Supplemental Figures

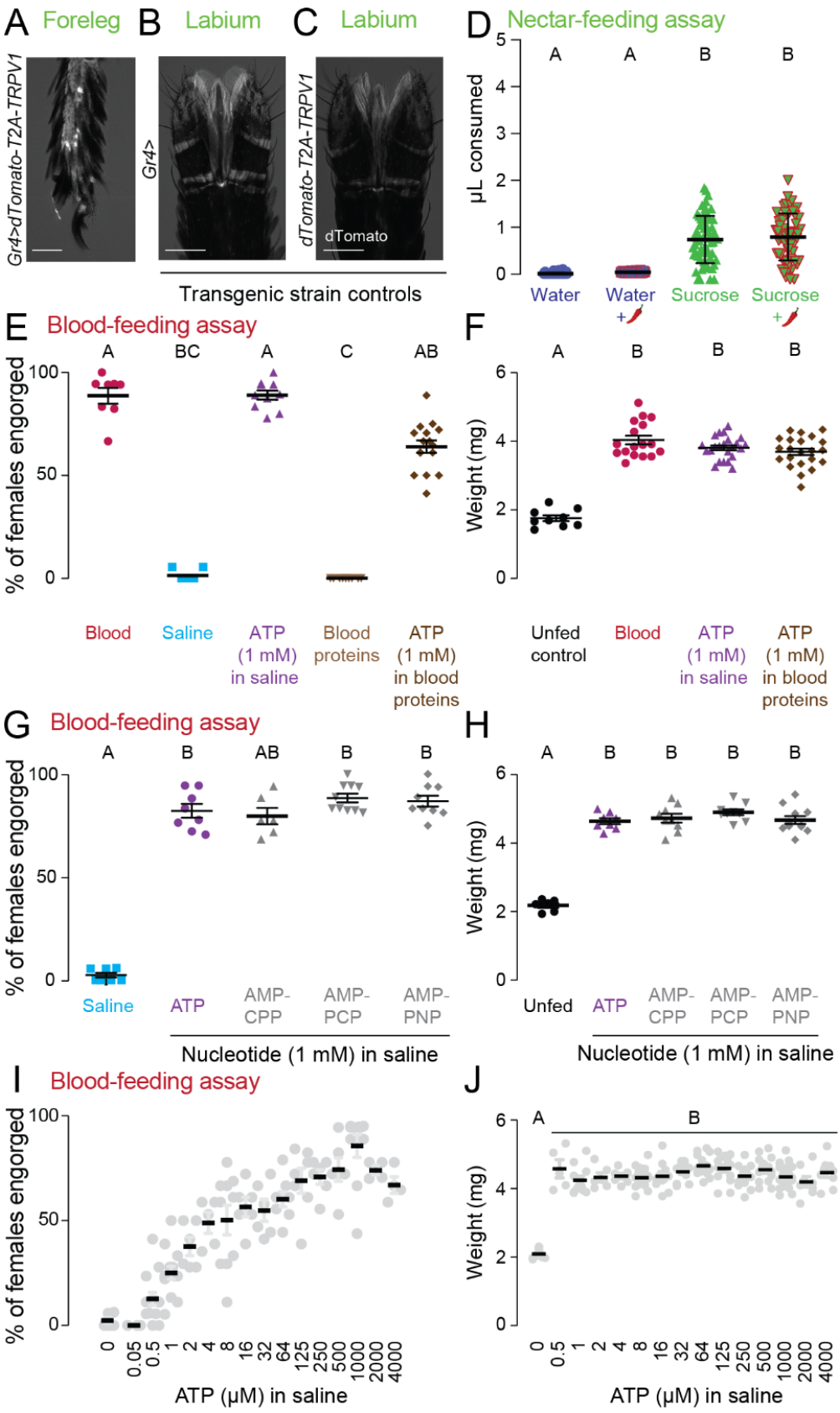

Figure S1. Features of Blood- and Nectar-feeding Behavior, Related to Figure 1

(A-C) Confocal image of dTomato expression with transmitted light overlay in *Gr4>dTomato-T2A-TRPV1* foreleg (A), *Gr4* control labium (B), and *dTomato-T2A-TRPV1* control labium (C). Scale bar: 50 μm.

(D) Volume of meal consumed by wild-type mosquitoes. Chili pepper cartoon indicates addition of 50 μM capsaicin. Each data point represents 1 female: N=58-60 females/meal (mean ± SD).

(E, G) Female engorgement on the indicated meal delivered via Glytube. Each data point denotes 1 trial with 15-20 females/trial: N=6-16 trials/meal**.**

(F, H, J) Sampled weight measurements from engorged females offered the indicated meal or unfed controls not offered any meal from data in (E, G, I), respectively. N=5-25 weight measurements. Data labeled with different letters are significantly different from each other (mean ± SEM; one-way ANOVA with Dunnett's multiple comparisons with a single pooled variance, $p < 0.05$).

(I) Female engorgement on the indicated concentration of ATP delivered in saline via Glytube. Each data point denotes 1 trial with 15-20 females/trial, N=4-14 trials/meal (mean ± SEM).

