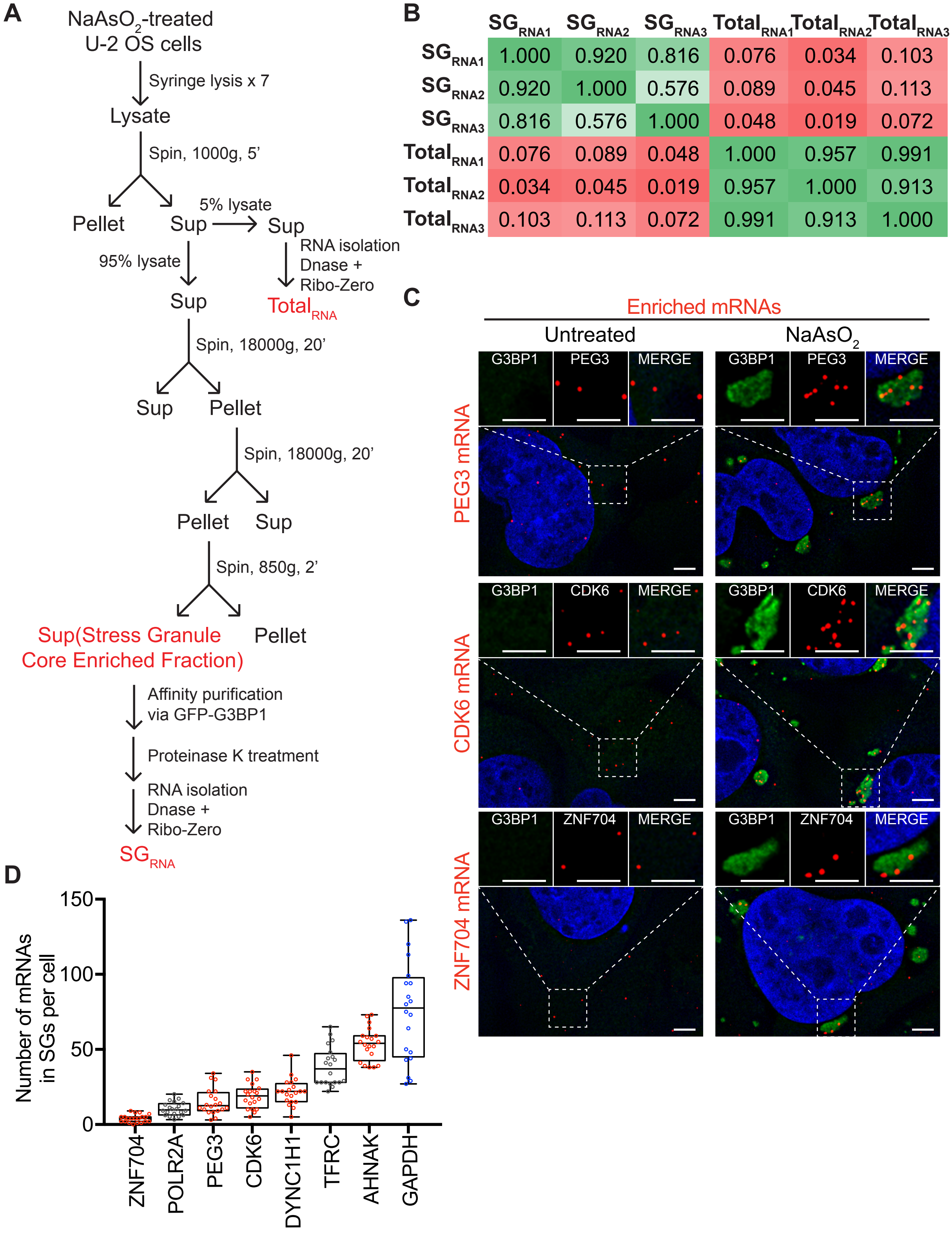
Figure S1



	SG_{RNA1}	SG_{RNA2}	SG_{RNA3}	$Total_{RNA1}$	$Total_{RNA2}$	$Total_{RNA3}$
SG_{RNA1}	1.000	0.920	0.816	0.076	0.034	0.103
SG_{RNA2}	0.920	1.000	0.576	0.089	0.045	0.113
SG_{RNA3}	0.816	0.576	1.000	0.048	0.019	0.072
$Total_{RNA1}$	0.076	0.089	0.048	1.000	0.957	0.991
$Total_{RNA2}$	0.034	0.045	0.019	0.957	1.000	0.913
$Total_{RNA3}$	0.103	0.113	0.072	0.991	0.913	1.000

