.

C) Flow cytometry analysis on GFP-expressing cells (gating strategy in fig. S3E) after intratracheal infection of wt mice with GFP-expressing Adenovirus and treated with either Saline (light green) or Bleomycin for 14 days (dark green). Empty Adenovirus was used as control and

sets GFP background baseline (black line). Percentages in the plots indicate the average proportion of each population among total GFP^+ cells in the AdenoGFP + Bleomycin group (dark green). Note that Alveolar macrophages have higher autofluorescence in the GFP channel. Flow cytometry detection of GFP-expressing cells after Adenovirus infection was repeated twice.

D) *Left*: Representative GFP histograms from lung flow cytometry analyses 10 days after Bleomycin treatment. Fra-$2^{\Delta AlvII*}$ mice treated with Saline were used as controls. Fra-2 deletion and gene activation, reported by GFP expression. *Right*: Individual values for MFI are plotted. * $p < 0.05$. Flow cytometry experiment was performed once.

Supplementary Figure 4. Fra-2 expression in macrophages is essential for Bleomycin-induced lung fibrosis (Related to Figure 4).

A) Representative GFP histograms from flow cytometry analyses of lung cells, 14 days after Saline or Bleomycin treatment. Fra-2 deletion and gene activation, reported by GFP expression, was studied in different myeloid subpopulations. MFI individual values are plotted. * $p < 0.05$. Note that, as in previous experiments, Alveolar macrophages have higher autofluorescence in the GFP channel. Doublets, dead cells and debris was excluded following the gating strategy as before. Flow cytometry experiment was repeated twice.

B) qRT-PCR analysis of *Fosl2* (primer set does not detect floxed *Fosl2* gene) in macrophages (as $B220^-$, $Ly6G^-$, $CD11b^+$, $F4/80^+$, Siglec-F^- cells) isolated 14 days after Bleomycin treatment. Average gene expression in Saline-treated Fra-$2^{\Delta mac}$ sorted cells is set to 1. * $p < 0.05$ (One way ANOVA; Bonferroni post-test).

C) Fra-2 Immunoblot in lung lysates 21 days after Bleomycin treatment. Control group was treated with Saline. Vinculin is used to control loading.

D) Lung blood vessel leakage and lung edema were assessed by Evans Blue permeability assay in 21 days after Bleomycin treatment. *Left*: Arrows on blue tissue point to local edema. Evans Blue is quantified on the *right* panel (n=3). Control group (black bar) was treated with Saline. * $p < 0.05$, ** $p < 0.01$ (One way ANOVA; Bonferroni post-test).

E) *Left*: Representative pictures of Sirius Red staining of lung sections from mice 21 days after under polarized light. Scale bar: 200µm, insert: 20µm. Sirius Red refractivity is proportional to collagen fiber packing with densities ranging from red (higher) to green (lower) and can be quantified. *Right panels*: Quantification of Collagen packing density. Fra-$2^{\Delta Mac}$ mice treated with Saline were used as controls. Mean±sem is plotted ($n \geq 3$ per group). The *left* plot shows the relative proportion while the *right* plot shows the same proportion normalized by the different collagen content in each group.

F) Lung resistance and Dynamic Compliance ratio after 21 days of either Saline or Bleomycin. * $p < 0.05$ compared to Saline-treated controls (Two-way ANOVA; Bonferroni post-test).

G) Immunoblot analysis of Pro-collagen I, collagen I and fibronectin in lung lysates. Relative densitometry quantification for each protein is shown as a ratio to vinculin density (loading control). Saline-treated control group is set to 1. Mean±sem are plotted. * $p < 0.05$, ** $p < 0.01$.

H) qRT-PCR analysis of macrophage marker genes in mouse lung tissue 21 days after Bleomycin treatment. Mean±sem is plotted. Relative expression in Saline-treated Fra-$2^{\Delta Mac}$ is set to 1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (One way ANOVA; Bonferroni post-test).

I) qRT-PCR analysis of fibrosis marker genes in mouse lung tissue 21 days after Bleomycin treatment. Mean±sem is plotted. Relative expression in Saline-treated Fra-$2^{\Delta Mac}$ is set to 1. * $p < 0.05$ (One way ANOVA; Bonferroni post-test).

Supplementary Figure 5. Fra-2 in macrophages induces the secretion of profibrotic factors but does not alter their alternative activation (Related to Figure 5).

A) qRT-PCR analysis of macrophage marker genes in Fra-$2^{f/f}$ and Fra-$2^{\Delta mac}$ BMDMs at the end of the experiment. ** $p < 0.01$ (One-way ANOVA; Bonferroni post-test).

B) qRT-PCR analysis of macrophage-related pro-fibrotic genes in Fra-$2^{f/f}$ and Fra-$2^{\Delta mac}$ BMDM cells. In all panels, bars=mean±sem. Relative expression in untreated Fra-$2^{f/f}$ is set to 1. * $p < 0.05$ (One-way ANOVA; Bonferroni post-test).

C) qRT-PCR analysis of classically activated macrophage marker genes and *Fosl2* gene in Fra-$2^{f/f}$ and Fra-$2^{\Delta mac}$ BMDMs stimulated with 1 ng/mL of lipopolysaccharide (LPS). * $p < 0.05$ (Unpaired t-test; two-tailed).

D) Crystal violet assay for cell viability of wt lung fibroblasts cultured with Fra-$2^{f/f}$ and Fra-$2^{\Delta mac}$ BMDM-conditioned media. Cells maintained in serum-free medium or treated with 10% FBS media were used as controls. Cell viability of fibroblasts with CM from untreated Fra-$2^{f/f}$ was considered 100%.

