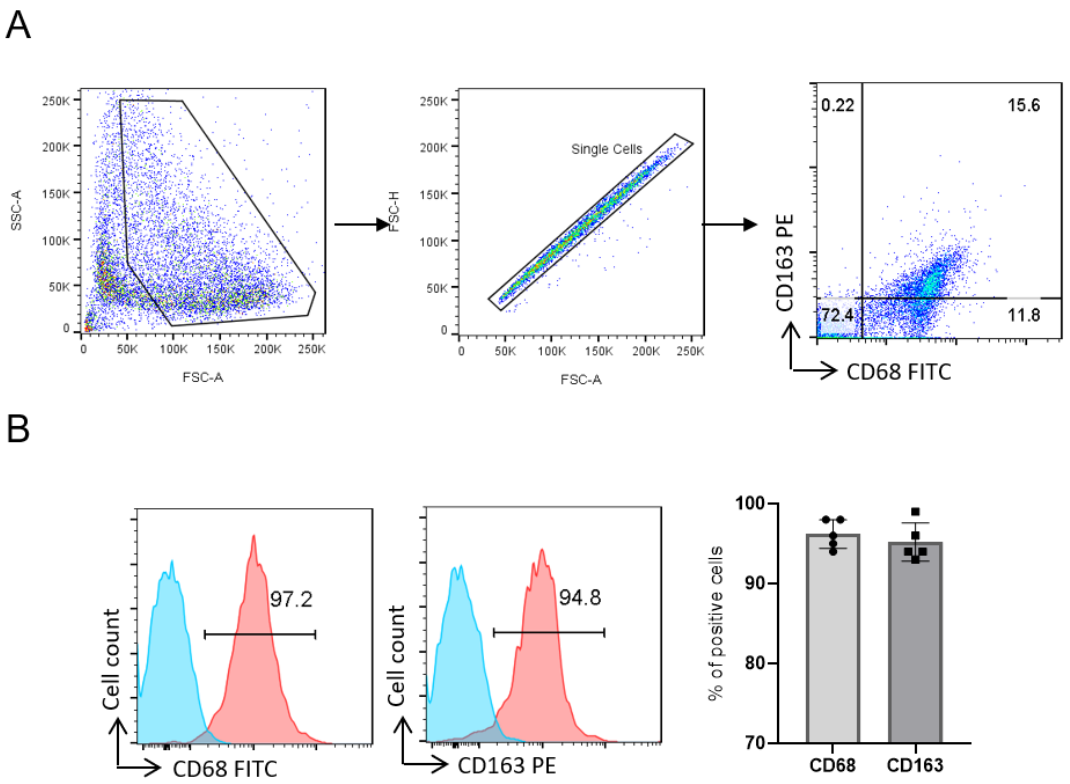
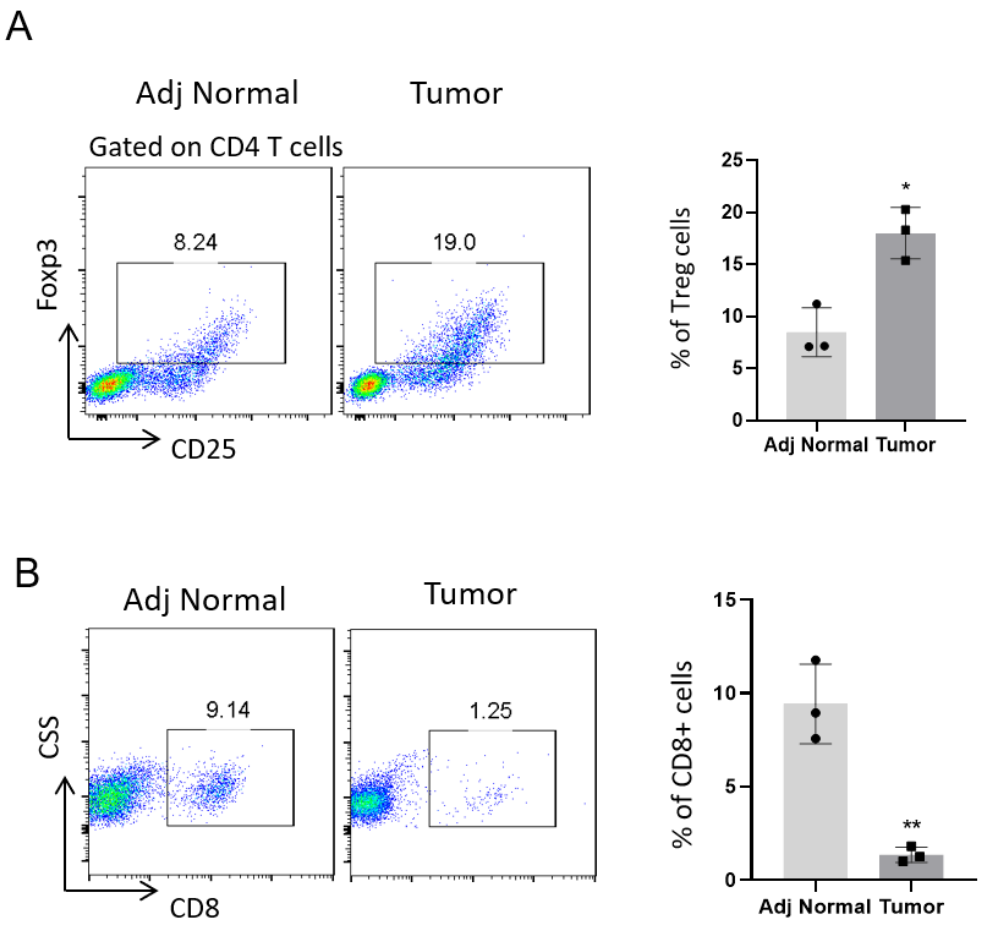
Supplemental Figures and legends

