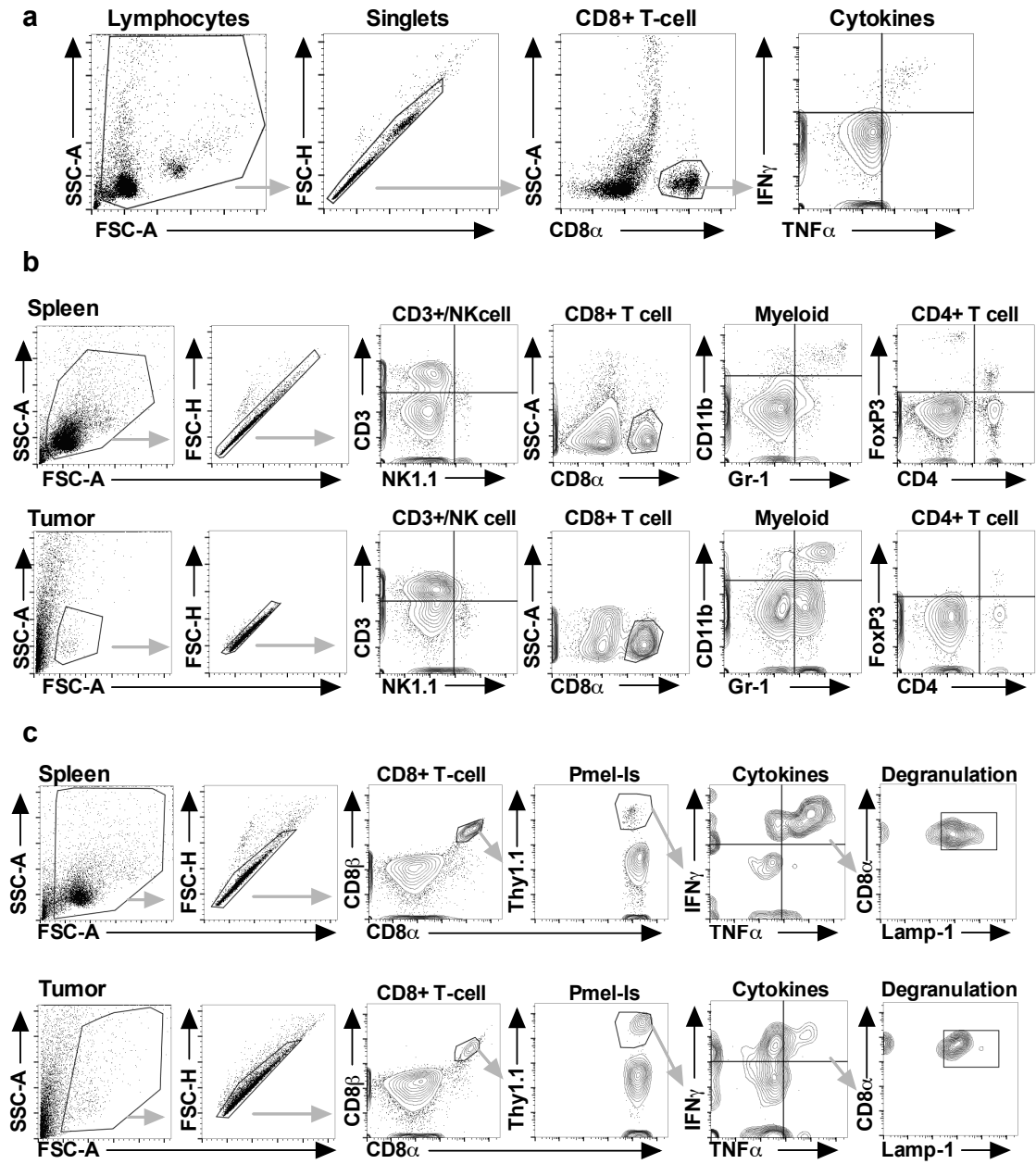
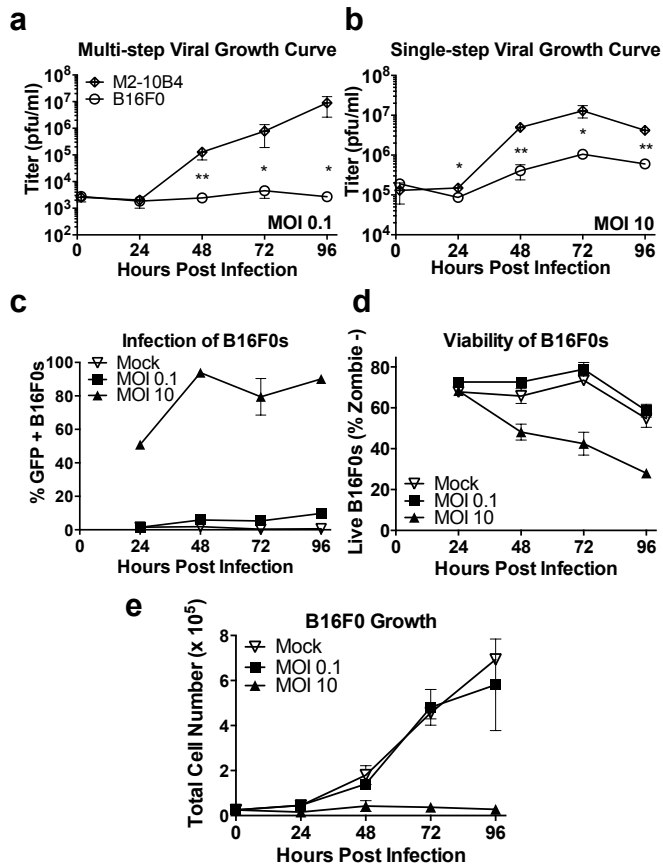


