Supplemental Material to:

Widespread CD4+ T-cell reactivity to novel hTERT epitopes following vaccination of cancer patients with a single hTERT peptide GV1001

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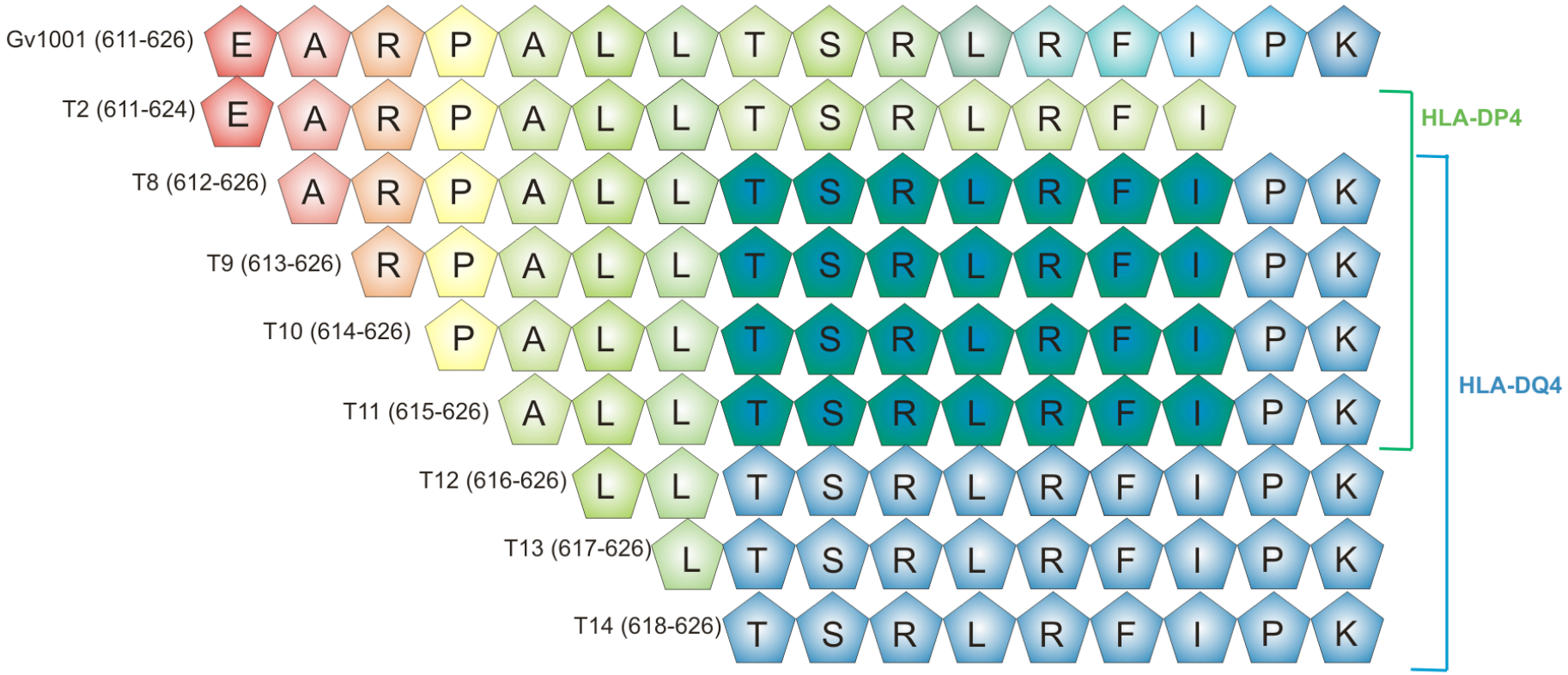
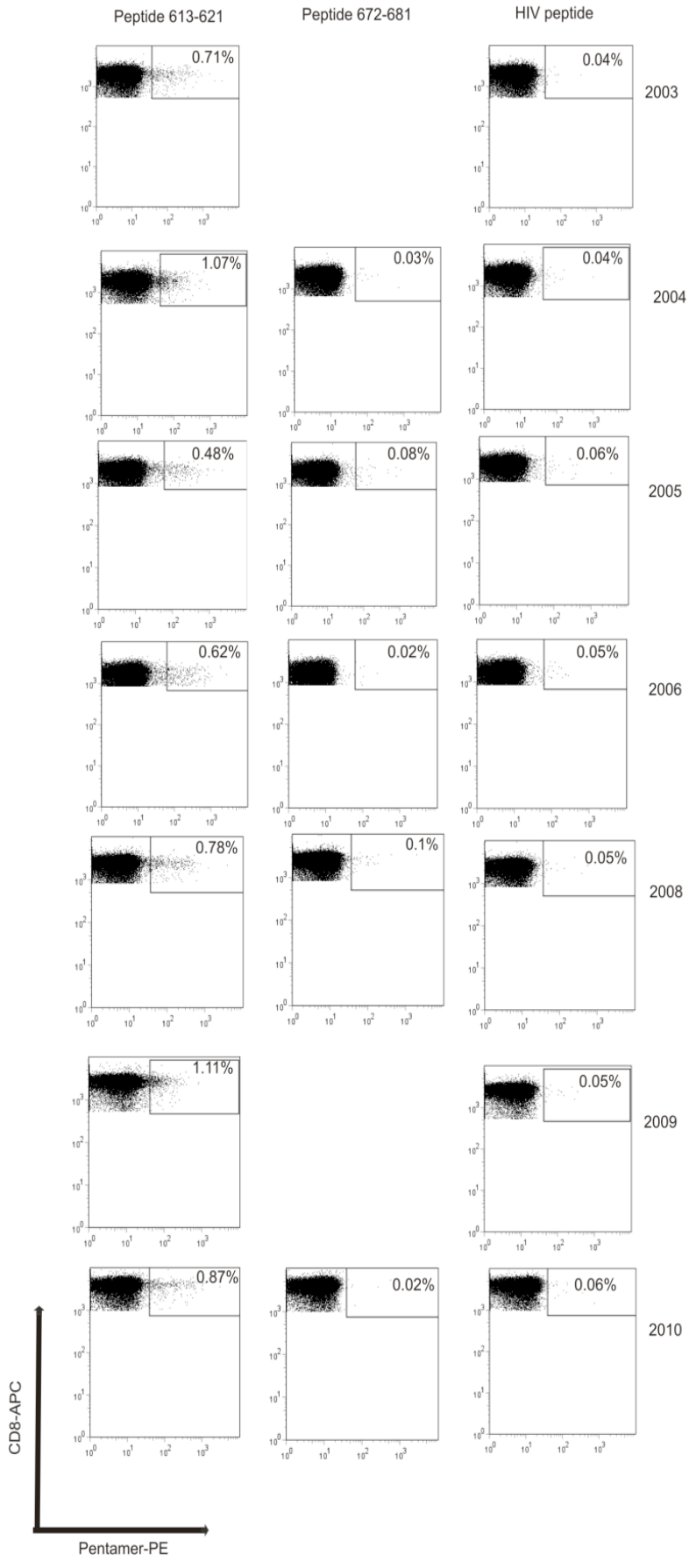
