Supporting Information

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SI Materials and Methods

Plasmids and Reagents. The JTO sequence used in this paper is a full-length lambda light chain (LC) using the variable region sequence from the JTO protein (1), fused to the constant region of ALLC (2). The ALLC and JTO sequences were chemically synthesized and purified by Integrated DNA Technologies and supplied in the pIDTSMART vector flanked by a 5′ BamHI and 3′ EcoRI digestion site. ALLC and JTO were inserted into the pENTR1A vector (Invitrogen) using the aforementioned restriction sites, and then ALLC was shuttled into pDEST-14 (for bacterial expression), pTREx-DEST30, pcDNA-DEST40, or pLENTI4/TO vectors as appropriate, using LR clonase II (Invitrogen) recombination. ALLC and JTO were also cloned from the pENTR1A vector into a pCMV1 vector containing a signal sequence and FLAG tag (Sigma) by amplification with the following primers (ALLC: 5′-gaccacaagcttgccagttttatgctggctcagcc and 5′-agctgggtctagatatctcgagtgcg, JTO: 5′-tgctttaagcttaactttatgctgaaccagccgc and 5′-aataaagcggccgctatgaacattctgtaggggccactg) followed by digestion with HindIII and NotI. All constructs were sequenced to confirm their identity. ERSE-Firefly luciferase reporter was cloned into a vector suitable for mammalian cell selection by transferring ERSE-FLuc from ERSE.FLuc.pGL3 (3) into a promoterless pcDNA3.1 vector using Xba1 and Not1 restriction sites.

All compounds were dissolved in sterile dimethyl sulfoxide (DMSO). Thapsigargin (Tg) was obtained from A.G. Scientific. Doxycycline hydrochloride (Dox) and Trimethoprim (TMP) were obtained from Fischer Scientific. MG-132, Bortezomib, Chloroquine, and Eeyarestatin I were obtained from Sigma-Aldrich. GSK2606414 was obtained from Merck Millipore.

Cell Culture and Transfections. HEK293T-Rex (Invitrogen) cells were cultured in complete DMEM (CellGro), and Tet-On ARPE-19 cells (4) were cultured in high-glucose DMEM/F12 50:50 (Gibco) media, both supplemented with 10% FBS (CellGro) and penicillin/streptomycin (CellGro). Transient transfection of ALLC and JTO into HEK293^{DAX} cells was performed by calcium phosphate transfection. Lentiviruses encoding ALLC, ALLC-GLuc, and GLuc were transduced into HEK293T-Rex or ARPE-19 cells using 1–5 mL of virus in media containing 5 mg/mL polybrene. Stable cell lines were selected by culturing in blasticidin (10 μg/mL) and zeocin (50 mg/mL), before characterization. Creation and maintenance of HEK293^{DAX} cells has been described previously (5). HEK293T-Rex cells containing ER stress responsive element-firefly luciferase (ERSE-FLuc) reporter were created by transfection with ERSE.FLuc.pcDNA3.1 by calcium phosphate followed by culturing in geneticin sulfate (G-418, 500 μg/mL) before single colony selection. All cells were cultured under typical tissue culture conditions (37 °C, 5% CO_2).

Virus Production. Vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped lentiviral particles were produced by cotransfecting Lenti-X 293T cells (Clontech) with the structural plasmids necessary for virus production (Rev, RRE, and VSVG) along with the pLentiV5-Dest lentivirus construct. Lenti-X 293T cells were transfected using Fugene-6 (Roche) for 24 h, after which the media was removed and replaced with fresh media. Media containing viral particles was collected at 48 h and again at 72 h posttransfection. Viral particles were concentrated by centrifugation at 40,000 × g for 2 h at ambient temperature. The supernatant was removed, and the pellet containing viral particles was resuspended in Hank's buffered salt solution at 1/100th of the initial volume. Virus was then aliquoted and stored at −80 °C until use. To transduce ARPE-19 or HEK293-TRex cells, the cells were plated at ∼100,000 cells/well in a 12-well plate and infected for 48 h with 1–5 mL of virus in media containing 5 mg/mL polybrene.

Western Blot Analysis. Cells were lysed in 50 mM Tris buffer, pH 7.5 containing 0.1% TritonX (Fisher Scientific) and supplemented with protease inhibitor mixture (Roche). Protein lysate concentrations were normalized by Bradford assays (Bio-Rad). Lysates or media were boiled for 10 min in Laemmli buffer + 100 mM DTT before loading onto SDS-PAGE gel. Proteins were transferred from gel slabs to nitrocellulose, and the Odyssey Infrared Imaging System (Li-Cor Biosciences) was used to detect proteins of interest.

Immunoprecipitation. Cells were washed with PBS and then cross-linked with 0.5 mM Dithiobis(succinimidiyl propionate) (DSP) for 30 min at room temperature. The reaction was quenched by addition of 100 mM Tris pH 7.5, and then radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 0.5% deoxycholate) containing 10 mM $CaCl_2$ was added for cell lysis. Proteins were immunopurified using anti-FLAG M1 agarose beads (Sigma). Protein was eluted by boiling in Laemmli buffer + 100 mM DTT, and samples were separated by SDS-PAGE as previously described.

