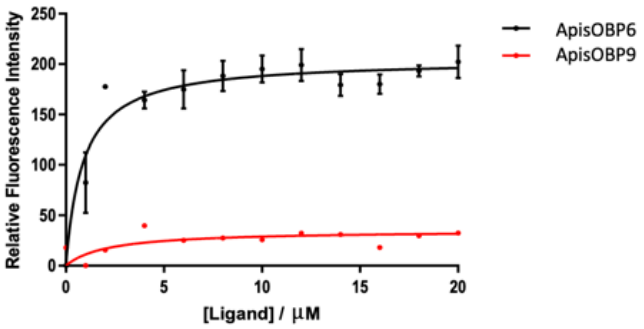
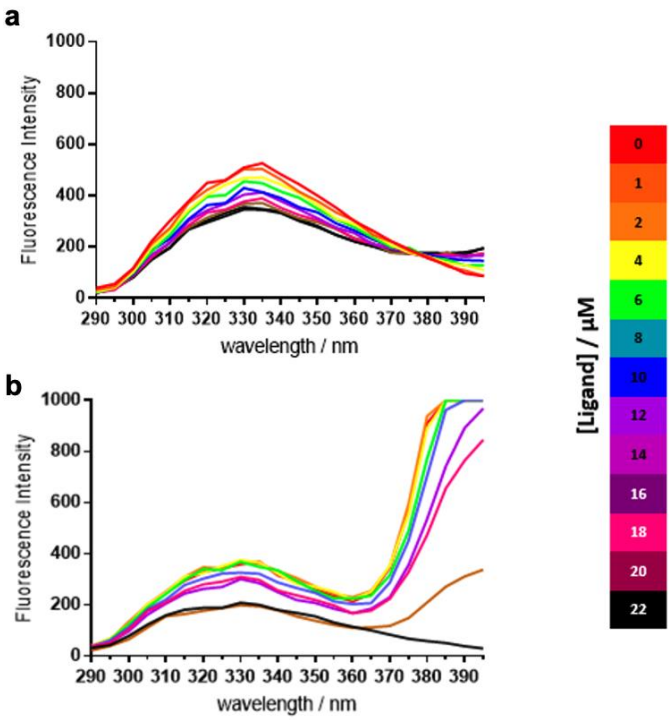
Appendix A. Supplementary Data

