

# **G-quadruplex DNA drives genomic instability and represents a targetable molecular abnormality in ATRX-deficient malignant glioma**

Wang Y et al.

## **Supplementary Information**

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| Target               | Merchant, catalog number       | Application                               |
|----------------------|--------------------------------|-------------------------------------------|
| ATRAX                | Santa Cruz (H-300): sc-15408   | Western Blots (1:500), IF (1:100)         |
| VINCULIN             | Sigma Aldrich (hVIN-1), V9131  | Western Blots (1:5000)                    |
| phospho-KAP1 Ser 824 | Abcam, ab70369                 | Western Blots (1:1000)                    |
| phospho-CHK1 Ser 317 | Cell Signaling, 2344           | Western Blots (1:500)                     |
| phospho-CHK1 Ser 345 | Cell Signaling, 2348           | Western Blots (1:500)                     |
| G-quadruplex, 1H6    | Gift from Dr. Peter Lansdorp   | IF (1:100)                                |
| G-quadruplex, BG4    | Millipore, MABE917             | IF (1:100)                                |
| γ-H2AX               | Millipore (JBW301), 05-636     | IF (1:500), ChIP (2 μg), IHC (Fr) (1:500) |
| 53BP1                | Novus Biologics, NB100-304     | IF (1:1000)                               |
| Ki-67                | Santa Cruz, sc-23900           | IHC (P) (1:100)                           |
| BLM                  | Bethyl laboratories, A300-110A | IF (1:100), ChIP (5 μg)                   |

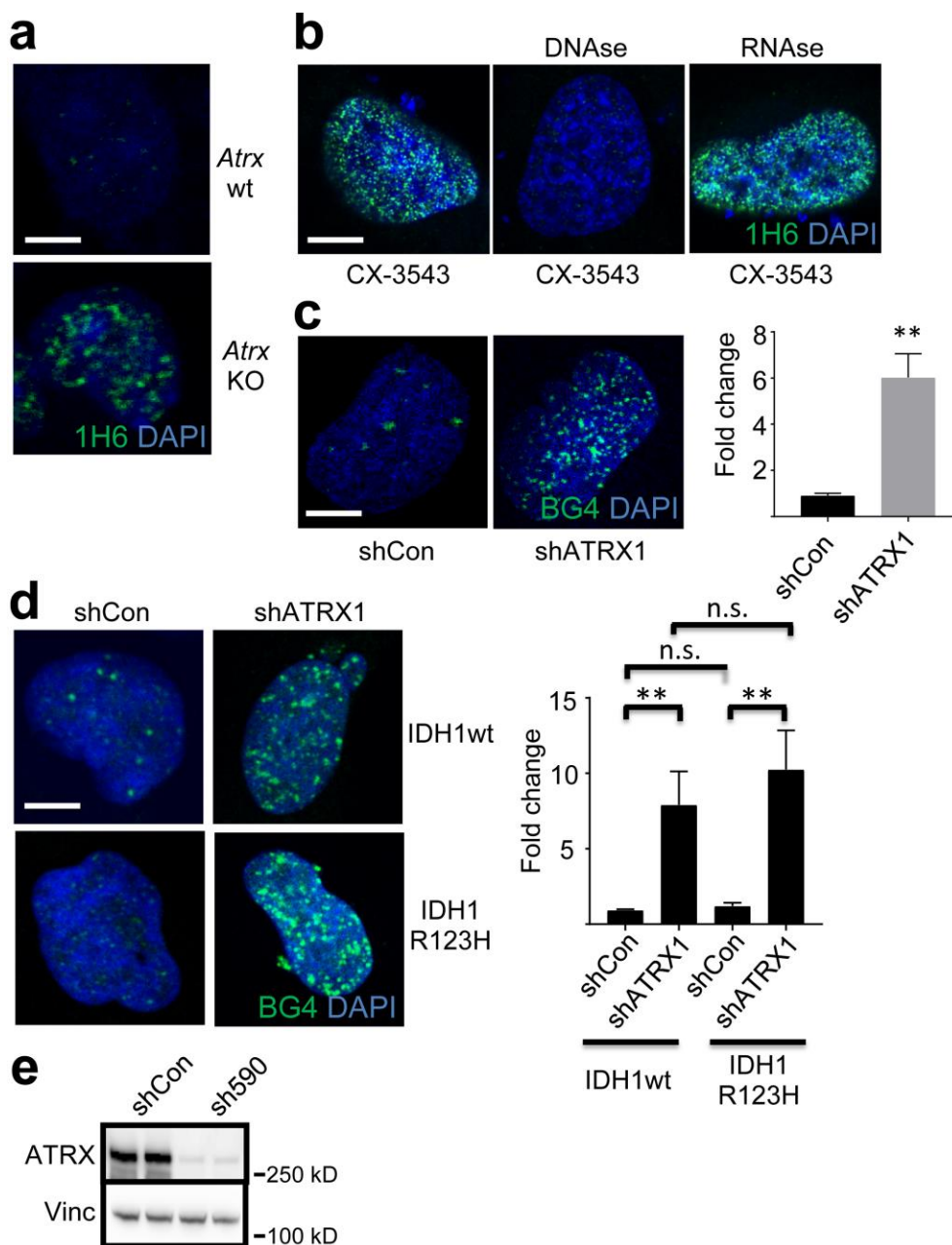
**Supplementary Table 1:** Commercially available antibodies used in this study.

|           |                        |
|-----------|------------------------|
| shCon-1   | ATCTCGCTTGGGCGAGAGTAAG |
| shATRAX-1 | GGAAGTAGCTCTTCAGAAA    |
| shCon-2   | CAACAAGATGAAGAGCACCAA  |
| shATRAX-2 | GGAAAGATGATAAAGGAAA    |
| shSCR     | CAACAAGATGAAGAGCACCAA  |
| sh590     | CGACAGAACTAACCCTGTAA   |

