

Figure S1: Gle1 is phosphorylated in the N-terminal region. (A) K252a treatment inhibits Gle1 phosphorylation. HeLa cells were treated with indicated doses of K252a for 60 min, then either maintained at 37°C or heat shocked at 45°C for 60 min. Cells lysates were resolved by phos-tag SDS-PAGE and analyzed by immunoblotting using anti-Gle1 antibodies. (B) HeLa cells were transfected with plasmids expressing either the N-terminal region of Gle1 (residues 1-400; GFP-Gle1¹⁻⁴⁰⁰), C-terminal region of Gle1 (residues 401-698; GFP-Gle1⁴⁰¹⁻⁶⁹⁸) or Gle1 lacking the first 120 residues (GFP-Gle1¹²¹⁻⁶⁹⁸). 24 h post transfection, cells were heat shocked at 45°C, exposed to 0.5mM sodium arsenite at 37°C for 60 min in the presence or absence of K252a, or left untreated at 37°C. Cell lysates were resolved by phos-tag SDS-PAGE and immunoblotted with anti-GFP antibodies.

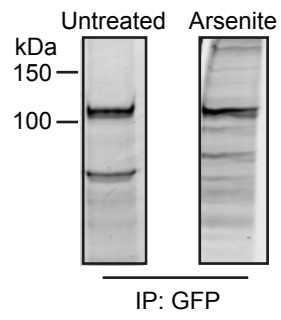


Figure S2: GFP-Gle1A is enriched from HeLa cells by immunoprecipitation. (A-B) HeLa cells transfected with *GFP-GLE1A* were either (A) untreated or (B) treated with 0.5mM sodium arsenite at 37°C for 60 min. and immunoprecipitated using GFP beads. Immunisolated complexes were resolved by SDS-PAGE and stained with colloidal blue.

