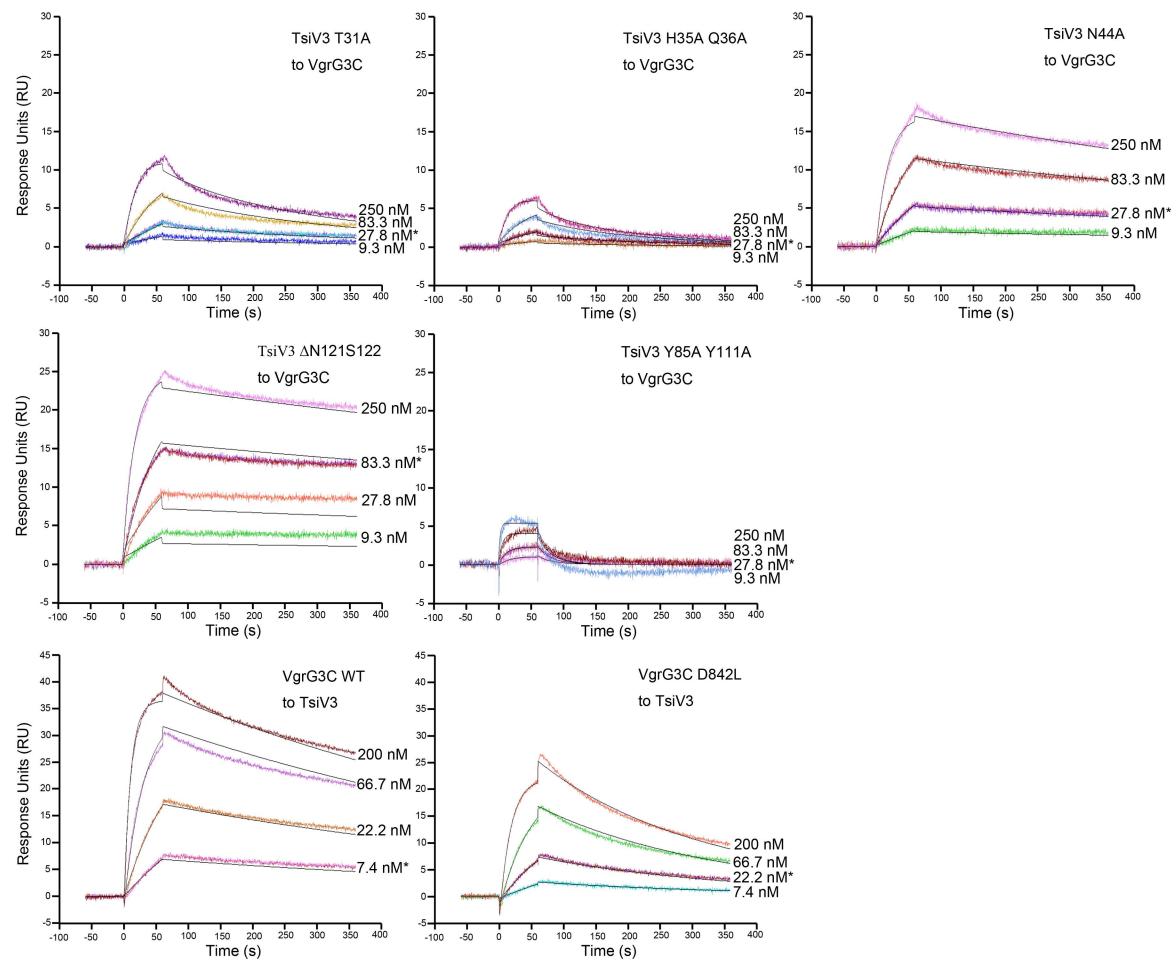
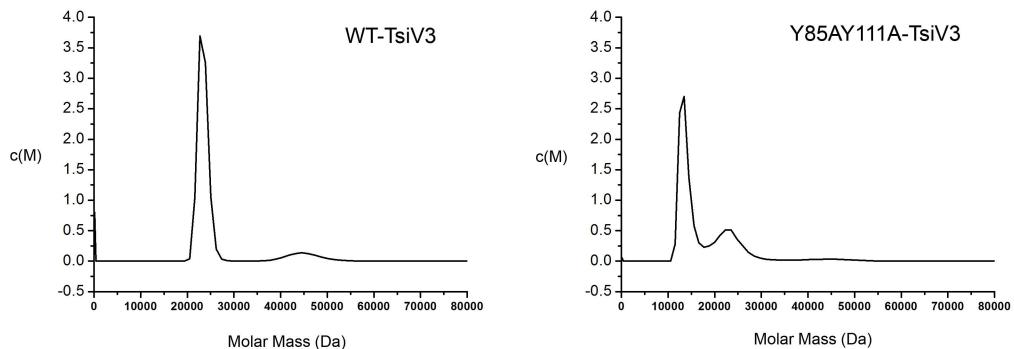


**Supplemental Figure 1.** (A) Domain architecture of *Vibrio cholerae* VgrGs and T4 bacteriophage spike. Various domains were indicated in different colors. ACD, actin cross linking domain; PGB, peptidoglycan binding domain. (B) A translocation model of VgrG3 during bacterial competition. The C-terminal extension of VgrG3 effector is associated with TsIV3 dimers to block its enzymatic activity in the periplasm of *Vibrio cholerae*.

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**Supplemental Figure 2.** BIACore data of VgrG3C to TsiV3 wide type and mutant proteins. Gradient concentrations of analytes were injected through flow cells immobilized with ligands. The BIACore traces and kinetic profiles are shown. The binding affinity ( $K_d$ ) were calculated in Biacore T100 evaluation software kinetic analysis by 1:1 Langmuir binding model.



**Supplemental Figure 3. Analytical ultracentrifugation data of the TsiV3 wide type and Y85A/Y111A mutant.** Protein samples were diluted to an OD 280 nm of 0.8 in 20 mM Hepes, pH7.5, 150mM NaCl. Data were collected at 60,000 r.p.m. every 3 min at a wavelength of 280nm. Interference sedimentation coefficient distributions,  $c(M)$ , were calculated from the sedimentation velocity data by using SEDFIT.