**Supplementary data**

**Aiolos collaborates with Blimp-1 to regulate the survival of multiple myeloma cells**

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**Supplementary Materials and Methods**

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## SUPPLEMENTARY MATERIALS AND METHODS

### Mass spectrometric analysis

The immunoprecipitated Blimp-1 complexes were separated by NuPAGE Tris-Acetate 3%-8% Pre-Cast Gel System (Invitrogen), and then the gel was subjected to silver stain (G-Biosciences). The excised bands were destained and used for trypsin digestion, followed by Nano-liquid chromatography–tandem mass spectrometry (Nano-LC-MS/MS). The procedures and condition of Nano-LC-MS/MS were described as previously [1]. For data analysis, all MS/MS spectra were converted to the mxXML format from the RAW file format by *MM File Conversion Tools* and the database search was then carried out by *MassMatrix* (http://www.massmatrix.net).

### Immunoprecipitation and immunoblot analysis

Lysates harvested from 293T cells transfected with various amounts of expression plasmids (1 to 2 μg) by PEI method as described previously [2]. Pre-cleared nuclear extracts or total extracts prepared according to a previous protocol [2] were immunoprecipitated by the indicated antibodies, followed by incubation with protein G or protein A beads (Santa Cruz Biotechnology). Precipitated protein complexes were eluted by boiling in Laemmli buffer. Primary antibodies used in this study are: mouse anti-Blimp-1 antibody (1:500 dilution)[3], mouse anti-FLAG antibody (1: 2,000 dilution; Sigma), rabbit anti-HA antibody (1:1,000 dilution; Sigma), rabbit anti-Aiolos antibody (1:1,000 dilution; Santa Cruz), mouse anti-β-actin antibody (1:5,000 dilution; Sigma), anti-His antibody (1:1,000 dilution; Bioman), anti-GST (1:2,500 dilution; Amersham Biosciences), anti-CUL4A antibody (1:1000 dilution; Abcam), anti-Ikaros antibody (1:200 dilution; Santa Cruz), anti-CRBN (1:1000 dilution; Abcam) and anti-Ub (1:400 dilution; Santa Cruz). Secondary antibodies used in this study are goat anti-mouse IgG HRP conjugate (1:5,000 dilution; Sigma) and goat anti-rabbit IgG HRP conjugate (1:10,000 dilution; Sigma). Actin blot was used as the loading control. The immunoreactive proteins were detected by the enhanced chemiluminescence system (Amersham Biosciences) according to the manufacturer's protocol. Chemiluminescent signal images were captured using the Fujifilm LAS 3000 system.

### ChIP and re-ChIP

A total of $1 \times 10^7$ H929 cells were used per Aiolos (Santa Cruz Biotechnology), or Blimp-1 ChIP assay. Anti–Blimp-1 was generated by inoculating a rabbit with a protein fragment spanning the N-terminal region of Blimp-1 (residues 1–217) followed by affinity purification

of the antisera by LTK BioLaboratories in Taiwan. The detailed ChIP procedures have been described [2]. For re-ChIP assays, $3 \times 10^7$ H929 cells were used in the first ChIP with anti–Blimp-1. Precipitated chromatin complexes were eluted with a buffer containing 50 mM Tris-HCl, 10 mM EDTA, 10 mM dithiothreitol, and 0.2% SDS at 37°C for 30 min and then diluted 20 fold in a buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5 mM EGTA, 100 mM NaCl, protease inhibitor cocktail (Roche), and 1% (w/v) Triton X-100. The second IP was performed with 5 μg anti-Aiolos. DNA in the IP was quantified with real-time PCR (QPCR) using the SYBR green method on an ABI Prism 7300 system using primers that specifically amplified fragments encompassing the Blimp-1–binding site or within 100 bp of the Blimp-1–binding site at each individual gene locus. Data obtained from QPCR were normalized to input chromatin. In some experiments, a *CIITA* 3′ untranslated region (UTR) was amplified as a negative control locus. The primer sequences used in this study are: CIITA forward, 5’-GCCACCTTGCAGGGAGAGT-3’ and reverse, 5’-AAGCTAAGCCAACATGCAAAGAA-3’, CIITA 3'UTR forward, 5’-CCATCATGTCTGGCTAATTTTTCA-3’ and reverse, 5’-GGATCACCTGAGGTCAAGAGTTTG-3’, ASK1 forward, 5’-GTACGGGAACGATGACATACCA-3’ and reverse, 5’-CATCACATGGCATGCAAATTAA-3’, TRAIL forward, 5’-TAATGATCTGACTATTGCC-3’ and reverse, 5’-CCTTAAACACTCTTTGAAGG-3’, Noxa forward, 5’-TTGCATCAGACGATTATACG-3’ and reverse, 5’-ACGCCCCAGGAGCTCTA-3’, KLF10 forward, 5’-GGATAGAAATGCTATTTGC-3’ and reverse, 5’-TGTCTCTCCTTCGAGCAC-3’, IFIT3 forward, 5'-GGACCCCAGCTTTTCAGAACT-3’ and reverse, 5'-ATCAGAGAAGCAGGGACTATTTACCT-3’, PARP8 forward, 5'-CCCCTTCGGCTCCCTCTAG-3’ and reverse, 5'- GAATCCGAGGAATCCCTTCTCT-3’, BLNK forward, 5’-TGACCAGCCAGAAACTTGTCCTA-3’ and reverse, 5’-TCTTTCCAGCTGCTTTCTTTTACA-3’, HDAC7 forward, 5’-ATATATGGCAAAGCCTGG-3’ and reverse, 5’-AAGGCCATCCCATTCCTC-3’, IRF2 forward, 5’-TTCAGCCAAGTACGAGCAAGTG-3’ and reverse, 5’-TTCCTCGACGTGTGCAAGAC-3’, FBXL11 forward, 5’-TTCTGATTACCGGTGAGAGAGAACT-3’ and reverse, 5’-CCAGGTGAAGCACCACTGAA-3’.

