

1 **An octopamine receptor confers selective toxicity of amitraz on**
2 **honeybees and *Varroa* mites**

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Abstract

The *Varroa destructor* mite is a devastating parasite of *Apis mellifera* honeybees. They can cause colonies to collapse by spreading viruses and feeding on the fat reserves of adults and larvae. Amitraz is used to control mites due to its low toxicity to bees; however, the mechanism of bee resistance to amitraz remains unknown. In this study, we found that amitraz and its major metabolite potently activated all four mite octopamine receptors. Behavioral assays using *Drosophila* null mutants of octopamine receptors identified one receptor subtype Oct β 2R as the sole target of amitraz *in vivo*. We found that thermogenetic activation of *oct β 2R*-expressing neurons mimics amitraz poisoning symptoms in target pests. We next confirmed that the mite Oct β 2R was more sensitive to amitraz and its metabolite than the bee Oct β 2R in pharmacological assays and transgenic flies. Furthermore, replacement of three bee-specific residues with the counterparts in the mite receptor increased amitraz sensitivity of the bee Oct β 2R, indicating that the relative insensitivity of their receptor is the major mechanism for honeybees to resist amitraz. The present findings have important implications for resistance management and the design of safer insecticides that selectively target pests while maintaining low toxicity to non-target pollinators.

Introduction

The western honeybee, *Apis mellifera*, is the most common and important pollinator. They visit flowering plants to collect nectar, and move pollen between

flowers. However, large colony losses have been reported worldwide over the last few decades in large part due to a syndrome called colony collapse disorder (Ratnieks and Carreck, 2010; Tylianakis, 2013). Factors that appear to contribute to colony collapse disorder and global bee decline include land-use intensification, pesticides (Martin et al., 2012; Woodcock et al., 2017), and the parasitic mite *Varroa destructor*, which transmits pathogens (Stokstad, 2019; Wilfert et al., 2016).

Varroa mites colonize beehives and are the most destructive pest of managed honeybee colonies. Commercial beekeepers rely on chemical control agents against *Varroa* mites. However, it is quite difficult to selectively kill mites without impacting on the insect hosts. Currently, only a few synthetic insecticides/acaricides are employed for this purpose such as tau-fluvalinate, coumaphos and amitraz (Johnson et al., 2013). Excessive dependence on tau-fluvalinate and coumaphos have led to high levels of resistance and loss of effectiveness (González-Cabrera et al., 2016; Higes et al., 2020). In contrast, wide-ranging amitraz resistance is rare. Consequently, amitraz is still effective in controlling *Varroa* populations in commercial beekeeping operations (Kamler et al., 2016; Rinkevich, 2020).

Many commonly used insecticides, such as neonicotinoids and pyrethroids, are safe for mammals. However, most of them are toxic to bees since they act on

ion channels and receptors which are usually highly conserved in insects. Therefore, it is important to find out why tau-fluvalinate, coumaphos and amitraz are all effective for *Varroa* mites but have low toxicity to honeybees. Previous studies found that the P450 inhibitor piperonyl butoxide elevated the toxicity of tau-fluvalinate and coumaphos in bees (Johnson et al., 2006, 2009) by inhibiting three P450 enzymes that belong to the CYP9Q family (Mao et al., 2011). On the other hand, no metabolite enzyme inhibitors enhance the toxicity of amitraz to honeybees (Johnson et al., 2013), indicating that pharmacological differences of the molecular target rather than metabolic detoxication may account for the bee's resistance to amitraz.

Amitraz is particularly effective against mites and ticks, as well as first instar larvae of lepidoptera (Hollingworth, 1976). Like other formamidines, amitraz can mimic the actions of octopamine (OA), the invertebrate analog of norepinephrine (Evans and Gee, 1980; Hollingworth and Murdock, 1980). It acts as a high-affinity agonist of OA receptors which belong to the family of rhodopsin-like G protein coupled receptors (GPCRs). In invertebrates, there are at least four classes of OA receptors: Oct α 1R (α 1-adrenergic-like octopamine receptor, also referred to as OAMB or OA1), Oct α 2R (α 2-adrenergic-like octopamine receptor, also referred to as OA3), Oct β R (β -adrenergic-like octopamine receptor, also referred to as OA2), and Oct-TyrR (octopamine/tyramine receptor, also referred to as TAR1) (Qi et al., 2017). Thus, it is not known which one is the molecular target of amitraz *in vivo*. Amitraz is also used to control the cattle tick,

79 *Rhipicephalus microplus*, and point mutations in the Oct β 2R or Oct-TyrR were
80 found in resistant strains (Baron et al., 2015; Chen et al., 2007; Corley et al.,
81 2013), suggesting that modification of these receptors could be the underlying
82 mechanism.

83
84 In this study, we identified four *Varroa* OA receptors which are all activated by
85 amitraz and its main metabolite, N²-(2,4-Dimethylphenyl)-N¹-methyformamidine
86 (DPMF), in the nanomolar range. We then used two behavioral assays to screen
87 six *Drosophila melanogaster* OA receptor mutants and found that only *Oct β 2R*
88 knockout flies were insensitive to amitraz. Artificial activation of *oct β 2R* neurons
89 also induced amitraz-like poisoning symptoms. Therefore, Oct β 2R is the sole
90 molecular target of amitraz *in vivo*. We further confirmed that VdOct β 2R from
91 *Varroa* mites was more sensitive to amitraz and DPMF than AmOct β 2R from
92 honeybees. Homology modeling and sequence alignment uncovered three
93 amino acids in the predicted binding site of Oct β 2R which confers the selective
94 toxicity of amitraz in both pharmacological assays and transgenic flies. Our
95 results reveal the molecular target of amitraz *in vivo* and key residues involved in
96 the selectivity of amitraz between *Varroa* mites and honeybees. We suggest that
97 our findings will be very useful for resistance management and the design of
98 bee-friendly insecticides.

Results

Amitraz and DPMF can activate multiple *Varroa* octopamine receptors

We identified four candidate *Varroa* OA receptors, which have amino acid homology to the *Drosophila* counterparts VdOAMB (33% identical; 81% coverage), VdOct α 2R (61% identical; 44% coverage), VdOct-TyrR (56% identical; 52% coverage), and VdOct β 2R (56% identical; 63% coverage) (Figure 1A). Therefore, we tested whether these receptors are sensitive to amitraz and DPMF in a heterologous expression system. When expressed in HEK293 cells, all receptors were functional and showed dose-dependent responses to OA (Figure 1—figure supplement 1). Dose response analysis showed that VdOct β 2R (EC_{50} = 72.8 nM) and VdOct α 2R (EC_{50} = 90.2 nM) were more sensitive to amitraz compared to VdOAMB (EC_{50} = 499.5 nM) and VdOct-TyrR (EC_{50} = 548.2 nM) (Figure 1C). However, VdOct β 2R (EC_{50} = 6.9 nM) and VdOAMB (EC_{50} = 5.2 nM) were more sensitive to DPMF compared to VdOct-TyrR (EC_{50} = 49.3 nM) and VdOct α 2R (EC_{50} = 190.2 nM) (Figure 1D). Therefore, we could not exclude any of these proteins as amitraz or DPMF receptors since they all were activated in the nanomolar range. In addition, although three out of four of these *Varroa* receptors showed higher potency and efficacy to DPMF than amitraz, both chemicals were very effective.

An I61F amino acid substitution in the Oct β 2R of the cattle tick, *R. microplus*, is associated with amitraz resistance (Corley et al., 2013). We expressed the wild-type and I61F mutant of RmOct β 2R in HEK293 cells, but they were not

functional (Figure 1—figure supplement 2). We found that this residue in transmembrane domain 1 (TM1) is conserved in all representative invertebrate species (Figure 1B). Therefore, we generated a I40F (equivalent to I61F in RmOct β 2R) mutant of VdOct β 2R and tested whether the point mutation caused insensitivity to amitraz. However, there was no significant difference in the EC₅₀ between the wild-type and mutant receptors (Figure 1C, 1D, and Figure 1—figure supplement 1), suggesting that the resistance found in cattle ticks may not be attribute to the I61F mutation in RmOct β 2R. In addition, it is still not clear whether Oct β 2R is the molecular target of amitraz.

Amitraz affects *Drosophila* aggression and locomotion through Oct β 2R

Since genetic manipulation of *Varroa* mites have not been established, we took advantage of genetic tools in *Drosophila* to study the mode of action of amitraz, which has been proven useful in elucidating the molecular targets of several insecticides (Douris et al., 2016; Nesterov et al., 2015). We hypothesized that if amitraz acts on a specific OA receptor in flies, then disruption of the receptor gene would render the mutants insensitive to amitraz. We found that amitraz has low toxicity to wild-type flies, as even 5 mM amitraz caused no significant lethality after they were exposed to an amitraz diet for four days (Figure 2—figure supplement 1). Thus, we used behavioral assays to compare the effects of amitraz on control and mutant flies.

OA is both a neuromodulator and neurotransmitter and therefore influences a range of behaviors in insects, including aggression (Hoyer et al., 2008), locomotion (Yang et al., 2015), sleep (Deng et al., 2019) and others (Kim et al., 2017). It is reported that treatment with chlorodimeform, another formamidine insecticide and octopaminergic agonist, reduces fighting latency and increases the lunging frequency in socially grouped flies (Zhou et al., 2008). We first tested whether feeding with 1 mM amitraz would lead to similar effects in flies (Figure 2A and 2B). In comparison to control flies, treatment of group-housed wide-type Canton-S flies with amitraz resulted in more aggressive behaviors. Males markedly increased their lunging frequency and decreased their fighting latency (Figure 2C and 2D). We then applied amitraz to six OA receptor mutants, and found that all but the *octβ2R* null allele *octβ2R^{f05679}* showed robust male-male aggression, as seen in the wide-type animals (Figure 2C and 2D). We also combined *octβ2R^{f05679}* with a corresponding deficiency (Df) that uncovers this gene, and found that these males were also not affected by amitraz (Figure 2C and 2D). However, heterozygous control males were still responsive to amitraz (Figure 2C and 2D).

To further confirm that amitraz affects flies through Octβ2R rather than other receptors, we continuously fed flies with amitraz and measured locomotor behavior using an automated monitoring system (Chiu et al., 2010). When we added 100 μM or 1 mM amitraz to the diet of agarose-sucrose medium (2% agarose and 5% sucrose), wide-type flies exhibited hyperactivity (Figure 3A).

Same as above, *octβ2R*^{f05679} flies showed no elevation in locomotor activity, while other receptor mutants exhibited an increase in locomotion upon amitraz treatment (Figure 3B-3G). Taken together, these results suggest that *octβ2R* mutants showed behavioral resistance to amitraz, and the deletion of any other OA receptor genes had no impact on their sensitivity to amitraz. Therefore, Octβ2R is the only receptor that mediates the effects of amitraz *in vivo*.

