# Novel GS5 sericin mitigate UVA-induced photoaging by activating Nrf2 and inhibiting the JAK-STAT pathway

## Supplementary Figures

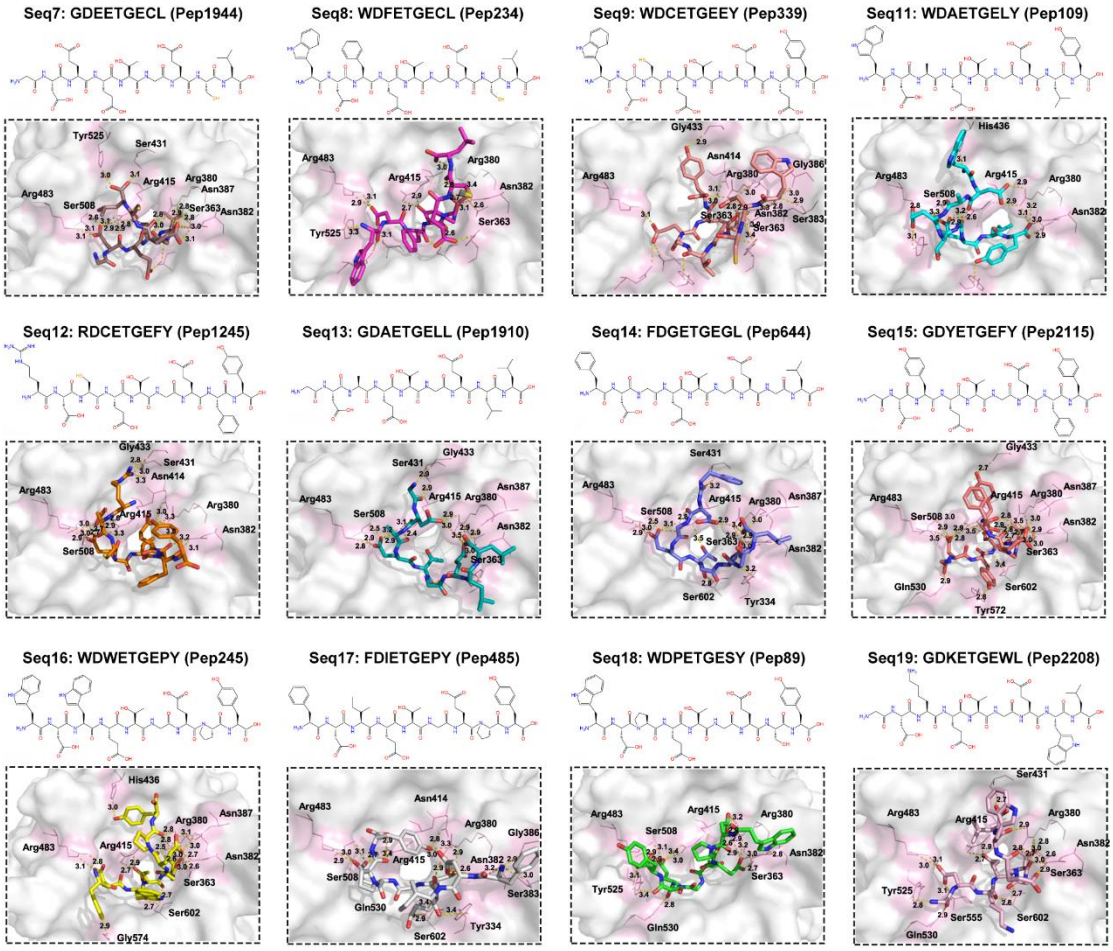


Fig. S1. Visualization of the interactions between the candidate peptide and the Keap1 complex after molecular docking.

