

Expanded View Figures

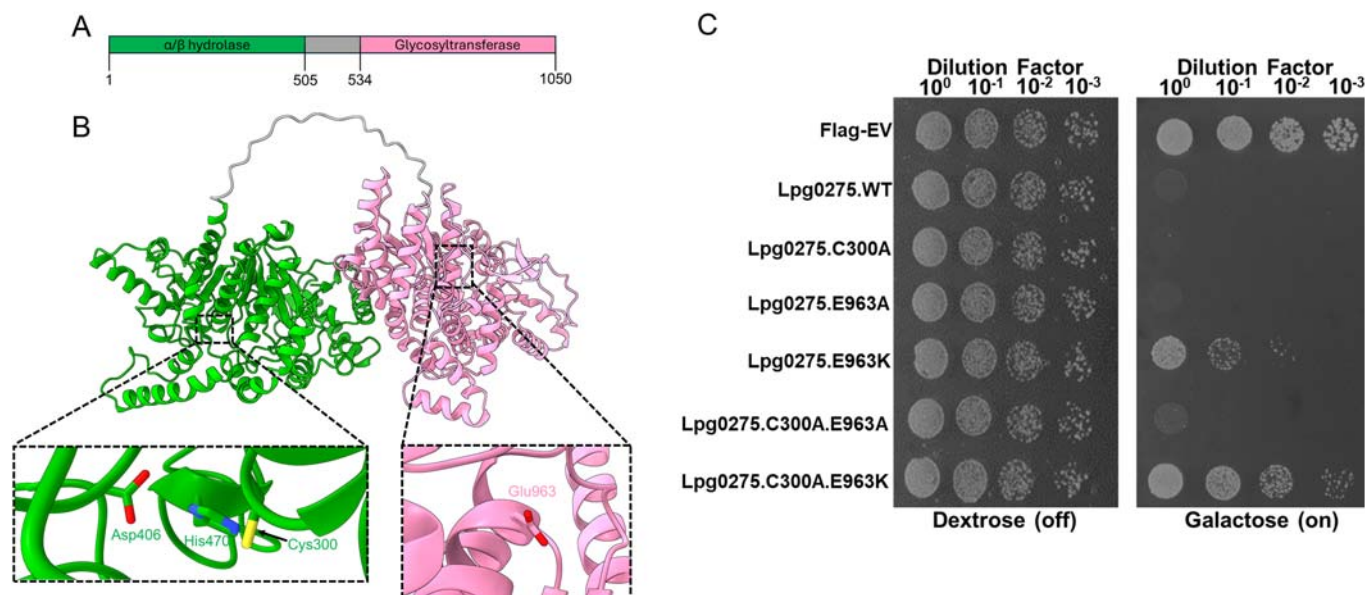


Figure EV1. Both domains of Lpg0275/SdbA are involved in yeast toxicity.

(A) Schematic of the domain organization of Lpg0275/SdbA. The N-terminal domain shown in green corresponds to the hydrolase domain, whereas the pink indicates the glycosyltransferase domain. (B) AlphaFold2 model of Lpg0275/SdbA. The green represents the hydrolase domain, and the pink represents the glycosyltransferase domain. Below is a zoomed-in view of the predicted catalytic residues of each predicted enzymatic domain. (C) Yeast toxicity panel of strains expressing FLAG-tagged constructs of full-length wildtype Lpg0275 and its variants.

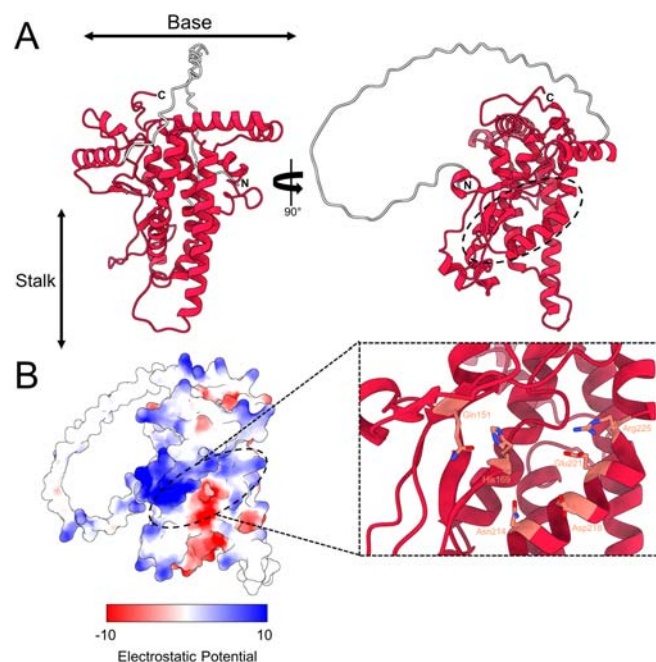


Figure EV2. Lpg1154/RavQ forms a unique "T" shape containing a highly conserved groove that may serve as an active site.

(A) The Lpg1154/RavQ model (residues 59–389, red) shows the base and stalk that form the "T" shape. (B) An electrostatic potential surface representation of a potential active site cavity of Lpg1154/RavQ, followed by a zoom-in of the conserved residues identified in Lpg1154/RavQ and its orthologs from the *Legionella* genus, which are arranged in a potential active site.

