Expanded View Figures

Figure EV1. Cell-type variability in ATRX binding is associated with differential transcription.

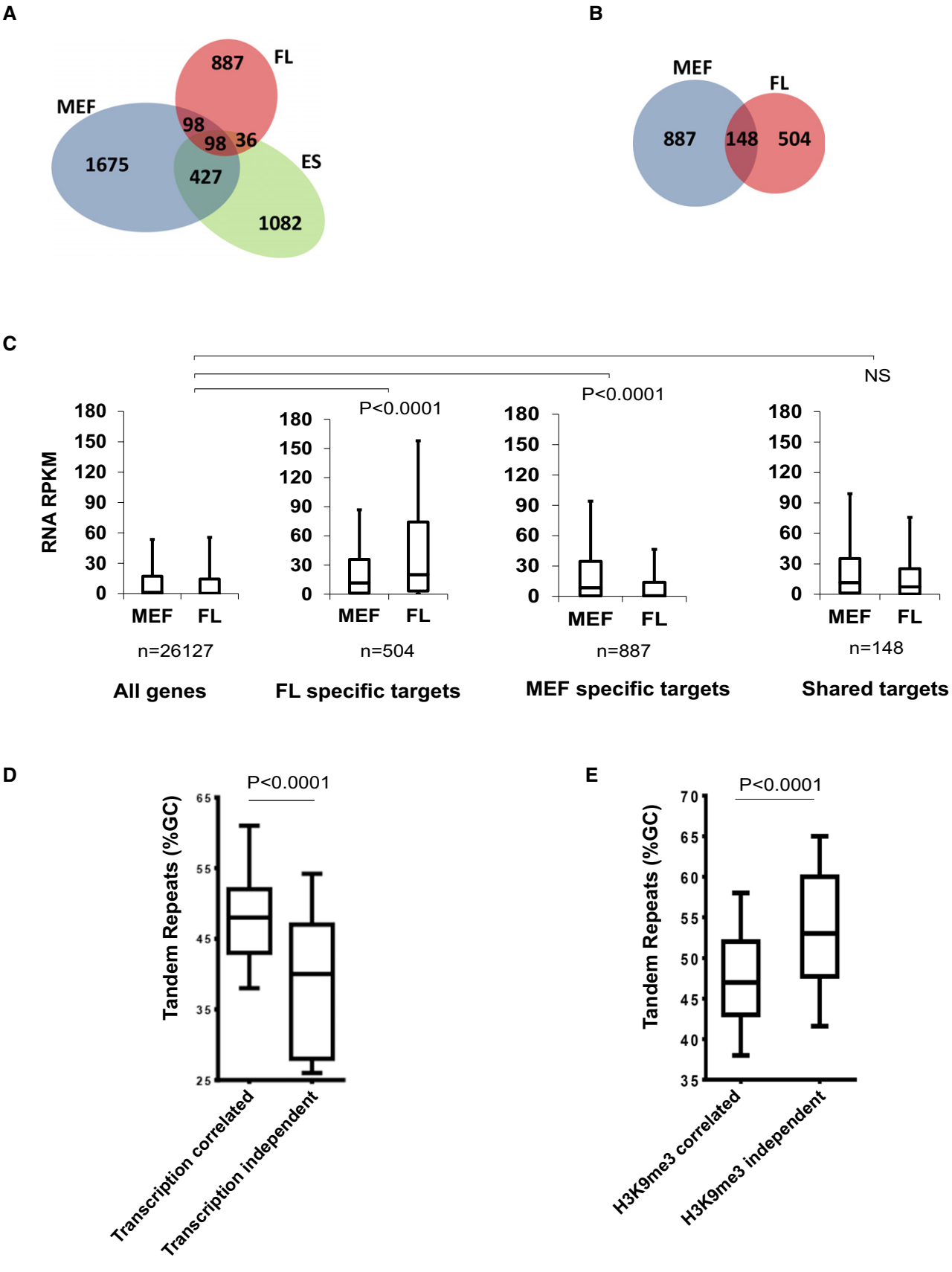
A Overlap between ATRX binding sites in mouse embryonic stem cells (ES), embryonic fibroblasts (MEF) and erythroid foetal liver (FL) cells.

