The non-apoptotic role of caspase-2 in cell cycle regulation and DNA repair

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Background

Caspase-2 is most well-known for initiating the intrinsic apoptosis pathway and as a tumor suppressor. Despite its pro-apoptotic role, caspase-2-deficient tumors have comparable basal apoptosis levels to wild-type tumors. This suggests a non-apoptotic role for caspase-2 could drive its tumor suppression function. It has long been noted that caspase-2-deficient cells proliferate faster, and studies have shown that caspase-2 induces cell cycle arrest following cytokinesis failure. However, whether caspase-2 plays an active role in regulating the cell cycle has up until now been unclear.

Caspase-2 limits proliferation

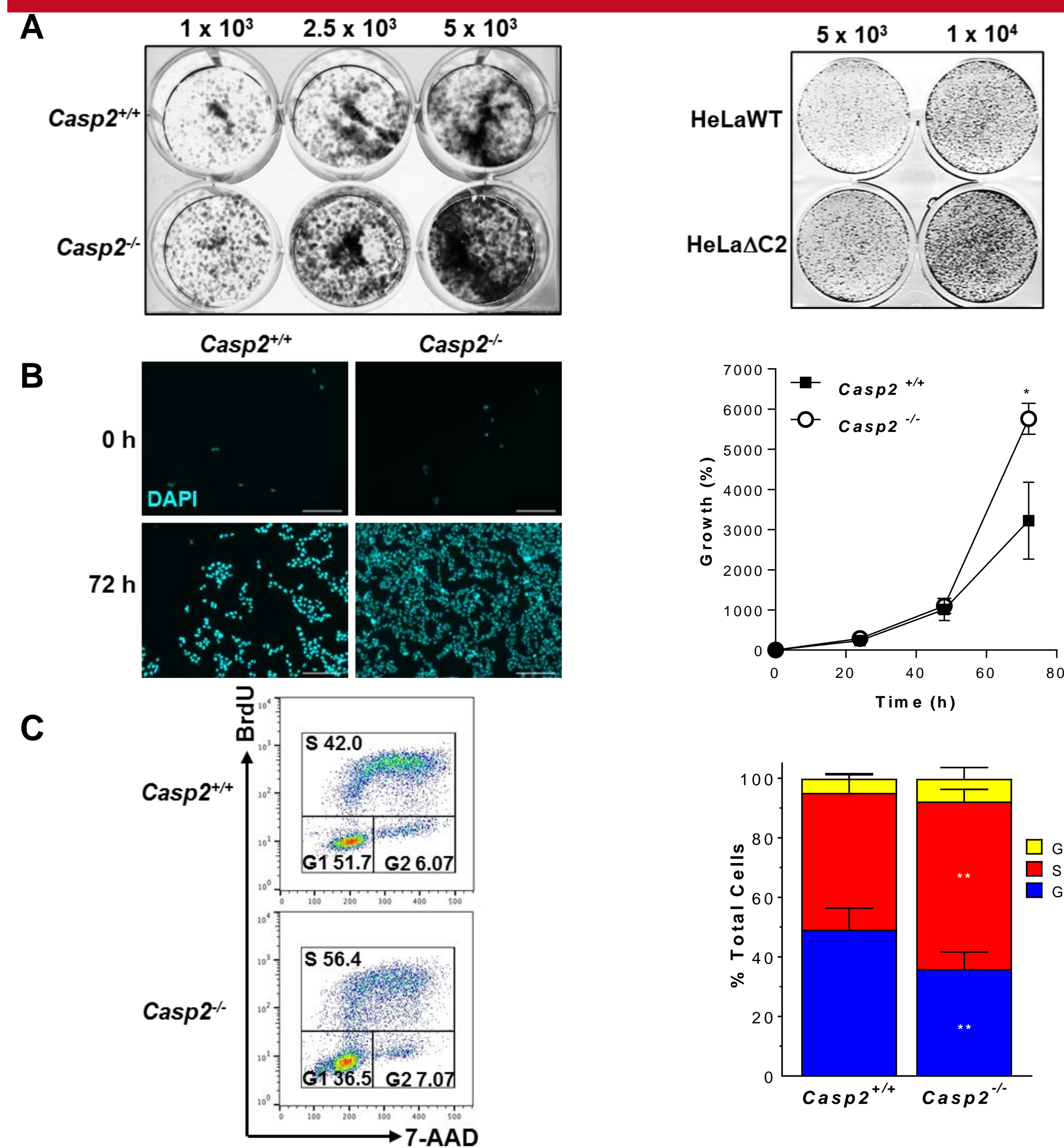


Fig 1: A. Casp2+/+ and Casp2−/−E1A/Ras transformed mouse embryonic fibroblasts (MEF) (left) and wild type (WT) or CRISPR/Cas9 generated caspase-2 deficient (ΔC2) HeLa cells were plated at the indicated cell number. Viable cells were stained with methylene blue 4 days after plating. Representative images are shown from 3 independent experiments. **B.** Casp2+/+ and Casp2−/− E1A/Ras transformed MEF were stained with Hoechst 33342 and imaged at the indicated time points. Representative images are shown from the 0 h and 72 h time points. Nuclei are shown in cyan. Bar, 200 µm. At least 30 images per well were used to determine cell number. Results are shown as the percent increase in cell growth from 0 h and are the average of 3 independent experiments +/- SD. *p < 0.05. **C.** Cycling Casp2+/+ and Casp2−/− E1A/Ras MEF were stained with anti-BrdU-FITC/7-AAD. Representative flow plots and the percentage of cells in G1, S, or G2/M phase are shown. Results are the average of 7 independent experiments +/- SD. **p < 0.01.

