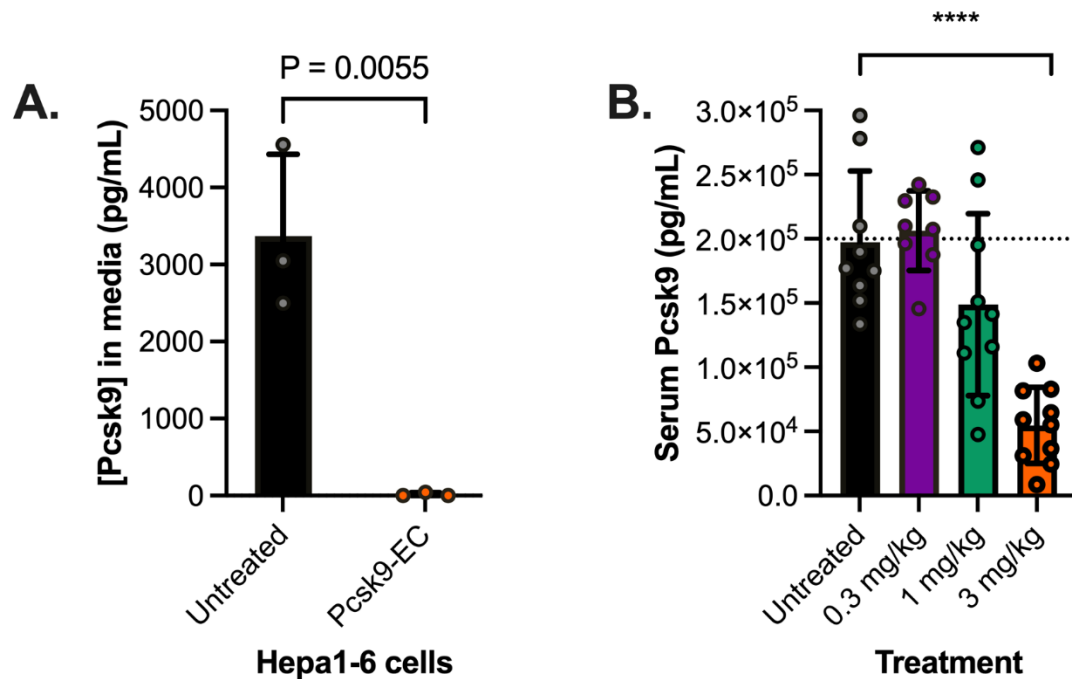


Programmable mRNA Therapeutics for Controlled Epigenomic Modulation of Single and Multiplexed Gene Expression in Diverse Diseases