Ligands: saline = 110 mM NaCl and 20 mM $NaHCO_3$; blood proteins = 15 mg/mL gamma-globulin, 8 mg/mL hemoglobin, 102 mg/mL albumin in 110 mM NaCl and 20 mM $NaHCO_3$ (Duvall et al., 2019; Kogan, 1990); AMP-CPP (α,β-methyleneadenosine 5'-triphosphate lithium salt), AMP-PNP (β,γ-imidoadenosine 5'-triphosphate lithium salt hydrate), AMP-PCP (β,γ-methyleneadenosine 5′-triphosphate disodium salt). In (D,E,G) data labeled with different letters are significantly different from each other (Kruskal-Wallis test with Dunn's multiple comparison, $p < 0.05$).

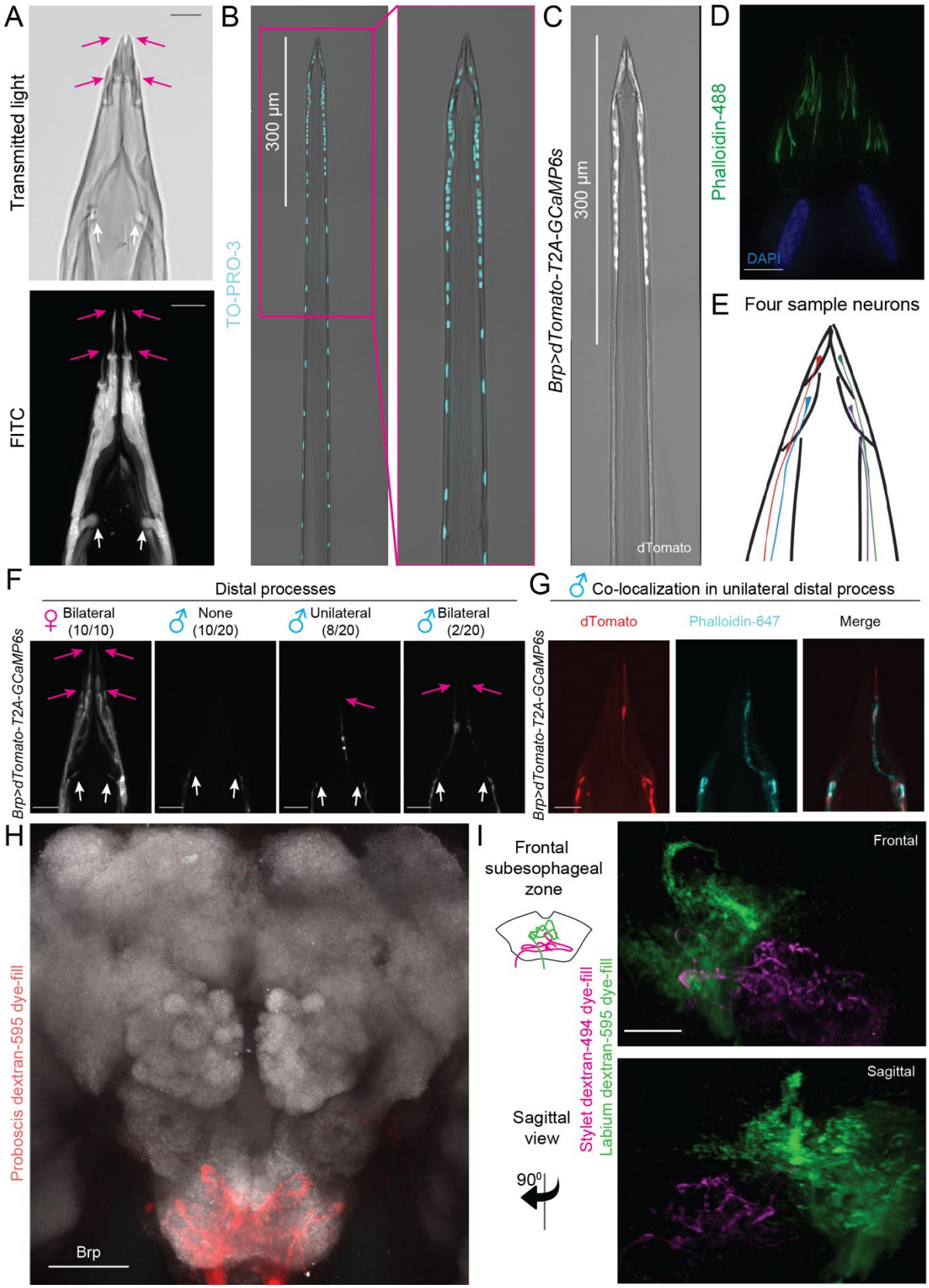

Figure S2. Sexual Dimorphism in the Stylet, Related to Figure 3

(A) Confocal images of transmitted light (top) and FITC counterstain (bottom) outline the female stylet chemosensory (pink arrows) and mechanosensory (white arrows) sensillar structure.

