

Supplementary Materials for
**Whole-animal multiplexed single-cell RNA-seq reveals transcriptional shifts
across *Clytia* medusa cell types**

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Supplementary Methods
Legends for tables S3 and S5

Other Supplementary Material for this manuscript includes the following:

Tables S3 and S5

Figures

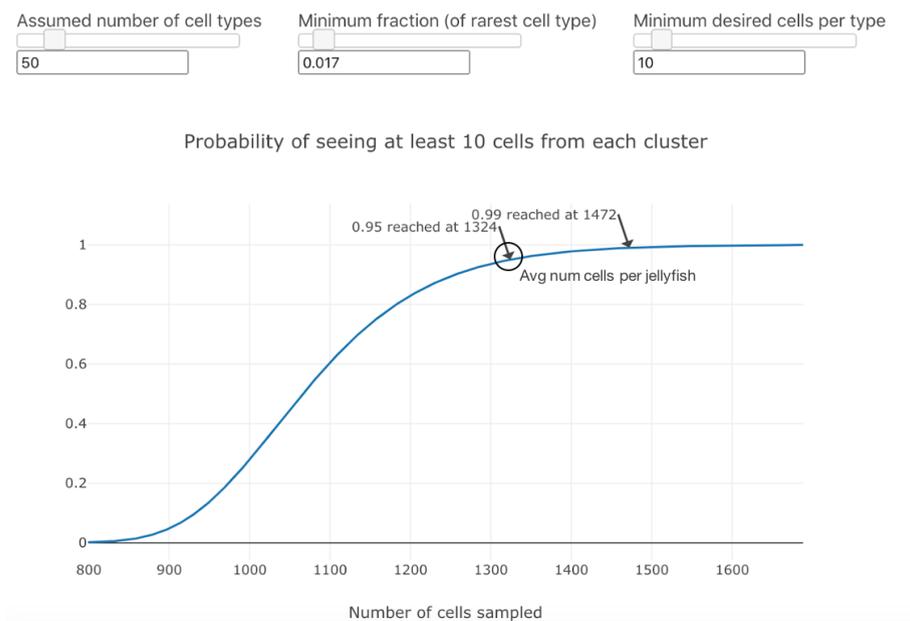


Fig. S1. Cell Type Limit of Detection for Starvation Experiment. Cumulative distribution function displaying likelihood of detecting at least 10 cells from rare cell types which make up 0.017 of the total population of cells, assuming ~50 cell types, over a possible range of number of cells sampled in the given sequencing experiment. The average number of cells per jellyfish used in the starvation experiment is denoted by the circle. Plot generated using the Satija Lab howmanycells calculator.

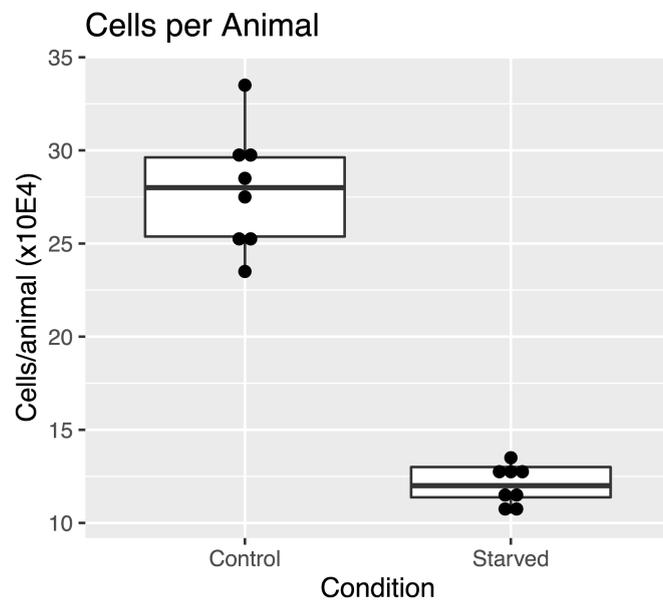


Fig. S2. Fold Change in Cells per Organism Under Starvation. Hemocytometer cell counts for jellyfish in control and starved conditions (n = 4 per condition), with two measurements per animal (cells for each sample counted in two 16 square grids; see Methods). Box-and-whisker plot displayed, with mean line shown and whiskers denoting 1.5 IQR.



Fig. S3. Egg Cell Production Under Starvation. Number of egg cells released in the daily spawning cycle from 5 control and 5 starved medusae on day four of starvation. Animals used are not the same as the individuals in the sequencing experiment, however the same protocol as in Methods was used to conduct the starvation. Error bars denote standard deviation.

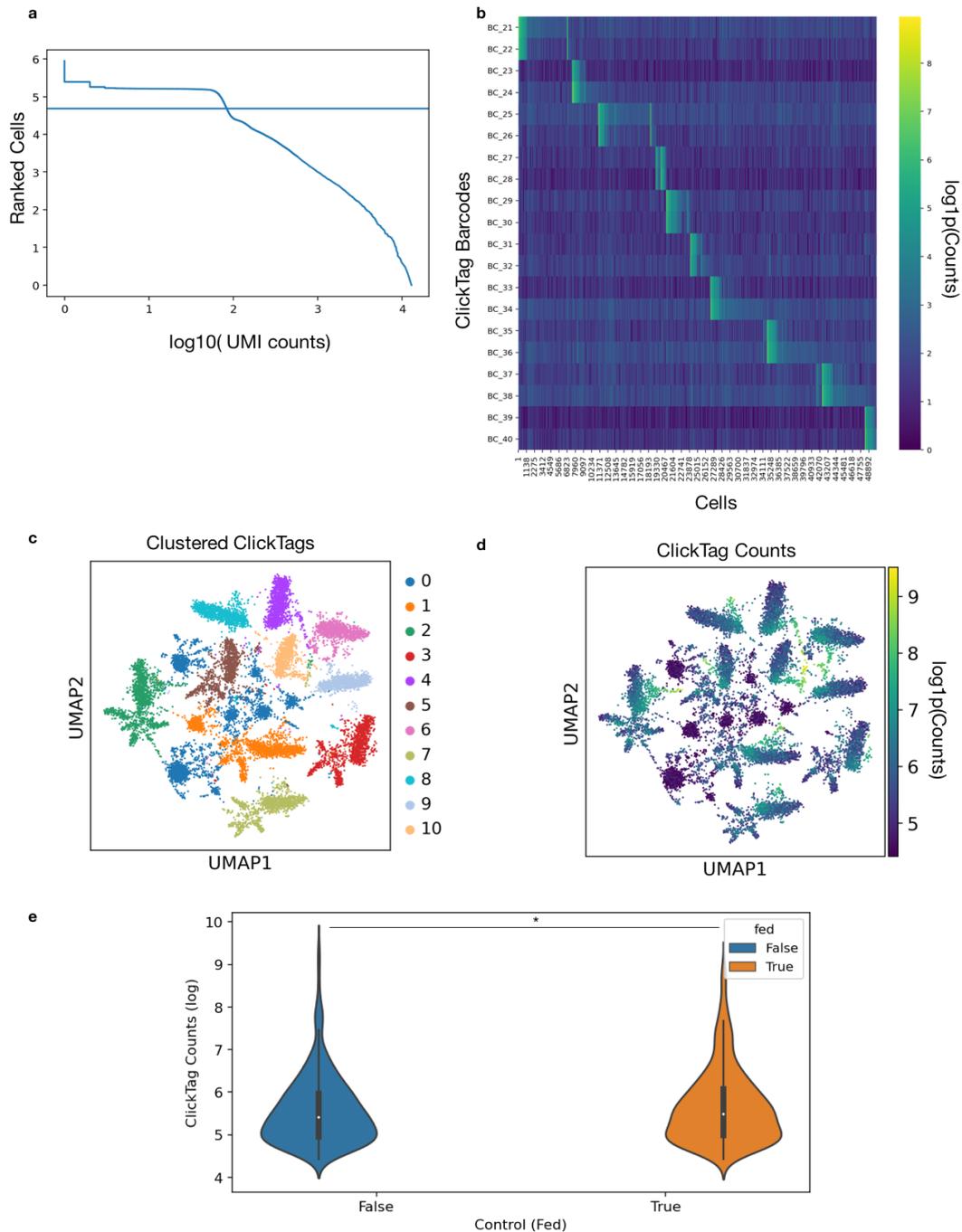


Fig. S4. Cell Barcode Filtering from ClickTag Clustering. **a)** Knee-plot for ranked cell barcodes (ranked according to number of UMIs) versus ClickTag UMI counts per cell barcode. Line denotes filtering/selection of the top 50,000 cells. **b)** Heatmap of counts for ClickTag barcodes associated with cell barcodes. Pairs of barcodes correspond to the ClickTags added to each organism's samples (Table S2). **c)** Louvain clustering of ClickTags based on counts across cells, corresponding to the 10 labeled organisms. **d)** Log counts of ClickTags. High count clusters correspond to cells of the 10 dual-barcoded animals. **e)** ClickTag counts per cell in control (fed) versus starved cells, subsetted to oocytes. * denotes p -value < 0.05 from a one-sided t-test, with alternative hypothesis Control ClickTag Counts $>$ Starved ClickTag Counts.

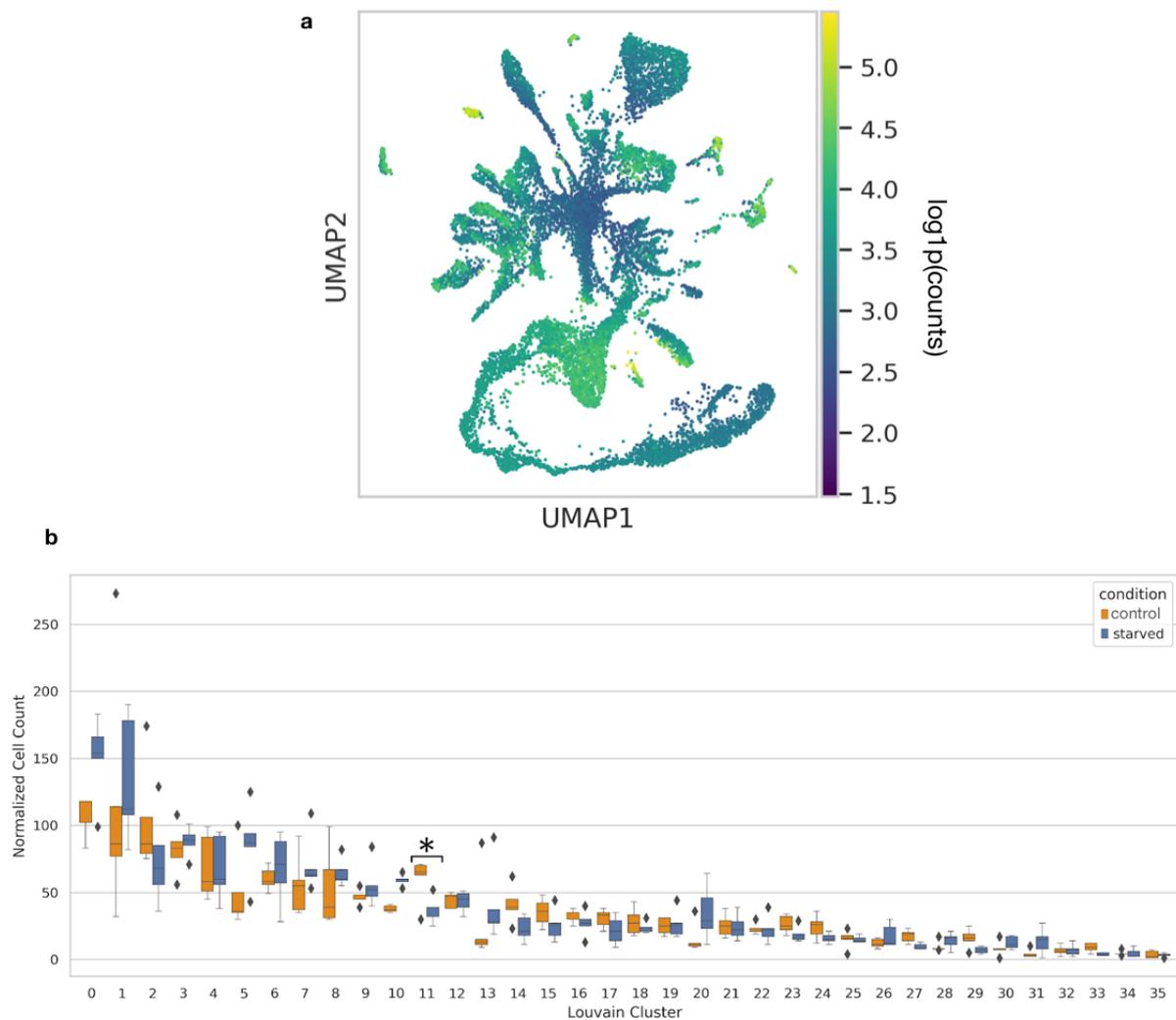


Fig. S5. Distribution of Cell Counts between Starvation Conditions. a) UMI counts shown on log scale across cells on the UMAP embedding. b) One-way ANOVA on log cell counts within each cell type (per each of the 5 individuals). Whiskers denote 1.5 IQR and diamonds denote outlier points. Pval obtained with an F test. * = p -value < 0.05, adjusted with Benjamini-Hochberg for FDR correction. Early nematoblasts (cluster 11) show significant differences in numbers between individuals in starved vs control conditions.

