Science Advances
AAAS

Supplementary Materials for

APOBEC3A induces DNA gaps through PRIMPOL and confers gap-associated therapeutic vulnerability

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Sci. Adv. **10**, eadk2771 (2024)
DOI: 10.1126/sciadv.adk2771

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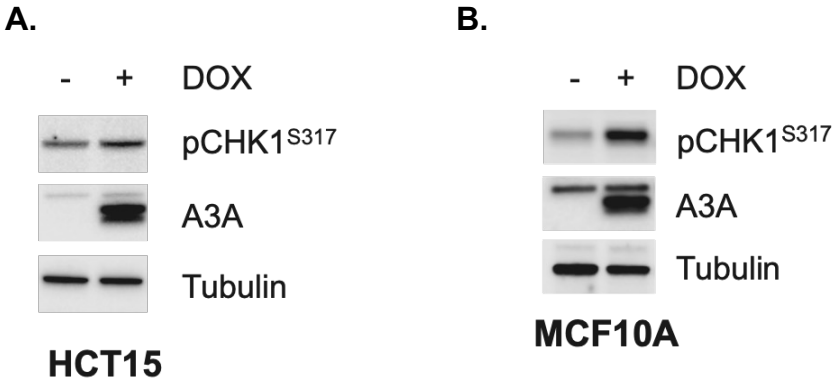

Fig. S1. pCHK1 activation in HCT15 and MCF10A inducible cell lines expressing A3A.
(A, B) HCT15 (in A) and MCF10A cells (in B) were treated with 200 ng/mL DOX to induce A3A expression for 48 hours prior to harvesting for Western Blot analysis. In (B), pCHK1 was probed on a different blot as A3A and Tubulin.

