Supporting Information

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SI Methods

Torsin A Structure Prediction, Electrostatic Surface and Hexamer Modeling. A TorsinA (TorA) model was created using Swissmodel in automated mode (1), using ClpB (PDB entry 1QVR, chain C) (2) as template. Predictions of electrostatic surface potential of the TorsinA monomer were carried out using the San Diego Supercomputing Center online server PDB2PQR v1.8 (http://kryptonite.nbcr.net/pdb2pqr/) (3) with PROPKA pK_a calculation software at pH 7.0 (4) and APBS Tools in PyMol (5). A hexameric model of Torsin A was generated by superimposing its structure with that of each monomer in the hexameric AAA (ATPases associated with a variety of cellular activities) ATPase p97 (PDB entry 1R7R) (6) in Coot (7). All structure representations were made using Pymol (5).

Immunofluorescence. For immunofluorescence, 25,000 HeLa cells were seeded on a coverslip and transfected after 24 h using X-tremGENE 9 (Roche). Immunofluroescence labeling was performed on HeLa cells 24 h posttransfection. Cells were washed with PBS and fixed with 4% paraformaldehyde for 20 min at room temperature. After permeabiliziation with 0.1% Triton X-100/PBS for 10 min at room temperature, the coverslips were blocked with 4% BSA/PBS for 20 min at room temperature. Coverslips were incubated with primary antibody (1:500) in 4% BSA/PBS overnight at room temperature. Coverslips were then washed and blocked with 4% BSA/PBS for 20 min, and incubated with Alexa488/568-conjugated secondary antibodies (Invitrogen) (1:700) for 45 min in the dark. Following three washes in PBS, coverslips were mounted using Fluoromount-G. Images were acquired using a Zeiss microscope, model Observer D1, and 63×/1.4 oil lens.

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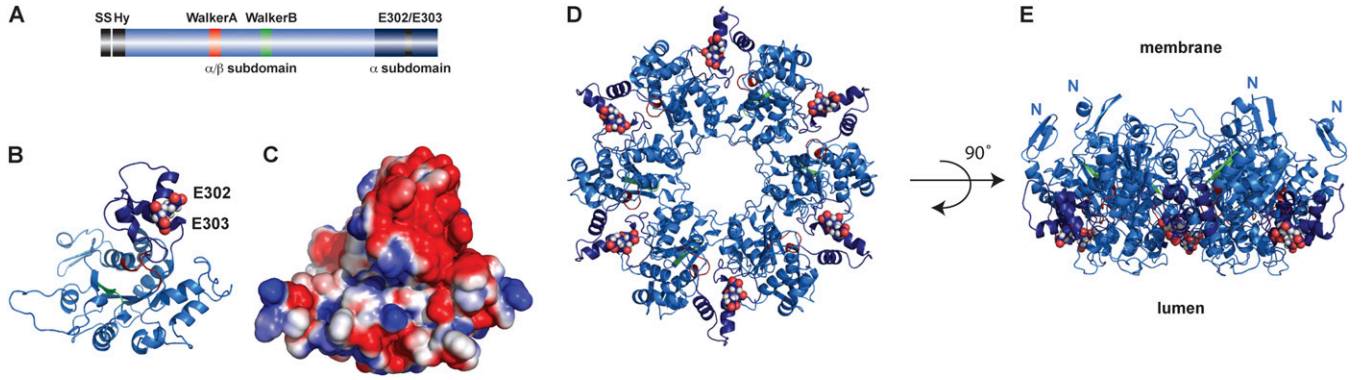

Fig. S1. A structural model for the TorsinA. (*A*) Domain organization of TorsinA. SS, cleavable signal sequence. Hy, hydrophobic domain. Note that the color code corresponds to the structure model depicted in *B*. (*B*) Cartoon representation of TorsinA. Glutamate residues implicated in primary dystonia are shown in space fill representation. (*C*) Electrostatic surface potential representation of TorsinA with kT/e ± 1. (*D*) A hexameric model of TorsinA. Note that the hydrophobic domain, which is only partially represented in this model (see main text), would be appended to the N termini (four of six are highlighted), which are facing upwards in the orientation shown in *E*.

