Supplement to:

Quantitative Interactome Proteomics Reveals a Molecular Basis for ATF6-Dependent Regulation of a Destabilized, Amyloidogenic Protein

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Running Title: Defining Amyloidogenic Light Chain Quality Control

Keywords: ER quality control; ER proteostasis; Unfolded Protein Response (UPR); ATF6; XBP1s; quantitative proteomics; ER chaperones

SUPPLEMENTAL FIGURE LEGENDS

Figure S1 (Supplement to Figure 1)

A. Amino acid alignment of the germline λ light chain (LC), non-amyloidogenic, energetically-normal LC JTO, and the destabilized, amyloidogenic LC ALLC used in this study.

B. Immunoblot of Flag M1 immunopurifications (IP) prepared from HEK293^{DAX} cells transiently transfected with ^{FT}JTO, ^{FT}ALLC, untagged ALLC, or mock, as indicated. DSP crosslinking (0.5 mM, x-link) was added to cells prior to lysis where indicated. IPs were washed with either high-detergent RIPA or the more gentle lysis buffer (20 mM Hepes pH 7.5 100 mM NaCl 1% Triton X100), as indicated. Notice that the addition of crosslinker allows IPs to be washed with high-detergent RIPA buffer while retaining interactions with ER proteostasis buffers that are lost in the absence of crosslinking. Lysate inputs are shown as controls.

C. Immunoblot of Flag M1 immunopurifications (IP) prepared from HEK293^{DAX} cells transiently transfected with ^{FT}ALLC or mock, as indicated. Cells were crosslinked with the indicated concentration of DSP prior to lysis and IP. Lysate inputs are shown as controls.

Figure S2 (Supplement to Figure 2)

A. Comparison of the unnormalized TMT intensities for ^{FT}ALLC quantified in the Vh, XBP1s, and ATF6 channels of multiple replicate TMT-quant AP-MS experiments. All 7 biological replicates from this experiment are shown.

B,C. Pairwise correlations of interaction fold changes between ^{FT}ALLC and high-confidence interactors for all 7 individual biological replicates in response to XBP1s (**B**) or ATF6 activation (**C**). The lines show linear least-square fits and individual correlation coefficients (R) and mean correlation coefficient are plotted.

D. Schematic of the SILAC-quantification based AP-MS workflow to identify interactome changes of ^{FT}ALLC under conditions of stress-independent activation of XBP1s or ATF6. HEK293^{DAX} cells grown in either light $^{12}C/^{14}N$ media, or heavy $^{13}C/^{15}N$-labeled media were transfected with ^{FT}ALLC, treated with Dox or TMP to activate XBP1s or ATF6 and cross-linked in situ with DSP. Cells lysates from light and heavy cells were then mixed in equal ratios and subjected to immunoprecipitation with anti-M1 FLAG

agarose beads. Protein elutions were then processed and analyzed by MuDPIT LC-MS and peptides were quantified based on the intensities of the respective heavy and light precursor ions in the MS1 chromatograms.

E,F. Volcano plots displaying interactions changes of ^{FT}ALLC measured by SILAC-quantification AP-MS after stress-independent activation of XBP1s (**E**; n=4 biological replicates) or ATF6 (**F**; n=5 biological replicates). Shown in red are negative interaction changes and in green are positive interaction changes with secretory proteins.

G. Correlation of interactions changes observed after ATF6 (blue) or XBP1s (red) activation using TMT quantification (n=7 biological replicates) and SILAC quantification (n=4 or 5 biological replicates).

H. Comparison of interaction fold changes between ^{FT}ALLC and selected proteostasis factors in response to XBP1s activation as quantified by three independent methods: TMT-based q-AP-MS (n=7 biological replicates), SILAC-based q-AP-MS (n= 4 or 5 biological replicates), or Co-immunoprecipitation followed by quantitative immunoblotting (IP:IB; n=3-6 biological replicates).

I. Number of independent co-IP affinity purification samples required for the TMT-based q-AP-MS or the SILAC-based q-AP-MS analysis. The number of processed biological replicates (n) is indicated.

J. Mass spectrometry instrument time consumed during the TMT-based q-AP-MS or the SILAC-based q-AP-MS analysis to determine interaction changes for ^{FT}ALLC in response to ATF6 and/or XBP1s activation. The number of processed biological replicates (n) is indicated.

K. Comparison of quantified protein IDs across replicates highlights the improved detection of interaction partners using the TMT quantification approach in contrast to SILAC quantification. Highlighted in grey are proteins identified in a particular biological replicate MS experiment, and the red line shows the cumulative number of proteins quantified for the given number of replicates. For clarity of comparison, the number of proteins quantified in at least 3 replicates is listed.

L. Heatmap displaying the percentage of biological replicates where a given high-confidence interactor of ^{FT}ALLC was quantified in the TMT-based q-AP-MS or the SILAC-based q-AP-MS analysis.

Figure S3 (Supplement to Figure 3)

A. Plot showing TMT interaction ratio vs. q-value (Storey) for high confidence ALLC interacting proteins that co-purify with ^{FT}ALLC and/or ^{FT}JTO from untreated HEK293^{DAX} cells (n=3 biological replicates). Secretory proteins are shown in red. Full data available in **Supplemental Table 3**.

