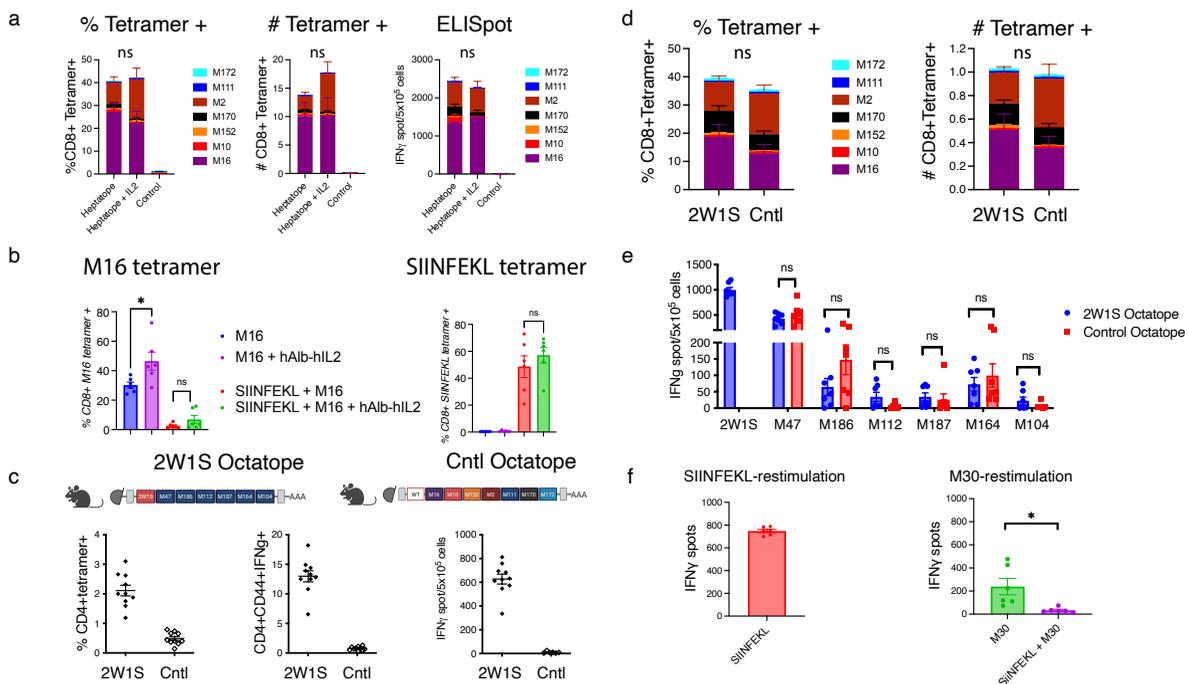


Extended Data Figure 2



Extended Data Fig. 2. T cell competition is not mediated by the limited bioavailability of IL-2 or limitations in CD4+ T cell help

a, C57BL/6 mice were immunized with heptatope RNA-LPX in the presence and absence of exogenous IL-2 (15,000 units i.p daily). Following three immunizations, spleens were removed and neoantigen-specific T cell responses were evaluated by pMHC-I tetramer staining and IFN-gamma ELISpot. Data are shown as pMHC-I tetramer+ cells as a percentage of total CD8+ T cells, total number of neoantigen-specific CD8+ T cells or IFN spot count per 105 splenocytes. n=10. b Mice were immunized with monotype vaccines encoding M16 and SIINFEKL, either alone or mixed together. Mice immunized with the M16 monotype or a mixture of M16 + SIINFEKL received half-life extended human IL-2 delivered in LNP (3ug) intravenously. M16 or SIINFEKL neoantigen-specific T cell responses were evaluated in the spleen 7 days after the last immunization using pMHC-I tetramer+ staining and are shown as a percentage of total CD8+ T cells. c-e, The heptatope RNA-LPX vaccine was modified to include 2W1S ("octatope"), or a non-immunogenic control antigen. Mice were immunized three times and neoantigen-specific T cell responses were evaluated in the spleen 5 days after the last immunization. c, The 2W1S CD4+ T cell response was confirmed by pMHC-II tetramer staining and production of IFN γ after ex vivo restimulation of spleen cells by 2W1S peptide n=10. Neoantigen-specific CD8+ T cell responses were quantified in the spleen by pMHC tetramer+ staining in the presence and absence of the 2W1S CD4+ T cell responses. Data are shown as pMHC-I tetramer+ cells as a percentage of total CD8+ T cells and total number of neoantigen-specific CD8+ T cells n=4-7. e, We generated a novel heptatope consisting of 6 neoantigens from the MC38 model that generate CD4+ T cell responses and a seventh CD4+ T cell antigen, 2W1S. Mice were immunized three times and neoantigen-specific CD8+ T cell responses were evaluated in the spleen 5 days later. Neoantigen-specific CD8+ T cell responses were quantified by IFN ELISpot and are shown as IFN spots per 105 splenocytes n=7. f, RNA-LPX monotype vaccines encoding either SIINFEKL or the M30 neoantigen, or both monotype vaccines combined were used to immunize mice 3 times. Neoantigen-specific T cell responses were quantified in the blood 5 days after the third immunization. n=6. Unpaired, two-tailed t-test. ns; not significant *p<0.5.