

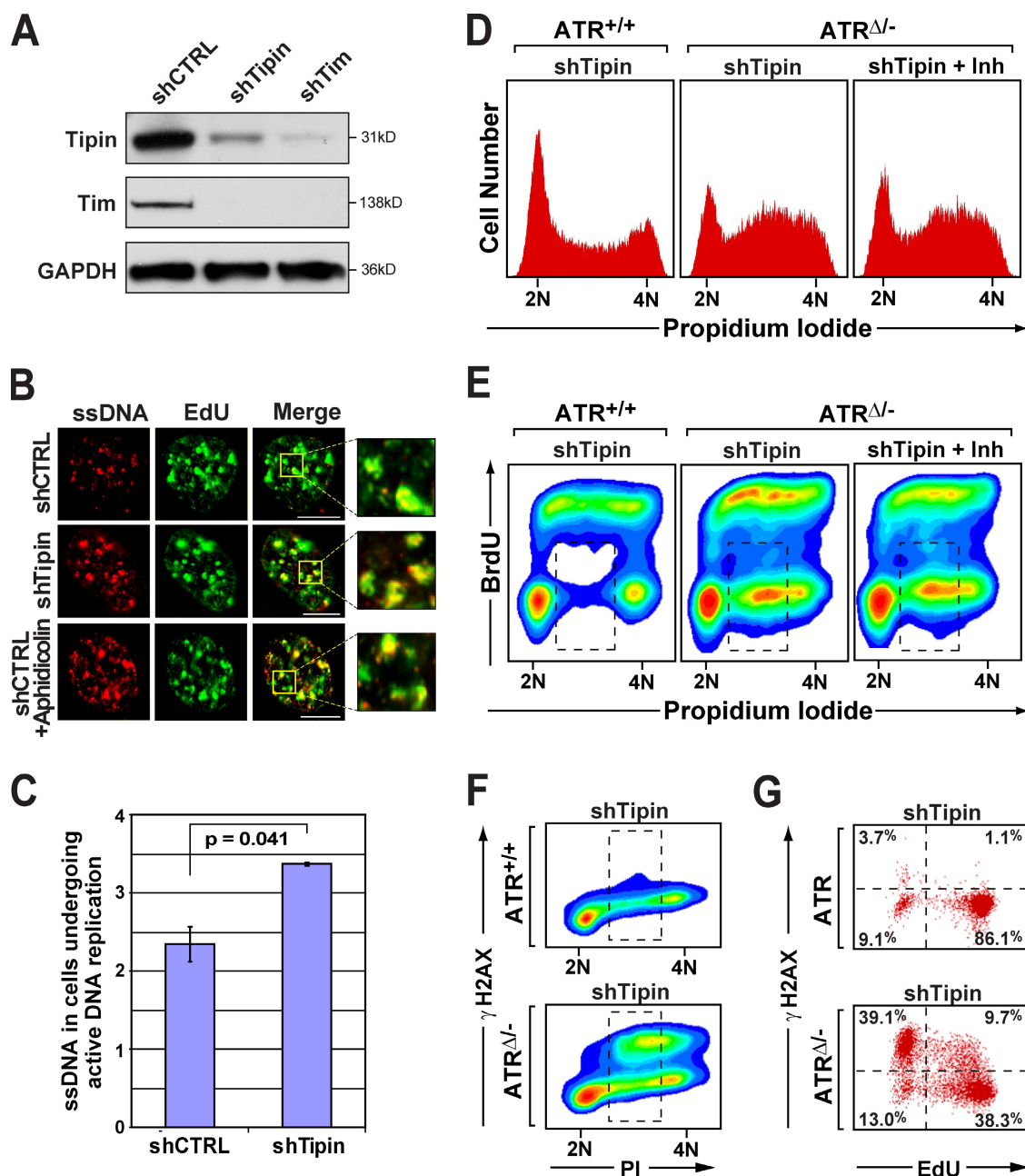
Smith et al., <http://www.jcb.org/cgi/content/full/jcb.200905006/DC1>

Figure S1. **Tipin reduction causes increased ssDNA generation and an increased reliance on ATR.** (A) Tim and Tipin reduction after shRNA-mediated suppression. Tim and Tipin were detected by Western blotting lysates from NIH3T3 cells 72 h after infection with shRNA-expressing lentiviruses. (B) ssDNA detection at sites of active DNA replication after Tipin reduction, performed as described in Fig. 1. Insets show higher magnification of IdU–EdU overlapping regions (yellow). (C) Quantification of ssDNA levels represented in B. Error bars represent SEM. (D and E) Flow cytometric detection of DNA content and BrdU incorporation in ATR/Tipin-deficient and control cells performed as described in Fig. 2. (E) Dashed boxes indicate cells with S-phase DNA content that did not incorporate BrdU. (F and G) Quantification of  $\gamma$ -H2AX and active DNA synthesis in ATR/Tipin-deficient cells by flow cytometry. Experiments were performed as described in Fig. 4. Cells with S-phase DNA content (dashed boxes; F) were analyzed by  $\gamma$ -H2AX staining and EdU incorporation and quantified by dot plot. Inset numbers represent quadrant means. CTRL, control. Bars, 10  $\mu$ m.

