

Supplemental information

Arginine demethylation of G3BP1 promotes stress granule assembly.

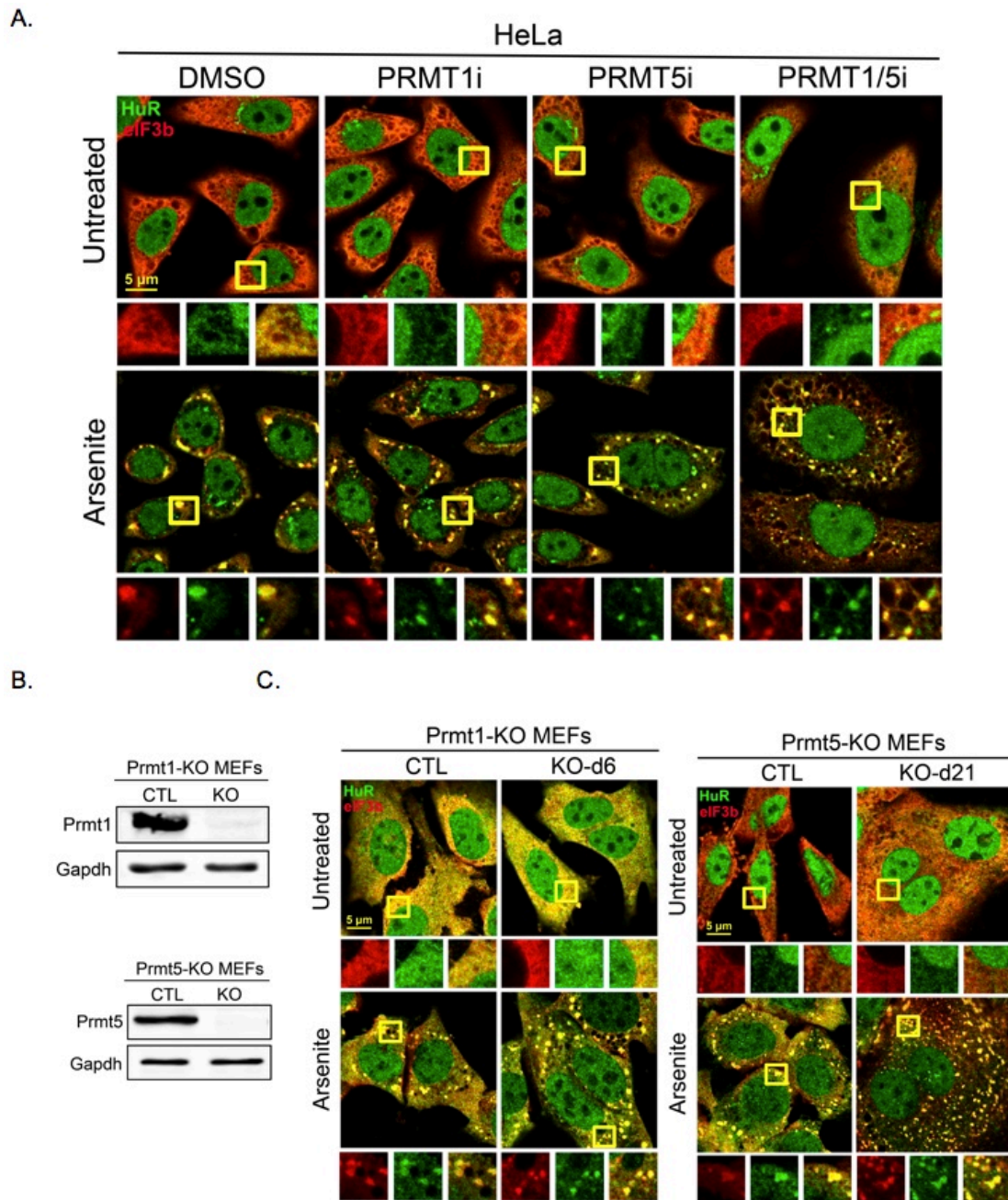
Wei-Chih Tsai¹, Sitaram Gayatri², Lucas C. Reineke¹, Gianluca Sbardella³, Mark T. Bedford², and Richard E. Lloyd¹

¹ Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA. ² Department of Epigenetics and Molecular Carcinogenesis, University of Texas M. D. Anderson Cancer Center, Science Park, P.O. Box 389, Smithville, TX 78957, USA. ³ Epigenetic Med Chem Lab, Dipartimento di Farmacia, Università degli Studi di Salerno, Via Giovanni Paolo II 132, I-84084 Fisciano, Salerno, Italy.