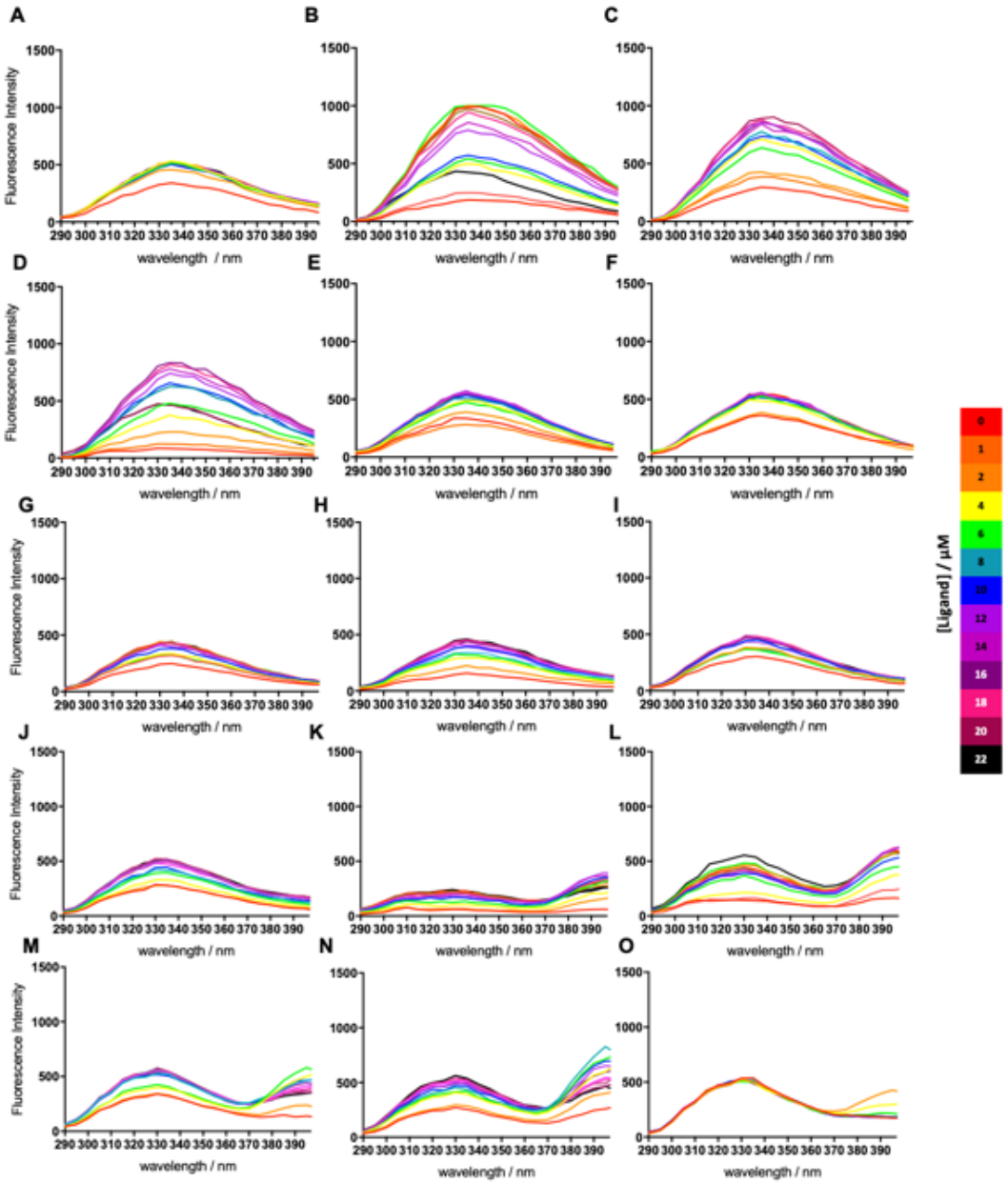
Supplementary Figures



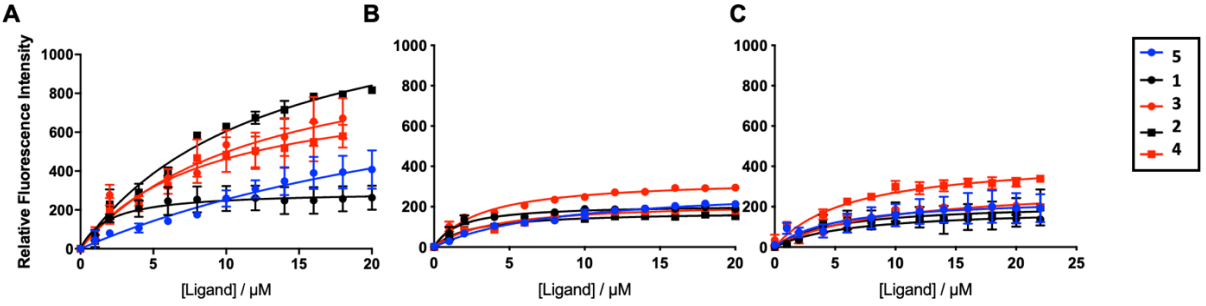
Supplementary Figure 1: Binding curves of ApisOBP6 (black) and ApisOBP9 (red) with (4aS,7S,7aR)-nepetalactone **1**. A large response can be seen be from ApisOBP6, but the fluorescence intensity of ApisOBP9 is too low to determine an accurate binding curve.



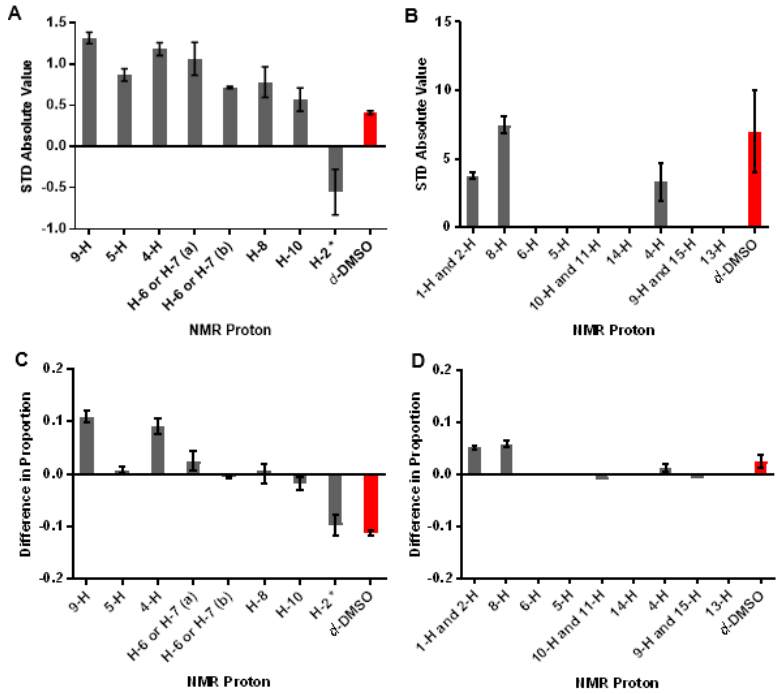
Supplementary Figure 2: Tryptophan fluorescence (280 nm excitation) of **(A)** ApisOBP6 and **(B)** ApisOBP9 with a titration of 1-NPN to final concentrations of 0-16 µM with 0 µM 1-NPN in red and 16 µM 1-NPN in black.



