Supplemental Information

Functional specialty of CD40 and dendritic cell surface lectins for exogenous antigen presentation to CD8$^+$ and CD4$^+$ T cells

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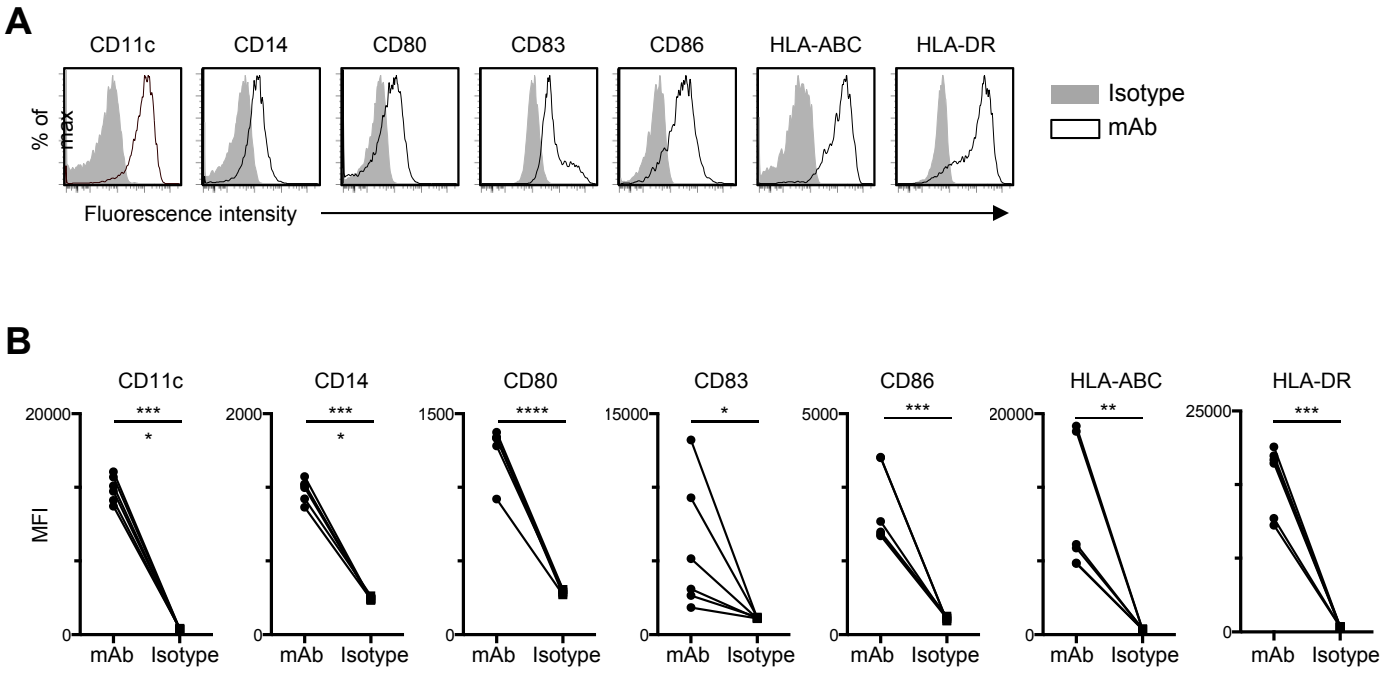
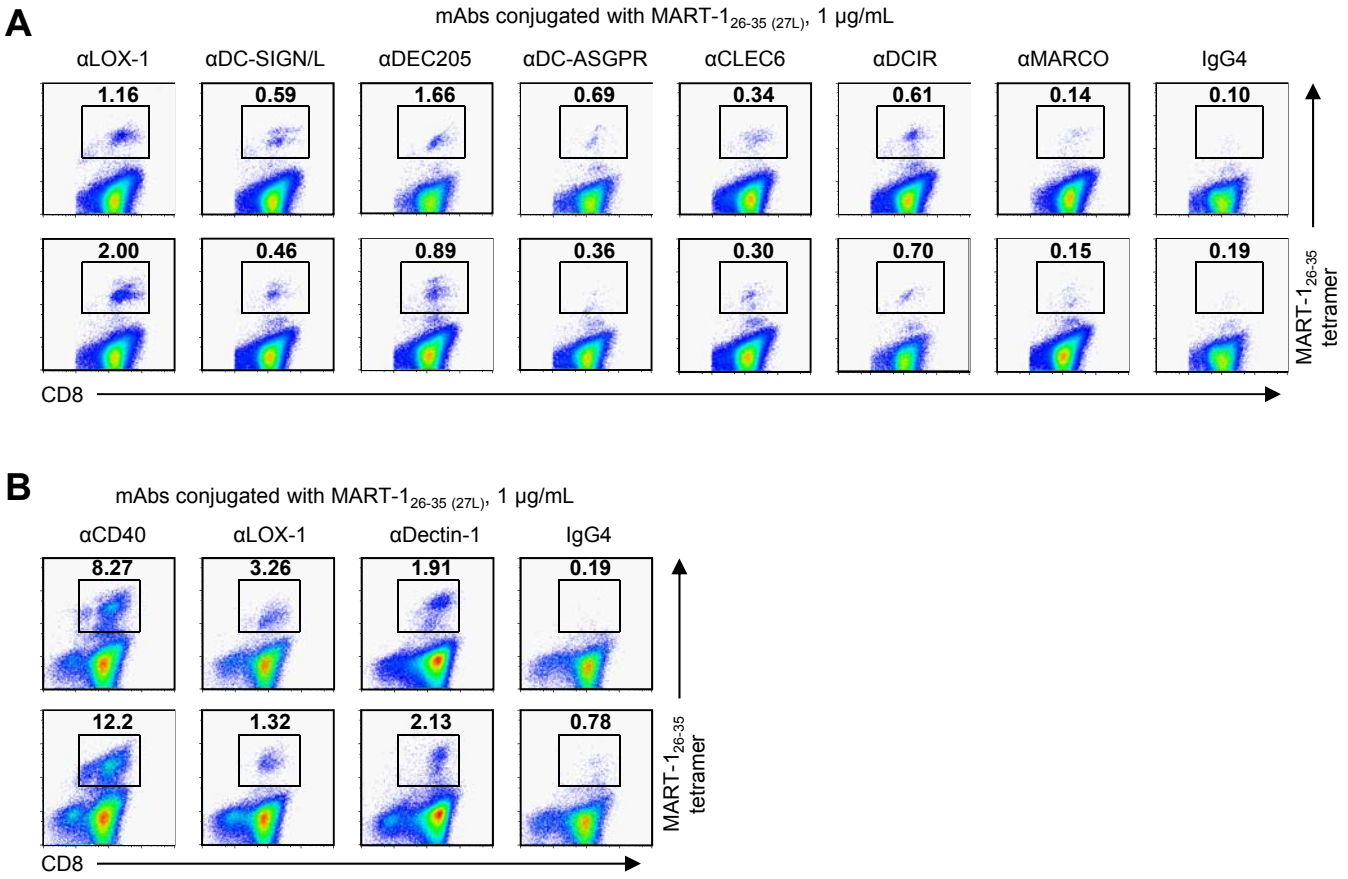
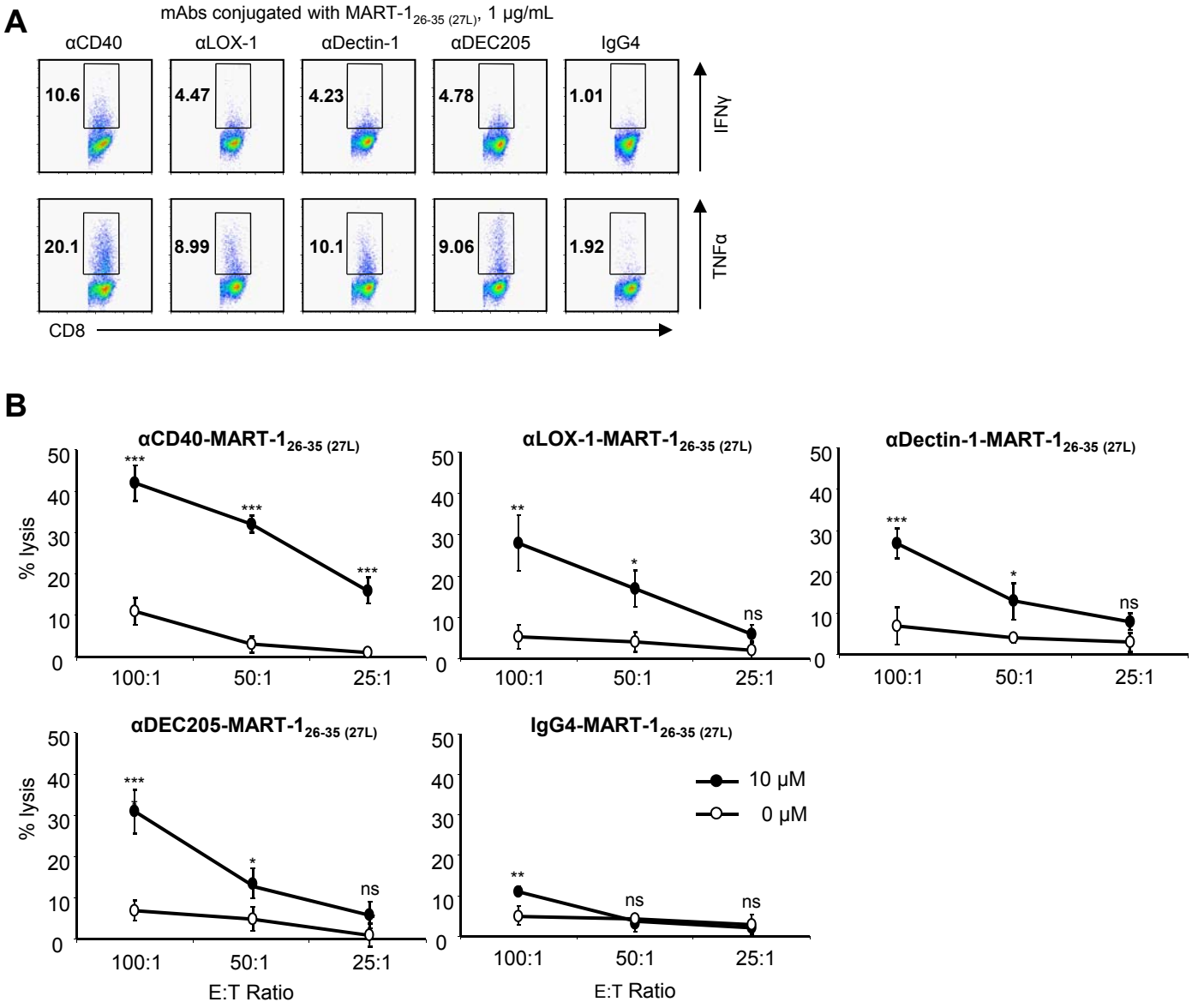
Contents



