SUPPLEMENTARY MATERIAL

Figure S1. The FANCD2-CtIP interaction is FA core complex and DNA-damage independent and very low CtIP protein levels are sufficient to stabilize FANCD2

(**A**) WCE were prepared from PD221+FANCA (lane 1) and PD221 (lane 2) cells and subjected to IP using rabbit IgG (lanes 3 and 4, neg. control), or CtIP antibodies (lanes 5 - 8) in presence of EtBr. WCE and equal volumes of IP samples were analyzed for the presence of CtIP, FANCD2, and tubulin. Immunoblot signals for FANCD2 levels interacting with CtIP were analyzed by densitometry and normalized again CtIP signals using ImageJ software. [***N.B***.: lanes 1 and 2 represent samples run on the same gel as lanes 3-8, but are derived from shorter western blot exposures.] (**B**) PD20+D2 cells were untreated (lanes 1) or treated with 30 μM APH for 6 h or 24 h (lanes 2 and 3, respectively). WCE from PD20+D2 cells were subjected to IP with rabbit IgG (lane 4 and 5, negative control) or CtIP-1 antibody (lane 6-8) in presence of EtBr. WCE and IP samples were analyzed for presence of FANCD2, CtIP and tubulin by western blotting. Immunoblot signals for FANCD2 levels interacting with CtIP were analyzed by densitometry and normalized again CtIP signals using ImageJ software. (**C**) *Left panel*: WCE were prepared from PD20+D2 cells that had been treated with high concentrations (300 nM) of siCon or siCtIP. Cells were collected at the indicated time points and analyzed for the presence of FANCD2, CtIP and GAPDH by western blot. *Right panel*: Immunoblot signals for CtIP and FANCD2 shown in the left panel were analyzed by densitometry and normalized against GAPDH signals using ImageJ software. The graph shows the percentage of FANCD2 and CtIP protein levels in siCtIP-treated compared to siCon-treated cells.

Figure S2. CtIP foci formation is strictly FANCD2-dependent

(**A**) PD20+D2 and PD20 were untreated or treated with 30 μM APH for the indicated time points. Nuclear CtIP foci formation was analyzed by fluorescence microscopy and representative images were taken for each time point. (**B**) FANCD2-proficient (PD20+D2) and -deficient cells (PD20) were untreated or treated with 2 mM HU for the indicated times (30 min, 2 h, 6 h and 24

h) and cellular nuclei were analyzed for the presence of CtIP foci. Nuclei with > 5 foci were considered positive for CtIP foci formation. **(C)** PD20+D2 and PD20 were treated with 10 Gy for 3 h. Nuclear CtIP foci formation was analyzed by fluorescence microscopy and representative images were taken. Nuclei with > 5 foci were considered positive for CtIP foci formation. **(D)** *FANCD2 and CtIP colocalize in nuclear foci in unperturbed cells and following APH-triggered fork collapse*. FANCD2-proficient cells (PD20+D2) cells were untreated or treated with 30 μM APH, followed by immunofluorescence staining with anti-FANCD2 and anti-CtIP antibodies. Nuclei were counterstained with DAPI.

Figure S3. MRE11 and CtIP are involved in the same pathway for replication fork recovery

(**A**) WCE were prepared from human HCT 116 $CtIP^{+}/^{+}$ cells that had been treated with control siRNA (siCon) or MRE11 siRNA (siMRE11) for the indicated time points and analyzed for MRE11 and FANCD2 protein levels. Tubulin, loading control. (**B**) WCE were prepared from HCT 116 $CtIP^{+}/^{-}$ that had been treated with control siRNA (siCon) or MRE11 siRNA (siMRE11) for the indicated time points and analyzed for MRE11 and FANCD2. Tubulin, loading control. [***N.B.*** *As previously described by Roques et al. (EMBO J.,2009), knockdown of MRE11 causes FANCD2 protein levels to drop significantly (1)].* Based on MRE11 knockdown efficiencies, the following replication restart assay (Figure S4C) was performed 72 h after siRNA treatment. (**C**) The efficiency of replication restart in wild-type, CtIP-, MRE11- and CtIP/MRE11 double-deficient cells was measured as the number of restarted replication forks after APH-mediated fork stalling (DigU→BioU tracts), compared with the total number of DigU-labeled tracts (DigU plus DigU→BioU); ***$P < 0.0001$.

Figure S4. FANCD2 is dispensable for BRCA1-CtIP-MRN complex formation

WCE from PD20+D2 and PD20 cells were subjected to IP with rabbit IgG or anti-CtIP in presence of EtBr. WCE and IP samples were analyzed for presence of CtIP, BRCA1 and MRE11 by western blotting.