**DNA Pull-down Assay**

The DNA pull-down assay was performed essentially as reported [4] using bacterially expressed recombinant His-fusion proteins including His6–tBlimp-1 (△1–505) and His6-Aiolos. The purified recombinant proteins were incubated with biotinylated wild-type or mutated oligonucleotides coupled with streptavidin-conjugated Dynabeads (Invitrogen) overnight at 4°C in a buffer containing 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM EDTA, 1 mM $MgCl_2$, 5% glycerol, and 0.3% (w/v) NP40. The DNA-protein complexes were collected with a magnetic apparatus and further analyzed with immunoblotting. The oligonucleotide sequences are: *MYC*-Wt, 5'-TGCACTTTCACTAGTATTCAGAAAAAATTG-3', *MYC*-Mutant, 5'-TGCAAGCATCATAGTATTCAGAAAAAATTG-3', *CIITA*-Wt, 5'-CACAGTAAGGAAGTGAAATTAATTTCAGAG-3', and *CIITA*-Mutant, 5'-CACAGTAAGGAATGATGCTTAATTTCAGAG-3'.

**Luciferase reporter assay**

In general, luciferase reporter assay was essentially performed as previously described [2]. Briefly, we transfected luciferase reporter construct driven by *CIITA* promoter III (*CIITA*-pIII -Luc) with Blimp-1 or Aiolos expression vectors, plus *Renilla* luciferase reporter driven by the tk promoter (RL-tk) into 293T cells. The transfection procedure was performed by the polyethylenimine (PEI; Sigma) method [2]. After 48 h, cells were used for firefly luciferase and *Renilla* luciferase assays using the dual-luciferase reporter assay kit (Promega). The luminescence was measured by TopCount NXT. Repression was calculated by normalization of the firefly luciferase/*Renilla* luciferase ratios to the ratio obtained from empty vector control transfection.

**Lentiviral vector preparation and transduction**

MM cell lines, including H929, IM9 and U266, were transduced with lentiviral vector at a multiplicity of infection (MOI) of 5 to 10 in the presence of 5 μg/ml polybrene (Sigma-Aldrich) according to a previous established protocol [5]. The percentage of cells expressing GFP in transduced populations reached over 95% 3 days after infection as determined by flow cytometric analysis. Lentiviral vector producing shRNA against Blimp-1 was as described [6]. Lentiviral vectors producing shRNA against Aiolos (target sequence, 5-GCCAATGAAGATGAAGACATA-3'), was produced using previously described protocols

[6]. shRNAs against CUL4A (clone #1, TRCN0000320827 and clone #2, TRCN0000320896) were obtained from National RNAi Core Facility, Academia Sinica.

**GST pull-down assay**

Glutathione S-transferase (GST)-Blimp-1 and its deletion constructs and GST-Aiolos and its deletion constructs were generated by subcloning various forms of Blimp-1 and Aiolos, respectively, into pGEX-4T3 (Amersham Biosciences). His6-Δ1−526 Blimp-1 was generated by subcloning Δ1−526 Blimp-1 into pRSET-b (Invitrogen). Recombinant GST or His tagged fusion proteins were expressed in *Escherichia coli* strain BL21 and then purified according to a previous report [2]. Various GST fusion proteins were incubated with His6-tagged proteins for 2 h at 4°C, followed by washing three times with binding buffer (20 mM Tris [pH 7.5], 150 mM NaCl, 1% [wt/vol] NP-40, and protease inhibitor cocktails). Pull-down protein complexes were eluted by boiling in Laemmli buffer.

