Online Supplemental Data

Supplemental Figure 1. Acute misfolded secretory protein stress does not necessarily disrupt or significantly perturb ER structure. Cos7, MDCK, U2-OS, and HepG2 cells transiently transfected 16h with ER-GFP were untreated, treated with indicated amounts of DTT or Tm for indicated times and imaged live by confocal microscopy. Only Cos7 cells tolerated 10mM DTT, while MDCK cells contracted at 1h and rounded up and died by 3h. 5mM DTT was well tolerated by MDCK, U2-OS, and HepG2 cells for 1h. 3h of 5mM DTT tended to lead to HepG2 cell contraction. Cell morphology was not significantly altered at 5h of Tm treatment. Scale bar = 10μm.

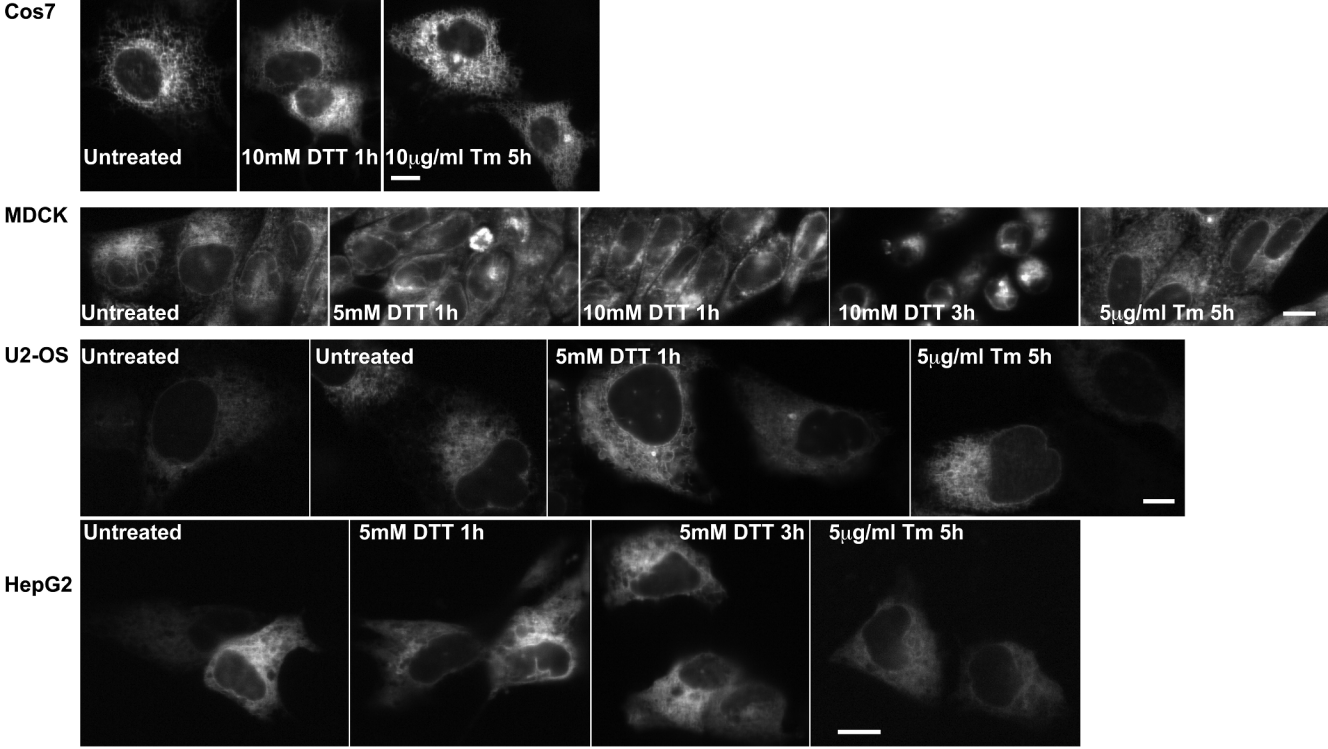
Supplemental Figure 2. Calreticulin-GFP availability does not decrease with Tm treatment. HeLa cells were transiently transfected overnight with Calreticulin-GFP (Crt-GFP) and ER-RFP or Crt-GFP and BiP-mCherry. FRAP analysis of both proteins in the red and green channels in the same photobleach ROI was followed with diffusion analysis. (A) Plot of *D* values of Crt-GFP and ER-RFP in untreated (filled squares) and Tm (5μg/ml for 5h) (open circles). Each symbol represents a single cell. Tm treatment has little affect on ER-RFP mobility, but significantly increases Crt-GFP mobility, consistent with a loss of glycosylated substrates for calreticulin. (B) Plot of *D* values of Crt-GFP and BiP-mCherry cells untreated (open diamonds) or treated with Tm (5μg/ml for 5h) (filled triangles). As in A, Crt-GFP mobility increases, but BiP-mCherry mobility significantly decreases following Tm treatment. Thus, the decrease in BiP-mCherry is specific to this ER chaperone, because the neither the inert ER-RFP or other ER chaperone Crt-GFP exhibit corresponding decreases in mobility following Tm treatment.

