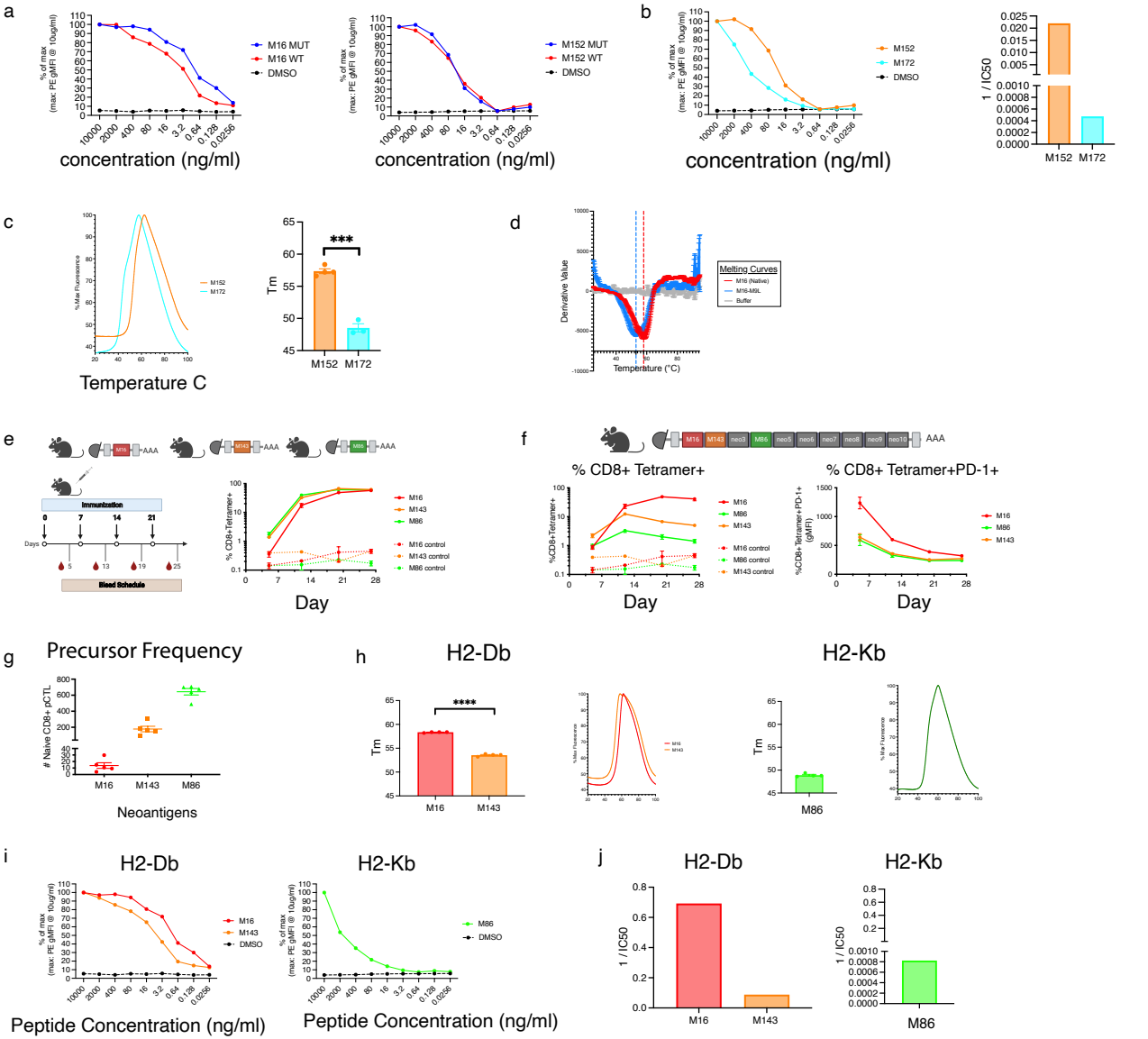
Extended Data Figure 3



Extended Data Fig. 3. Neoantigen-MHC class I stability partially determines immunodominance

a, pMHC affinity for each neoantigen was assessed by flow cytometric quantification of H2-Db or H2-Kb stabilization for both wild type (WT) or neoantigen mutant (MUT) peptides on TAP-deficient EL4 cells. Graph shows percent of maximum response.b, pMHC affinity for each neoantigen was assessed by flow cytometric quantification of H2-Kb stabilization of TAP-deficient EL4 cells. Percent of maximum response over a range of neoantigen concentrations is shown and the graph represents the calculated 1/EC50 for each specificity. A representative graph of three independent experiments is shown. c, pMHC complex stability (Koff) for each H2-Kb neoantigen was measured by differential scanning fluorimetry following thermal denaturation of soluble pMHC-I complexes. Melting curves were normalized to the minimum and maximum fluorescence values and Tm was calculated using the negative first derivative of RFU values over temperature. n=3.d, Differential scanning fluorimetry analysis of H-2Db/M16 9red) and H2-Db/M16-9L (blue) and buffer control (grey). Curves show the derivative of fluorescence over temperature, with dotted lines marking the melting temperature (Tm) on the x-axis. Each trace represents triplicate measurements. e, C57BL/6 mice were immunized four times on days 0, 7, 14 and 21 with either RNA-LPX monotope vaccines encoding a single neoantigen, decatope RNA-LPX vaccine encoding 10 neoantigens or RNA-LPX encoding F-luciferase was used as a control. Experimental design and flow cytometric quantification of CD8+ T cells with pMHCI-tetramer staining was performed in the blood 5 days after each immunization. Neoantigen specific CD8+ T cell responses as a percentage of total CD8+ T cells n=5. f, Neoantigen specific CD8+ T cell responses as a percentage of total CD8+ T cells is shown. Graph shows PD1 (gMFI) expression on neoantigen-specific CD8+ T cells=5. g, Naïve precursor CD8+ T cells (pCTL) were enriched from non-immunized mice and quantified. Graph shows the total number of pCTL for each specificity. Each dot represents an individual mouse n=5. h, pMHC complex stability (Koff) for each neoantigen was measured by differential scanning fluorimetry following thermal denaturation of soluble pMHC-I complexes. Melting curves were normalized to the minimum and maximum fluorescence values and Tm was calculated using the negative first derivative of RFU values over temperature. n=4 independent studies. i,j pMHC affinity for each neoantigen was assessed by flow cytometric quantification of H2-Db stabilization of TAP-deficient EL4 cells. Graph shows percent of maximum response and graph shows 1/EC50. ns not significant, *p<0.5, **p<0.01, ***p<0.001.<0.01, ***p<0.001.