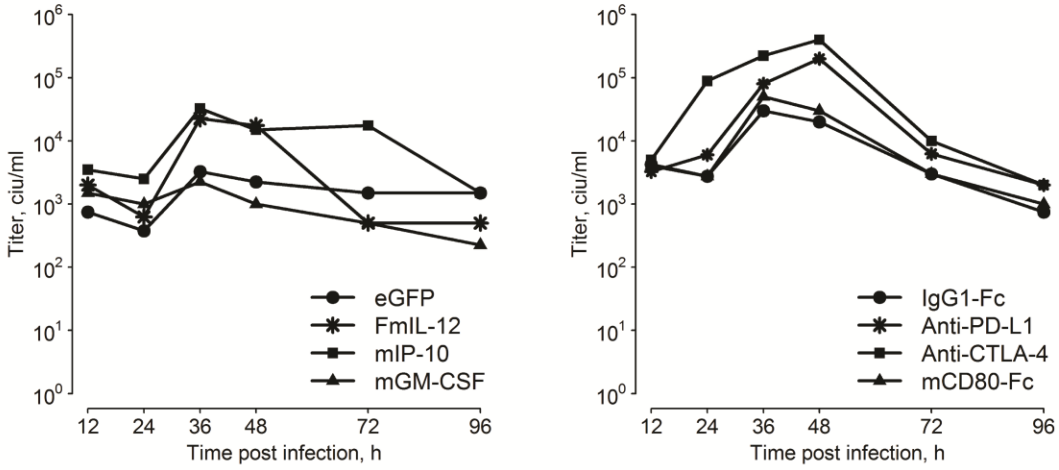
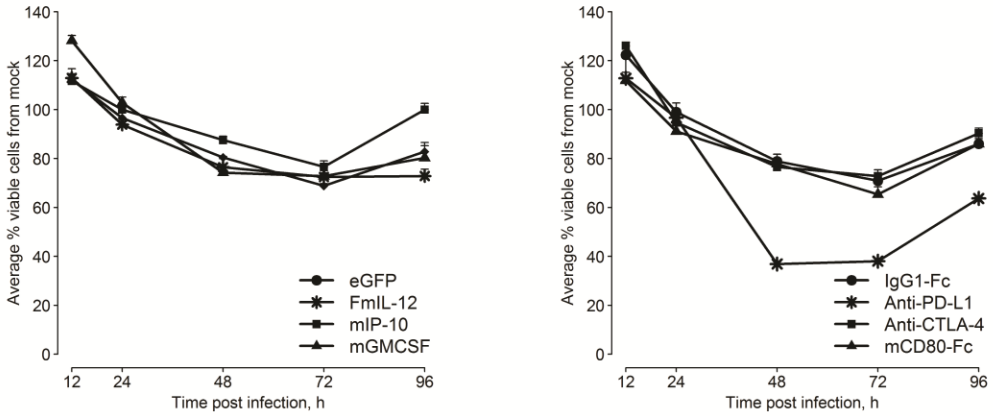
