

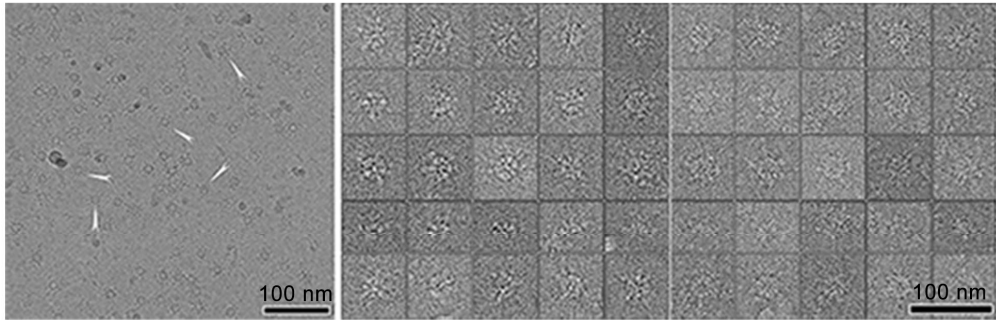
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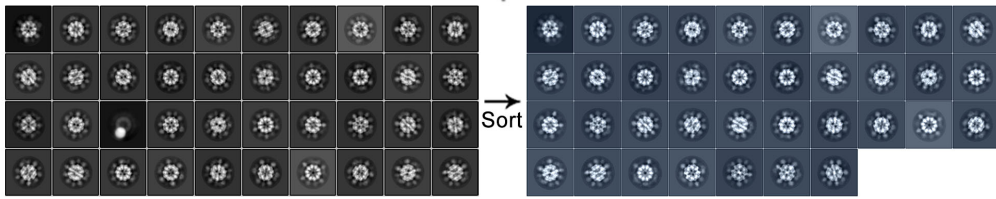
**Supporting information for article:**

## **Structural insights into stressosome assembly**

**Eunju Kwon, Deepak Pathak, Han-ul Kim, Pawan Dahal, Sung Chul Ha, Seung Sik Lee, Hyeongseop Jeong, Dooil Jeong, Hyeun Wook Chang, Hyun Suk Jung and Dong Young Kim**