**Supplementary Table 2:** sequences of shRNA against ATRX

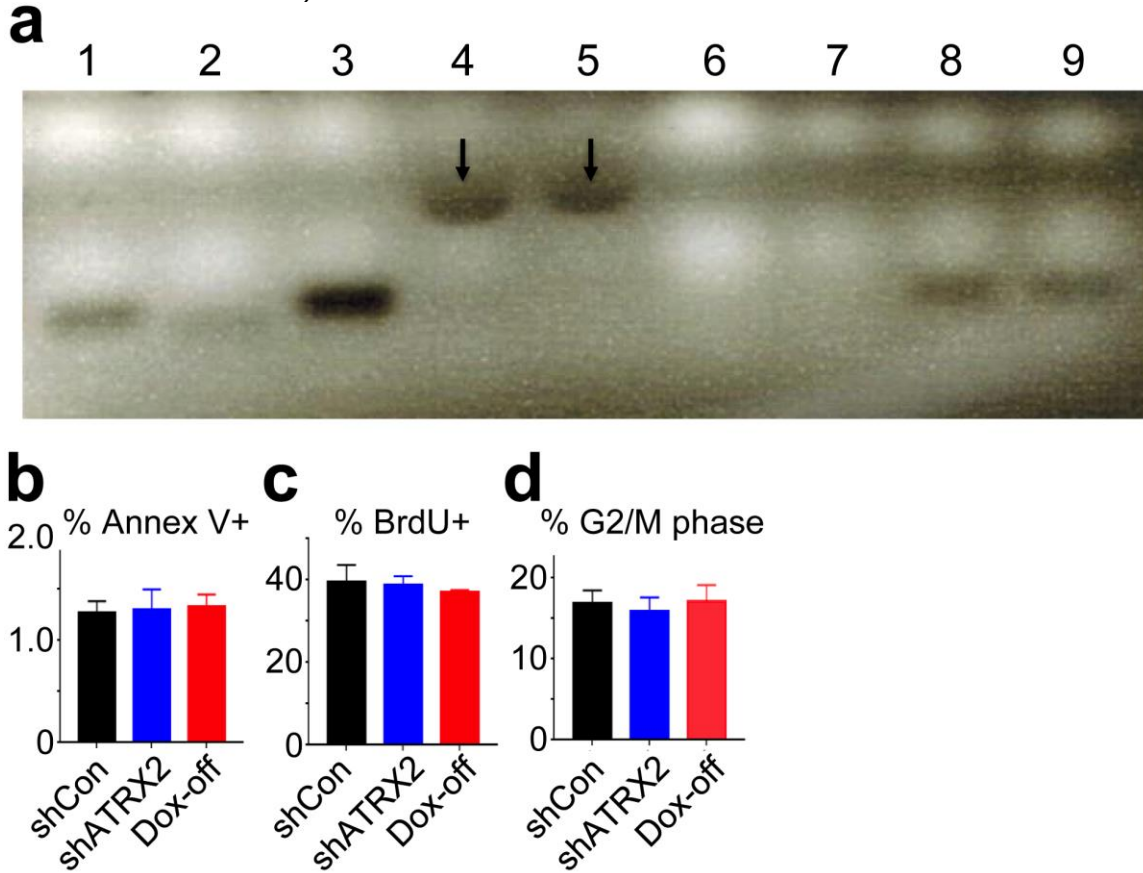
|                 |                                         |
|-----------------|-----------------------------------------|
| Tel 1/2 - Tel 1 | GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT   |
| Tel 1/2 - Tel 2 | TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA |
| Tel 2-F         | CAAGTTTAAGGTTGTGTTTGTAC                 |
| Tel 2-R         | AAATGAGTTGCAACAGGTACAAT                 |
| Tel X-F         | TGTCTGGGTCTTTGGAGAGG                    |
| Tel X-R         | CCTAACCCATCTGCTGGTTC                    |
| GAPDH CHIP-F    | CGGGATTGTCTGCCCTAATTAT                  |
| GAPDH CHIP-R    | GCACGGAAGGTCACGATGT                     |
| Myc-F           | AGGGCTTCTCAGAGGCTTG                     |
| Myc-R           | GCTGGAATTACTACAGCGAGTT                  |
| ZNF618-F        | CGACGCCACCTAGAGGATAC                    |
| ZNF618-R        | AATCTCTTACCCCTCCACTGC                   |
| ESR1-F          | GCAGATCCAAGCTGTCTTTACTCA                |
| ESR1-R          | GGTGGGCAGAAGAAATCCTTT                   |

**Supplementary Table 3: sequences of primers used in this study**

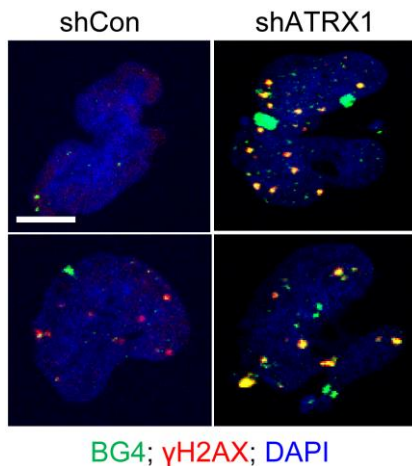


**Supplementary FIG. 1: ATRX deficiency promotes G4 formation.** **a:** G4 immunostaining (1H6) in primary murine neural progenitor cells harboring either intact or inactivated *Atrx*<sup>44</sup>. **b:** NHAs treated with 50nM CX-3543 alone (left), or with either DNase (middle), or RNase (right) to confirm the specificity of the 1H6 antibody for DNA structures. **c:** G4 immunostaining (BG4) in constitutive shATRX NHA lines (DAPI counterstain); quantified relative G4 signal intensity also shown (50 nuclei counted in all cases). **d:** G4 immunostaining (BG4) in constitutive shATRX NHA lines expressing either wild type (IDHwt) or R132H-mutant (IDH1 R132H) IDH1 (DAPI counterstain). Scale bars represent 10  $\mu$ m; quantified relative G4 signal intensity also shown (50 nuclei counted in all cases). **e:** Western blot confirms robust ATRX knockdown in sh590-expressing TS 543 GSCs relative to controls (shCon, Vinculin control, duplicate loading). Where

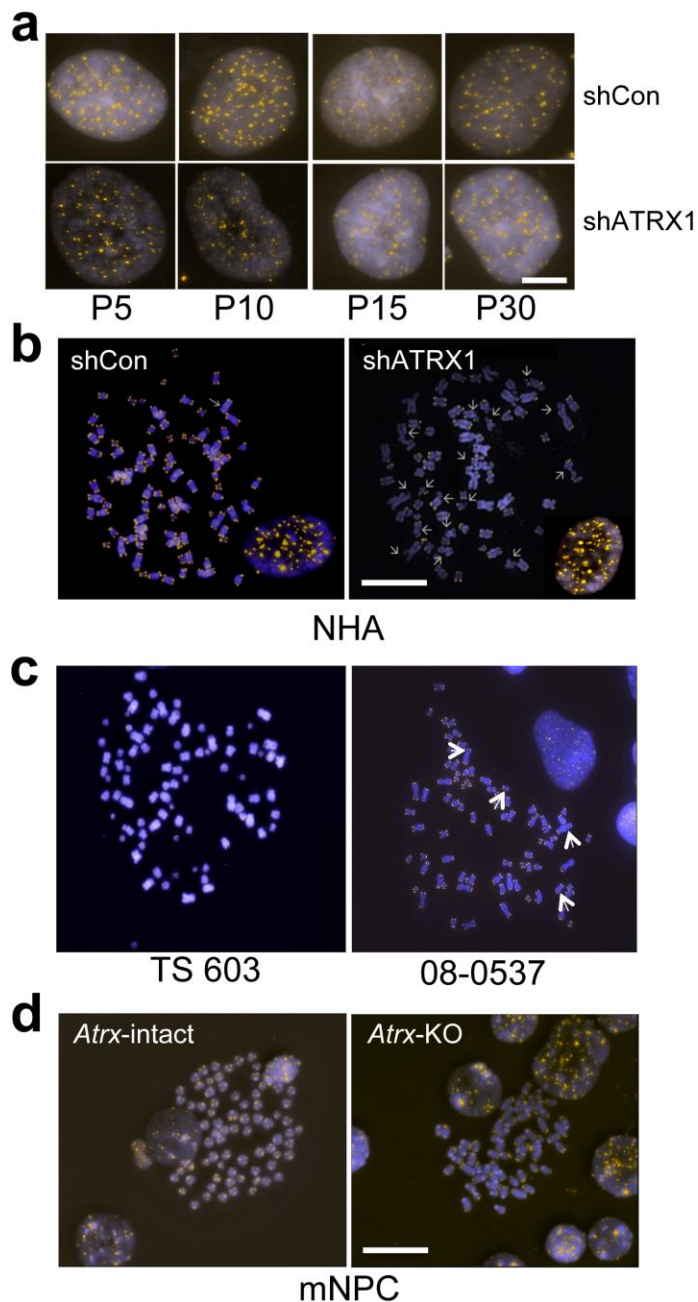
applicable, error bars reflect SEM;  $P$  values determined by unpaired, two-tailed t-test (\*\*:  $P < 0.01$ , n.s.:  $P > 0.05$ ).