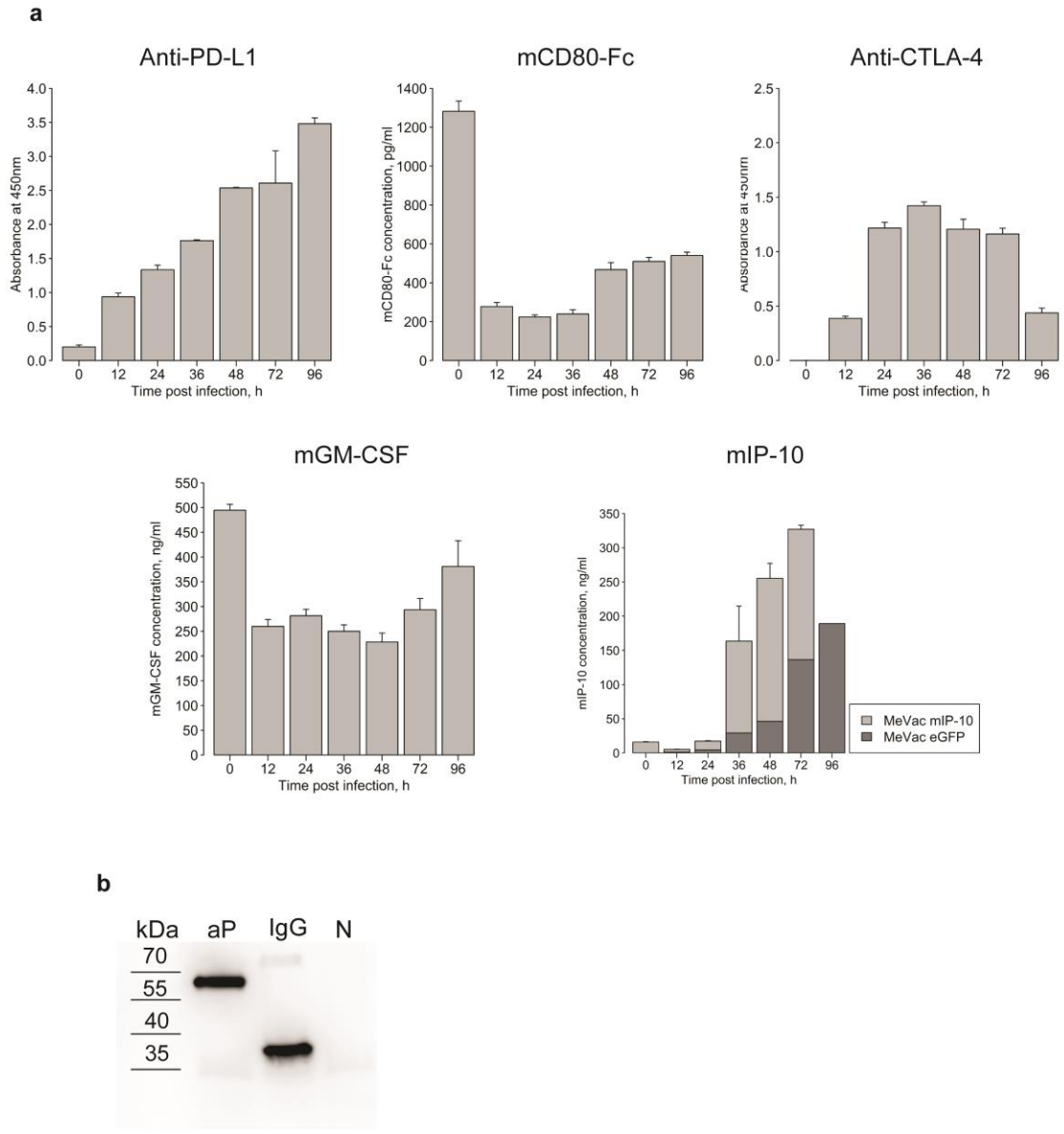