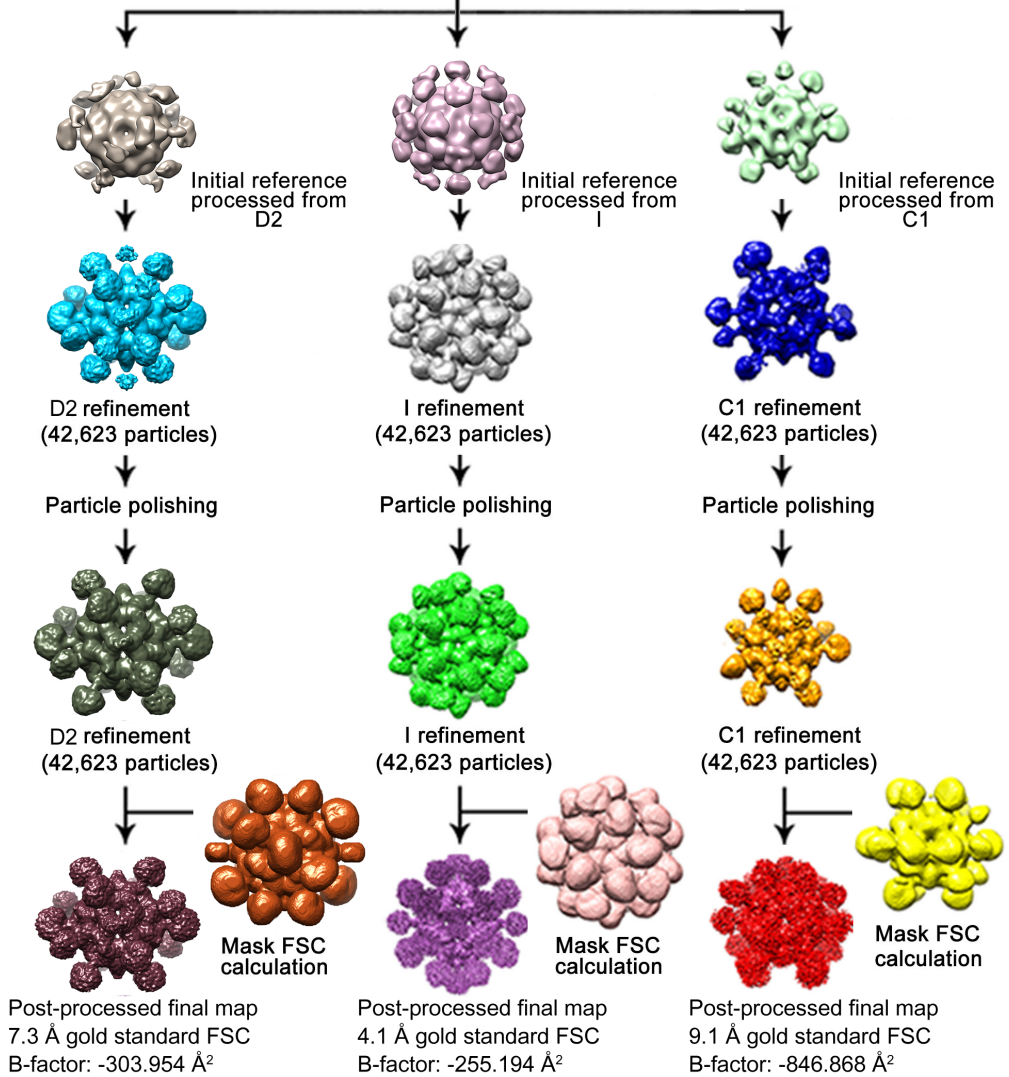
Particle picking & windowing



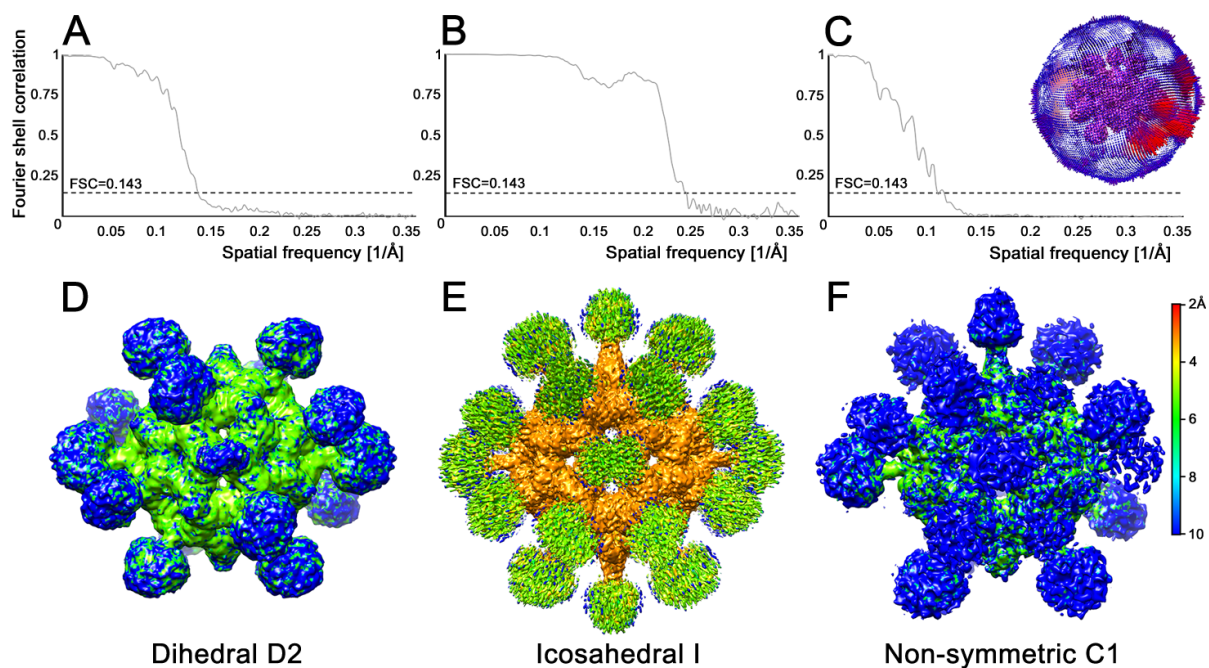
2D classification (54,798 particles)

Selected 2D class averages (42,623 particles)

