

## Supporting Information

### Functional disparity of three pheromone-binding proteins to different sex pheromone components in *Hyphantria cunea* (Drury)

Xiao-Qing Zhang<sup>a,b†</sup>, Ding-Ze Mang<sup>c†</sup>, Hui Liao<sup>b†</sup>, Jia Ye<sup>a</sup>, Jia-Li Qian<sup>a</sup>, Shuang-Lin Dong<sup>b</sup>, Ya-Nan Zhang<sup>d</sup>, Peng He<sup>e</sup>, Qing-He Zhang<sup>f</sup>, Endang R. Purba<sup>g</sup>, Long-Wa Zhang<sup>a\*</sup>.

<sup>a</sup> Anhui Provincial Key Laboratory of Microbial Control,

Engineering Research Center of Fungal Biotechnology, Ministry of Education School of Forestry & Landscape Architecture, Anhui Agricultural University, Hefei, 230036, China

<sup>b</sup> Education Ministry, Key Laboratory of Integrated Management of Crop Diseases and Pests, College of Plant Protection, Nanjing Agricultural University, Nanjing, China

<sup>c</sup> Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei 2-24-16, Tokyo 184-8588, Japan

<sup>d</sup> College of Life Sciences, Huaibei Normal University, Huaibei, China

<sup>e</sup> State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025 China

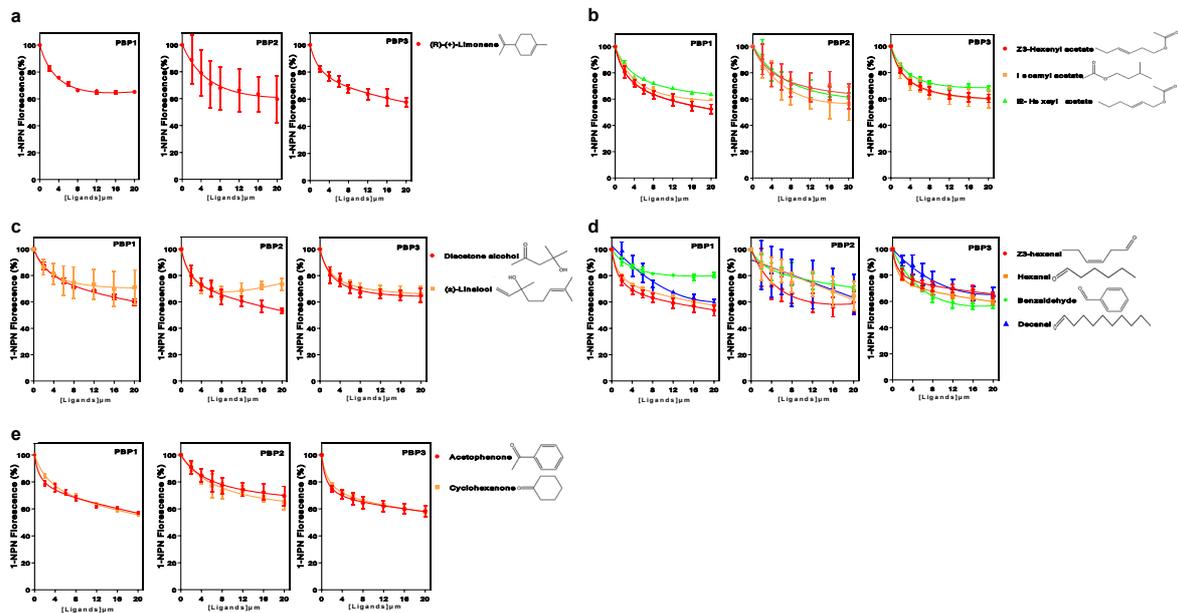
<sup>f</sup> Sterling International, Inc., Spokane, WA 99216, USA

<sup>g</sup> Structural Cellular Biology Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Okinawa, 904-0495, Japan

†: These authors contributed equally to this work.

\*Correspondence to: zhanglw@ahau.edu.cn

Long-Wa Zhang, School of Forestry & Landscape Architecture, Anhui Agricultural University, No. 130, Changjiang West Road, Hefei 230036, P. R. China.



