Datasheet for 600-401-394S

Mcl-1 Antibody

Overview

Description:	Anti-Mcl-1 (RABBIT) Antibody - 600-401-394S
Item No.:	600-401-394S
Size:	25 µL
Applications:	ELISA, WB, FC, IF, IHC, IP
Reactivity:	Mouse
Host Species:	Rabbit

Product Details

Background:	Regulated apoptosis is essential for both the development and the subsequent maintenance of the immune system. Interleukins, including IL-2, IL-4, IL-7 and IL-15, heavily influence lymphocyte survival during the vulnerable stages of VDJ rearrangement and later in ensuring cellular homeostasis, but the genes specifically responsible for the development and maintenance of lymphocytes have not been identified. The Anti apoptotic protein Mcl-1 (myeloid cell leukemia sequence 1 (BCL2-related)) is an attractive candidate, as it is highly regulated, appears to enhance short-term survival and functions at an apical step in genotoxic deaths. However, Mcl-1 deficiency results in peri-implantation lethality. Mice, conditional for Mcl-1, display a profound reduction in B and T lymphocytes when Mcl-1 is removed. Deletion of Mcl-1 during early lymphocyte differentiation increases apoptosis and arrests the development at pro-B-cell and double negative T-cell stages. Induced deletion of Mcl-1 in peripheral B- and T-cell populations results in their rapid loss. Moreover, IL-7 both induces and requires Mcl-1 to mediate lymphocyte survival. Mcl-1 is essential both early in lymphoid development and later on in the maintenance of mature lymphocytes.
Synonyms:	rabbit anti-Mcl-1 antibody, Mcl1, Mcl 1, Bcl 2 related protein EAT/mcl1 antibody, Bcl2 related antibody, EAT antibody, Induced myeloid leukemia cell differentiation protein Mcl-1 antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	Mcl1

Reactivity:	Mouse
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was purified from whole rabbit serum prepared by repeated immunizations with a synthetic peptide corresponding to an internal region of mouse Mcl-1 conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	Anti-Mcl-1 affinity purified antibody was prepared from monospecific antiserum by immunoaffinity chromatography using synthetic peptide coupled to agarose beads followed by cross adsorption to remove any unwanted reactivity. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum. This antibody detects mouse Mcl-1 and is not expected to cross react with the human sequence. Cross reactivity with Mcl-1 from other sources is unknown.
Relevant Links:	• GeneID - 17210 • NCBI - P97287.3 • UniProtKB - P97287

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	FC, IF, IHC, IP (Based on references)
Application Note:	Anti-Mcl-1 Antibody has been tested by ELISA and western blot and is suitable for immunoprecipitation. This antibody detects mouse Mcl-1 and is not expected to cross react with the human sequence. Cross reactivity with Mcl-1 from other sources is unknown. This antibody is highly specific, showing no bands in null cell lysates from Mcl-1 knockout mice even when grossly over-exposed. Mouse Mcl-1 (myeloid cell leukemia sequence 1) is composed of 331 amino acids and is reported to be 35.2 kDa in size. The human ortholog consists of 351 amino acids and is reported to be 37.3 kDa in size.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
WB:	1:10,000

Formulation

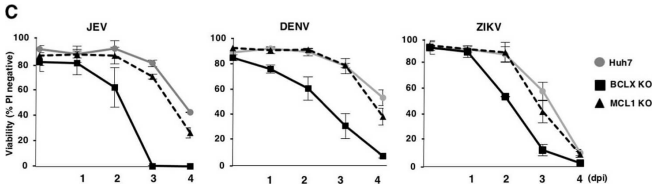
Physical State:	Liquid (sterile filtered)
Concentration:	0.95 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

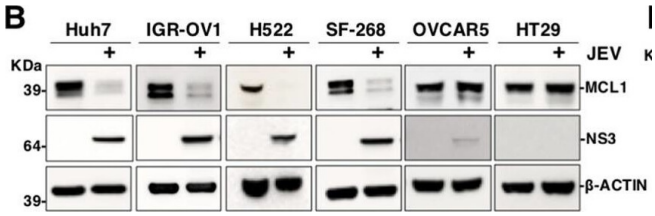
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Western Blot
BCLXL is a critical survival factor against flavivirus infection. (A) Expression of BCLXL, MCL1, BAK, and BAX in parental, BCLXKO and MCL1KO Huh7 cells was determined by immunoblotting. (B) Parental, BCLXKO and MCL1KO Huh7 cell lines were infected with lentiviruses expressing the indicated BIM-mutants, then cultured in the presence of puromycin. Remaining adherent cells were visualized by Giemsa staining. (C) Deficiency of BCLXL accelerates cell death upon infection with flaviviruses. Parental, BCLXKO and MCL1KO Huh7 cell lines were infected with JEV (MOI = 5), DENV (MOI = 3) or ZIKV (MOI = 1). Cell viability was assessed at the indicated time points. (D) Parental and BCLXKO Huh7 cell lines were infected with JEV (100 FFU), DENV (100 FFU) or ZIKV (500 FFU). At 2 days post-infection, the cells were stained with methylene blue or Giemsa staining. Images are representative of two independent experiments performed with a culture representative of each cell line (A,B,D). The data represent the mean ± SD of three independent experiments performed with a culture representative of each cell line (C). Figure provided by CiteAb. Source: PLoS Pathog, PMID: 30261081.