Hyperactivating *octβ2R*-expressing neurons mimics amitraz poisoning symptoms

The way insects react when they are exposed to formamidines is very unusual. At sublethal doses, these insecticides cause abnormal behaviors like dispersal from plants and detachment of ticks from their host, presumably induced by higher motor activity. At higher doses, this hyperactivity can induce tremors that lead to death (Evans and Gee, 1980; Stone et al., 1974). Octβ2R is coupled to G_s, which typically leads to neuronal activation. We then wondered whether artificial activation of *octβ2R* neurons would induce amitraz-like poisoning symptoms. Thus, we used the thermosensitive cation channel *Drosophila* TRPA1 (Hamada et al., 2008) to acutely hyper-stimulate these neurons. We found that expressing *trpA1* in *octβ2R-Gal4* neurons strongly induced hyperactivity behavior at 32 °C, and eventually led to paralysis (Video 1, Video 2), which is similar to the amitraz-induced behavior phenotype in insects and mites (Roeder, 2005). We also expressed *trpA1* in *oamb-Gal4*, *oct-tyrR*^{G4}, and *octα2R-Gal4* neurons and

found that activation of *oamb-Gal4* and *oct-tyrR^{G4}* neurons did not show any behavioral defects (Figure 4). Although activation of *octa2R-Gal4* neurons also induced knock-down effects, flies were directly paralyzed without hyperactivity (Video 1, Figure 4). Since Octa2R is a G_i-coupled receptor, to decrease cAMP upon activation (Qi et al., 2017), which is associated with neuronal silencing, we chose to use *UAS-Shibire^{ts}* (Kitamoto, 2001) to inhibit *octa2R* neurons. However, silencing of *octa2R-Gal4* neurons produced a “stop” behavior rather than hyperactivity or paralysis. The flies exhibited almost no translational or rotational body movement (Video 3). Therefore, thermogenetic activation of *octβ2R*-expressing neurons in a short time window phenocopies the action of amitraz in target pests, which demonstrates that pharmacological activation of Octβ2R by amitraz *in vivo* leads to toxicity and finally to death.

Honeybee Octβ2R is less sensitive to amitraz than the *Varroa* mite Octβ2R

Previous studies indicated that the differences in receptors may confer the different sensitivities of honeybees and *Varroa* mites to amitraz (Johnson et al., 2013). Therefore, we compared the effects of amitraz and DPMF on Octβ2Rs from both species, as well as fruit flies. We found that there were no differences in the AmOctβ2R, VdOctβ2R, and DmOctβ2R OA EC₅₀s (Figure 5—figure supplement 1). Notably, compared to VdOctβ2R, we found that AmOctβ2R was 16-fold less sensitive to amitraz (EC₅₀ = 1.2 μM) and 6-fold less sensitive to DPMF (EC₅₀ = 43.4 nM; Figure 5A and 5B).

In the case of DmOct β 2R, it was 3-fold less sensitive to amitraz (EC_{50} = 242.1 nM), but 5-fold more sensitive to DPMF (EC_{50} = 1.4 nM) than VdOct β 2R (Figure 5A and 5B), suggesting that metabolic detoxication may contribute to amitraz tolerance in flies. Actually, we found that both piperonyl butoxide (PBO, a cytochrome P450s inhibitor) and S,S,S-tributylphosphorotrithioate (DEF, a model carboxylesterase inhibitor), increased the toxicity of amitraz in survival assays. Diethyl maleate (DEM, a model glutathione-S-transferase inhibitor) did not significantly change the toxicity of amitraz (Figure 5—figure supplement 2). Thus, we suggest that the fruit fly and honeybee employ different mechanisms to resist amitraz.

We also tested the scenario that amitraz activates multiple OA receptors in *Varroa* mites to cause lethality, but only activates Oct β 2Rs in honeybees and flies. Therefore, we cloned the three remaining receptors from fruit flies and honeybees and tested whether these receptors are sensitive to amitraz and DPMF *in vitro*. Notably, all but DmOAMB showed responses to amitraz and DPMF in nanomolar concentrations (Figure 5—figure supplement 3). These results indicate that amitraz and DPMF can bind all four OA receptors in fruit flies and honeybees, further emphasizing that the relative insensitivity of AmOct β 2R to amitraz is critical for selective toxicity.

Three residues contribute to resistance of the honeybee Oct β 2R to amitraz

We next investigated the molecular mechanism governing the pharmacological differences in Oct β 2Rs described above. Amitraz is effective against mites and ticks, but safer for honeybees and for the bumblebee, *Bombus terrestris* (Marletto et al., 2003). We reasoned that species-specific sequences of Oct β 2Rs, especially around the ligand binding pocket, may determine their sensitivity to amitraz. To identify putative amino acid residues that affect the amitraz response, we generated models of the honeybee and mite Oct β 2R by homology modeling using the crystal structure of the carazolol-bound β -adrenergic receptor (Huang et al., 2016a). The models included placement of amitraz into the ligand-binding domain (Figure 5C-5E). Many residues were predicted to be involved in amitraz binding. However, we found three amino acids (E208, I335, I350) within the potential ligand-binding domain were unique to bees (Figure 5F; highlighted in green). We therefore generated a “*Varroa* version” of AmOct β 2R, in which the three amino acids were replaced with corresponding amino acids in VdOct β 2R, and examined its pharmacological profile in cell-based assays. Interestingly, the engineered receptor (AmOct β 2R^{E208V, I335T, I350V}, abbreviated to AmOct β 2R^{V3X}) was more sensitive (EC₅₀ = 440.0 nM) to amitraz than the wide-type AmOct β 2R (EC₅₀ = 1.2 μ M) (Figure 5A), while the OA responses were not affected (Figure 5—figure supplement 1). In contrast, AmOct β 2R^{V3X} showed no significant change in DPMF sensitivity (Figure 5B). These results indicate that the three residues are responsible for the resistance of honeybees to amitraz.

256

257 To test the role of these three amino acid substitutions *in vivo*, we generated
258 transgenic flies that express AmOct β 2R, AmOct β 2R^{V3X}, and VdOct β 2R, under
259 the control of the pan-neuronal *elav-GAL4* driver in the *oct β 2R* null mutant
260 background. As expected, expression of AmOct β 2R or VdOct β 2R rescued the
261 aggressive and locomotor behaviors induced by amitraz (Figure 6). Importantly,
262 ‘*Varroa* flies’ showed more aggression and hyperactivity (Figure 6A, 6C-6F),
263 while there was no significant effect on the fighting latency (Figure 6B). In
264 addition, flies expressing AmOct β 2R^{V3X} displayed a robust increase in the lunging
265 frequency and locomotion than the ‘honeybee flies’ (Figure 6A, 6C-6F).
266 Therefore, these three amino acid changes in honeybee Oct β 2R partially
267 phenocopy the amitraz-sensitive properties of the *Varroa* mites.

268

269 **Discussion**

270 Although the modes of action of most insecticides are known ([www.irac-](http://www.irac-online.org)
271 [online.org](http://www.irac-online.org)), for many the exact molecular targets remain elusive. In order to
272 ascribe whether a candidate protein is indeed the target for an insecticidal effect
273 *in vivo*, it is not sufficient to demonstrate an *in vitro* biochemical interaction
274 between an insecticide and a protein. Genetic evidence demonstrating an effect
275 due to mutation of the candidate receptor is critical before it is possible to
276 conclude that a given protein is the target of an insecticide. In some cases,
277 identification of the genetic basis for resistance to a specific insecticide can

implicate a putative target (Douris et al., 2016; Van Leeuwen et al., 2012). A previous study found that a single mutation (I61F) in the cattle tick Oct β 2R is associated with amitraz resistance (Corley et al., 2013). However, we found that mutation in the equivalent site in *Varroa* Oct β 2R did not reduce the potency of amitraz and DPMF. In addition, this isoleucine residue is in TM1 and is unlikely to be involved in agonist binding since the traditional orthosteric site of class A GPCRs is close to a region that includes TM3, TM5, TM6, and TM7 (Chan et al., 2019). Thus, more evidence was required to identify the exact molecular target.

Reverse genetics using *Drosophila melanogaster* has been a powerful approach to identify protein targets for insecticides (Douris et al., 2016; French-Constant et al., 1993; Scott and Buchon, 2019). In cases in which an insecticide is not toxic to the flies, behavioral assays can be employed to characterize potential targets for insecticides. For example, a recent study used climbing assays to identify a *Drosophila* TRPV channel as the target for two insecticides, pymetrozine and pyrifluquinazon (Nesterov et al., 2015). In this study, we used two behavioral paradigms to reveal that Oct β 2R but not other OA receptors is the molecular target for amitraz. We further showed that transient artificial activation of *oct β 2R*-expressing neurons is sufficient to induce amitraz-like poisoning symptoms in flies. Since Oct β 2R is conserved in invertebrates but absent in vertebrates, it could be an ideal target for pest control.

300 Bees are exposed to a great number of xenobiotics, including plant secondary
301 metabolites that serve as defense compounds against herbivores, various toxins
302 produced by fungi and bacterial, pesticides used in agriculture and other
303 environmental contaminants. Thus, it is not surprising that bees can tolerate
304 some toxic chemicals that occur naturally in their environment. So far, P450-
305 mediated detoxification is the only mechanism known. For instance, the CYP9Q
306 family can not only metabolize the flavonoid, quercetin, but can also detoxify the
307 insecticides coumaphos and tau-fluvalinate (Mao et al., 2011), as well as N-
308 cyanoamidine neonicotinoids (Manjon et al., 2018). Interestingly, bumblebees
309 may employ a different mechanism since a voltage-gated sodium channel, which
310 is the molecular target of pyrethroids, is resistant to tau-fluvalinate (Wu et al.,
311 2017). Here we found that target-site insensitivity is the major mechanism for
312 bees to resist amitraz. The VdOct β 2R from *Varroa* is more sensitive to amitraz
313 and DPMF than AmOct β 2R from honeybees. Pharmacological and genetic
314 studies revealed that three amino acids replacements increased amitraz
315 sensitivity without a cost to OA sensitivity.

