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A single amino acid mutation in an ABC transporter gene causes resistance to Bt toxin Cry1Ab in the silkworm, *Bombyx mori*

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AUTHOR SUMMARY

Toxins produced by *Bacillus thuringiensis* (Bt) are widely used for controlling insect pests as an insecticidal constituent in agricultural chemicals and transgenic crops. Expanding use of Bt insecticides and widespread cultivation of Bt crops have raised concerns about the potential accelerated development of Bt resistance in field populations (1). Despite the broad use of Bt toxin and discovery of molecules involved in Bt resistance in agricultural pests such as the tobacco budworm, *Heliothis virescens*, the diamondback moth, *Plutella xylostella,* and the pink bollworm, *Pectinophora gossypiella*, its mode of action is not fully understood (2).

The domesticated silkworm, *Bombyx mori*, in which this bacterial pathogen was first reported, shows various levels of susceptibility to Bt toxin among inbred strains. Taking advantage of recent advances in genome databases (3) and high density genetic maps (4) for map-based cloning, together with transgenic techniques (5) for the study of gene function, we initiated cloning of a silkworm gene conferring resistance to Bt toxin Cry1Ab. In these studies we used two strains differing nearly 300-fold in LC_{50}: Rin, a susceptible strain (LC_{50} 0.002 μg protein/cm^2), and C2, a resistant strain (0.567 μg protein/cm^2). F_1 hybrids were susceptible, indicating that resistance was recessive. We used single nucleotide polymorphism (SNP)-based PCR products to determine the linkage group carrying Bt resistance. Backcross (BC_1) progeny from a cross between an F_1 female (C2 female x Rin male) and a C2 male that survived Bt toxin screening were expected to carry homozygous alleles for Bt resistance. Only linkage group (chromosome) 15 among 28 linkage groups showed homozygosity in all tested progeny, indicating that the resistance locus was on this chromosome; all other chromosomes exhibited some heterozygotes. We examined linkage of other genes reported to be associated with Bt resistance including genes of *cadherin-like protein*, *aminopeptidase Ns*, *alkaline phosphatase*, and *glycosyltransferases*, but none were located on chromosome 15, indicating that this was a different form of resistance. Additionally, we detected no difference in the digestion of protoxin (130 kDa) into active toxin (60 kDa) between resistant C2 and susceptible Rin strains, indicating that enzymatic midgut toxin activation was unrelated to resistance.

Subsequently, we performed map-based (positional) cloning of the Bt resistance gene on chromosome 15 using BC_1 progeny between a C2 female and an F_1 male (C2 female x Rin male). We conducted 3 rounds of chromosome mapping on 44, 32, and 15 larvae selected after Bt toxin and SNP marker screening on several thousand BC_1 progeny. Using KAIKObase (http://sgp.dna.affrc.go.jp/KAIKObase/) (3), we found 6 candidate genes in a chromosome region ultimately narrowed to 82 Kb. These 6 genes were reduced to 4 genes because of incorrect assignment in the database, among which 2 genes were not expressed in the midgut. We determined the sequences of the remaining 2 candidate genes in C2 and Rin strains. One of them showed no difference; however, we found significant polymorphism between the two strains in the other gene, *BGIBMGA007792-93*, which was annotated as an ATP-binding cassette (ABC) family C transporter gene and was the most plausible candidate for Bt resistance. Upon examining 6 additional resistant and 9 susceptible strains for sequence polymorphisms, we found that a single, common amino acid (tyrosine) deletion/insertion distinguished susceptible vs. resistant strains.

We introduced the ABC transporter gene from the susceptible strain Rin into resistant strain w1-pnd, which is routinely used in silkworm transgenesis. We crossed the transformed UAS lines SS16-1 and SS16-3 with GAL4-line 52-2, which expresses GAL4 in the midgut. We used offspring selected for dual marker proteins EGFP for UAS and DsRed2 for GAL4 for Bt toxin screening. Examination in 2nd and 4th instar larvae, which are expected to

possess the endogenous ABC transporter genes (resistant) in both sister chromosomes and a transformed one (susceptible) in one of the sister chromosomes, revealed that they were susceptible to Bt toxin. We confirmed expression of the transgene and endogenous genes by RT-PCR. This is the first published study demonstrating that germline introduction of a functional form of a gene conferring resistance to Bt toxin can convert an insect from resistant to susceptible.

The tyrosine deletion/insertion site was on the 2nd outer loop in a predicted 12 transmembrane structure (Fig. P1, magenta dot). The involvement of an ABC transporter orthologous to the present *B. mori* gene was recently implicated in Cry1Ac resistance of *H. virescens* (6) and 2 other lepidopteran pests (7), but without direct functional confirmation. Here, we demonstrated that a mutation in the ABC transporter caused an alteration of susceptibility to Cry1Ab toxin. Homologous ABC transporter gene (subfamily C) to this *Bombyx* gene is known to work for human multidrug resistance. However, considering the recessive trait of the gene for Bt resistance, a more plausible mechanism for its mode of action might be that the ABC transporter acts in the midgut in conjunction with a toxin receptor such as a cadherin-like protein or aminopeptidase N or in insertion of the toxin into cells. The allelic forms of the silkworm gene will provide tools for critical functional studies of the transporter in the mechanism of Bt action in arthropods.

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Fig. P1. Map-based (positional) cloning scheme of Bt resistance gene from silkworm. Two strains, resistant (blue) C2 and susuceptible (yellow) Rin, were crossed and surviving silkworms after Bt toxin screeing in the BC_1 generation were used for chromosome linkage analysis and positional cloning. The susuceptible allele of the candidate gene for Bt resistance (the ABC transporter gene of the strain Rin) was introduced into a resistant strain (w1-pnd) that has been used for transgeneis. The transformed strain SS16 (UAS line) was tested after crossing with a GAL4-line, indicating the introduction of the transgene converted the resistant w1-pnd strain to susceptible. A mutation (deletion/insertion in susceptible/resistant strains) was found in the second outer loop of the predicted ABC transporter.

A single amino acid mutation in an ABC transporter gene causes resistance to Bt toxin Cry1Ab in the silkworm, *Bombyx mori*

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Abstract

Bt toxins derived from the arthropod bacterial pathogen, *Bacillus thuringiensis*, are widely used for insect control as insecticides or in transgenic crops. Bt resistance has been found in field populations of several lepidopteran pests and in laboratory strains selected with Bt toxin. Widespread planting of crops expressing Bt toxins has raised concerns about the potential increase of resistance mutations in targeted insects. Using *Bombyx mori* as a model, we identified a candidate gene for a recessive form of resistance to Cry1Ab toxin on chromosome 15 by positional cloning. *BGIBMGA007792-93*, which encodes the ABC transporter similar to human multidrug resistance protein 4 and orthologous to genes (*ABCC2*) associated with recessive resistance to Cry1Ac in *Heliothis virescens* and 2 other lepidopteran species, was expressed in the midgut. Sequences of 10 susceptible and 7 resistant silkworm strains revealed a common tyrosine insertion in an outer loop of the predicted membrane-bound structure of resistant alleles. We confirmed participation of this ABC transporter gene in Bt resistance by converting a resistant silkworm strain into a susceptible one using germline transformation, the first direct demonstration of Bt resistance gene function by transgenesis in insects.

Bacillus thuringiensis, a bacterial pathogen for many insects, produces insecticidal proteins which are used as selective orally-ingested insecticides. The genes of the insecticidal toxin are also introduced into Bt resistant crops. Increasing use of the toxins has threatened to increase the prevalence of Bt resistance in insect pest populations since its first discovery in 1985 in *Plodia interpunctella* (1). A key problem in agricultural production is how to avoid the development of Bt resistant pest populations (2, 3).

A number of Bt resistance mechanisms have been reported, including mutations in cadherin and aminopeptidase genes (4). The most common type of resistance is "Mode I," characterized by recessive inheritance, high resistance level, and reduced binding of toxin to a putative midgut receptor (5). Some lepidopteran pests, e.g., *Plutella xylostella* and *Heliothis virescens*, show characteristics of Mode I resistance. However, Bt resistance was not fully explained by these mutations and the molecular basis for this type of resistance has not been unequivocally established in these pest species (6). Elucidation of Bt resistance genes, especially those involved in the resistance of major pest populations, is of great importance for understanding the detailed mode of action and practical use of these environmentally safe molecules.

Recently, a mutation in a class of ATP-binding cassette (ABC) transporters was proposed to be associated with Bt resistance in a laboratory population of *Heliothis virescens* (7). This study utilized the *Bombyx* genetic map (8, 9) and genome sequence, aided by the results of a reported chromosomal linkage analysis of the *Bombyx* Bt resistance gene (10) and a high level of chromosome synteny between these two species. Although mutations in the orthologous ABC transporters (ABCC2) were reported to be associated with Bt resistance in *Trichoplusia ni* and *Plutella xylostella* (11), without direct functional assays on the

mechanism of resistance, the evidence that this ABC transporter is involved in Bt resistance of this pest remains circumstantial. This raises two important research issues. One is to confirm that mutation of the ABC transporter gene *ABCC2* is causally-related to Bt resistance, and the second is to explore the function of this gene in the resistance mechanism.

Here, we report direct evidence that Bt resistance is caused by a mutation in an orthologous ABC transporter in *B. mori* by introducing a Bt susceptible allele into a resistant silkworm using transgenesis. That a positional cloning study to seek the Bt resistance gene in *B. mori* was performed independently from the *Heliothis* study (7) using available *Bombyx* genome information clearly indicates this gene (*ABCC2*) is the causal agent of Bt resistance. Further, resistance in the transporter gene seems to be attributable to a single tyrosine insertion in an outer loop of the predicted transmembrane protein, a surprisingly drastic effect which promises to yield new insights into the function of this protein.

Results

Insecticidal screening of silkworm strains using Cry1Ab. We tested susceptibility to Cry1Ab toxin in 133 inbred silkworm strains and found a wide concentration range required for lethality. We chose two strains in which the LC_{50} of newly hatched larvae differed by 315-fold, Chinese No. 2 (C2; resistant, LC_{50} 0.5664 μg protein/cm^2) and Ringetsu (Rin; susceptible; 0.0018 μg protein/cm^2). The genetic basis of resistance in C2 was shown to be inherited as a single major recessive gene by crossing experiments. C2 resistant strain was susceptible to Cry1Aa toxin (LC_{50} 0.0310 μg protein/cm^2) as well as Rin susecptible strain (0.0122 μg protein/cm^2), showing no cross-resistance between Cry1Ab and Cry1Aa. The

resistance gene was mapped to linkage group 15 (chromosome 15) among 28 linkage groups using restriction fragment length polymorphisms (10).

Linkage analysis using SNP markers. We initiated map-based cloning of the resistance locus using these two strains, C2 and Rin, based on the completed silkworm genome sequence (12-14) and an integrated physical-genetic map (8, 9). Taking advantage of the lack of chromosomal crossing over in females, we first confirmed the linkage assignment of the resistance trait by single nucleotide polymorphism (SNP) marker-based analysis (8) using surviving progeny from a backcross (BC_1) between an F_1 female (C2 female x Rin male) and a C2 male. The yield of BC_1 survivors at a preliminary-defined dose (0.031 μg protein/cm^2) expected to kill 100% of susceptible larvae was 48.9%, consistent with resistance being under the control of a single recessive gene (Table 1). We extracted DNA from 19 surviving 5th instar larvae and amplified the DNA using primers corresponding to the genome region previously shown to have SNP(s) in the two strains, C2 and Rin (8). Genotypes (C2/C2 or C2/Rin) were determined by sequencing the PCR products (Fig. S1). All of the surviving larvae were homozygous (C2/C2) only for chromosome 15 (Table S1), indicating that the Bt-resistance gene locates on the chromosome 15.

Comparing with previously known Bt resistace genes. To date several genes have been implicated in Bt resistance in lepidopteran pests and in the nematode, *Caenorhabditis elegans*. To ascertain whether the strain C2 resistance gene corresponded to any of these potential candidates, we examined their chromosome assignments in silkworm using KAIKObase (http://sgp.dna.affrc.go.jp/KAIKObase/) (15). Glycosyltransferase genes of *B. mori* were PCR amplified, cloned and sequenced using newly desined primers (Bre-primers

in Table S2). None of the genes for cadherin-like peptide (16), aminopeptidases (17, 18), glycosyltransferases (Bre-2–5) (19, 20), alkaline phosphatase (21), chlorophyllide-binding protein (22), α-amylase (23) or mitogen-activated protein kinase p38 (24) were located on chromosome 15 (Table S3), indicating the presence of a different form of Bt resistance.

Protoxin activation and toxin digestion. Gut protease is required to activate Bt protoxin and lack of major gut proteases is associated with a form of toxin resistance (25, 26); conversely, high enzymatic activity may quickly digest toxin, resulting in low susceptibility. Therefore, we compared midgut enzyme activity between strains C2 and Rin. Gut enzyme extracts from both strains digested Cry1Ab protoxin protein (130 kDa) to the active toxin protein form (60 kDa) with no marked differences in the protoxin digestion profiles (Fig. S2), indicating that the resistance in C2 was not related to the gut enzyme digestion process.

Binding ability of toxins to the midgut brush border membrane vesicles (BBMV) was examined in susceptible Rin and resistant C2 strains, because recessive resistance might be related to defect of midgut receptors, to which Bt toxins bind. Cry1Ab toxin was biotinylated and incubated with BBMV prepared from two silkworm strains. The toxin bound to the BBMV was detected using streptavidin-peroxidase and chemiluminescence detection system. Specific binding of the toxins was evaluated with excess amount of unlabeled toxin. Cry1Ab specifically bound to BBMV of both susceptible Rin and resistant C2 strains (Fig. S3) and no visible difference was detected between two strains, indicating that initial Cry1Ab binding to midgut receptor(s) occurs in both strains.

Map-based cloning. We carried out map-based cloning for the resistance gene on chromosome 15 in three stages using progeny from male informative backcrosses (BC_1), that were those between a C2 female and an F_1 male (C2 female x Rin male). We first determined a broad candidate region for the resistance locus using 44 larvae that survived toxin treatment at a discriminating dose (>0.031 μg protein/cm^2) using 17 SNP markers on chromosome 15. As before, we expected the surviving larvae to be homozygous for resistance (C2/C2), and heterozygous larvae (C2/Rin) to be susceptible. We determined the homozygous or heterozygous state for all SNP marker regions by direct sequencing of PCR products (Table S4). The homozygous region among all 44 samples was located between markers 15-016 and 15-089 on chromosome 15, which we estimated to be located at 11.4 cM in the genetic map (Fig. 1).

To narrow down the location of the Bt resistance mutation, we performed two more rounds of mapping experiments. We obtained 400 new DNA samples from resistant larvae of the male informative backcross generation and sequenced the PCR products from two SNP-PCR markers, 15-016 and 15-089. We sought samples that showed homozygosity for one marker and heterozygosity for the other, indicating a crossover had occurred between these two primers in one of the sister chromosomes. In a second round of mapping (Table S5), we used 10 PCR-SNP markers on 32 larvae to narrow the candidate region to about 1.0 cM, which corresponded to a physical distance of about 1 Mb located between SNP markers 15-011 and 15-089 (Fig. 1). In a third round of mapping (Table S6), we screened another set of 1,365 resistant backcross larvae; from these we selected 15 samples that were homozygous in one marker region (15-011 or 15-089) and heterozygous in another. Using 17 PCR-SNP markers including 16 newly designed ones (Table S2), we finally narrowed the candidate region to approximately 82 kb between markers 15-327-4 and 15-218 (Fig. 1).

Determination of the candidate gene. Six genes, *BGIBMGA007735*, *007793*, *007736*, *007792*, *007791* and *007737*, were predicted in the 82 kb candidate region by gene models in KAIKObase ver. 2.1.0 (Fig. 1, Table 2; http://sgp.dna.affrc.go.jp/KAIKObase/) (15); the marker 15-327-4 was located inside the predicted gene, *007735*. Of these six genes we found *007735*, *007793*, *007736*, and *007792* were expressed in the midgut of C2 and Rin by RT-PCR, excluding *007791* and *007737* as candidates (Fig. 2). Determination of the cDNA sequences of the four expressed genes revealed that *007792* and *007793* belonged to a single gene and *007736* was present in the intron region of *007792-93*. We concluded that *007736* was annotated incorrectly as its PCR product seemed to correspond to an immature mRNA. Consequently, we predicted two *bona fide* candidate genes in the narrowed region, *007735* and *007792-93*, both of which showed homology to members of the ATP-binding cassette (ABC) transporter superfamily.

The nucleotide sequences of *007735* were identical between the two silkworm strains in the region inside the critical SNP markers (accession number AB621548), suggesting this gene was unlikely to be responsible for Bt resistance. In contrast, the sequences of *007792-93* were significantly different between C2 and Rin (Fig. S4, accession numbers AB620074 and AB620075, respectively): 39 different nucleotides in the protein coding region yielded 13 different amino acid residues, including one insertion/deletion. From these results we concluded that *007792-93* was the most plausible candidate gene for the Bt resistance and further examined its expression in 11 silkworm organs and tissues by RT-PCR. We found that *007792-93* was expressed in the midgut, but not in the fat body, silk glands, Malpighian tubules, haemocytes, testis, ovary, or integument (Fig. S5), a pattern consistent with a role in conferring resistance to Bt toxin.

We determined the nucleotide sequences of gene *007792-93* in 6 additional Bt-resistant and 9 susceptible silkworm strains (Table S7) to determine which sequence differences in the coding region were responsible for Bt resistance. Strains that showed a dominant genetic trait in the original toxin survey and preliminary genetic studies were excluded. Although the sequence comparison among the 17 strains revealed many polymorphisms, only one showed a fixed difference between resistant and susceptible strains (Fig. 3; Fig. S6): the insertion of three consecutive nucleotides encoding tyrosine in the *007792-93* gene product in resistant strains. The presence of this common polymorphism in a predicted ABC transporter expressed in larval midgut strongly implicated this gene in contributing to Bt resistance.

Introduction of susceptible gene into the resistant strain. To confirm that *007792-93* was the causative agent of the Bt resistance, we introduced a copy of the gene from susceptible strain Rin (*Rin-007792-93*) into a resistant strain. The recipient resistant strain was the non-diapausing white-eyed silkworm strain (w1-pnd), derived from a strain (w1-c) and used for transgene expression (27). We established two transgenic strains (SS16-1 and SS16-3) expressing *Rin-007792-93* under the upstream activating sequence (UAS) together with *EGFP* as a selectable marker. SS16-1 had two transfered genes on the chromosomes 15 and 23 and SS16-3 on the chromosome 25 (Fig. S7). We crossed these males with females of a previously established GAL4 driver strain carrying *DsRed2* (52-2) (28) and selected offspring that possessed both *Gal4* and *Rin-007792-93* by examining eye colors derived from DsRed2 and EGFP at a late embryonic stage (Fig. S8).

We tested the resistance levels of the transgenic silkworms at the 2nd and 4th larval instars by feeding Cry1Ab toxin on mulberry leaf disks and recording mortality after 4 days. We first examined the parent (Rin and C2), recipient (w1-c and w1-pnd), and GAL4-driver

(52-2) strains at 2nd instar for susceptibility (Table 3). The susceptible strain, Rin, had an LC_{50} of 0.006 μg toxin/cm^2, in contrast with the LC_{50} of the resistant strain, C2, which was greater than 17.6 μg toxin/cm^2. The recipient and driver strains had LC_{50} values of 1.9–22 μg toxin/cm^2. We then tested the two transgenic strains, SS16-1 and SS16-3, at two larval stages. The LC_{50}s of 2nd instar larvae from crosses between 52-2 and SS16-1 or SS16-3 were 0.0054 and 0.0033 μg toxin/cm^2, respectively (Table 3, Fig. 4), showing susceptibility to Bt toxin. As controls, offspring from crosses between w1-c females and the SS16 transgenic strains showed high resistance to toxin (LC_{50} values 48.7 and > 800 in SS16-1 and SS16-3, respectively). Crosses between the 52-2 GAL4 driver strain and the original w1-c strain also produced resistant offspring (LC_{50} value 3.9). We obtained similar results for 4th instar larvae, confirming that introducing *Rin-007792-93* into Bt-resistant silkworm strains made them highly susceptible to Cry1Ab toxin (Table 3).

