Archived source document. Publication links are updated; scientific text and reported values are retained. Raw and complete data remain in the unpublished Zenodo preparation package.

AeroSense experimental protocols

Record assembled11October2026 from the website's documented methods. This is not a claim that every specified assay was executed. Status and missing metadata remain adjacent to each protocol. Shared microscopy record: WL-MIC-01. The acquisition date/operator and delivery method have not been recovered.

Reporter correction, 13 October 2026: The team identifies the TH recording as "5137;TH" (TH-GFP), using membrane-targeted mCD8::GFP rather than a calcium indicator. The 11 October methods record is otherwise retained; the live methods page carries later execution-status updates.

Live methods page · Measurement and calibration audit · Results and source data

A01

Molecular cloning

Maintain sample identity from a bacterial colony to the plasmid preparation used downstream.

Three colony-plate photographs and four DNA gels document the bacterial preparation track.

1. 01**Transform**Introduce the plasmid and retain plate identity
2. 02**Miniprep**Recover plasmid DNA from a selected colony
3. 03**DNA gel**Inspect preparation quality with a documented lane map

Transformation and colony selection

The existing specification uses *E. coli* DH5α, LB and ampicillin selection (100 µg/mL), with overnight propagation at 37 °C and 180 rpm. The supplied plate photographs retain the original labels and marked colonies. Competent-cell preparation, DNA input, transformation and recovery settings, and the corresponding negative-control record are not yet documented. View the three original plate photographs.

Plasmid miniprep

Link each picked colony to its construct and preparation identifier. Record the purification method, culture input, elution volume, concentration and purity. The current source does not identify the miniprep kit or provide a measured yield. The earlier endotoxin-free maxiprep specification remains a separate requirement for mammalian transfection-quality DNA.

DNA gel electrophoresis

Undigested plasmids were loaded as paired sample lanes for ORCO, GCaMP6f and ten OR labels. All four gels used the same 1 kb ladder. Adjacent lanes sharing a construct name are grouped in the figure, retaining their original left-to-right order.

The original TIFFs are retained. Each panel uses a contiguous crop and a small rigid rotation to align the wells. The original 0–255 intensity range is unchanged; no lanes are spliced and no bands are retouched. Crop coordinates and rotation angles accompany the figure downloads.

These are undigested plasmids: their migration depends on conformation as well as length. The ladder product is unknown, so individual bands are not assigned kb values. Gel concentration, running buffer, voltage and loading amount are not recorded. Dates and lane maps are retained in the Notebook. [1]

Construct provenance and sequence checks

The earlier preparation specification names chemically synthesised, pre-cloned pcDNA3.1(+) constructs from GenScript. Its construct count must be reconciled with the Parts — wiki page publication pending record and the labels in the image dataset. Compare returned sequences with the designs, retain both insertion junctions and internal Sanger coverage, and include a read across the $(GGGGS)_3$ linker. The specification advises against PstI diagnostic digests because of internal coding-sequence sites. These are verification requirements, not a declaration that every supplied colony passed.

Construct design — wiki page publication pending · Parts — wiki page publication pending · Notebook

Read the corresponding evidence →

B01

Mammalian cell culture

Maintain the mammalian host under defined growth, wash, and detachment conditions.

> **Executed, details incomplete.** HEK293 cultures were imaged; passage, seeding history and a dated operator record for this batch remain unresolved.

Key parameters

Parameter	Condition
Cell line	HEK293 (team-confirmed mammalian experimental host)
Growth medium	DMEM (high glucose) + 10 % FBS + 1 % penicillin–streptomycin

Parameter	Condition
Incubation	37 °C, 5 % CO_2
Passaging	At ~80 % confluence
Detachment	TrypLE Express — 1 mL per T-25, 3 mL per T-75; 37 °C, 3–5 min
Wash before detachment	Ca^{2+}/Mg^{2+}-free PBS

Method

1. Grow cells in high-glucose DMEM with 10 % FBS and 1 % penicillin–streptomycin at 37 °C, 5 % CO_2.
2. Passage at about 80 % confluence.
3. Before detachment, wash with Ca^{2+}/Mg^{2+}-free PBS.
4. Lift with TrypLE Express (1 mL per T-25, 3 mL per T-75; 37 °C, 3–5 min).