Antibodies. Blots were probed with the following primary antibodies: rabbit polyclonal anti-human lambda LC (1:1,000, Bethyl Laboratories A90-112A), mouse monoclonal anti-Grp78 (1:500, Santa Cruz Biotechnology sc-166490), rabbit polyclonal anti-Grp94 (1:1,000, GeneTex GTX103203) and mouse monoclonal anti β-actin (1:10,000, Sigma).

Luciferase Assays. ***GLuc assay.*** Conditioned media (50 μL) was added to a well of a flat-bottomed, black 96-well plate (Costar, Corning Inc.). A solution of 50 nL coelenterazine substrate in 10 μL neat GLuc assay buffer (BioLux Gaussia luciferase assay kit, New England Biolabs) was added to each well, and luminescence activity was measured in a Safire II microplate reader (Tecan).

FLuc reporter assay. HEK293T-Rex cells stably incorporating the ERSE-Firefly Luciferase Reporter were plated ∼20,000 cells per well in flat-bottomed, black 96-well assay plates (Costar, Corning Inc.) overnight before compound administration. Cells were treated with compounds (1−10 μM) for 18 h, and then the plates were equilibrated to room temperature and 50 μL of SteadyLite (PerkinElmer) was added to each well. Luminescence activity was measured in a Safire II microplate reader (Tecan) after 10 min of incubation.

High-throughput screening. ALLC-GLuc and GLuc Tet-ON ARPE19 cells were plated at 5,000 cells/well in flat-bottomed, white, 384-well plates (Costar 3570, Corning) in a total media volume of 20 μL (containing 1 μg/mL Dox to induce expression of the protein of interest). Approximately 6 h after seeding, cells were treated with compounds [10 μM final concentration in DMSO (0.5% final volume)] from the LOPAC dispensed via a Biomek FX pintool instrument (100 nL pintool) and then incubated at 37 °C/5% CO_2 for 24 h. GLuc substrate and buffer (10 nL substrate per well diluted with 2.2 μL of buffer/media) were dispensed directly into the 384-well plate via a BioRAPTR flying reagent dispenser (Beckman Coulter). Luminescence was measured immediately after reagent addition with an EnVision Multilabel Reader (PerkinElmer) using a 100-ms integration time.

Toxicity Assay. HEK293^{DAX} cells transfected with either GFP or ss.FT.ALLC.pCMV1 plasmids were seeded into 96-well plates. After 6 h, a 2× solution of vehicle (DMSO), Tg (500 nM), or Arsenite (100 μM) was added to the cells for 15 h or 24 h. Then 10× Resazurin was added directly to the wells (10 μL) and the plates were incubated for 1 h before measuring fluorescence (excitation 530, emission 590).

Quantitative RT-PCR. Cells were treated as described at 37 °C, harvested by trypsinization, and washed with Dulbecco's PBS (Gibco), and then RNA was extracted using the RNeasy Mini Kit (Qiagen). qPCR reactions were performed on cDNA prepared from 500 ng of total cellular RNA using the QuantiTect Reverse Transcription Kit (Qiagen). The FastStart Universal SYBR Green Master Mix (Roche), cDNA, and appropriate primers purchased from Integrated DNA Technologies (5) were used for amplifications (45 cycles of 2 min at 95 °C, 10 s at 95 °C, 30 s at 60 °C) in an ABI 7900HT Fast Real Time PCR machine. Primer integrity was assessed by a thermal melt to confirm homogeneity and the absence of primer dimers. Transcripts were normalized to the housekeeping gene Rplp2, and all measurements were performed in triplicate. Data were analyzed using the RQ Manager and DataAssist 2.0 softwares (ABI). qPCR data are reported as mean ± 95% confidence interval as calculated in DataAssist 2.0.

LC ELISA. The ELISA was performed in 96-well plates (Immulon 4HBX, Thermo Scientific). Wells were coated overnight at 37 °C with rabbit anti-human lambda LC polyclonal antibody (Bethyl Laboratories) at a 1:1,000 dilution in 50 mM sodium carbonate (pH 9.6). In between all incubation steps, the plates were rinsed extensively with Tris-buffered saline containing 0.05% Tween-20 (TBST). Plates were blocked with 5% nonfat dry milk in TBST for 1 h at 37 °C. ALLC or JTO containing analytes (lysate or conditioned media) were diluted between fivefold and 20-fold in 5% nonfat dry milk in TBST, and 100 μL of each sample was added to individual wells. LC standards ranging from 3 ng/mL to 1,000 ng/mL were prepared from purified human Bence Jones λ LC (Bethyl Laboratories). Plates were incubated at 37 °C for 2 h while shaking. Finally, HRP-conjugated goat anti-human lambda LC antibody (Bethyl Laboratories) was added at a 1:10,000 dilution in 5% nonfat dry milk in TBST, followed by a 2-h incubation of the plates at 37 °C. The detection was carried out with 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 0.18 mg/mL) and 0.03% hydrogen peroxide in 100 mM sodium citrate pH 4.0. Detection solution (100 μL) was added to each well, and the plates were incubated at room temperature. The absorbance was recorded at 405 nm, and the values for the LC standards were fitted to a four-parameter logistic function. LC concentrations were averaged from three independent replicates under each treatment and then normalized to vehicle conditions.

