Supporting Information

Brown et al. 10.1073/pnas.1415271111

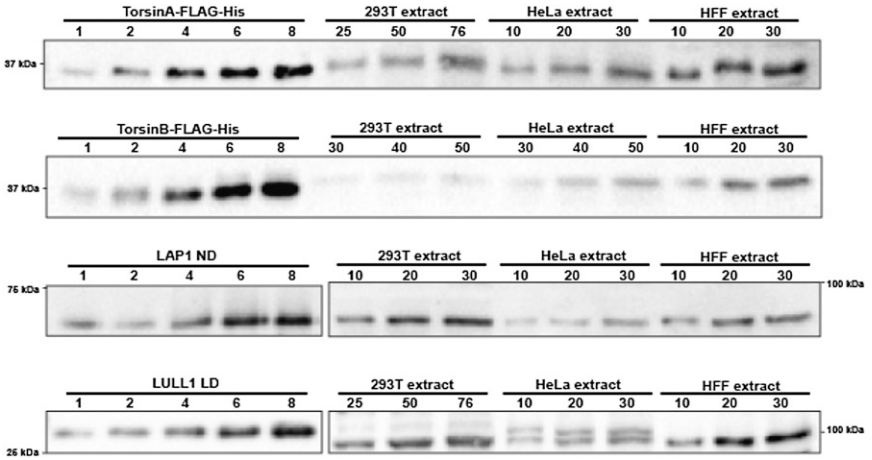

Fig. S1. Copy number determination for Torsins, LAP1, and LULL1 in three human cell lines. Blots shown were used to arrive at the cellular copy numbers listed in Table 1. Numbers given above blots represent nanograms of purified protein or cellular extract loaded in each lane. All standard curves are from the same blot as the cellular extracts and were used to determine the number of protein molecules present in a given quantity of cellular extract. Because it was not possible to purify soluble full-length LAP1 and LULL1 due to their transmembrane domains, the N-terminal cytosolic domain for LAP1 and the ER luminal domain for LULL1 were used as standards and do not run at the same molecular mass on the blot as full-length LAP and LULL in the cellular extracts. HFF, Human Foreskin Fibroblast.

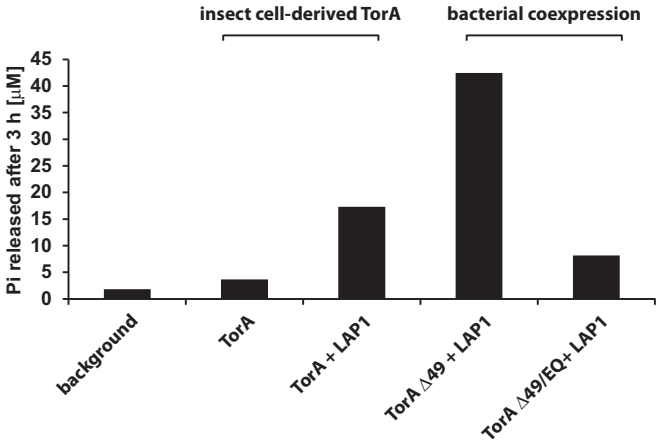

Fig. S2. Bacterially expressed TorA is active. (*Left*) As a positive control, 3 μM insect cell-derived TorA, 3 μM LAP1^{LD}, and 2 mM ATP were incubated at 37 °C. ATP hydrolysis was measured by monitoring Pi-release after 3 h. Insect cell-derived TorA served as a negative control. (*Right*) Bacterially expressed, purified complex of MBP-TorAΔ49/LAP1^{LD} was subjected to proteolytic cleavage to release the MBP tag quantitatively. Following MBP removal, the activity of an equal concentration of bacterially coexpressed material was determined. A hydrolysis-deficient mutant TorA–Δ49EQ/LAP1 complex served as a negative control to rule out the formal possibility of a contaminating ATPase activity. EQ, TorA E171Q Walker B mutant.

