Supporting Information

The novel function of an orphan pheromone receptor reveals the sensory specializations of two potential distinct types of sex pheromones in noctuid moth

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Table S1 Type II sex pheromones used in this study.

Chemical component	Short name	Purity	CAS number
3Z,6Z,9Z-nonadecatriene	3Z,6Z,9Z-19:H	95.3%	89353-62-8
3Z,6Z,9Z-heneicosatriene	3Z,6Z,9Z-21:H	93.6%	87255-15-0
3Z,6Z,9Z-tricosatriene	3Z,6Z,9Z-23:H	94.5%	102673-51-8
3Z,6Z,9Z,12Z,15Z-pentacosapentaene	3Z,6Z,9Z,12Z,15Z-25:H	73.6%	854201-96-0

Table S2 Primers used in this study.

Primer	Forward (5' to 3')	Reverse (5' to 3')
Primers for gene clone		
HarmOrco	ATGATGACCAAGGTGAAGGC	TTACTTGAGCTGTATCAATACC
HarmOR11	ATGCATCTTGCAGGCAATGC	TTAAAACGTGCGTAGAAAAGC
Primers for pT7Ts vector construction		
HarmOrco	TCAGATATCGCCACCATGATGACCAAGGTGAAGGC	TCAGCATGCTTACTTGAGCTGTATCAATACC
HarmOR11	TCAGGGCCCGCCACCATGAGCTTTAAAAAATTTC	TCAGCGGCCGCTTAAAACGTGCGTAGAAAAGC
Primers for *in situ* hybridization		
HarmOR11	TCAAAGCTTTTCTGGAGCTGGGACAGTTG	TCAGAATTCTTTCACTCATAGTGCCGATGG
Primers for OR11 sgRNA synthesis and mutant screening		
OR11-sgRNA	GAAATTAATACGACTCACTATAGCTATCTCAGAAATATAGAG	TTCTAGCTCTAAAACCTCTATATTTCTGAGATAGC
OR11-mutant	CTGGAGCTGGGACAGTTGTATATTG	TGAGACAGAGGAATAAGTCAAACATCG

Table S3 Information for molecular dynamic stimulations in this study.

Molecular dynamic simulations		
System	**Ligand**	**MD times**
HarmOR11 (State 1) × 2 : HarmOrco × 2	apo	1000 ns × 2
HarmOR11 (State 2) × 2 : HarmOrco × 2	3Z,6Z,9Z-21:H	1000 ns × 2
HarmOR13 × 2: HarmOrco ×2	apo	1000 ns × 2
HarmOR13 × 2: HarmOrco × 2	Z11-16:Ald	1000 ns × 2

Fig. S1 Linear regression analysis between the contents and the integrated areas of Z3,Z6,Z9–21:H and Z3,Z6,Z9–23:H in GC/MS.

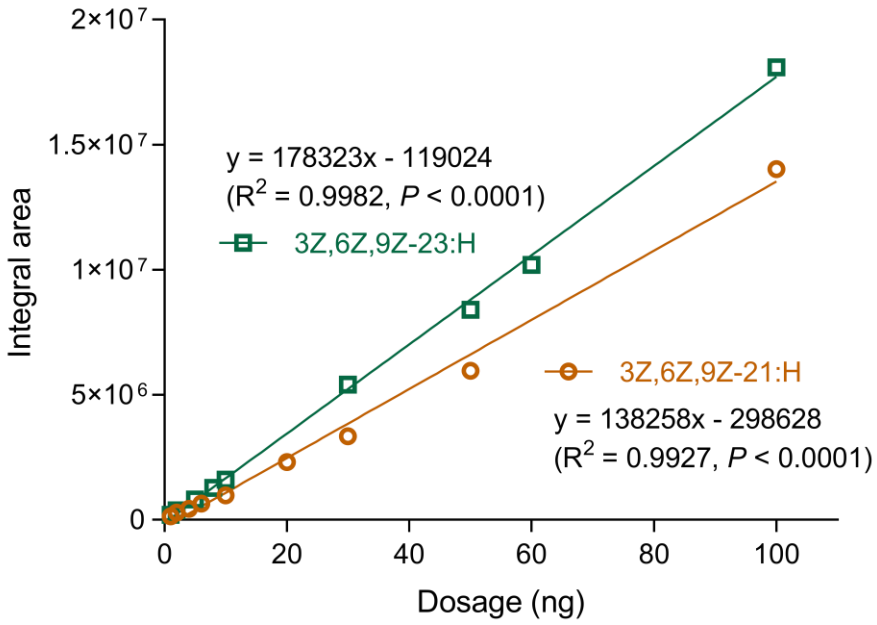


Fig. S2 Molecular Dynamics Simulations of $(HarmOrco)_2$/$(HarmOR11)_2$ with 3Z,6Z,9Z-21:H. Each simulation involved two replicas of the pheromone-bound OR, resulting in a total of four replicas from two one-microsecond MD simulations. The figure shows trajectories and conformational distributions of ligand-receptor center distance components ($Dist_x$, $Dist_y$, and $Dist_z$) and ligand-membrane angle (θ). Additionally, two representative binding modes are shown.