B. Representative immunoblot of anti-FLAG IPs from HEK293^{DAX} cells transiently transfected with ^{FT}JTO, ^{FT}ALLC, or untagged ALLC. A graph is included showing the relative recovery of ER proteostasis factors in in ^{FT}JTO (grey) or ^{FT}ALLC (red) is shown. Error bars represent n = 2 independent experiments.

C. Heatmap displaying the observed interactions changes between either ^{FT}ALLC (n=7 biological replicates) or ^{FT}JTO (n=6 biological replicates) and high confidence ER proteostasis network components following stress-independent ATF6 activation. Interactors are organized by pathway or function.

Figure S4 (Supplement to Figure 4).

A. Graph showing changes in total protein levels (open symbols) or ^{FT}ALLC interactions (solid bars) for DNAJC3 in HEK293^{DAX} cells following stress-independent XBP1s (red), ATF6 (blue), or XBP1s and ATF6 (green) activation. Error bars show SEM for n>3 individual replicates.

B. Graph showing changes in total protein levels (open symbols) or ^{FT}ALLC interactions (solid bars) for BiP in HEK293^{DAX} cells following stress-independent XBP1s (red), ATF6 (blue), or XBP1s and ATF6 (green) activation. Error bars show SEM for n>3 individual replicates.

C. Graph showing changes in total protein levels (open symbols) or ^{FT}ALLC interactions (solid bars) for GRP94 in HEK293^{DAX} cells following stress-independent XBP1s (red), ATF6 (blue), or XBP1s and ATF6 (green) activation. Error bars show SEM for n>3 individual replicates.

Figure 5 (Supplement to Figure 5).

A. Representative autoradiogram of [^{35}S]-labeled ^{FT}ALLC immunopurified from lysates or media collected from HEK293^{DAX} cells overexpressing mock, BiP, GRP94, or ERdj3 at the indicated time following metabolic labeling. In this experiment, cells were labeled for 30 min with [^{35}S] then incubated in label free media for 0, 2 or 4 h, as described in *Supplemental Materials and Methods*.

B. Graph showing normalized fraction [^{35}S]-labeled ^{FT}ALLC secreted at 0, 2 or 4 h in HEK293^{DAX} cells overexpressing mock, BiP, GRP94, or ERdj3. Fraction secreted was calculated using the following formula: fraction secreted = [^{35}S]-labeled ^{FT}ALLC in media at time t / ([^{35}S]-labeled ^{FT}ALLC in lysate at t= 0 + [^{35}S]-labeled ^{FT}ALLC in media at t= 0). Fraction secreted was normalized to mock transfected cells at each time point. Representative autoradiograms are shown in **Fig. S5A**. Error bars show SEM for n > 3 independent experiments. *indicates p<0.05; **indicates p<0.01; and ***indicates p<0.005 for unpaired t-tests.

C. Graph showing fraction [^{35}S]-labeled ^{FT}ALLC remaining at 0, 2 or 4 h in HEK293^{DAX} cells overexpressing mock, BiP, GRP94, or ERdj3. Fraction remaining was calculated using the following formula: fraction secreted = ([^{35}S]-labeled ^{FT}ALLC in media at time t + [^{35}S]-labeled ^{FT}ALLC in lysates at time t) / ([^{35}S]-labeled ^{FT}ALLC in lysate at t= 0 + [^{35}S]-labeled ^{FT}ALLC in media at t= 0). Representative autoradiograms are shown in **Fig. S5A**. Error bars show SEM for n > 3 independent experiments.