Figure S5. FANCD2-CtIP pathway model (text in Figure)

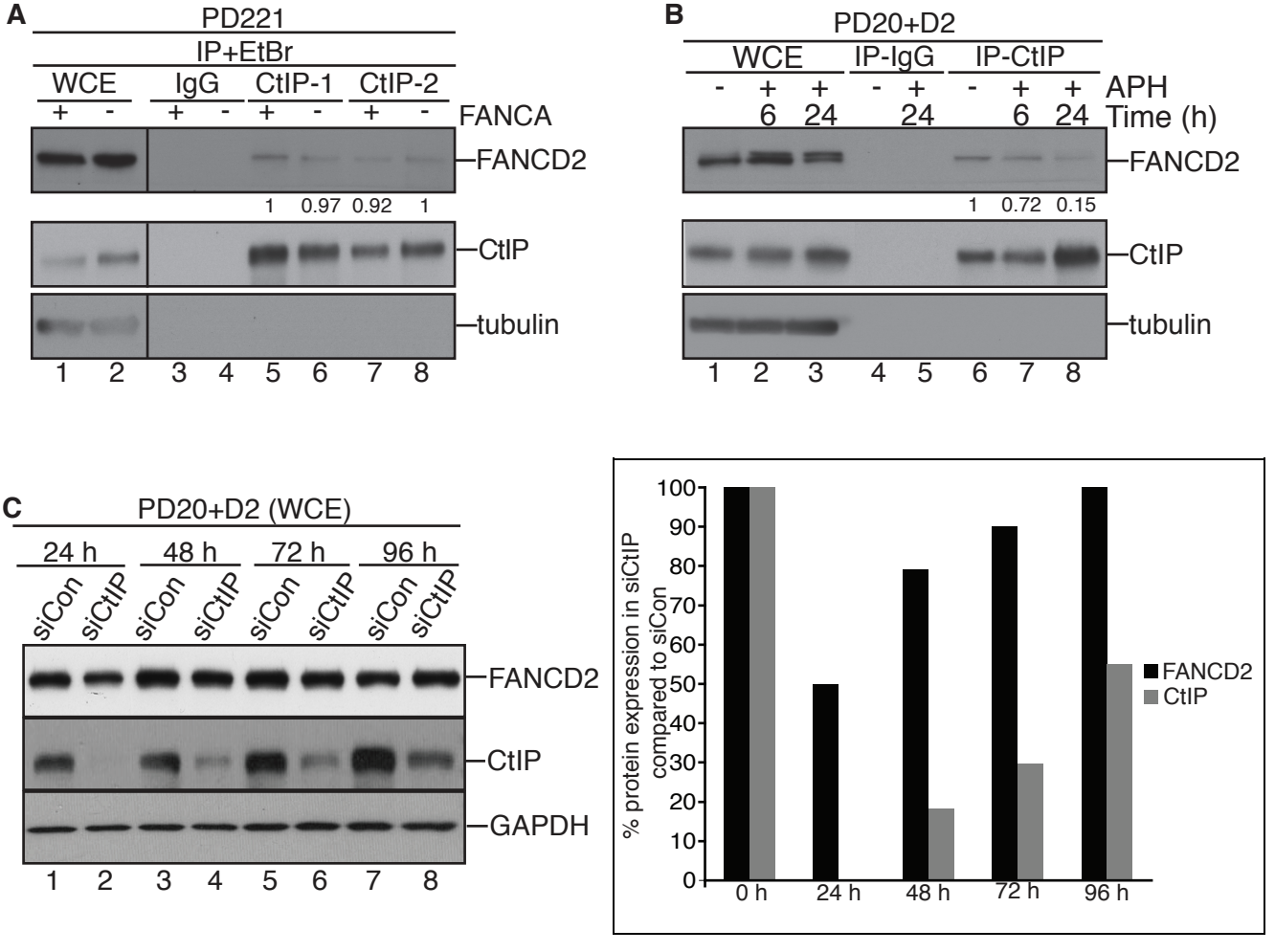
Figure S6. Construction of a human CtIP$^{+/-}$ heterozygous somatic cell line

The CtIP$^{+/-}$ heterozygous HCT116 cell line was constructed with the aid of recombinant adeno-associated virus (rAAV) mediated gene-targeting technology (2, 3). In order to inactivate CtIP, a knockout vector utilizing the SEPT:Neo selection cassette was designed to target exon 2 of the CtIP genomic locus such that it creates a frameshift mutation in the open reading frame when deleted. After the initial round of gene targeting, correctly targeted CtIP$^{Neo/+}$ clones were transiently transfected with a Cre-expressing plasmid (pGKCre) to excise the SEPT:Neo cassette, which yielded the heterozygous CtIP$^{+/-}$ HCT116 cell line.

