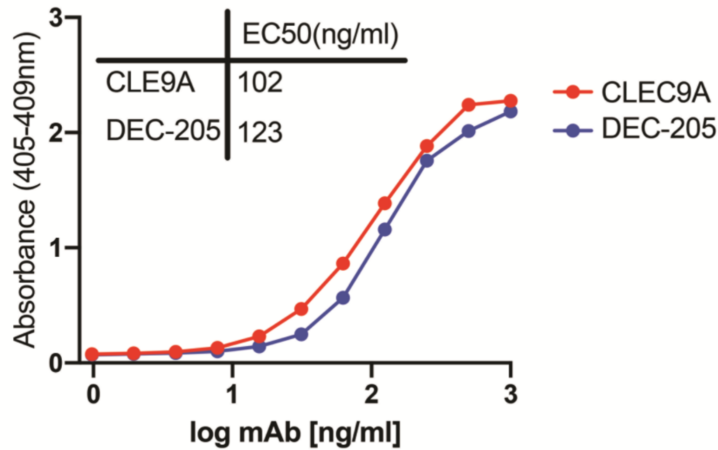
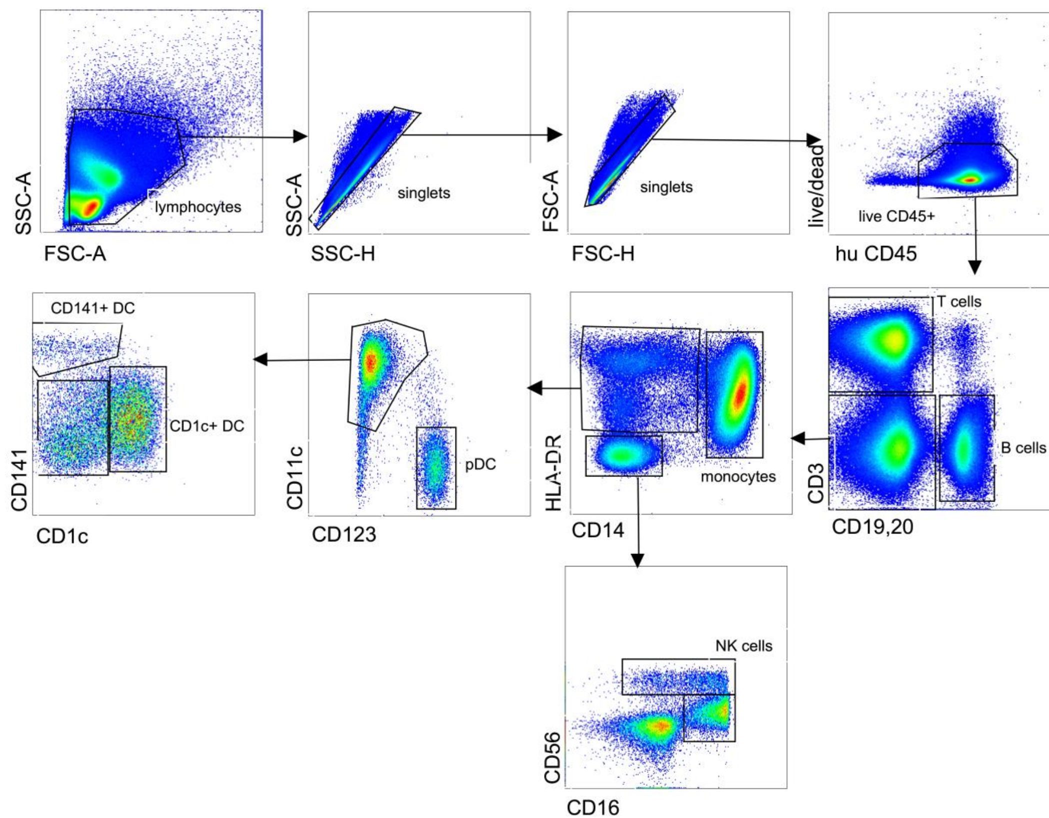