**Figure S1.** Binding affinities of 12 plant volatiles to HcunPBP1, HcunPBP2 and HcunPBP3. Plant volatiles: alkenes (a), esters (b), alcohols (c), aldehydes (d) and ketones (e).

```

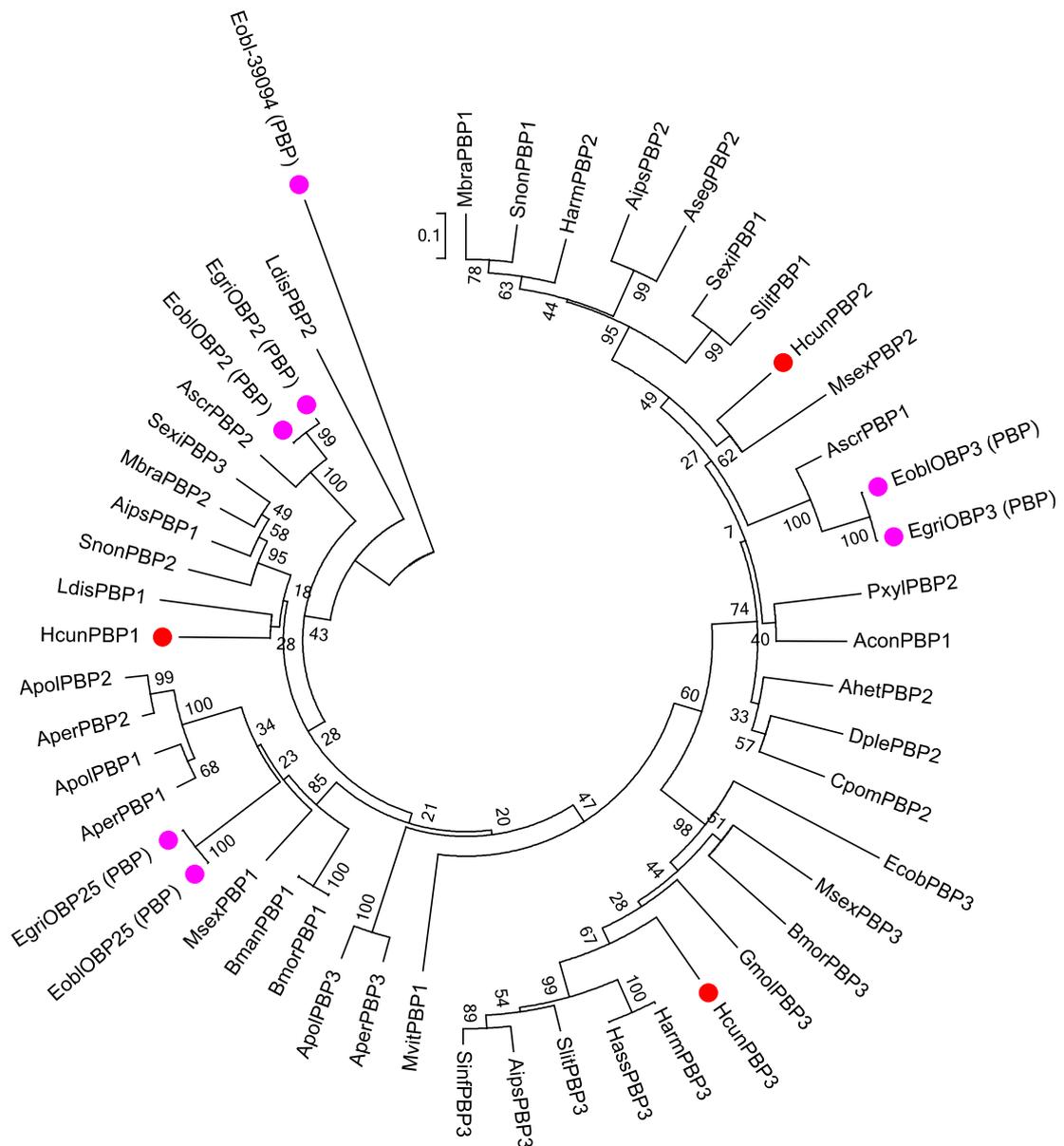
HcunPBP1  -----MSVK-----LAILVAACLAIRVETSQDVIKQMAINFVKPLEACKKEMDIPET  47
HcunPBP2  -----MAANTKWRAVLLICAAISLKEVVTGSQEVMMKLTGGFAKVLKESCKNELTIGDS  52
HcunPBP3  MIVFIDFSFKMEASKICLSFILLAISIRRTPEPSKDGMYISSGFVKVLEECKHELNLDPDH  60
          ...      :  ::  *      ..  *::  : *  ::  . * . * ** * * : :  :

HcunPBP1  VIQDFYNFWKEGYELTNRQMGCAILCMSSKLELIDGEMNLHHGNAQEFAKKHGADDAMAK  107
