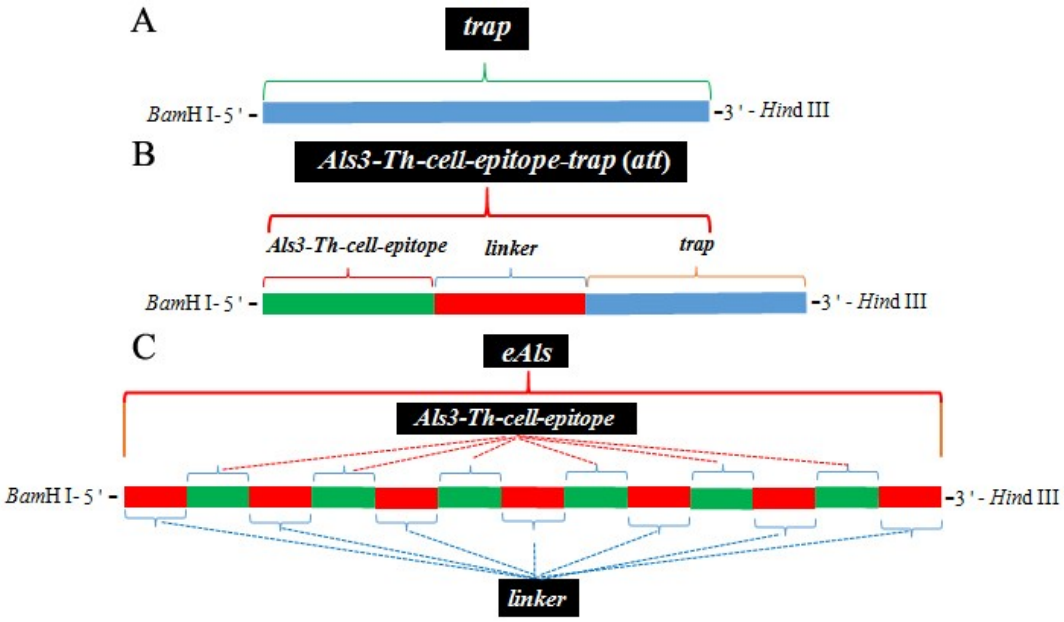

Figure 1. The structural cassette of target gene.

A. The cassette of *trap* gene. The *trap* gene involves in 498 bp at 5 ' end and 3' end with *Bam*H I, *Hin*d III restriction endonuclease, respectively. **B. The cassette of *att* gene.** The *att* gene includes *Als3-Th-cell-epitope*, *linker* and *trap*, three parts of 558 bp with 5 ' end of *Bam*H I and 3' end of *Hin*d III restriction endonuclease. **C. The cassette of *eAls* gene.** The *eAls* gene consists of 426 bp, containing *Als3-Th-cell-epitope* repeated for 6 times and the nucleotide sequence of flexible linker repeated for 7 times with 5 ' end of *Bam*H I and 3' end of *Hin*d III restriction endonuclease.

