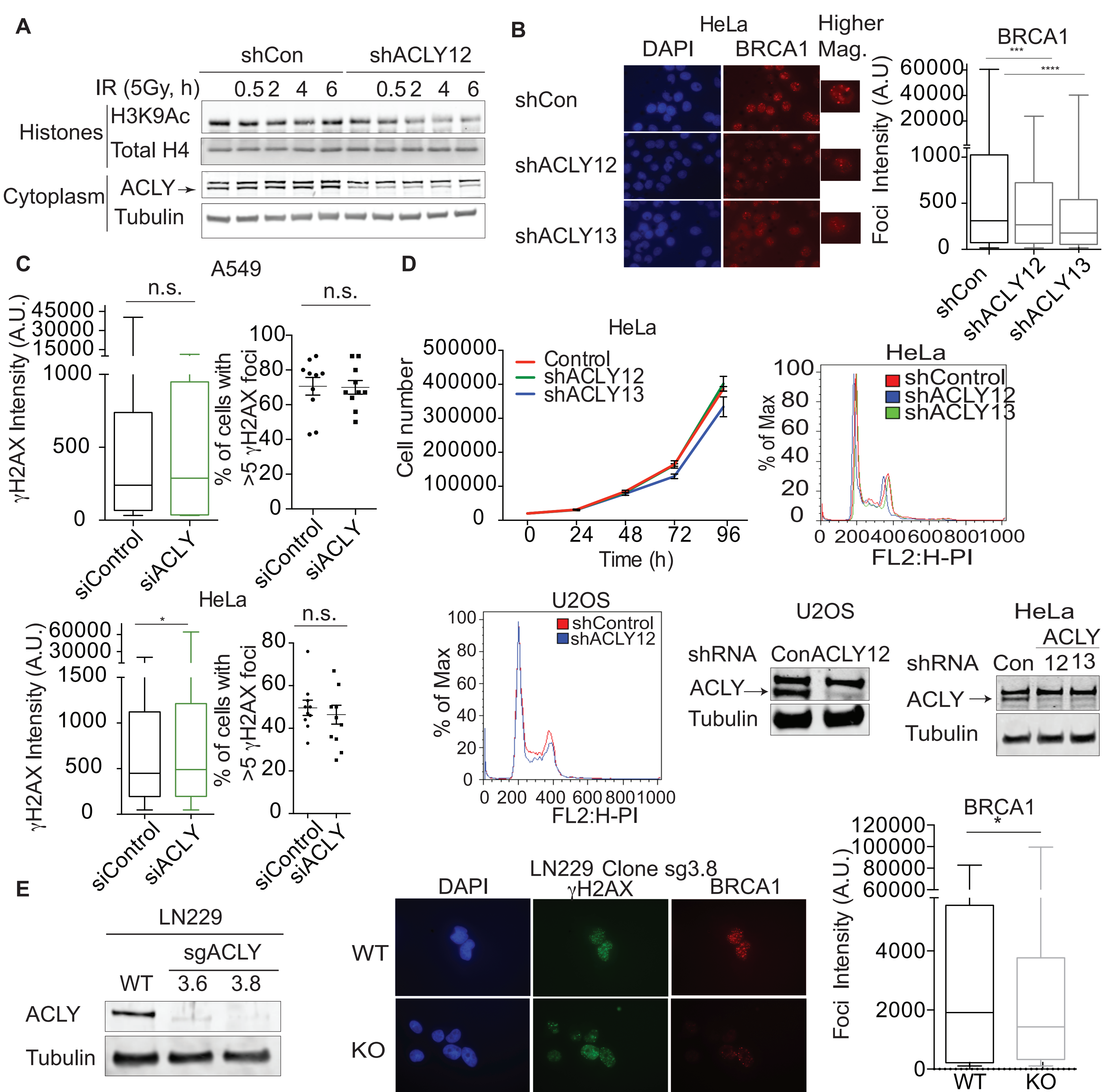

Figure S1, Related to Figure 1. Validation of ACLY's role in BRCA1 recruitment and cell-cycle progression in ACLY silenced cells.

(A) Histone acetylation was examined in cells after knockdown of ACLY with shRNA. Histones were acid extracted after indicated time following 5Gy IR and specific proteins examined by western blot. Cytoplasmic fraction was used to verify knockdown.

