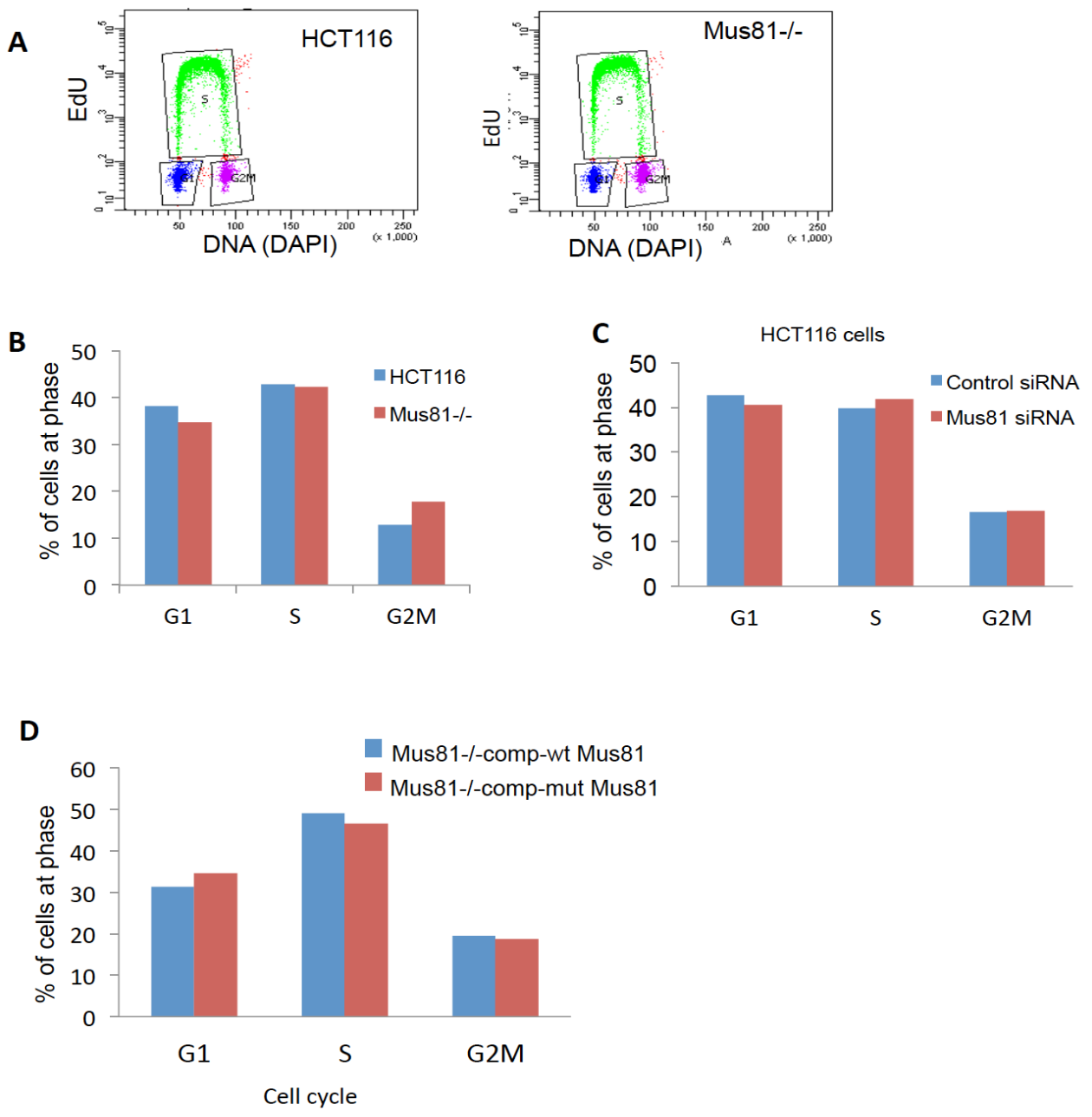
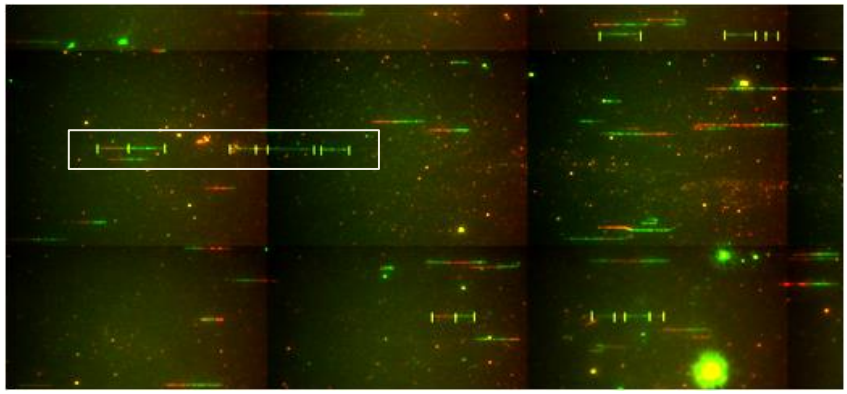
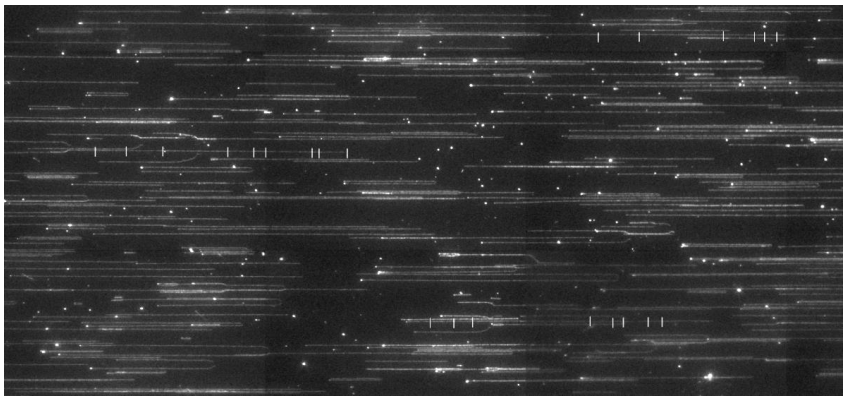


