Figure S1. related to Figure 1.

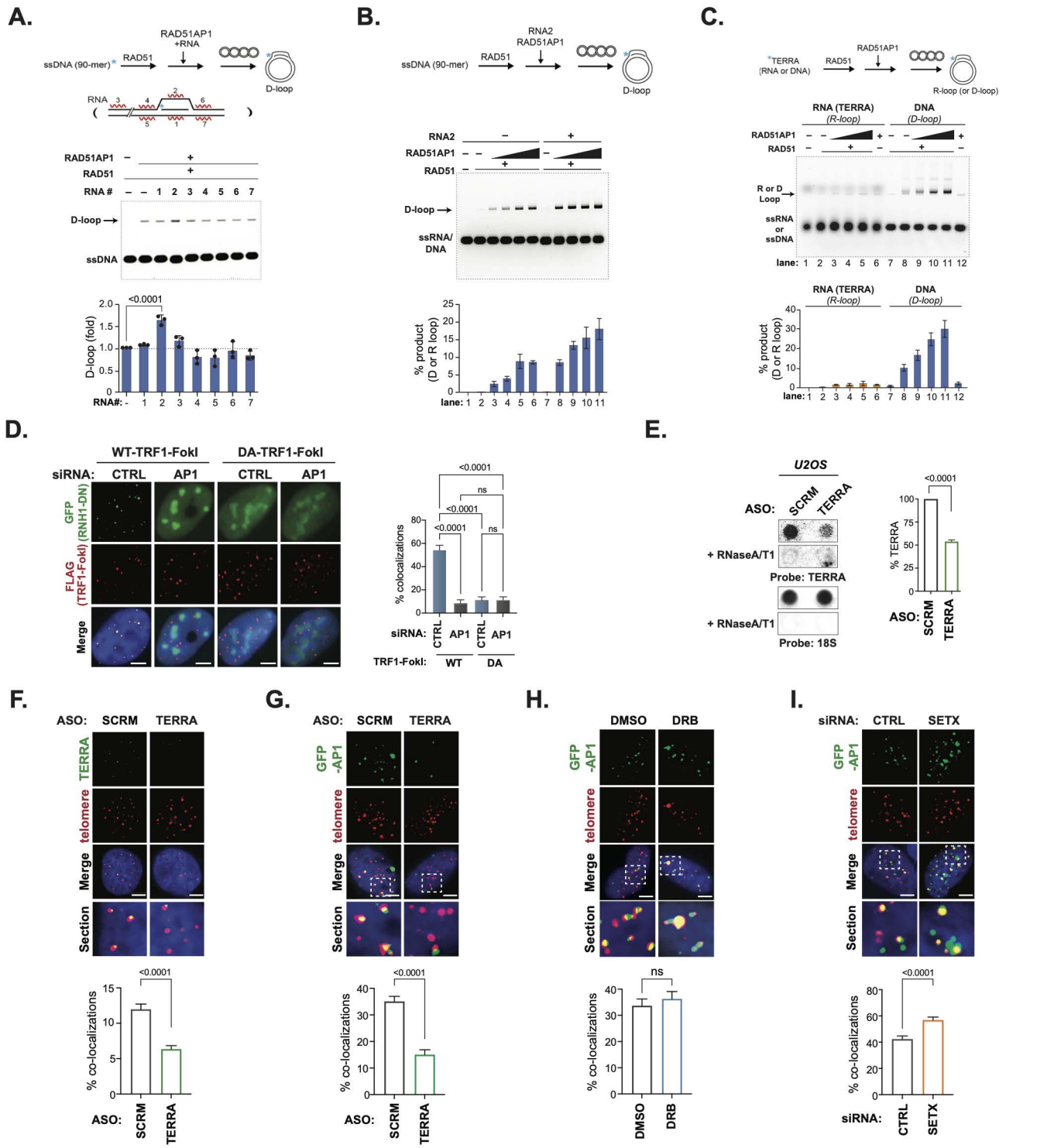

Figure S1. TERRA stimulates RAD51AP1-RAD51 mediated D- and R- loop formation.
(A) Top. Schematic of preparation of substrates and placement of RNAs (1-6) within D- loops. Middle. EMSA of D-loop formation after incubation with RAD51AP1 and RNAs complementary to different areas around the D-loop. Below. Quantification of products generated. Data represent mean ± S.D, n=3. **(B)** Top. Schematic of the preparation of substrates for examination of RAD51AP1 and RNA 2 on D-loop products. Middle. EMSA of RNA2-dependent D-loop formation with increasing concentrations of RAD51AP1. Below. Quantification of products generated. Data represent mean ± S.D, n=4. **(C)** Top. Schematic of the preparation of substrates for examination of R- or D- loop products. Middle. EMSA measuring stimulation of RAD51 dependent left; R- loop and right; D-loop formation after incubation with RAD51AP1. Below. Quantification of products generated. Data represent mean ± S.D, n=3. **(D)** Representative images and quantification of GFP-RNaseH1-D210N localizing to telomeric double-stranded breaks (t-DSBs) (FLAG-WT-TRF1-FokI) after knockdown of RAD51AP1 in U2OS cells. Data represent mean ± SEM, n=2. **(E)** Northern dot-blot and quantification of TERRA and 18S rRNA in U2OS cells transfected with scrambled (SCRM) and TERRA anti-sense oligos (ASOs). Data represent mean ± SEM, n=2. **(F-G)** Representative images and quantification of **(F)** TERRA and **(G)** GFP-RAD51AP1 at telomeres in SCRM and TERRA ASO transfected U2OS cells. Data represent mean ± SEM, n=3. **(H)** Representative images and quantification of GFP-RAD51AP1 localization at telomeres in DRB-treated (10μM, 4hrs) U2OS cells. Data represent mean ± SEM, n=2. **(I)** Representative images and quantification of GFP-RAD51AP1 localization at telomeres in SETX-depleted U2OS cells. Data represent mean ± SEM, n=3. p values are indicated and generated by Students t-tests (A-C, E-I) and One way ANOVA (D). All scale bars, 5μm.

Figure S2. Related to Figure 2.

