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Supplemental Information

Manipulating TLR Signaling Increases the Anti-tumor T Cell Response Induced by Viral Cancer Therapies

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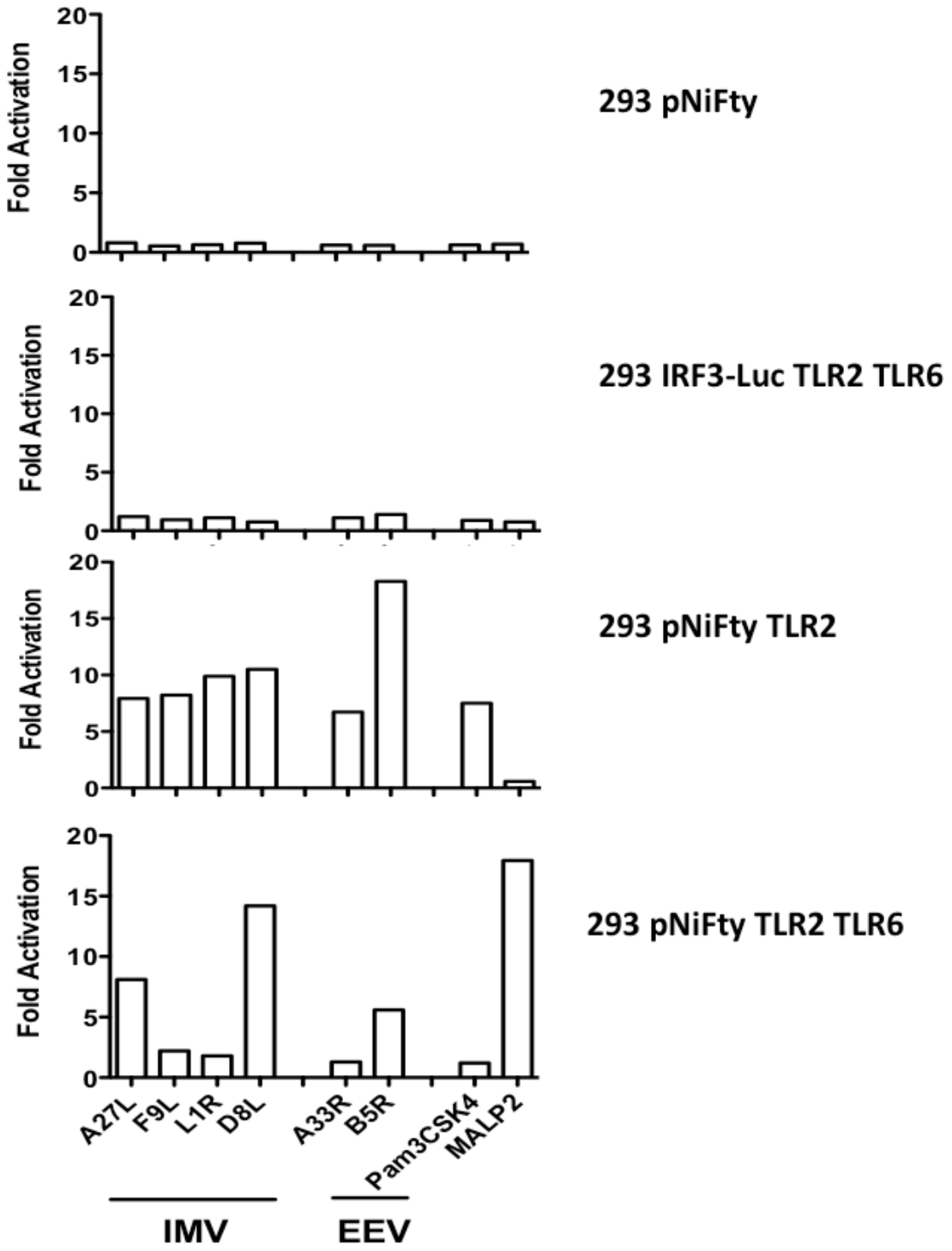
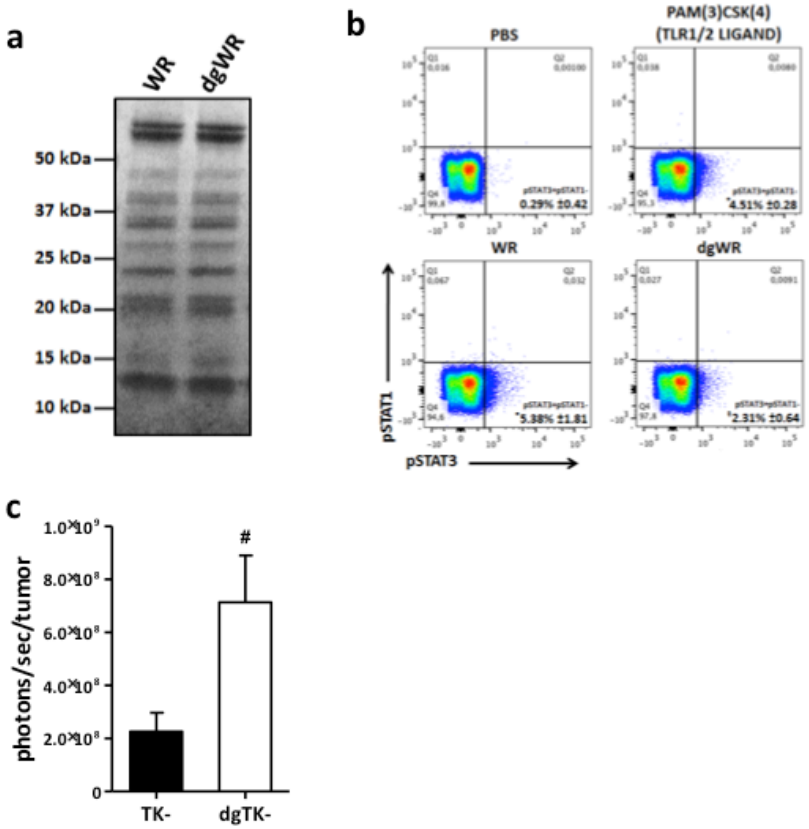
Supplemental Figure 1



