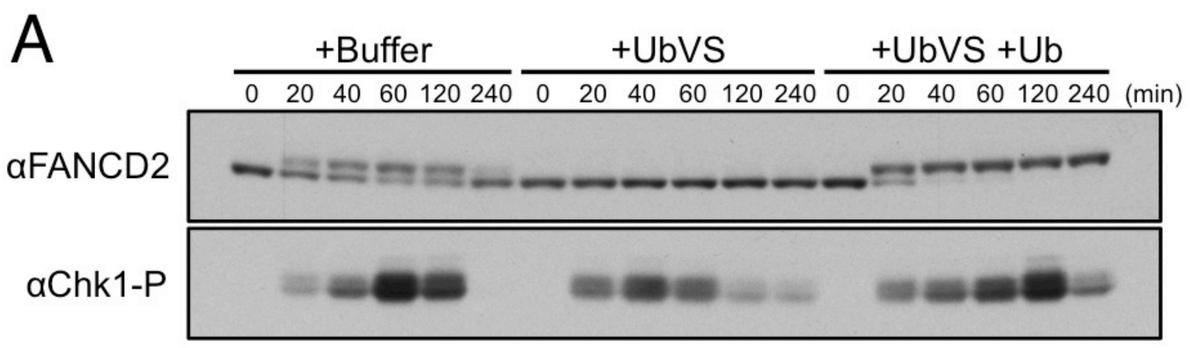
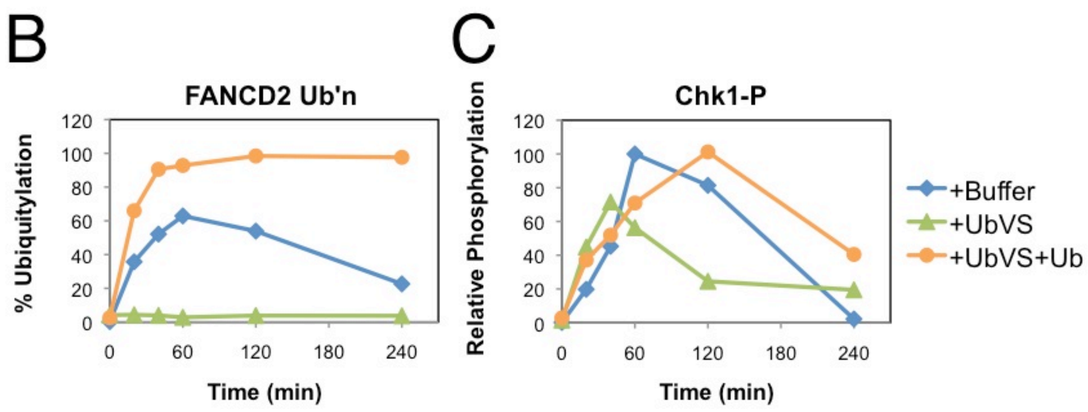
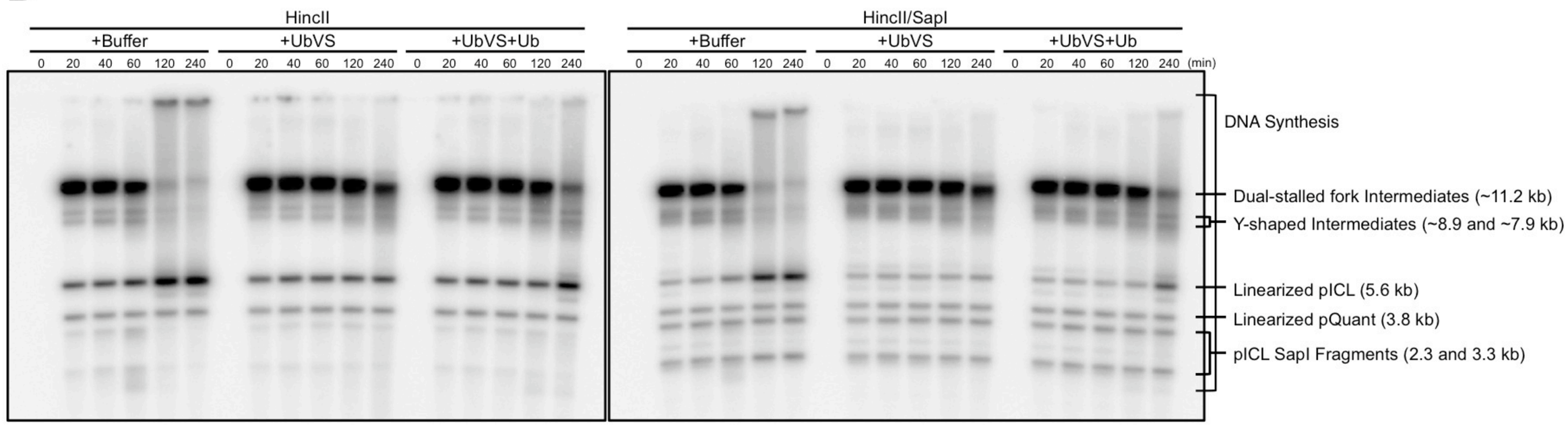
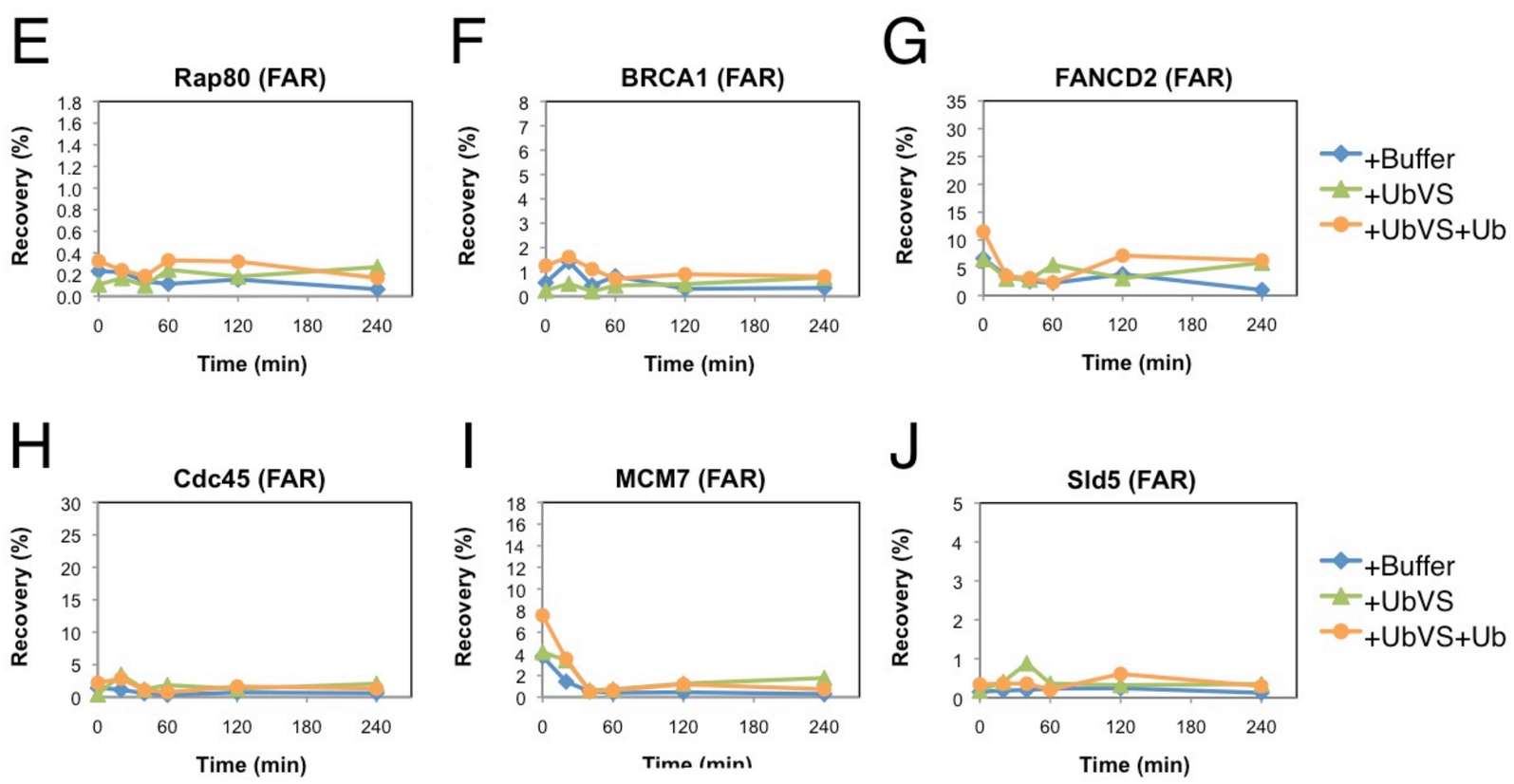
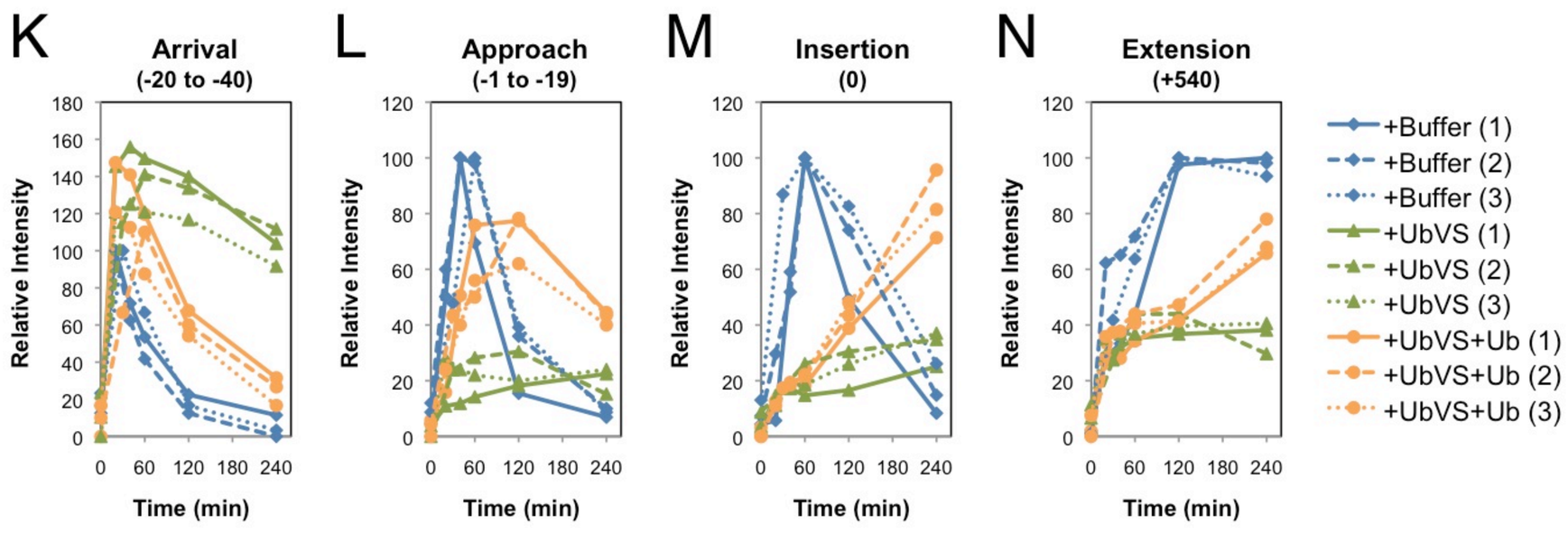
Figure S1


