SUPPLEMENTARY FIGURE 1

Analyses from TILs in Figure 1 showing the expression of CD137 on: (A) CD4+FOXP-3+ regulatory T cells; (B) NK cells (CD3-CD49b+) and NKT cells (CD3+CD49b+).
SUPPLEMENTARY FIGURE 2

CD137 expression in other leukocyte subsets infiltrating CT26 tumors

(A) gated CD19+ B cells, (B) F4/80+ CD11b+ macrophages, (C) GR1+ CD11b+ F4/80- myeloid suppressor cells; and (D) CD11c+ CD45+ dendritic cells.
Supplementary Figure 3

Transplanted and spontaneous solid tumors are hypoxic. (A and B) Positron emission tomography (PET) imaging of mice injected with $^{18}$F-MISO probe that accumulates in and marks hypoxic tissue. As indicated in the Figure, a syngeneic tumor-free control and a tumor-bearing mouse are included for the tumors derived from each cell line (A) and for tumor-bearing MMTV-Her2-Neu female mice (B). Tumor areas are marked by a dotted gate. Images from one representative mouse out of 6 per group are presented. (C) Computer-assisted 3D reconstruction of the tumor associated $^{18}$F-MISO emission from a representative CT26-bearing mouse. L marks liver, B bladder and G gut.
SUPPLEMENTARY FIGURE 4.

CD137 expression under hypoxia on isolated T cells and T reg cells.

(A) CD137 induction in normoxia and hypoxia in magnetically sorted CD4 and CD8 T cells in experiments as those as in figure 3 but with negatively isolated T cell subsets instead of total splenocytes. (B) CD137 expression on Spleen Foxp-3+CD4+ Tregs following 48 hour co-cultures with CD3CD28-coated beads in 21% vs 1% O2.
SUPPLEMENTARY FIGURE 5

Hypoxia up-regulates surface CD137 on human T cells. (A) Buffy coat PBMC were stimulated by plate-bound anti-CD3 mAb in normoxic conditions or under 1% O₂. Cells harvested 48h later were immunostained, gated for CD4 and CD8 events (dot plots) and analyzed for CD137 expression. A representative case of high and low up-regulation is shown while a series of 6 individuals from a single experiment is shown in B. (C) Experiments as in A but comparing cultures in the presence or absence of 0.2 mM DMOG. Results from a representative individual are presented.
SUPPLEMENTARY FIGURE 6

T cell activation cultures started under hypoxia to ascertain the need for continuous hypoxia to sustain CD137 expression.

Experiments as those in figure 3 in which splenocytes were cultured with CD3CD28-coated beads and kept under 1% O2 for the duration of the experiment or retrieved from the hypoxia chamber after the first 24 h. Experiments were performed with HIF-1α-/- (blue) and control (red) T cells and data are presented for gated CD4 (A) and CD8 (B).
SUPPLEMENTARY FIGURE 7

Tumor-naive

Rechallenge

SUPPLEMENTARY FIGURE 7

Tumor re-challenge 3 months following complete tumor rejection in the mice from Figure 6 cured by i.t. injections of CD137 mAb and i.p. injections of anti-B7-H1. As a control, tumor naive mice developed lethal tumors in every case.
Lack of expression of CD137 on CD4 and CD8 T cells in draining and non-draining lymph nodes.

CD137 immunostaining of lymph node cell suspensions of BALB/c mice bearing CT26 established tumors. Results shown are from lymph node cells pooled from seven mice. CD137 was readily detected in the TILs of these mice.
SUPPLEMENTARY FIGURE 9

Intratumoral anti-CD137 mAb at low doses elicits changes in TDLN and TILs.

Absolute numbers of mononuclear cells in tumor-draining lymph nodes and tumor tissue from mice bearing established CT26 tumors treated intratumorally with 5 μg of 1D8 (αCD137) or control antibody (Rat IgG). (B to E) Qualitative analyses of TILs from CT26 tumors treated intratumorally with 5 μg of 1D8 or control antibody (Rat IgG) to study percentage and absolute numbers of T cell subsets (B, C, D) and activation...
SUPPLEMENTARY FIGURE 10

Intratumoral low-dose αCD137 mAb enhances cytokine production. Tumor infiltrating CD8+ T cells

(A) Mice challenged with $5 \times 10^5$ CT26 cells and treated intratumoraly with 5 μg of 1D8 or control mAb on days 7, 9 and 11 were sacrificed on day 13. Tumor mononuclear cells were restimulated with AH1 peptide (10 μg/ml) for 1 hour and incubated overnight in the presence of brefeldin-A (10 μg/ml). Cells were fixed and analyzed by flow cytometry for intracellular IFN-γ and TNF-α production on CD8+ T lymphocytes. (B) Dot plots showing TNFα and IFNγ in gated CD8+ T cells of a representative case. (C) Data from 7 mice per experimental group.