(B,C) Tiled confocal image with transmitted light overlay of TO-PRO-3 nuclear staining (B, cyan) in a wild-type female stylet and dTomato expression (C, gray) in a *Brp>dTomato-T2A-GCaMP6s* female stylet. Right panel in (B) is an enlargement of the magenta-boxed area in the left panel.
(D) Super-resolution structured illumination image of phalloidin-488 actin stain (green) and DAPI nuclear stain (blue) in the female stylet tip. Scale bar: 5 μm.
(E) Schematic of 4 sample neurons with dendrites, each innervating 1 chemosensory sensillum at the tip of the stylet. The exact numerology of stylet sensory neurons / sensillum is unknown and this schematic shows only a single neuron/dendrite example per sensillum for clarity.
(F) Confocal image of dTomato expression in the female (left) and male (remaining 3 panels) stylet tip of *Brp>dTomato-T2A-GCaMP6s* animals. From left to right: 10/10 females examined have extensive bilateral distal processes, 10/20 males examined have no distal processes, 8/20 males examined have sparse unilateral distal processes, and 2/20 males examined have sparse bilateral distal processes.
(G) dTomato expression (left) and phalloidin-647 actin staining (middle) co-localize in the *Brp>dTomato-T2A-GCaMP6s* male stylet. Right panel is a merge of left and middle panel.
(H) Proboscis neuron projection pattern (red) is restricted to the suboesophageal zone as revealed by dextran-595 dye-fill. The proboscis consists of the stylet and labium, neuropil stained with anti-*Drosophila* Brp (gray). Scale bar: 50 μm.
(I) Subesophageal zone after dual dye-fill with bilateral stylet dextran-494 (magenta) and unilateral labium dextran-595 (green). Bottom panel is a 90º optical rotation from the sagittal perspective (See also Video 2). Scale bar: 25 μm.
In (A,F,G) Scale bar: 10 μm.