A
+Buffer
+UbVS
+UbVS +Ub
0 20 40 60 120 240 0 20 40 60 120 240 0 20 40 60 120 240 (min)
αFANCD2
αChk1-P


B
FANCD2 Ub'n
% Ubiquitylation
Time (min)
C
Chk1-P
Relative Phosphorylation
Time (min)
+Buffer
+UbVS
+UbVS+Ub


D
HincII
HincII/SapI
+Buffer
+UbVS
+UbVS+Ub
0 20 40 60 120 240 (min)
DNA Synthesis
Dual-stalled fork Intermediates (~11.2 kb)
Y-shaped Intermediates (~8.9 and ~7.9 kb)
Linearized pICL (5.6 kb)
Linearized pQuant (3.8 kb)
pICL SapI Fragments (2.3 and 3.3 kb)


E
Rap80 (FAR)
F
BRCA1 (FAR)
G
FANCD2 (FAR)
Recovery (%)
Time (min)
+Buffer
+UbVS
+UbVS+Ub
H
Cdc45 (FAR)
I
MCM7 (FAR)
J
Sld5 (FAR)


K
Arrival
(-20 to -40)
L
Approach
(-1 to -19)
M
Insertion
(0)
N
Extension
(+540)
Relative Intensity
Time (min)
+Buffer (1)
+Buffer (2)
+Buffer (3)
+UbVS (1)
+UbVS (2)
+UbVS (3)
+UbVS+Ub (1)
+UbVS+Ub (2)
+UbVS+Ub (3)

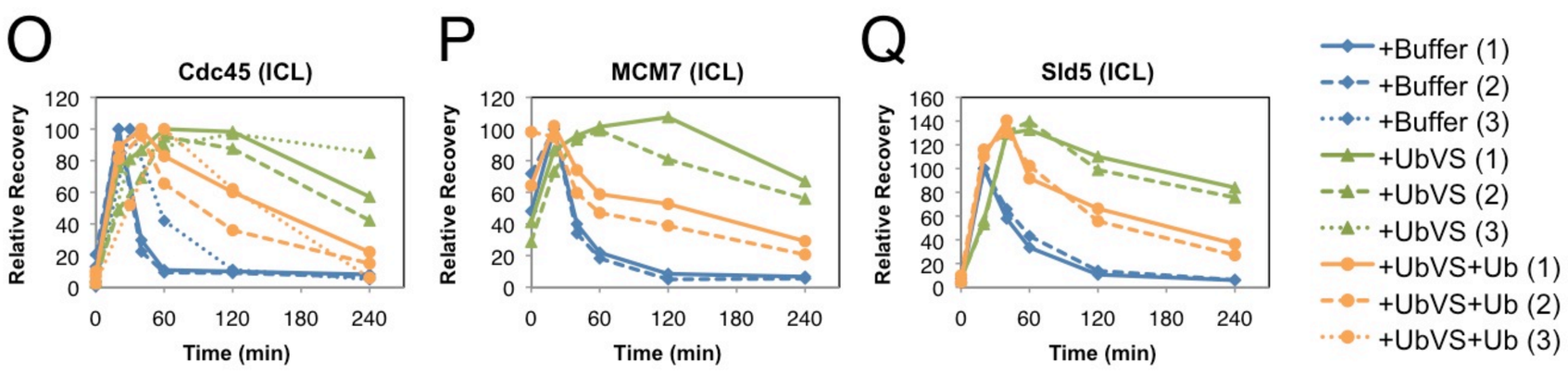
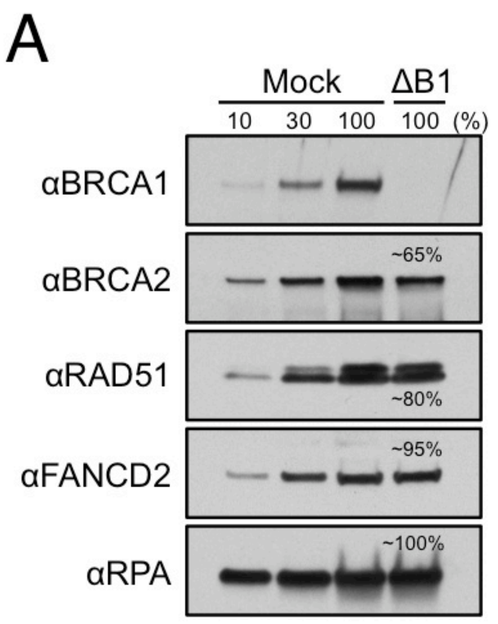
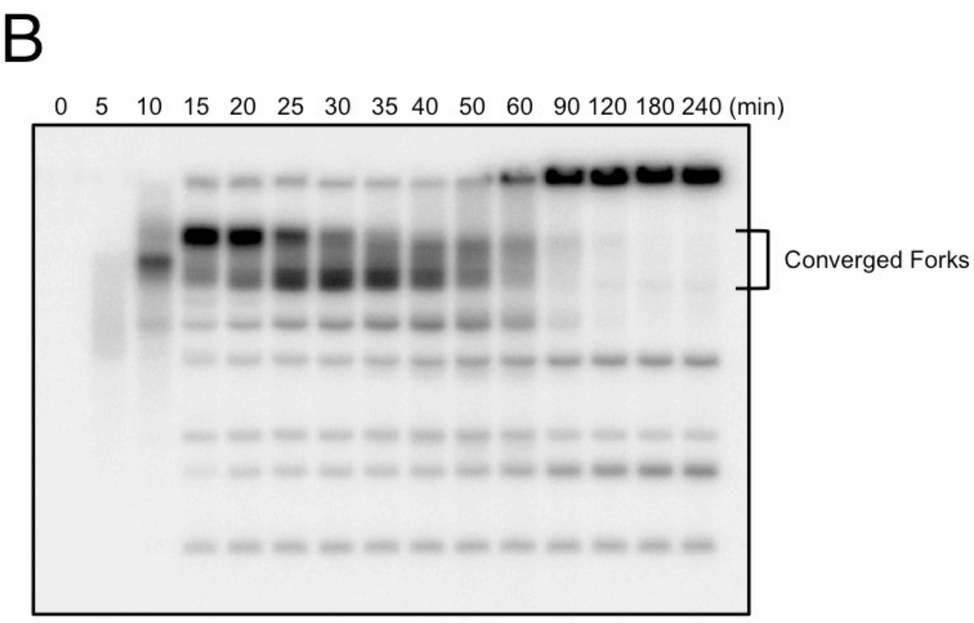
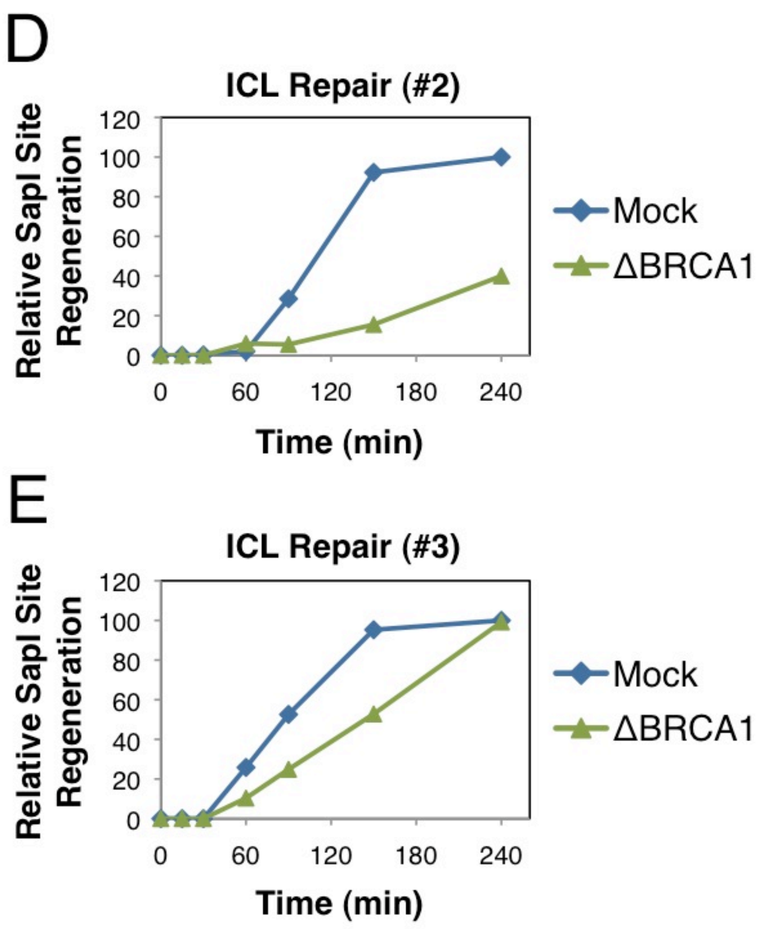
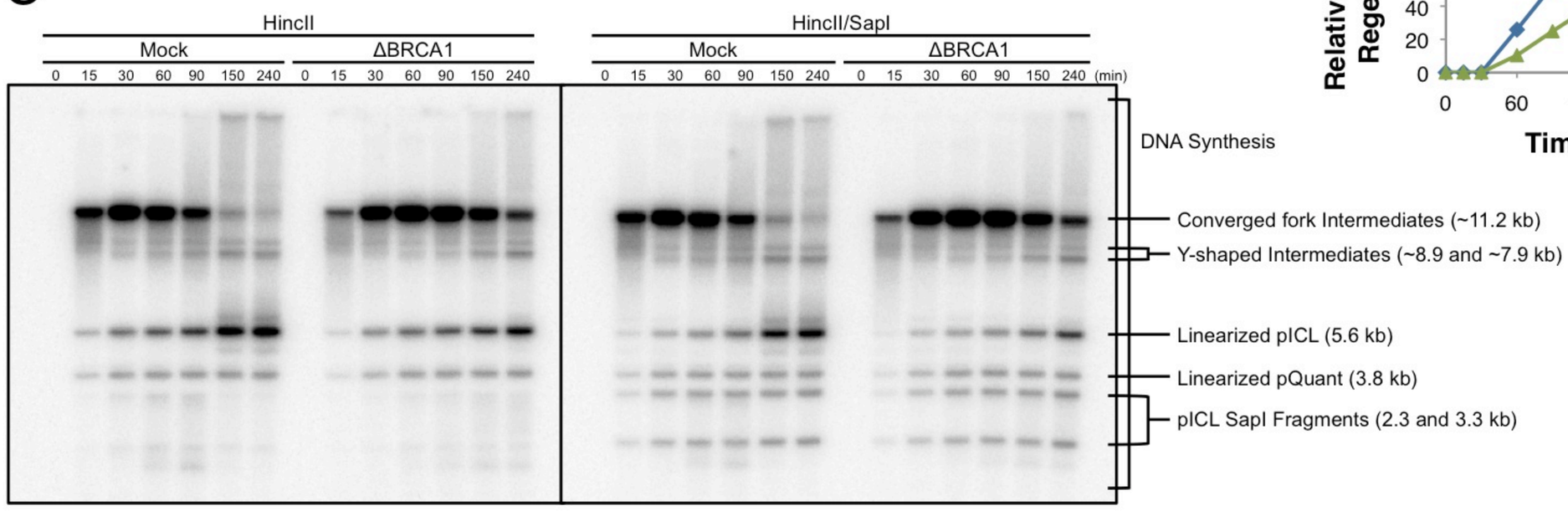

