Mapping Macrophage–Fibroblast Interactions and Mitochondrial Communication

Within most tissues, macrophages and fibroblasts do not function as isolated cell types but instead form closely associated functional units within shared tissue microenvironments. Spatial imaging and lineage-tracing studies have shown that macrophages frequently localize adjacent to stromal fibroblasts, particularly in barrier tissues and sites of active remodeling, where sustained bidirectional communication contributes to tissue homeostasis, repair, and inflammation. Consistent with these observations, our preliminary studies demonstrate that direct physical association between macrophages and fibroblasts is critical for regulating both macrophage homeostasis and inflammatory responses. These findings raise two fundamental questions: How do macrophages and fibroblasts establish physical interactions, and what forms of intercellular communication occur once these contacts are formed?

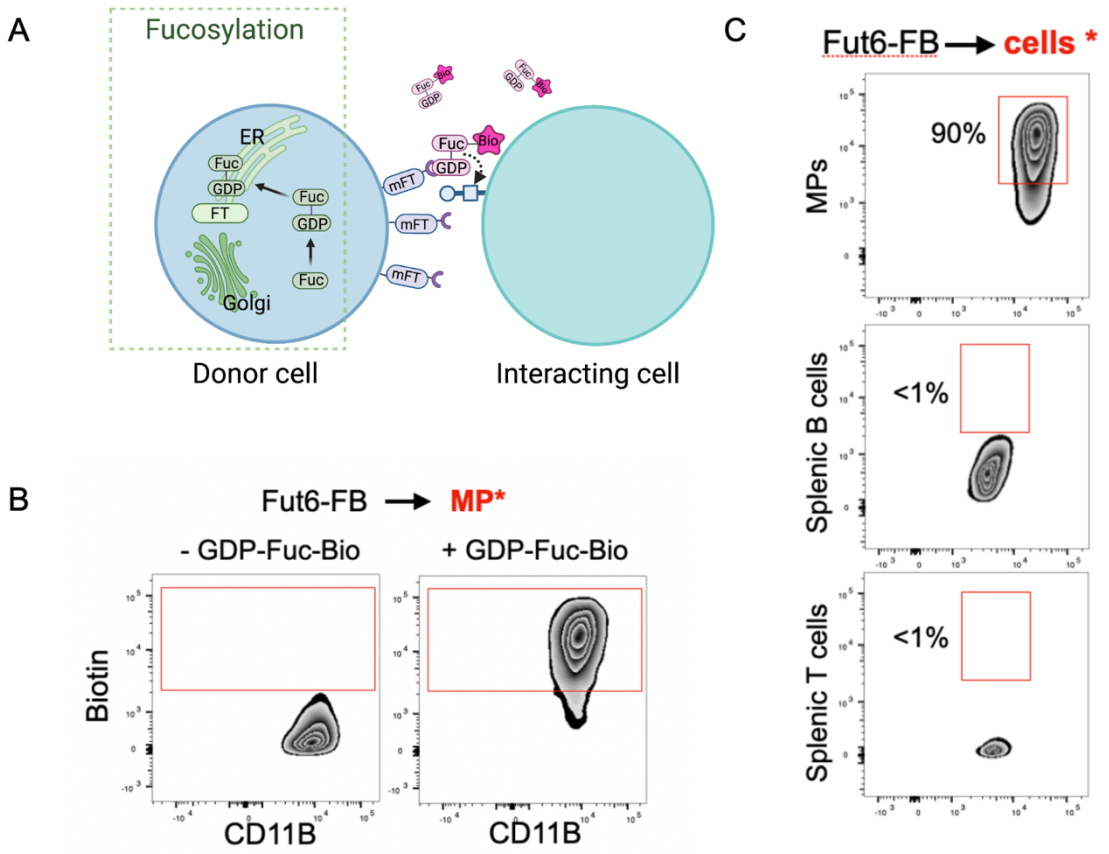
To address the first question, we collaborated with the laboratory of Peng Wu at The Scripps Research Institute to apply a recently developed chemical-genetic cell-contact labeling strategy based on enzymatic fucosylation (Fig1A). This approach enables selective labeling and quantification of cells that have undergone direct physical interaction. Using this platform, we found that macrophage–fibroblast interactions are substantially more robust than interactions between fibroblasts and other immune cell types (Fig1B-C), highlighting the unique nature of this cellular partnership.