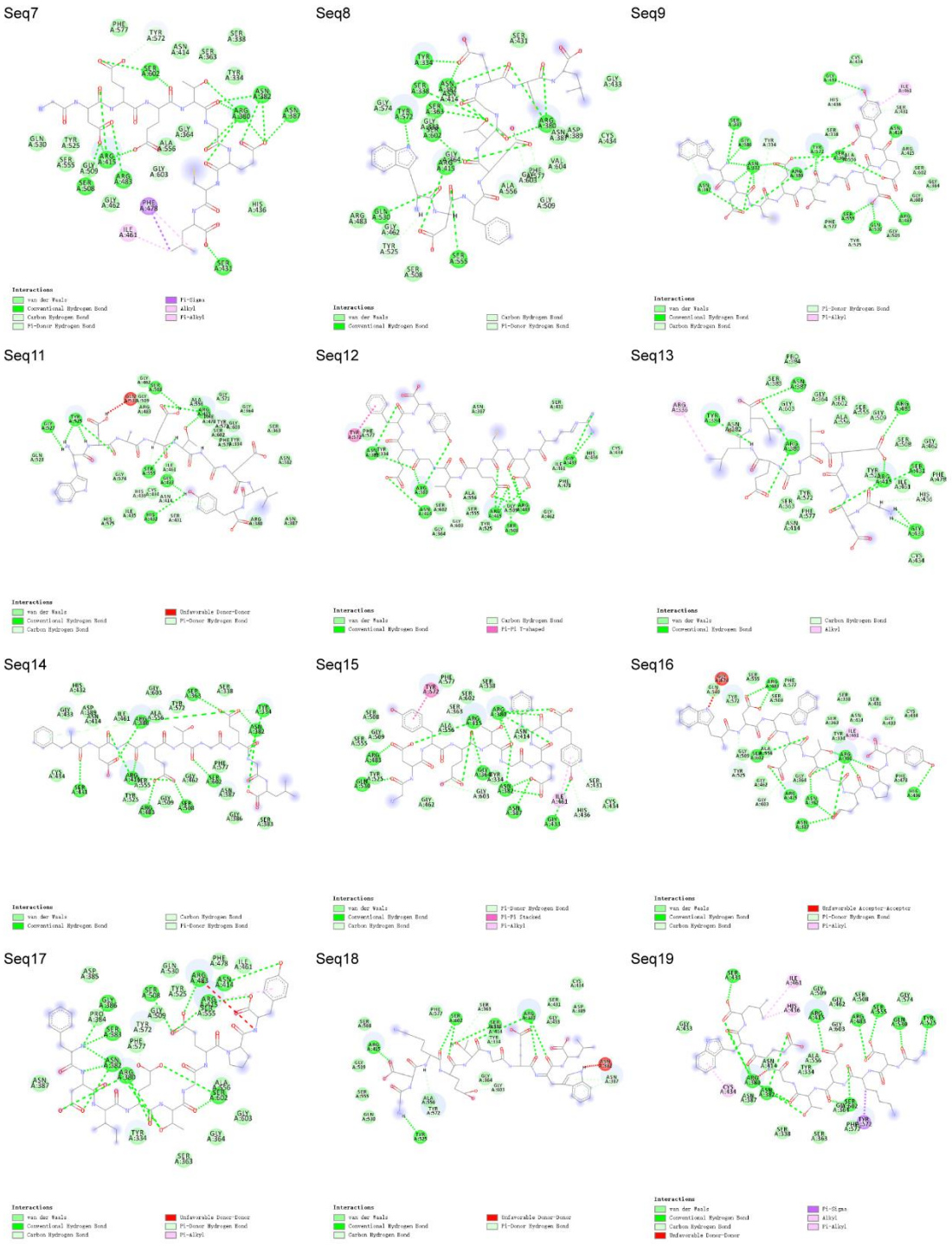


Fig. S2. 2D binding schematic of candidate peptides interacting with Keap1, highlighting the precise non-covalent interactions between them.