Expression of the introduced gene in transgenic silkworms. We confirmed expression of the introduced gene into the transgenic silkworms by realtime RT-PCR. Since the transgenic silkworms possessed an endogenous *007792-93* gene, we used primers designed for the 3' region that included mismatched nucleotides for distinguishing the expression of the endogenous and exogenous genes separately (Fig. S9; Table S2). We successfully quantified expression of the genes in the midgut of 4th instar C2 and Rin larvae (Fig. 5A). We also quantified expression in three groups of transgenic animals, 52-2 x SS16 (GAL4 x UAS), w1-c x SS16 (no-GAL4 x UAS), and 52-2 x w1-c (GAL4 x no-UAS) by realtime RT-PCR using the two effector strains, SS16-1 (Fig. 5B) and SS16-3 (Fig. 5C). The exogenous *Rin-007792-93* gene was highly expressed in 52-2 x SS16-1 and 52-2 x SS16-3 (GAL4 x UAS) (a in Fig. 5B and 5C). Although we could not compare directly the

expression levels of the introduced gene, *Rin-007792-93*, and the endogenous gene, *w1-c-007792-93*, because of different PCR efficiency using different primers, the expression level of *Rin-007792-93* was apparently as high as that of *w1-c-007792-93* in both SS16-1 and SS16-3. Notably, *Rin-007792-93* was expressed at a low level even in the absence of GAL4 (b in Fig. 5B and 5C), indicating leaky expression of the introduced gene. However, the effect of the leaky expression on the resistance level was unclear (w1-c x SS16 in Table 3).

Structure of ABC transporter gene. The gene *007792-93* showed high homology to human ABC transporter gene ABCC4, which is known to be involed in multidrug resistance (Fig. S10). Two ATP-binding cassette domains were predicted including Walker A, WalkerB, and C-motifs. Two transmembrane domains each consisting of 6 transmembrane regions (TM) were also predicted (Fig. S10). The insertion of tyrosine was predicted in or on the edge of the second outer loop between TM 3 and TM4 (Fig. 6; Fig. S11).

Discussion

This is the first published study demonstrating that germline introduction of a functional form of a gene conferring resistance to Bt toxin can convert an insect from resistance to susceptibility. It confirms a central role for *007792-93* gene in Bt toxin action. The achievement of the gene cloning and confirmation of function of the cloned gene was accomplished using three main research platforms. First, the success of the the map-based cloning was much owed to a well-maintained genome database (http://sgp.dna.affrc.go.jp/KAIKObase/) (15) and a high density of SNP markers on the genetic map (http://sgp.dna.affrc.go.jp/LinkageMap/cgi-bin/index.cgi) (8, 9). Second, the

353 transformation technique that was first devoloped in lepidopteran insects (29) clearly
354 demonstrated that the candidate *007792-93* gene played a key role in the Bt toxin response.
355 Finally, selection of two suitable Bt resistant/susceptible strains for map-based cloning and
356 determination of the site of the mutation using many resistant and susceptible strains were
357 achieved by using silkworm strains maintained in the Genetic Resource Center of National
358 Institute of Agrobiological Sciences
359 (http://www.gene.affrc.go.jp/databases_en.php?section=animal). Successful use of these
360 resources illustrates that the silkworm is an excellent research model for lepidopteran insects.
361 To confirm the ABC transporter gene is responsible for the resistance/susceptibility to
362 Bt toxin, susceptible Rin gene was introduced into resistant strain w1-pnd. The transgenesis
363 was not performed in reverse direction, resistant C2 gene into a susceptible strain, because
364 the present Bt resistance showed reccessive trait. The introduction of the resistance gene into
365 a susceptible strain does not alter the susceptiblity into resistance, because endogeneous
366 susceptible gene is dominant. Introduction of *Rin-007792-93* sucessfuly altered the Bt
367 responsibility into susceptible trait.
368 Sequence analyses of 7 resistant and 10 susceptible strains illustrated that only one
369 nucleotide insertion/deletion was responsible for the change in function of the *Bombyx* Bt
370 resistance gene *007792-93.* This gene possesses domains required for the functions of an
371 ABC transporter (Fig. S10) and shows high homology to the human mutidrug resistance gene
372 *ABCC4* (30). This gene was recently reported as a candidate for Bt resistance in *H. virescens*
373 and named as *ABCC2* (7). However, its function in Bt resistance is still unclear. Two
374 plausible alternatives may be considered as Bt resistance mechanisms. One is that the protein
375 is involved in binding and/or insertion of Cry1Ab toxin into the midgut membrane, working
376 as a receptor in a mechanism similar to those proposed for a cadherin-like protein (16) or

aminopeptidases (31), or as a membrane channel (7), and the insertion of tyrosine in the second loop outside the membrane may interfere with these processes. Another possibility is that the ABC transporter works to detoxify the Bt protein by excluding it from cells, in a manner analogous to that used by members of ABC transporter subfamily C in drug resistance (32). However, the second resistance machanism is irreconcilable with the fact that the resistance is recessive trait. If the ABC transporter would work for detoxification of Bt toxin, resistance trait should dominant because detoxification would be expected to occur in heterozygous (R/S) silkworm. If the ABC transporter gene works for a toxin binding or transfer, both genes in the sisiter chromosomes should have mutation (i.e. homozygous) for resistance.

Gahan *et al*. (7) recently reported that a frameshift mutation in an ABC transporter of *H. virescens*, which is orthologous to silkworm gene *007792-93* (*ABCC2*) and located in a syntenic chromosome region, is linked genetically with resistance to Cry1Ac. The *H. virescens* mutation is accompanied by reduced binding of Cry1Ac and Cry1Ab toxins to midgut membranes. There is a possibility that the exposed loop region where the tyrosine insertion occurred in *Bombyx mori* is a toxin binding region (Fig. 6). However, Cry1Ab bound to the BBMV from both susecptible and resistance strains (Fig. S3). Since Cry1A toxins are shown to bind cadherin-like protein(33) and aminopeptidase N (34) in *Bombyx mori*, no marked difference may not be observed in the toxin binding assay between two strains. Another unknown resistance mechanism, which will explain the recesive trait of resistance, also cannot be excluded in this *Bombyx* Bt toxin resistance. Studies on the impact of other amino acid variants on the degree of resistance or susceptibility among silkworm strains may help identify additional critical regions of the *007792-93* protein and elucidate their roles in Bt toxin action. Further, that the function of this gene appears to have been

401 conserved in lepidopteran species belonging to different superfamilies (Bombycoidea and
402 Noctuoidea) which diverged at least several million years ago supports the value of
403 comparative studies between the silkworm model and members of this large and highly
404 pestiferous insect clade.

405 The nearest wild ancestor to the silkworm, *B. mandarina*, had only 3 amino acid
406 differences in the predicted sequence of homologous ABC tranposrter (reference or accession
407 #) from those of the *B. mori* reported here, including the deletion of tyrosine (Fig. S12). *B.*
408 *mandarina* is expected to be susceptible to Bt toxin. The origin of the resistant gene
409 possessing a tyrosine insertion is unclear. Rearing of the domestic silkworms takes place in a
410 relatively controlled and hygienic environment. Although *B. thuringiensis* may be present on
411 mulberry leaves grown in the field and routinely fed to laboratory and commercial strains, the
412 likelihood that this subjects larvae to strong selection pressure against Bt toxin is small. The
413 finding of several sequence polymorphisms which were not correlated with resistance or
414 susceptibility to Cry1Ab supports this idea. Both resistant and susceptible genes have likely
415 been maintained under non-selective conditions for a long period of time in the domestic
416 silkworm strains. Preliminary phylogenetic analysis based on the nucleotide sequences of the
417 ABC transporter gene suggests that resistant strains are included in a single clade, but
418 additional variants will be needed for a well-supported evolutionary scenario.

419 A high resistance level appears to be conferred by a reduction in binding by mutation of
420 the target, which is shown in cadherin-like proteins expressed in the midgut (35). In the
421 present study, a single amino acid mutation in an ABC transporter gene appear to be
422 responsible for a high level of Bt toxin resistance in *B. mori*. Relatively large deletions in the
423 homologous transporter genes were reported in Bt resistant strains of *H. virescens* (7) and *P.*
424 *xylostella* (11). Thus, a critical role of the ABC transporter gene in Bt resistance in field pest

populations is apparent. Functional studies of this gene in the silkworm and other species are warranted because of the importance of Bt toxin as a pest control tactic and of the implication that mutant transporter genes of this type may become prevalent in important pests. The ability of the present *Bombyx* ABC transporter alleles to confer Bt resistance or susceptibility based on a single amino acid difference suggests they will be good candidates for studying the detailed resistance mechanism and mode of action of Bt toxin.

Materials and Methods

Silkworm strains used. Two *B. mori* strains, Chinese No.2 (C2, resistant to Cry1Ab toxin, race 401, http://www.gene.affrc.go.jp/ex-nises/bombygen/indexJ4-eng.html) and Ringetsu (Rin, susceptible to Cry1Ab toxin, race 606, http://www.gene.affrc.go.jp/ex-nises/bombygen/indexJ6-eng.html) were used for map-based cloning; they were reared on mulberry leaves or artificial diet (Nosan Corporation) at room temperature. The strains used for transgenesis were reared on artificial diet. They included the recipient, w1-pnd, a white eye-color and non-diapausing mutant strain of diapausing strain w1-c, a GAL4 driver strain, 52-2, which expresses the GAL4 protein in the midgut and DsRed2 in the eyes (28, 36), and SS16-1 and SS16-3, two newly established UAS strains expressing EGFP. *Bombyx* strains used are listed in Table S7.

Insecticidal screening by Cry1Ab toxin. The Cry1Ab toxin from *B. thuringiensis* subsp. kurstaki HD-1 was expressed in *Escherichia coli* (37). Bacteria expressing the toxin were centrifuged and protoxin inclusions were recovered by the method of Lee *et al.* (38). The protein content of the suspension of protoxin inclusions was estimated by a Lowry assay (39) using bovine serum albumin (Wako Pure Chemical) as a standard. The protoxin content in the suspension was estimated by a modified method of Brussock and Currier (40). The protoxin was eluted using 7% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the content of 130 kDa protein was measured by image analyzing software, Quantity one (Bio-Rad).

For Bt toxin screening, a mulberry leaf fragment (2 x 4 cm) coated with 80 μl of diluted suspension of Cry1Ab protoxin was fed to 15 newly-hatched larvae for 2 days. The dose (usually >0.03 μg /cm^2) was determined to be high enough to kill 100% neonate larvae of susceptible strains that were homozygous or heterozygous for susceptible genes, but not to kill those homozygous for resistance (Table 1). Larvae were fed fresh mulberry leaves for 2 days after toxin exposure, and mortality was recorded 4 days after the initial application of Bt toxin.

Transgenic silkworms were tested individually for susceptibility to the Bt toxin. Second or 4th instar larvae were allowed to feed on treated mulberry leaf discs (1 cm x 1 cm or 1 cm x 2 cm) in 24 well plastic plates or six well plastic plates. Larvae were allowed to feed for 2 days on leaves treated with Cry1Ab toxin at several different doses. A fresh leaf was added and mortality was recorded 4 days after initial exposure to toxin. Probit analysis was carried out using SPSS Statistics software ver. 7.5.1J (SPSS Japan Inc.) to determine LC_{50}.

Toxin digestion by gut enzymes. Cry1Ab toxin inclusion expressed in *E. coli* was incubated in 0.1M Na2CO3/NaHCO3 (pH 10.2) with 10 mM DTT for 1 h on ice. After centrifuged for 20 min at 5,000 xg, the supernatant was filtrated using 0.45 μm filter and dialyzed overnight at 4°C. The protoxin solution was precipitated with ammonium sulfate and washed with sterile distilled water. Pre-starved fifth instar larvae were frozen and thawed; the midgut was dissected and the liquid that leached out of the midgut was collected. The midgut juice was diluted two-fold with bicarbonate buffer (50mM Na_2CO_3/HCl pH 11) and the Cry1Ab protoxin was added. After incubation at 25°C for 0 min, 1 min, 10 min, and 120 min, the solution was inactivated with heat and analyzed with SDS-PAGE (e-PAGEL, ATTO). The gel was stained with Coomassie Brilliant Blue. The toxin molecules were also visualized by Western blot analysis. Proteins in the PAGE gel was transferred onto polyvinylidene fluoride (PVDF) membrane (Hybond-P, GE Healthcare) by Trans-blot SD (Bio-Rad). After bloking with skim milk, the membrane was incubated with rabbit antiserum against Cry1A toxin (41), then with peroxidase labeled anti-rabit antibody (GE Healthcare), and visualized using HistMark TrueBlue Peroxidase System (KPL).

Toxin binding. Protoxin (130 kDa) prepared as described above was activated with trypsin (42) and the activated toxin (60 kDa) was labeled with biotin using ImmunoProbe Biotinylation Kit (Sigma). Midgut BBMV was prepared from C2 and Rin strains as described by Wotfersberger (43). The BBMV suspension in 0.01 M PBS (inluding 0.15 M NaCl) were stored at -80°C until use. BBMV preparation was evaluated by aminopeptidase activity (31). BBMV (25 μg protien) was incubated in the PBS with 0.1 μg of biotin-labeled Cry1Ab for 1 h at 25°C. Excess amount of unlabeled Cry1Ab (1 or 10 μg) was also added for competition assay of toxin binding. The BBMV were collected by centrifugation (10,000 x g, 10 min) and washed three times with the PBS at 4°C. The toxin bound to the BBMV was examined with SDS-PAGE (e-PAGEL) and stained with Ez Stain Silver Kit (ATTO). Proteins on the gel were blotted onto PVDF membrane (Hybond-P) and incubated with streptavidine-horseradish peroxidase conjugate (GE Healthcare) for 1 h at 25°C. The biotin labeled toxin was detected with ECL plus (GE Healthcare) using Lumino Imaging Analyzer FAS-1000 (TOYOBO).

Cloning and sequencing. Glycosyltransferase genes were cloned and sequenced using total RNA of C2 strain. cDNA was synthesized using SUPERSCRIPT II (Invitrogen, San Diego) and oligo(dT) primer. The cDNA was amplified with PCR primers (Table S2) and 3'-rapid amplification of cDNA ends (3'-RACE) was performed. PCR products were cloned into a pGEM-T vector (Promega). DNA amplified from the clones by colony PCR was used for sequencing reactions. The sequence analysis was performed with an ABI Prism 3730 using BigDye Terminator (Applied Biosystems). Cloning and sequencing of other genes in this study was carried out in a similar manner. PCR products amplified with SNP-PCR primers were directly sequenced (without cloning) using the SNP-PCR primers from both ends.

Linkage analysis and positional cloning of the resistance gene. SNP-based linkage analysis and recombination mapping were performed by PCR amplification of the SNP region and sequencing the PCR products (8, 9). Genomic DNA was isolated from an anterior leg of adult moths of grand-parental strains (C2 female and Rin male) and parental F_1 individuals using DNAzol (Invitrogen). For the BC_1 generation, genomic DNA was isolated

from legs of 5th instar larvae. Nineteen segregant BC_1 larvae that survived after screening using Cry1Ab were used for the linkage analysis. Thirty SNP markers, including three markers for chromosome 15 and a single marker for each of the other 27 chromosomes (9), were used. The PCR products were directly sequenced by BigDye terminator cycle sequencing (Applied Biosystems) using the same PCR primers. The homozygous (A) or heterozygous (H) state of each linkage group was determined (Fig. S1). A pair of sister chromosomes for each of the 28 linkage groups should be composed of the two same chromosomes originated from C2, or different chromosomes from C2 and Rin. Larvae that possessed a pair of homozygous sister chromosomes should show resistance to Bt toxin if the resistance gene was located on this chromosome. Therefore, the chromosome (linkage group) carrying the resistance trait should be homozygous in all resistant larval samples examined.

To determine the locus of the resistance gene on chromosome 15, F_1 males (C2/Rin) were crossed with C2 females (C2/C2). Since chromosomal crossing over (recombination) occurs in silkworm males but not in females (44, 45), reciprocal backcrosses were used for chromosome linkage assignment and positional cloning. Recombination between sister chromosomes was used to find the homozygous region in chromosome 15 of Bt-resistant BC_1 larvae. In addition to already known SNP markers (PCR primers) on chromosome 15, new SNP-PCR primers that could distinguish C2 and Rin were designed after sequencing the corresponding region of the two strains.

Transgenesis. An established silkworm GAL4/UAS system (27) was used for transgenesis. Two piggyBAC vector constructs were used: a driver construct (GAL4 line) BmA3-0052-2 (52-2) containing *GAL4* and *DsRed2* genes that was used previously (28, 36), and a new effector construct (UAS line) containing a *Rin-007792-93* gene and an *EGFP* gene (Fig. S8A). The coding sequence of the *Rin-007792-93* gene was amplified from cDNA from the midgut of Rin using primers with cutting site *Xba* I (Table S2) and cloned into pGEM-T. The insert DNA was digested with *Xba* I and subcloned downstream of the *GAL4* binding site of UAS (*Bln* I site) of the plasmid pBacMCS[UAS-3xP3-EGFP] (46). The insert sequence of the resultant effector vector was confirmed by DNA sequencing.

Transgenesis was performed as described previously (27, 29). The eye-color mutant strain, w1-pnd, a non-diapausing mutant strain of diapausing w1-c, was used as recipient. Two EGFP positive UAS lines (SS16-1 and SS16-3) were established and maintained by crossing with w1-c. Females of 52-2, which expresses the GAL4 protein in the midgut and DsRed2 in the eyes, were crossed with males of the UAS lines (SS16-1 and SS16-3). The DsRed- and EGFP-positive offspring were selected at a late embryonic stage. As experimental controls, offspring from crosses between w1-c females and SS16-1 or SS16-3 males and between 52-2 females and w1-c males were used.

Southern blot analysis and inverse PCR. Copy number of the PiggyBAC vector bearing *Rin-007792-93* gene was exmined by genomic Southern blot analysis. Genomic DNA was prepared from the embryos using a DNeasy Blood & Tissue Kit (QIAGEN) or from adults as reported previously (27). About 2 μg each of genomic DNA was digested with *Pst* I, *Hpa* I or *Eco* RV. DNA was blotted onto a nylon membrane (Hybond-N, GE Helthcare) after agarose gel electrophoresis. EGFP gene fragment (672 bp) amplified with primers KS113 and KS248 (Table S2) was labeled using Alkphos direct labeling and detection system (GE Helthcare) and used as a probe. The insertion sites of the vector on the chromosmes was determined by inverse-PCR. Two pairs of primers for 1st and 2nd PCR were designed on both the left and right arms of the vector (Table S2). After sequencing the 2nd PCR products using 2nd primers, blast search of the sequences was done against genome sequence in the KAIKObase.