Supplemental Figures:



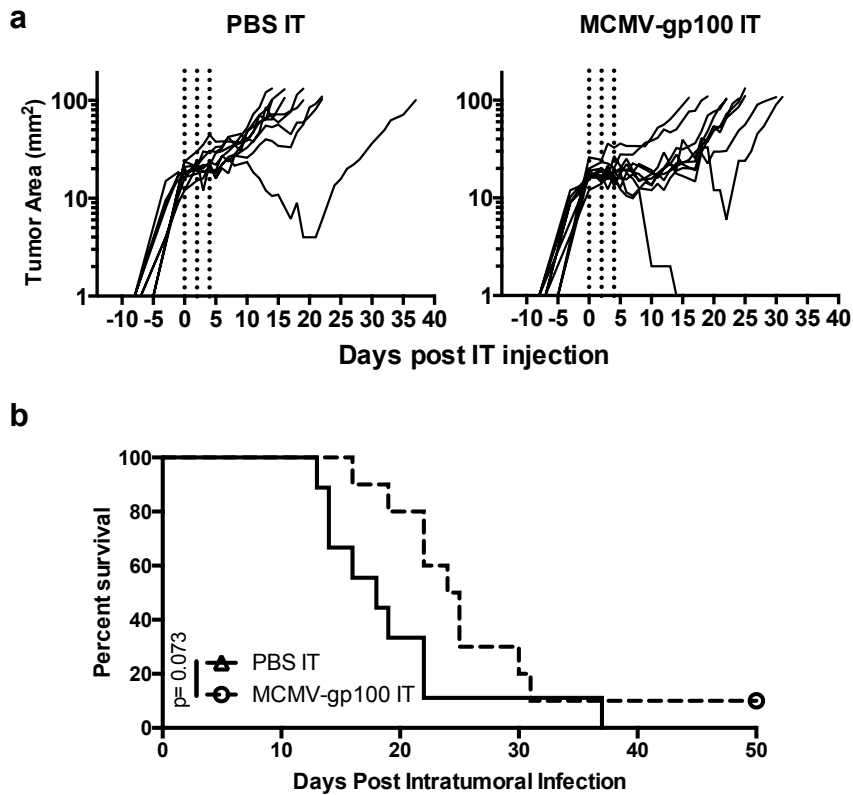
Supplemental Figure 1

Supplemental Figure 1: Representative gating strategies for CD8⁺ T cells recovered from blood, tumors and spleens. (a) Identification and functional analysis of CD8⁺ T cells from the blood was accomplished as shown. (b) Representative gating of lymphocytes in the spleen (top panel) and tumors (bottom panel). (c) Gating strategy for identification of Pmel-Is (Thy1.1⁺) in the tumor and spleen and the subsequent cytokine production (IFN γ ⁺, TNF α ⁺) and degranulation (Lamp-1/CD107a⁺) of IFN γ ⁺, TNF α ⁺ Pmel-Is.