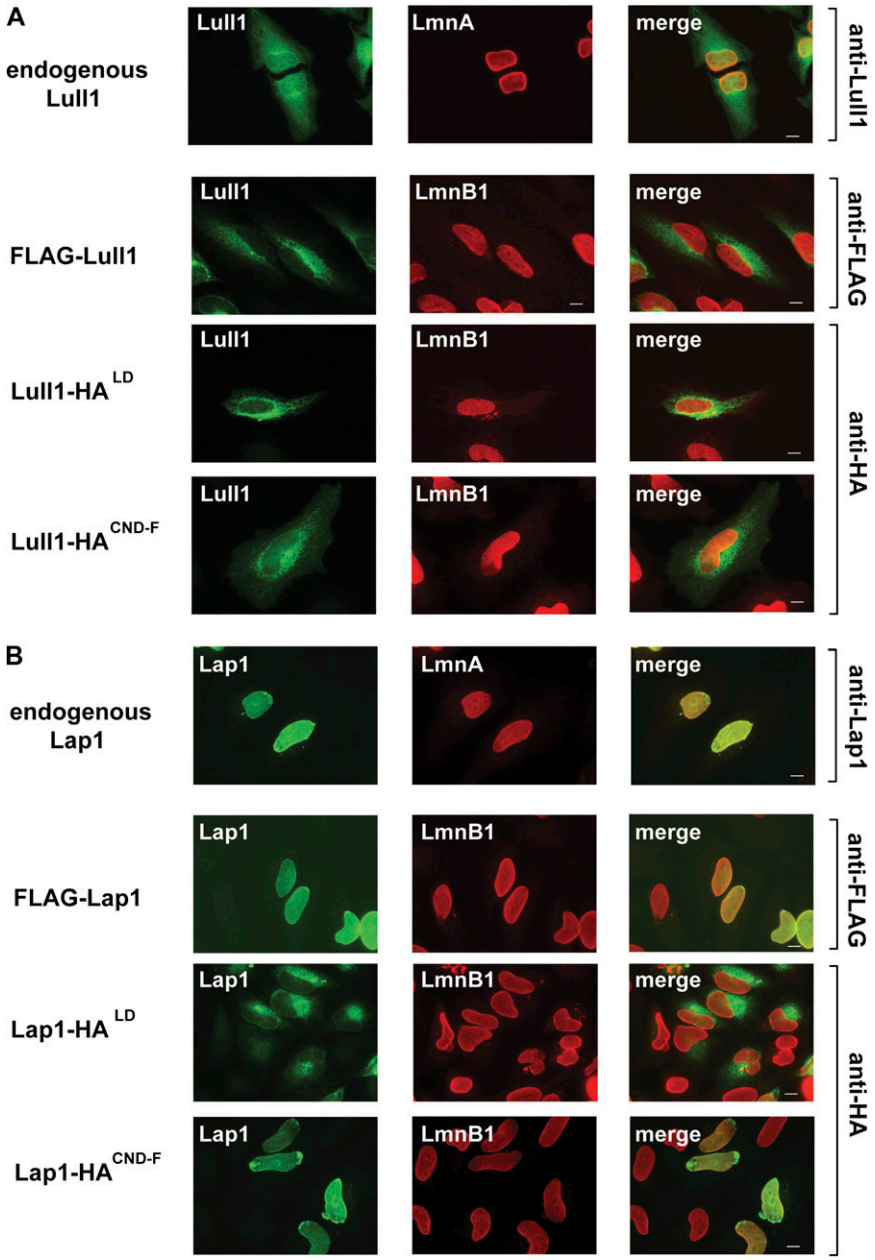

Fig. S2. LULL1^{LD} and LAP1^{LD} (LD, luminal domain) constructs localize to the endoplasmic reticulum (ER) lumen. (*A* and *B*) Subcellular localization of LAP1 (lamina-associated polypeptide) and LULL1 (which features a LAP1-like luminal domain). HeLa cells were transfected with a vector control or the indicated constructs depicted in Fig. 1 *C* and *D*. Twenty-four hours posttransfection, cells were processed for immunofluorescence microscopy using the indicated antibodies. Cells were costained against LaminA or LaminB1 to mark the nuclear envelope.