Supplementary Figure 1. Cell cycle analysis *via* flow cytometry. (A) Example dot plots of cell-cycle analyses by EdU staining. (B) Cell-cycle profiles of wild-type and Mus81-deficient HCT116 cells. (C) Cell-cycle profiles of HCT116 cells treated with siRNA directed against Mus81 or control siRNA. (D) Cell-cycle profile of Mus81-deficient cells expressing wild type or mutant Mus81.

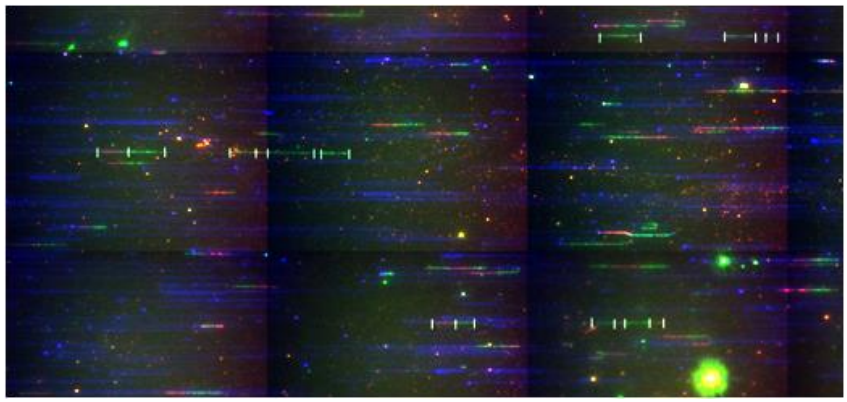