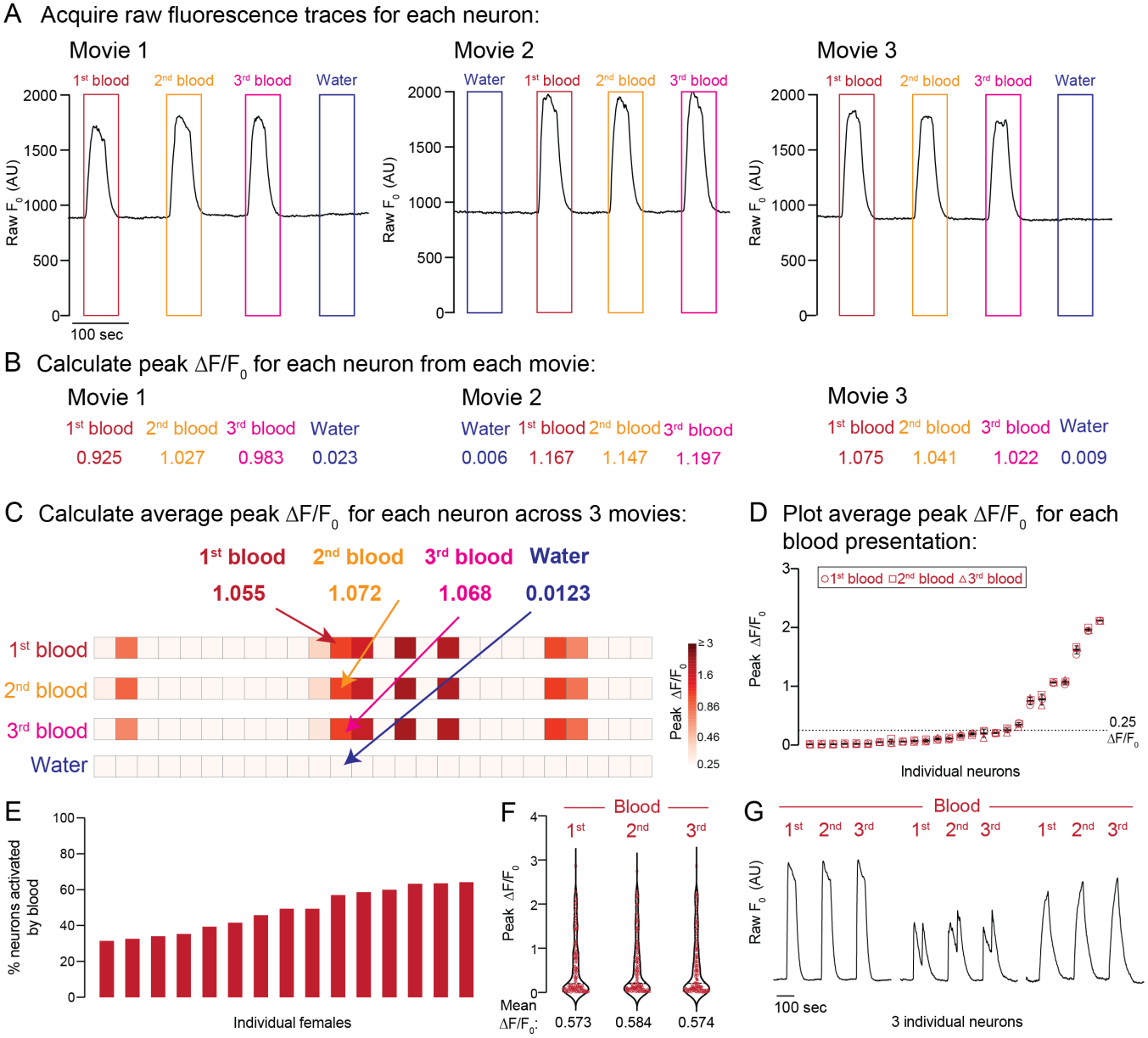

Figure S3. Analysis and Validation of Calcium Imaging Data, Related to Figure 4

(A-D) These panels provide more information on how the values presented in Figure 4G were calculated by showing measurements acquired from 1 individual female in this dataset. (A) Raw fluorescence traces in response to indicated ligand recorded from 1 neuron for 3 replicate movies; each ligand (water, 1st blood, 2nd blood, 3rd blood) is delivered once per movie. (B) For each ligand, the peak ΔF/F is calculated per neuron for each of the 3 movies. (C) Next, the average peak ΔF/F per neuron is calculated by averaging peak ΔF/F from each movie in (B). The average peak ΔF/F to a given ligand is represented as 1 square per neuron in the heatmap. Each column represents 1 neuron and each row represents the response to the indicated ligand for all neurons from 1 individual female from Figure 4G. Neurons are ordered from proximal to distal. (D) For each neuron in (C), the average peak ΔF/F to 1st, 2nd, and 3rd blood is represented as a circle, square, and triangle respectively. Data points (mean ± SD) are sorted by peak ΔF/F0.

(E) Summary of % neurons with ≥ 0.25 peak $\Delta F/F_0$ to blood for all females in Figure 4G, Figure 5D, and Figure S4A (N=15 females). Each column represents 1 female and columns are sorted by % neurons activated by blood (average across all samples = 49.05%).
(F) Summary of peak $\Delta F/F_0$ data for all neurons from the 6 females in Figure 4G (N=161 neurons). Data is shown as median with range (1^{st} blood vs 2^{nd} blood, $p = 0.05$; 1^{st} blood vs 3^{rd} blood, $p > 0.99$; Friedman's test with Dunn's multiple comparisons).
(G) A subset of traces for 3 neurons from 1 individual in Figure 4G, y axis scale: arbitrary units of raw fluorescence.

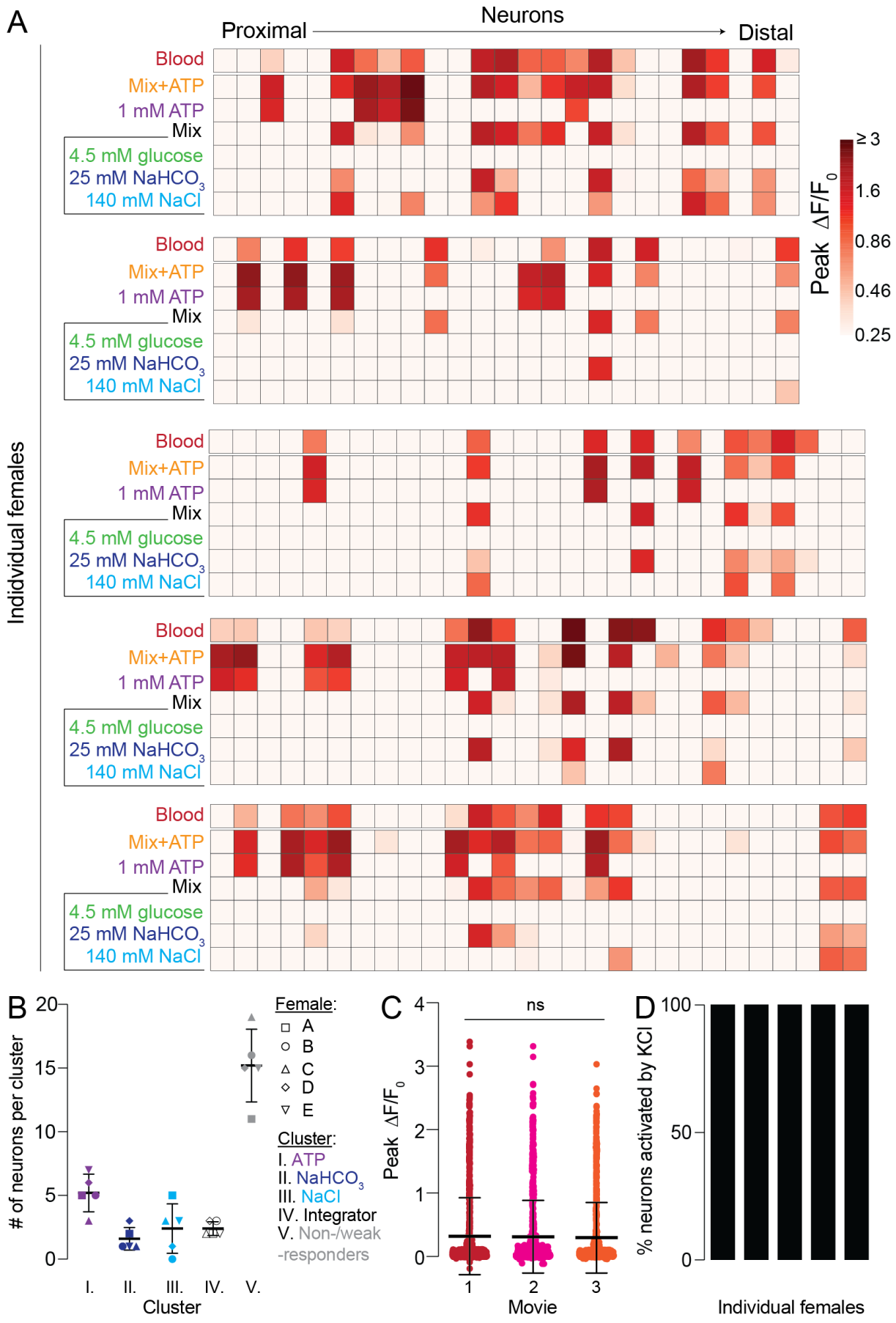
A
Proximal
Neurons
Distal
Blood
Mix+ATP
1 mM ATP
Mix
4.5 mM glucose
25 mM NaHCO3
140 mM NaCl
Individual females
Peak ΔF/F0
≥ 3
1.6
0.86
0.46
0.25
B
# of neurons per cluster
Cluster
I.
II.
III.
IV.
V.
Female:
A
B
C
D
E
Cluster:
I. ATP
II. NaHCO3
III. NaCl
IV. Integrator
V. Non-/weak -responders
C
Peak ΔF/F0
ns
Movie
1
2
3
D
% neurons activated by KCl
Individual females

