Jiang et al., http://www.jcb.org/cgi/content/full/jcb.200810084/DC1

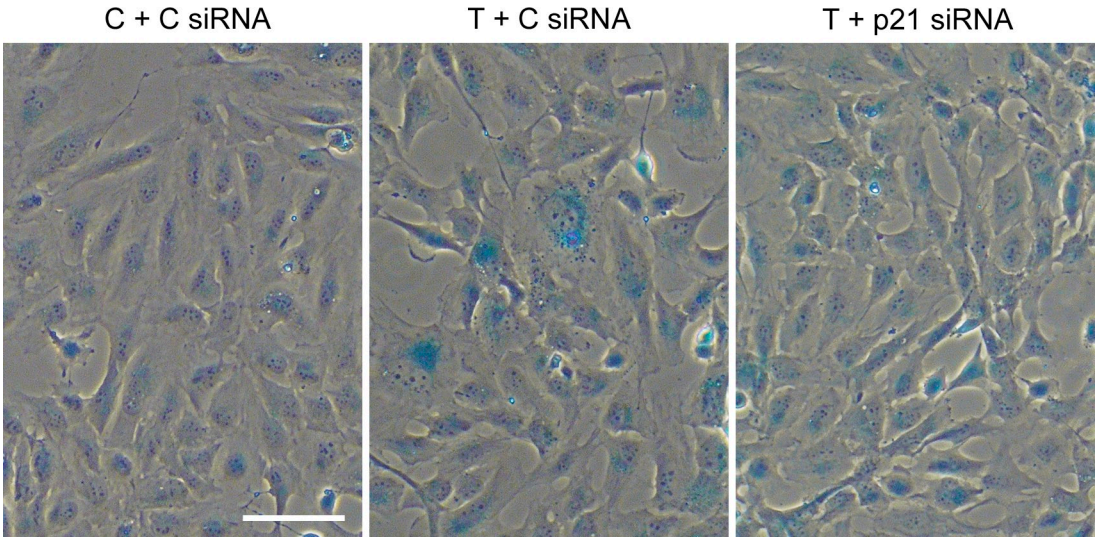

Figure S1. **Induction of a senescent phenotype in SV40-immortalized ALT cells upon treatment with SV40T siRNA.** SA-β-gal staining of IIICF-T/B3 cells treated with indicated combinations of siRNAs for 3 d. Strong SA-β-gal expression was found in cells treated with SV40T and control (T + C) siRNAs, but to a much lesser extent in cells treated with T + p21-7 or C + C siRNAs. Bar, 100 μm.

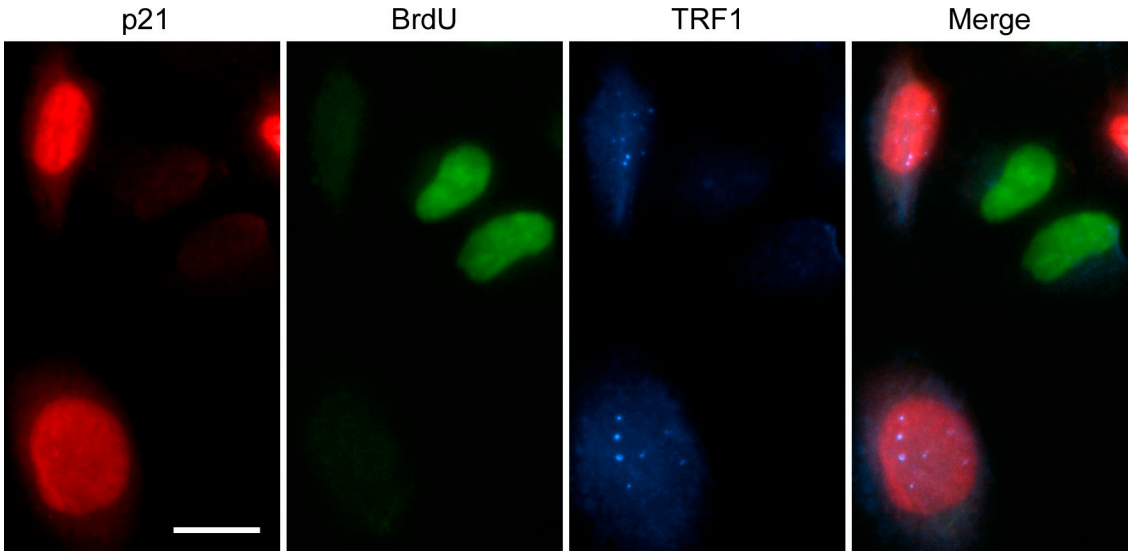

Figure S2. **Association of APB induction with p53/p21-mediated growth arrest/senescence.** Triple immunostaining of TRF1, p21, and BrdU in IIICF-T/B3 cells treated with SV40T siRNA for 4 d (BrdU was added 25 h before the end of siRNA treatment). APBs were detected mainly in cells positive for p21 and negative for BrdU. Bar, 20 μm.