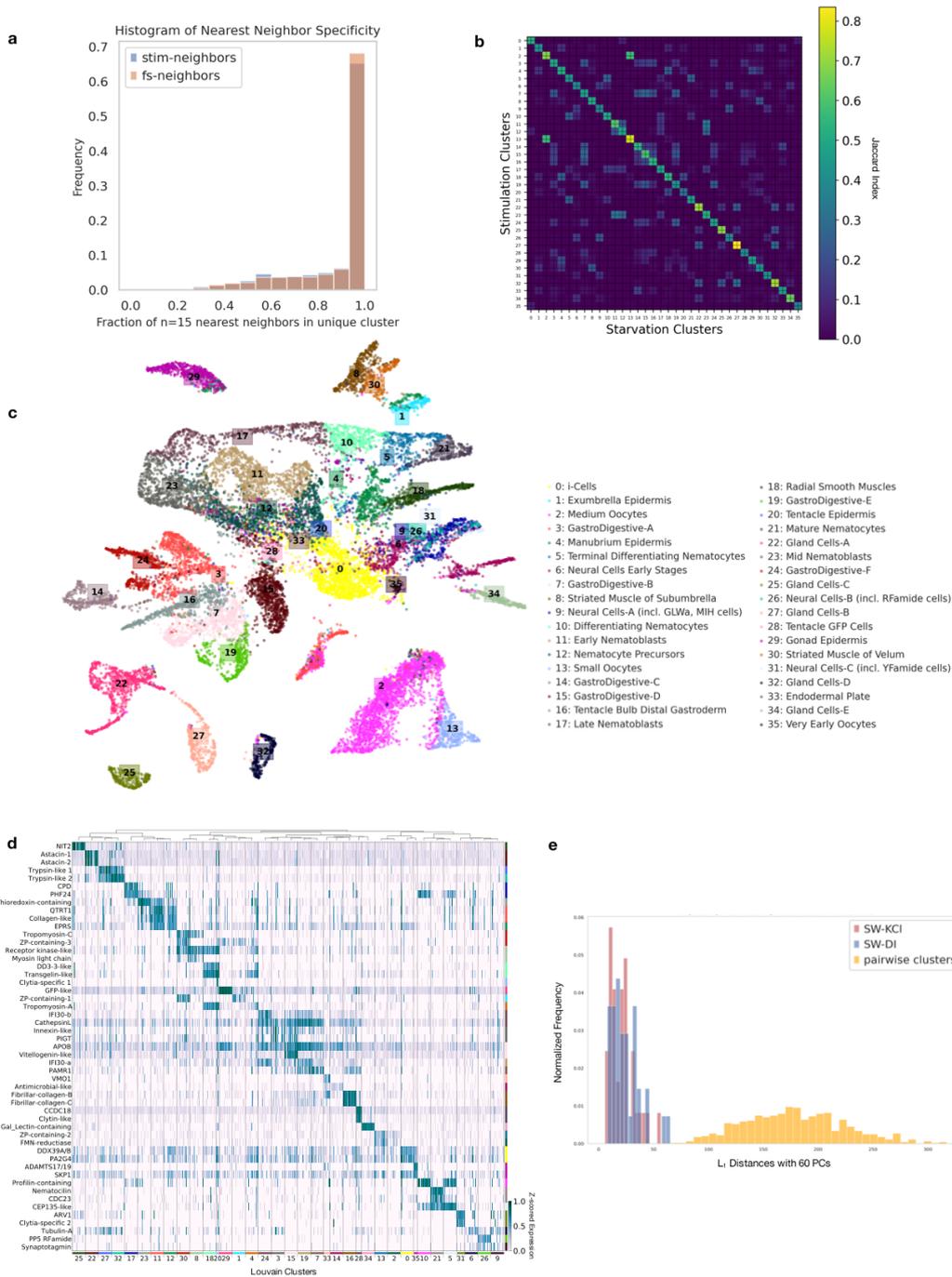


Fig. S6. Concordance of Cell Types Between Two Distinct Multiplexed Experiments. a) Starvation data (training set) used to select nearest neighbors from left-out starvation data and all the stimulation data. For each selection, the fraction of nearest neighbors that had the same cell type label is reported (see Methods). **b)** Top 100 markers from the stimulation experiment with cluster labels applied from K-Nearest Neighbors (see Methods) compared to top 100 markers per cell type from starvation clustered data. **c)** UMAP/PAGA embedding of stimulation data with applied cell type labels. **d)** Heatmap of markers from Fig. 2b shown for stimulation data. **e)** Inter- and intra-cluster distances shown for control (SW) versus perturbed cells for each perturbation (see Methods). The cell type with largest internal distance (but non-overlapping) is 20 (tentacle epidermis).

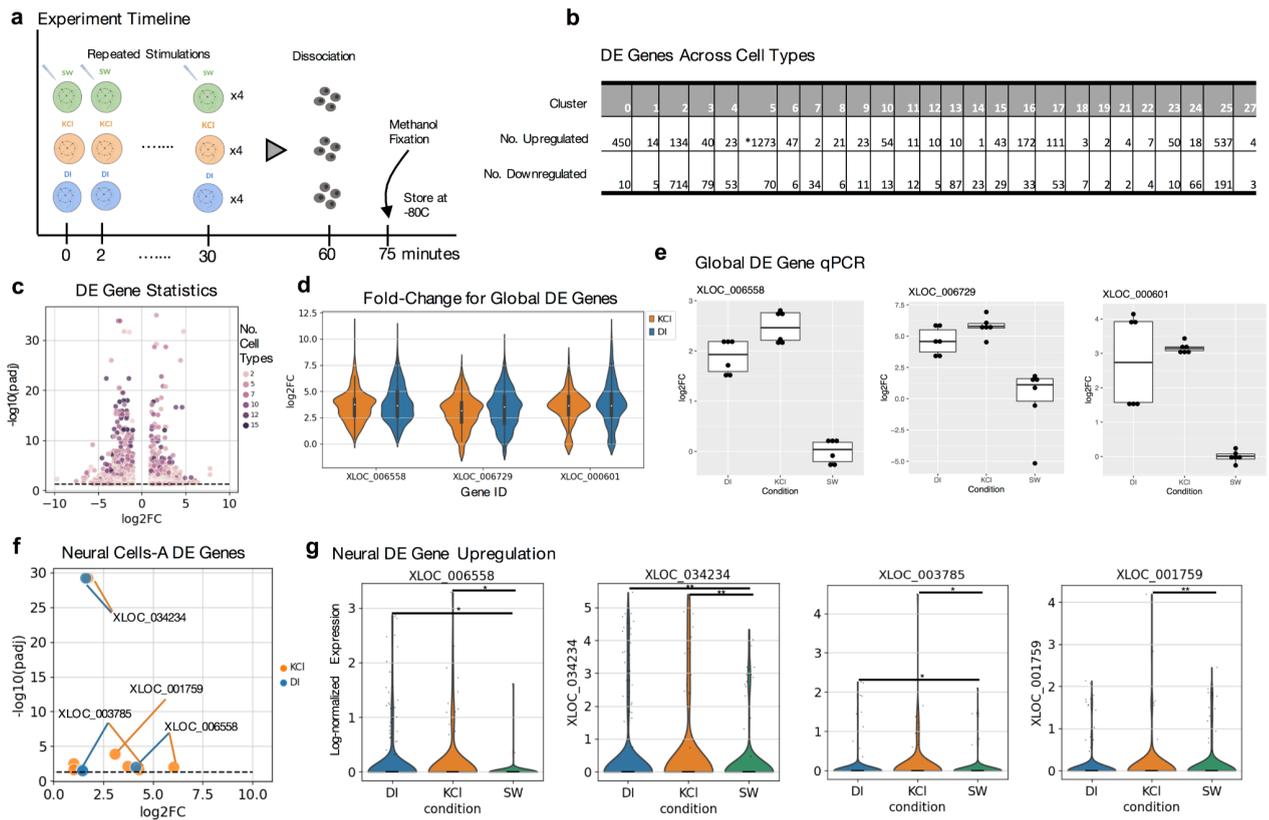


Fig. S7. Cell-Type Specific Gene Expression Changes Following KCl and DI Treatment.

a) Diagram of the ‘Stimulation’ experiment. Four biological replicates (animals) used for each condition. SW denotes seawater (control), DI denotes deionized water addition, and KCl denotes potassium chloride addition (see Methods). 30 minutes following the last stimulation, animals were dissociated and fixed in methanol. **b)** Summary table of numbers of up- and down-regulated genes in each cluster (cell type). *Denotes highest number of DE genes in 5, terminal differentiating nematocytes. **c)** Volcano plot of p -value and fold-change from DeSeq2 analysis (see Methods) for DE gene candidates. Dashed line denotes 0.05 alpha cutoff applied during selection of DE genes (see Methods). Colors indicate the number of cell types a gene is found to be differentially expressed in. Genes appear multiple times for each cell type (if applicable). **d)** Fold-change per condition across all cells for global (DE in many cell types), ‘IEG’-like candidates. **e)** qPCR for DE gene candidates shown in panel d in both conditions (see Methods). **f)** Volcano plot for selection of upregulated DE genes in Neural Cells-9 (cluster 9), which consists of the majority of neural cells, colored by perturbation condition. Gene names denote selected candidates. **g)** Expression for cells in each condition of upregulated DE genes found in Neural Cells-9 (cluster 9) using the non-parametric, Wilcoxon test. P -values adjusted for multiple testing with Benjamini-Hochberg correction. * denotes p -value < 0.01 , ** denotes p -value < 0.001 .

