Supporting Information

Abdel-latief *et al.* 10.1073/pnas.0801559105

SI Text

Preparation of (±)-[$^{13}C_{18}$]-*Erythro*-12,13-Epoxy-9*Z*-Octadecenoic acid ((±)-[$^{13}C_{18}$]-Vernolic Acid). Ten milliliters of methanol and 1 ml of acetyl chloride were stirred under dry nitrogen at 0°C for 10 min. One hundred milligrams of (0.34 mmol) [$^{13}C_{18}$]-linoleic acid (Campro Scientific) was added and the solution was stirred for 1 h at room temperature. Subsequently, the mixture was diluted with 5 ml of a 5% $NaHCO_3$ solution and extracted twice with 50 ml of hexane. The hexane phase was washed with brine, dried with Na_2SO_4, and evaporated under reduced pressure. The crude ester was dissolved in 8 ml of CH_2Cl_2 and 58.6 mg (0.34 mmol) of *m*-chloroperbenzoic acid was added and stirred overnight. Sixty milligrams (1.5 mmol) of CaO was added to the solution and subsequently stirred for 20 min, filtrated, and evaporated.

The mixture of reaction products was subjected to flash chromatography with 30 g of silica gel (Merck; 0.015–0.04 mm) with gradient elution using a mixture of petroleum benzene and ethyl acetate (200 ml of 95:5, 100 ml of 92.5:7.5, and 200 ml of 90:10). The elution sequence and amount of products was: (*i*) [$^{13}C_{18}$]-methyl linoleate 30 mg, (*ii*) (±)-[$^{13}C_{18}$]-*erythro*-12,13-epoxy-9*Z*-octadecenoic acid methyl ester ((±)-[$^{13}C_{18}$]-vernolic acid methyl ester) 7 mg, (*iii*) (±)-[$^{13}C_{18}$]-*erythro*-9,10-epoxy-12*Z*-octadecenoic acid methyl ester ((±)-[$^{13}C_{18}$]-coronaric acid methyl ester) 7 mg, 4) 9,10,12,13-diepoxystearic acid methyl ester 35 mg. 7 mg (23 μmol) of (±)-[$^{13}C_{18}$]-vernolic acid methyl ester was dissolved in 100 μl of acetonitrile, 0.55 mg (46 μmol) of LiOH was added and stirred for 48 h at room temperature. The solution was neutralized with 1 N HCl, stirred for 1 h, diluted with 10 ml, water, acidified to pH 3 with 1 N HCl, and finally extracted with diethyl ether (3 × 30 ml). The combined ether phase was washed with brine, dried over Na_2SO_4, and evaporated. The crude acid was applied to a LC column containing 5 g of silica gel (Merck, 0.063–0.2 mm) and eluted with 200 ml of petroleum benzene:ethyl acetate:acetic acid 94:5:1 to yield 5 mg of pure (±)-[$^{13}C_{18}$]-*erythro*-12,13-epoxy-9*Z*-octadecenoic acid. GC-MS-analysis of the product was done with the methyl ester by derivatization with trimethylsulfonium hydroxide (Macherey & Nagel). The ^{13}C-labeled compound coeluted with an unlabeled reference chemical (vernolic acid methylester, purity 99%, Sigma–Aldrich). The mass spectrum of the labeled product is shown in Fig. S4.

Fig. S1. Nucleotide and deduced amino acid sequences of the putative *Nasonia vitripennis* epoxide hydrolase gene *Nasvi-EH1*. The sequences are numbered at the left. Stop codon indicated by asterisk. The *Nasvi-EH1* N terminus (EHN) is coded in italics, the abhydrolase_1 region is encoded in bold. Nucleotide sequences (5–3′) of primers that were used in the gene amplification are underlined and written in italics. The primer names are given above the respective sequences.

```
Athro-JHEH          MTILKYSLIGVGLIVGISYLFNQGGNQKAPKLGDQWWGPGKE-QKIVKDVVPFKVNFSKG 59
Nasvi-EH1*          MGVLLWGFLGIVLIGGATIFISPP-VQQPPKLTDVYWGPGDEPKKTDTSVRPFKITFQKE 59
Apime-EH            --MYYFHFI---------YLFSVN-ELNVPTLPETNWGSKKN-EKESIEIRPFKIDVSKS 47
                                             * *    **      *      ***    *

Athro-JHEH          DIEDLKTRLKNTRNLTPALENAGWTYGVDGKFVPKIVDYWLNKYDFKKREQYLNQYDQFV 119
Nasvi-EH1*          MLDDLRNRLKNTRKMQPPLENVGWTYGLSGDFVPVIVDHWLNKYDFKKREAHLNQYPQFI 119
Apime-EH            VLDDLKYRLAHRRTFKKPLENVGWTYGISTTYLNTVLDYWRDKYNWTERQALLNKYPQFM 107
                       **  **   *     *** *****          * *  **    *   ** * **

Athro-JHEH          TNIQGLNIHFLHVR---PKNSGGKRVLPLLIQHGWPGSVVEFYKIIPMLTTPRDDYDFVF 176
Nasvi-EH1*          TNVQGLDIHFIHVKPQLPKNSK-IQVLPLYLQHGWPGSIVEFQKIIPMLTTPRSDRNFVF 178
Apime-EH            TTIQGLDIHFYHVKPNLPNNKN-LKVLPLLMLHGWPGSVVEFQKIIPMLTKPWPNQNFVF 166
                    *  *** *** **    * *     ****   ****** *** ******* *     ***

Athro-JHEH          EVIAPSLPGFGFPSGAVRPGLGAAQIAVVLKNLMLRLGFNKFYTQGGDWGAIITAHMAVL 236
Nasvi-EH1*          EVVAPSLPGFGYSQAASKAGLGAVQMAHLMKNLMLRLGFDKFYTQGGDWGAVIGANMASM 238
Apime-EH            EVIVPSLPGYGFSEGAVRPGMANAQIAVIFKNLMQRLGFEKFYVQGGDWGSVIASDMAVL 226
                    **  ***** *    *   *    * *   **** **** *** ******  *   **

Athro-JHEH          FPEHVLGIHSNMCAVLQPQTFFTTYLYSYWPSLLVPDEDYHLMYPLSKKWSRTIEETGYF 296
Nasvi-EH1*          YPQHVLGMHSNMCLVVRPWTWLKIAAYSLMPSLLPEDE-RQLMFPLSTKLALGIEETGYL 297
Apime-EH            FPEKIIGLHNNMCTSLNLSNLFWLFVGTYFPSLIGANEHYSKFFPVSEILSFLIEESGYF 286
                     *    * * ***                 ***    *      * *      *** **

Athro-JHEH          HIQATKPDTLGAALADSPAGLAAWILEKFSTGTNPELRFKEDGGLFDIHSPDELLDNVML 356
Nasvi-EH1*          HLQATKPDTIGIGVSDSPAGLAAYILEKMAYCTKPDNKFTDDGNLLEKFTMDELIDNLMM 357
Apime-EH            HIQATKPDTIGAALTASPDALAAYILEKFSVWTNKTYKKQDDGGITEKFVLDELLDNIMI 346
                    * ******* *     **  *** ****    *        **        *** ** *

Athro-JHEH          YWMPNSMTTAIRIYAETFSAANRALRMDYVPIEVPSACAQFPHEISYQPPSLLSARYKKL 416
Nasvi-EH1*          YWAPNKASSSFRIYAESFNKQTFGYKMDNVPVTVPSACAQFPYEIIFQSANFLRDRFVNL 417
Apime-EH            YWITNSITTSVRLYAENYTSSYRSLKIDQLPIKVFTACAVFPNEILVLPESLLKQKYPNI 406
                    **  *      * ***           *  *  *  *** ** **       *

Athro-JHEH          IRARKMPKGGHFAAFEQPQLLADEVWTSIGIMEADKKQRE-DKLKKSA- 463
Nasvi-EH1*          LRVTKMPRGGHFAVLEEPQLLADDIWASVQAFRDYYAAQEQERLEKQKS 466
Apime-EH            IQYNIISRGGHFAAFEEPRLLADDIFSFVKKIENLTSKSS--------- 446
                           *****  * * ****
```

