SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Quantitative RT-PCR

The relative mRNA expression levels of target genes were measured using quantitative RT-PCR using previously reported primers to *TTR* and *RiboP* (Shoulders et al., 2013). RNA was extracted using the RNeasy Mini Kit (Qiagen) and converted to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen). Amplification was measured with FastStart Universal SYBR Green Master Mix (Roche) with the program 6 min at 95 ^{O}C, 45 cycles of 10 s at 95 ^{O}C, 30 s at 60 ^{O}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 *RiboP* and all measurements were performed in quadruplet. Data were analyzed using the RQ Manager and DataAssist 2.0 softwares (ABI). qPCR data are reported as mean $\pm$ 95% confidence interval as calculated in DataAssist 2.0.

Gel Filtration Chromatography of ^{FT}TTR variants in HEK293^{DAX} conditioned media.

Conditioned media or media with recombinant ^{FT}TTR^{WT} was first passed through a 0.22 µm syringe filter and 500 µl of filtered media was loaded by AKTA FPLC (GE Healthcare) onto Superdex 200 column (10 × 300 mm, GE Healthcare) at flow rate of 0.5 mL/min at ambient temperature. The column was pre-equilibrated with DPBS and calibrated with NativeMark Unstained Protein Standard (Life Technologies). Elution fractions of 1 ml were collected and analyzed by immunoblotting.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1 – Supplement to Figure 1

A. Representative autoradiograms of ^{FT}TTR variants immunoisolated from media and lysates prepared from HEK293^{DAX} transfected with the indicated ^{FT}TTR variant in the absence or presence of ATF6 preactivation (TMP; 10 μM, 16 h), as indicated. These gels are representative of those quantified to prepare **Figure 1B-H** and **Figure 3A**. The [^{35}S] labeling protocol used for these experiments is shown above the autoradiograms.

B-C. Quantification of fraction secreted [^{35}S]-labeled ^{FT}TTR^{D18G} (**B**) or ^{FT}TTR^{A25T} (**C**), as in **Figures 1B-C** but on the same scale to that used for other ^{FT}TTR variants shown in **Figures 1D-H**.

Figure S2 – Supplement to Figure 3

A. Graph showing the fraction intracellular of ^{FT}TTR variants in HEK293^{DAX} following a 4 h chase in the absence (gray) or presence (blue) of ATF6 preactivation (TMP; 10 μM, 16 h). Fraction intracellular was calculated using the equation: Fraction Intracellular = {Total ^{FT}TTR Signal in Lysate at Time = 4h} divided by {Total ^{FT}TTR Signal in Lysate at Time = 0} + {Total ^{FT}TTR Signal in Media at Time = 0}. Error bars represent SEM from biological replicates ($n \geq 3$).

B. Representative autoradiograms of ^{FT}TTR^{D18G} immunoisolated from HEK293^{DAX} media and lysates in the absence or presence of ATF6 pre-activation (TMP; 10 μM, 16 h) quantified to prepare **Figure 3C** and **Figure S2C**. The proteasome inhibitor MG132 (10 μM) and/or the lysosomal inhibitor NH_4Cl (25 mM) were added during the pulse and subsequence chase, as indicated. The [^{35}S] labeling protocol used for these experiments is shown.

C. Plot showing fraction remaining for [^{35}S]-labeled ^{FT}TTR^{D18G} in HEK293^{DAX} media and lysates in the absence of ATF6 chased in the presence of vehicle (black), MG132 (orange), NH_4Cl (green) or both (purple), from autoradiograms as shown in **Figure S2B**. Fraction remaining was calculated as described in **Figure 3** (Shoulders et al., 2013). Error bars represent SEM from biological replicates ($n \geq 3$).

D. Representative autoradiogram and plot showing fraction secreted of [^{35}S]-labeled ^{FT}M-TTR from HEK293^{DAX} in the absence (black) or presence (blue) of ATF6 preactivation (10 μM TMP, 16 h). Fraction secreted was calculated as in **Figure 1** (Shoulders et al., 2013). Error bars represent SEM from biological replicates ($n = 6$).