Supplemental Figure 3. BiP-GFP expression level does not affect *D* values. All of the cells used in Figure 5D were analyzed for both their *D* values and the microscope camera gain settings (intensities) used to capture each cell image. Image acquisition time and laser power settings were not changed between samples. Gain is a measure of how much camera sensitivity needs to be amplified to image a cell. Higher gain is needed to image dimmer samples. To emphasize the inverse relationship between gain and fluorescence intensity, the Y-axis of the plot has been inverted. The bins used in Figure 6A were also used in this figure, for untreated up through the 41-60min bins. After *D* values had stabilized, all later *D* values were binned into a single group (61-130 min). For all times, similar *D* values can be observed over a wide range of intensity settings. In addition, different *D* values can be detected for cells with the same intensity value. During the time of decrease in BiP-GFP *D* values in Tm treated cells (21-60 min), low intensity cells (high gain) do not exhibit decreased *D* relative to more intense cells (low gain). Even the dimmest cells exhibit a wide range of *D* values. Therefore, our data are inconsistent with a correlation between BiP-GFP mobility and expression levels. Thus, variability in BiP-GFP mobility in different cells supports the interpretation that BiP-GFP mobility corresponds to differences in unfolded protein burdens in each cell.

Supplemental Figure 4. Induction of BiP upregulation with a range of Tm concentrations. MDCK cells were treated with 0-500ng/ml of Tm for 24h, lysed, separated by SDS-PAGE, and immunoblotted with an anti-BiP antibody or anti-α tubulin

antibody. While comparable levels of tubulin are observed in all lanes, all Tm treated lanes exhibit a significant increase in BiP levels, indicative of a cellular response to ER stress. Surprisingly, BiP expression for low doses of Tm is similar to the highest doses of Tm used. Note that most groups use ten times as much Tm (5000ng/ml). Molecular weight marker positions are indicated to left of blots.

