Expanded View Figures

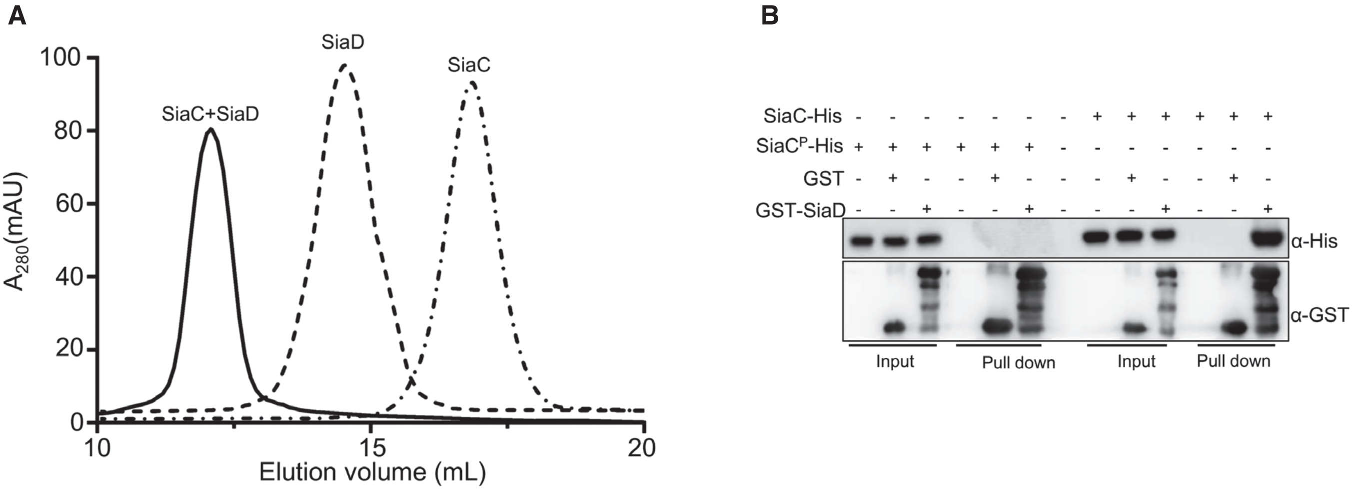

Figure EV1. Interactions of SiaD with SiaC or SiaC^P.

A Size exclusion chromatography elution profile of the purified SiaC and SiaD on a superdex 200 10/300 column.

B GST pull-down assays showed that SiaD interacts with SiaC but not SiaC^P. Purified SiaC or SiaC^P was incubated with GST, GST-PA0170 individually, and protein complexes were captured by glutathione beads.

Source data are available online for this figure.

