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Article

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Posted Date: September 1st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10626944/v1>

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Additional Declarations: No competing interests reported.

Matricytosis is a phenotype-specific ECM internalization program dysregulated across macrophages and fibroblasts in pulmonary fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is characterized by persistent extracellular matrix (ECM) accumulation, yet the mechanisms governing ECM clearance remain poorly understood. In this study we investigate matricytosis, a program of ECM uptake and lysosomal degradation, and its status in disease. We show that matricytosis is highly phenotype-dependent in human macrophages and fibroblasts: disease-associated macrophages have impaired ECM internalization, while myofibroblasts demonstrate increased uptake. Inhibition studies, single-cell RNA sequencing pathway analysis and spatial transcriptomics reveal distinct uptake mechanisms for each cell type and highlight macrophages as dominant uptake-proficient cells in both healthy and fibrotic lung tissue, while fibroblast matricytosis is linked to invasive programs in IPF. Using human decellularized precision-cut lung slices, we demonstrate that fibrotic matrix affects matricytosis and modulates cell behavior. In this work, we identify matricytosis as a fibrosis-relevant mechanism shaped by cell type, phenotype, and matrix environment, pointing to a potential therapeutic axis.

Keywords

Pulmonary fibrosis, extracellular matrix remodeling, macrophages, fibroblasts, spatial transcriptomic analysis, live cell imaging

Introduction

Idiopathic pulmonary fibrosis (IPF) is a devastating interstitial lung disease marked by progressive deposition of extracellular matrix (ECM), destruction of lung architecture, and steady decline in respiratory function [1]. While it is well established that myofibroblasts produce excessive ECM in IPF, the role of ECM degradation has received far less attention [2,3]. As a result, the field largely views fibrosis as a disease of overproduction rather than a failure of clearance [4]. However, ECM homeostasis is inherently a balance between synthesis and degradation, and distortion of either arm can drive pathological accumulation, leading to pulmonary dysfunction [3]. Consistent with this, ECM-derived fragments have emerged as biomarkers in IPF, supporting the clinical relevance of matrix turnover in disease [5,6]. Classical ECM degradation is attributed primarily to extracellular proteases, including matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs), cathepsins, and other matrix-cleaving enzymes [7]. Yet increasing evidence suggests that cell-mediated internalization represents a parallel and potentially equally important mechanism of matrix turnover [8,9]. Notably, matrix uptake is not governed by a single route but instead involves multiple pathways and a degree of receptor specificity. These receptors recognize distinct ECM components, thereby enabling selective trafficking of collagen, fibronectin, or other matrix proteins. These matrix cargos are routed to the lysosomal compartment for enzymatic degradation following internalization [4,9–11]. Spatial transcriptomic analyses revealed that these ECM-receptor interactions are the primary mode of communication in the fibrotic niche, and such receptor engagement may represent the upstream cue that initiates ECM internalization in IPF [12]. However, ECM internalization has not been systematically studied in lung fibrosis, nor is it known whether this process exists broadly across cell types, how it varies with phenotype, or how it becomes dysregulated in IPF.

Tissue remodeling is governed by macrophages and fibroblasts, which coordinate ECM turnover and the processes of tissue repair and resolution [13–16]. Macrophages orchestrate clearance of apoptotic cells through efferocytosis, as well as pathogens via phagocytosis, along with processing released matrix [16]. Fibroblasts synthesize, assemble, and remodel ECM [3]. Yet whether macrophages and fibroblasts directly engulf ECM, and whether this capacity is influenced by activation state, remains unknown in IPF. Notably, macrophages in this disease context display impaired efferocytosis and phagocytosis alongside increased apoptotic cell and bacterial burden [17–19], suggesting that altered ECM clearance by phagocytes may contribute to net matrix accumulation. Beyond macrophages, increasing evidence indicates that subsets of fibroblasts in fibrotic disease adopt programs reminiscent of invasive stromal cells, with actin-rich protrusive structures [20–23]. Such invasion-

associated phenotypes imply that fibroblasts are not only depositors but also active handlers of ECM, capable of internalizing matrix components as part of a broader remodeling program.

Here, we use the term matricytosis to denote the internalization and lysosomal degradation of ECM. By establishing an imaging-based quantitative assay to measure matricytosis using decellularized ECM (dECM), native ECM proteins and human decellularized precision-cut lung slices (dPCLS), we reveal that matricytosis is a phenotype-specific and disease-modulated process of ECM clearance in both macrophages and fibroblasts. We show that matricytosis is impaired in disease-associated macrophages but enhanced in myofibroblasts. Mechanistically, this process diverges across cell types and is further sculpted by the fibrotic extracellular matrix.

Together, these findings establish matricytosis as a systematically defined, cross-cell-type ECM-clearance program whose magnitude and mechanism depend on effector phenotype, disease state, and matrix context. By integrating these *in vitro* signatures with single-cell and spatial transcriptomic datasets, we demonstrate that matricytosis maps onto *ex vivo* fibrotic niches and aligns with macrophage and fibroblast states enriched in IPF. Finally, we show that matricytosis is pharmacologically responsive, positioning this pathway as a modifiable and previously underrecognized contributor to ECM turnover in lung fibrosis. This reframes fibrosis as a disease not only of excessive ECM synthesis but also of imbalanced clearance, with matricytosis emerging as promising therapeutic axis.

Results

Matricytosis is a conserved but phenotype-specific ECM internalization program in macrophages and fibroblasts

To determine whether human macrophages and lung fibroblasts internalize ECM, we first established a quantitative fluorescence-based matricytosis assay using pH-sensitive labeling of *in vitro*-derived decellularized ECM (dECM) produced by myofibroblasts and purified collagen I. Live-cell imaging revealed robust accumulation of pHrodo fluorescence in macrophages and fibroblasts over time, indicating uptake of ECM cargo and trafficking into acidic compartments (Supplementary Figure S1 a-f). Confocal microscopy demonstrated strong co-localization between intracellular pHrodo signal and LysoTracker, confirming lysosomal targeting of engulfed ECM (Figure 1 a, d) in both cell types. Together, these observations establish matricytosis as a bona fide lysosomal ECM degradation pathway.

We validated specificity and interpretability of the assay in several ways. Inhibition of actin-dependent internalization with cytochalasin D substantially reduced pHrodo accumulation in both macrophages and fibroblasts, establishing that the assay readout

reflects active intracellular uptake rather than passive association (Supplementary Figure S1 a-f). Imaging of dECM in the absence of cells confirmed fluorophore labeling and allowed discrimination of extracellular substrate signal from cell-associated fluorescence. pHrodo-labeled dECM exhibited minimal fluorescence at neutral pH (pH 7.4) and increased fluorescence under acidic conditions (pH 4.5), consistent with detection of cargo delivered to endo-lysosomal compartments (Supplementary Figure S1 g). Furthermore, quantitative assessment of collagen I fiber morphology revealed that cellular presence altered substrate architecture in terms of fiber length, consistent with active cell-matrix engagement during uptake (Supplementary Figure S1 h, i). Collectively, these validation steps establish a robust platform to quantify ECM internalization across diverse cell states.

We further characterized the dECM produced by myofibroblasts under control and transforming growth factor beta 1 (TGF- β 1) conditions using immunostaining for homeostasis- and disease-relevant matrix proteins and (myo-)fibroblast markers (Supplementary Figure S2). TGF- β 1 treatment increased markers consistent with myofibroblast differentiation (e.g., alpha smooth muscle actin (α SMA), collagen triple helix repeat-containing protein 1 (CTHRC1)) and altered matrix composition, including increased fibrillar matrix components (e.g., collagens, fibronectin, tenascin C); and reduced basement membrane components (collagen IV, laminin). Quantification of matrix staining supported these qualitative trends and confirmed that fibroblast activation produces a distinct ECM environment relevant to fibrotic lung remodeling [24]. Proteomic characterization of the ECM further supported overlap between the *in vitro*-derived matrix and *ex vivo* ECM signatures reported in IPF relevant resources [24] (Supplementary Figure S3 a). Pathway enrichment analyses highlighted ECM organization and collagen related pathways among regulated components, and mapping deregulated proteins onto the Naba matrisome [25] confirmed alignment with fibrotic ECM (Supplementary Figure S3 b, c). Mapping the signature of the *in vitro* produced dECM to a spatial transcriptomic atlas of healthy and IPF tissue [12] demonstrated correspondence with the fibrotic niche and its adjacent environments (Supplementary Figure S3 d, e).

Using this validated assay, we next asked whether matricytosis differs across disease-relevant phenotypes in macrophages and fibroblasts. Macrophages internalized both fibroblast-derived dECM and collagen I; however, uptake capacity was strongly phenotype-dependent (Figure 1 b, c). Control macrophages showed efficient ECM uptake, whereas disease-associated polarization states, including pro-inflammatory macrophages induced by lipopolysaccharide (LPS) or pro-fibrotic macrophages induced by combination of interleukin-4 (IL-4), IL-13 and tumor necrosis factor alpha (TNF- α) [26], displayed markedly reduced matricytosis, as reflected by diminished uptake kinetics and reduced area under the curve (AUC) after 24 h. This reduction parallels known impairments in efferocytosis and phagocytosis in

IPF-associated macrophages [17–19], suggesting shared polarization-dependent vulnerabilities in clearance programs. High-resolution imaging further confirmed intracellular localization of pHrodo signal in macrophages and co-localization with lysosomal compartments (Figure 1 a). Fibroblasts likewise internalized dECM and collagen I but exhibited the opposite phenotype-dependency. TGF- β 1-activated fibroblasts (myofibroblasts) tended to show higher matricytosis capacity than control fibroblasts on dECM, whereas collagen I uptake was significantly increased, as reflected by greater pHrodo signal accumulation over time and increased AUC values after 24 h (Figure 1 e, f). Confocal imaging demonstrated co-localization of engulfed ECM with LysoTracker in fibroblasts, confirming endo-lysosomal routing analogous to macrophage uptake (Figure 1 d). These findings align with the invasive, ECM-remodeling behavior of myofibroblasts and highlight matricytosis as a component of their pro-fibrotic state.

Since differences in dECM uptake across phenotypes could theoretically reflect variability in extracellular cleavage rather than uptake per se, we examined whether protease abundance mirrors matricytosis capacity. Spatial transcriptomics revealed that ADAMs, MMPs and tissue inhibitors of metalloproteinases (TIMPs) exhibit compartment- and disease-dependent expression patterns in lung tissue (Supplementary Figure S4 a, b). Proteomic and secretomic profiling similarly identified phenotype-dependent differences in matrix-processing enzymes in both macrophages and fibroblasts (Supplementary Figure S4 c, d). However, phenotype-specific differences in matricytosis persisted even when using pre-cleaved ECM fragments (collagen I, collagen IV, laminin, and fibronectin), suggesting that uptake programs substantially shape matricytosis capacity, in addition to any contribution from extracellular proteolysis (Figure 1 c, f; Supplementary Figure S5 a-c and f-h). Using these purified ECM components, we also found that both macrophages and fibroblasts displayed qualitatively similar uptake across substrates. This demonstrates that matricytosis reflects a broader ECM-internalization program and is not restricted to specific ECM substrates.

Finally, because macrophage dysfunction in IPF is frequently framed in terms of impaired phagocytosis and efferocytosis, we compared these classical uptake pathways directly to matricytosis. Macrophages retained robust efferocytotic and phagocytotic activity across polarization states, with cargo-independent variations consistent with established macrophage specialization (Supplementary Figure S5 d, e). In contrast, fibroblasts displayed minimal phagocytosis or efferocytosis relative to macrophages, with no significant change between the TGF- β 1 and control condition (Supplementary Figure S5 i, j). These observations reinforce that fibroblast matricytosis does not represent a generalized acquisition of professional phagocyte-like functions, but rather a distinct ECM-handling behavior.

