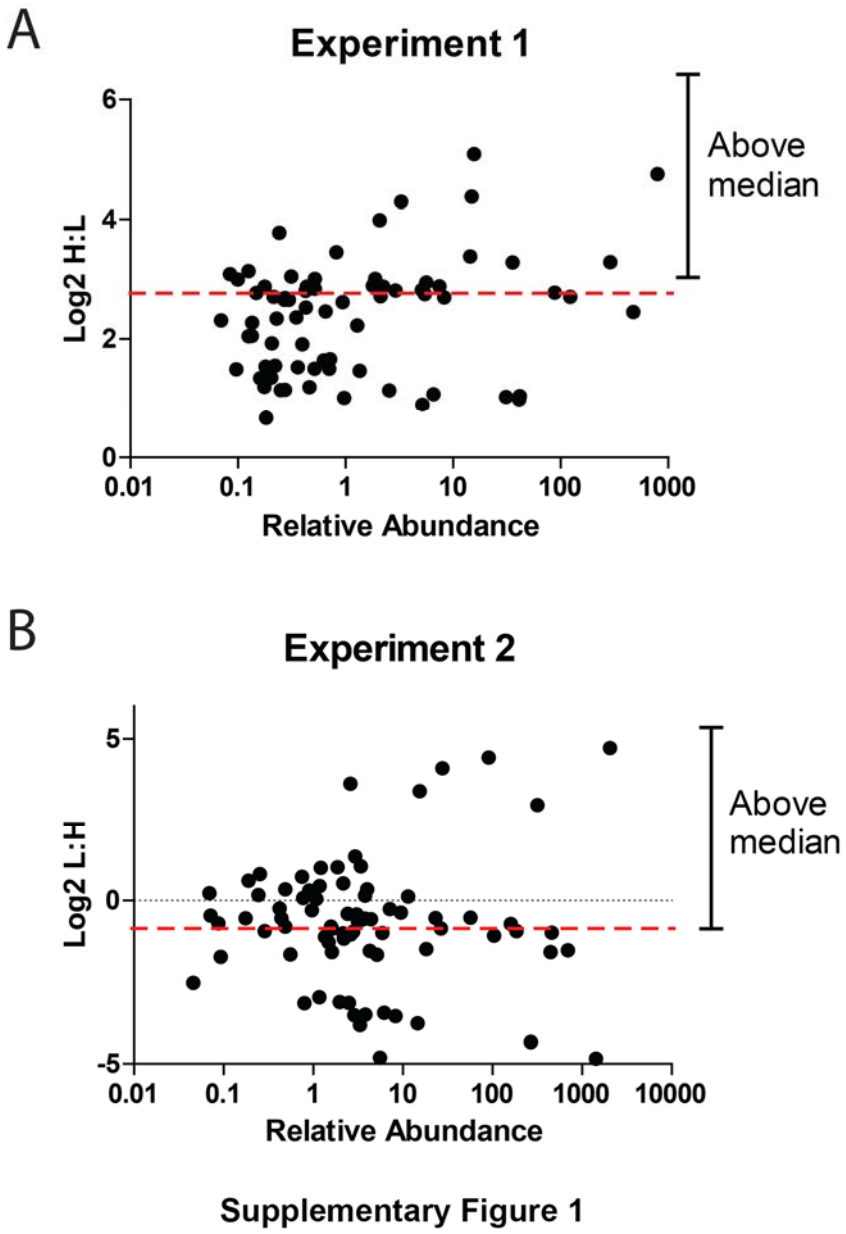
Supplemental Materials

