Expanded View Figures

Figure EV1. Compartment disassembly by osmotic stress, temperature change, and increasing salt concentration is specific for 53BP1.

A U-2 OS cells were irradiated with 0.5 Gy in absence or presence of 0.4 M sorbitol, fixed 1 h later, and γH2AX foci were quantified by QIBC.
B U-2 OS cells were irradiated with 0.5 Gy in absence or presence of 0.4 M sorbitol, fixed 1 h later, and MDC1 foci were quantified by QIBC.
C U-2 OS cells were irradiated with 0.5 Gy, exposed to the indicated temperature during recovery, fixed 1 h later, and 53BP1 foci were quantified by QIBC.
D For the same cells in (C), γH2AX foci were quantified by QIBC.
E U-2 OS cells were irradiated with 0.5 Gy in the presence of increasing NaCl concentrations (0, 100, 125, 150, 175 mM), fixed 1 h later and 53BP1 foci were quantified by QIBC.
F For the same cells in (E), γH2AX foci were quantified by QIBC.

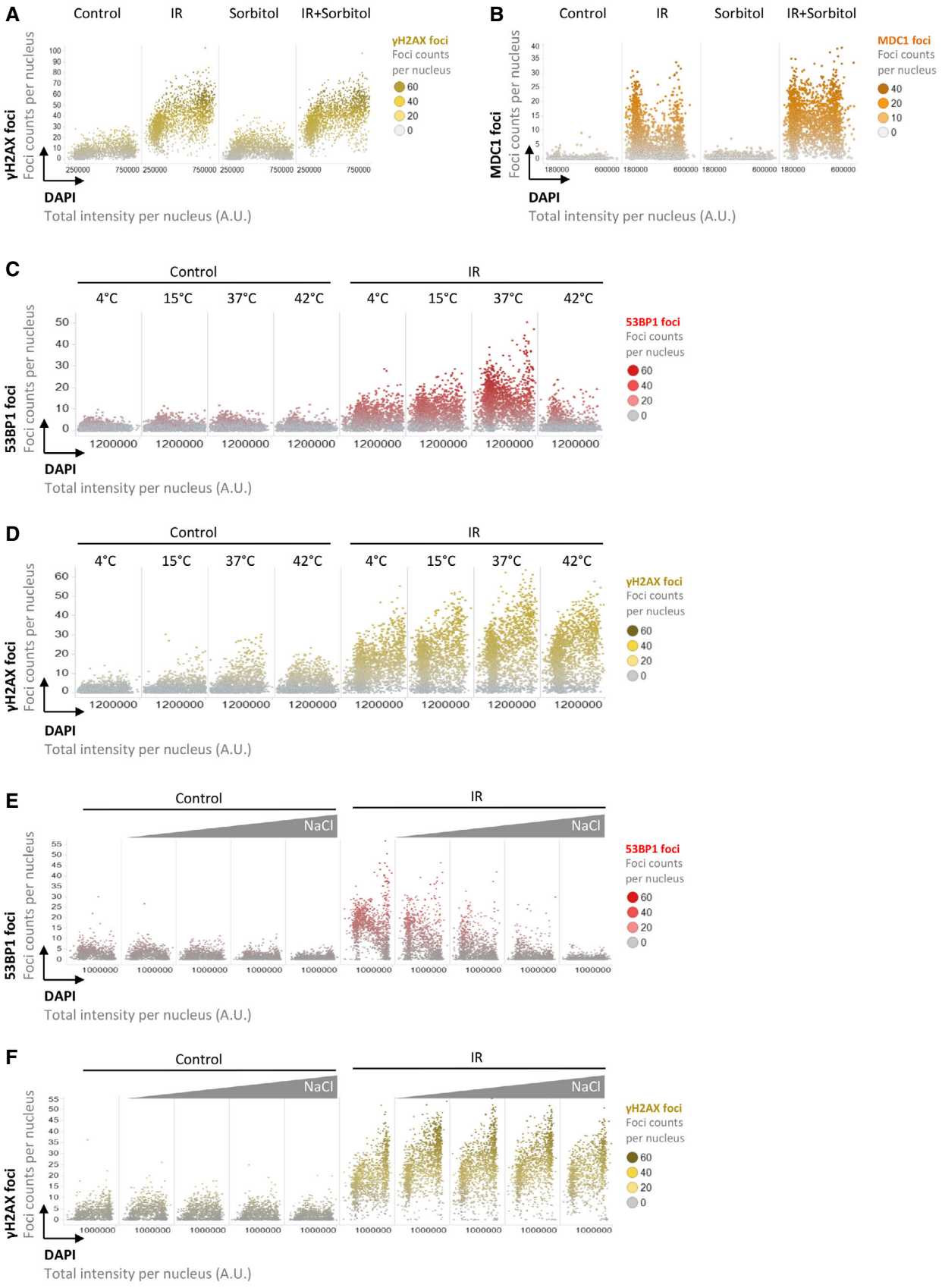

Figure EV1.

Figure EV2. 53BP1 optoDroplet formation.

A The indicated Cry2-mCherry-fusion constructs were transfected into U-2 OS cells, activated by blue light, and optoDroplet formation was followed by time-lapse microscopy at 15-s intervals. OptoDroplets before (pre) and 6 min after light induction (post) were quantified by single-cell QIBC analysis of 100–250 cells per condition from 2 to 3 independent experiments with mean (solid line) and standard deviation from the mean (dashed lines) indicated.

B Additional examples for full-length wild-type Cry2-mCherry-53BP1 clustering upon light activation.

C Cry2-mCherry-MDC1 was transfected into U-2 OS cells, cells were exposed to NCS (25 ng/ml), fixed 1 h later, and stained for 53BP1 and γH2AX.

D The indicated Cry2-mCherry-fusion constructs were transfected into U-2 OS cells, exposed to HCl-adjusted medium at pH 5.5, and analyzed for optoDroplet formation 5 min later.

E The indicated Cry2-mCherry-fusion constructs were transfected into U-2 OS cells, exposed to HCl-adjusted medium at pH 5.5, and analyzed for optoDroplet formation 5 min later.

F The indicated Cry2-mCherry-fusion constructs were transfected into U-2 OS cells, activated by blue light, and optoDroplet formation was followed by time-lapse microscopy at 15-s intervals. Red bars indicate the part of 53BP1 that was expressed as Cry2-mCherry fusion.

Data information: Scale bars, 10 μm.

