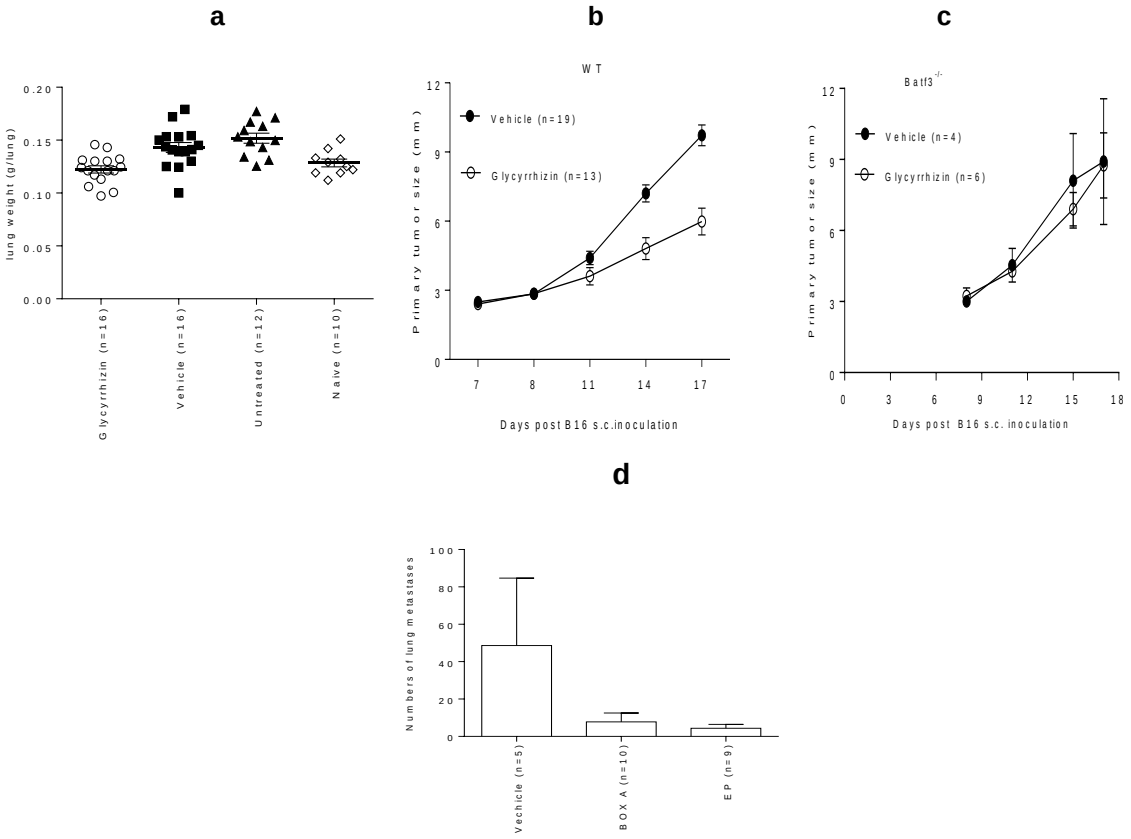
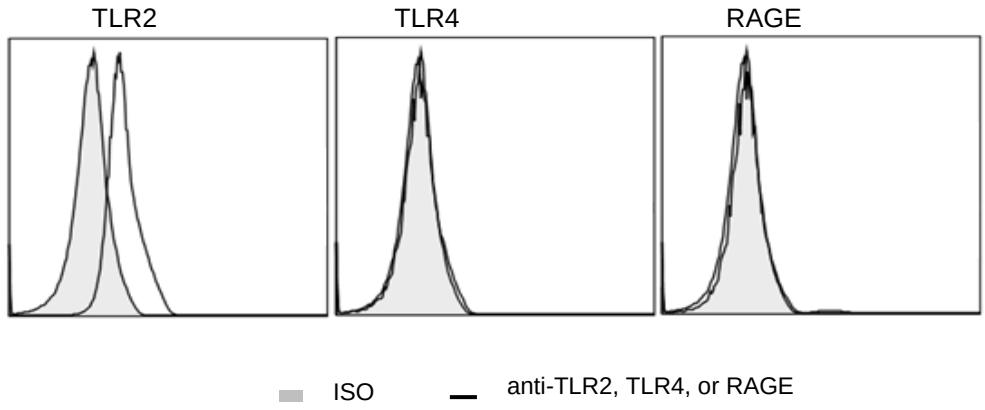
Supplemental Materials