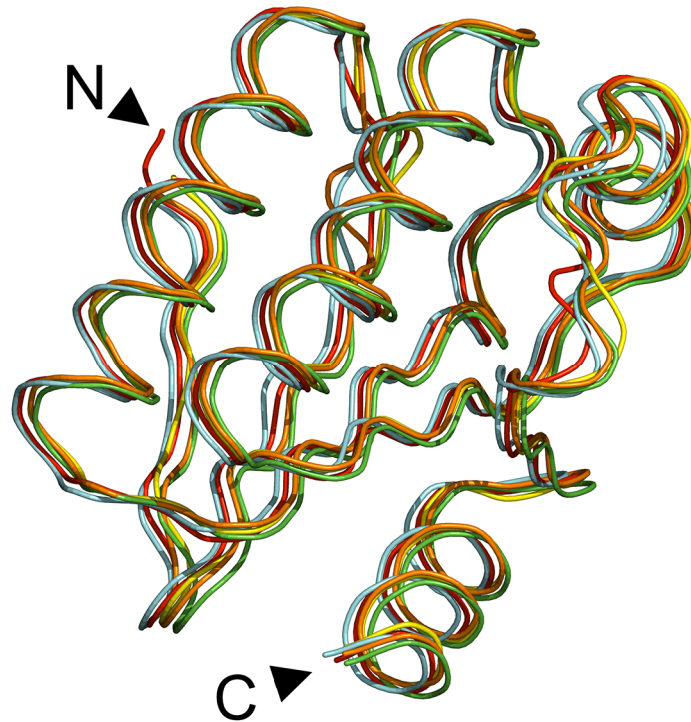
42,623 individuals



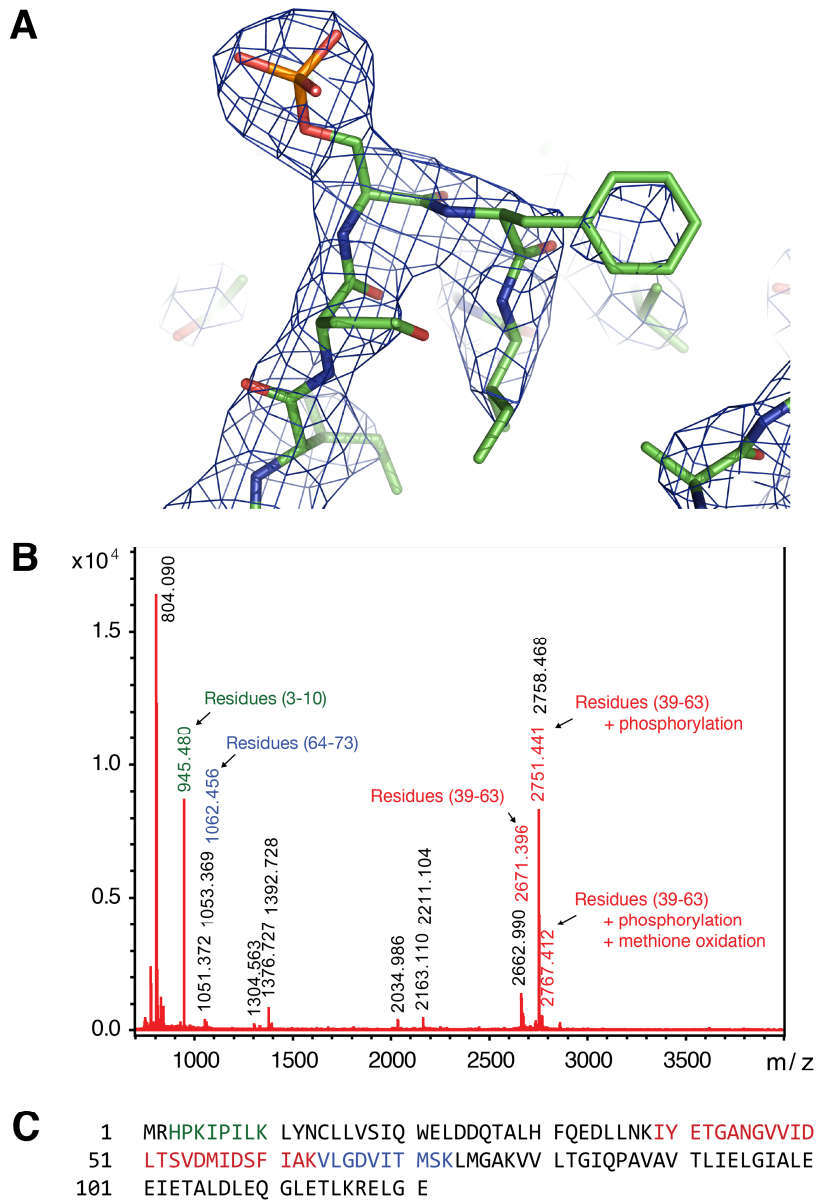
**Figure S1. Details of Cryo-EM data processing.** The workflow that represents 3D reconstruction of RsbRA/RsbS complex. Cryo-EM envelope structures of RsbRA/RsbS complex were refined with dihedral (D2), icosahedral (I), and non-symmetric (C1) restraints.



