Macrophage Cx43 Is Necessary for Fibroblast Cytosolic Calcium and Lung Fibrosis After Injury

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Macrophages are paracrine signalers that regulate tissular responses to injury through interactions with parenchymal cells. Connexin hemichannels have recently been shown to mediate efflux of ATP by macrophages, with resulting cytosolic calcium responses in adjacent cells. Here we report that lung macrophages with deletion of connexin 43 (MacΔCx43) had decreased ATP efflux into the extracellular space and induced a decreased cytosolic calcium response in co-cultured fibroblasts compared to WT macrophages. Furthermore, MacΔCx43 mice had decreased lung fibrosis after bleomycin-induced injury. Interrogating single cell data for human and mouse, we found that P2rx4 was the most highly expressed ATP receptor and calcium channel in lung fibroblasts and that its expression was increased in the setting of fibrosis. Fibroblast-specific deletion of P2rx4 in mice decreased lung fibrosis and collagen expression in lung fibroblasts in the bleomycin model. Taken together, these studies reveal a Cx43-dependent profibrotic effect of lung macrophages and support development of fibroblast P2rx4 as a therapeutic target for lung fibrosis.

Keywords: P2RX4, lung fibrosis, fibroblast, macrophage, calcium imaging

INTRODUCTION

Tissue fibrosis is a universal feature of the response to injury and is associated with high mortality in pulmonary fibrosis, liver cirrhosis, chronic heart failure, and chronic kidney disease. In most cases, the inflammatory response is marked by large numbers of monocyte-derived macrophages, which have been found to be necessary for lung fibrosis (1–3). Our recent work profiled the fibrotic phase of lung injury at the single-cell level, revealing a subset of Cx3cr1+ macrophages that localize to clusters of activated fibroblasts and exert a profibrotic effect (3). With respect to the profibrotic mechanisms of these macrophages, one emerging view is that they activate surrounding fibroblasts...
by secreting cytokines and growth factors, such as TNFα, TGFβ, and PDGF (2–6). However, the full repertoire of signals deriving from macrophages is not known.

Previous in vitro results have shown that extracellular ATP activates purinergic receptors on fibroblasts, leading to calcium influx and collagen expression (7, 8). ATP is a known damage associated molecular pattern that binds to and activates membrane channels for calcium entry in a range of inflammatory contexts (9). Recent reports demonstrate that connexin 43 (Cx43) hemichannels are a conduit for ATP efflux by macrophages, with paracrine effects on calcium transients in bystander cells (10–12). However, this biology has not been explored in reference to macrophage-fibroblast crosstalk.

In this study, we found lung fibroblast calcium is increased by co-culture with lung monocyte-derived macrophages. Furthermore, macrophages were found to secrete ATP in a Cx43-dependent manner after injury, and the fibroblast cytosolic calcium response was decreased in co-culture with Cx43 KO macrophages. In vivo studies indicated that the ATP receptor P2rx4 expressed by fibroblasts was necessary for lung fibrosis, revealing a novel profibrotic interaction of macrophages and fibroblasts in lung injury.

MATERIALS AND METHODS

Mice
Ai14 (Rosa26-LSL-tdTomato), Cx3cr1-CreERT2 (Cx3cr1tm2.1 (cre/ERT2))Jung), Cx43 floxed, Rosa26-LSL-Salsa6f, and Macblue (Csf1r-GAL4/VP16,UAS-ECFP)1Hume) mice were obtained from the Jackson Laboratory. Col1a2-CreERT2 mice were obtained from Bin Zhou (13). Pdgfrb-Cre (14) and P2rx4 floxed (15) mice were in the possession of the investigative team. Mice with tamoxifen-inducible alleles were administered 2 mg tamoxifen (Sigma, T5648) dissolved in olive oil (Sigma, 01514) intraperitoneal injection every other day for Cre induction. All experiments were balanced for gender, and mice were used for experiments between the ages of 6 and 10 weeks. Mice were maintained in specific-pathogen-free conditions in the Animal Barrier Facility of the University of California, San Francisco.

