Wan et al., http://www.jcb.org/cgi/content/full/jcb.201409073/DC1

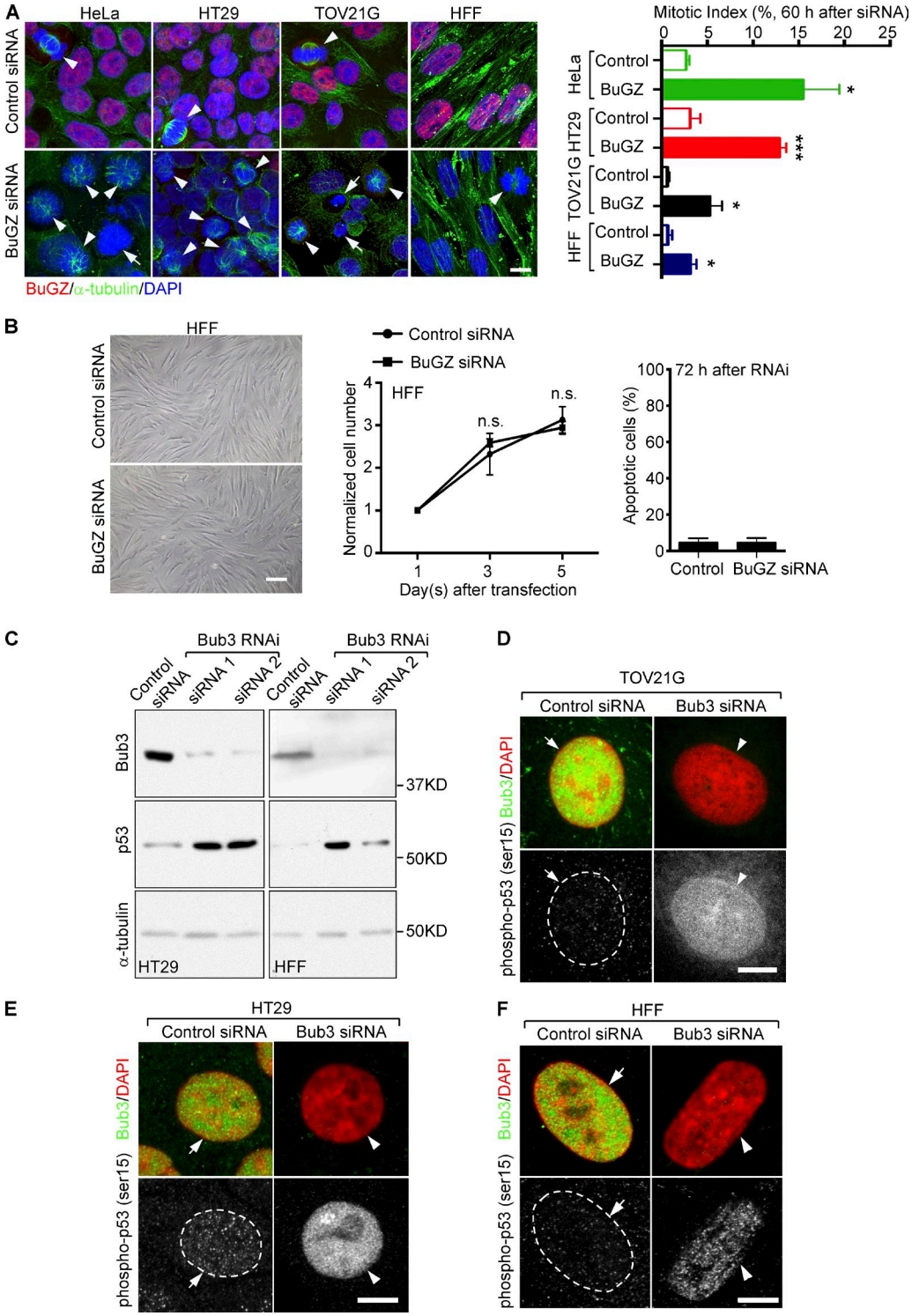

Figure S1. **Analyses of the effect of BuGZ and Bub3 depletion in interphase and mitosis.** (A and B) The effect of BuGZ reduction by siRNA treatment on mitotic index and cell proliferation. (A) Mitotic cells (arrowheads, judged by condensed chromosomes and mitotic spindles) and dead cells (arrows, judged by existence of pyknotic nuclei) in cell populations treated by control or BuGZ siRNA for 60 h. A quantification of mitotic index is shown to the right. Bar, 10 µm. Error bars indicate SEM. Student's *t* test: *, $P < 0.05$; ***, $P < 0.001$ from three independent experiments. (B) Depletion of BuGZ in HFFs did not affect cell proliferation or cause apoptosis within the first 5 d after siRNA treatment. Representative images of cells (left), quantifications of cell numbers (middle; Student's *t* test: n.s., not significant), and apoptosis (right) are shown. Bar (left), 100 µm. Error bars indicate SD from three independent experiments. (C–F) Bub3 depletion leads to p53 activation. (C) Depletion of Bub3 by two different siRNAs in HT29 cells and HFFs resulted in p53 accumulation. The cells were assayed 48 h after transfection. (D–F) Depletion of Bub3 resulted in increased p53 phosphorylation on serine 15 in TOV21G cells (D), HT29 cells (E), and HFFs (F). Bars, 10 µm. Bub3-positive cells (arrows) have lower phospho-p53 (broken circles) than cells depleted of Bub3 (arrowheads).

