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## Supplementary Materials for

### **Engineered probiotics biofilm enhances osseointegration via immunoregulation and anti-infection**

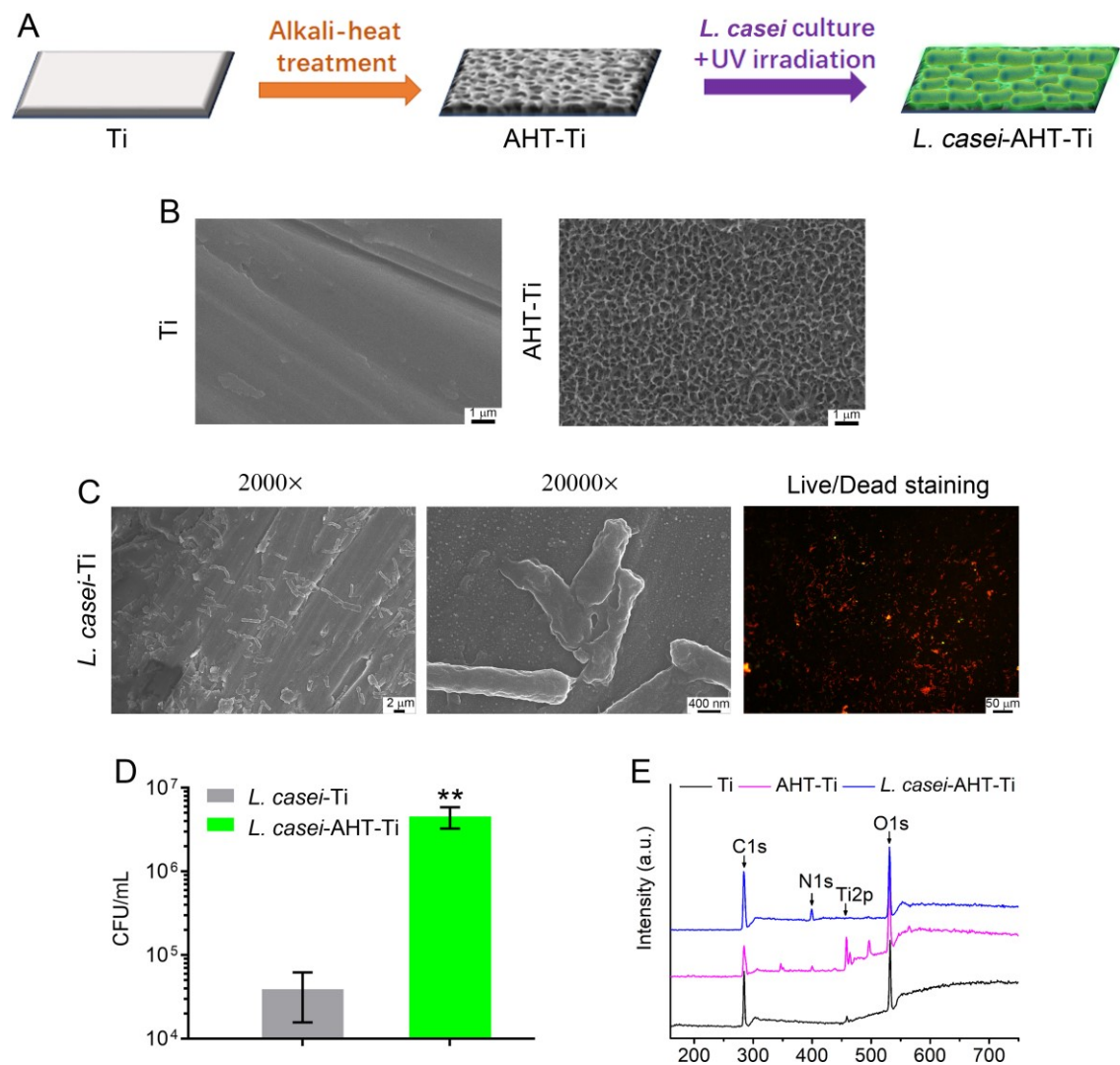
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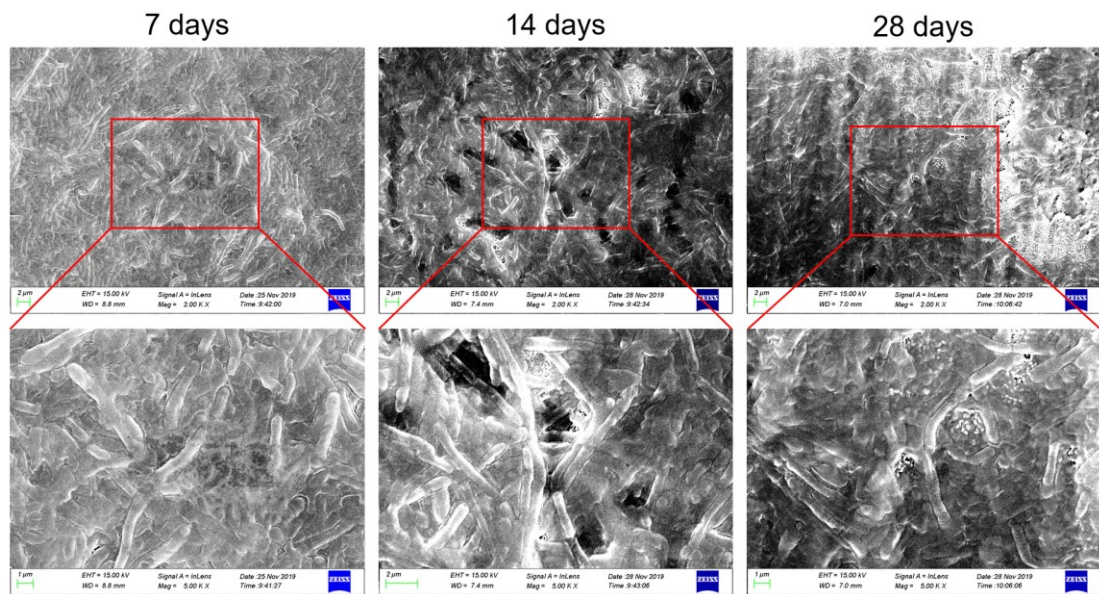
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#### **This PDF file includes:**

