Supporting Information

Draskovic et al. 10.1073/pnas.0907689106

SI Text

Western Blotting Analyses. Twenty million VA13 and HT1080 cells were transfected with GFP-ICP0* (pAL30) or GFP alone (pAL39) using Lipofectamine 2000. Twenty-four hours post-transfection cells were collected by trypsinization and subjected to fluorescence activated cell sorting (FACS) on a Vantage apparatus to obtain the GFP-positive and -negative cell populations. Sorted cells were washed three times in PBS and frozen at −80 °C. Cell pellets were re-suspended in the RIPA buffer, incubated on ice for 30 min, and centrifuged for 30 min at 12,000 × *g* at 4 °C. Twenty micrograms of supernatant proteins were fractionated by SDS/PAGE and transferred to nitrocellulose. Blots were blocked overnight at 4 °C in 5% milk in PBS/0.1% Tween-20, incubated with primary antibodies overnight at 4 °C (antibodies are listed below), followed by horseradish peroxidase-conjugated donkey anti-mouse or anti-rabbit IgG (Dako, 1/1,000) for 1 h at RT. Bound antibodies were detected with the enhanced chemiluminescence (GE Healthcare) using the Fujifilm LAS-1000 camera and Fujifilm Intelligent Dark Box II.

1. Bischof O, et al. (2001) Regulation and localization of the Bloom syndrome protein in response to DNA damage. *J Cell Biol* 153:367–380.

2. Lombard DB, Guarente L (2000) Nijmegen breakage syndrome disease protein and MRE11 at PML nuclear bodies and meiotic telomeres. *Cancer Res* 60:2331–2334.