Realtime RT-PCR. To confirm the expression of the exogenous transformed gene, the endogenous and exogenous genes were detected using primers that amplified each of the genes separately. Since sequences of both genes were similar and it was difficult to design specific primer pairs for the open reading frame, the primers were designed in the 3' region of the genes (Fig. S9; Table S2). The primers included mismatched nucleotides with the corresponding sequences of cDNA to ensure the differential amplification between the two genes. Both genes in transgenic silkworms were quantified on a real-time thermal cycler (LightCycler® 480 Real-Time PCR System, Roche Diagnostics). The midguts were

dissected from 4th instar larvae and total RNA was extracted using an RNeasy Mini Kit (Qiagen). cDNA was synthesized from the RNA with an oligo (dT) primer using a PrimeScript RT reagent Kit (Takara Bio) in a 10-μl reaction volume. The reaction mixture was then diluted 10-fold with MilliQ water. Realtime RT-PCR was carried out in 20-μl reaction volumes containing 5 μl of template cDNA or standard DNA, 0.75x SYBR Green PCR premix (Roche Diagnostics), and 10 pmole of each primer. PCR conditions were 95 °C for 5 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 15 s. The absence of undesirable by-products was confirmed by automated melting curve analysis. The expressed transcript levels were standardized to that of the ribosomal protein L32 (AY769302) (47).

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Figure legends

Fig. 1. The process of mapping of the Bt resistance gene on chromosome 15. Three rounds of mapping analyses were performed using SNP markers on 44, 32, and 15 Bt-resistant BC_1 larvae for the 1st, 2nd, and 3rd mapping screens. Homozygosity (C2/C2) or heterozygosity (C2/Rin) for each marker site was determined by sequencing PCR products. Markers in magenta were used as boundaries for the subsequent mapping round or gene prediction after the three screens. Six genes were predicted in final 82 kb region in KAIKObase (http://sgp.dna.affrc.go.jp/KAIKObase/).

Fig. 2. Expression of the six predicted genes in the midgut. RT-PCR products of *BGIBMGA007735*, *007793*, *007736*, *007792*, *007791* and *007737* (1–6) are shown in midgut from Rin and C2. *BGIBMGA007735–007792* (1–4) showed PCR products of expected size in both Rin and C2. M, DNA marker.

Fig. 3. Sequence alignment of putative amino acids deduced from a portion of gene *007792-93* (from residues 223 to 246 in C2; Fig. S4 and S6). Seven Bt resistant strains (upper) and 10 susceptible strains (lower) are shown. Tyrosine is present in the resistant strains and lack in the susceptible strains.

Fig. 4. Examples of Bt toxin bioassay of silkworms transformed with a Bt-susceptible gene. (A–D) Toxin screening results for 2nd instar larvae after two-day-toxin administration followed by two-day-rearing without toxin. (A, B) offspring from 52-2 (GAL4 driver strain with a w1-c genetic background) x SS16-3 (UAS strain with the susceptible *Rin-007792-93* gene); (C, D) offspring from w1-c (recipient strain) x SS16-3; (A, C) control without toxin; (B, D), 0.275 $\mu g/cm^2$ toxin protein applied on the leaf disk for the first 2 days. The silkworms to which Bt-susceptible gene (*Rin-007792-93*) was introduced and the gene was activated by GAL4 (B) were mostly dead and the leaf disks remain uneaten.

773 Fig. 5. Expression of Bt-susceptible gene introduced into transformant silkworms.
774 Susceptible (*Rin-007792-93*) and resistant (*C2-* or *w1-c-007792-93*) genes were individually
775 detected by realtime RT-PCR. Midguts of 4th instar larvae were individually tested.
776 Expression levels relative to those of a ribosomal protein gene (*RpL32*) are shown with
777 standard errors. The number of larvae tested is shown above the columns. Closed and open
778 boxes indicate susceptible and resistant genes, respectively. The asterisk indicates no
779 expression. (A), Parent strains, Rin and C2, expressed their endogeneous genes,
780 *Rin-007792-93* and *C2-007792-93*, respectively; this real-time PCR method differentially
781 detects the susceptible and resistant genes. (B) and (C), Gene expression level in offspring
782 of SS16-1 and SS16-3, respectively; expression of the exogenous (*Rin-007792-93*) and
783 endogenous (*w1-c-007792-93*) genes is shown in closed and open boxes; a, offspring from
784 52-2 x SS16; b, offspring from w1-c x SS16; c, offspring from 52-2 x w1-c; the offspring from
785 52-2 x SS16 showed expression of the introduced susceptible gene as well as endogenous
786 gene and leaky expression of the susceptible gene is observed in the offspring from w1-c x
787 SS16.
788
789 Fig. 6. Schematic structure of the ABC transporter. Twelve transmembrane domain
790 structures were predicted based on the amino acid sequence of *BGIBMGA007792-93* (Fig.
791 S10) using TMHMM ver. 2.0. The tyrosine residue (magenta dot) was predicted to be
792 located on the second outer loop (Fig. S11).
793
794
795 **Legends for supplemental figures**
796 Fig. S1. An example of linkage analysis using SNP markers. Homozygosity (C2/C2) or
797 heterozygosity (C2/Rin) was determined by direct sequencing of PCR products amplified
798 using a pair of marker primers. The heterozygous type shows two peaks in the SNP site.
799
800 Fig. S2. Digestion of Bt protoxin by crude midgut enzymes. A, Polyacryl amide gel
801 electrophoresis of proteins; B, Western blot analysis using polyclonal antibody against Cry
802 toxin. We used protoxin Cry1Ab expressed in *E. coli*. Protoxin (130 kDa, open arrow head)

803 was mainly digested into active toxin (60 kDa, closed arrow head). M, molecular marker;
804 Lane 1, protoxin protein purified from *E. coli* homogenate; 2 and 7, protoxin 0min after
805 adding C2 or Rin midgut homogenate, respectively; 3 and 8, 1 min; 4 and 9, 10 min; 5 and
806 10, 120 min; 6 and 11, C2 or Rin midgut homogenate, respectively.
807
808 Fig. S3. Cry1Ab toxin binding assay to brash border membrane vesicles (BBMV). Activated
809 cry1Ab toxin by tripsin (60 kDa, open arrow head) was incubated with BBMV of midgut from
810 resistant C2 and susceptible Rin strains. Biotinylated toxin were incubated with BBMV (25 μg
811 protein) for 1 h at 25°C and resolved by electrophoresis (A) and blotted onto membrane and
812 detected by chemiluminescent-coupled streptoavidine peroxidase (B). M, molecular marker;
813 Lane 1, mixture of labeled toxin (0.1 μg) and unlabeled toxin (10 μg); 2 and 6, supernatant of
814 BBMV solution of C2 or Rin incubated with labeled toxin (0.1 μg), respectively (centrifuged at
815 10,000 x g, 10 min); 3 and7, pellet of 2 and 6, respectively; 4 and 8, pellet of BBMV solution
816 of C2 or Rin incubated with labeled toxin (0.1 μg) plus unlabeled toxin (20 μg); 5 and 9, pellet
817 of BBMV of C2 or Rin. Biotinylated toxin was recovered in the pellet of BBMV (lane 3 and 7)
818 and binding specificity was assessed in lane 4 and 8 by incubating with 20 fold amount of
819 unlabeled toxin. The toxin solution included degraded toxin fragments or impurity that were
820 also biotinylated (lane 1; 10–45 kDa).
821 This preliminary figure will be replace by another one, because we are now carrying out
822 anther binding assays to confirm the binding.
823
824 Fig. S4. Amino acid sequence alignment of *BGIBMGA007792-93* from C2 and Rin. Thirteen
825 deduced amino acids were different between the two strains.
826
827 Fig. S5. Expression of *BGIBMGA007792-93* gene in tissues of C2 and Rin. Expression of
828 *BGIBMGA007792-93* (upper) was observed in the anterior (amg), middle (mmg) and
829 posterior (pmg) midgut but not in the thorax fat body (afb), abdominal fat body (pfb), silk
830 glands (sg), Malpighian tubules (mt), haemocytes (hc), testis (te), ovary (ov), or integument
831 (int). An actin gene (lower) used as a constitutive control was expressed in all tissues.
832

833 Fig. S6. Alignment of predicted amino acid sequences of *BGIBMGA007792-93* from 7
834 resistant strains (upper) and 10 susceptible strains (lower). A tyrosine residue in amino acid
835 234 in the resistant strains was deleted in the susceptible strains.
836
837 Fig. S7. Copy number and the insetion site of the susceptible Rin gene in the chromosomes
838 of two transgenic effector strains (SS16-1 and SS16-3). A, Southern blot analysis of the
839 gene using genomic DNA. A EGFP sequence was used as probe for detecting
840 pBacMCS[UAS-3xP3-EGFP]. SS16-1 genome, digested by *Pst* I (P) or *Hpa* I (H), shows two
841 bands and SS16-3 genome, digested by *Pst* I (P) or *Eco* RV (E), one band (asterisks). B,
842 The chromosome position of the *piggyBAC* vector was determined by inverse PCR of
843 genomic DNA using primers designed for vector arm sequences (Table S2). SS16-1 has the
844 genes on chromosome 15 and 23, and SS16-3 on chromosome 25.
845
846 Fig. S8. Transgenesis in the silkworm. A. Driver construct containing *GAL4* and *DsRed2*
847 genes (36) and a newly fabricated effector construct with the Bt resistance gene (Bt-r) and
848 *EGFP*. The driver construct (strain 52-2) expressed GAL4 in the midgut; the effector
849 construct (strain SS16) possessed *Rin-BGIBMGA007792-93*. B. Three strains, UAS-Bt-r
850 (SS16, male), GAL4 driver (52-2, female) and w1-c, were crossed. Susceptible offspring
851 were expected to arise from the cross between SS16 male x 52-2 female.
852
853 Fig. S9. Primers designed for realtime RT-PCR to distinguish susceptible (*Rin*) and resistant
854 (*w1-c*) genes in transgenic silkworms. Primers, Rin_92-93 and C2_92-93 (Table S2), were
855 designed in the 3' non-coding region. Primer binding sites are shown in blue for the
856 exogenous Rin gene and red for the endogenous w1-c gene. Primer sequences are in Table
857 S2.
858
859 Fig. S10. Predicted structure of *Bombyx* ABC transporter gene *BGIBMGA007792-93*
860 (*C2-007792-93*) aligned with human ABC transporter gene *ABCC4*. WalkerA and B
861 sequences, C-Motif, and transmembrane domains (TM) are shown. The transmembrane
862 regions were predicted by TMHMM ver. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) based

863 on the sequences. TM9 of *BGIBMGA007792-93* and *ABCC4* were not predicted by
864 YMHMM.
865
866 Fig. S11. Prediction of the structural location of tyrosine of *C2-007792-93* near the
867 transmembrane domain (TM) 3 and 4 by three programs. The transmembrane regions (TM)
868 were predicted by TMHMM (blue) ver. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), SOSUI
869 (green) ver. 1.11 (http://bp.nuap.nagoya-u.ac.jp/sosui/), and phobius (magenta)
870 (http://phobius.sbc.su.se/). TMHMM predicted that tyrosine located at the end of TM3,
871 SOSUI in the outer loop between TM3 and TM4, and phobius at the beginning of the outer
872 loop.
873
874 Fig. S12. Alignment of predicted amino acid sequences of BGIBMGA007792-93 from
875 *Bombyx mandarina* and *B. mori,* strains C2 and Rin. Three deduced amino acids in *B.*
876 *mandarina* (green) differed from *B. mori* strains. Red, amino acid (indel) distinguishing
877 resistant and susceptible strains.
878

879
880 Table 1. Bioassay of Cry1Ab toxin in two strains.

Race/cross	No. tested	No. survived	% survived
C2	60	60	100.0
Ringetsu	60	0	0.0
F1: C2 x Ringetsu	60	0	0.0
BC1: (C2 x Ringetsu) x C2	135	66	48.9

881
882 For each assay, 15 first instar larvae were reared on a 2 x 4 cm mulberry applied with 0.031
883 $\mu g/cm^2$ of Cry1Ab protoxin was applied. A fresh leaf was provided after 2 days and surviving
884 larvae were recorded after 4 days.
885

Table 2. Genes in the 82 kb region on chromosome 15 predicted in KAIKObase

Gene name	Strand	Position	Size	Exon size	Description
BGIBMGA007735	+	8912489–8944193	31705	3807	ABC transporter
BGIBMGA007793	-	8949057–8952178	3122	999	ABC transporter
BGIBMGA007736	+	8952469–8952919	451	229	undefined
BGIBMGA007792	-	8956687–8966706	10020	2150	ABC transporter
BGIBMGA007791	-	8969410–8981067	11658	5418	undefined
BGIBMGA007737	+	8992602–8992829	228	228	undefined

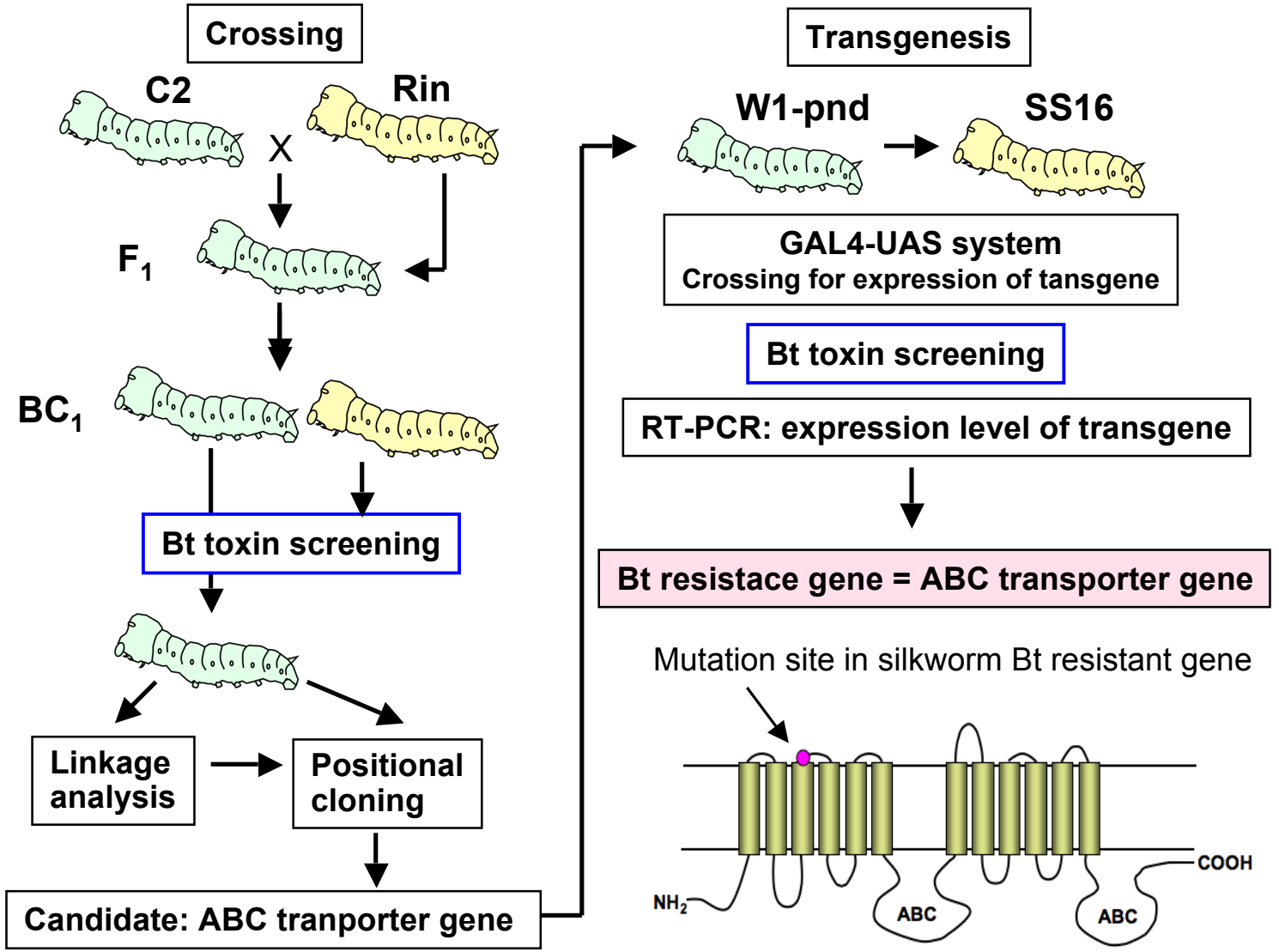
Six genes were predicted. cDNA and genome sequence analyses indicated that *BGIBMGA007793* and *007792* were parts of the same gene and *BGIBMGA007736* was located in one of the intron regions of the gene. Therefore, four genes, *BGIBMGA007735*, *7792-93*, *007791* and *007737* were actually predicted.

Table 3. Susceptibility to Cry1Ab toxin in transgenic silkworms

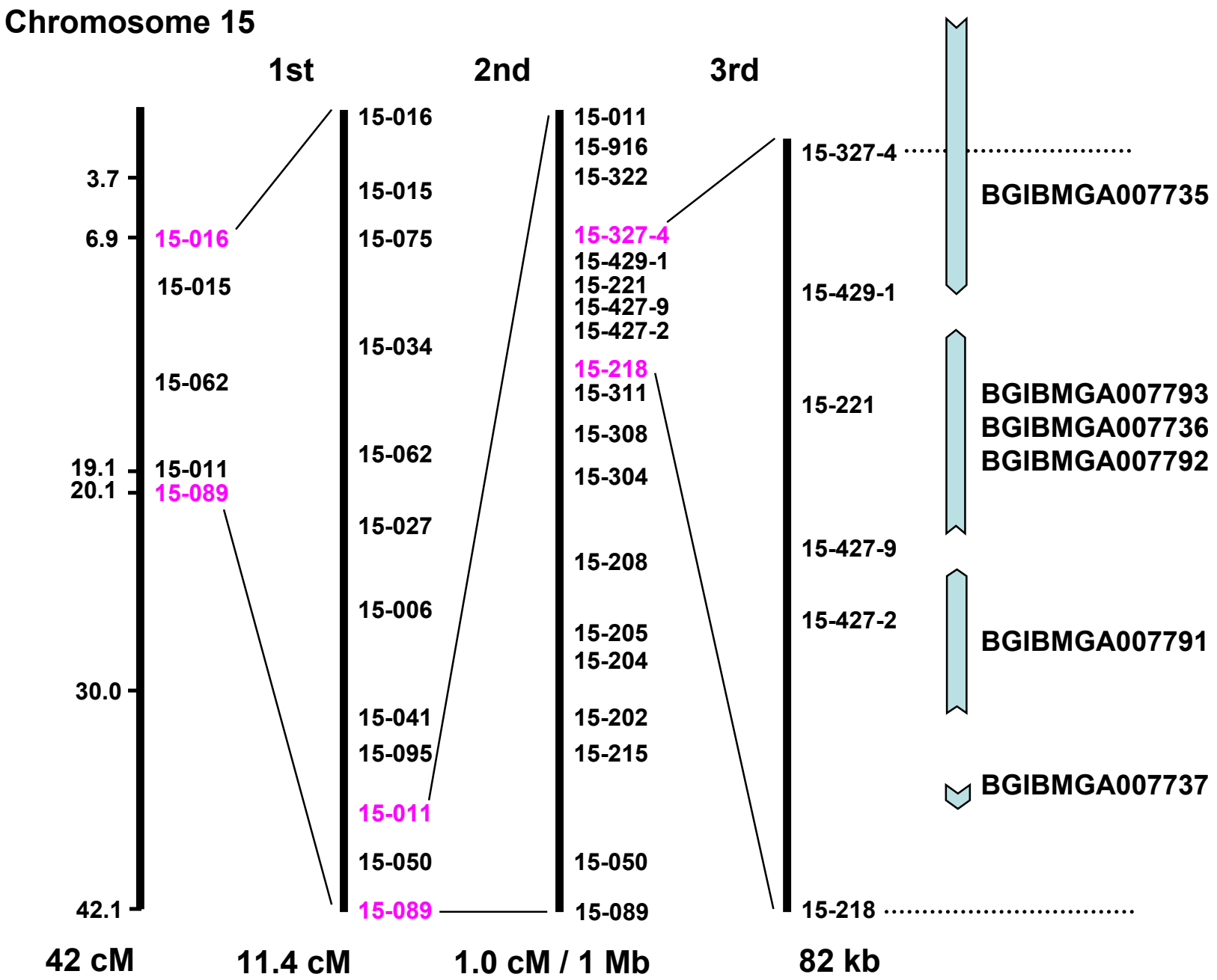
Strains Cross (female x male)	No. tested	LC_{50} (μg protein/cm^2) *	95% FL**	Slope ± SE***
Original strains, 2nd instar				
Ringetsu (Rin)	168	0.00616	0.0027 – 0.0147	1.85 ± 0.27
Chinese 2 (C2)	168	>17.6	–	–
w1-c	168	1.94	1.13 – 3.54	1.10 ± 0.16
w1-pnd	144	22.1	10.8 – 123	1.23 ± 0.33
52-2	168	12.7	2.56 – 37300	0.74 ± 0.15
Gal4 x UAS, 2nd instar				
52-2 x SS16-1	144	0.00543	0.0040 – 0.0074	3.62 ± 0.76
w1-c x SS16-1	144	48.7	19.4 – 4230	1.33 ± 0.48
52-2 x SS16-3	168	0.00329	0.0001 – 0.0024	1.38 ± 0.19
w1-c x SS16-3	144	846	–	–
52-2 x w1-c	144	3.89	2.01– 9.69	0.89 ± 0.17
Gal4 x UAS, 4th instar				
52-2 x SS16-3	144	0.00942	0.0067 – 0.0129	4.67 ± 1.03
w1-c x SS16-3	108	131	20.1 – 11.0 x $10^{\prime}$	0.69 ± 0.27
52-2 x w1-c	90	2.36	1.01 – 7.12	0.87 ± 0.21

* LC_{50} = median lethal concentration; ** 95% FL = 95% confidence limit; *** Slope ± SE = Slope calculated by probit analysis. w1-c, diapausing recipient strain used for maintaining the transgenic strains; w1-pnd, non-diapausing recipient strain in which the susceptible gene (*Rin-007792-93*) was introduced; 52-2, GAL4 driver strain with *DsRed2*; SS16-1 or SS16-3, transgenic strains expressing *EGFP* and *Rin-007792-93*.