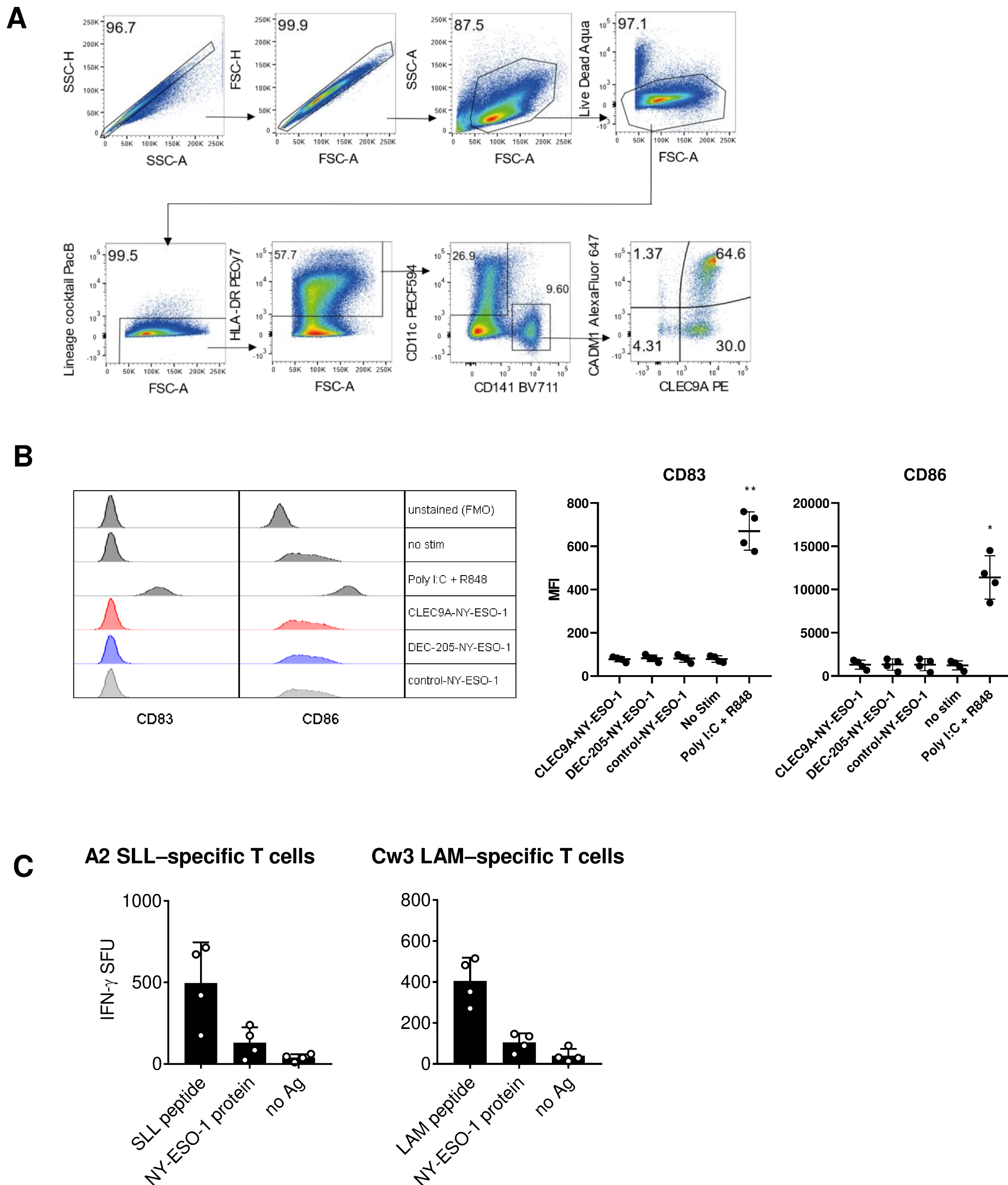
A