316
317 Many insecticides are actually proinsecticides which are transformed into active
318 forms inside insects (Casida, 2017). Amitraz has been considered to be a pro-
319 acaricide/insecticide for many years since it can be quickly metabolized to
320 DPMF, 2,4-dimethylformanilide (DMF), 2,4-dimethylaniline (DMA) and others
321 (Knowles and Hamed, 1989; Schuntner and Thompson, 1978). Thus, it is
322 thought that amitraz undergoes bioactivation *in vivo* to produce the active

metabolite DPMF, although both drugs are active *in vivo* (Davenport et al., 1985). In this study, we found that both amitraz and DPMF are potent Oct β 2R agonists while DMF and DMA are not (Figure 5—figure supplement 4). DPMF is 11 times more potent than amitraz on VdOct β 2R from *Varroa* mites, which is consistent with a previous report using the silkworm orthologue (Kita et al., 2016). On the other hand, DPMF is 28 times more potent than amitraz on AmOct β 2R from honeybees. Amitraz content decreases rapidly to about 1/5 the level of DPMF in ticks and caterpillars (Knowles and Hamed, 1989; Schuntner and Thompson, 1978). However, it remains at a high level in honeybees even after 24 hours (Hillier et al., 2013). Therefore, metabolic differences between mites and bees may further amplify the differential pharmacological effects and contribute to the bee's tolerance to amitraz.

Growing evidence indicates that sublethal doses of insecticides may affect the physiology, cognitive function, and behavior of bees (Johnson, 2014). Genetic studies on *Drosophila* have revealed that Oct β 2R is involved in behaviors ranging from learning and memory (Burke et al., 2012; Wu et al., 2013) to ovulation (Lim et al., 2014), foraging (Koon et al., 2011) and sleep (Deng et al., 2019). Further research is needed to examine whether chronic exposure of amitraz affects these bee behaviors negatively. Another major concern is about possible synergism due to exposure of bee colonies to amitraz and other pesticides. Amitraz significantly synergizes the toxic effects of tau-fluvalinate and coumaphos on honeybees (Johnson et al., 2013). Amitraz also shows selective

synergistic effects for neonicotinoids and pyrethroids against mosquito larvae (Ahmed and Matsumura, 2012). Therefore, our identification of the target receptor for amitraz may help clarify the molecular basis of these synergistic effects so that they can be predicted and avoided.

Finally, there is great demand for safer and more selective insecticides that spare beneficial insects. Since the structure and pharmacology of Oct β 2R is different between mites and bees, our findings will help target-based screening and the design of novel chemicals acting on this unique molecular target. Some plant-derived essential oils are insecticidal and act on OA receptors (Jankowska et al., 2018). Therefore, it will be interesting to test them on *Varroa* mites and Oct β 2R.

Materials and Methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Genetic reagent (<i>Drosophila melanogaster</i>)	Canton-S	Shanghai Institute of Biochemistry and Cell Biology	Cat#BCF47	
Genetic reagent (<i>Drosophila</i>)	<i>w¹¹¹⁸</i>	Bloomington Drosophila Stock	Cat#5905 RRID:BDSC_5905	

<i>melanogaster</i>)		Center		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>elav-Gal4</i>	Blooming ton Drosophila Stock Center	Cat#8765 RRID:BDSC_8765	
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>octβ2R-Df</i>	Blooming ton Drosophila Stock Center	Cat#56254 RRID:BDSC_56254	
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>octβ3R</i> ^{MB04794}	Blooming ton Drosophila Stock Center	Cat#24819 RRID:BDSC_24819	
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>octβ1R</i>	(Koon and Budnik, 2012)		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>octβ2R</i> ^{T05679}	(Lim et al., 2014)	Cat#18896 RRID:BDSC_18896	
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>oamb</i> ^{del}	(Deng et al., 2019)		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>oamb-Gal4</i>	(Zhou et al., 2012)		
Genetic	<i>octa2R</i> ^{attp}	(Deng et		

reagent (<i>Drosophila melanogaster</i>)		al., 2019)		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>octα2R-Gal4</i>	(Deng et al., 2019)		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>octβ2R-Gal4</i>	(Deng et al., 2019)		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-trpA1</i>	(Hamada et al., 2008)		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-Shibire^{ts}</i>	(Kitamoto, 2001)		
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>oct-tyrR^{Gal4}</i>	This paper		Mutant allele; Materials and Methods, “Fly strains”
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-VdOctβ2R</i>	This paper		Mutant allele; Materials and Methods, “Fly strains”
Genetic reagent (<i>Drosophila</i>)	<i>UAS-AmOctβ2R</i>	This paper		Mutant allele; Materials and Methods, “Fly strains”

<i>melanogaster</i>)				
Genetic reagent (<i>Drosophila melanogaster</i>)	<i>UAS-AmOctβ2R^{V3X}</i>	This paper		Mutant allele; Materials and Methods, “Fly strains”
Chemical compound, drug	Amitraz	Sigma	Cat#45323	
Chemical compound, drug	N ² -(2,4-Dimethylphenyl)-N ¹ -methyformamide	Sigma	Cat#BP641	
Chemical compound, drug	2,4-dimethylaniline	Sigma	Cat#301493	
Chemical compound, drug	(±)-octopamine hydrochloride	Sigma	Cat#68631	
Chemical compound, drug	2,4-dimethylformanilide	AccuStandard	Cat#P-1100S-CN	
Chemical compound, drug	Piperonyl butoxide	Aladdin	Cat#P113864	
Chemical compound, drug	S,S,S-tributylphosphorotrithioate	Aladdin	Cat#T114221	
Chemical compound, drug	Diethyl maleate	Aladdin	Cat#D104017	
Chemical compound, drug	Poly-D-lysine	Sigma	Cat#P0296	
Chemical compound, drug	Sucrose	Sinopharm	Cat#10021418	
Chemical compound, drug	Agarose	Sinopharm	Cat#63005518	
Cell lines	HEK 293	The Cell	Cat#GNHu4	https://www.cellbank.org

		Bank of Type Culture Collection of Chinese Academy of Sciences	3	g.cn/
Recombinant DNA	Plasmid: pcDNA3.1-VdOAMB	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-VdOct β 2R	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-VdOct α 2R	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-VdOct-tyrR	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-AmOAMB	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-AmOct β 2R	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-AmOct β 2R ^{V3X}	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-AmOct α 2R	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-AmOct-tyrR	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-DmOAMB	This paper		See “Construction of expression plasmids”
Recombinant DNA	Plasmid: pcDNA3.1-DmOct β 2R	This paper		See “Construction of expression plasmids”
Recombinant	Plasmid: pcDNA3.1-	This paper		See “Construction of expression plasmids”

DNA	DmOcta2R			
Recombi nant DNA	Plasmid: pcDNA3.1- DmOct-tyrR	This paper		See “Construction of expression plasmids”
Recombi nant DNA	Plasmid: pcDNA3.1- RmOctβ2R	This paper		See “Construction of expression plasmids”
Recombi nant DNA	Plasmid: pcDNA3.1- RmOctβ2R ^{I40F}	This paper		See “Construction of expression plasmids”
Software	SoftMax Pro software (v. 7.1.2.0)	Molecula r Devices		https://www.molecular evices.com/
Software	Molecular Operating Environments (MOE, 2015.10)	Chemical Computi ng Group		https://www.chemcomp .com/
Software	Prism 7.0	GraphPa d	GraphPad Prism, RRID:SCR_ 002798	
Other	DMEM media	ThermoF isher Scientific	Cat#105660 16	
Other	Lipofectamine 2000	ThermoF isher Scientific	Cat#116680 19	
Other	96 well polystyrene microplates	ThermoF isher Scientific	Cat#165305	
Other	0.25% Trypsin- EDTA	ThermoF isher Scientific	Cat#252000 72	
Other	Fura 2-AM and Pluronic® F-127	Dojindo Molecula r Technolo gies	Cat#F025	

363

364 **Fly strains**

Flies were maintained and reared on conventional cornmeal-agar-molasses medium at 25 ± 1 °C, 60% \pm 10% humidity with a photoperiod of 12 hours light: 12 hours night (lights on at 7 AM). For experiments using *UAS-trpA1* and *UAS-Shibire^{ts}* transgenes, flies were reared at 21 °C. The Canton-S strain was used as wide-type for aggression assays. For locomotion assays, the *w¹¹¹⁸* strain was crossed to Canton-S so that the X chromosome was *w⁺* while the other chromosomes were from *w¹¹¹⁸* (*w⁺*; *w¹¹¹⁸*).

The following stains were sourced from the Bloomington Stock Center (Indiana University): *elav-Gal4* (#8765), *oct β 2R-Df* (#56254), *oct β 3R^{MB04794}* (#24819), *UAS-trpA1* (Hamada et al., 2008), *UAS-Shibire^{ts}* (Kitamoto, 2001), *oct β 2R^{f05679}* (Lim et al., 2014). *oct β 1R* was a gift from Dr. Vivian Budnik (Koon and Budnik, 2012) (University of Massachusetts Medical School). *oamb^{del}*, *oamb-Gal4*, *octa2R^{attp}*, *octa2R-Gal4*, and *oct β 2R-Gal4* were gifts from Dr. Yi Rao (Deng et al., 2019) (Peking University). *oct β 3R^{MB04794}* has an insertion of a transposable element that disrupts the gene (Zhang et al., 2013).

We generated the *oct-tyrR^{Gal4}* mutant by ends-out homologous recombination, in which the first exon of the gene was replacement by gene encoding the Gal4 transcription activator and the *mini-white* reporter gene as described previously (Huang et al., 2016b). We PCR amplified two 3 kb genomic DNA fragments from isogenic *w¹¹¹⁸*, corresponding to the 5' end of the start codon and the 3' side of

387 the first exon of the *oct-tyrR* gene, as homologous arms, then subcloned into the
388 pw35Gal4 (Moon et al., 2009). The transgenic flies were generated by randomly
389 inserted transgenes, mobilizing transgenes and screening for targeted
390 insertions. The following primers were used for mutant confirmation:

391 *oct-tyrR*^{Gal4}-F: 5'-CTGTTTGTAAATGTCACCACAACGG-3'

392 *oct-tyrR*^{Gal4}-R: 5'-CGCCCCAGGATCGAGTAA-3'

393

394 To generate the *UAS-VdOctβ2R* and the *UAS-AmOctβ2R* transgenes, we
395 subcloned the *Varroa* and honeybee *Octβ2R* cDNA sequences into the pUAST-
396 attp vector, respectively. The mutated version of *AmOctβ2R* was directly
397 synthesized by GenScript. The Octβ2R-pUAST construct with φC31-mediated
398 transgenesis targeted the attp40 specific-site on the second chromosome. The
399 following primers were used for generation of transgenes:

400 *UAS-AmOctβ2R*-F: 5'-GGCCGCGGCTCGAGGATGACGACGATCGTGACGAG-
401 3'

402 *UAS-AmOctβ2R*-R: 5'-

403 AAAGATCCTCTAGAGTCAGAGGCTGCTACCGTACTCG-3'

404 *UAS-AmOctβ2R*^{V3X}-F: 5'-

405 GGCCGCGGCTCGAGGATGACCACAATCGTGACCAGC-3'

406 *UAS-AmOctβ2R*^{V3X}-R: 5'-

407 AAAGATCCTCTAGAGTCAAAGCTTCAGGCTAGAGCCATACT-3'

408 *UAS-VdOctβ2R*-F: 5'-GGCCGCGGCTCGAGGATGTCTGTGGAGGCTGGAGC-
409 3'

410 UAS-VdOct β 2R-R: 5'-

411 AAAGATCCTCTAGAGTCAAAGCTTTGTCACCAGGGTCTTATATGTAC-3'

412

413 **Identification of OA receptors in *V. destructor***

414 To identify members of the OA receptor gene family in *Varroa*, we performed a
415 two-step analysis: 1) we used the *Drosophila* OA receptor protein sequences as
416 queries to perform BLASTp search against *Varroa* genome (Vdes_3.0) and 2)
417 we verified the candidate genes by BLASTp again without a limit of species as
418 previous described (Guo et al., 2020). We took all screened genes that were
419 Reciprocal Best Hits with the *Drosophila* OA receptor gene family and then
420 renamed *Varroa* OA receptors.

