Supporting Information

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SI Materials and Methods

Identification of a Geranylgeranyl Diphosphate Synthase in the White Pine Weevil, *Pissodes strobi* (Peck). The white pine weevil [WPW, *P. strobi* (Peck)] is a major pest of several spruce and pine species in North America (1). Adult male weevils produce the monoterpenoid compound grandisol, *cis*-2-isopropenyl-1-methylcyclobutaneethanol (2, 3). Grandisol (Fig. 1) is a pheromone component in WPW (2, 3), other *Pissodes* species (3–5), and other weevil genera (6). Although the biosynthetic origin of grandisol has not been determined in WPW, it does originate de novo from the mevalonate pathway in the cotton boll weevil (*Anthonomus grandis*) (7) and is derived from geraniol and/or its isomer nerol (8). A putative full-length IDS cDNA clone PST046_L05 was identified from a sequence assembly of WPW larval and adult cDNA libraries (NCBI TSA accession no. GAEO01002907) by BLASTx and tBLASTn comparisons with known insect isoprenyl diphosphate synthases (IDSs), was fully sequenced, and was deposited in GenBank [accession no. KC464331 (*PstrGGPPS*, PST046_L05)].

Cloning, Expression, and Purification of IDSs. The full ORFs of the putative IDSs were amplified from pDNR-Lib plasmids (9) using InFusion primers (Table S1) and were inserted into the *Nco*I- and *Xho*I-digested pET28b(+) vector (Novagen) using In-Fusion Dry Down PCR cloning (Clontech), resulting in C-terminal 6xHis-tagged enzymes. PCR amplifications were completed in a 100-μL final volume containing 2 μL of 10 mM dNTPs, 10 μM of each primer, 10 ng template, 20 μL 5xHF Phusion buffer, and 2 U of Phusion DNA polymerase (New England Biolabs). The cycling profile was 3 min at 98 °C, 35 cycles of 10 s at 98 °C, 20 s at 63 °C, 1.5 min at 72 °C, and a 5-min extension at 72 °C. Each pET28b(+) construct was confirmed by sequencing. Recombinant IDSs were expressed in *Escherichia coli* C41 (DE3) cells (Lucigen) containing the pRARE2 plasmid (Novagen), which encodes rare tRNA codons. An overnight culture grown in LB [kanamycin/chloramphenicol (Kan/Cam)] was diluted into 500 mL of TB(Kan/Cam) in a 2-L culture flask. Cells were grown at 37 °C and 250 rpm in a shaking incubator (New Brunswick Scientific) to an OD_{600} of 0.8; then the cultures were cooled to 16 °C, 0.2 mM isopropyl β-D-1-thiogalactopyranoside was added, and cultures were grown for 17 h at 16 °C and 225 rpm. Cell pellets were lysed by sonication and were centrifuged for 15 min at 12,000 × *g*. The supernatant was applied a 1-mL HisTrap HP Ni-affinity column, and the recombinant proteins were purified using an ÄKTApurifier 10 fast protein liquid chromatograph (GE Healthcare). Eluted His-tagged proteins then were desalted using Econo-Pac 10DG columns (Bio-Rad) into 50 mM Hepes, 5 mM $MgCl_2$, and 10% glycerol, pH 7.2. Protein concentrations were determined by absorbance at 280 nm and the calculated extinction coefficients.

Results of the Identification and Functional Characterization of a Geranylgeranyl Diphosphate Synthase in the White Pine Weevil, *P. strobi* (Peck). The complete cDNA sequence of the putative IDS from *P. strobi* was 1,499 bp long and included 79 bp and 499 bp of 5′ and 3′ UTRs, respectively. The deduced amino acid sequence corresponds to a 306-aa protein with a theoretical mass of 36.6 kDa, and a pI of 6.13. Heterologous expression of PST046_L05 and in vitro assays with isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) as substrates produced geranylgeranyl diphosphate (GGPP) with only a trace of geranyl diphosphate (GPP) (Fig. S3). We thus designate this gene as *P. strobi* geranyl diphosphate synthase (*PstrGGPPS*). When assayed with a range of IPP:DMAPP substrate ratios between 5:1 and 1:5, PstrGGPPS produced GGPP with only a trace of GPP. *PstrGGPPS* would readily take IPP and GPP or farnesyl diphosphate as substrates to make GGPP. We determined through protein cross-linking experiments that PstrGGPPS formed mixtures of multimers of up to eight units. PstrGGPPS was 68% identical to the putative GGPPS from *Tribolium castaneum* and was 77% identical to DponGGPPS.