See Safety · See Notebook

Read the corresponding evidence →

B02

Transfection

Deliver OR and Orco plasmids into HEK293 cells and preserve the delivery record for each image set.

> **Executed, details incomplete.** The imaged cells were transfected. The earlier electroporation specification and Lipofectamine/P3000 workbook are not enough to identify the delivery method used for WL-MIC-01.

Method provenance

The earlier protocol specification describes electroporation. The supplied transfection workbook — Zenodo (publication pending) also contains P3000 and Lipofectamine 3000 reagent tables, but does not establish a dated link between those mixtures and each microscopy set. The electroporation parameters retained below are therefore a prior specification, not evidence of the delivery method used for the current images. The executed method, reagent amounts and sample mapping remain to be confirmed from the experimental record.

Key parameters

Parameter	Value
Earlier protocol specification	Electroporation in suspension; execution for the imaged samples is unconfirmed
Cells per reaction	1–5 × 10^6, log phase, > 90 % viability
DNA per reaction	~5 µg total. OR plasmid : Orco plasmid DNA mass ratio 2:1 (not protein stoichiometry)
Buffer	Low-conductivity electroporation/nucleofection buffer, 100 µL
Recovery	Pre-warmed DMEM + 10 % FBS, no antibiotics

Method

The earlier specification calls for suspension electroporation using the DNA mass, plasmid DNA mass ratio, buffer volume, and recovery medium in the table. Pulse voltage, pulse number, instrument programme, cuvette, and named buffer are not specified in this source.

Zboray *et al.* used FuGene HD into HEK293 stable lines [2]. That is not substituted for the AeroSense transfection specification above.

Controls & critical notes

- Buffer-only pulse — electroporation toxicity.
- GFP-only plasmid — transfection efficiency.
- Genetic control Orco–GCaMP6f alone, without a tuning OR — should respond to VUAA1 but not to VOCs.

Use endotoxin-free, low-salt, concentrated DNA. Use healthy log-phase cells and minimise time in electroporation buffer.

Recover immediately in warm antibiotic-free medium. Omit penicillin–streptomycin during electroporation and for the following 24 h.

See Design — wiki page publication pending · See Notebook

Read the corresponding evidence →

B03

Stable-line selection

Select G418-resistant mammalian lines after transfection.

> **Partially executed.** A separate G418 batch was reported in progress; no selection trajectory or endpoint dataset is archived.

Current experimental status. The supplied fluorescence images were acquired approximately 44–48 h after transfection, before G418 selection. A separate batch is undergoing G418 selection. Consequently, these images cannot establish enrichment or survival after selection, and the schedule below remains a protocol target rather than a confirmed result for the imaged batch.

Current protocol target — not a validated kill curve

Protocol target timeline. Day 0 transfection with antibiotic-free recovery. G418 at 100 micrograms per millilitre starts 24 to 48 hours later and runs for 2 weeks. Concentration is then raised to 150 for 1 week, then maintenance at about 75 ongoing. These doses are a literature-informed starting condition, not an AeroSense-confirmed kill-curve result.

1. Day 0 Transfection Suspension transfection. Recovery in DMEM + 10 % FBS, no antibiotics for 24 h.
2. 24–48 h G418 100 µg/mL Selection start, 2 weeks. Not added at seeding. Days 1–2: little visible effect; 3–5: bulk death; 10–14: resistant colonies.
3. After 2 weeks G418 150 µg/mL Selection raised, 1 week.
4. Thereafter G418 ~75 µg/mL Maintenance, ongoing.

Figure 2. G418 selection timeline (protocol target).

Current protocol target / literature-informed starting condition: G418 started 24–48 h after transfection at 100 µg/mL for 2 weeks, then 150 µg/mL for 1 week, then maintenance at ~75 µg/mL. This is not an AeroSense-validated kill-curve result.

Key parameters

Stage	G418 concentration	Duration
Selection start (24–48 h post-transfection)	100 µg/mL	2 weeks
Selection raised	150 µg/mL	1 week
Maintenance	~75 µg/mL	ongoing

Method

1. Before transfection, run a G418 kill curve on untransfected host cells (draft range 0–800 µg/mL) for that cell lot, confluence, and G418 batch. Do not treat the table below as a confirmed effective dose.
2. After transfection, allow 24–48 h recovery. Do not add G418 at seeding.
3. Start selection at 100 µg/mL G418 for 2 weeks. Refresh selection medium every 2–3 days.
4. Raise G418 to 150 µg/mL for 1 week.