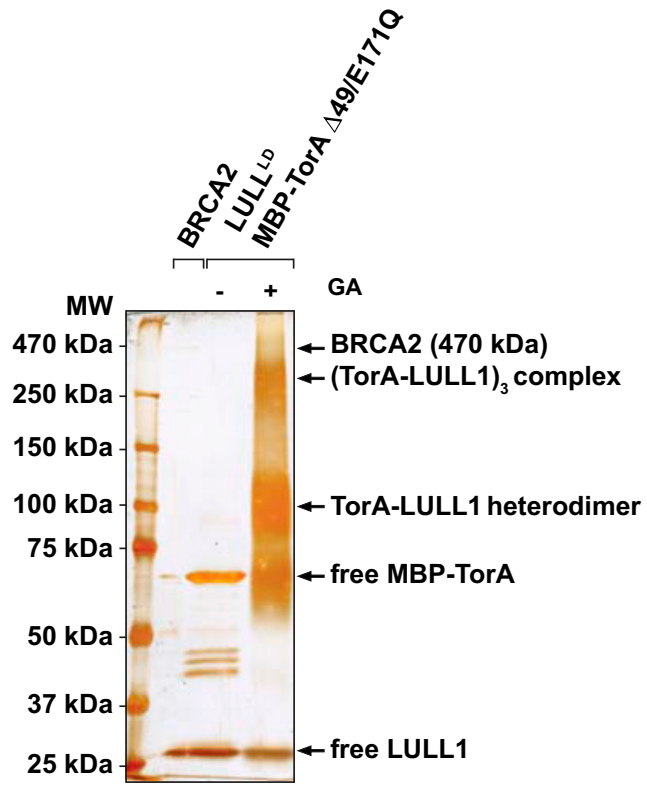

Fig. S3. TorA and LULL1 can form a hexameric complex. The size exclusion chromatography-purified TorA and LULL1 complexes were incubated at room temperature for 10 min in the absence or presence of 0.1% (wt/vol) glutaraldehyde (GA) and resolved by gradient SDS/PAGE (6–9%), followed by silver staining. Purified breast cancer type 2 susceptibility protein (BRCA2) (470 kDa) was used as an additional molecular mass reference.

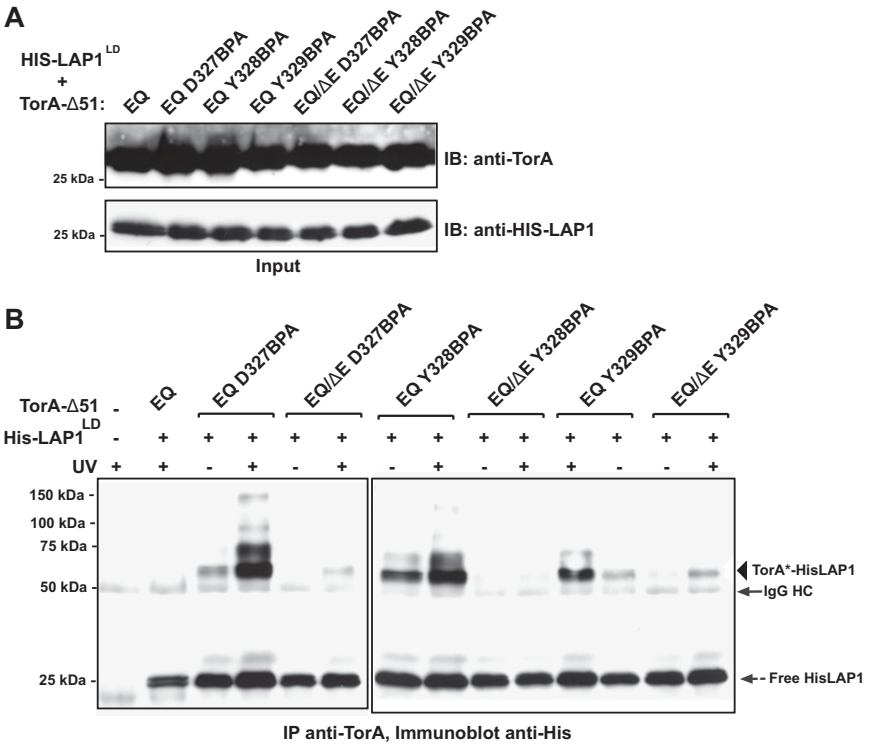

Fig. S4. Copurified His-LAP1^{LD} and TorA input protein. (*A*) SDS/PAGE and Western blots of 3 μL of copurified His-LAP1^{LD} and TorA Δ51 E171Q or TorA Δ51 E171Q/ΔE. Anti-TorA antibody was used to detect TorA, and anti-His was used to detect LAP1. (*B*) Full gel from Fig. 3*C* shows free HisLAP1. IB, immunoblot; IP, immunoprecipitate.