Together, these results define matricytosis as a regulated, phenotype-dependent ECM-handling program present in macrophages and fibroblasts. Disease-associated macrophage states exhibit impaired matricytosis, whereas pro-fibrotic fibroblasts show enhanced ECM internalization, underscoring distinct and opposing contributions to matrix turnover across cell types.

Distinct molecular pathways mediate matricytosis in macrophages and fibroblasts

To identify the molecular pathways that mediate matricytosis in macrophages and fibroblasts, we performed targeted perturbation studies using collagen I as a model substrate (Figure 2 a, b). These experiments revealed cell type-specific sensitivities to pathway interference. Moreover, treatment with the anti-fibrotic drug Nintedanib significantly reduced matricytosis in both macrophages and fibroblasts. These findings show that ECM internalization is drug-responsive and that common druggable nodes exist. To place these functional data into a disease context, we used scRNA-seq-based pathway scoring to map uptake mechanism signatures across compartments and macrophage/fibroblast subsets in healthy and fibrotic lung tissue based on a PF-ILD atlas [12] (Figure 2 c-f). These analyses supported compartment- and phenotype-specific enrichment of uptake programs, reinforcing the idea that matricytosis is mediated through distinct, cell type biased molecular modules that can be altered in disease.

Macrophages are dominant engulfing cells in healthy and fibrotic lung tissue, while fibroblast matricytosis aligns with invadosome-like transcriptional programs enriched in IPF

Next, we sought to investigate matricytosis in health and disease. To this end, we examined scRNA-seq and spatial transcriptomic datasets from healthy and IPF lungs [12] and applied an uptake score consisting of genes responsible for cargo uptake and lysosomal processing. Uptake pathway scoring revealed that macrophages consistently exhibit the highest uptake scores across lung compartments, consistent with professional scavenging specialization (Figure 3 a-c, e, f). Spatial transcriptomics confirmed the presence of tissue regions enriched in uptake-associated macrophage signatures, reinforcing macrophages as dominant engulfing cells *ex vivo* (Figure 3 c, f). These findings align with the notion that macrophages contribute significantly to ECM processing in tissue homeostasis and that alteration of this role may contribute to pathological ECM accumulation in fibrosis. Importantly, both scRNA-seq and spatial transcriptomic analyses showed that macrophages retain high uptake signatures not only in healthy lungs but also in IPF tissue (Figure 3 b, d, f), indicating that macrophages remain major engulfing cells even under fibrotic conditions. More precisely, spatial analysis revealed that under healthy conditions, alveolar macrophages scored highest and are evenly

spread throughout the tissue. In IPF, alveolar macrophages are still uptake-proficient shown by the scoring. However, the regions with the highest uptake score co-localize with the airway macrophage niche (Figure 3 d), which harbors pro-fibrotic macrophage populations and is adjacent to the fibrotic niche with areas of active remodeling, as previously shown by Mayr *et. al* [12].

In addition, we evaluated both an invadosome and fibrosis score based on Lebel *et. al* [22] using scRNA-seq (Figure 4 a, d, e) and spatial transcriptomics data (Figure 4 b, c, f) and observed enrichment of invasive remodeling programs in IPF fibroblast states and fibrotic regions. Importantly, spatial mapping revealed that areas with high uptake-associated signatures co-localized with regions exhibiting high fibrosis and high invadosome scores, indicating that uptake-competent cell populations are positioned within the most pathologically remodeled niches of the IPF lung (Figure 3 d, Figure 4 c). Moreover, these invasive signatures showed overlap with uptake-associated pathways (including Endo180 linked modules, see Figure 2 c), consistent with a coupled program of invasion and ECM handling. Across these datasets, MMP2 within the invadosome signature was elevated in IPF conditions (Figure 4 e). This enzyme is mainly responsible for cleavage of type IV and type V collagen, laminin, fibronectin, and elastin. MMP2 is frequently deregulated in fibrotic diseases and cancer, driving excessive ECM remodeling that contributes to fibrosis and tumor progression [27]. *In vitro*, MMP2 promoted dECM internalization in fibroblasts and enhanced matrix remodeling, as reflected by increased pHrodo labeled fiber signal accumulation and altered collagen IV fiber area/architecture (Supplementary Figure S6 a-f). This suggests that ECM breakdown and uptake are mechanistically linked within an invasive myofibroblast program, which is facilitated by MMP2. To link lung myofibroblast behavior to the invasive properties of mesenchymal cancer cells, we mapped the signature of cancer-associated fibroblasts (CAFs) on the spatial transcriptomic atlas from healthy and IPF lungs [12] (Supplementary Figure S6 g). The signature of these CAFs showed strong alignment to the fibrotic niche and to the fibroblast and mixed smooth muscle/adventitial/mesothelial niche. Expanding upon these findings, we further examined the cellular signature of fibroblasts and macrophages after matricytosis derived from post-uptake proteomics (matricytosis signature; Supplementary Figure S7). Spatial projection analysis indicated a preferential association of the matricytosis signature of both cell types with the fibrotic niche, with additional signal detected in the mixed smooth muscle/adventitial/mesothelial niche in IPF tissue, comparable to CAFs. Within the analyzed samples, this matricytosis signature was exclusively observed in IPF tissue, whereas it was not detected in healthy lung samples.

Together, these findings demonstrate that ECM engulfment triggers a shared matricytosis program in fibroblasts and macrophages, and that this program maps predominantly to fibrotic

niches in IPF, linking matrix uptake to the spatially organized cellular states that drive progressive lung fibrosis.

IPF-derived matrix modulates matricytosis and phenotype-specific behavior

To evaluate matricytosis within physiologically relevant matrix architecture, we developed a matricytosis assay using pHrodo labeled decellularized precision-cut lung slices (dPCLS) from healthy and IPF lungs. These tissue-scale matrices showed massive ECM accumulation and altered composition in IPF dPCLS, including increases in collagen I, fibronectin, and region-specific changes in basement membrane integrity (Supplementary Figure S8). Macrophages displayed a trend towards higher matricytosis on IPF dPCLS compared to healthy slices (Figure 5). Notably, the polarization-dependent differences observed *in vitro* were only visible when using healthy ECM, and less pronounced with the fibrotic ECM. This indicates that ECM architecture and composition override classical macrophage polarization cues, suggesting that the fibrotic matrix microenvironment is a dominant determinant of macrophage uptake behavior. TGF- β 1-activated fibroblasts exhibited higher matricytosis, especially on IPF dPCLS, suggesting that fibrotic matrix may reinforce fibroblast matricytosis. These findings support a model in which matricytosis is governed by both cellular and matrix state, and that disease-derived ECM imposes constraints and cues that reshape ECM uptake behavior in a cell type-specific manner. Moreover, it validates matricytosis as a physiologically relevant process in fibrotic lung tissue.

Discussion

While ECM internalization is a well-described process in invasive cancer cell biology [28–31], its contribution to IPF is largely unexplored. However, evidence from murine models indicates that collagen uptake pathways do influence fibrotic progression, as shown by studies demonstrating that impaired collagen internalization exacerbates lung fibrosis [32,33]. Here, we examine this ECM-uptake program, referred to as matricytosis, in macrophages and fibroblasts and characterize how it is modulated across disease-relevant phenotypes and IPF-derived matrix environments. Our findings broaden the current understanding of ECM turnover in IPF, a field that has historically focused on excessive synthesis rather than impaired or imbalanced intracellular degradation [2–4]. By demonstrating that ECM uptake is an active, regulated process that is modulated by both cell-intrinsic phenotype and the extracellular matrix microenvironment, our work reframes fibrosis as a disease in which disrupted clearance contributes substantially to pathological matrix accumulation.

Our study confirms that matricytosis is a bona fide lysosomal degradation pathway distinct from classical proteolytic mechanisms relying on extracellular degradation [34–36]. The *in vitro*

generated dECM provides a relevant approximation of the remodeled matrix environment in IPF, capturing key compositional features of diseased tissue while allowing controlled interrogation of ECM uptake. In addition to increased fibrillar matrix components, the reduction in basement membrane proteins such as collagen IV and laminin points to a loss of homeostatic matrix architecture and a shift towards a pathologic fibrotic state [24,37]. This remodeling may shape matricytosis by altering both substrate availability and cell-matrix interactions, thereby influencing how different cell types engage with and internalize ECM. Both macrophages and fibroblasts internalized dECM and purified ECM proteins, with intracellular trafficking confirmed through pH-sensitive labeling and co-localization with lysosomal compartments. This aligns with earlier reports that ECM internalization contributes to matrix turnover in other tissues, including cancer and stromal remodeling [8,9,35,38,39]. In cancer, endocytosis-based collagen degradation has been reported in invasive fibroblasts, where the collagen receptor Endo180 (also designated: urokinase plasminogen activator receptor-associated protein (uPARAP)) directs uptake; mannose receptor 1 (MRC1)-dependent collagen internalization has been described for macrophages in the tumor milieu [9,28–31,39,40]. However, in lung fibrosis, ECM uptake has not previously been systematically analyzed.

A major finding of our study is the strong phenotype-dependency of matricytosis. Disease-associated macrophage states, whether pro-inflammatory or pro-fibrotic, displayed markedly reduced ECM uptake *in vitro*, paralleling described defects in efferocytosis and phagocytosis in IPF macrophages [17–19]. This suggests a broader polarization-dependent defect in cellular clearance programs that may contribute to ECM accumulation in the fibrotic lung. In contrast, TGF- β 1-activated myofibroblasts exhibited robustly increased matricytosis, consistent with their invasive and matrix-remodeling states [21–23]. This cell-type divergence underscores that macrophages and fibroblasts do not simply contribute additively to ECM turnover but rather exhibit opposing alterations in IPF that may together exacerbate pathological matrix deposition.

We further demonstrate that matricytosis is driven by distinct molecular modules in macrophages versus fibroblasts. Pathway enrichment and perturbation analyses highlight cell type-specific dependencies on distinct routes. These data suggest that matricytosis represents not a single pathway but a family of ECM-internalization programs whose composition varies with cell identity and activation state. The sensitivity of matricytosis to Nintedanib further suggests that anti-fibrotic therapies may act in part by modulating ECM uptake, a possibility that warrants future mechanistic investigation. The integration of scRNA-seq and spatial transcriptomics offers important physiological context for these mechanistic insights.

Macrophages emerged as the dominant uptake-competent cells across lung compartments in healthy and fibrotic tissue, supporting their long-recognized role in clearance and tissue homeostasis [41–43]. Notably, macrophages retained high uptake signatures in IPF lungs despite reduced uptake capacities *in vitro*, suggesting that cues from the fibrotic ECM niche influence cellular behavior *in vivo*, either by maintaining uptake activity or enriching for uptake-competent subsets. In addition, spatial mapping indicated a redistribution of macrophage populations in IPF, as previously reported [12], which is captured by the applied uptake score: In a healthy state, alveolar macrophages are shown to be broadly distributed in the tissue and exhibited the highest uptake score. In contrast, IPF tissue showed an expansion of the disease-specific airway macrophage niche adjacent to fibrotic remodeling regions [12] which displayed the strongest enrichment of the uptake score. Moreover, regions with high fibrosis scores aligned with areas exhibiting high uptake-associated signatures and invadosome-like programs [20–23]. This pattern may reflect increased ECM abundance, enrichment of myofibroblasts with intrinsically higher matricytosis capacity, or coupling of matrix uptake to disease-promoting remodeling programs. Together, these observations place uptake capacity and invasive remodeling within discrete fibrotic microenvironments. The invasive fibroblast phenotype was accompanied by enhanced MMP2 activity, linking extracellular matrix cleavage, internalization, and remodeling within a coordinated program. The convergence of these lung myofibroblasts with CAFs suggests that key aspects of CAF biology, such as invasiveness, may be mirrored in fibrotic lung disease. This reinforces the concept that myofibroblasts are not merely matrix producers but also active modifiers and consumers of ECM. To better understand whether matricytosis is associated with beneficial or disease-promoting programs in IPF, we next evaluated the cellular signatures post matricytosis. Both cell types indicated a preferential association of the resulting matricytosis signature with the fibrotic niche. The absence of a corresponding signature in healthy tissue indicates that this program is specifically induced by the fibrotic microenvironment. These observations support a potential feed-forward mechanism whereby myofibroblast-mediated ECM deposition and remodeling increase matrix availability for uptake; matricytosis in turn generates bioactive cues that could sustain pro-fibrotic activation, promote chemotaxis, and support cellular retention within fibrotic regions.