Figure S4. Blood Component Responses of Individual Females, Related to Figure 5 and Figure S5

(A) Heat maps of peak $\Delta F/F_0$ response to the indicated ligand for individual females prior to clustering in Figure 5G. Each square is the average of 3 ligand exposures. Each column represents one neuron and each row represents the response to the indicated ligand for all neurons from 1 individual female. Neurons are ordered from proximal to distal. N=5 individual females.

(B) Number of neurons per cluster in Figure 5G. All females have neurons in every cluster with the exception of Female D, which has neurons in all clusters except for the NaCl cluster.

(C) For all neurons in Figure 5G and Figure S4A, peak $\Delta F/F_0$ to every perfusion ligand tested in movie 1 (red dots), compared to movie 2 (pink dots) and movie 3 (orange dots). Each data point denotes the response from 1 neuron to 1 ligand, 6 ligands were presented to N=161 neurons (ns: not significant, $p > 0.05$, Friedman test with Dunn's multiple comparisons).

(D) For all females in Figure 5G and Figure S4A, summary of % neurons with ≥ 0.25 peak $\Delta F/F_0$ to the positive control, KCl. Each column represents 1 female, N=5 females.

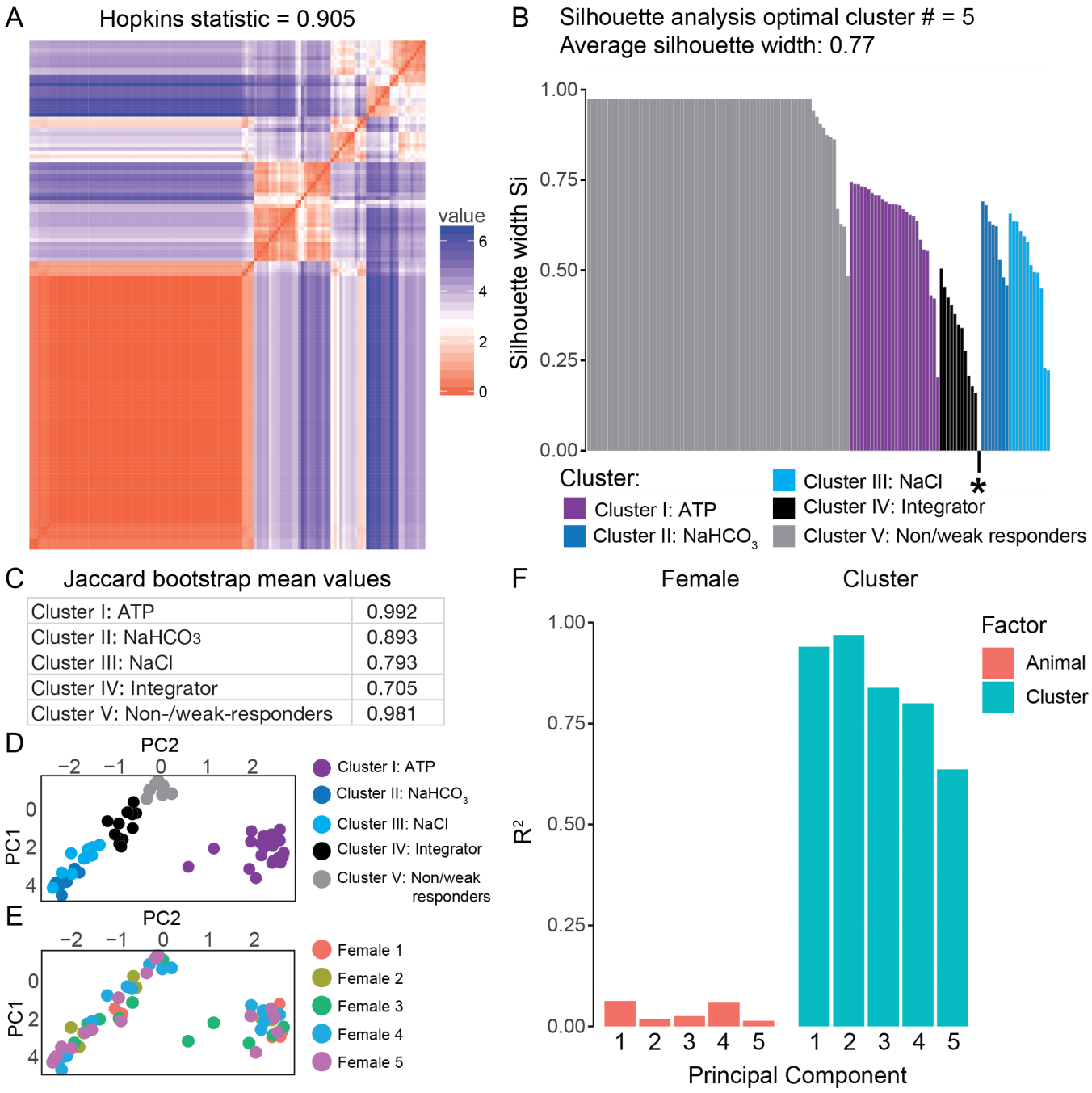

Figure S5. Statistical Analysis of Neuronal Hierarchical Clustering Method, Related to Figure 5 and Figure S4

(A) Clustering tendency in neuronal responses was assessed by calculating the Hopkins statistic using the factoextra R package (https://CRAN.R-project.org/package=factoextra). In the ordered dissimilarity matrix the color level is proportional to the value of dissimilarity between observations and objects belonging to the same cluster are displayed in consecutive order.
