Supplementary Figure 1: TNFR1 deficiency strongly enhances the anti-PD-1 response in experimental melanoma. C57BL/6 wild-type (WT) and TNFR1-deficient mice were intradermally and bilaterally grafted with 3x10^5 B16K1 melanoma cells prior to intraperitoneal injection of anti-PD-1 antibodies (αPD-1, 10 mg kg⁻¹) or a relevant isotype control (Iso, 10 mg kg⁻¹) at days 6, 9 and 13 (n=6 mice per group). a, Tumor volumes were determined with a calliper. Individual curves are depicted for each tumor. Numbers indicate how many tumors regressed out of total tumors. Results are representative of two experiments. b, Values determined at day 27 for individual tumors are depicted. Bars represent mean values ± s.e.m and are representative of two experiments. A Mann-Whitney U test was used and differences were considered to be statistically significant when p<0.05 (*p<0.05; **p<0.01; ***p<0.001). c, Cumulative survival curves. At day 60, surviving mice were challenged with a second injection of B16K1 cells as indicated by the arrow. For statistical analysis of survival, the log-rank test was used and differences were considered to be statistically significant when p<0.05 (**p<0.01; ***p<0.001).
Supplementary Figure 2: anti-TNFR1 blocking antibodies potentiate anti-PD-1 efficacy. C57BL/6 WT mice were intradermally and bilaterally grafted with $3\times10^5$ B16K1 melanoma cells. Mice received two injections of anti-TNFR1 and anti-PD-1 antibodies at days 6 and 9 (10 mg kg$^{-1}$) alone or in combination. Alternatively, mice were injected with isotype control. Tumor volumes were determined with a calliper at the indicated days. a, Individual curves are depicted for each tumor. Numbers indicate how many tumors regressed out of total tumors. b, Data are means ± s.e.m. of at least 4 mice per group of one experiment. A two-way Anova with Tukey multiple comparison test was used and differences were statistically significant (*p<0.05; **p<0.01; *** p<0.001) at the indicated times.
Supplementary Figure 3: Deficiency in TNF or TNFR1 potentiates anti-PD-1 therapy in established Lewis lung carcinoma (LLC) tumors. a, MHC-I expression level on LLC was evaluated using flow cytometry: blue histogram, MHC-I staining; grey histogram, isotype control. Numbers indicate the mean fluorescence intensity. C57BL/6 wild-type (WT), TNF-deficient (TNF-/−) and TNFR1-deficient (TNFR1-/−) mice were intradermally and bilaterally grafted with 4x10^5 LLC cells prior to intraperitoneal injection of anti-PD-1 antibodies (αPD-1, 10 mg kg\(^{-1}\)) or a relevant isotype control (Ctrl, 10 mg kg\(^{-1}\)) at days 6, 9 and 13. b, Tumor volumes were determined with a calliper at the indicated days. Data are mean ± s.e.m of at least 4 mice per group analysed in one experiment. A two-way Anova with Tukey multiple comparison test was used and differences were statistically significant (*p<0.05; **p<0.01; *** p<0.001) at day 21. c, Values determined at day 21 for individual tumors are depicted. Bars represent mean values ± s.e.m. A Mann-Whitney U test was used and differences were considered to be statistically significant when p<0.05 (*p<0.05; **p<0.01; ***p<0.001) (b, c).
Supplementary Figure 4: TNF deficiency did not modify cell proliferation in CD4+ and CD8+ TIL upon anti-PD-1 therapy. C57BL/6 wild-type (WT) and TNF-deficient (TNF/-) mice were injected as described in the legend to Fig. 2. At day 10, the TIL content was analysed by flow cytometry. The proportion of CD8+ and CD4+ TILs expressing KI67 was evaluated. Left panels: representative stainings. Right panels: values from one experiment measured in 6 tumors per group are represented as Tukey boxes. No significant differences were found between both groups when using a Mann-Whitney U test.
