SUPPLEMENTAL DATA

Priming of qualitatively superior human effector CD8$^+$ T cells using TLR8 ligand combined with FLT3L

Running title: Enhanced T cell priming in humans

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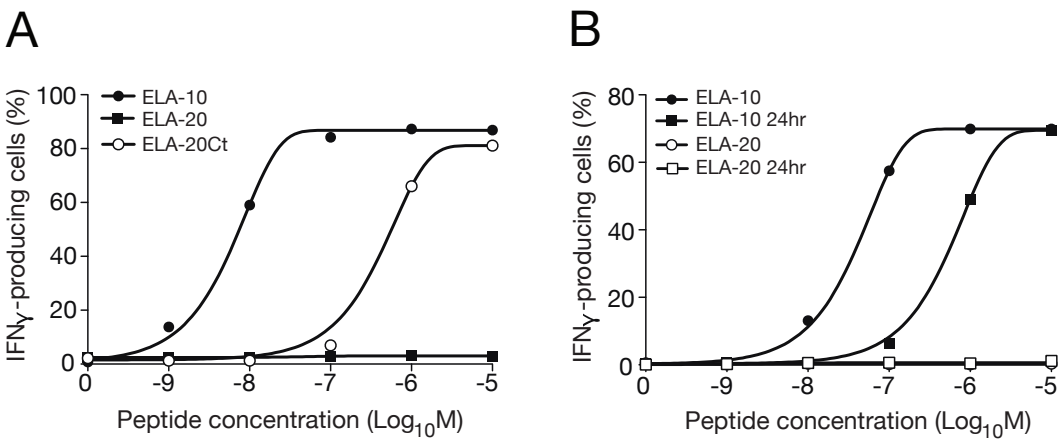
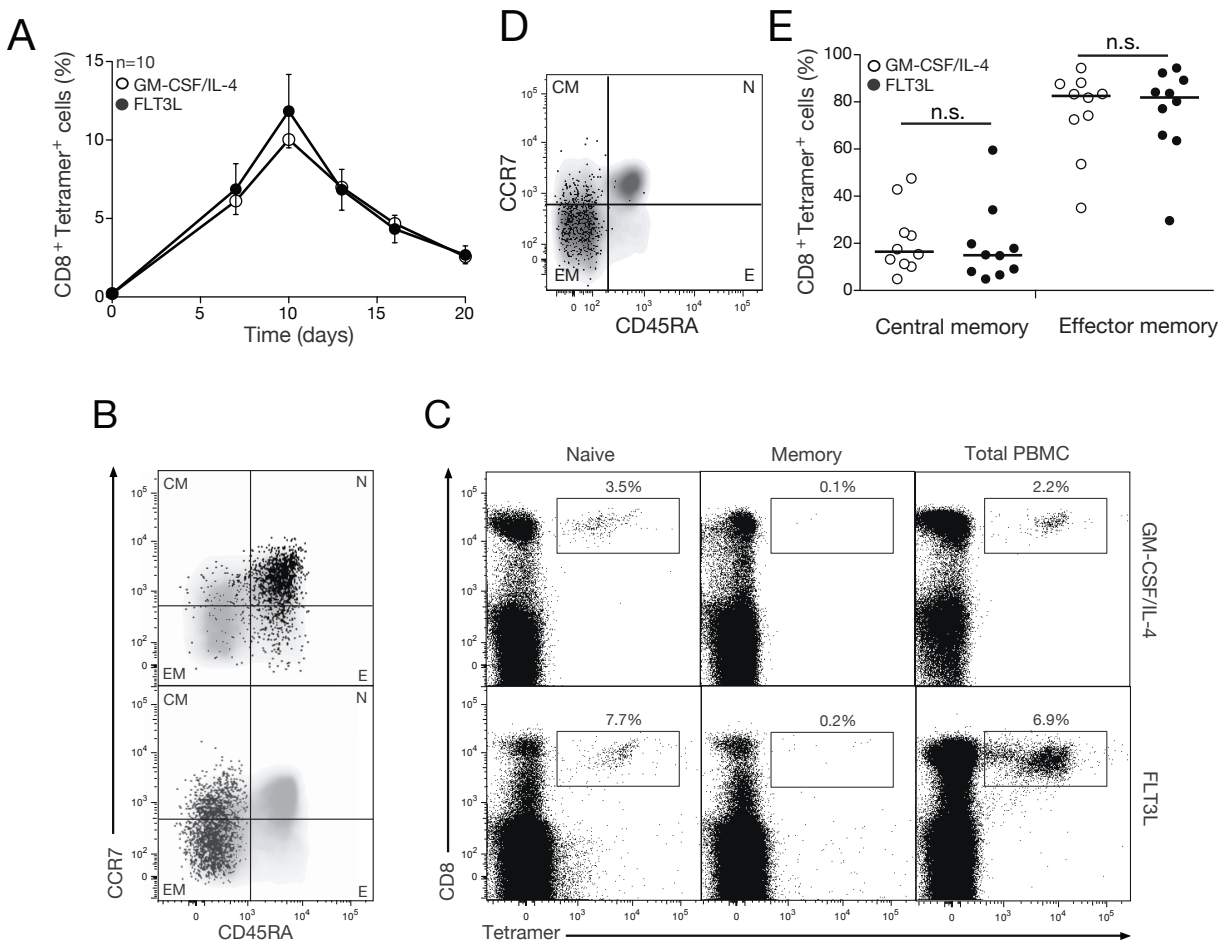
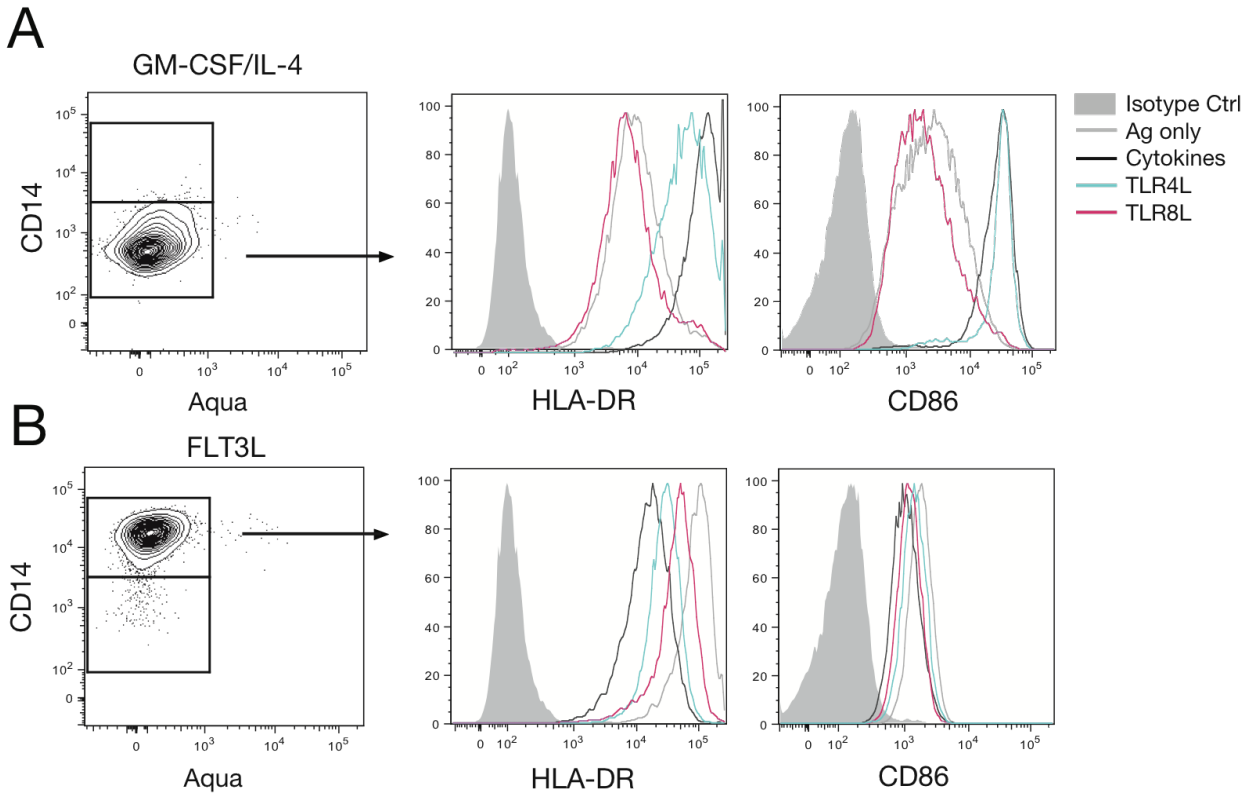


