Unfolded Protein Response-Induced ERdj3 Secretion Links ER Stress to Extracellular Proteostasis

Joseph C. Genereux, Song Qu, Minghai Zhou, Lisa M. Ryno, Shiyu Wang, Matthew D. Shoulders, Randal J. Kaufman, Corinne I. Lasmézas, Jeffery W. Kelly, and R. Luke Wiseman

SUPPLEMENTAL FIGURE LEGENDS

Figure S1 (Supplement to Figure 1).

A. Representative immunoblot of media and lysates collected from HEK293T cells overexpressing ERdj3^{WT} and treated with Brefeldin A (BFA, 100 ng/ml), as indicated. Cells were pretreated with BFA for 1 h, followed by 6 h conditioning of fresh media containing BFA.

B. Quantification of the fold change of ERdj3 in lysates and media collected from HEK293T cells overexpressing ERdj3^{WT} and treated with the indicated concentration of BFA in fresh media as in **Figure S1A**. ** indicates a p-value < 0.01; (n = 3), *** indicates a p-value < 0.001; (n = 6).

C. Immunoblot of media and lysates collected from Huh7, HepG2, SH-SY5Y, HEK293T, and HeLa cells as indicated. In each case, 10^5 cells were seeded and allowed to attach overnight. Cells were then incubated in fresh media for 24 h prior to collection.

D. Immunoblot of media and lysates collected from Huh7 cells treated with the indicated concentration of Tg in fresh media for 16 h.

E. Immunoblot of sera collected from strain 129 mice with recombinant ERdj3 standard. The Ponceau S-stained blot is included as a loading control. The difference in mobility between ^{R}ERdj3 and endogenous murine ERdj3 is due to glycosylation of the latter.

F. Representative immunoblot of sera collected from mice fed a high fructose diet (60%) for the indicated period of time.

G. Quantification of immunoblots as described in **Figure S1F**. *indicates a p-value < 0.05; n = 3 for each time-point.

H. Immunoblot of lysates of hepatic cells isolated from mice fed either a control or high fructose diet for the indicated period of time.

Figure S2 (Supplement to Figure 2)

A. qPCR analysis of *ERdj3* in HEK293^{DAX} cells following XBP1s (induced by 1 μg/mL doxycycline for 6 h), ATF6 (activated by 10 μM TMP for 6 h), or XBP1s/ATF6 activation. The increased expression following Tg treatment (1 μg/mL, 6 h) is shown as a control. Error bars represent the mean ± 95% confidence interval as calculated in DataAssist 2.0 (n = 3).

B. Total synthesized ERdj3 in HEK293^{DAX} cells following XBP1s, ATF6, or XBP1s/ATF6 activation as determined by [^{35}S] incorporation quantified from autoradiograms as shown in **Figure 2B**. Total synthesized ERdj3 at t = 0 h was normalized to the amount of [^{35}S] labeled ERdj3 in vehicle-treated controls. *indicates a p-value < 0.05; n = 3.

C. Immunoblot of lysates and media collected from HEK293^{DAX} cells treated with TMP (10 μM to activate ATF6) and/or doxycycline (1 mg/mL to activate XBP1s) as indicated for 48 h. *indicates a non-specific band detected by the HYOU1 antibody.

D. Immunoblot of lysates and media collected from HEK293^{DYG} cells, a clonal control line allowing regulation of DHFR.YFP and GFP in an analogous manner to the HEK293^{DAX} cell line (Shoulders et al, 2013), following 16 h of activation of GFP (by 1 μg/mL doxycycline) and/or DHFR.YFP (by 10 μM TMP), as indicated.

E. Immunoblot of ERdj3 in lysates and media collected from HepG2 cells following transduction with adenovirus carrying GFP, XBP1s, or ATF6 overexpression constructs, as indicated.

Figure S3 (Supplement to Figure 3)

A. qPCR of the UPR target genes *ERdj4* and *BiP* from HEK293T-Rex cells stably expressing tet-inducible ERdj3^{WT} or ERdj3^{KDEL}. Cell lines were pretreated with doxycycline (1 μg/mL, 16 h) to induce expression of tet-inducible ERdj3. Fresh media with doxycycline and Tg (1 μM, 6 h) was then added as indicated. Error bars represent the mean ± 95% confidence interval as calculated in DataAssist 2.0 (n = 3). A schematic showing the experimental protocol is shown above.

B. Bar graph quantifying cellular metabolic activity, as measured by resazurin fluorescence, of HEK293T-Rex cells stably expressing tet-inducible ERdj3^{WT} or ERdj3^{KDEL} following a 13 h Tg treatment at the indicated concentration. Cells were pre-treated with doxycycline (1 μg/mL, 5 h) to induce expression of tet-inducible ERdj3. Error bars represent s.e.m. (n = 4). A schematic showing the experimental protocol is shown above.

C. Far UV-vis circular dichroism spectrum of ^{R}ERdj3 at 8 μM in 50 mM K_2HPO_4/KH_2PO_4, pH 7.5, 300 mM KCl, 10 mM $MgCl_2$, 10% glycerol.

D. Quantification of ATP consumed by ^{R}BiP and ^{R}ERdj3 separately and in combination. ^{R}ERdj3 promotes a 1.7-fold increase in ATP consumption by ^{R}BiP, consistent with the reported value for ERdj3-dependent stimulation of BiP ATPase activity of 1.8-fold (Shen & Hendershot, 2005). Error bars show s.e.m. (n=3).

E. Representative immunoblot of ^{R}ERdj3 affinity purified with quenched resin (QS), or native (N) or GdnHCl/DTT-denatured (D) RNAse A covalently coupled to sepharose resin, indicating ^{R}ERdj3 selectively binds denatured proteins.

F. Plot showing the time-dependent increase in ThioT fluorescence of $A\beta_{1-40}$ incubated (10 μM, 37 ^{O}C, pH 7.2) with regular agitation in the presence of native or denatured ^{R}ERdj3 (0.37 μM). ^{R}ERdj3 was denatured by boiling in the presence of 100 mM DTT for 5 min, as indicated. All traces represent the average of three replicates.

Figure S4 (Supplement to Figure 4)

A. Representative immunoblot of conditioned media collected from HEK293T-Rex cells overexpressing ERdj3^{WT}, non-silencing shRNA (NS) or *ERdj3* shRNA. These conditioned media were used for the experiments described in **Figure 4A-C** and **Figure S4B,C**.