Supplemental Figure 5. BiP-GFP stable and transient transfection. (A) Confocal micrograph of Maximum Intensity projection of MDCK cells expressing exceptionally high levels of BiP-GFP. Clusters of fluorescence accumulate on the nuclear envelope and perinuclear structures. Cells exhibiting nuclear envelope accumulations were not used for FRAP studies. Scale bar = 10μm. (B) Comparison of *D* values of individual moderate to low expressing MDCK cells (filled circles) transiently transfected with BiP-GFP for 24h or stably transfected. The differences in the mean *D* values (graph bars) were not statistically significant using a student's *t*-test (p=0.1301, variances were not significantly different by F test).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10mM DTT 1h
10μg/ml Tm 5h
MDCK
Untreated
5mM DTT 1h
10mM DTT 1h
10mM DTT 3h
5μg/ml Tm 5h
U2-OS
Untreated
Untreated
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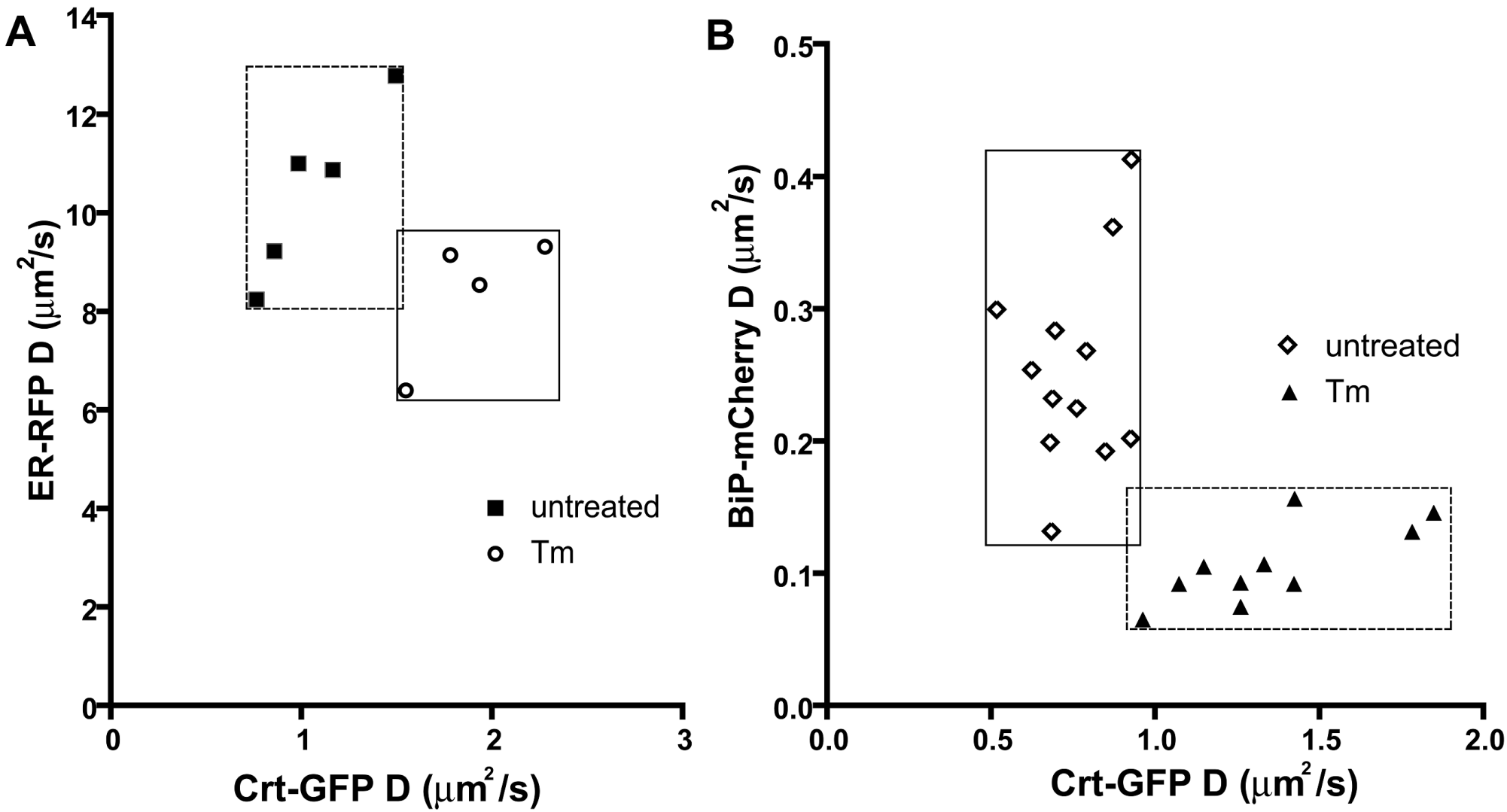
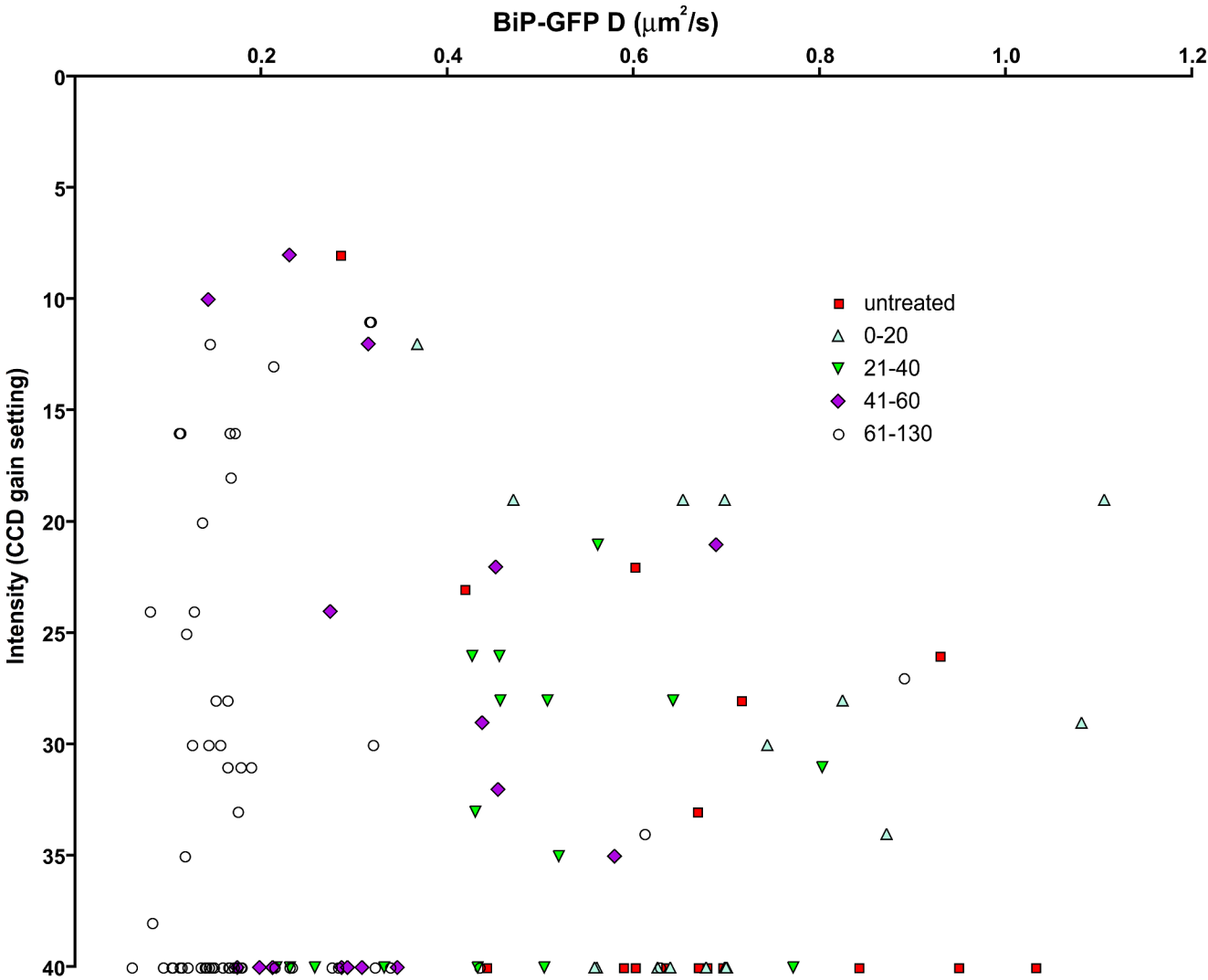
5μg/ml Tm 5h
HepG2
Untreated
5mM DTT 1h
5mM DTT 3h
5μg/ml Tm 5h


A
ER-RFP D (μm²/s)
Crt-GFP D (μm²/s)
untreated
Tm
B
BiP-mCherry D (μm²/s)
Crt-GFP D (μm²/s)
untreated
Tm


BiP-GFP D (μm²/s)
0.2
0.4
0.6
0.8
1.0
1.2
