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Supplemental Material to:

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JNK signaling is needed to tolerate chromosomal instability

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Supplementary Figures

Figure S1

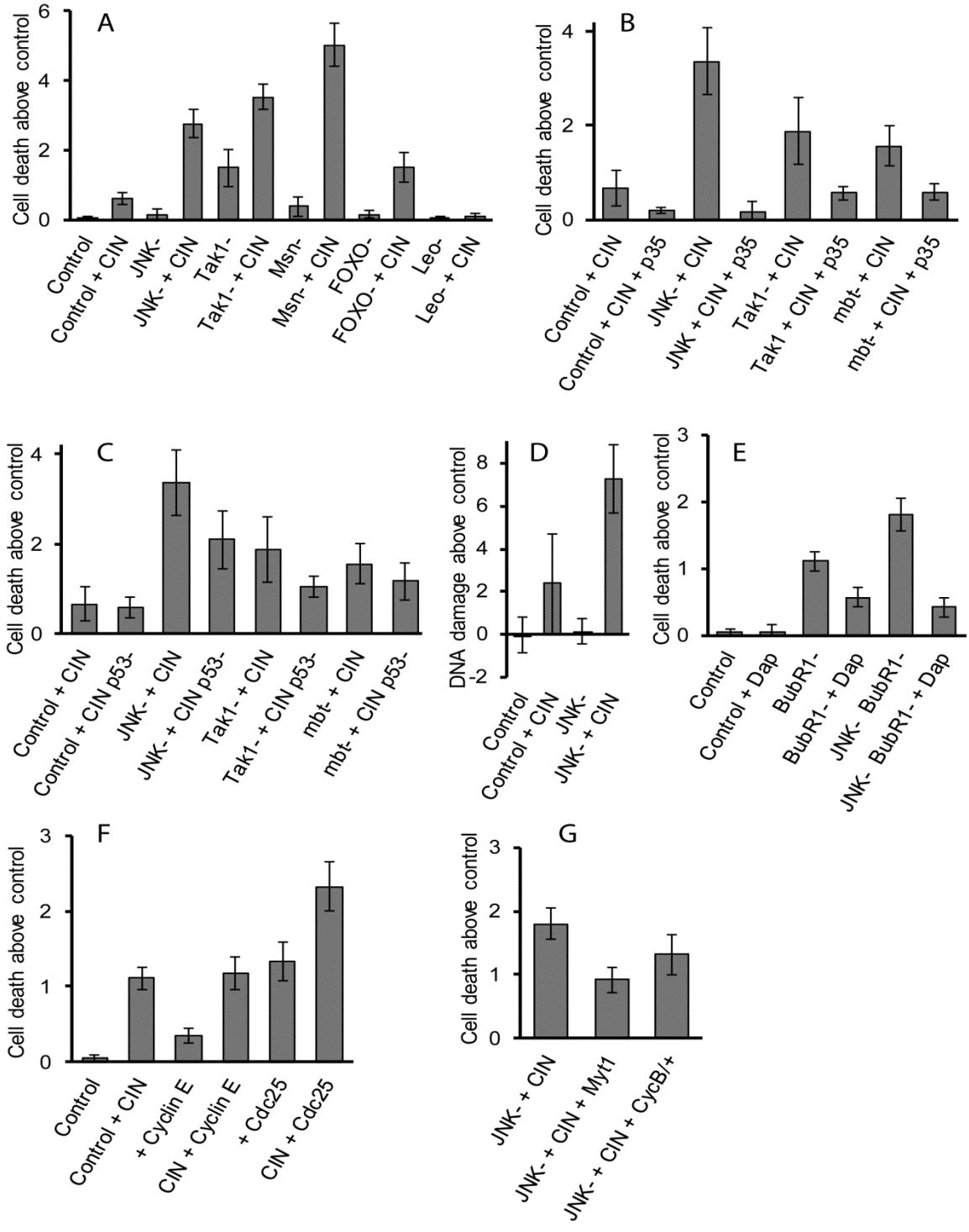

Figure S1: Quantitation of the cell death and DNA damage stainings of wing discs shown in Figures 1 to 5. In each disc, the average signal from the wild type posterior half has been subtracted from the average signal in the affected anterior half to give a normalized average for that disc. More than ten discs were scored for each genotype. Error bars represent 95% confidence intervals.

