Supplement

Fig. S1. Effect of puromycin on aggresome formation in MCF10A cells is distinct from effects of other inhibitors of protein synthesis. MCF10A cells stably expressing Syn-GFP were incubated, as indicated, with or without 10 μM MG132, 7.35 μM (4 μg/ml) puromycin, 10 μM emetine and 71 μM cycloheximide for 2 hours and the images were gained with a fluorescence microscope. The aggresomes are seen as bright foci in perinuclear area with nuclei seen as Syn-GFP exclusion zones. The scale bar is 20 μm.

Fig. S2. Effect of puromycin on aggresome formation in HeLa cells is distinct from the effects of other inhibitors of protein synthesis. HeLa cells stably expressing Syn-GFP were incubated with the indicated additions (same concentrations as in (Fig. 1A)) for 3 hours and a fraction of cells with aggresomes was counted.

Fig. S3. Viral deubiquitinase PLpro prevents distribution of mRFP-Ub to aggresomes. HeLa cells stably expressing mRFP-Ub were transiently co-transfected with two plasmids encoding PLpro and GFP. The cells which accumulated GFP were considered transfected and expressing PLpro (*), the cells without the reporter were considered untransfected (**O**) and used as a control. Cells were incubated with or without 10 μM MG132 for 6 (for mRFP-Ub) and images were gained with a fluorescence microscope. The scale bar is 20 μm.

Fig. S4. Comparison of aggresomes and stress granules. MCF10A cells stably expressing Syn-GFP were incubated, as indicated, with 0.5 mM sodium arsenite, an inducer of stress granule, for 75 min or with 10 μM MG132 with or without 2 μM hippuristanol for 3 hours and then fixed. To detect stress granules, we immuno-stained fixed cells with antibody against a translation factor eIF3, which is a reliable marker of these bodies. Cells were analyzed with a fluorescence microscope. The proteasome inhibition led to appearance of aggresomes, but did not trigger stress granules; exposure to arsenite promoted efficient formation of the stress granules, which did not show any co-localization with the few aggresomes, which appeared under these conditions. Hippuristanol also robustly triggered stress granules, but completely prevented MG132-unduced formation of the aggresomes. These data exclude the possibility that stress granules are related to the aggresome formation. The scale bar is 20 μm.

Fig. S5. Concurrence of active translation and proteasome inhibition is required for an aggresome-triggering signal. MCF10A cells stably expressing Syn-GFP were incubated with 5 μM MG132 with or without 71 μM cycloheximide for indicated times. Then some of the samples were thoroughly washed and left in a regular medium to reach 3 hours of cumulative incubation.

Fig. S6. Exposure to puromycin increases the cellular pool of DRiPs and lowers their length. HeLa cells were incubated under indicated conditions for 3.5 hrs, and the levels of ubiquitinated species were assessed in cell lysates by immunoblotting.

Fig. S7. Balance between inhibition of proteasome and inhibition of translation determines the extent of aggresome formation. MCF10A cells stably expressing Syn-GFP were incubated with

indicated concentrations of MG132 and emetine for 3.5 hrs, and the cells with aggresomes were counted.

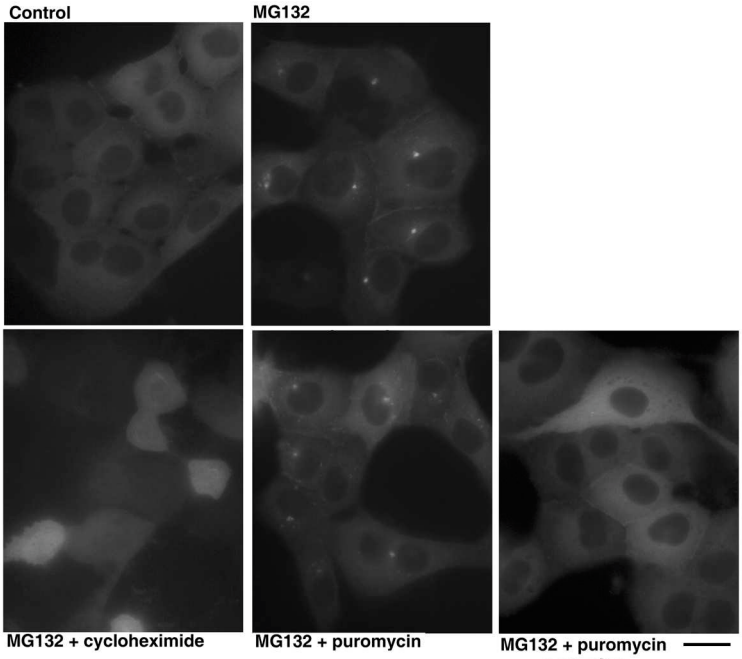
Control
MG132
MG132 + cycloheximide
MG132 + puromycin
MG132 + puromycin
+ emetine