To systematically identify the molecular regulators governing macrophage–fibroblast engagement, we developed an unbiased CRISPR knockout screening platform coupled with the fucosylation-based interaction assay. We constructed a customized guide RNA (gRNA) library targeting approximately 2,600 macrophage membrane proteins (Fig2A-B) and screened for genes whose disruption altered macrophage–fibroblast interactions. This approach identified dozens of candidate regulators that either enhanced or impaired cell-cell engagement, providing a valuable resource for dissecting the molecular mechanisms underlying stromal-immune communication (Fig3A-B).

While characterizing these interactions, we made an unexpected observation that led to a new direction of investigation. We discovered that fibroblasts transfer mitochondria directly to macrophages. Using organelle-specific fluorescent reporters expressed in fibroblasts, we found that mitochondria were robustly transferred to macrophages during co-culture, whereas transfer of the nucleus, endoplasmic reticulum, and lysosomes was minimal (Fig4A). Live-cell imaging independently confirmed active mitochondrial movement between the two cell types (Fig4B). Importantly, this phenomenon was highly cell type-specific: mitochondrial transfer occurred efficiently to macrophages but was rarely detected in B cells, $CD4^+$ T cells, $CD8^+$ T cells, or peritoneal macrophage subsets cultured under identical conditions (Fig4C). Furthermore, separating macrophages and fibroblasts using a transwell system completely abolished mitochondrial transfer (Fig4D), demonstrating that direct cell-cell contact is required. Together, these findings establish contact-dependent mitochondrial transfer as a selective feature of macrophage–fibroblast communication and suggest a novel mechanism by which stromal cells may regulate macrophage metabolism and function during inflammation.

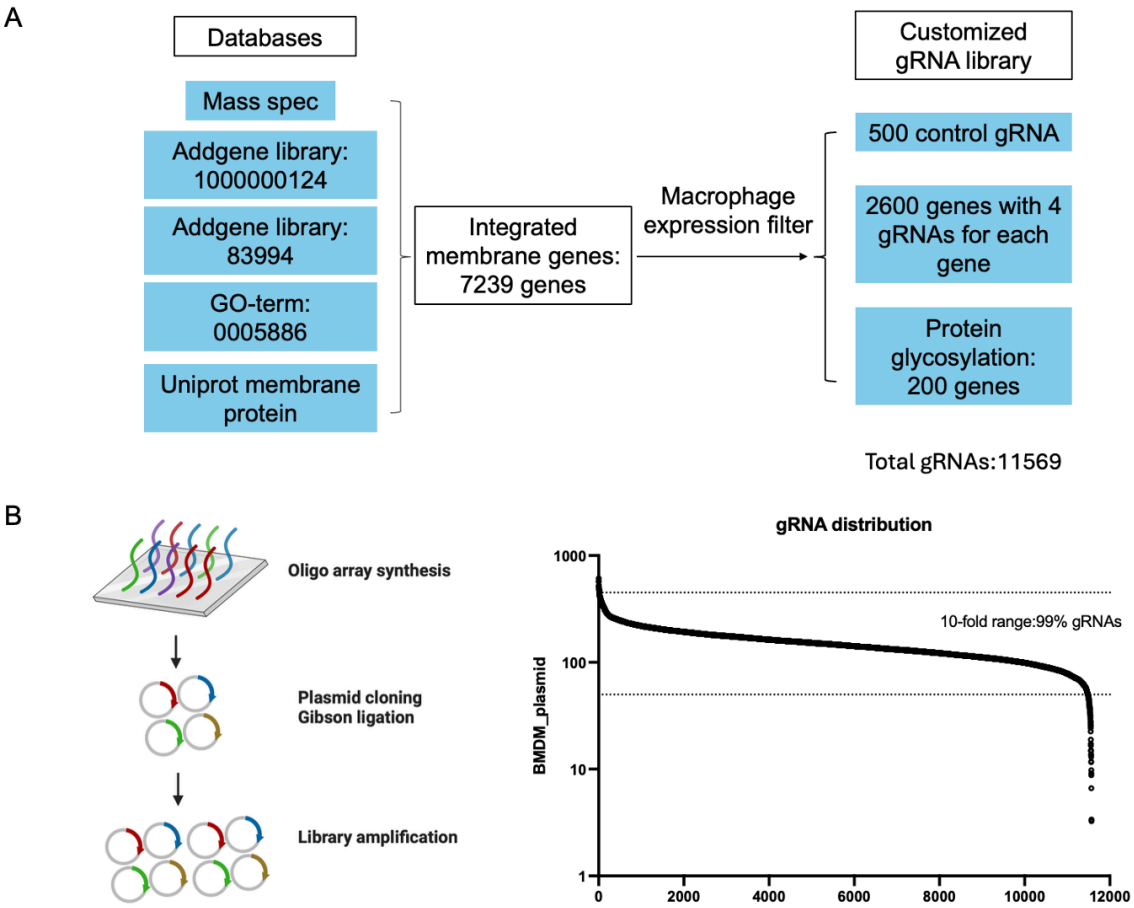
To further extend these findings beyond imaging-based assays, we collaborated with Kyle Kimler to develop MitoPaseo, a computational framework for detecting and interpreting intercellular mitochondrial transfer from single-cell RNA sequencing data. As a proof of concept, macrophages isolated from CD1 mice were co-cultured with fibroblasts from C57BL/6 mice. Because the mitochondrial genomes of these two strains differ by three naturally occurring single nucleotide polymorphisms (SNPs) (Fig5A), transferred mitochondria can be distinguished based on their genetic signatures. Following co-culture, single-cell RNA sequencing successfully captured these strain-specific mitochondrial SNPs, demonstrating that MitoPaseo can identify mitochondrial exchange between interacting cell populations (Fig5B-C). This framework provides a scalable approach for studying mitochondrial communication in complex tissues and existing single-cell datasets without requiring genetically encoded fluorescent reporters.