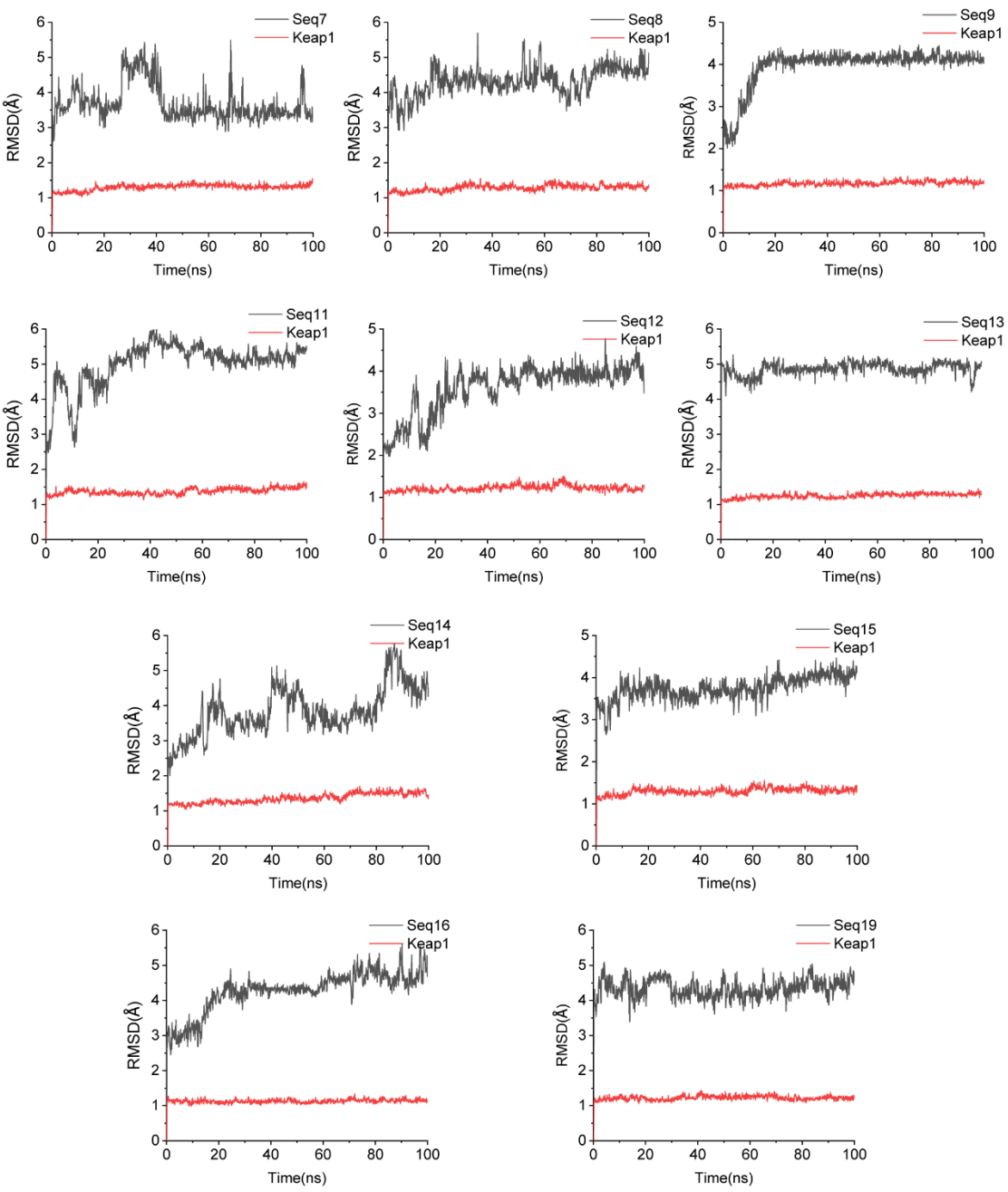


Fig. S3. RMSD curve of the MD simulation of the peptide-Keap1 complex.

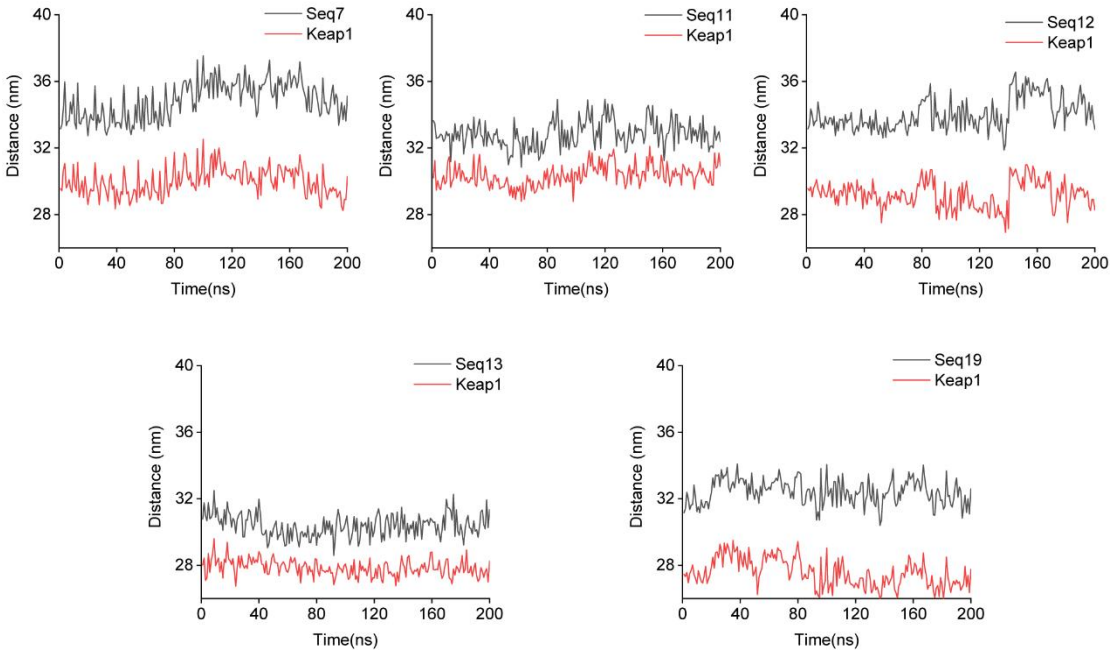


Fig. S4. The variation of distance between the peptide and Keap1 complex over time as observed in MD simulations.

