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Supplemental Material to:

Zheng Wang, Hao Yin, Lei Lv, Yun Huang, Xiaohua Jiang, Hanwei Jiang, Ihtisham Bukhari, Howard J Cooke, and Qinghua Shi

Unrepaired DNA damage facilitates elimination of uniparental chromosomes in interspecific hybrid cells

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Figure S1

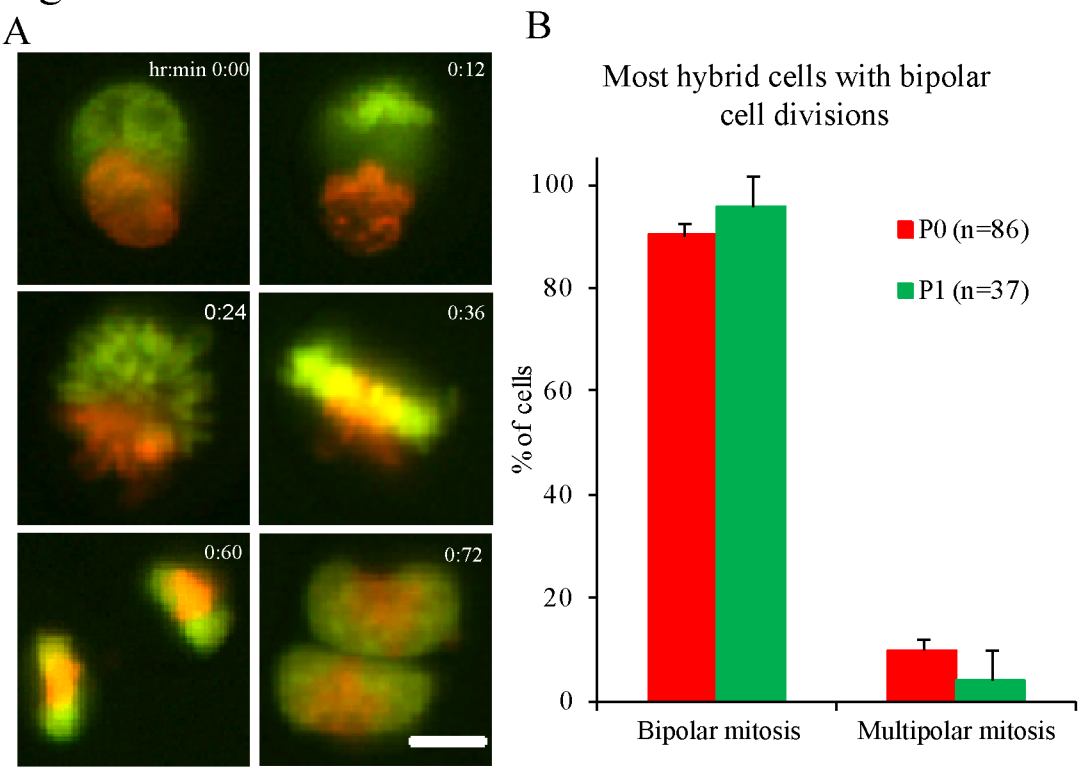

Figure S1. Most hybrid cells enter into bipolar mitosis. (A) Representative images of hybrid cells entering into bipolar mitosis detected by live cell imaging. Green, Mouse nucleus; Red, Human nucleus. Bars= 20 μm. (B) Statistical analysis on polarity of hybrid cells during first (P0) or second (P1) cell division. n= the number of cell division counted. Mean ± SD, from three independent experiments.