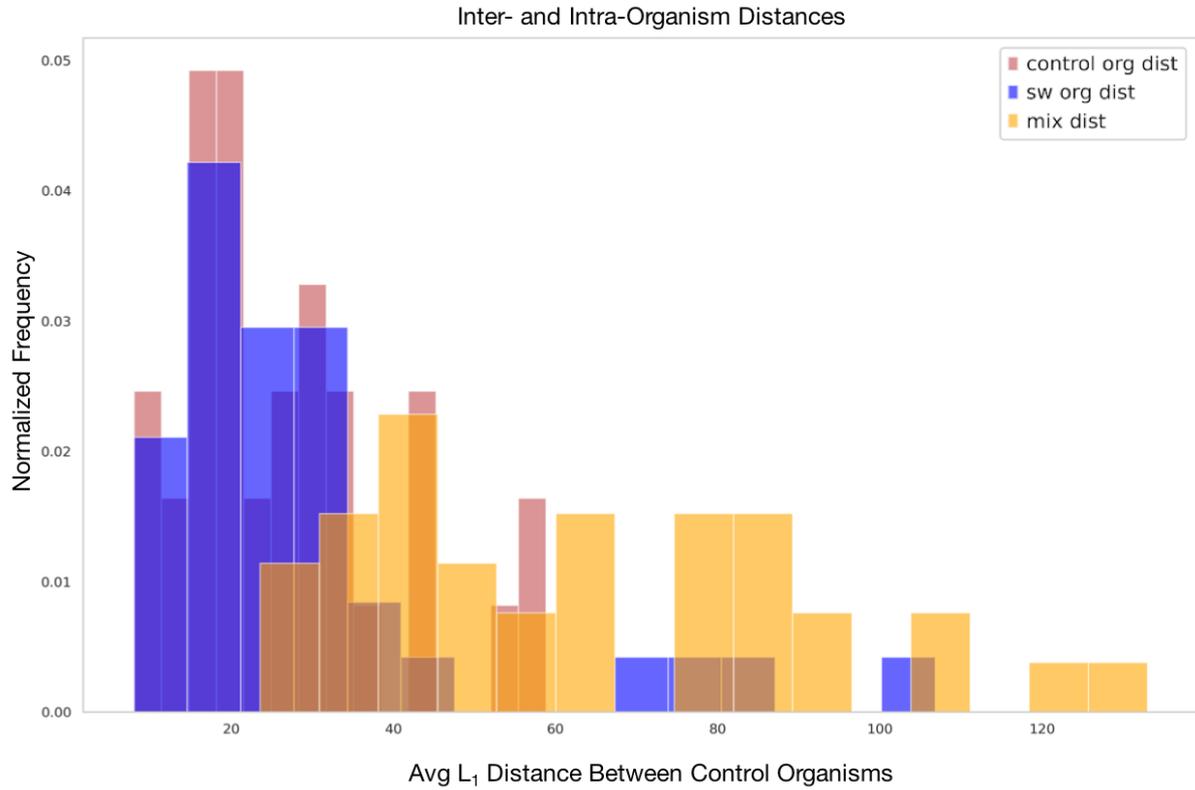


Fig. S8. Distances between Control Cells in each Multiplexed Experiment. Histograms of average pairwise L_1 distances between cell types of control animals within (red and blue) and across (yellow) experiments for visualization of batch effect (see Methods).

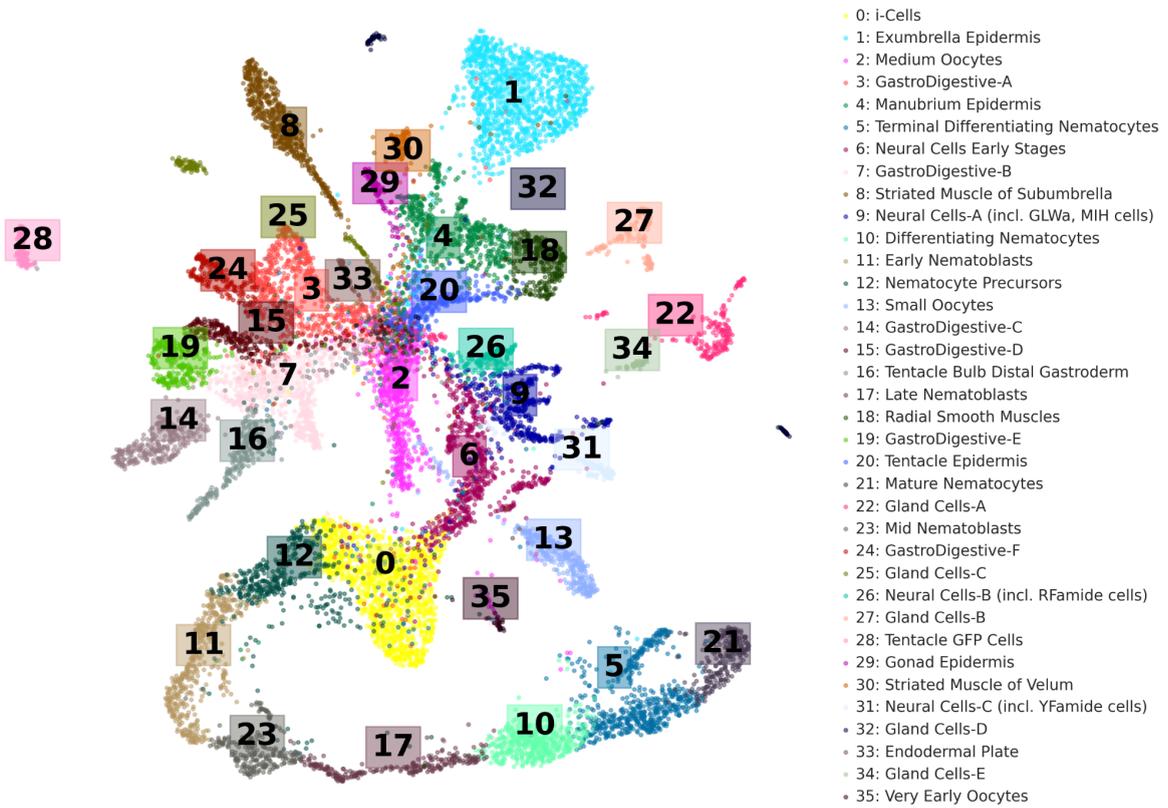


Fig. S9. High Resolution Clustering Annotation of Cell Atlas from Starvation Dataset. Cell Atlas with all 36 clusters/cell types labeled with UMAP/PAGA embedding from the starvation experiment data (see Methods).

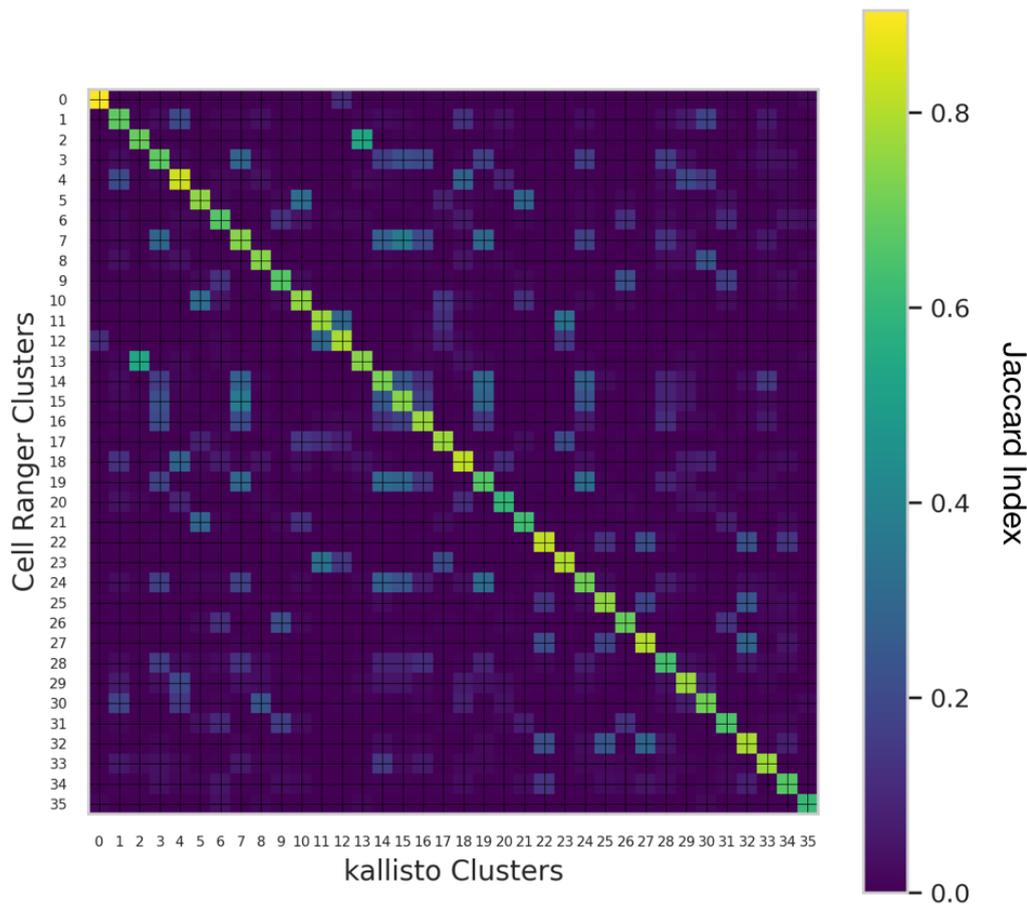


Fig. S10. Marker Gene Overlaps for Cell Types in Cell Ranger versus kallisto Read Quantifications. Pairwise overlap of the top 100 marker genes from the original 36 Cell Ranger clusters applied to kallisto-bustools processed starvation data versus the top 100 markers from the original Cell Ranger clustered data. The coloring reflects the Jaccard Index of each top marker gene set (number of genes in the intersection divided by the number of genes in the union).

GENERAL IDENTITY	CLUSTER	KEY DIAGNOSTIC GENES	CELL TYPE
Epidermal / muscle	1	TPMA, GFP3 ³⁹	Exumbrella epidermis
	4	ST MyHCa, ST MyHCb, GFP4 ³⁹	Manubrium epidermis
	8	ST MyHCa, TPMB	Striated muscle of subumbrella
	18	TPMA	Radial smooth muscle
	20	TPMA, ST MyHCa, Wnt2 ³⁵ ,	Tentacle epidermis
	29	TPMA, ST MyHCb, GFP4 ³⁹	Gonad epidermis
Gastroderm	30	ST MyHCa , TPMB, Peroxidase	Striated muscle of velum
	3	CathepsinL	GastroDigestive A
	7	CathepsinL, Nucleoside hydrolase	GastroDigestive B
	14	CathepsinL, BP10-like , Fibulin	GastroDigestive C
	15	CathepsinL, VCBS repeat protein	GastroDigestive D
	19	CathepsinL, FibCdom-2	GastroDigestive E
	24	CathepsinL, DDAH2	GastroDigestive F
Bioluminescent cells	16	FibColl-2	Tentacle bulb distal gastroderm
	33	FibColl-2, FibColl-1, Fibulin	Endodermal plate
Stem cell /Germ cells	28	GFP2b ³⁹	Tentacle GFP cells
	0	Nanos1 ³⁴ , Piwi ³⁴ , Vasa ³⁴ , Znf845	I-cells
	35	Nanos1 ³⁴ , Piwi ³⁴ , Vasa ³⁴ , Scyp1 ⁸	Very early oocytes
	13	Nanos1 ³⁴ , Piwi ³⁴ , Vasa ³⁴ , GFP2a ³⁹ , Clytin2 ³⁹	Small oocytes ^a
Gland cells	2	Nanos1 ³⁴ , Piwi ³⁴ , Vasa ³⁴ , GFP2a ³⁹ , Clytin2 ³⁹	Medium oocytes ^a
	22	ShKT-TrypB	Gland cells A
	27	C3-Lipase , Tryp-like , Fibulin	Gland cells B
	25	FibCdom-1	Gland cells C
	32	C3-Lipase	Gland cells D
Nematocytes	34	Chitinase	Gland cells E
	12	Znf845, Mos3, Minicollagen 3/4 ³⁶ , NOWA ³⁶	Nematocyte precursors
	11	Mos3, Minicollagen 3/4 ³⁶ , NOWA ³⁶	Early nematoblasts
	23	Minicollagen 3/4 ³⁶ , NOWA ³⁶ , DKK3 ³⁶	Mid nematoblasts
	17	Minicollagen 3/4 ³⁶ , NOWA ³⁶ , DKK3 ³⁶ , M14-peptidase, Whirlin ⁴⁵	Late nematoblasts
	10	M14-peptidase, Whirlin ⁴⁵ , Harmonin ⁴⁵	Differentiating nematocytes
	5	Whirlin ⁴⁵ , Harmonin ⁴⁵ , Sans ⁴⁵ , PKD-2 like	Terminal differentiating nematocytes
Neural	21	Whirlin ⁴⁵ , Harmonin ⁴⁵ , Sans ⁴⁵ , PKD-2 like, Nematocilin ⁴⁴	Mature nematocytes
	6	ELAV, PP3 ⁵ , PP19 ⁵	Neural cell early stages
	9	ELAV, PP11 ⁵ , Opsin9 ⁶⁰	Neural cells A (inc. GLWamide & MIH cells)
	26	ELAV, PP5 ⁵	Neural cells B (inc. RFamide cells)
	31	ELAV, PP9 ⁵	Neural cells C (inc. YFamide cells)

Fig. S11. Examples of Diagnostic Genes for Assigning Cell Type Identities.

