Supplementary Figure legends

Supplementary Figure 1. RAD51 and RAD51 paralogs are enriched spontaneously onto the S-phase chromatin during DNA replication. (A) Chromatin fractionation was carried out as described in materials and methods. H2AX was used as marker for chromatin while α-tubulin and Lamin A served as marker for cytosolic and nuclear fractions, respectively. (B) U2OS cells were synchronized by serum starvation (G0/G1), thymidine-aphidicolin block (S) and nocodazole (G2/M). Whole cell extracts were prepared at indicated cell cycle stages and analysed for RAD51 and RAD51 paralogs. Asyn, asynchronous cells (C) HeLa cells were synchronized with nocodazole and at the indicated stages of the cell cycle, cells were harvested and chromatin extract was prepared and analyzed for indicated proteins. Asyn, asynchronous cells.

Supplementary Figure 2. RAD51 paralogs associate with S-phase chromatin in a CDK-dependent manner. (A) HeLa cells were synchronized in G0/G1 phase with serum starved media and cells were harvested at indicated time points and chromatin extract was prepared to analyze indicated proteins. (B) G0/G1 synchronized HeLa cells were harvested without or with addition of CDK inhibitor (last 1 h) at indicated time points and chromatin extract was prepared to analyze indicated proteins.

Supplementary Figure 3. RAD51 paralogs supress HU induced toxicity. (A) Experimental design for nascent strand pull-down of active replication forks. HeLa cells were labelled with IdU for indicated times. Cells were cross-linked, and the chromatin fraction was isolated (input) and subjected to IP using anti-IdU antibody (Idu-IP) or only beads (Beads control). Fractions were probed for indicated proteins with H3 as loading control. (B) Replication fork velocity as a measure of CldU tract length in RAD51 paralogs deficient cells compared to respective parental cells. A minimum of 100 tracts were measured per

experiment. Mean values for three independent experiments are shown±SD. (C) Cellular sensitivity of RAD51C, XRCC2 and XRCC3 deficient cells to continuous exposure of HU, CPT and APH as measured by colony formation assay. Results shown are mean±SD of three independent experiments.

Supplementary Figure 4. RAD51 paralogs associate with CFSs following replicative stress. (A) Experimental design for pull-down of nascent DNA behind fork during template damage. HeLa cells were labelled with IdU for 30 min, followed by UV-C irradiation ($25J/m^2$) and a *chase* into thymidine-containing medium for the indicated times. Cells were cross-linked, and the chromatin fraction was isolated (input) and subjected to IP using anti-IdU antibody. Fractions were probed for indicated proteins. (B) Genomic organization of the FRA3B and FRA16D region. Primer sets of distal (FDR) and central (FCR) region within the FRA3B locus, and for FRA16D are indicated. Experimental design for ChIP analysis. HeLa cells were either untreated or treated with aphidicolin (0.5μM) for 24 h and then processed for ChIP followed by semi-quantitative PCR using RAD51C, XRCC2 and XRCC3-specific antibody. A representative gel of the amplified DNA immunoprecipitated with indicated antibodies. Input represents 1% of the cross-linked chromatin used for ChIP. (C) Quantification of cross-linked FRA3B-FCR and FRA3B-FDR chromatin immunoprecipitated from HU treated HeLa cells using indicated antibodies. Fold enrichment over goat IgG was determined and is shown for each primer pair for the ChIP. Results are expressed as means±SD for at least three independent experiments.

Supplementary Figure 5. RAD51 paralogs are crucial for spontaneous chromatin loading of RAD51 in S-phase. (A) CDK inhibition disrupts RAD51 paralogs enrichment on the fragile sites. HeLa cells were either untreated or treated with aphidicolin (0.5 μM) for 24 h and then processed for ChIP using RAD51, RAD51C, XRCC2 and XRCC3-specific antibody after adding CDK inhibitor for last 1 h. Representative gel images are shown. Fold

enrichment over goat IgG was determined and is shown for each primer pair for the ChIP. Results are expressed as means±SD for at least three independent experiments. (B) Representative images of RAD51 foci formed spontaneously in asynchronous and S-phase V79B cells. Quantification of spontaneous RAD51 foci in S-phase in the indicated cells. At least 50 cells were counted and results show the mean of two experiments.

Supplementary Figure 6. RAD51 paralogs assemble at stalled replication forks in distinct complexes and regulate the loading of RAD51. (A) Experimental design for nascent strand pull-down of HU-mediated stalled replication forks in RAD51 paralogs deficient cells. Indicated cells were labeled with IdU for 15 min followed by addition of HU for 45 min. Cells were cross-linked, and the chromatin fraction was isolated (input) and subjected to IP using anti-IdU antibody. Fractions were probed for RAD51 and RAD51 paralogs with Histone H3 (input loading control). (B) RAD51 paralogs are critical for RAD51 recruitment to CFSs. Quantification of fold enrichment of RAD51 at FRA3B and FRA16D sites from the indicated cells. The indicated hamster cells or HeLa cells treated with control or RAD51C, XRCC2 and XRCC3 shRNA for 24 h were exposed to aphidicolin (0.5μM) for the next 24 h, and analyzed by ChIP. Data are means ±SD (n=3).

Supplementary Figure 7. Cells deficient in RAD51 paralogs undergo increased replication fork collapse following replicative stress. (A) RAD51 paralog depleted or control HeLa cells were either untreated or treated with aphidicolin (24 h) and analysed by ChIP for the γ-H2AX accumulation at the indicated CFSs (FRA16D and FRA3B) or control genomic regions (β-actin). Data are means ±SD (n=3). (B) Quantification of 53BP1 positive nuclei from 100 V79B and U2OS cells after treatment with HU for indicated time periods. Cells having 8 or more foci were considered as positive. (C) Cell cycle profile after HU and aphidicolin treatment in U20S cells.

Supplementary Figure 8. FA-BRCA pathway contributes to maintenance of stalled forks and genomic stability. (A) Cellular sensitivity to continuous exposure of PARP inhibitor (ABT-888) and 4 h of HU in BRCA2 deficient V-C8 cells. (B) Chromosomal aberrations after 4 h of HU in BRCA2 deficient V-C8 cells. (C) Measurement of IdU labelled DNA fiber in BRCA2 defective V-C8 and parental V79 cells. (D) Measurement of IdU labelled DNA fiber in I-BR3 and V79B cells treated with FANCD2 shRNA. Western blots show depletion of FANCD2 in indicated cells.

Supplementary Figure 9. RAD51 paralogs, in parallel with FA-BRCA pathway, contribute to maintenance of stalled forks and genomic stability. (A) Measurement of IdU labelled DNA fiber in BRCA2-defective V-C8 cells with or without depletion of RAD51paralogs. Bar graph represents median IdU tract lengths of 100 DNA fibers. (B) Measurement of IdU labelled DNA fiber in RAD51 paralog deficient cells with or without depletion of FANCD2. (C) Epistatic roles of RAD51 paralogs with BRCA2 in DSB repair. Cellular sensitivity to Zeocin in BRCA2 deficient V-C8 cells following depletion of RAD51 paralogs. (D) Fold enrichment of MUS81 at FRA3B-FCR locus from RAD51 paralog deficient cells. Cells were treated with aphidicolin (0.5 μM) for 24 h, and analyzed by ChIP. Data are means ±SD (n=3). (E) Fold enrichment of SLX4 at FRA3B-FCR locus from RAD51 paralog deficient cells. Cells were treated with aphidicolin (0.5 μM) for 24 h, and analyzed by ChIP. Data are means ±SD (n=3). (F) Fold enrichment of MUS81 at FRA3B-FCR locus from CL-V4B cells treated with control/FANCM shRNA or WRN inhibitor. Cells were treated with aphidicolin (0.5 μM) for 24 h, and analyzed by ChIP. Data are means ±SD (n=3).

Supplementary Figure 10. RAD51C and XRCC3 are critical for fork restart and unperturbed S-phase progression. Confocal microscopy images of representative S-phase cells during stalled or recovering replication. Cells were stained with anti-IdU (red) and anti-XRCC2 (A), anti-RAD51C (B) or anti-XRCC3 (C) antibodies. (D) Experimental protocol for

measuring S-phase progression. U2OS cells were pulse-labeled with 50 μM BrdU for 40 min, washed (W), and then treated with 4 mM HU for 2 h. HU was removed, and the S-phase population was monitored immediately after treatment (0h) and at the indicated times after removal of HU (1-24 h). FACS profiles for control and RAD51 paralogs depleted cells. (E) Spontaneously arising micro-nucleated cells and their quantification from indicated cells, as determined by propidium iodide (red) and α-tubulin (green) staining, ($n \geq 100$ asynchronous cells per experiment). Results shown are mean±SD ($n=3$). P values are obtained for paralog deficient cells in comparison with parental cells. (F) Quantification of spontaneously arising micro-nuclei in CL-V4B cells overexpressing wt or indicated RAD51C mutants.

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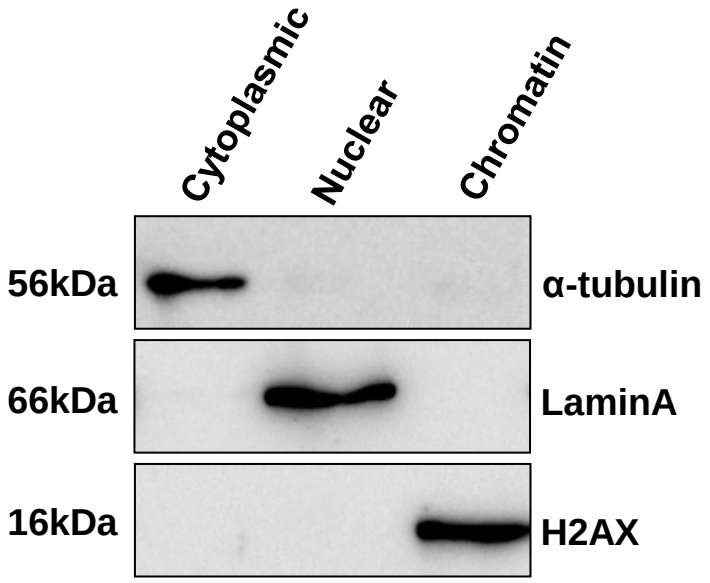

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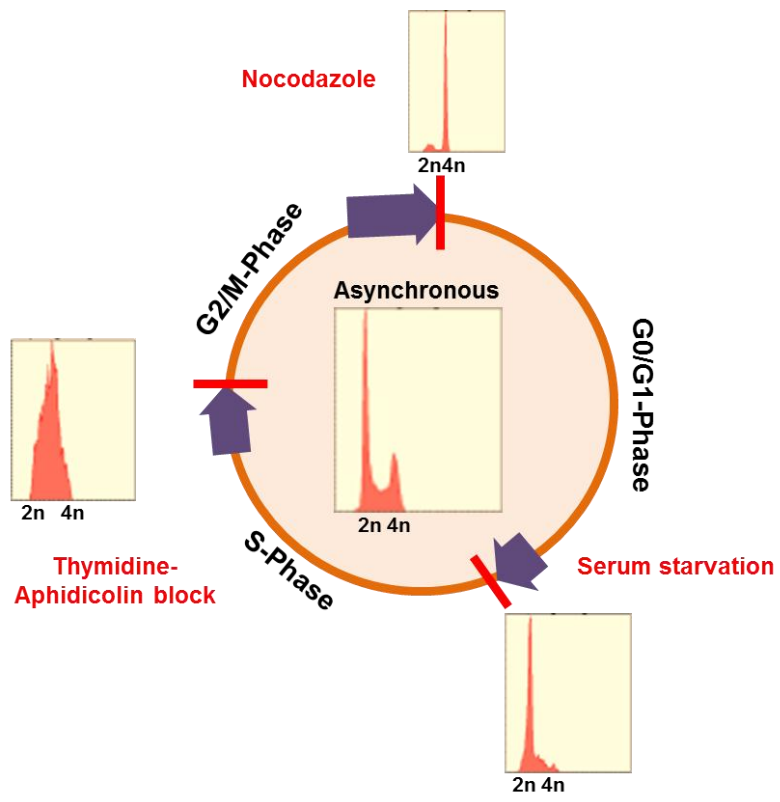