**p < 0.01; *p < 0.05.

Figure S3 – Supplement to Figure 4

A. Representative autoradiogram of [^{35}S]-labeled ^{FT}TTR^{WT} or ^{FT}TTR^{A25T} immunoisolated using the M2 anti-FLAG antibody from HepG2^{DA} media and lysates in the absence or presence of ATF6 preactivation (100 μM TMP; 16 h) used to prepare **Figures 4C-F**. Endogenous TTR^{WT} co-purifies with ^{FT}TTR variants, as previously reported (Sekijima et al., 2005; Shoulders et al., 2013), which is shown by the arrows.

B. Graph showing the fraction ^{FT}TTR^{A25T} remaining at 2 h in HepG2^{DA} cells in the absence of ATF6 preactivation. The proteasome inhibitor MG132 (10 μM; orange), the lysosomal inhibitor NH_4Cl (25 mM; green) or MG132 and NH_4Cl (purple) were added to the labeling and chase media, as indicated. Fraction remaining was calculated as described in **Figure 3**. Error bars represent standard deviation from biological replicates (n = 2).

C. Graph showing the fraction ^{FT}TTR^{A25T} remaining at 2 h in HepG2^{DA} cells in the presence of ATF6 preactivation (TMP; 100 μM, 16 h). The proteasome inhibitor MG132 (10 μM; orange), the lysosomal inhibitor NH_4Cl (25 mM; green) or MG132 and NH_4Cl (purple) were added to the labeling and chase media, as indicated. Fraction remaining was calculated as described in **Figure 3**. Error bars represent standard deviation from biological replicates (n = 2).

D. Graph showing the relative recovery of endogenous TTR^{WT} in ^{FT}TTR^{A25T} immunopurifications from [^{35}S] labeled HepG2^{DA} cells following a 4 h chase in the presence or absence of ATF6 preactivation (TMP; 100 μM, 16 h). A representative autoradiogram of this experiment is shown in **Figure S3A**. Relative endogenous TTR^{WT} recovery was calculated using the equation {recovered [^{35}S]-labeled endogenous TTR^{WT} in anti-FLAG immunopurifications of media at t=4 h} divided by {recovered [^{35}S]-labeled ^{FT}TTR^{A25T} in anti-FLAG immunopurifications of media at t=4 h} for experiments as shown in **Figure S3A** (n=5).

Figure S4 – Supplement to Figure 5

A. Representative autoradiograms of [^{35}S]-labeled ^{FT}TTR^{L55P} isolated from HEK293^{DAX} media and lysates in the absence or presence of ATF6 preactivation (10 μM TMP; 16 h) quantified to prepare **Figure 5B,C**. Either

vehicle or the small molecule TTR kinetic stabilizer Taf (10 μM) was included throughout the experiment, as indicated. The experimental protocol is shown above.

B. Representative autoradiogram of [^{35}S]-labeled ^{FT}TTR^{A25T} isolated from HEK293^{DAX} media and lysates in the absence or presence of ATF6 preactivation (10 μM TMP; 16 h) quantified to prepare **Figure 5E,F**. Either vehicle or the small molecule TTR kinetic stabilizer Taf (10 μM) was included throughout the experiment, as indicated. The experimental protocol was performed as in **Figure S4A**.

Figure S5 – Supplement to Figure 6

A. Immunoblot of ^{FT}TTR variants in fractions from conditioned media (CM) prepared on HEK293T cells (18 h) overexpressing ^{FT}TTR^{A25T} or ^{FT}TTR^{WT} and separated on a Superdex 200 gel filtration column. The fractionation of recombinant ^{FT}TTR^{WT} (^{RecFT}TTR^{WT}) on the same gel filtration column is shown as a control. The Input lanes represent 4% of injected volume. A molecular weight ladder for the elution volumes is shown above the images.

B. Immunoblot (IB) of Clear-Native (CN)-PAGE separation of conditioned media prepared on HEK293^{DAX} cells expressing ^{FT}TTR^{WT}, ^{FT}TTR^{A25T}, ^{FT}TTR^{V30M}, or ^{FT}TTR^{L55P} for 24 h in the absence or presence of Taf (10 μM). Immunoblots (IB) were performed using the anti-TTR antibody. An SDS-PAGE/immunoblot (IB) for identical samples is shown as a control for total ^{FT}TTR protein levels in the conditioned media.

