Supplemental Information

The Opposing Effect of Type I IFN on the T Cell

Response by Non-modified mRNA-Lipoplex Vaccines

Is Determined by the Route of Administration

Lien Van Hoecke, Kenny Roose, Marlies Ballegeer, Zifu Zhong, Niek N. Sanders, Stefaan De Koker, Xavier Saelens, and Sandra Van Lint

Figure S1. T cell response upon s.c. delivery of mRNA-lipoplexes. Wild type, CD11c-Cre^{+/-}IFNAR^{fl/fl} mice and CD4-Cre^{+/-}IFNAR^{fl/fl} mice were immunized s.c. with 10 μg CleanCap OVA mRNA-lipoplexes (DOTAP/DOPE or RNAiMAX). Two weeks after the last immunization the induced T cell responses were determined. Spleens were isolated and the splenocytes were stimulated *in* vitro with OVA peptide for 16h. (A) The number of OVA-specific IFN–γ-secreting splenocytes was determined by enzyme linked immunosorbent spot (ELISpot). Data represent individual mice and the mean (n = 6 mice per group). (B) A mixture of CFSE-labeled cells pulsed with control (CFSE^{low}) or OVA peptide (CFSE^{high}) were adoptively transferred in a 1:1 ratio to mRNA-lipoplex immunized mice (s.c. route). Specific killing was analyzed 2 days later by flow cytometry. (n = 6 mice per group). Statistical analyses on data sets were performed by one-way ANOVA followed by Tukey's post hoc test. (ns, non significant; **, p < 0.01; ****, p < 0.0001).

Figure S2. T cell response upon i.v. delivery of mRNA-lipoplexes. Wild type mice, CD11c-Cre^{+/-}IFNAR^{fl/fl} mice and CD4-Cre^{+/-}IFNAR^{fl/fl} mice were immunized i.v. with 10 μg OVA mRNA-lipoplexes (DOTAP/DOPE or RNAiMAX). Two weeks after the last immunization the induced T cell responses were determined. Spleens were isolated and the splenocytes were stimulated *in* vitro with OVA peptide for 16h. (A) The number of OVA-specific IFN–γ-secreting splenocytes was determined by an ELISpot. Data represent individual mice and the mean (n = 6 mice per group). (B) A mixture of CFSE-labeled cells pulsed with control (CFSE^{low}) or OVA peptide (CFSE^{high}) were adoptively transferred in a 1:1 ratio to mRNA-lipoplex immunized mice (i.v. route). Specific killing was measured 2 days later by flow cytometry. Data represent individual mice and the mean (n = 6 mice per group). Statistical analyses on data sets were performed by one-way ANOVA followed by Tukey's post hoc test. (ns, non-significant; *, p < 0.05).

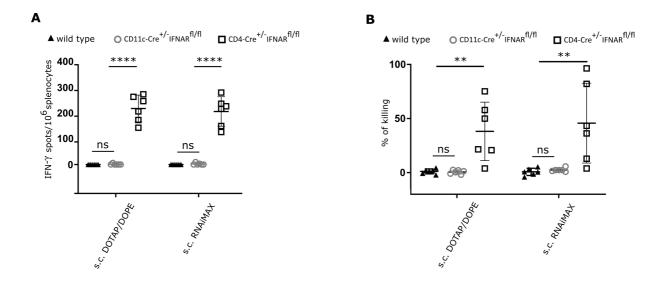


Figure S1. T cell response upon s.c. delivery of mRNA-lipoplexes.

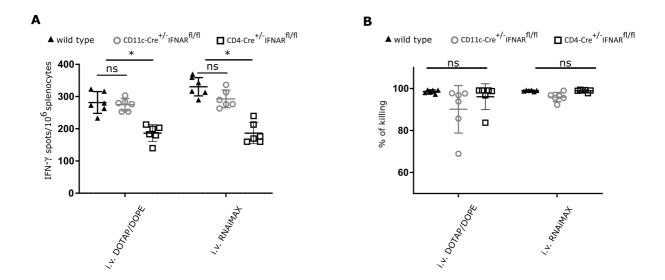


Figure S2. T cell response upon i.v. delivery of mRNA-lipoplexes.