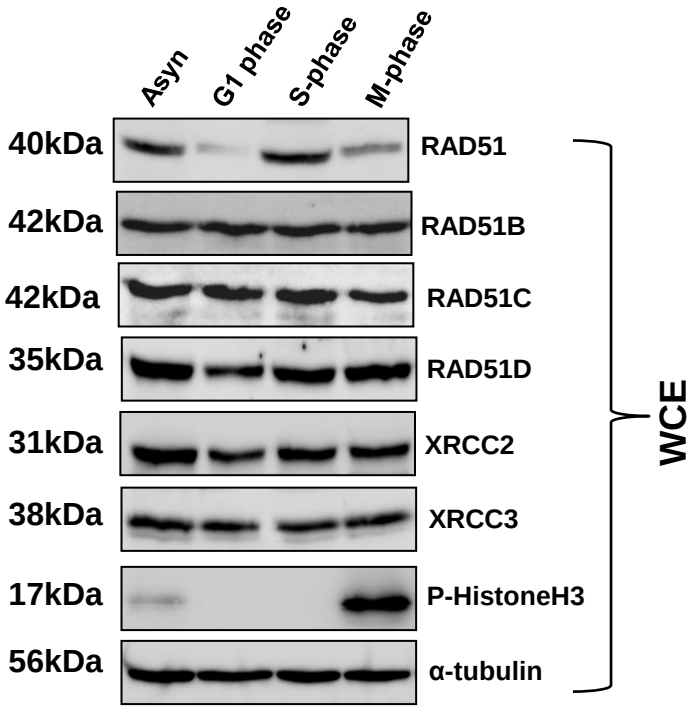

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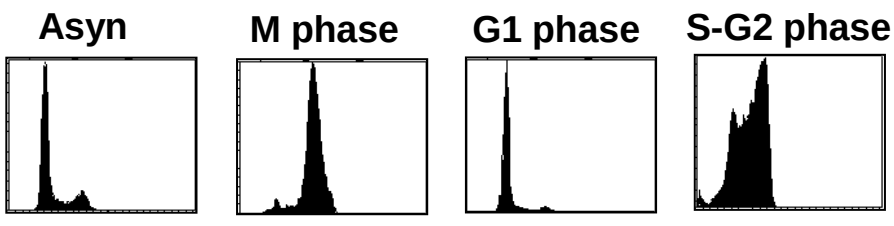

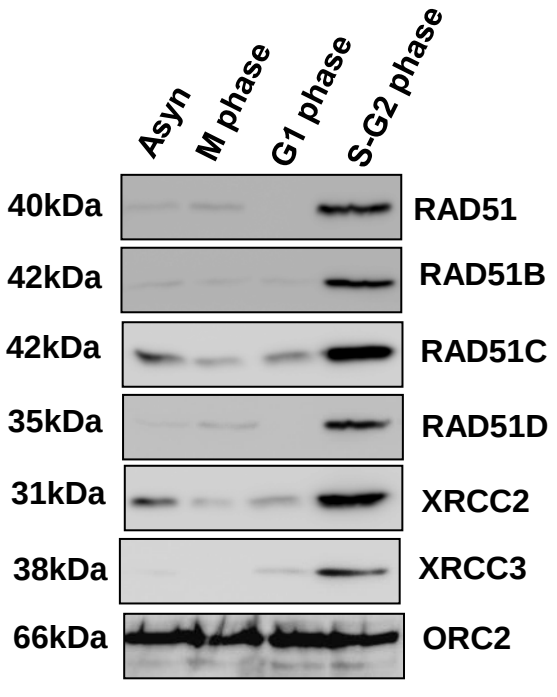

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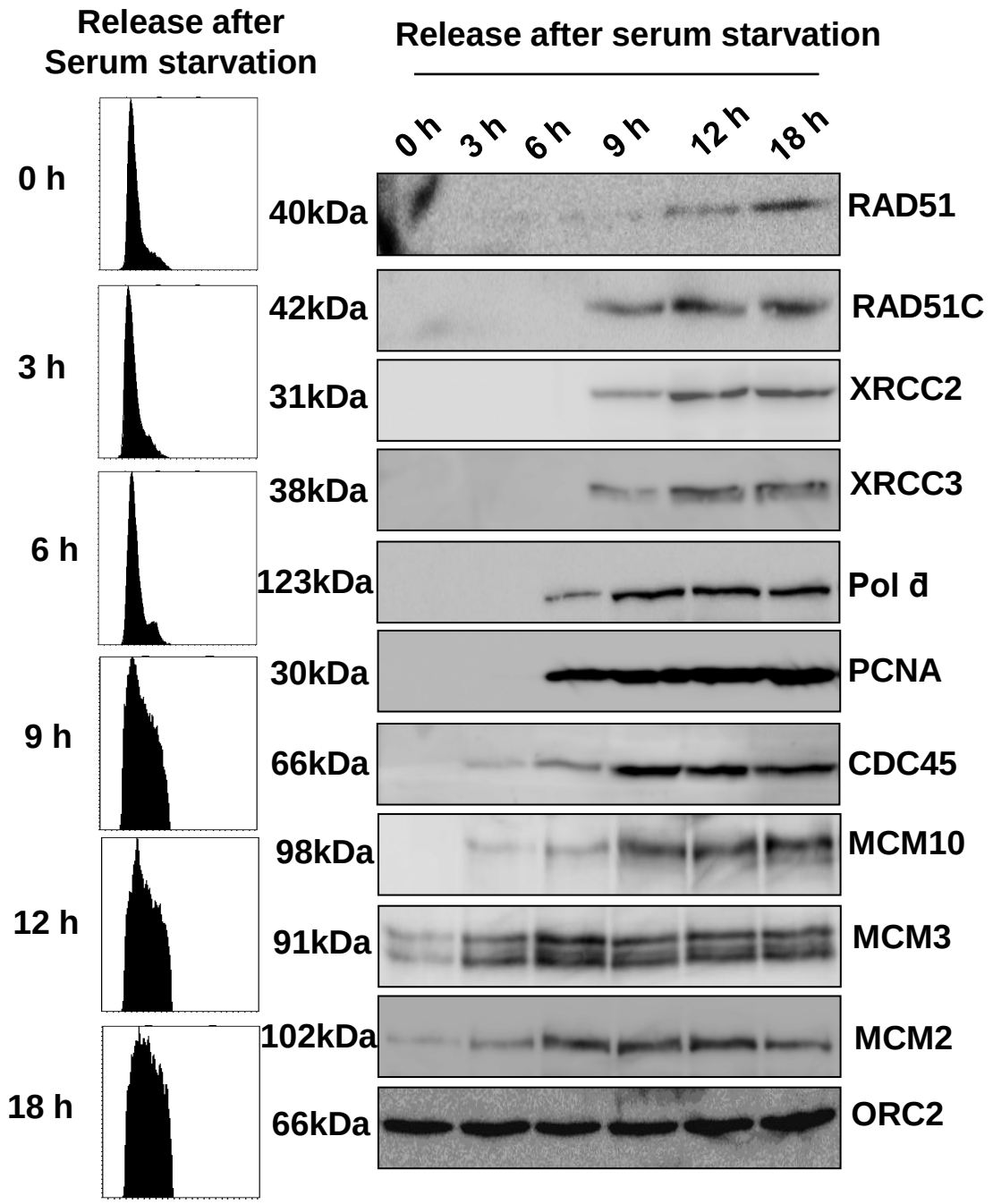

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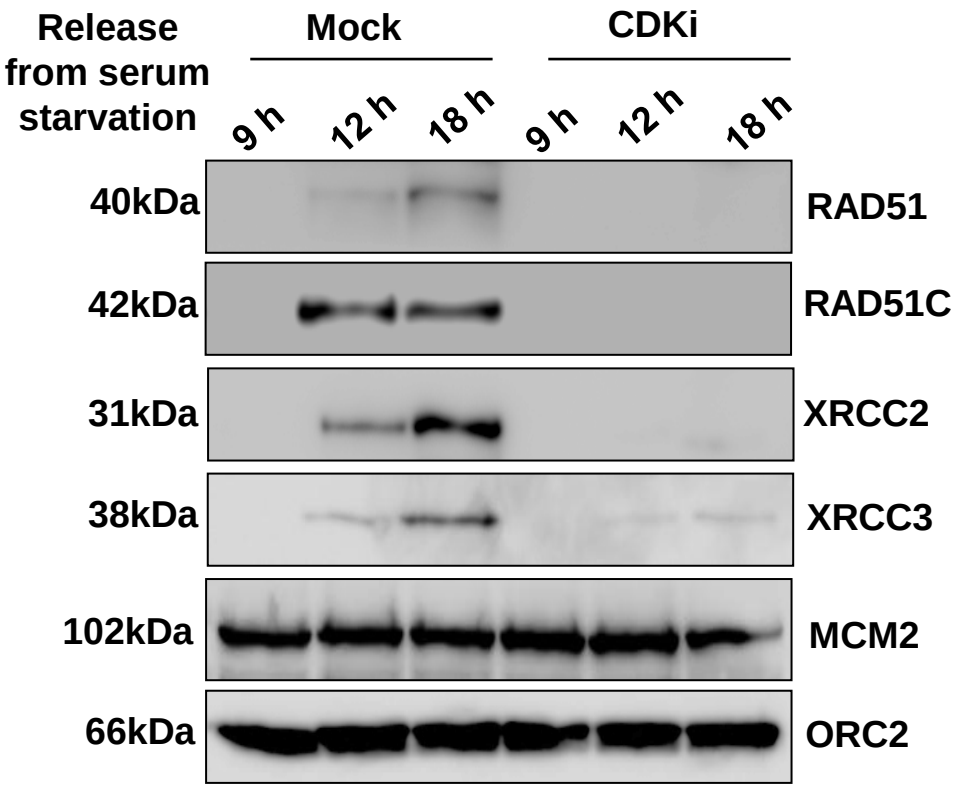

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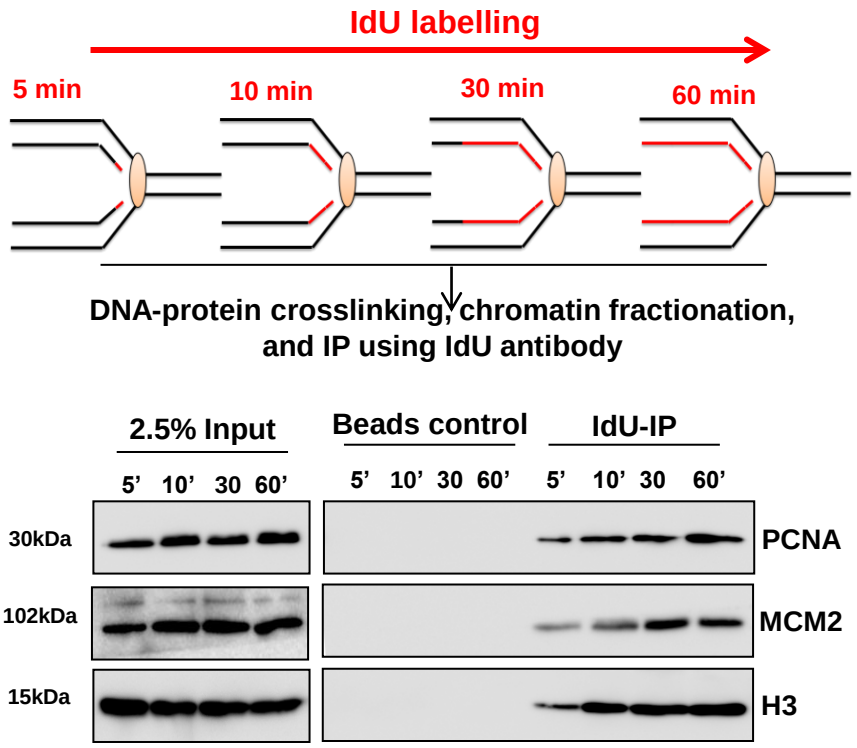

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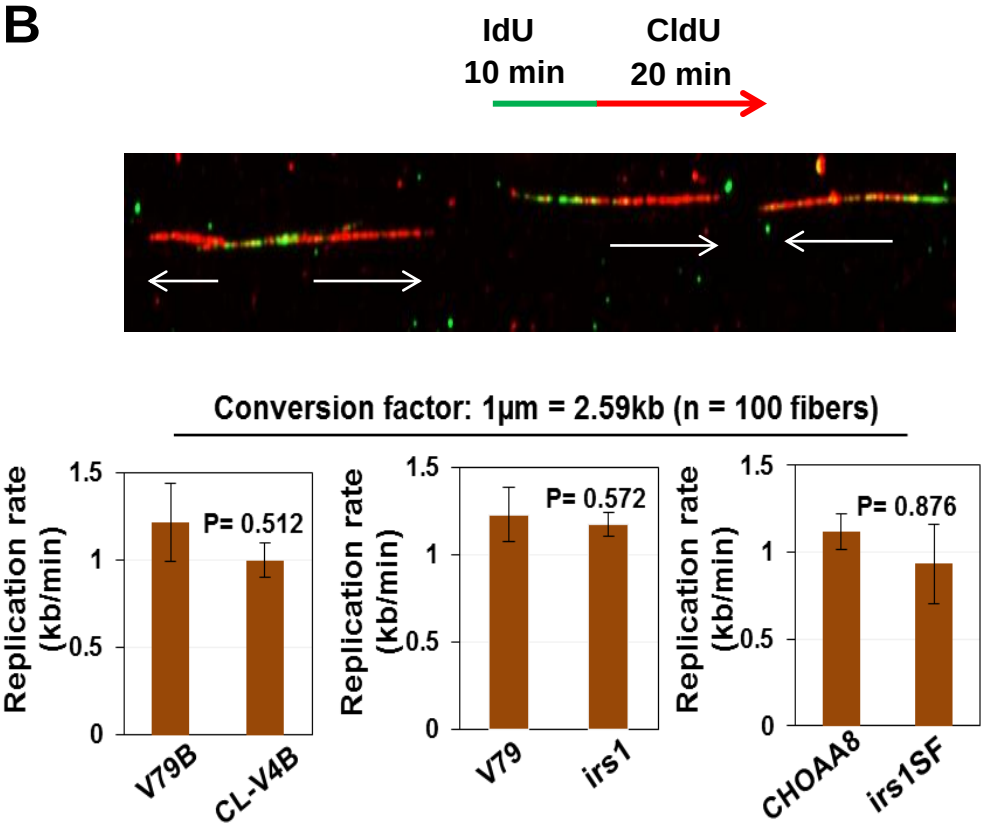