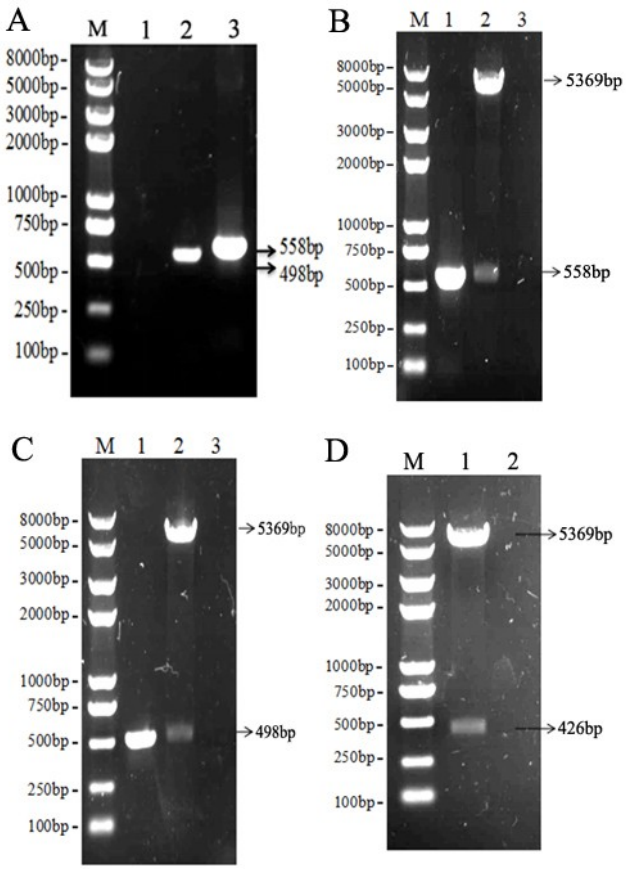

Figure 2. Acquisition of target fragments and construction of recombinant plasmids

A. Acquisition of target fragments. The fragments of 498 bp and 558 bp were obtained by using PCR method, respectively. **B. The identification of the pET-28a (+)-*att* plasmids.** The analytical results showed that the 558 bp fragments were obtained with PCR, enzyme digestion method, respectively. **C. The identification of the pET28a-(+)-*trap* plasmids.** The 498 bp fragments were exhibited with PCR and digestion method, respectively. **D. The confirmation of the pET-28a (+)-*eAls* plasmids.** The 426 bp segments were displayed in figure D by using digestion method.

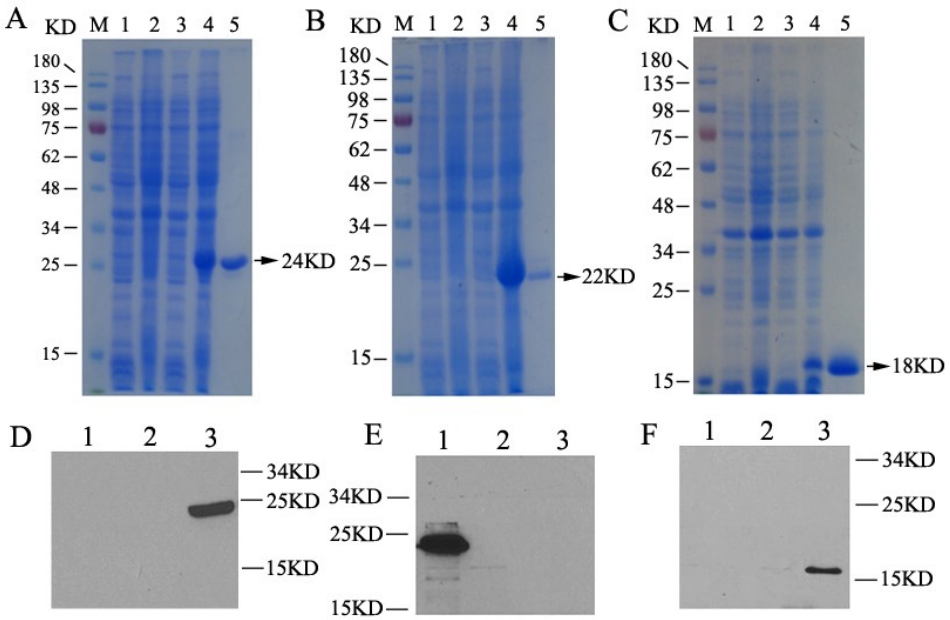

Figure 3. Analysis of protein expression.

