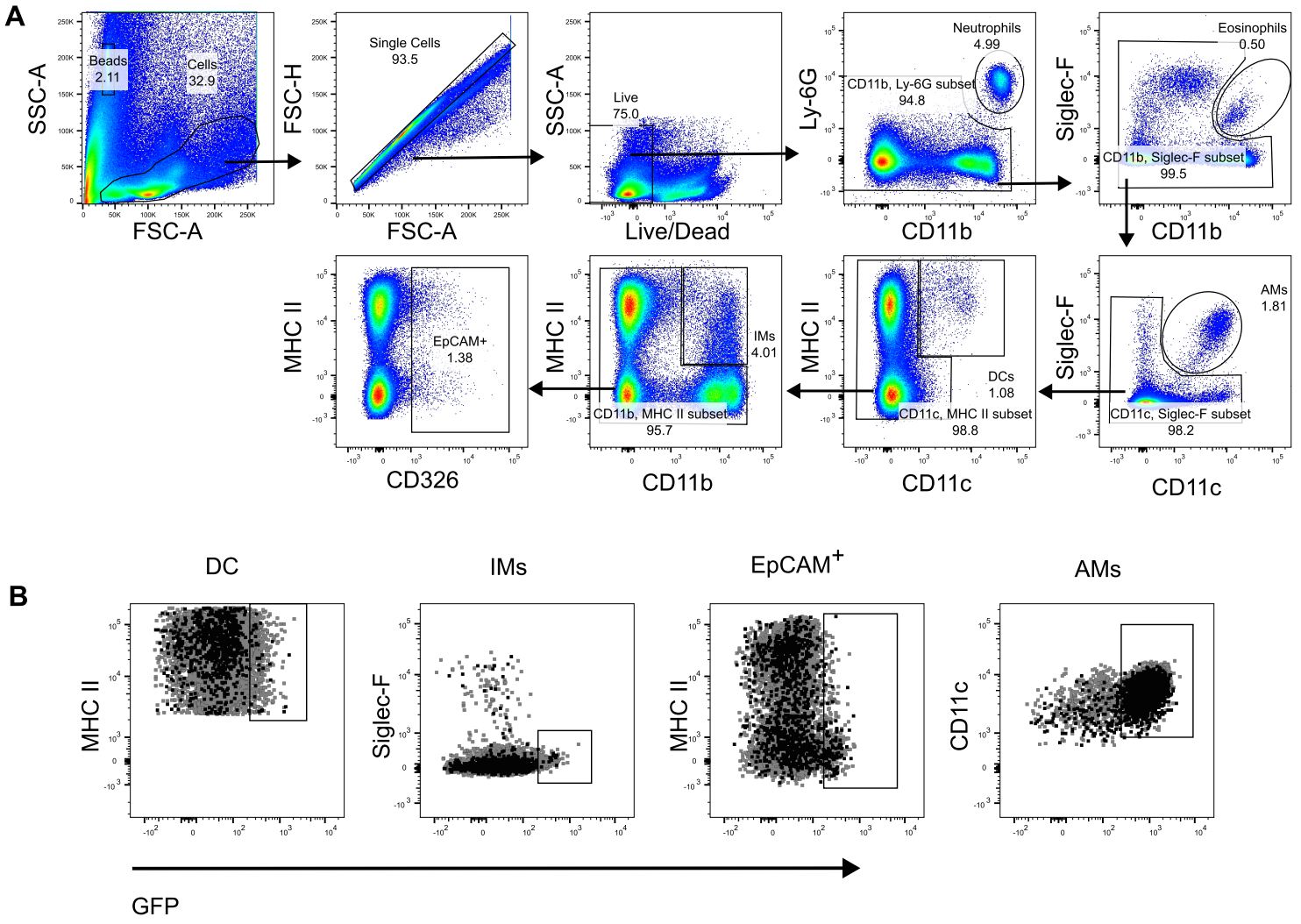
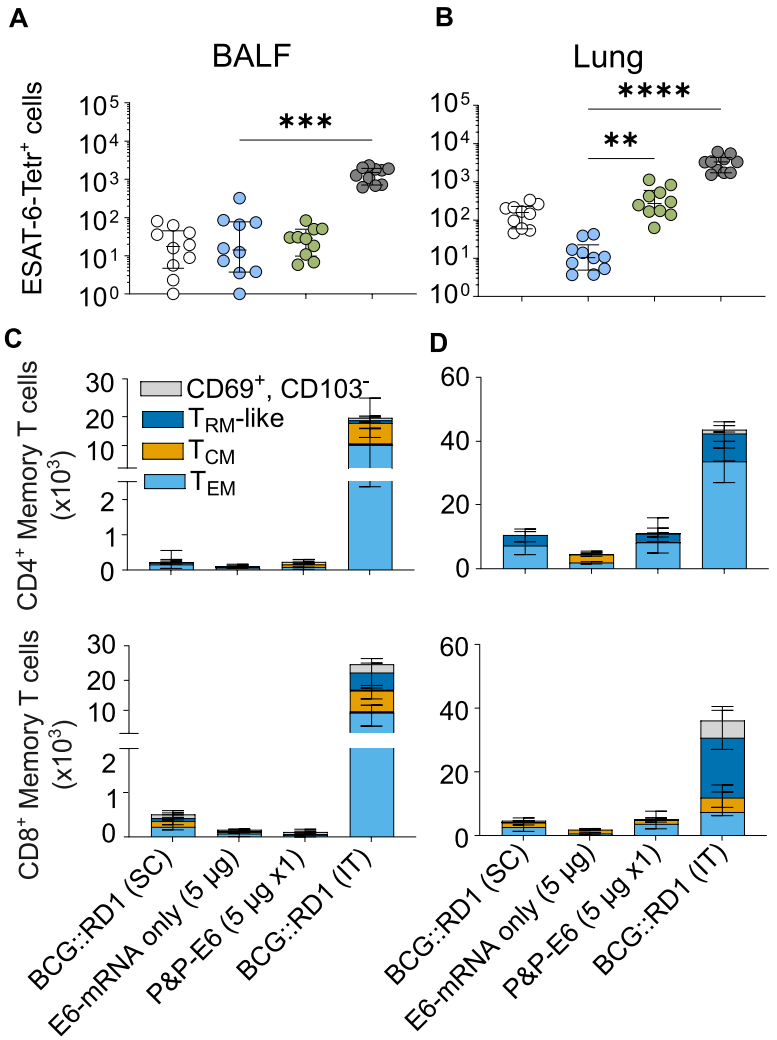
