Regulation of cGAS activity by RNA-modulated phase separation

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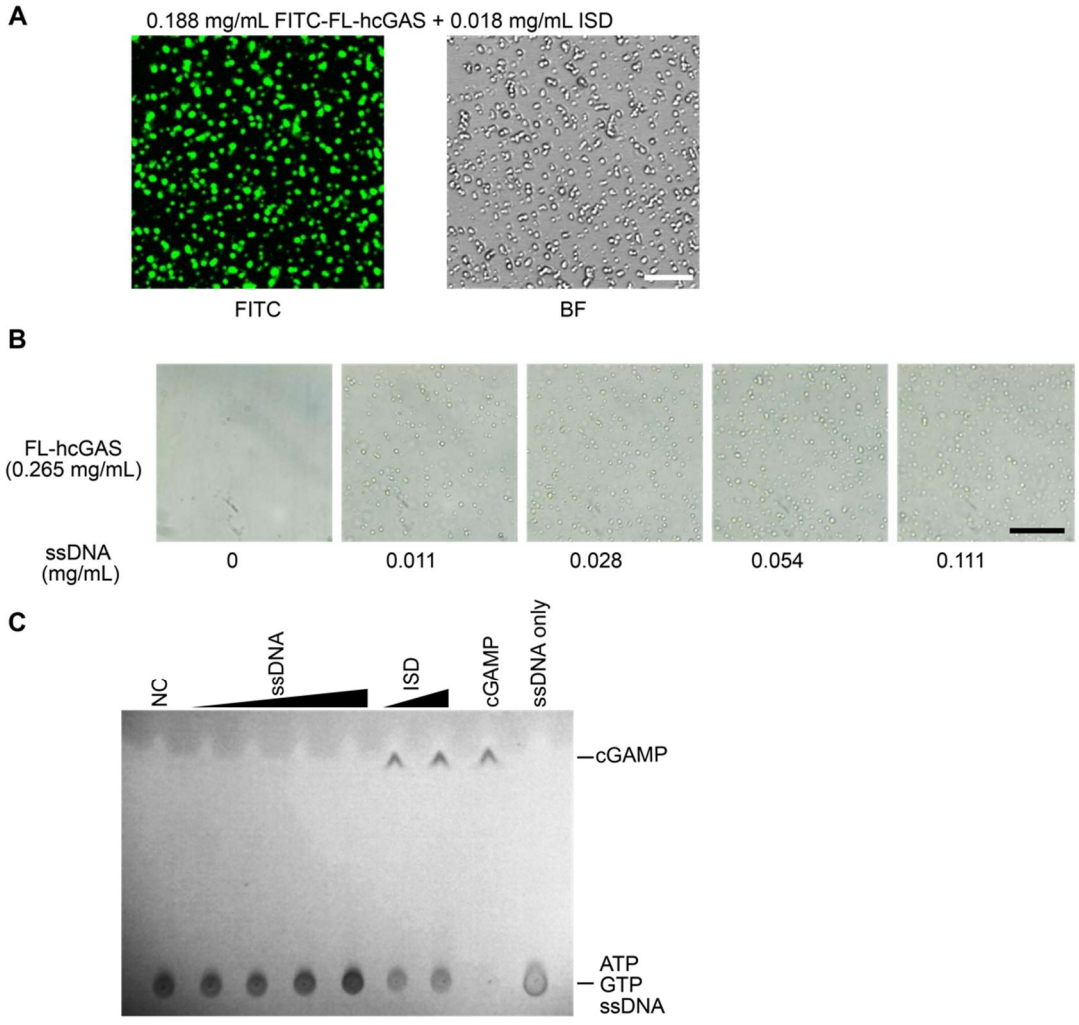
CONTENT