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Supplementary Document

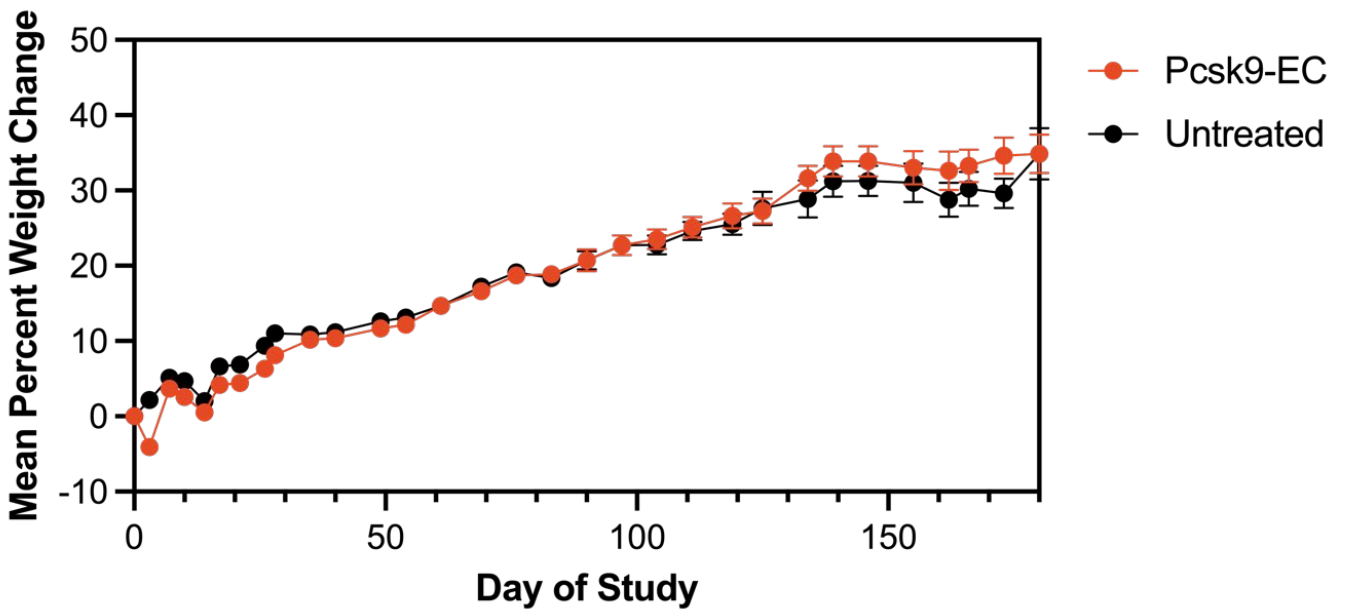
Supplementary Figure 1



Supplementary Figure 1. Pcsk9 downregulation in Hepa1-6 cells

A. Pcsk9-EC was validated on the murine hepatic cell line Hepa1-6. After 5 days post-transfection, we observed near complete reduction of Pcsk9 in Hepa1-6 cells. Data represents the mean of 3 biological replicates from independent experiments ($P=0.0055$, unpaired, two-tailed t-test). **B.** A dose-range pilot murine study treating C57BL/6 mice with a single administration of Pcsk9-EC (0.1 mg/kg, $n=8$; 1 mg/kg, $n=10$; 3 mg/kg, $n=10$) via tail vein injection identified a reduction in levels of serum Pcsk9 seven days following treatment with a 3 mg/kg dose compared to control untreated mice ($n=9$, **** $P<0.0001$, one-way ANOVA). Source data are provided as a Source Data file.

Supplementary Figure 2

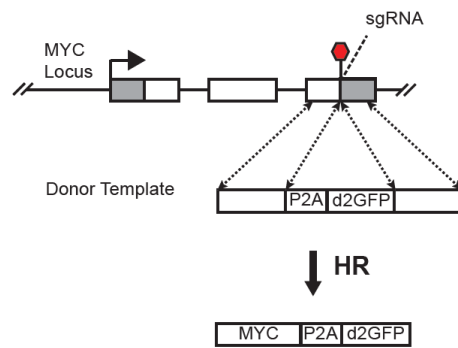


Supplementary Figure 2. In vivo EC treatment does not affect mouse body weight through 180 days

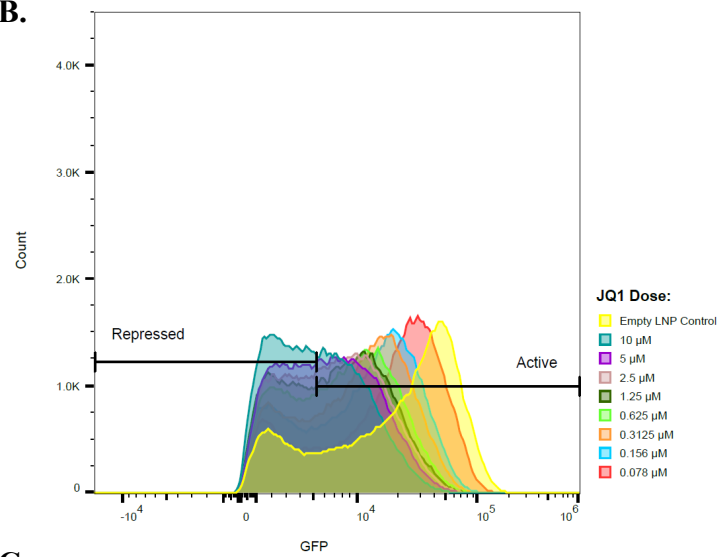
The mean percent weight change in mice after either treatment with Pcsk9-EC or PBS is shown for the duration of the study. No statistically significant difference was observed between the two groups. The data represents the mean $\pm$ standard deviation (SD) of all mice at each time point per group. Each group started with n=70 mice with periodic samplings for analysis. On day 14, n=60 mice per group remained. On day 28, n=50 mice per group remained. On day 60, n=40 mice per group remained. On day 90, n=30 mice per group remained. On day 120, n=20 mice per group remained. On day 150, n=10 mice per group remained until the end of the study. Source data are provided as a Source Data file.