Supplemental Figure 1. Surface presentation and stability of long versus short synthetic ELA peptides. A. IFNγ production by the Mel9916 ELA-specific $CD8^+$ T cell line in response to HLA-$A2^+$ B-LCL target cells pulsed with the optimal ELA-10 peptide or two different versions of the 20mer peptide (ELA-20 or ELA-20Ct) over a range of concentrations was measured via intracellular cytokine staining. The ELA-20Ct peptide, which contains the ELA epitope sequence at the C-terminal end, partially activated cognate $CD8^+$ T cells without the need for processing and was subsequently eliminated from the study. The ELA-20 peptide did not bind directly to HLA-A2. **B.** The ELA-20 peptide was tested in a stability assay to determine if non-specific cleavage by enzymes present in serum could liberate the optimal ELA-10 epitope. IFNγ production by the Mel9916 ELA-specific $CD8^+$ T cell line was measured as in (A). Prior to B-LCL pulsing, peptides were either handled normally or incubated with 10% v/v FCS for 24 hours at 37°C. Incubation with 10% v/v FCS reduced the antigenicity of the ELA-10 peptide by 1 $\log_{10}$M, but did not generate functionally active fragments from the ELA-20 peptide.



Supplemental Figure 2. *In vitro* priming of ELA-specific CD8$^+$ T cells from naïve precursors. **A.** Comparison of ELA-specific CD8$^+$ T cell priming kinetics from HLA-A2$^+$ PBMC samples (n=10) using either GM-CSF/IL-4 or FLT3L in combination with a cocktail of inflammatory cytokines (TNF, IL-1β, PGE2 and IL-7). Error bars indicate SEM. **B.** Phenotypic profiles of purified naïve (top plot) or memory (bottom plot) CD8$^+$ T cell subsets (black dots) overlaid on the phenotypic distribution of peripheral blood CD8$^+$ T cells (grey density plot). CM, central memory; E, effector; EM, effector memory; N, naïve. Naïve (CCR7$^+$ CD45RA$^+$) and memory (CCR7$^-$ CD45RA$^-$) CD8$^+$ T cell populations were isolated from HLA-A2$^+$ PBMCs prior to reconstitution with CD8-depleted PBMCs in priming experiments. Unmanipulated PBMCs from the same donor were primed in parallel. **C.** Representative flow cytometry plots showing ELA/HLA-A2 tetramer$^+$ CD8$^+$ T cells on day 10 post-priming in the presence of either GM-CSF/IL-4 (top row) or FLT3L (bottom row) together with the inflammatory cytokine cocktail. Percentages of ELA/HLA-A2 tetramer$^+$ cells within the total CD8$^+$ gate are indicated. **D.** Representative phenotypic analysis of ELA-specific CD8$^+$ T cells on day 10 post-priming. ELA/HLA-A2 tetramer$^+$ CD8$^+$ events are shown as black dots superimposed on density plots depicting the phenotypic distribution of

peripheral blood $CD8^+$ T cells. **E.** Phenotypic characterization of ELA/HLA-A2 $tetramer^+$ $CD8^+$ T cells primed from the donors shown in (A). Horizontal bars indicate median values. Statistical comparisons between groups were performed using the Wilcoxon signed rank test.



Supplemental Figure 3. Tracing the expression of CD14, HLA-DR and CD86 on purified monocytes, exposed to different maturation conditions. Expression of CD14, HLA-DR and CD86 on monocytes (initially purified with magnetic microbeads, on the basis of CD14 expression) differentiated *in vitro* using GMCSF/Il-4 (**A**) or FLT3L (**B**), and cultured for two days with ELA antigen alone (grey lines) or in the additional presence of the following maturation factors: cytokines (TNF-a, PGE2, IL-1b and IL-7; black lines), TLR4L (blue lines) or TLR8L (pink lines).