Supporting Information
Otsuki, et al.

SI Appendix

SI Materials and Methods

Silkworm strains and insect cultured cells. The non-diapausing silkworm strain *w1-pnd* was used to generate transgenic silkworms. The commercial hybrid strain *Kinshu x Showa* (*KS* strain; Ueda San-shu, Ueda, Japan) was also used in this study. Silkworm larvae were reared on an artificial diet (Aseptic Sericulture System Laboratory, Kyoto, Japan) at 25 °C under aseptic conditions.

Silkworm *Bombyx mori*-derived BM-N and *Spodoptera frugiperda*-derived Sf21 cells were cultured at 27 °C in Grace's medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; MP Biomedicals, Santa Ana, CA, USA).

cDNA cloning of pierisin-1A (P1A) from *Pieris rapae* and P1A activity assay. All plasmids used in this study were confirmed by DNA sequencing. The oligonucleotide sequences are summarized in *SI Appendix*, Table S2. A genomic DNA clone encoding a sequence homologous to pierisin-1 was identified by screening using a pierisin-1 DNA probe from a previously constructed genomic DNA library (1). A cDNA clone was obtained by PCR using a primer coding for the newly identified gene named *pierisin-1A* (*P1A*; accession no. LC200434). The resulting fragment was inserted into the *Eco*RI-*Pst*I site of the pCold TF DNA vector (pCold TF-P1A; Takara Bio, Ohtsu, Japan) and confirmed by sequencing.

To express the P1A protein, *in vitro* transcription and translation were carried out as previously described (2). A PCR-amplified DNA fragment coding the T7 promoter plus P1A cDNA was obtained from pCold TF-P1A and transcribed using a MEGAscript® T7 Kit (Thermo Fisher Scientific, Waltham, MA, USA). The P1A protein was then translated from the obtained RNA using the rabbit reticulocyte lysate (Retic Lysate IVT™; Thermo Fisher Scientific, Waltham, MA, USA). The translation efficiencies were confirmed by autoradiography of SDS/PAGE gels with [^{35}S]methionine-incorporated products.

Analysis of the DNA ADP-ribosylating activity of P1A. The DNA ADP-ribosylating activity was determined as previously described (3,4). Briefly, calf thymus DNA (5 μg),

18.5 kBq [^{32}P]NAD and 10 μM *β*-NAD were incubated with 0.1 μl trypsinized *in vitro* translated protein (5 μl) in 50 μl reaction buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 50 mM NaCl, 1 mM DTT) for 30 min at 37 °C. The DNA was then recovered and digested with micrococcal nuclease (Wako Pure Chemicals, Osaka, Japan) and phosphodiesterase II (Wako Pure Chemicals, Osaka, Japan) before spotting onto CEL 300 PEI polyethyleneimine-cellulose TLC sheets (Macherey-Nagel, Duren, Germany). After development, the sheets were exposed to Fuji Imaging Plates (Fujifilm, Tokyo, Japan) and detected with a Bio-Image Analyzer (Fujifilm, Tokyo, Japan).

The products obtained by the reaction of *in vitro* translated P1A or pierisin-1 with DNA and *β*-NAD were examined by HPLC, as previously described (5). The reaction products were analyzed by HPLC with a LC-10A system (Shimadzu, Kyoto, Japan) armed with a SPD 10Avp photodiode array detector (Shimadzu, Kyoto, Japan) and a Develosil RPAQUEOUS column (4.6 ×250 mm; Nomura Chemical, Seto, Japan).

Construction of vectors. A DNA fragment lacking the P1A start codon (amino acids 2–853) was cloned into the pENTR/D-TOPO vector using the TOPO® Cloning Kit (Invitrogen, Carlsbad, CA, USA) to obtain the pENTR/D-P1A vector. The *P1A* sequence in pENTR/D-P1A was transferred into pDEST-N-H1 (6) using the GATEWAY™ PCR cloning system (Invitrogen, Carlsbad, CA, USA) to obtain pDEST-N-H1/P1A, which encoded a fusion protein consisting of P1A fused at the N-terminus to the H1 α-helix (polyhedron-encapsulation signal) from the cypovirus polyhedrin (amino acids 1–30; see ref. no. 6). pIZ-H1/P1A269/FLAG was obtained by inserting a PCR-amplified DNA fragment from the template DNA sequence in pDEST-N-H1/P1A into the *Bam*HI–*Xho* I site and by inserting a fragment encoding a DYKDDDDK peptide (FLAG) into the *Xho* I-*Xba* I site of pIZ/V5-His (Invitrogen, Carlsbad, CA, USA). The insert encoding the protein designated P1A269 in pIZ-H1/P1A269/FLAG had an N-terminal H1 plus the putative DNA ADP-ribosylating activity domain (2–269) of P1A and a C-terminal FLAG (*SI Appendix*, Figs. S1 and S3). Similarly, pIZ-H1/P1AFull/FLAG expressing the protein designated P1AFull, which included an H1/P1A fusion protein with a C-terminal FLAG, was obtained by inserting H1/P1A into pIZ/V5-His with the FLAG sequence (*SI Appendix*, Figs. S1 and S3). The oligopeptide tags N-terminal H1 and C-terminal FLAG were added for protein encapsulation into cypovirus polyhedra and simple immunological detection, respectively (6). A DNA fragment for the *B. mori* fibroin heavy-chain (FibH) promoter (nucleotides –860 to +10 in the FibH gene; accession no. AADK01000575) was amplified and cloned into *Kpn* I–*Bam*HI of pIZ-H1/P1A269/FLAG to construct

pIZ-FibHPro-H1/P1A269/FLAG. pFastBac-H1/P1A269/FLAG was constructed by inserting a PCR-amplified fragment for H1/P1A269/FLAG into *Bam*HI-*Pst* I of pFastBac1 (Thermo Fisher Scientific, Waltham, MA, USA); and a recombinant AcNPV named Ac-H1/P1A269/FLAG, for the production of P1A269 in Sf21 cells under the control of the polyhedrin promoter, was obtained using the Bac to Bac system (Thermo Fisher Scientific, Waltham, MA, USA). To construct the pBacMCS[FibHPro-H1/P1A269/FLAG,3xP3-egfp] vector (*SI Appendix*, Fig. S6), the fusion sequence for P1A269 along with the upstream FibH promoter and downstream OpIE2 3' untranslated region of pIZ/V5-His was amplified and inserted into the *Bln*I site in the pBacMCS[UAS, 3xP3-egfp] plasmid (7). Plasmids were purified using a Plasmid Midiprep Kit (QIAGEN) in accordance with the manufacturer's instructions.

