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Supplemental information

TZAP overexpression induces telomere dysfunction and ALT-like activity in ATRX/DAXX-deficient cells

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Supplemental information

Supplementary Figure 1: Further characterization of HT-1080 hTR cells and TZAP overexpression in U-2 OS cells. Related to Figure 1.

A) TRF analysis of HT-1080 cells overexpressing either empty vector (EV) or the telomerase RNA component hTR. **B)** Representative immunofluorescence maximum intensity projection images of U-2 OS cells overexpressing either EV or Myc-TZAP showing the nuclear localization of RPA70 and TRF1. White arrows point to RPA70-TRF1 colocalizations. Scale bar 10 μm. **C)** Scatter plot showing quantification of experiment described in B). A total of at least 55 nuclei were analyzed for each sample. Data are from a single experiment. Centre line, mean. n shows number of cells analyzed. **D)** Cell cycle profiles of asynchronous or G2-arrested U-2 OS overexpressing either EV or Myc-TZAP (TZ) from experiment shown in Figure 1E-F.

Supplementary Figure 2: Effects of TZAP overexpression in ATRX/DAXX deficient telomerase positive cells. Related to Figure 2.

A) Western blot analysis of the proteins ATRX, DAXX and GAPDH in the indicated ALT$^+$ and telomerase$^+$ cell lines. **B)** Cell cycle profiles of HeLa 1.2.11 cells stably expressing FLAG-TZAP and treated with the indicated siRNAs. 3 experiments were performed (upper panel). Scatter plots with bars showing the mean percentage of the cells described in B in G1, S and G2/M phases of the cell cycle. Bars represent mean with SD. Data from 3 independent experiments (lower panel). **C)** Western blot analysis of the indicated proteins in HT-1080 hTR cells overexpressing either empty vector (EV) or FLAG-TZAP. **D)** TCA of the cells described in C). The arrow points to the products of rolling circle amplification of TTAGGG-rich circular extrachromosomal DNA. The experiment was repeated 3 times and one representative experiment is shown.

Supplementary Figure 3: TZAP overexpression in ATRX/DAXX deficient cells induces an ALT-like pathway dependent on the BTR complex. Related to Figure 3.

