Figure S1

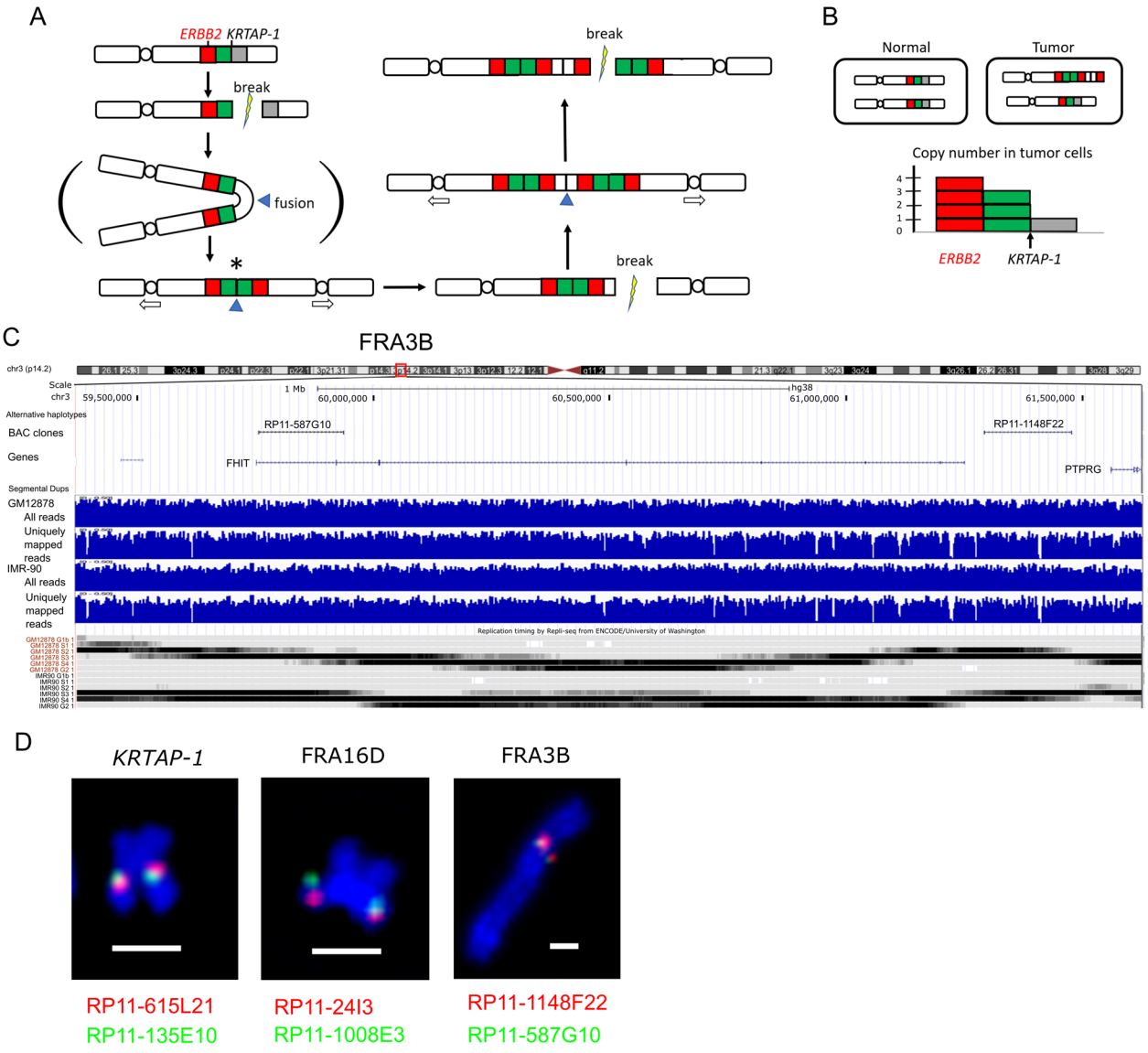

Figure S1. Breaks at *KRTAP-1* trigger palindromic duplication and BFB cycles for the amplification of *ERBB2*. (**A**) An early stage of the BFB cycle for the amplification of *ERBB2* that is initiated by a break at *KRTAP-1* (model). The genomic segment harboring the *ERBB2* gene (red), the centromeric (green), and the telomeric segment (gray) that flank *KRTAP-1* are shown. The initiating break at *KRTAP-1* leads to a chromatid fusion (blue triangles) and an inverted duplication (*) followed by *ERBB2* gene amplification. (**B**) The BFB cycles can result in the gradient of copy-number increases (model). Illustrated is a normal cell with two normal chromosomes and a tumor cell with a chromosome generated by the BFB cycles in Figure S1A with a normal homolog. A copy-number transition is observed at *KRTAP-1* with the gradient of the copy-number increase toward the *ERBB2* gene (red) in the tumor cell. (**C**) FRA3B and its flanking regions in hg38. The locations of alternative haplotypes, BAC clones for the FISH