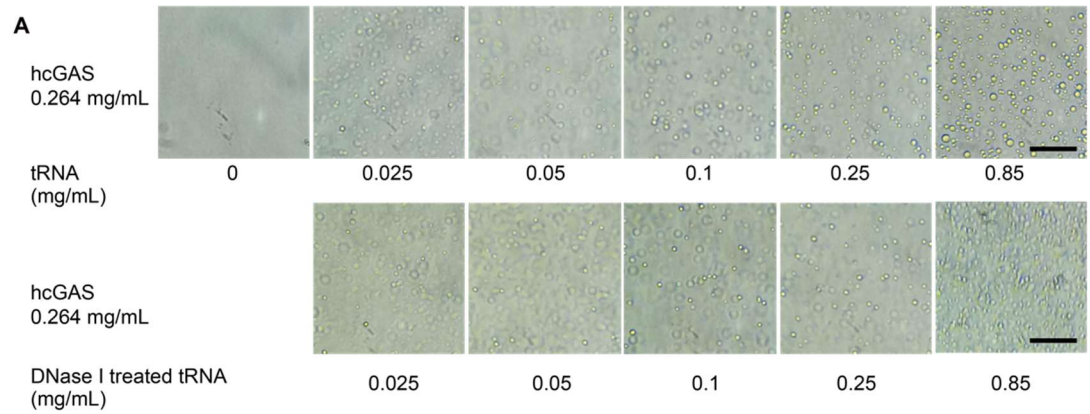

A
0.188 mg/mL FITC-FL-hcGAS + 0.018 mg/mL ISD
FITC
BF
B
FL-hcGAS
(0.265 mg/mL)
ssDNA
(mg/mL)
0
0.011
0.028
0.054
0.111
C
NC
ssDNA
ISD
cGAMP
ssDNA only
cGAMP
ATP
GTP
ssDNA

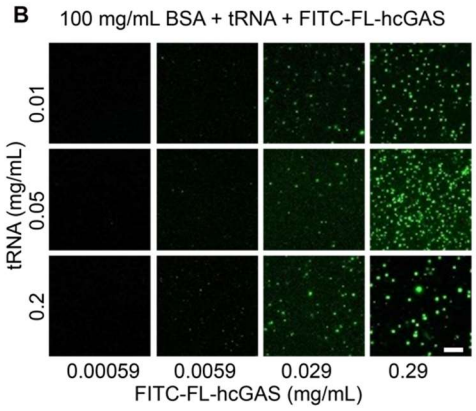
Appendix Figure S1. ssDNA mediates phase separation of FL-hcGAS.

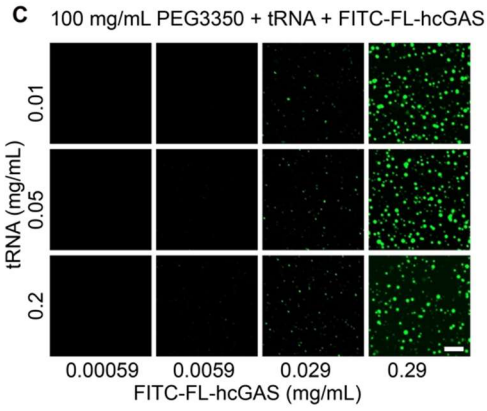
A. Fluorescence and bright field photographs showing phase-separated granules of FITC-labeled FL-hcGAS induced by the interferon stimulatory dsDNA (ISD) (n=3, biological replicates, data from one representative independent biological replicate are shown). The ISD has the sequence 5 -TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACA-3. The FITC-labelled FL-cGAS concentration was 0.188 mg/mL and the ISD concentration was 0.018 mg/mL. The scale bar represents 20 μm.

B. Bright field photographs showing FL-hcGAS (0.265 mg/mL) mixed with a 48 nt ssDNA at the indicated concentrations (n=2, biological replicates, data from one representative independent biological replicate are shown). The scale bar represents 40 μm. The 48 nt ssDNA has the sequence 5 -AGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG-3.

C. TLC analysis of the cGAMP production, which is indicative of cGAS activation (n=2, biological replicates, data from one representative independent biological replicate are shown). The FL-hcGAS concentrations was 0.265 mg/mL. The ssDNA concentrations from low to high were 0.011, 0.028, 0.054 and 0.111 mg/mL. NC indicates negative control which has only the FL-hcGAS. The ISD concentrations from low to high were 0.055 and 0.111 mg/mL. Samples were prepared in 20 mM HEPES at pH 7.5 and 150 mM NaCl.