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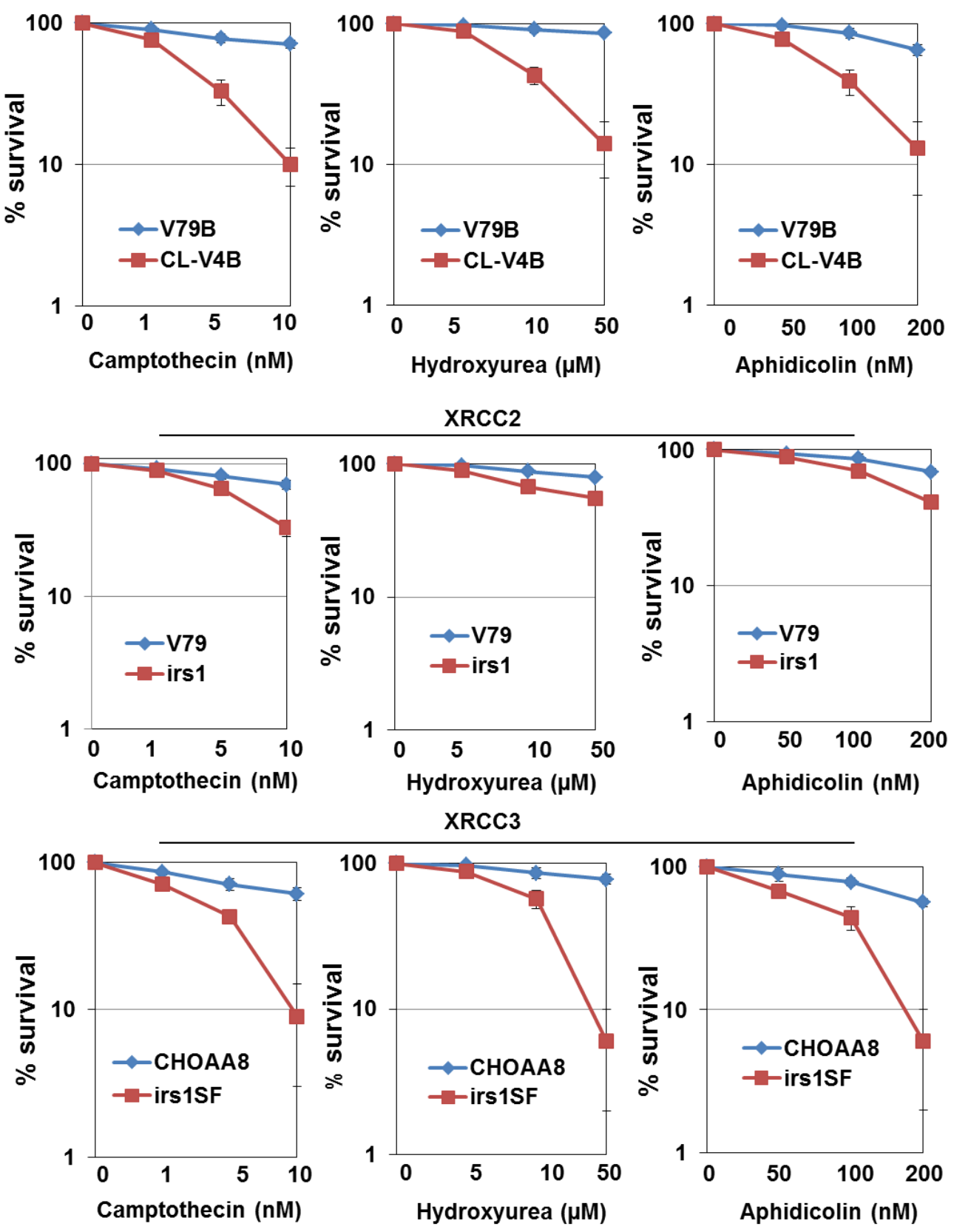

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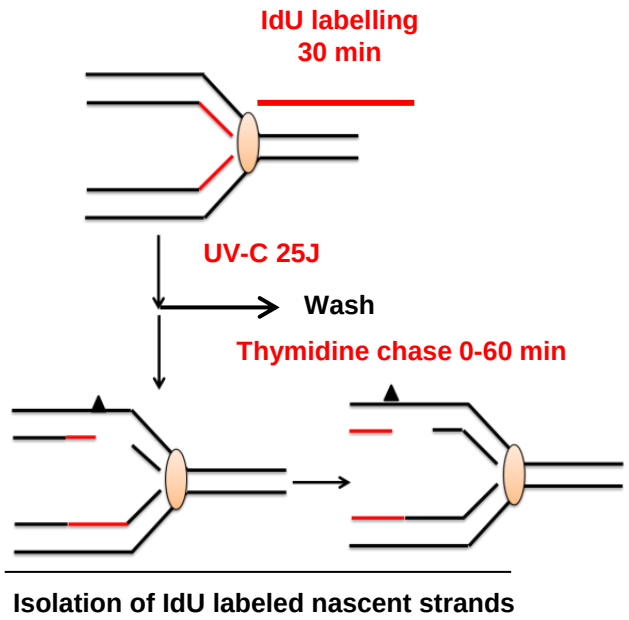

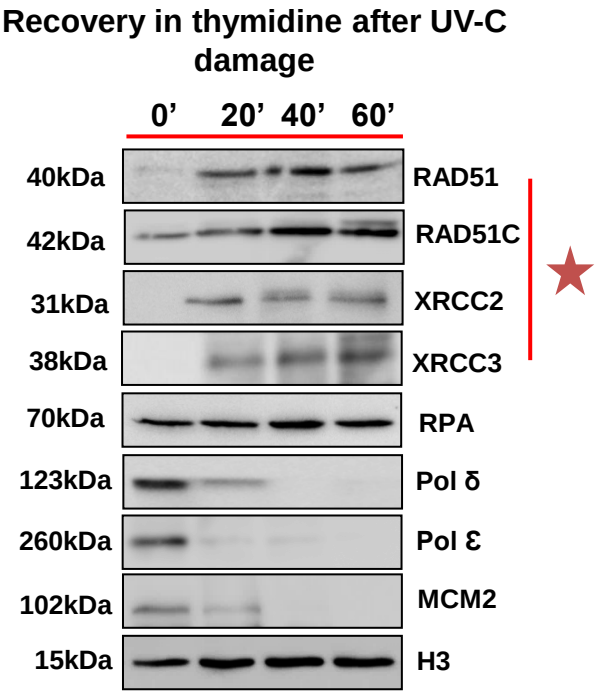

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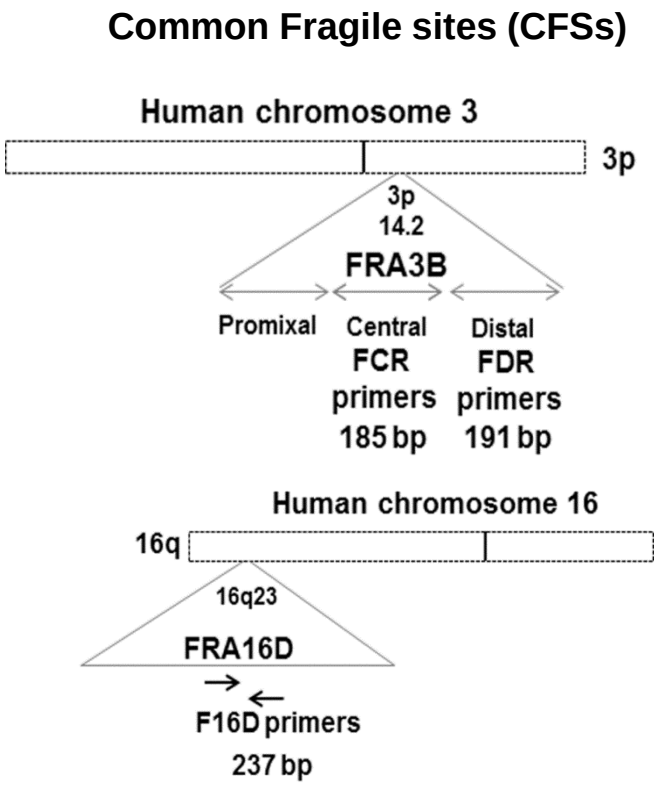