B. Representative images under 136X magnification of PK1 cells incubated with control conditioned media collected from HEK293T-Rex cells expressing either non-silencing shRNA (NS) or shRNA directed against *ERdj3* in the presence or absence of TPrP (600 ng/mL), as shown in **Figure 4A**. Insets show 544X magnification of a typical region of the image.

C. Quantification of vacuole formation from images as shown in **Figure S4B**. Equal area images comprising ~1200 cells per well were counted for each sample. Counting of cells and of cells with identifiable vacuoles was done using the Fiji image processing package (Schindelin et al, 2012). ** indicates a p-value < 0.01 (n = 2).

Figure S5 (Supplement to Figure 5)

A. Immunoblot of M2 anti-FLAG immunopurifications from media conditioned for 24 h on HEK293T cells overexpressing ^{FT}TTR^{WT}, ^{FT}TTR^{A25T}, and/or ERdj3^{WT}, either as a co-incubation or co-expression as

described in **Figure 5A**. In these experiments, beads were washed in a less stringent 1% Triton X100, 50 mM Tris pH 7.5, 150 mM NaCl buffer prior to elution.

B. Representative immunoblot of M2 anti-FLAG immunopurifications from media conditioned for 24 h on SH-SY5Y cells overexpressing $^{FT}TTR^{A25T}$ or $^{FT}TTR^{WT}$ as indicated. Media inputs (1:300) are shown as a control.

C. Quantification of ERdj3 in M2 anti-FLAG immunopurifications from media conditioned for 24 h on SH-SY5Y cells overexpressing $^{FT}TTR^{A25T}$ or $^{FT}TTR^{WT}$. ERdj3 levels were normalized to the mock transfection condition. ** indicates a p-value < 0.01 (n = 3).

D. Immunoblot of M2 anti-FLAG immunopurifications from media conditioned for 24 h on HepG2 cells overexpressing $^{FT}TTR^{A25T}$ or $^{FT}TTR^{WT}$ as indicated. Media input (1:400) are shown as a control.

E. Immunoblot of M1 anti-FLAG immunopurification from lysates harvested from HEK293T cells overexpressing $^{FT}TTR^{D18G}$ and ERdj3. Cells were treated with the cell-permeable, reversible crosslinker DSP (1 mM) in PBS for 30 min at ambient temperature, quenched for 15 min with 100 mM Tris pH 8.0 prior to lysis to immortalize transient substrate-chaperone interactions. Lysate inputs (1:100) are shown as a control.

F. Immunoblot of M2 anti-FLAG immunopurifications from the conditioned media collected from HEK293T cells overexpressing $^{FT}TTR^{WT}$, $^{FT}TTR^{A25T}$, and CLU^{3xHA} as indicated, either as a co-incubation or co-expression, in a similar manner as shown in **Figure 5A**. Beads were washed in RIPA buffer prior to elution. Media inputs (1:450) are shown as a control.

G. Immunoblot of M2 anti-FLAG immunopurifications from the conditioned media collected from HEK293T cells co-expressing $^{FT}TTR^{D18G}$, with Mock (GFP), CLU^{3xHA} or ERdj3 as indicated. Beads were washed in RIPA buffer prior to elution. Media inputs (1:450) are shown as a control.

H. qPCR analysis of *TTR* in HEK293T cells overexpressing $^{FT}TTR^{D18G}$ with Mock (GFP), CLU^{3xHA} or ERdj3 as in **Figure S5G**. Error bars represent the mean ± 95% confidence interval as calculated in DataAssist 2.0 (n = 3).

Figure S6 (supplement to Figure 6)

A. Representative immunoblot of M2 anti-FLAG immunopurifications from media conditioned for 24 h on HEK293T-Rex cells overexpressing $^{FT}TTR^{A25T}$ and treated with Tg at the indicated concentrations.

B. Bar graph depicting the quantified ratio of ERdj3 to ^{FT}TTR in FLAG immunopurifications with Tg treatment, as in **Figure S6A**, normalized to the ERdj3/TTR ratio for the vehicle-treated condition. Error bars represent standard error from biological replicates. * indicates a p-value < 0.05 (n = 3).

C. Immunoblot of lysate inputs (1:200), conditioned media inputs (1:2000) and M1 anti-FLAG immunopurification of media or lysates from HEK293T cells co-overexpressing $^{FT}TTR^{A25T}$ and/or $ERdj3^{WT}$, as indicated. Media was conditioned for 24 h prior to harvesting.

D. Representative immunoblot of M2 anti-FLAG immunopurification of media conditioned for 16 h on HEK293T cells co-overexpressing $^{FT}TTR^{A25T}$ and CLU^{3xHA}, with mock (GFP) or BiP, as indicated. Media inputs (1:450) are shown as a control. Lysates are also shown to confirm BiP overexpression.

E. Bar graph depicting the quantified ratio of CLU^{3xHA} to ^{FT}TTR in FLAG immunopurifications with BiP overexpression, as in **Figure S6C**, normalized to the CLU/TTR ratio for the mock-transfection condition. Error bars represent standard error from biological replicates (n = 3).