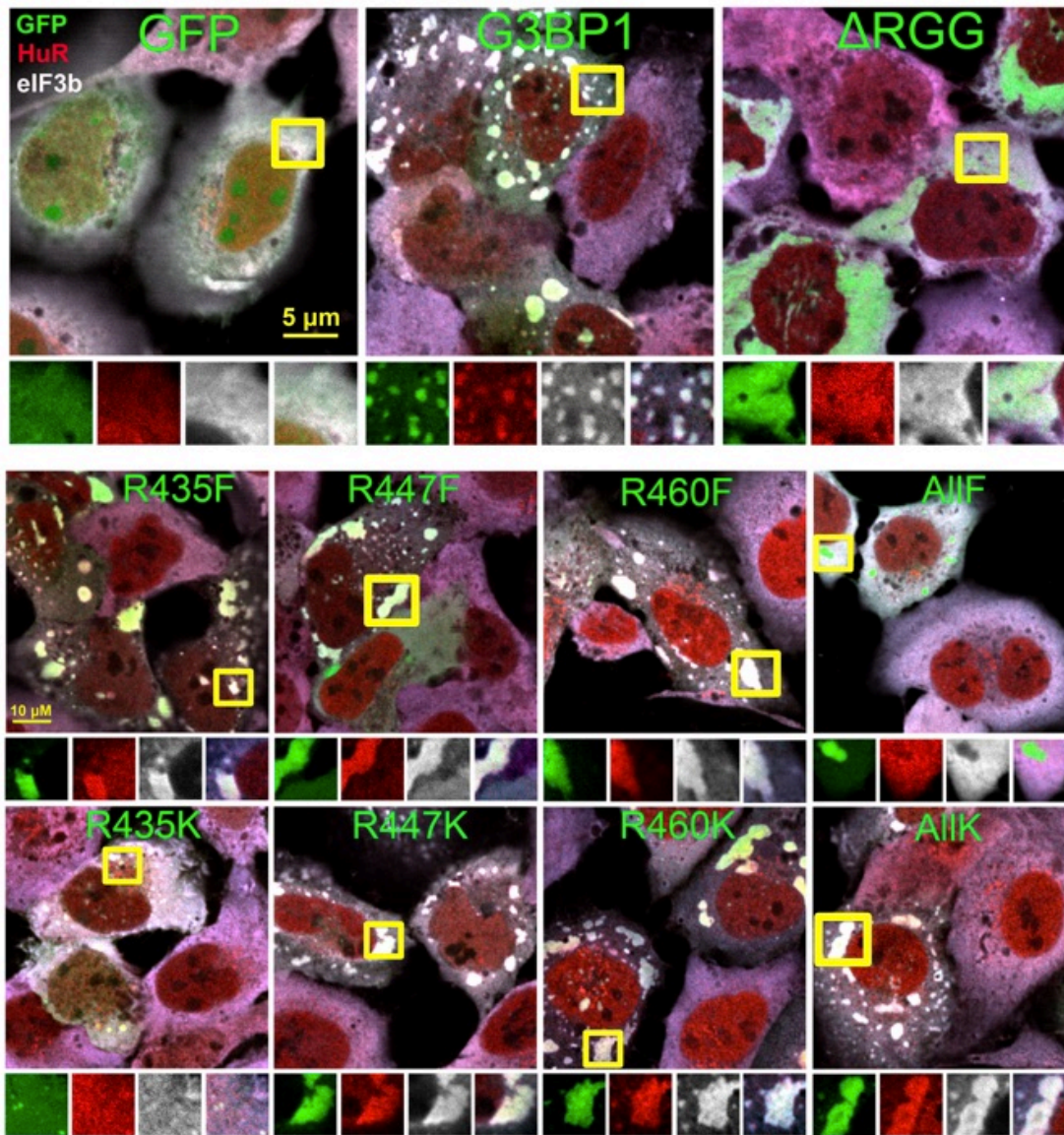
Supplemental Methods

Ribopuromycilation assay – Cells were grown as described in the main text, and RPA was conducted essentially as described previously (1) using the 12D10 antibody directed against puromycin (Millipore, #MABE343).