Figure S2

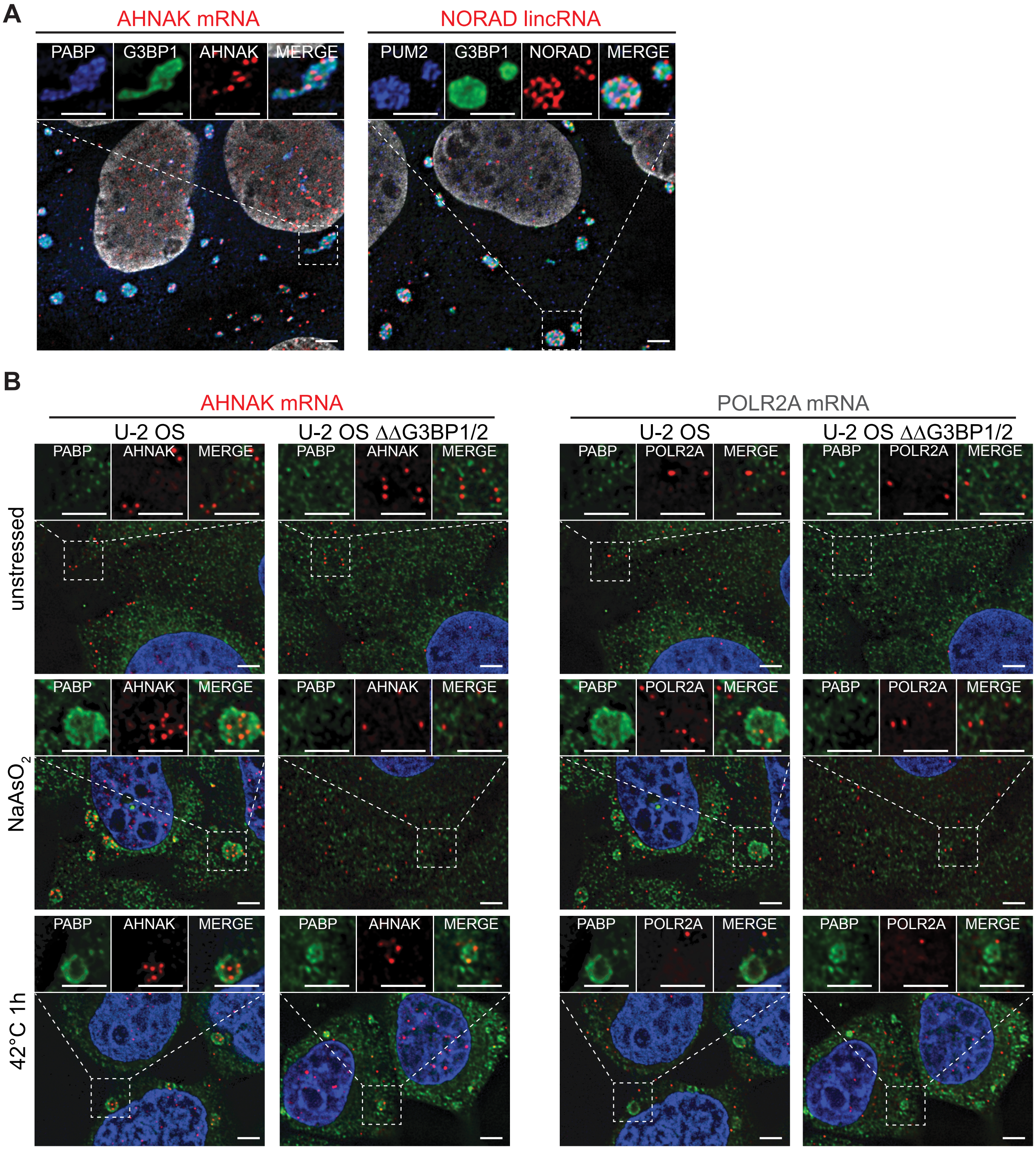

A
AHNAK mRNA
PABP
G3BP1
AHNAK
MERGE
NORAD lincRNA
PUM2
G3BP1
NORAD
MERGE
B
AHNAK mRNA
U-2 OS
U-2 OS ΔΔG3BP1/2
POLR2A mRNA
U-2 OS
U-2 OS ΔΔG3BP1/2
unstressed
NaAsO2
42°C 1h
PABP
AHNAK
MERGE
PABP
POLR2A
MERGE