Functional analysis of recombinant P1A expressed in cultured insect cell lines. pIZ-H1/P1A269/FLAG and pIZ-H1/P1AFull/FLAG (0.1 μg) were transfected into the insect cell lines BM-N and Sf21 (0.2×10^6) using the XtremeGENE HP reagent (Roche Diagnostic, Indianapolis, IN, USA) in accordance with the manufacturer's instructions. The empty vector pIZ/V5-His (Invitrogen, Carlsbad, CA, USA) was used as a control. Following incubation, morphological changes in the cells were examined using an Olympus IX71 light microscope. The effector caspase activity of each transfectant (22.5 μg protein from each sample), as indicated by hydrolysis of the caspase-3 substrate DEVD-AMC in the APOPCYTO Caspase-3 Fluorometric Kit (MBL, Nagoya, Japan) for 1 h at 37 °C, was measured using a Fluoroskan Ascent™ Microplate Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The caspase activity of each sample was then calculated using an AMC standard curve.

The reporter plasmid pIZ-actin-pro-5'UTR-luci (0.05 μg; see ref. no. 8) expressing luciferase was co-transfected into BM-N cells (0.2×10^6 cells per well) in 24-well plates (AGC Techno Glass, Shizuoka, Japan) with 0.1 μg of an empty vector, pIZ-H1/P1A269/FLAG or pIZ-H1/P1AFull/FLAG. Cell lysates (4 μL containing 1 μg/μL protein) were mixed with 20 μL of the substrate mixture Steady Glo® (Promega, Madison, WI, USA) and then incubated for 1 h at 25 °C. The luciferase activity of each lysate was then measured using a GloMax 20/20 luminometer (Promega, Madison, WI, USA).

Generation of transgenic silkworm lines. To generate transgenic silkworms, we performed micro-injections as previously described (9,10). The donor (pBacMCS[FibHPro-H1/P1A269/FLAG,3xP3-egfp]; *SI Appendix*, Fig. S6) and helper

plasmids for the production of transposase were injected into embryos at the pre-blastoderm stage. A transgenic silkworm line carrying the P1A269 gene was generated and screened for traits of the vector-derived marker, including EGFP expression in the eyes and central nervous system of G1 first instar larvae.

Transgenesis of the gene was confirmed using the following inverse PCR method. Genomic DNA from the silk glands of marker positive, G2 fifth-instar larvae was purified using a standard phenol treatment after overnight incubation with proteinase K. The purified genomic DNA was then digested with *Sau*3AI and circularized by overnight ligation at 16 °C using Ligation High version 2 (Toyobo, Osaka, Japan). The DNA fragments were amplified from the circularized template by PCR amplification of the fragment in the vicinity of both the upstream and downstream inverted terminal repeats (ITRs) of the *piggyBac* sequence (*SI Appendix*, Table S1). The transgenic line *w1-pnd*$^{P1A269/P1A269}$ carrying the homogenous allele *P1A269/P1A269* was further established by repeated sib matings of selected individuals that had EGFP expression in their eyes.

The *w1-pnd*$^{P1A269/P1A269}$ line was mated with the commercial strain *KS* allowing the hybrid *KW*$^{P1A269/P1A269}$ line with the genotype *P1A269/P1A269* to be established from the repeated sib matings.

SDS/PAGE analysis of commercial sericin. Commercial Pure Sericin (Wako Pure Chemicals, Osaka, Japan) was dissolved in water (1 % [W/V]). The Pure Sericin (10 μg) and intact sericin solution (4 μg) obtained from sericin cocoons in this study (*Materials and Methods*) were then denatured in an SDS/PAGE sample buffer, electrophoresed on a 5% gel and detected by Coomassie Brilliant Blue staining.

Preparation of proteins encapsulated in cypovirus polyhedra. To prepare purified cypovirus polyhedron-encapsulated P1A269, Sf21 cells were co-inoculated with Ac-H1/P1A269/FLAG and the recombinant baculovirus AcCP-H29/PDI expressing polyhedra plus protein disulfide-isomerase, as previously described (11,12). Similarly, to produce polyhedron-encapsulated human Leukemia inhibitory factor (LIF), Sf21 cells were co-inoculated with AcCP-H29/PDI and a recombinant AcNPV expressing human LIF fused with N-terminal H1, as previously described (13). Sf21 cells were also inoculated with AcCP-H29/PDI to prepare the empty polyhedra. The infected cells were cultured for 10 days at 27 °C, harvested in a test tube, and treated with an ultrasonic homogenizer (Titec, Nagoya, Japan) at 6% power for 30 s. Cell homogenates were centrifuged at 4 °C and the supernatant was removed. After repeated sets of these

treatments, the polyhedron suspensions were adjusted to the same density (5×10^4 cubes/μL) and stored at 4 °C in distilled water containing 100 units/mL penicillin and 100 μg/mL streptomycin.

Quantitative RT-PCR analysis of the P1A269 and actin A3 mRNAs. The P1A269 and *B. mori* cytoplasmic actin A3 mRNA levels in PSGs were analyzed using quantitative RT-PCR described in *Materials and Methods*. The primer sequences used in this analysis are listed in *SI Appendix*, Table S2.

SI Results

Expression of truncated P1A in insect culture cells. To investigate the function of P1A in insect cultured cells, recombinant full-length P1A (P1AFull) and the P1A N-terminal portion containing the DNA ADP-ribosyltransferase domain (P1A269) were transiently expressed by vector transfection of two insect cell lines, *B. mori*-derived BM-N and *S. frugiperda*-derived Sf21 (*SI Appendix*, Fig. S3). P1A269 expression was confirmed in both cell lines by immunoblotting using an antibody that was specific for the C-terminal tag (P1A269, *SI Appendix*, Fig. S7). Microscopy showed the presence of apoptotic cell fragments in Sf21 cells expressing P1AFull and P1A269 up to 48 h post-transfection, whereas no morphological changes were observed in Sf21 cells transfected with the empty vector, indicating that P1A lacking the C-terminal domain can induce DNA ADP-ribosylation and apoptosis in these cells (*SI Appendix*, Fig. S4). By contrast, the expression of P1A269 and P1AFull in BM-N cells did not induce any morphological changes characteristic of apoptosis until 96 h, but there was an increase in the number of flattened cells (*SI Appendix*, Fig. S4). The effector caspase activities of Sf21 cells expressing P1A269 and P1AFull at 24 and 48 h post-transfection were much higher (30- and 24-fold, respectively, at 24 h; 13- and 17-fold, respectively, at 48 h; $p < 0.001$) than those treated with an empty vector, whereas such increased activities were not observed in BM-N cells expressing P1A269 and P1AFull until 96 h (*SI Appendix*, Fig. S5*A*).

