SUPPLEMENTARY FIGURE LEGENDS:

Supplementary Figure 1. Increased peripheral blood CD8$^+$ T cell responses in IT MVA-TAA-4-1BBL immunized tumor-bearing mice. Related to *Figure 1*. **(A)** Representative dot plots and frequency of peripheral blood CD44$^+$ $OVA_{257-264}$ Dex$^+$ CD8$^+$ T cells 3 days after last IT PBS, MVA-OVA or MVA-OVA-4-1BBL immunization of B16.OVA tumor-bearing mice (n= 5 mice/group). **(B)** Representative dot plots and frequency of $p15E_{604-611}$ peptide restimulated peripheral blood CD44$^+$ IFNγ^+ CD8$^+$ T cells 3 days after last IT PBS, MVA-Gp70 or MVA-Gp70-4-1BBL immunization of B16.F10 tumor-bearing mice (n= 5 mice/group). **(C)** Representative dot plots and frequency of $AH1_{6-14}$ peptide restimulated peripheral blood CD44$^+$ IFNγ^+ CD8$^+$ T cells 3 days after last IT PBS, MVA-Gp70 or MVA-Gp70-4-1BBL immunization of CT26.WT tumor-bearing mice (n= 5 mice/group). (**D**) Representative picture of vitiligo development in IT MVA-TAA-4-1BBL cured C57BL/6 mice. (**E**) Pie charts displaying vitiligo incidence of C57BL/6 mice cured from melanoma after IT MVA-TAA (data combined from MVA-OVA and MVA-Gp70) and MVA-TAA-4-1BBL (data combined from MVA-OVA-4-1BBL and MVA-Gp70-4-1BBL combined) treatment, respectively. (**A-E**) Data are representative of at least two independent experiments. (**A-C**) Data expressed as Mean ± SEM. One-way ANOVA was performed. ***$p < 0.005$, ****$p < 0.001$. ANOVA, analysis of variance; IT, intratumoral; MVA, modified vaccinia Ankara; OVA, ovalbumin; PBS, phosphate buffered saline; SC, subcutaneous; SEM, SE of the mean; WT, wild type.

Supplementary Figure 2. Direct CD8$^+$ T cell activation by MVA-OVA-4-1BBL infected B16.F10 melanoma cells. Related to *Figure 2*. B16.F10 cells were infected with MVA-OVA or MVA-OVA-4-1BBL at MOI 10 for 18 hours. Infected tumor cells were harvested and cocultured with OT-I transgenic CD8$^+$ T cells at a ratio of 1:5 for 48 hours. After 48 hours, OT-I CD8$^+$ T cells were analyzed by flow cytometry and culture supernatants were collected for cytokine concentration analysis by Luminex. **(A)** Representative dot plots and frequency of Granzyme B$^+$ or IFNγ^+ OT-I$^+$ CD44$^+$ CD8 T cells

48 hours after co-culture with B16.F10 cells infected with MVA-OVA or MVA-OVA-4-1BBL. **(B)** Frequency of Granzyme B^+ or $IFN\gamma^+$ $OT\text{-}I^+$ $CD44^+$ CD8 T cells of living cells 48 hours after co-culture with B16.F10 cells infected with MVA-OVA or MVA-OVA-4-1BBL. **(C)** IFNγ, TNFα and GM-CSF in culture supernatants (pg/ml) 48 hours after co-culture with B16.F10 cells infected with MVA-OVA or MVA-OVA-4-1BBL. Data are representative of two independent experiments. **(B,C)** Data are represented as Mean ± SEM. One-way ANOVA was performed. *p < 0.05, **p < 0.005, ***p < 0.0005, ****p < 0.0001. ANOVA, analysis of variance; MVA, modified vaccinia Ankara; MOI, Multiplicity Of Infection; OVA, ovalbumin; SEM, SE of the mean.