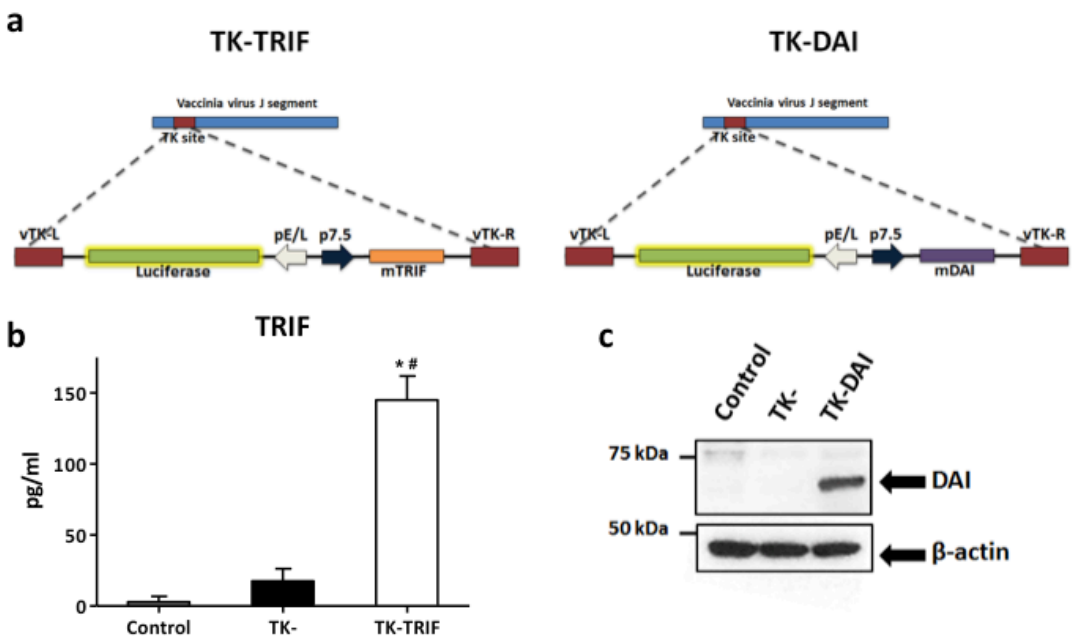
Supplementary Figure 1. Related to Figure 1. Determination of (a) TLR2:TLR6 and (b) TLR2:TLR2 activation (determined by pNiFty luciferase assay of NF-kB activation, with IRF3-luciferase assay used as a control) in 293 cells transfected to express hTLR2 or hTLR2 and hTLR6. Bioluminescence was measured by IVIS200 (Perkin Elmer) 6h after exposure to different recombinant vaccinia proteins. 293 cells not expressing any TLR were used as a control and proteins are divided into those found on the surface of either the IMV or the EEV form of the virus. PAM3CSK4 is a TLR2 homodimer ligand, and MALP2 is a ligand for TLR2:TLR6 heterodimers.

