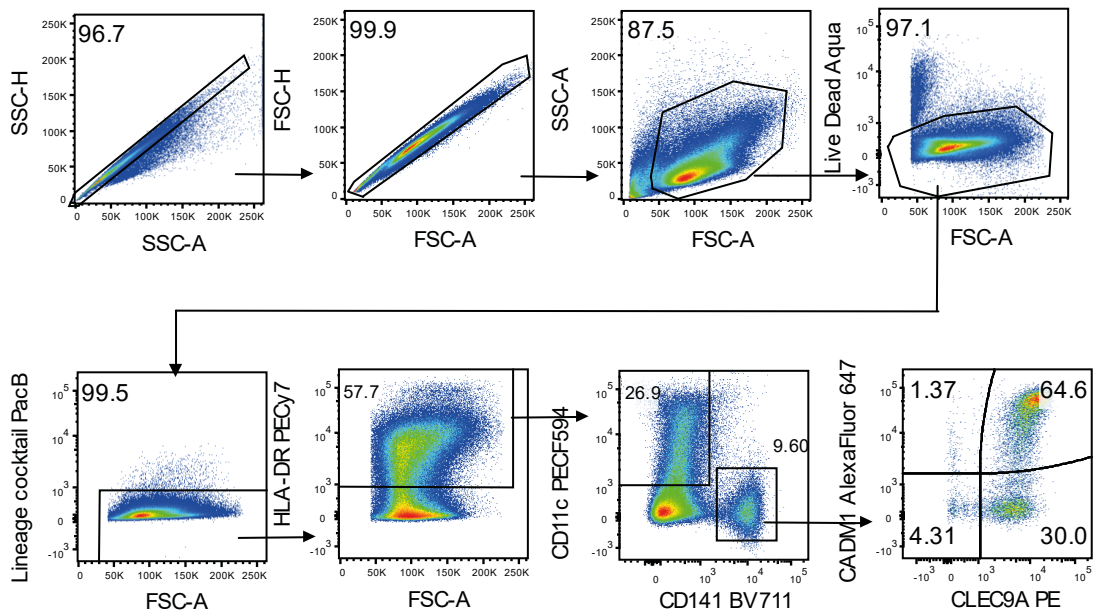
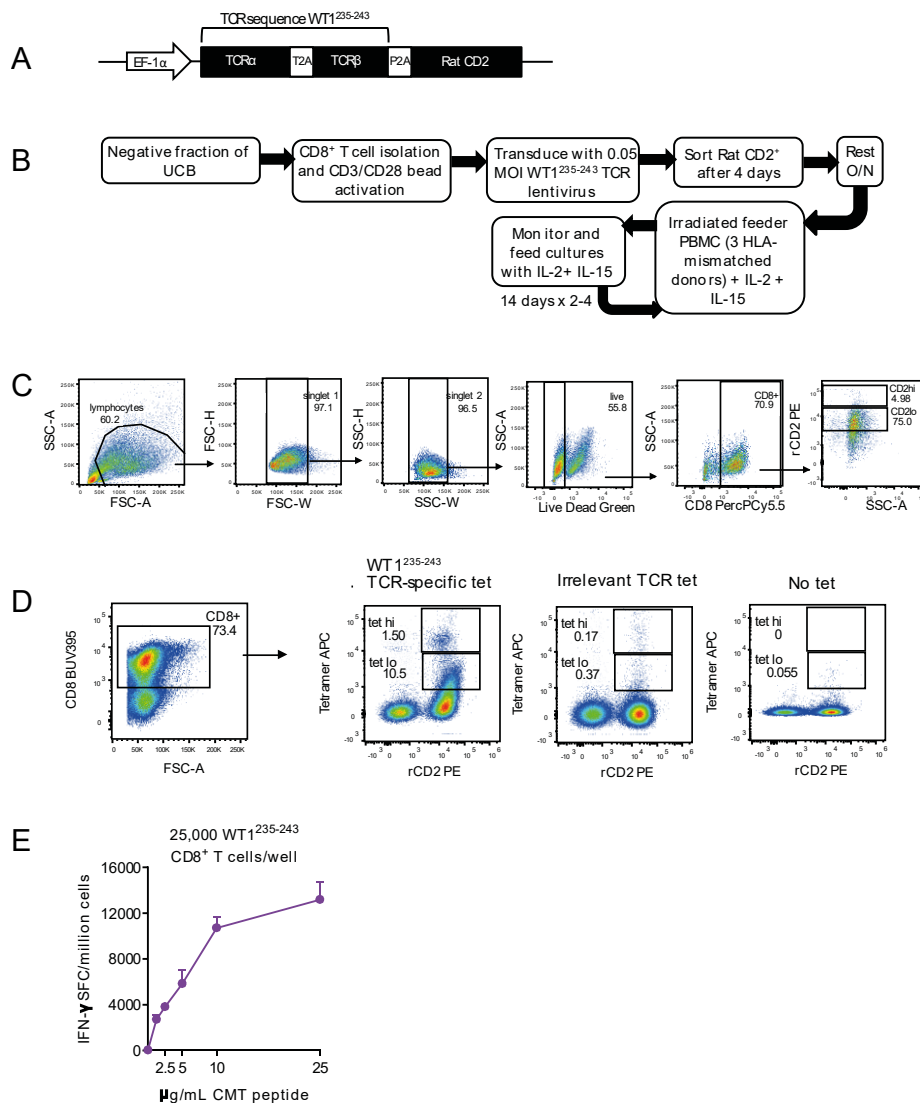


Supplementary figure 1 (Related to manuscript Figure 1): Validation of chimeric CLEC9A and DEC-205 and β-gal (control) Ab specificity.