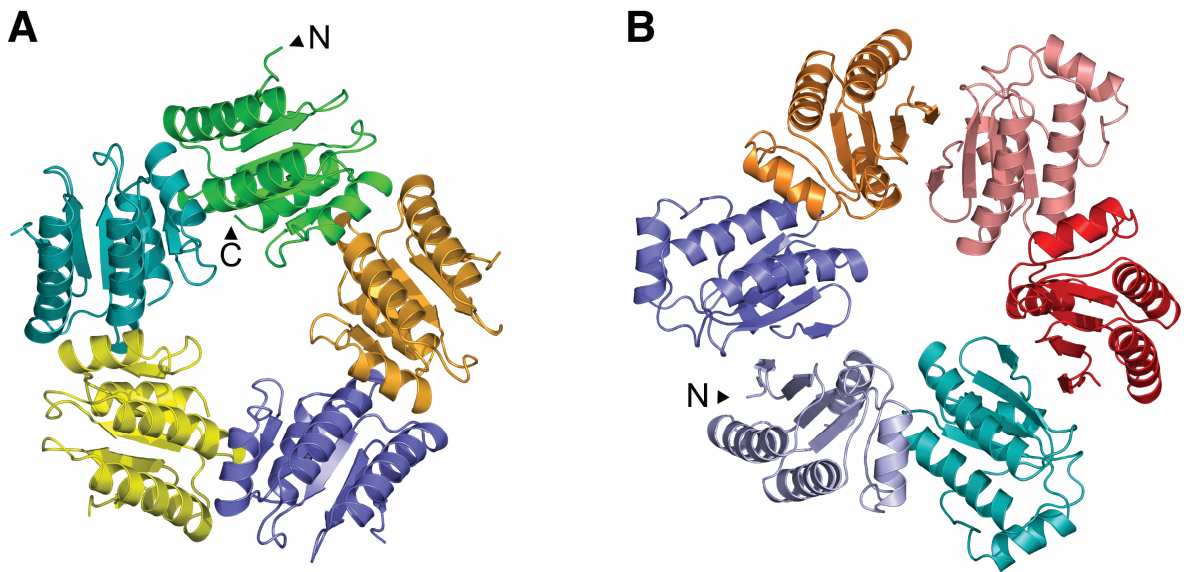
**Figure S2.** Fourier shell correlation (FSC) and local resolution distributions of Cryo-EM envelope structure. (A-C) FSC curves of D2- (A), I- (B) and C1-envelopes (C) indicates final resolution of 7.3 Å, 4.1 Å, and 9.1 Å, respectively. The resolution was estimated by FSC = 0.143 criterion. The inset image in (C) shows the angular distribution of particles contributing to the final reconstruction of C1-envelope. (D-E) The distribution of local resolution in D2- (D), I- (E), and C1-envelopes (F).



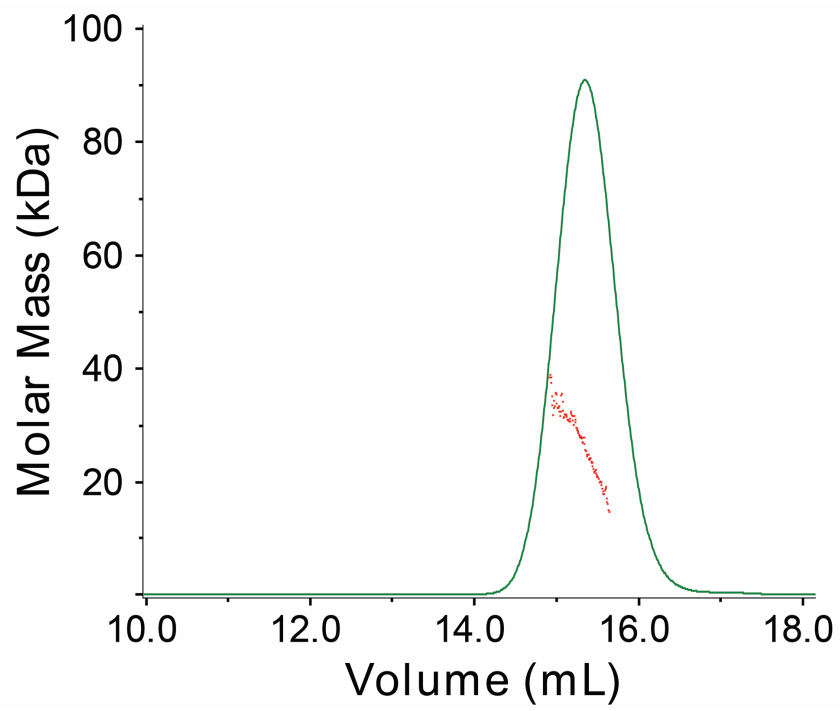
**Figure S3. The superposition of five RsbS monomers in an asymmetric unit.**  $\text{C}\alpha$  trace models of the five RsbS monomers were superposed together at the same orientation as Fig 1C. Each monomer is colored differently. The N- and C-termini are labeled as N and C, respectively.



