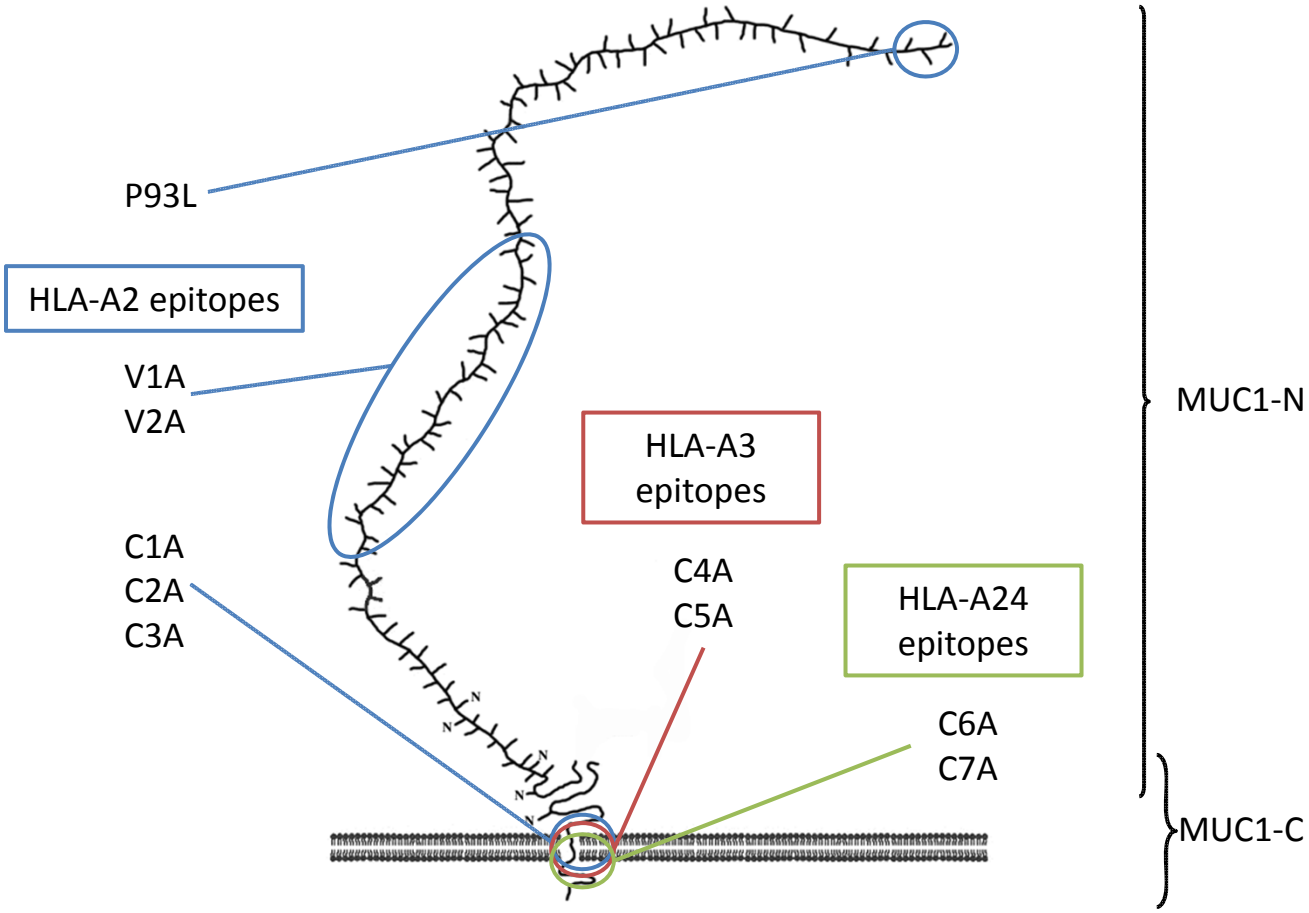
