4-Vinylanisole is an aggregation pheromone in locusts

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Locust plagues threaten agricultural and environmental safety throughout the world[1,2]. Aggregation pheromones have a crucial role in the transition of locusts from a solitary form to the devastating gregarious form and the formation of large-scale swarms[3,4]. However, none of the candidate compounds reported[5–7] meet all the criteria for a locust aggregation pheromone. Here, using behavioural assays, electrophysiological recording, olfactory receptor characterization and field experiments, we demonstrate that 4-vinylanisole (4VA) (also known as 4-methoxystyrene) is an aggregation pheromone of the migratory locust (*Locusta migratoria*). Both gregarious and solitary locusts are strongly attracted to 4VA, regardless of age and sex. Although it is emitted specifically by gregarious locusts, 4VA production can be triggered by aggregation of four to five solitary locusts. It elicits responses specifically from basiconic sensilla on locust antennae. We also identified OR35 as a specific olfactory receptor of 4VA. Knockout of OR35 using CRISPR–Cas9 markedly reduced the electrophysiological responses of the antennae and impaired 4VA behavioural attractiveness. Finally, field trapping experiments verified the attractiveness of 4VA to experimental and wild populations. These findings identify a locust aggregation pheromone and provide insights for the development of novel control strategies for locusts.

A previous study on body and faeces of solitary and gregarious locusts of both sexes at different developmental stages identified several volatile compounds[8], but their biological functions remain largely unknown. Phenylacetonitrile was recently confirmed to be an olfactory aposematic signal and cyanide precursor in the migratory locust[9]. Although sequences of 141 olfactory receptors and the odorant receptor co-receptor *Orco* have been identified[10,11], the matching ligands remain uncharacterized. The establishment of *Orco*-mutant locusts using CRISPR–Cas9[12] has enabled assignment of olfactory behavioural traits to *Orco*, which is a co-receptor of all locust olfactory receptors, suggesting that olfactory attraction and olfactory receptors are involved in locust aggregation.

Locusts exhibit attraction to 4VA

Among the 35 volatiles that we identified derived from the body and faeces of the locust[8], we found six compounds—phenylacetonitrile, benzeneacetaldehyde, 4-vinylanisole (4VA), 2,5-dimethylpyrazine, phenethyl alcohol and anisole—that showed significantly higher emissions in gregarious locusts than in solitary locusts (Fig. 1a). Using a dual-choice arena system[9] to evaluate the preferences of locusts on the basis of the total duration and distance moved in each zone (Fig. 1b), we found that only 4VA exhibited significant attractiveness to gregarious locusts, whereas phenylacetonitrile induced repellent responses in gregarious locusts (Fig. 1c, Extended Data Fig. 1a). The gregarious locusts did not show any preference for benzeneacetaldehyde, dimethylpyrazine, phenethyl alcohol or anisole in comparison to the control (mineral oil) (Fig. 1c, Extended Data Fig. 1a).

Next, we conducted behavioural assays of putative cohesion pheromones including guaiacol, phenol, veratrole and mixtures of these three volatiles, as previously reported[6,8]. Of these, guaiacol was found to be a behavioural inhibitor for the gregarious locusts, whereas phenol and veratrole were neutral in the tests (Extended Data Fig. 1b, c); the two mixtures elicited repellent responses in the gregarious locusts (Extended Data Fig. 1b, c). Thus, only 4VA shows significant attractiveness to conspecifics, indicating a potential role in locust aggregation.

4VA attraction across phase, age and sex

We further evaluated locust behaviours across developmental stages, phases and sexes. Gregarious locust 3rd-to-5th-instar nymphs (G3, G4 and G5) and adults showed a significant preference for remaining and moving in the zone suffused with 4VA (Fig. 1d, Extended Data Fig. 1d). At the 5th stadium, the gregarious locusts showed attraction to 4VA at 8 h, 24 h, 48 h and 72 h after ecdysis (Extended Data Fig. 1e, f). In addition, the gregarious locusts displayed a significant preference for 4VA at concentrations ranging from 0.5 to 5,000 ng μl^{-1} (Fig. 1e, Extended Data Fig. 1g). The attraction to 4VA did not show sex bias in either locust nymphs or adults (Fig. 1f, Extended Data Fig. 1h). Furthermore, the behavioural preferences of solitary locusts in response to 4VA were almost the same as those of the gregarious locusts in the

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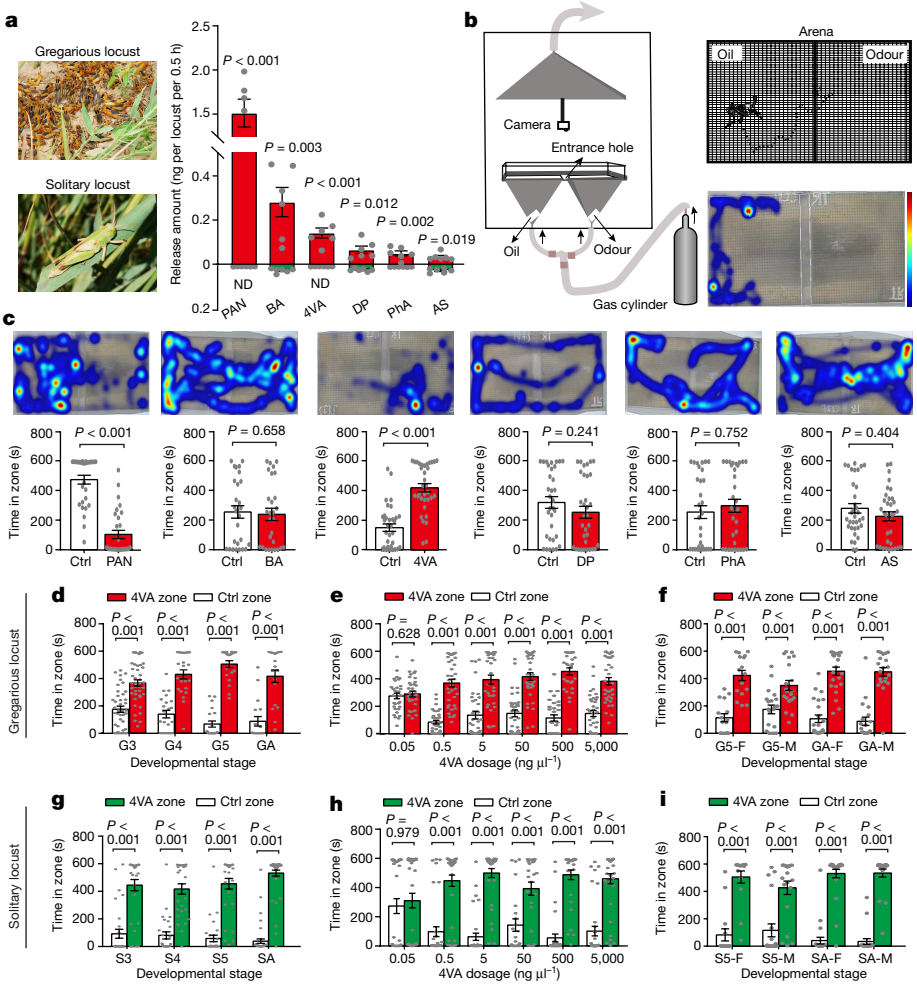

Fig. 1 | 4VA exhibits strong attractiveness to the migratory locust. **a**, Released amounts of phenylacetonitrile (PAN), benzeneacetaldehyde (BA), 4-vinylanisole (4VA), 2,5-dimethylpyrazine (DP), phenethyl alcohol (PhA) and anisole (AS) in gregarious and solitary locusts, respectively. Data are mean ± s.e.m. $n = 6$ biological replicates. *P* values determined by two-tailed unpaired *t*-test. **b**, Setup for behavioural tracing of a locust in a dual-choice arena system. **c**, The responses of gregarious locusts to the six compounds in **a**. $n = 30$ (PAN), 28 (BA), 34 (4VA), 33 (DP), 32 (PhA) and 34 (AS) locusts, respectively. Ctrl, control. **d**, **e**, The behavioural responses of gregarious locusts to 4VA at different stadiums ($n = 41$ (G3), 22 (G4), 20 (G5) and 18 (GA) locusts) (**d**), concentrations ($n = 32$ (0.05 ng µl^{-1}), 36 (0.5 ng µl^{-1}), 35 (5 ng µl^{-1}), 34 (50 ng µl^{-1}), 30 (500 ng µl^{-1}) and 36 (5,000 ng µl^{-1}) locusts) (**e**) and sexes ($n = 16$ (G5-F), 20, (G5-M), 23 (GA-F) and 20 (GA-M) locusts) (**f**). **g**–**i**, The behavioural responses of solitary locusts to 4VA at different stadiums ($n = 30$ (S3), 27 (S4), 28 (S5) and 44 (SA) locusts, respectively) (**g**), concentrations ($n = 32$ (0.05 ng µl^{-1}), 36 (0.5 ng µl^{-1}), 34 (5 ng µl^{-1}), 24 (50 ng µl^{-1}), 32 (500 ng µl^{-1}) and 35 (5,000 ng µl^{-1}) locusts) (**h**) and sexes ($n = 15$ (S5-F), 20 (S5-M), 24 (SA-F) and 20 (SA-M) locusts) (**i**). Behavioural data are mean ± s.e.m. *P* values in behavioural assays were determined by Wilcoxon signed-rank test. F, female; M, male; ND, not detected.

same behavioural paradigm (Fig. 1g–i, Extended Data Fig. 1i–m). Thus, 4VA is an attractive pheromone to the locusts regardless of age, sex and density-dependent phase.

4VA is released by gregarious locusts

On the basis of the emission amounts and dynamics, we found that 4VA is emitted from both the body and faeces, and specifically in gregarious locusts (Fig. 2a). 4VA constituted approximately 4.64% of the volatiles released from the body and 0.58% of volatiles released from the faeces of the gregarious locusts (Extended Data Fig. 2a). 4VA is detectable in almost all tissues and is stored at relatively high levels in the tergum and front, middle and hind legs; the hind legs released more than 56% of the 4VA released from the whole body (Extended Data Fig. 2b). In addition, the 4VA content increased gradually from first- to fifth-instar nymphs of gregarious locusts, and did not show any sex bias within each stadium (Fig. 2b). The release rates of 4VA showed a single major peak and the highest concentration occurred 24 h after ecdysis in the fifth-instar nymphs, when 4VA concentration was 43-fold that in the first 1 h (Extended Data Fig. 2c).