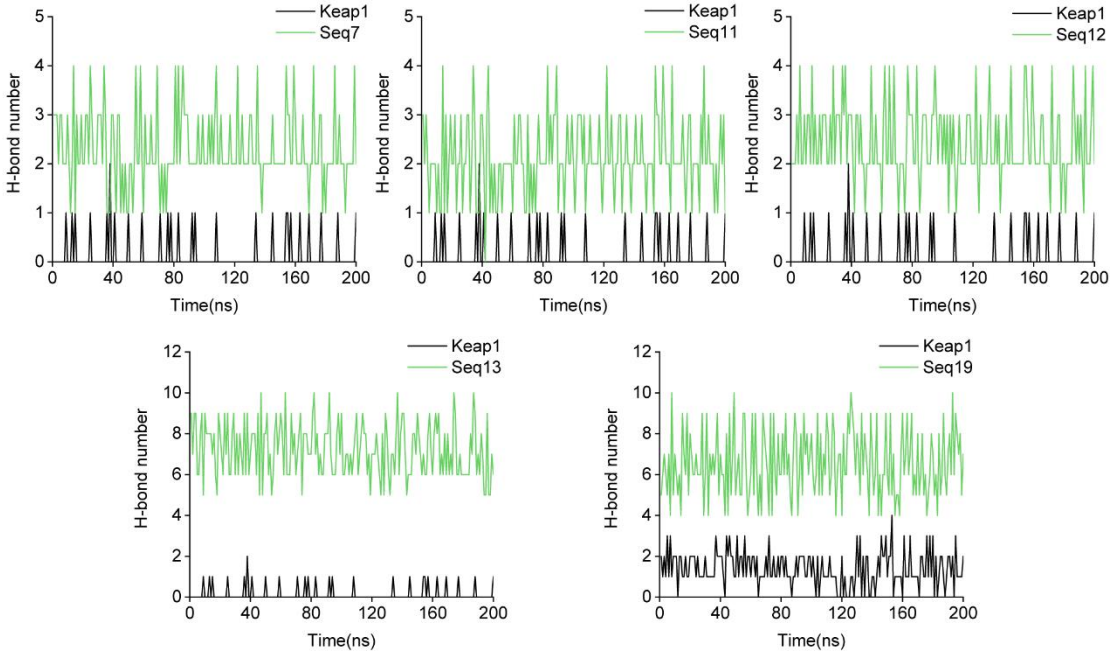


Fig. S5. The variation of hydrogen bonds in the MD simulation of the peptide-Keap1 complex over time.

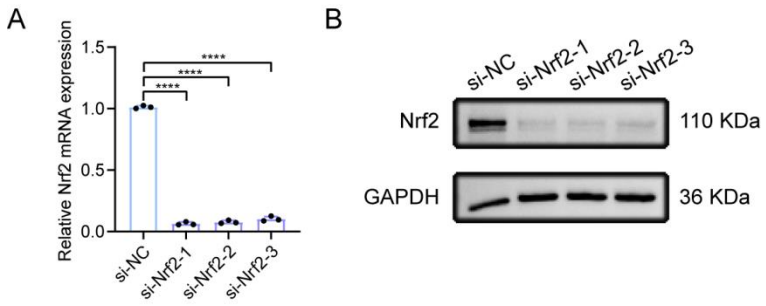


Fig. S6. Verification of Nrf2 Knockdown. (A) Detection at the mRNA level; (B) Detection at the protein level.