C. Immunoblot of CN-PAGE for conditioned media prepared on HEK293^{DAX} cells expressing ^{FT}TTR^{D18G}, ^{FT}TTR^{A25T}, ^{FT}TTR^{L55P}, and ^{FT}TTR^{V30M} for 24 h in the absence or presence of ATF6 activation (TMP; 10 μM).

Figure S1

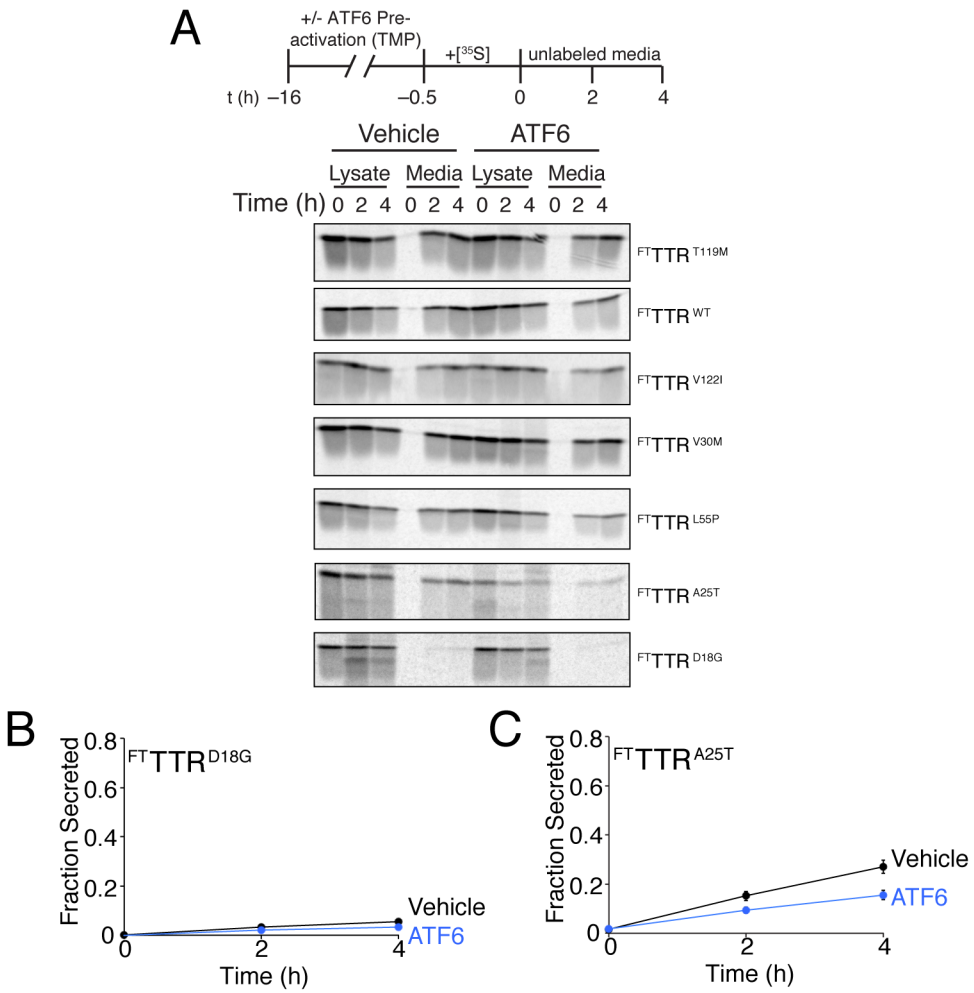
A
+/- ATF6 Pre-activation (TMP)
+[35S]
unlabeled media
t (h) –16
–0.5
0
2
4
Vehicle
ATF6
Lysate
Media
Lysate
Media
Time (h) 0 2 4 0 2 4 0 2 4 0 2 4
FTTTR T119M
FTTTR WT
FTTTR V122I
FTTTR V30M
FTTTR L55P
FTTTR A25T
FTTTR D18G
B
FTTTR D18G
Fraction Secreted
0.8
0.6
0.4
0.2
0
0
2
4
Time (h)
Vehicle
ATF6
C
FTTTR A25T
Fraction Secreted
0.8
0.6
0.4
0.2
0
0
2
4
Time (h)
Vehicle
ATF6