Protein Expression and Purification. LC proteins were expressed as inclusion bodies using a modified version of the protocol described by Rognoni et al. (6). BL21 (DE3) *Escherichia coli* transformed with LC plasmids were grown at 37 °C in ZYP-glucose media and induced overnight with 0.1 mM IPTG. Cells were harvested and lysed, and inclusion bodies were washed three times with phosphate buffered saline (PBS: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) containing 1% Triton-X and once in PBS. The inclusion bodies were dissolved in 6 M guanidine hydrochloride, 5 mM DTT. Insoluble material was removed by centrifugation, and then the protein was refolded by dropwise dilution into 50 mM Tris·Cl (pH 8.5 on ice) containing 5 mM reduced glutathione and 0.5 mM oxidized glutathione. After overnight refolding on ice with stirring, ammonium sulfate was added to 50% saturation and allowed to equilibrate. Insoluble material was removed by centrifugation, and the supernatant, which contained the soluble, folded protein, was filtered and loaded onto a phenyl Sepharose column (GE) equilibrated with 2 M ammonium sulfate. The protein was eluted in 50 mM Tris·Cl pH 8.5. The eluent was concentrated and then purified by size exclusion chromatography on a Superdex 75 column (GE). All LCs eluted at a volume consistent with a dimer of around 45 kDa. Protein purity was assessed by SDS-PAGE; when necessary, the proteins were further purified by anion exchange on a Source 15Q column (GE) at pH 8.5, eluting with a 0–0.5 M NaCl gradient. Protein solutions were filter sterilized and stored at 4 °C and dialyzed into appropriate buffers for further analysis. Protein concentrations are given in milligrams per milliliter, to avoid ambiguity between monomer and dimer concentrations.

Circular Dichroism Spectroscopy. Proteins were dialyzed into 50 mM sodium phosphate buffer, pH 7, and diluted to 0.2 mg/mL. CD spectra were recorded on an Aviv 420SF spectrapolarimeter at 25 °C with a resolution of 1 nm. Aliquots of the protein stocks were unfolded in 8 M urea, and their tryptophan fluorescence intensity (excitation 280 nm, emission 300–400 nm) used to normalize the CD spectra. This allows direct comparison of the secondary structure content of the proteins, and bypasses inaccuracies in determining their concentration by absorbance spectroscopy. Mean residue ellipticity $[\Theta]_{MRE}$ was calculated with the formula:

$$[\Theta]_{MRE} = \frac{\Theta_{obs} \times M_r/(n-1)}{c \times d}.$$

Θobs is the measured ellipticity in millidegrees, M_r is the molecular weight of the protein, n is its number of amino acids, c is its concentration in milligrams per milliliter, and d is the cuvette pathlength in millimeters.

Urea Denaturation Titrations. LC proteins were incubated overnight at 25 °C in 50 mM sodium phosphate buffer, pH 7, containing varying urea concentrations. Protein concentration was approximately 0.1 mg/mL. Intrinsic tryptophan fluorescence was measured on an Aviv ATF 105 spectrofluorimeter at 25 °C, using an excitation wavelength of 280 nm and recording emission spectra between 300 nm and 400 nm. Each LC monomer has three tryptophan residues, two of which are quenched in the native state by packing against the disulfide bond in each domain. To assess spectral changes as a function of urea concentration, we calculated the average wavelength, <λ> (7):

$$\langle\lambda\rangle = \frac{\sum_i \lambda_i \cdot I_i}{\sum_i I_i}$$

where λ and I are the wavelength and intensity at that wavelength, calculated across the emission spectrum. The data were fit to a two-state equilibrium unfolding equation (8):

$$\frac{(a+bx)e^{\frac{G-mx}{RT}} + (c+dx)}{1+e^{\frac{G-mx}{RT}}}$$

where x is the urea concentration (M), G is the free energy of unfolding (kJ/mol), m is the denaturant dependence of the unfolding reaction (kJ/mol/M), R is the ideal gas constant, T is the temperature in K, a and c are the fluorescence of the folded and unfolded states, and b and d are the denaturant dependence of the fluorescence of the folded and unfolded states, respectively. We were unable to quantitatively refold either LC protein from its urea-denatured state, as assessed by tryptophan fluorescence (Fig. S3*A*). This may be due to aggregation or misfolding of the dimer. Therefore, we assume that the protein is not truly at equilibrium, so the stabilities measured here should be treated with caution. We report only the unfolding midpoint urea concentration, C_m, calculated from the fit by $m \times G$.

Aggregation Kinetics. ALLC was filtered with a 0.22-μm syringe filter then serially diluted 1.2-fold from 1.5 mg/mL to 0.25 mg/mL concentration in PBS containing 0.5 M $MgSO_4$ (9). The protein solutions were aliquoted onto a 96-well microplate in triplicate, and the plate was sealed with Crystal Clear tape (Hampton). Aggregation was assessed by turbidity. The plate was incubated at 44 °C, and absorbance at 405 nm was measured in a Molecular Devices SpectraMax 250 plate reader (44 °C is the maximum temperature that the plate reader can maintain). Incubation at 37 °C or without $MgSO_4$ for up to 8 d did not produce aggregates. Aggregation t_{50} was determined from the individual absorbance traces by determining the average initial and final absorbances and then fitting to a double-exponential function and finding the root of the fit equation at the midpoint of the initial and final absorbance values. We were unable to fit the traces to a mechanistic model, which prevented a more detailed analysis of lag times or growth rates.