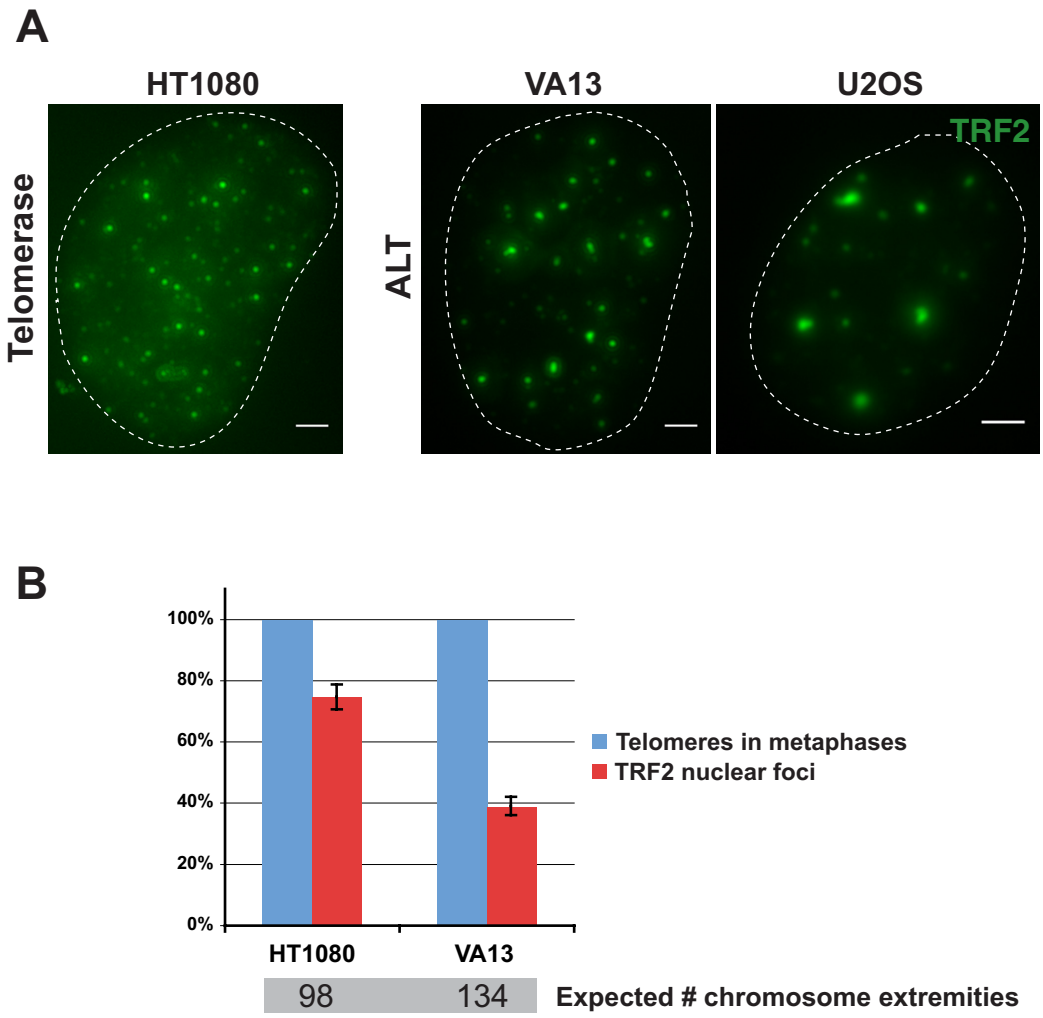


Fig. S1. ALT cell lines display a surprisingly low number of telomeric foci. (*A*) TRF2 forms large aggregates in ALT cells (VA13, U20S) but not in telomerase-positive HT1080 as detected by anti-TRF2 antibody (green). (Scale bars, 2 μm.) (*B*) Quantification of TRF2 foci in VA13 and HT1080 cells as detected in (*A*) in comparison with the number of telomeric extremities detected in metaphase chromosomes by a PNA FISH. VA13 = 134 extremities, HT1080 = 98 extremities.