Figure S3

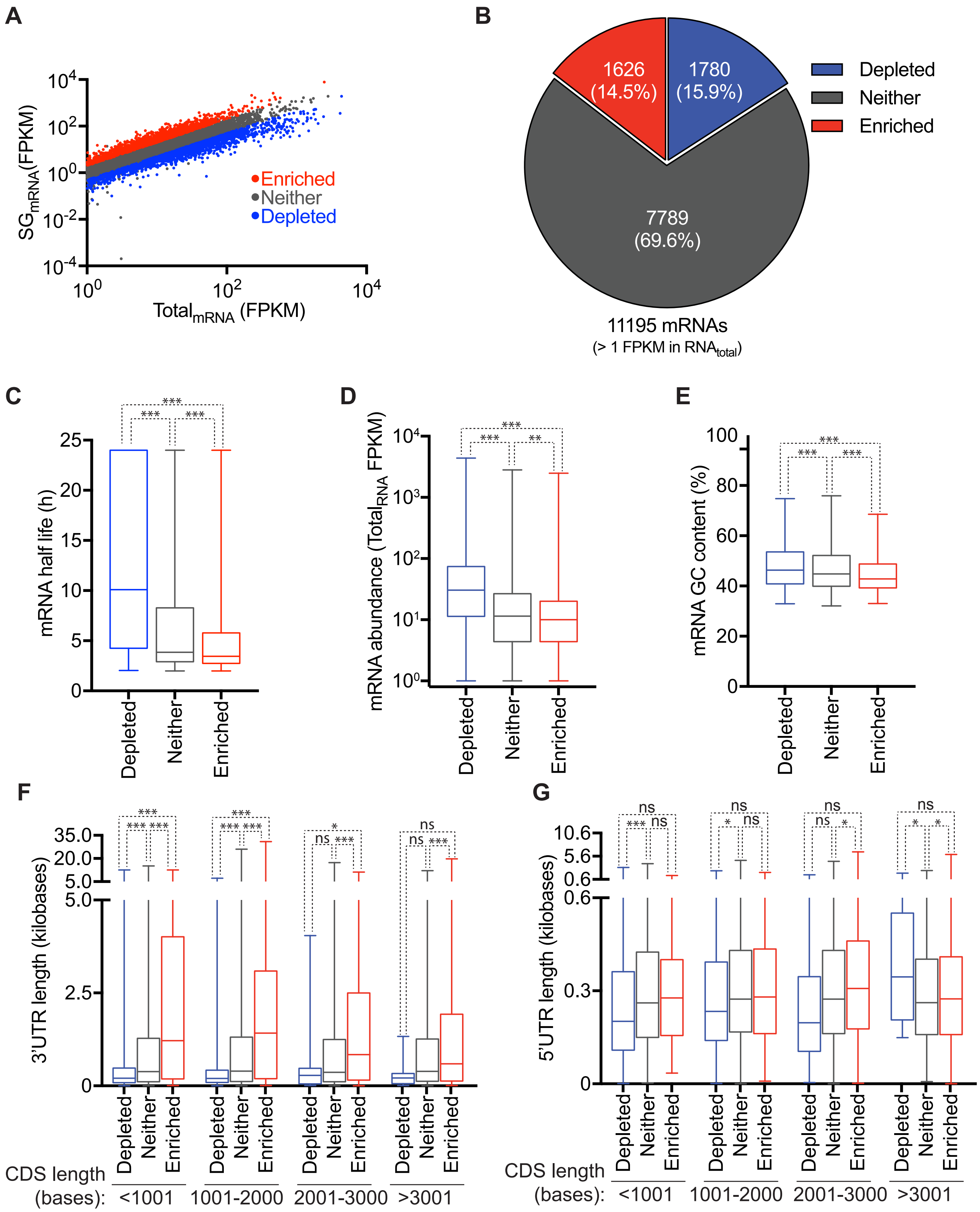
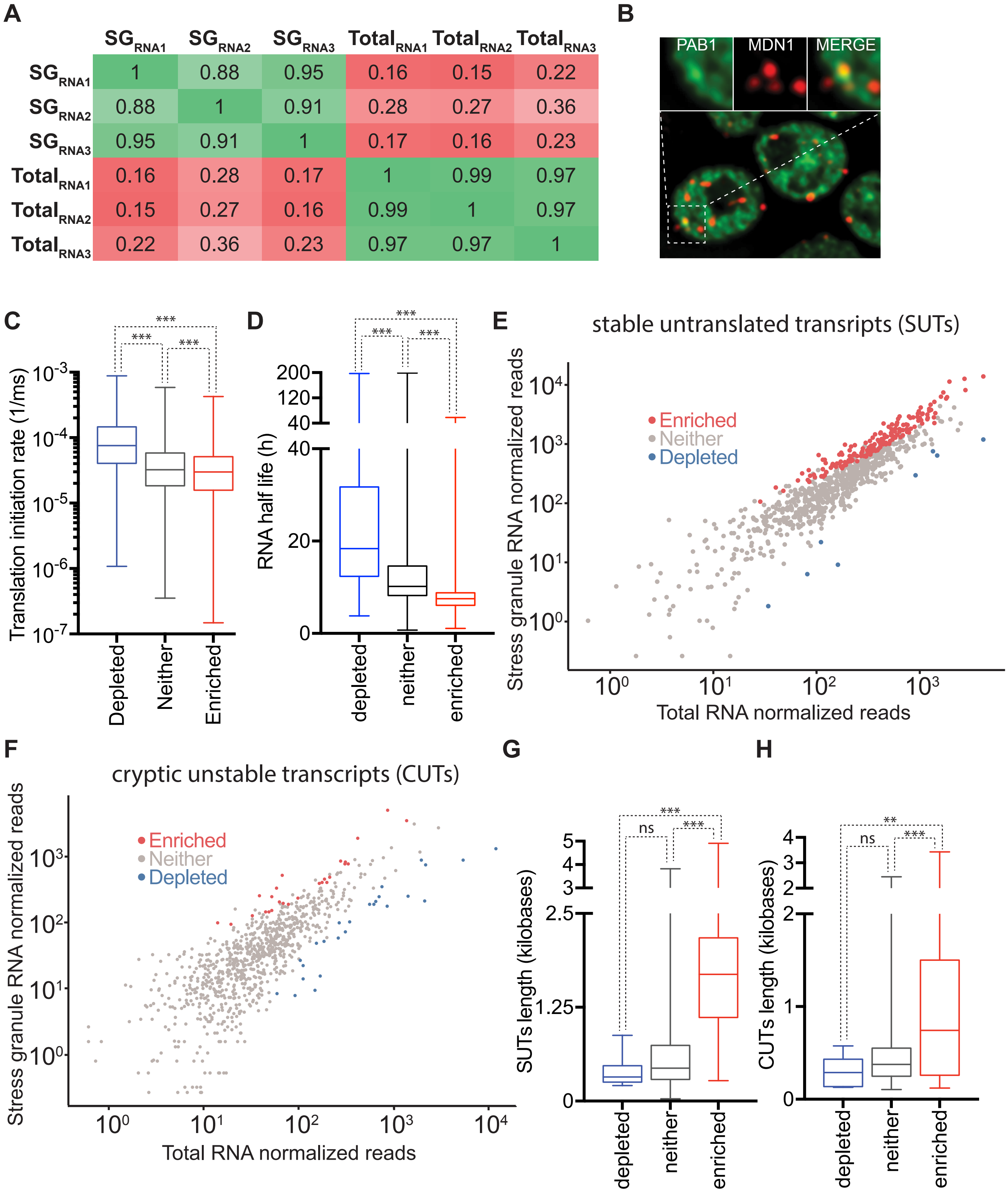