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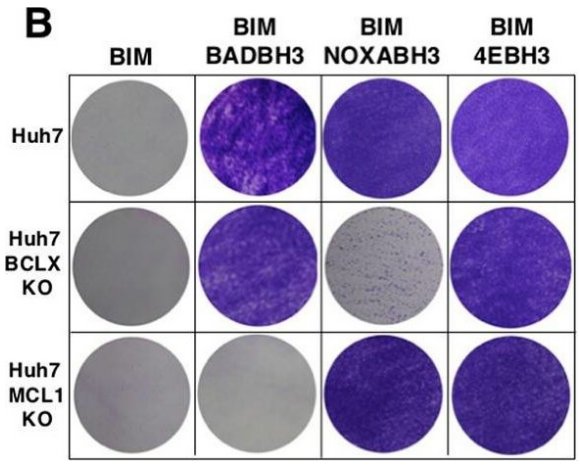
Viral propagation is required for the induction of cell death through suppression of MCL1 expression.(A) Susceptibility of human cell lines to JEV. Human cell lines were infected with JEV (MOI = 5) and infectious titers were determined by focus-forming assay at 3 days post-infection. (B) JEV-susceptible cell lines induce suppression of MCL1 expression. upon infection. Lysates from human cell lines infected with JEV (MOI = 5) were subjected to immunoblotting at 2 days post-infection. (C) Treatment with ABT-737 induces cell death in the susceptible cell lines infected with JEV. Human cell lines were infected with or without JEV (MOI = 5) in the absence or presence of ABT-737 (1 μM) and cell viability was assessed at 3 days post-infection. (D) Dose-dependent suppression of MCL1 expression by JEV infection. Huh7 cells infected with JEV, at an MOI of 10 to 100, were incubated with the caspase inhibitor, QVD-OPH (20 μM), for 1 day. Cell lysates were subjected to immunoblotting. (E) Interferon treatment attenuates JEV-induced suppression of MCL1 expression Huh7 cells infected with JEV (MOI = 5) were treated with interferon-α (IFNα). Cell lysates were subjected to immunoblotting at 2 days post-infection. (F) Interferon treatment attenuates JEV-induced cell death. Huh7 cells were infected with JEV (MOI = 5) and treated with ABT-737 (1 μM) and IFNα for 3 days. Cell viability was assessed at 3 days post-infection. (G, H) U937 cells were infected with JEV (MOI = 20), DENV (MOI = 20) and ZIKV (MOI = 20). (G) Infectious titers were determined by focus-forming assay at indicated time points, and (H) expression of MCL1 and NS3 was determined by immunoblotting at 2 days post-infection. (I) U937 cells infected with ZIKV (MOI = 20) were treated with/without ABT-737 (1 μM) or A-1331852 (1 μM) and cell viability was determined at 3 days post-infection. The data represent the mean ± SD of two (A, C, F, G) or three (I) independent experiments. Images are representative of two independent experiments (B, D, E). Significant differences were determined using Student's t-test and are indicated with asterisks (*P<0.05) and double asterisks (**P<0.01). Figure provided by CiteAb. Source: PLoS Pathog, PMID: 30261081.



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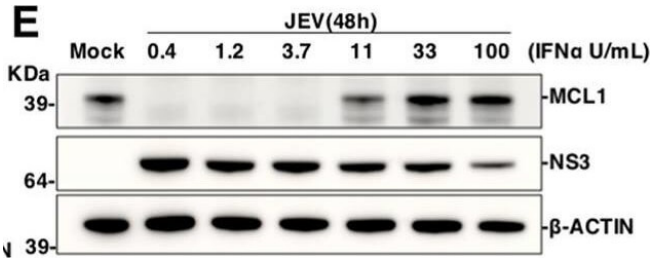