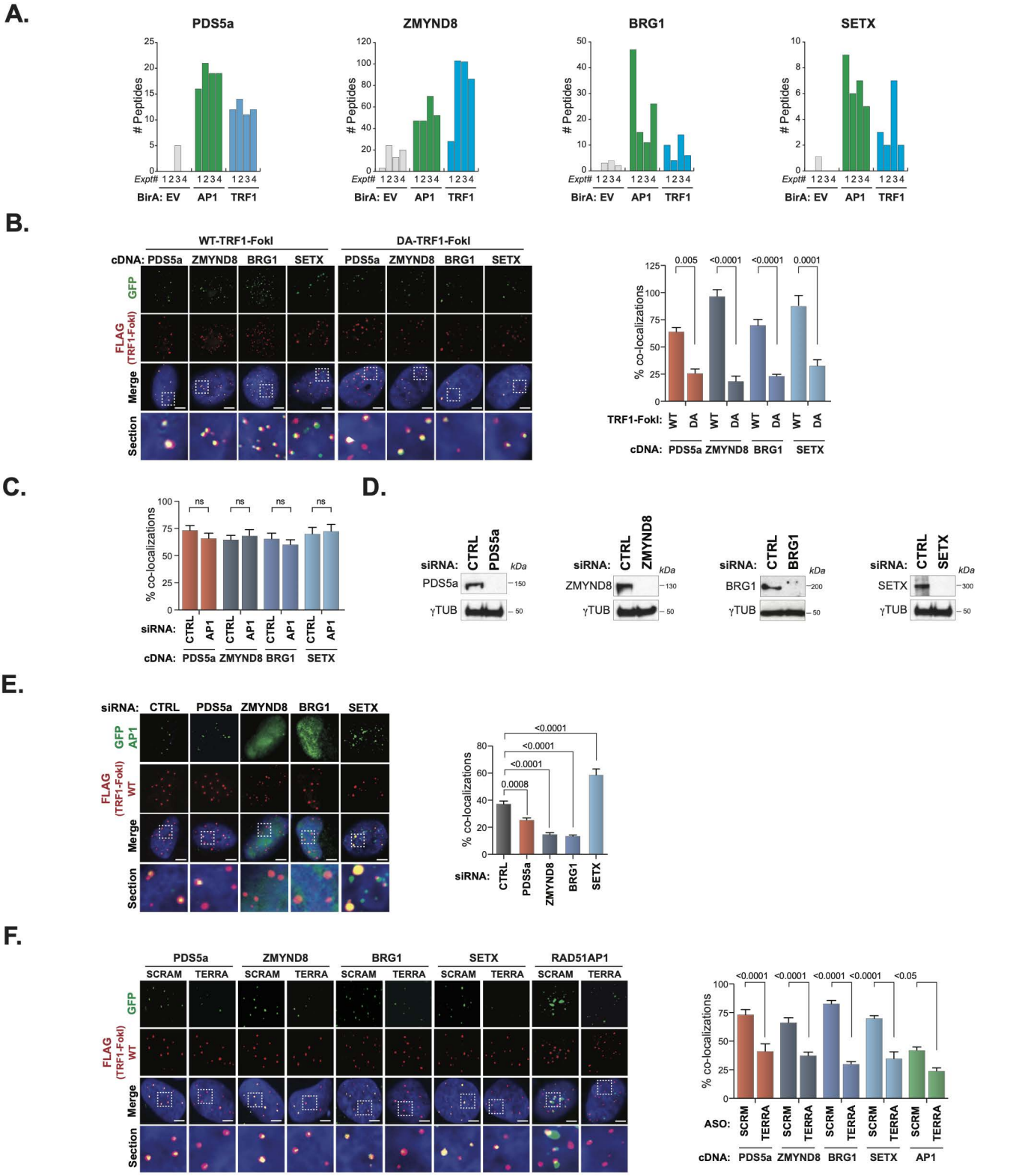

Figure S2. Influence of TERRA and chromatin remodelers on RAD51AP1 localization to telomeres.
(A) Graphs of peptides of PDS5a, ZMYND8, BRG1 and SETX recovered in four independent BioID experiments with BirA, BirA-AP1 (RAD51AP1) and BirA-TRF1. **(B)** Representative images and quantification of GFP-tagged PDS5a, ZMYND8, BRG1 and SETX localizing to WT and DA-TRF1-FokI sites in U2OS cells. All data represent mean ± SEM, n=2 **(C)** Quantification of GFP-tagged PDS5a, ZMYND8, BRG1 and SETX localizing to WT-TRF1-FokI t-DSBs in control and RAD51AP1 siRNA transfected U2OS cells. All data represent mean ± SEM, n=2 **(D)** Western blot verification of PDS5a, ZMYND8, BRG1 and SETX depletion by knockdown with the corresponding siRNAs. γTubulin was blotted as a loading control. **(E)** Representative images and quantification of GFP-tagged RAD51AP1 localizing to t-DSBs in PDS5a, ZMYND8, BRG1 and SETX depleted U2OS cells. **(F)** Representative images and quantification of GFP-tagged PDS5a, ZMYND8, BRG1 and SETX localizing to t-DSBs in TERRA depleted U2OS cells. All data represent mean ± SEM, n=3. p values are indicated and generated by One way ANOVA. All scale bars, 5μm.

Figure S3. Related to Figure 2.