A
10^4
10^2
10^0
10^{-2}
10^{-4}
SG_{mRNA}(FPKM)
10^0
10^2
10^4
$Total_{mRNA}$ (FPKM)
●Enriched
●Neither
●Depleted
B
1626
(14.5%)
1780
(15.9%)
7789
(69.6%)
Depleted
Neither
Enriched
11195 mRNAs
(> 1 FPKM in RNA_{total})
C
mRNA half life (h)
25
20
15
10
5
0
***
***
***
Depleted
Neither
Enriched
D
mRNA abundance ($Total_{RNA}$ FPKM)
10^4
10^3
10^2
10^1
10^0
***
***
**
Depleted
Neither
Enriched
E
mRNA GC content (%)
100
80
60
40
20
0
***
***
***
Depleted
Neither
Enriched
F
3'UTR length (kilobases)
35.0
20.0
5.0
5.0
2.5
0
***
*** ***
***
*** ***
*
ns ***
ns
ns ***
Depleted
Neither
Enriched
CDS length
(bases):
<1001
1001-2000
2001-3000
>3001
G
5'UTR length (kilobases)
10.6
5.6
0.6
0.6
0.3
0
ns
*** ns
ns
* ns
ns
ns *
ns
* *
Depleted
Neither
Enriched
CDS length
(bases):
<1001
1001-2000
2001-3000
>3001

Figure S4



	SG_{RNA1}	SG_{RNA2}	SG_{RNA3}	$Total_{RNA1}$	$Total_{RNA2}$	$Total_{RNA3}$
SG_{RNA1}	1	0.88	0.95	0.16	0.15	0.22
SG_{RNA2}	0.88	1	0.91	0.28	0.27	0.36
SG_{RNA3}	0.95	0.91	1	0.17	0.16	0.23
$Total_{RNA1}$	0.16	0.28	0.17	1	0.99	0.97
$Total_{RNA2}$	0.15	0.27	0.16	0.99	1	0.97
$Total_{RNA3}$	0.22	0.36	0.23	0.97	0.97	1

