# Supplementary Information

## Supplementary Methods

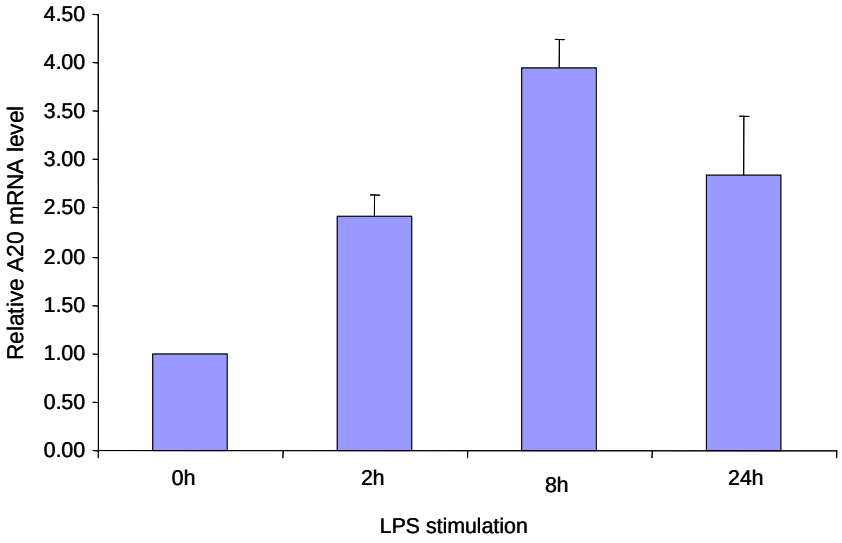
**Antibodies:** Cells were stained with FITC, PE, allophycocyanin (APC) mAbs in PBS containing 0.1% $NaN_3$ and 2% FCS. mAbs specific for mouse CD8 (53-6.7), CD11c (HL3), CD40 (3/23), CD80 (16-10A1), CD86 (GL1), CCR7, MHC-II and matched isotype controls were purchased from BD PharMingen or eBioscience. PE-conjugated mGITRL-specific mAb was purchased from BioLegend.

**Quantitative RT-PCR and enzyme-linked immunospot (ELISPOT) assays.** The relative expression of A20 was evaluated by quantitative real-time RT-PCR, as described previously[32]. Pre-developed primer/probe sets for mouse A20 and 18S ribosomal control (VIC) were purchased from Applied Biosystems (primers for A20, 5'- TTTGCTACGACACTCGGAAC-3' and 5'- TTCTGAGGATGTTGCTGAGG-3'. and the hybridization probes, 5'-CACCTGCAGCCCGAAGTGGA-3'), and pre-established primer/probe sets for Q-RT-PCR of mouse IL6 and TNFα were purchased from SuperArray (Frederick, MD). ELISPOT assays of isolated $CD8^+$ and $CD4^+$ T-cells and splenocytes were performed as described in our previous studies[32]. Antigen-specific peptides were used for T-cell stimulation. T cells were isolated from splenocytes by using MACS CD4 (L3T4) or MACS CD8 (Ly-2) MicroBeads (Miltenyi Biotec, Auburn CA).

