Supplemental Figure 1: Flow Gating Strategy 1 (Global Populations)
Supplemental Figure 2: Flow Gating Strategy 2 (Memory CD8+ T Cells)
Supplemental Figure 3: Flow Gating Strategy 3 (Checkpoint Molecules)
Supplemental Figure 4: Flow Gating Strategy 4 (Checkpoint Ligands)
Supplemental Figure 5: NM600 can be utilized for diagnostic and therapeutic applications. A) TRAMP-C1 Tumor-bearing mice were injected with $^{88}$Y-NM600 and imaged via serial PET/CT at the indicated time points. Representative maximum intensity projections are shown. H = heart, L = lungs, K = kidneys, T = tumor. B, C) Individual growth curves from the experiment in Figure 1M-P. D, E) Summary growth curves from TRAMP-C1 and Myc-CaP tumors in repeat of experiments shown in Figure 1 M-P. F, G) Survival to 2000 mm$^3$ or death from the same experiment. * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$ via linear-mixed effects model with Benjamini-Hochberg adjustment of pairwise tests. Error bars represent mean +/- standard error. Results shown are from one experiment.
Supplemental Figure 6: 90Y-NM600 increased CD8+ T cell infiltration into Myc-CaP prostate tumors but also increased checkpoint molecule expression. Myc-CaP tumor-bearing animals (n=3 per group) were treated with IgG alone, low-dose TRT, or high-dose TRT. Tumors were digested and collected for flow cytometry analysis for A) CD3+CD8+ T cells as a percentage of CD45+ cells. MFI of B) PD-1, C) CTLA-4, D) LAG-3 on CD8+ T cells. E) MFI of PD-L1 on CD45- cells. * denotes p < 0.05, ** denotes p < 0.01, *** denotes p < 0.001 via 2-way ANOVA with Benjamini-Hochberg adjustment of pairwise tests. Error bars represent mean +/- standard error. Results shown are from one experiment.
Supplemental Figure 7: Combination of PD-1/PD-L1 blockade and ⁹⁰⁴-NM600 TRT was ineffective regardless of radiation dose or antibody timing. TRAMP-C1 tumor-bearing mice (n=7 per group) were given IgG alone or TRT with anti-PD-1, anti-PD-L1, or IgG and followed for tumor growth. Antibody treatments were given on Day 0 or 6 post-TRT or Day 0, 3, and 6 post-TRT as indicated. A) Summary growth curves from high-dose treated mice. B) Summary growth curves from low-dose treated mice. C) Individual growth curves from all treatment schedules and doses. D) Myc-CaP tumor-bearing mice (n=10 per group) were treated with 165 µCi of ⁹⁰⁴-NM600 or no treatment in combination with 3 doses of anti-PD-1 or IgG control given on Day 0, 3, and 6 post-TRT, then followed for tumor growth. * denotes p < 0.05, ** denotes p < 0.01, *** denotes p < 0.001 via linear-mixed effects model with Benjamini-Hochberg adjustment of pairwise tests. Error bars represent mean +/- standard error. Results shown are from one experiment.
Supplemental Figure 8: Treg depletion via anti-CTLA-4 therapy improved anti-tumor immunity in combination with ^{90}Y-NM600. A) Individual growth curves from experiment shown in Figure 6A and D. B) Summary growth curves from Myc-CaP tumors (n=8 per group) treated with the same experimental setup as in Fig 6. Results shown are from one experiment.
Supplemental Figure 9: Conditional Treg depletion was necessary for efficacy of anti-PD-1 + ⁹⁰Y-NM600 combination. DEREG mice (n=3 per group) were treated with PBS or 1 μg diptheria toxin (DT) i.p. for two consecutive days. The day after the second DT treatment, spleens were harvested and analyzed for CD4+CD25+FoxP3+ cells via flow cytometry. A) Representative flow plots B) Quantification of A. TRAMP-C1 tumors were implanted into DEREG mice (n=5/group) and then treated with high-dose TRT. On Day -2 and -1 before TRT, 1 μg of diptheria toxin was administered i.p. to deplete Tregs. 200 μg anti-PD-1 or IgG was administered i.p. on the same day as TRT. C) Summary growth curves D) Individual growth curves * denotes p < 0.05, ** denotes p < 0.01, *** denotes p < 0.001 via linear mixed-effects model with Benjamini-Hochberg adjustment of pairwise tests. Results shown are from one experiment.