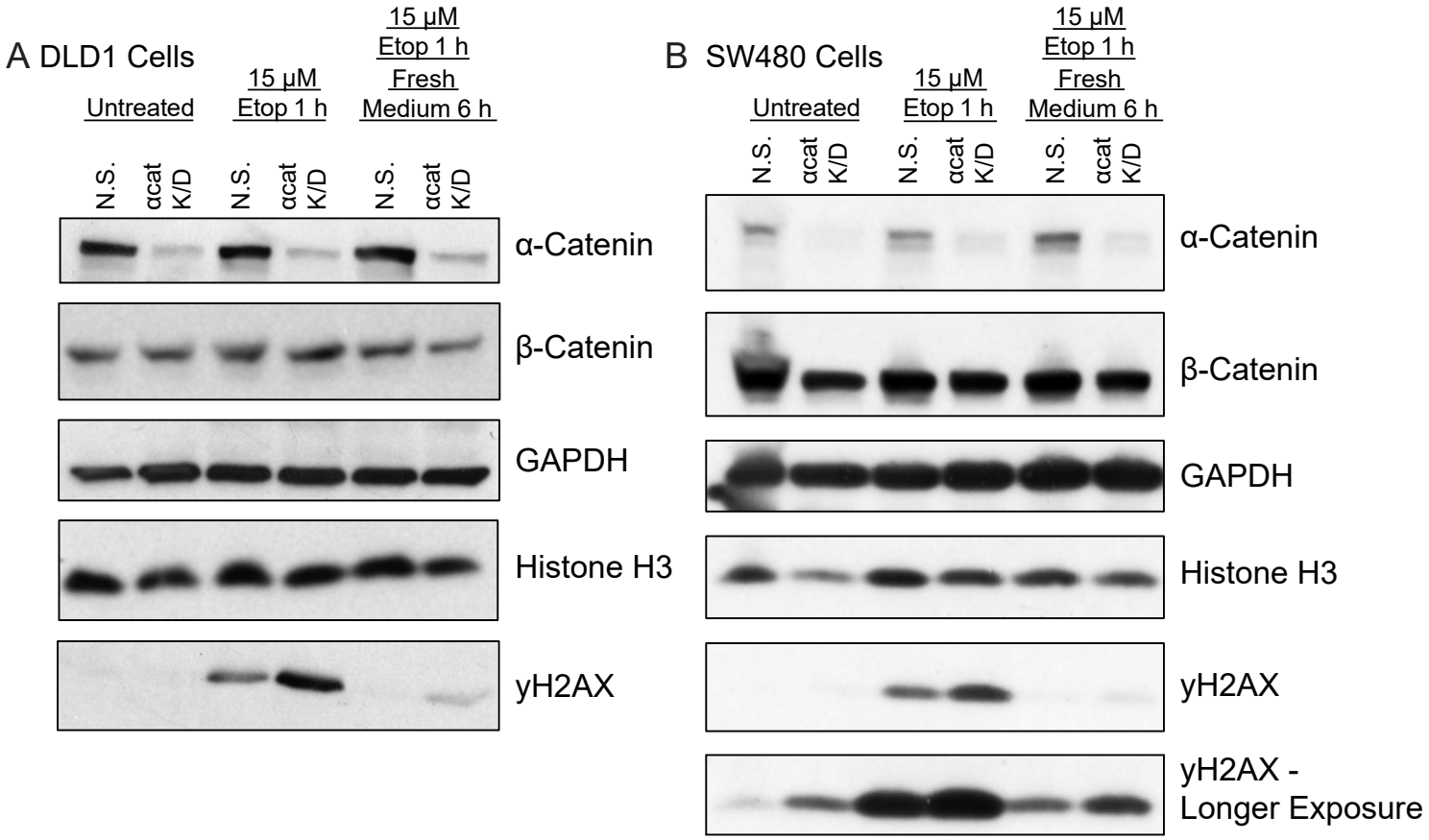
SUPPLEMENTARY FIGURES



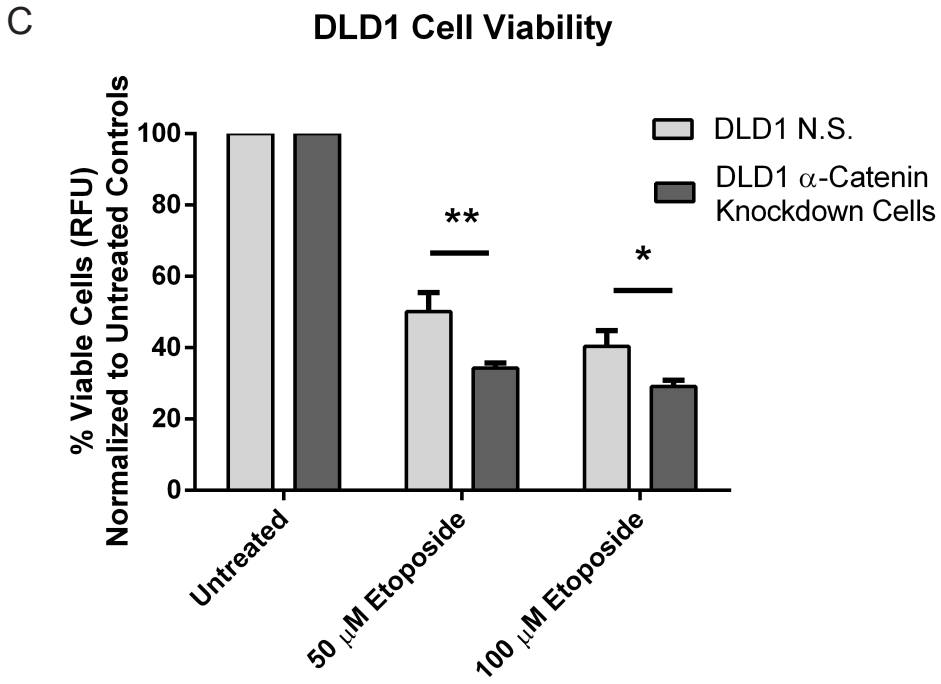