probes, genes, read depth of GM12878, IMR-90 whole genome sequencing, and the replication timings of GM12878 and IMR-90 cells from Repli-seq (ENCODE data) are shown. (**D**) Hybridization of flanking probes for *KRTAP-1*, FRA16D, and FRA3B with metaphase chromosomes of IMR-90 cells. Scale bars = 2 μm.

Figure S2

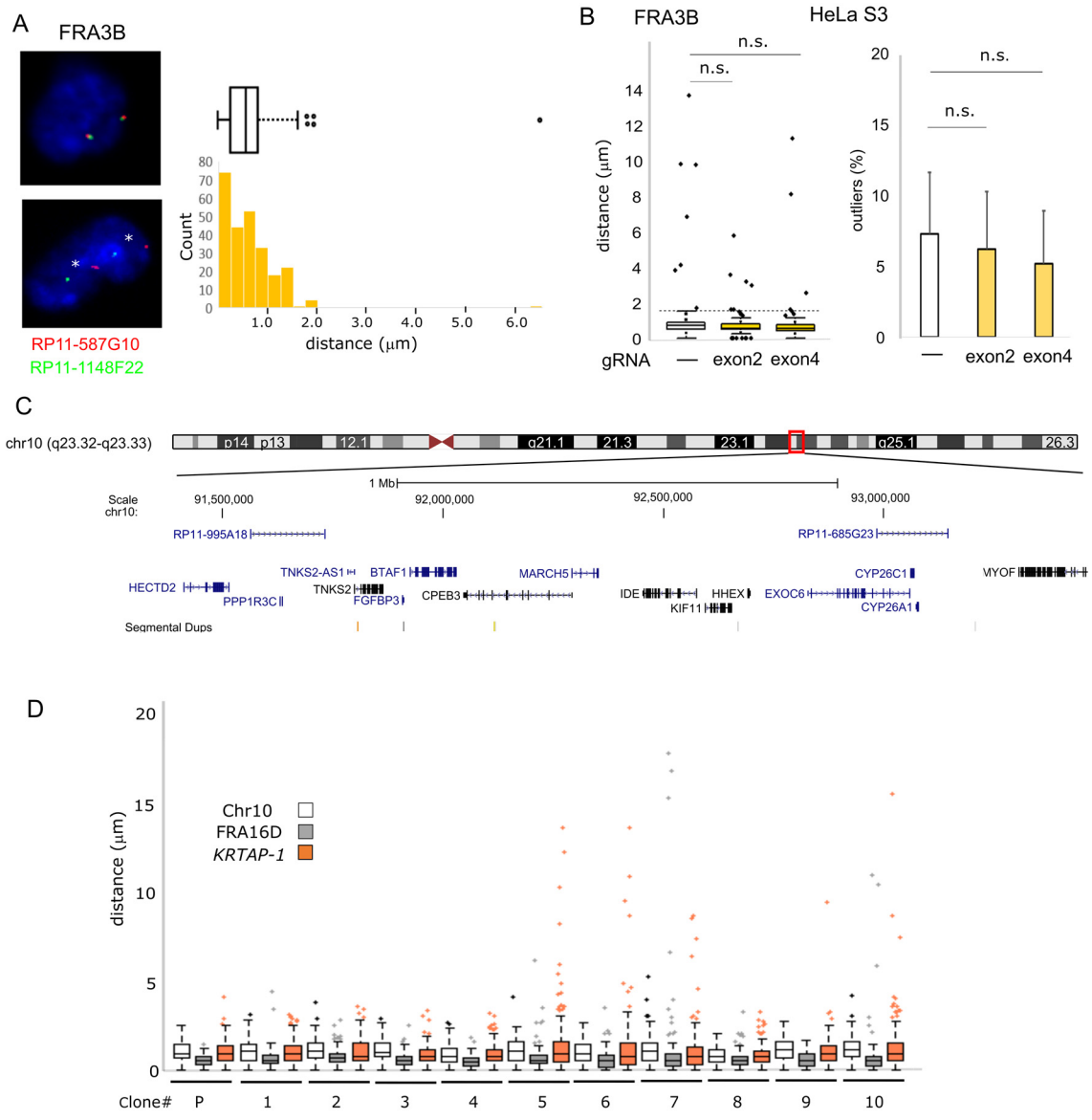

Figure S2. Measuring breaks by Fluorescence in situ hybridization. (**A)** Measurements of the distance between both ends of FRA3B. Representative photomicrographs of FISH of the nuclei of IMR-90 are shown. The asterisks (*) show that the distance between the probe pair is greater than the sum of the third quartile (Q3) and 1.5 times the interquartile range (IQR). The distributions of the distances (n=250) are depicted by box plots and histograms. Outliers are plotted at their exact lengths. (**B**) (left) The distribution of distances between probes for non-targeted FRA3B before and after targeting FRA16D by CRISPR/Cas9 in HeLa S3 cells. The box plots depict distributions of the distances (n=100), with outliers plotted at their exact distances. P-values were determined by the one-tailed Mann-Whitney U test. The horizontal dotted lines indicate thresholds for outliers determined by the cells transfected with the empty vector. The histograms depict the frequencies of outliers. Error bars represent 95% confidence intervals. P-

values were determined by Fisher's exact test. n.s.: not significant. (**C**) FRA10A and its centromeric flanking regions in hg38. The locations of BAC clones for the Break-apart FISH probes, genes, and segmental duplications are shown. (**D**) The box plot depicts distributions of distances between the probes (*n*=160) for *KRTAP-1* and FRA16D or Chr10 (the region centromeric to a rare fragile site FRA10A). (P): parental TK6 cell population.