Finally, our data show that ECM architecture and composition shape matricytosis in an *ex vivo* matrix scaffold context. Using decellularized precision-cut lung slices, we found that fibrotic ECM altered macrophage uptake behavior, overriding polarization-dependent differences and directing matricytosis. This highlights the dominant regulatory influence of the ECM microenvironment in IPF, consistent with the notion that both matrix stiffening and compositional shifts feed back onto cell behavior [37,44–48]. In this context, the prominence

of ECM-receptor interactions in the fibrotic niche [12,49] may represent a key upstream driver of matrix uptake pathways, potentially integrating mechanical and biochemical cues that initiate matricytosis. The apparent discrepancy between controlled *in vitro* assays and tissue-level observations likely reflects the integration of intrinsic uptake programs with environmental constraints. *In vitro*, matricytosis can be isolated as a cell-intrinsic property. However, in tissue contexts, macrophages operate within a niche, where both ECM abundance, its composition and microenvironmental cues shape effective uptake behavior. Thus, the reduced uptake capacity observed in disease-associated macrophages may coexist with increased total ECM internalization at the tissue level; caused by overwhelming substrate availability, altered ECM architecture, and spatial enrichment of uptake-competent cell subsets.

Despite these insights, several considerations temper the interpretation of our findings. First, while our *in vitro* matricytosis assay allows controlled quantification of ECM uptake, it cannot fully recapitulate the dynamic multicellular interactions present *in vivo*. Second, although dECM and dPCLS systems preserve aspects of native matrix architecture, they lack cellular feedback loops, mechanical forces, and immune-stromal crosstalk that influence ECM turnover in intact tissue [50,51]. Third, our analyses focus on macrophages and fibroblasts, but other cell types, such as epithelial and endothelial cells may also contribute to ECM internalization and warrant future investigation. Fourth, our uptake score panel is not restricted to matricytosis-specific factors. Many receptors and pathway constituents included in the signature are shared across diverse forms of cargo uptake (e.g., apoptotic cells, microbial components, lipoproteins, debris, and ECM), which is particularly relevant for macrophages whose uptake machinery is inherently multifunctional [9,52–56]. As such, the score reflects a broader engulfment competency rather than a purely matricytotic program. Finally, while our scRNA-seq and spatial transcriptomic analyses map uptake-related signatures across tissue niches, they cannot directly quantify ECM internalization *in vivo*.

Together, our findings position matricytosis as a central and previously underappreciated mechanism of ECM turnover in the context of IPF. Impaired intrinsic macrophage matricytosis in defined *in vitro* states, combined with enhanced fibroblast matricytosis, may shift the balance of ECM handling in IPF. However, high uptake signatures observed in fibrotic tissue and with IPF-derived matrix indicate that these programs are strongly shaped by niche context. Our findings point to therapeutic opportunities in uptake-associated pathways and fibroblast invasive remodeling programs, while underscoring the importance of understanding how the fibrotic niche modulates macrophage matricytosis. Overall, we define matricytosis as a context- and phenotype-dependent ECM internalization program whose functional consequences depend on matrix availability, cellular state, and microenvironmental

constraints. Importantly, our findings also raise important open questions: How does matrix uptake reshape the phenotypic state of individual cells, and to what extent does this influence the behavior of neighboring cells within the fibrotic niche and beyond? Moreover, is matricytosis ultimately beneficial or detrimental for IPF progression, and does its impact depend on which cell type engages in matrix internalization? Answering these questions is key to identifying ways to target matricytosis for new fibrosis treatments.

Methods

Ethical approval

Lung tissue samples from patients were obtained from the German Centre for Lung Research (DZL/BREATH) at the Hannover Medical School (MHH), Germany to generate precision-cut lung slices. All experimental procedures involving human participants and human-derived tissues were reviewed and approved by the Ethics Committee of the German Centre for Lung Research (DZL/BREATH; approval number 10194_B0_K_2022). Human lung tissue samples were obtained through Hannover Medical School (MHH), Germany. All methods and experimental procedures were performed in accordance with the Declaration of Helsinki and all relevant institutional, national, and international guidelines and regulations. Written informed consent was obtained from all participants for the use of their biological material in research. No organs or tissues used in this study were procured from prisoners. In this study, tumor resections were defined as portions of resected tissue characterized as healthy lung parenchyma by pathologists (MHH) based on macroscopic assessment.

Cell culture of macrophages and fibroblasts

To generate human induced pluripotent stem cells (hiPSC)-derived macrophages (iDMs), we used a previously published protocol using the human iPSC line 201B7 (Cellosaurus; CVCL_A324) [57]. This generated cell line is an established human iPSC line that was used in accordance with the conditions and ethical approvals described in the original publication. During experiments, macrophages were cultured in DMEM (cat. 31966021, Gibco), supplemented with 10 % heat-inactivated fetal bovine serum (HI-FBS; cat. 16140071, Gibco), 1 % non-essential amino acids (NEAA; cat. 11140035, Gibco), 1 % Penicillin-Streptomycin (Pen/Strep; cat. 15140-122, Gibco) and 50 ng/ml Macrophage colony-stimulating factor (M-CSF; cat. 130-096-493, Miltenyi Biotec). Detachment was carried out with a cell scraper (cat. 83.1831, Sarstedt).

Normal HLFs (NHLFs; Lonza, cat. CC-2512) and IPF HLFs (Lonza, cat. CC-7231) were obtained commercially from Lonza and used according to the supplier's terms and conditions. No additional donor-use restrictions relevant to this study were reported by the supplier. The

cells were thawed at passage 4 (P4) and used for experiments between P5-P7. Cells were expanded in T75 (cat. 156499, Thermo Fisher Scientific) or T175 flasks (cat. 10127340, Thermo Scientific) using Fibroblast Basal Medium (FBM; cat. CC-3131, Lonza) supplemented with the Fibroblast Growth Medium (FGM) FGM®-2 SingleQuots® Supplement Pack (FGM, cat. CC-3132; Lonza) containing 2 % HI-FBS, Insulin, human fibroblast growth factor (hFGF) and Gentamicin Sulfate-Amphotericin (GA-1000). Cells were maintained in a humidified incubator at 37 °C and 5 % CO₂. For experimental use, fibroblasts were washed with 1x phosphate-buffered saline (PBS; cat. 10010023, Gibco) and detached with Accutase (cat. 00-4555-56, Invitrogen) for 1-2 min at 37 °C. Detachment was stopped by adding FGM.

Cell tracking and lysosomal staining

To enable tracking in uptake assays, cells were resuspended in respective serum-free complete medium containing 1 µM CellTracker™ Green CMFDA Dye (cat. C7025, Invitrogen) and incubated for 45 min at 37 °C and 5 % CO₂. To stain lysosomes, 50 nM LysoTracker™ Deep Red (cat. L12492, Invitrogen) was added to cell cultures in cell medium (1 h, 37 °C), followed by a washing step with 1x DPBS.

Phenotype induction in macrophage and fibroblasts

After cell labeling, macrophages were seeded in 96-well plates (cat. 161093, Thermo Scientific) with 15,000 cells/well, or in 6-well UpCell plates (cat. 174901, Thermo Scientific) at 600,000 cells/well. Macrophages were treated with either LPS, IL4+IL13 or IL4+IL13+TNFα as outlined in Table 1 for 24 h. Untreated macrophages were used as control, while M-CSF was contained in every condition to maintain the macrophage's functionality.

To induce fibroblast-to-myofibroblast transition (FMT), cells were seeded at 200,000 cells/well in 6-well plates (cat. 140675, Thermo Scientific), 6,000 cells/well in 96-well plates, or 1,500 cells/well in 384-well plates (cat. 6057328, Revvity). After 24 h of culture, fibroblasts were washed three times with a Hanks' Balanced Salt Solution (HBSS; cat. 14025092, Gibco) buffer, supplemented with 10 mM HEPES (cat. 15630080, Gibco) and 0.0375 % sodium bicarbonate (cat. 15424879, Thermo Scientific), and then and serum-starved in FBM for 24 h. The cells were then stimulated with recombinant human TGF-β1 (Table 1) in FBM for 72 h.

453 **Table 1. Reagents used to induce cell phenotypes.**

Treatment	Concentration	Catalog#	Manufacturer
LPS (<i>Salmonella enterica</i>)	10 ng/ml	L6143-1MG	Sigma-Aldrich
IL4	10 ng/ml	6507-IL/CF	R&D Systems
IL13	10 ng/ml	213-ILB/CF	R&D Systems
TNF α	10 ng/ml	10291-TA/CF	R&D Systems
TGF- β 1	5 ng/ml	240-B-010	R&D Systems

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455 **Production of decellularized ECM**

456 To generate decellularized ECM, fibroblasts were cultured in 384-well (cat. 6057500 , Revvity)
 457 or 96-well (cat. 6005710, Revvity) poly-D-lysine (PDL)-coated ViewPlates. Wells were seeded
 458 with 1,500 cells in 50 μ l of FGM (384-well plates), or 6,000 cells in 100 μ l FGM (96-well plates)
 459 and incubated for 16-24 h at 37 °C. The following day (day 1), cells were washed three times
 460 using a BioTek microplate washer (Agilent) with a HBSS buffer, supplemented with 10 mM
 461 HEPES and 0.0375 % sodium bicarbonate. Subsequently, cells were starved by adding 60 μ l
 462 of FBM supplemented with GA-1000 per well, followed by incubation for 16-24 h. On day 2,
 463 the cells were treated with 5 ng/ml TGF- β 1 to FMT and support matrix synthesis and
 464 deposition. To promote matrix accumulation at the well bottom, Ficoll 70 (cat. 17-0310-10,
 465 Cytiva) and Ficoll 400 (cat. 17-0300-10, Cytiva) were diluted in FBM to final concentrations of
 466 37.5 mg/ml and 25.0 mg/ml, respectively. For enhanced matrix synthesis, 200 μ M L-ascorbic
 467 acid 2-phosphate sesquimagnesium salt (Vitamin C; cat. A8960, Sigma-Aldrich) was added.
 468 After medium removal using an automated washer (BioTek), 20 μ l of the prepared stimulation
 469 medium was added to each well and then incubated for 72 h. The synthesized ECM was then
 470 either fixed with 80 μ l of 100 % ice-cold methanol (Sigma-Aldrich) for imaging analysis or
 471 washed three times with 1X DPBS using the washer in preparation for decellularization. For
 472 decellularization, 20 μ l of pre-warmed extraction buffer (1X DPBS containing 0.5 % Triton X-
 473 100 (cat. 93443, Sigma Aldrich) and 20 mM ammonium hydroxide (NH₄OH; cat.221228,
 474 Sigma Aldrich)) was added per well and incubated for 5 min at 37 °C. Plates were
 475 subsequently washed three times with 1x DPBS. Decellularized plates were either used
 476 immediately for downstream staining procedures or stored at 4 °C for up to four weeks.