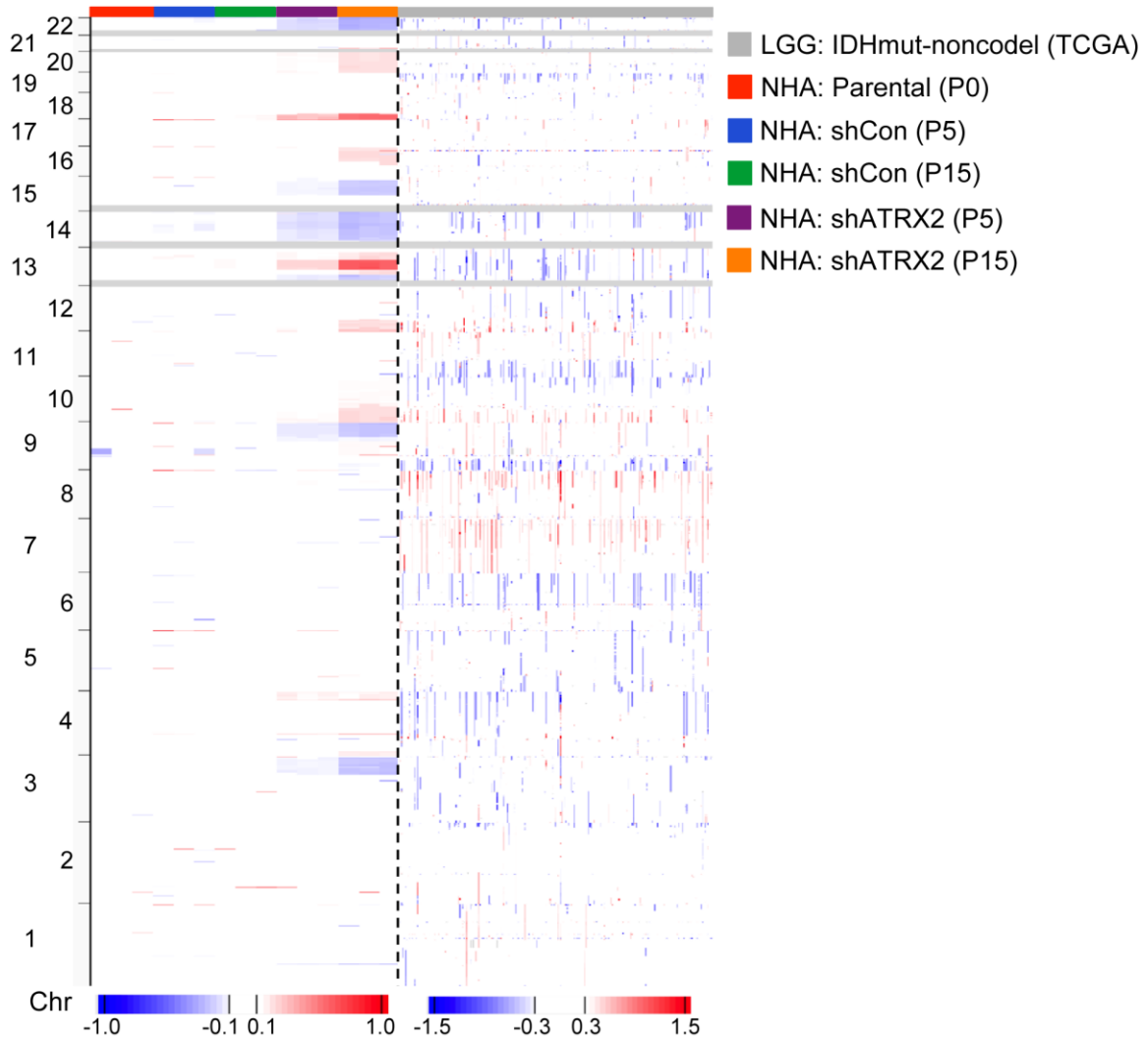
**Supplementary FIG. 2:** **a:** Confirmation of specificity for Hf2 G4 pulldowns using kit2 G4 nucleotides. Hf2 antibodies were incubated with kit2+random ssDNA mixture, no elution (lane 4), kit2+random dsDNA, no elution (lane 5), ssDNA alone (lane 6), dsDNA alone (lane 7), kit2+ssDNA, eluted (lane 8) and kit2+dsDNA, eluted (lane 9). Kit2 (lane 1), ssDNA (lane 2) and dsDNA (lane 3) were also included in the electrophoresis. Samples were run on a 3% agarose gel, non-denaturing. Arrows showed gel shift due to binding of pulldown antibodies. **b-d:** Inducible NHA lines (3 replicates each) showed no effects of ATRX inactivation on either apoptosis (measured by Annexin V positive population, **b**) or proliferation (measured by BrdU incorporation, **c**; G2/M-phase content (4n), **d**). Error bars reflect SEM; scale bars represent 10  $\mu$ m.



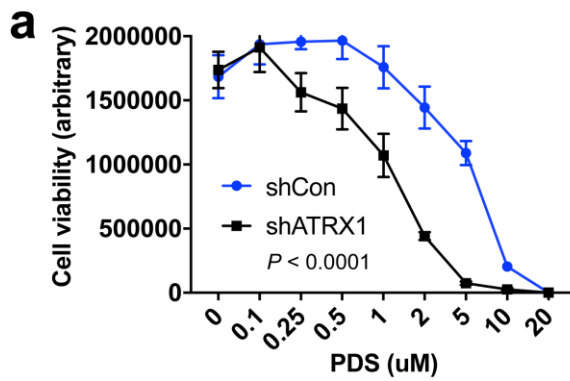
**Supplementary FIG. 3: G4 and DNA damage sites colocalize in the setting of ATRX deficiency.** Constitutive shATRX NHAs were double stained for G4 (BG4) and  $\gamma$ -H2AX. Two examples are shown for shCon and shATRX1 NHAs (DAPI counterstain). Scale bars represent 10  $\mu$ m.