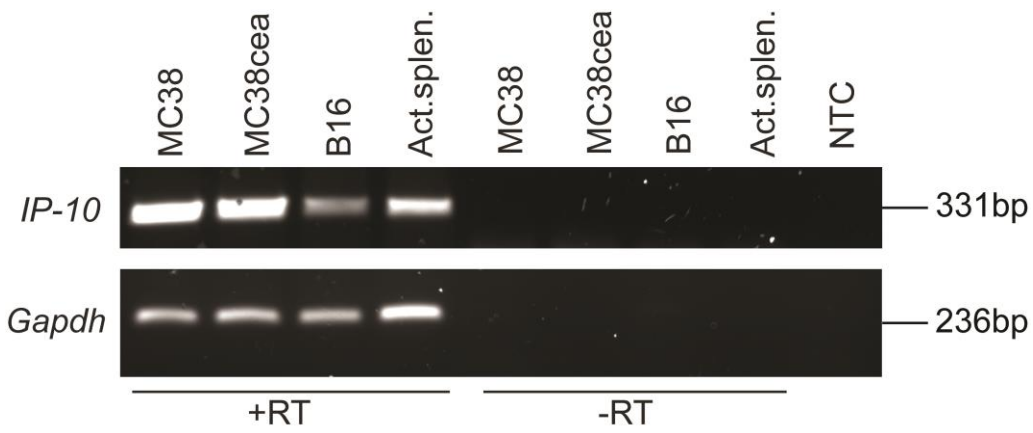

**Supplementary Figure S1**. Replication kinetics in the murine MC38cea cell line. One-step growth curves were constructed by transduction of MC38cea cells with MeVac vectors encoding the respective transgenes at a multiplicity of infection = 3. Cells were scraped in their medium at the depicted time points and titers were assessed by serial dilution titrations and expressed as cell infectious units (ciu) per ml.



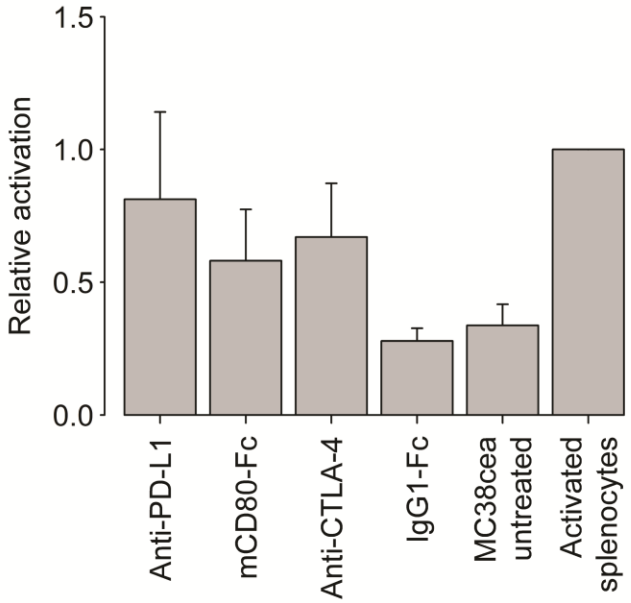

**Supplementary Figure S2**. Cytotoxic effects in the murine MC38cea cell line. MC38cea cells were infected at multiplicity of infection = 5 with MeVac encoding the respective transgenes. An XTT cell viability assay was carried out at the depicted time points. Results are presented as percentage of viable cells compared to mock (= 100%). Mean values from triplicate infections with standard errors of the mean are shown.