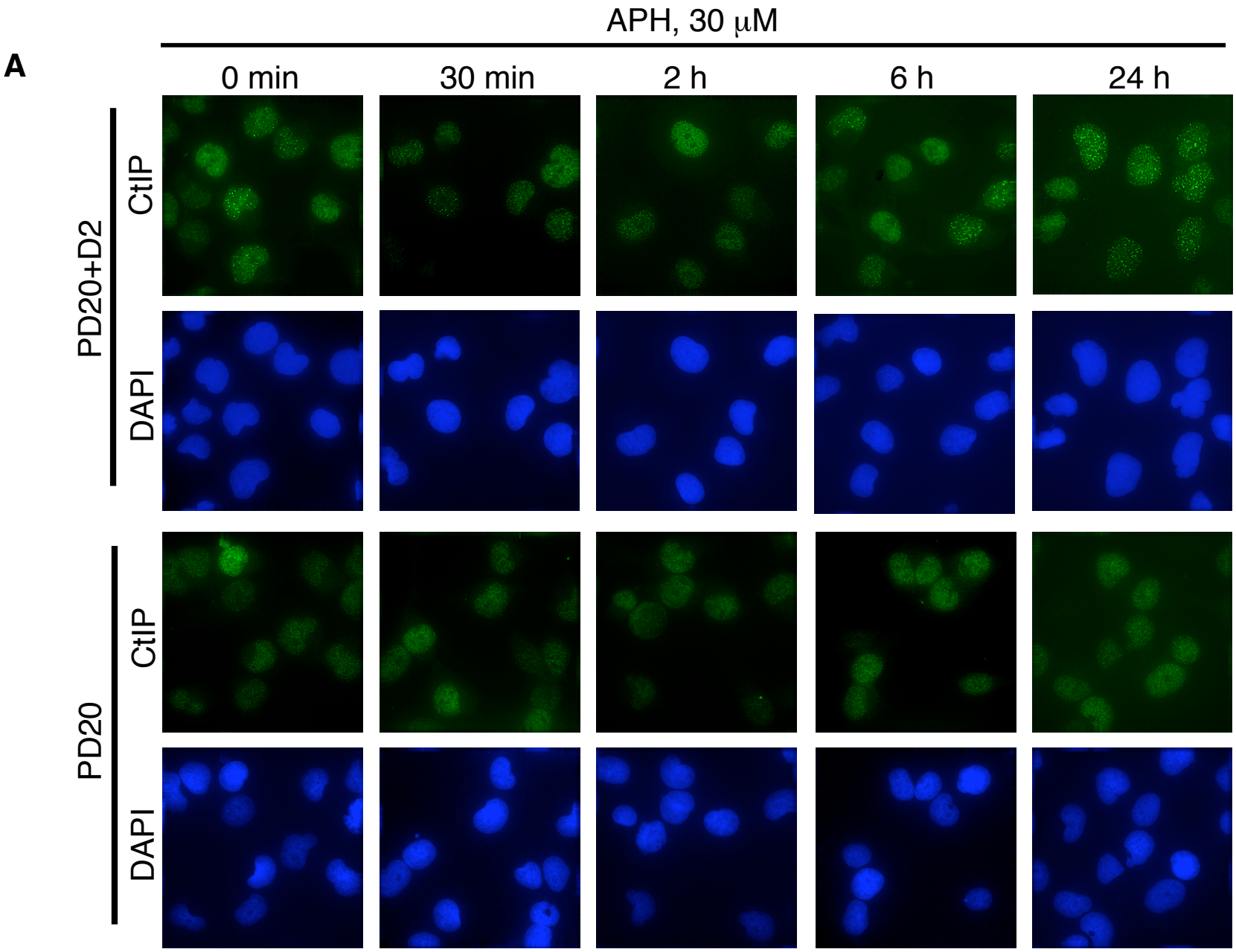
REFERENCES IN SUPPLEMENTARY MATERIAL

1. Roques C., Coulombe Y., Delannoy M., Vignard J., Grossi S., Brodeur I., Rodrigue A., Gautier J., Stasiak A.Z., Stasiak A., *et al* (2009) MRE11-RAD50-NBS1 is a critical regulator of FANCD2 stability and function during DNA double-strand break repair. *EMBO J.*, **28**, 2400-2413.
2. Kohli M, Rago C, Lengauer C, Kinzler KW, & Vogelstein B. (2004) Facile methods for generating human somatic cell gene knockouts using recombinant adeno-associated viruses. *Nucleic. Acids. Res.*, **32**, e3.
3. Russell DW & Hirata RK. (1998) Human gene targeting by viral vectors. *Nat. Genet.,* **18**, 325-330.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Fig. 2 (continued)

