

Supplementary Materials

Combination oncolytic virus, radiation therapy, and immune checkpoint inhibitor treatment in anti-PD-1-refractory cancer

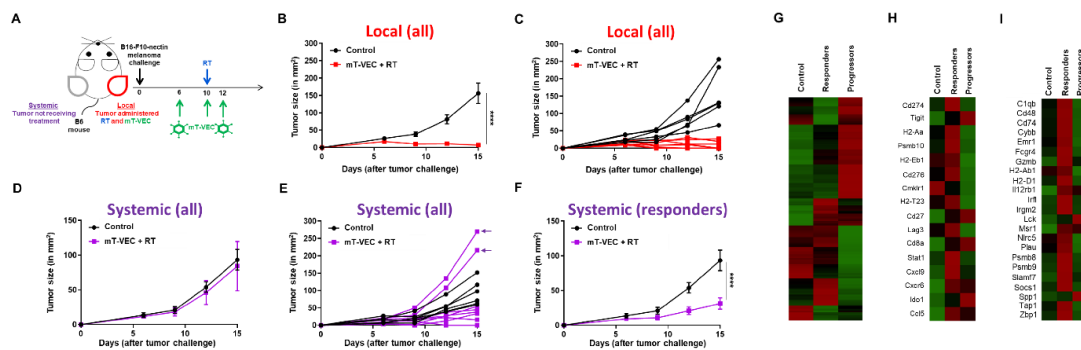
Supplementary Figure S1

Supplementary Figure S2

Supplementary Figure S3

Supplementary Figure S4

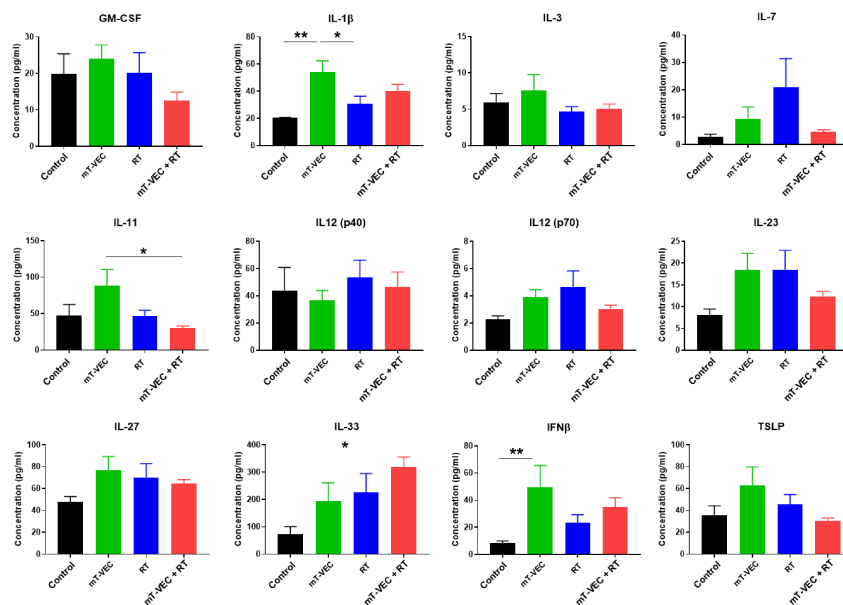
Figure S1. Combination Oncolytic Virus and Radiation Therapy Induces Systemic Anti-Tumor Immunity.



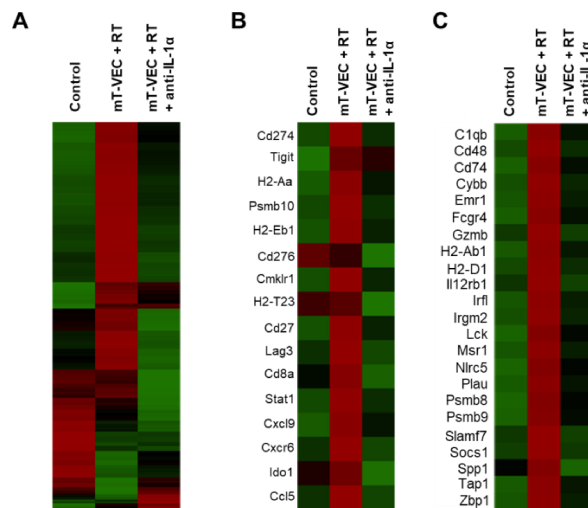
Supplementary Figure S1. Combination Oncolytic Virus and Radiation Therapy Induces Systemic Anti-Tumor Immunity.

