

Airway Delivery of Encapsulated Cytokine-Secreting Cells for Local Immunomodulation in Inflammatory Lung Diseases

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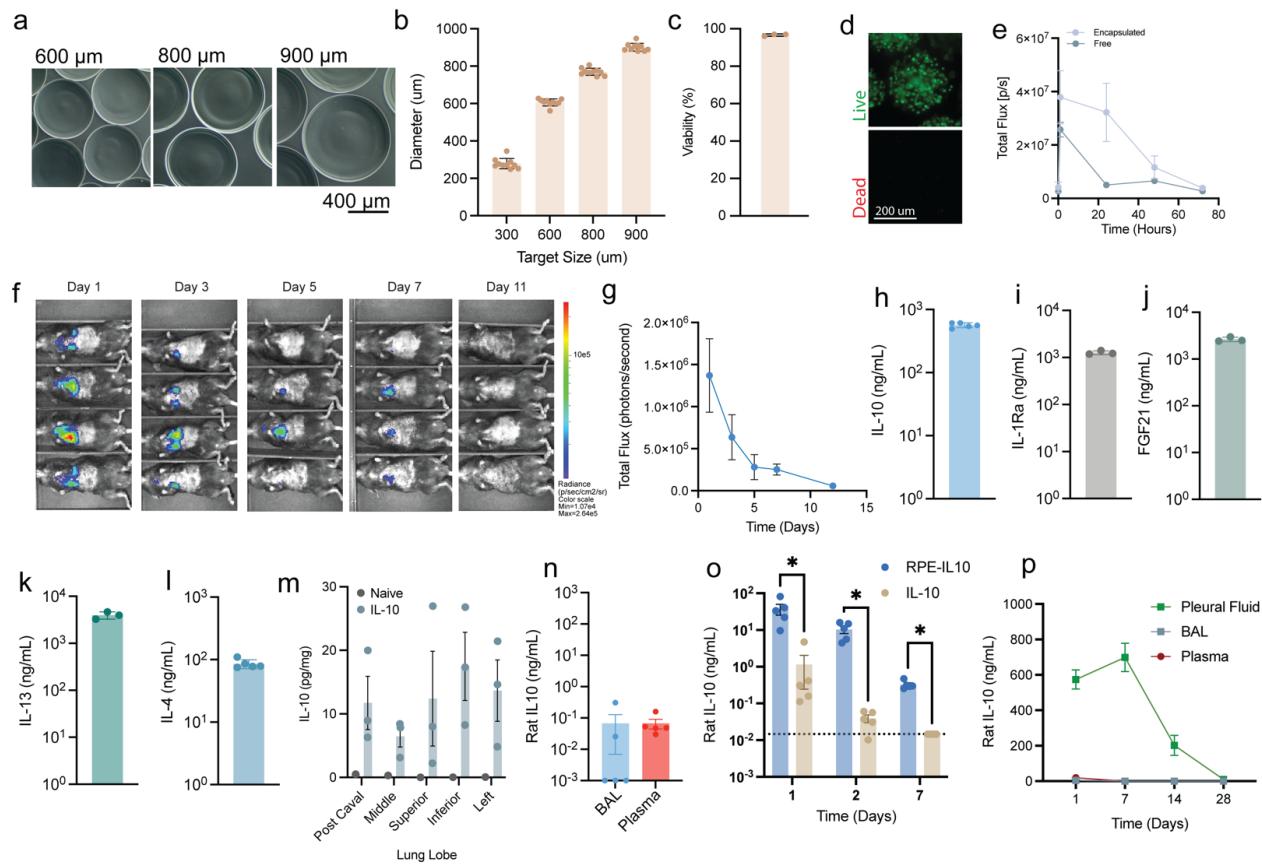
