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Supplemental Information

Efficient treatment of a preclinical inflammatory bowel disease model with engineered bacteria

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Figure S 1

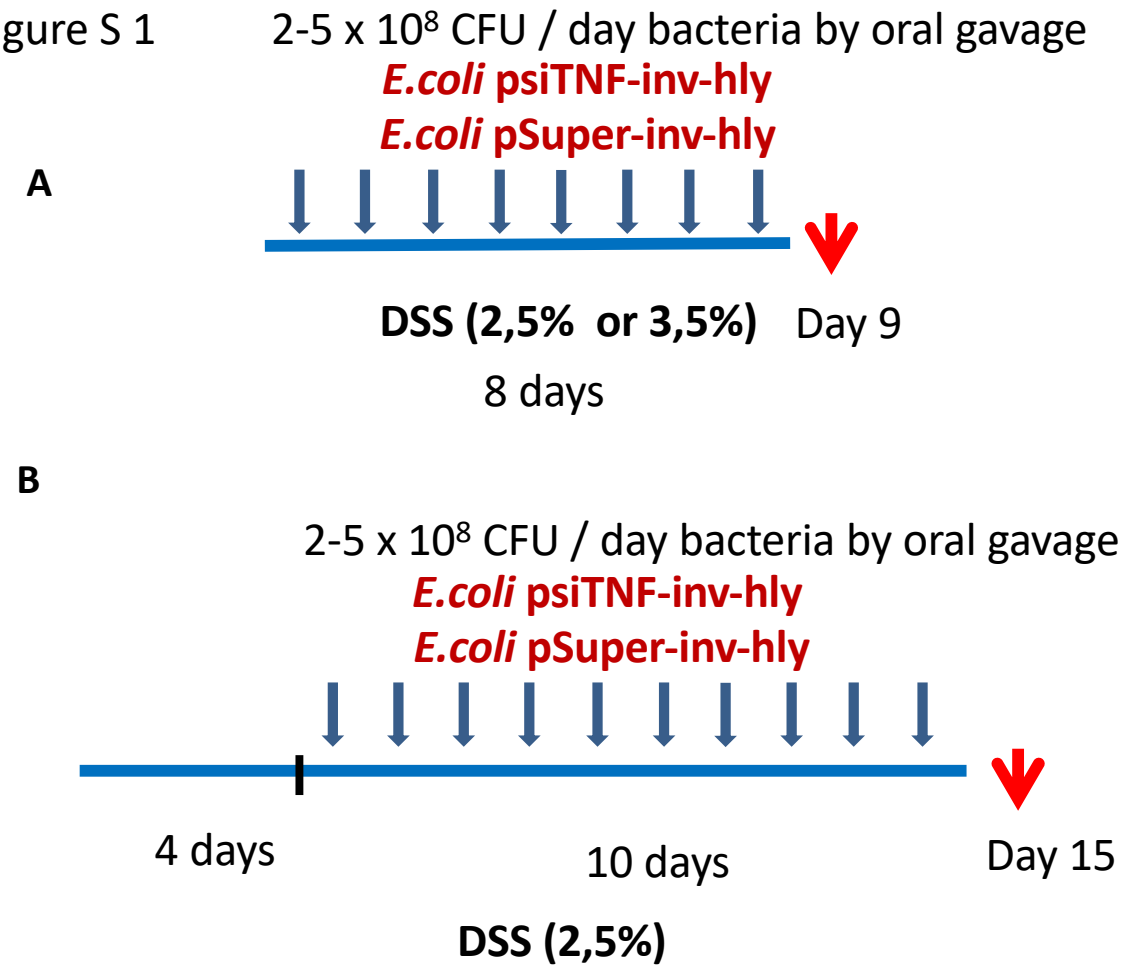

Figure S 1: Treatment protocols. Experimental animals were treated according to two types of treatment protocols. A) Animals were treated with 2.5% or 3.5% DSS added to the drinking water for eight days. The engineered bacterial cells (2-5x 10^8 CFU) and vehicle (sterile tap water) was administered daily by oral gavages. At the end of the experiment (day 9) animals were anesthetized and sacrificed. B) Animals were treated with 2.5% DSS added to the drinking water for four days. Animals continued to be treated with 2.5% DSS added to the drinking water for ten more days but during this period they also received engineered bacterial cells (2-5x 10^8 CFU) and vehicle (sterile tap water) daily by oral gavages. At the end of the experiment (day 15) animals were anesthetized and sacrificed.