To further investigate the relationship between P1A function and cellular protein synthesis, a luciferase reporter plasmid was co-transfected into BM-N cells with P1A-expressing vectors and the expressed luciferase activities were compared with cells co-transfected with an empty vector. Luciferase expression was significantly repressed in BM-N cells co-transfected with the luciferase reporter and vectors expressing P1A269 and P1AFull (97.6 and 98.6% lower, respectively; $p < 0.001$) compared with cells co-transfected with the empty vector up to 96 h (*SI*

Appendix, Fig. S5*B*). These results indicated that ectopic gene expression of P1A269 and PIAFull leads to the non-apoptotic effects on silkworm BM-N cells that are characterized by an increase in the number of flattened cells and repression of protein synthesis. Meanwhile, P1A269 and PIAFull induced apoptosis in transfected Sf21 cells, suggesting that different kinds of cells activate different cellular pathways in response to DNA ADP-ribosylation.

SI References

1. Yamamoto M, et al. (2011) Nucleotide sequence and chromosomal localization of the gene for pierisin-1, a DNA-ribosylating protein, in the cabbage butterfly *Pieris rapae*. *Genetica* 139(10):1251-1258.
2. Watanabe M, et al. (1999) Molecular cloning of an apoptosis-inducing protein, pierisin, from cabbage butterfly: possible involvement of ADP-ribosylation in its activity. *Proc Natl Acad Sci USA* 96(19):10608-10613.
3. Matsumoto Y, et al. (2008) Distribution of cytotoxic activity and DNA ADP-ribosylating activity in crude extracts from the butterflies among the family Pieridae. *Proc Natl Acad Sci USA* 105 (7):2516-2520.
4. Nakano T, et al. (2006) Purification and molecular cloning of a DNA ADP-ribosylating protein, CARP-1, from the edible clam Meretrix lamarckii. *Proc Natl Acad Sci USA* 103 (37): 13652-13657.
5. Yamamoto M, et al. (2009) Molecular cloning of apoptosis-inducing Pierisin-like proteins, from two species of white butterfly, Pieris melete and Aporia crataegi. *Comp Biochem Physiol B Biochem Mol Biol* 154(3): 326-333.
6. Ijiri H, et al. (2009) Structure-based targeting of bioactive proteins into cypovirus polyhedra and application to immobilized cytokines for mammalian cell culture. *Biomaterials* 30(26):4297-4308.
7. Sakudoh T, et al. (2007) Carotenoid silk coloration is controlled by a carotenoid-binding protein, a product of the Yellow blood gene. *Proc Natl Acad Sci USA* 104(21):8941-8946.
8. Kotani E, Muto S, Ijiri H, Mori H (2015) *Bombyx mori* nucleopolyhedrovirus nucleic acid binding proteins BRO-B and BRO-E associate with host T-cell intracellular antigen-1 homologue BmTRN-1 to influence protein synthesis during

infection. *J Gen Virol* 96(7):1947-1956.

9. Tamura T, et al. (2000) Germline transformation of the silkworm *Bombyx mori* L. using a *piggyBac* transposon-derived vector. *Nat Biotechnol* 18(1):81-84.
10. Tamura T, Kuwabara N, Uchino K, Kobayashi I, Kanda T (2007) An improved DNA injection method for silkworm eggs drastically increases the efficiency of producing transgenic silkworms. *J Insect Biotechnol Sericol* 76(3):155–159.
11. Matsumoto G, et al. (2012) Bone regeneration by polyhedral microcrystals from silkworm virus. *Sci Rep* 2:935.
12. Matsumoto G, et al. (2014) Control of angiogenesis by VEGF and endostatin-encapsulated protein microcrystals and inhibition of tumor angiogenesis. *Biomaterials* 35(4):1326-1333.
13. Nishishita N, et al. (2011) The use of leukemia inhibitory factor immobilized on virus derived polyhedra to support the proliferation of mouse embryonic and induced pluripotent stem cells. *Biomaterials* 32(14):3555-3563.
14. Pallen M J, Lam A C, Loman N J, McBride A (2001) An abundance of bacterial ADP-ribosyltransferases--implications for the origin of exotoxins and their human homologues, *Trends Microbiol* 9(7):302-307; discussion 308.
15. Bell C E, Eisenberg D (1996) Crystal structure of diphtheria toxin bound to nicotinamide adenine dinucleotide. *Biochemistry* 35(4):1137-1149.
16. Molloy S S, Bresnahan P A, Leppla S H, Klimpel K R, Thomas G (1992) Human furin is a calcium-dependent serine endoprotease that recognizes the sequence Arg-X-X-Arg and efficiently cleaves anthrax toxin protective antigen. *J Biol Chem* 267(23):16396-16402.
17. Sphyris N, Lord J M, Wales R, Roberts L M (1995) Mutational analysis of the Ricinus lectin B-chains. Galactose-binding ability of the 2 gamma subdomain of *Ricinus communis* agglutinin B-chain. *J Biol Chem* 270(35):20292-20297.

SI Figures

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P1     1 MADRQPYM--TNGIQAAVVEWIRALDLEIISLLLSRAWPMALLATSELRWRPTVLTDTDNVVRLDRRQRLVRWDRRPPNEIFLDGFVPIVTRENPDWEETDLYGFAKNNHPSIFVSTVKT
P1A    1 ..N.G.PNPI.....L....S.....V..S...SI.DI..P.....EV.....I.....E.....D.....S....F.....

