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Supplemental information

BRCA1 mediates protein homeostasis through the ubiquitination of PERK and IRE1

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. High level of unfolded proteins was detected in BRCA1-def breast cancer cells, Related to Figure 1.

(A-B) HCC1937 ($^{+}$ or ^{def}) breast cancer cells were treated with TPE-MI and analyzed by microscopy (A) or flow cytometry (B). Scale = 20 μM.

(C) quantitative analysis of the signal intensity of TPE-MI fluorescence in labeled cells.

(D) quantitative analysis of the TPE-MI-high cell population detected by flow cytometry.

Data are displayed as means ± SD.

TPE = TPE-MI. Con-A = concanavalin-A. $^{+}$ = BRCA1-replete. ^{def} = BRCA1-deficient.

Figure S2. Confocal microscopic images of BRCA1 and DERL1 in MDA-MB-231 breast cancer cells, Related to Figure 1.

(A) Upper panel, single-color channels of cancer cells in white background. Lower panel**,** series of z-stack images in black background. Z depth between scans was 0.6 μm.

All cells were counterstained with DAPI (blue). Scale = 5 μM. Green = GFP-KDEL.

Figure S3. Confocal microscopic images of BRCA1 and DERL1 in MCF7 breast cancer cells, Related to Figure 1.

(A) Top panel, **a,** cancer cells were immunostained with BRCA1 (red) and DERL1 (yellow) antibodies. ***b,*** cells were immunostained with secondary antibodies conjugated with fluorophores as control. Middle panel**,** single-color channels in white background. Lower panel, series of z-stack images of MCF7 cancer cells in black background. Z depth between scans was 0.6 μm.

All cells were counterstained with DAPI (blue). Scale = 5 μM. Green = GFP-KDEL.

Figure S4. **BRCA1 does not mediate ubiquitination of SEL1L and DERLIN-1,** Related to Figure 1.

(A) Reverse IP assays using SEL1L antibodies to co-IP BRCA1 protein from cytoplasmic extract isolated from MCF7 cells.

(B) Reverse IP assays using DERL1 antibodies to co-IP BRCA1 protein from cytoplasmic extract isolated from MCF7 cells. IB = immunoblotting.

Ig = non-specific immunoglobulin fragments. IgG(H) & IgG(L) = heavy and light chains. BRCA1 IB: ** = truncated BRCA1. *** = delta11q isoform.

(C-D) Ubiquitination analysis on SEL1L and DERL1 using protein extract from control or BRCA1 siRNA-transfected MCF7 cells (**C**) or MDA-MB-231 cells (**D**). Co-IP reaction was carried out using agarose beads conjugated with Ubiquitin (Ub) monoclonal antibody and immunoblotted with either SEL1L or DERL1 antibodies.

DERL1 = DERLIN-1. IP = immunoprecipitation. IB = immunoblotting.

Figure S5. Protein and mRNA levels of PERK and IRE1 in BRCA1 under- or over-expressing breast cancer cells, Related to Figure 2.

(A-C) Quantitative analysis of PERK and IRE1 protein levels in transient transfected cells from Fig. 2A-C. A, control or BRCA1-depleted MDA-MB-231 cells. B, control or BRCA1-depleted MCF7 cells. C, untreated MDA-MB-436 BRCA1+ and BRCA1-def cells. Protein expression signal intensities were quantified by ImageJ and normalized by expression of GAPDH.

(D-E) qRT-PCR analysis of BRCA1, PERK, IRE1 and BARD1 mRNA expression in breast cancer cells . D, cDNAs were generated from mRNAs isolated from control or BRCA1-depleted cancer cells. E, cDNAs were generated from mRNAs isolated from control, BRCA1-overexpressing or BARD1-overexpression MDA-MB-436 BRCA1-def cancer cells. cDNA levels were normalized by expression of housekeeper gene GAPDH.

Data are displayed as means ± SD. P value was calculated by Student's two-tailed, unpaired *t*-test. (* < 0.05, ** < 0.01, *** < 0.001).

Figure S6. BRCA1 protein interacts with and ubiquitinates PERK and IRE1, Related to Figure 2.

(A) Reverse IP assays using PERK or IRE1 antibodies to co-IP BRCA1 protein from cytoplasmic extract isolated from MCF7 cells. IRE1 IB: * = possible ubiquitinated IRE1.

(B) **a**, Western blot analysis of proteins isolated from nuclear and ER subcellular fractions. 15 μg of nuclear and ER proteins were used for each lane. p300 = nuclear marker. GRP94 = ER marker. **b-d**, Co-IP assays

using protein isolated from MCF7 ER microsomes show BRCA1 protein interacts with PERK and IRE1. BRCA1 IB: * = hyperphosphorylated BRCA1, ** = truncated BRCA1. *** = delta11q isoform.C
(C) Quantitative analysis of ubiquitination level of PERK and IRE1 proteins in BRCA1-depleted breast cancer cells.
(D) Western blot analysis of total protein extract isolated from control or BRCA1-depleted cells, with or without Bortezomib treatment. Equal amounts of total protein extract were loaded into each lane.
(E) In vitro Ubiquitination Assay of NSP15 protein with BRCA1/BARD1 as E3 ligase. Full-length NSP15 was incubated with E1, UBE2J1, BRCA1, BARD1 and/or Ubiquitin. Ubiquitin or ubiquitinated protein was detected with an anti-Ub antibody. NSP protein was detected using anti-Strept-tactin antibody.
Data are displayed as means $\pm$ SD. P value was calculated by Student's two-tailed, unpaired *t*-test. (* < 0.05, ** < 0.01, *** < 0.001).
IP = immunoprecipitation. IN = Input. Ig = non-specific immunoglobulin fragments. Bortez = Bortezomib. Ub = Ubiquitin. IB = immunoblotting. Ub = ubiquitin.

Figure S7. Lower BARD1 protein level was detected in BRCA1-def cancer cells compared to BRCA1-replete cells, Related to Figure 2.
(A) Western blot analysis of BARD1 protein expression in BRCA1-replete (+) and BRCA1-deficient (-def) breast cancer cells.
(B) quantitative analysis of BARD1 protein expression normalized with GAPDH.
436+ = MDA-MB-436 BRCA1-replete. 436-def = MDA-MB-436 BRCA1-deficient. 1937+ = HCC1937 BRCA1-replete. 1937-def = HCC1937 BRCA1-deficient.
Data are displayed as mean $\pm$ SD. P value was calculated by Student's two-tailed, unpaired *t*-test. (** < 0.01).
IB = immunoblotting.