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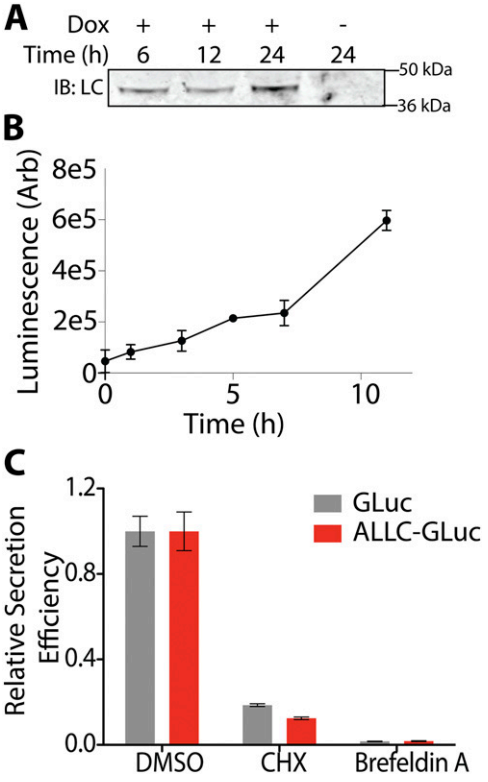

Fig. S1. ALLC-GLuc is efficiently secreted from mammalian cells. (*A*) Immunoblot of media conditioned on HEK293T-Rex cells transiently transfected with Doxycycline (Dox)-inducible ALLC-GLuc for the indicated time. Dox (1 μg/mL) was added for 24 h, and then the media was replaced with fresh media and secreted ALLC was measured by SDS-PAGE/immunoblot at the indicated time. (*B*) Plot showing the GLuc luminescence of media conditioned on HEK293T-Rex cells transiently transfected with Dox-inducible ALLC-GLuc for the indicated time. Dox (1 μg/mL) was added for 24 h, and then the media was replaced with fresh media and secreted ALLC was measured by GLuc luminescence assay at the times indicated. Error bars show SEM for $n = 3$. (*C*) Graph showing the secretion of ALLC-GLuc or GLuc from HEK293T-Rex cells transiently transfected with Dox-inducible ALLC-GLuc or Dox-inducible GLuc. Dox (1 μg/mL) was added to the cells for 15 h, and then the media was replaced by media containing vehicle (DMSO), cycloheximide (CHX, 5 μM), or Brefeldin A (5 μg/mL) as indicated. After 6 h incubation, secretions of ALLC-GLuc and GLuc were measured by GLuc luminescence assay, and values were normalized to vehicle control. Error bars represent SEM for biological replicates ($n = 3$).

