

advances.sciencemag.org/cgi/content/full/6/42/eabb8941/DC1

Supplementary Materials for

cGAS suppresses genomic instability as a decelerator of replication forks

Hao Chen, Hao Chen, Jiamin Zhang, Yumin Wang, Antoine Simoneau, Hui Yang, Arthur S. Levine,
Lee Zou, Zhijian Chen, Li Lan*

*Corresponding author. Email: llan1@mgh.harvard.edu

Published 14 October 2020, *Sci. Adv.* **6**, eabb8941 (2020)
DOI: [10.1126/sciadv.abb8941](https://doi.org/10.1126/sciadv.abb8941)

This PDF file includes:

Figs. S1 to S5
Table S1

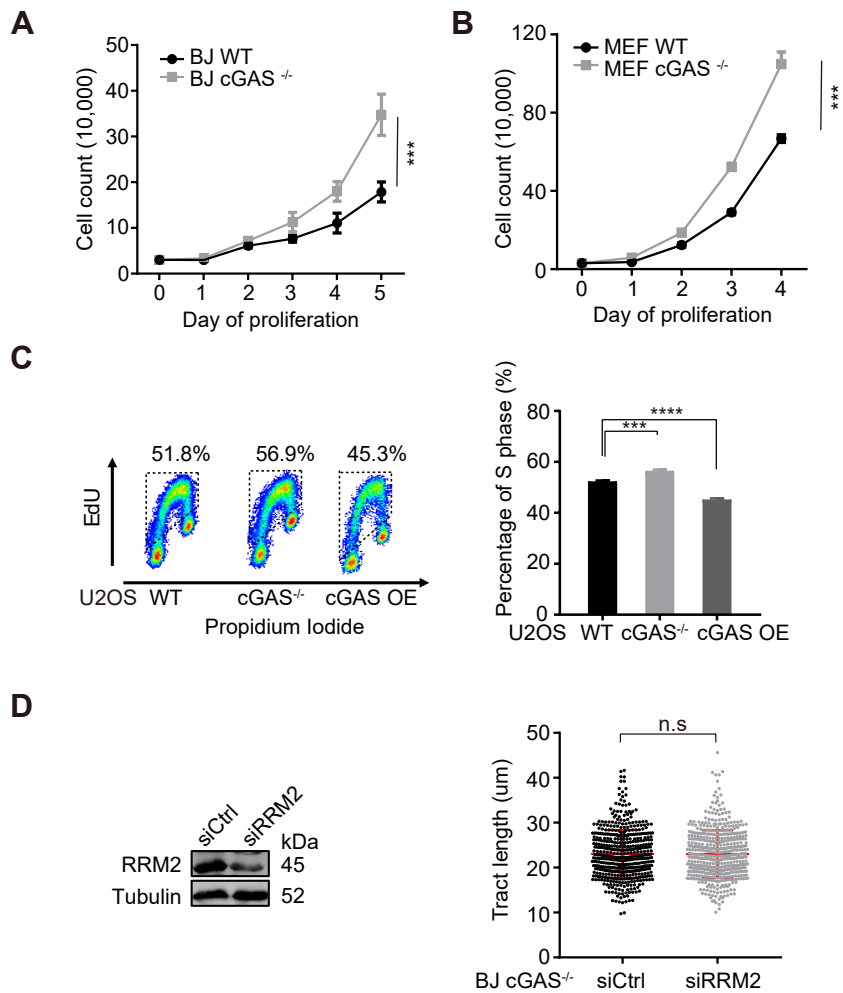


Figure S1 | cGAS prevents fast proliferation and accelerated replication.

(A-B) Proliferation curve based on the number of BJ WT/cGAS^{-/-} (A) and MEF WT/cGAS^{-/-} (B) cells on the indicated day. (C) The staining of EdU and PI in U2OS WT, corresponding cGAS^{-/-}, and overexpression cells were analyzed by flow cytometry. The percentage of S phase from flow-cytometry analysis is shown. (D) Analysis of nascent DNA tract length (n=500) in BJ cGAS^{-/-} cells of siCtrl and siRRM2. WB of RRM2 siCtrl and siRRM2 in BJ cGAS^{-/-} cells is shown.