Figure S3

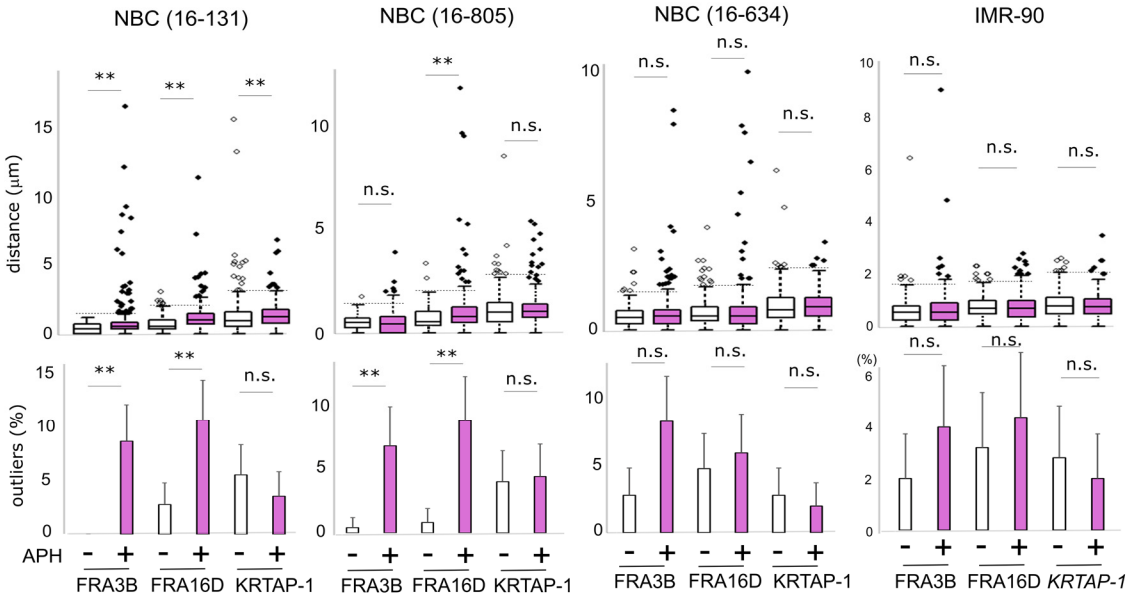

Figure S3. Aphidicolin-induced breaks at *KRTAP-1*. The box plots (top) showing the distribution of distances between the flanking probes for *KRTAP-1*, FRA16D, and FRA3B in primary human mammary epithelial cells (NBC 16-131, 16-805, and 16-634), and IMR90 primary human lung fibroblast with or without aphidicolin treatment (24 hours). For each locus, the distances between the pair of probes were measured for 250 pairs. P-values were determined by the Mann-Whitney U test. The horizontal dotted lines indicate thresholds for the outliers in cells without aphidicolin