B Overlap between ATRX binding sites at genes in isogenic MEF and FL cells.

C Reads per kilobase of transcript per Million mapped reads (RPKM) of RNA-seq data in MEF and FL cells at: all genes in the genome; genes which bind ATRX in FL cells but not MEFs; genes which bind ATRX in MEFs but not FL cells; genes which bind ATRX in both MEF and FL cells (shared targets). Boxes represent the 25^{th}, median and 75^{th} percentiles. Whiskers represent the 10^{th} and 90^{th} percentiles. Statistical significance was determined using a Mann–Whitney *U*-test on the difference in expression between FL and MEFs (MEF-FL) in each group compared to (MEF-FL) values of all genes in the genome.

D %GC content of tandem repeats underlying ATRX peaks which were transcription correlated or transcription independent in MEF and FL cells. Transcription-independent ATRX TRs (n = 35) had average %GC content lower than transcription-correlated (n = 169) ATRX TRs. Boxes represent the 25^{th}, median and 75^{th} percentile of %GC content. Statistical significance was assessed using a two-tailed Mann–Whitney test.

E %GC content of tandem repeats underlying ATRX peaks which were H3K9me3 correlated or H3K9me3 independent in MEF and ES cells. H3K9me3-independent ATRX TRs (n = 83) had average %GC content higher than H3K9me3-correlated (n = 622) ATRX TRs. Boxes represent the 25^{th}, median and 75^{th} percentile of %GC content. Statistical significance was assessed using a two-tailed Mann–Whitney test (see also Table EV1).


A
887
FL
MEF
98
98
36
1675
427
ES
1082
B
MEF
FL
887
148
504
C
NS
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P<0.0001
RNA RPKM
180
150
120
90
60
30
0
MEF
FL
n=26127
All genes
n=504
FL specific targets
n=887
MEF specific targets
n=148
Shared targets
D
P<0.0001
Tandem Repeats (%GC)
65
55
45
35
25
Transcription correlated
Transcription independent
E
P<0.0001
Tandem Repeats (%GC)
70
65
60
55
50
45
40
35
H3K9me3 correlated
H3K9me3 independent

Figure EV1.