**Microarray analysis**

Total RNA from control, Aiolos knockdown or Blimp-1 knockdown H929 cells were isolated by RNeasy spin column (Qiagen) and GeneChip expression analysis using the GeneChip Human Genome U133 Plus 2.0 array (Affymetrix) was performed by the Affymetrix Gene Expression Service Laboratory at Institute of Plant and Microbial Biology, Academia Sinica. The microarray data were analyzed by GeneSpring GX11 software (Agilent) and 166 genes with up- or down- regulation for over 4 fold in Aiolos knockdown or Blimp-1 knockdown were selected to plot the heatmap by software of package 'pheatmap' of R.

**Primers for RT-QPCR analysis**

TRAIL forward, 5’-GCCCCTGCTGGCAAGTC-3’ and reverse, 5’-GGTTCTCAAAATCATCTTTCTAACG -3’, Noxa forward, 5’-CTCCAGCAGAGCTGGAAG-3’ and reverse, 5’-TTCTGCCGGAAGTTCAGTTTG -3’, KLF10 forward, 5’-GGTGCCTCTCTCCAGCAGACT-3’ and reverse, 5’-CTTTTGGCCTTTCAGAAATCATTT -3’, CIITA forward, 5’-CCAGGGAGGCTTATGCCAAT-3’ and reverse, 5’-GCTGGGAGTCCTGGAAGACA-3’, Aiolos forward, 5’-CAAATGCGGAACTGAAAAGCA-3’ and reverse, 5’-CAAAACCGCTGCACTTTCTG-3’, Blimp-1 forward, 5’-CGAAATGCCCTTCTACCCTG-3’ and reverse, 5’-GCGTTCAAGTAAGCGTAGGAGT

-3', PPIA forward, 5'-CCCTCCACCCCATTTGC-3' and reverse, 5'-GAACTGCAGCGAGAGCACAA-3', CUL4A forward, 5'-GAATGAGCGGTTCGTCAACCTG-3' and reverse, 5'-CTGTGGCTTCTTTGTTGCCTGC-3', Ikaros forward, 5'-GCTGCCACAACTACTTGGAAAGC-3', reverse, 5'-AGTCTGTCCAGCACGAGAGATC-3', CRBN forward, 5'-ACACCAGTCTGCCGACATCACA-3', reverse, 5'-GAAGAACTGGAATCACCTGACAG-3', Aiolos forward, 5'-GCCAAAGAAGAGATGCGCTCAC-3', reverse, 5'-GGCGTTATTGATGGCTTGGTCC-3'.

**Statistics**

Statistical significance was performed by two-tailed student's t test.

## SUPPLEMENTAL REFERENCES


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3. Chang DH, Cattoretti G, Calame KL. The dynamic expression pattern of B lymphocyte induced maturation protein-1 (Blimp-1) during mouse embryonic development. *Mech Dev* 2002, **117**(1-2)**:** 305-309.

