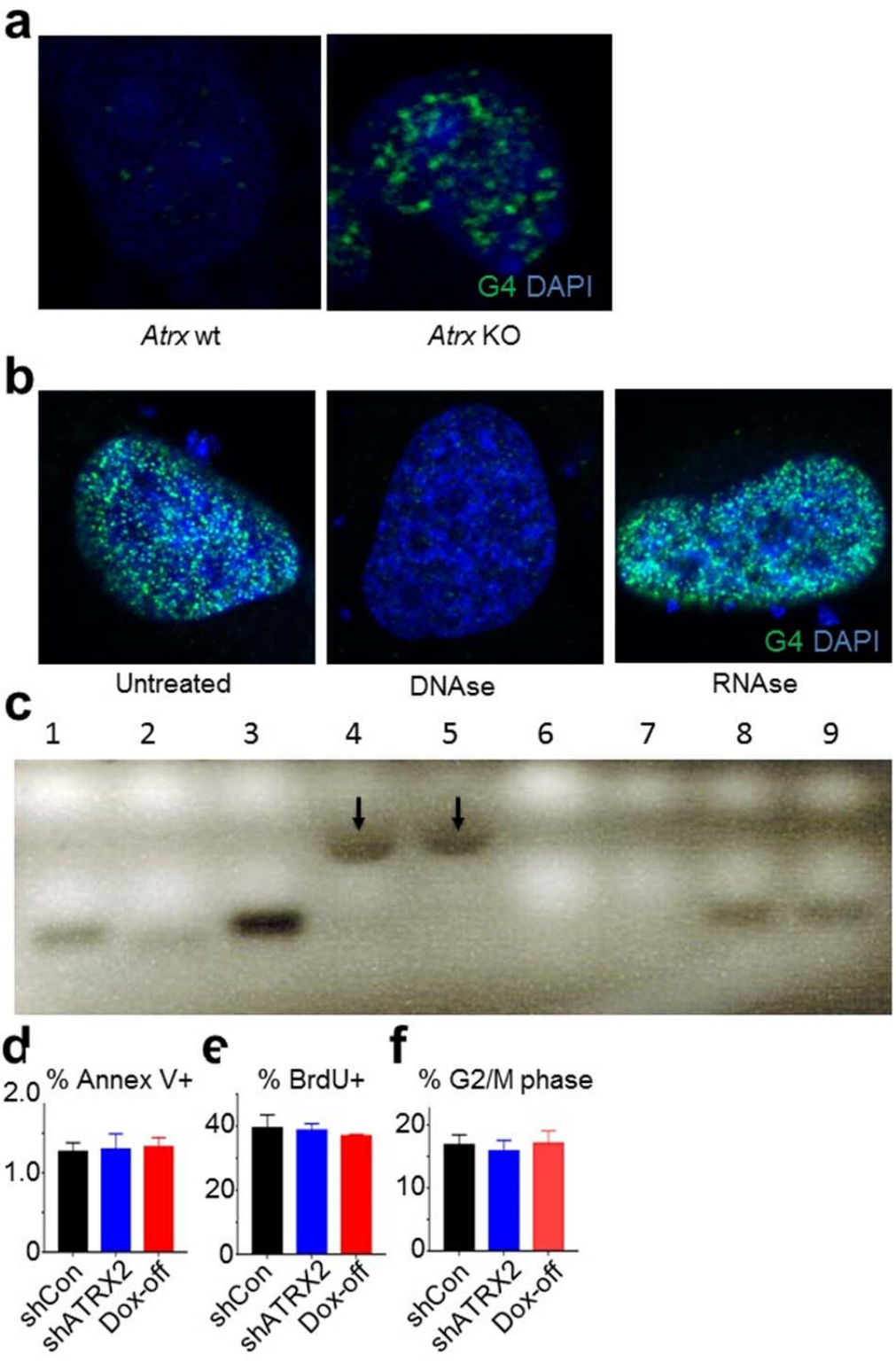