Fig. S2. Amino acid sequence alignment of *Nasvi-EH1* from *Nasonia vitripennis* with epoxide hydrolase genes from *Apis mellifera* (*Apime-EH*) and *Athalia rosae* (*Athro-JHEH*), using CLUSTAL W analysis. Name of gene sequences are given to the left and amino acids are numbered to the right. Identical amino acid positions are indicated by asterisks.

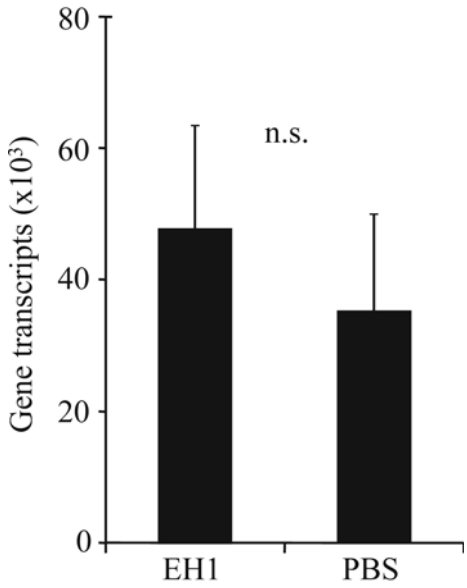

Fig. S3. Effect of the injection of dsRNA corresponding to *Nasvi-EH1* (EH1) on the quantity of gene transcripts of the nontarget gene *Nasvi-EH2* in male rectal vesicles as estimated by quantitative real time RT-PCR analysis. Control males were injected with 1× DEPC-PBS buffer. No significant differences were found (*t* test for independent samples).