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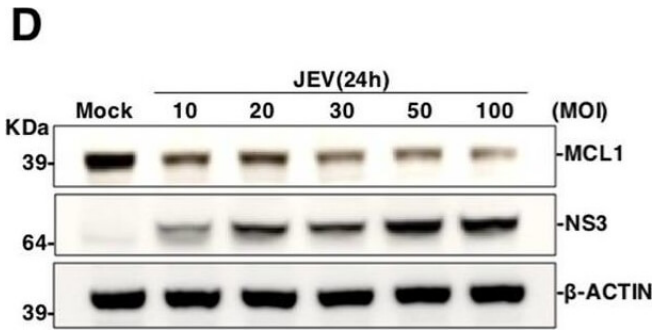

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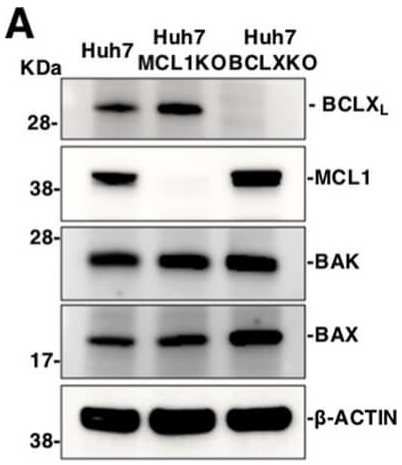

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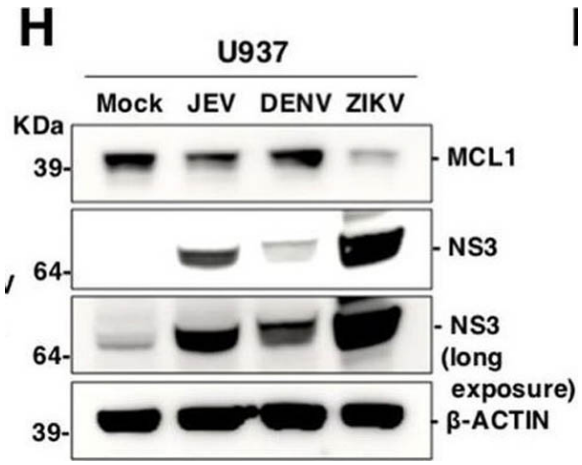

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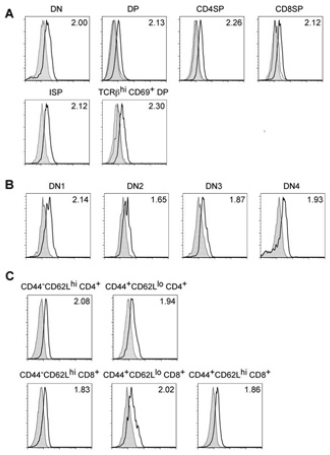
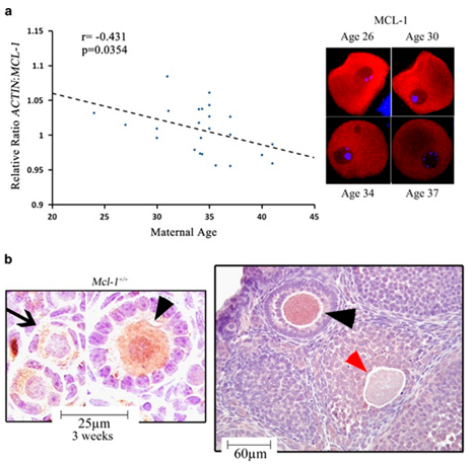


