Supplementary Figure 1. Reovirus-induced pro-inflammation. BMDCs, splenocytes or tumor cells were incubated in the presence of 1 PFU/cell of reovirus or PBS, while WT C57BL/6 and SCID mice were injected with 5 x 10^8 and 1 x 10^7 PFU of reovirus, respectively. After 24 h, culture supernatants or sera were analyzed using Antibody-based Qualitative and Quantitative Cytokine Arrays. A representative example of Quantitative Cytokine Array shows the profile of 40 cytokines in quadruplicates, with rectangles and elliptical circles indicating increased or decreased expression respectively, in reovirus-stimulated BMDC supernatant as compared to that of PBS-stimulated sample. A cytokine map indicates the location of respective cytokines on an array.
Supplementary Figure 2

A. Reovirus-induced activation of DCs *in vivo*

B. Reovirus-induced activation of BMDCs *in vitro*

Supplementary Figure 2. Reovirus-initiated maturation of APCs *in vivo and in vitro*. a. APCs from spleen and tumors of PBS- or reovirus-treated tumor bearing mice were harvested and directly stained for CD86. Histogram represented as: Filled – PBS-injected; solid line – reovirus-injected. b. BMDCs were cultured in the presence of ten fold dilutions of live or UV-inactivated reovirus (10 - 0.0001 PFU/cell) for 18-24 h and then stained. Dot-plots show the expression of CD86, CD80, CD40 or MHC class II on CD11c+ gated cells after exposure to 1 PFU/cell of live and UV-inactivated reovirus. Histograms represented as: Filled – PBS-stimulated; dotted line – UV-reovirus; solid line – live reovirus.
**Supplementary Figure 3.** Tumor antigen presentation by APCs *in vivo* in LLC C57BL/6 model. CD11c+ DCs were enriched from the spleens, LNs and tumors of PBS- or reovirus-treated LLC or LLC-ova tumor bearing mice (injected as per protocol in Fig. 2b) and then evaluated for their capacity to activate CD8 T cells using B3Z assay.
**Supplementary Figure 4.** Activation of OT-1 T cells *in vivo* in native B16 tumor model. Naïve OT-1 splenocytes were labeled with 1 μM of CFSE and then cultured in the presence of enriched DCs collected from the spleens, LNs and tumors of PBS- or reovirus-treated native B16 tumor bearing B6.PL-Thy1a/CYJ mice (injected as per Fig. 2b). After 5 days cells were stained with anti-CD3 and anti-thy1.2 antibodies, and then analyzed in flow cytometry for the deconvolution of CFSE fluorescence as a measure of proliferation.
Supplementary Figure 5A

Cell division index (C.D.I.)

LN

Tumor

PBS reovirus

PBS reovirus

PBS reovirus

PBS reovirus

SIINFEKL UV reovirus B16 lysate
Supplementary Figure 5C

Supplementary Figure 5. Oncotherapy-initiated anti-tumor T cell responses in vivo. a. Lymphocytes were collected from spleen, LN and tumor of PBS/reovirus treated B16-ova tumor bearing WT C57BL/6 mice (Fig. 1B), labeled with 1 μM of CFSE and then cultured in the presence of BMDCs pulsed with 5 μg/mL of SIINFEKL peptide, UV-reovirus or ConA (5 μg/mL, positive control). After 5 days, cells were stained with anti-CD3 antibodies and analyzed for CFSE fluorescence to define proliferation as shown in ModFit LT histograms. b. Cumulative data from 4 independent experiments (n = 5-7 mice in each group) representing the anti-tumor and anti-viral T proliferation. c. Lymphocytes isolated from LNs and stimulated as in Fig. 4a were cultured in the presence of anti-CD107a antibodies for 6 h, stained with anti-CD3, anti-CD8 and anti-IFN-γ antibodies and analyzed. Cumulative data from 3 independent experiment (n = 5 - 7 mice per group) for IFN-γ and CD107a producing CD3+, CD8+ T cells in LNs of PBS-reovirus treated tumor bearing mice is shown.
Supplementary Figure 6. The phenotype of BMDCs used for anti-cancer immunotherapy. BMDCs were stimulated with IL-1β (10 ng/mL), TNF-α (10 ng/mL), IL-6 (1000 U/mL) and PGE2 (1 µg/mL) in the presence of SIINFEKL peptide for 2 days before using them as immunotherapeutic agents. Histograms represented as: Filled – unstimulated; solid line – stimulated.