Supplemental Fig. 1. Surface phenotype of Mo-DCs.
A and **B**. Mo-DCs were stained with fluorescence-labeled mAbs specific to the indicated surface markers or with their isotype-matching controls. **A**. Representative flow cytometric data and **B**. summarized data on mean fluorescence intensities (MFIs) of each marker are shown. Dots represent data generated with cells from individual healthy donors (n=6). Significance was determined using a paired *t* test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ****, $P < 0.001$.

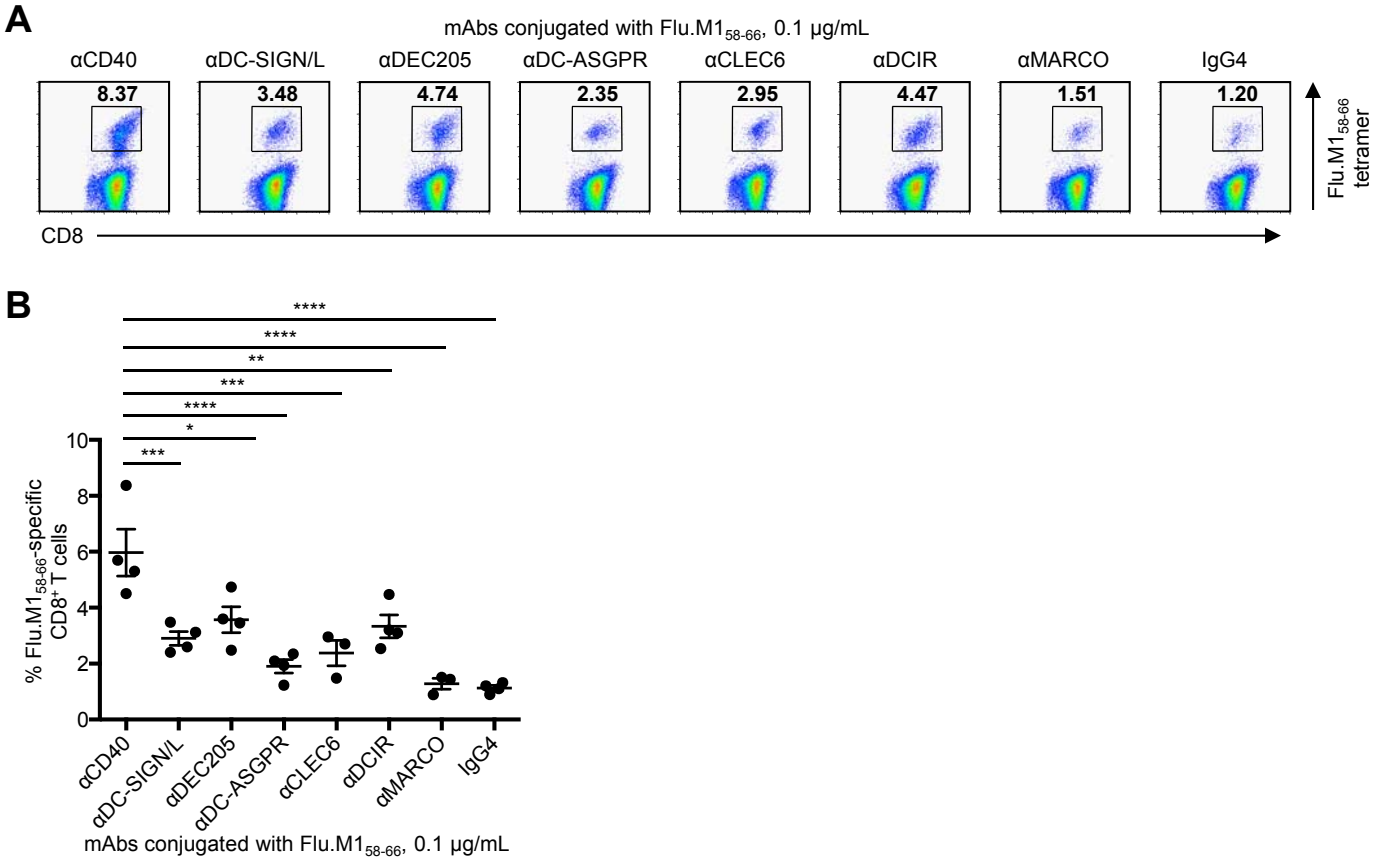


Supplemental Fig. 2. Targeting antigen to DCs via CD40 can efficiently prime CD8⁺ CTLs.
A and **B**. Purified naïve $CD8^+$ T cells were co-cultured for 9 days with Mo-DCs loaded with 1 µg/mL of the indicated mAb-MART-$1_{26\text{-}35\ (27L)}$ conjugates. $CD8^+$ T cells were then stained with HLA-A*A0201-MART-$1_{26\text{-}35}$ tetramer. Representative flow cytometric data from duplicate assays (top and bottom panels) are shown.



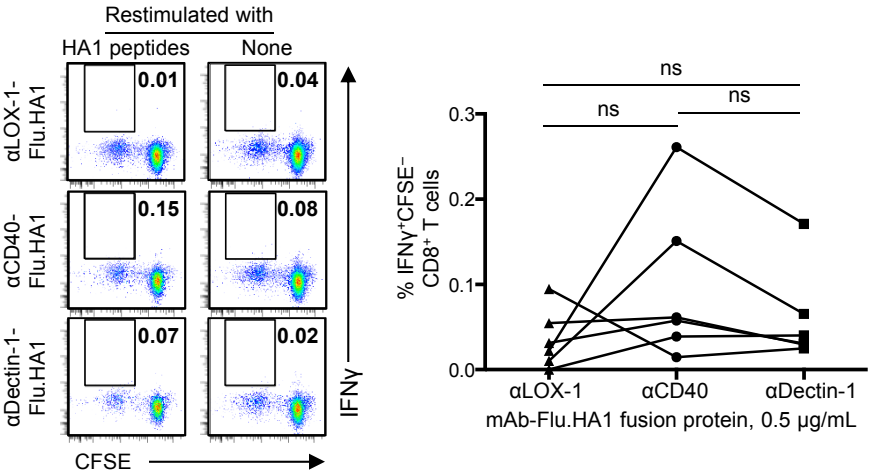
Supplemental Fig. 3. CD8⁺ CTLs primed by targeting antigens to CD40 are functional.

