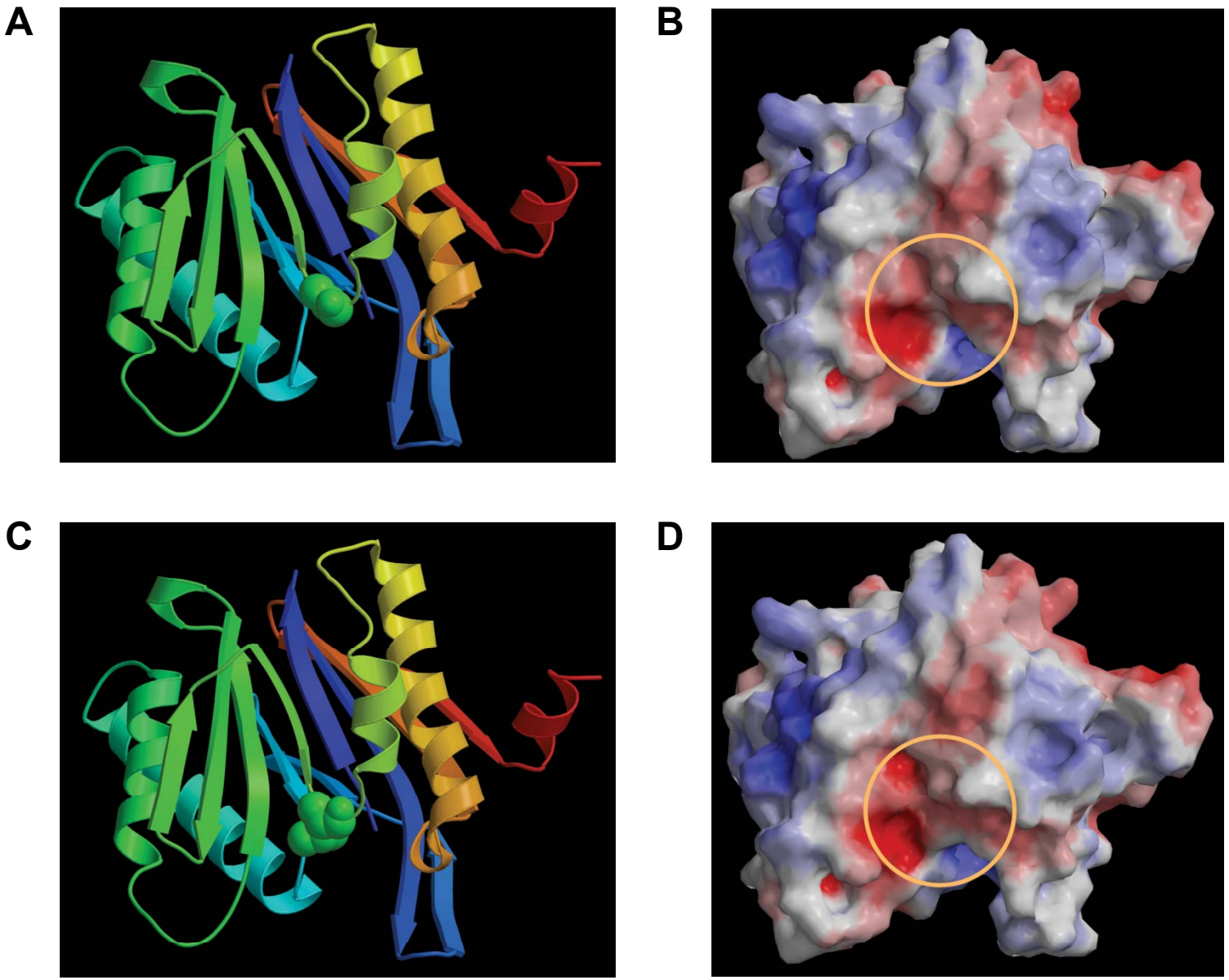
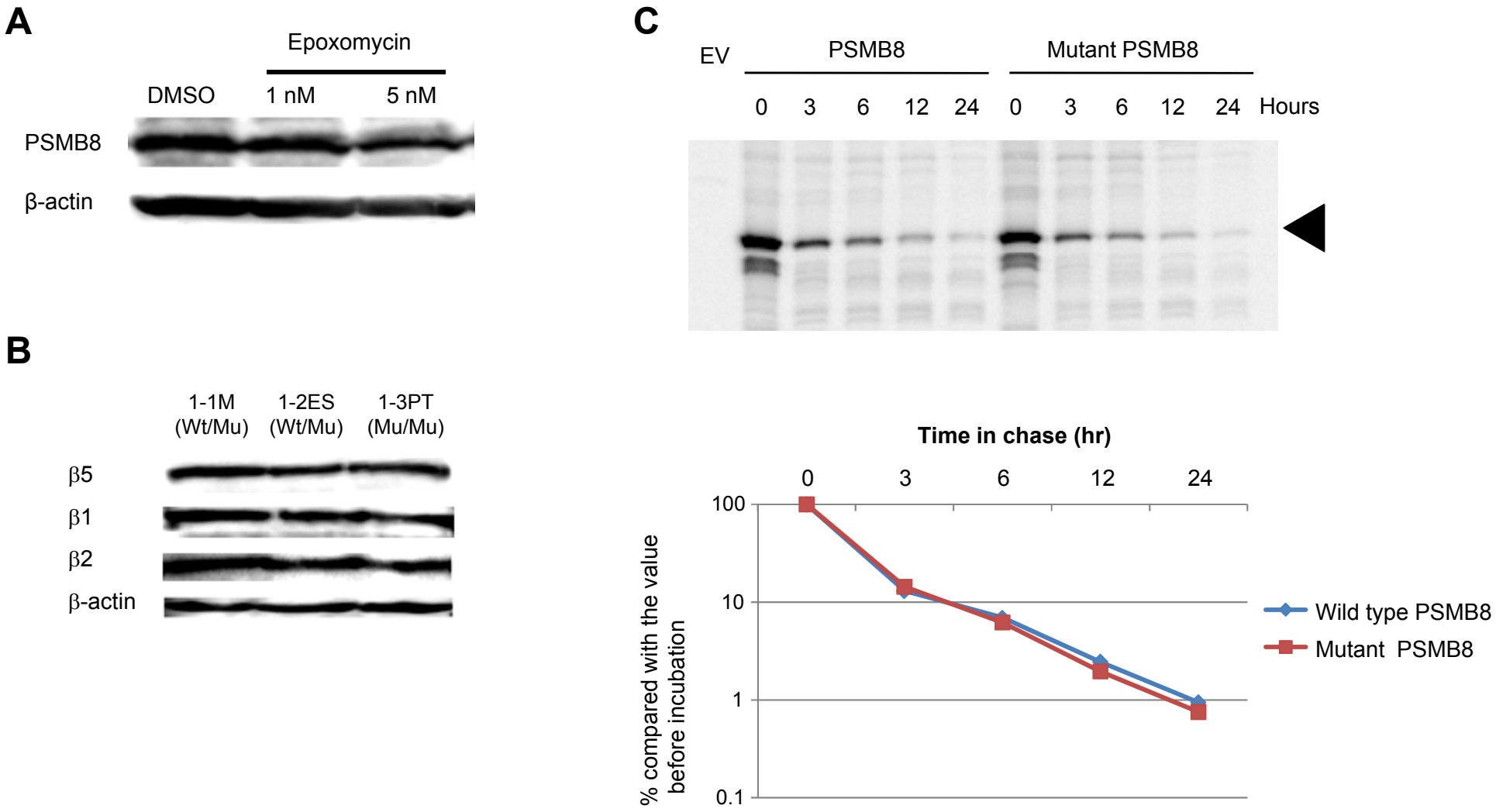
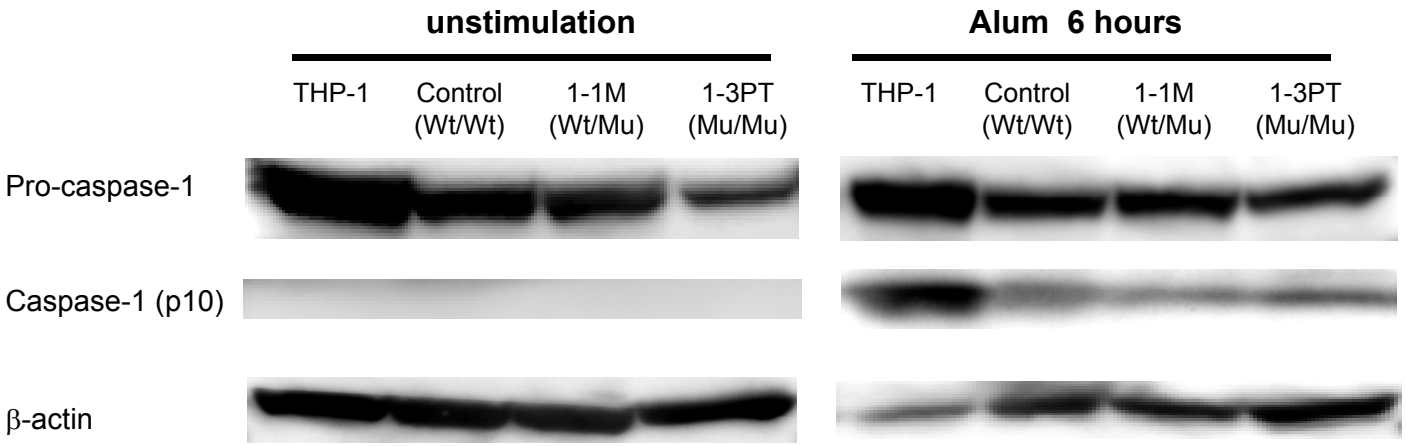
Supplementary Figure 1. Kitamura *et al.*