A
hcGAS
0.264 mg/mL
tRNA
(mg/mL)
0
0.025
0.05
0.1
0.25
0.85
hcGAS
0.264 mg/mL
DNase I treated tRNA
(mg/mL)
0.025
0.05
0.1
0.25
0.85

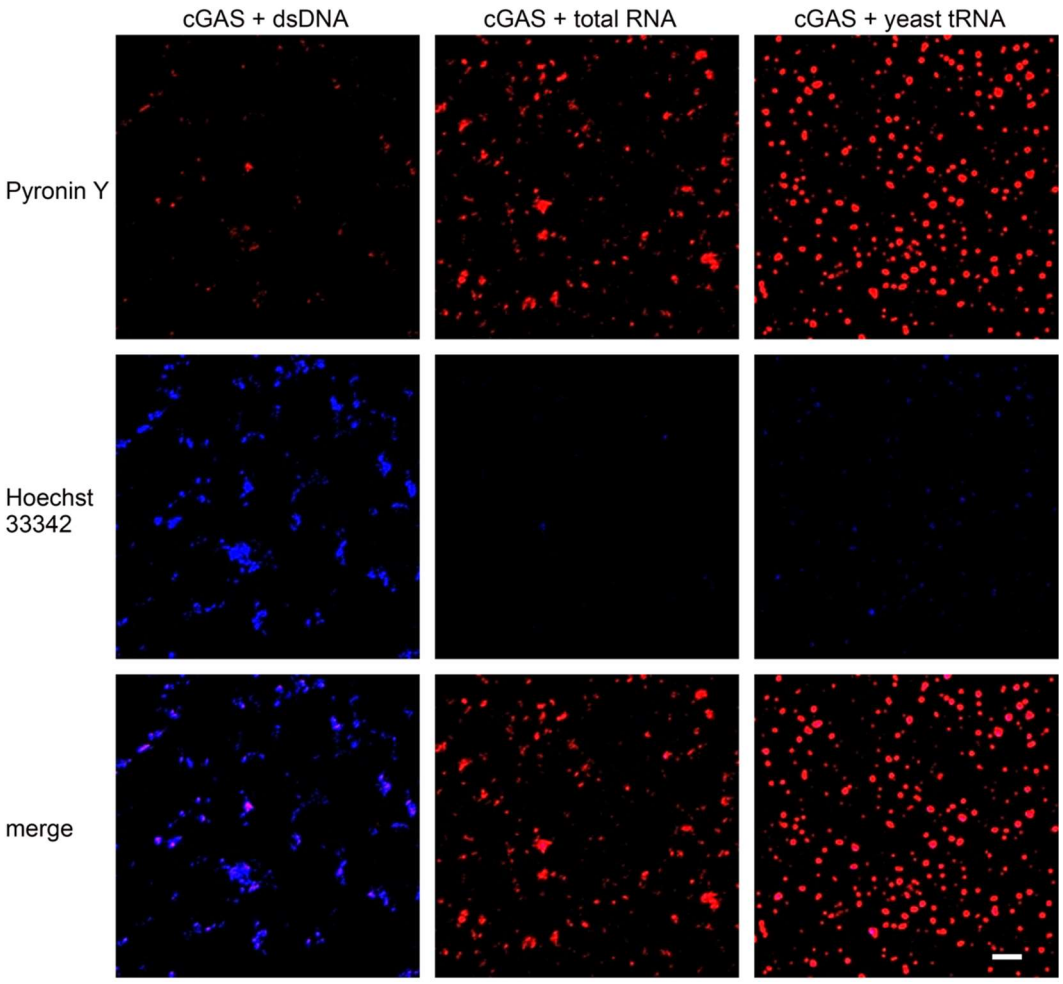

B
100 mg/mL BSA + tRNA + FITC-FL-hcGAS
tRNA (mg/mL)
0.01
0.05
0.2
0.00059
0.0059
0.029
0.29
FITC-FL-hcGAS (mg/mL)


C
100 mg/mL PEG3350 + tRNA + FITC-FL-hcGAS
tRNA (mg/mL)
0.01
0.05
0.2
0.00059
0.0059
0.029
0.29
FITC-FL-hcGAS (mg/mL)