Caspase-2 is activated in divding cells in G1

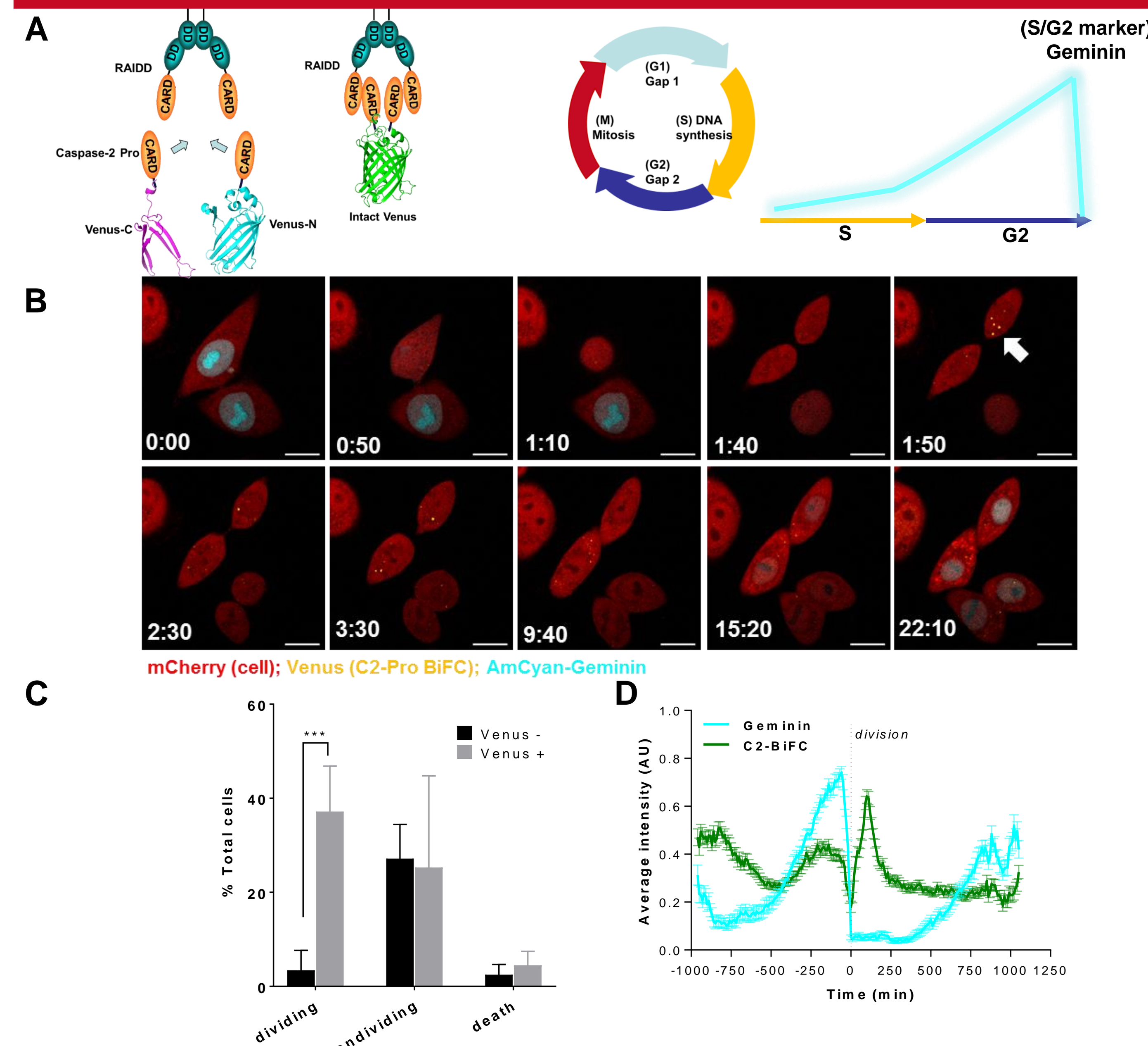


Fig 2: A. Diagram of applied method Bimolecular Fluorescence Complementation (BiFC) (left) and the timing of the peak of expression of Geminin (S/G2 marker) during the cell cycle (right). **B.** HeLa.C2 Pro-BiFC cells stably expressing AmCyan-Geminin were imaged every 10 min for 24 h. Frames from the time-lapse show representative cells undergoing BiFC (yellow) relative to geminin expression as measured by Cyan fluorescence. Bar, 10 µm. **C.** The percentage of mCherry-positive cells that became Venus+ or remained Venus- were categorized into those with observed division, no division, or cell death. At least 100 cells per experiment were measured. Results are the average of 4 independent experiments +/- SD. ***p < 0.001. **D.** Graph of the dividing cells that became Venus-positive and Cyan-positive is shown. Each point on the Cyan graph is scaled and aligned to each point on the caspase-2 BiFC graph (green) that represents the average intensity of Cyan or Venus respectively in the cell at 10 min intervals where time = 0 is cell division. The peaks of Cyan intensity represents G2-phase of the cell cycle and the peaks of Venus intensity represent caspase-2 activation as measured by C2-BiFC (green). Error bars represent SEM of 94 individual cell divisions.