Supplementary Figure 3

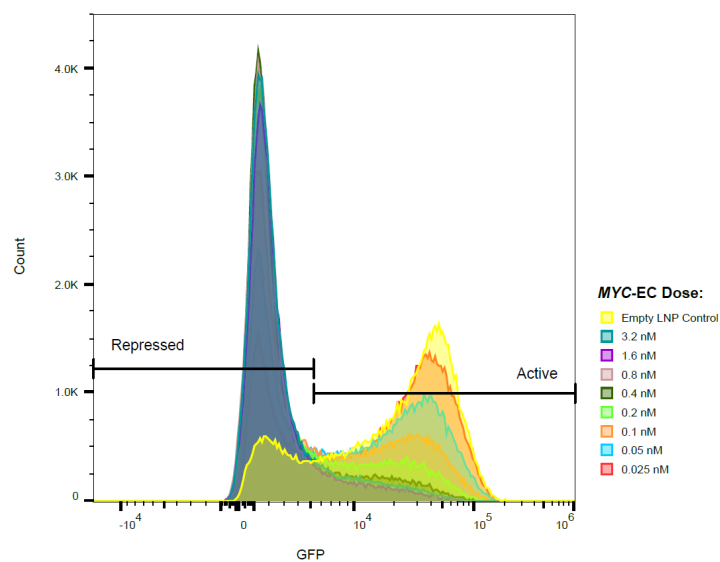
A.



B.



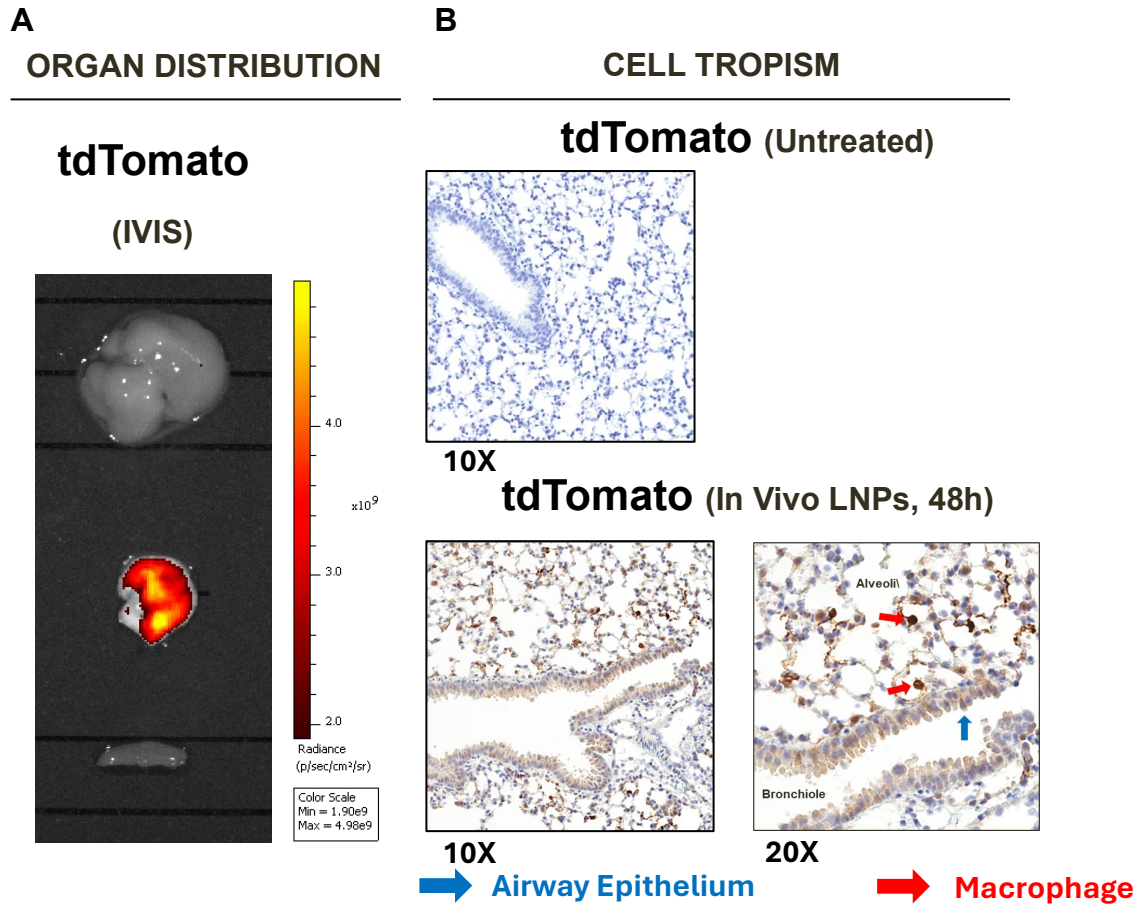
C.