5. Maintain lines at ~75 µg/mL G418 thereafter.

During the first selection window, little visible effect is expected for 1–2 days, bulk cell death at days 3–5, and discrete resistant colonies against a cleared background by days 10–14. Those timings are operational expectations, not recorded AeroSense outcomes.

Controls & critical notes

- Negative control Untransfected well at the same G418 dose throughout selection — every cell in it should die.

Add G418 24–48 h after transfection, not at seeding, so cells can recover and express NeoR.

The 100 → 150 → ~75 µg/mL schedule is a current protocol target / literature-informed starting condition, not an experimentally confirmed AeroSense kill curve.

See Design — wiki page publication pending · See Notebook

Read the corresponding evidence →

B04

Expression / functional validation

Confirm cassette transcription and Orco/GCaMP function before VOC assays.

> **Partially executed.** Fixed-cell endpoint imaging was acquired; the localization and time-resolved functional tests below remain assay specifications.

Current microscopy record

The team confirms that the supplied HEK293 images were acquired approximately 44–48 h after transfection and before G418 selection. Cells were fixed with 2% paraformaldehyde before DAPI staining, without a permeabilization step. Blue images show DAPI. Files sharing the same field number refer to the same field of view across channels; exposure was held constant within each channel across groups, and blank calibration was performed. Fixation duration, numerical exposure settings and the complete acquisition log have not been supplied.

The available fields are technical observations from one experimental batch, with no independent biological repeats and no live/dead assay. DAPI provides a nuclear denominator for an image-based estimate after segmentation quality control; it does not establish viability. Dense or overlapping nuclei require explicit uncertainty handling. Report the number of analyzable fields per sample and distinguish a nuclear-normalized reporter-positive fraction from confirmed live-cell transfection efficiency. GCaMP6f resting fluorescence and mCherry expression are interpreted separately from stimulus-dependent response and cell viability.

Image analysis, sample-level estimates and limitations · Original preparation workbook — Zenodo (publication pending)

Key parameters

Parameter	Value
mCherry microscopy	ex ~587 nm / em ~610 nm
GCaMP6f rest fluorescence	ex ~485 nm / em ~510 nm; dim at rest is expected, not a failure
VUAA1	Synthetic Orco agonist used as a functional positive-control stimulus [3]
Western blot	Optional / implementation details pending
Ionomycin	Optional / implementation details pending

Method

1. Record mCherry (ex ~587 nm / em ~610 nm) versus mock / untransfected cells. Red fluorescence reports that the OR cassette is transcribed. It does not report OR folding or plasma-membrane trafficking.
2. After an expression gate, compare vehicle versus VUAA1 on cells that should contain Orco–GCaMP6f. VUAA1 is a synthetic Orco agonist used as a functional positive-control stimulus [3] Jones et al., 2011 — full entry in References. . A VUAA1 response supports functionality of the Orco/GCaMP sensing chain. It does not, by itself, prove membrane trafficking.

Controls & critical notes

- Expression control Mock / untransfected cells for mCherry autofluorescence.
- Functional positive control VUAA1 versus vehicle. VUAA1 is a synthetic Orco agonist used as a functional positive-control stimulus, not a proof of membrane trafficking.
- Optional / implementation details pending Ionomycin (reporter isolated from channel) and anti-His western blot of the RSET leader. Concentration, solvent, incubation, antibody, and whether either assay is in the AeroSense SOP are not specified.

GCaMP6f is dim at rest. Absence of bright green is expected, not a failure.

See Design — wiki page publication pending · See Results · See Notebook

Read the corresponding evidence →

B05

Endpoint microscopy and image analysis

Reproduce the DAPI-object fluorescence screen reported in Results.

> **Executed analysis.** All 25 fields and all 2,624 eligible objects are retained in the published data tables and reproducibility package.