ATP Measurement
To isolate macrophages, we sorted tdTomato+ cells using a Sony SH 800 flow cytometer with freshly isolated lung cell suspensions from tamoxifen-induced Cx3cr1-CreERT2: Rosa26-loxp-STOP-loxP-tdTomato mice following lung dissociation with 2000 U/ml DNase I (Roche, 4716728001), 0.1 mg/ml Dispase II (Sigma, 4942078001) and 0.2% Collagenase (Roche, 10103586001), with gating as previously described (3). Cells were passed through a 70-µm filter prior to FACS (Sony SH800) of DAPI- (live), tdTomato+ cells followed by 24-hour culture. Supernatants were then collected for ATP measurement using a luciferase assay as described in the manufacturer’s protocol (ATP Determination Kit, ThermoFisher Scientific, A22066). Bioluminescence was quantified using a Biotek H1 Plate Reader. The ATPase inhibitor ARL 67156 (Sigma-Aldrich, 67156) was used to reduce extracellular ATP degradation.

Macrophage-Fibroblast Co-Culture
tdTomato+ cells were isolated as above by FACS of freshly isolated cell suspensions from Cx3cr1-CreERT2:Ai14 or Cx3cr1-CreERT2: Ai14: Cx43 fl/fl mice. Primary fibroblasts were freshly isolated from mouse lung cell suspensions from Col1a2-CreERT2: Rosa26-LSL-Salsa6f mice by negative selection of endothelial (CD31), leukocyte (CD45), epithelial (Epcam), vascular endothelial, pericytes & smooth muscle (CD146) and red blood (Ter119) cells by biotin-labeled antibodies and Dynabeads MyOne Streptavidin T1 (Thermo Fisher Scientific, 65601) as per Tsukui et al. (16). Sorted macrophages and isolated fibroblasts were co-cultured at 1:1 in µ-slide plates (Ibidi, 81506) for 24 hours. Fluorescence images were captured by confocal microscopy with a Leica CTR 6500 microscope, and images were analyzed using Imaris software.

Lung Slice Preparation and Calcium Imaging by Two-Photon Microscopy
Precision-cut lung slices were obtained from mouse lungs according to the protocol described (17). Mice at day 7 after injury with bleomycin were euthanized, and the trachea was cannulated followed by inflation of the lung with 1 mL of 2% low melting point agarose warmed to 37°C (Lonza, 21083-027). Left lung lobes were isolated and precision-cut transversely in a bath of ice-cold PBS to generate 400-µm slices using a vibratome (Leica VT1200). Slices were placed in a 24-well plate in Leibovitz’s media until imaging.

During imaging, slices were housed in an HP Ultra Quiet Imaging Chamber ( Warner Instruments, JG23W/HP) attached to a PM-1 Chamber platform and Series 20 stage adapter. 1X Hank’s Balanced salt solution (HBSS, Gibco, 14025-076) was bubbled with a carbogen gas mixture (95% O2/5% CO2) in a 39°C water bath and pumped with a mini-variable flow pump at a rate of approximately 1.5 mL/minute through an in-line heater into the imaging chamber, maintaining the temperature of the sample at 35-37°C with a dual-chamber temperature controller. Live calcium imaging was performed by two-photon microscopy using an upright LSM 7 MP INDIMO system (Carl Zeiss Microscopy), customized with four GaAsP detectors and a Z-Deck motorized stage (Prior). Samples were imaged with a W Plan-Apochromat 20X/1.0 N.A. water-immersion objective. To minimize the spectral overlap of CFP and GFP, these fluorophores were sequentially excited at different wavelengths. Specifically, one Chameleon Ultra II laser (Coherent) was tuned to a wavelength of 870nm to excite CFP, while the other Chameleon Ultra II laser was tuned to a wavelength of 970 nm to excite both GFP and tdTomato. Samples were imaged with two ‘tracks’ with rapid line switching of laser emissions by acousto-optic modulators (Carl Zeiss Microscopy). Emission filters were 472/30 (Semrock) for CFP, 525/50 (Chroma) for GFP, and 605/70 (Chroma) for tdTomato. Images were collected with ZEN Black software (Carl Zeiss Microscopy), and image
analysis was performed with Imaris software (Bitplane). Mean GFP intensity from the entire duration of the video was plotted for all the individual tdTomato+ cells in the field of view.