Figure S2

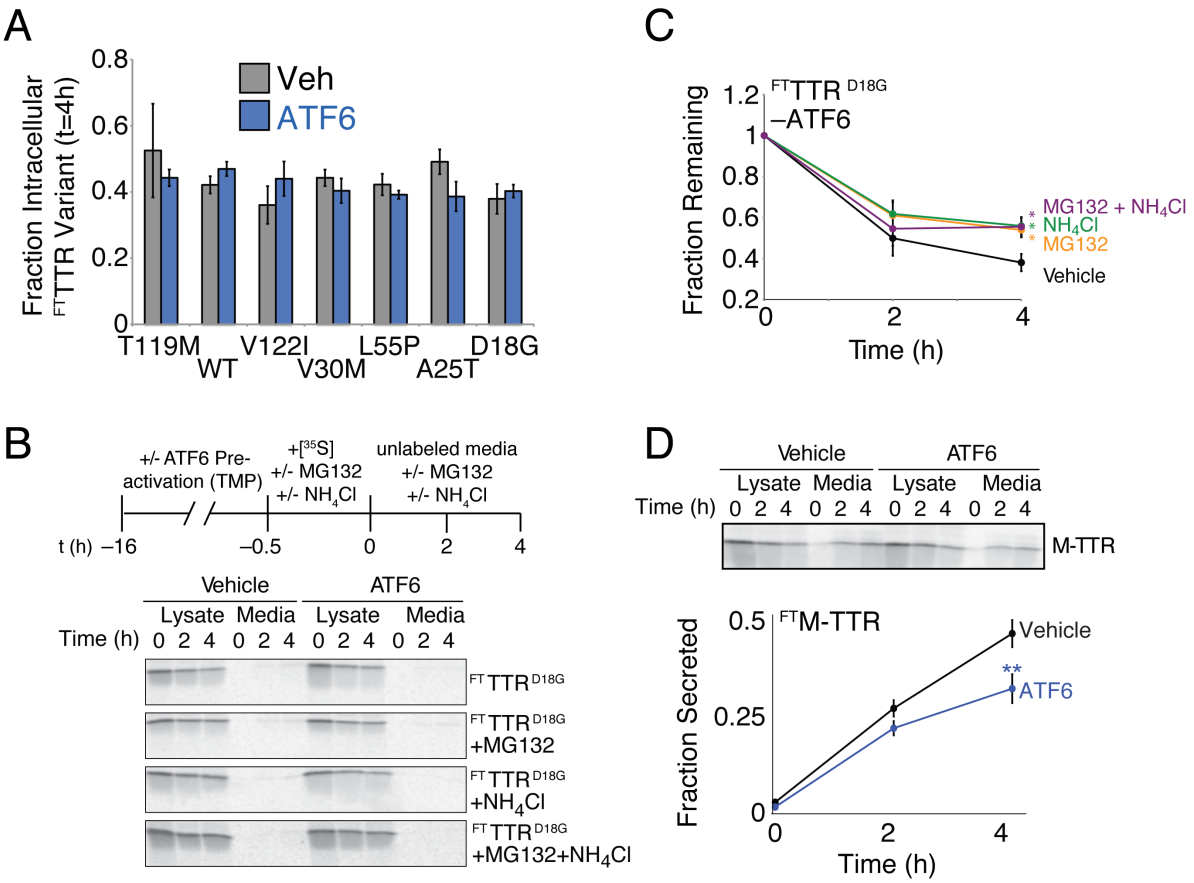
A
Fraction Intracellular FTTTR Variant (t=4h)
0.8
0.6
0.4
0.2
0
Veh
ATF6
T119M
WT
V122I
V30M
L55P
A25T
D18G
C
Fraction Remaining
1.2
1
0.8
0.6
0.4
0.2
FTTTR D18G
–ATF6
MG132 + NH4Cl
NH4Cl
MG132
Vehicle
0
2
4
Time (h)
B
+/- ATF6 Pre-activation (TMP)
+[35S] +/- MG132 +/- NH4Cl
unlabeled media +/- MG132 +/- NH4Cl
t (h) –16
–0.5
0
2
4
Vehicle
ATF6
Lysate Media Lysate Media
Time (h) 0 2 4 0 2 4 0 2 4 0 2 4
FTTTR D18G
FTTTR D18G +MG132
FTTTR D18G +NH4Cl
FTTTR D18G +MG132+NH4Cl
D
Vehicle
ATF6
Lysate Media Lysate Media
Time (h) 0 2 4 0 2 4 0 2 4 0 2 4
M-TTR
Fraction Secreted
0.5
0.25
0
FTM-TTR
Vehicle
**
ATF6
0
2
4
Time (h)