HcunPBP2  IVQEFYNYWREDYALVNRDLGCMIIICMASKLDLLTDEMKNHHNNAHEFAKAHGADDDTAK  112
HcunPBP3  IIGDLFHFWKLEYDHLNRDTGCIICMSKKLDLIDASGKLHHGNAKEFAMKHGAEEFIAS  120
          ::  : : : : : *  ** : * * * : : . * : : . : * . * * * * * * * : : * .

HcunPBP1  QLTDIHNCVQTSPEAPDDPCQKTLNTVICFKAIEIHKLNWAPNPELLVGEELLAETK--  163
HcunPBP2  QLVSIFRECE-SAPTTEDDPCLRALEFSKCFRSRIHELKWAPSMVEVLEEVMTEGKVT  168
HcunPBP3  KLVSAHLGCE-TKNEGNADDECLRALEMAKCFRTLVDLEWSPKVEYIVEEVLTEV--  174
          : * . . : : *  :      * * : : * :   * : : : : * . * * * . * : : * : : : .

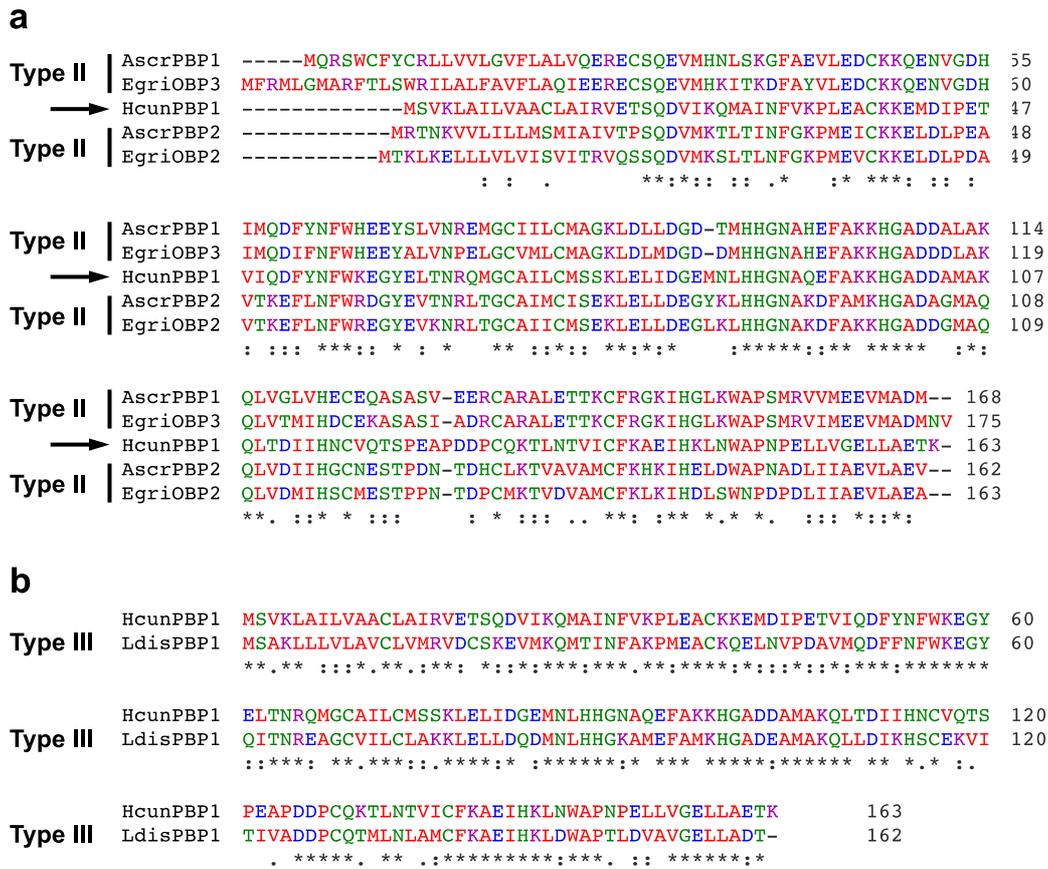
```