E) qRT-PCR analysis of macrophage marker genes in wt and Fra-2^{tg} BMDMs at the end of the experiment. Relative expression in untreated wt is set to 1. Mean±sem is plotted (n=5 per group). * $p < 0.05$, *** $p < 0.001$ (One-way ANOVA; Bonferroni post-test). n.s.= not significant.

F) qRT-PCR analysis of macrophage-related pro-fibrotic genes in wt and Fra-2^{tg} BMDM cells. Relative expression in untreated wt is set to 1. Mean±sem is plotted (n=5 per group). * $p < 0.05$ (One-way ANOVA; Bonferroni post-test).

Supplementary Figure 6. Type VI collagen expression in lung fibrosis mouse models (Related to Figure 6).

A) Scheme of the experimental design for exosomes isolation from BALF and subsequent analysis by label-free LC MS/MS.

B) Nanosight® analysis of number and size of particles corresponding to exosome-like vesicles before ultracentrifugation in wt, Fra-2^{tg} and Bleomycin-treated wt mice. Individual mouse values and mean±sem are plotted. * $p < 0.05$. ** $p < 0.01$ (One-way ANOVA; Bonferroni post-test).

C) Venn diagram of the proteins identified by LC MS/MS in wt, Fra-2^{tg} and Bleomycin-treated wt mice.

D) Cellular Component Gene Ontology (GO) analysis of the 261 proteins identified in the three experimental groups. FDR = false discovery rate.

E) Enrichment analysis for the proteins identified only in exosomes from fibrotic lungs compared to macrophage- and granulocyte-specific gene signatures. Normalized enrichment score (NES), False discovery rate (FDR) and p value are indicated.

F) Normalized intensity for ColVI fragments in LC MS/MS analysis of BALF extracted from 12 week-old wt mice, Fra-2^{tg} mice and wt mice after 12 days of Bleomycin treatment.

G) Heat-maps for ColVI genes in wt and Fra-2^{tg} lungs (6 weeks, RNA-seq, n=3/3).

H) *Left:* Confocal microscopy images of fluorescent immunohistochemistry (F-IHC) analysis of lungs from wt mice treated with either Saline or Bleomycin for 21 days, and MHV68-infected *Ifngr*$^{-/-}$ mouse. Co-staining for Fra-2 (green), Ym1 (red) and ColVI (purple). Arrows point to triple positive cells. Nuclei are counterstained with DAPI (blue). Scale bar = 25µm. *Right*: Lung hydroxyproline content in lungs from MHV68-infected and non-infected *Ifngr*$^{-/-}$ mouse. * $p < 0.05$ (Paired t-test; two-tailed).

I) Quantification of the positive area for type VI collagen immunohistochemistry of lungs, 14 days after Bleomycin. Data from two independent experiments are plotted as mean±sem and individual values. * $p < 0.05$. ** $p < 0.01$ (Unpaired t-test; one-tailed).

Supplementary Figure 7. Type VI collagen gene expression in macrophages and fibroblasts (Related to Figure 6).

A) ColVI chains gene expression in different lung cell populations sorted out from wt mice 14 days after Saline or Bleomycin treatment. Lung macrophages are included in the $CD45^+$; $CD11b^+$; $Ly6G^-$;$F4/80^+$ population. Fibroblasts are included in the $CD45^-$; $EpCAM^-$; $PDGFR\alpha^+$ population. $EpCAM^+$ population includes mostly epithelial cells. Data are expressed as ΔCt values normalized to the cell number in each population. * $p < 0.05$. ** $p < 0.01$ (Unpaired t-test; two-tailed).

B) qRT-PCR analysis of ColVI genes in Fra-$2^{f/f}$ and Fra-$2^{\Delta mac}$ BMDMs at the end of the experiment described in Figure 6A. Relative expression in untreated wt is set to 1. * $p < 0.05$, *** $p < 0.001$ (One-way ANOVA; Bonferroni post-test).

C) qRT-PCR analysis of ColVI genes and *Fosl2* in untreated and TGF-β1-treated (1 ng/mL) Fra-$2^{f/f}$ primary lung fibroblasts, pre-treated with empty and Cre-expressing Adenovirus for control and Fra-2 loss-of-function, respectively. Relative expression in untreated Fra-$2^{f/f}$ lung fibroblasts pretreated with empty Adenovirus is set to 1.

D) qRT-PCR analysis of ColVI genes in untreated and TGF-β1-treated (1 ng/mL) Fra-$2^{+/+}$ and Fra-$2^{-/-}$ mouse embryonic fibroblasts (MEFs). Relative expression in untreated Fra-$2^{+/+}$ MEFs is set to 1. * $p < 0.05$, *** $p < 0.001$ (One-way ANOVA; Bonferroni post-test).

E) Scheme of the probable transcription regulatory sequence and coordinates used for the ChIP study on the different Col6 chain gene promoter regions.

Supplementary Figure 8. Type VI collagen deficiency in lung fibrosis (Related to Figure 6).

A) qRT-PCR analyses of *Acta2* expression in primary wt lung fibroblasts cultured on plates coated with either fibronectin (Fn), type I collagen (ColI) or ColVI. Individual values are from six independent primary fibroblast cultures. * $p < 0.05$ (Paired t-test; two-tailed).

B) qRT-PCR analysis of macrophage marker genes in wt and ColVI$^{-/-}$ BMDMs at the end of the experiment, either treated with IL-4 or untreated. Relative expression in untreated wt is set to 1. * $p < 0.05$, *** $p < 0.001$ (One-way ANOVA within each gene; Bonferroni post-test).

C) Immunoblot analysis of pro-collagen I and α-smooth muscle actin (αSMA) in lysates from primary wt lung fibroblasts Relative densitometry quantification for each protein is shown as a ratio to vinculin density (loading control). Individual values and mean±sem are plotted. * $p < 0.05$ (Unpaired t-test; One-tailed).