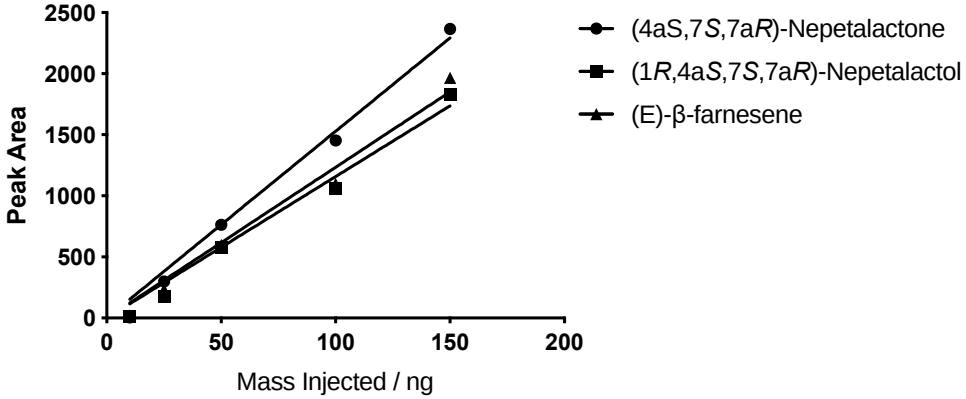
Supplementary Figure 3: Tryptophan fluorescence of **(A-E)** ligands (4aS,7S,7aR)-nepetalactone **1 (A)**, (1R,4aS,7S,7aR)-nepetalactol **2 (B)**, (4aR,7R,7aS)-nepetalactone **3 (C)**, (1S,4aR,7R,7aS)-nepetalactol **4 (D)** and (*E*)-β-farnesene **5 (E)** to final concentrations of 0-22 µM**; (F-J)** ApisOBP6 at 2 µM with 2 µM 1-NPN titrated with with ligands **1 (F)**, **2 (G)**, **3 (H)**, **4 (I)** and **5 (J)** to final concentrations of 0-22 µM; **(K-O)** ApisOBP9 at 2 µM with 2 µM 1-NPN titrated with with ligands **1 (K)**, **2 (L)**, **3 (M)**, **4 (N)** and **5 (O)** to final concentrations of 0-22 µM. For all data, the lowest concentration of each ligand (0 µM) can be seen in red and the highest concentration (22 µM) in black.



Supplementary Figure 4: Binding curves of **(A)** ApisOBP6 in a probe-free assay with (4aS,7S,7aR)-nepetalactone **1**, (4aR,7R,7aS)-nepetalactone **3**, (1R,4aS,7S,7aR)-nepetalactol **2**, (1S,4aR,7R,7aS)-nepetalactol **4** and (*E*)-β-farnesene **5**; **(B)** ApisOBP6 in a fluorescence assay with NPN and ligands **1-5; (C)** ApisOBP9 in an fluorescence assay with NPN and ligands. Bind curves were used to determine final binding constant values (K_D in µM) by non-linear regression for both OBP6.



Supplementary Figure 5: STD absolute values[59] and changes in relative intensity of different protons in the STD NMR spectrum of **(A, C)** ApisOBP6 and (4a*S*,7*S*,7a*R*)-nepetalactone **1** and (**B, D**) ApisOBP6 and (*E*)-β-farnesene **5**.



Supplementary Figure 6: Calibration of gas-chromatography data for each observed ligand (4a*S*,7*S*,7a*R*)-nepetalactone **1**, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactone **2** and (*E*)-β-farnesene **5**.