4VA responds to locust density changes

The amount of 4VA emitted from gregarious nymphs increased with the population density (Fig. 2c). Moreover, crowding of solitary locusts (30 locusts per cage, 10 cm × 10 cm × 10 cm) led to the rapid production of 4VA for 24 h, and a gradual increase in production after 48 h and 72 h (Extended Data Fig. 2d). To determine the threshold of 4VA release, we evaluated 4VA release in association with the crowding of solitary locusts at different densities for 72 h. The solitary locusts began to release 4VA when four or five individuals were placed in a cage (Fig. 2d). Thus, the emission of 4VA by locusts is sensitive to changes in population density, and solitary locusts are able to initiate 4VA release when they form groups with very low population density. Thus, 4VA exhibits typical characteristics of aggregation pheromones in locusts.

4VA activates basiconic sensilla

To detect the responses of the peripheral nervous system to 4VA, we carried out single-sensillum recording to test the potentials of four types

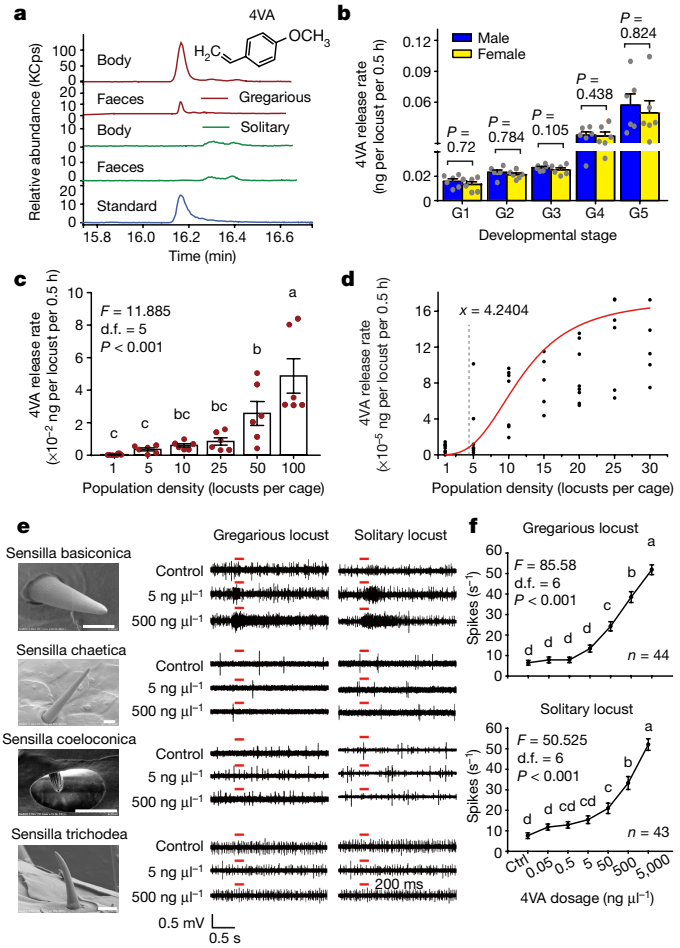

Fig. 2 | Density-dependent 4VA elicits responses specifically from basiconic sensilla. a, Chromatograms of 4VA in locust body and faecal volatiles. **b**, 4VA production from first-instar (G1) to fifth-instar (G5) nymphs. $n = 6$ biological replicates. P values determined by two-tailed unpaired t-test. **c**, 4VA emitted by gregarious locusts at different population densities. $n = 6$ biological replicates. **d**, 4VA emitted by solitary locusts after crowding at different densities. $n = 8$ (1 per cage), 7 (5 per cage), 7 (10 per cage), 5 (15 per cage), 6 (20 per cage), 5 (25 per cage) and 6 (30 per cage) biological replicates. **e**, Representative spike traces of basiconic, chaetic, coeloconic and trichoid sensilla in response to 4VA. The experiments were performed three times. Scale bar, 4 μm. **f**, The responses of basiconic sensilla to 4VA at different concentrations in gregarious ($n = 44$ sensilla) and solitary ($n = 43$ sensilla) locusts. Different letters indicate statistically significant differences between groups using one-way ANOVA (Tukey's multiple comparisons test, $P < 0.05$) (**c**, **f**). Data in **b**, **c**, **f** are mean ± s.e.m.

of sensilla (basiconic, chaetica, coeloconic and trichoid) in response to 4VA. We found strong responses of the basiconic sensilla to 4VA in both gregarious and solitary locusts, but no similar responses in the chaetic, coeloconic or trichoid sensilla (Fig. 2e, Supplementary Table. 1). We recorded and quantified the potentials in the basiconic sensilla using the same concentration gradient used in the behavioural experiments. The basiconic sensilla showed concentration-dependent responses to 4VA in both gregarious and solitary locusts (0.05 to 5,000 ng μl^{-1}) (Fig. 2f). Thus, 4VA elicits responses specifically from basiconic sensilla.

OR35 tunes 4VA with attractive behaviour

Thirty-one migratory locust odorant receptors[10,11] were tested for responses to a panel of 28 odorants using electrophysiological recording in the *Xenopus* oocyte system. In the odorant receptor–odorant matrix, OR35 showed the highest responses to 4VA among the tested receptors (Fig. 3a). The 4VA response of OR35 was approximately 19-fold and 90-fold higher than those of OR64 and OR55, respectively (Fig. 3a). Moreover, OR35 shows the highest responses to 4VA among the locust volatiles reported previously[8,13] (Fig. 3a). Thus, OR35 is a specific olfactory receptor of 4VA in locusts.

We performed immunohistochemistry to determine the location of OR35, and found that OR35 is housed in the basiconic sensilla on locust antennae (Extended Data Fig. 3a, b). To determine whether OR35 mediates 4VA attraction to locusts, we established an *Or35*$^{-/-}$ line using CRISPR–Cas9. An sgRNA targeting the first exon of *Or35* introduced a 2-bp deletion, resulting in a truncated OR35 protein (Extended Data Fig. 4a–d). Protein level of OR35 and responses of basiconic sensilla and antennae decreased significantly in the *Or35*$^{-/-}$ line compared with the wild type (Fig. 3b, Extended Data Fig. 4e, f), indicating the successful establishment of the *Or35*$^{-/-}$ line. Moreover, the homozygous mutant locusts lost their attraction to 4VA compared with the wild-type locusts (Fig. 3c, Extended Data Fig. 4g). In sum, these results indicate that OR35 is a necessary and specific receptor for locust attraction and aggregation.

4VA attracts locusts in the fields

To verify the aggregation effect of 4VA on locusts in the field, we designed a series of behavioural assays and sticky trap experiments. We conducted dual-choice experiments by releasing laboratory locusts on outdoor turf. Twenty lures with volatilized 4VA (4 mg per lure) and 20 lures containing CH_2Cl_2 were placed in a dual-choice arena (1.5 m × 1 m) (Extended Data Fig. 5a). In all 8 arenas, more than 65% of the gregarious locusts moved into the 4VA area; on average, approximately 80% of the gregarious locusts chose the 4VA area (Extended Data Fig. 5b). We then used sticky boards with 4VA or control lures to test the effect of trapping on the outdoor turf. The sticky boards with lures were positioned around the release points (Extended Data Fig. 5c). An average of 26 locusts were trapped by the 4VA lures and an average of 3 locusts were trapped by the control lures 12 h after release; there was no sex bias effect on the attraction to the sticky boards (Extended Data Fig. 5d).

Next, we extended the trapping experiments to the field, in a natural breeding area of the migratory locust in northern China. Across six blocks selected on the basis of vegetation composition and topography, we quantified the background locust density and numbers of locusts captured on 4VA and control (CH_2Cl_2) sticky boards (Fig. 4a, b). According to the linear model Count ∝ Treatment × Block × Locustdensity, locust density and the interaction between treatment and locust density did not significantly contribute to count ($P = 0.8865$ and $P = 0.6124$, respectively), indicating that the background population density of locusts does not affect the efficiency of 4VA attraction. We then applied an updated linear model, Count ∝ Treatment + Block (Extended Data Fig. 7), in which treatment contributed significantly to count ($P = 0.0001$) and explained 7.87% of the sum of squares ($P = 0.00011$) and block significantly contributed to count ($P = 0.0408$) and explained 2.16% of the sum of squares ($P = 0.0408$). The regression line of 4VA was consistently higher than that of the control, demonstrating the attractiveness of 4VA to the wild populations of locusts (Fig. 4c). Furthermore, a generalized linear mixed model (GLMM), Count ∝ Treatment + (1|Block)was applied to estimate the fixed effects of the treatments and the random effect of block on count, as the data follow a Poisson distribution. Treatment significantly contributed to the differences between counts at two levels (4VA versus control, $P = 5.41 \times 10^{-9}$). In addition, the average number of captured locusts per board in the 4VA groups from six blocks was significantly higher than that in the control groups (Fig. 4d). Together, these results show that 4VA is able to aggregate the wild populations of locusts, suggesting its potential for application in locust prediction and management.