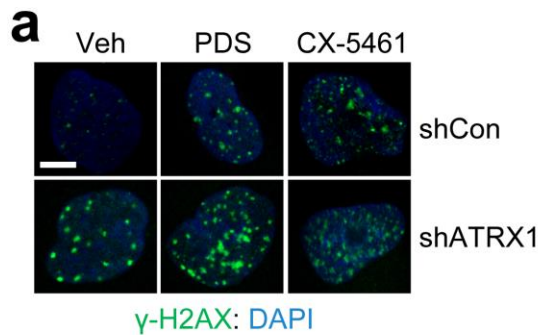
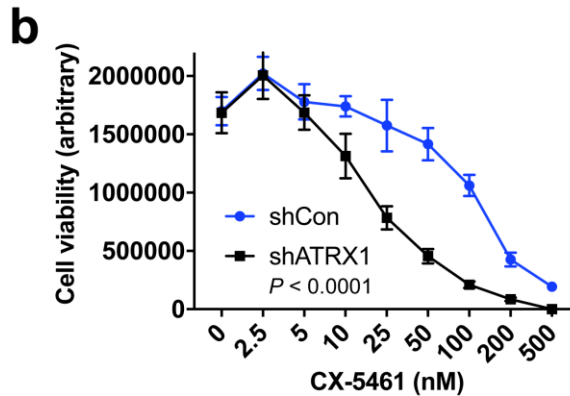
**Supplementary FIG. 4: ATRX knockdown induces chromosome breaks, but not ALT in NHAs.** **a:** TEL-FISH of NHAs at passages 5, 10, 15 and 30 after ATRX knockdown. No ultrabright foci, normally indicative of ALT, are apparent. **b:** ATRX deficient NHAs (shATRX1, passage 15) showed significantly increased chromosome breaks by cytogenetic analysis (white arrows). Despite these chromosome abnormalities, Tel-FISH (yellow) showed no change in telomere signal (Scale bar: 10  $\mu$ m). **c-d:** cytogenetic analysis for chromosome breaks in *ATRX*-wild type (TS 603) and *ATRX*-mutant (08-0537) GSCs (c) and Tel-FISH in *Atrx*-intact and *Atrx*-KO mNPCs (d; Scale bar: 25  $\mu$ m; white arrows indicate chromosome breaks in field of view).



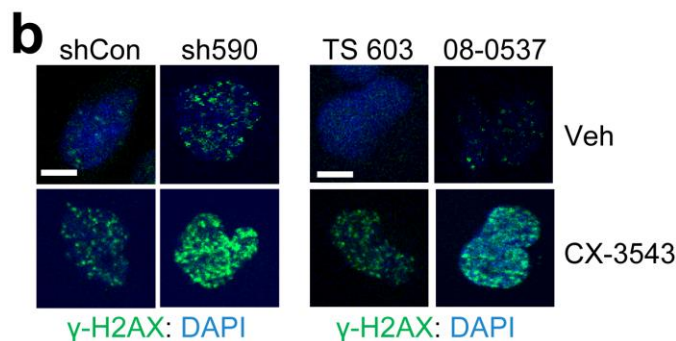
**Supplementary FIG. 5: ATRX deficiency induces CNAs in NHAs and is associated with a distinct CNA profile in gliomas.** Segmented SNP array data for parental NHAs, shCon NHAs (P5 and P15), shATRX1 NHAs (P5 and p15) and IDHmut-noncode1 LGG patients (TCGA) visualized by IGV. Color scales indicate  $\log_2$  copy number.

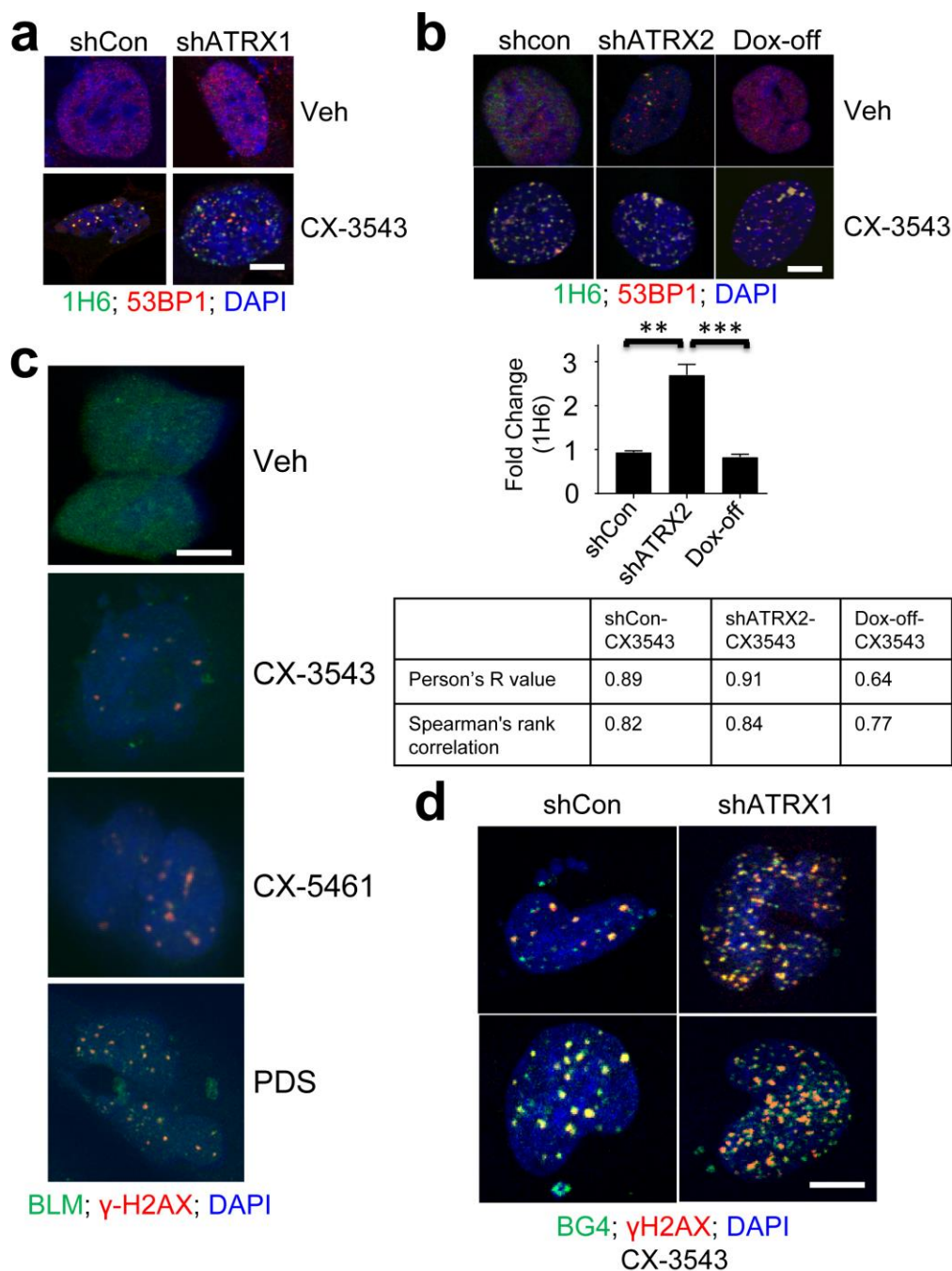


**Supplementary FIG. 6: ATRX-deficient NHAs are selectively sensitive to G4 stabilization. a-b:** Cell viability (CellTiter-Glo) of constitutive shCon and shATRX1 NHAs (3 replicates each) treated with either PDS (**a**) or CX-5461 (**b**) at indicated concentrations. Error bars reflect SEM; *P* values determined by two-way ANOVA.



