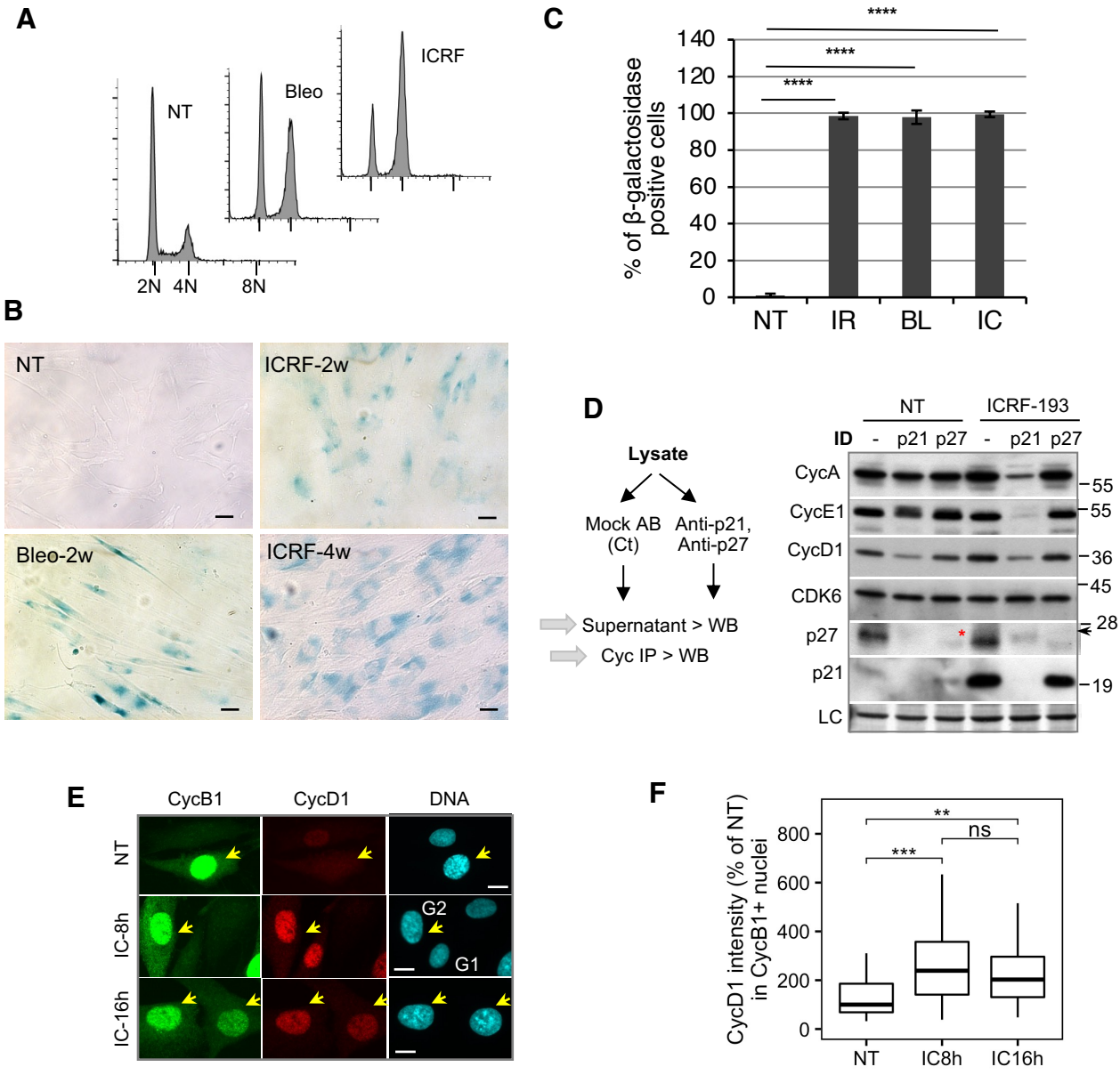



**Fig. S1. DNA damage-induced cell cycle arrest in G2 leads to cell cycle exit and senescence**

**A.** DNA content profiles obtained by flow cytometry of the asynchronously growing non-treated (NT) and HDFs exposed to bleomycin (Bleo) or ICRF-193 (ICRF) for 48 h (n=2).

**B.** Representative phase-contrast images showing β-galactosidase staining of non-treated (NT) and HDFs exposed to ICRF-193 (ICRF) and bleomycin (Bleo) for 2 and 4 weeks (n=2). NT, non-treated cells. Scale bar, 5 μm.

**C.** Quantification of β-galactosidase staining in HDF exposed to γ irradiation (IR, 10 Gy), ICRF-193 (IC) or bleomycin (BL) for two weeks. NT, non-treated cells. Data are mean +/- s.d. of two independent experiments. *P* values were calculated with two-tailed paired Student's *t*-test; ****$P \leq 0.0001$.

**D.** p21/p27 immunodepletion (ID) experiment. Left: experimental design. Right: Immunoblots showing CycE1, CycA, CycD1 and CDK6 levels before (mock-treated: Ct (-)) or after immunodepletion (ID) of p21 or p27 from the lysates prepared from NT (non-treated) HDFs and HDFs exposed to ICRF-193 for 16 h (n=2). LC, loading control is Amido Black-stained membrane. Arrow indicates position of p27 band. Red asterisk indicates immunoblot artefact.

**E.** Representative immunofluorescence images (n=2) showing co-localization of CycB1 and CycD1 in non-treated (NT) HDFs and HDFs exposed to ICRF-193 (IC) for 8 h and 16 h. Scale bars, 10 μm. Arrows points at cells expressing nuclear CycB1. Pro, prophase.

**F.** Quantification of CycD1 intensity in CycB1-positive HDFs exposed to ICRF-193 (IC) for indicated times (expressed as a percentage of CycD1 intensity in NT). Cells (n>100) were pooled from two independent experiments. The box represents the 25–75th percentiles, central line indicates the median, and whiskers indicate the 10th and 90th percentiles. ns, not significant, **$P \leq 0.01$; ***$P \leq 0.001$. *P* values were calculated with two-tailed unpaired Student's *t*-test.

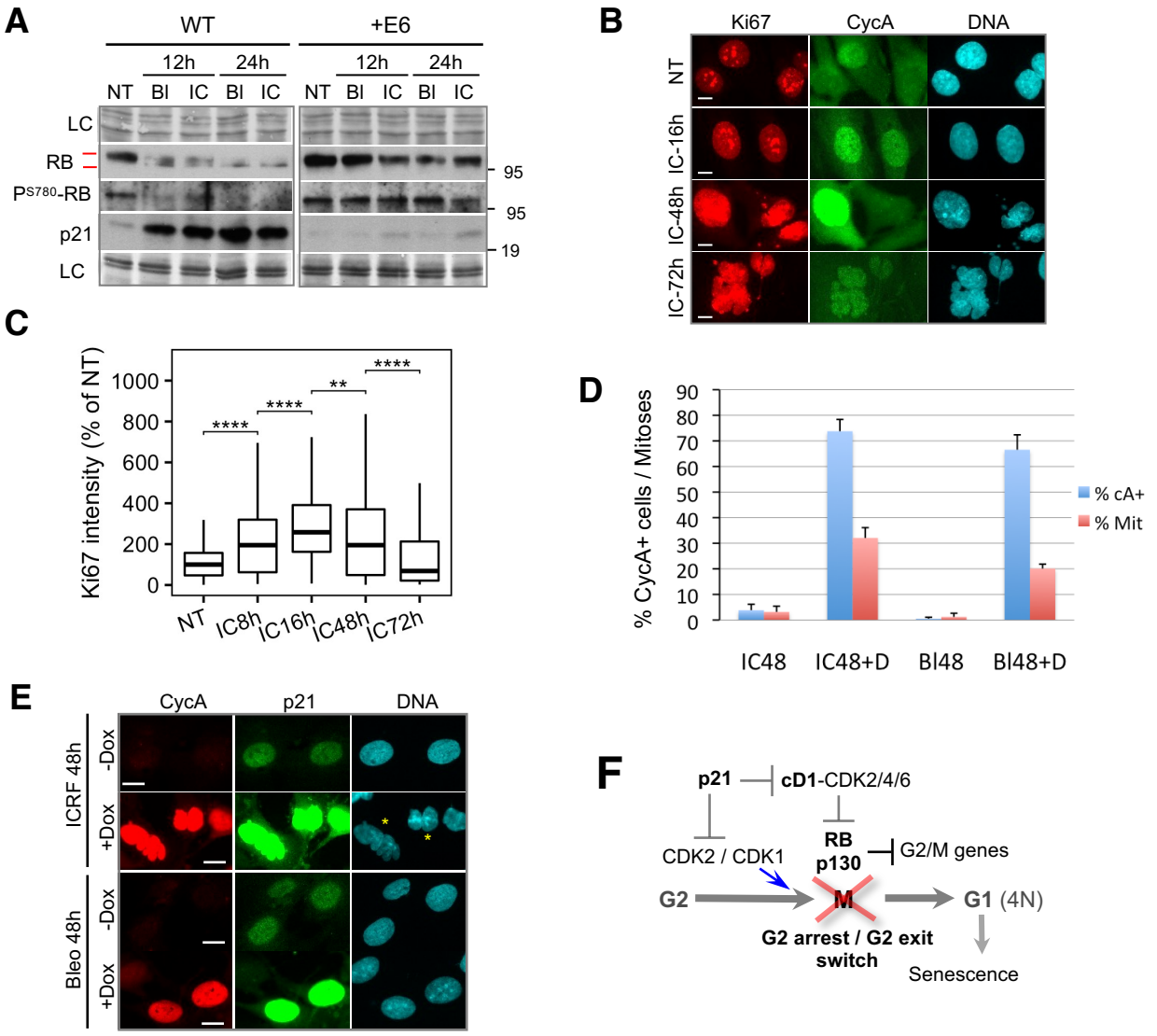



**Fig. S2. HPV-E6-mediated p53 degradation and inactivation of pocket proteins by the $T_{121}$ mutant of SV40 oncogene compromises G2 exit in human fibroblasts**

**A.** Immunoblots showing persistent CycD1-CDK-specific RB phosphorylation ($P^{S780}$) in p21-deficient HPV16-E6 expressing HDFs (E6) exposed to bleomycin (BI) or ICRF-193 (IC) for 12 and 24 h. Loading controls (LC) were Amido Black-stained membranes. Red bars indicate RB phosphorylation shift (n=2).

**B.** Representative immunofluorescence images (n=2) showing expression of Ki67 and CycA in HDF-E6 cells exposed to ICRF-193 (IC) for indicated times. Scale bars, 10 μm.

