Figure legend

Figure 1 Solubilization and activation test of Cyt2Aa2 wild type (WT) and mutant toxins. Lane M: Protein marker. Inclusion bodies (I), the pellet (P) after solubilization in 50mM carbonate buffer at 37 ^{o}C for 1 hour. The soluble fractions (S) after solubilization in 50mM carbonate buffer. Activated toxins (A) were obtained from incubation of the soluble fraction with proteinase K at 37^{o}C for 1 hour.

Figure 2 Cyt2Aa2 toxin detection by western blotting using anti-Cyt2Aa2 of protoxin A) and activated toxins B) of wild type (WT) and mutants.

Figure 3 Membrane binding and complex formation of Cyt2Aa2 wild type and mutant toxins on rat RBC membrane. Activated toxin was mixed with 2% rat RBC in PBS pH 7.4 in total volume 0.4 ml and incubated for 2 hours at room temperature. The membrane-bound toxin complexes were visualized by western blotting using anti-Cyt2Aa2. Rat RBCs incubated without toxin was used as a negative control.

Figure 4 Hemoglobin release assay of rat RBCs treated with Cyt2Aa2 wild type and Cyt2Aa2 mutant toxins at room temperature for 2 hours. The percentage of hemolysis was calculated from the absorption of hemoglobin at 540 nm. Supernatant from 2% rat RBCs mixed with 0.1% Triton X-100 used as a 100% hemolysis control and a supernatant from 2% rat RBCs in PBS buffer was used as a blank.

Figure 1

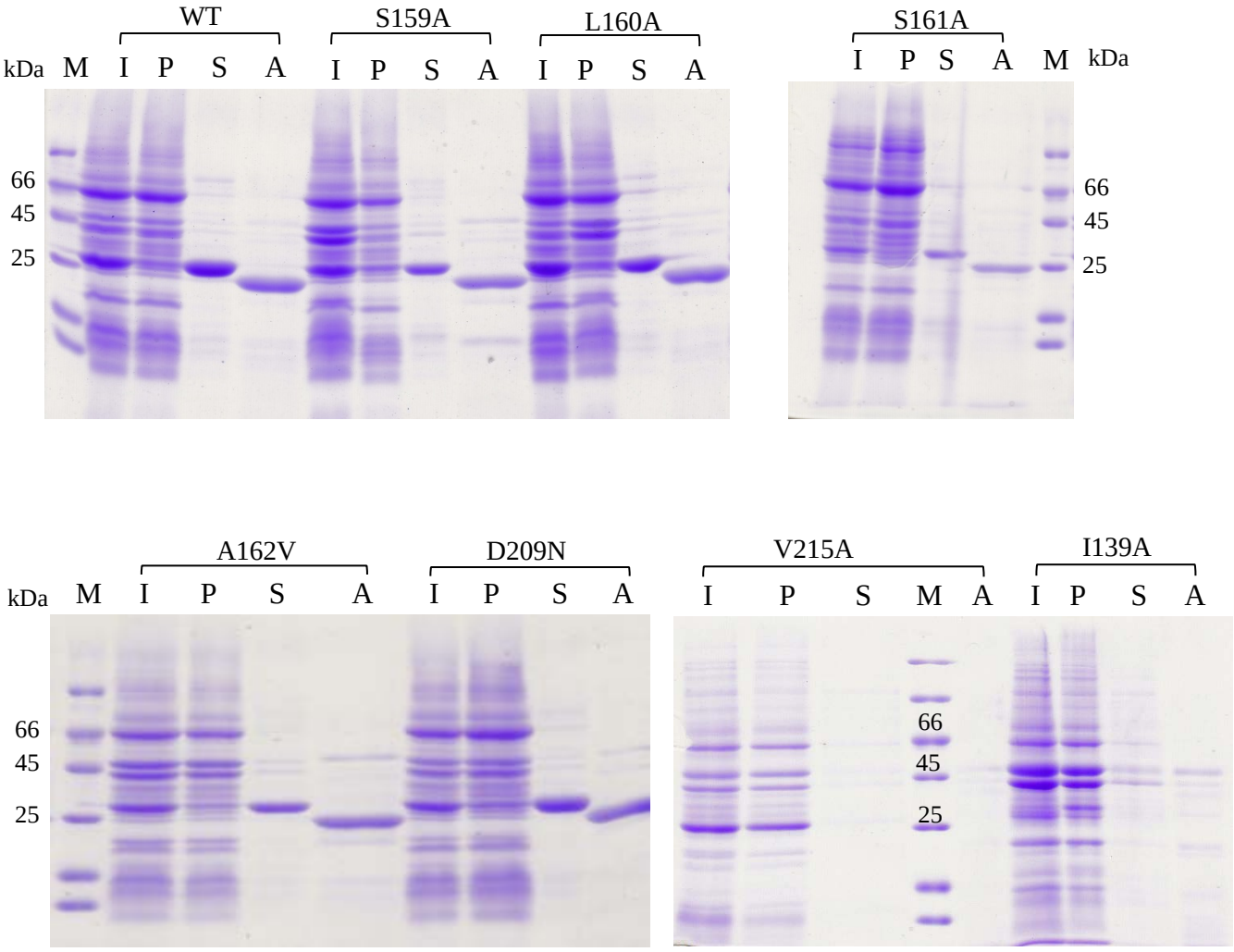
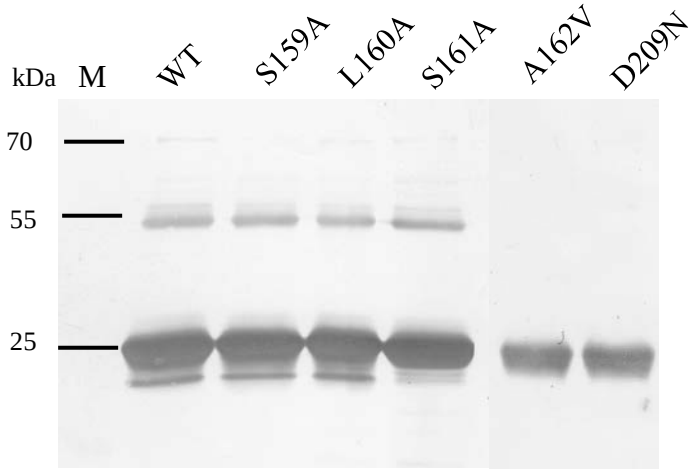