O
Cdc45 (ICL)
P
MCM7 (ICL)
Q
Sld5 (ICL)
Relative Recovery
Time (min)
+Buffer (1)
+Buffer (2)
+Buffer (3)
+UbVS (1)
+UbVS (2)
+UbVS (3)
+UbVS+Ub (1)
+UbVS+Ub (2)
+UbVS+Ub (3)

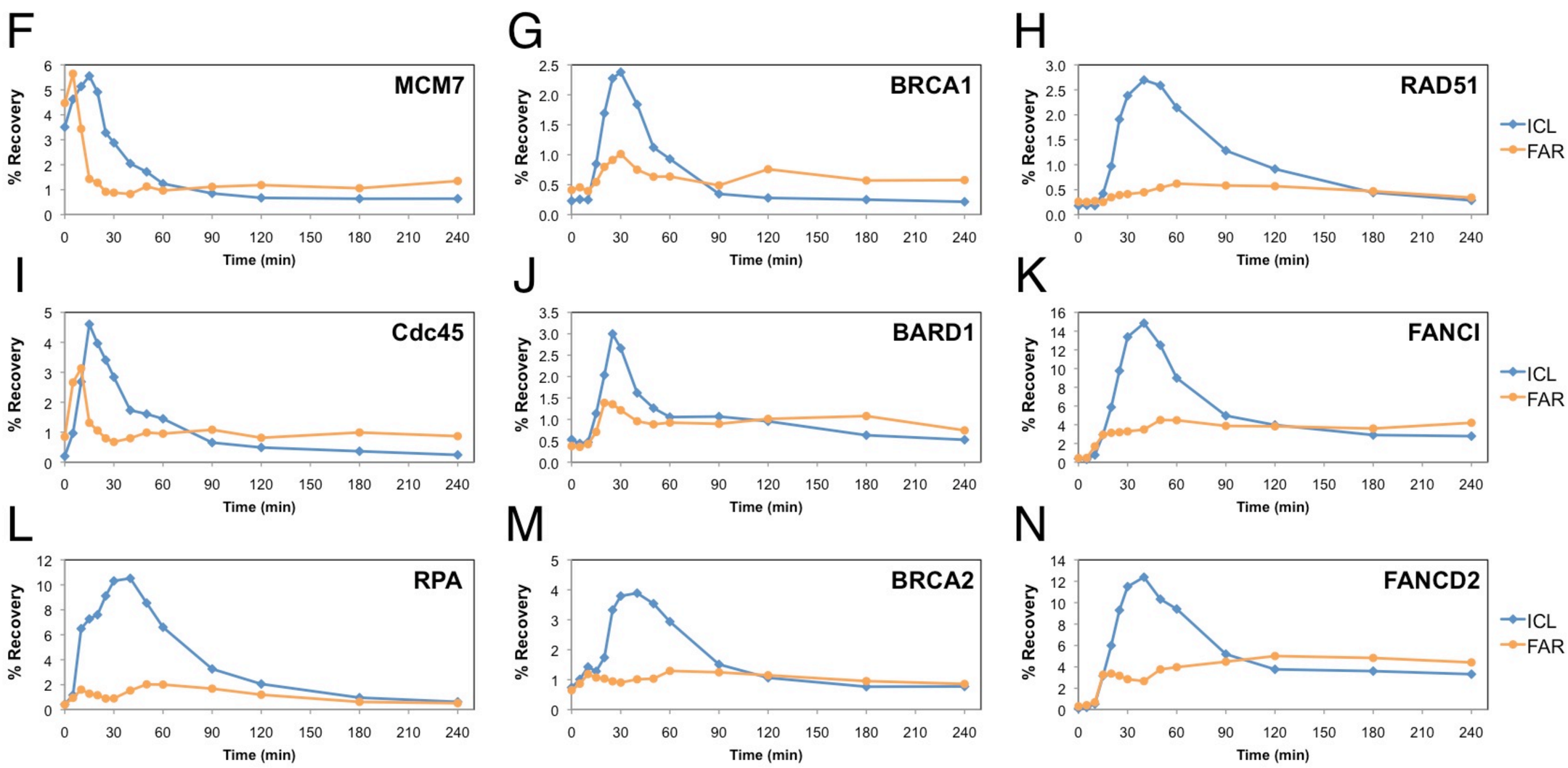
Figure S2


A
Mock
ΔB1
10 30 100 100 (%)
αBRCA1
αBRCA2
~65%
αRAD51
~80%
αFANCD2
~95%
~100%
αRPA


B
0 5 10 15 20 25 30 35 40 50 60 90 120 180 240 (min)
Converged Forks


D
ICL Repair (#2)
Relative SapI Site Regeneration
Time (min)
Mock
ΔBRCA1
E
ICL Repair (#3)
Relative SapI Site Regeneration
Time (min)
Mock
ΔBRCA1


C
HincII
HincII/SapI
Mock
ΔBRCA1
0 15 30 60 90 150 240 (min)
DNA Synthesis
Converged fork Intermediates (~11.2 kb)
Y-shaped Intermediates (~8.9 and ~7.9 kb)
Linearized pICL (5.6 kb)
Linearized pQuant (3.8 kb)
pICL SapI Fragments (2.3 and 3.3 kb)


F
MCM7
G
BRCA1
H
RAD51
% Recovery
Time (min)
ICL
FAR
I
Cdc45
J
BARD1
K
FANCI
L
RPA
M
BRCA2
N
FANCD2

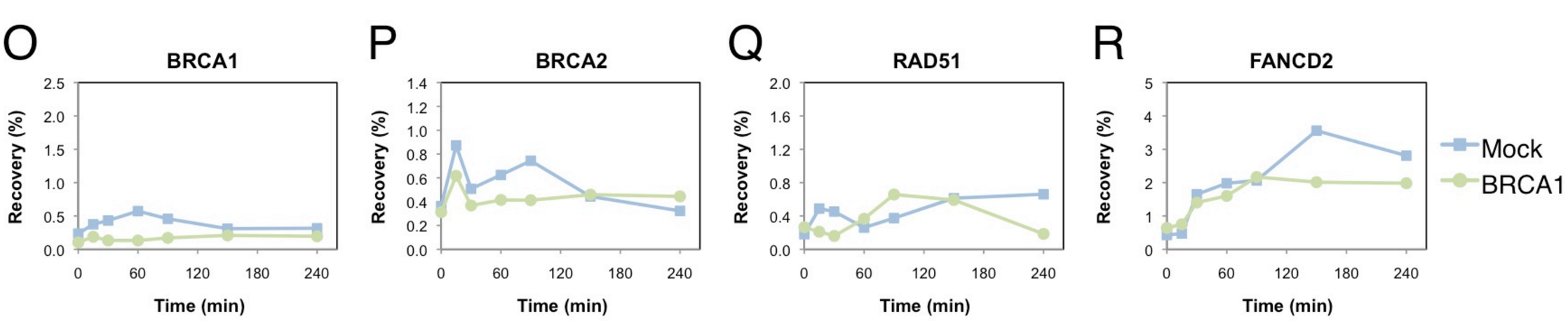
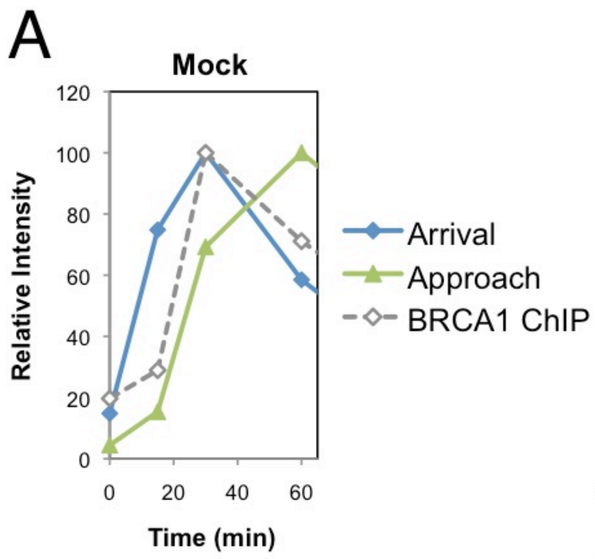
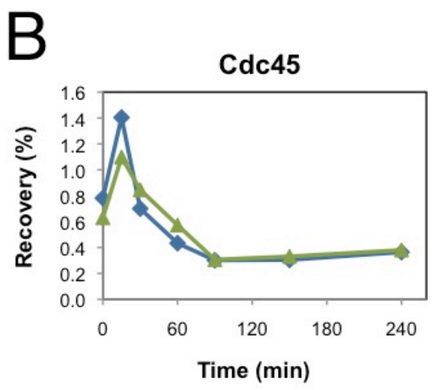
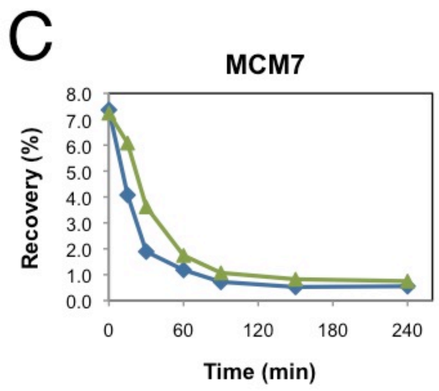
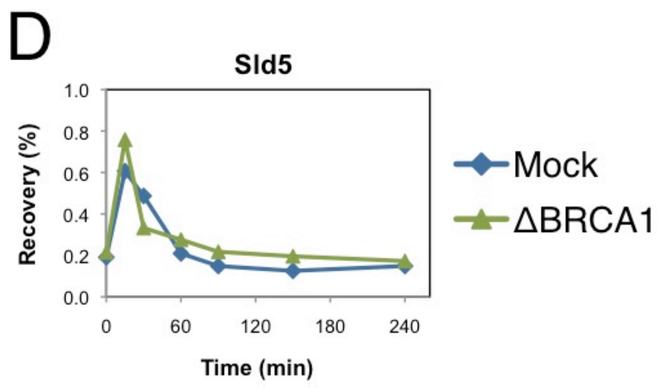

O
BRCA1
P
BRCA2
Q
RAD51
R
FANCD2
Recovery (%)
Time (min)
Mock
BRCA1

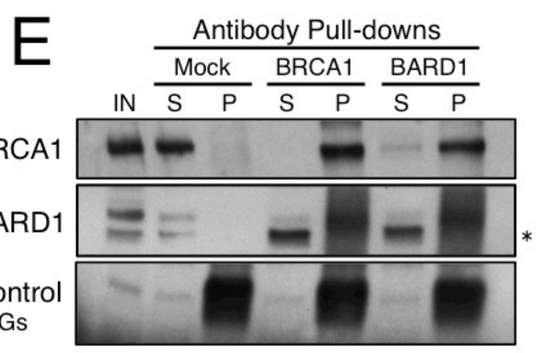
Figure S3

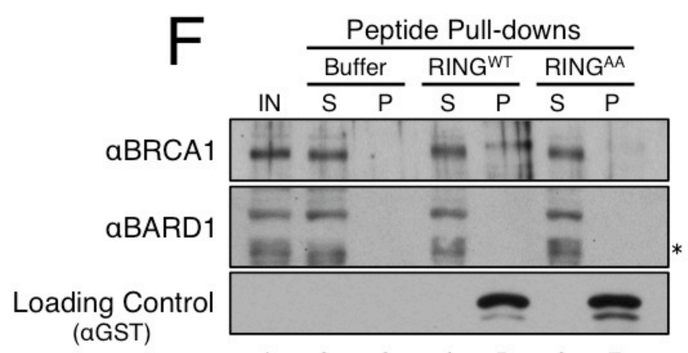

A
Mock
Relative Intensity
Time (min)
Arrival
Approach
BRCA1 ChIP

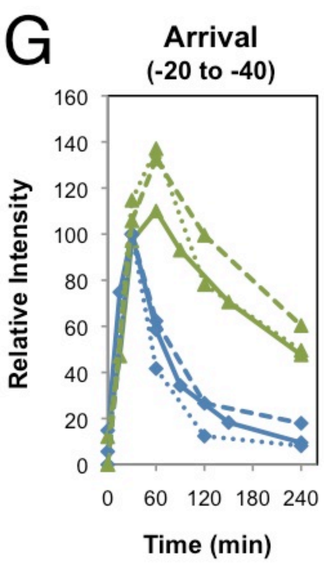

B
Cdc45
Recovery (%)
Time (min)