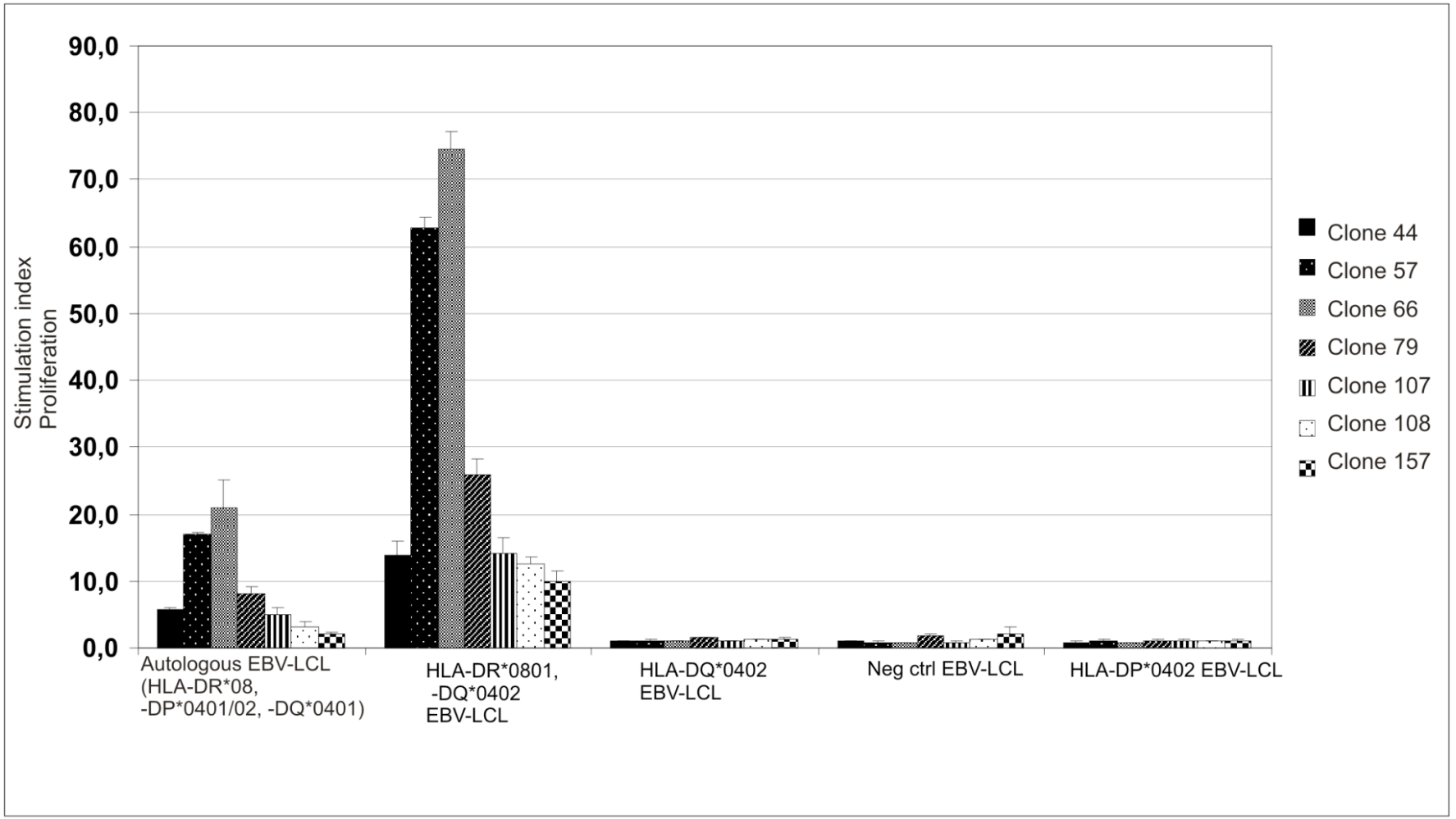


Supplementary figure 1. Minimal peptide sequences recognised by vaccine-specific CD4+ T cell clones.T cell clones specific for the GV1001 peptide were tested against a set of truncated peptides T2, T8-T14 with amino acid sequences in parentheses. The top line shows the GV1001 sequence. Amino acids sequence in green shows the required sequence for recognition by HLA-DP*04 restricted T cell clones and sequence in blue the minimal sequence for recognition by HLA-DQ*04 restricted, cytotoxic T cell clones.