Figure S 2

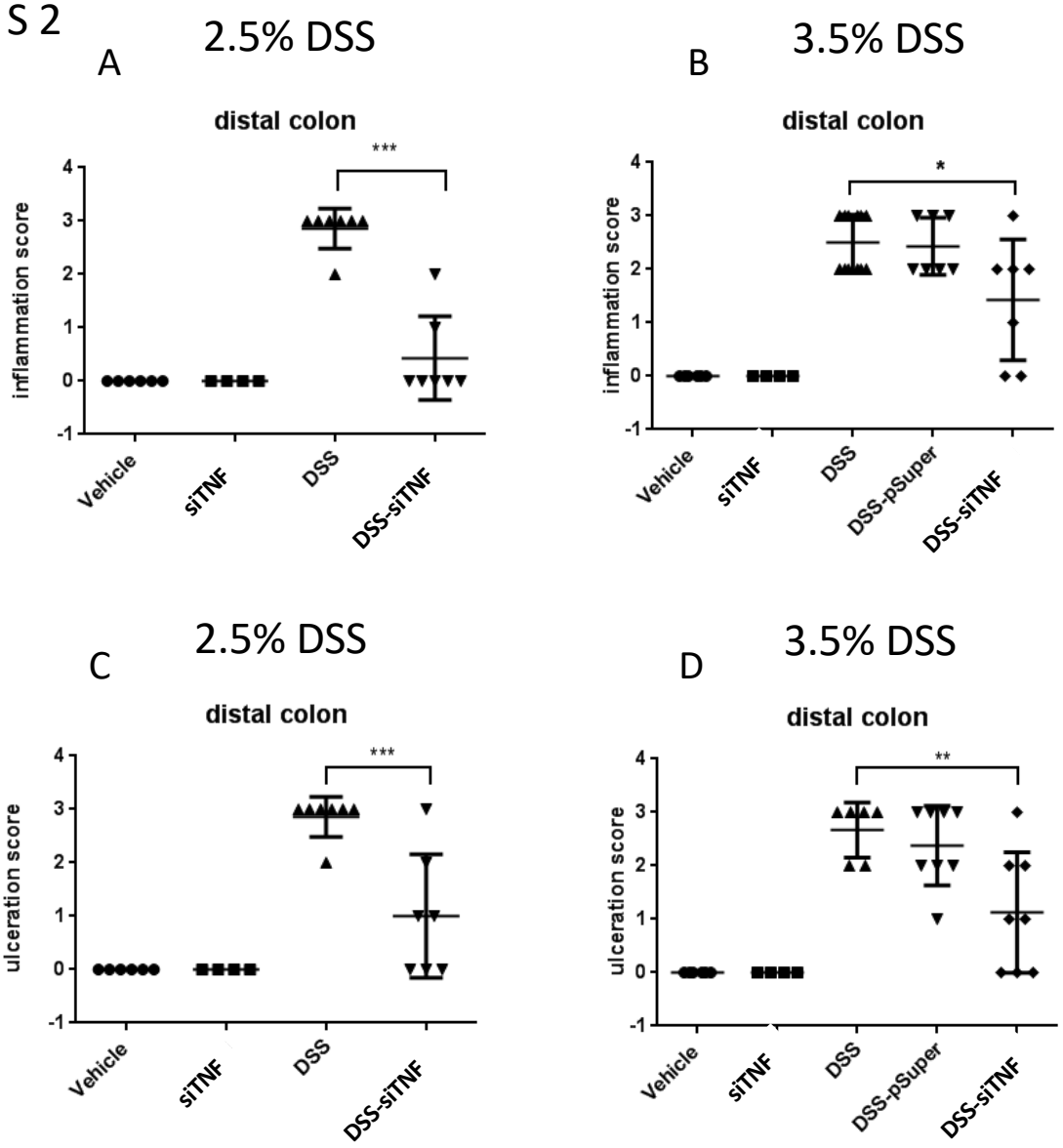

Figure S 2: The inflammatıon score and ulceration scores of the 2,5% and 3,5% DSS administered animals after 8 days of treatment. Histological analysis was carried out on FFPE colon samples from experimental groups and the levels of inflammation and ulceration was scored as described in the methods. A: inflammation score of the distal colon for the various treatment groups with 2,5% DSS. B: inflammation score of the distal colon for the various treatment groups with 3,5% DSS. C: ulceration scores of the distal colon for the various treatment groups with 2,5% DSS., D: ulceration scores of the distal colon for the various treatment groups with 3,5% DSS. Vehicle: Tap water administered animal group, siTNF: animals treated by modified *E. coli* MDS42 strains containing psiTNF and invasive plasmids, DSS: colon specimen of the DSS treated animals, DSS-siTNF: animals administered DSS and treated modified *E. coli* MDS42 strains containing