Loss of caspase-2 leads to S-phase defects

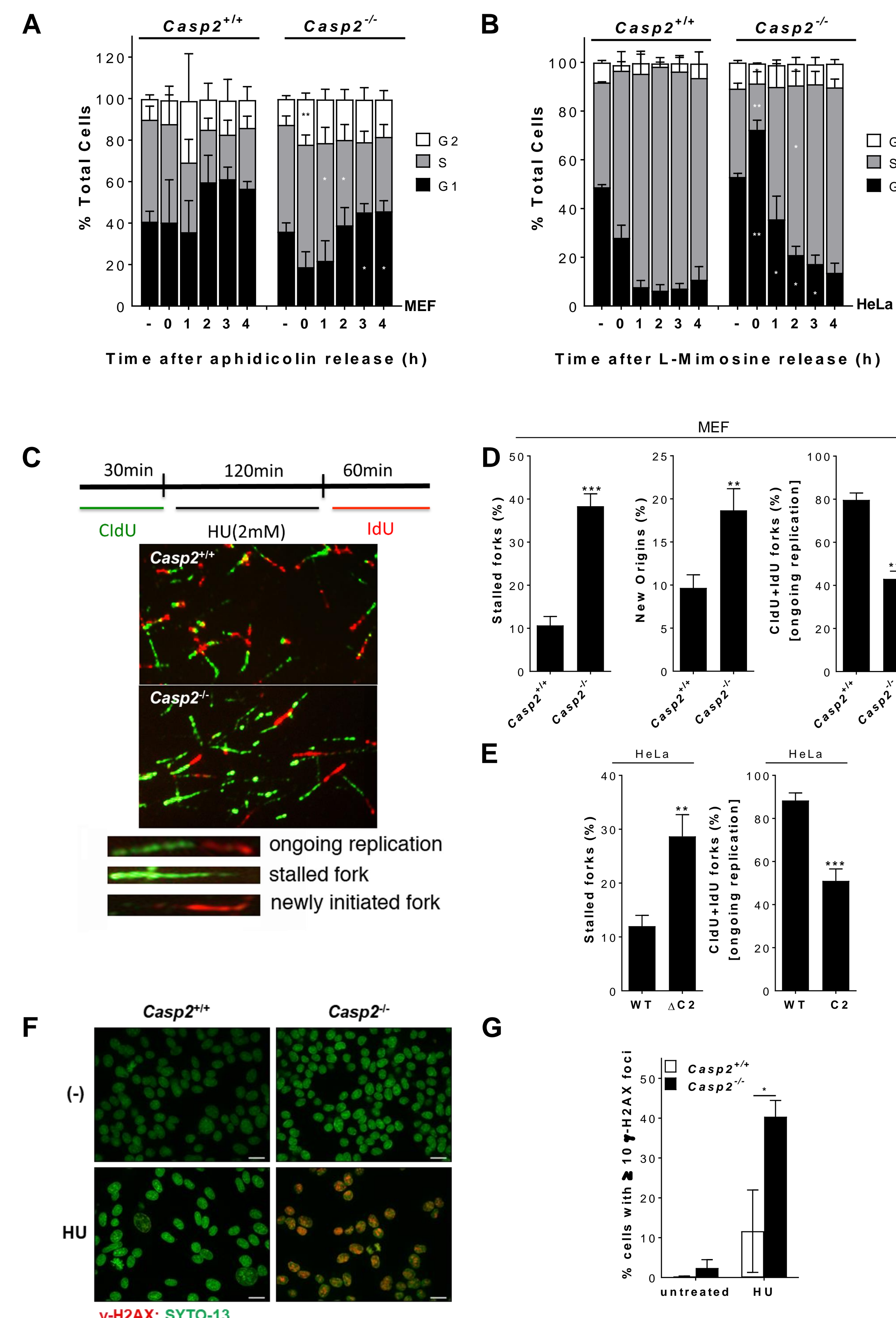


Fig 3: A. Casp2+/+ and Casp2−/− E1A/Ras transformed MEF were either left untreated (−) or treated with aphidicolin (1 µM). After 16 h, the treatment was removed and replaced with fresh media (0 h). Cells were harvested following a 30 min BrdU pulse at the indicated time points after aphidicolin release and stained with anti-BrdU-FITC/7-AAD. The proportion of cells in S-, G1- and G2/M-phase was determined by flow cytometry. Results are the average of 5 independent experiments +/- SD. *p < 0.05; **p <0.01 . **B.** HeLaWT or HeLaΔC2 cells were treated with L-mimosine (0.5 mM) for 24 h. L-mimosine was removed and cells were stained as in (A). Results are the average of 3 independent experiments +/- SD. *p < 0.05, **p <0.01. **C.** A scheme for dual labeling of DNA fibers to evaluate replication restart/recovery following hydroxyurea (HU)-induced replication fork stalling. Representative images of replication tracts from Casp2+/+ and Casp2−/− E1A/Ras-transformed MEF are shown. **D.** The percentage of stalled forks, new origins, and tracts with both CldU and IdU labels are shown. About 300 fibers were analyzed for each condition. **p < 0.01; ***p < 0.001. **E** The percentage of stalled forks and tracts (CldU+IdU) are shown from HeLaWT and HeLaΔC2 cells treated and analyzed as in (C&D). **F.** Casp2+/+ and Casp2−/− E1A/Ras transformed MEF treated with DMSO (-) or HU (2 mM) for 2 h followed by recovery for 24 h were stained for γ-H2AX. Representative images are shown as maximum intensity projections of 5 image Z stacks, with nuclei shown in green and γ-H2AX foci in red. Bar, 50 µm. **G.** γ-H2AX foci were counted per cell and the percentage of cells with ≥10 foci was calculated from at least 30 images per treatment. Results are the average of three independent experiments +/- SD. *p < 0.05.