A



Green-IdU
Red-CldU



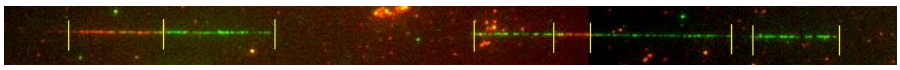
ssDNA-blue



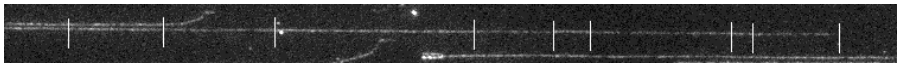
Merged

B

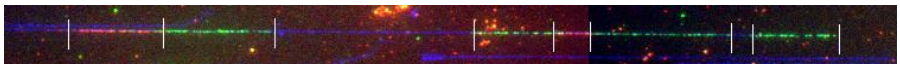
Zoom in



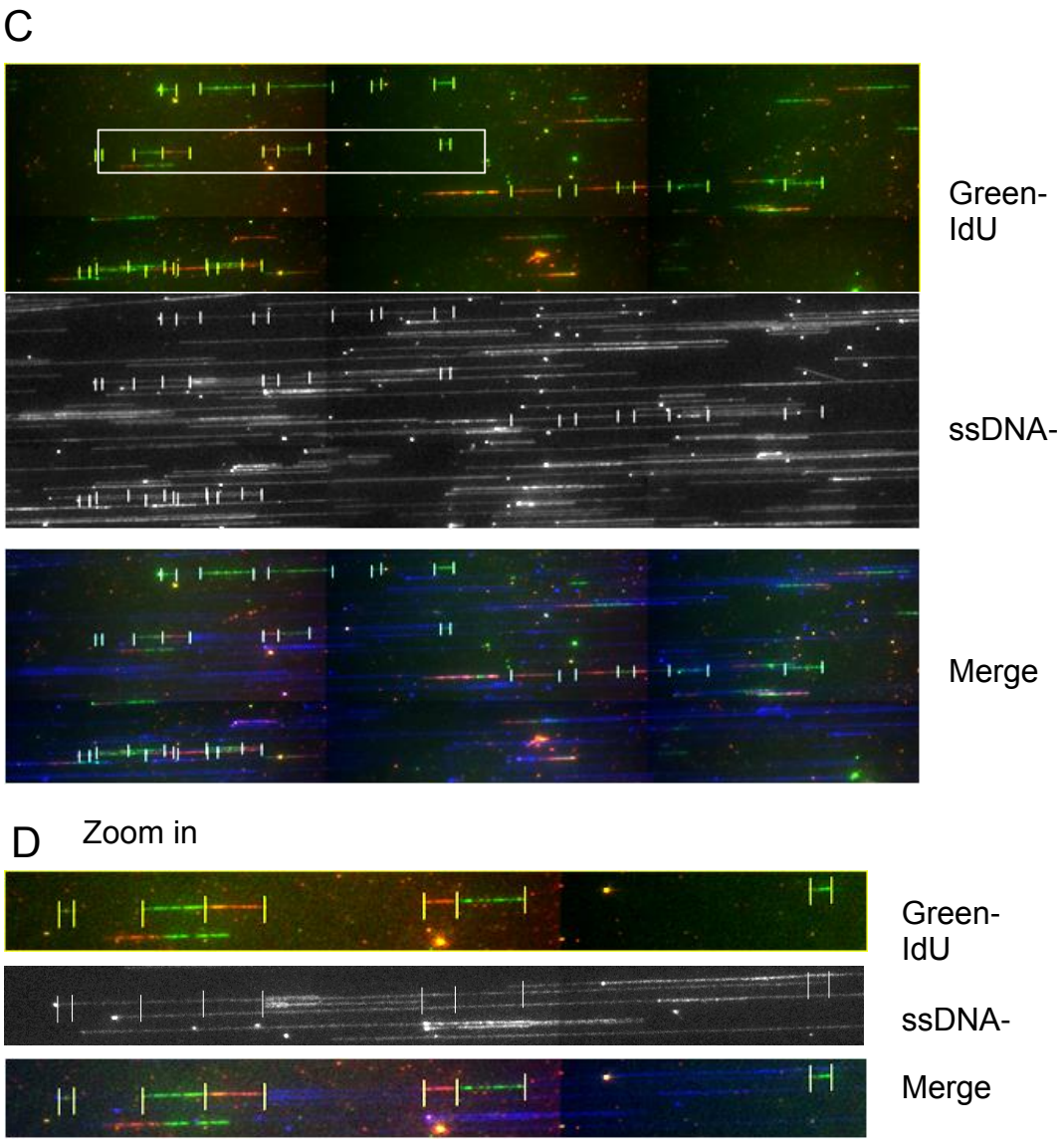
Green-IdU
Red-CldU



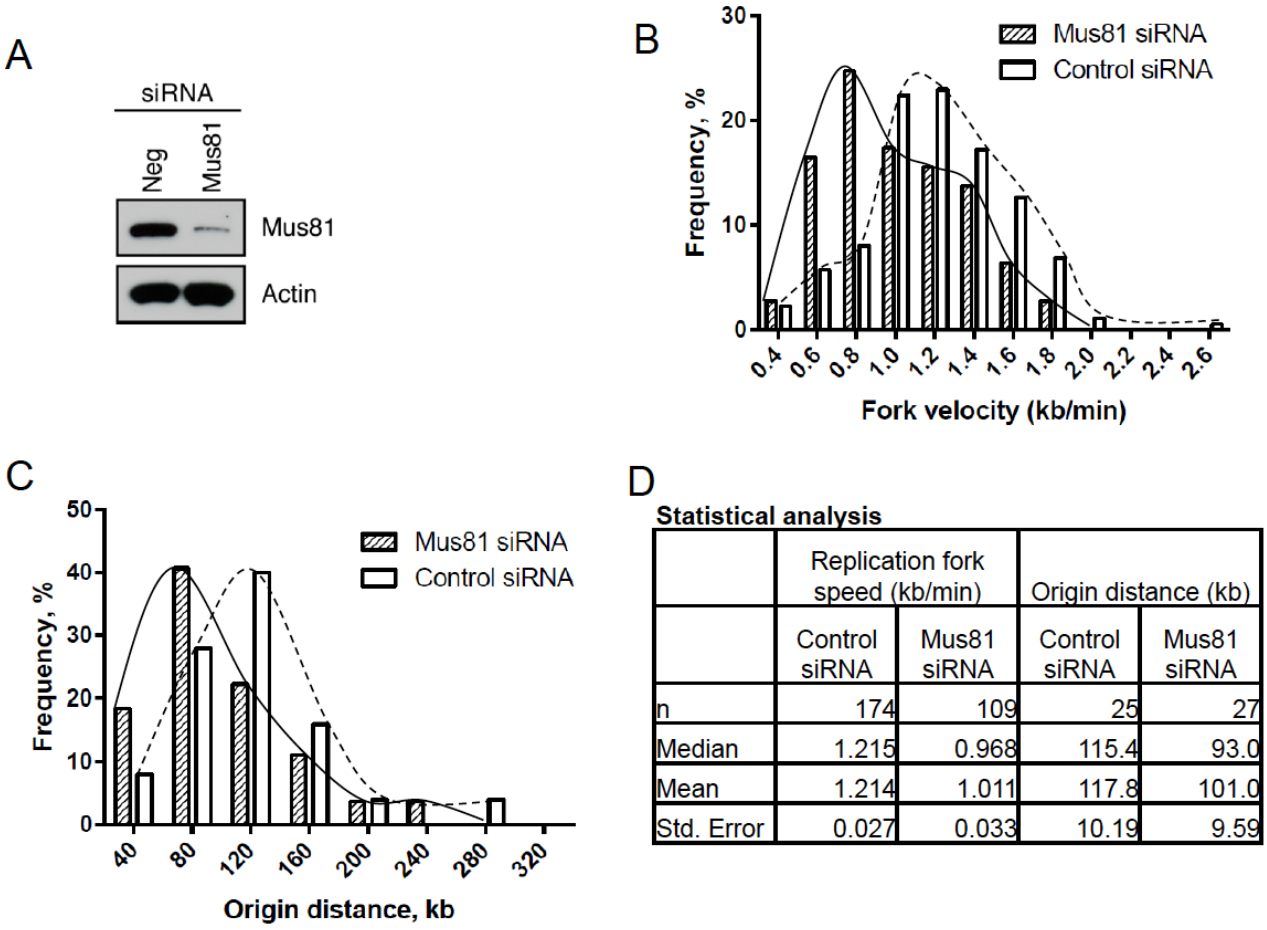
ssDNA-blue



Merged

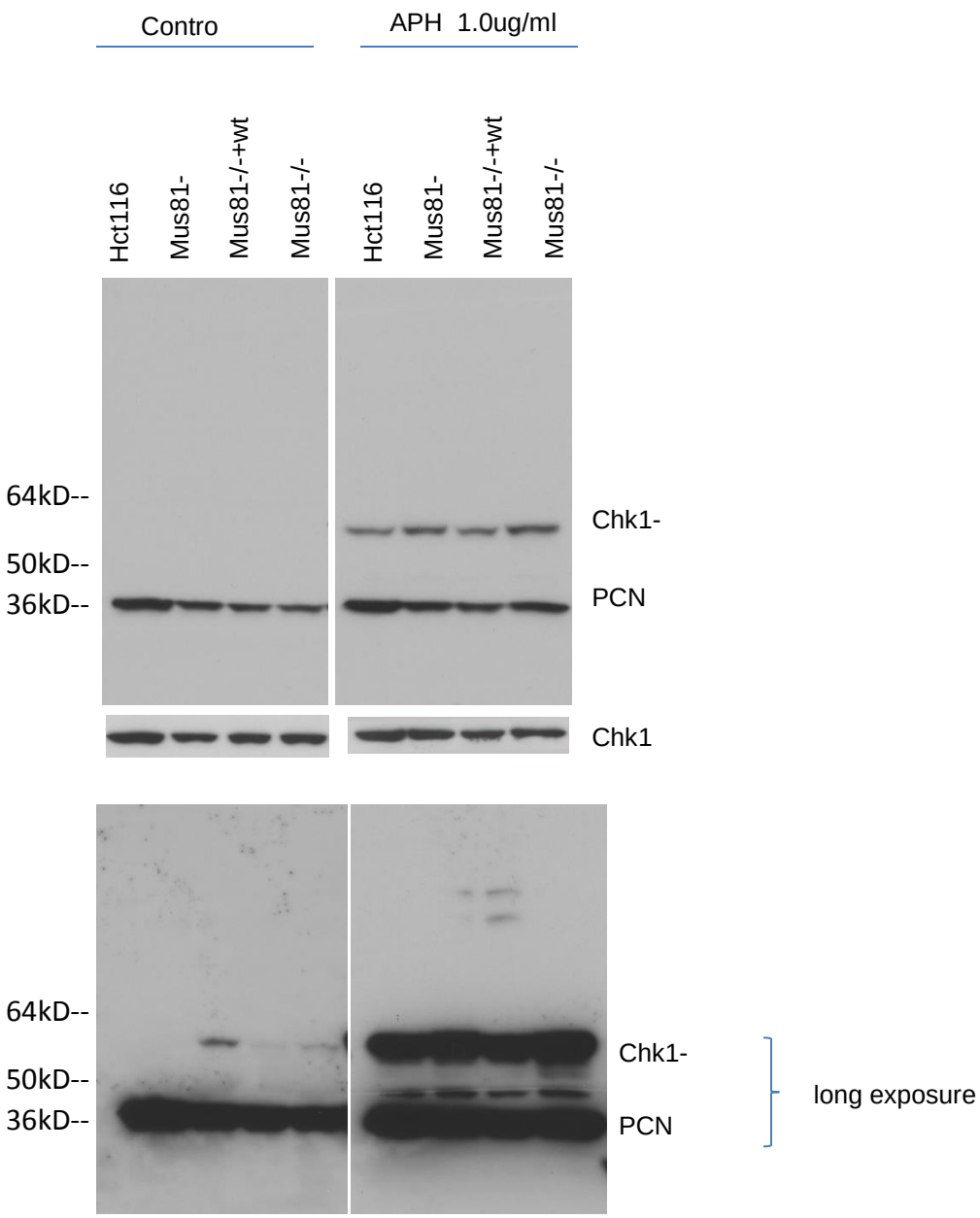


Supplementary Figure 2. Representative images of DNA combing. See Figure 1B for detailed explanations of the signals. This larger image shows a typical field with many DNA combing signals. (A, C) An image containing multiple microscopic fields to visualize long DNA fibers. (B, D) Zoom-in the rectangle area of (A) for a higher resolution. The vertical lines are examples of marks set to measure the length of the green, red and gaps between green/red signals on the same fiber, annotated by ImageJ.