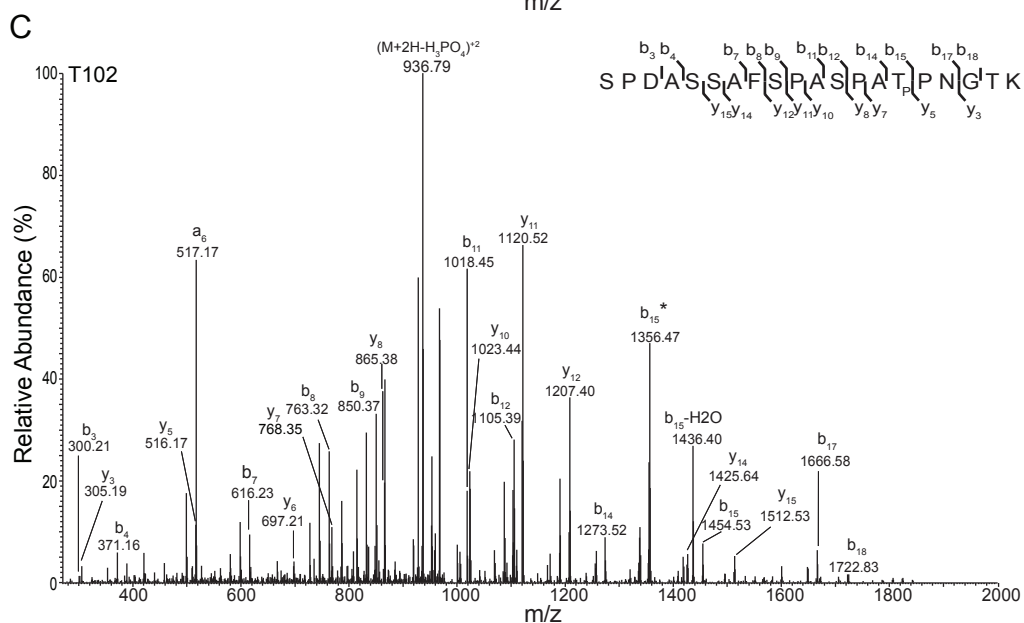
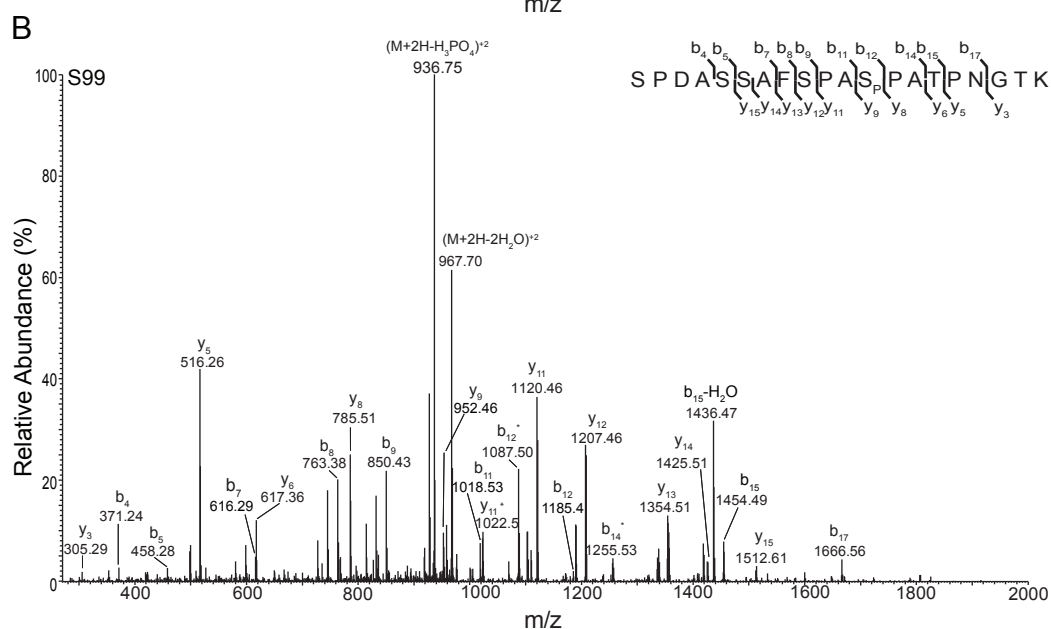
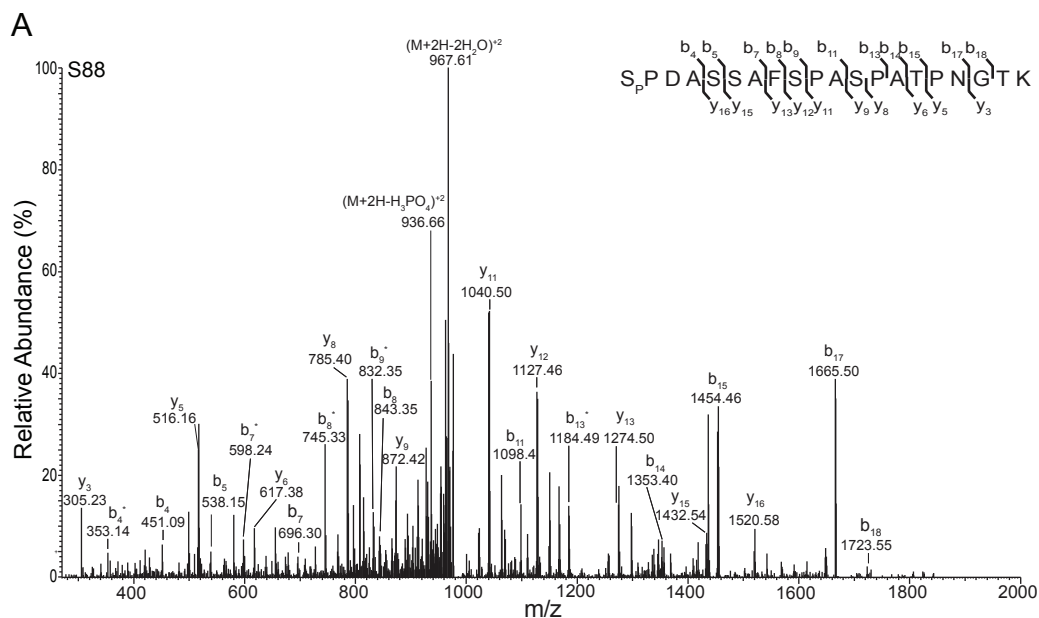


Figure S3: Gle1A is phosphorylated at S88, S99 and T102. A tandem mass spectrum of a Gle1 peptide spanning residues 88-107 shows phosphorylation at (A) S88, (B) S99 and (C) T102. The subscript “p” in the identified amino acids denotes the phosphorylated serine or threonine residue, and brackets indicate sites of collision-induced dissociation (CID) at the peptide backbone. Observed b- and y-type product ions are assigned to their corresponding m/z peaks in the mass spectrum, and asterisks (*) above certain peaks in the spectrum denote product ions that have undergone neutral loss of H_3PO_4 .