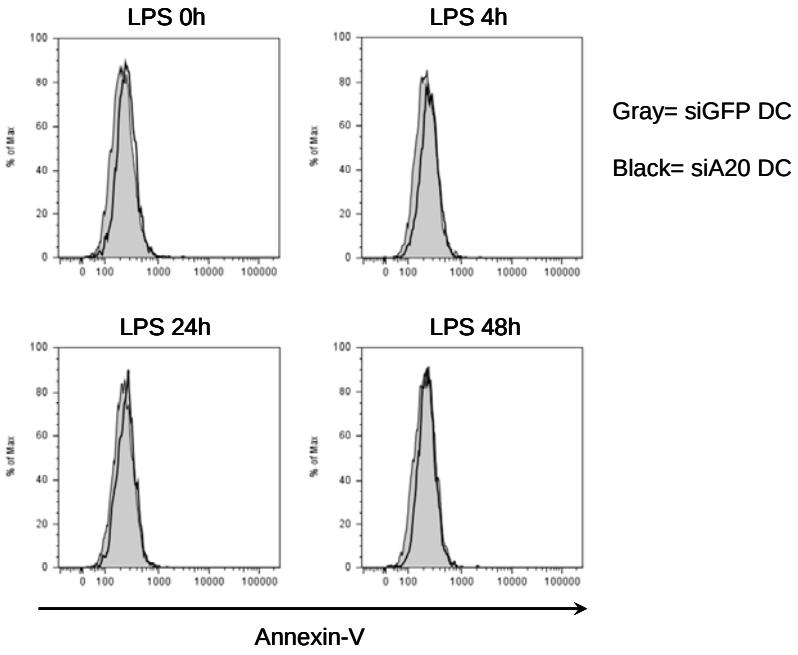
**Isolation and analysis of TILs.** Groups of mice were inoculated in the flank i.d. at day 0 with $4x10^5$ B16-OVA tumor cells in 200 μl of a collagen matrix (Matrigel). Mice were immunized with OVA-pulsed siA20-DC or control DC (footpad) with or without 100 μg anti-CD25 (i.p.) days 1 and 5 prior to DC immunization, followed by *in vivo* polyI:C stimulation. Mice were immunized with OVA-pulsed siA20-DC or control DC again 5 days after the first DC immunization. On different days after DC immunization, mice were sacrificed, and TILs were isolated from tumor cell suspensions after a Ficoll gradient was performed to eliminate dead cells. TILs were analyzed by flow cytometry for the surface expression of CD4 and CD8, and intracellular staining of $Foxp3^+$, and IFN-γ, and TNFα. The total number of infiltrating $CD8^+$ or $CD4^+$ T cells/gram of tumor was obtained by multiplying the percentage of $CD8^+$ or $CD4^+$cells by the total number of lymphocytes obtained from Ficoll and dividing that number by the weight (g) of the tumors. For intracellular cytokine staining, TILs were restimulated *in vitro* for 4 hr and then stained for flow-cytometric analysis of intracellular IFN-γ, IL-6, or TNFα using a Cytofix/Cytoperm kit according to the manufacturer's instructions (BD Biosciences-Pharmingen).

## Supplementary Figures

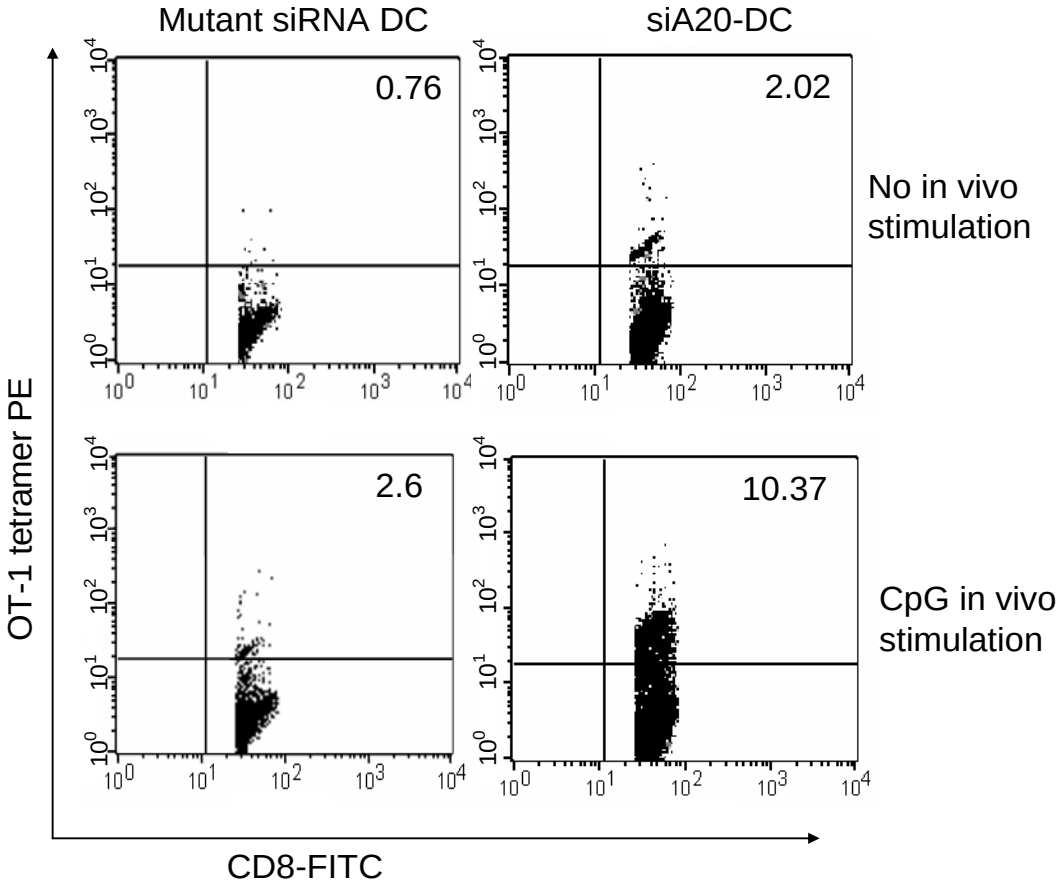
**Supplementary Fig. 1.** Q-RT-PCR analysis of A20 mRNA levels in bone marrow-derived DCs following LPS stimulation for 0, 2, 8 and 24 hours.