**Figure S4. Phosphorylation of S59.** (A) Electron density map around S59 of RsbS. RsbS are drawn as a stick model. 2Fo-Fc electron density map is shown at 1.5  $\sigma$  contour level. (B) Peptide mass fingerprinting (PMF). The peptides from purified RsbS were analyzed by MALDI-TOF and identified by peptide mass fingerprinting. (C) The peptide containing S59 (red) was phosphorylated.

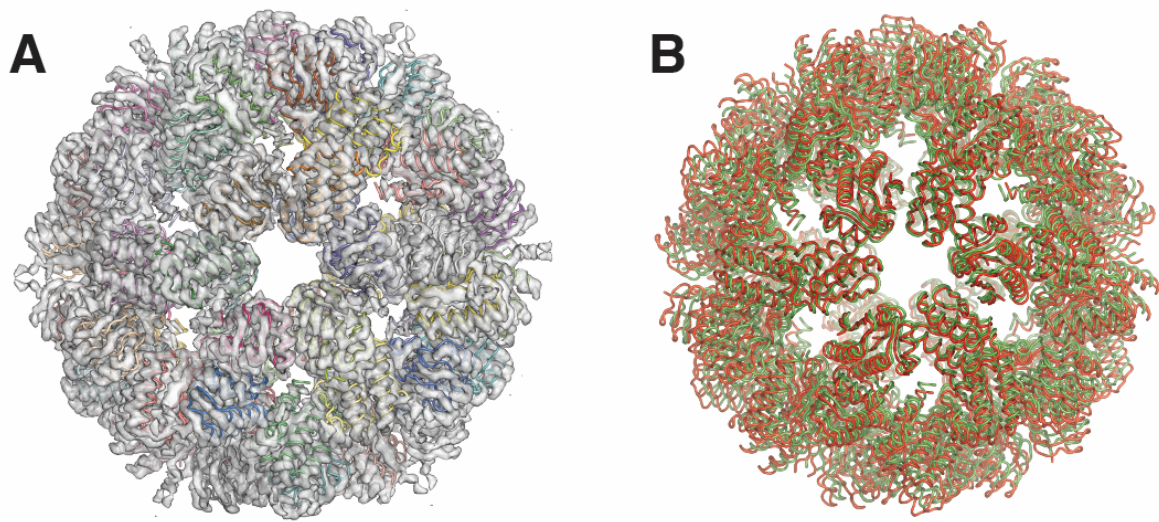


**Figure S5. Oligomerization of RsbS shown in the crystal.** (A) Ribbon diagram of RsbS pentamer in the asymmetric unit. The pentamer is formed by the “head to tail” interactions of RsbS. Each RsbS monomer is colored differently. (B) RsbS hexamer (trimer of RsbS dimer) generated by crystallographic symmetry.