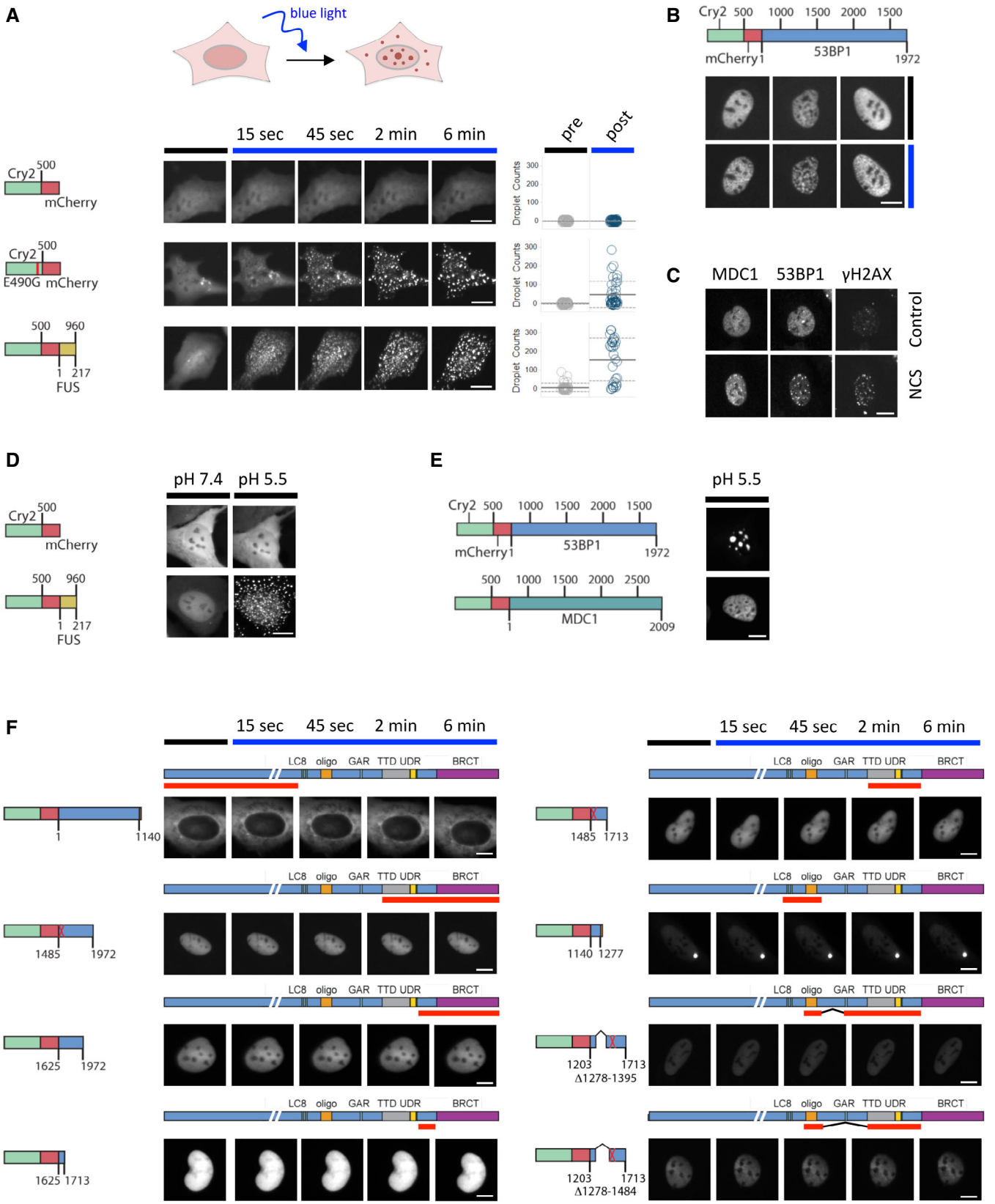
A
blue light
15 sec
45 sec
2 min
6 min
pre
post
Cry2 500 mCherry
Cry2 500 E490G mCherry
500 960 1 217 FUS
Droplet Counts
B
Cry2 500 1000 1500 2000 1500
mCherry 1
53BP1
1972
C
MDC1
53BP1
γH2AX
Control
NCS
D
pH 7.4
pH 5.5
Cry2 500 mCherry
500 960 1 217 FUS
E
pH 5.5
Cry2 500 1000 1500 2000 1500 mCherry 1 53BP1 1972
500 1000 1500 2000 2500 1 MDC1 2009
F
15 sec
45 sec
2 min
6 min
LC8 oligo GAR TTD UDR BRCT
1 1140
1485 1972
1625 1972
1625 1713
1485 1713
1140 1277
1203 1713 Δ1278-1395
1203 1713 Δ1278-1484

Figure EV2.

