Supplementary Figures

Figure S1.

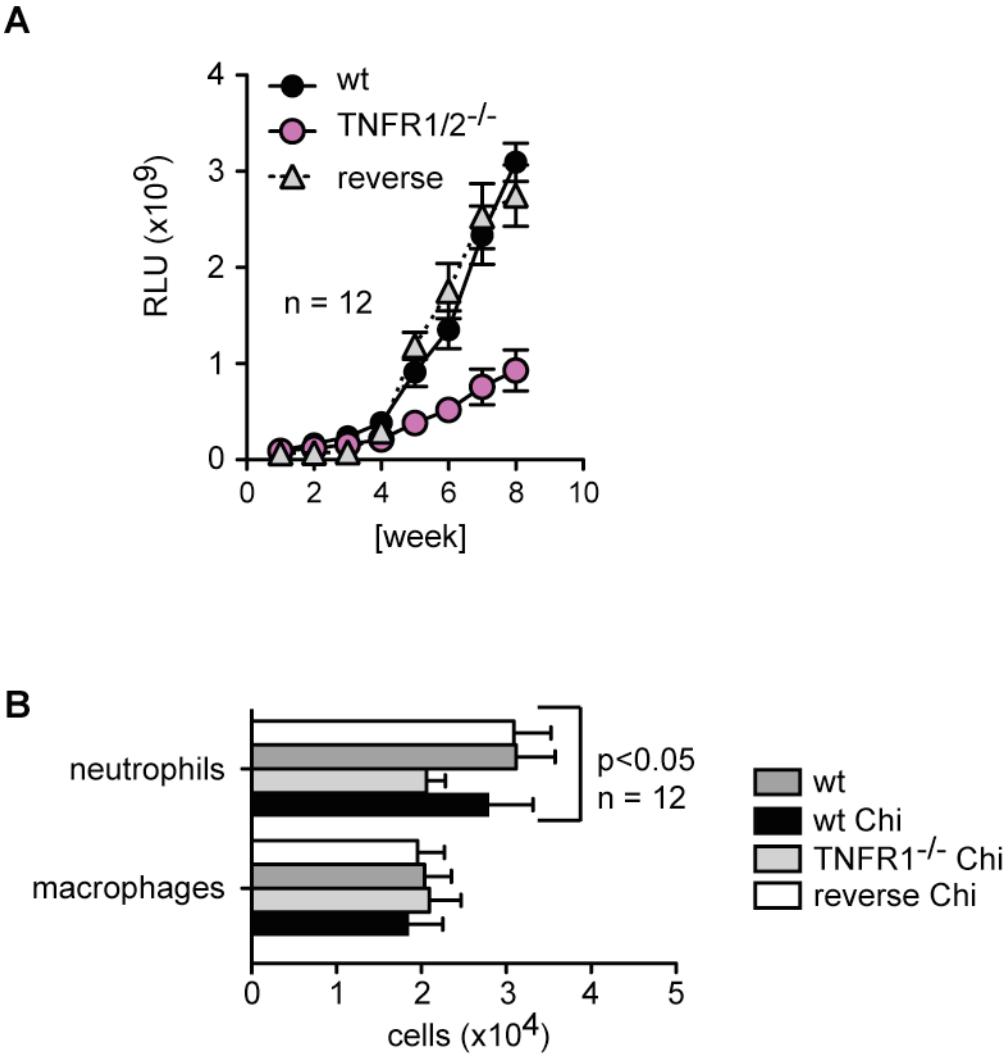


Figure S2.

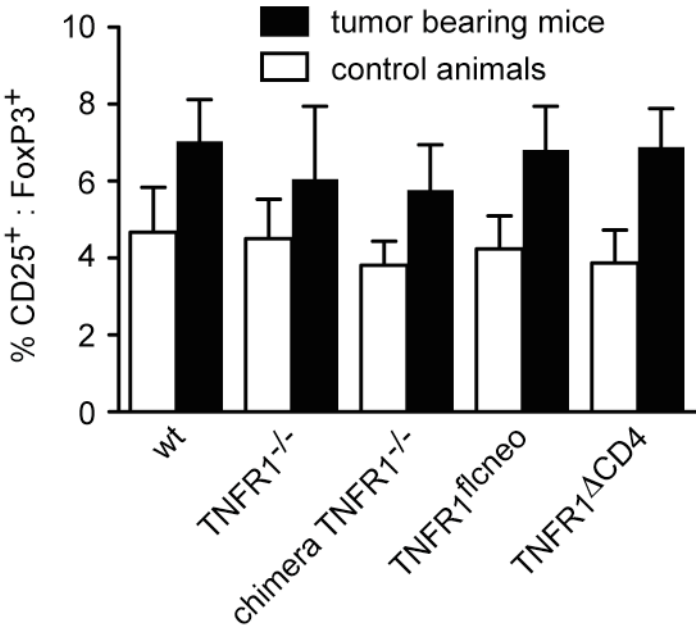

tumor bearing mice
control animals
% CD25+ : FoxP3+
10
8
6
4
2
0
wt
TNFR1-/-
chimera TNFR1-/-
TNFR1flcneo
TNFR1ΔCD4

Figure S3.