1. Humble LM, Humphreys N, VanSickle GA (1994) Distribution and hosts of the white pine weevil, *Pissodes strobi* (Peck), in Canada. *White Pine Weevil: Biology, Damage and Management*, eds Alfaro RI, Kiss G, Fraser RG (Canadian Forest Service, Victoria, Canada), pp 68–75.
2. Hibbard BE, Webster FX (1993) Enantiomeric composition of grandisol and grandisal produced by *Pissodes strobi* and *P. nemorensis* and their electroantennogram response to pure enantiomers. *J Chem Ecol* 19(10):2129–2141.
3. Booth DC, et al. (1983) Aggregation pheromone components of two species of *Pissodes* weevils (Coleoptera: Curculionidae): Isolation, identification, and field activity. *J Chem Ecol* 9(1):1–12.
4. Marques FA, et al. (2011) Identification of (*1R,2S*)-grandisal and (*1R,2S*)-grandisol in *Pissodes castaneus.* Male-produced volatiles: Evidence of a sex pheromone. *J Braz Chem Soc* 22(6):1050–1055.
5. Sang-Zi Z, Zheng-Liang Y, Zhen Z, Hui-Fen M (2010) Identification and bioassay of aggregation pheromone components of *Pissodes punctatus* (Coleoptera: Curculionidae). *Acta Entomol. Sin* 53(3):293–297.
6. Tumlinson JH, et al. (1969) Sex pheromones produced by male boll weevil: Isolation, identification, and synthesis. *Science* 166(3908):1010–1012.
7. Mitlin N, Hedin PA (1974) Biosynthesis of grandlure, the pheromone of the boll weevil, *Anthonomus grandis*, from acetate, mevalonate, and glucose. *J Insect Physiol* 20(9): 1825–1831.
8. Thompson AC, Mitlin N (1979) Biosynthesis of the sex pheromone of the male boll weevil from monoterpene precursors. *Insect Biochem* 9(3):293–294.
9. Keeling CI, et al. (2012) Transcriptome and full-length cDNA resources for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major insect pest of pine forests. *Insect Biochem Mol Biol* 42(8):525–536.