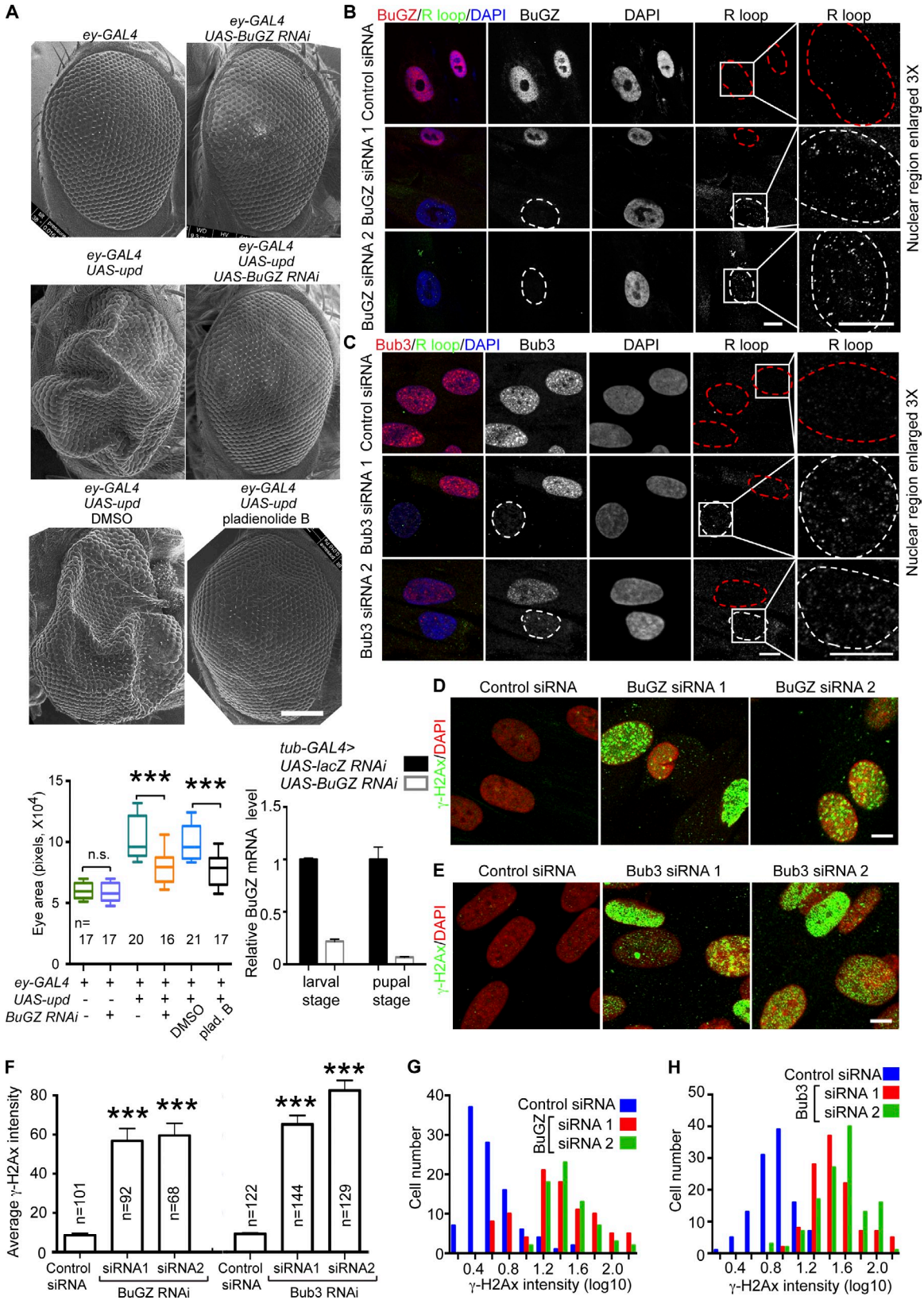

Figure S2. **The effects of BuGZ or Bub3 depletion or pladienolide B treatment in vivo and in vitro.** (A) BuGZ RNAi or pladienolide B treatment significantly repressed the hyperproliferation-induced rough eye phenotype in *Drosophila*. The eye-specific expression of *upd* (ligand of the *Jak–Stat* pathway) and/or BuGZ shRNA was achieved through *ey*-driven Gal4 expression. The hyperplasia of *Drosophila* eyes due to *upd* expression (as shown in the scanning electron micrographs) was measured by the pixels in the eye area. BuGZ RNAi efficiency was measured using flies ubiquitously expressing BuGZ shRNA or LacZ shRNA, achieved by *tub*-driven Gal4 expression. Fly eyes (*n*) analyzed were from three independent experiments. Bar, 100 μm. Student's *t* test: ***, $P < 0.001$; n.s., not significant. (B and C) Depletion of BuGZ (B) or Bub3 (C) in HFFs resulted in increased R-loop formation as detected by the S9.6 antibody. The indicated nuclei (white squares) were enlarged by 3× to show R-loop staining. Red broken circles mark the cells with BuGZ or Bub3 expression, whereas white broken circles mark the BuGZ- or Bub3-depleted nuclei. Bars, 10 μm. (D and E) Depletion of BuGZ (D) or Bub3 (E) in HFFs resulted in increased γ-H2Ax foci (green) in the nucleus (DAPI, red). Bars, 10 μm. (F–H) Quantification for γ-H2Ax intensities in individual cells (F) and distributions of the γ-H2Ax intensities in control, BuGZ-depleted, or Bub3-depleted cells (G and H). Error bars indicate SEM. Student's *t* test: ***, $P < 0.001$. Cell numbers analyzed (*n*) were from three independent experiments.