treatments. The histograms (bottom) depict the frequencies of outliers. Error bars represent 95% confidence intervals. P-values were determined by Fisher's exact test. *: $p<0.05$, **: $p<0.01$, n.s.: not significant.

Figure S4

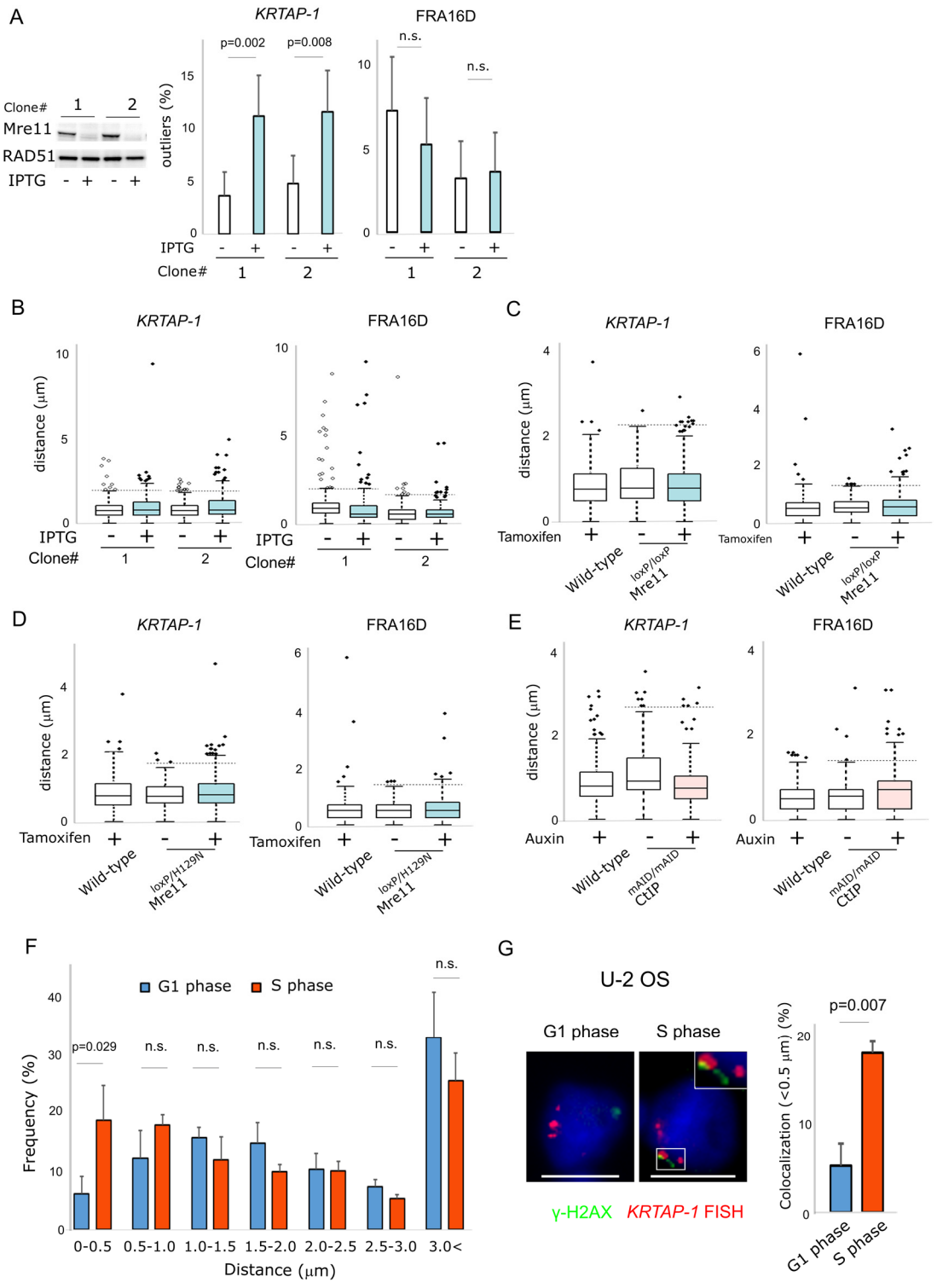
A
KRTAP-1
FRA16D
Clone#
Mre11
RAD51
IPTG
outliers (%)
p=0.002
p=0.008
n.s.
B
distance (μm)
C
Tamoxifen
Wild-type
loxP/loxP Mre11
D
loxP/H129N Mre11
E
Auxin
mAID/mAID CtIP
F
G1 phase
S phase
p=0.029
Frequency (%)
0-0.5
0.5-1.0
1.0-1.5
1.5-2.0
2.0-2.5
2.5-3.0
3.0<
Distance (μm)
G
U-2 OS
γ-H2AX
KRTAP-1 FISH
p=0.007
Colocalization (<0.5 μm) (%)