Supplementary Figure 3. Fluorescent reporter co-expression system and transcriptional downregulation of MYC locus

(A) CRISPR engineering strategy for knock-in reporter cell line that couples expression of a destabilized Green Fluorescent Protein variant (d2GFP) to *MYC* in K-562 cells (K-562 MYC/d2GFP). Flow cytometry analysis of JQ1 (B) and MYC-EC dose escalation (C) in K-562 MYC/d2GFP cells compared to control (yellow). Cellular *MYC* levels, represented by GFP fluorescence, progressively shift lower as a population as the dose increases after JQ1 treatment, whereas MYC-EC treatment induces a repressed population with roughly equivalent repression. Active and repressed gates shown are used for cellular classification in Figure 2E. Data shown is representative of 3 independent experiments.

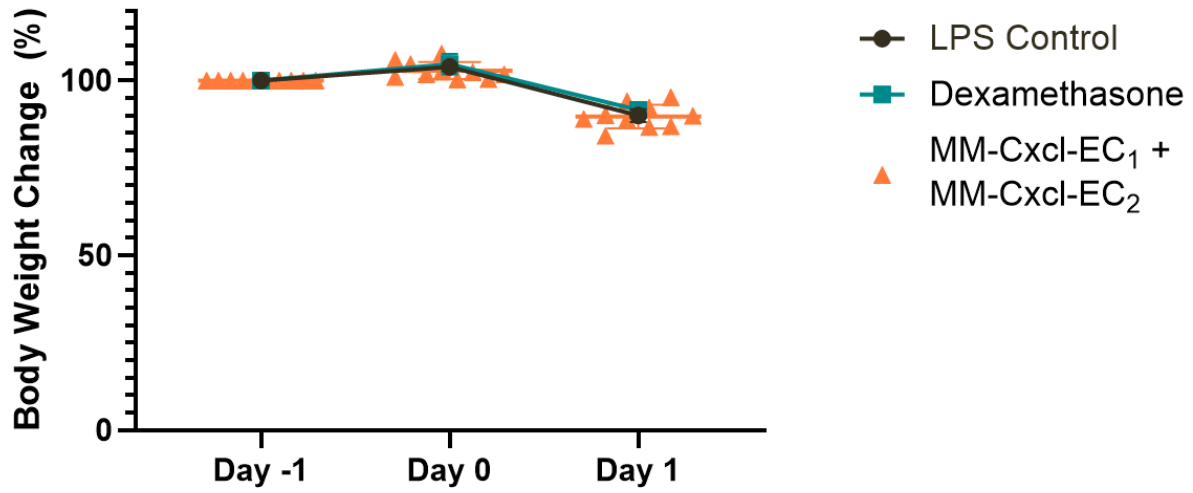
Supplementary Figure 4



Supplementary Figure 4. LNPs targeting lung tissue

Ai14 mice (B6.Cg-*Gt(ROSA)26Sor*^{tm14(CAG-tdTomato)Hze/J}) were dosed intravenously with 3 mg/kg of Cre mRNA (TriLink, L-7211) loaded into in vivo LNPs for overall assessment of LNP biodistribution. **A.** Ex-vivo IVIS imaging shows tdTomato expression specifically in the lung as compared to common LNP distribution tissues such as liver (top) and spleen (bottom). **B.** Lungs were sectioned for IHC and stained for the tdTomato reporter. Airway epithelial cells and macrophages showed the highest reporter expression of cell types identified. Images are representative of a cohort of n=3 mice.