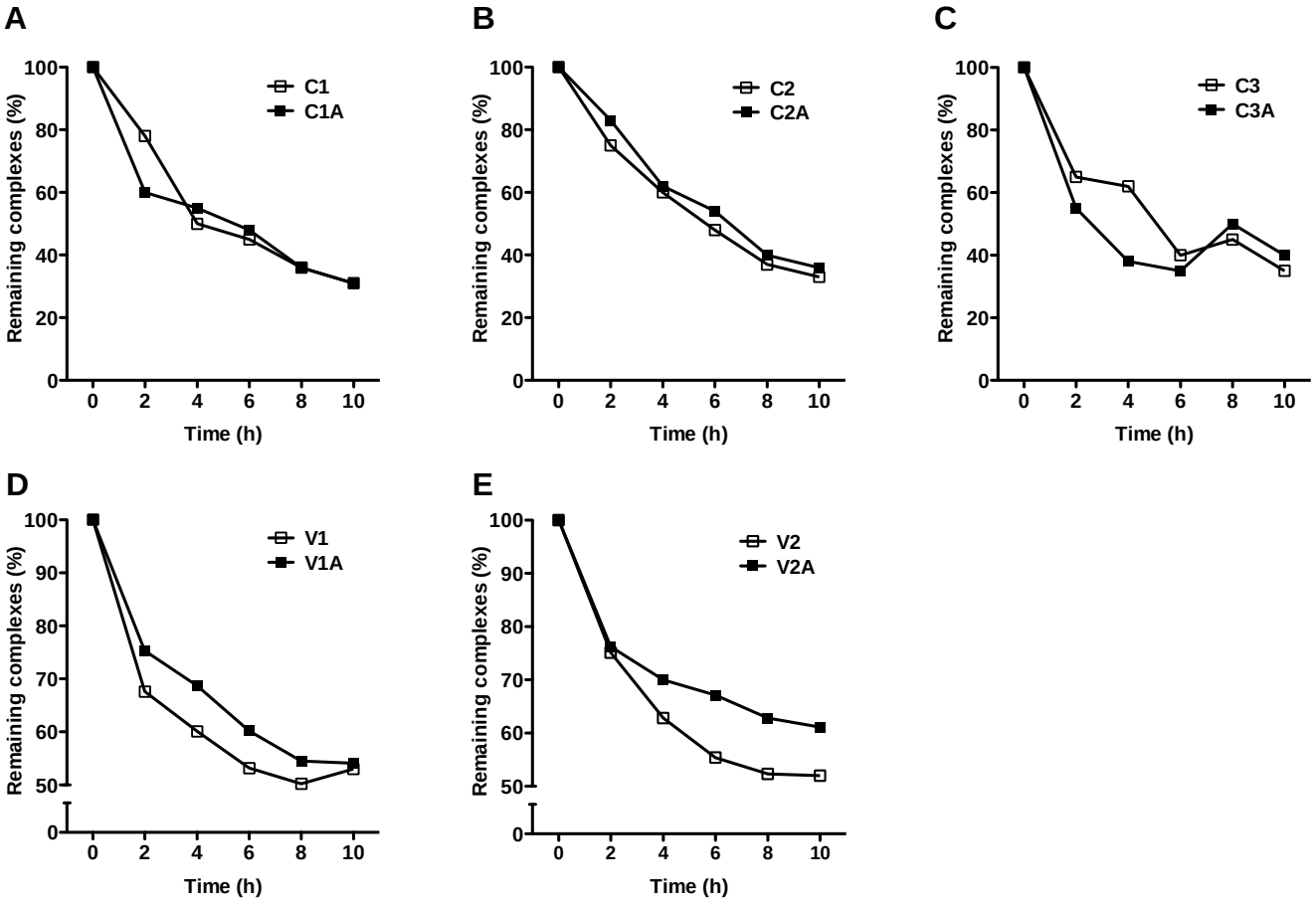
Supplemental Figure 1