We tested susceptibility to Cry1Ab toxin at 2nd instar for SS16-1 and SS16-3 and at 4th instar for SS16-3. We tested individual larvae by providing a leaf applied with Bt toxin in 24 well plates at 2nd instar or 6 well plates at 4th instar. We fed a fresh leaf after 2 days and recorded the number of surviving larvae after 4 days.



Summary figure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2nd
3rd
3.7
6.9
15-016
15-015
15-062
19.1
15-011
20.1
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30.0
42.1
15-016
15-015
15-075
15-034
15-062
15-027
15-006
15-041
15-095
15-011
15-050
15-089
15-011
15-916
15-322
15-327-4
15-429-1
15-221
15-427-9
15-427-2
15-218
15-311
15-308
15-304
15-208
15-205
15-204
15-202
15-215
15-050
15-089
15-327-4
15-429-1
15-221
15-427-9
15-427-2
15-218
BGIBMGA007735
BGIBMGA007793
BGIBMGA007736
BGIBMGA007792
BGIBMGA007791
BGIBMGA007737
42 cM
11.4 cM
1.0 cM / 1 Mb
82 kb

Figure 1

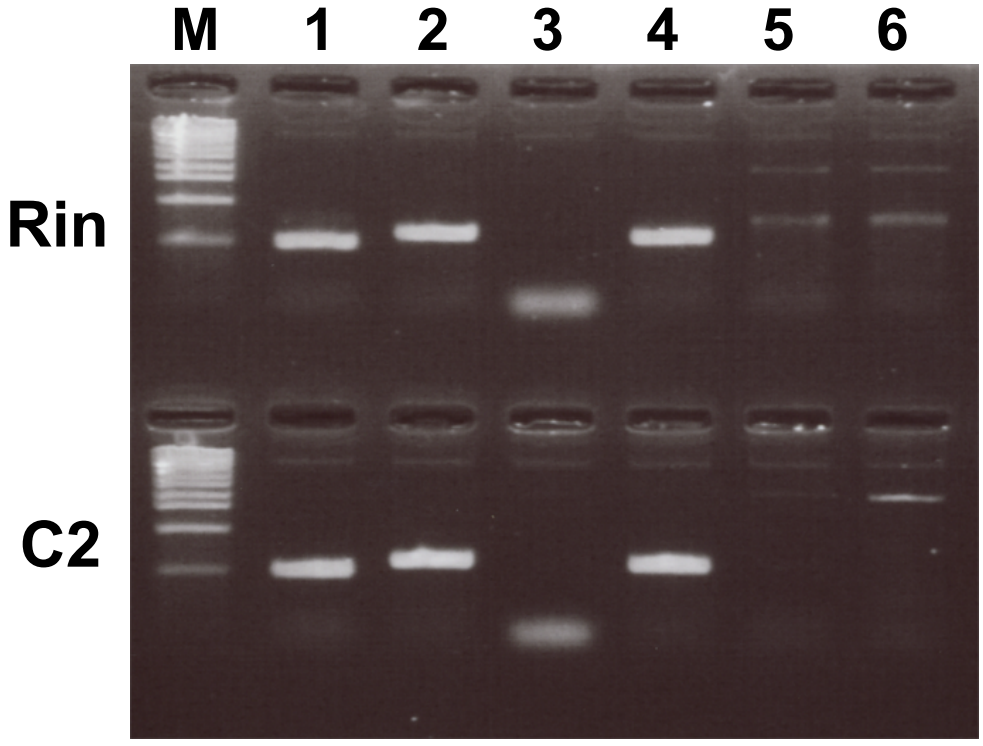
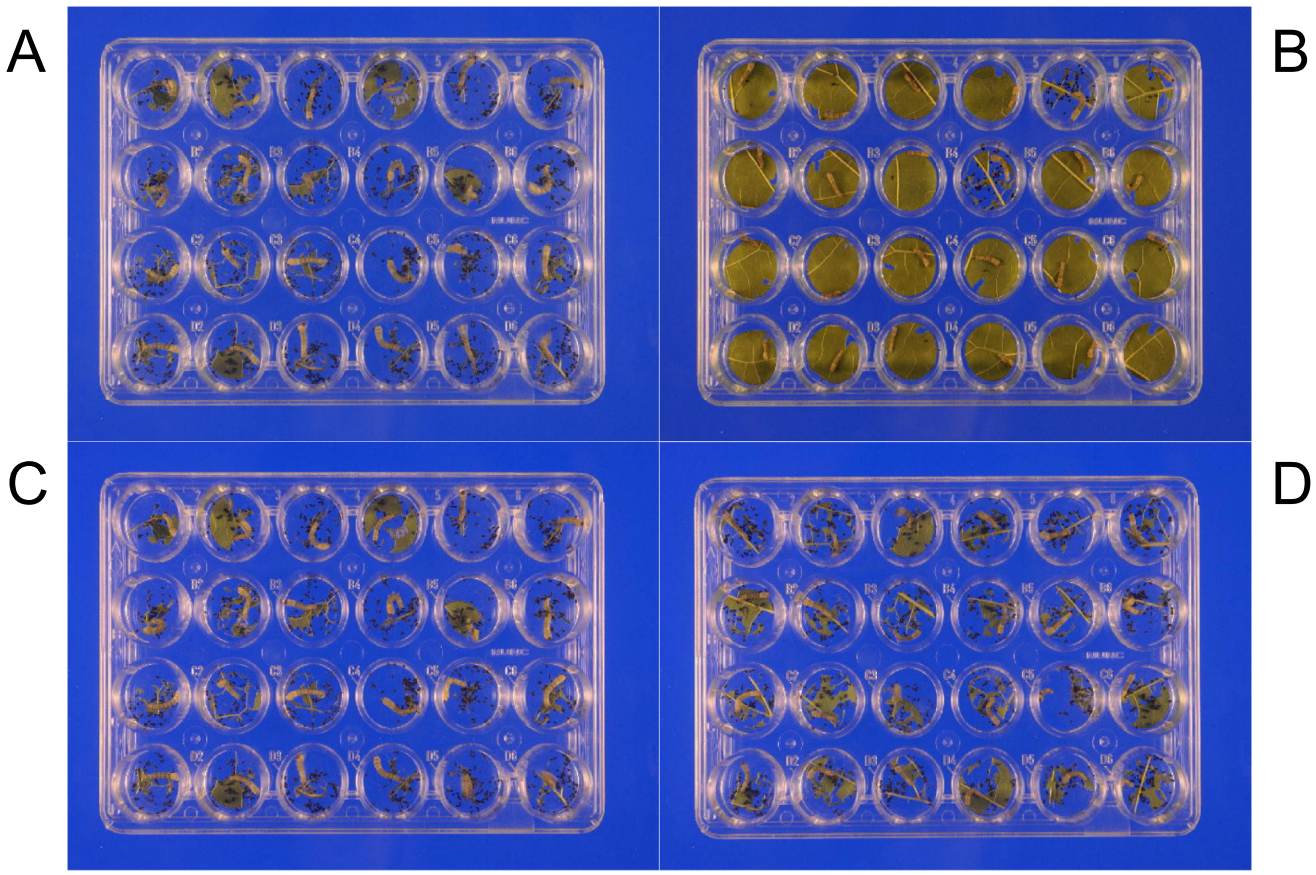


Figure 2


J1_R VQAAVVLYFLYYISAGYAPFVGFF
Ki_R VQAAVVLYFLYYISAGYAPFVGFF
Be_R VQAAVVLYFLYYISAGYAPFVGFF
C2_R VQAAVVLYFLYYISAGYAPFVGFF
C7_R VQAAVVLYFLYYISAGYAPFVGFF
Csek_R VQAAVVLYFLYYISAGYAPFVGFF
N15_R VQAAVVLYFLYYISAGYAPFVGFF
Yosh_S VQAAVVLYFLY-ISAGYAPFVGFF
Bag_S VQAAVVLYFLY-ISAGYAPFVGFF
N65_S VQAAVVLYFLY-ISAGYAPFVGFF
Eu12_S VQAAVVLYFLY-ISAGYAPFVGFF
Ann_S VQAAVVLYFLY-ISAGYAPFVGFF
CamM_S VQAAVVLYFLY-ISAGYAPFVGFF
My_S VQAAVVLYFLY-ISAGYAPFVGFF
PMy_S VQAAVVLYFLY-ISAGYAPFVGFF
Rin_S VQAAVVLYFLY-ISAGYAPFVGFF
e21_S VQAAVVLYFLY-ISAGYAPFVGFF

Figure 3



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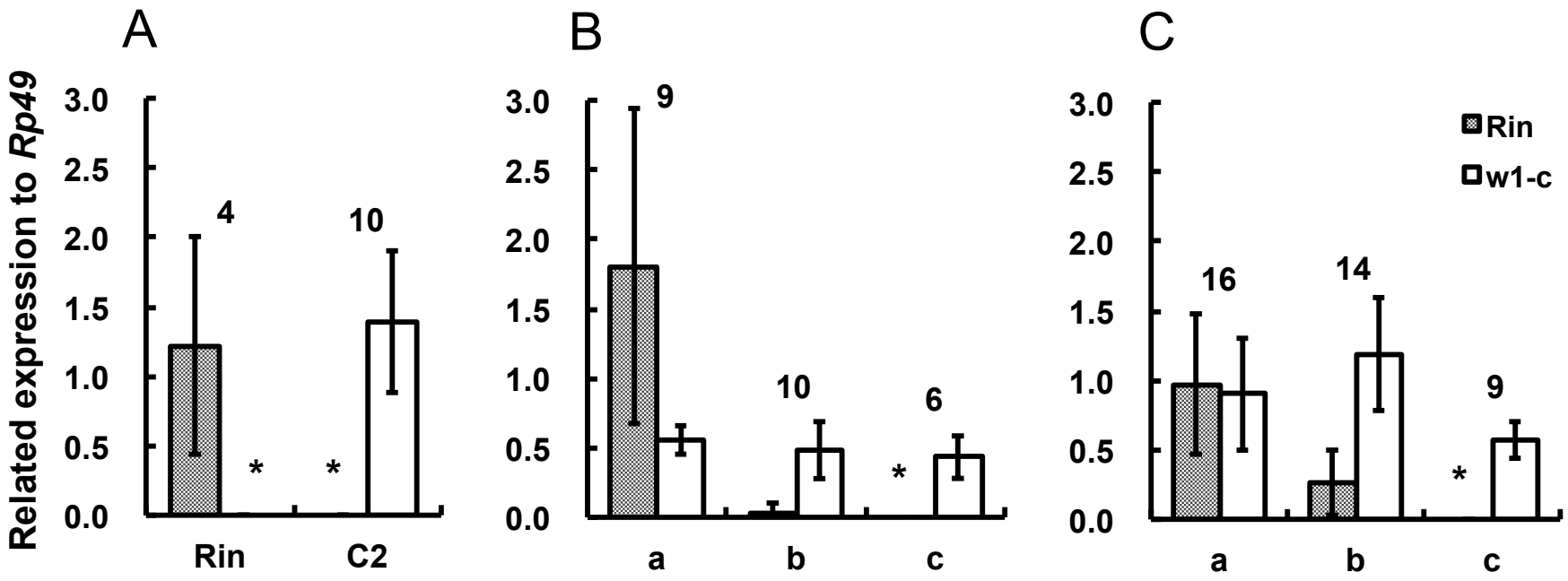

A
B
C
Related expression to Rp49
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4
10
9
10
6
16
14
9
*
Rin
C2
a
b
c
Rin
w1-c

Figure 5

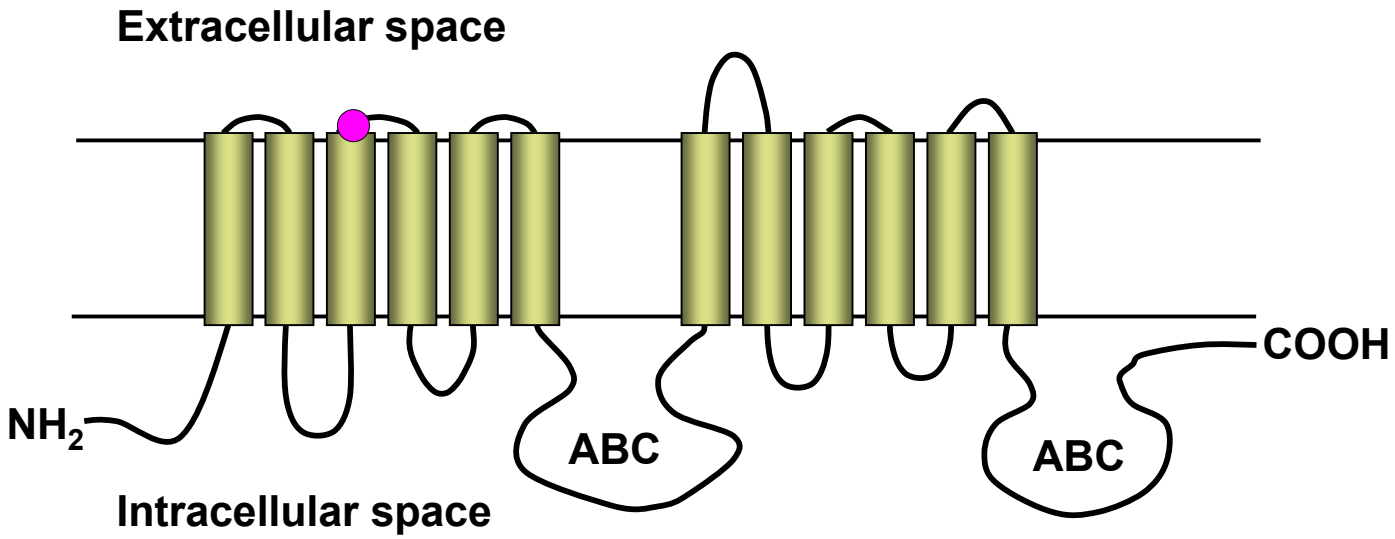


Figure 6

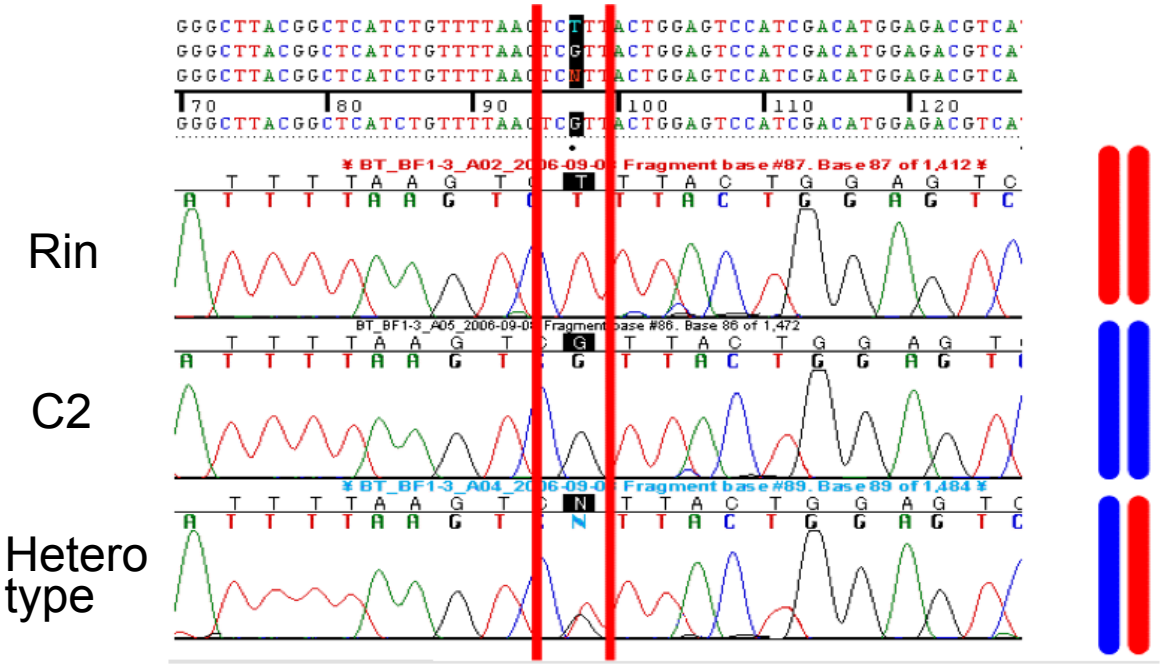


Figure S1

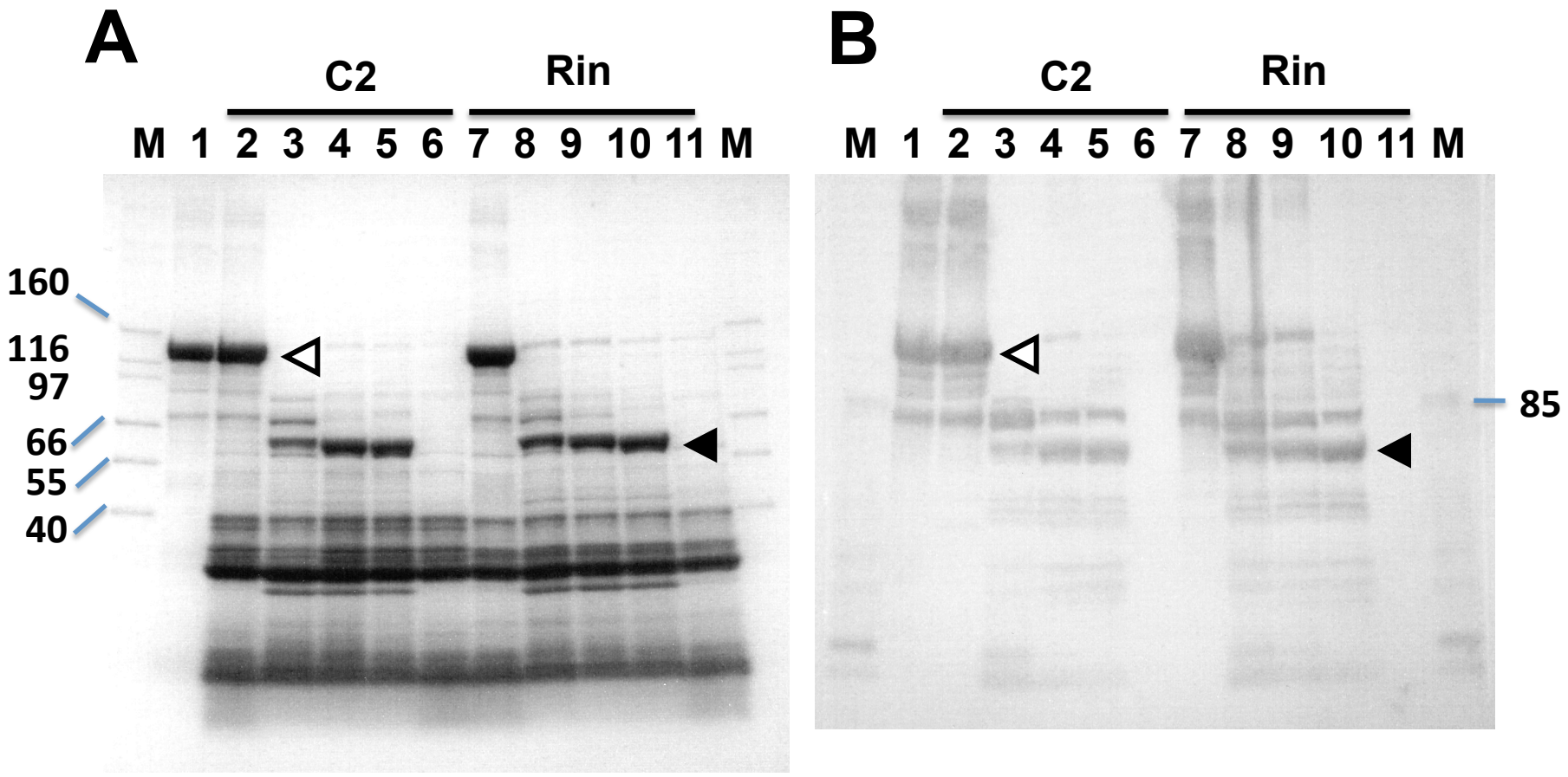

A
C2
Rin
M 1 2 3 4 5 6 7 8 9 10 11 M
160
116
97
66
55
40
B
C2
Rin
M 1 2 3 4 5 6 7 8 9 10 11 M
85