Figure S2

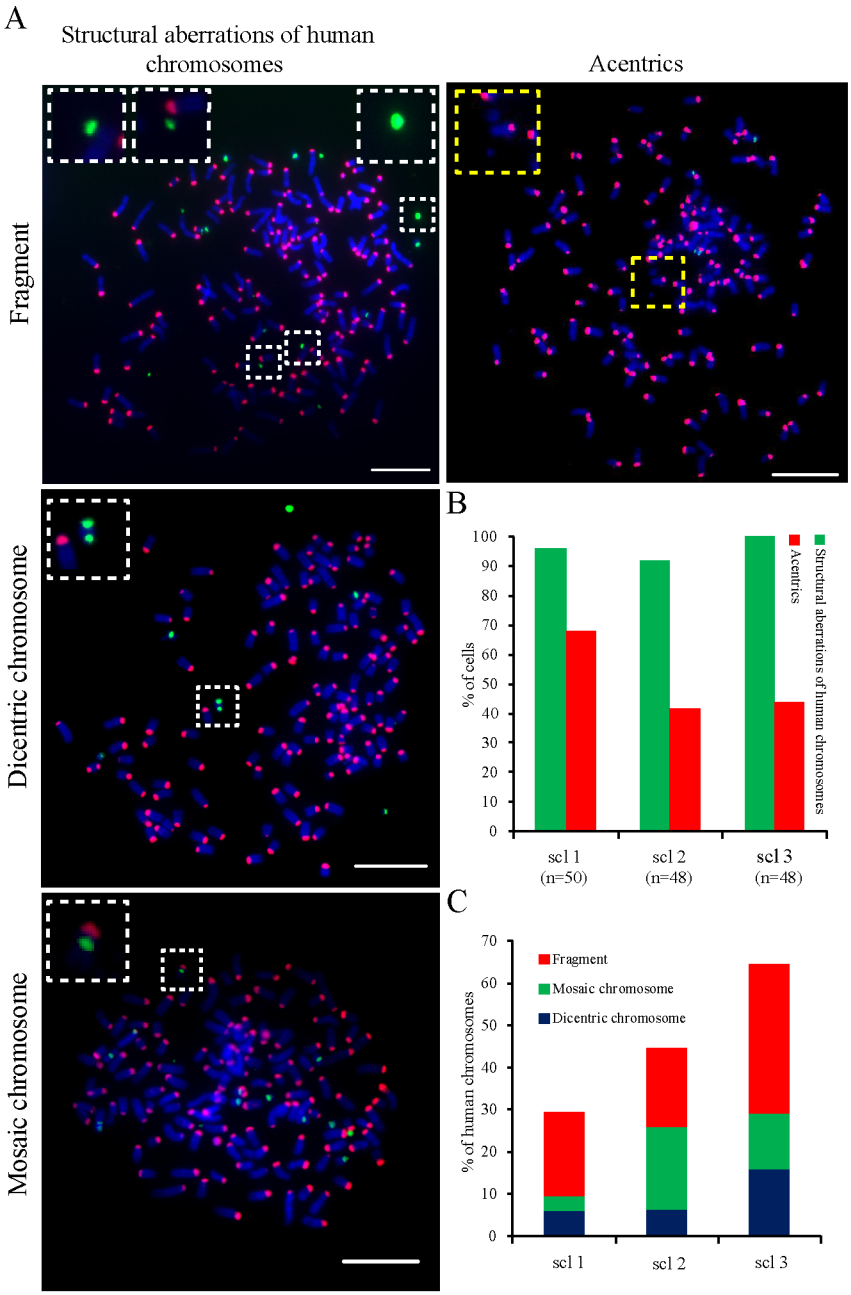

Figure S2. Structural chromosome aberrations in metaphase hybrid cells from 3 randomly selected cell clones. (A) Representative images show acentrics (bottom) and structural aberrations of human chromosomes (top) such as fragments (left), mosaic chromosomes (middle) and dicentric chromosomes (right). Red, Mouse centromeres; Green, Human centromeres; Blue, DNA; Bars= 20 µm. (B) Percentage of cells with each type of structural chromosome aberrations. (C) The number of structural aberrations per human chromosomes.