FIGURE S1

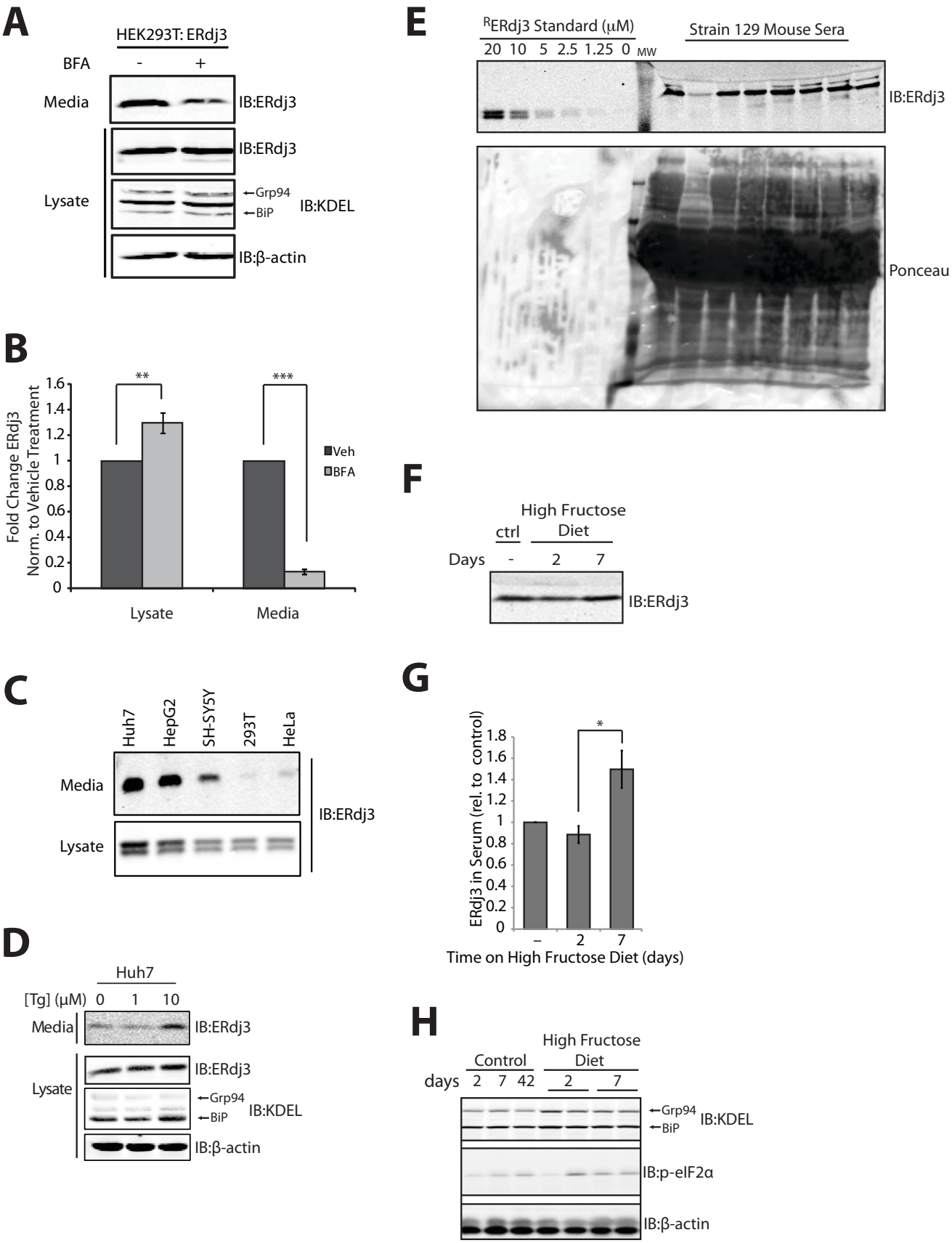

A
HEK293T: ERdj3
BFA - +
Media
IB:ERdj3
IB:ERdj3
Lysate
←Grp94
←BiP
IB:KDEL
IB:β-actin
B
Fold Change ERdj3 Norm. to Vehicle Treatment
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
***
Veh
BFA
Lysate
Media
C
Huh7
HepG2
SH-SY5Y
293T
HeLa
Media
Lysate
IB:ERdj3
D
Huh7
[Tg] (μM) 0 1 10
Media
IB:ERdj3
Lysate
IB:ERdj3
←Grp94
←BiP
IB:KDEL
IB:β-actin
E
RERdj3 Standard (μM)
20 10 5 2.5 1.25 0 MW
Strain 129 Mouse Sera
IB:ERdj3
Ponceau
F
High Fructose
ctrl Diet
Days - 2 7
IB:ERdj3
G
ERdj3 in Serum (rel. to control)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
–
2
7
Time on High Fructose Diet (days)
H
High Fructose
Control Diet
days 2 7 42 2 7
←Grp94
←BiP
IB:KDEL
IB:p-eIF2α
IB:β-actin

