Figure S1 Brockmann L. et al.

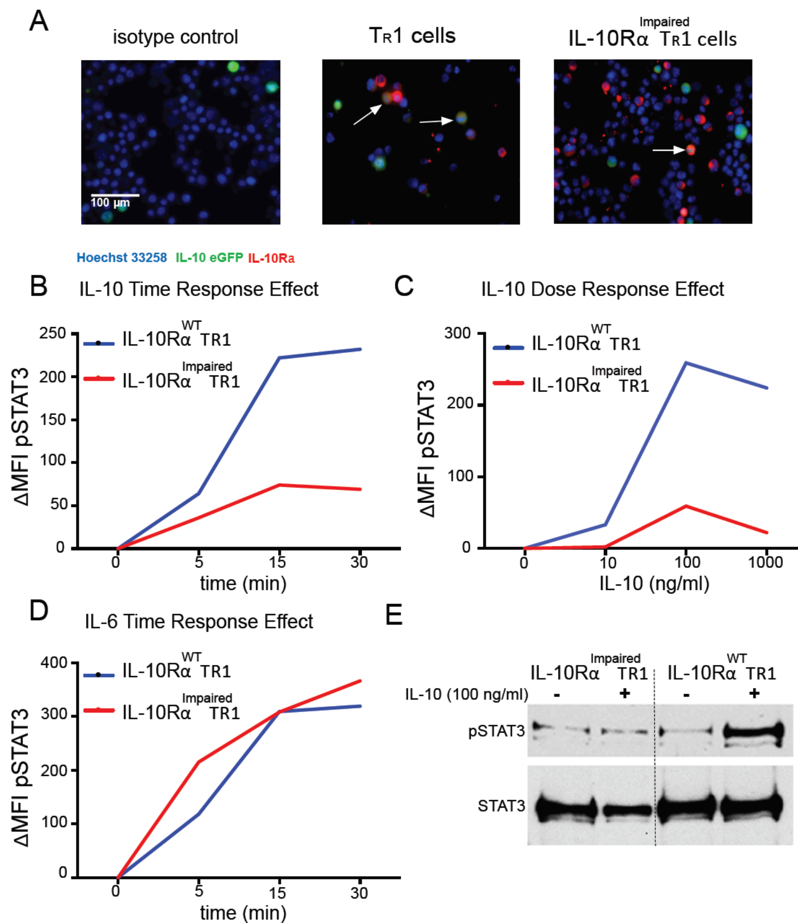

Fig. S1. ***In vitro*** **differentiated T_R1 cells can respond to IL-10.** (**A**) Immunofluorescence staining of IL-10Rα expression on *in vitro* differentiated IL-10Rα^{WT} or IL-10Rα^{Impaired} T_R1 cells. Data are representative of two independent experiments. (**B-C**) Naïve CD4^{+} T cells were isolated from wild type (IL-10Rα^{WT}) or CD4-DNIL-10R transgenic (IL-10Rα^{Impaired}) Foxp3^{RFP} IL-10^{eGFP} double reporter mice and cultured under T_R1 polarizing conditions. FACS-sorted T_R1 cells (CD4^{+}IL-10^{+}Foxp3^{-}) were re-stimulated in the presence or absence of IL-10. ΔMFI (compared to unstimulated cells) of pSTAT3 level as assessed by flow cytometry are shown. Cells were stimulated with IL-10 (**B**) or IL-6 (**C**) for indicated time points. (**D**) IL-10Rα^{WT} or IL-10Rα^{Impaired} T_R1 cells were stimulation for 20 min with the indicated concentrations of IL-10. Data are representative of two independent experiments, and were confirmed using Immunoblotting (three independent experiments) (**E**).