Figure S3

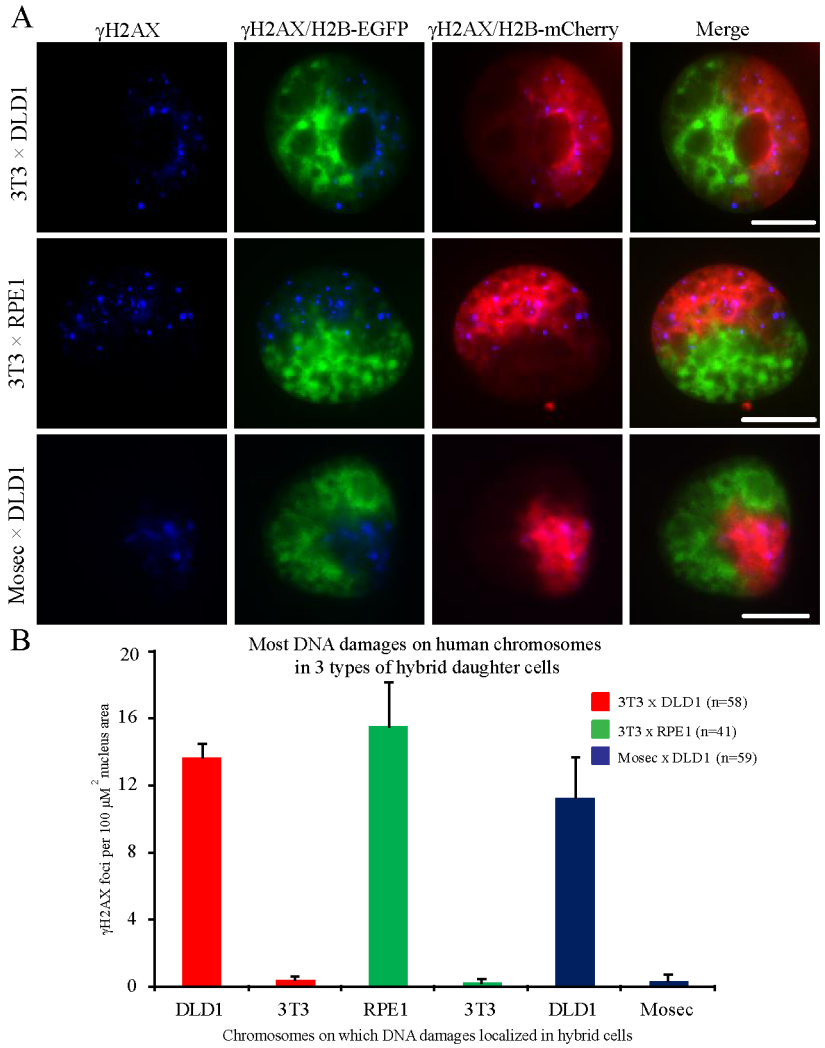

Figure S3. DNA damage sites exhibit distinctive distribution in several types of hybrid daughter cells. (A) Representative images and (B) statistical results of γH2AX staining in 3 types of human-mouse somatic hybrid cells. 3T3 or Mosec x DLD1 or RPE1: representing hybrid cells of 3T3 or Mosec H2B-EGFP cells fused with DLD1 or RPE1 H2B-mCherry cells. Green, Mouse genome; Red, Human genome; Blue, γH2AX staining. Bars= 20 μm. Mean ± SD, from two independent experiments.