477 **Generation of human decellularized precision cut lung slices**

478 Precision-cut lung slices (PCLS) from resections or IPF donors were prepared using human
 479 agarose-filled lung tissue samples utilizing the Krumdieck tissue slicer MD6000 (Alabama
 480 Research and Development) for cutting, after being loaded with 500 ml of cold PCLS medium
 481 comprising DMEM/F-12 (cat. 11039021, Gibco), 0.1 % HI-FBS (cat. 16140071, Gibco), and
 482 1 % Antibiotic-Antimycotic (cat. 15240062, Gibco). Lung cylinders with a diameter of 5 mm

were generated using a tissue coring press, and the ends were precisely trimmed with a scalpel. Slices with a thickness of 220 μm were then produced and transferred to 6-well plates (cat. 140675, Thermo Scientific) equipped with net inserts and filled with PCLS medium pre-warmed to 37°C. The PCLS were washed five times for 60 min each and subsequently incubated overnight at 37°C and 5 % CO_2 to ensure effective removal of agarose. On the following day, decellularization was commenced, with all procedures conducted under sterile conditions. The tissue slices were collected in a 50 ml tube filled with 50 ml of deionized water. The tube was placed on a tube roller and incubated at room temperature for 10 min. Following this, the water was removed and replaced with fresh deionized water, and the tube was again rolled slowly for 16 hours at 4°C to ensure thorough washing. Next, the deionized water was replaced with 50 ml of Decel-Solution A (0.1 % sodium dodecyl sulfate (cat. L3771, Sigma-Aldrich) in deionized water, warmed to 37°C), and the tube was incubated while rolling at room temperature for 2 h. This step was repeated by replacing with fresh Decel-Solution A and incubating under the same conditions for an additional 2 h. The slices were then washed twice with 50 ml sterile deionized water for 10 min each, followed by incubation in 50 ml of Decel-Solution B (1 M NaCl (cat. 73575250G, Honeywell) in deionized water) while rolling at 4°C for 16 h. After removal of Decel-Solution B, the tissue was washed with 50 ml deionized water for 10 min, then with 50 ml PBS for 10 min. Subsequently, 50 ml DNase (cat. 04716728001, Roche) was added at a final concentration of 100 $\mu\text{g}/\text{ml}$ diluted in 5 mM MgCl_2 (cat. 60-047-233, Honeywell) in deionized water. The tube was incubated with slow shaking at 37°C for 2 h, and then washed twice with 50 ml deionized water for 10 min each. Finally, the decellularized PCLS were transferred into storage solution consisting of 1 % Pen/Strep in PBS and stored in 48-well plates (cat. 152640, Thermo Scientific) at 4 °C until further use.

Labeling of matrix proteins, dECM and dPCLS with pHrodo™ dye

Matrix was labeled using the pH-sensitive fluorescent dye pHrodo™ iFL Red STP Ester (cat. P36011, Thermo Scientific), which exhibits increased fluorescence intensity under acidic conditions. For labeling of matrix proteins, protein solutions were prepared at a final concentration of 1 mg/ml in PBS supplemented with 100 mM bicarbonate buffer (pH 8.5; cat. J62808.AP, Thermo Scientific), maintained on ice. The required volume of pHrodo™ dye was calculated based on manufacturer's instructions with a molecular ratio (dye to protein) of 15 and the reference values provided in Table 2. Incubation with pHrodo™ was performed on ice for 1.5 h following a clean-up to remove unbound dye using Zeba™ Dye and Biotin Removal Spin Columns (cat. A44299, Thermo Scientific). The labeled proteins were diluted to a final concentration of 25 $\mu\text{g}/\text{ml}$ in respective medium with 100 $\mu\text{l}/\text{well}$ using 96-well plates, and 40 $\mu\text{l}/\text{well}$ using 384-well plates.

518 **Table 2. Utilized matrix proteins in matricytosis assays.**

Protein	Molecular weight	Catalog#	Manufacturer
VitroCol® Type I human Collagen	300 kDa	5007	Advanced Biomatrix
Human Collagen Type IV	550 kDa	C5533	Sigma-Aldrich
iMatrix-511 Recombinant Laminin	150 kDa	AMS.892012	AMSBIO
Human Fibronectin	440 kDa	F0895	Sigma-Aldrich

519

520 To label dECM or dPCLS, a 1 μ M stock solution of pHrodo™ dye was prepared in PBS
 521 containing 100 mM bicarbonate buffer (pH 8.5). dPCLS placed in 48-well plates were
 522 incubated with 300 μ l of labeling solution. For dECM in 384-well plates and 96-well plates, 40
 523 μ l or 100 μ l of the dye solution were added per well, respectively. dPCLS and dECM were
 524 incubated for 1.5 h at room temperature protected from light, following three washing steps
 525 with 1x DPBS to remove unbound dye. Polarized macrophages were added at 20,000 cells in
 526 100 μ l/well complete medium with 50 ng/ml M-CSF to labeled dECM plates, fibroblasts at
 527 5,000 cells (96-well) or 1,500 cells (384-well) with 100 μ l/well or 40 μ l/well FBM medium,
 528 respectively. Labeled dPCLS were incubated with 250,000 cells per dPCLS in HI-FBS-coated
 529 cryotubes slowly rolling for 5 h at 37°C, to enable adherence of cells to the matrix.

530 **Phagocytosis and efferocytosis assay**

531 To measure the phagocytotic activity, pHrodo™ Red *E. coli* BioParticles™ (cat. P35361,
 532 Invitrogen) were added to the plate with pre-stimulated and CellTracker™ Green labeled
 533 macrophages or fibroblasts at a final concentration of 100 μ g/ml in the respective cell medium.

534 Apoptotic B cell lymphoma Raji cells (cat. CCL-86, ATCC) cells were used as prey cells for the
 535 efferocytosis assay. These cells were grown in T-75 flasks with RPMI-1640 medium (cat.
 536 61870-010, Gibco) with 10 % HI-FCS and 1 % Pen/Strep at 37 °C and 5 % CO₂, and
 537 maintained by addition of fresh medium or replacement of medium by centrifuging at 250 x g
 538 for 6 min. To induce apoptosis, Raji cells were exposed to UV-C light for 7 min in a 10 cm petri
 539 dish (cat. 263991, Thermo Scientific) at 1x10⁶ cells per ml. To track the internalization of
 540 apoptotic prey cells, the IncuCyte® pHrodo® Red Cell Labeling Kit for Phagocytosis (cat. 4649,
 541 Sartorius) was used according to the manufacturer's instructions with a dye concentration of
 542 250 ng/ml and incubation for 1 h at 37°C. After the labeling procedure, cells were centrifuged
 543 (250 x g, 6 min), and added at a ratio of 6:1 to the macrophages or fibroblasts in the respective
 544 cell medium.

545 **Modulation of uptake capacity**

546 In order to validate the established matricytosis assay, cells were treated with 1 μ M
 547 Cytochalasin D (cat. 1233, Tocris) for 1 h before addition of pHrodo-labeled matrix proteins,

or to addition of cells to pHrodo-labeled dECM. To test modulation of matrix uptake capacity, cells were treated with the following compounds for 2 h prior to addition of pHrodo-labeled Collagen I: BTT-3033 (cat. 4724, Tocris; 1 μ M) targeting α 2 β 1 integrin, Dynasore (cat. HY-15304, MedChem Express; 10 μ M) targeting Dynamin GTPase, SB 202190 (cat. S7067, Sigma-Aldrich; 30 μ M) targeting p38 MAP kinase and Nintedanib (Boehringer Ingelheim; 100 nM) targeting VEGFR, PDGFR and FGFR. While untreated macrophages were used in this setup, TGF- β 1 pre-treated fibroblasts were used, which gained matricytotic capacities upon FMT. To assess the role of MMPs in dECM degradation, 500 ng/ml of either MMP-1 (cat. 420-01-1MG, Proteintech) or MMP-2 (cat. 420-02-1MG, ProteinTech) was added per well to pHrodo-labeled dECM. Plates were pre-incubated (2 h, 37 °C) prior to fibroblast seeding to initiate the matricytosis assay.

Live-cell imaging and quantification of matricytosis, phagocytosis and efferocytosis

Uptake of different cargo was imaged using the Sartorius IncuCyte S3 system over a time course of up to 48 h, with imaging every hour using a 10 $\times$ objective. The uptake was quantified by the IncuCyte 2023A software by calculating the total red integrated intensity (RCU $\times$ μ m²/image), which was then normalized to the green object count based on CellTracker™ labeled cells.

To measure the uptake of labeled dPCLS, dPCLS with attached cells were transferred to the well center of HI-FCS pre-coated ultra-low attachment-coated plates (Revvity, #6055802) containing 50 μ l of respective medium. To ensure stable positioning and contact with the well bottom, stainless steel tubes with an inner diameter of 3 mm were placed into each well to secure and weigh down the dPCLS, followed by addition of 100 μ l of respective medium. Confocal imaging was performed using the high-content imager Opera Phenix™ (Revvity) operated via Harmony software (v5.2) in a kinetic mode using the live-cell chamber of the Opera Phenix™ at 37 °C and 5 % CO₂. Stack measurements were carried out by imaging 75 planes using a 20 $\times$ (NA 1.0) water-immersion objective in confocal mode with a binning factor of 2, spanning a total depth of 650 μ m. Autofocus was set to “Two Peak” mode. Alexa Fluor 488 (CellTracker™ Green) and Alexa Fluor 568 (pHrodo™ red) channels were acquired separately to prevent spectral overlap. Advanced flatfield correction was applied to ensure uniform illumination across the dataset. Image analysis was conducted using signals Image Artist (SImA) software, version 1.3 (Revvity). Quantification of uptake was performed by measuring pHrodo spot intensities in CellTracker™ labeled cells.

Immunocytochemistry (ICC) and imaging of fibroblast cultures

To assess the qualitative and quantitative presence of specific proteins or structures, fibroblast cultures were stained by immunocytochemistry (ICC) and imaged by high-content confocal

microscopy using the Opera Phenix™ (Revvity). Cells were washed with 1x DPBS prior to fixation with either 4 % paraformaldehyde (PFA) or ice-cold 100 % methanol (for αSMA staining), depending on the sample type. Following fixation for 15-30 min, samples were washed three times with 1x DPBS (15 min each). For storage, 1x DPBS was added to each well, and plates were sealed and stored at 2-8 °C until further use. Permeabilization was performed with 0.3 % Triton X-100 (cat. 93443, Sigma-Aldrich) in 1x DPBS for 15 min, followed by three washes. To block non-specific antibody binding, samples were incubated with 5 % bovine serum albumin (BSA; cat. A5611, Sigma Aldrich) in PBS for 1-2 h.

Primary antibodies (Table 3) were diluted in blocking buffer and incubated with the samples for 1-2 h. After incubation, samples were washed three times with 1x DPBS for 15 min per wash. Secondary antibodies (Table 4), Hoechst 33342 (cat. H13991, Invitrogen; 1:1000) for nuclear staining and CellMask™ Green (cat. C37608, Invitrogen; 1:50000) were diluted in 1x DPBS and incubated for 1 h protected from light. Finally, plates were washed again three times and stored at 2-8 °C until used for confocal imaging.

Table 3. Primary antibodies used to label fibroblast cultures.