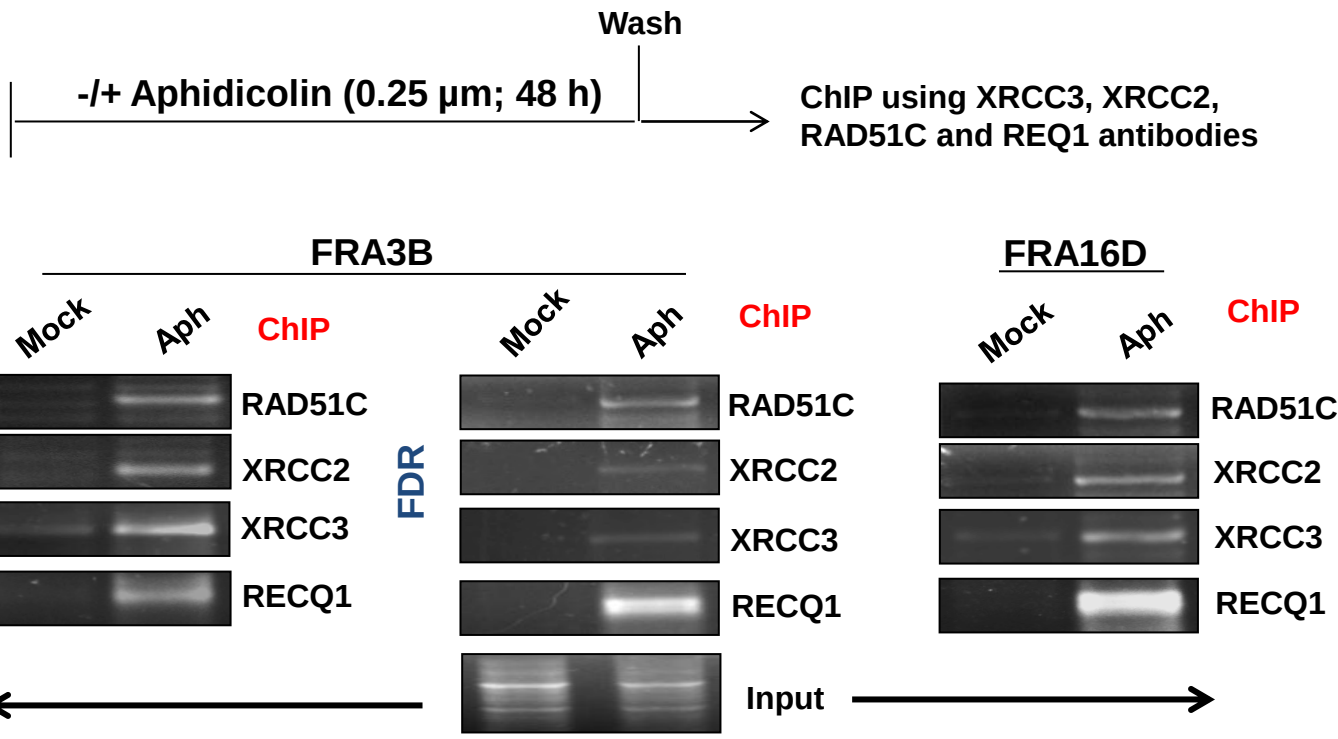

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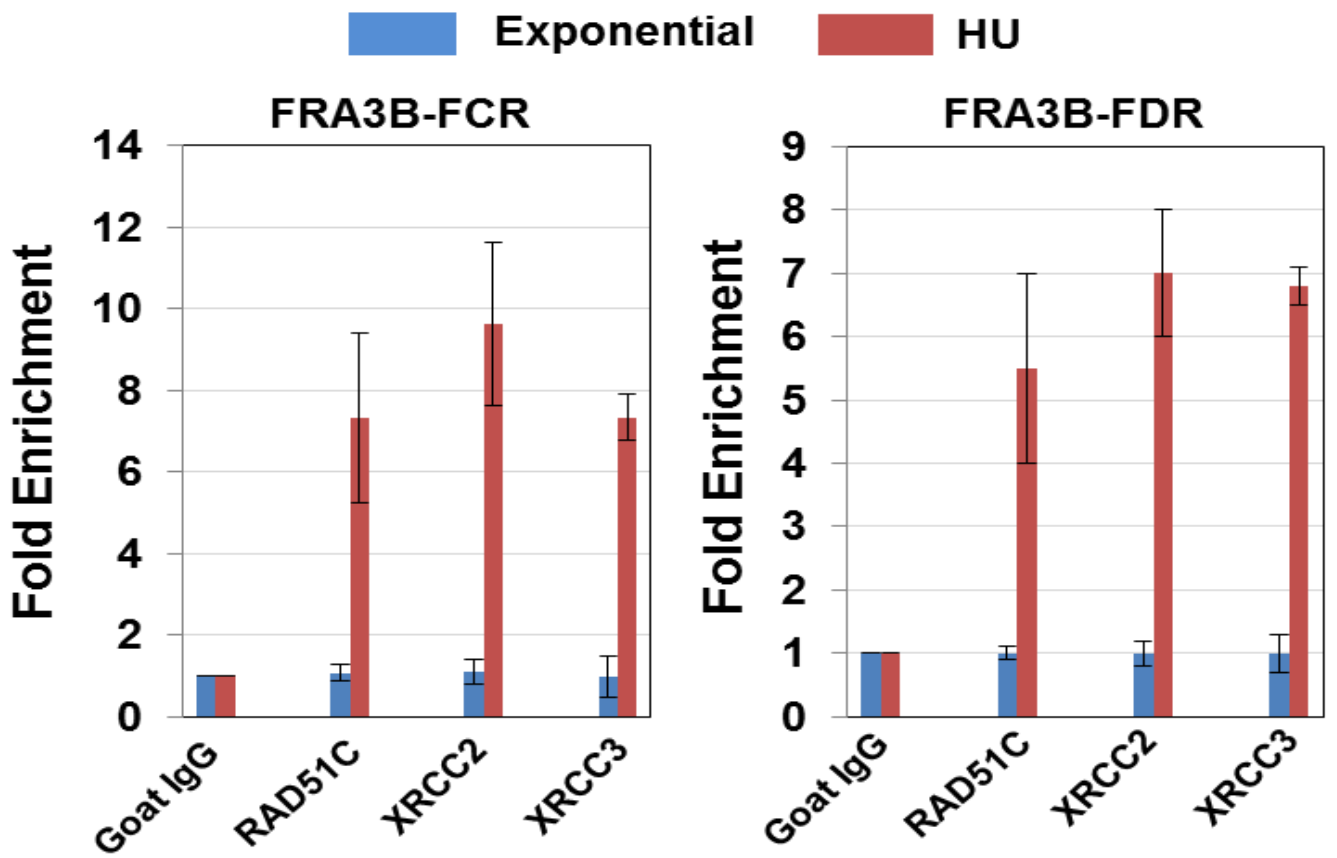

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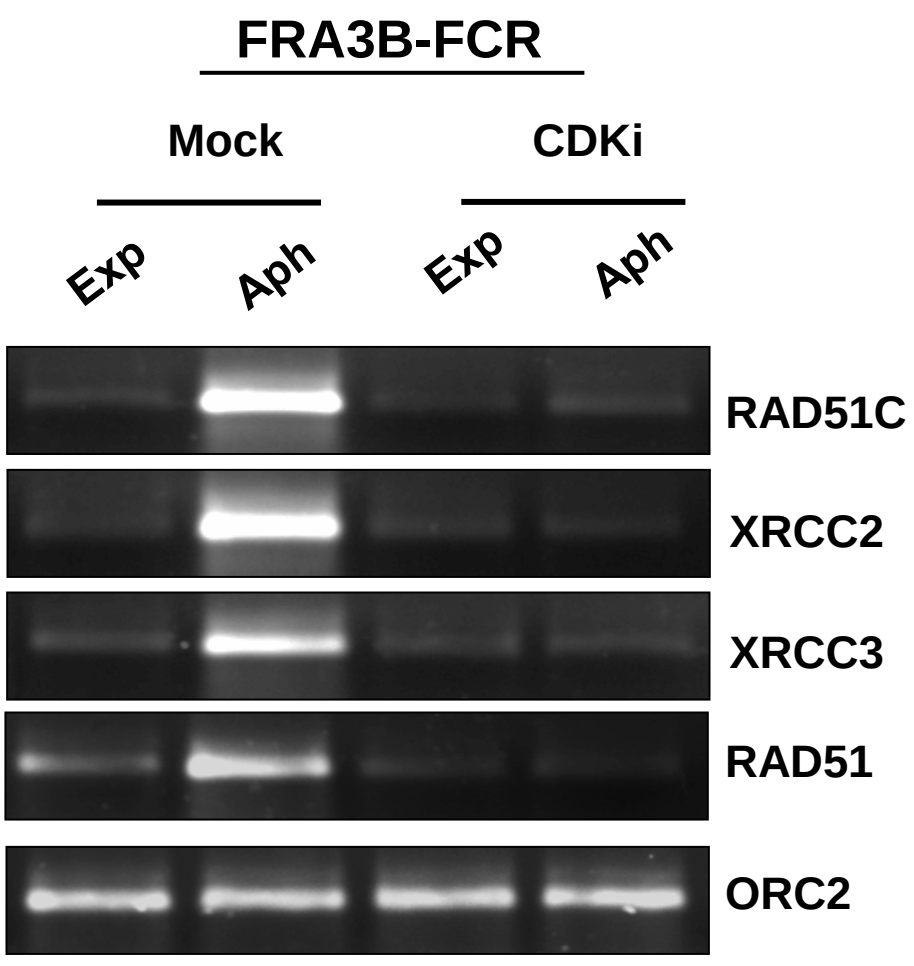

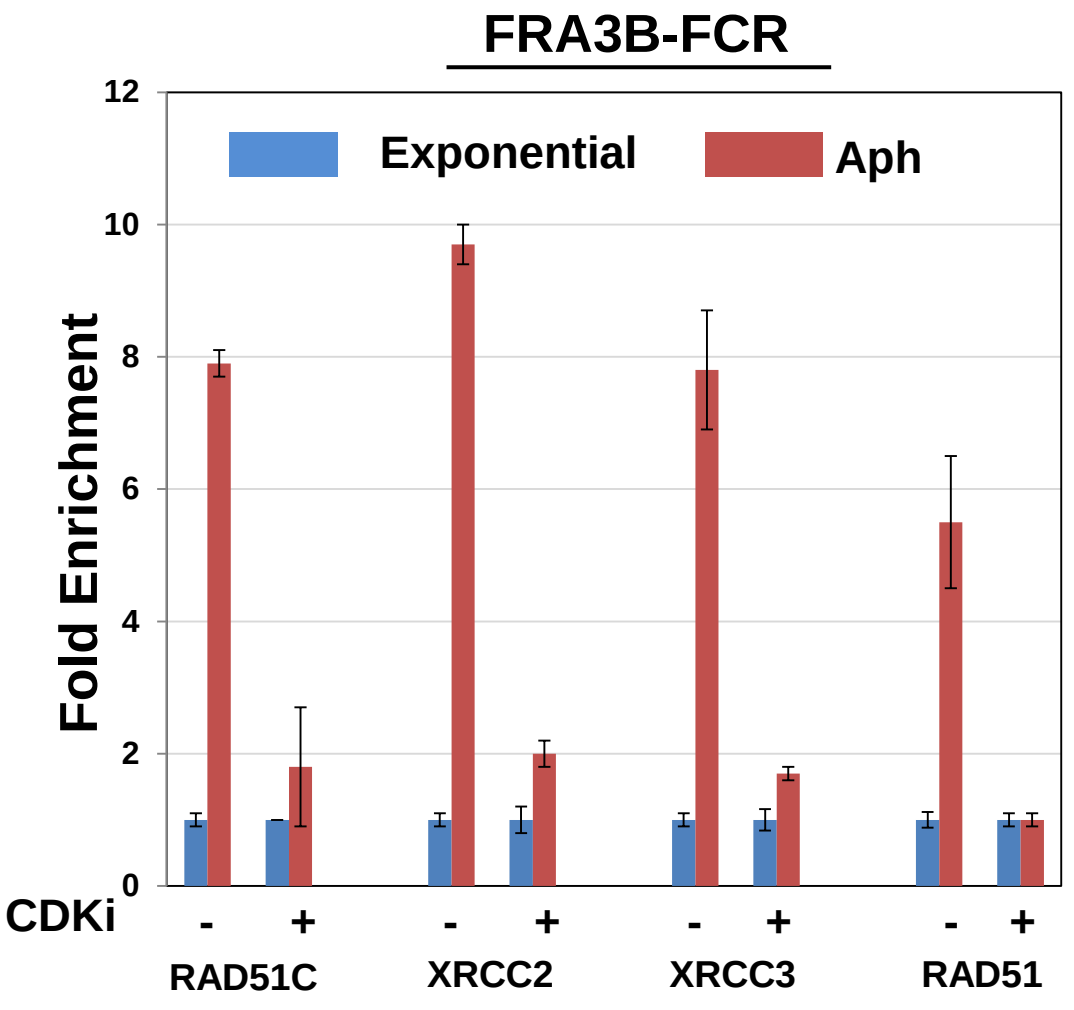

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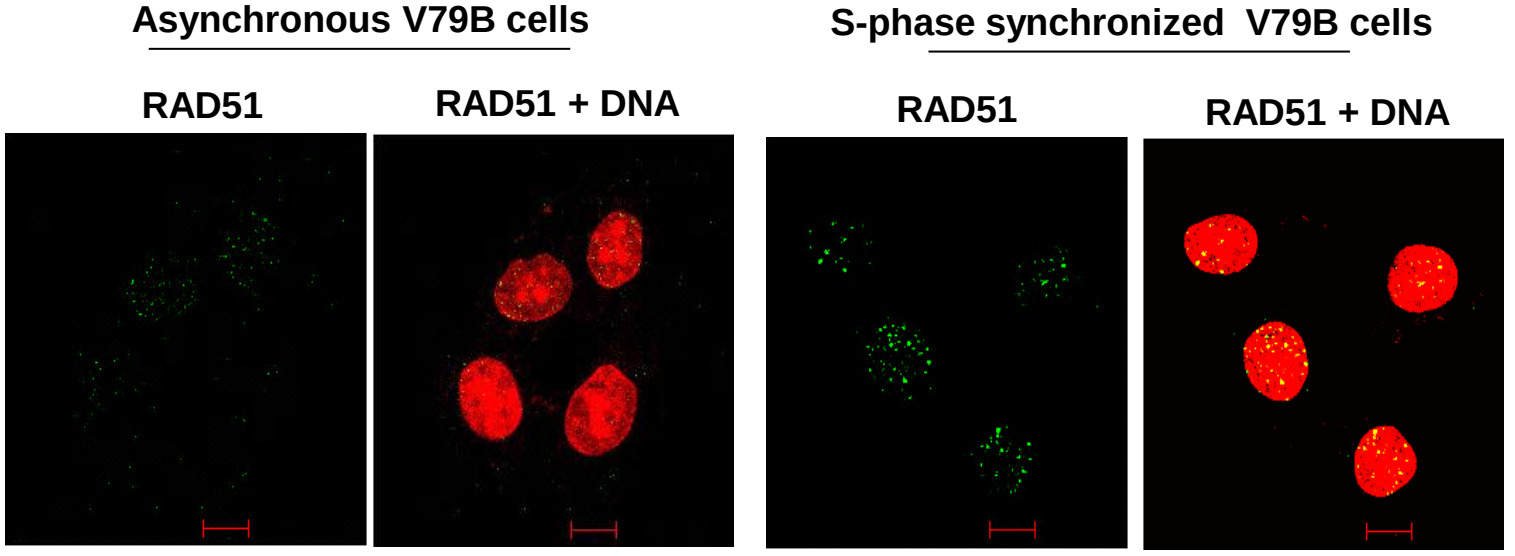