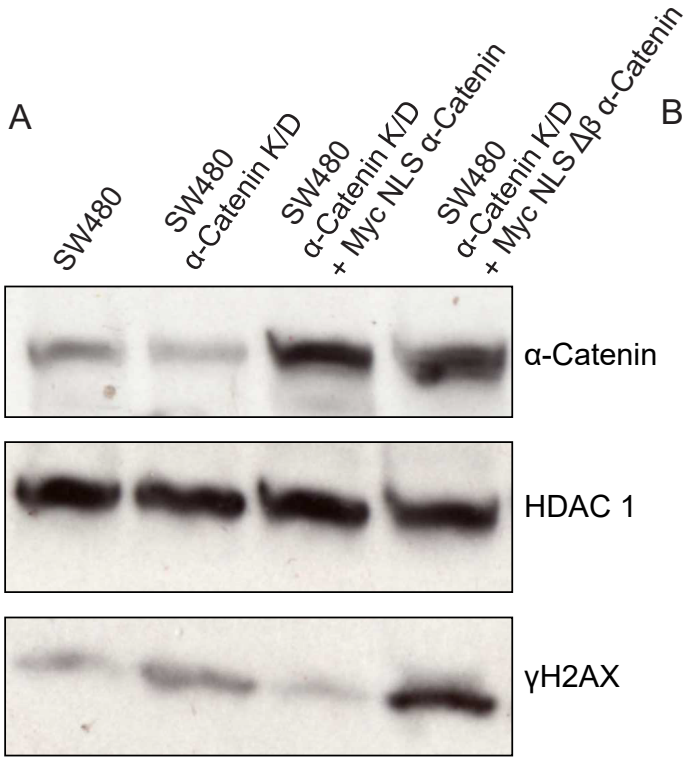


Figure S1: Loss of α-catenin increases γH2AX levels and toxicity in colon cancer cells.