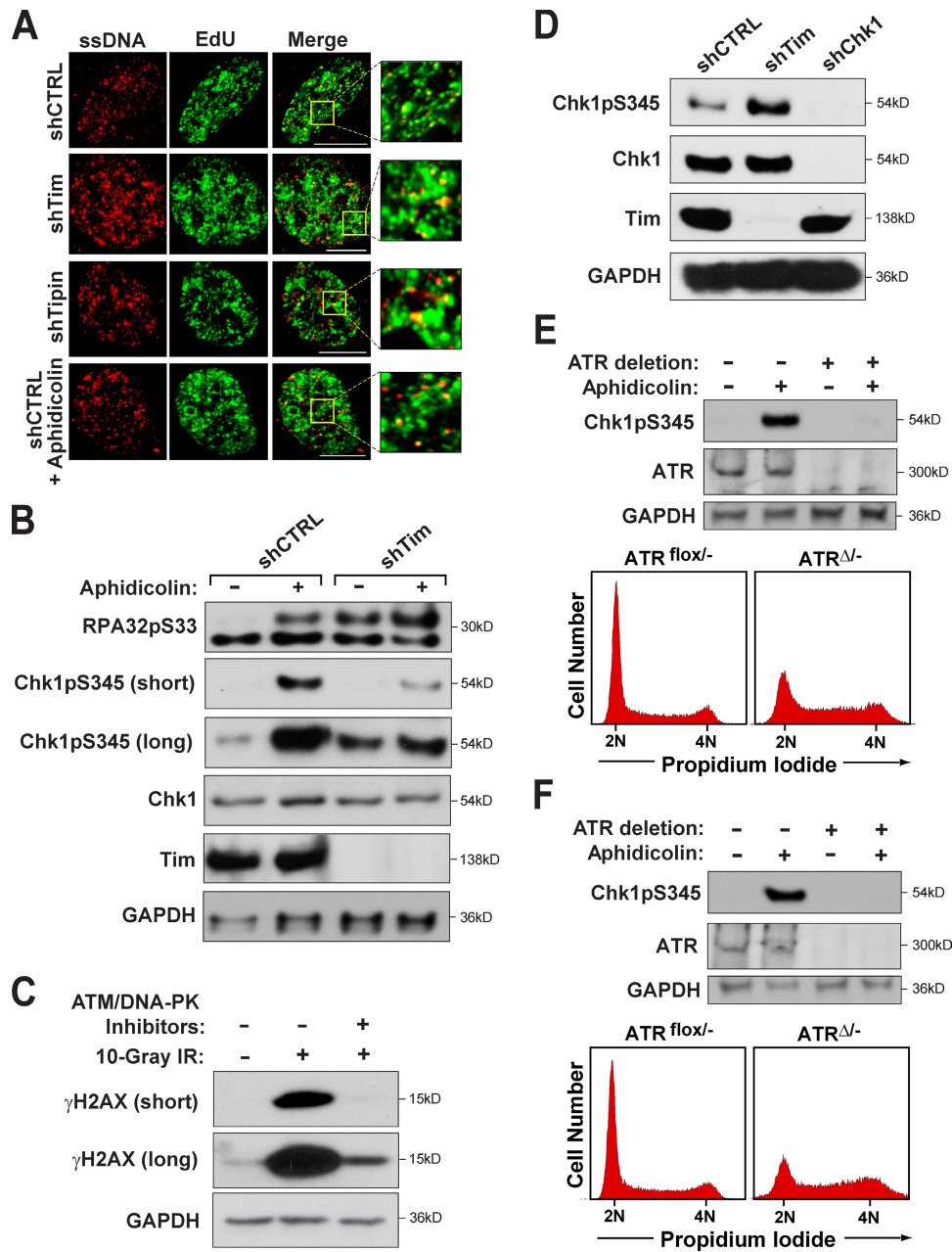


Figure S2. **Additional control experiments.** (A) Tim reduction also increased ssDNA in cells exhibiting more numerous replication foci, performed as described in Fig. 1. Insets show higher magnification of IdU–EdU overlapping regions (yellow). (B) Tim–Tipin facilitates ATR-mediated Chk1 phosphorylation but not RPA phosphorylation. Phospho-S345 Chk1 (Chk1pS345) and phospho-S33 RPA32 (RPA32pS33) were detected by Western blotting of lysates from NIH3T3 cells expressing control (CTRL) or Tim shRNA. Synchronized cells enriched and controlled for S phase (60–70% S-phase DNA content) were treated with 5  $\mu$ M aphidicolin for 1 h to activate ATR before collection. Chk1 and GAPDH were detected as loading controls. (C) ATM/DNA-PK inhibitors prevent H2AX phosphorylation in response to IR. Cells treated or left untreated with ATM and DNA-PK inhibitors 60 min before 10 Gy IR were harvested and detected for phospho-S139 H2AX ( $\gamma$ -H2AX) by Western blotting. Long and short exposures are shown. (D) shRNA-mediated Chk1 depletion produces undetectable levels of Chk1 or phospho-Chk1 during unperturbed S phase. Phospho-S345 Chk1, Chk1, and Tim were detected by Western blotting 48 h after infecting ATR<sup>+/+</sup> cells with the indicated shRNA-expressing lentiviruses. (E and F) The levels of ATR protein depletion and functional deficiency achieved 48 (E) and 72 h (F) after Cre-mediated ATR deletion. Lysates from control and ATR-deleted cells were detected for ATR by Western blotting. To assess ATR functional deficiency, cells were treated with 5  $\mu$ M aphidicolin for 1 h before collection, and phospho-S345 Chk1 was detected. Chk1 and GAPDH are shown as loading controls. Bars, 10  $\mu$ m.