Supplementary Figure 1

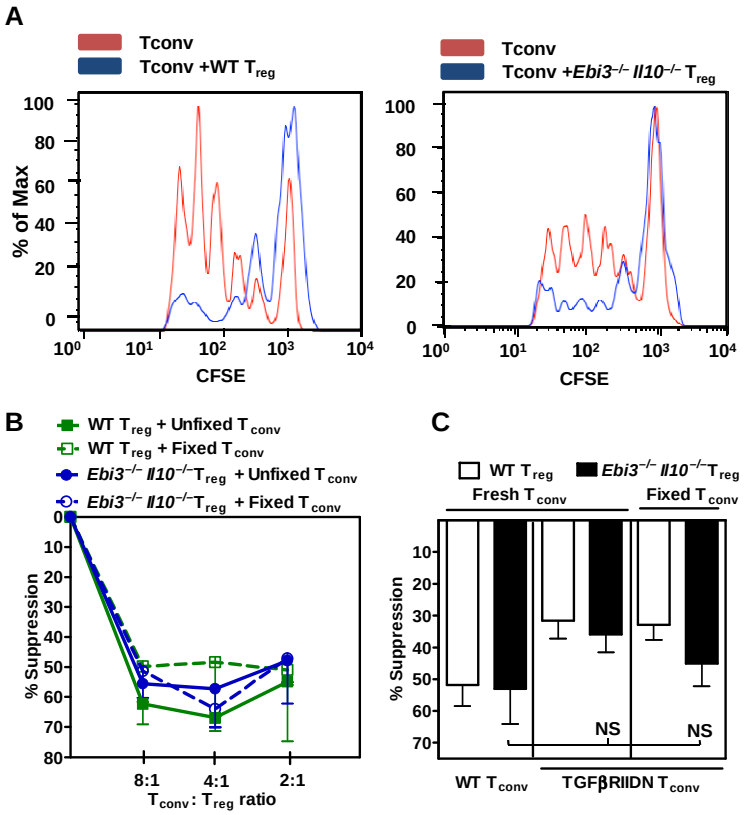

Figure S1. *Ebi3*$^{-/-}$*Il10*$^{-/-}$ T_{regs} are suppressive *in vitro*. (A) Wild type or knock out T_{reg} were cultured with CFSE-labeled wild type T_{conv} cells and anti-CD3 and anti-CD28 coated latex beads. The cells were analyzed 72h later on a FACS calibur and CFSE dilution was assessed. Data is representative of 2 independent experiments. (B) Wild type or knock out T_{reg} were cultured either with fixed or fresh wild type T_{conv} cells in the inserts of a Transwell™ culture plate in presence of anti-CD3 and anti-CD28 coated latex beads. Third party, wild type responder T_{conv} were activated in the bottom chamber of the plate. Proliferation of responder cells was determined by [^{3}H]-thymidine incorporation. Proliferation ranged from 30,000- 60,000 cpm. Data represents the mean ± SEM of 2-4 independent experiments. (C) Wild type or knock out T_{regs} purified by FACS were cultured with naive fresh or fixed wild type T_{conv} cells in the inserts of a Transwell™ culture plate in presence of anti-CD3 and anti-CD28 coated latex beads. in the presence of anti-CD3 and anti-CD28 coated beads for 72 h. Freshly purified wild type or TGFβ RII dominant negative responder T_{conv} cells were activated in the bottom chamber of a Transwell™ culture plate. Proliferation of responder cells was determined by [^{3}H]-thymidine incorporation. Results are mean ± SEM of 3 independent experiments. Statistical analysis was carried out with students t test, NS: Not significant.

