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Supplemental Information

Nanoparticles versus Dendritic Cells as Vehicles to Deliver mRNA Encoding Multiple Epitopes for Immunotherapy

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Supplemental Figures

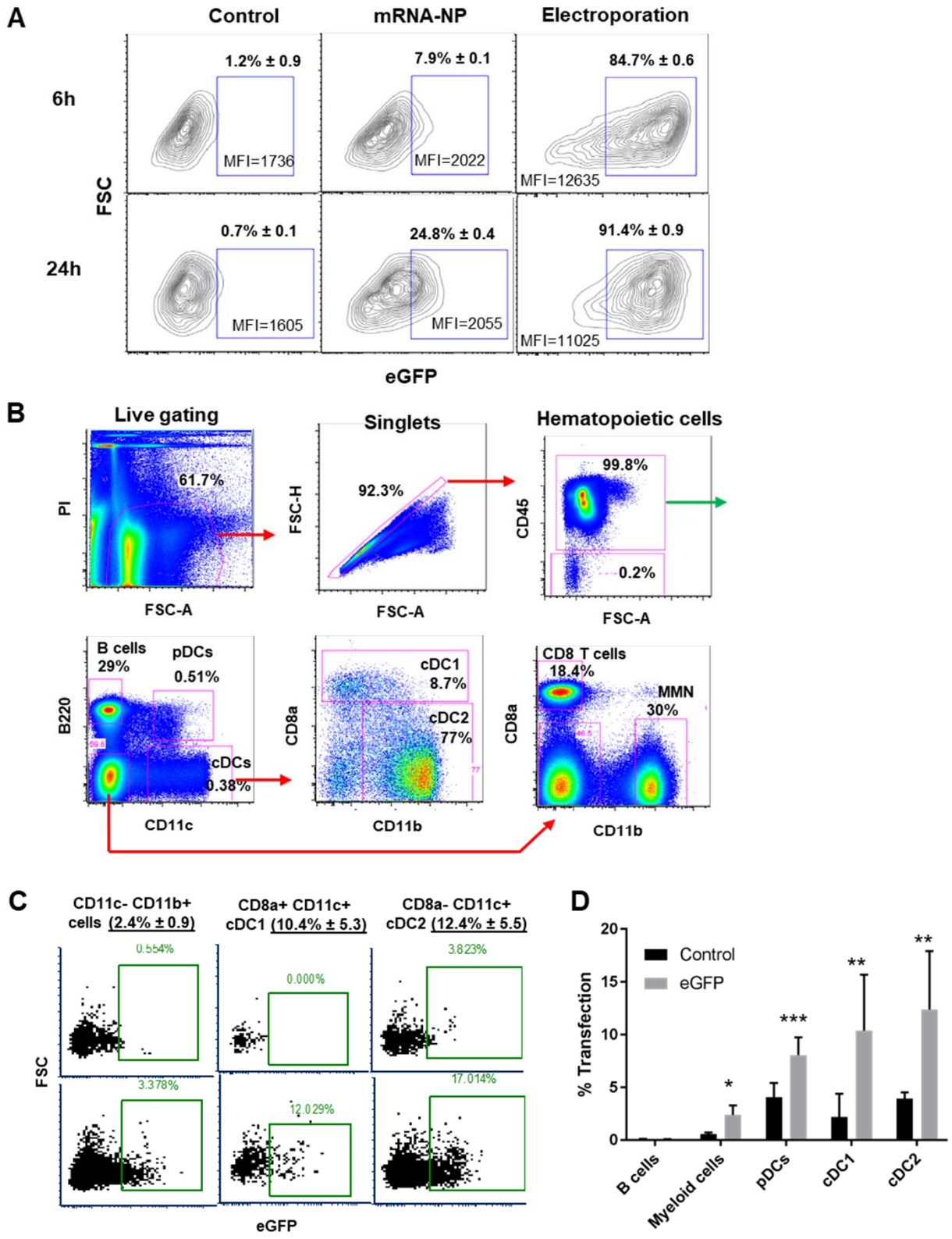
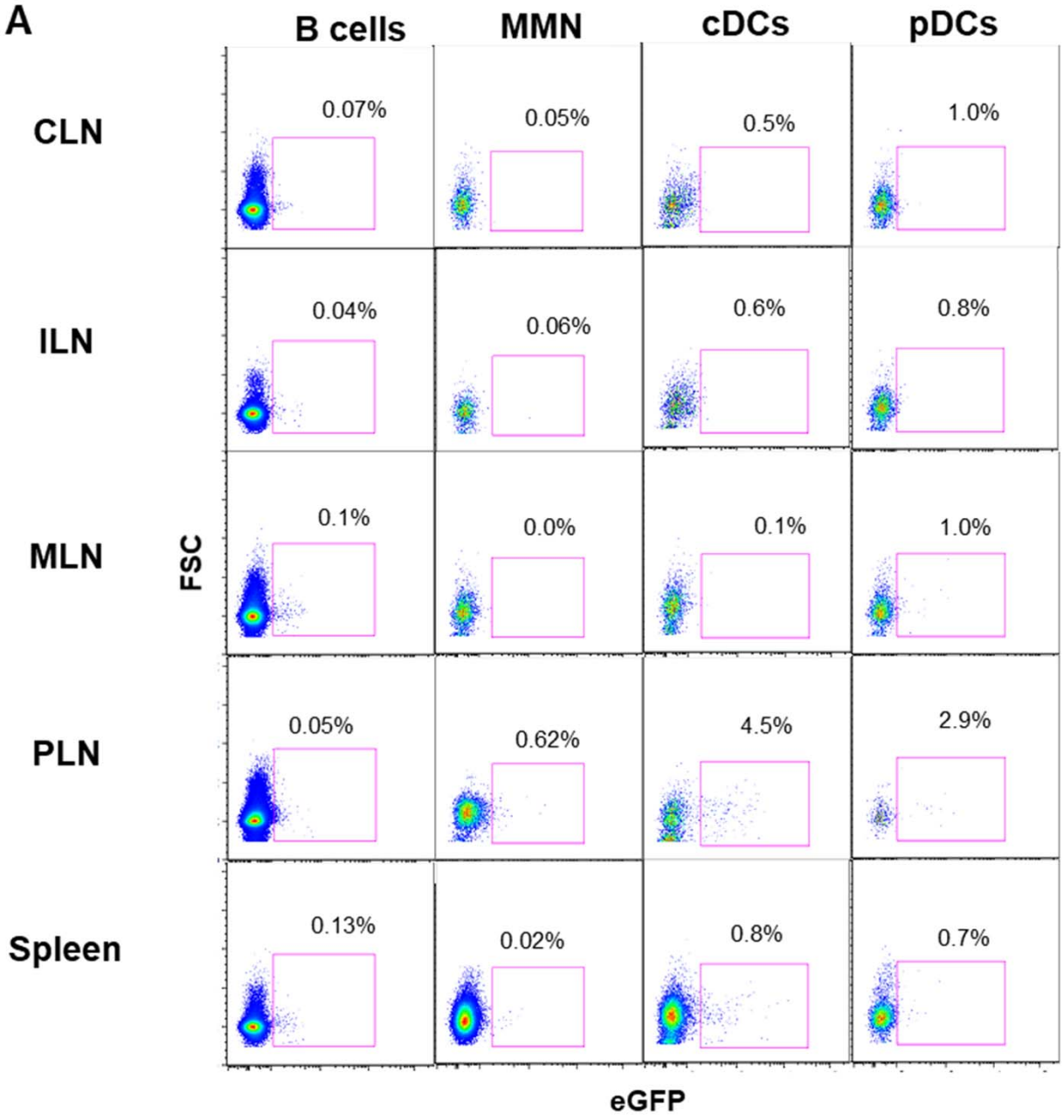