Overview of cluster identities deduced from markers including the key diagnostic genes listed. These include *Clytia* genes whose expression in the medusa has been previously characterised (34-36, 39, 58) and genes with clear homology to genes with clear cell type specificity in other animals (Scyp1= XLOC_008881, ortholog to *Hydra* Scyp1/Sc4wPfr_899.g22607 (8); Sterovillus components Harmonin/USH-IC =XLOC_003773, Whirlin=XLOC_011922 and Sans/USH-1G=XLOC_039341 (43). For all other genes cited, *in situ* localization profiles, UMAP embedding and XLOC identifiers are shown in Fig. 2C , Fig. 3, Fig. S14, Fig. S18, and Table S3.

^a Clusters 2 and 13 have overlapping profiles including known oocyte specific genes such as GFP 2 (XLOC_004150) and ones highly expressed in published *Clytia* “Growing” and “Fully grown“ oocyte transcriptomes (38) .

^b Nematogenesis occurs in two distinct phases with distinct transcriptional profiles expressing genes associated either with the nematocyst (Clusters 12, 11 and 23) or with nematocil formation (Clusters 10, 5 and 21) . Cluster 17 includes two cell subpopulations preferentially expressing either nematocyst components such as Minicollagen3/4 (XLOC_004102) (36) or structural genes associated with the mechanosensory cnidocil. It is thus interpreted as a transition phase between distinct phases of nematogenesis.

^c Details of neuropeptide precursors in Table S4.

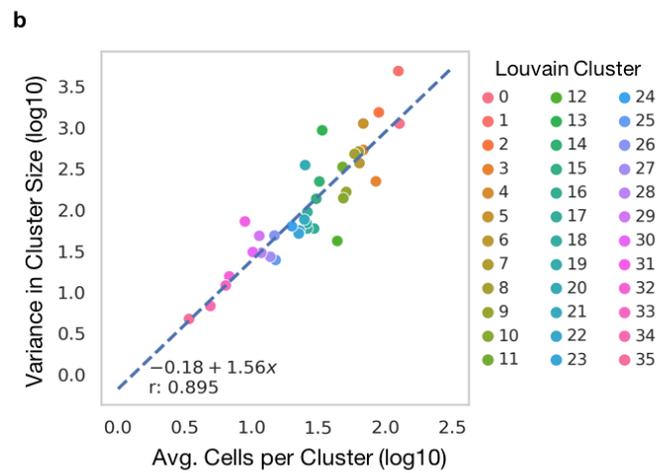
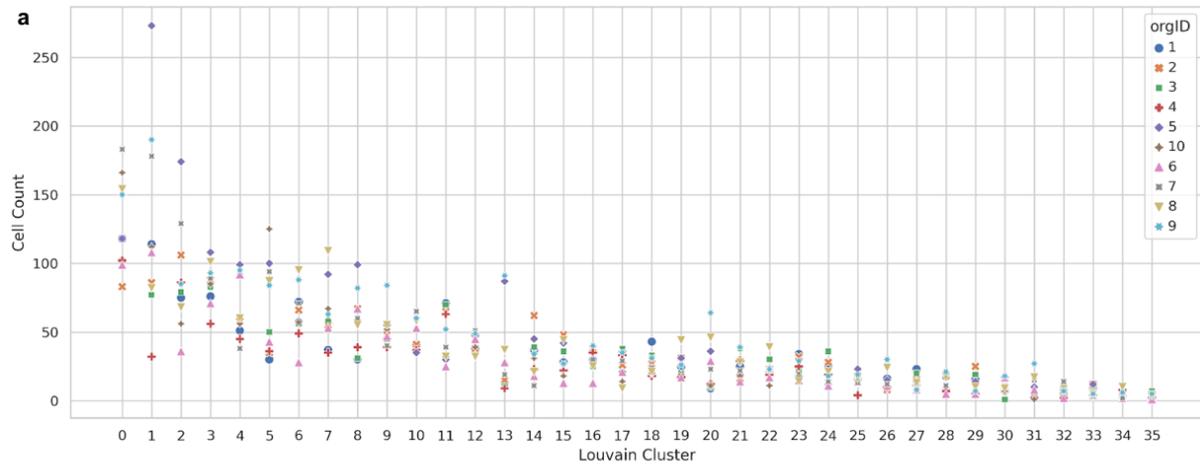


Fig. S12. Cell Counts per Individual Across Cell Types. a) Cell count for each of the 36 cell types and each organism. **b)** Linear log-log relationship between variance in cluster size (cell count) across individuals and average size (cell count), for all 36 cell types.

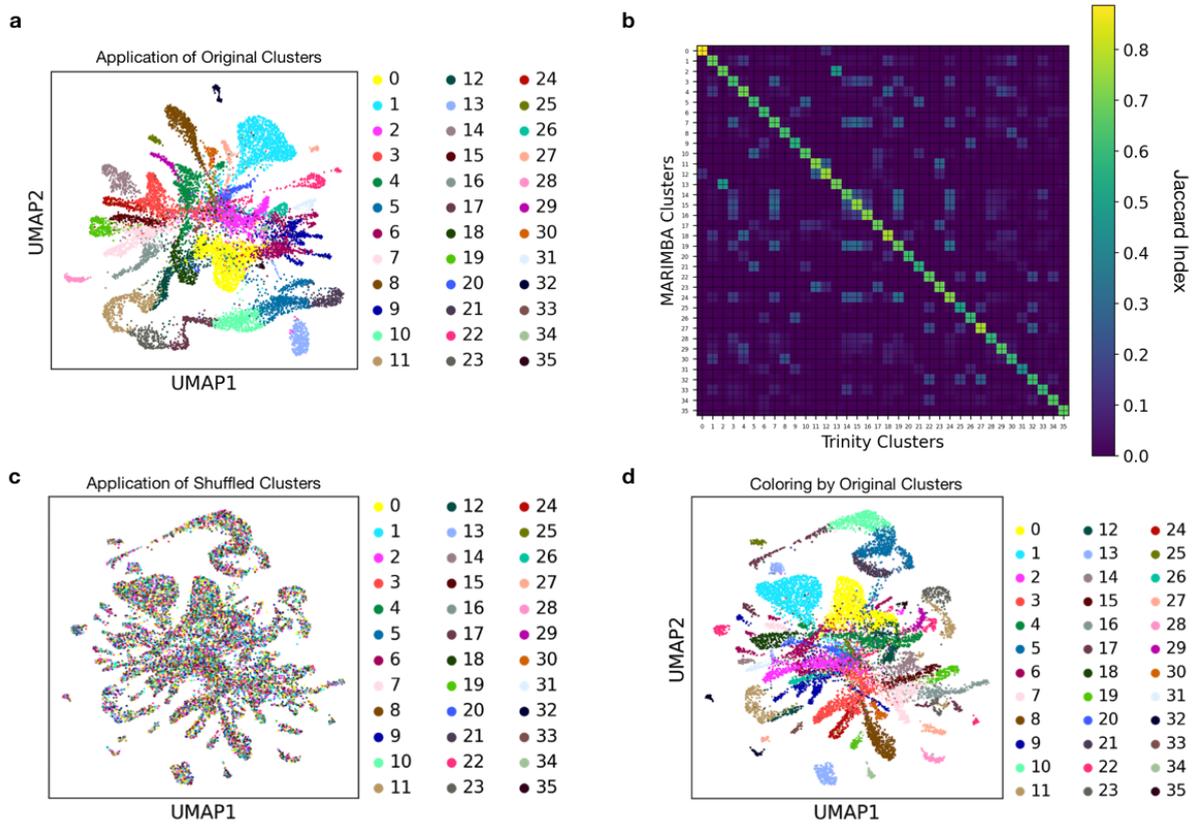


Fig. S13. Cell Type Comparisons from Read Quantifications using MARIMBA Transcriptome versus Trinity-assembled Transcriptome. **a)** UMAP/PAGA embedding of the cell-by-gene counts derived from processing the scRNA-seq data with respect to MARIMBA v.1 with the previously derived 36 cell type labels applied to the cells (see Methods). **b)** The Jaccard index for the top 100 markers from the MARIMBA v.1 annotation derived clusters versus from the Trinity/Cuffcompare annotation derived clusters. **c)** UMAP/PAGA embedding of the count matrices derived from MARIMBA v.1 annotations with scrambled cell type labels used as PAGA input. **d)** Embedding from **c** colored according to the cell types derived from Trinity/Cuffcompare annotations.

Gastroderm

GFP cells

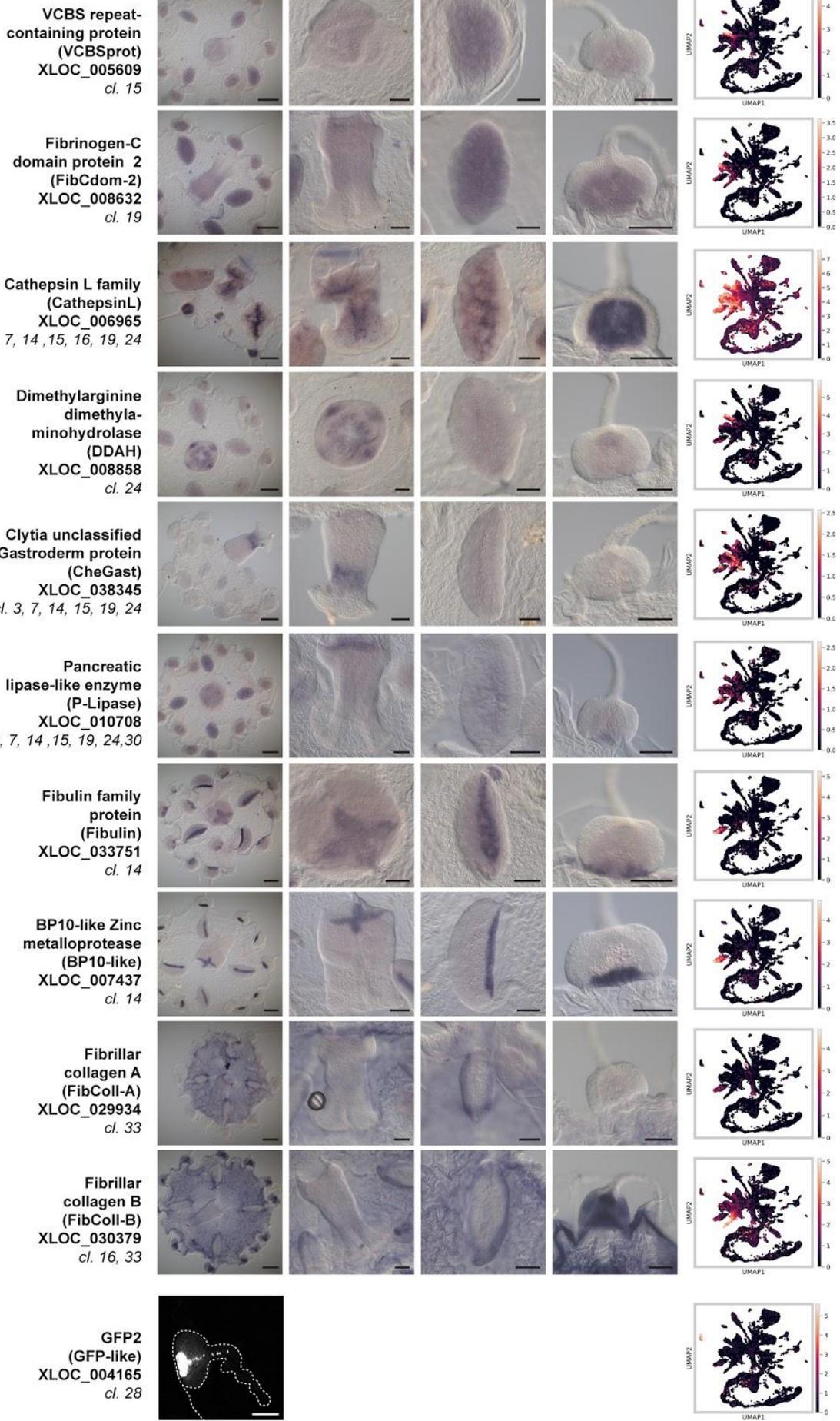


Fig. S14. Continued.