Supplementary Figure 3. Loss of exhaustion markers and reduction of T_{reg} after IT MVA-OVA-4-1BBL. Related to *Figure 2*. C57BL/6 mice received $5x10^5$ B16.OVA cells subcutaneously in the flank. Ten days later when tumor volumes were around 80 mm^3, mice were grouped and IT injected with either PBS, $2x10^8$ $TCID_{50}$ MVA-OVA or MVA-OVA-4-1BBL. One, three and seven days after immunization, mice were sacrificed for further analysis (n= 5-11 mice/group). **(A)** Number of $CD4^+$ T cells per mg tumor; **(B)** Number of $CD4^+$ T cells per TdLN; **(C)** GMFI of PD-1 in $CD44^+$ $OVA_{257-264}$ Dex^+ $CD8^+$ T cells in the tumor on day 7; **(D)** GMFI of Lag3 in $CD44^+$ $OVA_{257-264}$ Dex^+ $CD8^+$ T cells in the tumor on day 7; **(E)** Percentage of T_{reg} ($CD4^+Foxp3^+$) of $CD4^+$ T cells in the tumor on day 7; **(E)** Ratio of $CD44^+$ $OVA_{257-264}$ Dex^+ $CD8^+$ T cells to T_{reg} in the tumor on day 7. Data are representative of two independent experiments. Data in A-F expressed as Mean ± SEM. A-B Two-way ANOVA comparing cell numbers in analyzed organs upon treatment. **, p < 0.005; ***, p < 0.0005; n.s. non-significant. **C-F** One-way ANOVA was performed. **, p < 0.005; ***, p < 0.0005; ****, p < 0.0001. ANOVA, analysis of variance; GMFI, Geometric Mean Fluorescence Intensity; IT, intratumoral; MVA, modified vaccinia Ankara; n.s., non-significant; OVA, ovalbumin; PBS, phosphate buffered saline; SC, subcutaneous; SEM, SE of the mean; T_{reg}, regulatory T cells.

Supplementary Figure 4. $CD4^+$ T cell and NK cell depletion in IT MVA-Gp70-4-1BBL treated mice.

Related to *Figure 2*. **(A,B)** $CD4^+$ T cell depletion. When B16.F10 tumor volumes were above 60 mm^3, mice received PBS or were immunized IT with $5x10^7$ $TCID_{50}$ of MVA-Gp70-4-1BBL. IT immunization was repeated on day 5 and 8 after the first immunization (dotted lines). Mice received 200 µg of either IgG2b or anti-CD4 antibody IP at day -1, 3, 6 and 10 after immunization; **(A)** Tumor size follow-up (n= 8 mice/group) and **(B)** overall survival (n= 8 mice/group). **(C,D)** NK cell depletion. When B16.F10 tumor volumes were above 60 mm^3, mice received PBS or were immunized IT with $5x10^7$ $TCID_{50}$ of MVA-Gp70-4-1BBL. IT immunization was repeated on day 5 and 8 after the first immunization (dotted lines). Mice received 200 µg of either IgG2a or anti-NK1.1 IP at day -1, 3, 6 and 10 after immunization; **(C)** Tumor size follow-up (n= 5-8 mice/group) and **(D)** overall survival (n= 5-8 mice/group). Log-rank test on mouse survival was performed for Figures B and D. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. IP, intraperitoneally; IT, intratumoral; MVA, modified vaccinia Ankara; PBS, phosphate buffered saline; SC, subcutaneous.

Supplementary Figure 5. MVA localization upon IT MVA injection and liver $CD8^+$ T cell infiltration upon IT MVA-Gp70-4-1BBL

Related to *Figure 3*. **(A)** Seven days after SC B16.F10 tumor inoculation, mice were grouped (n=5 mice/group) and administered IT either with saline or with $1x10^8$ $TCID_{50}$ MVA-OVA-huFlt3L. 6 hours after IT injection tumor, TdLN and NdLN were homogenized, digested and cultured for 16 hours. Graph shows ELISA detection of human Flt3L production in supernatants after 16h culture. **(B-F)** Assessment of liver damage. **(B-F)** Briefly, when B16.F10 tumor volumes were above 60 mm^3, mice were injected IT with $2x10^8$ $TCID_{50}$ of MVA-Gp70-4-1BBL on days 0, 5 and 8. As positive control naive C57BL/6 mice received 500 µg of anti-4-1BB antibody IV twice per week. Mice were sacrificed 20 days after treatment start. Naïve, non-treated C57BL/6 mice were included as negative controls. Livers were analyzed. **(B)** Liver weight in mg. **(C)** Total cell number and **(D)** number of $CD8^+$ T cells per

liver is shown. **(E)** Percentage of Granzyme B^+ and **(F)** $Ki67^+$ cells gated on $CD8^+$ T cells is shown. Data in A -F expressed as Mean ± SEM. **(A)** Two-way ANOVA was performed. *, $p < 0.05$; ***, $p < 0.005$; ****, $p < 0.0001$. **(B-F)** One-way ANOVA was performed. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0005$, ****, $p < 0.0001$. ANOVA, analysis of variance; IT, intratumoral; IV, intravenously; MVA, modified vaccinia Ankara; PBS, phosphate buffered saline; SC, subcutaneous; SEM, SE of the mean.