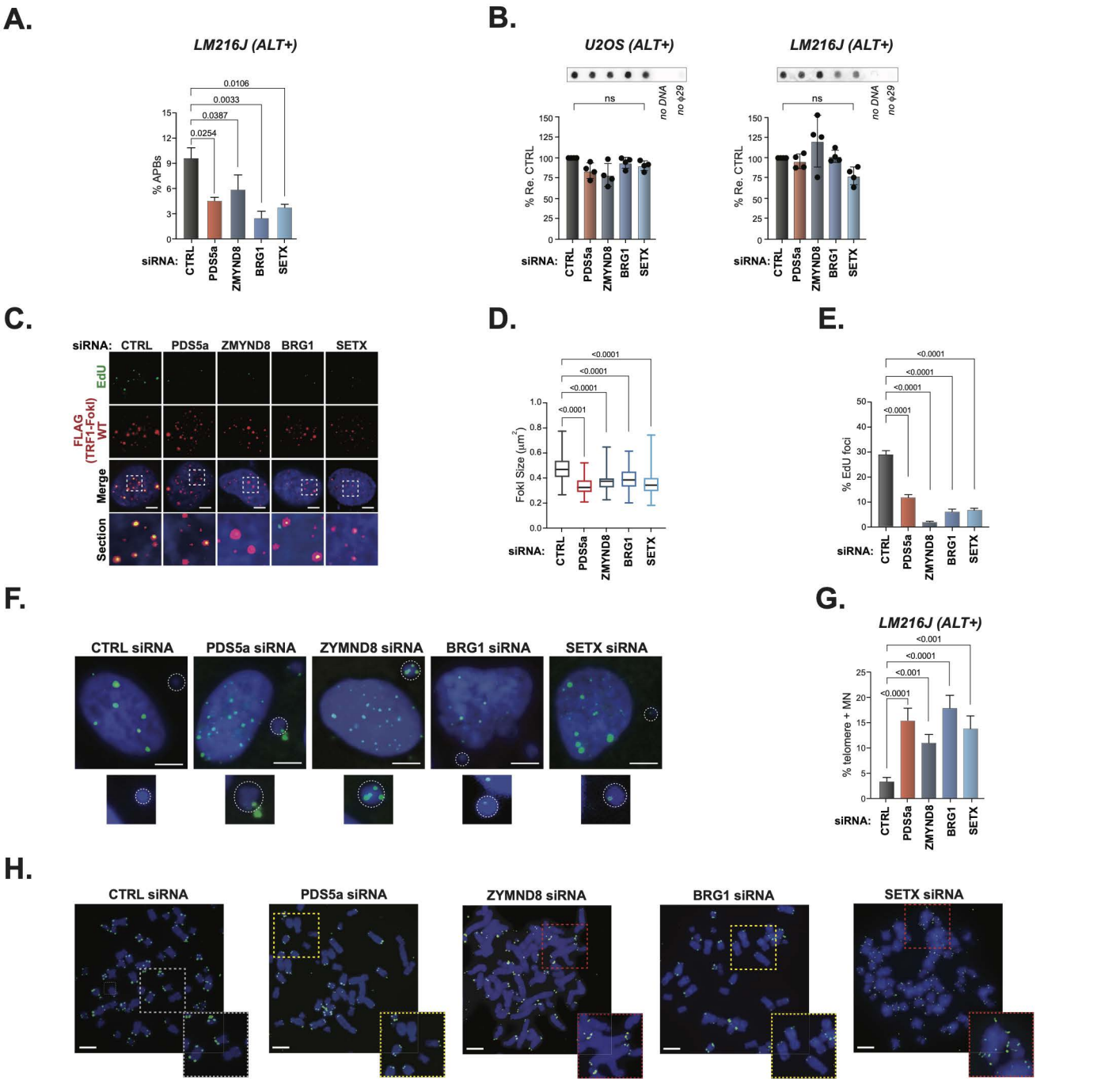

Figure S3. Chromatin-directed TERRA suppression is a feature of ALT.
(A) Quantification of APBs in PDS5a, ZMYND8, BRG1 and SETX depleted LM216J cells. All data represent mean ± SEM, n=2. **(B)** Southern dot-blot detection of C-circles with a P32-labelled telomere oligo and quantification of C-circles in PDS5a, ZMYND8, BRG1 and SETX depleted U2OS and LM216J cells. All data represent mean ± SEM, n=3. **(C)** Representative images of break-induced telomere synthesis (EdU and FLAG-TRF1-FokI localization) after siRNA knockdown of the indicated proteins in U2OS cells. All data represent mean ± SEM, n=3. **(D)** Quantification of telomere clustering by measurement of t-DSB focus size (μm). Box and whiskers plots show the interquartile and min-max ranges. The median is represented by the horizontal black line. Data from triplicate independent experiments are shown. **(E)** Break -induced telomere synthesis as inferred by the accumulation of EdU foci at t-DSBs generated by WT-TRF1-FokI following siRNA knockdown of the indicated proteins in U2OS cells. Data represent mean ± SEM, n=3. **(F)** Examples of micronuclei containing telomere fragments in control and PDS5a, ZMYND8, BRG1 and SETX siRNA knockdown U2OS cells. The grey dotted circle box displays the enlarged insert. **(G)** Quantification of micronuclei with TTAGGG FISH signals after siRNA knockdown of the indicated proteins in LM216J cells. All data represent mean ± SEM, n=2. **(H)** Examples of telomere alterations observed in FISH of metaphase chromosomes prepared from control and PDS5a, ZMYND8, BRG1 and SETX siRNA knockdown U2OS cells. The grey dotted box displays features of telomeres observed in control cells including fragility, signal-free and heterogenous TTAGGG FISH signals i.e., varying telomere lengths). The yellow dotted box display TTAGGG FISH signal-free metaphases following PDS5a and BRG1 siRNA knockdown. The red dotted box displays telomere fragility (i.e., multiple distinct TTAGGG FISH signals per single chromatid) observed following ZMYND8 and SETX siRNA knockdown. p values are indicated and generated by One way ANOVA. All scale bars, 5μm.

Figure S4. Related to Figure 3.