Figure S8. Depleting UPR components is lethal in the BRCA1-def cancer cells, Related to Figure 3.
(A) Depletion of CypB induced severe synthetic lethality in SUM149PT and UWB1.289 BRCA1-def cancer cells. Left panels, Western blot analysis. Middle panels, representative images of colony forming units (CFUs) from control or CypB knock-down cancer cells after 12 days culturing. Right panels**,** quantitative analysis of the clonal colony formation assay.
(B) Western blot analysis of the UPR or ERAD markers in cells targeted by control or gene specific siRNA. a, MDA-MB-436 BRCA1+ (lanes 1-3) and MDA-MB-436 BRCA1-def (lanes 4-6) cells. b, HCC1937 BRCA1-def (lanes 1-2) and SUM149PT BRCA1-def (lanes 3-4) cells. c, HCC1937 BRCA1-def cells. d, MDA-MB-436 BRCA1-def (left), HCC1937 BRCA1-def (Middle) and SUM149PT BRCA1-def (right) cells. e, MDA-MB-436 BRCA1+ cells. f, MDA-MB-436 BRCA1-def (lanes 1-2), HCC1937 BRCA1-def (lanes 3-4) and SUM149PT BRCA1-def (lanes 5-8). BiP = GRP78. CypB = cyclophilin B. DERL1 = DERLIN-1.
Results were calculated as means $\pm$ SD. P value was calculated by student's t-test. (*** < 0.001).

Figure S9. Analysis of UPR components protein and mRNA level in untreated, DMSO-treated or CsA-treated MDA-MB-436 breast cancer cells, Related to Figure 5.
BRCA1-replete (+) and BRCA1-deficient (-def) breast cancer cells were either untreated, treated with DMSO (vehicle) or treated with 1 μM CsA for 2 days. Cells were harvested for (A) protein or (B) mRNAs analysis. p-PERK = phospho-PERK. p-IRE1 = phospho-IRE1. p-eIF2a = phospho-eIF2a. sp XBP1 = spliced XBP1. IB = immunoblotting.
Results were calculated as means $\pm$ SD. P value was calculated by student's t-test.

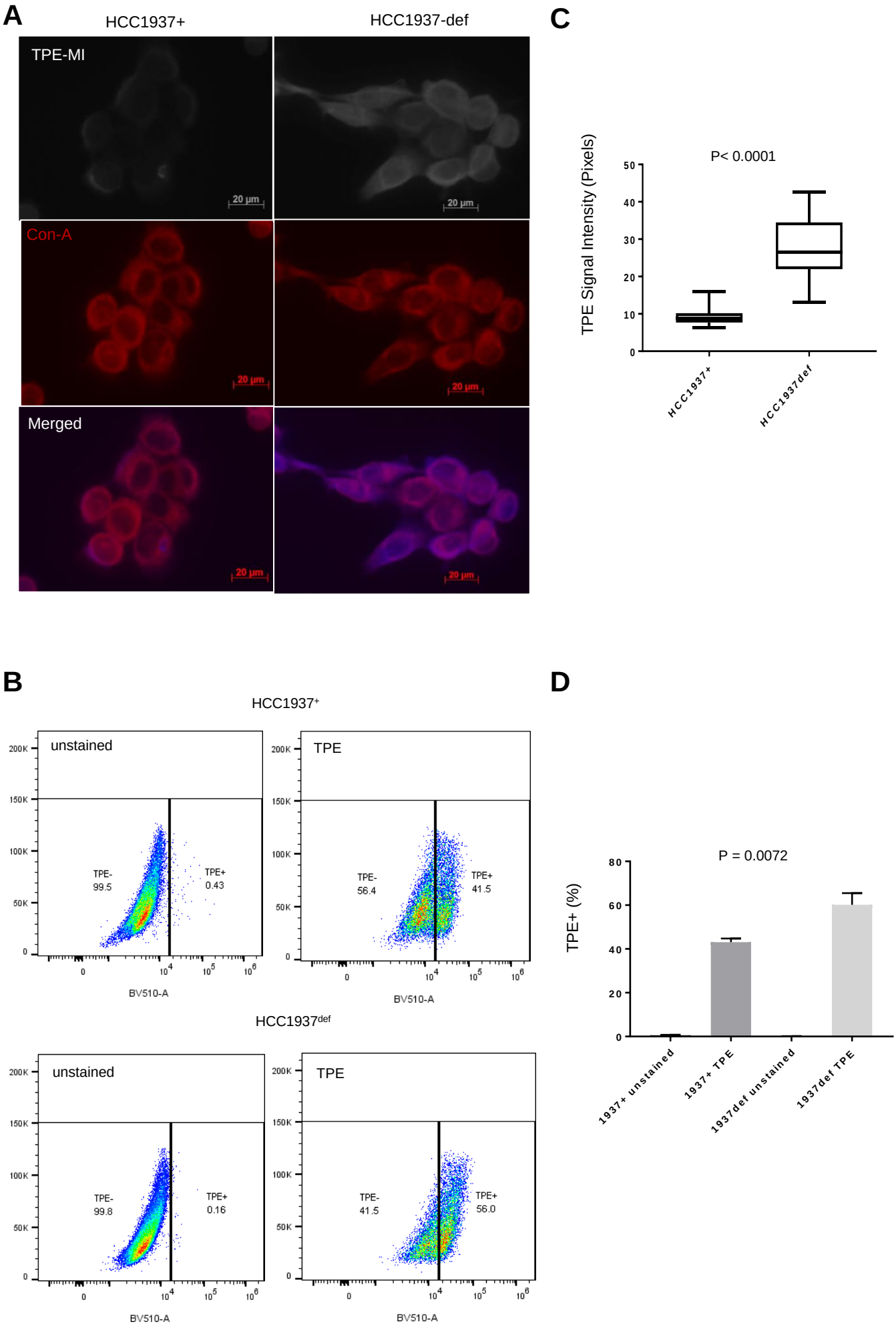

A
HCC1937+
HCC1937-def
TPE-MI
Con-A
Merged
20 µm
C
P< 0.0001
TPE Signal Intensity (Pixels)
HCC1937+
HCC1937def
B
HCC1937+
unstained
TPE
TPE-
99.5
TPE+
0.43
TPE-
56.4
TPE+
41.5
HCC1937def
TPE-
99.8
TPE+
0.16
TPE-
41.5
TPE+
56.0
SSC-A
BV510-A
D
P = 0.0072
TPE+ (%)
1937+ unstained
1937+ TPE
1937def unstained
1937def TPE

Figure S1

Figure S1. High level of unfolded proteins was detected in the BRCA1-def breast cancer cells, Related to Figure 1.

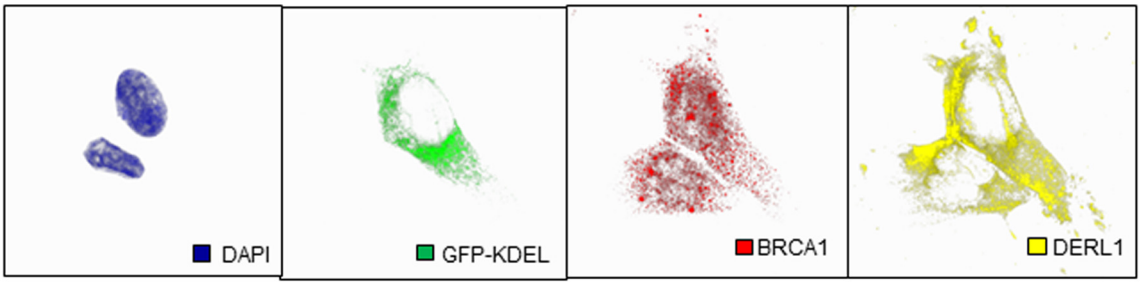
(A-B) HCC1937 (+ or def) breast cancer cells were treated with TPE-MI and analyzed by microscopy (A) or flow cytometry (B). Scale = 20 μM.