421

422 We next built a phylogenetic tree to show the evolutionary relationships between
423 these OA receptor genes among different species. The *Drosophila* FMRamide
424 receptor (DmFR) was used as the outgroup. All the amino acid sequences were
425 aligned by Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). A
426 Neighbor-joining tree was performed by MEGA X with default parameters, 1000
427 bootstrap replications, and substitution with JTT model (Kumar et al., 2018). The
428 screened genes and sequences containing previous accessions were list in
429 supplement source data.

430

431 **Construction of expression plasmids**

Varroa full-length cDNAs for all predicted OA receptors in genome annotation were cloned from a single mite or synthesized by GenScript. We inserted the Kozak consensus sequence before protein translation initiation site and subcloned with NheI-HindIII sites into the pcDNA3.1(-)-myc-His A vector for expression in mammalian cells.

Drosophila and honeybee OA receptors synthesized by GenScript were cloned into the pcDNA3.1(-)-myc-His A vector for expression in mammalian cells. We synthesized a wild-type *R. microplus* β -adrenergic OA receptor (accession number: AFC88978.1) and its I61F mutant.

Cell culture and transfection

HEK293 cells were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China, Cat#GNHu43) and the identity (STR profiling) and mycoplasma contamination status of this cell line was tested by the supplier (<https://www.cellbank.org.cn/search-detail.php?id=770>). Cells were grown in DMEM media (ThermoFisher Scientific, Cat#10566016) supplemented with 10% Fetal Bovine Serum according standard protocol at 37 °C and 5% CO₂. Cells were seeded on 60-mm Petri dishes until 80% - 90% confluent at transfection. Cells were transiently transfected, using Lipofectamine 2000 Transfection Reagent (ThermoFisher Scientific, Cat#11668019), according to the manufacturer's protocol, with 2 μ g of each OA receptor-expressing

plasmid and $G\alpha_{16}$ -expressing plasmid (Offermanns and Simon, 1995), except OAMB, which is coupled to G_q protein endogenously and can be directly activated to increase intracellular Ca^{2+} levels. The $G\alpha_{16}$ can convert G_i/G_s -coupled receptors to phospholipase C pathway (Offermanns and Simon, 1995).

50 μ L of 50 μ g/mL poly-D-lysine (Sigma-Aldrich, Cat#P0296) were added to each well of a 96 well polystyrene microplates (ThermoFisher Scientific, Cat#165305) for 4-5 hours and then washed with deionized water for 3 times. Transfected cells were washed with PBS and dissociated with 200 μ L 0.25% Trypsin-EDTA (ThermoFisher Scientific, Cat#25200072) for 30 seconds at room temperature. Cells were equally seeded into 96 well plates and incubated with DMEM media containing 10% Fetal Bovine Serum for 24-30 hours.

Calcium mobilization assay

To monitor intracellular Ca^{2+} levels, cells in 96 well microtiter plates were washed 3 times with 100 μ L saline solution containing 152 mM NaCl, 5.4 mM KCl, 0.8 mM $CaCl_2$, 1.8 mM $MgCl_2$, and 5.5 mM glucose, 10 mM Hepes (pH 7.4) (Qi et al., 2016) and loaded with 2 μ mol/L Fura 2-AM and 0.05% Pluronic[®] F-127 (Dojindo Molecular Technologies, Cat#F025) at 50 μ L/well for 40 minutes in saline solution at 37 °C and 5% CO_2 . After washing 3 times in saline solution, we pipetted 200 μ L of fresh saline solution into each well of a 96 well plate containing the loaded cells, and incubated for 15 minutes at 37 °C and 5% CO_2 .

476

477 Calcium mobilization experiments were performed on a FlexStation 3 Multi-Mode
478 Microplate Reader (Molecular Devices). All compounds were prepared freshly 1-
479 2 hour prior to assays at 5x concentration in saline buffer into a 96 well plate.
480 The FlexStation 3 instrument was set up using a SoftMax Pro software
481 according to the recommended experimental protocol. Calcium flux was
482 monitored using the 340/380 nm excitation ratio channel and 510 nm emission
483 wavelength. PMT sensitivity set up medium and 3 flashes/read. 50 μ L of
484 compounds were transferred to each well containing 200 μ L of fresh saline
485 solution at 30 seconds and fluorescence were recorded every 4 seconds for 3
486 minutes. Normalization of fluorescence data to zero baseline and SoftMax Pro
487 software (v. 7.1.2.0) (Molecular Devices) was used to calculate the ratio of the
488 340/380 nm wavelengths. Dose-response curve and data analysis were done in
489 GraphPad Prism 7 (GraphPad).

490

491 **Bioassay**

492 We introduced ten 5-7 old females into fly vials containing 5 mL 2% (wt/vol)
493 agarose and 5% (wt/vol) sucrose, supplemented with a final amitraz
494 concentration of 0 mM to 5 mM. Amitraz concentrations ≥ 10 mM cannot be
495 prepared because of insolubility. The control and each treatment were
496 performed in triplicate. Female mortality rates were monitored for 8 days.
497 For the enzyme inhibitor assay, we introduced 10 females into a new vial
498 containing sucrose solution supplemented with 5mM amitraz and 1mM inhibitor.

499

500 **Aggression assay**

501 10-15 newly eclosed male flies were reared for 5-7 days in fly vials containing
502 conventional cornmeal-agar-molasses medium. They were transferred to new
503 vials consisting of filter paper with 300 μ L 5% sucrose solution and 1% DMSO
504 (control) or an appropriate concentration of amitraz for 2 hours prior to
505 performing behavior assays. Aggressive behavior was assayed in a “8-well”
506 cylindrical arena, with the dimension of 11 mm and the volume of 1.6 mL. Each
507 arena contained 1 mL of fresh sucrose-agarose food solution (apple juice (Wei
508 Chuan Foods Corporation) consisting of 2.25% (w/v) agarose and 2.5% (w/v)
509 sucrose) (Asahina et al., 2014). A drop of yeast solution was added to the center
510 of each well and allowed to air dry. Aggression assays were performed between
511 7:00-11:00 AM and 4:00-7:00 PM, because the flies were most active during
512 these periods (see Locomotion assay). Two male flies were gently introduced
513 into the arenas by aspiration through a small hole in the lid. We then transferred
514 other flies by sliding the lid. Control and drug-treated flies were placed in the
515 arenas at the same time and recorded with a video camera for 15 minutes. The
516 total number of lunges, which is a stereotyped behavioral pattern in which the
517 winner rears up on its hind legs and uses its front legs snap on the loser, were
518 measured to assess fighting behavior (Huang et al., 2016b; Zhou et al., 2008).
519 The latency time was the time that elapsed between initiation of the video
520 capture and when the first lunge was completed (Zhou et al., 2008).

521

Locomotion assay

Locomotor behavior was performed using the *Drosophila* activity monitoring system (DAMS, Trikinetics) as briefly described previously (Yang et al., 2015). Individual 5-7 days old female flies were gently anesthetized and introduced into tubes (5 mm x 65 mm) containing drug medium or no drug medium for 3 days. The tubes were then inserted and fixed firmly in the DAMS. Flies were allowed to adapt to the new environment for 1 day before sampling midline crossing activity every minute and binning data to 30 minutes. Average daily activities were calculated based on 2-days of testing.

Thermogenetic activation and silencing assays

Flies for TRPA1-mediated thermogenetic activation and Shibire-mediated silencing experiments were collected upon eclosion and reared in vials containing standard food medium at 21 °C for 5-8 days. For thermogenetic activation with the *UAS-trpA1* transgene, 10 flies were transferred to new empty vials by gently inspiration, and then the assays were performed at 23 °C and 32 °C for 10 minutes. The percentage of paralysis behavior, in which the animal lies on its back with little effective movement of the legs and wings, was measured. For silencing assays, *UAS-Shibire^{ts}* transgene was used and flies were also transferred to fly vials at 23 °C and 32 °C for 10 minutes. The “stop” behavior

was defined as a condition that the animal exhibits almost no translational or rotational body movement.

Molecular docking

Models of the honeybee and *Varroa* Oct β 2R-LBD were built using the Molecular Operating Environments (MOE, 2015.10) using the β 2-adrenergic receptor and carazolol-bound crystal structures (PDB ID: 5D5A) as homology templates as described (Hu et al., 2017). The models were evaluated using Ramachandran plots and the UCLA-DOE server. During molecular docking calculations water molecules were deleted, 3D protonation was added, and the energy of the protein models was minimized using the MOE algorithm with default parameters. The MOE-dock program was used for docking compounds, and energies were allowed to minimize. To search for the correct conformations during the calculations, the ligands were kept flexible. The default parameters were set according to the rigid receptor docking protocol. Thirty conformations containing the docked poses and scores were output at the end of the dock operation. A lower binding free energy in the docking simulation means a better binding interaction between the receptor and the ligand.

Statistical Analysis

All statistical analyses were performed using Graphpad Prism 7 (Graphpad Software). Data for aggressive behavior were analyzed with nonparametric tests. For comparisons of more than three groups, we first used the Kruskal-Wallis

test. If the null hypothesis that the median of all experimental groups is the same was rejected, a *post hoc* Mann-Whitney U test was used for statistical analysis between the relevant pair of genotypes. Data for locomotion were considered to be normally distributed since these data passed the D'Agostino–Pearson omnibus test. Therefore, one-way or two-way ANOVA was performed to test the null hypothesis. If the null hypothesis was rejected, we then used a *post hoc* Bonferroni correction for multiple comparisons. *P* values were indicated in the figures. Details of other statistical methods are reported in the figure legends.