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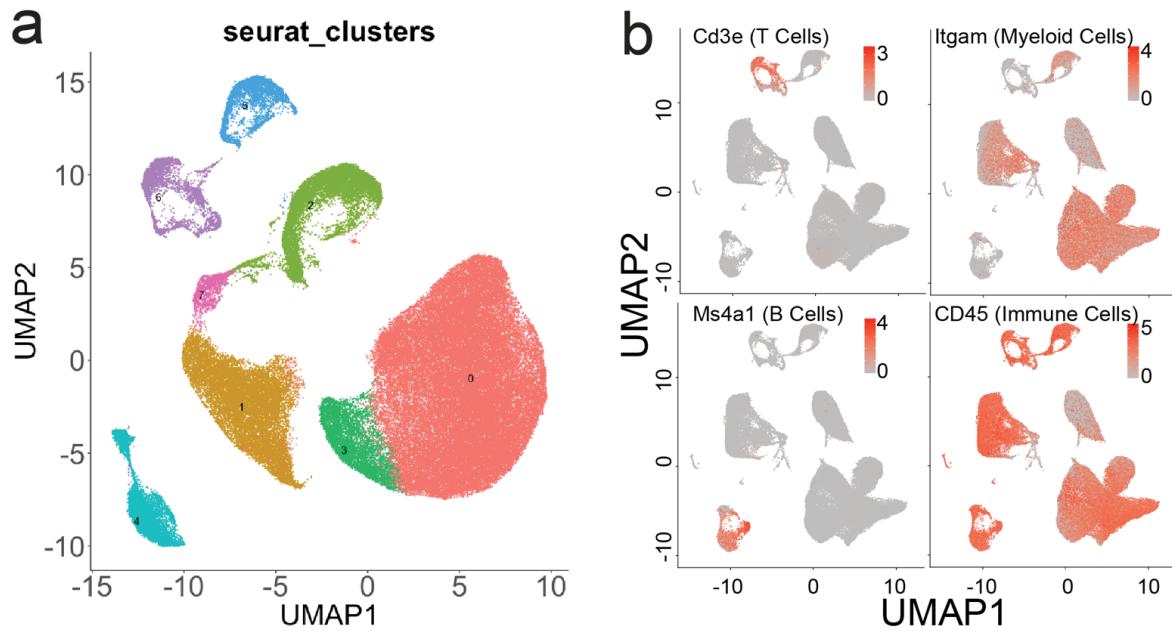
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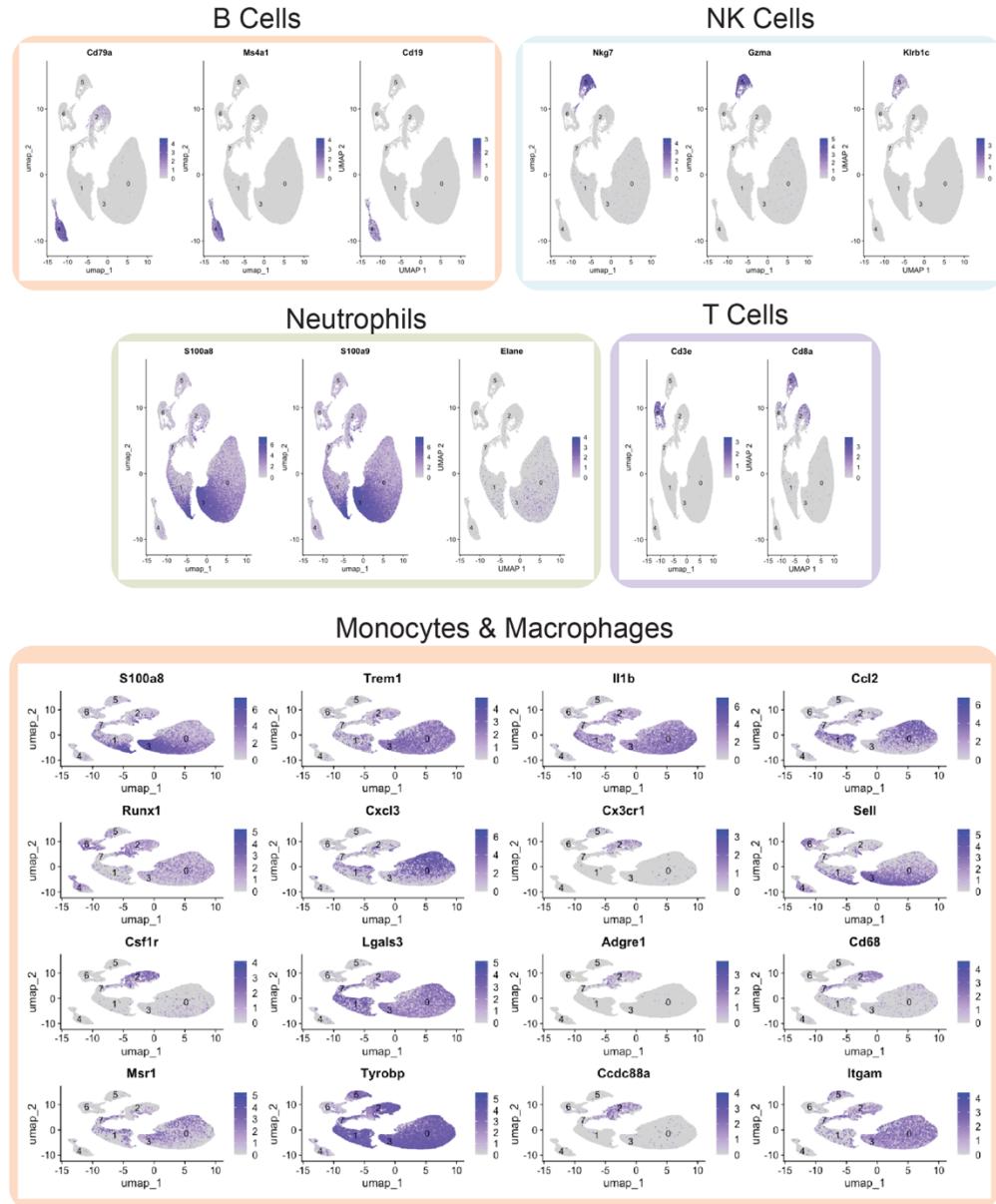
Supplemental Figures