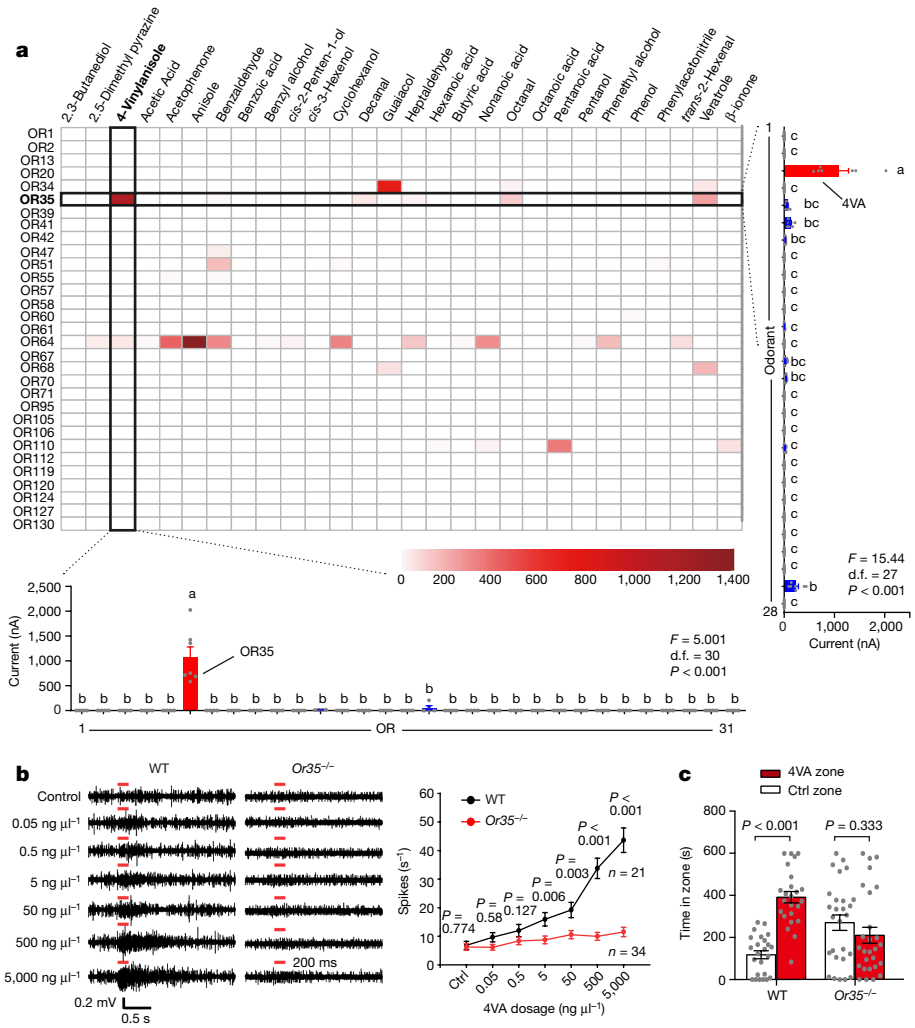

Fig. 3 | OR35 tunes 4VA and mediates the attractiveness to locusts. a, Inward current responses of 31 olfactory receptors stimulated with 28 locust odorants. $n = 7$ frog oocytes per condition. Different letters indicate statistically significant differences between groups using one-way ANOVA (Tukey's multiple comparisons test, $P < 0.05$). **b**, The responses of basiconic sensilla significantly decreased in *Or35*$^{-/-}$ locusts. $n = 21$ (wild-type) and 34 (*Or35*$^{-/-}$) sensilla, respectively. *P* values determined by two-tailed unpaired *t*-test. **c**, The *Or35*$^{-/-}$ locusts lost attraction to 4VA. $n = 24$ (wild-type) and 27 (*Or35*$^{-/-}$) locusts. *P* values were determined by Wilcoxon signed-rank test. Data in **a**–**c** are mean ± s.e.m. WT, wild type.

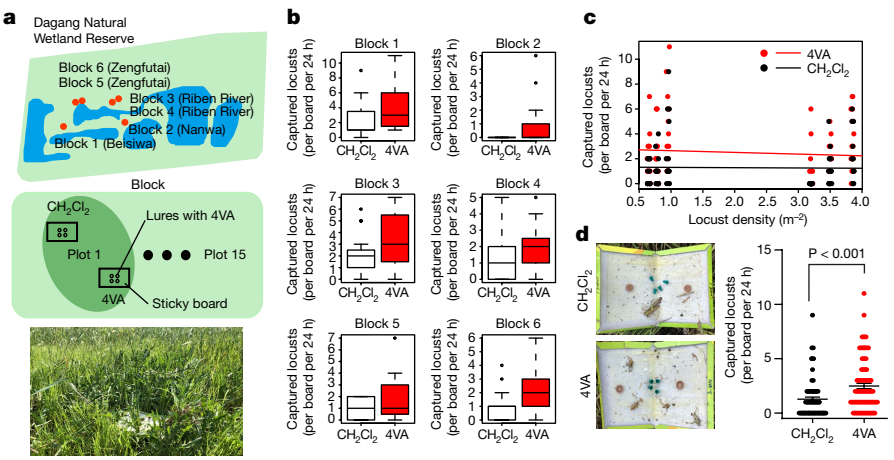

Fig. 4 | 4VA attracts locusts in natural habitats. a, Schematic of the field trapping experiment in natural habitats. **b**, The number of captured locusts in the control (CH_2Cl_2) and 4VA groups in six blocks. In the box plots, the centre line represents the median, whiskers represent maximum and minimum values, and box boundaries represent 75th and 25th percentiles. **c**, The regression line for the 4VA group was stably higher than the line for the control group. **d**, The average number of captured locusts per board in the 4VA group ($n = 90$ boards) was higher than in the control group ($n = 90$ boards). Data are mean ± s.e.m. *P* values determined by two-tailed unpaired *t*-test.

Discussion

Here we report a biological function of 4VA in locusts. The presence of 4VA has previously been reported in locusts[8] and a few plant species[14,15], but has not been widely studied in animals, probably owing to its very low titres in animals other than locusts. The specificity and sensitivity of 4VA sensing in locusts probably facilitate the location of other individuals, aggregation and swarm formation. The other four compounds that were enriched in gregarious locusts in the present study and previously proposed potential aggregation pheromones[6] were unable to attract locust individuals, further supporting the uniqueness of 4VA function in the aggregation of the migratory locusts.

4VA elicited responses specifically from basiconic sensilla. In *Drosophila*, there are several functional classes of basiconic sensilla, which express different olfactory receptor proteins[16,17]. There are 20–50 sensory neurons below the hair base of each basiconic sensillum[18], and the neurons under the different basiconic sensilla are expected to express different members of the 141 olfactory receptor protein family in the migratory locust. It has been suggested that, as in other insects, there is functional heterogeneity among the different basiconic sensilla; however, this needs further investigation. Nevertheless, a previous study on *Orco*-mutant locusts suggested that the responses of coeloconic sensilla is mediated by ionotropic receptors[12]. Indeed, the broadly tuned receptor LmigOR3—which is expressed in the trichoid sensilla of the migratory locust—responds to ketones, esters and heterocyclic compounds[19]. The chaetic sensilla are thought to function as taste and mechanoreceptors in the locust[18].

In this study, we have identified OR35 as the olfactory receptor for 4VA and demonstrated its critical role in attracting locusts to 4VA. The high sensitivity of OR35 for 4VA provides locusts with a specific and sensitive means to search for and join groups of other locusts. A specific molecule and a dedicated circuit developed in locust aggregation indicate the ecological importance of aggregation in locust evolution. Although the antennae of the OR35 mutant line retained weak responses to 4VA, they lost the aggregative behaviour. The remaining response may arise from the binding of 4VA to broadly or non-specifically tuned olfactory receptors. OR64 and OR55 are potential broadly tuned and nonspecific receptors for 4VA.

As the most widely distributed and one of the most dangerous locust species[20], the migratory locust remains a serious threat to agriculture worldwide[21]. Our present findings, in particular the 4VA emission threshold, the identification of the olfactory receptor for 4VA and successful field trapping provide clues towards the development of novel approaches for prediction and control of locust outbreaks. First, synthetic 4VA or its analogues could be deployed to attract locusts in trapping belts, where they could be killed using a fungal pathogen[22,23] and pesticides, thereby avoiding broad utilization of chemical insecticides. Spraying of 4VA antagonist could prevent locust aggregation and migration. Moreover, 4VA could be used to monitor the population dynamics of locusts in the field to warn against locust outbreaks. Finally, OR35 mutant locusts could be generated using CRISPR–Cas9 and released into the field to establish non-gregarious locust populations for long-term population management, subject to biosecurity evaluation.

Our present results suggest that 4VA acts as an aggregation pheromone for the migratory locust, although *L. migratoria* and desert locusts (*Schistocerca gregaria*) share similar odour composition[6,8,24] and morphological and molecular characteristics of olfactory circuits[10,18,25,26]. The two locust species belong to different subfamilies (Oedipodinae and Cyrtacanthacridinae) and different reaction norms of phase polyphenism in these two species are thought to have evolved independently[27], which may explain the specific adoption of 4VA by *L. migratoria*. Alternatively, the very low release rate of 4VA and the threshold sensitivity of the chromatographic and mass spectrometric methods and instruments used in previous studies may have contributed to the inability to detect 4VA. Thus, conservation and/or divergence of 4VA emission and decoding in different locust species needs further investigation.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2610-4.

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Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Insects

The gregarious and solitary locusts (*Locusta migratoria*) used in the experiments were maintained in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China. In brief, the gregarious locusts were reared in cages (30 cm × 30 cm × 30 cm) with 800 to 1,000 first-instar insects per cage in a well-ventilated room. The solitary locusts were raised in another room, each in a separate ventilated cage (10 cm × 10 cm × 25 cm). The gregarious and solitary locusts were maintained for at least three generations before the experiments were conducted. All locusts were cultured under the following conditions: a 14 h:10 h light:dark photoperiod, temperature of 30 ± 2 °C, relative humidity of 60 ± 5%, and a diet of fresh greenhouse-grown seedlings and bran[28].

Xenopus laevis

The frogs (female adults more than 3 years old) were raised in a box with purified water at 18–21 °C and fed on pig liver. All experiments were conducted under the license of the Animal Experimental Committee of the Institute of Zoology (AECIOZ), Chinese Academy of Science (IOZ20170071).