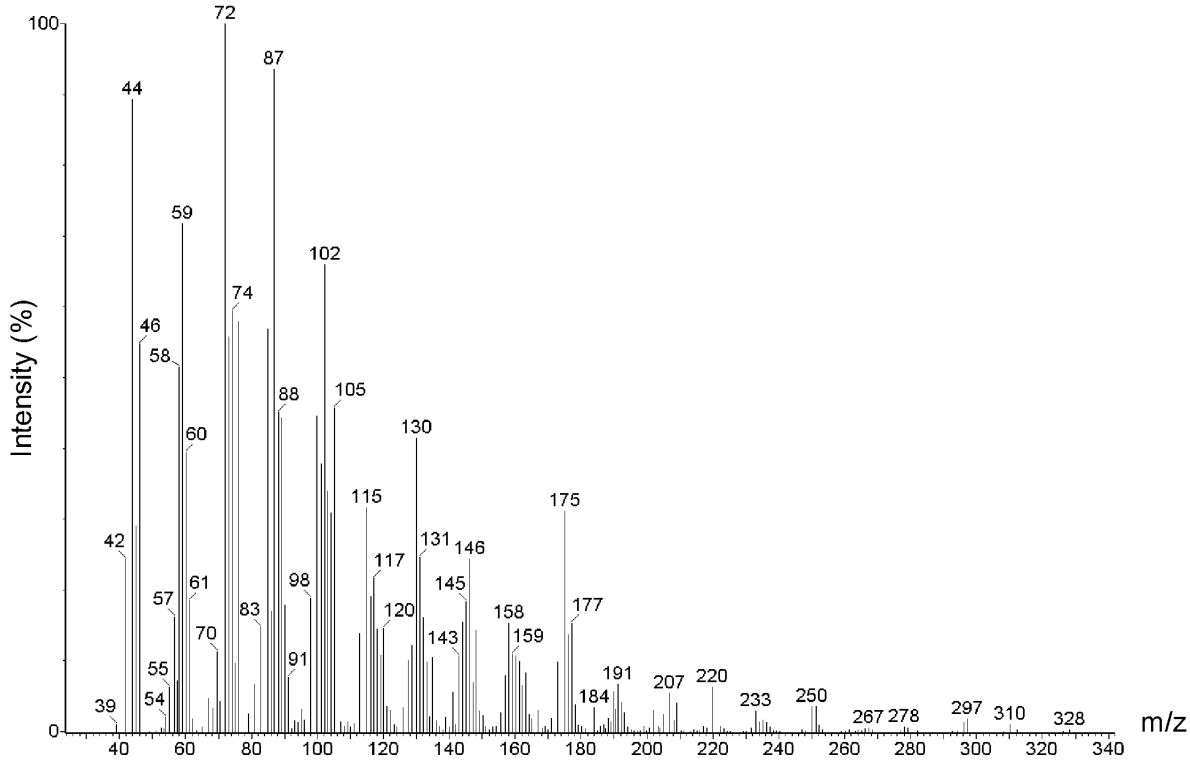

Fig. S4. Mass spectrum (EI, 70 eV) of synthetic (±)-[$^{13}C_{18}$]-vernolic acid methylester.