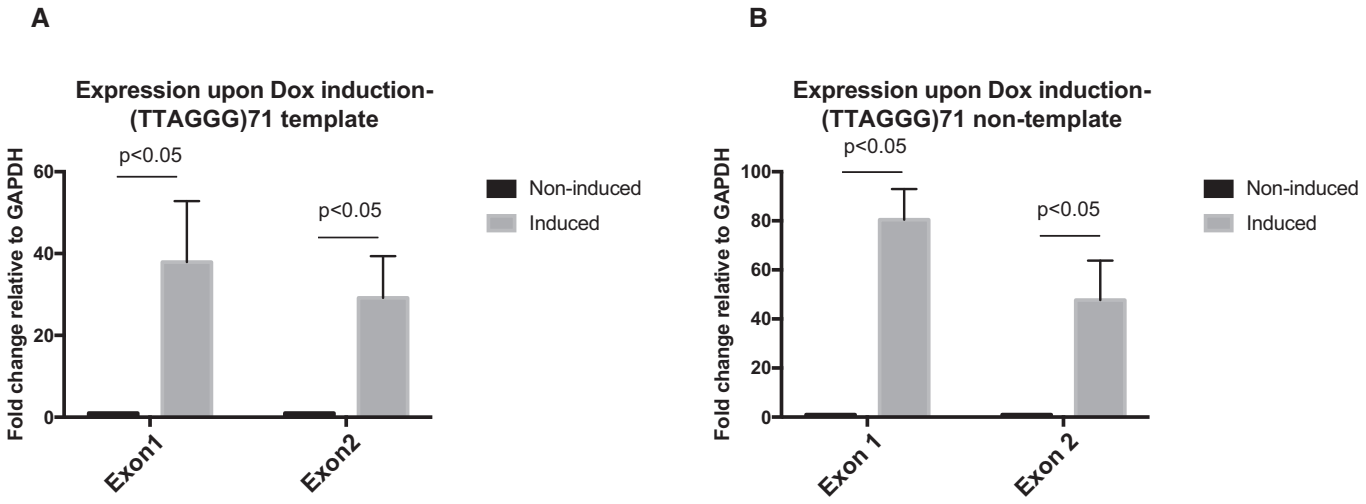

Figure EV2. Expression of GFP exon 1 and exon 2 upon doxycycline induction.

A, B Reverse transcription quantitative PCR showing that both GFP exon 1 and exon 2 in (A) the $(TTAGGG)_{71}$ clones with G-rich strand the template and (B) the reverse orientation clones with G-rich strand the non-template are expressed upon transcription induction by doxycycline. Data are presented as fold change of expression relative to GAPDH. "No reverse transcriptase" (no RT) controls were included in each experiment. Data bars represent the mean of at least four biological repeats (± SEM). Statistical significance was determined using Student's *t*-test.