Figure S1. In vitro mRNA-NP transfection efficiency in various antigen-presenting cells. (**A**) In vitro transfection efficiency of mRNA-NPs in BM-DCs as compared to mRNA electroporation after 6h (p=0.0013 for electroporation vs mRNA-NP) and 24h (p=0.0019 for electroporation vs mRNA-NP). The mean fluorescence intensity of eGFP among mRNA-NP transfected cells is about 5-7 times lower than that of electroporated cells. (**B**) Gating strategy for analysis of mRNA-NP uptake and expression by both hematopoietic CD45+ cells and non-hematopoietic CD45- cells. MMN (Monocytes, macrophages or neutrophils) are within the B220- CD11c- CD8- CD11b+ cells. (**C,D**) In vitro transfection efficiency of mRNA-NPs in splenocytes, presented as representative dot plots (**C**) and bar graph (**D**). The cells were harvested 24h post-transfection with 0.1 µg/well eGFP-encoding mRNA formulated as mRNA-NP. The bar graph shows the mean ± SD from technical triplicates and the significant differences indicated are for B cells relative to other cell types in the eGFP-treated group.



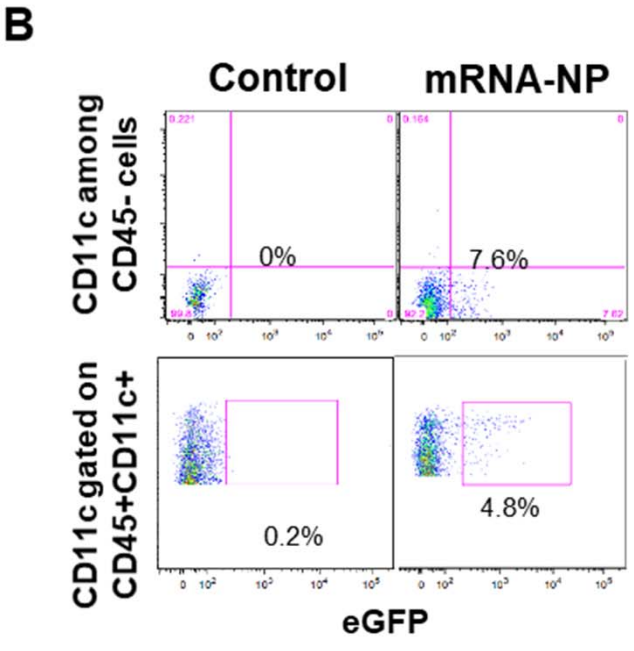


Figure S2: In vivo mRNA-NP transfection efficiency in various antigen-presenting cells. (**A**) In vivo transfection efficiency by eGFP mRNA-NPs in different APCs from various lymphoid tissues. (**B**) Expression of eGFP in both CD45+ CD11c+ cells and non-hematopoietic CD45- cells from PLN. MMN: monocytes / macrophages / neutrophils. For both experiments (**A,B**), mRNA-NPs were injected i.p. at a dose of 20 µg of mRNA eGFP/mouse and APC subsets were analyzed 48h later and data are presented as representative plots from three biological replicates.

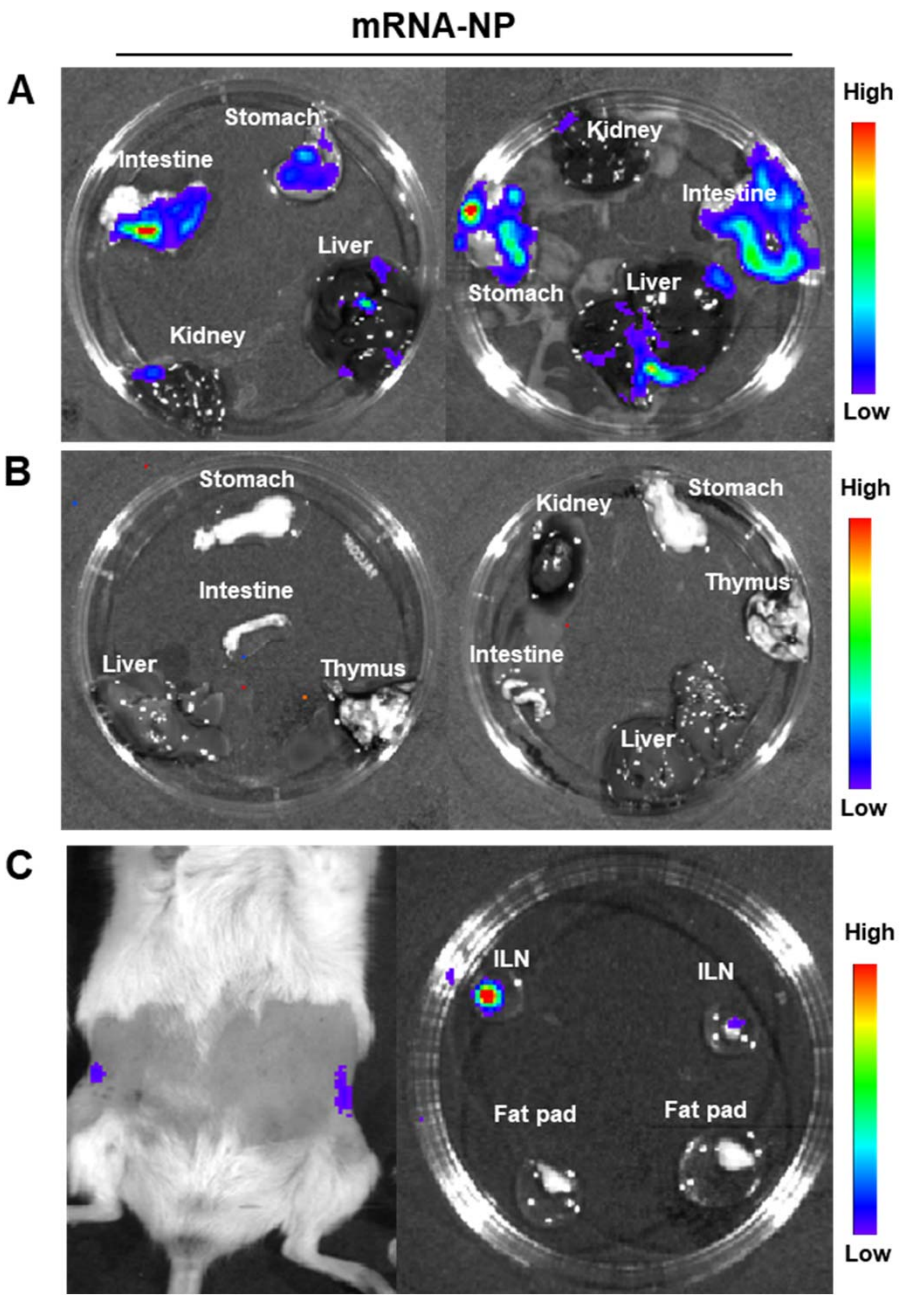


Figure S3. In vivo expression of luciferase from mRNA-NP in various organs. Luciferase signals 8h after i.p. (**A**), i.v. (**B**) and i.n. (**C**) administration. The data are representative of three biological replicates.