(B) BRCA1 recruitment was examined in HeLa cells with two different shRNA targeting *ACLY*. Quantification was performed in 10 fields. ***, $p<0.001$, ****, $p<0.0001$. A.U.- Arbitrary Units.

(C) γH2AX levels and foci intensity was quantified under experimental conditions used in Figure 1C; left panel- bar and whisker (min to max) plot; right panel- mean +/- SEM; *, $p<0.05$. n.s.- not significant.

(D) Cell cycle profile in *ACLY*-silenced HeLa cells and U2OS cells as examined using propidium iodide staining. Proliferation curve (mean +/- SEM of triplicates) was performed by examining cell counts using hemocytometer.

(E) BRCA1 foci was quantified in control and ACLY KO LN229 cells from 5-10 fields; *, $p<0.05$. A.U.- Arbitrary Units. Data from 1 clone is depicted and was reproducible with both clones.

Figure S2, Related to Figure 2. BRCA1 recruitment is mediated by ACLY.

(A) BRCA1 protein levels were examined in U2OS cells following *ACLY* knock-down.

(B) Validation of 53BP1 deficiency in the U2OS-reporter sg53BP1 clones, using immunofluorescence staining following induction of DNA damage using shield-1 and 4-OHT.

(C) Confirmation of co-localization γH2AX and Fok1 in the U2OS-TRF1 Fok1 cell line following 4-OHT treatment.

(D) Immunofluoresce staining for BRCA1 in U2OS-TRF1 Fok1 cell line with and without *ACLY* knockdown. Quantification of at least 10 fields per condition; mean+/- SEM. *p<0.05.

(E) Verification of ACLY knockdown in the DR-GFP reporter line with 2 independent siRNAs targeting *ACLY* (upper panel). Validation of specificity of human siACLY for human ACLY in cells overexpressing Flag-tagged mACLY (lower panel). Vertical line indicates that samples are from the same membrane with intervening bands cropped out.

(F) *ACSS2* was silenced using siRNA in the U2OS DSB reporter; damage was induced using shield-1 and 4-OHT for 5h following 48h knockdown. Quantification was performed on 15 fields using ImageJ. n.s.- not significant; A.U.- Aribitrary units.

(G) HR assay was performed in DR-GFP cells following ACLY knockdown. 5 mM acetate was added to the medium at the time of Sce-1 transfection, and samples were harvested 48-72h. GFP+ cells were scored, mean +/- SEM of triplicates, **, p<0.01.