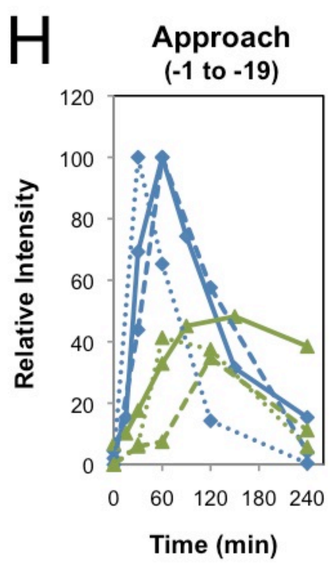

C
MCM7
Recovery (%)
Time (min)

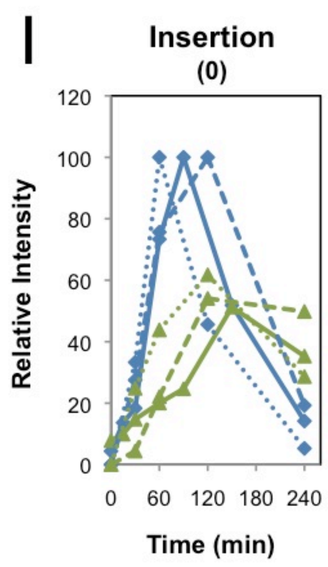

D
Sld5
Recovery (%)
Time (min)
Mock
ΔBRCA1

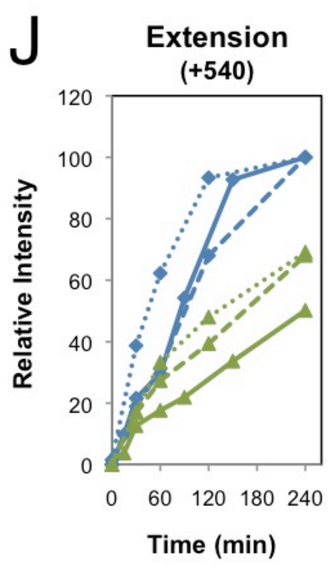

E
Antibody Pull-downs
Mock
BRCA1
BARD1
IN
S
P
αBRCA1
αBARD1
Loading Control
αRabbit IgGs
*
1 2 3 4 5 6 7

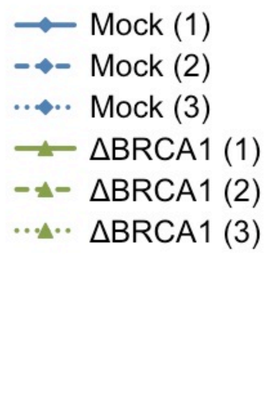

F
Peptide Pull-downs
Buffer
RING^WT
RING^AA
IN
S
P
αBRCA1
αBARD1
Loading Control
(αGST)
*
1 2 3 4 5 6 7

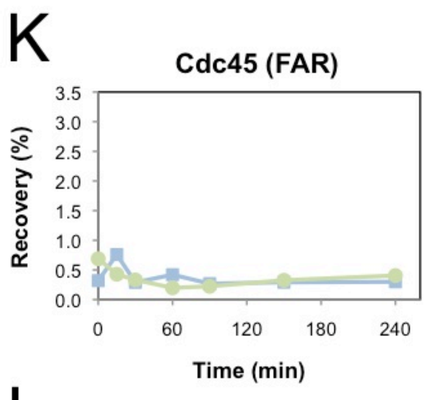

G
Arrival
(-20 to -40)
Relative Intensity
Time (min)

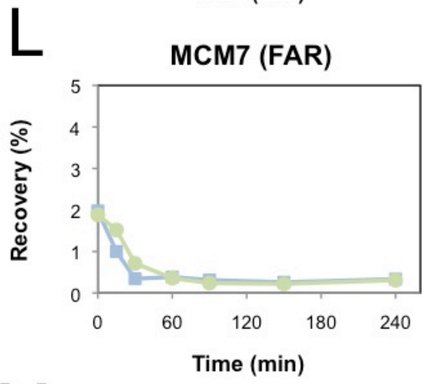

H
Approach
(-1 to -19)
Relative Intensity
Time (min)

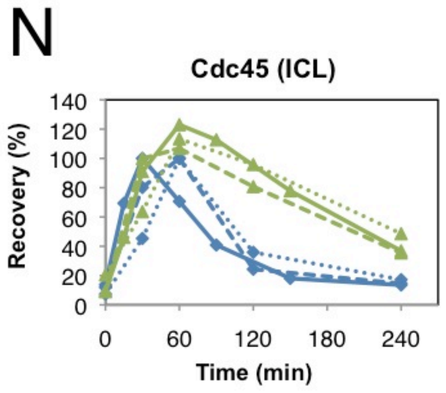

I
Insertion
(0)
Relative Intensity
Time (min)

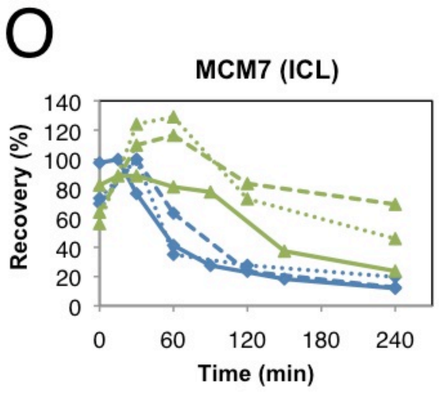

J
Extension
(+540)
Relative Intensity
Time (min)

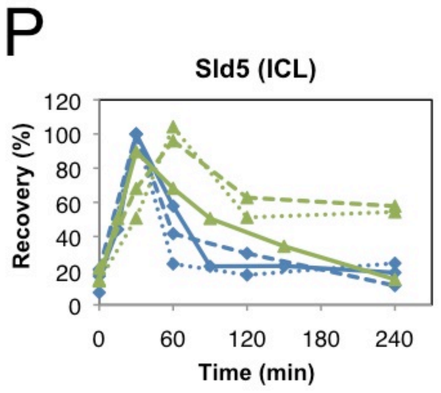

Mock (1)
Mock (2)
Mock (3)
ΔBRCA1 (1)
ΔBRCA1 (2)
ΔBRCA1 (3)

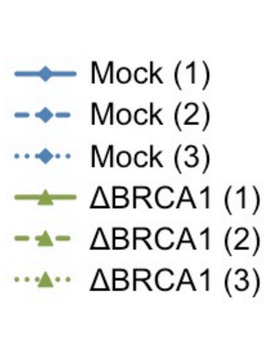

K
Cdc45 (FAR)
Recovery (%)
Time (min)
Mock
ΔBRCA1


L
MCM7 (FAR)
Recovery (%)
Time (min)
Mock
ΔBRCA1


N
Cdc45 (ICL)
Recovery (%)
Time (min)


O
MCM7 (ICL)
Recovery (%)
Time (min)


P
Sld5 (ICL)
Recovery (%)
Time (min)


Mock (1)
Mock (2)
Mock (3)
ΔBRCA1 (1)
ΔBRCA1 (2)
ΔBRCA1 (3)

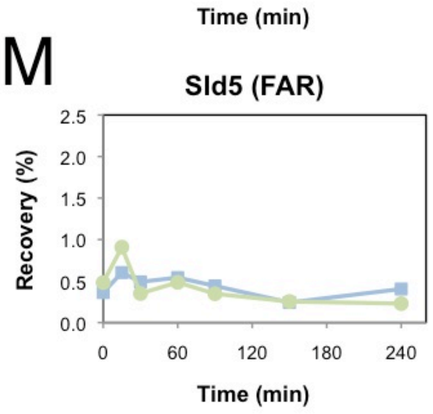
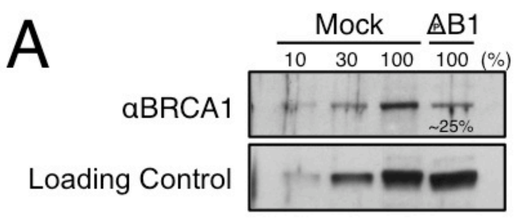
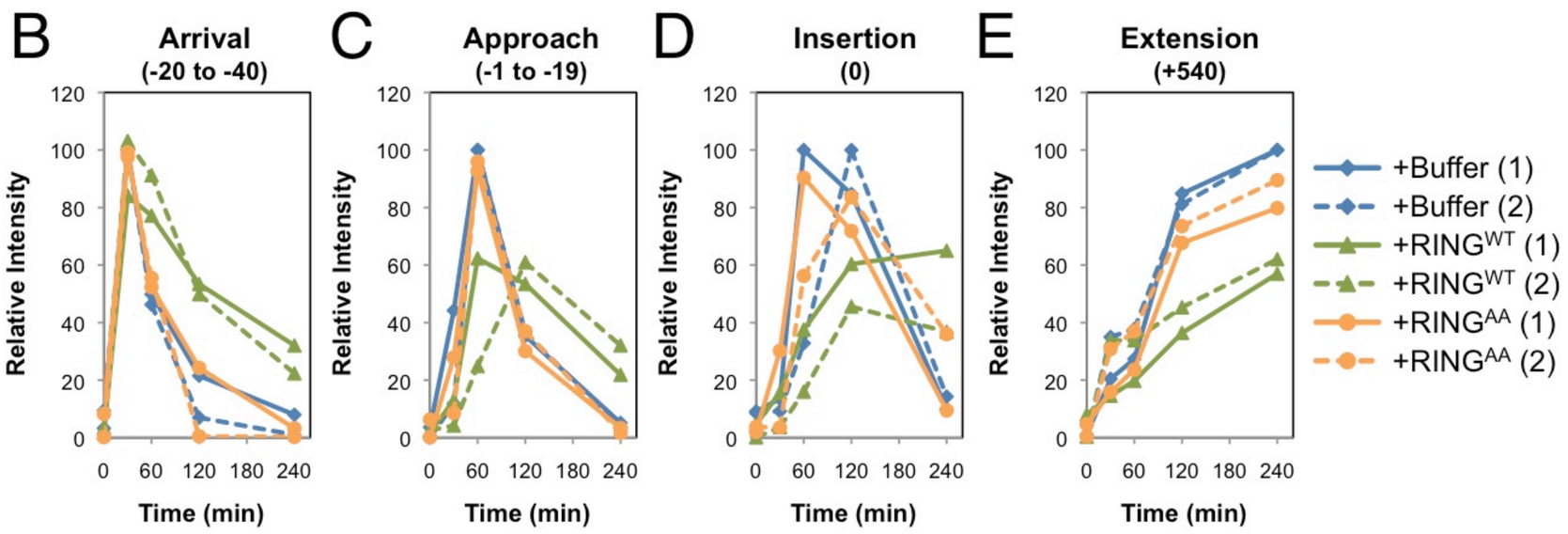
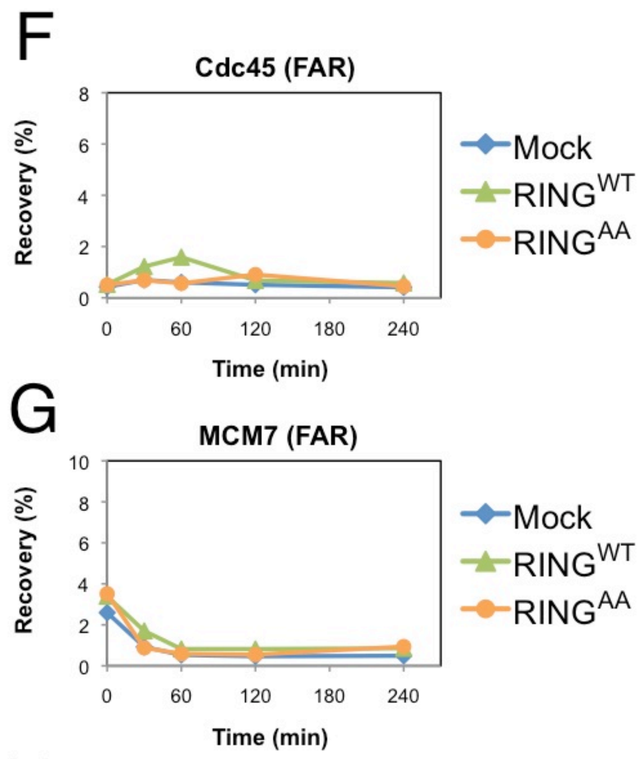
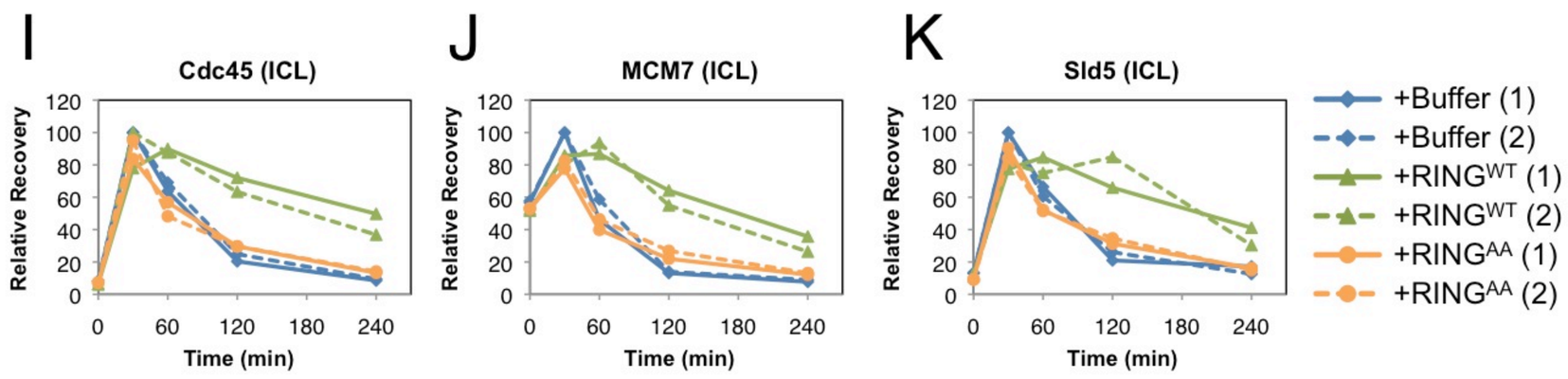