Figure S5

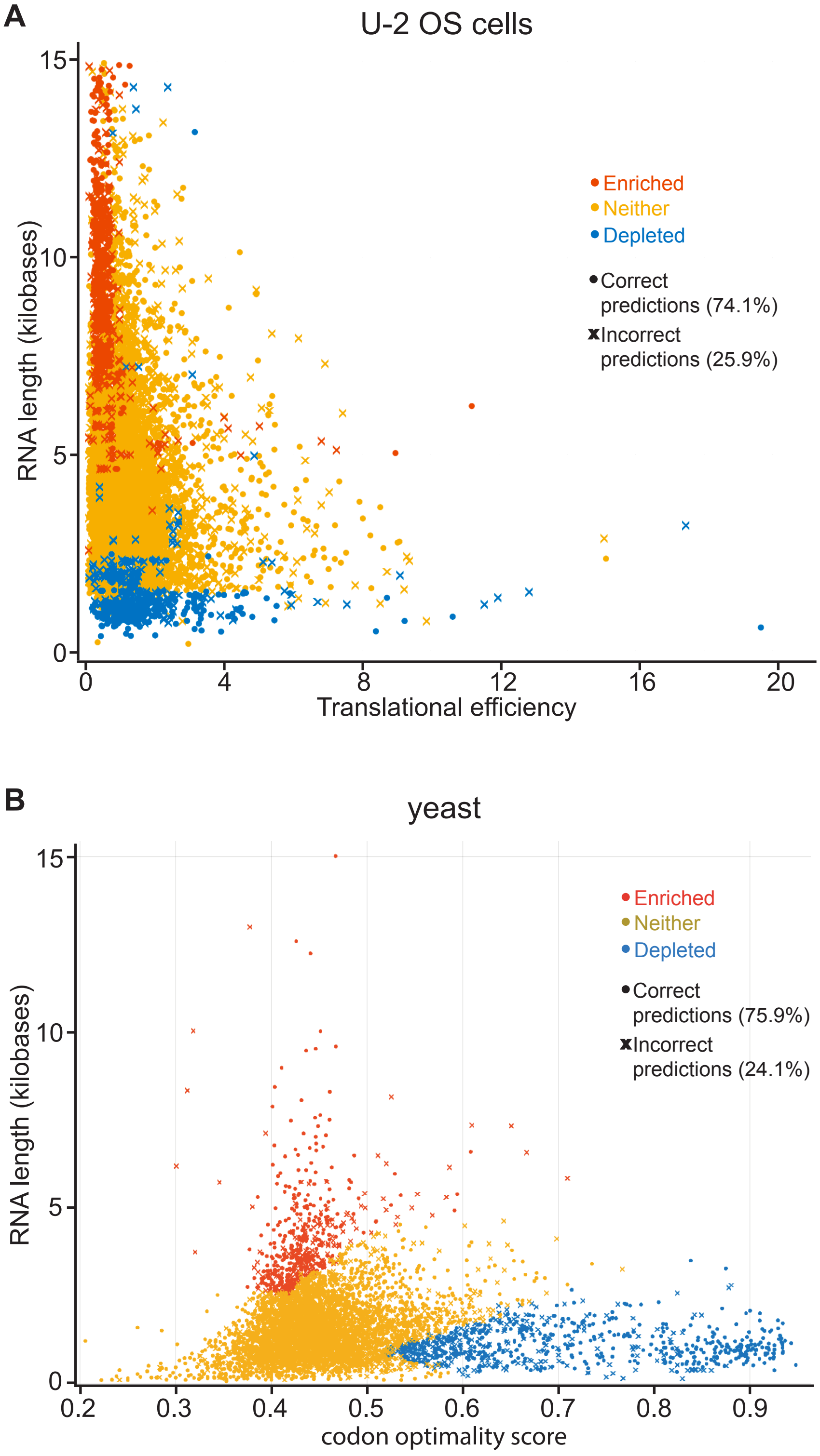

A
U-2 OS cells
RNA length (kilobases)
15
10
5
0
0
4
8
12
16
20
Translational efficiency
● Enriched
● Neither
● Depleted
● Correct predictions (74.1%)
✕ Incorrect predictions (25.9%)
B
yeast
RNA length (kilobases)
15
10
5
0
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
codon optimality score
● Enriched
● Neither
● Depleted
● Correct predictions (75.9%)
✕ Incorrect predictions (24.1%)