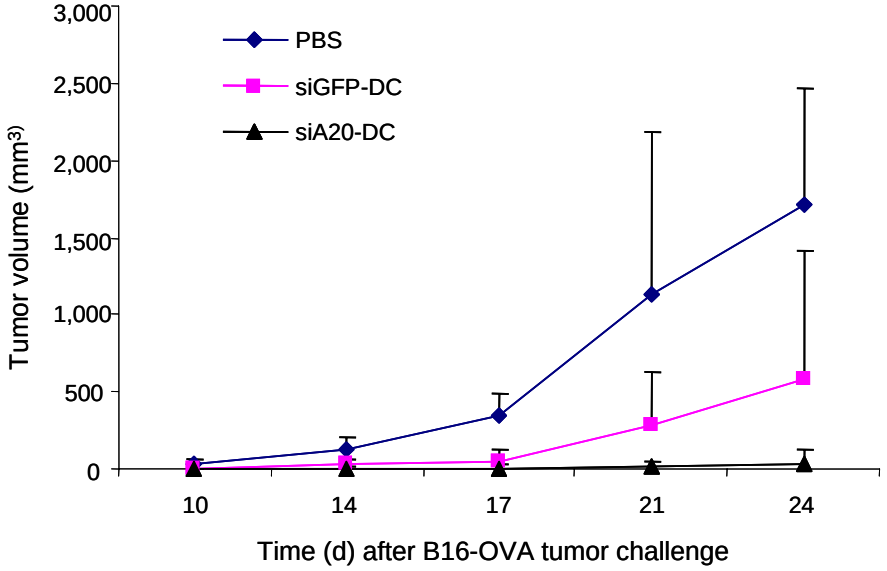

**Supplementary Fig. 2**. Apoptotic cell death of siA20-DC and control siGFP-DC (BM-derived) stained with anti-Annexin-V (BD PharMingen) at different time points after LPS stimulation *in vitro*.



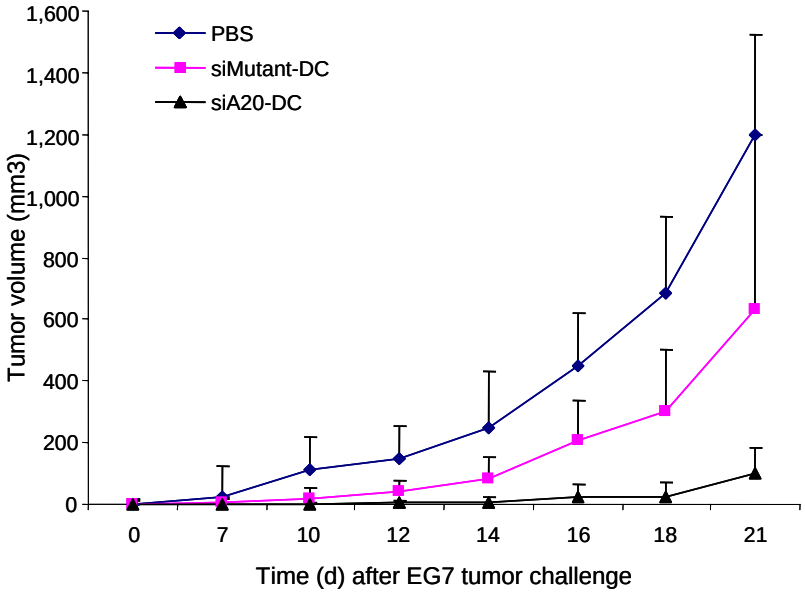
**Supplementary Fig. 3.** Enhanced ability to induce $CD8^+$ and $CD4^+$ T cell responses by siA20 oligo-transfected DCs. BM-DCs at day 5 of culture were transfected with synthetic A20 siRNA duplexes (Dharmacon) by GenePorter, pulsed with OVA (50 μg/ml) and then matured with LPS *ex vivo* (100 ng/ml) for 12 hr. C57BL/6 mice were immunized twice with transfected DCs (1 $x10^6$/mouse) followed by no or *in vivo* polyI:C stimulation. Two weeks later, splenocytes pooled from 2 or 3 immunized mice from each group were subjected to tetramer staining. OT-I $tetramer^+$ T-cell percentages in the $CD8^+$ T-cells (**sFig. 5**) are shown from one of three independent experiments.