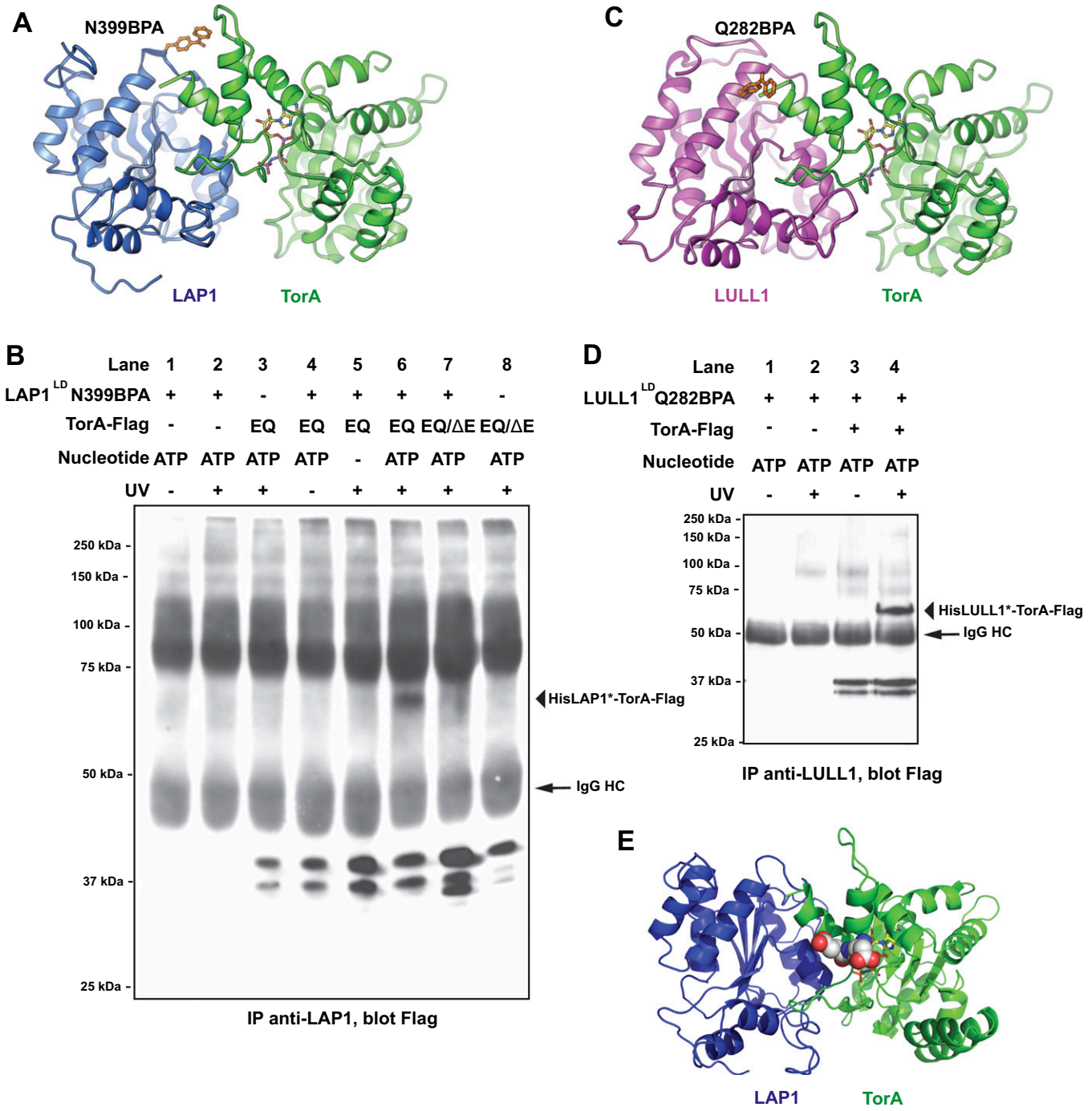

Fig. S5. Site-specific cross-linking of LAP1^{LD} and LULL1^{LD} with TorA. (*A*) Cross-linker pBPA (orange) was installed on His-LAP1^{LD} (blue) at N399 at the predicted interface with TorA (green). Adenylylimidodiphosphate (AMPPNP) is shown in stick representation. (*B*) LAP1 N399BPA (5 μM) was incubated with or without 5 μM TorA E171Q or TorA E171Q/ΔE in the presence or absence of 2 mM ATP. Complexes were incubated at 30 °C for 5 min, cooled to 20 °C, and UV-irradiated for 15 min. Cross-linked species were immunoprecipitated with a LAP1 antibody and analyzed by Western blot using a FLAG antibody to detect TorA. (*C*) Cross-linker pBPA (orange) was installed on His-LULL1^{LD} (magenta) at Q282 at the predicted interface with TorA (green). AMPNP is shown in stick representation. (*D*) LULL1 Q282BPA (5 μM) was incubated with or without TorA E171Q (5 μM) in the presence of ATP (2 mM). Cross-linking was performed as described in *B* but was immunoprecipitated with a LULL1 antibody. (*E*) LAP1^{LD} (blue) and TorA (green) interface highlighting both TorA E302 and E303 as spheres because the ΔE occurs in one of either two consecutive glutamate residues. HC, heavy chain.