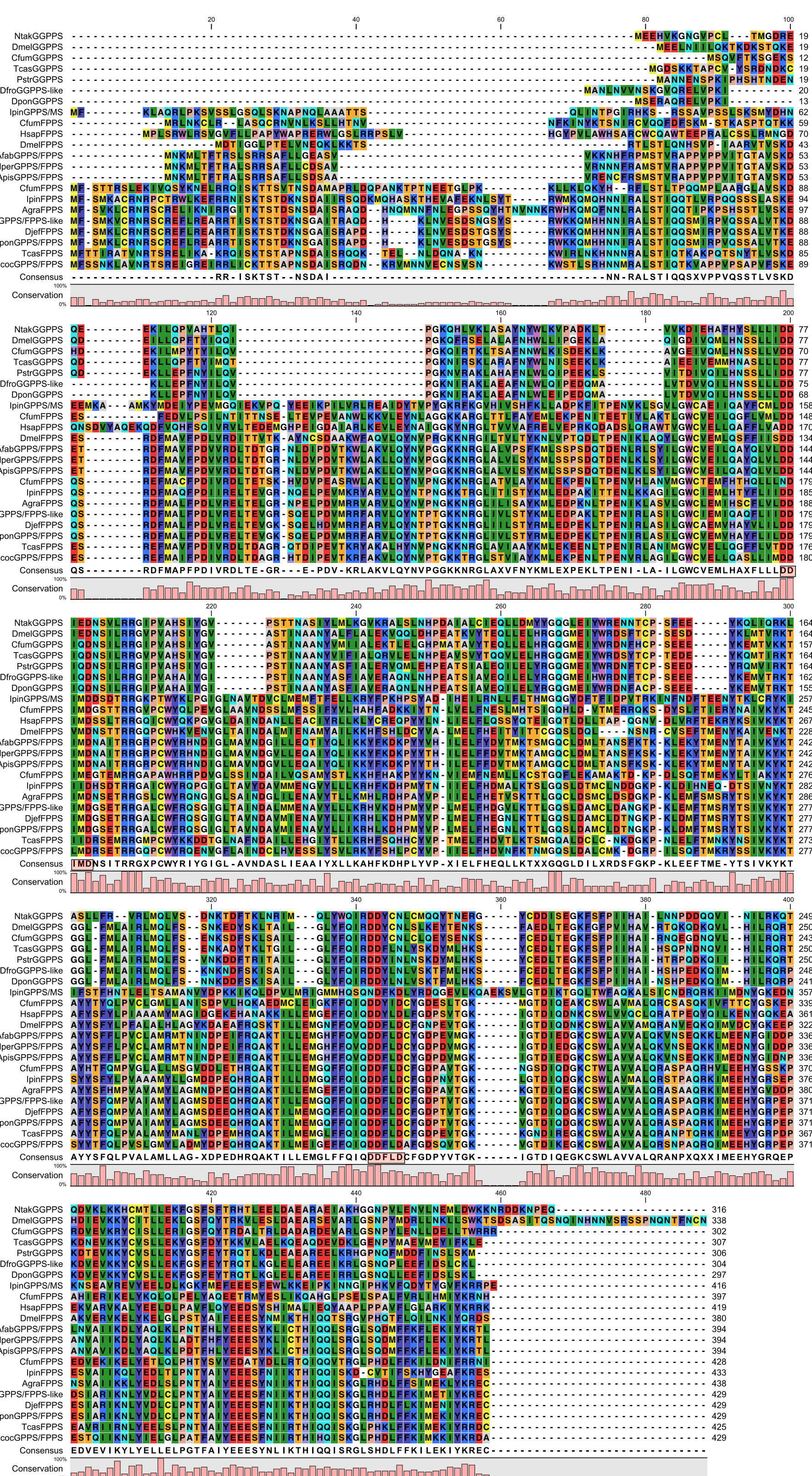

Fig. S1. Alignment of insect IDSs. Protein National Center for Biotechnology Information accession numbers are indicated in Fig. S5. The conserved aspartate-rich motifs are indicated by boxes in the consensus sequence. Species abbreviations: Afab, *Aphis fabae*; Agra, *Anthonomus grandis*; Apis, *Acyrthosiphon pisum*; Cfum, *Choristoneura fumiferana*; Dfro, *Dendroctonus frontalis*; Dmel, *Drosophila melanogaster*; Djef, *Dendroctonus jeffreyi*; Dpon, *Dendroctonus ponderosae*; Hsap, *Homo sapiens*; Ipin, *Ips pini*; Mper, *Myzus persicae*; Ntak, *Nasutitermes takasagoensis*; Pcoc, *Phaedon cochleariae*; Pstr, *P. strobi*. HsapFPPS was used as an out-group.