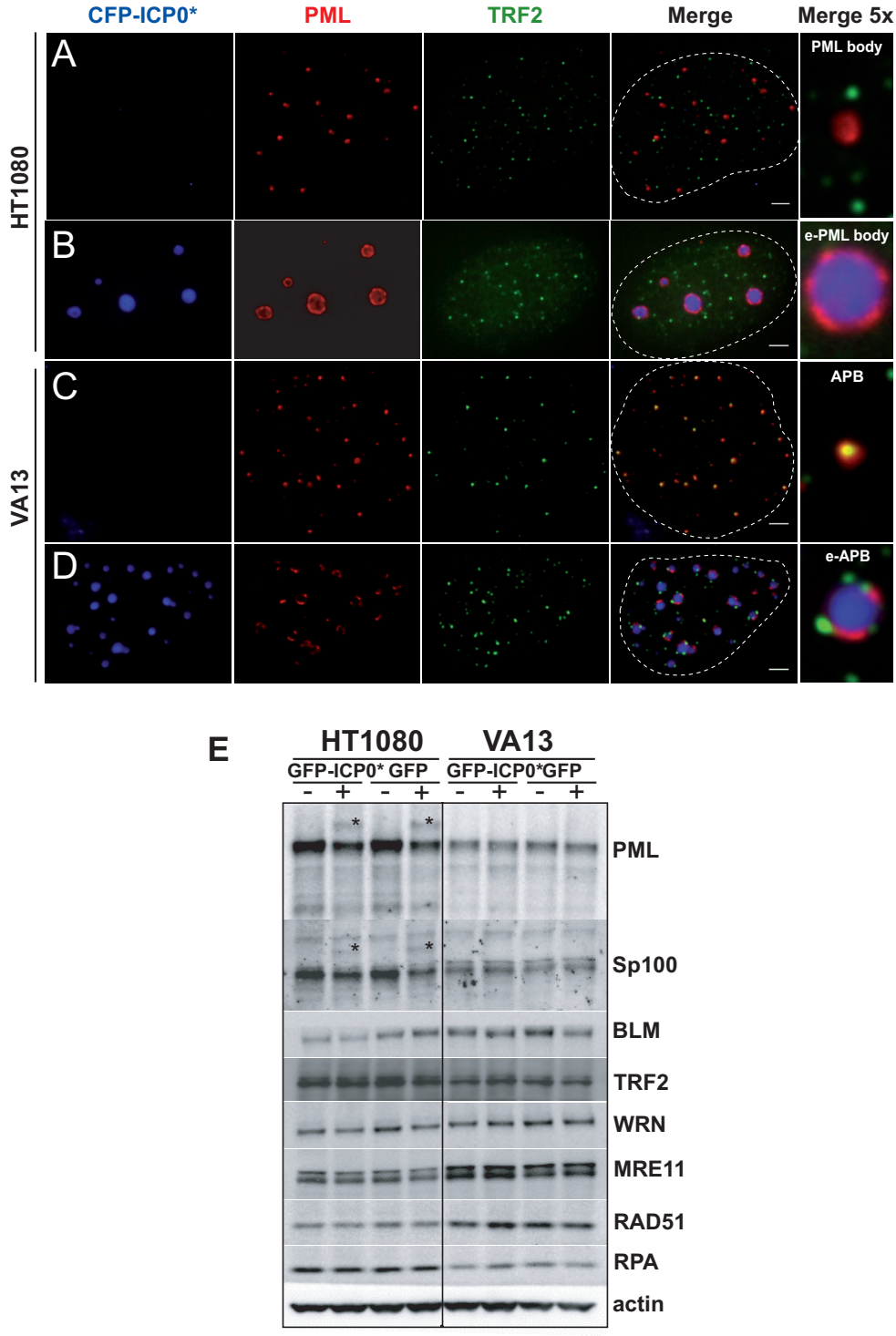


Fig. S2. Model of enlarged (e-) APBs using a viral protein ICP0*. (*A–D*) Deconvolved images of nuclei. (Scale bars, 2 μm.) Immunofluorescence analysis of fixed HT1080 (telomerase+) and VA13 (ALT+) cells stained with anti-PML (red) and anti-TRF2 (green). Twenty-four hours before fixation cells were transfected with CFP-ICP0* (blue). Non-transfected nuclei from the same experiment are also shown. (*A*) PML bodies in HT1080. TRF2 foci are not associated with PML bodies. (*B*) Enlarged PML bodies (e-PML) are not associated with TRF2. Accumulation of ICP0* does not induce association of TRF2 foci with e-PML bodies. (*C*) ALT-associated PML bodies or APBs in native VA13 cells. Co-localization of PML and TRF2 indicates presence of APBs. (*D*) Enlarged APBs (e-APBs) reveal TRF2 clusters. Twenty-four h post-transfection with CFP-ICP0* (blue) APBs in VA13 cells are enlarged by infiltration of ICP0*, revealing multiple TRF2 foci surrounding the PML scaffold. (*E*) ICP0* does not induce protein degradation of PML bodies or APBs components HT1080 and VA13 cells were transfected with GFP-ICP0* or GFP and cell sorted into GFP positive (+) and GFP negative (-) cell populations 24 h post-transfection. Twenty micrograms of protein extract from each cell population were analyzed by immunoblotting against various components of PML bodies and APBs. Protein isoforms due to transfection with GFP are marked (*).