Epidermal / Muscle



Gland cells

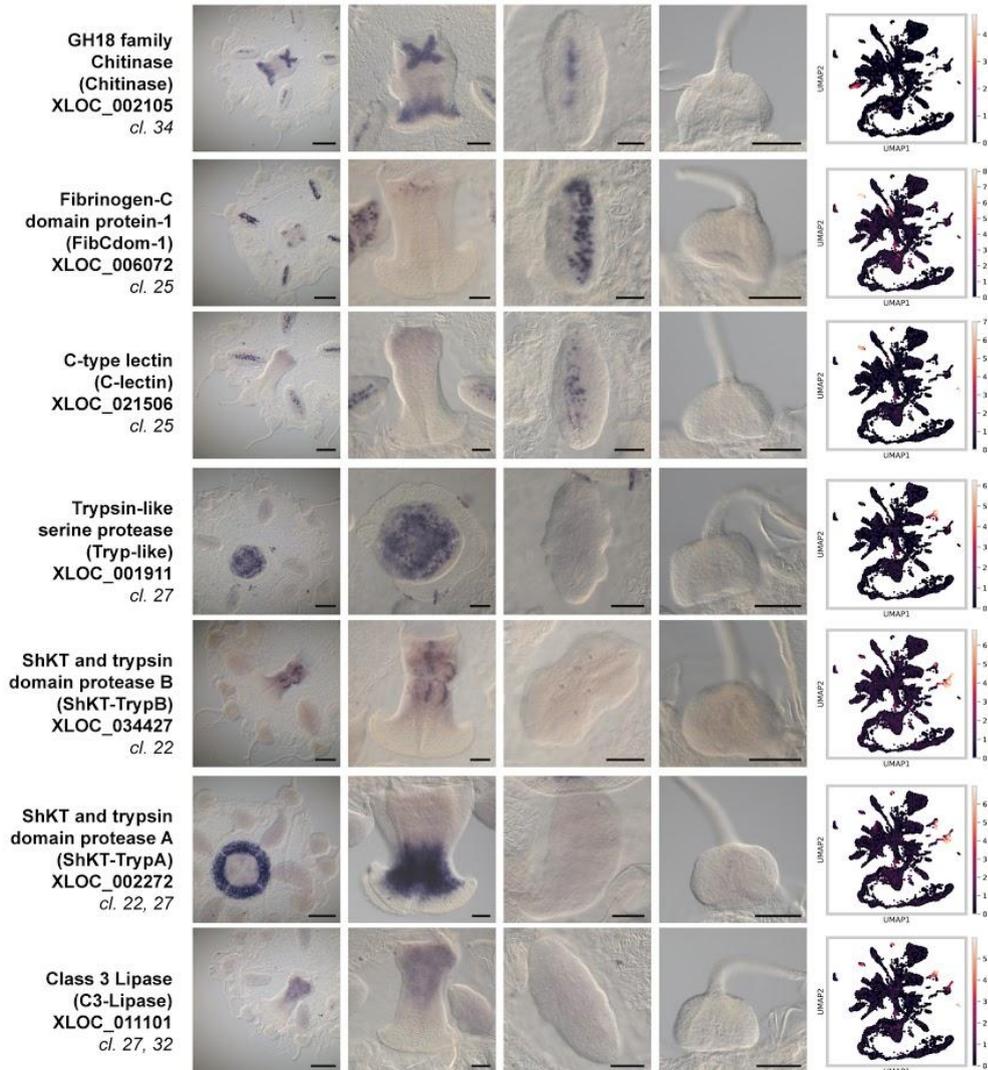


Fig. S14. Continued.

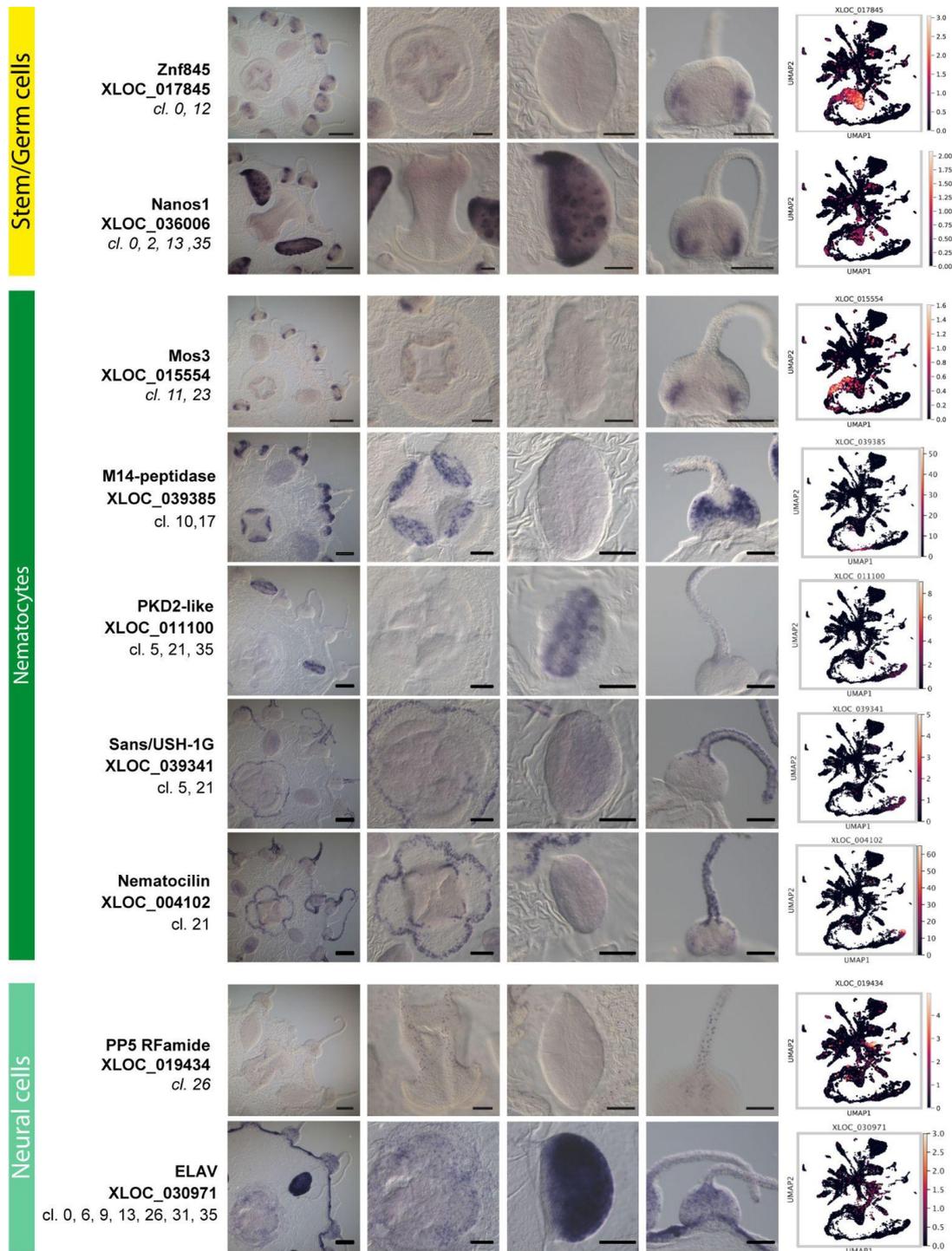


Fig. S14. *In situ* Hybridizations of Cell Type Marker Genes. *In situ* hybridization patterns for a selection of cluster marker genes providing spatial location of expression on the animal. Cluster IDs (cl.) indicated beneath the gene names/XLOC identifiers were assigned directly using the marker gene lists and/or by comparing gene expression profiles in the merged-experiment dataset (see Methods). Images from left to right: whole medusa, manubrium, gonad, tentacle bulb. Right column: Gene expression for each of the marker genes represented in red in the single-cell atlas. Scale bars in the whole medusa images represent 200 μ m; in the manubrium, gonad and tentacle bulb images they represent 100 μ m.

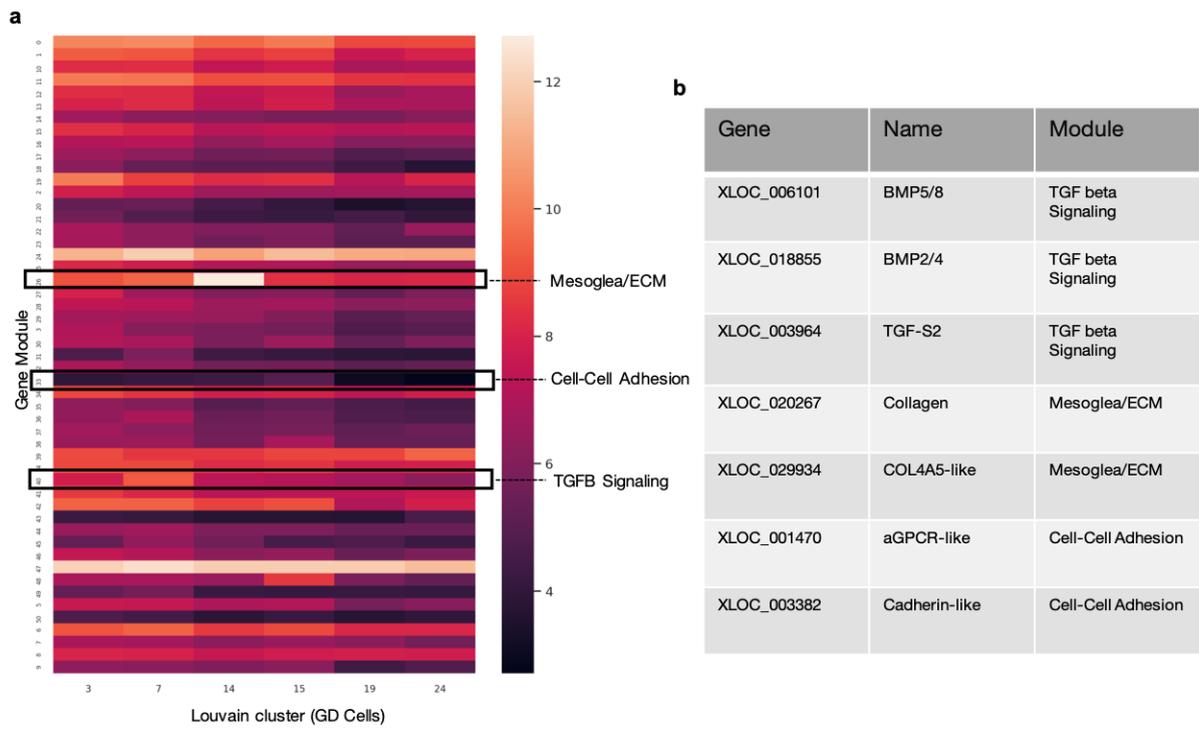


Fig. S15. Specialization of Gastrodermal Cell Types in Gene Modules. **a)** Modules of co-expressed genes with average expression of each module ($n = 50$) in each GD cell type (see Methods). **b)** Gene IDs for selected genes of the designated modules.