Source data and experimental context

The host was HEK293, as confirmed by the team. Imaging occurred approximately 44–48 h after transfection and before G418. The team fixed cells with 2% paraformaldehyde (PFA), performed no permeabilization step, and then stained with DAPI. The source workbook contains plate-layout and reagent-calculation records, including DAPI/G418 layouts and lipid-reagent mixtures. These records do not independently establish experimental execution or an assay outcome; the VUAA1/odor grids contain labels without response measurements. The workbook does not supply objective, numerical aperture, camera model, gain, acquisition software, DAPI concentration, fixation duration, or field coordinates. Neither the source TIFF tags nor the workbook establishes these missing acquisition details. The biological preparation and transfection record is linked from Experiments; undocumented settings are not reconstructed from a literature protocol.

Conservative DAPI segmentation

The BLUE plane was extracted from each exported TIFF. A 3 × 3 median filter was used for segmentation only, followed by a common threshold of >128 on the 0–255 export scale and 8-connected labeling. The bottom-right annotation rectangle (x ≥85% of image width and y ≥90% of image height) was excluded equally in every field. Eligible objects had area 200–2200 pixels, bounding-box aspect ratio ≤2.5, and object area/bounding-box area ≥0.4. Objects within 49 pixels of a frame edge or whose measurement neighborhood intersected the annotation exclusion were rejected. No watershed split was imposed on merged objects.

These rules define a conservative image-analysis subset, not a validated nuclear census. Segmentation has been visually inspected, but not benchmarked against independently annotated cell boundaries. Small touching nuclei or nuclear fragments can still pass these geometric filters, so an accepted object is not guaranteed to equal one cell. DAPI is a nuclear counterstain commonly used with fixed preparations. Here, fixation is confirmed and no permeabilization was performed; completeness and uniformity of nuclear labeling were not independently assessed. Fixed DAPI images do not recover the cells' viability before fixation. [4]

Reporter measurement and positivity threshold

The full Measurement record adds an explicit statistical specification, all three same-batch blank-field holdouts and the area-versus-object audit. These additions leave the original segmentation and primary thresholds unchanged.

For each eligible object, the signal region contains its nuclear mask plus pixels within 12 pixels, assigned to the nearest blue connected component to limit sharing between adjacent objects. The local background is sampled 24–48 pixels from that object, excluding blue foreground; at least 100 background pixels are required. For RED and GREEN separately, the score is the signal region's 90th percentile intensity minus the background median. Measurements use the original exported channel values, before the display adjustments.

This nearest-DAPI expansion does not segment cell boundaries. Excluding blue foreground from the background ring also does not exclude every neighboring cell's cytoplasm. Fluorescent cytoplasm within that ring can raise the estimated background and reduce an object's score, particularly in dense fields.

The primary positivity threshold is the maximum of the three blank-field 99th percentiles of these object scores: **RED >16.60 DN and GREEN >11.95 DN**. DN denotes the 8-bit exported digital value. Alternative P95 and P99.5 blank thresholds are reported for sensitivity analysis. Thresholds are calibrated and evaluated using the same blank set, so the blank fraction is a calibration diagnostic, not an independent false-positive validation.

The reported fraction is 100 × positive eligible objects / all eligible objects. Condition summaries pool these counts across fields; they do not average percentages with different denominators. “Both” indicates both channel criteria within one object's measurement region. It does not prove two-plasmid delivery, protein localization, or co-expression in a viable cell.

Image processing and presentation

Original TIFF and workbook files were opened read-only and verified by SHA-256 checksums. New figures use fixed linear display windows across all conditions: DAPI 0–255, GREEN 0–120 and RED 0–180; brightfield remains 0–255. The enhanced display can clip bright exported values and is used only for viewing. Original-level views remain available. No sharpening, deconvolution, content removal, generative enhancement or reconstruction of missing detail was applied. The same-field RGB merges combine the original channel positions; no registration correction was imposed. [5] [6]

Web previews are resized with Lanczos resampling and saved as lossless WebP. The original scale bars remain within the full field; they were not used to infer pixel size. Object overlays mark classifications on a dimmed merge for legibility and have full-resolution PNG versions for audit.

Microscopy results and all-field gallery · Scripts, data and source files · Reproduce the control comparison

Read the corresponding evidence →

B06

Calcium imaging assay

Measure VOC-evoked GCaMP6f fluorescence in a 384-well plate with a matched vehicle injection.

