Supplementary Information

# Leucine 232 and hydrophobic residues at the ribosomal P stalk binding site are critical for biological activity of ricin

Yijun Zhou, Xiao-Ping Li, Jennifer N. Kahn, John E. Mclaughlin and Nilgun E. Tumer

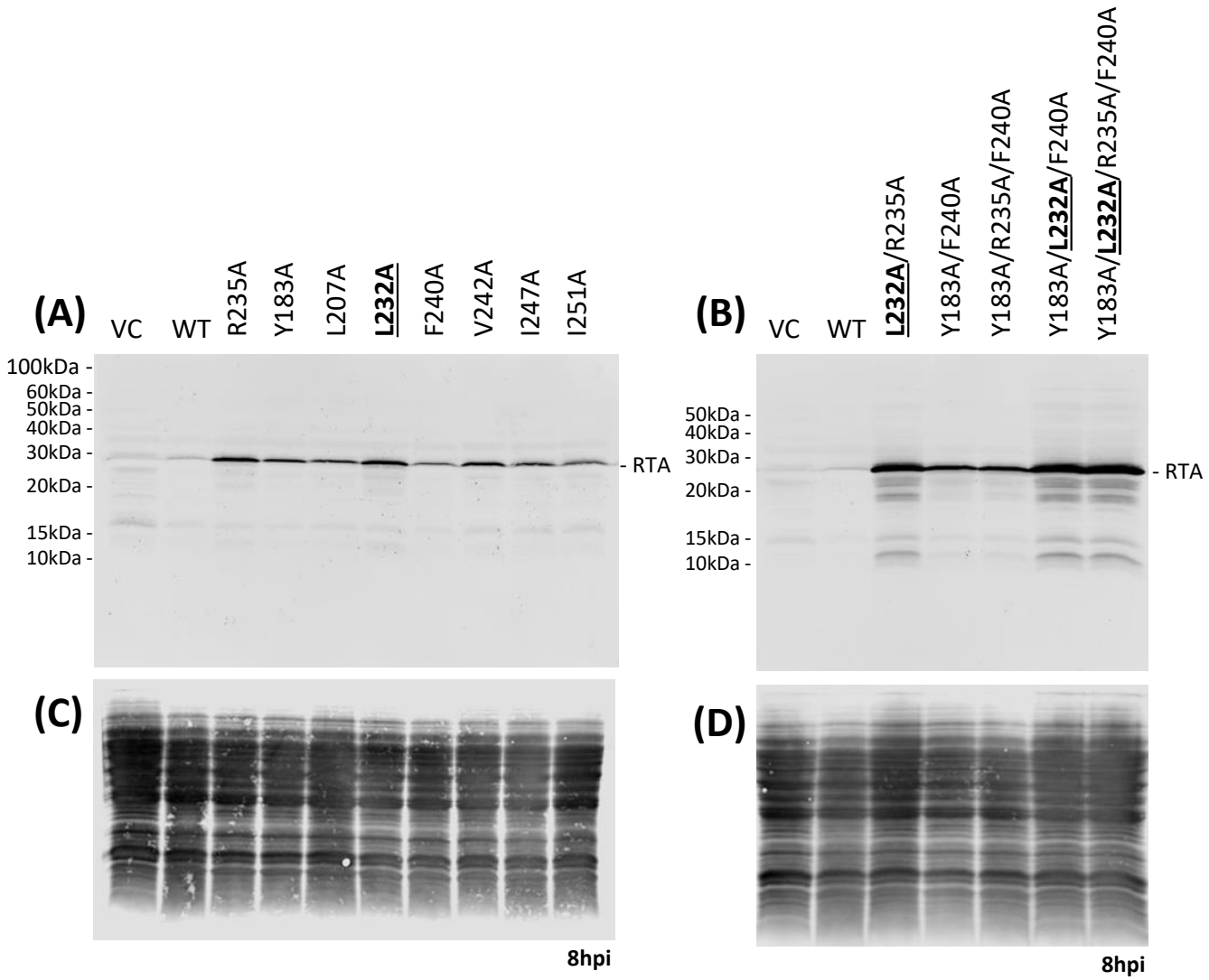
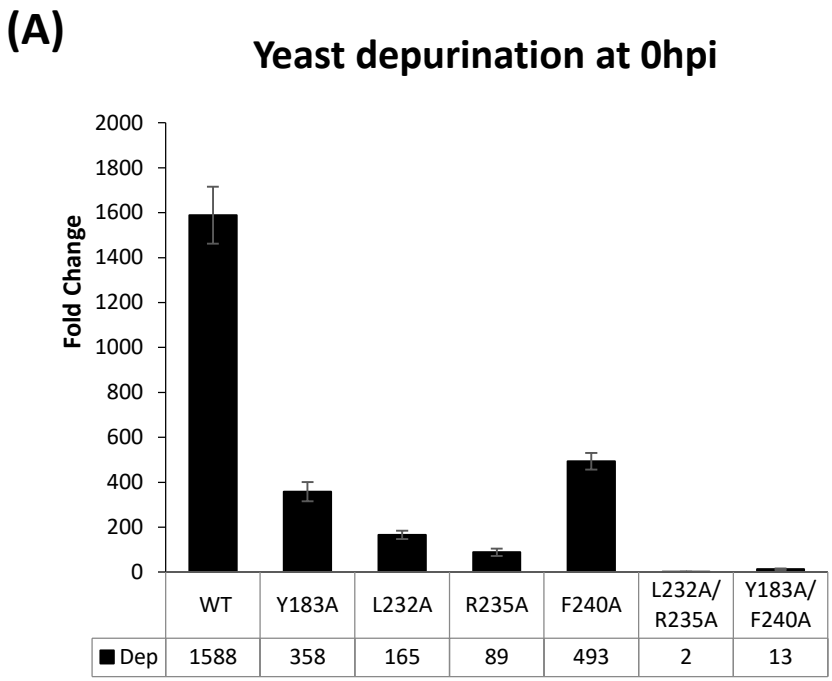