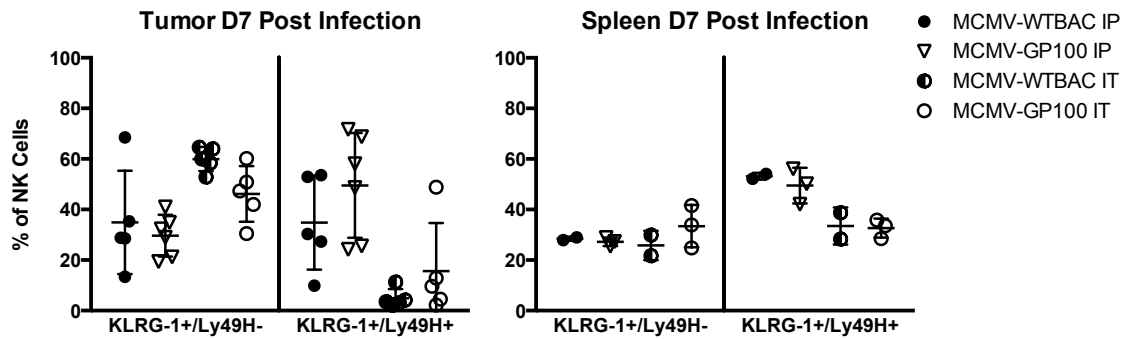
Supplemental Figure 2

Supplemental Figure 2: MCMV-gp100 infection of B16F0s *in vitro* induced cell death. (a-e) B16F0s were infected *in vitro* at the indicated MOI and data are represented as the mean +/- the SD. Shown is the growth of virus after infection of B16F0s or M2-10B4s with low (a) or high (b) MOI, the proportion of B16s that were infected (c), the viability of B16s after infection (d) and the growth of B16s after infection (e) after low or high MOI. Data are representative of at least two independent experiments. Error bars indicate standard deviation from replicate samples (n=2). Significance was assessed by an unpaired t-test, $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$, $p < 0.0001 = ****$.



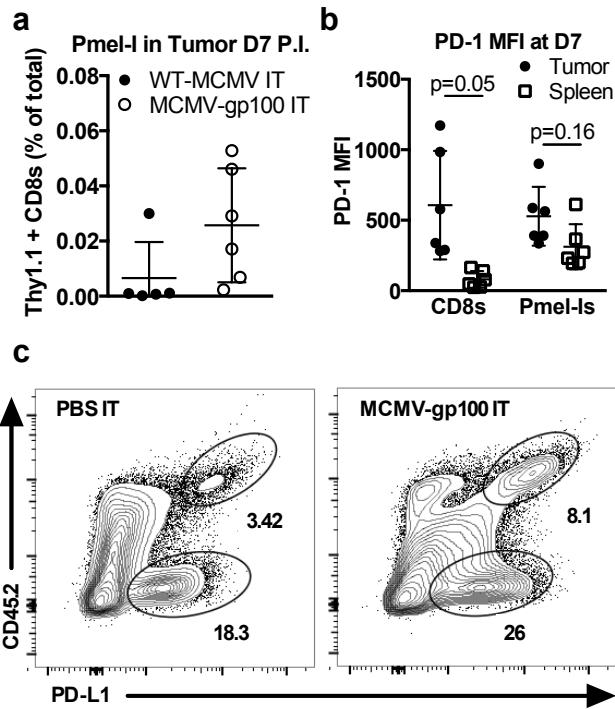
Supplemental Figure 3

Supplemental Figure 3: MCMV IT therapy prolongs survival of MC38-tumor bearing animals. C57BL/6 mice were subcutaneously implanted with 5×10^5 MC38s and treated with MCMV IT or PBS IT as described in fig. 3, when tumors were 20 mm². (a) Tumor growth, represented as change in tumor area (mm²) over time, is shown from the day of the first intra-tumoral injection. (b) Kaplan Meier survival curve of the MCMV IT versus PBS IT treated animals from day of tumor implantation until tumors were above 100 mm². Significance was assessed by a logrank test, $p < 0.05 = *$.



Supplemental Figure 4

Supplemental Figure 4: MCMV IT therapy induces more activated NK cells in tumors that do not recognize m157 on CMV infected cells than systemic infection on D7 post infection. Mice received 1×10^4 Pmel-Is one day prior to tumor implantation. Recipients were vaccinated by the IP route on day 5 after tumor implantation or by the IT route when the tumor reached 20 mm^2 . For all groups, tumors and spleens were collected for analyses on day 7 after either infection. Expression of KLRG1 and Ly49H was assessed on NK cells (NK1.1+, CD3-) cells in tumors (left) and spleens (right).



Supplemental Figure 5

Supplemental Figure 5: Tumor antigen-specific CD8⁺ T cells in the tumor were PD-1^{hi} and dysfunctional after MCMV IT infection. The same mice from Figure S4 were used here. (a) Shown is the frequency of Pmel-I in the tumor 7 days after the initial MCMV infection (MCMV-gp100 IT, n=6; WT-MCMV IT, n=5). Data are combined from 2 independent experiments and represented as the mean +/- the SD. (b) Mean fluorescence intensities of PD-1 on total CD8⁺ T cells and Pmel-I and represented as the mean +/- the SD. Significance was assessed by a paired t-test, $p < 0.05 = *$. (c) Representative FACS plots of PD-L1 by CD45.2 expression in tumors after PBS IT versus MCMV-gp100 IT injection (tumor from animals in Fig. 3).