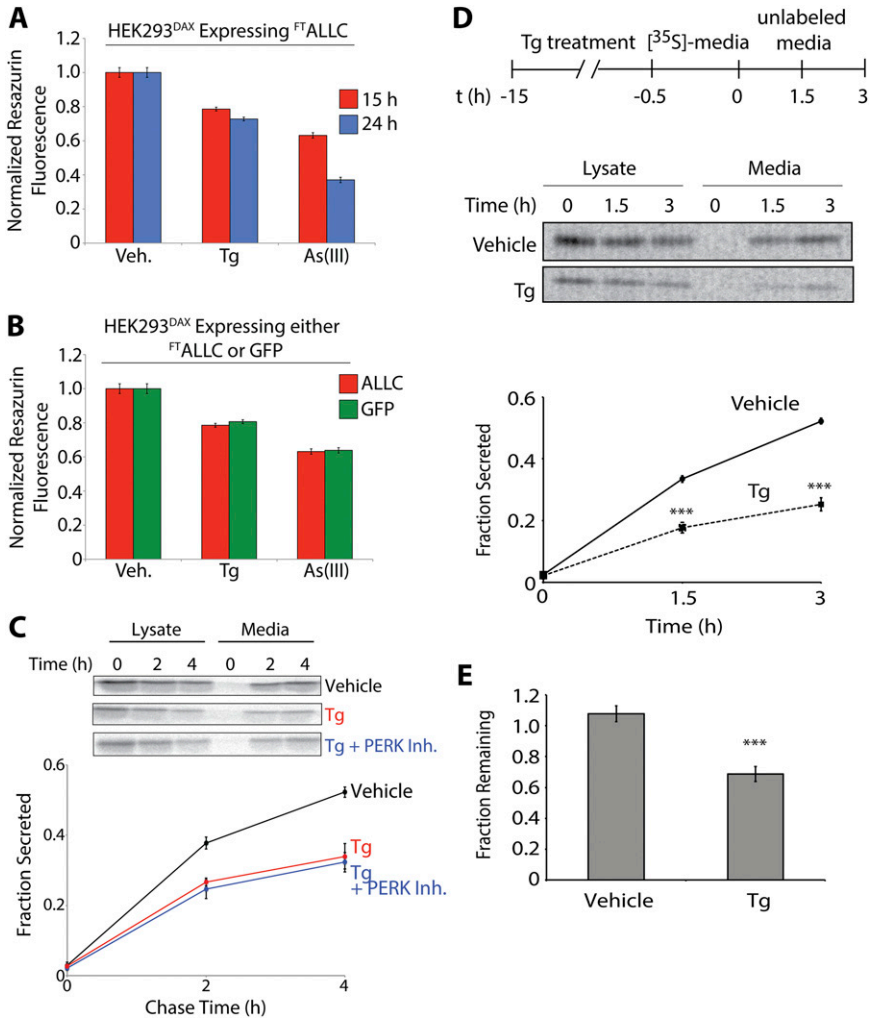

Fig. S2. Tg reduces the secretion and increases the degradation of destabilized ALLC. (*A*) Bar graph showing the viability of HEK293^{DAX} cells treated with thapsigargin (Tg; 500 nM) for 15 or 24 h measured by resazurin fluorescence. Highly toxic arsenite [As(III); 100 μM] was included as a control. These results demonstrate that the treatment of HEK293^{DAX} cells with Tg for 15–24 h does not dramatically reduce the viability of these cells. Error bars show SEM for biologic replicates ($n = 3$). (*B*) Bar graph showing the viability of HEK293^{DAX} cells expressing ^{FT}ALLC or GFP (as a control) treated with thapsigargin (Tg; 500 nM) for 15 (red) or 24 (blue) h measured by resazurin fluorescence. Highly toxic arsenite (As(III); 100 μM) was included as a control. These results demonstrate that the overexpression of ^{FT}ALLC does not significantly increase the sensitivity of HEK293^{DAX} cells to Tg-induced ER stress. Error bars show SEM for biologic replicates ($n = 3$). (*C*) Autoradiogram and graph depicting the fraction secreted of [^{35}S]-labeled ^{FT}ALLC immunopurified with M1 FLAG antibody from media and lysates collected from transfected HEK293^{DAX} cells following a 1-h pretreatment with GSK2606414 (30 nM) and then a 15-h preactivation with Tg (500 nM). GSK2606414 was added during the Tg incubation as well. Fraction secreted was calculated by normalizing the [^{35}S] signal in the media at each time point to the total amount of labeling at t = 0 as described in *Materials and Methods*. Error bars represent SEM from biological replicates ($n = 2$). (*D*) Autoradiogram and graph depicting the fraction secreted of [^{35}S]-labeled ALLC immunopurified with anti-human lambda LC antibody from media and lysates collected from transfected HEK293^{DAX} cells following a 15-h preactivation with Tg (500 nM). The labeling protocol is shown. Fraction secreted was calculated by normalizing the [^{35}S] signal in the media at each time point to the total amount of labeling at t = 0 as described in *Materials and Methods*. Error bars represent SEM from biological replicates ($n = 6$). ***$P < 0.005$. (*E*) Graph depicting total [^{35}S]-labeled ALLC remaining at 3 h in HEK293T-Rex cells following a 15-h pretreatment with 500 nM Tg ($n = 6$). The fraction remaining was calculated as described in *Materials and Methods*. ***$P < 0.005$.