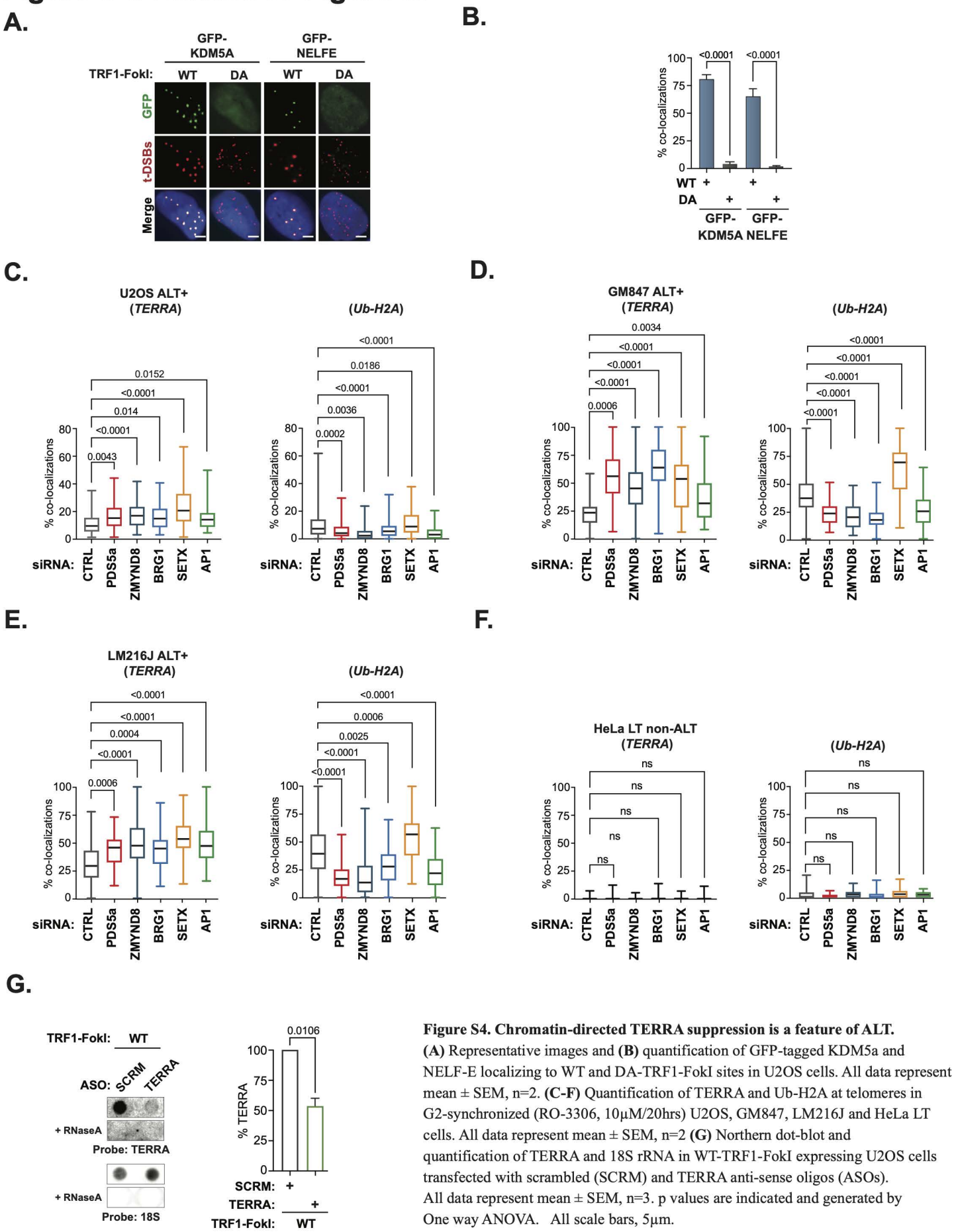

Figure S4. Chromatin-directed TERRA suppression is a feature of ALT.
(A) Representative images and **(B)** quantification of GFP-tagged KDM5a and NELF-E localizing to WT and DA-TRF1-FokI sites in U2OS cells. All data represent mean ± SEM, n=2. **(C-F)** Quantification of TERRA and Ub-H2A at telomeres in G2-synchronized (RO-3306, 10μM/20hrs) U2OS, GM847, LM216J and HeLa LT cells. All data represent mean ± SEM, n=2 **(G)** Northern dot-blot and quantification of TERRA and 18S rRNA in WT-TRF1-FokI expressing U2OS cells transfected with scrambled (SCRM) and TERRA anti-sense oligos (ASOs). All data represent mean ± SEM, n=3. p values are indicated and generated by One way ANOVA. All scale bars, 5μm.