Figure S4. Depletion of Mre11 induces breakage in *KRTAP-1*. (**A**) IPTG-inducible Mre11 knockdown in two independent clones of RKO cells. Western blot shows the Mre11 protein level before and after 10 days of IPTG treatment. The RAD51 protein level is shown as a control. The histograms depict the frequencies of outliers. Error bars represent 95% confidence intervals. P-values were determined by Fisher's exact test. n.s.: not significant. (**B** – **E**) The box plots showing the distribution of distances between both ends of *KRTAP-1* and FRA16D: (**B**) IPTG-inducible Mre11 knockdown in two independent clones of RKO cells, (**C**) TK6 cells with the tamoxifen-inducible knockout of Mre11, (**D**) TK6 cells with the tamoxifen-inducible knockout of wild-type Mre11 for the exclusive expression of the nuclease -deficient Mre11 (H129N), and (**E**) Auxin-inducible depletion of CtIP in TK6 cells expressing CtIP fused with mAID. For each locus, the distances between the probes were measured for 250 pairs. Outliers are plotted at the exact distance. The horizontal dotted lines indicate thresholds for the outliers for cells without treatments for induction. (**F**) The histogram depicts ranges of the distance between a Mre11 focus and *KRTAP-1* on the x-axis and the fraction of nuclei for each distance range on the y-axis. The averages of three experiments are shown. Error bars represent standard deviation. P-value was determined by a two-tailed t-test. n.s.: not significant. (**G**) Representative photomicrographs of the nuclei of U-2 OS cells in the G1 phase and S phase after staining with anti-Phosphorylated histone H2AX at serine 139 (γ-H2AX) antibodies (green) and hybridization of FISH probes for *KRTAP-1* (red). Scale bars = 10 μm. The histogram depicts the fraction of nuclei with the co-localization of γ-H2AX foci and *KRTAP-1*. The averages of three experiments are shown. p-value was calculated with a two-tailed t-test.