1. A, B, C. **The expression of target protein.** The desired proteins, ATT (A), TRAP (B) and eAls (C) were exhibited with the molecular weight of 24 KD, 22KD and 18KD by using SDS-PAGE assay, respectively. (A, B, C) : M lane: protein Marker; 1 lane: The non-induced recombinant bacteria with PET-28a (+) plasmids ; 2 lane: The induced recombinant bacteria with PET-28a (+) plasmids. (A) : 3 lane: The non-induced recombinant bacteria with PET-28a (+)-*att* plasmids. 4 lane: The induced recombinant bacteria with PET-28a (+)-*att* plasmids. 5 lane: The purified ATT proteins. (B): 3 lane: The non-induced recombinant bacteria with PET-28a (+)-*trap* plasmids. 4 lane: The induced recombinant bacteria with PET-28a (+)-*trap* plasmids. 5 lane: The purified TRAP proteins. (C): 3 lane: The non-induced recombinant bacteria with PET-28a (+)-*eAls* plasmids. 4 lane: The induced recombinant bacteria with PET-28a (+)-*eAls* plasmids. 5 lane: The purified eAls proteins. **2. D, E, F. Analysis of the desired protein expression.** The protein expression was confirmed with Western blot method. (D): 1 lane: The induced *E. coli* strain BL21; 2 lane: The induced *E. coli* strain BL21 with PET-28a (+) plasmids. 3 lane: The induced *E. coli* strain BL21 with PET-28a (+) –*att* plasmids. (E): 1 lane: The induced *E. coli* strain BL21; 2 lane: The induced *E. coli* strain BL21 with PET-28a (+) plasmids. 3 lane: The induced *E. coli* strain BL21 with PET-28a (+)–*trap* plasmids. (F): 1 lane: The induced

E. coli strain BL21; 2 lane: The induced *E. coli* strain BL21 with PET-28a (+) plasmids. 3 lane: The induced *E. coli* strain BL21 with PET-28a (+) –*eAls* plasmids.

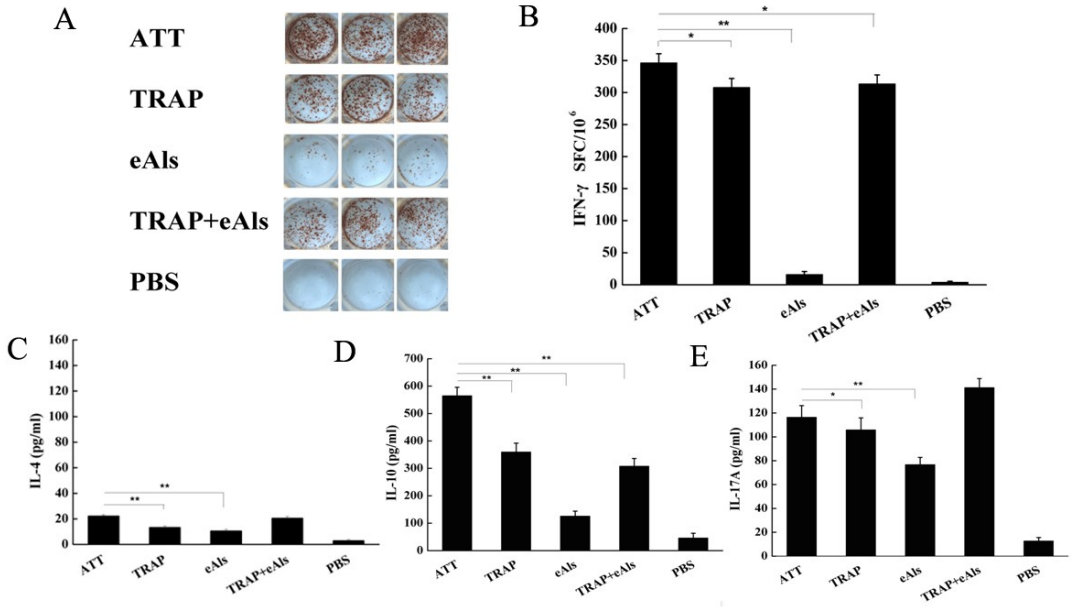

Figure 4. Evaluation of cytokine production.