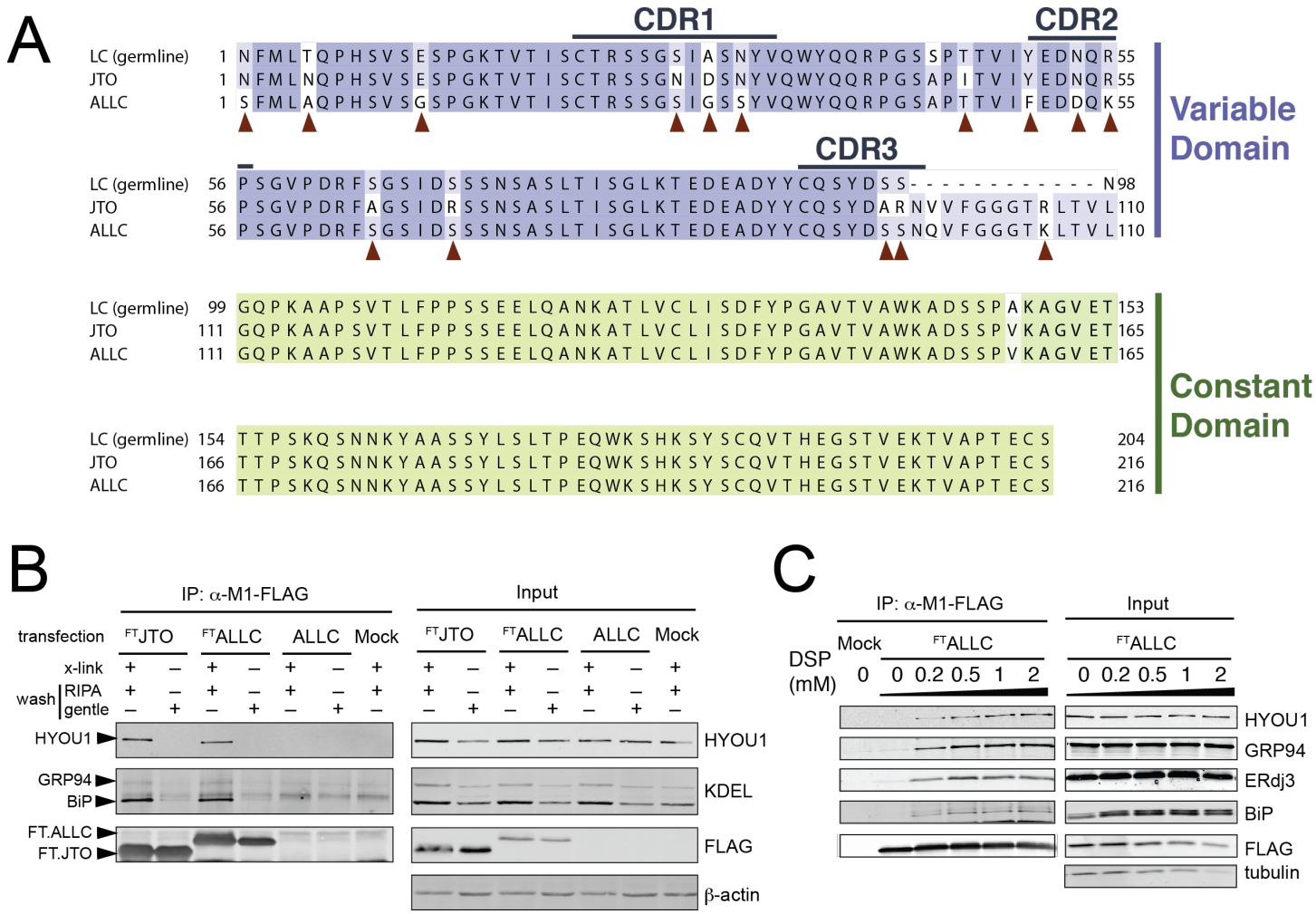
Figure 6 (Supplement to Figure 6)

A. Representative autoradiogram of [^{35}S]-labeled ^{FT}ALLC immunopurified from lysates or media collected from HEK293^{DAX} cells overexpressing mock or BiP and pretreated for 16 h with trimethoprim (TMP; 10 μM) to activate DHFR.ATF6. In this experiment, cells were labeled for 30 min with [^{35}S] then incubated in label free media for 0, 2 or 4 h, as described in *Supplemental Materials and Methods*.

B. Graph showing fraction [^{35}S]-labeled ^{FT}ALLC secreted at 0, 2, or 4 h in HEK293^{DAX} cells overexpressing mock of BiP and pretreated for 16 h with trimethoprim (TMP; 10 μM) to activate DHFR.ATF6 in these cells. Fraction secreted was calculated as described in **Fig. S5B**. Error bars show SEM for n=2 independent experiments.

C. Graph showing normalized fraction [^{35}S]-labeled ^{FT}ALLC secreted at 0, 2 or 4 h in HEK293^{DAX} cells overexpressing mock or BiP and pretreated for 16 h with trimethoprim (TMP; 10 μM) to activate DHFR.ATF6 in these cells. Fraction secreted was calculated as described in **Fig. S5B**. Fraction secreted was normalized to mock transfected cells at each time point. Representative autoradiograms are shown in **Fig. S6A**. Error bars show SEM for n=2 independent experiments.

FIGURE S1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CDR2
CDR3
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JTO 1 NFMLNQPHSVSESPGKTVTISCTRSSGNIDSNYVQWYQQRPGSAPITVIYEDNQR 55
ALLC 1 SFMLAQPHSVSGSPGKTVTISCTRSSGSIGSSYVQWYQQRPGSAPTTVIFEDDQK 55
LC (germline) 56 PSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSS- - - - - - - - - - - - N 98
JTO 56 PSGVPDRFAGSIDRSSNSASLTISGLKTEDEADYYCQSYDARNVVFGGGTRLTVL 110
ALLC 56 PSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNQVFGGGTKLTVL 110
Variable Domain
LC (germline) 99 GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPAKAGVET 153
JTO 111 GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVET 165
ALLC 111 GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVET 165
LC (germline) 154 TTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS 204
JTO 166 TTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS 216
ALLC 166 TTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS 216
Constant Domain
B
IP: α-M1-FLAG
Input
transfection
FTJTO
FTALLC
ALLC
Mock
x-link
wash
RIPA
gentle
HYOU1
GRP94
BiP
FT.ALLC
FT.JTO
KDEL
FLAG
β-actin
C
IP: α-M1-FLAG
Input
Mock
FTALLC
DSP (mM)
0 0 0.2 0.5 1 2
HYOU1
GRP94
ERdj3
BiP
FLAG
tubulin