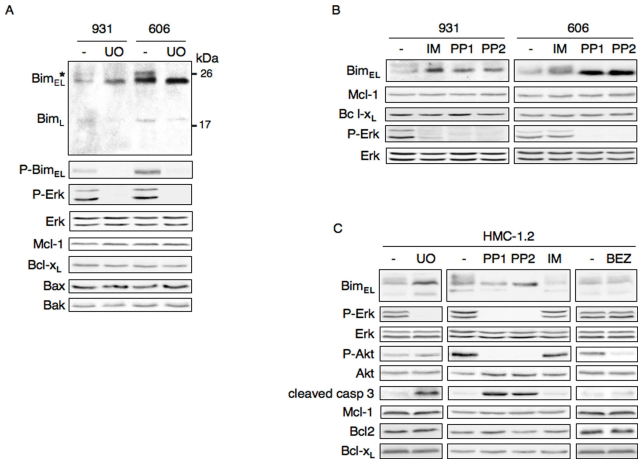
Flow Cytometry

A. Expression of Mcl-1 (thick lines) in double-negative CD4−CD8−CD3− (DN), double-positive CD4+CD8+ (DP), CD4 single-positive CD4+CD8−TCRβhi (CD4SP), CD8 single-positive CD4−CD8+TCRβhi (CD8SP), immature single-positive CD4−CD8+TCRβ − (ISP), and positively selected DP CD4+CD8+CD69+TCRβhi thymocytes as assessed by FACS. B. Expression of Mcl-1 (thick lines) in different DN subpopulations: DN1 – CD44+CD25− DN2 – CD44+CD25+; DN3 – CD44−CD25+; DN4 – CD44−CD25− as assessed by FACS. C. Expression of Mcl-1 (thick lines) in peripheral T cells: naïve (CD44−CD62Lhi) CD4+ or CD8+ T cells; activated/effector memory (CD44+CD62Llo) CD4+ or CD8+ T cells and central memory (CD44+CD62Lhi) CD8+ T cells as assessed by FACS. (A–C) The shaded histograms depict isotype control staining. For DP, CD4SP, CD8SP, and positively selected DP cells the thin lines represent staining for Mcl-1 in these cells from Mcl-1f/fCD4-Cre mice. The numbers represent the ratios of the fluorescence intensity of Mcl-1 staining divided by the fluorescent intensity of the isotype control staining.
Data are representative of four individual experiments. Mcl-1 was detected with polyclonal rabbit anti-Mcl-1 antibody (p/n 600-401-394) at 0.1 µg/106 cells in permeabilization buffer for 1 h on ice, with normal rabbit immunoglobulin as an isotype control, and donkey anti-rabbit-FITC as the secondary antibody. Fig 1.
PMID: 18566418



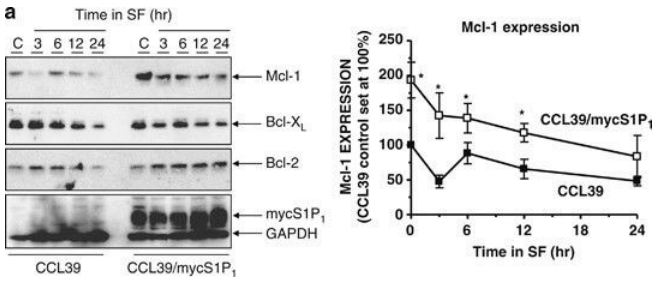
Immunohistochemistry

Patterns of MCL-1 expression in human and murine oocytes. (a) Age-associated changes in MCL-1 transcript (left) and MCL-1 protein immunoreactivity (right) in human GV oocytes from different patients. Pearson analysis revealed a negative correlation between MCL-1 transcript level and maternal age (n=24; r=−0.431; P=0.035). (b) Immunohistochemical staining of MCL-1 (brown) in 3-week ovarian sections of wild-type mouse ovaries, counterstained with hematoxylin (blue). PMF oocytes (arrows), PF (PF) and PA follicles (arrowheads), and oocytes undergoing early stages of atresia (red arrowhead). At least three ovaries were stained per age group with identical expression patterns. Fig 1. PMID: 25950485



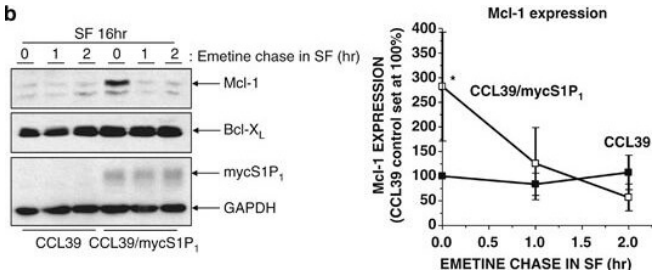
Western Blot

The down-regulation of Bim protein occurs downstream of the activation of Erk1/2 and Kit mutant in Kit-driven leukemic cells.(A) Whole cell extracts from 606HS2 and 931HS2 cells treated or not (–) for 4 h with the Mek inhibitor UO126 (10 µM) were fractionated by SDS-PAGE and immunoblotted using antibodies raised against the phosphorylated forms of Erk1/Erk2(Thr202-Tyr204) and Bim (Ser65) and the constitutive form of Bim, Erk1/Erk2, Mcl1 and BclXL,. *indicate the band corresponding to the phosphorylated form of BimEL. Molecular weight standards are indicated on the right. (B) Whole cell extracts from 606HS2 and 931HS2 cells treated or not (–) for 4 h with Kit inhibitors PP1 (4 µM), PP2 (4 µM) and Imatinib Mesilate (IM: 0.75 µM) were immunoblotted using indicated antibodies. Data are a representative experiment out of three. (C) Whole cell extracts from human HMC-1.2 cells treated or not (–) for 4 h with Mek inhibitor UO126 (15 µM) or Kit inhibitors IM (10 µM), PP1 (10 µM) and PP2 (10 µM) or PI3K inhibitor NVP-BEZ235 (25 nM) were analyzed by immunoblotting using indicated antibodies. Data are a representative experiment out of three. Figure provided by CiteAb. Source: PLoS One, PMID: 23145067.



