Supplemental figures

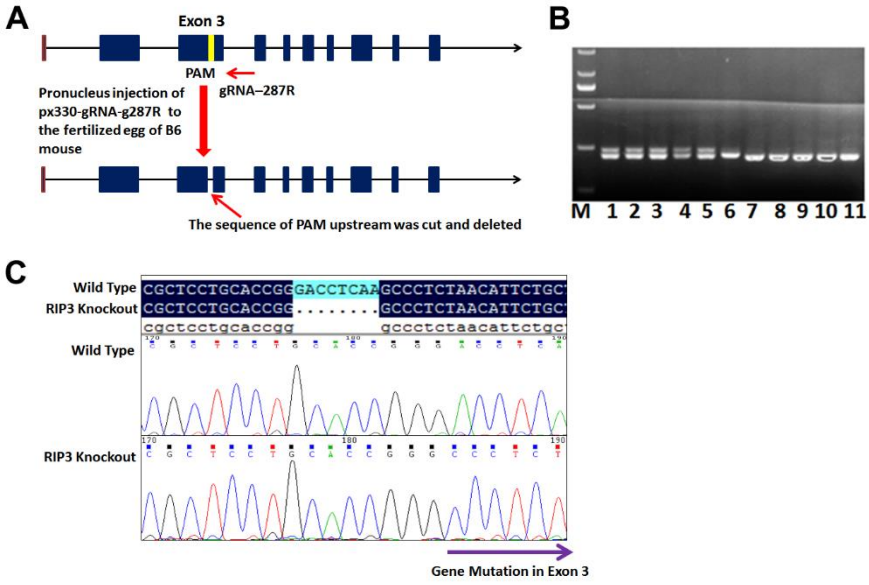

Figure S1. CRISPR/Cas9-induced deletion of mouse RIP3 gene. (A) The design of the strategy to delete the mouse RIP3 gene; gRNA-287R/cas9 was designed to target the third exon of the RIP3 gene, and eight nucleotides were deleted. Yellow strip, protospacer adjacent motif (PAM) sequence. **(B)** PCR-based genotyping. The F1 mice were detected by the primers that bind the targeted sites of the RIP3 gene. M, DL2000 DNA marker; RIP3 heterozygous mice, lanes 1, 2, 3, 4 and 5; WT mice, lane 6; RIP3 homozygousmice, lanes 7, 8, 9, 10 and 11. **(C)** Gene Blast was implemented to determine the sequences of the targeted and wild type alleles, and eight nucleotides were deleted in the RIP3 gene on the targeted allele.

