**Supplemental Table S1**

Plasmids and oligonucleotides used in this study.

| Plasmid | Description | |
| --- | --- | --- |
| pIDTSMART-KAN: SynGene18\_aTt471620Vint | The Vint domain from gene TTHERM\_00471620  The gene of Vint protein (512-680) from *Tetrahymena* *thermophila* SB210 was chemically synthesized based on the protein sequence Vint protein (515-680) (XM\_001033046, aTt\_00471620 [‎1]) for E. coli codons and purchased from Integrated DNA technologies as a plasmid. This plasmid was used as a template for creating other plasmids. | |
| pBHRSF114 | H6-SUMO-Vint domain, Vint(512-680) from *Tetrahymena thermophila* SB210  The wild-type Vint domain, Vint(512-680) was genetically fused with the N-terminal SUMO domain, including the three-residue N-terminal junction sequence of "SGG". The purchased plasmid pIDTSMART-KAN: SynGene18\_aTt471620Vint was digested with two restriction enzymes, BamHI and KpnI. The Vint domain-containing gene fragment was ligated into pHYRSF53 with the same restriction sites. This resulted in a bacterial expression vector for protein expression in E. coli encoding the Vint domain with an N-terminally hexahistidine-tagged SUMO domain [‎2]. | |
| pBHRSF123 | H6-SUMO-Vint(512-680) with C1A mutation for structural analysisThe first residue Cys of the Vint domain was mutated to Ala by amplifying the gene with the two oligonucleotides I733: 5’-AAAGGATCCGGAGGTG-CTATGCATGGTGATTCAC and HK029: 5’-gctagttattgctcagc-gg by PCR. The amplified gene was cloned into pHYRSF53 using BamHI/HindIII sites. | |
| pJEJRSF267 | H6-SUMO-Vint(512-680) with H67A mutation  The mutation of H67A in the Vint domain was introduced by PCR and Gibson cloning [‎3]. The two DNA fragments were amplified from the vector pBHRSF114 as a template using two primers, M035: 5’-CTGATCTGTGGA-TTACTCCAAAGGCTCCTATTAGAGTTAATG and J541: 5’-CAGCGGT-TTCTTTACCAGACTCG, or M036:5’-AATCCACAGATCAGTACCCAG-CTG and J502: 5’-TAAGCTTGCGGCCGCACTC. The two DNA fragments were assembled by Gibson cloning [‎3]. | |
| pJEJRSF258 | H6-SUMO-Vint(512-680) with C110A mutation  The mutation of C110A in the Vint domain was introduced by PCR and Gibson cloning [‎3]. The two DNA fragments were amplified from the vector pBHRSF114 as a template using two oligonucleotides, L699:5’-CAGACCCCTGAAGATTTGG and M024: 5'- CTTGAAAGTTATGACCC-AAACAGATAGCTTCATAACCACCAAT**,** or L225: 5'-ACCACCAATC-TGTTCTCTGTGAG and M023: 5'- TTGGGTCATAACTTTCAAGAACG-TGTTGC. The two DNA fragments were assembled by Gibson cloning. | |
| pJEJRSF260 | H6-SUMO-Vint(512-680) with C112A mutation  The mutation of C112A in the Vint domain was introduced by PCR and Gibson cloning [‎3]. The two DNA fragments were amplified from the vector pBHRSF114 as a template using two oligonucleotides, L699 and M026: 5’-CGTTCTTGAAAGTTATGACCCAAAGCGATACATTCATAACC, or L225 and M023. The two DNA fragments were assembled by Gibson cloning. | |
| pJEJRSF264 | H6-SUMO-Vint(512-680) with C110A and C112A mutations  The mutations of C110A and C112A in the Vint domain in pBHRSF114 were introduced by PCR using two oligonucleotides, L699 and M027: 5'- CTTGA-AAGTTATGACCCAAAGTGATAGCTTCATAACCACCAATA, or L225 and M023 and subsequently by Gibson cloning. | |