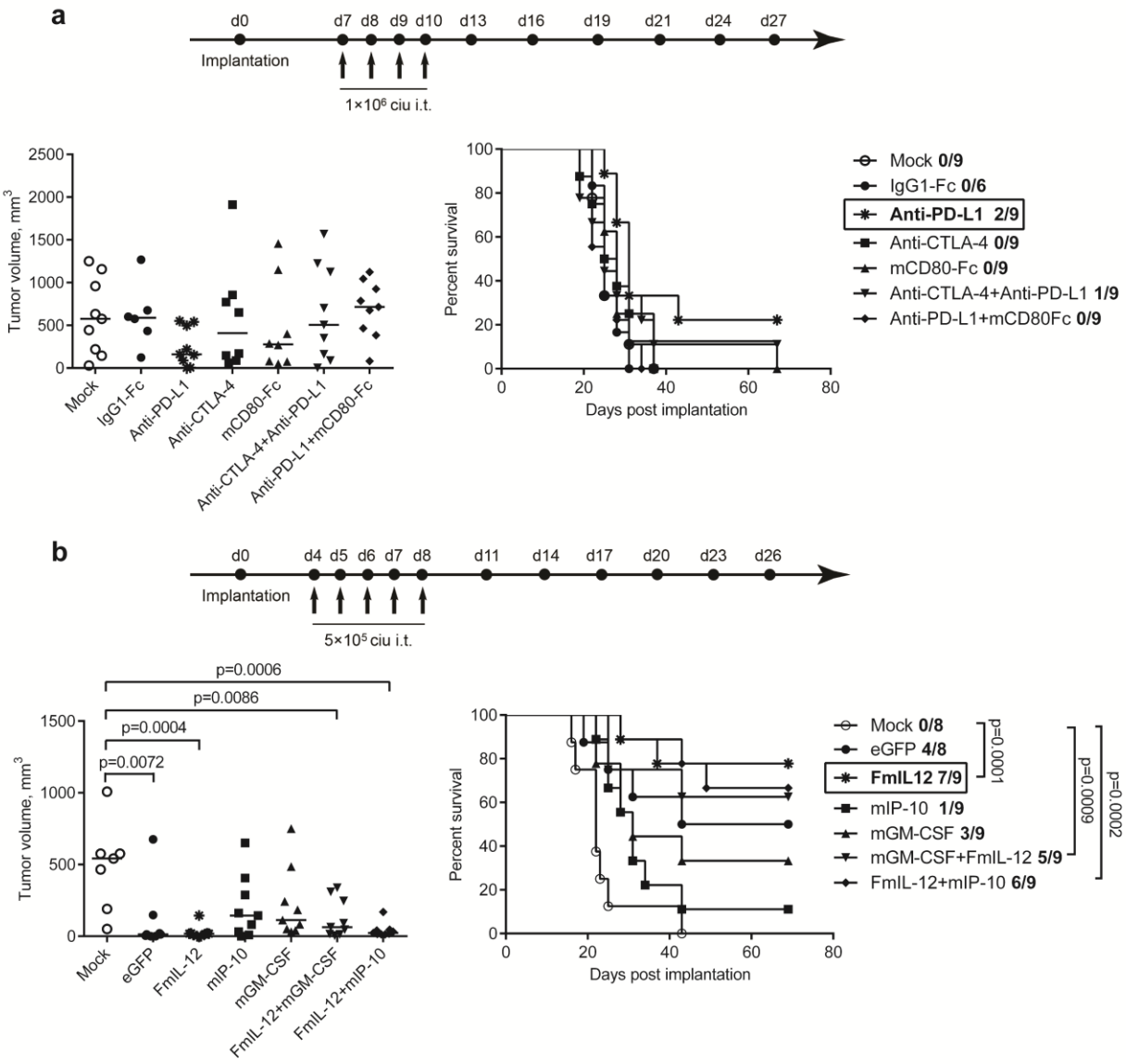

**Supplementary Figure S3.** Expression kinetics of MeVac-encoded immunomodulators. (**a**) Murine MC38cea cells were transduced with MeVac vectors encoding the respective immunomodulators and with MeVac eGFP or MeVac IgG1-Fc as controls at a multiplicity of infection = 3. Supernatants were collected from the infected cells at the depicted time points and the concentrations of the respective immunomodulators were assessed by ELISA. Supernatants from cells infected with MeVac IgG1-Fc (anti-CTLA-4, mCD80-Fc, anti-PD-L1) or MeVac eGFP (mGM-CSF, mIP-10) served as background controls. In case of mIP-10 a specific signal was observed also in the MeVac eGFP control (depicted in dark gray). Mean values from triplicate infections per time point with standard errors of the mean are shown. (**b**) Expression of anti-PD-L1 and IgG1-Fc. Supernatants were collected from Vero-αHis cells infected with MeVac encoding anti-PD-L1 (aP) or IgG1-Fc (IgG) and western blot analysis was carried out. DMEM+10% FCS was used as a negative control (N).