Figs. S1 to S8  
Table S1

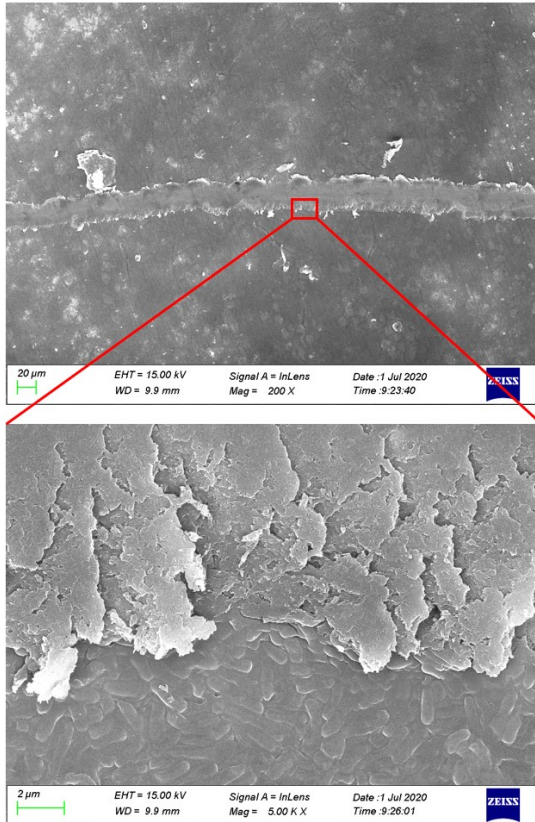


**Fig. S1. Preparation procedure and surface characterization of *L. casei* biofilm.** (A) Preparation procedure of *L. casei* biofilm modified Ti implants. (B) Surface morphology of Ti and AHT-Ti. (C) Surface morphology and Live/dead (green/red) staining of *L. casei* biofilm modified Ti. (D) Number of bacterial colonies on the surface of Ti and AHT-Ti. (E) XPS of Ti, AHT-Ti and *L. casei*-AHT-Ti.  $n = 3$  independent experiments per group,  $**P < 0.01$ .

