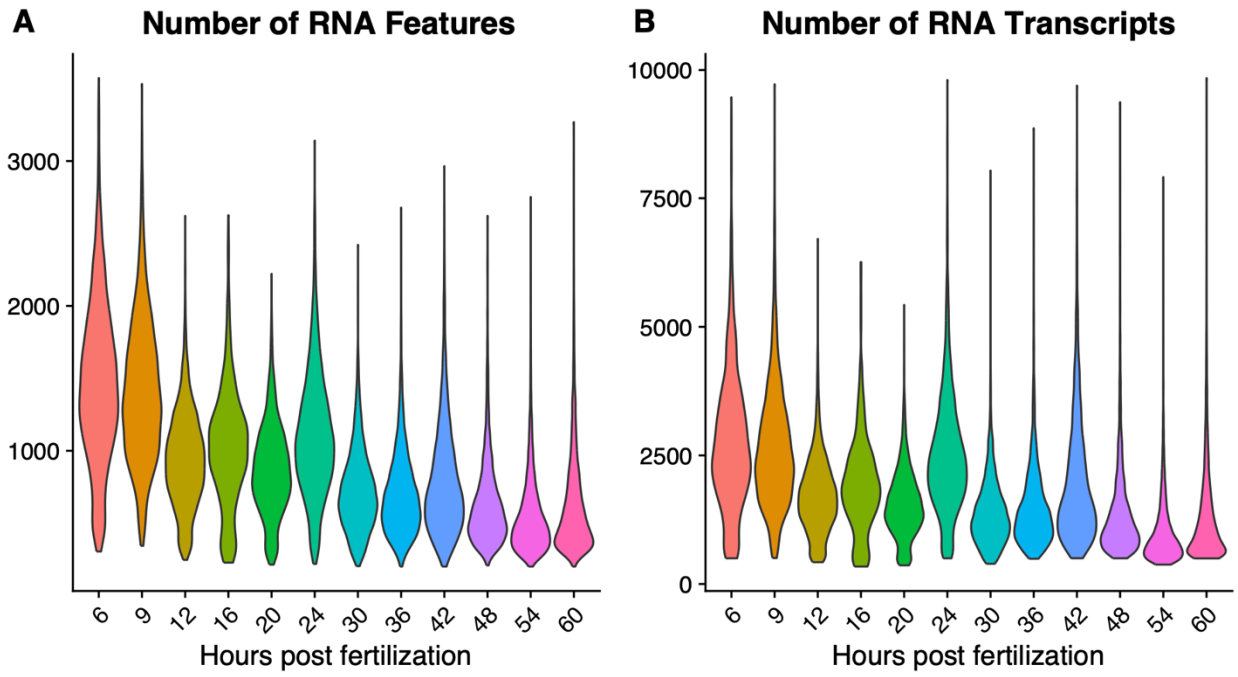


Fig. S1. Quality control plots for the *He* scRNA-seq atlas. (A) Violin plots showing the distribution of the number of RNA features (unique gene transcripts) in each cell for each sample time point. (B) Violin plots showing the distribution of the number of RNA transcripts in each cell for each sample time point.