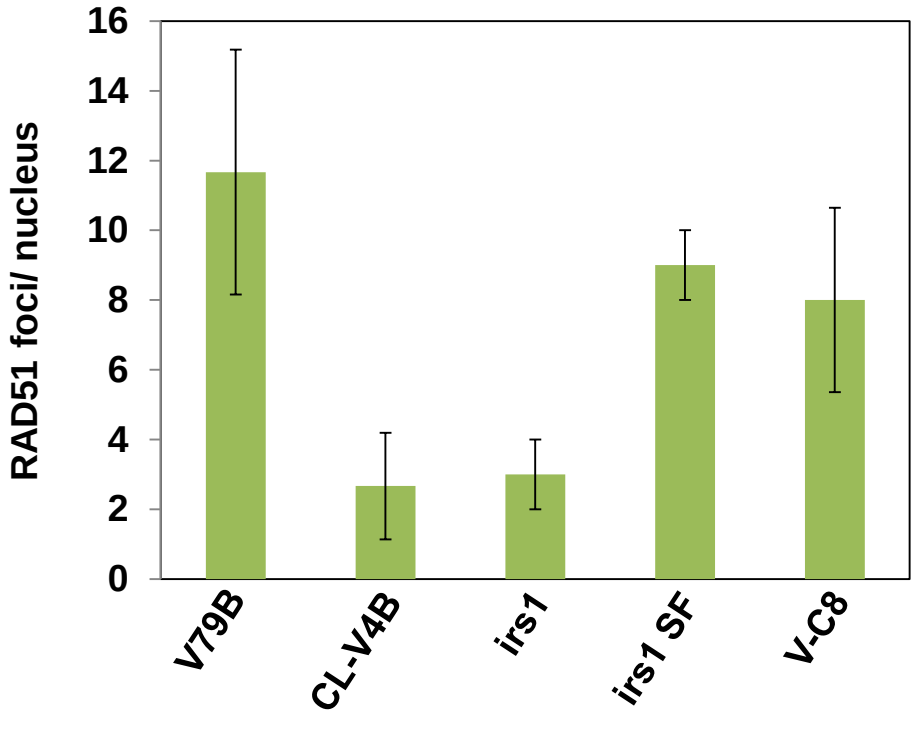

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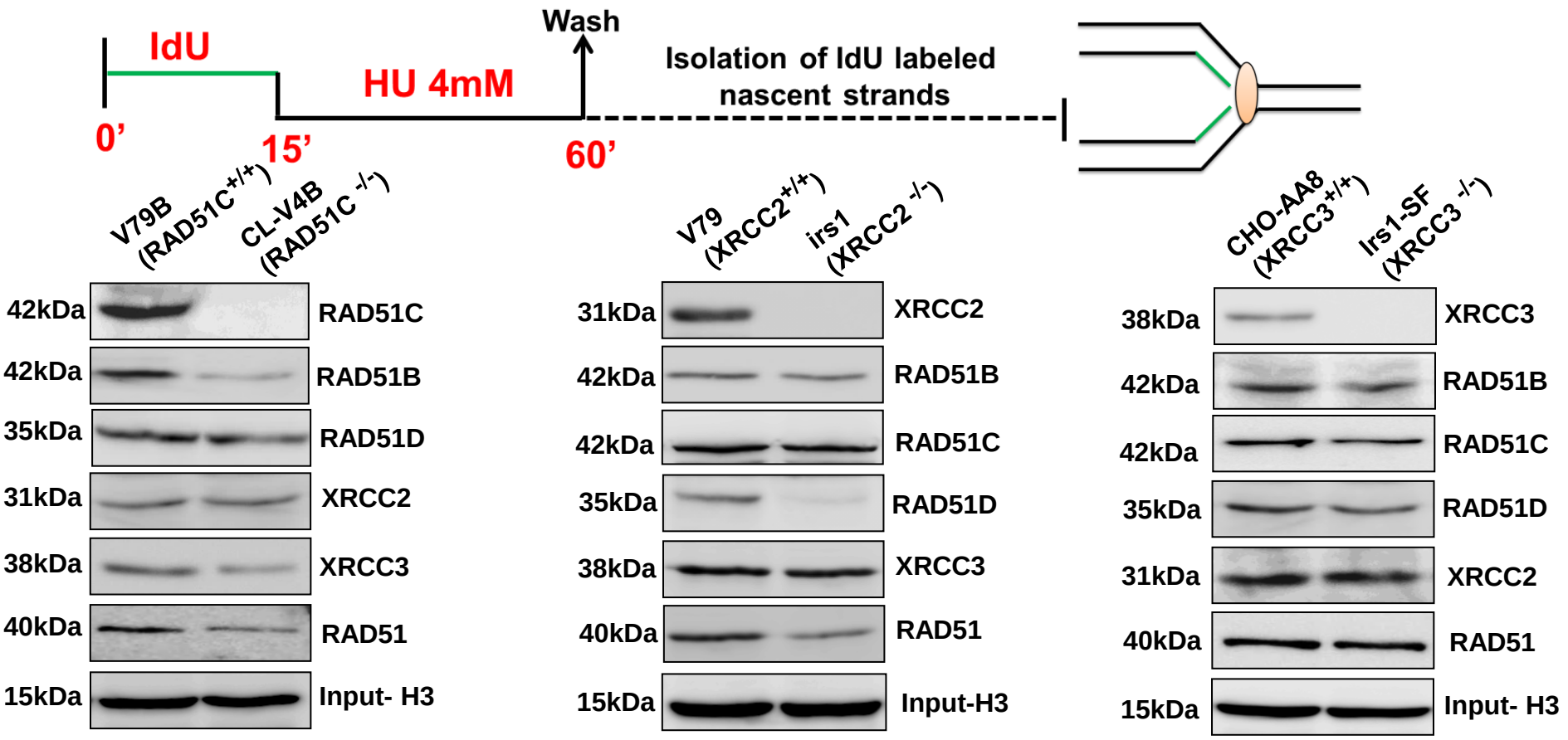

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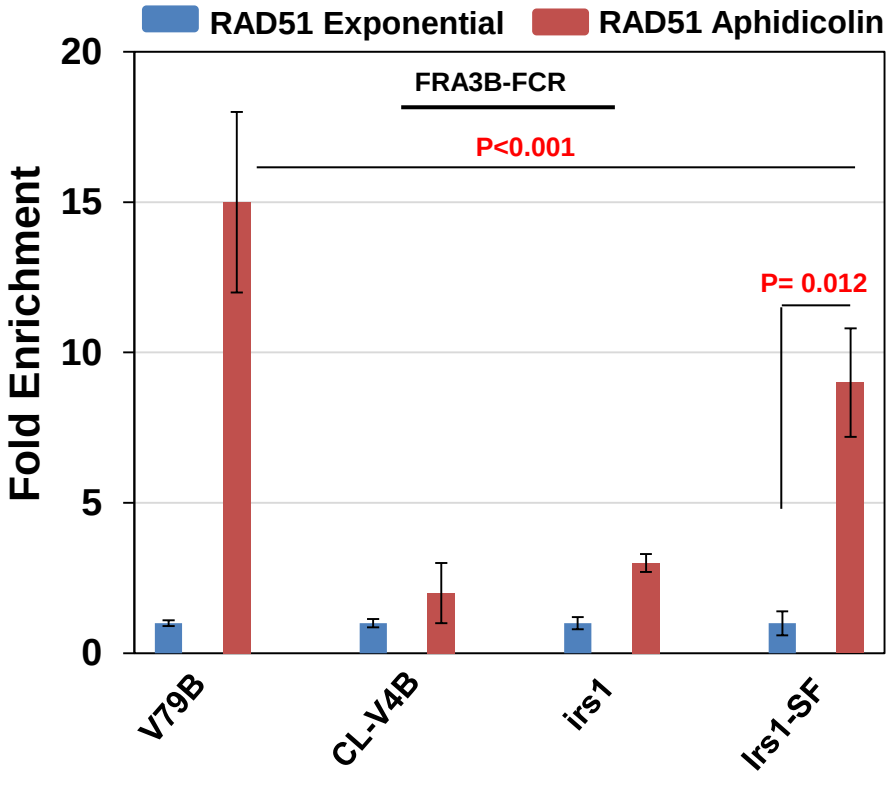

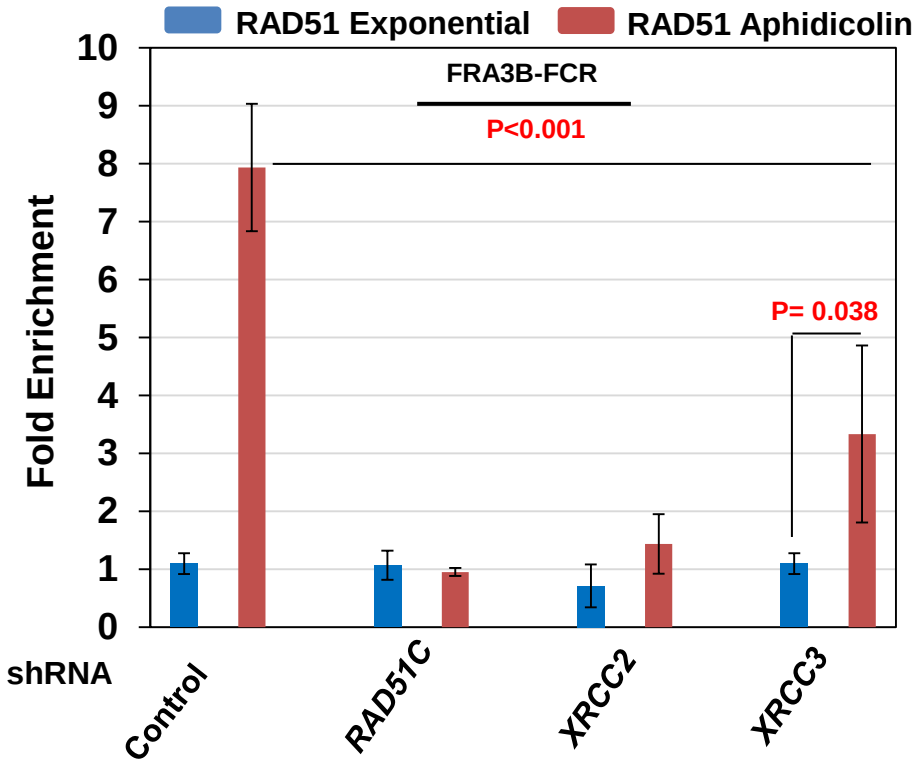

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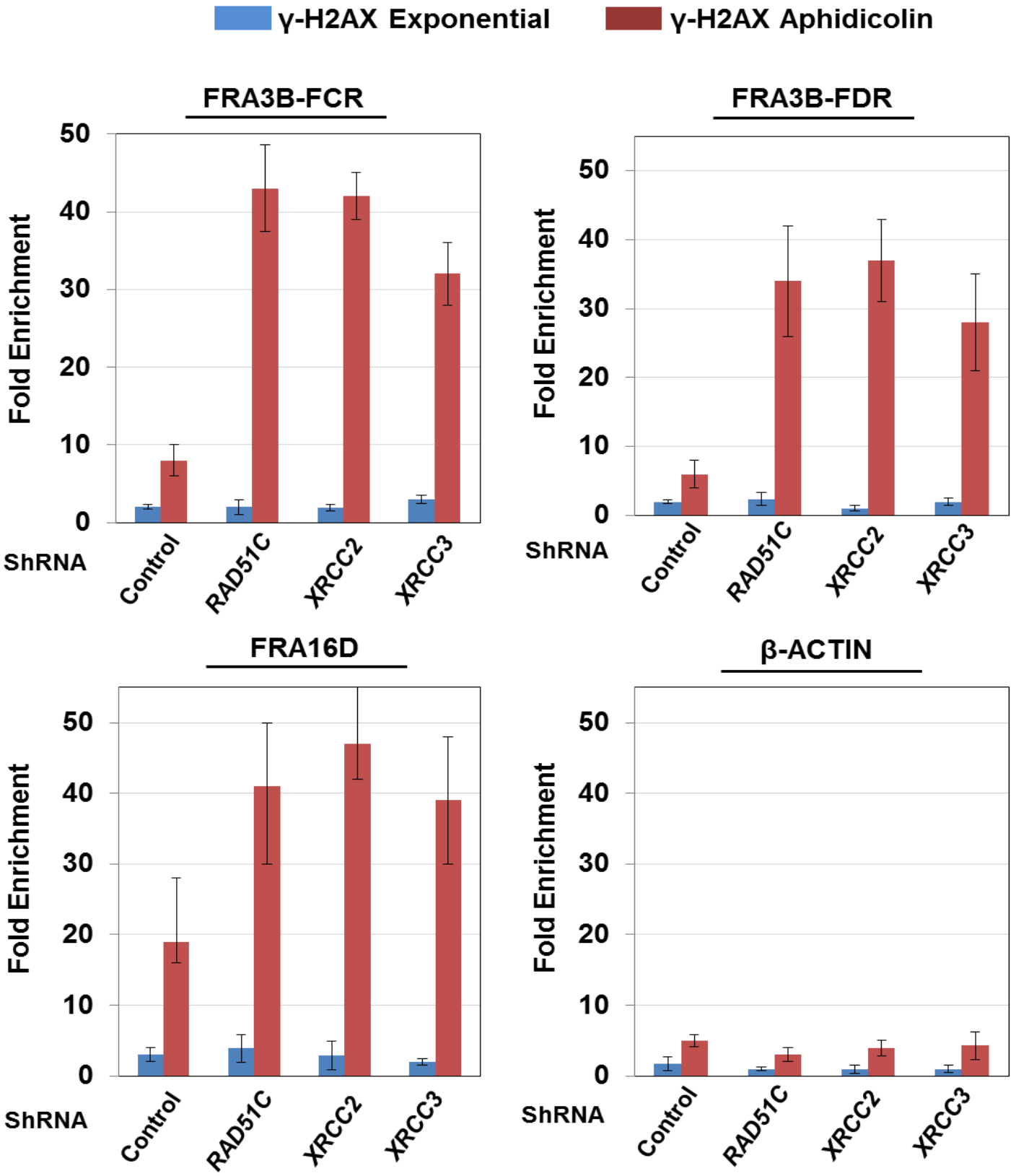

