c-IAP1 monoclonal antibody (1E1-1-10)

All members of the inhibitor of apoptosis proteins (IAP) family contain at least one, but usually three copies of baculovirus IAP repeat (BIR), a ~70-aa zinc binding domain, and upon overexpression suppress apoptosis. The BIR motif is capable of interacting with caspases and occluding their catalytic pockets. Certain IAPs also possess C-terminal RING domains. RING-containing proteins often act as E3 ubiquitin ligases and can catalyse the degradation of both, themselves and selected target proteins through ubiquitylation. So far, eight human IAPs have been identified: Apollon (Bruce), c-IAP1 (HIAP2; MIHB), c-IAP2 (HIAP1; MIHC), ILP-2 (Ts-IAP), Livin (ML-IAP; KIAP), NAIP, Survivin (TIAP) and XIAP (ILP-1; MIHA). c-IAP1 has been shown to inhibit specific caspases.

This antibody is covered by our Worry-Free Guarantee.

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ALX-803-335-C100	100µg

Manuals, SDS & CofA View Online »

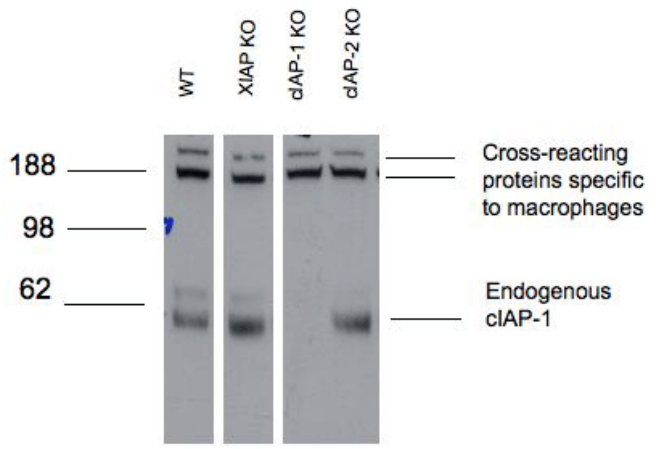


Figure 1: Western blot analysis of c-IAP1 in mouse bone marrow-derived macrophages of the indicated strains lysed in DISC buffer (150mM sodium chloride, 2mM EDTA, 1% Triton X-100, 10% glycerol, 20mM Tris pH 7.5) using MAb to c-IAP1 (1E1-1-10) (Prod. No. ALX-803-335) followed by HRP-anti rat antibody and developed by ECL.Molecular weight marker (kD) is shown at the left. Lanes have been digitally excised from a single blot to aid comparison.

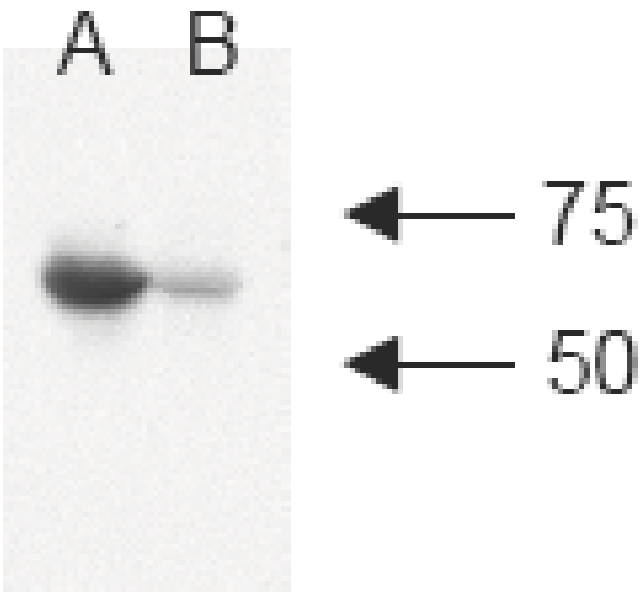


Figure 2: Western blot analysis of human c-IAP1 using MAb to c-IAP1 (1E1-1-10)A: MDA-MB-231 cell lysateB: MDA-MB-231 +LBW 1µM/1h cell lysate (LBW causes degradation of c-IAP1; A. Gaither, et al., 2007)(Image courtesy of Dr. Pascal Meier, The Institute of Cancer Research, London.)