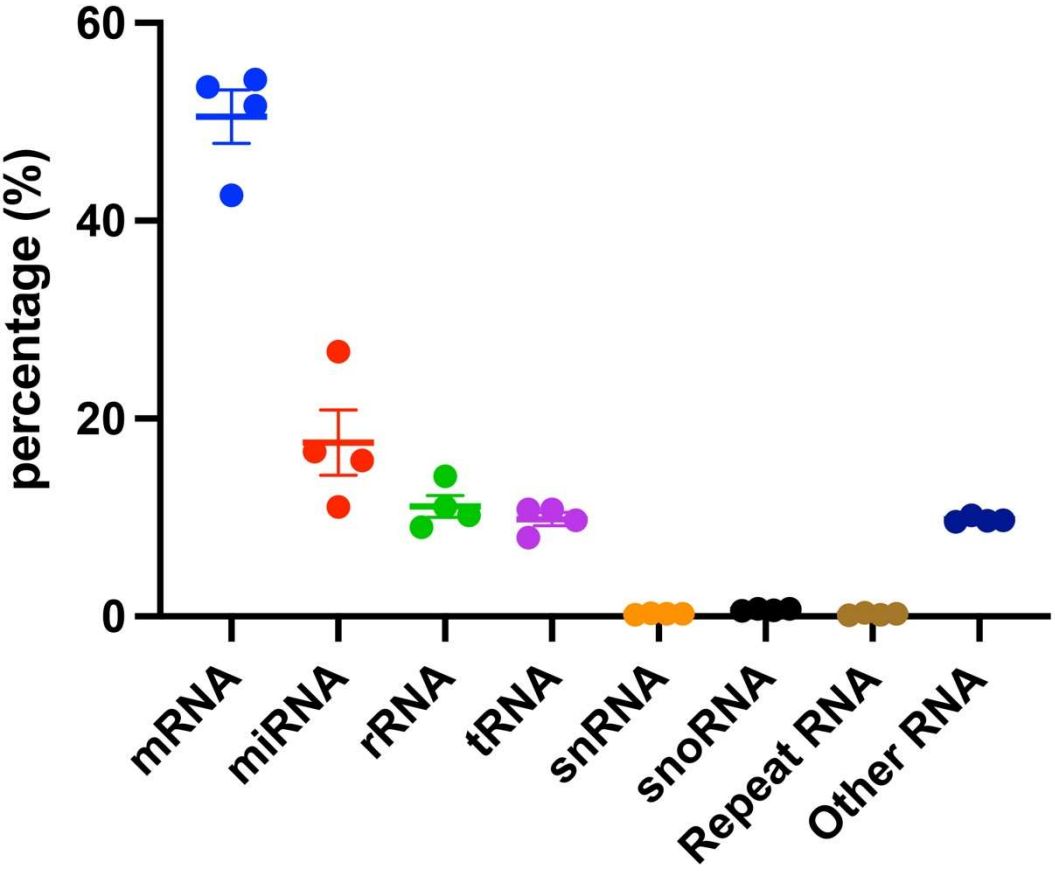
Appendix Figure S2. RNA mediated phase separation of cGAS.

A. Upper: Bright field photographs of samples of FL-hcGAS (0.264 mg/mL) and yeast tRNA at the indicated concentrations (n=2, biological replicates, data from one representative independent biological replicate are shown). Lower: Bright field photographs of samples of FL-hcGAS (0.264 mg/mL) and DNase I-treated yeast tRNA at the indicated concentrations (n=2, biological replicates, data from one representative independent biological replicate are shown). The scale bars represent 40 μm.

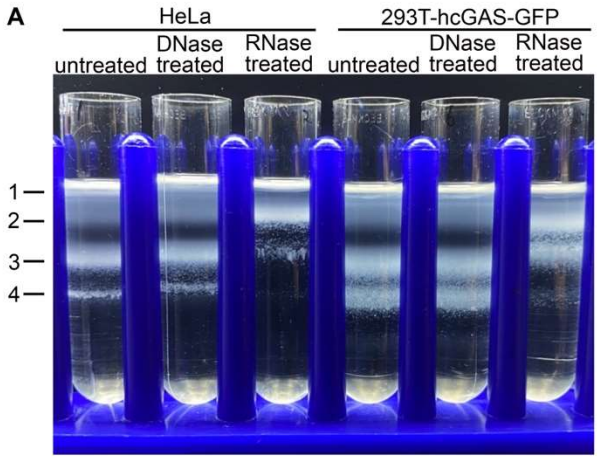
B and C. Fluorescence images of FITC-labeled FL-hcGAS and yeast tRNA in presence of 100 mg/mL **B)** BSA or **C)** PEG3350 (n=2, biological replicates, data from one representative independent biological replicate are shown). Samples were prepared in 20 mM HEPES at pH 7.5 and 150 mM NaCl. The scale bars represent 20 μm.