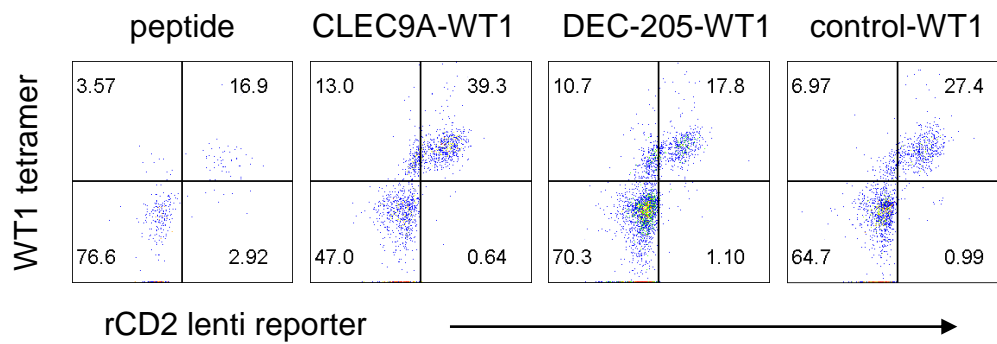
A. Binding of CLEC9A-WT1 (i) or DEC-205-WT1 (ii) chimeric Abs to mammalian 293F cells transiently transfected with full-length CLEC9A (blue), DEC-205 (red) or untransfected (grey). Chimeric Ab binding was detected using anti-human IgG4-biotin (Life technologies) and streptavidin-PE (BD Pharmingen) by flow cytometry. **B.** Binding of CLEC9A-WT1 (i), DEC205-WT1 (ii) and β-gal (control)-WT1 (iii) to respective CLEC9A, DEC-205 or β-gal recombinant proteins or peptides by ELISA, detected using anti-human IgG4-biotin and streptavidin-HRP (GE Healthcare) and visualised with ABTS as previously described (Tullett et al, [JCI Insight](https://doi.org/10.1172/jci.insight.87102). 2016 May 19;1(7):e87102. doi: 10.1172/jci.insight.87102). **C.** Gating strategy for identification of immune cell subsets from (i) PBMC (ii) mDC-enriched PBMC from healthy peripheral blood for validation of chimeric Ab binding.



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.



Supplementary figure 3: Generation and validation of a CD8⁺ T cell line specific for WT1₂₃₅₋₂₄₃. CD8⁺ T cells expressing a TCR specific for WT1₂₃₅₋₂₄₃ were generated using a third generation lentiviral system. **A.** Lentiviral expression cassette encoding the TCR- α and β genes of the HLA-A*2402-restricted WT1₂₃₅₋₂₄₃ specific CTL clone TAK-1 (Ohminami H et al Blood 2000 Jan 1;95(1):286-93) and the rat CD2 reporter gene under control of mammalian Elongation Factor-1 α promoter in the pELN vector. T2A and P2A cleavage sequences allow separate co-expression of TCR- α , - β and rat CD2. Lentiviral particles were generated by co-transfection of HEK 293T cells with the pELN transfer vector and packaging plasmids pVSVg (envelope), pMDLg/pRRE (gag/pol) and pRSV.rev (rev) in Lipofectamine 2000. Virus was concentrated by ultracentrifugation from supernatants harvested 24-72 hr after infection. **B.** Schematic of the generation of WT1₂₃₅₋₂₄₃ T cell lines. CD8⁺ T cells were isolated from cord blood by negative selection using an EasySep Human CD8⁺ T cell isolation kit (Stemcell) and cultured overnight with anti-CD3/CD28 Dynabeads (ThermoFisher), prior to infection with the WT1₂₃₅₋₂₄₃ TCR encoding lentivirus in the presence of Polybrene (Sigma) and 200U/mL IL-2. Rat CD2^{hi} CD8⁺ T cells were sorted by FACS four days later using the gating strategy shown in **C** and rested overnight. CD2^{hi} sorted cells were expanded with 2-4 x 14 day cycles of stimulation with HLA-mismatched irradiated PBMC from 3 separate donors, 1 μ g/mL phytohaeagglutinin (PHA, Remel, ThermoFisher), 25 ng/mL IL-15 and 200IU/mL IL-2. Fresh media and cytokines were replenished every 2-3 days **D.** Expression of rat CD2 and the WT1₂₃₅₋₂₄₃ TCR on the surface of the expanded CD8⁺ T cell line using a WT1₂₃₅₋₂₄₃-specific tetramer. **E.** Specificity of the WT1₂₃₅₋₂₄₃-specific CD8⁺ TCR line confirmed by IFN γ production following stimulation with titrating concentrations of cognate peptide (CMTWNQMNL) by ELISPOT.



Supplementary figure 4 (Related to manuscript Figure 4). Representative dot plots showing expansion of CD2⁺ WT1₂₃₅₋₂₄₃-specific TCR⁺ CD8⁺ T cells (gated on the CD3⁺ CD8⁺ T cell population) following expansion of humanized mouse splenocytes 8 days after incubation with cognate peptide or chimeric antibodies in the presence of poly I:C and R848.