**Figure S1. Expression of hydrophobic mutants in yeast**.
**(A)** The full gel of Fig.1E showing western blot analysis of single RTA mutants in yeast at 8 hpi. **(B)** The full gel of Fig. 2C showing western blot analysis of double, triple and quadruple RTA mutants in yeast at 8 hpi. **(C)** Total protein stain of A. **(D)** Total protein stain of B.



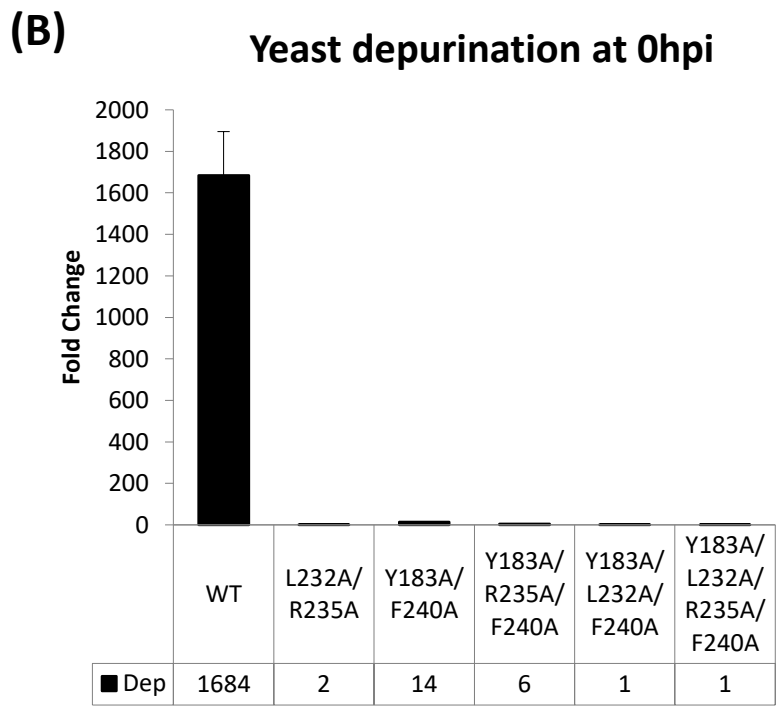




**Figure S2**. **Depurination of RTA variants in yeast.**
Yeast cells transformed with VC, WT RTA or RTA mutants were cultured overnight in GLU-LEU medium for 20 hours (0 hpi). Depurination was measured by qRT-PCR. **(A)** Depurination of WT RTA, single and double RTA mutants at 0 hpi. **(B)** Depurination of WT RTA, double, triple and quadruple RTA mutants at 0 hpi. Fold change in depurination is shown relative to the VC.