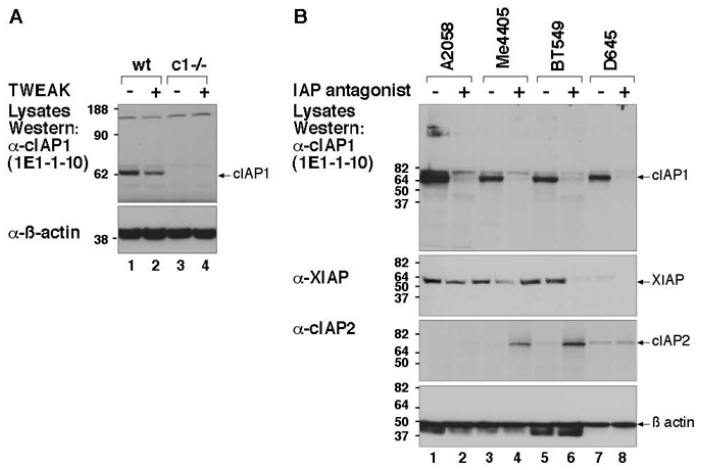
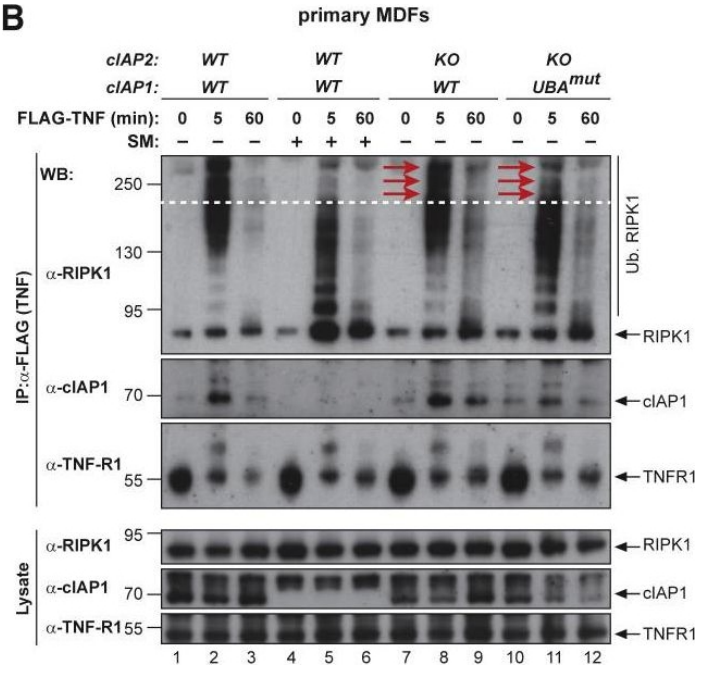
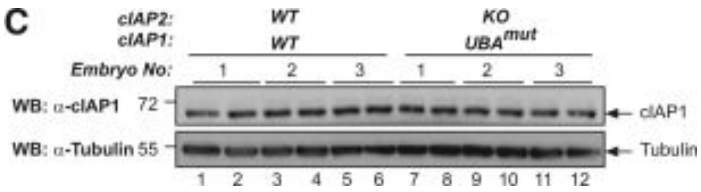


Figure 3: MAb to c-IAP1 (1E1-1-10) is specific for mouse and human c-IAP1. **A)** Wild type and c-IAP1-/- MEFs were treated +/- TWEAK for 6 hours and whole cell lysates prepared and separated on an SDS-PAGE gel, transferred to a PVDF membrane and probed with MAb to c-IAP1 (1E1-1-10). TWEAK causes a partial loss of c-IAP1. **B)** 4 human cell lines were treated overnight with an IAP antagonist that induces rapid degradation of c-IAP1. Cells were lysed with DISC lysis buffer and separated on an SDS-PAGE gel, transferred and probed with the MAb to c-IAP1 (1E1-1-10) as in A. XIAP and c-IAP2 blots demonstrate the specificity of the c-IAP1 antibody.



