Expanded View Figures

Figure EV1. G3BP1 engages cGAS in a condensed state.

A   Bright-field microscope images of indicated groups (left) and a quantitative analysis of total area of the condensates. $n = 3$ biological replicates. 10 μM cGAS, 5 μM G3BP1, 5 μM BSA and 500 nM dsDNA were used in these assays.

B   Coomassie blue staining of purified recombinant cGAS-mCherry protein.

C   Fluorescent images of liquid droplets after mixing 10 μM cGAS-mCherry with 1 μM FAM-labeled dsDNA.

D   Immunofluorescent staining of cGAS in WT and $cGAS^{-/-}$ cells. Images were acquired with Leica TCS SP8 Confocal Microscopy.

E, F   Immunoblotting of WT and $G3BP1^{-/-}$ cells with indicated antibodies.

G   Immunofluorescent staining of cGAS and G3BP1 in both WT and $G3BP1^{-/-}$ HeLa cells. Images were acquired with Leica TCS SP8 Confocal microscopy. The white arrows indicate the cGAS-G3BP1 condensates.

H   Immunofluorescent staining of cGAS and G3BP1 in both WT and $G3BP1^{-/-}$ HeLa cells. 3D images were reconstituted by Leica LAS X software.

I   Quantitative analysis of total cGAS puncta number (left) and volume (right) per cell of (H). $n = 21$ cells.

J   Immunoblotting of fractionated lysates from WT and $G3BP1^{-/-}$ HeLa cells. GAPDH and Lamin B1 blots were used as controls for cytoplasmic and nuclear fractions, respectively.