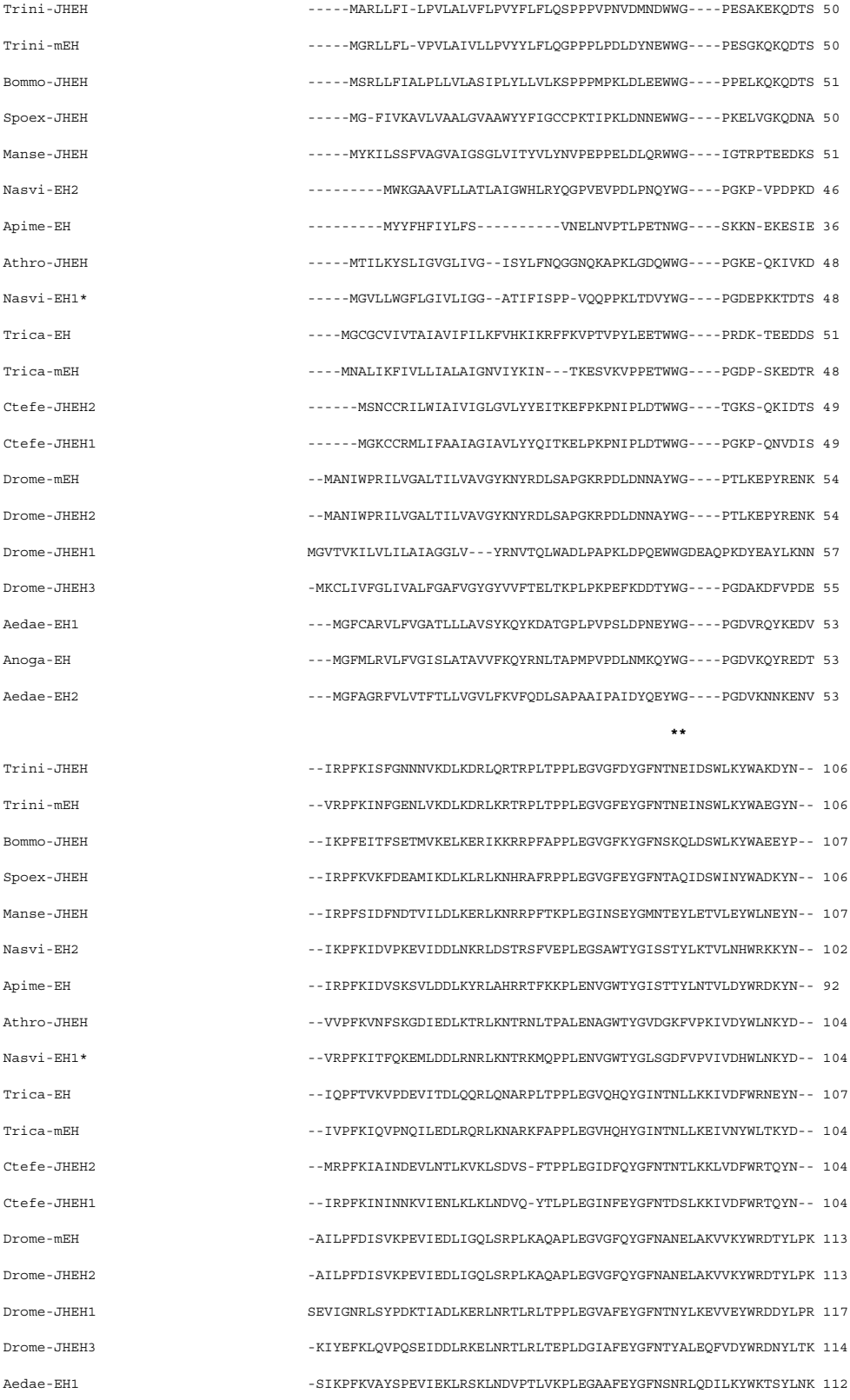

Fig. S5. CLUSTAL W amino acid sequences alignment of the epoxide hydrolase genes of *N. vitripennis* (*Nasvi-EH1**), (*Nasvi-EH2* = GenBank accession no. XP_001602953), *Apis mellifera* (*Apime-EH*), *Athalia rosae* (*Athro-JHEH*), *Manduca sexta (Manse-JHEH*), *Bombyx mori* (*Bommo-JHEH*), *Spodoptera exigua* (*Spoex-JHEH*), *Trichoplusia ni* (*Trini-mEH*, and *EH*), *Drosophila melanogaster* (*Drome-JHEH1*, *2*, and *3* and *mEH*), *Aedes aegypti* (*Aedae-JHEH1*, and *2*), *Ctenocephalides felis* (*Canfe-EH1*, and *2*), and *Tribolium castaneum* (*Trica-EH*, and *mEH*). Gene names are given to the left, Identical amino acid positions are indicated by asterisks.

```
Anoga-EH                    -SIKPFKVSYDAAVIEKLRGKLTDVPKLTPPLDGVAFEYGFNTNRLQDILKYWRTTYLDK 112
Aedae-EH2                   -EVKSFELNYGEDVIGKLRNRLDDVPKFAEPLEGTAFEYGFNSKKLGEILKYWRSDYLER 112
                                         *           *     **          *   *

Trini-JHEH                  FKERETFLNQFPQFKTNIQGLDIHFIRVTPKVPQG---VEVVPLLLLHGWPGSVREFYEA 163
Trini-mEH                   FKERETFLNQFPQFKTNIQGLDIHFIKVTPKVPAG---VQVVPMLLLHGWPGSVREFYES 163
Bommo-JHEH                  FAERQKFLNQYPHFKTNIQGLNIHFMRITPKVPKD---VEIVPLLLLHGWPGSVREFYEA 164
Spoex-JHEH                  FSEREAFLNKFPHFKTNIQGLDIHFIRVKPEVPKN---VEVLPLLMIHGWPGSVREFYEA 163
Manse-JHEH                  FKKRAELLNKFPHYKTRIQGLDLHFIRVKPEIKEG---VQVLPLLMMHGWPSSSKEFDKV 164
Nasvi-EH2                   WSQRQALLNKYPQFKTKIQGLDIHFYHVKPQVPKDRK-VRVLPLLMLHGWPGSIVEFQKI 161
Apime-EH                    WTERQALLNKYPQFMTTIQGLDIHFYHVKPNLPNNKN-LKVLPLLMLHGWPGSVVEFQKI 151
Athro-JHEH                  FKKREQYLNQYDQFVTNIQGLNIHFLHVR---PKNSGGKRVLPLLIQHGWPGSVVEFYKI 161
Nasvi-EH1*                  FKKREAHLNQYPQFITNVQGLDIHFIHVKPQLPKNSK-IQVLPLYLQHGWPGSIVEFQKI 163
Trica-EH                    WKERETFLNKLPQFTVSVQGLRLHYIHVKP--EKT-DGLKVLPLLMLHGWPGSVREFYEI 164
Trica-mEH                   WRERENFLNQYPQFKTNIQGLDVHFIHVKP--KNVPSGVKTQPLLLVHGWPGSVREFYEI 162
Ctefe-JHEH2                 WREREALLNKYPHFKTNIQGLDIHYVHIKPQVSKN---IHVLPMIMVHGWPGSFVEFYKI 161
Ctefe-JHEH1                 WREREALLNKYPHFKTNIQGLDIHYVHIKPQVSKN---IEVLPLVMIHGWPGSFVEFYKI 161
Drome-mEH                   WSEREEYLKKLDHYQTEIQGLKIHFIHAKPSQVKGQKPKKVLPLLLMHGWPGTVREFYDF 173
Drome-JHEH2                 WSEREEYLKKLDHYQTEIQGLKIHFIHAKPSQVKGQKPKKVLPLLLMHGWPGTVREFYDF 173
Drome-JHEH1                 WREREVFLWQFNHFTTDIQGLRTHFLHLMVYDDNKVG-KKHYPVLLLHGWPGSVREFYDF 176
Drome-JHEH3                 WDERQELFNSFKQYKTEIQGLNIHYIHEKVSEEAKEK-KHVYPLLLLHGWPGSVREFFDF 173
Aedae-EH1                   WTEREAFLNKFPHFKTQIQGLNIHFIHVKPKVPAGTK---VLPLLLLHGWPGSVREFYDV 169
Anoga-EH                    WSEREKFLNKFPHFQTQIQGLNIHYIHVKPKVPAGTK---VLPLLLLHGWPGSVREFYDI 169
Aedae-EH2                   WDERQKYLNRFPQFKTQIQGLDIHFLRVKPEVQNPKR---IVPLLMLHGWPGSVQKFYEI 169
                              *             ***  *                 *    ****     *

Trini-JHEH                  IPLLTAV--SKDRDFAFEVIVPSLPGYGFSDPAVRPGLGAPQIGVVMKNLMSRLGYKQFY 221
Trini-mEH                   IPLLTAV--SKDRDFALEVIVPSLPGYGFSDGAVRPGMGAPHIGIIMRNLMNRLGYKRYF 221
Bommo-JHEH                  IPHLTAV--SRDRNFALEIIAPSLPGYGFSDAAVRPGLAAAEVAVIFKNLMARLGYKQYY 222
Spoex-JHEH                  IPLLTRQ--TAGYNFVFELIIPSIPGYGFSDPAVRPGLGMPQVAVIFRNLMNRLGHKKYY 221
Manse-JHEH                  IPILTTP--KHEYNIVFEVVAVDLPGYGFSEGTNKPGLNPVQIGVMMRNLMLRLGFEKFY 222
Nasvi-EH2                   IPMLTTA--KPDENFVFELIIPSLPGYGFSQAAARPGLGPAQMAVVFKNLMQRLGFEQFY 219
Apime-EH                    IPMLTKP--WPNQNFVFEVIVPSLPGYGFSEGAVRPGMANAQIAVIFKNLMQRLGFEKFY 209
Athro-JHEH                  IPMLTTP--RDDYDFVFEVIAPSLPGFGFPSGAVRPGLGAAQIAVVLKNLMLRLGFNKFY 219
Nasvi-EH1*                  IPMLTTP--RSDRNFVFEVVAPSLPGFGYSQAASKAGLGAVQMAHLMKNLMLRLGFDKFY 221
Trica-EH                    IPLLTKP--QPGRDFVFEVIAPSLPGYGFSEAAVRPGLGAIQMAVLFKNFMNRLGFQKYY 222
Trica-mEH                   IPLLTTV--QKDKKFVFEVIIPSLPGYGFSQAAVRPGLGAHQTAVIFKNLMKRLGFDRYY 220
Ctefe-JHEH2                 IPMLTTP--RTDYNFVFELILPSIPGYGFSQAAAKPGLGATQIAVIMHNLMDRIGFKKYY 219
Ctefe-JHEH1                 IPMLTTP--RAGYNFVFELILPSIPGYGFSQAAAKPGLGSTQVAVIMRNLMERIGFKKYY 219
Drome-mEH                   IPLLTTP--SDKSDYVFEVIAPSLPGYGWSQGSSKTGFGVAQVAVVMRNLMLRVGFDKFL 231
Drome-JHEH2                 IPLLTTP--SDKSDYVFEVIAPSLPGYGWSQGSSKTGFGVAQVAVVMRNLMLRVGFDKFL 231
```

