S. Figure 1. *In vitro* B16F10 internalization of VAX014 leads to rapid oncolysis. A) Flow cytometric analysis of β1 (blue) and α5 (red) integrin expression levels on B16F10 cells. Cells stained with secondary antibody only and isotype-matched antibody were used as negative controls. **B)** B16F10 cell internalization of CFSE-stained VAX-I rBMCs (PFO^-, Inv^+) after a 2-hour co-incubation. Untreated cells were used as a negative control. **C)** Potency of a titration of VAX014 or VAX-I rBMCs against B16F10 cells (MOI= ratio of rBMCs to plated mammalian cell). Viability was determined by Prestoblue™ viability assay (Thermo Fisher). Performed in triplicate. **D)** B16F10 LDH release as a measure of oncolytic activity after a 2-hour co-incubation with a titration of VAX014 and VAX-I rBMCs. LDH activity in supernatants was measured using TOX-7 kit (Sigma). Performed in triplicate. **E)** B16F10 propidium iodide uptake to assess plasma membrane permeabilization after a 2-hour co-incubation with VAX014 or VAX-I rBMCs. Heat treated cells were used as a positive control. **F)** Decrease in B16F10 mitochondrial potential using Mitotracker™ Red CMXRos (Thermo Fisher) following treatment with VAX014 for 5 hours. **G)** B16F10 nuclear condensation (pyknosis) after treatment with VAX014. Cell nuclei were stained with DAPI. Images captured using EVOS microscope system. Scale bar= 100 µm.

S. Figure 2. Ulceration occurs in i.d. B16F10 tumors shortly after cresting 4 mm in length. A) Intradermal B16F10 tumors prior to treatment initiation. Red arrows indicate location of i.d. tumors prior to ulceration. B) Ulcerated i.d. B16F10 tumors > 4 mm in length.

S. Figure 3. Individual tumor growth rates of single i.d. B16F10 tumors treated via i.t. route weekly with VAX014 or saline. A-B) Individual tumor growth and CR rates of B16F10 tumor bearing mice treated weekly via the i.t. route with **A)** saline (*n*= 35) or **B)** VAX014 (*n*= 38) compared to the mean tumor growth rate of saline treated control (black line). **C)** Individual tumor growth rates of B16F10 tumor-naïve mice as a control for tumor growth in B16F10 rechallenge experiments (*n*= 5). No additional treatment was given. Where present, error bars represent +/- SEM.

S. Figure 4. Weekly i.t. treatment of a single i.d. CT26 tumor with VAX014 causes $CD8^+$ T cell-dependent tumor clearance and protective immunologic memory. **A-B)** Individual tumor growth and CR rates of i.d. CT26 tumors after weekly i.t. treatment with **A)** saline (*n*= 5) or **B)** VAX014 (*n*= 20) compared to the mean tumor growth rate of saline treated control (black line). Treatments (30 µL) were initiated when tumors approached 5 mm in length. **C)** Mean tumor growth rates. **D)** Comparative survival curves. **E)** Survival curves of CT26 tumor-bearing mice treated weekly via the i.t. route with VAX014 ± depletion of $CD8^+$ T cells (*n*= 8/treatment group). **F)** Individual tumor growth rates of CT26 tumor-naïve mice as a control for tumor growth in CT26 rechallenge experiments (*n*= 5). **G)** Individual tumor growth and rejection (RJ) rates of i.d. CT26 tumors following rechallenge in mice that had previously achieved a CR in response to weekly i.t. treatment with VAX014 (*n*= 7). Tumor rechallenge was performed 25-42 days post CR and compared to the mean tumor growth rate of naïve control (black line). No additional treatment was given. **H)** Mean tumor growth rate of CT26 rechallenge tumors compared to the mean tumor growth rate of naïve control. **I)** Comparative survival curves of CT26 rechallenged mice and naïve controls. Student's *t*-test was used to determine significance

between tumor growth rates and Log-rank test was used to determine significance between survival curves. Where present, error bars represent +/- SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