Figure S3

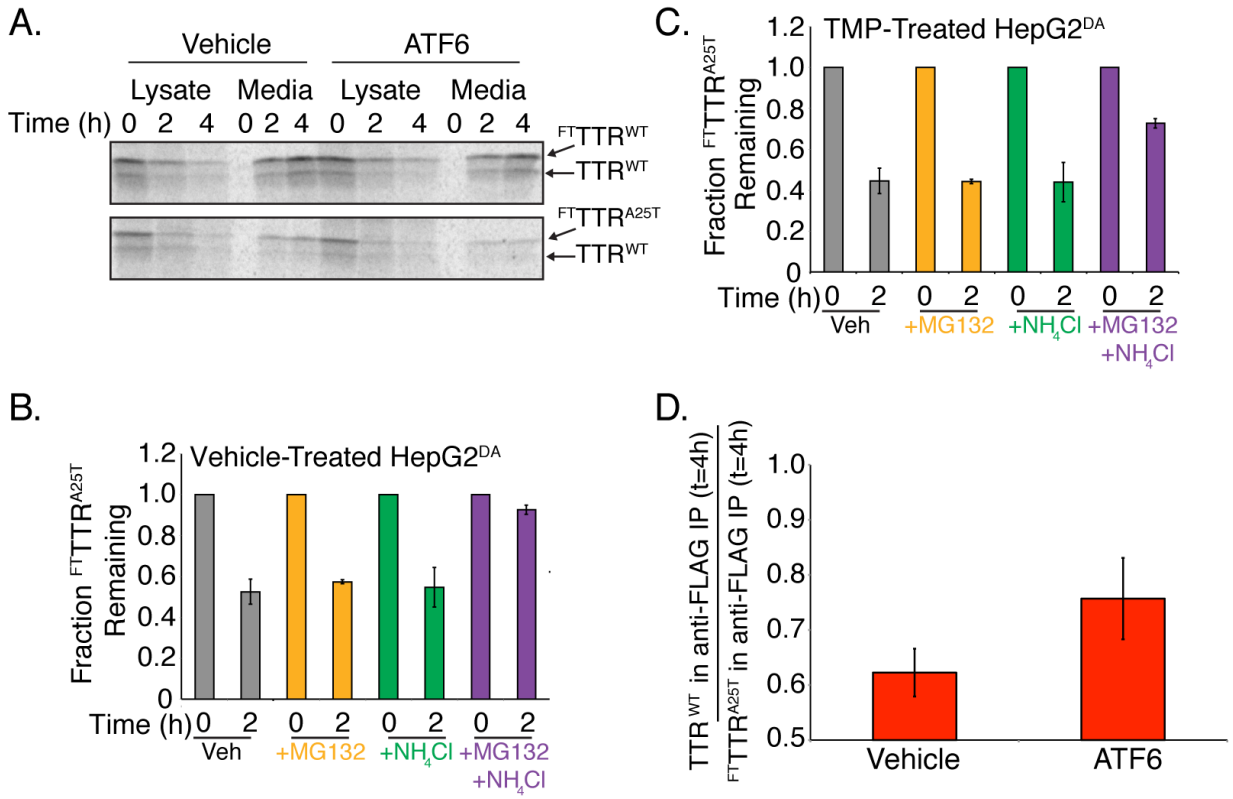
A.
Vehicle
ATF6
Lysate
Media
Lysate
Media
Time (h) 0 2 4 0 2 4 0 2 4 0 2 4
FTTTRWT
TTRWT
FTTTRA25T
TTRWT
B.
Vehicle-Treated HepG2DA
Fraction FTTTRA25T Remaining
Time (h) 0 2 0 2 0 2 0 2
Veh
+MG132
+NH4Cl
+MG132 +NH4Cl
C.
TMP-Treated HepG2DA
Fraction FTTTRA25T Remaining
Time (h) 0 2 0 2 0 2 0 2
Veh
+MG132
+NH4Cl
+MG132 +NH4Cl
D.
TTRWT in anti-FLAG IP (t=4h) / FTTTRA25T in anti-FLAG IP (t=4h)
Vehicle
ATF6