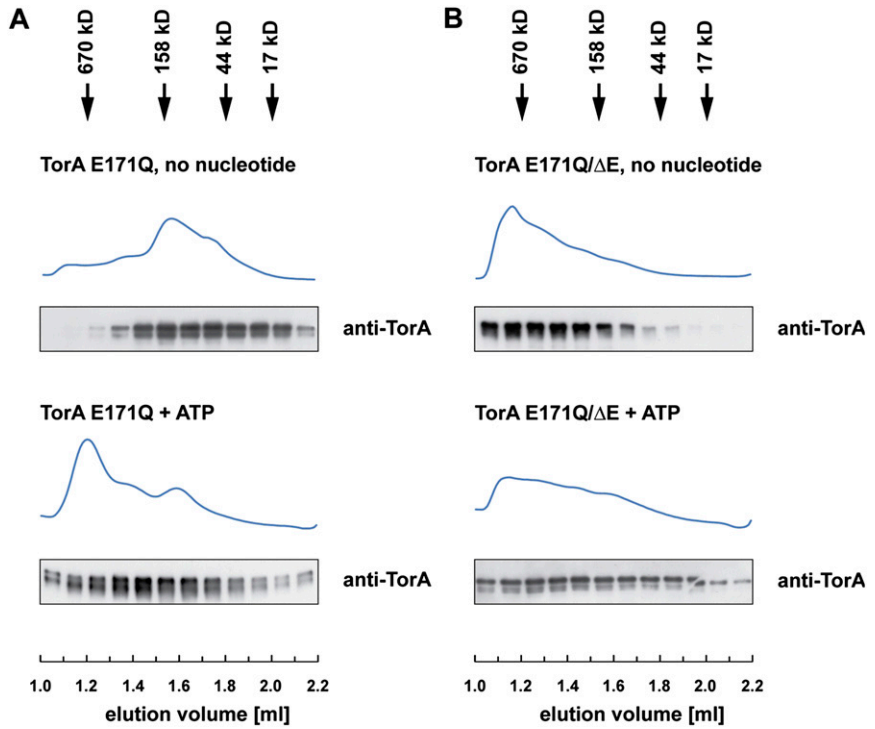

Fig. S3. Additional size-exclusion chromatography controls for Fig. 2. (*A*) Chromatogram of TorA E171Q in the absence (*Upper*) or presence (*Lower*) of ATP. TorA E171Q was incubated with 2 mM ATP and subjected to a Superdex 200 PC 3.2/30 column preequilibrated in 500 μM ATP. UV traces are shown in blue, elution positions of size markers are indicated by arrows on top. Elution fractions were subjected to immunoblotting using the indicated antibodies. (*B*) Chromatogram of TorA E171Q/ΔE in the absence (*Upper*) or presence (*Lower*) of ATP.

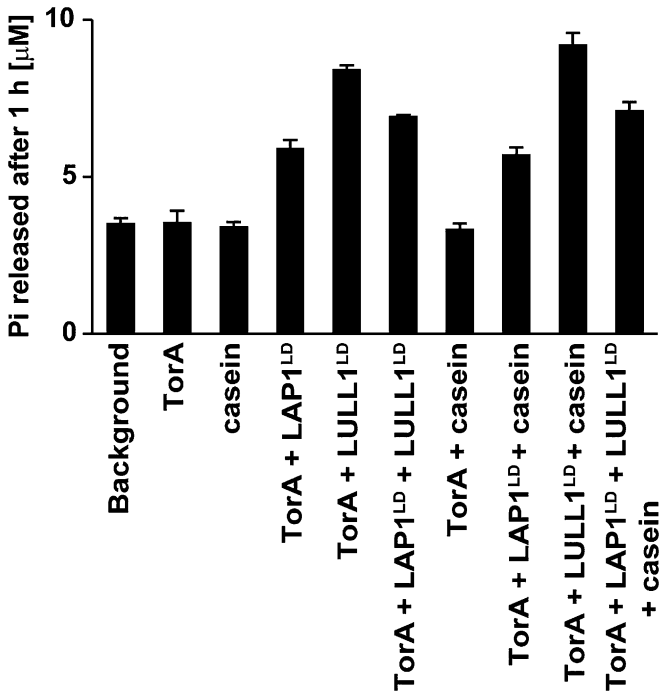

Fig. S4. TorA ATPase activity was not stimulated by α-casein. Proteoliposomes containing 3 μM TorA were incubated with 0.25 mg/mL (11 μM) α-casein, in the absence or presence of 3 μM $Lap1^{LD}$ or $Lull1^{LD}$ or mixture of $Lap1^{LD}$ and $Lull1^{LD}$ (each 1.5 μM) in the ATPase assay. For comparison, TorA ATPase activities in the absence or presence cofactors were included.