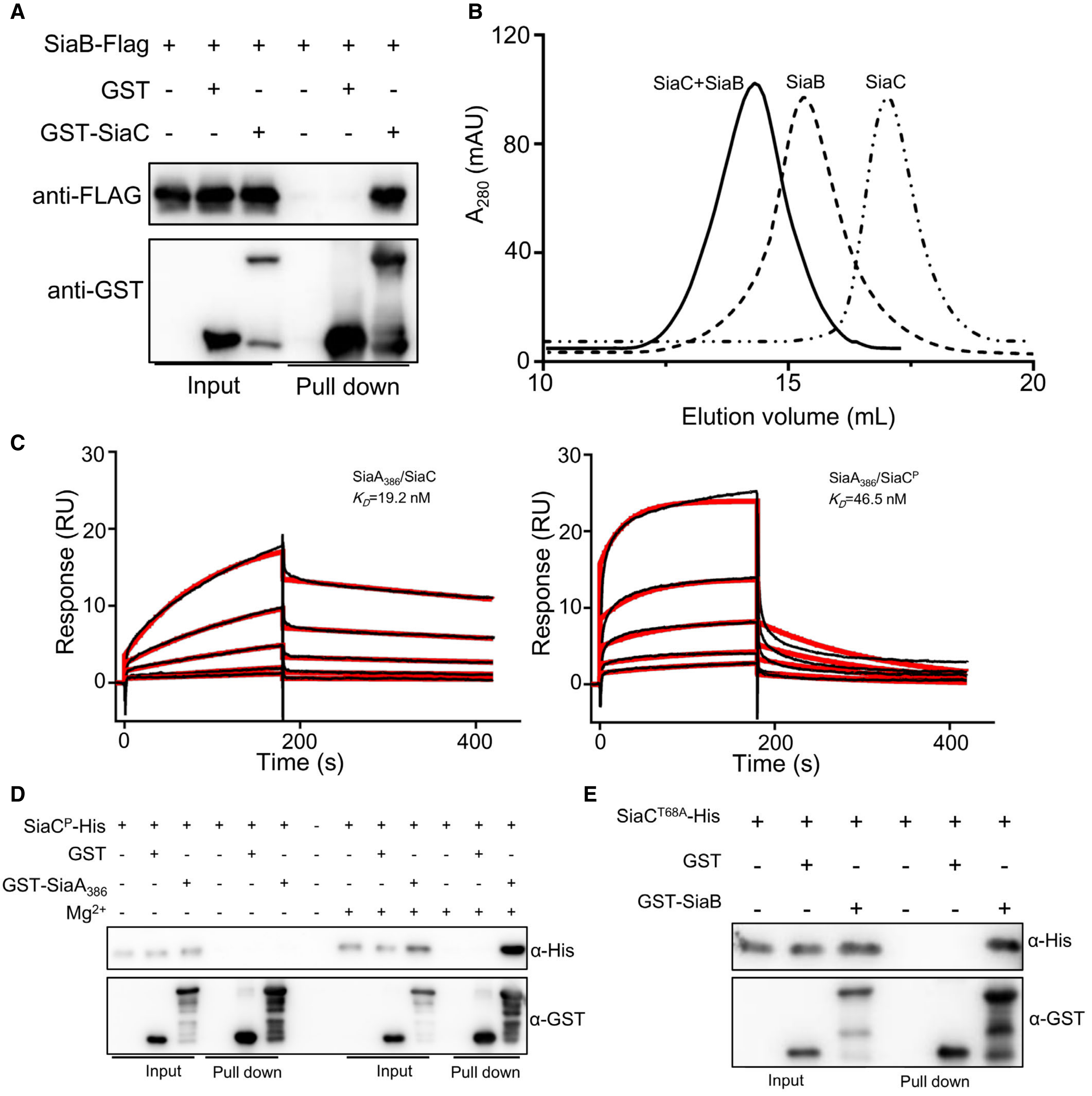

Figure EV2. SiaC interacts with SiaA or SiaB.

A GST pull-down shows direct interaction between SiaC and SiaB. Cell lysates of *Pseudomonas aeruginosa* containing pMM67EH-*siaB*-Flag were incubated with GST and GST-SiaC individually, and protein complexes were captured by glutathione beads.

B Size exclusion chromatography elution profile of the purified SiaB and SiaC on a superdex 200 10/300 column.

C SPR measurements of SiaC or SiaC^P binding at varying concentrations to SiaA$_{386}$. SiaC-His or SiaC^P-His specifically interacted with SiaA$_{386}$ with a K_D of 19.2 or 46.5 nM. Shown are measured binding responses (black) and curve fits to a 1:1 interaction (red). Plots are representative of two experiments with similar results. RU, response units; K_D, dissociation constant.

D GST pull-down shows direct interaction between SiaC^P and SiaA$_{386}$. Purified SiaC^P was incubated with GST and GST-SiaA$_{386}$ individually in buffers with or without 10 mM MgCl$_2$, and protein complexes were captured by glutathione beads.

E GST pull-down shows direct interaction between SiaC^{T68A} and SiaB. Purified SiaC^{T68A}-His was incubated with GST, GST-SiaB individually, and protein complexes were captured by glutathione beads.

Source data are available online for this figure.