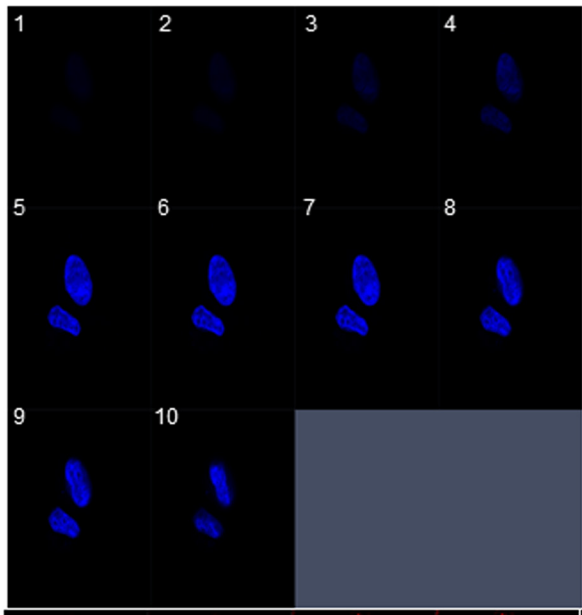
(C) quantitative analysis of the signal intensity of TPE-MI fluorescence in labeled cells.

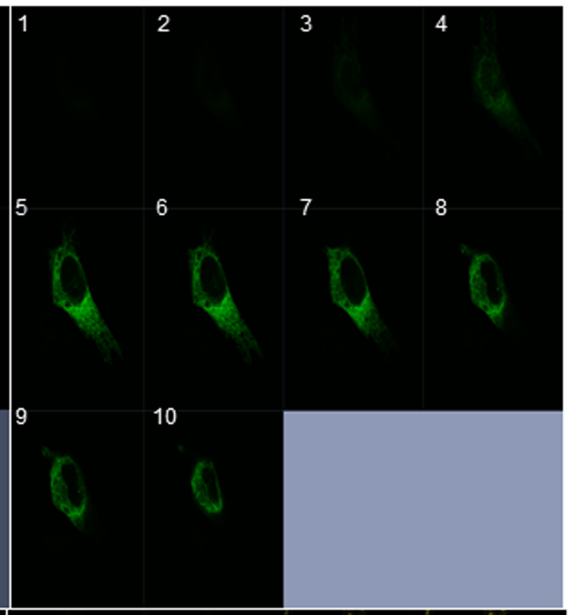
(D) quantitative analysis of the TPE-MI-high cell population detected by flow cytometry.

Data are displayed as means ± SD.

TPE = TPE-MI. Con-A = concanavalin-A. + = BRCA1-replete. Def = BRCA1-deficient.


DAPI
GFP-KDEL
BRCA1
DERL1


1
2
3
4
5
6
7
8
9
10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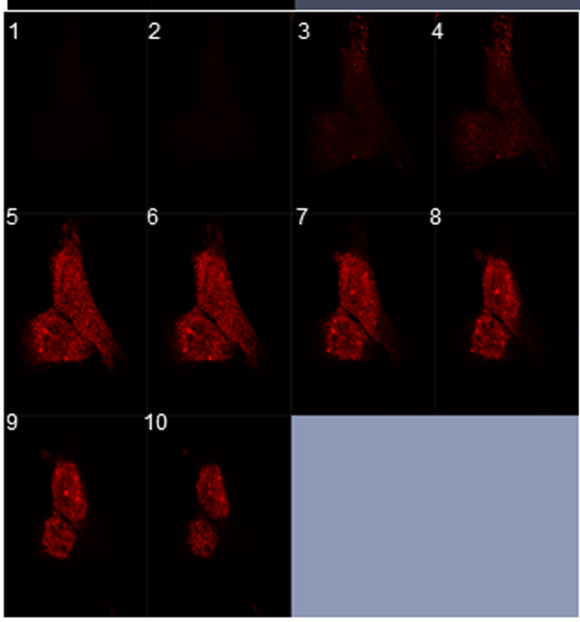
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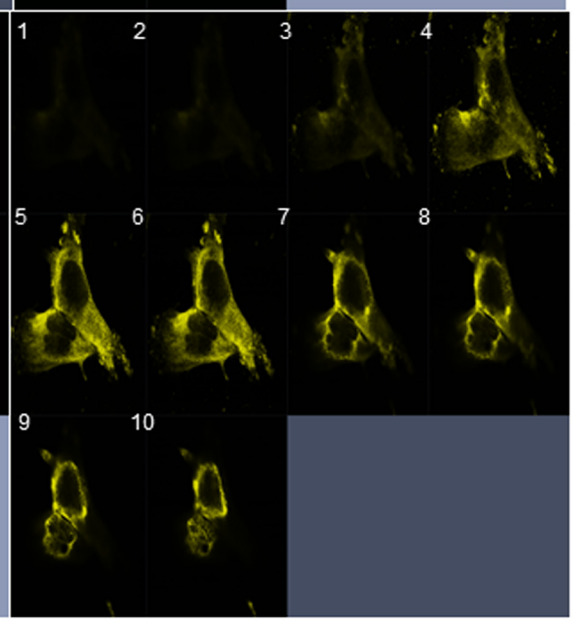

1
2
3
4
5
6
7
8
9
10

Figure S2

Figure S2. Confocal microscopic images of BRCA1 and DERL1 in MDA-MB-231 breast cancer cells, Related to Figure 1.

(A) Top panel**,** single-color channels of cancer cells in white background.

Bottom panel, series of z-stack images in black background. Z depth between scans was 0.6 μm.

All cells were counterstained with DAPI (blue). Scale = 5 μM. Green = GFP-KDEL.

