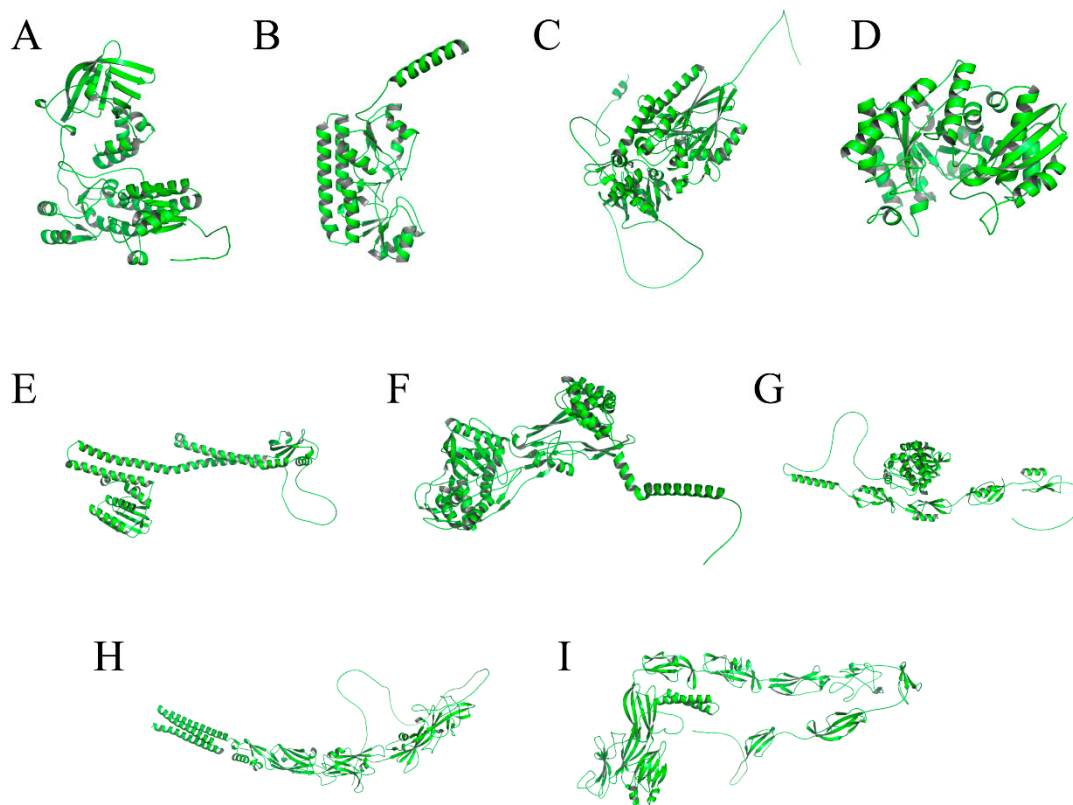


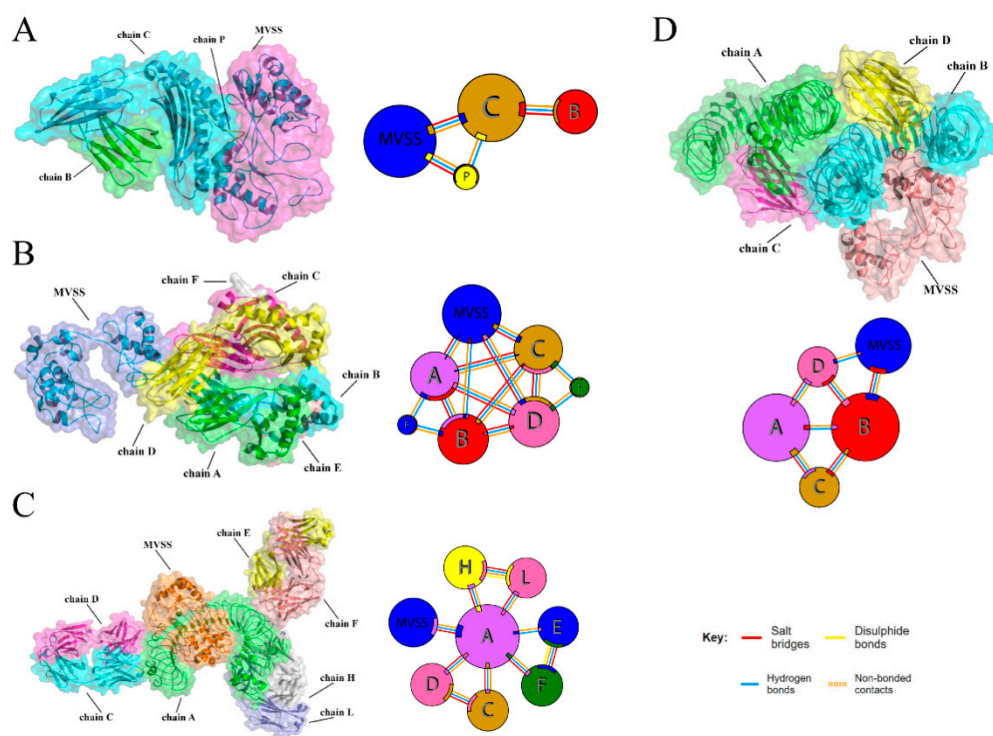
**Table S1** Primers used in this study

Primers	Sequence (5'-3')	Function	Reference
MVSS-F	GGATCCGGGCCAGGTCCCGGAT	A fragment for recombinant protein MVSS gene	This study
MVSS-R	GAGCTCGAGTCGTGTTCCAGCG		
T7-F	TAATACGACTCACTATAGGG	A fragment for plasmid	[1]
T7-R	TGCTAGTTATTGCTCAGCGG	pET-28a gene	
GAPDH-F	TGCACCACCAACTGCTTAG	A fragment for murine	
GAPDH-R	GGATGCAGGGATGATGTTC	housekeeping marker gene	
IL-1 $\beta$ -F	TCCAGGATGAGGACATGAGCAC	A fragment for murine IL-1 $\beta$	
IL-1 $\beta$ -R	GAACGTCACACACCAGCAGGTTA	gene	
IL-6-F	CCACTTCACAAGTCGGAGGCTTA	A fragment for murine IL-6	
IL-6-R	GCAAGTGCATCATCGTTGTTCATAC	gene	
TNF $\alpha$ -F	GGTGCCTATGTCTCAGCCTCTT	A fragment for murine TNF $\alpha$	
TNF $\alpha$ -R	GCCATAGAAGTATGAGAGGGAG	gene	
IL-2-F	GCGGCATGTTCTGGATTGACTC	A fragment for murine IL-2	
IL-2-R	CCACCACAGTTGCTGACTCATC	gene	
IL-10-F	CGGGAAGACAATAACTGCACCC	A fragment for murine IL-10	
IL-10-R	CGGTTAGCAGTATGTTGTCCAGC	gene	