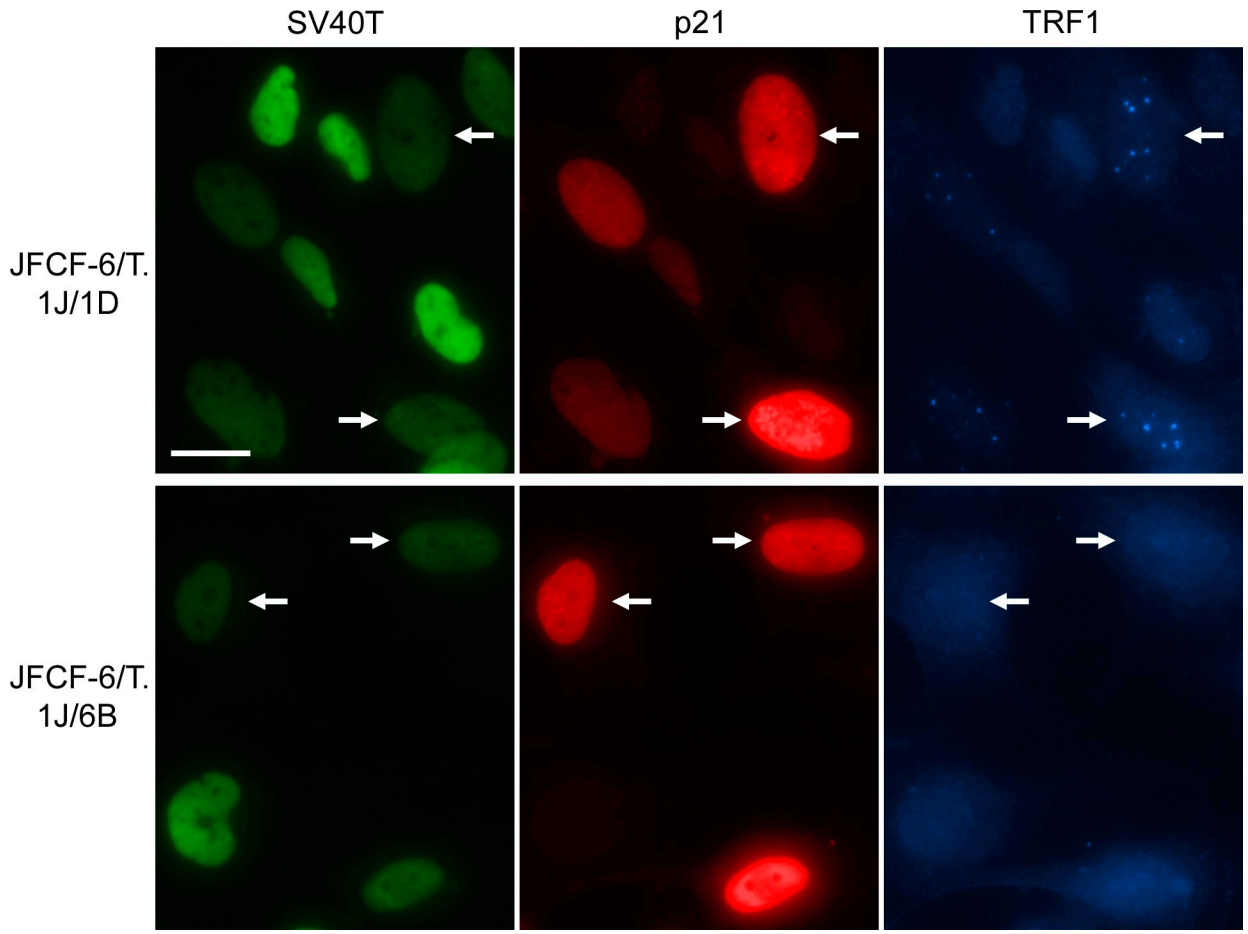

Figure S3. **No APB induction in SV40-immortalized telomerase-positive cells.** Triple immunostainings of TRF1, p21, and SV40T in JFCF-6/T.1J/1D (ALT) and JFCF-6/T.1J/6B (telomerase) cells treated with SV40T siRNA for 4 d. APBs were detected in ALT cells but not telomerase-positive cells. The arrows indicate cells depleted of SV40T. Bar, 20 µm.

