

## S1 Reverse-transcriptase polymerase chain reaction (RT-PCR)

S 2.1 Methods Total RNA was extracted from spleen and liver tissue specimens (20–30 mg) using RNAPure Tissue Kit (Beijing CoWin Bioscience Co., Ltd., Beijing, China) according to the manufacturer's instructions. Two point five  $\mu$ l of RNA was then reverse transcribed using the Takara PrimeScript™ reagent Kit (Perfect Real Time) (Takara, Kyoto, Japan). To analyze the expression levels of genes, the oligonucleotide primers reported by Cho et al. <sup>1</sup> were as follows:

TNF- $\alpha$ : 5- AGCACAGAAAGCATGATCCG-3 (forward),

5- CTGATGAGAGGGAGGCCATT-3 (reverse);

IL-1  $\beta$ : 5- ACCTGCTGGTGTGTGACGTT-3 (forward),

5- TCGTTGCTTGGTTCTCCTTG-3 (reverse);

IL-6: 5-GAGGATACCACTCCCAACAGACC-3 (forward),

5-AAGTGCATCATCGTTGTTTCATACA-3 (reverse);

$\beta$  -actin: 5-ATCACTATTGGCAACGAGCG -3 (forward),

5-TCAGCAATGCCTGGGTACAT-3 (reverse).

The PCRs were performed in 25  $\mu$ l system and contained SYBR Premix Ex Taq™ II (Takara) as recommended by the manufacturer. The reactions were performed using the IQ5 (BIO-RAD) system. Cycling conditions were as follows: one cycle at 95°C for 30 s; 45 cycles at 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 60 s; and a dissociation step at 95°C for 15 s, 60°C for 30 s. All samples were analyzed in triplicate and normalized against the  $\beta$  -actin gene. Relative quantification based on the expression of a target gene versus the  $\beta$ -actin gene were determined by the  $(2^{-\Delta\Delta CT})$

method described previously.

## S 2.2 Results

Table S1 The expression levels of cytokine genes in spleen and liver of mice treated or untreated with punicalagin.

Sample	Paramter	Gene expression		
		<i>Sal</i>	<i>Sal</i> +250µg/ml	<i>Sal</i> +500µg/ml
Live	IL-6	1	0.61±0.02**	0.62±0.05**
	IL-1β	1	0.64±0.05**	0.79±0.07*
	TNF-α	1	0.5±0.03*	0.48±0.02*
Spleen	IL-6	1	0.43±0.24*	0.28±0.13*
	IL-1β	1	0.40±0.26*	0.19±0.10**
	TNF-α	1	0.47±0.11**	0.42±0.09**

\* comparing with the group that only infected with *S. Typhimurium*

## References

1. E. J. Cho, J. S. Shin, Y. S. Noh, Y. W. Cho, S. J. Hong, J. H. Park, J. Y. Lee and K. T. Lee, *Journal of Ethnopharmacology*, 2011, 136, 428-435.