

SUPPLEMENTAL MATERIAL

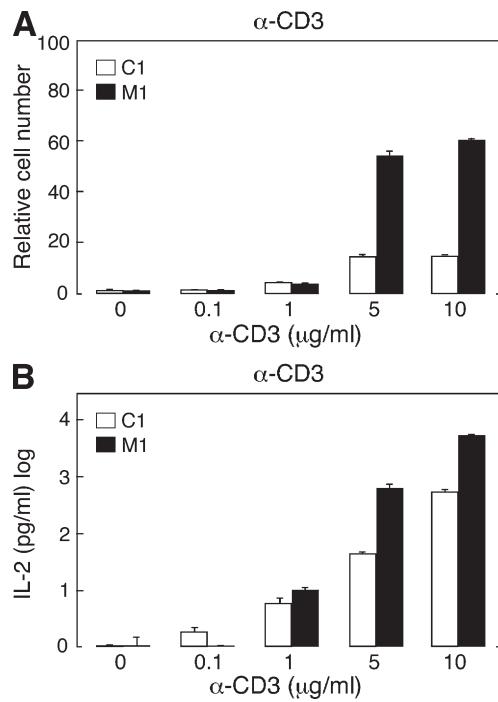
Dougan et al., <http://www.jem.org/cgi/content/full/jem.20101123/DC1>

Figure S1. Response of anti-CD3-stimulated CD4⁺ T cells to IAP antagonists. 10⁵ mouse CD4⁺ T cells were isolated and stimulated with anti-CD3 at the indicated concentration for 72 h in the presence of M1 or control compound (C1) at 500 nM. (A) Relative cell numbers were determined as in Fig. 1 and normalized to unstimulated cultures treated with C1. (B) IL-2 was measured by ELISA. (A and B) Error bars represent SEM. Results are representative of two independent experiments. P < 0.005 for 5 and 10 μg/ml.

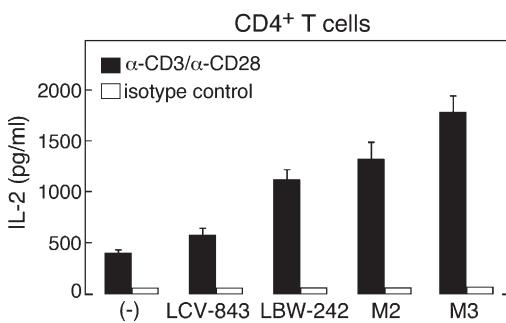


Figure S2. Multiple IAP antagonists augment cytokine secretion from CD4⁺ T cells. 10⁵ mouse CD4⁺ T cells were isolated and stimulated for 48 h in the presence of vehicle (−), a control compound (LCV-843), or IAP antagonists (LBW-242, M2, or M3). IL-2 was measured by ELISA. Error bars represent SEM. Results are representative of three independent experiments. LBW-242, P = 0.02; M2, P = 0.008; M3, P = 0.008.

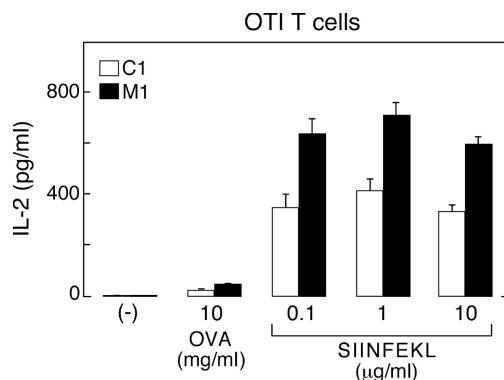


Figure S3. IAP antagonists augment peptide-specific responses. 10^5 OTI cells were stimulated by fixed bone marrow-derived DCs pulsed with whole OVA protein or SIINFEKL peptide at the indicated concentrations. M1 was used at 500 nM. Cytokines were measured by ELISA after 24 h. Error bars represent SEM. Results are representative of two independent experiments. 0.1 μ M SIINFEKL, $P = 0.0005$; 1.0 μ M SIINFEKL, $P = 0.002$; 10 μ M SIINFEKL, $P = 0.004$; OVA, $P = 0.02$.

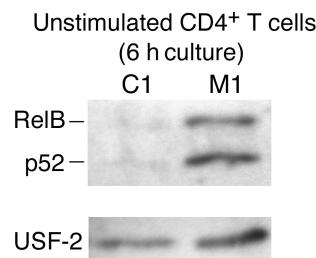


Figure S4. NF- κ B induction in unstimulated CD4 $^{+}$ T cells by IAP antagonist treatment. Immunoblot using the indicated antibodies on nuclear lysates from isolated mouse CD4 $^{+}$ T cells cultured for 6 h with M1 or control compound. Results are representative of two independent experiments.

