

Supplementary Information

accompanying

14-3-3 Activated Bacterial Exotoxins AexT and ExoT Share Actin and the SH2 Domains of CRK Proteins as Targets for ADP-Ribosylation

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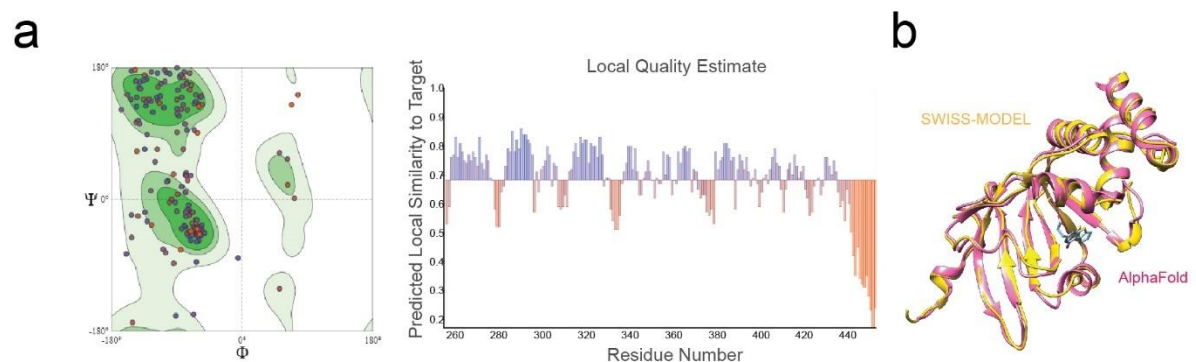


Figure S1 (pertaining to Figure 1 of the main text): Quality of the AexT-ART homology model constructed with SWISS-MODEL. **(a)** Ramachandran plot and per-residue quality estimate with global quality score (QMEANDisCo) of 0.68 represented as the average of the local quality estimate. **(b)** Superimposition with the AlphaFold model. RMSD across all 198 C α atom pairs: 1.403Å (0.772Å between 182 pruned atom pairs).