M
Sld5 (FAR)
Recovery (%)
Time (min)
Mock
ΔBRCA1

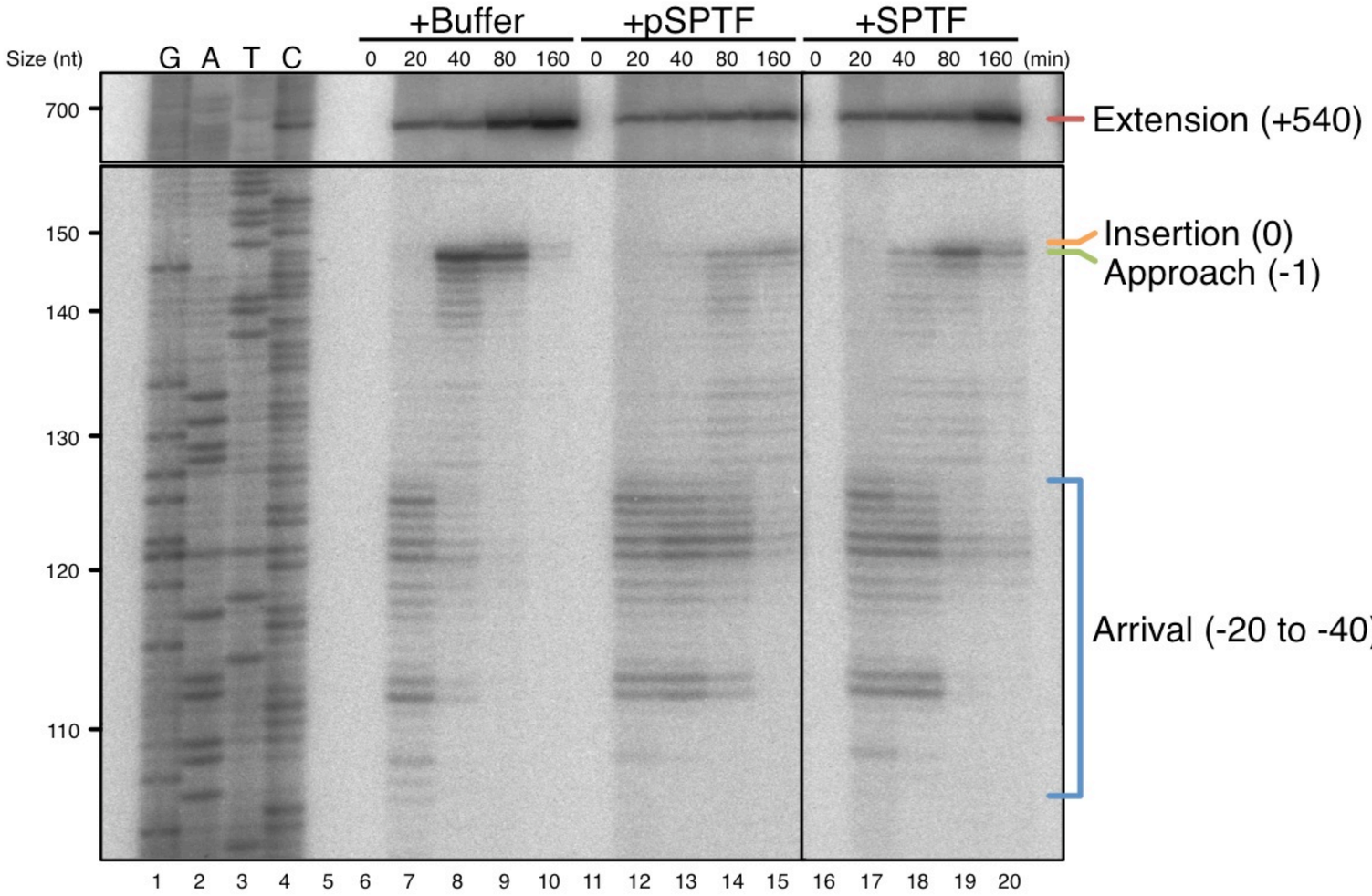
Figure S4

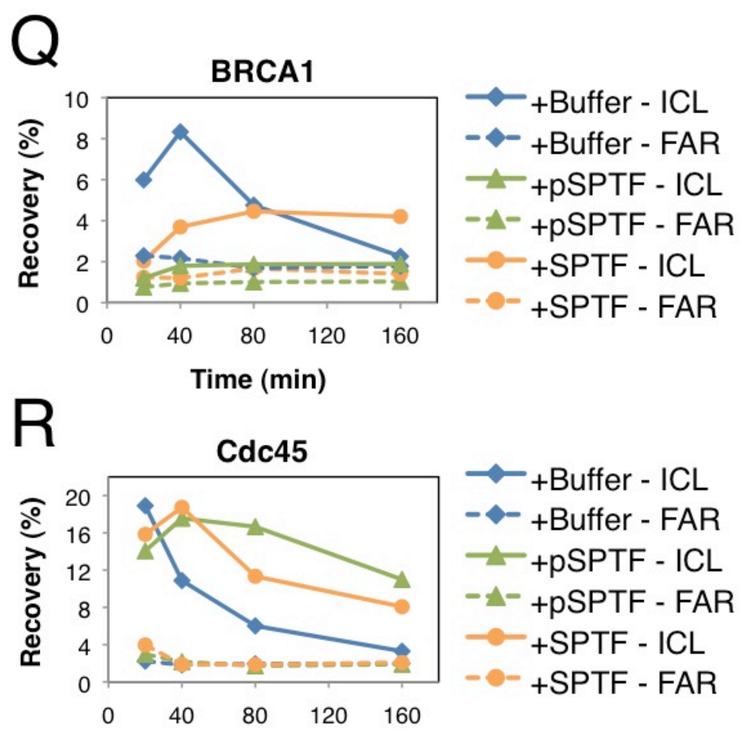

A
Mock
ΔB1
10 30 100 100 (%)
αBRCA1
~25%
Loading Control


B
Arrival
(-20 to -40)
C
Approach
(-1 to -19)
D
Insertion
(0)
E
Extension
(+540)
Relative Intensity
Time (min)
+Buffer (1)
+Buffer (2)
+RINGWT (1)
+RINGWT (2)
+RINGAA (1)
+RINGAA (2)


F
Cdc45 (FAR)
G
MCM7 (FAR)
H
Sld5 (FAR)
Recovery (%)
Time (min)
Mock
RINGWT
RINGAA


I
Cdc45 (ICL)
J
MCM7 (ICL)
K
Sld5 (ICL)
Relative Recovery
Time (min)
+Buffer (1)
+Buffer (2)
+RINGWT (1)
+RINGWT (2)
+RINGAA (1)
+RINGAA (2)


L
+Buffer
+pSPTF
+SPTF
Size (nt)
G A T C
0 20 40 80 160 0 20 40 80 160 0 20 40 80 160 (min)
700
150
140
130
120
110
Extension (+540)
Insertion (0)
Approach (-1)
Arrival (-20 to -40)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20


Q
BRCA1
R
Cdc45
S
MCM7
Recovery (%)
Time (min)
+Buffer - ICL
+Buffer - FAR
+pSPTF - ICL
+pSPTF - FAR
+SPTF - ICL
+SPTF - FAR

