Supporting Information

Ho et al. 10.1073/pnas.0811928106

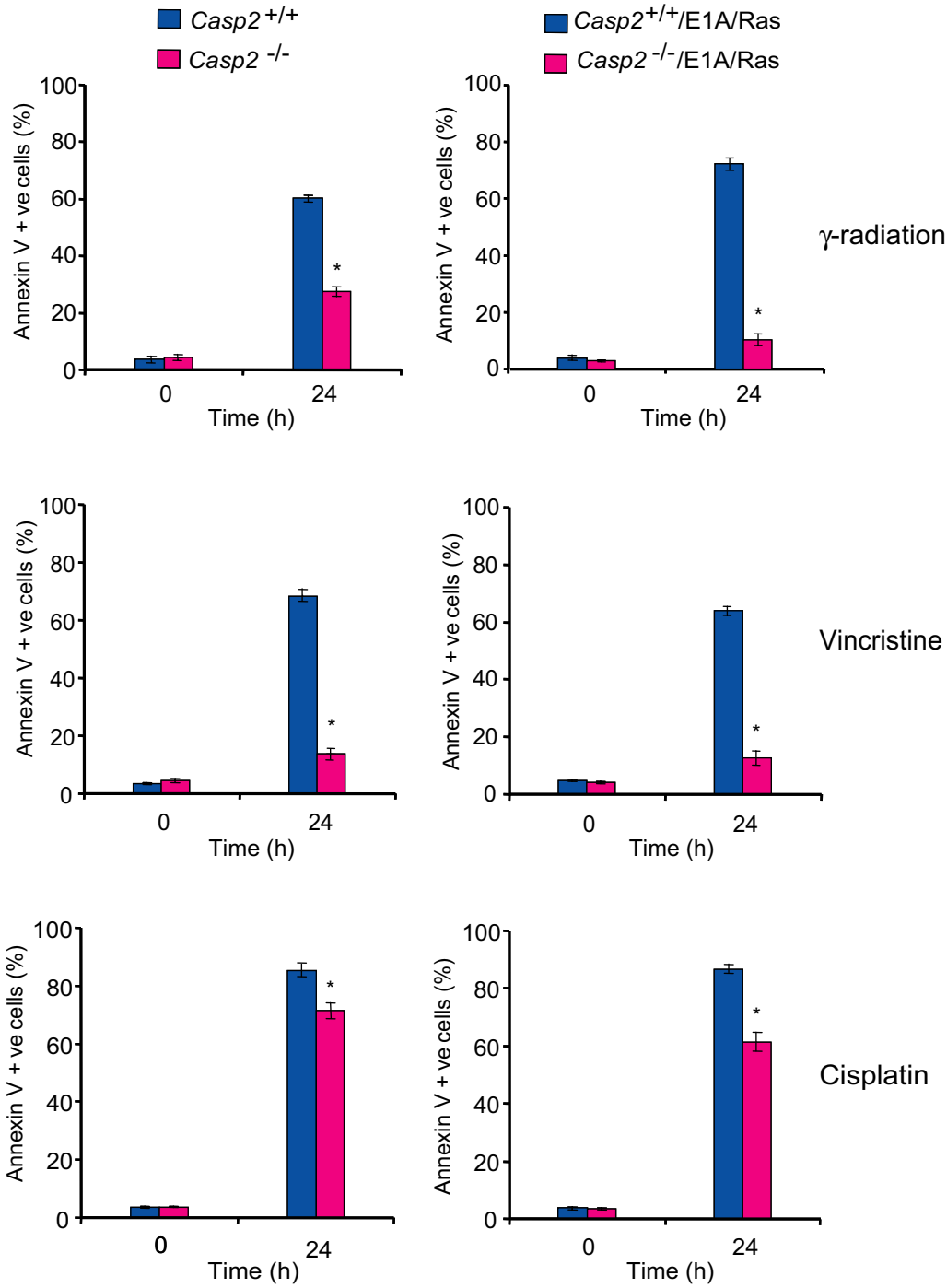

Fig. S1. The loss of caspase-2 results in reduced apoptosis in nontransformed and E1A/Ras-transformed MEFs. Primary nontransformed *caspase-2*$^{+/+}$ and *caspase-2*$^{-/-}$ MEFs, and E1A/Ras-transformed *caspase-2*$^{+/+}$ and *caspase-2*$^{-/-}$ MEFs were exposed to γ-radiation (10 Gy), vincristine (50 μg/ml), or cisplatin (30 μM) for the indicated times and apoptosis analyzed by Annexin V staining. Results are mean ± SEM from 3 experiments (*$P < 0.05$).