Figure S2F

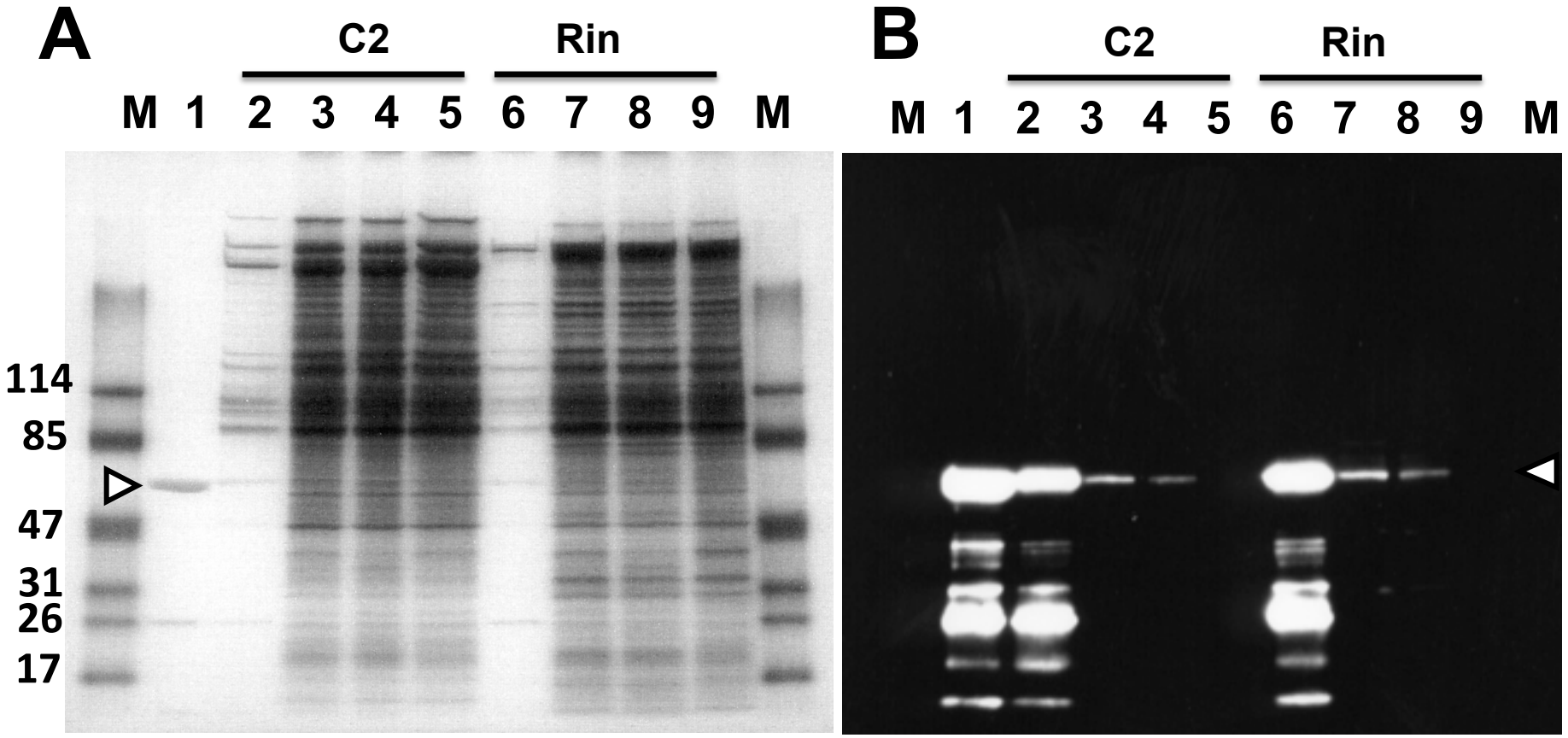
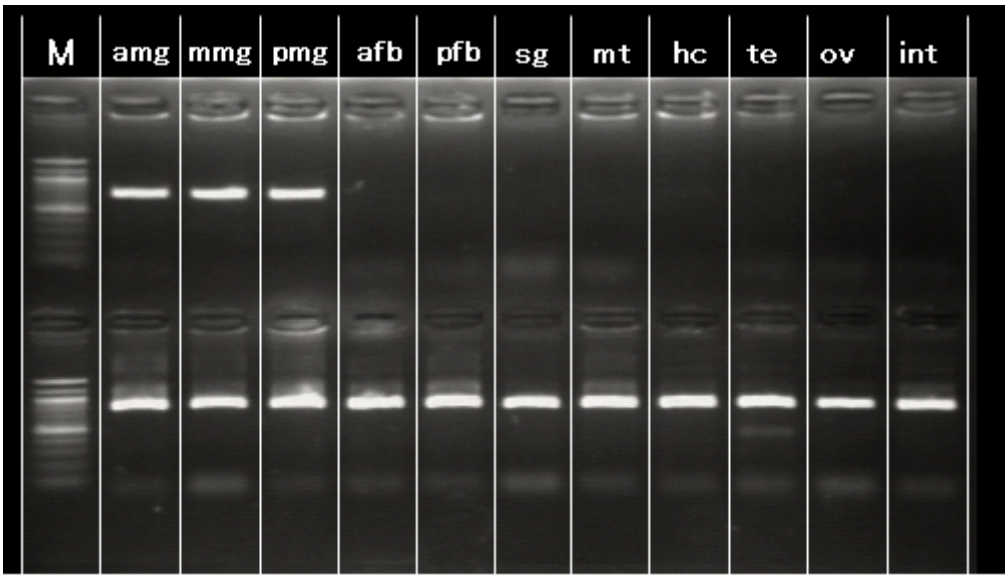


Figure S3

```
C2       1 MNSDGRAGENSSAETRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSE    60
Rin      1 MNSDGRAGENSSAETRRKPHKPNILSRIFLWWMCPVLVKGNQRDTVEDDLIIPKKSFNSE    60
           ****************************************** ***************

C2      61 NQGEYLERYWLQEYEAAIKEKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFA   120
Rin     61 NQGEYLERYWLQEYEAAIKEKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFA   120
           ************************************************************

C2     121 ELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNSLFVARFGLKVKVACSSLVYRK   180
Rin    121 ELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNSLFVARFGLKVKVACSSLVYRK   180
           ************************************************************

C2     181 LLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGYA   240
Rin    181 LLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGYA   239
           ************************************************** ******

C2     241 PFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPF   300
Rin    240 PFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPF   299
           ************************************************************

C2     301 QAIVKVARNFEMIALRKSIFIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQ   360
Rin    300 QAIVKVARNFEMIALRKSIFIRSVFLGFMLFTERSIIFITCLTFLLTGNLVTATLIYPIQ   359
           ****************************************** ***************

C2     361 QYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDEREDVQITPKSYGDDNRLIFNN   420
Rin    360 QYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDEREDVQITPKSYGDDNRLIFNN   419
           ************************************************************

C2     421 KASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTSE   480
Rin    420 KASLGPQNEIIPKKYLATDGQLASTLTNEPVLSTDPAVCDYPIELSKVDATWSSSTDTSE   479
           ******************************** ************************

C2     481 MTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLF   540
Rin    480 MTLRNITLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLF   539
           ****** ************************************************

C2     541 PATVRENILFGLPYESQKYHEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINL   600
Rin    540 PATVRENILFGLPYDSQKYHEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINL   599
           *************** ************************************************

C2     601 ARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRGRTCVLVTHQIHYLKAADIIVI   660
Rin    600 ARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRGRTCVLVTHQIHYLKAADIIVI   659
           ************************************************************

C2     661 LNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDNE   720
Rin    660 LNEGAIENVGSYDDLVNTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDNE   719
           *************** ************************************************

C2     721 KVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQ   780
Rin    720 KVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVVTLVITQGCATFIDYWLSFWTNQ   779
           ************************************ ********* ************

C2     781 VDEYEQSLAEGEEPSTSLDTQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRAS   840
Rin    780 VDEYEQSLAEGEEPSTSLDTQAGAFTLGVYLWTYGGVILILIVISHVRILTFVITTMRAS   839
           ************************ ************************************

C2     841 SNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMDEFLPRSLFETVQMYLTLCSIL   900
Rin    840 SNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMDEFLPRSLFETVQMYLTLCSIL   899
           ************************************************************

C2     901 ILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLSTI   960
Rin    900 ILNAIALPWTLIPTAVLLILFFVLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLSTI   959
           ********************** ************************************

C2     961 RSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFST  1020
Rin    960 RSSNSQGRLLQMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFST  1019
           ********** ************************************************

C2    1021 LIPVGSVGLAVSQSMVLTMMLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLP  1080
Rin   1020 LIPVGSVGLAVSQSMVLTMMLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLP  1079
           ************************************************************

C2    1081 KDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKVGVVGRTGAGKSSMISALFRLY  1140
Rin   1080 KDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKVGVVGRTGAGKSSMISALFRLY  1139
           ************************************************************

C2    1141 DLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALEQ  1200
Rin   1140 ELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALEQ  1199
            ************************************************************

C2    1201 VELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQ  1260
Rin   1200 VELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQ  1259
           ************************************************************

C2    1261 TTIRREFASCTVITIAHRLNTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRET  1320
Rin   1260 TTIRREFASCTVITIAHRLNTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRET  1319
           ************************************************************

C2    1321 GDTMSKVLFRVAEDKHLGRNTEK                                       1343
Rin   1320 GDTMSKVLFRVAEDKHLGRNTEK                                       1342
           ************************
```

Figure S4

C2



Rin

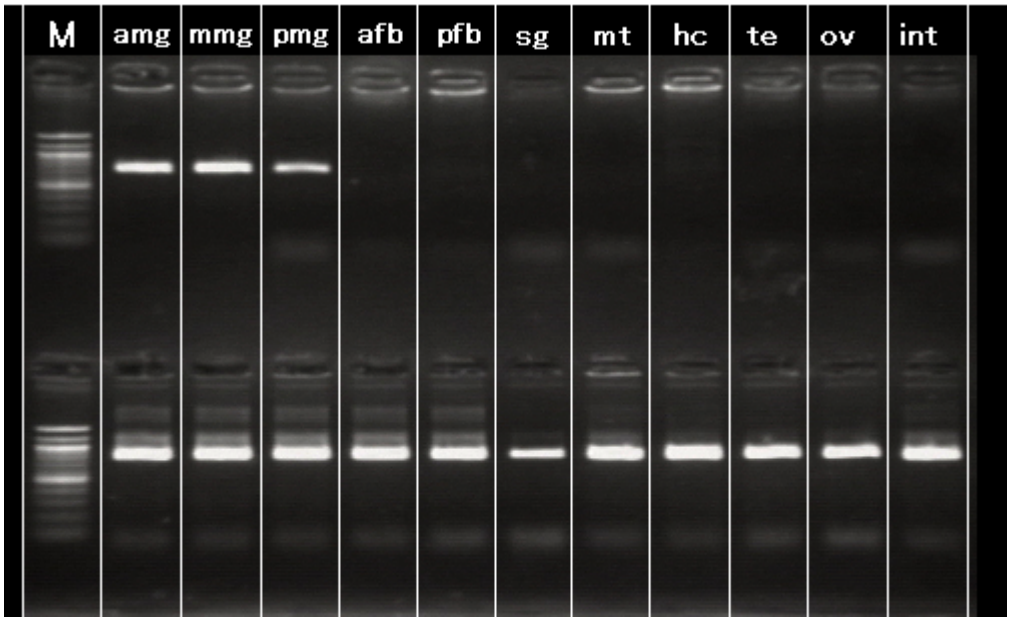
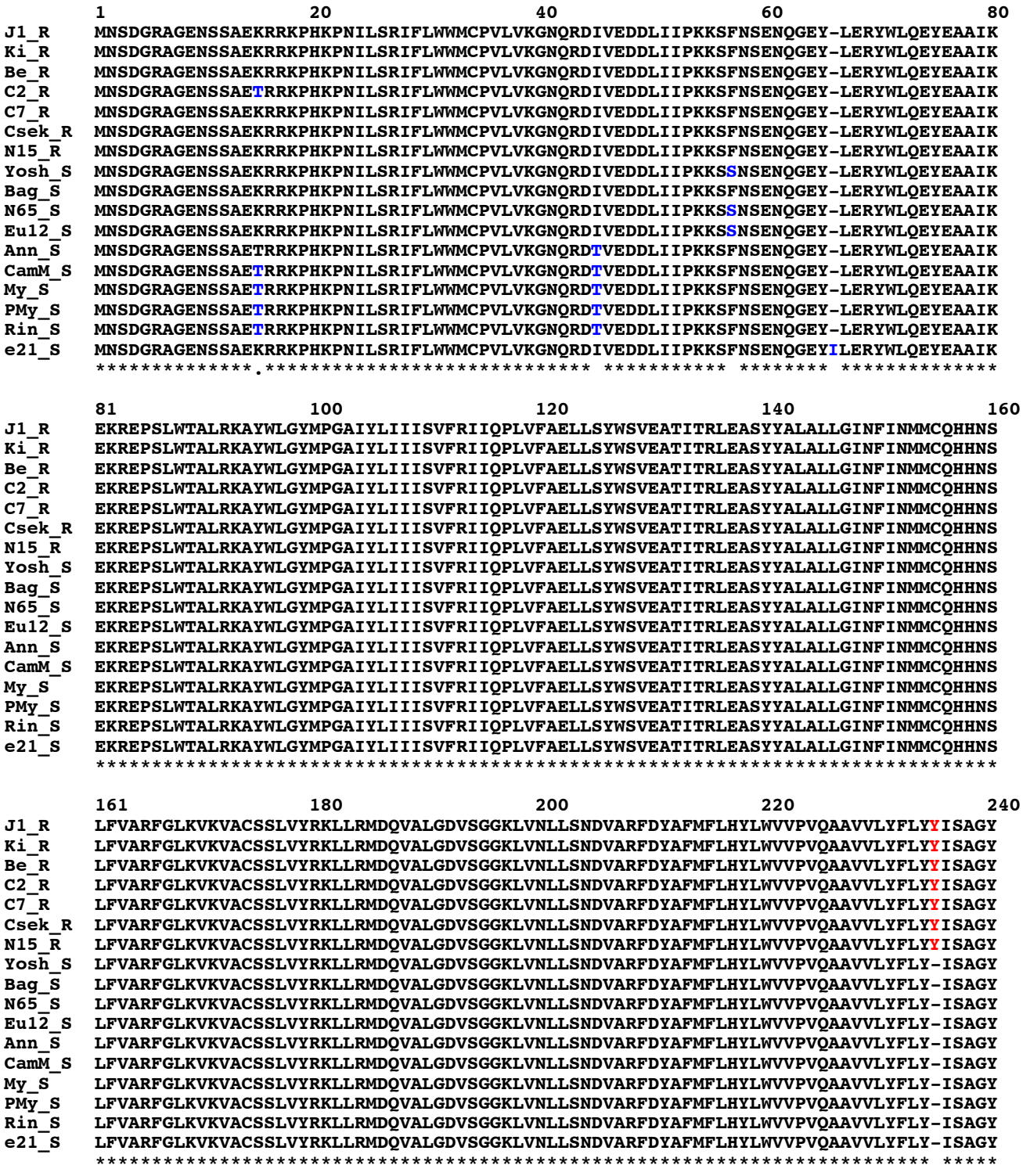


Figure S5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Be_R MNSDGRAGENSSAEKRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
C2_R MNSDGRAGENSSAETRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
C7_R MNSDGRAGENSSAEKRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
Csek_R MNSDGRAGENSSAEKRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
N15_R MNSDGRAGENSSAEKRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
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Bag_S MNSDGRAGENSSAEKRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
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Eu12_S MNSDGRAGENSSAEKRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSSNSENQGEY-LERYWLQEYEAAIK
Ann_S MNSDGRAGENSSAETRRKPHKPNILSRIFLWWMCPVLVKGNQRDTVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
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PMy_S MNSDGRAGENSSAETRRKPHKPNILSRIFLWWMCPVLVKGNQRDTVEDDLIIPKKSFNSENQGEY-LERYWLQEYEAAIK
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e21_S MNSDGRAGENSSAEKRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEYILERYWLQEYEAAIK
81 100 120 140 160
J1_R EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Ki_R EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Be_R EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
C2_R EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
C7_R EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Csek_R EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
N15_R EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Yosh_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Bag_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
N65_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Eu12_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Ann_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
CamM_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
My_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
PMy_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
Rin_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
e21_S EKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYYALALLGINFINMMCQHHNS
161 180 200 220 240
J1_R LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGY
Ki_R LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGY
Be_R LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGY
C2_R LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGY
C7_R LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGY
Csek_R LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGY
N15_R LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLYYISAGY
Yosh_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
Bag_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
N65_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
Eu12_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
Ann_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
CamM_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
My_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
PMy_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
Rin_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY
e21_S LFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDYAFMFLHYLWVVPVQAAVVLYFLY-ISAGY

