

Supplement materials

Boosting Mouse Defense against Lethal *Toxoplasma gondii* Infection with Full-Length and Soluble SAG1 Recombinant Protein

Xiang Li ^{1,2,†}, Wei Yuan ^{1,2,†}, Ting He ^{1,2}, Ruiying Guo ^{1,2}, Xiuxian Du ^{1,2}, Yanhong He ^{1,2}, Xuan Li ^{1,2}, Saeed El-Ashram ³, Ebtesam M. Al-Olayan ⁴, Na Yang ^{1,2,*} and Xiaoyu Sang ^{1,2,*}

1 Key Laboratory of Livestock Infectious Diseases, Shenyang Agricultural University, Ministry of Education, Shenyang 110866, China; lx19980405@163.com (X.L.); yuanwei110923@163.com (W.Y.); 2021240709@stu.syau.edu.cn (T.H.); fruiming@stu.syau.edu.cn (R.G.); 2021220598@stu.syau.edu.cn (X.D.); 2022220595@stu.syau.edu.cn (Y.H.); 13081305895@163.com (X.L.)

2 College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, China

3 Zoology Department, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt; saeed_elashram@yahoo.com

4 Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; eolayan@ksu.edu.sa

* Correspondence: dayangna@syau.edu.cn (N.Y.); xysang2016@syau.edu.cn (X.S.)

† These authors contributed equally to this work.

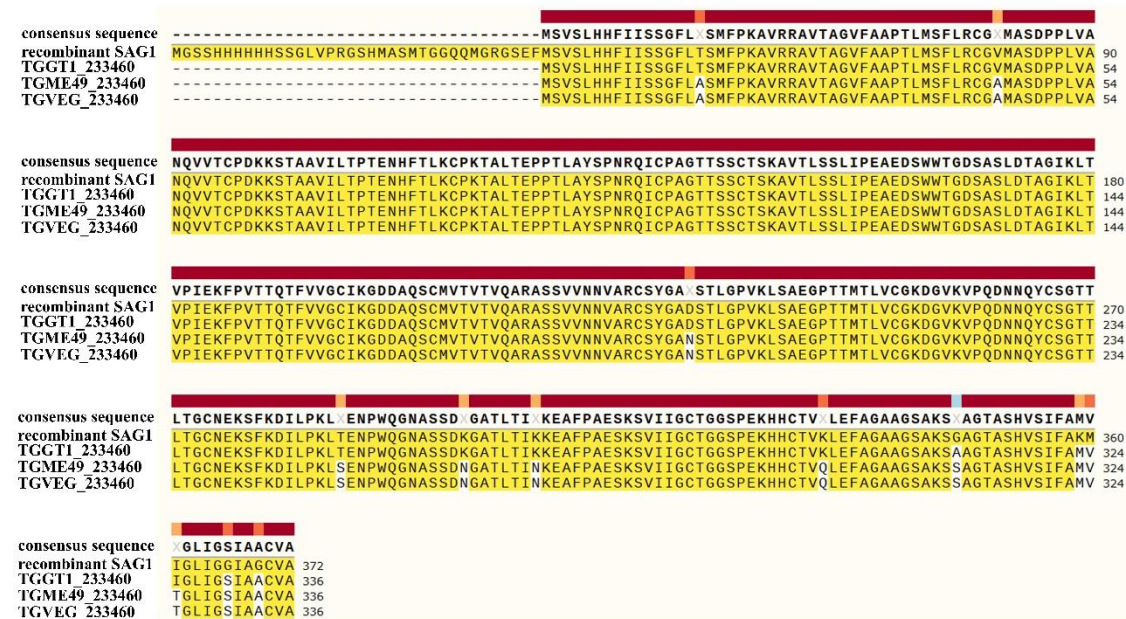


Figure S1. Sequence alignment between recombinant SAG1 protein and native SAG1 proteins of *T. gondii* types I and II strains. The amino acid sequence of the recombinant SAG1 was translated from the pET-28a-SAG1 sequence. The amino acid sequences of three native SAG1 were loaded from ToxoDB data.

