Supplemental Materials

Molecular Biology of the Cell

Aditi et al.

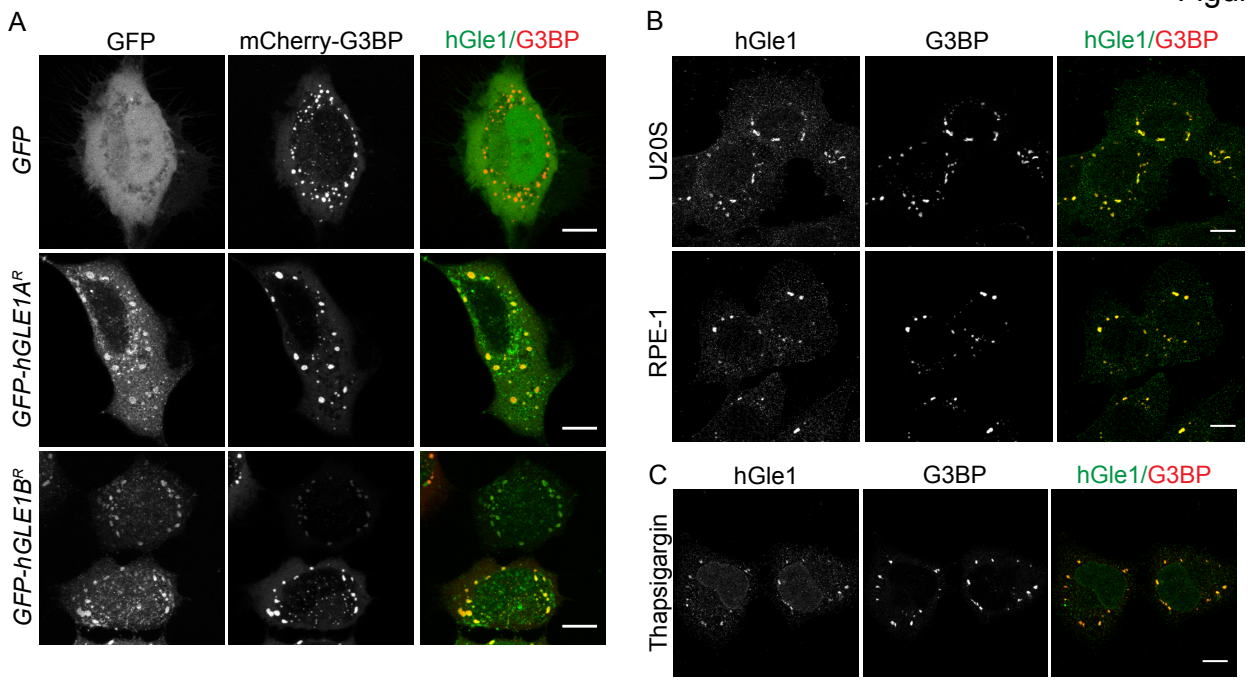

Figure S1. Endogenous hGle1 and exogenously expressed hGle1A and hGle1B are recruited to SGs upon stress. (A) Both hGle1A and hGle1B are recruited to SGs upon heat shock. Plasmids expressing EGFP, EGFP-hGle1A^R or EGFP-hGle1B^R and mCherry-G3BP proteins were co-expressed in HeLa cells. Cells were heat shocked at 45°C for 45 min and imaged live by confocal microscopy. Scale bar =10µm. (B) hGle1 localization in SGs is not cell type specific. U2OS and RPE-1 cells were heat shocked at 45°C for 60 min and processed for immunofluorescence using anti-hGle1 and G3BP antibodies. Scale bar =10µm. (C)hGle1 localization in SGs is not stress type specific. HeLa cells were treated with 10µM thapsigargin for 60 min at 37°C and processed for immunofluorescence using anti-hGle1 and G3BP antibodies. Scale bar =10µm.