Figure S5

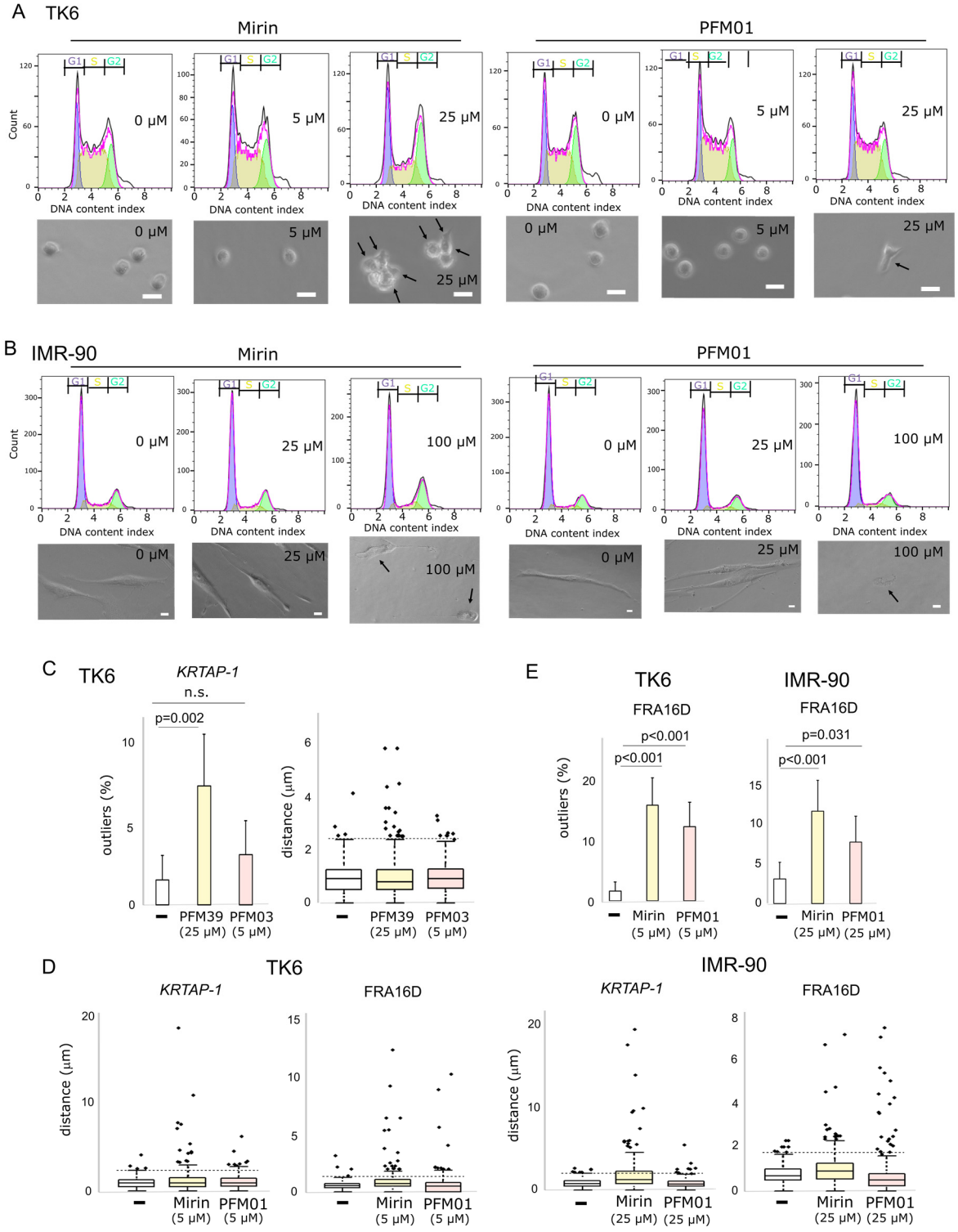
A
TK6
Mirin
PFM01
G1
S
G2
Count
DNA content index
0 μM
5 μM
25 μM
B
IMR-90
Mirin
PFM01
0 μM
25 μM
100 μM
C
TK6
KRTAP-1
n.s.
p=0.002
outliers (%)
distance (μm)
PFM39 (25 μM)
PFM03 (5 μM)
D
TK6
KRTAP-1
FRA16D
IMR-90
distance (μm)
Mirin (5 μM)
PFM01 (5 μM)
E
TK6
IMR-90
FRA16D
p<0.001
p=0.031
outliers (%)
Mirin (25 μM)
PFM01 (25 μM)

Figure S5. Mre11 exonuclease activity protects *KRTAP-1* from breaks. (**A and B)** Cell cycle profiles and phase-contrast images of TK6 (A) and IMR-90 (B) cells treated with a serial dose of Mre11 inhibitors Mirin and PFM01. Cells with morphological abnormalities are shown by arrows. Scale bars = 20 μm. (**C**) Distinct effects of an exonuclease (PFM39) and an endonuclease (PFM03) inhibitor of Mre11 on breaks in *KRTAP-1* in TK6 cells (*n*=250). The histogram depicts frequencies of outliers at *KRTAP-1*. Error bars represent 95% confidence intervals. P-values were determined by Fisher's exact test. n.s.: not significant. The box plots show the distribution of distances between the flanking probes for *KRTAP-1*. Outliers are plotted at the exact distance. The horizontal dotted lines indicate thresholds for outliers for untreated populations. (**D**) Effects of an exonuclease (Mirin) and an endonuclease (PFM01) inhibitor of Mre11 on the distances of *KRTAP-1* and FRA16D in TK6 (left) and IMR-90 cells (right) (*n*=250). The box plots show the distribution of distances between the flanking probes for *KRTAP-1* and FRA16D. Outliers are plotted at the exact distance. The horizontal dotted lines indicate thresholds for outliers for untreated populations. (**E**) Effects of an exonuclease (Mirin) and an endonuclease (PFM01) inhibitor of Mre11 on breaks in FRA16D in TK6 and IMR-90 cells (*n*=250). The histograms depict frequencies of outliers at FRA16D. Error bars represent 95% confidence intervals. P-values were determined by Fisher's exact test.

