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Supplemental Data

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**Supplemental Figure Legends.**

**Supplemental Figure 1. Expression of CTLA4 by intratumoral NK cells.**

(A) CT26 cells were transplanted s.c. and on day 9 post tumor transplantation mice were treated with anti-CTLA4 (clone 9H10) or isotype (Isotype) control. Graphs show the frequency of NK cells (Live CD45+CD19-Ly6G-CD3-NKp46+) that display CD107a on their surface on day 14 as determined by flow cytometry. (B) Representative contour plots and corresponding graphs showing expression of NKp46 versus IC or surface CTLA4 (sCTLA4) by Live CD45+CD19-CD11c-Ly6G- cells infiltrating CT26 tumors 18 days post transplantation. Isotype control staining for the sCTLA4 antibody is also shown. To determine statistical significance t-test for two groups was used. \*\*\*\* indicates p< 0.0001.

**Supplemental Figure 2. Expression of sCTLA4 on T cells infiltrating murine tumors.**

(A-B) Flow cytometry was used to visualize expression of sCTLA4 on intratumoral T cell subpopulations. Histograms show sCTLA4 expression on the following intratumoral T cell (Live CD45+B220-CD11b-CD11c-Ly6G-CD3+) subsets: Tregs (CD4+FOXP3+, open black histogram), CD4 effector T cells (CD4+FOXP3-, open blue histogram), CD8 T cells (CD8+, open red histogram) and splenic Tregs (Spl Tregs, black dotted histogram), Filled gray histograms show background from cells similarly stained with isotype control. (A) sCTLA4 expression by B16-infiltrating T cell subsets 19 days post tumor transplantation. (B) sCTLA4 expression by YUMM1.7-infiltrating T cell subsets 45 days post tumor transplantation. (C) Flow cytometry was used to determine expression of sCTLA4 on sCTLA4+ Tregs (Live CD45+TCRβ+CD4+FOXP3+), CD4 effector (Live CD45+TCRβ+CD4+FOXP3-) and CD8 (Live CD45+TCRβ+CD8+) T cells infiltrating conditionally-induced, autochthonous melanoma tumors from the *Braf*V600E/*Pten*-/- mouse model. (D) Flow cytometry was used to determine expression of sCTLA4 on peripheral, skin and intratumoral Tregs (Live CD45+TCRβ+CD4+FOXP3+) from conditionally-induced *Braf*V600E/*Pten*-/- melanoma mouse model. To determine statistical significance t-test for two groups was used. For multiple groups of data analysis ANOVA with Dunnett’s post-hoc multiple comparison was used to determine statistical significance between two groups. n.s. indicates non-significant, \* indicates p<0.05, \*\*\* indicates p<0.001, \*\*\*\* indicates p< 0.0001.

**Supplemental Figure 3. Proposed Model. Anti-CTLA4 opsonizes intratumoral Tregs leading to the activation of intratumoral NK cells, which are able to mediate killing of opsonized Tregs.**

The figure depicts anti-CTLA4 binding to sCTLA4 on intratumoral Tregs (Step1). The Fc region of anti-CTLA4 then interacts with FcRs on NK cells leading to NK cell activation (Step 2). These activated NK cells then degranulate (Step 3), leading to depletion of intratumoral Tregs (Step 4). The NK cell degranulation results in CD107a being expressed on the surface of NK cells (Step 3 and 4).

**Supplemental Figure 4. Ipilimumab induces activation and degranulation of human NK cells.**

Human NK cells from donor PBMCs were cultured overnight in RPMI plus 10% FBS, non-essential amino acids, sodium pyruvate and penicillin-streptomycin-glutamine supplemented with 50 ng/ml recombinant IL15 (NIH biorepository). These IL15 stimulated NK cells were then washed and transferred to pre-coated plates. To precoat plates 100 μl of Ipilimumab at 100 μg/ml or PBS was added overnight to 96 well plates (costar) pre-coated overnight. Plates were then washed three times with RPMI plus 10% FBS and then blocked for 20 minutes with RPMI plus 10% FBS. 100,000 NK cells were added per a well in the presence or absence of plate-bound Ipilimumab. Cells were cultured for 6 hours with monensin solution (Biolegend) being added at 1 hour. Flow cytometry was used to determine the frequency of CD69+ cells (A) or surface CD107a expression (B) on CD56+CD3- NK cells.