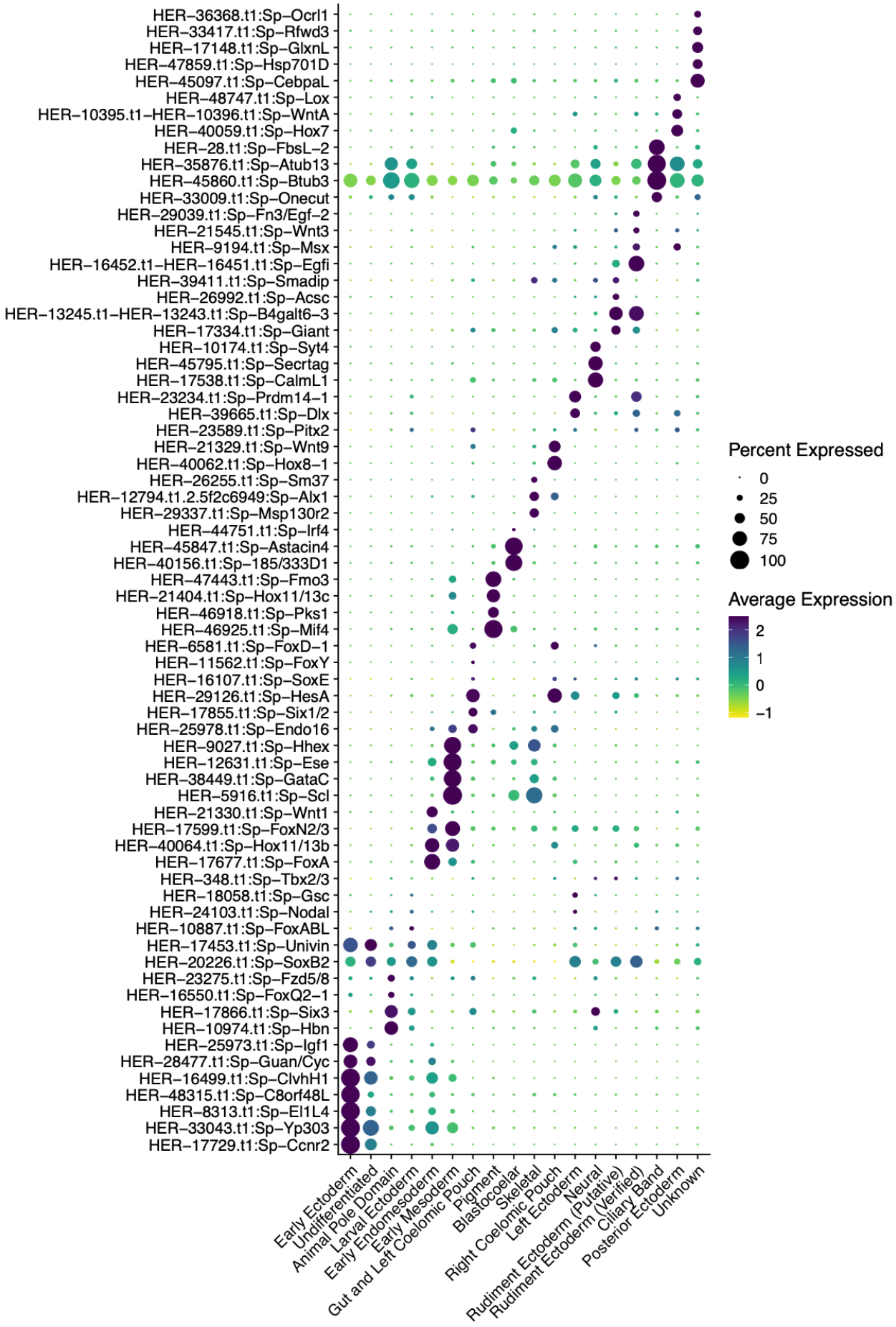

HER–36368.t1:Sp–Ocrl1
HER–33417.t1:Sp–Rfwd3
HER–17148.t1:Sp–GlxnL
HER–47859.t1:Sp–Hsp701D
HER–45097.t1:Sp–CebpaL
HER–48747.t1:Sp–Lox
HER–10395.t1–HER–10396.t1:Sp–WntA
HER–40059.t1:Sp–Hox7
HER–28.t1:Sp–FbsL–2
HER–35876.t1:Sp–Atub13
HER–45860.t1:Sp–Btub3
HER–33009.t1:Sp–Onecut
HER–29039.t1:Sp–Fn3/Egf–2
HER–21545.t1:Sp–Wnt3
HER–9194.t1:Sp–Msx
HER–16452.t1–HER–16451.t1:Sp–Egfi
HER–39411.t1:Sp–Smadip
HER–26992.t1:Sp–Acsc
HER–13245.t1–HER–13243.t1:Sp–B4galt6–3
HER–17334.t1:Sp–Giant
HER–10174.t1:Sp–Syt4
HER–45795.t1:Sp–Secrtag
HER–17538.t1:Sp–CalmL1
HER–23234.t1:Sp–Prdm14–1
HER–39665.t1:Sp–Dlx
HER–23589.t1:Sp–Pitx2
HER–21329.t1:Sp–Wnt9
HER–40062.t1:Sp–Hox8–1
HER–26255.t1:Sp–Sm37
HER–12794.t1.2.5f2c6949:Sp–Alx1
HER–29337.t1:Sp–Msp130r2
HER–44751.t1:Sp–Irf4
HER–45847.t1:Sp–Astacin4
HER–40156.t1:Sp–185/333D1
HER–47443.t1:Sp–Fmo3
HER–21404.t1:Sp–Hox11/13c
HER–46918.t1:Sp–Pks1
HER–46925.t1:Sp–Mif4
HER–6581.t1:Sp–FoxD–1
HER–11562.t1:Sp–FoxY
HER–16107.t1:Sp–SoxE
HER–29126.t1:Sp–HesA
HER–17855.t1:Sp–Six1/2
HER–25978.t1:Sp–Endo16
HER–9027.t1:Sp–Hhex
HER–12631.t1:Sp–Ese