The UBA Directly Regulates RIPK1 Ubiquitylation(A and B) Purification of the TNF-R1 signaling complex (complex-I) from primary MEFs (A) and MDFs (B). Cells of the indicated genotypes were treated with FLAG-TNF for 0, 5, and 60 min. Cell lysates were then subjected to FLAG IP followed by western blot analysis with the indicated antibodies. Representative images of three independent experiments are shown.(C) Purification of the TNF-R1 signaling complex (complex-I) from immortalized cIAP2−/− and cIAP1UBAmut MEFs reconstituted either with empty vector (control) or a doxycycline-inducible construct encoding cIAP1UBAmut.



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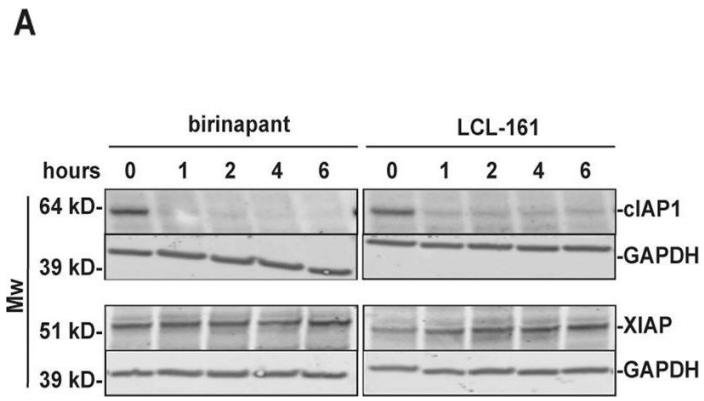
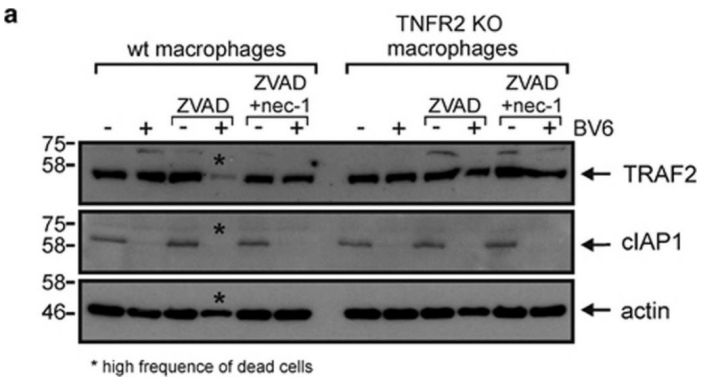
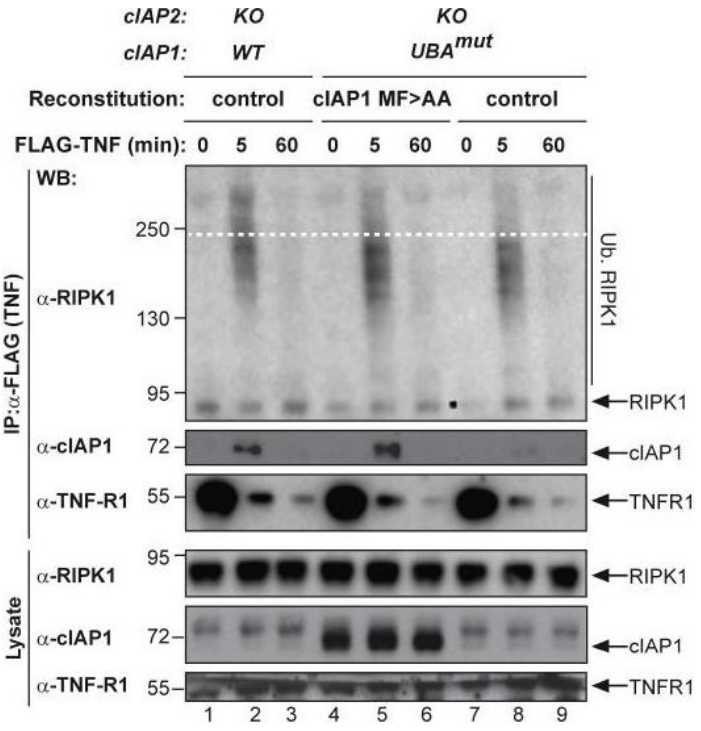


