Supplementary Information

Rapid induction of Alternative Lengthening of Telomeres by depletion of the histone chaperone ASF1

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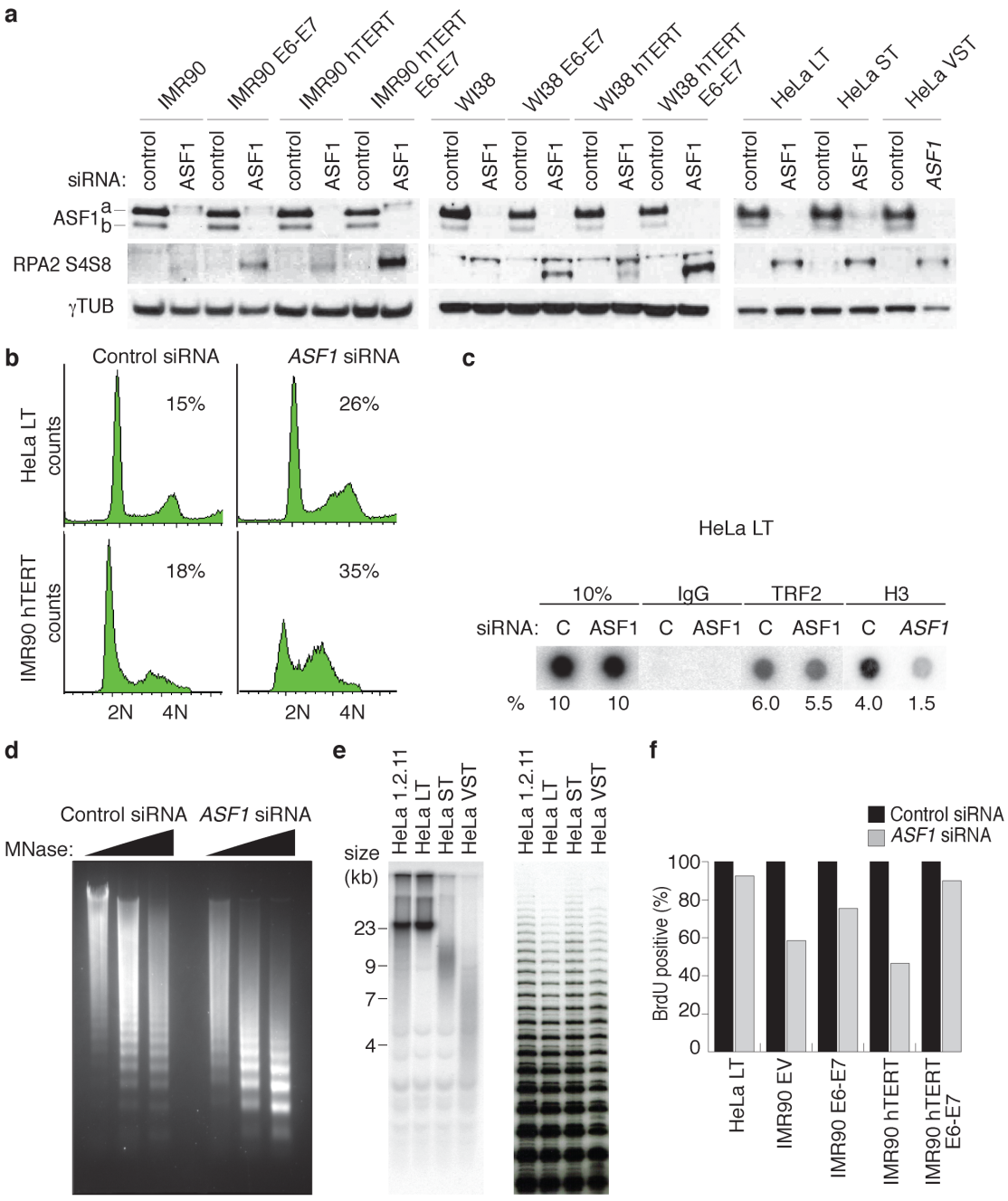
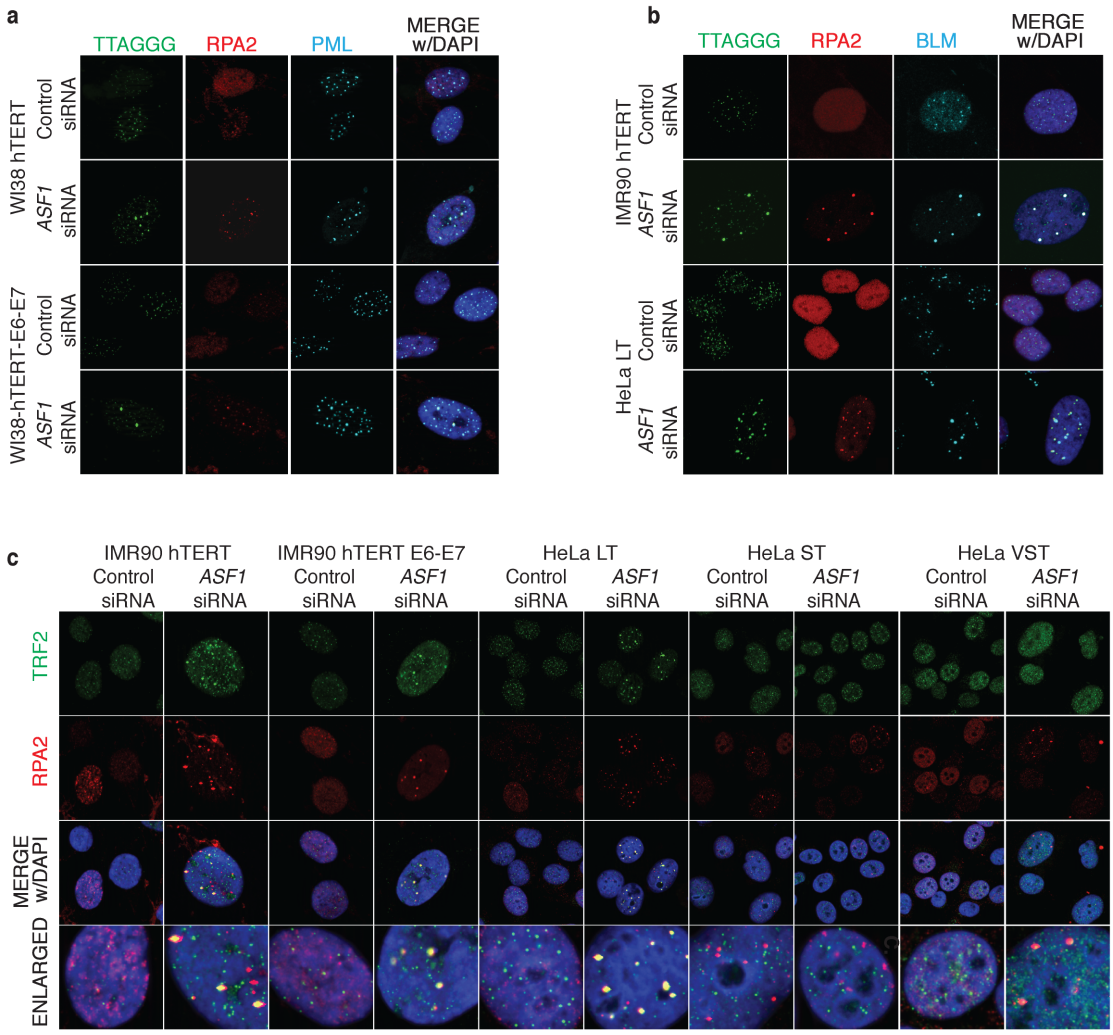
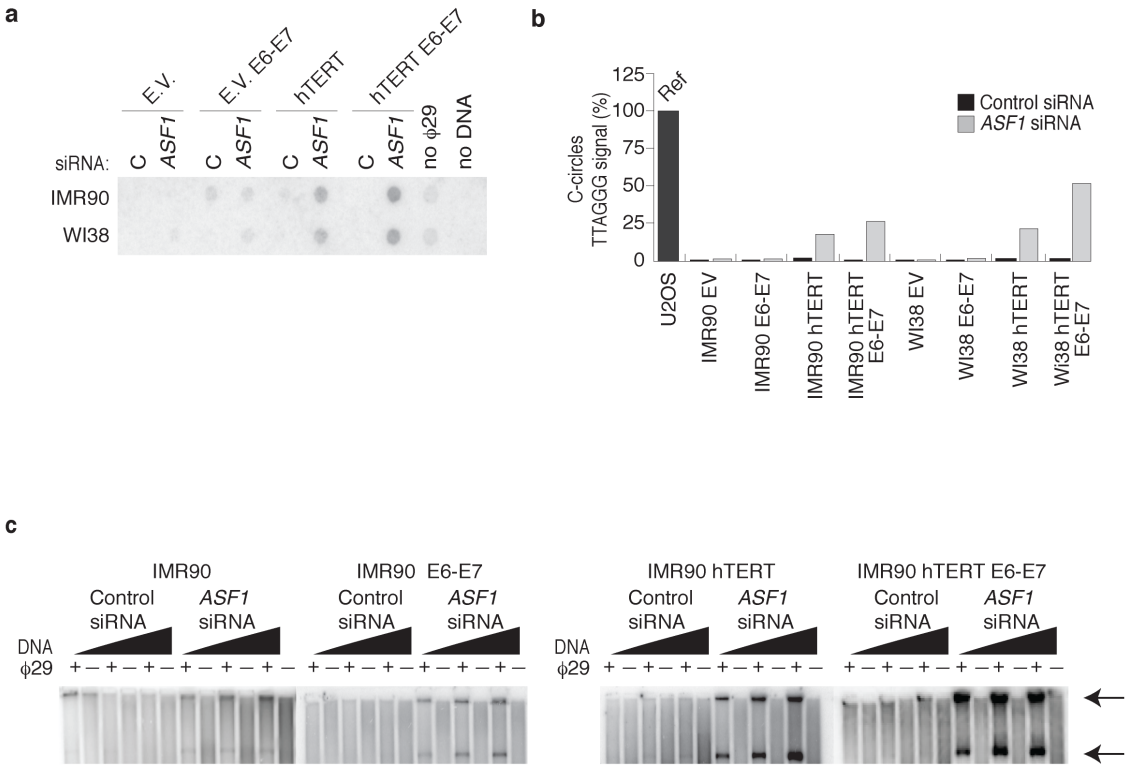


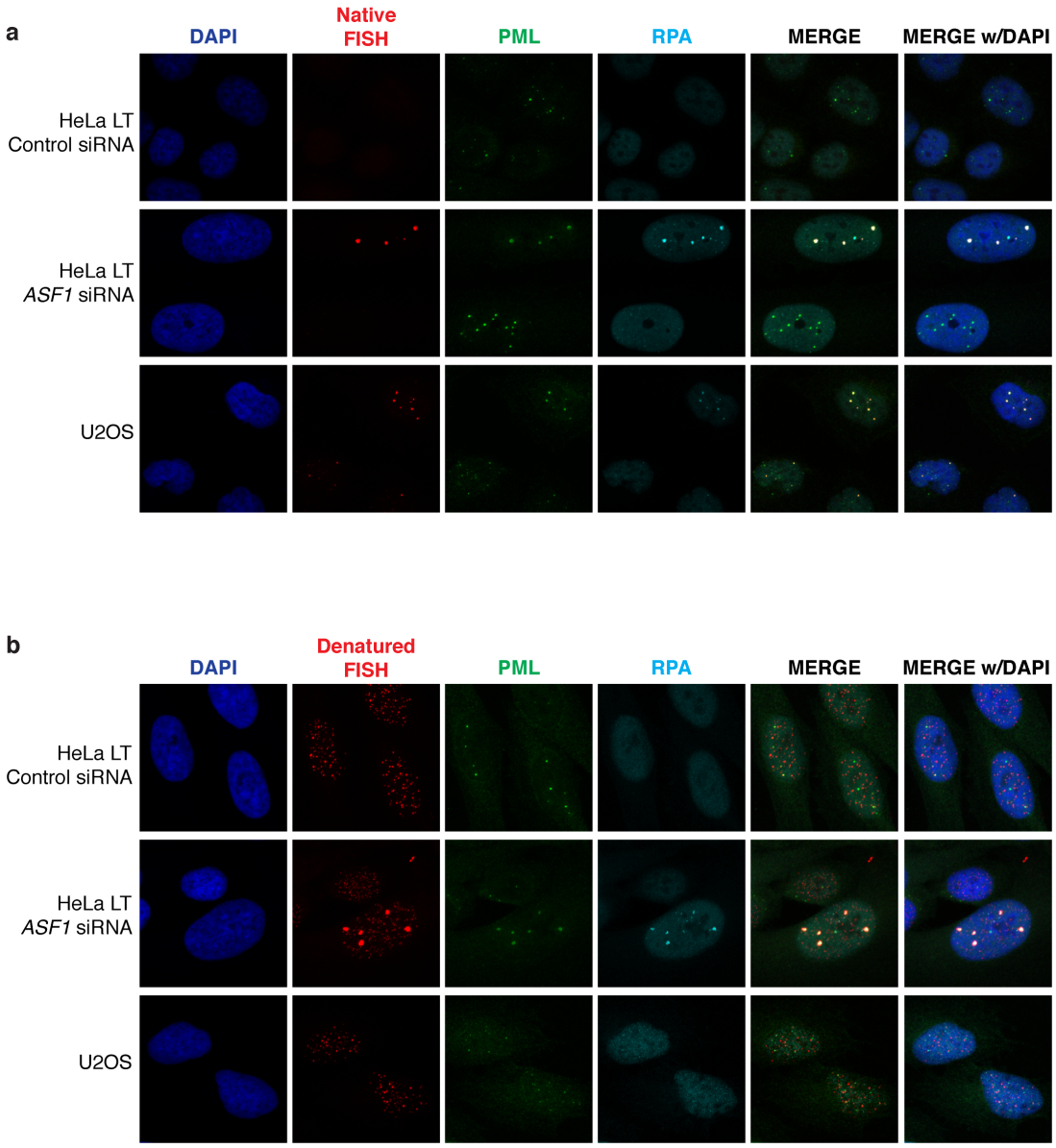
Supplementary Figure 1. Effects of ASF1 suppression. (**a**) Western blot analysis of ASF1 expression and RPA2 S4/S8 phosphorylation following transfection with control and ASF1 siRNAs in IMR90, WI38 and HeLa cells. γTubulin was used as loading control. (**b**) FACS analysis of control and ASF1 transfected Hela LT and IMR90-hTERT cells. Average percentage of cells in S-phase are indicated from 3 independent experiments. (**c**) Chromatin Immuno-precipitation (ChIP) of TRF2 and histone H3 at telomeres in control (c) and ASF1 (ASF1) siRNA treated HeLa cells. Numbers displayed beneath the panels are % telomeric DNA precipitated relative to the IgG control IP. (**d**) Micrococcal Nucleasae (MNase) digestion of chromatin from control siRNA and ASF1 siRNA treated HeLa LT cells. Increasing amounts of MNase (2, 4 and 8 units) were used in 10 min digestions. 5μg of purified digested DNA was electrophoresed on a 1.2% TAE gel. (**e**) Telomere length and telomerase activity of HeLa 1.2.11, Hela LT, ST and VST cells. Telomerase activity was determined by TRAP assay using 1μg of input soluble protein extract. (**f**) Quantification of FACS analysis of BrdU incorporation in control and ASF1 transfected Hela LT and IMR90 cells. Percentages of BrdU positive cells are shown and derived from 3 independent experiments.



