Supplementary Figure Legends

Supplementary Figure S1. Improved cytokine polyfunctionality in T cells following co-blockade of RANKL with PD-1 and CTLA4. Groups of BALB/c WT mice (n= 5-10/group) were injected with 2 x 10^5 CT26 colon carcinoma cells. On day 10 after tumor inoculation, mice were randomized into groups bearing equivalent median tumor size and were treated i.p with a single dose of antibody as indicated: cIg (200 µg), anti-CTLA4 (9D9, mIgG2a isotype, 50 µg), anti-PD1 (200 µg), anti-RANKL (200 µg) or indicated combinations Three days after treatment, tumors were harvested and processed for flow cytometry gating on live CD45.2 cells of leukocyte morphology. **A:** Wet masses of CT26 s.c. tumors at day 13 after tumor inoculation according to indicated treatment group, with four experiments combined. Statistical differences in tumor mass were determined by Kruskal-Wallis test with Dunn's post-test (**p< 0.01, ****p< 0.0001). # indicates the following significant comparisons for both cIg and anti-RANKL: ** (vs anti-CTLA4 + anti-RANKL), ** (vs anti-PD1 + anti-CTLA4), **** (vs anti-PD1 + anti-CTLA4 + anti-RANKL). **B:** Representative FACS contour plots for tumors included in Figure 5A, gated on $CD8^+$ T cell TILs. **C:** Representative FACS contour plots for tumors included in Figure 5B, gated on $CD4^+$ T cell TILs. **D:** Representative FACS contour plots for tumors included in Figure 5C, gated on $CD8^+$ T cell TILs.

Supplementary Figure S2. No significant differences in proliferative status or gp70 antigen specificity of $CD8^+$ T cell TILs with co-blockade of RANKL with PD-1 and CTLA4. Groups of BALB/c WT mice (n= 5-10/group) were injected with 2

x 10^5 CT26 colon carcinoma cells. On day 10 after tumor inoculation, mice were randomized into groups bearing equivalent median tumor size and were treated with a single dose of antibody as indicated: cIg (200 µg), anti-CTLA4 (9D9, mIgG2a isotype, 50 µg), anti-PD1 (200 µg), anti-RANKL (200 µg) or indicated combinations. Mice were culled on day 13 after tumor inoculation and their tumors were harvested and processed for flow cytometry. Mean ± SEM proportion of (**A**) $CD8^+$ T cell TILs of total live single $CD45.2^+$ cells, (**B**) $CD8^+$ T cell TILs positive for Ki67, and (**C**) $CD8^+$ T cell TILs positive for gp70 tetramer are shown. Data pooled from three experiments.

Supplementary Figure S3. Anti-RANKL when combined with immune checkpoint blockade may modulate PD-L1 expression. For experiment depicted in Figure 6E, representative FACS contour plots for CT26 tumors depicted for indicated treatment groups.

Supplementary Figure S4. RANKL identifies $PD1^{hi}$ expressing T cells whose PD1 expression can be modulated. (**A-F**) Groups of BALB/c wild type (WT) mice (n=10/group) were injected s.c. with 2 x 10^5 CT26 colon carcinoma cells. On day 10 after tumor inoculation, mice were randomized into groups bearing equivalent median tumor size and were treated i.p with a single dose of antibody as indicated: cIg (200 µg), anti-CTLA4 (9D9, mIgG2a isotype, 50 µg), anti-PD1 (200 µg) or the indicated combinations. Three days after treatment, tumors were harvested and processed for flow cytometry gating on live CD45.2 cells of leukocyte morphology. (**A**) Proportion of $RANKL^+$ (black bars) or $RANKL^-$ (grey bars) $CD8^+$ T cell TILs expressing PD1, (**B**) expression level of PD1 (expressed as geometric MFI, gMFI) by $RANKL^+$ (black bars) or $RANKL^-$ (grey bars) $CD8^+$ T cell TILs, and (**C**) proportion of $RANKL^+$ (black bars) or

RANKL$^-$ (grey bars) CD8$^+$ T cell TILs expressing Ki67 displayed for indicated treatment groups. (**D**) expression level of PD1 (expressed as geometric MFI, gMFI) by RANKL$^+$ (black bars) or RANKL$^-$ (grey bars) gp70-specific CD8$^+$ T cell TILs displayed for indicated treatment groups. (**E**) proportion of RANKL$^+$ (black bars) or RANKL$^-$ (grey bars) CD4$^+$ T cell TILs expressing PD1, and (**F**) expression level of PD1 (gMFI) by RANKL$^+$ (black bars) or RANKL$^-$ (grey bars) CD4$^+$ T cell TILs displayed for indicated treatment groups. Means ± SEM are shown. Statistical differences were determined by one way ANOVA with Tukey's post-test analysis, except in (**C**), where Mann-Whitney test was used to compare within-treatment groups (*p< 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001).

Supplementary Figure S5. PD1, CTLA4 and RANKL co-expression on gp70 tetramer specific CD8$^+$ cells. For experiments depicted in Figure 6A-C, representative dot plots of CD8$^+$ T cell TILs for CT26 tumors depicted for indicated treatment groups.