HER–38449.t1:Sp–GataC
HER–5916.t1:Sp–Scl
HER–21330.t1:Sp–Wnt1
HER–17599.t1:Sp–FoxN2/3
HER–40064.t1:Sp–Hox11/13b
HER–17677.t1:Sp–FoxA
HER–348.t1:Sp–Tbx2/3
HER–18058.t1:Sp–Gsc
HER–24103.t1:Sp–Nodal
HER–10887.t1:Sp–FoxABL
HER–17453.t1:Sp–Univin
HER–20226.t1:Sp–SoxB2
HER–23275.t1:Sp–Fzd5/8
HER–16550.t1:Sp–FoxQ2–1
HER–17866.t1:Sp–Six3
HER–10974.t1:Sp–Hbn
HER–25973.t1:Sp–Igf1
HER–28477.t1:Sp–Guan/Cyc
HER–16499.t1:Sp–ClvhH1
HER–48315.t1:Sp–C8orf48L
HER–8313.t1:Sp–El1L4
HER–33043.t1:Sp–Yp303
HER–17729.t1:Sp–Ccnr2
Early Ectoderm
Undifferentiated
Animal Pole Domain
Larval Ectoderm
Early Endomesoderm
Gut and Left Coelomic Pouch
Early Mesoderm
Pigment
Blastocoelar
Skeletal
Right Coelomic Pouch
Left Ectoderm
Neural
Rudiment Ectoderm (Putative)
Rudiment Ectoderm (Verified)
Ciliary Band
Posterior Ectoderm
Unknown
Percent Expressed
0
25
50
75
100
Average Expression
2
1
0
–1

Fig. S2. Dot plot of cell type marker gene expression patterns. ~3-6 enriched marker genes (see Table S1) for each cell type in the scRNA-seq atlas were selected, and the average expression of the genes in each cell type was plotted.