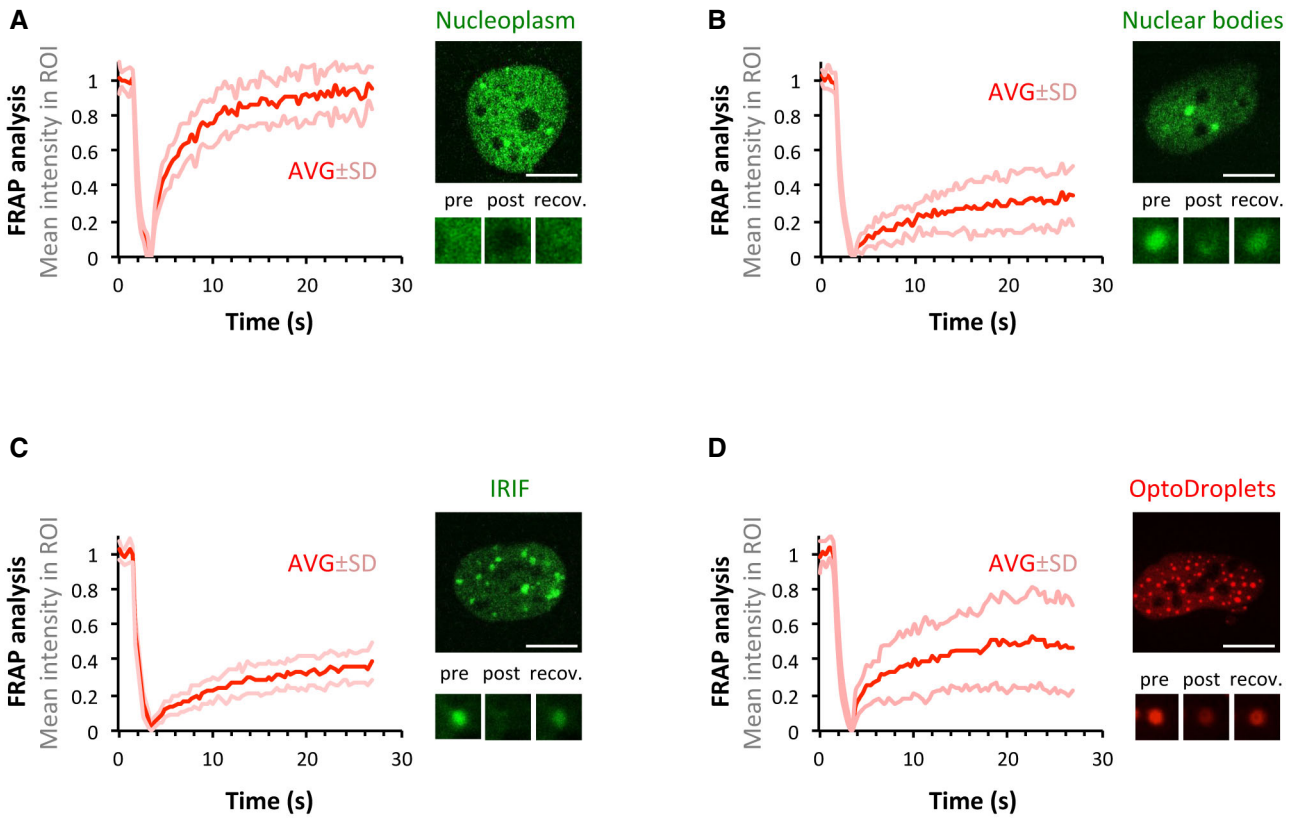

Figure EV3. FRAP analysis of 53BP1 nuclear bodies, IR-induced foci, and optoDroplets.

A U-2 OS cells stably expressing GFP-53BP1 were bleached in the nucleoplasm and fluorescence recovery was monitored.

B U-2 OS cells stably expressing GFP-53BP1 were bleached at individual replication stress-associated 53BP1 nuclear bodies and fluorescence recovery was monitored.

C U-2 OS cells stably expressing GFP-53BP1 were irradiated with 1 Gy, allowed to recover for 1–2 h, bleached at individual 53BP1 foci, and fluorescence recovery was monitored.

D U-2 OS cells transfected with Cry2-mCherry-53BP1 (1203–1972 W1495A) were light-activated to induce optoDroplets, individual droplets were bleached and fluorescence recovery was monitored. Between 12 and 20 cells were analyzed for each condition.