D) qRT-PCR analysis of *Col6a1* gene in the lungs of wt and ColVI$^{-/-}$ mice after Saline or Bleomycin treatment. Relative expression in wt mice treated with Saline is set to 1. Mean, maximum and minimum are plotted with individual values. * $p < 0.05$. ** $p < 0.01$ (Mann-Whitney test).

E) Type I collagen immunohistochemistry of lungs from wt and ColVI$^{-/-}$ mice 14 days after either Saline or Bleomycin treatment. Nuclei are counterstained with hematoxylin. Scale bar= 100 μm.

F) Immunoblot analysis of pro-collagen I, fibronectin and Fra-2 in lung lysates from wt and ColVI$^{-/-}$ mouse lungs after Saline or Bleomycin treatment. Vinculin is used to control loading. Relative densitometry quantification for each protein is shown as a ratio to vinculin density. Median, maximum and minimum are plotted with individual values. Data derived from two independent experiments. * $p < 0.05$. ** $p < 0.01$ (Unpaired t-test; two-tailed).

G) ColVI$^{-/-}$ PCR products in a 2% agarose gel. DNA isolated from bone marrow (BM) of wt mice 6 weeks after transplantation with either wt BM (WT→WT) or ColVI$^{-/-}$ BM (KO→WT), and from tails of ColVI$^{-/-}$ and ColVI$^{+/+}$ mice as controls.

H) Quantification of Sirius Red-positive area in lungs from Saline- and Bleomycin-treated wt mice transplanted with either wt BM (WT→WT) or ColVI$^{-/-}$ BM (KO→WT), 21 days after Bleomycin. Data from two independent experiments are plotted as mean±sem and individual values. * $p < 0.05$. ** $p < 0.01$ (One-way ANOVA; Bonferroni post-test).

Supplementary Figure 9. AP-1 inhibition reverses lung fibrosis in Bleomycin and Fra-2^{tg} mouse models of lung fibrosis. (Related to Figure 7).

A) Scheme for AP-1 inhibitor T-5224 therapeutic protocol in Saline- and Bleomycin-treated wt mice. Plethysmography for lung function assessment was performed at 0, 14 and 21 days after fibrosis induction (green asterisks). All mice that showed fibrosis in the plethysmograph after 14 days of Bleomycin, were orally treated every day with either T-5224 or vehicle (PVP) for 7 consecutive days.

B) Lung hydroxyproline content at the end of the experiment. Individual values and mean±sem are plotted. * $p < 0.05$ (Unpaired t-test; One-tailed).

C) Quantification of the positive area for type VI collagen immunohistochemistry of lungs 21 days after either Saline or Bleomycin. Data from one experiment is plotted as mean±sem and individual values. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$ (One-way ANOVA; Bonferroni post-test).

D) Longitudinal analyses of respiratory function of Saline- and Bleomycin-treated wt mice with either T-5224 or PVP. LR/DC ratios are plotted. Note that the T-5224 or vehicle treatment started 14 days after fibrosis induction, when animal respiratory function was already compromised (arrow). * $p < 0.05$, *** $p < 0.001$ (Two-way ANOVA; Bonferroni post-test).

E) Relative (percentage) mouse body weight variation upon T-5224 or vehicle over the treatment period. Mean±sem is plotted (n=2 for wt; n>6 for Fra-2^{tg}). * $p < 0.05$ respect the wt + PVP group (Two-way ANOVA; Bonferroni post-test).

F) Representative pictures of Sirius Red staining of whole lung sections from experimental groups. The red staining is quantified as the collagen area in Figure 2B. Scale bar = 1mm.

G) qRT-PCR analysis of fibrosis marker genes in the lungs of Fra-2^{tg} and wt controls treated with T-5224 or PVP at the end of the experiment. Mean, maximum and minimum are plotted with individual values. Relative expression in wt + PVP is set to 1. * $p < 0.05$ (Mann-Whitney test).

H) qRT-PCR analysis of *fosl2* and *il4* gene expression in lungs from Fra-2^{tg} and wt controls treated with T-5224 or PVP at the end of the experiment. Mean, maximum and minimum are plotted with individual values. * $p < 0.05$, *** $p < 0.001$ (One-way ANOVA; Bonferroni post-test).

I) IL-4 quantification in lung lysates by ELISA. Individual values and mean±sem are plotted.

J) qRT-PCR analysis of AAM marker gene expression in lungs from Fra-2^{tg} and wt controls treated with T-5224 or PVP at the end of the experiment. Mean, maximum and minimum are plotted with individual values. * $p < 0.05$, *** $p < 0.001$ (One-way ANOVA; Bonferroni post-test).

Supplementary Table S1: qRT-PCR and ChIP primers for mouse genes:

	Forward primer	Reverse primer
Fra-2	CCAGCAGAAGTTCCGGGTAG	GTAGGGATGTGAGCGTGGATA
Fra-2(Ex3-Ex4)	CGCTCACATCCCTACAGTCC	CCTGCAGCTTCTCTGTCAGC
Acta2	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
S100a4	TCCACAAATACTCAGGCAAAGAG	GCAGCTCCCTGGTCAGTAG
Tgfb1	CCACCTGCAAGACCATCGAC	CTGGCGAGCCTTAGTTTGGAC
Serpine1	TTCAGCCCTTGCTTGCCTC	ACACTTTTACTCCGAAGTCGGT
Postn	CTCATTCCTGATTCTGCCAA	ATTGTAGAGGTCGCTAAGGCC
IL-4	ACAGGAGAAGGGACGCCAT	GAAGCCCTACAGACGAGCTCA
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Mrc1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
Retnla	CACAAGCATCCAGTCACA	GGAAGCTCTCAGTCGTCAAGA
Chi3l3	TCTGGGTACAAGATCCCTGAA	TCATATGGAGATTTATAGAGGGGACT
Tnf	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Ccl17	TGCTTCTGGGGACTTTTCTG	GAATGGCCCTTTGAAGTAA
Ccl22	TCTTGCTGTGGCAATTCAGA	GAGGGTGACGGATGTAGTCC
Ccl22	TCTTGCTGTGGCAATTCAGA	GCAGAGGGTGACGGATGTAG
Il12a	CCATCAGCAGATCATTCTAGACAA	CGAACTTCTGATTCAGAGACTG
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Irf5	ATGTAGTAGCCACAACCCAG	CTGAGTGGTTCATGGCAAAG
Cd86	GCATATGACCGTTGTGTGTG	CTAGGCTGATTCGGCTTCTT
Mmp12	CTGCTCCCATGAATGACAGTG	AGTTGCTTCTAGCCCAAAGAAC
Timp1	GCAACTCGGACCTGGTCATAA	CGGCCCGTGATGAGAAACT
Col1a2	GTAACTTCGTGCCTAGCAACA	CCTTTGTCAGAATACTGAGCAGC
Col3a1	ACGTAGATGAATTGGGATGCAG	GGGTTGGGGCAGTCTAGTG
Col6a1	CAGGATTTGATGGCATTCAAG	TGAGTTCCCTGGTCTCCCAG
Col6a2	CCATTGCCAAGGACCCCAAG	GTTGACTCGCTCGTCGTCCA
Col6a3	AGGGTTCAGGCCCTGTATCA	CCCACATCCAAGCTCTCCAC
Fn1	GATGTCCGAACAGCTATTTACCA	CCTTGCGACTTCAGCCACT
Rps29	ATGGGTCACCAGCAGCTCTA	GCCTATGTCCTTCGCGTACT
Rpl4	CCGTCCCCTCATATCGGTGTA	GCATAGGGCTGTCTGTTGTTTTT

ChIP primers

Col6a1 TRE	CCTTGAAGAGAAAAAGGCCC	CTGAGTGGGTAAACATCCCA
Col6a2 TRE	GATTTCATGAATGGACAAAA	AGAGGCCTCTAAGGAGGTC
Col6a3 TRE	CAGTTCCCTTAAGCATCCTC	CCAGTTGCAGGGCTGGGCC

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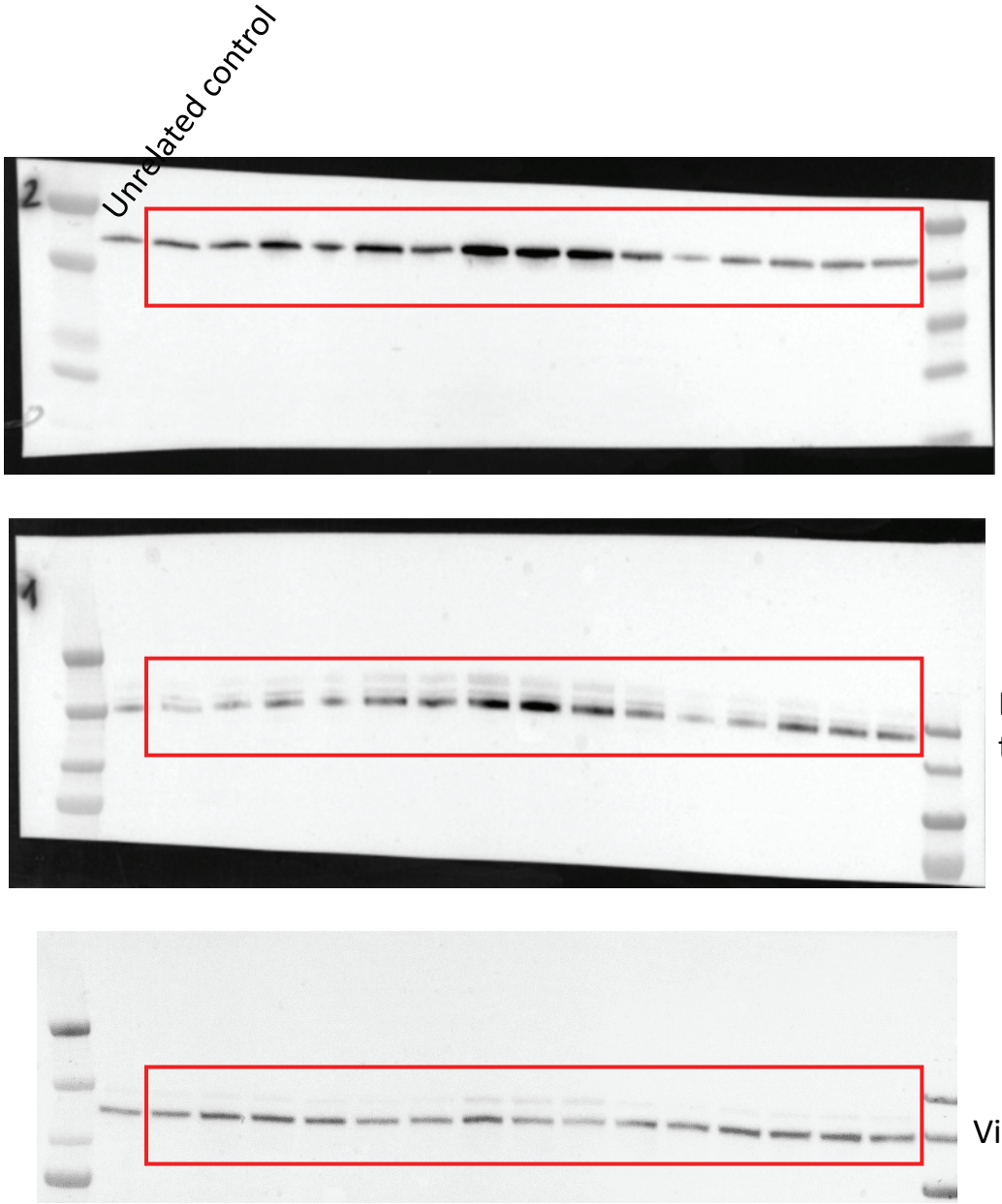