Volatile collection of gregarious and solitary locusts

The volatiles of fifth-instar gregarious and solitary nymphs were collected by static solid phase microextraction (SPME) for a short period (30 min), as described in our previous study[8]. In brief, a fibre (PDMS/DVB 65 μm) was introduced into a glass jar (10.5 cm high × 8.5 cm internal diameter) approximately 1 cm above a stainless steel lid (9 cm in diameter with holes of 2 mm diameter and 2 mm apart), which served as a barrier to confine a group of 10 nymphs in the first or second instar or 5 individual locusts in the third to fifth instar from different locust colonies. To determine the relative amounts of odours from bodies and faeces, five 5th-instar gregarious or solitary nymphs, or 2 g of faeces from 5th-instar gregarious and solitary nymphs were placed in the bottom of the jar to avoid direct contact with the fibre. The SPME volatiles collected from an empty glass jar for 30 min served as a control. The fibres with adsorbed odours were subjected to chemical analyses.

4VA was extracted from various locust tissues or organs via solvent extraction in accordance with a previously described method[9]. In brief, fifth-instar gregarious nymphs at 48 h postecdysis were used in all experiments. Approximately 20–100 mg of fresh tissue sample was transferred into a 1.5-ml Eppendorf tube containing 0.2 ml of ultrapure water and homogenized thoroughly for 30–60 s with an electric pestle (Kontes). Cuticle tissues were homogenized in liquid nitrogen. Hexane (200 μl) containing 4 ng of 4-methyl PAN was added to each sample as an internal standard. The samples were agitated for 10 min at 4 °C and then centrifuged at 13,000 rpm for 10 min. The top layer of the hexane phase was subsequently transferred to a 150-μl insert tube in a 2-ml screw cap vial (Waters) with a Teflon or rubber septum and stored at −20 °C until further analysis.

To determine the level of 4VA emissions by the fifth-instar nymphs at densities of 1–100 individuals, gregarious locusts were cultured in Perspex cages (15 cm × 15 cm × 10 cm). To determine the threshold of 4VA production in the solitary locusts during crowding, we crowded 30 solitary 5th-instar nymphs after ecdysis in a small cage (10 cm × 10 cm × 10 cm) for 24 h, 48 h or 72 h. Then we crowded solitary nymphs at different densities of 5, 10, 15, 20, 25, and 30 for 72 h. The SPME volatiles collected from a solitary nymph for 30 min served as a control. The fibres with adsorbed odours were subjected to chemical analysis. Equal numbers of females and males were pooled together in each sample for chemical analysis.

Chemical analysis

A Bruker GC system (456-GC) coupled with a triple quadrupole (TQ) mass spectrometer (Scion TQ MS/MS, Bruker Daltonics) equipped with a DB-1MS column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness, Agilent Technologies) was used to quantify the volatile compounds in the SPME samples. The same thermal program was adopted as described above for chemical analysis. The gas chromatography with tandem mass spectrometry electron impact source was operated in multiple-reaction monitoring mode with the mass spectrometry source temperature at 200 °C, manifold temperature at 40 °C, transfer line temperature at 250 °C and collision-induced dissociation with argon as the collision cell gas at a pressure of 2.0 Torr. The injector temperature was maintained at 250 °C with a constant flow rate of 1.0 ml min^{-1} of helium. The fibre was inserted into the inlet operated in splitless injection mode and held for 1 min. A Bruker chemical analysis MS workstation (MS Data Review, Data Process v.8.0) was used to analyse and process the data. Mixed samples consisting of standard compounds standards (purities ≥ 95%, Sigma-Aldrich) at different dosages (0.1, 1, 10 and 100 ng μl^{-1}) were used as external standards to develop the standard curves to quantify the volatiles. The same thermal program and multiple-reaction monitoring method were used.

Locust behavioural responses and video-tracking system

We used a vertical airflow olfactometer as described in a previous study[9]. In brief, two square tapered stainless steel funnels with a bottom length of 28 cm were inverted into stainless steel platforms. The bottom of each funnel was equipped with stainless steel sheets of uniform size and 28 cm in length. These plates had small holes 2 mm in diameter and 5 mm in interval. An organic glass chamber (60 cm × 30 cm × 30 cm) was used to enclose the area between the two plates. The table was placed in an observation room (150 cm × 100 cm × 180 cm) with a ventilation system and a video camera installed on the ceiling. Six 22-W white fluorescent tubes were located on the side wall to provide uniform lighting. Air conditioning was used to keep the indoor temperature at 30 ± 2 °C at all times. Each funnel was connected to an air purification system by a Tygon tube (internal diameter, 0.7 cm). The behavioural device provides two choices for locusts to be tested: clean vertical airflow in the control zone and adjacent vertical airflow filled with synthetic chemicals in the odour area. For the series of behavioural tests, locusts entered the arena through a small door in the middle of the Plexiglas chamber and were allowed to stay in the olfactometer for 10 min in the presence of different odours. The diluted odorant was coated on filter paper (3 cm × 3 cm; Whatman No. 1), and mineral oil was used as a control agent in another funnel. After testing 10–15 individuals, the positions of both sides of the funnel were reversed to prevent position deviation. Then the funnel was heated to 180 °C for 2 h to remove odorous residues. By using a video camera (Panasonic), combined with VCR software (v.2, Noldus Information Technology), we can capture locust behavioural activities within 10 min at 25 frames s^{-1}. Video was analysed using EthoVision XT software (v.11.5, Noldus Information Technology) to objectively measure the total time spent on each side (unit: s) and the total distance of movement (moving distance, unit: cm).

The gregarious and solitary nymphs at 48 h postecdysis were used in all comparisons. The concentrations of different odorants (50 ng μl^{-1}), including 4-vinylanisole (4VA), phenylacetonitrile (PAN), benzeneacetaldehyde (BA), phenethyl alcohol (PhA), veratrole (VT), 2,5-dimethylpyrazine (DP), anisole (AS), guaiacol (GA), phenol (PN) Mix 1 (GA: PN: VT, 13:83:4), and Mix 2 (GA: PN: VT, 27:70:3), close to the physiological emission amounts of gregarious locusts in the reared cages, were chosen to conduct the behavioural experiments. Equal numbers of females and males were tested in each behavioural assay.

Single-sensillum recordings

To investigate the response of the antennae to 4VA, we conducted the single-sensillum recordings (SSRs) of fifth-instar nymphs. Each locust was placed in a plastic tube 1 cm in diameter, and its antennae were fixed with dental wax. A tungsten wire electrode was electrolytically sharpened by 10% $NaNO_2$. The recording electrode was inserted into the bottom of the sensilla (one to four lengths from the top of the antenna) through a micromanipulator (Narishige). The reference electrode was inserted into the eye. Recording electrodes were connected to amplifiers (IDAC4, Syntech). The frequency variation of each pulse at 0.2 s was recorded and calculated using Autospike32 (v.3.9, Syntech). Chemical substances as SSR stimulants included mineral oil as the blank, which was used to dilute 4VA by 1/10 (v/v). A piece of filter paper (Whatman) was placed in a 15-cm Pasteur glass tube and 10 µl of volatile solution was added to the filter paper. Equal numbers of females and males were used for the electrophysiological recording.

Scanning of sensilla using electron microscope

Antennae of locusts from each sex were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS; 0.1 M, pH 7.2) for 4 h at 4 °C then rinsed with PBS (three 15-min washes). Subsequently, the antennae were post-fixed in 1% osmium tetroxide for 1 h at 4 °C before being dehydrated in a graded ethanol series (30, 50, 70, 80, 90 and 100%, 15 min each) and ultrasonically cleaned for 20 s in 100% ethanol. After immersion in the transitional solvent isoamyl acetate for 30 min, the antennae were dried using a CO_2 critical point drier (model HCP-2, Hitachi) and then sputter-coated with gold (model IB-5 ion sputterer, Hitachi). Antennal sensilla were scanned using scanning electron microscopy (model S-4800, Hitachi).

Expression of odorant receptors in *Xenopus laevis* oocytes and electrophysiological recordings

Vectors (pT7TS) with full-length olfactory receptor (*Or*) sequences were used to synthesize complementary RNAs (cRNAs) with the mMESSAGE mMACHINE T7 kit (Ambion) following the manufacturer's instructions. The primers for *Or* full-length amplification and expression were listed in Supplementary Table 2.

Frogs were anaesthetized by bathing in a mixture of ice and water for 30 min, and the oocytes were surgically collected before the experiments. Each 5 ml of stage V–VII oocytes was treated with 20 mg of collagenase type I (GIBCO) in 10 ml of washing buffer (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$ and 5 mM HEPES, pH 7.6) for 0.5–1 h at room temperature. After culture overnight in an incubator at 18 °C, oocytes were microinjected with 25 ng *Or* cRNA and 25 ng *Orco* cRNA. The 31 *Or* cRNAs were individually expressed with *Orco* in oocytes. The injected oocytes were incubated for 2 days at 18 °C in 1 × Ringer buffer (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, 0.8 mM $CaCl_2$ and 5 mM HEPES, pH 7.6) supplemented with 5% dialysed horse serum, 50 mg ml^{-1} tetracycline, 100 mg ml^{-1} streptomycin and 550 mg ml^{-1} sodium pyruvate. The two-electrode voltage-clamp technique was used to record whole-cell currents. Signals were amplified with a two-electrode voltage clamp. The responses of all ORs to 28 locust odorants were recorded and analysed. Data acquisition and analysis were carried out with Digidata 1440A and pCLAMP 10.6 software (Axon Instruments). The details of all odorants are listed in Supplementary Table 3.

Immunofluorescence of OR35 in the locust antennae

The dissected antennae were embedded in freezing medium Tissue-Tek O.C.T. Compound (Sakura Finetek) and rapidly frozen at −70 °C. Sections (10 µm) were prepared at −20 °C (Leica CM1950), thaw mounted on SuperFrost Plus slides (Menzel-Gläser) and air dried for 15 min. The immunofluorescence was performed according to the previously described protocol[29] with slight modifications. After fixation in 4% formaldehyde at room temperature for 1 h, the sections were washed with 0.1 M PBS (pH 7.2) twice for 15 min each, and then incubated in 0.1 M PBS containing 5% normal goat serum (NGS, Boster) for 1 h at room temperature. The primary anti-OR35 antibody (custom made, see 'Protein preparation and Western blot analysis' for details) was diluted at 1:500 in 0.1 M PBS containing 2% NGS. Incubation with primary antibodies lasted for 24 h. The tissues were washed with 0.1 M PBS three times for 15 min each and subsequently incubated with the secondary antibody, Goat anti-rabbit antibody Alexa Fluor 488 (1: 500, Life Technology, A11034) for 1 h at room temperature. After washing three times, the nucleus of locust antennae was labelled by Hoechst33342 (Life Technology). After washing three times, the tissues were mounted in anti-fade fluorescence mounting medium. The negative serum of OR35 from rabbit was used as the negative control. The fluorescence was detected using a Zeiss LSM 710 confocal microscope (Zeiss).