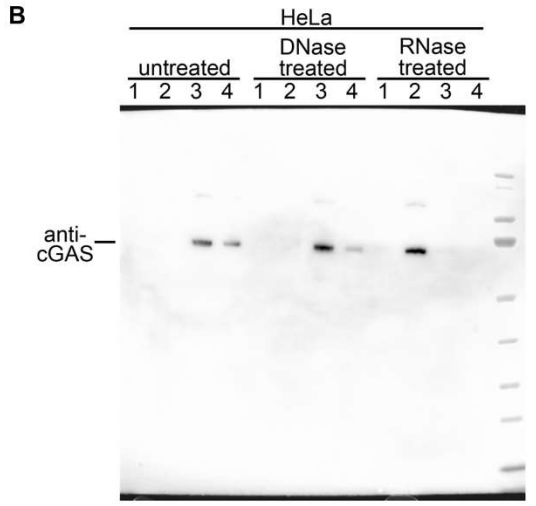

cGAS + dsDNA
cGAS + total RNA
cGAS + yeast tRNA
Pyronin Y
Hoechst 33342
merge

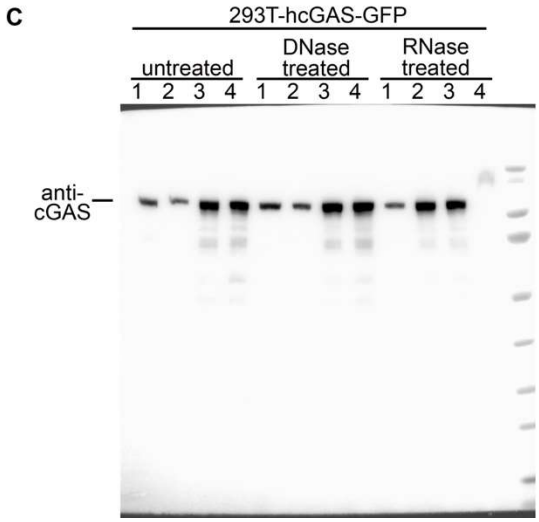
Appendix Figure S3. Differential staining of dsDNA and RNA in the cGAS involved phase separations by Hoechst 33342 and pyronin Y. Confocal microscopy images of FL-hcGAS incubated with 55-bp dsDNA, extracted total RNA from HeLa cells, or yeast tRNA and stained with 2 μg/mL Hoechst 33342 and 4 μg/mL pyronin Y (n=2, biological replicates, data from one representative independent biological replicate are shown). The concentration of FL-hcGAS was 0.2 mg/mL. The concentrations of 55-bp dsDNA, total RNA and yeast tRNA were all 0.1 mg/mL. The scale bar represents 10 μm.


60
40
20
0
percentage (%)
mRNA
miRNA
rRNA
tRNA
snRNA
snoRNA
Repeat RNA
Other RNA

Appendix Figure S4. RNA sequencing results of the cGAS-containing cytoplasmic fractions from the OptiPrep gradient. Libraries were generated from band 3 and band 4 of the Opti-prep density gradient (Figure 2H) for RNA sequencing. The preparation of the samples was repeated 2 times. A total of 4 samples were sent for RNA sequencing (n=4, biological replicates). The histograms show the percentage of sequencing reads for different RNA species. Data are presented as means ± SEM.