Figure S6

```
          241                 260                 280                 300                 320
J1_R      APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Ki_R      APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Be_R      APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
C2_R      APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
C7_R      APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Csek_R    APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
N15_R     APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Yosh_S    APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Bag_S     APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
N65_S     APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Eu12_S    APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Ann_S     APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
CamM_S    APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
My_S      APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
PMy_S     APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
Rin_S     APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
e21_S     APFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSI
          **********************************************************************************

          321                 340                 360                 380                 400
J1_R      FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Ki_R      FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Be_R      FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
C2_R      FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
C7_R      FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Csek_R    FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
N15_R     FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Yosh_S    FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Bag_S     FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
N65_S     FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Eu12_S    FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Ann_S     FIRSVFLGFMLFTERSIIFITCLTFLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
CamM_S    FIRSVFLGFMLFTERSIIFITCLTFLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
My_S      FIRSVFLGFMLFTERSIIFITCLTFLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
PMy_S     FIRSVFLGFMLFTERSIIFITCLTFLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
Rin_S     FIRSVFLGFMLFTERSIIFITCLTFLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
e21_S     FIRSVFLGFMLFTERSIIFITCLTLLLTGNLVTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDE
          ************************:**************************************************************

          401                 420                 440                 460                 480
J1_R      REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
Ki_R      REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
Be_R      REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
C2_R      REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
C7_R      REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
Csek_R    REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
N15_R     REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
Yosh_S    REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
Bag_S     REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
N65_S     REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
Eu12_S    REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
Ann_S     REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLSTDPAVCDYPIELSKVDATWSSSTDTS
CamM_S    REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLSTDPAVCDYPIELSKVDATWSSSTDTS
My_S      REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLSTDPAVCDYPIELSKVDATWSSSTDTS
PMy_S     REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLSTDPAVCDYPIELSKVDATWSSSTDTS
Rin_S     REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLSTDPAVCDYPIELSKVDATWSSSTDTS
e21_S     REDVQITPKSYGDDNRLIFNNKASLGPQNEIIPKKYLATDGQLASTLTNEPVLQTDPAVCDYPIELSKVDATWSSSTDTS
          ************************************************.**************************
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Figure S6 cont'd

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          481                 500                 520                 540                 560
J1_R      EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
Ki_R      EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
Be_R      EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
C2_R      EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
C7_R      EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
Csek_R    EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
N15_R     EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
Yosh_S    EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
Bag_S     EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
N65_S     EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
Eu12_S    EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
Ann_S     EMTLRNITLRIGRGKHCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYDSQKY
CamM_S    EMTLRNITLRIGRGKHCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYDSQKY
My_S      EMTLRNITLRIGRGKXCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYDSQKY
PMy_S     EMTLRNITLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYDSQKY
Rin_S     EMTLRNITLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYDSQKY
e21_S     EMTLRNISLRIGRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKY
          *******:******* ************************************************************:****

          561                 580                 600                 620                 640
J1_R      HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Ki_R      HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Be_R      HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
C2_R      HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
C7_R      HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Csek_R    HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
N15_R     HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Yosh_S    HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Bag_S     HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
N65_S     HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Eu12_S    HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Ann_S     HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
CamM_S    HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
My_S      HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
PMy_S     HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
Rin_S     HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
e21_S     HEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFDGCIKGYLRG
          **********************************************************************************

          641                 660                 680                 700                 720
J1_R      RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Ki_R      RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Be_R      RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
C2_R      RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
C7_R      RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Csek_R    RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
N15_R     RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Yosh_S    RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Bag_S     RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
N65_S     RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Eu12_S    RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Ann_S     RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVNTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
CamM_S    RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVNTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
My_S      RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVNTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
PMy_S     RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVNTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
Rin_S     RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVNTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
e21_S     RTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEKNFLRAISKISTKSVEDPDN
          ************************************:**************************************************
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Figure S6 cont'd

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          721                 740                 760                 780                 800
J1_R      EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Ki_R      EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Be_R      EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
C2_R      EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
C7_R      EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Csek_R    EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
N15_R     EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Yosh_S    EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Bag_S     EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
N65_S     EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Eu12_S    EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Ann_S     EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVVTLVITQGCATFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
CamM_S    EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVVTLVITQGCATFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
My_S      EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVVTLVITQGCATFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
PMy_S     EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVVTLVITQGCATFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
Rin_S     EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVVTLVITQGCATFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
e21_S     EKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFIDYWLSFWTNQVDEYEQSLAEGEEPSTSLD
          ************************************.********* ******************************

          801                 820                 840                 860                 880
J1_R      TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Ki_R      TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Be_R      TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
C2_R      TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
C7_R      TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Csek_R    TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
N15_R     TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Yosh_S    TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Bag_S     TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
N65_S     TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Eu12_S    TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Ann_S     TQAGAFTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
CamM_S    TQAGAFTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
My_S      TQAGAFTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
PMy_S     TQAGAFTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
Rin_S     TQAGAFTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
e21_S     TQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRASSNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMD
          *****:**********************************************************************

          881                 900                 920                 940                 960
J1_R      EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Ki_R      EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Be_R      EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
C2_R      EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
C7_R      EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Csek_R    EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
N15_R     EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Yosh_S    EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Bag_S     EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
N65_S     EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Eu12_S    EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Ann_S     EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFVLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
CamM_S    EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFVLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
My_S      EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFVLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
PMy_S     EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFVLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
Rin_S     EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFVLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
e21_S     EFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLST
          *******************************************.************************************
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Figure S6 cont'd

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          961                 980                 1000                1020                1040
J1_R      IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Ki_R      IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Be_R      IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
C2_R      IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
C7_R      IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Csek_R    IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
N15_R     IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Yosh_S    IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Bag_S     IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
N65_S     IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Eu12_S    IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Ann_S     IRSSNSQGRLLQMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
CamM_S    IRSSNSQGRLLQMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
My_S      IRSSNSQGRLLQMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
PMy_S     IRSSNSQGRLLQMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
Rin_S     IRSSNSQGRLLQMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
e21_S     IRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTM
          **********:**************************************************************************

          1041                1060                1080                1100                1120
J1_R      MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Ki_R      MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Be_R      MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
C2_R      MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
C7_R      MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Csek_R    MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
N15_R     MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Yosh_S    MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Bag_S     MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
N65_S     MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Eu12_S    MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Ann_S     MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
CamM_S    MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
My_S      MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
PMy_S     MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
Rin_S     MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
e21_S     MLQMAARFTADFLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKV
          **************************************************************************************

          1121                1140                1160                1180                1200
J1_R      GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Ki_R      GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Be_R      GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
C2_R      GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
C7_R      GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Csek_R    GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
N15_R     GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Yosh_S    GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Bag_S     GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
N65_S     GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Eu12_S    GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Ann_S     GVVGRTGAGKSSMISALFRLYELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
CamM_S    GVVGRTGAGKSSMISALFRLYELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
My_S      GVVGRTGAGKSSMISALFRLYELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
PMy_S     GVVGRTGAGKSSMISALFRLYELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
Rin_S     GVVGRTGAGKSSMISALFRLYELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
e21_S     GVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALE
          **********************:************************************************************
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Figure S6 cont'd

```
         1201                1220                1240                1260                1280
J1_R     QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Ki_R     QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Be_R     QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
C2_R     QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
C7_R     QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Csek_R   QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
N15_R    QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Yosh_S   QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Bag_S    QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
N65_S    QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Eu12_S   QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Ann_S    QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
CamM_S   QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
My_S     QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
PMy_S    QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
Rin_S    QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
e21_S    QVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRL
         ********************************************************************************

         1281                1300                1320                1340
J1_R     NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Ki_R     NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Be_R     NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
C2_R     NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
C7_R     NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Csek_R   NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
N15_R    NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Yosh_S   NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Bag_S    NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
N65_S    NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Eu12_S   NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Ann_S    NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
CamM_S   NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
My_S     NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
PMy_S    NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
Rin_S    NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
e21_S    NTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFRVAEDKHLGRNTEK
         ************************************************************
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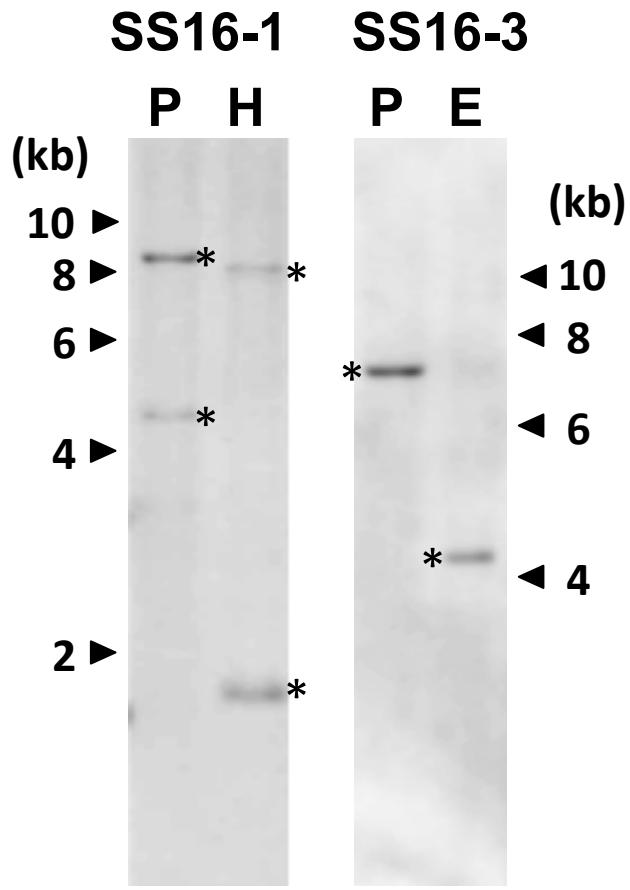
Figure S6 cont'd

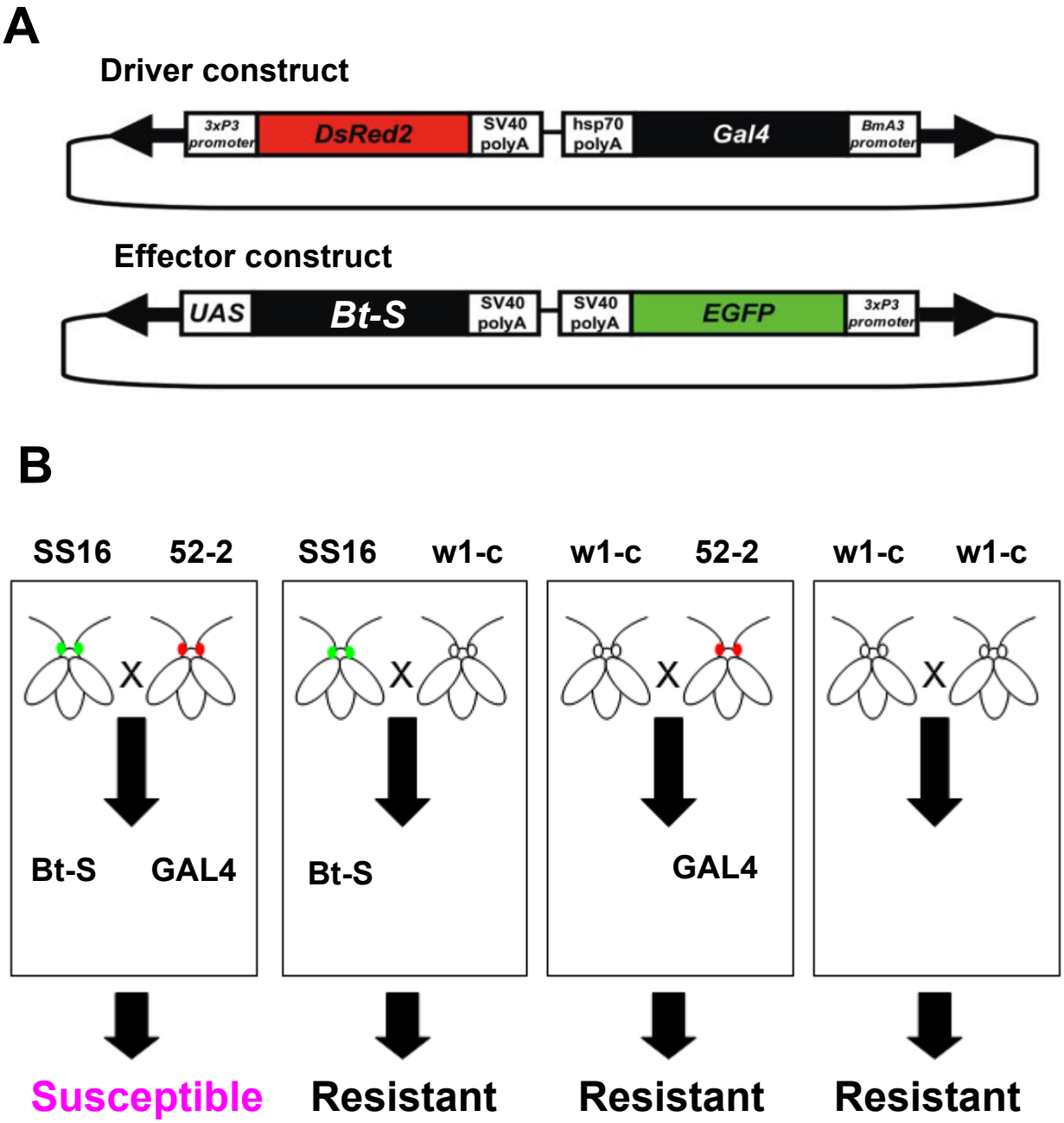
A



B

Strain name	*piggyBac* arm	Chromosome position
SS16-1	L-arm	chr15:14891445
	R-arm	chr15:14891448
	L-arm	chr23:22339304
	R-arm	chr23:22339307
SS16-3	L-arm	chr25:8683927
	R-arm	chr25:8683929

Figure S7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DsRed2
SV40 polyA
hsp70 polyA
Gal4
BmA3 promoter
Effector construct
UAS
Bt-S
SV40 polyA
SV40 polyA
EGFP
3xP3 promoter
B
SS16
52-2
SS16
w1-c
w1-c
52-2
w1-c
w1-c
X
X
X
X
Bt-S
GAL4
Bt-S
GAL4
Susceptible
Resistant
Resistant
Resistant

Figure S8

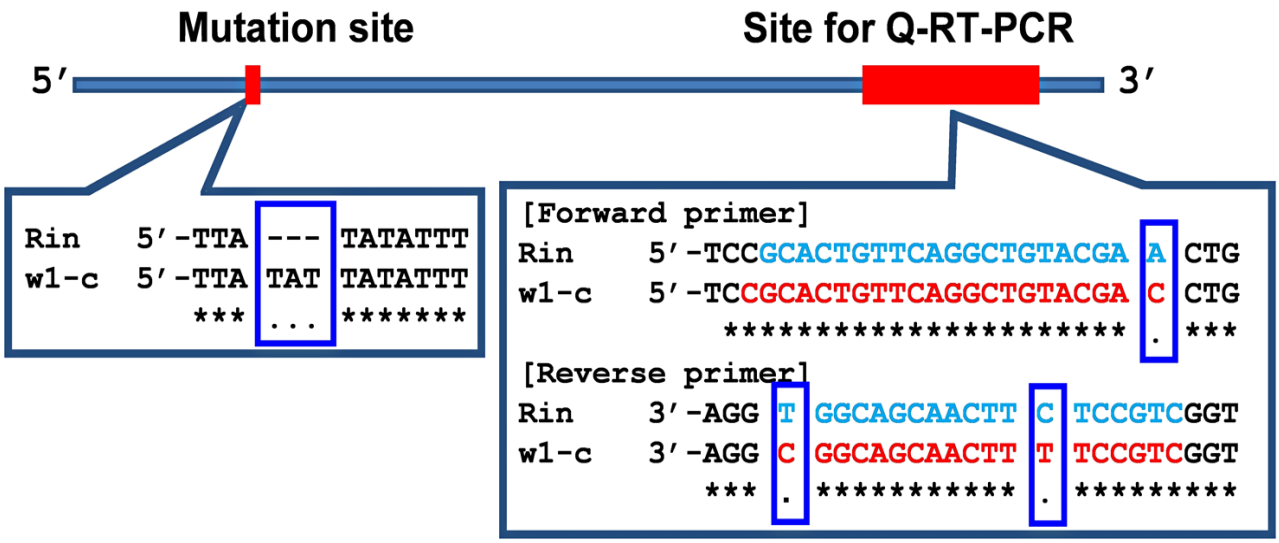
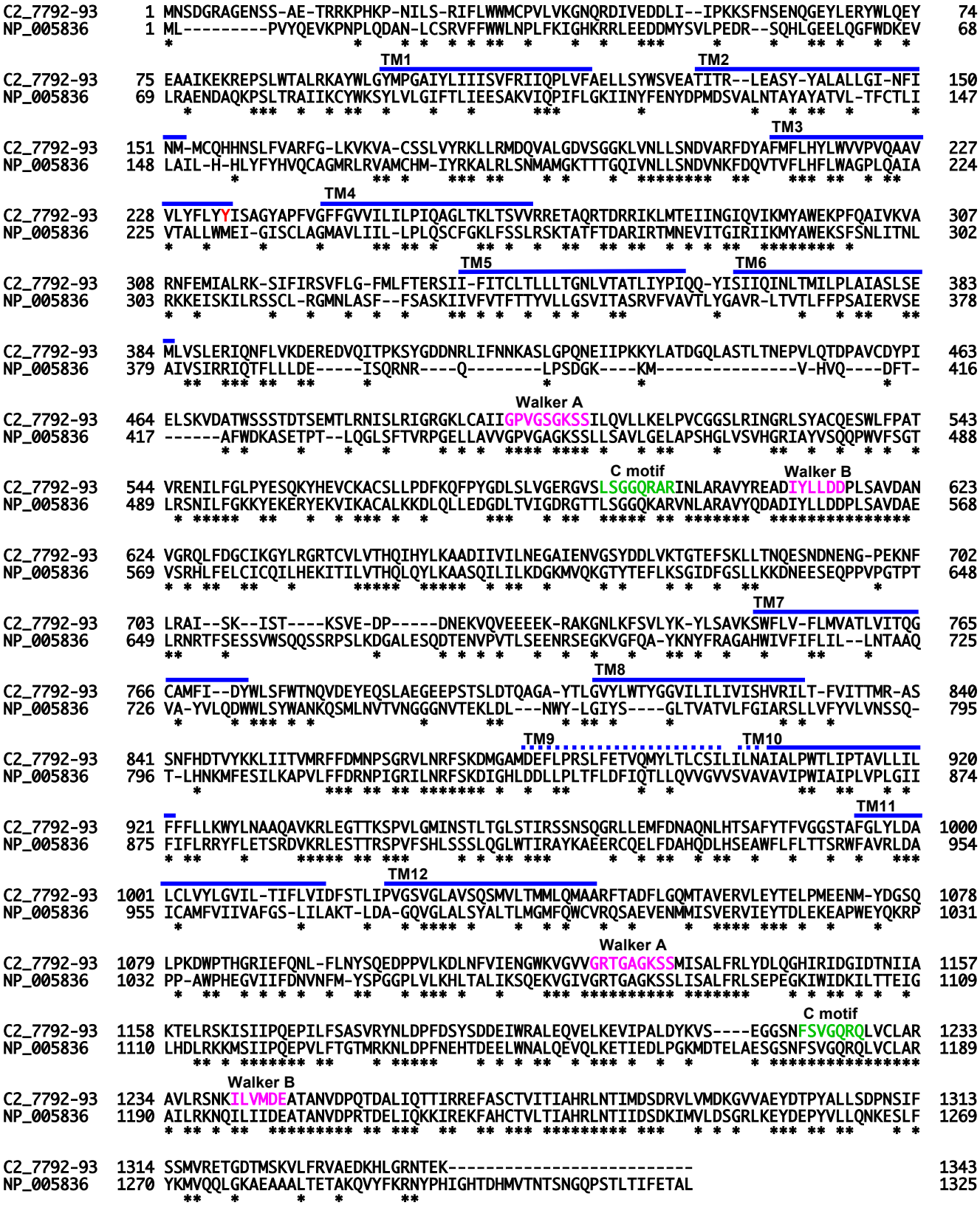