Figure S4

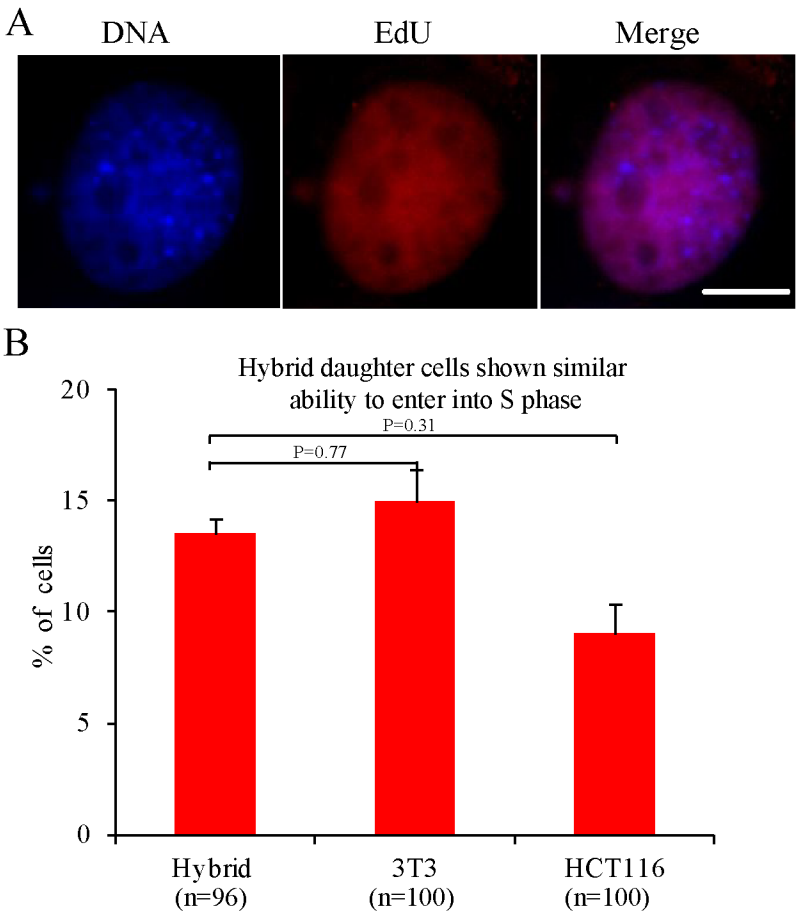

Figure S4. Hybrid daughter cells from first cell division are normally capable to enter next S phase. (A) Representative images of positive-EdU hybrid cells after 2 h adding EdU. Bars= 20 µm. (B) Percentage of EdU-positive cells in hybrid cells and two control cells.

