## Supplementary methods

*Characterization of the myeloid dendritic cell product*

Purity of the isolated CD1c (BDCA-1)$^+$/CD141 (BDCA-3)$^+$ myDC product was analyzed by flow cytometry (BD LSR Fortessa) using the following fluorescently-labeled monoclonal antibodies: CD45 PE (clone REA747, Miltenyi Biotec), 7-AAD (Invitrogen), CD14 FITC (clone 61D3, Biolegend), CD141 (BDCA-3) PE-Cy7 (clone JAA17, eBioscience), CLEC9A APC (clone 8F9, Biolegend), CD123 APC-Vio770 (clone REA918, Miltenyi Biotec), CD11c AF700 (clone B-ly6, BD Biosciences), CD1c (BDCA-1) BV510 (clone F10/21A3, BD Biosciences) and FcεR VioBlue (clone CRA1, Miltenyi Biotec). Cells were first gated based on FSC/SSC characteristics, followed by gating on single cells. Next, dead cells were excluded and CD45$^+$ cells were gated, followed by the exclusion of CD14$^+$ cells. On this gate, pDC were identified as CD11c$^{-/dim}$ CD123$^+$ cells. On the non-pDC gate, CD141 (BDCA-3)$^+$ myDC (or cDC1) were identified as CD11c$^{low/+}$ CD141$^+$ cells and CLEC9A expression by these cells was analyzed. On the non-cDC1 gate, CD1c (BDCA-1)$^+$ myDC (or cDC2) were identified as CD11c$^+$ CD1c$^+$ FcεR$^+$ cells. Data were analyzed using FlowJo version 7.6. Predefined release criteria were a cell viability and a purity of >50%.

Maturation status of the isolated CD1c (BDCA-1)$^+$/CD141 (BDCA-3)$^+$ myDC product was analyzed by flow cytometry (BD LSR Fortessa) using the following fluorescently-labeled monoclonal antibodies: CD11c Alexa Fluor 700 (clone B-ly6, BD Biosciences), CD1c BV510 (clone F10/21A3, BD Biosciences), CD141 PE/Cy7 (clone JAA17, eBioscience), CD274 PE-Dazzle594 (clone EH12.2H7, Biolegend), CD86 BV421 (clone IT2.2, BD Biosciences), CD83 PE (clone HB15E, BD Biosciences), CD40 APC (clone 5C3, BD Biosciences), CD80 PE-Cy5 (clone L307.4, BD Biosciences), HLA-ABC FITC (clone G46 2.6, BD Biosciences) and Zombie yellow (Biolegend). Cells were first gated based on FSC/SSC characteristics, followed by gating on single cells. Next, dead cells were excluded and DCs were gated as CD11c$^+$ CD1c$^+$ (cDC2) and CD11c$^{low}$ CD141$^+$ (cDC1). On each DC subtype, the expression of the maturation markers was assessed. Data were analyzed using FlowJo version 7.6.

*Determination of lymphocyte subsets in peripheral blood*

Lymphocyte subsets in peripheral blood were assessed using the BD Multitest 6-color TBNK kit (BD Biosciences). Briefly, whole blood was stained with the BD Multitest 6-color TBNK reagent (containing CD3 FITC, CD16 PE, CD56 PE, CD45 PerCP-Cy5.5, CD4 PE-Cy7, CD19 APC and CD8 APC-Cy7), followed by red blood cell lysis and acquisition on a BD FACS Lyric flow cytometer. Flow cytometry data were analyzed using the BD FACSSuite software (version 1.5). For analysis, cells were gated on FSC/SSC characteristics to remove debris followed by gating on SSC-$A^{lo}$ $CD45^{+}$ cells as a first step to select the lymphocyte population. Next, monocytes were excluded by their $CD3^{-}$ $CD4^{lo}$ expression. On the monocyte-excluded lymphocyte population, B- and T lymphocytes were gated using CD19 and CD3 respectively. NK cells were identified as $CD19^{-}$ $CD3^{-}$ $CD56^{+}$/$CD16^{+}$ cells and a small population of $CD3^{+}$ NK-T cells was identified as $CD3^{+}$ $CD56^{+}$/$CD16^{+}$ cells. The $CD3^{+}$ T lymphocytes have been further subdivided into a $CD4^{+}$, $CD8^{+}$, $CD4^{-}CD8^{-}$ and $CD4^{+}CD8^{+}$ population.