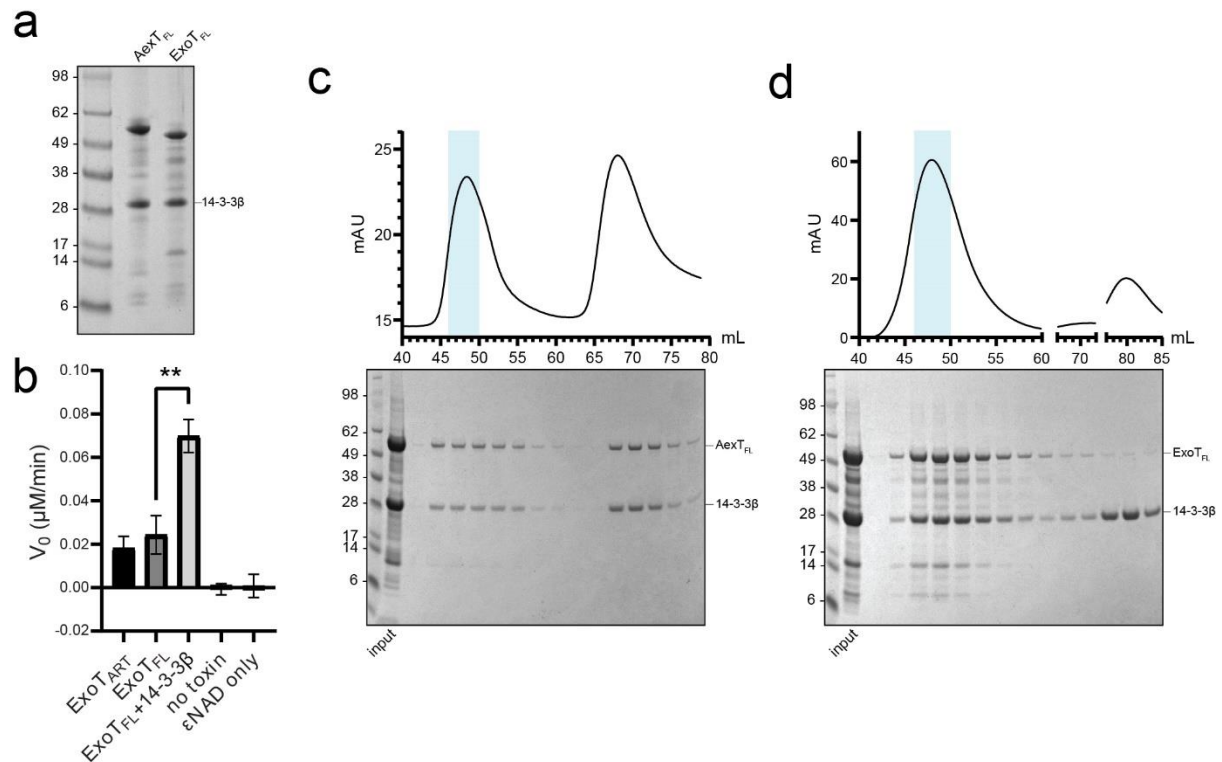


Figure S2 (pertaining to Figure 2 of the main text): Steps in the recovery of full-length toxins from *E. coli* inclusion bodies. **(a)** Coomassie stained SDS-PAGE gel showing the purity of the refolded exotoxins before size exclusion chromatography. **(b)** The addition of 14-3-3β during the refolding process significantly improves the activity of refolded ExoT. Processing rates were determined in assays containing 200 nM toxin, 500 nM 14-3-3β, 5 μM CRK SH2 domain, and 40 μM εNAD⁺. **, adjusted P value = 0.002, determined with ordinary one-way ANOVA. **(c, d)** Typical SEC profiles of (c) AexT and (d) ExoT in complex with 14-3-3β. A substantial amount of protein elutes in the void volume of the column (around 48 ml, indicated in blue; column: Hiload 16/600 Superdex 200 pg). The complex of interest elutes around 70 ml, followed by excess 14-3-3β.

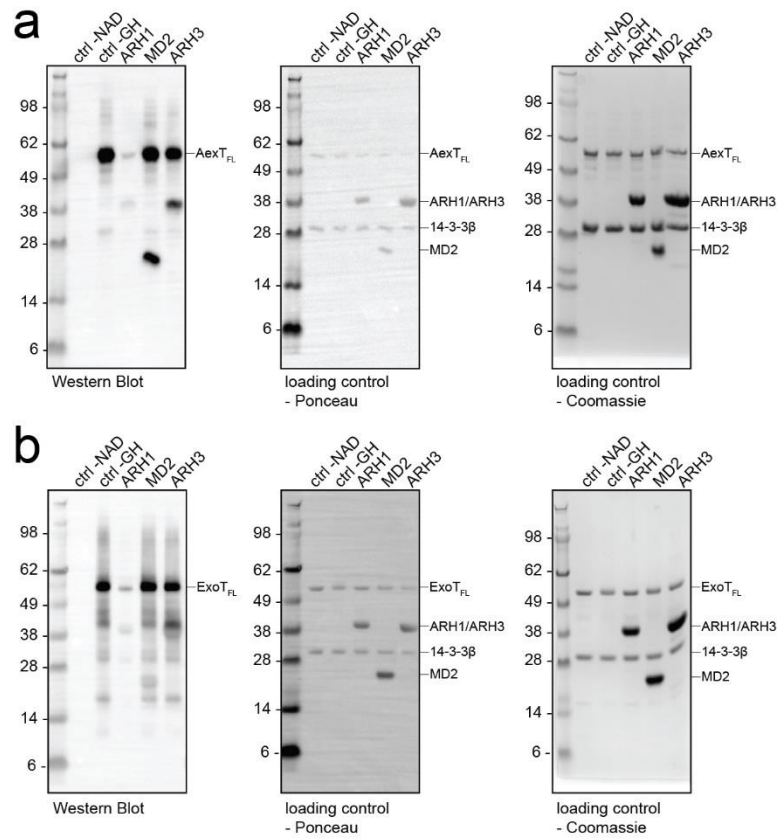


Figure S3 (pertaining to Figure 4 a,b of the main text): Uncropped Western blot images and loading controls for Figure 4a,b. (a) AexT; (b) ExoT.