Figure1.



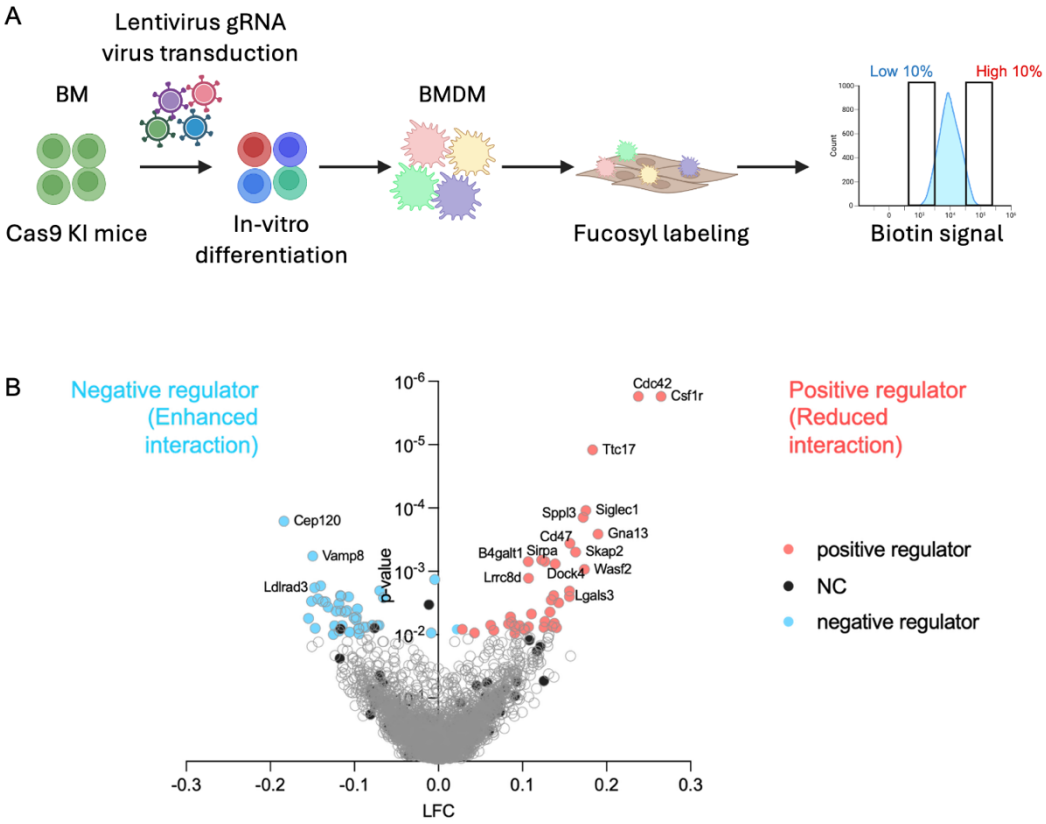
A) Diagram of fucosyl-labeling to study the interactions between MPs and FBs. A mammalian fucosyltransferase was engineered to the plasma membrane (mFT) by fusion of the enzyme with the transferrin receptor N-terminal domain. Fucosyltransferases (FTs) catalyze the conjugation of fucose onto glycosylated proteins. Endogenous FTs are restricted to the Golgi apparatus. Only cells expressing mFT can tag the glycosylated proteins on the interacting cells in the presence of the synthetic ligand GDP-fucose-biotin, allowing us to quantify direct cell-cell interactions by the biotin signal. B-C) in vitro labeling of MP-FB interactions (B) and immune-FB interactions (C) analyzed by flow cytometry and immunofluorescence.