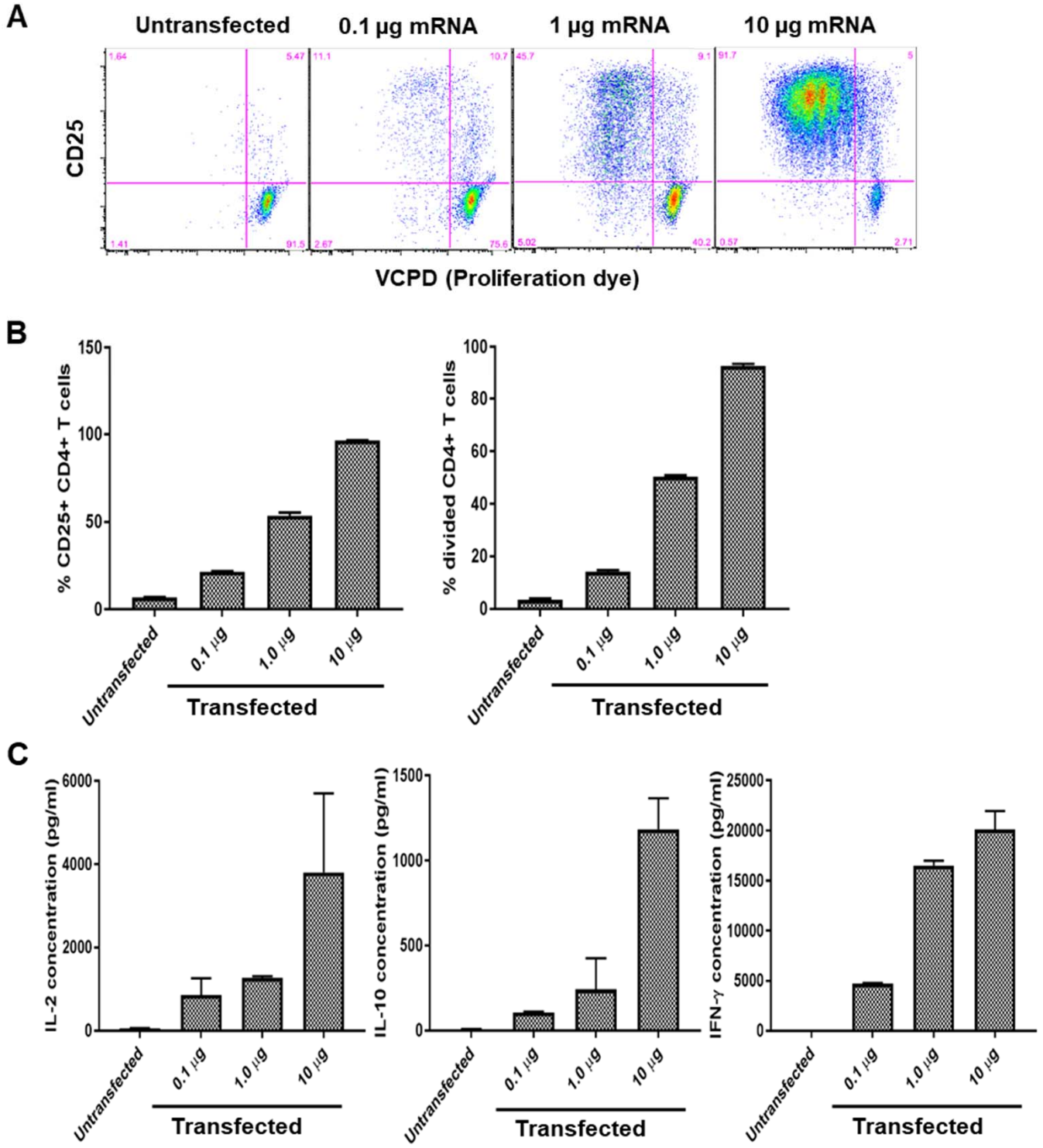


Figure S4: Assessment of BDC2.5 CD4+ T-cell responses to antigen mRNA-NPs in vitro. (**A,B**) Data show proliferation and CD25 upregulation by activated CD4+ T cells presented as representative plots (**A**) and bar graphs (**B**). (**C**) Cytokine secretion in culture supernatant from stimulated BDC2.5 CD4+ T cells. The bar graphs show the mean ± SD from technical triplicates.

