Fig. S1

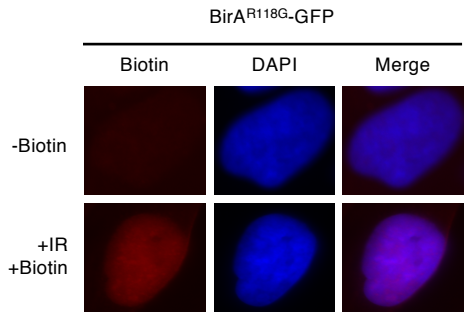
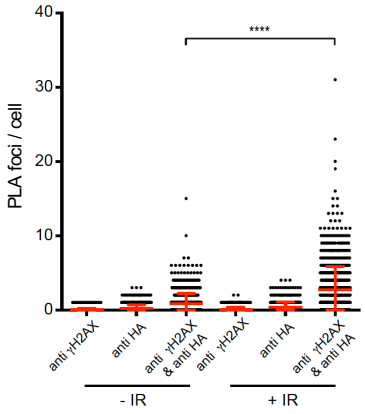


Fig. S1

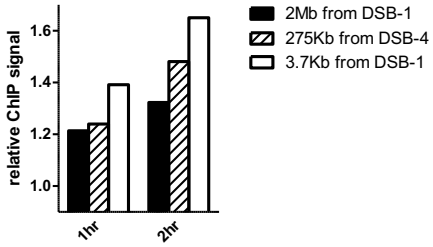
Figure S1. BirA^{R118G}-GFP does not promote localized protein biotinylation at DNA damage sites. U2OS cells stably expressing BirA^{R118G}-GFP were either irradiated (10 Gy) or left undamaged on coverslips. The cells were recovered for 1 hr and then incubated with exogenous biotin for 4 hr. The cells were then fixed and stained for biotin followed by analysis by immunofluorescence.

Fig. S2

A



B



C

Uniprot accession code	Description	Relative accumulation (Max = 1.00)		
		Fork	Stalled Fork (HU)	Chase
Q8ND82	Zinc finger protein 280C (ZPET)	0	0.96	1.00
P12004	Proliferating cell nuclear antigen (PCNA)	1.00	0.52	0.17
P62805	Histone H4 (H4)	0.23	0.50	1.00

D

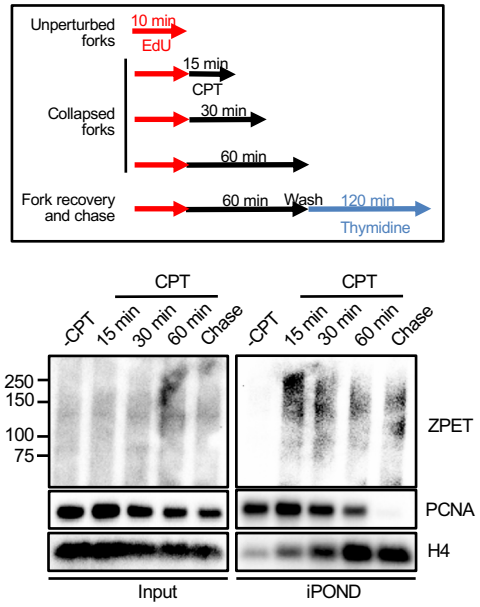


Fig. S2

Figure S2. ZPET is recruited to DSBs and stalled replication forks. (A) U2OS cells expressing HA-ZPET were treated with 10 Gy of IR or left untreated, and analyzed by PLA using the indicated antibodies 1 hr after IR. Levels of PLA foci were quantified in 1,000 cells (n=1,000). Error bar: S.D. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ (unpaired Student's *t* test). **(B)** The relative increases of GFP-ZPET at an AsiSI site and two control loci distal to DSBs were compared at 1 and 2 hr after DSB induction. The level of GFP-ZPET at each site before DSB formation were defined as 1. **(C)** As shown in Fig. 2E, HEK 293T cells were incubated with EdU for 15 min and either left untreated (fork) or treated with HU (2 mM) for 2 hr. Cells treated with HU were either harvested (stalled fork) or washed and chased for 1 hr. iPOND was then conducted, followed by Mass Spec analysis. The relative abundance of the indicated proteins in –HU, +HU and chase samples are shown. **(D)** HEK 293T cells were incubated with EdU for 10 min and either left untreated (fork) or treated with the CPT (1 μM) for the indicated durations. For the chase sample, following the incubation with EdU, cells were treated with CPT for 1 hr, washed, incubated with thymidine for 2 hr, and prepared for iPOND as in C.