A. The scanned images of ELISpot membranes. Representative images of spot forming cells (SFC) for IFN-γ secretion in response to TRAP were exhibited. **B. ELISpot analysis.** Data represented the mean of spot-forming cells (SFCs) per million splenocytes from three mice/group with the standard deviation (SD). **C, D, E. The measurement of ELISA.** IL-4 (C), IL-10 (D) and IL-17A (E) cytokines from supernatants of splenocytes were detected by ELISA. Data were shown as means ± SD (n = 3). Statistical differences between groups are shown as p^* <0.05 or ** p < 0.01.

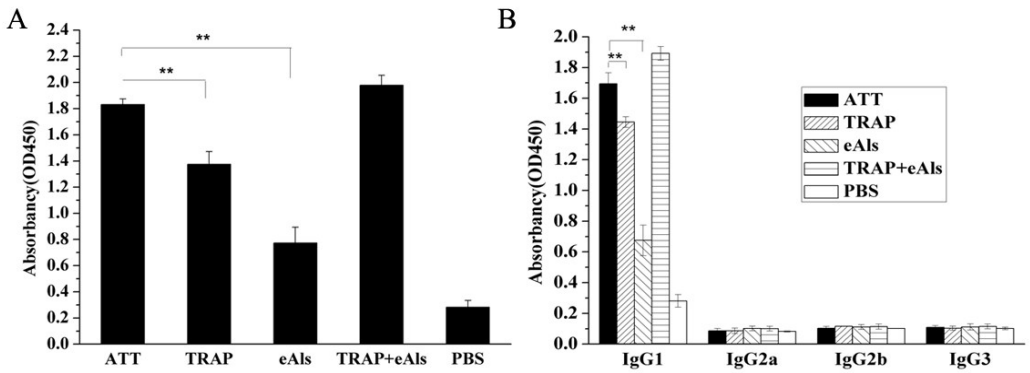

Figure 5. Determination of antibody in serum.

A, B. The level of IgG and its subclasses (IgG1, IgG2a, IgG2b and IgG3) against

TRAP proteins are showed in figure A and B, respectively. The IgG antibody level against TRAP was assessed with ELISA. The OD_{450nm} value was shown as means ± SD (n = 3), and the significant differences were expressed as ** p <0.01.

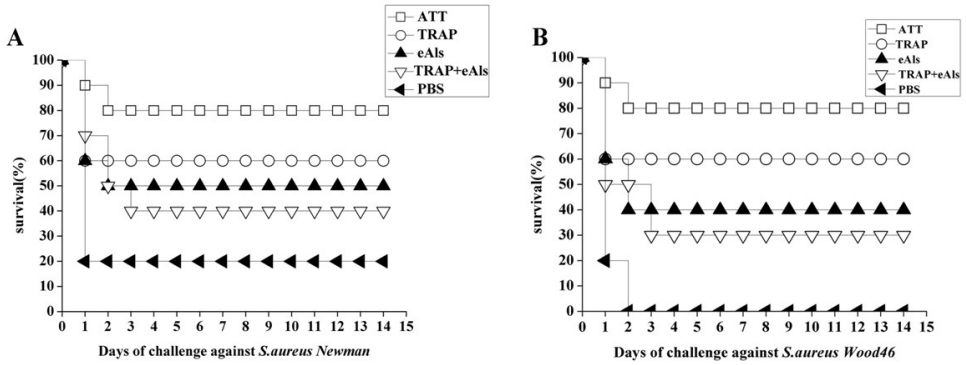

Figure 6. Survival rate of mice after challenge.

A, B. Percentage survival after infection with the lethal doses of *Staphylococcus aureus* strain Newman (A) and *S. aureus* strain Wood46 (B), respectively. The survival of mice was recorded for 14 days after infection.