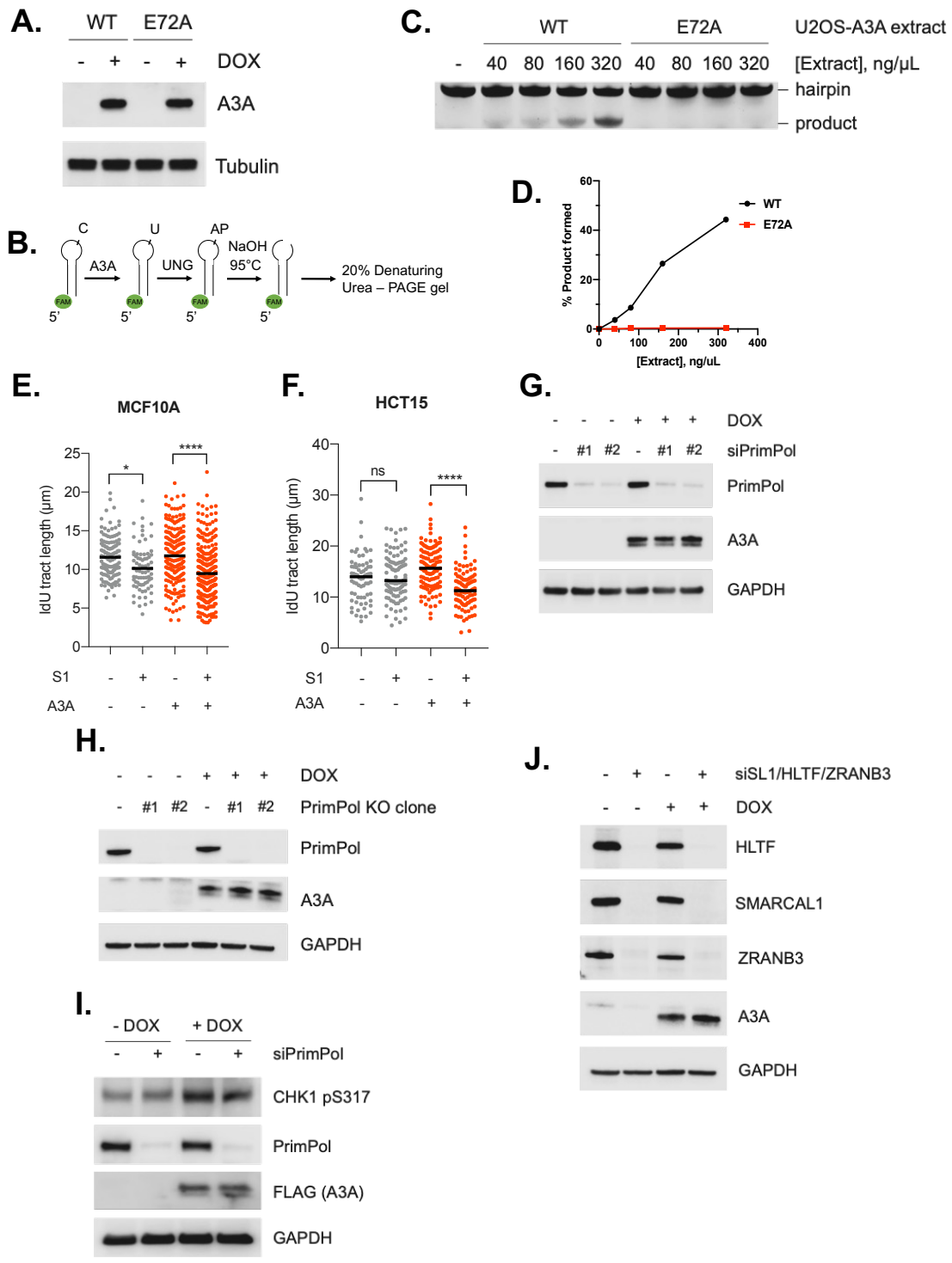

Fig. S2. Expression and activity of A3A in inducible cells, formation of A3A-induced gaps and expression levels of PrimPol and fork reversal factors.

(A) Western blot analysis indicating the expression levels of WT and E72A mutant of A3A after 48 hours of DOX induction. **(B)** Schematic of the deaminase activity assay. 5'-FAM-labeled

hairpin oligonucleotide containing a cytosine in the loop is incubated with U2OS cell extracts expressing either $A3A^{WT}$ or $A3A^{E72A}$ for 1 hour. Deamination of cytosine to uracil is followed by generation of AP sites by UNG. The resulting AP sites are cleaved in the presence of NaOH at 95 degrees C resulting in a strand break. The reaction is then electrophoresed under denaturing conditions to separate the substrate from the cleaved product. **(C)** Representative gel image showing the increasing conversion of the hairpin substrate (top band) into a cleaved product (bottom band) with increasing concentrations of extracts expressing $A3A^{WT}$ but not $A3A^{E72A}$. **(D)** Quantification of the deaminase activity observed in C. **(E, F)** Quantification of IdU tract length showing A3A expression leads to S1-sensitive shortening of IdU tracts in MCF10A in (E) and HCT15 cells in (F). **(G-J)** Western blots showing the level of knockdown for proteins in Fig. 2.

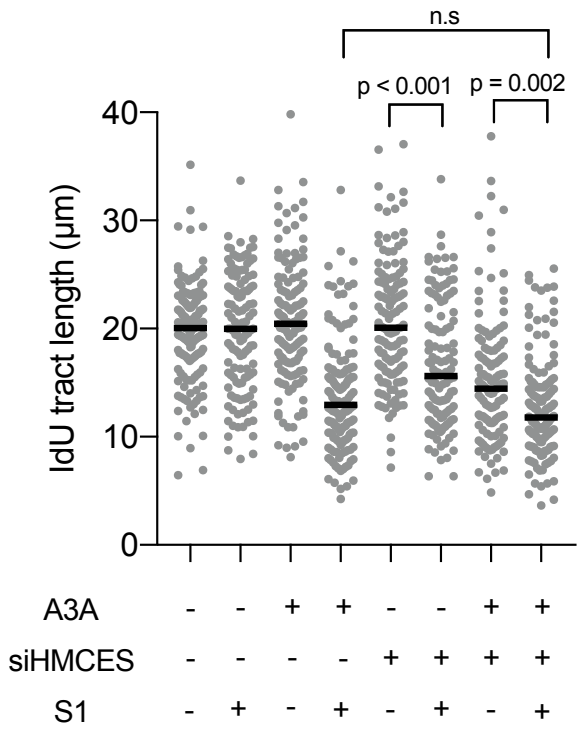

Fig. S3. Loss of HMCES slows down forks in A3A-expressing cells but does not lead to more ssDNA gaps.