(B) The optimal number of clusters was assessed by performing Silhouette analysis (Rousseeuw, 1987) using the NbClust R package (Charrad et al., 2014) with potential cluster numbers in the range of 2 to 10. 5 was selected based on the highest mean silhouette value across clusters (* indicates that 1 neuron in Cluster IV: “Integrator” can be considered mis-clustered with a silhouette width less than 0).

(C) Cluster stability was evaluated by assessing the bootstrap distribution of the Jaccard coefficient of resampled versus original data (Hennig, 2007, 2008). The Jaccard bootstrap mean for each cluster and average across clusters was calculated using the fpc R package's clusterboot function (https://CRAN.R-project.org/package=fpc) with 100 bootstraps.
(D,E) Principal component analysis for individual neurons colored by cluster membership (D) or female (E).
(F) Correlation between each principal component and female identity (orange, left) or cluster membership (cyan, right) was assessed in FactoMineR (Lê et al., 2008).

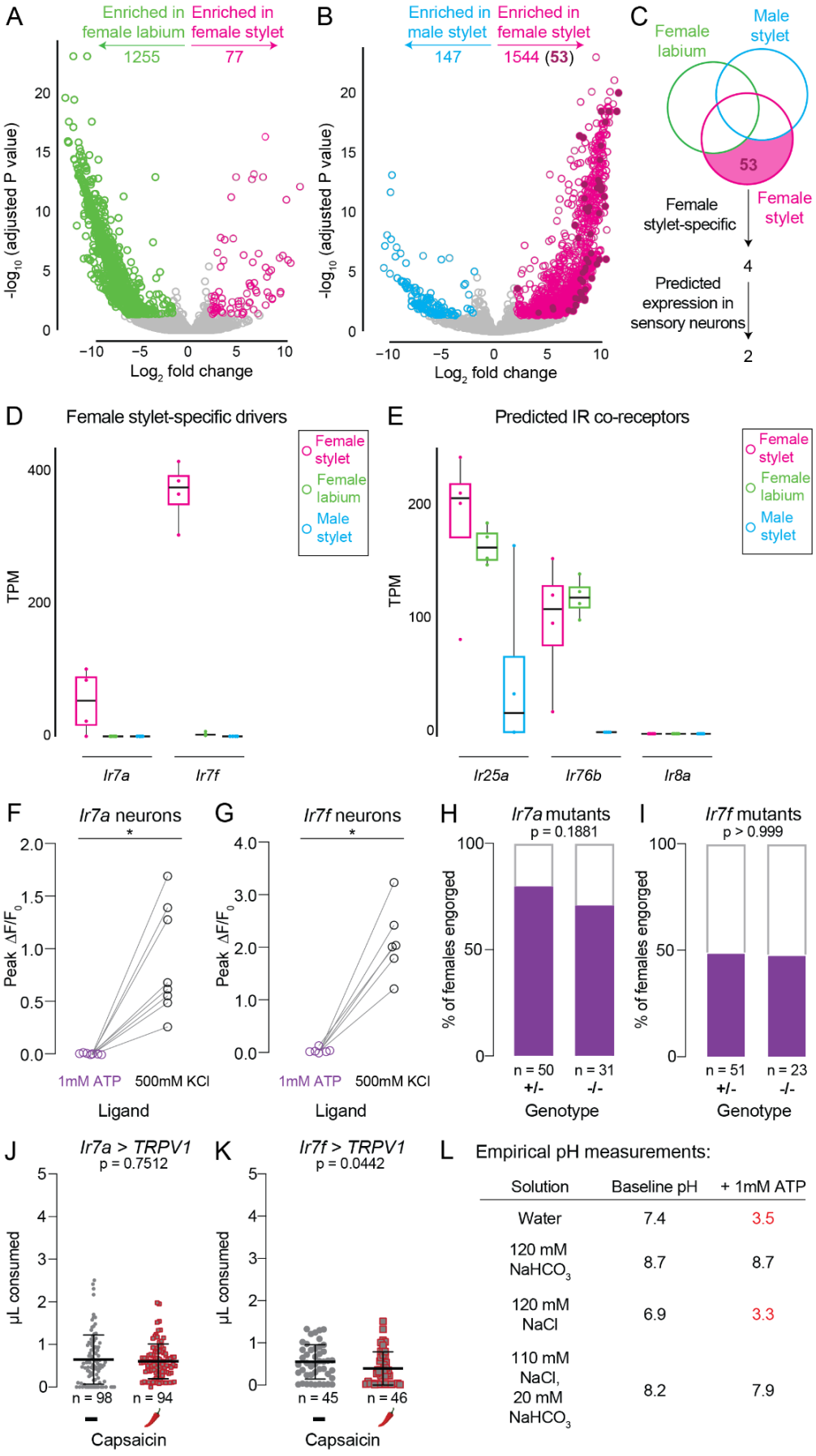
A
Enriched in female labium
1255
Enriched in female stylet
77
-log10 (adjusted P value)
Log2 fold change
B
Enriched in male stylet
147
Enriched in female stylet
1544 (53)
C
Female labium
Male stylet
53
Female stylet-specific
Female stylet
4
Predicted expression in sensory neurons
2
D
Female stylet-specific drivers
TPM
Ir7a
Ir7f
Female stylet
Female labium
Male stylet
E
Predicted IR co-receptors
Ir25a
Ir76b
Ir8a
F
Ir7a neurons
Peak ΔF/F0
1mM ATP
500mM KCl
Ligand
G
Ir7f neurons
H
Ir7a mutants
p = 0.1881
% of females engorged
n = 50
+/-
n = 31
-/-
Genotype
I
Ir7f mutants
p > 0.999
n = 51
+/-
n = 23
-/-
J
Ir7a > TRPV1
p = 0.7512
µL consumed
n = 98
n = 94
Capsaicin
K
Ir7f > TRPV1
p = 0.0442
n = 45
n = 46
L
Empirical pH measurements:
Solution | Baseline pH | + 1mM ATP
Water | 7.4 | 3.5
120 mM NaHCO3 | 8.7 | 8.7
120 mM NaCl | 6.9 | 3.3
110 mM NaCl, 20 mM NaHCO3 | 8.2 | 7.9