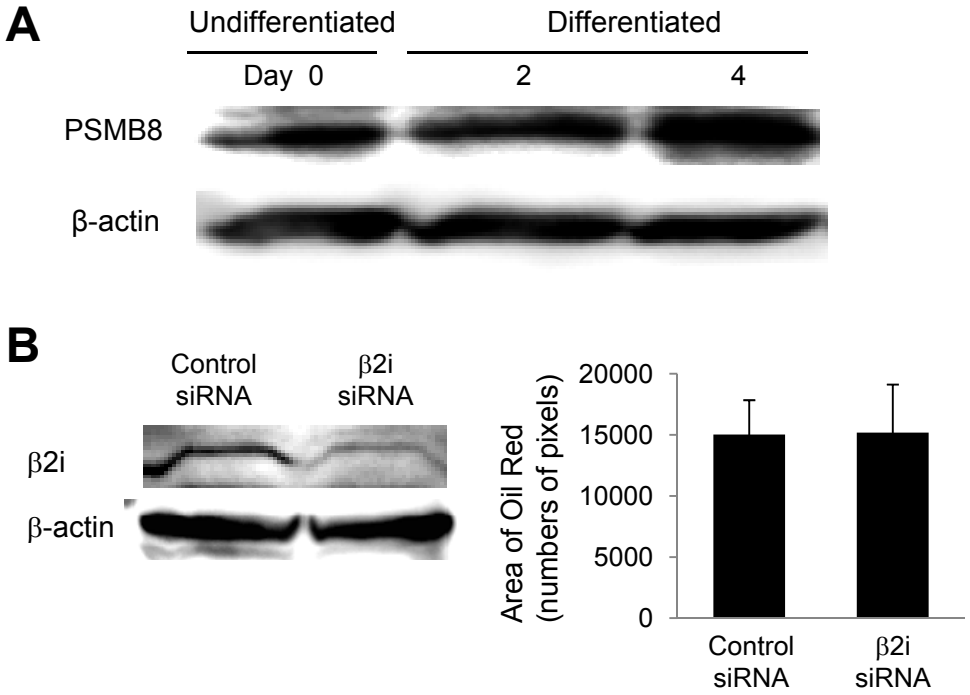
Supplementary Figure 2. Kitamura *et al.*