Birinapant and LCL-161 trigger TNF-dependent cell death in pre-OC.Pre-OC were treated with 1 μM birinapant or LCL-161 at the indicated time points, and cell lysates were analyzed for cIAP1 and XIAP protein levels by immunoblotting. GAPDH is loading control, shown for corresponding membranes. One representative of three donors is shown (A). Pre-OC were treated with birinapant (B) or LCL-161 (C) alone or in combination with 25 ng/ml TNF for 18 h and analyzed for cell death. 5 donors, mean, and SD are shown. Pre-OCs were treated with 1 μM of birinapant (D) or LCL-161 (E) in combination with the TNF-blocking antibody infliximab (0.1 μg/ml) and analyzed for cell death. Cell death was measured by LDH-release (B–E), 6 donors, mean and are shown (D–E). Viability of hOCP after treatment with indicated concentrations of LCL-161 (F) and birinapant (G) alone or in combination with 25 ng/ml TNF for 18 h. Results are technical triplicates from one experiment. Cell viability was measured with the Cell Titer Glo assay. Asterisks indicate groups significantly different from control (B, C, F, G) or between indicated groups (D, E, F, G) ($p < 0.05$, One-way ANOVA).



BV6 induces necroptosis in murine macrophages. (a) Wild type and TNFR2-deficient macrophages derived from HoxB8-immortalized MPCs were challenged for 7 h with the indicated combinations of BV6 (10 μM), zVAD-fmk (20 μM) and necrostatin-1 (45 μM). Cells were finally analyzed by western blotting for the presence of TRAF2 and cIAP1. Please note, wild-type cells treated with BV6 and zVAD-fmk were already largely dead when cells were harvested for Western blot analysis. (b) Macrophages derived from HoxB8-immortalized MPCs were stimulated in triplicates (technical replicates) with the indicated concentrations of BV6 in the presence and absence of 20 μM zVAD-fmk and analyzed for viability after 36 h. One of four representative experiments is shown. (c) HoxB8-immortalized MPC-derived macrophages were challenged with the indicated mixtures of 10 μM BV6, 20 μM ZVAD-fmk and 45 μM necrostatin-1 and analyzed for viability after 36 h. Shown are data points with S.E.M. of five independent experiments. (d–f) Macrophages derived from Hoxb8 immortalized MPCs of wild type, TNF- (d), TNFR1- (e) and TNFR2-deficient mice (f) were stimulated with the indicated combinations of BV6 (10 μM) and zVAD-fmk (20 μM). After 36 hours cell viability was quantified using MTT assay or crystal violet staining. Data points derived from six (d and e) or four (f) independent experiments together with mean±S.E.M. are depicted. ***p<0.001



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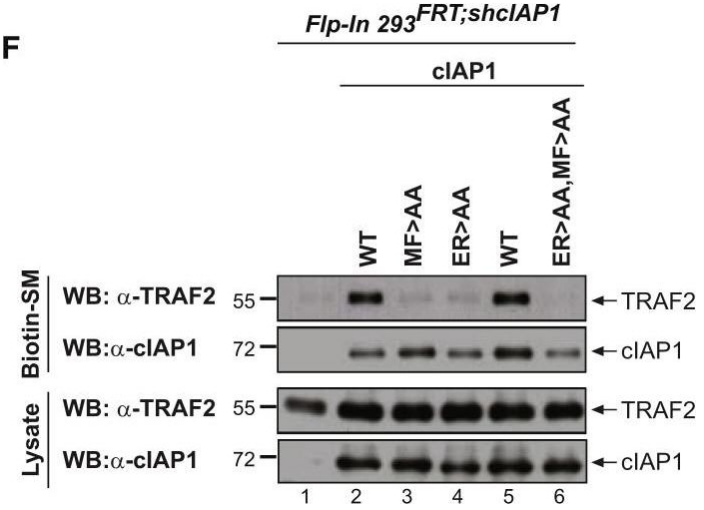
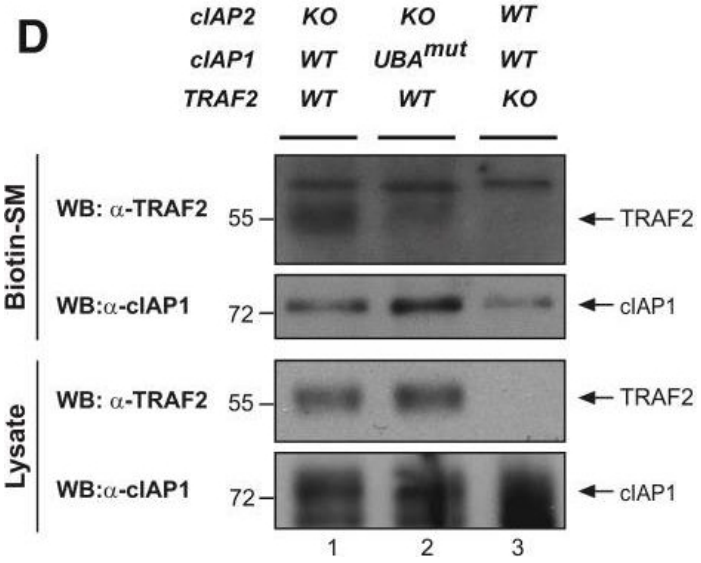