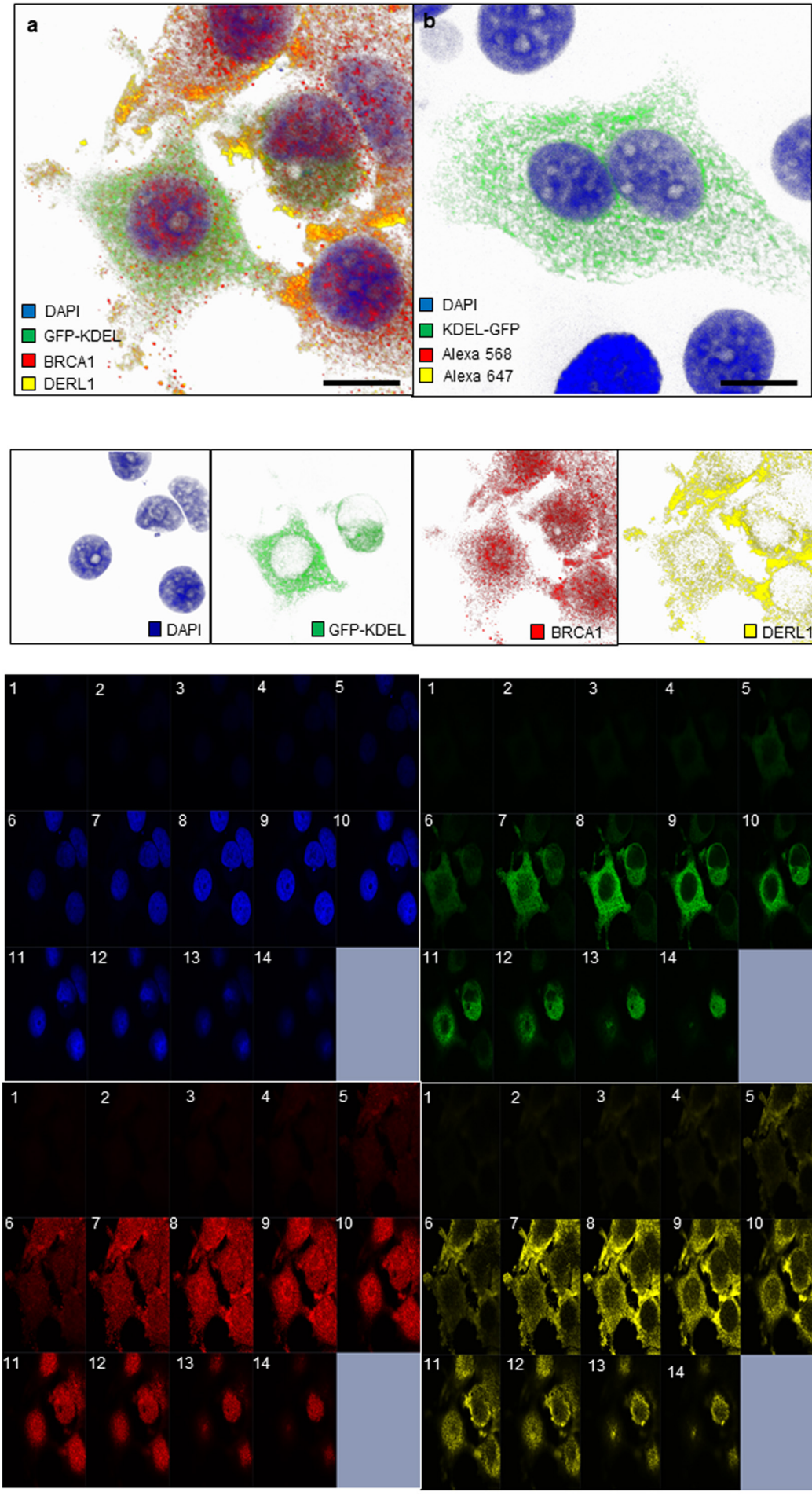

a
DAPI
GFP-KDEL
BRCA1
DERL1
b
DAPI
KDEL-GFP
Alexa 568
Alexa 647
DAPI
GFP-KDEL
BRCA1
DERL1
1 2 3 4 5
6 7 8 9 10
11 12 13 14

Figure S3

Figure S3. Confocal microscopic images of BRCA1 and DERL1 in MCF7 breast cancer cells, Related to Figure 1.

(A) Top panel*,* **a,** *cancer* cells were immunostained with BRCA1 (red) and DERL1 (yellow) antibodies. **b***,* cells were immunostained with secondary antibodies conjugated with fluorophores as control. Middle panel**,** single-color channels in white background. Bottom panel, series of z-stack images in black background. Z depth between scans was 0.6 μm.

All cells were counterstained with DAPI (blue). Scale = 5 μM. Green = GFP-KDEL.

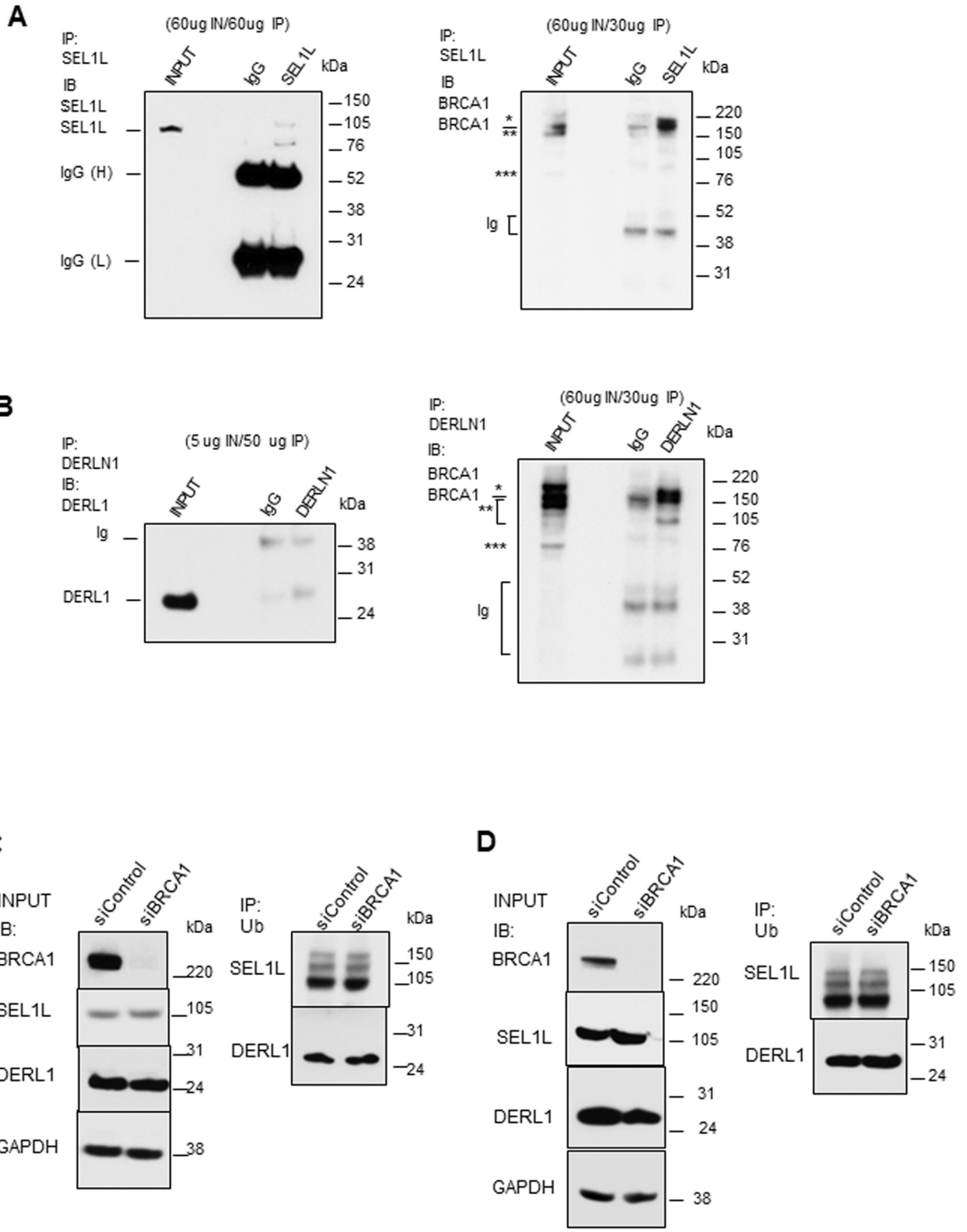

A
(60ug IN/60ug IP)
IP:
SEL1L
IB
SEL1L
SEL1L
IgG (H)
IgG (L)
INPUT
IgG
SEL1L
kDa
150
105
76
52
38
31
24
(60ug IN/30ug IP)
IP:
SEL1L
IB
BRCA1
BRCA1
*
**
***
Ig
220
150
105
76
52
38
31
B
(5 ug IN/50 ug IP)
IP:
DERLN1
IB:
DERL1
Ig
DERL1
INPUT
IgG
DERLN1
kDa
38
31
24
(60ug IN/30ug IP)
IP:
DERLN1
IB:
BRCA1
BRCA1
*
**
***
Ig
C
INPUT
IB:
BRCA1
SEL1L
DERL1
GAPDH
siControl
siBRCA1
kDa
220
105
31
24
38
IP:
Ub
SEL1L
DERL1
150
105
31
24
D
INPUT
IB:
BRCA1
SEL1L
DERL1
GAPDH
220
150
105
31
24
38
IP:
Ub
SEL1L
DERL1
150
105
31
24