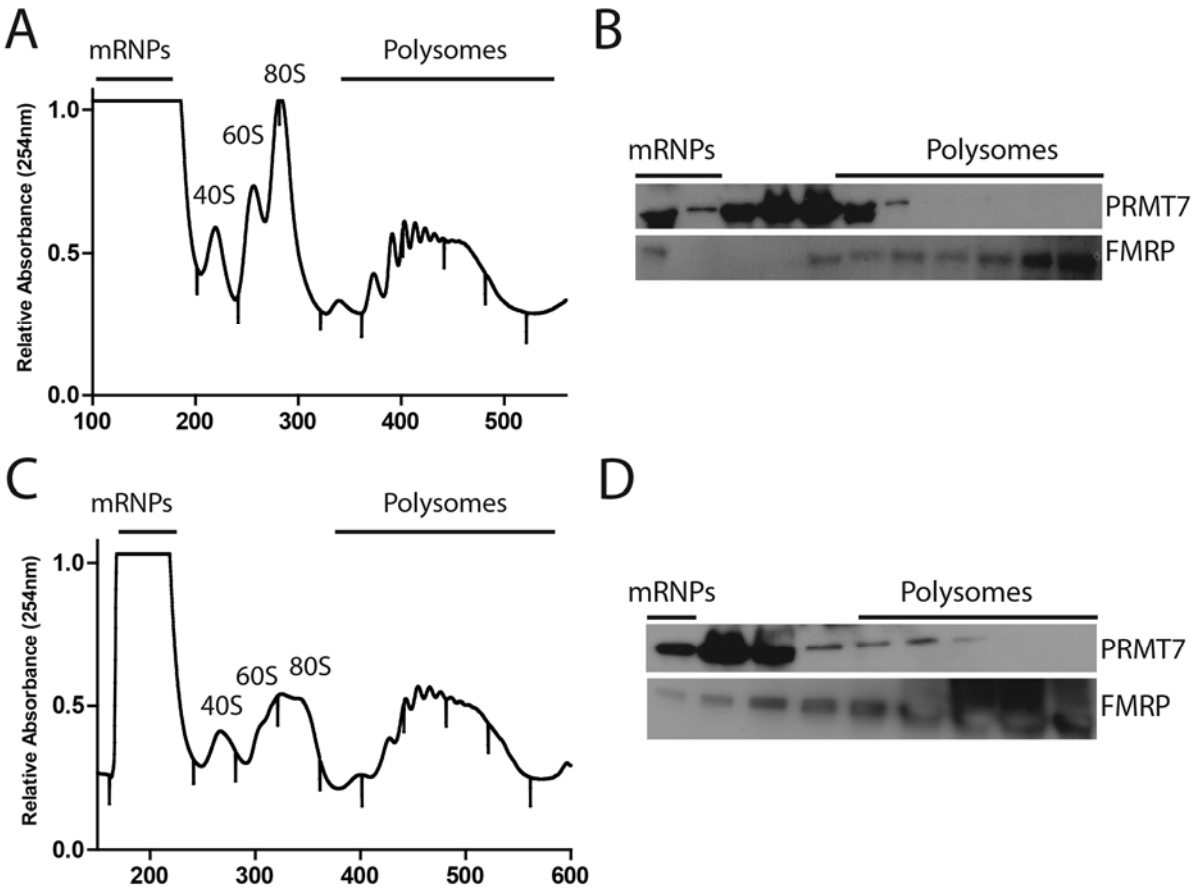
Molecular Biology of the Cell

Haghandish et al.