Supplementary Figure 2

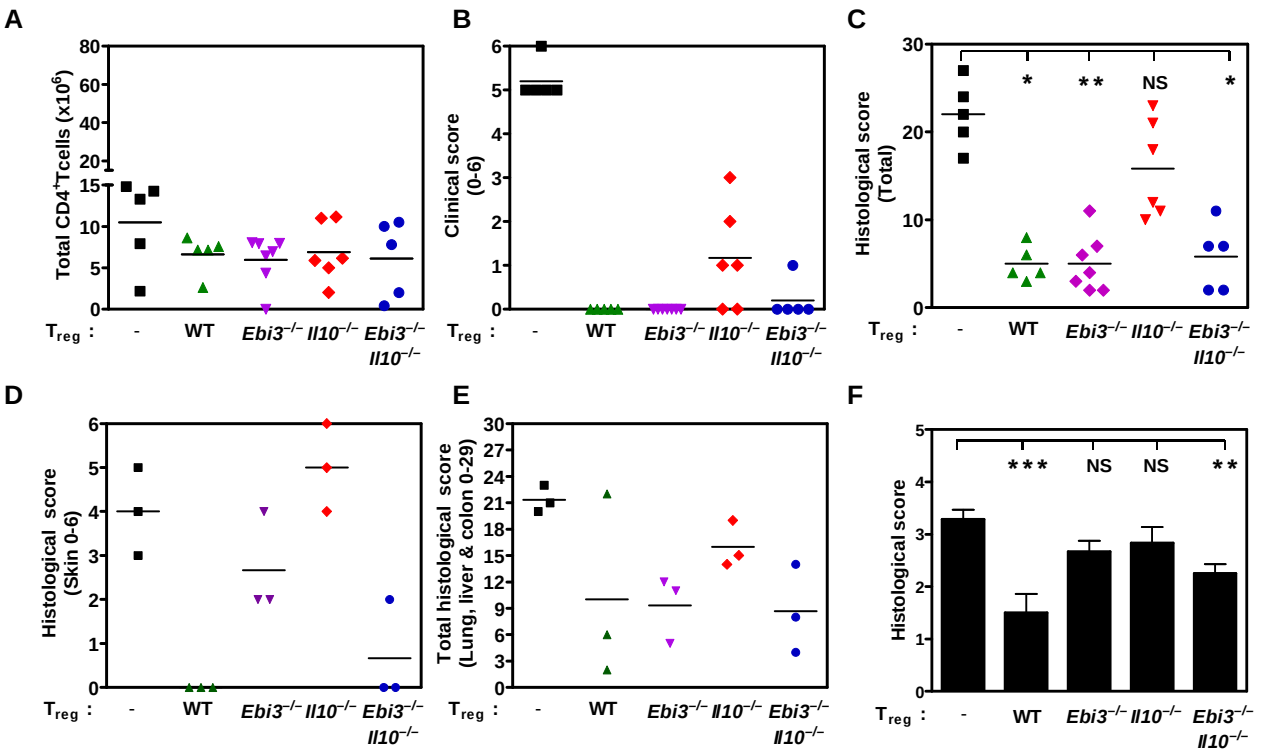

Figure S2. *Ebi3*$^{-/-}$*Il10*$^{-/-}$ T_{regs} can rescue *Foxp3*$^{-/-}$ phenotype and alleviate inflammation in an inflammatory bowel disease model. Wild type or knock out T_{regs} ($1x10^6$) were injected in to 2-3 day old *Foxp3*$^{-/-}$ pups. (A) CD4 T cell numbers were analyzed in the spleen 4 weeks after injection. (B) Clinical score of the pups were determined as described in the methods. (C) Lung, liver and ear pinna were collected at the time of analysis, sectioned and stained with H& E. The tissue sections were analyzed in a blinded manner as described in methods and total score is plotted. Data represents the mean of 5-7 mice per group. One way Anova., Dunn's Multiple comparison test, p value., **<0.005, *<0.05. Bone marrow from wild type or knock out T_{regs} were mixed in a 1:1 ratio with *Foxp3*$^{-/-}$ bone marrow and injected into sub lethally irradiated *Rag*$^{-/-}$ mice. The tissue sections were collected 4-5 weeks following injection and histological scores are plotted. (D) Histological score of the skin and (E) total histological score of lung, liver and colon are plotted. Data represents the mean of 3 mice per group.(F) Wild type T_{conv} cells ($0.5x10^6$) were injected into *Rag1*$^{-/-}$ mice. The weight of the mice was monitored weekly. Once the mice had lost 5% of its body weight, wild type or knock out T_{regs} were injected. Colonic tissue was collected and stained with H&E 4 weeks after T_{reg} injection . The tissue sections were analyzed in a blinded manner and the histological scores are plotted. Data represents the mean ±SEM three independent experiments with 6-12 mice per group. Statistical analysis was carried out with students t test, p value: *** <0.0005, **<0.005