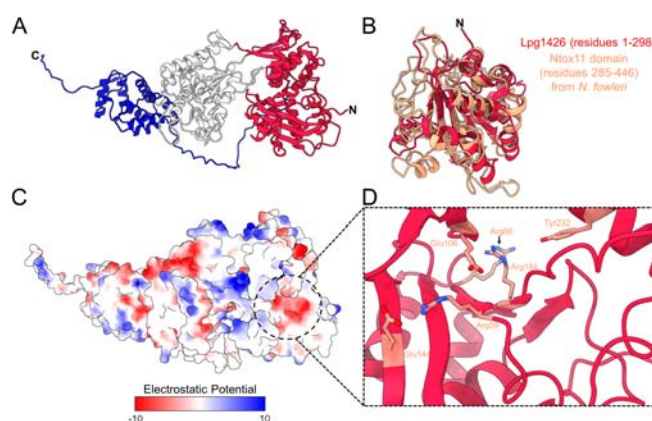


Figure EV3. Lpg1426/VpdC has a cryptic domain on the N-terminus that has structural similarity to the Ntox11 putative toxin found in human pathogenic amoeba.

(A) Cartoon representation of the Lpg1426/VpdC AlphaFold2 model. The cryptic domain is found on the N-terminus (red), followed by a central phospholipase domain (white) and the C-terminal helical bundle involved in interactions with ubiquitin. (B) Structural alignment of the Lpg1426/VpdC cryptic domain (residues 1–298, red) onto the Ntox11 AlphaFold2 model (residues 285–446, salmon) from *N. fowleri*. (C) Surface representation of the electrostatic potential of the Lpg1426/VpdC model that also shows a conserved negatively-charged pocket (dotted circle) is present in the cryptic domain. (D) Zoom in on the positively charged region where highly conserved residues (salmon sticks), which are present in the *Legionella* orthologs of Lpg1426/VpdC, form a pocket.

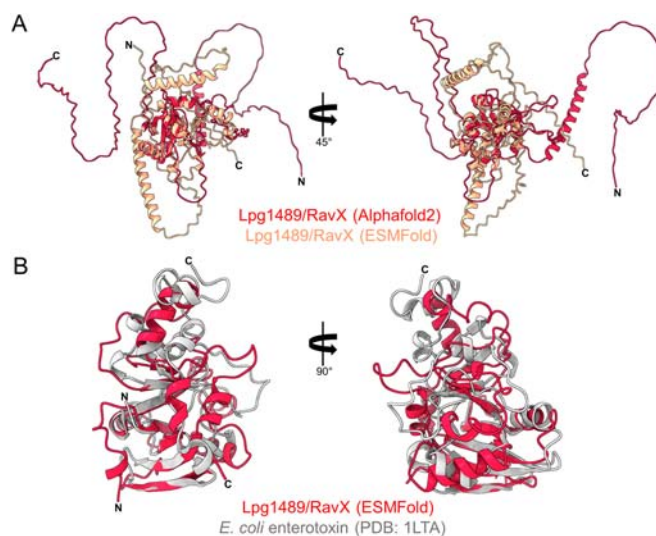


Figure EV4. Lpg1489/RavX has a central globular cryptic domain surrounded by disordered loops.

(A) Structural alignment of the full-length Lpg1489/RavX models generated by AlphaFold2 (red) and ESMFold (salmon). (B) ESMFold model of the Lpg1489/RavX cryptic domain (residues 83-263, red) onto the *E. coli* enterotoxin (PDB: [1LTA](#), residues 1-181, gray).

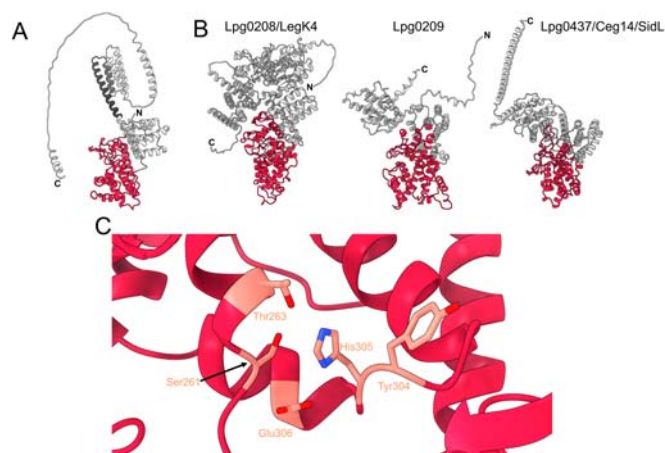


Figure EV5. The cryptic domain of Lpg2527/LnaB is present in other *L. pneumophila* effectors, harboring a conserved set of residues that resemble a potential active site.

(A) AlphaFold2 model of Lpg2527/LnaB which highlights the cryptic domain (red) and the helical bundle that was previously shown to be important in the activation of the NF- κ B pathway (dark gray) (Losick et al, 2010). (B) Representation of the Lpg2527/LnaB cryptic domain (shown in red) that is present in other *L. pneumophila* effectors (Lpg0208/LegK4, Lpg0209, and Lpg0437/Ceg14/SidL). (C) Zoomed-in image of the putative active site of Lpg2527/LnaB that is also conserved in its *Legionella* orthologs and other *L. pneumophila* effectors containing this domain.