WT
S159A
L160A
S161A
kDa
M
I
P
S
A
66
45
25
A162V
D209N
V215A
I139A

Figure 2

A)


kDa
M
WT
S159A
L160A
S161A
A162V
D209N
70
55
25

B)

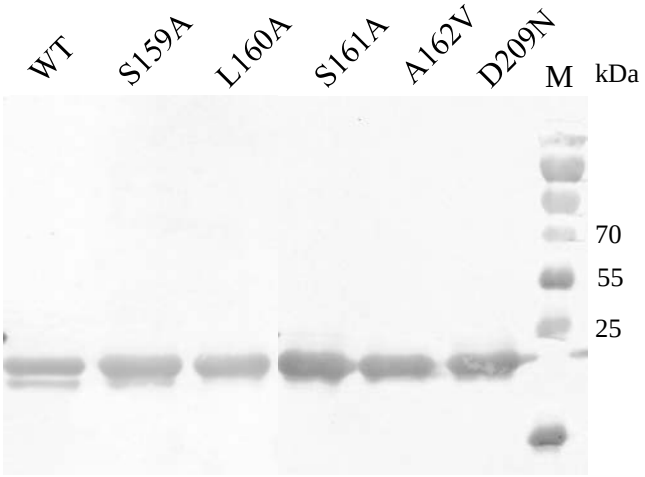

WT
S159A
L160A
S161A
A162V
D209N
M
kDa
70
55
25

Figure 3

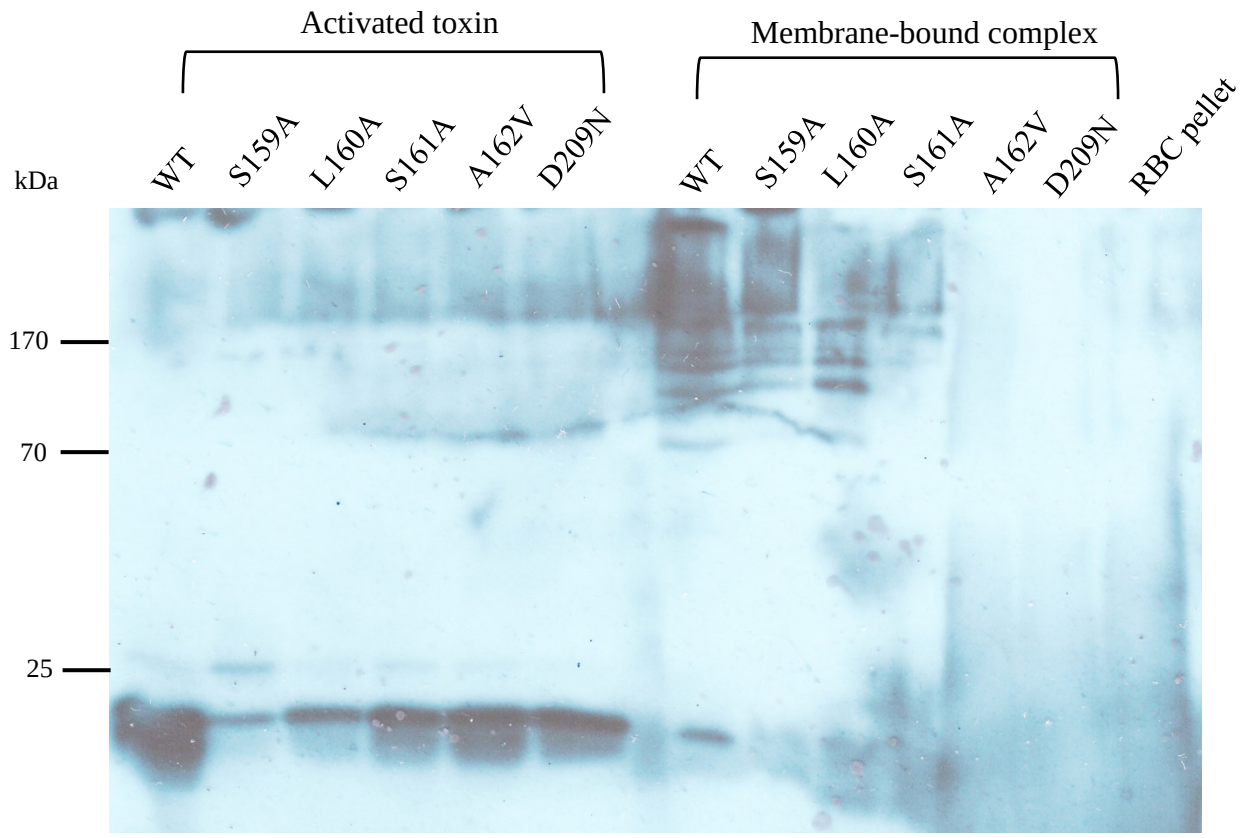