Supplementary Figure 2



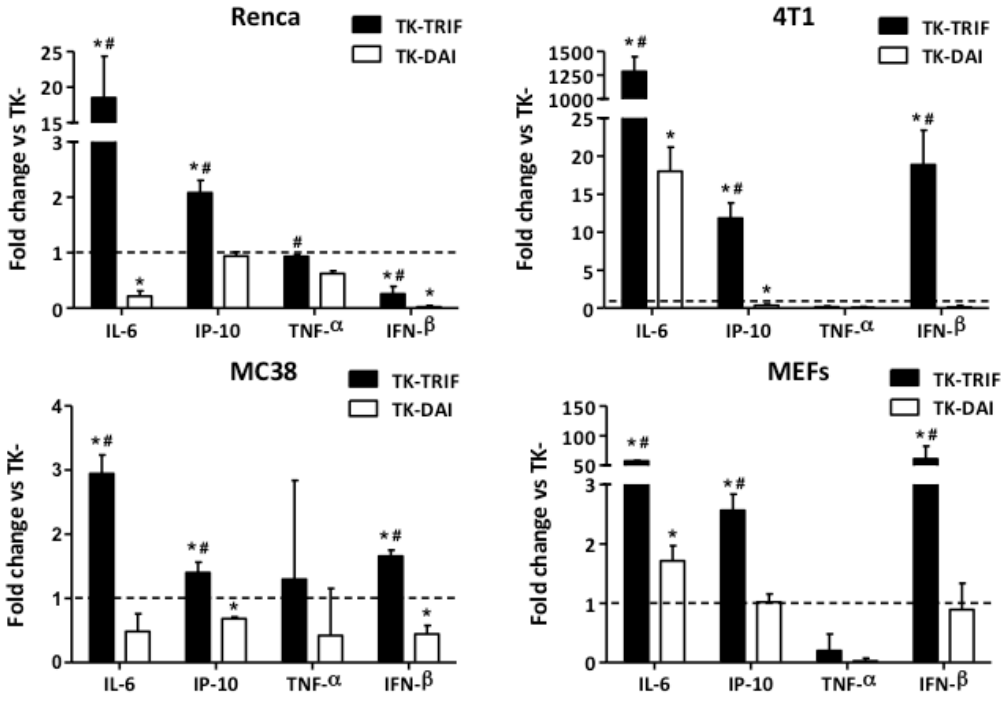
Supplementary Figure 2, Related to Figure 1. (a) Total protein content in disrupted Vaccinia virus preparations. Coomassie-blue staining was used as a control for the amount of protein loaded for blotting in figure 1a. (b) Representative distributions of pSTAT1+ and pSTAT3+ populations within splenic lymphocytes. Splenocytes from C57/BL6 mice treated as indicated were stained as in figure 1d for intracellular levels of pSTAT1 and pSTAT3 and analyzed by flow cytometry. (c) Luciferase levels from within MC38 tumors at day 3 after virus injection. C57/BL6 mice bearing subcutaneous xenografts of MC38 cells were injected intravenously with a dose of $1x10^8$ pfu per mouse of TK- or dgTK-. Viral luciferase expression was determined at day 3 after virus injection by bioluminescence imaging. Mean values of 10-12 animals +SD are plotted.