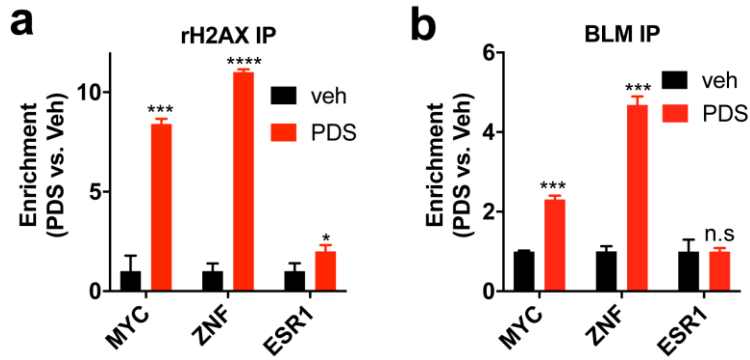
**Supplementary FIG. 7: G4 stabilization markedly enhances DNA damage in the ATRX-deficient context. a:** γ-H2AX immunofluorescence in constitutive shATRX NHAs showing increased DNA damage with either PDS (2 μM) or CX-5461 (50 nM) treatment, particularly in the setting of ATRX knockdown (DAPI counterstain). **b:** γ-H2AX immunofluorescence in ATRX-intact (shCon) and ATRX-knockdown (sh590) TS543 GSCs and in ATRX-wild type (TS 603) and ATRX-mutant (08-0537) GSCs treated with 100 nM CX-3543. Scale bars represent 10 μm.