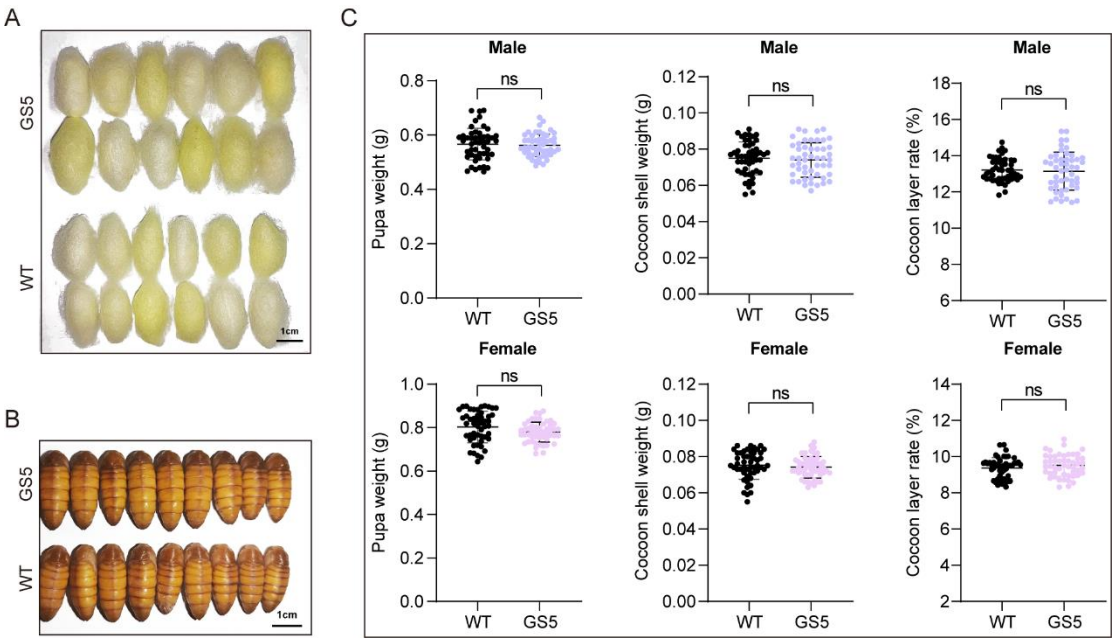


Fig. S7. Economic shape evaluation of transgenic silkworms. (A-B) Representative images of cocoons and pupae from WT silkworms and GS5 transgenic silkworms. (C) Statistical analysis of pupal weight, cocoon layer weight, and cocoon layer rate for male and female GS5 transgenic silkworms.