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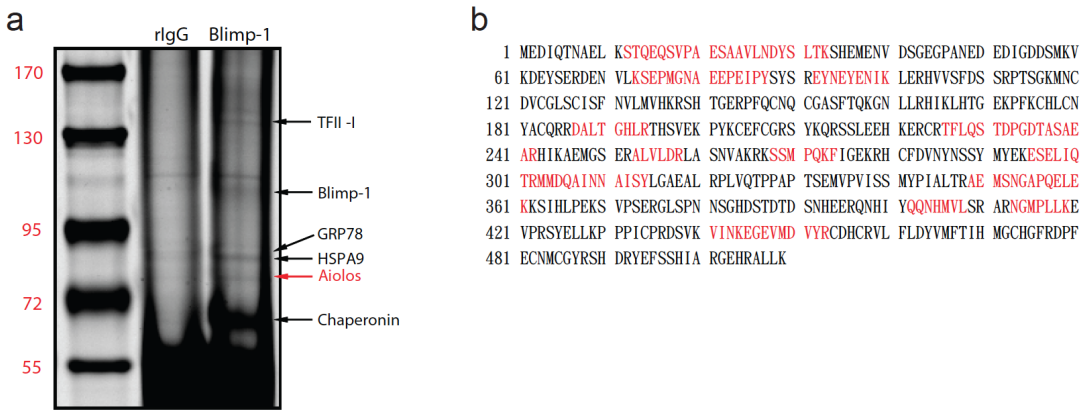
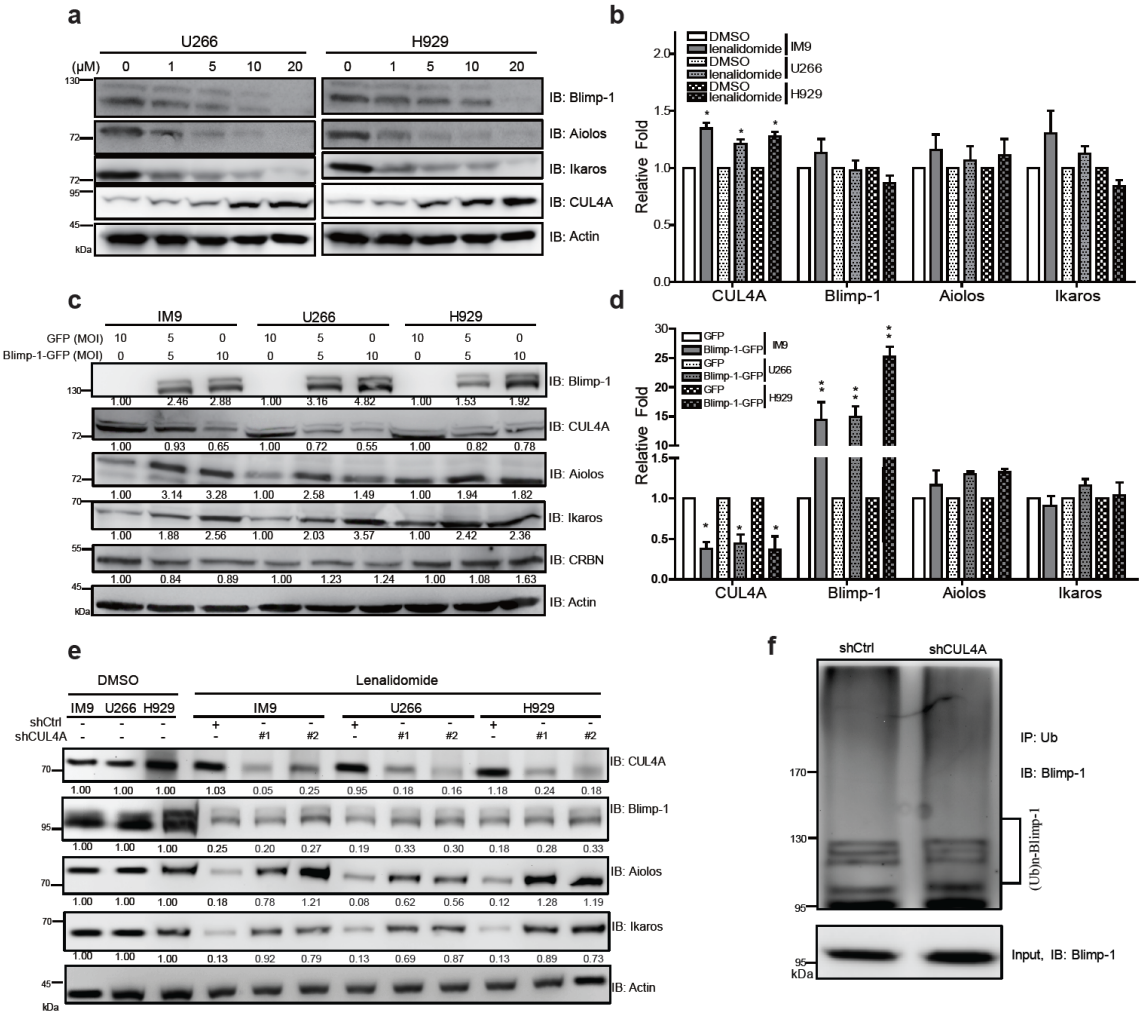
5. Lin FR, Huang SY, Hung KH, Su ST, Chung CH, Matsuzawa A*, et al.* ASK1 promotes apoptosis of normal and malignant plasma cells. *Blood* 2012, **120**(5)**:** 1039-1047.