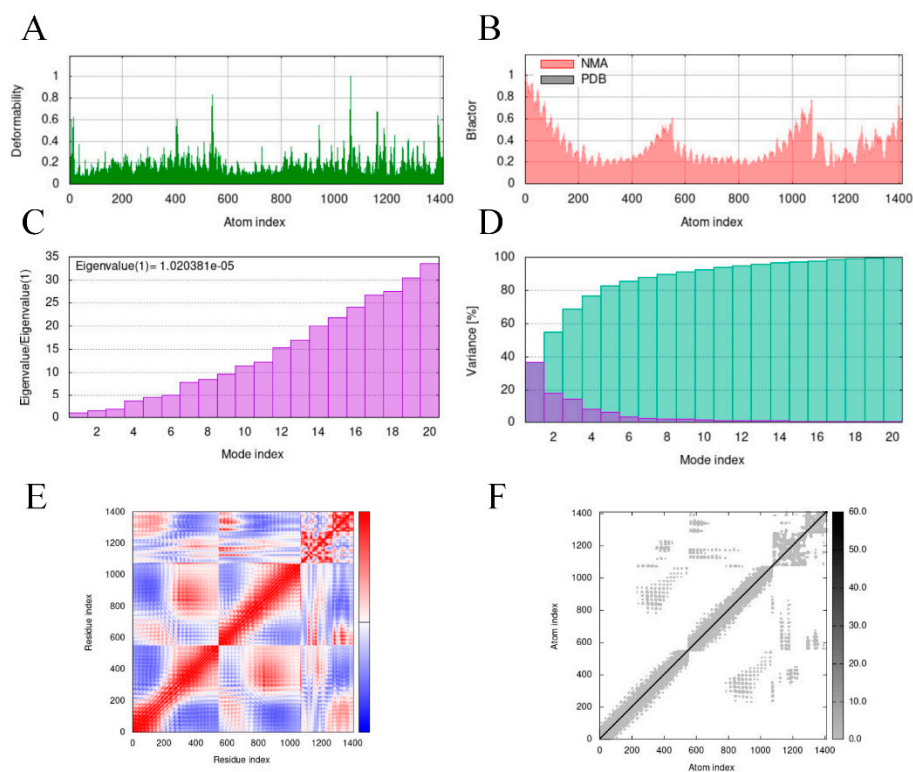


**Figure S1** Three-dimensional modeling of candidate proteins. (A) CP024974\_1\_00171, (B) CP024974\_1\_00407, (C) CP024974\_1\_01634, (D) enolase, (E) CP024974\_1\_01033, (F)

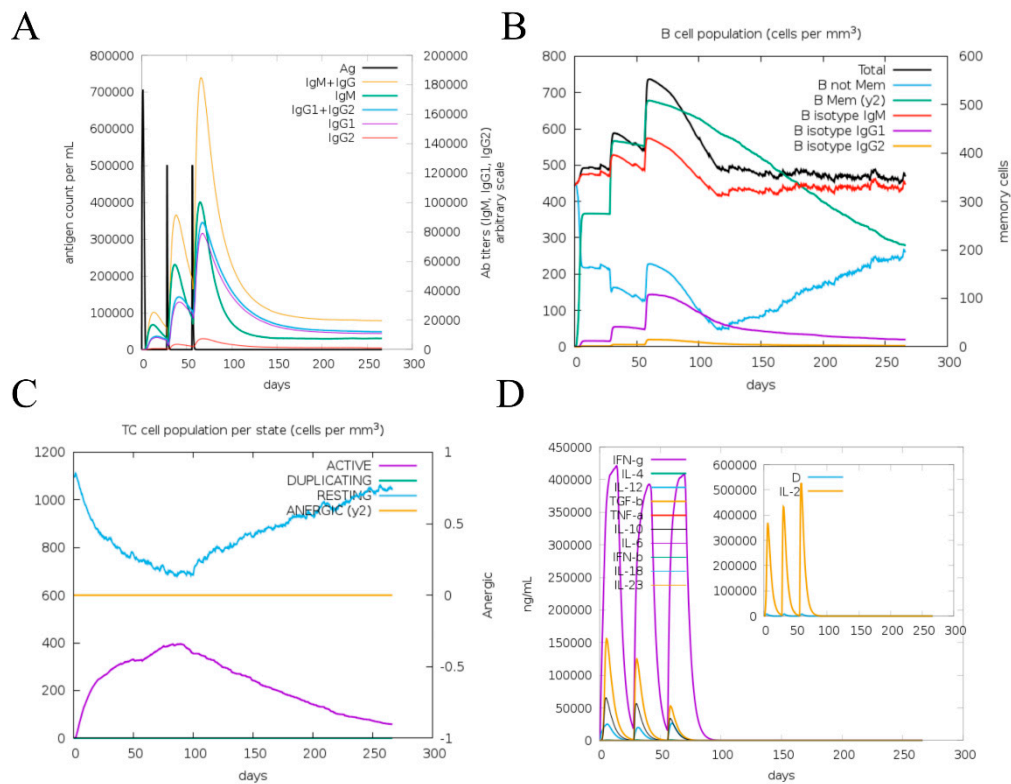




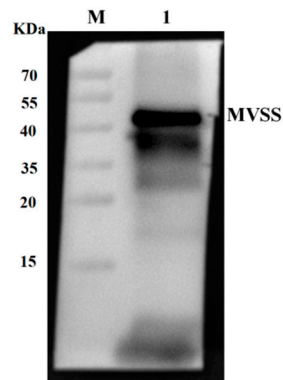
**Figure S3** Molecular docking of MVSS and human immune receptors. Schematic illustration of interaction power between MVSS-MHC I (A), MVSS-MHCII (B), MVSS-TLR 3 (C) and MVSS-TLR4 (D) complexes.