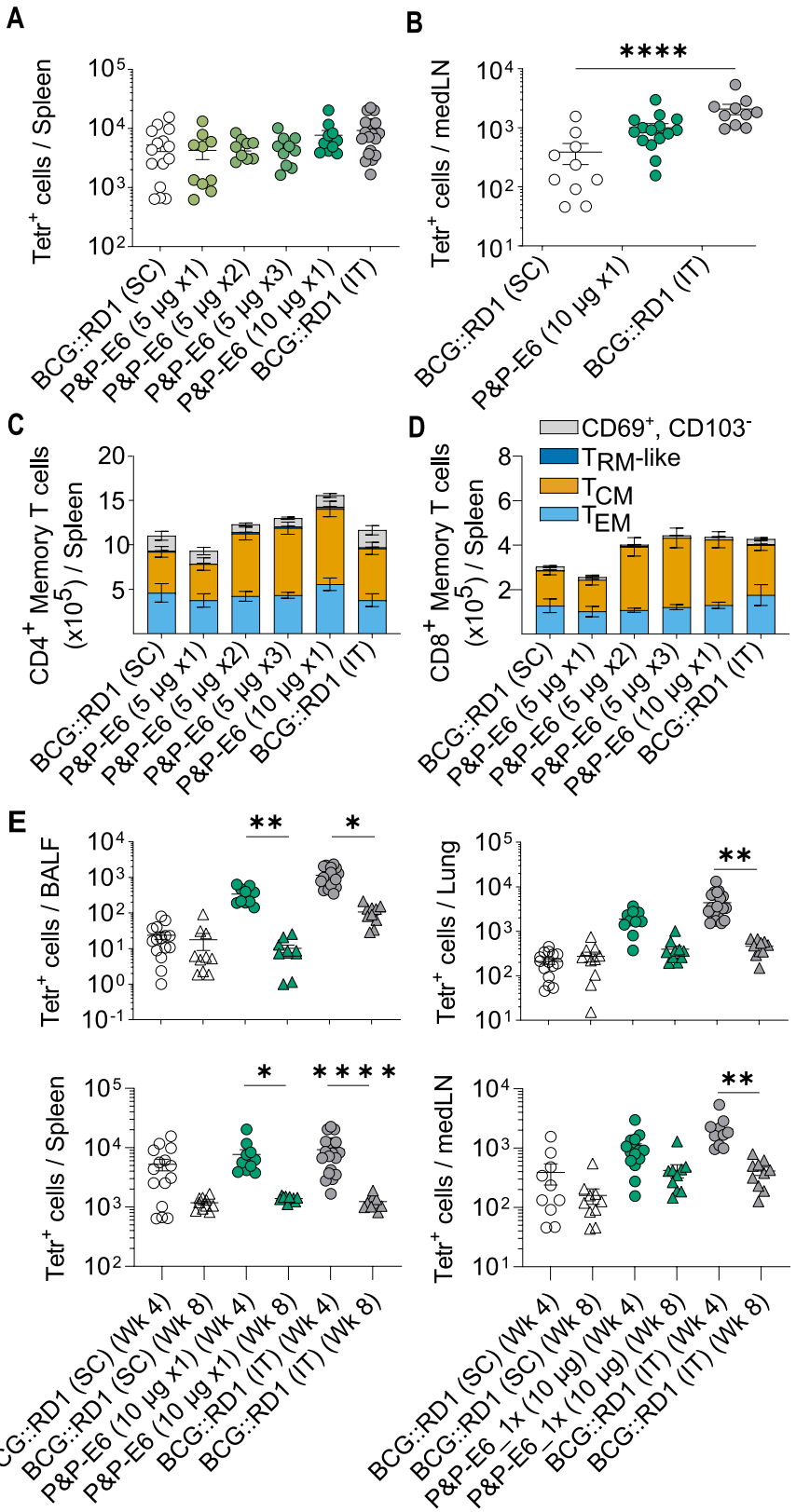