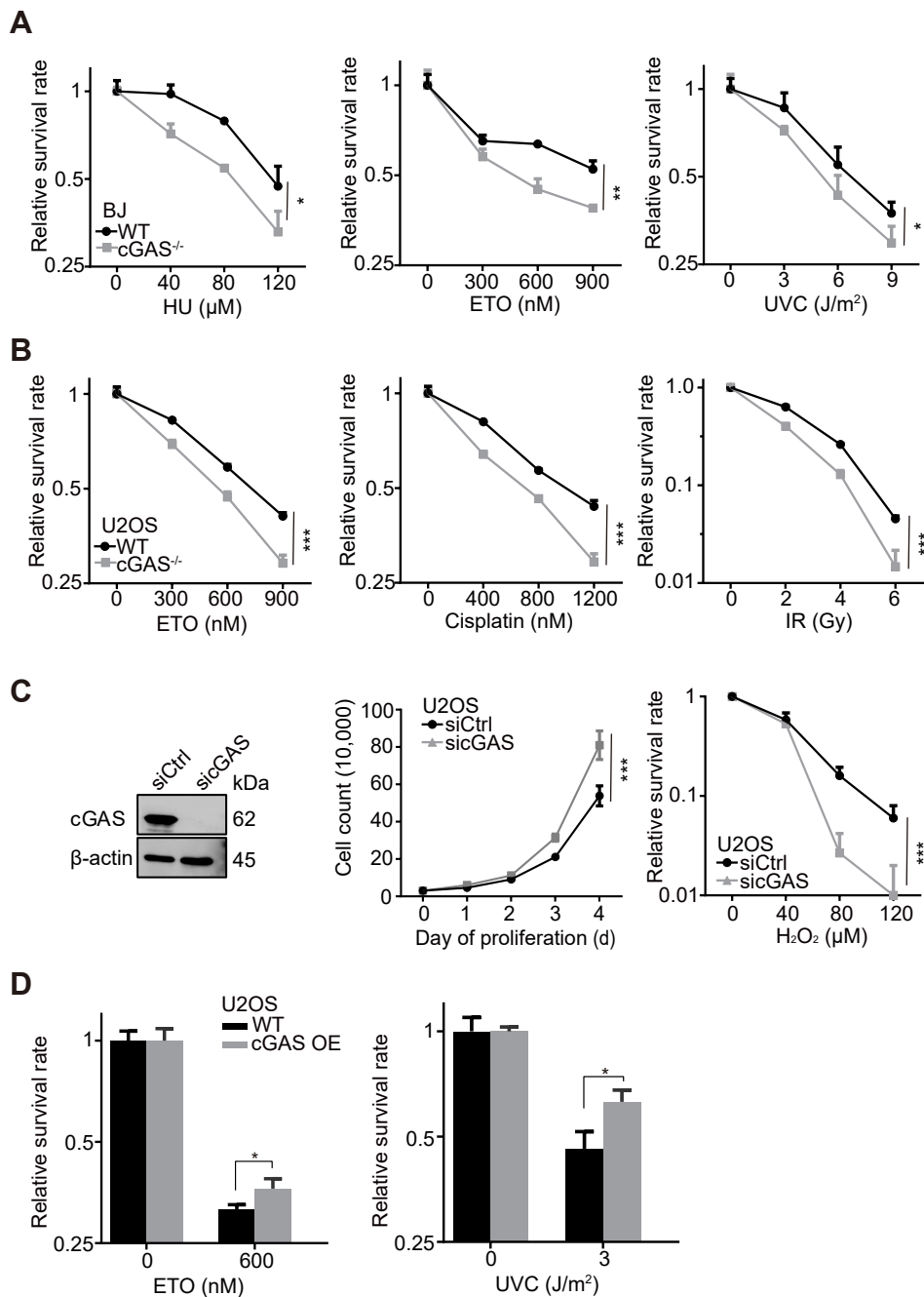


Figure S2 | cGAS protects cells from various types of damage.

(A) Colony formation assay for BJ WT and cGAS^{-/-} cells with indicated dose of hydroxyurea (HU), etoposide (ETO), or ultraviolet C (UVC). (B) Colony formation assay for U2OS WT and cGAS^{-/-} cells with indicated dose of ETO, cis-platin, or IR. (C) Cell proliferation rate and cell survival via colony formation assay of U2OS siCtrl and sicGAS with indicated dose of H₂O₂. WB of cGAS siCtrl and sicGAS in U2OS cells is shown. (D) Colony formation assay for U2OS WT and cGAS -OE cells with indicated dose of ETO and UVC.

