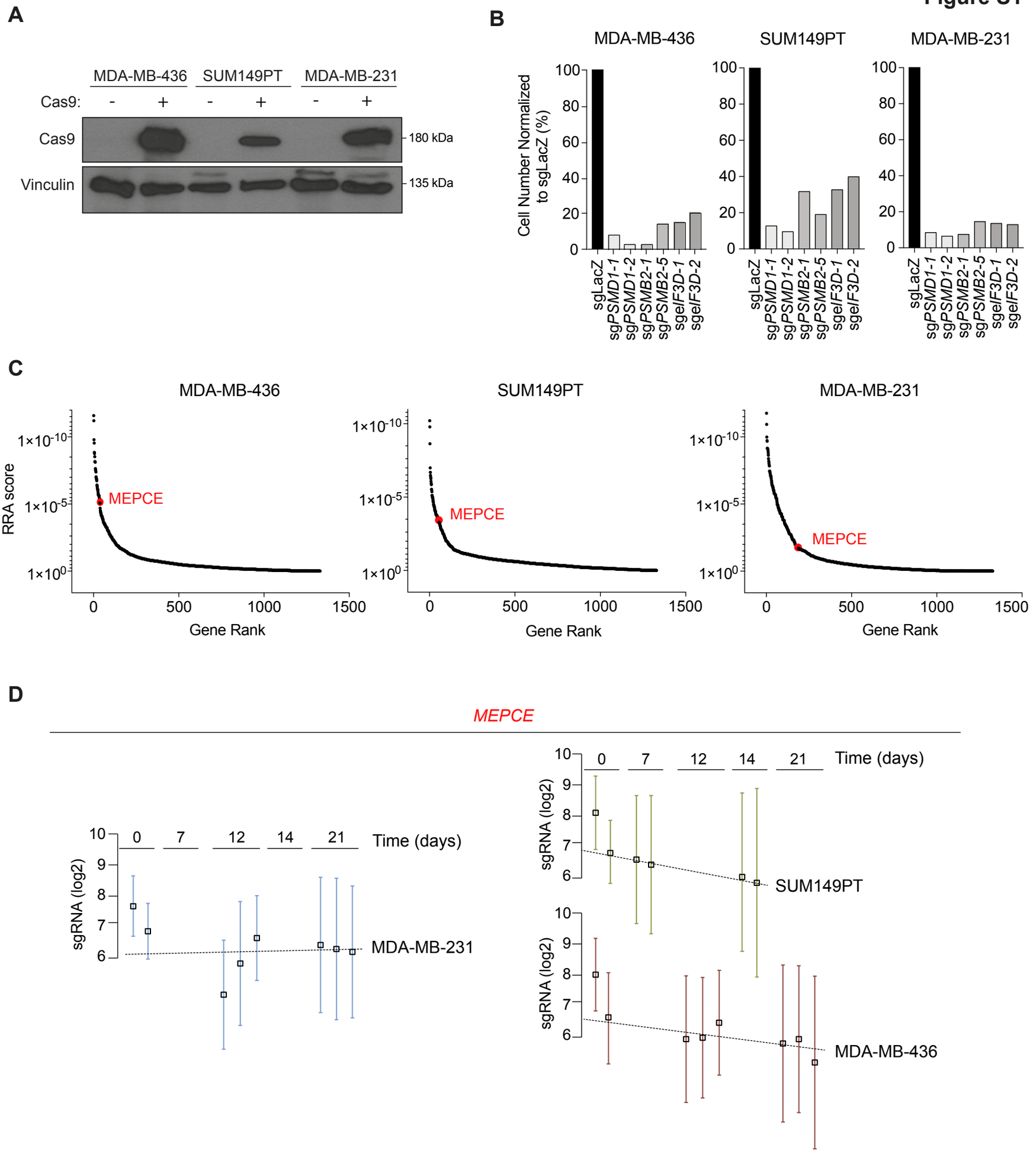
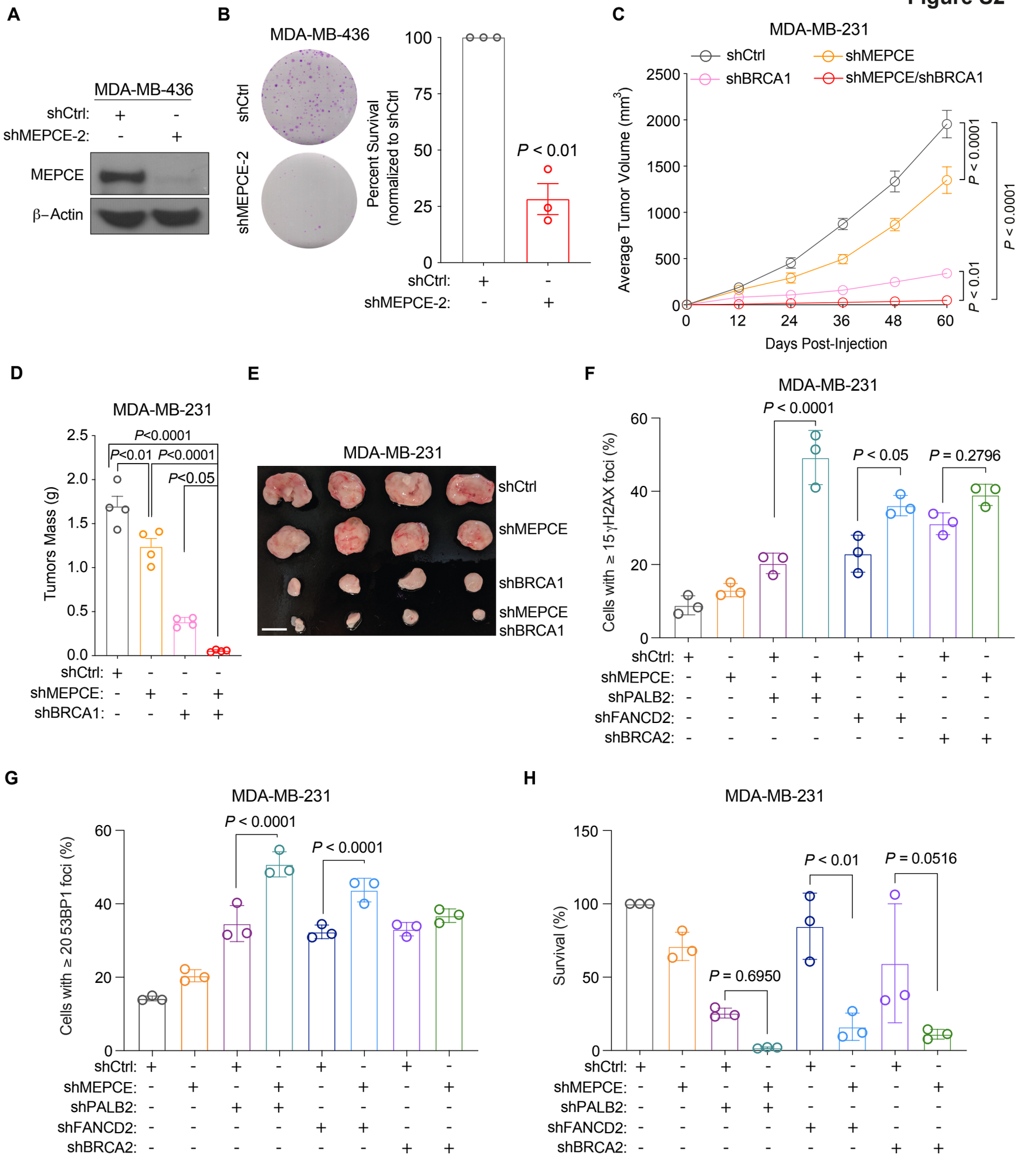
Figure S1