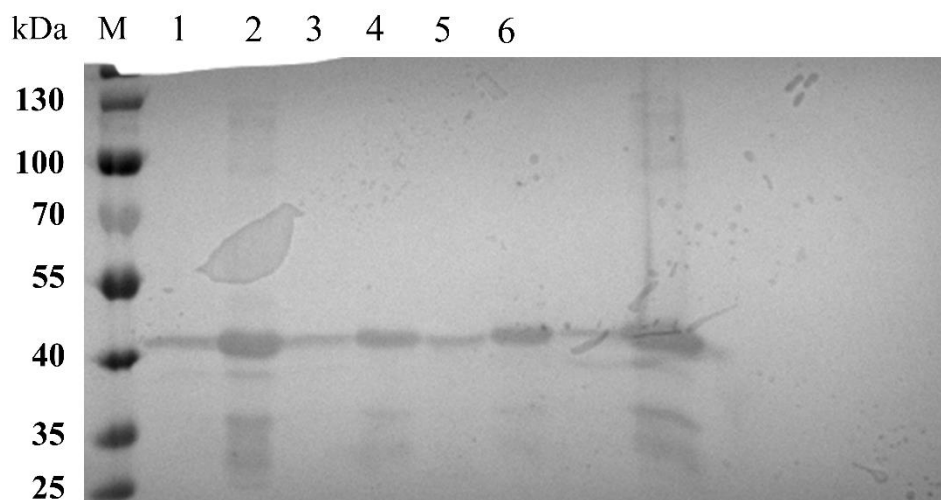


Figure S2. The whole images of Figure 1 B. Identification of SAG1 recombinant protein expressed in *E. coli* Transetta (DE3) cells by western blot. 1,3,5: The soluble protein of interest in the cell lysates supernatant from three randomly selected single colonies ; 2,4,6: The insoluble protein of interest in cell lysate precipitation from three randomly selected single colonies; M: Stander protein marker.

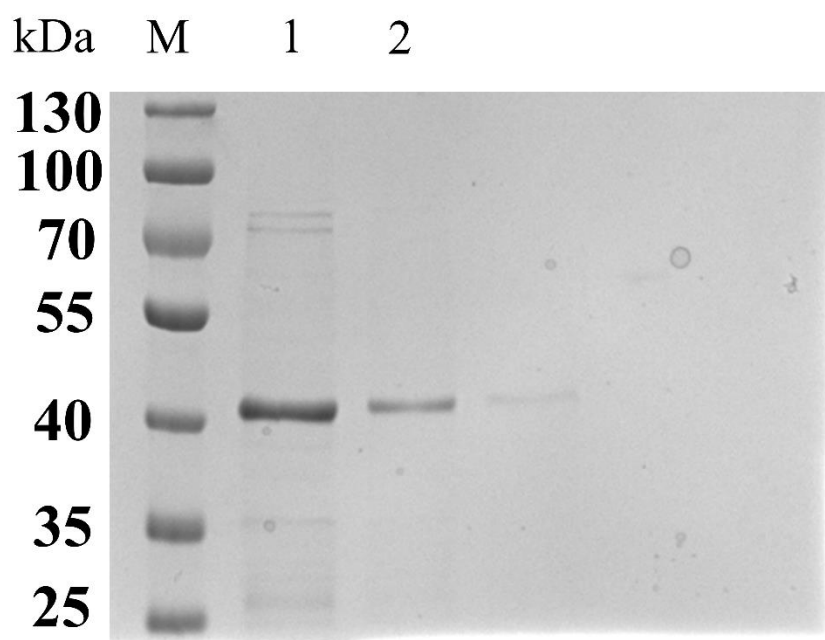


Figure S3. The whole images of Figure 1 D. (D) Purified SAG1 recombinant protein identified by SDS-PAGE. 1,2: Target protein was in eluent buffer; M: Stander protein marker.