Supplementary Figure 3

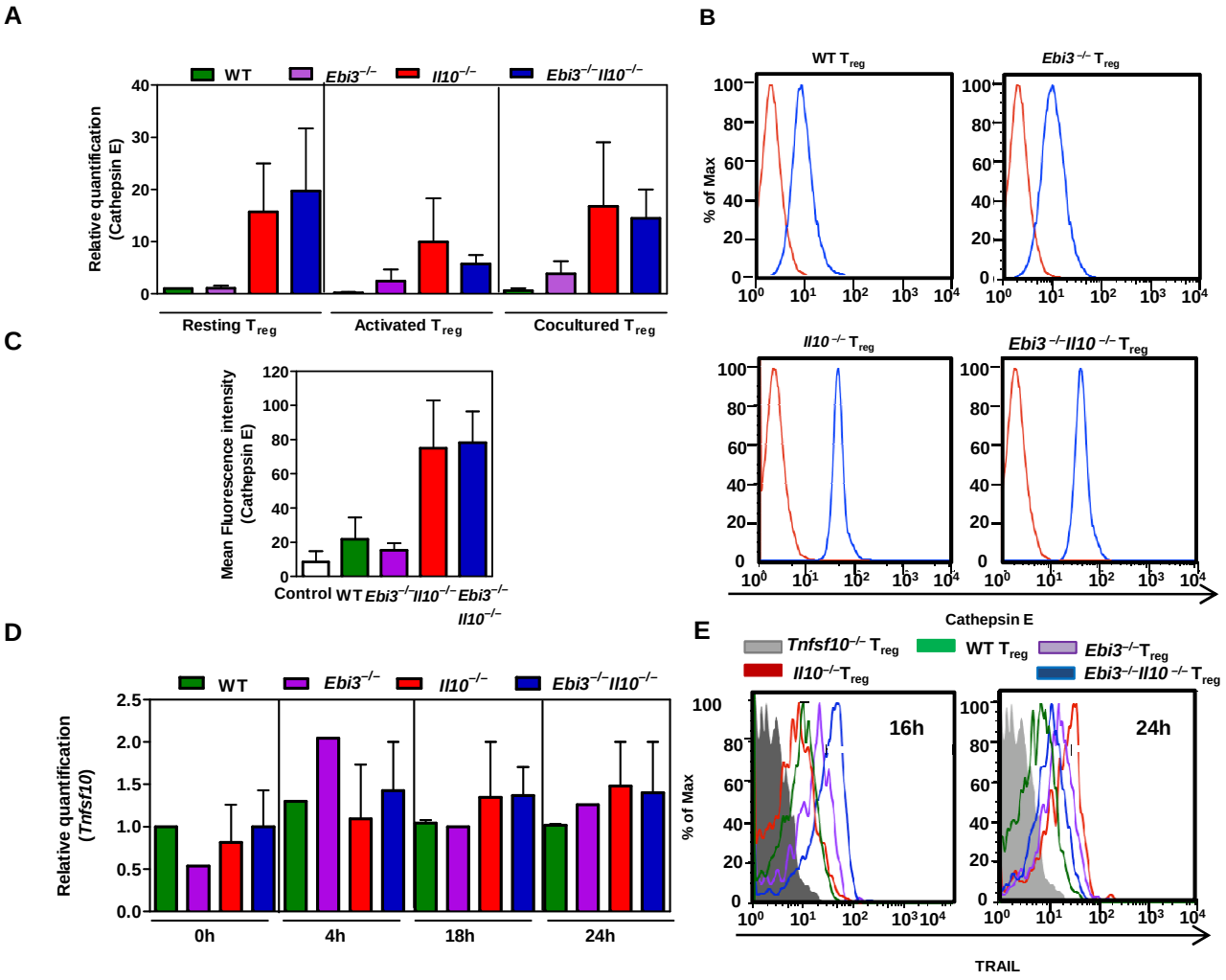

Figure S3. *Ebi3*$^{-/-}$*Il10*$^{-/-}$ T_{reg} expresses higher levels of Cathepsin E and TRAIL. Wild type or knock out T_{regs} were purified by FACS. (A) RNA was isolated from resting, activated or cocultured T_{regs} as described in the methods. cDNA was synthesized and qPCR was carried out for Cathepsin E with specific primers and probe. Relative expression of cathepsin E compared to wild type resting T_{reg} is shown. Data represent the mean ± SEM of 2-3 independent experiments. Statistical analysis was carried out with Anova, p=<0.05. (B) Wild type or knock out T_{regs} purified by FACS were stained for intracellular cathepsin E. Secondary antibody staining alone is shown in red and cathepsin E in blue. Representative histograms from 2-3 independent experiments are shown. (C) Mean fluorescence intensity of cathepsin E intracellular expression from 2-3 independent experiments is shown. Anova, p=<0.05. (D) Wild type or knock out T_{regs} were activated with anti-CD3 and anti-CD28 coated beads in presence of rIL2. mRNA was isolated and qPCR was carried out. Statistical analysis was carried out with Anova, NS (E) *Tnfsf10*$^{-/-}$, *Ebi3*$^{-/-}$, *Il10*$^{-/-}$, *Ebi3*$^{-/-}$*Il10*$^{-/-}$ T_{regs} were stained for TRAIL following activation for 16 and 24 h respectively. The cells were analyzed on a FACS calibur. *Tnfsf10*$^{-/-}$ T_{regs} were used as a control for staining. Representative histograms from three independent experiments are given.