A
HeLa
293T-hcGAS-GFP
untreated
DNase treated
RNase treated
untreated
DNase treated
RNase treated
1
2
3
4


B
HeLa
untreated
DNase treated
RNase treated
1 2 3 4 1 2 3 4 1 2 3 4
anti-cGAS

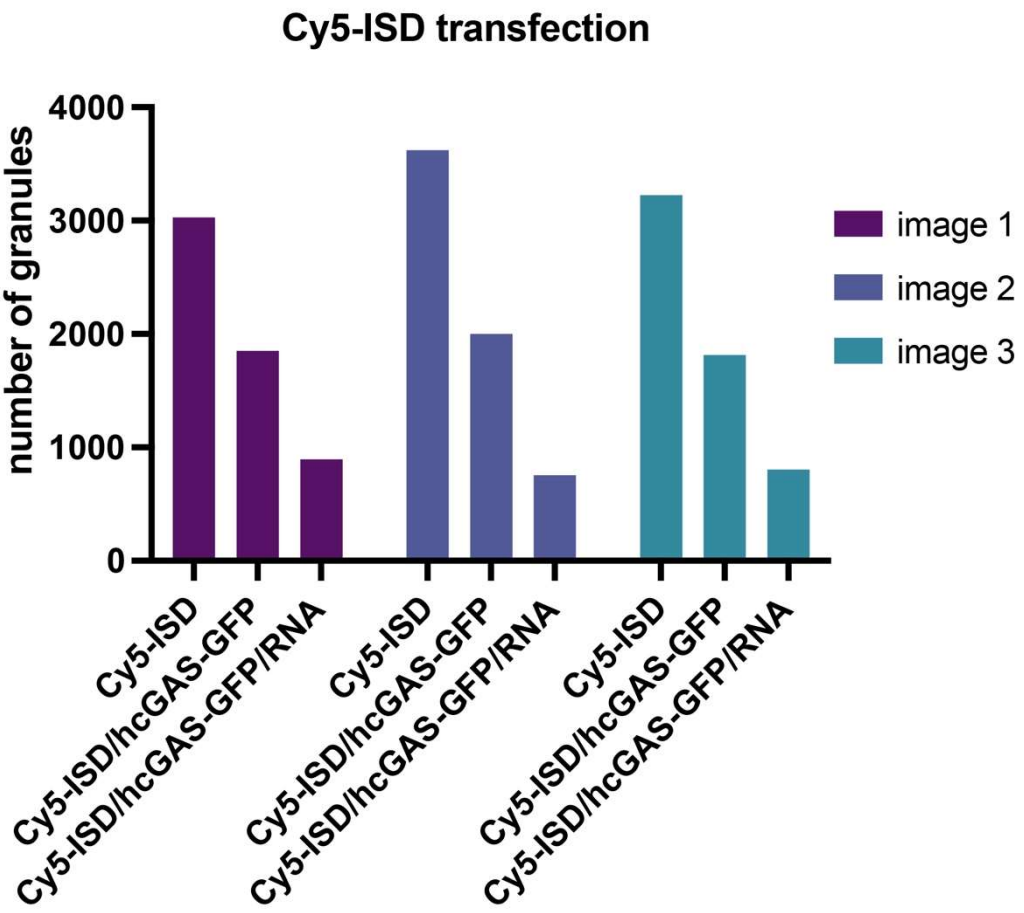

C
293T-hcGAS-GFP
untreated
DNase treated
RNase treated
1 2 3 4 1 2 3 4 1 2 3 4
anti-cGAS

Appendix Figure S5. OptiPrep density gradient analyses of endogenous cGAS in HeLa cells and the overexpressed cGAS-GFP in HEK293T cells.