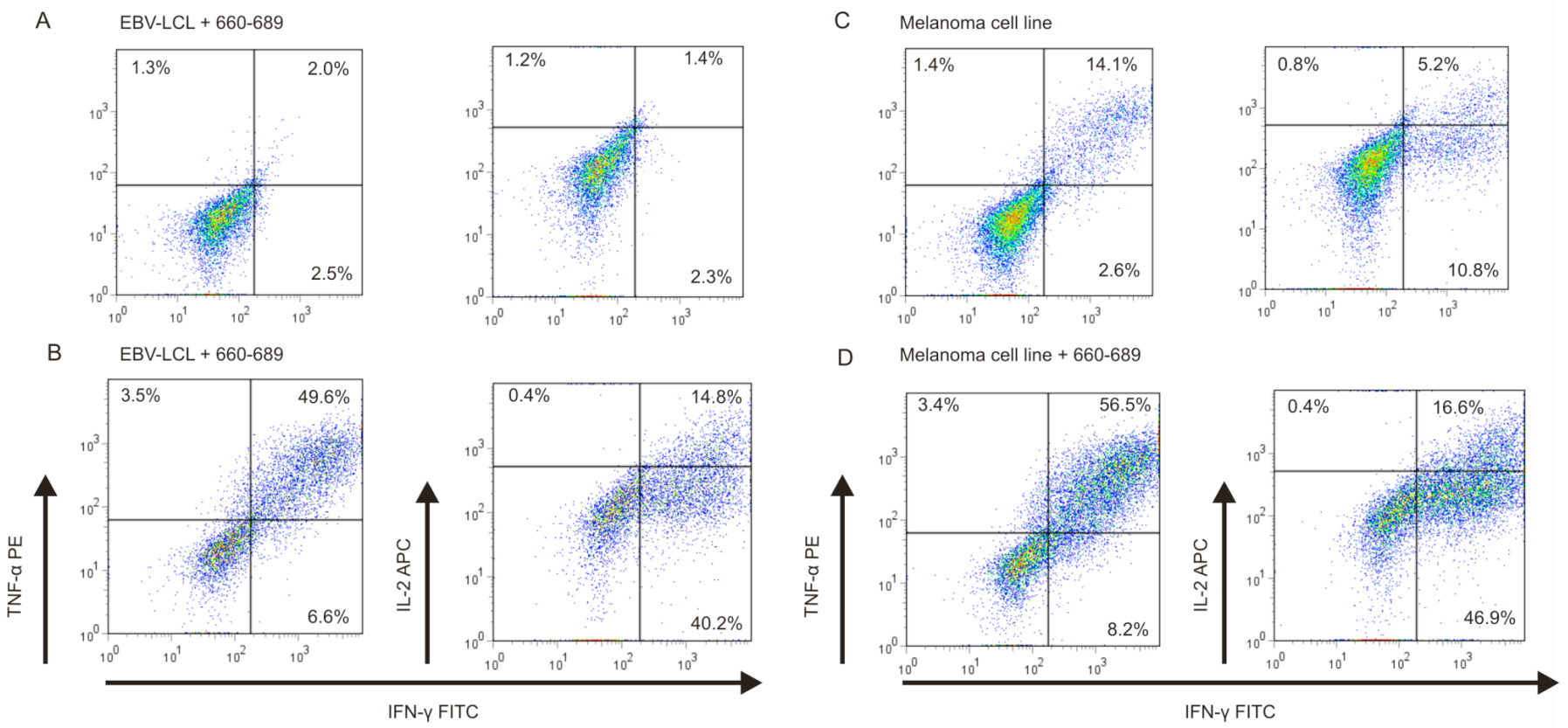


Supplementary figure 2

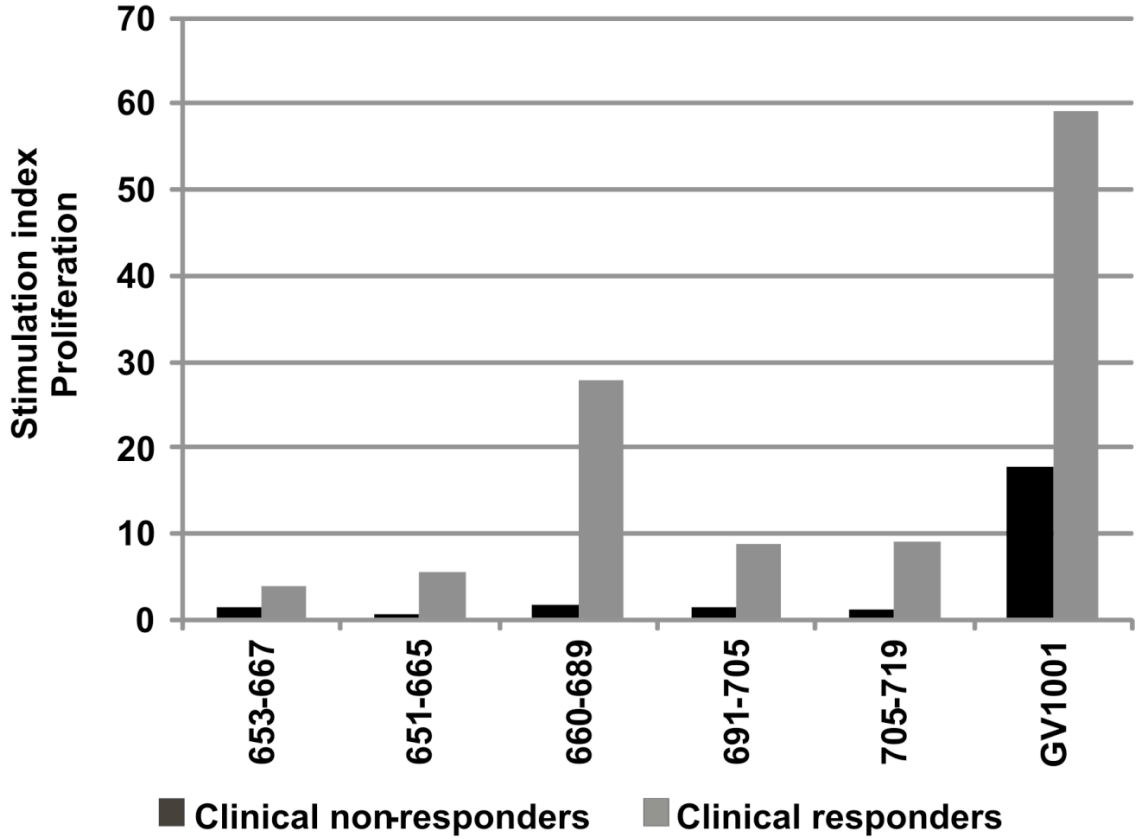
Supplementary figure 2. Pentamer analysis of hTERT-specific HLA-B*0702 restricted CD8+ T cells in post-vaccination samples. PBMCs were stained *ex vivo* with two pentamers with novel hTERT peptides 613-621 (left panels) and 672-681 (middle panels) which were nested epitopes of GV1001 and 660-689, respectively. A HIV pentamer was used as a negative control at each time point tested (right panels). Vaccination started in 2002 and post vaccination samples from the years indicated above were tested.



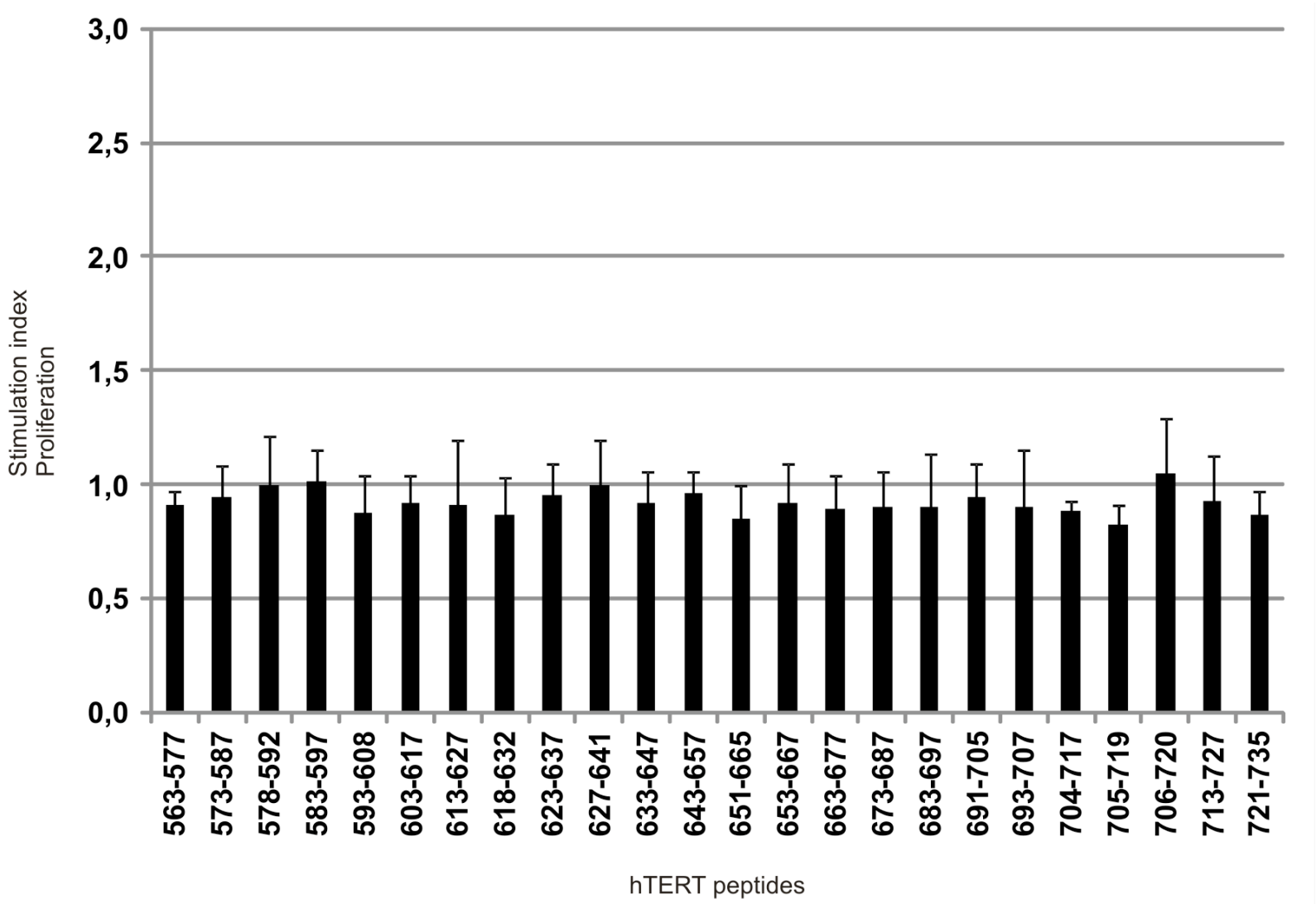
Supplementary figure 3. CD4+ T cell clones specific for hTERT peptide 660-689 are HLA-DR*0801 restricted. HLA-restriction of CD4+ T cell clones was tested using a panel of EBV-LCL homozygous for various HLA-alleles. The EBV-LCL were loaded with hTERT peptide 660-689 The x-axis shows the different EBV-LCL and the HLA alleles these have in common with the patient and the Y axis the stimulation index (SI). The different T cell clones tested are listed in the legend.