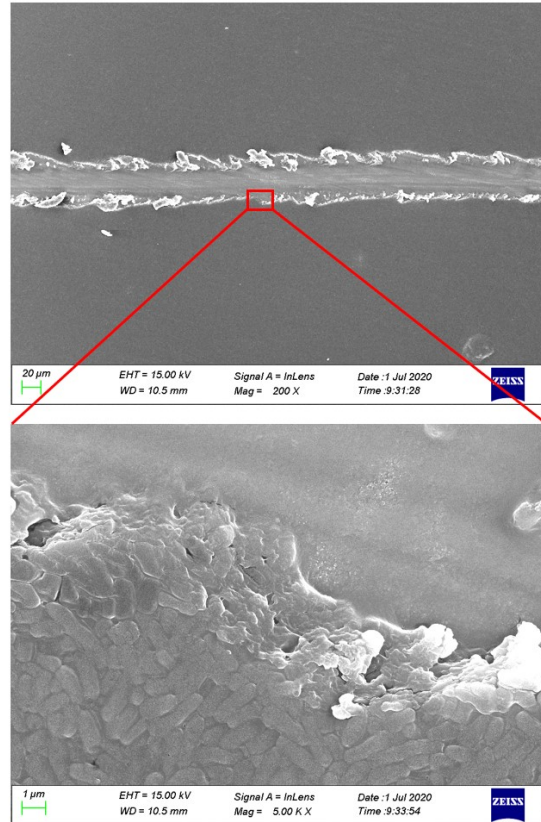


**Fig. S2.** SEM images of *L. casei*-AHT-Ti after immersing in PBS solution for 7, 14 and 28 days.

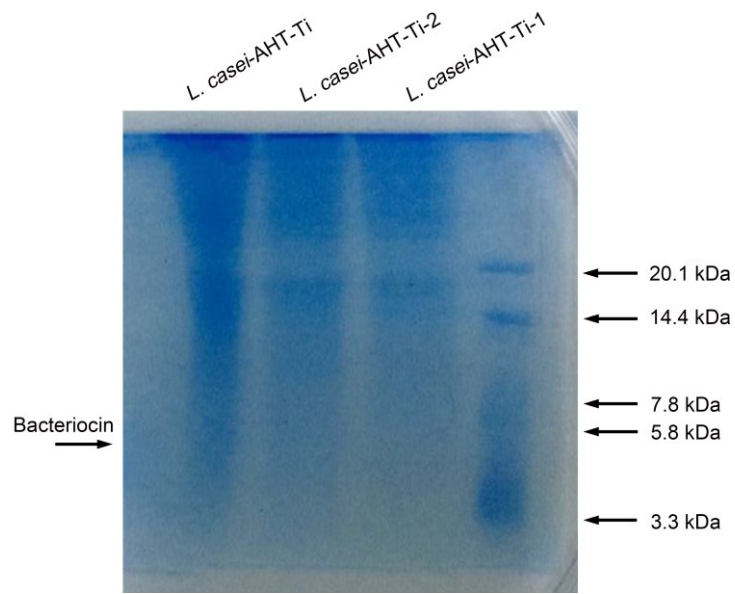
Pipette tip scratch