A. Purified naïve CD8⁺ T cells were co-cultured with Mo-DCs loaded with 1 μg/mL mAb-MART-$1_{26\text{-}35\ (27L)}$ conjugates for 9 days. CD8⁺ T cells were then restimulated with 1 μM MART-$1_{26\text{-}35}$ and stained for intracellular IFNγ and TNFα expression. Two independent experiments showed similar results. Representative flow cytometric data are shown. **B**. A 5-hr ^{51}Cr release assay using T2 cells loaded with 0 or 10 μM MART-$1_{26\text{-}35}$ peptide. MART-$1_{26\text{-}35}$-specific CD8⁺ T cells in **A** were used as effector cells. Data are presented as mean ± SD of triplicate assays, and significance was determined using an ANOVA test. Two independent experiments resulted in similar data. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ****, $P < 0.001$; ns, not significant.



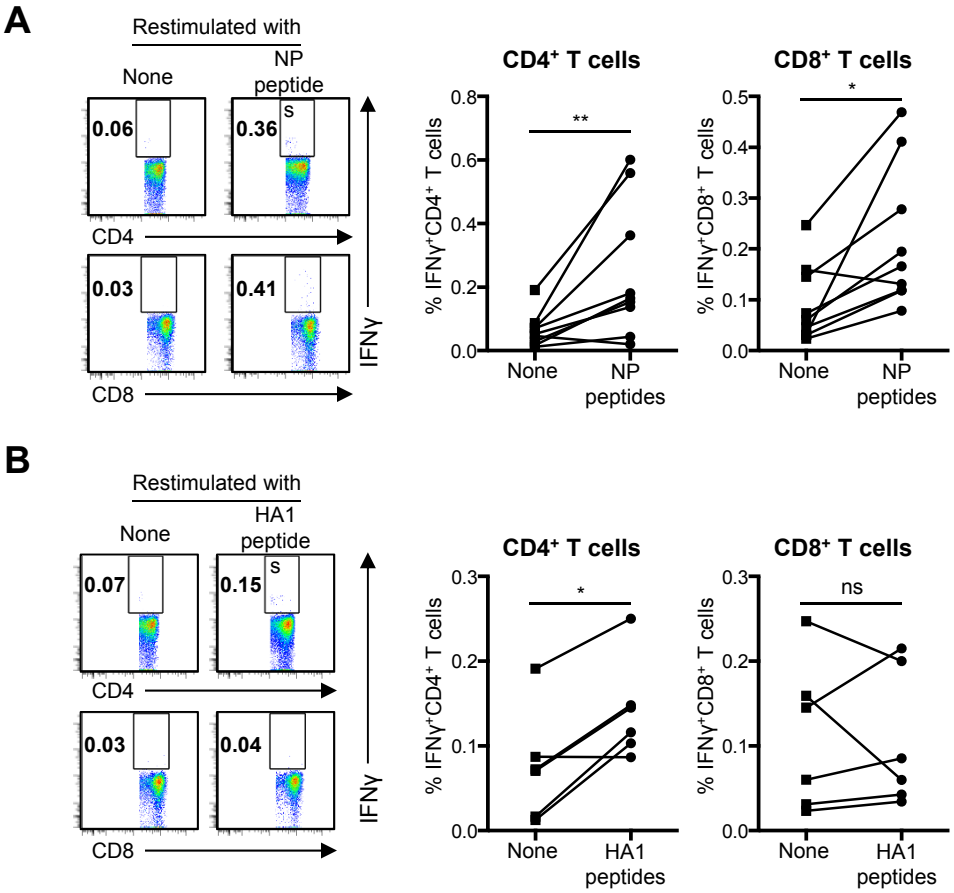
Supplemental Fig. 4. Targeting antigen to DCs via CD40 can efficiently activate antigen-specific memory CD8$^+$ CTLs.