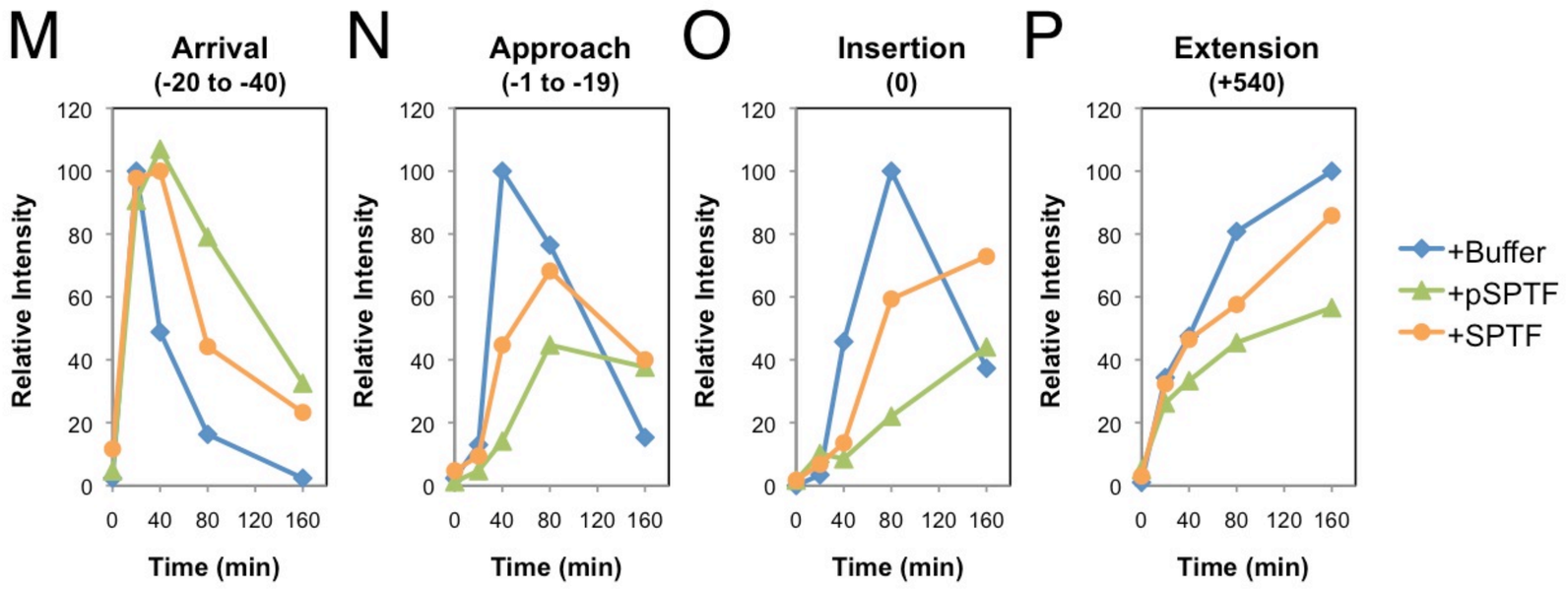
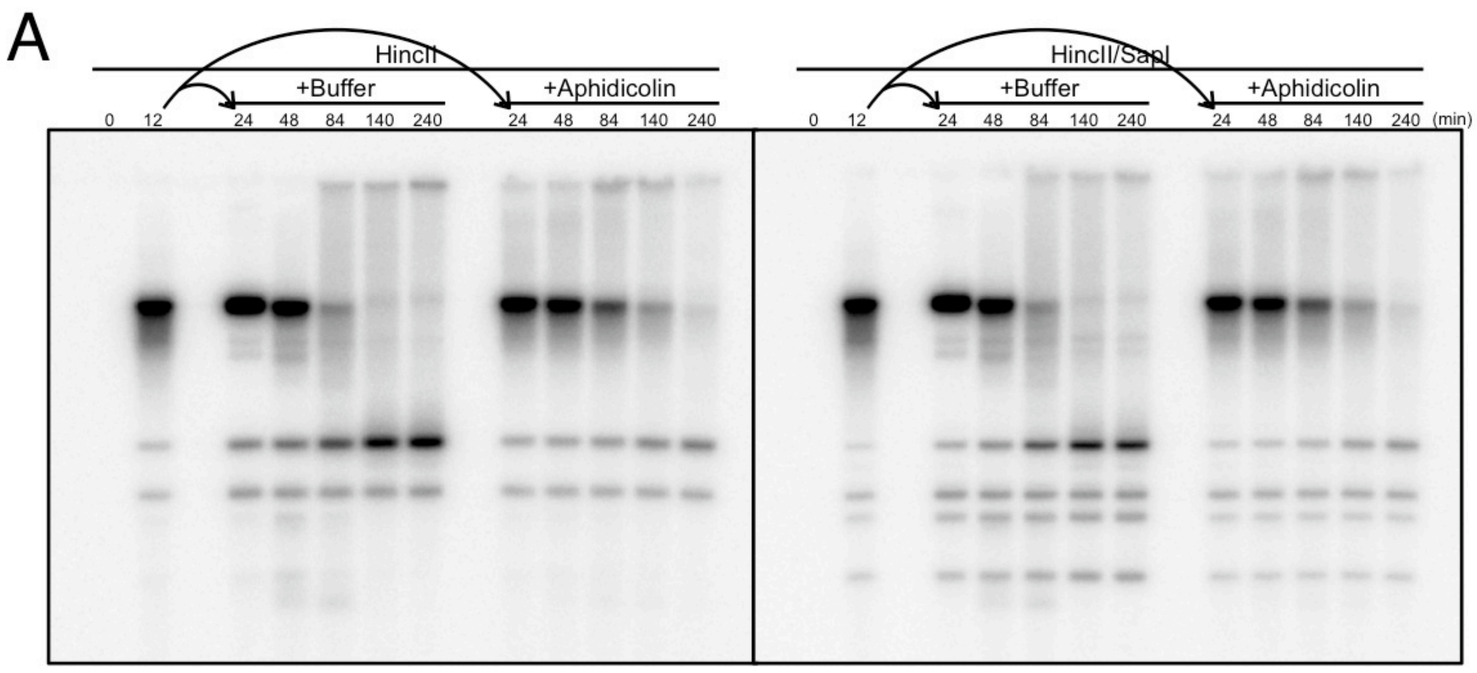
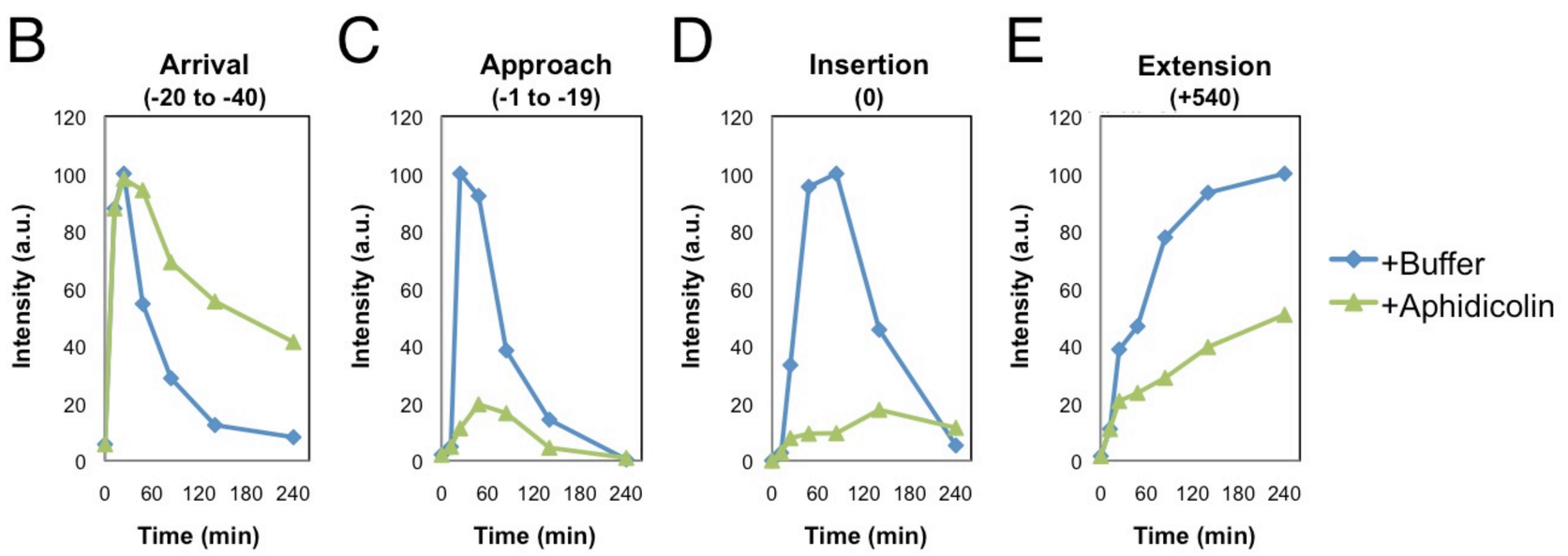
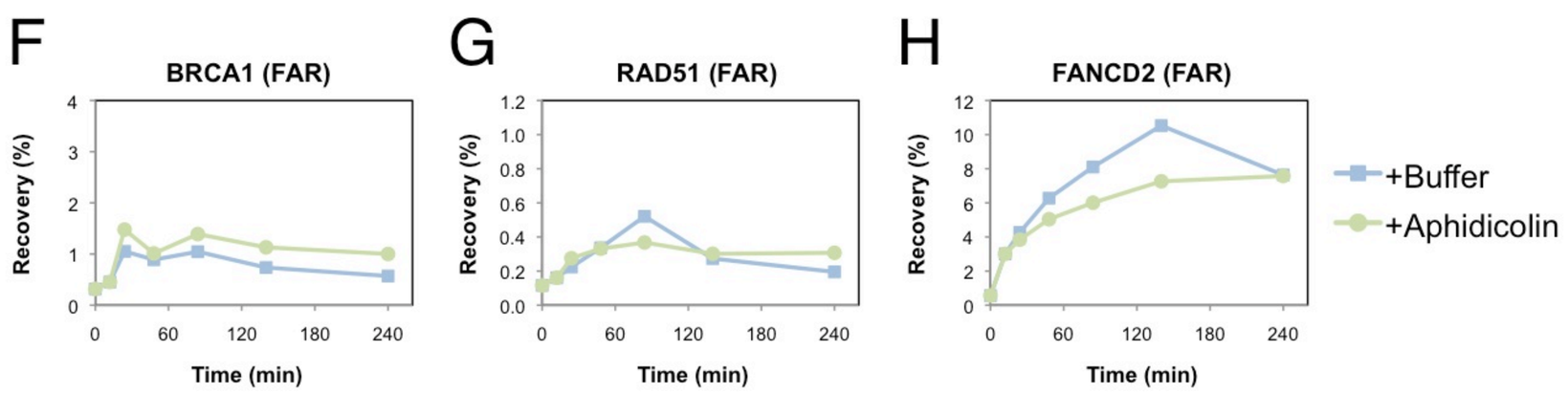
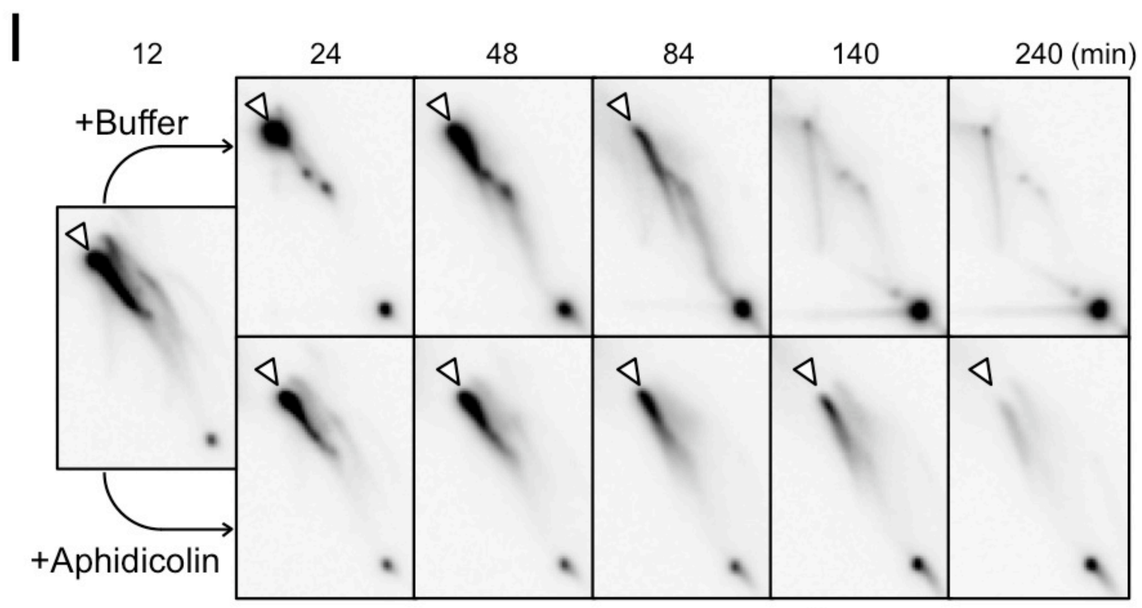