Supplementary Figure 5: Analysis of cell death in TILs. Mice were intradermally and bilaterally grafted with $1 \times 10^6$ B16K1 melanoma cells. At day 10, tumors were collected and dissociated, and tumor cell content was analysed by using flow cytometry after cell incubation with Live/dead reagents, anti-CD45, anti-Thy1, anti-CD4 and anti-CD8 antibodies. The gating strategy of TIL was as follows: (i) cell debris were first excluded from the analysis by using FSC-A and SSC-A parameters; (ii) analysis was next restricted to single cells by using FSC-A and FSC-H parameters; (iii) CD4+ TILs and CD8+ TILs were next gated among CD45+Thy+ cells; (iv) Live/dead reagent staining was analysed as indicated to determine the proportion of dead cells among CD4+ and CD8+ TILs.
Supplementary Figure 6: Chemokine mRNA expression analysis. C57BL/6 wild-type (WT) and TNF-deficient (TNF-/-) mice were intradermally and bilaterally grafted with 1x10^6 B16K1 melanoma cells prior to intraperitoneal injection of anti-PD-1 antibodies (αPD-1, 10 mg kg^-1) at day 7. Chemokine transcript levels were quantified in tumors from 6 mice per group and relative expression was normalized to housekeeping gene. Data from one experiment are represented as Tukey box. No significant differences were found between both groups by using a Mann-Whitney U test.
Supplementary Figure 7: IFN-γ and granzyme B production in wild-type and TNF-deficient mice. C57BL/6 wild-type (WT), TNF-deficient (TNF-/−) and CD8-deficient (CD8-/−) mice were injected as described in the legend to Fig. 2. a and b, At day 10, TNF (a) and IFN-γ (b) transcript levels were quantified in tumors. Data are means ± s.e.m. of at least 4 tumors per group analysed in one experiment. (Mann-Whitney U test: *p<0.05; **p<0.01; ***p<0.001). c and d, The TIL content was analysed by flow cytometry after 4 hours incubation with PMA and ionomycin. The proportion of CD8+ TILs expressing IFN-γ (c) and that of CD8+ TILs expressing granzyme B (GRZ) (d) were determined. FMO: Fluorescence Minus One controls. Upper panels: representative stainings. Lower panels: values measured in 5-12 tumors per group from two experiments are represented as Tukey boxes. (Student’s t test: *p<0.05; **p<0.01; ***p<0.001).
Supplementary Figure 8: TNF induces PD-L1 and/or PD-L2 expression on TILs and DC.
WT and TNF-deficient (TNF-/-) mice were injected as described in the legend to Fig. 2. TILs were analysed by flow cytometry on tumors developed at day 10. **a**, Representative staining for PD-L1 (left panels) and PD-L2 (right panels) on CD8+ and CD4+ TILs from WT and TNF-deficient mice injected with anti-PD-1. Grey histograms: isotype control. **b**, Mean of fluorescence intensity (MFI) for the PD-L1 staining on CD8+ TILs (left panel) and CD4+ TILs (right panel). **c**, Percentage of tumor DCs among total cells. **d-f**, Representative plots showing the expression of PD-L1 (left panel) and PD-L2 (right panel) on DCs in tumors from WT and TNF-deficient mice (d). Mean of fluorescence intensity (MFI) of PD-L1 (e) or PD-L2 (f) staining on DCs. **b, c, e and f**, Values measured in at least 6 tumors per group from one (out of two) representative experiment are represented as Tukey boxes (Mann-Whitney U test: *p<0.05; **p<0.01).
Supplementary Figure 9: TNF deficiency impairs anti-PD-1-treated TIM-3 up-regulation on T lymphocytes. a and b, WT and TNF-deficient (TNF-/−) mice were injected as described in the legend to Fig. 2. TIM-3 expression on CD8+ (a) and CD4+ TILs (b) was determined by flow cytometry on tumors developed at day 10. Upper panels: representative staining on TILs from WT (blue histogram) and TNF-deficient (red histogram) mice injected with anti-PD-1. Lower panels: Mean Fluorescence Intensity (MFI) measured in at least 11 tumors per group from two independent experiments are represented as Tukey boxes (Mann-Whitney U test: *p<0.05; **p<0.01).