S. Figure 5. Weekly i.t. treatment of a single i.d. MB49 tumor with VAX014 causes complete tumor clearance and protective tumor-specific immunologic memory. A-B) Individual tumor growth and CR rates of MB49 tumors treated weekly via the i.t. route with **A)** saline (n= 11) or **B)** VAX014 (n= 12) compared to the mean tumor growth rate of saline treated control (black line). Treatments (30 µL) were initiated when tumors approached 5 mm in length. **C)** Mean tumor growth rates. **D)** Comparative survival curves. **E)** Individual tumor growth rates of i.d. MB49 tumors in tumor-naïve control mice (n= 4). **F)** Individual tumor growth and RJ rates of B16F10 tumors (left flank) and MB49 rechallenged (RC) tumors (right flank) implanted in mice that previously achieved CR of a single MB49 tumor in response to weekly i.t. treatment with VAX014 (n= 5). B16F10 and MB49 tumor installation was performed 27-34 days post CR and compared to the mean tumor growth rate of naive control (black line). No additional treatment was given. **G)** Mean tumor growth rates of B16F10 and MB49 tumors following rechallenge compared to the mean tumor growth rate of naive control. Student's t-test was used to determine significance between mean tumor growth rates and Log-rank test was used to determine significance between survival curves. Where present, error bars represent +/- SEM. $^{****}p < 0.0001$.

S. Figure 6. Comparative MHC-I expression in B16F10 cells versus MB49 cells *in vitro*. Flow cytometric analysis of MHC-I expression levels in **A)** B16F10 cells and **B)** MB49 cells. Cells were enzymatically dissociated from flasks, centrifuged, and washed prior to staining with anti-MHC-I or isotype-matched antibodies. **C**) Addition of IFN-γ induced MHC-I expression in B16F10 cells. B16F10 cells were pre-incubated with 50 ng/mL mIFN-γ overnight prior to analysis.

S. Figure 7. Comparative MHC gene expression in immunotranscriptomes from saline treated i.d. MB49 tumors compared to i.d. B16F10 tumors. A) Complete hierarchical list of fold changes in individual gene transcripts involved in MHC expression in i.d. MB49 tumors versus i.d. B16F10 i.d. tumors analyzed 24 hours following i.t. treatment with saline (*n*= 2-3/group). Red arrows highlight MHC-I gene transcripts. Log2 FC = Log2 fold change.

S Figure 8. Comparative immunotranscriptomes of immune-excluded MB49 tumors versus immune desert B16F10 tumors. A-B) Comparative heat map of **A**) immunotranscriptomes from total RNA and **B**) TGF-β pathway genes, a hallmark of immune-excluded tumors, in i.d. B16F10 tumors (*n*=2) versus i.d. MB49 tumors (*n*=3) treated with saline. Immunotranscriptome analysis was performed 24 hours following i.t. treatment.

S. Figure 9. Peripheral monocytic MDSCs (Mo-MDSCs) increase as B16F10 tumor burden increases. A single subcutaneous B16F10 tumor was implanted in female C57BL/6 mice on Day 0 with an inoculum of $2x10^5$ cells in 50 µL volume. Tumors were allowed to grow until the

indicated size, at which time single cell PBMC suspensions from blood were stained with anti-CD11b and anti-Ly6C and analyzed by flow cytometry. Matching isotype antibodies were used to set gates. Black box highlights $CD11b^{+}LyC^{+}$ Mo-MDSCs. *n*= 3/group.

S. Figure 10. Peripheral polymorphonuclear MDSCs (PMN-MDSCs) increase as B16F10 tumor burden increases. A single subcutaneous B16F10 tumor was implanted in female C57BL/6 mice on Day 0 with an inoculum of $2x10^{5}$ cells in 50 µL volume. Tumors were allowed to grow until the indicated size, at which time single PBMC cell suspensions from blood were stained with anti-CD11b and anti-Ly6G and analyzed by flow cytometry. Matching isotype antibodies were used to set gates. Black box highlights $CD11b^{+}LyG^{+}$ PMN-MDSCs. *n*= 3/group.

S. Figure 11. Intratumoral treatment of a single i.d. B16F10 tumor with VAX014 leads to disparate treatment responses. A) Individual tumor growth rates of single i.d. B16F10 tumors following weekly i.t. treatment with VAX014 (*N*= 38) were categorized into treatment response groups defined as either CR (*n*= 22), Rs tumors (*n*= 13), or Rb tumors (*n*= 3) in comparison to the mean tumor growth rate of saline treated control (*n*= 35, black line). **B)** Individual tumor growth rates of B16F10 tumors treated with a single VAX014 i.t. dose (*N*= 21) grouped into CR (*n*= 6), Rs tumors (*n*= 10), or Rb tumors (*n*= 5) in comparison to the mean tumor growth rate of saline treated control (*n*= 10, black line).