**Bleomycin Lung Injury and Hydroxyproline Measurement**

Animals were euthanized 21 days after injury with bleomycin (Fresenius; 3 U/kg), and lungs were perfused with 1X PBS and snap frozen in liquid nitrogen. These samples were then homogenized and incubated with 50% Trichloroacetic acid (Sigma, T6399) on ice for 20 mins followed by an overnight incubation at 110°C in 12N HCl (Fisher, A144). Samples were reconstituted in distilled water with constant shaking for 2h. Aliquots of these samples were allowed to react with 1.4% Chloramine T (Sigma, 85739) and 0.5 M sodium acetate (Sigma, 241245) in 10% 2-propanol (Fisher, A416). The samples were incubated with Ehrlich’s solution (Sigma, 03891) for 15 min at 65°C. Hydroxyproline content was measured by the analyzing absorbance at 550 nm with respect to the standard curve generated from purchased hydroxyproline (Sigma, H5534).

**Analysis of Published Mouse and Human Lung scRNAseq Data Sets**

Data were downloaded from GSE132771 (16), who performed 10x-based single cell sequencing of lungs from 3 Idiopathic Pulmonary Fibrosis patients and 3 healthy controls as well as 10x-based single cell sequencing of lungs from 3 Idiopathic Pulmonary Fibrosis patients and 3 healthy controls. For human data analysis, we used the lineage-negative data from Pulmonary Fibrosis patients and 3 healthy controls as well as 10x-based single cell sequencing of lungs from 3 Idiopathic Pulmonary Fibrosis patients and 3 healthy controls. RNA was purified using RNeasy Kit (Qiagen,74106) with on-column DNaseI digestion. iScript reverse transcription super mix (Bio-Rad, 4309155). Gene expression was normalized to human 18S rRNA.

**qPCR of Primary Mouse Lung Fibroblasts**

Primary mouse lung fibroblasts were freshly isolated by negative selection from lung cell suspensions as above. RNA was purified using RNeasy Kit (Qiagen, 74106) with on-column DNaseI digestion. iScript reverse transcription super mix (Bio-Rad, 1708841) was used for first-strand synthesis, followed by qPCR using SYBR Green super mix (Applied Biosystems, 4309155). Gene expression was normalized to Gapdh. The following primers were used (5’ to 3’):

- **Mouse Col1a1 Forward:** AGTATGACTCCACTCACGGCAA
- **Mouse Col1a1 Reverse:** TGTAGCCAGGAGACACGTTGTG
- **Mouse Col3a1 Forward:** GCTTTCAGGAGATGGCAGTGGA
- **Mouse Col3a1 Reverse:** CAAGACCTCGTGCTCCAGTTAG
- **Mouse Cx43 Forward:** CCTCAGGGTATTGCTGGACAAC
- **Mouse Cx43 Reverse:** GAGAGATGGGGAAGGACTTGT
- **Mouse Gapdh Forward:** AGTATGACTCCACTCACGGCAA
- **Mouse Gapdh Reverse:** TCTCGCTCCTGGGAAGATGGG

**ATPγS Treatment of Human and Mouse Fibroblasts**

Normal adult human lung parenchyma was collected from lobectomy specimens from resections performed for primary lung cancer or from normal lungs not used for transplantation. Lung tissue was considered “normal” if the pulmonary function was normal. Informed consent was obtained from all study participants as part of an approved ongoing research protocol by the University of California San Francisco Committee on Human Research in full accordance with the declaration of Helsinki principles. Lung fibroblasts isolated were cultured by the explant technique (19) and used P1 to P4. Primary mouse lung fibroblasts were freshly isolated by negative selection from lung cell suspensions as above.

In both cases (human and mouse), cells were serum starved for 1 hour and, in some cases, treated with ATPγS (Sigma, A1388). Cells were lysed, and RNA was purified using RNeasy Kit (Qiagen,74106) with on-column DNaseI digestion. iScript reverse transcription super mix (Bio-Rad, 1708841) was used for first-strand synthesis, followed by qPCR using SYBR Green super mix (Applied Biosystems, 4309155). Gene expression was normalized to 18S rRNA.