Figure S2

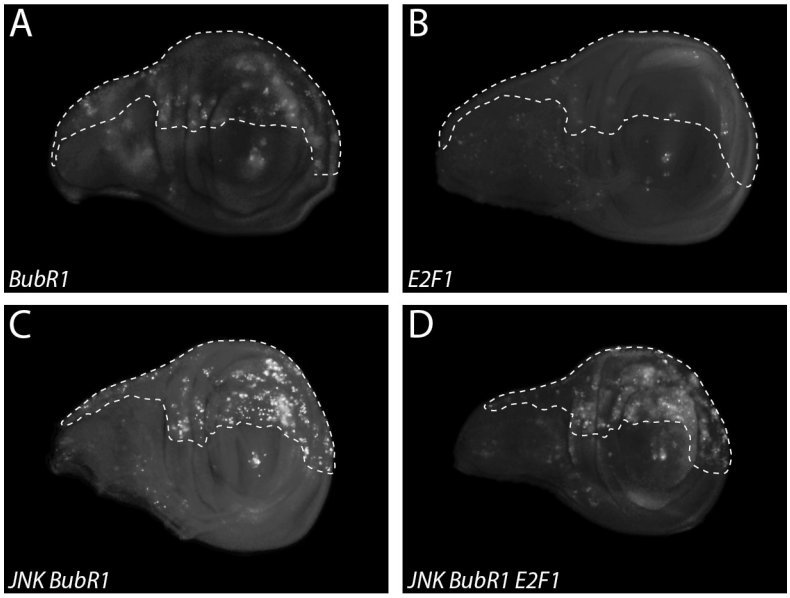

Figure S2: The cell death observed when JNK is depleted in CIN cells is partly independent of E2F1. Wing discs were stained with Acridine Orange to show cell death. In every disc, the unmarked region does not express RNAi constructs, while the dashed line shows the area affected by CIN (*BubR1-RNAi*) and/or depletion of E2F1. Control wings (A, B) show little cell death when CIN is induced (A) or when E2F1 is depleted in CIN cells (B). (C) Imaginal disc in which JNK has been depleted in CIN cells, giving rise to high levels of cell death. (D) Imaginal disc showing that cell death is reduced but not eliminated by depletion of E2F1 in CIN cells depleted for JNK.

Figure S3

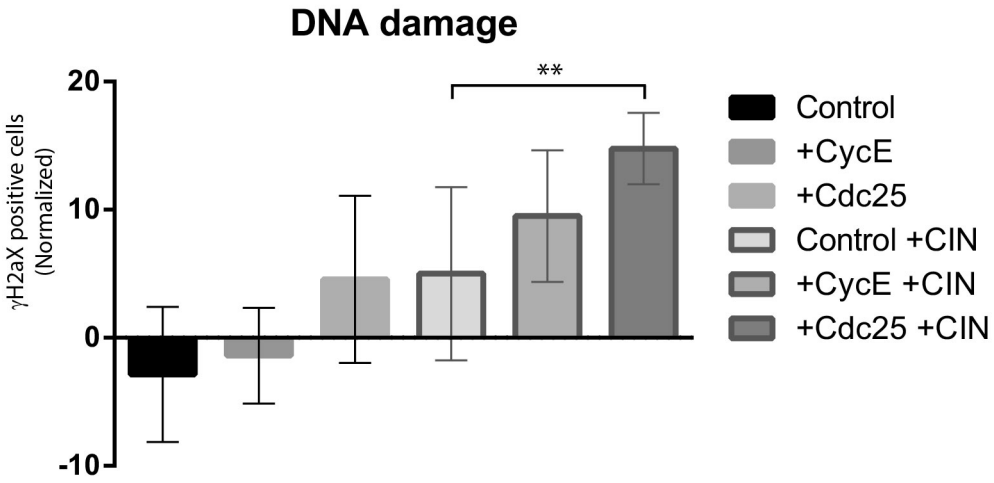

Figure S3: The effect of overexpressing cell cycle regulators on DNA damage in CIN cells. The graph shows the average number of γH2aX (anti-H2AvD pSer137) positive cells in the posterior compartment of third instar imaginal discs of the indicated genotypes. The signal was normalized by subtracting the signal from the wild type anterior compartment of each disc. Overexpression of Cyclin E had no significant effect on the level of DNA damage observed, even in CIN cells (*mad2*-RNAi). Overexpression of Cdc25 significantly increased the level of DNA damage observed in CIN cells (t test, $p<0.05$). Error bars indicate the 95% CI.