Figure S4

Figure S4. BRCA1 does not mediate ubiquitination of SEL1L and DERLIN-1, Related to Figure 1.

(A) Reverse IP assays using SEL1L antibodies to co-IP BRCA1 protein from cytoplasmic extract isolated from MCF7 cells.

(B) Reverse IP assays using DERL1 antibodies to co-IP BRCA1 protein from cytoplasmic extract isolated from MCF7 cells. IB = immunoblotting.

Ig = non-specific immunoglobulin fragments. IgG(H) & IgG(L) = heavy and light chains. BRCA1 IB: ** = truncated BRCA1. *** = delta11q isoform.

(C-D) Ubiquitination analysis on SEL1L and DERL1 using protein extract from control or BRCA1 siRNA-transfected MCF7 cells (**C**) or MDA-MB-231 cells (**D**). Co-IP reaction was carried out using agarose beads conjugated with Ubiquitin (Ub) monoclonal antibody and immunoblotted with either SEL1L or DERL1 antibodies.

DERL1 = DERLIN-1. IP = immunoprecipitation. IB = immunoblotting.

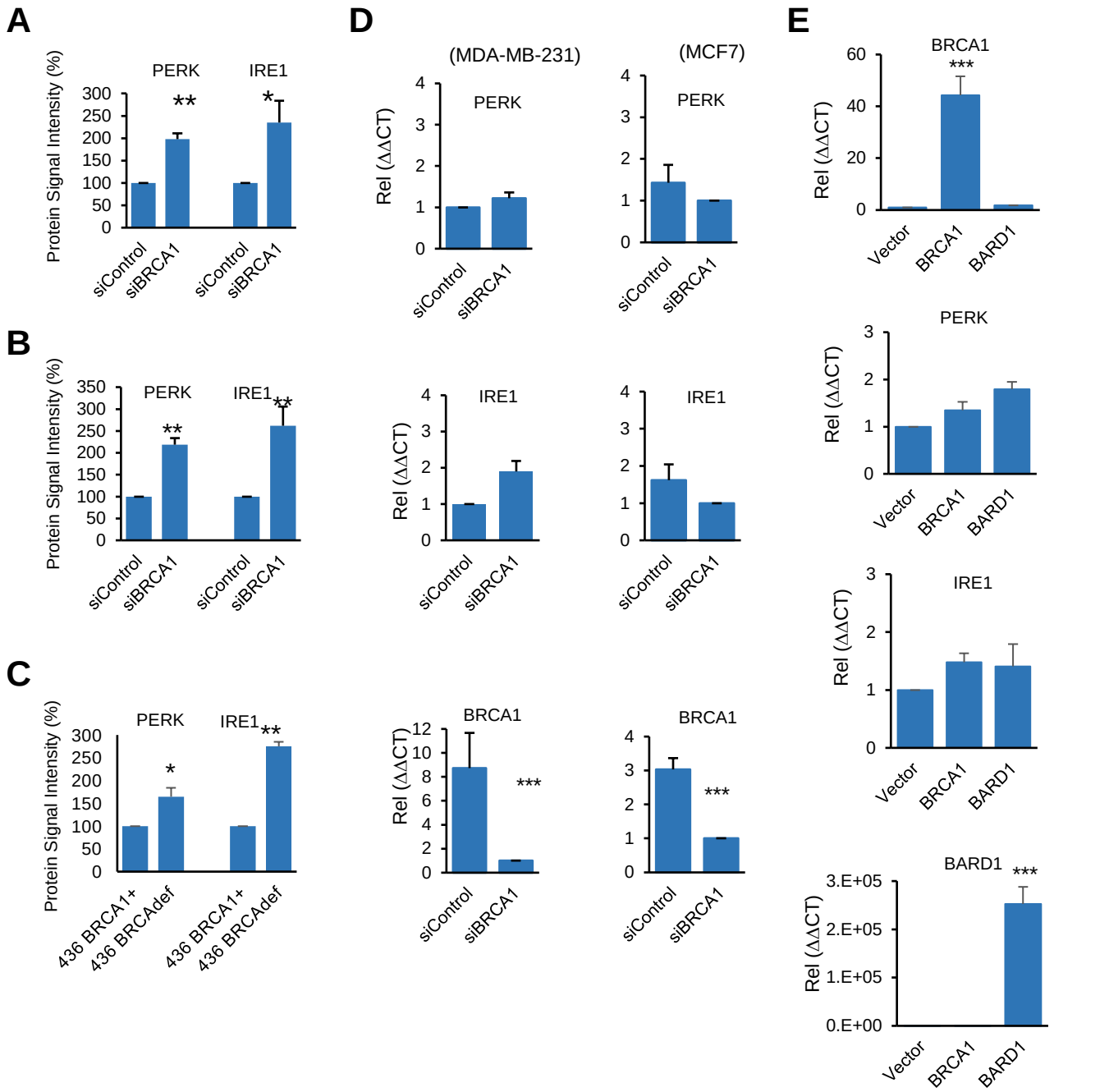

A
Protein Signal Intensity (%)
PERK
IRE1
**
*
siControl
siBRCA1
siControl
siBRCA1
B
Protein Signal Intensity (%)
PERK
IRE1
**
**
siControl
siBRCA1
siControl
siBRCA1
C
Protein Signal Intensity (%)
PERK
IRE1
*
**
436 BRCA1+
436 BRCAdef
436 BRCA1+
436 BRCAdef
D
(MDA-MB-231)
(MCF7)
PERK
PERK
Rel (ΔΔCT)
siControl
siBRCA1
siControl
siBRCA1
IRE1
IRE1
Rel (ΔΔCT)
siControl
siBRCA1
siControl
siBRCA1
BRCA1
BRCA1
Rel (ΔΔCT)
***
***
siControl
siBRCA1
siControl
siBRCA1
E
BRCA1
***
Rel (ΔΔCT)
Vector
BRCA1
BARD1
PERK
Rel (ΔΔCT)
Vector
BRCA1
BARD1
IRE1
Rel (ΔΔCT)
Vector
BRCA1
BARD1
BARD1
***
Rel (ΔΔCT)
Vector
BRCA1
BARD1

Figure S5

Figure S5. Protein and mRNA levels of PERK and IRE1 in BRCA1 under- or over-expressing breast cancer cells, Related to Figure 2.