Figure S4

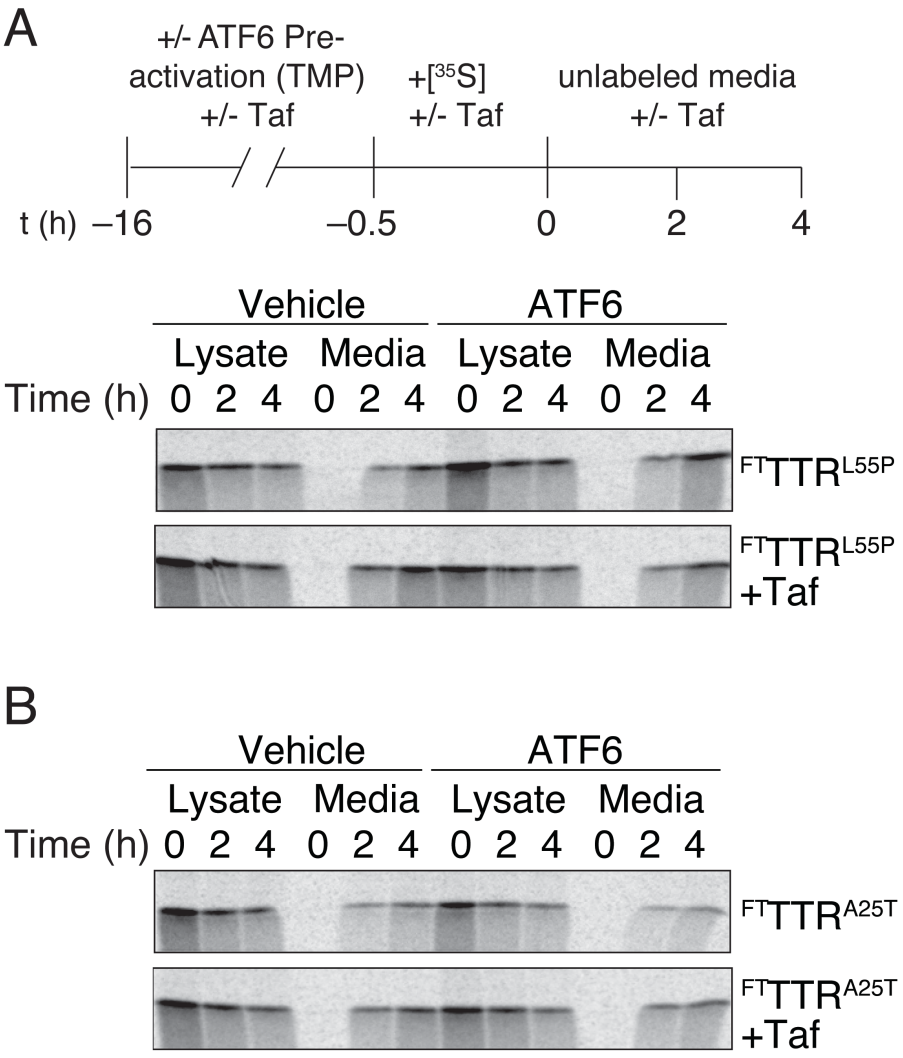
A
+/- ATF6 Pre-activation (TMP) +/- Taf
+[35S] +/- Taf
unlabeled media +/- Taf
t (h) –16
–0.5
0
2
4
Vehicle
ATF6
Lysate
Media
Lysate
Media
Time (h) 0 2 4 0 2 4 0 2 4 0 2 4
FTTTRL55P
FTTTRL55P +Taf
B
Vehicle
ATF6
Lysate
Media
Lysate
Media
Time (h) 0 2 4 0 2 4 0 2 4 0 2 4
FTTTRA25T
FTTTRA25T +Taf