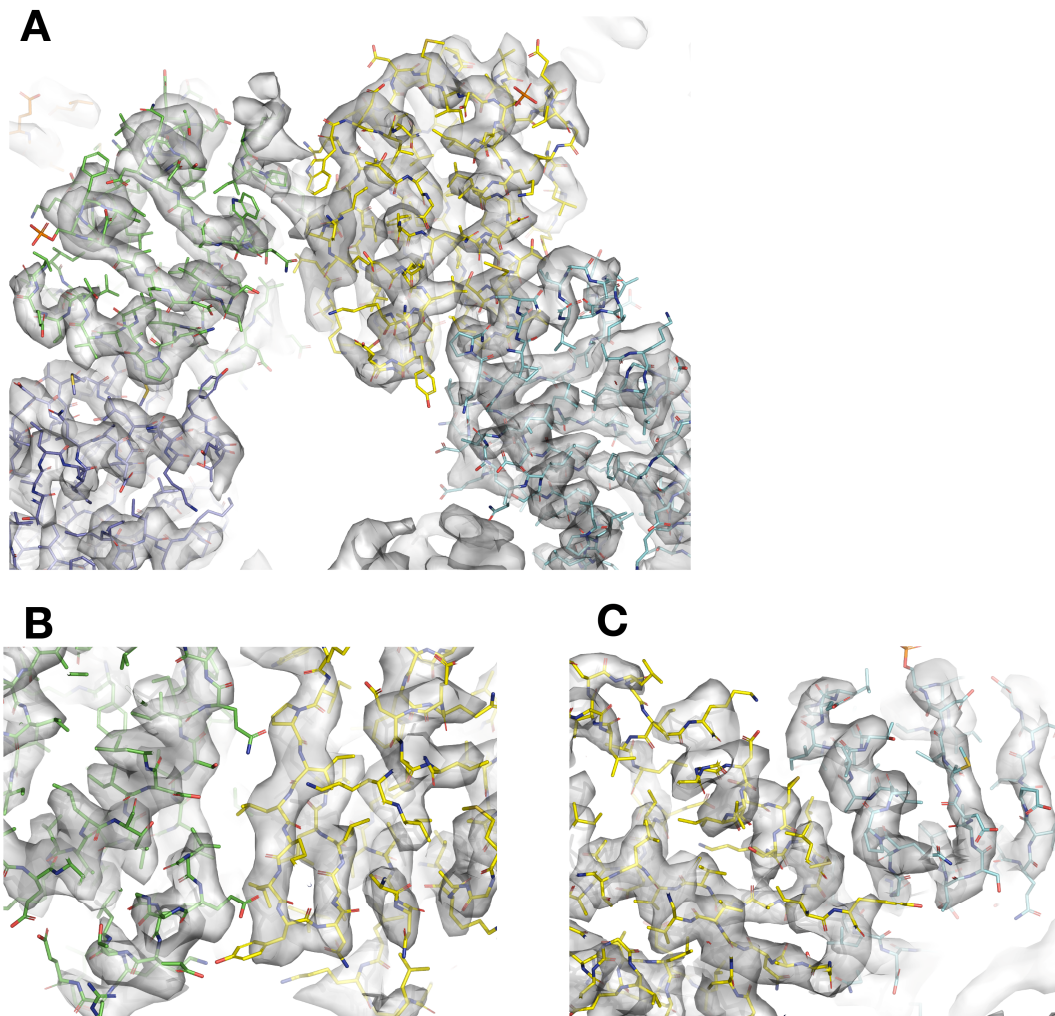


**Figure S6. SEC-MALS of RsbS-S59A mutant.** The calculated molecular mass of RsbS S59A was 26.8 kDa, which is close to that of a dimer (molecular weight of a monomer: 13.3 kDa).