**C.** Quantification of Ki67 intensity in the nuclei of HDF-E6 cells exposed to ICRF-193 (IC) for indicated times (expressed as a percentage of Ki67 intensity in NT). Cells (n>100) were pooled from two independent experiments. The box represents the 25–75th percentiles, central line indicates the median, and whiskers indicate the 10th and 90th percentiles. *P* values were calculated with two-tailed paired? Student's *t*- test; $^{**}P \leq 0.01$; $^{****}P \leq 0.0001$. NT, non-treated cells.

**D.** CycA positive (cA+) cells and mitotic cells (Mit, DAPI) were scored from immunofluorescence images taken at the indicated time points after the drug addition. $T_{121}$-expressing fibroblasts were exposed to bleomycin (BI) or ICRF-193 (IC) for 48 h. Doxycycline (D) was added 12 h prior to drug treatment (+). More than 100 cells were analyzed in each experiment. Data are mean +/- s.e.m. of two independent experiments.

**E.** Representative immunofluorescence images (n=2) showing co-expression of CycA and p21 in the presence of ICRF-193 (ICRF) and bleomycin (Bleo) in $T_{121}$-expressing fibroblasts. Asterisks denote binuclear cells generated by unsuccessful cytokinesis after mitosis. Experimental conditions were as above. Dox, doxycycline. Scale bars, 10 μm.

**F.** Model proposing the role of p21-mediated CycD1-CDK2/4 inhibition in the G2-arrest-G2-exit switch preceding senescence (cD1, cyclin D1).

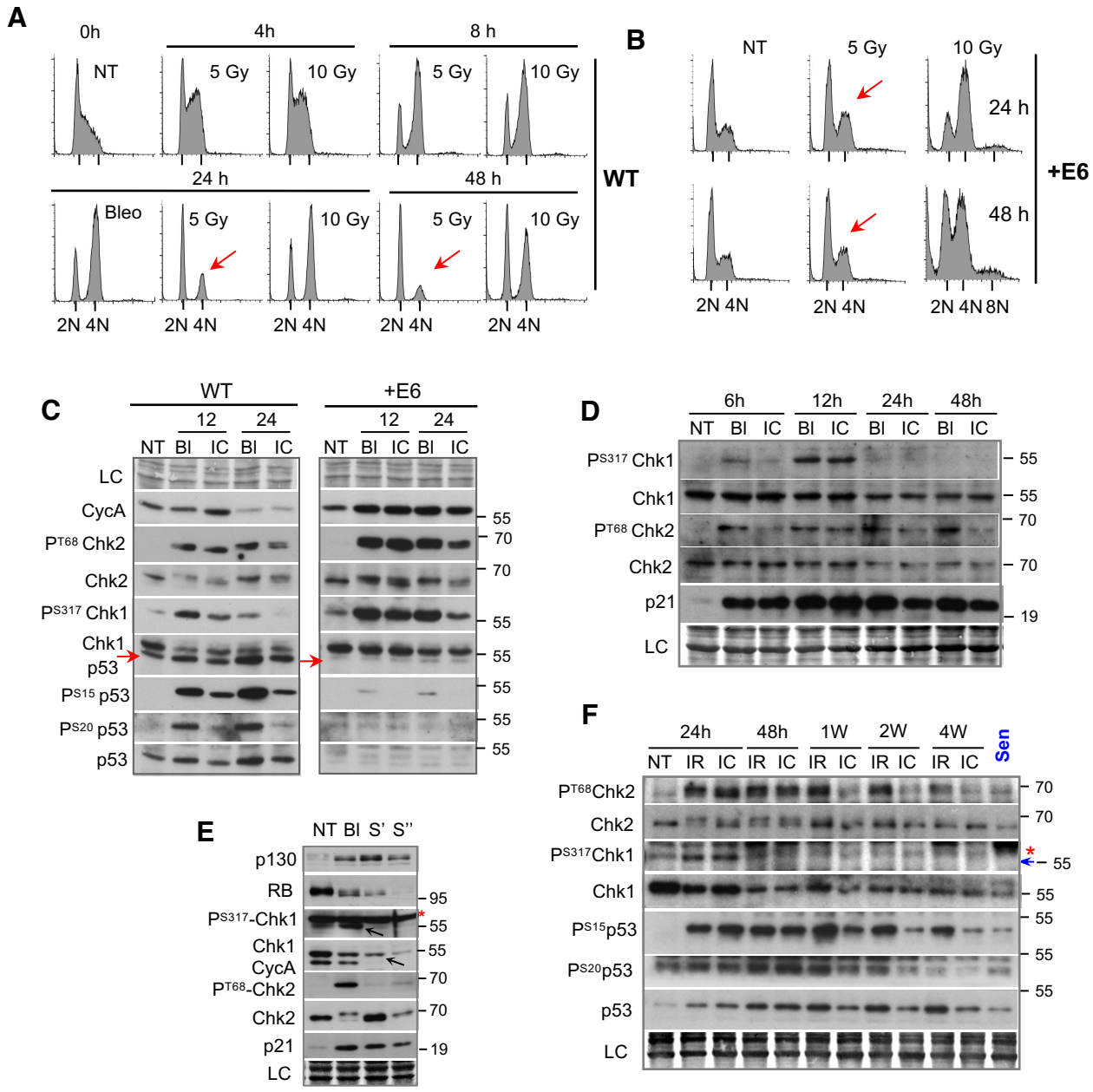



**Fig. S3. G2 exit correlates with p53-dependent downregulation of Chk1 signaling**

**A.** DNA content profiles obtained by flow cytometry of wild-type (WT) HDFs at indicated times after irradiation (n=3). Cells were irradiated (5 or 10 Gy) or exposed to bleomycin (Bleo, 24 h) 16 h after release from quiescence by contact inhibition. Arrows indicate lower amount of cells arrested in G2. NT, non-treated cells (0 h time-point).

**B.** DNA content profiles obtained by flow cytometry of HDFs expressing HPV16-E6 (+E6) 24 and 48 h after irradiation (n=2). Asynchronous cells were exposed to 5 or 10 Gy. NT, non-treated control.

**C.** Immunoblots showing phosphorylation of Chk1 ($P^{S317}$), Chk2 ($P^{T68}$) and p53 ($P^{S15}$, $P^{S20}$) as well as p21 induction in wild-type (WT) HDFs and HDFs expressing HPV16-E6 (+E6) exposed to ICRF193 (IC) or bleomycin (Bl) for indicated times (n=2). NT, non-treated cells; LC, loading control. Red arrows show p53 band.

**D.** Immunoblots showing down-regulation of Chk1 phosphorylation ($P^{S317}$-Chk1) and sustained Chk2 ($P^{T68}$-Chk2) phosphorylation in HDFs exposed to ICRF-193 (IC) or bleomycin (Bl) at indicated times (n=?). NT, non-treated cells; LC, loading control.

**E.** Immunoblots of the indicated cell cycle regulators and DNA damage signalling proteins in extracts of early passage (population doubling (PD) 30) HDFs, non-treated (NT) or exposed to bleomycin (Bl-12h), and senescent HDFs (S' - PD 74; S'' - PD 84) (n=2). Arrows: $P^{S317}$-Chk1 and Chk1 bands; Red asterisk: nonspecific band. LC, loading control.

**F.** Immunoblots showing down-regulation of Chk1 phosphorylation ($P^{S317}$-Chk1) and sustained Chk2 ($P^{T68}$-Chk2) phosphorylation in HDFs upon γ irradiation (IR, 10 Gy) or treatment with ICRF-193 (IC) for indicated times (n=2). NT, non-treated cells; Sen, senescent cells. LC, loading control. Arrow: $P^{S317}$-Chk1 band; Red asterisk: nonspecific band.

In all immunoblots loading controls (LC) were Amido Black-stained membranes.

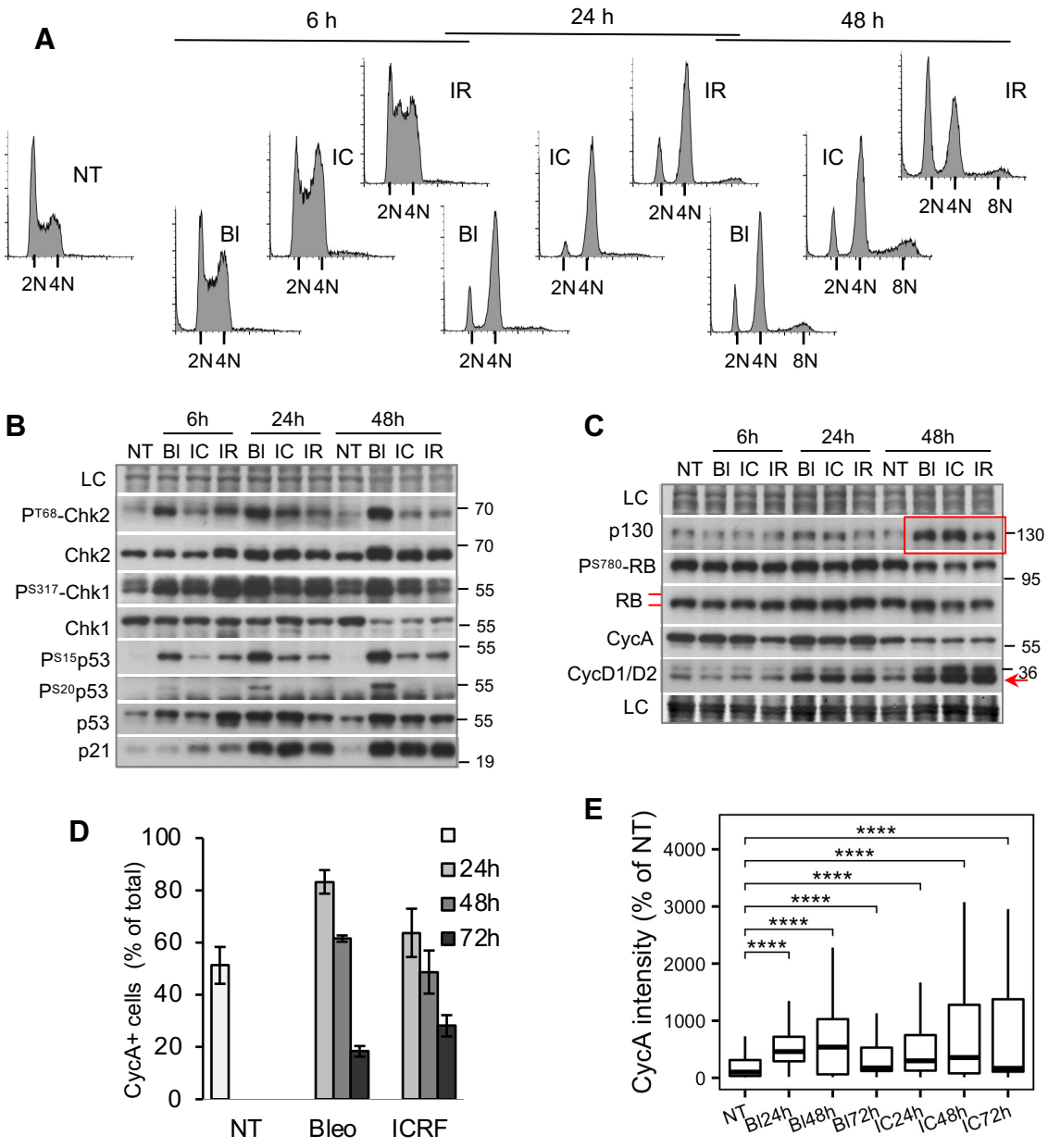



**Fig. S4. Sustained Chk1 activity in G2-arrested U2OS cells coincides with altered mitotic bypass and delayed G2 exit**

**A.** DNA content profiles obtained by flow cytometry of non-treated (NT) U2OS cells and cells exposed to bleomycin (BI), ICRF-193 (IC) or irradiation (IR, 10 Gy) for indicated times (n=2).