Figure 2.



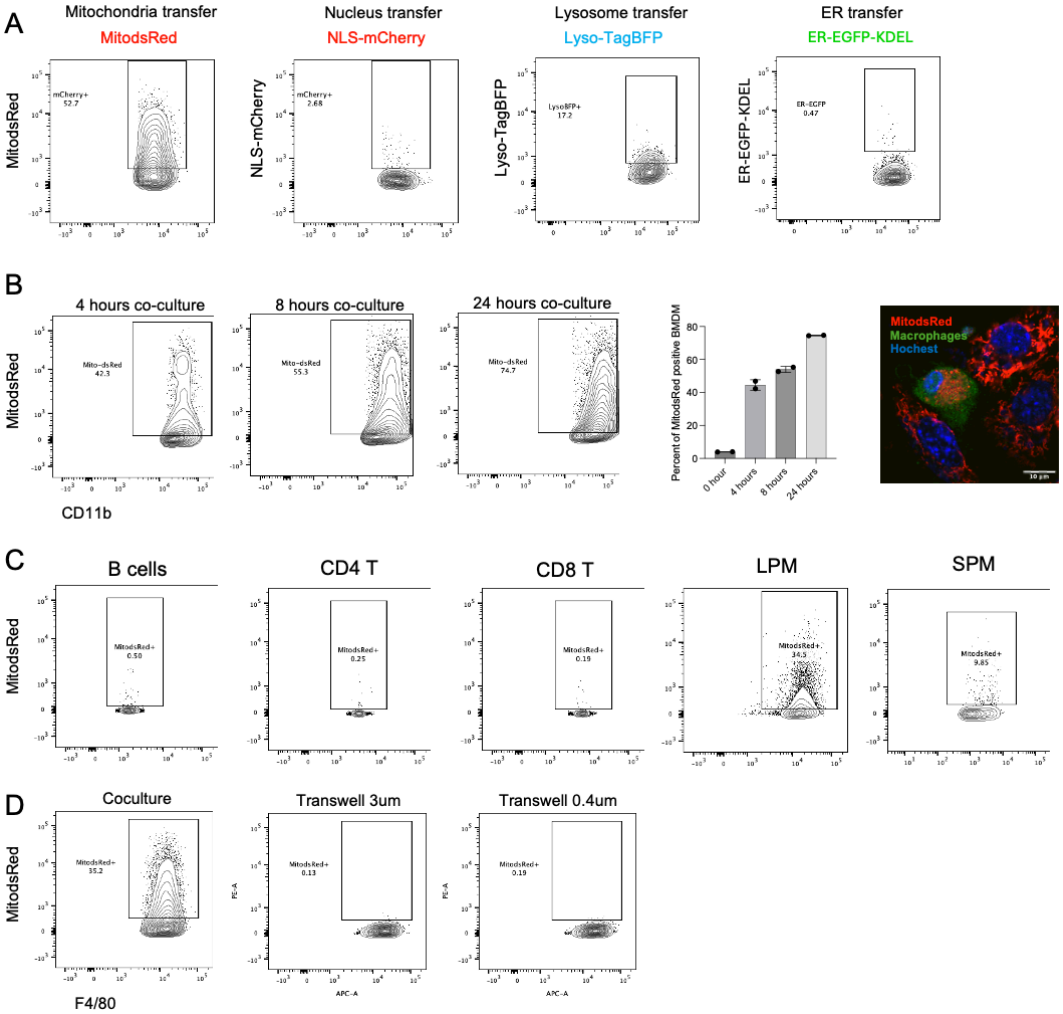
A) Macrophage membrane proteins were selected from different data base and filtered with macrophage transcription data. B) Construction of gRNA library. Oligo were synthesized and cloning into plasmid by Gibson ligation. Abundance of different gRNAs were analyzed by high-through put sequencing.