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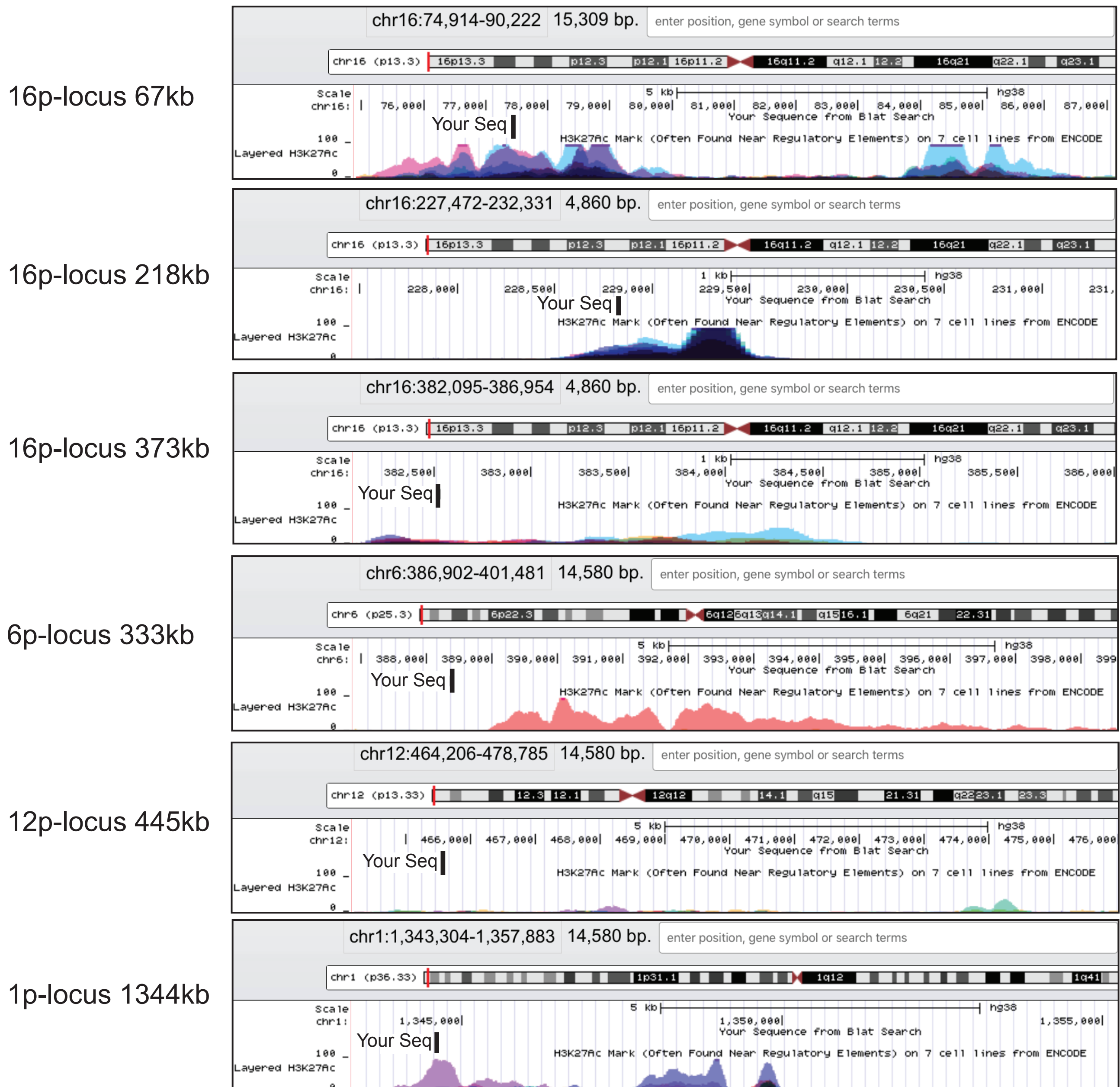


Figure S3, Related to Figure 2. Schematic of primer design used in the U2OS TRF1-Fok1 ChIP. (A) Location of the primer sequences (designated as Your Seq) used in the ChIP experiments generated using the UCSC Genome Browser. Peaks corresponding to ENCODE H3K27Ac is shown.