Supplementary figure 4. HLA-DR*0801 restricted CD4+ T cell clone specific for novel hTERT epitope recognizes melanoma cell line. Intracellular cytokine staining of the hTERT-specific HLA-DR*08 restricted CD4+ T cell clone 109 was performed after overnight stimulation with autologus EBV-LCL -/+ peptide 660-689 (A,B) or the HLA-DR*0801 melanoma cell line, ESTDAB-100 -/+ peptide 660-689 (C,D).



Supplementary figure 5. T-cell reactivity against hTERT in GV1001-vaccinated patients is significantly higher and broader in long term compared with short term surviving patients. Five of the most frequently recognized hTERT peptides from our overlapping peptide library (653-667, 651-665, 660-689, 691-705, 705-719) were tested for their capacity to stimulate T cells in ten melanoma and lung cancer patients who had responded immunologically against the GV1001 vaccine but displayed below median survival (clinical non-responders). The T cell responses were compared to those of four clinical responders/long term survivors who also responded immunologically against the GV1001 vaccine. Black bars represent the mean stimulation indexes for each peptide tested for clinical non-responders (n=10) and grey bars for clinical responders (n=4). The non-parametric Mann-Whitney U test showed that the difference in mean stimulation index (SI) between clinical responders and non-responders was statistically significant for all peptides: 653-667 p=0.028, 651-665 p=0.007, 691-705 p=0.011, 660-689 p=0.005, 705-719 p=0.011, and GV1001 p=0.048.



Supplementary figure 6. Healthy donors do not display T-cell responses against overlapping hTERT peptide library. PBMCs from six healthy donors were pre-stimulated once with the overlapping TERT peptide library and tested for proliferation against single peptides in triplicates. Each bar represents the mean stimulation index (SI) for each peptide for six donors +/- standard deviation.