Quantification of IdU tract lengths in A3A-expressing cells with or without HMCES depletion. Black horizontal line indicates median IdU tract length from at least 125 fibers quantified from two independent experiments (n>125). Statistical significance was determined by one-way ANOVA using Tukey's multiple comparisons test.

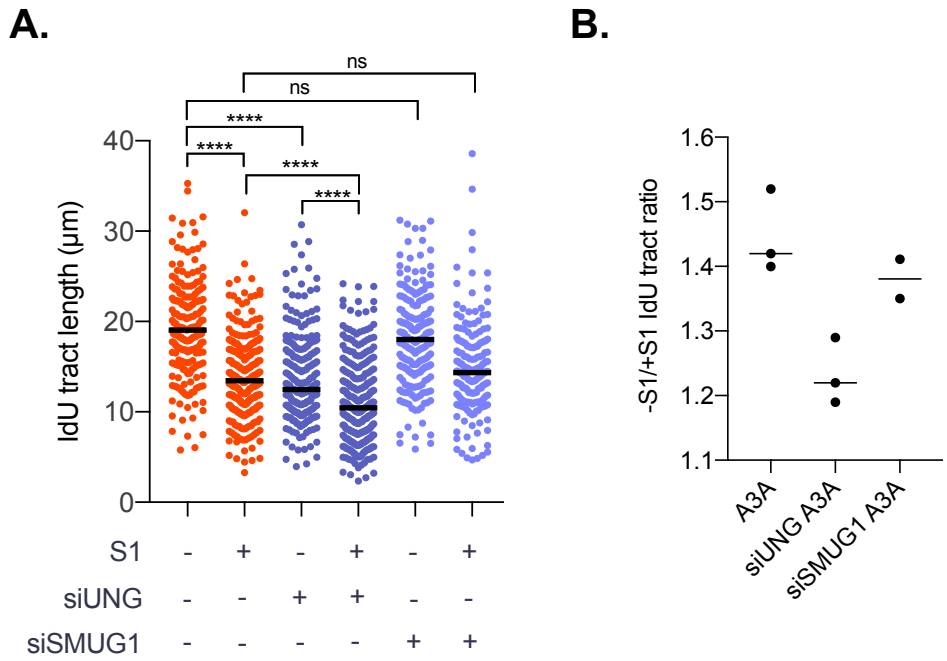

Fig. S4. A3A-induced gaps are formed from abasic sites formed after UNG activity.
(A) Quantification of IdU tract lengths in A3A-expressing cells with or without UNG depletion of SMUG1 depletion. Black lines indicate median IdU tract lengths. **(B)** Ratio of median IdU tracts for samples untreated with S1 over median IdU tracts for samples treated with S1. Higher ratio indicates more level of gaps.

