

1 **Supplemental Figure Legends:**

2 **Supplemental Figure 1 Legend. NKTR-214/RT therapy increases survival and immune**

3 **activation compared to rhIL-2/RT therapy.** CT26 tumors were implanted into bilateral flanks

4 on day 0. Established tumors were treated with NKTR-214/RT or rhIL-2 on day 10 post-

5 implantation and blood draws performed on day 17 post-implantation in order to assess

6 systemic treatment effects. Tumor growth and overall survival were assessed (A). T cell subset

7 and NK cell frequencies as well as Treg, CD4<sup>+</sup> Teff, and CD8<sup>+</sup> T cell activation, proliferation, and

8 exhaustion markers in peripheral blood at day 17 are shown (B). Data is shown from a single

9 experiment (n = 5-7 mice/group). Statistics show a one-way ANOVA Dunnett's test comparing

10 each group to NKTR-214/RT combination therapy group. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001,

11 \*\*\*\*p<0.0001.

12 **Supplemental Figure 2 Legend. Increased CD8<sup>+</sup> T cell levels correlate with decreased**  
13 **tumor burden while increased Treg levels correspond with increased tumor burden in**  
14 **NKTR-214 treated animals.** Correlation between each cell type and the total tumor burden  
15 (treatment and abscopal side) measured on day 17, limited to those mice that received either  
16 NKTR-214 monotherapy (blue) or NKTR-214/RT combination therapy (red). All plots show data  
17 from two independent experiments (n = 14).  
18

19 **Supplemental Figure 3 Legend. NKTR-214/RT combination treatment induces an**  
20 **increased percentage of CD8<sup>+</sup> T cells that correlates with reduction in tumor size.** CT26  
21 tumors were implanted into bilateral flanks on day 0. Established tumors were treated (NKTR-  
22 214 +/- RT) day 10 post implant and tumors were harvested 17 days post implant. Percentages  
23 of CD8<sup>+</sup> T cell **(A)**, Teff (FoxP3<sup>-</sup>) **(B)**, or Treg cell (FoxP3<sup>+</sup>) **(C)** phenotypes in treatment side  
24 (circles) and abscopal side (squares) tumors. Spearman's correlations of each cell subset from  
25 either treatment side (T) or abscopal side (A) with the corresponding tumor size on harvest day.  
26 For all box and whisker plots, statistics show a one-way ANOVA comparing each group to  
27 treatment side NKTR-214/RT combination \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001. For  
28 Spearman's correlations color represents r<sub>s</sub>, statistics indicated by p-value.

29 **Supplemental Figure 4 Legend. NKTR-214/RT combination treatment induces increased**  
30 **Nur77<sup>+</sup> CD8<sup>+</sup> T cell intratumoral density but does not increase the percent of Nur77<sup>+</sup> CD8<sup>+</sup>**  
31 **T cells.** CT26 tumors were implanted into bilateral flanks of Nur77-GFP transgenic mice on day  
32 0. Established tumors were treated (NKTR-214 +/- RT) day 10 post implant and tumors were  
33 harvested 17 days post-implant. Nur77<sup>+</sup> CD8<sup>+</sup> T cell, T effector cell (FoxP3<sup>-</sup>), or T regulatory cell  
34 (FoxP3<sup>+</sup>) densities (top row) or percentages (bottom row) in treatment side (circles) and  
35 abscopal side (squares) tumors. Spearman's correlations of each cell subset from either  
36 treatment side (T) or abscopal side (A) with the corresponding tumor size on harvest day. For all  
37 box and whisker plots, statistics show a one-way ANOVA comparing each group to treatment  
38 side NKTR-214/RT combination \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001. For Spearman's  
39 correlations color represents r<sub>s</sub>, statistics indicated by p-value.

40 **Supplemental Figure 5 Legend. NKTR-214/RT combination therapy increases chemokine**  
41 **receptor expression on the CD45<sup>-</sup> cells.** CT26 tumors were implanted into bilateral flanks on  
42 day 0. Established tumors were treated with NKTR-214 or NKTR-214/RT on day 10 and tumors  
43 were harvested on day 17 post-implantation. DC, TAM, and MDSC populations were initially  
44 gated as CD45<sup>+</sup>, CD11b<sup>+</sup>, and Ly6C<sup>-</sup> before differentiation using Ly6G<sup>+</sup> (MDSCs) or MHC class  
45 II<sup>+</sup> with CD24<sup>+</sup> (DCs), or F4/80<sup>+</sup> (TAM). Frequency and density of MDSC, TAM, DC, and CD45<sup>-</sup>  
46 populations within the tumor were assessed as well as the frequency of cells expressing CSFR1  
47 and CX3CR1. Data is shown from a single experiment (n = 8 mice/group). Statistics show a  
48 one-way ANOVA Dunnett's test comparing each group to NKTR-214/RT combination therapy  
49 group. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.