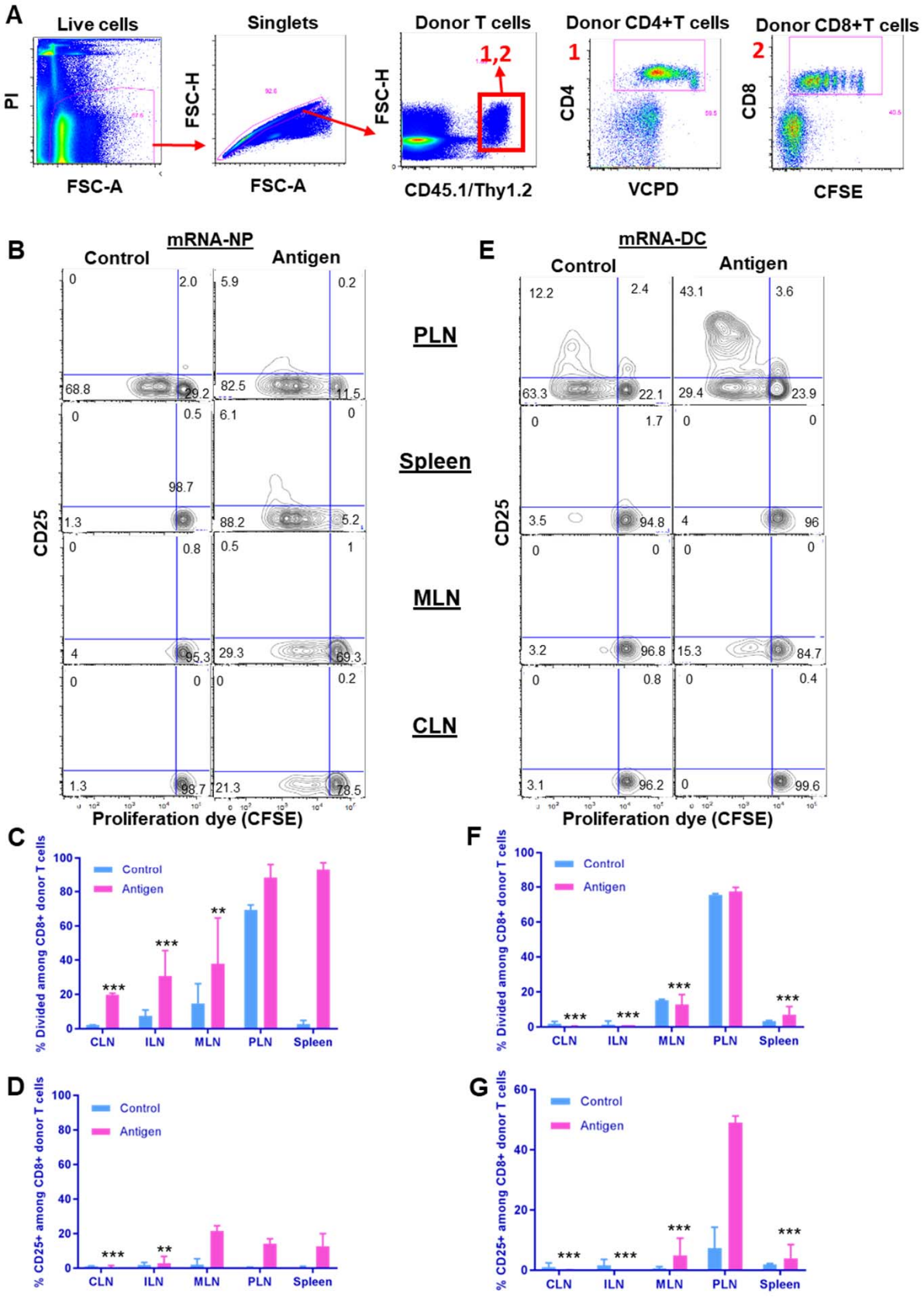


Figure S5. Gating strategy and antigen-specific CD8+ T-cell responses induced by mRNA-NPs and mRNA-DCs after i.p. administration. (**A**) Gating strategy for analysis of T-cell responses in adoptive transfer model. (**B-G**) Responses of transferred NY8.3 CD8+ T cells to mRNA-NPs at a dose of 5 µg mRNA/mouse (**B-D**) and mRNA-DCs at a dose of 2 µg/1x10^6 electroporated DCs/mouse (**E-G**) were analyzed in CLN, ILN, PLN, MLN and spleen, and the results are depicted as representative dot plots (**B**,**E**), proliferation (percentage divided) (**C,F**) and CD25 upregulation (**D**,**G**). eGFP were used as a control in both modalities and the data are presented as mean ± SD from at least three biological replicates. The significant differences indicated for several lymphoid tissues are relative to PLN in the antigen-treated group.