Figure S6

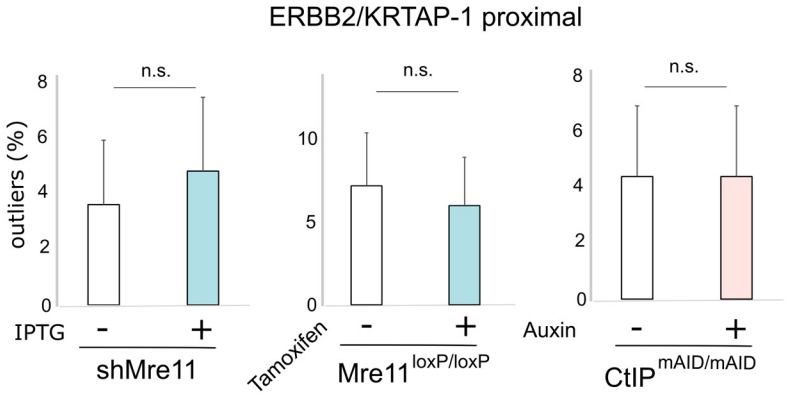

Figure S6. Lack of induction of breaks between *ERBB2* and *KRTAP-1* after depletion of Mre11
Frequency of outliers before and after depletion of Mre11 in the IPTG-inducible Mre11 knockdown in RKO cells (left) and the Cre/loxP-inducible Mre11 knockout in TK6 cells (middle), and the mAID-inducible depletion of CtIP in TK6 cells (right). The histograms depict frequencies of outliers between *ERBB2* and KRTAP-1 proximal (RP11-94L15 and RP11-615L21 in Figure 5A, respectively). Error bars represent 95% confidence intervals. P-values were determined by Fisher's exact test. n.s.: not significant.