Figure S5

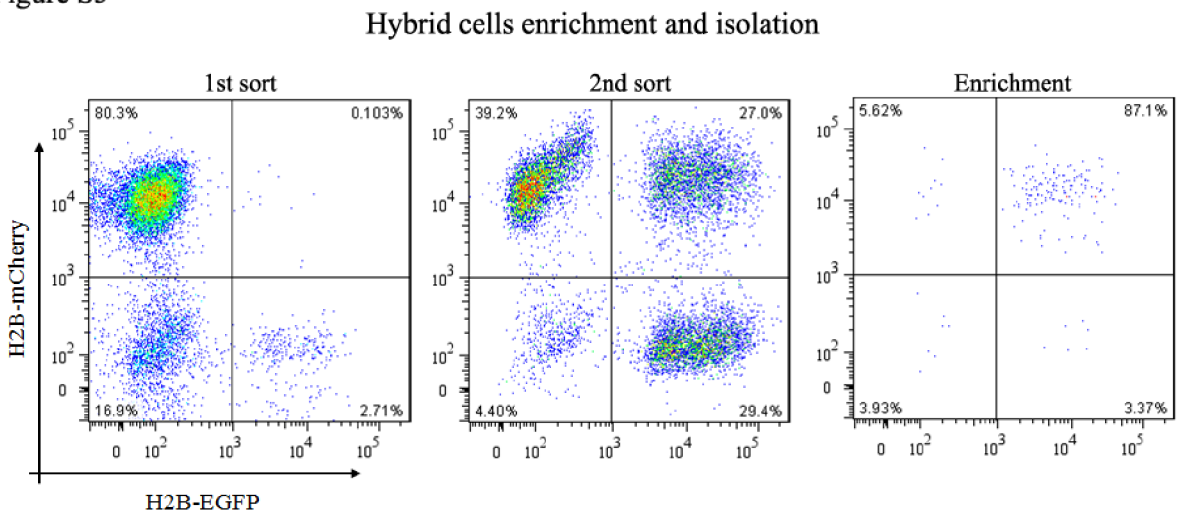

Figure S5. Hybrid cells enrichment and isolation by FACS. Representative FACS plots for hybrid cells enrichment and isolation at 10 h after 3T3 H2B-EGFP cells fused with HCT116 H2B-mCherry cells. From left to right: EGFP+ mCherry+ hybrid cells first sort, second sort, enrichment.

Figure S6

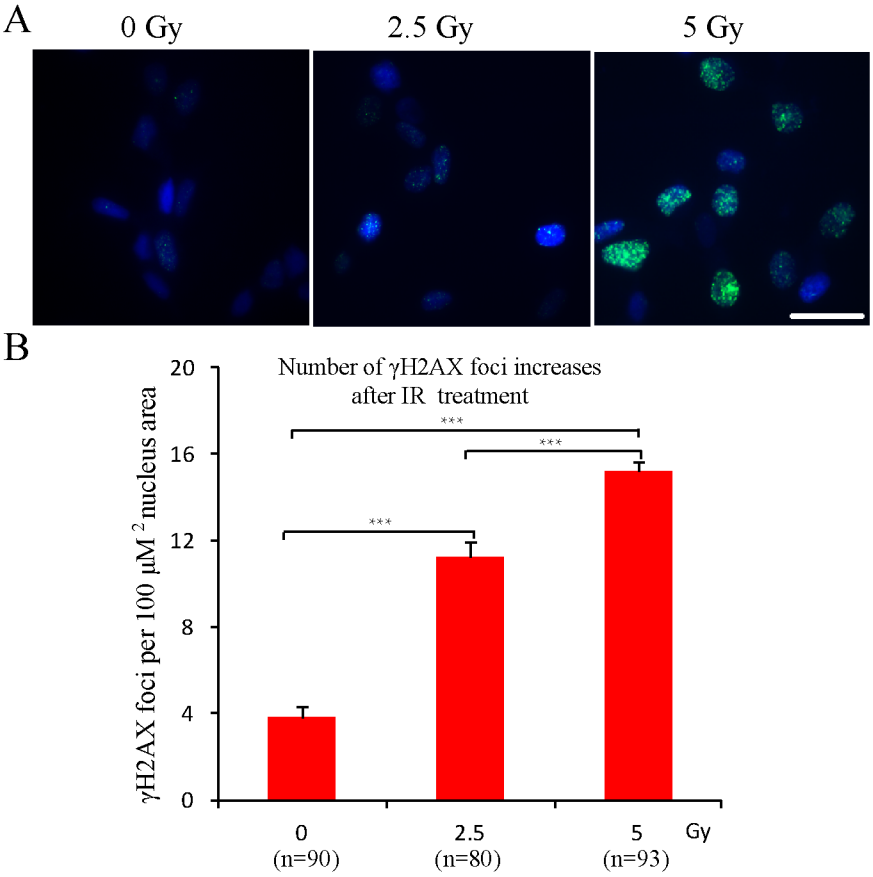

Figure S6. DNA damages in HCT116 H2B-mCherry cells after treatment with different doses of ionizing radiation (IR). (A) Representative images and (B) statistical results for γH2AX staining in HCT116 H2B-mCherry cells after the different doses of IR treatment at 3-4 h before cell fusion. Bars= 10 μm. Mean ± SD, from two independent experiments.