Establishment of the OR35 mutant line using CRISPR–Cas9

The establishment of OR35 mutant locusts by CRISPR–Cas9 was performed as previously described[12]. In brief, the embryos of locusts were collected from egg pods, washed with 75% ethanol, and were placed on 1% agarose gel. The purified Cas9 protein and guided RNA were mixed to final concentrations of 400 and 150 ng μl^{-1}, respectively (13.8 nl), and were injected in the embryos using a nanolitre injector (World Precision Instruments) with a glass micropipette tip under an anatomical lens. Then, the embryos were placed in a 30 °C incubator for approximately 14 days until the locusts hatched. The first-instar nymphs were placed in the cages with a 14 h:10 h light:dark cycle and sufficient food. We collected part of adult legs and lysed them with a 45 µl NAOH buffer (50 mM) at 95 °C in a PCR machine for 30 min and added 5 µl Tris-HCL (pH 8.0, 1 M). Then, we used a 2 µl template to amplify the targeted fragments and sequenced the fragments to identify whether the mutants were generated. In 88 detected locust individuals, 8 locust mutants were found in exon 1 (mutation efficiency: 9.09%). To further investigate the exact mutation models, we performed TA cloning and Sanger sequencing for PCR amplification of all mutation locust individuals and found five deleting base types and one insertion type (Extended Data Fig. 4a). The mRNA of OR35 showed a 2-bp deletion (Extended Data Fig. 4b). The locusts were bred to generate a homozygous mutant line. Mutations in G0 locusts were evaluated by using PCR-based genotyping. G0 mutants were crossed with the wild type to obtain G1 offspring. G1 locusts, one DNA strands containing 2 bp-deleted modifications, were in-crossed to establish stable lines. For the expanding mutant population, 2 bp-deleted homozygotes of G2 locusts were in-crossed to generate homozygotes G3 animals. Finally, OR35 homozygous mutant lines were established successfully (Extended Data Fig. 4c).

Protein preparation and western blot analysis

Six samples of antennae (1 pair per sample) of wild type and OR35 mutants were collected and homogenized in TRIzol reagent (Life Technology) and protein for western blot analysis was extracted following manufacturers' instructions, respectively. For protein analyses, affinity-purified polyclonal antibodies against OR35 (rabbit) was developed (ABclonal). The protein samples (100 µg) were separated by gel electrophoresis and then transferred onto polyvinylidene difluoride membranes (Millipore). Non-specific binding sites on the membranes were blocked with 5% bovine serum albumin. The blots were separately incubated with the primary antibody (rabbit anti-OR35 antibody, 1:500; rabbit anti-GAPDH antibody, 1:5,000) in TBS-T overnight at 4 °C. After incubation, the membranes were washed, incubated with anti-rabbit IgG secondary antibody (1:5,000) (EASYBIO Technology) for 1 h at room temperature, and then washed again. Immunological blot was subsequently detected by an ECL kit (Thermo Fisher, 34096). The intensities of the western blot signals were quantified using densitometry.

Electroantennograms

We conducted electroantennograms (EAGs) of OR35 mutant locusts and wild type. The antennae of nymphs were cut off at the bases of flagella and fixed between two electrodes by electrode gel Spectra 360 (Parker). The signal was sent from an amplifier (IDAC4, Syntech) to a computer. Syntech EAG software v.2.6c was used to record and analyse the response. A stimulus controller (Syntech CS-05) could generate 30 ml s^{-1} continuous air flow. The stimulation time was 1 s. The blanks were used at the beginning and end of the stimulus series. The average blank EAG amplitude was subtracted from the stimulus EAG amplitude. The stimuli used in EAG were the same as SSR. Equal numbers of females and males were used for the electrophysiological recording.

Field trapping experiment

On artificial turf, we designed a series of behavioural assays and sticky trap experiments. In a dual-choice arena (1.5 m × 1 m), we placed 4VA traps (20 lures) and control traps (20 lures). The locusts were released at the release zone. After 30 min, we counted the numbers of locusts in each zone. Eight independent behavioural assays were conducted at different locations.

For the trap experiments on artificial turf, we used 10 sticky traps (5 with 4VA lures and 5 with control lures), there was a distance of 2 m between every 2 sticky traps. The locusts were released at the centre point. After 12 h, each trapping board was checked carefully, and the sexes and numbers of locusts were recorded. These behavioural assays were independently repeated 8 times at different locations.

The location of field experiments is a breading area of the migratory locust and locate in the natural wetland reserve of North Dagang area (38° 36′ N–38° 57′ N, 117° 11′ E–117° 37′ E, approximately 660 ha), southeast of Tianjin, China. We selected 6 blocks in 4 sites for our field experiments. Beisiwa (38° 46′ 32″ N, 117° 16′ 29″ E) (1 block) is mainly covered with *Setaria viridis*, *Aeluropus sinensis* and a small proportion of *Sonchus wightianus*. Zengfutai (38° 47′ 24″ N, 117° 17′ 54″ E) (2 blocks) is mainly covered with *S. viridis* and *Phragmites australis*. Nanwa (38° 47′ 31″ N, 117° 18′32″ E) (1 block) is mainly covered with *P. australis*, *S. viridis* and small proportion of *Artemisia scoparia*. Riben River (38° 47′ 3″ N, 117° 18′ 11″ E) (2 blocks) is mainly covered with *S. viridis*, *P. australis* and a small proportion of *Cucumis bisexualis*. Our experiments were carried out in August, when the second generation occurred. First, the population density of each site was investigated by counting the individual locusts and sampled in a square plot (1 m^2) covered by a sampler composed of two halves of a square. Each edge and middle part of one piece of nylon mesh 1 m high × 2 m wide were fixed on 1.2-m-high bamboo sticks to form a half of sampler. Two investigators held one half of the sampler and four investigators could randomly combine a square together to form a plot where locusts were enclosed. We randomly sampled 20 plots in an area approximately 30 m wide × 100 m long and calculated the population density of each site.

The sticky boards (30 cm in length and 20 cm in width) with 4 4VA lures or 4 control lures were used to investigate extent of attractiveness to locusts. The traps were randomly positioned in each plot in the field. One plot consisted of one 4VA traps and one control trap in 2 m apart. Fifteen plots were located in each block. After 24 h, each trapping board was checked carefully, and the sexes, numbers, and development stages of each locust were recorded.

Each lure (1/10,000 release rate) was added with 4 mg 4VA (40 μg μl^{-1} 4VA in 100 μl CH_2Cl_2), volatilized for 30 min and stored in a sealed bottle. This dosage was determined according to the densities of locust swarms, the physiological emission levels of 4VA in gregarious locusts, and the area of the field experiments. The control lures with 100 μl CH_2Cl_2 were volatilized for 30 min and stored separately with 4VA lures to avoid contamination.

Statistical analysis

The content differences between gregarious and solitary locusts, and between females and males at different developmental stages, are compared using two-tailed unpaired *t*-test. The content differences across different tissues, different densities and different durations after ecdysis in gregarious locusts, and across different durations after crowding of the solitary locusts were analysed using one-way ANOVA with Tukey's multiple-comparisons test. The behavioural data of locusts were analysed for statistical significance using the Wilcoxon signed rank test (mean ± s.e.m.). The differences in SSR responses between the control and 4VA at different concentrations were compared using one-way ANOVA with Tukey's multiple-comparisons test. The data from the oocyte recordings were analysed using one-way ANOVA with Tukey's multiple-comparisons test. The differences in EAG and SSR between wild-type and OR35 mutant were analysed with two-tailed unpaired *t*-test. The statistical analysis of olfactory preference on artificial turf was performed according to previous work, and the *G*-test for goodness of fit was used to determine the significance of the divergence from an expected 50% decision for the 4VA or control treatment. The counts of locusts preferring the volatile or air control were used for this analysis. The average data from 8 arenas was presented as $p \pm$ s.e.m. The standard error (SE) of locusts' preference was calculated as $\sqrt{p\ (1-p)/n}$, in which p is the proportion of locusts that were attracted by 4VA and n is the number of locusts that were tested. The differences between 4VA and the control of the trapping experiments on artificial turf were analysed using two-tailed unpaired *t*-test. Differences were considered significant at $P < 0.05$. All statistics were analysed using SPSS 18.0 (SPSS). The fitting curve for calculating the threshold of 4VA production was conducted using Software Origin 2018 (OriginLab). The logistic fit was chosen for the fit curve based on related index. The fit formula and related indexes are listed in Supplementary Table. 4.

A linear model (Count ∝ Treatment × Block × Locustdensity) was applied for analysing the field data. The coefficients of Treatment, Block, Locust density, and the interaction of treatment and background were all estimated. Then an updated linear model, Count ∝ Treatment + Block,including the significant coefficients Treatment and Block, was applied to evaluate the contribution of these two factors to the variance. The residuals were calculated for the evaluation of this linear model (Extended Data Fig. 7). A generalized linear mixed model (GLMM) was also applied for data analysis, as the count data exhibited a Poisson distribution. These analyses were performed using the R platform (v.3.2.3). All the statistical results throughout this study are listed in Supplementary Table 4.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Sequence data of *Or35* that supporting the findings of this study have been deposited in GenBank with the accession code KP843355. All data supporting the findings of this study are available within the Article, the Extended Data and the Supplementary Information files. Source data are provided with this paper.