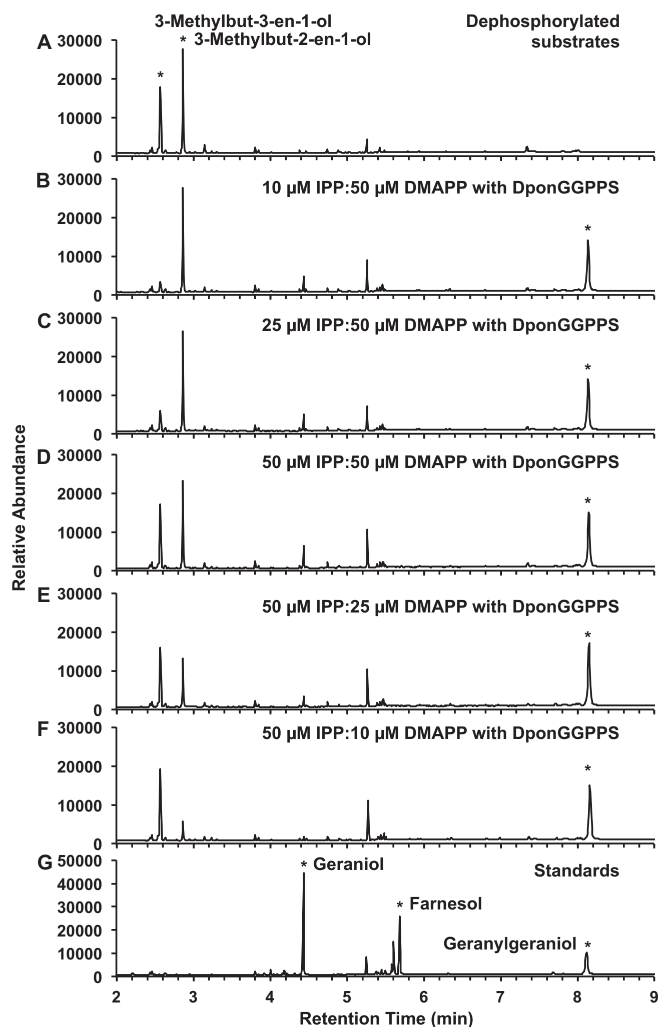

Fig. S2. In vitro assays of recombinant DponGGPPS with varying IPP and DMAPP concentrations. DB-WAX GC/MS traces of the dephosphorylated IDS assay products when the Ni-affinity–purified recombinant DponGGPPS was incubated with DMAPP and IPP varying between 10 and 50 µM. (*A*) Substrates DMAPP and IPP after dephosphorylation. (*B–F*) Dephosphorylated assay products of DponGGPPS incubated with the substrate concentrations indicated. (*G*) Standards of the potential dephosphorylated products. Single-ion monitoring of *m/z* 69, 81, 86, 93, and 204 were summed for the traces. Asterisks indicate product peaks.

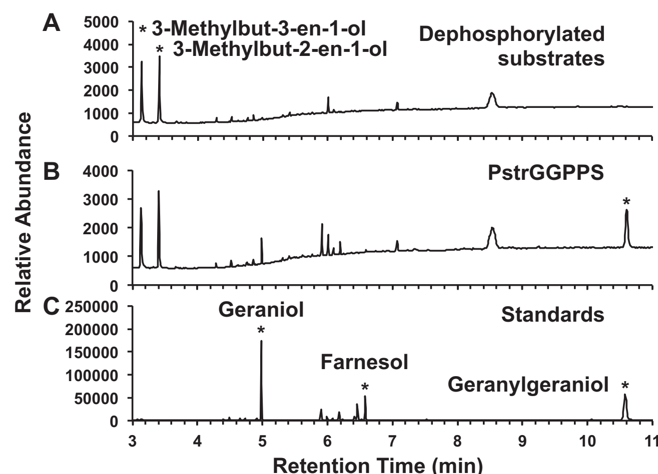

Fig. S3. In vitro assays of recombinant PstrGGPPS. DB-WAX GC/MS traces of the dephosphorylated IDS assay products when the Ni-affinity–purified recombinant PstrGGPPS was incubated with 50 µM DMAPP and 50 µM IPP. (*A*) Substrates DMAPP and IPP after dephosphorylation. (*B*) Dephosphorylated assay products of PstrGGPPS. (*C*) Standards of the potential dephosphorylated products. Total ion current traces are shown for substrates and standards; single-ion monitoring of *m/z* 69, 81, 86, 93, and 204 were summed for PstrGGPPS trace. Asterisks indicate product peaks.

