Supplementary data 1. (A) 4EGI disrupts eIF4E-4GI association as assessed by cap-binding assays. HeLa cells were left untreated or were treated with 4EGI (250 μM) for 7h. Cells were lysed and then incubated with m^7GTP-Sepharose beads. Eluted proteins were analysed by Western blot using specific antibodies. Total = 1% of the input used for cap pull-down. (B-C) Rescue experiments. HeLa cells were treated with eIF4E-selective siRNAs then transfected with a pcDNA vector encoding murine eIF4E fused to 2 Flags. (B) Cells were then treated with 150 μM arsenite, fixed, permeabilized and then processed for immunofluorescence using anti-eIF4E (green) and anti-G3BP (red) antibodies. Cells overexpressing Flag-eIF4E are shown. (C) Cells were lysed and protein extracts were prepared and analyzed by Western blot to detect eIF4E. (D-F) Depletion of either eIF4E or mTOR impairs formation of SG in MCF-7. MCF-7 cells were treated with either non-specific siRNAs or eIF4E-selective siRNAs or mTOR-specific siRNA for 96 h. (D) Cells were then treated with 250 μM arsenite for 1 h. Cells were fixed, permeabilized and then processed for immunofluorescence to detect SG using anti-FMRP and anti-G3BP1 antibodies. Pictures were taken using a 63X objective with a 1.5 zoom. The percentage of cells harboring SG (> 3 granules/cell) from 5 different fields and 5 different experiments containing a total of 2 x 10^3 cells is indicated at the bottom of the merged images. (E-F) Cells were lysed and protein extracts were prepared and analyzed by Western blot to detect eIF4E (E) and mTOR (F). Tubulin serves as a loading control. The percentage of eIF4E and mTOR knockdown was determined as above by densitometry quantification of the film signal and expressed as a percentage of total eIF2α. The results are representative of 3 different experiments.

Supplementary data 2. *Localization of mTOR in SG*. Cells were treated with non-specific or mTOR-selective siRNAs for 96 h and then were incubated with either 150 μM arsenite for 1 h or with 2 μM bortezomib for 4 h. Cells were fixed, permeabilized and then processed for immunofluorescence to detect SG using anti-FMRP. Anti-mTOR antibodies were used to assess mTOR localization (left panels) and its depletion (right panels). The percentage of cells harboring SG (> 3 granules/cell) from 5 different fields and 5 different experiments containing a total of 2 x 10^3 cells is indicated at the bottom of the merged images.

Supplementary data 3. *PP242 and Torin1inhibit SG formation*. (A-B) MCF-7 cells were treated with either pp242 (2. 5 μM) for 7 h, arsenite (250 μM) for 1 h, or were incubated with pp242 (2.5 μM) for 6 h before adding arsenite (250 μM) for an additional 1 h. (A) Cells were then processed for immunofluorescence to detect SG using anti-FMRP and anti-G3BP1 antibodies. Pictures were taken using a 63X objective with a 1.5 zoom. The percentage of cells harboring SG (> 3 granules/cell) from 5 different fields and 5 different experiments containing a total of 2 x 10^3 cells is indicated at the bottom of merged images. (B) MCF-7 cells were lysed and protein extracts were prepared and analyzed by Western blot for 4E-BP1 and phospho-4E-BP1 (S65). The results are representative of three different experiments. (C-D) HeLa cells were pretreated either with Torin 1 (0.1 μM) for 4 h and then incubated with 2 μM bortezomib for 4 h (Torin 1+Bortezomib). As controls, cells were treated with 2 μM bortezomib for 4 h (Bortezomib). (C) Cells were processed for immunofluorescence to detect SG using anti-FMRP and anti-G3BP1 antibodies. Pictures were taken using a 63X objective with a 1.5 zoom. The percentage of cells harboring SG (> 3 granules/cell) from 5 different fields

and 3 different experiments containing a total of 1 x 10^3 cells is indicated at the bottom of merged images. (D) Cells were lysed and protein extracts were prepared and analyzed by Western blot to detect 4E-BP1. Tubulin serves as a loading control. The results are representative of 3 different experiments.

Supplementary data 4. *The SG inhibitor pp242 has a modest effect on general translation initiation*. HeLa were left untreated (left panels) or were treated with either pp242 (2.5 μM) for 6 h, arsenite (150 μM) for 1 h, or bortezomib (2 μM) for 4 h, or were incubated with pp242 (2.5 μM) for 6 h before adding arsenite (150 μM) for an additional 1 h, or were incubated with pp242 (2.5 μM) for 2 h before adding bortezomib (2 μM) for an additional 4 h. (A) Sucrose gradient analysis of polysomes. Cytoplasmic extracts were prepared and sedimented through sucrose density gradients, which were then processed as described in "Material and Methods". The results are representative of 3 different experiments. (B) Western blot analysis of the input used in polysome analysis. The indicated proteins were detected using their corresponding antibodies.