P1   119 QRNKK---KYVWTPRNANRGIVYQYEIYAPGGVDVNDSFSDASPWPNQMEVAFPGGIQNIYIRSARELHNGRIQRIWINPNFLDPGDLEPIVSSSRTPQVIWRMNHPDGGHR--DQRSER
P1A  121 ...R.NNK.....S.....E.LLHE.R.TT.....R.....RE..N.T.....N....R.....NKDS.G.LK.

P1   234 SASSYDDLMYGGTGNVQEDTFGDEPNNPKPIAAGEFMIESIKDKNSFLDLSKNVNGGVIHSNLYSGGDNQIWVFSYDDNKKAYRIQSYQNSYLYLSWDSNASSKEMILRGYTNSGSNNQY
P1A  241 .T..D.....A.D.E..S....V.-...FPD.....N.....TQ.EL..IV..HV.N.....T..E.....K.I.K...S.L...N...T...I.....E...D..C

P1   354 WQIEQTGKNYRLRNLLNLDMIITAQDKPSAFGGKEVIVNTEISNSNTKISQEWKMIPFDFRPIIDGDYNIFNVDLSNQVVDFSNQPDLLVHGHIFCDNENQTWHFTYNSTYHAYKIWSGR
P1A  360 .RF...DDY.....FV.....N.S.....K....TPDE.S..A..IN.VS.QT.P....H...L.V.DN.I...T.N.F.I..QNYM.....K.K...D.GSR....C..L

P1   474 KSNLLLTWDSNAASKEMVVRAYTESRSKNQYWRIEQTGSKSYKVRNLENSSMILGLTRVSTPYGGLNLMVEDDSDGHSDLHSDWDIKPIFYQDIPDGDYNIFNDNFPNIAIDFTNQEGSL
P1A  480 R.....S.N.....L..N...G.....R..DE..RL...Q.L.L..M..ES.S.....I.QN...INTN...N.N.....Y....E.H.....VV.....D..

P1   594 IHGHNFCSNNNQKWSFVFDGKRKAYRIKSGVRSNLWLSWDSNASSKEMVLRAYTESGSSNQYWRLDEANDGSYRIRNLQDYYKLIALTNKNTPYGGKELIVSDNKESGNTWYLKKLGEVP
P1A  600 ...Y.YFGT.....A.IY.ENKQ..K..NDASL---...N...KPQ.F.....T.....PV.....L...ISF.RS....DEDS....RK....TGT.N.....EI.K.A

P1   714 LPNRKFRIATKLNYKKVIDSSTSYNLIITHDLNFASSIWELVYDSSKKAYNIYSSDINNLGWIYQNKNFFVKLGNIDGPDHGDLRYFWTIEYSMQTGCYLIRSLHDPANAVGYTDSESVI
P1A  717 V..G.....NQNND...SEN..LLT.E...K....R.....V.....GY.....N...W.....S.....E.SH....S.TNA..

P1   834 TDTSTYSDNQLFHFILM
P1A  837 .....PI
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Fig. S1. Alignment of the deduced amino acid sequences of newly identified pierisin-1 (P1A) homologous with the previously reported pierisin-1 (P1; see ref. no. 2). The consensus arginine residue in the β-1 *s*trand (14), serine residue in the β-2 strand (14) and glutamic acid residue in the NAD binding site (15) are boxed in black. The R-x-x-R consensus motif recognised by furin (16) is underlined. The QxW motif or its equivalent consensus motif in each ricin lectin subdomain (17) is highlighted in gray.

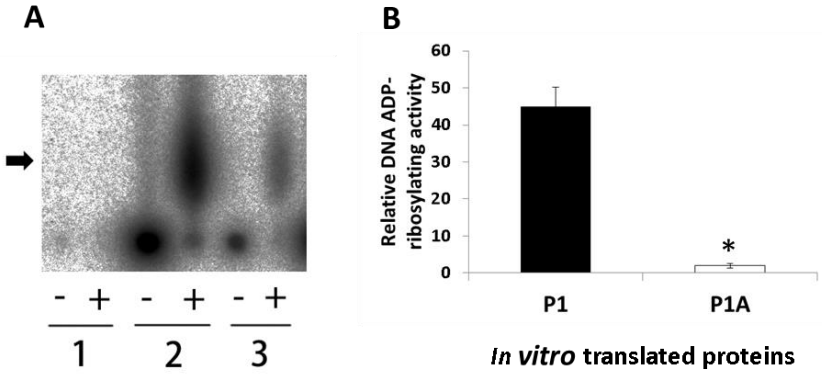

Fig. S2. Detection of ADP-ribosylated DNA adducts. (A) *In vitro* translated proteins were incubated with calf thymus DNA and [^{32}P]NAD, spotted onto TLC sheets with (+) or without (–) nuclease digestion, developed and then detected by autoradiography: sample 1, no protein (negative control); sample 2, *in vitro* translated pierisin-1 lysate; and sample 3, *in vitro* translated P1A lysate. Arrow indicates an Rf value of 0.05. (B) Relative DNA ADP-ribosylating activity of *in vitro* translated pierisin-1 (P1) or P1A that was analyzed by the HPLC method. Data are means ± SD; n = 3 independent samples. *$p < 0.001$ versus lysate of *in vitro* translated pierisin-1.

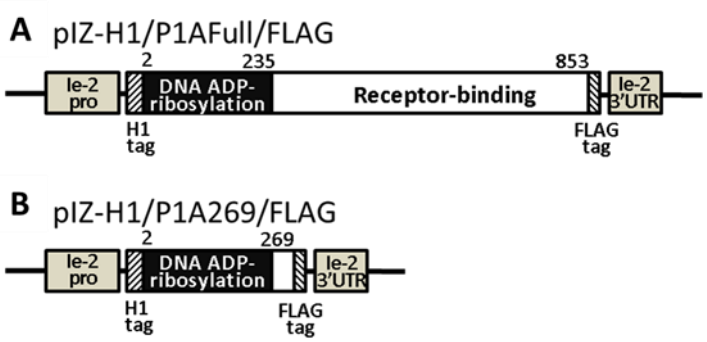

Fig. S3. Schematic diagrams of the plasmids for transient expression in the insect culture cells. (A) pIZ-H1/P1AFull/FLAG encoding amino acids 2–853 of P1A fused with N-terminal H1- and C-terminal FLAG-tag, P1AFull; P1AFull harbors the DNA ADP-ribosyltransferase domain and the receptor-binding domain. (B) pIZ-H1/P1A269/FLAG encodes amino acids 2–269 of P1A with the tags, P1A269; P1A269 harbors the DNA ADP-ribosyltransferase domain. Note: The expression of both proteins was controlled by the vector-derived Ie-2 promoter (Ie-2 pro) and both transcripts were designed to include the vector-derived Ie-2 3' untranslated region (Ie-2 3'UTR).