Figure S6. Female Stylet-Specific Transcripts, Related to Figure 6

(A-E) RNA-seq dataset comparing the female stylet (pink), female labium (green), and male stylet (blue). N=4 replicates/tissue. For more details on RNA-seq experiment, see Data File 1.

(A,B) Volcano plot of transcripts enriched in the female stylet (pink) or female labium (green) in (A), and female stylet (pink) or male stylet (blue) in (B). 53 transcripts (fuchsia) were enriched in the female stylet compared to both female labium and male stylet. Transcripts were identified as significantly enriched in indicated tissue if Log_2 fold change > 2 and adjusted p value < 0.05, as determined by DESeq2 differential expression analysis.

(C) Venn diagram schematizing filters for identifying female stylet-specific transcripts.

(D,E) Transcripts per million (TPM) data represented as box plots for putative female stylet-specific transcripts selected as driver lines in (D) and predicted Ionotropic Receptor (IR) co-receptors in (E). Median indicated by black line, bounds of box represent first and third quartile, whiskers are 1.5 times the inter-quartile range, and dots represent TPM value from each biological replicate. In (E) the outlier is denoted by a dot without whisker.

(F,G) Peak $\Delta F/F_0$ in response to 1mM ATP (purple) or positive control (500 mM KCl, black) for *Ir7a*- (F, N=8) and *Ir7f*-expressing (G, N=6) neurons. Each data point denotes the response from 1 neuron and responses from the same neuron are connected by a line (* $p < 0.05$, Wilcoxon matched-pairs signed rank test).

(H,I) Female engorgement on 1 mM ATP in 110 mM NaCl and 20 mM $NaHCO_3$ delivered via Glytube (statistical significance determined using Fisher's exact test).

(J,K) Volume of sub-optimal meal consumed by the indicated genotypes. Sub-optimal meal consists of 1 mM ATP in 20 mM $NaHCO_3$, with addition of DMSO vehicle (gray) or 50 µM of capsaicin (gray with red outline). Each data point represents 1 female: *Ir7a>TRPV1* N = 94- 98, *Ir7f>TRPV1* N = 45-46 females/meal (mean ± SD, p values calculated using Mann-Whitney test).

(L) pH of the indicated solution immediately before and after the addition of 1 mM ATP. See also Data File 1.