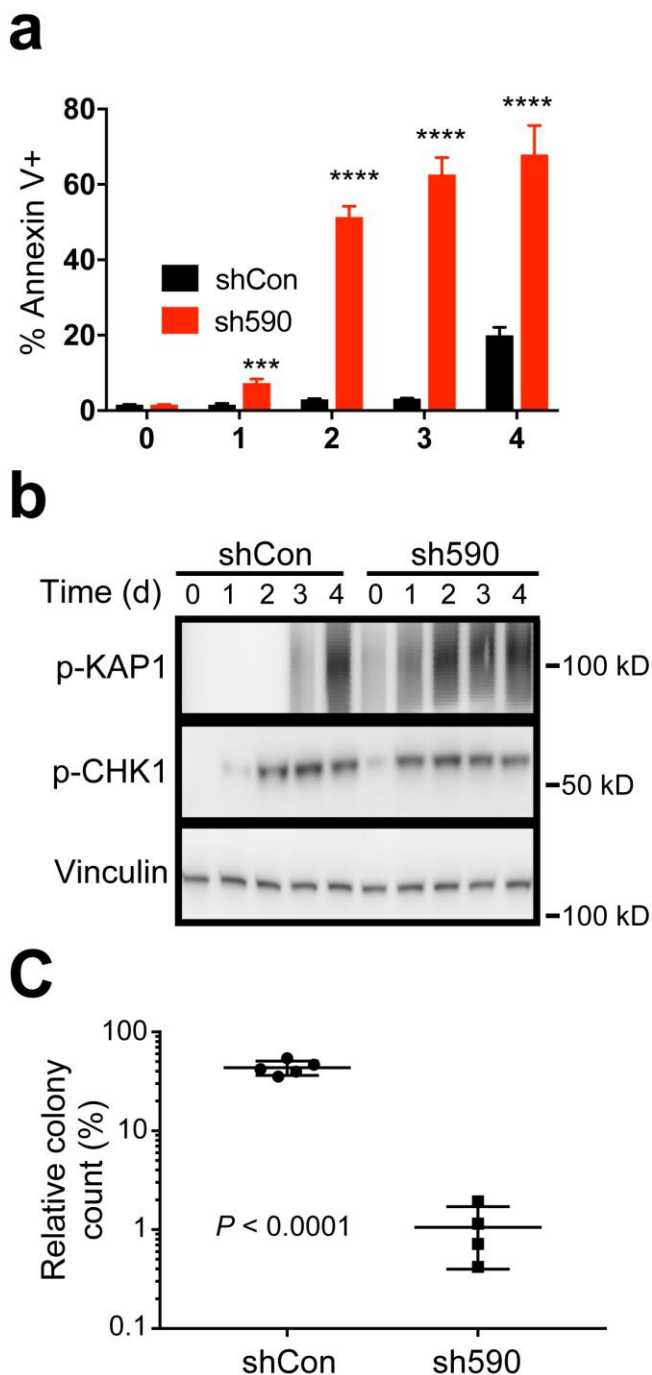


**Supplementary FIG. 8: G4s colocalize with DNA damage foci induced by CX-3543.**

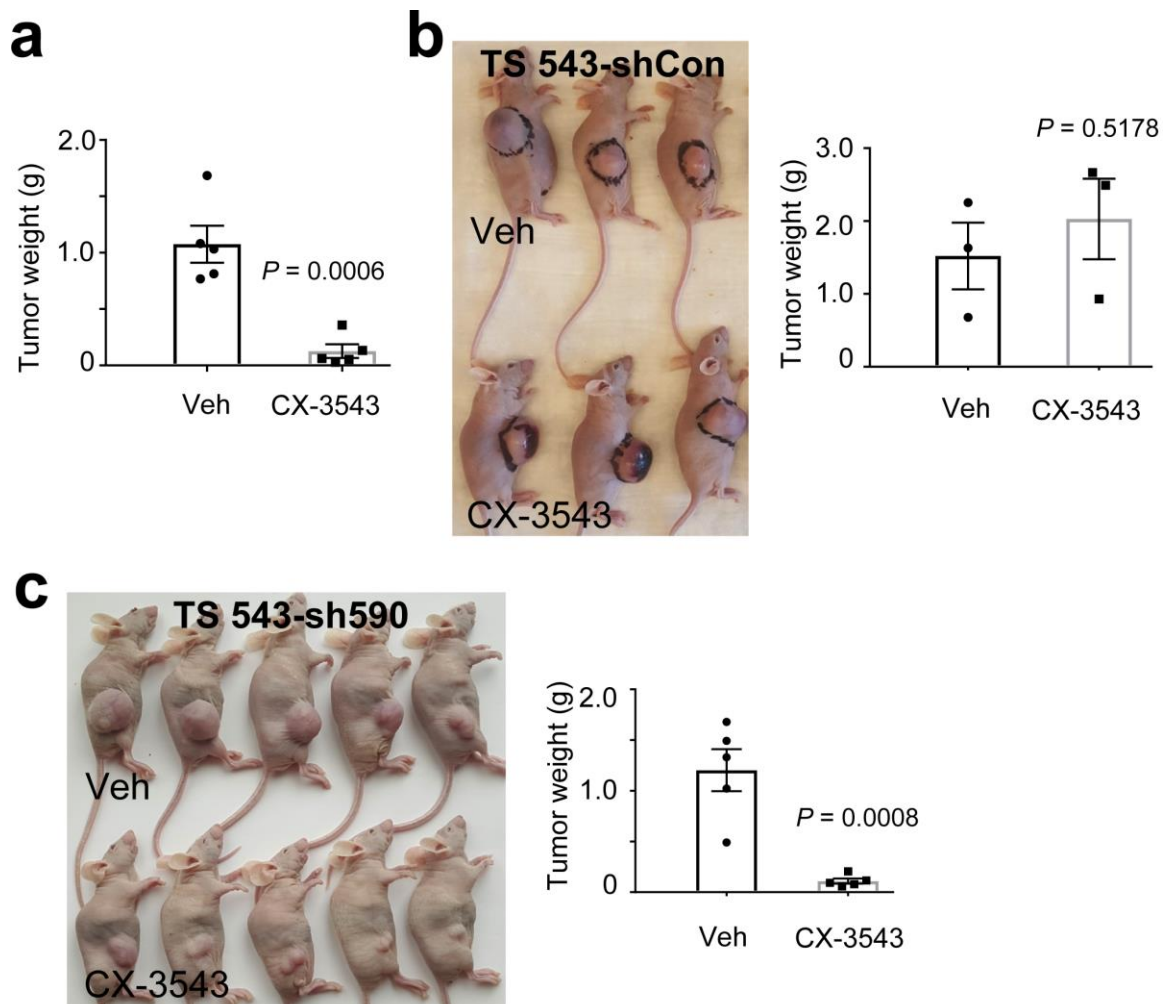
G4 and 53BP1 coimmunofluorescence of constitutive (a) and inducible (b) shATRX NHAs treated with 100 nM CX-3543 (DAPI counterstain); quantified relative G4 signal intensity shown for inducible NHAs (50 nuclei counted in all cases), along with coefficients for spatial correlations between 1H6 and 53BP1 IF. c: Coimmunofluorescence for BLM and  $\gamma$ -H2AX reveals extensive colocalization in shATRX1 NHAs treated with either CX-3543 (100 nM), CX-5461 (50 nM), and PDS (2  $\mu$ M). d: Coimmunofluorescence for G4 (BG4) and  $\gamma$ -H2AX in shATRX1 NHAs treated with 100 nM CX-3543. Two examples shown for treated and untreated cells. Scale bars represent 10  $\mu$ m. *P* values determined by unpaired two-tailed t-test (\*\*: *P* < 0.01, \*\*\*: *P* < 0.001).



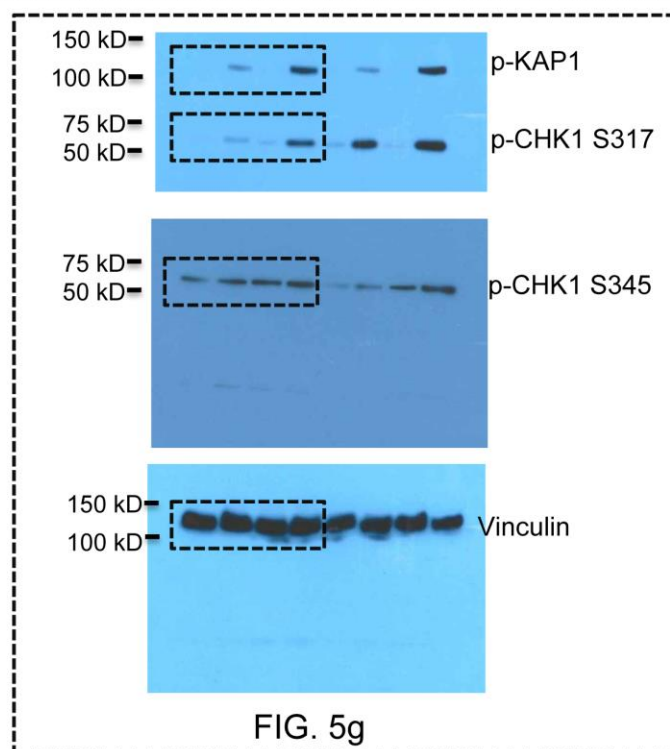
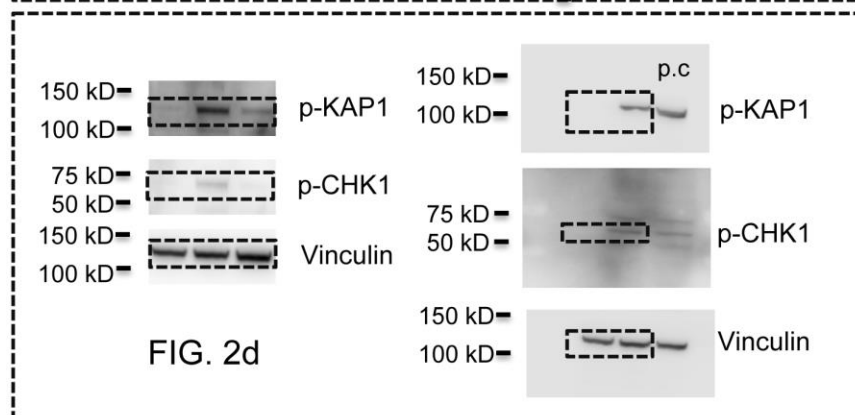
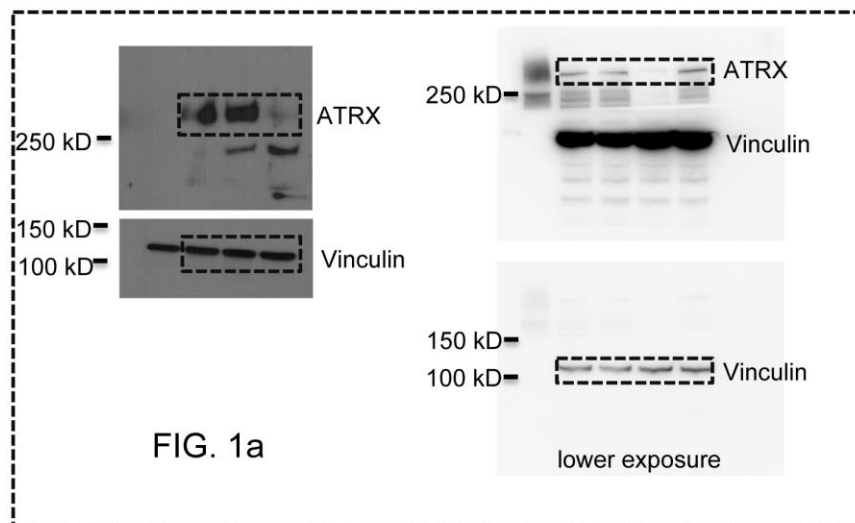
**Supplementary FIG. 9: G4 stabilization enhances DNA damage at putative G4 sites.**  $\gamma$ -H2AX (**a**) and BLM (**b**) ChIP in shATRX1 NHAs (3 replicates each) reveals highly significant qPCR enrichment at putative G4 sites in the promoter regions of *MYC* and *ZNF618* (ZNF). Enrichment at a control site (ESR1) is either modest ( $\gamma$ -H2AX) or not significant (BLM). Error bars reflect SEM; *P* values determined by unpaired two-tailed t-test (\*:  $P < 0.05$ , \*\*\*:  $P < 0.001$ , \*\*\*\*:  $P < 0.0001$ , n.s.:  $P > 0.05$ ).