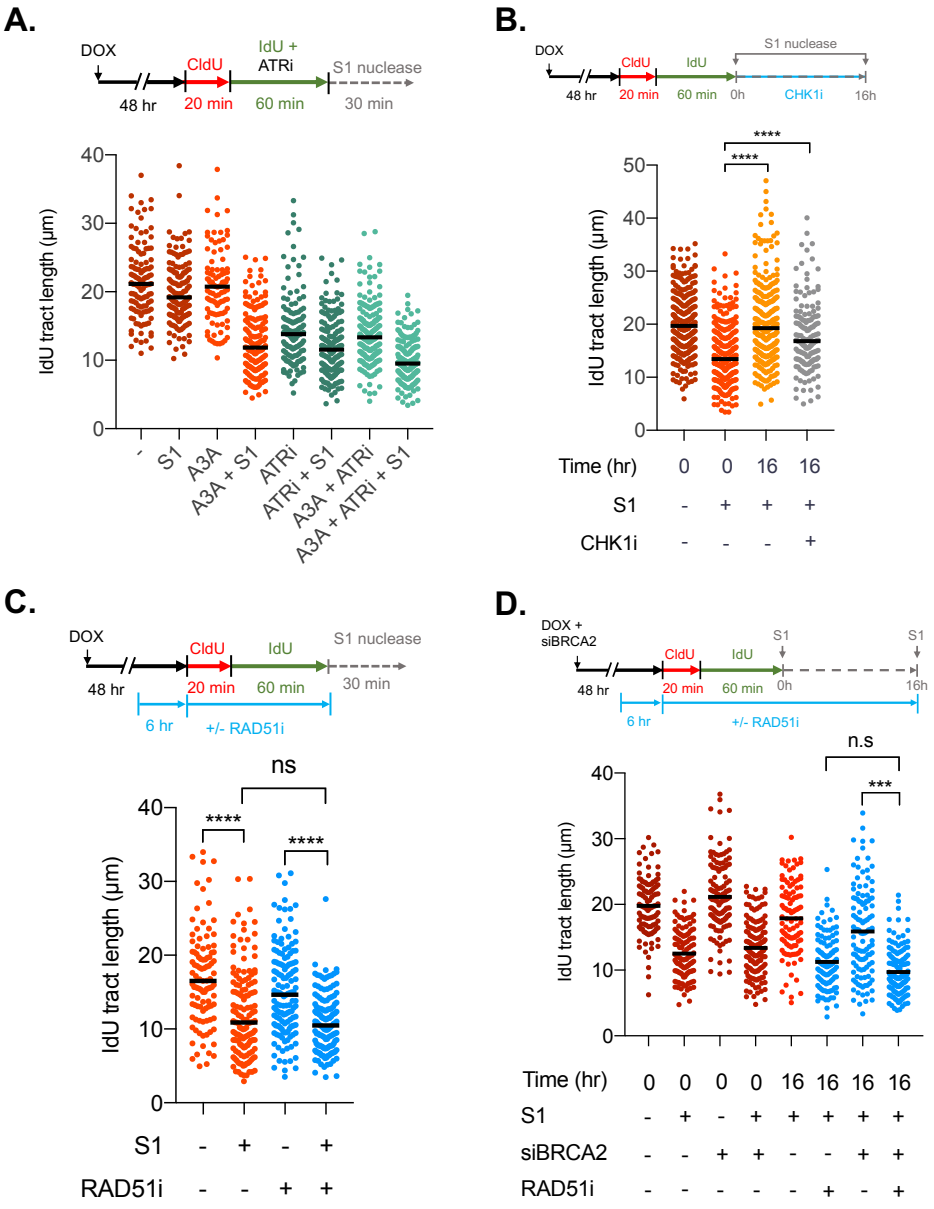

Fig. S5. Effect of ATRi on gap formation, CHK1i on gap repair and RAD51i on gap formation.

(A) Cells were treated with 5 µM VE-821 during the IdU labeling period. Quantification of IdU tract lengths showing the effect of ATRi on formation of A3A-induced ssDNA gaps. **(B)** CHK1 has a modest effect on repairing A3A-induced gaps. Cells were treated with 5 µM MK-8776 during the recovery period of 16 hr before harvesting cells for DNA fiber analysis. **(C)** RAD51i does not lead to an increase in gap formation in A3A-expressing cells. Cells were treated with 25 µM B02 during the labeling period and shortening of the IdU tract lengths was monitored. **(D)** 20 nM siRNA was used to deplete BRCA2. Cells were treated with RAD51i as mentioned above.