Supplementary data 5. (A-B) *Depletion of 4E-BP1 rescues bortezomib-SG formation and eIF4E-4GI interaction during mTOR inhibition*. (A) HeLa cells were left untreated or were treated either with pp242 (2.5 μM) for 6 h, or with bortezomib (2 μM) for 4 h, or were incubated with pp242 (2.5 μM) for 2 h before adding bortezomib (2 μM) with pp242 (2.5 μM) for an additional 4 h. Cells were then processed for immunofluorescence to detect SG using anti-FMRP (green). Depletion of 4E-BP1 was assessed using anti-4E-BP1 antibodies (red). Pictures were taken using a 63X objective with a 1.5 zoom. The percentage of cells harboring SG (> 3 granules/cell) from 5 different fields and 3 different experiments containing a total of 1 x 10^3 cells is indicated at the bottom of merged

images. (B) Cells were treated with non-specific or 4E-BP1-selective siRNAs for 96 h. Following treatment with pp242 (2.5 μM) for 6 h, cells were lysed and the cell extract was incubated with m^7GTP-Sepharose beads. Eluted proteins were analyzed by Western blot using anti-eIF4E and anti-eIF4GI antibodies. Depletion of 4E-BP1 was assessed using anti-4E-BP1 antibodies. Total= 1% of the input used for cap pull-down. (C-E) *Depletion of 4E-BP2 does not rescue SG formation during mTOR inhibition*. (C) HeLa cells were left untreated or were treated either with pp242 (2.5 μM) for 7 h, or with arsenite (150 μM) for 1 h, or were incubated with pp242 (2.5 μM) for 6 h before adding arsenite (150 μM) with pp242 (2.5 μM) for an additional hour. Cells were then processed for immunofluorescence to detect SG using anti-FMRP (green) and anti-G3BP1 (red) antibodies. Pictures were taken using a 63X objective with a 1.5 zoom. The percentage of cells harboring SG (> 3 granules/cell) from 5 different fields and 3 different experiments containing a total of 1 x 10^3 cells is indicated at the bottom of merged images. (D) Depletion of 4E-BP2 was assessed qRT-PCR of 4E-BP2 mRNA. Cells were collected and total RNA content was then isolated. The amount of 4E-BP2 was quantified by qRT-PCR relative to GAPDH mRNA using the ΔΔCt method. Results are expressed as the mean ± SEM (error bars) of triplicate measurement. (E) Cells were treated with non-specific or 4E-BP2-selective siRNAs for 96 h. Following treatment with pp242 (2.5 μM) for 6 h, cells were lysed and the cell extract was incubated with m^7GTP-Sepharose beads. Eluted proteins were analyzed by Western blot using anti-eIF4E, anti-eIF4GI, and anti-4E-BP1 antibodies. Total= 1% of the input used for cap pull-down. (F) *4E-BP1 antagonizes eIF4E-eIF4GI association*. HeLa cells were transfected with either wild type (WT) HA-4E-BP1 or HA-4E-BP1-4A, or with HA-4E-BP1-Δ4E for 48 h. Cells were

lysed and then incubated with m^7GTP-Sepharose beads. Eluted proteins were analysed by Western blot using specific antibodies. Total= 1% of the input used for cap pull-down. (G) HeLa cells were transfected with either wild type HA-4E-BP1 or with pcDNA. Cells were then left untreated, or were treated with arsenite (150 μM) for 1h before been harvested and lysed to prepare cytoplasmic (C) and nuclear (N) fractions. Western blot analysis was performed to detect eIF4E and 4E-BP1 using specific antibodies. The anti-tubulin (cytoplasmic marker), -Histone3 (nuclear marker) antibodies were used to assess equal protein loading among cellular fractions.

Supplementary data 6. *Depletion of eIF4GI impairs formation of SG*. HeLa Cells were treated with non-specific or eIF4G-I-specific siRNAs for 96 h. Cells were treated with 150 μM arsenite for 1 h. Cells were fixed, permeabilized and then processed for immunofluorescence to detect SG using anti-FMRP antibodies. Anti-eIF4GI antibodies were used to assess eIF4GI depletion. Circle denotes eIF4GI-depleted cells. Pictures were taken using a 63X objective with a 1.5 zoom. The percentage of cells harboring SG was calculated as described in Figure 2.

Supplementary data 7. *PP242 sensitizes MCF-7 cells to bortezomib-mediated apoptosis in vitro*. (A) MCF-7 cells were incubated with either pp242 (2.5 μM), bortezomib (2 μM), or both compounds for 18 h. Cells were then lysed and proteins resolved on SDS-PAGE and analyzed by Western blot for p21 expression. Tubulin serves as a loading control and 4E-BP1 was used to verify mTORC1 inactivation with pp242. (B) qRT-PCR of p21 mRNA. Following treatment with bortezomib (2 μM), pp242 (2.5 μM), or both, cells were collected and total RNA content was then isolated. The amount of p21 was quantified by real time-PCR relative to GAPDH mRNA using the ΔΔCt method. Results

are expressed as the mean ± SEM (error bars) of triplicate measurements. (C) Cells were treated as in (A), collected and then stained with annexin V-FITC and PI, and analyzed by flow cytometry. The percentage of total dead or dying cells (indicated at the top of each panel) was defined as the sum of early (*lower right box*) and late (*upper right box*) apoptosis and corresponds to the mean ± SEM from three independent experiments. V: viable cells; E.A: early apoptosis; L.A: late apoptosis.