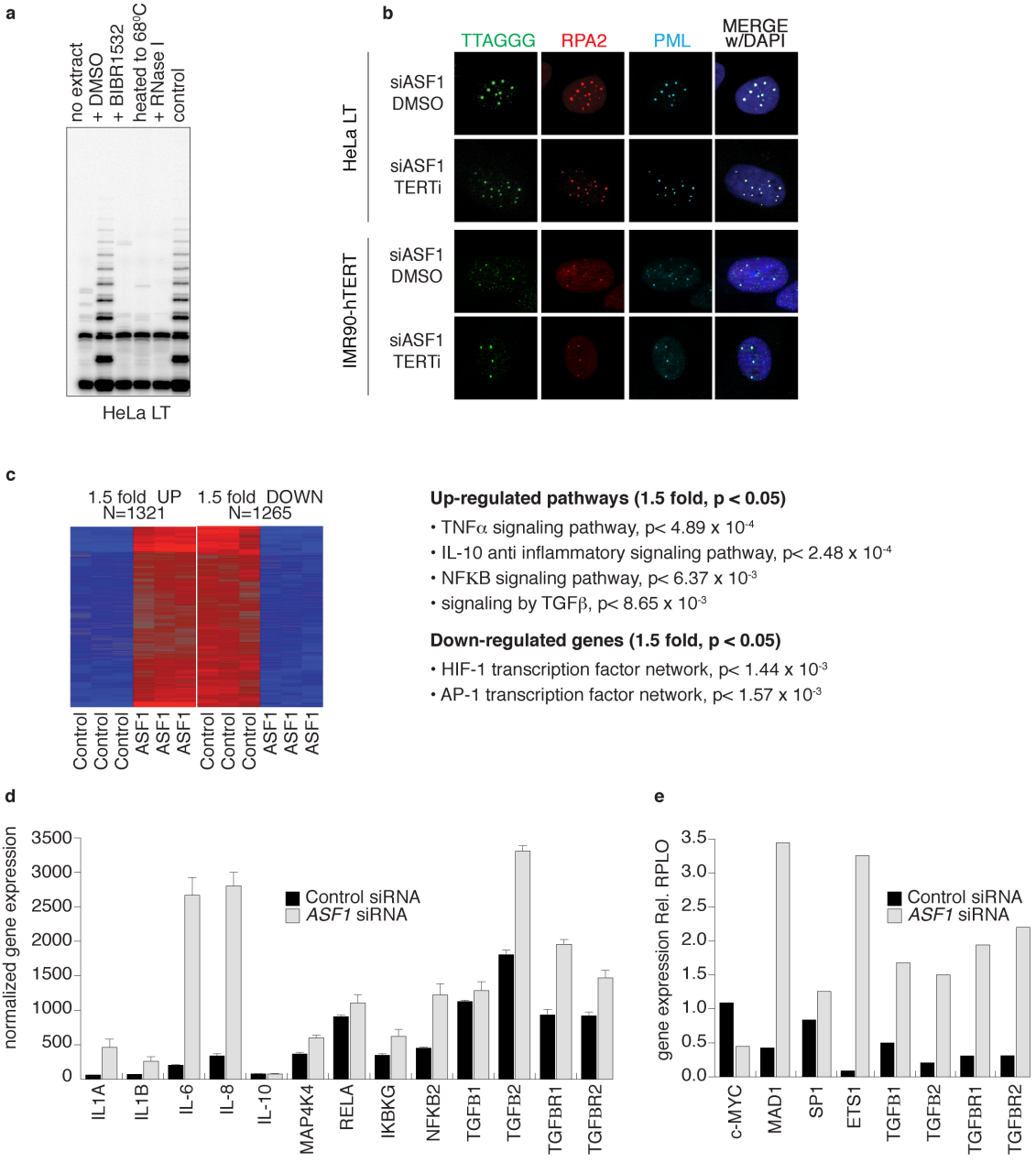
Supplementary Figure 2. ASF1 suppression causes APB formation. (**a**) Confocal IF-FISH imaging of RPA2 (Red), PML (Cyan), TTAGGG-FISH (Green) co-localization in control siRNA and ASF1 siRNA treated WI38-hTERT and WI38-hTERT-E6-E7. Images are maximum intensity projections of ~10 stacks captured with a 63X objective lens. All panels include the merged channels with DAPI. (**b**) Confocal IF-FISH imaging of RPA2 (Red), BLM (Cyan), TTAGGG-FISH (Green) co-localization in control siRNA and ASF1 siRNA treated IMR90-hTERT and HeLa LT. Images are maximum intensity projections of ~10 stacks captured with a 63X objective lens. All panels include the merged channels with DAPI and enlarged sections of the merge with DAPI. (**c**) Confocal-IF imaging of TRF2 (Green) and RPA2 (Red) co-localization in control siRNA and ASF1 siRNA treated IMR90-hTERT, IMR90-hTERT-E6-E7, HeLa LT, ST and VST cells. As in Fig 1A, TRF2/RPA2 co-localization in large foci in IMR90 hTERT, IMR90-hTERT-E6-E7 occurs in ~5% of the cellular population. Otherwise, images of all control siRNA treated cells and ASF1 depleted HeLa ST represent the general cell population.