Data information: Representative images are shown (A, C–H, and J). Error bars, mean with s.d. (A and I). Scale bars, 10 μm (A), 14 μm (C), 5 μm (D, G and H). WT, wild type; IB, immunoblotting; NS, non-significant. Hoechst (blue), nuclear staining. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, two-tailed *t*-test.
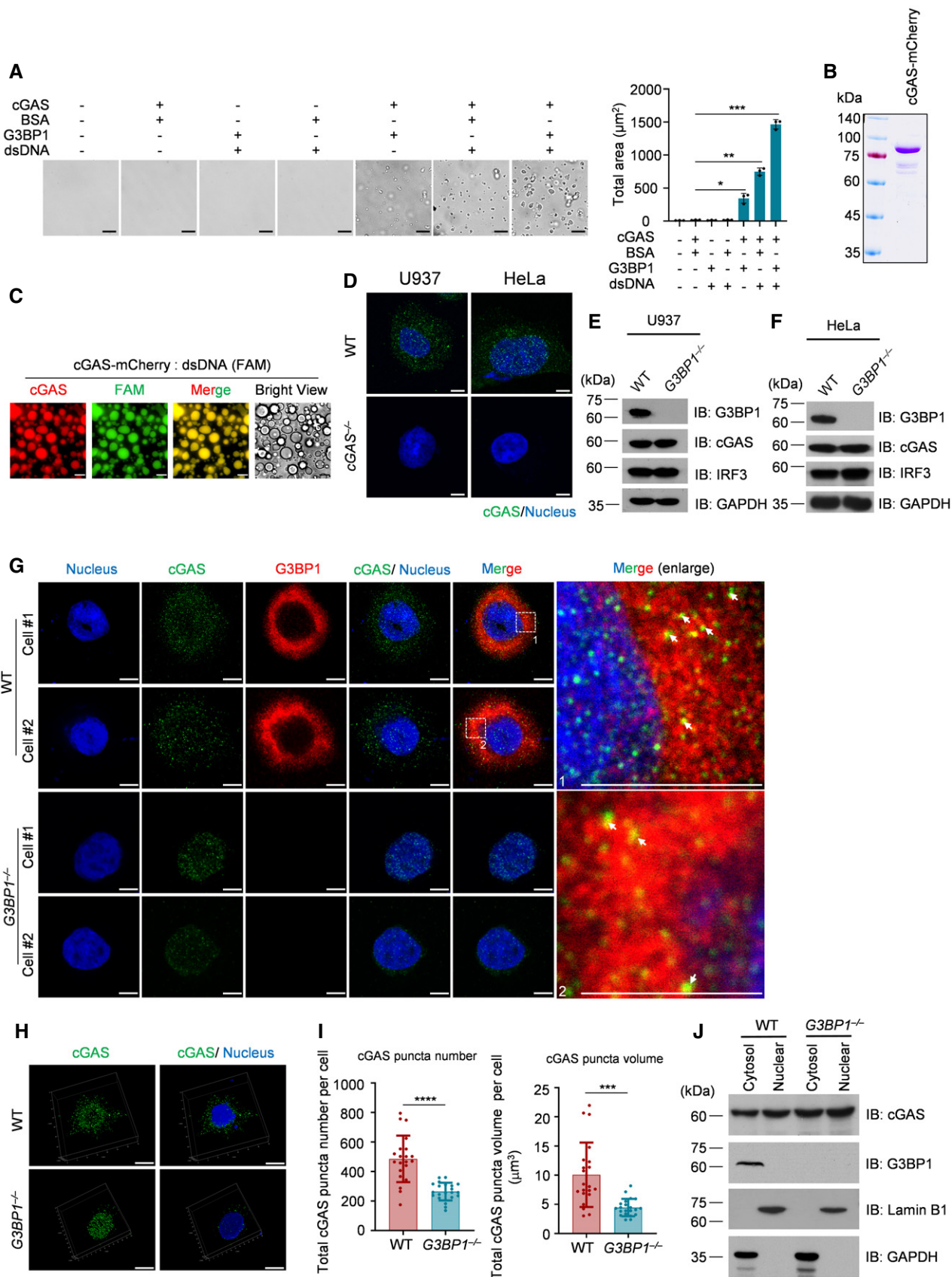
Source data are available online for this figure.
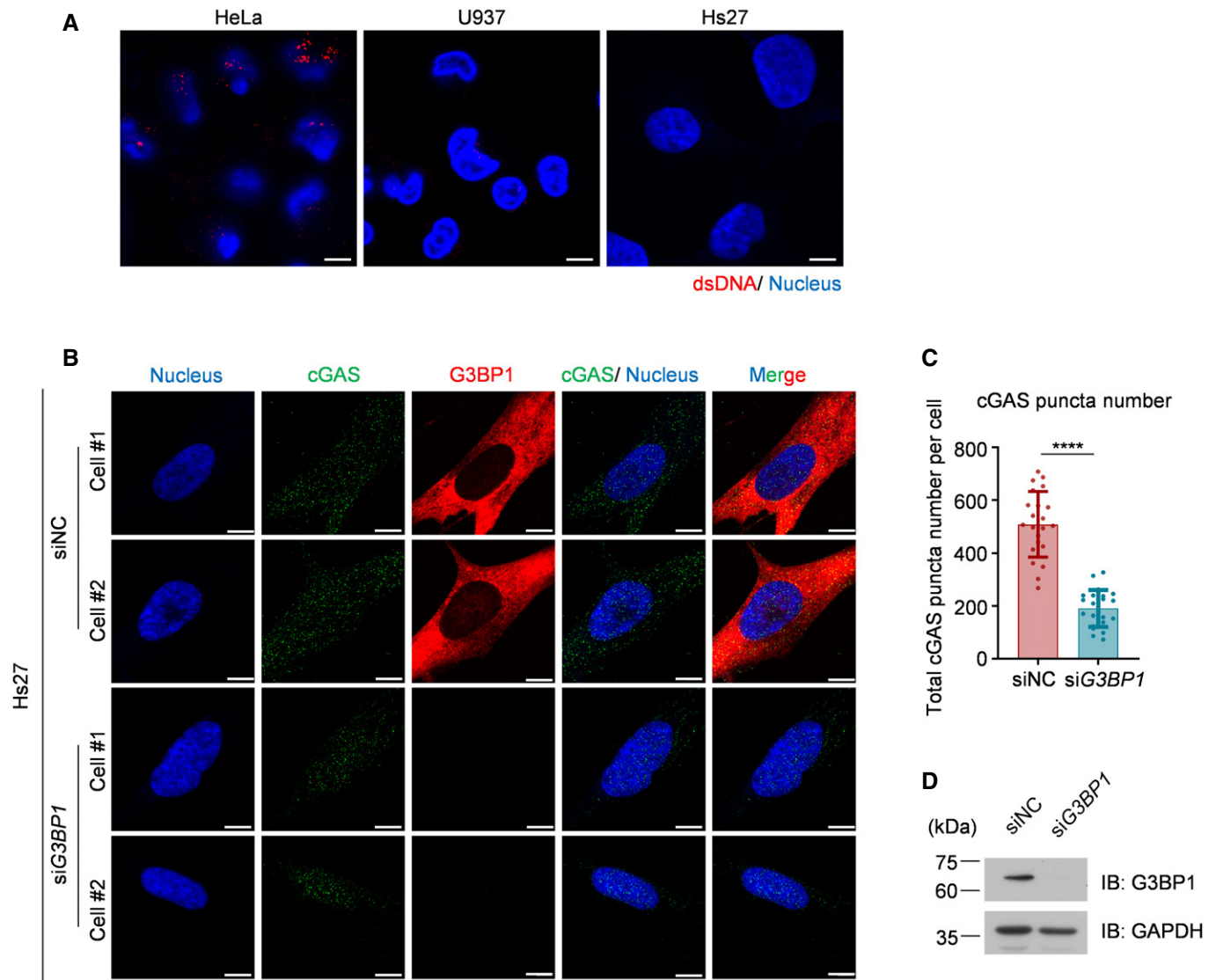
Figure EV1.

