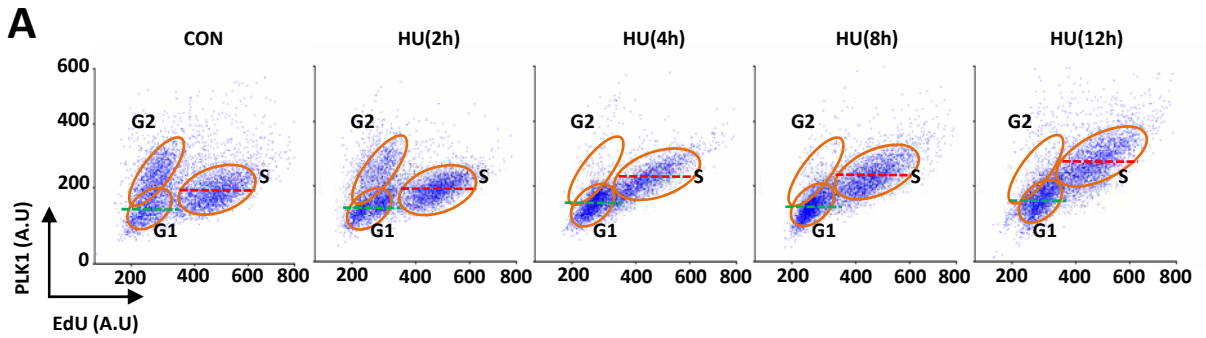
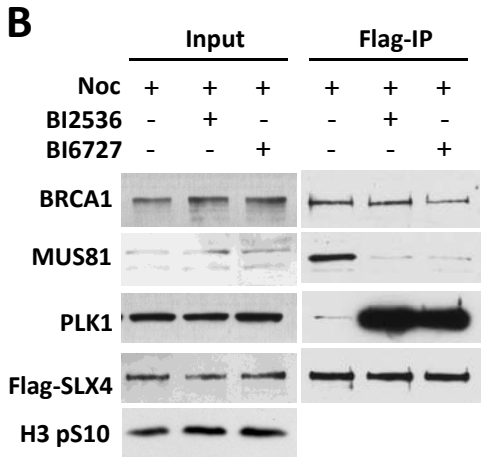
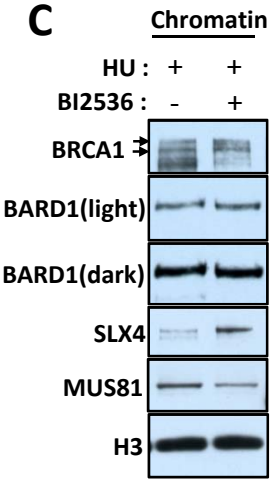
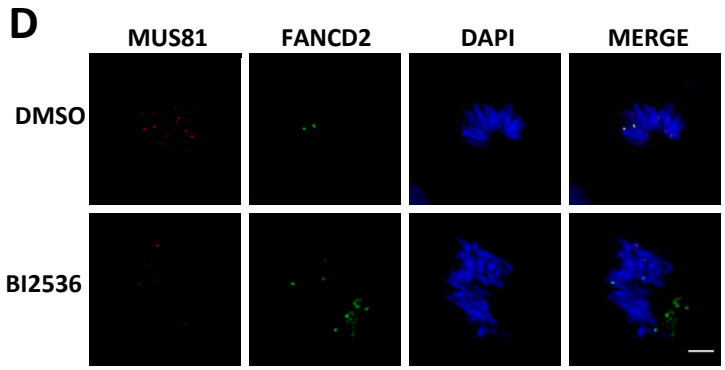
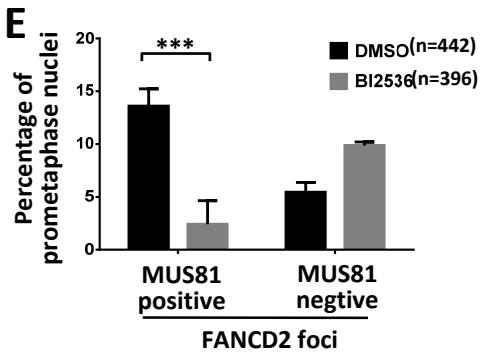
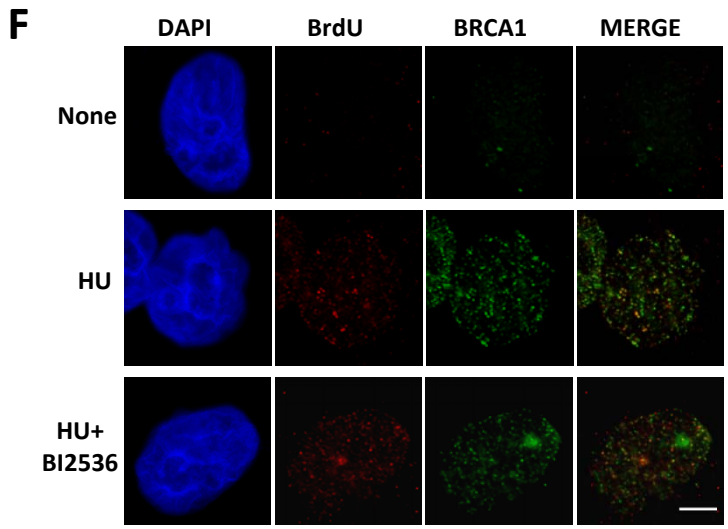

