

Supplementary Materials

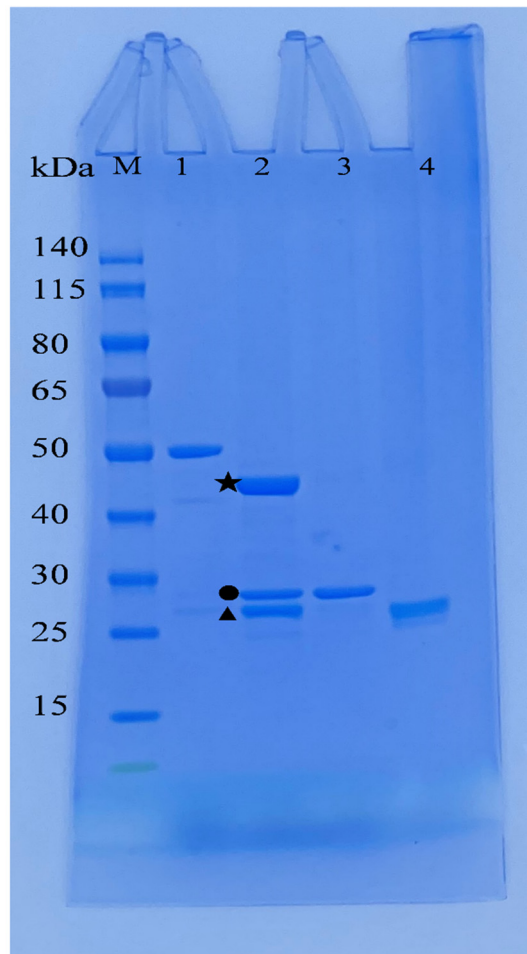


Figure S1. SDS-PAGE analysis of purified and cleaved proteins. Lane M: protein marker; Lane 1: purified GST-EQ8-ScfF fusion protein (~50 kDa); Lane 2: cleavage mixture after PreScission protease digestion (★: GST-tagged PreScission protease at ~44 kDa; ●: untagged EQ8-ScfF at ~28–30 kDa; ▲: released GST tag at ~26 kDa); Lane 3: purified untagged EQ8-ScfF protein after secondary purification (~28–30 kDa); Lane 4: purified GST tag protein (~26 kDa).

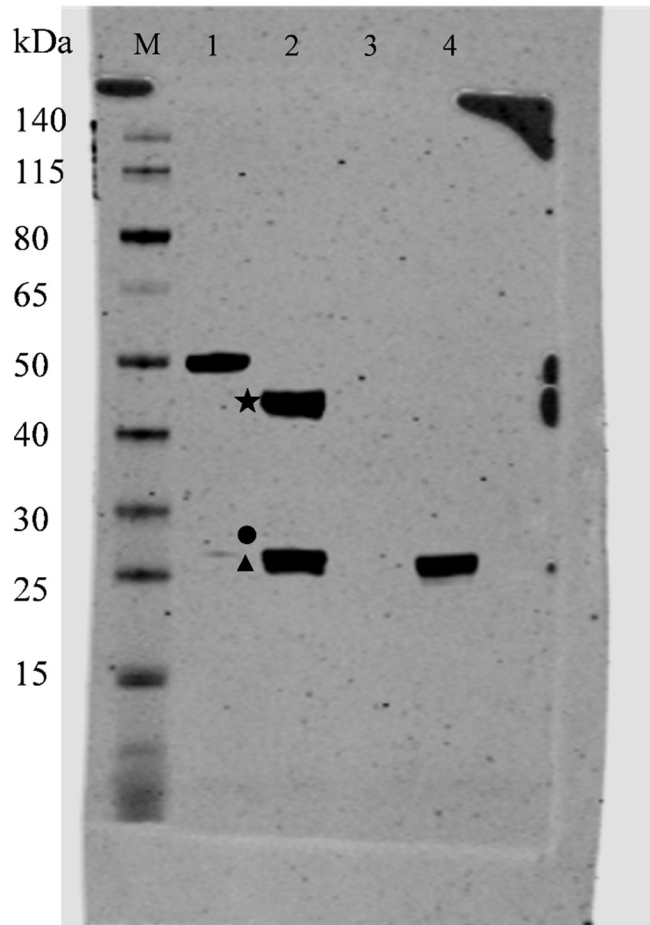


Figure S2. Western blot probed with strangles-positive equine serum. Lane M: protein marker; Lane 1: purified GST-EQ8-SclF fusion protein (~50 kDa); Lane 2: cleavage mixture after PreScission protease digestion (★: GST-tagged PreScission protease at ~44 kDa; ●: untagged EQ8-SclF at ~28–30 kDa; ▲: released GST tag at ~26 kDa); Lane 3: purified untagged EQ8-SclF protein after secondary purification (~28–30 kDa); Lane 4: purified GST tag protein (~26 kDa). The results confirmed that the untagged EQ8-SclF protein retained strong immunoreactivity.