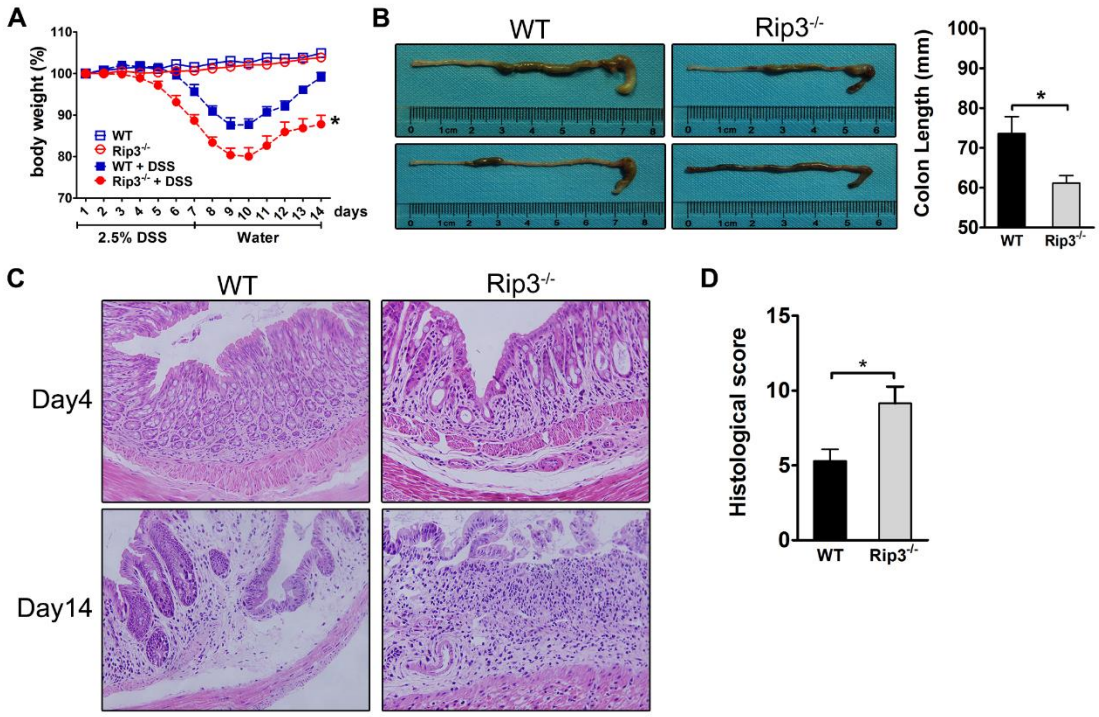

Figure S2. RIP3 is required to maintain the mucosal integrity. (**A-D)** Acute colitis was induced in Rip3$^{-/-}$ mice and WT littermates by administering 2.5% DSS in the drinking water for 7 days followed by recovery for 7 days on normal drinking water. Changes in body weight **(A)**, colon shortening **(B)**, and mucosal histology **(C)** were examined in WT and Rip3$^{-/-}$ mice on days 4 and 14 using H&E staining (original magnification, ×200), and colitis severity scores **(D)** were determined in a double-blinded manner. All experiments were repeated at least twice. In A, B, and D, data are presented as means ± SEM (n = 7 animals per group). *$p < 0.05$ compared with WT mice treated with DSS.

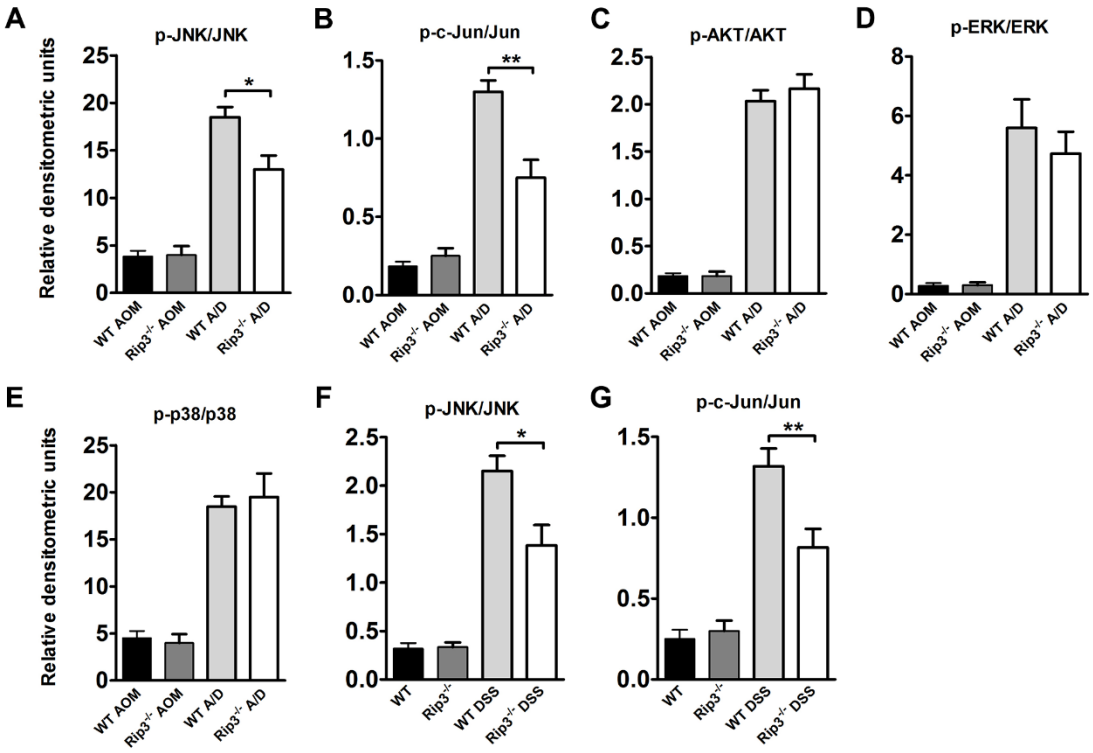

Figure S3. Densitometry analysis of immunoblots. (A-E) Densitometry analysis of p-JNK **(A)**, p-c-Jun **(B)**, p-AKT **(C)**, p-ERK **(D)**, and p-p38 **(E)** levels in western blots of protein extracts from the colons of AOM/DSS-treated wild type and Rip3$^{-/-}$ mice. **(F-G)** Densitometry analysis of p-JNK **(F)** and p-c-Jun **(G)** levels in western blots of colon tissues from control mice and the mice treated with DSS for 7 days. Data are means ± SEM (n = 5), *$p < 0.05$, **$p < 0.01$.