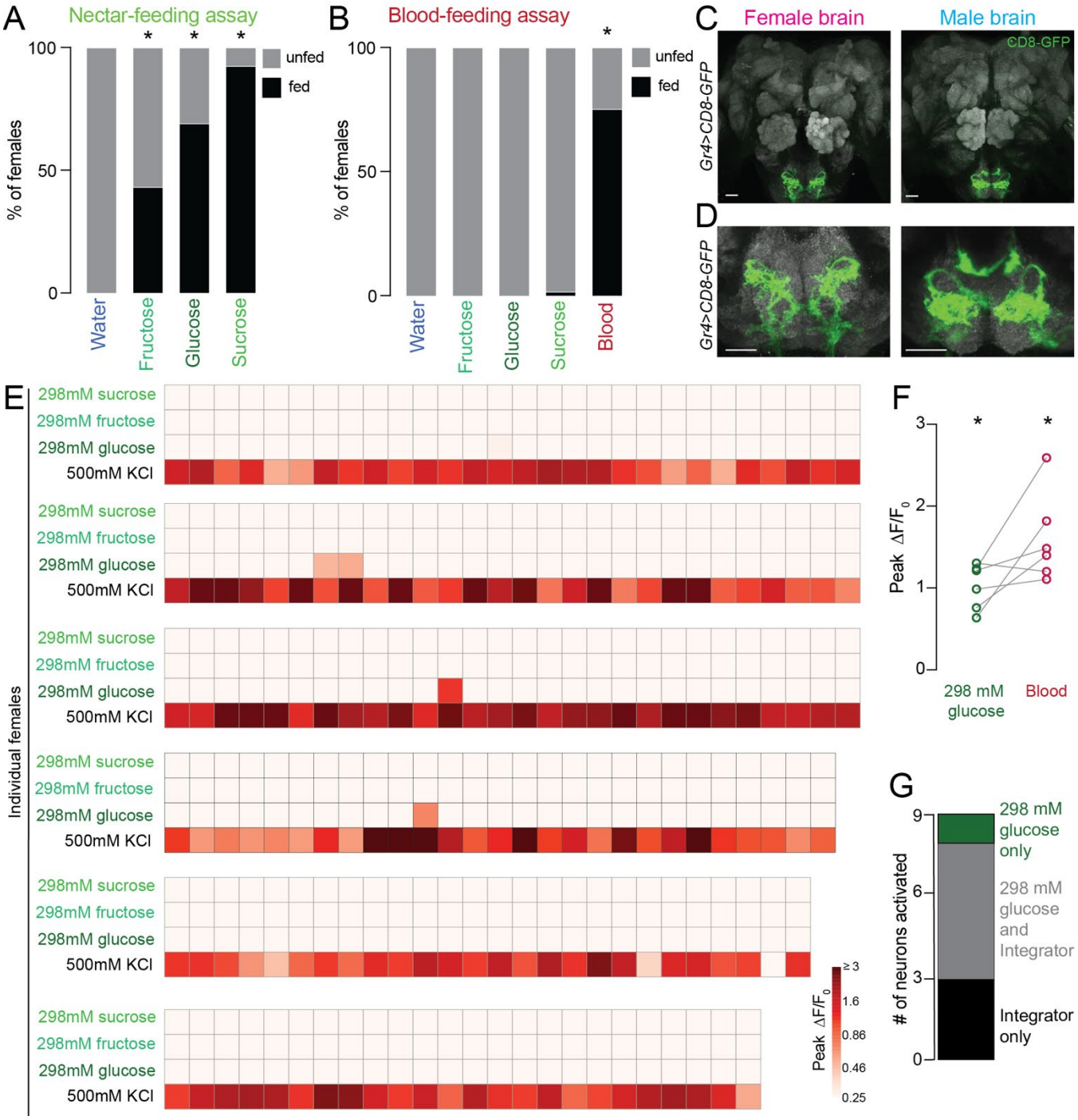

Figure S7. 298 mM-Sensitive Neurons Intersect with Integrator Neurons, Related to Figure 7

(A,B) % of females feeding on the indicated meal offered in the nectar-feeding (A) and blood-feeding (B) assay, based on µL consumed measurements in Figure 7B,C. Unfed and fed females consumed ≤0.05 µL and >0.05 µL, respectively, in Figure 7B,C (water N=36-40; sucrose N=53–60; fructose N=40-74; glucose N=55-59; blood N=76 females). Groups labeled with * are significantly different from water ($p < 0.001$, Fisher's exact test with Bonferroni correction).

(C,D) mCD8:GFP expression (green) of *Gr4>mCD8:GFP* in female (left) and male (right) brain (C) and subesophageal zone (D). Neuropil labeled with anti-*Drosophila* Brp (gray). Brain and subesophageal zone images were acquired from two different individuals.
(E) Heat maps of peak $\Delta F/F_0$ response to the indicated ligand for individual females in Figure 7G. Each square is the average of 3 ligand exposures. Each column represents one neuron and each row represents the response to the indicated ligand for all neurons from 1 individual female. Neurons are ordered from proximal to distal. N=6 individual females.
(F) For 298 mM-sensitive neurons (response to 298 mM glucose ≥ 0.25 peak $\Delta F/F_0$), peak $\Delta F/F_0$ to 298 mM glucose, compared to blood. Each data point denotes the response from 1 neuron and responses from the same neuron are connected by a line (N=6 neurons, mean ± SD, * indicates $p < 0.05$, one-sample Wilcoxon signed rank test).
(G) A dataset from N=6 females was filtered for all 298 mM-sensitive neurons and Integrator neurons to compare the intersection of 298 mM-sensitive neurons and Integrator neurons (N=9 neurons; 1/9 = 298 mM glucose only, 5/9 = 298 mM glucose and Integrator, 3/9 = Integrator only).