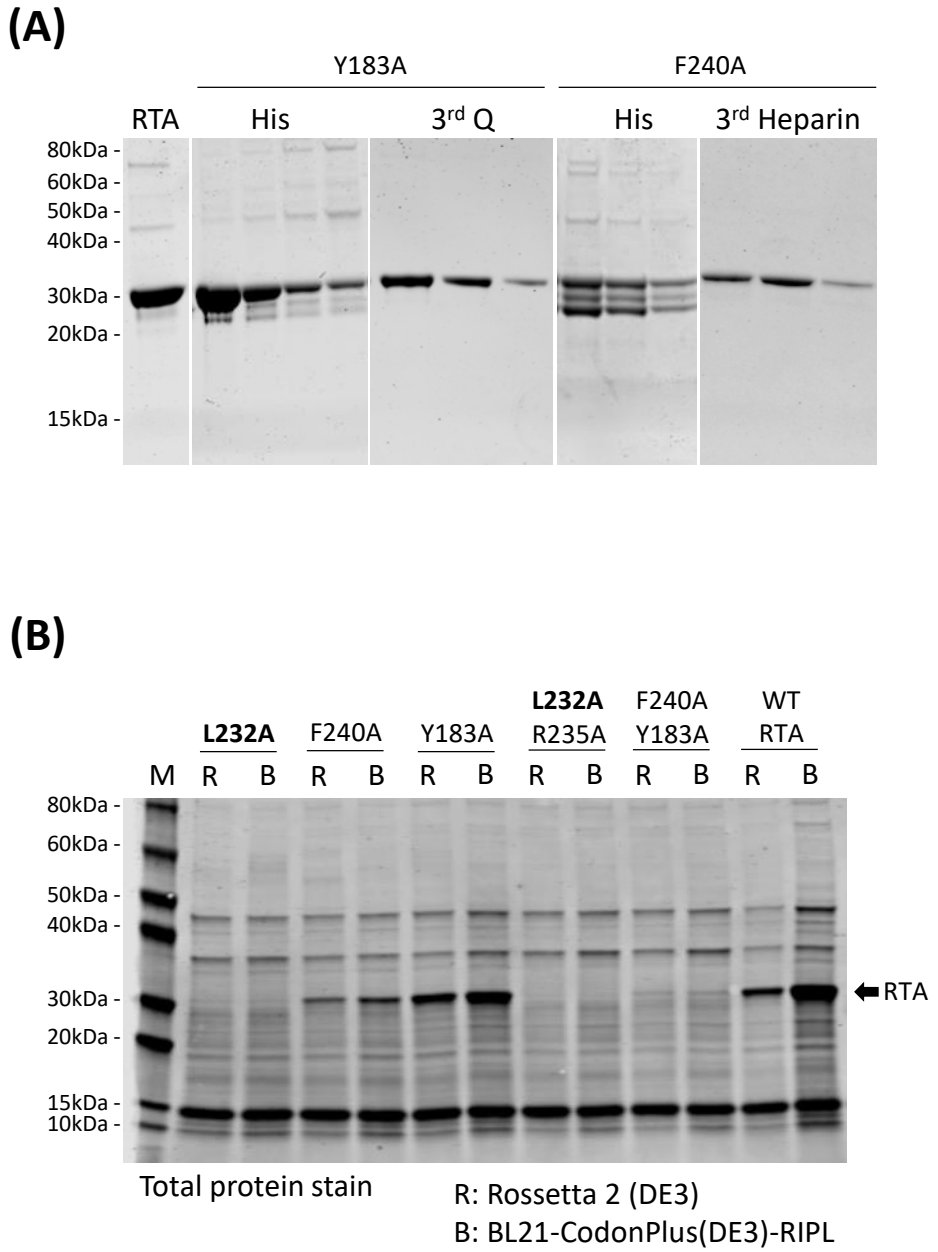



**Figure S3. Purification of hydrophobic mutants.**
**(A)** Eluted fractions from His (2nd) and Q (3rd) column purification of Y183A and His (2nd) and Heparin (3rd) column purification of F240A are shown. Purified RTA is loaded as control. **(B)** Hydrophobic mutants were expressed in two different *E.coli* expression strains, Rossetta 2 (DE3) (R) and BL21-CodonPlus(DE3)-RIPL (B), as described in the Materials