Fig. S3

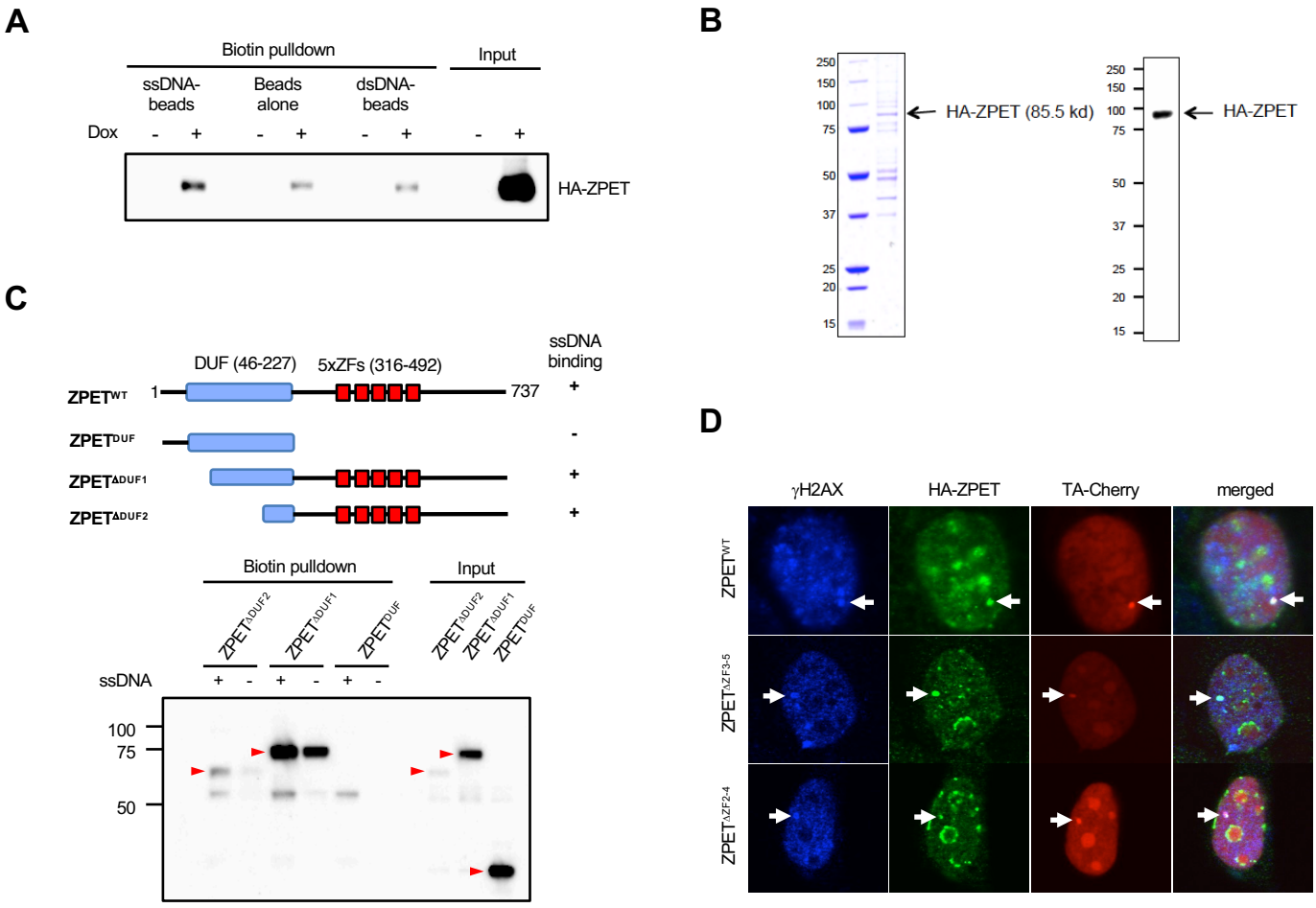

A
Biotin pulldown
Input
ssDNA-beads
Beads alone
dsDNA-beads
Dox
HA-ZPET
B
HA-ZPET (85.5 kd)
HA-ZPET
C
DUF (46-227)
5xZFs (316-492)
ssDNA binding
ZPETWT
ZPETDUF
ZPETΔDUF1
ZPETΔDUF2
Biotin pulldown
Input
ssDNA
D
γH2AX
HA-ZPET
TA-Cherry
merged
ZPETWT
ZPETΔZF3-5
ZPETΔZF2-4

Fig. S3

Figure S3. ZPET is a ssDNA-binding protein. (A) ZPET KO U2OS cells reconstituted with Dox inducible HA-ZPET were either left untreated or treated with Dox. Chromatin fractions were generated and then incubated with either ssDNA or dsDNA bound to streptavidin conjugated magnetic beads or beads alone. The ZPET captured was visualized by western blot. **(B)** HA-ZPET was partially purified using the HA tag. A Coomassie blue stained gel of the HA-ZPET preparation is shown in the left panel (the HA-ZPET band contains ~230 ng protein), and a Western blot of HA-ZPET is shown in the right panel. **(C)** HA-tagged wild-type ZPET and three ZPET fragments were tested for ssDNA binding. The domain structures of these ZPET variants are shown the in the upper panel. In the lower panel, biotinylated ssDNA was used to capture proteins from cell extracts. The levels of the ZPET variants on ssDNA and in input extracts were analyzed by Western. **(D)** U2OS cells carrying a TRE/I-SceI array were transfected with plasmids expressing I-SceI, TA-Cherry and HA-tagged ZPET variants. The array was visualized by TA-Cherry. The localization of ZPET variants was detected by immunostaining with anti-HA antibody.