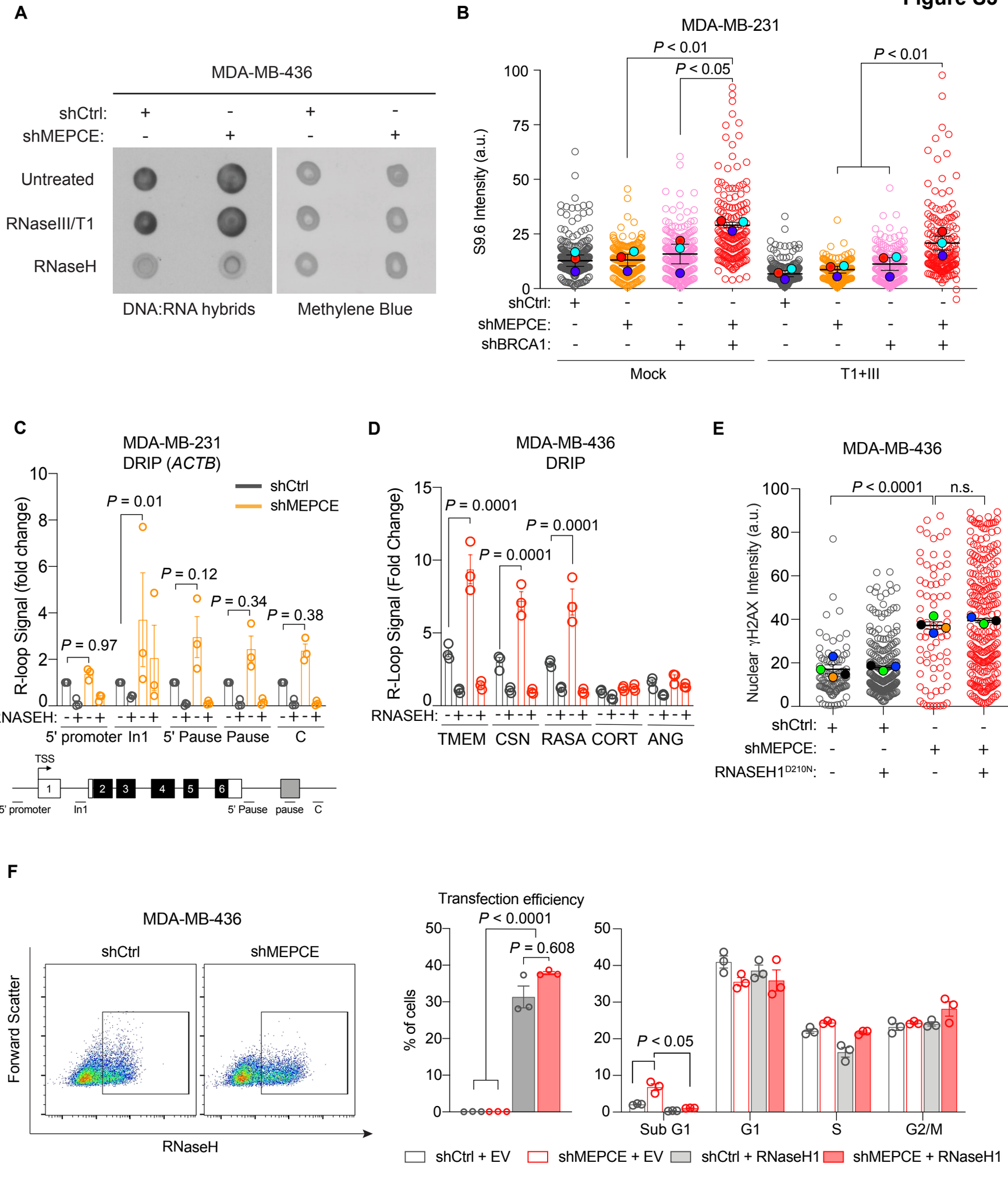

A
MDA-MB-436
SUM149PT
MDA-MB-231
Cas9:
- + - + - +
Cas9
Vinculin
180 kDa
135 kDa
B
MDA-MB-436
SUM149PT
MDA-MB-231
Cell Number Normalized to sgLacZ (%)
sgLacZ
sgPSMD1-1
sgPSMD1-2
sgPSMB2-1
sgPSMB2-5
sgIF3D-1
sgIF3D-2
C
MDA-MB-436
SUM149PT
MDA-MB-231
RRA score
MEPCE
Gene Rank
D
MEPCE
sgRNA (log2)
Time (days)
0
7
12
14
21
MDA-MB-231
SUM149PT
MDA-MB-436

Supplementary Figure S1. Validation of Cas9 functionality in breast cancer cell lines used in CRISPR- Cas9 dropout screen and screen analysis. (A) Western blot showing the overexpression of Cas9 in MDA-MB-436, SUM149PT and MDA-MB-231 clones stably transduced with the lentiviral Lenti-Cas9-2A-Blast construct. Vinculin was used as a loading control. **(B)** Validation of CRISPR-Cas9 gene editing efficiency in Cas9-expressing MDA-MB-436, SUM149PT and MDA-MB-231 cells. Bar graph representing cell proliferation 9-10 days post- transduction with a control sgRNA construct (sgLacZ) or sgRNAs targeting essential genes *PSMD1*, *PSMB2* and *EIF3D*. Cell number is normalized to control sgRNA (sgLacZ). **(C)** Scatter plot representing gene rank vs RRA score calculated by the MAGeCK-VISPR pipeline for each cell line. *MEPCE* is highlighted. **(D)** Analysis of *MEPCE* gRNAs in indicated cell lines used for CRISPR screening. RRA: robust rank aggregation.