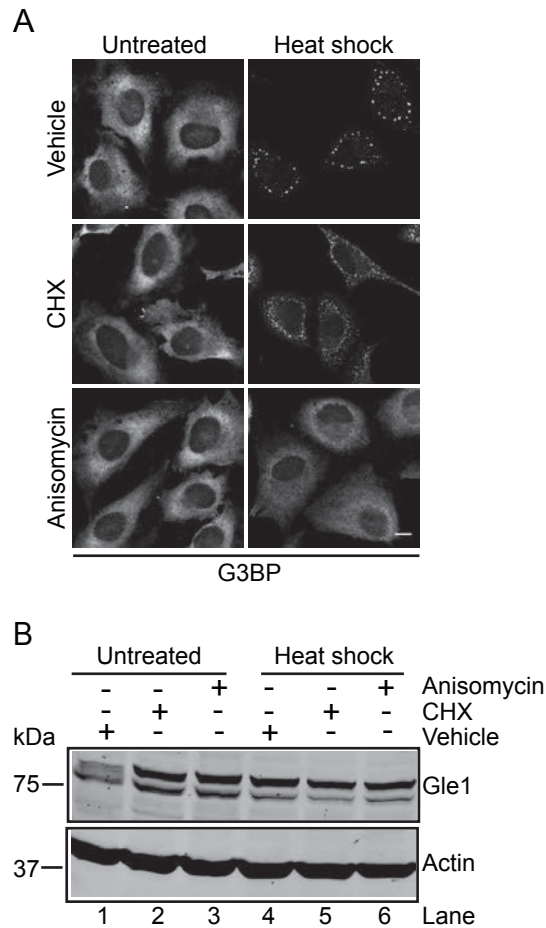


Figure S4: Gle1 phosphorylation is stimulated by translation inhibition independent of SG assembly. (A) Treatment of HeLa cells with cycloheximide or anisomycin inhibits SG formation. Cells were either untreated or heat shocked at 45°C for 60 min in the presence of vehicle, cycloheximide (CHX) or anisomycin, and processed for immunofluorescence using anti-G3BP antibodies. Scale bar represents 10µm. (B) Gle1 is phosphorylated in response to translation inhibition irrespective of stress. HeLa cells treated as in (A) were lysed and analyzed by immunoblotting using anti-Gle1 and anti-actin antibodies.

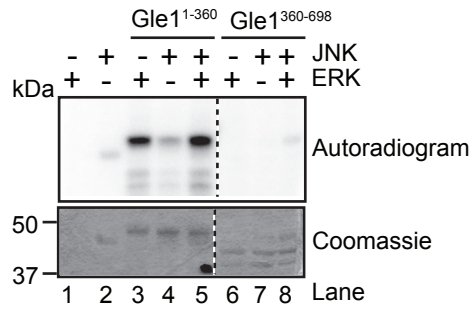


Figure S5: The Gle1 C-terminal region is not phosphorylated by MAPK kinases. Bacterially expressed and purified recombinant Gle1¹⁻³⁶⁰ or Gle1³⁶⁰⁻⁶⁹⁸ proteins were incubated with recombinant active ERK or/and JNK kinases in the presence of radioactive [γ -³²P]-ATP. Reactions were terminated by addition of Laemmli sample denaturation buffer and resolved on a SDS-PAGE gel. Radioactivity incorporation was measured by autoradiography. Coomassie stained gel demonstrates loading of proteins.