> **Specification; not run.** This 384-well assay has no executed plate-level dataset in the current archive. The earlier qualitative VUAA1 observation does not verify this protocol.

The calcium-response assay was adapted from Zboray *et al.* [2] Zboray et al., 2023 — full entry in References. . AeroSense-specific construct, transfection, and validation procedures are documented separately above. Plate geometry, buffer volumes, and the two-round injection sequence below follow that published assay design.

Key parameters

Parameter	Value
Plate format	Black-wall, clear-bottom 384-well
Seeding	12,000–18,000 cells per well, 48–72 h before measurement
Assay volume	20 µL assay buffer per well
Vehicle injection	7 µL plain VOC buffer
Odorant injection	9 µL of VOC at 4× final concentration
Final well volume	36 µL (20 + 7 + 9)
VOC concentrations	1, 10 and 100 µM final
VUAA1 reference	50 µM on every plate for every cell line
Instrument	Multimode plate reader with a two-channel injector (model not specified)
Readout	ex 485 nm / em 535 nm
Sampling	F_0 for 5 s; then 30 s at 1 read/s after each injection

Buffers

Component	Assay buffer	VOC buffer
NaCl	140 mM	140 mM
KCl	5 mM	5 mM
$CaCl_2$	2 mM	2 mM
$MgCl_2$	1 mM	1 mM
HEPES-Na	5 mM	5 mM
Glucose	20 mM	31 mM

Component	Assay buffer	VOC buffer
DMSO	—	0.1 %
pH / osmolarity	7.3 / 340 mOsm	7.3 / 340 mOsm

Method

Read on a multimode plate reader with a two-channel injector, ex 485 nm / em 535 nm.

1. Seed cells at 12,000–18,000 per well in black-wall, clear-bottom 384-well plates, 48–72 h before measurement.
2. On the day of measurement, replace growth medium with 20 µL assay buffer per well.
3. Round 1 — vehicle: record baseline F_0 for 5 s, inject 7 µL plain VOC buffer, record 30 s at 1 read/s.
4. Round 2 — odorant: into the same wells, inject 9 µL of VOC at 4× final concentration, same recording protocol.
5. Layout: one compound per row; up to eight cell lines per 24-column plate. Use three wells per cell line and measure each VOC–OR pair on at least three separate days.

The volumes are self-consistent: 20 + 7 = 27 µL, then + 9 = 36 µL; 9/36 = 1/4, so a 4× stock dilutes exactly to 1× final. The vehicle injection simultaneously establishes the volume for the second round and measures the solvent and injection artefact alone.

Well volume and two-round sequence

Three well volumes. First: 20 microlitres assay buffer. Second: 20 plus 7 microlitres vehicle equals 27. Third: 27 plus 9 microlitres of 4 times VOC equals 36. Nine divided by 36 equals one quarter, so a 4 times stock becomes 1 times final.

20 µL assay buffer
20 µL
+ 7 µL vehicle 20 µL assay buffer
= 27 µL
+ 9 µL 4× VOC 7 µL vehicle 20 µL assay buffer
= 36 µL

9 / 36 = 1/4 → 4× stock dilutes to 1× final

1. Round 1 F_0 5 s Inject 7 µL vehicle Record 30 s at 1 read/s
2. Round 2 Same wells Inject 9 µL 4× VOC Record 30 s at 1 read/s

Figure 3. Two-round calcium assay volumes.

20 µL assay buffer + 7 µL vehicle = 27 µL; then + 9 µL of 4× VOC = 36 µL. 9/36 = 1/4, so a 4× stock becomes 1× final concentration. Geometry adapted from Zboray *et al.* [2].

Reagent preparation VUAA1 stock and VOC handling

VUAA1. Dissolve in DMSO to a 50 mM stock (18.4 mg/mL). Aliquot and store at −20 °C protected from light. Prepare the 4× working dilution fresh in VOC buffer.

VOCs. Dilute directly in VOC buffer, or first in DMSO for water-insoluble compounds. Prepare working solutions fresh daily. Store concentrated stocks in amber glass with PTFE-lined caps.