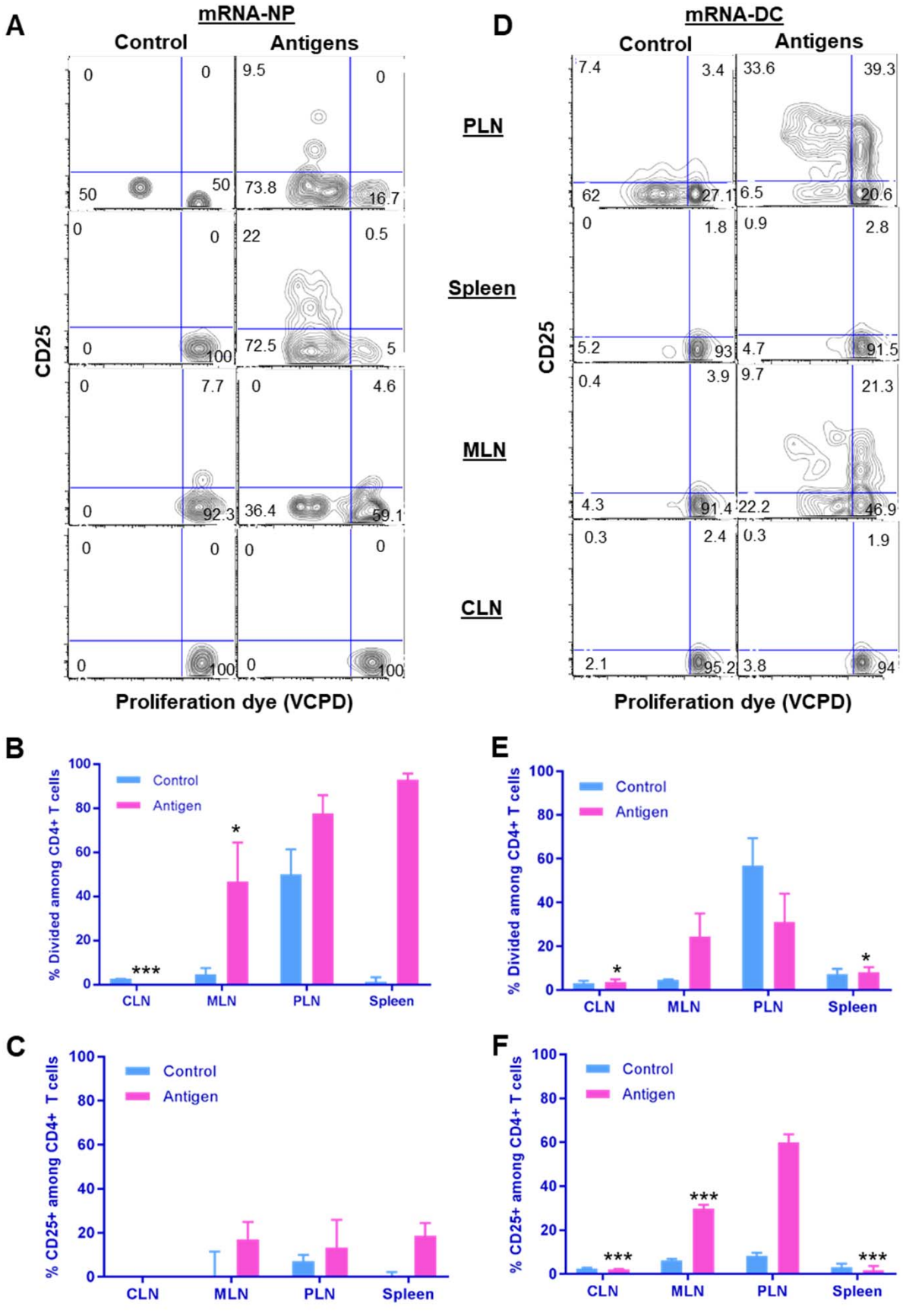


Figure S6. Antigen-specific BDC12-4.1 T-cell response to mRNA-NPs or mRNA-DCs. (**A-F**) Antigen-specific BDC12-4.1 T-cell responses to mRNA-NPs (**A-C**) and mRNA-DCs (**D-F**) in various lymphoid tissues after i.p. administration. mRNA-NPs and mRNA-DCs were used at a dose of 5 µg mRNA/mouse and 0.6 µg/ $1x10^6$ electroporated DCs/mouse, respectively. eGFP was used as a control in both modalities and the data are presented as mean ± SD from three biological replicates. The significant differences indicated for several lymphoid tissues are relative to PLN in the antigen-treated group.