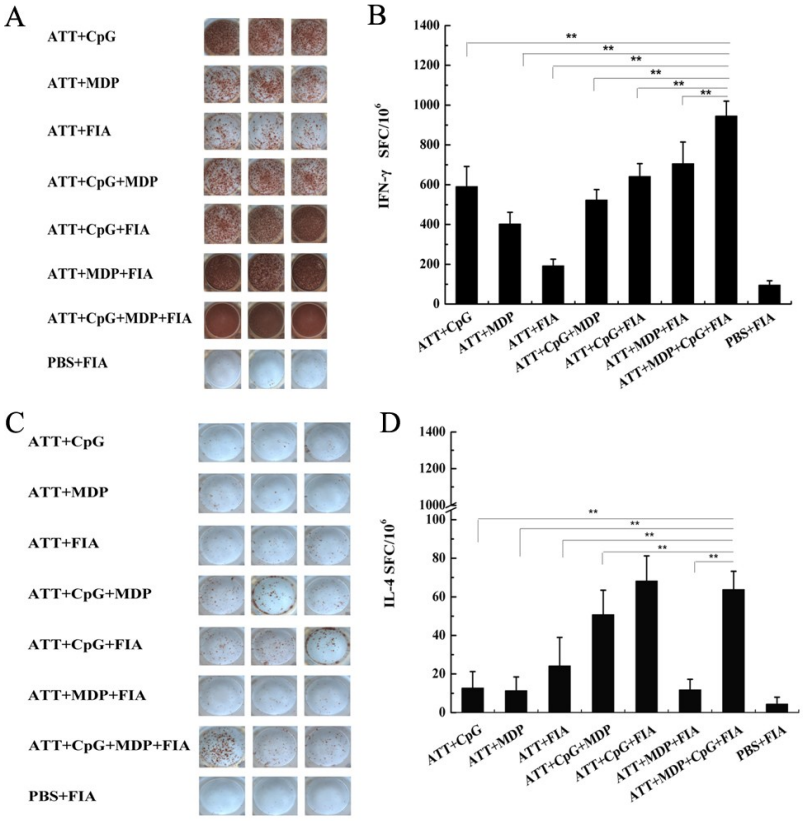

Figure 7. Analysis of cytokine secretion.

A, C. The representative scanned images of ELISpot. IFN-γ and IL-4 secretion

of splenocytes from each group was determined by ELISpot assay. The representative scanned images of ELISpot membranes about IFN-γ (A) and IL-4 (C) secretion. **B, D. Analysis of data.** Data represented the mean of TRAP-specific spot-forming cells (SFCs) for IFN-γ (B) and IL-4 (D) secretion per million splenocytes from three mice per group. Data were shown as means ± SD (n=3). Statistical differences between groups are shown as ** $p < 0.01$.

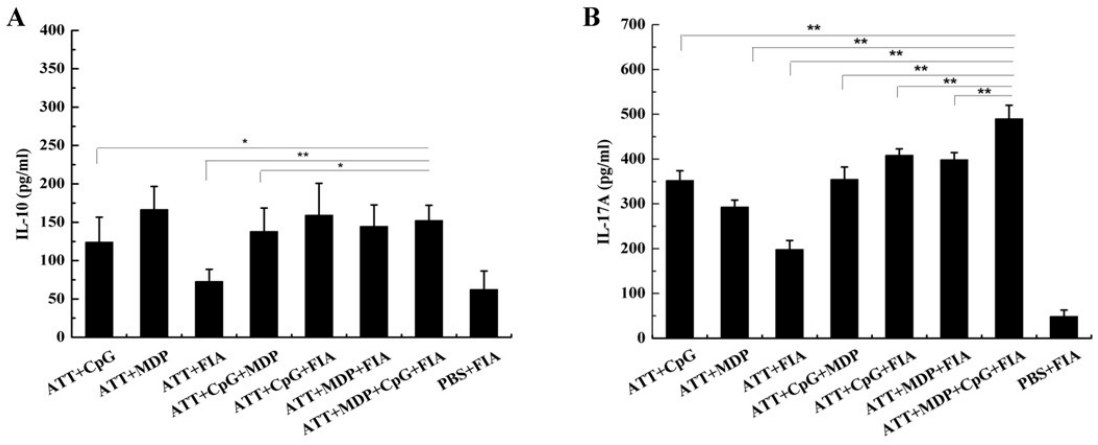

Figure 8. Evaluation of cytokine production.

