

Supplemental figure S1. Determination of TIDC cytotoxicity using Chromium release assay or Annexin V / 7-Amino-Actinomycin D labeling.

(A) TIDC or CD8⁺ T cells isolated from CT26 tumors of untreated or CTX-BCG treated mice were incubated with CT26 cells for 6h in a standard ⁵¹Cr release assay at effector / target ratios of 25/1 and 5/1. Results of the chromium release assay are represented as means ± SEM of triplicate wells from one representative experiment out of two. (B) TIDC isolated from tumors of untreated or CTX-BCG treated rats were incubated with GFP-PROb cells at a 1/1 effector / target ratio for 48 h. Cytotoxic effect on PROb cells was determined using Annexin V-PE and 7-AAD labeling on GFP⁺ cells.

Supplemental figure S2. TIDC from CTX-BCG treated animals present tumor antigens.

(A) FACS analysis of costimulatory molecules on CD45⁺ CD11c⁺ CD11b⁺ MHC-II⁺ TIDC from untreated or treated CT26 tumor-bearing mice. (B) 3.10⁵ CD11b⁺ TIDC isolated from CT26 tumors of control mice or mice treated by CTX-BCG were injected into footpad of naïve mice. Five days later draining popliteal lymph node was excised and 5.10⁵ cells were stimulated alone or with 1.10⁴ killed CT26 cells. Supernatants were harvested three days later and IFN-γ was determined by ELISA. (C) 3.10⁵ CD11b⁺ TIDC isolated from CT26 tumors from mice treated by CTX, BCG or the association were injected into the footpads of naïve mice. Seven days and 20 days later, 5.10⁵ CT26 and TS/A tumor cells were injected s.c. and tumor growth was monitored during 50 days. n=5 mice in each group. (D) 1.10⁴ TIDC isolated from untreated or from combined treated PROb tumor-bearing rats were incubated with 1.10⁵ specific antitumor T cells. Three

days later, coculture supernatants were collected and IFN- γ concentration was determined by ELISA. (E) $5 \cdot 10^5$ TIDC isolated from untreated or treated PROb tumor bearing animals were injected into the footpad of naïve rats. Seven days later $1 \cdot 10^6$ PROb were injected s.c. and tumor growth was monitored during 80 days.

Supplemental figure S3: TRAIL expression elicited by BCG on DC is dependent on TLR 2, 4 and 9 and MyD88 but not on TRIF signaling.

Mouse BM-DC derived from TLR2, TLR4, Myd88 or TRIF deficient mice were incubated overnight in the presence or absence of BCG ($8 \cdot 10^5$ CFU/ml). In addition, mouse BM-DC from wild-type mice were incubated with an inhibitory oligodeoxynucleotide (IRS 954) to block TLR9 signaling and stimulated as described above. TRAIL mRNA expression was determined by quantitative RT-PCR. BM-DC incubated with IFN- γ represent a positive control of TRAIL mRNA expression. Each value is expressed as fold increase from medium control after normalization with GAPDH. Data are representative of two experiments, (* $p < 0.05$ / medium from WT group).

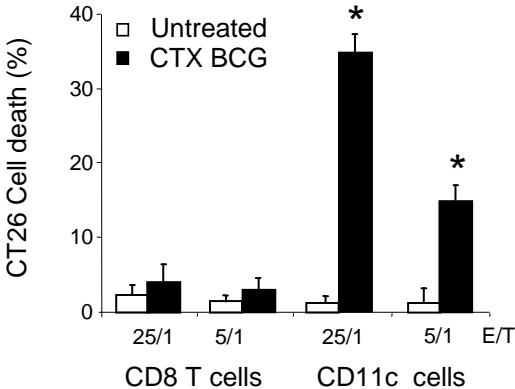
Supplemental figure S4: Foxp3 expression increases sensitivity of Jurkat cells to mafosfamide.

(A) $1 \cdot 10^5$ CD4⁺ CD25⁺ (Treg) or $1 \cdot 10^5$ CD4⁺ CD25⁻ (Tconv) T cells isolated from the spleen of CT26 tumor-bearing mice were incubated for 2 h with mafosfamide at the

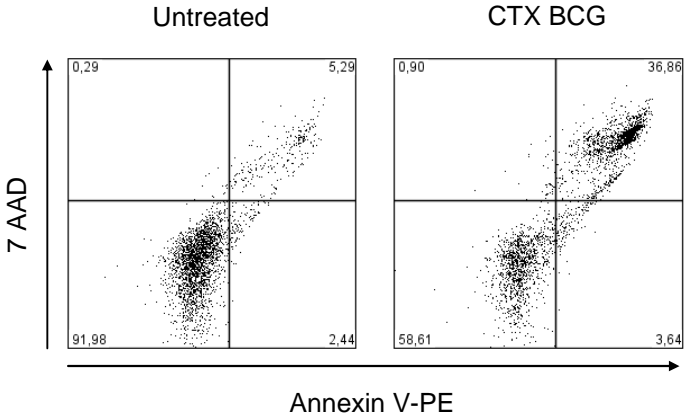
indicated concentrations. Apoptosis was determined 24 h later using Hoechst 33342 chromatin staining and fluorescence microscopy. **(B)** 10^5 Jurkat cells transfected with Foxp3 (Foxp3) or an empty vector (control) were incubated for 2 h with mafosfamide at the indicated concentrations. Apoptosis was determined 24 h later using Hoechst 33342 chromatin staining. Data represent means \pm SEM, * $p < 0.05$.