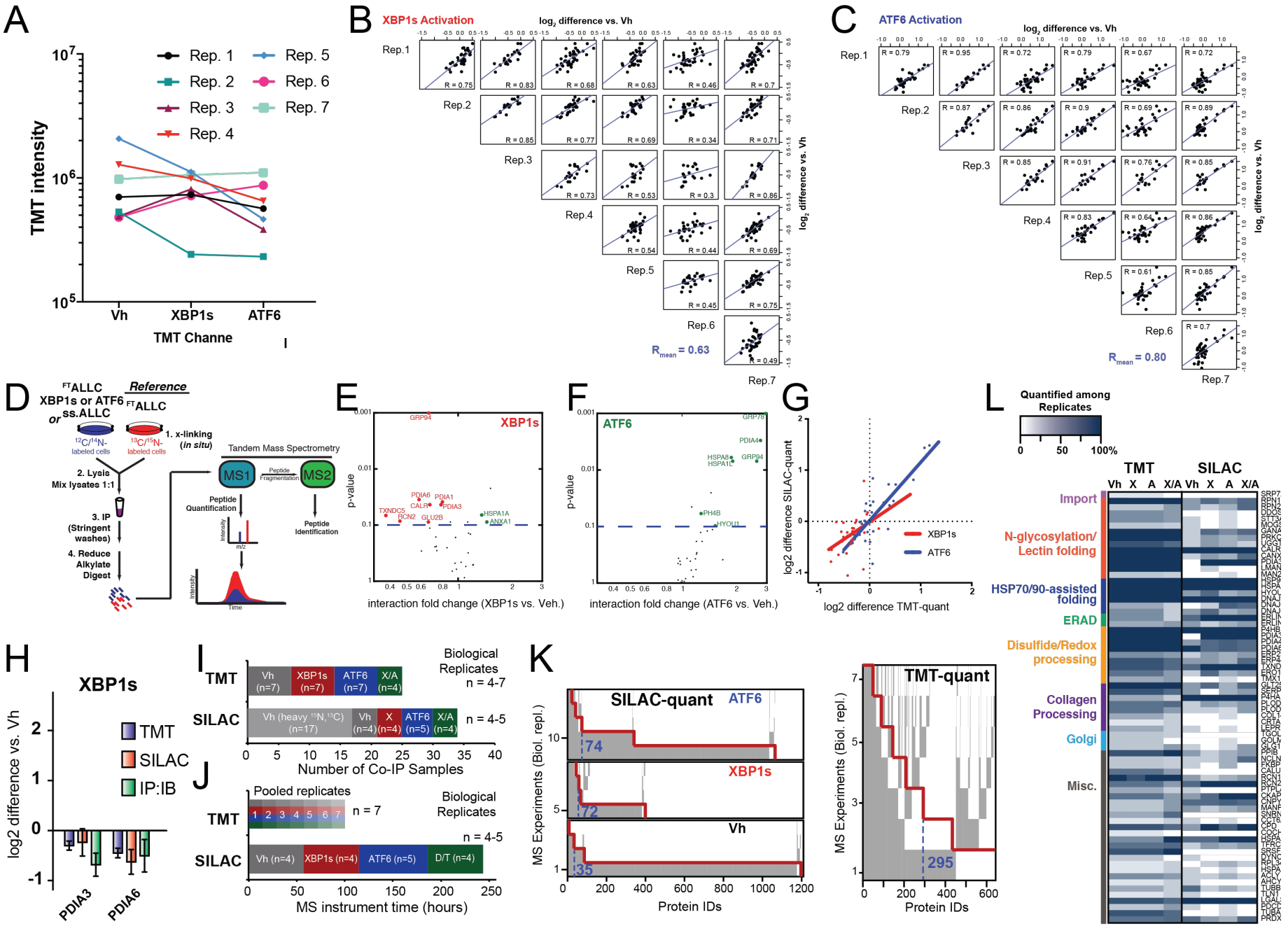

A
Rep. 1
Rep. 2
Rep. 3
Rep. 4
Rep. 5
Rep. 6
Rep. 7
TMT intensity
Vh
XBP1s
ATF6
TMT Channe l
B
XBP1s Activation
log2 difference vs. Vh
Rep.1
Rep.2
Rep.3
Rep.4
Rep.5
Rep.6
Rep.7
R = 0.75
R = 0.83
R = 0.68
R = 0.63
R = 0.46
R = 0.7
R = 0.85
R = 0.77
R = 0.69
R = 0.34
R = 0.71
R = 0.73
R = 0.53
R = 0.3
R = 0.86
R = 0.54
R = 0.44
R = 0.69
R = 0.45
R = 0.75
R = 0.49
Rmean = 0.63
log2 difference vs. Vh
C
ATF6 Activation
log2 difference vs. Vh
R = 0.79
R = 0.95
R = 0.72
R = 0.79
R = 0.67
R = 0.72
R = 0.87
R = 0.86
R = 0.9
R = 0.69
R = 0.89
R = 0.85
R = 0.91
R = 0.76
R = 0.85
R = 0.83
R = 0.64
R = 0.86
R = 0.61
R = 0.85
R = 0.7
Rmean = 0.80
log2 difference vs. Vh
D
FTALLC
XBP1s or ATF6
or ssALLC
Reference
FTALLC
12C/14N-labeled cells
13C/15N-labeled cells
1. x-linking (in situ)
2. Lysis
Mix lysates 1:1
3. IP (Stringent washes)
4. Reduce Alkylate Digest
Tandem Mass Spectrometry
MS1
Peptide Fragmentation
MS2
Peptide Quantification
Peptide Identification
Intensity
m/z
Time
E
XBP1s
p-value
GRP94
PDIA6
PDIA1
CALR
PDIA3
TXNDC5
PCN2
GLU2B
HSPA1A
ANXA1
interaction fold change (XBP1s vs. Veh.)
F
ATF6
p-value
GRP78
PDIA4
HSPA8
HSPA1L
GRP94
PH4B
HYOU1
interaction fold change (ATF6 vs. Veh.)
G
log2 difference SILAC-quant
XBP1s
ATF6
log2 difference TMT-quant
L
Quantified among Replicates
0
50
100%
TMT
SILAC
Vh X A X/A Vh X A X/A
Import
N-glycosylation/ Lectin folding
HSP70/90-assisted folding
ERAD
Disulfide/Redox processing
Collagen Processing
Golgi
Misc.
H
XBP1s
log2 difference vs. Vh
TMT
SILAC
IP:IB
PDIA3
PDIA6
I
TMT
Vh (n=7)
XBP1s (n=7)
ATF6 (n=7)
X/A (n=4)
Biological Replicates
n = 4-7
SILAC
Vh (heavy 15N,13C) (n=17)
Vh (n=4)
X (n=4)
ATF6 (n=5)
X/A (n=4)
n = 4-5
Number of Co-IP Samples
J
Pooled replicates
1 2 3 4 5 6 7
n = 7
Biological Replicates
n = 4-5
TMT
SILAC
Vh (n=4)
XBP1s (n=4)
ATF6 (n=5)
D/T (n=4)
MS instrument time (hours)
K
SILAC-quant
ATF6
74
XBP1s
72
Vh
35
MS Experiments (Biol. repl.)
Protein IDs
TMT-quant
295
MS Experiments (Biol. repl.)
Protein IDs