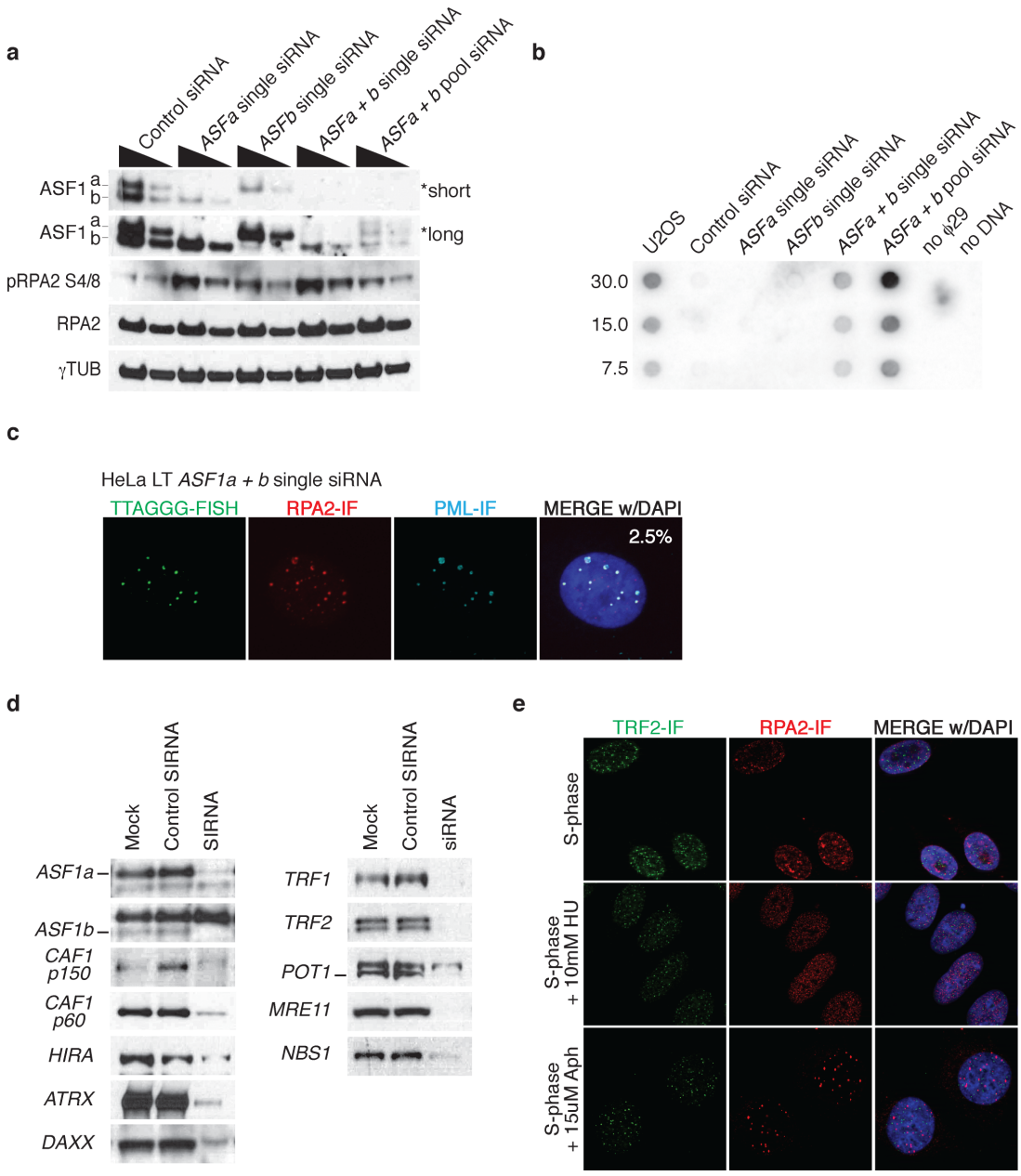
Supplementary Figure 3. ASF1 suppression leads to ECTR generation. (**a**) C-circle assay in control and ASF1 depleted human primary IMR90/WI38-Empty Vector (EV), IMR90/WI38-E6-E7, IMR90/WI38-hTERT and IMR90/WI38-hTERT-E6-E7 cells. Note that the transformed IMR90 cells displayed here were derived from independent retroviral infections and differ from those shown in Fig. 2. Negative controls are reactions lacking ϕ29 and DNA. (**b**) Quantification of C-circles in control (black bar) and ASF1 depleted (light grey bar) cells shown in (a). The ALT positive U2OS control was used as reference for C-circles (dark grey bar), against which IMR90 and WI38 data was calculated. Data represent means ±SDs of at least 3 experiments. (**c**) T-circle assay in following ASF1 depletion. Increasing amounts (0.25, 0.5 and 1 μg) of digested genomic DNA from control and ASF1 depleted human primary IMR90, IMR90 E6-E7, IMR90-hTERT and IMR90-hTERT E6-E7cells. Arrows indicate T-circle products. Negative controls are reactions lacking ϕ29.