(**A**) DLD1 cells stably transfected with non-specific (N.S.) and α-catenin (α cat K/D) shRNAs were left untreated, treated with 15 μM etoposide for 1 hour, or treated with etoposide for 1 hour and left to recover for 6 hours in fresh medium. Cells were then extracted in hot SDS and Western blots were performed for the indicated proteins. Increased γH2AX levels in etoposide treated α-catenin knockdown cells were observed relative to wild type. (**B**) SW480 cells stably transfected with non-specific (N.S.) and α-catenin (α cat K/D) shRNAs were treated and prepared as in (A). (**C**) DLD1 cells stably transfected with non-specific (N.S.) and α-catenin shRNAs were treated for 48 hours with the indicated concentrations of etoposide. The percentage of cells that remained viable was calculated relative to untreated controls. Mean + SEM is shown (N = 4; * $P < 0.05$, ** $P < 0.01$ by 2-way ANOVA).

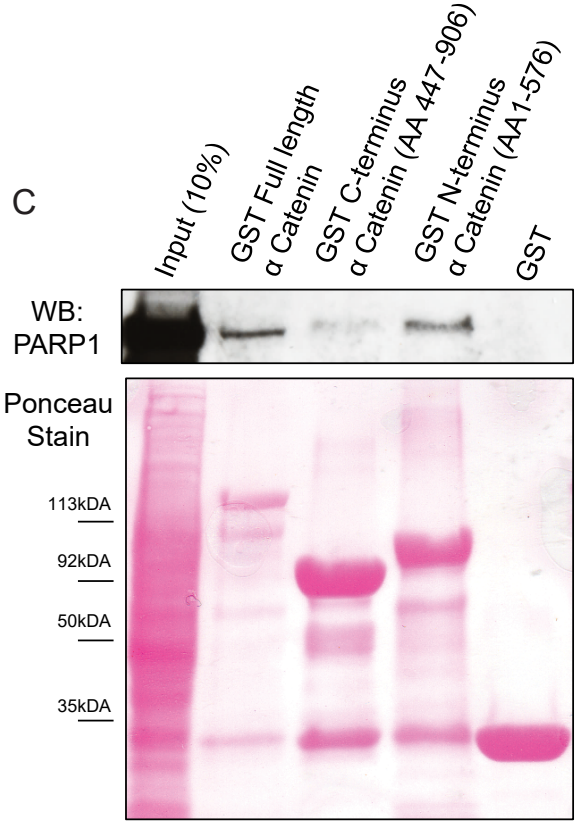
A



B

Identified Proteins	Molecular Weight	Scaffold Identification Probability	Unique # peptides
Catenin alpha-1	100 kDa	100%	69
Glutathione S-transferase P	23 kDa	100%	12
Catenin beta-1	85 kDa	100%	18
ATP-dependent DNA helicase 2 subunit 2 (Ku80)	83 kDa	100%	5
Nucleophosmin	33 kDa	100%	4
ATP-dependent DNA helicase 2 subunit 1 (Ku70)	70 kDa	100%	5
Poly [ADP-ribose] polymerase 1 (PARP1)	113 kDa	100%	4
Nuclease-sensitive element-binding protein 1	36 kDa	100%	2
Catenin alpha-2	105 kDa	100%	2