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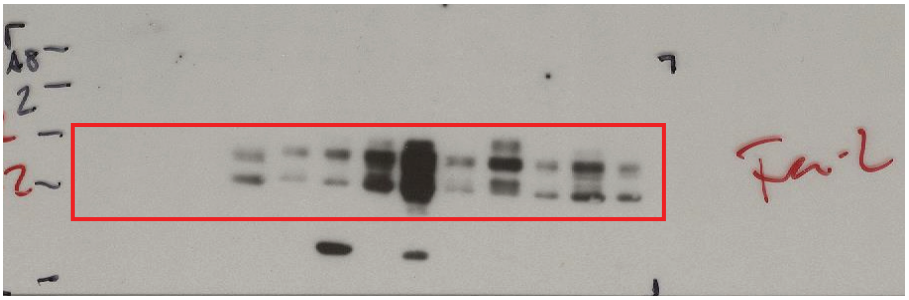

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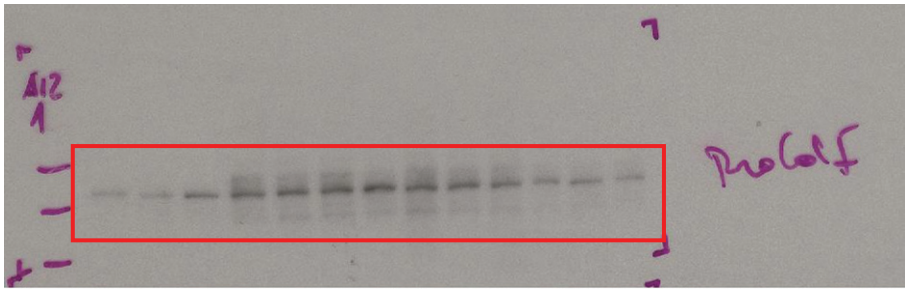

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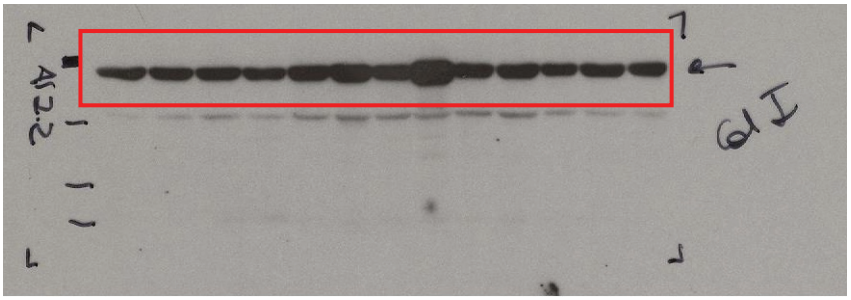

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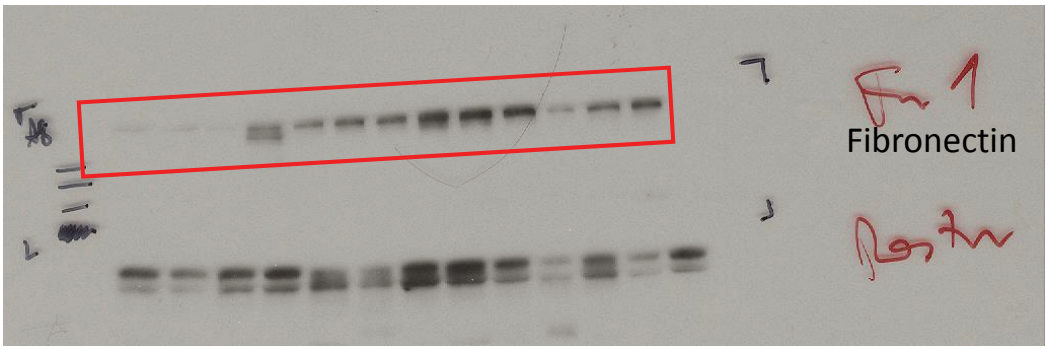