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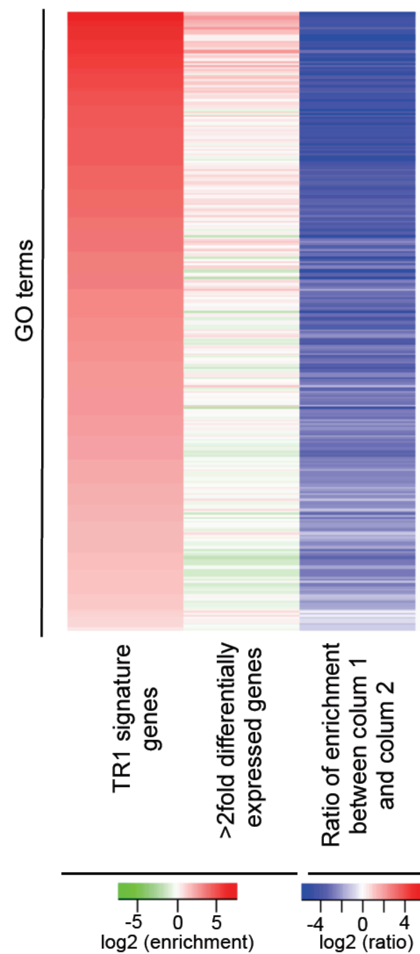

Fig. S2. More than two fold differentially expressed genes are not enriched over GO terms significantly enriched for T_R1 signature genes. Enrichment scores of T_R1 signature genes and >2-fold differentially expressed genes over GO terms significantly enriched for T_R1 signature genes. Enrichment scores (columns 1 and 2) are shown in log-scale for visualization. Column 3 shows the ratio in enrichment >2-fold differentially expressed genes versus T_R1 signature genes in log-scale for visualization.

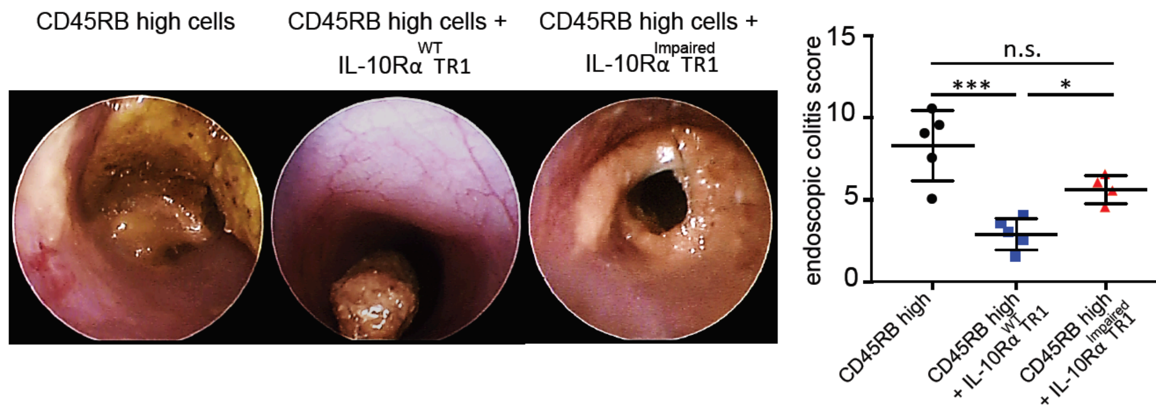

Fig. S3. IL-10 signaling in T_R1 cells is essential to prevent $CD4^+Foxp3^-CD45RB^{hi}$ T cell mediated colitis. *In vitro* differentiated IL-10Rα^{WT} or IL-10Rα^{Impaired} T_R1 were injected alone or together with $CD4^+Foxp3^-CD45RB^{hi}$ T cells into *Rag1*$^{-/-}$ mice. Endoscopy score 5 weeks upon transfer of two independent experiments are shown (CD45RB^{hi} n=5, CD45RB^{hi}+WT T_R1 n=5, CD45RB^{hi}+ IL-10Rα^{Impaired} T_R1n=4). One-way ANOVA (post-test Tukey) was used to calculate significance (* $p < 0.05$; *** $p<0.001$).