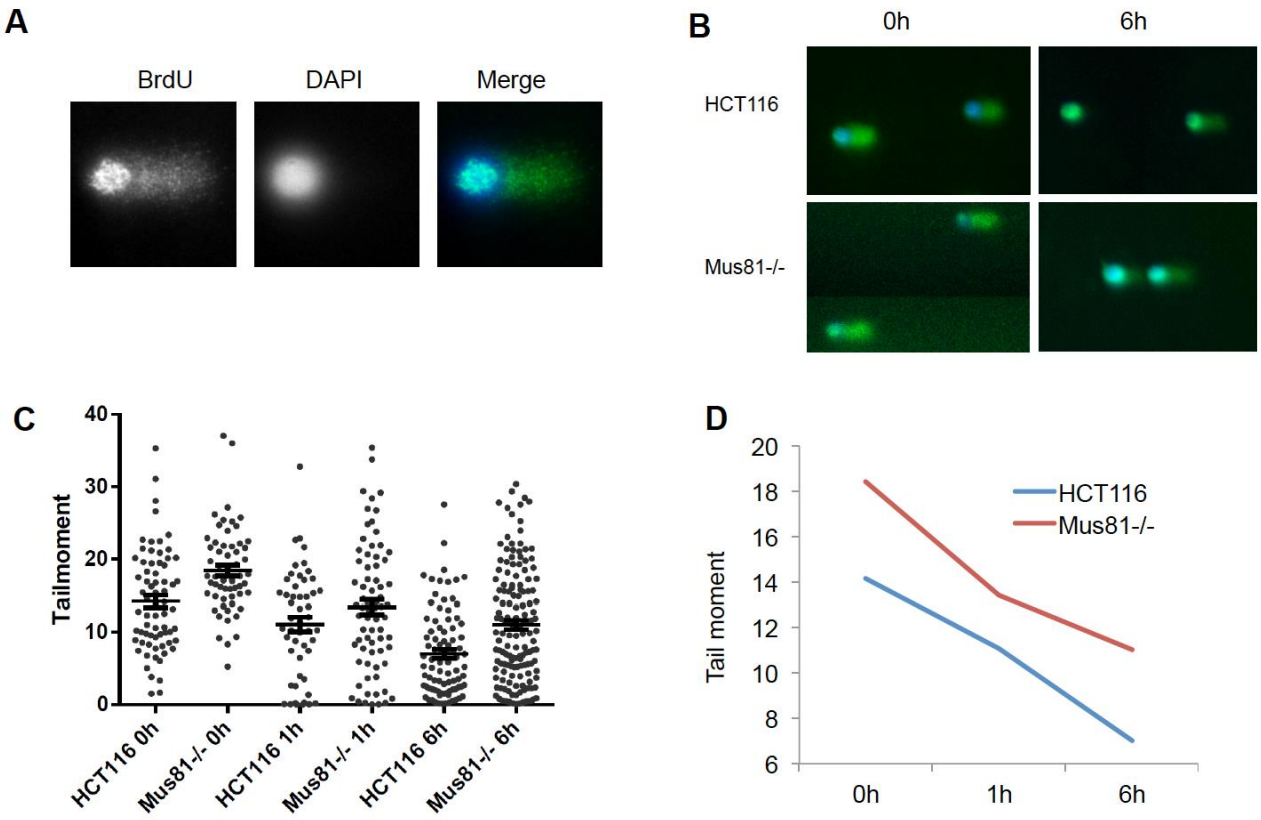


	Replication fork speed (kb/min)		Origin distance (kb)	
	Control siRNA	Mus81 siRNA	Control siRNA	Mus81 siRNA
n	174	109	25	27
Median	1.215	0.968	115.4	93.0
Mean	1.214	1.011	117.8	101.0
Std. Error	0.027	0.033	10.19	9.59

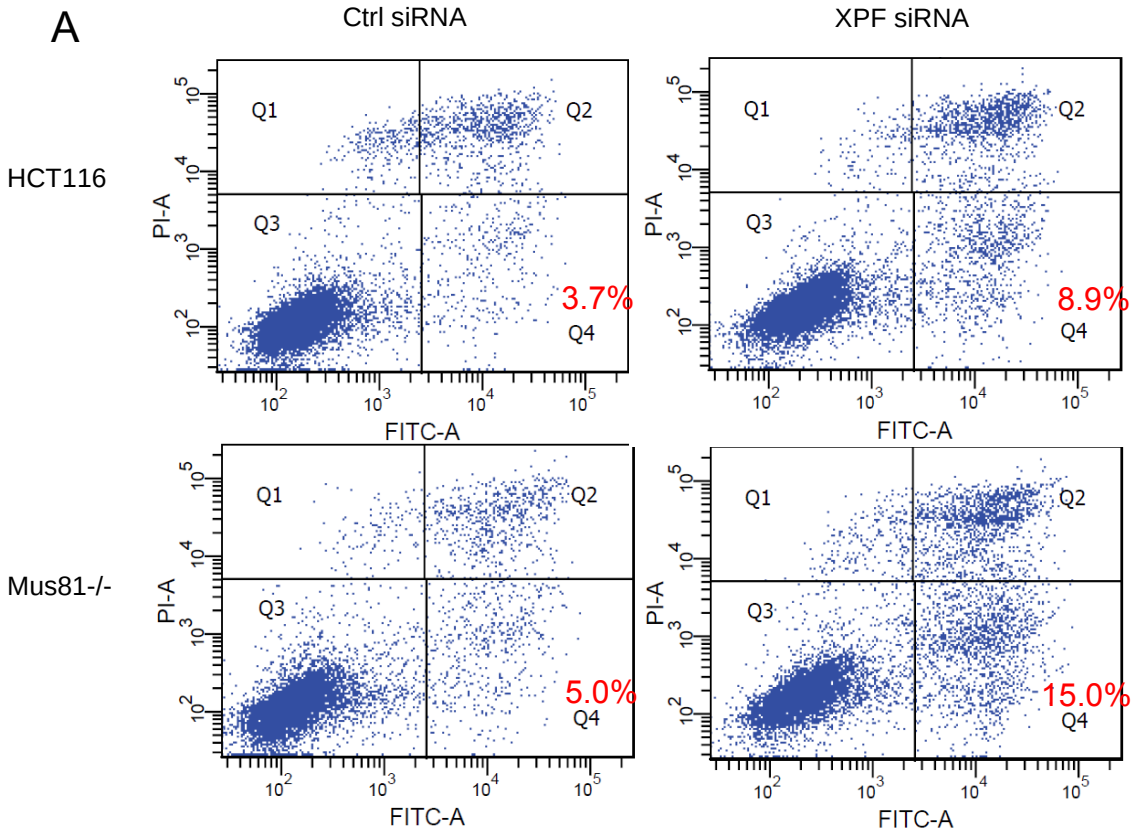
Supplementary Figure 3. Acute mus81 depletion slows replication and elevates the frequency of initiation in MDA-MB-231 Cells. (A) MDA-MB-231 cells were transfected with control siRNA or siRNA directed against Mus81 for 48 hours. Western-blot analysis confirmed the knockdown efficiency of the Mus81 siRNA. . Single fiber replication analyses were used to measure rates of replication fork progression (B) and inter-origin distances (C). Statistical analyses are shown in (D).