S. Figure 12. Immune cell analyses of large and small i.d. B16F10 saline injected tumors and splenic CTL specificity for MHC-I restricted TRP-2/gp100 melanoma peptides

following weekly i.t. treatment of B16F10 tumors with VAX014. A) Percentage of $CD45^+$ leukocytes and lymphocytes in small (≤ 5 mm) versus large (≥ 7 mm) saline treated B16F10 tumors (*n*= 3/group) on Day 12-19 post tumor installation. **B)** TRP-2 specific MHC-I restricted dextramer staining of tumor draining lymph nodes (DLN), splenocytes, and tumor associated lymphocytes from B16F10 tumor-bearing mice treated weekly via the i.t. route with VAX014 (*n*= 4) versus saline treated control (*n*= 2). **C)** Mean splenic CTL activity from B16F10 tumor-bearing mice following weekly i.t. treatment with VAX014 (*n*= 4). CTL assays were performed at E:T ratios of 100:1, 50:1 and 10:1 against EL4 thymoma cells pulsed with either TRP-2 or gp100 peptides. Statistical significance of dextramer staining and mean CTL activity was analyzed using Student's *t* test. Where present, error bars represent ± SEM. $**p<0.01$, $*p<0.05$.

S. Figure 13. Weekly i.t. treatment of i.d. MB49 tumors with VAX014 causes immune-dependent tumor clearance in injected and distal noninjected MB49 tumors. A-B) Individual tumor growth and CR rates of both injected and distal noninjected i.d. MB49 tumors in the bilateral MB49 tumor model from **A)** saline treated control mice (*n*= 13) or **B)** VAX014 treated mice (*n*= 21) compared to the mean tumor growth rate of saline treated control (black line). **C)** Mean tumor growth rates of injected and distal noninjected i.d. MB49 tumors following weekly i.t. treatment with VAX014 versus saline treated control. **D)** Comparative survival curves of bilateral i.d. MB49 tumor bearing mice treated weekly via the i.t. route with VAX014 versus saline treated control. **E)** Comparative survival curves of bilateral i.d MB49 tumor bearing mice treated weekly with VAX014 ± depletion of $CD8^+$ T cells or $CD4^+$ T cells (*n*= 9-13/group). Student's *t*-test was used to determine significance between mean tumor growth rates and Log-

rank test was used to determine significance between survival curves. Where present, error bars represent +/- SEM. ****$p< 0.0001$, ***$p< 0.001$, **$p\leq 0.01$.

S. Figure 14. Tumor response following VAX014 i.t. treatment is specific for the injected tumor type (MB49) in mice bearing a MB49 tumor and a second contralateral noninjected B16F10 tumor. A-B) Individual tumor growth and CR rates of injected i.d. MB49 (right flank) and distal noninjected i.d. B16F10 tumors (left flank) from **A)** saline treated control mice ($n= 4$) or **B)** VAX014 treated mice ($n= 6$) compared to the mean tumor growth rate of saline treated control (black line). **C)** Individual B16F10 tumor growth rates of tumor-naïve mice ($n= 5$) as a control for B16F10 tumor growth in the bilateral model compared to the mean tumor growth rate of saline treated B16F10 control mice (black line). **D)** Mean tumor growth rates of injected i.d. MB49 and distal noninjected i.d. B16F10 tumors from weekly VAX014 treated mice compared to saline treated and B16F10 tumor-naïve control. **E)** Mean splenic CTL response against MB49 target cells versus B16F10 target cells following weekly i.t. treatment of MB49 tumors in the bilateral MB49/B16F10 model ($n= 6$). CTL assays were performed at E:T ratios of 50:1, 25:1, and 10:1 against MB49 (solid line) or non-specific B16F10 target cells (dotted line). Student's *t*-test was used to determine significance between mean tumor growth rates and CTL activity. Where present, error bars represent +/- SEM. ****$p< 0.0001$, **$p\leq 0.01$, *$p\leq 0.05$.