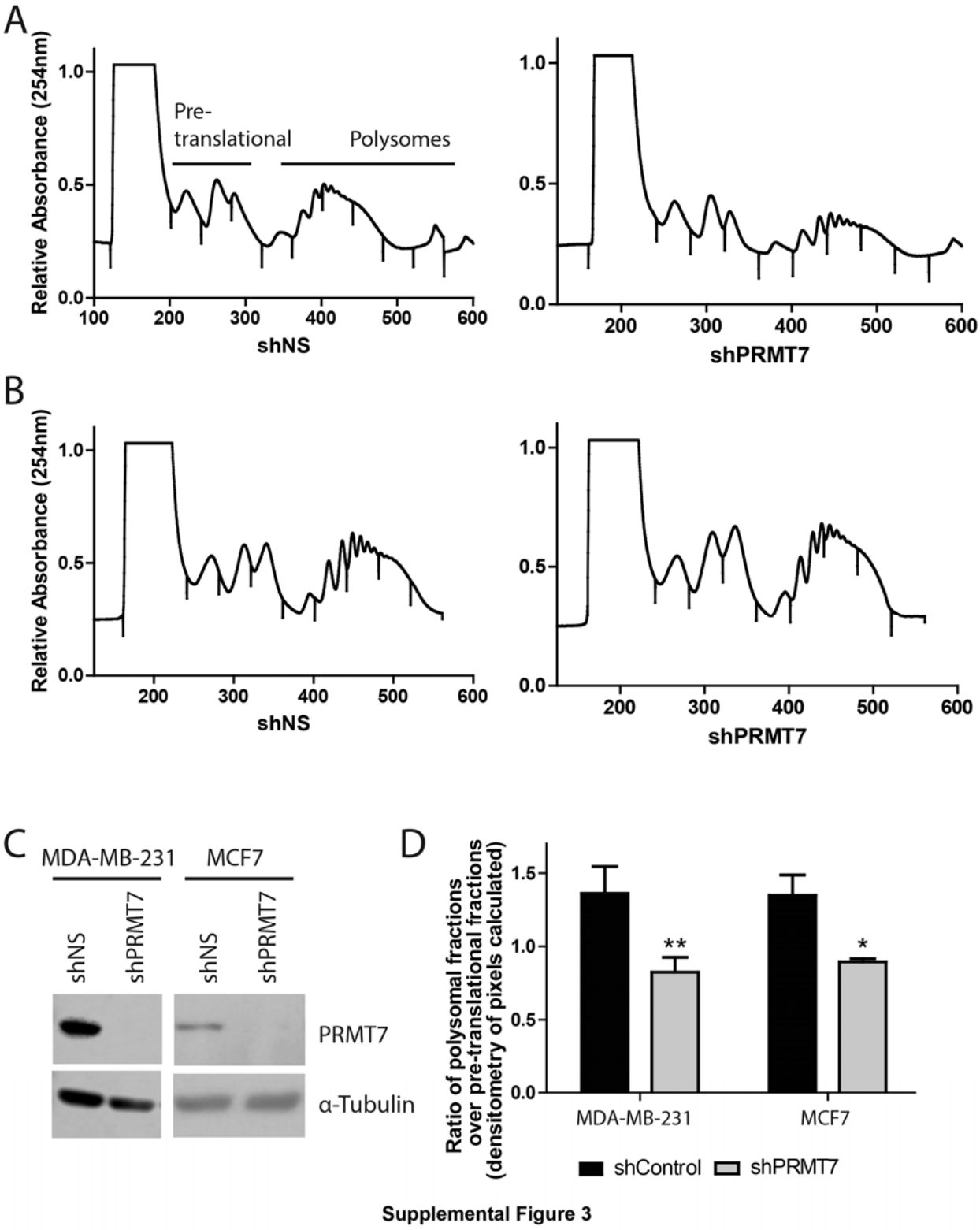
Supplementary Figure 1

Supplemental Figure 1. Analysis of SILAC-based mass spectrometry results. (A) Scatter plot showing comparison of the SILAC H:L ratio of proteins to their relative abundance within the mass spectrometry experiment (experiment 1). The median is marked by a dashed line. (B) Similar comparison but for experiment 2 (reverse labeling; SILAC ratio is L:H).