Supplemental Figure 1



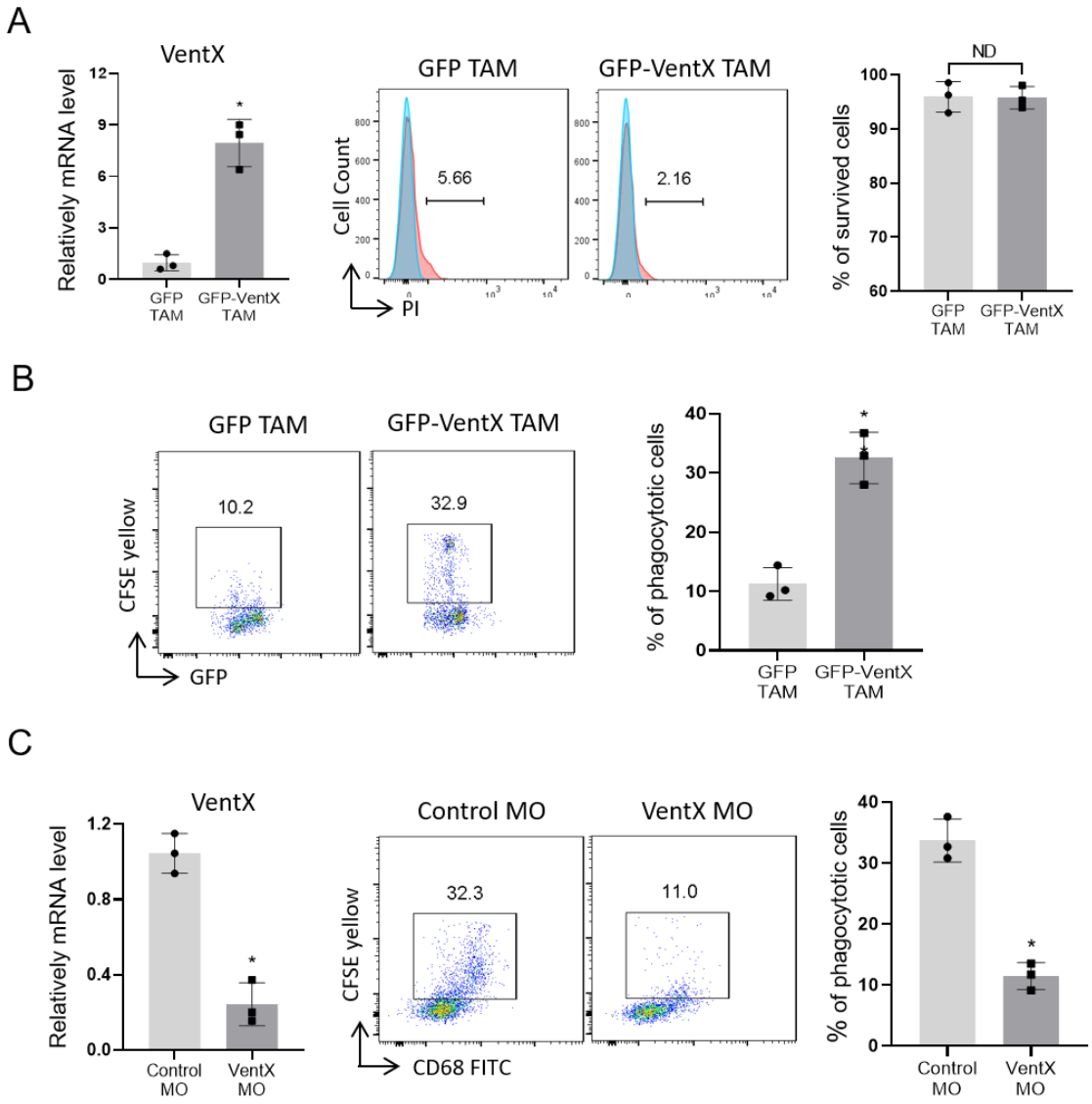
Supplemental Figure 1. Gating strategy and characterization of PDA-TAMs. (A) Gating strategy of FACS analysis. Mononuclear cells were gated on the basis of FCS-A versus SSC-A and then were sequentially gated to select single cells by FSC-A versus FSC-H. Subsequently, flow cytometry plots for the various markers were shown in biexponential format. **(B)** Purity of isolated TAMs. Mononuclear cells from tumor were used for TAM isolation process according to Material and Methods and purity of TAMs was determined by FACS analysis on indicated microphage markers. Data shown are means ± SD of 5 independent experiments