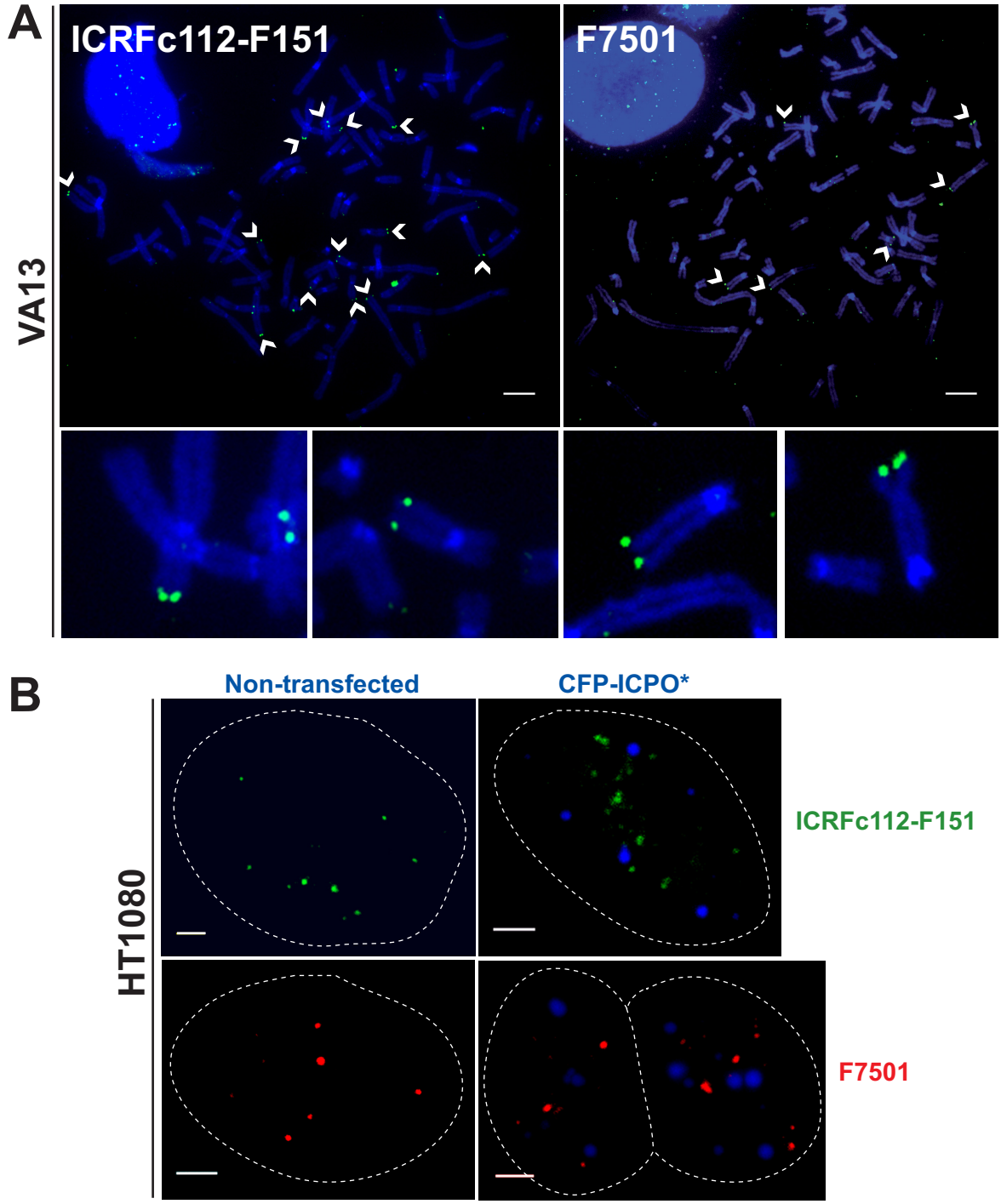


Fig. S3. Subtelomeric probes label chromosome ends. (*A*) Subtelomeric probes are specific for a subset of chromosomes. FISH analysis of VA13 metaphase spreads using ICRFc112-F151 and F7501 subtelomeric probes (green). Chromosomes were counterstained with DAPI. ICRFc112-F151 labels chromosome extremities 1p, 5q, 6q, 17q, and 20p and less frequently 6p, 7p, 8p, 9q, 11p, 15q, and 19p. F7501 probe detects chromosome extremities 3q, 15q, and 19p and less frequently 9q, 11p, and 16pq. (Scale bars, 5 μm.) VA13 cell nuclei were hybridized with these two subtelomeric probes to demonstrate the association of chromosome ends with APBs (see Fig. 1*C*). (*B*) FISH analysis of HT1080 nuclei using two subtelomeric probes ICRFc112-F151 (green) and F7501 (red) 24 h post-transfection with CFP-ICP0* (blue). In telomerase-positive cells subtelomeric sequences do not associate with e-PML bodies infiltrated by ICP0*. Non-transfected nuclei are also shown.