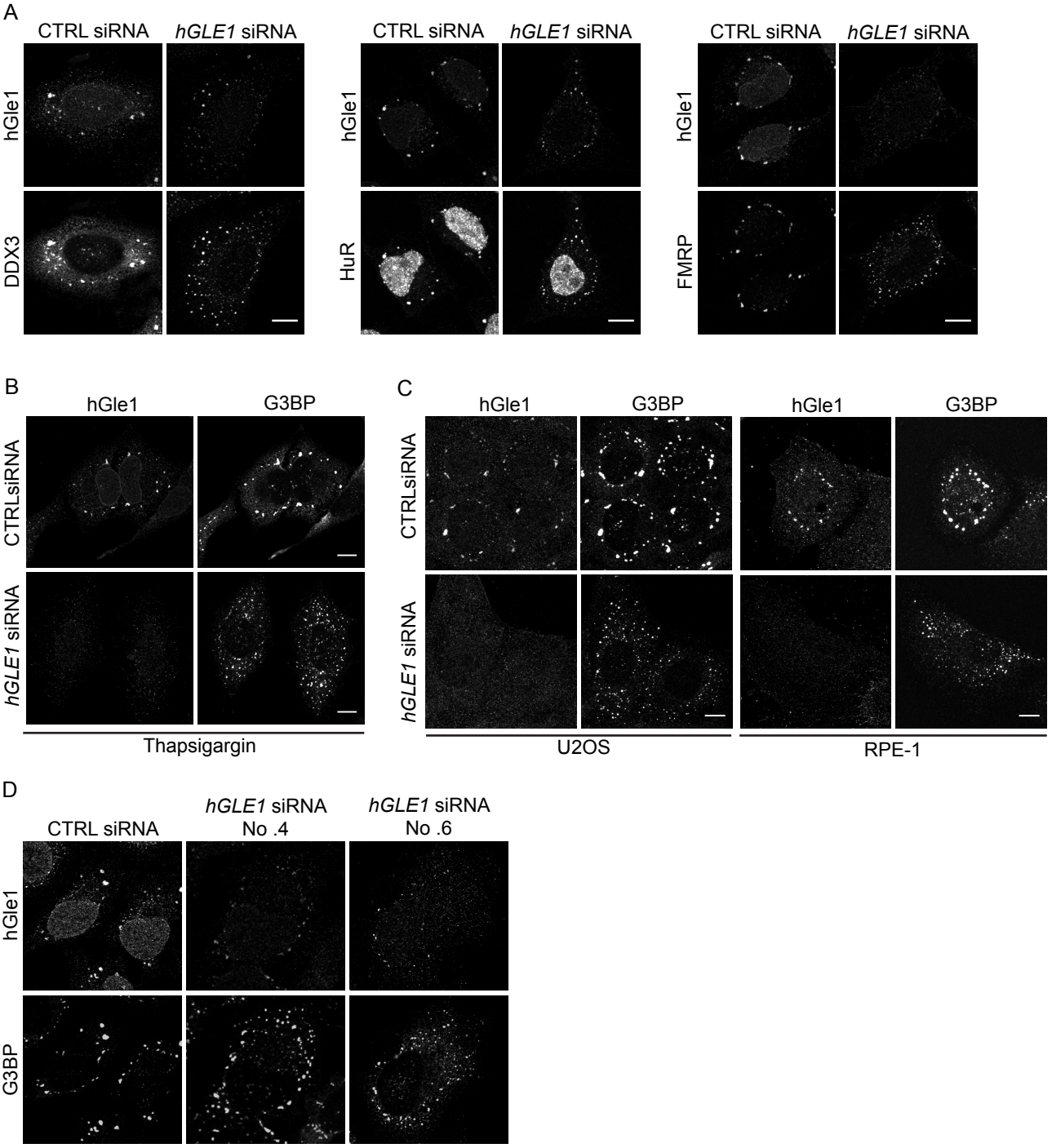

Figure S2. hGle1-dependent SG defects are not stress or cell type specific. (A) hGle1-dependent SG defects are not limited to G3BP. CTRL or *hGLE1* transfected HeLa cells were heat shocked at 45°C for 60 min and processed for immunofluorescence with anti-hGle1 and DDX3, HuR, or FMRP. Scale bar =10μm. (B) hGle1-depleted cells exhibit SG defects in response to thapsigargin treatment. CTRL or *hGLE1* siRNA-treated HeLa cells were incubated with 10 μM thapsigargin for 60 min at 37°C. Following treatment, cells were processed for immunofluorescence using anti-hGle1 and G3BP antibodies. Scale bar =10μm. (C) hGle1-dependent SG defects are not limited to HeLa cells. U20S and RPE-1 cells were transfected with *hGLE1* and CTRL siRNAs. After 72 h siRNA transfection, cells were heat shocked at 45°C for 60 min and processed for immunofluorescence with anti-hGle1 and G3BP antibodies. Scale bar =10μm. (D) SG defects in hGle1-depleted cells are not due to off targets effects of siRNAs. HeLa cells transfected with either CTRL, *hGLE1* siRNA No.4 or *hGLE1* siRNA No.6. Cells were heat shocked at 45°C for 60 min and processed for immunofluorescence with anti-hGle1 and G3BP antibodies. Scale bar=10μm