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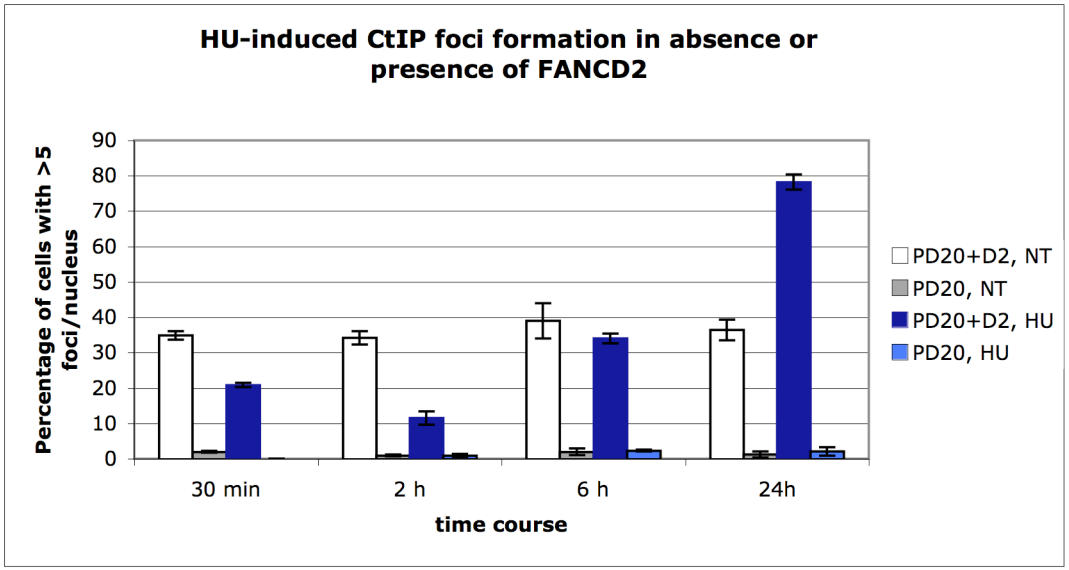

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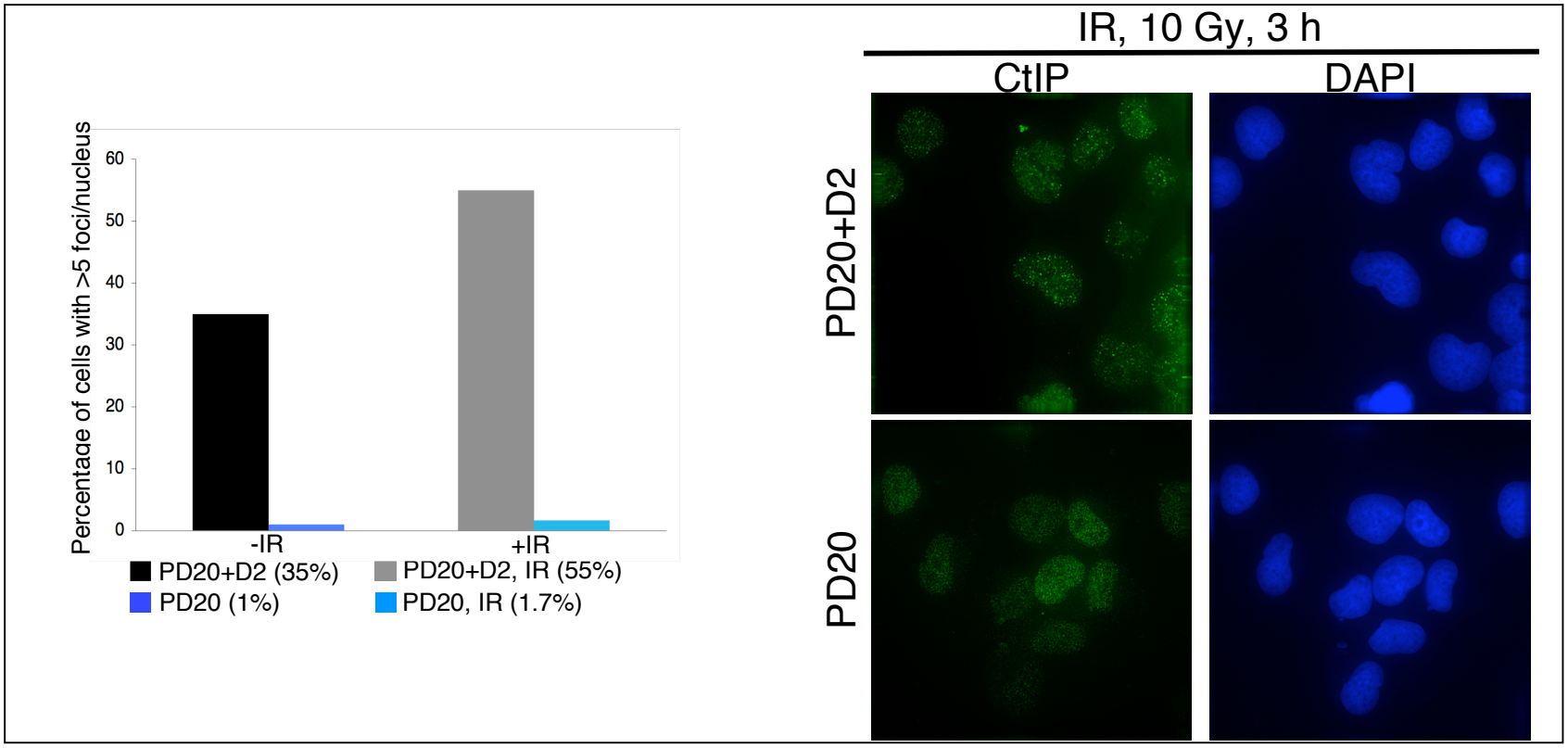