Acknowledgments

We thank Yi Rao (Peking University), Vivian Budnik (University of Massachusetts), Zhefeng Gong, Liming Wang and Jianhua Huang (Zhejiang University) for fly stocks; Huoqin Zheng (Zhejiang University) for *Varroa* cDNAs; Yinjun Tian (Zhejiang University) for assistance with the locomotion assays; Xueping Hu (Zhejiang University) for assistance with the molecular docking. We also thank Xiao-Wei Wang (Zhejiang University) for helpful comments on the manuscript. Stocks obtained from the Bloomington *Drosophila* Stock Center (NIH P40OD018537) were used in this study.

Funding

Funder	Grant reference number	Author
National Natural Science	31572039; 32072496	Jia Huang

Foundation of China		
Zhejiang Provincial Fund for Distinguished Young Scholars	LR19C140002	Jia Huang
National Institute on Deafness and Other Communication Disorders	DC007864; DC016278	Craig Montell
The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.		

585

586 **Competing interests**

587 The authors declare no competing interests.

588

589 **Source data files**

590 The raw data for all plots in figures and accession numbers for sequences are
591 provided in source data files.

592

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Figure legends

Figure 1. Amitraz and its main metabolite DPMF can activate *Varroa* multiple OA receptors *in vitro*. (A) Phylogenetic tree of OA receptors from *Varroa destructor*, *Apis mellifera*, *Rhipicephalus microplus*, and *Drosophila melanogaster*. The values on the branches represent the bootstrap support. The candidate *Varroa* receptors are in bold. (B) The Isoleucine40 in the TM1 of Oct β 2R, which is associated with amitraz resistance in the cattle tick *Rhipicephalus microplus*, is highly conserved in Arachnida and Insecta. (C and D) Dose-response curves of amitraz (C) and DPMF (D) against the *Varroa* OA receptors. EC₅₀ values were calculated using log(agonist) versus response nonlinear fit, mean \pm SEM, n = 3 trials, 3 replicates per trial.

Figure 2. Amitraz affects *Drosophila* aggression through Oct β 2R. (A) Preparation of test flies. In brief, group-housed male flies (~10-15) were fed 1% DMSO plus 5% sucrose (control) or 1 mM amitraz plus 5% sucrose for 2 hours (see Methods). (B) The 8-well aggression arena used in this behavioral test. (C and D) Effects of 1 mM amitraz on the number of lunges (C) and latency to initiate fighting (D) in different OA receptor mutants and control flies. *P* values, Mann-Whitney U tests were performed to analyze statistically significant differences between treatment with 1% DMSO versus 1 mM amitraz in the indicated genotypes, mean \pm SEM, n = 12-34.

Figure 3. Amitraz affects *Drosophila* locomotion through Oct β 2R. (A-G) Effects of amitraz on midline crossing activity in flies of the indicated genotypes. One 5-7-day-old female fly was gently introduced into each tube, which contained 1% DMSO (control), 100 μ M amitraz or 1mM amitraz added to the agarose-sucrose medium (2% agarose and 5% sucrose) at one end. The other end was sealed with a cotton plug. The tubes were placed in a *Drosophila* Activity Monitor System (see Methods). Black and white bars represent the night and day periods of the 12:12 LD cycle. Yellow boxes indicate the two-day window of daily crossing activity test. *P* values, one-way ANOVA and *post hoc* Bonferroni correction, mean \pm SEM, n= 16-32.

Figure 4. The percentage of paralysis behavior, in which four OA receptor-expressing neurons were thermally hyperactivated with *UAS-trpA1*. The following transgenes were used: *oamb-Gal4>UAS-trpA1*; *oct-tyrR^{Gal4}>UAS-trpA1*; *oct α 2R-Gal4>UAS-trpA1*; *oct β 2R-Gal4>UAS-trpA1*. n = 50-100.

Figure 5. Triple amino acids differences determine amitraz sensitivity in Oct β 2R *in vitro*. (A and B) The predicted ligand-binding domain of amitraz in the *Varroa* (A) and the honeybee (B) Oct β 2R. Amitraz and three amino acids mutated in this study are shown. (C) Superposition of the predicted ligand-binding domain of the honeybee (golden cartoon) and the *Varroa* (blue cartoon) Oct β 2R structures. (D) Amino acid substitution in the ligand-binding domain (TM5-TM7) of Oct β 2R in

representative species from arachnida and hymenoptera. The predicted amino acids involved in the binding of amitraz are indicated in red. Three amino acids (E208, I335, I350) highlighted in green are conserved among species of bees. Abbreviations: EL: extracellular loop; TM: transmembrane domain. (E and F) Dose-response curve of amitraz (E) and DPMF (F) against the indicated OA receptors. EC₅₀ was calculated using log(agonist) versus response nonlinear fit, mean ± SEM, n = 3-4 trials, 3 replicates per trial.

Figure 6. Transgenic flies expressing Octβ2R variants show different sensitivities to amitraz. (A and B) Number of lunges (A) and latencies before initiating fighting (B) in the *Octβ2R* null mutant expressing VdOctβ2R, AmOctβ2R or AmOctβ2R^{V3X}. Changes were compared to the AmOctβ2R flies. AmOctβ2R^{E208V, I335T, I350V}, abbreviated to AmOctβ2R^{V3X}. Genotype: *elav-gal4/UAS-XXOctβ2R; octβ2R^{f05679}/octβ2R^{f05679}*. *P* values, Kruskal-Wallis and *post hoc* Mann-Whitney U tests, mean ± SEM, n = 18-24. (C-E) Midline crossing activity in Octβ2R null mutants expressing VdOctβ2R, AmOctβ2R or the AmOctβ2R^{V3X}. (F) Daily crossing activities exhibited by *Octβ2R* null mutants expressing VdOctβ2R, AmOctβ2R or the AmOctβ2R^{V3X}. Changes were compared to the AmOctβ2R flies. Genotype: *elav-gal4/UAS-XXOctβ2R; octβ2R^{f05679}/octβ2R^{f05679}*. *P* values, two-way ANOVA and *post hoc* Bonferroni correction, mean ± SEM, n = 16.

Supplementary figure legends

Figure 1—figure supplement 1. Dose-response curves of OA against the indicated OA receptors. EC₅₀ was calculated using log(agonist) versus response nonlinear fit, mean ± SEM, n = 3 trials, 3 replicates per trial.

Figure 1—figure supplement 2. Effects of OA and amitraz on the *Rhipicephalus microplus* Octβ2R expressed in HEK293 cells. mean ± SEM, n = 3 trials.

Figure 2—figure supplement 1. Adult survival of flies reared on diets containing 1 % DMSO or a range of amitraz concentrations. In brief, 10 wild-type female flies were allowed to feed on diets with 1% DMSO or a series of amitraz concentrations ranging from 1 mM to 5 mM. Data are shown as mean ± SEM. n = 3 biological replicates.

Figure 5—figure supplement 1. Dose-response curves of OA against the indicated OA receptors. EC₅₀ values were calculated using log (agonist) versus response nonlinear fit, mean ± SEM, n = 3 trials, 3 replicates per trial.

Figure 5—figure supplement 2. Dose-response curves of OA, amitraz, and DPMF against the fly (A-C) and honeybee (D-F) OA receptors. EC₅₀ values were calculated using log (agonist) versus response nonlinear fit, mean ± SEM, n = 1-3 trials, 3 replicates per trial.

Figure 5—figure supplement 3. Adult survival when reared on sucrose solution containing 5 mM amitraz and 1 mM detoxicative enzyme inhibitor. Abbreviations: PBO: piperonyl butoxide; DEF: S,S,S-tributylphosphorotrithioate; DEM: diethyl maleate. One way ANOVA and *post hoc* Mann-Whitney U tests, mean ± SEM, n = 3-9 biological replicates. * p<0.05; ** p<0.01.

Figure 5—figure supplement 4. Effects of DMA (2,4-dimethylaniline) and DMF (2,4-dimethylformanilide) on VdOctβ2R *in vitro*. mean ± SEM, n = 5 trials.

Videos

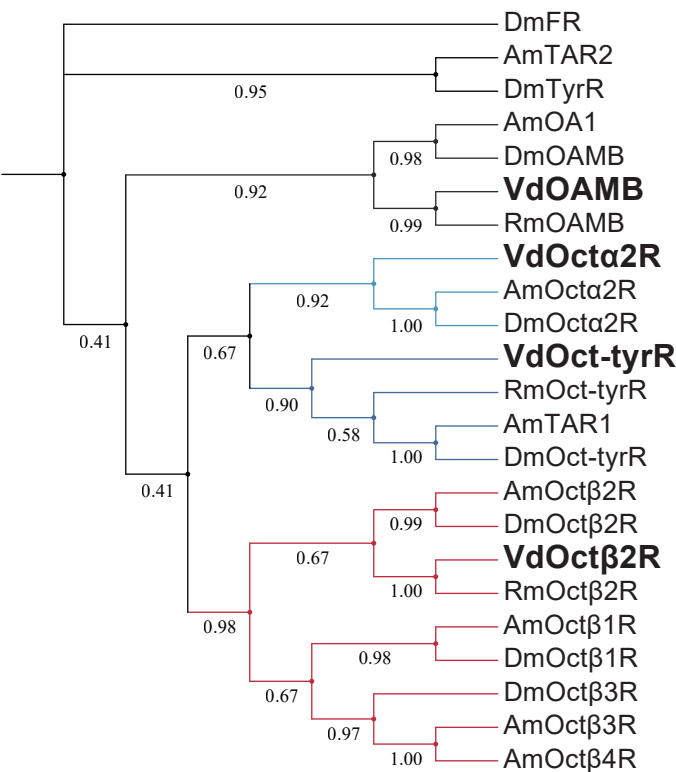
Video 1. Thermogenetic activation of four OA receptor-expressing neurons using *UAS-trpA1* induces paralysis behavior (related to Figure 4). The following transgenes were used: *oamb-Gal4>UAS-trpA1*; *oct-tyrR^{G4}>UAS-trpA1*; *octα2R-Gal4>UAS-trpA1*; *octβ2R-Gal4>UAS-trpA1*. The movie was speeded up 2x.

Video 2. Thermogenetic activation of *octβ2R-Gal4* neurons using *UAS-trpA1* induces paralysis behavior (related to Figure 4). The following transgenes were used: *octβ2R-Gal4>UAS-trpA1*.

Video 3. Silencing of *octα2R-Gal4* neurons using *UAS-Shibire^{ts}* decreases activity. The following transgenes were used: *octα2R-Gal4> UAS-Shibire^{ts}*. The movie was speeded up 2x.