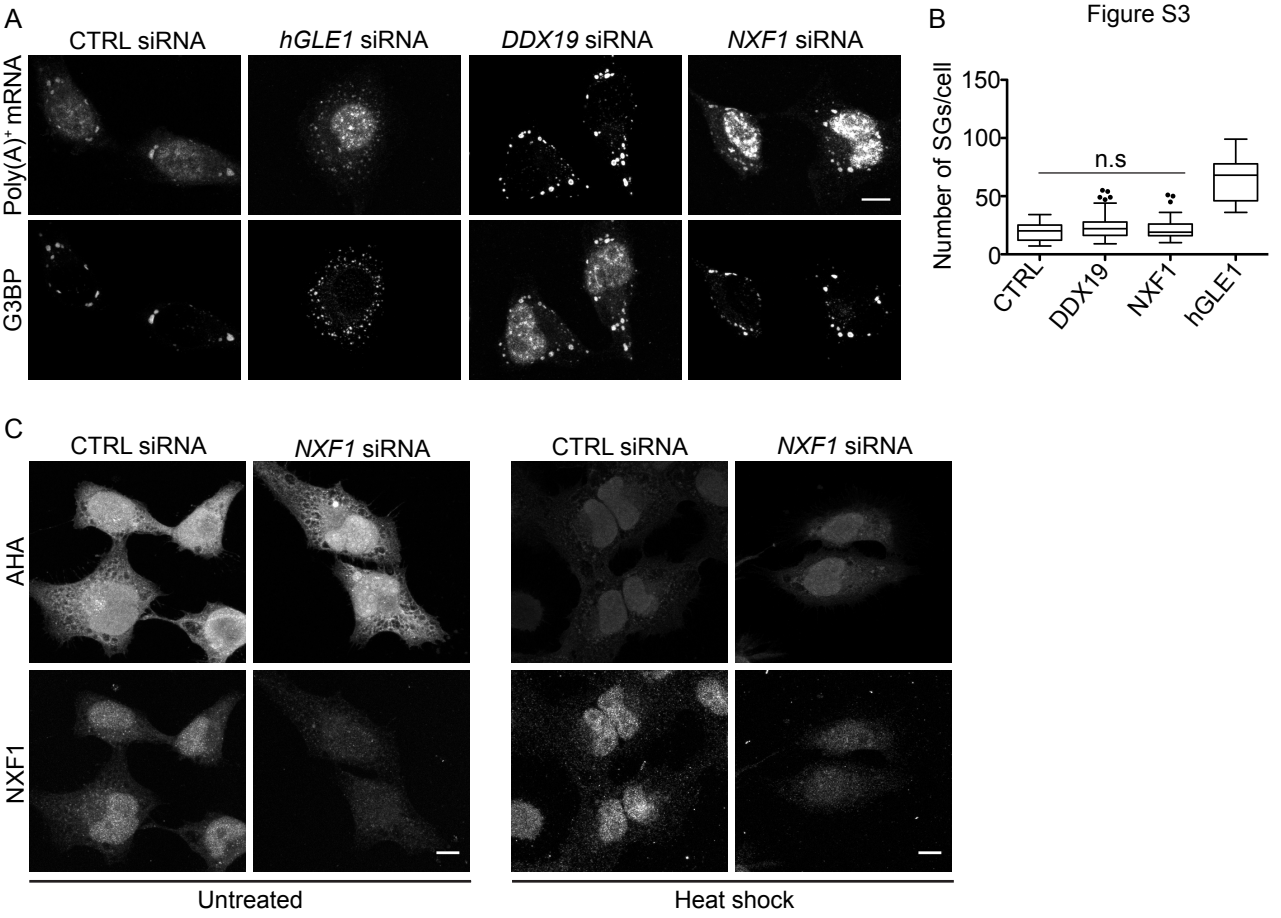

Figure S3. SG defects or translation defects in hGle1-depleted cells are not due to mRNA export defects. (A) HeLa cells were treated with CTRL, *hGLE1, NXF1,* or *DDX19B* siRNAs for 48 h. After siRNA treatments, cells were heat shocked at 45°C for 60 min and processed for immunofluorescence with anti-G3BP antibody followed by in situ hybridization using Cy3 oligo-dT probe. Scale bar =10μm. (B) NXF1 knockdown does not cause deregulation in translation similar to hGle1-depleted cells. HeLa cells were transfected with either CTRL or *NXF1* siRNAs. Cells were either left untreated or heat shocked at 45°C for 15 min followed by metabolically labeling with AHA at either 37°C or 45°C for 30 