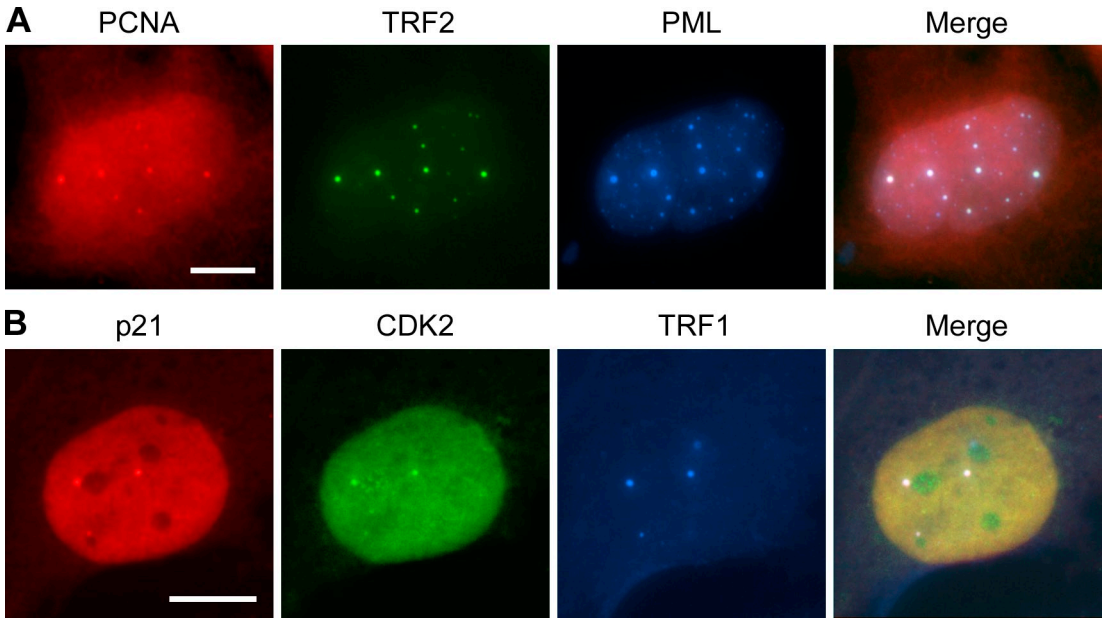

Figure S4. **Presence of p21, PCNA, and Cdk2 within APBs.** (A) Triple immunostainings of PCNA, TRF2, and PML in untreated IIICF/c cells (p53 null). PCNA foci colocalized with APBs. (B) Triple immunostainings of p21, Cdk2, and TRF1 in IIICF-T/B3 cells treated with SV40T siRNAs for 4 d. p21 and CDK2 were colocalized in APBs. Bars, 20 μm.