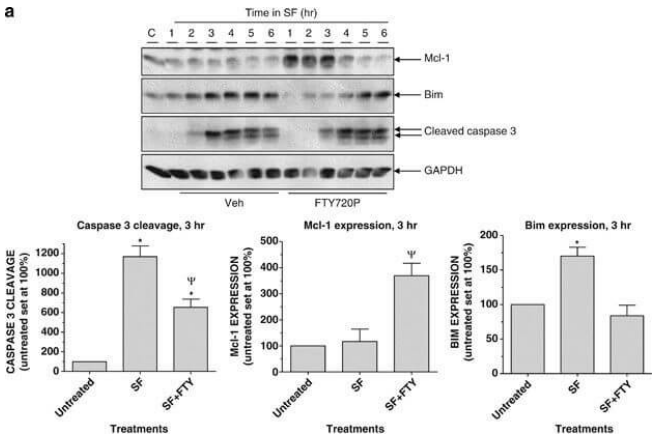
Western Blot

S1P1 regulates expression of pro-survival protein Mcl-1. (a) Control and S1P1-expressing CCL39 cells were switched to serum-free medium (SF) for the indicated times before preparation of detergent-soluble cell extracts. Samples were equalised for protein content before fractionation via SDS-PAGE and subsequent immunoblotting with the indicated antibodies. Quantitation of Mcl-1 expression normalised to GAPDH in control and S1P1-expressing CCL39 cells is presented as mean values±S.E. for n=3 separate experiments. *P<0.05 versus CCL39 control cells at the indicated time point. (b) Control and S1P1-expressing CCL39 cells were switched to either SF medium or fresh growth medium (C) for 16 h in the presence of LY294002 (LY, 20 μM) or GF109203X (5 μM) either alone or in combination, or a vehicle control for 16 h before preparation of cell extracts, fractionation via SDS-PAGE and subsequent immunoblotting with the indicated antibodies. Quantitation of Mcl-1 expression normalised to GAPDH in control and S1P1-expressing CCL39 cells is presented as mean values±S.E. for n=3 separate experiments. *P<0.05 versus CCL39 control cells, ΨP<0.05 versus vehicle-treated CCL39/mycS1P1 cells Figure provided by CiteAb. Source: Cell Death Dis, PMID: 24263101.



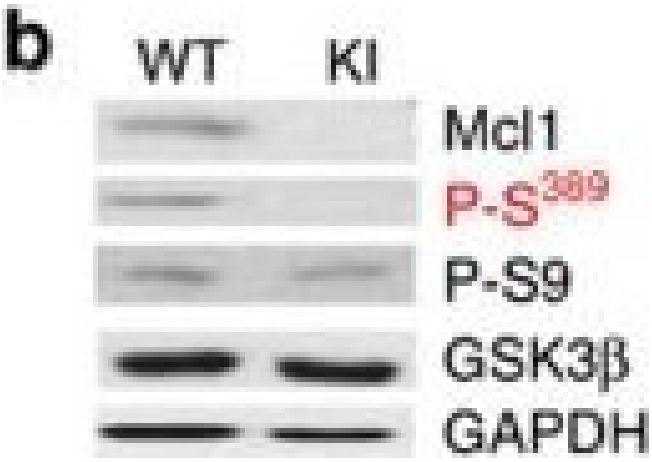
Western Blot

The enhanced survival capacity of S1P1-expressing cells requires new protein synthesis and is lost upon Mcl-1 downregulation. (a) Control and S1P1-expressing CCL39 cells were switched to serum-free medium (SF) for 16 h before washing and incubation in SF for the indicated times in the presence of protein synthesis inhibitor emetine (100 µM). Cell extracts were then prepared for fractionation via SDS-PAGE and subsequent immunoblotting with the indicated antibodies. Quantitation of cleaved caspase-3 and Bim expression normalised to GAPDH in control and S1P1-expressing CCL39 cells is presented as mean values±S.E. for n=3 separate experiments. *P<0.05 versus CCL39 control cells. (b) Control and S1P1-expressing CCL39 cells were switched to SF medium for 16 h before washing and incubation in SF for the indicated times in the presence of protein synthesis inhibitor emetine as described in (a). Cell extracts were then prepared for fractionation via SDS-PAGE and subsequent immunoblotting with the indicated antibodies. Quantitation of Mcl-1expression normalised to GAPDH in control and S1P1-expressing CCL39 cells is presented as mean values±S.E. for n=3 separate experiments. *P<0.05 versus CCL39 control cells Figure provided by CiteAb. Source: Cell Death Dis, PMID: 24263101.