M
Arrival
(-20 to -40)
N
Approach
(-1 to -19)
O
Insertion
(0)
P
Extension
(+540)
Relative Intensity
Time (min)
+Buffer
+pSPTF
+SPTF

Figure S5


A
HincII
+Buffer
+Aphidicolin
0 12 24 48 84 140 240 24 48 84 140 240
HincII/SapI
+Buffer
+Aphidicolin
0 12 24 48 84 140 240 24 48 84 140 240 (min)


B
Arrival
(-20 to -40)
C
Approach
(-1 to -19)
D
Insertion
(0)
E
Extension
(+540)
Intensity (a.u.)
Time (min)
+Buffer
+Aphidicolin


F
BRCA1 (FAR)
G
RAD51 (FAR)
H
FANCD2 (FAR)
Recovery (%)
Time (min)
+Buffer
+Aphidicolin


I
12 24 48 84 140 240 (min)
+Buffer
+Aphidicolin
Converged Forks

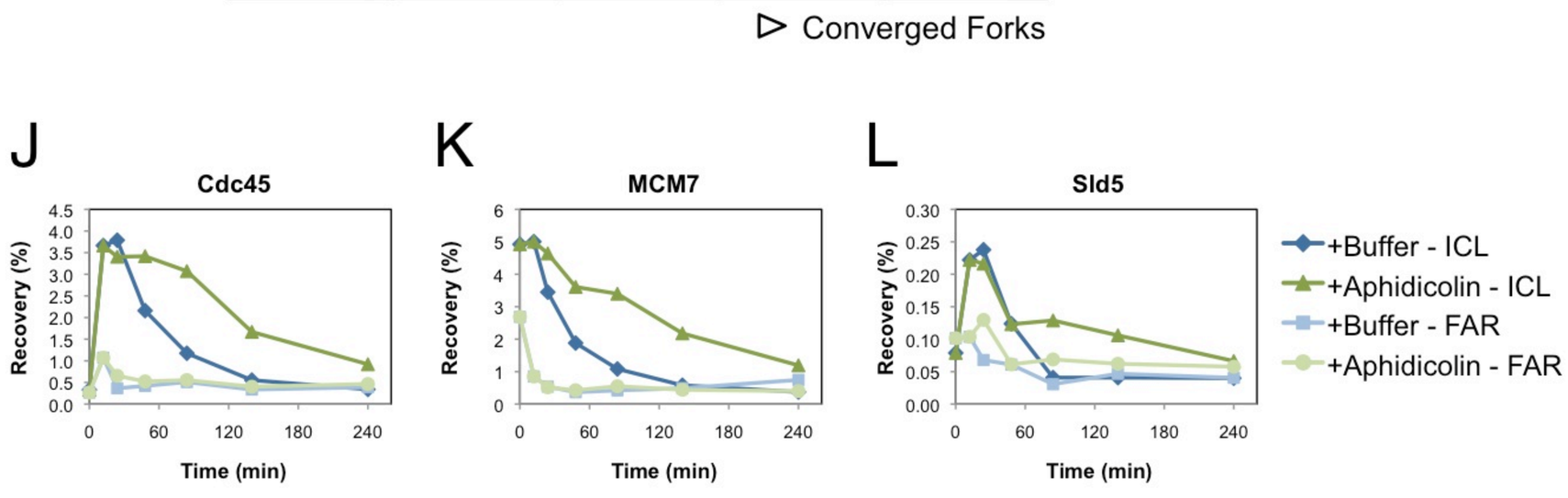
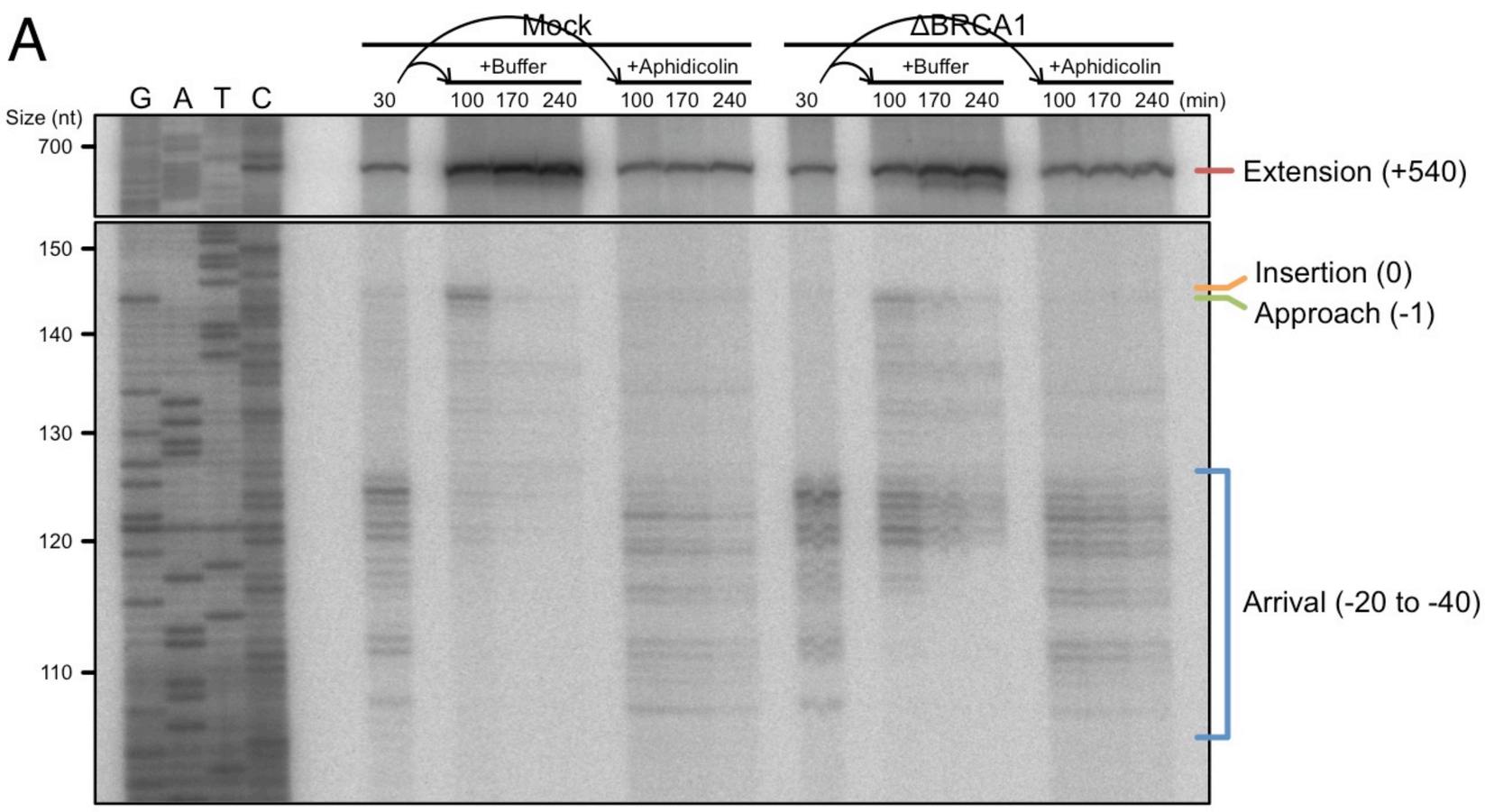

J
Cdc45
K
MCM7
L
Sld5
Recovery (%)
Time (min)
+Buffer - ICL
+Aphidicolin - ICL
+Buffer - FAR
+Aphidicolin - FAR

Figure S6


A
Mock
ΔBRCA1
+Buffer
+Aphidicolin
G A T C
30 100 170 240 100 170 240 (min)
Size (nt)
700
150
140
130
120
110
Extension (+540)
Insertion (0)
Approach (-1)
Arrival (-20 to -40)