Figure 1

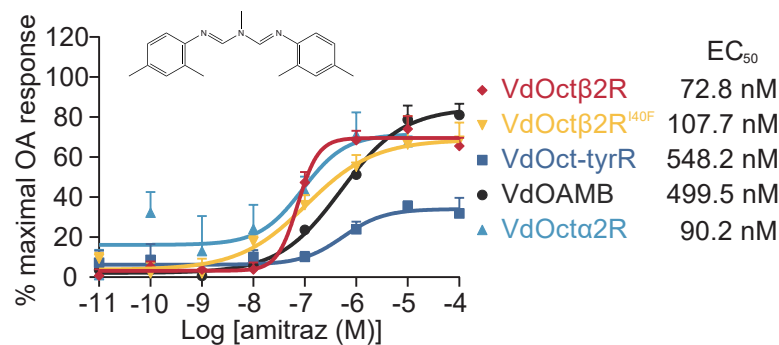
A



B



C



D

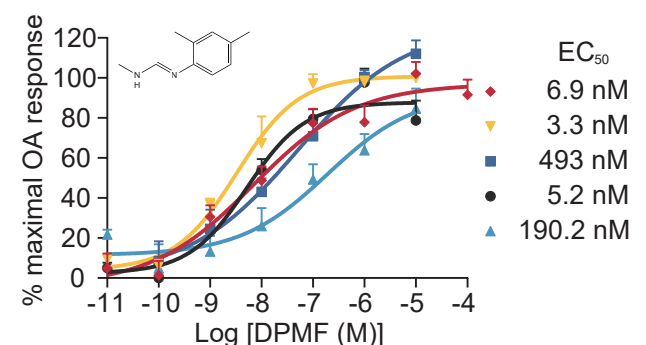
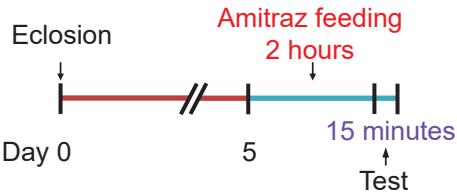
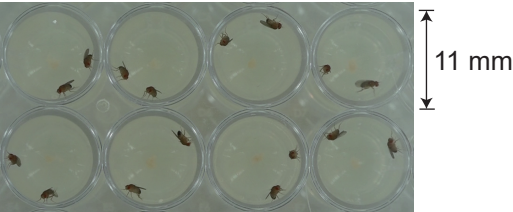


Figure 2

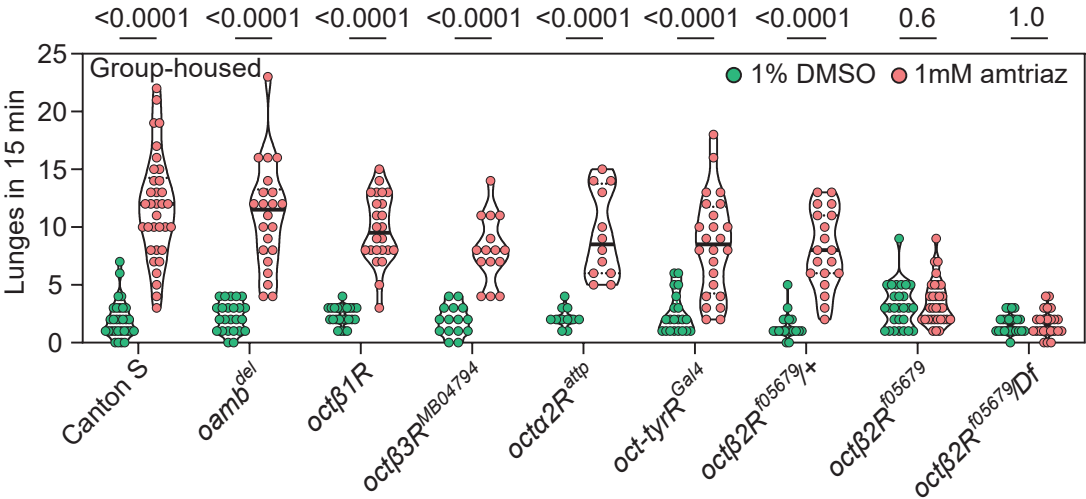
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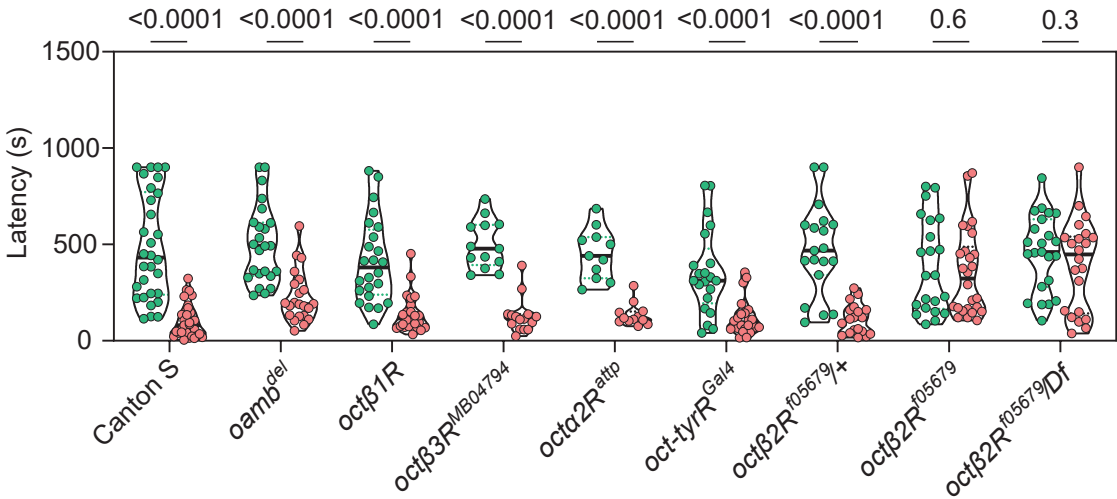