FIGURE S2

FIGURE S3

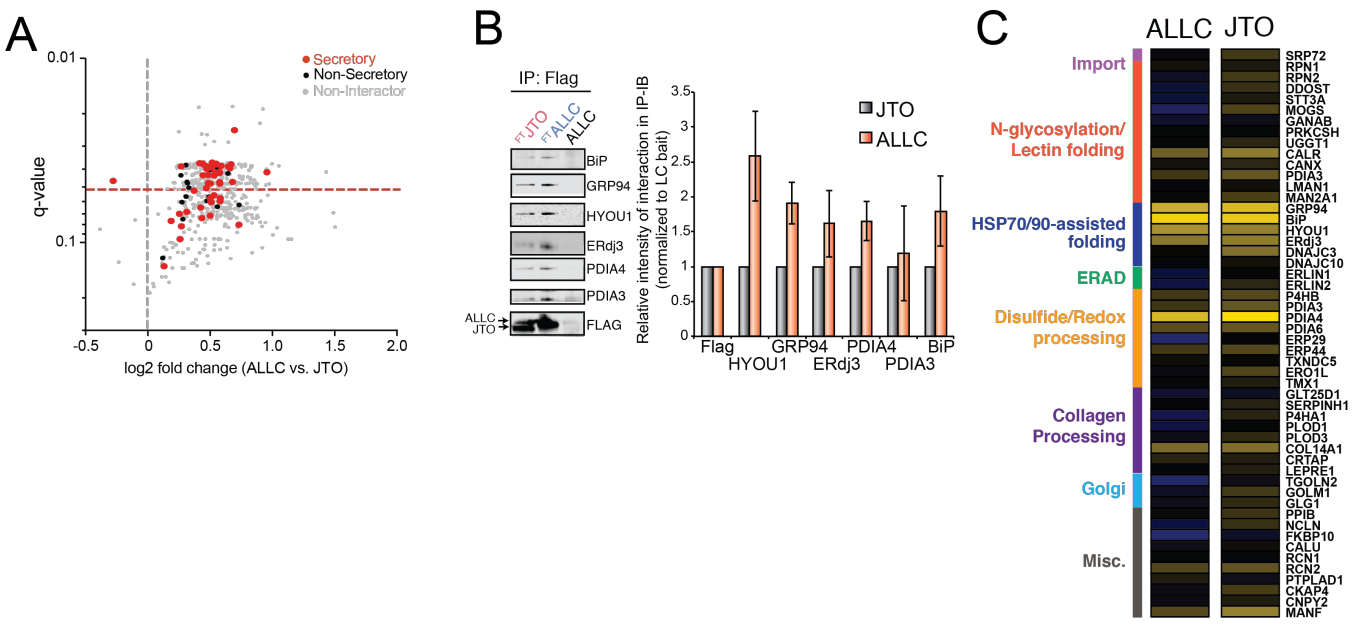
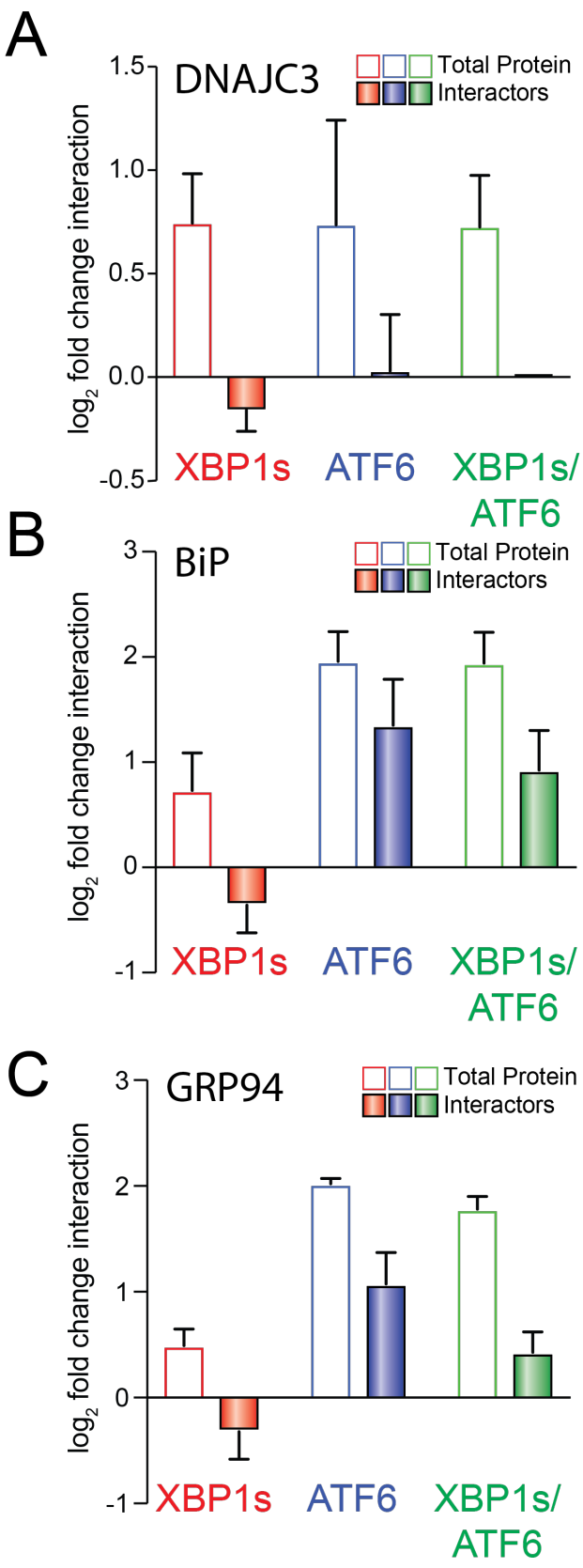

A
Secretory
Non-Secretory
Non-Interactor
q-value
0.01
0.1
-0.5
0
0.5
1.0
1.5
2.0
log2 fold change (ALLC vs. JTO)
B
IP: Flag
FT JTO
FT ALLC
ALLC
BiP
GRP94
HYOU1
ERdj3
PDIA4
PDIA3
FLAG
ALLC
JTO
Relative intensity of interaction in IP-IB
(normalized to LC bait)
JTO
ALLC
Flag
HYOU1
GRP94
ERdj3
PDIA4
PDIA3
BiP
C
ALLC
JTO
Import
N-glycosylation/
Lectin folding
HSP70/90-assisted
folding
ERAD
Disulfide/Redox
processing
Collagen
Processing
Golgi
Misc.
SRP72
RPN1
RPN2
DDOST
STT3A
MOGS
GANAB
PRKCSH
UGGT1
CALR
CANX
PDIA3
LMAN1
MAN2A1
GRP94
BiP
HYOU1
ERdj3
DNAJC3
DNAJC10
ERLIN1
ERLIN2
P4HB
PDIA3
PDIA4
PDIA6
ERP29
ERP44
TXNDC5
ERO1L
TMX1
GLT25D1
SERPINH1
P4HA1
PLOD1
PLOD3
COL14A1
CRTAP
LEPRE1
TGOLN2
GOLM1
GLG1
PPIB
NCLN
FKBP10
CALU
RCN1
RCN2
PTPLAD1
CKAP4
CNPY2
MANF

FIGURE S4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DNAJC3
Total Protein
Interactors
log2 fold change interaction
1.5
1.0
0.5
0.0
-0.5
XBP1s
ATF6
XBP1s/
ATF6
B
BiP
Total Protein
Interactors
log2 fold change interaction
3
2
1
0
-1
XBP1s
ATF6
XBP1s/
ATF6
C
GRP94
Total Protein
Interactors
log2 fold change interaction
3
2
1
0
-1
XBP1s
ATF6
XBP1s/
ATF6