Supplementary Figure 4

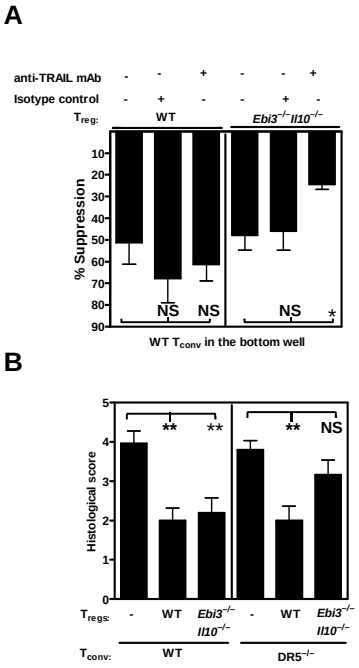

Figure S4. Abrogating TRAIL function with an anti-TRAIL mAb or TRAIL-R deficient target cells inhibits the suppressive capacity of *Ebi3*$^{-/-}$ *Il10*$^{-/-}$ T_{regs} *in vitro* and *in vivo*. (A) Wild type or knock out T_{regs} purified by FACS were cultured with wild type T_{conv} cells in the top compartment of a Transwell ™ culture plate. A neutralizing antibody to TRAIL was added to the wells at 10µg/ml concentration. Freshly purified wild type responder T_{conv} were activated in the bottom chamber of the plate. Proliferation of responder cells was determined by [^{3}H]-thymidine incorporation. Data represents the mean± SEM of 3 independent experiments. (B) Wild type or DR5 $^{-/-}$ T_{conv} cells ($0.5x10^6$) were injected into *Rag1*$^{-/-}$ mice. The mice was monitored weekly for weight loss. Once the mice had lost 5% of its body weight, wild type or *Ebi3*$^{-/-}$*Il10*$^{-/-}$ T_{regs} were injected. Colonic tissue was collected and stained with H&E 4 weeks after T_{reg} injection . The tissue sections were analyzed in a blinded manner and the histological scores are plotted. Statistical analysis was carried out with students t test. * < 0.05, **<0.005