**B.** Immunoblot analysis showing changes in DNA damage response effectors in U2OS cells after γ-radiation (IR, 10 Gy) or exposure to bleomycin (BI) or ICRF-193 (IC) for indicated times (n=2). NT, non-treated cells. LC, loading control.

**C.** Immunoblot analysis showing changes in cell cycle control regulators in U2OS cells after γ-radiation (IR, 10 Gy) or exposure to bleomycin (BI) or ICRF-193 (IC) for indicated times (n=2). Red bars indicate RB phosphorylation shift, red arrow shows CycD2 band and red rectangle shows accumulation of hypo-phosphorylated p130. In all immunoblots loading controls (LC) are Amido Black-stained membranes.

**D.** Quantification of CycA positive cells (expressed as a percentage of total cell numbers) in immunofluorescence images taken at the indicated time-points after exposure to bleomycin (Bleo) or ICRF-193 (ICRF). Data are mean +/- s.e.m. of three independent experiments. More than 100 cells were analysed in each experiment.

**E.** Quantification of nuclear CycA intensity (obtained by immunofluorescence) in U2OS cells exposed to bleomycin (BI) or ICRF-193 (IC) for indicated times (expressed as a percentage of CycA intensity in NT). Cells (n>100) were pooled from two independent experiments. The box represents the 25–75th percentiles, central line indicates the median, and whiskers indicate the 10th and 90th percentiles. *P* values were calculated with two-tailed unpaired Student's *t*-test; ****$P \leq 0.0001$. NT, non-treated cells.

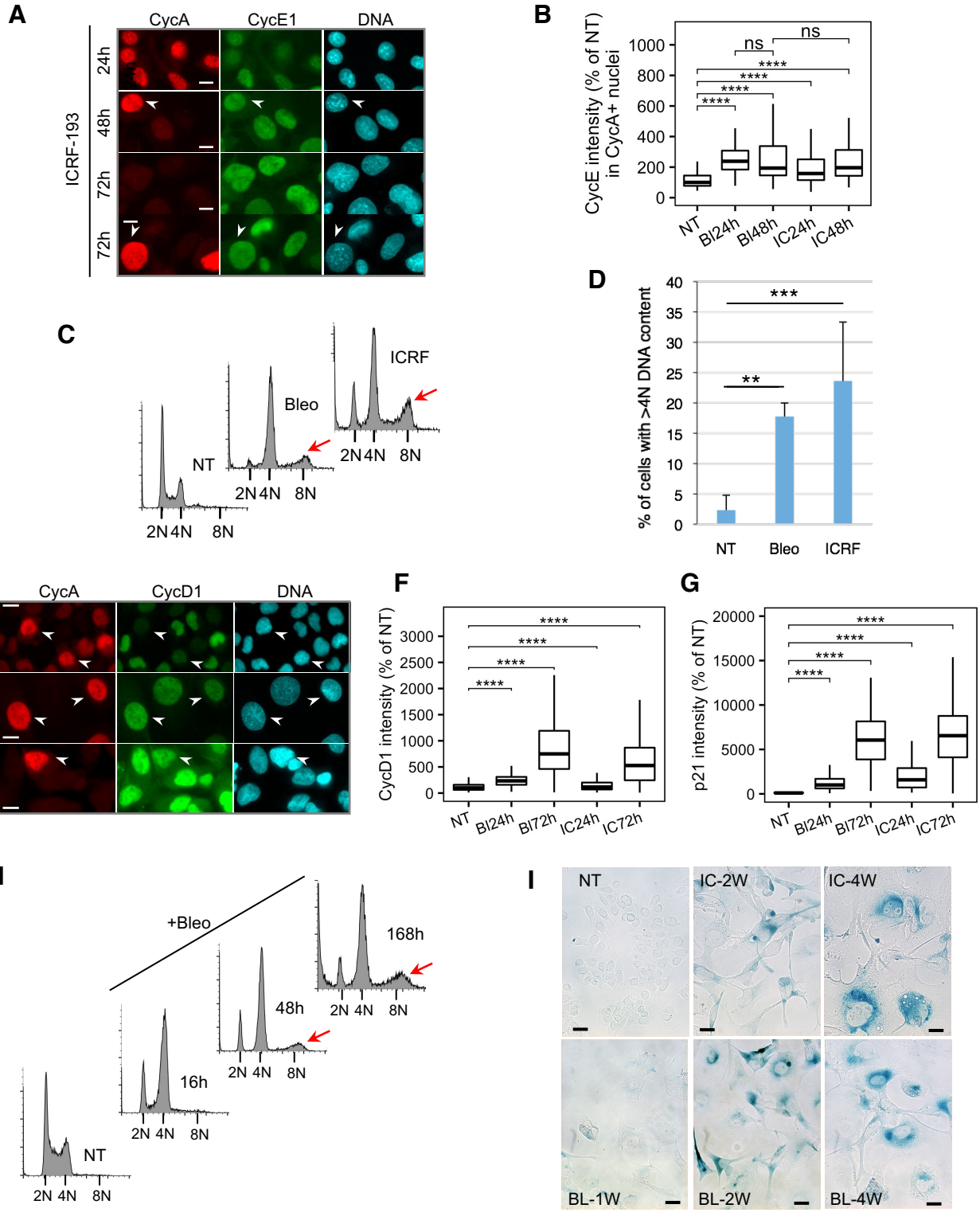


**Fig. S5. DNA damage-induced G2 arrest in U2OS leads to aberrant upregulation of G1 cyclins coinciding with p21 induction, genome re-duplication and delayed senescence onset in G2.**

**A.** Representative immunofluorescence images (n=2) showing CycA/CycE1 co-expression in U2OS cells exposed to ICRF-193 for indicated times. Arrowheads show CycA-positive cells with high CycE1 expression. Scale bars, 10 μm.
**B.** Quantification of CycE1 intensity in CycA-positive nuclei in non-treated (NT) U2OS cells or cells exposed to bleomycin (Bl) or ICRF-193 (IC) for 24 or 48 h (expressed as a percentage of CycE1 intensity in NT). Cells (n>200) were pooled from two independent experiments. The box represents the 25–75th percentiles, central line indicates the median, and whiskers indicate the 10th and 90th percentiles. *P* values were calculated using two-tailed unpaired Student's *t*-test. ns, not significant, ****$P \leq 0.0001$.
**C.** DNA content profiles obtained by flow cytometry of non-treated (NT) U2OS cells and cells exposed to bleomycin (Bleo) and ICRF-193 (ICRF) for 48 h. Red arrows indicate 8N population as a result of DNA re-replication in G2 arrested cells.
**D.** Percent of cells with >4N DNA content in non-treated (NT) U2OS cells and cells exposed to bleomycin and ICRF-193 (ICRF) for 48 h. Data are mean +/- s.d. of five independent experiments. *P* values were calculated with two-tailed paired Student's *t*-test; **$P \leq 0.01$, ***$P \leq 0.001$.
**E.** Representative immunofluorescence images (n=2) showing CycA/CycD1 co-expression in U2OS cells exposed to bleomycin (Bleo) for indicated times. Arrowheads show CycA-positive cells with high CycD1 expression. Scale bars, 10 μm.
**F.** Quantification of nuclear CycD1 intensity in U2OS cells exposed to ICRF-193 or bleomycin for indicated times (expressed as a percentage of CycD1 intensity in NT). Cells (n>100) were pooled from two independent experiments. The box represents the 25–75th percentiles, central line indicates the median, and whiskers indicate the 10th and 90th percentiles. *P* values were calculated with two-tailed unpaired Student's *t*-test. ****$P \leq 0.0001$. NT, non-treated cells.
**G.** Quantification of nuclear p21 intensity in U2OS cells exposed to bleomycin (Bl) or ICRF-193 (IC) for indicated times (expressed as a percentage of p21 intensity in NT). Cells (n>100) were pooled from two independent experiments. The box represents the 25–75th percentiles, central line indicates the median, and whiskers indicate the 10th and 90th percentiles. *P* values were calculated with two-tailed unpaired Student's *t*-test. ****$P \leq 0.0001$. NT, non-treated cells.
**H.** DNA content profiles obtained by flow cytometry of non-treated (NT) U2OS cells and cells treated with bleomycin (Bleo) for 16, 48 and 168 h. Red arrows indicate the cells that underwent re-replication after prolonged DNA damage (8N).
**I.** Representative phase-contrast images showing β-galactosidase staining of non-treated (NT) U2OS cells and cells exposed to ICRF-193 (ICRF) or bleomycin (Bleo) for 1 week (W), 2 weeks and 4 weeks. Scale bars, 10 μm.