Supplementary Figure 6. MVA-induced inflammation and immunogenic cell death. Related to *Figure 3*. **(A)** C57BL/6 mice received $5x10^5$ B16.OVA cells. When tumor volumes reached 60 mm^3, mice were grouped (n=3 mice/group) and administered IT either with PBS or with $2x10^8$ $TCID_{50}$ of the indicated MVA constructs. 6 hours after IT injection tumors were extracted and tumor lysates processed. Concentration (pg/ml) of indicated cytokines/chemokines in tumor lysates are shown. **(B)** B16.OVA and CT26.WT tumor cell lines as well as bone marrow derived macrophages (BMDM) were infected with the indicated viruses at a MOI of 10 for 20 hours. Then, cells were analyzed for their viability by flow cytometry. Percentage of Dead cells (Zombie Aqua™) is shown. **(C)** B16.OVA and CT26.WT tumor cell lines as well as BMDM were infected with the indicated viruses at a MOI of 10 for 20 hours. HMGB1 release was determined by ELISA in supernatants 20 hours after infection. Data are representative of two independent experiments. Data in **A-C** expressed as Mean ± SEM. **A-C** One-way ANOVA was performed. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; n.s., non-significant. ANOVA, analysis of variance; BMDM; Bone Marrow Derived Macrophages; HMGB1, High Mobility Group Box factor 1; IT, intratumoral; MOI, Multiplicity Of Infection; MVA, modified vaccinia Ankara; n.s., non-significant; OVA, ovalbumin; PBS, phosphate buffered saline; SC, subcutaneous; SEM, SE of the mean; WT, wild type.

Supplementary Figure 7. Intratumoral MVA-Gp70-4-1BBL immunotherapy confers protection from local tumor re-challenge. Related to *Figure 5*. Naïve C57BL/6 mice or long-term survivors of Figures

1C and 1D were rechallenged SC into the tumor-naïve flank of cured mice with $5x10^5$ B16.F10 cells. Peripheral blood was analyzed by flow cytometry before (day -6) and after (day 7) after rechallenge. Blood, spleen, NdLN and TdLN were analyzed on day 42 after tumor cell inoculation. **(A)** Percentage of tumor-free mice over time is displayed (n=5-11 mice/group). Number of tumor-free mice per group is shown. **(B)** Frequency of peripheral blood $CD44^+$ $p15E_{604-611}$ $Pent^+$ $CD8^+$ T cells pre and post B16.F10 cell rechallenge. **(C)** Frequency of $CD62L^-$ $CD127^+$ $p15E_{604-611}$ $Pent^+$ $CD8^+$ T cells (T_{EM}) in blood, spleen, NdLN and TdLN. **(D)** Frequency of $CD62L^+$ $CD127^+$ $p15E_{604-611}$ $Pent^+$ $CD8^+$ T cells (T_{CM}) in blood, spleen, NdLN and TdLN. **(E)** Frequency of $CD62L^-$ $CD127^+$ $CD69^+$ $p15E_{604-611}$ $Pent^+$ $CD8^+$ T cells (T_{RM}) in blood, spleen, NdLN and TdLN. **(A-E)** n=5-11 mice/group. **(B-E)** Data are expressed as Mean ± SEM. **(B)** Two-way ANOVA was performed **, $p < 0.005$; ns, non-significant. **(C-E)** One-way ANOVA was performed **, $p < 0.005$; ***, $p < 0.0005$. ANOVA, analysis of variance; IT, intratumoral; MVA, modified vaccinia Ankara; NdLN, non-draining lymph node; n.s., non-significant; SC, subcutaneous; SEM, SE of the mean; TdLN, tumor-draining lymph node.