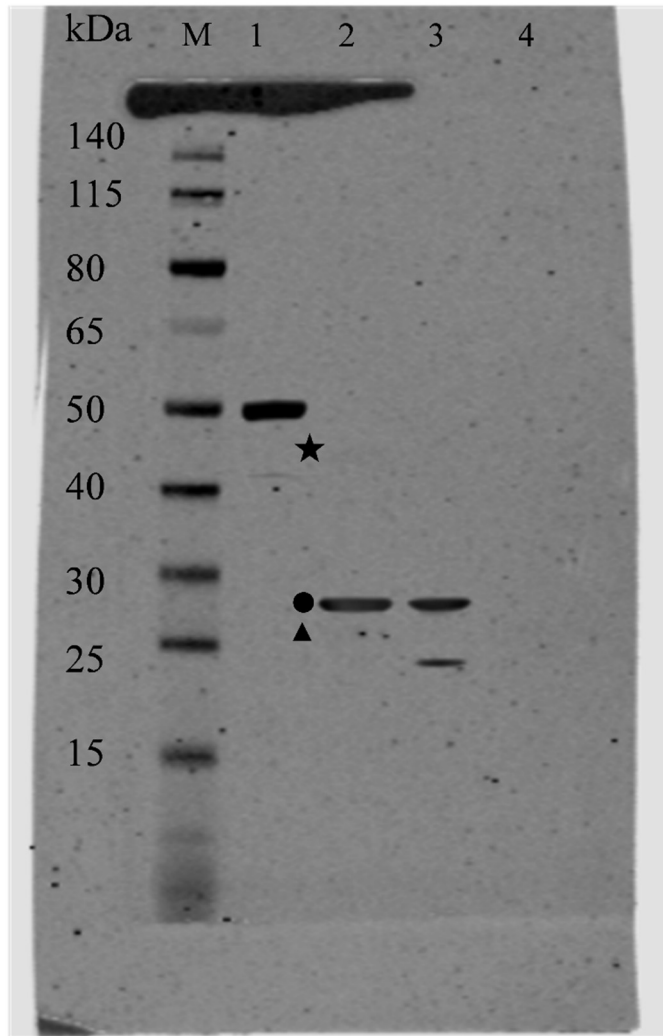


Figure S3. Western blot probed with anti-GST antibody. Lane M: protein marker; Lane 1: purified GST-EQ8–ScIF fusion protein (~50 kDa); Lane 2: cleavage mixture after PreScission protease digestion (★: GST-tagged PreScission protease at ~44 kDa; ●: untagged EQ8–ScIF at ~28–30 kDa; ▲: released GST tag at ~26 kDa); Lane 3: purified untagged EQ8–ScIF protein after secondary purification (~28–30 kDa); Lane 4: purified GST tag protein (~26 kDa). The results confirmed that the final purified EQ8–ScIF protein was free of GST tag contamination.