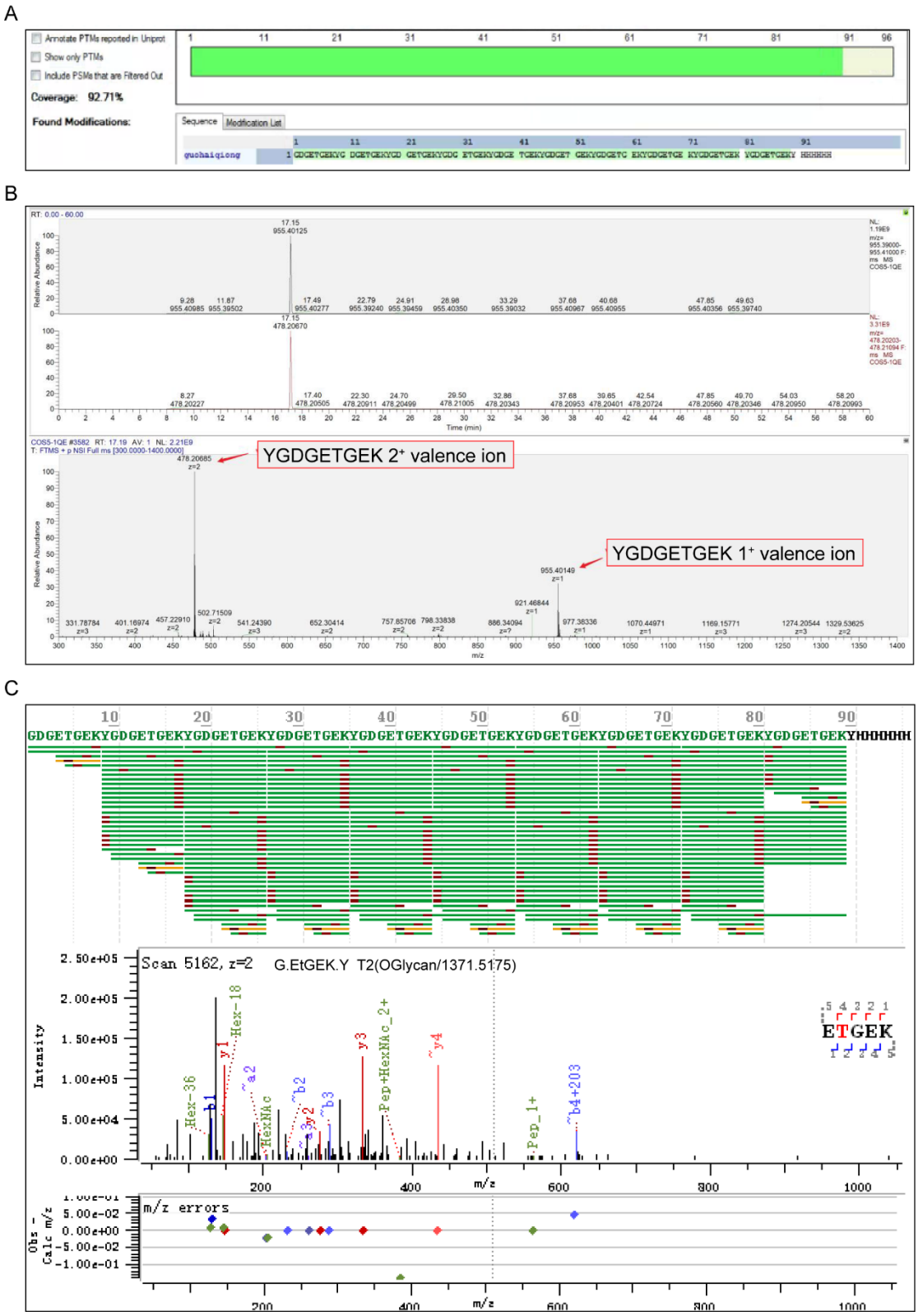


Fig. S8. Mass spectrometric identification of GS5 protein expressed in transgenic silkworms. (A) The amino acid sequence matching degree of the target protein. (B) Mass spectrometry of the characteristic peptide segment of the target protein. (C)

Identification of O-glycosylation modification sites.

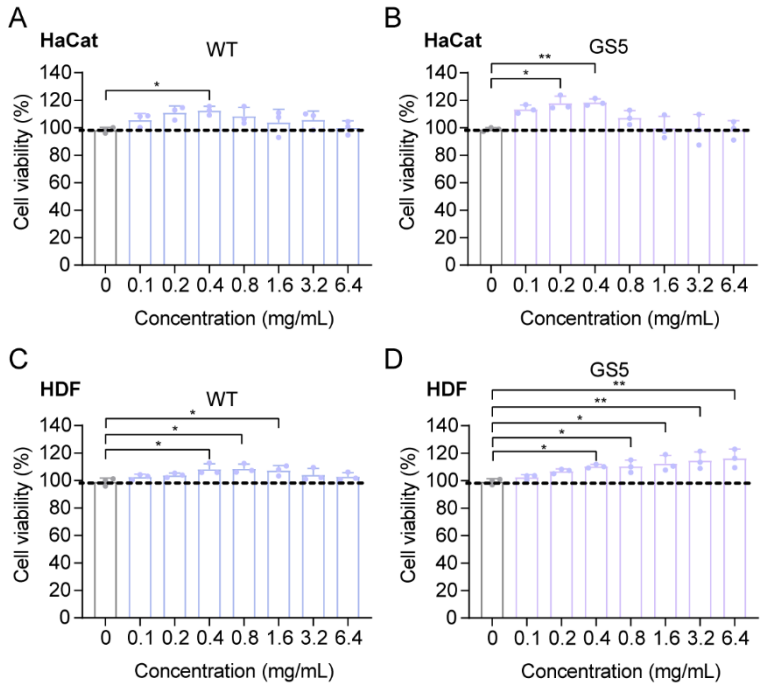


Fig. S9. Effects of WT or GS5 sericin on cell viability. (A-B) Cell viability of HaCaTs treated with specified concentrations of WT or GS5 for 24 h. (C-D) Cell viability of HDFs treated with specified concentrations of WT or GS5 for 24 h.

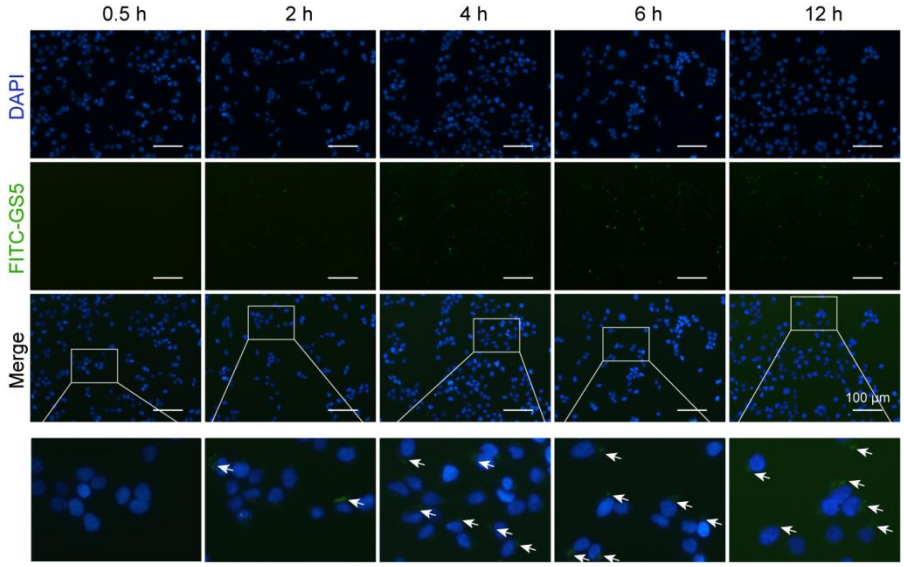


Fig. S10. Representative images of HaCaTs uptake of GS5 at different time points.