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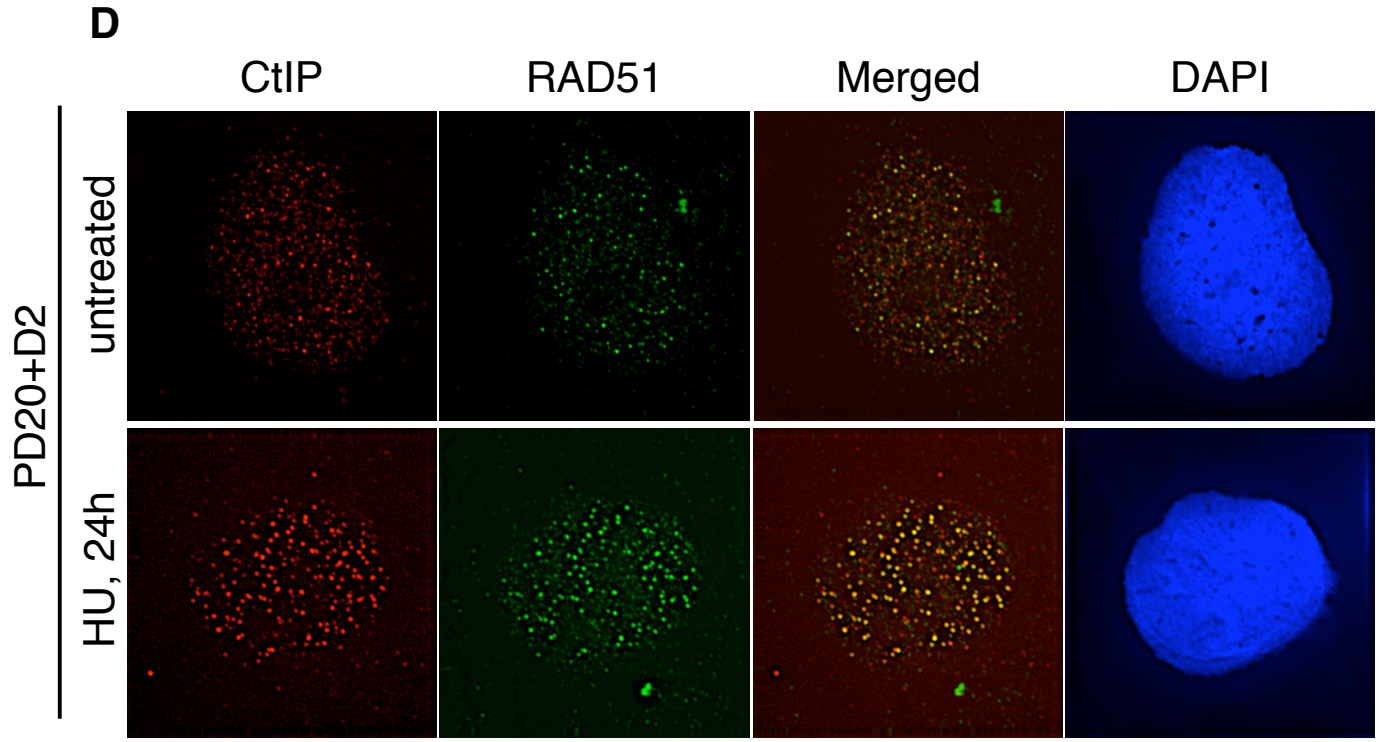
