Supplementary methods

Fluorescent microscopy

For the uptake of tumor antigen by ascites macrophages, adherent ascites cells from mice bearing GFP^+ ID8-VEGF tumors were collected and stained with PE-conjugated anti-F4/80 antibody (clone CI:A3-1, Biologend) for evaluating F4/80 and eGFP co-localization. DAPI was used for nuclear staining. All images were acquired on a Nikon Eclipse Ti2-E, 40x-60x magnification.

Immunoaffinity purification of HLA peptides

Briefly, W6/32 and HB145 monoclonal antibodies were purified from the supernatants of HB95 (ATCC® HB-95™) and HB145 cells (ATCC® HB-145™) using protein-A sepharose 4B (Pro-A) beads (Invitrogen, California, USA), and antibodies were then cross-linked to Pro-A beads. Cells were lysed and the lysates cleared by centrifugation at 4°C, 50 min at 21,191 x *g*. Snap-frozen and cryopreserved tissues were homogenized on ice with Ultra Turrax homogenizer (IKA) at maximum speed, and the lysates cleared by high-speed centrifugation (25,000 rpm, Beckman Coulter, JSS15314) at 4°C, 50 min. For HLA immunopurification, Waters Positive Pressure-96 Processor (Waters) and 96-well microplates with 3µm glass fibers and 10µm polypropylene membranes (Seahorse Bioscience) were used. The lysates were passed sequentially through protein-A sepharose 4B to deplete autoantibodies, followed by pan-HLA-I antibody-crosslinked beads and finally through pan-HLA-II antibody-crosslinked beads at 4°C. Then, the beads were washed twice with 20 mM Tris-HCl pH 8. Sep-Pak tC18 100 mg Sorbent 96-well plates (Waters) were used for purifying can concentrating HLA-I and HLA-II peptides. The HLA-complexes and bound peptides were directly eluted from the affinity plate with 1% trifluoroacetic acid (TFA, Sigma-Aldrich, Missouri, USA). After washing the C18 sorbents with 0.1% TFA, HLA-I peptides were eluted with 28% acetonitrile (ACN; Sigma-Aldrich, Missouri, USA) in 0.1% TFA, and

HLA-II peptides were eluted with 32% ACN in 0.1% TFA. Recovered HLA-I and -II peptides were dried using vacuum centrifugation (Concentrator plus, Eppendorf) and stored at -20°C.

LC-MS/MS analyses

The LC-MS/MS system consisted of an Easy-nLC 1200 (Thermo Fisher Scientific) connected to a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific). Peptides were separated on a 450 mm analytical column (8 µm tip, 75 µm inner diameter, PicoTipTMEmitter, New Objective) packed with ReproSil-Pur C18 (1.9 µm particles, 120 Å pore size, Dr. Maisch GmbH). The separation was performed at a flow rate of 250 nL/min by a 120 min or 90 min gradient of 0.1% formic acid (FA) in 80% ACN in 0.1% FA in water for HLA-I peptides and HLA-II peptides, respectively. The mass spectrometer was operated in the data-dependent acquisition (DDA) mode. Full MS spectra were acquired in the Orbitrap from m/z = 300-1650 with a resolution of 60,000 (m/z = 200) and an ion accumulation time of 80 ms. The auto gain control (AGC) was set to 3e6 ions. MS/MS spectra were acquired in a data-dependent manner on the 10 most abundant precursor ions (if present) with a resolution of 15,000 (m/z = 200), an ion accumulation time of 120 ms and an isolation window of 1.2 m/z. The AGC was set to 2e5 ions, the dynamic exclusion was set to 20 s, and a normalized collision energy (NCE) of 27 was used for fragmentation. No fragmentation was performed for HLA-I peptides with assigned precursor ion charge states of four and above or for HLA-II peptides with an assigned precursor ion charge state of one, or six and above. The peptide match option was disabled.

Identification of HLA binding peptides

The MaxQuant computational platform version 1.5.5.1 was used to search the peak lists against the UniProt databases (Human 42,148 entries, March 2017) and a file containing 247 frequently observed

contaminants [47]. Methionine oxidation (15.99491 Da) was set as a variable modification. The enzyme specificity was set as unspecific and the second peptide identification option in Andromeda was enabled. A peptide spectrum match (PSM) false discovery rate (FDR) of 0.05 and no protein FDR were set. The initial allowed mass deviation of the precursor ion was set to 6 ppm and the maximum fragment mass deviation was set to 20 ppm.