FIGURE S2

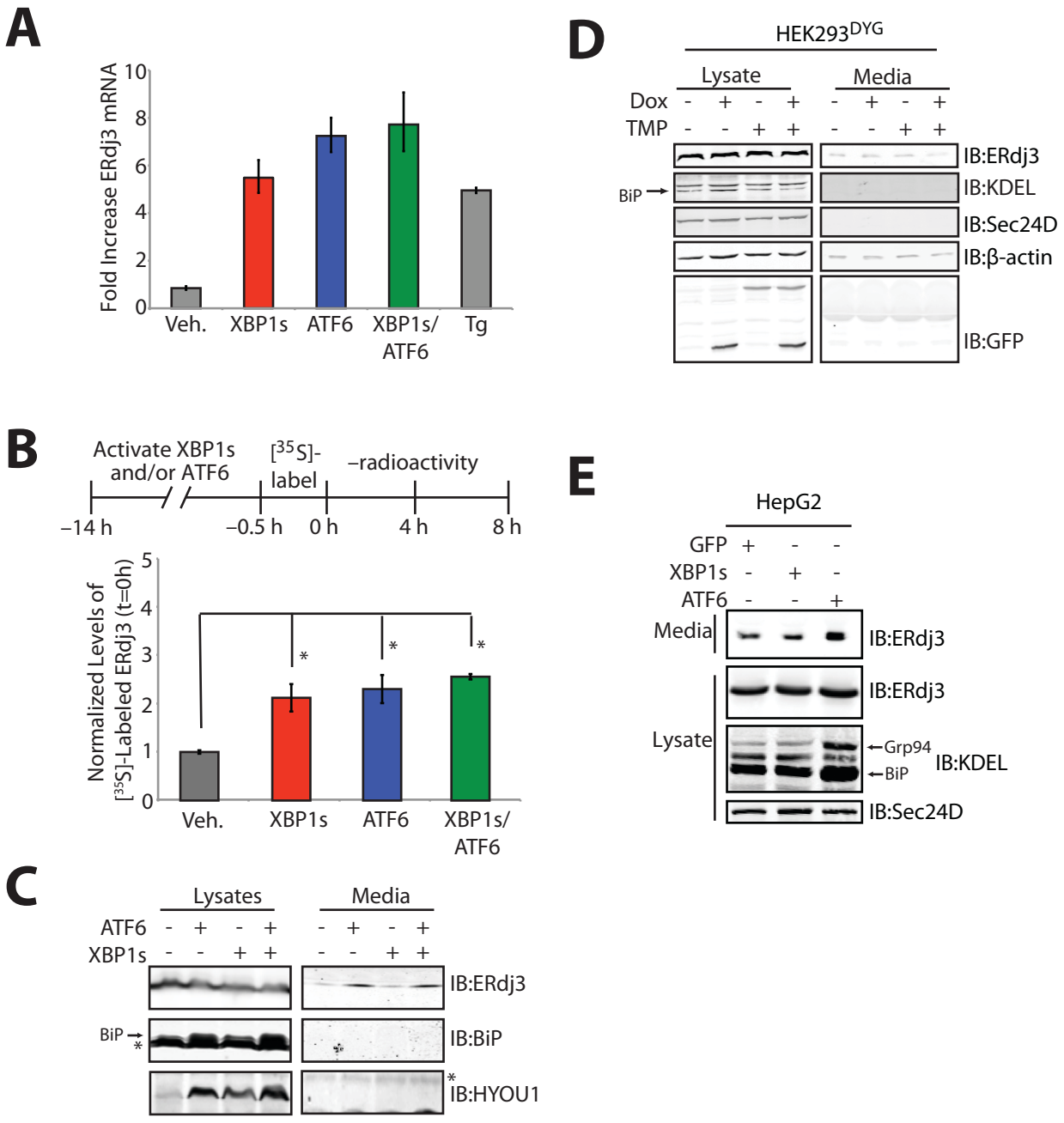

A
Fold Increase ERdj3 mRNA
10
8
6
4
2
0
Veh.
XBP1s
ATF6
XBP1s/ ATF6
Tg
B
Activate XBP1s and/or ATF6
[35S]- label
–radioactivity
–14 h
–0.5 h
0 h
4 h
8 h
Normalized Levels of [35S]-Labeled ERdj3 (t=0h)
5
4
3
2
1
0
*
*
*
Veh.
XBP1s
ATF6
XBP1s/ ATF6
C
Lysates
Media
ATF6 - + - + - + - +
XBP1s - - + + - - + +
IB:ERdj3
BiP
*
IB:BiP
*
IB:HYOU1
D
HEK293DYG
Lysate
Media
Dox - + - + - + - +
TMP - - + + - - + +
IB:ERdj3
BiP
IB:KDEL
IB:Sec24D
IB:β-actin
IB:GFP
E
HepG2
GFP + - -
XBP1s - + -
ATF6 - - +
Media
IB:ERdj3
IB:ERdj3
Lysate
Grp94
BiP
IB:KDEL
IB:Sec24D

FIGURE S3

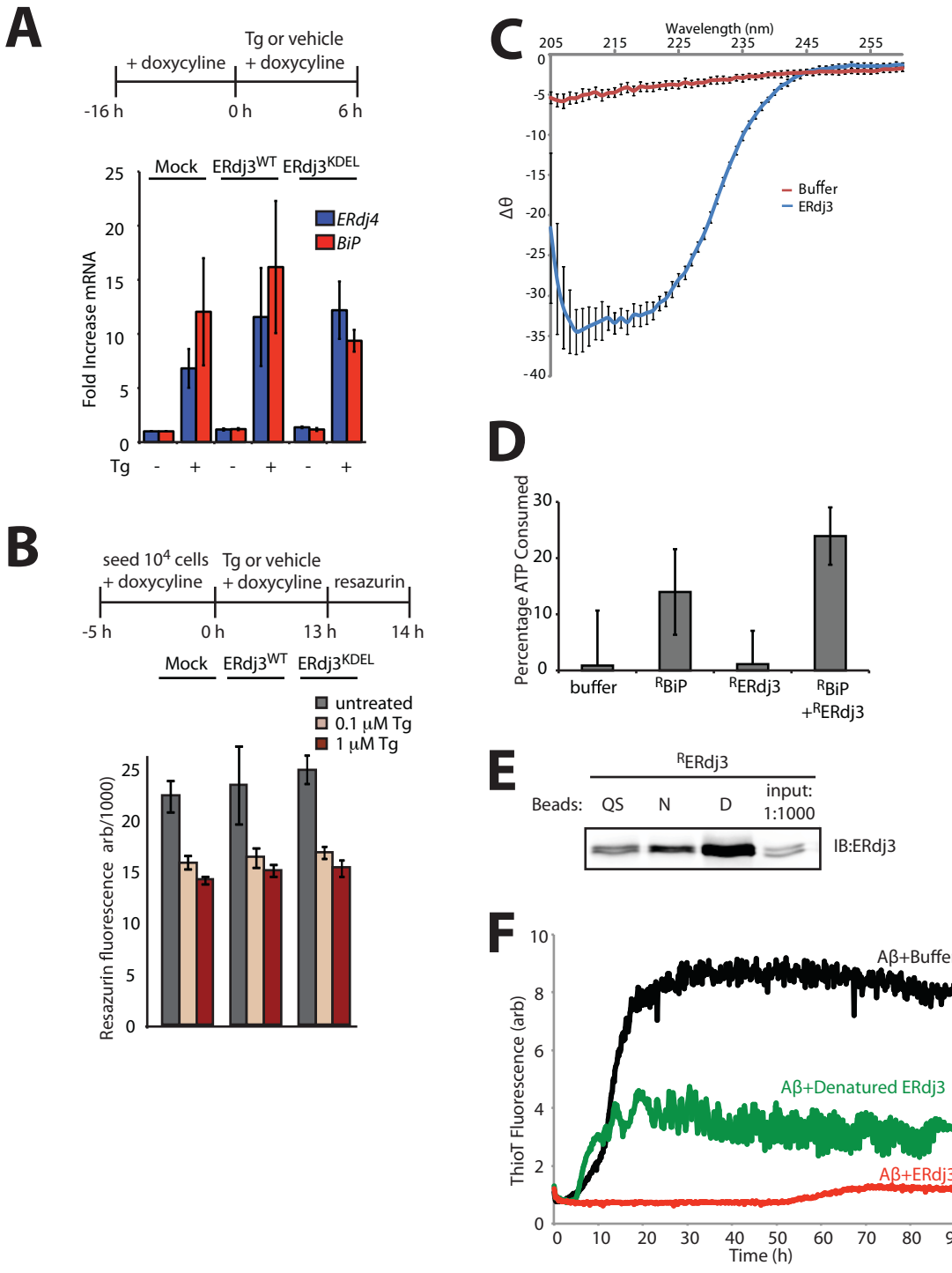

A
Tg or vehicle
+ doxycyline
+ doxycyline
-16 h
0 h
6 h
Mock
ERdj3WT
ERdj3KDEL
ERdj4
BiP
Fold Increase mRNA
Tg
B
seed 10^4 cells
+ doxycyline
Tg or vehicle
+ doxycyline
resazurin
-5 h
0 h
13 h
14 h
Mock
ERdj3WT
ERdj3KDEL
untreated
0.1 μM Tg
1 μM Tg
Resazurin fluorescence arb/1000)
C
Wavelength (nm)
Δθ
Buffer
ERdj3
D
Percentage ATP Consumed
buffer
RBiP
RERdj3
RBiP
+RERdj3
E
RERdj3
Beads:
QS
N
D
input:
1:1000
IB:ERdj3
F
ThioT Fluorescence (arb)
Aβ+Buffer
Aβ+Denatured ERdj3
Aβ+ERdj3
Time (h)