The role of caspase-2 in cell division is distinct from its apoptotic pathway

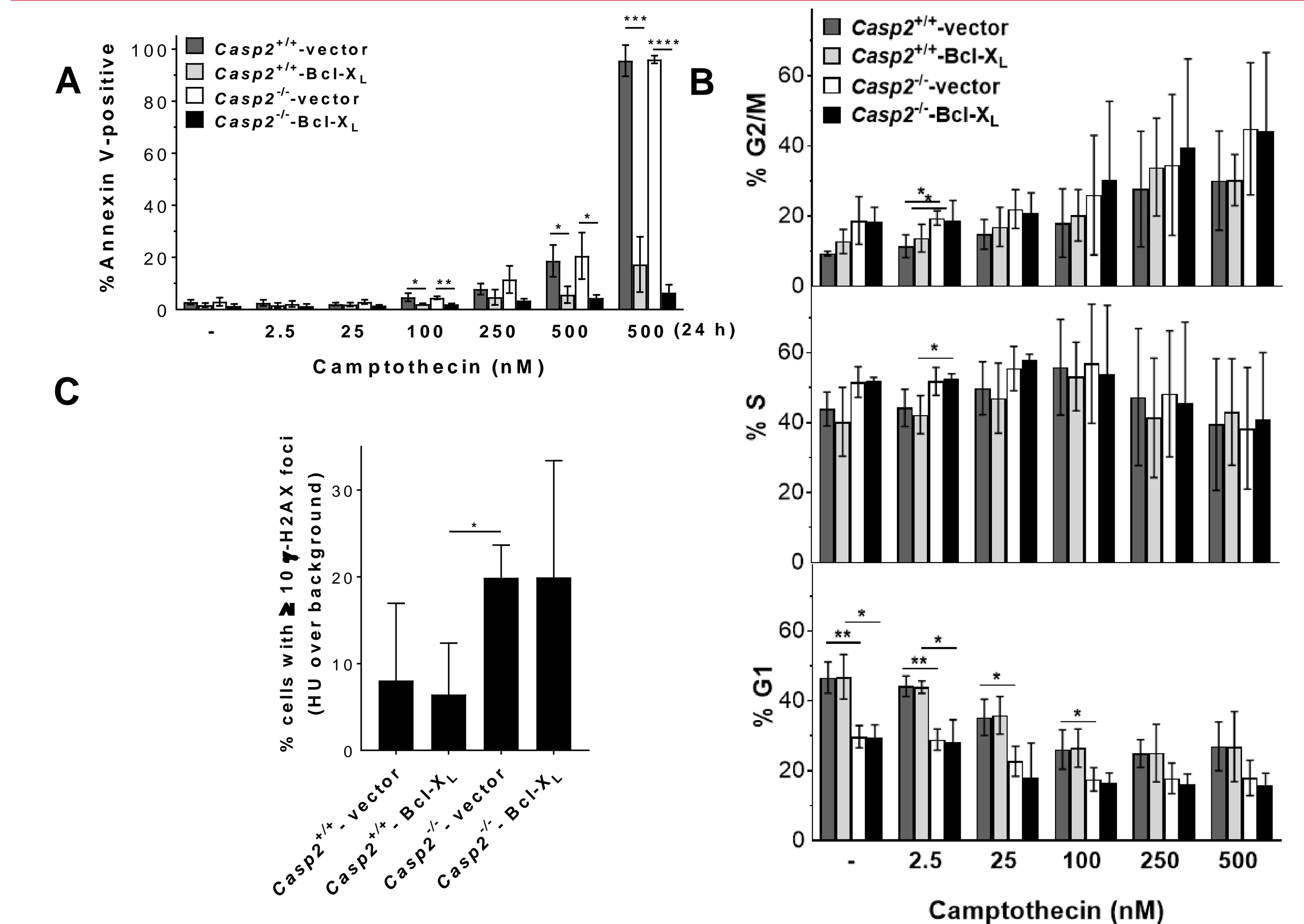
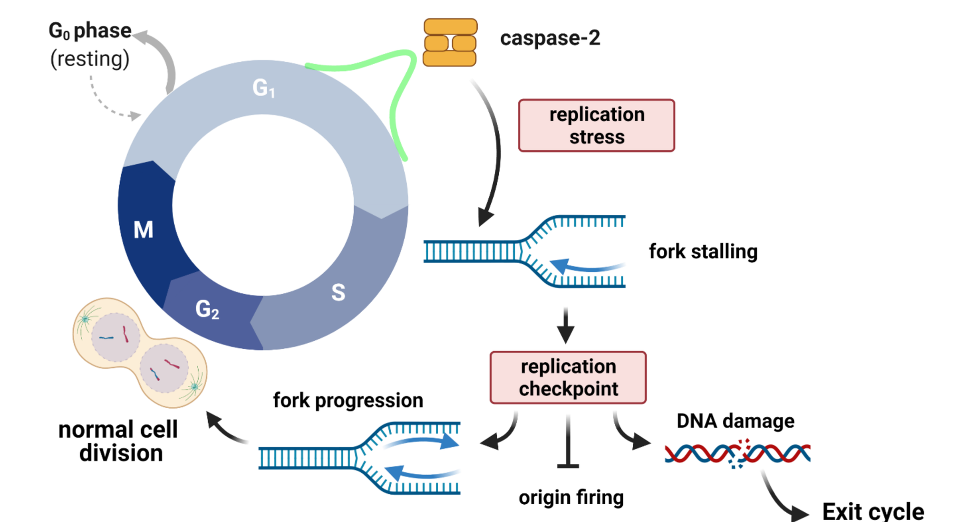


