Supplemental Figure 1. BEMPEG+NKTR-262 combination therapy significantly reduces EMT6 tumor growth. (A) EMT6 tumor growth depicted for individual animals on the right side treated tumor (top row, solid lines), the left side non-treated tumor (middle row, dashed lines), and averages of the right and left side tumor growth for each treatment group (bottom row). *p<0.05, Students T test. (B) Probability of survival for BEMPEG+NKTR-262 (red), BEMPEG+RT (blue), BEMPEG (yellow), NKTR-262 (black triangle), RT (black circle), and control (grey). For A and B, N=4-8 from one experiment. *p<0.05, Log-rank test.

Supplemental Figure 2. NK cells do not contribute to BEMPEG+NKTR-262 efficacy. (A) Confirmation of cellular depletion using PBL drawn 5 days post-delivery of depleting antibody. (B) CT26 tumor growth depicted for individual animals after depletion on the right side (treated) tumor (top row, solid lines) and the left side, non-treated tumor (middle row, dashed lines). (C) MCA-205 tumor growth in C57BL/6 mice after BEMPEG+NKTR-262 therapy in the absence of CD8 T cells (green) or NK T cells (purple). *p<0.05, Repeated measures ANOVA with Dunnett's multiple comparison test (each colored asterisk denotes difference between that group and vehicle control). (D) Probability of survival for control (grey), BEMPEG+NKTR-262 (red), BEMPEG+NKTR-262+aCD8 (blue), and BEMPEG+NKTR-262+aNK1.1 (purple). *p<0.05, Log-rank test. N=7 from one experiment.

Supplemental Figure 3. Minimal changes to lymphocytes in LN on day 7 post treatment. (A) Heat map displaying the fold change in phenotype frequencies using control as baseline. (B) Flow cytometry data of a subset of CD8+ T cell phenotypes displaying the differences across treatment groups. N=5 from one experiment. One-way ANOVA with Šídáks multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Supplemental Figure 4. CD4+ T cell immune cell phenotyping 7 days post treatment. (A) PBL immune phenotypes were determined by flow cytometry. Box and whisker plots represent the min and max (whiskers), the quartiles (box) and median (line). Each point represents an individual mouse. For Teff, Treg, Ki-67, N = 15, from two independent experiments; for CD62L, CD122, CD25, ICOS, and PD-1, N =4-8 one representative experiment. One-way ANOVA with Šídáks multiple comparisons test. (B) Luminex CBA assay of serum from PBL. Data shown are the cytokines that were significantly down (left) or up (right) comparing BEMPEG+NKTR-262 with BEMPEG+RT. *p<0.05.

Supplemental Figure 5. IFN/ signaling contributes to efficacy of BEMPEG+NKTR-262 therapy. CT26 tumor growth depicted for individual animals on the right side treated tumor (top row, solid lines), and the left side non-treated tumor (bottom row, dashed lines). On each row, squares indicate IFNAR-1 antibody treated mice. Arrow indicates mice whose non-treated tumors grew out to cut-off size in the IFNAR-1 mice. Two independent experiments are shown.

Supplemental Figure 6. BEMPEG+NKTR-262 induces conventional type 1 DCs. CT26 tumors were harvested 3-days post therapy and analyzed by flow cytometry. (A) UMAP distribution of DCs isolated 3-days post therapy. (B) FlowSOM unbiased clustering of the DC UMAP distribution and the proportion of DCs in each cluster after therapy. (C) UMAP distribution of cluster 1 after BEMPEG+RT (blue) or BEMPEG+NKTR-262 (red) and histogram distribution of MHCII, CD103, PD-L1, and iNOS from cluster 1. (D) Frequency of CD103+ DCs. One-way ANOVA with Šídáks multiple comparisons test. *p<0.05, **p<0.01,

***p<0.001, ****p<0.0001. For A-C, represents 1 of 2 independent experiments, N=5/treatment group. For D, two independent experiments are shown, N=8-10.

Supplemental Figure 7. BEMPEG+NKTR-262 favors M1 macrophage polarization. CT26 tumors were harvested 1- or 3-days post treatment and analyzed by flow cytometry. (A) Frequency of macrophages (F4/80+MHC-II+ of CD11b+Ly6C-) (left), ratio of M1 (iNOS+) macrophages to M2 (arginase+) macrophages (middle), and frequency of PD-L1+ macrophages (right). (B) Frequency of Mo- or PMN-MDSCs 1-day post treatment. One-way ANOVA with Šídáks multiple comparisons test. *p<0.05, **p<0.01, ****p<0.0001. Day 1 data from one experiment, day 3 data from two independent experiments.

Supplemental Figure 8. Tumor CD4+ T cell immune phenotyping 7 days post-treatment. CT26 tumors harvested 7 days post therapy and analyzed by flow cytometry. (A) Percent (left) and density (right) of CD4+ T effector (Teff) cells in the tumor. N=8-14, from three or four independent experiments (B) Percent (left) and density (right) of regulatory Foxp3+CD4+ T (Treg) cells in the tumor. N=8-14, from three or four independent experiments. (C) Activation markers expressed on Teff cells in the tumor. (D) Activation markers expressed on Treg cells in the tumor. N=8-13 from two experiments; one-way ANOVA with Šídáks multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001. T=treated tumor, NT=non-treated tumor.

Supplemental Figure 9. NKTR-262 monotherapy recruitment of Nur77+CD8+ T cells correlate with tumor size; RT monotherapy does not. Correlation between percent of Nur77+ of CD8+ T cells and tumor area for either RT monotherapy (left graphs) or NKTR-262 monotherapy (right graphs). CD8+ T cells from the treatment side tumors represented on top graphs, non-treatment tumors on bottom graphs. N=8 (NKTR-262) or N=10 (RT) from two independent experiments. Simple linear regression.