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Supplementary Information for PrimeFlow™ Assay for Cell Type–Specific Co-detection of Transgene RNA and Protein in Mouse Spleens From Preclinical Studies

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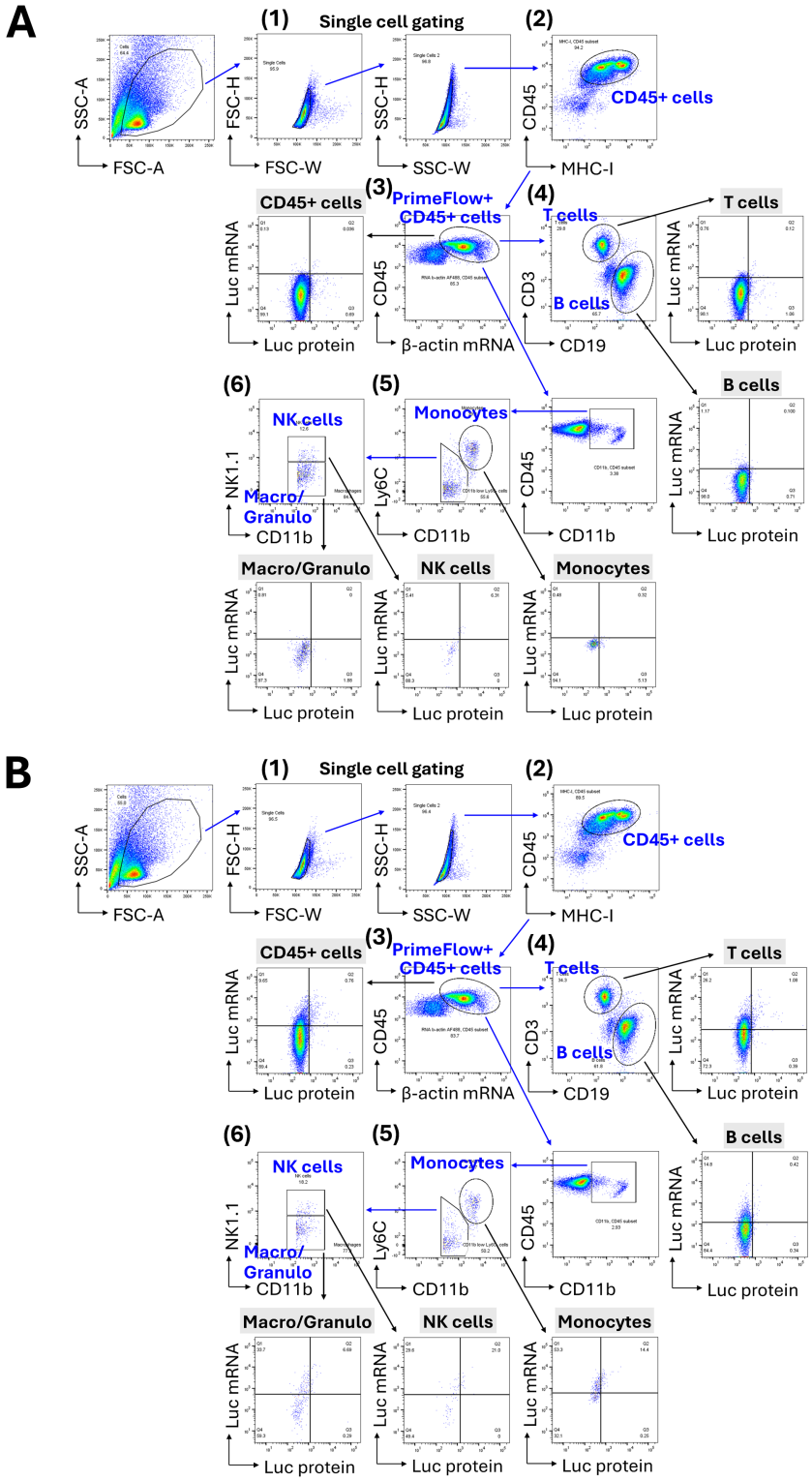

Figure S1. Example of gating strategy for immunophenotyping and luciferase RNA and protein co-detection in isolated mouse splenocytes transfected with mRNA-LNP ex vivo. Cryopreserved C57BL/6 mouse splenocytes (Cell Biologics, catalog number: C57-6321F) were plated and cultured in complete cell culture medium (Cell Biologics, catalog number: M5567; supplemented with 2% FBS, 0.1% epidermal growth factor (EGF), 0.1% insulin, 0.1% hydrocortisone) and analyzed using PrimeFlow™ protocol 18 h after transfection with mRNA-LNP. Representative pseudocolor scatterplots for ex vivo–transfected C57BL/6 mouse splenocytes, with untreated samples (A) and LNP-treated samples (B) illustrating hierarchical gating strategy. As stated in the main text, the single-cell gates were generated (1). Then, using MHC-I vs. CD45 labeling, the CD45+ (hematopoietic) cell gate was created (2). Then, "PrimeFlow+ CD45+" cells were defined using β-actin mRNA vs. CD45 labeling signals (3). This population was used to gate "T cells" and "B cells" based on mutually

exclusive CD3 and CD19 signals (4), and a CD11b+ population from which “Monocytes” were gated based on CD11b and Ly6C staining (5). The CD11b low and Ly6C- population was used to gate macrophages and granulocytes (“Macro/Granulo”) and natural killer cells (“NK cells”), based on the CD11b and NK1.1 signal (6). As stated in the main text, median fluorescence intensity and % positive cells (using bivariate plots of protein vs. mRNA signal) for luciferase mRNA and protein were assessed in each cell population. SSC: side scatter, FSC: forward scatter, A: area, H: height, W: width.