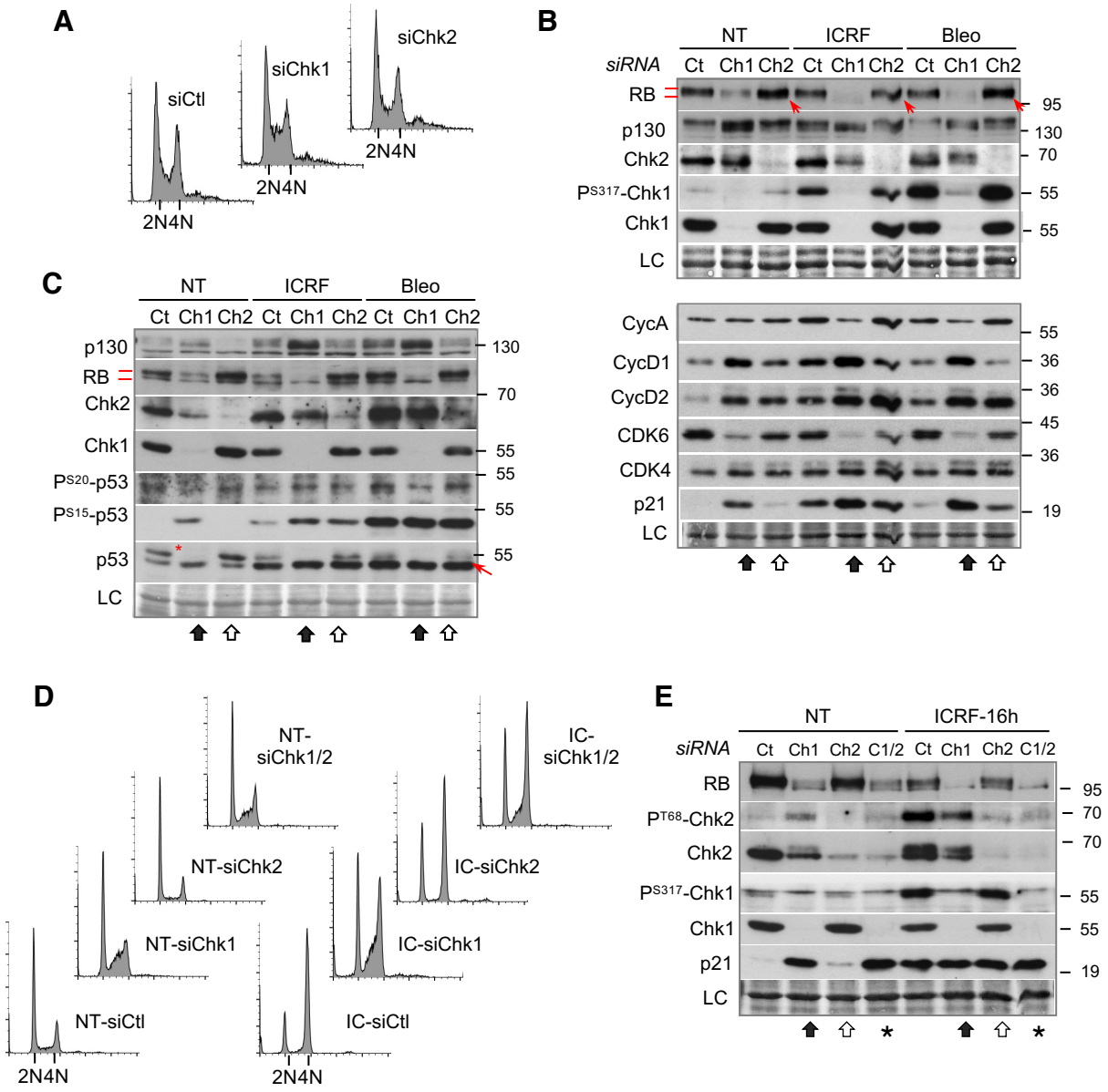



**Fig. S6. Chk1 knockdown accelerates whereas Chk2 knockdown delays DNA damage-induced cell cycle exit.**

**A.** DNA content profiles obtained by flow cytometry of asynchronously growing U2OS cells 24 h after siRNA-mediated Chk1 (siChk1) or Chk2 (siChk2) knockdown (n=2).

**B.** Immunoblots showing effects of Chk1 (Ch1, bottom black arrow) and Chk2 (Ch2, bottom white arrow) knockdown on RB and p130 phosphorylation (upper panel) and expression of cell cycle regulators (lower panel) in non-treated (NT) U2OS cells and cells exposed to ICRF-193 (ICRF) or bleomycin (Bleo) for 16 h (n=2). Red arrows: increased RB levels after siChk2. LC, loading control.

**C.** Immunoblots showing effects of Chk1 (Ch1, bottom black arrow) and Chk2 (Ch2, bottom white arrow) knockdown on p53 phosphorylation ($P^{S20}$, $P^{S15}$) in non-treated (NT) U2OS cells and cells exposed to ICRF-193 (ICRF) or bleomycin (Bleo) for 48 h (n=2). Red arrow: p53 band. Red asterisk: Chk1 band. LC, loading control.

**D.** DNA content profiles obtained by flow cytometry of asynchronously growing non-treated (NT) HDFs and HDFs exposed to ICRF-193 (IC, 16 h) 24 h after siRNA-mediated Chk1 (siChk1), Chk2 (siChk2) or double Chk1/Chk2 (siChk1/2) knockdown (n=2).

**E.** Immunoblots showing effects of Chk1 KD (Ch1, bottom black arrow), Chk2 (Ch2, bottom white arrow) and double Chk1/Chk2 (C1/2, asterisk) knockdown on RB phosphorylation and p21 induction in non-treated (NT) HDFs and HDFs treated with ICRF-193 (ICRF) for 16 h (n=2). LC, loading control.

In all immunoblots loading controls (LC) are Amido Black-stained membranes.

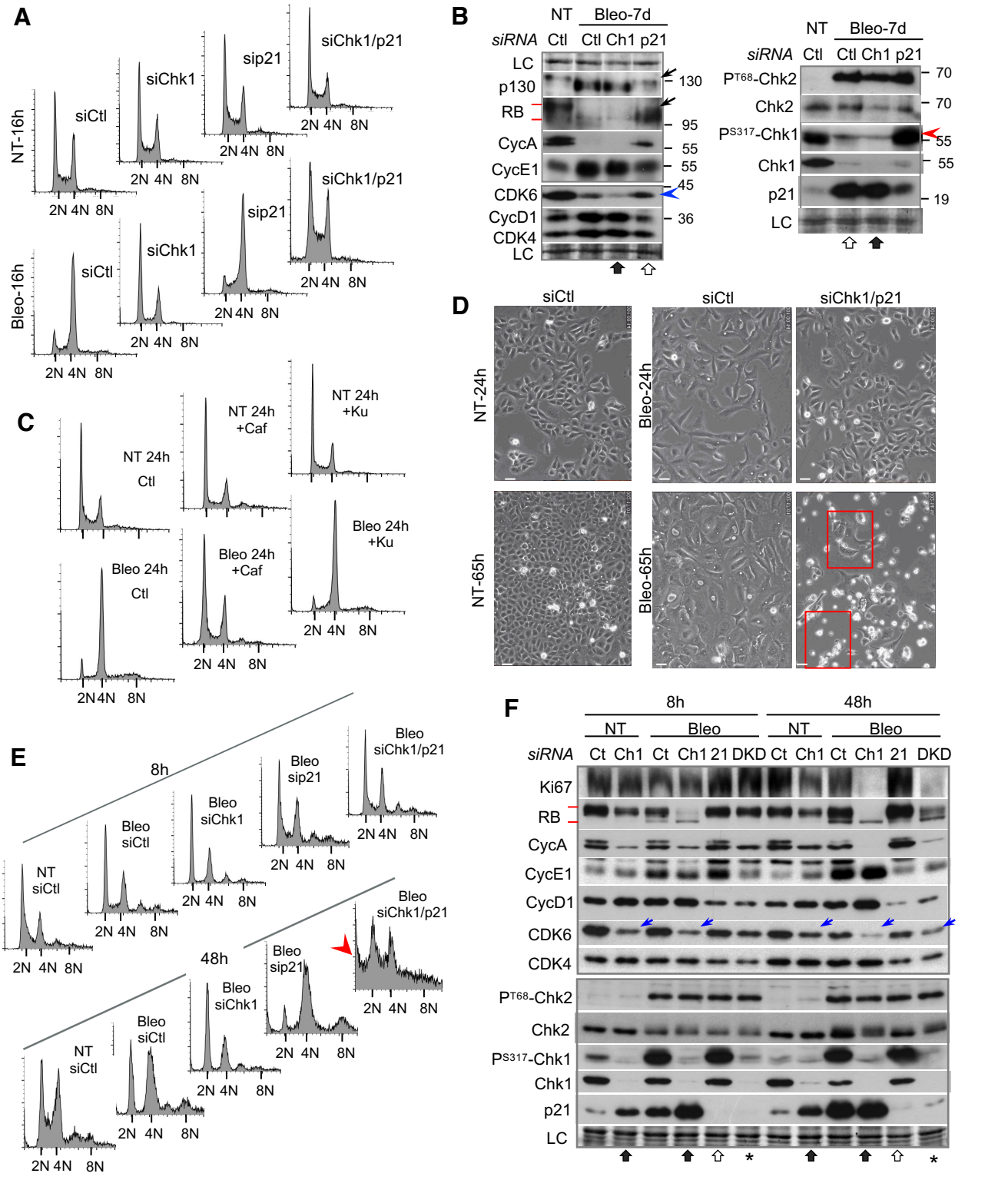


**Fig. S7. Chk1 knockdown in cancer cells accelerates cell cycle exit by inducing p21 and downregulating CDK6**

**A.** DNA content profiles obtained by flow cytometry of non-treated control (siCtl) and bleomycin-treated U2OS cells depleted for Chk1 (siChk1), p21 (sip21) or both proteins (siChk1/p21) after 16 h (n=2).
**B.** Immunoblots showing effects of Chk1 (Ch1- bottom black arrow) or p21 knockdown (bottom white arrow) on pocket protein phosphorylation and expression of different cell cycle regulators in non-treated (NT) U2OS cells and cells exposed to bleomycin (Bleo) for 7 days (n=2). Black arrows: elevated p130 and pRb hyper-phosphorylation after p21 depletion. Red arrowhead: strong PS317-Chk1 signal in the absence of p21. Blue arrowhead indicates low Cdk6 levels. Red bars indicate RB phosphorylation shift. LC, loading control.
**C.** DNA content profiles obtained by flow cytometry of non-treated (NT) U2OS cells and cells exposed to bleomycin for 24 h. Caffeine (Caf) and KU-55933 (Ku) were added one hour before treatment (n=2).
**D.** Representative phase contrast images from a single video-microscopy sequence showing bleomycin-treated control (siCtl) and double Chk1/p21 knock down (siChk1/p21) U2OS cells at different times after exposure to the drug. Red rectangles show the inserts presented in Figure 7C. Scale bars, 20 μm.
**E.** DNA content profiles obtained by flow cytometry of non-treated control (siCtl) and bleomycin-treated HCT-116 cells depleted for Chk1 (siChk1), p21 (sip21) or both (siChk1/p21) proteins after 8 and 48 h (n=2). Red arrow indicates the presence of sub-G1 population associated with dying cells after 48 h in bleomycin-treated siChk1/p21 cells.
**F.** Immunoblots showing effects of Chk1 (Ch1- bottom black arrow), p21(bottom white arrow) or double p21/Chk1 (DKD-asterisk) knockdown on Ki67, RB phosphorylation, expression of different cell cycle regulators and DNA damage response ($P^{T68}$-Chk2, $P^{S317}$-Chk1, p21) in non-treated (NT) HCT-116 cells and cells exposed to bleomycin (Bleo) for 8 and 48 h (n=2). Blue arrows indicate low Cdk6 levels after Chk1 depletion. LC, loading control.
In all immunoblots loading controls (LC) are Amido Black-stained membranes. Red bars indicate RB phosphorylation shift.