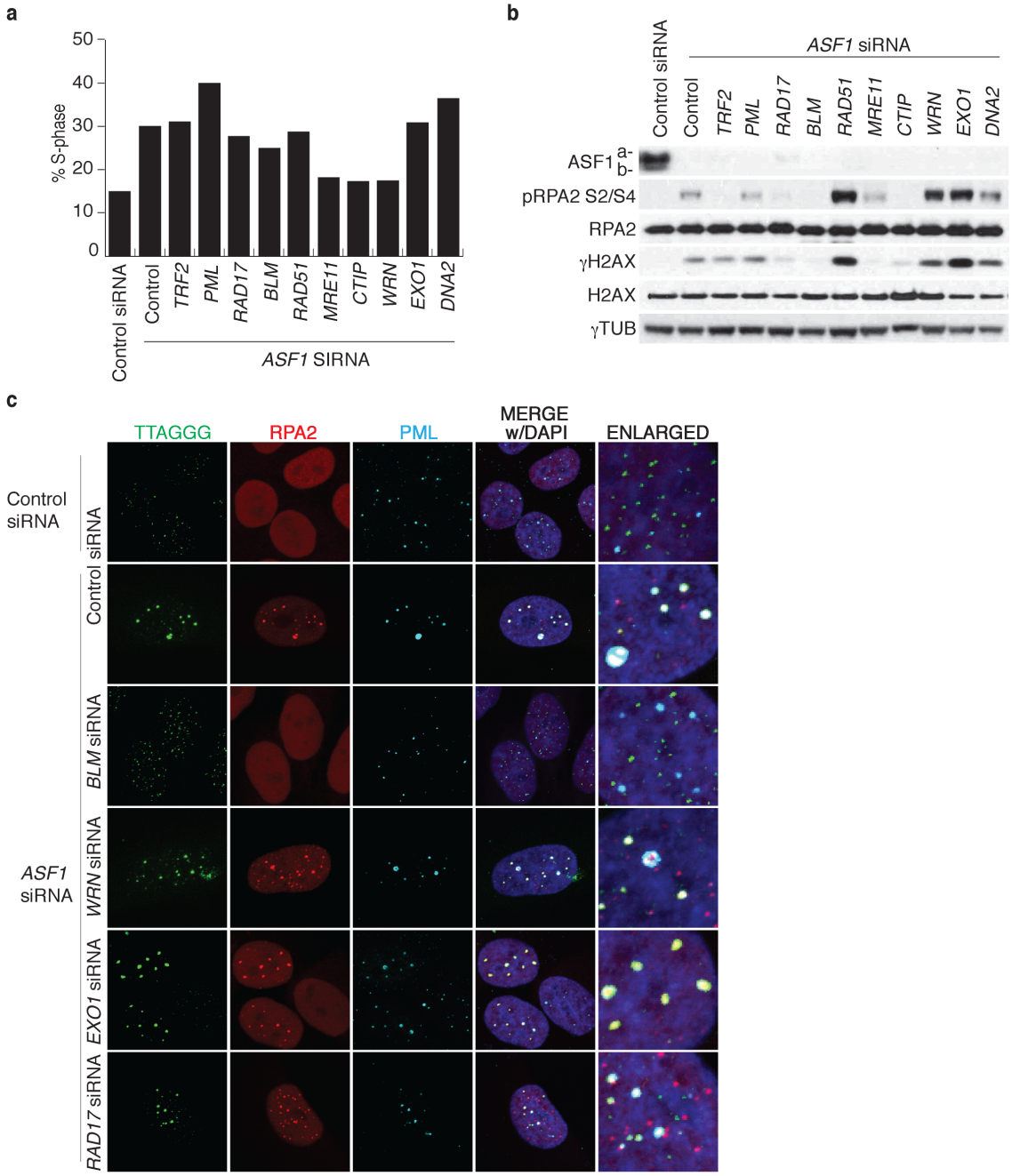
Supplementary Figure 4. ASF1 suppression leads to C-circle formation. (**a**) Confocal IF-FISH imaging of RPA2 (Cyan), PML (green), ssTTAGGG-FISH (Red) co-localization in control siRNA and ASF1 siRNA treated HeLa LT. Images are maximum intensity projections of ~10 stacks captured with a 63X objective lens. All panels include the merged channels with and without DAPI. (**b**) As in (a) with the exception that the coverslips were denatured so as to allow detection of dsTTAGGG repeats.