Figure EV2. G3BP1 engages cGAS in a condensed state in human fibroblast cell line.

A   Immunofluorescent staining of cytosolic dsDNA in indicated cells.
B   Immunofluorescent staining of cGAS in both WT and *G3BP1*-knockdown Hs27 cells.
C   Quantitative analysis of total cGAS puncta number per cell in (B). n = 22 cells. Error bar, mean with s.d. ****P < 0.0001, two-tailed t-test.
D   Knockdown effect of G3BP1 was analyzed by immunoblotting.

Data information: Representative images are shown (A, B and D). Scale bars, 5 μm (A and B). Hoechst (blue), nuclear staining. WT, wild type; IB, immunoblotting; NC, negative control.
Source data are available online for this figure.

Figure EV3. The engagement with DNA, but not with RNA, leads to the dissociation of G3BP1 from cGAS.

A   Time-lapse imaging of a single cGAS-G3BP1 droplet (left). A quantitative analysis of the time course of the droplet fluorescence intensity is shown (right). $n = 20$ condensates.

B   cGAS-G3BP1 interaction analysis. cGAS was precipitated with anti-cGAS antibodies in U937 cells treated with HT-DNA as indicated. IgG served as control. The cGAS-G3BP1 interaction was further analyzed with immunoblotting (IB).

C   Time-lapse imaging of a single cGAS-DNA droplet in the presence of G3BP1-mEGFP (left). A quantitative analysis of the time course of the droplet fluorescence intensity is shown (right). $n = 20$ condensates.

D   Time-lapse imaging of a single droplet formed by cGAS, G3BP1, and dsRNA (left). A quantitative analysis of the time course of the droplet fluorescence intensity is shown (right). $n = 20$ condensates.

E   cGAS, G3BP1, and Cy5-labled ssRNA were incubated. Indicated concentrations of Cy5-labeled ssRNA were used.

F   Time-lapse imaging of condensates formed by cGAS, G3BP1, and ssRNA.

G   cGAS, G3BP1, and Cy5-ssRNA were incubated. A quantitative analysis of a representative cGAS-DNA condensate is shown. Along the white line on the merged image, the fluorescence intensity of G3BP1, cGAS, and ssRNA channels were recorded.

H   Time-lapse imaging of a single droplet formed by cGAS, G3BP1, and ssRNA (left). A quantitative analysis of the time course of the droplet fluorescence intensity is shown (right). $n = 20$ condensates.

