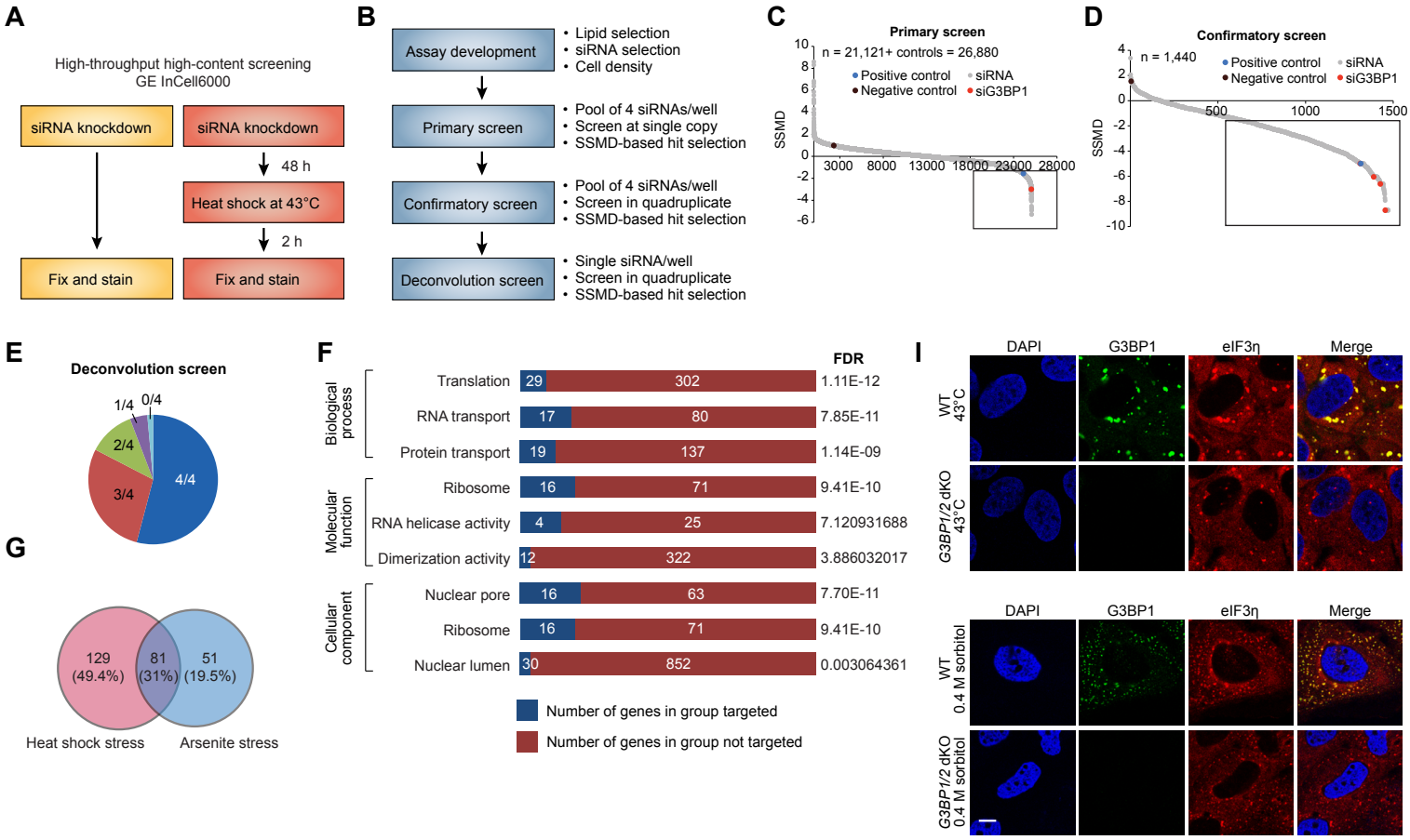
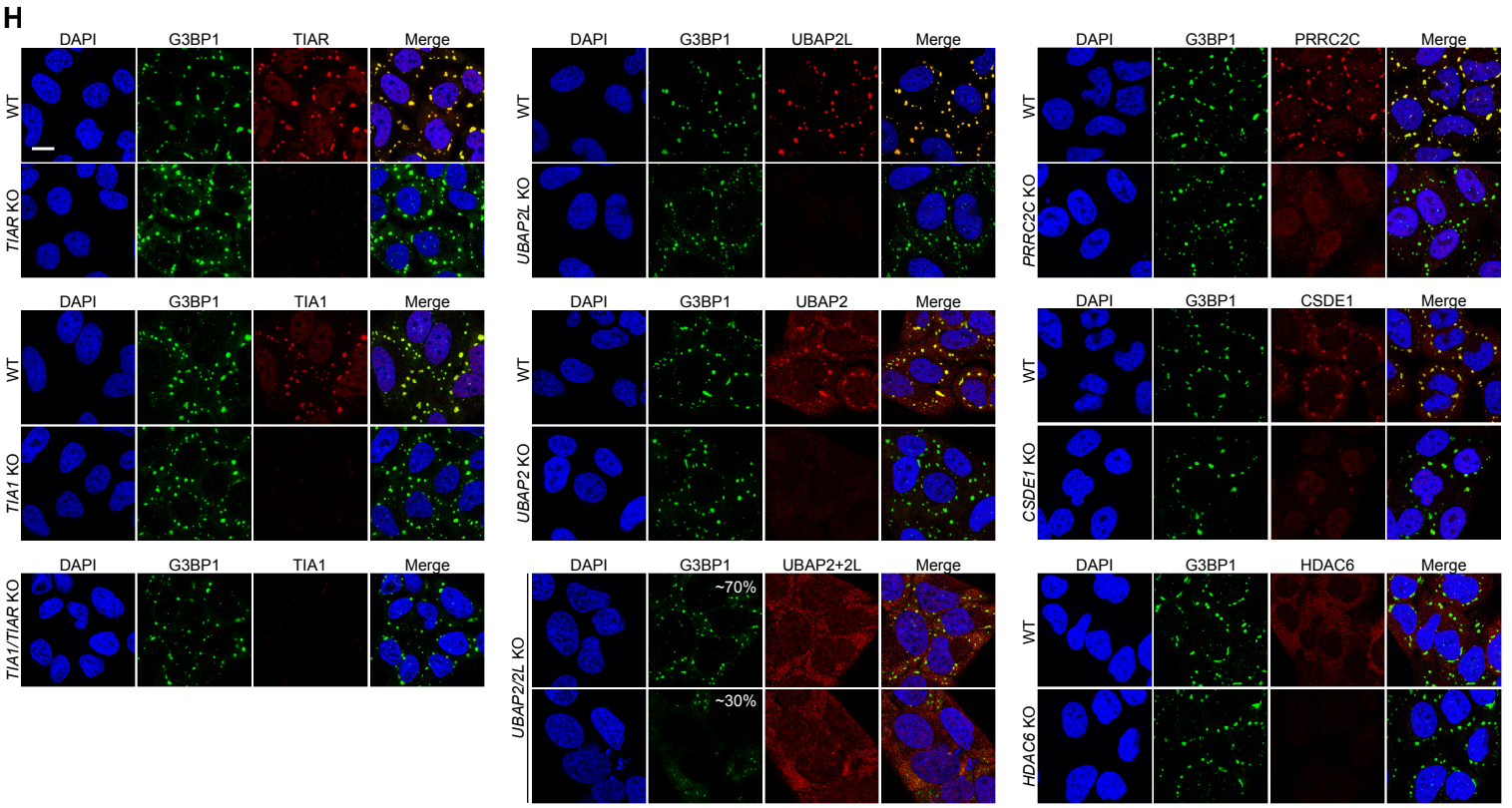
Figure S1


A
High-throughput high-content screening
GE InCell6000
siRNA knockdown
siRNA knockdown
48 h
Heat shock at 43°C
2 h
Fix and stain
Fix and stain
B
Assay development
• Lipid selection
• siRNA selection
• Cell density
Primary screen
• Pool of 4 siRNAs/well
• Screen at single copy
• SSMD-based hit selection
Confirmatory screen
• Pool of 4 siRNAs/well
• Screen in quadruplicate
• SSMD-based hit selection
Deconvolution screen
• Single siRNA/well
• Screen in quadruplicate
• SSMD-based hit selection
C
Primary screen
n = 21,121+ controls = 26,880
Positive control
siRNA
Negative control
siG3BP1
SSMD
D
Confirmatory screen
n = 1,440
Positive control
siRNA
Negative control
siG3BP1
SSMD
E