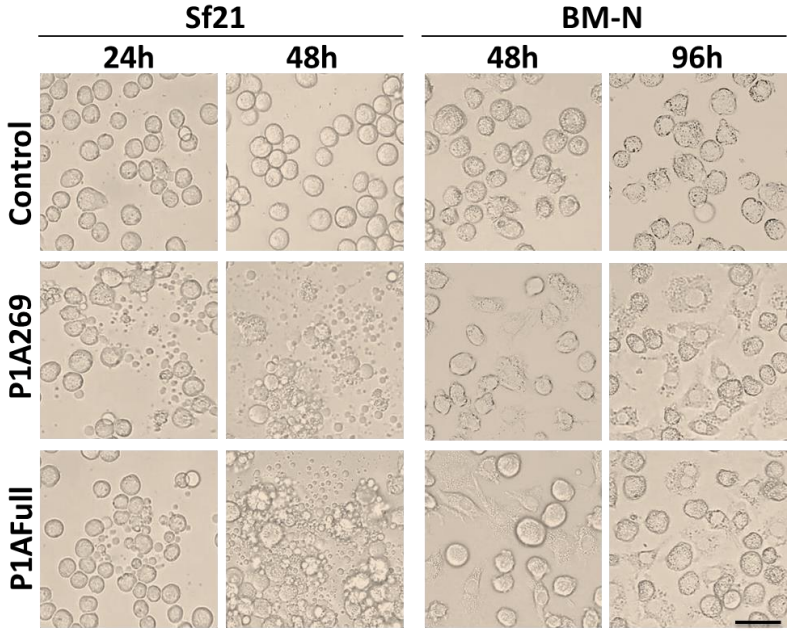

Fig. S4. Morphological changes in Sf21 or BM-N cells transfected with vectors for P1A269 or P1AFull. Both cell types were also transfected with empty vectors as a control. Cells were incubated for the indicated periods (hours) after transfection and then observed under a microscope (20× objective lens). Bar, 50 µm.

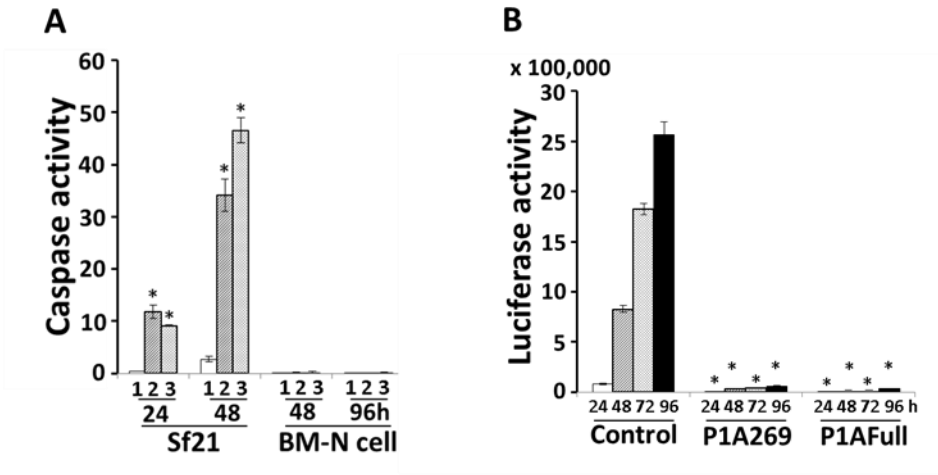

Fig. S5. Responses of insect cell lines expressing P1A. (A) Caspase activities expressed as nanomoles of free AMC produced following 1 h of incubation at 37 °C with 22.5 µg proteins from the lysates of Sf21 (24 and 48 h post-transfection) and BM-N (48 and 96 h post-transfection) cells transfected with empty vectors (column 1), vectors for P1A269 (column 2) or vectors for P1AFull (column 3). Data are means ± SD; n = 3 independent samples. *p < 0.001 versus empty vector-transfected cells. (B) Luciferase activity (photon counts/second produced by 4 µg protein from each sample) derived from the luciferase reporter plasmid in BM-N cell lysates 24, 48, 72 and 96 h post-transfection co-transfected with empty vectors (Control), vectors for P1A269 or vectors for P1AFull. Data are means ± SD; n = 3 independent samples. *p < 0.001 versus empty vector-transfected cells at the corresponding sampling times.

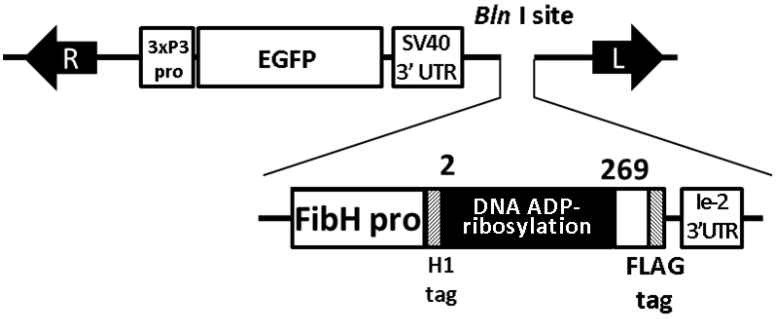

Fig. S6. Schematic diagram of the donor plasmid pBacMCS[FibHPro-H1/P1A269/FLAG,3xP3-egfp] that was used to establish *w1-pnd*$^{P1A269/P1A269}$. The expression of P1A269 was controlled by the FibH promoter (FibH pro). The expression of marker EGFP was controlled by the 3xP3 promoter (3xP3 pro) and the EGFP transcript was designed to include the vector-derived SV40 3' untranslated region (SV40 3'UTR). The transgene was coded between the left (L) and right (R) inverted repeat of *piggyBac*.