**Sirius Red With Fast Green**

Staining with Sirius red (Electron Microscopy Science, 26357-02) and Fast Green FCF (Fisher Scientific, F99-10) was performed on sections prepared from mouse lungs frozen in OCT. Sections were stained with Sirius Red/Fast Green solution (0.125% Fast Green FCF and 0.1% Sirius Red in saturated Picric Acid) for 60 minutes followed by a 2-minute 0.01 N HCl wash, rinsed in 70% EtOH, and air dried overnight prior to imaging. A brightfield microscope (Zeiss Axiolab scan. Z1) was used for acquiring images, which were quantified using ImageJ. At least three different regions of interest were used for analysis of each sample.

**Western Blot**

Fibroblasts isolated from mice were lysed with Pierce RIPA buffer (Thermo Fisher Scientific, 89901) and Halt protease inhibitor cocktail (Thermo Fisher Scientific, 1861278). 25 micrograms of protein was subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Bio-rad, 4561034) and transferred to a PVDF membrane (Thermo Fisher Scientific, 88520). Membranes were blocked with 5% BSA in 1X PBST (1X PBS+0.01%Tween 20) for 2h at room temperature. Thereafter, the membranes were incubated with primary antibodies: Phospho-p38 MAPK (1:1000, Cell Signaling Technology, 9211) and p38 MAPK (1:1000, Cell Signaling Technology, 9212). After washing with PBST, membranes were incubated with secondary antibody, peroxidase-conjugated goat anti-rabbit (1:20000, Anaspec, AS28177). Membranes were developed using SuperSignal West Pico Chemiluminescent substrate (Thermo Fisher Scientific, 34080) and scanned using ChemiDoc XRS+ gel imaging system (Bio-rad). Quantification of bands was done using ImageJ as described earlier (20).
Statistical Analysis
GraphPad Prism version 8 was used for statistical analysis and linear regression. For categorical variables, Student’s t-test or Mann-Whitney test was used to calculate P values. For comparing more than 2 populations, 1-way ANOVA or 2-way ANOVA was used followed by Sidak’s or Kruskal Wallis multiple-comparisons testing. A P value of less than 0.05 was considered significant.

RESULTS
Recent studies have shown that Cx43 hemichannels serve as a conduit for ATP release from macrophages, leading to paracrine effects on other cells in the microenvironment (10–12). To probe the potential significance of macrophage ATP efflux in fibrosis, we employed the bleomycin model of lung fibrosis (21). We first measured ATP in conditioned media of cultured tdTomato+ cells isolated from the lungs of bleomycin-injured Cx3cr1-CreERT2: Rosa26-LSL-tdTomato mice (largely monocytes and monocyte-derived macrophages) and found that Cx43 KO macrophages released less ATP into the extracellular space than wild type (Figure 1A).

Extracellular ATP is known to bind to membrane-bound purinergic receptors that mediate extracellular calcium entry into the cytosol. First, to test whether fibroblast cytosolic calcium is elevated in injury, we imaged precision-cut lung slices by two-photon laser scanning microscopy. Slices were prepared from mice with the fibroblast-specific Col1a2-CreERT2 allele that we previously generated (15) to the MacBlue allele (23) was included for visualization of moMacs. These data indicated that fibroblast cytosolic calcium was increased in injured mice compared to uninjured controls (Figures 1B, Supplementary Videos 1, 2). We then explored the role of macrophage Cx43 by fibroblast calcium imaging in a co-culture system. Co-culture with WT macrophages was associated with higher fibroblast cytosolic calcium than co-culture with Cx43 KO macrophages, or fibroblasts alone (Figure 1C). Of note, our previous bulk RNAseq data indicated an increase in Cx43 expression in lung monocyte-derived macrophages compared with tissue-resident alveolar macrophages after bleomycin injury (3). Therefore, to test the functional role of Cx43 in lung fibrosis resulting from injury we used the Cx3cr1-CreERT2, a Cre driver that has been used for functional study of monocyte-derived macrophages in multiple contexts in vivo (3, 24, 25). We measured lung collagen by hydroxyproline assay and found that the increase of lung collagen after bleomycin injury was markedly reduced with Cx43 deletion in moMacs compared to wild type (Figures 2A, B). Taken together, these data suggest the hypothesis that macrophages efflux ATP into the extracellular space via Cx43, with a resulting calcium response that is necessary for lung fibrosis after injury.