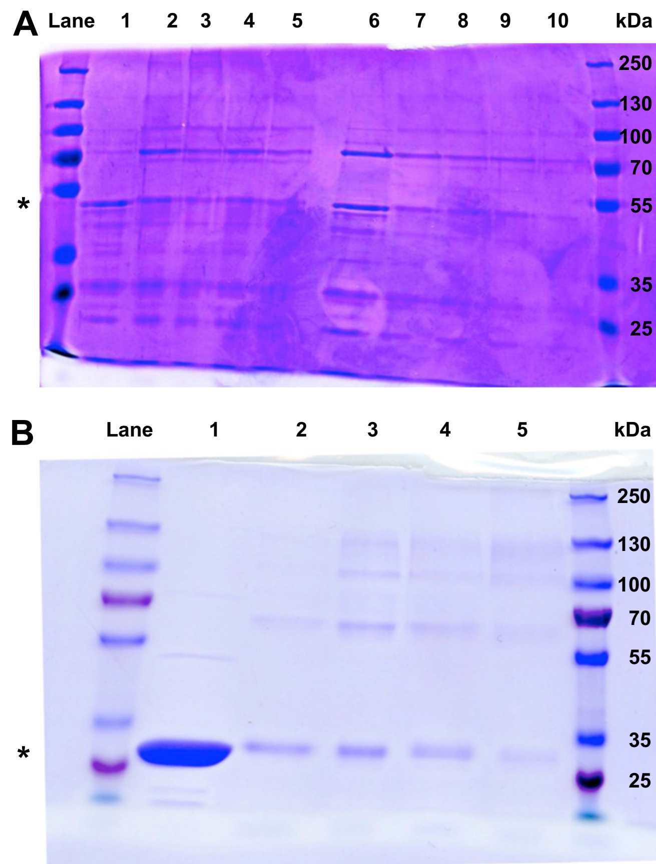

Fig. S4. Cross-linking with dimethyl suberimidate (DMS). Cross-linking experiments were carried out as described in *Materials and Methods* for DMS quantities and reaction times indicated. Asterisks indicate the location of expressed proteins. (*A*) SDS/PAGE of expressed DponGPPS/FPPS before and after reaction with DMS. Lanes 1 and 6, untreated enzyme (no DMS); lanes 2 and 7, enzyme treated with 5 μg DMS for 30 min; lanes 3 and 8, enzyme treated with 5 μg DMS for 1 h; lanes 4 and 9, enzyme treated with 10 μg DMS for 30 min; lanes 5 and 10, enzyme treated with 10 μg DMS for 1 h. After DMS reaction, samples in lanes 1–5 were denatured for 30 min at 55 °C, and samples in lanes 6–10 were denatured for 10 min at 90 °C before SDS/PAGE. (*B*) SDS/PAGE of expressed DponGGPPS before and after cross-linking. Lane 1, untreated enzyme (no DMS); lane 2, enzyme was treated with 5 μg DMS for 30 min; lane 3, enzyme treated with 5 μg DMS for 1 h; lane 4, enzyme treated with 10 μg DMS for 30 min; lane 5, enzyme treated with 10 μg DMS for 1 h. Samples were denatured for 10 min at 90 °C before SDS/PAGE.