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Author contributions L.K., X.G. and J.W. conceived and designed the experiments. X.G., Q.Y. and J.Y. performed behavioural assays in the laboratory. X.G., Q.Y., J.W. and J.Y. performed chemical collection and analysis. Q.Y. performed electroantennograms and single sensillum recordings. D.C. performed scanning of sensilla using electron microscopy, and electrophysiological recording in *Xenopus* oocyte system. X.G. performed immunohistochemistry of OR35 in locust antennae. X.G., Q.Y., D.C. and X.W. performed experiments on OR35 mutant locusts. X.G., Q.Y., J.W., J.Y. and X.W. performed experiments in artificial turf and fields. X.G., Q.Y., P. Y. and L.K. contributed to data analysis and interpretation. X.G., Q.Y., J.W. and L.K. wrote the manuscript.

Competing interests The authors declare no competing interests.

Additional information

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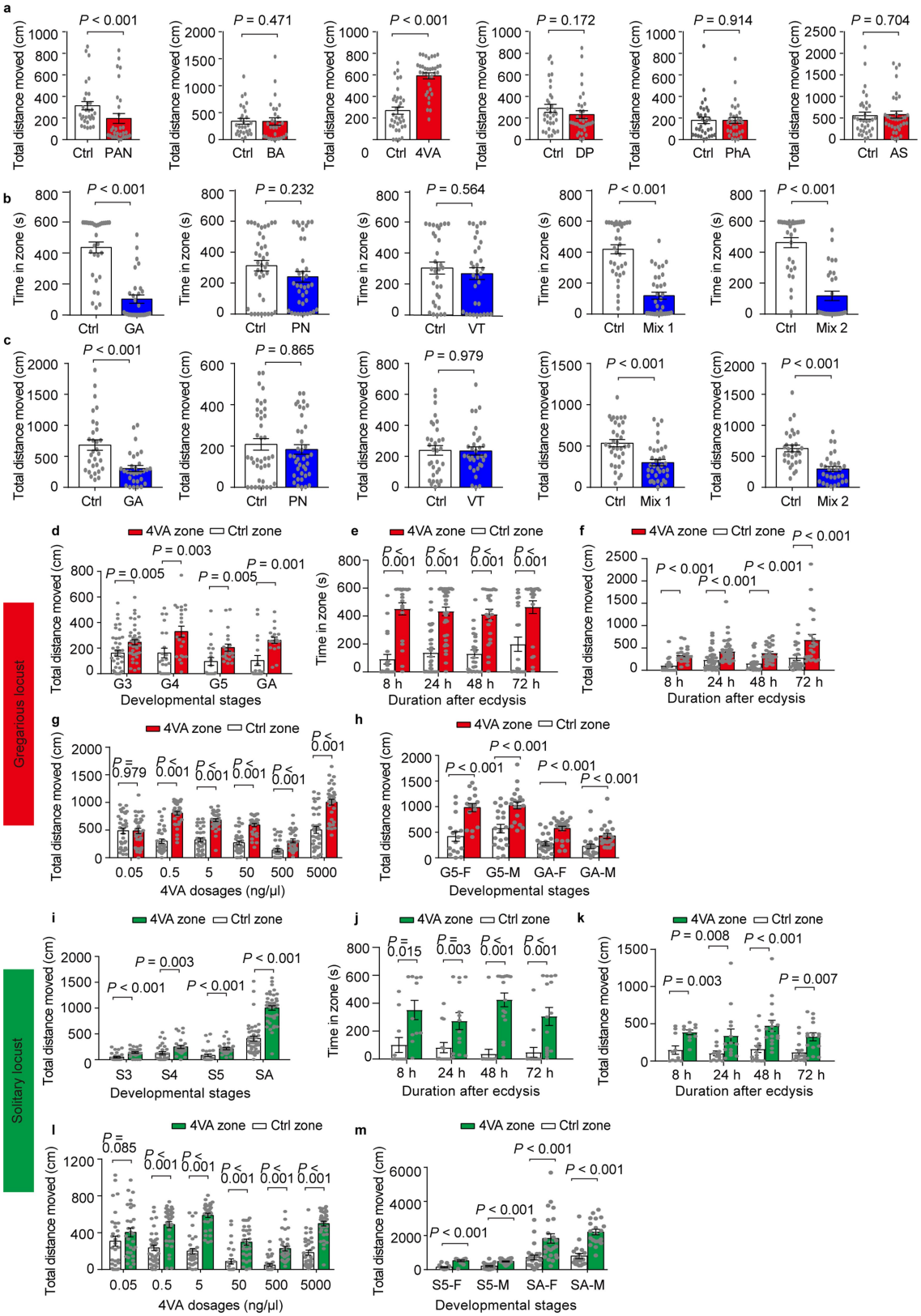
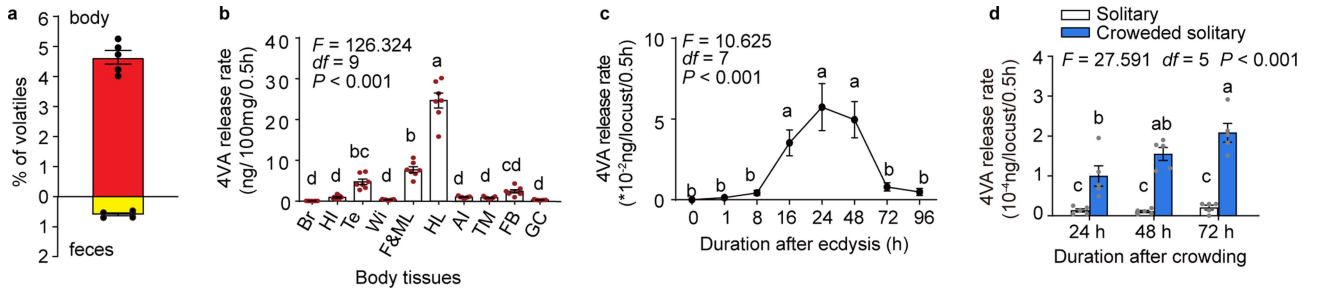
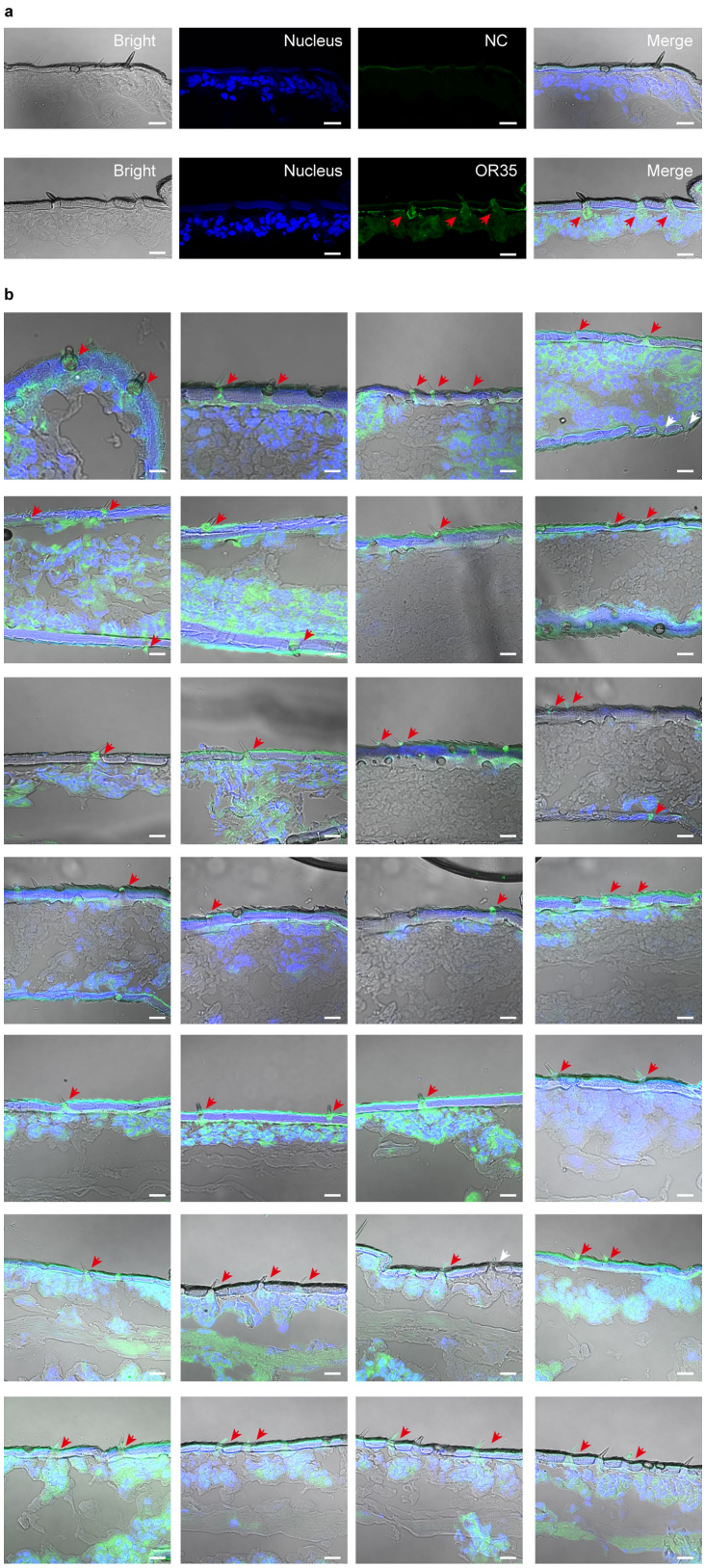


Extended Data Fig. 1 | See next page for caption.