Supplemental Figure 2



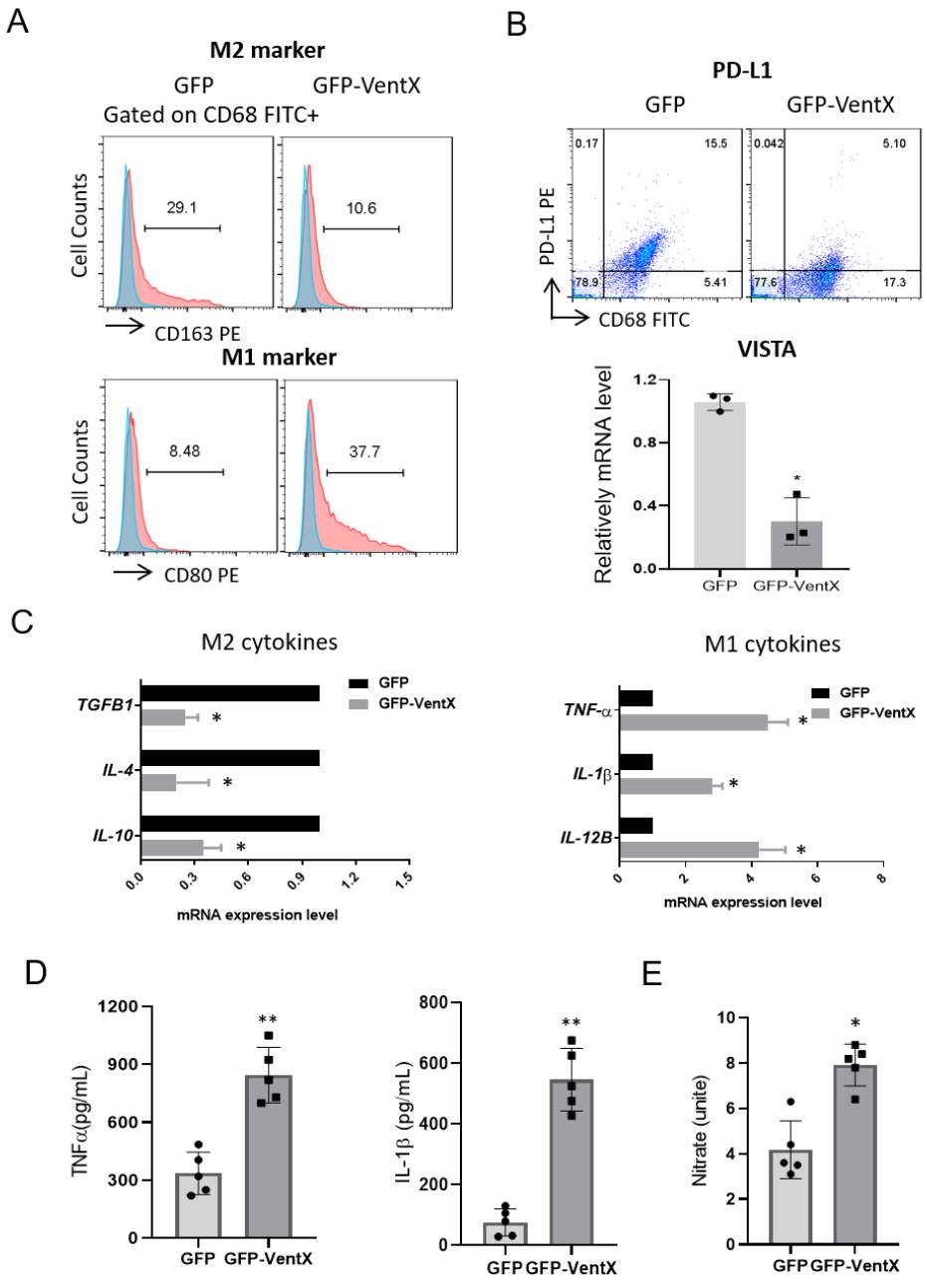
Supplemental Figure 2. Characterization of Treg and CD8 cells isolated from pancreatic tumors and adjacent normal pancreatic tissues. (A) Percentage of Treg cells in PDA and adjacent normal pancreatic tissues. Isolated tissue mononuclear cells were stained with anti-CD4-FITC, CD25-PE, Foxp3-APC and subjected to FACS analysis. The percentage of Treg (CD4+, CD25+, Foxp3+) cells was shown. **(B)** Percentage of CD8 cells in PDA and adjacent normal pancreatic tissues. Cells were stained with anti-CD8-PE and then subjected to FACS analysis. Data shown are means ± S.D. of three independent experiments, and paired Student's *t* test was performed. * P <0.05, ** P <0.01.