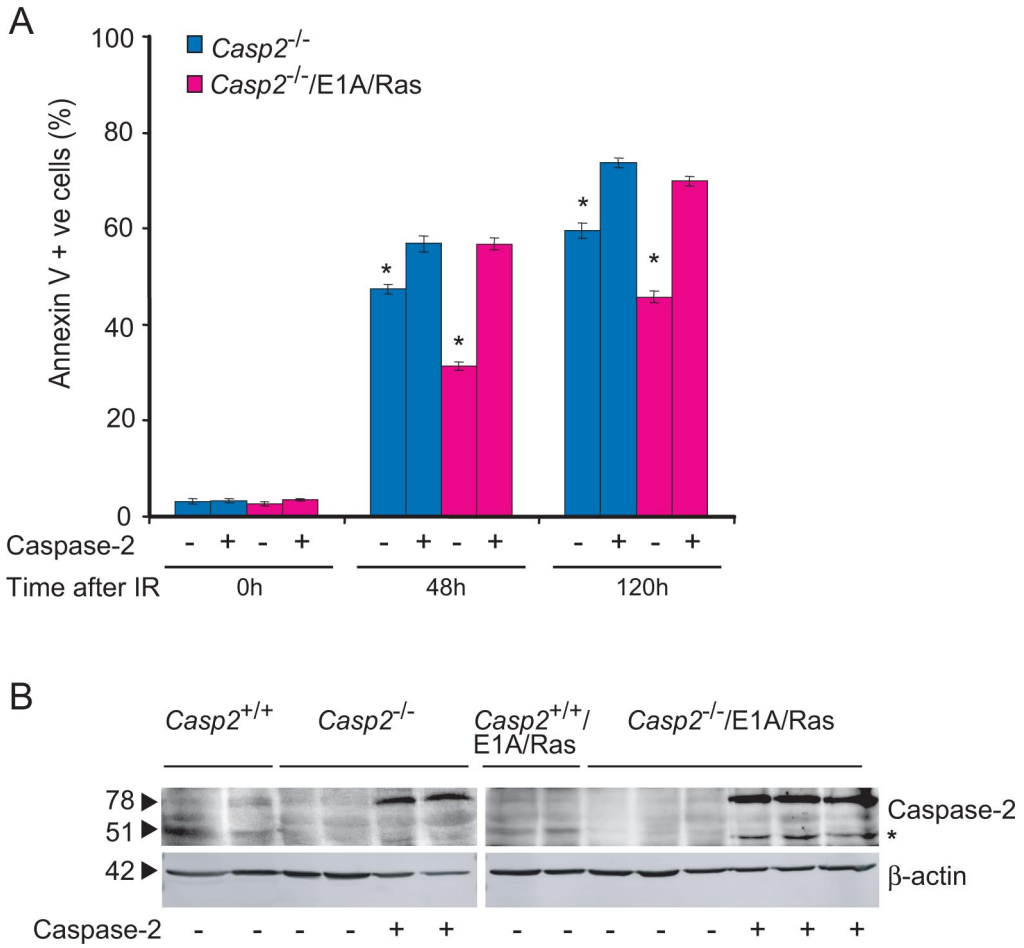

Fig. S2. Caspase-2 contributes to apoptosis induced by IR. (*A*) Apoptosis level of nontransformed and E1A/Ras-transformed *caspase-2*$^{-/-}$ MEFs following restoration of caspase-2 expression via transfection of caspase-2-GFP. Cell were γ-irradiated (IR) and then cultured for 48 or 120 h before apoptosis analysis. Results are expressed as mean $\pm$ SEM from 3 experiments (*$P < 0.05$). (*B*) Immunoblots showing levels of caspase-2 protein following transfection of *caspase-2*$^{-/-}$ MEFs and E1A/Ras-transformed *caspase-2*$^{-/-}$ MEFs with the caspase-2-GFP expression construct. Data from 2–3 separate transfection experiments are shown. Asterisk indicates a band that presumably represents caspase-2 from which GFP has been cleaved off. The relative positions of caspase-2-GFP (78 kDa), caspase-2 (51 kDa), and β-actin (42 kDa) are indicated by arrowheads. Same blots were sequentially probed with a caspase-2 antibody and a β-actin antibody.