**Figure S2.** Comparison of the amino acid sequences of HcunPBP1, HcunPBP2 and HcunPBP3.



**Figure S3.** Molecular phylogeny comparing HcunPBPs with PBPs from twenty-seven other insect species. 3 PBPs (HcunPBP1, 2 and 3) from *H. cunea* and PBPs from *Ectropis obliqua* (Eobl); *Ectropis grisescens* (Egri); *Ascotis selenaria cretacea* (Ascr); *Agrotis ipsilon* (Aips); *Agrotis segetum* (Aseg); *Antheraea pernyi* (Aper); *Antheraea polyphemus* (Apol); *Bombyx mori* (Bmor); *Helicoverpa armigera* (Harm); *Lymantria dispar* (Ldis); *Mamestra brassicae* (Mbra); *Manduca sexta* (Msex);





**Figure S5.** Comparison of the amino acid sequences of HcunPBP1 with pheromone-binding proteins from different species of Lepidoptera. Pheromone-binding proteins from Type II pheromone releasing species (a) and Type III pheromone releasing specie (b).

**Table S1** Primers of *H. cunea* PBP genes used for RT-qPCR

Primer name	Forward (5'–3')	Reverse (5'–3')
HcunPBP1	CCAAGATTTCTACA ACTTCTG	CCAAGATTTCTACA ACTTCTG
HcunPBP2	CCAGGACTTTAGCGAATC	CCAGGACTTTAGCGAATC
HcunPBP3	CGCTAAAGAATTTGCTATGA	CGCTAAAGAATTTGCTATGA
EF1-a	CAAGGCTGATGGTAAATG	ACCAGGTTTAAGGATACC

**Table S2** Plant volatiles used in the binding assays of HcunPBPs

Chemicals	Purity (%)	Source
$\beta$ -Ocimene	95%	Sigma-Aldrich, St. Louis, MO, USA
Palmitic acid	$\geq 99\%$	Macklin, Shanghai, China
E2-Hexenol	95%	Nimord Inc, Jiangsu, China
Z3-Hexenol	98%	Sigma-Aldrich, St. Louis, MO, USA
Z2-Penten-1-ol	95%	Nimord Inc, Jiangsu, China
2,4-dimethyl-3-pentanol	98%	Nimord Inc, Jiangsu, China
(+)-Cedrol	$\geq 99\%$	Fluka™, New Jersey, USA
Nerolidol	98%	Sigma-Aldrich, St. Louis, MO, USA
(-)-Menthol	$> 99\%$	Macklin, Shanghai, China
E2-hexenal	96%	Nimord Inc, Jiangsu, China
Nonanal	95%	Nimord Inc, Jiangsu, China
6-methyl-5-hepten-2-one	96%	Nimord Inc, Jiangsu, China
(R)-(+)-Limonene	$\geq 99\%$	Sigma-Aldrich, St. Louis, MO, USA
Z3-Hexenyl acetate	$\geq 98\%$	SAFC, St. Louis, MO, USA
Isoamyl acetate	97%	Sigma-Aldrich, St. Louis, MO, USA
E2-Hexenyl acetate	98%	Sigma-Aldrich, St. Louis, MO, USA
Diacetone alcohol	98%	Nimord Inc, Jiangsu, China
( $\pm$ )-Linalool	$\geq 95\%$	Fluka™, New Jersey, USA
Z3-hexenal	95%	Sigma-Aldrich, St. Louis, MO, USA
Hexanal	98%	Sigma-Aldrich, St. Louis, MO, USA
Benzaldehyde	$\geq 99\%$	Sigma-Aldrich, St. Louis, MO, USA
Decanal	97%	Nimord Inc, Jiangsu, China
Acetophenone	99%	Sigma-Aldrich, St. Louis, MO, USA
Cyclohexanone	98%	Nimord Inc, Jiangsu, China

**Table S3** Primers of *H. cunea* PBP genes used for prokaryotic expression (PE)

Primer name	Forward (5'–3')	Reverse (5'–3')
HcunPBP1	<u>GGATCCTCGCAGGATGTTATTA</u> A GCAGAT	<u>CTCGAGTTATTTAGTTTCAGCCAA</u> CAGTTCT
HcunPBP2	GCCATGGCTGATATC <u>GGATCCTCG</u> CAGGAGGTTATGAAGAAGTT	GTGGTGGTGGTGGT <u>GCTCGAGCT</u> AAGTTTTCACTCCCGTCATAACC
HcunPBP3	<u>GGATCCTCTAAAGATGGCATGAA</u> GTATATAA	<u>CTCGAGTCAA</u> ACTTCTGTTAGTAC TTCCTCT

The restriction sites are underlined.