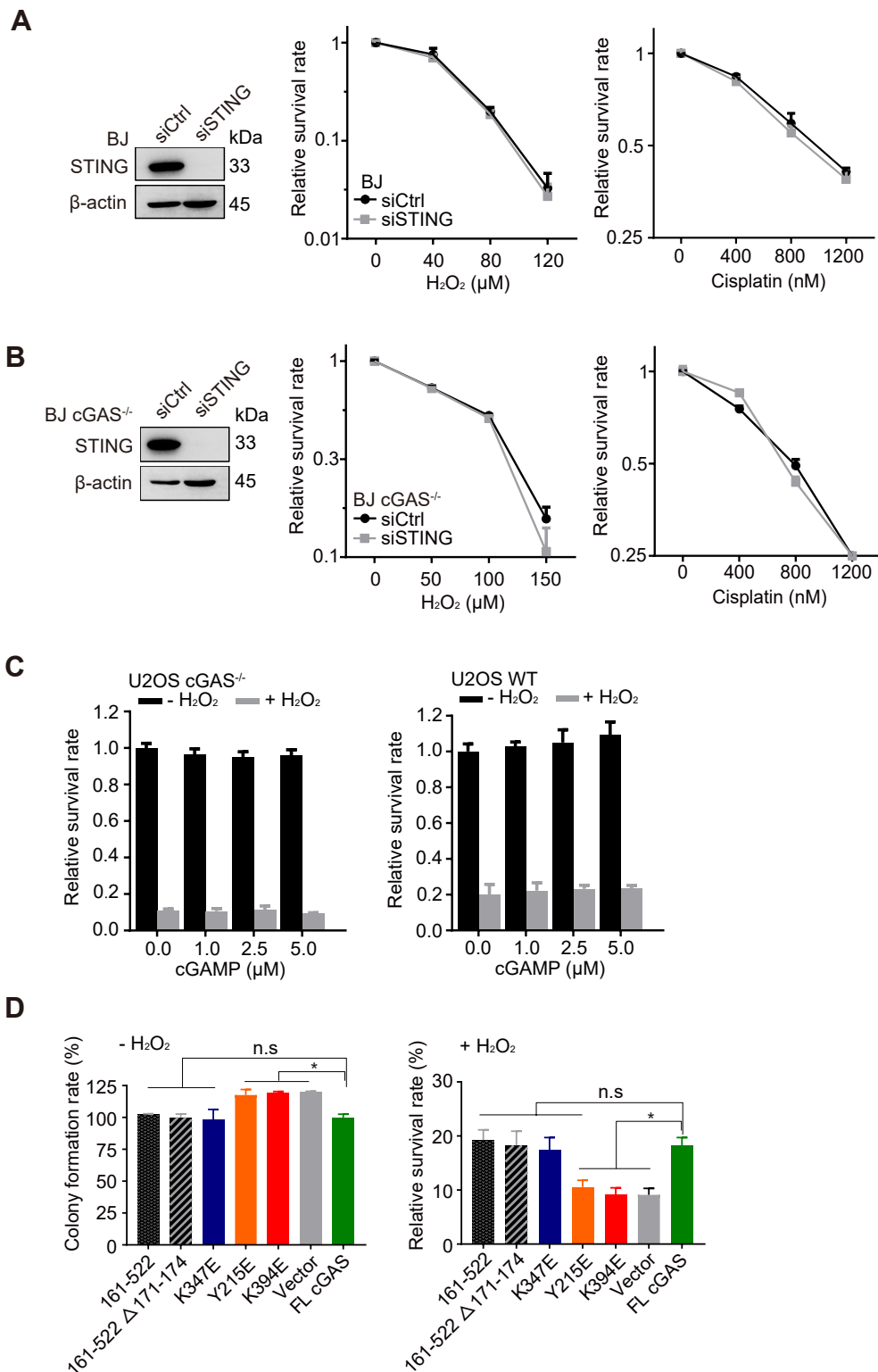


Figure S3 | cGAS depletion sensitizes cells to IR and chemo-drugs independently of STING and cGAMP.

(A) Expression of STING in siCtrl and siSTING transfected BJ WT cells. Colony formation assay for siCtrl and siSTING transfected cells with the indicated dose of cisplatin. (B) Expression of STING in siCtrl and siSTING transfected BJ cGAS^{-/-} cells. Colony formation assay for siCtrl and siSTING transfected cells with the indicated dose of H₂O₂ and cisplatin. (C) Colony formation assay for U2OS WT and cGAS^{-/-} cells incubated with cGAMP at the indicated concentration and cultured in the presence or absence of 100 μM H₂O₂ for 7-10 days. (D) Colony formation assay for U2OS cGAS^{-/-} cells expressing the indicated cGAS mutants without and with 100 μM H₂O₂ treatment.