Mutation site
Site for Q-RT-PCR
5′
3′
Rin 5′-TTA --- TATATTT
w1-c 5′-TTA TAT TATATTT
*** ... *******
[Forward primer]
Rin 5′-TCCGCACTGTTCAGGCTGTACGA A CTG
w1-c 5′-TCCGCACTGTTCAGGCTGTACGA C CTG
********** . ***
[Reverse primer]
Rin 3′-AGG T GGCAGCAACTT C TCCGTCGGT
w1-c 3′-AGG C GGCAGCAACTT T TCCGTCGGT
*** . ********** . *********

Figure S9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TM1
TM2
C2_7792-93 75 EAAIKEKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITR--LEASY-YALALLGI-NFI 150
NP_005836 69 LRAENDAQKPSLTRAIIKCYWKSYLVLGIFTLIEESAKVIQPIFLGKIINYFENYDPMDSVALNTAYAYATVL-TFCTLI 147
TM3
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NP_005836 148 LAIL-H-HLYFYHVQCAGMRLRVAMCHM-IYRKALRLSNMAMGKTTTGQIVNLLSNDVNKFDQVTVFLHFLWAGPLQAIA 224
TM4
C2_7792-93 228 VLYFLYYISAGYAPFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKLMTEIINGIQVIKMYAWEKPFQAIVKVA 307
NP_005836 225 VTALLWMEI-GISCLAGMAVLIIL-LPLQSCFGKLFSSLRSKTATFTDARIRTMNEVITGIRIIKMYAWEKSFSNLITNL 302
TM5
TM6
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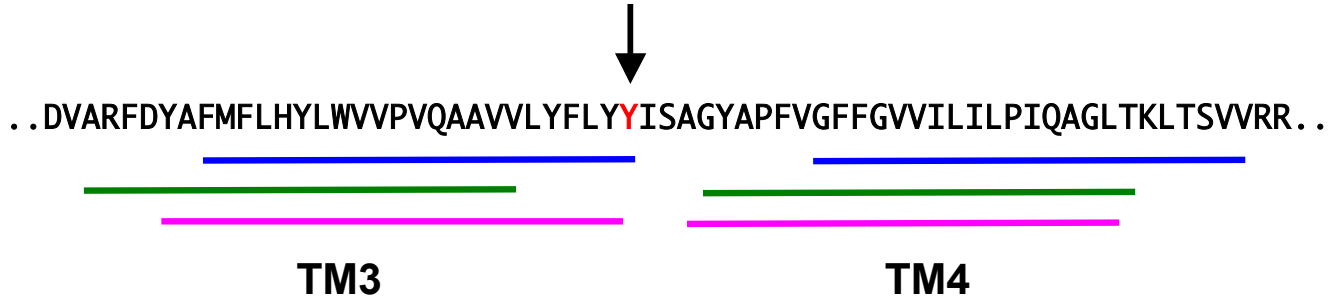
C motif
Walker B
C2_7792-93 544 VRENILFGLPYESQKYHEVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDAN 623
NP_005836 489 LRSNILFGKKYEKERYEKVIKACALKKDLQLLEDGDLTVIGDRGTTLSGGQKARVNLARAVYQDADIYLLDDPLSAVDAE 568
C2_7792-93 624 VGRQLFDGCIKGYLRGRTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENG-PEKNF 702
NP_005836 569 VSRHLFELCICQILHEKITILVTHQLQYLKAASQILILKDGKMVQKGTYTEFLKSGIDFGSLLKKDNEESEQPPVPGTPT 648
TM7
C2_7792-93 703 LRAI--SK--IST----KSVE-DP-----DNEKVQVEEEEK-RAKGNLKFSVLYK-YLSAVKSWFLV-FLMVATLVITQG 765
NP_005836 649 LRNRTFSESSVWSQQSSRPSLKDGALESQDTENVPVTLSEENRSEGKVGFQA-YKNYFRAGAHWIVFIFLIL--LNTAAQ 725
TM8
C2_7792-93 766 CAMFI--DYWLSFWTNQVDEYEQSLAEGEEPSTSLDTQAGA-YTLGVYLWTYGGVILILIVISHVRILT-FVITTMR-AS 840
NP_005836 726 VA-YVLQDWWLSYWANKQSMLNVTVNGGGNVTEKLDL---NWY-LGIYS----GLTVATVLFGIARSLLVFYVLVNSSQ- 795
TM9
TM10
C2_7792-93 841 SNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMDEFLPRSLFETVQMYLTLCSILILNAIALPWTLIPTAVLLIL 920
NP_005836 796 T-LHNKMFESILKAPVLFFDRNPIGRILNRFSKDIGHLDDLLPLTFLDFIQTLLQVVGVVSVAVAVIPWIAIPLVPLGII 874
TM11
C2_7792-93 921 FFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLSTIRSSNSQGRLLEMFDNAQNLHTSAFYTFVGGSTAFGLYLDA 1000
NP_005836 875 FIFLRRYFLETSRDVKRLESTTRSPVFSHLSSSLQGLWTIRAYKAEERCQELFDAHQDLHSEAWFLFLTTSRWFAVRLDA 954
TM12
C2_7792-93 1001 LCLVYLGVIL-TIFLVIDFSTLIPVGSVGLAVSQSMVLTMMLQMAARFTADFLGQMTAVERVLEYTELPMEENM-YDGSQ 1078
NP_005836 955 ICAMFVIIVAFGS-LILAKT-LDA-GQVGLALSYALTLMGMFQWCVRQSAEVENMMISVERVIEYTDLEKEAPWEYQKRP 1031
Walker A
C2_7792-93 1079 LPKDWPTHGRIEFQNL-FLNYSQEDPPVLKDLNFVIENGWKVGVVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIA 1157
NP_005836 1032 PP-AWPHEGVIIFDNVNFM-YSPGGPLVLKHLTALIKSQEKVGIVGRTGAGKSSLISALFRLSEPEGKIWIDKILTTEIG 1109
C motif
C2_7792-93 1158 KTELRSKISIIPQEPILFSASVRYNLDPFDSYSDDEIWRALEQVELKEVIPALDYKVS----EGGSNFSVGQRQLVCLAR 1233
NP_005836 1110 LHDLRKKMSIIPQEPVLFTGTMRKNLDPFNEHTDEELWNALQEVQLKETIEDLPGKMDTELAESGSNFSVGQRQLVCLAR 1189
Walker B
C2_7792-93 1234 AVLRSNKILVMDEATANVDPQTDALIQTTIRREFASCTVITIAHRLNTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIF 1313
NP_005836 1190 AILRKNQILIIDEATANVDPRTDELIQKKIREKFAHCTVLTIAHRLNTIIDSDKIMVLDSGRLKEYDEPYVLLQNKESLF 1269
C2_7792-93 1314 SSMVRETGDTMSKVLFRVAEDKHLGRNTEK-------------------------- 1343
NP_005836 1270 YKMVQQLGKAEAAALTETAKQVYFKRNYPHIGHTDHMVTNTSNGQPSTLTIFETAL 1325

Figure S10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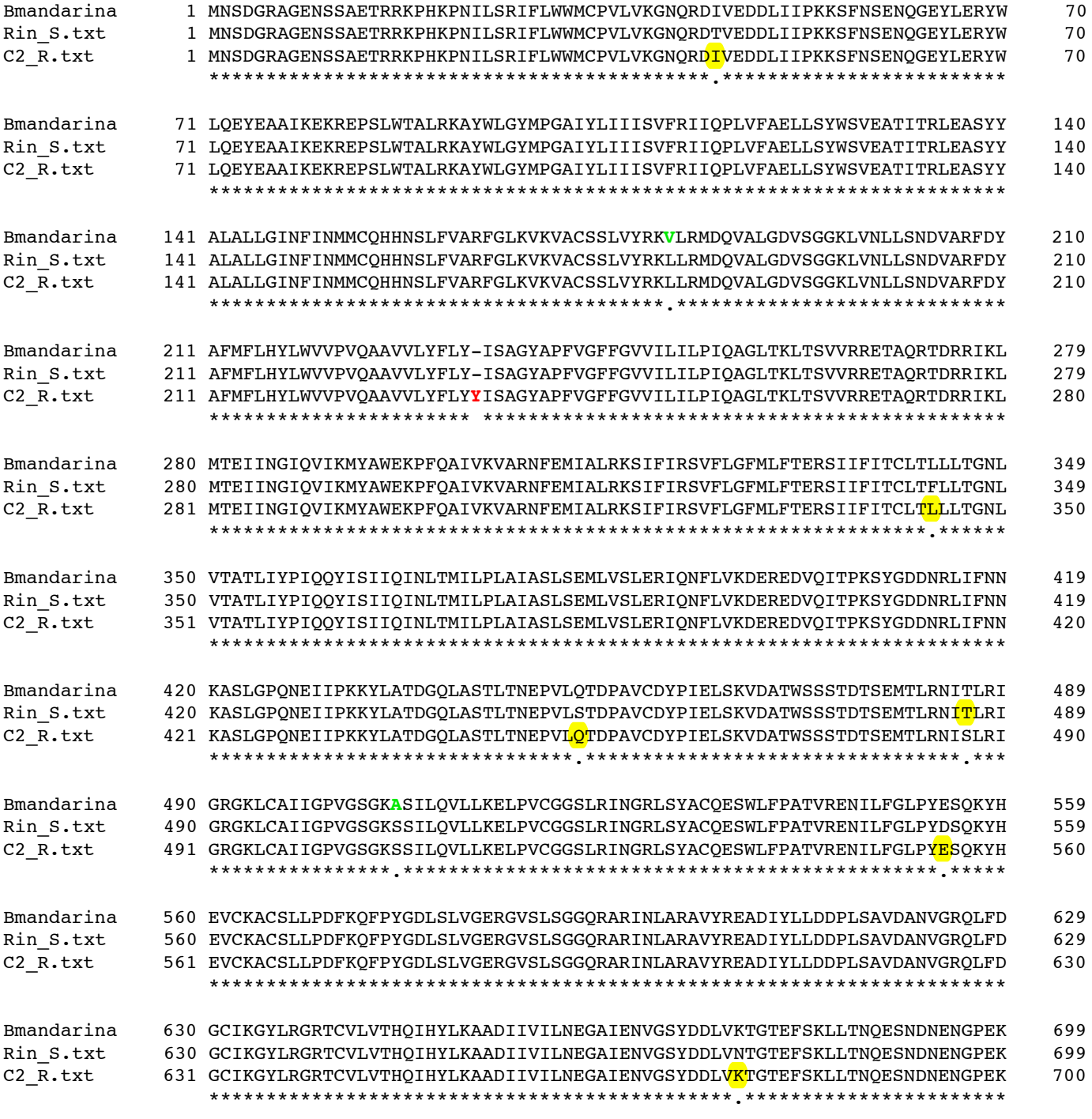
TM3
TM4

Figure S11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Rin_S.txt 1 MNSDGRAGENSSAETRRKPHKPNILSRIFLWWMCPVLVKGNQRDTVEDDLIIPKKSFNSENQGEYLERYW 70
C2_R.txt 1 MNSDGRAGENSSAETRRKPHKPNILSRIFLWWMCPVLVKGNQRDIVEDDLIIPKKSFNSENQGEYLERYW 70
Bmandarina 71 LQEYEAAIKEKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYY 140
Rin_S.txt 71 LQEYEAAIKEKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYY 140
C2_R.txt 71 LQEYEAAIKEKREPSLWTALRKAYWLGYMPGAIYLIIISVFRIIQPLVFAELLSYWSVEATITRLEASYY 140
Bmandarina 141 ALALLGINFINMMCQHHNSLFVARFGLKVKVACSSLVYRKVLRMDQVALGDVSGGKLVNLLSNDVARFDY 210
Rin_S.txt 141 ALALLGINFINMMCQHHNSLFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDY 210
C2_R.txt 141 ALALLGINFINMMCQHHNSLFVARFGLKVKVACSSLVYRKLLRMDQVALGDVSGGKLVNLLSNDVARFDY 210
Bmandarina 211 AFMFLHYLWVVPVQAAVVLYFLY-ISAGYAPFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKL 279
Rin_S.txt 211 AFMFLHYLWVVPVQAAVVLYFLY-ISAGYAPFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKL 279
C2_R.txt 211 AFMFLHYLWVVPVQAAVVLYFLYYISAGYAPFVGFFGVVILILPIQAGLTKLTSVVRRETAQRTDRRIKL 280
Bmandarina 280 MTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSIFIRSVFLGFMLFTERSIIFITCLTLLLTGNL 349
Rin_S.txt 280 MTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSIFIRSVFLGFMLFTERSIIFITCLTFLLTGNL 349
C2_R.txt 281 MTEIINGIQVIKMYAWEKPFQAIVKVARNFEMIALRKSIFIRSVFLGFMLFTERSIIFITCLTLLLTGNL 350
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Rin_S.txt 350 VTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDEREDVQITPKSYGDDNRLIFNN 419
C2_R.txt 351 VTATLIYPIQQYISIIQINLTMILPLAIASLSEMLVSLERIQNFLVKDEREDVQITPKSYGDDNRLIFNN 420
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Bmandarina 490 GRGKLCAIIGPVGSGKASILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKYH 559
Rin_S.txt 490 GRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYDSQKYH 559
C2_R.txt 491 GRGKLCAIIGPVGSGKSSILQVLLKELPVCGGSLRINGRLSYACQESWLFPATVRENILFGLPYESQKYH 560
Bmandarina 560 EVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFD 629
Rin_S.txt 560 EVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFD 629
C2_R.txt 561 EVCKACSLLPDFKQFPYGDLSLVGERGVSLSGGQRARINLARAVYREADIYLLDDPLSAVDANVGRQLFD 630
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C2_R.txt 631 GCIKGYLRGRTCVLVTHQIHYLKAADIIVILNEGAIENVGSYDDLVKTGTEFSKLLTNQESNDNENGPEK 700

Figure S12

```
Bmandarina    700 NFLRAISKISTKSVEDPDNEKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCATFI    769
Rin_S.txt     700 NFLRAISKISTKSVEDPDNEKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVVTLVITQGCATFI    769
C2_R.txt      701 NFLRAISKISTKSVEDPDNEKVQVEEEEKRAKGNLKFSVLYKYLSAVKSWFLVFLMVATLVITQGCAMFI    770
                  ************************************************************.**

Bmandarina    770 DYWLSFWTNQVDEYEQSLAEGEEPSTSLDTQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRAS    839
Rin_S.txt     770 DYWLSFWTNQVDEYEQSLAEGEEPSTSLDTQAGAFTLGVYLWTYGGVILILIVISHVRILTFVITTMRAS    839
C2_R.txt      771 DYWLSFWTNQVDEYEQSLAEGEEPSTSLDTQAGAYTLGVYLWTYGGVILILIVISHVRILTFVITTMRAS    840
                  ********************************.***********************************

Bmandarina    840 SNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMDEFLPRSLFETVQMYLTLCSILILNAIALPWT    909
Rin_S.txt     840 SNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMDEFLPRSLFETVQMYLTLCSILILNAIALPWT    909
C2_R.txt      841 SNFHDTVYKKLIITVMRFFDMNPSGRVLNRFSKDMGAMDEFLPRSLFETVQMYLTLCSILILNAIALPWT    910
                  ************************************************************************

Bmandarina    910 LIPTAVLLILFFFLLKWYLNAAQAVKRLEGTAKSPVLGMINSTLTGLSTIRSSNSQGRLLEMFDNAQNLH    979
Rin_S.txt     910 LIPTAVLLILFFVLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLSTIRSSNSQGRLLQMFDNAQNLH    979
C2_R.txt      911 LIPTAVLLILFFFLLKWYLNAAQAVKRLEGTTKSPVLGMINSTLTGLSTIRSSNSQGRLLEMFDNAQNLH    980
                  ************.******************.****************************.*********

Bmandarina    980 TSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTMMLQMAARFTAD   1049
Rin_S.txt     980 TSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTMMLQMAARFTAD   1049
C2_R.txt      981 TSAFYTFVGGSTAFGLYLDALCLVYLGVILTIFLVIDFSTLIPVGSVGLAVSQSMVLTMMLQMAARFTAD   1050
                  ************************************************************************

Bmandarina   1050 FLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKVG   1119
Rin_S.txt    1050 FLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKVG   1119
C2_R.txt     1051 FLGQMTAVERVLEYTELPMEENMYDGSQLPKDWPTHGRIEFQNLFLNYSQEDPPVLKDLNFVIENGWKVG   1120
                  ************************************************************************

Bmandarina   1120 VVGRTGAGKSSMISALFRLYELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYS   1189
Rin_S.txt    1120 VVGRTGAGKSSMISALFRLYELQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYS   1189
C2_R.txt     1121 VVGRTGAGKSSMISALFRLYDLQGHIRIDGIDTNIIAKTELRSKISIIPQEPILFSASVRYNLDPFDSYS   1190
                  ********************.***************************************************

Bmandarina   1190 DDEIWRALEQVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQ   1259
Rin_S.txt    1190 DDEIWRALEQVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQ   1259
C2_R.txt     1191 DDEIWRALEQVELKEVIPALDYKVSEGGSNFSVGQRQLVCLARAVLRSNKILVMDEATANVDPQTDALIQ   1260
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Bmandarina   1260 TTIRREFASCTVITIAHRLNTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFR   1329
Rin_S.txt    1260 TTIRREFASCTVITIAHRLNTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFR   1329
C2_R.txt     1261 TTIRREFASCTVITIAHRLNTIMDSDRVLVMDKGVVAEYDTPYALLSDPNSIFSSMVRETGDTMSKVLFR   1330
                  ************************************************************************

Bmandarina   1330 VAEDKHLGRNTEK                                                          1342
Rin_S.txt    1330 VAEDKHLGRNTEK                                                          1342
C2_R.txt     1331 VAEDKHLGRNTEK                                                          1343
                  *************
```