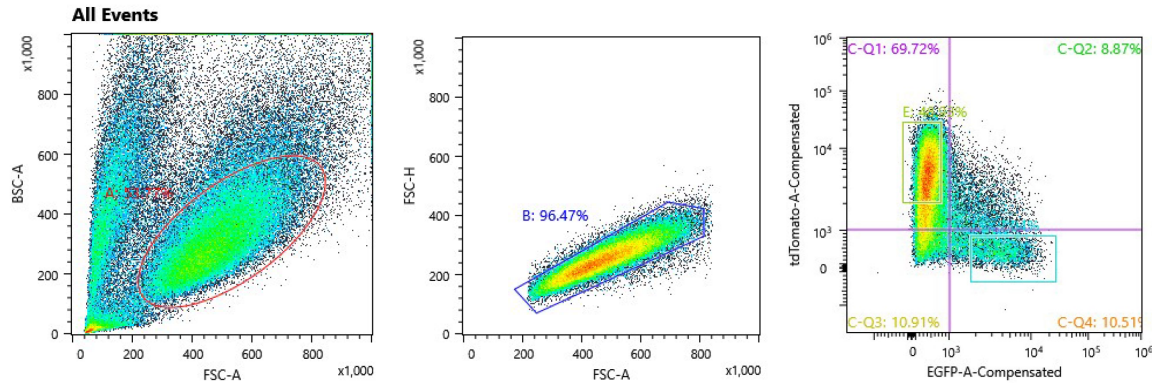
Supplementary Figure 5



Supplementary Figure 5. Body weights for animals treated with MM-Cxcl-EC₁ + MM-Cxcl-EC₂

Body weights for treatment groups were measured on the day of treatment and 1-day pre and post treatment, with no changes in weight between the groups (n=10 animals per group). The data is presented as the mean $\pm$ standard deviation (SD). Source data are provided as a Source Data file.

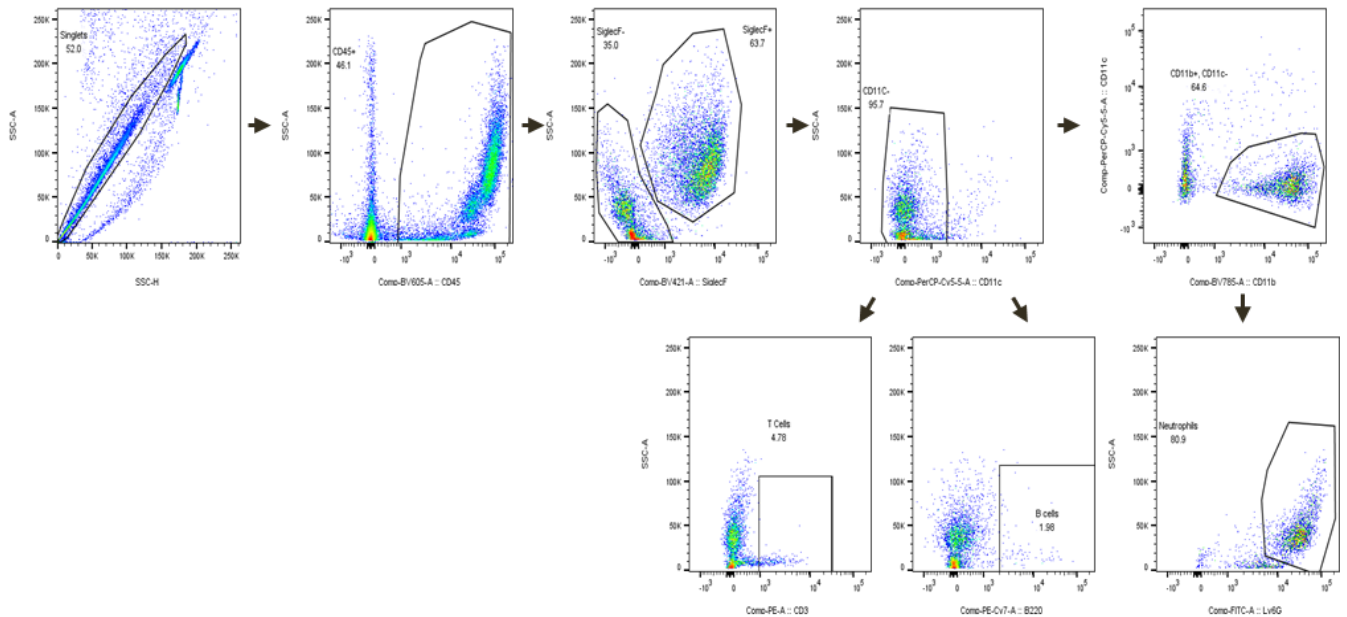
Supplementary Figure 6



Supplementary Figure 6. FACS gating strategy

Schematic for sorting cells in Figure 2F.

Supplementary Figure 7



Neutrophils: CD45+, Siglec F-, CD11b+, CD11c-, Ly-6G+

T cells: CD45+, Siglec F-, CD11c-, CD3+

B cells: CD45+, Siglec F-, CD11c-, B220+

Supplementary Figure 7. FACS gating strategy

Schematic for identifying cell types in Figure 5A.