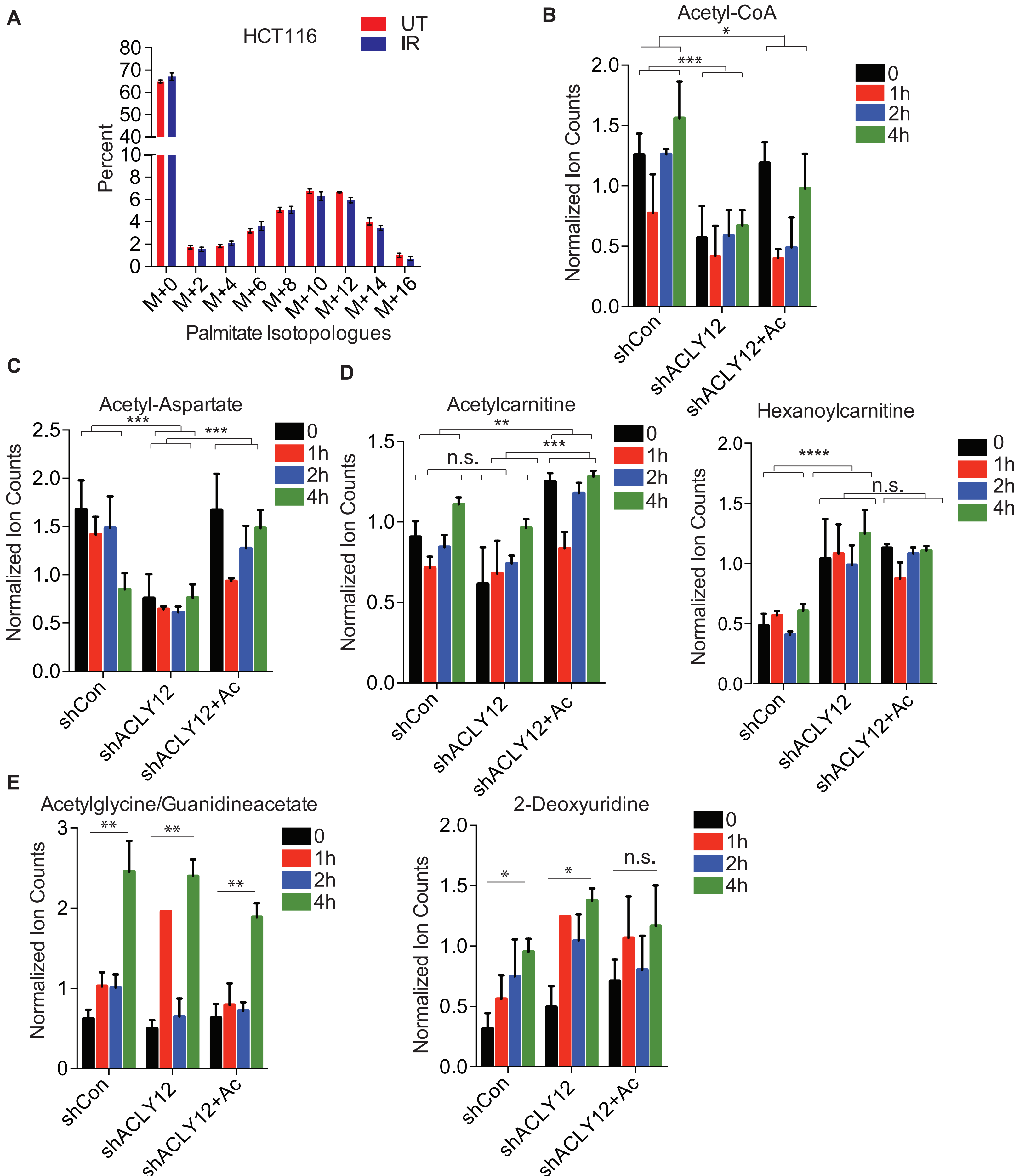


Figure S4, Related to Figure 3. IR-dependent metabolic reprogramming does not account for ACLY's role in DNA repair.

(A) HCT116 cells were plated in triplicate and treated with IR, followed by 24h 13C-glucose labeling. Enrichment of palmitate was examined by fatty-acid methyl-ester analysis, mean +/- SEM of triplicates.

(B-E) Metabolites analyzed post 5Gy IR at indicated time points. *, p<0.05, **, p<0.01, ***, p<0.001, ****, p<0.0001 n.s.-not significant, mean+/- SEM of triplicates.