cIAP2 Requires a Functional UBA Domain to Efficiently Interact with TRAF2(A–C) Binding of the indicated cIAP2 fragments to TRAF2 was measured by isothermal titration calorimetry. KD, binding constant. Note the data shown in (A) are from Zheng et al. (2010) and are shown for the purposes of comparison only.(D) Schematic diagram of the Flp-InTMT-RExTM-HEK293shcIAP1 cell system in which endogenous cIAP1 was knocked down via inducible expression of mir30-based shRNA targeting cIAP1's 3′ UTR. These cells also carry a single FRT site that allows Flp-mediated integration of transgenes into the same transcriptionally regulatable genomic locus. Expression of the transgene and the mir30-based shcIAP1 are induced following treatment with doxycycline (Dox). TRE, tetracycline response element; UBC, ubiquitin promoter; FRT, flippase recognition target; Tet Op, tetracycline operator; Tet-R, tet repressor protein; rtTA3, reverse Tet transactivator (rtTA3).(E) Western blot analysis of Flp-In cells treated for 72 hr with Dox (100 ng/mL), to allow expression of the indicated transgenes, followed by treatment with the SMAC mimetic (SM) compound A (100 nM) for 6 hr.(F and G) Biotinylated SM was used to purify IAPs from lysates of Flp-In cells that were treated with Dox for 72 hr. TRAF2-binding was then assessed by immunoblotting. In parallel, expression levels of cIAP1 and TRAF2 were controlled by immunoblotting total cell lysates with the respective antibodies. Representative immunoblots are shown of three independent experiments.



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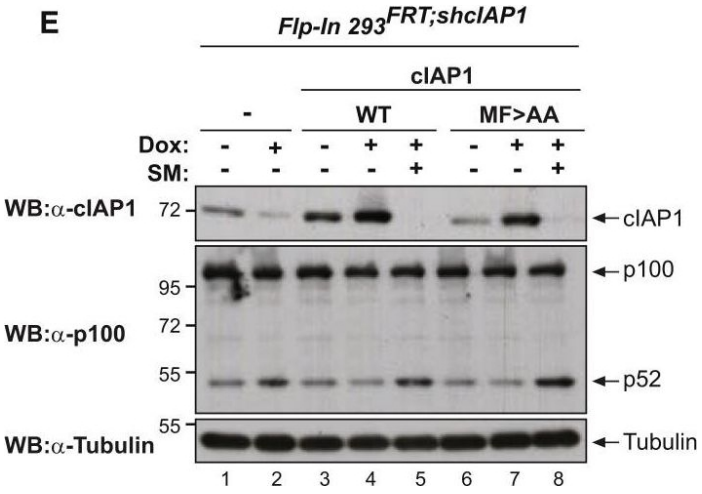

