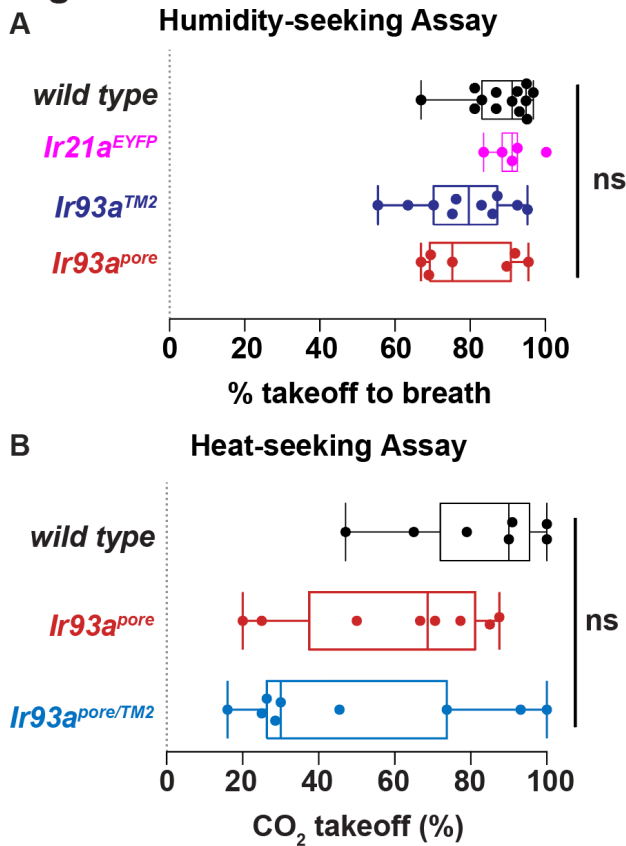


1
 2 **Figure S1 related to Figure 3: *AgIr93a>CD8:GFP* expression in mosquito antennae. (A) *AgIr93a^{pore-RFP/+>}CD8:GFP* expression is detected in all 13 flagellomeres. (B) Expression in neurons innervating**
 3 **sensilla ampullacea in flagellomere 2. (C-D) Expression in neurons innervating trichoid sensilla in**
 4 **flagellomere 11 (C) and flagellomeres 3 to 5 (D). Arrowheads mark GFP+ trichoid sensilla, asterisk**
 5 **marks entrance to sensilla ampullacea. (E) *AgIr93a^{pore-RFP/+>}mCD8:GFP* expressing neurons that**
 6 **innervate trichoid sensilla co-express Orco. Yellow arrowheads mark soma. (F) The number of cells per**
 7 **flagellomere in which *AgIr93a^{pore-RFP/+>}CD8:GFP* expression was detected in the female antenna.**
 8
 9