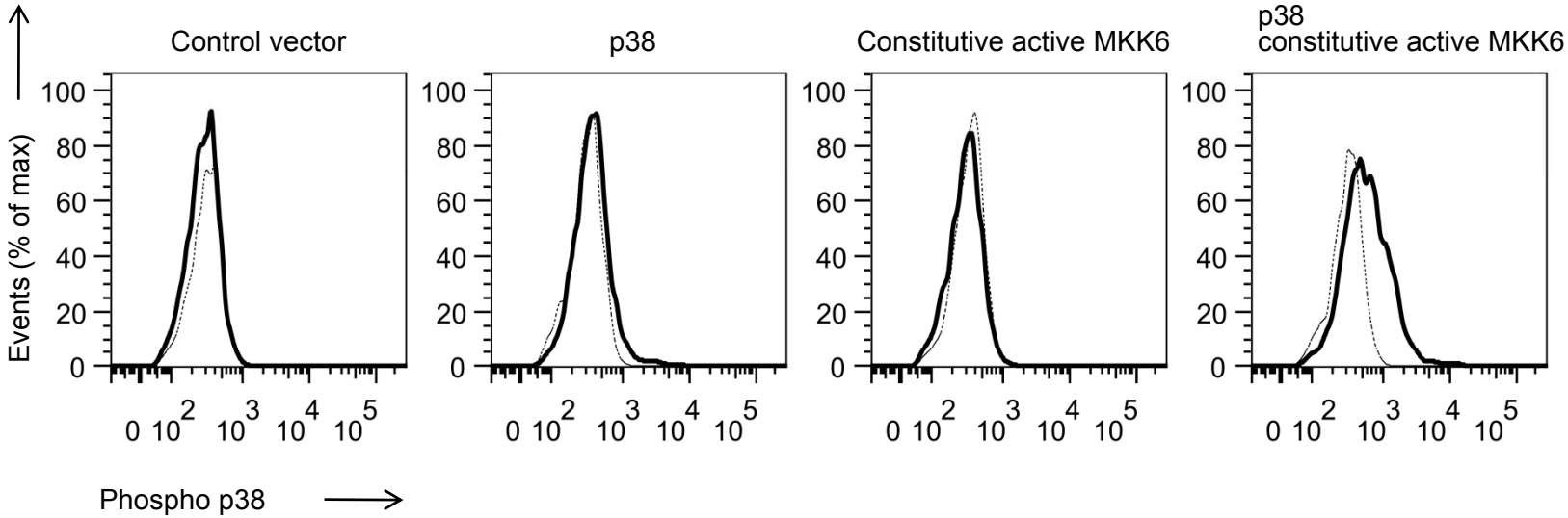
Supplementary Figure 3. Kitamura *et al.*