C



D

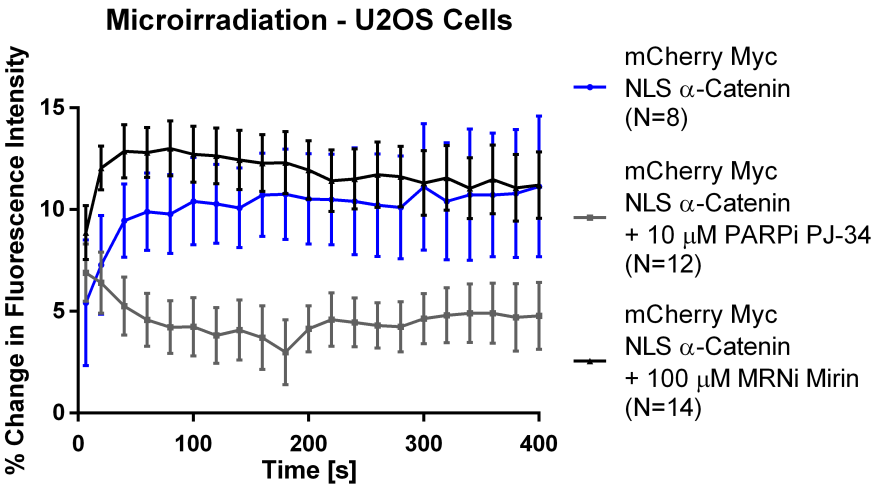
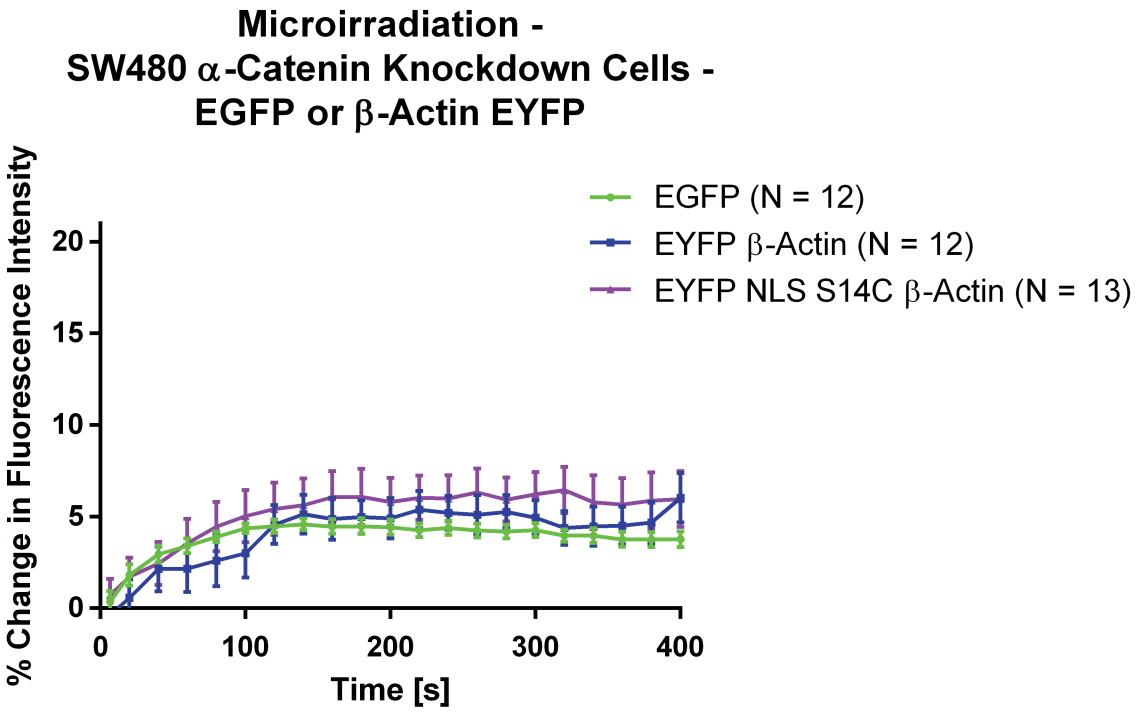


Figure S2: Nuclear α-catenin interacts with several DNA damage proteins.