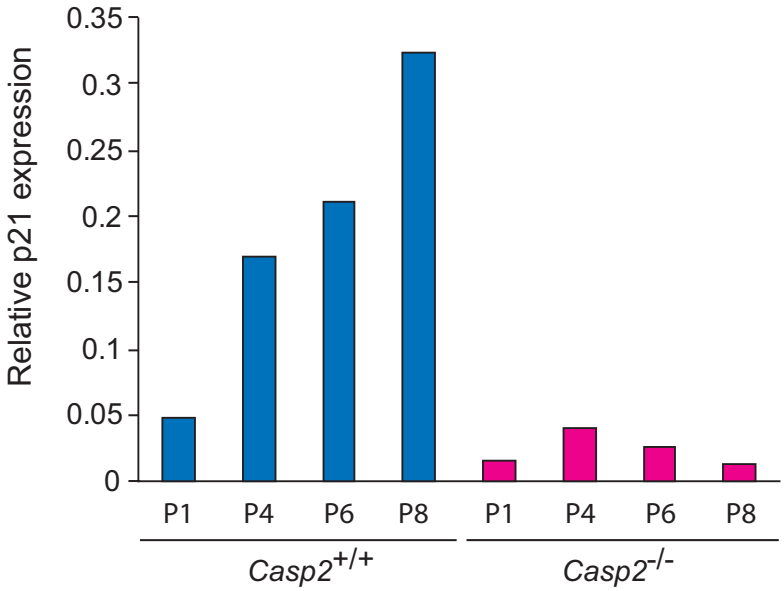

Fig. S3. Reduced levels of *p21* expression in late-passage *caspase-2*$^{-/-}$ MEFs. Wild-type (*casp2*$^{+/+}$) and *caspase-2*$^{-/-}$ (*casp2*$^{-/-}$) MEFs were grown in high-glucose DMEM and RNA extracted at passages P1, P4, P6, and P8, as indicated. Levels of *p21* transcript were measured in triplicate reactions using real-time qPCR and expressed relative to the internal control gene *β-actin*. The data shown are averages from a single experiment. Similar data were obtained in 2 independent experiments.