Supplementary Figure 2

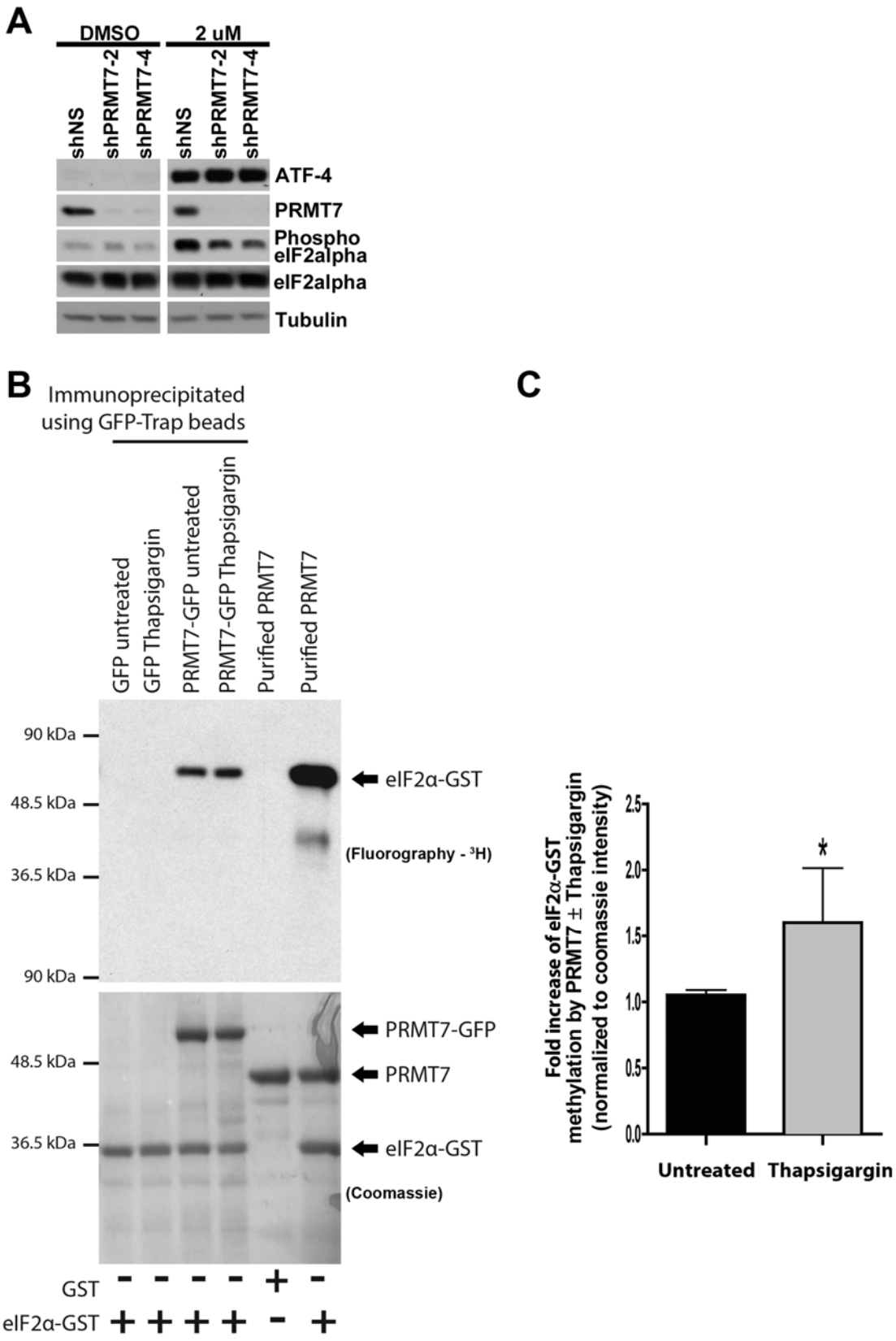
Supplemental Figure 2. PRMT7 is present in translational machinery within breast cancer cell lines.Polyribosome profiles and fractions of parental MCF7 cells (A, B) and parental MDA-MB-231 cells (C, D). Relative absorbance of RNA was read at 254nm. PRMT7 is found within both polysomes and pre-translational fractions – more so in cancer cells. FMRP was used as a positive control for pre-translational and polysomes.