FIGURE S5

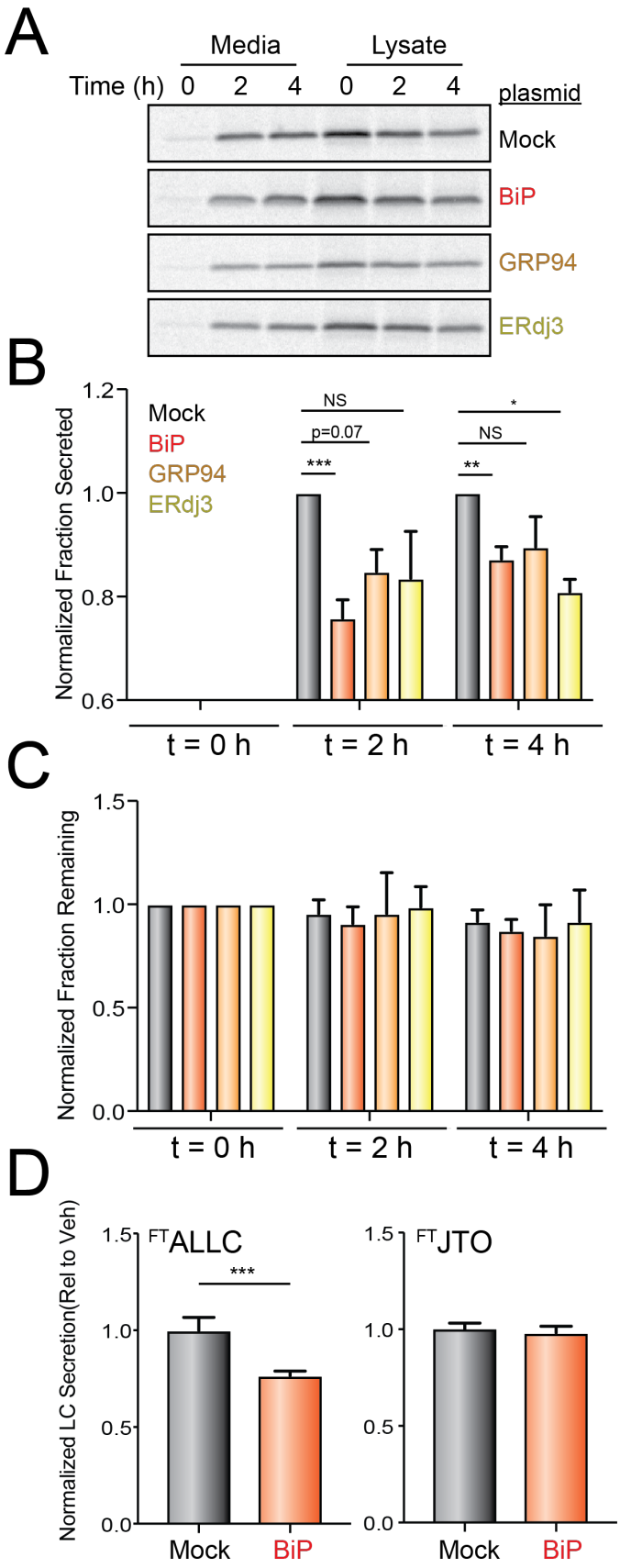
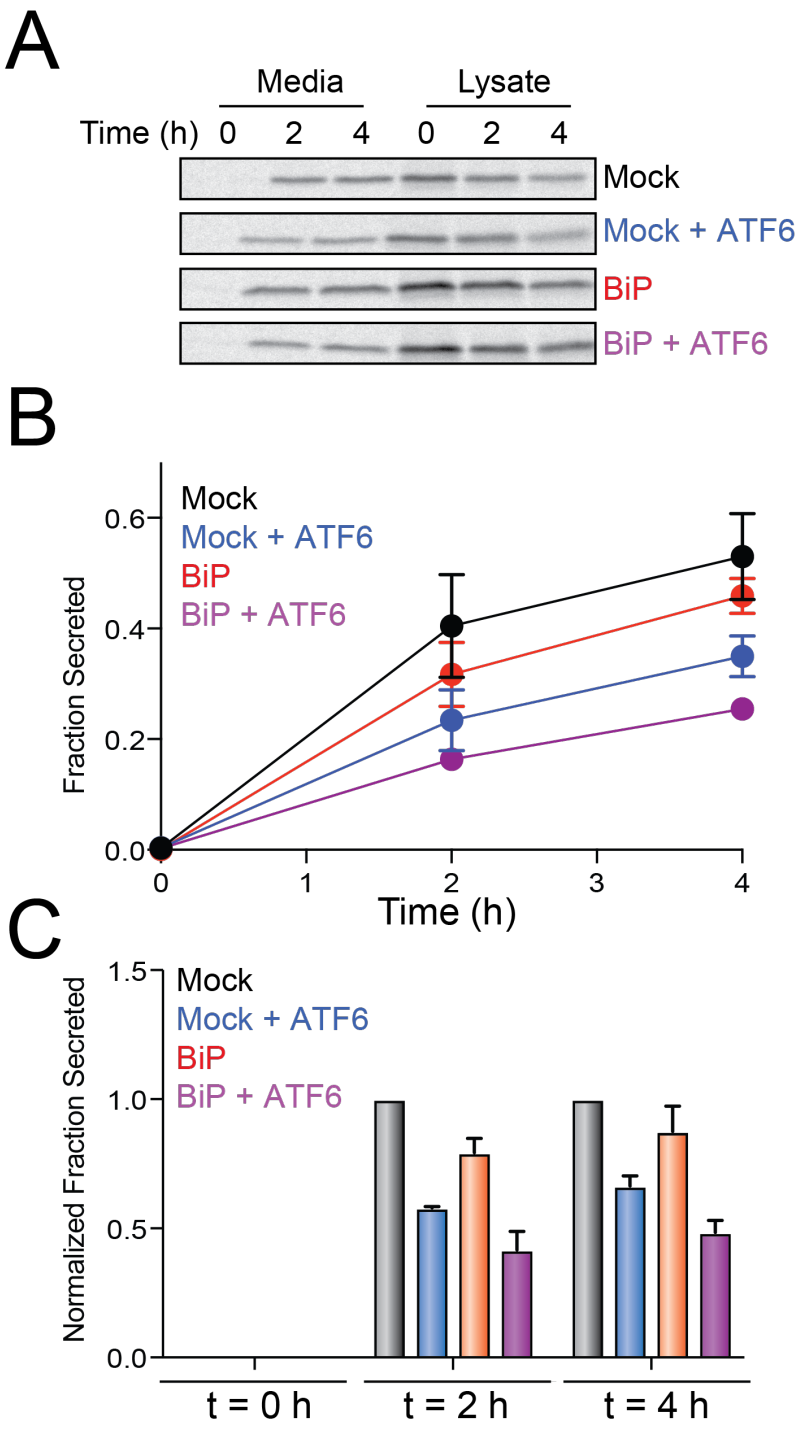