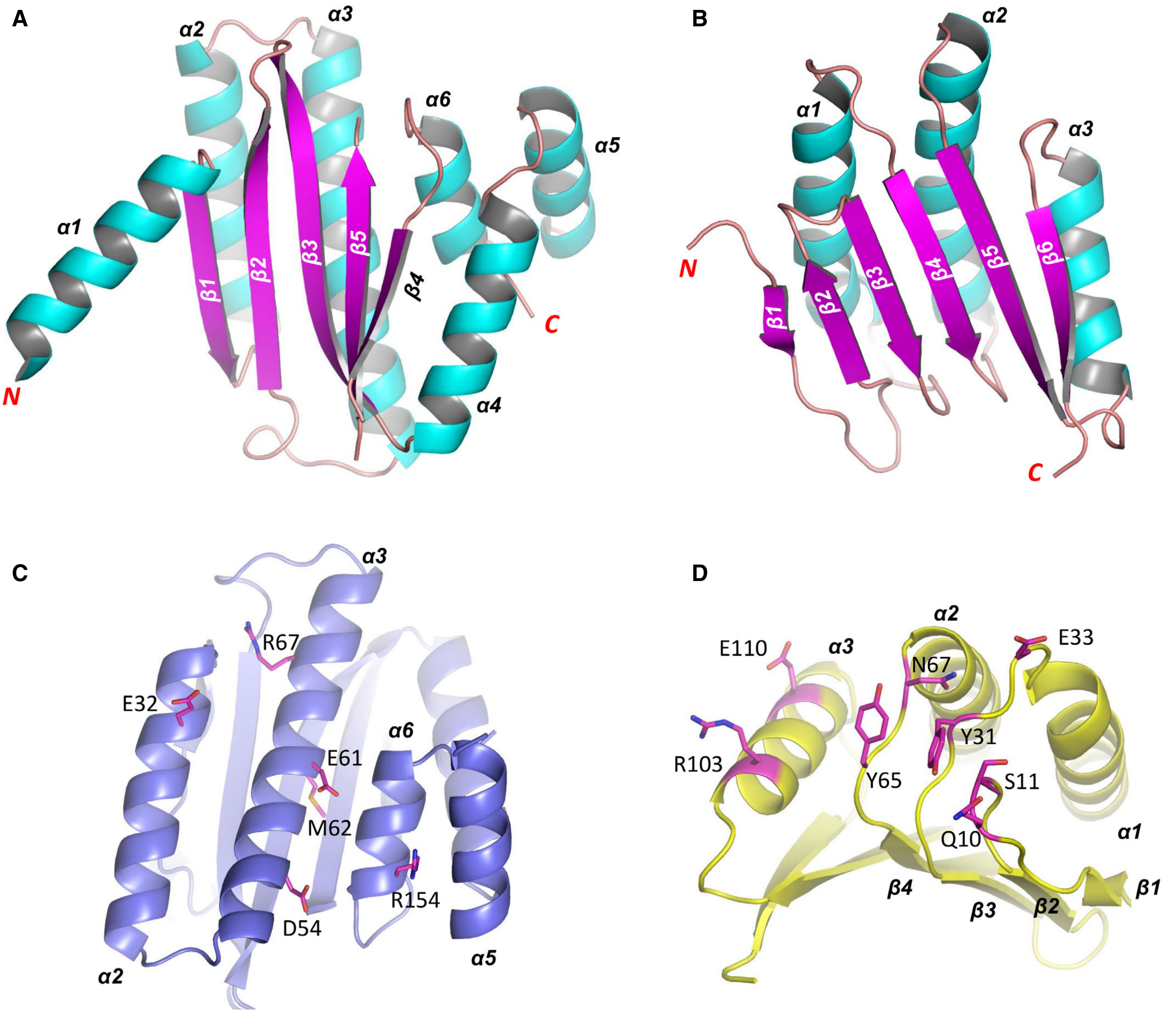

Figure EV3. The structures of SiaB and SiaC.

A, B The overall fold of SiaB and SiaC monomers.

C, D The relative orientations of residues involved in SiaB-SiaC interactions.

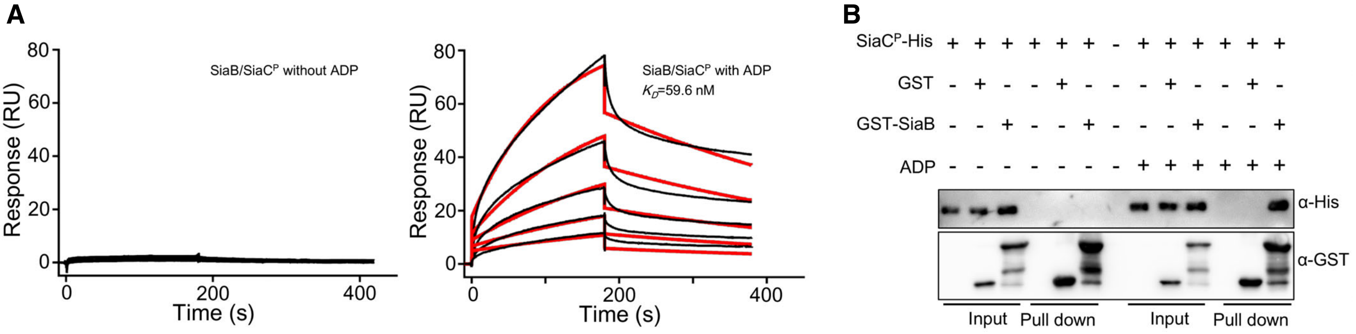

Figure EV4. SiaB interacts with SiaC^P in the presence of ADP.

A SPR measurements of SiaC^P binding at varying concentrations to SiaB with or without 2 mM ADP. SiaC^P-His specifically interacted with SiaB with a K_D of 59.6 nM in the presence of ADP. Shown are measured binding responses (black) and curve fits to a 1:1 interaction (red). Plots are representative of two experiments with similar results. RU, response units; K_D, dissociation constant.

B GST pull-down shows direct interaction between SiaC^P and SiaB in the presence of 2 mM ADP. Purified SiaC^P was incubated with GST and GST-SiaB individually in buffers supplemented with or without 2 mM ADP, and protein complexes were captured by glutathione beads.