Fig. S1

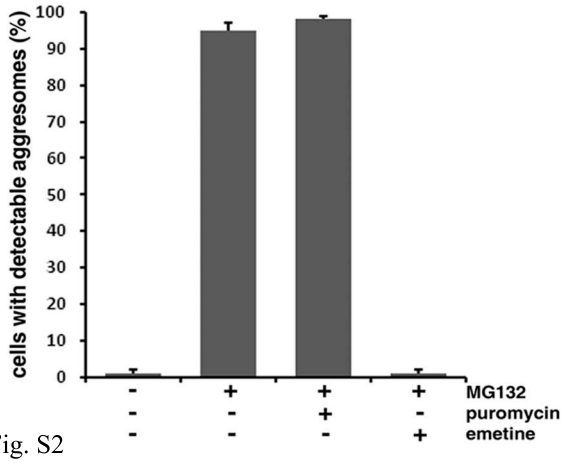
cells with detectable aggresomes (%)
100
90
80
70
60
50
40
30
20
10
0
- + + +
MG132
- - + -
puromycin
- - - +
emetine

Fig. S2

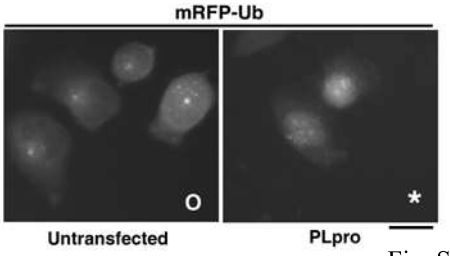

Fig. S3

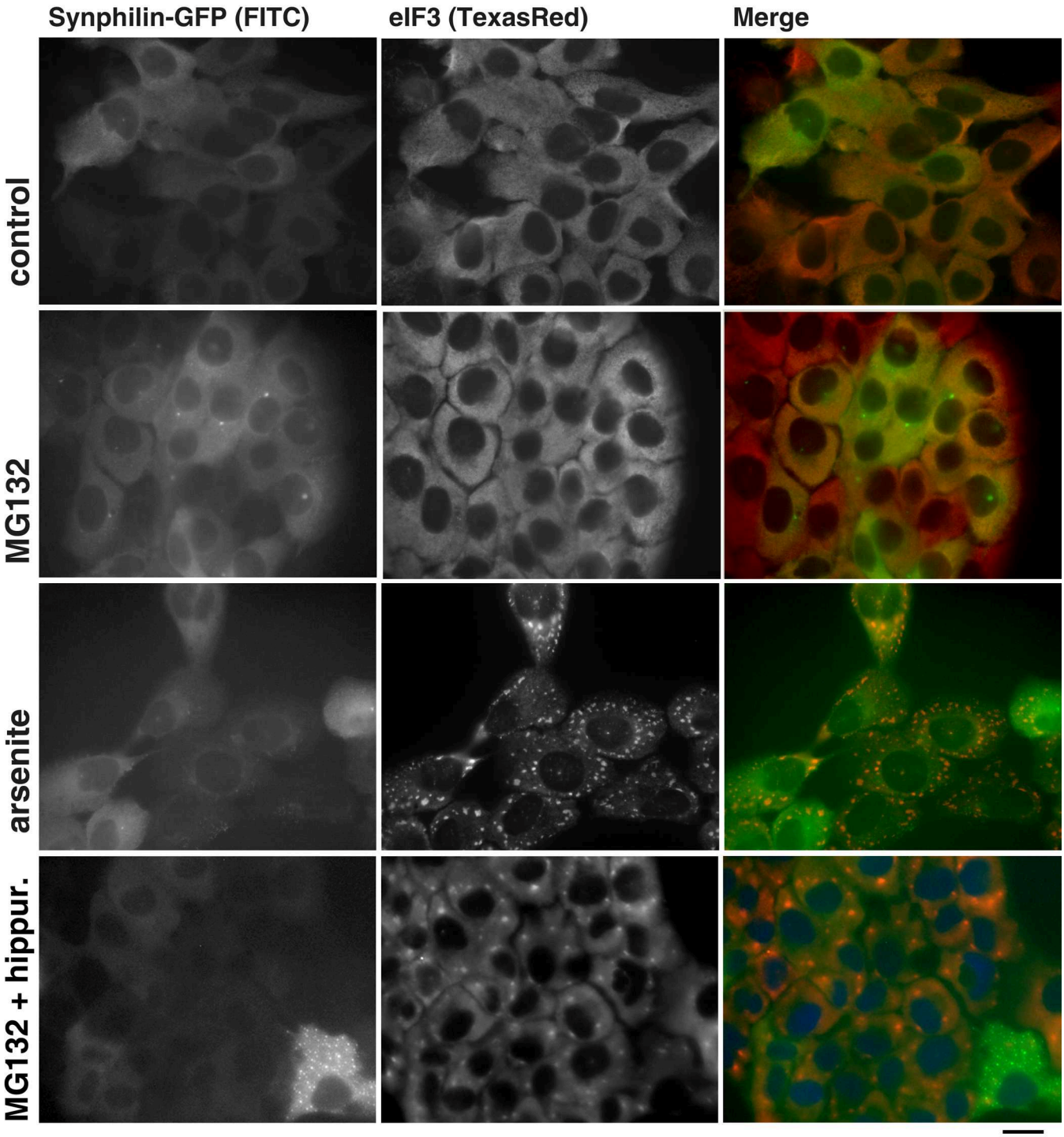
Synphilin-GFP (FITC)
eIF3 (TexasRed)
Merge
control
MG132
arsenite
MG132 + hippur.