Figure S6

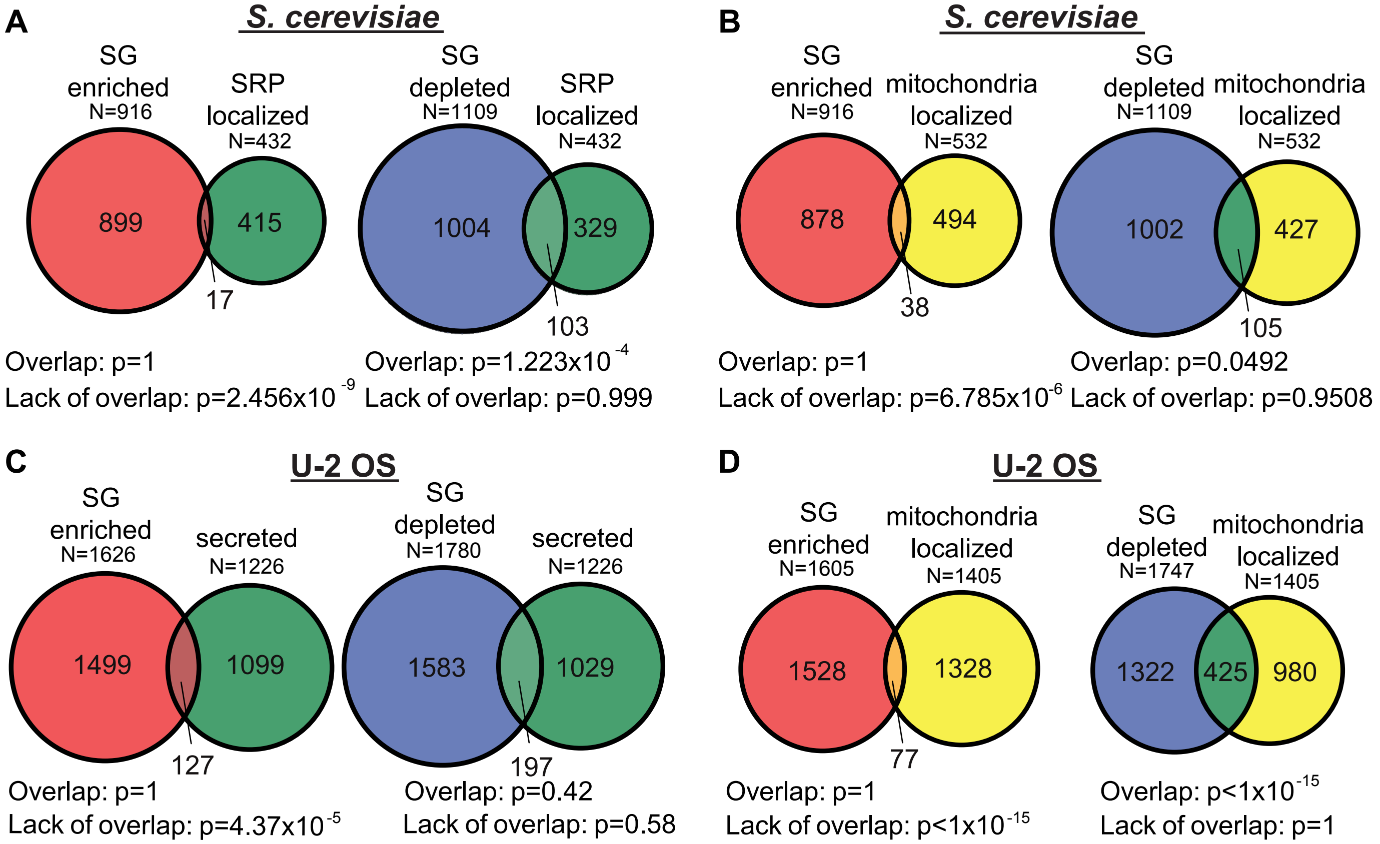

A
S. cerevisiae
SG enriched N=916
SRP localized N=432
899
415
17
Overlap: p=1
Lack of overlap: p=2.456x10^-9
SG depleted N=1109
SRP localized N=432
1004
329
103
Overlap: p=1.223x10^-4
Lack of overlap: p=0.999
B
S. cerevisiae
SG enriched N=916
mitochondria localized N=532
878
494
38
Overlap: p=1
Lack of overlap: p=6.785x10^-6
SG depleted N=1109
mitochondria localized N=532
1002
427
105
Overlap: p=0.0492
Lack of overlap: p=0.9508
C
U-2 OS
SG enriched N=1626
secreted N=1226
1499
1099
127
Overlap: p=1
Lack of overlap: p=4.37x10^-5
SG depleted N=1780
secreted N=1226
1583
1029
197
Overlap: p=0.42
Lack of overlap: p=0.58
D
U-2 OS
SG enriched N=1605
mitochondria localized N=1405
1528
1328
77
Overlap: p=1
Lack of overlap: p<1x10^-15
SG depleted N=1747
mitochondria localized N=1405
1322
425
980
Overlap: p<1x10^-15
Lack of overlap: p=1

Table S1A: Most enriched cytosolic mRNAs in SGs. Related to Figures 1-2.