Protein	Catalog#	Manufacturer	Dilution
αSMA	A2547	Sigma Aldrich	1:700
Collagen I	SAB4200678	Sigma Aldrich	1:500
CTHRC1	ab256458	Abcam	1:200
Laminin	MA1-06100	Invitrogen	1:250
Fibronectin	F6140	Sigma Aldrich	1:250
Tenascin C	33352S	Cell Signaling	1:200
Collagen III	ab7778	Abcam	1:200
Collagen IV	ab6586	Abcam	1:200

Table 4. Secondary antibodies used to label fibroblast cultures and dPCLS.

Secondary antibody	Fluorophore	Catalog#	Manufacturer	Dilution
Donkey anti-Mouse IgG (H+L)	Alexa Fluor™ 488	A21202	Invitrogen	1:500
Donkey anti-Rabbit IgG (H+L)	Alexa Fluor™ 488	A21206	Invitrogen	1:500
Donkey anti-Goat IgG (H+L)	Alexa Fluor™ 568	A1057	Invitrogen	1:500
Goat anti-Guinea Pig IgG (H+L)	Alexa Fluor™ 568	A11075	Invitrogen	1:500
Goat anti-Mouse IgG (H+L)	Alexa Fluor™ 555	A21422	Invitrogen	1:500
Goat anti-Rabbit IgG (H+L)	Alexa Fluor™ 555	A21428	Invitrogen	1:500
Donkey anti-Rat IgG (H+L)	Alexa Fluor™ 647	A78947	Invitrogen	1:500
Donkey anti-Mouse IgG (H+L)	Alexa Fluor™ 647	A31571	Invitrogen	1:500
Donkey anti-Rabbit IgG (H+L)	Alexa Fluor™ 647	A31573	Invitrogen	1:500

Confocal imaging was performed using the high-content imager Opera Phenix™ (Revvity) operated via Harmony software (v5.2). Measurements were carried out using a 20× (NA 1.0) or 40× (NA 1.1) water-immersion objective in confocal mode with a binning factor of 2. Autofocus was set to “Two Peak” mode. Alexa Fluor 488 (CellMask™ Green), Alexa Fluor 568 and Alexa Fluor 647 channels were acquired separately to prevent spectral overlap. Advanced flatfield correction was applied to ensure uniform illumination across the dataset. Image analysis was conducted using signals Image Artist (SImA) software, version 1.3 (Revvity). Nuclei segmentation was based on the Hoechst 33342 channel using “Method A”. To assess cell morphology, the cytoplasm was defined using CellMask™ surrounding each nucleus, applying “Method A or B” with an individual threshold of 0.15. Mean fluorescence intensities for Alexa Fluor 488, 568, and 647 were calculated both within the nuclear region and the cytoplasmic area. Morphological parameters such as fiber length (μm) and fiber area (μm²) were quantified using standard analysis methods based on the fiber segmentation. All mean values were exported and statistically analyzed using GraphPad Prism (v10.4.1).

Immunocytochemistry (ICC) and imaging of dPCLS

To assess the differential presence of matrix proteins in healthy and IPF conditions, dPCLS were stained by ICC and imaged by EVOS™ M7000 (Invitrogen). dPCLS were transferred to 48-well plates (cat. 150687, Thermo Scientific) for stainings and kept on a shaker during incubation times. Slices were blocked for 1 h at room temperature with 10 % BSA in DPBS, and then incubated with primary antibodies (Table 5) diluted in 1 % BSA in DPBS overnight at 4°C. After three washing steps for 10 min each with DPBS, the slices were incubated with secondary antibodies (Table 4) diluted in 1 % BSA in DPBS for 2 h at room temperature. Following three washing steps à 10 min with DPBS, dPCLS were stored in DPBS until being imaged.

Table 5. Primary antibodies used to label dPCLS.

Protein	Catalog#	Manufacturer	Dilution
Collagen I	SAB4200678	Sigma Aldrich	1:100
Laminin	MA1-06100	Invitrogen	1:200
Fibronectin	66042-1-Ig	Proteintech	1:100
Collagen IV	ab6586	Abcam	1:200

Mass spectrometry analysis

Cell pellets of macrophages and fibroblasts were lysed with GuHCl lysis buffer (6 M guanidine-hydrochloride, 10 mM Tris(2-carboxyethyl)phosphine, 30 mM 2-chloroacetamide in 10 mM Tris-HCl, pH 8.5) and incubated at 95°C for 10 min. Cell culture supernatants were also

incubated at 95°C for 10 min after collection. The samples were centrifuged for 10 min at maximum speed and transferred into fresh tubes. Protein concentrations were determined with the Pierce 660 nm assay (cat. 22662, Thermo Scientific). Protein digestion was carried out with an in-house modified and automated protein aggregation capture protocol [58]. In brief, 10 µg of protein were captured on magnetic hydroxyl-modified beads (Resyn) by adding 100 % acetonitrile to a final acetonitrile concentration of 70 % and washed twice with 70% acetonitrile and 70 % ethanol, respectively. Next, 30 µl of trypsin/Lys-C (Promega) in 100 mM Tris-HCl, pH 8.5 was added (enzyme-to-protein ratio = 1:100) and digested at 37°C overnight. The following day, formic acid was added to a final concentration of 0.5 % and tryptic peptides were transferred onto a fresh 96-well PCR plate (Eppendorf). After determining peptide concentrations on a Nanodrop (Thermo), 200 ng of peptides were loaded onto Evotip Pure trap columns (Evosep) following the manufacturer's protocol and stored until liquid chromatography mass spectrometry (LC-MS) measurement.

All samples were measured in data-independent acquisition (DIA) mode on an Evosep One (Evosep) ultra-high performance liquid chromatography Orbitrap Astral (Thermo) mass spectrometry (MS) system. Peptides were separated by reversed-phase chromatography using a binary buffer system (A: 0.1 % formic acid in LC-MS-grade water, B: 0.1 % formic acid in acetonitrile) and the pre-installed 40 samples-per-day Whisper Zoom method (32-min effective gradient). An Aurora Elite analytical column (IonOpticks) was used for peptide separation. Eluting peptides were ionized by electrospray ionization (ESI). Full MS spectra were acquired in the Orbitrap analyser operated at a resolution of 240,000. The scan range was set to 380 – 980 m/z with a relative AGC target of 300 % and an injection time of 5 ms. MS2 DIA scans were acquired in the Astral analyser operated at a resolution of 80,000. The scan range was set to 380 – 980 m/z with 299 DIA windows of 2 m/z width. The AGC target was set to 300% with a maximum injection time of 3 ms. Fragmentation was achieved by higher collisional dissociation (HCD) set to 25 %. The loop control was set to 600 ms.

MS files were processed with DIA-NN (version 2.1.0, Academia) in the library-free mode [59]. For spectra prediction, the Homo sapiens Swissprot database (version downloaded from www.uniprot.org on 2024-08-01) was used. Mass accuracies were set to 2e-6 for MS1 and 1.4e-5 for MS2 spectra; the scan radius was set to 5. Peptide and protein FDRs were set to 0.01 with MBR activated and MaxLFQ normalization used for protein group assembly.

The statistical analysis was performed using Perseus [60]. LFQ protein group intensities were log2-transformed and filtered to ensure data completeness for at least one stimulation condition. Missing values were replaced by downshift imputation from a 1.8 standard deviation downshifted 0.3 standard deviations broad normal distribution. Significantly regulated protein

groups were identified by Welch's t-testing with multiple testing correction ($S_0 = 0.1$, permutation-based FDR = 0.05, 250 randomizations). Enriched functional terms, e.g., GO ontology terms, were identified by 1D annotation enrichment analysis (Benjamini-Hochberg FDR = 0.02) [61]. We further included categories of ECM and matrisome proteins in the 1D annotation enrichment analysis based on Naba et. al [25].

Human single-cell sequencing and spatial transcriptomics analysis

Gene signatures (Table 6) and proteomic gene signatures (see defining proteomic gene signatures section) were scored onto an integrated scRNA-seq dataset, as well as a VisiumSD for formalin-fixed paraffin-embedded tissue (FFPE) spatial transcriptomics dataset, from control and IPF patients, as previously published [12]. In brief, publicly available scRNA-seq datasets of interstitial lung disease (ILD) were integrated into a large atlas of PF-ILD patients, which is available on Zenodo (10.5281/zenodo.10015169). For this study, the data were subsetted to include only IPF and control patients, resulting in a dataset comprising 292,096 cells. Single genes were visualized as UMAPs or dot plots using Scanpy (v1.10.4) [62].

Defining gene signatures

Differentially abundant proteins (DAPs) were identified in each lysate contrast by selecting proteins whose abundance was significantly changed, using a 5 % FDR (false discovery rate) threshold. Protein IDs were mapped to Ensembl gene IDs using pybiomart [63]. The mapped gene IDs were used for gene signature scoring (see gene signature scoring section). For Univariate Linear Model (ULM) scoring, each gene was assigned a weight equal to its log2 fold change.

Gene signature scoring

To score gene signatures in human control and IPF scRNA-seq and spatial transcriptomics data, we used Hotspot [64] and ULM [65] methods. To calculate Hotspot scores, we used the `compute_scores` function from Hotspot [64]. Briefly, gene expression was modeled using a normal noise model, the values for each gene were z-scored (zero mean, unit variance), and then smoothed using the k-nearest neighbor graph of each cell. Finally, the smoothed expression values for all genes in the signature were summarized to a single score using the first principal component (PCA). We used log1p-transformed gene expression values normalized to 10,000 counts per cell, 30 neighbors, and a neighborhood factor of 3. For single-cell RNA-seq and spatial transcriptomics datasets, X_scVI and spatial coordinate embeddings were used, respectively, to construct the neighbor graphs. The scores were visualized as UMAPs or dot plots using Scanpy (v1.10.4) [62].

Gene signature activity was estimated using the ULM method as implemented in decoupleR (run_ulm function [65]). Briefly, for each cell and gene signature, a univariate linear regression was fit with the cell's gene expression profile as the response variable and the assigned gene weights as the predictor. Gene weights were derived from the differential protein abundance analysis. The resulting t-statistic of the regression slope served as the gene signature activity score, with positive values indicating higher and negative values indicating lower gene signature activity.

Table 6. Literature-based gene panels of individual signature scores used for human control and IPF scRNA-seq and spatial transcriptomic analyses. Selection of major endocytic, internalization, fibrosis-related pathways with representative genes involved in their regulation and execution, in addition to signature genes of cancer-associated fibroblasts.

Signature score	Gene panel	
Invadosome	MMP2 MMP14 ADAM12 VIM	SH3PXD2A SH3PXD2B LPA LPAR1
Fibrosis	ACTA2 COL3A1 CTHRC1	MMP11 TNC POSTN
Uptake	CD14 CD44 CLTC CTSB CTSD CTSS CTSZ GALC ITGAV ITGB1 ITGB3 TREM2 STAB1 TLR4	CD36 LAIR1 LAMP1 LAMP2 LRP1 MARCO MERTK1 MRC1 MRC2 OSCAR PDL3 PSAP TLR2
Integrin-mediated phagocytotic uptake	ITGA2 ITGA3 ITGA5 ITGB1 GSN MYH9	VAV2 RAC1 MLCK RAP1A RAP1B
MFGE8-pathway	ITGB3 ITGB5	ITGAV MFGE8
Endo180-dependent (clathrin-mediated) uptake	MRC2 CLTC	FLOT1 FLOT2
Phagosomal machinery	RAB7A LAMP1 LAMP2 CTSK CTSD	ADAM8 ADAMTS1 ADAMTS4 LAMTOR1 LAMTOR2

	LGMN CAPN1 CAPN2	LAMTOR3 LAMTOR4 LAMTOR5
Macropinocytosis	RAC1 RAS CDC42 RHOA PIK3CA SRC PLCG1	PKC ABI1 ACTN4 ANKFY1 ARF6 CD14
Cancer-associated fibroblasts	COL1A1 COL1A2 COL5A1 ACTA2 FGF2 FAP LRP1 CD248 COL6A1 COL6A2	COL6A3 CXCL12 FBLN1 LUM MFAP5 MMP3 MMP2 PDGFRB PDGFRA FN1

710

711 **Reactome pathway analysis**

712 Pathway analysis was performed against the curated database Reactome, version 95
713 (<https://reactome.org/>) with default settings, using only significantly deregulated proteins of a
714 secretome data set derived from fibroblasts treated with TGF- β 1 vs. ctrl. Out of this analysis,
715 the 10 most relevant pathways sorted by *p*-value were selected.