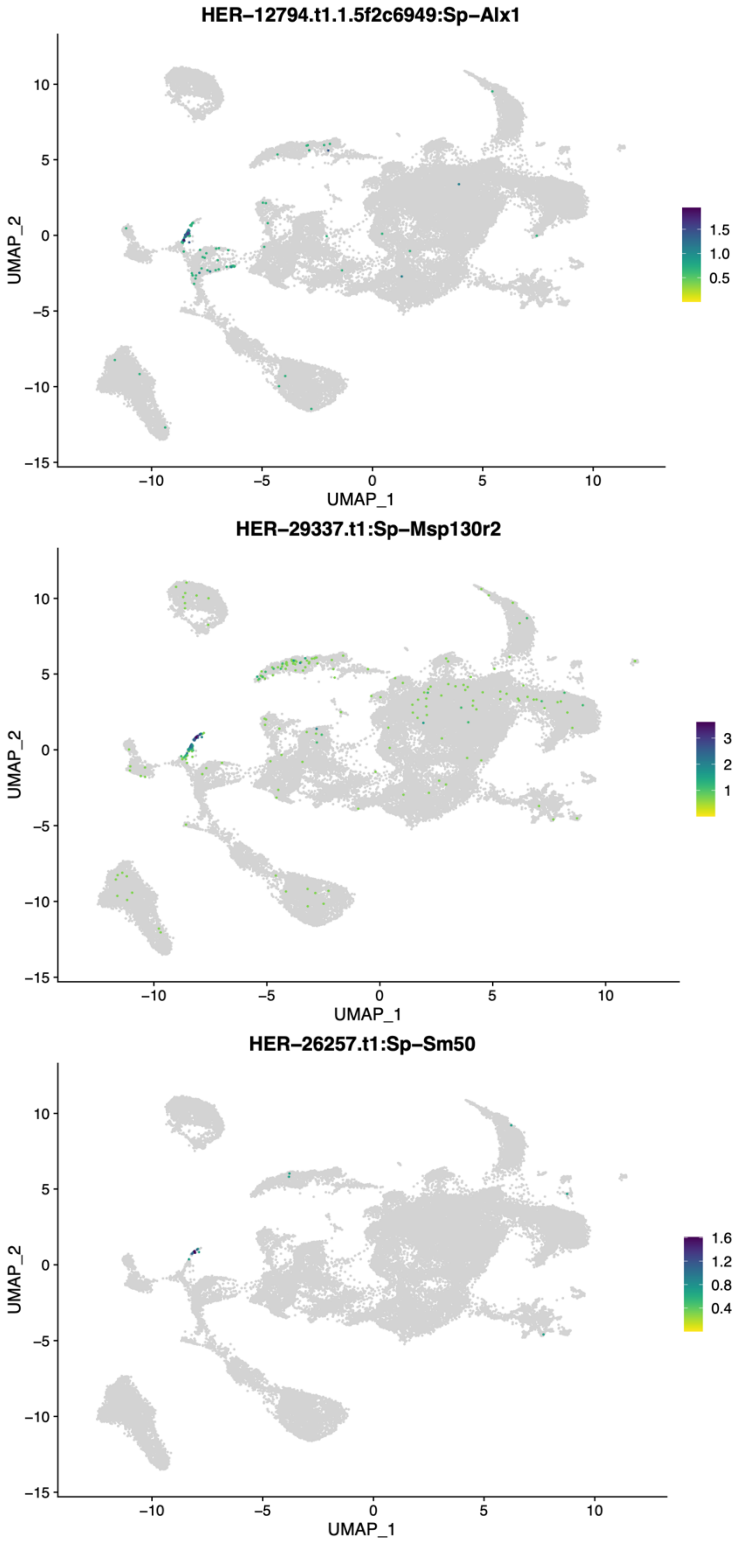


Fig. S3. Expression patterns of skeletogenic cell marker genes. These UMAPs show the expression patterns of three of the marker genes used to annotate the skeletogenic cell lineage in the *He* scRNA-seq atlas.

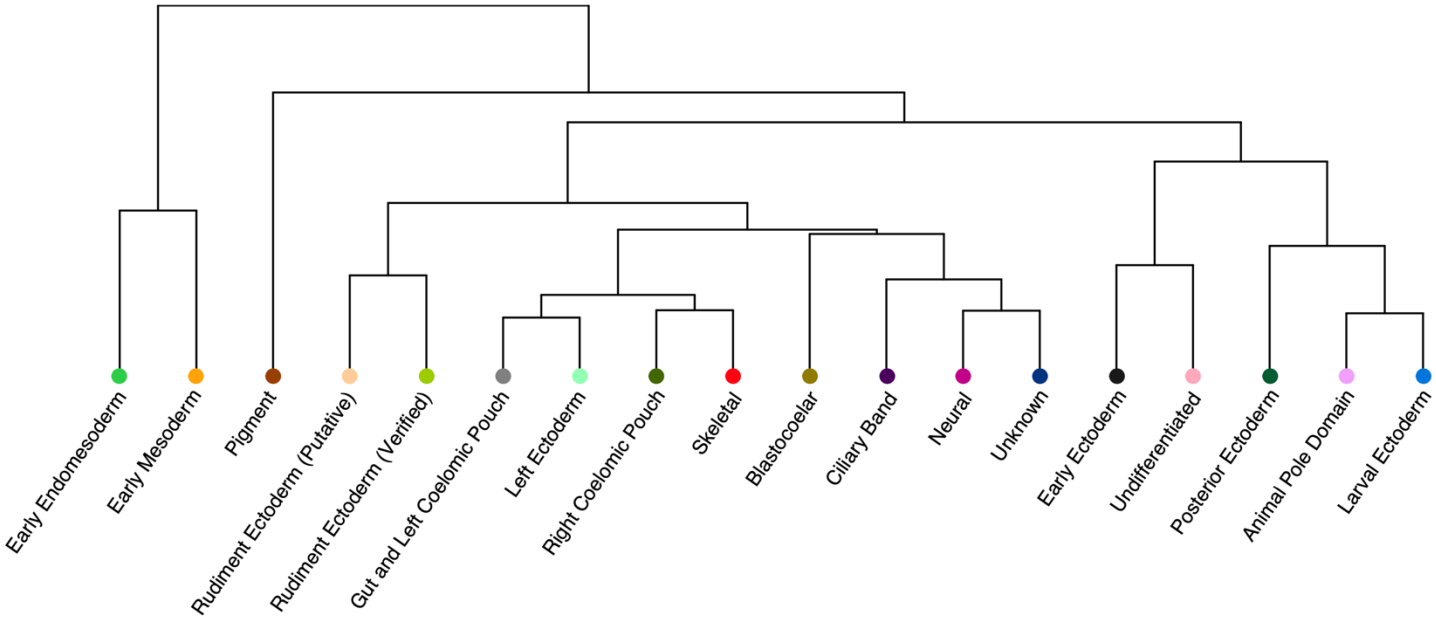


Fig. S4. Phylogenetic tree of cell types in the scRNA-seq atlas. This plot was generated using Seurat's BuildClusterTree function, which generates the phylogenetic tree using pairwise distances between the cell types in gene expression space.