Supplemental Figure 3



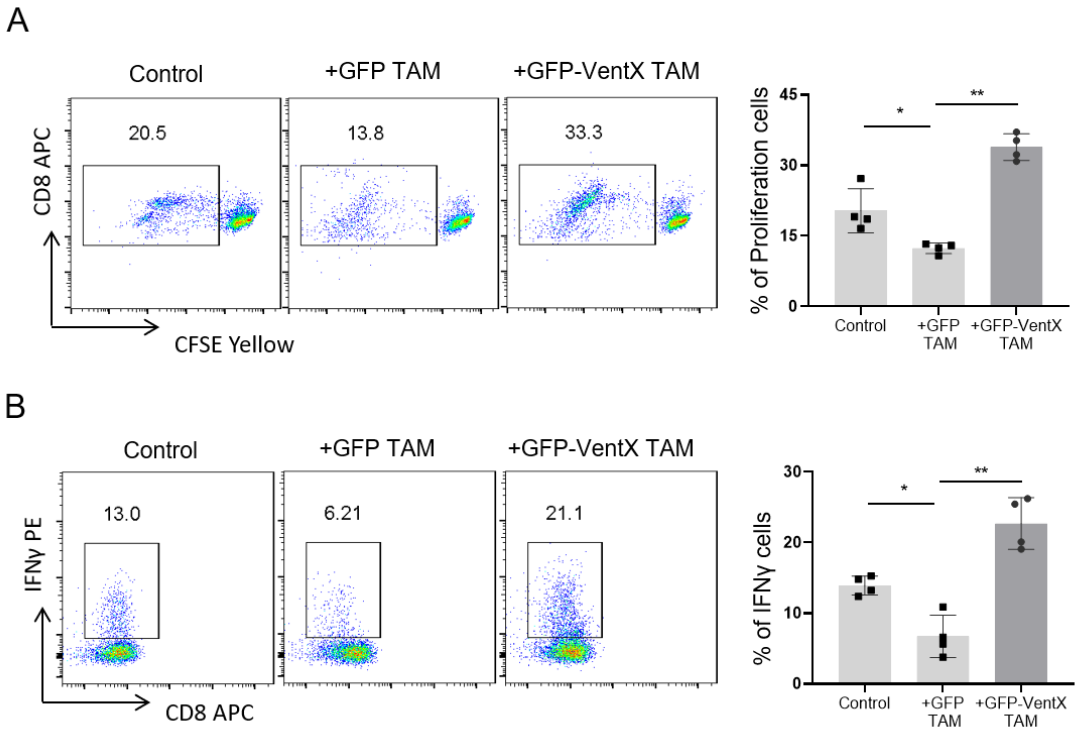
Supplemental Figure 3. VentX is required in TAMs for phagocytosis of leukemia cells.
(A) TAMs were transfected with GFP-VentX or GFP control. The VentX expression was determined by real-time PCR and cell viability was determined by PI staining. **(B)** TAMs were transfected with GFP-VentX or GFP control, and then incubated with CFSE-Yellow labelled leukemia cells at 1:2 ratio for 24 hours. Flow cytometric analysis were performed. Data represent the means ± SD from 3 independent experiments. *$p < 0.05$, by Student's *t* test. **(C)** Normal tissue macrophages were transfected with VentX-morpholino or morpholino control. The efficacy of VentX knock down was determined by qRT-PCR. The ratio of phagocytosis was determined by flow cytometry. Data shown are the means ± SD from 3 independent experiments. *$p < 0.05$, by paired Student's *t* test.

