Supplementary Information

The protective role of m^1A during stress-induced granulation

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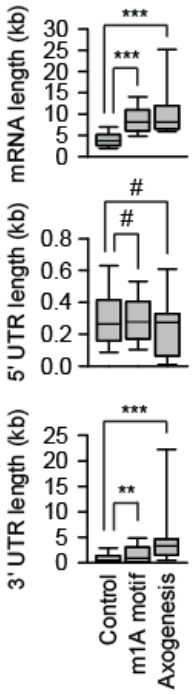
This file includes Supplementary Figures S1-S5

A

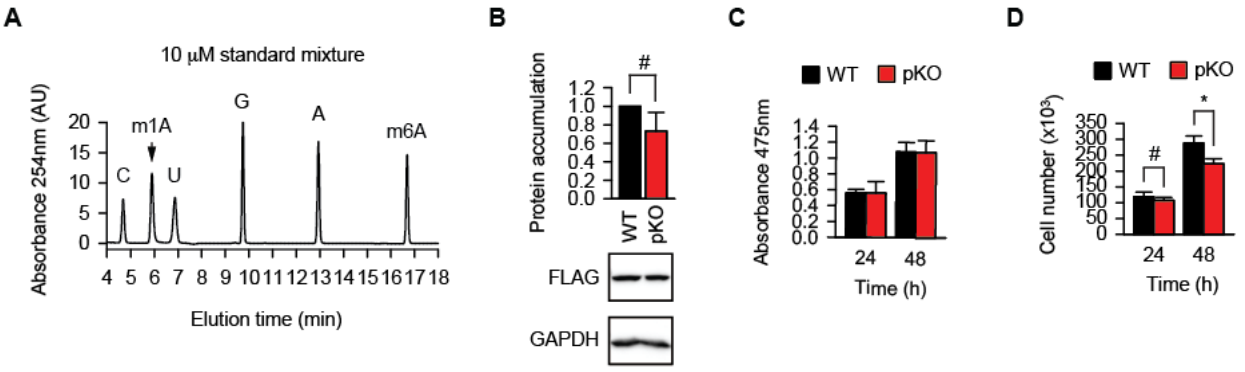
Gene	Protein	m^{1}A motif
ADCY1	Adenylate cyclase type 1	C
ALS2	Alsin	C
ARHGAP35	Rho GTPase-activating protein 35	3'
BCL11B	B-cell lymphoma/leukemia 11B	3'
CELSR3	Cadherin EGF LAG G-type receptor 3	C
EPHA4	Ephrin type-A receptor 4	C
FLRT2	LRR transmembrane protein FLRT2	3'
GLI3	Transcriptional activator GLI3	3'
KIF5A	KIF5A Kinesin heavy chain isoform 5A	C
MAP1B	Microtubule-associated protein 1B	3'
PARD3	Partitioning defective 3 homolog	C
SPTAN1	Spectrin alpha chain, non-erythrocytic 1	C
SPTBN2	Spectrin beta chain, non-erythrocytic 2	C

B



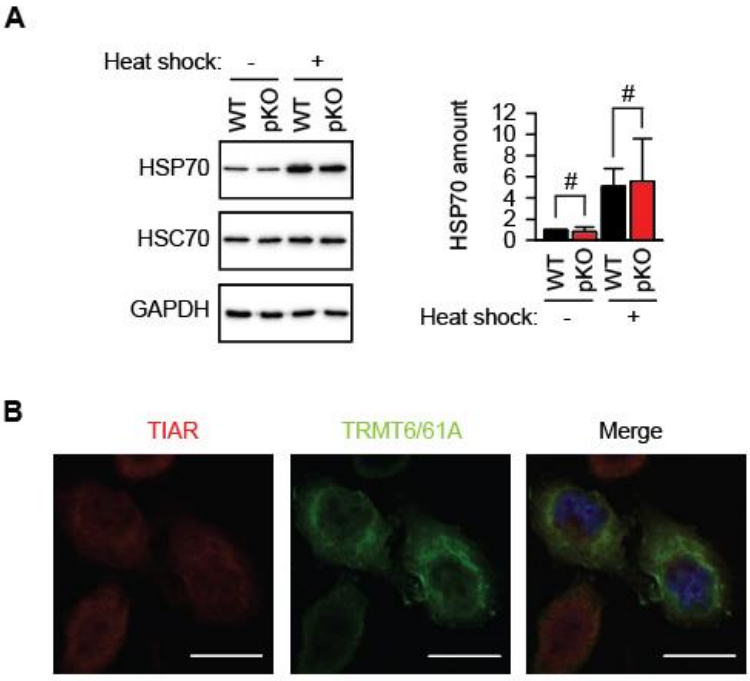
Supplementary Figure S1 Bioinformatic analysis of stress granule transcripts.