FIGURE S4

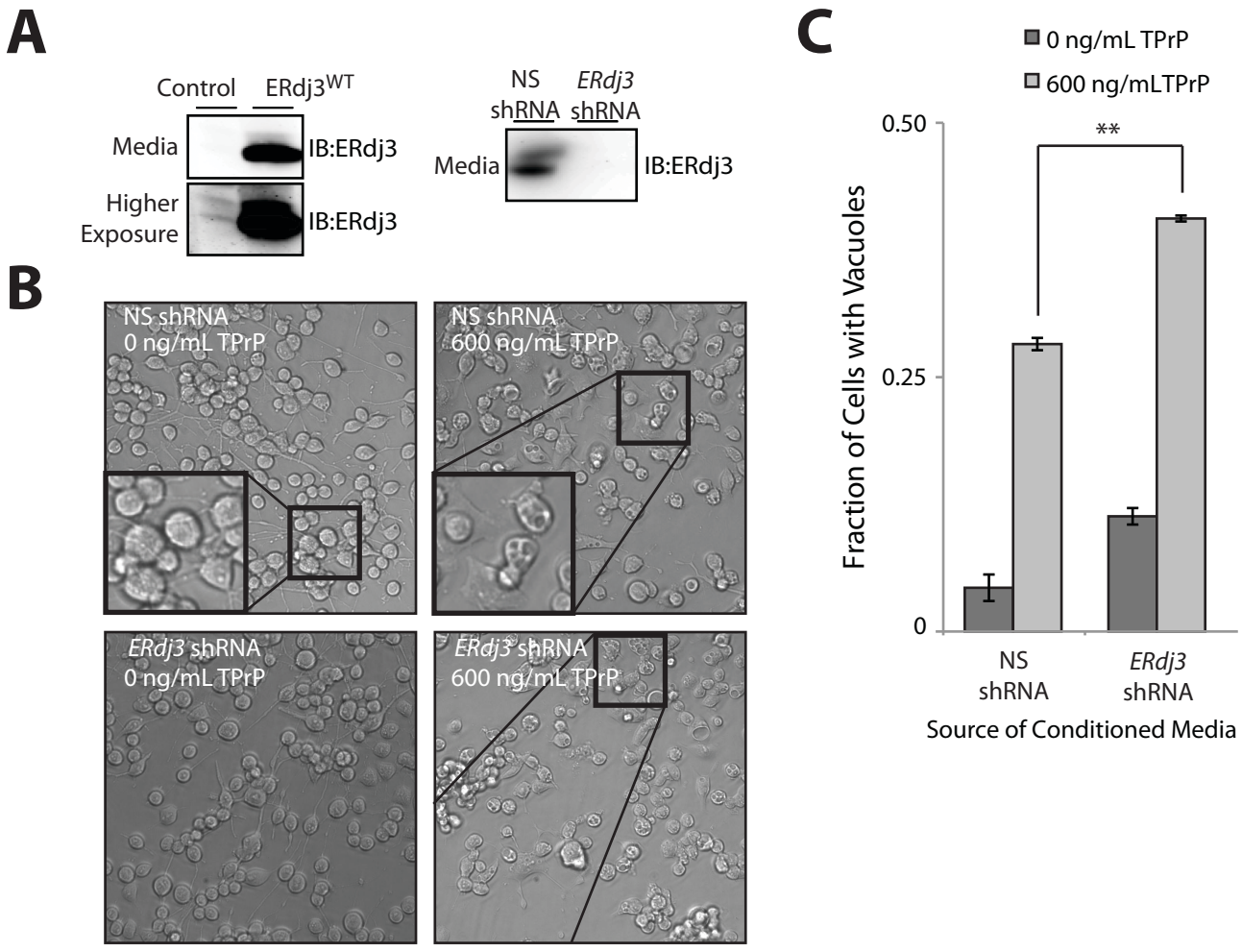

A
Control
ERdj3WT
Media
IB:ERdj3
Higher Exposure
IB:ERdj3
NS shRNA
ERdj3 shRNA
Media
IB:ERdj3
B
NS shRNA 0 ng/mL TPrP
NS shRNA 600 ng/mL TPrP
ERdj3 shRNA 0 ng/mL TPrP
ERdj3 shRNA 600 ng/mL TPrP
C
0 ng/mL TPrP
600 ng/mLTPrP
**
0.50
0.25
0
Fraction of Cells with Vacuoles
NS shRNA
ERdj3 shRNA
Source of Conditioned Media