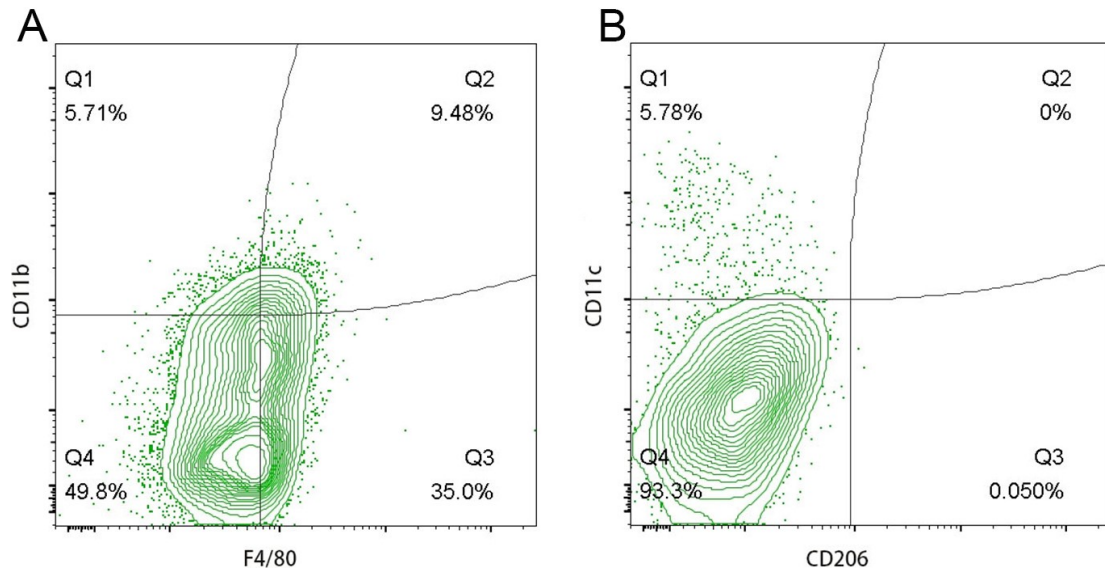
Syringe needle scratch



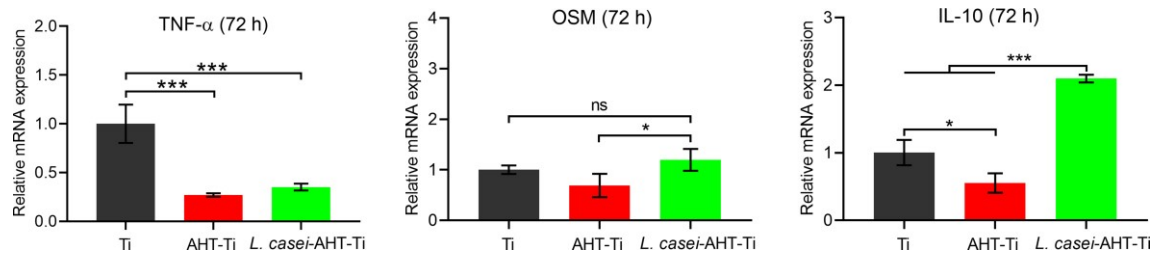
**Fig. S3.** SEM images of *L. casei*-AHT-Ti scratched by a 200  $\mu$ L pipette tip and a 5 mL syringe needle, respectively.