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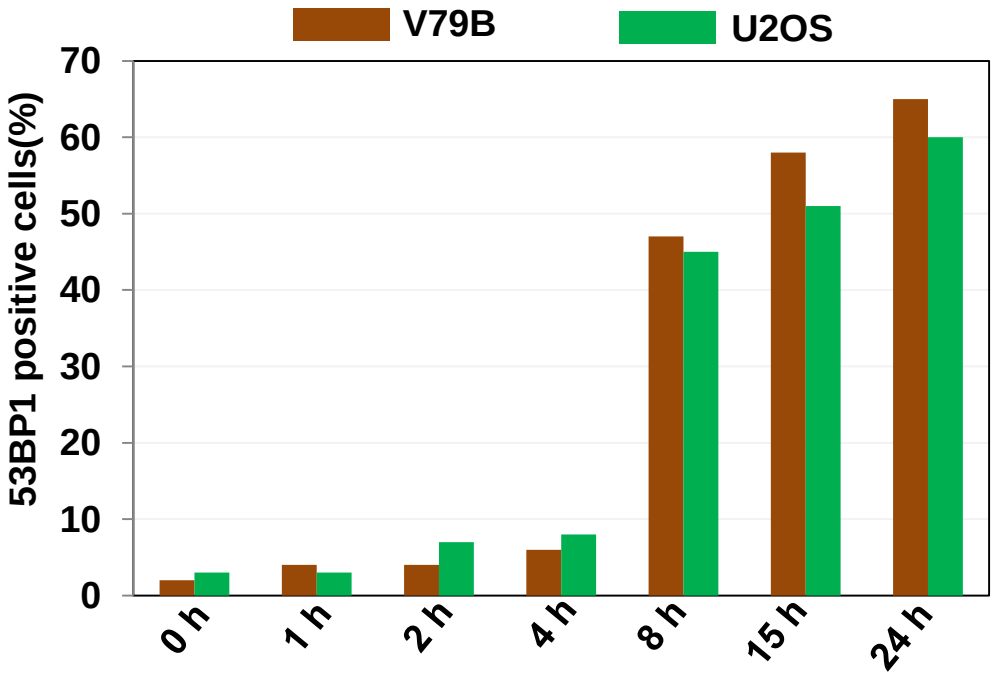

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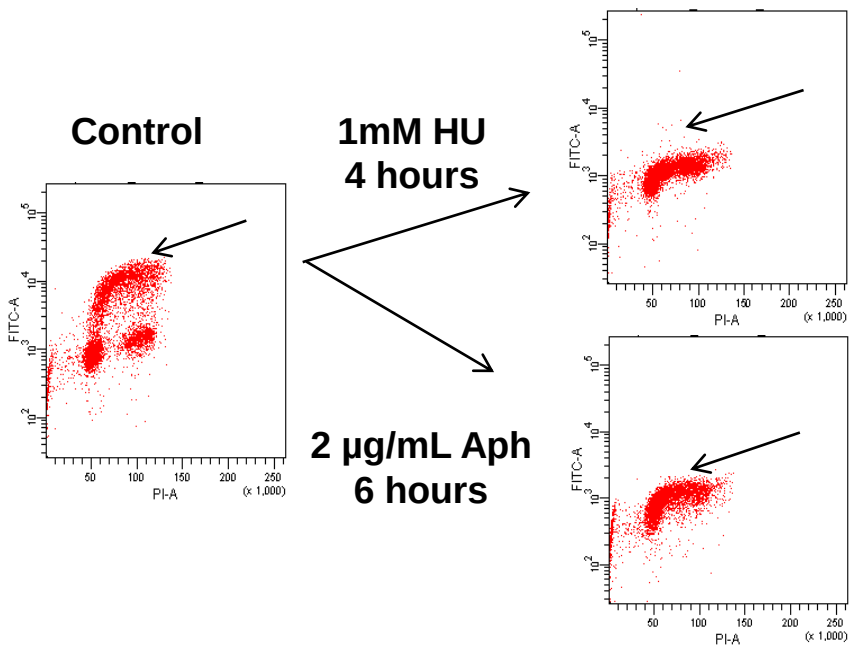

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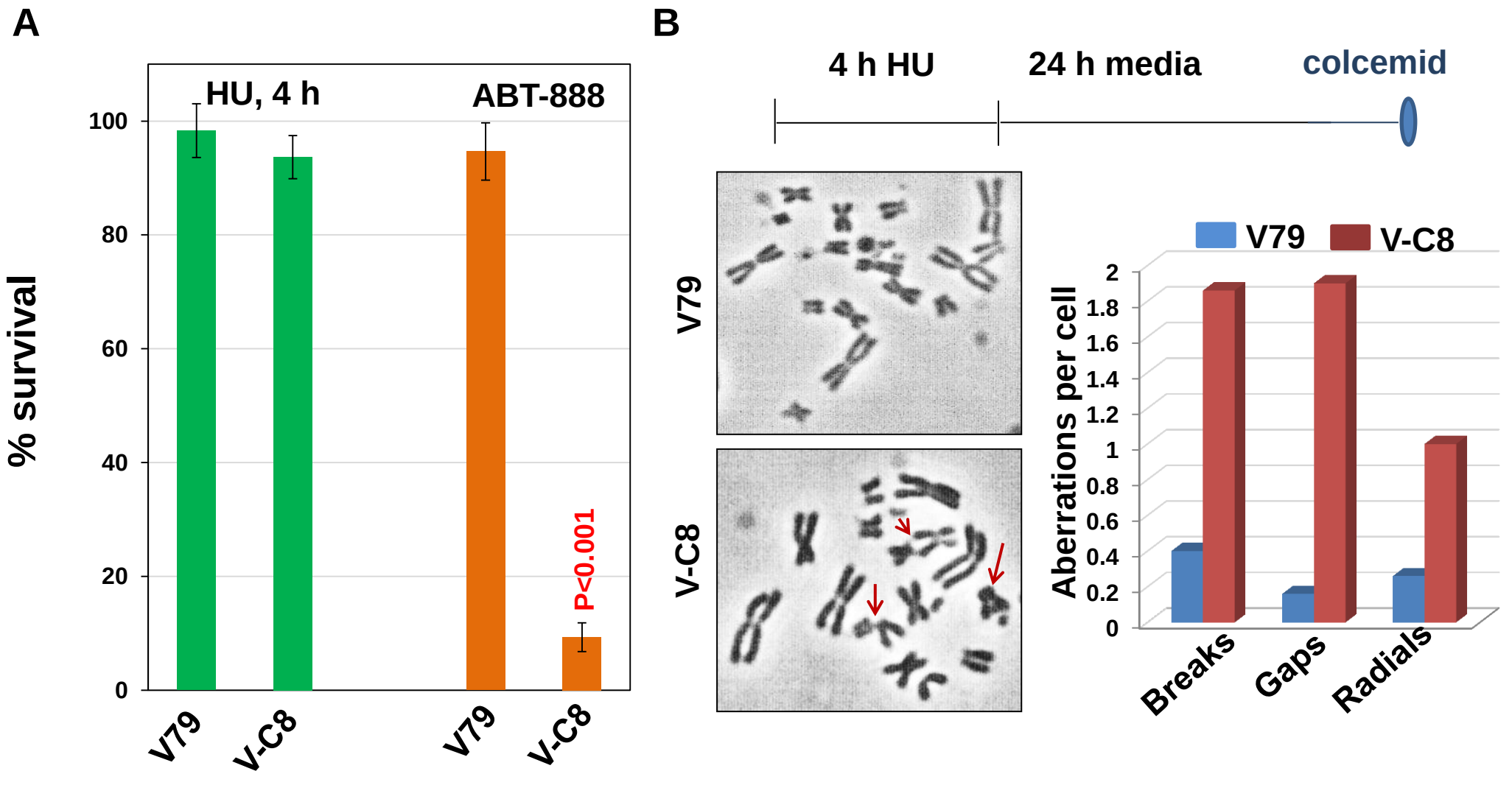
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% survival
HU, 4 h
ABT-888
P<0.001
V79
V-C8
V79
V-C8
B
4 h HU
24 h media
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V79
V-C8
V79
V-C8
Aberrations per cell
Breaks
Gaps
Radials
C
Control
HU
Median IdU tract length (μm)
V79
V-C8
D
Control shRNA
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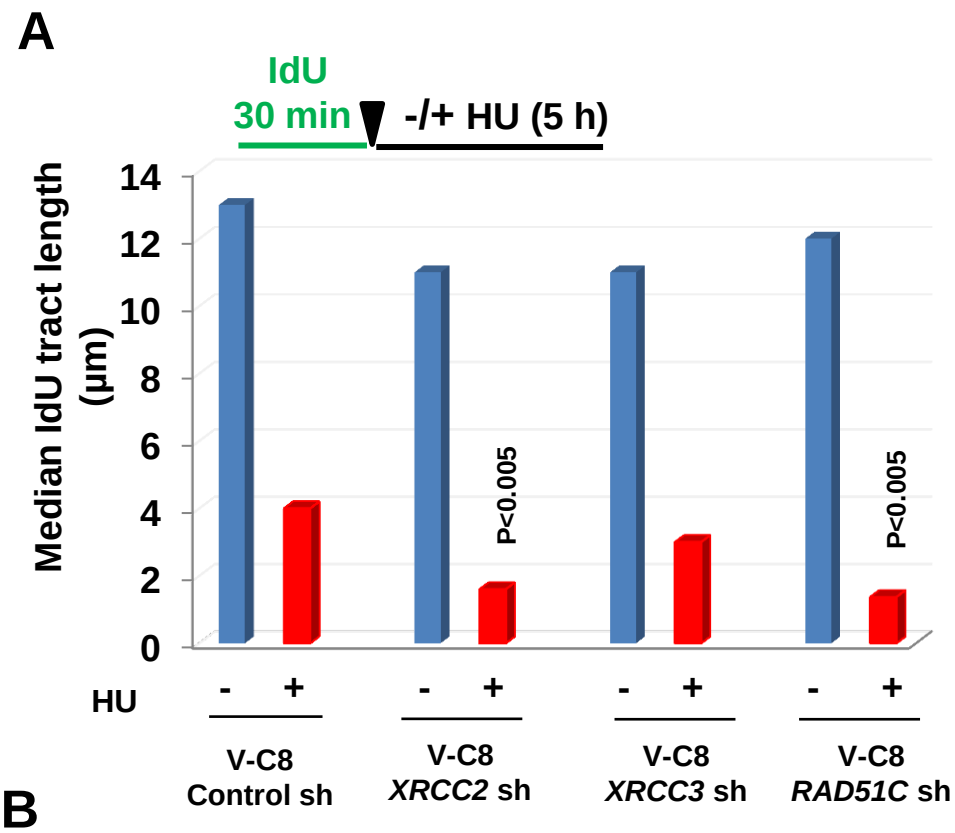
Median IdU tract length (μm)
IBR3
V79