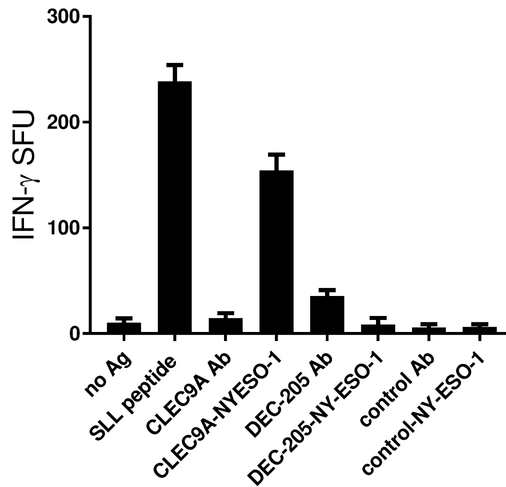
B



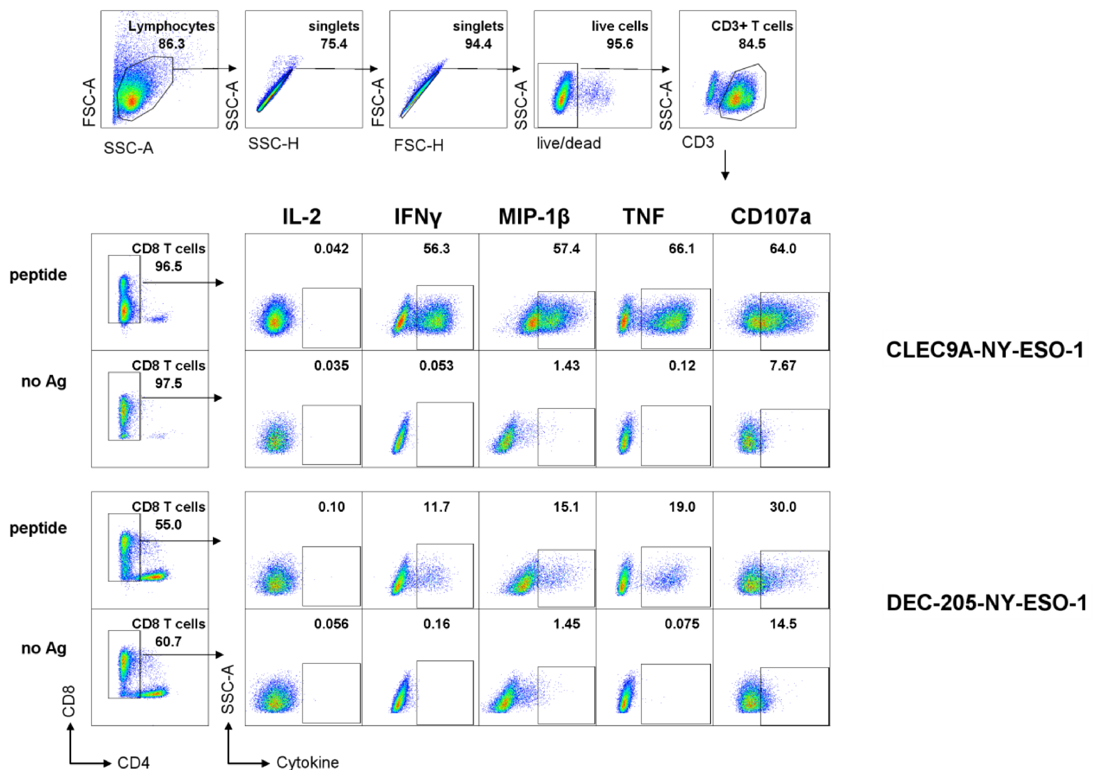
Supplementary Figure 1 (related to Figure 1): **A**. Binding affinity of chimeric CLEC9A and DEC-205 antibodies to respective recombinant protein targets by ELISA. **B.** Gating strategy to identify cell subsets within PBMC.



Supplementary Figure 2 (Related to manuscript Figure 2): Generation and isolation of CD141⁺ DC from CD34⁺ HSC *in vitro*. A. CD141⁺ DC were identified and sorted as live, singlet cells that were lineage cocktail (CD2, CD14, CD16, CD19, CD20)⁻, HLA-DR⁺, CD11c^{low} and CD141⁺. The majority of the CD141+ cells expressed CD141+ DC-specific markers CLEC9A and CADM1. **B.** Upregulation of CD83 and CD86 on CD141+ DC after overnight incubation alone, with targeting antibodies or with poly I:C + R848. One representative (right) and mean fluorescence intensity (MFI) from 5 donors is shown (right). **C.** Activation of SLL and Cw3-specific T cell lines by IFNγ ELISPOT following stimulation with HLA-matched CD141⁺ DC pulsed with saturating concentrations of cognate peptide or recombinant NY-ESO-1 protein. Each data point represents the mean of triplicate wells from an individual donor. Bars represent compiled means +/-SD from 4 donors.



Supplementary Fig 3: Related to Figure 3. PBMCs from a melanoma patient with known NY-ESO-1 SLL-specific response were stimulated no Ag, SLL peptide, unconjugated or NY-ESO-1- conjugated anti-CLEC9A, DEC-205 or control (β –gal) Ab. IFNγ production was measured by ELISPOT 18 hr later. Data shown are the mean ± SD from triplicate wells.



Supplementary Figure 4. Related to Figure 6: Gating strategies and controls for intracellular cytokine staining