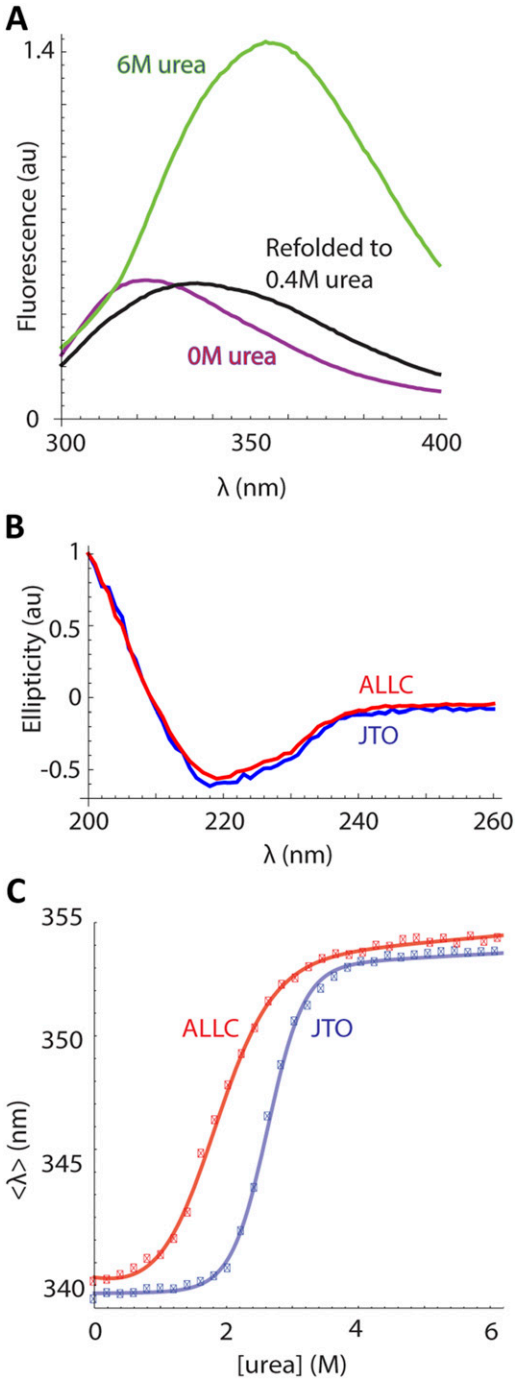

Fig. S3. Stability of recombinant ALLC and JTO. (*A*) Intrinsic tryptophan fluorescence spectra of recombinant ALLC in native (purple trace) and denaturing (green trace, 6 M urea) conditions showing the characteristic fluorescence quenching by the disulfide bonds in each domain. Protein refolded from 6 M urea by dilution to 0.4 M urea under nonoxidizing conditions (black trace) did not regain a native-like fluorescence spectrum, demonstrating that recombinant ALLC unfolding is not reversible. (*B*) Circular dichroism spectra of ALLC (red) and JTO (blue) show characteristic and indistinguishable β-sheet structures. (*C*) Urea titrations of ALLC (red) and JTO (blue) show that JTO has a higher apparent stability than ALLC. Spectroscopic data were measured in 50 mM sodium phosphate buffer and urea concentrations ranging from 0 M to 6 M at pH 7, 25 °C.

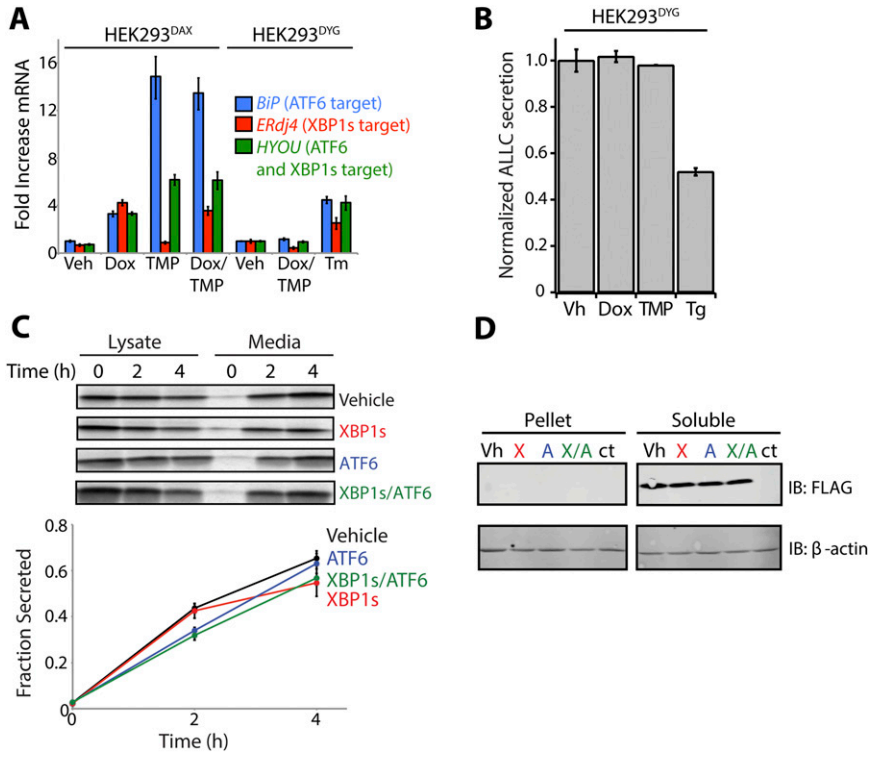

Fig. S4. Stress-independent activation of XBP1s and/or ATF6 in HEK293^{DAX} cells does not significantly influence secretion of the stable LC JTO. (*A*) qPCR analysis of XBP1s and ATF6 target genes in HEK293^{DAX} and HEK293^{DYG} cells following a 15-h treatment with Dox (1 μg/mL), TMP (10 μM), or the ER stressor tunicamycin (Tm, 1 μg/mL). Data are shown normalized to vehicle-treated HEK293^{DYG} cells. Error bars show ±95% confidence interval for $n = 3$. HEK293^{DAX} cells stably express DHFR-ATF6 and Dox-inducible XBP1s (5). HEK293^{DYG} cells stably express DHFR-YFP and Dox-inducible GFP (5). Thus, we only observe the selective induction of XBP1s and/or ATF6 target genes in HEK293^{DAX} cells treated with the appropriate activating ligand, clearly indicating our ability to sensitively activate the XBP1s and/or ATF6 transcriptional programs in HEK293^{DAX} cells. (*B*) ALLC levels by ELISA in HEK293^{DYG} cells expressing ^{FT}ALLC following a 15-h treatment with Dox (1 μg/mL), TMP (10 μM), or thapsigargin (Tg, 500 nM). Error bars represent SEM from biological replicates ($n = 3$). These results show that the reduction in ^{FT}ALLC secretion afforded by stress-independent XBP1s and/or ATF6 activation in HEK293^{DAX} cells requires the activity of these UPR-associated transcription factors. (*C*) Representative autoradiogram and quantification of [^{35}S]-labeled ^{FT}JTO immunopurified from media and lysates collected from transfected HEK293^{DAX} cells following a 15-h preactivation of XBP1s (Dox; 1 μg/mL), ATF6 (TMP; 10 μM), or both. Fraction secreted was calculated as described in *Materials and Methods*. Error bars represent SEM from biological replicates ($n = 3$). (*D*) Immunoblots measuring soluble and insoluble (pellet) levels of ^{FT}ALLC or untagged ALLC (Ct) following a 15-h preactivation of XBP1s (X; Dox; 1 μg/mL), ATF6 (D; TMP; 10 μM), or both (X/A). Lysates and pellets were harvested from cells and the protein from the pellets extracted in 8 M urea before SDS-PAGE and immunoblotting.