**Figure S4** Molecular dynamics simulations of the MVSS-TLR2 complex. (A) Deformability map of the docking complex. (B) Calculation of the B-factor by NMA. (C) Eigenvalues of docked complexes. (D) NMA variance. (E) Docking complex amino acid residue atom pair covariance matrix plot. (F) Docking complex elastic network model connection spring diagram.

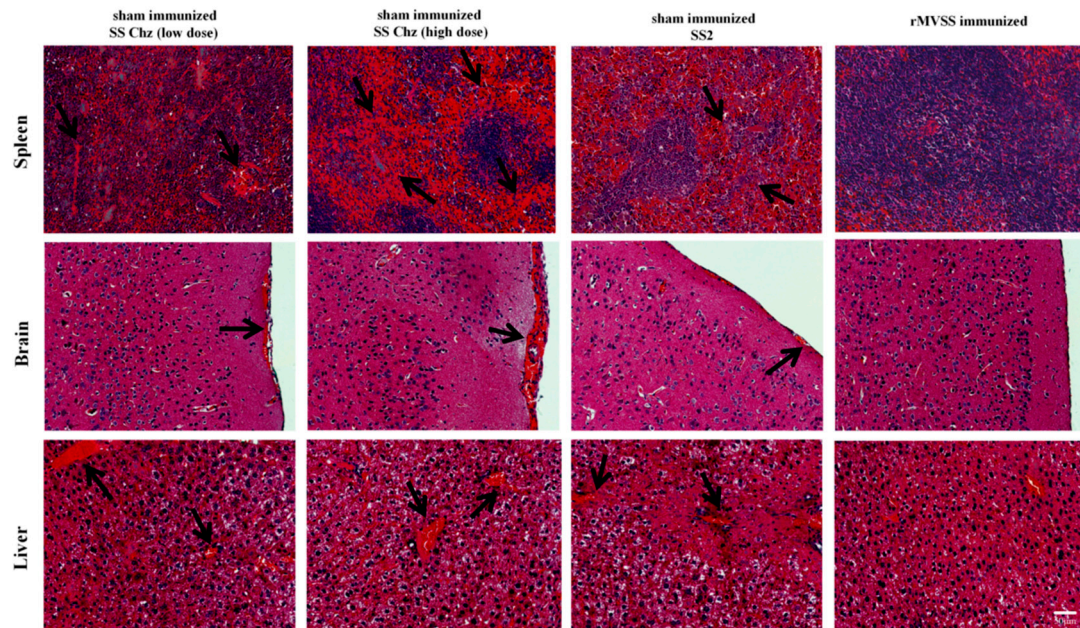


**Figure S5** Simulation of the immunological response of the host following immunization with the multi-epitope vaccine *in silico*. (A) Significantly elevated antibody levels after vaccination with the multi-epitope vaccine. (B) B cells and memory immunoglobulin levels rise following immunization. T-lymphocyte (C) and cytokine (D) numbers rose after vaccination and persisted at elevated levels throughout the exposure period.



**Figure S6** Western blot analyses of MVSS. M indicated Marker, lane 1 meant western blot to detect the reactivity of anti-rMVSS serum with rMVSS.





**Figure S7** Histopathological damage assessment of spleen, brain and liver tissues of rMVSS-immunized and sham-immunized mice after being challenged by various serotypes of *S. suis*. Black lines indicated bleeding areas.

#### Reference

- [1] Zhang, Y.; Liang, S.; Zhang, S.; Zhang, S.; Yu, Y.; Yao, H.; Liu, Y.; Zhang, W.; Liu, G. Development and Evaluation of a Multi-Epitope Subunit Vaccine against Group B Streptococcus Infection. *Emerg. Microbes Infect.* 2022, 11, 2371–2382, doi:10.1080/22221751.2022.2122585.