Supplementary Methods

Yeast two-hybrid screening. Yeast two-hybrid screening was performed using a method described previously[1]. Briefly, BZEL or DeSI-1 cDNA was cloned in-frame with the GAL4 DNA-binding domain of pGBKT7 and the resulting plasmids were employed as bait for the screening of 1×10^7 colonies of a murine T-cell lymphoma cDNA library (Clontech). Colonies that grew in the absence of histidine and showed detectable β-galactosidase staining within 2 h of incubation were selected as positives. All other yeast two-hybrid assay methods were according to the manufacturer's protocol (Stratagene). To generate the bait for the yeast two-hybrid assays, the full open reading frames of BZEL or DeSI-1 were amplified by PCR using 5' and 3' primers bearing an *Nde*I and *Bam*HI site extension, respectively. The PCR products were then digested with *Nde*I/*Bam*HI and ligated in-frame with the transcriptional activation domain of GAL4 into the pGBKT7 vector.

Plasmids. For expression plasmids encoding FLAG- or Myc-tagged murine DeSI-1 and DeSI-2, the corresponding fragment was amplified by PCR and subsequently inserted into the p3XFLAG-CMV or pCDNA 3.1 vector. The expression plasmid encoding DeSI-1^{C108S} was generated by mutagenesis using a Quick-change Mutagenesis Kit (Stratagene). To express DeSI-1, SENP1C and SENP6C as GST-fusion proteins, corresponding fragments were obtained by PCR and subcloned into pGEX-4T2 (Amersham Pharmacia) using *Eco*RI/*Bam*H1, *Eco*RI/*Not*I or *Bam*H1/*Not*I sites, respectively.

Isolation of murine tissue proteins. Tissues were isolated from C57 BL/6 mice and homogenized on ice in RIPA buffer (2 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% Na-deoxycholate), supplemented with complete protease inhibitors (1 mM PMSF, 1 μg/ml Aprotinin, 2 μg/ml leupeptin, 1 μg/ml Pepstatin A, 1 μg/ml $NaOVa_4$). The homogenates were then centrifuged at 12,000 rpm for 20 min at 4℃ , and the resulting supernatant fraction was transferred to a fresh tube and used for subsequent experiments.

Construction of shRNA-expressing plasmids. The pSUPER plasmid was used to synthesize small hairpin RNAs (shRNAs). Three 19-mer sequences corresponding to nucleotides (nts) 28-46 (GCTCTACGTGTACGACCTG), 95-113 (CTGGAAGGCATCTGGCACA), 133-151 (GGATGAGTTCT TCTTCGGC) in the coding sequence of DeSI-1 were selected as targets for the construction of shRNA-expressing constructs in pSUPER plasmids. To examine the effects of the shRNAs, the expression plasmids encoding DeSI-Myc and each shRNA were co-transfected into 293 cells. Subsequent analysis of DeSI levels by immunoblotting with an anti-Myc Ab revealed that the more effective construct were encoding the sequence of nts 28-46 and 133-151. Therefore, these construct were used for subsequent experiments. The control shRNA of DeSI-1 was generated by site directed mutagenesis using the following oligonucleotides: 5'-GAATCACTTCTTCAAGGGC-3' and 5'-GCCCTTGAAGAAGTGATTC-3'.

Immunofluorescent microscopy. To visualize the subcellular localization of DeSI-1 and -2, immunofluorescence microscopy was performed as described previously[2]. Briefly, cells were fixed in 4% paraformaldehyde and permeabilized in 0.5% Triton X-100 in PBS for 10 min, and then incubated with primary Ab overnight at 4℃. Subsequently, cells were stained with Allex 568 fluorescein-conjugated (Pierce) mouse

secondary Ab for 30 min at RT. Immunostained cells were visualized with a Zeiss LSM510 confocal microscope.

Immunoprecipitation and immunoblot analysis. Cells were lysed in RIPA buffer supplemented with protease inhibitors, as described above. After incubation for 1h on ice, insoluble material was removed by centrifugation at 12,000 rpm for 20 min at 4℃. Subsequently, supernatant samples were precleared for 1 h with normal mouse serum and protein G agarose, and subjected to immunoprecipitation with appropriate antibodies and protein G agarose. Subsequent procedures for immunoprecipitation and immunoblot analysis were described previously[2].

