Supplemental Figure 7. Characterization of CD14^+CD1c^- DC and CD14^+CD1c^- monocytes/macrophages in the TME of HPV16^IR- and HPV16^IR+ OPSCC tumors.

Freshly dissociated OPSCC tumor tissue from 9 HPV16^+ OPSCC patients was analyzed by 13-parameter flow cytometry analysis with antibodies directed against CD3/CD19/CD20/CD56, CD11c, HLA-DR, CD14, CD11b, CD163, CD141, CLEC9A, CD1c, CD16, CD123, CD36 and CD32B. A, B) The gating strategy is depicted for a representative OPSCC sample. A) Dot plot showing expression of CD14 and CD1c within lineage-negative (LIN-), CD11c^+ and HLA-DR^- myeloid cells. Singlets were gated on FSC-H/FSC-A properties, after which dead cells were excluded through gating on yellow amine reactive dye-negative cells. Next, CD3-CDC19-CD20-CD56^-HLA-DR^-CD11c^- myeloid cells were selected, which were subsequently divided based on CD14 and CD1c expression. B) Histogram plots showing CD16, CD163, CD32B, CD36, CD123, CD141 and CLEC9A expression for CD14^-CD1c^- (black), CD14^+CD1c^- (blue) and CD14^+CD1c^+ cells (red). C) Histogram plots showing CD40 and CD86 expression for CD45^lin^- (black), CD11c^-HLA-DR^- (blue) and CD14^-CD11c^-HLA-DR^- cells (red). D) Box plots depicting the distribution of the identified CD14^+CD1c^- DC (left) and CD14^-CD1c^- monocytes/macrophages (right) among HPV16^IR^- (blue, n=3) and HPV16^IR+ (green, n=6) OPSCC tumors. Data is represented as percentage of live cells.