Supplemental Figure 1

A

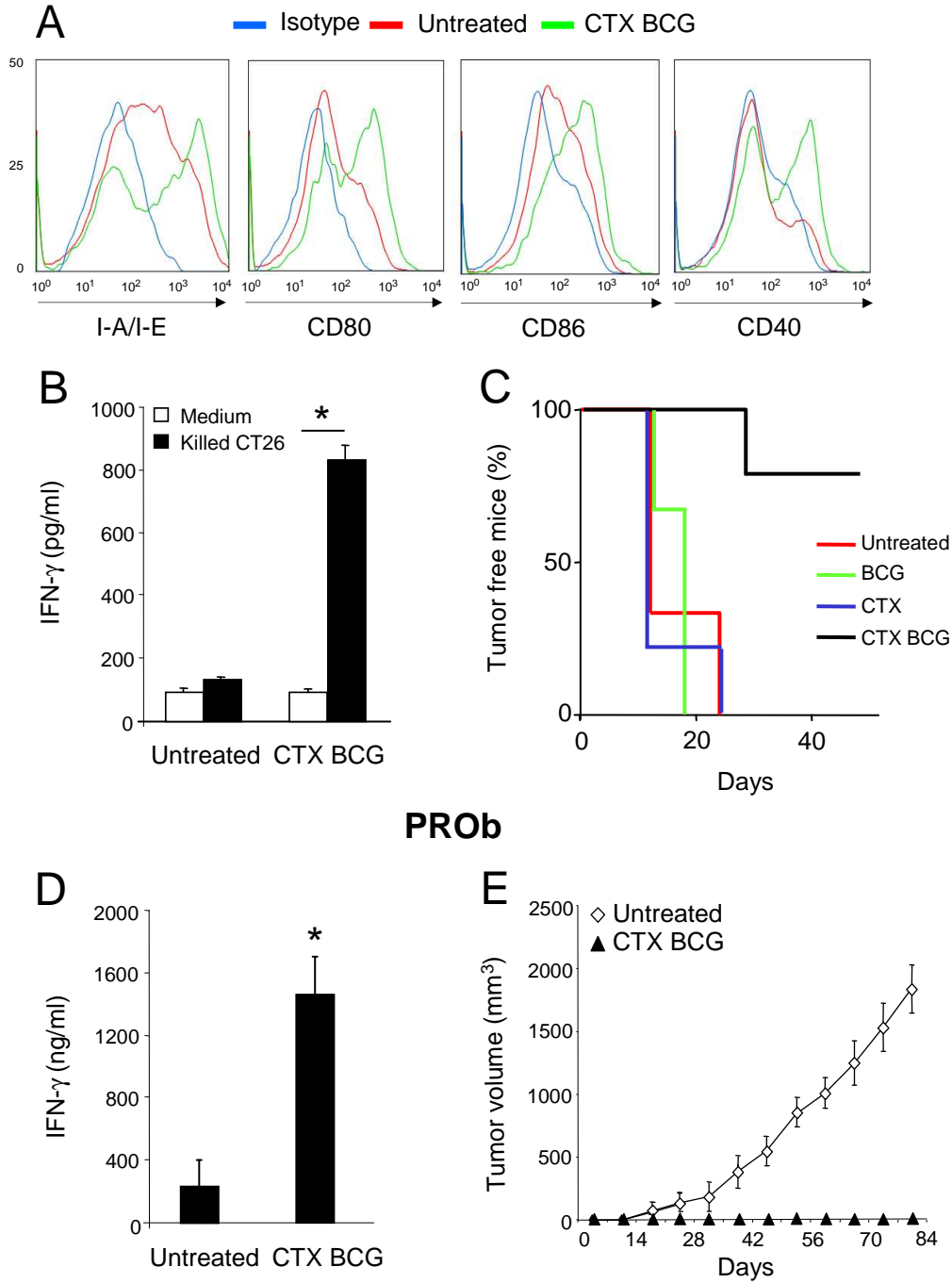


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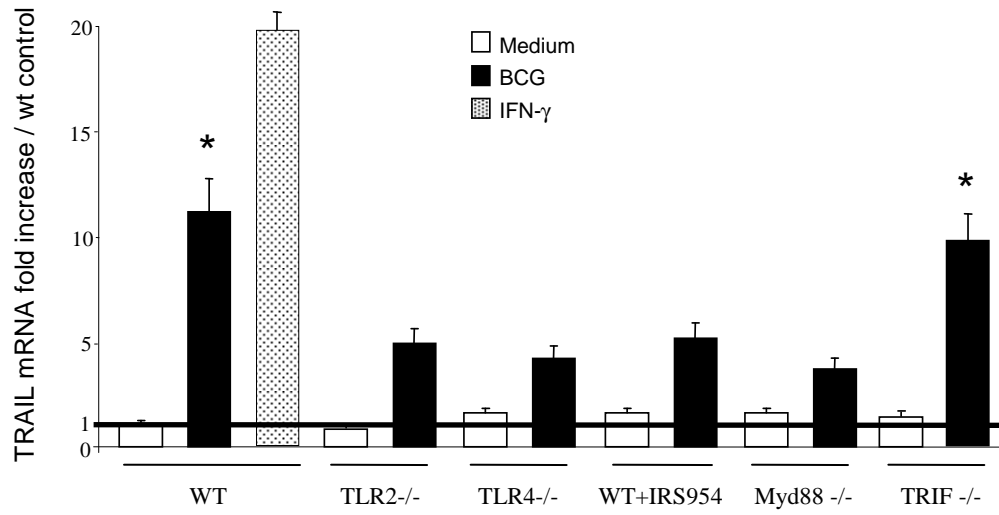


Supplemental Figure 2

CT26



Supplemental Figure 3



Supplemental Figure 4

