Supplementary Figures 1. Regulatory T cell responses following belinpectin + pembrolizumab.

Fresh whole blood was stained with mAbs to CD45, CD3, CD4, CD8, CD25, CD127, Ki-67, and HLA-DR. Following RBC lysis and washing, A) Treg (CD3⁺CD4⁺CD8⁻CD25⁺CD127lo) phenotypes were determined by flow cytometry. Each line represents an individual patient and graphs depict the median (center line), the interquartile range (box), and the minimum and maximum values (whiskers) of n=9-11 patients per cohort. Green indicates an increase post- vs. pre-treatment, blue indicates no change, and orange indicates a decrease. *P<0.05, **P<0.01. NR=non-responder (white bars); R=responder (gray bars).
Supplemental Figure 2. Increased pro-inflammatory cytokines and chemokines in patients responding to belapectin + pembrolizumab. Pre- versus post-treatment levels of IP-10/CXCL10, IL-12p70, and IL-18 were determined by multiplex ELISA. Each line represents an individual patient and graphs depict the median (center line), the interquartile range (box), and the minimum and maximum values (whiskers) of n=9 patients per cohort. *P<0.05, **P<0.01. NR=non-responder (white bars); R=responder (gray bars).
Supplemental Figure 3. Tumor galectin-3 expression does not correlate with the efficacy of anti-PD-1 therapy. Bulk RNA-seq data from patients with advanced melanoma (adapted from Gide et al. (62)) were re-analyzed to assess the correlation between galectin-3 or PD-L1 expression and the efficacy of PD-1 blockade. A) Gene expression of LGALS3 (galectin-3) and CD274 (PD-L1) from pre-treatment biopsies. N=73 (anti-PD-1; n=41, anti-CTLA-4 + anti-PD-1; n=32) B) PFS based on the LGALS3 cutoff. Gal-3 low (blue): PFS for the patient group with lower LGALS3 expression (< median, n=21). Gal-3 high (red): PFS for the patient group with higher LGALS3 expression (> median, n=20). P = 0.9223. C) PFS based on the CD274 cutoff. PD-L1 low (blue): PFS for the patient group with CD274 expression below the median (< median, n=21). PD-L1 high (red): PFS for the patient group with CD274 expression above the median (n=20). P = 0.0005 (Log-rank test).
Supplemental Figure 4. Gating strategy for effector memory (EM) T cells and the expression of Ki-67 and ICOS. Representative flow cytometry data and gating strategy to identify CD4+ and CD8+ EM T cells are shown. The insert shows the levels of Ki-67 or ICOS for CD4+ or CD8+ EM T cells before and after (at the peak) treatment from one representative subject with PR.
Supplemental Figure 5. Identification of MDSCs. M-MDSCs were defined as lineage (CD3, CD7, CD19, and CD20) negative, CD15^CD11b^CD33^CD14^HLA-DR^{low} and PMN-MDSCs as lineage (CD3, CD7, CD19, and CD20) negative and CD15^CD11b^CD33^CD14^HLA-DR^{low}. The insert is shown to illustrate the