716 **Matrisome analysis**

717 Matrisome of significantly deregulated proteins of a secretome data set derived from
718 fibroblasts treated with TGF- β 1 vs. ctrl was analyzed using Matrisome Analyzer
719 [66] (<https://matrinet.shinyapps.io/MatrisomeAnalyzer/>).

720 **Statistical analysis**

721 All analyses were carried out using GraphPad Prism v10.4.1 (GraphPad Software, Inc.). Data
722 are presented as the mean value for each experimental group with variation represented as $\pm$
723 standard error of mean (s.e.m.) or $\pm$ standard deviation (s.d.) from at least three independent
724 experiments or different donors. Equal numbers of NHLFs and IPF HLFs were included, as
725 previous analyses demonstrated no significant differences in their marker expression or assay
726 behavior, allowing both fibroblast types to be used interchangeably for these experiments. The
727 number of experiments or donors (*n* number) is indicated in the respective figure legend
728 throughout the manuscript. Statistical differences were determined by ANOVA or Student's *t*-
729 test where appropriate, and *p* values < 0.05 were considered statistically significant. Group
730 differences were assessed using two-sided Dunn's post-hoc tests following Kruskal-Wallis

analysis. For comparisons against a single control, Dunnett's tests were applied. All tests are standard implementations in GraphPad Prism v10.4.1. The normality of the data distribution was assessed using the Kolmogorov-Smirnov or Shapiro-Wilk test ($\alpha = 0.05$) prior to applying parametric tests. All images in one experiment were acquired and edited with identical settings across conditions to enable direct comparison. Schematic figures were designed using BioRender templates available on Biorender.com.

Statement of AI-assisted language editing

The authors declare that Microsoft Copilot (<https://copilot.microsoft.com>) was used solely to support language editing, grammar checking, proofreading, and improvement of clarity in the manuscript. The tool was not used to generate scientific content, interpret data, draw conclusions, or alter the intellectual content of the work. All scientific content, analyses, interpretations, and conclusions were developed and verified by the authors, who take full responsibility for the final manuscript.

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Acknowledgements

We thank Danny Jonigk, Mark Kühnel, Jan-Christopher Kamp, Lavinia Neubert, Regina Engelhardt, Christina Petzold-Mügge and Mareike Knoll from Hannover Medical School to provide and prepare human lung tissue samples. We acknowledge Jochen Drescher, Dennis Schäfer and Jennifer Bruggesser for preparation of PCLS, Alexis Efstratiadis and Ronja Joos for supporting in vitro uptake experiments, and scientific advice from Vincent Fiore.

Author contributions

S.G., B.M.C. designed the experiments with scientific guidance from W.S.-W. and F.H. S.G. and B.M.C. carried out the majority of the experiments. S.K. and F.M. carried out proteomic measurements and analysis. C.M. and L.B. carried out scRNA-seq and spatial transcriptomic analyses. S.G. and B.M.C. carried out the majority of formal analysis, curated the datasets and visualized the data. S.G. and B.M.C. wrote the manuscript, with scientific input from M.J.T. All authors reviewed the manuscript. W.S.-W. and F.H. supervised the overall study.

Data availability

The proteomics data have been deposited with the ProteomeXchange Consortium via the PRIDE partner repository under dataset identifier PXD077197 (<https://www.ebi.ac.uk/pride/archive/projects/PXD077197>). The remaining data supporting the findings of this study are available in the manuscript and its Supplementary Information. Additional data are available from the corresponding author upon reasonable request.

Additional information

All authors were contracted and funded by Boehringer Ingelheim Pharma GmbH & Co. KG. S.G., B.M.C., L.B., M.J.T., W.S.-W., F.E.H. are employees of Boehringer Ingelheim Pharma GmbH & Co. KG at which the current work was performed and conceptualized. S.K. and F.M. are researchers at the University of Bonn. This relationship has no bearing or relevance to the current work.

This work was funded by Boehringer Ingelheim Pharma GmbH & Co. KG and is the result of a collaboration between researchers at Boehringer Ingelheim and the University of Bonn, which is funded in part by a cooperation contract.

Figure legends

Figure 1. Matricytosis is a phenotype-specific ECM internalization program in macrophages and fibroblasts. (a) High-resolution fluorescence imaging with a 40x objective for pHrodo™ (magenta), LysoTracker™ (cyan) and CellTracker™ (green) in different macrophage subsets after 24 h (n = 3 independent experiments; scale bar, 20 μm). (b, c) Quantification of uptake kinetics shown by area under the curve (AUC) for pHrodo™-labeled in vitro-generated decellularized extracellular matrix (dECM; n = 5 independent experiments; b) and collagen I (n = 8 independent experiments; c) in macrophage subsets measured via IncuCyte® live-cell imaging for 24 h. (d) High-resolution fluorescence imaging with a 40x objective for pHrodo™ (magenta), LysoTracker™ (cyan) and CellTracker™ (green) of TGF-β1-induced myofibroblasts vs. control after 24 h (n = 6 donors; scale bar, 20 μm). (e, f) Quantification of uptake kinetics (AUC) of dECM (n = 5 donors; e) and collagen I (n = 5 donors; f) internalization in TGF-β1-induced myofibroblasts measured via IncuCyte® live-cell imaging for 24 h. Data are presented as mean ± s.d. (standard deviation). *p < 0.05 is considered to be statistically significant. (b, c) ANOVA/Šidák correction, (e, f) two-tailed unpaired Welch's t test.

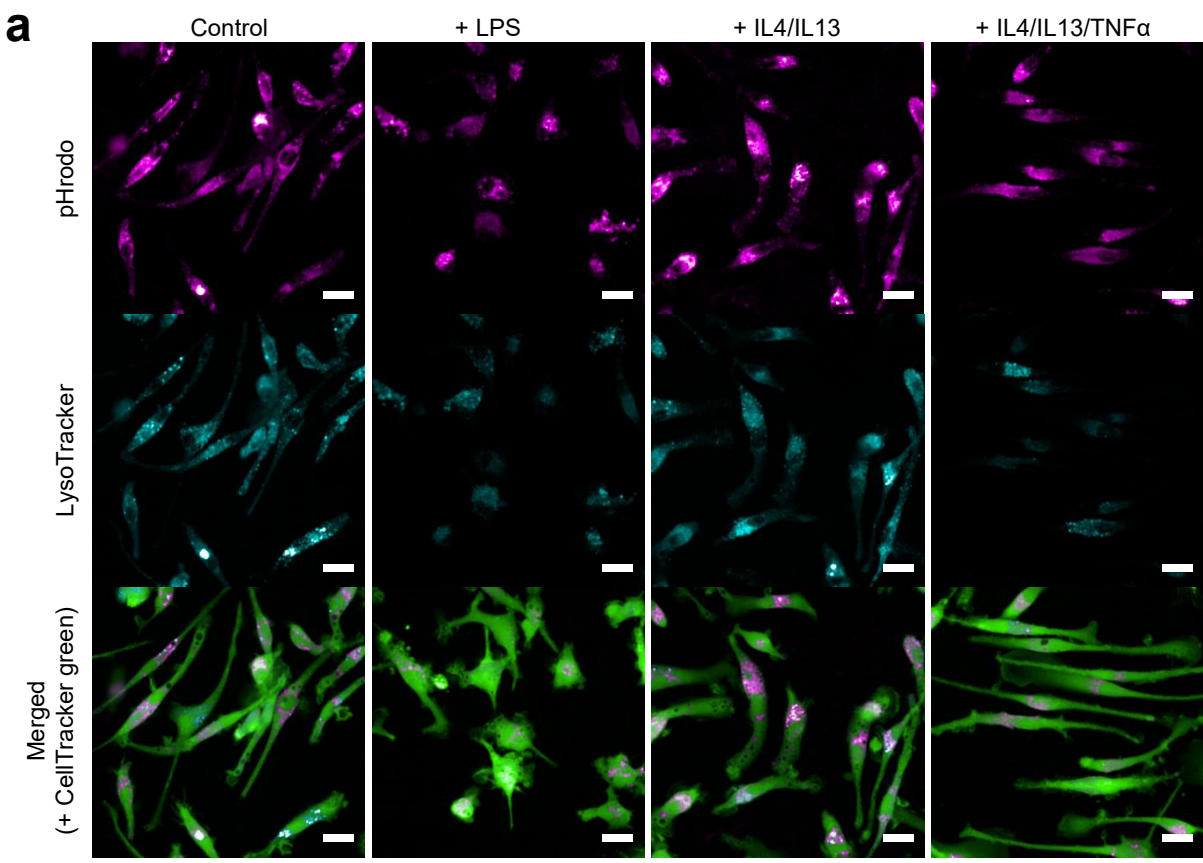
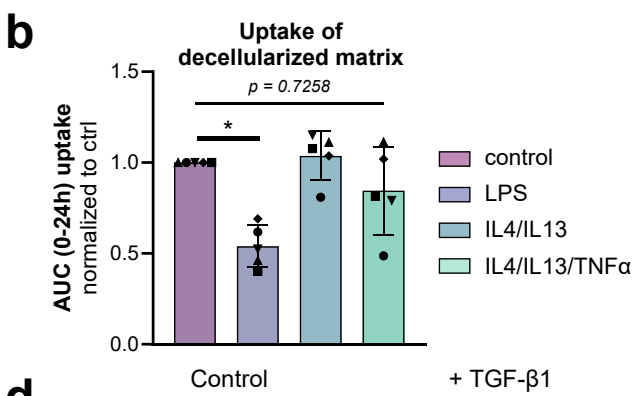
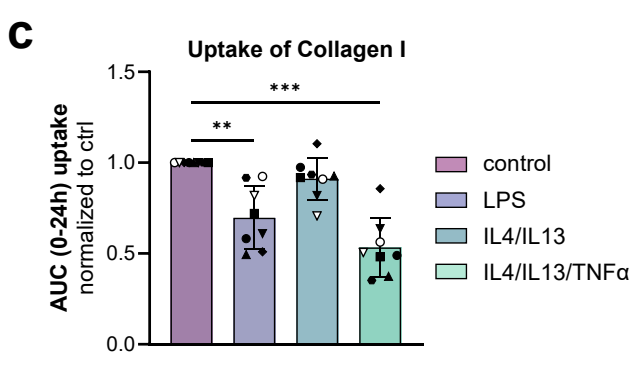
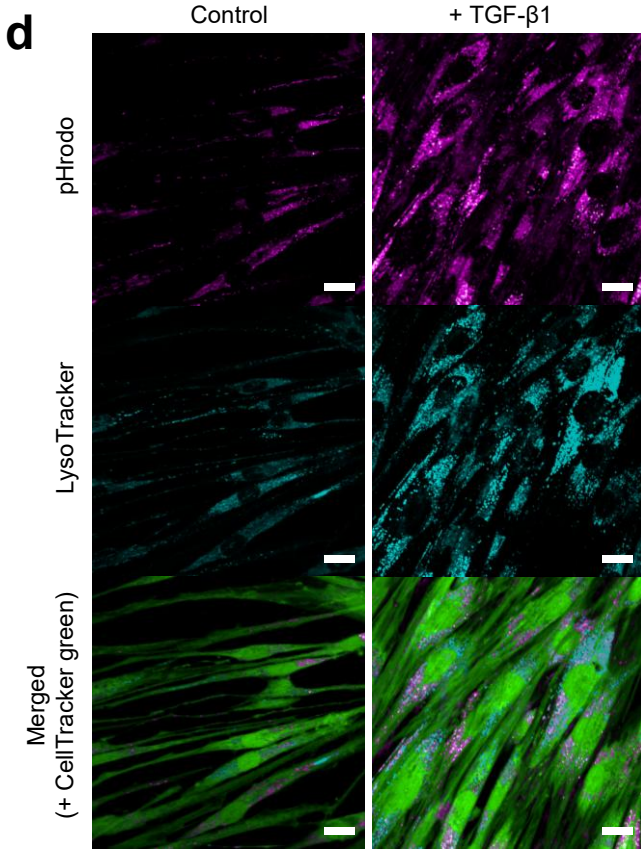
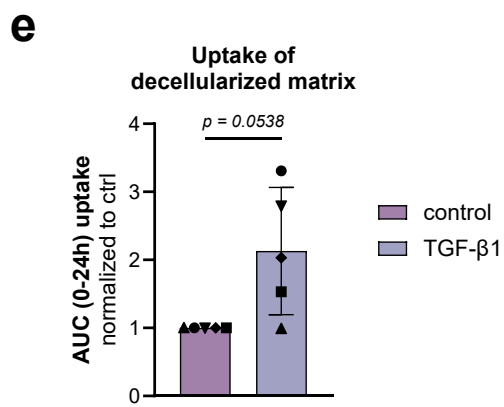
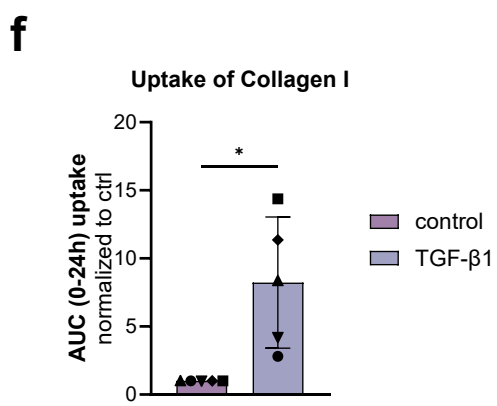
Figure 2. Distinct molecular pathways mediate matricytosis in macrophages and fibroblasts. (a, b) Quantification of collagen I internalization kinetics (AUC) in fibroblasts (n = 6 donors; a) and macrophages (n = 5-7 independent experiments; b) upon pharmacological