Figure S2

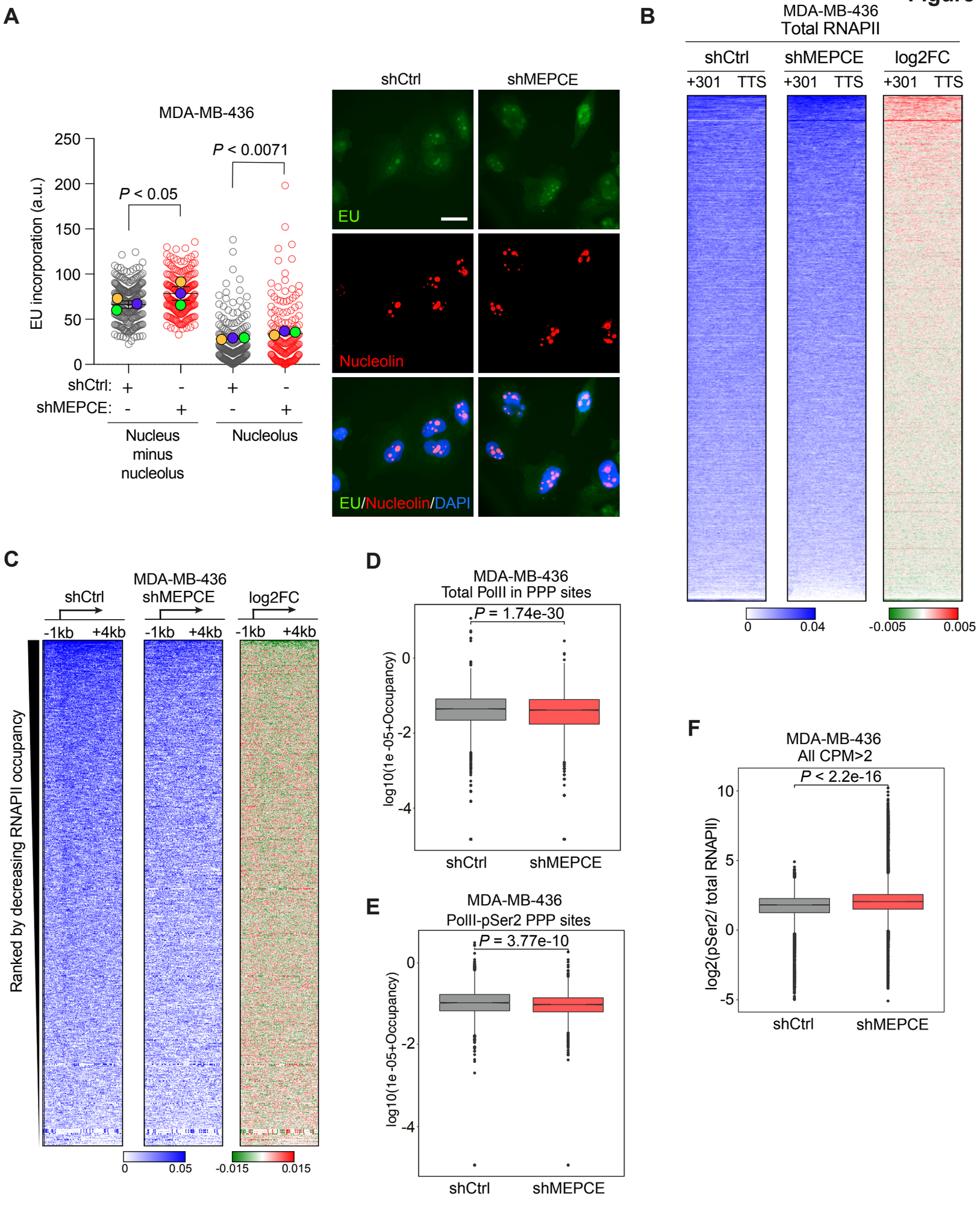

A
MDA-MB-436
shCtrl: + -
shMEPCE-2: - +
MEPCE
β–Actin
B
MDA-MB-436
shCtrl
shMEPCE-2
Percent Survival (normalized to shCtrl)
100
75
50
25
0
P < 0.01
shCtrl: + -
shMEPCE-2: - +
C
MDA-MB-231
shCtrl
shMEPCE
shBRCA1
shMEPCE/shBRCA1
Average Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0 12 24 36 48 60
Days Post-Injection
P < 0.0001
P < 0.01
P < 0.0001
D
MDA-MB-231
Tumors Mass (g)
2.5
2.0
1.5
1.0
0.5
0.0
P<0.0001
P<0.01 P<0.0001
P<0.05
shCtrl: + - - -
shMEPCE: - + - +
shBRCA1: - - + +
E
MDA-MB-231
shCtrl
shMEPCE
shBRCA1
shMEPCE shBRCA1
F
MDA-MB-231
Cells with ≥ 15 γH2AX foci (%)
60
40
20
0
P < 0.0001
P < 0.05
P = 0.2796
shCtrl: + - + - + - + -
shMEPCE: - + - + - + - +
shPALB2: - - + + - - - -
shFANCD2: - - - - + + - -
shBRCA2: - - - - - - + +
G
MDA-MB-231
Cells with ≥ 20 53BP1 foci (%)
60
40
20
0
P < 0.0001
P < 0.0001
shCtrl: + - + - + - + -
shMEPCE: - + - + - + - +
shPALB2: - - + + - - - -
shFANCD2: - - - - + + - -
shBRCA2: - - - - - - + +
H
MDA-MB-231
Survival (%)
150
100
50
0
P = 0.6950
P < 0.01
P = 0.0516
shCtrl: + - + - + - + -
shMEPCE: - + - + - + - +
shPALB2: - - + + - - - -
shFANCD2: - - - - + + - -
shBRCA2: - - - - - - + +

Supplementary Figure S2. Loss of MEPCE in *BRCA1*-null and some HR-defective cells causes severe growth defect *in vivo,* leads to elevated DNA damage, and R-loop accumulation. **(A)** Western blot of MEPCE expression in indicated cells. **(B)** Representative images of colony forming assay and quantification in indicated MDA-MB-436 cells. **(C)** *In vivo* growth curve of MDA-MB-231 control (shCtrl), shMEPCE, shBRCA1 and shMEPCE/shBRCA1 xenografts depicting changes in tumor volume over the duration of the experiment measured by an external caliper. **(D)** Quantification of tumor mass post-resection. **(E)** Representative images of tumors at day 60 post-injection (n = 4 per condition; bar = 1 cm). **(F)** Quantification of γH2AX foci in indicated MDA-MB-231 cells. (n = 3). **(G)** Quantification of 53BP1 foci in indicated MDA-MB-231 cells. (n = 3). **(H)** Quantification of survival in indicated MDA-MB-231 cells. B, Student's *t*-test. C, two-way ANOVA (Tukey's multiple comparisons test). D, F, G, and H, one-way ANOVA (Tukey's multiple comparisons test). For B, D, F, G, and H, mean ± SEM is shown.


A
MDA-MB-436
shCtrl: + - + -
shMEPCE: - + - +
Untreated
RNaseIII/T1
RNaseH
DNA:RNA hybrids
Methylene Blue
B
MDA-MB-231
P < 0.01
P < 0.05
P < 0.01
S9.6 Intensity (a.u.)
shCtrl:
shMEPCE:
shBRCA1:
Mock
T1+III
C
MDA-MB-231
DRIP (ACTB)
shCtrl
shMEPCE
R-loop Signal (fold change)
P = 0.97
P = 0.01
P = 0.12
P = 0.34
P = 0.38
RNASEH:
5' promoter
In1
5' Pause
Pause
C
TSS
D
MDA-MB-436
DRIP
R-Loop Signal (Fold Change)
P = 0.0001
P = 0.0001
P = 0.0001
RNASEH:
TMEM
CSN
RASA
CORT
ANG
E
MDA-MB-436
P < 0.0001
n.s.
Nuclear γH2AX Intensity (a.u.)
shCtrl:
shMEPCE:
RNASEH1D210N:
F
MDA-MB-436
shCtrl
shMEPCE
Forward Scatter
RNaseH
Transfection efficiency
P < 0.0001
P = 0.608
% of cells
P < 0.05
Sub G1
G1
S
G2/M
shCtrl + EV
shMEPCE + EV
shCtrl + RNaseH1
shMEPCE + RNaseH1