A
Media
Lysate
Time (h) 0 2 4 0 2 4
plasmid
Mock
BiP
GRP94
ERdj3
B
Normalized Fraction Secreted
1.2
1.0
0.8
0.6
Mock
BiP
GRP94
ERdj3
NS
p=0.07
***
*
NS
**
t = 0 h
t = 2 h
t = 4 h
C
Normalized Fraction Remaining
1.5
1.0
0.5
0.0
t = 0 h
t = 2 h
t = 4 h
D
Normalized LC Secretion(Rel to Veh)
1.5
1.0
0.5
0.0
FTALLC
***
FTJTO
Mock
BiP
Mock
BiP

FIGURE S6


A
Media
Lysate
Time (h) 0 2 4 0 2 4
Mock
Mock + ATF6
BiP
BiP + ATF6
B
Mock
Mock + ATF6
BiP
BiP + ATF6
Fraction Secreted
0.0
0.2
0.4
0.6
0
1
2
3
4
Time (h)
C
Mock
Mock + ATF6
BiP
BiP + ATF6
Normalized Fraction Secreted
0.0
0.5
1.0
1.5
t = 0 h
t = 2 h
t = 4 h

SUPPLEMENTAL TABLE LEGENDS

Supplemental Table 1 (Supplement to Figure 1). Excel spreadsheets including the interactome data comparing the interactions between ER proteostasis factors and either ^{FT}LC (combined replicates of ^{FT}ALLC and ^{FT}JTO) or untagged ALLC. Two sheets are included within this file: 1) a summary sheet including only the final TMT ratios and significance and 2) a sheet containing all of the raw data for the included analyses.

Supplemental Table 2 (Supplement to Figure 2). Excel spreadsheets describing the interactome data comparing interactions between ER proteostasis factors and ^{FT}ALLC following stress-independent XBP1s and/or ATF6 activation in HEK293^{DAX} cells. Two sheets are included within this file: 1) a summary sheet including only the final TMT ratios and significance and 2) a sheet containing all of the raw data for the included analyses.

Supplemental Table 3 (Supplement to Figure 3). Excel spreadsheet describing the interactome data comparing the interactions between ER proteostasis factors and ^{FT}ALLC and ^{FT}JTO in HEK293^{DAX} cells or ^{FT}JTO in HEK293^{DAX} cells following stress-independent ATF6 activation. Four sheets are included within this file: 1) a summary sheet including only the final TMT ratios and significance comparing the interaction ratios between ^{FT}ALLC and ^{FT}JTO and 2) a sheet containing all of the raw data used to compare the interactomes of ^{FT}ALLC and ^{FT}JTO, 3) a summary sheet including only the final TMT ratios and significance comparing the interaction ratios for ^{FT}JTO in the absence or presence of ATF6 activation in HEK293^{DAX} cells and 4) a sheet containing all of the raw data used to compare the interactome ^{FT}JTO in the presence or absence of ATF6 activation.

Supplemental Table 4 (Supplement to Figure 4). Excel spreadsheets comparing changes in the mRNA or protein levels and ^{FT}ALLC interactions for high confidence LC interacting proteins in HEK293^{DAX} cells following stress-independent activation of ATF6, XBP1s, or ATF6 and XBP1s co-activation. This table contains four sheets. Data for changes in mRNA or protein levels in HEK293^{DAX} cells following these treatments is from (Plate et al., 2016; Shoulders et al., 2013).