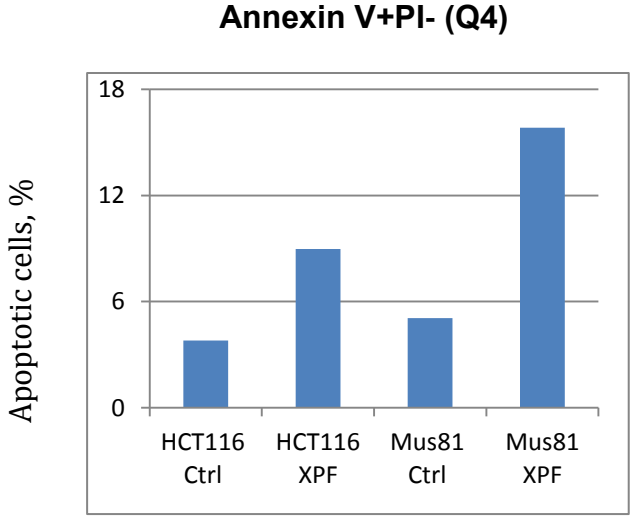


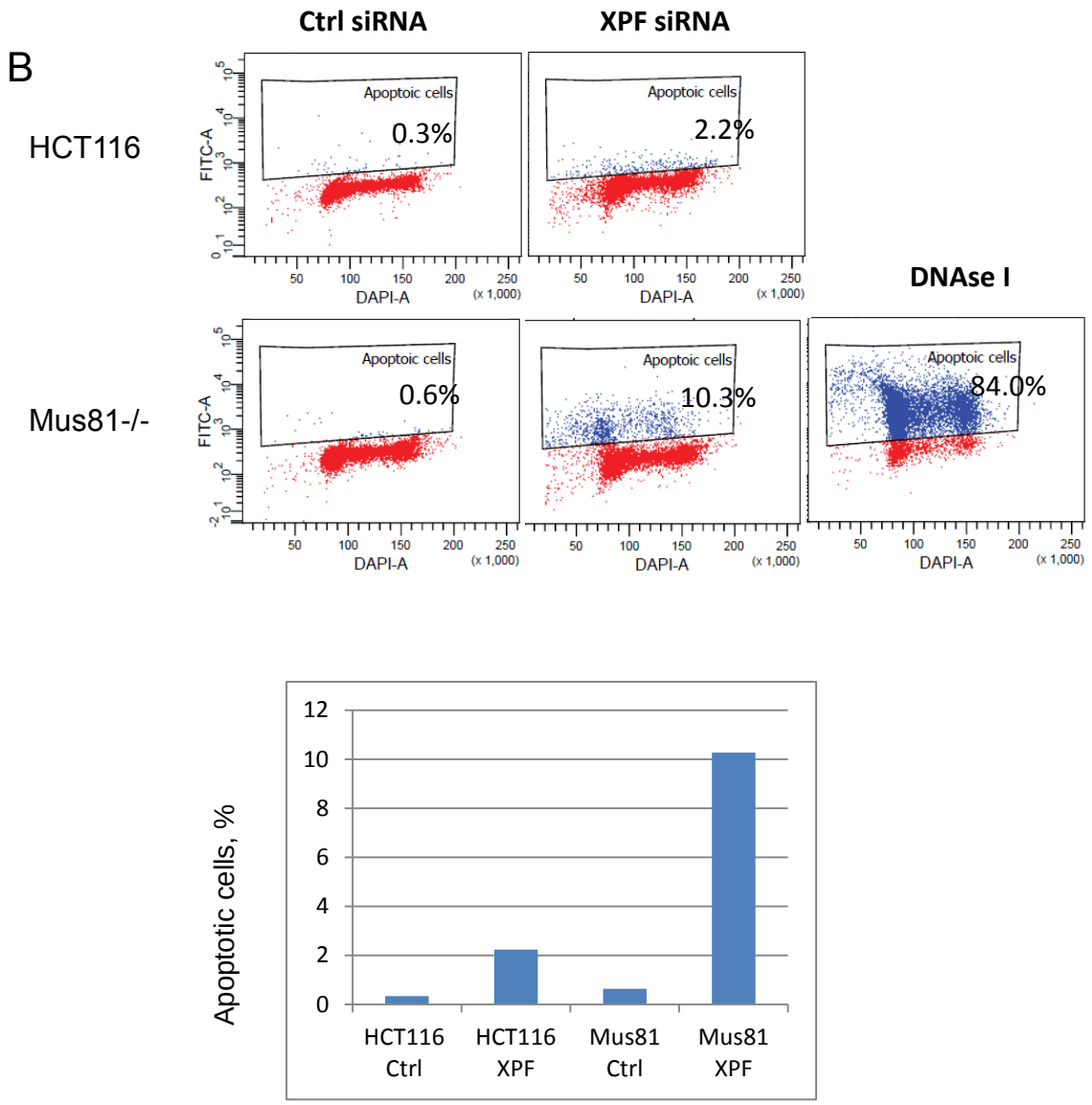
Supplementary Figure 4. Chk1-317 phosphorylation. Chk1-p317 was tested in Mus81-proficient and deficient HCT116 cells, Mus81-deficient cells complemented with wild type Mus81 (wt) and endonuclease dead Mus81 (mut). Cells were treated with or without APH for 80 minutes and protein was extracted by adding 1X SDS loading buffer directly to cells. Total Chk1 and PCNA were used as loading control.



