Supporting Information

Jackson et al. 10.1073/pnas.1304097110

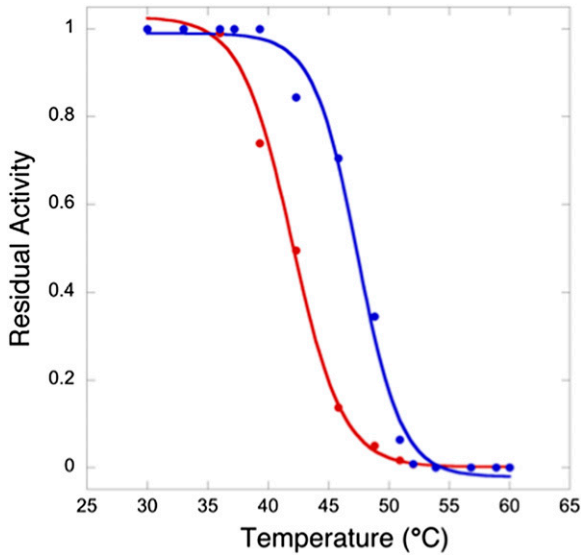

Fig. S1. Plot of residual activity of *Lucilia cuprina* (*Lc*αE7; red) and LcαE7-4 (blue). Points are averages of three measurements; protein was incubated at the temperatures indicated for 15 min before being placed on ice. Activity was measured with 4-nitrophenyl butyrate and converted to a percentage of maximum activity.

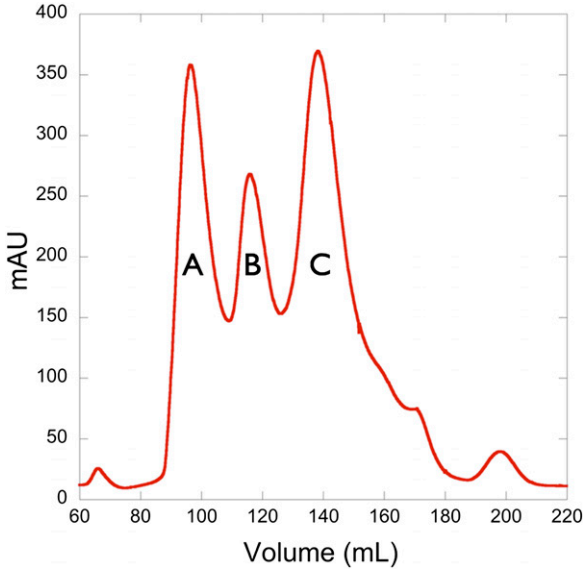

Fig. S2. Size exclusion chromatography of *Lc*αE7-4. Pure LcαE7-4 protein (postaffinity chromatography) was loaded onto a Sephacryl S300 column. Protein eluted in three peaks. Peak A corresponds to a high-molecular weight aggregate and did not crystallize in any condition tested. Peak B crystallized as dimeric *Lc*αE7-4. Peak C crystallized as monomeric *Lc*αE7-4.