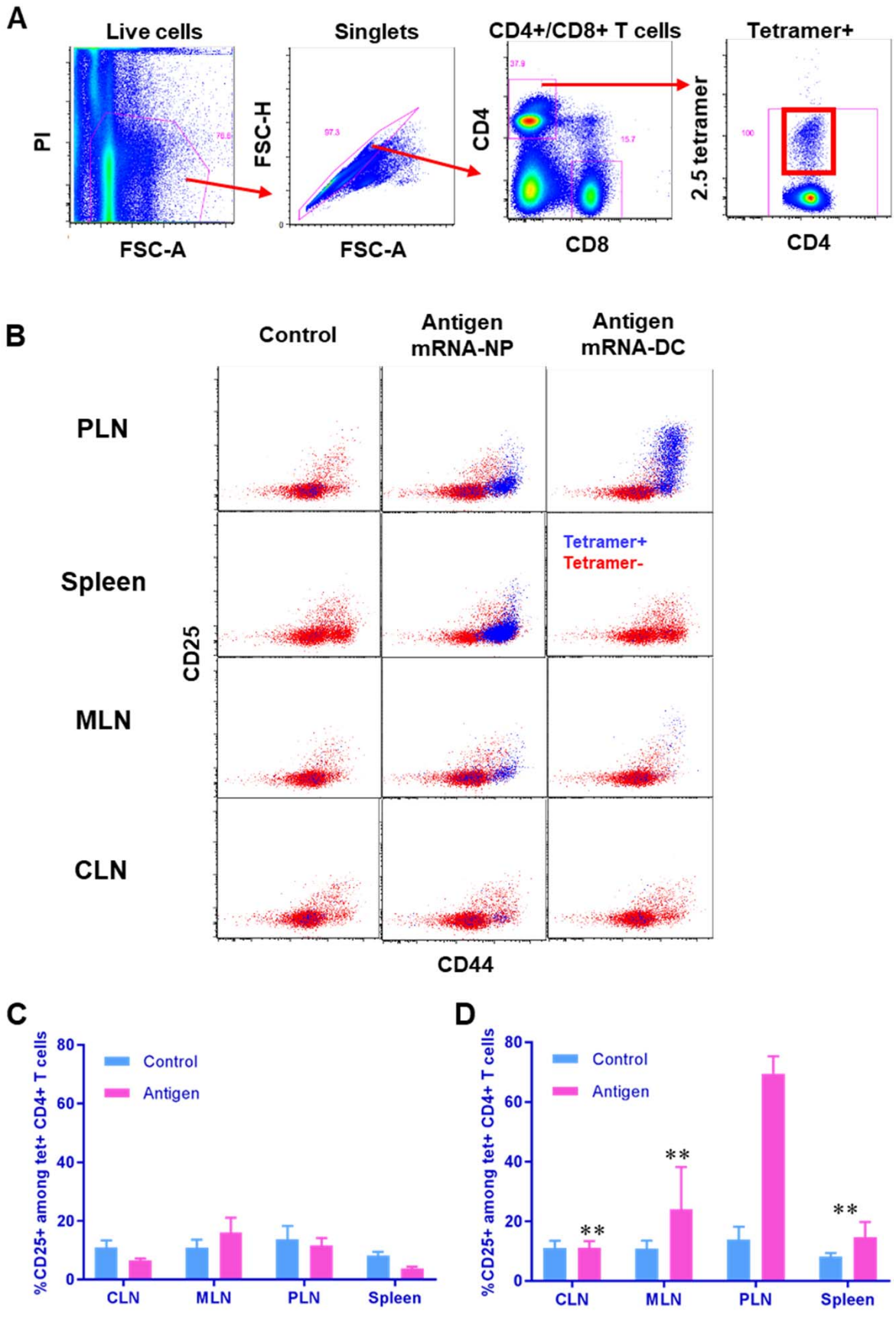


Figure S7: Response of antigen-specific endogenous CD4+ T cells to mRNA-NPs or mRNA-DCs. (**A**) Gating strategy for analysis of endogenous antigen-specific T-cell responses using 2.5 MHC tetramers. (**B-D**) Endogenous antigen-specific CD4+ T-cell responses to mRNA-NPs (**B,C**) and mRNA-DCs (**B,D**), in various lymphoid tissues 2.5 days after i.p. administration. Mice were injected with mRNA-NPs (5 µg/mouse) expressing multiple epitopes (or mCherry as control) or 0.6 µg/10^6 electroporated DCs/mouse. The bar graphs show the mean ± SD from biological triplicates. The significant differences indicated for several lymphoid tissues are relative to PLN in the antigen-treated group.

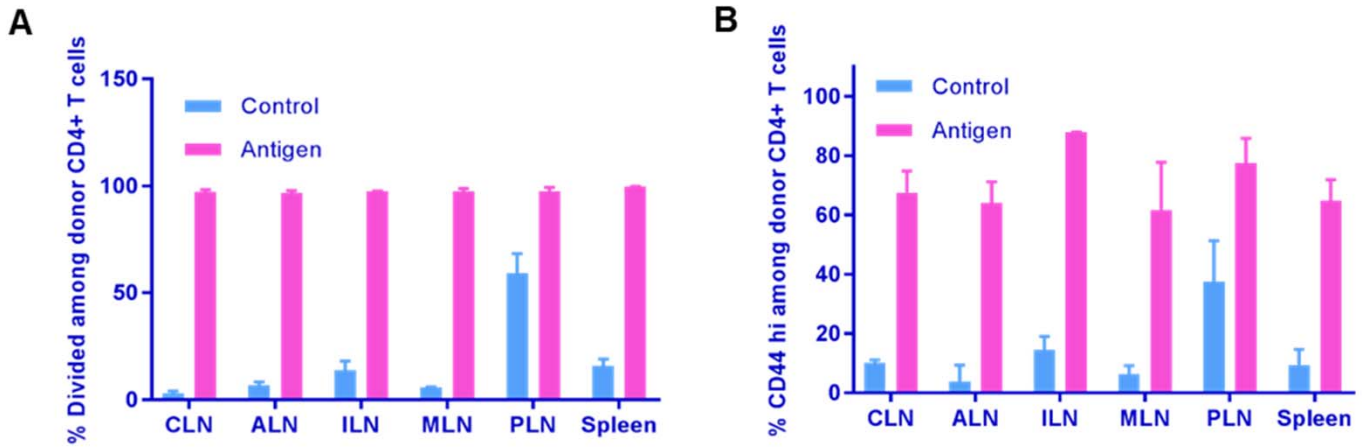


Figure S8. Immune responses induced by mRNA-NPs after i.v. delivery. Response of adoptively transferred BDC2.5 CD4+ T cells as proliferation (**A**) and CD44 upregulation (**B**) induced by mRNA-NPs ("Antigen") in various lymphoid tissues after i.v. injection. Mice in "Control" group were treated with saline. The data are presented as mean ± SD from at least 3 mice per group.