Supplementary Figure 10: TNF does not induce TIGIT, LAG-3 and CTLA-4 expression on CD8+ TILs. The mean of fluorescence intensity (MFI) of TIGIT, LAG3 and CTLA-4 staining on CD8+ TILs was determined by flow cytometry on tumors developed at day 10 in WT and TNF-deficient (TNF-/-) mice that had received one injection of anti-PD-1 antibodies (αPD-1, 10 mg kg⁻¹) or vehicle (PBS) at day 7. Values measured in at least 11 tumors per group from two independent experiments are represented as Tukey box. A Mann-Whitney U test was used (ns: not significant; *p<0.05; **p<0.01).
Supplementary Figure 11: IFN-γ and Granzyme B expression in TIM-3+ and TIM-3-CD8+ TILs. WT and TNF-deficient (TNF-/−) mice were injected as described in the legend to Fig. 2. TILs from tumors developed at day 10 were stimulated with PMA/ionomycin in the presence of brefeldin A and the proportion of CD8+ TILs expressing granzyme B (GRZ) and IFN-γ was analysed by flow cytometry. a, Representative stainings; values indicate the proportion of cells in the different quadrants. b, Quantification of the proportion of GRZ+ and IFN-γ+ cells among the TIM-3+ and TIM-3- CD8+ TILs. c, Proportion of cell death of the indicated populations among CD8+ T cells. Values measured in 5-6 mice per group from one experiment are represented as Tukey boxes (b and c: Two-way Anova with Tukey’s multiple comparison test: *p<0.05; ***p<0.001).
Supplementary Figure 12: TNF dose-dependently induces TIM-3 up-regulation on activated CD8+ T lymphocytes in vitro. a, Activated CD8+ T cells from WT mice were incubated with murine TNF for 2 days. The mean fluorescence intensity (MFI) of TIM-3, LAG3 and TIGIT was next analysed by flow cytometry. Data are means ± s.e.m. of three independent experiments. b, Similar experiment as described in "a" was conducted by incubating cells with 1 µM LY294002 (PI3K inhibitor), 10 µM PD98059 (MEK inhibitor), 1 µM SB203580 (p38 MAPK inhibitor) or vehicle (DMSO) in the presence or absence of 2.5 ng mL⁻¹ TNF. Percentages of CD8+TIM-3+ T cells were next determined by flow cytometry. Data are means ± s.e.m. of four independent experiments. A Mann-Whitney U test was used and differences were statistically significant (***p<0.01, TNF vs vehicle; ###p<0.01, LY294002 or PD98059 vs vehicle). c, Human CD8+ T cells were incubated with different concentrations of human TNF for 2 days. TIM-3 expression was next analysed by flow cytometry. Histograms represent the MFI after subtracting basal fluorescence intensity. Data are means ± s.e.m. of two experiments. d, TILs from one human melanoma metastasis (patient 2) were cultured as described in Figs 4b and c with increasing concentrations of human recombinant TNF for 48h. TIM-3 expression on re-stimulated CD8+ TILs was analysed by flow cytometry.
Supplementary Figure 13: anti-TNF antibodies overcome the resistance to anti-PD-1 therapy in experimental breast carcinoma. 1x10^5 4T1 breast carcinoma cells were orthotopically injected into the mammary fat pad of WT Balb/c mice. Mice were next injected with vehicle, isotype control, anti-TNF, anti-PD-1 or the combination of anti-PD-1 and anti-TNF (10 mg kg⁻¹ of each antibody). A two-way Anova with Tukey multiple comparison test was used and differences were statistically significant (*p<0.05; *** p<0.001) at the indicated times between the group of mice, which received the combo anti-PD-1 and anti-TNF and the isotype control group. Data are mean values ± s.e.m. of 6 mice per group from one experiment.