min. After fixation, CTRL and *NXF1* siRNA-treated cells were processed for detection of AHA–labeled proteins using Alkyne-488 followed by immunofluorescence with anti- NXF1 antibodies. Scale bar =10μm.

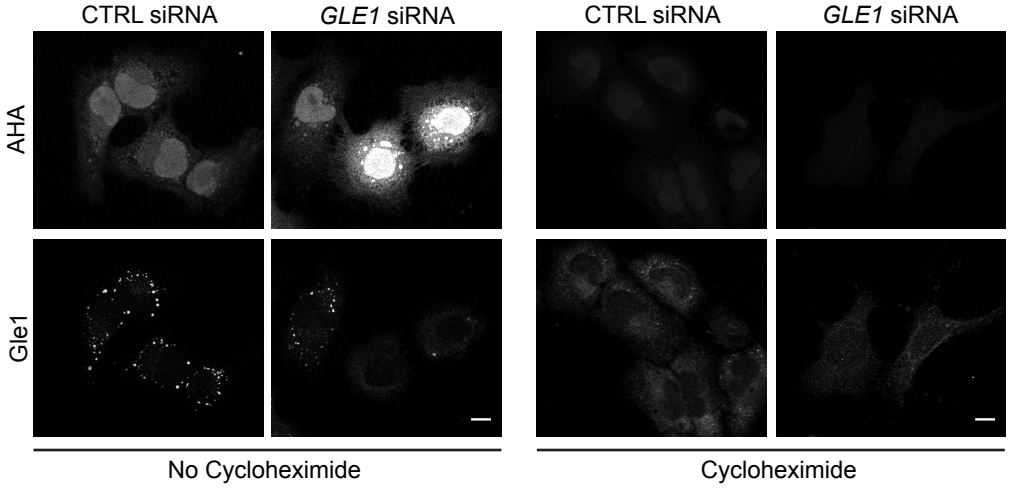

Figure S4. AHA incorporation in hGle1-depleted cells is due to nascent protein synthesis. HeLa cells were transfected with either CTRL or hGLE1 siRNAs. Cycloheximide was added to cells and cells were heat shocked at 45°C for 15 min followed by metabolically labeling with AHA for 30 min at 45°C. After fixation, hGLE1 and CTRL siRNA-treated cells were processed for detection of AHA –labeled proteins using Alkyne-488 followed by immunofluorescence with anti-hGle1 antibodies. Scale bar =10μm.