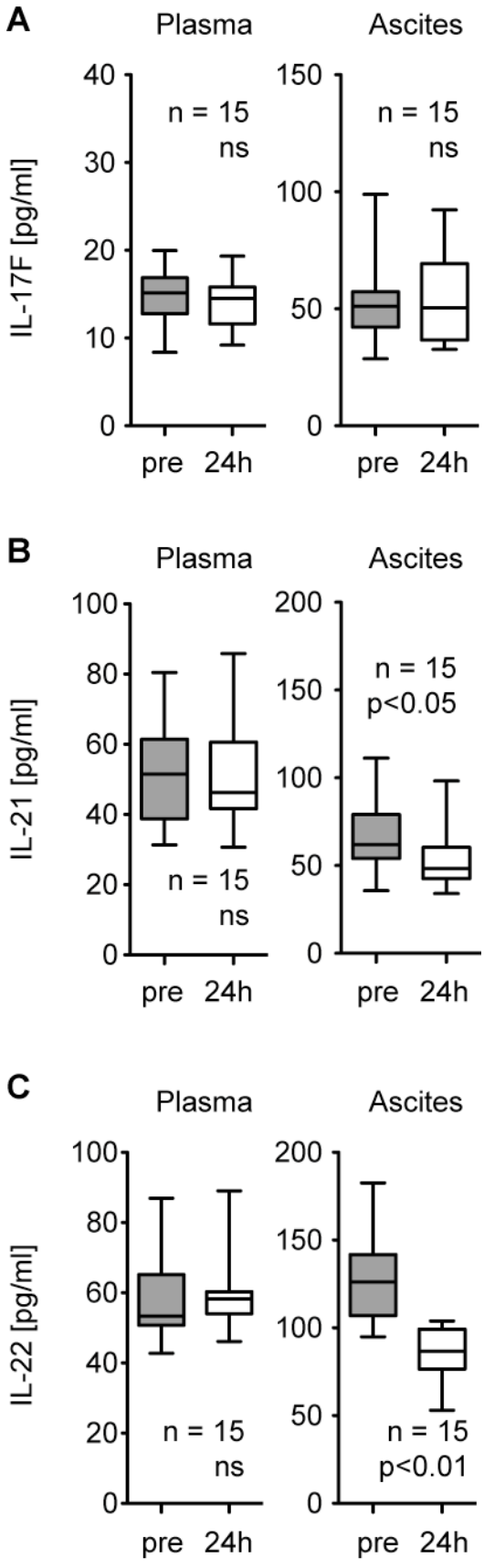
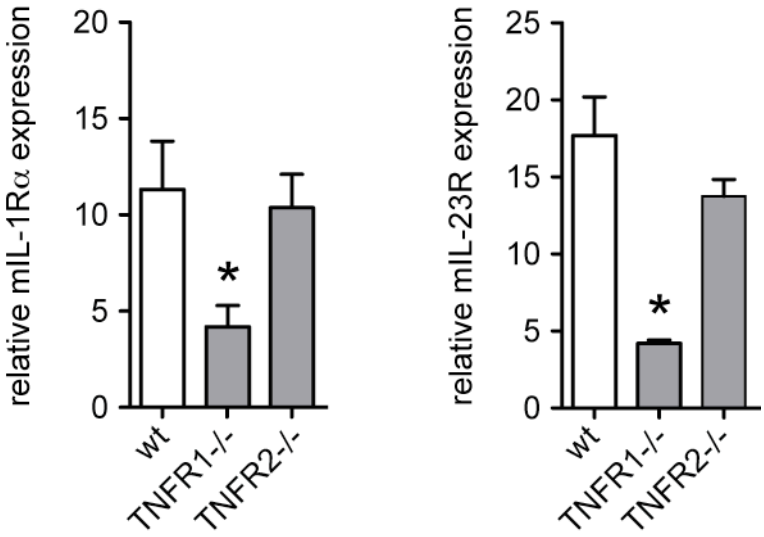