Antibodies. Polyclonal rabbit anti-DeSI-1 and -2 sera were raised against GST-fusion proteins expressed in *E.coli*, according to a method described previously[3]. The other antibodies used in this study were obtained from commercial sources and include anti-Myc (Abcam), anti-FLAG (Sigma), anti-PML (Santa Cruz Biotechnology, Inc.), anti-SUMO-1 (Cell signaling), anti-GST (Cell signaling) and anti-SUMO-2/3(Abcam) antibodies.

Expression and purification of recombinant proteins from *E.coli*. Full-length mouse BZEL, DeSI-1, DeSI-2 , PML, ΔNp63, SENP1C and SENP6C were produced in the *E. coli* BL21 (DE3) RIG strain (Novagen) as a GST-fusion protein. Bacterial lysates were sonicated in buffer A, which was composed of 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 1 mM dithiothreitol. Cleared lysates were loaded onto a column containing GST-Bind resin (Novagen) and eluted with buffer A, supplemented with 10 mM reduced glutathione, After the removal of the N-terminal GST using TEV protease (Invitrogen), HiTrap Q and HiLoad 26/60 Superdex 75 columns (Amersham Pharmacia) were used for further purification. Human full-length SUMO-1,-2, -3 with a $(His)_6$-tag at its C-terminus were also produced in the *E. coli* BL21 (DE3) RIG strain (Novagen). The proteins were purified using a Ni-NTA column (QIAGEN) and subsequent HiTrap Q and HiLoad 26/60 Superdex 75 columns.

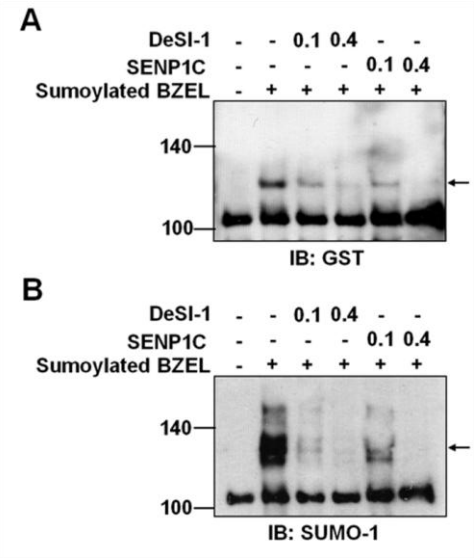
Supplemental References

1. Park D, Park I, Lee D, Choi YB, Lee H, Yun Y (2007) The adaptor protein Lad associates with the G protein beta subunit and mediates chemokine-dependent T-cell migration. *Blood* **109:** 5122-5128

2. Ahn E, Lee H, Yun Y (2006) LIME acts as a transmembrane adapter mediating BCR-dependent B-cell activation. *Blood* **107:** 1521-1527

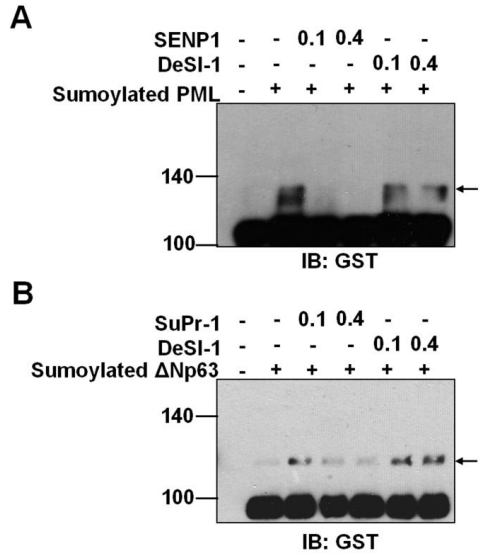
3. Hur EM, Son M, Lee OH, Choi YB, Park C, Lee H, Yun Y (2003) LIME, a novel transmembrane adaptor protein, associates with p56lck and mediates T cell activation. *J Exp Med* **198:** 1463-1473