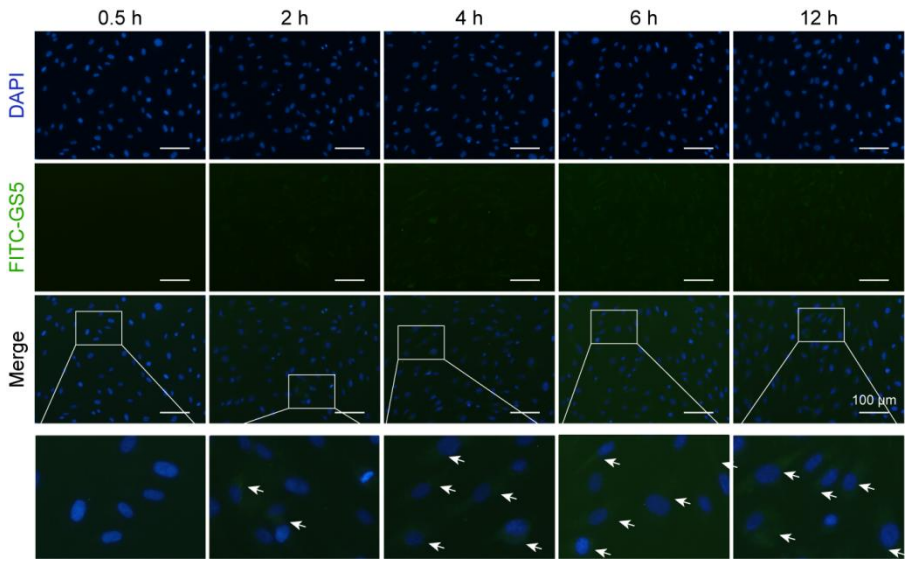


Fig. S11. Representative images of HDFs uptake of GS5 at different time points.

## Supplementary Tables

Table S1. Detailed information on High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) of in-house peptides.

| Seq. NO. | MW (g/mol) | | HPLC purity (%) | Appearance |
|---|---|---|---|---|
| | Theoretical | Observed | | |
| Seq7 | 993.9 | 993.3 | 98.7 | White lyophilized powder |
| Seq8 | 1141.2 | 1140.6 | 98.0 | White lyophilized powder |
| Seq9 | 1173.1 | 1172.8 | 95.1 | White lyophilized powder |
| Seq10 | 996.9 | 996.8 | 95.9 | White lyophilized powder |
| Seq11 | 1125.1 | 1125.0 | 97.9 | White lyophilized powder |
| Seq12 | 1161.20 | 1160.8 | 97.8 | White lyophilized powder |
| Seq13 | 945.9 | 945.6 | 95.8 | White lyophilized powder |
| Seq14 | 965.9 | 965.2 | 98.3 | White lyophilized powder |
| Seq15 | 1122.10 | 1121.6 | 98.9 | White lyophilized powder |
| Seq16 | 1224.2 | 1224.8 | 98.4 | White lyophilized powder |
| Seq17 | 1112.1 | 1111.2 | 97.5 | White lyophilized powder |
| Seq18 | 1125.1 | 1124.4 | 96.0 | White lyophilized powder |
| Seq19 | 1076.1 | 1076.8 | 96.6 | White lyophilized powder |
| Seq20 | 1078.1 | 1077.6 | 97.6 | White lyophilized powder |

MW: molecular weight.

Table S2. Sequences of the siRNA for RNA interference

| Gene | Sequence |
|---|---|
| si-Nrf2-1 | F: 5′-CAGUCUUCAUUGCUACUAA-3′ |
| | R: 5′-UUAGUAGCAAUGAAGACUG-3' |
| si-Nrf2-2 | F: 5′-CAUUGAUGUUUCUGAUCUA-3′ |
| | R: 5′-UAGAUCAGAAACAUCAAUG-3' |
| si-Nrf2-3 | F: 5′-GACAGAAGUUGACAAUUAU-3′ |
| | R: 5′-AUAUUAGUCAACUUCUGUC-3' |

siRNA: Small interfering RNA

Table S3 Details of antibodies.