Fig. S5. (*Continued*)

```
Drome-JHEH1                    IHLLHQTNLDNNNKYIFNVVVPSLPGYGWSQGTSRKGLGPAQVAVMMRNLMLRLGYNKFF 236
Drome-JHEH3                    IPMLTKH--SNITDYAFEVVAPSLVGYGWSDAATRPGFNAAEMATVMRNLMLRLGHKKFF 231
Aedae-EH1                      IPKLTTK--SDDKDFVFEVVVPSLPGYGWSEGASKQGLSPSRIAVIMKNLMDRVGHKKFY 227
Anoga-EH                       IPLLTTS--SKDKDFVFEVIVPSLPGYGWSQGSAKKGLSPSEVAIVMKNLMSRVGFEQFY 227
Aedae-EH2                      IPRLVAR--SDDKEYVFEVIVPSLPGYGFSQGASKQGLSPAKIAVIMRNLMARLGFKKYY 227
                               *  *                    * *       *          * * * *

Trini-JHEH                     LQGGDWGALIGNCIVTLFPKDILGYHTNMPIVMSAKSTLFELLGSVFPSLILEDMSTYER 281
Trini-mEH                      VQGGDWGSVIGTSLATFFPEEVLGYHANIGLVLSTKAMVWQAIGSVWPSLIMDDLSLVDR 281
Bommo-JHEH                     VQGGDWGALIGSAMATSFPKEIIGFHSYMALTLSPAATFLEFVGALFPSLIVEP-ELANR 281
Spoex-JHEH                     VQGGDWGAGIVSTMSTLFPEDILGHHSNMLFTQHTCATVRTLVGAFLPSLIIEE-HLASR 280
Manse-JHEH                     IQAGDWGSQCATHMATLFPDQVLGLHTNMPLSSRPLSTVKLFIGALFPSLIVDA-KYMDR 281
Nasvi-EH2                      TQGGDWGSLITANMAVLYPKKVIGTHLNMCFIESHKAHFLSLVGAYIPSLVVDS-EHYSK 278
Apime-EH                       VQGGDWGSVIASDMAVLFPEKIIGLHNNMCTSLNLSNLFWLFVGTYFPSLIGAN-EHYSK 268
Athro-JHEH                     TQGGDWGAIITAHMAVLFPEHVLGIHSNMCAVLQPQTFFTTYLYSYWPSLLVPD-EDYHL 278
Nasvi-EH1*                     TQGGDWGAVIGANMASMYPQHVLGMHSNMCLVVRPWTWLKIAAYSLMPSLLPED-E-RQL 279
Trica-EH                       IQGGDWGAVITQHMATLYPEKILGLHSNMCFINTLKSQVKLFLFSFYPTLIVKQ-EHVNK 281
Trica-mEH                      VQGGDWGSAVTSAMALYYPDRVKGIHLNMCVSNSYLAKLKLLAGSVWPSLVVEE-KQKHK 279
Ctefe-JHEH2                    VQGGDWGSRIVSAMSTLFPENVLGHHSNLCFLNTLSSNIKSFVGSLFPEWFAGK-QNVHK 278
Ctefe-JHEH1                    VQGGDWGSMIISAMSTLFPENVLGQHSNMCFVNTPSSNIKAIIGSFFPESFAGT-GNAHK 278
Drome-mEH                      VQGGDWGSIIGSNVASLFPENVLGYHSNMCGNNSPMGQLKMVLASFFPSWFVDSE-YADF 290
Drome-JHEH2                    VQGGDWGSIIGSNVASLFPENVLGYHSNMCGNNSPMGQLKMVLASFFPSWFVDSE-YADF 290
Drome-JHEH1                    IQGGDWGSIIGSNIATLYPENVLGYHSNMCNNLSPKSLAKGLVAEFWPSLFVPSG-FEDF 295
Drome-JHEH3                    IQGGDWGSIIGSNLATLYPENVIGYHSNMCVLHTPLAILKGIYGSFFPEKYLPSRFFVDH 291
Aedae-EH1                      VQGGDWGSLIANLISTLYQDNVLGVHMNMCGANGLQAILKSIIASFRPSMFIEEK-YVDY 286
Anoga-EH                       IQGGDWGSLIGNYIATYFQSNVLGVHLNMCSIMTPLSYPKMFLAALKPSLFIDEQ-YTDF 286
Aedae-EH2                      VHGGDWGSVIGNLMATFFQDEVLGVHLTMCMNTAPIGTLKNILGAVAPSLVVESQ-YKDF 286
                                  ****               * *                   *

Trini-JHEH                     LYPLSTRFANLLRETGYMHIQSTKPDTVGVALSDSPAGLLAYILEKFATWTRPDLMSKPN 341
Trini-mEH                      IYPLSKTLSFQVRESGYLHIQASKPDTVGVALTDSPAGLLAYIVEKFSIWTRPELTSKPN 341
Bommo-JHEH                     LYPLSEKYSTLLEELGYMHIQATKPDTVGIGLTDSPAGLLAYILEKFSTWTNPDLRSKED 341
Spoex-JHEH                     IYPLSSFFAYVLEEFGYMHIQATKPDTVGVPLSDSPAGLLAYILEKFSTWTKKEYKFKAG 340
Manse-JHEH                     IYPLKNLFSYILRETGYFHIQATKPDTIGVALTDSPAGLAGYLIEKMAICSNRDQLDTPH 341
Nasvi-EH2                      MYPLSYHFGRLIEETGYLHIQATKPETVGAALTDSPAGLAAYILEKFSTWTNPDYRFRDD 338
Apime-EH                       FFPVSEILSFLIEESGYFHIQATKPDTIGAALTASPDALAAYILEKFSVWTNKTYKKQDD 328
Athro-JHEH                     MYPLSKKWSRTIEETGYFHIQATKPDTLGAALADSPAGLAAWILEKFSTGTNPELRFKED 338
Nasvi-EH1*                     MFPLSTKLALGIEETGYLHLQATKPDTIGIGVSDSPAGLAAYILEKMAYCTKPDNKFTDD 339
Trica-EH                       VYPLSSKFAKTLLETGYMHLQATKPDTVGVALNDSPIGLAAYILEKFITWTNPAWRDLED 341
Trica-mEH                      IYPLSNYFSNALLEFGYMHLQATKPDTIGVALNDSPVGLAAYIIEKFTTWTNPEWKNRAD 339
Ctefe-JHEH2                    IYPLSEHFFTLLEESGYFHIQATKPDTVGVALRDSPAGLAAYILEKFSTGTNKAWRSAKD 338
```