(A-C) Quantitative analysis of PERK and IRE1 protein levels in transient transfected cells from Fig. 2A-C. A**,** control or BRCA1-depleted MDA-MB-231 cells. B, control or BRCA1-depleted MCF7 cells. C**,** untreated MDA-MB-436 BRCA1+ and BRCA1-def cells. Protein expression signal intensities were quantified by ImageJ and normalized by expression of GAPDH.

(D-E) qRT-PCR analysis of BRCA1, PERK, IRE1 and BARD1 mRNA expression in breast cancer cells . D, cDNAs were generated from mRNAs isolated from control or BRCA1-depleted cancer cells. E, cDNAs were generated from mRNAs isolated from control, BRCA1-overexpressing or BARD1-overexpression MDA-MB-436 BRCA1-deficient cancer cells. cDNA levels were normalized by expression of housekeeper gene GAPDH.

Data are displayed as means $\pm$ SD. P value was calculated by Student's two-tailed, unpaired *t*-test. (* < 0.05, ** < 0.01, *** < 0.001).

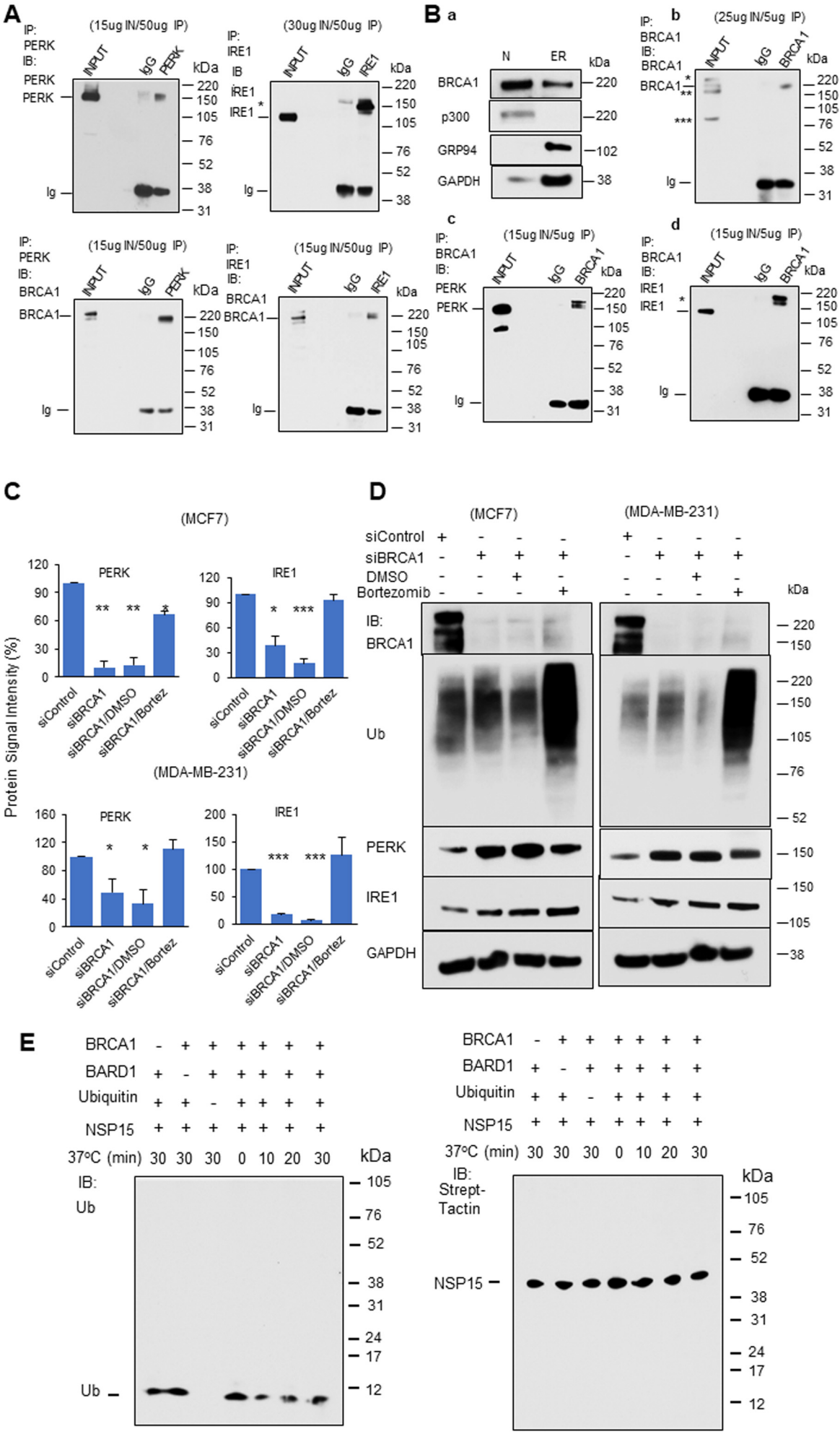

A
IP: PERK
IB: PERK
(15ug IN/50ug IP)
INPUT
IgG
PERK
kDa
220
150
105
76
52
38
31
Ig
IP: IRE1
IB: IRE1
(30ug IN/50ug IP)
IRE1
IP: PERK
IB: BRCA1
(15ug IN/50ug IP)
BRCA1
IP: IRE1
IB: BRCA1
(15ug IN/50ug IP)
B
a
N
ER
BRCA1
p300
GRP94
GAPDH
102
b
IP: BRCA1
IB: BRCA1
(25ug IN/5ug IP)
c
IP: BRCA1
IB: PERK
(15ug IN/5ug IP)
d
IP: BRCA1
IB: IRE1
(15ug IN/5ug IP)
C
(MCF7)
PERK
IRE1
Protein Signal Intensity (%)
siControl
siBRCA1
siBRCA1/DMSO
siBRCA1/Bortez
(MDA-MB-231)
D
(MCF7)
(MDA-MB-231)
siControl
siBRCA1
DMSO
Bortezomib
IB: BRCA1
Ub
PERK
IRE1
GAPDH
E
BRCA1
BARD1
Ubiquitin
NSP15
37°C (min) 30 30 30 0 10 20 30
IB: Ub
Ub
IB: Strept-Tactin
NSP15
105
76
52
38
31
24
17
12

Figure S6

Figure S6. **BRCA1 protein interacts with and ubiquitinates PERK and IRE1,** Related to Figure 2.

(A) Reverse IP assays using PERK or IRE1 antibodies to co-IP BRCA1 protein from cytoplasmic extract isolated from MCF7 cells. IRE1 IB: * = ubiquitinated IRE1.

(B) **a**, Western blot analysis of proteins isolated from nuclear or ER subcellular fractions. 15 μg of nuclear or ER proteins were used for each lane. P300 = nuclear marker. GRP94 = ER marker. **b-d**, Co-IP assays show BRCA1 protein interacts with PERK and IRE1 in MDA-MB-231 breast cancer cells. IB = immunoblotting.

(C) Quantitative analysis of ubiquitination level of PERK and IRE1 proteins in BRCA1-depleted breast cancer cells.