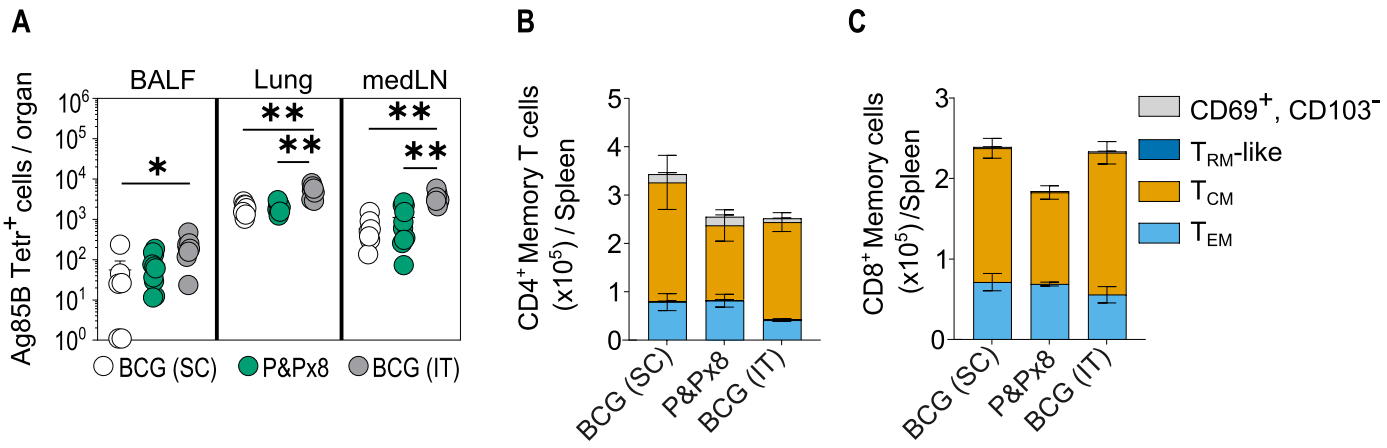

**Supplementary Figure 1. Gating strategy and representative GFP expression in pulmonary cell populations following intratracheal administration of GFP-mRNA-sLNPs. A.** Flow cytometry gating strategy used to identify neutrophils, dendritic cells (DCs), interstitial macrophages (IMs), alveolar macrophages (AMs), and EpCAM⁺ epithelial cells from lung single-cell suspensions. **B.** Representative GFP fluorescence plots for DCs, IMs, AMs, EpCAM⁺ epithelial cells, and neutrophils from PBS-treated mice (black dots) and mice analysed 48 h following intratracheal administration of 10 µg GFP-mRNA-sLNPs (grey dots). GFP fluorescence is shown following gating on the indicated pulmonary cell populations. Interpretation of GFP expression in AMs and EpCAM⁺ epithelial cells was limited by intrinsic autofluorescence within the GFP detection channel.



