Supplementary Figure 1. Efficacy of MVA-mIL12 in CT26 peritoneal metastatic model. (A) C57BL/6 mice were challenged SC with MC38 cells. After 7 days, mice with established tumors were randomized and treated IT 3 times with MVA empty at 10^7 IFU alone or in combination with αPD1, with MVA empty at $6x10^5$ IFU alone or in combination with αPD1 or leave untreated. Tumor volume was monitored over time. Percentages on the graphs indicate the response rate (n ≥ 6) (B) C57BL/6 mice were challenged SC with MC38 cells. After 7 days, mice with established tumors were randomized and treated IT one time or two times with MVA-mIL12 $6x10^5$ IFU in combination with αPD1. Tumor volume was monitored over time. Lines in the graphs represent each individual tumor (full lines, responder tumors; dot lines, non-responder tumors). Percentages on the graphs indicate the response rate (n = 10). (C) BALB/c mice were injected in the peritoneum with CT26 cells. After 3 days, mice were treated IP with MVA-mIL12 at 10^7 IFU, with MVA-mIL12 at $6x10^5$ IFU alone or in combination with αPD1, with MVA empty 10^7 IFU or αPD1 as control. Mice survival was monitored over time (n = 8). (D) Mice that survived in Figure S1B were re-challenged IP with CT26 cells and survival was monitored over time (n ≥ 6). Comparison of survival curves with Gehan-Breslow-Wilcoxon test. ****, $P < 0.0001$.

Supplementary Figure 2. Mechanism of action of MVA-mIL12 in CT26 peritoneal metastatic model. (A-D) BALB/c mice were injected in the peritoneum with CT26 cells. After 3 days, mice were treated IP with MVA-mIL12 at 10^7 IFU or with MVA empty at day 0, 2 and 4. At different time points as in the scheme same animals of all the groups were sacrificed and PEC were collected and analyzed by Flow Cytometry (n ≥ 3). Graph bars represent the percentage of (A) F480+ CD11b+ Macrophages, (B) MHCII+ CD206- M1 Macrophages and CD206+ MHCII- M2 Macrophages at day 14, (C) CD11c+ Dendritic Cells, CD103+ XCR1+ cDC1 (D) CD8+ and CD4+ lymphocytes. Data are presented as median values + SEM; n ≥ 3 mice. (E) BALB/c mice were injected in the peritoneum with CT26 cells. After 3 days, mice were treated IP with MVA-mIL12 10^7 IFU alone or in combination with αCD4 or αCD8 depletion antibody. (F) TREG Flow Cytometry analysis at day 3 of the experiment in (A-D). Graph bars represent the percentage of FOXP3+ CD4+ Lymphocytes. (G) C57BL/6 mice were challenged SC with MC38 cells. After 7 days, mice with established tumors were randomized and treated intra-muscular (IM) as in the scheme with MVA-mIL12 (lower dose) $6x10^5$ IFU in combination with αPD1. Tumor volume was monitored over time. Lines in the graph represent each individual tumor. Percentages on the graphs indicate the response rate. One-way ANOVA test with Fisher's LSD test was performed to obtain P value. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$; ****, $P < 0.0001$.

Supplementary Figure 3. RT-PCR validation of gene expression signature. (A) C57BL/6 mice were challenged SC with MC38 cells. After 7 days, mice with established tumors were randomized and treated (as in figure 5) with the combination of MVA-IL12 ($6x10^5$ IFU) with αPD1, with MVA-IL12 ($6x10^5$ IFU) alone, with αPD1 or with MVA empty ($6x10^5$ IFU). Tumors from all the groups were collected and analyzed by RT-PCR. One-way ANOVA test with Fisher's LSD test was performed to obtain P value. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.