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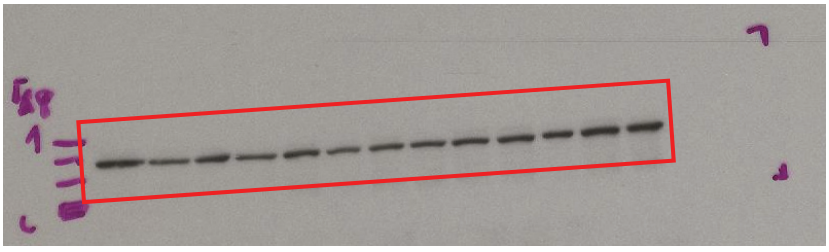
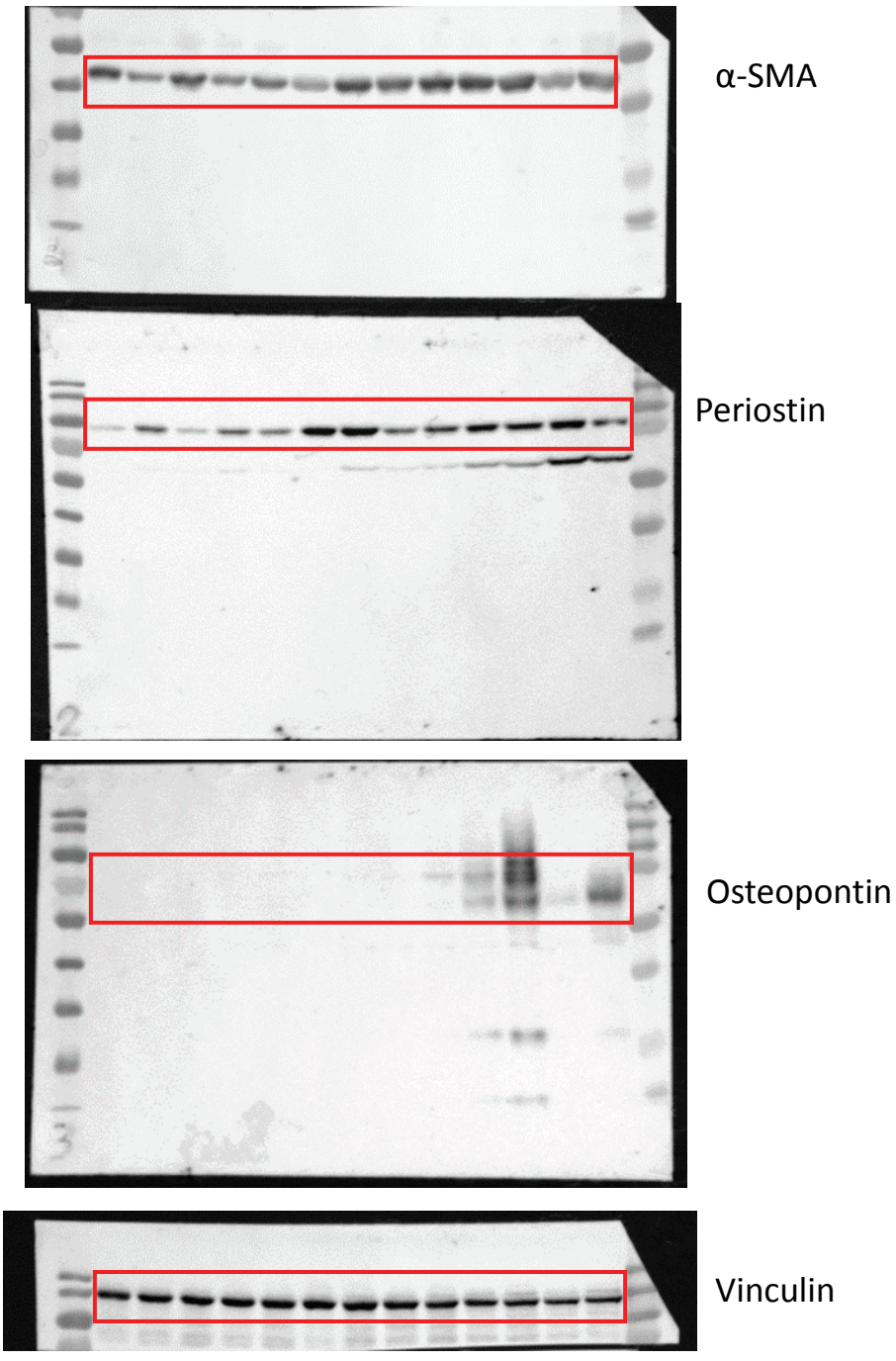


Vinculin

C



Full unedited gel for Supplementary Figure 1

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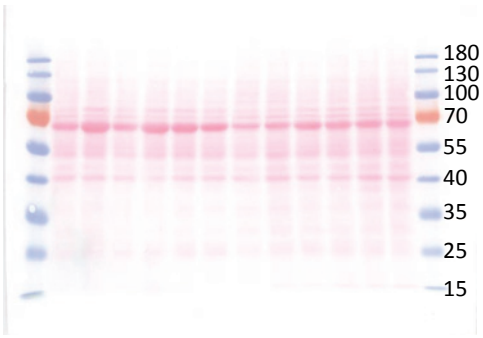