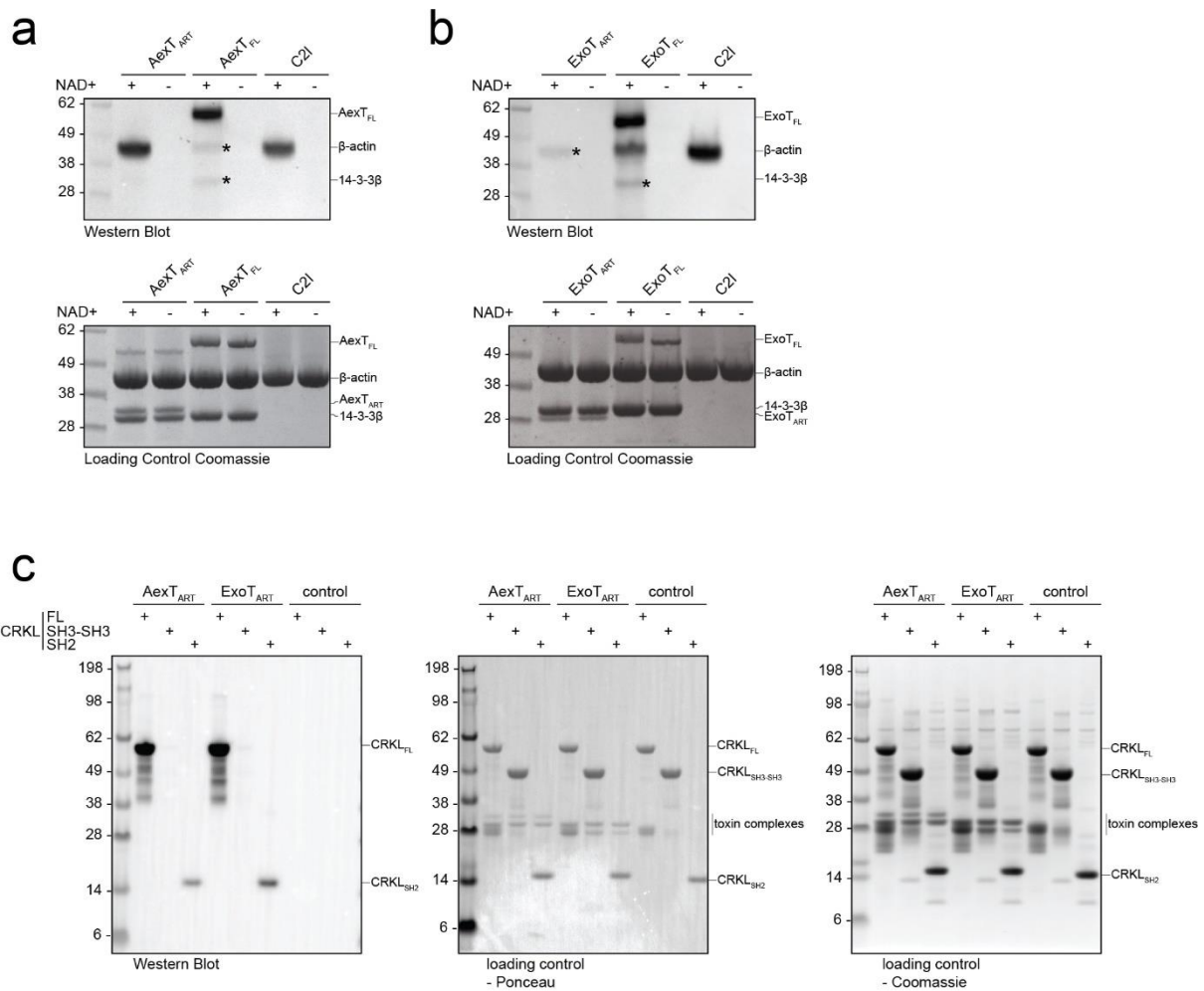


Figure S4 (pertaining to Figure 5 of the main text): Uncropped Western blot images and loading controls for Figure 5 a,b,c.