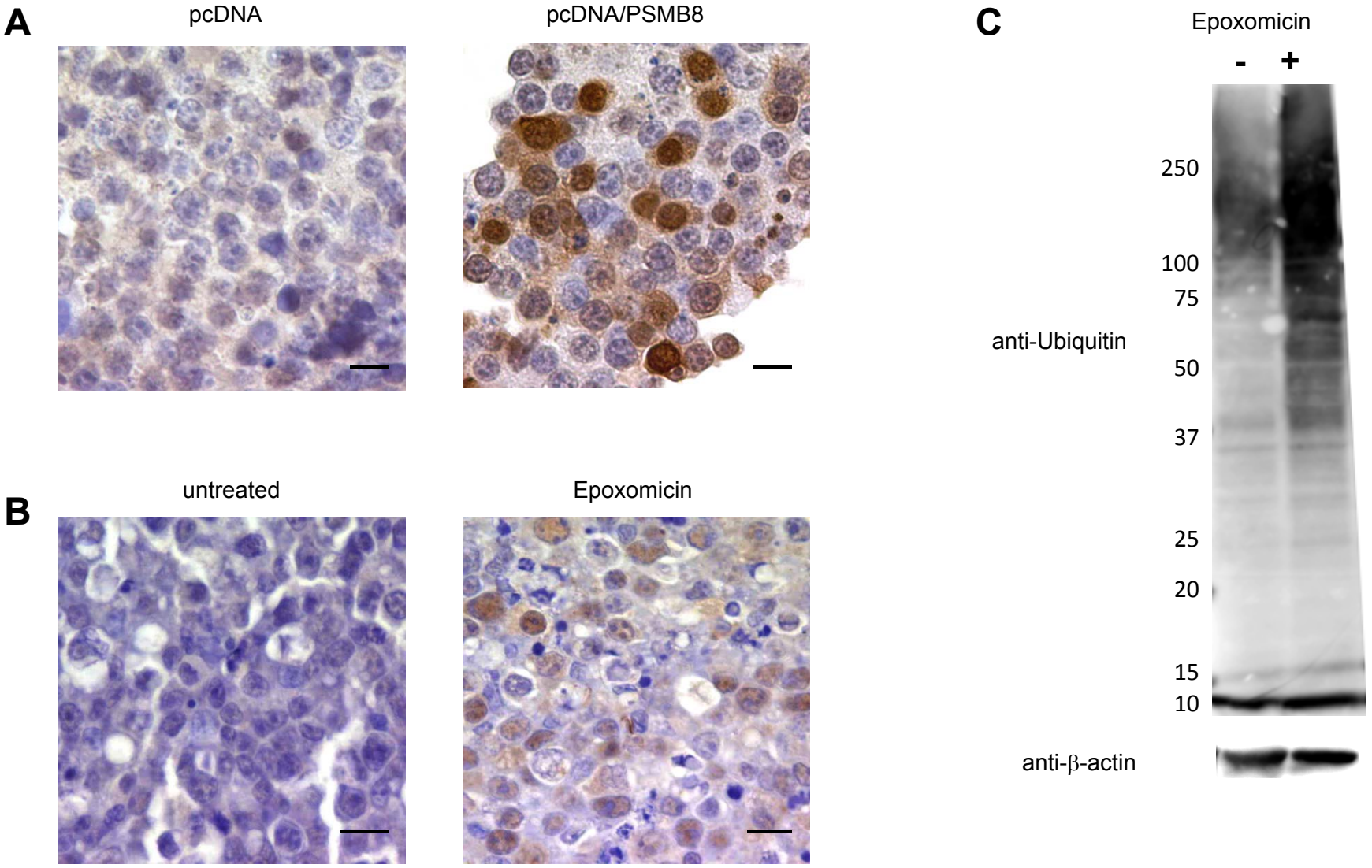
Supplementary Figure 4. Kitamura *et al.*



Supplementary Figure 5. Kitamura *et al.*



Supplementary Figure 6. Kitamura *et al.*



Supplementary Table 1. Clinical and laboratory findings of JASL patients

	1-3PT	2-2B	2-1S
Sex	Female	Male	Female
Parental consanguinity	Yes	Yes	Yes
Family history	No	Brother of Patient 2-1S	Sister of Patient 2-2B
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive
Age of onset (autoinflammation)	1M	3Y	3Y
Age of onset (lipodystrophy)	7Y	12Y	6Y
Age of death	63Y (respiratory failure)	47Y (heart failure)	54Y (respiratory failure)
Recurrent skin eruption	nodular erythema , frostbitten hands	nodular erythema , frostbitten hands	nodular erythema , frostbitten hands
Periodic fever	over 40 ºC	over 40 ºC	over 40 ºC
Deformities of fingers	Yes	Yes	Yes
Lipodystrophy	Partial (face, upper body, extremities)	Partial (face, upper body, extremities)	Partial (face, upper body, extremities)
Muscular atrophy	Yes	Yes	Yes
Joint contraction	Fingers, toes, mandibular joint	Fingers, toes	Fingers, toes
Hepatomegaly	Yes	Yes	Yes
Splenomegaly	No	Yes	No
Calcification of the basal ganglia	No	Yes	Yes
Cardiac insufficiency	No	Arrhythmia (Premature ventricular contraction)	Arrhythmia (ST segment depression)
Hypertension	No	No	No
Dyslipidemia	No	No	No
Diabetes mellitus	No	No	No
Mental retardation	No	No	IQ < 60
Macroglossia	Yes	Yes	Yes
Hyper γ globurinemia	IgG, A (2000, 700 mg/dl)	IgG, A (3140, 508 mg/dl)	IgG, A (2800, 652 mg/dl)
IgG (870-1700), IgA (110-410)			
Elevation of CRP and ESR	Yes	Yes	Yes
Autoantibody	Negative (anti-dsDNA, Jo-1, SSA, SSB, Scl70)	Negative (anti-dsDNA, mitochondria, smooth muscle)	Negative (anti-dsDNA, mitochondria, smooth muscle)
Complement	Normal (C1-C9, CH50)	Normal (C3, C4, CH50)	Normal (C3, C4, CH50)
CPK	Normal	Normal	Normal

Supplementary Table 2. Clinical and laboratory findings of JASL or JASL-like syndromes published in Japanese Journal.