Figure S2



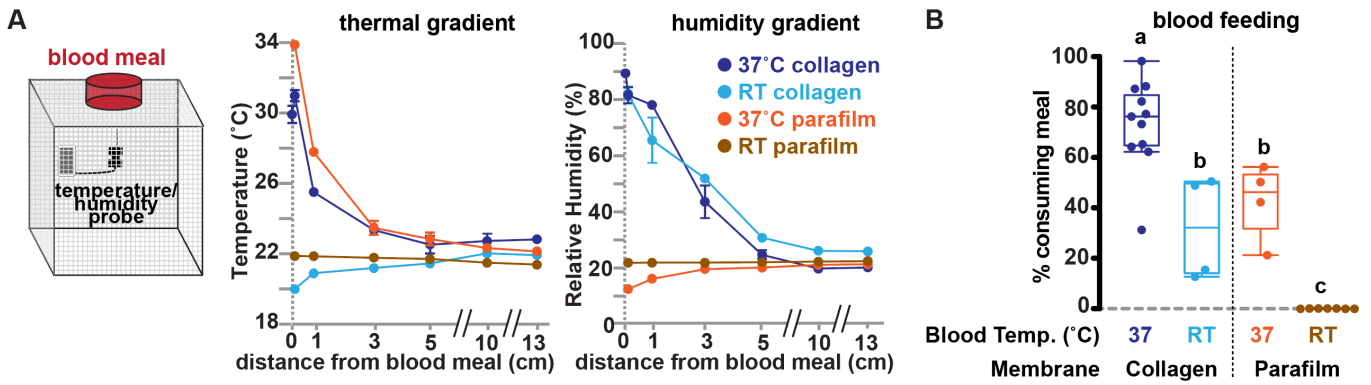
11

12 **Figure S2 related to Figure 5: Behavioral assay female activation rates. (A)** Percent of females (not
 13 gravid) taking flight within 2 minutes after breath exposure at end of humidity-seeking assay. Kruskal-
 14 Wallis: $H = 5.527$, $df = 3$, $p = 0.137$. Genotypes: *wild type*, G3. *AgIr21a^{EYFP}/AgIr21a^{EYFP}*. *AgIr93a^{TM2-}*
 15 *RFP/AgIr93a^{TM2-EYFP}*. *AgIr93a^{pore-RFP/AgIr93a^{pore-EYFP}}*. **(B)** Percentages of animals in heat-seeking assays
 16 that took flight after the initial pulse of 4% CO₂. ANOVA [$F(2,21) = 3.00$, $p = 0.07$]. Genotypes: *wild*
 17 *type*, G3. *AgIr93a^{pore-RFP/AgIr93a^{pore-EYFP}}*. *AgIr93a^{pore-EYFP/AgIr93a^{TM2-RFP}}*.

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Figure S3



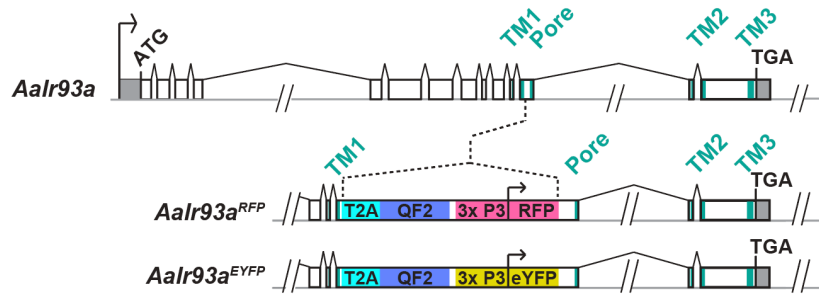
20

21 **Figure S3 related to Figure 6: Using different membranes and blood meal temperatures induces**
 22 **different levels of blood feeding and creates different temperature and humidity gradients.** (A) Left
 23 panel: Temperature and humidity probe was placed inside cage at multiple distances from blood meal.
 24 Center and Right panel: Temperature and relative humidity measurements at defined distances from blood
 25 meal presented using either a collagen (water vapor permeable) or parafilm (water vapor impermeable)
 26 feeding membrane. Mean \pm SEM. N = 3 measurements. Where SEM was smaller than dot, no error bar
 27 shown. (B) Wild type *Anopheles* (G3) blood feeding from meals at different temperatures and using
 28 different membranes. Letters denote distinct statistical groups, Tukey HSD, alpha = 0.05.

29

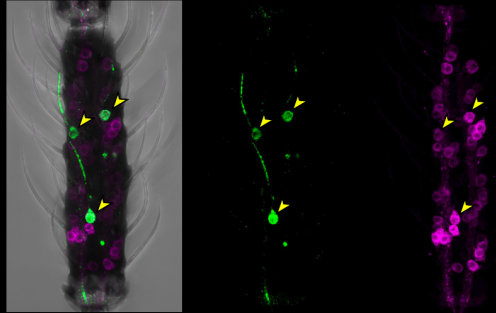
Figure S4

A



B

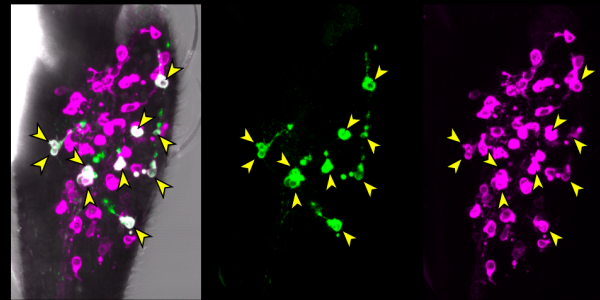
Ae. aegypti *Ir93a^{RFP}*; *QUAS-CD8:GFP*
 CD8:GFP + anti-Orco CD8:GFP anti-Orco



flagellomere 10

C

Ae. aegypti *Ir93a^{RFP}*; *QUAS-CD8:GFP*
 CD8:GFP + anti-Orco CD8:GFP anti-Orco



maxillary palp

D

Ae. aegypti *Ir93a^{RFP}*; *QUAS-CD8:GFP*

| flagellomere # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | max palp |
|----------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------|
| average | 15.1 | 2.3 | 0.6 | 0.4 | 0.3 | 0.6 | 1.0 | 1.3 | 0.3 | 2.1 | 0.3 | 2.1 | 4.3 | 7.6 |
| SEM | 0.5 | 0.3 | 0.2 | 0.2 | 0.1 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.5 |
| median | 15.5 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 2 | 4 | 7 |
| minimum | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 4 | 6 |
| maximum | 18 | 5 | 3 | 2 | 2 | 4 | 3 | 4 | 2 | 4 | 1 | 4 | 5 | 10 |
| flagellomeres scored | 16 | 18 | 18 | 18 | 18 | 17 | 15 | 16 | 13 | 15 | 12 | 12 | 12 | 8 |