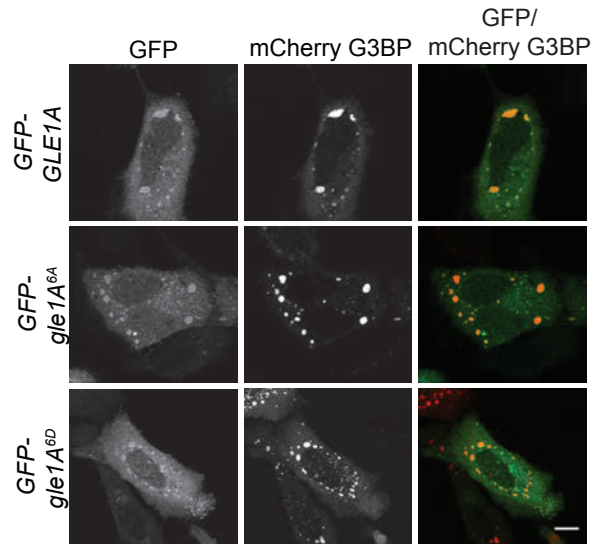


Figure S6: *gle1A^{6A}* and *gle1A^{6D}* are recruited to SGs in response to heat shock. HeLa cells were transfected with plasmids expressing *GFP*, *GFP-gle1A^{6A}* or *GFP-gle1A^{6D}* and *mCHERRY-G3BP*. Cells were heat shocked at 45°C for 45 min and imaged live by confocal microscopy. Images shown are representative of three independent experiments. Scale bar, 10 μ m.

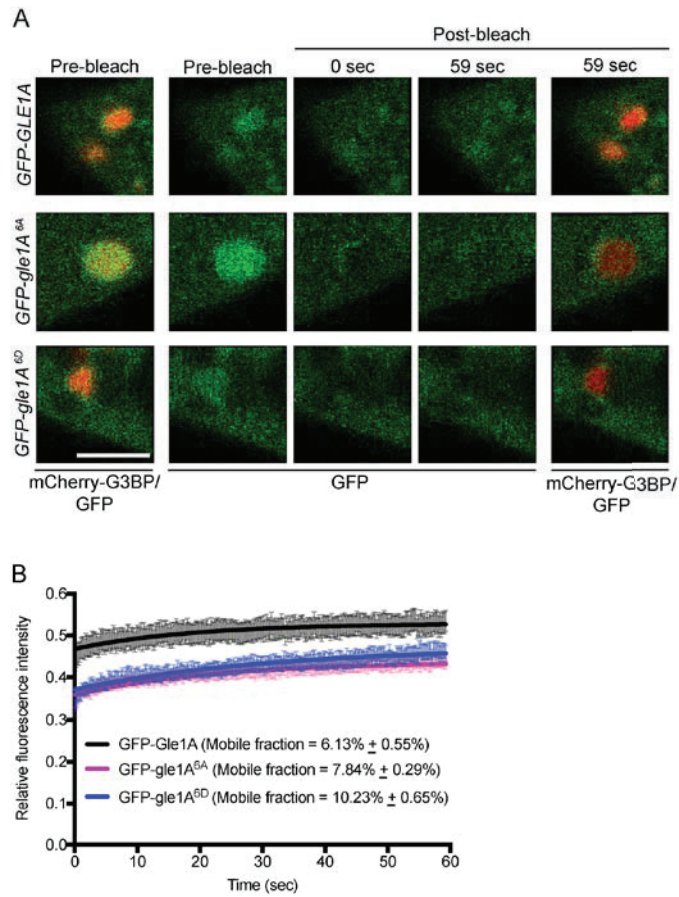


Figure S7: GFP-Gle1A is a stable component of SGs. (A-B) FRAP analysis of Gle1A was conducted in heat shocked HeLa cells co-transfected with *mCHERRY-G3BP* and *GFP-GLE1A*, *GFP-gle1A^{6A}* or *GFP-gle1A^{6D}* plasmids. Cells were heat shocked at 45°C for 45 mins prior to FRAP assay at 45°C. Stress granules containing mCherry-G3BP and either GFP-Gle1A, GFP-gle1A^{6A} or GFP-gle1A^{6D} were bleached using Argon488 laser and imaged for recovery up to 59 sec. Images are representative of three independent experiments, data was quantified for at least 19 cells per condition, and analyzed as in Figure 6. Error was calculated as the standard error of the mean. Scale bar, 5µm.