**Supplemental figure legends**

**Figure S1**: Reproducibility of the methodology. (A) Purity of Melan-A sorted and amplified T cells was assessed on three independent assays performed on the same PBMC derived from a HLA-A\*0201 healthy donor. At the end of the amplification period, double labeling with Melan-A/A2 specific tetramer and anti-CD8 antibody was performed on 2.105 T cells and analyzed by flow cytometry. (B) Vß repertoire diversity of the three Melan-A specific T cell populations was documented by flow cytometry using a panel of 24 Vß antibodies. Each Vß subtype is indicated for each assay by the same pattern.

**Figure S2**: PD-1 and TIGIT expression on total peripheral CD8 T cells. PD-1 (upper panel), TIGIT (middle panel) expression and PD-1/TIGIT co-expression (lower panel) were assessed on CD8 T cells from 7 melanoma patients, before PD-1 treatment, through multiple labeling with anti-CD8, anti-PD-1 and anti-TIGIT specific antibodies. After PD-1 treatment, PD-1 expression was detected using the same anti-PD-1 antibody together with a mouse anti-human IgG recognizing Nivolumab antibody bound on CD8 T cells. Statistical analyses were performed using a non parametric Mann-Whitney t test.

**Figure S3:** PD-1 and TIGIT expression, PD-1/TIGIT co-expression were assessed on all the main Vß subtypes, from all Melan-A specific T cell populations, at rest, through multiple labeling with anti-Vbeta, anti-CD25 and specific antibodies. For each Vß subfamily, PD-1 and TIGIT expressions were evaluated on the CD25 negative fraction at rest. Empty circles represent pre-existing non-amplified Vß subtypes (T0, n=19). Light grey and dark grey circles illustrate respectively amplified pre-existing (n=5), and emerging Vß subtypes (n=8). Statistical analyses were performed using a non parametric Kruskal-Wallis test, followed by a t test (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, two-tailed p value).

**Figure S4:** 41-BB, PD-1, TIGIT, PD-1/TIGIT expression was assessed on all the main Vß subtypes, upon TCR activation, through multiple labeling with anti-Vbeta, anti-CD25 and specific antibodies. Empty circles represent Vß subfamilies not amplified upon PD-1 therapy (T0, n=19). Light grey and dark grey circles illustrate respectively pre-existing subpopulations amplified after therapy (n=5), and emerging subpopulations only detected after treatment (n=8). For each molecule, the mean value of expression was calculated and reported on the figure (horizontal bars). This mean value was further used to define PD-1low/TIGITlow and PD-1high/TIGIThigh subgroups.