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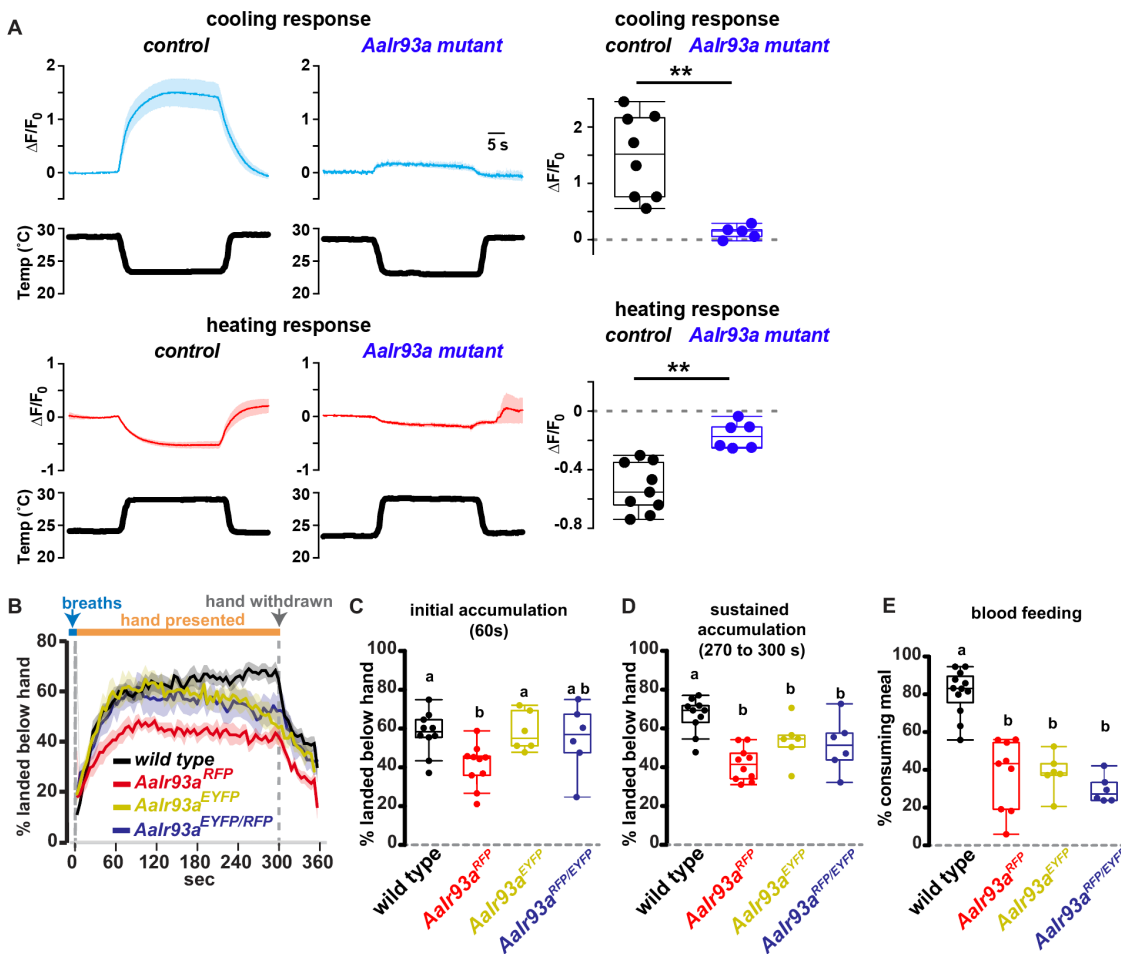
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Figure S4 related to Figure 7: *Aalr93a* QF2 knock-ins and *Aalr93a*>*CD8:GFP* expression in antenna and maxillary palp. (A) Top depicts *Aalr93a* locus organization. Exonic regions encoding the three transmembrane domains and ion pore are denoted in blue-green. 5' and 3' UTR are gray. Bottom panels depicts gene structure in *Aalr93a^{RFP}* and in *Aalr93a^{EYFP}* alleles. (B-C) *Aalr93a^{RFP/+>CD8:GFP}* expression in Orco-positive neurons that innervate trichoid sensilla in flagellomere 10 (B) and neurons that innervate olfactory sensilla in the maxillary palp (C). Yellow arrowheads, *Aalr93a^{RFP/+>CD8:GFP}* expressing neurons. (D) The number of cells in which *Aalr93a^{RFP/+>CD8:GFP}* expression was detected in the female antenna and maxillary palp.