	Our cases	Nakajo [a)]		Nishimura [b)]			Horikoshi [c)]	Kitano [13)]	Hayashi [d)]	Kasagi [12)]
Age of death	47-63Y	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sex	F, M	M	F	F	F	M	F	M	M	F
Parental consanguinity	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Family history of syndrome	Yes or No	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Age at onset of inflammation	1M-3Y	2Y	6M	5Y	2Y	4Y	3M	2M	< 5Y	10M
Age at onset of lipodystrophy	6Y-12Y	< 10	N.D.	6Y	7Y	N.D.	12Y	4Y	N.D.	2Y
Recurrent skin eruption	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Periodic fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N.D.	Yes	Yes
Steroid sensitive	Yes	Not used	Not used	Not used	Not used	Not used	Yes	N.D.	Yes	Yes
Deformities of fingers	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lipodystrophy	Yes	Partial	N.D.	Partial	Partial	Partial	Partial	Partial	Partial	Partial
Hepatomegaly	Yes	Yes	N.D.	No	No	No	Yes	N.D.	No	N.D.
Splenomegaly	Variable	Yes	N.D.	No	No	No	Yes	N.D.	No	N.D.
Joint contraction	Yes	No	No	No	No	No	Yes	No	Yes	Yes
Muscular atrophy	Yes	Yes	N.D.	Yes	Yes	Yes	Yes	Yes	Yes	No
Calcification of the basal ganglia	No	N.E.	N.E.	N.E.	N.E.	N.E.	Yes	N.E.	Yes	Yes
Hypertension	No	N.E.	N.E.	N.E.	N.E.	N.E.	Yes	N.D.	N.D.	N.D.
Dyslipidemia	No	N.E.	N.E.	N.E.	N.E.	N.E.	Yes	No	N.D.	N.D.
Diabetes mellitus	No	N.E.	N.E.	No	No	No	No	No	No	N.D.
Cardiac insufficiency	Variable	N.E.	N.E.	No	No	No	No	Arrhythmia (ST segment depression)	No	No
Mental retardation	Variable	Yes	N.D.	N.D.	Yes	N.D.	Yes	No	Yes	No
Macroglossia	Yes	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Hyper γ globurinemia	Yes	Hyper-proteinemia	N.E.	Yes	Yes	Yes	Yes	No	Yes	Yes
Elevation of CRP and ESR	Yes	ESR elevated	N.E.	ESR elevated	ESR elevated	ESR elevated	Yes	Yes	Yes	Yes
Autoantibody	Negative	N.E.	N.E.	N.E.	N.E.	N.E.	No	N.D.	No	Yes
Complement	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	CH50 normal	N.D.	Normal	Normal
CPK	Normal	N.E.	N.E.	N.E.	N.E.	N.E.	Normal	N.D.	N.D.	N.D.

N.E.; not examined N.D.; not described

a. Nakajo A. Hypertrophic osteoperiostosis with pernio (in Japanese). J Dermatol Venereol 1939;45:77-86.
b. Nishimura N, Deki T, Kato S. Hypertrophic osteoperiostosis with pernio-like skin lesions observed in two families (in Japanese). J Dermatol Venereol 1950;60:136-141.
c. Horikoshi A, Iwabuchi S, Iizuka Y, et al. A case of partial lipodystrophy with erythema, dactylic deformities, calcification of the basal ganglia, immunological disorders and low IQ level(in Japanese). Clin Neurol 1980; 20:173-180.
d. Hayashi Y, Shiraishi T, Tani J, et al. A case of hereditary inflammation disorder with calcification of basal ganglia similar to lipodystrophy(in Japanese). J Pediatr Practice 1989; 3:561-565.

Supplementary Table 3 Clinical and laboratory findings of JASL and other lipodystrophic syndromes

	JASL	Congenital generalized lipodystrophy		Mandibuloacral dysplasia with lipodystrophy		Familial partial lipodystrophy		Barraquer-Simons syndrome	Juvenile Dermatomyositis
		Type 1	Type 2	Type A	Type B	Dunnigan variety	PPARG mutations		
Gene Involved	*PSMB8*	*AGPAT2*	*BSCL2*	*LMNA*	*ZMPSTE24*	*LMNA*	*PPARG*	No	No
Inheritance	AR	AR	AR	AR	AR	AD	AD	Sporadic	Sporadic
Autoinflammation	Yes	No	No	No	No	No	No	No	No
Age of onset (lipodystrophy)	6-12Y	Neonate	Neonate	Neonate	Neonate	After puberty	After puberty	Childhood	Childhood
Age of death	Middle age	Young adult	Young adult	Middle age	Middle age	Middle age	Variable	Variable	Variable
Skin	Nodular erythema	Acanthosis nigricans	Acanthosis nigricans	Atrophy	Atrophy	Acanthosis nigricans	Acanthosis nigricans	Acanthosis nigricans	Erythema
Periodic fever	Yes	No	No	No	No	No	No	No	No
Deformities of fingers	Yes	No	No	No	No	No	No	No	No
Lipodystrophy	Partial	Generalized	Generalized	Partial	Partial	Partial	Partial	Partial	Partial
Muscular atrophy	Yes	No	No	Yes	Yes	No	No	No	Yes
Bone deformity	Joint contraction	No	No	Mandibular hypoplasia, Dysplastic clavicals	Mandibular hypoplasia, Dysplastic clavicals	No	No	No	Joint contraction
Hepatomegaly	Yes	Yes	Yes	No	No	Yes	No	No	Variable
Splenomegaly	Variable	Yes	Yes	No	No	No	No	No	Yes
Calcification of the basal ganglia	Variable	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cardiac insufficiency	Arrhythmia	hypertrophic cardiomyopathy	hypertrophic cardiomyopathy	No	No	Variable	No	No	No
Hypertension	No	Yes	Yes	No	No	No	Yes	Variable	No
Dyslipidemia	No	Yes	Yes	Yes	Yes	Yes	Yes	Variable	Yes
Diabetes mellitus	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Mental retardation	Variable	Variable	Variable	Normal	Normal	Normal	Normal	Normal	Normal
Macroglossia	Yes	No	No	No	No	No	No	No	No
Hyper γ globulinemia	Yes	No	No	No	No	No	No	No	No
Elevation of CRP and ESR	Yes	No	No	No	No	No	No	Unknown	Yes
Autoantibody	Negative	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Variable	Positive
Complement	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Low C3	Normal
CPK	Normal	Normal	Normal	Normal	Normal	Normal	Normal	variable	Elevated