psiTnfa and invasive plasmids, DSS-pSuper: animals administered DSS and treated by modified *E. coli* MDS42 strains containing pSuper (empty vector) and invasive plasmids. Data represent the mean (SD). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure S 3

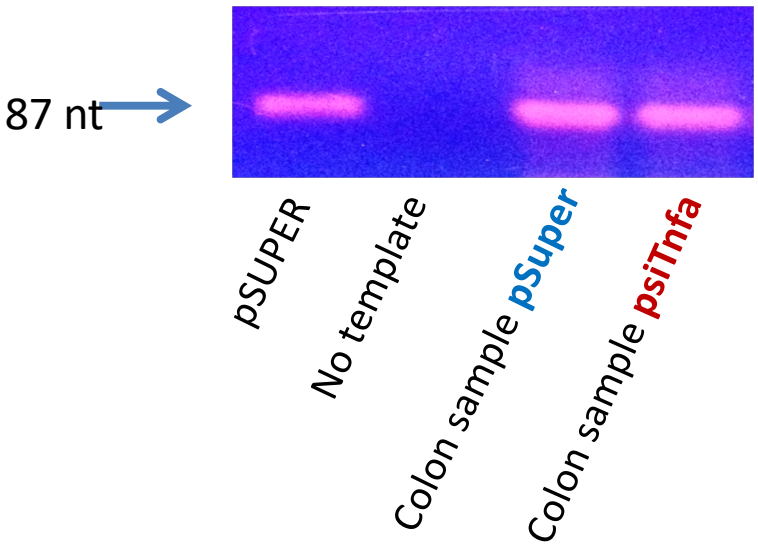

Figure S 3: Total RNA was isolated and cDNA synthesis was performed from the distal colon samples. The *gfp* expression in the colon tissue was demonstrated by PCR using *gfp* specific oligonucleotid primers. The PCR product was separated and stained on 2% agarose gel. pSUPER: intact pSUPER plasmid served as PCR template, colon sample pSUPER: cDNA from animals administered 2,5% DSS and treated by modified *E. coli* MDS42 strains containing pSuper (empty vector) and invasive plasmids served as PCR template, colon sample psiTNF: cDNA from animals administered 2,5% DSS and treated by modified *E. coli* MDS42 strains containing psiTNF silencer and invasive plasmids served as PCR template