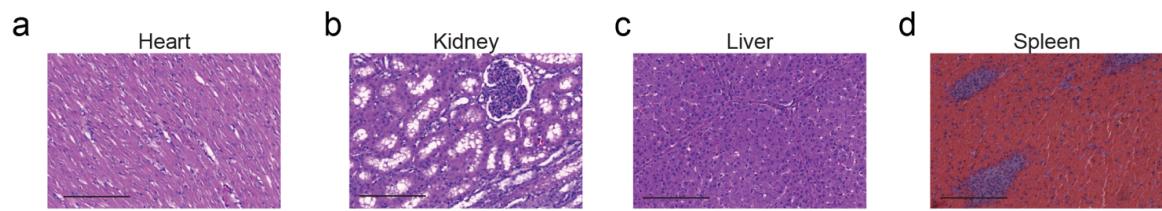
Supplemental Figure 1 | Microcapsule platform characterization. A) Representative brightfield images of alginate microcapsules with varying diameters. B) Quantification of capsule diameters confirming size distribution. C) Cell viability 24 hours post-encapsulation, expressed as a percentage. D) Representative live/dead staining images of encapsulated cells. E) Quantification of total luminescent flux over time to evaluate retention and activity of microcapsules in rats. F) Luminescent (IVIS) imaging of mice following intratracheal instillation of alginate microcapsules containing RPE cells expressing luciferase. G) Quantification of total luminescent flux over time to evaluate retention and activity of microcapsules in mice. H-L) ELISA measurements of IL-10, IL-1Ra, FGF21, IL-13, and IL-4 released from microcapsules after 24 hours in vitro M) Distribution of IL-10 across lung lobes 24 hours post-instillation of IL-10-producing microcapsules. N) IL-10 levels in BAL fluid and plasma 100 days post-instillation. O) Quantification of BAL and plasma concentrations of IL-10 at 0, 1, 2-, and 7-days post-instillation with free IL-10 protein. Dashed line represents basal levels (n=5) P) Quantification of BAL, pleural fluid, and plasma concentrations of IL-10 at 1, 7-, 14- and 28-days post-pleural implant of IL-10 capsules (n=5). All data are represented as mean \pm SEM.



Supplemental Figure 2 | Non-annotated cell clusters. **A)** Integrated clusters across four conditions: healthy rat lung, rat lung exposed to LPS, rat lung exposed to LPS and blank capsules, and rat lungs exposed to LPS and capsules producing IL-10. There are 6 clusters with each color representing a different cell cluster. **B)** FeaturePlot of canonical gene expression across clusters. Red is more highly expressed while grey is lower expressed.



Supplemental Figure 3 | UMAP of cluster-specific genes for B cells, Natural Killer (NK) cells, Neutrophils, T cells, and Monocytes & Macrophages. Integrated clusters across four conditions: healthy rat lung, rat lung exposed to LPS, rat lung exposed to LPS and blank capsules, and rat lungs exposed to LPS and capsules producing IL-10. Genes are plotted for specific cell types (B cells, NK cells, neutrophils, T cells, monocytes & macrophages). Purple dots indicate cells with higher gene expression while gray dots represent cells with lower gene expression.



Supplemental Figure 4 | H&E-stained sections of the **A**) heart, **B**) kidney, **C**) liver, and **D**) spleen showing no change in morphology. (n=3)