1. Reineke, L. C., Dougherty, J. D., Pierre, P., and Lloyd, R. E. (2012) Large G3BP-induced granules trigger eIF2 α phosphorylation. *Mol Biol Cell.* **23**, 3499–3510

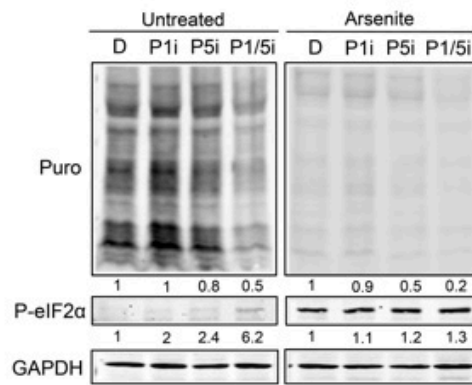


Supplemental figure 1. HuR and eIF3b colocalize with SGs in PRMT inhibitor-treated and PRMT KO cells. (a) The enzymatic activities of PRMTs in HeLa cells were inhibited by PRMT1 inhibitor (PRMT1i), PRMT5 inhibitor (PRMT5i) or both together (PRMT1/5i), then cells were arsenite treated and were stained for HuR in green and eIF3b in red. (b) PRMT1-KO and PRMT5-KO MEFs were assessed for knockout efficiency, (c) untreated or arsenite treated cells were stained for HuR(green) and eIF3b (red). Yellow squares indicate regions in vignettes. Original magnification: 63X.

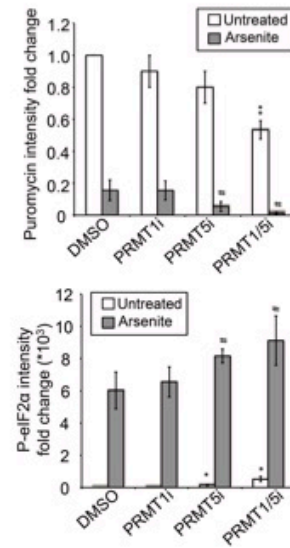


Supplemental figure 2. HuR and eIF3b colocalize with SGs formed during overexpression of G3BP1 methylation mutants. G3BP1 KO U2OS cells were transfected with GFP-tagged G3BP1 methylation mutants and processed for IFA to visualize G3BP1-induced SGs. Cells were stained for SGs with HuR in red and eIF3b in Gray. Images were captured by confocal microscopy with magnification of 63X. Yellow squares indicate regions depicted in vignettes.