Sup Figure 8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IdU
30 min
-/+ HU (5 h)
Median IdU tract length (µm)
14
12
10
8
6
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P<0.005
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V-C8 XRCC3 sh
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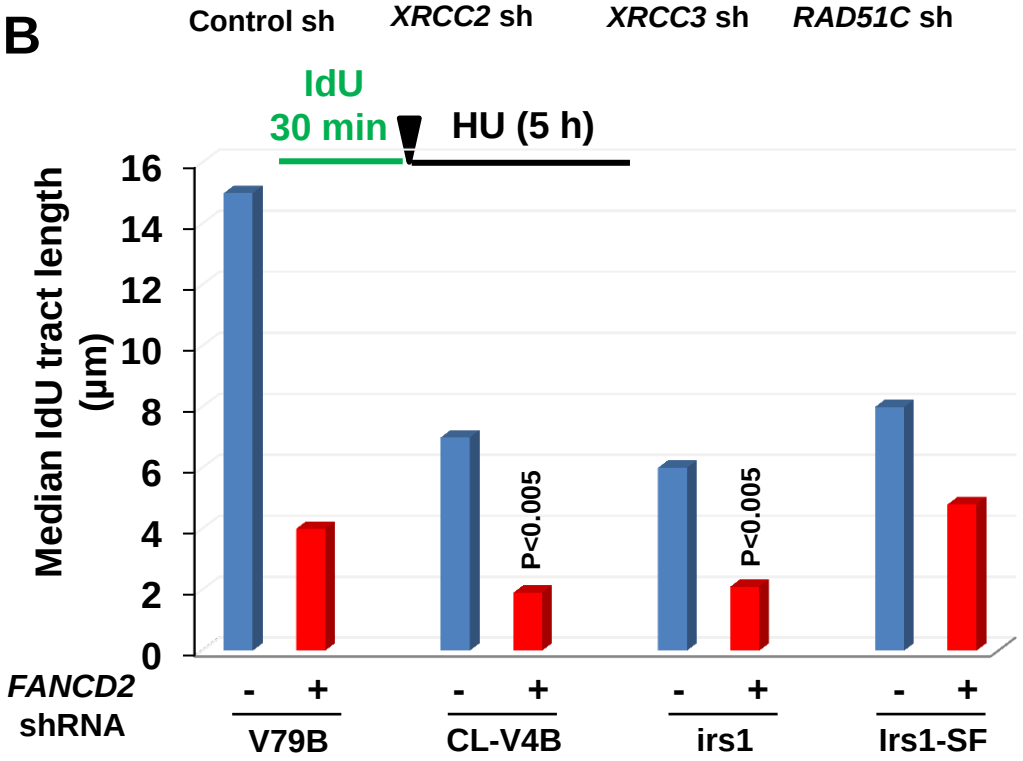
B
IdU
30 min
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Median IdU tract length (µm)
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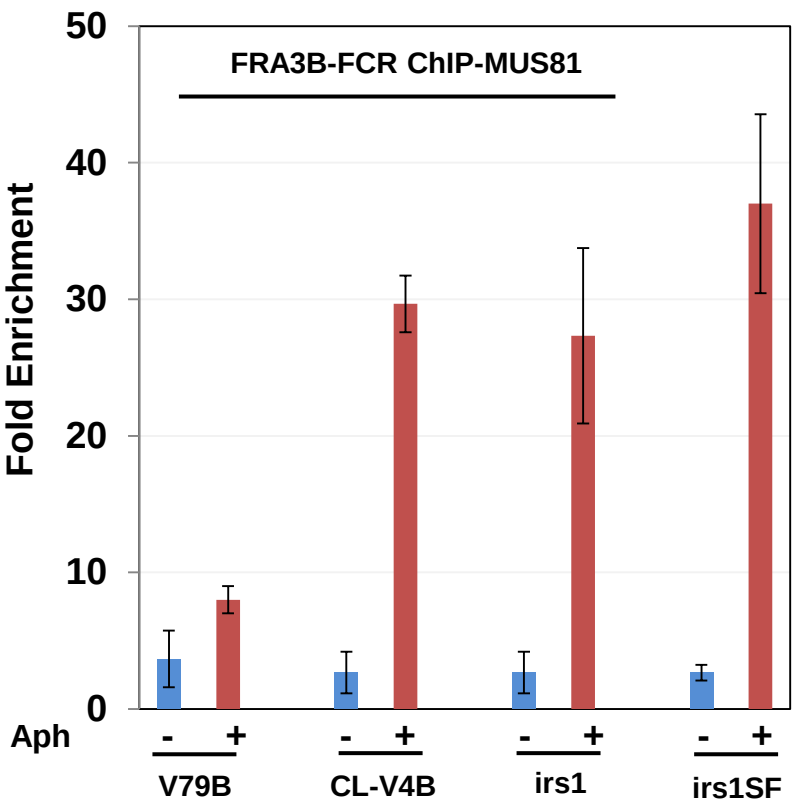
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V79B
CL-V4B
irs1
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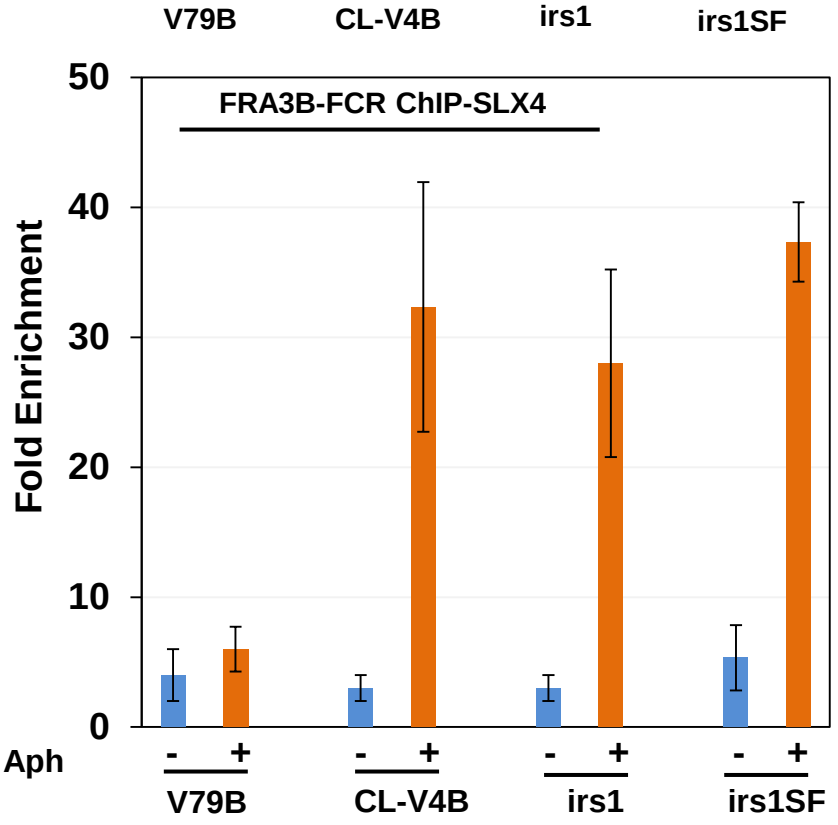
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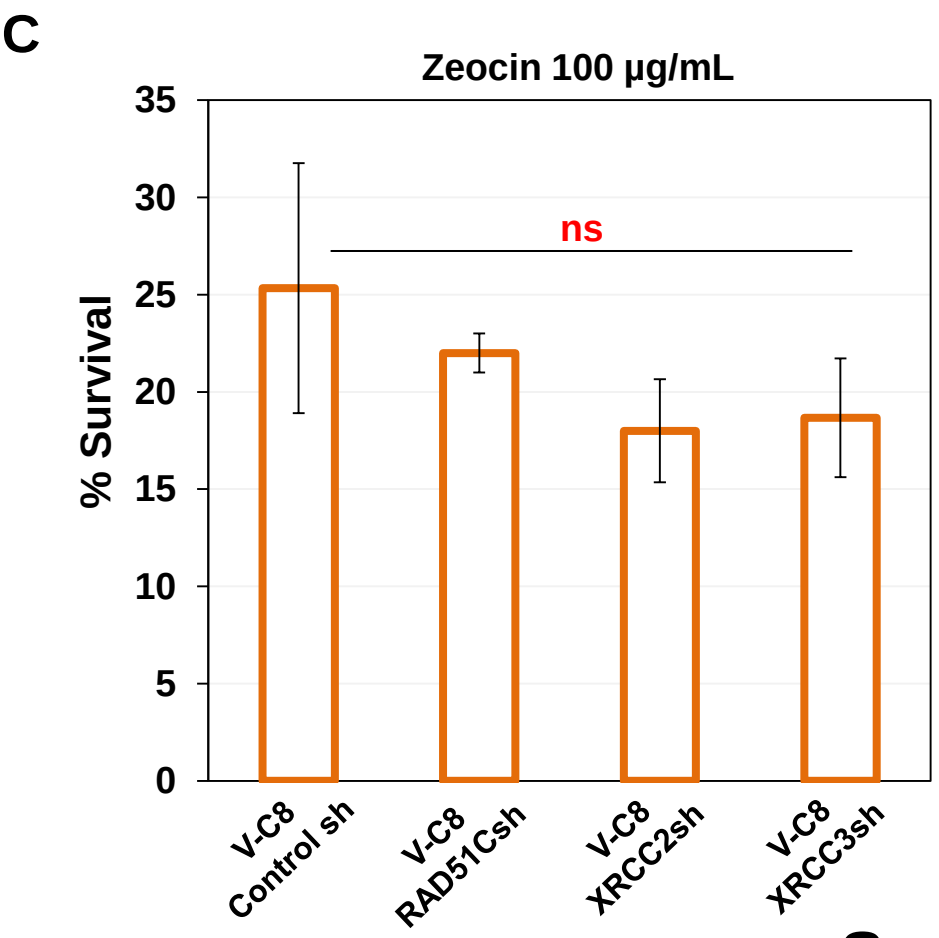
C
Zeocin 100 µg/mL
% Survival
35
30
25
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15
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ns
V-C8 Control sh
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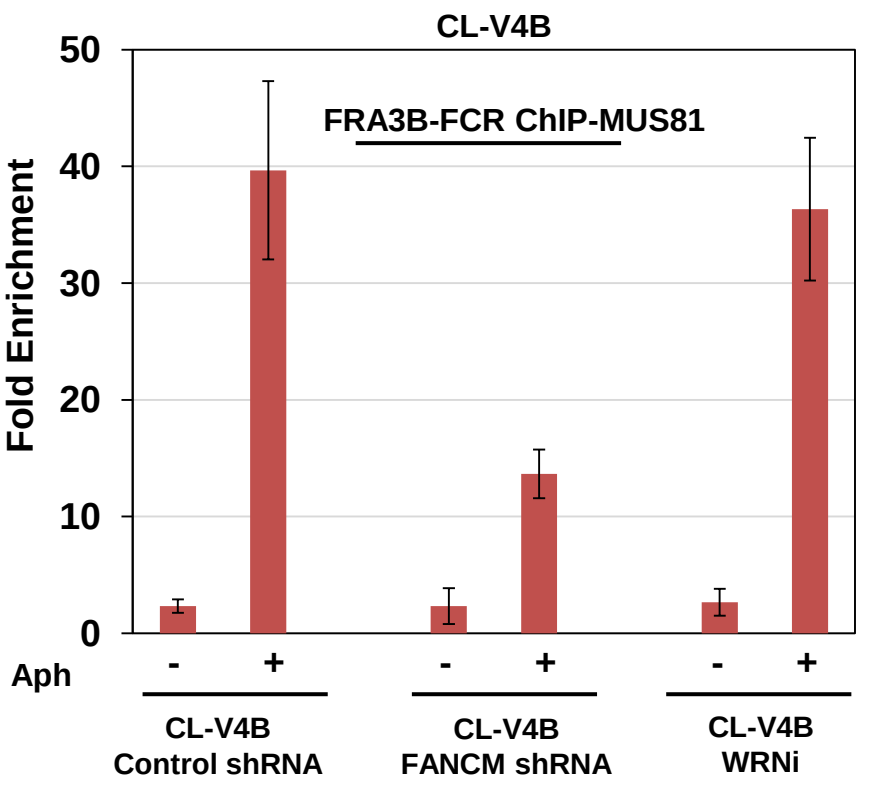
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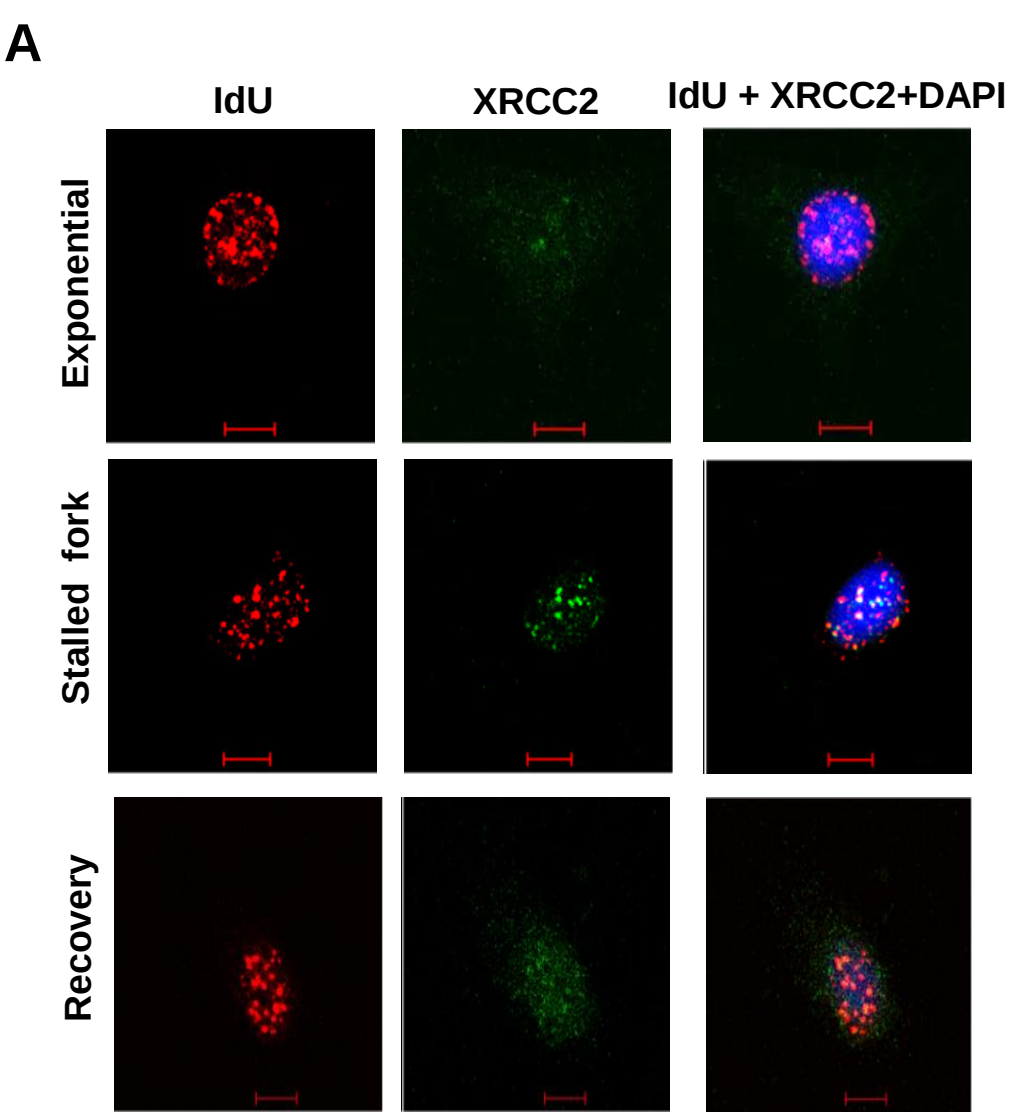
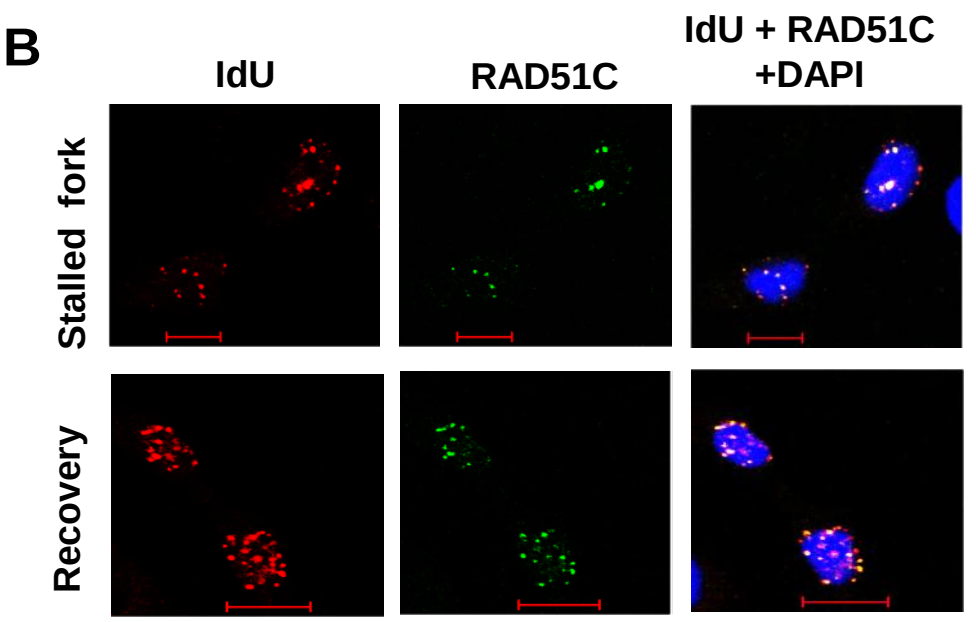
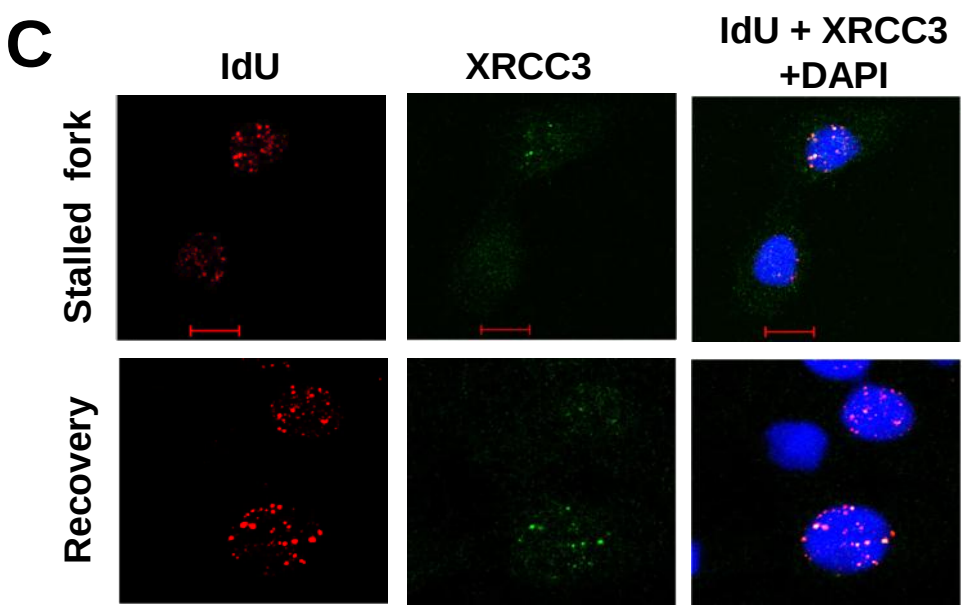
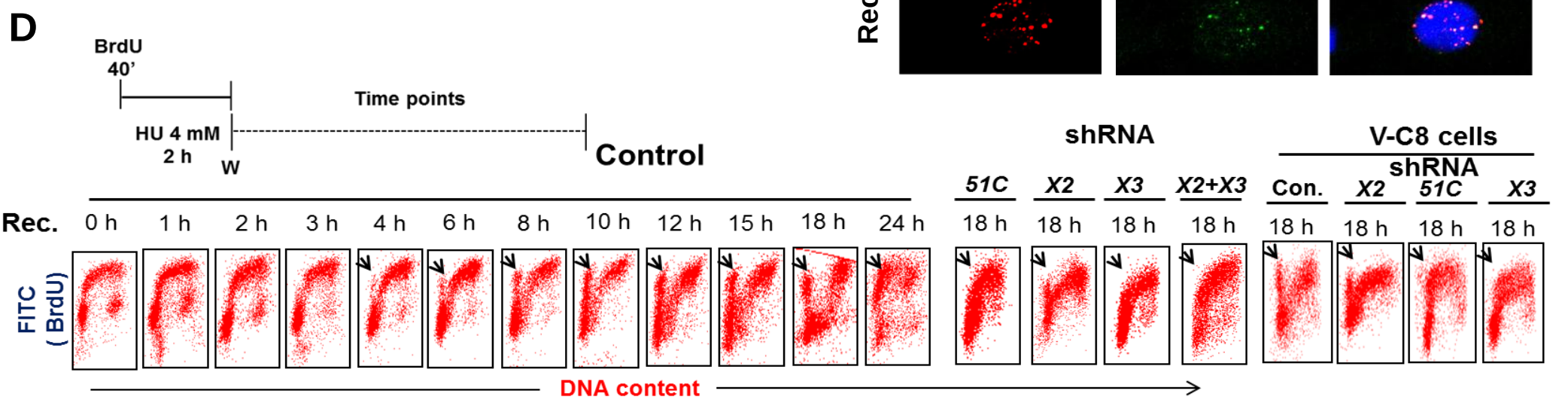
CL-V4B WRNi