Figure S5

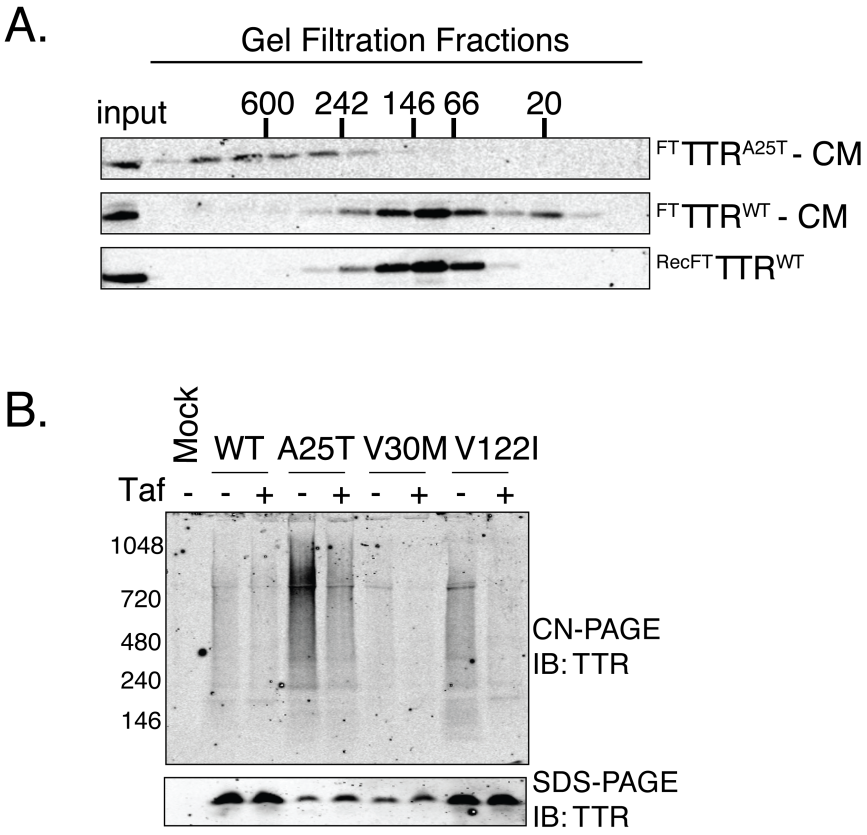
A.
Gel Filtration Fractions
input
600
242
146
66
20
FTTTRA25T - CM
FTTTRWT - CM
RecFTTTRWT
B.
Mock
WT
A25T
V30M
V122I
Taf - - + - + - + - +
1048
720
480
240
146
CN-PAGE
IB: TTR
SDS-PAGE
IB: TTR
C.
Mock
D18G
A25T
L55P
V30M
TMP - - + - + - + - +
1048
720
480
240
146
CN-PAGE
IB:M2 FLAG

Table S1 – Supplement to Figure 2: Table showing the biophysical parameters for the TTR variants used in this manuscript. These parameters include the midpoint for urea denaturation (C_m), the $t_{1/2}$ for TTR tetramer dissociation/unfolding, the optimal pH for fibril formation and the combined stability score. These parameters were previously reported in (Sekijima et al., 2005).

	Urea Denaturation Midpoint (C_m)	Tetramer Dissociation/ Unfolding ($t_{1/2}$; h)	Optimal pH for Fibril Formation	Combined Stability Score
T119M	3.7	1533.5	3.5	1.16
WT	3.4	41.3	4.2	1
V122I	3.4	19	4.2	0.97
V30M	2	67.9	4.4	0.79
L55P	2.3	4.41	5	0.75
A25T	1.96	0.035	5	0.55
D18G	1.93	0.0000278	5.4	0.32

SUPPLEMENTAL REFERENCES

Sekijima, Y., Wiseman, R.L., Matteson, J., Hammarstrom, P., Miller, S.R., Sawkar, A.R., Balch, W.E., and Kelly, J.W. (2005). The biological and chemical basis for tissue-selective amyloid disease. Cell *121*, 73-85.
Shoulders, M.D., Ryno, L.M., Genereux, J.C., Moresco, J.J., Tu, P.G., Wu, C., Yates, J.R., 3rd, Su, A.I., Kelly, J.W., and Wiseman, R.L. (2013). Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER proteostasis environments. Cell Rep *3*, 1279-1292.