Figure S12 cont'd

Table S1. Linkage analysis of the Bt resistance gene in C2

Linkage group	SNP marker	SNPs	Genotype																			No. of H	No. of A	% of H
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19			
1	01-030	AC382	A	H	A	H	A	A	A	A	A	H	H	A	H	A	H	H	A	H	A	8	11	42.1
2	02-001	TA738	H	A	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	A	H	16	3	84.2
3	03-048	TA212	A	A	A	A	H	A	H	H	A	H	A	H	A	H	A	A	A	A	H	7	12	36.8
4	04-018	TA227	A	H	A	A	A	H	A	H	A	H	A	H	H	A	H	A	H	A	A	8	11	42.1
5	05-034	GT366	A	H	H	A	A	H	H	H	H	A	A	A	A	H	A	H	A	A	H	6	13	31.6
6	06-047	CT243	H	A	H	H	A	A	A	H	H	H	H	H	A	H	H	A	H	H	A	12	7	63.2
7	07-009	CT95	H	A	H	A	A	H	H	A	H	H	H	H	A	A	A	H	A	H	H	11	8	57.9
8	08-018	CT154	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	A	H	A	H	13	6	68.4
9	09-048	AC215	H	A	H	H	H	H	H	H	H	A	H	A	H	A	A	H	H	H	A	13	6	68.4
10	10-007	CT130	A	A	H	H	A	A	H	H	A	A	A	A	A	H	A	H	H	A	H	8	11	42.1
11	11-039	CT271	A	A	A	A	H	A	H	H	H	H	H	A	H	H	H	H	H	A	H	12	7	63.2
12	12-026	AG170	A	H	H	H	A	A	H	H	A	A	H	A	H	A	H	A	A	A	H	9	10	47.4
13	13-071	CG338	H	A	A	A	A	A	H	A	A	H	A	A	H	A	H	A	H	H	A	7	12	36.8
14	14-004	TA439	A	H	A	H	H	H	H	H	A	A	H	H	A	H	H	A	A	A	H	11	8	57.9
15	15-001	CG590	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0	19	0.0
15	15-057	TC282	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0	19	0.0
15	15-056	TC355	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0	19	0.0
16	16-022	CT369	H	A	H	A	H	H	A	H	A	H	A	A	A	A	A	A	A	H	H	8	11	42.1
17	17-025	INS283(CGT)	H	H	H	H	A	A	A	H	A	A	A	A	H	A	A	A	A	H	H	8	11	42.1
18	18-029	TA203	A	A	A	H	H	A	A	A	A	H	A	A	A	A	H	A	H	H	H	7	12	36.8
19	19-002	CT192	A	H	H	H	H	H	A	H	A	H	A	H	A	H	H	A	A	H	H	12	7	63.2
20	20-020	INS387(G)	A	A	A	A	H	A	A	A	H	A	A	H	A	A	H	H	H	H	H	8	11	42.1
21	21-008	CG323	H	A	H	H	A	H	A	A	H	A	H	A	H	A	H	H	A	A	A	9	10	47.4
22	22-014	TC473	H	H	A	H	H	H	A	H	A	H	A	A	A	A	A	H	H	A	A	9	10	47.4
23	23-024	TC227	H	H	A	H	H	H	H	H	H	A	A	A	H	H	H	A	H	A	H	13	6	68.4
24	24-030	AT439	H	A	H	A	A	A	H	A	A	A	A	H	H	A	H	H	H	H	A	9	10	47.4
25	25-013	TC445	A	A	H	A	H	H	H	H	H	A	A	H	H	A	A	H	H	A	A	10	9	52.6
26	26-004	CT200	A	H	H	H	A	A	A	H	H	H	A	A	A	H	H	H	H	H	A	11	8	57.9
27	27-034[a]	CT222	H	H	A	H	H	A	A	H	A	H	A	A	H	A	A	H	A	H	H	10	9	52.6
28	28-002[a]	GA214	A	A	H	A	A	H	A	H	A	H	A	A	H	H	A	A	H	H	H	9	10	47.4

Nineteen BC_1 larvae (1–19) that survived after Bt toxin exposure were screened with 30 SNP markers (three for linkage group 15 and one each for the remaining 27 linkage groups). Homozygosity (C2/C2, A) or heterozygosity (C2/Rin, H) was determined for each marker by sequencing. A, homozygous genotype; H, heterozygous genotype. The shaded area shows the region where all markers were homozygous.

[a] SNP markers, 27-034 and 28-002, correspond to B034 and A002, respectively, in Yamamoto et al. (9)

Table S2. Primer list

Primer name	Sequences	Expected size (bp)
Glycosyltransferase gene cloning		
Bre-2-F4	5'–AGTGTGGCAATCACAATAGCAATC–3'	
Bre-2-R3	5'–ACGTTTTTCTGATGACAAGCCTG–3'	
Bre-2-F6	5'–TAGTTTCCTCCAACCCCTTG–3'	
Bre-2-R6	5'–TGTACGACTTGCGGAAACTG–3'	
Bre-2-GSP2	5'–TTAGCACAGAGGAGACAGAG–3'	
Bre-2-NGSP2	5'–TCGTTTACTGGAAGCAGCAC–3'	
Bre-3-F6	5'–GTGCTTATTGATGATGAAGTCAGG–3'	
Bre-3-R6	5'–CGCCTTGAATAAAGCTCCAG–3'	
Bre-3-F3	5'–TTCAGAATGATCAGAGGGCG–3'	
Bre-3-R3	5'–TCGTTGCTGCATGAAATCCC–3'	
Bre-3-F4	5'–ATATGGGCAAGCTAAGGCTG–3'	
Bre-3-R5	5'–CAATGTGCAGTTACTAGCAAAGAG–3'	
Bre-3-GSP2	5'–TGGGTAACACTCCCACTATC–3'	
Bre-3-NGSP2	5'–GCTTGTGGATTTACAGCAGC–3'	
Bre-4-F5	5'–ATGGGACAATTTCACCGGAC–3'	
Bre-4-R5	5'–TAACGCTTCTCCACCCAATC–3'	
Bre-4-F3	5'–TCTTCGGCTCCATACTGGAC–3'	
Bre-4-R3	5'–TGGTACGTCTTGCTCGTTTG–3'	
Bre-4-F7	5'–ATTCCACGACATCGATCTGC–3'	
Bre-4-R6	5'–AACGCTGATGTTACCTGTCG–3'	
Bre-4-GSP2	5'–GTCCGCTTCCATAGACAAAC–3'	
Bre-4-NGSP2	5'–CTCTAACGTTGGAGCAGTTC–3'	
Bre-5-F3	5'–CCTGTGTCCATCAGTTCTTC–3'	
Bre-5-R3	5'–AGTAGTGTTGAGCTTCAGCG–3'	
Bre-5-F2	5'–ATCGCGAATCTCAGTGTACG–3'	
Bre-5-R2	5'–AGTAAACGCGCGACTCATAC–3'	
Bre-5-GSP2	5'–AATACGTTAGCGACGTGACG–3'	
Bre-5-NGSP2	5'–GCGAAAGAGACGGAATACTG–3'	
Mapping		
15-016_F	5'–AATGCCAATGTGGTTAATAAGTTT–3'	705
15-016_R	5'–TGCTGCTGTTTATATATGAGGGC–3'	
15-089_F	5'–CAGCAATAGCATGTGCCAAC–3'	603

15-089_R	5'–TTCGCGCAGTTTTGTTTACT–3'	
15-011_F	5'–ACGTCGATCATGACTTTCCC–3'	629
15-011_R	5'–ATCGCGAATTGCTAATGCTT–3'	
15-916_F	5'–TCGACTGATAGTAGGACCGC	587
15-916_R	5'–GATTAACGAGATCCGGTAGG	
15-322_F	5'–TACCAAAATGTCGGGACAGC	612
15-322_R	5'–AGTTCCAGTTCCATTCCCAC	
15-327-4_F	5'–GCTAAATTGATTCTGGCCCG–3'	721
15-327-4_R	5'–ATGGCCAGTGTTGTCACATC–3'	
15-429-1_F	5'–AACACGCTGACATTGCTGAC–3'	377
15-429-1_R	5'–AGGCACTTAAGACAGGTGAG–3'	
15-221_F	5'–TTTGGTCAAGACCGGAACAG	613
15-221_R	5'–ACAGTTAACGGATGTCCACC	
15-427-9_F	5'–CGAGTAAGATTGCAGACCTG–3'	563
15-427-9_R	5'–AGACAAGGAATCGCAAGTGC–3'	
15-427-2_F	5'–GCTTGGAGATATAGGTTCGC–3'	600
15-427-2_R	5'–ACAATCTGGTCAGCCTGTTG–3'	
15-218_F	5'–GGACGAAACATAGGTCCATC –3'	432
15-218_R	5'–TCGTGCCTGTTTCCTCTAAG –3'	
15-311_F	5'–CGGGTATTGTTTGCAACACG–3'	509
15-311_R	5'–ATAGCCCACCTGGTGTTAAG–3'	
15-308_F	5'–AAGGCCAGTGCTAGAACTAC–3'	639
15-308_R	5'–AGAACTCTAAGCCTGCTCCT–3'	
15-304_F	5'–TAACCACTCACCACCAGTTG–3'	678
15-304_R	5'–TTCCGATCATTGCTGGAGTG–3'	
15-208_F	5'–AGCCTATCAAAGCCGCAATG–3'	434
15-208_R	5'–GCCGAACAAGATGTTCTGTC–3'	
15-205_F	5'–ACAGGCTACTTTGCTTTGGG–3'	516
15-205_R	5'–CCGATCACTAACACAGTTCC–3'	
15-204_F	5'–TTCAGATCACTGCCAGATCC–3'	578
15-204_R	5'–CCATTTACTTACCAGCTCCC–3'	
15-202_F	5'–ATACGGAGTGTATTTCGCCG–3'	584
15-202_R	5'–AACCCATTCAGTTCTTCCGG–3'	
15-215_F	5'–TTCATGTTTGGACCAGGACC–3'	609
15-215_R	5'–GCACAAACACCACAGTACTC–3'	
RT-PCR		
007735_F	5'–TAGATGTTCAAGTGGGACTGTT–3'	429
007735_R	5'–GTTCCCTCCAATCTTTTAATGC–3'	

007793_F	5'–AACTTCCCAAGGACTGGCCTA–3'	513
007793_R	5'–CTGTCGCCTCGTCCATTACAA–3'	
007736_F	5'–ATGTTTAGAACCTACATATACATAG–3'	210
007736_R	5'–CTAGCCTCCTAGTCCTCCTA–3'	
007792_F	5'–AGAGGACGTCCAAATAACACCA–3'	472
007792_R	5'–CTGTGATTCGTACGGCAAACCA–3'	
007791_F	5'–TCATAGAAGATCGGAACGGGTA–3'	547
007791_R	5'–TCCCTCAGCTTCTTGAGTTCTT–3'	
007737_F	5'–ATGGTCAGTGGAAATAAAGACG–3'	227
007737_R	5'–TACTTTTCAAGCCGATCACCAAG–3'	
Vector construction		
BTRCG-F-XbaI[b]	5'–TCTAGAATGAATAGTGATGGGAGAGCCGGA–3'	4042
BTRCG-R-XbaI[b]	5'–TCTAGATTCATTTTTCTGTATTTCTACCAA–3'	
Probe for Southern		
KS113	5'– ATGGTGAGCAAGGGCGAGGAGCTGT–3'	672
KS248	5'– GAACTCCAGCAGGACCATGTGAT–3'	
Inverse PCR		
L-arm 1st PCR		
KS129	5'–AAATCAGTGACACTTACCGCATT–3'	
KS133	5'–ACTATAACGACCGCGTGAGTCAA–3'	
L-arm 2nd PCR		
KS130	5'–CGACTGAGATGTCCTAAATGCAC–3'	
KS395	5'–TTATCGATACCGTCGACCTCGAC–3'	
R-arm 1st PCR		
KS125	5'–GCGCCATAAAAGTTTTGTTACTT–3'	
KS398	5'–TCGAATTCGCTTCGGTTTATATG–3'	
R-arm 2nd PCR		
KS396	5'–AGACCGATAAAACACATGCGTCA–3'	
KS397	5'–GGGTCCGTCAAAACAAAACATC–3'	
Realtime RT-PCR		
Rin_92-93_F	5'–GCACTGTTCAGGCTGTACGAA–3'	212
Rin_92-93_R	5'–GACGGAAAAGTTGCTGCCG–3'	
C2_92-93_F	5'–CGCACTGTTCAGGCTGTACCAC–3'	268
C2_92-93_R	5'–GACGGAAAAGTTGCTGACG–3'	
Bm_rp49-3[a]	5'–CAGGCGGTTCAAGGGTCAATAC–3'	267
Bm_rp49-4[a]	5'–TGCTGGGCTCTTTCCACGA–3'	

Bre-2, Bre-3, Bre-4, and Bre-5 represent genes for β-1,3-galactosyltransferase, β-1,4-mannosyltransferase, β-1,4-N-acetylgalactosaminiltransferase and

β-1,3-N-acetylglucosaminyltransferase, respectively. GSP2 and NGSP2 primers were used for 3'-RACE.

RT-PCR primers were designed in exon regions; each one of them was on the other side of an intron except 007737.

[a] Primers used to amplify ribosomal protein L32 gene (44)

[b] Primers used to amplify *Rin-007792-93* for UAS vector construct

Table S3. Chromosome location of Bt resistance related genes in *Bombyx mori*

Gene name	Accession No.	.Gene name	Chromosome No. : position
Alkaline phosphatase	NM_001044071	BGIBMGA008818	chr3:18166325–18168639
Aminopeptidase N class1	AF084257	BGIBMGA008059	chr9:893313–917498
Aminopeptidase N class2	AB011497	BGIBMGA008017	chr9:1039491–1064313
Aminopeptidase N class3	AF352574	BGIBMGA008059	chr9:893313–917498
Aminopeptidase N class4	AB013400	BGIBMGA008060	chr9:921362–937045
Alpha-Amylase	GQ344953	BGIBMGA003057	chr4:16330723–16336955
Cadherin-like protein	NM_001044217	BGIBMGA013616	chr6:2723247–2750142
Chlorophyllide-binding protein	AM113746	BGIBMGA004806	chr25:3744988–3784275
Glycosyltransferase Bre-2	AB620070	BGIBMGA004619	chr27:12179554–12181239
Glycosyltransferase Bre-3	AB620071	BGIBMGA001169	chr13:10537381–10538713
Glycosyltransferase Bre-4	AB620072	BGIBMGA007485	chr3:3992963–4001297
Glycosyltransferase Bre-5	AB620073	BGIBMGA005534	chr17:542185–543174
Mitogen-activated protein kinase	XM_001653191	BGIBMGA003561	chr5:15798629–15807846

Accession numbers correspond to the query sequences used to search the chromosome position using KAIKObase (http://sgp.dna.affrc.go.jp/KAIKObase/).

Aminopeptidase N class 1 (N1) and 3 (N3) were mapped to the same gene, because the anterior half of BGIBMGA008059 had the sequence of aminopeptidase N1 and the posterior half that of N3. Aminopeptidases N1-N8 (16) all mapped on chromosome 9. Glycosyltransferase genes were newly identified in *B. mori*, based on the sequence reported in *C. elegans*.

Table S4. Chromosome mapping of Bt resistance gene – part I

SNP marker	Genotype																																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
15-001	H	A	H	A	A	A	H	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
15-073	H	A	H	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
15-040	H	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	-	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
15-030	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
15-071	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
15-016	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
15-015	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-062	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-011	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-089	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A
15-020	A	A	A	H	A	A	A	A	H	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A
15-060	A	A	A	H	A	A	A	-	H	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A
16-037	A	A	A	H	A	A	A	-	H	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A
15-074	A	A	A	H	A	A	A	A	H	A	H	A	H	A	A	H	A	A	A	A	H	A	A	H	A	A	A	A	H	A	H	A	A	H	H	A	H	A	H	A	A	H	A	H
15-009	A	A	A	H	A	A	A	A	H	A	H	A	H	A	A	H	A	A	A	A	H	A	A	H	A	A	A	A	H	A	H	A	A	H	H	A	H	A	H	A	A	H	A	H
15-057	A	H	A	H	A	A	A	A	H	A	H	A	H	A	A	H	A	A	H	A	H	H	A	H	A	A	H	A	H	A	H	A	A	H	H	H	H	A	H	A	A	H	A	H
15-056	A	H	A	H	A	A	A	A	H	H	H	H	H	A	A	H	A	A	H	A	H	H	A	H	H	A	H	A	H	A	H	A	A	H	H	H	H	A	H	A	A	H	A	H

Forty-four BC_1 larvae (1–44) that survived after Bt toxin screening were subjected to analyses using 17 SNP markers on Chromosome 15 (9). Homozygosity (C2/C2, A) or heterozygosity (C2/Rin, H) was determined for each marker site by sequencing the marker regions. The resistance gene was predicted to be located between markers 15-016 and 15-089.

Table S5. Chromosome mapping of Bt resistance gene – part II

SNP	Genotype																															
marker	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
15-016	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
15-015	A	H	H	A	H	H	H	A	A	H	A	A	A	H	A	A	H	A	A	A	H	H	A	A	H	A	A	H	H	A	H	H
15-075	A	H	H	A	H	H	H	A	A	H	A	A	A	H	A	A	H	A	A	A	H	H	A	A	H	A	A	H	H	A	H	H
15-034	A	H	H	A	H	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	H	H	A	A	H	A	A	H	A	A	A	H
15-062	A	H	H	A	H	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	H	H	A	A	H	A	A	H	A	A	A	H
15-027	A	H	H	A	H	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	H	H	A	A	H	A	A	H	A	A	A	H
15-006	A	H	H	A	H	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	H	H	A	A	H	A	A	H	A	A	A	H
15-041	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-095	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-011	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-050	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-089	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Thirty-two BC_1 larvae (1–32) that survived after Bt toxin screening were subjected to analyses using 12 SNP markers on Chromosome 15 (9). Homozygosity (C2/C2, A) or heterozygosity (C2/Rin, H) was determined at each site by sequencing the marker regions. The resistance gene was predicted to be located between markers 15-011 and 15-089.

Table S6. Chromosome mapping of Bt resistance gene – part III

SNP marker	Genotype														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15-011	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A
15-916	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A
15-322	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A
15-327-4	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A
15-429-1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-221	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-427-9	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-427-2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
15-218	A	H	A	H	H	H	H	A	A	A	H	H	A	A	A
15-311	A	H	A	H	H	H	H	A	A	A	H	H	A	A	A
15-308	A	H	A	H	H	H	H	A	A	A	H	H	A	A	A
15-304	A	H	A	H	H	H	H	A	A	A	H	H	A	A	A
15-208	A	H	A	H	H	H	H	A	A	A	H	H	A	A	A
15-205	H	H	A	H	H	H	H	H	A	A	H	H	A	A	A
15-204	H	H	A	H	H	H	H	H	A	A	H	H	A	A	A
15-202	H	H	H	H	H	H	-	H	A	A	H	H	A	A	A
15-215	H	H	H	H	H	H	H	H	A	H	H	H	A	A	H
15-050	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H
15-089	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H

Fifteen BC_1 larvae (1–15) that survived after Bt toxin screening were scored with 19 SNP markers on Chromosome 15. Sixteen markers (15-916–15-215) were newly designed (table S2). Homozygosity (C2/C2, A) or heterozygosity (C2/Rin, H) was determined for each site by sequencing the marker regions. The resistance gene was predicted to be located between markers 15-327-4 and 15-218.

Table S7. Silkworm strains used

Strain	Strain name	Race No.	Origin / character
Bt resistant strains			
J1_R	Japanese no. 1	204	Japanese, improved
Ki_R	Kiuki	212	Japanese, improved
Be_R	Benishina	302	Chinese, native
C2_R	Chinese no. 2	401	Chinese, improved
C7_R	Chinese no. 7	404	Chinese, improved
Csek_R	C sekko	418	Chinese, improved
N15_R	N15	—	From Chinese no. 342
Bt susceptible strains			
Yosh_S	Yoshi N	217	Japanese, improved
Bag_S	Bagdad	504	European, native
N65_S	No.65	516	European, native
Eu12_S	European no. 12	555	European, improved
Ann_S	Annam	601	Tropical
CamM_S	Cambodia	603	Tropical
My_S	Mysore	604	Tropical
PMy_S	Pure Mysore	605	Tropical
Rin_S	Ringetsu	606	Tropical
e21_S	e21	—	From mutant race no.912
Strains used or generated in transgenesis			
w1-c		—	Egg and eye-color mutant, white
w1-pnd		—	Non-diapausing mutant of w1-c
52-2		—	GAL4 driver strain
SS16-1		—	UAS effector strain
SS16-3		—	UAS effector strain

Strains with race number are maintained in the Genetic Resource Center of NIAS (http://www.gene.affrc.go.jp/databases_en.php?section=animal). N15_R and E21_S were established from NIAS strains and provided from O. Ninagi.