Fig. S4

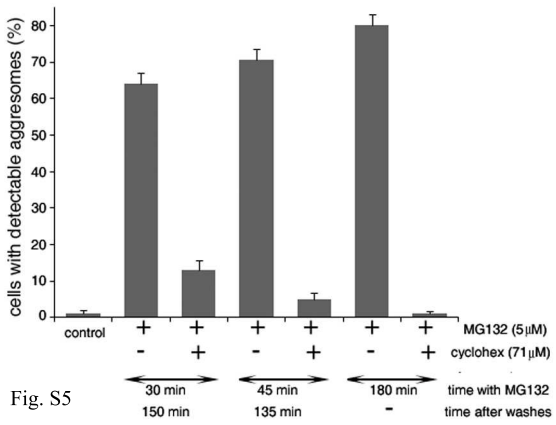
cells with detectable aggresomes (%)
80
70
60
50
40
30
20
10
0
control
MG132 (5 μM)
cyclohex (71 μM)
30 min
150 min
45 min
135 min
180 min
-
time with MG132
time after washes

Fig. S5

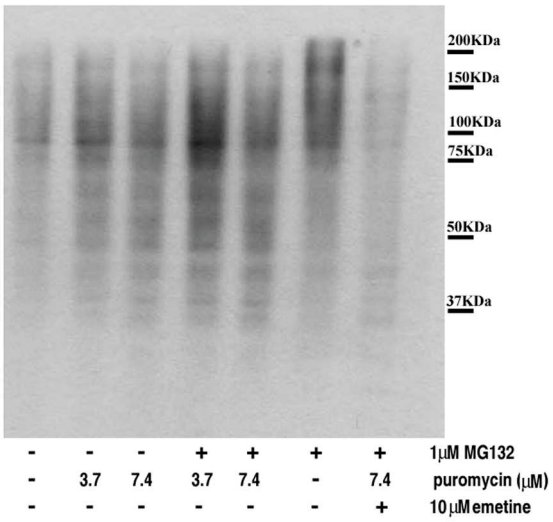
200KDa
150KDa
100KDa
75KDa
50KDa
37KDa
- - - + + + + 1μM MG132
- 3.7 7.4 3.7 7.4 - 7.4 puromycin (μM)
- - - - - - + 10μM emetine

Fig. S6

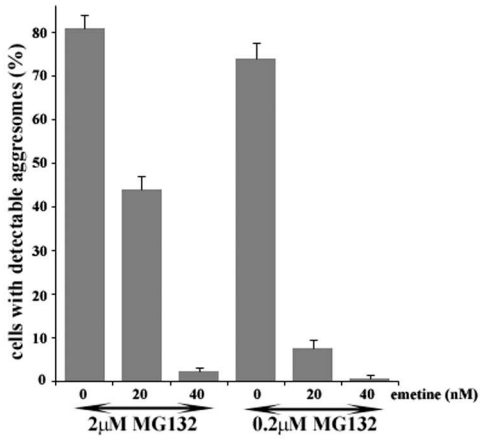
cells with detectable aggresomes (%)
80
70
60
50
40
30
20
10
0
0
20
40
0
20
40
emetine (nM)
2μM MG132
0.2μM MG132

Fig. S7