Figure S 4

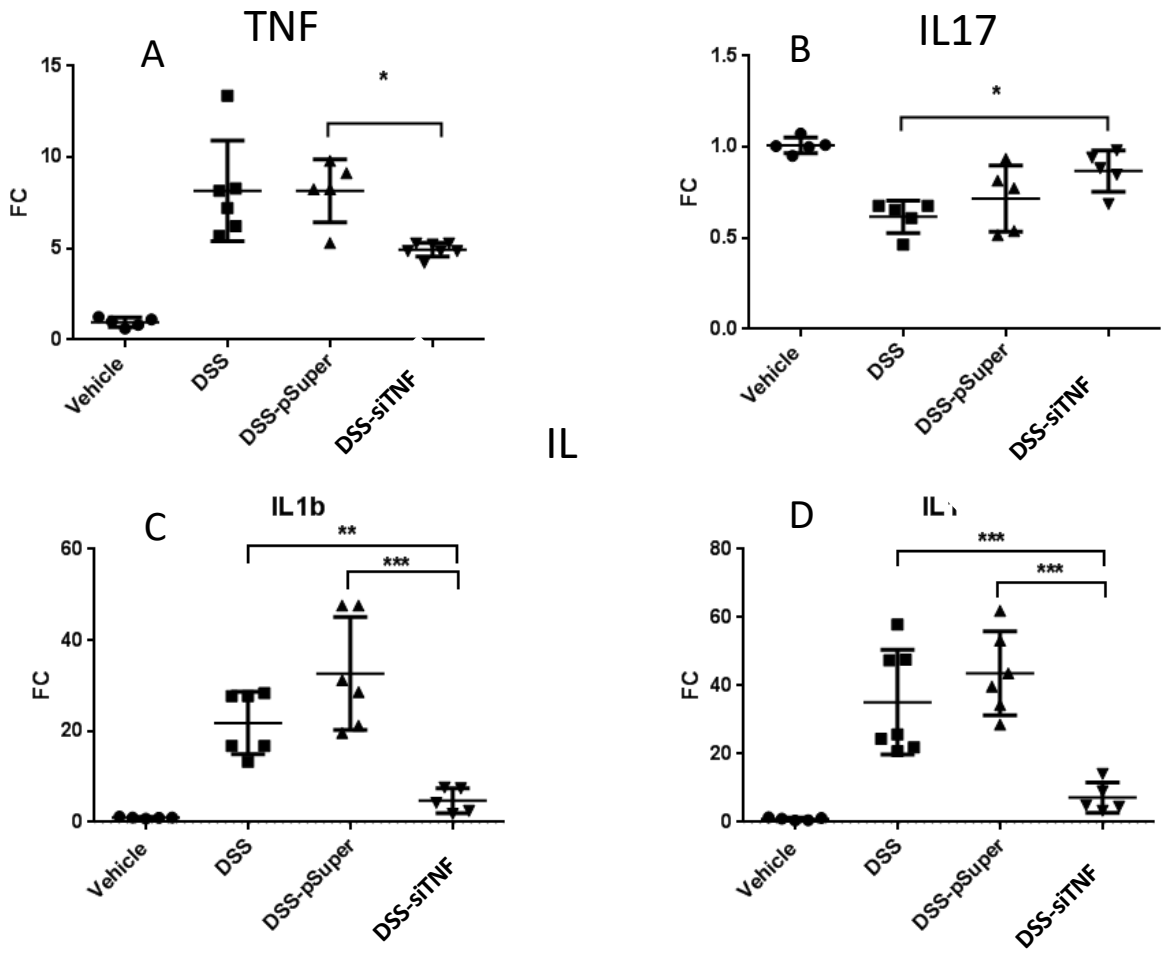
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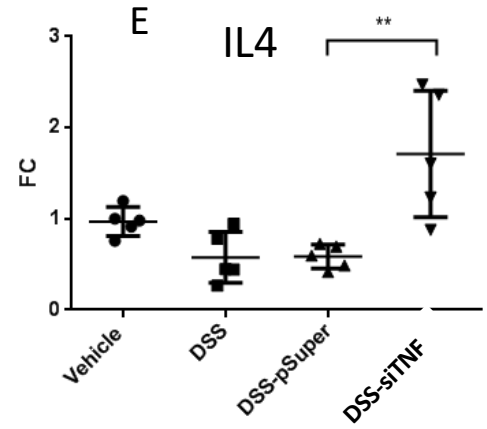
Figure S 4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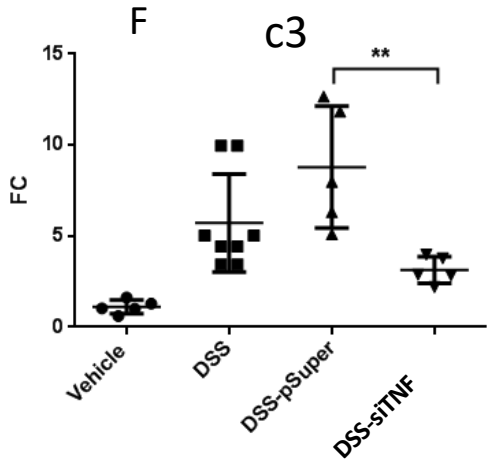