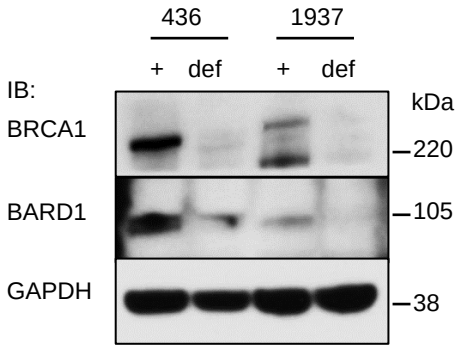
(D) Western blot analysis of total protein extract isolated from control or BRCA1-depleted cells, with or without Bortezomib treatment. Equal amounts of total protein extract were loaded into each lane.

(E) In vitro Ubiquitination Assay of NSP15 protein with BRCA1/BARD1 as E3 ligase. Full-length NSP15 was incubated with E1, UBE2J1, BRCA1, BARD1 and/or ubiquitin. Ubiquitin or ubiquitinated protein was detected with an anti-Ub antibody. NSP protein was detected using anti-Strept-tactin antibody.

Data are displayed as means $\pm$ SD. P value was calculated by Student's two-tailed, unpaired *t*-test. (* < 0.05, ** < 0.01, *** < 0.001).

IP = immunoprecipitation. IN = Input. Ig = non-specific immunoglobulin fragments. Bortez = Bortezomib. Ub = Ubiquitin. IB = immunoblotting. Ub = ubiquitin.

A


436
1937
+ def + def
IB:
BRCA1
BARD1
GAPDH
kDa
220
105
38

B

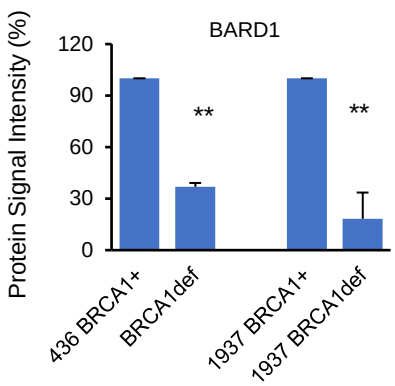

BARD1
Protein Signal Intensity (%)
120
90
60
30
0
**
**
436 BRCA1+
BRCA1def
1937 BRCA1+
1937 BRCA1def

Figure S7

Figure S7. Lower BARD1 protein level was detected in BRCA1-def cancer cells compared to BRCA1-replete cells, Related to Figure 2.

(A) Western blot analysis of BARD1 protein expression in BRCA1+ and BRCA1-def breast cancer cells.

(B) quantitative analysis of BARD1 protein expression normalized with GAPDH.

436+ = MDA-MB-436 BRCA1-replete. 436def = MDA-MB-436 BRCA1-deficient. 1937+ = HCC1937 BRCA1-replete. 1937def = HCC1937 BRCA1-deficient.

Data are displayed as mean $\pm$ SD. P value was calculated by Student's two-tailed, unpaired *t*-test. (** < 0.01).

IB = immunoblotting.

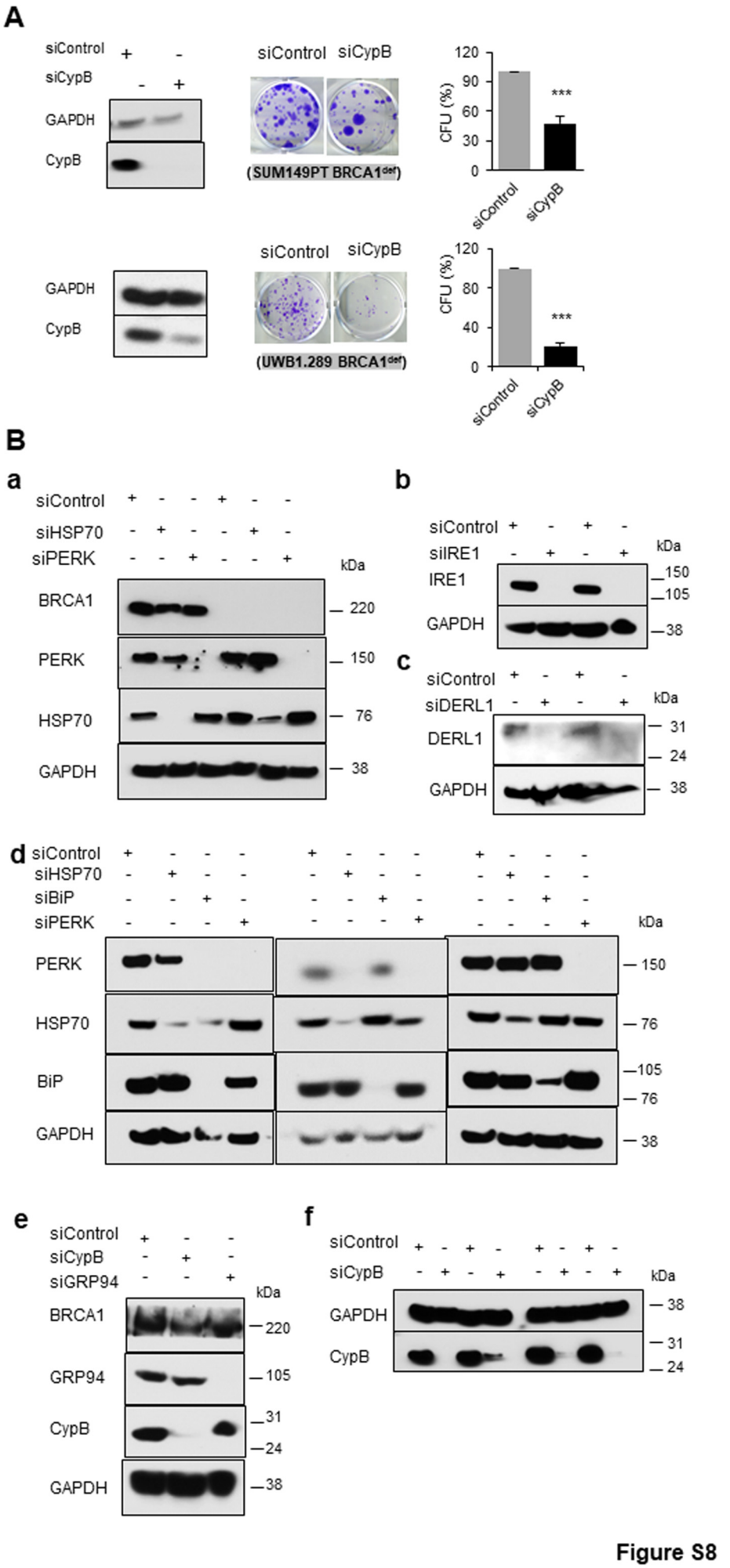

A
siControl
siCypB
GAPDH
CypB
siControl siCypB
(SUM149PT BRCA1def)
CFU (%)
120
90
60
30
0
***
siControl
siCypB
GAPDH
CypB
siControl siCypB
(UWB1.289 BRCA1def)
CFU (%)
120
80
40
0
***
siControl
siCypB
B
a
siControl
siHSP70
siPERK
kDa
BRCA1
220
PERK
150
HSP70
76
GAPDH
38
b
siControl
siIRE1
kDa
IRE1
150
105
GAPDH
38
c
siControl
siDERL1
kDa
DERL1
31
24
GAPDH
38
d
siControl
siHSP70
siBiP
siPERK
kDa
PERK
150
HSP70
76
BiP
105
76
GAPDH
38
e
siControl
siCypB
siGRP94
kDa
BRCA1
220
GRP94
105
CypB
31
24
GAPDH
38
f
siControl
siCypB
kDa
GAPDH
38
CypB
31
24

Figure S8

Figure S8. Depleting UPR components is lethal in the BRCA1-def cancer cells, Related to Figure 3.