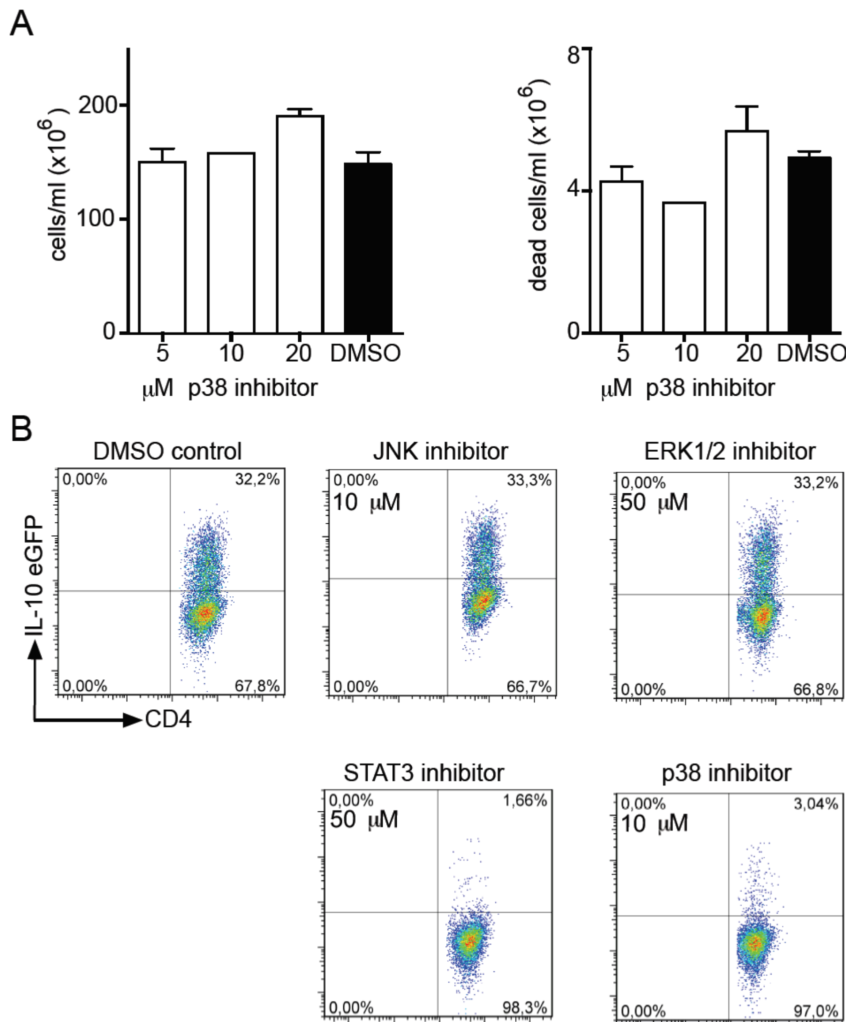

Fig. S4. p38 MAPK inhibition does not result in an increased proliferation of IL-10 negative cells and blocks the differentiation of T_R1 cells *in vitro.* (**A**) $CD4^+$ T cells were isolated from wild type $Foxp3^{RFP}$ $IL\text{-}10^{eGFP}$ double reporter mice, cultured under T_R1 polarizing conditions and re-stimulated for 48 hours with or without SB 203580 (p38 inhibitor). Number of total cells in the culture and number of dead cells are shown. (**B**) $CD4^+$ T cells were isolated from wild type $Foxp3^{RFP}$ $IL\text{-}10^{eGFP}$ reporter mice and cultured under T_R1 polarizing conditions with or without JNK inhibitor II, PD 98059 (ERK1/2 inhibitor), STAT3 inhibitor VI or SB 203580 (p38 inhibitor). Representative dot plots of two independent experiments are shown.