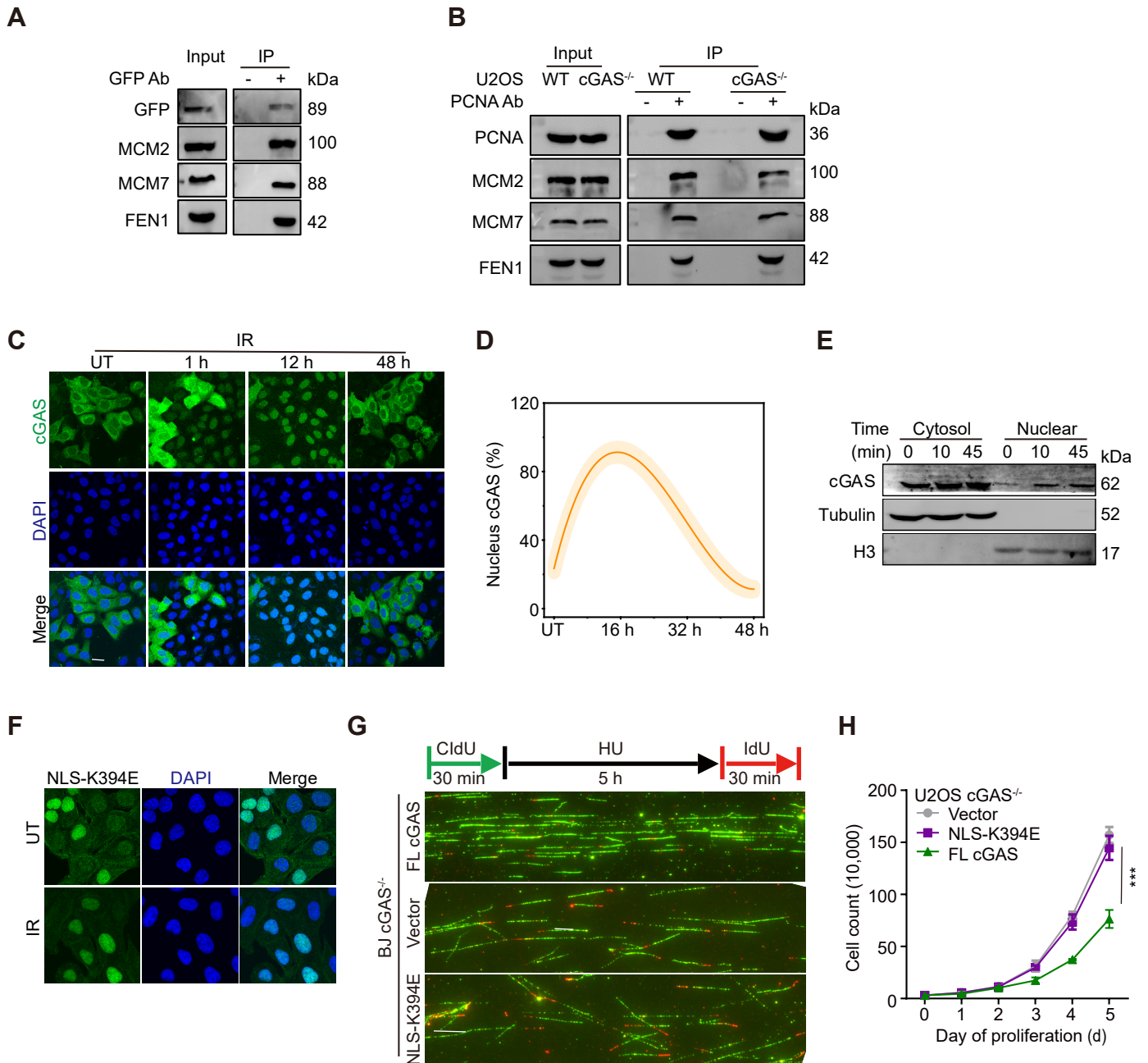


Figure S4 | cGAS is translocated into the nucleus after damage.

(A) Interaction of cGAS and MCM2/MCM7/FEN1 detected with Co-IP. GFP-cGAS overexpression U2OS cells were used for Co-IP analysis with or without anti-GFP antibody. (B) Interaction of PCNA and MCM2/MCM7/FEN1 detected with Co-IP with or without cGAS. U2OS WT and cGAS^{-/-} cells were used for Co-IP analysis with or without anti-PCNA antibody. (C-D) Immunostaining of endogenous cGAS in U2OS cells before and after treatment with 1 mM H₂O₂ for the indicated time (scale bar: 10 μm). The percentage change of cGAS expression in the nucleus is fitted by Python package NumPy. (E) Immunoblot estimation of cGAS in nuclear/cytosolic fractions of U2OS cells treated with 1 mM H₂O₂ for the indicated time. β-actin and H3 are nuclear and cytosolic markers, respectively. (F) Immunostaining of cGAS in U2OS cGAS^{-/-} cells transfected with NLS-flag-K394E mutant before and after treatment with 2 Gy IR (scale bar: 10 μm). (G) The replication fork status of BJ cGAS^{-/-} cells transfected with flag-tagged cGAS NLS-K394E mutant treated with 4 mM HU for 5 h. Representative images of fiber assay are shown (scale bar: 10 μm). (H) Thirty-thousand cells were seeded into each well of a 6-well plate. The number of U2OS cGAS^{-/-} cells transfected with NLS-K394E and flag-vector plasmids was counted every day until reaching high density.