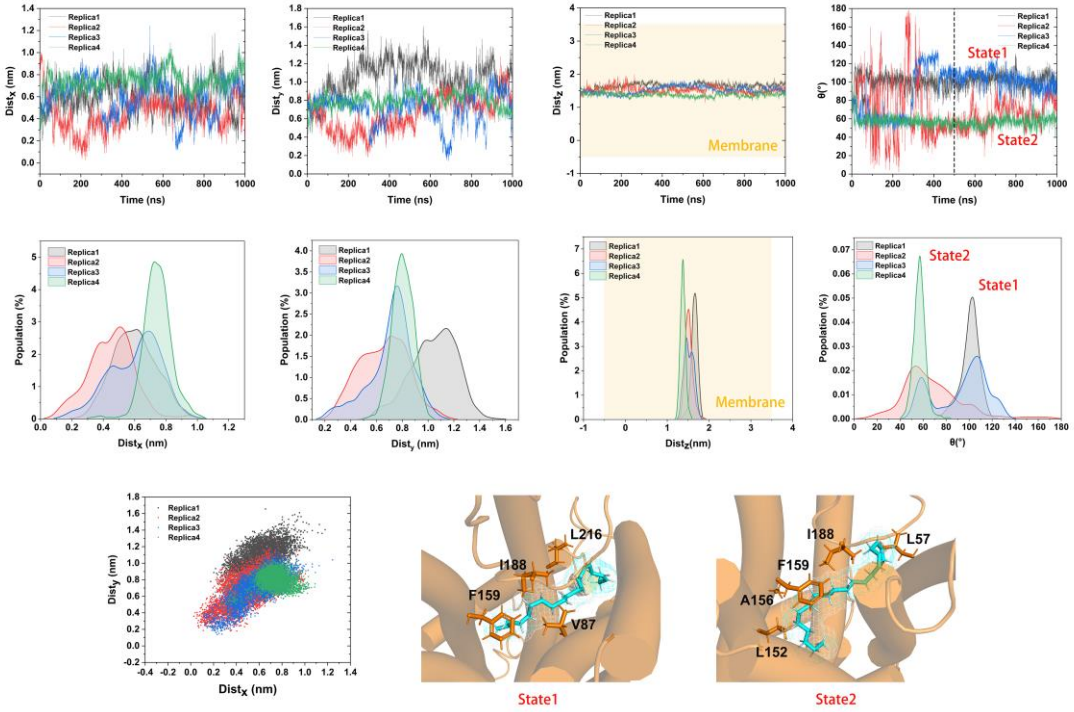


Fig. S3 Molecular Dynamics Simulations of $(HarmOrco)_2/(HarmOR13)_2$ with Z11-16:Ald. Each simulation involved two replicas of the pheromone-bound OR, resulting in a total of four replicas from two one-microsecond MD simulations. The figure shows trajectories and conformational distributions of ligand-receptor center distance components ($Dist_x$, $Dist_y$, and $Dist_z$) and ligand-membrane angle (θ).