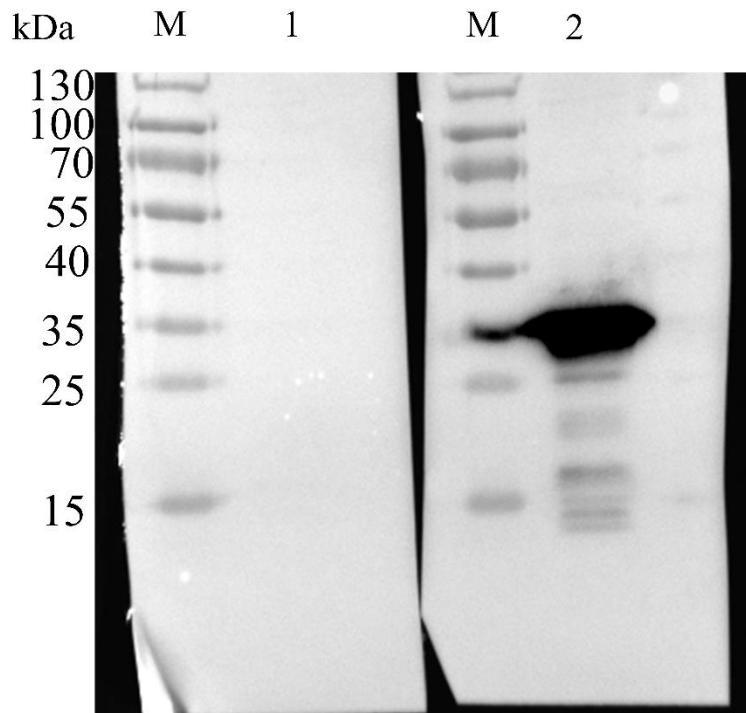


Figure S4. The whole images of Figure 2A. Western blot analysis for native SAG1 in TLA with the experimental mouse sera. 1: The reaction of TLA with the sera isolated from mice in the control group; 2: The reaction of TLA with the sera isolated from mice in the immunize group; M: Stander protein marker.

Table S1. Peptides with high score at C terminal of SAG1 protein binding to MHC class II molecules predicted by IEDB^a.

Allele ^b	Start	End	Peptide ^c	Score ^d
H2-IAAd	175	189	TVQARASSVNNVAR	0.519
H2-IAAd	308	322	AKSAAGTASHVSIFA	0.4901
H2-IAAd	171	185	MVTVTVQARASSVNN	0.4661
H2-IAAd	296	310	TVKLEFAGAAGSAKS	0.4398
H2-IAAd	169	183	SCMVTVTVQARASSV	0.4304
H2-IAAd	170	184	CMVTVTVQARASSVV	0.4233
H2-IAAd	307	321	SAKSAAGTASHVSIF	0.4184
H2-IAAd	174	188	VTQARASSVNNVA	0.4077
H2-IAAd	113	127	KAVTLSSLIPEAEDS	0.3972
H2-IAAd	302	316	AGAAGSAKSAAGTAS	0.3781
H2-IAAd	240	254	EKSFKDILPKLTENP	0.3711
H2-IAAd	168	182	QSCMVTVTVQARASS	0.3714
H2-IAAd	295	309	CTVKLEFAGAAGSAK	0.3736

H2-IAd	306	320	GSAKSAAGTASHVSI	0.3635
H2-IAd	297	311	VKLEFAGAAGSAKSA	0.3525
H2-IAd	63	77	KKSTAAVILTPTEH	0.3298
H2-IAd	61	75	PDKKSTAAVILTPTE	0.3298
H2-IAd	301	315	FAGAAGSAKSAAGTA	0.3227
H2-IAd	25	39	RAVTAGVFAAPTLMS	0.3234
H2-IAd	62	76	DKKSTAAVILTPTEN	0.3172

^aThe Immune Epitope Database (<http://tools.immuneepitope.org/mhcii>);

^b H2-IAd alleles (mouse MHC class II molecules);

^c 15 amino acids were chosen for analysis;

^d High score indicates high level binding.