Data information: Representative images are shown (A, C–H). 45 μM cGAS-mCherry, 10 μM G3BP1-mEGFP, 2 μM dsDNA, dsRNA ssRNA were used in the assays. Error bars, mean with s.d. (A, C, H) and s.e.m. (D). Scale bars, 0.28 μm (A), 1 μm (C, D and H), 10 μm (E and F), 5 μm (G).
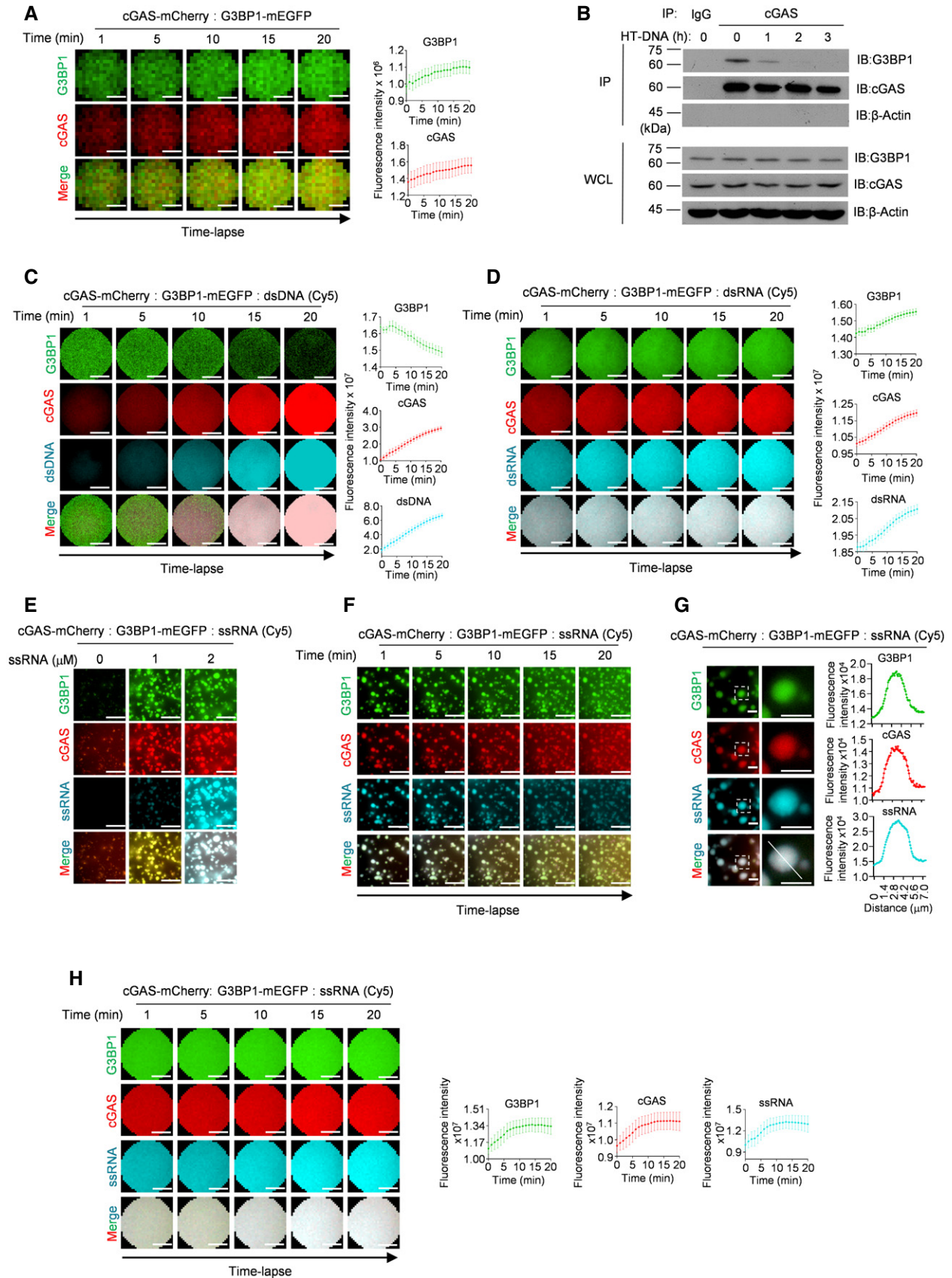Source data are available online for this figure.