Tregs in peripheral blood were assessed by a flow cytometric stain-lyse-wash procedure. 50 µl of peripheral blood was stained with CD3 FITC (BD Biosciences, clone SK7, 10µl); CD4 APC (BD Biosciences, clone SK3, 3µl); CD25 BV786 (BD Biosciences, clone M-A251, 3µl) and CD45 V500 (BD Biosciences, clone 2D1, 3µl) and incubated for 10 min at room temperature. Red blood cell lysis was performed by adding 200 µL of Optilyse C (Beckman Coulter). After washing with 2 ml FACSFlow buffer (sheath fluid, BD Biosciences), the cells were resuspended in 0.5 ml FACSFlow buffer. Analysis was performed on a FACSLyric (BD) flow cytometer. Lymphocytes were gated as SSC-$A^{lo}$ $CD45^{+}$, with exclusion of contaminating monocytes by a $CD3^{-}CD4^{weak+}$ gate. Within the monocyte-excluded lymphocyte population, Tregs were identified as $CD3^{+}CD4^{+}CD25^{hi}$ cells.

Absolute cell numbers were calculated based on the whole blood cell (WBC) count and percentage of lymphocyte subsets (obtained by an Abbott Alinity hq hematology analyzer).

Finally, we evaluated the evolution of DC subsets and memory T cell subsets in PBMC. For characterization of DC subsets, PBMC were stained with the following fluorescently-labeled antibodies: 7-AAD (Invitrogen), CD14 FITC (clone 61D3, Biolegend), CD3 APC-Fire750, CD19 APC-

Fire750, CD11c AF700 (clone B-ly6, BD Biosciences), CD141 (BDCA-3) PE-Cy7 (clone JAA17, eBioscience), CD123 PE-Cy5, HLA-DR BV785 and CD1c (BDCA-1) BV510 (clone F10/21A3, BD Biosciences). Samples were acquired on a BD FACSSymphony A1 flow cytometer (BD Biosciences). To remove debris, cell were first gated on FSC/SSC characteristics, followed by gating on single cells and exclusion of dead cells. Next, total DCs were gated as $CD14^-$ $CD19^-$ $CD3^-$ $HLA\text{-}DR^+$ cells. On the total DC gate, pDC were identified as $CD11c^-$ $CD123^+$ cells. On the non-pDC gate, CD141 $(BDCA\text{-}3)^+$ myDC (or cDC1) were identified as $CD11c^{low/+}$ $CD141^+$ cells. On the non-cDC1 gate, CD1c $(BDCA\text{-}1)^+$ myDC (or cDC2) were identified as $CD11c^+$ $CD1c^+$ cells. DC frequencies are shown as percentage of total DCs. Data were analyzed using FlowJo version 10.9.0. To determine the memory T cell subsets, PBMC were stained with Zombie yellow (Biolegend), CD3 Viogreen, CD4 Alexa Fluor 700, CD8 VioBlue, CD45RA VioBright 515, CD45RO APC-Vio770, CD197 (CCR7) APC, CD27 PE-Vio770 and CD62L PE and analyzed on a BD FACSSymphony A1 flow cytometer (BD Biosciences). Exclusion of debris pas performed by gating on FSC/SSC characteristics, followed by gating out dead cells. Next, total T cells were gated as CD3+ cells, followed by gating $CD4^+$ T cells ($CD4^+$ $CD8^-$) and $CD8^+$ T cells ($CD4^-$ $CD8^+$). On both the $CD4^+$ and $CD8^+$ T cells, memory T cell subsets were analyzed. Naïve T cells were defined as $CD45RA^+$ $CD45RO^-$ $CCR7^+$ $CD62L^+$ $CD27^+$ cells. Stem cell memory T cells (SCM) were characterized as $CD45RA^+$ $CD45RO^+$ $CCR7^+$ $CD62L^+$ CD27+. Central memory T cells (CM) were identified as $CD45RA^-$ $CD45RO^+$ $CCR7^+$ $CD62L^+$ $CD27^+$ cells. Effector memory T cells (EM) consisted of $CD45RA^-$ $CD45RO^+$ $CCR7^-$ $CD62L^-$ $CD27^{+/-}$ cells. Finally, terminally differentiated effector T cells (TE) were determined as $CD45RA^+$ $CD45RO^-$ $CCR7^-$ $CD62L^-$ $CD27^-$ cells. Frequencies of the different subsets are shown as percentage of $CD4^+$ and $CD8^+$ T cells respectively. Data were analyzed using FlowJo version 10.9.0.