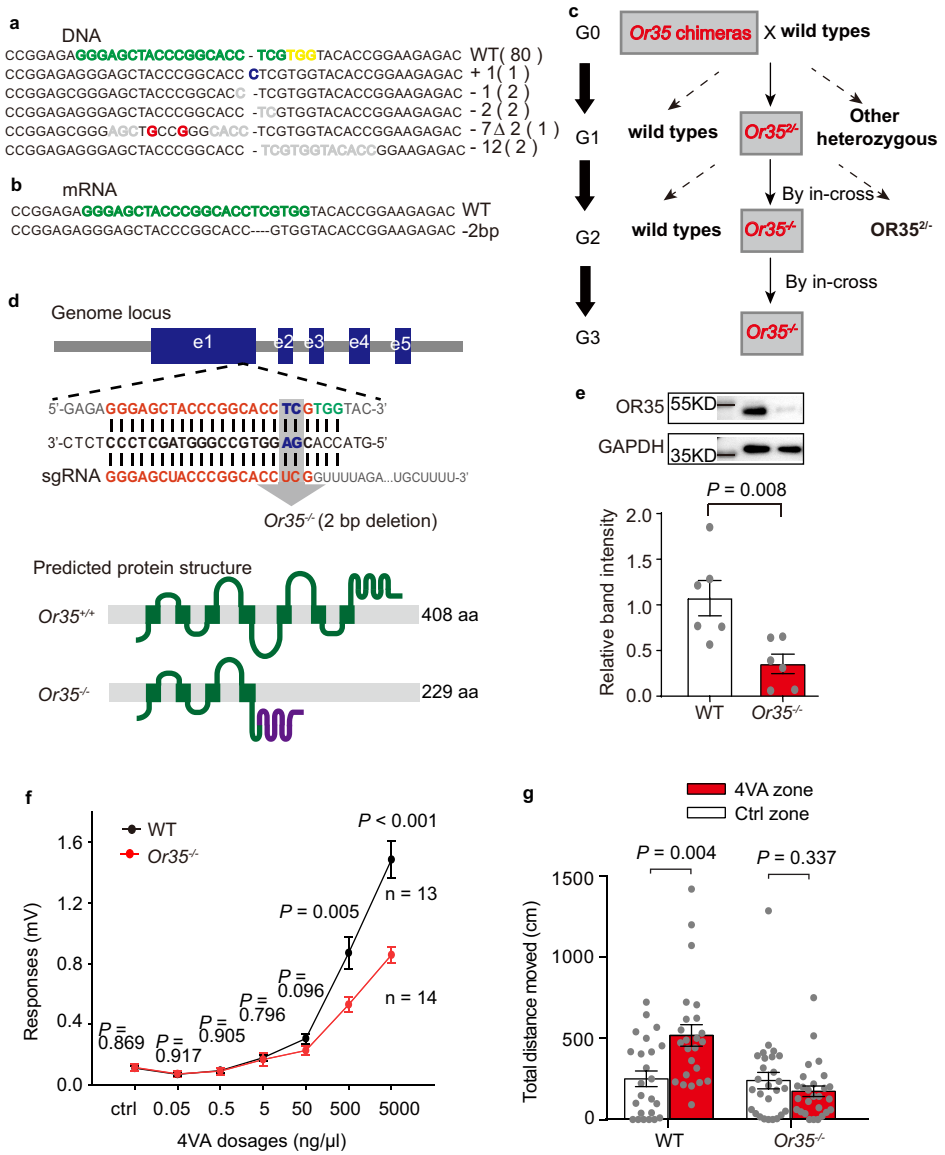
Extended Data Fig. 1 | 4VA has strong attractiveness to the migratory locust. a, The total movement distances of gregarious locusts in PAN, BA, 4VA, DP, PhA, and AS zone, compared with those in control zone. *n* = 30, 28, 34, 33, 32, and 34 locusts, respectively. **b**, **c**, The total duration and movement distances of gregarious locusts in GA, PN, VT, Mix 1, and Mix 2 zone, compared with those in control zone. *n* = 30, 39, 32, 35, and 30 locusts, respectively. **d**, The total movement distances of gregarious locusts at different stadiums in 4VA zone. *n* = 41, 22, 20, and 18 locusts, respectively. **e**, **f**, The duration and the total movement distances of gregarious locusts at 8 h, 24 h, 48 h and 72 h after ecdysis in 4VA zone, compared with those in control zone. *n* = 21, 37, 25 and 22 locusts, respectively. **g**, The total movement distances of gregarious locusts in 4VA zone with different concentrations. *n* = 32, 36, 35, 34, 30, and 36 locusts, respectively. **h**, The total movement distances of gregarious locusts across different sexes in 4VA zone. *n* = 16, 20, 23, and 20 locusts, respectively. **i**, The total movement distances of solitary locusts at different stadiums in 4VA zone. *n* = 30, 27, 28, and 44 locusts, respectively. **j**, **k**, The duration and the total movement distances of solitary locusts at 8 h, 24 h, 48 h, and 72 h after ecdysis in 4VA zone, compared with those in control zone. *n* = 11, 14, 17, and 15 locusts, respectively. **l**, The total movement distances of solitary locusts in 4VA zone with different concentrations. *n* = 31, 36, 34, 29, 32, and 35 locusts, respectively. **m**, The total movement distances of solitary locusts across different sexes in 4VA zone. *n* = 15, 20, 24, and 20 locusts, respectively. Behavioural data are presented as mean ± s.e.m. *P* values in behavioural assays were determined by Wilcoxon signed rank test.



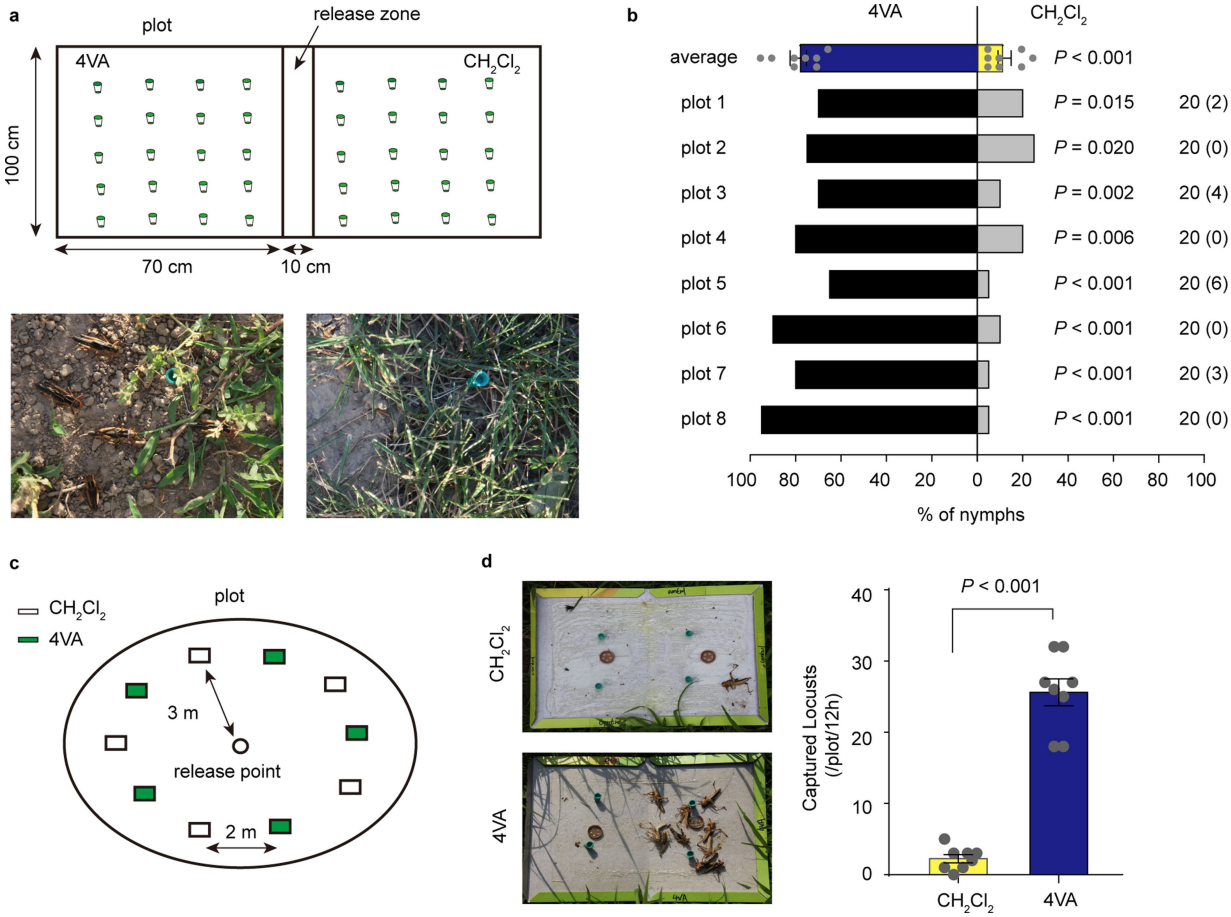
Extended Data Fig. 2 | The emission traits of 4VA in locusts. a, The relative amounts of 4VA in the body and fecal volatiles of gregarious locusts, respectively. $n = 5$ biological replicates. **b**, 4VA emission amount in brain (Br), head integument (HI), tergum (Te), wing (Wi), foreleg and midleg (F&ML), hindleg (HL), abdominal integument (AI), thorax muscle (TM), fat body (FB), gastric caeca (GC) of gregarious locusts. $n = 7$ biological replicates. **c**, The 4VA fluctuation in gregarious locusts after ecdysis. $n = 5$ biological replicates. **d**, The emission amounts of 4VA in the solitary locusts after crowding for different durations. $n = 5$ biological replicates. Data are presented as mean ± s.e.m. Different letters indicate statistically significant differences between groups using one-way ANOVA (Tukey's multiple comparisons test, $P < 0.05$) (**b**–**d**).