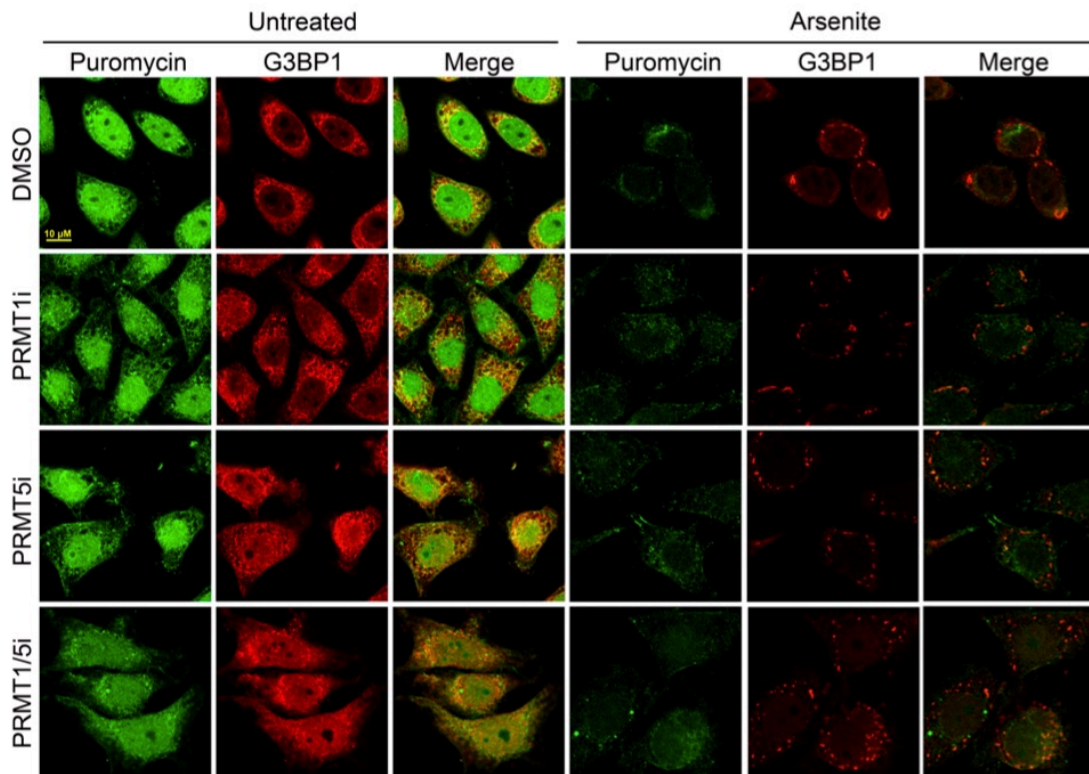
a.



b.

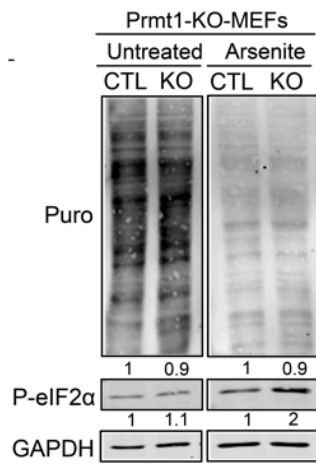


c.

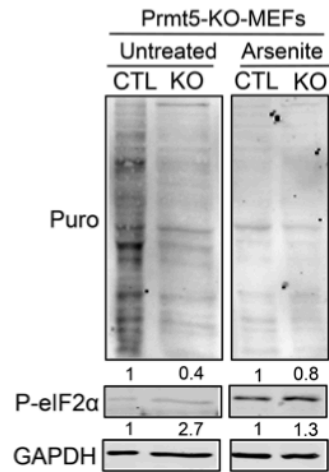


Supplemental figure 3. Enzymatic activities of PRMT1 and PRMT5 affect protein synthesis in cells. (a) The enzymatic activity of PRMTs in HeLa cells were inhibited with PRMT1 inhibitor (PRMT1i), PRMT5 inhibitor (PRMT5i) or both together (PRMT1/5i). HeLa cells were arsenite treated and pulse-labeled with puromycin for 5 mins followed by western blotting to detect puromycin and p-eIF2α. (b) Quantification of puromycin (b, upper panel) and p-eIF2α (b, bottom panel) from western blots. The results shown are representative of 3 independent experiments. *P<0.05, and **P<0.01 versus DMSO untreated cells. #P<0.05 versus DMSO arsenite treated cells. (c) HeLa cells were treated with the indicated PRMT inhibitors then stained for IFA with puromycin in green and G3BP1 in red. Original magnification: 63X.

a.



b.



Supplemental figure 4. Knockout of PRMT1 and PRMT5 repressed protein synthesis in cells. PRMT1-KO MEFs (a) or PRMT5-KO MEFs (b) were harvested after arsenite treated following by 5 mins of puromycin pulse labeling followed by western blotting to detect puromycin and p-eIF2α in cells.