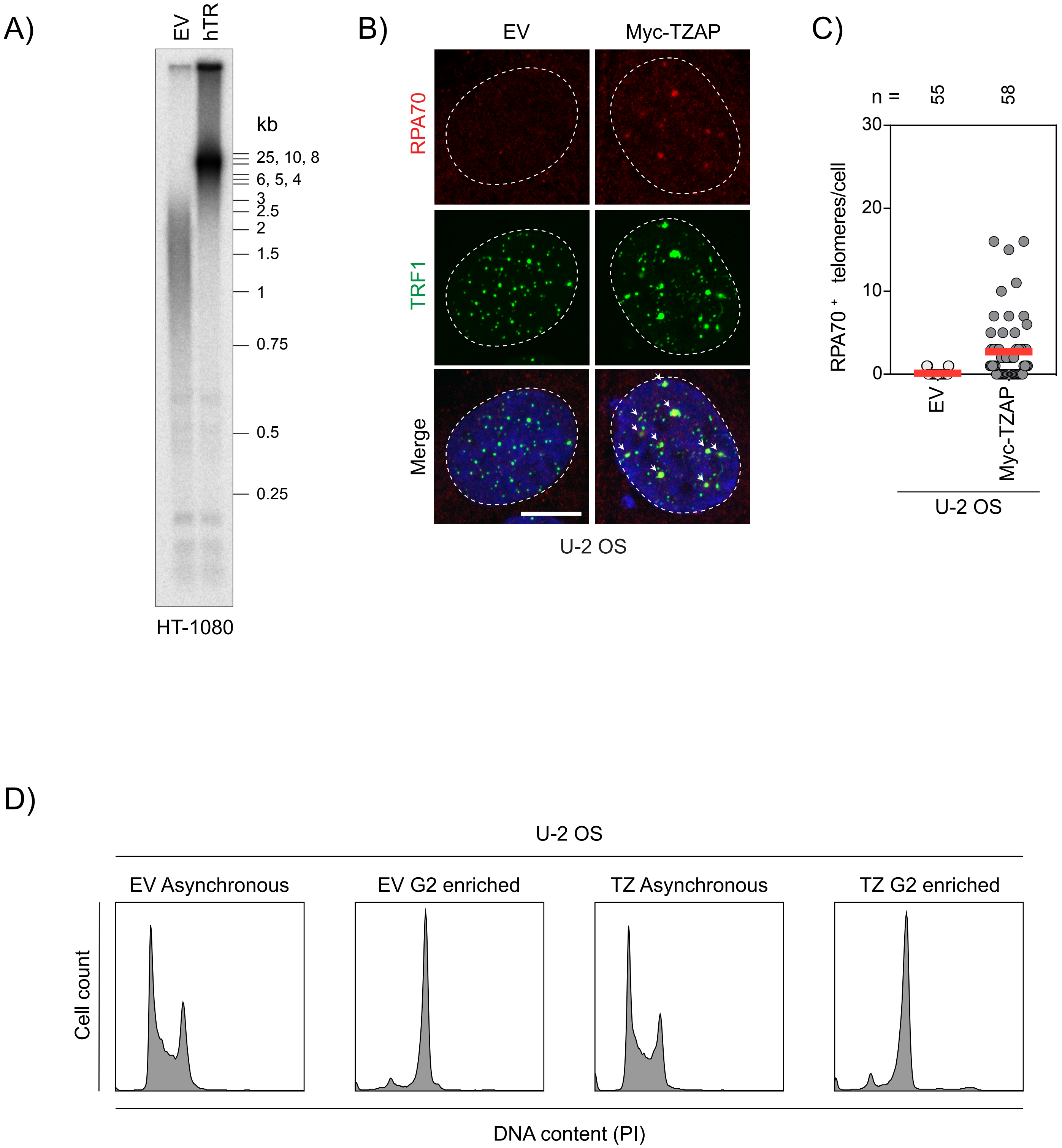
A) TRF analysis of parental U-2 OS cells, as well as RMI1-/- (1A8, 1B5) and BLM-/- (2C8, 3D1) KO clones. **B)** Representative immunofluorescence images of

HeLa-LT cells overexpressing Myc-TZAP and treated with the indicated siRNAs, showing the nuclear localization of BLM and TRF2. White arrows point to BLM-TRF2 colocalizations. Scale bar 10 μm. **C)** Scatter plot showing quantification of experiment described in B). A total of at least 279 nuclei were analyzed for each sample. Data are representative of two independent experiments. Centre line, mean. n shows number of cells analyzed. *** $P < 0.001$, ns: non-significant, one-way ANOVA test was used to calculate the p-values. **D)** CCA of HeLa-LT cells overexpressing Myc-TZAP and treated with the indicated siRNAs. **E)** Quantification of CCA signals in D) was performed relative to si-scramble treated HeLa-LT cells overexpressing Myc-TZAP. Bars represent the mean ± SD of 3 independent experiments. *** $P < 0.001$, ns: non-significant, one-way ANOVA test was used to calculate the p-values.

Supplementary Figure 4: Proposed model to explain the consequences of TZAP overexpression in ATRX/DAXX deficient cells.

Rapid decompaction of over-elongated telomeres takes place upon loss of ATRX/DAXX. This in turn facilitates the efficient loading of overexpressed TZAP onto telomeres, which causes telomere dysfunction by a still unknown mechanism and activation of an ALT-like pathway. In result, cECTRs (c-circles and t-circles) are generated in a BTR dependent manner.

Supplementary Figure 1: Further characterization of HT-1080 hTR cells and TZAP overexpression in U-2 OS cells. Related to Figure 1.