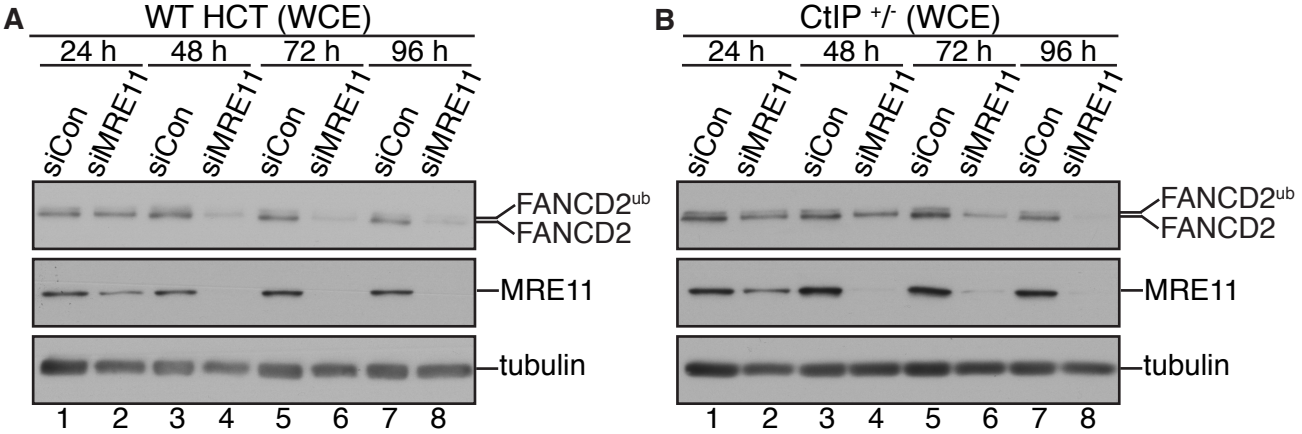
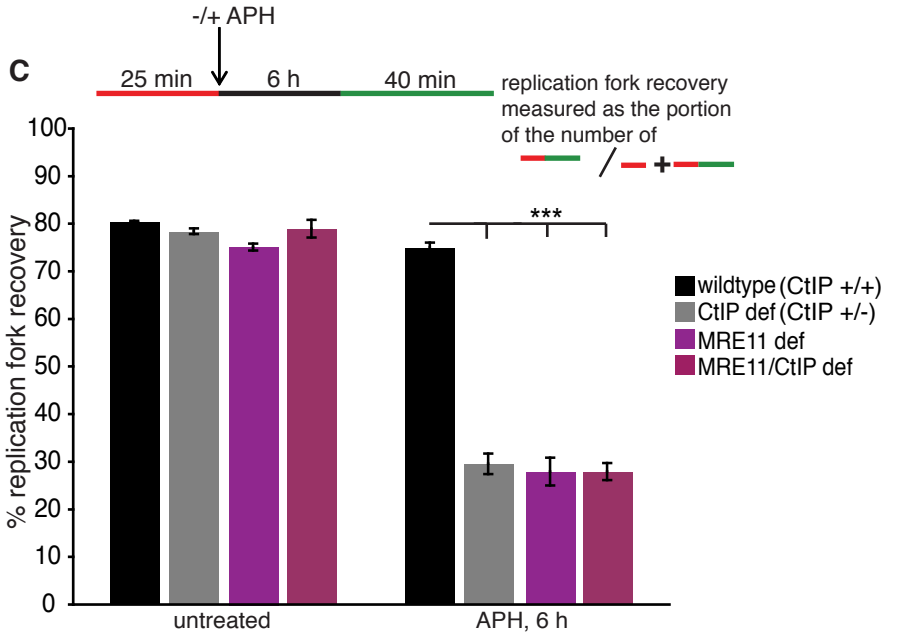
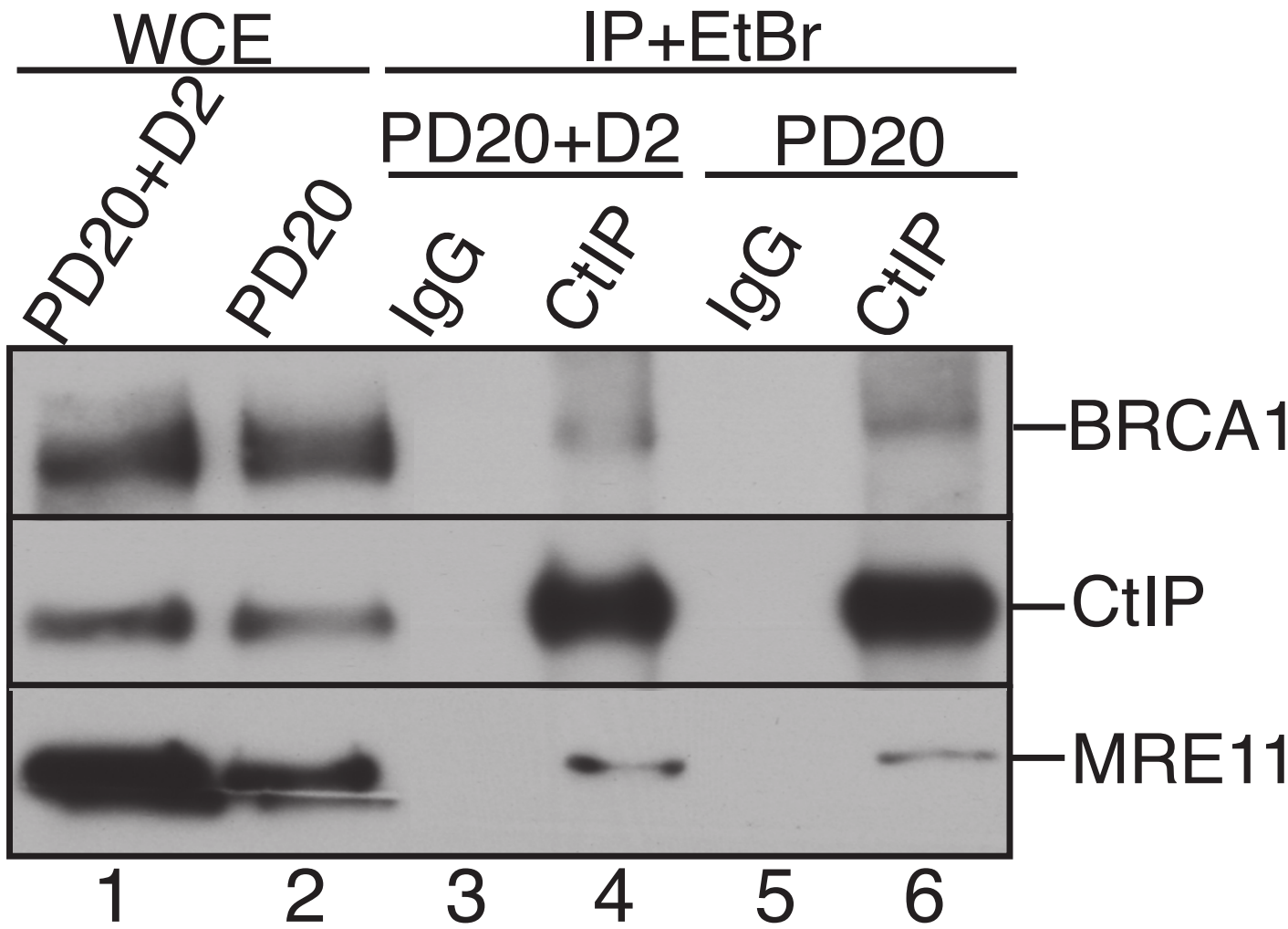