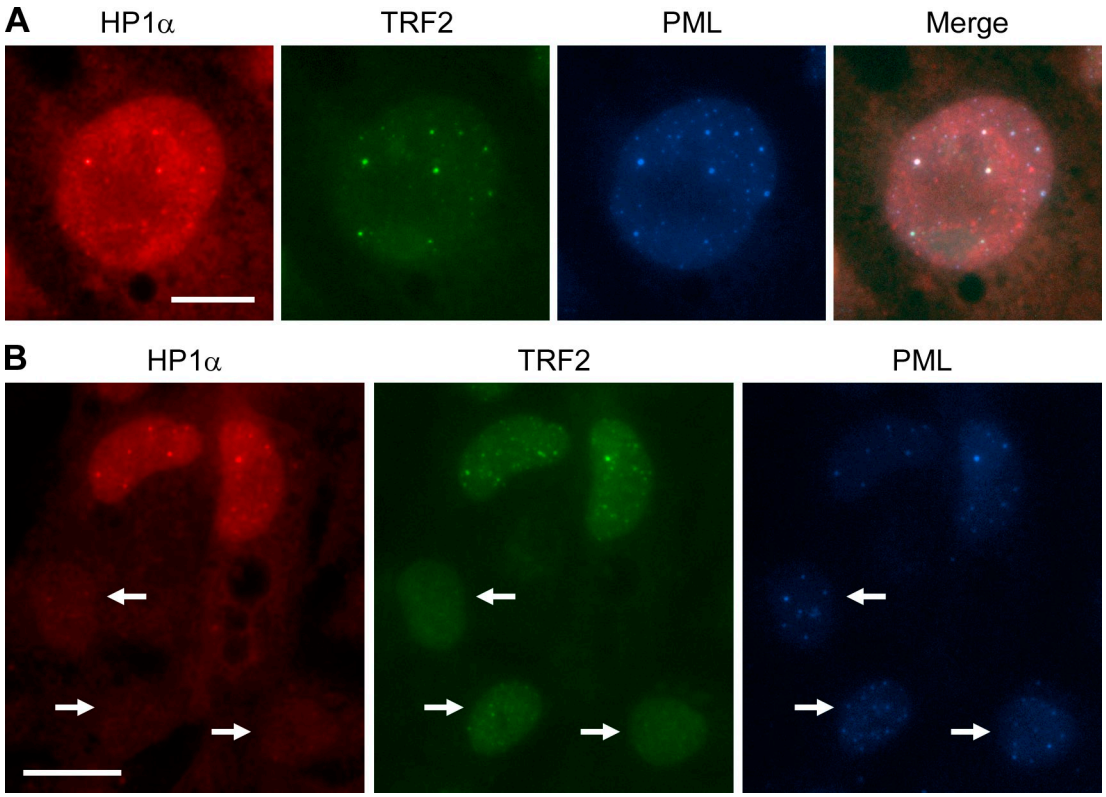

Figure S5. **Requirement of HP1 for APB formation in p53-negative IIICF/c cells.** (A) Triple immunofluorescence showed colocalization between HP1α and APBs in IIICF/c cells maintained under normal growth conditions. (B) Triple immunostainings for HP1α, TRF1, and PML in IIICF/c cells treated for 48 h with siRNAs HP1α-2 and HP1γ-6 against HP1α and HP1γ, respectively, then subjected to methionine restriction and a repeated siRNA transfection for 4 d. Simultaneous depletion of HP1α and HP1γ prevented induction of APBs in methionine-restricted IIICF/c cells. The arrows indicate cells depleted of HP1α. Bars, 20 μm.

Table S1. **Proportion of APB-positive cells after methionine starvation**

Cell lines	Treatment[a]	APB+/total (%)
IIICF-T/B3	Control	12/159 (7.5)
IIICF-T/B3	Met−	86/165 (52.1)
IIICF-402DE/D2	Control	8/162 (5.3)
IIICF-402DE/D2	Met−	81/167 (48.5)

[a]Cells were maintained in methionine-deficient medium (Met−) for 4 d before being fixed for immunostaining.

Table S2. **Proportion of APB+ cells containing p21+ APBs after induction of p53**

Cell lines (treatment[a])	Total APB + cells counted	Cells with p21 + APBs (%)	Antibodies used for p21 detection
IIICF-T/B3 (SV40T-siRNA)	322	95 (29.5)	Goat anti-p21
IIICF-T/B3 (SV40T-siRNA)	318	110 (34.6)	mAb anti-p21
C7 (4OHT)	253	67 (26.5)	Goat anti-p21
C7 (4OHT)	256	61 (23.8)	mAb anti-p21

[a]Cells were treated with 1 μM 4OHT or 10 nM SV40T siRNA for 4 d.

Table S3. **Proportion of APB-positive IIICF/c cells after siRNA treatment and methionine restriction**

siRNA treatment[a]	Total[b]	APB+ (%)
Control (20 nM)	202	117 (57.9)
Sp100-1 (20 nM)	204	130 (63.7)
HP1α-2 (20 nM)	207	68 (32.9)
HP1α-2 + HP1γ-6 (10 nM + 10 nM)	211	39 (18.5)

[a]Cells were treated with siRNAs for 2 d, then subjected to methionine restriction and a repeated siRNA transfection for 4 d.
[b]Only cells that were negative by immunostaining for the knocked-down protein were examined for APBs, with the exception of control siRNA.