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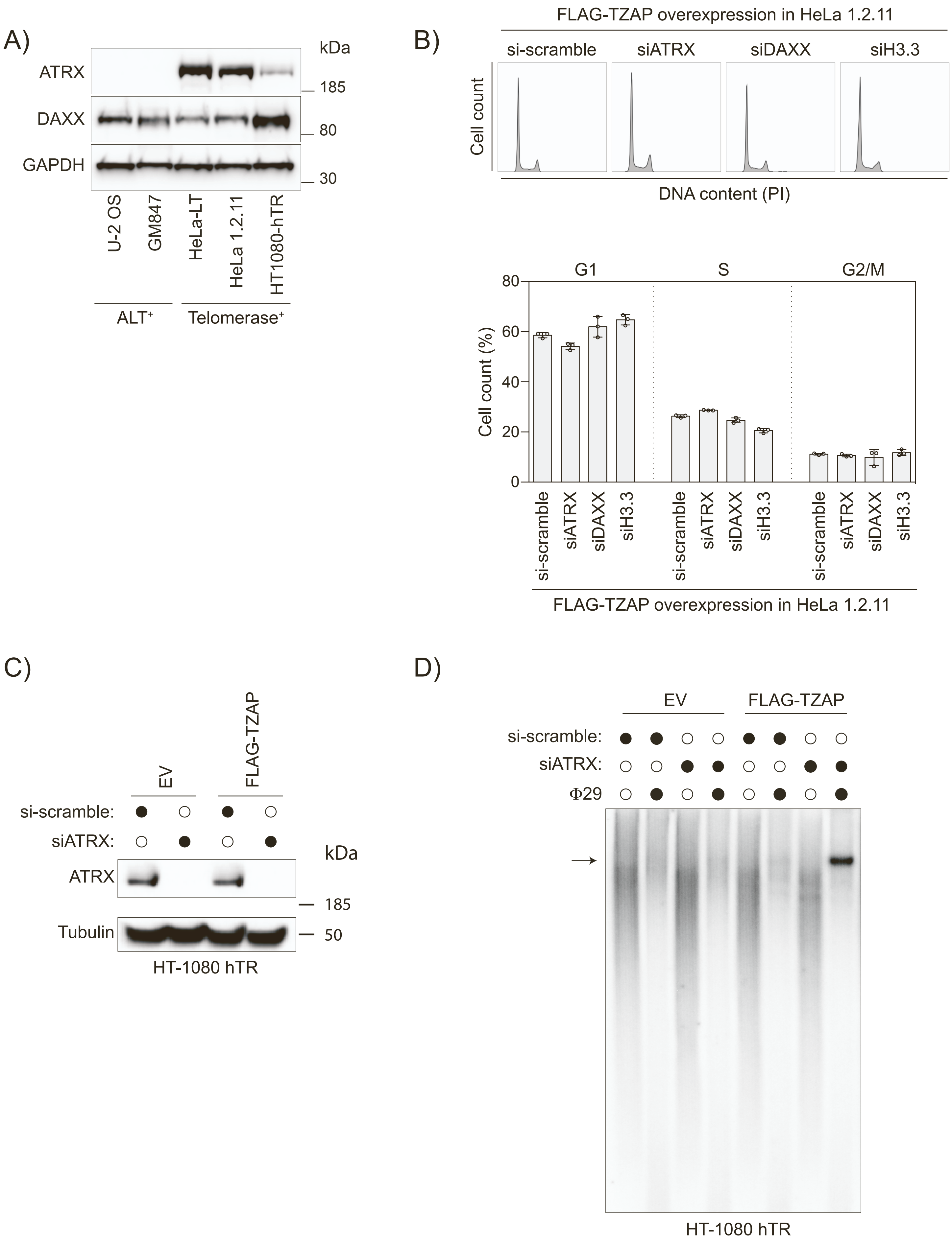