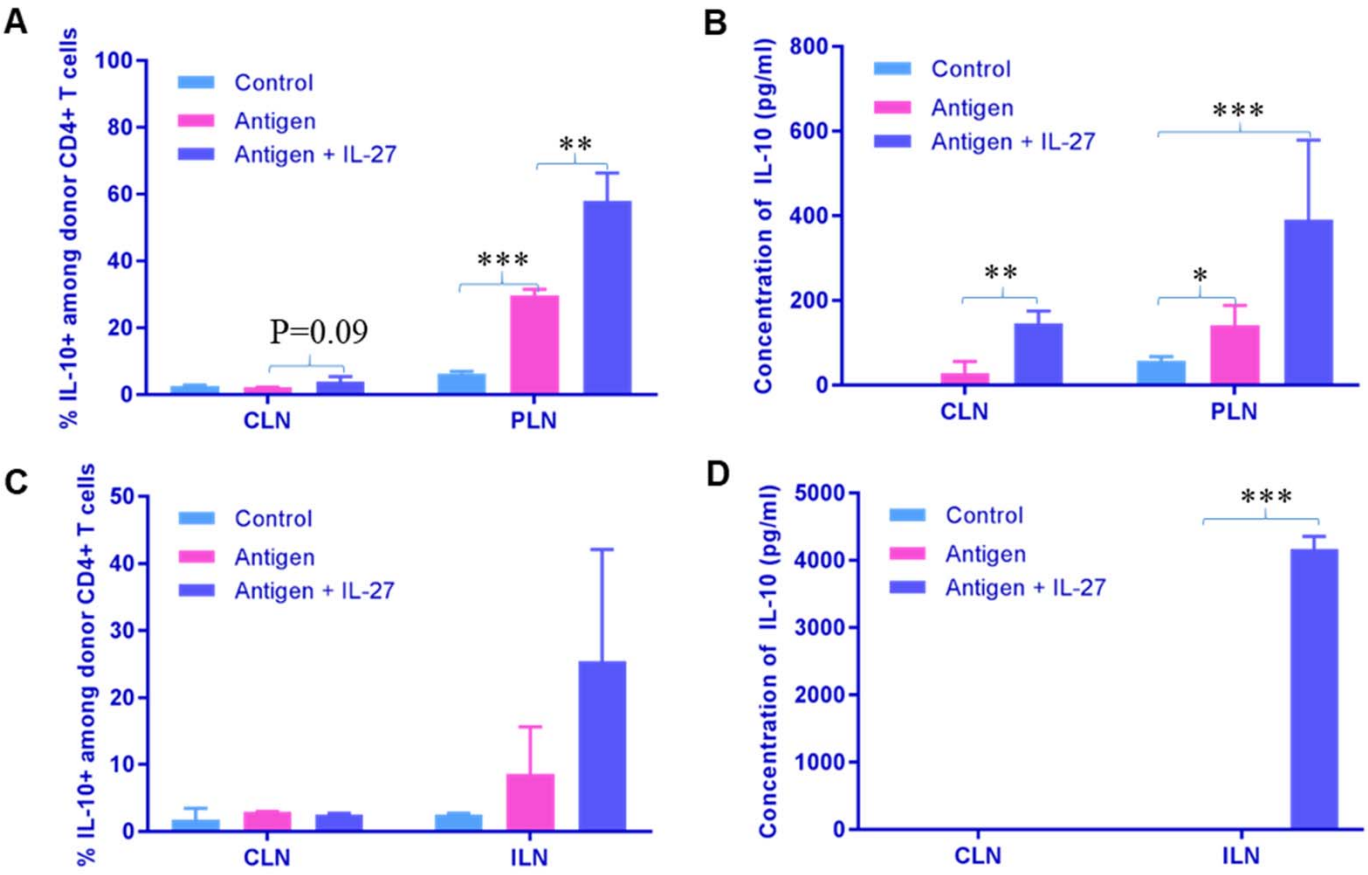


Figure S9. Enhancement of IL-10 production with co-delivery of IL-27 in mRNA-NPs and mRNA-DCs. IL-10 production in response to mRNA-NPs at a dose of 5 µg of antigen-encoding mRNA co-delivered with 25 µg of GFP mRNA ("Antigen") or with 25 µg of IL-27 mRNA ("Antigen + IL-27") per mouse through the i.p. route (**A-B**) and to mRNA-DCs at a dose of 0.6 µg antigen-encoding mRNA with 5.4 µg of GFP mRNA ("Antigen") or IL-27 mRNA ("Antigen + IL-27") per $1x10^6$ electroporated DCs per mouse through the i.d. route (**C-D**). Measurement of IL-10 was performed by intracellular cytokine staining (**A,C**) or by ELISA after 3 days of culture ex vivo (**B,D**). Mice in "Control group" were treated with saline. The data are presented as mean ± SD from at least 3 mice per group.