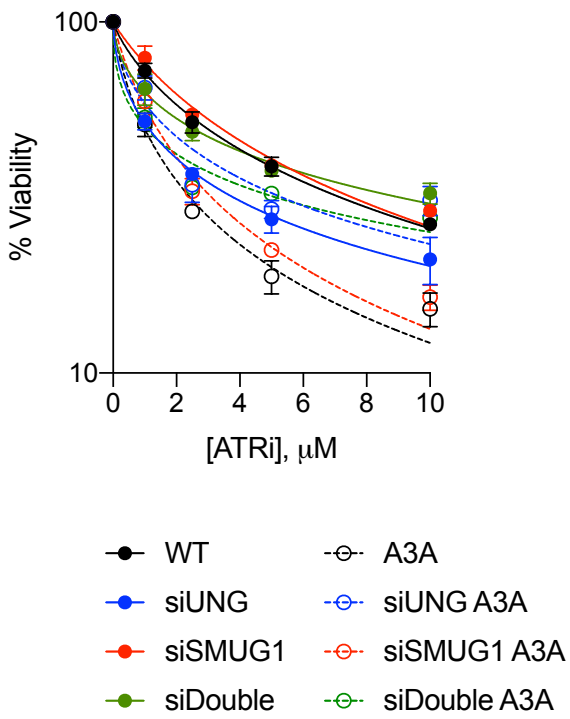

Fig S6. Viability of A3A-expressing cells after UNG and SMUG1 double-depletion. Cells were treated with DOX to induce A3A expression and 10 nM siUNG and/or 2 nM siSMUG1. After 48 hr cells were treated with ATRi, VE-821. Viability was measured after 5 days using Cell Titre Glo 2.0 viability reagents. % Viability was normalized to the untreated samples for each condition.