Data information: Scale bars, 10 μm.

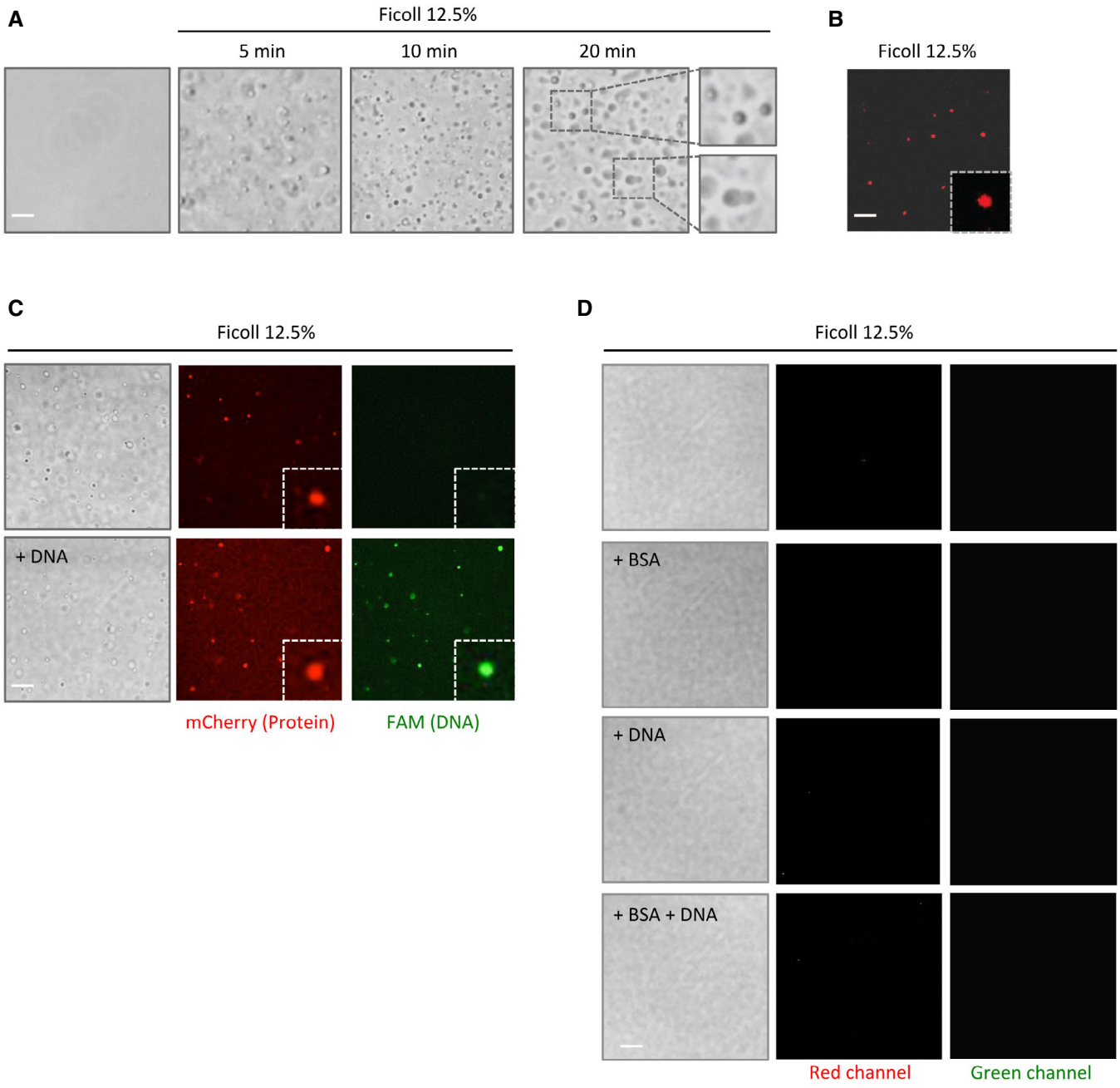

Figure EV4. The 53BP1 C-terminus phase separates *in vitro*.