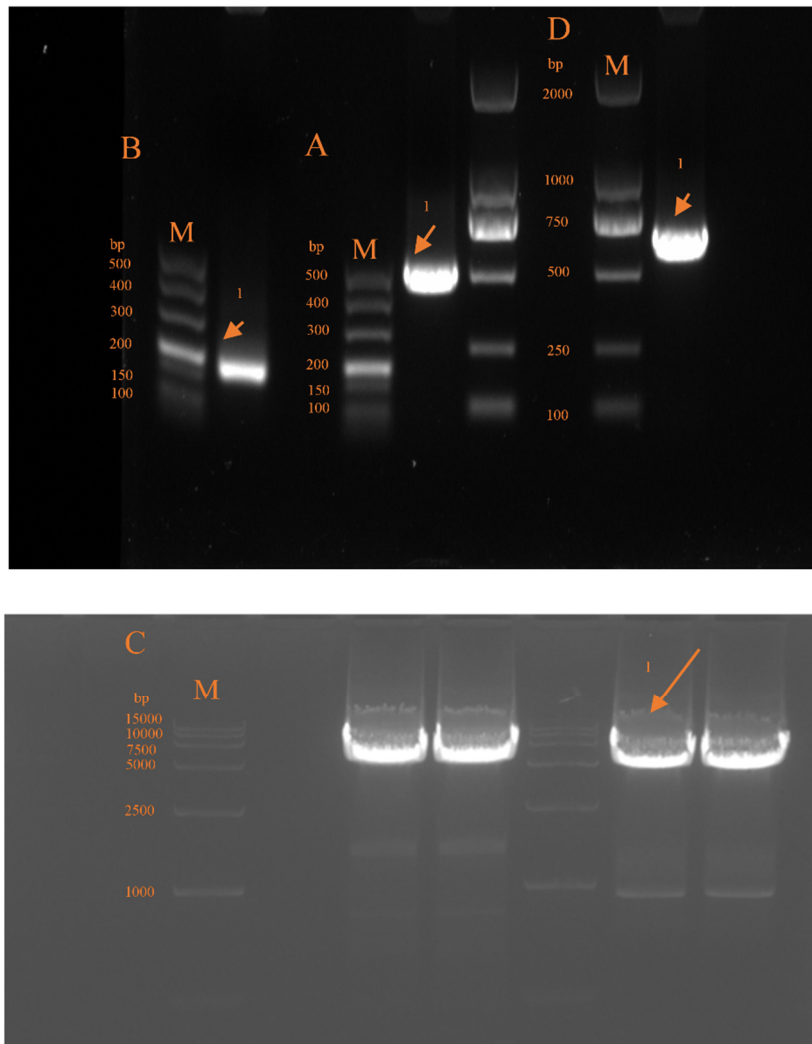


Figure S4. PCR amplification and restriction digestion verification of the recombinant plasmid pGEX-6p-1-EQ8-ScIF. (A) PCR amplification of the EQ8 fragment. Lane M: DL500 DNA marker; Lane 1: EQ8 amplicon (450 bp). (B) PCR amplification of the ScIF fragment. Lane M: DL500 DNA marker; Lane 1: ScIF amplicon (132 bp). (C) Linearized pGEX-6p-1 vector fragment. Lane M: DL15000 DNA marker; Lane 1: linearized pGEX-6p-1 vector (4984 bp). (D) Double digestion verification of the recombinant plasmid with *Bam*HI and *Xho*I. Lane M: DL2000 DNA marker; Lane 1: released EQ8-ScIF insert fragment (618 bp). The expected sizes of the amplified and digested fragments were consistent with the design, confirming successful construction of the recombinant plasmid.

Supplementary Table S1. Complete data for inter-assay reproducibility of the indirect ELISA. Twenty-four serum samples covering different antibody levels (negative, weakly positive, and strongly positive) were tested on two independently coated ELISA plates, with each sample run in duplicate. OD₄₅₀₋₁ and OD₄₅₀₋₂ represent the absorbance values obtained from the two independent plates. CV (%) was calculated as $SD/mean \times 100\%$.

Sample No.	OD ₄₅₀₋₁	OD ₄₅₀₋₂	CV (%)
1	0.077	0.083	5.30
2	0.079	0.085	5.17
3	0.092	0.084	7.07
4	0.091	0.087	3.16
5	0.084	0.082	1.69
6	0.103	0.092	7.78
7	0.092	0.086	4.72
8	0.099	0.094	3.63
9	0.213	0.226	4.18
10	0.095	0.092	2.24
11	0.089	0.092	2.31
12	0.091	0.092	0.78
13	0.089	0.079	8.33
14	0.084	0.083	0.84
15	0.080	0.085	4.22
16	0.109	0.098	7.48
17	0.105	0.092	9.29
18	1.856	1.906	1.88
19	1.687	1.621	2.82
20	3.268	3.316	1.03
21	1.647	1.634	0.18
22	2.001	2.011	0.35
23	2.888	2.890	0.05
24	1.979	1.836	5.30

Note: Twenty-four serum samples covering different antibody levels (negative, weakly positive, and strongly positive) were tested on two independently coated ELISA plates, with each sample run in duplicate. OD₄₅₀₋₁ and OD₄₅₀₋₂ represent the absorbance values obtained from the two independent plates. CV (%) was calculated as $SD/mean \times 100\%$.