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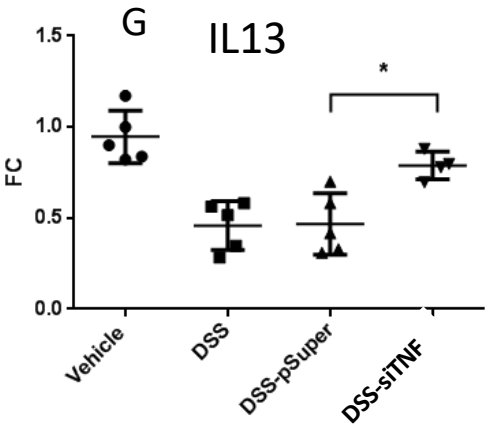
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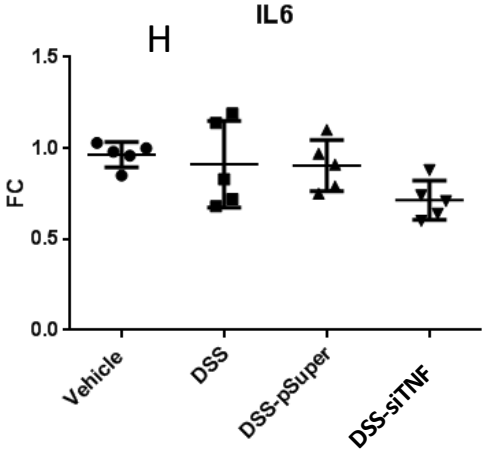
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DSS
DSS-pSuper
DSS-siTNF