Figure 3

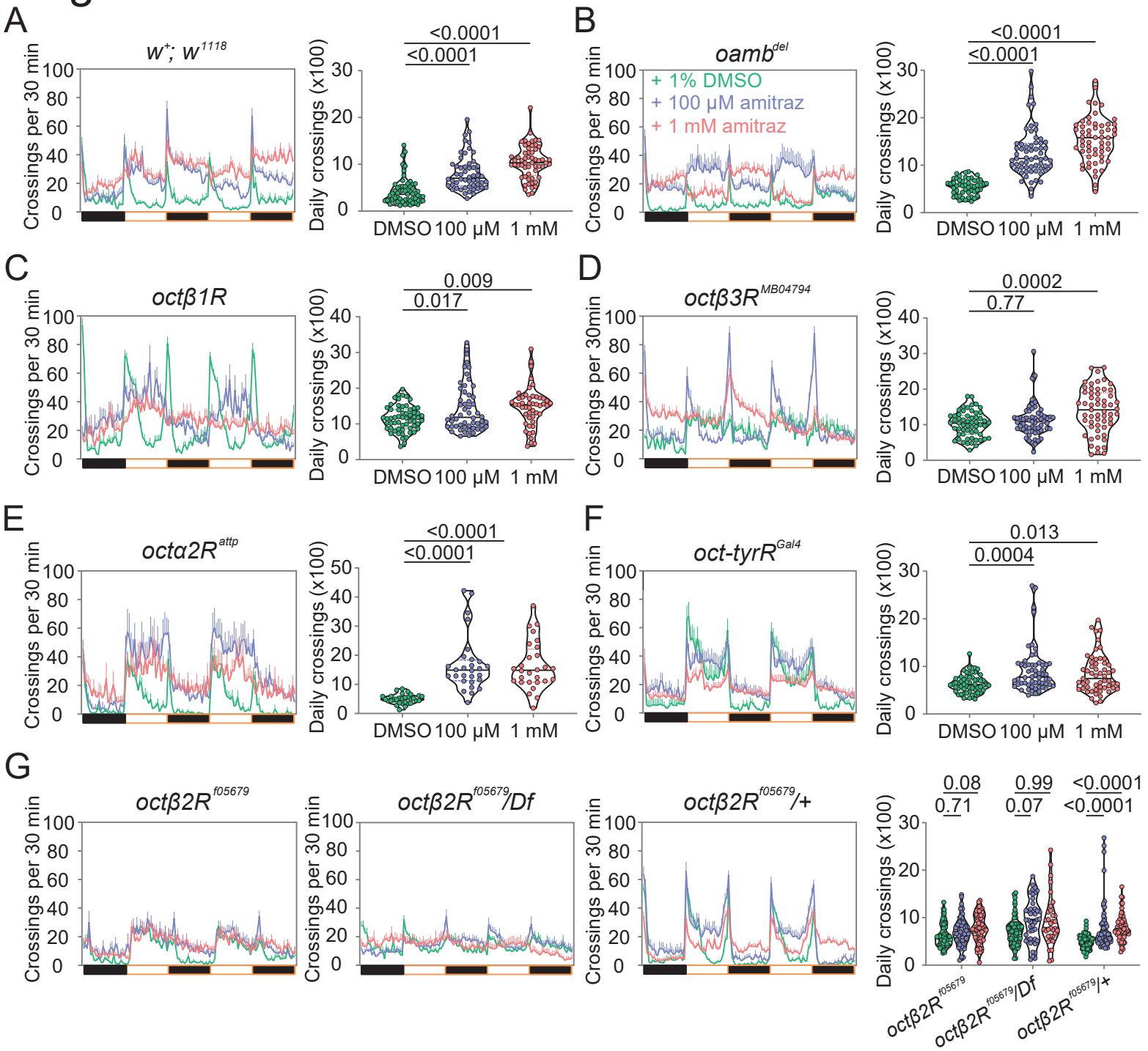


Figure 4

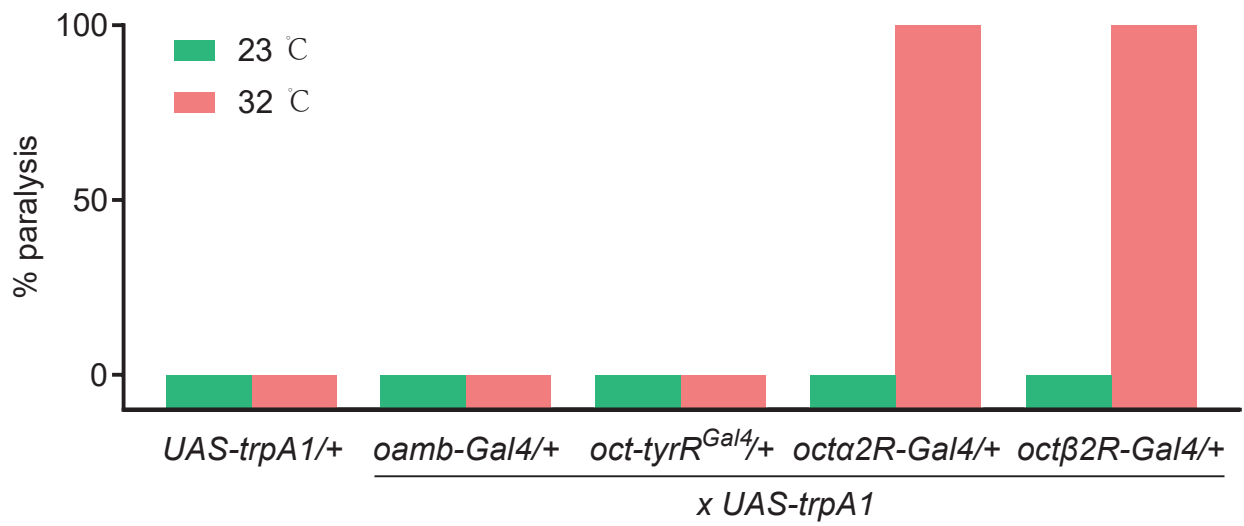


Figure 5

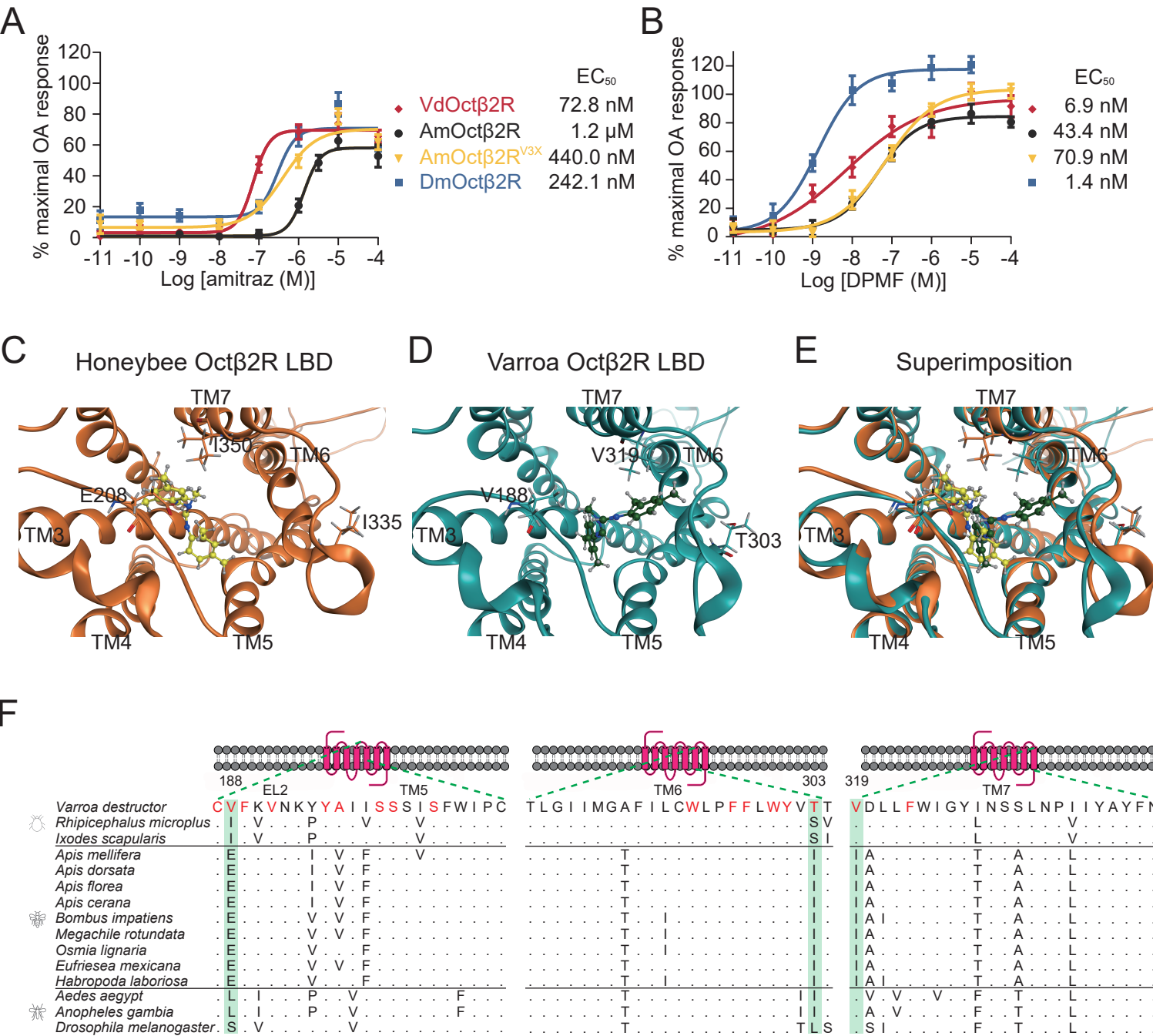


Figure 6

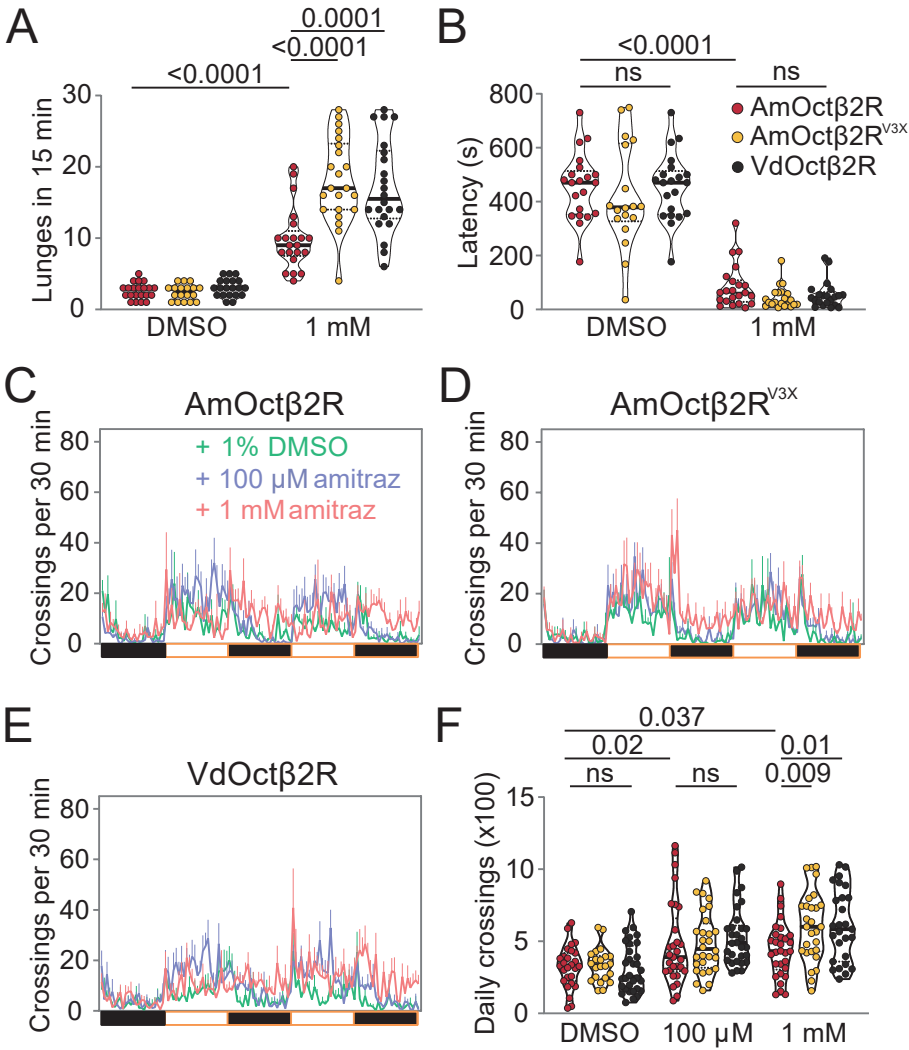


Figure 1—figure supplement 1

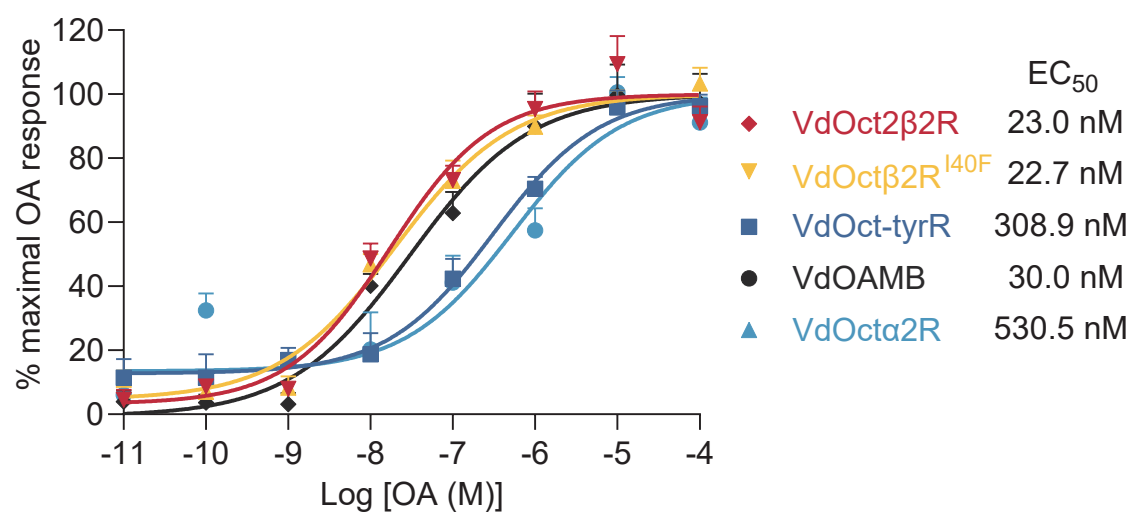


Figure 1—figure supplement 2

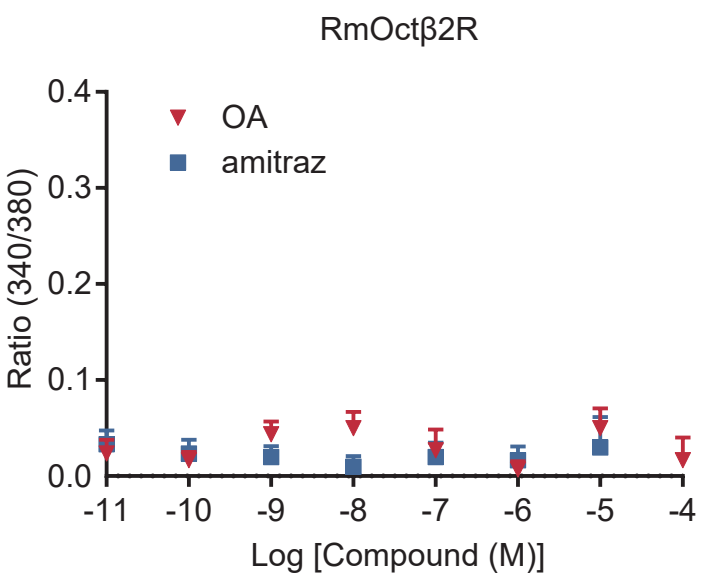