(A) Depletion of CypB induced severe synthetic lethality in SUM149PT and UWB1.289 BRCA1-def cancer cells. Left panels, Western blot analysis. Middle panels, representative images of colony forming units (CFUs) from control or CypB knock-down cancer cells after 12 days culturing. Right panels**,** quantitative analysis of the clonal colony formation assay.

(B) Western blot analysis of the UPR or ERAD components in cells targeted by control or gene specific siRNA. **a**, MDA-MB-436 BRCA1+ (lanes 1-3) and MDA-MB-436 BRCA1-def (lanes 4-6) cells. **b**, HCC1937 BRCA1-def (lanes 1-2) and SUM149PT BRCA1def (lanes 3-4) cells. **c**, HCC1937 BRCA1-def cells. **d**, MDA-MB-436 BRCA1-def (left), HCC1937 BRCA1-def (Middle) and SUM149PT BRCA1-def (right) cells. **e**, MDA-MB-436 BRCA1+ cells. **f**, MDA-MB-436 BRCA1-def (lanes 1-2), HCC1937 BRCA1-def (lanes 3-4) and SUM149PT BRCA1-def (lanes 5-8). BiP = GRP78. CypB = cyclophilin B. DERL1 = DERLIN-1.

Results were calculated as means $\pm$ SD. P value was calculated by student's t-test. (*** < 0.001).

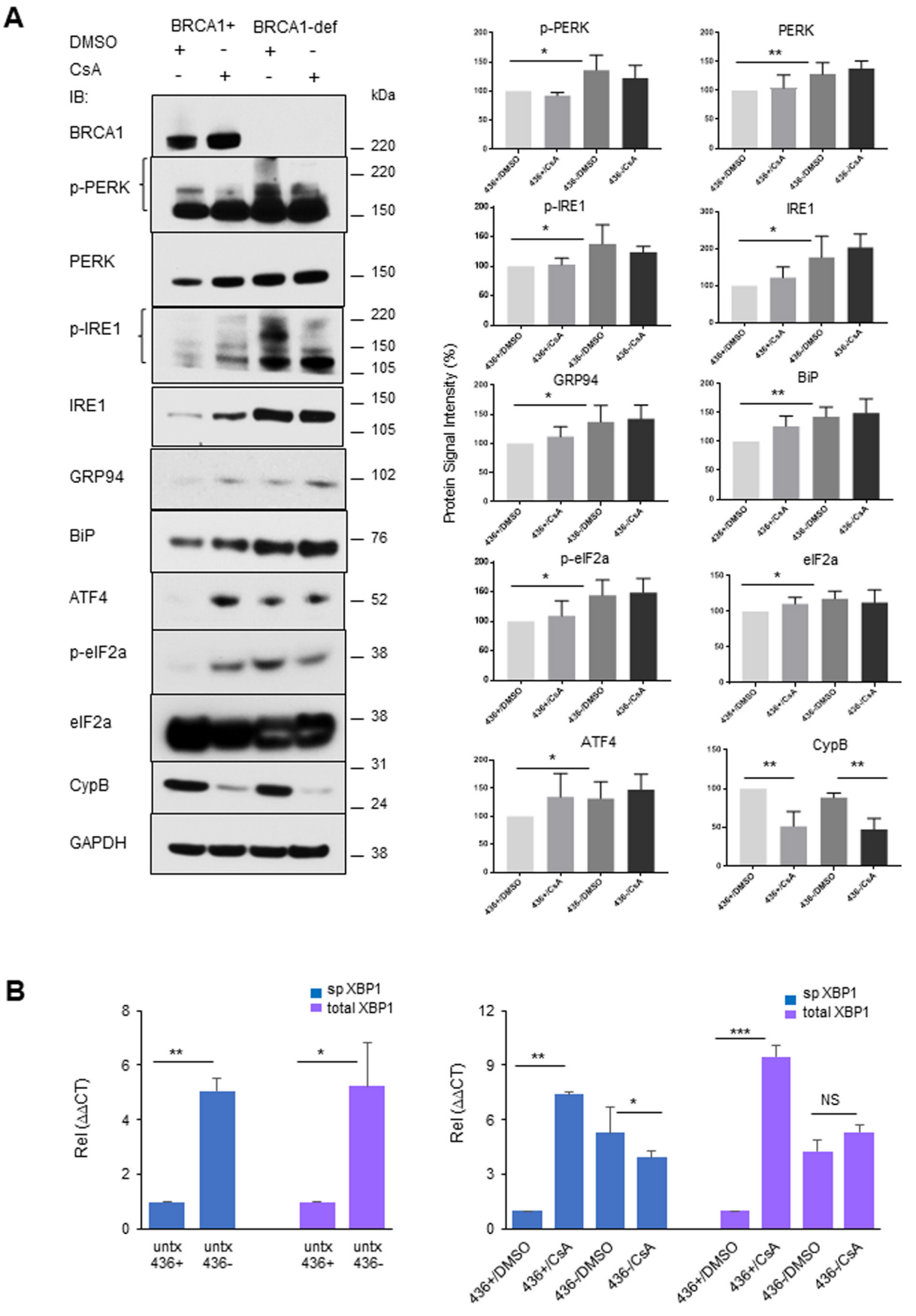

A
BRCA1+
BRCA1-def
DMSO
CsA
IB:
kDa
BRCA1
p-PERK
PERK
p-IRE1
IRE1
GRP94
BiP
ATF4
p-eIF2a
eIF2a
CypB
GAPDH
220
220
150
150
220
150
105
150
105
102
76
52
38
38
31
24
38
Protein Signal Intensity (%)
p-PERK
PERK
p-IRE1
IRE1
GRP94
BiP
p-eIF2a
eIF2a
ATF4
CypB
436+/DMSO
436+/CsA
436-/DMSO
436-/CsA
B
sp XBP1
total XBP1
Rel (ΔΔCT)
untx 436+
untx 436-
NS

Figure S9

Figure S8. Analysis of UPR components protein level in untreated, DMSO-treated or CsA-treated MDA-MB-436 breast cancer cells, Related to Figure 5.

BRCA1-replete (+) and BRCA1-deficient (def) breast cancer cells were either untreated, treated with DMSO (vehicle) or treated with 1 μM CsA for 2 days. Cells were harvested for (A) protein or (B) mRNAs analysis. p-PERK = phospho-PERK. p-IRE1 = phospho-IRE1. p-eIF2a = phospho-eIF2a. sp = spliced XBP1. IB = immunoblotting.

Results were calculated as means ± SD. P value was calculated by student's t-test.