Supplementary Figure 3





Supplementary Figure 4



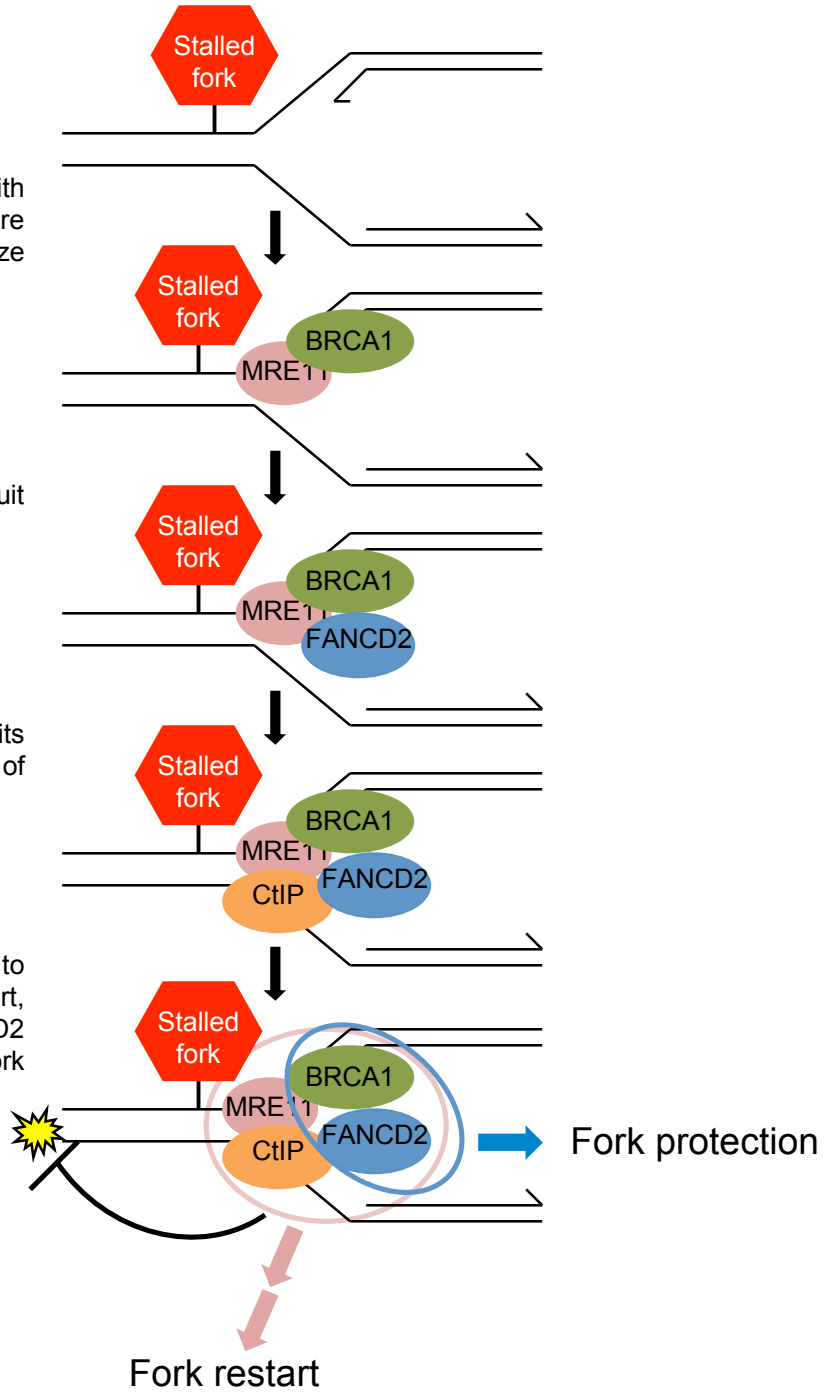
Supplementary Figure 5

1. Upon replication fork stalling with APH or HU, MRE11 and BRCA1 are recruited to chromatin and stabilize each other at sites of stalled forks.

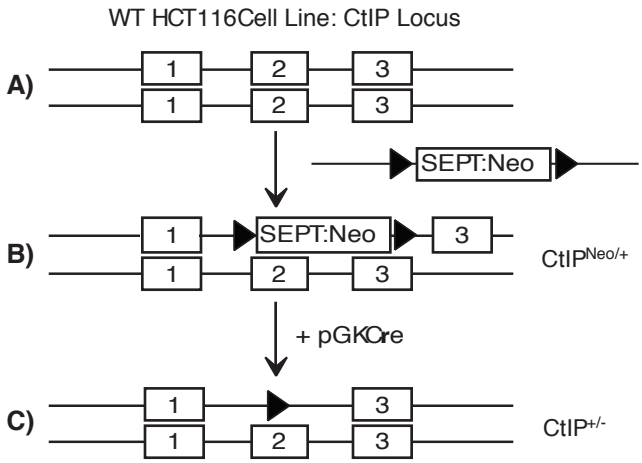
2. MRE11 and BRCA1 recruit FANCD2 to the stalled fork.

3. Subsequently, FANCD2 recruits CtIP in a manner independently of FANCD2 monoubiquitination.

4. All four proteins act in concert to promote replication fork restart, whereas only BRCA1 and FANCD2 are crucial to protect the stalled fork from nucleolytic degradation.



Supplementary Figure 6



Supplementary Table 1

Cell line	Treatment	Fiber analyzed	# fibers	# experiments	Median total	*p-value (two-tailed)	Figure #
1.Wild type (siCon)	media	Bio-dUTP	600	3	5.85	<0.0001 vs. 2 0.37 vs. 5	3
2. CtIP def (siCtIP)	media	Bio-dUTP	600	3	4.58	0.18 vs. 6	3
3. HCT CtIP $^{+/+}$	media	Bio-dUTP	400	2	9.32	<0.0001 vs.4	3