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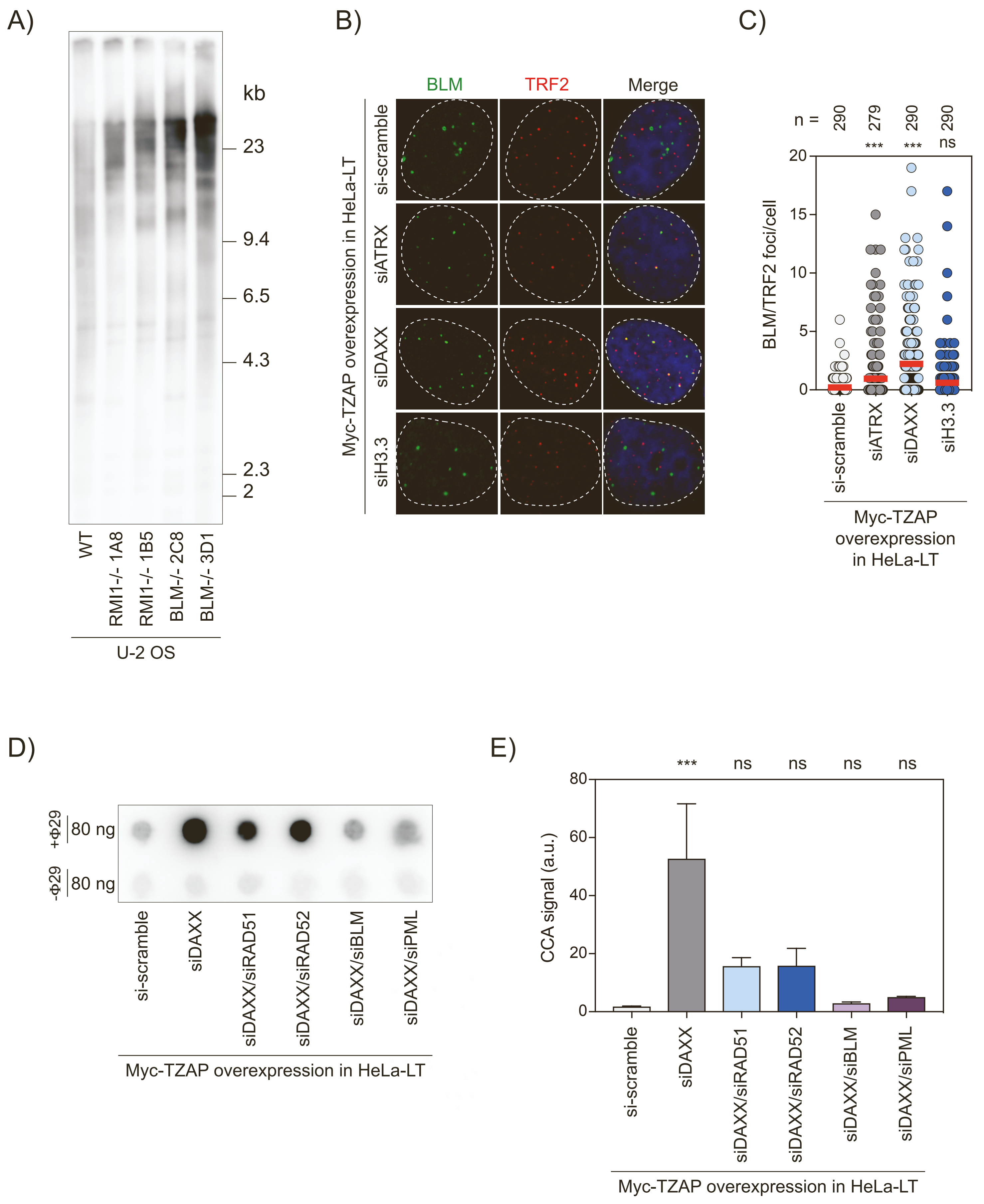

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