Supplemental Figure 3

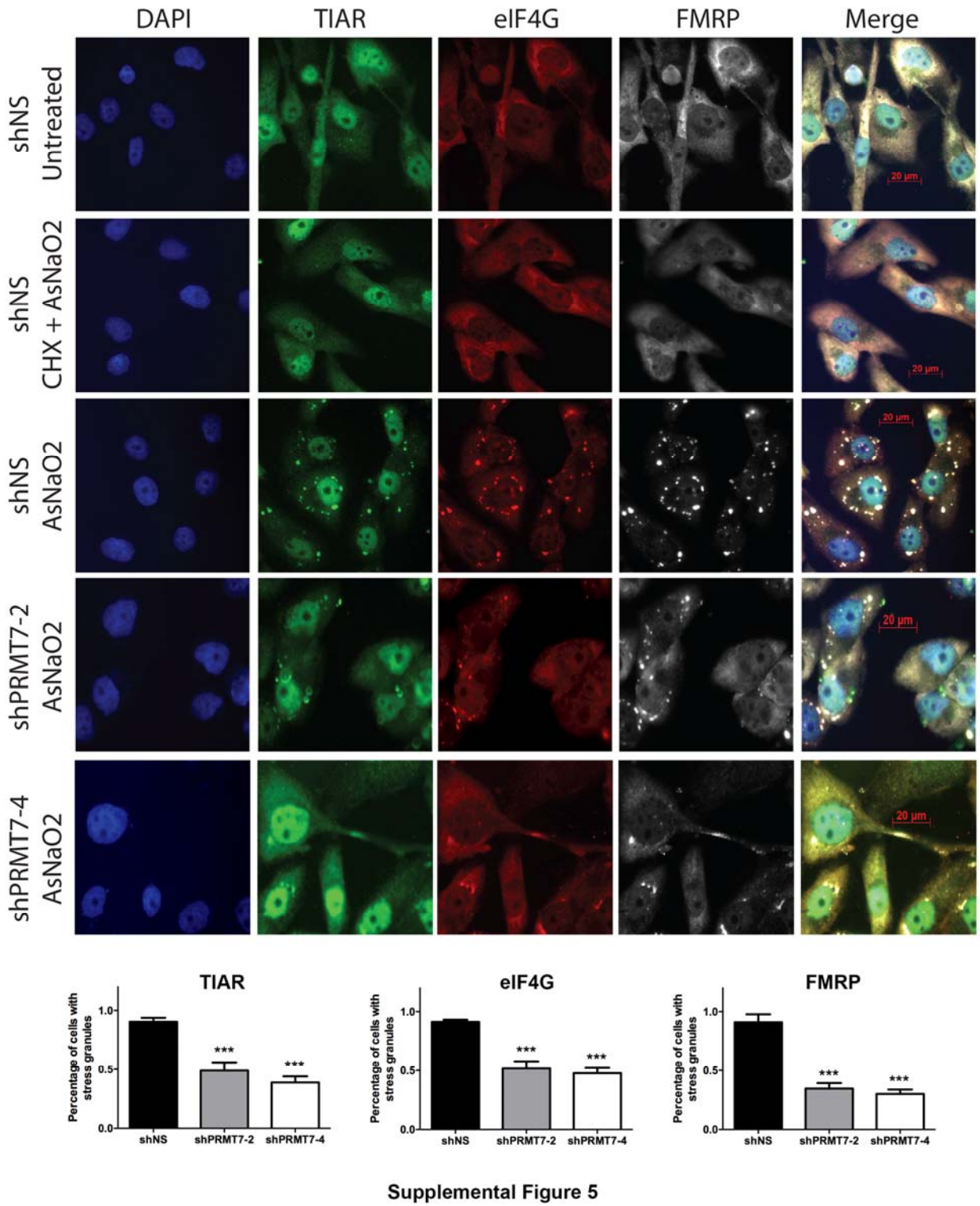
Supplemental Figure 3. PRMT7 knockdown does not drastically affect polyribosome profile. (A) Representative polyribosome profiles of MDA-MB-231 cells 48 h post-infection with either control or PRMT7-targeting shRNAs. (B) Representative polyribosome profiles of MCF7 cells

48 h post-infection with either control or PRMT7-targeting shRNAs. Relative absorbance of RNA was read at 254nm. (C) Western blots depict extent of PRMT7 knockdown in both cell types. (D) Quantification of the profiles revealed a statistically significant reduction of 36% in MDA-MB-231 cells and 32% s in MCF7 cells, upon PRMT7 knockdown (**p=0.01; *p=0.03, n=3, ANOVA; presented as mean ± SEM).



