Supplementary figure legends:

Supplementary Figure 1. Peptides identified by mass-spectrometry. A) Total unique peptides identified by mass spectrometry (left) their protein sources following mapping to the mouse proteome (right) from the indicated cell populations.

Supplementary Figure 2. Bia in observed cross-presentation of tumor cell-derived peptides. A) Ranked representation of each amino acid in 1740 MHC-I peptides presented by B16F10 reported by Schuster et al. B) Weblogo plots of amino acid preference per position showing bias toward anchor peptides for 8-mers (top) and 9-mers (bottom). C) Ranked coverage of MHC-I peptides reported by Schuster et al for the four amino acids selected for heavy isotope labeling. D) Percent peptides harboring the indicated isotope labeled amino acids in tryptic digest of total cell lysates (black) and in MHC-I-bound peptides isolated from SILAC B16F10 cells. E) Residual variance analysis following linear regression analyses of cell compartment proportions on endogenous B16 vs endogenous BM-DC (left), Endogenous B16 vs Cross-presented, representing all B16-derived peptides (center), or Endogenous BM-DC vs Cross-presented, representing all peptides isolated from BM-DC (right). F) Histograms (left) and percent positive (right) of B16F10 and B16 $B2M^{-/-}$ cells stained for $H\text{-}2K^{b}$ after 24 h IFN-γ stimulation. G) Stacked plots of plasma membrane protein domains that served as sources for endogenous or cross-presented peptides isolated from BM-DC (left) or for endogenous peptides isolated from BM-DC vs B16F10 (right). NA, not accessible.

Supplementary Figure 3. Flow cytometry analysis of B16-cyto and B16-mem. A) Histograms of ZsGreen fluorescence in B16-cyto, B16-mem, and parental B16F10 following establishment and sorting of cell lines. B) Proliferation of OT-I T cells following coculture with BM-DC pulsed for 18 h with the indicated number of irradiated $B2M^{-/-}$ B16-cyto or B16-mem cells. Representative example shown. C) Representative gating strategy used while sorting cDC1 and cDC2/moDC from spleens and lymph nodes of naïve C57BL/6 mice. D) Representative plots of negative (cultured with cDC1 or cDC2/moDC only) and positive (cultured with cDC1 or cDC2/moDC with SIIN peptide) OT-I T cell proliferation controls.

Supplementary Figure 4. Analysis of patient derived data. A) SNV in cytoplasmic proteins (left) and plasma membrane proteins (right) plotted per patient with respect to their duration of clinical response reported by Nathanson et al. B) SNV in cytoplasmic proteins (left) or plasma membrane proteins (right) normalized to each patient's total SNV plotted with respect to their duration of clinical response. C) Patients from the OpACIN-Neo trial grouped by their clinical response with mean z-score of the T cell inflammation score per patient plotted. D) SNV analyses of the patient described in Linnette et al. Stacked plot indicates SNV in each subcellular compartment for each

lesion sequenced (left). SNV in cytoplasmic and plasma membrane proteins summarized for one early and one late lesion (right). SQ, subcutaneous lesions. PM, pulmonary metastasis. RM, retroperitoneal metastasis.

Supplementary Figure 5. Analysis of BM-DC maturation status post co-culture with tumor debris. A) CD80 expression of BM-DC post co-culture with tumor debris generated by either irradiation or treatment with indicated drug. B) CD86 expression of BM-DC post co-culture with tumor debris generated by either irradiation or treatment with indicated drug. Expression is represented by geometric mean fluorescence intensity values normalized to the values of immature BM-DC. Shown are mean with s.e.m. of pooled data from 2 independent experiments.