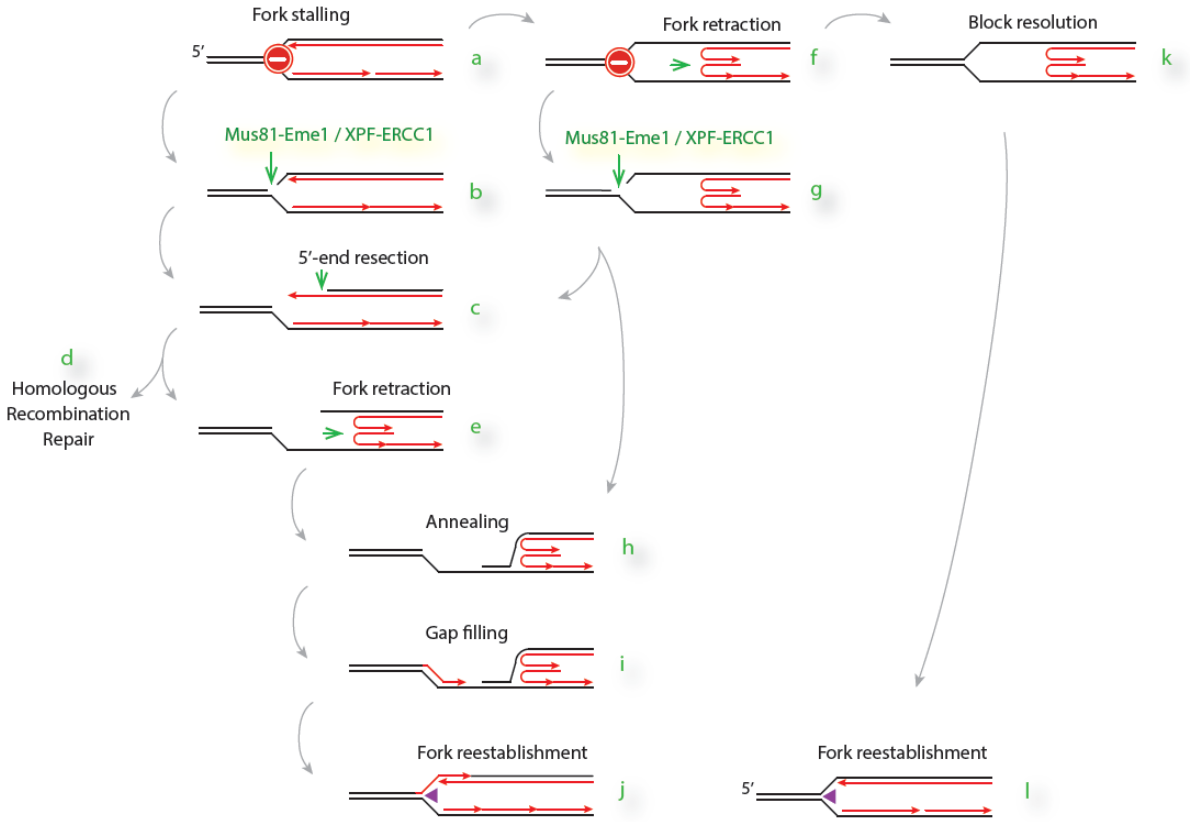
Supplementary Figure 5. Nascent strands/Okazaki fragments processing measured by BrdU comet assay. Mus81-proficient and deficient HCT116 cells were pulsed with BrdU for 30 minutes and chased with fresh medium for 0, 1 and 6 hours. Cells were collected for a regular alkaline comet assay and newly replicated DNAs were detected by anti-BrdU antibody. The amount of DNA in BrdU comet tail (newly replicated, non-ligated DNA) were analyzed to see if there were any differences in disappearing speed of BrdU comet tail, in other word, Okazaki fragment processing speed. (A) Representative images of BrdU incorporated DNA for newly replicated DNA, DAPI for total DNA and merged image with 20X objective. (B) Representative images for BrdU comet without chase or 6 hours chase after 30 minute of BrdU pulse in both HCT116 and Mus81-/- cells (10X objective). (C) BrdU tail moment of cells chased for 0, 1 and 6 hours after 30 minute of BrdU pulse. (D) Disappearing speed was similar in HCT116 cells and Mus81-/- cells.


A
Ctrl siRNA
XPF siRNA
HCT116
Mus81-/-
Q1
Q2
Q3
Q4
PI-A
FITC-A
3.7%
8.9%
5.0%
15.0%


Annexin V+PI- (Q4)
Apoptotic cells, %
18
12
6
0
HCT116 Ctrl
HCT116 XPF
Mus81 Ctrl
Mus81 XPF

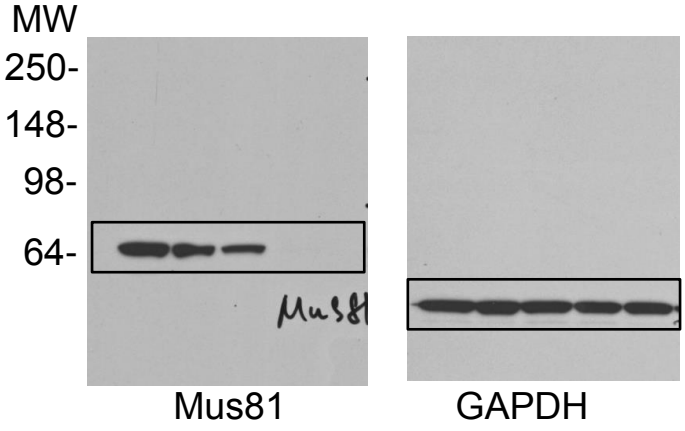


Supplementary Figure 6. Apoptosis in XPF depleted HCT116 cells and Mus81-deficient HCT116 cells detected by Annexin V assay and TUNEL assay. Cells were transfected with control siRNA or XPF siRNA twice with 72 hours interval, then cells were collected at 72 hours after the second transfection for: (A) Annexin V assay by FACS, the percentages of early apoptotic cells (Annexin V positive PI negative, Q4 in the dot plots) are shown as a histogram; (B) TUNEL assay by FACs, the percentages of apoptotic cells were shown in the FACs dot plot and summarized in a histogram.

