**Supplementary information**

**Supplementary Materials and Methods**

*Reagents and antibodies:* Immunohistochemistry on melanoma was performed with MECA79 polyclonal antibody (hybridoma provided by Dr. Girard, IPBS institute, Toulouse, France), anti-mouse CD8 (BD Pharmingen), anti-mouse CD31 (Abcam). Secondary antibodies coupled to Alexa Fluor dyes were from Invitrogen. Flow cytometry analyses were performed using anti-mouse CD45 (eBioscience, eFluor450), anti-mouse CD3 (Biolegend, APC), anti-mouse CD8 (BD Pharmingen, A700), anti-mouse CD4 (eBioscience, PerCP eFluor 710), anti-mouse CD69 (BD Pharmingen, FITC), anti-mouse MHC-I (mouse Y3 hybridoma), anti-mouse Foxp3 (eBioscience, PE), anti-Gr1 (Biolegend, PE), anti-CD11b (BD Pharmingen, APC), anti-mouse TNF-R1 (Biolegend, APC), anti-mouse TNF-R2 (BD Pharmingen) antibodies, MHC-I dextramers (H-2Kb /SVYDFFVWL) (Immudex, 1:100, APC). Isotype controls and live-dead reactive dyes for flow cytometry were from BD Pharmingen and Invitrogen, respectively. Etanercept was provided by Dr. L. Astudillo (CHU, Toulouse, France). Mouse TNF was purchased from Peprotech. LPS was from Tebu-bio. Mouse TNF concentration was determined by ELISA, following manufacturer's instructions.

*Cell lines:* Melanoma B16-BL6 and Yumm cell lines were kindly provided by Drs. I. Escargueil-Blanc (Sanofi, Toulouse, France) and S. Tartare-Deckert (INSERM U1065 Nice, France), respectively. Cells were cultured in DMEM medium containing 10% heat-inactivated fetal calf serum (FCS).

*In vitro B16 melanoma growth:* 1x104 B16 cells (100 μL) were cultured in 96-well plates overnight before addition of 25 to 100 ng/mL TNF. Cells were further incubated for 24 to 72h and counted (Beckman Coulter). Alternatively, cells were grown as spheroids by culturing 3x103 B16 in 1% agar-coated 96-well plates in the presence or absence of 50 ng/mL TNF. Pictures of spheroids were taken from day 2 to day 12 and spheroid volume was quantified by Image J software.

*Analysis of the immune response in draining lymph nodes:* One million B16K1 cells were intra-dermally and bilaterally injected in WT or TNF-/- C57BL/6 mice. At day 12, mice were sacrificed and draining lymph nodes were collected from each flank, lymph node cells were pooled for each mouse, counted (Beckman Coulter) and incubated with the indicated antibodies and live-dead reactive dye. Cells were analysed by flow cytometry (BD LSRFortessa), analyses being restricted to viable cells. Alternatively, lymph node cells were re-stimulated with 120 Gy-irradiated B16K1 for 3 days, and cytokines secreted into the culture medium were measured by cytometric bead arrays (BD Cytometric Bead Assay, mouse Th1/Th2/Th17), according to the manufacturer’s instructions.

*Analysis of Treg and MDSC content in tumors:* One million B16K1 cells were intra-dermally and bilaterally injected in WT or TNF-/- C57BL/6 mice. At day 12, mice were sacrificed and tumors were collected and digested with collagenase D (Roche Diagnostics) for 30 min at 37°C. Cells were stained with the indicated antibodies and live-dead reagents prior to flow cytometry analysis.

*In vivo tumorigenesis:* 3x105 B16-BL6or1x106 Yumm cells (*BrafV600E/+*, *Pten*-/-, *Cdkn2a*-/- C57BL/6 primary mouse melanoma cells) were intra-dermally injected in WT and TNF-/- C57BL/6 mice. 3x105 B16K1 cells were intra-dermally and bilaterally injected in WT, TNF-R1-/- and TNF-R2-/- C57BL/6 mice. Tumor volume was calculated using a calliper at the indicated days.

*In vivo validation of Etanercept*: WT mice were intraperitoneally injected, or not, with LPS (10 μg) every third day. As indicated, mice were intraperitoneally injected with Etanercept (3 mg/Kg) 3 days before LPS injection. One hour after LPS injection, blood sample was collected and TNF serum concentration was determined by ELISA.

*Spleen cell-mediated cytotoxicity of B16K1 cells:* One million B16K1 cells were intra-dermally and bilaterally injected in WT or TNF-/- C57BL/6 mice. At day 10-12, mice were sacrificed and spleens were collected. After lysis of red blood cells, spleen cells were stained with CFSE (5 μM in PBS for 15 min at 37 °C) in order to distinguish these cells (effectors) from B16K1 target cells. Then, effector spleen cells were co-incubated for 4h with B16K1 target cells at different Target/Effector cell ratios. Immediately before FACS analysis, 7-AAD was added to each sample to stain dead cells. Analysis of dead cells was restricted to target cells (CFSE negative).

**Legend to Supplementary Figures**

**Supplementary Figure S1: TNF does not modulate *in vitro* B16 proliferation.** **A**, B16F10 and B16K1 cells were analysed for TNF-R1 and TNF-R2 expression by flow cytometry. **B**, B16F10 and B16K1 cells were incubated in the presence or absence of the indicated TNF concentrations. Cell proliferation was analysed at the indicated times by counting cells. Data are means ± sem of triplicate determinations and are representative of 3 independent experiments. **C**, B16K1 cells were cultured under conditions allowing growth of spheroids in the presence or absence of 50 ng/mL TNF. Data are means ± sem of 5 independent experiments. Right, micrographs depict representative spheroids after 12 days culture.