inhibition with 1 μ M integrin $\alpha 2\beta 1$ inhibitor (BTT-3033), 30 μ M p38/MAPK inhibitor (SB 202190), 10 μ M dynamin GTPase inhibitor (Dynasore) or 100 nM Nintedanib, measured via IncuCyte[®] live-cell imaging for 24 h. **(c)** Visualization of MFGE8-, Endo180-, integrin-, phagosomal machinery-, and macropinocytosis-associated uptake signatures on UMAPs across epithelial, endothelial, lymphoid, mesenchymal, and myeloid compartments using scRNA-seq scoring on the PF-ILD atlas [12]. **(d-f)** Dot plot visualization of scRNA-seq-derived uptake pathway scores across mesenchymal and myeloid compartments **(d)**, fibroblast subsets **(e)**, and macrophage populations **(f)** in control and IPF lungs. Dot size reflects the fraction of cells (%) expressing the signature, and colour intensity indicates mean expression in group. Quantification of matricytosis is presented as mean $\pm$ s.d.; * p < 0.05 is considered to be statistically significant. **(a, b)** ANOVA/Dunnett's correction.

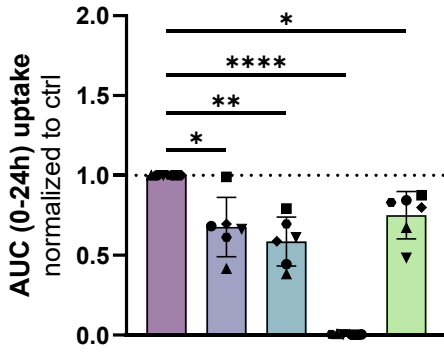
Figure 3. Macrophages are the dominant uptake-proficient cells in both healthy and fibrotic lung tissue. **(a)** scRNA-seq-based uptake signature scoring on UMAPs across epithelial, endothelial, lymphoid, mesenchymal, and myeloid compartments using scRNA-seq scoring on the PF-ILD atlas [12]. **(b)** Dot plot visualization of scRNA-seq-derived uptake score in the myeloid compartment. **(c-f)** Spatial transcriptomic-based uptake signature scoring in healthy and fibrotic lung tissue shown as UMAP **(c)**, exemplary spatial plots **(d)**, quantification of uptake scoring across cell compartments **(e)** and across spatial niches and cell subsets in healthy and IPF tissue **(f)** [12]. Dot size indicates the fraction of cells (%) expressing each signature within the respective group, and colour intensity reflects mean expression in group. Arrows in **(d)** point on alveolar macrophages. SMCs_Adv_Meso: smooth muscle/adventitial/mesothelial niche.

Figure 4. Fibroblast matricytosis aligns with invadosome-like programs enriched in IPF. **(a)** Invadosome and fibrosis scoring scRNA-seq-based across cell compartments in healthy and fibrotic lung tissue utilizing a published PF-ILD atlas [12] shown as UMAPs. **(b, c)** Invadosome and fibrosis scoring across niches using a spatial transcriptomic atlas from healthy and IPF lungs [12] shown as UMAP **(b)** and on exemplary spatial plots **(c)**. **(d)** Dot plots show invadosome and fibrosis scoring on PF-ILD atlas across different fibroblast subtypes (adventitial, alveolar and myofibroblasts) in control and IPF condition. **(e)** Scoring of individual markers associated with invasion (*ADAM12*, *LPA*, *LPAR1*, *MMP14*, *MMP2*, *SH3PXD2A*, *SH3PXD2B*) and fibrosis (*ACTA2*, *COL3A1*, *CTHRC1*, *MMP11*, *POSTN*, *TNC*) [12,22] across compartments in the PF-ILD atlas. **(f)** Scoring of invadosome and fibrosis gene signature across niches and cell subsets based on a spatial transcriptomic atlas from healthy and IPF lungs [12]. Dot size represents the fraction of cells (%) expressing each gene within the compartment, and colour intensity reflects mean expression. SMCs_Adv_Meso: smooth muscle/adventitial/mesothelial niche.

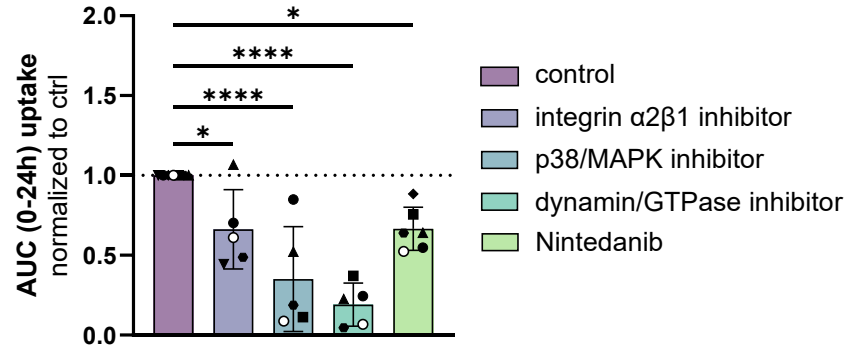
Figure 5. IPF-derived matrix modulates matricytosis and phenotype-specific behavior.
(a, b) Quantification of dPCLS internalization kinetics (AUC) by macrophages (IL4/IL13/TNF α vs. control; $n = 2$ for healthy tissue, $n = 3$ for IPF tissue; **a**) and fibroblasts ($n = 3$ donors; **b**) using healthy and IPF-derived dPCLS. Data were generated using high-resolution microscopy across multiple imaging planes with an Opera Phenix for 24 h. Amount of pHrodo™ spots per cell were analyzed and normalized to control. Data are presented as mean $\pm$ s.d.; * $p < 0.05$ is considered to be statistically significant (ANOVA/Tukey correction). Exact p -values are indicated on the graph.

Fig 1

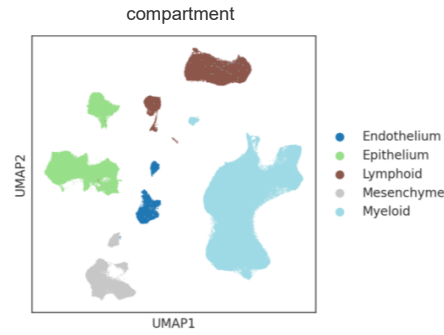
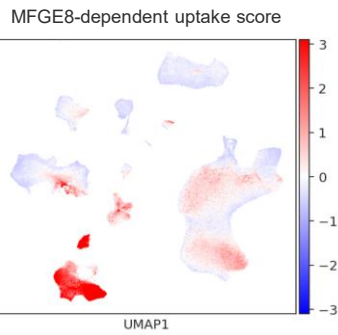
a Modulation of collagen I uptake
Fibroblasts



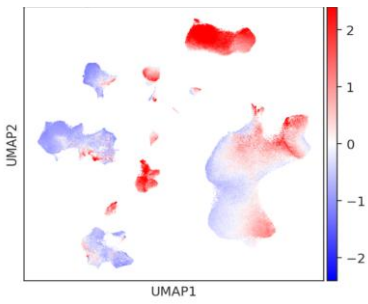
b Modulation of collagen I uptake
Macrophages



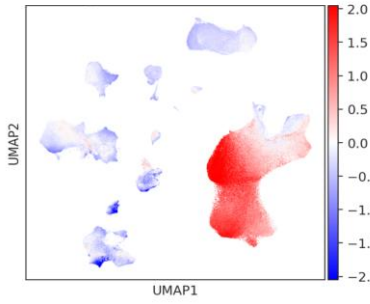
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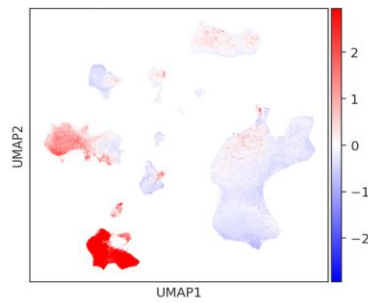
Integrin-mediated uptake score