Supplemental Figure 4

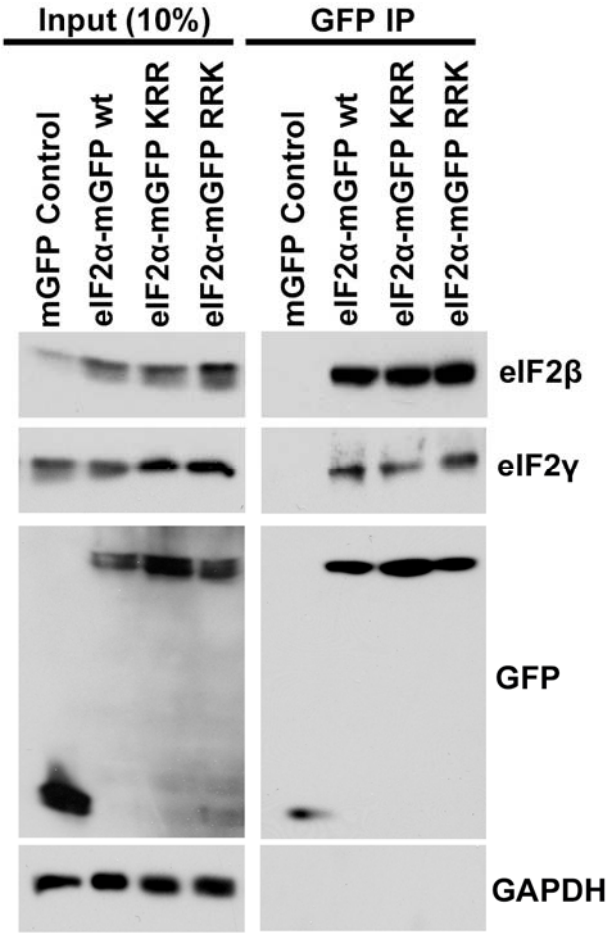
Supplemental Figure 4. PRMT7 is more active, methylatingeIF2α to a higher degree, when cells are stressed with thapsigargin. (A) Accumulation of eIF2α phospho-Ser51 following treatment with thapsigargin is reduced upon transient PRMT7 knockdown within MDA-MB-231 cells using two distinct shRNA targeting sequences. However, thapsigargin-induced accumulation of ATF4 is not affected upon PRMT7 knockdown.(B) PRMT7-Myc was transiently expressed in MDA-MB-231 cells and then affinity-purified using Myc-Trap beads. Affinity-purified PRMT7-Myc, from Mock- or thapsigargin-treated (2μM, 2 hours) was then used as a source of enzyme to perform *In vitro* methylation assays using ^{3}H-SAM as methyl-donor wild-type eIF2α-GST as a substrate. Immunoprecipiated Myc was used as a negative control. As a positive control, purified PRMT7 with either GST or wild-type eIF2α-GST was used. (C) Quantification of the methylation status of eIF2α was calculated in the thapsigargin treated cells and normalized to the amount of PRMT7-myc detected in the coomassie stain. A significant increase in methylation of eIF2α was observed in thapsigargin-treated cells; data are presented as mean ± SEM for n=5, *p=0.03, two-tailed t-test.



Supplemental Figure 5

Supplemental Figure 5. PRMT7 regulates stress granule formation. Representative immunofluorescent images of MDA-MB-231 cells depicting decreased stress granule formation upon transient knockdown of PRMT7 when exposed to $AsNaO_2$ (500µM for 30 minutes). Three stress granule markers were used: TIAR (green), eIF4G (red), and FMRP (white). Scale bar

20μm. Quantitation of stress granules from 3 independent experiments performed in triplicate when treated with $AsNaO_2$; data are presented as mean ± SEM, ***p=0.0001 (ANOVA). A cycloheximide control (CHX) was used to confirm the foci are stress granules as CHX prevents stress granule formation.



Supplemental Figure 6. Methylation of eIF2α by PRMT7 does not affects its incorporation into the ternary complex. MDA MB 231 cells were infected with lentivirus expressing either EGFP, eIF2α-mGFP wild type, KRR or RKK mutants. GFP-trap beads were used to affinity-purify EGFP and eIF2αalleles from cell lysates, followed by immunoblotting for the eIF2β and eIF2γ subunits.