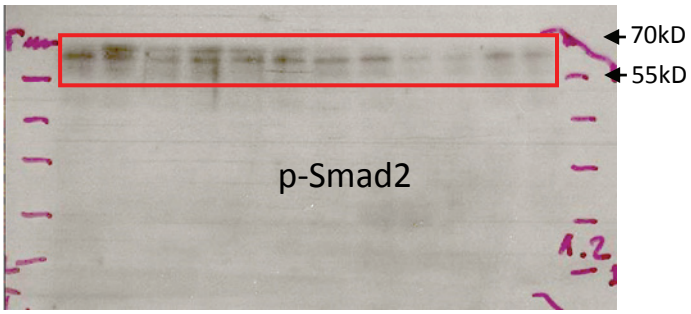

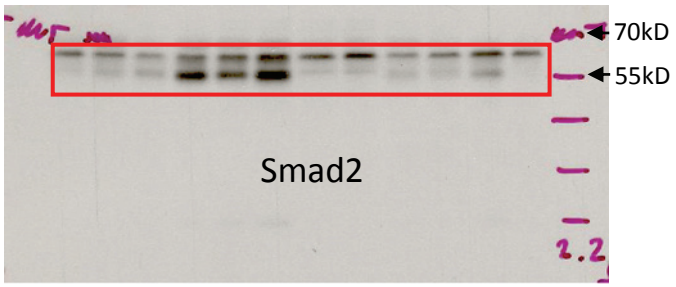

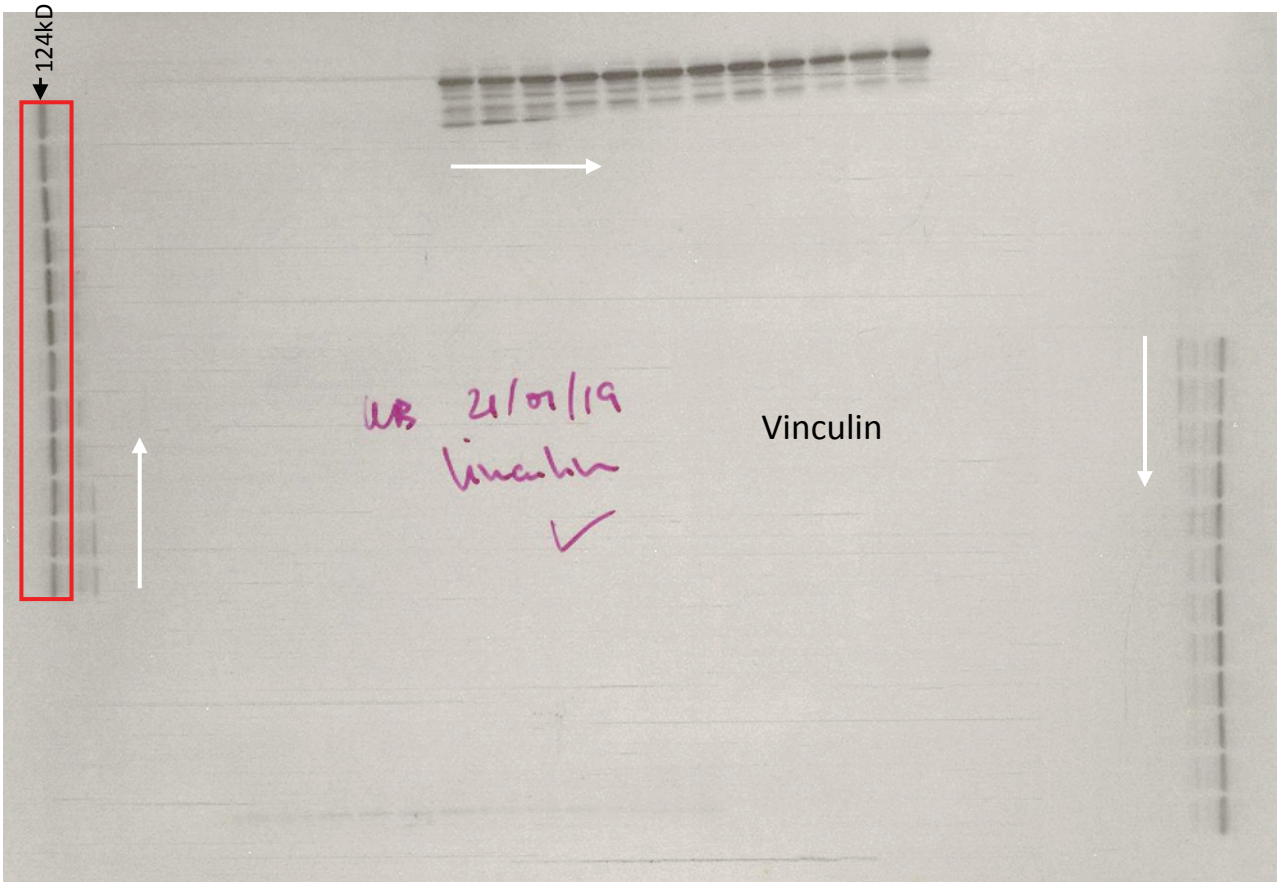

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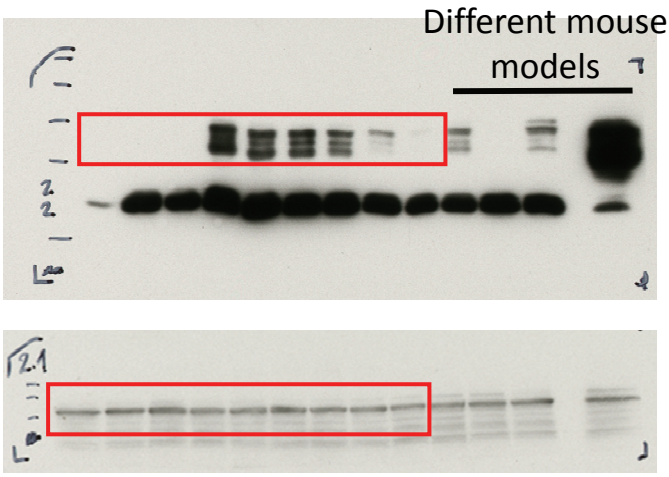

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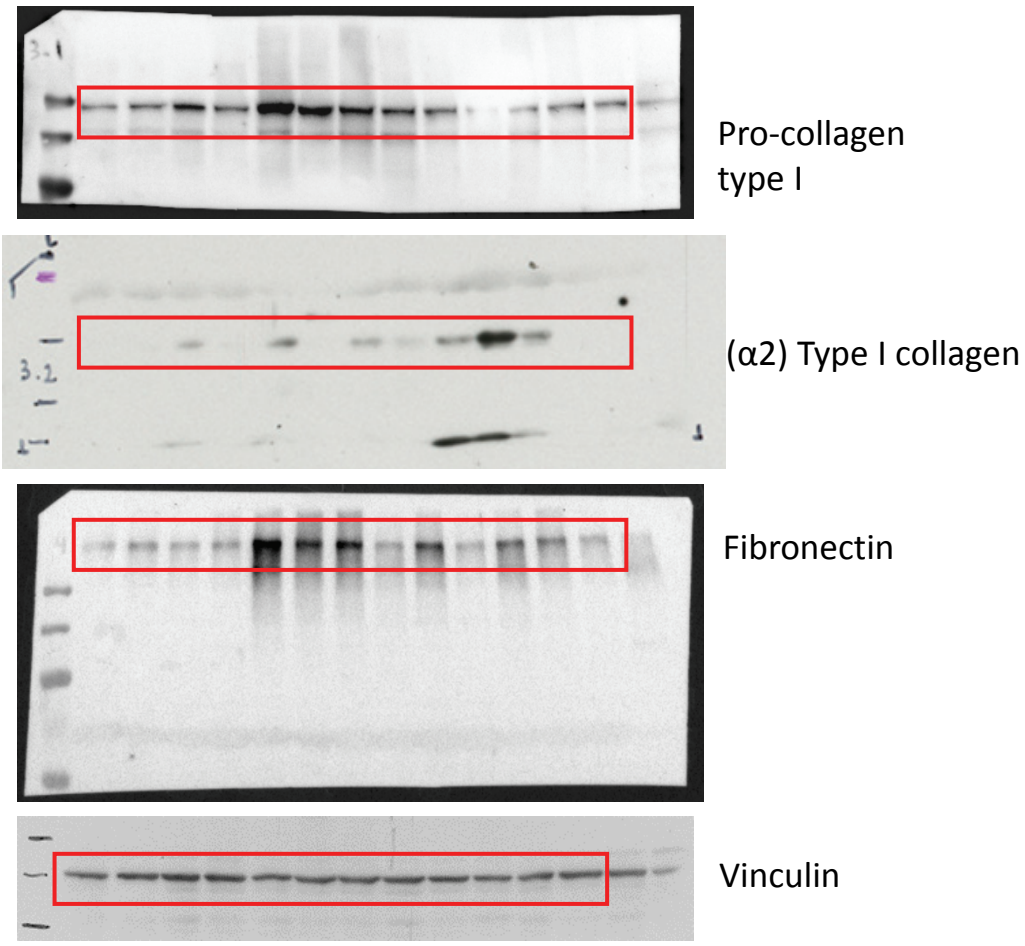