A and **B**. Purified CD8$^+$ T cells were co-cultured for 8 days with Mo-DCs loaded with 0.1 µg/mL of the indicated mAb-Flu.M1$_{58\text{-}66}$ conjugates. CD8$^+$ T cells were stained with HLA-A*A0201-Flu.M1$_{58\text{-}66}$ tetramer. **A**. Representative flow cytometric data and **B**. summarized data (mean ± SD) from healthy donors (n=4) are presented. Significance was determined using an ANOVA test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ****, $P < 0.001$.



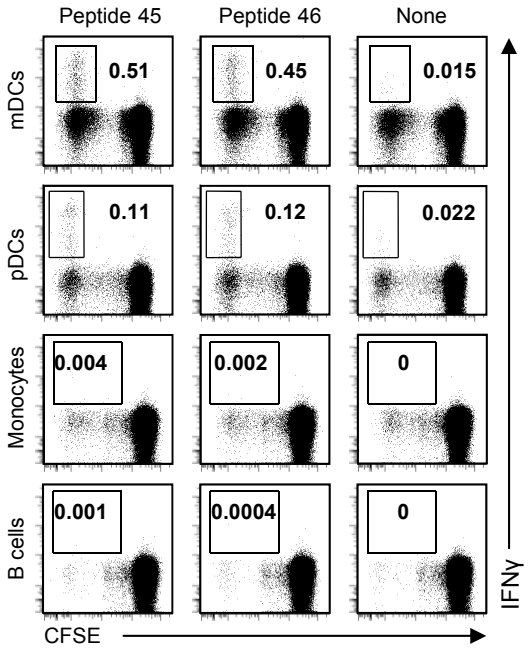
Supplemental Fig. 5. Flu.HA1-specific CD8⁺ T cell responses elicited by targeting Flu.HA1 to DCs via CD40, LOX-1, or Dectin-1.

CFSE-labeled PBMCs from healthy donors (n=6) were cultured in the presence of 1 □g/mL of the indicated mAb-Flu.HA1 recombinant fusion proteins for 8 days. T cells were then restimulated with Flu.HA1 peptide pools at 1 □M (each peptide) for 6 h and stained for intracellular IFNγ expression. Representative flow cytometric data (left) and summarized data (right) on are shown. Dots represent data generated with cells from individual healthy donors. Significance was determined using a paired *t*-test. ns, not significant.