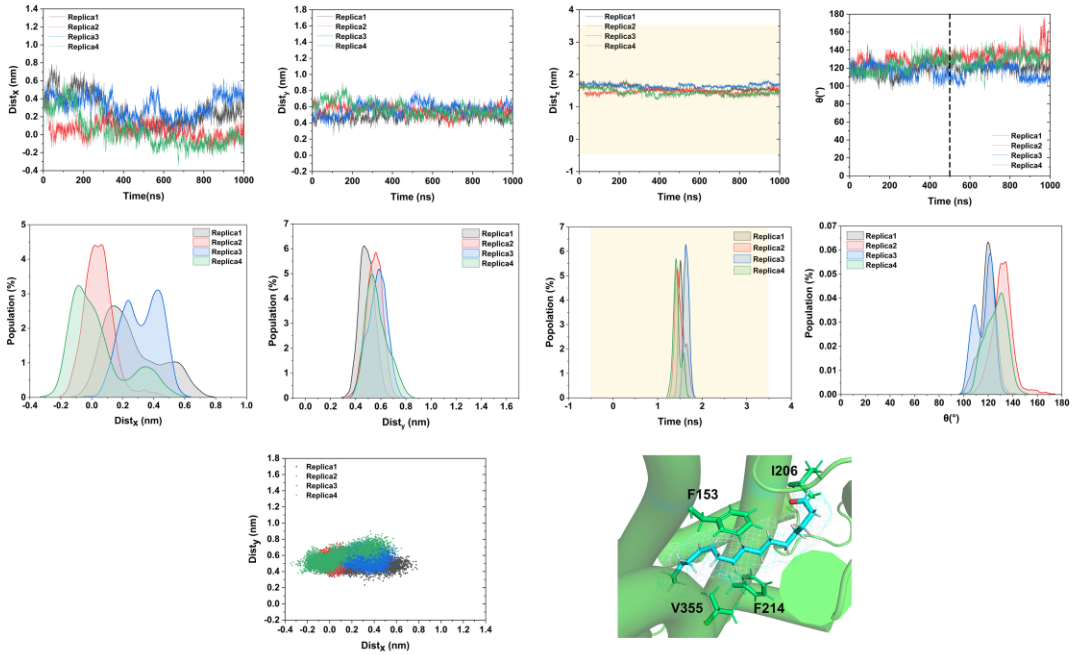
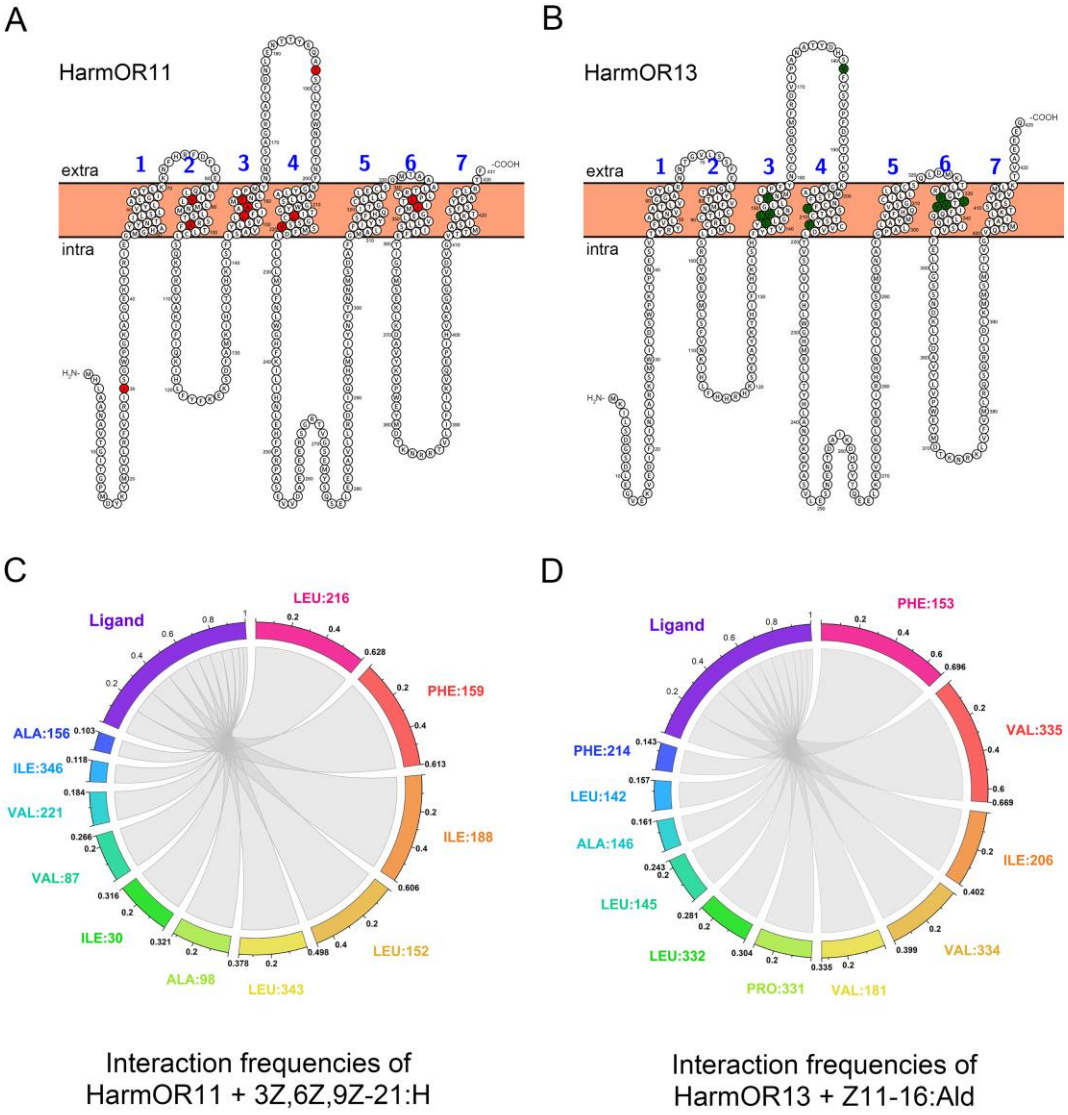


Fig. S4 Comparison of the transmembrane structures and interaction frequencies of HarmOR11 and HarmOR13 model. A and B) The key amino acid residues involved in ligand binding are marked in red and green in HarmOR11 and HarmOR13, respectively. C and D) Decimals surrounding the circle show the ratios of the time that each interaction exists to the whole MD simulation time. Only the ratios above 10% have been listed.



Interaction frequencies of HarmOR11 + 3Z,6Z,9Z-21:H

Interaction frequencies of HarmOR13 + Z11-16:Ald

Fig. S5 Representative binding modes and conformational distributions of ligand-receptor center distance components ($Dist_x$, $Dist_y$), ligand-membrane angle (θ), and radius of gyration of ligand (R_g) in the pheromone-bound OR systems. The simulation data of HarmOR14b and HarmOR16 were obtained from our previous work.