Gene symbol	Gene description	SG enrichment	mRNA length (bases)	Number of mRNAs in SGs/cell	Number of mRNAs in cell	% of mRNAs in SGs
AHNAK2	AHNAK nucleoprotein 2	11.00	18,335	5.1	4.0	>95*
RP11-438J1.1	Not available	10.84	2,831	1.4	1.1	>95*
BCL9L	B-cell CLL/lymphoma 9 like	9.50	10,005	11.5	10.4	>95*
MAP1A	microtubule associated protein 1A	9.46	10,553	5.9	5.3	>95*
MACF1	microtubule-actin crosslinking factor 1	9.40	24,828	34.7	31.4	>95*
CHD7	chromodomain helicase DNA binding protein 7	9.17	11,568	11.6	10.8	>95*
MAML1	mastermind like transcriptional coactivator 1	8.51	6,559	4.1	4.1	>95*
CDR1	cerebellar degeneration related protein 1	8.43	2,467	2.1	2.1	>95*
KMT2D	lysine methyltransferase 2D	8.33	19,419	11.2	11.4	>95*
HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2	7.90	15,337	15.9	17.1	>95*

*% of mRNAs in SGs greater than 95%

Table S1B: Most abundant cytosolic mRNAs in SGs. Related to Figures 1-2.

Gene symbol	Gene description	SG enrichment	mRNA length (bases)	Number of mRNAs in SGs/cell	Number of mRNAs in cell	% of mRNAs in SGs
ACTB	actin beta	0.44	2,245	178.2	3440	5.2
CHD4	chromodomain helicase DNA binding protein 4	3.56	6,554	159.2	381	41.8
EIF4G2	eukaryotic translation initiation factor 4 gamma 2	1.34	6,769	154.3	981	15.7
THBS1	thrombospondin 1	2.78	7,775	149.2	457	32.7
EEF2	eukaryotic translation elongation factor 2	0.89	3,164	142.9	1364	10.5
HSPA8	heat shock protein family A (Hsp70) member 8	0.98	2,463	106.3	929	11.4
PABPC1	poly(A) binding protein cytoplasmic 1	1.95	3,485	101.5	444	22.9
ERBB2IP	erbb2 interacting protein	4.76	8,647	100.9	181	55.9
PKM	pyruvate kinase, muscle	0.66	3,016	98.5	1264	7.8
COL1A1	collagen type I alpha 1 chain	2.08	6,727	77.2	317	24.4

Table S2: Most enriched mRNA in yeast. Related to Figure S6.

Gene symbol	Description	SG enrichment	RNA length (bases)
FIG2	Factor-induced gene	8.82	5721
DAN4	Delayed ANaerobic	8.28	3544
BNI1	Bud Neck Involved	6.64	6265
HKR1	Hansenula mrakii Killer toxin Resistant	5.83	5582
FLO1	FLOcculation	5.83	3228
NFT1	New Full-length MRP-type Transporter	5.32	3657
SAG1	Sexual AGglutination	5.25	2091
PRP8	Pre-mRNA Processing	5.21	7320
PMD1	Paralog of MDS3	5.12	5447
HSL1	Histone Synthetic Lethal	4.71	4912

Table S3A: Most enriched lincRNA in mammalian SGs. Related to Figure 5.

Gene symbol	SG enrichment	RNA length (bases)	Number of RNAs in SGs/cell	Number of RNAs in cell	% of RNAs in SGs
RP11-1100L3.8	8.10	687	0.79	0.83	95
RP11-123O10.4	5.05	192	0.99	1.64	59
CTC-444N24.11	4.99	4,258	1.38	2.35	58
CTC-459F4.1	4.99	3,666	0.47	0.81	58
LINC00657 (NORAD)	4.62	5,339	11.1	20.5	54
AC004449.6	4.34	433	0.65	1.29	51
CITF22-49D8.1	4.19	4,364	0.64	1.31	49
RP11-521L9.1	4.13	260	0.92	1.90	48
RP11-284F21.9	4.08	3,222	0.72	1.51	48
BAIAP2-AS1	4.07	4,465	3.30	6.97	48

Table S3B: Most enriched SUTs in yeast SGs. Related to Figure 6.