Supplemental Figure 4



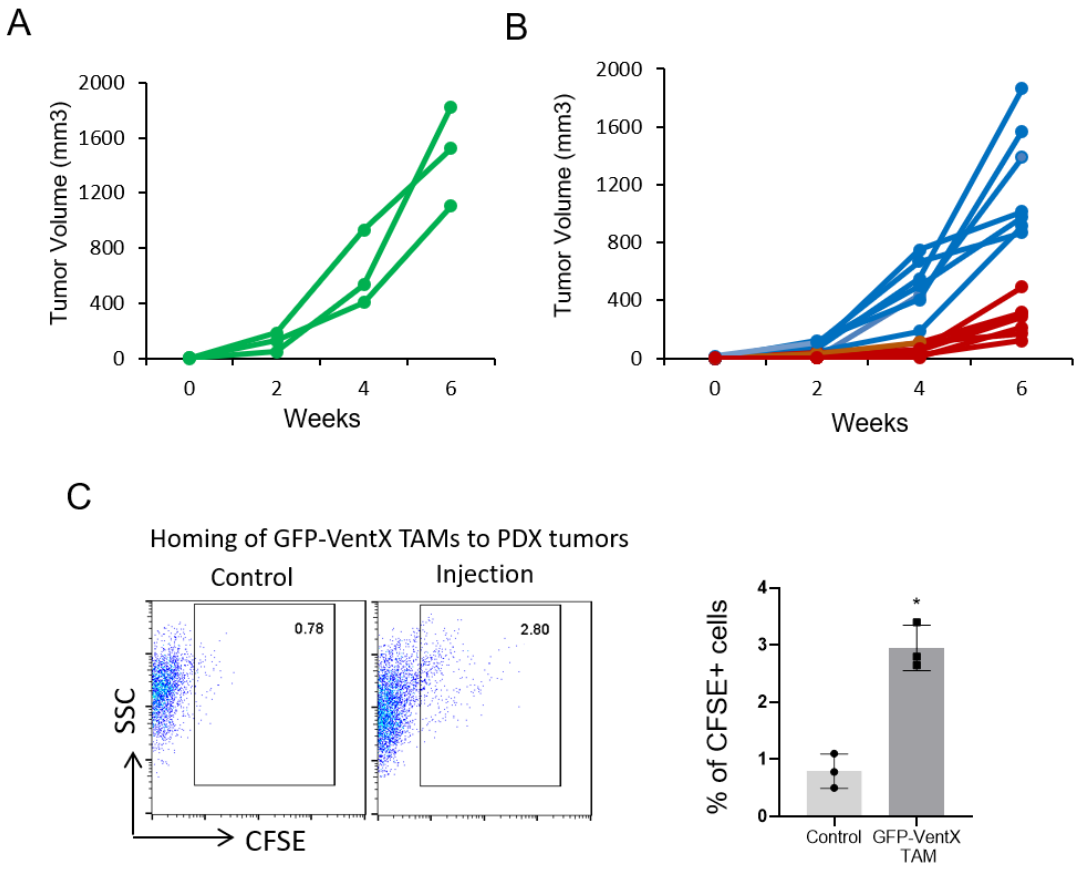
Supplemental Figure 4. Effects of VentX expression on TAM phenotype. Pancreatic TAMs were isolated and transfected with plasmids encoding GFP or GFP-VentX as described in material and methods. **(A)** The effects of VentX on the expression of M2 marker CD163 and M1 marker CD80 were determined by flow cytometry analysis. **(B)** The effects of VentX on the expression of PD-L1 and Vista was determined by FACS analysis and qRT-PCR respectively. **(C)** The effects of VentX on the expression of M2 and M1 cytokines as determined by qRT-PCR. Data represent means ± S.D. of three independent experiments, * $p < 0.05$. **(D)** TNF-α and IL-1β cytokine level and **(E)** Nitrate level from GFP-VentX or control GFP transfected TAMs. Results represent means ± S.D. of five independent experiments and paired Student's *t* test was used. * $p < 0.05$, ** $P < 0.01$ by Student *t* test.