A
CON
HU(2h)
HU(4h)
HU(8h)
HU(12h)
G2
S
G1
PLK1 (A.U)
EdU (A.U)


B
Input
Flag-IP
Noc
BI2536
BI6727
BRCA1
MUS81
PLK1
Flag-SLX4
H3 pS10


C
Chromatin
HU :
BI2536 :
BRCA1
BARD1(light)
BARD1(dark)
SLX4
MUS81
H3


D
MUS81
FANCD2
DAPI
MERGE
DMSO
BI2536


E
Percentage of prometaphase nuclei
***
DMSO(n=442)
BI2536(n=396)
MUS81 positive
MUS81 negtive
FANCD2 foci


F
DAPI
BrdU
BRCA1
MERGE
None
HU
HU+ BI2536

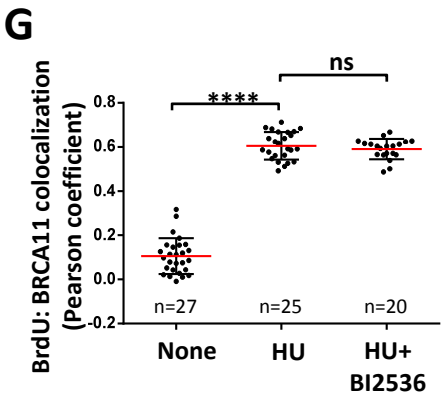

G
BrdU: BRCA11 colocalization (Pearson coefficient)
****
ns
n=27
n=25
n=20
None
HU
HU+ BI2536

Figure 6—figure supplement 1. PLK1 promotes the recruitment of MUS81 to stalled replication forks. (**A**) QIBC analysis of PLK1 expression after replication inhibition. Asynchronous HCT116 cells were treated with 2 mM HU as indicated time before fixing. The mean levels of PLK1 in the S and G1 phase were showed by red dash lines and green dash lines, respectively. (**B**) Immunoblot showing the immunoprecipitation of FLAG-tagged SLX4. Suspension HEK293 cells expressing FLAG-tagged SLX4 were treated with or without nocodazole (100 ng/ml) and PLK1 inhibitors (10 μM BI2536 or BI6727) for 17 hr and 5 hr before harvest, respectively. (**C**) Immunoblot showing protein levels of BRCA1, BARD1, SLX4 and MUS81 on the chromosome. Asynchronous cells were treated with or without 10 μM BI2536 and 5 mM HU for 16 hr before harvest. (**D-E**) Immunofluorescence (**D**) and its quantifications (**E**) showing colocalization of MUS81 and FANCD2 in condensed mitotic nuclei. Experiments were preformed as the workflow in Supplementary Figure 6C. PLK1 inhibitor (10 μM BI2536) was added after G2 phase release. (**F-G**) Immunofluorescence (**F**) and its quantifications (**G**) showing colocalization of BRCA1 and ssDNA. HeLa cells were labeled with BrdU for 24 hr and then treated with or without 2 mM HU and 10 μM BI2536 for 5 hr. The Pearson coefficient of colocalization between BRCA1 and BrdU was measured by Huygens Professional Analysis Software. The mean and s.d. from three independent experiments are shown. **** $P<0.0001$, *** $P<0.001$, ns $P>0.05$. Scale bar, 5 μm.