A, B. (A) IL-10 and (B) IL-17A cytokine level from the supernatants of splenocytes was detected by ELISA assay. Data were shown as means ± SD (n=3). Statistical differences between groups are shown as p* <0.05 or ** $p < 0.01$.

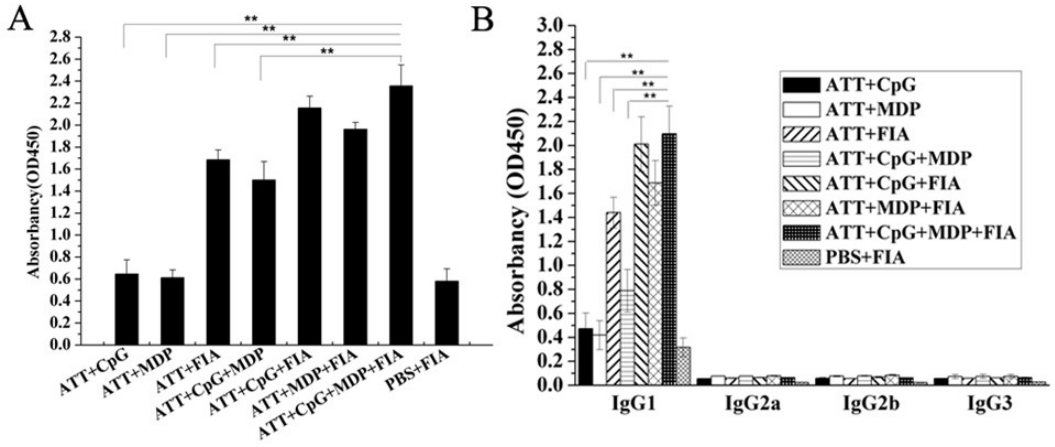

Figure 9. Detection of humoral immune response.

A, B. Serum antibody response in mice immunized with ATT plus the corresponding adjuvant. The levels of IgG (A) and IgG subclasses (B) (IgG1, IgG2a,

IgG2b and IgG3) against TRAP in serum were detected by ELISA. The data are shown as the mean ± SD (n=3).

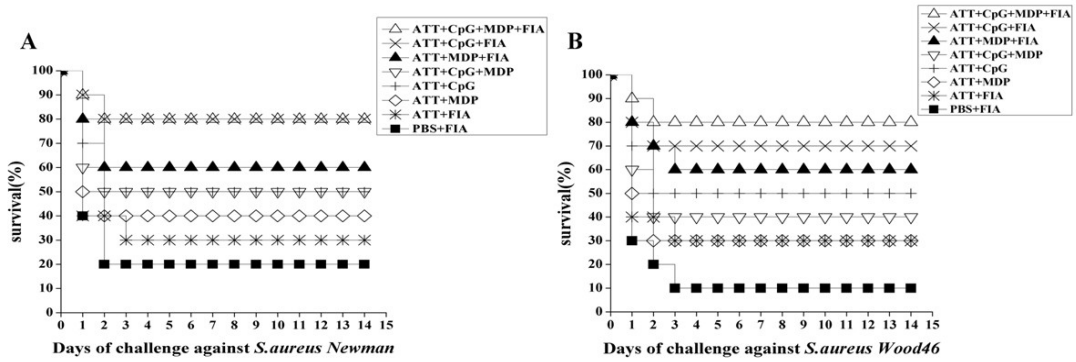

Figure 10. Determination of immunoprotective effect triggered with ATT plus the corresponding adjuvant.

A, B. The immunized mice were challenged with the lethal doses of *Staphylococcus aureus* strain Newman (A) and *S. aureus* strain Wood46 (B), respectively. The survival of mice was recorded and analyzed for 14 days after infection.