Figure S5

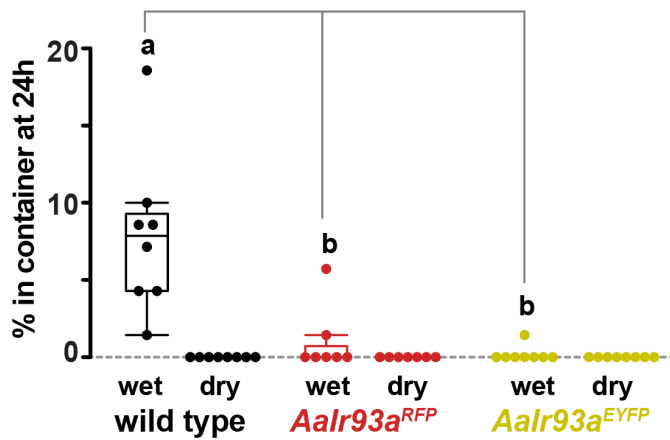


39 **Figure S5 related to Figure 7: A heteroallelic mutant combination confirms *Aalr93a*'s specific role**
 40 **in Cooling Cell thermotransduction, sustaining host attraction, and blood feeding. (A)** Left panels,
 41 trans-cuticular imaging of *Aalr93a*>*GCaMP7s* fluorescence changes in response to temperature changes.
 42 Mean +/- SEM. Control (*Aalr93a^{RFP/+}*), n=9 animals. *Aalr93a* mutant (*AgIr93a^{RFP}/AgIr93a^{EYFP}*), n=9.
 43 F_0 = average 5s to 1s pre-stimulus-switch. Right panels, fluorescence change upon switching between
 44 temperatures. $\Delta F = F(\text{average 15s to 25s post-switch}) - F_0$. ** p<0.01, t-test. **(B-D)** Hand-seeking assay.
 45 **(B)** Average +/- SEM. 50-63 females/assay. wild type (LVP), n=10 assays. *Aalr93a^{RFP/Aalr93a^{RFP}}*, n=10.
 46 *Aalr93a^{EYFP/Aalr93a^{EYFP}}*, n=6. *Aalr93a^{RFP/Aalr93a^{EYFP}}*, n=6. **(C)** Mosquitoes landed under hand at 60s.
 47 Letters denote distinct groups, Tukey HSD, alpha = 0.05. **(D)** Mosquitoes landed under hand 270-300s
 48 post-hand presentation. Letters denote distinct groups, Tukey HSD, alpha=0.05. **(E)** Blood meal
 49 consumption. 50-63 females/assay. wild type, n=12 assays. *Aalr93a^{RFP/Aalr93a^{RFP}}*, n=9.
 50 *Aalr93a^{EYFP/Aalr93a^{EYFP}}*, n=6. *Aalr93a^{RFP/Aalr93a^{EYFP}}*, n=6. Letters denote distinct groups, Tukey HSD,
 51 alpha=0.01.

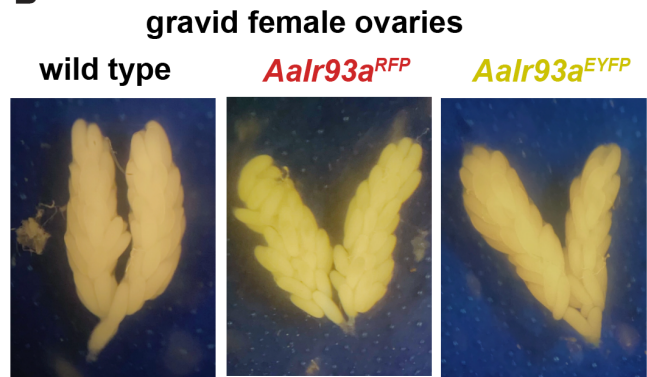
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Figure S6

A % females in container at assay end



B



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Figure S6 related to Figure 8: Presence of female mosquitoes within oviposition containers at the end of the site seeking assay and images of ovaries from gravid females. (A) Percentage of females found within each oviposition container at assay end. *wild type* (LVP), n=8 assays. *Aalr93a^{RFP}/Aalr93a^{RFP}*, n=7. *Aalr93a^{EYFP}/Aalr93a^{EYFP}*, n=8. Letters denote distinct statistical groups, comparing wet containers, Steel-Dwass, p<0.02. (B) Representative dissected ovaries from gravid females of indicated genotypes.