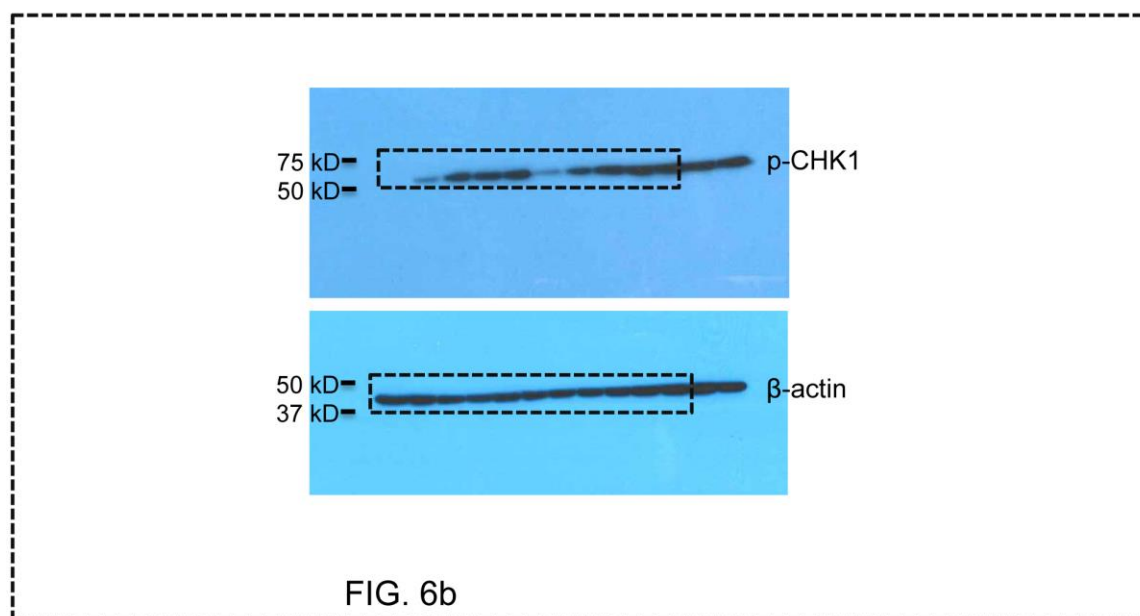
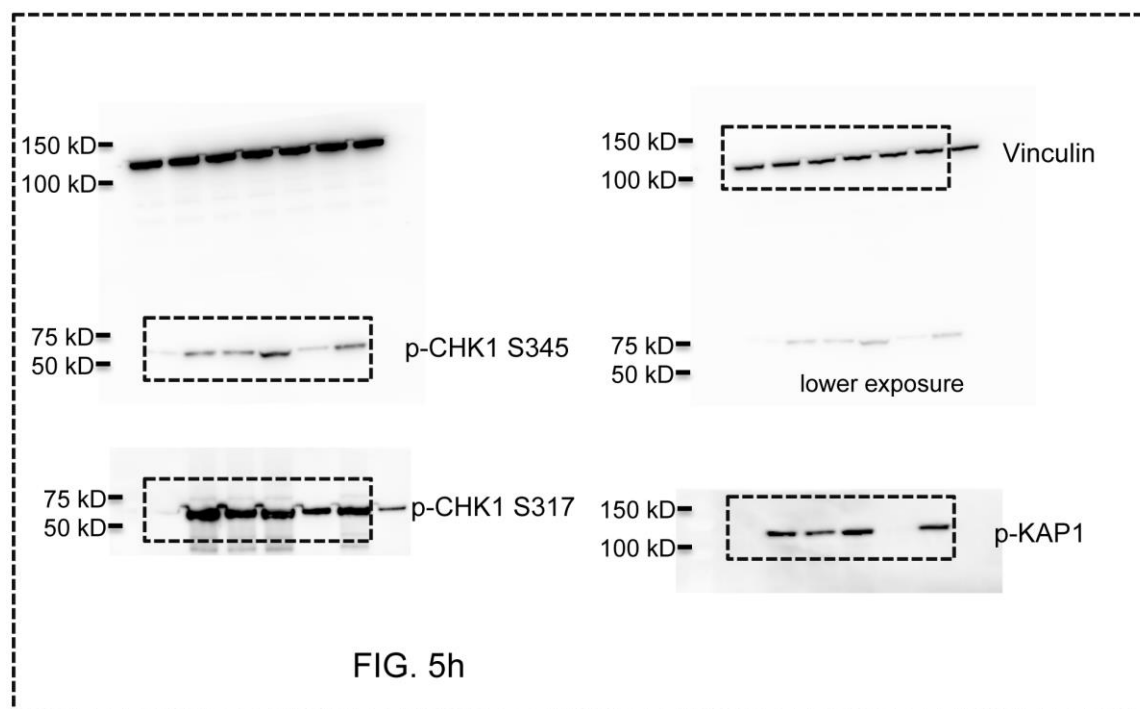