Source data are available online for this figure.

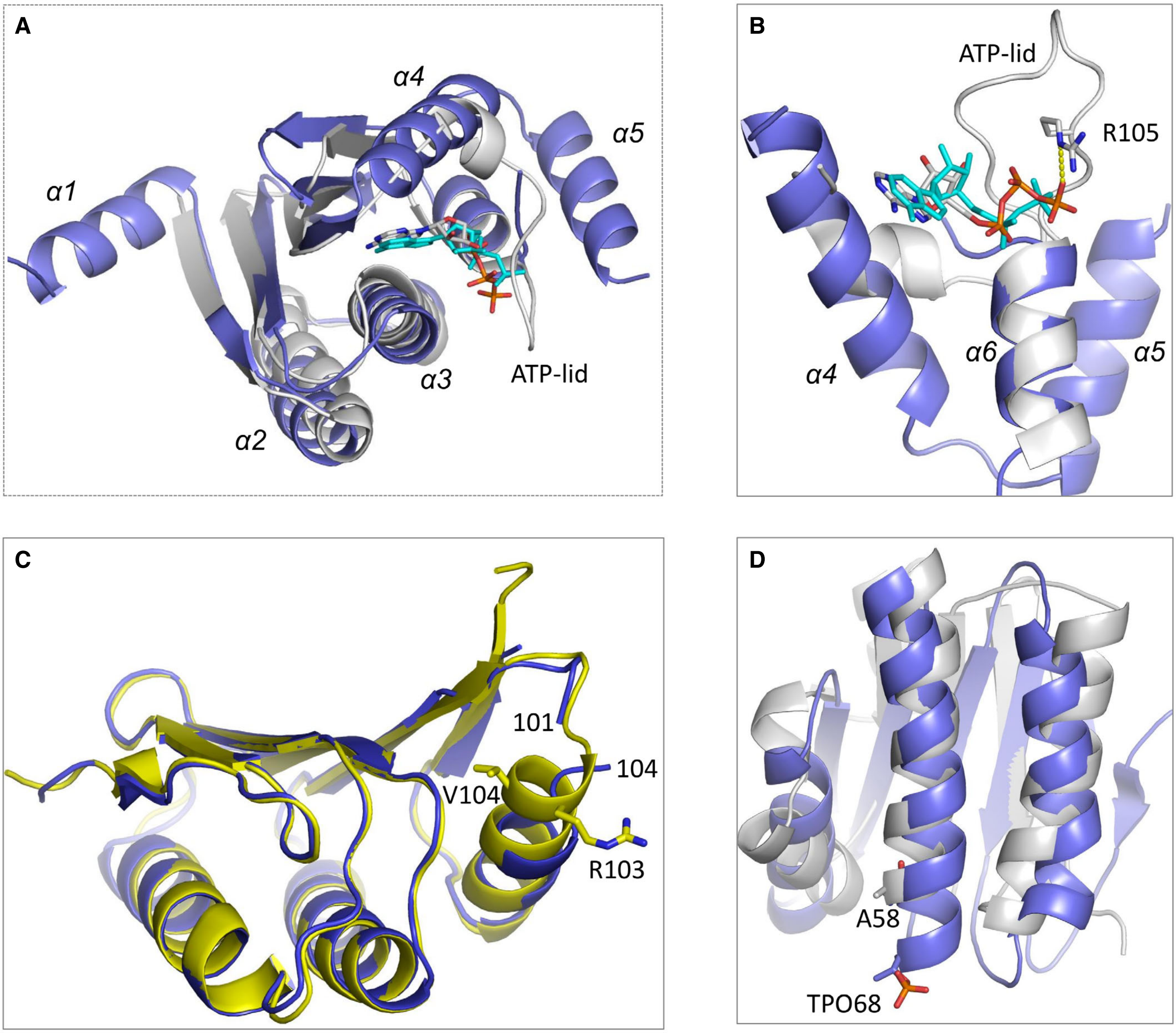

Figure EV5. Structural analysis of SiaB and SiaC.

A, B Comparison of SiaB and *Bs*SpoIIAB. SiaB and *Bs*SpoIIAB are shown as cartoon in blue and white, respectively. ADP in the SiaB/SiaC complex is shown as stick in cyan. ATP in the *Bs*SpoIIAB/*Bs*SpoIIAA complex is shown as sticks in atomic color (C, white; N, blue; O, red; P, orange).

C Superposition of SiaC in the complex and apo-structures, which are colored in yellow and blue, respectively.

D Superposition of SiaC and *Bs*SpoIIAA, which are colored in blue and white, respectively.