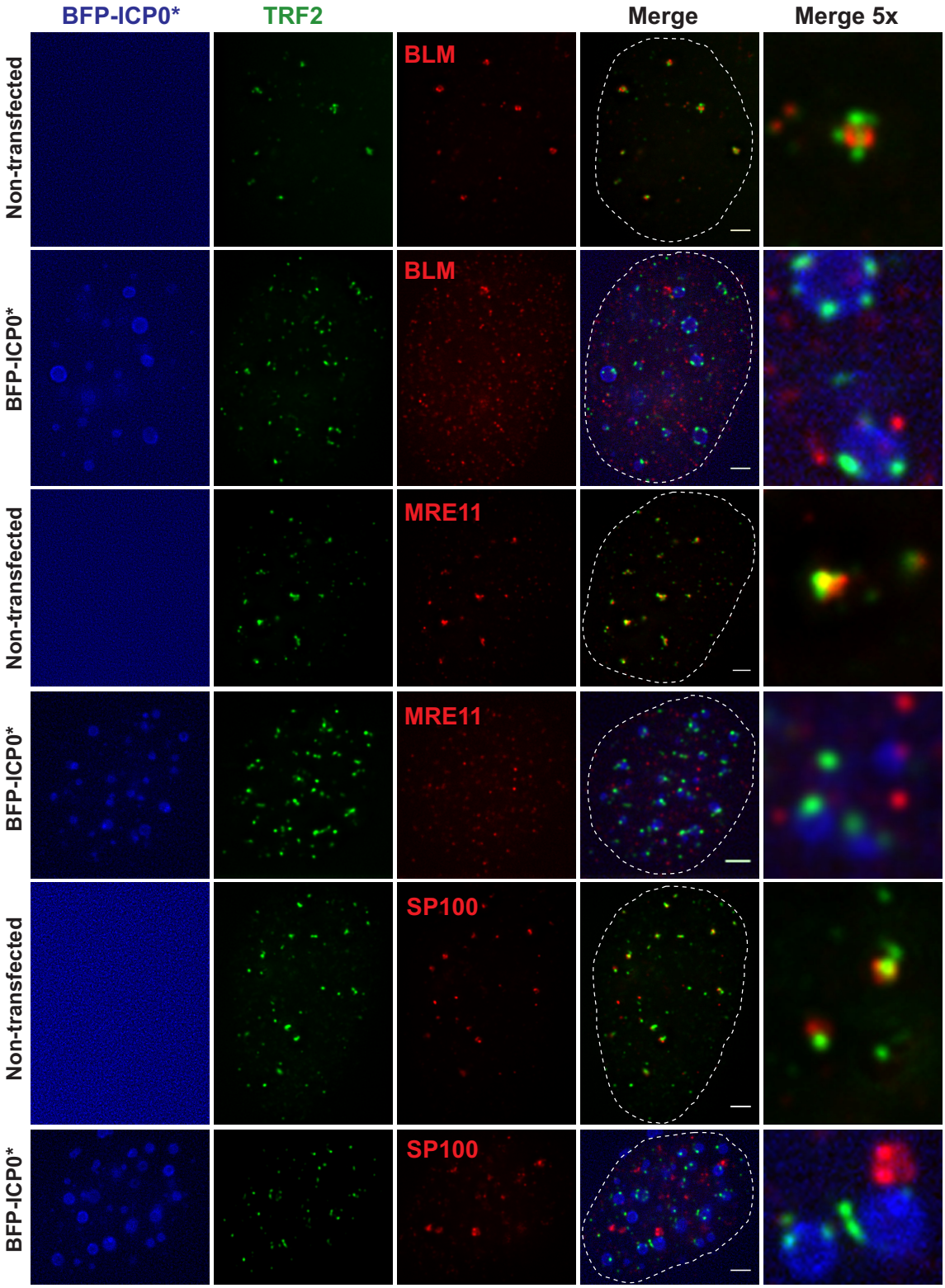