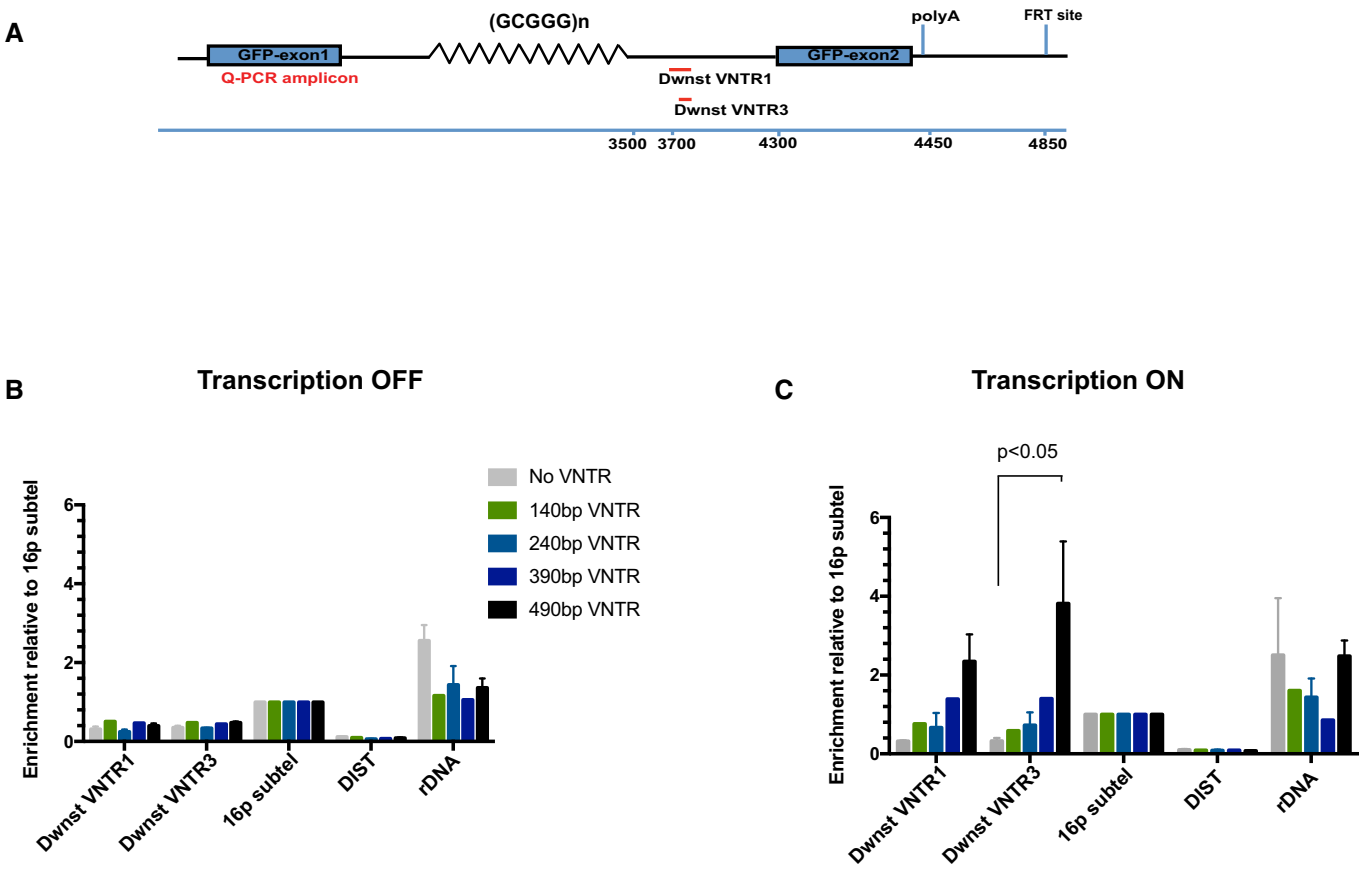

Figure EV3.

Figure EV3. ATRX recruitment to the G-rich ψζ VNTR is dependent on transcription and the length of repeats.

A Diagram shows locations of the qPCR amplicons used to assess ATRX enrichment at the ectopic telomere repeat region. Numbers indicate distance to the start of the ectopic cassette.

B ATRX ChIP analysis in stable clones containing ψζ VNTR of indicated sizes and the control without the VNTR when the ectopic gene is inactive (transcription off). The enrichment of ATRX is represented as % input normalised to that at 16p telomeric region (16ptel).

C ATRX ChIP analysis in stable clones containing ψζ VNTR of indicated sizes when the ectopic gene is switched on (transcription on) by addition of 1 μg/ml doxycycline for 48 h. The enrichment of ATRX is represented as % input normalised to that at 16p telomeric region (16ptel).

Data information: Data bars were plotted as the mean of two independent experiments for the 140 bp VNTR ($n = 2$) and 390 bp VNTR ($n = 2$), of three independent experiments for the NoVNTR ($n = 3$) and the 240 bp VNTR ($n = 3$), and of four independent experiments for the 490 bp VNTR ($n = 4$). Error bars represent standard error of the mean (SEM) where n is equal or greater than 3. DIST is a negative control, and ribosomal DNA (rDNA) is a positive control. Statistical significance was determined by unpaired Student's t-test.