Controls & critical notes

- Vehicle control Round-1 injection of plain VOC buffer — solvent and injection artefact.
- Normalisation reference 50 µM VUAA1 for each cell line on the same plate/day. Normalise to the mean VUAA1 response of that line, not a same-well VUAA1 injection.
- Specificity control Ca^{2+}-free condition. Zboray *et al.* showed that Ca^{2+}-free and Ca^{2+}-free + EGTA buffers abolish the VUAA1 response [2].

Keep DMSO identical in VOC, vehicle, and VUAA1 wells.

$CaCl_2$ in the assay buffer is required.

Use HEPES-Na rather than bicarbonate: the plate sits in ambient air.

Reproducibility metadata

Technical replicates
: Three wells per cell line, one compound per row, triplicate

Day replicates
: Each VOC–OR pair measured on at least three separate days

Instrument
: Multimode plate reader with a two-channel injector (model not specified)

Excitation / emission
: 485 nm / 535 nm

Sampling rate
: 1 read/s for 30 s after each injection; F_0 recorded for 5 s before injection

Analysis
: R then R_{norm}; see Data analysis

See Safety · See Results · See Notebook

Read the corresponding evidence →

Data analysis

Convert raw fluorescence into a VUAA1-normalised relative response for each receptor–stimulus pair.

> **Specification; not run.** These response metrics apply to future time courses; fixed-cell image fractions use the separate microscopy pipeline.

Relative-response definition, the ≤ 0.05 floor, plate/day VUAA1 mean normalisation, and Grubbs' outlier removal follow Zboray *et al.* [2] Zboray et al., 2023 — full entry in References. . Zboray *et al.* denote the relative response R as ΔF. This page uses R and R_{norm} to avoid mixing ΔF with $\Delta F/F_0$.

Key parameters

Parameter	Value
Raw fluorescence	Plate-reader intensity at ex 485 nm / em 535 nm
F_0	Baseline recorded for 5 s before the relevant injection
F_{max}	Peak fluorescence in the post-injection window
Raw relative response	$R = (F_{max} - F_0) / F_0$
VUAA1-normalised response	$R_{norm} = R_{VOC}$ / mean(R_{VUAA1}, corresponding cell line, same plate/day)
Zero floor	$R_{norm} \leq 0.05$ treated as zero, following [2]
Outliers	Removed by Grubbs' test, following [2] (α not specified here)

Method

1. Record raw fluorescence at ex 485 nm / em 535 nm. Take F_0 as the 5 s baseline before the relevant injection.
2. Take F_{max} as the peak in the post-injection window. Compute $R = (F_{max} - F_0) / F_0$.
3. Normalise each VOC response to the mean 50 µM VUAA1 response of the corresponding OR cell line measured on the same plate/day: $R_{norm} = R_{VOC}$ / mean(R_{VUAA1}, corresponding cell line, same plate/day). Do not use a same-well VUAA1 injection as the denominator.
4. Following [2], treat $R_{norm} \leq 0.05$ as zero, and remove outliers by Grubbs' test.

$R = (F_{max} - F_0) / F_0$

Raw relative response. Not an absolute Ca^{2+} concentration. Zboray *et al.* denote R as ΔF.

$R_{norm} = R_{VOC} / \text{mean}(R_{VUAA1}, \text{line, plate/day})$

VOC responses are normalized to the mean 50 µM VUAA1 response of the corresponding OR cell line measured on the same plate/day.

Controls & critical notes

VOC responses are normalized to the mean 50 µM VUAA1 response of the corresponding OR cell line measured on the same plate/day. Do not use a same-well VUAA1 injection as the denominator.

Reproducibility metadata

Excitation / emission
: 485 nm / 535 nm

Sampling
: F_0 for 5 s before the relevant injection

Analysis method
: R, then plate/day mean 50 µM VUAA1 R_{norm}; ≤ 0.05 floor and Grubbs' test following [2]

Downstream
: Multi-receptor vectors are intended for a sparse-coding classifier based on [7] Dasgupta et al., 2017 — full entry in References. . That classifier is not specified on this page. See Model.

See Model · See Results · See Notebook

Read the corresponding evidence →

Light-sheet imaging

Current fly preparation, acquisition timing and ROI analysis

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   Back to citation

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