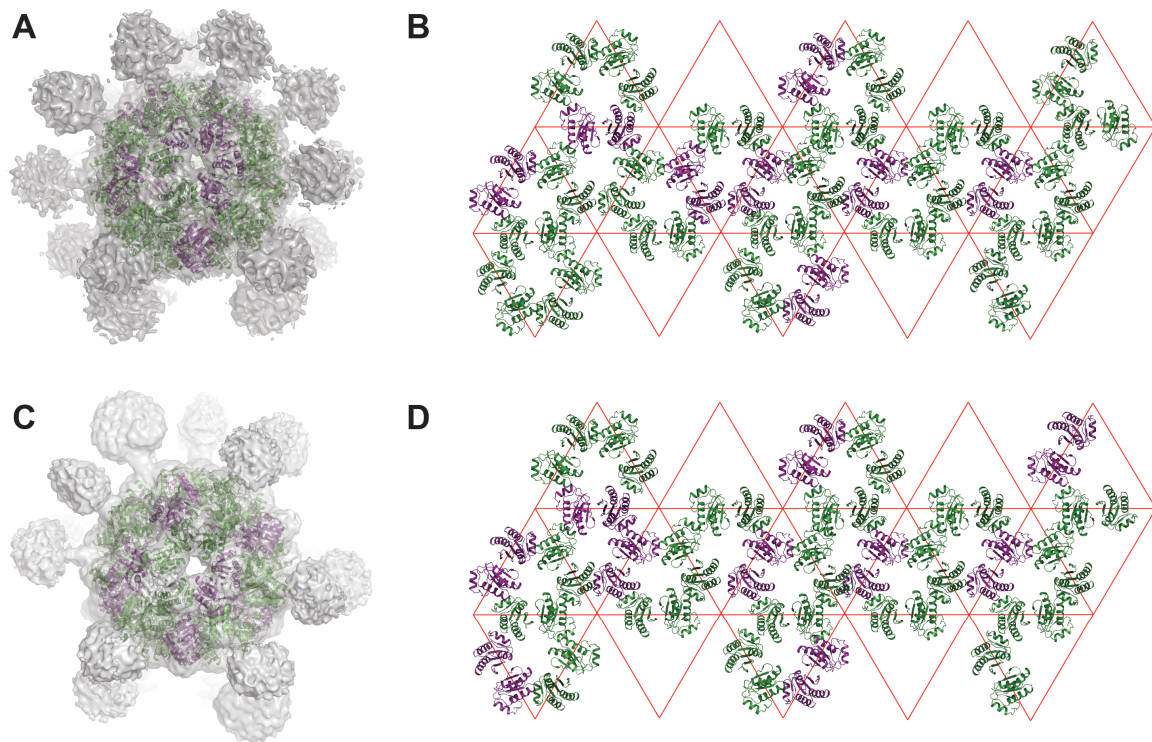




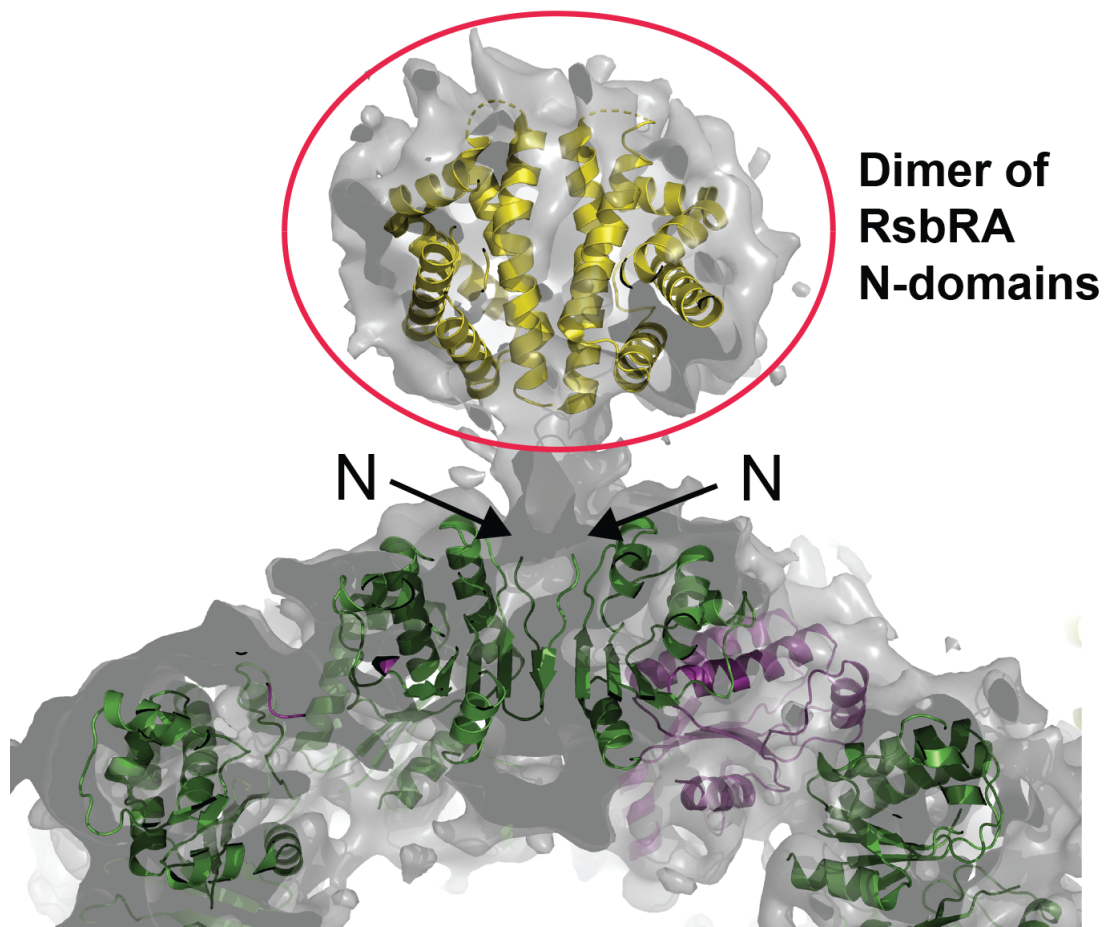
**Figure S7. Superposition of the crystal structure of RsbS icosahedron onto the icosahedral envelope.** (A) The crystal structure of RsbS icosahedron was drawn as ribbon diagram and was fitted into the cryo-EM envelope of RsbRA/RsbS complex determined with icosahedral symmetric restraint at 4.1 Å resolution. (B) Comparison of RsbS crystal structure and STAS icosahedron. To generate STAS icosahedron, each RsbS monomer from the RsbS crystal structure was fitted into the I-envelope, individually. RsbS crystal structure and STAS icosahedron were colored green and red, respectively.