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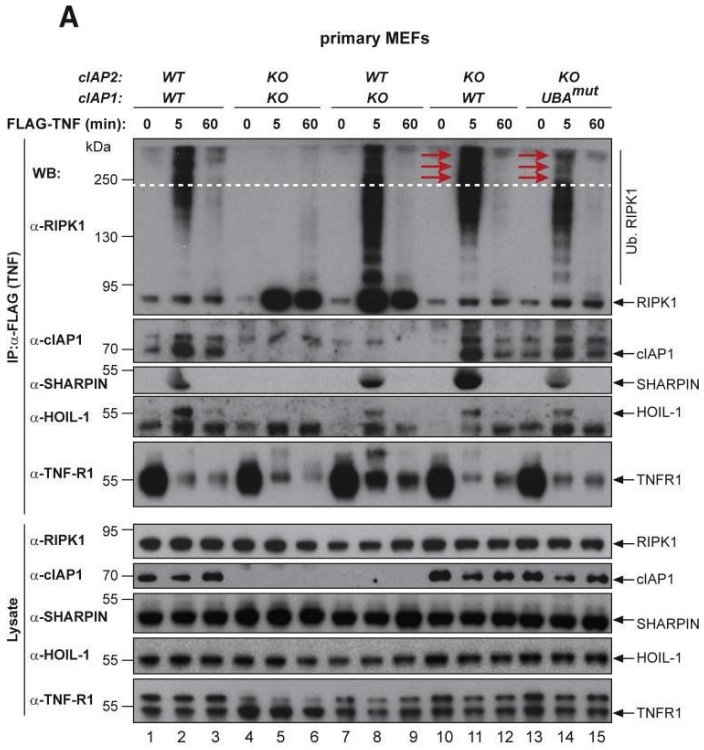

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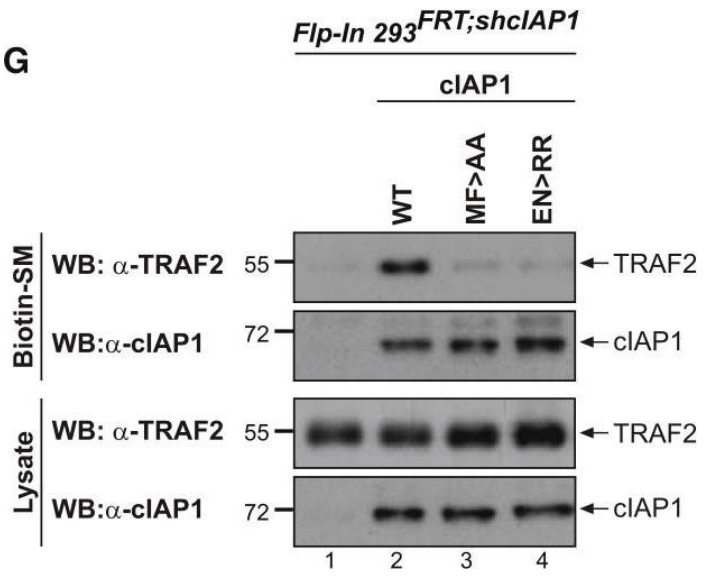

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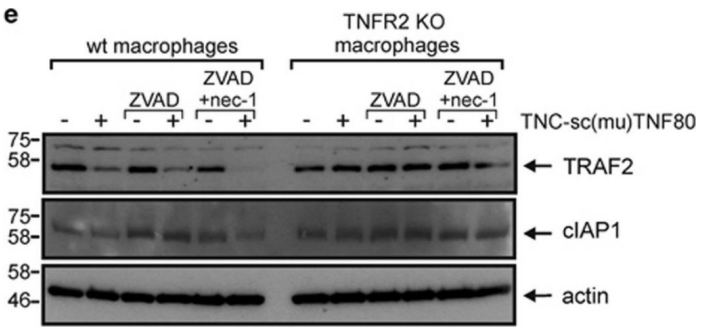
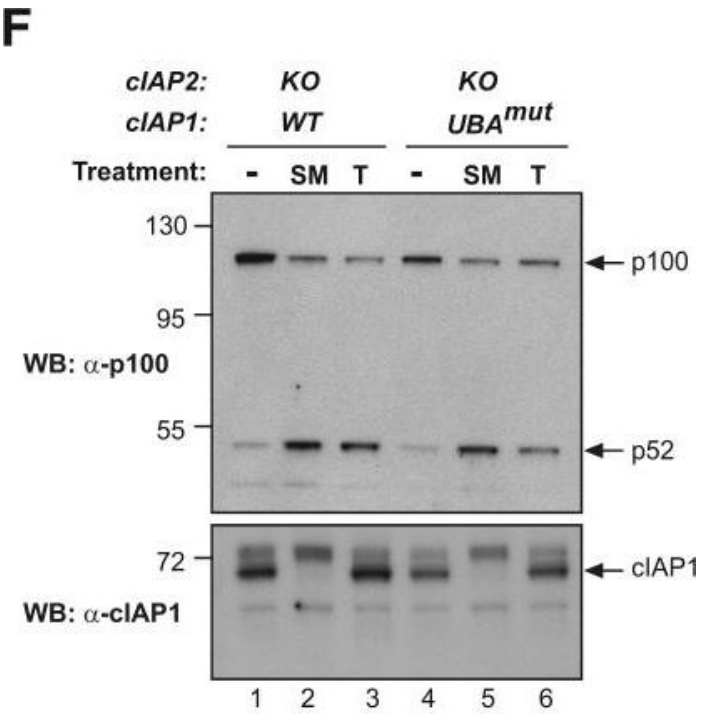