To pursue this hypothesis further, we first sought to elucidate the relevant ATP receptor in fibroblasts. We had previously identified 12 distinct subsets of collagen-producing cells from control mice and mice treated with bleomycin, as well as 6 distinct populations of collagen-producing cells from normal human lungs and lungs obtained at the time of transplant from patients with Idiopathic Pulmonary Fibrosis (16). Using these datasets, we interrogated expression of all 7 ionotropic ATP receptors in lung fibroblasts by single cell scRNAseq. We found that, among the detected ATP ionotropic receptors, P2rx4 was the most highly expressed in nearly all populations of collagen-producing cells as measured by scRNAseq or qPCR (Figures 3A–C); notably, the scRNAseq data indicated that P2rx4 was increased with bleomycin injury and in IPF lung samples relative to control. The increases in P2rx4 were the most pronounced in the cellular subclusters previously found (16) to express the highest levels of pathologic extracellular matrix proteins (clusters 8 in mice and 3 in humans).

Given the high expression of P2rx4 both at steady state and its even higher expression in pathologic fibrosis, we next tested the effect of fibroblast-specific P2rx4 deletion by crossing a P2rx4 floxed allele that we previously generated (15) to the Col1a2-CreERT2 allele (13). In the entire lung fibroblast population isolated from bleomycin-injured mice, there was a modest decrease in P2rx4 (Figure 4A); nonetheless, possibly because of focused expression of this collagen promoter-driven Cre in fibroblasts with high collagen production, fibroblasts from Col1a2-CreERT2/P2rx4 fl/fl mice had a pronounced decrease in collagen gene expression after bleomycin injury compared to wild type (Figure 4A). Conversely, collagen genes were induced by treatment of primary human and mouse lung fibroblasts with ATPγS (a non-hydrolyzable form of ATP; Figure 4B). Importantly, fibroblast-specific P2rx4 KO mice using two different Cre drivers, the Col1a2-CreERT2 and the Pdgfrb-Cre, had decreased lung collagen after bleomycin injury compared to wild type (Figure 4C). Moreover, we confirmed that P2rx4 signals in fibroblasts via p38 MAP kinase (Figure 4D), which has previously found to be necessary for P2rx4 signaling in other systems (26, 27). Taken together, these results suggest a model of ATP signaling via fibroblast P2rx4 as a positive regulator of tissue fibrosis after injury (Figure 4E).

DISCUSSION
How macrophages regulate tissue remodeling in homeostasis and injury is an area of active investigation (28), and much of the focus of the recent literature has been on the ontogeny of macrophages that subserve various functions in pathologic states. A major emerging theme is the role of moMacs, which support a range of pathologic phenotypes in multiple disease models (29, 30). Lung fibrosis is no exception, with monocyte-lineage cells being shown to be associated with poor outcomes in multiple clinical cohorts (31) and in mouse models including our own prior work showing a profibrotic effect of moMacs (1–3, 6). This pathologic effect of macrophages has been proposed to be related to macrophage-derived factors that both support fibroblast growth and lead to fibroblast activation, including TGFβ, PDGF, and other secreted factors (4–6).
Connexin hemichannel-mediated ATP efflux as a paracrine signaling mechanism has been described in multiple contexts (10, 11, 32). Here we found that Cx43 was necessary for lung moMac ATP efflux after bleomycin injury. Furthermore, deletion of Cx43 in moMacs decreased fibrosis, and we noted decreased cytosolic calcium in fibroblasts co-cultured with Cx43 KO moMacs. Both human and mouse lung fibroblasts had increased expression of collagen in response to ATPγS treatment. Thus, we tested the role in lung fibrosis of ATP receptor P2rx4, which we found was the most highly expressed ionotropic ATP receptor in both murine and human lung fibroblasts. Furthermore, fibroblast P2rx4 was upregulated in lung fibrosis and has previously been found both to have a pro-fibrotic role and to be necessary for fibroblast calcium in liver fibrosis (27). Using two different fibroblast Cre drivers, we found that fibroblast-specific deletion of P2rx4 decreased lung collagen in mice, both at the cellular and
whole-lung levels. Taken together, these results motivate enthusiasm for therapeutic development of inhibitors to P2rx4, some of which exist but are of relatively low potency (33). Moreover, they support a novel hypothesis of profibrotic macrophage-fibroblast interaction based on macrophage efflux of ATP via connexin hemichannels.