Figure 2—figure supplement 1

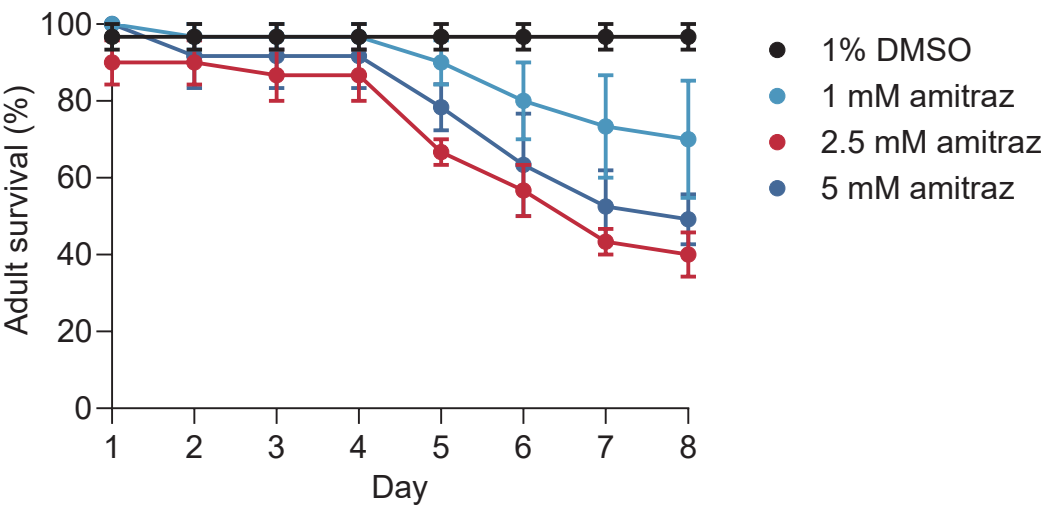


Figure 5—figure supplement 1

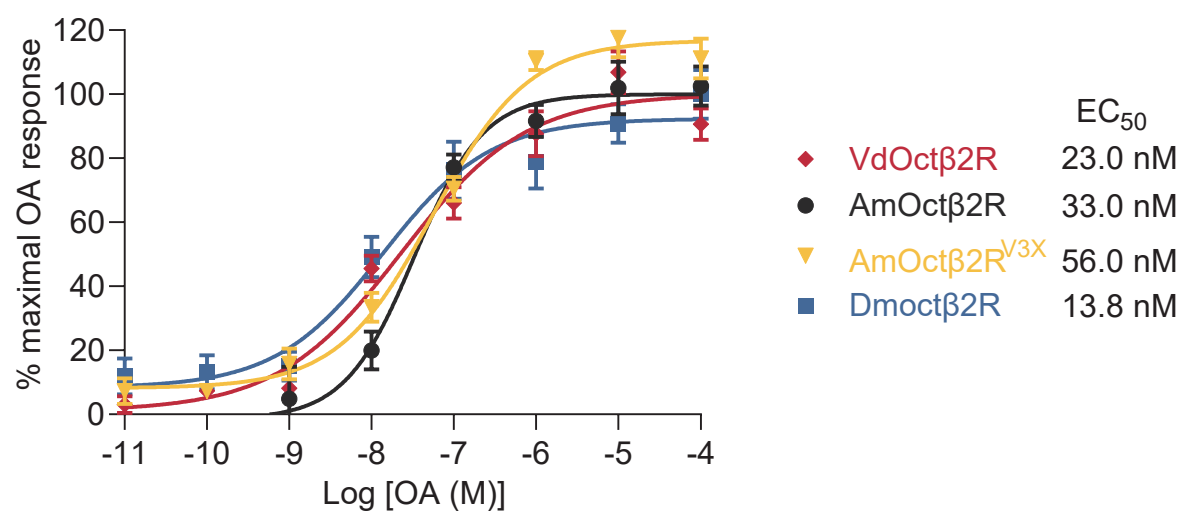


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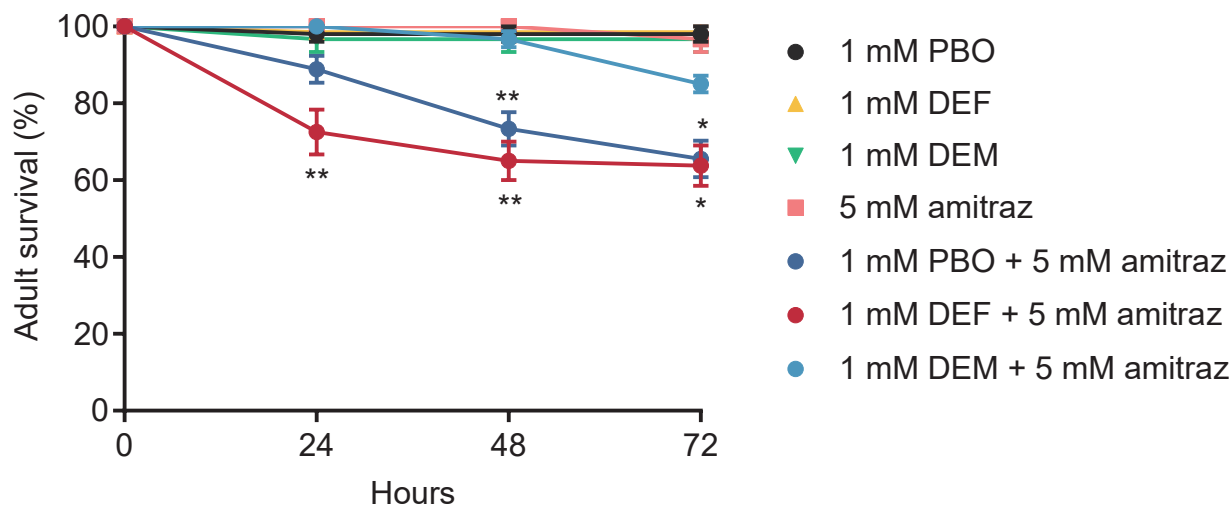


Figure 5—figure supplement 3

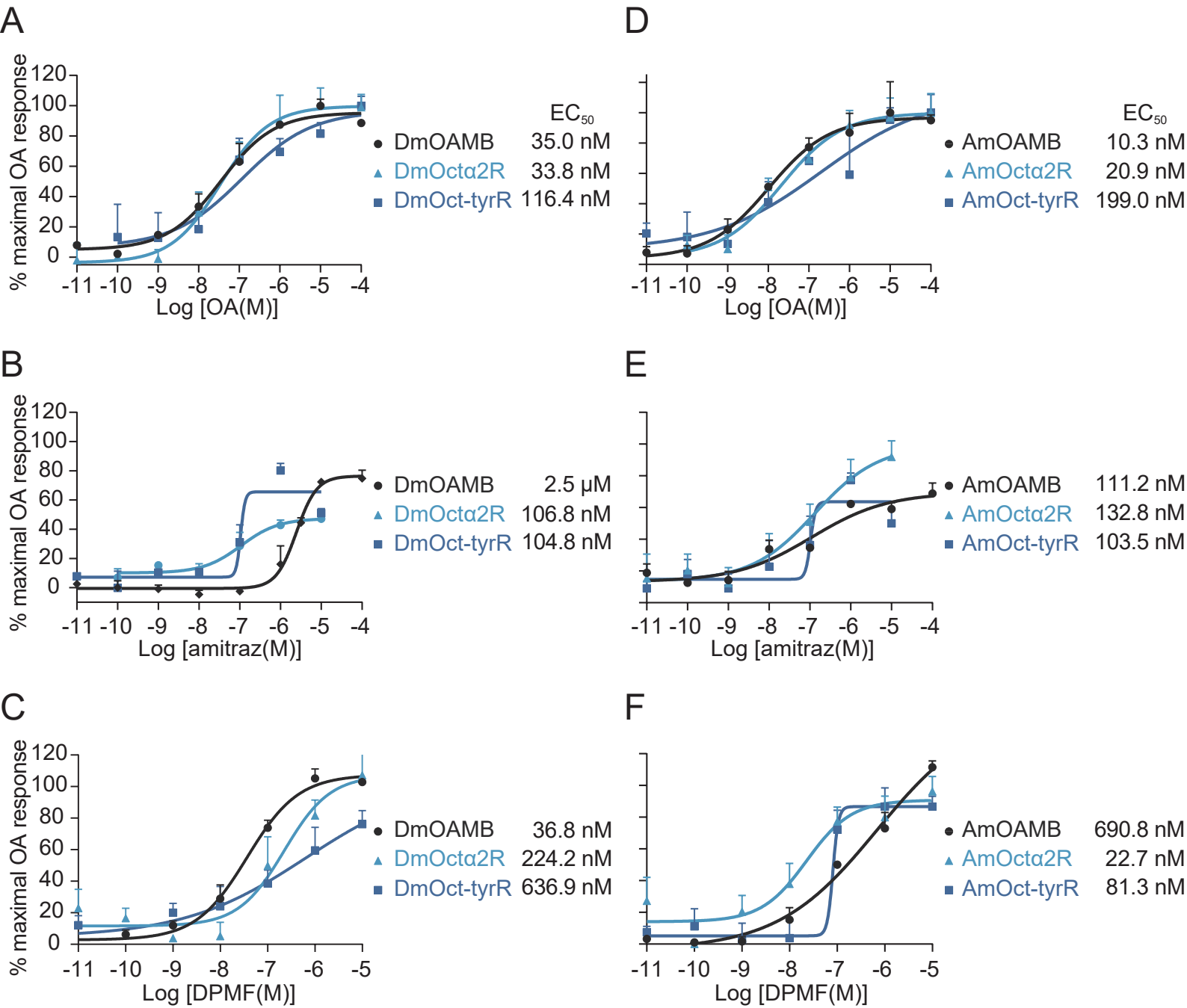


Figure 5—figure supplement 4