**Figure S8. Icosahedral envelope of the RsbRA/RsbS complex (A-C)** Envelope structure superposed with crystal structure of RsbS. The crystal structure of RsbS icosahedron was superposed on the icosahedral envelope calculated at 4.1 Å resolution. Individual RsbS monomer was then fitted into the envelope. RsbS monomers from the crystal structure are drawn as a stick model and differently colored. (B, C) Structure of binding interfaces.



**Figure S9. Cryo-EM structure of a stressosome** (A) Envelope structure of RsbRA/RsbS complex calculated at 9.1 Å resolution without symmetric restraint (C1-envelope). Ribbon diagram of RsbS icosahedron was superposed onto the C1 envelope. The positions of RsbRA-STAS domain and RsbS in stressosome core were distinguished by green and purple, respectively. (B) The flattened icosahedron that shows the arrangement of RsbRA and RsbS in the C1 envelope. (C) Envelope structure of RsbRA/RsbS complex calculated at 7.3 Å resolution under D2 symmetric restraint (D2-envelope). (D) The flattened icosahedron that shows the arrangement of RsbRA and RsbS in the D2 envelope.



**Figure S10. Superposition of domain models on C1 envelope of stressosome.** The STAS domains of RsbRA and RsbS and RsbRA N-domain are colored green, purple, and yellow, respectively.

## Supplementary Table S1. Genes and primers used

| Genes                    | UniProt accession number (www.uniprot.org) |
|--------------------------|--|
| RsbRA                    | P42409                                     |
| RsbS                     | P42410                                     |
| RsbT                     | P42411                                     |
| Primers                  | DNA sequence (5' → 3')                     |
| BsRsbS_F                 | ACTGGATCCATGAGACATCCGAAAATCCCGATCTT        |
| BsRsbS_R                 | AATGAATTCTATTCCCCAATCCCGCTTCAAT            |
| BsRsbRA_F                | ACTGGATCCATGATGTGCGAACCAGACTGTATACCA       |
| BsRsbRA_R                | ATGATAAGCTTTTATTCCCCAATGAAACGATTTTT        |
| BsRsbRA_R189A_F          | GTCAAACACGCGTCCCAAGTCGTGCTGATTGACATTACA    |
| BsRsbRA_R189A_R          | GACTTGGGACGCGTGTTTGACGACGCCATTAAGAAGGTT    |
| BsRsbRA_Q191A_F          | CACCGTCCGCGGTCGTGCTGATTGACATTACAGGTGTC     |
| BsRsbRA_Q191A_R          | CAGCACGACCGCGGAACGGTGTTTGACGACGCCATTAAG    |
| BsRsbRA_Q236A_F          | GAAATTGCTGCGACAATCGTCAACCTGGGGATTGATTTA    |
| BsRsbRA_Q236A_R          | GACGATTGTCGCGACAATTTCCGGACGGATTCCGGCCAG    |
| BsRsbRA_N252A_F          | ATAACTAAGGCGACCCTGCAAAAAGGAATTCAAACAGCA    |
| BsRsbRA_N252A_R          | TTGCAGGGTCGCCTTAGTTATAACTTGGGATAAATCAAT    |
| BsRsbRA_189Ato189A191A_F | CACGCGTCCGCGGTCGTGCTGATTGACATTACAGGTGTC    |
| BsRsbRA_189Ato189A191A_R | CAGCACGACCGCGGACGCGTGTTTGACGACGCCATTAAG    |
| BsRsbT_F                 | TGACATTACATATGAACGACCAATCCTGTGTAAGAAT      |
| BsRsbT_R                 | AACTATCACTCGAGCTACCGAAGCCATTTGATGGCTTGT    |