| Antibodies | Source | Product number |
|---|---|---|
| MMP-1 | Beyotime, Shanghai, China | AF0231 |
| MMP-9 | Beyotime, Shanghai, China | AF5234 |
| GAPDH | Beyotime, Shanghai, China | AF1186 |
| Lamin B1 | Beyotime, Shanghai, China | AF1408 |
| IL-1β | Cell Signaling Technology, Danvers, MA, USA | Cat No. 31202 |
| IL-6 | Beyotime, Shanghai, China | AF0201 |
| COX2 | Beyotime, Shanghai, China | AF1924 |
| iNOS | Beyotime, Shanghai, China | AG4771 |
| Phospho-Nrf2 | ABclonal, Wuhan, China | AP1498 |

| | | |
|---|---|---|
| Nrf2 | Proteintech, Wuhan, China | Cat No. 80593-1-RR |
| Keap1 | Proteintech, Wuhan, China | Cat No. 60027-1-Ig |
| NQO1 | Proteintech, Wuhan, China | Cat No. 67240-1-Ig |
| HO-1 | Proteintech, Wuhan, China | Cat No. 10701-1-AP |
| GCLM | Proteintech, Wuhan, China | Cat No. 14241-1-AP |
| COL1A2 | Proteintech, Wuhan, China | Cat No. 14695-1-AP |
| P21 | Proteintech, Wuhan, China | Cat No. 10355-1-AP |
| γH2AX | Beyotime, Shanghai, China | AF5836 |

48 Table S4 Primer sequences were utilized in this study.

| Primer name | Sequence |
|---|---|
| Nrf2 | F: 5′-GCCGCTTAGAGGCTCATCTC-3′ |
| | R: 5′-TGGGCGGCGACTTTATTCTT-3′ |
| HO-1 | F: 5′-CAAGCGCTATGTTCAGCGAC−3′ |
| | R: 5′-GCTTGAACTTGGTGGCACTG−3′ |
| NQO-1 | F: 5′-GGCATCCTGCGTTTCTGTG-3′ |
| | R: 5′-GGTTTCCAGACGTTTCTTCCAT-3′ |
| GCLM | F: 5′-TTGGAGTTGCACAGCTGGATTC-3′ |
| | R: 5′- TGGTTTTACCTGTGCCCACTG-3′ |
| MMP-1 | F: 5'-GATTCGGGGAGAAGTGATGTTC-3' |
| | R: 5'-CTCCTTTGGCTTCCCTAGAACT-3' |
| MMP-9 | F: 5'-GCACCACCACAACATCACCTAT-3' |
| | R: 5'-GATGACGAGTTGTGGTCCCTG-3' |
| COL3A1 | F: 5′-AGGACAAGAGGCATGTCTGGTT-3′ |
| | R: 5′-TTGCAGTGGTAGGTGATGTTCTG-3′ |
| IL-1β | F: 5′-TCCAGGATGAGGACATGAGCAC-3′ |
| | R: 5′-GAACGTCACACACCAGCAGGTTA-3' |
| TNF-α | F: 5′-ACTCCAGGCGGTGCCTATGT-3′ |
| | R: 5′-GTGAGGGTCTGGGCCATAGAA-3' |
| GAPDH | F: 5′-CAAGCGCTATGTTCAGCGAC−3′ |
| | R: 5′-GCTTGAACTTGGTGGCACTG−3′ |
| IL-6 | F: 5′-ACTCACCTCTTCAGAACGAATTG-3′ |
| | R: 5′-CCATCTTTGGAAGGTTCAGGTTG-3′ |
| P21 | F: 5′-CTGAGCCGCGACTGTGATGCG-3′ |
| | R: 5′-GGTCTGCCGCCGTTTTCGACC-3′ |
| P16 | F: 5′-GCAGCATGGAGCCTTCGGCT-3′ |
| | R: 5′-TGCAGCACCACCAGCGTGTC-3′ |
| JAK2 | F: 5′-CGAATGGTGTTTCTGATGTACC-3′ |
| | R: 5′-GTCTCCTACTTCTCTTCGTACG-3′ |
| SOCS1 | F:5′-TCTCACCTCTTGAGGGGGTC-3′ |
| | R: 5′-AGAGGTAGGAGGTGCGAGTT-3′ |
| C-Myc | F: 5′-CATCAGCACAACTACGCAGC-3′ |
| | R: 5′-GCTGGTGCATTTTCGGTTGT-3′ |

| | |
|---|---|
| STAT1 | F: 5′-TGTATGCCATCCTCGAGAGC-3′ |
| | R: 5′-AGACATCCTGCCACCTTGTG-3′ |
| STAT2 | F: 5′-CCGGGACATTCAGCCCTTTT-3′ |
| | R: 5′-CTCATGTTGCTGGCTCTCCA-3′ |
| IRF9 | F: 5′-TTCTTCAAGGCCTGGGCAAT-3′ |
| | R: 5′-CCTGGTGGCAGCAACTGATA-3′ |