**Table S1. List of antibodies used**

Click here to download Table S1

**Fig. S8. Blot transparency.** Red rectangles denote the blots shown in the figures.

Figure 1A

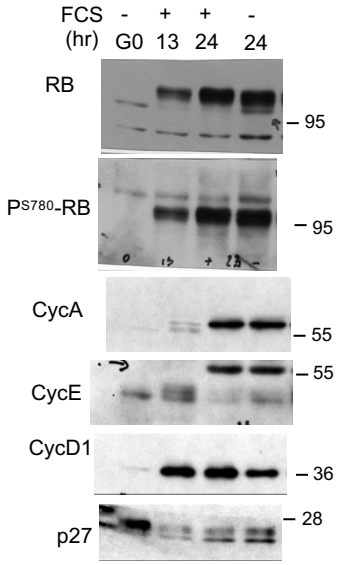



Figure 1B

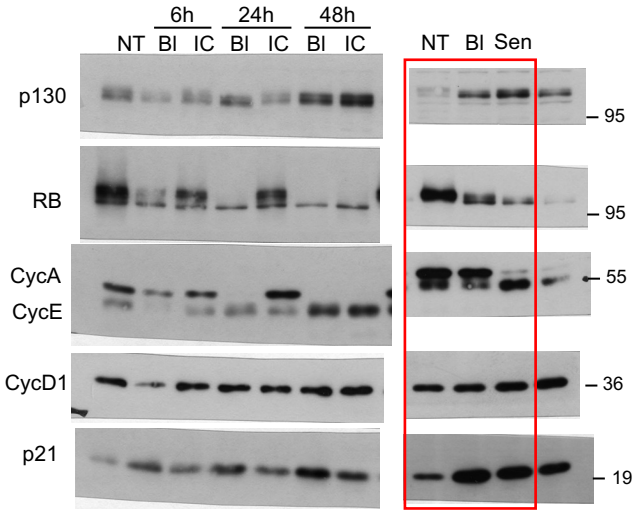



Figure 1E

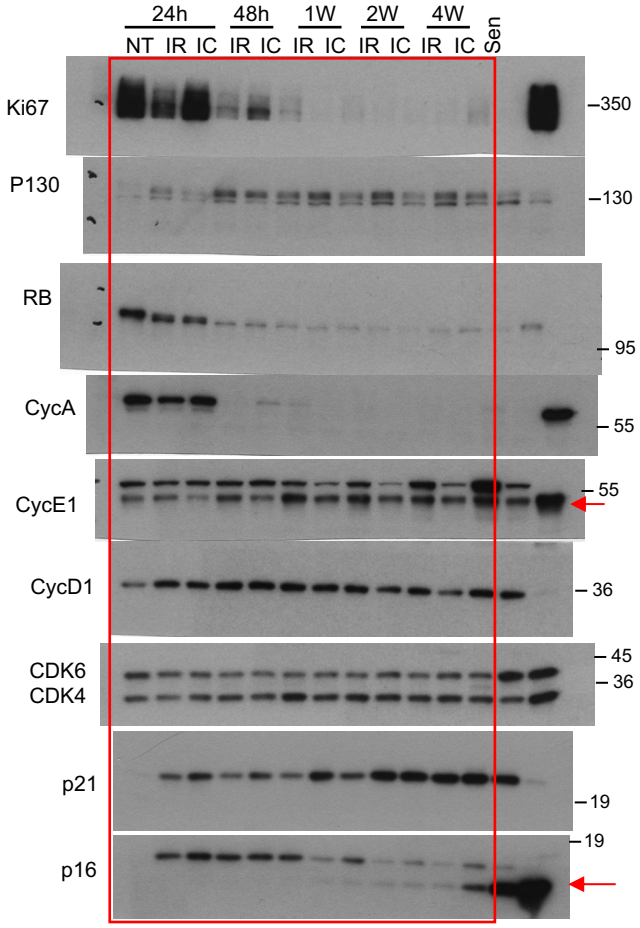
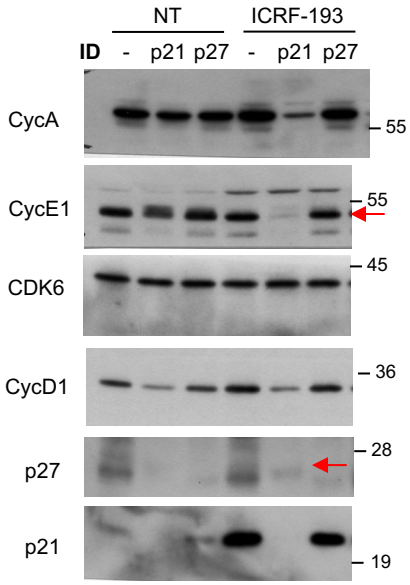



Figure 1S



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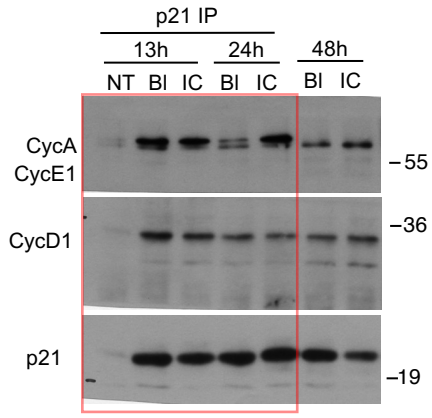
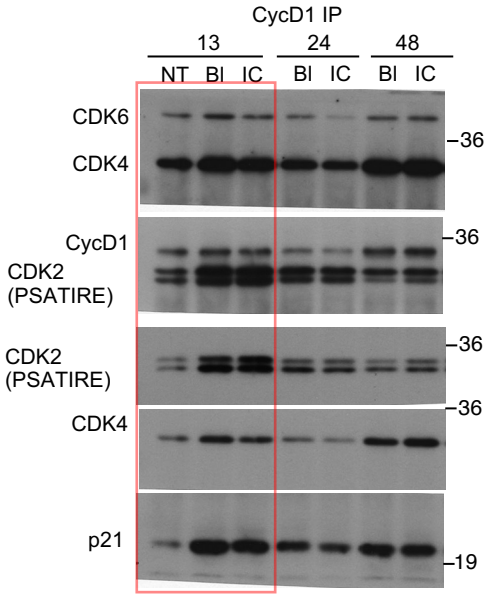



Figure 2F

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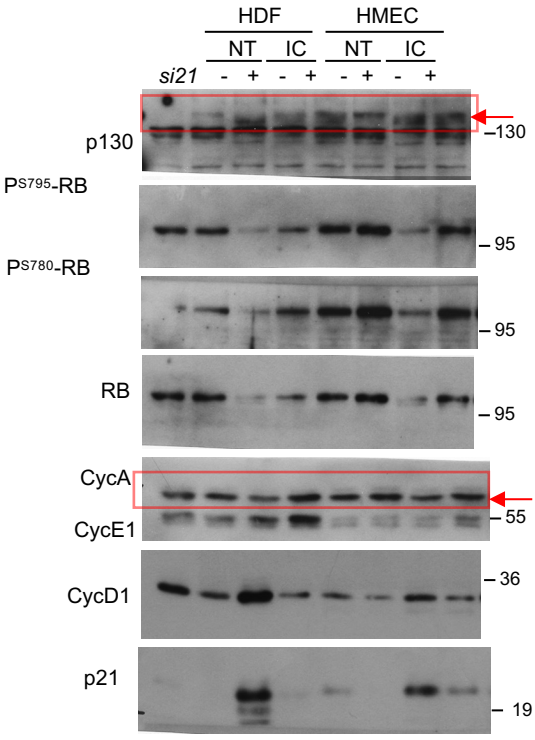


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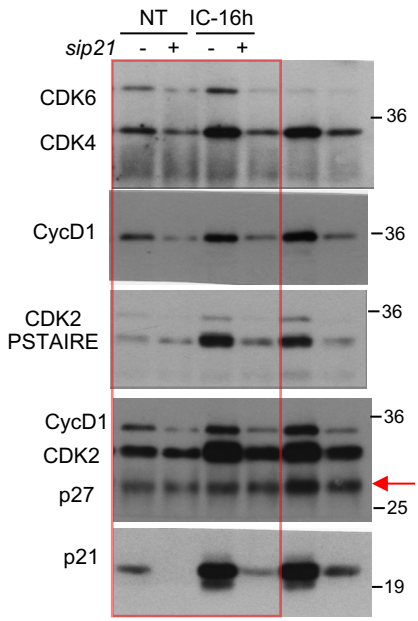

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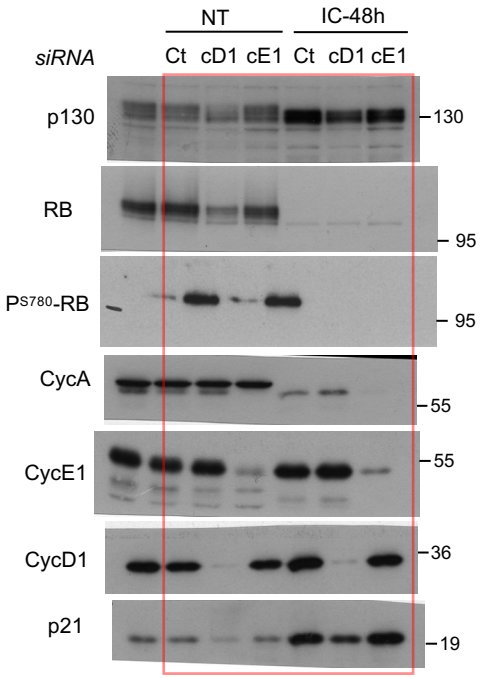