Fig. S4

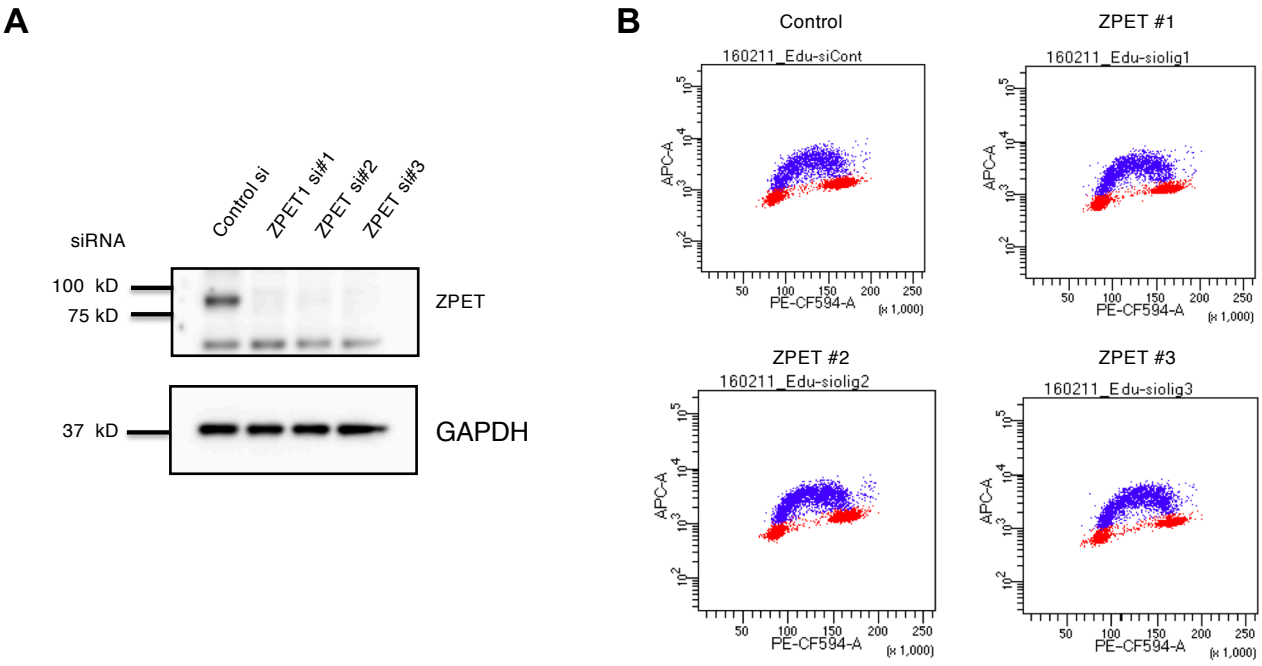

A
siRNA
Control si
ZPET1 si#1
ZPET si#2
ZPET si#3
100 kD
75 kD
ZPET
37 kD
GAPDH
B
Control
160211_Edu-siCont
ZPET #1
160211_Edu-siolig1
ZPET #2
160211_Edu-siolig2
ZPET #3
160211_Edu-siolig3
APC-A
PE-CF594-A
(x 1,000)

Fig. S4

Figure S4. ZPET knockdown does not influence the cell cycle. U2OS cells were transfected with either control siRNA or three independent siRNA's targeting ZPET. Three days post-transfection, the cells were either analyzed by Western blot to determine knockdown efficiency (A), or analyzed for cell-cycle profile (B). For cell-cycle analysis, cells were incubated with EdU for 30 min and stained with Propidium Iodide (PI).

Fig. S5

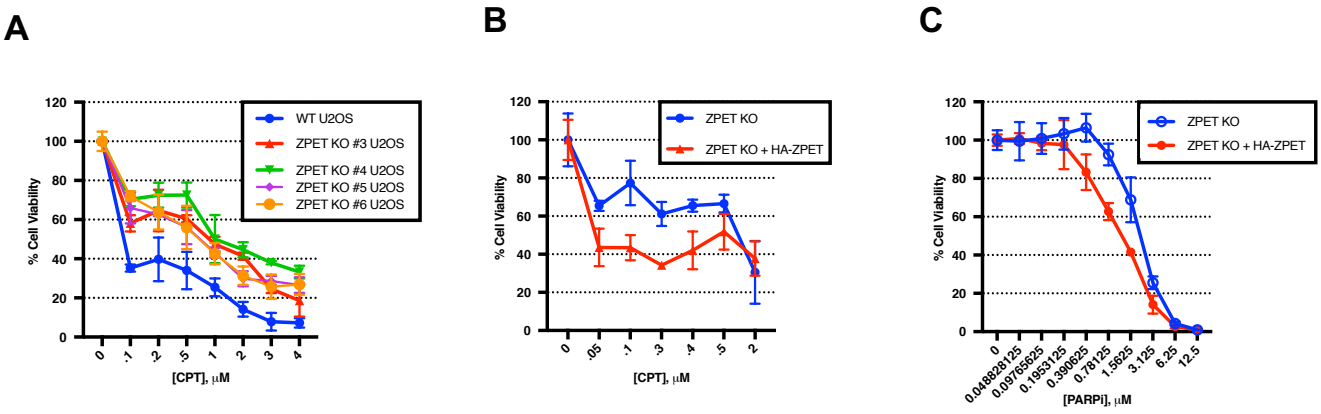

A
B
C
% Cell Viability
[CPT], μM
[PARPi], μM
WT U2OS
ZPET KO #3 U2OS
ZPET KO #4 U2OS
ZPET KO #5 U2OS
ZPET KO #6 U2OS
ZPET KO
ZPET KO + HA-ZPET

Fig. S5

Figure S5. ZPET increases cell sensitivity to CPT and Olaparib. (A) WT and ZPET KO U2OS cells were treated with the indicated high doses of CPT for 4 hr, washed, and cultured for 7 days. Cell viability was determined by the CellTiter-Glo assay. Error bars represent SD (n=3; technical triplicate). **(B)** ZPET KO U2OS cells reconstituted with Dox inducible HA-ZPET were cultured in the presence or absence of Dox, and cell sensitivity to high doses of CPT was analyzed as in (A). **(C)** ZPET KO U2OS cells reconstituted with Dox inducible HA-ZPET were cultured in the presence or absence of Dox, and treated with increasing doses of Olaparib. Cell viability was analyzed in 5 days.