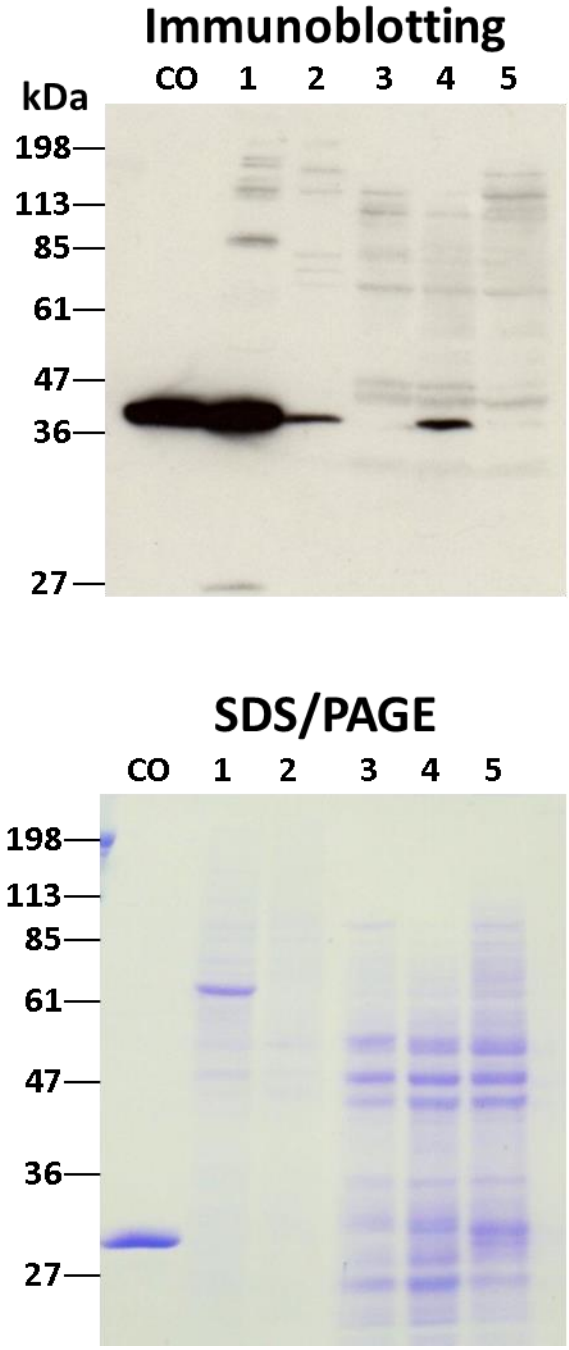

Fig. S7. Full gel image of the immunoblotting (upper panel) and SDS/PAGE (lower panel) of the same set of protein samples presented in Fig. 1*A*.

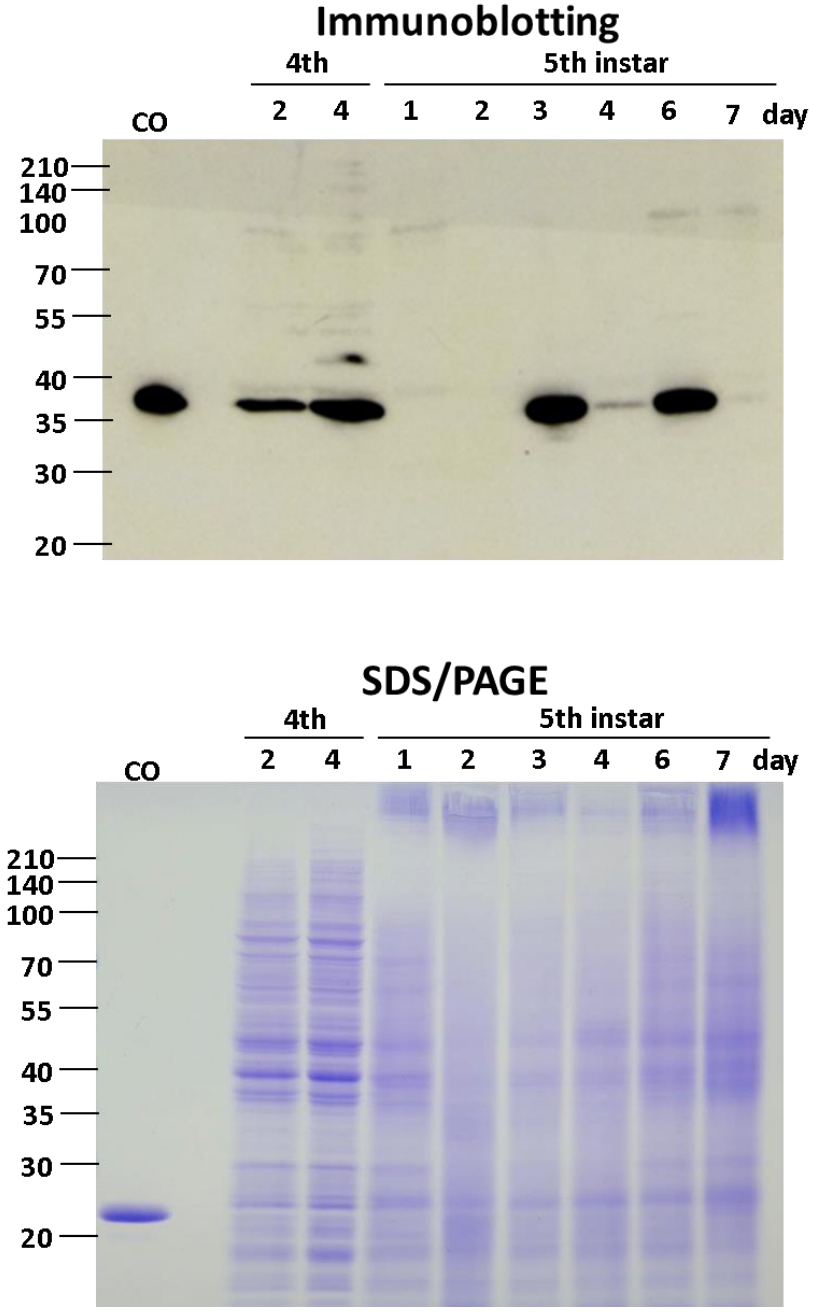

Fig. S8. Full gel image of the immunoblotting and SDS/PAGE of the same set of protein samples as presented in Fig. 1*B*.