Supplementary Figure 3

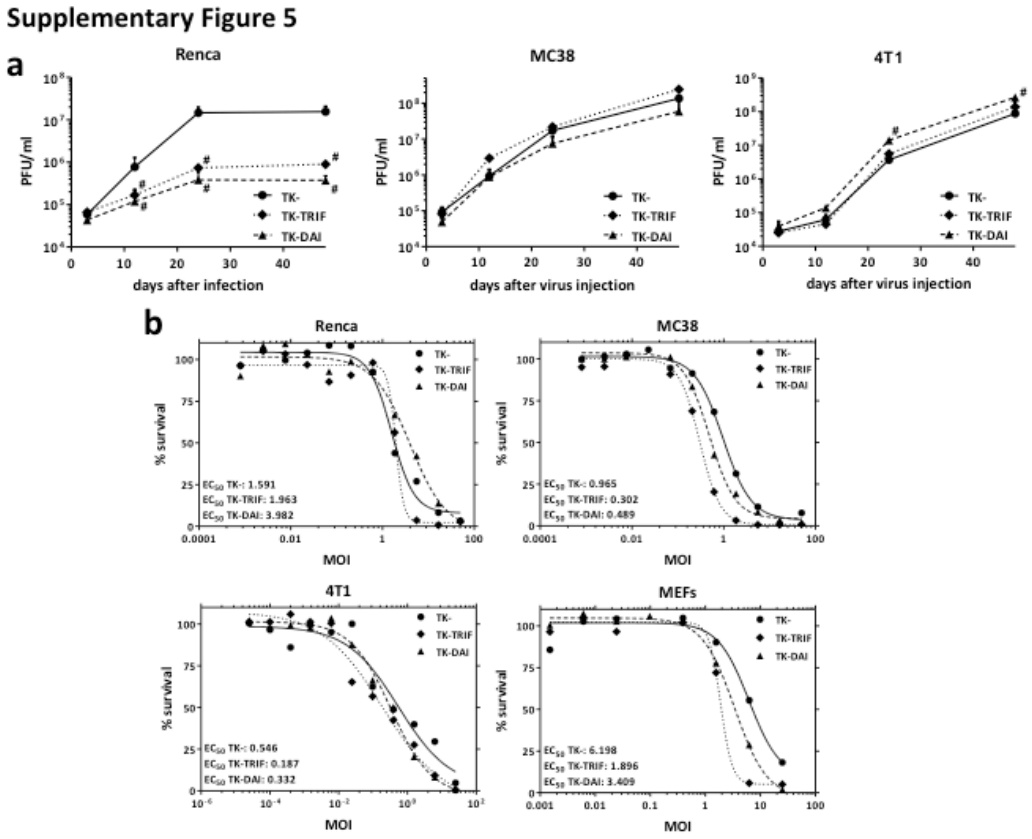


Supplementary Figure 3, Related to Figure 2. (a) Schematic diagram of TK-TRIF and TK-DAI recombinant viruses. mTRIF and mDAI, respectively, are expressed from the early/late vaccinia promoter p7.5 and cloned into the locus of the viral thymidine kinase gene. In addition, firefly luciferase gene is also expressed from the synthetic vaccinia promoter pE/L to monitor viral replication. Confirmation of mTRIF (b) and mDAI (c) expression was assessed by ELISA and Western-blot, respectively, after infection of HeLa cells (MOI of 1). *, significant $P<0.05$ compared with Control. #, significant $P<0.05$ compared with TK-.