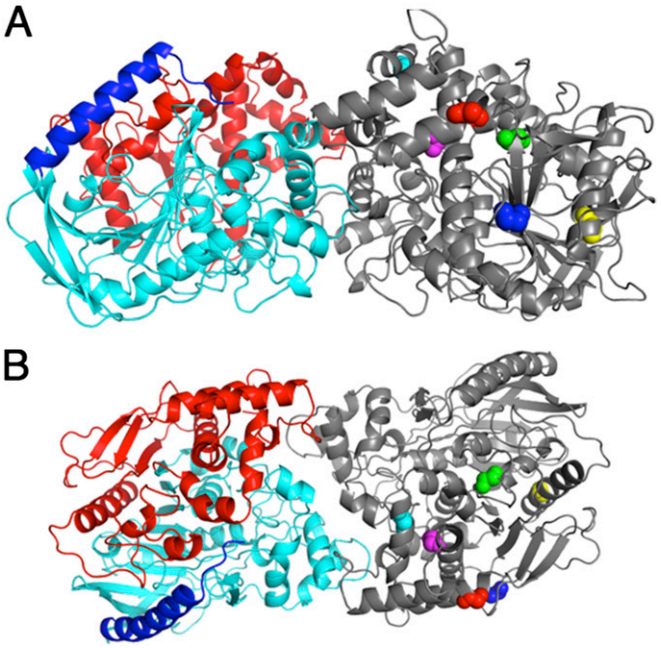

Fig. S3. Cartoon of the *Lc*αE7 dimer from (*A*) the side and (*B*) above the active site. The N-terminal membrane association helix is colored blue, the N-terminal one-half of the protein is colored cyan, and the C-terminal one-half is colored red. The locations of stabilizing mutations are shown as spheres (cyan, M364L; magenta, I419F; green, A472T; yellow, I505T; blue, K530E; red, D554G). The biological dimer is pictured, and the dimerization interface is highlighted.

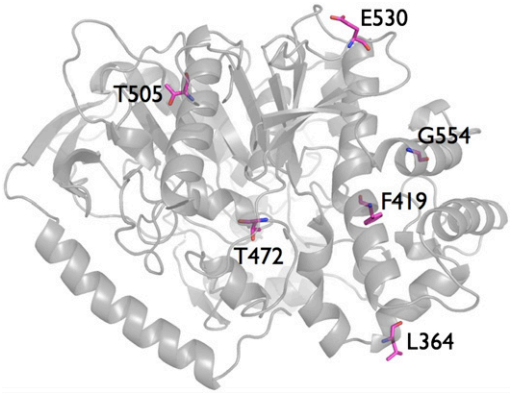

Fig. S4. Stabilizing mutations accumulated through directed evolution. The mutations that accumulated are represented as sticks. All mutations were located in the C-terminal one-half of the protein, with M364L, K530E, and D554G being surface-exposed. I419F, I505T, and A472T are located in the interior of the protein.

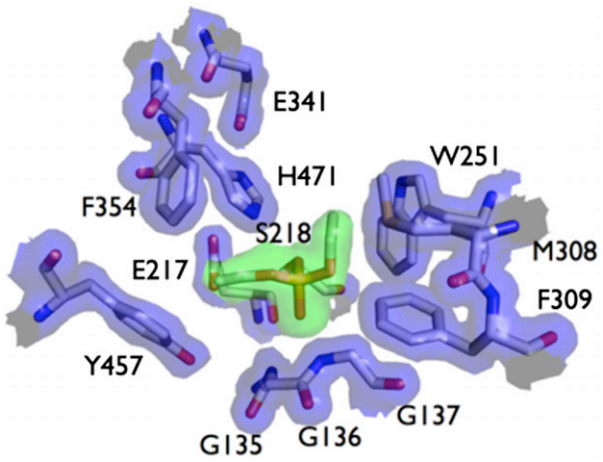

Fig. S5. The *Lc*αE7-diethylphosphate complex. Electron density around *Lc*αE7 is shown as a blue surface and contoured at 1.5 σ. Omit electron density corresponding to phosphorylation of Ser218 is shown as green surface and contoured at 3 σ.

Table S1. Primer sequences used in this work

Primer	Sequence (5′-3′)
E31	CGC AAG CTT CAT ATG AAT TTC AAC GTT AGT TTG
E32	CGG GTC GAC GAA TTC TTA CTA AAA TAA ATC TCT ATG
E33	GAA AAA TAG ACC CTC GTA TGA AGT GTT ACC CAT
E34	ATG GGT AAC ACT TCA TAC GAG GGT CTA TTT TTC
pET1	CGA CTC ACT ATA GGG AGA CCA CAA C
pET2	CCT TTC GGG CTT TGT TAG CAG