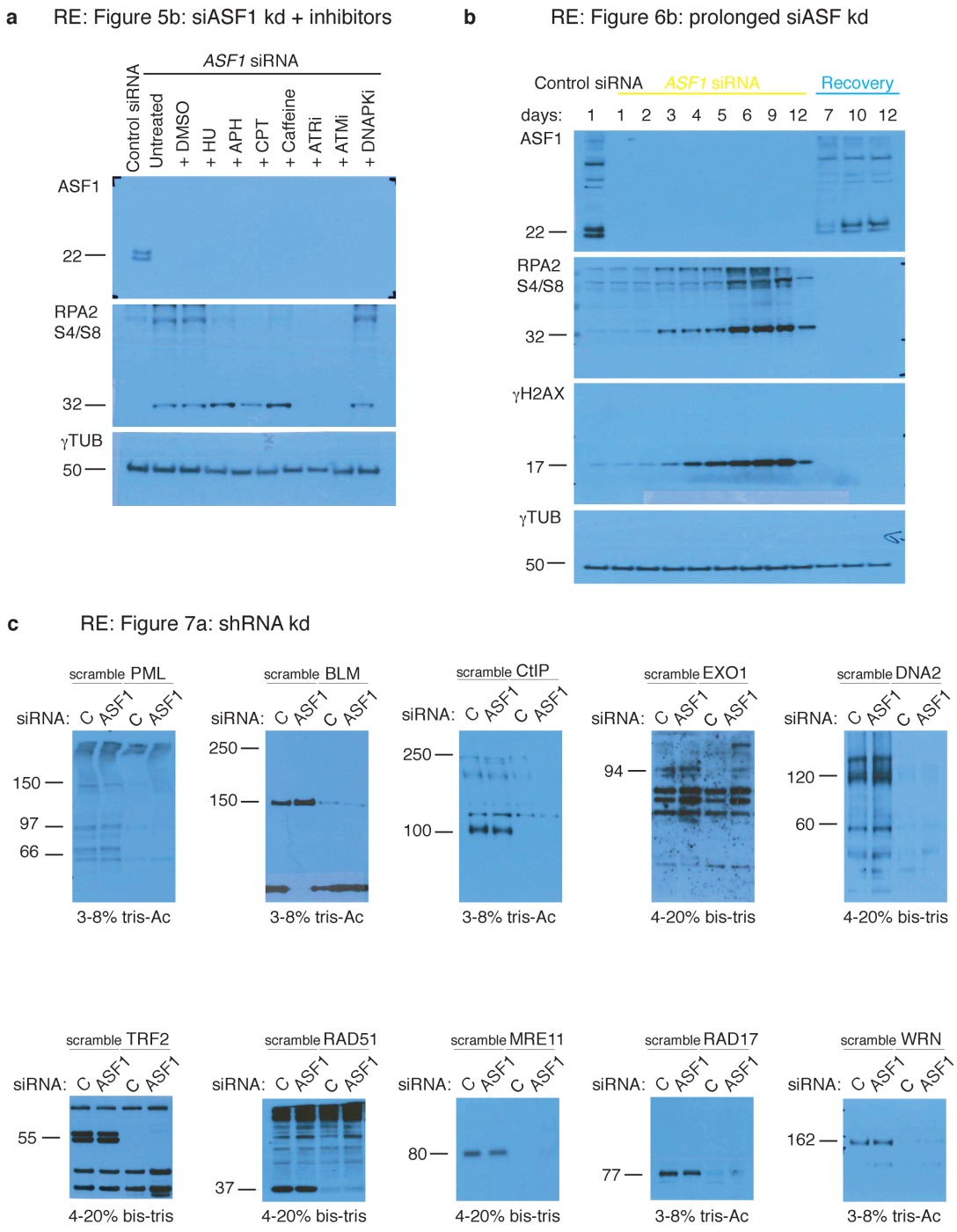
Supplementary Figure 5. ASF1 suppression inhibits telomerase activity. (**a**) Telomerase activity was determined by TRAP assay using 1μg of input soluble protein extract. Lanes i-vi show: (i) No extract control (ii) DMSO added to extract (iii) 10μM BIBR-1532 (TERTi) added to extract (iv) heat denatured control (v) RNase I treated extract (vi) positive control (mock treated HeLa LT extract). (**b**) Confocal IF-FISH imaging of RPA2 (Red), PML (Cyan), TTAGGG-FISH (Green) co-localization in ASF1 siRNA transfected HeLa LT and IMR90 hTERT cells treated with 10μM BIBR-1532 (TERTi). Images are maximum intensity projections of ~10 stacks captured with a 63X objective lens. All panels include the merged channels with DAPI. (**c**) Microarray heatmap of differentially expressed genes in ASF1 depleted HeLa LT. Red indicates up-regulated genes and blue indicates down-regulated genes. Selected enriched functional pathways are shown. (**d**) Expression of secretory cytokines (IL1A, IL1B, IL6, IL8), activators of NFKB signaling (IKBKG (*Nemo*), NFKB2 (p100)), and the TGFβ pathway. Data represent means ±SDs of 3 independent experiments. (**e**) qPCR of gene expression of regulators of TERT expression. Gene expression is normalized to that of the RPLO (ribosomal protein L15) gene. Data represent means of 3 independent experiments in which each PCR was conducted in triplicate.