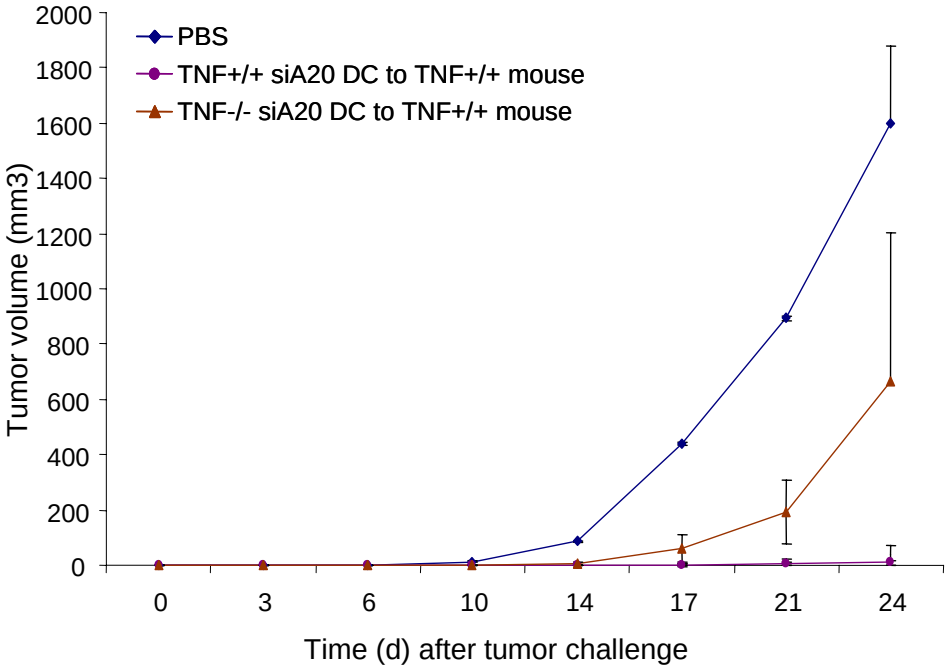
**Supplementary Fig. 4.** Inhibition of pre-established B16-OVA tumors. Groups of C57BL/6 mice were inoculated s.c. with B16-OVA tumor cells ($2.5x10^5$) and three days later, were immunized via the rear footpad with 1 $x10^6$ OVA-pulsed (50 μg/ml), LV-transduced DCs with *ex vivo* LPS maturation (100 ng/ml). One day after DC transfer, *in vivo* polyI:C was administered i.p. (30 μg/mouse) one time. Tumor growth curves (n=6 mice/group) represent one of three independent experiments. $P < 0.05$, siGFP-DC compared with siA20-DC.




**Supplementary Fig. 5.** Antitumor activity against EG.7 tumors by siA20 oligo-transfected DCs. C57BL/6 mice were inoculated s.c. with $OVA^+$ EG.7 tumor cells ($5x10^5$) and three days later were immunized with 1 $x10^6$ OVA-pulsed (50 μg/ml) siGFP oligo-DC or siA20 oligo-DCs with *ex vivo* LPS maturation (100 ng/ml) once without *in vivo* stimulation. Tumor growth curves (n=6 mice/group) represent one of repeated experiments. $P < 0.05$, siMutant-DC compared with siA20-DC.



**Supplementary Fig. 6.** Inability to inhibit pre-established tumors by TNFα-deficient siA20-DCs. Wt or TNFα KO (B6.129S6-Tnf$^{tm1Gkl}$/J) mice were inoculated s.c. with B16-OVA tumor cells. Three and ten days later the mice were immunized with 1.5 x10$^6$ OVA-pulsed, transduced wt or TNF KO DCs with *ex vivo* LPS maturation, followed by in vivo stimulation (i.p.) with poly(I:C) (50 μg). Tumor growth curves (n=6-8 mice/group) represent one of two independent experiments. $P < 0.01$, TNF KO siA20-DC compared with wt siA20-DC.




**Supplementary Fig. 7.** Inability to inhibit pre-established tumor in TNFR-deficient hosts or by TNFR-deficient siA20 DCs. Wt or TNFR KO (B6;129S-Tnfrsf1a$^{tm1Imx}$/J) mice were inoculated s.c. with B16-OVA tumor cells. Three and ten days later the mice were immunized with 1.5 x10$^6$ OVA-pulsed, transduced wt or TNFR KO DCs with *ex vivo* LPS maturation, followed by in vivo stimulation (i.p.) with poly(I:C) (50 μg). Tumor growth curves (n=6-8 mice/group) represent one of two independent experiments. $P < 0.01$, TNFR KO siA20-DC compared with wt siA20-DC.