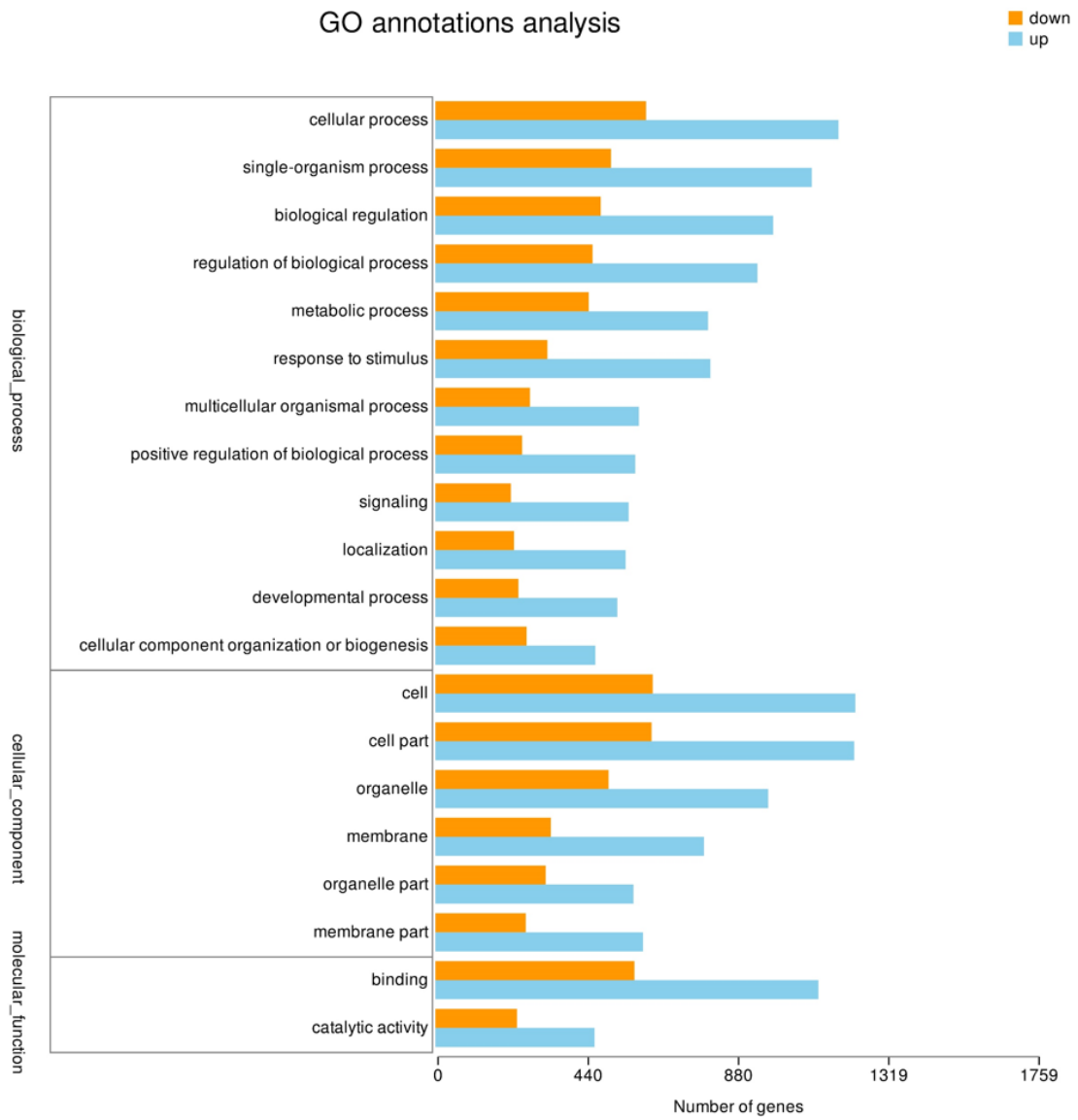
**Fig. S4.** The secretion of bacteriocin from the biofilm of *L. casei*-AHT-Ti-1 (1 day), *L. casei*-AHT-Ti-2 (2 days) and *L. casei*-AHT-Ti (3 days) by a Tricine-SDS-PAGE technique.



**Fig. S5. Original phenotypes of Raw 246.7 macrophages.** (A) Characterization of Raw 246.7 macrophages phenotypes *via* CD11b, F4/80, (B) CD11c and CD206 (gated on CD11b<sup>+</sup>F4/80<sup>+</sup>).