FIGURE S5

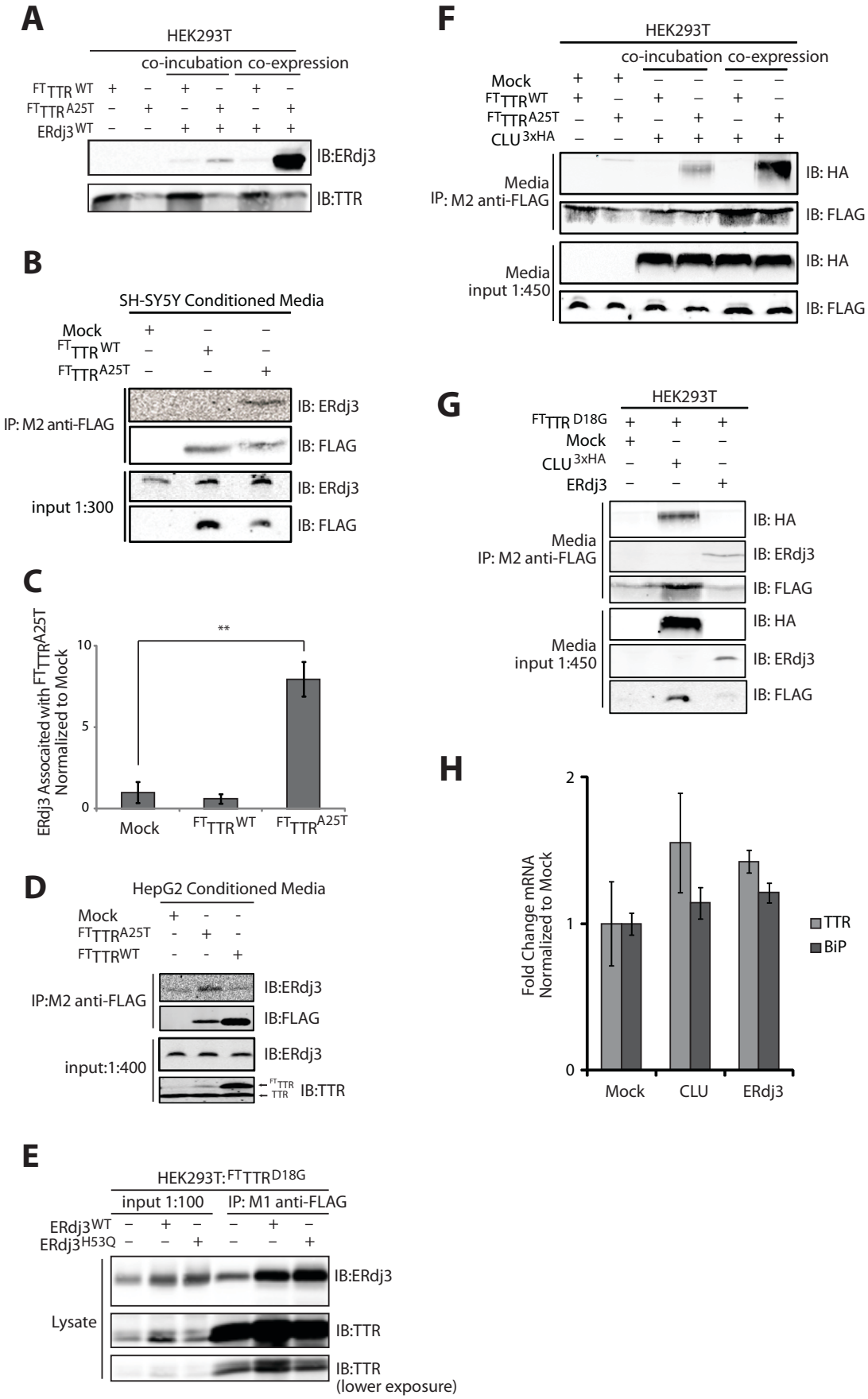
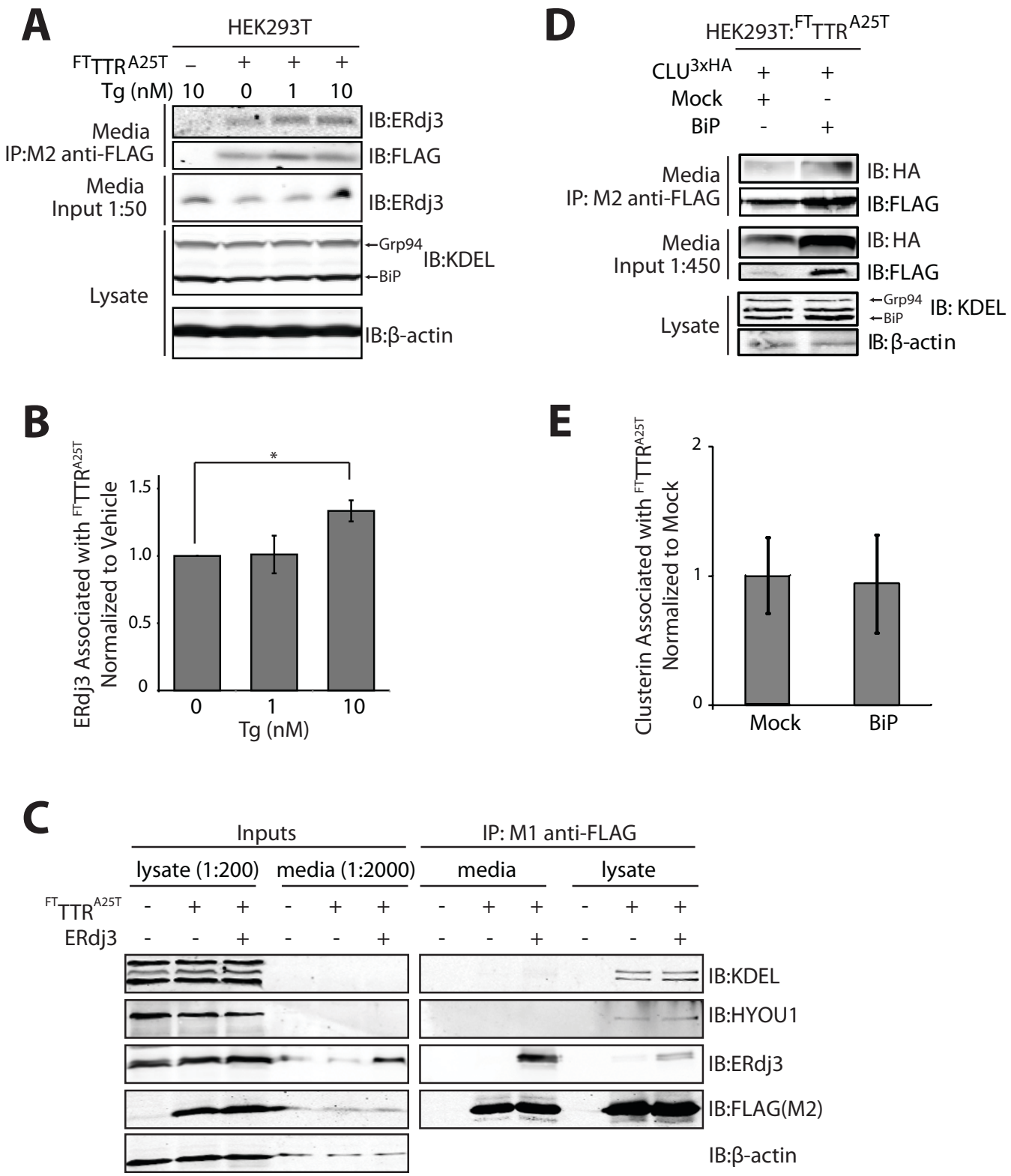