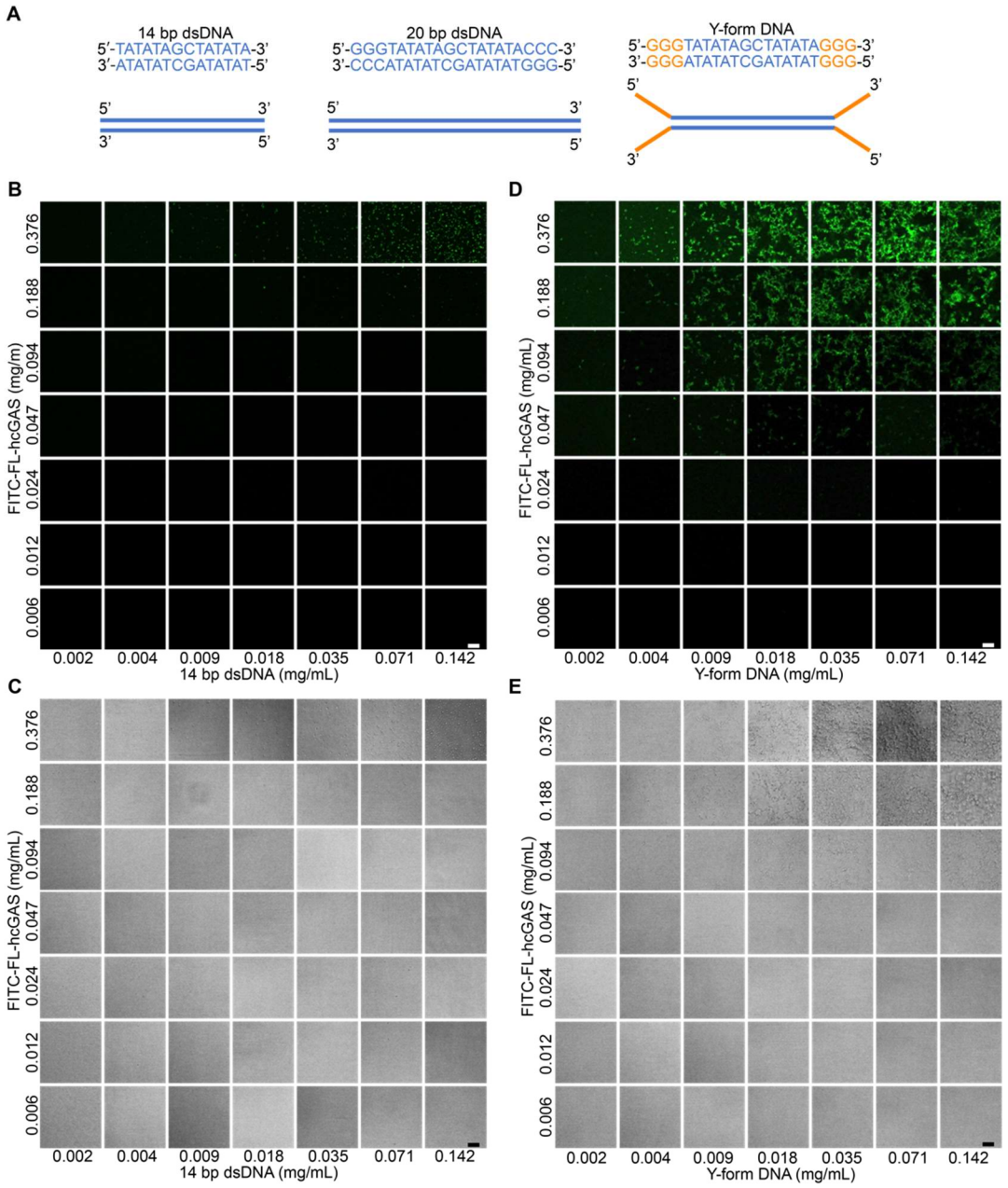
A. Opti-prep density gradient analyses of the HeLa cell cytoplasmic extracts and the HEK293T-hcGAS-GFP cell cytoplasmic extracts (n=2, biological replicates, data from one representative independent biological replicate are shown)**.**

B. Western blot analysis of the OptiPrep gradient fractions from the HeLa cell cytoplasmic extracts with or without DNase and RNase treatments (n=2, biological replicates, data from one representative independent biological replicate are shown).

C. Western blot analysis of the OptiPrep gradient fractions from the HEK293T-hcGAS-GFP cell cytoplasmic extracts with or without DNase and RNase treatments (n=2, biological replicates, data from one representative independent biological replicate are shown).


Cy5-ISD transfection
number of granules
4000
3000
2000
1000
0
image 1
image 2
image 3
Cy5-ISD
Cy5-ISD/hcGAS-GFP
Cy5-ISD/hcGAS-GFP/RNA
Cy5-ISD
Cy5-ISD/hcGAS-GFP
Cy5-ISD/hcGAS-GFP/RNA
Cy5-ISD
Cy5-ISD/hcGAS-GFP
Cy5-ISD/hcGAS-GFP/RNA

Appendix Figure S6. Statistics for the number of the granules with colocalized Cy5-ISD and hcGAS-GFP or Cy5-ISD, hcGAS-GFP and RNA (related to Fig 3B). Granules from 3 raw photographs that have approximately 50 cells per photograph were used for the statistics.