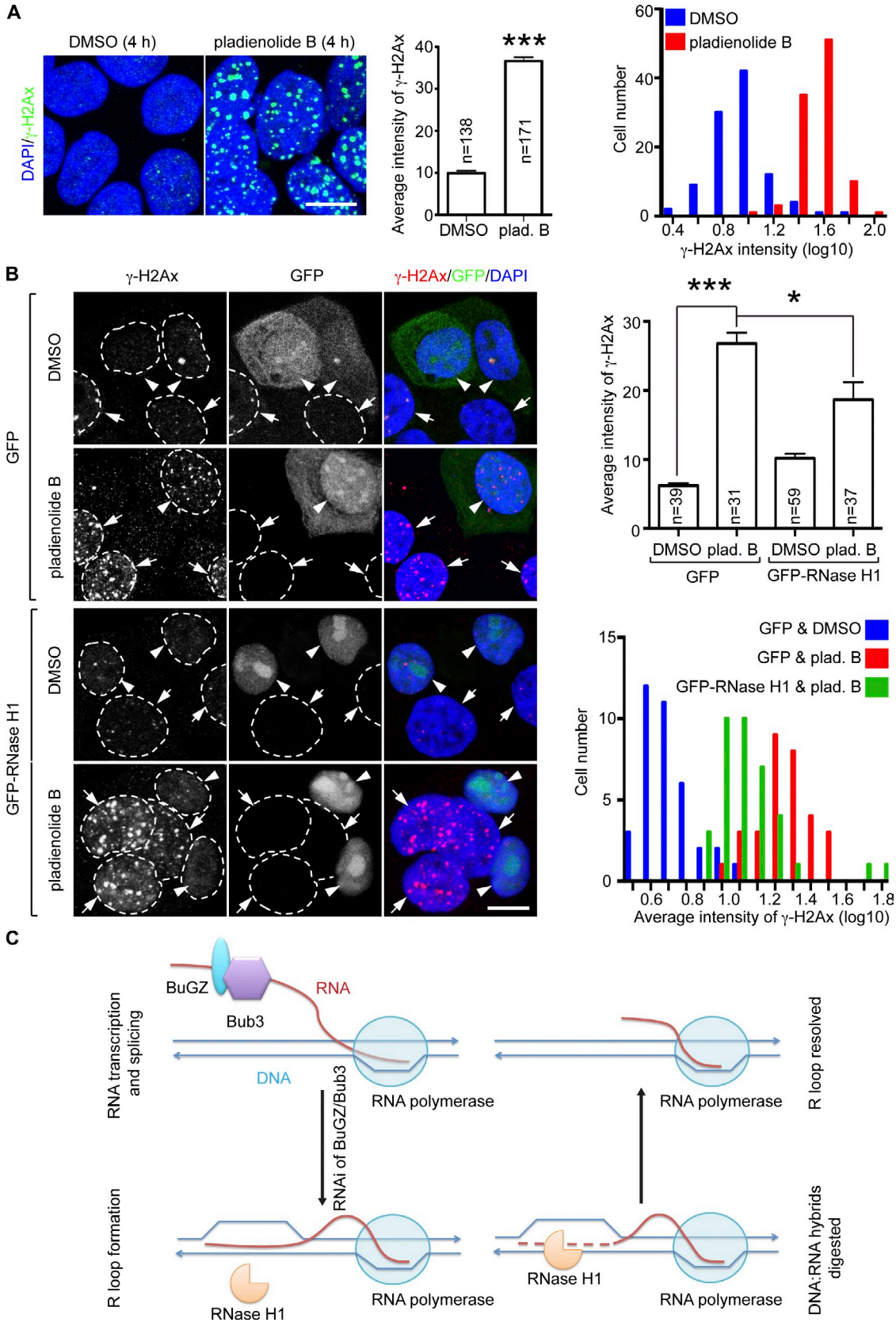

Figure S3. **DNA damage caused by splicing inhibitor pladienolide B can be rescued by forced expression of GFP-RNase H1 in HT29 cells.** (A) Pladienolide B caused increased DNA damage as judged by the increased mean γ-H2Ax intensity. The quantification of mean γ-H2Ax intensity and intensity distribution are to the right of the images. (B) Expression of GFP-RNase H1 in pladienolide B–treated cells decreased γ-H2Ax signals. Cells with or without GFP fluorescence are indicated by arrowheads or arrows, respectively. The broken circles indicate nuclear boundaries. The quantification of mean γ-H2Ax intensity and intensity distribution are shown to the right of the images. Bars, 10 μm. Error bars indicate SEM. Student's *t* test: *, $P < 0.05$; ***, $P < 0.001$. Cell numbers (*n*) analyzed were from three independent experiments. (C) A schematic model illustrating the formation of R-loops upon BuGZ or Bub3 depletion due to splicing defects and the resolution of R-loops by RNase H1. (C, left) Depletion of BuGZ or Bub3 could lead to the destabilization of the splicing machinery coating the nascent mRNAs, which in turn leads to the formation of RNA–DNA hybrids (R-loops). (C, right) degradation of the RNA (red line) in the RNA–DNA hybrids by RNase H1 would resolve R-loops and restore double-stranded DNA formation.

Table S1 gives a list of differential alternative splicing events caused by BuGZ RNAi, Bub3 RNAi, or pladienolide B treatment.

Table S2 gives the sources and sequences of mammalian expression plasmids, antibodies (with dilutions used), siRNA, shRNA, small molecules (with concentrations used), and cell lines.

Both are available as Excel files.