3. Single-cell epitope-transcriptomics reveal lung stromal and immune cell response kinetics to nanoparticle-delivered RIG-I and TLR4 agonists

Lung-resident and circulatory cells detect pathogens via pattern recognition receptors. Using single-cell RNA sequencing (CITE-seq), we analyzed early lung responses to nanoparticle-delivered PAMPs, specifically the RIG-I agonist PUUC, with or without TLR4 agonist MPLA, in mice. By 4 hours, ribosome-associated transcripts dropped, followed by strong interferon-stimulated gene expression. RNA velocity captured dynamic cytokine regulation in neutrophils. Co-delivery of MPLA and PUUC enhanced chemokine and antimicrobial protein synthesis across diverse cell types. These single-cell RNA findings reveal that RIG-I activation induces a broad antimicrobial state in lung cells, suggesting potential for intranasal therapies.