(A) Experimental design (in vivo) for determining systemic immunity utilizing a dual-flank B16 melanoma model, with the right flank tumor being treated and the left flank tumor remaining untreated. (n = 6-8 mice/group). (B) Cumulative tumor growth curve of the treated right flank tumor (local) response for all mice treated. (C) Individual mouse tumor growth curves of mice from Panel B. (D) Cumulative tumor growth curve of the untreated left flank tumor (systemic) response for all mice treated. (E) Individual mouse tumor growth curves of mice from Panel D. Arrows highlight two of eight mice which are progressors (i.e., non-responders) to the OncoVEC^{mGMCSF} (mT-VEC) and RT treatment. (F) Cumulative tumor growth curve of the untreated left flank tumor (systemic) response for all mice that were treatment responders (i.e., with the two progressors excluded). (G) Nanostring gene panel heatmap, with the OncoVEC^{mGMCSF} (mT-VEC) and RT treatment group responders and progressors shown individually, demonstrating differential gene expression among responders and progressors. (H) Nanostring gene panel heatmap, with inflammatory genes whose high expression has previously been shown to correlate with improved patient outcomes, particularly expressed in the responder group. (I) Nanostring gene panel heatmap with a unique set of genes highly expressed in the responder group. All error bars shown are S.E.M. Statistical significance assessment was performed using two-way ANOVA with Bonferroni correction for

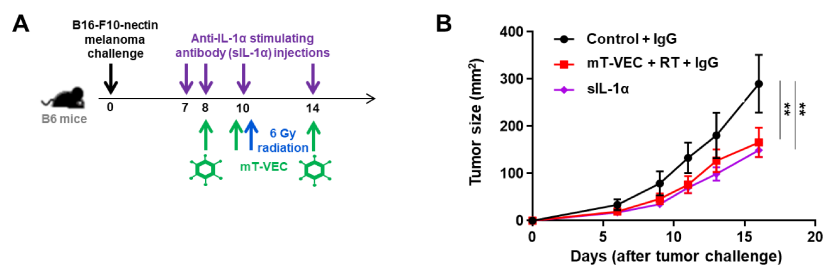
multiple comparisons (Panels B and C) and two-way ANOVA with Tukey correction for multiple comparisons (Panel E). ****, $P < 0.0001$.



Supplementary Figure S2. Cytokine Analysis with Combination Oncolytic Virus and Radiation Therapy. Series of 12 cytokines which do not show statistically significant expression in the OncoVEC^{mGMCSF} (mT-VEC) and RT group compared to the single treatments and control (PBS) treatment. (n = 5-7 mice/group). Error bars shown are S.E.M. Statistical significance assessment was performed using one-way ANOVA with Bonferroni's correction for multiple comparisons. *, P < 0.05; **, P < 0.01.



Supplementary Figure S3. IL-1 α Depletion Abrogates the Upregulated Gene Profile of Combination Oncolytic Virus and Radiation Therapy. (A) Nanostring gene panel heatmap, with the OncoVEC^{mGMCSF} (mT-VEC) and RT treatment group with or without IL-1 α depletion, demonstrating differential expression in the combination OncoVEC^{mGMCSF} (mT-VEC) and RT group (without IL-1 α depletion) compared to the OncoVEC^{mGMCSF} (mT-VEC) and RT with IL-1 α depletion group and similar gene expression in the IL-1 α depletion group compared to the control (PBS) group. (B) Nanostring gene panel heatmap, with inflammatory genes whose high expression has previously been shown to correlate with improved patient outcomes, particularly expressed in the combination OncoVEC^{mGMCSF} (mT-VEC) and RT group (without IL-1 α depletion). (C) Nanostring gene panel heatmap with a unique set of genes highly expressed in OncoVEC^{mGMCSF} (mT-VEC) and RT group (without IL-1 α depletion).



Supplementary Figure S4. Intratumoral Administration of stimulatory IL-1 α Reduces Tumor Growth. (A) Experimental design (in vivo) for determining the role of recombinant stimulatory IL-1 α (sIL-1 α) in altering tumor (B16-F10 melanoma) growth. (B) Tumor growth curve demonstrating the ability of intratumoral sIL-1 α to reduce tumor growth. (n = 5-7 mice/group). Error bars shown are S.E.M. Statistical significance assessment was performed using two-way ANOVA with Tukey correction for multiple comparisons. **, P < 0.01.