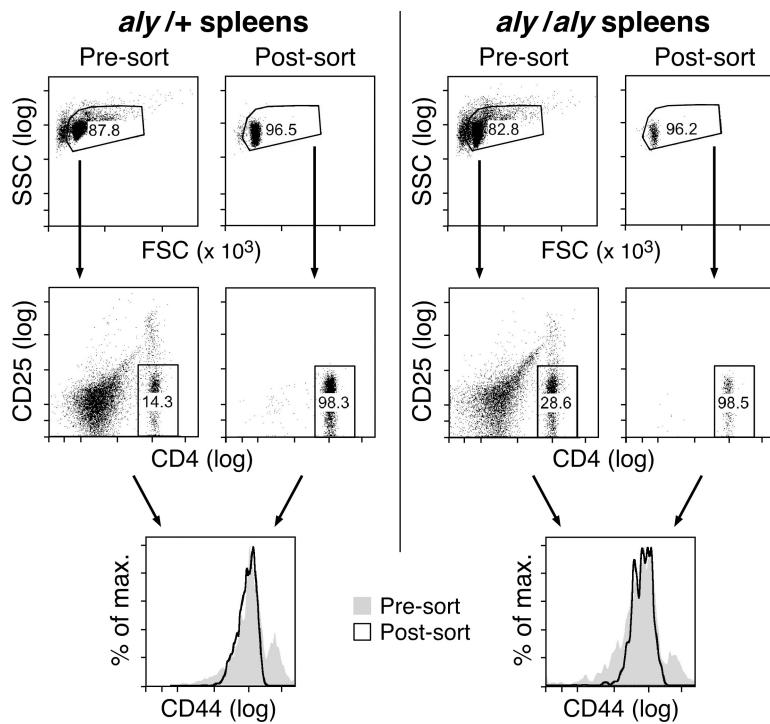


Figure S5. Schematic depicting the isolation of naive CD4⁺ T cells. Spleen cells from *aly/+* or *aly/aly* mice were isolated, stained with the indicated antibodies, and purified by fluorescence-activated cell sorting using the indicated gates. Cells were analyzed before (pre-sort) and after (post-sort) isolation. FSC, forward scattering.

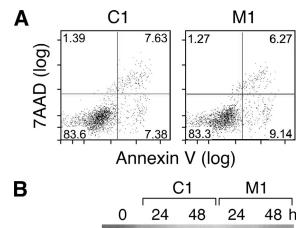


Figure S6. cIAP-1 and cIAP-2 degradation in unstimulated human CD4⁺ T cells treated with IAP antagonists. Immunoblot using the indicated antibodies on cytoplasmic lysates from unstimulated human CD4⁺ T cells exposed to M1 or a control compound for 2 h. Results are representative of at least two independent experiments.

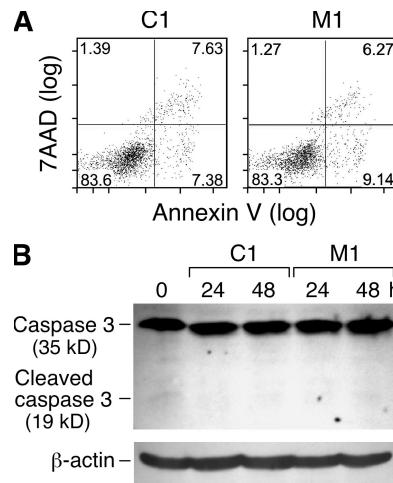


Figure S7. B16 cells are not responsive to IAP antagonist-mediated cytotoxicity in vitro. (A) B16 cells were irradiated with 3.5 krad and cultured for 24 h in the presence of IAP antagonists or control compound. Cells were analyzed by flow cytometry using staining with annexin V and 7AAD. (B) Immunoblot using the indicated antibodies on lysates from B16 cells irradiated and cultured as in A for the indicated period of time. Results are representative of two independent experiments.

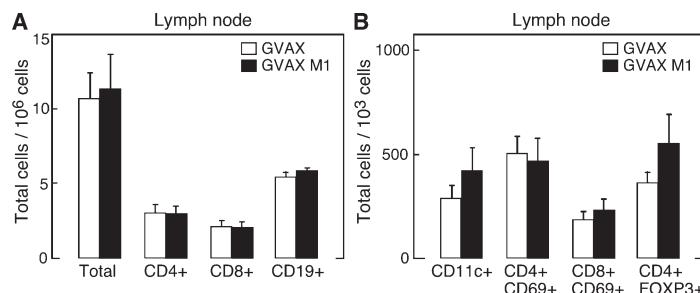


Figure S8. IAP antagonists do not lead to significant lymph node hyperplasia in combination with GVAX. Total cells and subpopulations identified by flow cytometry in the draining lymph nodes of mice vaccinated as in Fig. 8. Error bars represent SEM. Results are representative of two independent experiments with four mice per group.