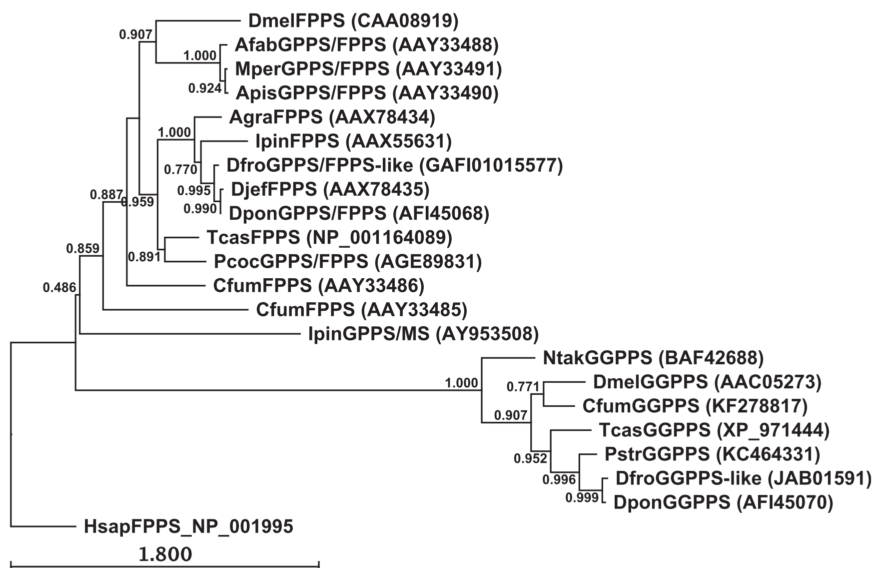

Fig. S5. Maximum-likelihood phylogeny of insect isoprenyl diphosphate synthases. Bootstrap values are indicated at branch points. HsapFPPS was used as an out-group. Abbreviations are the same as Fig. S1. Protein GenBank accession numbers are given in parentheses, except for DfroGPPS/FPPS-like, which is a nucleotide accession number.

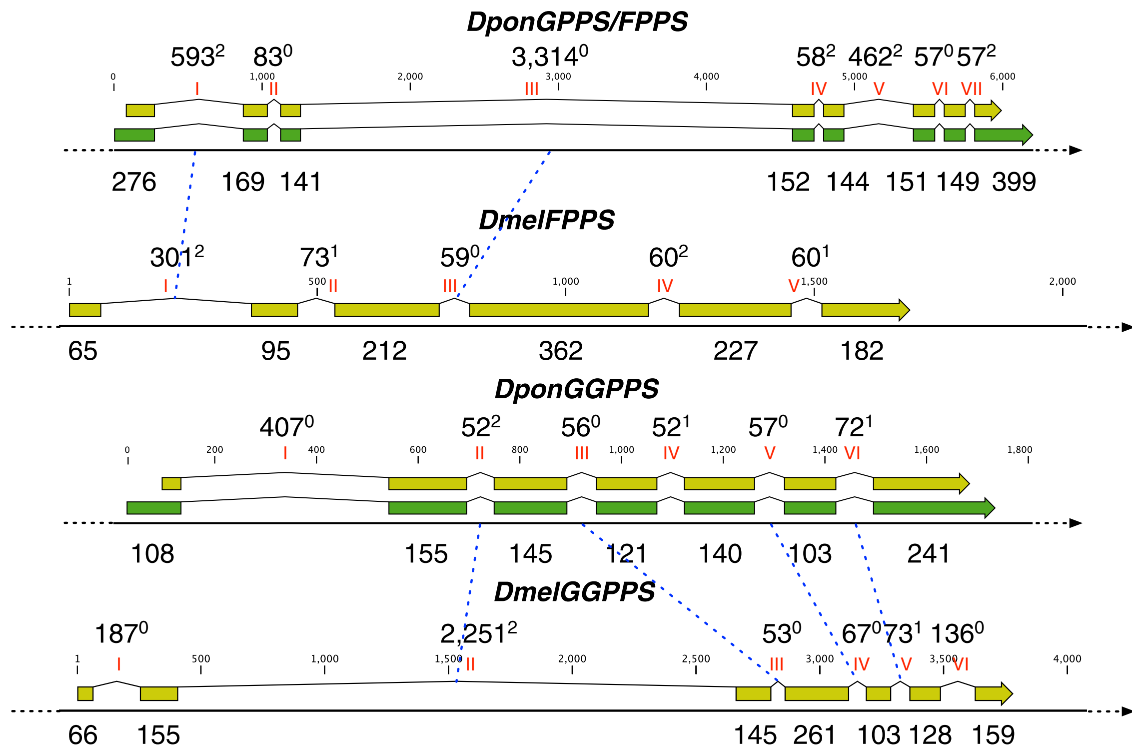

Fig. S6. Gene structure of *DponGPPS/FPPS* and *DponGGPPS*. Consistent gene models in both male and female draft genome assemblies (1) for *DponGPPS/FPPS* and *DponGGPPS* were compared with orthologous genes models in *Drosophila melanogaster* (*DmelFPPS*, FBgn0025373 and *DmelGGPPS*, FBgn0019662). The coding sequence is shown in yellow, and the mRNA sequence is shown in green. Numbers above and below the sequences indicate the intron and exon lengths (in base pairs), respectively. Superscripts after the intron lengths indicate the phase of the intron; red Roman numerals indicate intron numbers; and dashed blue lines indicate conserved splice sites between genes. There were no conserved splice sites between the two gene types.