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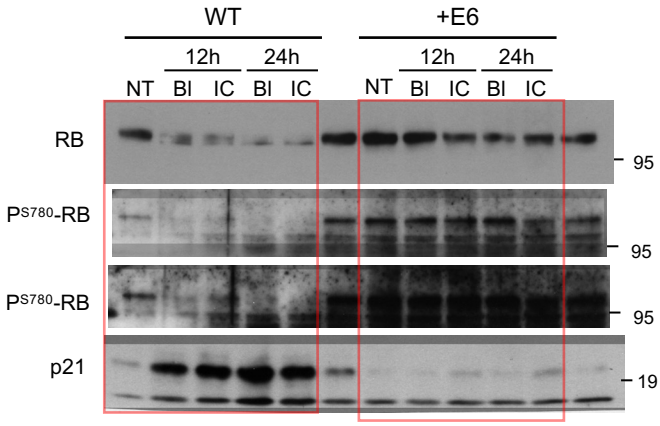
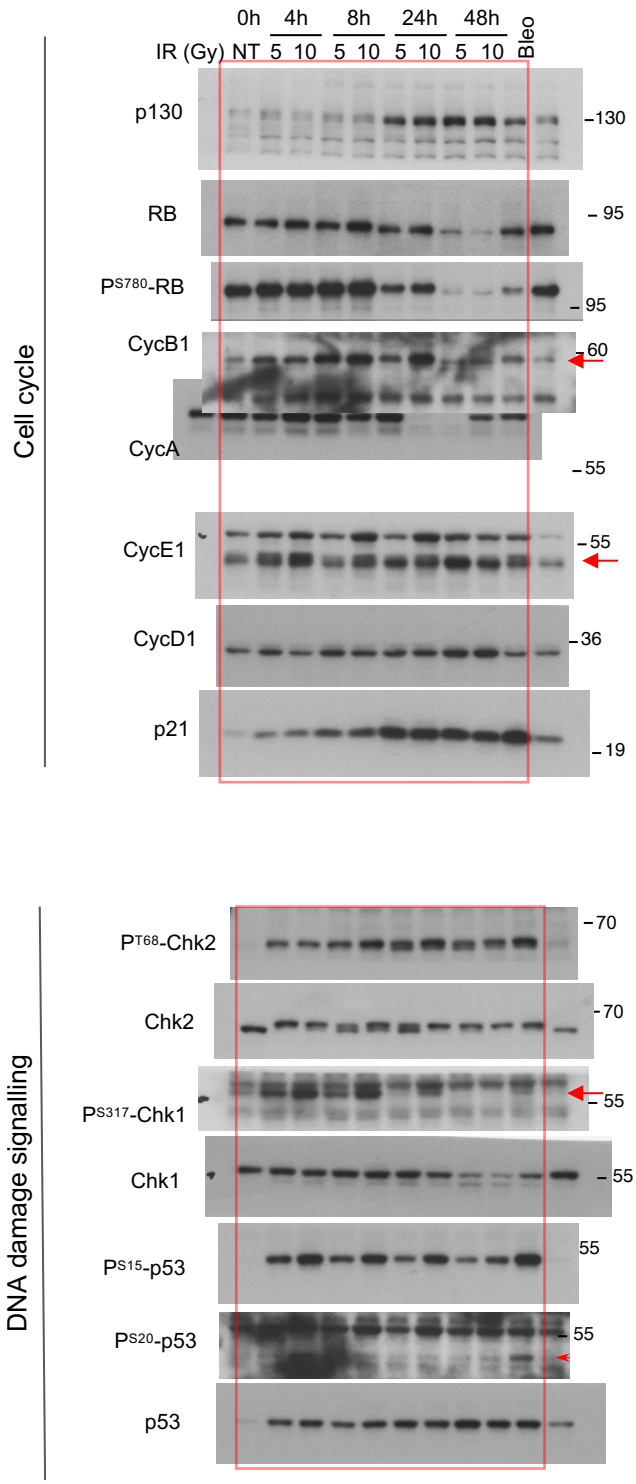


## Figure 3A

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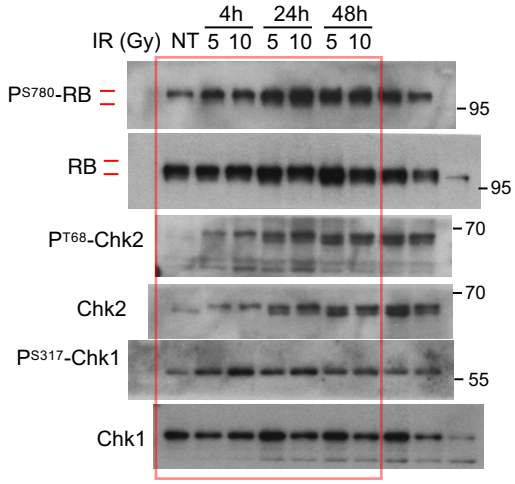


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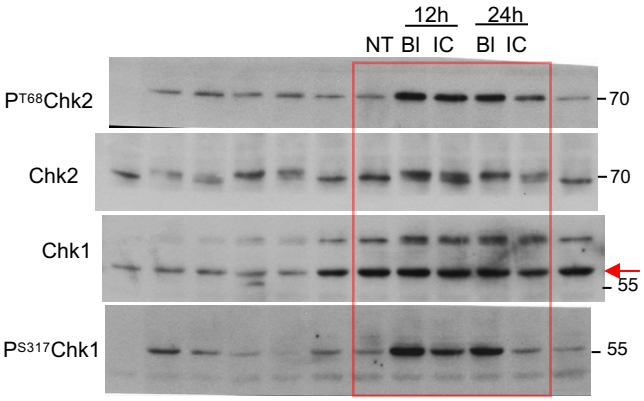

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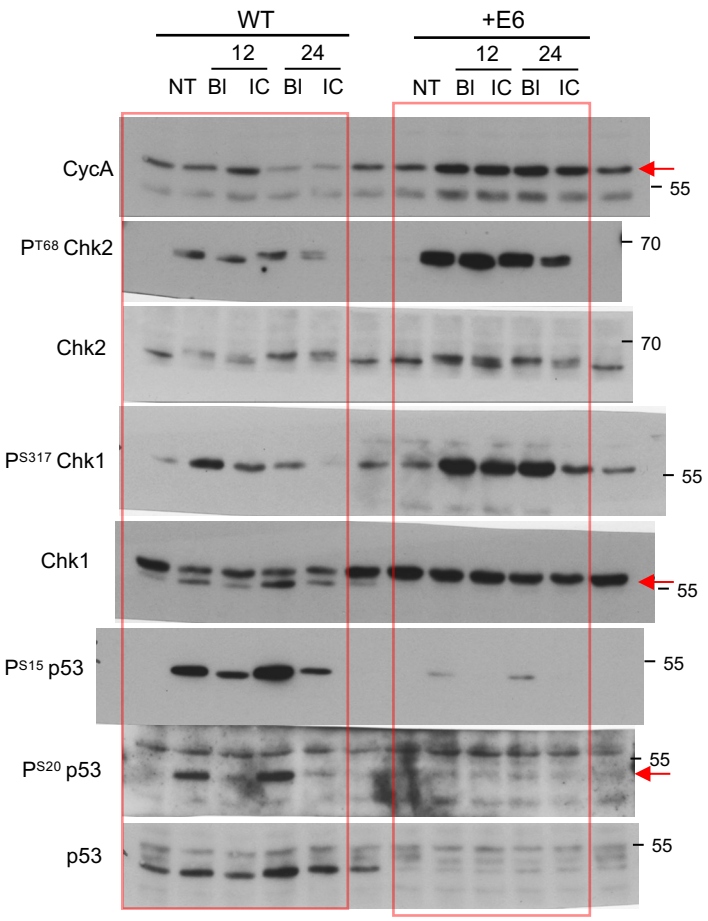


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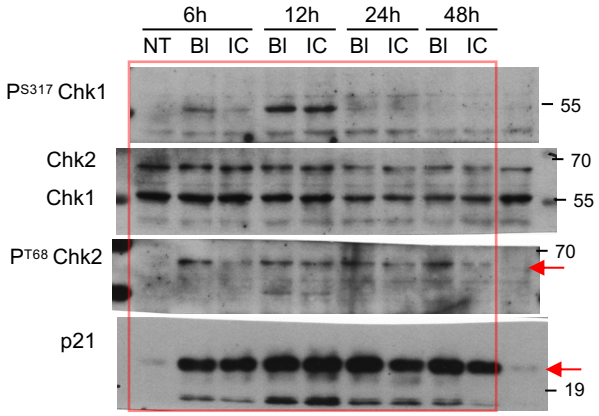


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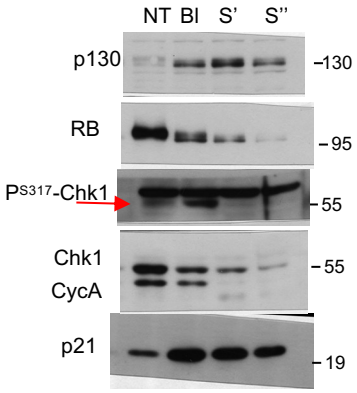


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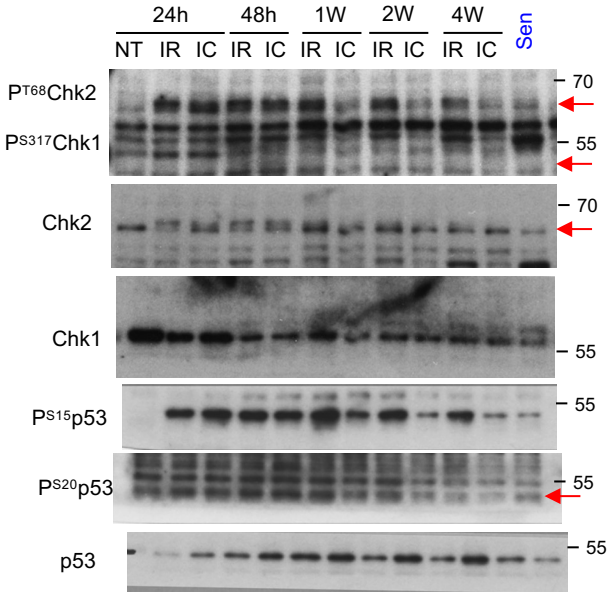