Figure S7

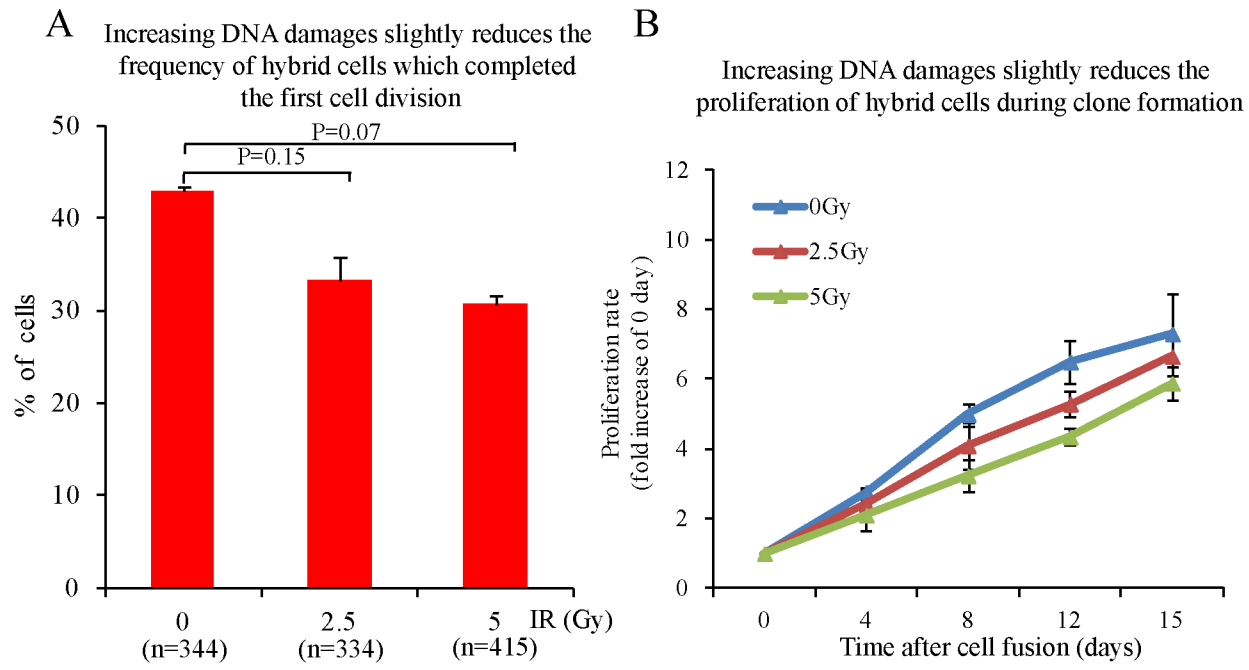

Figure S7. Increased DNA damages slightly disturb the proliferation of hybrid cells. (A) Percentage of hybrid binucleated cells which finished cell division at 13 h after cell fusion. n= the number of cells counted. (B) Different types of hybrid cells were generated following experimental scheme (Figure 6A), and the proliferation rate of these hybrid cells were analyzed during clone formation. Mean ± SD, from two independent experiments.