Figure S 4: mRNA expression of cytokines on 4+10 days 2,5% DSS induced colitis. Colitis and the modified *E. coli* MDS42 strains containing psiTNF and Invasive plasmid treatment induced cytokines mRNA level changes were measured by qPCR. A: TNF mRNA expression measured on distal colon samples, B: Il-17 mRNA expression measured on distal colon samples, C: Il-1b mRNA expression measured on distal colon samples, D: Il-1a mRNA expression measured on distal colon samples, E: Il-4 mRNA expression measured on distal colon samples, F: C3 complement mRNA expression measured on distal colon samples, G: IL13 mRNA expression measured on distal colon samples, H: IL6 mRNA expression measured on distal colon samples. Vehicle: Tap water administered animal group, DSS: colon specimen of the 2,5% DSS treated animals, DSS-siTNF: animals administered 2,5% DSS and treated by modified *E. coli* MDS42 strains containing psiTNF and invasive plasmids, DSS-pSuper: animals administered DSS and treated by modified *E. co*

Figure S 5

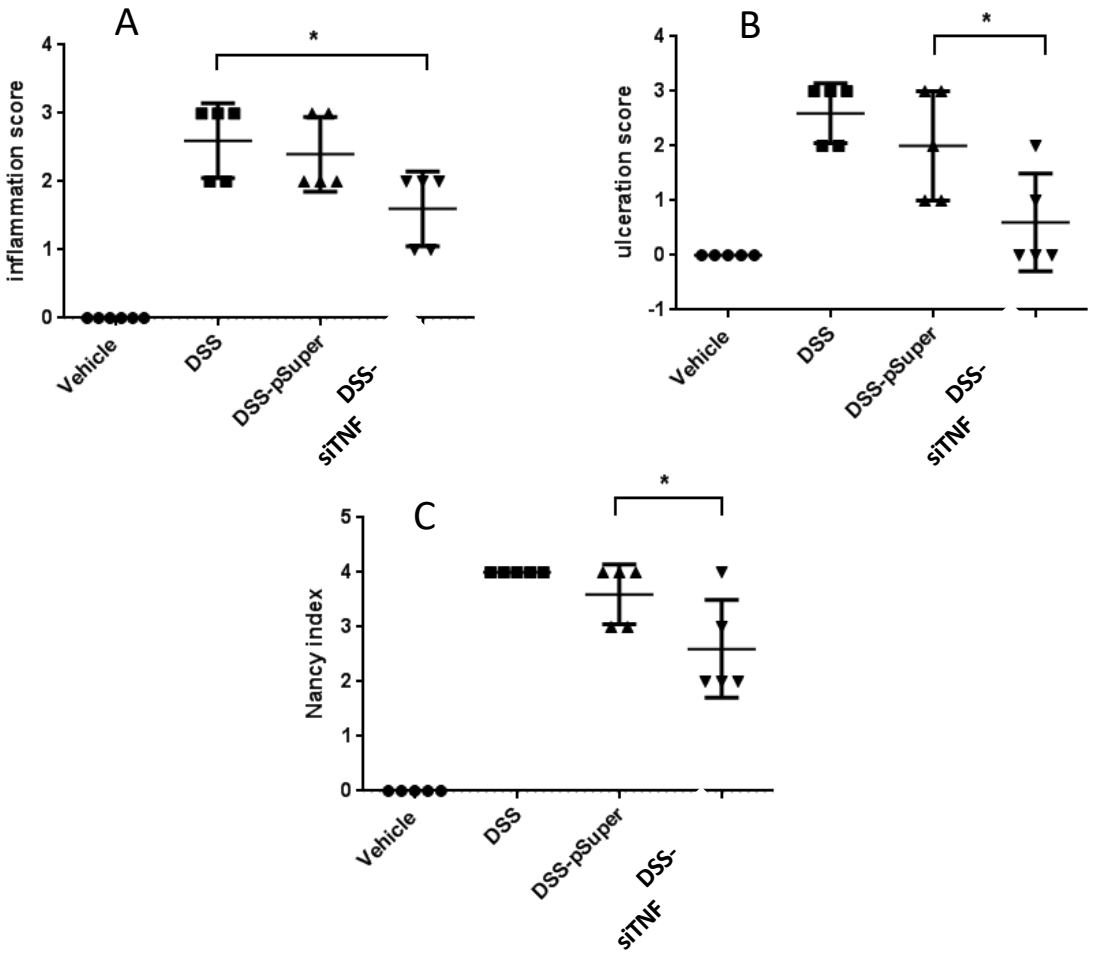

Figure S 5: The colon specimens inflammation score, ulceration score and Nancy histological index on 4+10 days 2,5% DSS induced colitis and treating them with the modified *E. coli* MDS42 strains containing psiTNF and invasive plasmid treatment. A: inflammation score of the distal colon for the various treatment groups with 2,5 % DSS, B: ulceration scores of the distal colon for the various treatment groups with 2,5 % DSS, C: Nancy histological index of the distal colon for the various treatment groups with 2,5% DSS. Vehicle: Tap water administered animal group, DSS: colon specimen of the DSS treated animals, DSS-siTNF: animals administered DSS and treated by modified *E. coli* MDS42 strains containing psiTNF and invasive plasmids. DSS-pSuper: animals administered 2,5 % DSS and treated with modified *E. coli* MDS42 strains containing pSuper (empty vector) and invasive plasmids. Data represent the mean (SD).* $P < 0.05$.

SM Table 1.: Primer sequences used in QRTPCR reactions.

GAPDH:	f: TGACGTGCCGCCTGGAGAAA, r: AGTGTAGCCCAAGATGCCCTTCAG
IL-13	f:ACAGCTCCCTGGTTCTCTCA, r: ACAGGGGAGTCTGGTCTTGT
TNF	f: CAGCCGATGGGTTGTACCTT, r: GGCAGCCTTGTGCCTTGA
IL-1b	f: GCCTCGTGCTGTCGGACCCA , r: TGAGGCCCAAGGCCACAGGT
IL-1a	f: CCATAACCCATGATCTGGAAGAG, r: GCTTCATCAGTTTGTATCTCAAATCAC
IL-6	f: CTCTGCAAGAGACTTCCATCC, r: AGTCTCCTCTCCGGACTTGT
C3	f: CCCTCACGCGCGTAGTGATT, r: TCGGCGTTGGAAGGCCGTAC
IL-4	f: AGCAACGAAGAACACCACAGA, r: AGCACCTTGGAAGCCCTA
IL-17	f: CCTGGCGGCTACAGTGAAG, r: GGAAGTCCTTGGCCTCAGTGT