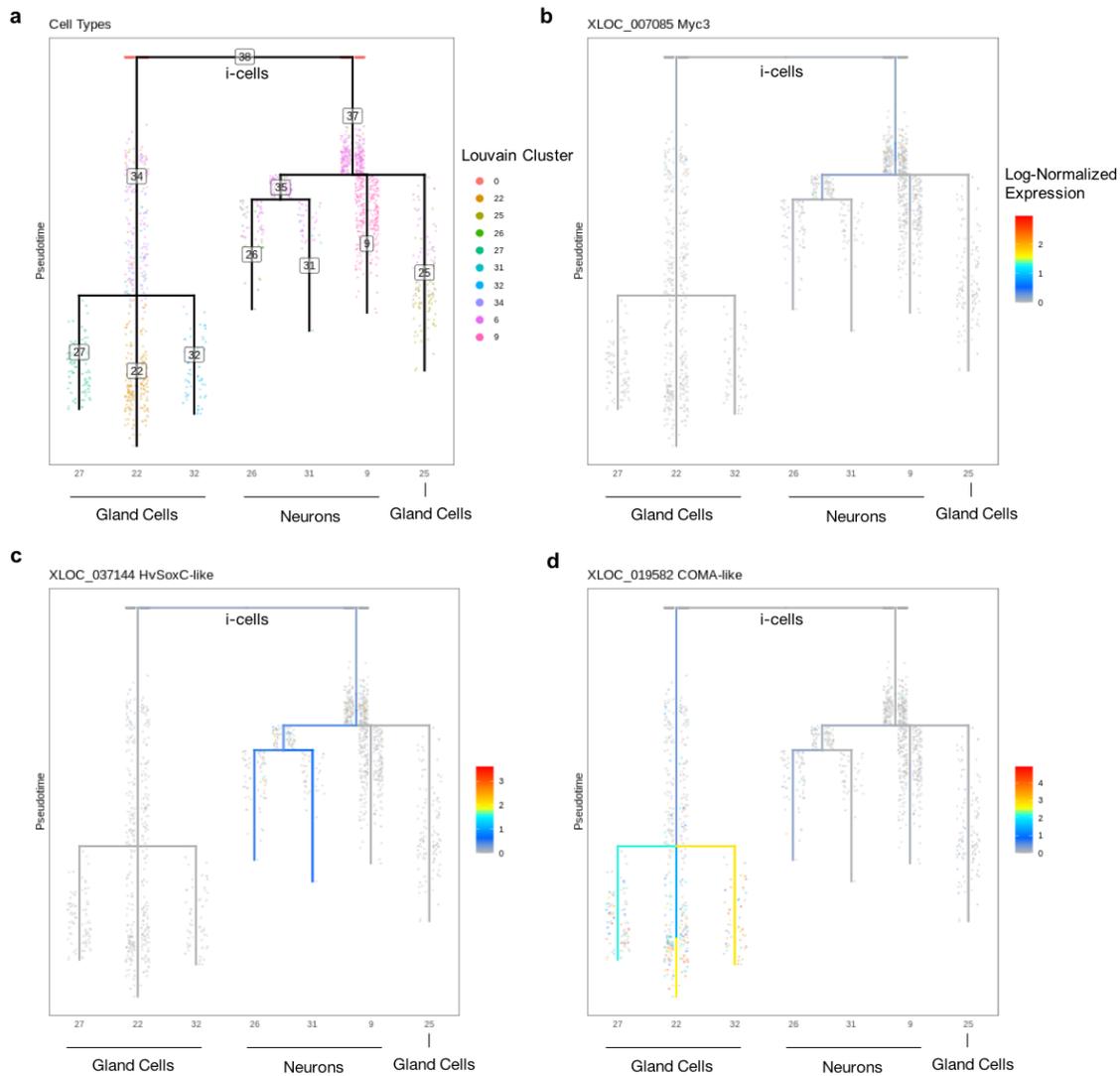


Fig. S16. Analysis of Gland Cell Relationships to Neural and i-cell Populations. **a)** URD tree diagram (78) of i-cell, gland cell, and neural populations with tree tips defined as 'terminal' populations. Branches are identified by numbers in boxes. Branch 34 consists of a mix of Cluster 34 cells and other gland cells. **b)** Expression of the *Clytia* ortholog of *Hydra Myc3* along the tree. The *Clytia Myb* ortholog has very low expression and is not included in these plots. **c)** Ortholog of *Hydra HvSoxC-like* marking the trajectory for Cluster 26 and 31 neurons. **d)** Expression of the *Clytia* ortholog of *Hydra COMA-like* (gland cell marker) in 4 of the 5 gland cell populations.

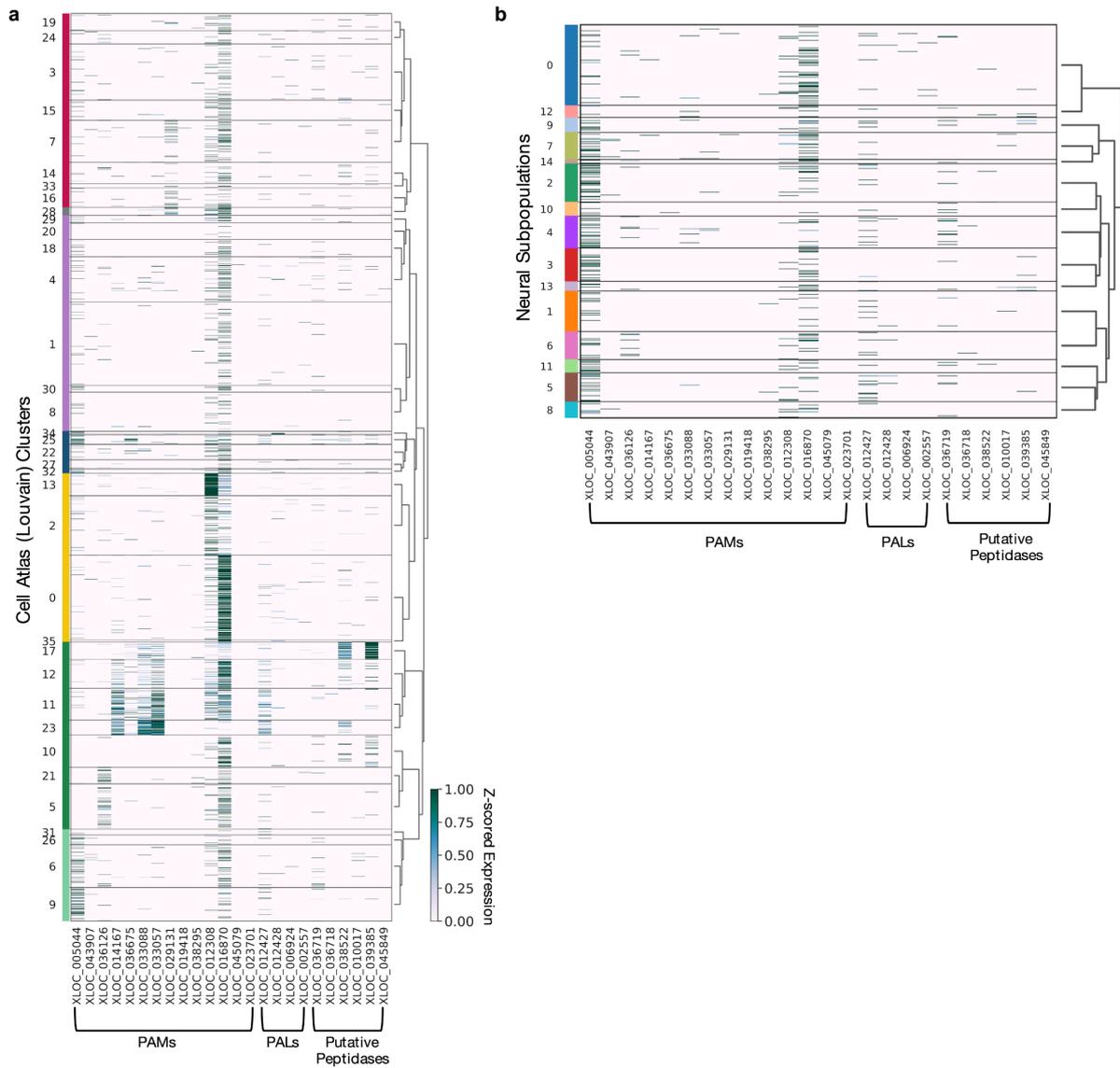


Fig. S17. Expression of Putative Neuropeptide Processing Genes. **a)** Expression profiles for putative neuropeptide processing genes, grouped into peptidylglycine alpha-amidating monooxygenases (PAMs), peptidyl-alpha-hydroxyglycine alpha-amidating lyases (PALs), and likely neuropeptide-processing carboxypeptidases. Expression shown across the cell atlas Louvain-derived clusters. **b)** Expression profiles for the same gene candidates across the neural subpopulations (see subpopulations in Fig. 4).

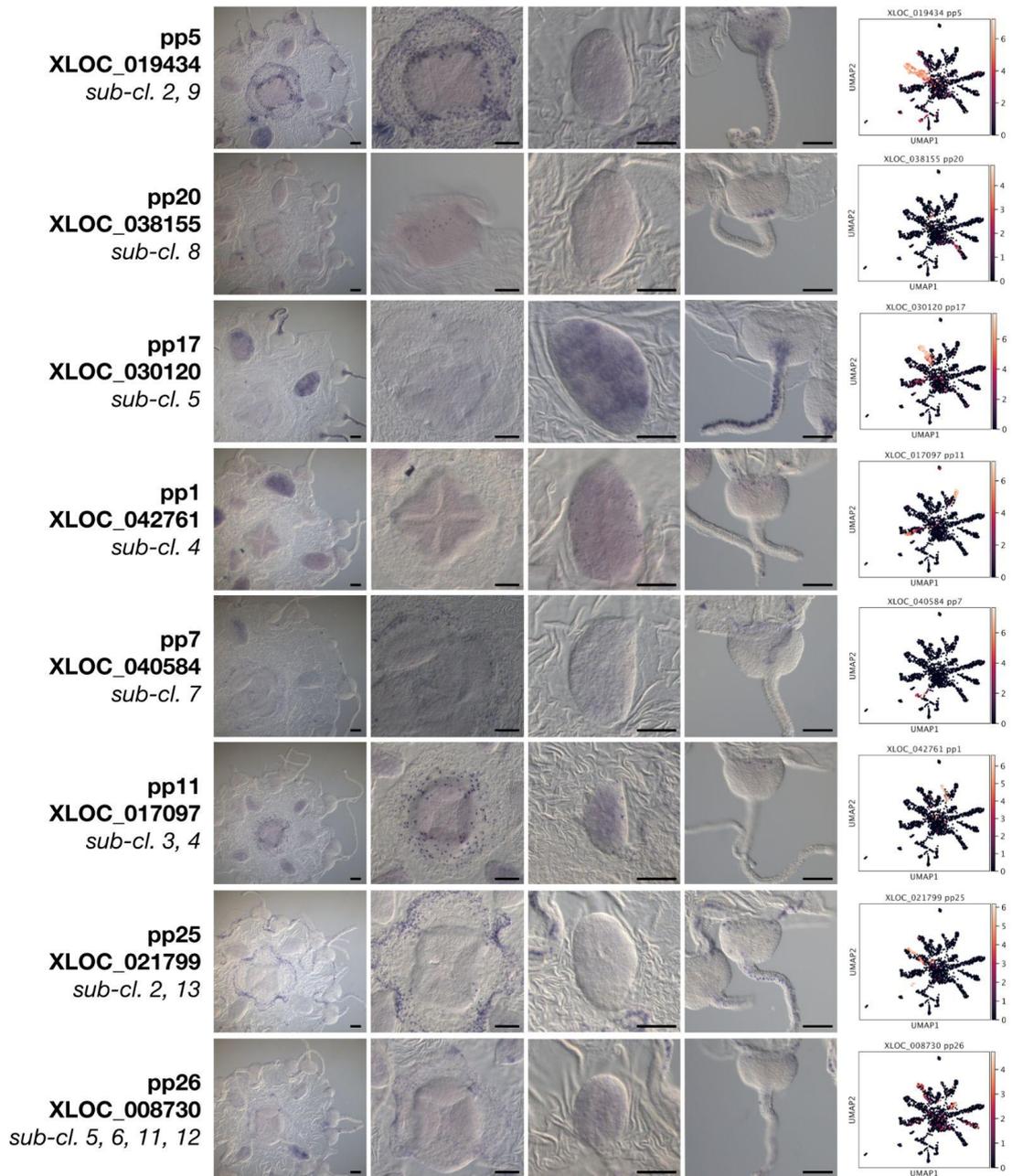


Fig. S18. *In Situ* Hybridizations for Neural Subpopulations. *In situ* hybridization for a selection of neuropeptide candidates. Sub-cluster IDs (sub-cl.) were assigned according to the expression of the markers in the UMAP plot. Images from left to right: whole medusa, manubrium, gonad, tentacle bulb. Right column: Expression for each of the neuropeptides represented in red in the neuron subpopulation atlas. Scale bars represent 100 μ m.