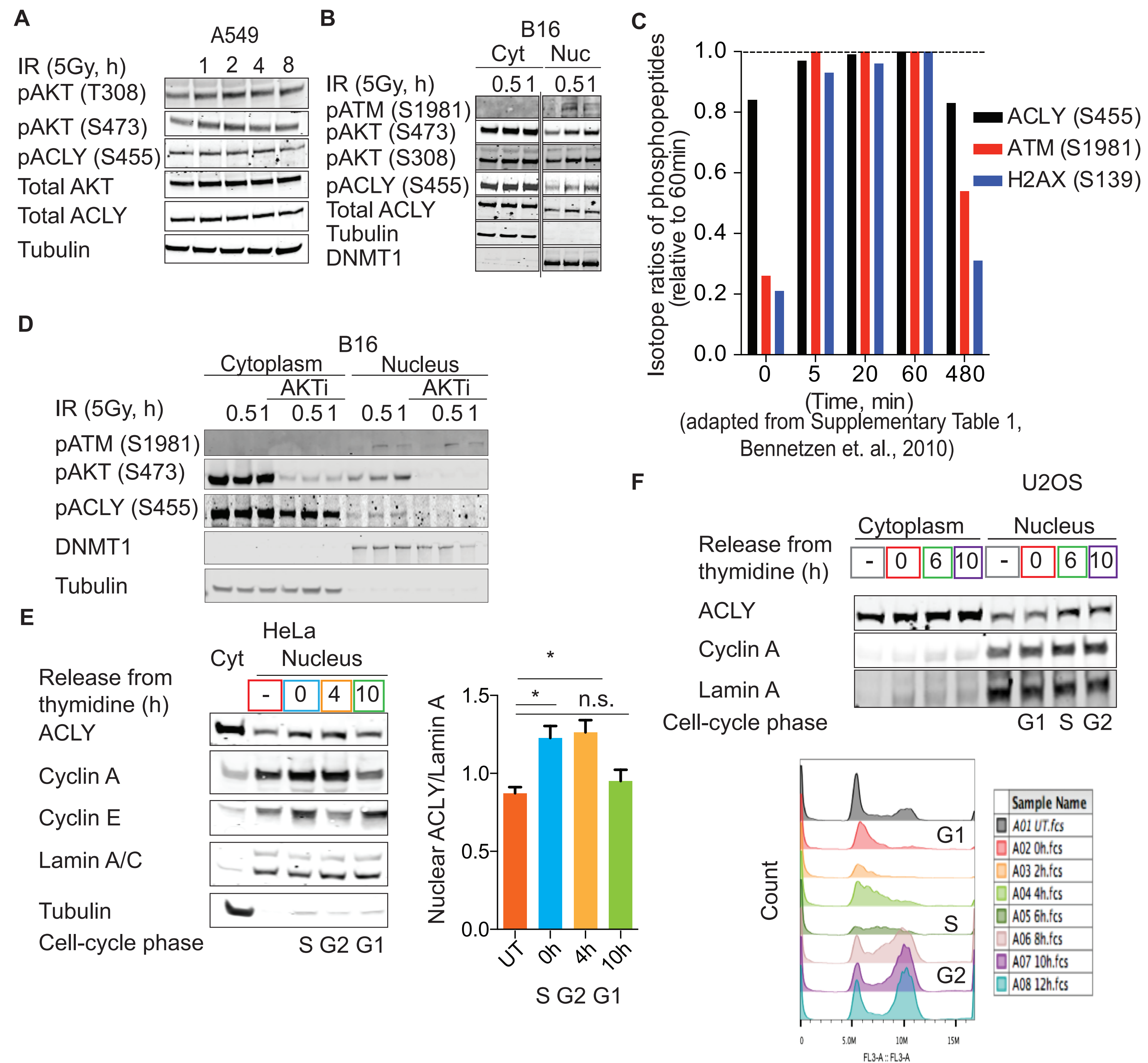


Figure S5, Related to Figure 4. Phosphorylation of ACLY in response to IR and cell cycle regulation of nuclear ACLY.

(A) A549 cells were treated with 5 Gy and whole cell lysates were harvested after indicated time points and protein levels were assayed by Western Blot.

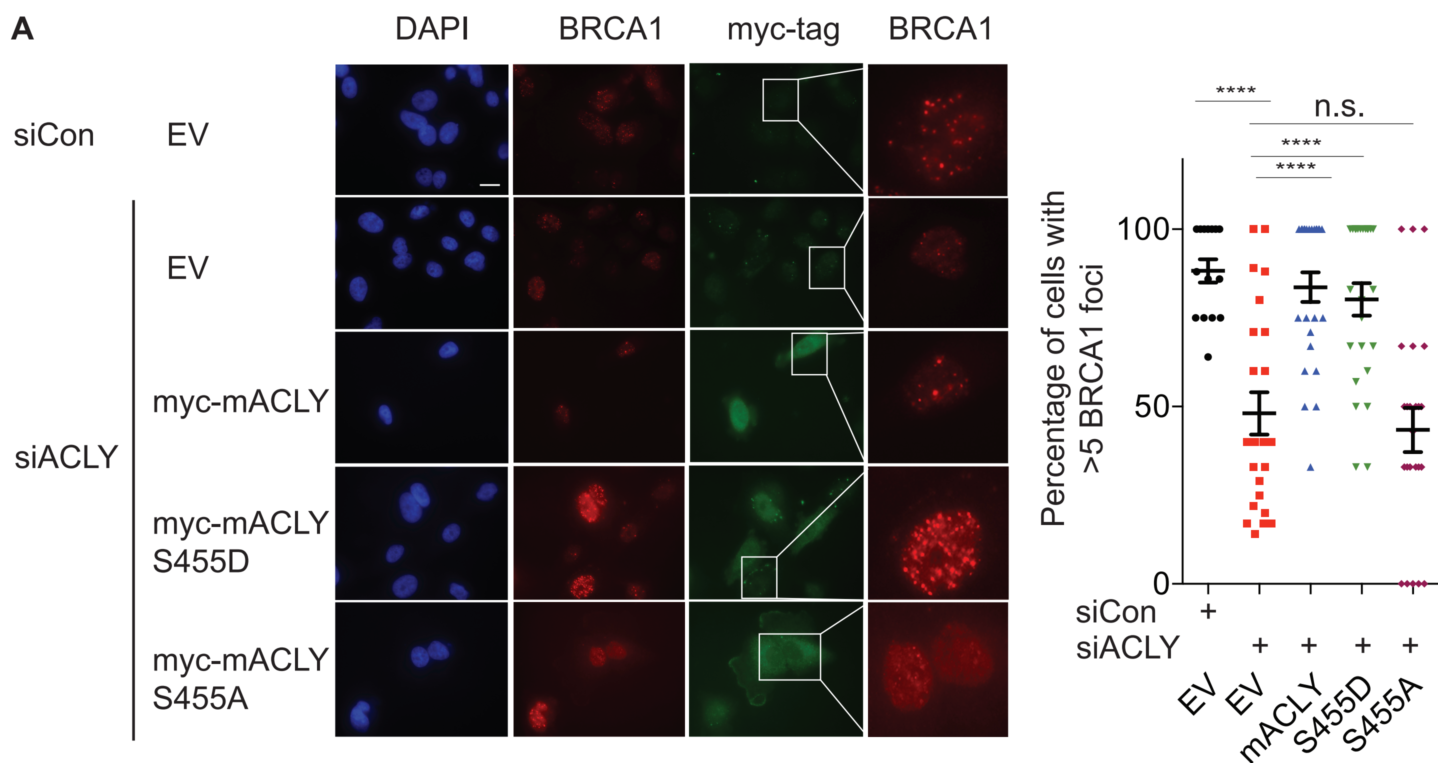
(B) B16 cells were treated with 5 Gy IR, fractionated and protein levels assayed by Western Blot.