Figure S4

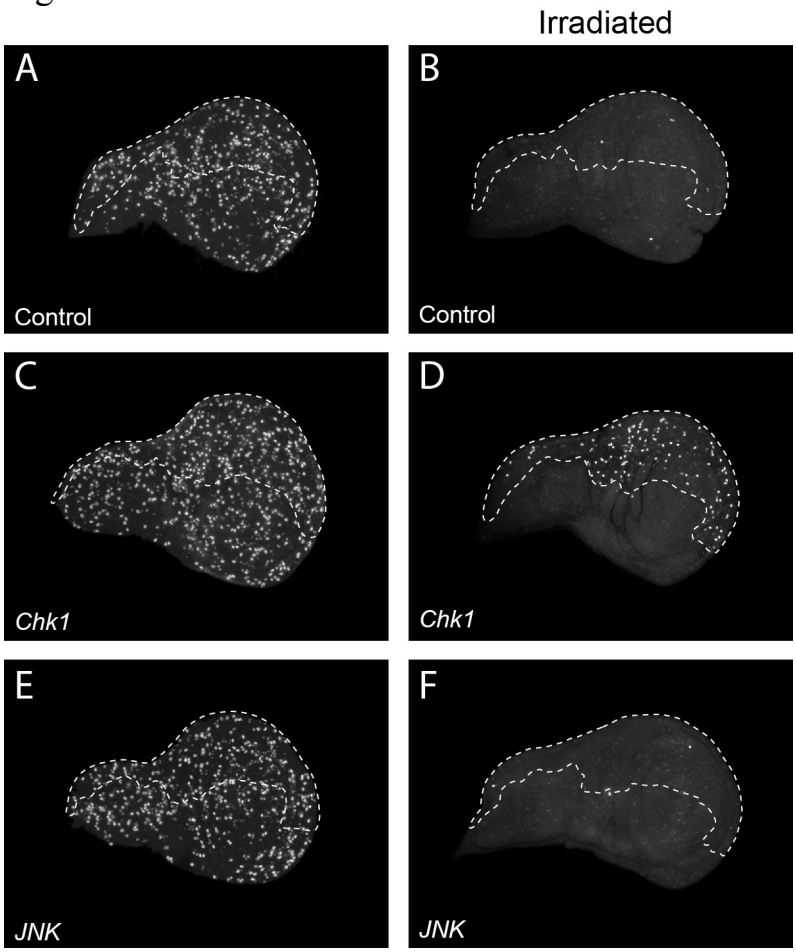

Figure S4: The DNA damage checkpoint is not affected by depletion of JNK. Third instar imaginal discs were stained for P-Histone 3 to indicate cells in mitosis. The dashed line indicates the posterior compartment expressing *chk1*-RNAi (C, D) or *JNK*-RNAi (E, F) while the unmarked anterior compartment is wild type in every disc. In un-irradiated discs (A, C, E), the frequency of mitotic cells is high. After gamma irradiation, then resting for one hour, the control disc (B) shows little mitosis, indicating a cell cycle arrest in response to the triggering of a functional DNA damage checkpoint. Many mitotic cells were found in the posterior compartment after irradiation when Chk1 was depleted (D), indicating the expected loss of checkpoint when this DNA damage response protein was depleted. When cells lacking JNK were irradiated (F), the frequency of mitosis was as low as wild type (B), indicating that JNK is not needed for a DNA damage checkpoint arrest in response to irradiation.

Figure S5

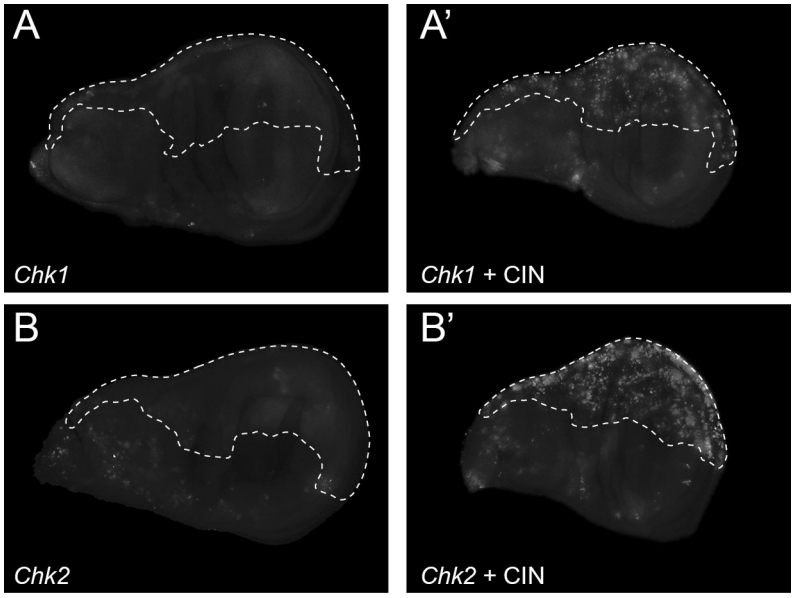

Figure S5: Depletion of DNA damage response proteins leads to cell death in CIN cells. Wing discs were stained with Acridine Orange to indicate cell death. In every disc, the unmarked region (anterior compartment) is the control tissue in which no RNAi constructs are expressed, while the dashed line shows the posterior compartment in which DNA damage response pathway members were depleted by RNAi expression with or without CIN induced by *mad2-RNAi* expression. (A, B) Discs in which Chk1 or Chk2 have been depleted in the posterior compartment (dashed), show little cell death. (A', B') Discs in which CIN has been induced (*mad2-RNAi*) in the posterior compartment along with depletion of the indicated DNA damage response protein. For both of these DNA damage response proteins, depletion in CIN cells causes cell death that is not seen in the normally dividing control cells