Fig. S5. (*Continued*)

```
Ctefe-JHEH1                    MYPMSEHFFTLLEEMGYLHLQATKPDTVGVALRDSPAGLAAYILEKFSTWTNRSWRSVKD 338
Drome-mEH                      YKGLGHLFSTIMEEMGYAHIQASKPDTIGNALIDNPTGLASYILEKFSTWTNTAFRSLPD 350
Drome-JHEH2                    YKGLGHLFSTIMEEMGYAHIQASKPDTIGNALIDNPTGLASYILEKFSTWTNTAFRSLPD 350
Drome-JHEH1                    FFPKSNEMRYLMEESGYFHIQATKPDTIGAALTDNPVGLAAYILEKFSTWTNPSYRSLPD 355
Drome-JHEH3                    HFPVWEKWLELLEESGYFHIQATKPDTIGAALTSSPVGLASYILEKFQTCTNPGLK-QDF 350
Aedae-EH1                      YYPSGPKFMQLLVESGYMHLQATKPDTIGTALVGNPVGLAAYIIEKFSTWTNPSYRQLAD 346
Anoga-EH                       YFPLSAKFANIIEETGYMHIQSTKPDTIGTVLQGNPVGLAAYILEKFSTWTNPAYRNLAD 346
Aedae-EH2                      YYPYLDRLKLLIAETGYMHIQATKPDTIGAVLTGNPVGLATYILEKFSTWTNPQYRSLAD 346
                                           * ** * *  ** * *      *  *     **

Trini-JHEH                     GGLDYRFTRDQLIDNLMMYWTNRAITPAMRLYAENFNKRTVEMKLDEIPTPVPTWGLQTK 401
Trini-mEH                      GGLDFRFTKDQLIDNLMMYWTSKSITTSVRLYAESFNIKVLGYQLDDIPTPVPSWFIQGK 401
Bommo-JHEH                     GGLSYRWTKDQLIDNLMLYWSTKSIVTSMRLYAESFSSRHFDLKLDEIQVQVPTWVLQAK 401
Spoex-JHEH                     GGLSNRFTKDQLIDNLMIYWSTNSITTSMRFYAENFSHKIMSLNLDQIPTDVPTWGLQAK 400
Manse-JHEH                     GGLEN-LNLDDVLDTVTINWINNCIVTSTRLYAEGFSWPEV-LIVHRIPSMVPTAGINFK 399
Nasvi-EH2                      GGLLEKFTMDELLDNLMVYWVTNSITTSQRLYAECFSKANRELGVDKMPIFVPTACANFP 398
Apime-EH                       GGITEKFVLDELLDNIMIYWITNSITTSVRLYAENYTSSYRSLKIDQLPIKVFTACAVFP 388
Athro-JHEH                     GGLFDIHSPDELLDNVMLYWMPNSMTTAIRIYAETFSAANRALRMDYVPIEVPSACAQFP 398
Nasvi-EH1*                     GNLLEKFTMDELIDNLMMYWAPNKASSSFRIYAESFNKQTFGYKMDNVPVTVPSACAQFP 399
Trica-EH                       GGLTKKYSYTSLLDNVMIYWVTNSITTSMRLYAETFNKNQTKLGVAKIPTTVPTACARFS 401
Trica-mEH                      GGLLERFTYDKILDNIMIYWVTNSITTSMRIYAESINK-ESNVFDDRAVITVPSACALFD 398
Ctefe-JHEH2                    GNLQSKFTFTELLDNVMIYYVTGSITTSMRIYAESYSWDHLSLNMDRVPTIVPTACAKFP 398
Ctefe-JHEH1                    GNLLLKYNIPELLDNVMIYYVTDSITTSMRLYAESFTKAHLALNLDRVRNHVPAACAKFP 398
Drome-mEH                      GGLTKRFTYDQLLDNVMIYYVTNSITTSMRLYSESMVASQFALAVDSVPIKAKAGCTRFA 410
Drome-JHEH2                    GGLTKRFTYDQLLDNVMIYYVTNSITTSMRLYSESMVASQFALAVDSVPIKAKAGCTRFA 410
Drome-JHEH1                    GGLTKRYKMDALLDNLMIYYLTNSITTSQRLYAEQYAQAQRDLHLDRVPTRVPTGCARFK 415
Drome-JHEH3                    GAIVTVFGLEAVLDNLMVYYLTNSATTAARFYLENVSKTYRDLQLDRVQSPVPMGCARFR 410
Aedae-EH1                      GGLEKYFTLDSLLDNIMIYYLTDSITTSQRIYYEAFSASEFALAIDRIPIRVPTACAKFK 406
Anoga-EH                       GGLEKYFSLDALLDNVMIYYLTDSITTSQRIYAEAFASDELKKEIDRIPTAVPAACAKFR 406
Aedae-EH2                      GGLEKYFTLDTLLDNIMIYYLSDSITTSQRLYAETFNIKELSRELDRIPTHVPAACAKFR 406
                               *           *              * * *

Trini-JHEH                     YELGYQPKYILKIKFPNLVGTTVLQEGGHFIAFELPEVFTNDVIKAVTEFRKLQKKNVKT 461
Trini-mEH                      YEIAYQPPFVLKLKYPNIVGVTVLDDGGHFFAFELPEVFSKDVLKAVTAFRKLQKNNEKT 461
Bommo-JHEH                     HELAYQPPCILKLKYTKLVNASVIEDGGHFLAFELPEIFAKDVLKAIGEFRKLK--NVKT 459
Spoex-JHEH                     EELFYQPPAVLSAKFKNLIGTTVLDDGGHFLAFELPQVLSADVFKAVKAFKEWHQANKKT 460
Manse-JHEH                     YEVLYQPDWILRDKFPNLVRSTVLDFGGHFAALHTPQALADDIFASAVQFLKFHDRKRNQ 459
Nasvi-EH2                      HELAYRSETILKERFTNLVQFTHPPRGGHFAAFEEPELLANDVWSFVHKLEQRLSDEKRQ 458
Apime-EH                       NEILVLPESLLKQKYPNIIQYNIISRGGHFAAFEEPRLLADDIFSFVKKIENLTSKSS-- 446
Athro-JHEH                     HEISYQPPSLLSARYKKLIRARKMPKGGHFAAFEQPQLLADEVWTSIGIMEADKKQRE-D 457
Nasvi-EH1*                     YEIIFQSANFLRDRFVNLLRVTKMPRGGHFAVLEEPQLLADDIWASVQAFRDYYAAQEQE 459
```