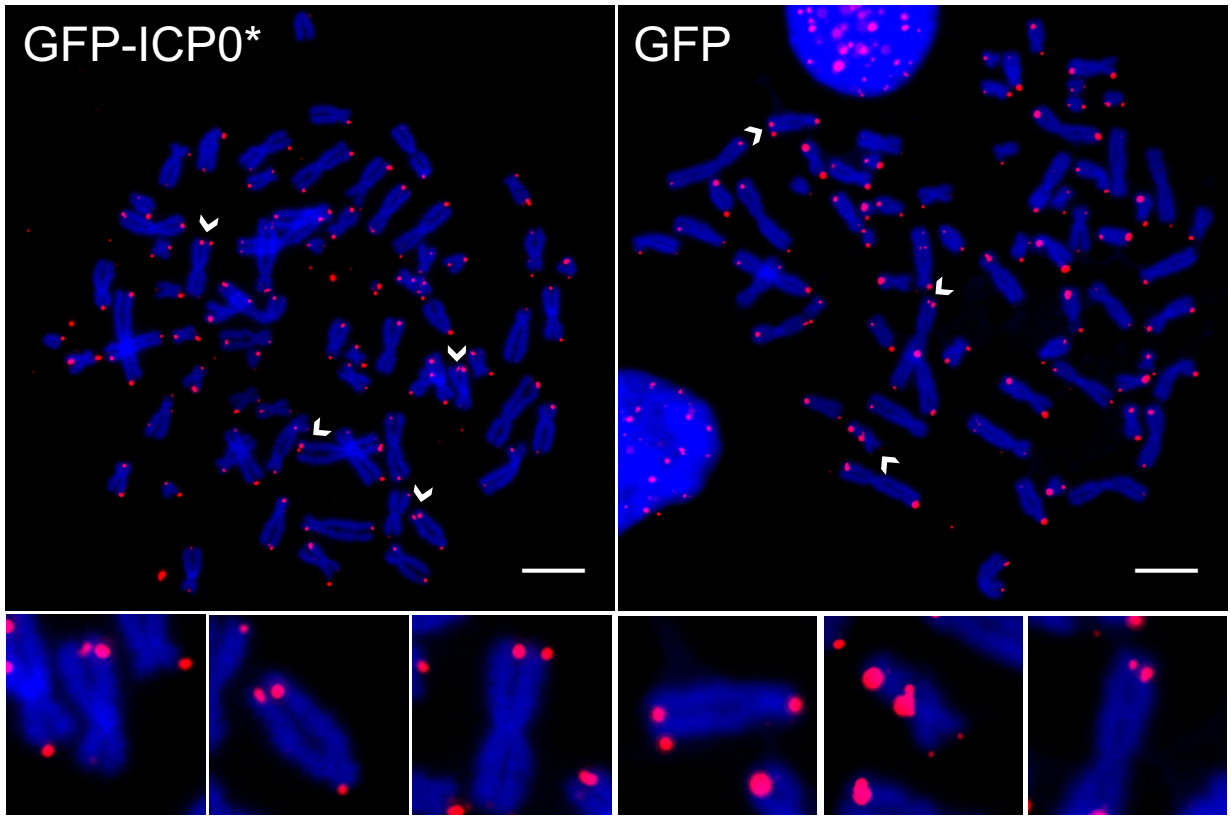