Figure EV3.

Figure EV4. G3BP1 promotes DNA-induced LLPS and activation of cGAS.

A, B Time-lapse imaging of droplets formed by recombinant cGAS (10 μM) and indicated amounts of Cy5-labeled dsDNA.

C Quantitative analysis of (A). *n* = 3 biological replicates.

D Time-lapse imaging of cGAS-DNA droplets in the presence of the indicated amounts of G3BP1. 10 μM cGAS and 200 nM Cy5-labeled dsDNA were used.

E Quantitative analysis of (D). *n* = 3 biological replicates.

F Time-lapse imaging of cGAS-DNA condensates in the presence of heparin affinity chromatography purified G3BP1. 10 μM cGAS and 500 nM Cy5-labeled dsDNA were used.

G Quantitative analysis of (F). *n* = 3 biological replicates.

Data information: Representative images are shown (A, B, D and F), scale bars, 10 μm. Error bars, mean with s.d. of (C, E and G). The partition coefficient was calculated as the total fluorescence intensity of droplets/bulk fluorescence intensity of background (C, E and G).
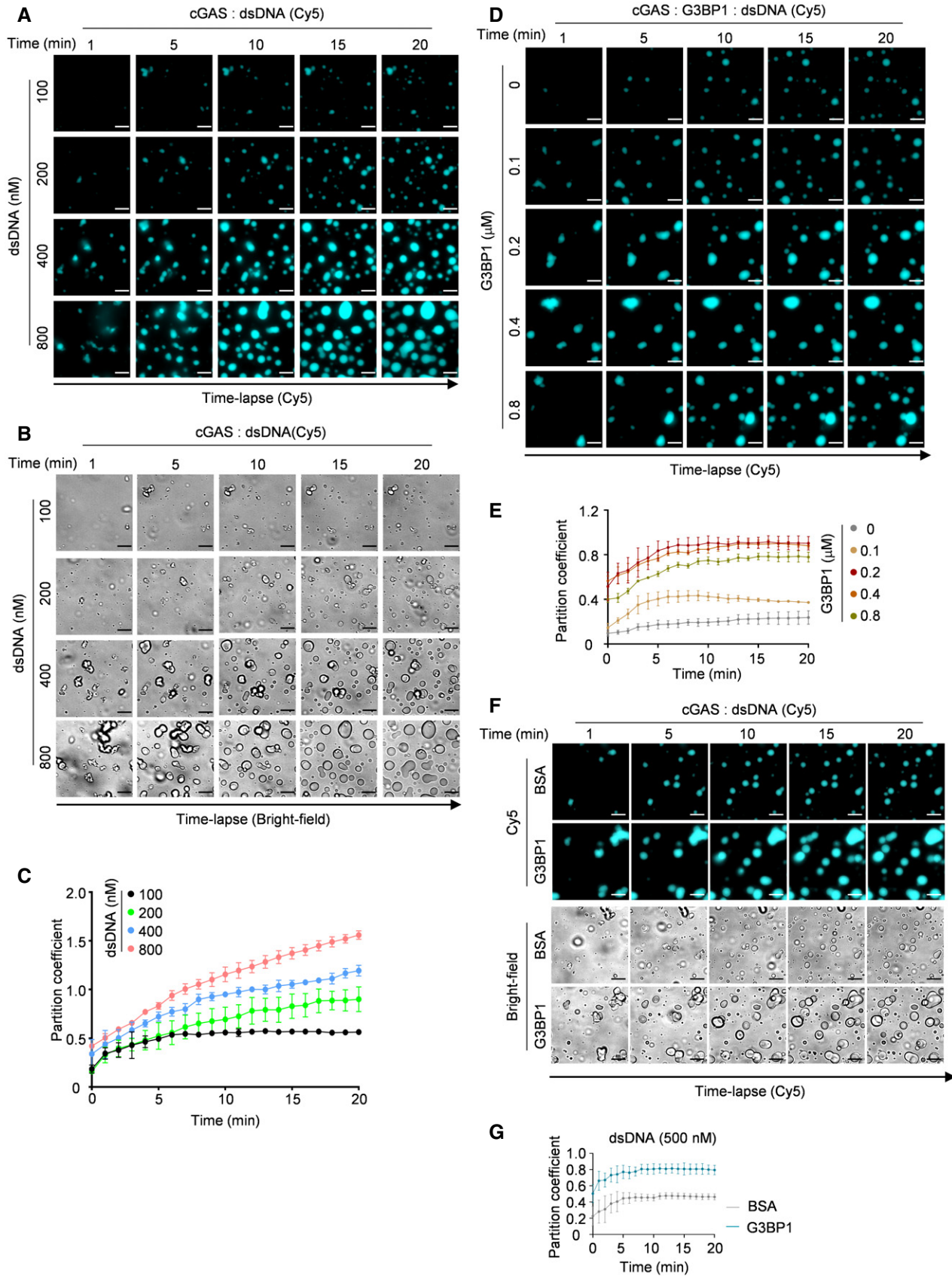Source data are available online for this figure.