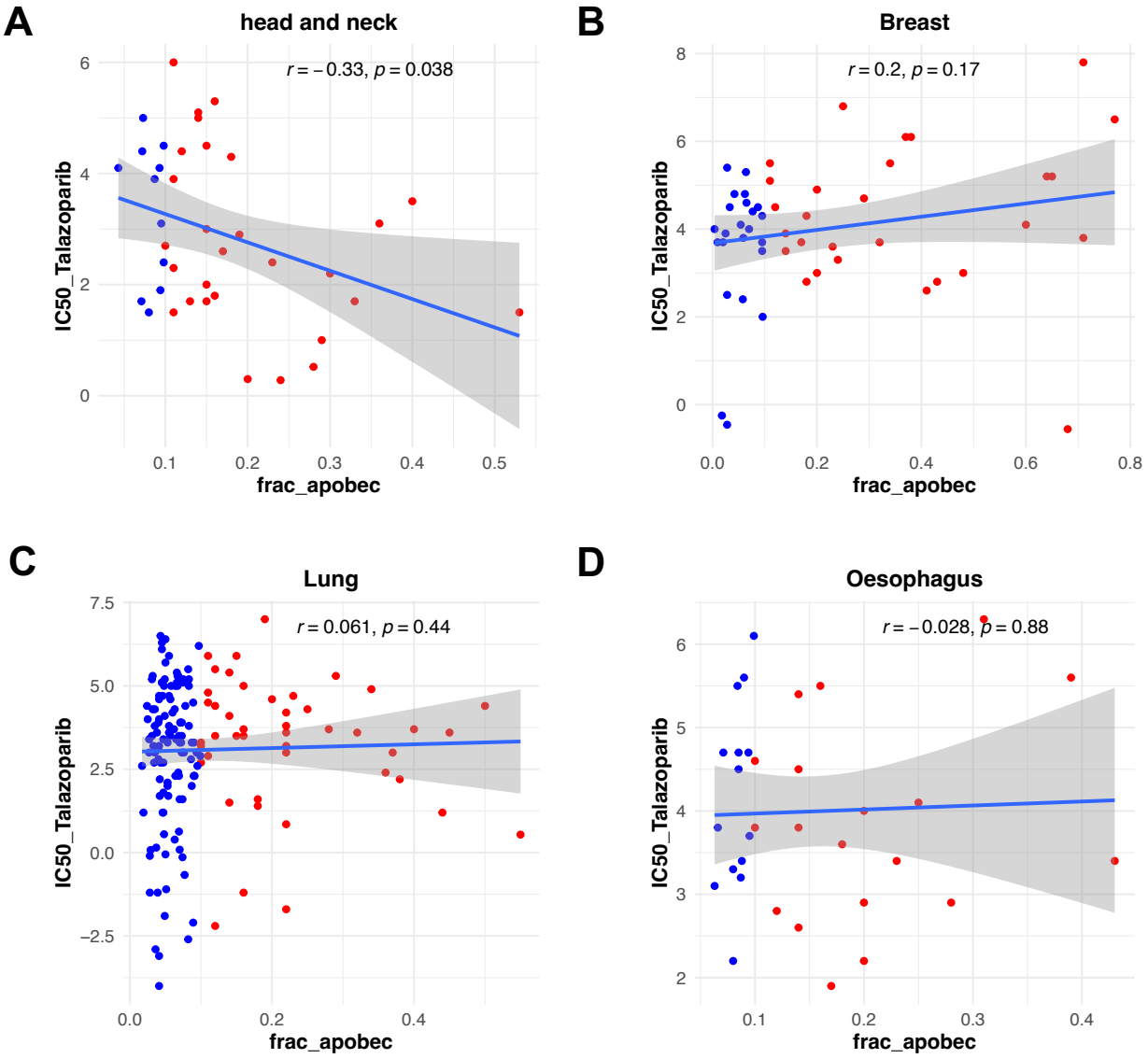

Fig S7. Correlation between APOBEC mutation signature and IC50 for Talazoparib across cell lines from 4 different tumor types. (A-D) Mutation signature analysis was performed using non-negative matrix factorization across different cell lines from head and neck (A), breast (B), lung (C) and oesophagial cancer (D). A negative correlation coefficient indicates inverse correlation between APOBEC mutation signature and Talazoparib IC50 for the given tumor types. For each tumor type, cell lines with 10% or more of the total mutations assigned to the APOBEC signature were considered as APOBEC+ samples shown in red whereas cell lines with less than 10% of the total mutations assigned to the APOBEC signature were considered as APOBEC- samples shown in blue.