Supplementary Figure 1



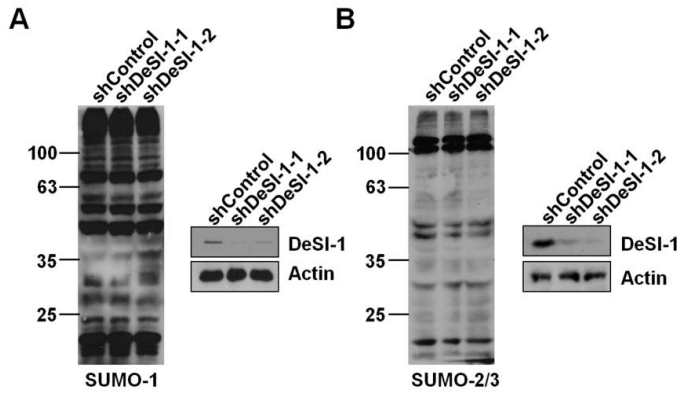
Purified GST-BZEL fusion protein from *E.coli* was sumoylated *in vitro*. Subsequently, sumoylated BZEL was incubated with 0, 0.1 or 0.4 μM of DeSI-1or SENPlC purified from *E.coli* for 1 h at 37℃. After incubation, the reaction mixture were analyzed by immunoblotting with anti-GST(A) and anti-SUMO-1(B) antibodies. The arrow indicates the position of sumoylated BZEL.

Supplementary Figure 2



(A) Purified GST-PML fusion protein from *E.coli* was sumoylated *in vitro*. Subsequently, sumoylated PML was incubated with 0, 0.1 or 0.4 μM of DeSI-1or SENPlC purified from *E.coli* for 1 h at 37℃. After incubation, the reaction mixture was analyzed by immunoblotting with anti-GST antibody. The arrow indicates the position of sumoylated PML.
(B) Same as (A), except that SuPr-1C was incubated instead of SENP1C, and the sumoylation of ΔNp63, instead of PML, was analyzed.

Supplementary Figure 3



(A) Analysis of the levels of SUMO-1 modified proteins upon reduction of DeSI-1 expression by the introduction of shRNA. 293T cells were transfected with the plasmids encoding two kinds of DeSI-1 shRNA, shDeSI-1-1 or shDeSI-1-2. Two days later, Cell lysates were subjected to immunoblot analysis with anti-SUMO-2/3 Ab(left). As a control for DeSI-1 expression, whole cell extract (WCE) was analyzed by immunoblotting with anti-DeSI-1 or anti-actin antibody(right).
(B) Analysis of the levels of SUMO-2/3 modified proteins upon reduction of DeSI-1 expression by the introduction of shRNA. The experiment was performed as described in (A) except that SUMO-2/3 was immunoblotted with anti-SUMO-2/3 antibody instead of SUMO-1.

Supplementary Table

List of potential substrates of DeSI-1 desumoylase identified through yeast Two-hybrid screening with DeSI-1 as bait. After screening of 1x107colonies of a murine T-cell lymphoma cDNA library, approximately 40 strong positives were rescued and subjected to nucleotide sequencing.

No.of colonies	Binding partners of DeSI-1
3	proliferating cell nuclear antigen (Pcna)
3	RNA binding motif protein 3 (Rbm3)
3	beta-2 microglobulin (B2m)
2	guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1 (Gnb2l1)
2	basigin (Bsg), transcript variant 1
2	H3 histone, family 3B (H3f3b)
2	ubiquitin B (Ubb)
2	stem-loop binding protein (Slbp)
1	Serpine1 mRNA binding protein 1 (Serbp1)
1	SFT2 domain containing 1 (Sft2d1)
1	zinc finger, DHHC domain containing 4 (Zdhhc4)
1	BTB (POZ) domain containing 1 (Btbd1)
1	NCK associated protein 1 like (Nckap1l)
1	SH3-domain kinase binding protein 1 (Sh3kbp1)
1	ubiquitin-activating enzyme E1-domain containing 1 (Ube1dc1)
1	ERGIC and Golgi 3 (Ergic3)
1	protein phosphatase 2, regulatory subunit B (B56), alpha isoform (Ppp2r5a)
1	DNA segment, Chr 15, Wayne State University 75, expressed (D15Wsu75e)
1	glycolipid transfer protein (Gltp)
1	eukaryotic translation initiation factor 3, subunit 6 interacting protein (Eif3s6ip)
1	adenosine kinase (Adk)
1	acidic ribosomal phosphoprotein P0 (Arbp)
1	citrate synthase (Cs) ,nuclear gene encoding mitochondrial protein
1	prohibitin (Phb)
1	translocase of inner mitochondrial membrane 23 homolog
1	budding uninhibited by benzimidazoles 1 homolog, beta (S. cerevisiae) (Bub1b)
1	ribosomal protein S4, X-linked (Rps4x)