Supplemental Figure 5



Supplemental Figure 5. VentX modulates TAM inhibition of CD8 TILs proliferation and activation. **(A)** Effects of VentX-modulated-TAMs on CD8 T cell proliferation. CFSE Yellow labelled CD8+ TIL T cells from PDA patients were stimulated with Dynabeads plus IL-2 and then cultured either alone or with autologous TAMs transfected with GFP or GFP-VentX at a T cells: TAMs ratio = 2:1 for 5 days. Representative results from 5 experiments were shown. The numbers on histograms represent the percentage of proliferating T cells; **(B)** the effects of VentX-modulated-TAMs on CD8 T cell activation was measured by FACS analysis of intracellular IFNγ. Data represent the means ± SD from 4 independent experiments. *$p < 0.05$, ** $p < 0.01$ by Student's *t* test.

Supplemental Figure 6



Supplemental Figure 6. Characterization of VentX-TAMs on tumorigenesis of PDA in NSG-PDX model of primary human pancreatic cancers. (A) Growth curve of individual primary human PDA in NSG-PDX mice. NSG-PDX models of primary human pancreatic cancers were generated by subcutaneous implantation of small pieces of primary human PDA on the dorsal side of NSG mice. The growth of the PDA in vivo was observed up to 6 weeks and the results of the PDA growth in each individual mice were shown, n=3 mice. **(B)** The NSG-PDX mice of primary human PDA were tail-vein injected with 0.5 million VentX-TAMs or control GPF-TAMs. The growth of PDA in each treated mice were shown individually. Red color lines indicated mice treated with VentX-TAMs. Blue color lines represented mice treated with control GFP-TAMs, n = 7 mice/group. **(C)** Accumulation of VentX-TAMs in PDA. The CSFE-labeled VentX transfected TAMs were tail-vein injected into NSG-PDX mice of human PDA. 72 hours post-injection, the PDAs were dissected out. The mononuclear cells were collected, and the presence of VentX-TAMs was revealed by CSFE positive cells. Data shown are means ± SD of 3 independent experiments, and paired Student's *t* test was performed. *$p < 0.05$.

Supplementary Table 1

Primer sequences used in this study

Gene name	Forward	Reverse
VentX-C*	AAGGCAATTAGGCGCTGCTT	ACAGAACAACTGAGTCCTCCA
VentX-R*	CCGTCAGCATCAAGGAGG	CTGGACCTCTGAGAGCTGC
IL1β	AAGCTGATGGCCCTAAACAG	AGGTGCATCGTGCACATAAG
GAPDH	AGAACGGGAAGCTTGTCATC	GCCTTCTCCATGGTGGTG
IL8	ATGACTTCCAAGCTGGCCGT	CCTCTTCAAAAACTTCTCCACA
IL12B	GCAGAGGCTCTTCTGACCCCCA	AGCTGACCTCCACCTGCCGA
TNFα	CGC CAC CAC GCT CTT CTG	GCC ATT GGC CAG GAG GGC
IL4	GCTTCCCCCTCTGTTCTTCC	CTGCTCTGTGAGGCTGTTCA
IL10	GATCCAGTTTTACCTGGAGGAG	CCTGAGGGTCTTCAGGTTCTC
IL13	CCTCTACAGCCCTCAGGGAG	ATCTTGGGAATCACCCACCC
TGFB1	TACCTGAACCCGTGTTGCTCTC	GTTGCTGAGGTATCGCCAGGAA
Vista	CCACCATGGCAACTTCTCCA	GAGTGGTGGTGCCTGATCTC
TLR2	TCTCCCATTTCCGTCTTTTT	GGTCTTGGTGTTCATTATCTTC
TLR9	CGCCAACGCCCTCAAGACA	GGCGCTTACATCTAGTATTTGC
MyD88	GAATCTTCCAAAGCGCAAAG	AGGATGACAAACTCCAAGCA
MAPKp38	AAGACTCGTTGGAACCCCAG	TCCAGTAGGTCGACAGCCAG
	C*: conventional PCR; R*: real-time PCR	