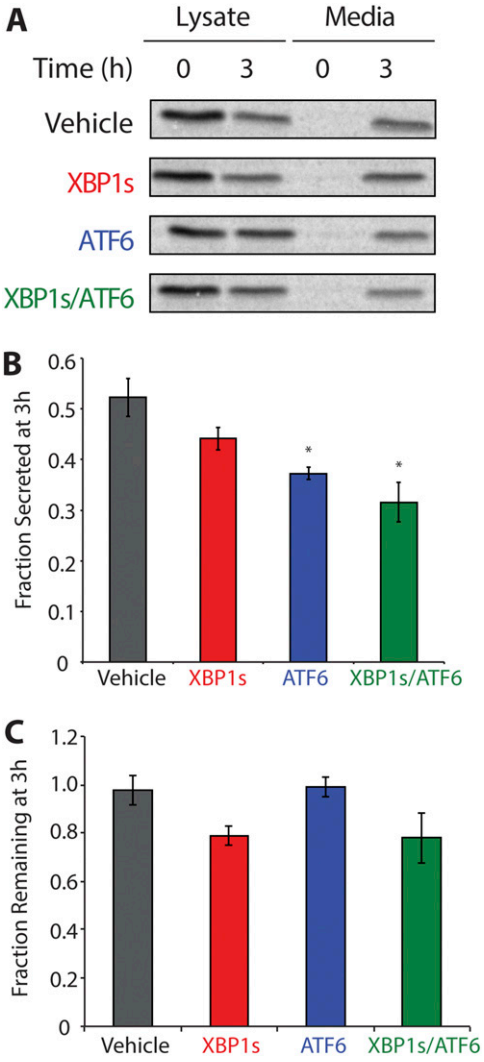

Fig. S5. XBP1s and ATF6 similarly affect the secretion and degradation of untagged ALLC. (*A*) Autoradiogram of [^{35}S]-labeled ALLC immunopurified from media and lysates collected from transfected HEK293^{DAX} cells following a 15-h preactivation of XBP1s (Dox; 1 μg/mL), ATF6 (TMP; 10 μM), or both. (*B*) Graph depicting the fraction secreted after 3 h of chase in *A*. Fraction secreted was calculated as described in *Materials and Methods*. *$P < 0.05$. (*C*) Graph depicting total [^{35}S]-labeled ^{FT}ALLC remaining at 3 h in *A*. The fraction remaining was calculated as described in *Materials and Methods*. All error bars represent SEM from biological replicates ($n = 3$).

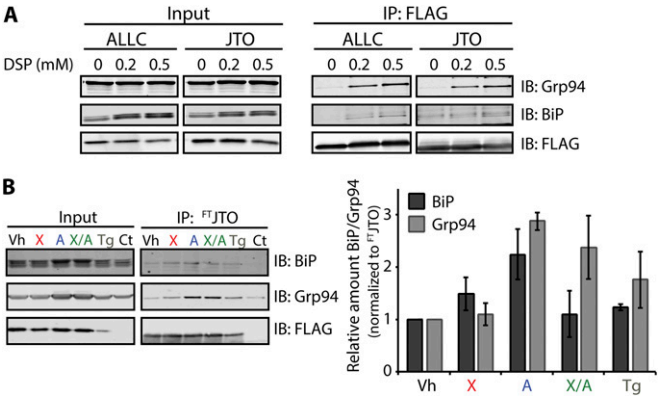

Fig. S6. Cross-linking allows for the quantification of intracellular interactions between LC proteins and ER chaperones. (*A*) Immunoblots of SDS-PAGE gels showing the inputs and FLAG immunopurifications of ^{FT}ALLC and ^{FT}JTO from HEK293^{DAX} cells treated in situ with increasing concentrations of the reversible cross-linker DSP [dithiobis(succinimidiyl propionate)]. The cross-linking and FLAG immunopurifications were performed as described in *SI Materials and Methods* and washed with high-detergent RIPA buffer. Note that the ER chaperones BiP and GRP94 are only recovered in our FLAG immunopurifications performed on lysates prepared from DSP-cross-linked cells, demonstrating that our cross-linking protocol allows for the recovery of intracellular interactions between LCs and ER chaperones without the complications arising from postlysis artifacts. (*B*) Immunoblot and quantification for FLAG immunopurifications of ^{FT}JTO isolated from HEK293^{DAX} cells following a 15-h treatment with Tg (500 nM) or preactivation of XBP1s (X), ATF6 (A), or both (X/A). The bar graph shows the recovery of BiP and GRP94 from these ^{FT}JTO immunopurifications quantified by comparing the signal for each of these ER chaperones under various conditions to vehicle and normalizing to the recovered ^{FT}JTO. HEK293^{DAX} cells expressing untagged ALLC was used as a control (Ct). All error bars represent SEM from biological replicates (n = 3).