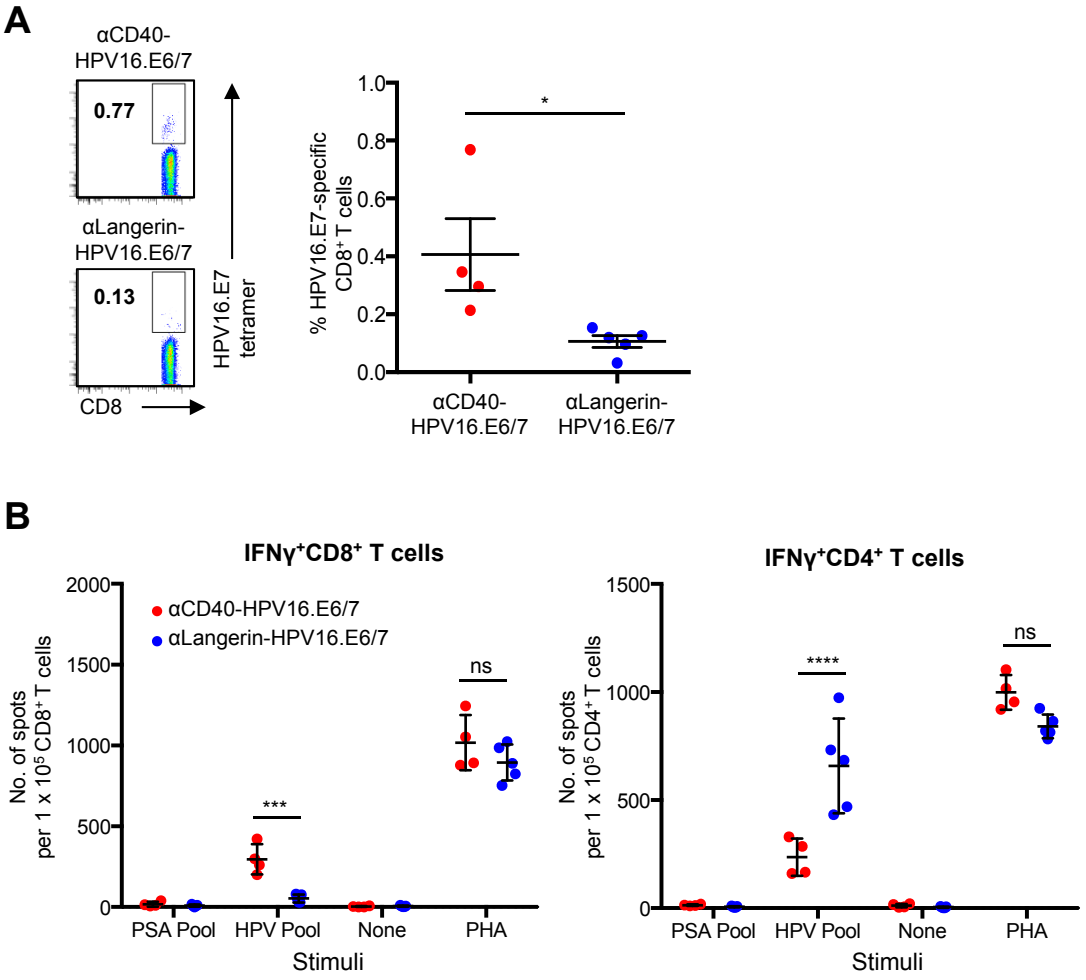
Supplemental Fig. 6. Frequencies of Flu.HA1- and Flu.NP-specific memory CD4⁺ and CD8⁺ T cells in healthy individuals.

A and **B**. PBMCs from healthy donors (n≥6) were stimulated with (**A**) Flu.NP or (**B**) Flu.HA1 peptide pools at 1 μM (each peptide) for 6 h. CD4⁺ and CD8⁺ T cells were stained for intracellular IFN□ expression. Representative flow cytometric data (left) and summarized data (right) are shown. Dots represent data generated with cells from individual healthy donors. Significance was determined using a paired *t* test. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.



Supplemental Fig. 7. DCs, particularly mDCs, are the major antigen-presenting cells to elicit Flu.HA1-specific CD4$^+$ T cell responses.

Blood mDCs, pDCs, CD14$^+$ monocytes, and CD19$^+$ B cells purified from the same donor were loaded with αDectin-1-HA1 at 1 μg/mL. They were then cultured for 7 days with CFSE-labeled purified autologous CD4$^+$ T cells. T cells were restimulated for 6 h with pre-determined Flu.HA1-derived peptides, GNLIAPWYAFALSRGFG (peptide 45) and WYAFALSRGFGSGIITS (peptide 46). Intracellular IFN□ expression was assessed. Two independent experiments showed similar data.



Supplemental Fig. 8. Antigen targeting to CD40 is more efficient than to Langerin at eliciting antigen-specific CD8⁺ T cell responses in hCD40Tg mice.

A. hCD40Tg animals were immunized s.c. with a combination of poly(I:C) (50 μg/dose) and αCD40-HPV16.E6/7 (30 μg/dose) or αLangerin-HPV16.E6/7 (30 μg/dose) in 100 μL PBS (n=4 per group). Animals were boosted twice with the same vaccine at two-week intervals and were sacrificed 7 days after the second boost. $CD8^+$ T cells in peripheral blood were stained with H-$2D^b$-$HPV16.E7_{RAHYNIVTF}$ tetramer. Left, representative flow cytometry data. Right, summarized data. **B**. IFNγ ELISpot assays were performed on $CD8^+$ (left) and $CD4^+$(right) T cells purified from splenocytes with HPV16.E6/7 peptide pool at 1 μM as stimulus. Dots represent data generated with individual animals. All data are presented as mean ± SD. Significance was determined using a *t* test in **A** or ANOVA test in **B**. *, $P < 0.05$; ****, $P < 0.001$; ns, not significant.