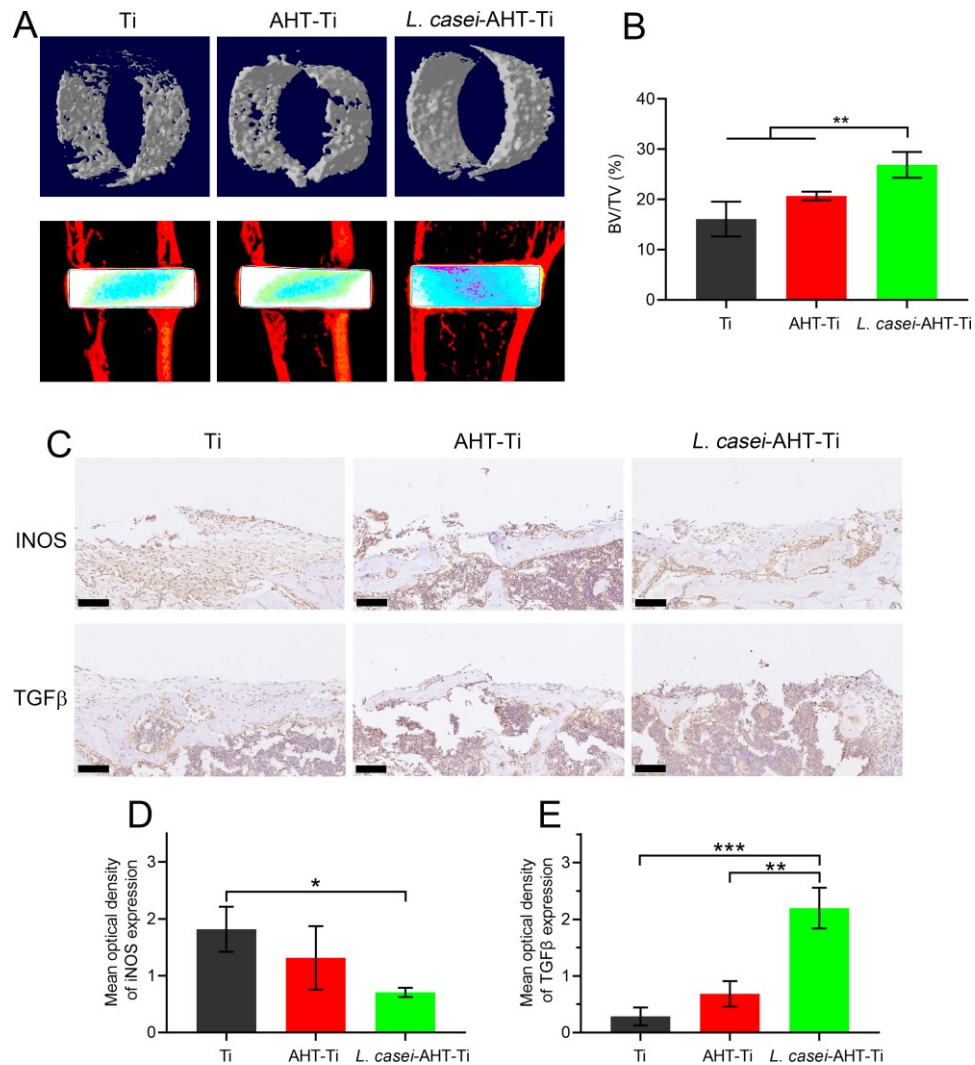


**Fig. S6.** Gene expression of TNF- $\alpha$ , OSM and IL-10 after 72 h treatments. n = 3 independent experiments per group, \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .



**Fig. S7.** Up-regulated and down-regulated genes GO enrichment analysis in *L. casei*-AHT-Ti.





**Fig. S8. Micro-CT results without MRSA infection and immunohistochemical staining for macrophages *in vivo*.** (A) Micro-CT results without MRSA infection. (B) Bone volume (BV) / tissue volume (TV) values of Ti, AHT-Ti and *L. casei*-AHT-Ti. (C) Immunohistochemical staining of iNOS (M1) and TGFβ (M2) for macrophages, respectively (Scale bar, 100 μm). (D) Mean optical density of iNOS and (E) TGFβ expression, respectively. The calculated area is 50 μm around the implant. n = 3 independent experiments per group, \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

Table S1. Primers of tested genes by quantitative polymerase chain reaction (qPCR).

Gene	Primers (F, Forward; R, Reverse)
TNF- $\alpha$	F: 5'-GACGTGGAAGTGGCAGAAGAG-3' R: 5'-TTGGTGGTTTGTGAGTGTGAG-3'
OSM	F: 5'-CCCGGCACAATATCCTCGG-3' R: 5'-TCTGGTGTTGTAGTGGACCGT-3'
IL-10	F: 5'-GCTCTTACTGACTGGCATGAG-3' R: 5'-CGCAGCTCTAGGAGCATGTG-3'
ALP	F: 5'-AGCGACACGGACAAGAAGC-3' R: 5'-GGCAAAGACCGCCACATC-3'
OCN	F: 5'-AAGCCCAGCGACTCTGAGTCT-3' R: 5'-CCGGAGTCTATTCACCACCTTACT-3'
RUNX2	F: 5'-AATGCCTCCGCTGTTATG-3' R: 5'-TTCTGTCTGTGCCTTCTTG-3'
COL-I	F: 5'-CCTGAGCCAGCAGATTGA-3' R: 5'-TCCGCTCTTCCAGTCAG-3'
GAPDH	F: 5'-GCCTCGTCTCATAGACAAGATGGT-3' R: 5'-GAAGGCAGCCCTGGTAACC-3'