AR: autosomal recessive, AD: autosomal dominant, N.D.: not detected

Supplementary Table 4. The target sequence for siRNA

siRNA	symbol	Sense	Antisense
Human PSMB8	hPSMB8-1	gaacaaggugauugagauuTT	aaucucaaucaccuuguucTT
	hPSMB8-2	ggucauggacaguggcuauTT	auagccacuguccaugaccTT
	hPSMB8-3	ggaagaugguugggugaaaTT	uuucacccaaccaucuuccTT
Mouse PSMB8	mPSMB8-1	ggaaaggaauguucaaauuTT	aauuugaacauuccuuuccTT
	mPSMB8-2	gggagaggcuguuggccaaTT	uuggccaacagccucucccTT
	mPSMB8-3	ccgcagagcuauugcuuauTT	auaagcaauagcucugcggTT
Negative control	#1	auccgcgcgauaguacguaTT	uacguacuaucgcgcggauTT
	#2	uuacgcguagcguaauacgTT	cguauuacgcuacgcguaaTT
	#3	uauucgcgcguauagcgguTT	accgcuauacgcgcgaauaTT
Mouse PSMB10	mPSMB10-1	ggacaaaagcugcgagaagTT	cuucucgcagcuuuuguccTT
	mPSMB10-2	gccaagcugcagagagcauTT	augcucucugcagcuuggcTT
	mPSMB10-3	ggccugagauguagaguuuTT	aaacucuacaucucaggccTT

Supplementary methods

Exome sequencing

A paired-end library was prepared from genomic DNA from two patients (1-3PT, 2-2B) and hybridized to biotinylated cRNA oligonucleotide baits from the SureSelect Human All Exon Kit (Agilent Technologies). The library was sequenced with paired-end 75 bp reads on one lane of an Illumina Genome Analyzer IIx. We aligned the sequence reads to the human reference sequence of the UCSC Genome Browser (hg18). The proportion of the entire targeted exome covered by > 10 reads was 90%. The targeted exon or exon-intron boundaries with fewer than 10 reads on the candidate region were sequenced using an ABI Prism 3730 sequencer (PE Applied Biosystems). DNA variants located in the candidate region were filtered and evaluated against dbSNP130 and missense, nonsense, frameshift, or splice-site alleles.

Cell culture and transfection conditions

The cDNAs for human *PSMB8* or mutant *PSMB8* were cloned into pcDNA3.1 or retroviral vector pKE004 that bicistonically expresses GFP. Peripheral blood mononuclear cells were obtained using Ficoll-Hypaque, and 2 - 3 x 10^6 cells were transformed using EB virus. Transformed B cells were cultured in RPMI1640 supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Retroviruses were constructed by transfecting Plat-A cells with vectors using the transfection reagent GeneJuice (Novagen, Darmstadt, Germany). Cells were infected with a retrovirus carrying cDNA for *PSMB8* and were stimulated with PMA (25 ng/mL) (Sigma-Aldrich, MO, USA) and ionomycin (1 μg/mL) (Sigma-Aldrich, MO, USA). In some experiments, an inhibitor for MEK1/2 (U0126; Cell Signaling Technology, MA, USA) (5 μM), p38 (SB203580; Sigma-Aldrich, MO, USA) (2 μM), JNK (SP600125; LC Laboratories, MA, USA) (0.22 μM) or a proteasome inhibitor (Epoxomicin, PEPTIDE INSTITUTE, INC., Japan) (5 nM) was added.

Mutation screening

The exons and exon-intron boundaries of the candidate genes were PCR amplified with specific intronic primers. Primer sequences for exon 5 of *PSMB8* were Fw, 5'-CCAATTTCAGCCTGAAATCTTTCATCT-3', and Rev, 5'-CGACTCCTCCCAGGCATGGT-3'. Gel-purified (QIAquick gel extraction kit) amplicons were subjected to bidirectional DNA sequencing with the BigDye terminator system on an ABI Prism 3730 sequencer (PE Applied Biosystems).

Real-time PCR

Total RNA was extracted from cells with TRIzol (Life Technologies Corporation, CA, USA) and cDNA was synthesized from one μg of total RNA using an Omniscript RT Kit (QIAGEN) and random hexamers. cDNA PCR amplification was performed using SYBR Premix Ex Taq II (TaKaRa, Shiga, Japan) on an Applied Biosystems 7500 real time system (Applied Biosystems) with primer pairs specific for *PSMB8* (F-GGGGAATGCAGCCCAC, and R-ACCTTGTTCACCCGTAAGGC), *IL6* (F-GGTACATCCTCGACGGCATC, and R-GTGCCTCTTTGCTGCTTTCAC) and *HPRT* (F-TGACCTTGATTTATTTTGCATACC, and R-CGAGCAAGACGTTCAGTCCT). Cycling conditions were as follows: 95°C for 30 sec; 95°C for 5 sec, 60°C for 34 sec for a total of 40 cycles. Each sample was analyzed in triplicate. For quantification, standard curves were obtained using serially diluted cDNA amplified in the same real-time PCR run. Results were normalized to *HPRT* mRNA levels. After the quantification procedure, the products were resolved by 2.5% agarose gel electrophoresis to confirm that the reaction had amplified the correct DNA fragments of known size.