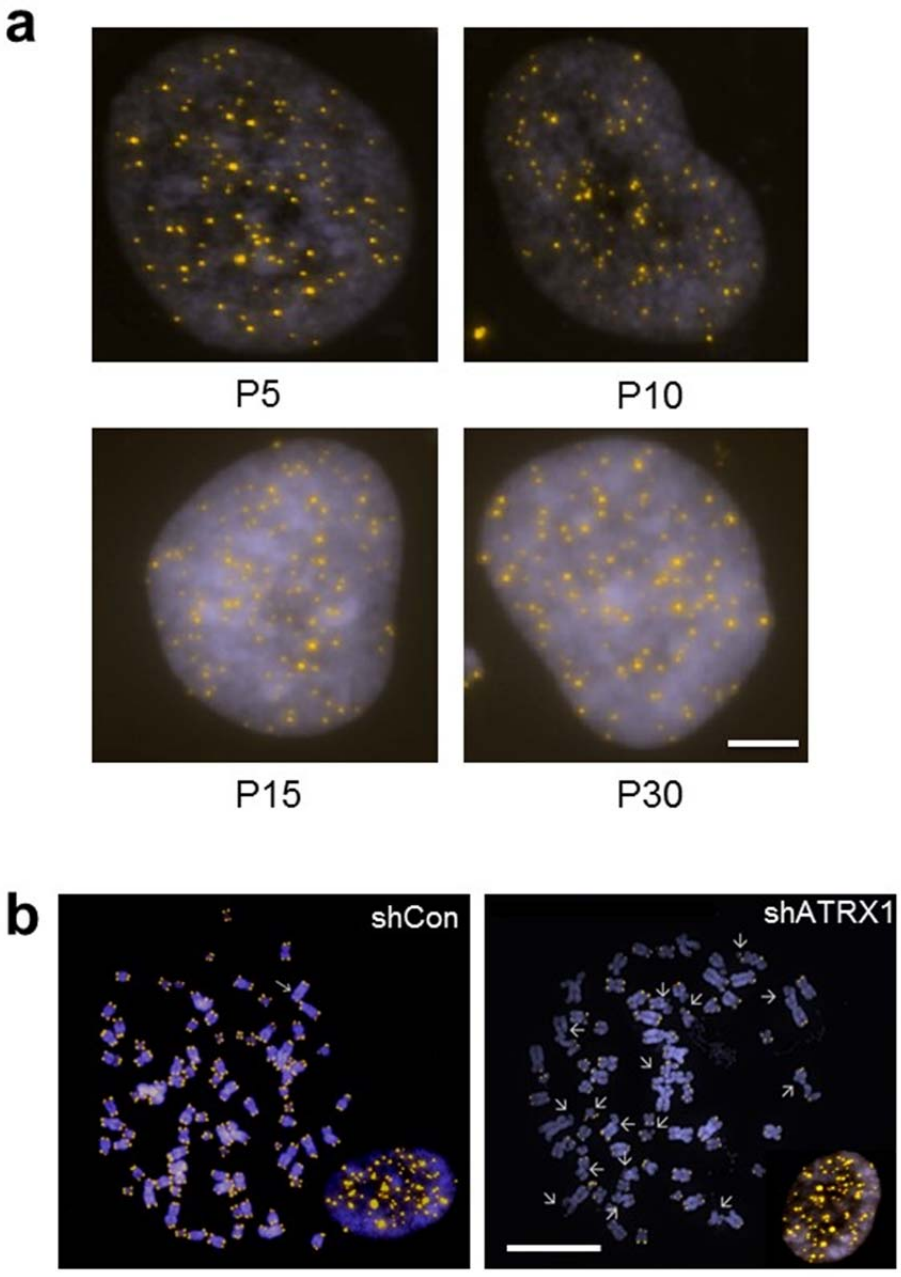