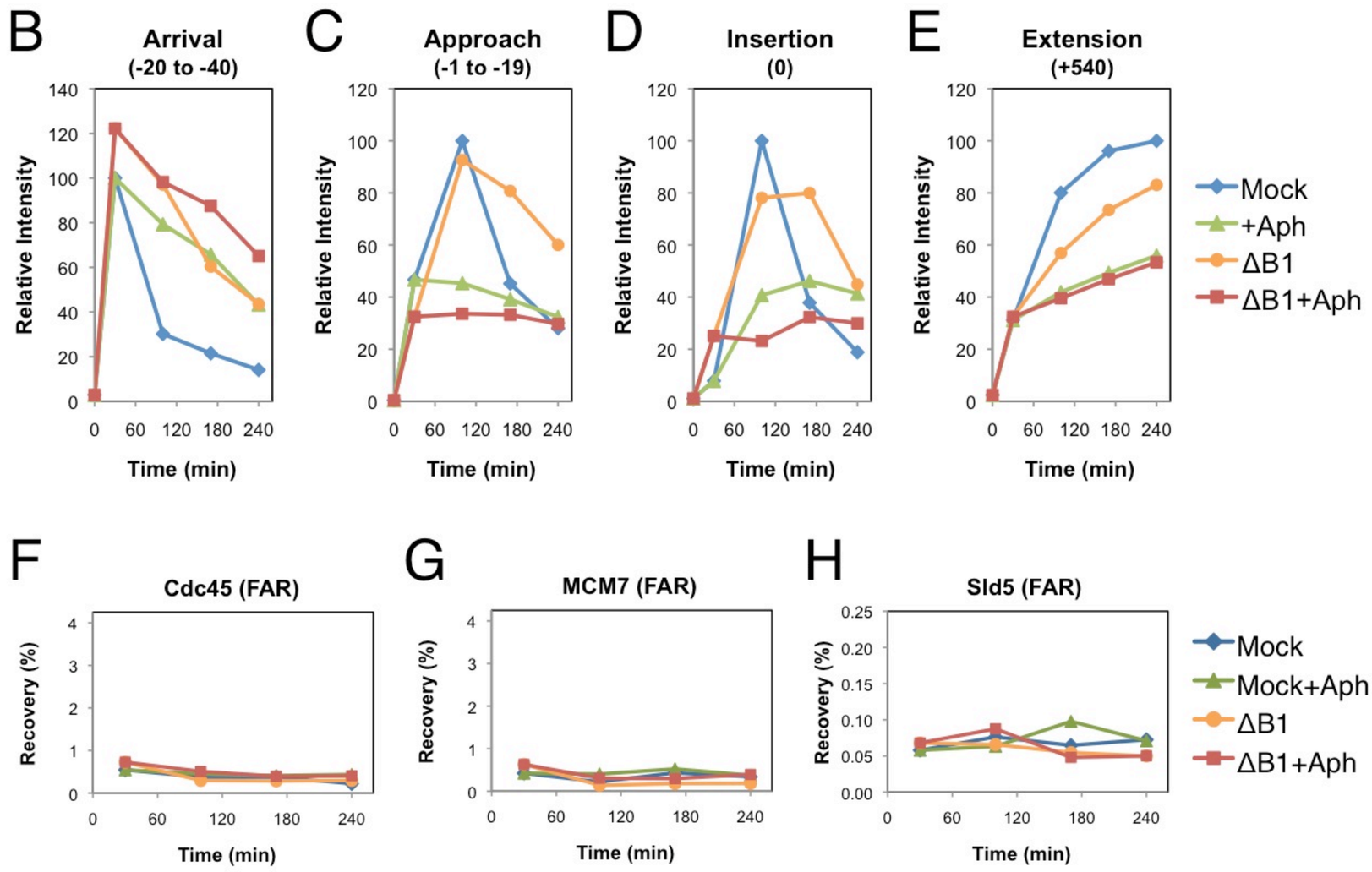
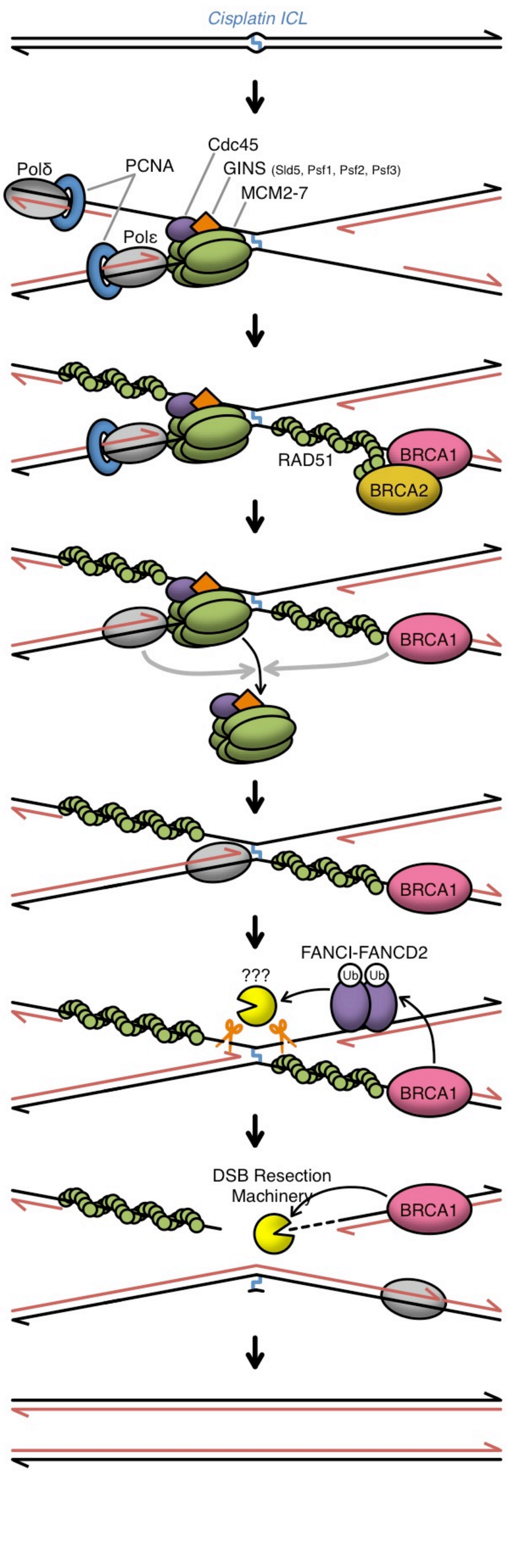

B
Arrival
(-20 to -40)
C
Approach
(-1 to -19)
D
Insertion
(0)
E
Extension
(+540)
Relative Intensity
Time (min)
Mock
+Aph
ΔB1
ΔB1+Aph
F
Cdc45 (FAR)
G
MCM7 (FAR)
H
Sld5 (FAR)
Recovery (%)
Mock
Mock+Aph
ΔB1
ΔB1+Aph

Figure S7



i. Fork Convergence

Replication forks converge on the ICL with leading strands stalling ~20 nucleotides from the crosslink due to the presence of the CMG replicative helicase complex.

ii. RAD51 Loading

Ubiquitin signaling promotes recruitment of BRCA1 and BRCA2 to ICL-stalled forks, followed by loading of RAD51 onto regions of ssDNA present at lagging strand gaps.

iii. Helicase Unloading

BRCA1 and the action of the polymerase cooperate to promote efficient unloading of the replicative helicase.

iv. Leading Strand Approach

Helicase eviction allows extension of leading strands to within 1 nucleotide of the ICL.

v. DNA Incisions

Leading strand extension creates a DNA structure that supports FANCI-FANCD2-dependent incisions by XPF-ERCC1 and other endonucleases.

vi. Lesion Bypass

Dual incisions unhook the ICL, creating a DSB in one sister chromatid and allowing lesion bypass to restore the other sister.

vii. HR and NER

Recombination repairs the DSB using the intact sister chromatid as a template for repair. The unhooked adduct is removed by excision repair.

Supplemental Figure Legends

Figure S1, related to Figure 2. (**A**) Samples from the reaction presented in Figure 2 were analyzed by Western blot with the indicated antibodies and quantified for (**B)** FANCD2 ubiquitylation and (**C**) Chk1 phosphorylation. (**D**) Primary data from which DNA Synthesis and ICL Repair was calculated for Figures 2A and B, respectively (described in *Methods*). pICL was replicated in extract supplemented with buffer (+Buffer), UbVS (+UbVS), or UbVS and ubiquitin (+UbVS+Ub) and DNA intermediates were digested with HincII or HincII and SapI. Samples were then separated by native agarose gel electrophoresis and visualized by phosphorimager. Intermediates used for quantification are indicated at right. (**E**-**J**) ChIP data from Figures 2C-E and 2H-J are shown with percent recovery values at the FAR locus. (**K**-**N**) Quantification of nascent strand products from Figure 2G (solid lines) is shown with two experimental replicates (dashed and dotted lines). Arrival (-20 to -40 position), Approach (-1 to -19), Insertion (0), Extension (+540). The abundance of each product is normalized to peak values in +Buffer samples. (**O**-**Q**) ChIP data from Figure 2H-J (solid lines) is shown with experimental replicates (dashed and dotted lines). Three replicates are shown for Cdc45 and two for MCM7 and Sld5.

Figure S2, related to Figure 3. (**A**) Western blot analysis of mock-depleted (Mock) and BRCA1-depleted (ΔB1) extracts. Relative protein levels in BRCA1-depleted extracts were determined by comparison with a dilution series of mock-depleted extract and indicated as a percentage in the figure. (**B**) Primary data from which Converged Forks accumulation was calculated for Figures 3A and B. pICL was replicated in extract supplemented with radioactive nucleotide to label nascent strands. Uncut DNA intermediates were separated by native agarose gel electrophoresis and visualized by phosphorimager. The radioactivity in the bands

encompassed by the bracket, which represent intact forks that have undergone varying degrees of resection, was quantified and graphed in Figures 3A and B. (**C**) Primary data from which DNA Synthesis and ICL Repair was calculated for Figures 3E and F, as in Figure S1. (**D** and **E**) ICL Repair data from two additional BRCA1 depletion experiments (#2 and #3, respectively). (**F**-**N**) ChIP data from Figure 3A-C are shown with percent recovery values for both ICL and FAR loci. (**O**-**R**) ChIP data from Figure 3G-J are shown with percent recovery values at the FAR locus.

Figure S3, related to Figure 5. (**A**) For the mock-depleted reaction from Figure 3, Arrival and Approach products (from *H* and *I*) were graphed relative to BRCA1 recruitment at the ICL as measured by ChIP (from Figure 3G). Mock- and BRCA1-depleted samples from Figure 3 were also used to analyze recruitment (by ChIP) of (**B**) Cdc45, (**C**) MCM7, and (**D**) Sld5 to an undamaged plasmid included in the replication reaction (pQuant, see *Methods*). (**E**) Pre-immune (Mock), BRCA1, or BARD1 antibodies were immobilized on protein A sepharose beads, then incubated with extract (input; IN). Beads were then pulled down and the supernatant (S) and pellet (P) fractions were blotted with the indicated antibodies. (**F**) Buffer, or the indicated BARD1 RING peptides were immobilized on glutathione sepharose beads, then incubated with extract, pulled down, and blotted as in *(E)*. Unlike the BRCA1 and BARD1 antibodies, which quantitatively precipitated both BRCA1 and BARD1, the $RING^{WT}$ peptide recovered only BRCA1, indicating that BARD1 was displaced from the complex. (**G**-**J**) Quantification of nascent strand products from Figure 5A (solid lines) is shown with two experimental replicates (dashed and dotted lines). The abundance of products at each time point is normalized to peak values in mock-depleted samples. (**K**-**M**) ChIP data from Figure 5B-D are shown with percent recovery values at the FAR locus. (**N**-**P**) ChIP data from Figure 5B-D (solid lines) are shown with two experimental replicates (dashed and dotted lines).

Figure S4, related to Figure 6. (**A**) Western blot analysis of mock-depleted (Mock) and partially BRCA1-depleted (ΔB1) extracts. The relative protein level was determined by comparison with a dilution series of mock-depleted extract. (**B**-**E**) Quantification of nascent strand products from Figure 6A (solid lines) is shown with two experimental replicates (dashed and dotted lines). The abundance of each product is normalized to peak values in +Buffer samples. (**F**-**H**) ChIP data from Figure 6B-D are shown with percent recovery values at the FAR locus. (**I**-**K**) ChIP data from Figure 6B-D (solid lines) are shown with an experimental replicate (dashed lines). pICL was replicated in egg extract for 18 minutes, then supplemented with buffer (+Buffer), phosphorylated SXXF peptide (+pSPTF), or non-phosphorylated SXXF peptide (+SPTF). At the indicated times, samples were then analyzed by denaturing polyacrylamide gel electrophoresis (**L; irrelevant lanes removed between lanes 15 and 16**) with strand products quantified in (**M**-**P**), and by ChIP with the indicated antibodies (**Q-S**).

Figure S5, related to Figure 7. (**A**) Primary data from which DNA replication (Figure 7B) and ICL repair (Figure 7G) were calculated. (**B**-**E**) Quantification of nascent strand products from Figure 7A. The abundance of products at each time point is normalized to peak values in +Buffer samples. (**F**-**H**) ChIP data from Figure 7C-E are shown with percent recovery values at the FAR locus. (**I**) Primary data from which Converged Forks accumulation (open arrowhead) was calculated (Figure 7F). (**J**-**L**) Samples from Figure 7A-G were analyzed by ChIP with the indicated antibodies at the ICL and FAR loci.

Figure S6, related to Figure 7. Primary data related to Figure 7H-J. (**A**) Nascent strand product formation in mock-depleted (Mock) and BRCA1-depleted (ΔBRCA1) extract supplemented with Buffer or Aphidicolin after 30 minutes. (**B**-**E**) Quantification of nascent strand products from *A*.

The abundance of products at each time point is normalized to peak values in the Mock+Buffer samples. (**F**-**H**) ChIP data showing Cdc45, MCM7, and Sld5 recovery at the FAR locus.

Figure S7, related to Figure 1. Schematic model of ICL repair in *Xenopus laevis* egg extracts. Parental DNA (black lines), nascent strands (red lines). HR (homologous recombination), DSB (double-strand break).