Western blotting

Cells were homogenized in lysis buffer (50 mM Hepes, 150 mM NaCl, 5 mM EDTA, 1% Triton-X, a protease inhibitor cocktail and 2 mM ATP). After centrifugation at 15,000 rpm for 20 min at 4°C, proteins were subjected to 10% SDS-PAGE and transferred onto a nitrocellulose membrane (ATTO). After one h blocking with PBS containing 1% BSA, blots were reacted with polyclonal anti-human PSMB8 (PW8355, ENZO Life Science), polyclonal anti-human β 5 subunit (PW8895, ENZO Life Science), monoclonal anti-β 1 subunit (PW8140, ENZO Life Science), monoclonal anti-β2 subunit (PW8145, ENZO Life Science), polyclonal anti-β-actin (3662-100, BioVision), polyclonal anti-ubiquitin mAb (Z0458, DAKO Co., CA, USA), polyclonal anti-caspase-1 (SC-514, Santa Cruz Biotech), or polyclonal anti-p38 (9212, Cell Signaling Technology). Blots were washed three times with TBS-Tween (20 mM Tris HCl, 4 mM Tris base, 140 mM NaCl, 1 mM EDTA, 0.1% Tween-20). After incubation with secondary HRP-conjugated goat anti-mouse (12-349, Upstate) or rabbit IgG antibodies (170-6515, BioRad) and subsequent washings, proteins were detected using a LAS-3000UVmini (FUJIFILM, Tokyo, Japan).

Adipocyte differentiation

Mouse 3T3-L1 preadipocyte cells were maintained in DMEM containing 10% FBS. For

adipocyte differentiation, the medium was replaced with DMEM containing 10% FBS, 10 μg/mL insulin, 0.5 mM 3-isobutyl-1-methylxantine and 2.5 μM dexamethasone two days post-confluence. Two days later, the medium was changed to DMEM containing 10 μg/mL insulin and 10% FBS and was replaced every three days. Oil Red staining of 3T3-L1 adipocytes was performed using a standard protocol and lipid accumulation was quantified by measuring Oil-Red positive pixels in ten fields. Human preadipocytes were purchased from Zen-Bio Inc. (NC, USA). For the differentiation of human preadipocytes, PM-1 (Zen-Bio Inc.) medium was replaced with DM-2 (Zen-Bio Inc.). Seven days later, the medium was changed to AM-1 (Zen-Bio Inc.). We transfected a cocktail of three siRNAs against murine *Psmb8*, *Psmb10* or control siRNA without any target genes (total 10 pmol) (B-Bridge, Cupertino, CA 95014 USA) into 3T3-L1 cells or a cocktail of three siRNAs against human *PSMB8* (total 10 pmol) or control siRNA without any target genes (total 10 pmol) (B-Bridge, Cupertino, CA 95014 USA) (Supplementary Table 4) into human preadipocytes using Nucleofector (Amaxa, Cologne, Germany). Cells were harvested and resuspended in Nucleofector solution at 2.5 x10^6 cells/100 μL. After addition of plasmids or siRNA, the cells were transfected using Nucleofector program T-030 or U-033, respectively. Transfected cells were plated in 12 well plates and used in differentiation experiments one or two days after the transfection. BALB/c mice were purchased from Japan SLC. (Hamamatsu, Japan) and housed in the Animal Research Center of the University of Tokushima under specific pathogen-free conditions. A cocktail of three siRNAs against murine *Psmb8* or negative control siRNA without any target sequence (B-Bridge, Cupertino, CA 95014 USA) (1 nmol/mouse) (Supplementary Table 4) was mixed with AteloGene Local (Koken, Japan) according to the manufacturer's protocol. The siRNA transfection mix was subcutaneously injected into shaved back skin of BALB/c mice three times at four day intervals.

Metabolic labeling

293T cells transfected with Flag-tagged *PSMB8* or mutant *PSMB8* were incubated in Dulbecco's modified Eagle's medium containing 100 μCi/mL L-[^{35}S]methionine/ L-[^{35}S] cysteine (EXPRESS Protein Labeling Mix, PerkinElmer Life Sciences) for 40 min at 37 °C, 5% CO_2. For immunoprecipitation, cell lysates were incubated with M2 anti-Flag monoclonal antibody (F1804, Sigma) for 90 min followed by incubation with Dynabeads Protein G (Invitrogen) for one h. The beads were washed four times, resuspended in 50 μL of 1x SDS-PAGE sample buffer. The samples were incubated at 95°C and cleared by centrifugation, and the supernatant was used for electrophoresis.

The gel was dried and exposed to the imaging plate for two days. The results were analyzed with Typhoon FLA9000 (GE Healthcare).

Histology

Biopsied skin and cultured cells were fixed in 10% formalin, embedded in paraffin, and sectioned. Tissue sections were stained with hematoxylin and eosin. Age-matched control skin was purchased from BioChain (CA, USA). Sections were also used for immunohistochemical analysis. Sections were deparaffinized with xylene, rinsed with ethanol solution, and endogenous peroxidase activity was blocked by incubating in 0.3% hydrogen peroxide for ten min at room temperature. Sections were incubated with a polyclonal anti-human PSMB8 (ab3329, Abcam) or a monoclonal anti-ubiquitin mAb (3936, Cell Signaling Technology) for one h at room temperature. Slides were washed and visualized using a ChemMate ENVISION kit/HRP (DAKO) with 3, 3'-diaminobenzidine tetrachloride as a chromogen. Staining was allowed to develop, the reaction was stopped in PBS, and sections were stained with hematoxylin. Hair follicles in 10000 μm^2 square (10 regions) were counted. The anti-PSMB8 antibody showed little positive staining of 293T cells by immunohistochemistry but showed strong staining of 293T cells transfected with cDNA for human PSMB8 (Supplementary Figure 6A). The anti-ubiquitin antibody showed only weak immunohistochemical staining of transformed B cells from control donors but showed strong staining of transformed B cells treated with epoxomycin (5 nM) (Peptide Inst., Osaka, Japan) for nine hours (Supplementary Figure 6B) and Western blotting (Supplementary Figure 6C).