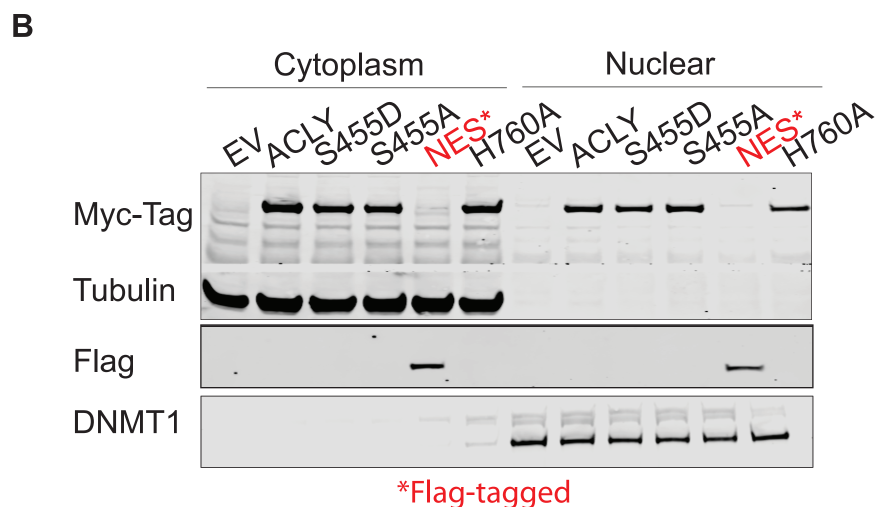
(C) Data re-graphed from Bennetzen et. al., 2010 depicting increase in phosphorylated levels of nuclear ACLY, ATM and γH2AX following damage.

(D) Cells were pre-treated with an Akt inhibitor, MK-2206, for 1 h followed by IR treatment and western blotting of lysates at indicated time points.

(E) HeLa cells were synchronized in S-phase (0h) using a double thymidine block and released from S-phase by changing media. Lysates were fractionated and protein levels were assessed in cytoplasmic and nuclear fractions (left panel). See Figure 4E for corresponding PI profiles. Quantification of three experiments mean +/-SEM, *$p<0.05$. n.s.- not significant (right panel).

(F) U2OS cells were synchronized in G1 (0h) as in E, lysates fractionated and protein levels assessed by Western Blot (top panel). Propidium idodine staining was used to confirm cell cycle stage (bottom panel).