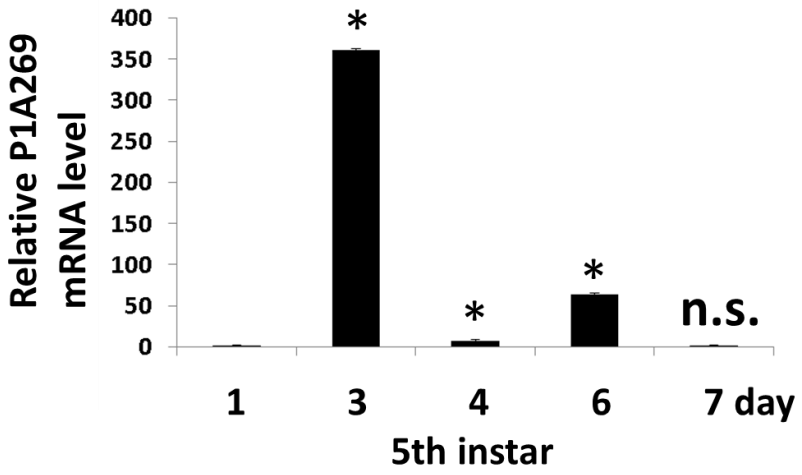

Fig. S9. Relative P1A269 mRNA levels in the PSGs from *w1-pnd*$^{P1A269/P1A269}$ larvae on days 1, 3, 4, 6 and 7 of the fifth instar as analyzed by quantitative RT-PCR. The values of expression levels for P1A269 mRNAs were normalized to that of 18S ribosomal RNA. Data are means ± SD of relative values obtained by comparing the value on day 1 (set as 1); $n = 3$ independent samples. *$p < 0.001$ and n.s. (not significant; $p > 0.05$) versus *w1-pnd*$^{P1A269/P1A269}$ larvae on day 1.

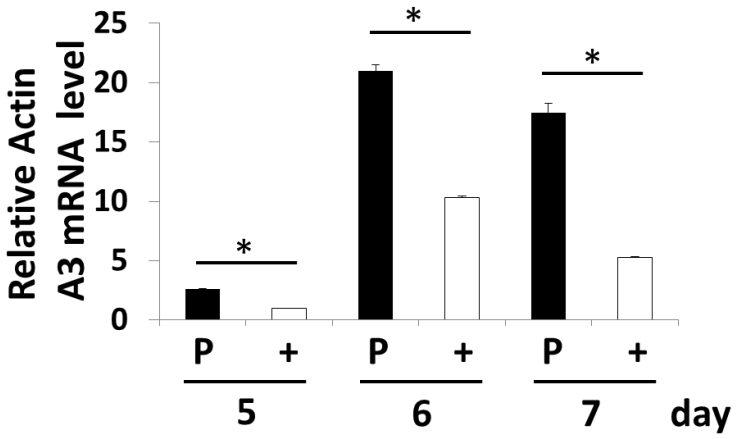

Fig. S10. Relative cytoplasmic actin A3 mRNA levels in the PSGs from *w1-pnd*$^{+/+}$ (+) and *w1-pnd*$^{P1A269/P1A269}$ (P) larvae on days 5, 6 and 7 of the fifth instar as analyzed by quantitative RT-PCR. Data are means ± SD of relative values. The values of expression levels for actin A3 mRNAs were normalized to that of 18S ribosomal RNA. Data are means ± SD of relative values obtained by comparing the value of *w1-pnd*$^{+/+}$ on day 5 of the fifth instar (set as 1); $n = 3$ independent samples. *$p <$ 0.001.

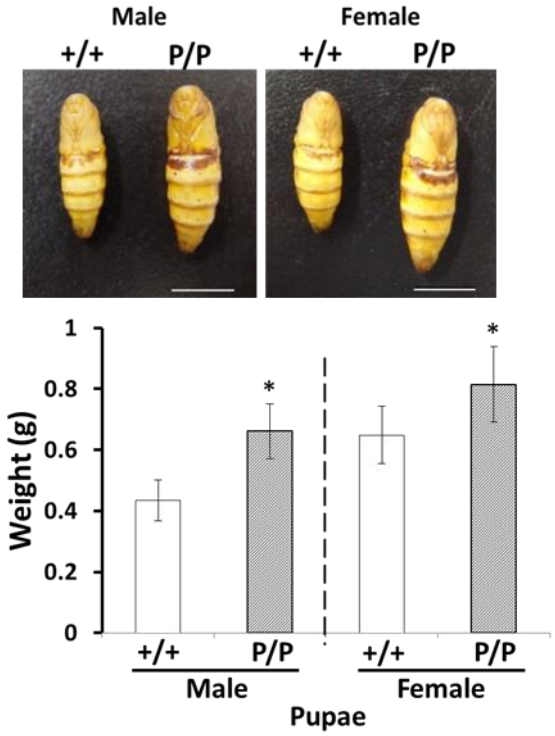

Fig. S11. Excess weight of *w1-pnd*$^{P1A269/P1A269}$ pupae. Pupae are shown in the upper panels, while a comparison of the weights of *w1-pnd*$^{+/+}$ (+/+) and *w1-pnd*$^{P1A269/P1A269}$ (P/P) pupae is provided in the lower panels. Data are means ± SD; male and female pupae of *w1-pnd*$^{+/+}$, n = 124 and 139, respectively; male and female pupae of *w1-pnd*$^{P1A269/P1A269}$, n = 173 and 172, respectively. *$p < 0.001$ versus *w1-pnd*$^{+/+}$ pupae. Bar, 10 mm.