Supplementary Figures Legends

Supplementary Figure 1. Homology models of PSMB8 WT and the G197V mutant.

(A) Space-filling representation of PSMB8 with G197 is shown. (B) The molecular surface of WT PSMB8 is colored as follows: red represents negative (blue, positive) electrostatic potential, and yellow represents hydrophobicity. The G197 region is framed by an orange circle. (C) Space-filling representation of PSMB8 G197V mutant model with V197 is shown. (D) The molecular surface of PSMB8 G197V colored as in (B), with the V197 region framed by an orange circle.

Supplementary Figure 2. Stability of β5i expression.

(A) Transformed B cells from a JASL patient (1-3PT) were incubated with Epoxomicin, a proteasome inhibitor (Peptide Ins., Osaka, Japan) (5 nM) for 48 h. The expression of PSMB8 and β-actin was examined by Western blotting. (B) The expression of β5, β1, and β2 subunits and β-actin in EBV-transformed B cells from 1-1M, 1-2ES or 1-3PT was evaluated by Western blotting. Genotypes of PSMB8 are shown. WT: wild type allele, Mu: mutant allele. (C) [^{35}S]-methionine-labeled cell lysates of 293T cells transfected with control vector (EV), Flag-tagged wild type PSMB8 or mutant PSMB8 were immunoprecipitated with anti-Flag antibody and subjected to SDS-PAGE (upper panel). The arrowhead indicates PSMB8. PSMB8 stability was calculated by measuring radioactivity (lower panel) (blue line: wild type PSMB8, red line: mutant PSMB8). The experiments in this figure are representative of three independent experiments.

Supplementary Figure 3. The mutation in PSMB8 does not affect activation of caspase 1.

Transformed B cells of a JASL patient (1-3PT), the mother (1-1M) and an unrelated healthy control were cultured in the absence or presence of Alum (Imject Alum, 400 μg/mL) (Thermo Fisher Scientific Inc. IL, USA) for six hours. As a positive control, THP-1 cells (kindly provided from Dr. Matsuo, University of Tokushima, Japan), a human monocyte cell line, were used. Cell lysates were obtained as described in Materials and Methods. Each cell lysate was subjected to SDS-PAGE, immunoblotted with polyclonal anti-caspase 1 or polyclonal anti-β-actin antibody and visualized as described in Materials and Methods. The experiments in this figure are representative of three independent experiments.

Supplementary Figure 4. Downregulation of PSMB8 expression affects adipocyte differentiation.

(A)The expression of PSMB8 and β-actin in 3T3-L1 cells zero, two and four days after differentiation to adipocytes was examined by Western blotting. (B) 3T3-L1 cells were transfected with siRNA against *Psmb10* (β2i) or control siRNA. Expression of β2i was evaluated by Western blotting (left panel). Induction of adipocytes was evaluated by staining cells with Oil-Red six days after induction and Oil-Red positive cells were quantified (right panel). Data are shown as means ± S.D. (C) RNA was isolated from the skin of mice that received *PSMB8* siRNA three times. The expression of *PSMB8* was examined by real-time PCR. Data are shown as means ± S.D. * indicates a statistical difference ($p<0.01$). (D) Skin sections in mice that received siRNA against *PSMB8* or control siRNA three times were stained with anti-PSMB8 antibody. Lower panel shows the enlarged figures of the square region of the upper panel. (E) Skin sections from mice that received siRNA against PSMB8 or control siRNA three times were stained with anti-FABP4 mAb. The experiments in this figure are representative of three independent experiments.

Supplementary Figure 5. High specificity of anti-phospho-p38 antibody

KOP cells, a murine p38-deficient fibroblast cell line (Riken Cell Bank, Tsukuba, Japan) were transfected with human p38 cDNA with or without cDNA of constitutively active MKK6. Cells were stained with isotype control polyclonal rabbit IgG or anti-phospho-p38 antibody followed by PE-conjugated anti-rabbit antibody one day after transfection and analyzed with the FACS Canto II. Dotted or Solid line indicates cells stained with isotype control antibody or anti-phospho-p38 antibody, respectively. The experiments in this figure are representative of three independent experiments.

Supplementary Figure 6. High specificity of anti-PSMB8 or ubiquitin antibody

(A) 293T cells were transfected with cDNA of human PSMB8 and then fixed in 10% formalin, embedded in paraffin, and sectioned. Sections from 293T cells transfected with pcDNA3.1 (pcDNA) or cDNA of human PSMB8 (pcDNA/PSMB8) were stained with anti-human PSMB8 as described in Materials and Methods (Histology section). (B) Transformed B cells from a healthy donor were incubated in the presence of epoxomycin, a proteasome inhibitor (5 nM) for nine hours and then fixed in 10% formalin, embedded in paraffin, and sectioned, or treated with lysing buffer. Cells treated or untreated with the proteasome inhibitor were stained with anti-ubiquitin antibody as described in Materials and Methods section (Histology section). (C) Cell lysates were subjected to Western blotting with anti-ubiquitin or β-actin antibody as descried in Materials and Methods section (Western Blotting). The experiments in this

figure are representative of three independent experiments.