Deconvolution screen
4/4
3/4
2/4
1/4
0/4
F
FDR
Biological process
Translation 29 302 1.11E-12
RNA transport 17 80 7.85E-11
Protein transport 19 137 1.14E-09
Molecular function
Ribosome 16 71 9.41E-10
RNA helicase activity 4 25 7.120931688
Dimerization activity 12 322 3.886032017
Cellular component
Nuclear pore 16 63 7.70E-11
Ribosome 16 71 9.41E-10
Nuclear lumen 30 852 0.003064361
Number of genes in group targeted
Number of genes in group not targeted
G
129 (49.4%)
81 (31%)
51 (19.5%)
Heat shock stress
Arsenite stress
I
DAPI
G3BP1
eIF3η
Merge
WT 43°C
G3BP1/2 dKO 43°C
WT 0.4 M sorbitol
G3BP1/2 dKO 0.4 M sorbitol


H
DAPI
G3BP1
TIAR
Merge
WT
TIAR KO
UBAP2L
UBAP2L KO
PRRC2C
PRRC2C KO
TIA1
TIA1 KO
UBAP2
UBAP2 KO
CSDE1
CSDE1 KO
TIA1/TIAR KO
UBAP2+2L
UBAP2/2L KO
~70%
~30%
HDAC6
HDAC6 KO