A Purified 53BP1 1203–1972 W1495A forms protein condensates in Ficoll.

B Purified unlabeled 53BP1 1203–1972 W1495A and an mCherry-labeled version were mixed at a 9:1 ratio, exposed to Ficoll and analyzed for mCherry-positive protein condensates.

C A 9:1 ratio of unlabeled to mCherry-labeled purified 53BP1 1203–1972 W1495A was mixed with FAM-labeled DSB-mimicking dsDNA, and co-occurrence of DNA in 53BP1 droplets was analyzed by confocal microscopy.

D As in (C) with the indicated specificity controls.

Data information: Scale bars 10 μm.

Figure EV5. 53BP1 phase separation is linked to p53 activation.

A GFP-53BP1 cells were transfected with wild-type Myc-53BP1, treated with IR (10 Gy) and sorbitol (0.4 M) for 2 h, GFP-53BP1 was immunoprecipitated, and the interaction between GFP-53BP1 and p53 and between GFP-53BP1 and Myc-53BP1 was analyzed by Western blot. Inputs and IP samples were run on the same gel. WCE, whole cell extract.

B U-2 OS cells were treated with IR (2 Gy) and sorbitol (0.4 M) for 1 h, released into fresh medium, allowed to recover for 7 h, and nuclear p53 levels were analyzed by QIBC. The mean from single-cell QIBC analysis of at least 3,500 cells per condition is indicated with a dashed white line.

C Western blot analysis of 53BP1 protein levels in MCF7 wild-type (WT) and 53BP1 knockout (KO) cells.

D MCF7 cells (WT and 53BP1 KO) were treated with IR (10 Gy), allowed to recover for 4 h, and induction of p53 was analyzed by Western blot.

E MCF7 cells were treated as in (D) and induction of p21 was analyzed by Western blot.

F U-2 OS cells were transfected with siRNA as indicated, exposed to IR (2 Gy), allowed to recover for 8 h, and cell cycle analysis was performed by QIBC using DNA content and nuclear Cyclin A levels to gate G1 versus S/G2 cells. Averages ± standard deviation and *P*-values from unpaired *t*-tests are provided from three replicates ($P \geq 0.05$ was considered not significant, N.S.).

G U-2 OS cells were treated with IR (10 Gy, 4 h) in absence or presence of 250 mM NaCl or 3% 1,6-hexanediol as indicated. Induction of p53 and p21 was analyzed by Western blot.

H Scheme of 53BP1 protein domains and their contribution for initial recruitment to DNA lesions, self-assembly and phase separation, and effector protein activation. Oligomerization domain (OD), 1231–1277; GAR, 1396–1403; TTD, 1484–1603; UDR, 1604–1631; BRCT, 1714–1972.