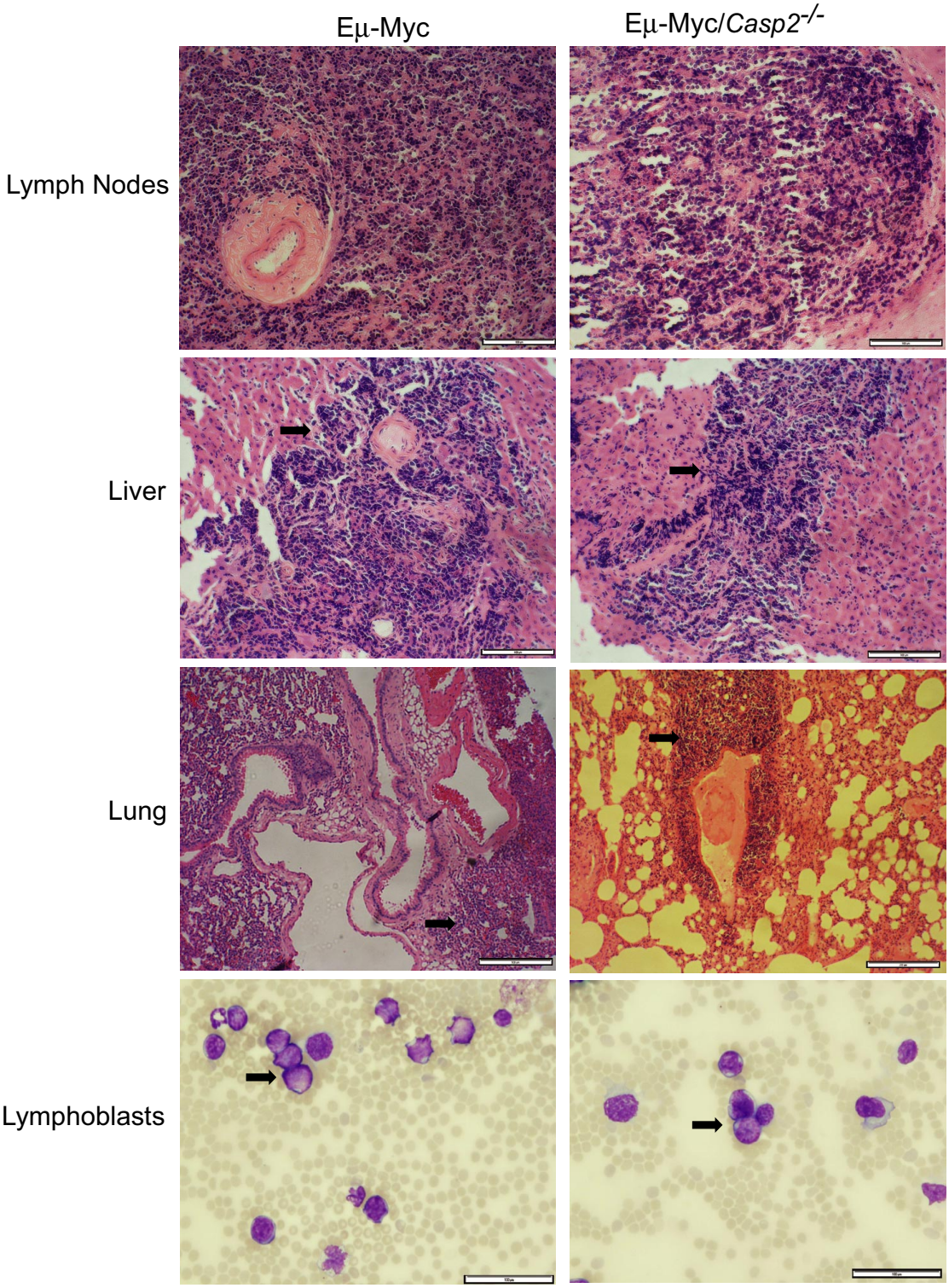

Fig. S4. Eμ-***Myc*** and *caspase-2*$^{-/-}$/Eμ-*Myc* mice show lymphocytic infiltrates characteristic of lymphoma. Representative histology of lymph nodes, liver, and lung from Eμ-*Myc* (LHS) and *caspase-2*$^{-/-}$/Eμ-*Myc* (RHS) mice showing (arrows) increased lymphocytic infiltrates and prominent lymphoblasts in blood smear. Sections were stained with H&E.