Fig. S4. Redistribution of APBs' proteins BLM, MRE11, and SP100 upon infiltration of ICP0*. VA13 cells transfected with BFP-ICP0* were fixed 24 h post-transfection and immunostained with anti-GFP antibodies to detect ICP0* (blue), anti-TRF2 antibodies (green), and anti-BLM, anti-MRE11 or anti-SP100, antibodies (red). Nuclei were visualized by transluminescence. Natural fluorescence of BFP alone was too weak to be visualized directly therefore we used the anti-GFP antibodies to detect the BFP-ICP0* fusion protein. In non-transfected cells SP100, BLM, and MRE11 were present in APBs while accumulation of ICP0* displaced all these proteins from e-APBs into the nucleoplasm. (Scale bars, 2 μm.)

A



B

VA13 cells expressing	# metaphases analyzed	# extremities with T-SCE	Range of T-SCE / metaphase
GFP-ICP0* (high level)	40	153/4160 (3,68%)	0-16
GFP-ICP0* (low level)	40	154/4290 (3,59%)	0-12
GFP (high level)	42	187/4337 (4,31%)	1-12

Fig. S5. Accumulation of ICP0* in APBs does not interfere with telomeric sister chromatide exchange (T-SCE). (*A*) VA13 cells were transfected with GFP-ICP0* or GFP control. Twenty-four hours posttransfection cells were FACS sorted into cells that express high levels of GFP or low levels of GFP. Sorted cells were maintained in culture for another 24 h in the presence of BrdU and BrdC before the preparation of metaphase spreads. T-SCEs were visualized by the CO-FISH procedure using a single telomeric C-rich PNA probe labeled with Cy3 (red) and chromosomes were counterstained with DAPI. Representative metaphase spreads from cells expressing high levels of GFP-ICP0* and GFP are shown. Arrowheads point to T-SCEs. (Scale bars, 5 μm.) (*B*) Quantification of results obtained in (*A*). Frequency of T-SCE in VA13 cells remains unchanged upon expression of ICP0*.

Table S1. Proteins present in PML bodies, APBs and e-APBs

Protein	Antibody	PML body	APB	e-APB
PML	Chemicon, AB1370	+	+	+
SP100	Chemicon, AB1380	+	+	–
BLM	ab2179	+	+	–
TopoIIIα	D6 (gift from F. Riou)	+	+	–
WRN	sc-5629	ND	–	–
BRCA1	ab2956, Calbiochem OP92T	ND	–	–
MRE11	ab397	ND	+	–
RAD9	sc-8324	ND	+	–
RAD51	Calbiochem, PC130	ND	+	+
RPA	GeneTex, RB-RPA32-UP100	ND	+	+
SMC5	Bethyl Laboratory, A300–236A	ND	–	ND
MMS21 (NSE2)	ab53485	ND	–	ND
TRF2	IMG-124A	–	+	+
TRF1	ab10579	–	+	+
Tel DNA	PNA probe	–	+	+
Coup-TF2 (NR2F2)	ab50487	–	+	+

Many proteins initially listed in the literature as specific for APBs have been later found to also be part of PML bodies and therefore may no longer be considered specific to APBs. For example, DNA repair and recombination proteins such as MRE11, RAD51 and RPA are also present in a substantial fraction of PML bodies in the absence of induced DNA damage (1, 2). Furthermore, we could not confirm localization of certain proteins, such as BRCA1, WRN, SMC5 and MMS21, reported to be associated with APBs in the VA13 cell line. These discrepancies could be due to differential temporal association of proteins with PML bodies in different ALT cell lines. However, caution should be exerted when localizing proteins to APBs by immunofluorescence since bona fide proteins such as PML, by their very high local concentration, often yield highly intense signals that can be falsely detected outside their specific wavelength.