(**A**) The list of human genes from GO category "Axonogenesis" that encode mRNA which are enriched in SG and contain an m^{1}A motif. The position of the motif in the transcript is indicated (C, coding region; 3', 3'-UTR). (**B**) Distribution among the indicated sets of mRNAs of total, 5'-UTR and 3'-UTR length. Control, mRNA set neither enriched nor depleted in SG; m1A motif, mRNAs enriched in SG and containing the m^{1}A motif; Axonogenesis, SG-enriched mRNA with the m^{1}A motif which belong to the GO category "Axonogenesis". ******p<0.01, *******p<0.001, Mann-Whitney test. #, not significant difference.



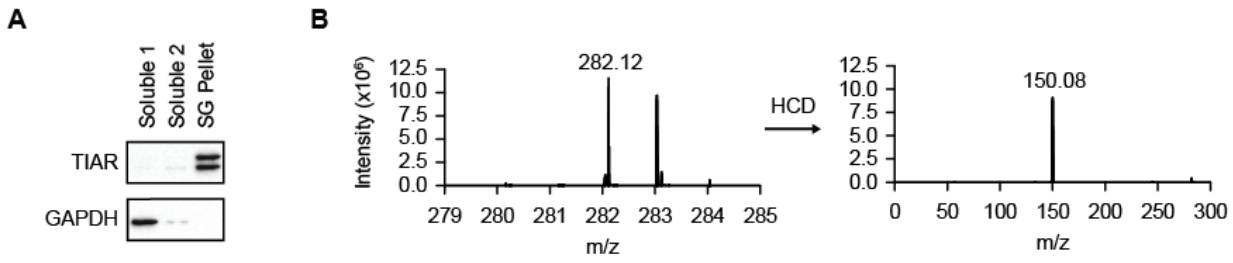
Supplementary Figure S2 Characterization of TRMT61A knock-down cells.

(**A**) HPLC analysis of the 10 μM mixture of the ribonucleosides cytidine (C), N1-methylated adenosine (m1A), uridine (U), guanosine (G), adenosine (A) and N6-methylated adenosine (m6A). (**B**) Protein synthesis is not affected significantly in TRMT61A partial knock-out cells as exemplified by the accumulation of Flag-NQO1 during 24 h after transfection. GAPDH was used as loading control. WT, wild-type HeLa cells; pKO, partial knock-outs of TRMT61A. #, not significant difference, two-tailed t-test; N=3 independent experiments (mean + SD). (**C**) Cell viability at 37°C is not affected significantly by the reduced level of TRMT61A as measured by XTT assay. WT, wild-type HeLa cells; pKO, partial knock-outs of TRMT61A. #, not significant difference, two-tailed t-test; N=3 independent experiments (mean + SD). (**D**) Cell proliferation at 37°C is not affected significantly by the reduced level of TRMT61A. WT, wild-type HeLa cells; pKO, partial knock-outs of TRMT61A. #, not significant difference, two-tailed t-test; N=3 independent experiments (mean + SD).



Supplementary Figure S3 Stress response of TRMT61A knock-down cells.

(**A**) Amounts of inducible (HSP70) and constitutive (HSC70) chaperones in wild-type (WT) and TRMT61A partial knock-out (pKO) cells. Cells were untreated (-) or heat shocked for 1 h at 45°C and left to recover at 37°C for 3 h (+) before lysis and analysis by western blotting. One representative from three independent experiments is shown. GAPDH was used as loading control. #, not significant difference; two-tailed t-test; N=3 independent experiments (mean + SD). (**B**) Localization of TRMT6/61A and TIAR in control cells. A representative image from three independent experiments. Scale bar 20 μm. Red, TIAR; green, TRMT6/61A.



Supplementary Figure S4 Mass spectrometry analysis of stress granule RNA.

(**A**) A representative western blot of isolated stress granules (SG pellet) used for mass spectrometry of methylated adenosine (N=3 independent experiments). GAPDH was used as cytosolic marker. Soluble 1, Soluble 2, supernatant fractions as described in Method details. (**B**) Spectra of the parent ion (m/z = 282.12) and fragment ion (m/z = 150.08) upon higher-energy collisional dissociation (HCD). Selected ion monitoring was performed at m/z = 282.12 ±4 Da.

Supplementary Figure S5 Analyses of m^1A during heat shock.

(**A**) Fractions of N1-methyladenine (m1A) and N6-methyladenine (m6A) in cytosolic mRNAs are slightly increased upon heat shock at 45°C for 1 h as measured by selective ion monitoring mass spectrometry. *****p<0.05, ******p<0.01, two-tailed t-test (N=3 independent experiments, mean + SD). (**B**) m^1A-motif in the 5'-untranslated region of the PRUNE1 gene and in the WT-UbE and MUT-UbE reporters is marked red. Asterisk indicates adenine methylated by TRMT6/61A. Translated sequence is underlined. (**C**) The amounts of reporter mRNA during recovery after heat shock do not differ significantly as determined by quantitative PCR. #, not significant difference, two-tailed t-test; N=3 independent experiments (mean + SD).