4. HCT CtIP $^{+/-}$	media	Bio-dUTP	400	2	6.32		3
5. FANCD2 def (PD20)	media	Bio-dUTP	400	2	5.91	<0.0001 vs. 6	4
6. FANCD2/ CtIP def (siCtIP,PD20)	media	Bio-dUTP	400	2	4.35		4
7. Wild type (HCC1937 +BRCA1)	media	Bio-dUTP	600	3	10.83	<0.001 vs.8	5
8. BRCA1 def (HCC1937)	media	Bio-dUTP	600	3	9.51		5
9. Wild type (siCon)	media	Dig-dUTP	600	3	6.36	0.65 vs. 10 <0.0001 vs. 15 <0.001 vs. 18	3
10. CtIP def (siCtIP)	media	Dig-dUTP	600	3	6.18	<0.0001 vs. 13	3
11. HCT CtIP$^{+/+}$	media	Dig-dUTP	400	2	11.08	<0.0001 vs.12	3
12. HCT CtIP $^{+/-}$	media	Dig-dUTP	400	2	9.58		3
13. CtIP/FANCD2 def (siCtIP,PD20)	media	Dig-dUTP	400	2	5.92	<0.01 vs.10	4
14. Wild type (siCon)	APH	Dig-dUTP	600	3	6.17	0.17 vs.15 0.86 vs. 9 <0.0001 vs.18	3
15. CtIP def (siCtIP)	APH	Dig-dUTP	600	3	6.06	<0.0001 vs.18	3
16. HCT CtIP$^{+/+}$	APH	Dig-dUTP	400	2	10.98	<0.0001 vs.17	3
17. HCT CtIP $^{+/-}$	APH	Dig-dUTP	400	2	9.54		3
18. CtIP/FANCD2 def (siCtIP,PD20)	APH	Dig-dUTP	400	2	4.32	<0.0001 vs.13	4

Median values are in μm. *Mann-Whitney test