Figure 3.



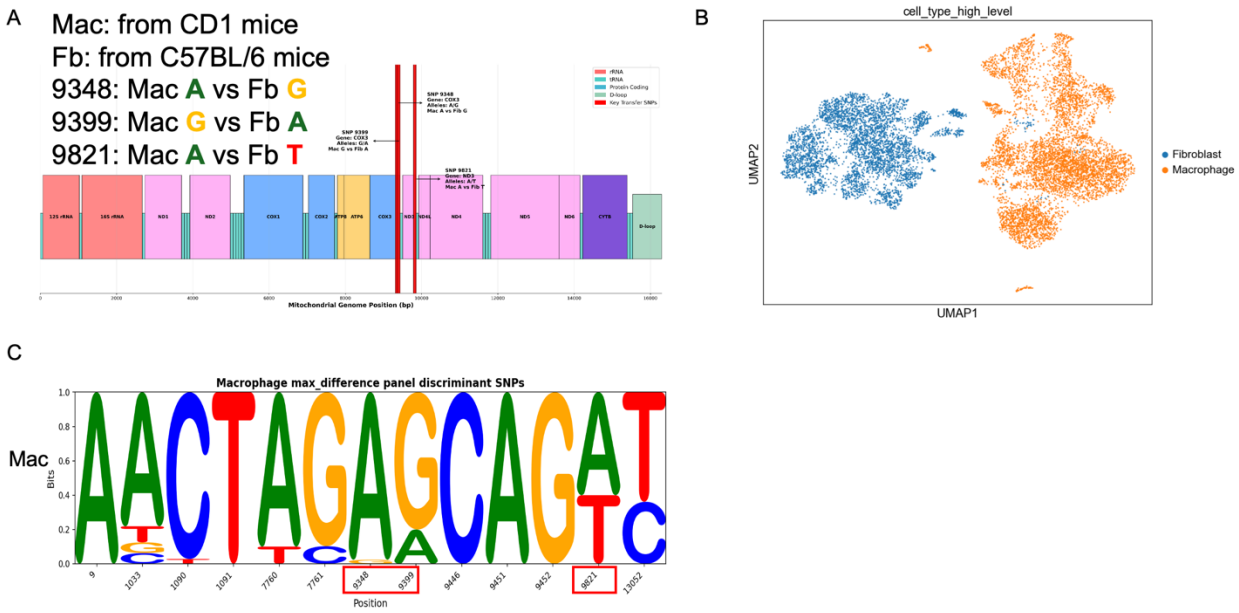
A) CRISPR Fucosyl-labeling screening. Volcano plot showing candidate regulators (negative regulator, blue; positive regulator, red) significantly enriched in altered MP-FB interaction. Candidates were selected with p-value<0.01. B) Scheme of Fucosyl-labeling based CRISPR screen of immune-FBs interactions in vitro.

Figure 4.



A) Measurement of different organelle transfer from fibroblasts to macrophages. This data demonstrated that mitochondria are robustly transferred to macrophages. B) (left) Dynamic of mitochondria transfer from fibroblast to macrophages. (right) Live cell imaging reveals that macrophages (green) contain genetically labeled mitochondria (red) that were derived from fibroblast carrying mitoDsRed. C) Mitochondria transfer from fibroblast to different immunocytes, analyzed by flow cytometry. This data showed the specificity of mitochondrial transfer in macrophages. D) Mitochondrial transfer from fibroblast to macrophages separated by a transwell. This data demonstrated that direct cell-cell contact is necessary for mitochondrial transfer.

Figure 5.



A) Identification of strain-specific mitochondrial SNPs distinguishing CD1 and C57BL/6 mice. B) t-SNE visualization of single-cell RNA-sequencing data showing the separation of fibroblasts and macrophages after co-culture. C) MitoPaseo identifies strain-specific mitochondrial SNPs, enabling detection of mitochondrial transfer between fibroblasts and macrophages.