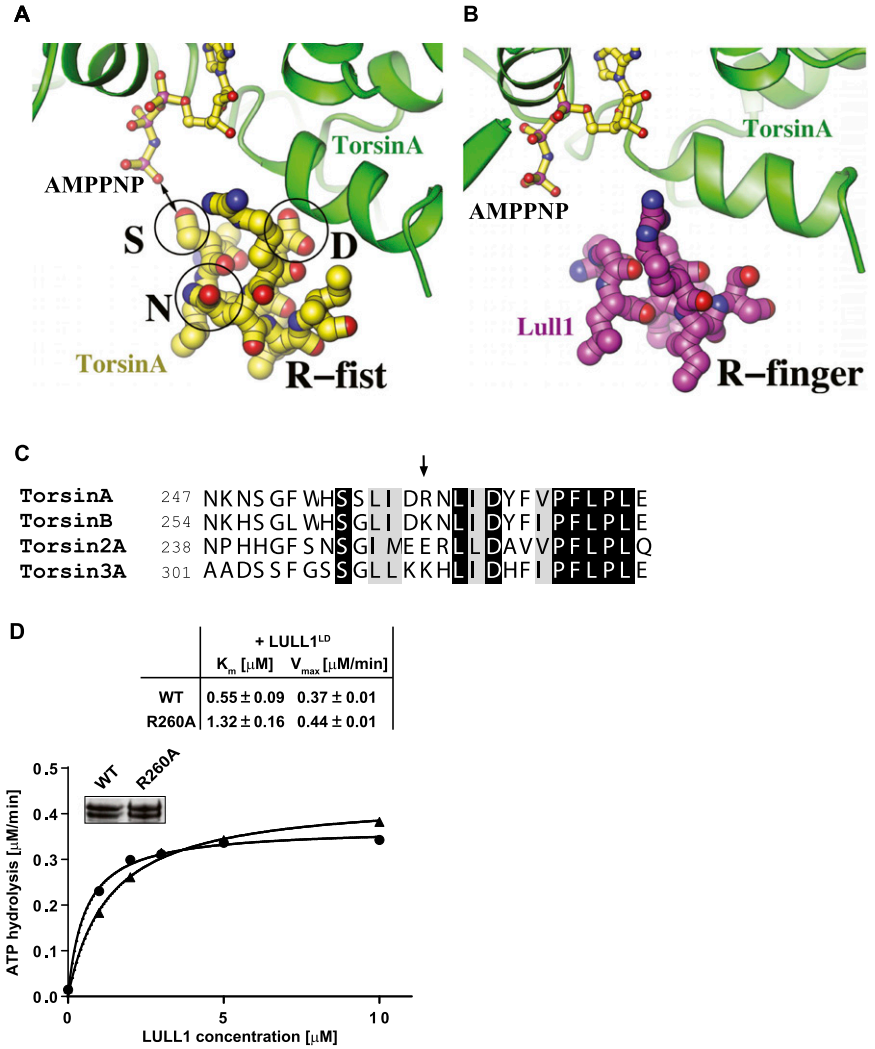

Fig. S6. TorA R260 is dispensable for ATPase activity. (*A*) Zoomed-in view of TorA–TorA interface from Fig. 1C. TorA (yellow) residues clash with the neighboring TorA (green) subunit, preventing R260 access to AMPPNP. Circled residues depict S265, D259, and N261. (*B*) Zoomed-in view of the TorA–LULL1 interface. LULL1 R449 (magenta) comes in close proximity to AMPPNP without clashing with TorA (green). (*C*) Sequence alignment of the Torsin family showing that TorA R260 (arrow) is not conserved. (*D*) Initial velocities of ATP hydrolysis were measured by monitoring Pi-release in the presence of 3 μM TorA and