Supplementary Figure 6. Effects of ASF1 suppression. (a) Western blot analysis of ASF1 and RPA2 expression and RPA2 S4/S8 phosphorylation following transfection with individual and mixed single siRNAs and *Smartpool* siRNAs against ASF1a and ASF1b in HeLa LT cells. Control siRNAs were also used in transfections. γTubulin was used as loading control. Black triangles above panels indicate 2X and 1X loading of whole cell extracts. **(b)** C-circle assays from siRNA transfected HeLa LT cells in (a). Negative controls are reactions lacking φ29 and DNA. Control siRNAs were also used in transfections. **(c)** Confocal-IF imaging of RPA2 (Red), PML (Cyan), TTAGGG-FISH (Green) co-localization in control siRNA and single ASF1 siRNA treated HeLa LT cells. Images are maximum intensity projections of ~10 stacks captured with a 63X objective lens. All panels include the merged channels with DAPI and enlarged sections of the merge with DAPI. **(d)** Western analysis to confirm siRNA knockdown. The target gene is indicated on the left, adjacent to each panel. To the right of each panel, the S-phase index (%) at the time of harvest (72hrs) of the transfections is indicated. **(e)** Standard IF of TRF2 (Green) and RPA2 (Red) localization after synchronization of HeLa LT cells in S-phase and chronic treatment with hydroxyurea (HU) (3mM) and aphidicolin (APH) (5μM) over 48hrs. Images. All panels were captured using a 63X objective lens on a Zeiss Axioplan II microscope and include the merged channels with DAPI and enlarged sections of the merge with DAPI.



Supplementary Figure 7. ALT Pathway analysis. (a) Quantitation of S-phase index of shRNA siC/ASF1 siRNA transfected cells. Data represents the average total S-phase population in cell cultures at 72hrs post siRNA transfection. **(b)** Western analysis of ASF1, phospho-RPA2 S4/S8, RPA2, γH2AX and H2AX. shRNA target is indicated above panels. γTUB is general loading control. **(c)** Confocal IF for RPA2 (red), PML (cyan) and TTAGGG FISH (green) in shRNA infected HeLa LT cells. From top to bottom the images are from shScramble and control siRNA, shScramble and ASF1 siRNA, BLM shRNA and ASF1 siRNA, WRN shRNA and ASF1 siRNA, EXO1 shRNA and ASF1 siRNA and RAD17 shRNA and ASF1 siRNA HeLa LT cells. Images shown are representative of those cells containing APB like structures that are observed as indicated in Fig. 4. For BLM shRNA and ASF1 siRNA where few APBs are observed the images shown are representative of the cell population. Note the absence of focal RPA2 formation in the absence of BLM. Images in are maximum intensity projections of ~10 stacks captured with a 63X objective lens. All panels include the merged channels with DAPI and enlarged sections of the merge with DAPI.



Supplementary Figure 8. Original blots of westerns in main figures. (**a**) Complete blots of westerns for ASF1, RPA2 S4S8 and γTubulin shown in Figure 5b. The molecular weight of the target protein is indicated. (**b**) Complete blots of westerns for ASF1, RPA2 S4S8, γH2AX and γTubulin shown in Figure 6b. The molecular weight of the target protein is indicated. (**c**) Complete blots of westerns confirming shRNA knockdown of factors shown in shown in Figure 7a. Top panel; left to right; PML, BLM, CtIP, EXO1, DNA2 and bottom panel; left to right; TRF2, RAD51, MRE11, RAD17 and WRN. The molecular weight of the target protein is indicated. The type and percent gradient of acrylamide in each gel is indicated beneath the image.