**Supplemental Figure 1. *Concomitant immune tolerance prevents an immunotherapeutic response at the distant tumor site, is observed with an 8 Gy x 3 fraction RT regimen, and requires a well-established macroscopic secondary tumor.* A)**Secondary tumor sizes are shown for mice whose primary tumor response is displayed in **Fig 1A**. Combined treatment of the primary tumor with RT+IT-IC did not affect secondary tumor growth compared to control treatments. **B)** C57BL/6 mice bearing a single or two B78 melanoma tumors (both tumors implanted ~ 4-5 weeks prior to RT) were treated as indicated with 8 Gy x 3 daily fractions or sham RT starting on day 1 and IT injected on days 6-10 with hu14.18-IL2 IC or control IgG. Results from the first of replicate studies are shown. Aggregate data demonstrate complete tumor regression in 50% (6/12) of animals bearing a single B78 melanoma following 8 Gy x 3 fractions + IT-IC compared to 0% complete regression of the primary tumor (0/14; *p* < 0.01) in mice bearing two tumors and receiving this same treatment combination at the primary tumor (secondary tumor is untreated). **C**) In mice bearing a primary and secondary B78 tumor in which all secondary tumors were injected 2 weeks after the primary, “outlier” mice with slow growing, barely palpable, but not grossly visible tumors (≤4 mm3) were compared with those bearing modest sized (~40 mm3) secondary tumors that were comparable to secondary tumors in A). Primary tumor response to combined treatment with 12 Gy and IT-hu14.18-IL2 IC is shown. The size of the secondary tumor appears to affect concomitant immune tolerance; very small sites of disease have a limited CIT effect on primary tumor response to combined RT + IT-IC. In these experiments, primary tumor complete response was observed in 42% (5/12) of mice with very small secondary tumors, compared to 0% (0/11, *p* = 0.02) of mice with established measurable macroscopic secondary tumors. n = number of mice per group. NS = non-significant, \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001.

**Supplemental Figure 2.** ***Cooperative effect of RT and IT-hu14.18-IL2 is observed in mice bearing a single syngeneic Panc02-GD2+ tumor.*** C57BL/6 mice bearing a single flank Panc02-GD2+ tumor were treated with 12 Gy or sham RT on day 1 and IT injected on day 6-10 with hu14.18-IL2 IC or control IgG. Results from the first of replicate experiments are shown. Aggregate data from replicate experiments demonstrate that combined treatment with 12 Gy and IT-hu14.18-IL2 IC was more effective than single agent treatments and resulted in 69% (9/13) complete tumor regression compared to 0% in all other groups (0/13 in each group; *p* < 0.001). We did not monitor overall survival in this Panc02 model because regardless of treatment group these tumors caused skin ulceration and nearly all mice (even those with complete tumor regression) were recommended for euthanasia by animal care staff ~ 30 days after RT due to skin wound rather than tumor progression or moribund behavior. n = number of mice per group. \*\*\* *p* < 0.001.

**Supplemental Figure 3.** ***The presence of an untreated secondary tumor influences the effect of RT on the ratio of tumor infiltrating CD8+:FoxP3+ cells at a radiated tumor site.* A)** RNA was isolated from B78 tumor samples on day 6 after RT, cDNA was generated, and real-time PCR was used to compare the ratio of the Treg-specific FoxP3 transcript with that of the tumor-specific GD2-synthase transcript. Importantly, we have previously demonstrated that RT does not affect the expression of GD2 in B78 melanoma cells ([10](#_ENREF_10)). In this figure, each bar is labelled A1, A2, A3 or A4, to correspond to the four conditions shown in **Fig 3A**. In samples from mice bearing a single tumor, RT decreased the relative fraction of FoxP3 to GD2-synthase (**A2**) compared to non-radiated tumor (**A1**). In mice bearing two tumors, where only the primary tumor was radiated, this decline in the FoxP3:GD2 ratio on day 6 (compared to values from non-radiated mice with only 1 tumor) was not observed in either the primary (**A3**) or secondary (**A4**) tumor. To evaluate this effect further, we performed immunohistochemistry to quantify changes in tumor infiltrating FoxP3+ and CD8+ cells over time following 12 Gy RT in tumors from mice bearing a single or two B78 tumors (both tumors implanted ~ 4-5 weeks prior to RT) in the absence of any IT-IC treatment. Box and whisker plots show individual data points (circles), middle two quartiles (box), local maximum and minimum (whisker bars), and median (line) values for tumor-infiltrating **B)** FoxP3+ and **C)** CD8+ cells at the indicated interval after 12 Gy in mice with a single tumor. Points outside the whisker bar are those extending beyond the middle quartile limits by > 1.5x the interquartile range. These mice were engrafted and treated at the same time as those in Fig 5 and these experiments share the same untreated controls, which were analyzed on the day corresponding to day 2 for the mice that received RT. **D)** The ratio of the mean number of CD8+: FoxP3+ tumor-infiltrating cells is displayed, as derived from B) and C). Similarly, whisker plots display data from quantification of tumor infiltrating **E)** FoxP3+ and **F)** CD8+ cells in the primary (irradiated) tumor and a secondary (non-irradiated) tumor at the indicated intervals after 12 Gy primary tumor RT in mice also bearing an untreated (non-irradiated) secondary tumor. **G)** The ratio of the mean number of CD8+: FoxP3+ tumor-infiltrating cells is displayed, as derived from E) and F). These data indicate that RT locally depletes FoxP3+ and CD8+ cells at one day after RT (day 2) in mice with one tumor, and the FoxP3+ cell depletion remains evident at least until day 6, with return of FoxP3+ cells as of Day 12 (B). Similarly, in mice with a distant untreated secondary tumor, the irradiated primary tumor also shows FoxP3+ cell depletion one day after RT (day 2), however the un-radiated secondary tumor shows no change in FoxP3+ cells (E). The rate of FoxP3+ cell repopulation of the radiated tumor microenvironment thereafter is greater in these mice with a secondary tumor, with return to baseline FoxP3+ cells in the irradiated primary tumor evident on days 4 and 6 (E). As for FoxP3+ cells, tumor irradiation causes CD8+ cell depletion 1 day after irradiation in mice with one tumor (C). However, in mice with only one tumor, CD8+ cell repopulation occurs more quickly following RT (C) than does repopulation of FoxP3+ cells (B), with CD8+ cell infiltrate exceeding untreated tumor levels on days 4 and peaking at day 12 (C) . Because of these changes over time, we observe an increase in the ratio of tumor infiltrating CD8+:FoxP3+ cells in a single radiated tumor peaking at day 6 after 12 Gy (D) but no such trend is observed in mice bearing a second un-radiated tumor (G). This is consistent with a shared sensitivity of both FoxP3+ and CD8+ cells to 12 Gy and a subsequent pro-inflammatory effect of RT resulting in increased CD8+ tumor infiltrate. In mice with a single radiated tumor this is followed by a relatively slow restoration of FoxP3+ tumor infiltrate by day 12 after RT and in mice with an untreated secondary tumor, this process occurs more rapidly. The values for the ratio of tumor infiltrating CD8+:FoxP3+ cells in the irradiated tumor are 3.0 and 3.5 on days 4 and 6 in mice with only 1 tumor (D), but only 1.3 and 1.2 on days 4 and 6 in the mice with 2 tumors (G).