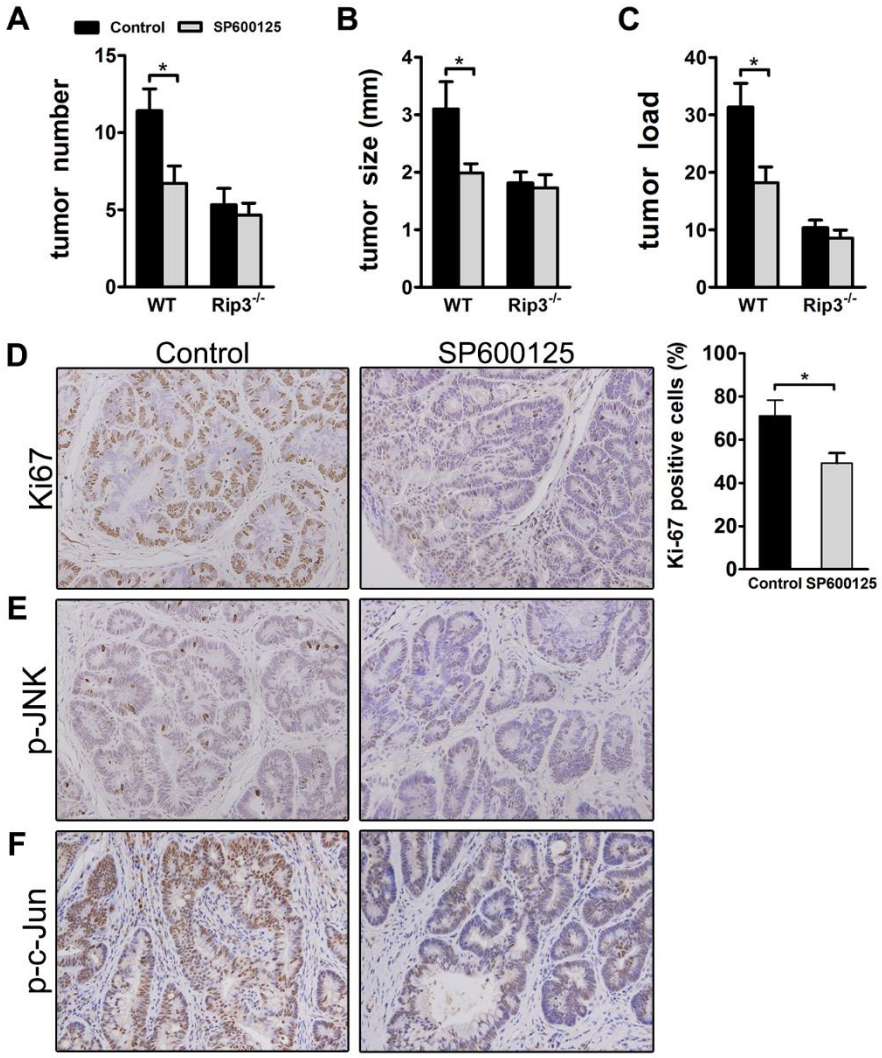

Figure S4. SP600125 suppresses the development of CAC in WT mice. CAC was induced in WT and Rip3$^{-/-}$ mice and animals were administered vehicle or SP600125 (40 mg/kg) by oral gavage for 100 days. **(A-C)** Tumor number **(A)**, tumor size **(B)**, and tumor load **(C)** were analyzed on day 100. **(D)** Proliferation was determined by Ki-67 staining, and the percentages of Ki-67-positive cells within colonic crypts were calculated. Original magnification, ×200. **(E-F)** Immunohistochemical staining for phospho-JNK **(E)** and phospho-c-Jun **(F)** in CAC tumors from WT mice treated with vehicle or SP600125. Original magnification,

×200. Data are presented as means ± SEM, n = 7–8 animals per group, *p < 0.05.