A
HEK293T
co-incubation co-expression
FTTTR WT
FTTTR A25T
ERdj3 WT
IB:ERdj3
IB:TTR
B
SH-SY5Y Conditioned Media
Mock
FTTTR WT
FTTTR A25T
IP: M2 anti-FLAG
IB: ERdj3
IB: FLAG
input 1:300
C
ERdj3 Associated with FTTTR A25T Normalized to Mock
**
Mock
FTTTR WT
FTTTR A25T
D
HepG2 Conditioned Media
IP:M2 anti-FLAG
IB:ERdj3
IB:FLAG
input:1:400
IB:TTR
E
HEK293T: FTTTR D18G
input 1:100
IP: M1 anti-FLAG
ERdj3 WT
ERdj3 H53Q
Lysate
IB:TTR
(lower exposure)
F
HEK293T
co-incubation co-expression
Mock
FTTTR WT
FTTTR A25T
CLU 3xHA
Media
IP: M2 anti-FLAG
IB: HA
IB: FLAG
Media
input 1:450
G
HEK293T
FTTTR D18G
Mock
CLU 3xHA
ERdj3
H
Fold Change mRNA Normalized to Mock
TTR
BiP
Mock
CLU
ERdj3

FIGURE S6


A
HEK293T
FTTTRA25T – + + +
Tg (nM) 10 0 1 10
Media
IP:M2 anti-FLAG
IB:ERdj3
IB:FLAG
Media
Input 1:50
IB:ERdj3
←Grp94
←BiP
IB:KDEL
Lysate
IB:β-actin
D
HEK293T:FTTTRA25T
CLU3xHA + +
Mock + -
BiP - +
Media
IP: M2 anti-FLAG
IB: HA
IB:FLAG
Media
Input 1:450
IB: HA
IB:FLAG
Lysate
←Grp94
←BiP
IB: KDEL
IB: β-actin
B
ERdj3 Associated with FTTTRA25T
Normalized to Vehicle
1.5
1.0
0.5
0
*
0
1
10
Tg (nM)
E
Clusterin Associated with FTTTRA25T
Normalized to Mock
2
1
0
Mock
BiP
C
Inputs
IP: M1 anti-FLAG
lysate (1:200)
media (1:2000)
media
lysate
FTTTRA25T - + + - + + - + + - + +
ERdj3 - - + - - + - - + - - +
IB:KDEL
IB:HYOU1
IB:ERdj3
IB:FLAG(M2)
IB:β-actin

SUPPLEMENTAL METHODS

Plasmid construction

ERdj3 was amplified from HEK293T cDNA with primers containing BamHI and EcoRI sites and cut into the pcDNA3.1(+) vector. A C-terminal KDEL was appended to ERdj3 using the reverse primer (IDT) 5'- ATG TTG AAT TCT ACA ACT CAT CTT TAT ATC CTT GCA GTC CAT TGT ATA CCT TCT G-3' and the EcoRI site. The ERdj3^{H53Q} construct was generated by site-directed mutagenesis using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA), and the primers 5'–CCT GCA GCT TCA GCC CGA CCG GAA CC–3' (H53Q forward), and 5'–GGT TCC GGT CGG GCT GAA GCT GCA GG–3' (H53Q reverse). Clusterin was amplified from HepG2 cDNA with primers containing Xho1 and Xba1 sites and cut into the pCI-neo.X3HA vector.

Immunoblotting

For analysis of protein levels by immunoblotting, cells were rinsed with PBS and scraped from plates in 2 mM EDTA in Tris-buffered saline (TBS). Cells were pelleted and lysed on ice for 10 min in RIPA buffer with fresh protease inhibitors (Roche). Cellular debris was pelleted by centrifugation at 16,000 *x g* for at least 15 min, followed by quantification of soluble protein by chromatographic assay (Bio-rad). Total protein lysate was boiled in Laemmli reducing buffer, separated by SDS-PAGE, transferred to nitrocellulose, and equivalent loading confirmed by Ponceau S staining (0.1% Ponceau/5% aq. AcOH). Blots were blocked in 5% milk/TBS for about 45 min, followed by thorough rinsing in TBST (TBS + 0.1% Tween 20) and incubation in primary antibody. After several washes with TBST, blots were incubated in 1:10000 fluorescent secondary antibody (Odyssey) in 5% milk/TBS. Blots were washed well in TBST, then TBS and water, then scanned on a Li-COR imager. Band intensities were quantified by densitometry. Error is presented as s.e.m. of at least three replicates, with p values determined by the Student's two-tailed t test.

Preparation and characterization of recombinant ERdj3 (^{R}ERdj3)

The *ERdj3* gene coding for the signal peptide cleavage product (23-458) was amplified and cloned into the pSMT3 vector using the BamHI and NotI cleavage sites. Upon reaching an O.D. (600 nm) of 0.6, SHuffle T7 *E. coli* cells (NEB) expressing this His_6 SMT3(SUMO)-ERdj3 chimera were induced overnight with 1 mM IPTG at

37 ^{O}C. SHuffle T7 E. coli cells were employed to allow the correct formation of two intramolecular disulfide bonds. Cells were suspended in HKM buffer (50 mM HEPES/KOH, pH 7.5, 500 mM KCl, 10 mM $MgCl_2$,10% glycerol) with 20 mM imidazole and protease inhibitor cocktail (Roche). After sonication and centrifugation, the soluble fraction was applied to a HisTrap FF column (GE Healthcare) and eluted in HKM buffer with 250 mM imidazole. His6-tagged SUMO protease 1 (ULP1, LifeSensors) was added to the eluent to cleave the SMT3 tag concurrently with overnight dialysis at ambient temperature in HKM buffer with 20 mM imidazole and 1 mM DTT. The post-digestion sample was then applied to the HisTrap FF column and the flow through purified on a Superdex 200 (GE) column in HKM buffer. Collected fractions were concentrated on a Millipore protein concentrator (MWCO 10K). Purity and identity were validated by SDS-PAGE separation followed by immunoblot and Coomassie staining, and by LC-MS. For circular dichroism, purified recombinant ERdj3 was dialyzed in 50 mM K_2HPO_4/KH_2PO_4, pH 7.5, 300 mM KCl, 10 mM $MgCl_2$, 10% glycerol overnight. Recombinant ERdj3 was diluted to 8 μM in the same buffer as determined by extinction coefficient. CD measurements were made with an Aviv 62A DS Circular Dichroism spectrometer, using quartz cuvettes with a 0.1 cm path length. CD spectra were obtained by monitoring molar ellipticity from 260 to 200 nm, with 15 second averaging times.