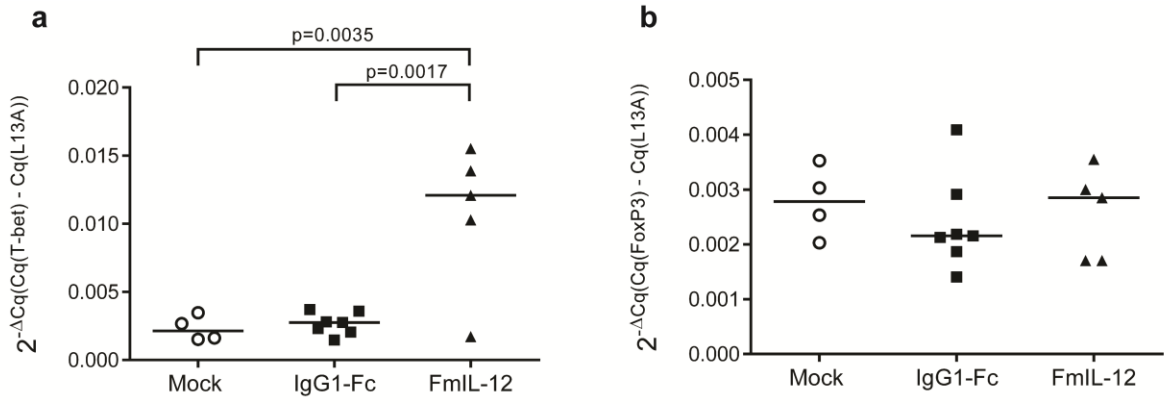

**Supplementary Figure S4**. Expression of mIP-10 in MC38cea cells. RT-PCR for murine IP-10 (*Cxcl10*) and *Gapdh* as a loading control was carried out using cDNA from the indicated cell lines and splenocytes stimulated with PMA and ionomycin (act.splen.) from a C57BL/6J mouse. +RT – cDNA samples; -RT – controls without reverse transcriptase; NTC – no template control.




**Supplementary Figure S5**. Counteraction of MC38cea-mediated immunosuppression with MeVac-encoded immunomodulators. MC38cea cells were treated with supernatants from Vero-αHis cells infected with MeVac encoding anti-PD-L1, mCD80-Fc, anti-CTLA-4 or IgG1-Fc and cocultured at a ratio of 2:1 with murine splenocytes in presence of PMA and ionomycin. After 24 h supernatants were collected and IFN-γ concentrations were measured by ELISA. Relative activation corresponds to the ratio of the optical density (absorbance at 450 nm minus 570 nm) of the respective samples to activated splenocytes. Average relative activation values with standard errors of the mean from three independent experiments are shown.




**Supplementary Figure S6**. Comparison of therapeutic efficacy *in vivo*. MC38cea cells were implanted subcutaneously (s.c.) into the right flank of C57BL/6J mice (6-9 animals per group). When tumors reached an average volume of 40-70 $mm^3$ mice received intratumoral (i.t.) injections (black arrows) with MeVac vectors encoding the respective transgenes on four consecutive days in 100 µl. Mice in the mock group received i.t. injections of 100 µl OptiMEM. Tumor volume distribution on day 19 (**a**) and day 16 (**b**) post implantation with dots representing individual mice and Kaplan-Meier survival analysis are shown. Complete tumor remission rates are shown for each group in Kaplan-Meier plots. ciu – cell infectious units.




**Supplementary Figure S7**. Changes in *T-bet* and *Foxp3* mRNA levels after MeVac therapy. MC38cea cells were implanted subcutaneously into C57BL/6J mice. When tumors reached an average volume of 120 $mm^3$ mice received intratumoral treatment with MeVac encoding the respective transgenes or the respective amount of OptiMEM (mock). Reverse transcription quantitative PCR analysis was carried out to assess *T-bet* (a) and *Foxp3* (b) mRNA levels using RNA from tumors explanted one day after the last treatment. *L13A* was used as a reference gene and results were expressed using the $2^{-\Delta Cq}$ method. Dots representing samples from individual mice and median values are shown. Cq – quantification cycle.