Supplementary figure 1



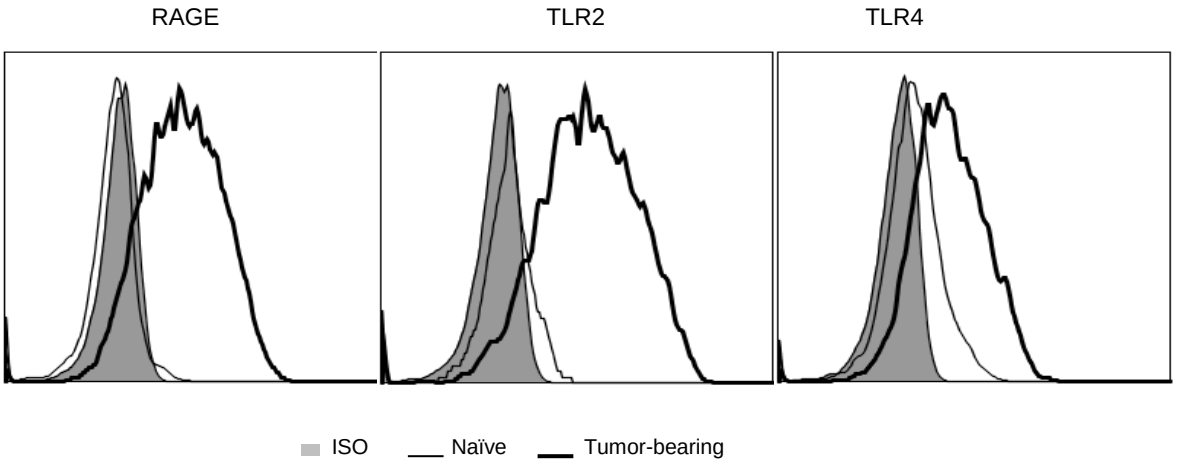
Supplementary figure 1: **a**) **I.t. inhibiting HMGB1 by GL in 4T1.2-Neu breast tumor reduces spontaneous lung metastases**. BALB/c mice were s.c. inoculated with 4T1.2-Neu. 8 d later, tumor-bearing mice were untreated or treated by GL or vehicle (Figure 5a). D 21 post tumor inoculation, lung metastases were determined by lung weights. Age-matched naïve mice served as controls. Data were statistically analyzed using a student's *t*-test. GL vs. vehicle or untreated: $p<0.01$; GL vs. naive: NS. **b-c) I.t. inhibiting HMGB1 by GL in B16 melanoma generates CD8α$^+$/CD103$^+$DC-dependent antitumor immunity**. B6-WT or -Batf3$^{-/-}$ mice were s.c. inoculated with B16. 8 d later, B16-bearing mice were treated by GL or vehicle and tumor growth was monitored (Figure 5a). Data were statistically analyzed using a student's *t*-test. GL vs. vehicle: $p<0.001$. **d**) **I.t. inhibiting HMGB1 by EP or Box A in 4T1.2-Neu breast tumor reduces spontaneous lung metastases**. Tumor-bearing BALB/c mice were untreated or treated as listed (Figure 5f). D 25 post tumor inoculation, lung metastases was determined by counting the numbers of tumor loci. Data were statistically analyzed by using a student's *t*-test. Box A or EP vs. vehicle: $p>0.05$.