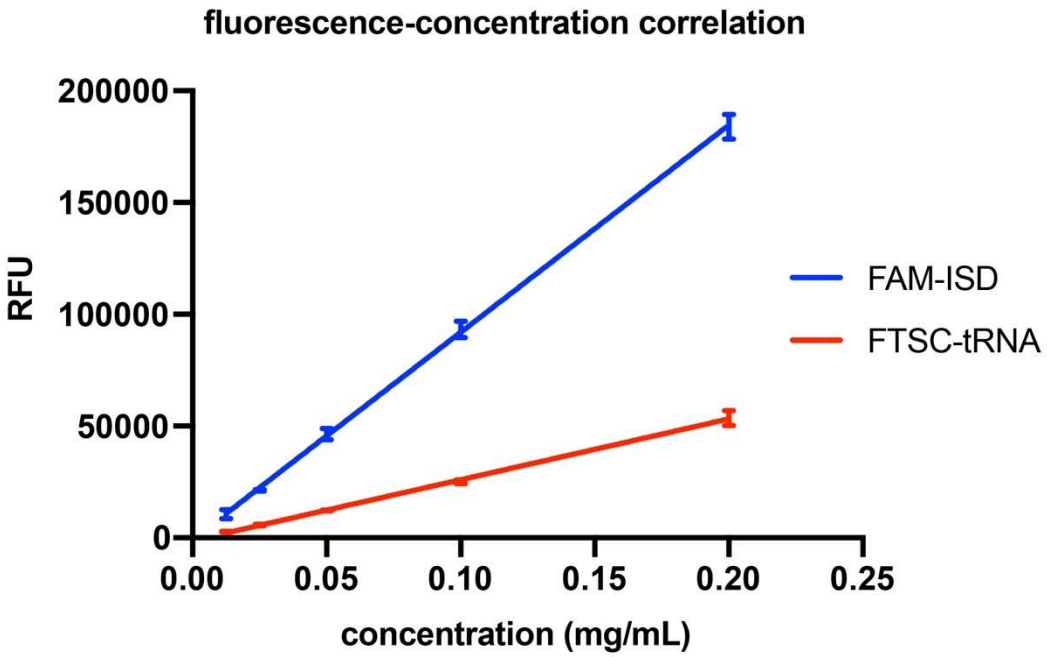

A
14 bp dsDNA
5′-TATATAGCTATATA-3′
3′-ATATATCGATATAT-5′
20 bp dsDNA
5′-GGGTATATAGCTATATACCC-3′
3′-CCCATATATCGATATATGGG-5′
Y-form DNA
5′-GGGTATATAGCTATATAGGG-3′
3′-GGGATATATCGATATATGGG-5′
5′
3′
B
C
D
E
FITC-FL-hcGAS (mg/mL)
0.376
0.188
0.094
0.047
0.024
0.012
0.006
0.002
0.004
0.009
0.018
0.035
0.071
0.142
14 bp dsDNA (mg/mL)
Y-form DNA (mg/mL)

Appendix Figure S7. Y-form DNA mediated phase separation of FL-hcGAS.

A. Sequences and structures of the 14-bp dsDNA, 20-bp dsDNA and Y-form DNA.

B and C. Phase diagrams of FITC-FL-hcGAS and the 14-bp dsDNA (n=2, biological replicates, data from one representative independent biological replicate are shown). **B)** Fluorescence photographs. **C)** Bright field photographs. The scale bars represent 20 μm.

D and E. Phase diagrams of FITC-FL-hcGAS and the Y-form DNA (n=2, biological replicates, data from one representative independent biological replicate are shown). **D)** Fluorescence photographs. **E)** Bright field photographs. The scale bars represent 20 μm.


fluorescence-concentration correlation
200000
150000
100000
50000
0
RFU
0.00
0.05
0.10
0.15
0.20
0.25
concentration (mg/mL)
FAM-ISD
FTSC-tRNA

Appendix Figure S8. Linear correlation between the concentrations and the fluorescence signals (RFUs) of the fluorophore-labeled ISD and tRNA. Data are presented as mean ± SD (n=3, technical replicates).