Fig. S5. (*Continued*)

```
Trica-EH                    YDLAYSPTAVLKEKYKNIVYESDYD-AGHFAAFEEPELLAKDIYKGVEKIEQFHKNNERL 460
Trica-mEH                   HEIIYQPVSIFKDRFAKLVQVNEYD-GGHFAAFEVPESLAKDIWLAVSKFEEPPKPNK-- 455
Ctefe-JHEH2                 HEIAYKTDFQLAEKYKTLLQSTIMPRGGHFAALEEPLLLAEDIFSAVKKFIDHHSKKDSK 458
Ctefe-JHEH1                 NELAYVTDCQLAEKYKTLLQSNDMPSGGHFAAFEEPGLLAEDIFTAVKKFKEFYSKK-AE 457
Drome-mEH                   HEITHFSDSVLANKFPNLVHSTHHRDGGHFPAFELPQQLYDDFVSFVQKANFS------- 463
Drome-JHEH2                 HEITHFSDSVLANKFPNLVHSTHHRDGGHFPAFELPQQLYDDFVSFVQKANFS------- 463
Drome-JHEH1                 SDIMQFLDVQLKDKYTNLVHSTYHKKGGHFAALEVPKVLYKDFIDFVETVERKFKIKTL- 474
Drome-JHEH3                 FDLASVTDWQLRDKFPNLTHSMYFQQGSHFAALEMPAMLFNDFTAFVGKIGLHGEKRK-- 468
Aedae-EH1                   YELMHTIDWALKDHFTNLVQSNHFDDGGHFAAMQLPDVLYKDFVEFVKKVEK-------- 458
Anoga-EH                    HELFQQIDWVLKDHFTNLVQSNHFEDGGHFAAMQLPEVLYKDFVAFIASIQK-------- 458
Aedae-EH2                   YELFQQTDWALRDHFRNLIQSKHYDDGGHFVAMQLPDVLYEDIVEFVNRLYRREDQ---- 462

                                                    **      *

Trini-JHEH                  DL---------- 463
Trini-mEH                   DL---------- 463
Bommo-JHEH                  EL---------- 461
Spoex-JHEH                  EL---------- 462
Manse-JHEH                  KSS--------- 462
Nasvi-EH2                   KQEAKKEKAKKA 470
Apime-EH                    ------------
Athro-JHEH                  KLKKSA------ 463
Nasvi-EH1*                  RLEKQKS----- 466
Trica-EH                    S----------- 461
Trica-mEH                   ------------
Ctefe-JHEH2                 NQENRDL----- 465
Ctefe-JHEH1                 SQKKADL----- 464
Drome-mEH                   ------------
Drome-JHEH2                 ------------
Drome-JHEH1                 ------------
Drome-JHEH3                 ------------
Aedae-EH1                   ------------
Anoga-EH                    ------------
Aedae-EH2                   ------------
```