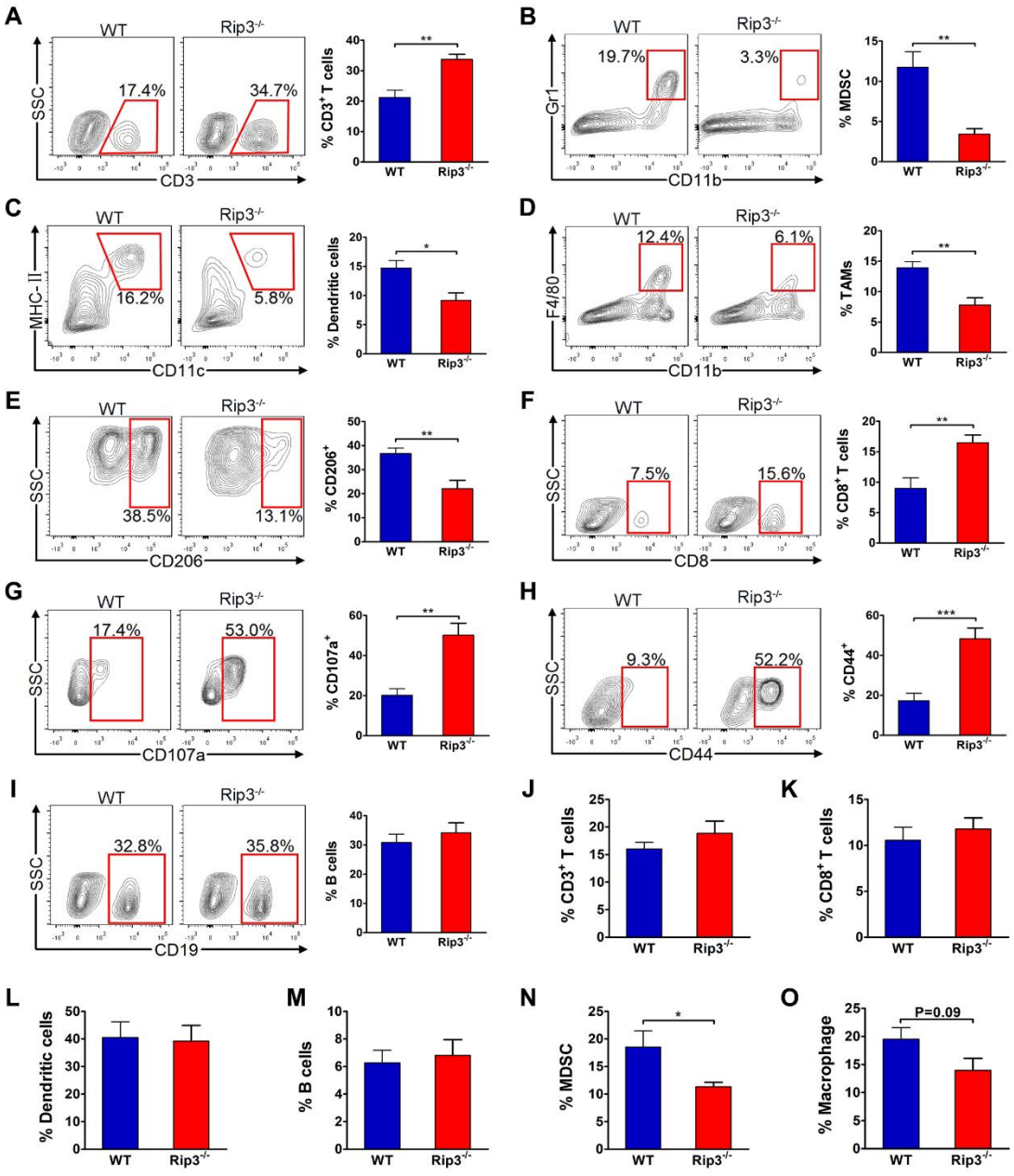

Figure S5. RIP3 deletion enhances the immunogenicity during acute colitis. (A-I) The mice of the indicated genotypes were treated with 2.5% DSS for 7 days and the cells that had been isolated from the colonic mucosa were subjected to flow cytometry analysis. **(A-E)** The fractions of $CD3^+$ T cells **(A)**, $Gr1^+$ $CD11b^+$ MDSC **(B)**, $F4/80^-$ $CD11c^+$ MHC II^+ dendritic cells **(C)**, and $Gr1^-$ $CD11b^+$ $F4/80^+$ TAMs **(D)**, and the expression of CD206 in TAMs **(E)** were assessed by flow cytometry. **(F-I)**