Schematic drawing of the MUC1 molecule with location of agonist peptides. Cartoon adapted from MR Price *et al.* [Price MR, Rye PD, Petrakou E, Murray A, Brady K, Imai S, Haga S, Kiyozuka Y, Schol D, Meulenbroek MF *et al*: Summary report on the ISOBM TD-4 Workshop: analysis of 56 monoclonal antibodies against the MUC1 mucin. San Diego, Calif., November 17-23, 1996. *Tumor Biol* 1998, 19 Suppl 1:1-20.] The agonist epitope P93L was previously characterized. (See Tsang KY, Palena C, Gulley J, Arlen P, Schlom J: A human cytotoxic T-lymphocyte epitope and its agonist epitope from the nonvariable number of tandem repeat sequence of MUC-1. *Clin Cancer Res* 2004, 10(6):2139-2149.)

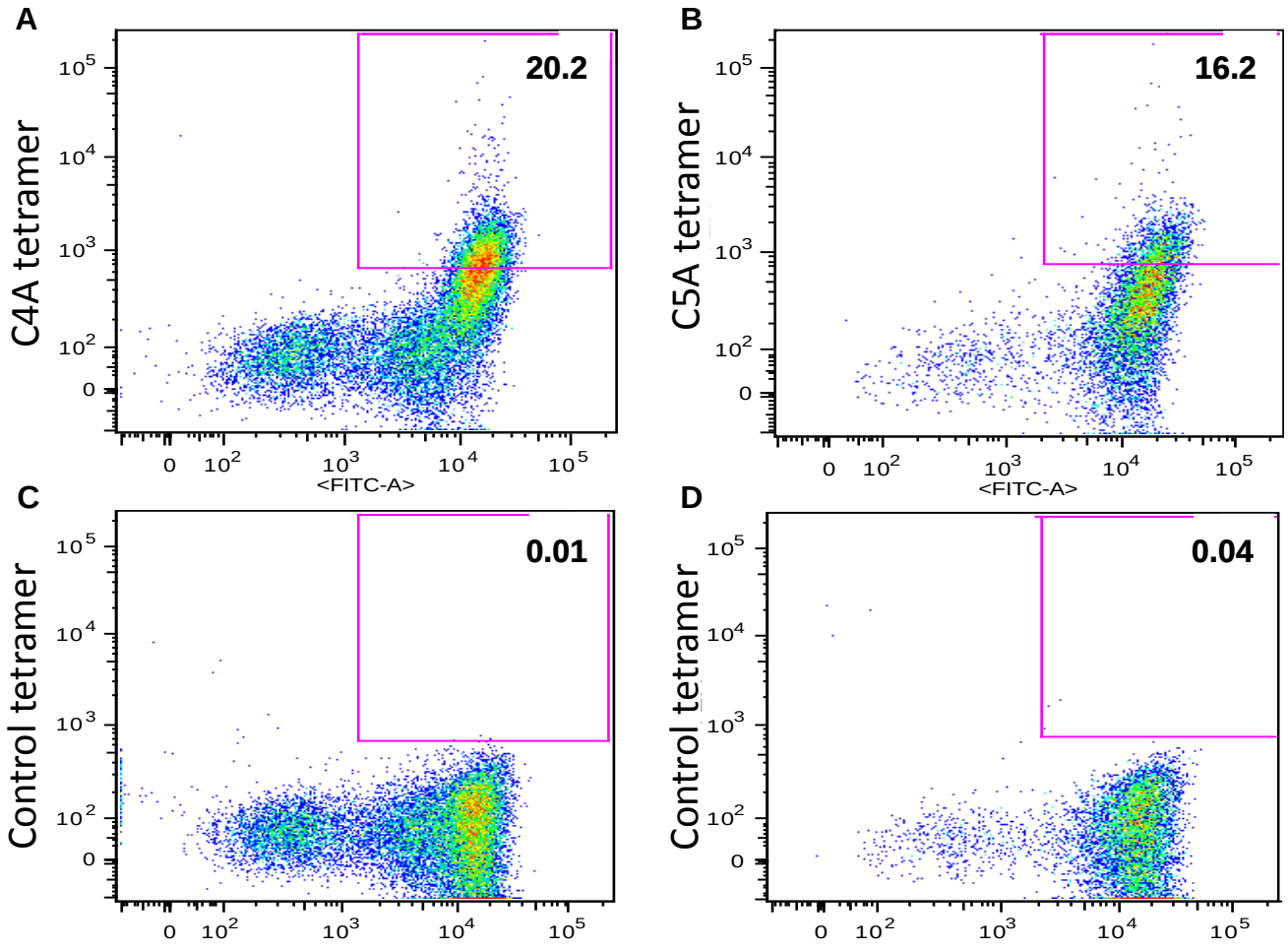
Supplemental Figure 2



Avidity assay for MUC1 native and agonist peptides.