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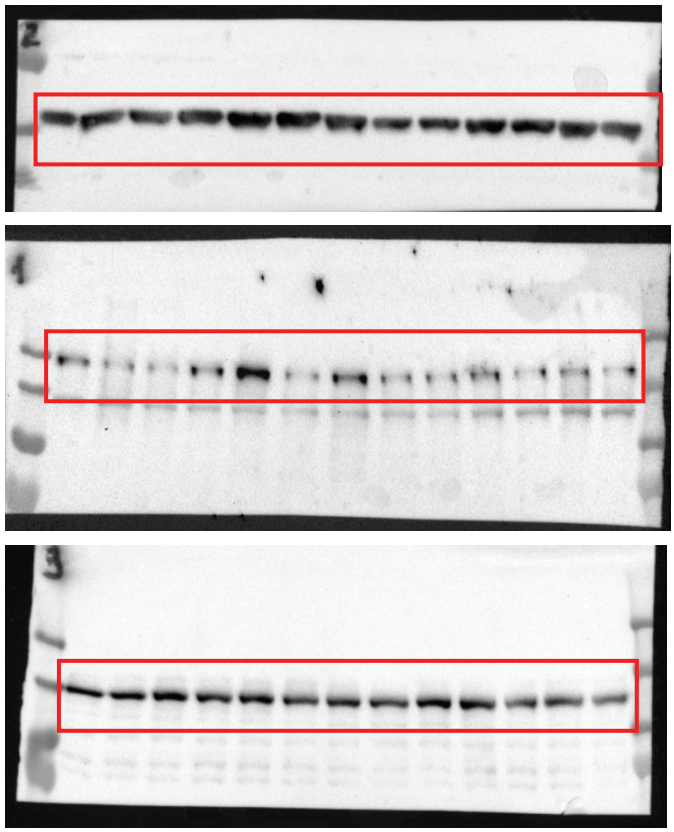

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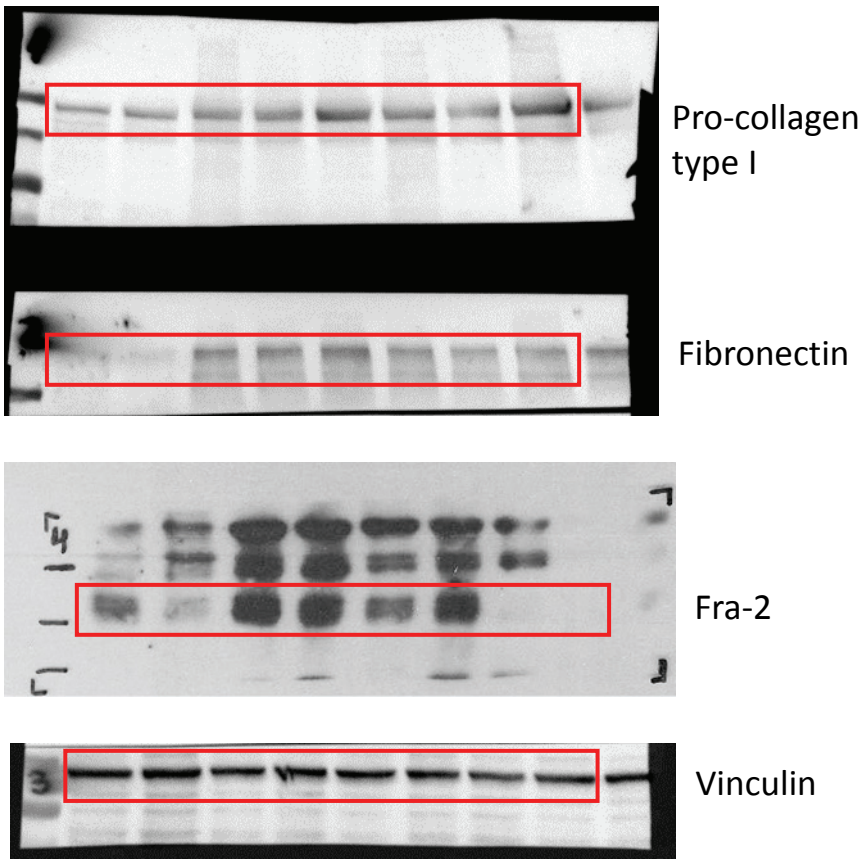

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