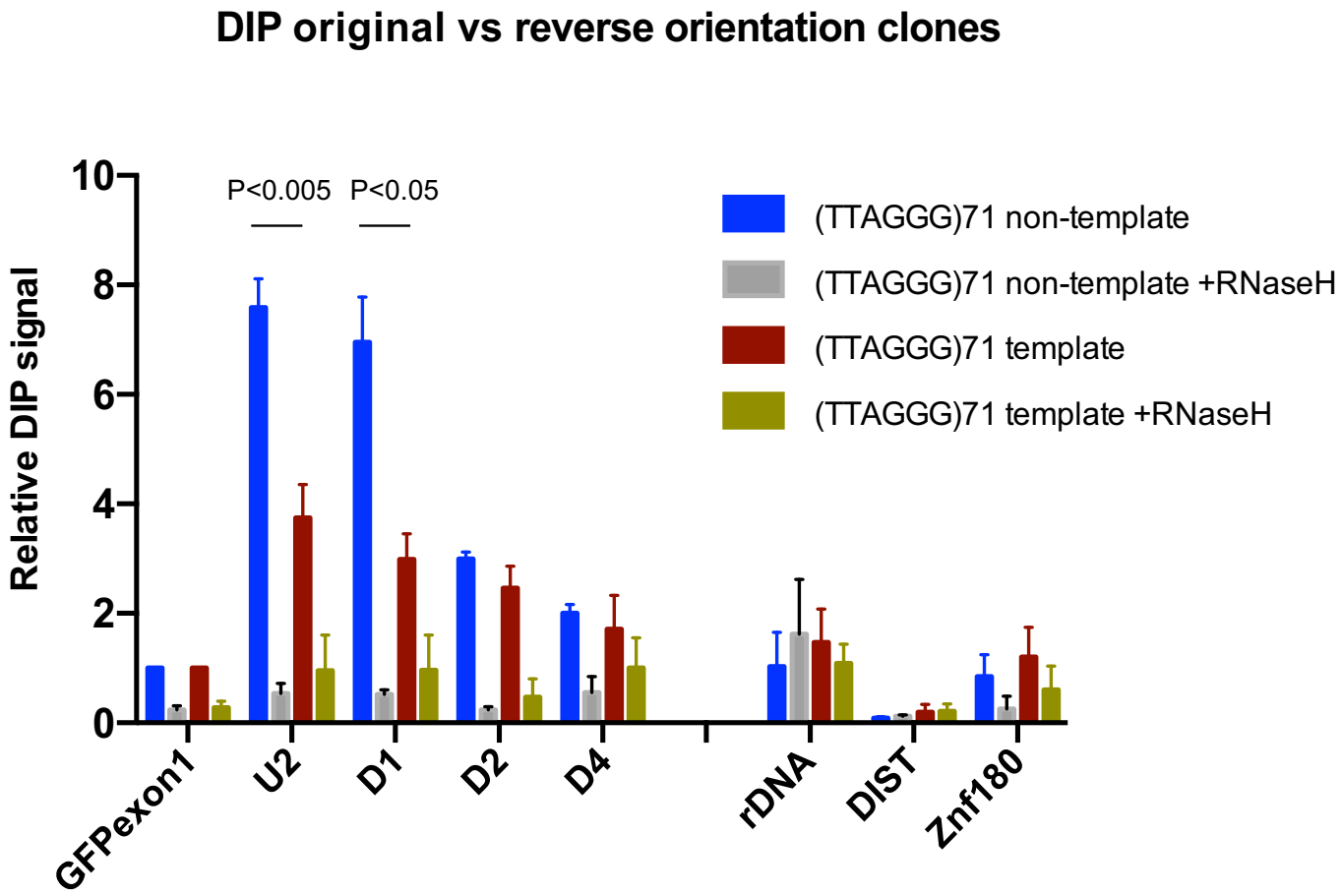

Figure EV4. R-loop formation preferentially occurs when the G-rich strand is the non-template strand.

DIP analysis in clones containing the ectopic telomeric sequence with the G-rich strand being the non-template strand and in clones containing the reverse oriented telomeric sequence with the G-rich strand being the template strand after activation of transcription by doxycycline. DIP samples were also treated with *E. coli* RNase H prior to immunoprecipitation with S9.6 antibody as a control. DIP signal is expressed as % input normalised to the non-repetitive region of GFP exon 1. Data bars represent the average value from four independent experiments ± SEM. Statistical significance was determined by unpaired Student's t-test.