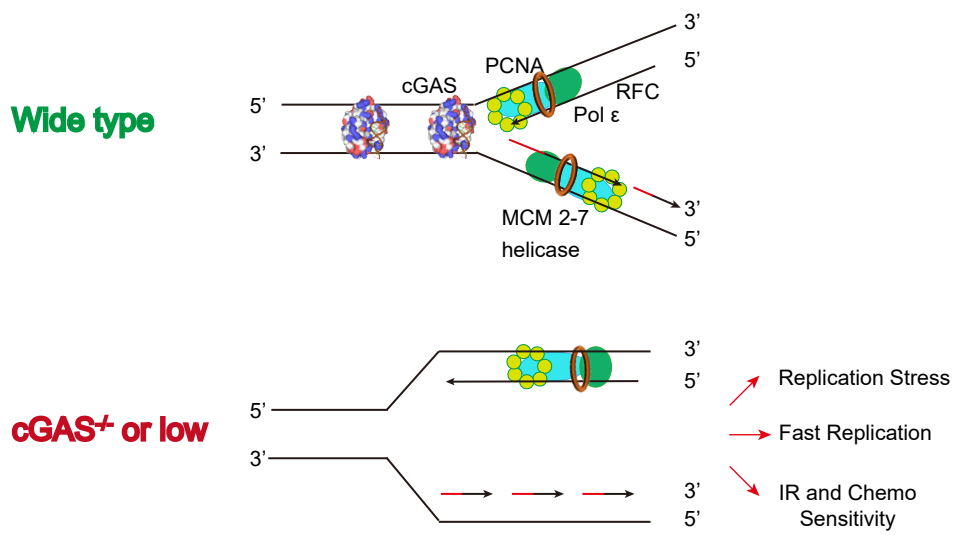


Figure S5 | Schematic diagram.

Schematic diagram of the role of cGAS in response to DNA damage via replication regulation.

Antibodies	Source	Identifier
anti-cGAS rabbit monoclonal antibody D1D3G	Cell Signaling Technology	Cat#15102S
anti-STING rabbit monoclonal antibody D2P2F	Cell Signaling Technology	Cat#13647S
anti-PCNA mouse monoclonal antibody	EMD Millipore	Cat# NA03
anti- β -Actin mouse monoclonal antibody 3700S	Cell Signaling Technology	Cat# 8H10D10
anti- GAPDH rabbit monoclonal antibody D16H11	Cell Signaling Technology	Cat# 5174S
anti- Tubulin hFAB Rhodamine antibody	BIO-RAD	Cat# 12004167
anti-p-ATR (Thr1989) rabbit monoclonal antibody	Cell Signaling Technology	Cat# 58014S
anti-ATR rabbit monoclonal antibody	Bethyl Laboratories Inc	Cat# A300-137A-M
anti-p-CHK1(Ser317) rabbit monoclonal antibody D7H2	Cell Signaling Technology	Cat# 8191S
anti-CHK1(2345) rabbit monoclonal antibody 2G1D5	Cell Signaling Technology	Cat# 2360
anti-FLAG M2 mouse monoclonal antibody	Sigma	Cat# F1804
anti-MCM2 rabbit affinity purified	Bethyl Laboratories Inc	Cat# A300-191A
anti-Fen 1 rabbit affinity purified	Bethyl Laboratories Inc	Cat# A300-255A
Anti-MCM7 (141) mouse monoclonal antibody	Santa Cruz Biotechnology	Cat# sc-9966
Mouse Anti- RRM2 (AA 1-110)	4A Biotech	Cat# ABIN562744
Goat Anti-Rabbit IgG StarBright Blue 520	BIO-RAD	Cat# 12005869
Goat Anti-Mouse IgG StarBright Blue 700	BIO-RAD	Cat# 1200415
Rabbit Anti-Mouse IgG H&L (HRP)	Abcam	Cat# ab6728
Goat Anti-Rabbit IgG H&L (HRP)	Abcam	Cat# ab6721

Table S1| List of Antibodies for Western blot.