Supplementary figure 2



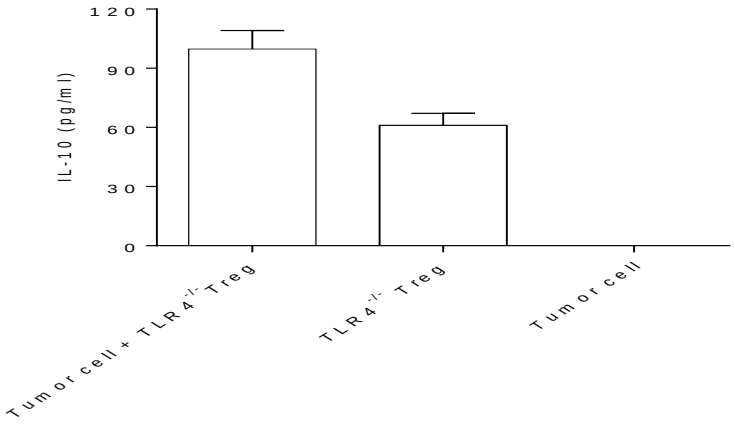
Supplementary figure 2: **Tumor cells express TLR2**. 4T1.2-Neu tumor cells were stained with anti-TLR2, -TLR4 or -RAGE and analyzed by flow cytometry (Figure 3a). Data showing the expression of TLR2, TLR4 or RAGE on tumor cells is representative of three independent experiments with a similar result.

Supplementary figure 3

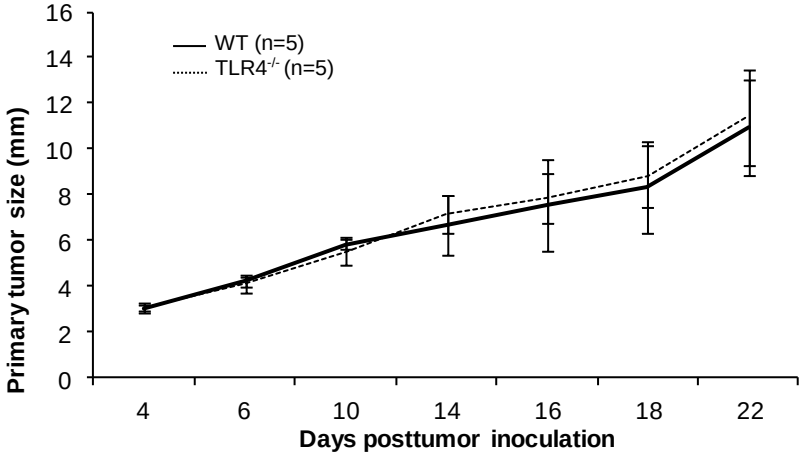


Supplementary figure 3: **Tumor progression induces the expression of HMGB1 receptors on tumor-associated Treg**. BALB/c or Foxp3-eGFP BALB/c mice were s.c. inoculated with 4T1.2-Neu tumor cells. 10 d later, single-cell suspensions of the TDLN (LN from naïve mice as control) were stained by anti-RAGE, -TLR2, or -TLR4 and analyzed by flow cytometry (Figure 3a). RAGE, TLR2 or TLR4 on Treg ($eGFP^+$) is shown. Results are representative of three independent experiments with a similar result.

Supplementary figure 4 **a**



b



Supplementary figure 4: **a**) **TLR4 on tumor-associated Treg is not required for tumor cell-enhanced IL-10 production**. TLR4$^{-/-}$-Treg were obtained from 4T1.2-Neu-bearing BALB/c-TLR4$^{-/-}$ mice [19]. Tumor TLR4$^{-/-}$-Treg ($2x10^5$) were cocultured with or without tumor cells in 200µl RPMI 1640 10%FBS at 37^0C, 5% CO_2 for 2 d (19). IL-10 in the supernatants was determined by ELISA. Data represents three independent experiments and were statistically analyzed by using student's *t* test. Tumor cell+TLR4$^{-/-}$Treg vs. TLR4$^{-/-}$Treg: $p<0.05$. **b**) **Deficiency of TLR4 does not affect tumor growth**. BALB/c-WT or-TLR4$^{-/-}$ mice were s.c. inoculated with 4T1.2-Neu tumor cells and tumor growth was monitored.