Supplementary Data

Sequence Data

>Acyrthosiphon pisum odorant-binding protein 6 (ApisOBP6) (with His tag)
MAHHHHHHVGTGSNDDDDKSPDPAGYDRTWILRQKRGTNDDECRTLLPSSEKKLPSCCQMPNILPNLDSTWEKCFETFKQFK
DKPETKEYKEMAHGKEPPCLFQCIFMQSGLTTSDGKLNEDAITKKMSEGINNDEKWKSIWQN
SLNKCFDDVKQEDKKQILIMNTPAGRLMKCFLRDMYMSCPKNVWVESSECLNMKDLVQKCPEMPPPVFKS
PPKLI

>Acyrthosiphon pisum odorant-binding protein 9 (ApisOBP9) (with His tag)
MAHHHHHHVGTGSNDDDDKSPDPIIKKTLLLSVFVLFGCLFSINKADDADAKDKELMSKLFTVVFKCFKDADWGTCGEMITTK
YDITQAKYKQCTCHMACAGEELGMINASGQPEPAKFLEYVNKINNPDIKSQLQLIYDKCQNVKGSEKCDLAEQFAICAFKESPAL
KERVSTLMEMLVKMKPKSK

Supplementary Table 1: The calculated K_D values for ApisOBP6 and ApisOBP9 with various ligands from different binding assays.

Ligand	**ApisOBP6**			**ApisOBP9**	
	Predicted *in silico* K_D / µM	**1-NPN Assay K_D / µM**	**Fluorescent Probe-Free Assay K_D / µM**	**Predicted *in silico* K_D / µM**	**1-NPN Assay K_D / µM**
(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol **2**	2.4	2.6 ± 0.6	12.7 ± 2.3	129.2	5.7 ± 1.7
(4a*S*,7*S*,7a*R*)-nepetalactone **1**	3.1	1.3 ± 0.6	1.9 ± 0.4	57.2	6.5 ± 1.6
(1*S*,4a*R*,7*R*,7a*S*)-nepetalactol **7**	2.3	2.7 ± 0.8	8.5 ± 1.6	97.4	6.3 ± 2.0
(4a*R*,7*R*,7a*S*)-nepetalactone **8**	2.7	4.4 ± 0.8	12.0 ± 4.2	65.7	9.7 ± 4.6
(*E*)-β-farnesene	11.5	10.1 ± 2.9	34.5 ± 10.9	188.8	8.3 ± 2.7

Supplementary Table 2: STD-NMR values for interactions between ApisOBP6 and (4a*S*,7*S*,7a*R*)-nepetalactone **1** and (*E*)-β-farnesene **5**. STD absolute values[59] and changes in relative intensity of different protons. STD absolute values were calculated by observing the change in proportions between the off-resonance spectrum and the final STD spectrum using the equation $(I_0-I_{STD})/I_0$.

(4a*S*,7*S*,7a*R*)-nepetalactone 1				**(*E*)-β-farnesene 5**			
δ / ppm	**Proton**	**STD Absolute Value**	**Difference in Proportion**	**δ / ppm**	**Proton**	**STD Absolute Value**	**Difference in Proportion**
1.21	9-H	1.68 ± 0.16	0.110 ± 0.010	1.48	1-H and 2-H	3.79 ± 0.21	0.052 ± 0.004
1.50-1.59	5-H	1.11 ± 0.05	0.008 ± 0.006	1.52	8-H	7.50 ± 0.64	0.058 ± 0.006
1.64	4-H	1.51 ± 0.14	0.009 ± 0.014	1.85	6-H	0	0
1.89-1.98	6-H or 7-H (a)	1.37 ± 0.21	0.025 ± 0.018	1.93	5-H	0	0
2.02-2.11	6-H or 7-H (b)	0.92 ± 0.02	-0.006 ± 0.002	2.05	10-H and 11-H	0	0

2.31-2.39	8-H	1.00 ± 0.21	0.000 ± 0.020	4.95	4-H	3.33 ± 1.38	0.012 ± 0.007
2.05	10-H	0.73 ± 0.24	-0.018 ± 0.012	5.02	9-H and 15-H	0	0
6.18-6.20	2-H	-0.70 ± 0.40	-0.113 ± 0.005	6.18	13-H	0	0
2.71	DMSO	0.52 ± 0.04	-0.097 ± 0.020	2.71	DMSO	7.02 ± 3.00	0.025 ± 0.013