and Methods. After separation on SDS-PAGE, proteins were transferred to a 0.2 μm nitrocellulose membrane and stained with REVERT total protein stain kit (Li-COR, Lincoln, NE, USA). Molecular weight markers are shown (M).

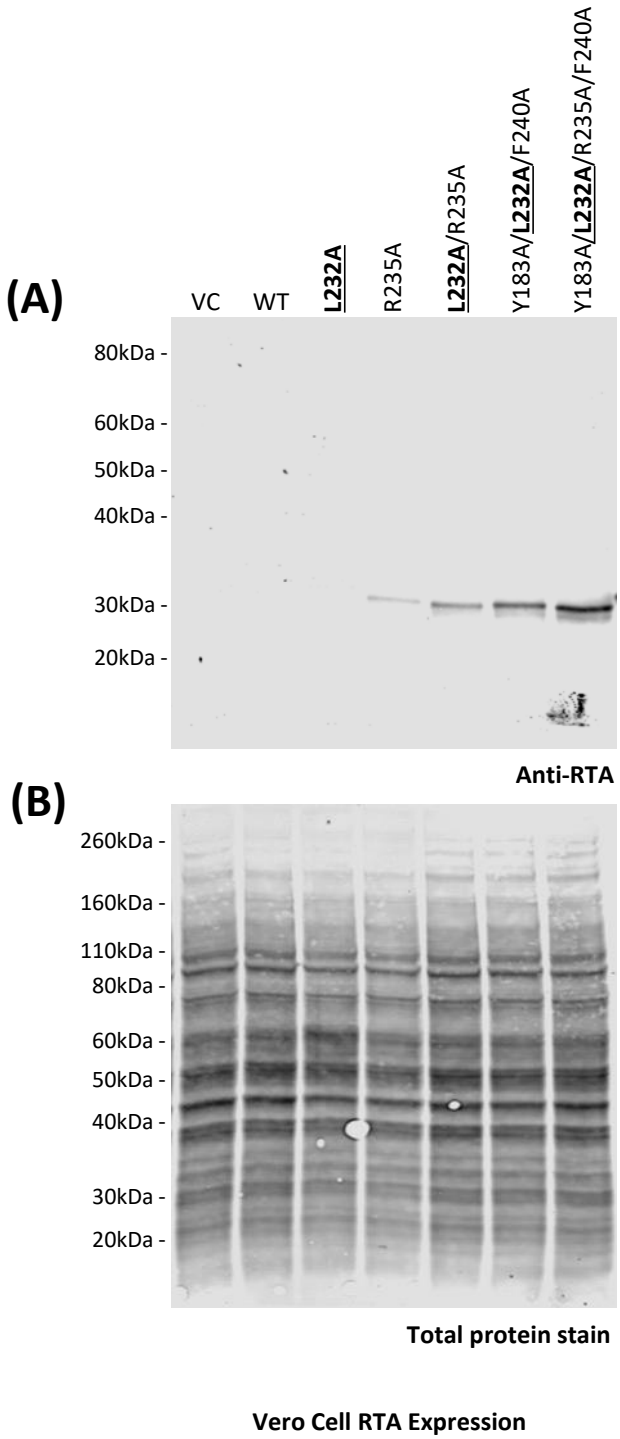



**Figure S4. Expression of RTA mutants in Vero cells.**
**(A)** The full gel of Fig. 5D showing western blot analysis of RTA mutants expressed in Vero cells. **(B)** Total protein stain of A.