The avidity of the native and agonist epitope peptides was investigated in an assay determining the stability of the peptide-HLA-A2 molecule complexes on T2 cells. The frequency of remaining complexes at different time points was measured as MFI by flow cytometry at 0, 2, 4, 6, 8 and 10 hours, and compared to the MFI at 0 hours. **A**: Peptides C1 and C1A. **B**: Peptides C2 and C2A. **C**: Peptides C3 and C3A. **D**: Peptides V1 and V1A. **E**: Peptides V2 and V2A.

Supplemental Figure 3

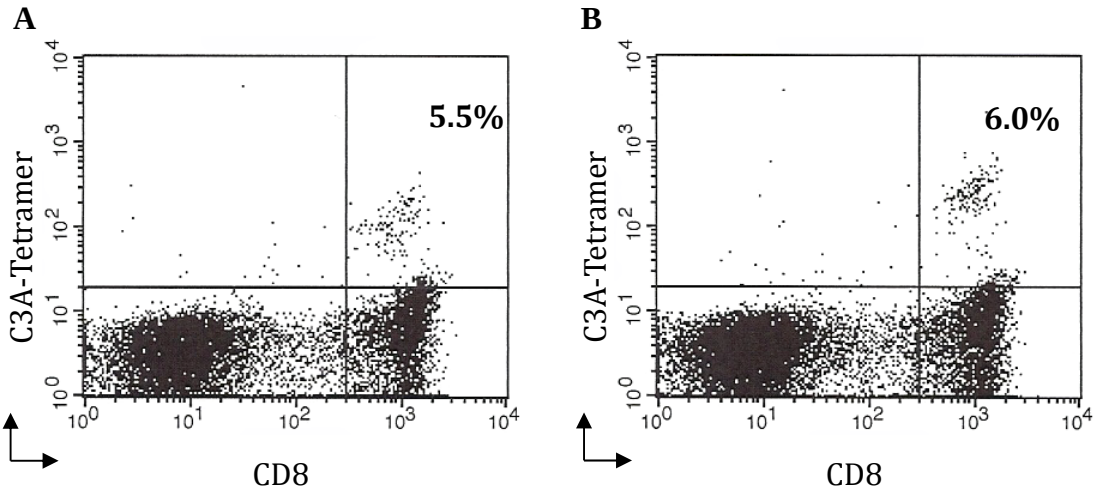


Stimulation with C4A and C5A produces a high percentage of tetramer positive cells.
Specific T-cell lines were established as described in materials and methods from a patient with colon carcinoma enrolled on the PANVAC vaccine trial for the native and agonist MUC1 peptides, and used at IVS6. The frequency of MUC1 peptide specific $CD8^+$ T cells was measured using tetramer analysis. The cells were stained with CD8-FITC and the corresponding agonist peptide tetramer-PE for 45 min. A negative tetramer was used as a control. $1x10^5$ cells were acquired on an LSRII (BD), and data was analyzed using FlowJo 9.0.1 software. Results are expressed as the percentage of $CD8^+tetramer^+$ cells. **A** and **C**: C4A specific T-cell line; C4A tetramer (**A**) and negative tetramer (**C**). **B** and **D**: C5A specific T-cell line; P483 tetramer (**B**) and negative tetramer (**D**).

Supplemental Table 1
Tetramer binding of PBMC stimulated with MUC1 native and agonist epitopes

Peptide	Patient #	Tetramer binding cells (%)
C1	1	1.53
C1A	**1**	**6.38**
C2	1	3.53
C2A	**1**	**6.18**
C3	2	5.5
C3A	**2**	**6.0**
V1	1	N/A
V1A	**1**	**5.4**
V2	1	N/A
V2A	**1**	**2.4**

Agonists are in bold.



MUC1 native and agonist epitopes were used to stimulate PBMCs from carcinoma patients enrolled in the PANVAC vaccine trial. The frequency of MUC1 peptide-specific $CD8^+$ T cells was measured after 2 IVS using tetramer analysis. The cells were stained with CD8-FITC and the corresponding agonist peptide tetramer-PE for 45 min. A negative tetramer was used as a control. $1x10^5$ PBMCs were acquired on an LSRII (BD), and data was analyzed using FlowJo 9.0.1 software. N/A: Non applicable. Lower panels: Representative FACS plots showing T cells from a patient stimulated with (**A**) native peptide C3 (5.5% tetramer positive cells) and (**B**) agonist peptide C3A (6.0% tetramer positive cells).