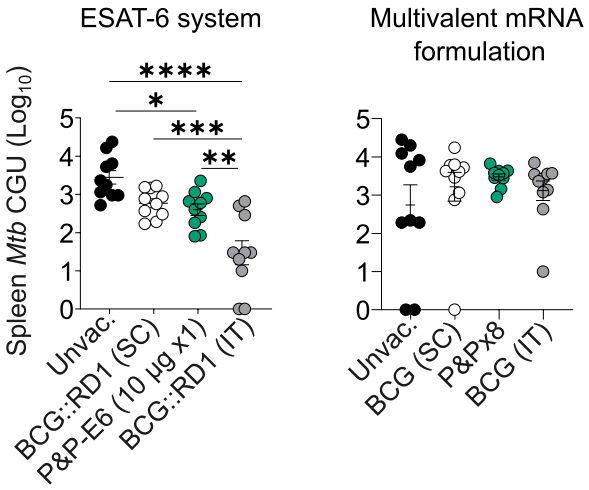

**Supplementary Figure 2. Pulmonary ESAT-6 mRNA boosting enhances antigen-specific and memory T-cell responses following BCG priming.** C57BL/6 mice were vaccinated with BCG::RD1 (SC), ESAT-6 mRNA alone (5 µg IT), prime-and-pull vaccination [P&P-E6 (5 µg × 1) or P&P-E6 (10 µg × 1)], or BCG::RD1 (IT) as described in Fig. 2. Antigen-specific and memory T-cell populations were analysed 28 days following primary vaccination. **A.** and **B.** Number of ESAT-6 tetramer$^+$ cells in BALF and lungs, respectively. **C-D.** Number of CD4$^+$ and CD8$^+$ memory T-cell populations, in BALF and lungs, including CD69$^+$CD103$^-$ cells, $T_{RM}$-like cells, $T_{CM}$ cells, and $T_{EM}$ cells. Data were analysed using the Kruskal-Wallis test with Dunn's multiple-comparison post-test. NE = 2-3 independent experiments; n = 4-5 mice per group. Symbols represent individual animals and bars indicate the median and IQR. *P<0.05, **P<0.01, ***P<0.001, ****P< 0.0001.




**Supplementary Figure 3. Prime-and-pull vaccination induces systemic and transient ESAT-6-specific T-cell responses.** C57BL/6 mice were vaccinated as described in Fig. 2. Antigen-specific and memory T-cell responses were analysed 28 or 56 days following primary vaccination. A**-D. Systemic T-cell responses at day 28. A.** Number of ESAT-6 tetramer$^+$ cells in the spleen. **B.** Number of ESAT-6 tetramer$^+$ cells in the medLN. **C-D.** Number of CD4$^+$ and CD8$^+$ memory T-cell populations, including CD69$^+$CD103$^-$ cells, $T_{RM}$-like cells, $T_{CM}$ cells, and $T_{EM}$ cells, in the spleen. **E.Durability of ESAT-6-specific T-cell responses.** Number of ESAT-6 tetramer$^+$ cells in BALF, lungs, spleen, and medLN at 28 and 56 days following vaccination. Data were analysed using the Kruskal-Wallis test with Dunn's multiple-comparison post-test. NE = 2-3 independent experiments; n = 4-5 mice per group. Symbols represent individual animals and bars indicate the median and IQR. *P<0.05, **P<0.01, ***P<0.001, ****P< 0.0001.




**Supplementary Figure 4. Ag85B-specific and memory T-cell responses following prime-and-pull vaccination with a multivalent mRNA formulation.** Hybrid F1 C57BL/6 × BALB/c mice were vaccinated as described in Fig. 3. Antigen-specific and memory T-cell responses were analysed 28 days following primary vaccination. **A.** Number of Ag85B tetramer⁺ cells in bronchoalveolar lavage fluid (BALF), lungs, and mediastinal lymph nodes (medLN). **B-C.** Number of spleen CD4⁺ and CD8⁺ memory T-cell populations, respectively. Data were analysed using the Kruskal-Wallis test with Dunn's multiple-comparison post-test. NE = 2-3 independent experiments; n = 4-5 mice per group. Symbols represent individual animals and bars indicate the median and IQR. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.




**Supplementary Figure 5. Prime-and-pull vaccination reduces splenic bacterial burden following *Mtb* challenge.** C57BL/6 mice (ESAT-6 model) and hybrid F1 C57BL/6 × BALB/c mice (multivalent mRNA formulation) were vaccinated as described in Fig. 4. Sixty days following vaccination, mice were challenged by very low-dose aerosol infection with *Mtb* H37Rv. Splenic bacterial burden was determined 45 days post-infection. **A.** Splenic bacterial burden in mice vaccinated with BCG::RD1 subcutaneously (SC), BCG::RD1 intratracheally (IT), or prime-and-pull vaccination using ESAT-6-mRNA-sLNPs [P&P-E6 (10 µg × 1)]. **B.** Splenic bacterial burden in mice vaccinated with BCG (SC), BCG (IT), or prime-and-pull vaccination using the multivalent mRNA formulation (P&Px8). Bacterial burden data were log10-transformed and analysed using one-way ANOVA with Tukey's multiple-comparison post-test. NE = 2 independent experiments; n = 5 mice per group. Symbols represent individual animals and bars indicate the mean ± SEM of log10-transformed data. *P<0.05, **P<0.01, ***P<0.001, ****P< 0.0001.