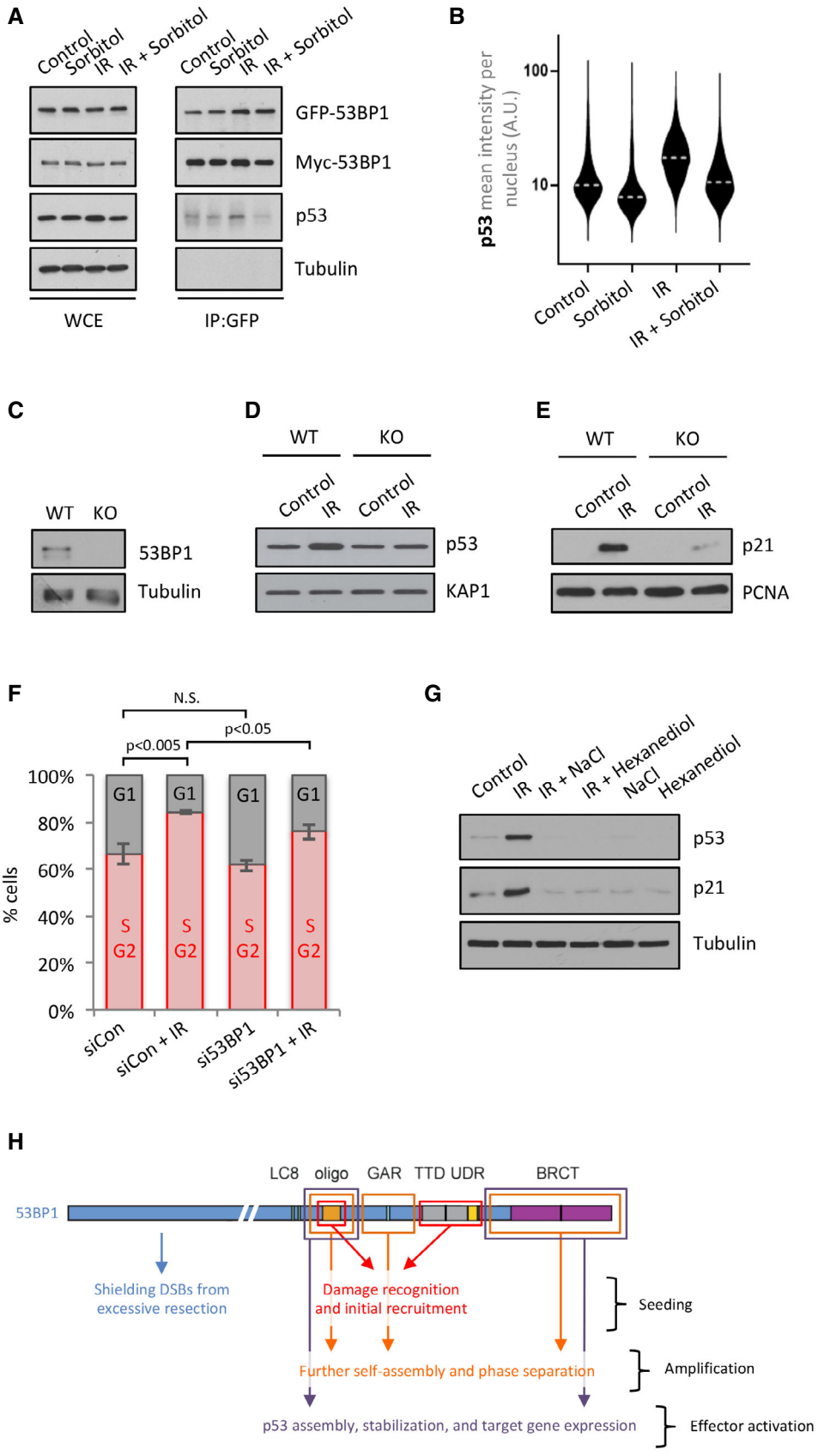
A
Control
Sorbitol
IR
IR + Sorbitol
GFP-53BP1
Myc-53BP1
p53
Tubulin
WCE
IP:GFP
B
p53 mean intensity per nucleus (A.U.)
100
10
Control
Sorbitol
IR
IR + Sorbitol
C
WT
KO
53BP1
Tubulin
D
WT
KO
p53
KAP1
E
p21
PCNA
F
N.S.
p<0.005
p<0.05
% cells
G1
S
G2
siCon
siCon + IR
si53BP1
si53BP1 + IR
G
IR + NaCl
IR + Hexanediol
NaCl
Hexanediol
H
LC8
oligo
GAR
TTD
UDR
BRCT
Shielding DSBs from excessive resection
Damage recognition and initial recruitment
Seeding
Further self-assembly and phase separation
Amplification
p53 assembly, stabilization, and target gene expression
Effector activation

Figure EV5.