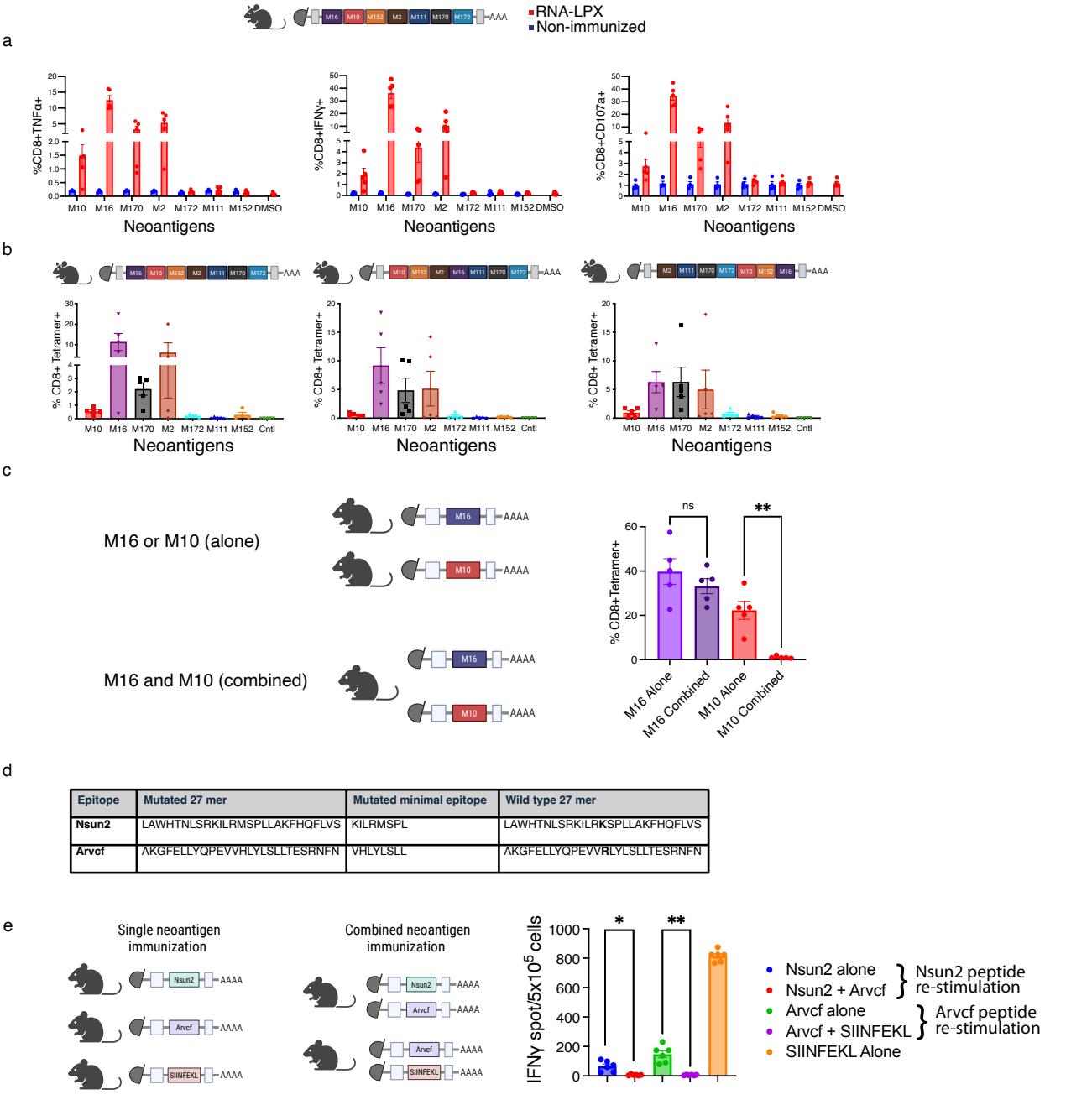
Extended Data Figure 1



Epitope	Mutated 27 mer	Mutated minimal epitope	Wild type 27 mer
Nsun2	LAWHTNLSRKILRMSPLLAKFHQFLVS	KILRMSPL	LAWHTNLSRKILR**K**SPLLAKFHQFLVS
Arvcf	AKGFELLYQPEVVHLYLSLLTESRNFN	VHLYLSLL	AKGFELLYQPEVV**R**LYLSLLTESRNFN

Extended Data Fig. 1. Distinct hierarchy of CD8+ T cell responses following multi-neoantigen RNA-LPX vaccination
a, C57BL/6 mice were immunized three times on days 0, 7 and 14 with heptatope RNA-LPX vaccine encoding all 7 neoantigens. Splenic T cells were harvested 5 days after the last immunization and restimulated with 27 mer peptides containing the neoantigen or non-peptide DMSO solvent controls. The magnitude of cytokine and CD107a responses were measured by flow cytometry and are shown as a percentage of total CD8+ T cells. Neoantigen specificities are indicated on the x-axis. n=3-5. b, As depicted, three different heptatope constructs were generated encoding 7 neoantigens in which the neoantigen position was varied across constructs. C57BL/6 mice were immunized three times on days 0, 7 and 14 and splenic T cells were harvested 5 days after the last immunization. Flow cytometric quantification of CD8+ T cells stained with pMHCI tetramers (specificities indicated on x-axis) was performed. n=5. c, Mice were immunized with monotope RNA-LPX vaccines encoding M16 or M10 alone or both monotype RNA-LPX vaccines were combined. Flow cytometric quantification of CD8+ T cells stained with pMHCI tetramers was performed in the blood 5 days after the third immunization. Clonal expansion was analyzed. n=5. d, Table depicts neoantigen and non-neoantigen wild type sequences of Nsun2 and Arvcf peptides identified in B16F10 tumors. e, Mice were immunized with monotope RNA-LPX vaccines encoding Nsun2, Arvcf or SIINFEKL in various combinations as depicted. Following three immunizations, splenocytes were assessed by IFNg ELISpot assay following peptide restimulation. One way anova or unpaired t-test. ns not significant, *p<0.5, **p<0.01, ***p<0.001.<0.01, ***p<0.001.