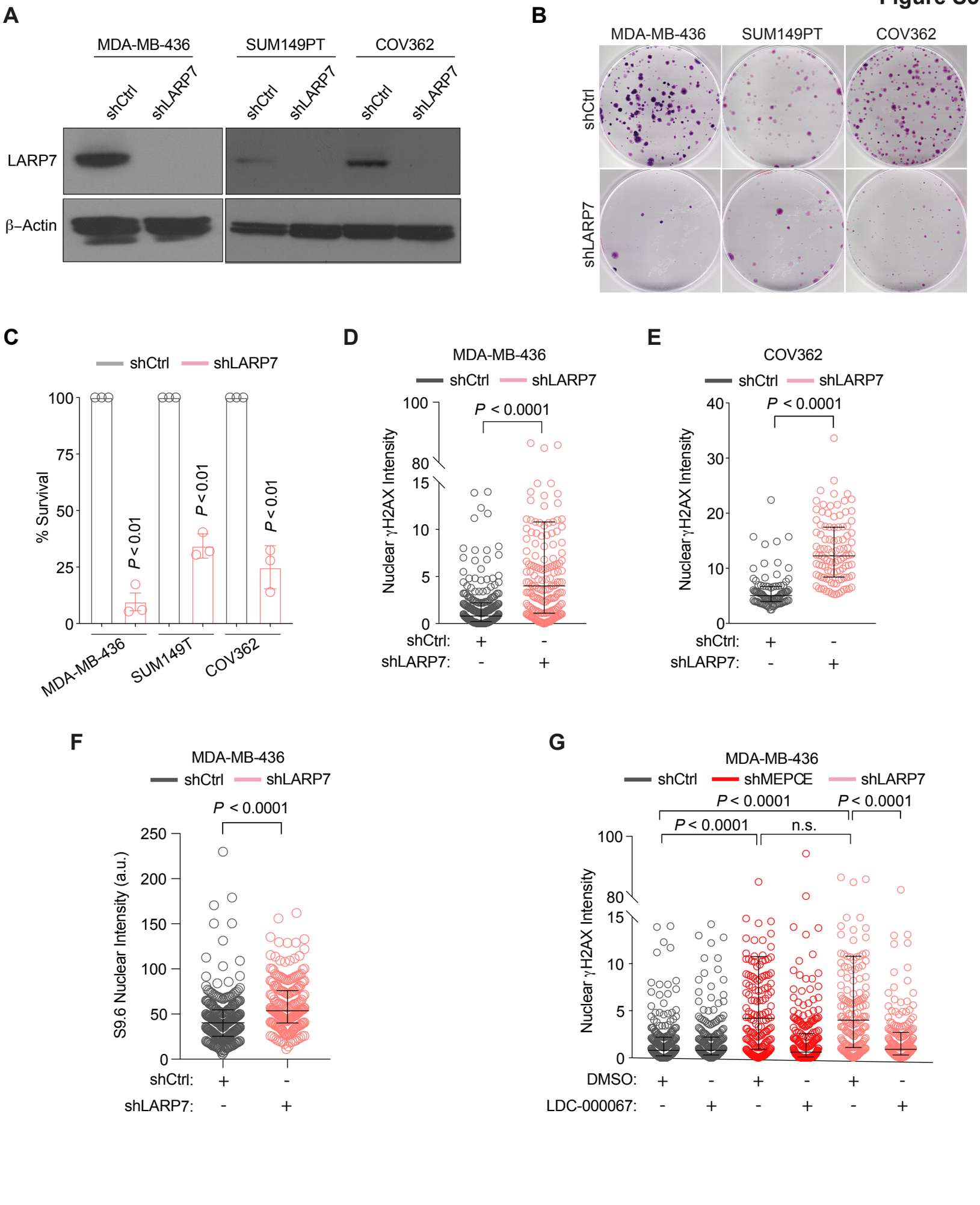
Supplementary Figure S3. MEPCE depletion increases R-loops in BRCA1-deficient cancer cells and short-term RNASEH1 overexpression does not significantly alter cell cycle profile in MEPCE-depleted or control cells. (A) Dot blot depicting S9.6 (DNA:RNA hybrid) levels in MDA-MB-436 cells. Methylene blue is used as loading control. **(B)** Quantification of anti-DNA-RNA hybrid (S9.6) immunofluorescence and quantification of nuclear and nucleolar hybrid levels in response to indicated treatment conditions (Mock, RNASET1 (T1), RNASEIII (III), and/or RNASEH (H)). **(C)** DRIP-qPCR using S9.6 antibody in indicated MDA-MB-231 cells in *ACTB* gene at the indicated loci. Enrichment is compared to negative control (IgG) followed by normalization to shCtrl cells (mean ± SEM). **(D)** DRIP-qPCR using S9.6 antibody in indicated MDA-MB-436 cells at the indicated loci. Enrichment is compared to negative control (IgG) followed by normalization to shCtrl cells (mean ± SEM). **(E)** Quantification nuclear γH2AX intensity in MEPCE-depleted MDA-MB-436 cells and control cells transiently transfected with RNASEH1-D210N **(F)** RNASEH1 transfection efficiency (FACS profile) and cell cycle profiles of MEPCE-depleted and control MDA-MB-436 cells as indicated. (n = 3). B-F, each dot represents a biological triplicate. B and E, one-way ANOVA (Tukey's multiple comparisons test), C and D, two-way ANOVA (Tukey's multiple comparisons test). F, one-way ANOVA (Tukey's multiple comparisons test).