Table S1. List of BAC clones

Location	BAC clone	Primer pair #1		PCR result	Primer pair #2		PCR result	FISH signal
		Forward #1	Reverse #1		Forward #2	Reverse #2		
ERBB2	RP11-94L15	GTGTGTGTGGTGGAGTGGAG	AGGAAAAGGCAGCAGAACAA	+	CTACGGCAGAGAACCCAGAG	ACACCATTGCTGTTCCTTCC	+	++
ERBB2 flanking (centromeric)	RP11-352B23	CCACGCACAAGAGTCAAAGA	CCCAGCATGTAGCTGAGACA	+	ACTGAGAGTGGGTGGGACAC	GTCCAGCACCTGGCTCTAAG	+	++
	RP11-614N11	CCACGCACAAGAGTCAAAGA	CCCAGCATGTAGCTGAGACA	+	ACTGAGAGTGGGTGGGACAC	GTCCAGCACCTGGCTCTAAG	+	NT
KRTAP-1 flanking (centromeric)	RP11-615L21	CAAGCCCACTTGGAAAAAGA	ATGGAGGGATTCCATGACAG	+	AGAGGTGGCTGTTCTCCTGA	TCTCCTGACATCCCTGATCC	+	++
KRTAP-1 flanking (telomeric)	RP11-135E10	CCTGAGGAAAACGCAGTCTC	TAGCTCTCTGCCCCAAGAAA	+	GCGGAGCTAGGAATTCAATG	GGCTGACCTCTTCACCTGAG	+	++
	RP11-156I3	CCTGAGGAAAACGCAGTCTC	TAGCTCTCTGCCCCAAGAAA	+	GCGGAGCTAGGAATTCAATG	GGCTGACCTCTTCACCTGAG	+	++ [a]
	RP11-156E5	CCTGAGGAAAACGCAGTCTC	TAGCTCTCTGCCCCAAGAAA	+	GCGGAGCTAGGAATTCAATG	GGCTGACCTCTTCACCTGAG	-	NT
FRA16D flanking (centromeric)	RP11-24I3	AGCCTGCTACCCTCAGAACA	TAATGGGATGGGGACGTAAA	+	GCTTGAACACCCTGGTTCAT	CCCATCCTCACTCAGTTGGT	+	++
	RP11-281J9	GCTTGAACACCCTGGTTCAT	CCCATCCTCACTCAGTTGGT	+	ATGTATGTGCGCCAGTGGTA	TTGTCACCACCCAAAAGTGA	+	++
	RP11-91O9	AACATGCCTTGGCACTAACC	ACAACCGTCGTTTTCTTTGG	+	ACAGGGAAAAAGGGTTTGCT	TTCCTTGAGCGAGGTTCTGT	+	++ [b]
FRA16D flanking (telomeric)	RP11-1008E3	GAGAGAAAGCGAGCAGGAGA	CATGGGCAGGTACAACAGTG	+	CACCACGAGAAATGCTGAGA	CGTAGCAGGCTTTCTGTTCC	+	+
	RP11-467I17	GAGAGAAAGCGAGCAGGAGA	CATGGGCAGGTACAACAGTG	+	CACCACGAGAAATGCTGAGA	CGTAGCAGGCTTTCTGTTCC	+	±
FRA3B flanking (telomeric)	RP11-587G10	TCTTTGGGGATTTTTGAGCA	TGGGCATTGAAGGAAGAATC	+	GAGGGAATCGTGTCAATGCT	CACAGTGAATTCTCGCTCCA	+	+
	RP11-449E4	TCTTTGGGGATTTTTGAGCA	TGGGCATTGAAGGAAGAATC	+	GAGGGAATCGTGTCAATGCT	CACAGTGAATTCTCGCTCCA	+	NT
FRA3B flanking (centromeric)	RP11-1148F22	AGTGGTAGTGCTGGGACAGG	TCCATTCTCCTTGCCCATAG	+	TCTTGACGCCTTTCTCCAGT	GGGTTTGGCAATACACTGCT	+	+
	RP11-273A23	CGTATGTGGCCTTCCAGAAT	TGTCAGCCACACCATTTGTT	+	GGACCTGCGGTGTACTGTTT	TTCAGCCACACAGCCAATAA	+	-
	RP11-137N22	CGTATGTGGCCTTCCAGAAT	TGTCAGCCACACCATTTGTT	+	GGACCTGCGGTGTACTGTTT	TTCAGCCACACAGCCAATAA	+	-
	RP11-1028J17	GGACCTGCGGTGTACTGTTT	TTCAGCCACACAGCCAATAA	+	GAAGTTGGCTGTGCTTCCTC	TTGCCACCAAATTAGCATCA	+	±
	RP11-960H3	GGACCTGCGGTGTACTGTTT	TTCAGCCACACAGCCAATAA	+	GAAGTTGGCTGTGCTTCCTC	TTGCCACCAAATTAGCATCA	+	±
	RP11-951G18	AGTGGTAGTGCTGGGACAGG	TCCATTCTCCTTGCCCATAG	-	AGTGGTAGTGCTGGGACAGG	TCCATTCTCCTTGCCCATAG	-	NT
Chr10 flanking (centromeric)	RP11-995A18	GCTTATCTGCTCCCAAGCAC	GAGGGAGAAATGCGGATACA	+	CTCTACTGGCCCTGGAATCA	ATGCCAAGTGAGCCAATCTC	+	++
	RP11-887N18	GCTTATCTGCTCCCAAGCAC	GAGGGAGAAATGCGGATACA	+	CTCTACTGGCCCTGGAATCA	ATGCCAAGTGAGCCAATCTC	+	NT
Chr10 flanking (telomeric)	RP11-685G23	CTGACCGCATACAGCAGAAA	TTGCAAATGACCACACAGGT	+	TTCGGGTTGCTCTGAAGACT	TCCTCCAAATGGAATGAAGC	+	++
	RP11-790D23	CTGACCGCATACAGCAGAAA	TTGCAAATGACCACACAGGT	+	TTCGGGTTGCTCTGAAGACT	TCCTCCAAATGGAATGAAGC	+	++

Table S1. BAC clones for FISH probes. A list of BAC clones tested for the present study. Two primer pairs that were used for PCR to validate each clone are shown. +: positive, ++: strong signals, ±: weak signals, -: negative, NT: not tested, a: the signal was too far from the signal from the paired probe in the majority of interphase cells.