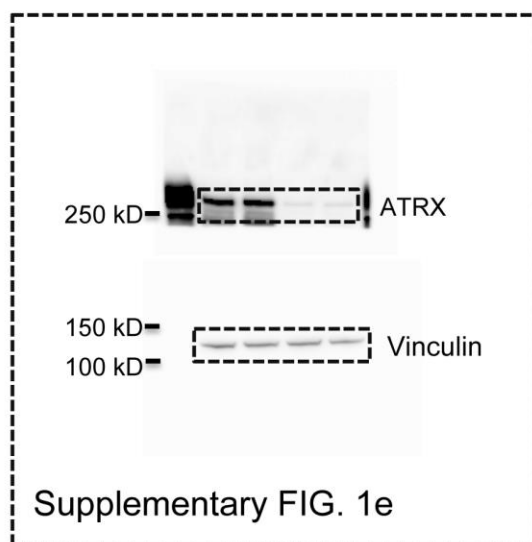
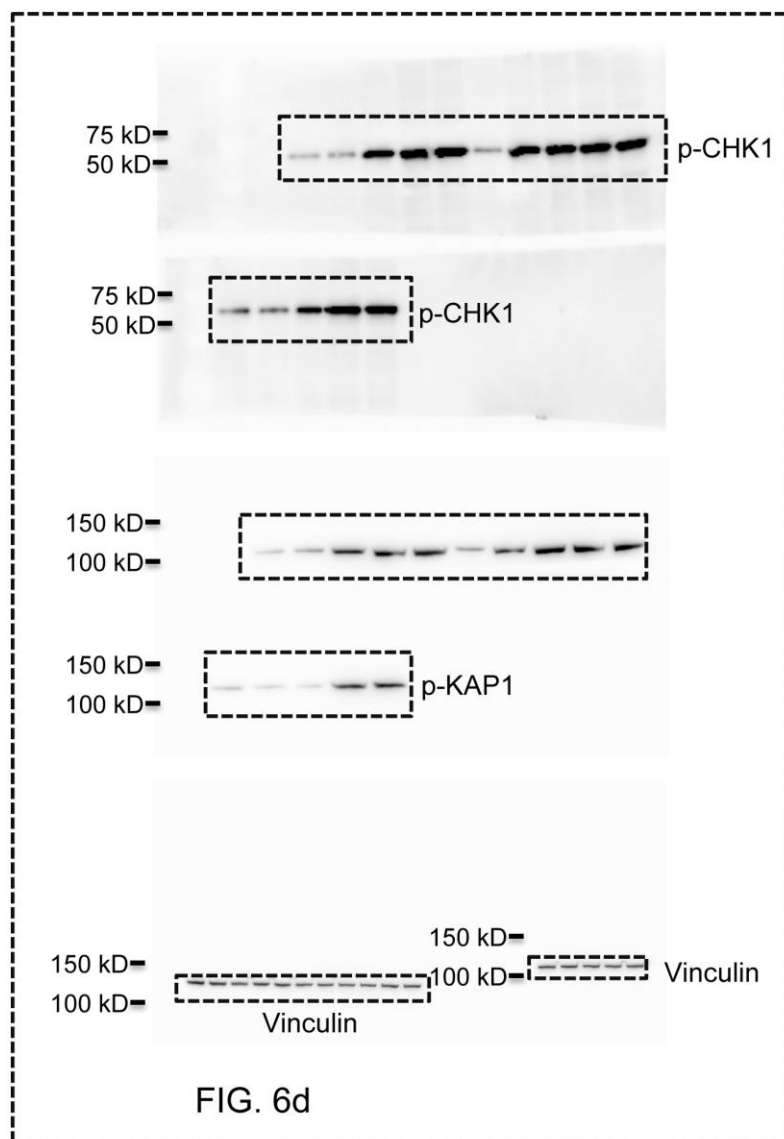
**Supplementary FIG. 10: G4 stabilization selectively promotes replication stress and apoptosis and in ATRX-deficient GSCs. a-b:** : Time course study in ATRX-intact (shCon) and ATRX-knockdown (sh590) TS 543 GSCs (4 replicates each) treated with 100 nM CX-3543 showing parallel kinetics of apoptosis (Annexin V positivity; a) and p-CHK1/p-KAP1 levels by western blot (b). **c:** ATRX-intact (shCon) and ATRX-knockdown (sh590) TS 543 GSCs (at least 4 replicates each) were subjected to soft agar colony formation assay and treated with radiation (2 Gy) and CX-3543 (50 nM). Colony number was quantified at 21 days, normalized relative to vehicle treated cells also subjected to 2 Gy, and compared to the number of colonies in unirradiated wells. Error bars reflect SEM; *P* values determined by unpaired two-tailed t-test (\*\*\*: *P* < 0.001, \*\*\*\*: *P* < 0.0001).

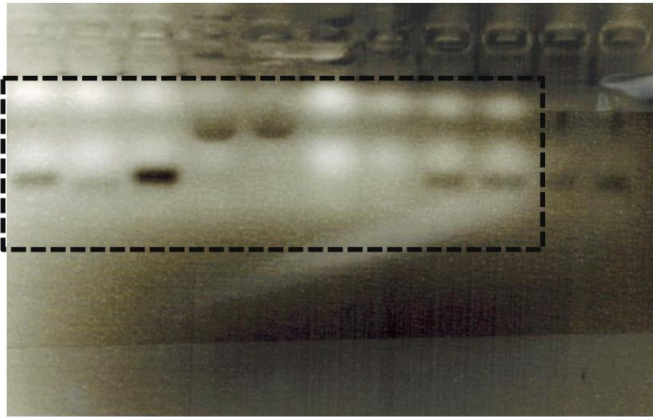


**Supplementary FIG. 11: CX-3543 markedly slows the growth of ATRX-mutant glioma xenografts *in vivo*.** **a:** Tumor weights of JHH-273 xenografts at study endpoint (See Materials and Methods) following treatment with either vehicle control (Veh) or 12.5 mg/kg CX-3543 (5 mice in each group). **b:** Representative image of mice bearing TS 543 (ATRX intact) xenografts following treatment with either vehicle (veh) or 12.5 mg/kg CX-3543 for 24 days. Tumor weights of TS 543-shCon xenografts at study endpoint (See Materials and Methods) following treatment with either vehicle control (Veh) or 12.5 mg/kg CX-3543 are also shown (3 mice in each group). **c:** Representative image of mice bearing TS 543 (sh590-ATRX knockdown) xenografts following treatment with either vehicle (veh) or 12.5 mg/kg CX-3543 for 19 days. Tumor weights of TS 543-sh590 xenografts at study endpoint (See Materials and Methods) following treatment with either vehicle control (Veh) or 12.5 mg/kg CX-3543 are also shown (5 mice in each group). Error bars reflect SEM;  $P$  values determined by unpaired two-tailed t-test.

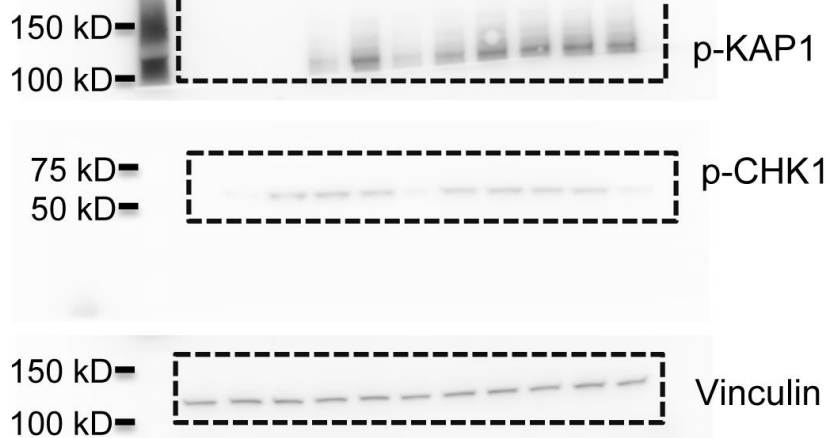








Supplementary FIG. 2a



Supplementary FIG. 10b

**Supplementary FIG. 12: Uncropped western blots used in figures.** Molecular markers and paper figures associated with blots are indicated, as is the extent of horizontal cropping (p.c.: positive control).