Figure S4

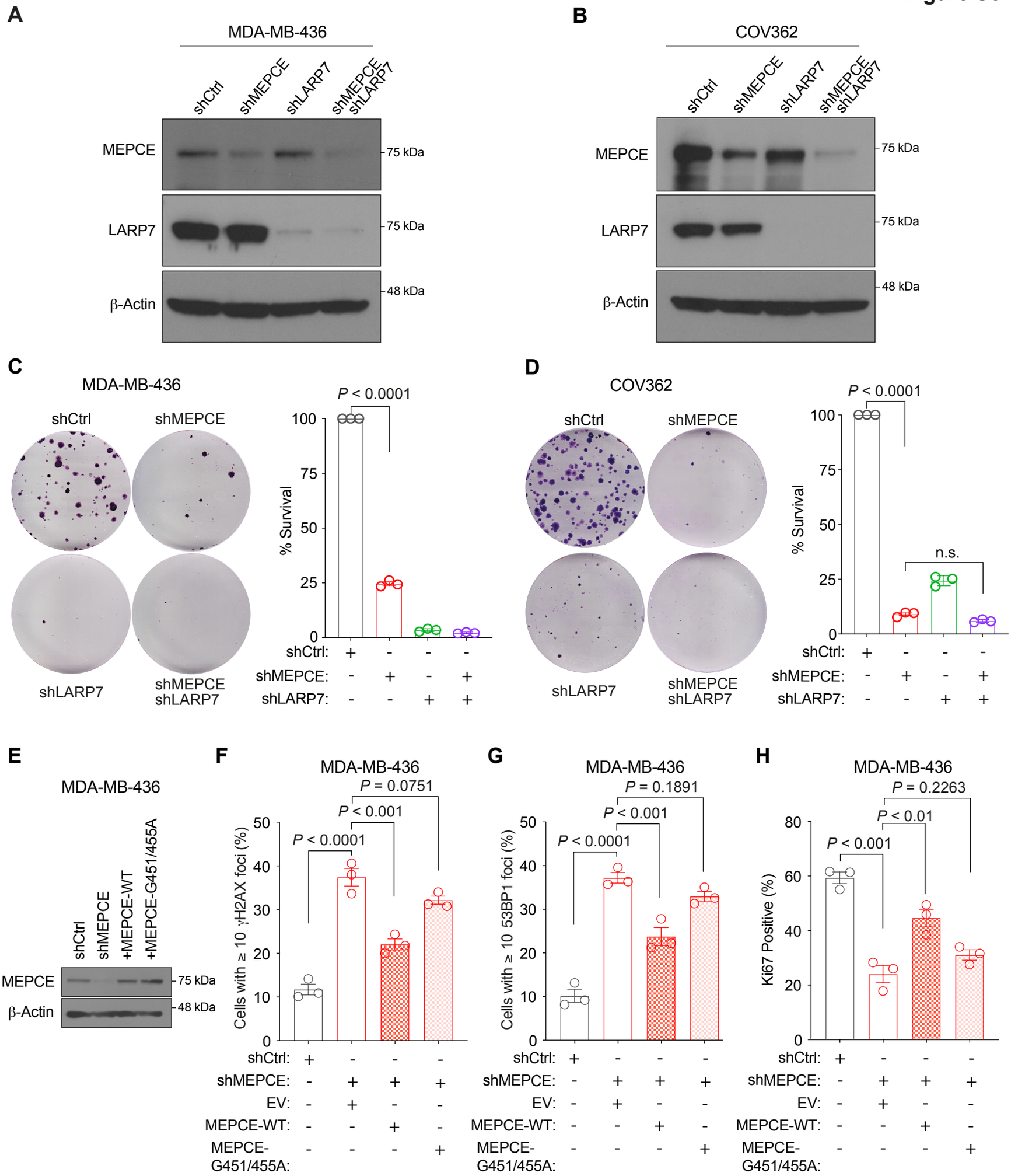

A
MDA-MB-436
EU incorporation (a.u.)
250
200
150
100
50
0
P < 0.05
P < 0.0071
shCtrl: + - + -
shMEPCE: - + - +
Nucleus minus nucleolus
Nucleolus
shCtrl
shMEPCE
EU
Nucleolin
EU/Nucleolin/DAPI
B
MDA-MB-436
Total RNAPII
shCtrl
shMEPCE
log2FC
+301 TTS
+301 TTS
+301 TTS
0 0.04
-0.005 0.005
C
MDA-MB-436
shCtrl
shMEPCE
log2FC
-1kb +4kb
-1kb +4kb
-1kb +4kb
Ranked by decreasing RNAPII occupancy
0 0.05
-0.015 0.015
D
MDA-MB-436
Total PolII in PPP sites
P = 1.74e-30
log10(1e-05+Occupancy)
0
-2
-4
shCtrl
shMEPCE
E
MDA-MB-436
PolII-pSer2 PPP sites
P = 3.77e-10
log10(1e-05+Occupancy)
0
-2
-4
shCtrl
shMEPCE
F
MDA-MB-436
All CPM>2
P < 2.2e-16
log2(pSer2/ total RNAPII)
10
5
0
-5
shCtrl
shMEPCE

Supplementary Figure S4. Loss of MEPCE in *BRCA1*-mutant cells alters transcription and occupancy of total RNAPII and pSer2 RNAPII. (A) Quantification and representative images of EU incorporation in indicated nuclear compartments in MEPCE-depleted MDA-MB-436 and control cells. (n = 3). Bar = 20 μm. **(B)** Heatmaps of total RNAPII occupancy in the +301bp to transcription termination site (TTS) in indicated MDA-MB-436 cells. Log2 fold change is shown. **(C)** PolII occupancy in -1kb and +4kb surrounding the transcription start site in genes with PRR>1 in shCtrl and shMEPCE MDA-MB-436 cells. Log2 fold change is shown. **(D)** Total PolII occupancy in the promoter region (-100bp to +300bp surrounding the transcription start site) which includes promoter proximal pause site. Log10 values are shown. **(E)** PolII-pSer2 occupancy in the promoter region (-100bp to +300bp surrounding the transcription start site) which includes promoter proximal pause site. Log10 values are shown. **(F)** Boxplots showing the ratio of pSer2 levels compared to total RNAPII enrichment in the promoter region in indicated MDA-MB-436 cells for all regions. A, D-F, Student's *t*-test.