Figure EV4.

Figure EV5. Full-length G3BP1 binds to cGAS and promotes LLPS of cGAS.

A   Bright-field microscope images of Fig 5D.
B   Turbidity (top) and bright-field microscope images (bottom) of indicated groups. 10 μM cGAS, 5 μM full length or truncated G3BP1 were used in the assay.
C   Quantitative analysis of (B) (top) were measured by absorbance at 600 nm. *n* = 3 biological replicates.
D   Quantitative analysis of total droplets area of (B) (bottom). *n* = 3 biological replicates.

Data information: Representative images are shown (A, B), scale bars, 10 μm. Error bars, mean with s.d. (C and D), **$P < 0.05$, ***$P < 0.001$, two-tailed *t*-test.
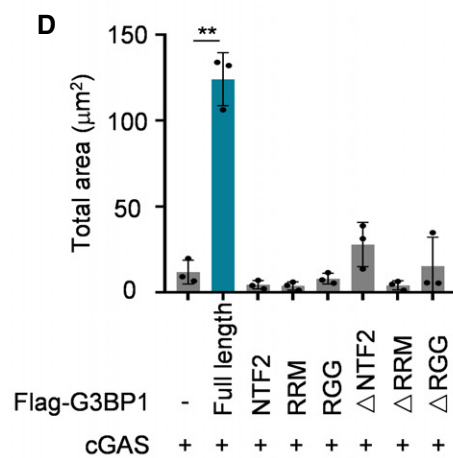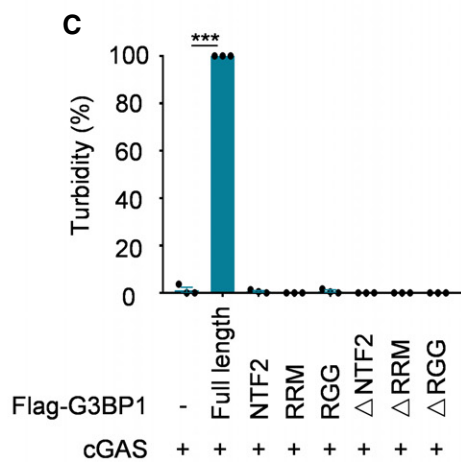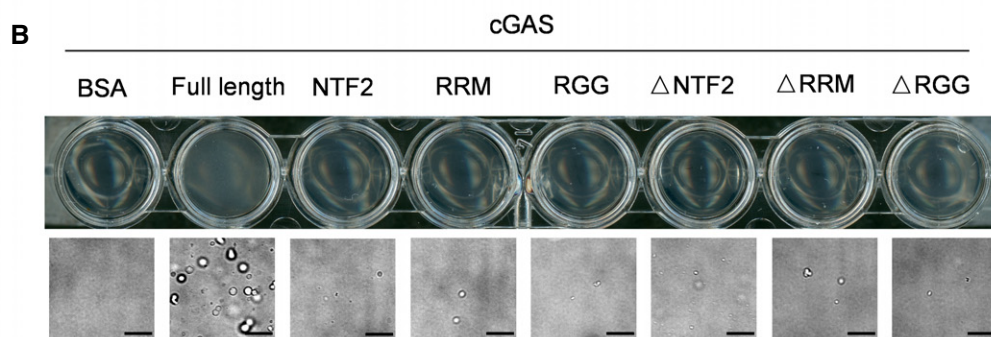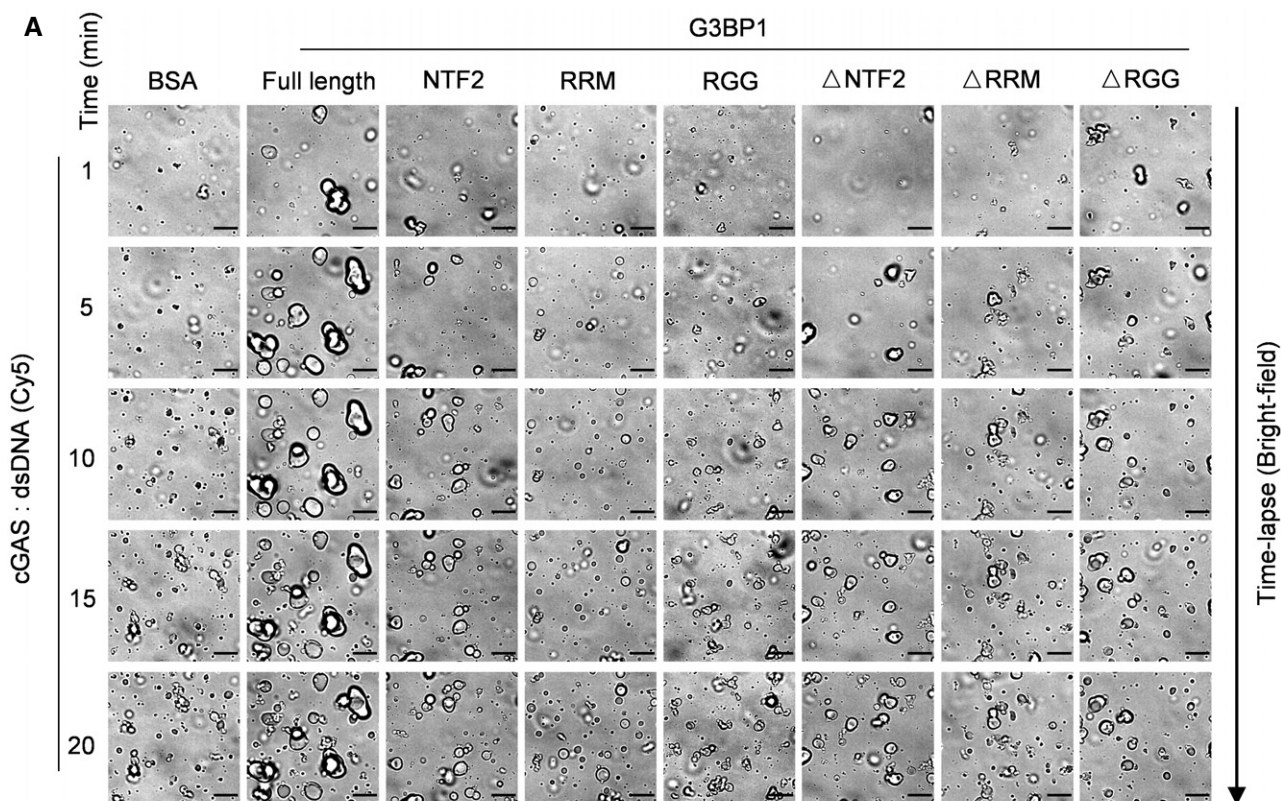Source data are available online for this figure.

Figure EV5.