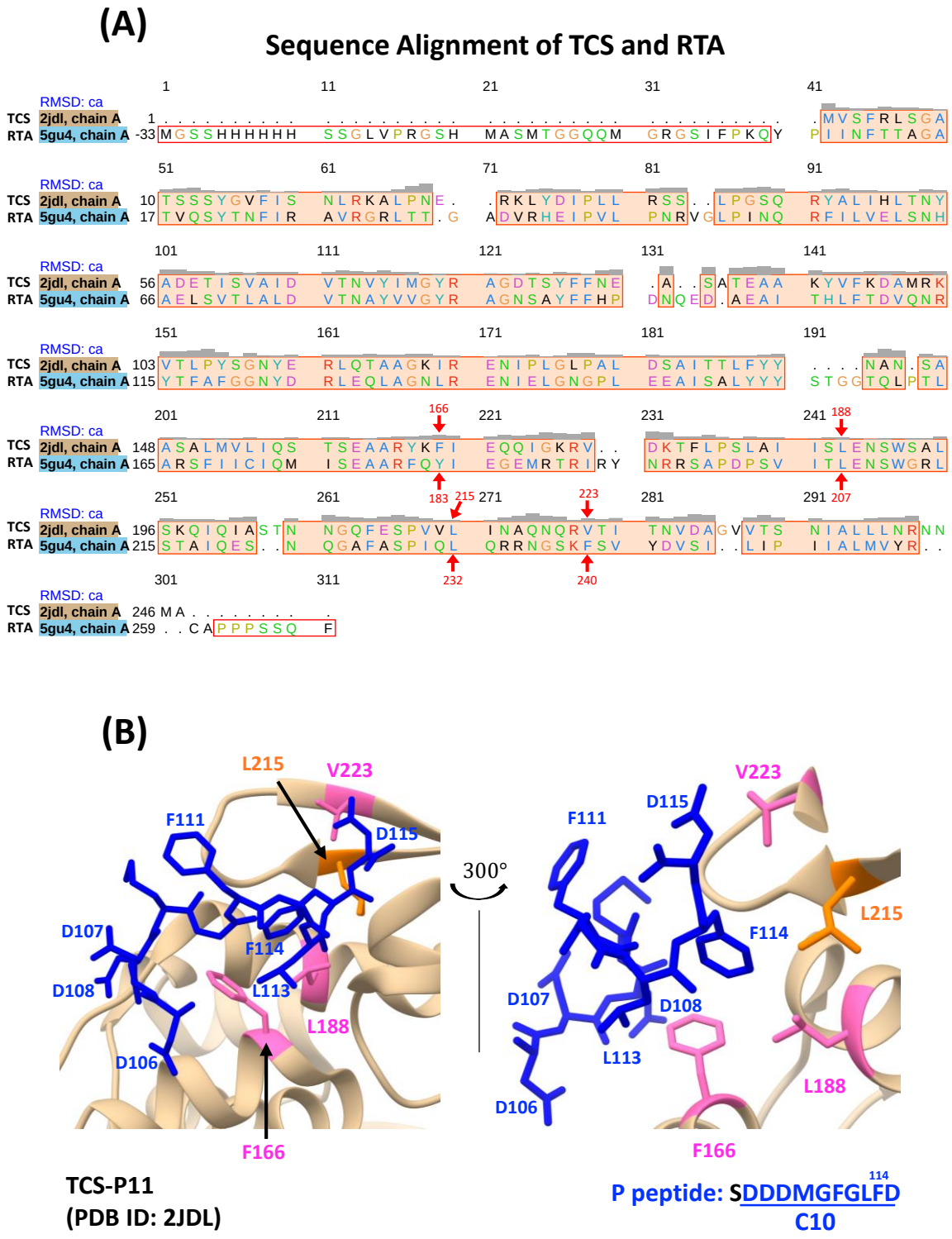



**Figure S5. Sequence alignment of TCS with RTA.**
**(A)** TCS (PDB ID: 2JDL) and RTA (PDB ID: 5GU4) were aligned using Chimera. Tyr183, Leu207, Leu232 and Phe240 of RTA and the corresponding residues in TCS, Phe166, Leu188, Leu215 and Val223 respectively, are indicated with arrows and residue numbers. **(B)** Structure of TCS-P11 (PDB ID:2JDL) visualized using Chimera. TCS is colored in tan, Val223, Leu188, and Phe166 are colored in pink. Leu215 is colored in orange. P11 peptide is colored in blue.