Figure S5

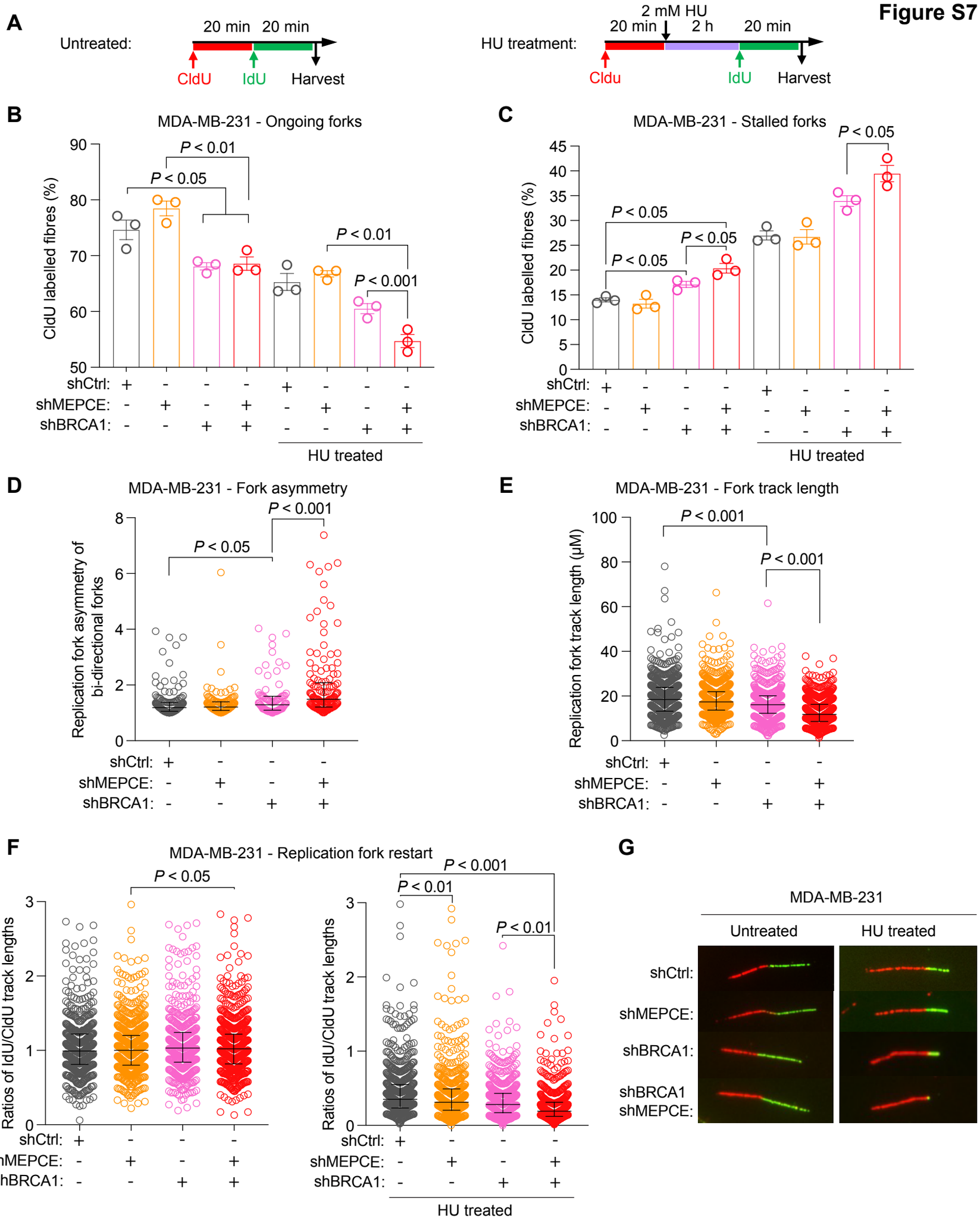

A
MDA-MB-436
SUM149PT
COV362
shCtrl
shLARP7
LARP7
β–Actin
B
MDA-MB-436
SUM149PT
COV362
shCtrl
shLARP7
C
shCtrl
shLARP7
% Survival
P < 0.01
P < 0.01
P < 0.01
MDA-MB-436
SUM149T
COV362
D
MDA-MB-436
shCtrl
shLARP7
P < 0.0001
Nuclear γH2AX Intensity
shCtrl:
shLARP7:
E
COV362
shCtrl
shLARP7
P < 0.0001
Nuclear γH2AX Intensity
shCtrl:
shLARP7:
F
MDA-MB-436
shCtrl
shLARP7
P < 0.0001
S9.6 Nuclear Intensity (a.u.)
shCtrl:
shLARP7:
G
MDA-MB-436
shCtrl
shMEPCE
shLARP7
P < 0.0001
P < 0.0001
P < 0.0001
n.s.
Nuclear γH2AX Intensity
DMSO:
LDC-000067:

Supplementary Figure S5. Depletion of LARP7 in *BRCA1*-mutant cells leads to growth defects and genomic instability. (A) Western blots indicating LARP7 expression in indicated *BRCA1*-mutated cells (MDA-MB-436, SUM149PT, COV362). **(B)** Representative images are shown for clonogenic growth in indicated *BRCA1*-mutated cells. 500 cells were seeded in 6-cm dishes. Cells were fixed and quantified after 21-28 days of growth. **(C)** Quantification from (B). **(D)** Quantification of γH2AX staining in indicated cells. **(E)** Quantification of γH2AX staining intensity in indicated COV362 cells. (**F)** Quantification of S9.6 staining in indicated cells. **(G)** Quantification of γH2AX staining in indicated cells treated with vehicle control or 10μM LDC-000067 for 2 hrs (median with interquartile range, >100 nuclei were scored per condition). C, D, E, and F, Student's *t*-test. G, one-way ANOVA.

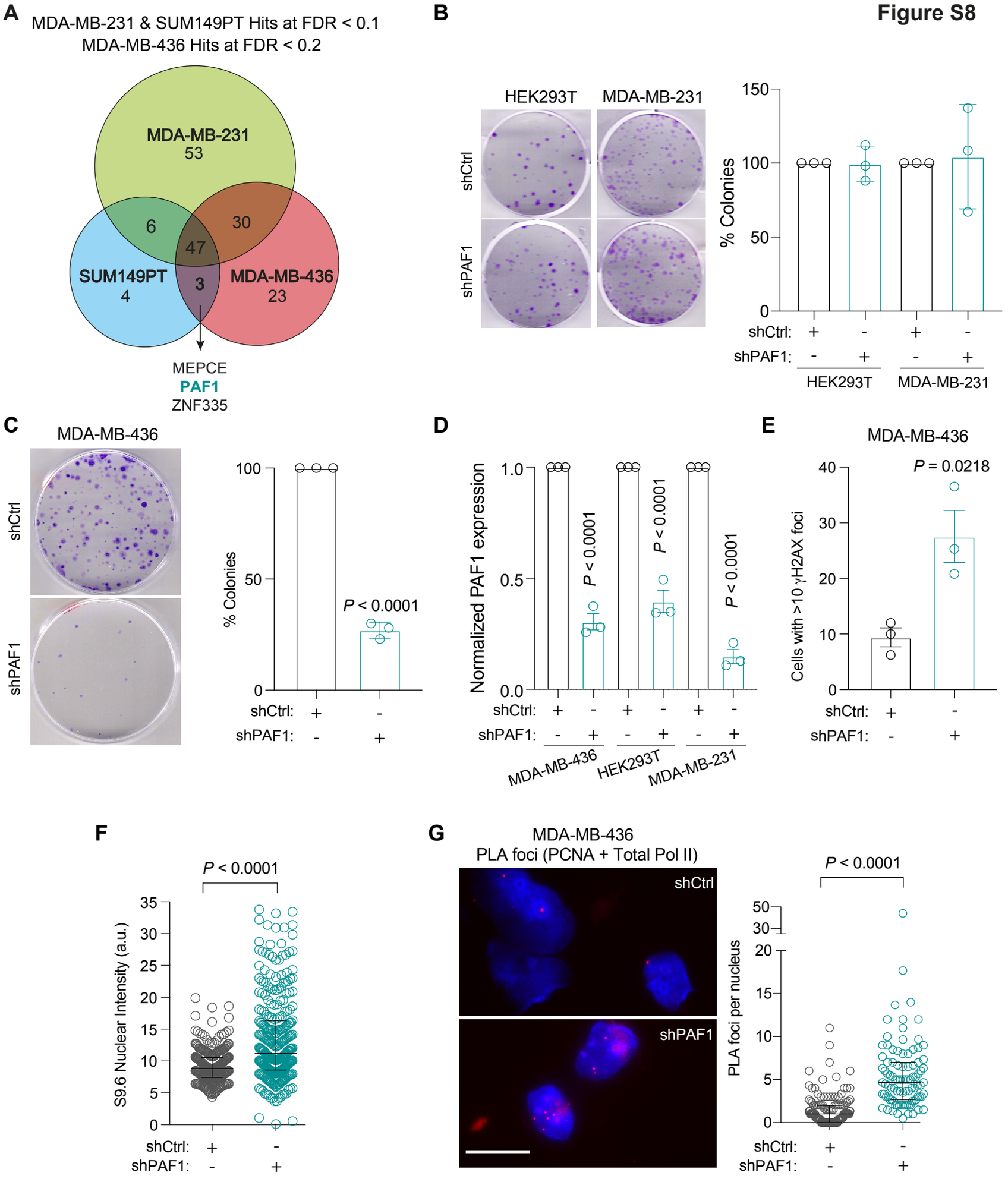

A
MDA-MB-436
shCtrl
shMEPCE
shLARP7
shMEPCE shLARP7
MEPCE
LARP7
β-Actin
75 kDa
75 kDa
48 kDa
B
COV362
shCtrl
shMEPCE
shLARP7
shMEPCE shLARP7
MEPCE
LARP7
β-Actin
75 kDa
75 kDa
48 kDa
C
MDA-MB-436
shCtrl
shMEPCE
shLARP7
shMEPCE shLARP7
P < 0.0001
% Survival
shCtrl:
shMEPCE:
shLARP7:
D
COV362
shCtrl
shMEPCE
shLARP7
shMEPCE shLARP7
P < 0.0001
n.s.
% Survival
shCtrl:
shMEPCE:
shLARP7:
E
MDA-MB-436
shCtrl
shMEPCE
+MEPCE-WT
+MEPCE-G451/455A
MEPCE
β-Actin
75 kDa
48 kDa
F
MDA-MB-436
P = 0.0751
P < 0.001
P < 0.0001
Cells with ≥ 10 γH2AX foci (%)
shCtrl:
shMEPCE:
EV:
MEPCE-WT:
MEPCE-G451/455A:
G
MDA-MB-436
P = 0.1891
P < 0.001
P < 0.0001
Cells with ≥ 10 53BP1 foci (%)
shCtrl:
shMEPCE:
EV:
MEPCE-WT:
MEPCE-G451/455A:
H
MDA-MB-436
P = 0.2263
P < 0.01
P < 0.001
Ki67 Positive (%)
shCtrl:
shMEPCE:
EV:
MEPCE-WT:
MEPCE-G451/455A:

Supplementary Figure S6. Concurrent loss of MEPCE and LARP7 in *BRCA1*-mutant cells does not leads to additive growth defects and MEPCE catalytic activity is important for suppressing DNA damage in *BRCA1*-null cells. (A, B) Western blots indicating MEPCE and LARP7 expression in MDA-MB-436 (A) and COV362 (B) cells transduced with shRNAs targeting a control, MEPCE, or LARP7 sequence as indicated. **(C, D)** Representative images and quantification of clonogenic growth of indicated MDA-MB-436 (C) and COV362 (D) cells. 500 cells were seeded in 6-cm dishes. Colonies were fixed and quantified after 21-28 days of growth (n = 3). (**E**) Western blot showing reconstitution of shMEPCE cells with MSCV-MEPCE-WT or MSCV-MEPCE-G451/455A. **(F)** Quantification of cells with equal to or greater than 10 γH2AX foci in MDA-MB-436 shCtrl cells or shMEPCE MDA-MB-436 cells reconstituted with EV (empty vector), full-length MEPCE-WT or catalytically inactive MEPCE-G451/455A mutant. (n = 3). **(G)** Quantification of cells with equal to or greater than 10 foci of 53BP1 in MDA-MB-436 shCtrl cells or shMEPCE MDA-MB-436 cells reconstituted with EV, full-length MEPCE-WT or catalytically inactive MEPCE-G451/455A mutant. (n = 3). **(H)** Quantification of Ki67 positive shCtrl MDA-MB-436 cells and shMEPCE MDA-MB-436 cells reconstituted with EV, full-length MEPCE-WT or catalytically inactive MEPCE-G451/455A mutant. (n = 3). C, D, F, G, and H, mean ± SEM are shown. Statistical significance was calculated using one-way ANOVA (Tukey's multiple comparisons test). n.s.: not significant.

Figure S7

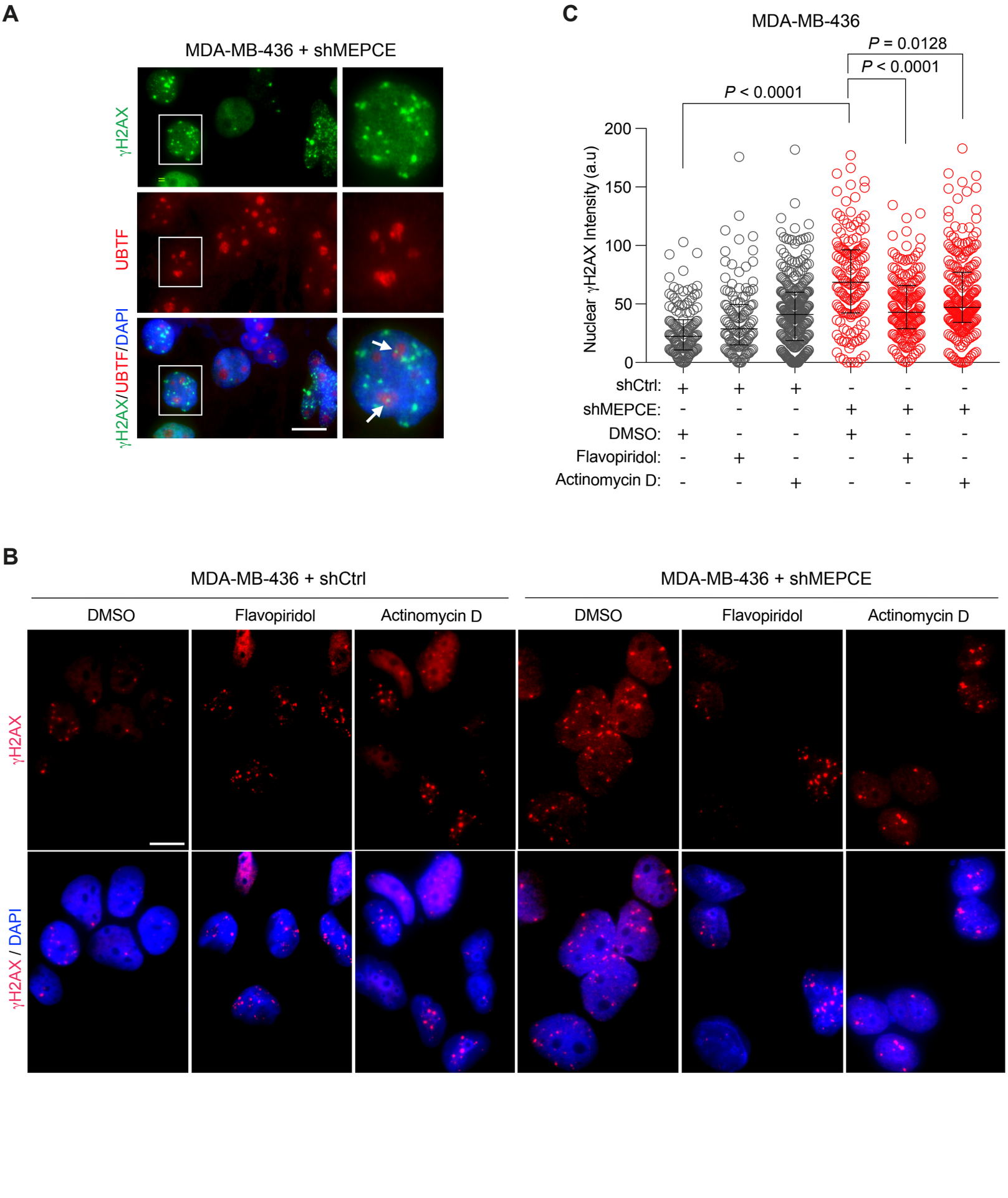

A
Untreated:
20 min
20 min
CldU
IdU
Harvest
HU treatment:
2 mM HU
20 min
2 h
20 min
CldU
IdU
Harvest
B
MDA-MB-231 - Ongoing forks
CldU labelled fibres (%)
P < 0.01
P < 0.05
P < 0.01
P < 0.001
shCtrl:
shMEPCE:
shBRCA1:
HU treated
C
MDA-MB-231 - Stalled forks
CldU labelled fibres (%)
P < 0.05
P < 0.05
P < 0.05
P < 0.05
shCtrl:
shMEPCE:
shBRCA1:
HU treated
D
MDA-MB-231 - Fork asymmetry
Replication fork asymmetry of bi-directional forks
P < 0.05
P < 0.001
shCtrl:
shMEPCE:
shBRCA1:
E
MDA-MB-231 - Fork track length
Replication fork track length (μM)
P < 0.001
P < 0.001
shCtrl:
shMEPCE:
shBRCA1:
F
MDA-MB-231 - Replication fork restart
Ratios of IdU/CldU track lengths
P < 0.05
P < 0.001
P < 0.01
P < 0.01
shCtrl:
shMEPCE:
shBRCA1:
HU treated
G
MDA-MB-231
Untreated
HU treated
shCtrl:
shMEPCE:
shBRCA1:
shBRCA1
shMEPCE:

Supplementary Figure S7. MEPCE is dispensable for replication fork progression and stability in BRCA1-proficient cells. (A) Schematic of conditions for DNA fibre experiments in figure B-G. **(B, C)** Quantification of the percentage of ongoing forks (B), and stalled forks (C) in indicated MDA-MB-231 cells. **(D)** Quantification of fork asymmetry in indicated MDA-MB-231 cells. **(E)** Quantification of replication fork track length in indicated MDA-MB-231 cells. **(F)** Quantification of replication fork restart in indicated cells under indicated conditions in indicated MDA-MB-231 cells. **(G)** Representative images of ongoing replication forks from F. In all cases data is representative of the average of 3 independent experimental repeats. For A and B, mean ± SEM are shown. At least 1200 CldU labelled replication fork structures were counted in total. For D, at least 90 bi-directional replication forks were analysed in total. For E and F, at least 580 ongoing replication fork structures were analysed in total. B and C, Students *t*-test was carried out. D, E, and F, Mann Whitney Rank Sum test.