(**A**) Nuclear extracts isolated from SW480 wildtype cells, α-catenin knockdown cells infected with non-specific hairpin shRNA lentivirus, and α-catenin knockdown cells rescued with lentivirus expressing Myc NLS α-catenin or Myc NLS Δβ α-catenin were blotted for γH2AX. Re-expression of Myc NLS full length α-catenin but not Myc NLS Δβ α-catenin resulted in γH2AX levels similar to wild type cells. HDAC1 was used as a loading control. (**B**) Nuclear extract from SW480 α catenin knockdown cells was incubated with GST α-catenin protein *in vitro* and GST pulldowns were performed. Mass spectrometry analysis identified several DNA damage pathway related proteins. Proteins with a 100% probability of identification and ≥ 2 identified peptides are reported. (**C**) Nuclear extract from SW480 α-catenin knockdown cells was incubated with GST full length α-catenin protein, GST N-terminus α-catenin, GST C-terminus α-catenin, or GST alone *in vitro* and GST pulldowns were performed. Western blotting for PARP1 shows a specific interaction with α-catenin mediated through its N-terminus. Ponceau stain is shown to indicate GST protein levels. (**D**) U2OS cells were transfected with mCherry fused to Myc NLS α-catenin for 48 hours. Cells were then treated with 30 mM LiCl, incubated with Hoechst 33342 (5 μg/mL) to sensitize the cells, and microirradiated to induce localized sites of DNA damage. Recruitment of mCherry Myc NLS α-catenin (N = 8) and mCherry Myc NLS α-catenin pre-treated with the PARP inhibitor, PJ34 (PARPi; N = 12), or Mre11-Rad50-Nbs1 (MRN) complex inhibitor, Mirin, for 1 hour (MRNi; N = 14) are shown (Mean ± SEM). PARP inhibition was able to reduce α-catenin recruitment. However, MRN, which is also responsible for the early recognition of DNA lesions and downstream signaling, was not identified in our proteomics screen and has no effect on α-catenin recruitment.

A



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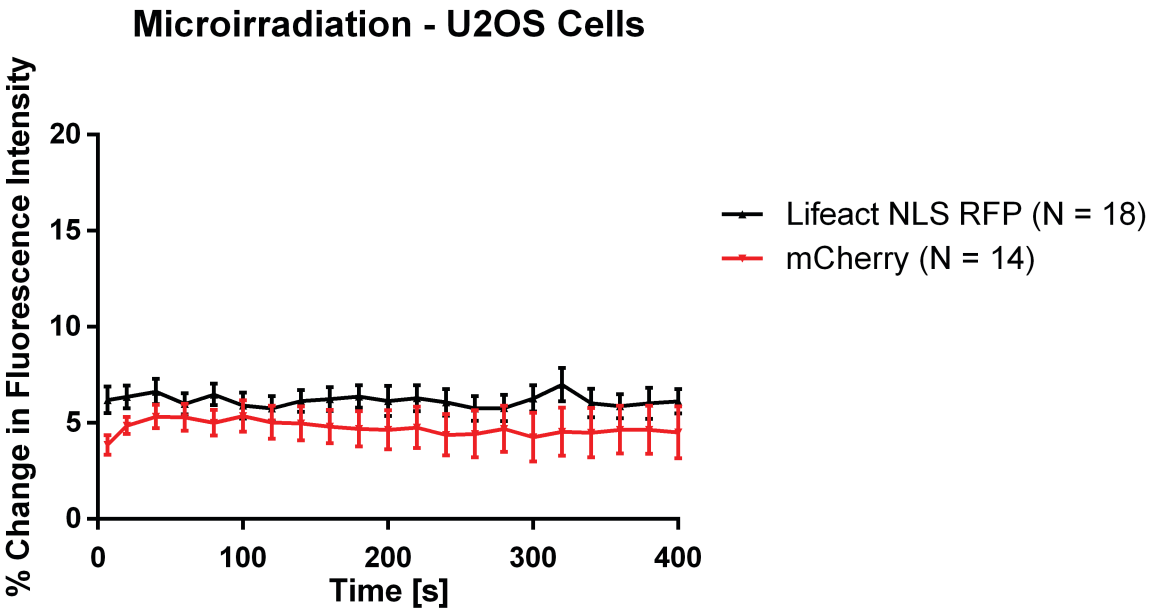

Figure S3: Nuclear actin is not enriched at sites of DNA repair.

(**A**) SW480 α-catenin knockdown cells transfected with EGFP alone, EYFP β-actin, or EYFP NLS S14C β-actin for 48 hours were pre-treated with Hoechst 33342 (5 μg/mL) and microirradiation experiments were performed. Mean ± SEM are shown. (**B**) U2OS cells transfected with mCherry alone or Lifeact NLS RFP for 24 hours were pre-treated with 30 mM LiCl, Hoechst 33342 (5 μg/mL), and microirradiation experiments were performed. Mean ± SEM are shown.