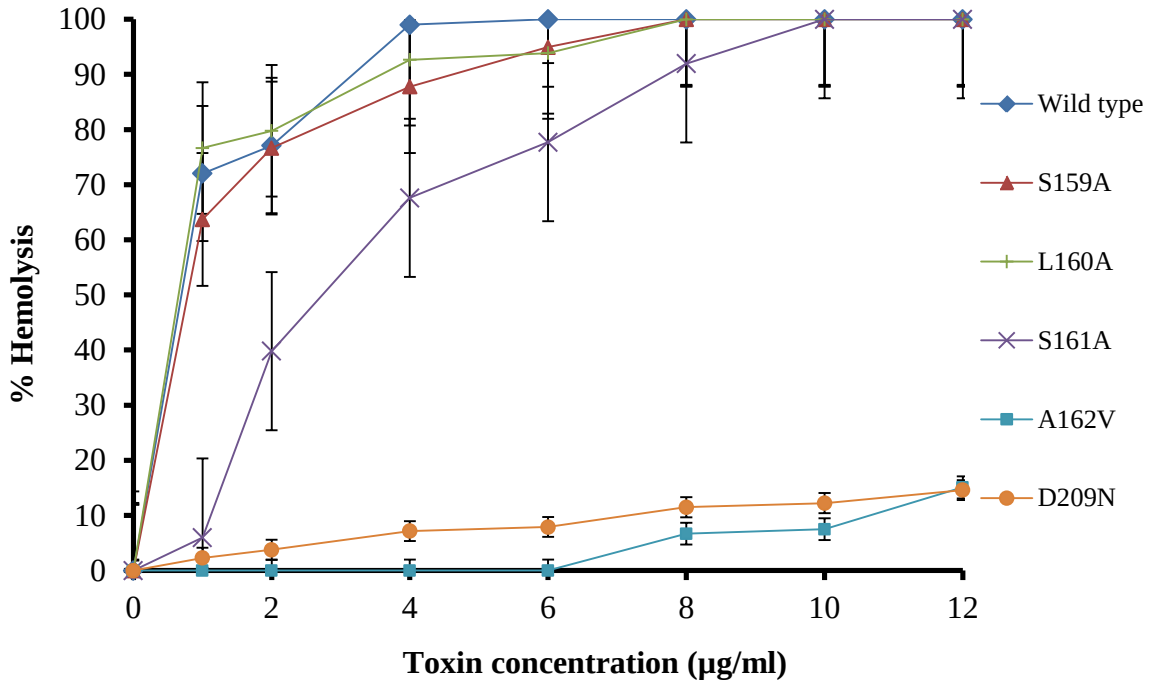

Activated toxin
Membrane-bound complex
WT
S159A
L160A
S161A
A162V
D209N
WT
S159A
L160A
S161A
A162V
D209N
RBC pellet
kDa
170
70
25

Figure 4


% Hemolysis
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
Toxin concentration (µg/ml)
Wild type
S159A
L160A
S161A
A162V
D209N