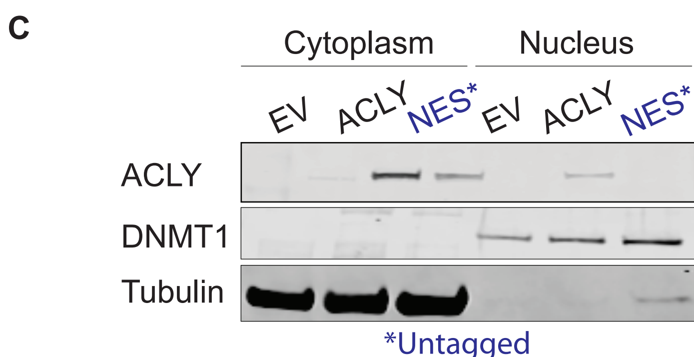
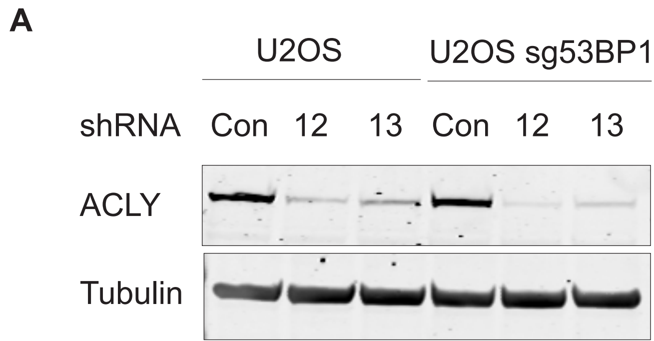


Figure S6, Related to Figure 5. Validation of mACLY constructs and rescue experiment.
(A) Immunofluoresce for BRCA1 and myc-tag following 2Gy IR in LN229 cells stably expressing mACLY constructs or EV with and without knockdown of hACLY using siRNA. Mean+/-SEM, ****p<0.0001, n.s.- not significant.
(B) Expression of mACLY constructs followed by fractionation and verification of localization of mACLY mutants to the nucleus and cytoplasm. Note that the flag tag version of mACLY-NES interfered with the nuclear exclusion of ACLY, thus untagged mACLY-NES was validated in panel C and subsequently used for experiments.
(C) Fractionation of cells in LN229 ACLY null cells depicting ACLY exclusion from the nucleus in cells expressing mACLY-tagged with NES.



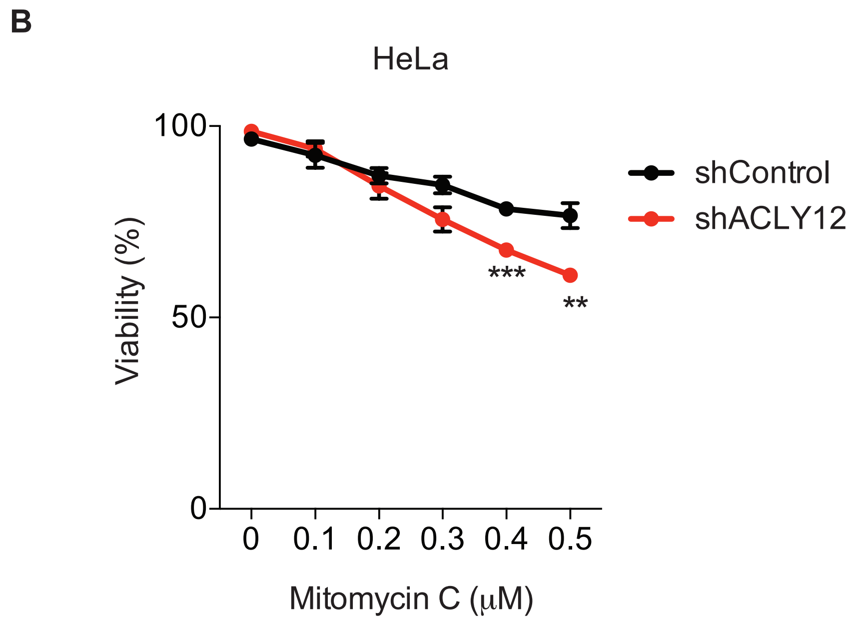


Figure S7, Related to Figure 6: Verification of ACLY knockdown and sensitivity to mitomycin C.
(A) Western blot depicting knockdown of ACLY in the U2OS cells.
(B) HeLa cells transduced with shControl or shACLY12 were treated with indicated concentration of mitomycin C for 48 h and cell viability determined by trypan blue exclusion. Mean +/- SEM. **, $p<0.01$, ***, $p<0.001$.