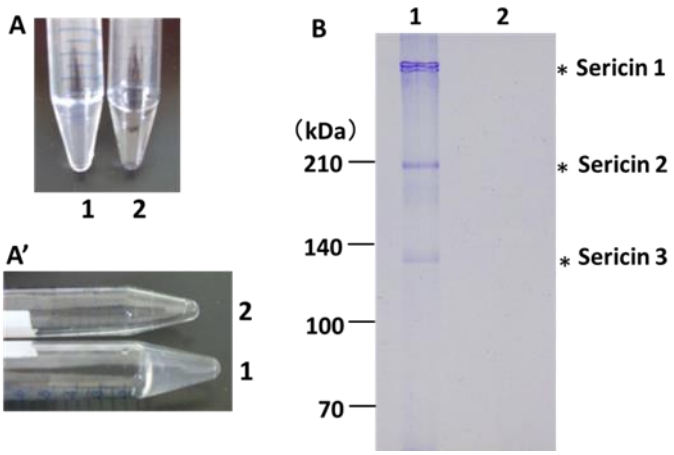

Fig. S12. Gel formation of sericin from the *w1-pnd*$^{P1A269/P1A269}$ cocoons. (A) Sericin hydrogel in a test tube, also shown lying horizontally (A'). One milliliter of a 1% (W/V) solution of intact sericin from *w1-pnd*$^{P1A269/P1A269}$ (tube 1) and a commercial sericin solution (tube 2) was mixed with 100 µL of ethanol in a test tube and then incubated for 1 h (*w1-pnd*$^{P1A269/P1A269}$ sericin) or 1 day (commercial sericin) at 4 °C. (B) Analysis of proteins in both solution by SDS/PAGE (5% gel) before ethanol addition; protein size markers are shown on the left.

SI Tables

Table S1. Genomic sequences bordering the transgene-inserted regions in the *w1-pnd*$^{P1A269/P1A269}$ line. Genomic DNA was isolated from the transgenic line *w1-pnd*$^{P1A269/P1A269}$ and the sequences bordering the transgene-inserted region were assessed by inverse PCR and database analysis using the KAIKOBLAST database (http://kaikoblast.dna.affrc.go.jp). The border sequence is listed with information about the chromosome and the clone name. The consensus sequence TTAA at the border of the *piggyBac*-driven transgene is indicated in bold.

Line	Sequence at the border of the transgene	Chromosome no. (clone name)
w1-pnd$^{P1A269/P1A269}$	CTTTCTGGCTTTTGTTACTTAGA**TTAA**- -(transgene)**TTAA**AGTACAATTTTGCCAACGTCGGG	Chromosome 16 (Bm_scaf4)

Table S2. The oligonucleotide primers used in this study. The authentic coding sequences for genes (*piggyBac,* FibH promoter, cytoplasmic actin A3 and 18S ribosomal RNA) and protein open reading frames (P1A, polyhedrin H1-helix, FLAG, FibH, and FibL) are underlined; restriction enzyme sites are indicated in bold.

Primers	Sequence (5'-3')	
(for cloning)		(constructed plasmids)
ECORI-P1A: *Eco*RI	AAA**GAATTC**ATGTCTAATCGAGGACC ACCA	pCold TF-P1A
PSTI-P1A: *Pst*I	AAA**CTGCAG**TAAATTGGAATAAAATGA AACAATT	pCold TF-P1A
P1A N-terminal	CACCGCTAATCGAGGACCACCAAACC	pENTR/D-P1A
P1A C-terminal	TTAAATTGGAATAAATGAAACAATTGA TTATCAGAGTATGTCG	pENTR/D-P1A
FLAG1: *Xho* I-*Xba* I	TTT**CTCGAG**CGATTACAAAGACGATG ACGATAAATAA**TCTAGA**TTT	pIZ-H1/P1A269/FLAG and pIZ-H1/P1AFull/FLAG
FLAG2: *Xba* I-*Xho* I	AAA**TCTAGA**TTATTTATCGTCATCGTC TTTGTAATCG**CTCGAG**AAA	pIZ-H1/P1A269/FLAG and pIZ-H1/P1AFull/FLAG
H1 5': *Bam*HI	TTT**GGATCC**AAAATGGCAGACGTAGC AG	pIZ-H1/P1A269/FLAG, pIZ-H1/P1AFull/FLAG, and pFastBac-H1/P1A269/FLAG

P1A(809) 3': *Xho* I	TTT**CTCGAG**CCATCTGGAAATGGTTTTGG	pIZ-H1/P1A269/FLAG
P1A(2558) 3': *Xho* I	TTT**CTCGAG**ATTGGAATAAAATGAAACAATTGGATTATCAGAGTATG	pIZ-H1/P1AFull/FLAG
FLG 3': *Pst* I	TTT**CTGCAG**TTATTTATCGTCATC	pFastBac-H1/P1A269/FLAG
FibHpro(-860): *Kpn* I	TTT**GGTACC**CAAAACTGCCACACGCATTTTTTTCTC	pIZ-FibHPro-H1/P1A269/FLAG
FibHpro(+10): *Bam*HI	TTT**GGATCC**TGCTGATTTGAAAAAGTTGAACC	pIZ-FibHPro-H1/P1A269/FLAG
FibHpro(-860): *Nhe*I	TTT**GCTAGC**ACCAAAACTGCCACACGCA	pBacMCS [FibHPro-H1/P1A269/FLAG, 3xP3-egfp]
OPIE2-3'UTR: *Nhe*I	TTT**GCTAGC**ACGCGCTTGAAAGGAGTGTG	pBacMCS [FibHPro-H1/P1A269/FLAG, 3xP3-egfp]
(for inverse PCR)		
PiggyBac right ITR 1: 5'	CGCATGATTATCTTTAACGTACGTCAC	
PiggyBac right ITR 2: 3'	GGGGTCCGTCAAAACAAAACATC	
PiggyBac left ITR 1: 5'	GAGTCTCTGCACTGAACATTGTCA	
PiggyBac left ITR 2: 3'	ATCAGTGACACTTACCGCATTGACA	

(for realtime PCR)	
18S rRNA-1	CGATCCGCCGACGTTACTACA
18S rRNA-2	GTCGGGCCTGGTGGTGAGATTT
FibL-1	CGTCATCAACCCTGGTCAAC
FibL-2	GCGGCTTCGAAGTCATAGAT
FibH-1	TCCGACGGTAACGAGTCCATTG
FibH-2	TACGTATGGCCCGCTCTGAGAA
Actin A3-1	GAAGCTGTGCTACGTCGCTC
Actin A3-2	CCGATGGTGATGACCTGACC
P1A269-1	CCATCTGGAAATGGTTTGG
P1A269-2	TGTATGGTGGTGCAGGTGAG
(for *in vitro* transcription of *P1A*)	
Transcription-1: 5'	TAATACGACTCACTATAGGGAGACCAC
	CATGCCTGCTGGAAAATACCTATACCG
Transcription-2: 3'	GACTATCGTCGTTGTTTATTTTGA