A
Plasma
Ascites
IL-17F [pg/ml]
40
30
20
10
0
150
100
50
0
n = 15
ns
n = 15
ns
pre
24h
pre
24h
B
Plasma
Ascites
IL-21 [pg/ml]
100
80
60
40
20
0
200
150
100
50
0
n = 15
ns
n = 15
p<0.05
pre
24h
pre
24h
C
Plasma
Ascites
IL-22 [pg/ml]
100
80
60
40
20
0
200
150
100
50
0
n = 15
ns
n = 15
p<0.01
pre
24h
pre
24h

Figure S4.


relative mIL-1Rα expression
20
15
10
5
0
*
wt
TNFR1-/-
TNFR2-/-
relative mIL-23R expression
25
20
15
10
5
0
*
wt
TNFR1-/-
TNFR2-/-

Supplementary methods.

Blood and ascites sample collection. Whole blood (10 ml) for plasma and serum was collected prior to and one hour after the end of each infliximab infusion, 1 hour (serum), 24 hour (plasma) and 48 hour (plasma) after the first infusion only and four weeks after the final infusion (plasma, end of study). All samples of serum or plasma were snap frozen in liquid nitrogen and stored at –80°C until analysis. Whole blood samples were collected into tubes containing preservative-free heparin (30 units/ml blood; Leo Laboratories Ltd., Princes Risborough, United Kingdom) and phytohaemagglutinin (PHA. 2 µg/ml blood; HA 16/17; Murex Biotech Ltd., Dartford, United Kingdom). Briefly, blood samples were incubated in presence of PHA at 5% CO2, 37°C for 24 hours, centrifuged at 1500 rpm for 10 minutes at 4°C to gain plasma samples, aliquoted and snap frozen.

Ascitic fluid was collected prior to treatment. Two hours after the infliximab administration, the drain was re-opened and ascitic fluid was collected at 24 hour intervals until end of drainage. Re-accumulated ascites was also collected during treatment or in the following 6 months after the completion of the trial.

Processing of ascitic fluid from patients with ovarian cancer. A 500 mL aliquot of ascitic fluid from each 24 hour interval was centrifuged at 1500 rpm for 5 min at 4°C. The ascitic drain was clamped after each paracentesis and not flushed to avoid infection complications. The ascitic fluid was snap frozen in liquid nitrogen and stored at –80°C until analysis. The remaining ascitic cell pellet was washed in RPMI-1640 medium and centrifuged at 1500 rpm for 5 min at

4°C. The ascitic cells were incubated with sterile erythrocyte lysis buffer (150mM NH4Cl, 10mM KHCO3, 100mM Na2EDTA, ph 7.4) for 5 min at room temperature and re-centrifuged. The ascitic cells were snap frozen in liquid nitrogen and stored at -80°C until analysis.

Supplementary Data

Figure S1. A. TNFR1/2$^{-/-}$ (double knock-outs) bone marrow, wt bone marrow and reverse (adoptive transfer of wt bone marrow in TNFR1/2$^{-/-}$ mice) chimeras and were i.p. injected with 10^7 ID8 cells/mouse. Tumor burden was monitored weekly *in situ* by bioluminescence. TNFR1/2$^{-/-}$ bone marrow chimeras mirrored the disease stabilization in TNFR1$^{-/-}$ chimeras ($p<0.01$). Data are represented as mean ± sem of n=12. **B.** Total neutrophil and macrophage count in the ascitic fluid of ID8 tumor bearing chimeras.

Figure S2. A. FACS analysis of the CD4+/CD25+/FoxP3+ population in control and tumor bearing wt, TNFR1$^{-/-}$, TNFR1^{flcneo} and TNFR1ΔCD4 mice.

Figure S3. Analysis of other Th17 cytokines in plasma and ascites samples of patients with ovarian cancer treated with infliximab. **A.** IL-17F protein levels did not significantly change in plasma and ascites. **B.** IL-21 protein levels were significantly reduced in the ascitic fluid 24h post infliximab infusion ($p<0.05$); however there was no difference in IL-21 plasma levels. **C.** IL-22 protein levels were significantly reduced in the ascitic fluid 24h post infliximab infusion ($p<0.01$); however there was no difference in IL-22 plasma levels. Data are represented as mean ± sd of n=15.

Figure S4. Quantitative real-time analysis of IL-1Rα and IL-23R expression on $CD4^+CD25^-$ T cells after TNF-α stimulation. Data are represented as mean ± sd of n=6; * indicates significance of $p<0.01$.