*Multiplex immunohistochemistry staining*

A CD4/CD8/CD20/Foxp3/CD68/SOX10 mIHC staining was performed manually on 4 µm FFPE biopsy sections using Opal reagents (Akoya Biosciences, Menlo Park, CA) in 3 batches including for each batch a 4 µm FFPE melanoma lymph node metastasis section as a staining and quantification quality control.

Briefly, the tissue sections mounted on Superfrost Plus slides (Menzel-Glaser) were deparaffinized with xylene, hydrated through an ethanol gradient and fixed in 10% neutral buffer formalin for 20 min. Heat-induced antigen retrieval was achieved in Antigen Retrieval (AR) 9 buffer (Akoya Biosciences) using a microwave (Panasonic with Inverter technology). The slides were brought to a boil at 100% power followed by 10% power for 15 min and then left to cool at least for 15 min. The tissue sections were blocked (Opal Antibody Diluent/Block, Akoya Biosciences) for 10 min and then incubated with the primary antibody for 1H30. The horseradish peroxidase (HRP)-conjugated secondary polymer (anti-mouse/anti-rabbit, Akoya Biosciences) was applied on tissue sections for 10 min and finally tissue sections were incubated with the HRP-reactive Opal fluorophore for 10 min (Akoya Biosciences). Heat-induced antibody removal was achieved in AR6 buffer (Akoya Biosciences) using the previous microwave treatment. The staining cycles were repeated until all markers were stained as described in **Supplementary Table 1.**

The slides were washed between each step in 0.05% Tris-Buffered Saline–Tween 20 three times at 2 min each. After the final staining cycle, the nuclei were counterstained with spectral DAPI (Akoya Biosciences) for 5 min and the slides were mounted in the ProLong Diamond Antifade Mountant (Invitrogen).

*Multiplex IHC Acquisition and Quantification (cont.)*

Quantification algorithms were created using the positive control MSI and 10 MSI of batch 1 containing positive cells for each marker. In general, tissue was segmented into three categories “no tissue”, “peritumoral” and “tumor” based on DAPI and Sox10 staining. Cell segmentation was based on DAPI staining and assisted by CD4, CD8, CD20 staining. Finally, cell phenotyping was performed by machine learning. InForm software was trained to recognize positive and negative cells for each marker. The phenotyping of CD4, CD20 and Sox10 were achieved in the same algorithm since positive staining for these markers is on different cells. For phenotyping of CD8, CD68 and FOXP3, one algorithm by marker was created while keeping the same tissue and cell segmentation settings.

As there may be staining variations between batches, the quantification algorithms created with batch 1 MSI were tested on positive control MSI of batch 2 and 3. Staining intensity and percentage of positive cells for each marker were compared between batch 1, 2 and 3 MSI from the same tissue areas. Similar staining intensity and percentage of positive cells were found for each marker between batch 1, 2 and 3 MSI expect for CD20 and CD8 staining of batch 2. For these two markers new quantification algorithms using the positive control MSI and MSI of batch 2 were created to improve cell detection.