Our study has several limitations. While the finding of elevated fibroblast cytosolic calcium dependent on macrophage co-culture was acquired in vitro, importantly, we also observed injury-induced calcium elevation in fibroblasts in precision-cut lung slices, a closer reflection of the biology of intact tissue. However, future studies should seek to confirm specifically within the context of lung slice imaging the dependence of this fibroblast calcium elevation on macrophages and on extracellular ATP, since other paracrine or cell autonomous factors may be relevant. Furthermore, our in vitro data do not rule out the

**FIGURE 2** MoMac Cx43 is required for lung fibrosis. (A) Schematic for testing the effect of macrophage-specific Cx43 deletion on lung collagen after bleomycin injury with Cx3cr1-CreERT2: Cx43 fl/fl mice and controls. (B) qPCR of Cx43 expression in sorted tdTomato+ macrophages from Cx3cr1-CreERT2: R26-LSL-tomato: Cx43 fl/fl mice and Cx3cr1-CreERT2: R26-LSL-tomato controls. N=3 mice per condition. P value is for unpaired one-tailed t-test (left). Hydroxyproline assay of whole lung collagen from Cx3cr1-CreERT2: Cx43 fl/fl mice and Cx3cr1-CreERT2 controls. N=4, 9, and 8 mice as shown. P value is for one-way ANOVA followed by Kruskal-Wallis multiple comparison test (right).

**FIGURE 3** P2rx4 is the most highly expressed ionotropic ATP receptor in lung fibroblasts. (A) UMAP plot of mouse fibroblasts from Tsukui et al. Dot plot shows expression of ionotropic ATP receptors. (B) UMAP plot of human fibroblasts from Tsukui et al. Dot plot shows expression of ionotropic ATP receptors. (C) qPCR of ionotropic ATP receptors in primary mouse lung fibroblasts isolated at steady state. N=3 mice.
possibility that macrophage Cx43 could have other effects separate from ATP efflux, including gap junctional communication or secretion of other pro-fibrotic mediators that may regulate cytosolic calcium in adjacent fibroblasts in a paracrine manner. We add that it remains to be determined what initiates ATP efflux by macrophages, and whether the resolution of fibrosis results from downregulation of this signaling. The use of two Cre drivers for our analysis of the effects of fibroblast P2rx4 also deserves comment. We observed modest P2rx4 knockout in fibroblasts in aggregate with the Col1a2-CreERT2, which is likely due to mosaic expression with this driver, although it was reassuring to find consistent effects with the Pdgfrb-Cre, which achieved more marked knockout in fibroblasts in aggregate. Finally, the pathways downstream of P2rx4 that drive expression of collagen genes in fibroblasts, including the role of calcium and p38 MAP kinase signaling, will require further elucidation. Despite these limitations, our results reveal a novel role for macrophages in paracrine regulation of fibroblast calcium, as well as a specific dependency on Cx43, and raise the profile of P2rx4 as a potential therapeutic target for lung fibrosis.
DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by UCSF Institutional Review Board. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by UCSF Institutional Animal Care and Use Program.

AUTHOR CONTRIBUTIONS

AB, PT, and PY performed all experiments under the guidance of MB. Imaging experiments were performed by PT and AB under the direction of JJ, X-ZT, and CA. KB and TC prepared breeding and MB. Imaging experiments were performed by PT and AB under the guidance of PT and MB. RM contributed

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.880887/full#supplementary-material

Supplementary Video 1 | 5-minute time-lapse video corresponding to Figure 1B, with images acquired every 5 seconds by two-photon calcium imaging of precision-cut lung slices from steady-state Col1a2-CreERT2: R26-LSL-Salsa6f (tdTomato–GCaMP); MacBlue mice. Scale is as for Supplementary Video 2.

Supplementary Video 2 | 20-minute time-lapse video corresponding to Figure 1B, with images acquired every 5 seconds by two-photon calcium imaging of precision-cut lung slices from Col1a2-CreERT2: R26-LSL-Salsa6f(tdTomato–GCaMP); MacBlue mice 7 days post-bleomycin injury. Scale is as for Supplementary Video 2.

SUPPLEMENTARY MATERIAL

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.
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