Figure S8

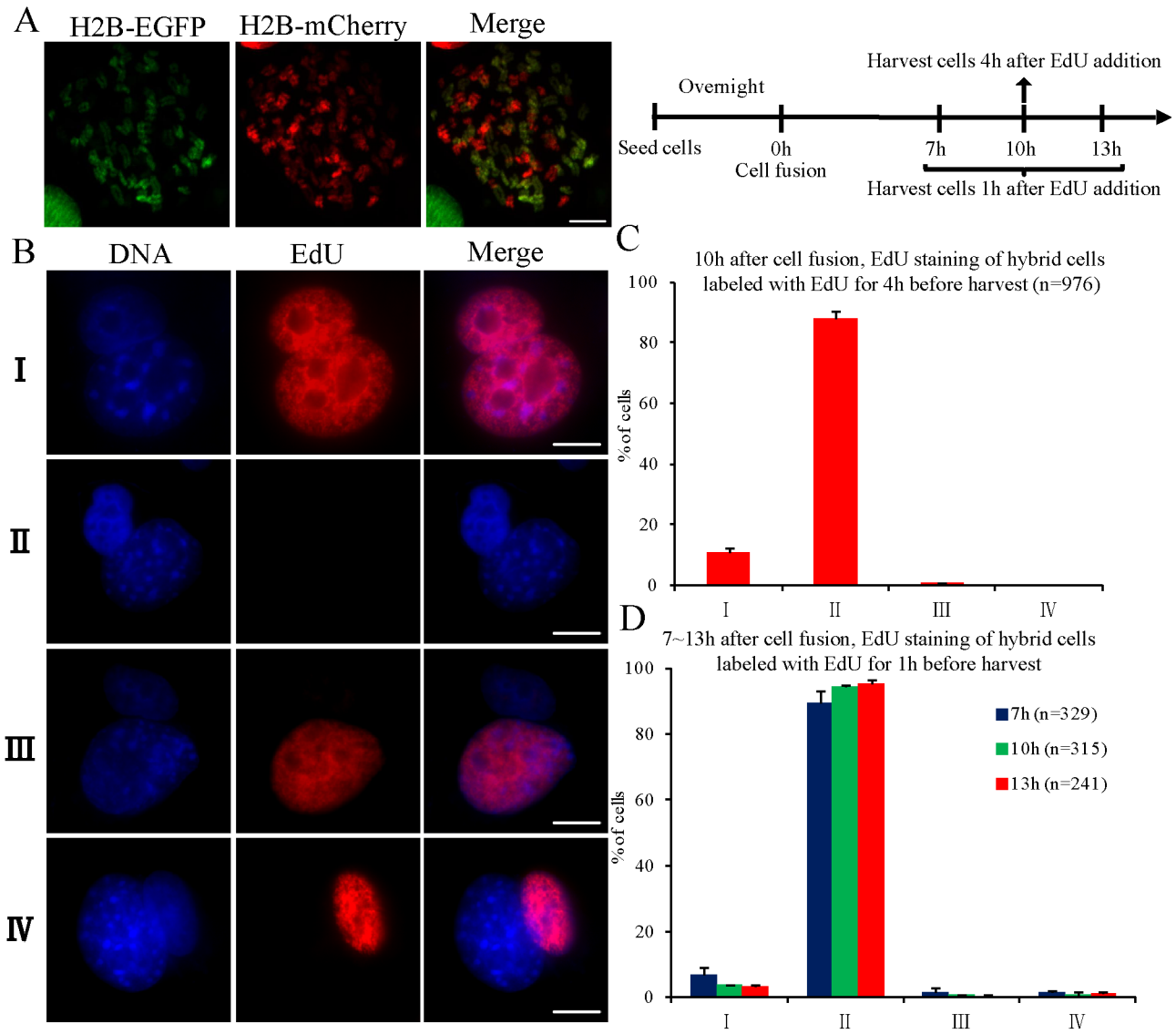

Figure S8. Most hybrid cells exhibit synchronized cell cycle. (A) Representative images of metaphase chromosomes spreads assay on hybrid binucleated cells. None (0/32) of hybrid binucleated cells exhibited premature chromosome condensation (PCC). Bar= 20 μm. (B) Representative images of 4 different EdU staining in hybrid binucleated cells. I: Positive EdU staining in both nuclei, II: Negative EdU staining in both nuclei, III: Positive EdU staining in mouse nucleus only, IV: Positive staining in human nucleus only. Red, EdU; Blue, DNA. Bar= 20 μm. (C) 6 h after cell fusion, EdU was added in culture medium, and harvest cells for EdU staining at 4 h after EdU addition. Percentage of cells with different types of EdU staining was analyzed. (D) At different time points (6, 9 and 12 h) after cell fusion, EdU was added into culture medium for 1 h, cells were then harvest for EdU staining. Percentage of cells with different types of EdU staining was analyzed. Mean ± SD, from two independent experiments.