Gene symbol	SG enrichment	Average SG reads	RNA length (bases)
ST4580	5.95	11511	4280
ST4796	5.29	991	1344
ST6623	4.99	2226	1880
ST6969	4.71	12772	3280
ST2060	4.67	2963	2728
ST6465	4.36	2678	1824
ST2371	4.08	4158	1824
ST1657	3.99	6353	3528
ST1280	3.82	2303	1664
ST5030	3.77	5183	2248

Figure S1 (related to Figure 1): Purification and analysis of the RNA content in mammalian SG cores

(A) Schematic depicting how SG cores were purified. In brief, SG formation was induced by sodium arsenite stress (.5mM, 1 hour) in a G3BP1-GFP tagged U-2 OS cell line. Cells were then subjected to syringe lysis, followed by a series of centrifugation steps. This stress granule core enriched fraction was then affinity purified via GFP-G3BP1 and proteinase K treated. RNA was isolated via trizol extraction and ribodepleted, yielding purified SG core RNA. (B) Pairwise R^2 correlations between individual replicates of SG purified RNA and total RNA. (C) smFISH validation of mRNAs enriched in SGs (PEG3, CDK6, and ZNF704). Scale bar = 2μm. (D) Quantification of the average number of each indicated transcripts that occur in SGs per cell. Twenty cells were counted for each experiment.

Figure S2 (related to Figure 1): Validation of the RNA-content in bonafide SGs

(A) Arsenite-treated U-2 OS cells co-stained for (Left) PABP protein, G3BP1 protein, and AHNAK mRNA or (Right) PUM2 protein, G3BP1 protein, and NORAD lincRNA. (B) Arsenite-treated wildtype U-2 OS cells and ∆∆G3BP1/2 U-2 OS cells were co-stained for PABP protein, (left) AHNAK mRNA, and (right) POLR2A mRNA. Scale bar = 2μm.

Figure S3 (related to Figure 4): Properties of differential mRNA recruitment to mammalian SGs

(A) Scatter plot depicting mRNA abundance (FPKM) in SG purified RNA vs. Total RNA. Red dots indicate mRNAs that are significantly enriched (Fold change > 2 and $p < .01$) in

SG purified RNA compared to Total RNA. Blue dots indicate mRNAs that are significantly depleted (Fold change <.05 and p >.01) in SG purified RNA compared to Total RNA. Gray dots indicate mRNAs that are either not significantly enriched or fail to meet the fold change requirement. (B) Pie chart depicting the relative contribution of each class of ncRNA (SG-enriched, SG-depleted, or neither) to the SG transcriptome. (C-E) Boxplots depicting (C) mRNA half-life, (D) mRNA abundance, and (E) GC content for the three classes of localization: SG enriched mRNAs, SG depleted mRNAs, or neither. (F) Boxplots illustrating 3' UTR length at various ORF lengths for each class of mRNA localization. mRNA ORF lengths were binned every kilobase. (G) Same as in F but for 5' UTR lengths. n.s., *, **, and *** = p value (Student's t-test) > 0.05, ≤0.05, ≤0.01, and ≤0.001 respectively.

Figure S4 (related to Figure 6): Purification and analysis of the RNA content in yeast SG cores

(A) Pairwise R^2 correlations between individual replicates of SG purified RNA from yeast and total yeast RNA. (B) RNA smFISH of MDN1 after 30 minutes of sodium azide treatment in Pab-GFP expressing yeast. (C) Boxplot showing predicted translation initiation rates for SG enriched RNA, SG depleted RNA, and RNA that shows no preference. (D) Boxplot depicting RNA half-lives for SG enriched RNA, SG depleted RNA, and RNA that shows no preference. (E) Scatterplot illustrating SUTs that are enriched in SGs (red), depleted from SGs (blue), or neither (gray). (F) Same as E but for CUTs. (G) Boxplot depicting transcript length of SUTs for each class of mRNA localization. (H) Same as G but for CUTs. n.s., **, and *** = p value (Student's t-test) > 0.05, ≤0.01, and ≤0.001 respectively.

Figure S5 (related to Figures 4 and 6): Machine learning model accurately predicts mRNA localization in SGs

(A) A machine learning model accurately predicts 74.1% of mammalian mRNA localization during stress based on ribosome density and transcript length alone. (B) A machine learning model accurately predicts 75.9% of yeast mRNA localization during stress based on fraction optimal codons and transcript length alone.

Figure S6 (related to Figures 4 and 6): Membrane targeting affects SG localization

(A) Venn diagram depicting the overlap between *(Left)* yeast SG-enriched mRNAs and mitochondrial-localized genes or *(Right)* yeast SG-depleted mRNAs and mitochondrial-localized genes. (B) Venn diagram illustrating the overlap between *(Left)* yeast SG-enriched mRNAs and SRP localized genes or *(Right)* SG-depleted mRNAs and SRP localized genes. (C) Venn diagram illustrating the overlap between *(Left)* human SG-enriched mRNAs and mitochondrial-localized genes or *(Right)* SG-depleted mRNAs and mitochondrial-localized genes. (D) Venn diagram illustrating the overlap between *(Left)* human SG-enriched mRNAs and SRP localized genes or *(Right)* SG-depleted mRNAs and SRP localized genes.