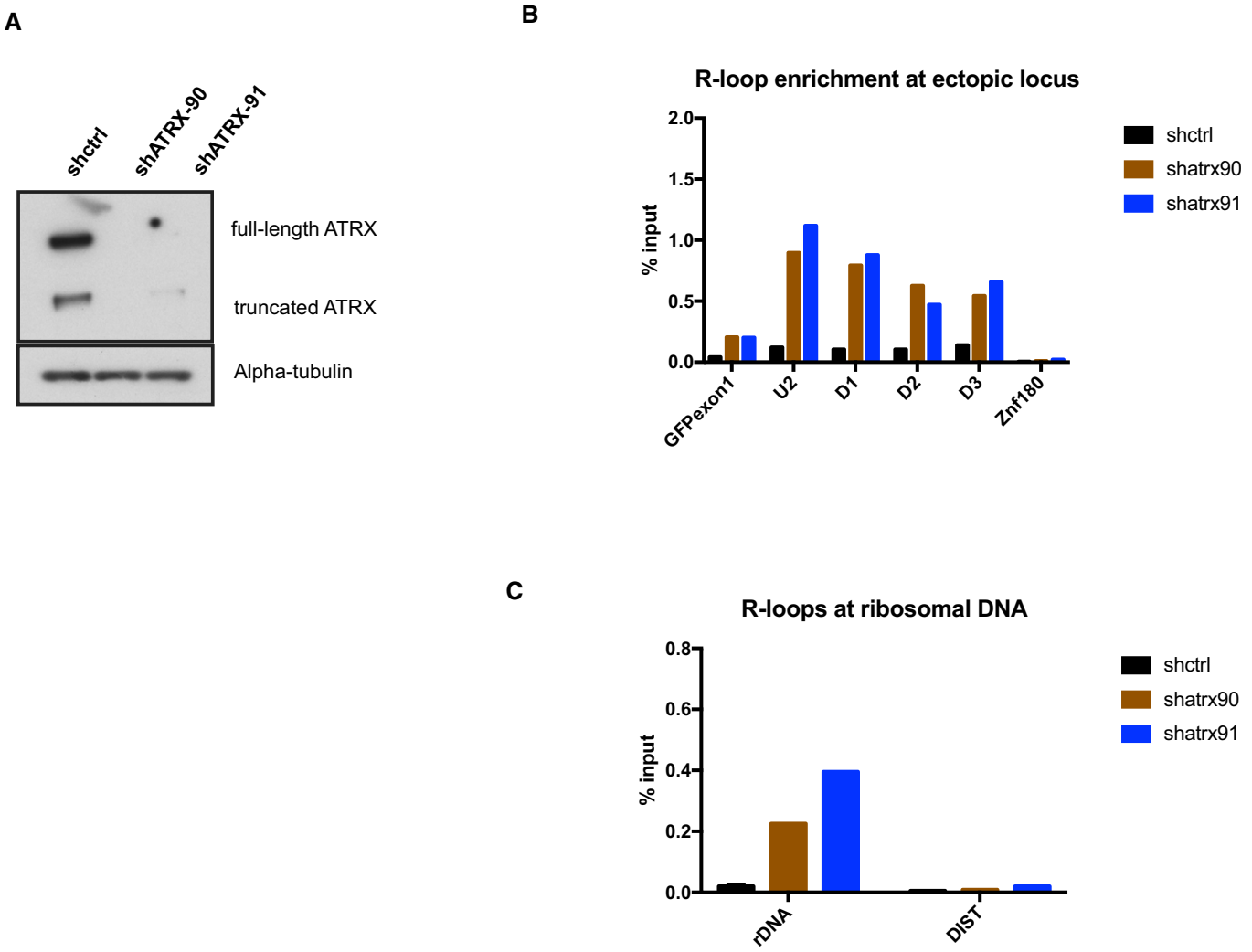

Figure EV5. ATRX modulates R-loops.

A Western blot analysis of whole-cell extract of cells with the ectopic telomeric sequence $(TTAGGG)_{71}$ treated with lentiviral shRNAs against ATRX (shATRX-90 and shATRX-91) or with shRNA control. The Western blot membrane was probed with anti-ATRX and anti-alpha-tubulin antibody.

B DIP analysis in clones treated with shRNA against ATRX or shRNA control, followed by transcription induction by addition of 1 μg/ml doxycycline for 24 h.

C DIP analysis showing an increase in R-loops at ribosomal DNA region in the ATRX knockdown cells. DIST is a negative control.

Data information: The enrichment of R-loops at the ectopic repeats was measured by qPCR. Data bars are the mean values from two independent experiments. Znf180 and DIST are negative controls.