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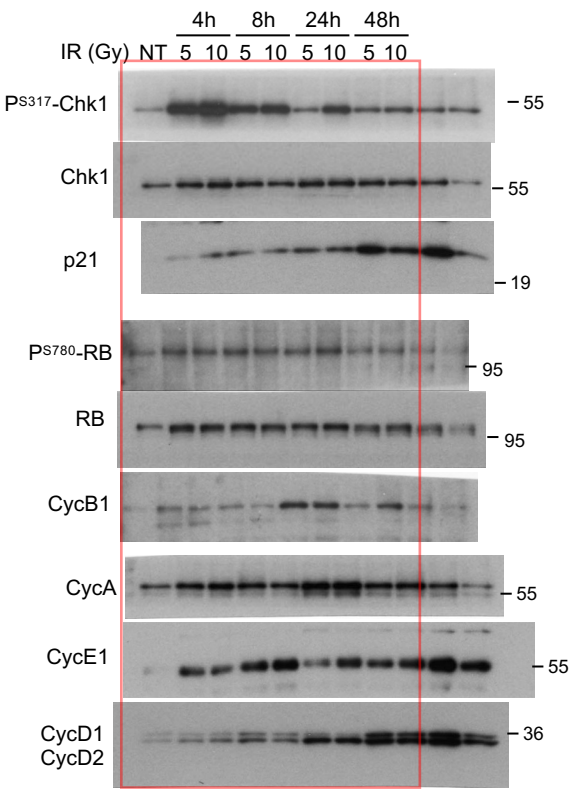


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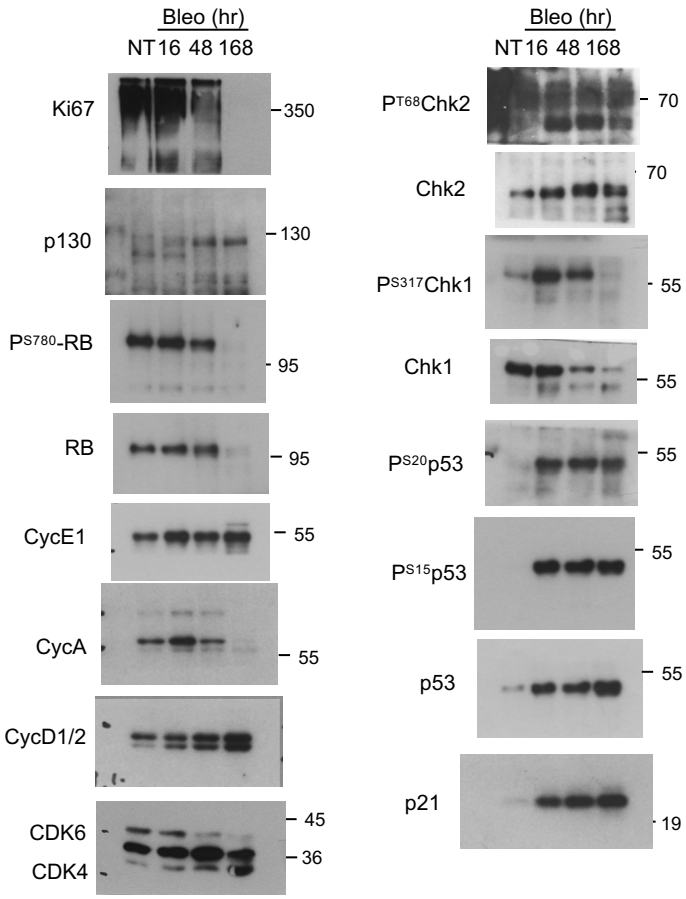
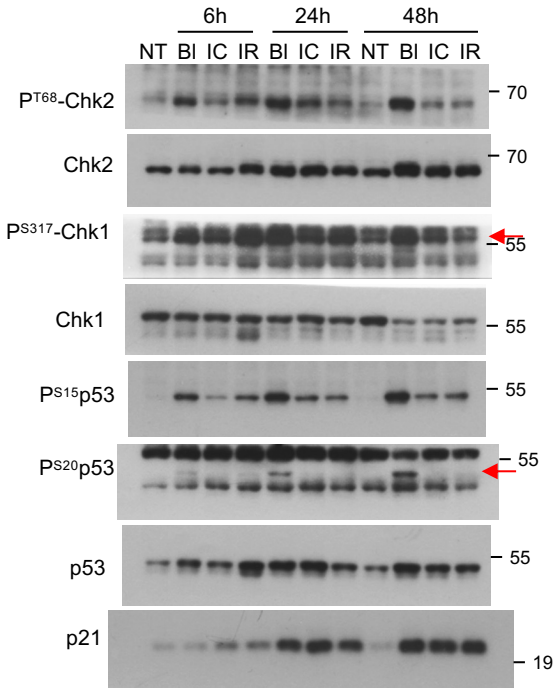



## Figure S4B




## Figure S4C

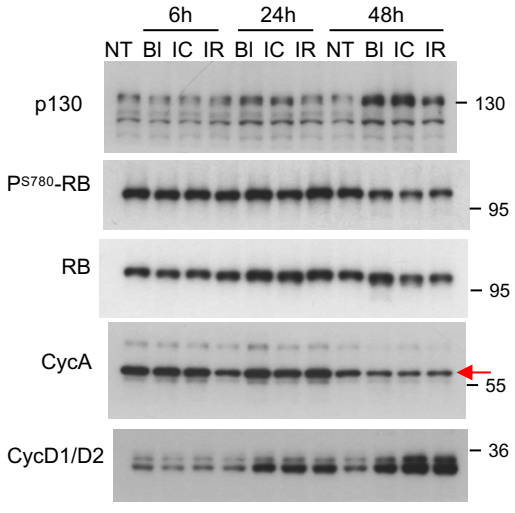


Figure 6D

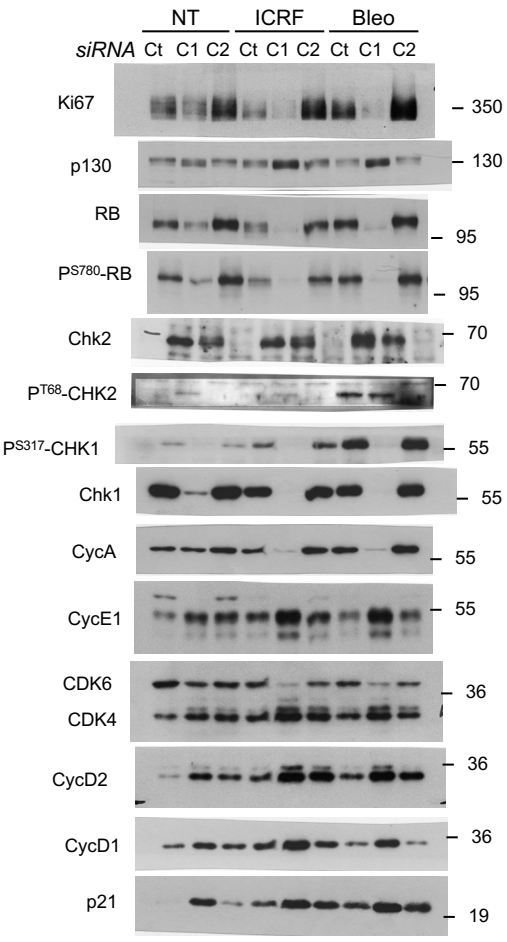



Figure 6E

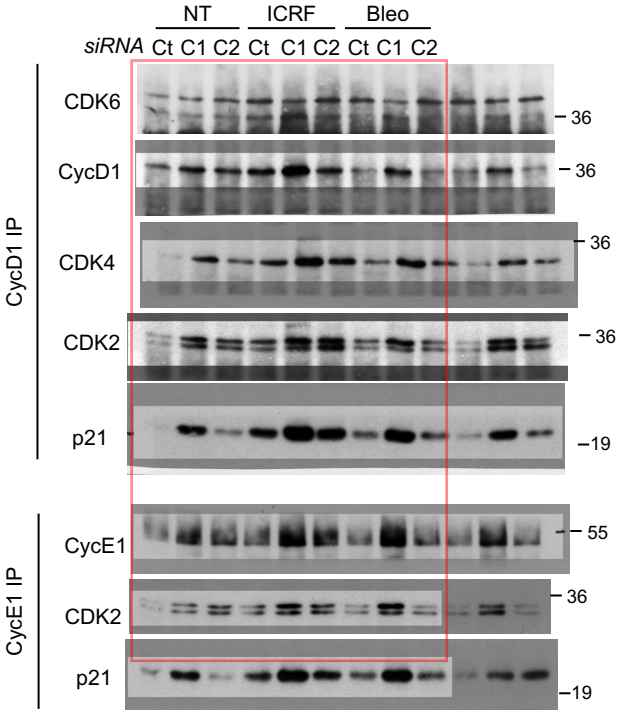
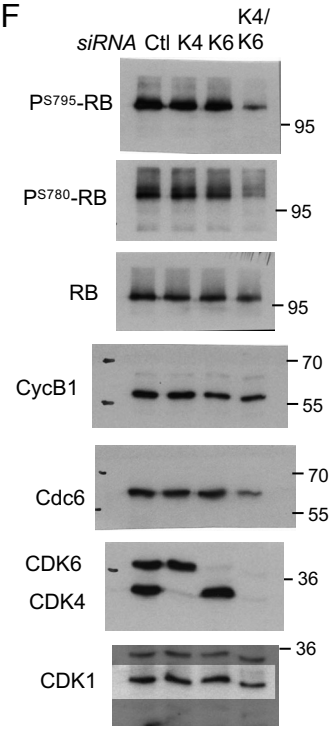
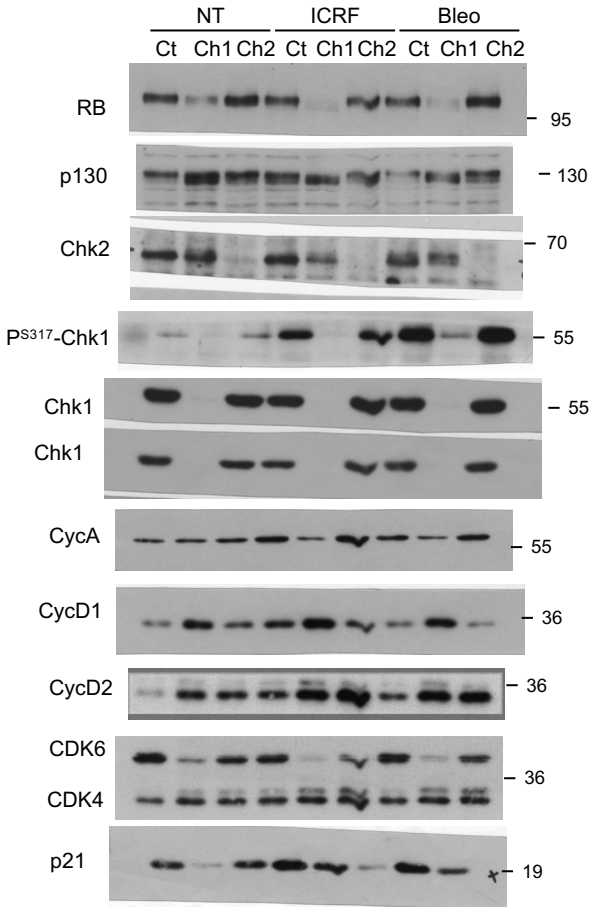