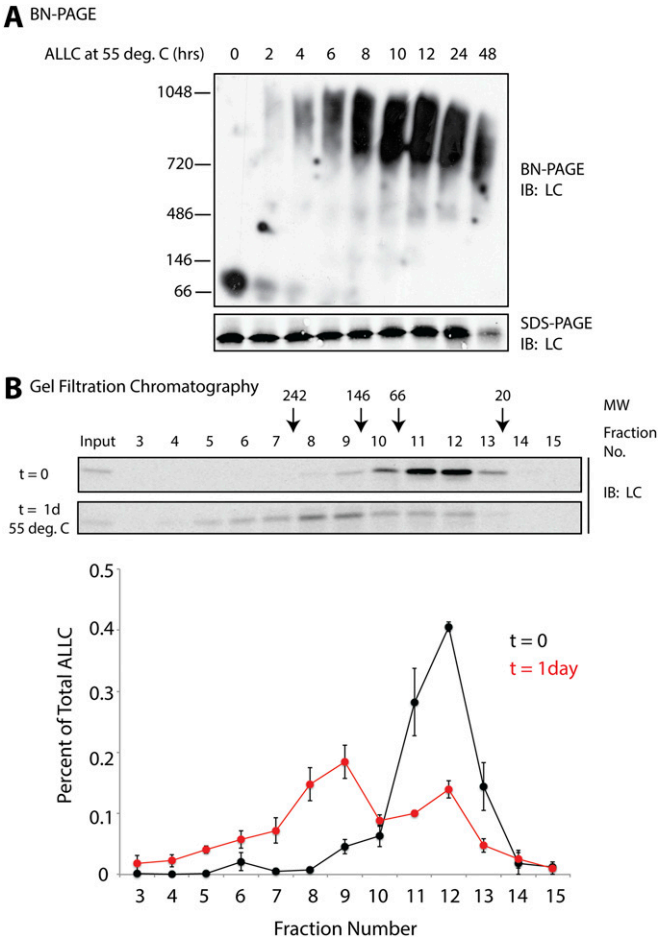

Fig. S7. ALLC aggregates into soluble oligomers in conditioned media upon heating. (*A*) Immunoblots of BN-PAGE and SDS-PAGE gels from media conditioned on HEK293 cells expressing ^{FT}ALLC following incubation at 55 °C for the indicated time. (*B*) Immunoblot and quantification from gel filtration fractions of media conditioned on HEK293 cells expressing ^{FT}ALLC following 24-h incubation at 55 °C. Fractions (1 mL) were collected and analyzed by SDS-PAGE and immunoblot for LC relative to MW standards. Quantification of the immunoblots is shown below. Percent ALLC protein represents the fraction ALLC detected in each fraction relative to the total ALLC detected in each run. Error bars represent SEM from biological replicates (n = 3).

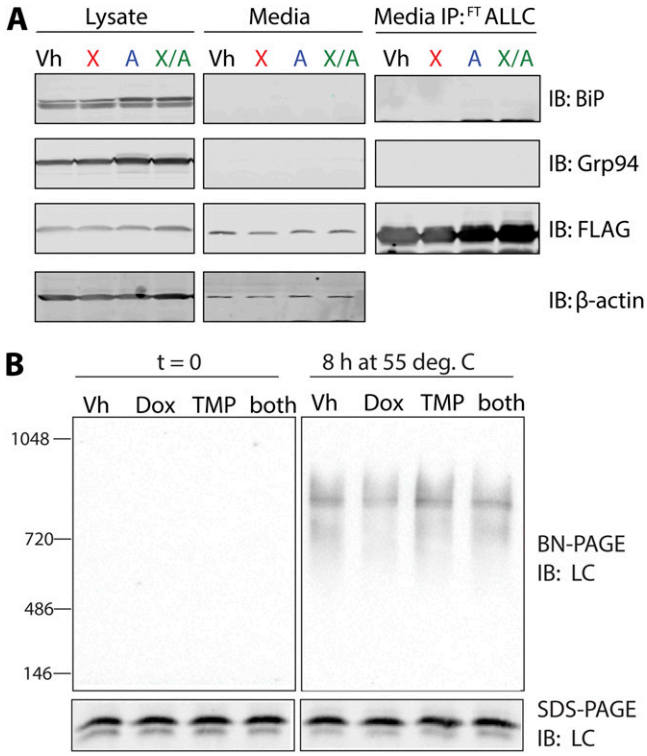

Fig. S8. Doxycycline and TMP do not affect ^{FT}ALLC aggregation in media conditioned on HEK293^{DYG} cells. (*A*) Immunoblot of lysates, media, and FLAG immunopurifications of the media prepared from HEK293^{DAX} cells expressing ^{FT}ALLC following a 15-h preactivation of XBP1s (X; Dox, 1μg/mL), ATF6 (A; TMP, 10 μM), or both XBP1s and ATF6 (X/A). We do not recover the ER chaperones BiP or GRP94 in the FLAG immunopurifications, demonstrating that the reduced aggregation of secreted ^{FT}ALLC cannot be attributed to the activity of increased extracellular populations of these ER chaperones. (*B*) Immunoblots of BN-PAGE and SDS-PAGE gels of media conditioned on HEK293^{DYG} cells transiently expressing ^{FT}ALLC following a 16-h treatment with Dox (1 μg/mL), TMP (10 μM), or both. After 16-h incubation of the media with ALLC-expressing cells, the media was incubated at 55 °C for 8 h, and soluble aggregates were measured by BN-PAGE.