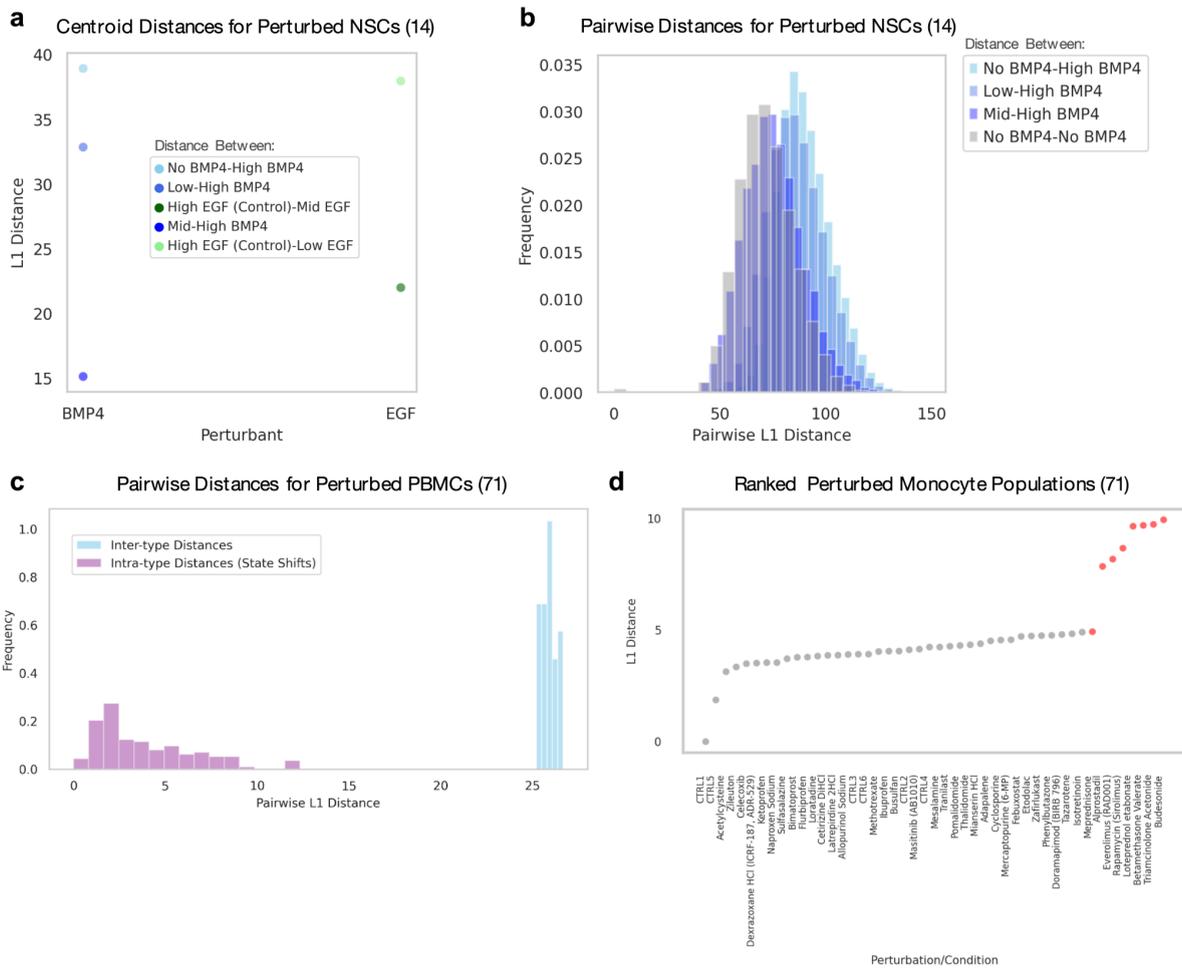


Fig. S19. Validation of L_1 Metric for Recapitulation of Perturbation States. **a)** L_1 distances between the centroids of control populations of NSCs (No BMP4 and High EGF) and their respective perturbed conditions. Higher BMP4 concentration, and lower EGF concentration, denote the most perturbed states respectively. (14) **b)** All pairwise L_1 distances between cells in BMP4 conditions, with lower distance between less distant conditions. (14) **c)** Intra-type distances (state shifts) in purple i.e. L_1 distances between the centroids of perturbed monocytes or T-cells and the control (CTRL1) cells' centroid (for monocytes or T-cells). Inter-type- distances in blue i.e. L_1 distances between the centroids of monocytes and T-cells in each control (CTRL). (71) **d)** Ranked L_1 distances between the centroids of perturbed monocytes to CTRL1 monocytes, as a measure of perturbation distance. Top ranked conditions (most perturbed conditions) from original study, highlighted in pink (see Methods for more details) (71).

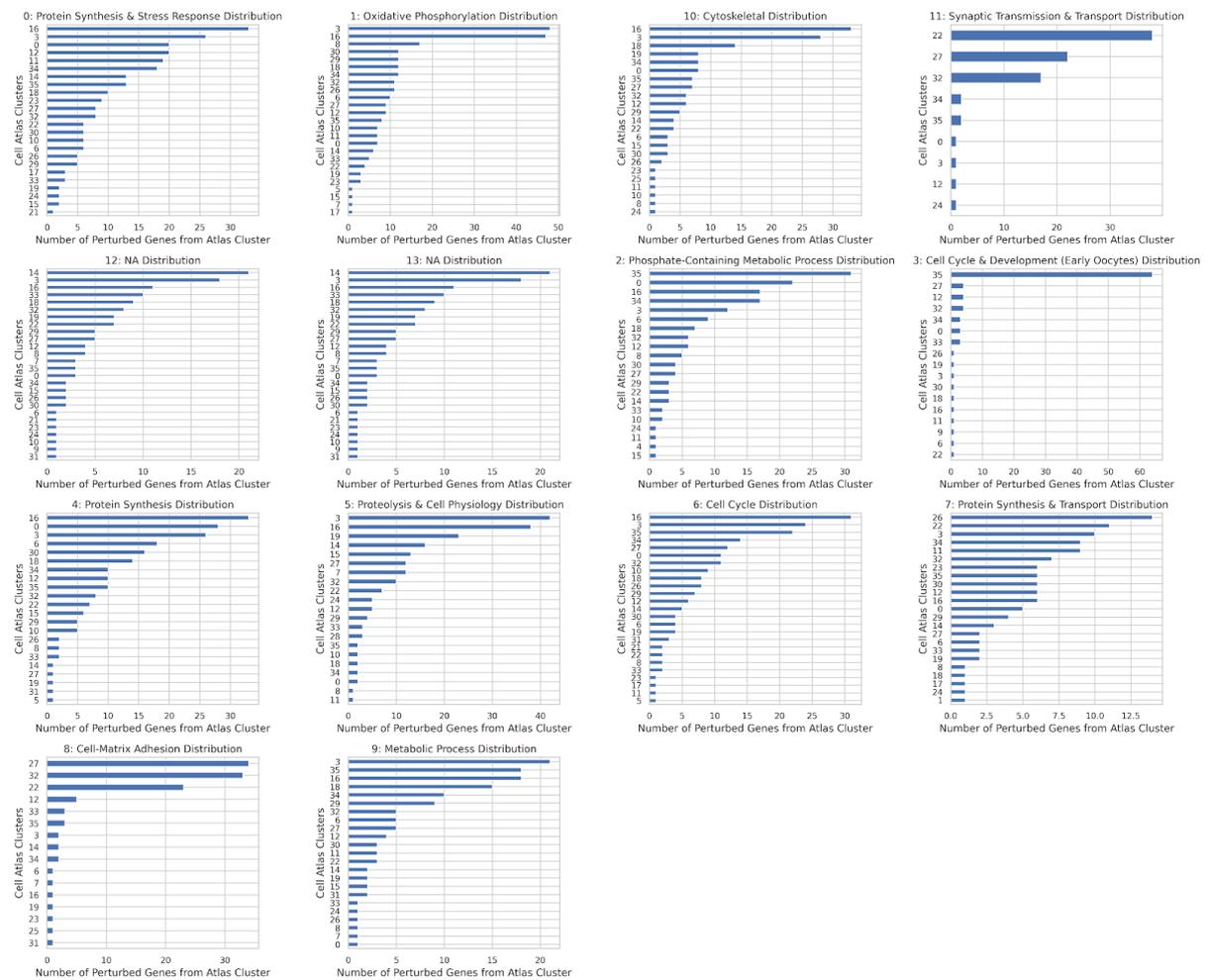


Fig. S20. Contribution of Genes each Perturbed Gene Module by Cell Type. The number of genes contributed by each of the 36 cell types to each of the gene modules. Names for gene modules were assigned based on gene ontology (GO) term enrichment with topGO (see Methods). 'NA' is assigned to gene modules with no significant GO terms.

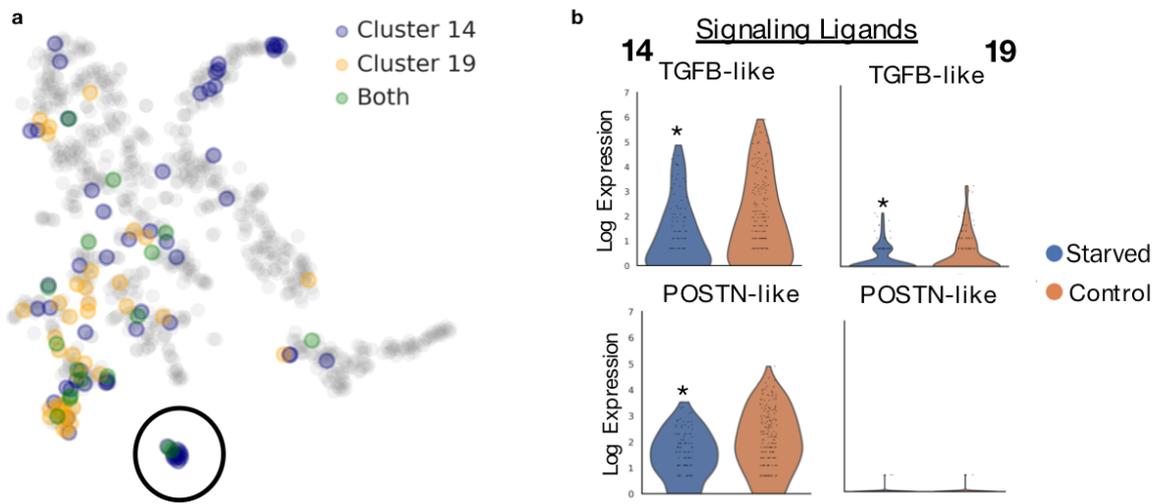


Fig. S21. Differential Expression of Signaling Ligands Under Starvation. **a)** Example of a gene module (module 13 from Fig. S20, circled) with shared and unique perturbed genes between 'internally-distant' GD cell types 14 and 19. **b)** Violin plots show expression of signaling ligand related genes in this module, with shared and divergent expression between the cell types. Both cell types show downregulation of the same TGFB-like (XLOC_035232) gene under starvation, however downregulation of a POSTN-like gene (XLOC_007437, related to cell adhesion and migration) is only present in 14, with low/no expression in 19 for either condition. * denote p -value < 0.05 for differential expression, using the non-parametric, Wilcoxon test.