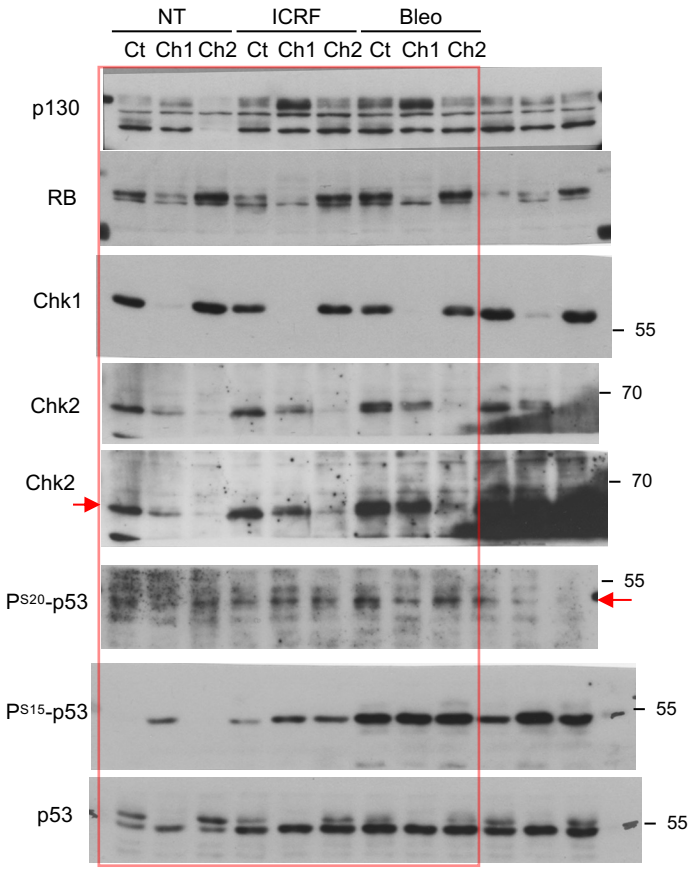

Figure 6F



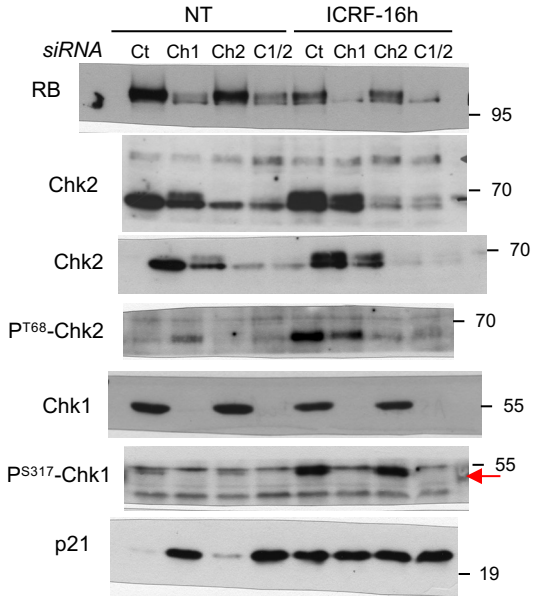
## Figure S6B



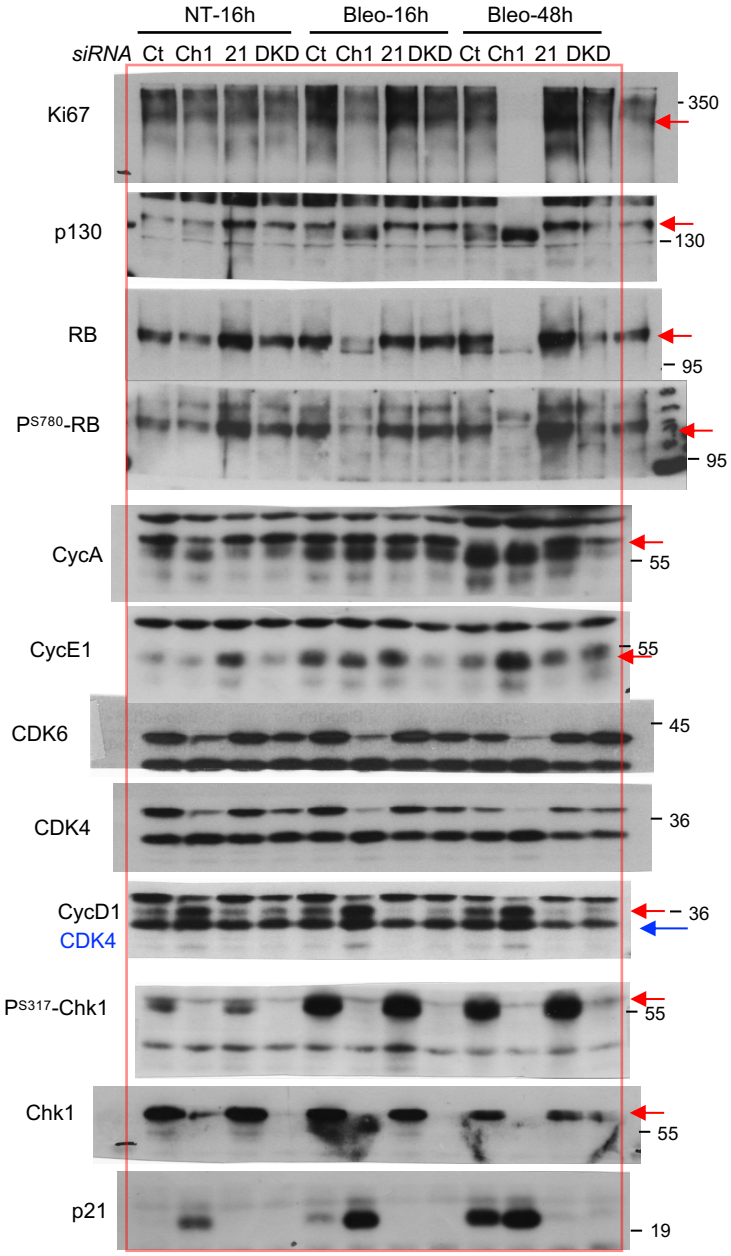

## Figure S6C



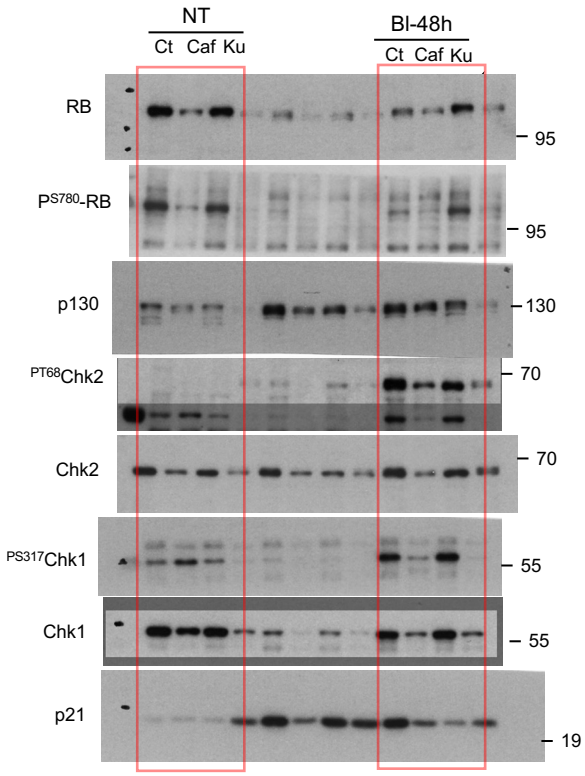

## Figure S6E



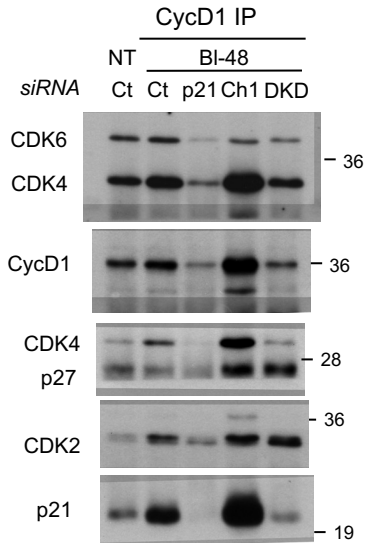
## Figure 7B



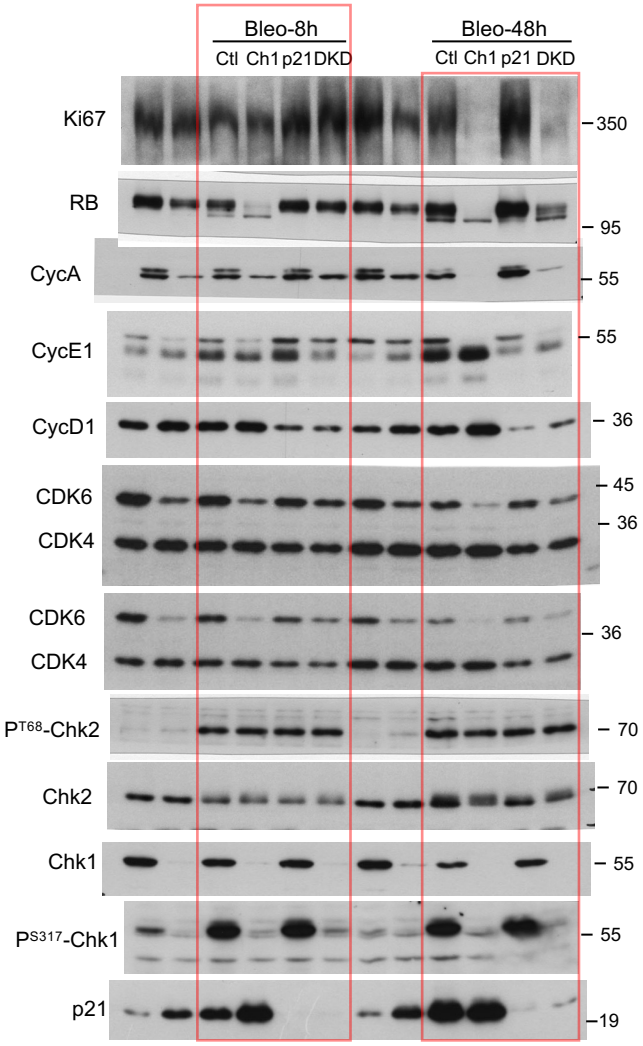

## Figure 7C




## Figure 7F



## Figures 7E and S7F




## Figure S7B