The fractions of $CD8^+$ T cells **(F)**, the expression of CD107a **(G)** and CD44 **(H)** on $CD8^+$ T cells, and the fraction of $CD19^+$ B cells **(I)** were also analyzed. **(J-O)** The fractions of $CD3^+$ T cells **(J)**, $CD8^+$ T cells **(K)**, $F4/80^-$ $CD11c^+$ MHC II^+ dendritic cells **(L)**, B cells **(M)**, $Gr1^+$ $CD11b^+$ MDSC **(N)**, and macrophages **(O)** in naive WT and $Rip3^{-/-}$ mice were assessed by flow cytometry. Flow cytometry data were reproduced in three separate experiments. Data are presented as means ± SEM, n = 7–8 animals per group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

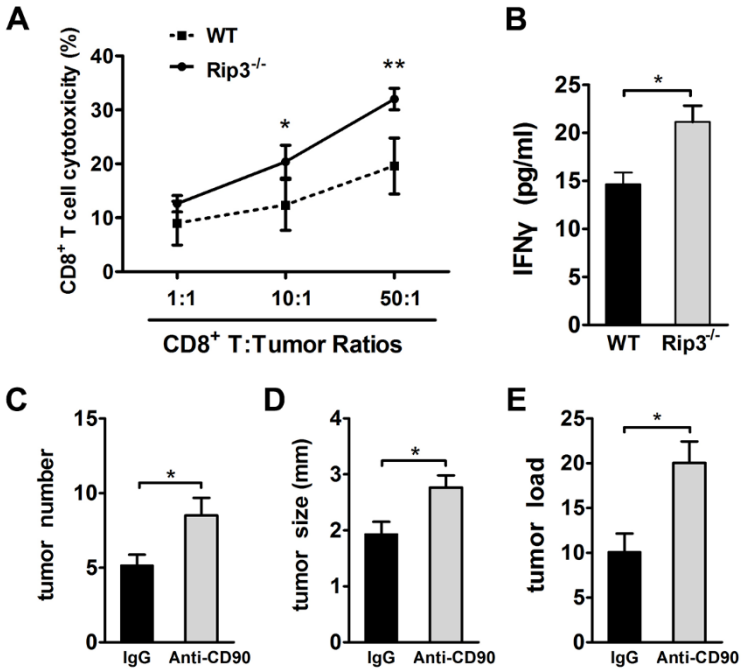

Figure S6. Activated CD8$^+$ T cells are tumor-protective in Rip3$^{-/-}$ mice. **(A)** The cytotoxicity of colonic CD8$^+$ T cells isolated from WT and Rip3$^{-/-}$ mice against tumor cells isolated from tumors of AOM/DSS-treated WT mice was determined as described in the Methods section. **(B)** Levels of IFNγ secreted from CD8$^+$ T cells cocultured with tumor cells. The ratio of CD8$^+$ T cells and tumor cells was 50:1. CD8$^+$ T cells isolated from WT and Rip3$^{-/-}$ mice and tumor cells isolated from tumors of AOM/DSS-treated WT mice were cocultured. **(C-E)** CAC was induced in Rip3$^{-/-}$ mice, and a neutralizing anti-CD90 monoclonal antibody or control antibody was intraperitoneally injected once a week for 14 weeks. Tumor number **(C)**, tumor size **(D)**, and tumor load **(E)** were analyzed on day 100. Data are presented as means ± SEM (n = 5). *$p < 0.05$; **$p < 0.01$.