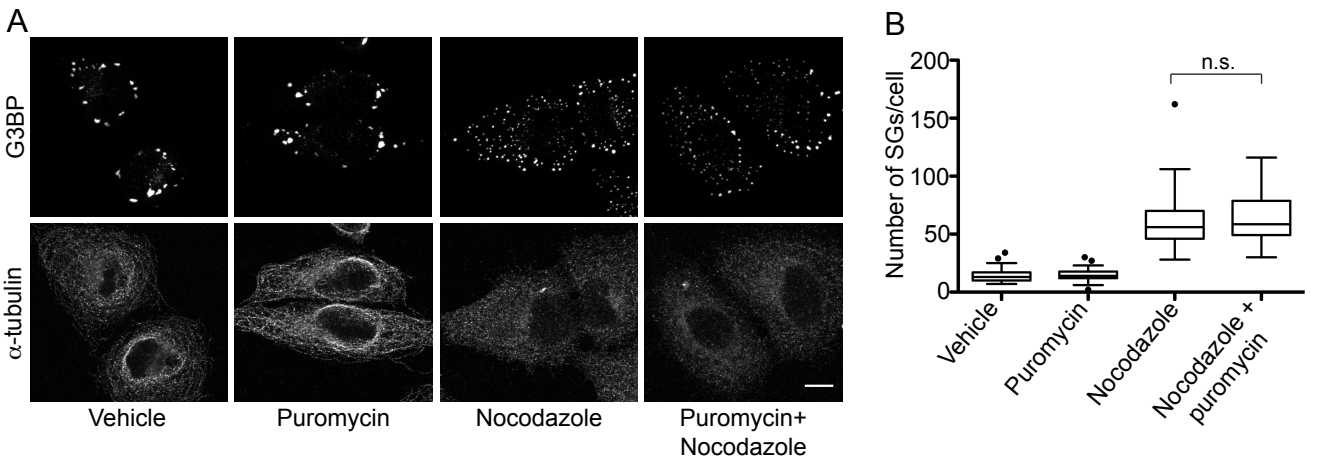

Figure S5. Puromycin does not rescue microtubule-dependent SG defects. (A-B) HeLa cells were pre-incubated with 5 μM nocodazole for 120 min at 37°C. Cells were treated with vehicle alone, 0.5 mg/ml puromycin, 5 μM nocodazole, or 0.5 mg/m puromycin and 5 μM nocodazole for 60 min at 45°C. Cells were processed for immunofluorescence using anti α-tubulin and G3BP antibodies. Scale bar =10μm.

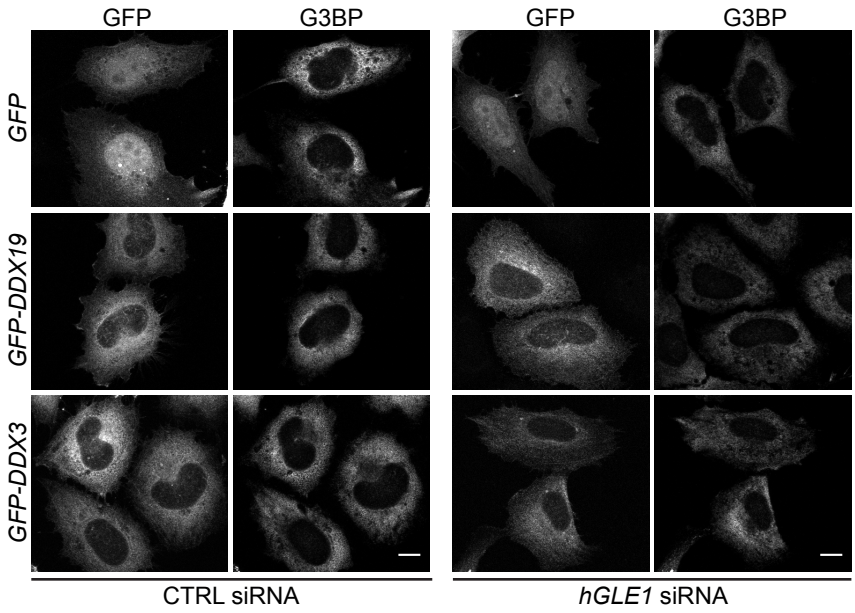

Figure S6. Expression of DDX3 in hGle1-depleted cells under non-stress conditions. CTRL or *hGLE1* siRNA-treated cells were transfected with *EGFP, EGFP-DDX19B,* or *DDX3-EGFP* plasmids. Cells were processed for immunofluorescence detection of G3BP and hGle1. Scale bar =10μm.