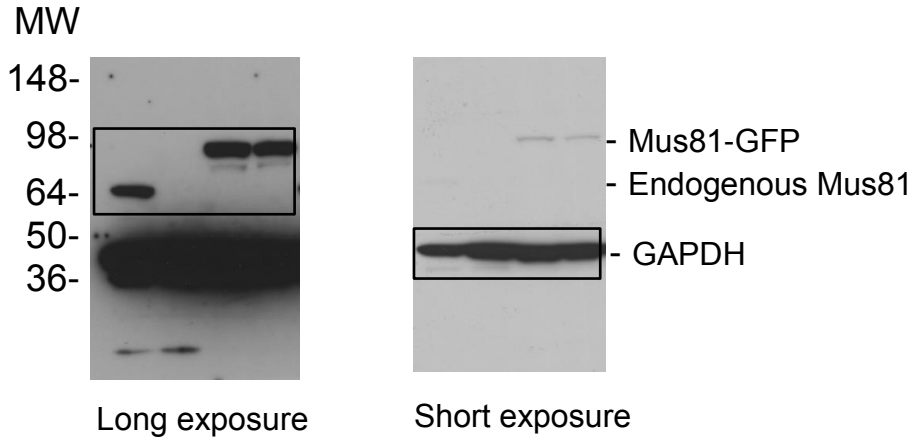


Supplementary Figure 7. A working model illustrating a potential role of Mus81 and XPF in resolving stalled replication forks. (a) A stalled replication fork showing a putative lesion (white bar in a red circle). (b) Cleavage of the stalled fork by Mus81-Eme1, Mus81-Eme2 or XPF-ERCC1 (Eme1 is shown for simplicity); (c) cleavage facilitates 5'-end exonucleolytic resection; (d) the resected products can be repaired by homologous recombination – detailed are not shown for simplicity; (e) Newly synthesized strands hybridize following cleavage, forming a "chicken-foot" structure; (f) Fork reversal can also occur in the absence of endonuclease cleavage, leading to a blocked replication fork; (g) endonuclease cleavage of a blocked reversed fork; (h) annealing of the template strands, which can occur on a cleaved reversed forks from either step (e) or step (g); (i) gap-filling of the annealed strands by DNA polymerases; and (j) fork reestablishment and replication restart. Retracted forks can also be resolved (k) and re-established (l) in a parallel process in the absence of endonuclease cleavage, however our working hypothesis is that endonuclease cleavage results in a faster resolution of the reversed forks.

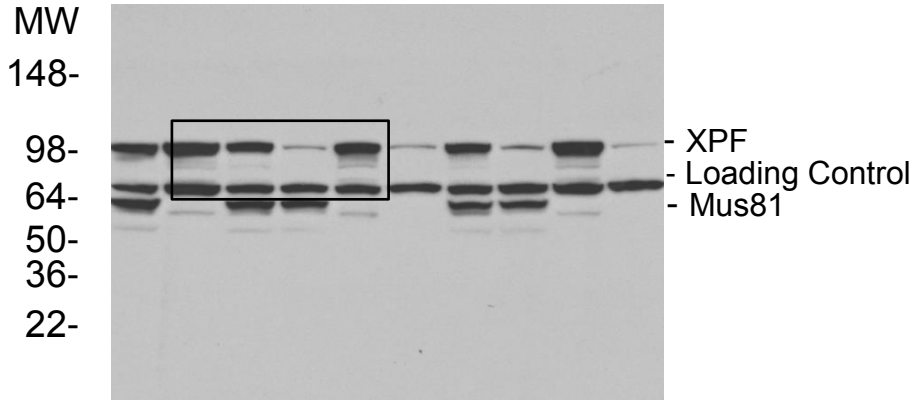
For Figure 3A



For Figure 5B



For Figure 8A



Supplementary Figure 8. Original full blots for all the Western blot figures.

Supplementary Table 1 Statistical analysis for Figure 1C ad 1D

	Fork speed (kb/min)		Origin distance (kb)	
	HCT116	Mus81-/-	HCT116	Mus81-/-
n	174	194	45	61
Median	1.74	1.42	161.2	124.8
Mean	1.9	1.59	180.3	135.7
Std. Error	0.06	0.06	15.72	8.09
p value*	<0.0001		<0.05	

* Mann Whitney test

Supplementary Table 2 Statistical analysis for Figure 3B and 3C

	Fork speed (kb/min)		Origin distance (kb)	
	Control siRNA	Mus81 siRNA	Control siRNA	Mus81 siRNA
n	481	322	52	67
Median	1.82	1.63	140.17	109.77
Mean	1.89	1.67	154.82	119.83
Std. Error	0.026	0.031	11.16	6.08
p value *	<0.0001		0.0134	

* Mann Whitney test.

Supplementary Table 3 Statistical analysis for Figure 5C and 5D

	Fork speed (kb/min)		Origin distance (kb)	
	Mus81-/-comp-WT	Mus81-/-comp-mut	Mus81-/-comp-WT	Mus81-/-comp-mut
n	672	736	101	86
Median	1.42	1.32	131.4	106.3
Mean	1.49	1.36	149.2	122.8
Std. Error	0.02	0.027	8.4	7.2
p value *	<0.0001		0.034	

* Mann Whitney test.

Supplementary Table 4 Statistical analysis for Figure 8B and 8C

Fork speed (kb/min)

	HCT116 ctrl	Mus81-/- ctrl	HCT116 XPF	Mus81-/- XPF
Number of values	274	283	354	258
Median	1.774	1.519	1.662	1.394
Mean	1.921	1.541	1.735	1.505
Std. Error of Mean	0.04584	0.02614	0.03142	0.03541

Origin distance (kb)

	HCT116 ctrl	Mus81-/- ctrl	HCT116 XPF	Mus81-/- XPF
Number of values	68	62	59	60
Median	166.5	141.8	143.3	117.5
Mean	178.6	152	160.3	144
Std. Error of Mean	9.227	8.844	10.15	11.25

Supplementary Table 5 Statistical analysis for Figure 9A

	HCT116 ctrl	HCT116 XPF	Mus81-/- ctrl	Mus81-/- XPF
G1	28.6	31	29.4	36.3
S	52.2	47.6	48.7	35.7
G2/M	15.4	18.2	18.2	19.2
SubG1	1.4	1.8	2.3	6.3