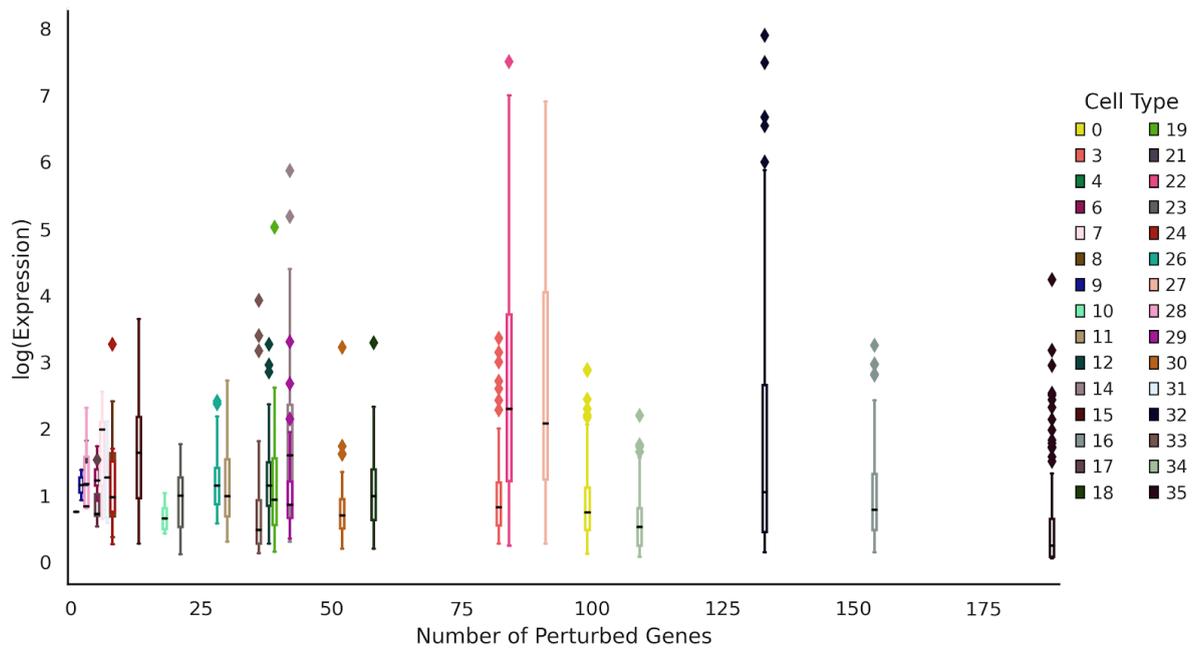


Fig. S22. Magnitude of Perturbation Responses Across Cell Types. Box-and-whisker plot showing expression level of perturbed genes (distribution across cells; mean indicated with horizontal bar) within each cell type versus the number of significantly perturbed genes found for each cell type. Whiskers denote 1.5 IQR and diamonds denote outliers.

Tables

Experiment	No. of conditions	Number of animals	Number of high quality cells	Total Sequenced Reads	Total cost	Unique reads in high quality cells	Median UMIs per cell	Median genes detected per cell	Technology used
Starvation	2	10	13,673	464,778,632	~\$12,000	67,374,740	1,802	676	10X Chromium V2.0 HiSeq 4000 MiSeq v3
Stimulation	3	12	18,921	676,754,269	~\$12,000	170,722,140	4,297	1,303	10X Chromium V3.0 HiSeq 4000 MiSeq v3

Table S1. Multiplexed experiment(s) overview. Table shows details for each multiplexed experiment including cost, technology, and animals used. Sequencing read output for each experiment is also reported.

Condition	Control (Fed)	Control	Control	Control	Control	Starved	Starved	Starved	Starved	Starved
Animal	Org 1	Org 2	Org 3	Org 4	Org 5	Org 6	Org 7	Org 8	Org 9	Org 10
Individual ClickTag	21,22	23,24	25,26	27,28	29,30	31,32	33,34	35,36	37,38	39,40

Table S2. Starvation experiment ClickTag assignments. Assignment of ClickTag barcodes to each animal used in the starvation experiment.

Table S3. Designed and Predicted Sequences. All designed and predicted sequences i.e. ClickTag barcodes, *in situ* primers (with EST sequences where applicable), predicted neuropeptide sequences, and qPCR primers. Gene IDs for gene markers in all Figures also included. See Auxiliary File 1.

Condition	SW	SW	SW	SW	DI	DI	DI	DI	KCl	KCl	KCl	KCl
Animal	Org 1	Org 2	Org 3	Org 4	Org 5	Org 6	Org 7	Org 8	Org 9	Org 10	Org 11	Org 12
Individual ClickTag	25	26	27	28	29	30	31	32	33	34	35	36
Condition ClickTag	37	37	37	37	38	38	38	38	39	39	39	39

Table S4. Stimulation ClickTag assignments. Assignment of ClickTag barcodes to each animal used in the stimulation experiment.

Table S5. All marker and differentially expressed (DE) genes. Contains genes marking cell types, low expression cell type markers, DE genes between starved and control cells,

neuron subpopulation markers, pseudotime markers, and DE genes between stimulation conditions with functional annotations where possible. See Auxiliary File 2.

Supplementary Methods

Full Protocol for ClickTag Sequencing

Starvation Single-cell Suspensions

- 1x PBS at 350 mM NaCl (hypertonic PBS solution)
 - MeOH (stored at -80)
 - LoBind Tubes (1.5 mL)
1. In six-well plates, transfer animals sequentially to three 4 C wash baths each with 25 mL filtered hypertonic PBS (1x PBS at 350 mM NaCl). Perform all subsequent steps at 4 C. Note: This is because seawater precipitates in methanol but hypertonic PBS does not. The washes thus both prepare the animals for fixation and remove precipitating sea salts
 2. After washing animals, transfer in ~400 uL to a 1 mL glass dounce homogenizer and pestle (loose) (Wheaton, USA).
 3. Homogenize animals by passaging 30 times with the dounce. First transfer samples in 200 uL aliquots to Eppendorf tubes, triturated 30 times with a P200 pipet, and transfer again to a dounce (passaging 30 times) .
 4. Bring volume to ~1.2 mL, and place sample on ice
 5. Process corresponding samples from the control or experimental group and centrifuge at 300 x g for 3 minutes at 4 C.
 6. Remove supernatant, leaving ~20 uL. Resuspend cells thoroughly in this volume using a P20 pipette tip.
 7. Add four volumes (~80 uL) ice-cold methanol to the sample and triturate for 30-60 seconds to reduce clumping during the early stages of MeOH fixation.
 8. Place samples at -20 C and store until sample labeling. Note: All samples were processed in this way, using pairs of experimental and control samples, alternating which sample was processed first, and tracking all animal characteristics with a known animal/barcode combination.
 9. In between sample pairs, clean and sterilize dounce/pestles with 20% bleach, rinsed thoroughly with water, and with 70% EtOH.

One-Pot Sample Labeling

1. For each sample, perform sample labeling according to (14):
Tags distributed as in Table S2.
 - a. 6 uL sample tag 1 (20uM)
 - b. 6 uL sample tag 2
 - c. 4 uL 1 mM NHS-TCO
 - d. Incubate 30 mins at room temperature on a rotating platform

- e. Quench by addition of Tris-HCl (to 10 mM final) and MTZ-DBCO (to 50 uM final) using 5 uL of a 200 mM Tris-HCl, 1 mM MTZ DBCO solution (DMSO, 50 mM MTZ-DBCO, 1 M Tris-HCl pH 7.5)
2. Pool samples and supplement with 10 uM "blocking oligo" to add excess DNA for quenching the reaction. Here we use the SoIvdAR primer at 100 uM stock (Table S3). 10 uL of resulting samples are combined with 9 uL PBS-BSA and 1 uL DAPI 400 ug/mL for counting.
3. Final combined volume of methanol-fixed cells is ~1mL. After mixing, split combined sample in two and add 500 uL PBS-BSA (0.1% BSA) to both samples.
4. Mix and spin (1000G for 5mins). Breakspeed 5
5. Remove supernatant and resuspend in 1.2 mL PBS-BSA
6. Wash again with with PBS-BSA (optional fourth wash if desired)
7. Supernatant removed, resuspend cells in 75 uL PBS-BSA and use 10 uL for counting on Countess.
8. Count fed and starved samples with a Countess automated cell counter by brightfield and DAPI fluorescence.
 - a. Using the Countess, cell concentrations are determined to roughly equate the contributions from both samples. Filter suspension with 100 micron filter before counting. Note: for this experiment, cell concentrations were determined to be 200,000 cells/mL for the starved sample and 1 M cells/mL for the fed sample
 - b. To proceed, equate contributions of the starved sample (~120,000-150,000 cells) to 200,000 cells (200 uL) from the fed sample.
9. Load 10X Chromium Controller with v2 chemistry (two lanes) following manufacturer's instructions.
10. Separate/process sample tag libraries after SPRI size-selection step as described in (14).
11. Sequence tag libraries on 2 lanes of an Illumina MiSeq sequencer using MiSeq v3 150 cycle kits (26 × 98-base-pair reads).
12. Pool and sequence the cDNA libraries on an Illumina HiSeq 4000 using two HiSeq 3000/4000 SBS 300 cycle kits (2 × 150-base-pair reads) (14).

Stimulation Protocol

- 150 mM KCl --> *KCl concentration of SW approx 10mM already*
- DI water
- 1x PBS at 350 mM NaCl (hypertonic PBS solution)
- MeOH (stored at -80)
- LoBind Tubes (1.5 mL)

Prepare samples using the same protocol as for the starvation experiment with two tags for each sample: one tag unique to each individual (1-12), and one tag unique to

each condition (DI, KCl, SW) (Fig. S7a; Table S4). The "blocking" oligos in this experiment are 5uM LCDBF and 5 uM LCDBR primers (simply to add excess DNA to the reaction) (Table S3).

The washing steps prior to Countess cell counting (steps 3-7) are replaced with the steps below.

1. Final combined volume of methanol-fixed cells is ~1 mL. After mixing, split combined sample in two, and add 1.4ml 3x SCC and 0.1% BSA to both samples
2. Mix and spin (1000G for 5mins). Breakspeed 5
3. Remove supernatant and resuspend in 4 ml 3x SCC and 0.1% BSA
4. Wash again with 4ml 3x SCC and 0.1% BSA
5. Supernatant removed, resuspend cells in 150 uL PBS and 0.1% BSA and use 10 uL for counting on Countess. Continue as written in step 8.

Run samples through two lanes on a 10X Chromium Controller with v3 chemistry following manufacturer's instructions. With resulting libraries, sequence on two Illumina HiSeq lanes with 3% tags in pooled mix (cDNA and ClickTags), and run a ClickTag only sample on two Illumina MiSeq lanes.