TCRα and TCRβ Sequencing and analysis

mRNA was isolated using the Dynabeads mRNA DIRECT purification kit (Life Technologies) and was then amplified using the MessageAmp II aRNA Amplification Kit (Ambion) with the following modifications: *in vitro* transcription was performed at 37°C for 16h. First strand cDNA was synthesized using the Superscript III (Thermofisher) and a collection of TRAV/TRBV specific primers. TCRs were then amplified by PCR (20 cycles with the Phusion from NEB) with a single primer pair binding to the constant region and the adapter linked to the TRAV/TRBV primers added during the reverse transcription. A second round of PCR (25 cycles with the Phusion from NEB) was performed to add the Illumina adapters containing the different indexes. The TCR products were purified with AMPure XP beads (Beckman Coulter), quantified and loaded on the MiniSeq instrument (Illumina) for deep sequencing of the TCRα/TCRβ chain. The TCR sequences were further processed using *ad hoc* Perl scripts to: (i) pool all TCR sequences coding for the same protein sequence; (ii) filter out all out-frame sequences; (iii) determine the abundance of each distinct TCR sequence. TCR with a single read were not considered for the analysis.

Flow cytometry

Phenotype of mouse ascites-derived APCs and T cells was evaluated using anti-mouse antibodies to CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), FoxP3 (FJK-16s), CD11b (M1/70), CD11c

(N418),CD14 (Sa2-8), CD45 (30-F11), F4/80 (BM8) [all eBioscience, Thermo Fisher Scientific, Massachusetts, USA], CD80 (16-10A1), CD83 (Michel-19) and CD86 (GL-1) [all Biolegend California, USA]. Anti-human antibodies included anti-CD14 (M5E2) , CD45 (HI30), CD11c (3.9), CD11B (ICRF44), CD40 (5C3), CD80 (2D10), CD83 (HB15e), CD86 (IT2.2), HLA-ABC (W6/32), HLA-DR (L243), CD3 (UCHT1), PD1(EH12.2H7), Lag3 (11C3C65), CD4 (OKT4), CD8 (RPA-T8), CD39 (A1), CD103 (Ber-ACT8), BTLA (MIH26), CTLA4 (BNI3), 41BB (4B4-1), CD28 (O323), CD25 (BC96), CD127 (A019D5), ICOS (C398.4A), OX40 (Ber-ACT35) [Biolegend, California, USA] and FOXP3 [PCH101, eBioscience, Thermo Fisher Scientific, Massachusetts, USA]. Zombie fixable dye was used to evaluate viability (Biolegend, California, USA) and eBioscience™ Foxp3/Transcription Factor Staining Buffer kit (Thermo Fisher Scientific, Massachusetts, USA) used for intranuclear FOXP3 staining.

Supplementary figure legends

Supplementary figure 1 A) eGFP expression in tumor cells, B cells, T cells and $CD11b^{+}F4/80^{+}$ cells from ascites of ID8-VEGF or ID8-VEGF-GFP inoculated mice. Tumors and ascites were induced by inoculating 6-8 weeks old female C57BL/6 mice (Charles Rivers) with $5x10^{6}$ tumor cells i.p. in a volume of 100µl of sterile saline. Animals were weighed at least twice weekly and were euthanized when they reached 30 g due to ascites accumulation. Ascites were harvested and GFP expression or loading was evaluated by flow cytometry. B) Fluorescent confocal microscopy showing GFP-positivity in CD45+F4/80+ ascites macrophages, indicating the uptake of ID8-GFP tumor in these cells.

Supplementary figure 2 Following ID8 tumor cells inoculation, $CD45^{+}$ ascites APCs were isolated from the ascites of mice and stimulated *ex vivo* for 48 hours with a combination of LPS, CpG-oligonucleotides and IL-10R Ab. Supernatant was harvested from post-stimulation and unstimulated

ascites APCs and analyzed by multiparameter analyte profiling (Myriad Rules Based Medicine Luminex multi-analyte profile).

Supplementary figure 3 Flow cytometry analysis at A) baseline of immune cells populations in the three OC patients used for the immunopeptidomic and *in vitro* coculture studies and B) purity of isolated $CD14^+$ cells from the three patients used for immunopeptidomic analysis.

Supplementary figure 4 TLR RNA expression (TPM) in tumor cells or $CD14^+$ cells from human ascites.

Supplementary figure 5 IFN-γ production upon 72 hours coculture of activated human TLRM and autologous T cells in presence or absence of ascites supernatant.

Supplementary file 1 List of identified HLA-I and HLA-II binding peptides in two OC patients.

Supplementary file 2 Expression values of genes expressed in two OC patients.