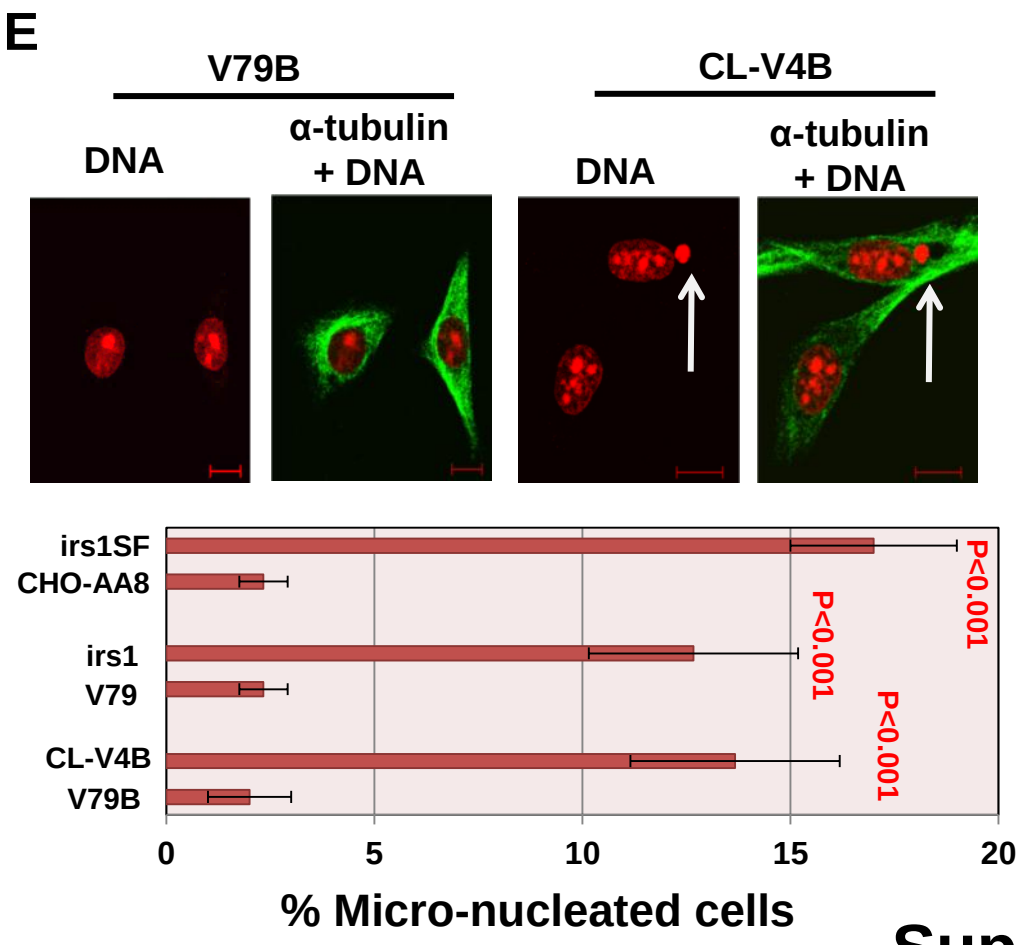
Sup Figure 9

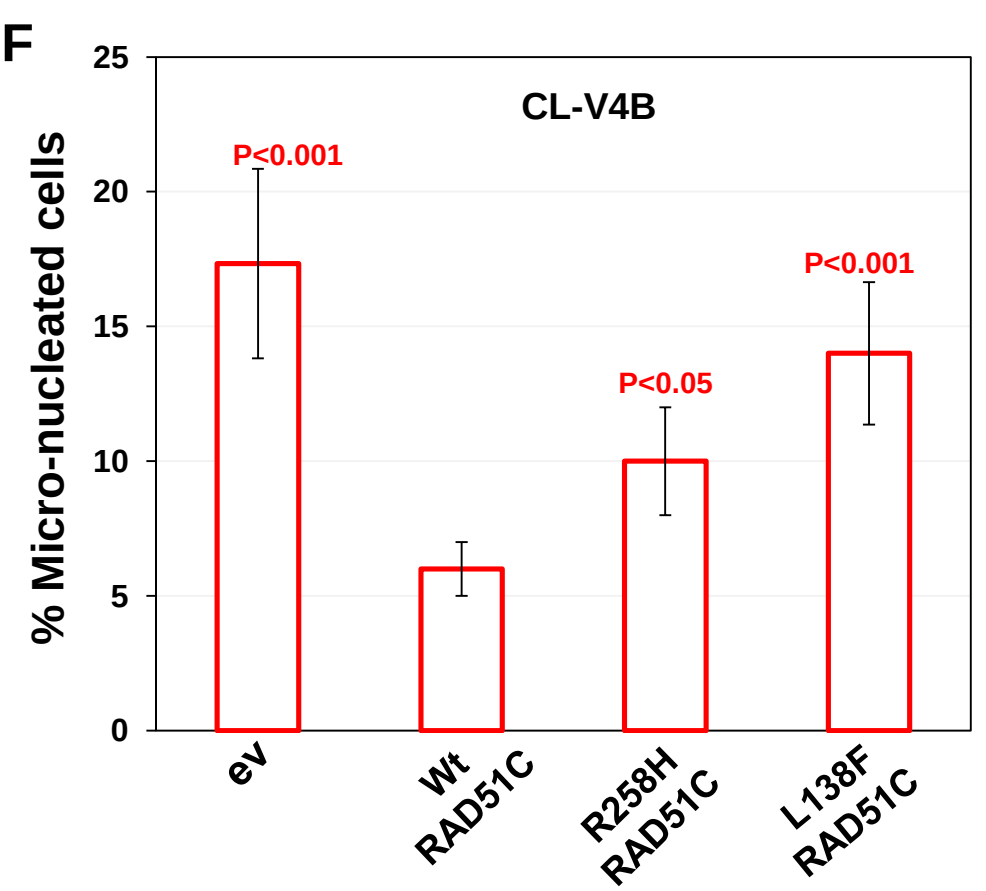












Sup Figure 10