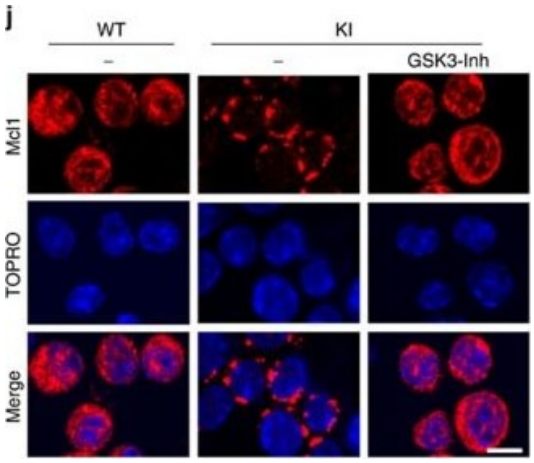
Western Blot

Activation of endogenous S1P receptors by FTY720P induces Mcl-1 to delay the onset of apoptosis in vascular ECs following growth factor withdrawal. (a) HUVECs were switched to serum- and growth factor-free medium in the presence or absence of FTY720P (0.1 μM) for the indicated times before preparation of detergent-soluble cell extracts. Samples were equalised for protein content and fractionated via SDS-PAGE for subsequent immunoblotting with the indicated antibodies. Quantitation of caspase-3 cleavage, Bim expression and Mcl-1 expression at the indicated times normalised to GAPDH is presented as mean values±S.E. for n=3 separate experiments. *P<0.05 versus cells maintained in normal growth medium, ΨP<0.05 versus growth factor-deprived cells treated with vehicle. (b) HUVECs were switched to serum- and growth factor-free medium in the presence or absence of FTY720P (0.1 μM) for 3 h before preparation of detergent-soluble cell extracts. Samples were equalised for protein content and fractionated via SDS-PAGE for subsequent immunoblotting with the indicated antibodies. Quantitation of caspase-3 cleavage and Mcl-1 expression normalised to GAPDH is presented as mean values±S.E. for n=3 separate experiments. *P<0.05 versus cells treated without FTY720P at the same time point, ΨP<0.05 versus corresponding control siRNA-treated samples Figure provided by CiteAb. Source: Cell Death Dis, PMID: 24263101.



Western Blot

Inactivation of GSK3β by p38 MAPK promotes accumulation of the prosurvival factor Mcl-1.(a,b) WT and GSK3β-KI B cells were activated for two days and the expression of β-catenin (a), Mcl-1, P-S389 GSK3β and total GSK3β (b) were examined by western blotting. GAPDH is shown as a loading control. (c) WT and GSK3β-KI B cells were activated as in a and Bclx, Bcl2, Bid, Bax and Bad levels were examined by western blotting. (d) Western blot analysis for PUMA in irradiated WT thymocytes (5 Gy) and activated WT and GSK3β-KI B cells. (e,f) B cells activated as in a were stained with MitoTracker (e) or TMRE (f) and analysed by flow cytometry. The number represents the percentage of cells within the gate. (g) WT and GSK3β-KI B cells were examined by western blotting for the expression of cleaved caspase-3 (activated caspase-3), full length RIPK1 (RIPK1) and cleaved RIPK1 (cleaved RIPK1). (h) WT and GSK3β-KI B cells were activated, after 2 days Nectrostatin-1s (Nec-1s) was added and cell viability was determined by cell counting 24 h later (n=3). (i) WT and GSK3β-KI B cells were activated for 3 days and phospho-MLKL was examined by western blotting. (j) WT and GSK3β-KI B cells in the presence or absence of the GSK3 inhibitor (GSK3-Inh) were activated and examined by immunostaining and confocal microscopy for Mcl-1 (red) and TOPRO nuclear stain (blue). Scale bar, 3 µm. (k) Mcl-1 levels in nuclear (Nuc) and mitochondrial (Mito) extracts from activated WT and GSK3β-KI B cells were determined by western blot analysis. Histone and CoxIV (Complex IV) were used as loading controls. (l) WT and GSK3β-KI B cells were transduced with either an empty retrovirus (E), a retrovirus expressing wildtype Mcl-1 (Mcl) or a retrovirus expressing a Ser140Ala mutant of Mcl-1 (mMcl). Three days after activation cell viability was determined by cell counting. (n=3) and *P value<0.05 as determined by t- test (h) or one-way ANOVA (l). Data are representative of three or more independent experiments. Figure provided by CiteAb.
Source: Nat Commun, PMID: 26822034.