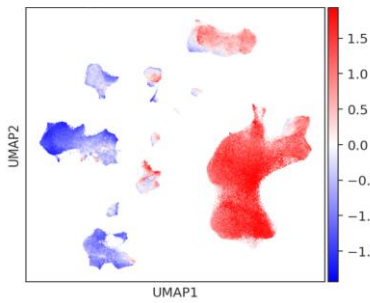
Phagosomal machinery score



Endo180-dependent uptake score

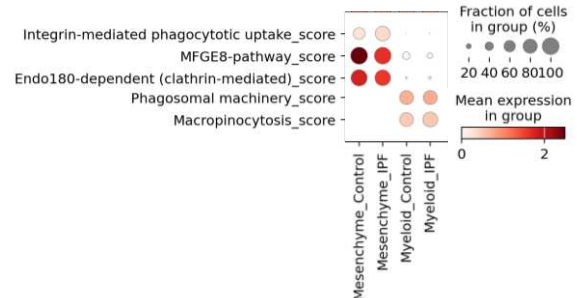


Macropinocytosis score



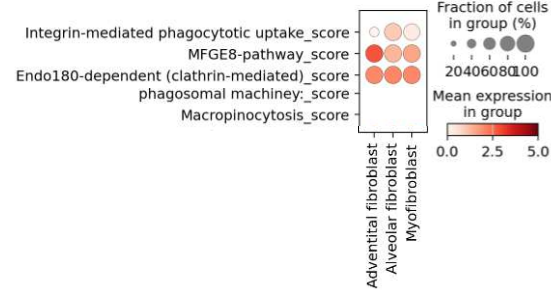
d

Scoring across compartments and disease



e

Scoring in fibroblast cell types



f

Scoring in macrophage cell types

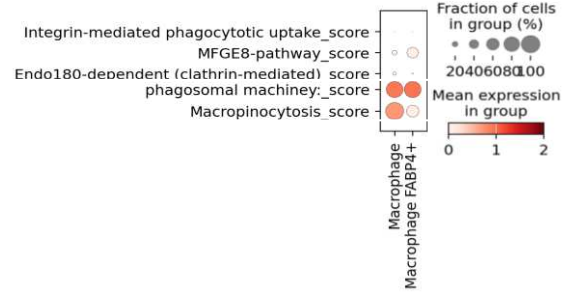
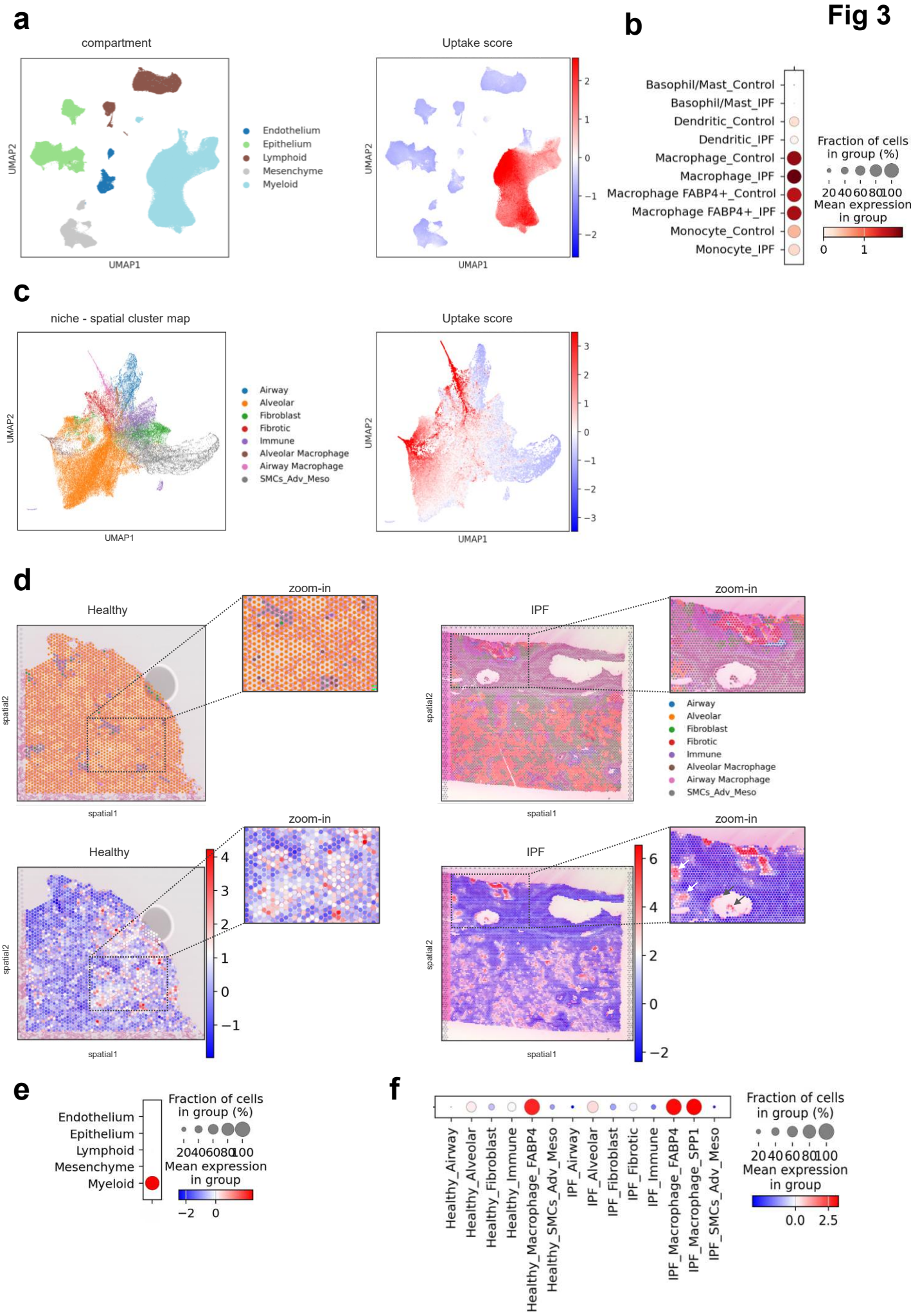
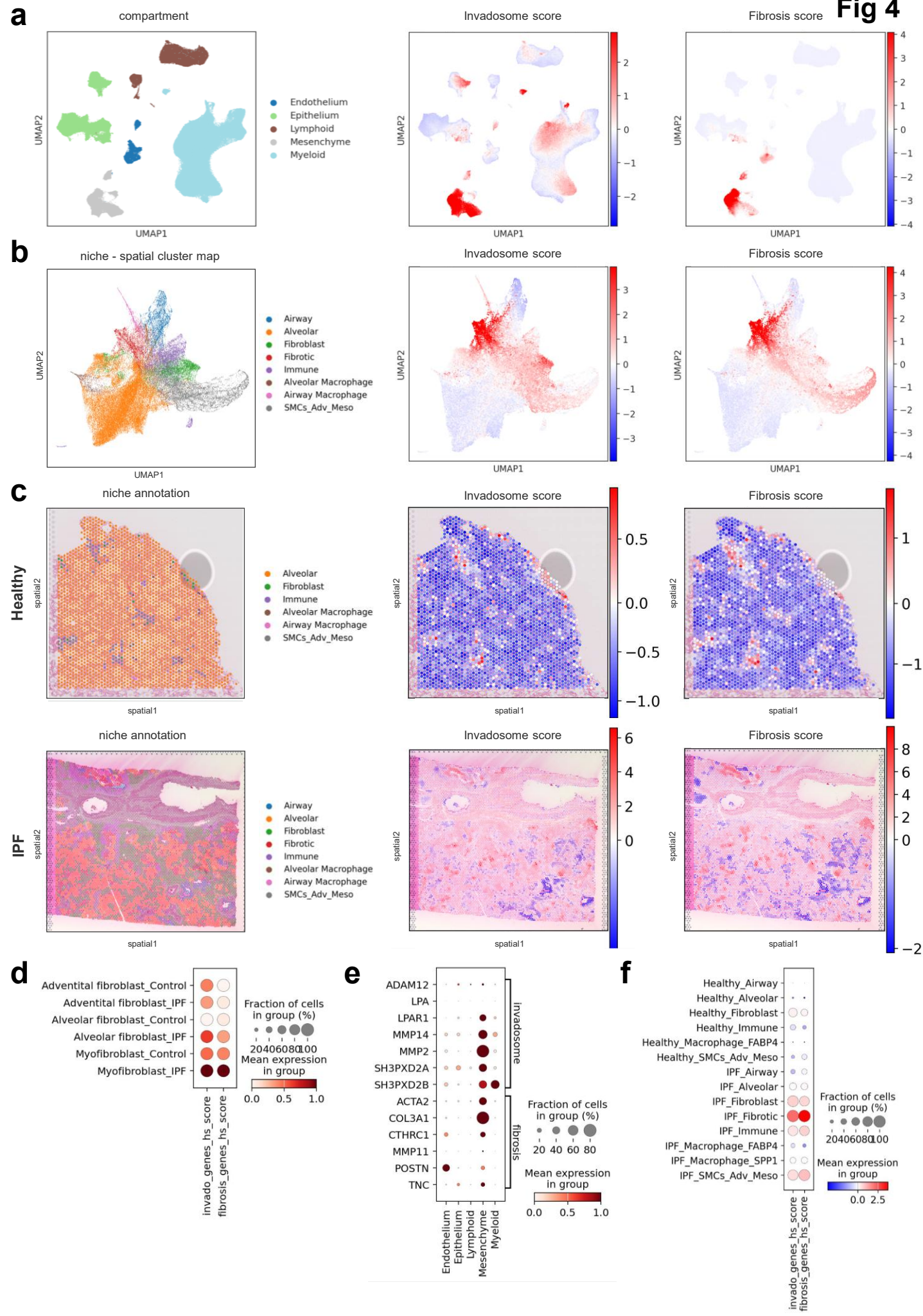
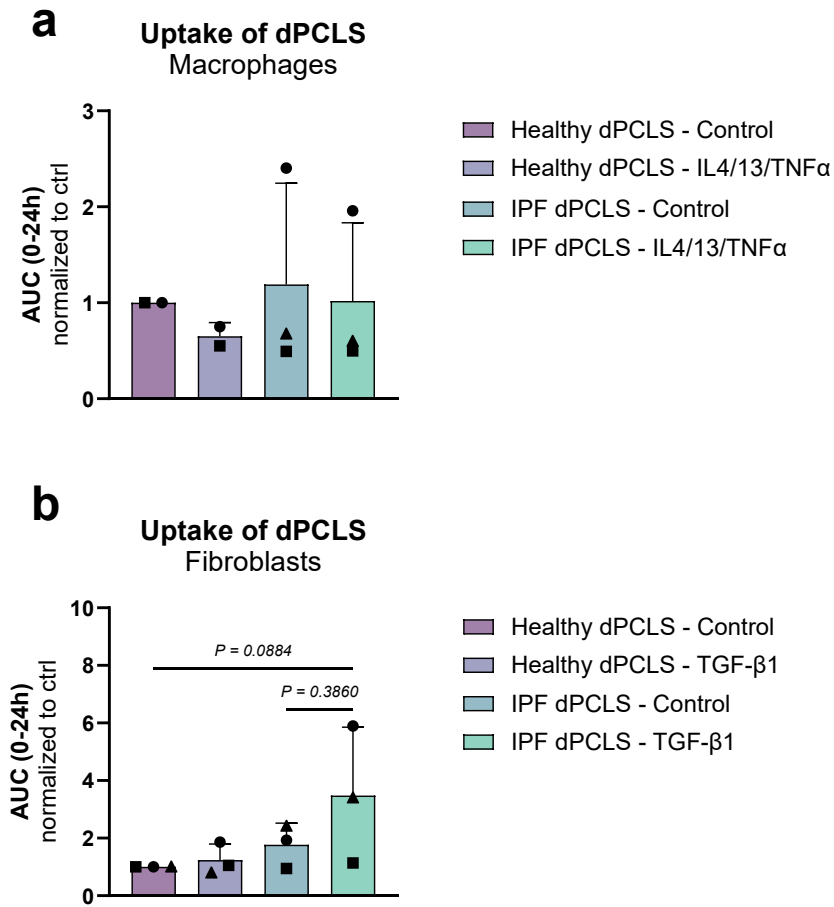


Fig 3







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