Fig. S5. (*Continued*)

(A)

```
Nasvi-EH1       MWKGAAVFLLATLAIGWHLRYQGPVEVP-DLPNQYWGPG-KPVPDPKDIKPFKIDVPKEV 58
Nasvi-EH2       MGVLLWGFLGIVLIGGATIFISPPVQQPPKLTDVYWGPGDEPKKTDTSVRPFKITFQKEM 60
                *      **   *  *       **  *  *   *****  *        ****   **

Nasvi-EH1       IDDLNKRLDSTRSFVEPLEGSAWTYGISSTYLKTVLNHWRKKYNWSQRQALLNKYPQFKT 118
Nasvi-EH2       LDDLRNRLKNTRKMQPPLENVGWTYGLSGDFVPVIVDHWLNKYDFKKREAHLNQYPQFIT 120
                 ***  **  **    ***   **** *         **  **    * * ** **** *

Nasvi-EH1       KIQGLDIHFYHVKPQVPKDRKVRVLPLLMLHGWPGSIVEFQKIIPMLTTAKPDENFVFEL 178
Nasvi-EH2       NVQGLDIHFIHVKPQLPKNSKIQVLPLYLQHGWPGSIVEFQKIIPMLTTPRSDRNFVFEV 180
                  ******* ***** **  *  ****   ******************   * *****

Nasvi-EH1       IIPSLPGYGFSQAAARPGLGPAQMAVVFKNLMQRLGFEQFYTQGGDWGSLITANMAVLYP 238
Nasvi-EH2       VAPSLPGFGYSQAASKAGLGAVQMAHLMKNLMLRLGFDKFYTQGGDWGAVIGANMASMYP 240
                  ***** * ****   ***  ***   **** ****  *********  * ****  **

Nasvi-EH1       KKVIGTHLNMCFIESHKAHFLSLVGAYIPSLVVDSEHYSKMYPLSYHFGRLIEETGYLHI 298
Nasvi-EH2       QHVLGMHSNMCLVVRPWTWLKIAAYSLMPSLLPEDERQ-LMFPLSTKLALGIEETGYLHL 299
                  * * * ***                  ***    *     * ***      ********

Nasvi-EH1       QATKPETVGAALTDSPAGLAAYILEKFSTWTNPDYRFRDDGGLLEKFTMDELLDNLMVYW 358
Nasvi-EH2       QATKPDTIGIGVSDSPAGLAAYILEKMAYCTKPDNKFTDDGNLLEKFTMDELIDNLMMYW 359
                ***** * *   **************    * **  * *** ********** *******

Nasvi-EH1       VTNSITTSQRLYAECFSKANRELGVDKMPIFVPTACANFPHELAYRSETILKERFTNLVQ 418
Nasvi-EH2       APNKASSSFRIYAESFNKQTFGYKMDNVPVTVPSACAQFPYEIIFQSANFLRDRFVNLLR 419
                  *    * * *** * *       *  *  ** *** ** *    *   *  ** **

Nasvi-EH1       FTHPPRGGHFAAFEEPELLANDVWSFVHKLEQRLSDEKRQKQEAKKEKAKKA 470
Nasvi-EH2       VTKMPRGGHFAVLEEPQLLADDIWASVQAFRDYYAAQEQERLEKQKS----- 466
                 *  *******  *** *** * *  *               *  *
```

(B)

```
        Nas.EH2.F1
  1 AAGATCGCGGCCTACAGCCTGATGCCGTCGCTTCTGCCGGAGGACGAGAGGCAGCTGATG
 61 TTCCCGCTGAGCACGAAACTGGCTTTGGGCATCGAGGAGACCGGTTACCTCCACTTGCAG
121 GCCACCAAGCCCGACACCATCGGAATCGGAGTGAGCGATTCTCCCGCTGGACTGGCGGCG
181 TACATCCTGGAAAAGATGGCTTACTGCACGAAACCCGACAACAAGTTCACCGACGACGGT
241 AACCTCCTGGAAAAGTTCACCATGGACGAGCTCATCGACAATCTCATGATGTACTGGGCG
301 CCGAACAAGGCTAGCAGCTCCTTCCGAATCTACGCCGAGAGTTTCAACAAGCAAACCTTC
361 GGCTACAAGATGGACAACGTTCCAGTAACGGTACCAAGCGCCTGCGCCCAGTTCCCCTAC
421 GAGATAATCTTCCAATCGGCCAACTTCCTTCGCGACCGTTTCGTCAACCTCCTCAGGGTA
481 ACGAAGATGCCTCGAGGTGGCCATTTCGCGGTCCTCGAAGAACCCCAGCTTCTCGCCGAC
                                    Nas.EH2.R
541 GACATCTGGGCTTCGGTACAGGCGTTCCGAGACTACTACGCCGCG
```

Fig. S6. (*A*) Amino acid sequence alignment of the putative EH genes *Nasvi-EH1* and *Nasvi-EH2* (GenBank accession no. XP_001602895) from *Nasonia vitripennis*, using CLUSTAL W analysis. Name of the gene sequences are given to the left, the amino acids are numbered to the right, and identical amino acids are indicated with asterisks. (*B*) Nucleotide sequence of the *Nasvi-EH2* dsRNA that was used in RNAi analysis. Primer nucleotide sequences are in bold and their names are given above.

Table S1. Exon/Intron splice sites prediction of the *Nasvi-EH1* DNA gene, using www.fruitfly.org/seq_tools/splice.html tools

Start	End	Score	Exon/Intron
53	67	0.65	atctcag**gt**atcaag
72	86	0.88	cgtggag**gt**acctga
165	179	0.89	aaaagag**gt**aatcga
393	407	0.75	gccccag**gt**acctaa
411	425	0.62	ccgcaag**gt**ccgtgt