A
MDA-MB-231 & SUM149PT Hits at FDR < 0.1
MDA-MB-436 Hits at FDR < 0.2
MDA-MB-231
53
SUM149PT
4
MDA-MB-436
23
6
30
47
3
MEPCE
PAF1
ZNF335
B
HEK293T
MDA-MB-231
shCtrl
shPAF1
% Colonies
150
100
50
0
shCtrl: + - + -
shPAF1: - + - +
HEK293T
MDA-MB-231
C
MDA-MB-436
shCtrl
shPAF1
% Colonies
100
50
0
P < 0.0001
shCtrl: + -
shPAF1: - +
D
Normalized PAF1 expression
1.0
0.5
0.0
P < 0.0001
P < 0.0001
P < 0.0001
shCtrl: + - + - + -
shPAF1: - + - + - +
MDA-MB-436
HEK293T
MDA-MB-231
E
MDA-MB-436
P = 0.0218
Cells with >10 γH2AX foci
40
30
20
10
0
shCtrl: + -
shPAF1: - +
F
P < 0.0001
S9.6 Nuclear Intensity (a.u.)
35
30
25
20
15
10
5
0
shCtrl: + -
shPAF1: - +
G
MDA-MB-436
PLA foci (PCNA + Total Pol II)
shCtrl
shPAF1
P < 0.0001
PLA foci per nucleus
50
30
20
15
10
5
0
shCtrl: + -
shPAF1: - +

Supplementary Figure S8. PAF1 deficiency in BRCA1-deficient cells leads to an increase in R-loop accumulation, genomic instability, and TRC. (A) Venn diagram representation of negatively selected genes in the indicated cell lines used for CRISPR-Cas9 screen. **(B, C)** Representative images and quantification of clonogenic growth of indicated cells. 500 cells were seeded and allowed to grow for 21-28 days. **(D)** *PAF1* mRNA expression normalized to 18S. **(E)** Quantification of cells with ≥ 10 spontaneous γH2AX foci in indicated cells. **(F)** Quantification of S9.6 immunofluorescence in indicated cells. >100 nuclei were scored per condition. **(G)** Representative images and quantification of PLA (PCNA + Total Pol II) foci. Bar = 20 μm. For B, C, D, and E, mean ± SEM is shown, and Student's *t*-test was conducted. F and G, median with interquartile range is shown and statistical significance was calculated using Mann-Whitney Rank Sum test.

Figure S9


A
MDA-MB-436 + shMEPCE
γH2AX
UBTF
γH2AX/UBTF/DAPI
C
MDA-MB-436
P = 0.0128
P < 0.0001
P < 0.0001
Nuclear γH2AX Intensity (a.u)
200
150
100
50
0
shCtrl: + + + - - -
shMEPCE: - - - + + +
DMSO: + - - + - -
Flavopiridol: - + - - + -
Actinomycin D: - - + - - +
B
MDA-MB-436 + shCtrl
MDA-MB-436 + shMEPCE
DMSO
Flavopiridol
Actinomycin D
DMSO
Flavopiridol
Actinomycin D
γH2AX
γH2AX / DAPI

Supplementary Figure S9. MEPCE depletion causes non-nucleolar and nucleolar DNA damage. **(A)** Representative images showing γH2AX and UBTF immunofluorescence in MEPCE-depleted MDA-MB-436 cells. Bar = 25 μM. **(B)** Representative images showing γH2AX immunofluorescence in indicated MDA-MB-436 cells after treatment with DMSO, Flavopiridol (1uM), and Actinomycin D (25ng/ml) for 1 hr. Bar = 25 μM. **(C)** Quantification of γH2AX immunofluorescence in cells from (B). A minimum of 100 cells were quantified per replicate. Three biological experiments were completed. Statistical significance was calculated using one-way ANOVA (Tukey's multiple comparisons test).

Supplementary Table 1. Full list of RT-qPCR primers.

ACTB 5'Promoter	5'-CCACCTGGGTACACACAGTCT-3'	5'-TGTCCTTGTCACCCTTTCTTG-3'
ACTB In1	5'-CGGGGTCTTTGTCTGAGC-3'	5'-CAGTTAGCGCCCAAAGGAC-3'
ACTB 5'Pause	5'-TTACCCAGAGTGCAGGTGTG-3'	5'-CCCCAATAAGCAGGAACAGA-3'
ACTB Pause	5'-GGGACTATTTGGGGGTGTCT-3'	5'-TCCCATAGGTGAAGGCAAAG-3'
ACTB C	5'-TGGGCCACTTAATCATTCAAC-3'	5'-CCTCACTTCCAGACTGACAGC-3'
GAPDH PPP	5'-CTCCTGTTCGACAGTCAGC-3'	5'-TTCAGGCCGTCCCTAGC-3'
GAPDH In5	5'-ATAGGCGAGATCCCTCCAA-3'	5'-TGAAGACGCCAGTGGAC-3'
GAPDH TES	5'-CCCTGTGCTCAACCAGT -3'	5'-CTCACCTTGACACAAGCC-3'
TEFM PPP	5'-CTTGGAGATGAGCGGGTCTG-3'	5'-GACAGACGGGAAATCACCCC-3'
TEFM In2	5'-TGGCCAATGTGGTGAAAGCC-3'	5'-GGGACTACAGGCCCACGCC-3'
TEFM TES	5'-ACCACATAGACTTTATGACAGAGAA-3'	5'-TCAATCCATGCTTGTGAAGCAAA-3'
18S	5'-CAGCCACCCGAGATTGAGCA-3'	5'-TAGTAGCGACGGGCGGTGTG-3'
PAF1	5'-CCTGACACCTACCGCATCG-3'	5'-TGTACTCTGTCTTTCGCATCCA-3'
TMEM242	5'-ATGCGCTATGTCTGTGGACC-3'	5'-GGCCCCAGTAAAGGTTAGCA-3'
CSNK1D	5'-GCGAGTTGAGTTCAGAGTCCA-3'	5'-CTGCCTACAGTTGGGACAGT-3'
RASA1	5'-AACCATGGGAAGCTCCCTCTA-3'	5'-TCCACAATGCAAGCACCTTC-3'
CORT	5'-TTCTCCAGAAGCAAGCGCAC-3'	5'-GAGCAATCAGTGCCCACAAA-3'
ANG	5'-GGGTGAGTAGCGTCTCTTTCG-3'	5'-TATCAGCAAGCAGGACAGGGT-3'