Intensity (CCD gain setting)
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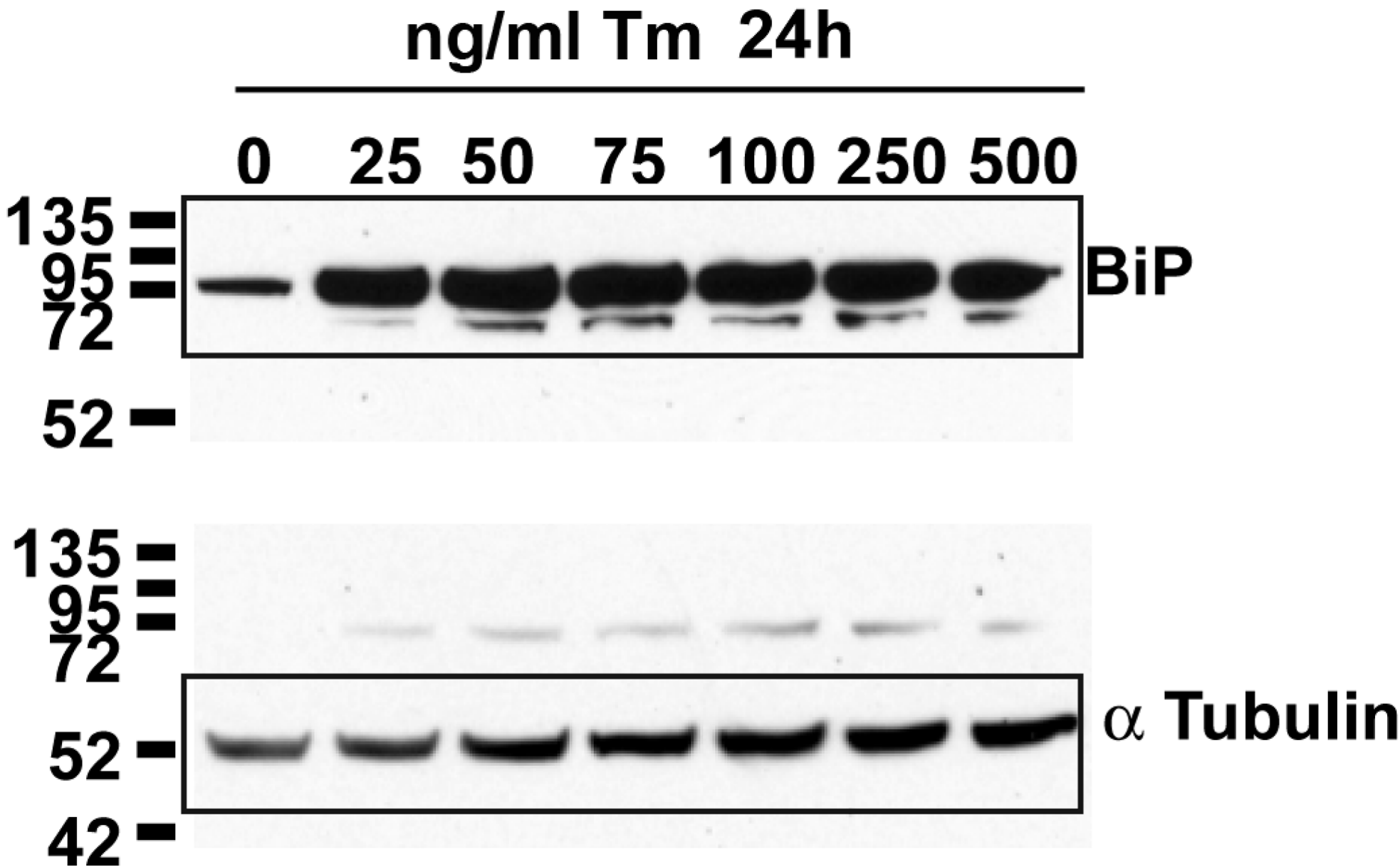
5
10
15
20
25
30
35
40
untreated
0-20
21-40
41-60
61-130


ng/ml Tm 24h
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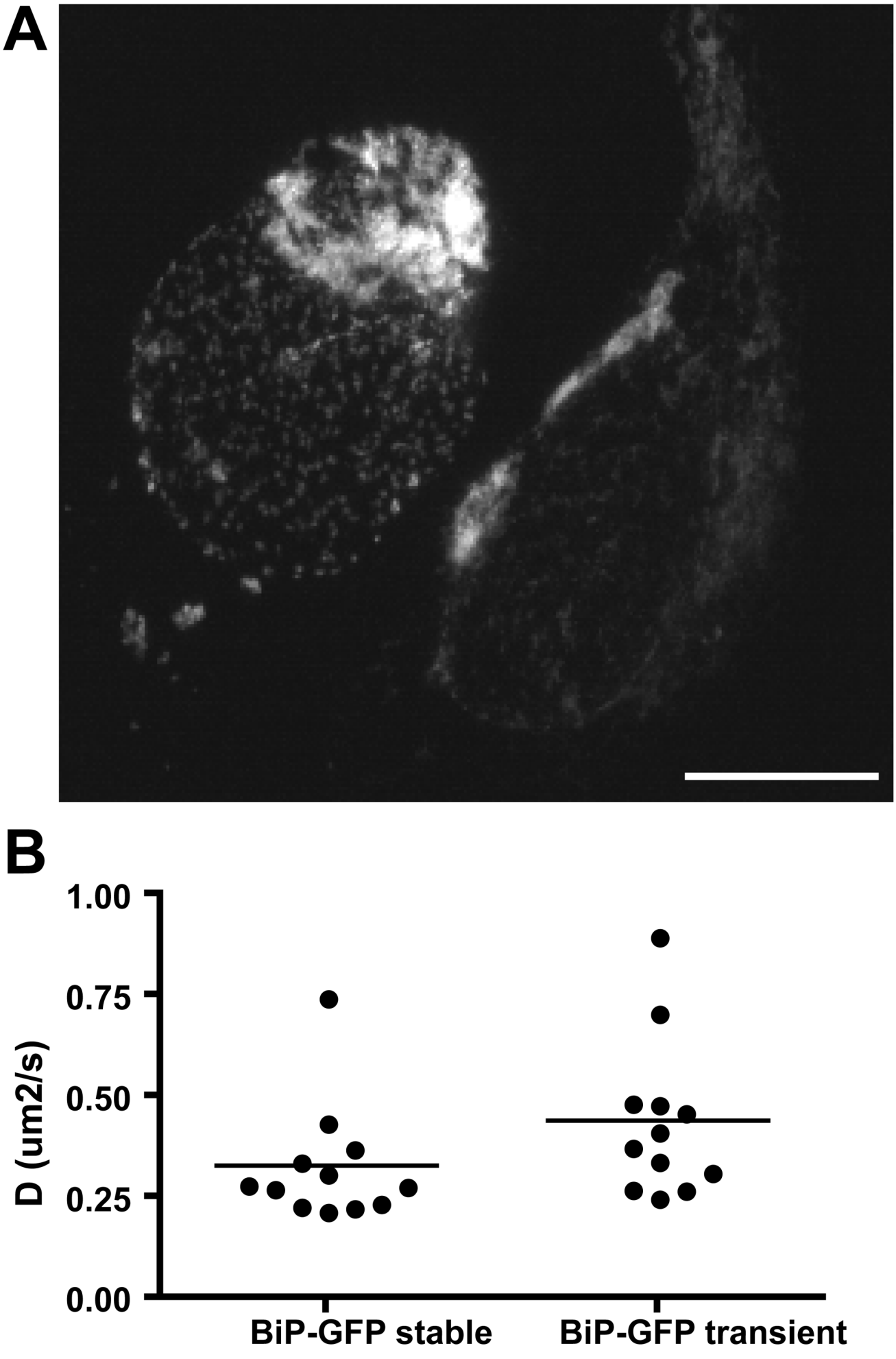
25
50
75
100
250
500
135
95
72
52
BiP
135
95
72
52
42
α Tubulin


A
B
1.00
0.75
0.50
0.25
0.00
D (um2/s)
BiP-GFP stable
BiP-GFP transient