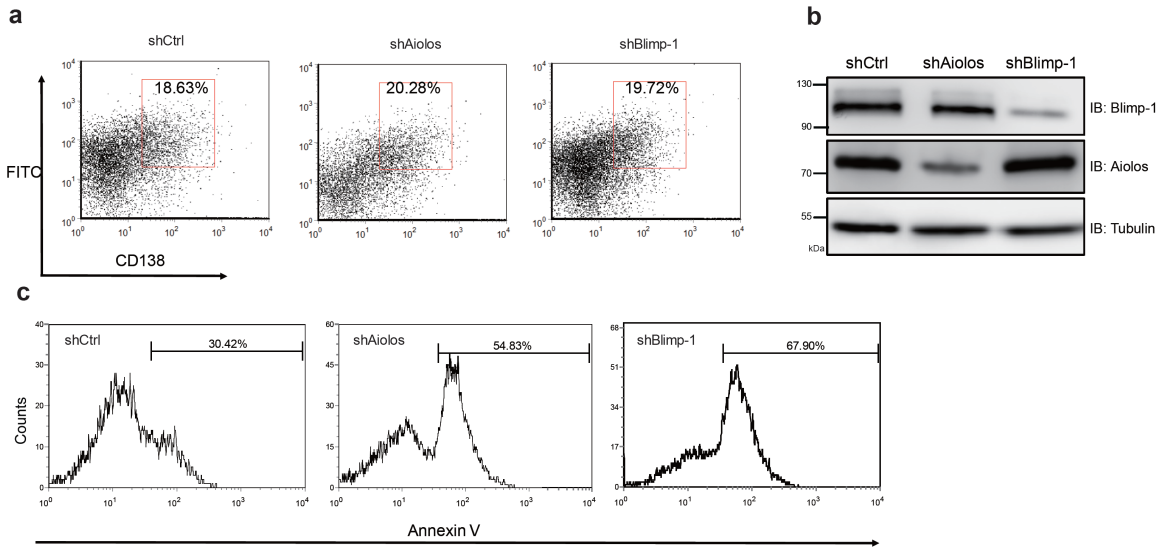
6. Lin FR, Kuo HK, Ying HY, Yang FH, Lin KI. Induction of apoptosis in plasma cells by B lymphocyte-induced maturation protein-1 knockdown. *Cancer Res* 2007, **67**(24)**:** 11914-11923.




**Supplementary Figure 1.** Affinity purification of the Blimp-1–interacting complex. (a) H929 nuclear extracts were used for IP using anti-Blimp-1 or rabbit IgG (rIgG). Immunoprecipitates were analyzed with SDS-PAGE followed by silver staining. The excised bands, as indicated, were destained and subjected to trypsin digestion and subsequent mass spectrometric analysis. Left lane: molecular weight markers (kDa). (b) Amino acid sequence of Aiolos. Peptide sequences labeled in red type represent the Aiolos fragments identified via mass spectrometry.




**Supplementary Figure 2.** The cytotoxic effects of lenalidomide in MM cells require Blimp-1. (a) IB shows the effects of indicated doses of lenalidomide on the expression of indicated proteins in three MM cells at 48 hours. (b) RT-QPCR shows the mRNA levels of indicated genes in IM9, U266 and H929 cells treated with lenalidomide for 72 hours as compared with solvent control DMSO treated cells. (c, d) IB (c) and RT-QPCR (d) show the levels of indicated proteins and genes in MM cells infected with Blimp-1-EGFP or EGFP lentiviral vectors at indicated MOI for 72 hours and treated with lenalidomide for 24 hours. (e) IB shows the expression of indicated proteins in MM cells after transduction with two different shRNAs (#1 and #2) against CUL4A for 72 hours and treatment with lenalidomide or DMSO for 24 hours. Data in (b) and (d) represent the mean ± SD (n = 3). (*$p < 0.05$; ** $p < 0.01$) (f) Co-IP shows the effect of knockdown of CUL4A on ubiquitination of Blimp-1. Pre-cleared nuclear extract from H929 cells transduced with shCtrl or shCUL4A for 72 hours, followed by treatment with MG132 (10 μM) for 12 hours, was used for IP with anti-Ub and IB analysis with anti-Blimp-1.




**Supplementary Figure 3.** Knockdown of Aiolos increased apoptosis in normal plasma cells. (a) Mouse splenic B cells were treated with lipopolysaccharide to induce the generation of $CD138^+$ plasma cells. Three days later, cells were transduced with lentiviral vectors expressing indicated shRNAs for two more days and the frequency of $CD138^+YFP^+$ cells was then analyzed by flow cytometric analysis. (b) IB shows the knockdown of Aiolos and Blimp-1 by shAiolos and shBlimp-1, respectively, in sorted $CD138^+YFP^+$ cells as shown in (a). (c) Frequency of annexin $V^+$ cells in $CD138^+YFP^+$ cells gated in (a) was determined by flow cytometric analysis.