BiP human cDNA was purchased from Invitrogen (clone ID# 5020098, Accession # BC02035) and cloned into pT7-7 vector using the polymerase incomplete primer extension (PIPE) method (Forward primer for BiP: 5' CACCATGAAAATTTGTATTTTCAAGGTGAGGAGGAGGACAAGAAGGAGGACGTG-3', Reverse primer for BiP: 5'- CAAAGATATTTCTTACAACTCATCTTTTTCTGCTGT-3'; Forward primer for pAB6xHis vector: 5' ACAGCAGAAAAAGATGAGTTGTAAGAAATATCTTTGCTCCCAGTTTC-3'; Reverse primer for pAB6xHis vector: 5' ACCTTGAAAATACAAATTTTCATGGTGATGGTGATGGTGATGGTGCATAT-3'). Mixed BiP gene and vector were mixed 1:1 and transformed into *E. coli* DH5α cells. Positive clones were picked and the plasmid was transformed into *E. coli* BL21 cells for expression. Cells were grown at 37 ^{O}C to an O.D. (600 nm) of 0.6 and induced with 1 mM IPTG for 4 h. Cells were lysed by sonication in 10 mM phosphate buffer, pH 7 with protease inhibitors (Roche) to collect supernatant. The supernatant was purified on a 2 mL bench-top Nickel-NTA agarose resin column (Qiagen), and eluted in PBS with 300 mM imidazole. The eluent was cleaved with His6-tagged ProTEV (Promega) to remove the His-tag and run over the Ni column to collect the flow through. BiP purity was confirmed by Coomassie staining and molecular weight confirmed by LC-MS. The BiP ATPase assay was performed using the ATPlite Luminescence assay system (PerkinElmer). Samples

were incubated with 20 mM ATP at 37 ^{O}C for 1 h, in a 96-well plate and luminescence recorded on a Safire2 scanner (Tecan). The final ATP concentration was calibrated to a standard curve from 0.2 μM to 2000 μM.

Denatured RNAse A Affinity Precipitation

About 100 μL RNAse A conjugated beads were denatured in 1.2 ml of 20 mM Tris pH 8.8, 6 M guanidinium hydrochloride (GdnHCl) and 100 mM DTT overnight. Denatured beads were washed with PBS buffer and then incubated in 100 μL of 50 mM Tris pH 8.0 with 18 μM iodoacetamide to block free thiol groups. Excess iodoacetamide was washed off with PBS. ERdj3 protein levels in media collected from cells overexpressing GFP (mock), ERdj3^{WT}, or ERdj3^{H53Q} were quantified by immunoblotting. Normalized media was divided in equal parts for incubation with either denatured RNAse A beads or native RNAse A beads at ambient temperature for 2 h. The beads were washed 4 times with 20 mM HEPES pH 7.0, 75 mM KCl, 5 mM $MgCl_2$ and 0.01% Tween 20, and eluted with 6 X reducing Laemmli for SDS-PAGE separation and immunoblotting. For experiments with recombinant ERdj3, 500μl of 10 μM ^{R}ERdj3 in HKM buffer was added to each condition (quenched sepharose, native RNAse A beads, and denatured RNAse A beads), and incubated for 1 h before washing.

Aβ aggregation assay

Aβ$_{1-40}$ peptide was prepared and purified as reported (Du et al, 2011), with the identity of the peptide confirmed using MALDI-TOF. Lyophilized Aβ $_{1-40}$ was dissolved in 15 mM NaOH and adjusted to pH 10.5 with 100 mM NaOH. The completely dissolved solution was sonicated in an ice-water bath for 1 h and passed through a 0.2 μM m PVDF syringe filter into an Ultra-2 10K filter (Amicon). The flow through was recovered after centrifugation at 4000 rpm. Aβ $_{1-40}$ concentration was determined by UV absorption after neutralization to pH 6.8. The final reaction mixture consisting of 10 μM Aβ $_{1-40}$ with the indicated ratio of ^{R}ERdj3 or BSA in 50 mM Na phosphate, pH 7.2, 150 mM NaCl, 1 mM EDTA and 20 μM Thioflavin T, was prepared and vortexed immediately before loading 100 μL per well into a 96-well plate (Costar #3631). The covered plate was loaded into a Gemini Spectra-Max EM fluorescence plate reader (MolecularDevices, Sunnyvale,CA) and Thioflavin T fluorescence (excitation at 440 nm, emission at 485 nm) was recorded at 37 ^{O}C from the bottom of the plate at 10 min interval with 5 s shaking before reading.

After the assay, the solution in the wells was recovered and either reserved or centrifuged at 16000 *x g* for 20 min to precipitate insoluble amyloid. 100 µL of 8 M GdnHCl was added to the emptied well, and the whole plate sonicated on ice to remove adherent amyloid stuck to the side wall of the well. All samples were brought up to the same volume of 8 M GdnHCl, sonicated on ice for 1 h, diluted in PBS and separated by SDS-PAGE for immunoblotting.

Resazurin assay

For metabolic assays, 10^4 cells in 50 µL of media were plated in each well of a 96-well clear flat-bottom plate (Corning 3603), and 1 µg/mL of doxycycline added to inducible cells lines for at least 5 h prior to treatment. 50 µL of media with Tg or vehicle was added to each well to achieve indicated final concentrations, and the plate incubated at 37 ^{O}C for 13 h. Resazurin stock was prepared at 500 µM in PBS and sterile-filtered. 10 µL of stock was added to each well and incubated at 37 ^{O}C for another hour before reading. The resazurin reading was taken with the TECAN Safire^2 plate reader, with excitation at 530 nM and emission recorded at 590 nM.

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