Supplementary Figure 4

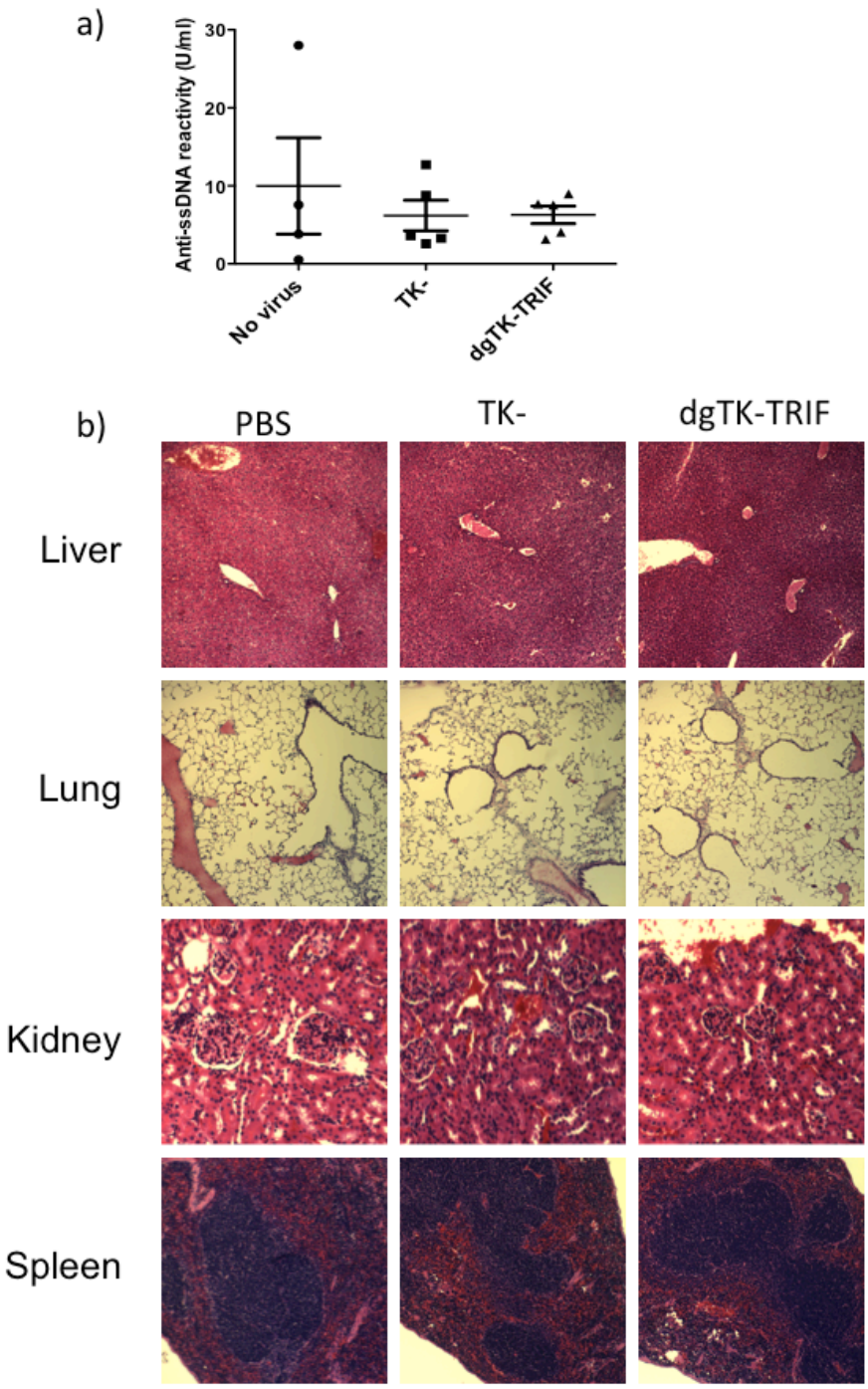


Supplementary Figure 4, related to Figure 2. Release of cytokines and chemokines *in vitro* after TK-TRIF and TK-DAI infection. IL-6, IP-10, TNF-α, and IFN-β concentrations in the supernatant of Renca, 4T1, MC38 and MEF cells were evaluated by Luminex assay 24 hours after infection with TK-, TK-TRIF and TK-DAI (MOI of 1). Data is depicted as fold change vs TK- +SD (2 independent experiments). Dashed lines indicate TK- concentrations.



Supplementary Figure 5, related to Figure 2. (a) Viral production of TK-TRIF and TK-DAI in mouse tumor cells. Different tumor cell lines were infected with viruses at an MOI of 1 and virus production was measured by plaque-assay at different time points. Viral yield was evaluated in quadruplicate for each cell line, by carrying out two independent experiments. Means +SD are plotted. (b) Comparative cytotoxicity of TK-TRIF and TK-DAI. Cells were infected with the indicated viruses at doses ranging from 75 to 0.00025 PFU/cell. EC_{50} values (MOI required to cause a reduction of 50% in cell culture viability) at day 4 after infection are shown. Four different replicates were quantified for each cell line and mean for each MOI is depicted.

Supplementary Figure 6



Supplementary Figure 6, related to Figure 5. BALB/c mice (n=5 per group) were treated with IV injection of PBS or 1e7 PFU of the indicated viruses. Mice were sacrificed after 21 days and (a) serum collected for quantification of the levels of circulating anti-ssDNA antibodies, and (b) other organs collected for determination of signs of toxicity and auto-immunity by H&E staining.