Immunocytochemistry

Inactivation of GSK3β by p38 MAPK promotes accumulation of the prosurvival factor Mcl-1.(a,b) WT and GSK3β-KI B cells were activated for two days and the expression of β-catenin (a), Mcl-1, P-S389 GSK3β and total GSK3β (b) were examined by western blotting. GAPDH is shown as a loading control. (c) WT and GSK3β-KI B cells were activated as in a and Bclx, Bcl2, Bid, Bax and Bad levels were examined by western blotting. (d) Western blot analysis for PUMA in irradiated WT thymocytes (5 Gy) and activated WT and GSK3β-KI B cells. (e,f) B cells activated as in a were stained with MitoTracker (e) or TMRE (f) and analysed by flow cytometry. The number represents the percentage of cells within the gate. (g) WT and GSK3β-KI B cells were examined by western blotting for the expression of cleaved caspase-3 (activated caspase-3), full length RIPK1 (RIPK1) and cleaved RIPK1 (cleaved RIPK1). (h) WT and GSK3β-KI B cells were activated, after 2 days Nectrostatin-1s (Nec-1s) was added and cell viability was determined by cell counting 24 h later (n=3). (i) WT and GSK3β-KI B cells were activated for 3 days and phospho-MLKL was examined by western blotting. (j) WT and GSK3β-KI B cells in the presence or absence of the GSK3 inhibitor (GSK3-Inh) were activated and examined by immunostaining and confocal microscopy for Mcl-1 (red) and TOPRO nuclear stain (blue). Scale bar, 3 µm. (k) Mcl-1 levels in nuclear (Nuc) and mitochondrial (Mito) extracts from activated WT and GSK3β-KI B cells were determined by western blot analysis. Histone and CoxIV (Complex IV) were used as loading controls. (l) WT and GSK3β-KI B cells were transduced with either an empty retrovirus (E), a retrovirus expressing wildtype Mcl-1 (Mcl) or a retrovirus expressing a Ser140Ala mutant of Mcl-1 (mMcl). Three days after activation cell viability was determined by cell counting. (n=3) and *P value<0.05 as determined by t- test (h) or one-way ANOVA (l). Data are representative of three or more independent experiments. Figure provided by CiteAb.
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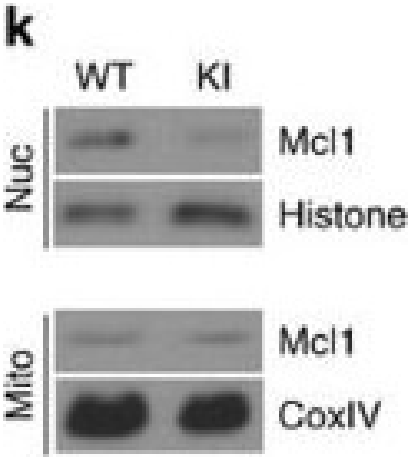

Western Blot

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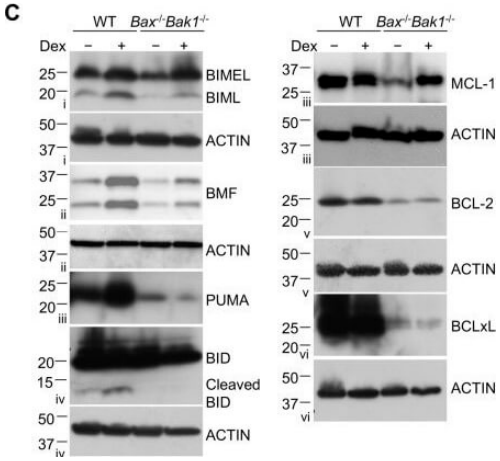
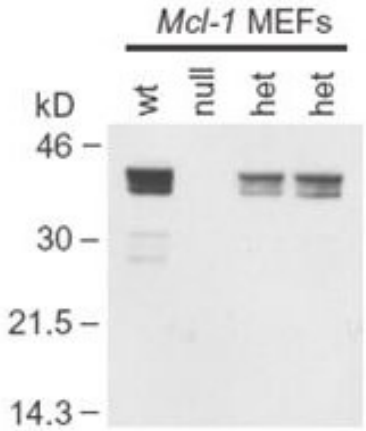