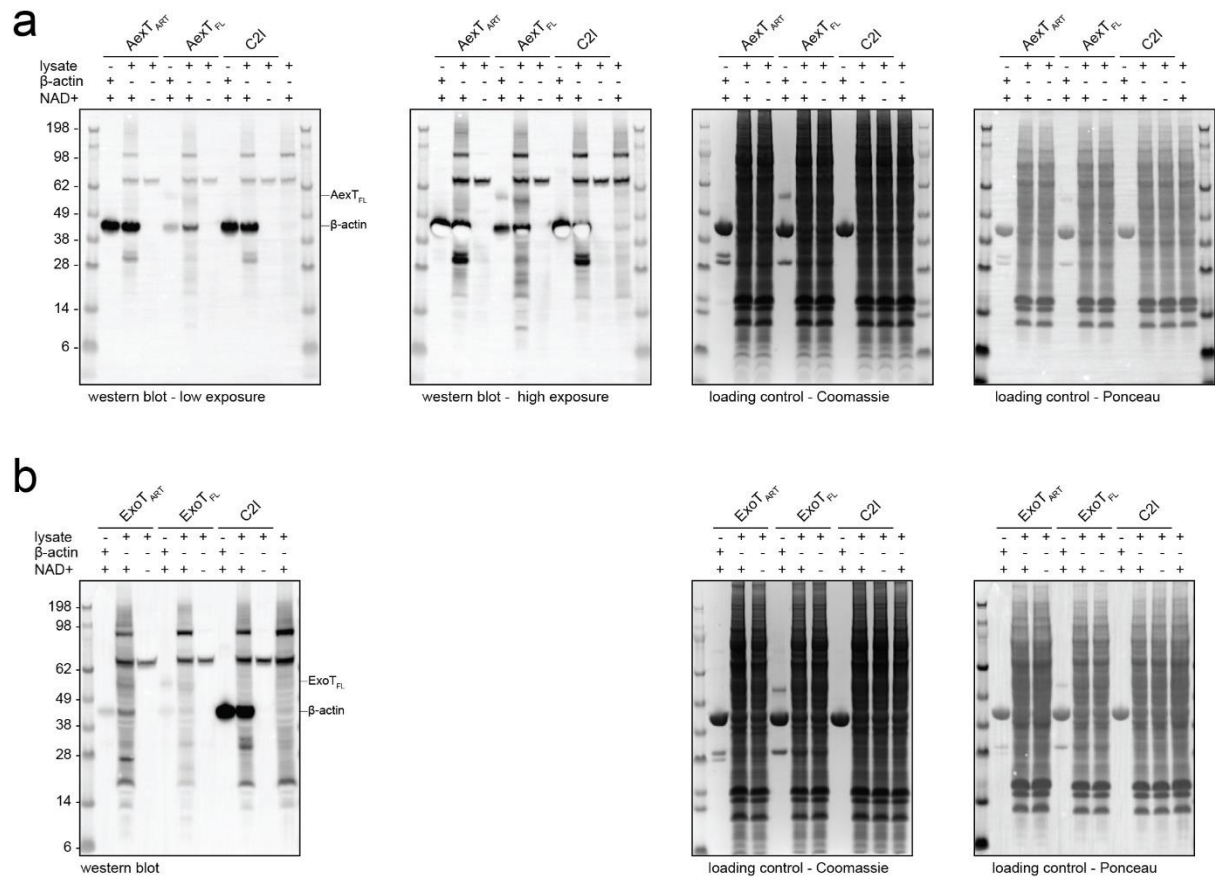


Figure S5 (pertaining to Figure 6 of the main text): Uncropped Western blot images, alternative exposures and loading controls for Figure 6 a,b.