a
G4 DAPI
Atrx wt
Atrx KO
b
G4 DAPI
Untreated
DNAse
RNAse
c
1 2 3 4 5 6 7 8 9
d
% Annex V+
2.0
1.0
0
shCon
shATRX2
Dox-off
e
% BrdU+
40
20
0
shCon
shATRX2
Dox-off
f
% G2/M phase
20
10
0
shCon
shATRX2
Dox-off

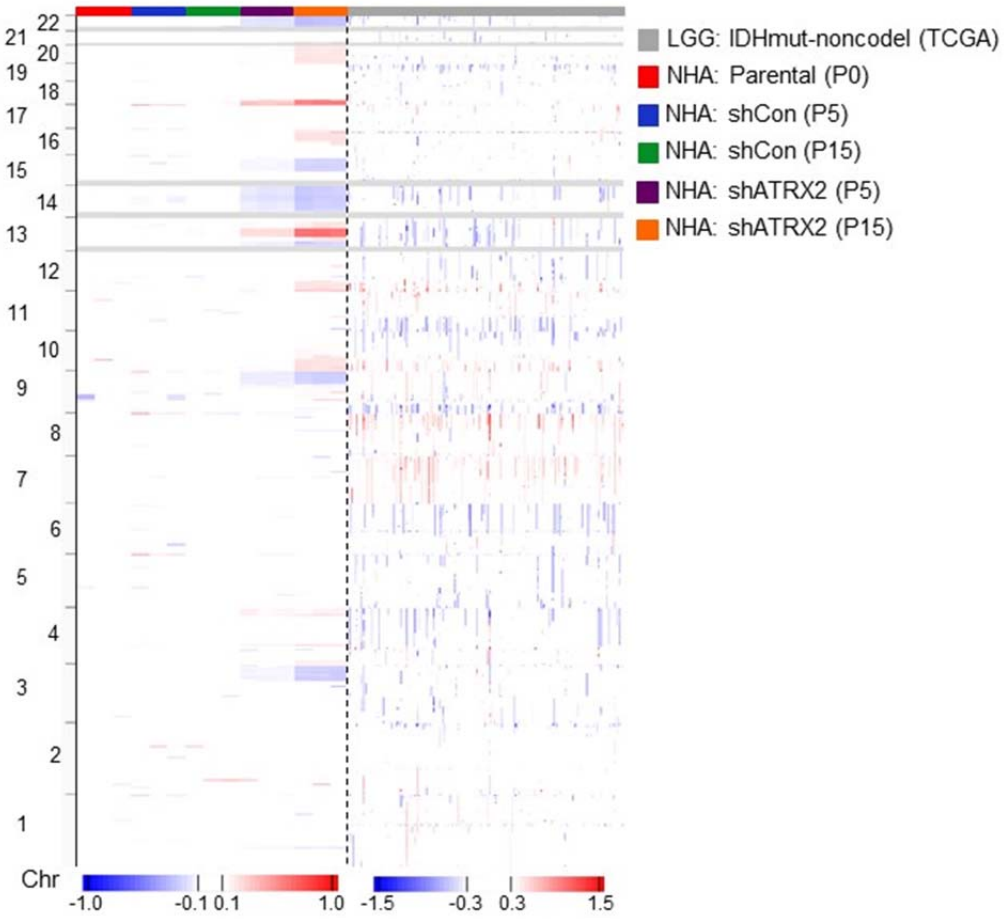
741

742

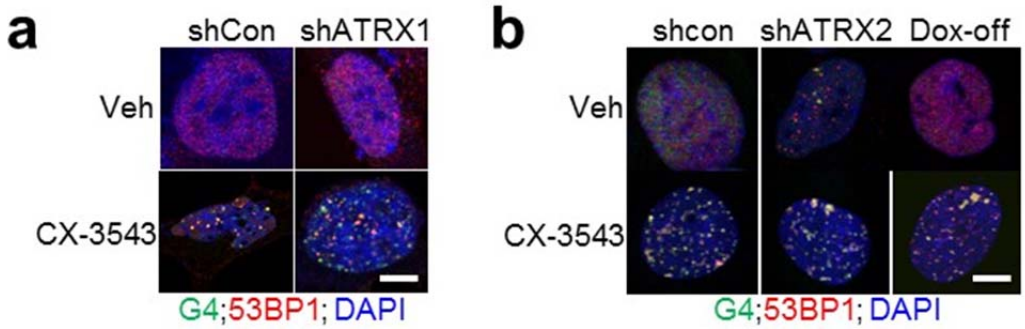
Supplementary FIG. 1: ATRX deficiency promotes G4 formation. a: G4 staining in primary murine neural progenitor cells harboring either intact or inactivated *Atrx*^{38}. **b:** NHAs treated with 50nM CX-3543 alone (left), or with either DNase (middle), or RNase (right) to confirm the specificity of the 1H6 antibody. **c:** 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. **d-f:** Inducible NHA lines showed no effects of ATRX inactivation on either apoptosis (measured by Annexin V positive population, d) or proliferation (measured by BrdU incorporation, e; G2/M-phase content (4n), f).



