**Supplementary Figure 1.**

**Expression of co-stimulatory and co-inhibitory markers in tumors and their expression in PD-1 negative biopsies.** A, Representative staining showing co-localization of VISTA with CD68, CD14, and CD8 in melanoma tumor. B, The number of positive cells for each marker from all biopsies (n=96) were summed and the percentage expressing CD3 was calculated. C, Representative images of VISTA expression on melanoma tumor cells in two individual patient biopsies. D, The number of positive cells for each marker per mm2 in PD-1 negative (≤1 cell/mm2) (n=18) tumors. E, Mean comparison in the expression of alternative checkpoint markers in PD-1 negative (≤1 cell/mm2) and PD-1 positive tumors (>1 cell/ mm2).

**Supplementary Figure 2.**

**Expression profile of checkpoint receptors at different stages of melanoma disease and site of disease in unmatched and matched patient specimens.** A, The number of GITR positive cells per 1mm2 in primary, in-transit metastases, lymph node metastases and distant metastases (unmatched). B, The proportion of intra-tumoral T cells expressing co-stimulatory (ICOS and OX4O) and co-inhibitory (PD-1, TIM-3, and VISTA) receptors per 1mm2 between matched primary and regional lymph node melanoma samples from the same patient (n=20). C, The proportion of intra-tumoral T cells expressing co-stimulatory (ICOS and OX4O) and co-inhibitory (PD-1, TIM-3, and VISTA) receptors per 1mm2 between matched primary and distant metastatic melanoma samples from the same patient (n=14).

**Supplementary Figure 3A.**

**Gating strategy for the identification of T cell and other immune cell subsets from CyTOF dataset.**

**Supplementary Figure 3B.**

**Expression and distribution of VISTA on immune cells in melanoma tumor.**  Distribution of VISTA on manually gated immune populations from n=20 patient tumor dissociates. Data is shown as the percentage of a population positive for that marker. Each dot represents a single tumor dissociate run through flow cytometry.

**Supplementary Figure 4A.**

**Tsne plots generated on CD3 positive T cells from all patients (60,000 events, concatenated) for EOMES, CD69, FOXP3, and CD4.**

**Supplementary Table 1**

**The mean and median values for checkpoint receptor expression in melanoma (positive cells per 1 mm2) for all biopsies (total, n=96).** The proportion of biopsies positive for each marker is also presented using a cut-off of >1cell and >5 cells per 1mm2 to define positive.

**Supplementary Table 2**

**Correlation between CD3 and checkpoint receptors from IHC data in all biopsies (n=96).**

**Supplementary Table 3**

**Clinical characteristics of melanoma biopsies used in CyTOF**

**Supplementary Table 4**

**Statistical summary of immune populations from CyTOF data**

**Supplementary Table 5**

**Cell surface antibodies used in CyTOF**

**Supplementary Table 6**

**Intracellular antibodies used in CyTOF**

**Supplementary Table 7**

**Fluorophore-conjugated Cell Surface antibodies**