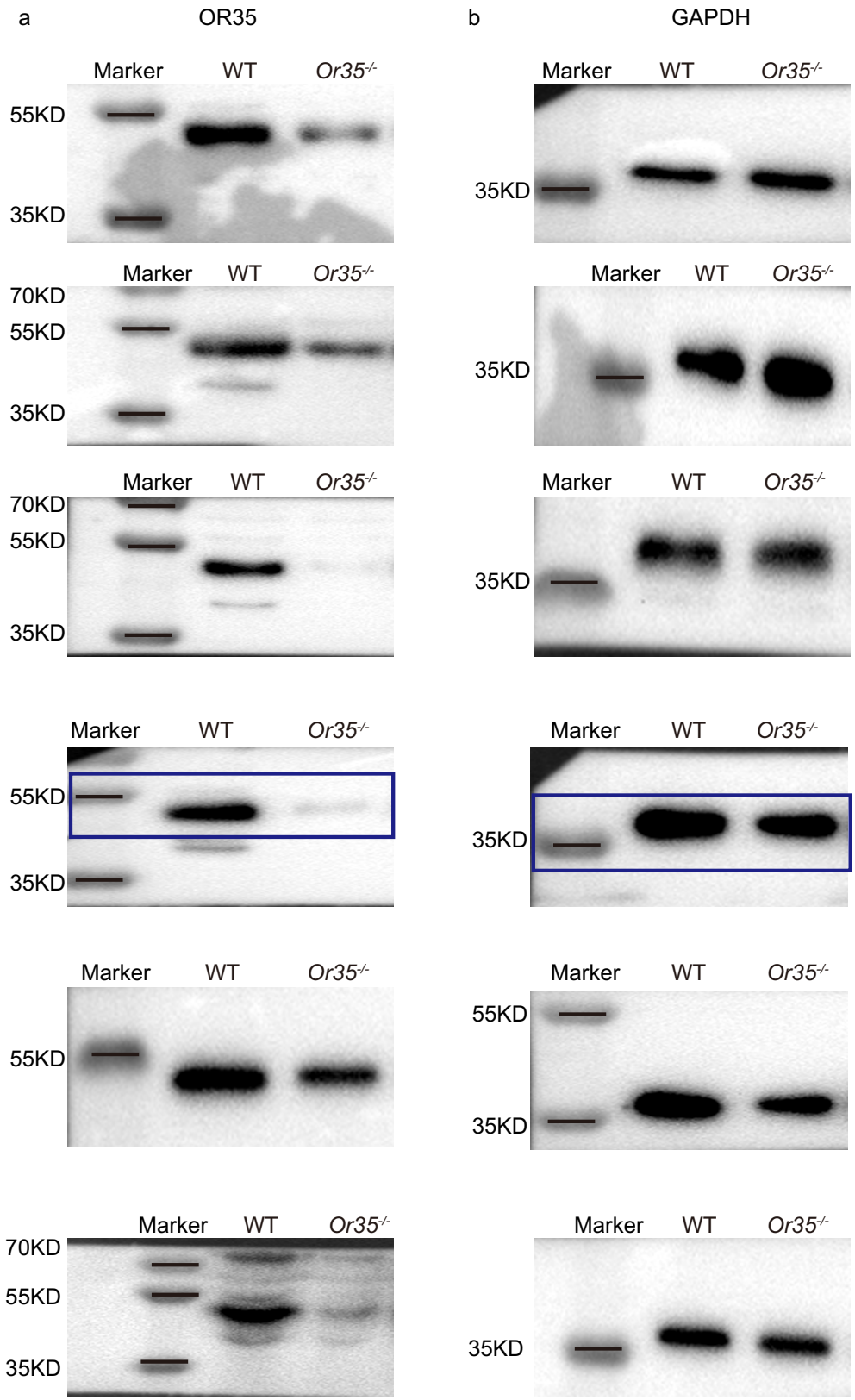
Extended Data Fig. 3 | OR35 located in most basiconic sensilla. a, Confocal microscopy images of OR35 location in locust antennae. 'Bright' indicated bright-field microscopy image. NC, negative control. **b**, In total 53 basiconic sensilla, 50 sensilla showed signals (Red arrow), and only 3 sensilla did not show signals (white arrow). These experiments in were performed three times. Scale bar, 20 μm.



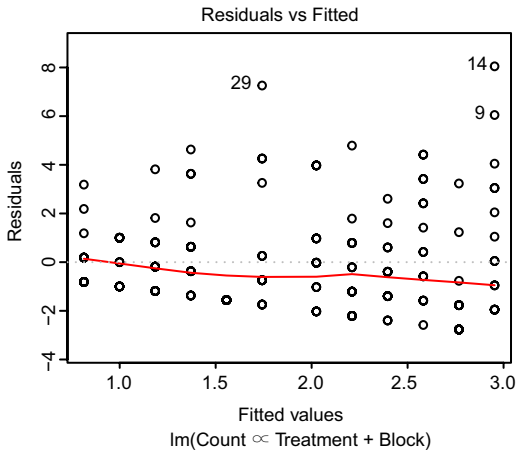
Extended Data Fig. 4 | Homozygous generation of OR35 mutant line. a, Five types of mutation of *Or35* was detected at the DNA level. **b**, A 2 bp-deletion of *Or35* was detected at the mRNA level. **c**, The establishment of OR35 mutant locusts through CRISPR–Cas9. **d**, Schematic of screening strategies to obtain 2 bp-deleted homozygous. $Or35^{2/-}$, one DNA strands with 2 bp-deletion; $Or35^{-/-}$, two DNA strands with 2 bp-deletion. **e**, The protein level of OR35 in mutant line significantly decreased compared with wild types. Data are presented as mean ± s.e.m. $n = 6$ biological replicates. *P* values in behavioural assays were determined by two-tailed unpaired *t*-test. For gel source data, see Extended Data Fig. 6. GAPDH were run on the same gel as loading controls. **f**, The EAG responses to 4VA at different concentrations (0.05 to 5,000 ng μl^{-1}) significantly decreased in OR35 mutant line. Data are presented as mean ± s.e.m. $n = 13$ (wild-type) and 14 ($Or35^{-/-}$) antennae. *P* values were determined by two-tailed unpaired *t*-test. **g**, The total movement distances of OR35 mutant locusts in 4VA and control zone. Behavioural data are presented as mean ± s.e.m. $n = 24$ (wild-type) and 27 ($Or35^{-/-}$) locusts. *P* values in behavioural assays were determined by Wilcoxon signed rank test. WT, wild type.



Extended Data Fig. 5 | 4VA attracts locusts in artificial turf. a, Schematic of the dual-choice bioassay in artificial turf. **b**, Locusts were present at higher frequencies in the 4VA zone than in the control zone. Data are presented as mean ± s.e.m. $n = 8$ biological replicates. P values were determined by G-test for goodness of fit. **c**, Schematic of trapping experiments in artificial turf. **d**, Locusts were captured in higher numbers in the 4VA traps than in the control traps. Data are presented as mean ± s.e.m. $n = 8$ biological replicates. P values were determined by two-tailed unpaired t-test.



Extended Data Fig. 6 | The full-size western blot scans of OR35 and GAPDH. **a**, The full-size western blot scans of OR35 in the antennae of wild type and OR35 mutant line. *n* = 6 biological replicates. **b**, The full-size western blot scans of GAPDH in the antennae of wild type and OR35 mutant line. *n* = 6 biological replicates. The bands in the blue frames are cropped and presented in Extended Data Fig. 4e. Protein marker (Thermo Fisher, 26619). WT, wild type.



Extended Data Fig. 7 | Plot of residuals for the linear model. Count ∝ Treatment + Block.

nature research

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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Behavioral assays were recorded by VCR software (Version 2, Noldus Information Technology). Syntech EAG software v2.6c and Syntech Autospike32 (V3.9) were used to record and analyze the EAG and SSR responses. The electrophysiological responses of oocytes were recorded and analyzed by Digidata 1440A and pCLAMP 10.6 software (Axon Instruments Inc., Union City, CA, USA).
Data analysis	Video was analyzed using EthoVision XT software (11.5 version, Noldus Information Technology). A Bruker chemical analysis MS workstation (MS Data Review, Data Process, version 8.0) was used to analyze and process the GC-MS Data. Data were analyzed using SPSS 18.0 (SPSS Inc.,Chicago, IL, USA), Origin 2018 (OriginLab, USA), and R platform (Version 3.2.3, R Development Core Team 2015).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequence data of OR35 that supporting the findings of this study have been deposited in Genbank with the accession codes KP843355. All data supporting the findings of this study are available within the article, the Extended data, and the supplemental files.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For behavioral assays, sample size was determined on the basis of previous studies (Ma et al., 2011; Wei et al., 2019). For chemical analysis, sample size were determined based on the previous studies in our laboratory (Wei et al., 2017, Wei et al., 2019). For electrophysiological recordings, sample size was determined based on the previous study (Li et al., 2016). For western blot, sample size was determined based on the previous studies (Guo et al., 2018; He et al., 2016).
Data exclusions	No data were excluded.
Replication	5-8 biological replicates were applied in this studies. All attempts to repeat the experiment were successful.
Randomization	The samples were allocated randomly into experimental groups.
Blinding	The investigators were blinded to group allocation during data collection and data analysis.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and*

	any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Affinity-purified polyclonal antibody against OR35 (rabbit) was developed in Abclonal Company, China. (1:500 in TBST) Rabbit anti-GAPDH antibody (1:5000 in TBST) Anti-rabbit IgG secondary antibody (1:5000 in TBST) (EASYBIO Technology, China, BE0101) Goat anti-rabbit antibody Alexa fluor 488 (1: 500 in PBS) (Life Technology, A11034)
Validation	The OR35 antibody were validated by Elisa and Western blot. The antibody against GAPDH (rabbit) was validated and applied in many published papers (Wang et al., 2013; Guo et al., 2018). Anti-rabbit IgG secondary antibody and goat anti-rabbit antibody Alexa fluor 488 are commercially available, and were validated by the manufacturer.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	*State the source of each cell line used.*
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	The gregarious and solitary locusts (Locusta migratoria, females and males, nymphs and adults) and frogs (Xenopus laevis, females adults older than 3 years) used in the experiments were maintained in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China.
Wild animals	For field trapping experiments, the migratory locusts (Locusta migratoria, females and males, nymphs and adults) were trapped to evaluate the attraction effect of aggregation pheromone 4VA.
Field-collected samples	The study did not involve samples collected from the field for laboratory work.
Ethics oversight	All experiments were conducted under the license of the Animal Experimental Committee of the Institute of Zoology (AECIOZ), Chinese Academy of Science (IOZ20170071)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*

Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*

Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
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Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g.*

Normalization template: *original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal: *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring: *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings: *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested: *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016): *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

Correction: *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity: *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis: *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis: *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*