S. Figure 15. Tumor response following VAX014 i.t. treatment is specific for the injected tumor type (B16F10) in mice bearing a B16F10 tumor and a second contralateral noninjected MB49 tumor. A-B) Individual tumor growth and CR rates of both injected i.d.

B16F10 (right flank) and distal noninjected i.d. MB49 tumors (left flank) from **A)** saline treated control mice (*n*= 5) or **B)** VAX014 treated mice (*n*= 5) compared to the mean tumor growth rate of saline treated control (black line). **C)** Mean tumor growth rates of injected i.d. B16F10 and distal noninjected i.d. MB49 tumors from weekly VAX014 treated mice compared to saline treated control. **D)** Comparative survival curves of bilateral i.d. B16F10/MB49 tumor bearing mice treated weekly via the i.t. route with VAX014 versus saline treated control in the B16F10 tumor. Student's *t*-test was used to determine significance between mean tumor growth rates and Log-rank test was used to determine significance between survival curves. Where present, error bars represent +/- SEM. $**p \leq 0.01$.

S. Figure 16. Individual tumor growth rates for immunotranscriptome analysis in the bilateral i.d. B16F10 tumor model. Individual tumor growth rates of injected and distal noninjected B16F10 tumors from the bilateral i.d. B16F10 model following weekly i.t. treatment with VAX014 monotherapy, systemic αCTLA-4 monotherapy, VAX014/αCTLA-4 combination, or tripartite VAX014/αCTLA-4/αPD-1 combination in comparison to the mean tumor growth rate of saline treated control (black line) prior to immunotranscriptome analysis on Day 13-14. Student's *t*-test was used to determine significance between tumor growth rates. Error bars represent +/- SEM.

S. Figure 17. Changes in T cell function gene expression in injected tumors treated with saline, VAX014 monotherapy, or VAX014/αCTLA-4 combination. A) Complete hierarchical list of fold change in individual gene transcripts involved in T cell function in injected tumors

following i.t. treatment with VAX014 as monotherapy versus saline treated control (*n*= 3/treatment group). **B)** Hierarchical list of fold change in individual gene transcripts in injected tumors following weekly i.t. treatment with VAX014/αCTLA-4 combination versus VAX014 as monotherapy (*n*= 3/treatment group). Log2 FC= Log2 fold change.

S. Figure 18. Individual tumor growth rates for TIL analysis of distal noninjected tumors in the bilateral i.d. B16F10 tumor model. Individual tumor growth rates of injected and distal noninjected B16F10 tumors from the bilateral i.d. B16F10 model following weekly i.t. treatment with VAX014 monotherapy, VAX014/αCTLA-4 combination, or tripartite VAX014/αCTLA-4/αPD-1 combination in comparison to the mean tumor growth rate of saline treated control (black line) prior to immune cell analysis (*n*= 5-6/treatment group) on Day 13-14. Student's *t*-test was used to determine significance between tumor growth rates. Error bars represent +/- SEM.

S Figure 19. Additional TIL analysis of distal noninjected B16F10 tumors treated with VAX014 ICB combination therapy. A) Percentage of cytotoxic effector $CD8^+$ T cells ($CD45^+CD3^+CD8^+GzmB^+$) among total lymphocytes. **B)** Percentage of $CD8^+$ central memory T_{CM} cells ($CD45^+CD3^+CD8^+CD44^+CD62L^+$) among total lymphocytes. **C)** Percentage of T regulatory (Tregs) cells ($CD45^+CD3^+CD4^+FoxP3^+$) among total lymphocytes. **D)** Percentage of T conventional (Tconv) cells ($CD45^+CD3^+CD4^+FoxP3^-$) among total lymphocytes. **E)** Percentage of Tregs ($CD45^+CD3^+CD4^+FoxP3^+$) among total $CD4^+$ lymphocytes. **F)** Percentage of Tconv cells ($CD45^+CD3^+CD4^+FoxP3^-$) among total $CD4^+$ lymphocytes. **G)** Ratio of Tconv cells to Tregs among total $CD4^+$ lymphocytes. **H)** Ratio of $CD8^+$ T cells to Tregs among total

lymphocytes. Median values from each treatment group are plotted (n= 5-6/treatment group). Mann-Whitney U-test was used to determine significance between treatment groups. **$p \leq 0.01$, *$p \leq 0.05$.