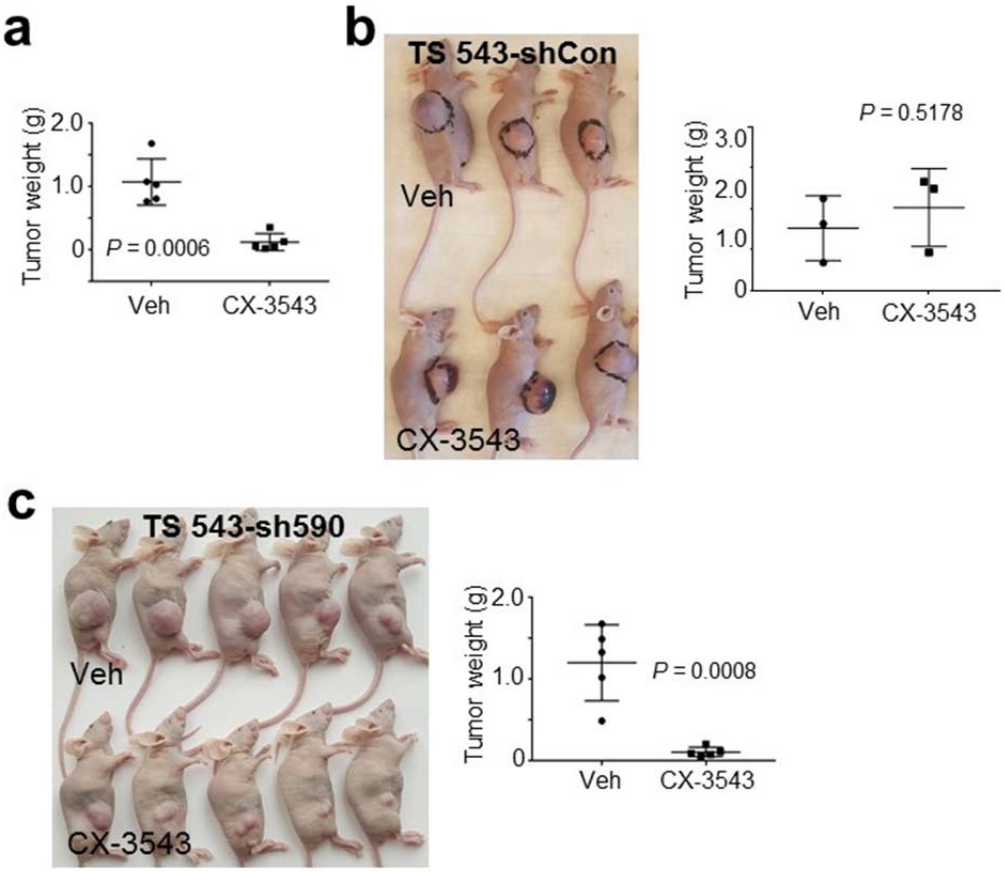
Supplementary FIG. 2: ATRX knockdown induces chromosome breaks, but not ALT in NHAs. **a:** TEL-FISH of NHAs at passages 5, 10, 15 and 30 after ATRX knockdown. **b:** ATRX deficient NHAs (shATRX1, passage 15) showed significantly increased chromosome breaks by cytogenetic analysis. Despite these chromosome abnormalities, Tel-FISH (yellow) showed no change in telomere signal.



Supplementary FIG. 3: 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-noncodel LGG patients visualized by IGV.



Supplementary FIG. 4: G4s colocalize with DNA damage foci induced by CX-3543. G4 and 53BP1 coimmunofluorescence of stable (a) and inducible (b) shATRX NHAs treated with 100 nM CX-3543 (DAPI counterstain).



Supplementary FIG. 5: 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. **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 TS543-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. **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 TS543-sh590 (c) 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.

shCon-1	ATCTCGCTTGGGCGAGAGTAAG
shATRX-1	GGAACTAGCTCTTCAGAAA
shCon-2	CAACAAGATGAAGAGCACCAA
shATRX-2	GGAAAGATGATAAAGGAAA
shCon-3	CAACAAGATGAAGAGCACCAA
sh590	CGACAGAAACTAACCCTGTAA

Table 1: sequences of shRNA against ATRX

Tel 1/2 - Tel 1	GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT
Tel 1/2 - Tel 2	TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA
Tel 2-F	CAAGTTTAAGGTTGTGTTTGTCAC
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

Table 2: primer sequences of G4-ChIP-qPCR