**Supplementary Figure S2: Tumor growth of B16-BL6 and Yumm melanoma cell lines in TNF-deficient mice. A,** B16-BL6 cells were intra-dermally injected in WT (n=11) and TNF-deficient (n=9) mice. **B,** Yumm primary mouse melanoma cells were intra-dermally injected in WT (n=8) and TNF-deficient (n=10) mice. **A-B**, left panels: basal MHC-I expression at the cell surface of the different cell lines was determined by flow cytometry (dashed line: secondary antibody alone; solid line: anti-H2Kb plus secondary antibody). Right panels: tumor volume was assessed at the indicated days. Data are means ± sem of two independent experiments.

**Supplementary Figure S3: Analysis of the immune response in spleen from WT and TNF-deficient mice.** B16K1 cellswere intra-dermally and bilaterally injected in WT (n=3) and TNF-deficient (n=5) C57BL/6 mice. Animals were sacrificed 12 days after. **A,** Spleen cells were analysed by flow cytometry. **B,** Spleen cells were stained with CFSE and co-cultured with B16K1 target cells. After 4 hours, cytotoxicity was evaluated by flow cytometry using 7-AAD uptake by target cells. Data are from three independent experiments performed in duplicate and are means ± sem.

**Supplementary Figure S4: Analysis of the immune response in draining lymph nodes from WT and TNF-deficient mice.** B16K1 cellswere intra-dermally and bilaterally injected in WT (n=7-10) and TNF-deficient (n=6-9) C57BL/6 mice. Animals were sacrificed 12 days after. **A** and **B**, Draining lymph nodes from both sides were collected, pooled, cells were counted and the indicated cell populations were numerated by flow cytometry. **C**, Draining lymph node cells were collected and incubated with irradiated B16K1 cells. Three days later, cytokine concentrations in the cell culture medium were determined by cytometric bead array technology. **A-C**, Bars represent mean values of 2 independent experiments.

**Supplementary Figure S5: Analysis of Treg and MDSC content in the tumors from WT and TNF-deficient mice.** B16K1 cells were intra-dermally injected in WT and TNF-deficient C57BL/6 mice, and mice were sacrificed 12 days after. **A,** The proportion of Treg (CD4+ Foxp3+ T cells) was determined by flow cytometry. Left panels are representative density plots and values are the percentages of Foxp3+ cells among the CD4+ T cells. Right panel, percentages of CD4+ Foxp3+ T cells among the total cells. Bars represent mean values from 4 to 6 mice. **B,** The proportion of MDSC (CD45+ Gr1+ CD11b+) was determined by flow cytometry. Left panels are representative density plots, and values are the percentages of MDSC among the CD45+ cells. Right panel, percentages of CD45+ Gr1+ CD11b+ cells among the total cells. Bars represent mean values from 5 mice of each group. A and B, Data are representative of 2 independent experiments.

**Supplementary Figure S6: Accumulation of CD8+ TIL in TNF-R1-deficient mice. A**, B16K1 cells were injected in WT, TNF-R1-/- or TNF-R2-/- mice. Tumor volume was determined at the indicated days. Left panels: data are means ± sem of 3 independent experiments and include 10 to 12 mice per group. Right panels: values determined at the indicated days for individual tumors are depicted. Bars represent mean values. **B-D,** B16K1 cellswere intra-dermally and bilaterally injected in WT (n=4), TNF-deficient (n=6) and TNF-R1-deficient (n=6) C57BL/6 mice, and mice were sacrificed 12 days after. **B,** The intratumor proportion of CD8+ T cells among total cells was determined by flow cytometry. **C,** The proportion of tumor-infiltrating CD8 T lymphocytes specific for a TRP2 peptide among total cells was evaluated by dextramer technology. **D,** Tumors from TNF-R1-deficient mice (n=6) were analysed by immunohistochemistry to evaluate CD31+MECA-79- (non-HEV) and CD31+MECA-79+ (HEV) vessels as well as CD8+ T cells. Five out of 6 tumors exhibited HEV (MECA-79+) vessels. Left panel: Representative picture; scale bar: 50 μm. Right panel: The number of CD8+ T cells was quantified in a 100 μm diameter region around the HEV and non-HEV vessels. Bars represent mean values.

**Supplementary Figure S7: Effect of Etanercept injection in mice. A,** WT mice (n=3) were intraperitoneally injected, or not, with LPS (10 μg) and Etanercept (3 mg/Kg) 3 days before LPS injection. One hour after LPS injection, TNF serum concentration was determined by ELISA. This protocol was carried out up to 4 weeks. The cumulative number of Etanercept injections throughout the protocol is indicated. Data are means ± sem of values determined in 3 mice and are representative of 2 independent experiments. **B,** Etanercept or vehicle was intraperitoneally injected 3 days before grafting 1 million B16K1 intra-dermally and bilaterally in WT mice (n=5-6). Etanercept and vehicle injection was next repeated every third day. At day 12, mice were sacrificed, and draining lymph nodes were collected, cells were analysed by flow cytometry to determine CD4+ and CD8+ T cell absolute numbers. Bars represent mean values. **C,** 3x105 B16K1 cells were intra-dermally injected in INFγ-deficient (left panel) and nude (right panel) mice. Tumor volume was determined at the indicated days. Left panels: data are means ± sem of values measured in 3 to 6 mice for each group and are representative of two independent experiments. **D,** One million B16K1 cells were intra-dermally injected in WT mice (n=3). Three days before sacrifice, a single intraperitoneal injection of Etanercept (3 mg/Kg) or vehicle was performed. At day 12, mice were sacrificed and tumors were collected and analysed by immunohistochemistry after staining CD31, MECA79 and CD8. HEVs surrounded by CD8+ T cells were found in 2 mice out of 3 injected with Etanercept. Scale bars: 50 μm.