Fig 4: A. Casp2+/+ and Casp2−/− E1A/Ras transformed MEF stably expressing vector or Bcl-XL were left untreated (-) or treated with the indicated doses of camptothecin for 24 h. Apoptosis was measured by flow cytometry for Annexin V staining. Results are the average of 3 independent experiments +/- SD. *p < 0.05; **p < 0.01 **B.** Cycling cells were left untreated (-) or were treated with the indicated doses of camptothecin for 4 h. Cells were harvested following a 30 min BrdU pulse and stained with anti-BrdU-FITC/7-AAD to determine the percentage of cells in G1-, S- or G2/M-phase of the cell cycle by flow cytometry. Results are the average of 3 independent experiments +/- SD. *p < 0.05; **p < 0.01. **C.** Casp2+/+ and Casp2−/− MEF stably expressing vector or Bcl-XL treated with DMSO or HU (2 mM) for 2 h followed by recovery for 24 h were stained for γ-H2AX. γ-H2AX foci were counted per cell and the percentage of cells with ≥10 foci/cell was calculated from at least 30 images per treatment. Results are shown as % cells with ≥10 foci/cell above untreated for each genotype. The average of three independent experiments +/- SD is shown. *p < 0.05.

Conclusions



For further Information

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Or see paper: Boice A. G. et al., Caspase-2 regulates S-phase cell cycle events to protect from DNA damage accumulation independent of apoptosis. Oncogene. 2022 Jan;41(2):204-219.

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