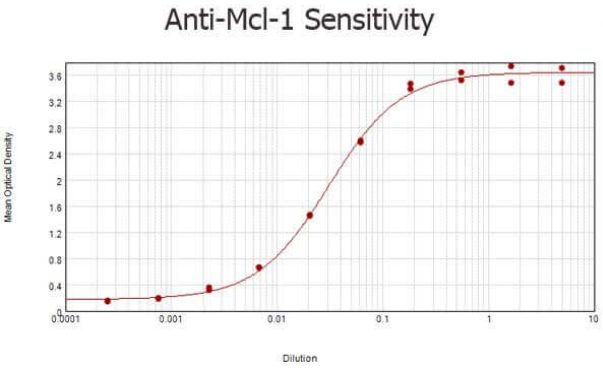
Western Blot

Characterization of clonal lymphoid lines mutant for combinations of pro-apoptotic BCL2 family proteins.a Whole-cell lysates from Bax−/−Bak1−/− and three independent Bax−/−Bak1−/−Bim−/− cell clones treated with 1 µM Dex treatment for 24 hrs were subjected to western blot analysis to detect BIM protein. Upper panel: WEHI7 mutant lines; lower panel: p53−/− T lymphoma mutant lines. bBax+/+Bak1+/+ WEHI7 cells expressing Cas9 were transduced with sgRNAs targeting mouse Bim, Bmf and Puma. Following treatment with doxycycline to induce sgRNA expression, clones were isolated and validated for absence of BIM, BMF, and PUMA by western blotting after 24 hrs treatment with 1 µM Dex. c Wild type (WT) and Bax−/−Bak1−/− WEHI7 cells were treated for 24 hrs with 1 µM Dex and lysates were run on replicate gels and analyzed by western blot using antibodies specific for the indicated BCL2 family proteins. Roman numerals to the left of blots (i–vi) indicate the membranes probed. d Whole-cell lysates from Bax+/+Bak1+/+ and Bax−/−Bak1−/−p53−/− T lymphoma cells treated with 1 µM Dex for 24 hrs were tested by western for expression of BIM. Note that the absence of protein in the Dex-treated WT p53−/− T lymphoma cells is due to the death of most of the cells by 24 hours. Figure provided by CiteAb. Source: Cell Death Dis, PMID: 32513923.

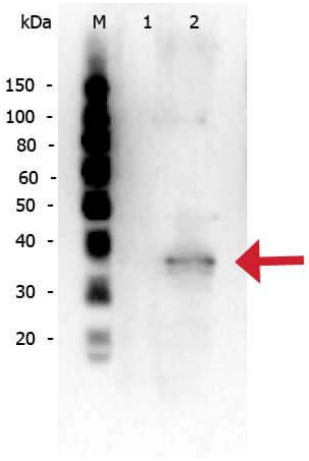


Western Blot

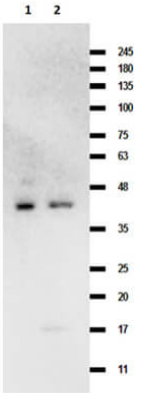
Western blot analysis using Rockland's anti-Mcl-1 antibody to detect Mcl-1 in MEF cell lysates (20 µg per lane). Anti-Mcl-1 was used at a dilution of 1:10,000 followed by reaction with HRP Anti-Rabbit IgG [H&L] MX10 (GOAT) used at a 1:2,000 dilution. Western Lightning Chemiluminescence Reagent Plus from Perkin Elmer was used for detection. The exposure time was exactly 30 seconds. This antibody detects 35 kDa mouse Mcl-1. Communicated with permission by J. Opferman and S. Korsmeyer. See Opferman et al (2003) for additional details.



ELISA
ELISA results of purified Rabbit anti-Mcl-1 Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of conjugate. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).



Western Blot
Western Blot of Rabbit anti-Mcl-1 antibody. Lane 1: MEF depleted Mcl-1 lysate. Lane 2: MEF WT Lysate. Load: 15µg per lane. Primary antibody: Mcl-1 antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:40,000 for 30 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting (MB-070) for 30 min at RT. Predicted/Observed size: 37 kDa, 37 kDa for Mcl-1.



Western Blot
Western Blot of Rabbit Anti-Mcl-1 Antibody. Lane 1: Ramos Lysate (p/n W09-001-GK4). Lane 2: Raji Lysate (p/n W09-001-368). Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Load: 10µg/lane. Primary Antibody: Anti-Mcl1 at 1µg/mL overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG Peroxidase (p/n 611-103-122) at 1:70,000 for 30 mins at RT. Block: Casein (p/n MB-082) for 30 mins at RT. Predicted MW: ~37kDa. Exposure: 30sec.

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