TNF and TNFR1 are required for zVAD-fmk/TNC-sc(mu)TNF80 induced cell death. (a) MPCs and macrophages derived thereof were analyzed by flow cytometry with for cell surface expression of the indicated proteins. (b,c) MPCs and macrophages derived from wild type, TNF- (b) and TNFR1-deficient mice (c) were stimulated with the indicated combinations of human TNF (50 ng/ml), TNC-sc(mu)TNF80 (200 ng/ml) and zVAD-fmk (20 μM). After 36 hours cell viability was quantified using the MTT assay or crystal violet staining. Data points derived from 9 (b) or 7 (c) independent experiments together with with mean±S.E.M. are depicted. (d) Macrophages derived from HoxB8-immortalized MPCs of wild type and TNFR2-deficient mice were stimulated overnight with the indicated combinations of TNC-sc(mu)TNF80 (200 ng/ml) and zVAD-fmk (20 μM). Tnf mRNA induction was analyzed by qPCR. Data points of four independent experiments together with mean±S.E.M. are shown. (e) Wild type and TNFR2-deficient macrophages were treated as indicated for 7 h with TNC-sc(mu)TNF80 (200 ng/ml), zVAD-fmk (20 μM) and necrostatin-1 (45 μM). Cells were analyzed by western blotting for the presence of TRAF2 and cIAP1. ***p<0.001



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Handling & Storage

Use/Stability	Stable for at least 12 months after receipt when stored at -80°C.
Handling	Avoid freeze/thaw cycles. After opening, prepare aliquots and store at -80°C.
Short Term Storage	+4°C
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	HIAP2, BIRC-2, Baculoviral IAP repeat-containing protein-2, Cellular inhibitor of apoptosis-1, Human inhibitor of apoptosis protein-2, Inhibitor of apoptosis protein-2
Application	WB
Application Notes	Detects a band of ~62kDa by Western blot. Not recommended for Immunohistochemistry or Immunoprecipitation.
Clone	1E1-1-10
Formulation	Liquid. 0.2µm-filtered solution in PBS containing 0.05% sodium azide.
Host	Rat
Immunogen	Synthetic peptide corresponding to aa 221-232 of mouse c-IAP1.
Isotype	IgG2a
Positive Control	Included. (ALX-803-335-POS. Wild type MEF lysate in SDS loading buffer). Negative control also included (ALX-803-335-NEG. c-Iap1-/- MEF lysate in SDS loading buffer.)

Recommendation Dilutions/Conditions	Western Blot (1:1,000-1:4,000 using ECL; suggested blocking and dilution buffer is PBST containing 0.1% Tween 20 and either 5% skim milk or 5% BSA. Suggested incubation time is 1 hour at room temperature or overnight at 4°C.)Suggested dilutions/conditions may not be available for all applications.Optimal conditions must be determined individually for each application.
Species Reactivity	Human, Mouse
UniProt ID	Q62210
Worry-free Guarantee	**This antibody is covered by our Worry-Free Guarantee .**

Last modified: May 29, 2024

ENZO LIFE SCIENCES, INC.
Phone: 800.942.0430
info-usa@enzolifesciences.com

European Sales Office
ENZO LIFE SCIENCES (ELS) AG
Phone: +41 61 926 8989
info-eu@enzolifesciences.com

Belgium, The Netherlands & Luxembourg
Phone: +32 3 466 0420
info-be@enzolifesciences.com

France
Phone: +33 472 440 655
info-fr@enzolifesciences.com

Germany
Phone: +49 7621 5500 526
info-de@enzolifesciences.com

UK & Ireland
Phone (UK customers): 0845 601 1488
Phone: +44 1392 825900
info-uk@enzolifesciences.com