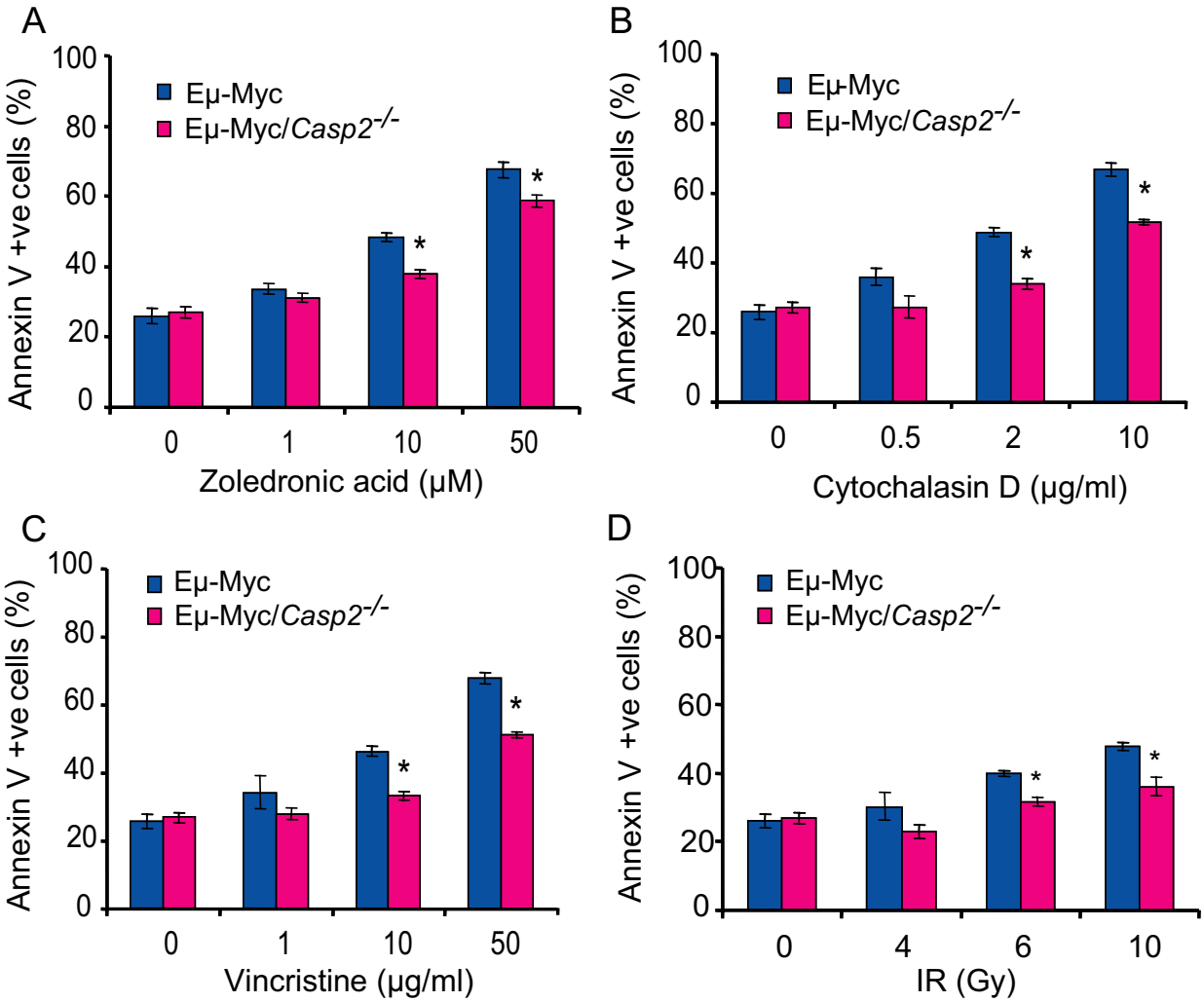

Fig. S5. The loss of caspase-2 results in reduced apoptosis in lymphoma cells. Eμ-*Myc* and *caspase-2*$^{-/-}$/Eμ-*Myc* lymphoma cells were treated with (*A*) zoledronic acid for 24 h, (*B*) cytochalasin D for 24 h, or (*C*) vincristine for 24 h, or (*D*) γ-irradiated and cultured for 48 h. Apoptosis was determined by Annexin V staining using flow cytometry. Results are expressed as mean ± SEM from 3–4 separate experiments (*$P < 0.05$).

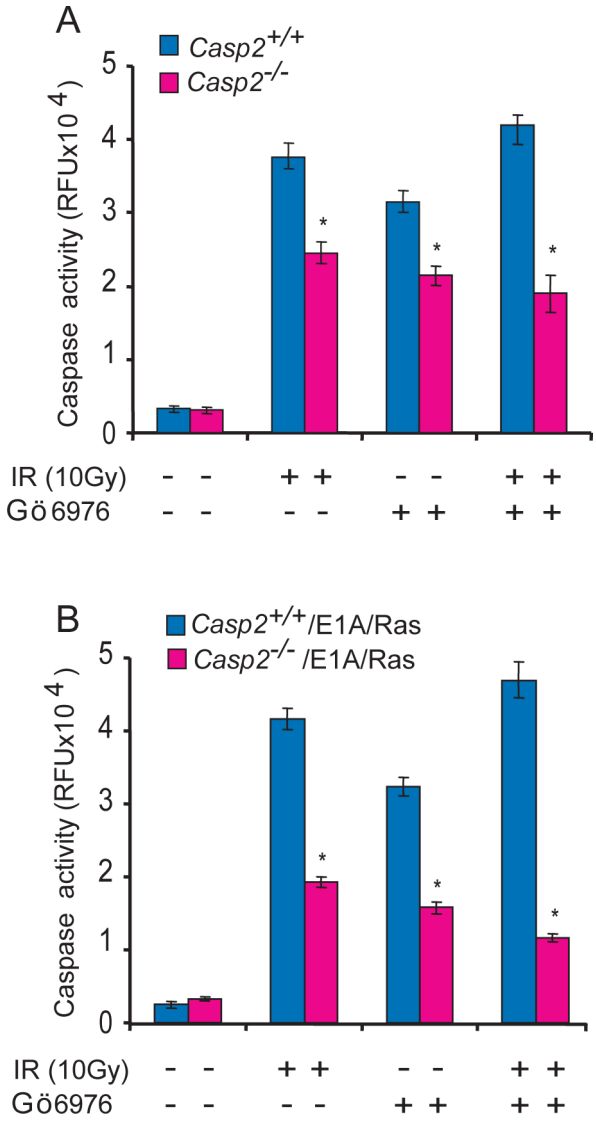

Fig. S6. VDVADase activity in cells undergoing IR-induced apoptosis following Chk1 inhibition. Caspase activity in *caspase-2*$^{+/+}$ and *caspase-2*$^{-/-}$ (*A*) and E1A/Ras-transformed *caspase-2*$^{+/+}$ and *caspase-2*$^{-/-}$ MEFs (*B*) was assessed by cleavage of VDVAD-AMC substrate by cell lysates. Results from 3 experiments are expressed as mean ± SEM relative fluorescence units (RFU). *$P < 0.05$.