| pLKRSF62 | H6-SUMO-Vint(512-114), DC52 variant of the Vint domain  The deletion of the C-terminal ARR/SRR region was introduced in pBHRSF114 by amplifying the gene with two oligonucleotides I780: 5'- CT-GAAGCTTAACCCAAACAGATACATTCATAAC and HK807: 5'- GGA-TAACGATATTATTGAG using PCR. The PCR product was cloned into pHYRSF53 using *Bam*HI/*Hind*III sites. | |
| pJTRSF18 | H6-SUMO-Vint(512-114), DC52 variant with C1A mutation  The C1A mutation in the C52 variant of the Vint domain was introduced by PCR using the two oligonucleotides I780 and I733: 5’-AAA-GGATCCGGAGGTGCTATGCATGGTGATTCAC The amplified DNA fragment from pBHRSF114 was cloned into pHYRSF53 using *Bam*HI/*Hind*III sites. | |
| pJTRSF25 | H6-SUMO-Vint(512-114), DC52 variant with C1A and C112A mutations  The C1A and C112A mutations in the C52 variant were introduced by PCR using the two oligonucleotides, I907: 5'-CTGAAGCTTAACCC-AAAGCGATACATTCATAACCAC and I733. The amplified DNA fragment from pBHRSF114 was cloned into pHYRSF53 using *Bam*HI/*Hind*III sites. | |
| pJLRSF7 | H6-SUMO-Vint(512-114), DC52 variant with C1A, C110A and C112A mutations  The C1A, C110A, and C112A mutations in the C52 variant were introduced by PCR using the two primers, I733 and J401: 5'- CGCAAGCTTAACCCAA-AGCGATAGCTTCATAACCACC. The DNA fragment amplified from pBHRSF114 was cloned into pHYRSF53 using *Bam*HI/*Hind*III sites. | |
| pJTRSF35 | H6-SUMO-Vint(512-680) with C1A and R154S mutation.  The mutation of R154S in the Vint domain was introduced in the vector pBHRSF123 by inverse PCR with two primers, I971: 5’-CATTCGTTCTCG-TGTAAGTGATCAGATTACTAATC and I972: 5’-GATTAGTAATCTGA-TCACTTACACGAGAACGAATG. | |
| pBHRSF218 | H6-SUMO-Vint(512-680) with C1A and R152M mutation.  The mutation of R152M in the Vint domain was introduced in the vector pBHRSF123 by inverse PCR with two primers, J007: 5’-AAGTCATCATTC-GTTCTATGGTACGTGATCAGATTAC and J008: 5’-GTAATCTGATCAC-GTACCATAGAACGAATGATGACTT. | |
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2. Guerrero F, Ciragan A, Iwaï H. Tandem SUMO fusion vectors for improving soluble protein expression and purification. Protein Expr Purif. 116, 42-49 (2015).
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**Supplemental Table S2**

Data collection and refinement statistics

|  |  |
| --- | --- |
|  | *Tth*Vint |
| Data collection |  |
| Wavelength | 0.9793 |
| Space group | P212121 |
| Molecules/a.u. | 1 |
| Unit cell a,=b, c (Å);   (°) | 35.89, 46.36, 115.05  90, 90, 90 |
| Resolution (Å) | 50.00-1.79 |
| Rmerge(%)† | 5.7 (64.3.) |
| Rpim(%) | 4.2 (45.5) |
| No. of reflections  (measured/unique) | 36668 /18399 |
| <I / I> | 11.33/2.13 |
| Completeness (%) | 99.4/95.91 |
| Redundancy | 2.0 (2.0) |
|  |  |
| Refinement |  |
| Resolution (Å) | 57.57-1.80 |
| No. of reflections  (refinement/ Rfree) | 18390/568 |
| R / Rfree‡18 | 18.86/22.58 |
| No. atoms |  |
| Protein | 1342 |
| Ligand/ion | 76 |
| Water | 92 |
| R.m.s. deviations from ideal |  |
| Bond lengths (Å) | 0.012 |
| Bond angles (°) | 1.30 |
| PDB code | 8R2C |