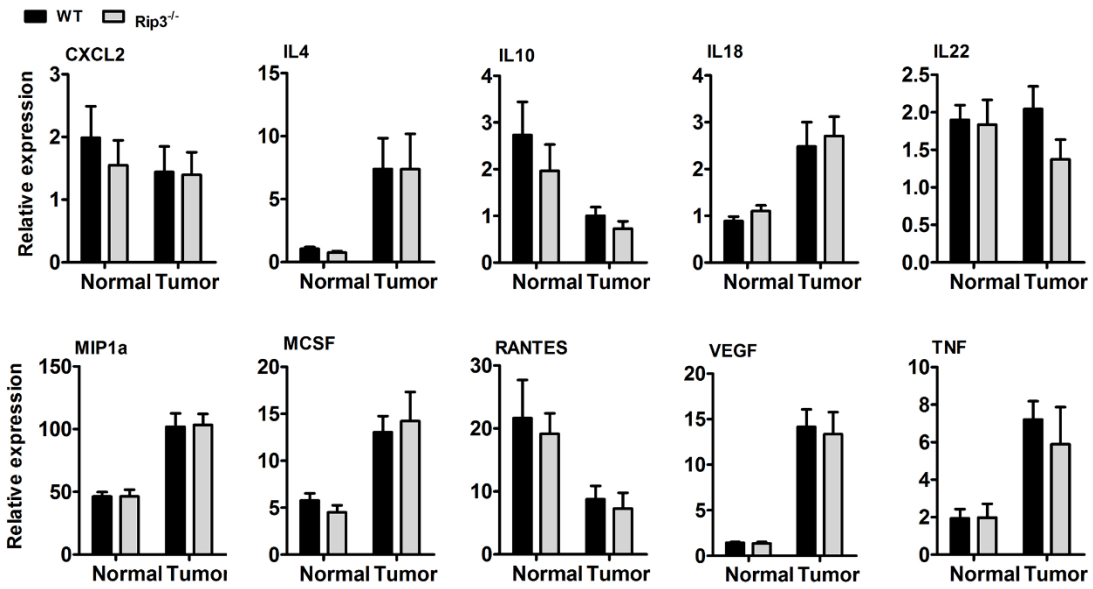

Figure S7. Cytokine expression in colonic tumors from AOM/DSS-treated WT and Rip3$^{-/-}$ mice. Relative mRNA expression of various cytokines in AOM/DSS-treated WT and Rip3$^{-/-}$ mice. Gene expression was normalized to GAPDH levels. One out of two representative experiments is shown. Data are presented as means ± SEM (n = 5 mice per group).

Table S1. Primer sequences used in this study.

Gene		Sequences	Gene		Sequences
RIP3	F	GAGATGGAAGACACGGCACT	CXCL1	F	CCACACTCAAGAATGGTCGC
	R	GGTGGTGCTACCAAGGAGTT		R	TCTCCGTTACTTGGGGACAC
IL1a	F	CGAAGACTACAGTTCTGCCATT	MCP1	F	AGGTGTCCCAAAGAAGCTGTA
	R	GACGTTTCAGAGGTTCTCAGAG		R	ATGTCTGGACCCATTCCTTCT
IFNγ	F	ACAGCAAGGCGAAAAAGGAT	TNF	F	CCCACTCTGACCCCTTTACT
	R	TGAGCTCATTGAATGCTTGG		R	TTTGAGTCCTTGATGGTGGT
IL6	F	CGGAGAGGAGACTTCACAGA	IL11b	F	GCAACTGTTCCTGAACTCAACT
	R	CCAGTTTGGTAGCATCCATC		R	ATCTTTTGGGGTCCGTCAACT
IL10	F	CTATGCTGCCTGCTCTTACTG	LIF	F	ATTGTGCCCTTACTGCTGCTG
	R	AACCCAAGTAACCCTTAAAGTC		R	GCCAGTTGATTCTTGATCTGGT
IL17a	F	TCCAGAAGGCCCTCAGACTA	IL4	F	CTGTGCTCCGGCAGTTCTA
	R	TGAGCTTCCCAGATCACAGA		R	ACGTACTCTGGTTGGCTTCC
IL18	F	CAGGCCTGACATCTTCTGCAA	CXCL2	F	GAGCTTGAGTGTGACGCCCCCAGG
	R	CTGACATGGCAGCCATTGT		R	GTTAGCCTTGCCTTTGTTCAGTATC
IL22	F	TTGAGGTGTCCAACTTCCAGCA	VEGF	F	GCACATAGAGAGAATGAGCTTCC
	R	AGCCGGACATCTGTGTTGTTA		R	CTCCGCTCTGAACAAGGCT
MIP1a	F	TTCTCTGTACCATGACACTCTGC	GAPDH	F	TTGATGGCAACAATCTCCAC
	R	CGTGGAATCTTCCGGCTGTAG		R	CGTCCCGTAGACAAAATGGT
Arg1	F	ACGGAAGAATCAGCCTGGTG	MCSF	F	ATGAGCAGGAGTATTGCCAAGG
	R	GTCCACGTCTCTCAAGCCAA		R	TCCATTCCCAATCATGTGGCTA
RANTES	F	GCTGCTTTGCCTACCTCTCC			
	R	TCGAGTGACAAACACGACTGC			