various concentrations of $LULL1^{LD}$. The data were fitted to Michaelis–Menten kinetics in Prism and yielded the indicated apparent K_m and V_{max} values. (*Inset*) Colloidal blue staining of 5 μg of TorA WT and TorA R260A (note that the double band reflects distinct glycosylation states).

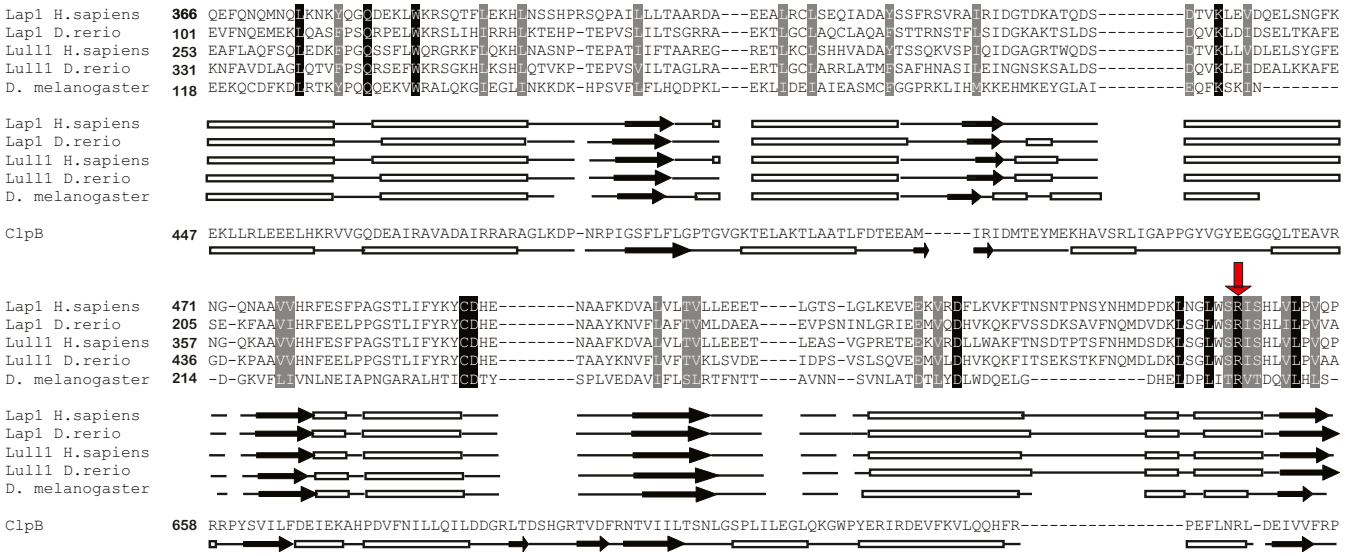

Fig. S7. LAP1 and LULL1 alignment to ClpB using predicted structural homology (as in Fig. 4*A* but showing more of the homology).

Dataset S1. LULL1 HHPred results

Dataset S1

Full-length LULL1 was used as the query sequence under default settings.

Dataset S2. LAP1 HHPred results (as in Dataset S1 but for LAP1)

Dataset S2