1. Keeling CI, et al. (2013) Draft genome of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major forest pest. *Genome Biol* 14(3):R27.

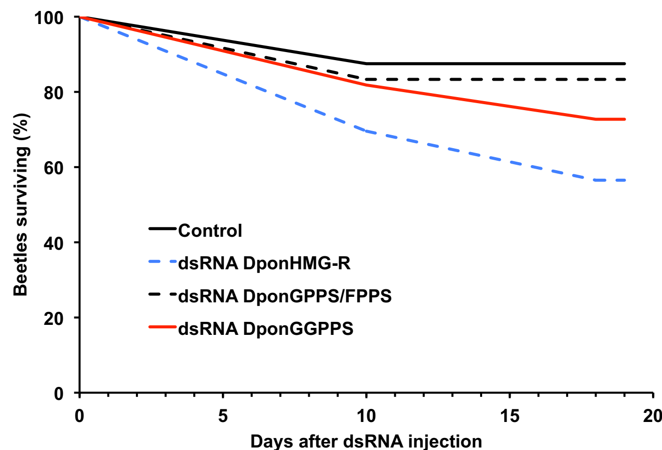

Fig. S7. Survivorship curves of dsRNA-injected beetles. The number of beetles surviving after injection of dsRNA compared with noninjected controls.

Table S1. Primers used for InFusion cloning into pET28b(+), quantitative RT-PCR (qRT-PCR), and dsRNA synthesis

Template	Forward primer, 5′ to 3′	Reverse primer, 5′ to 3′	Amplicon size, bp
	Cloning primers		
DponGGPPS (DPO061_E16)	GGAGATATACCATGTCTGAACGTGCCCAAAGAGAATTG	GTGGTGGTGCTCGAGCAATTTGGACAGCGAATCAATAAACTC	
DponGPPS/FPPS (DPO044_J12)	GGAGATATACCATGTTTTCGATGAAATTGTGTCGCAATCG	GTGGTGGTGCTCGAGACACTCGCGCTTGTAAATCTTCTCC	
PstrGGPPS (PST046_L05)	GGAGATATACCATGGCAAATAATGAAAACTCACCTAAAATACC	GTGGTGGTGCTCGAGCATTTTAGACAAAGAATTTATGAAGTCATCC	
DponHMG-R	CGTGCGTCTTCTGGAATA	GAAAAATGAAGGGTATGCAC	
	qRT-PCR primers for expression analysis		
DponTubulin	GACAATCTGGAGCAGGAAACAAT	TGCCTCTTTTCGCACAACATC	95
DponGPPS/FPPS	TGTGCTGGTTCCGCCAAT	GGTCTTTCAGGTGCCTTTTGACC	102
DponGGPPS	TGAACGTGCCCAAATATAATT	TCGTCTAGTTTATCTCGGATATT	106
	dsRNA primers		
DponHMG-R	TAATACGACTCACTATAGGGAGAAAAAACCAACGTCCATGCTC	TAATACGACTCACTATAGGGAGAGTAGTGGATCGGTTGTGCCT	459*
DponGPPS/FPPS	TAATACGACTCACTATAGGGTTGTCGACCATTCAGCAGAG	TAATACGACTCACTATAGGGGTTGACAGCAGTCAGACCGA	414*
DponGGPPS	TAATACGACTCACTATAGGGAACGTGCCCAAAGAGAATTG	TAATACGACTCACTATAGGGCCGTTTTTCGAGTGACCATT	459*
	qRT-PCR primers for expression analysis of RNAi targeted transcripts		
DponbTubulin	CCAGATTGGAGCTAAGTTTTGG	ACCGGATGCTTCGTTGTAAT	127
DponHMG-R	ACTTTGGGTGAATCATGCG	TGGCACTCCATCATCAGAAG	102
DponGPPS/FPPS	GGGCACCGATATCCAAGAC	TGGTTCGGGCCTTCCATAG	112
DponGGPPS	GGTCACTCGAAAAACGGGC	GAGATAATCGTCCCGAATTTGG	133

Restriction sites and adapter sequences are underlined.

*Excluding T7 adaptor sequences.