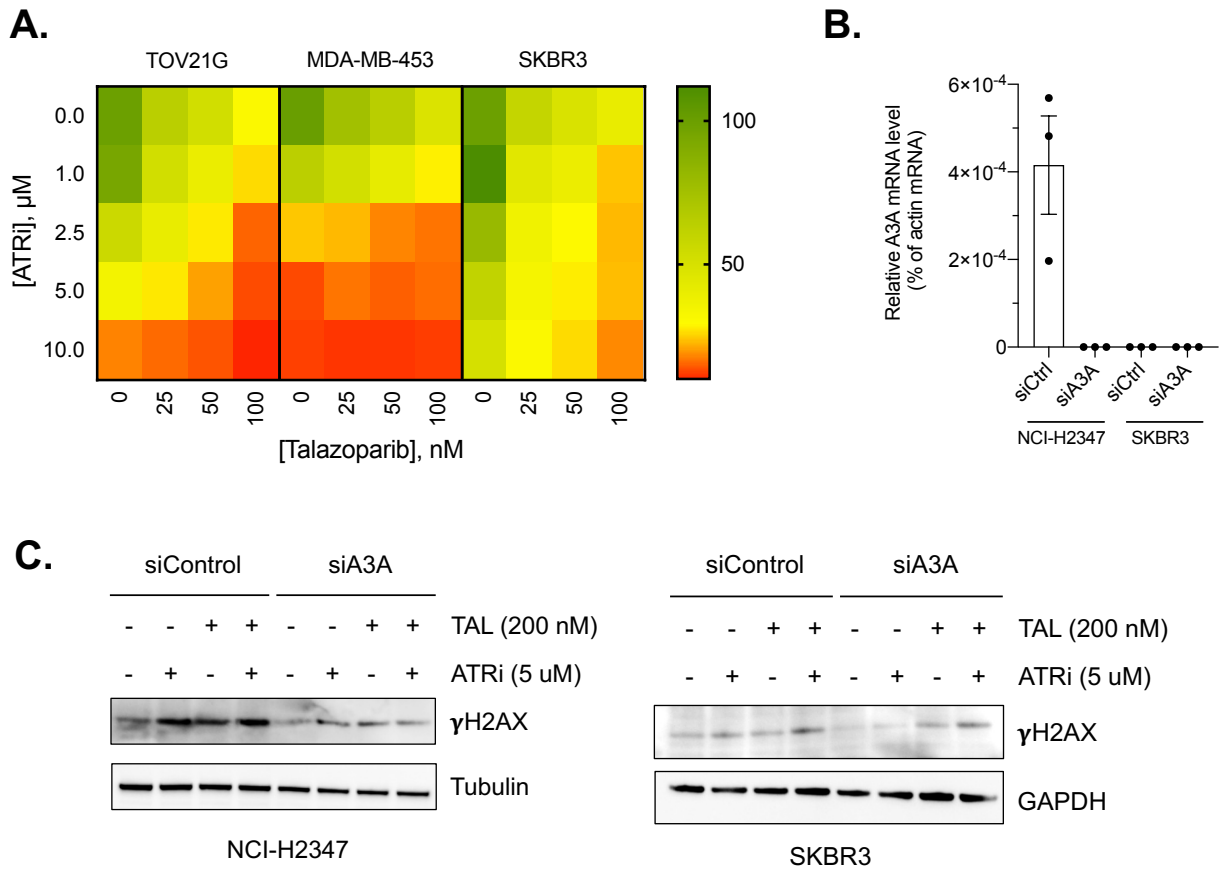

Fig S8. High endogenous A3A/B activities render cancer cells susceptible to a combination of ATRi and PARPi. (A) Cells were treated with high ATRi and PARPi for 5 days before measuring cell viability using Cell Titre Glo viability reagents. Viability values were normalized to the untreated samples for each cell line. Green indicates higher viability while red indicates lower viability. (B) 20 nM siRNA was used to deplete endogenous A3A. qPCR analysis was performed to confirm the expression level of endogenous A3A in NCI-H2347 cells and SKBR3 cells. (C) Cells were treated with appropriate concentrations of ATRi and PARPi for 8 hours before harvesting for western blotting. 20 nM siRNA was used to deplete endogenous A3A.

Supplementary Table S1: List of siRNAs used in the study.

Gene	Identifier
PrimPol#1	s47416
PrimPol#2	s47418
REV1	s28167
SMARCAL1	s531776
HLTF	s13138
ZRANB3	s224929
RAD51	s11735
UNG	s14679
SMUG1	s24135
RAD18	s32296
A3A	s47220
HMCES	s32442
BRCA2	s2085

Supplementary Table S2: List of antibodies used in the study.

Target	Source	Identifier	Dilution
Flag	Sigma-Aldrich	F3165	1:1000
GAPDH	Santa Cruz	sc-32233	1:2500
Tubulin	Cell Signaling	2125S	1:2500
PrimPol	Gift from Mendez Lab	NA	1:10
SMARCAL1	Santa Cruz	sc-376377	1:1000
HLTF	Santa Cruz	sc-398357	1:1000
ZRANB3	Novus Biologicals	NBP2-93301	1:1000
pCHK1 S317	Cell Signaling	2344S	1:500
IdU (Mouse Anti-BrdU)	BD	347580	1:100
CldU (Rat Anti-BrdU)	Abcam	ab264079	1:50
γH2AX (pS139)	EMD Millipore	JBW301	1:1000
anti-Mouse IgG Alexa Fluor 488	Jackson ImmunoResearch	715-545-151	1:100
anti-Rat IgG Alexa Fluor 594	Thermo Fisher Scientific	A11007	1:100
Anti-Rabbit-HRP (for WB)	Jackson ImmunoResearch	111-035-003	1:500 – 1:5000
Anti-Mouse-HRP (for WB)	Jackson ImmunoResearch	115-035-003	1:500 – 1:5000