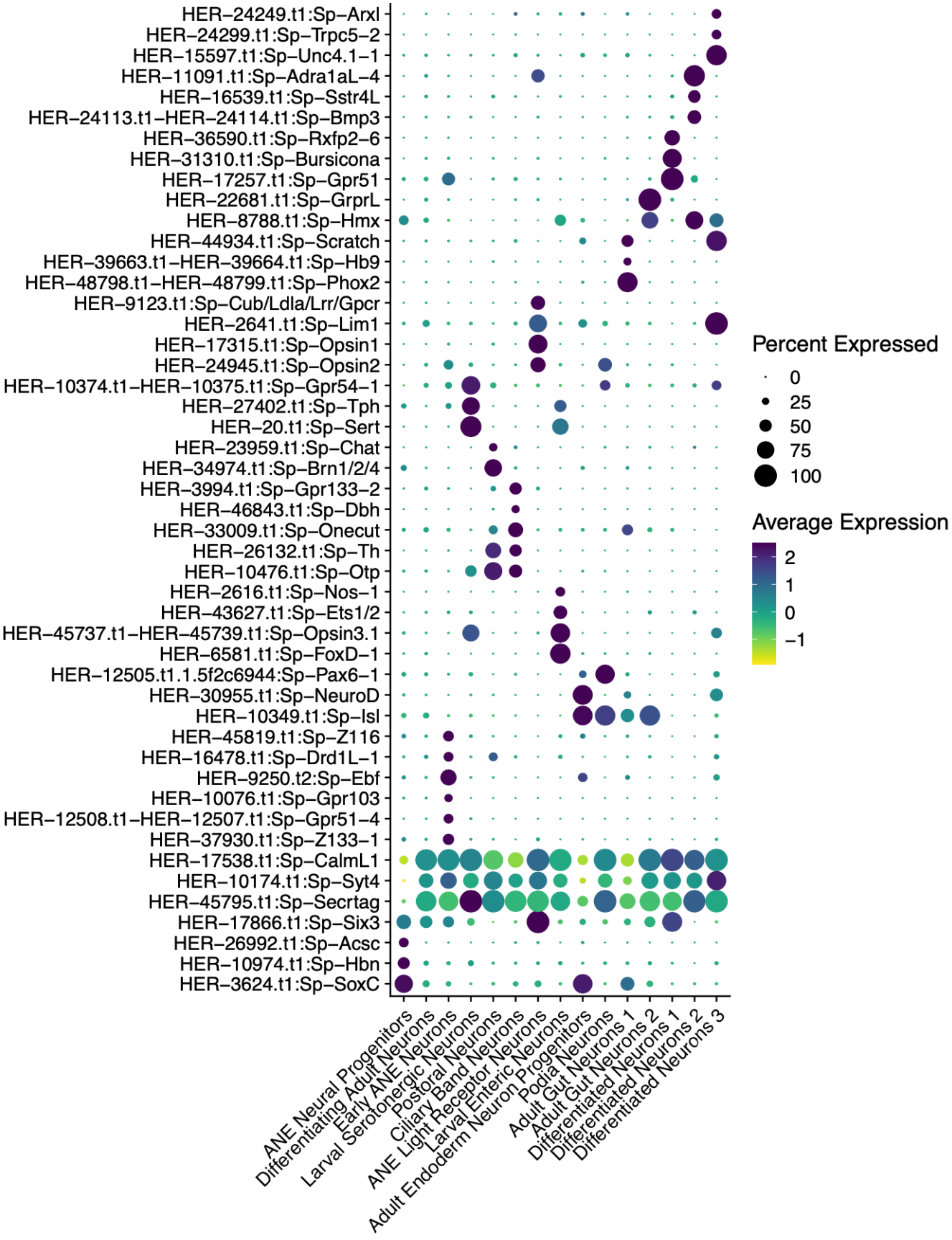
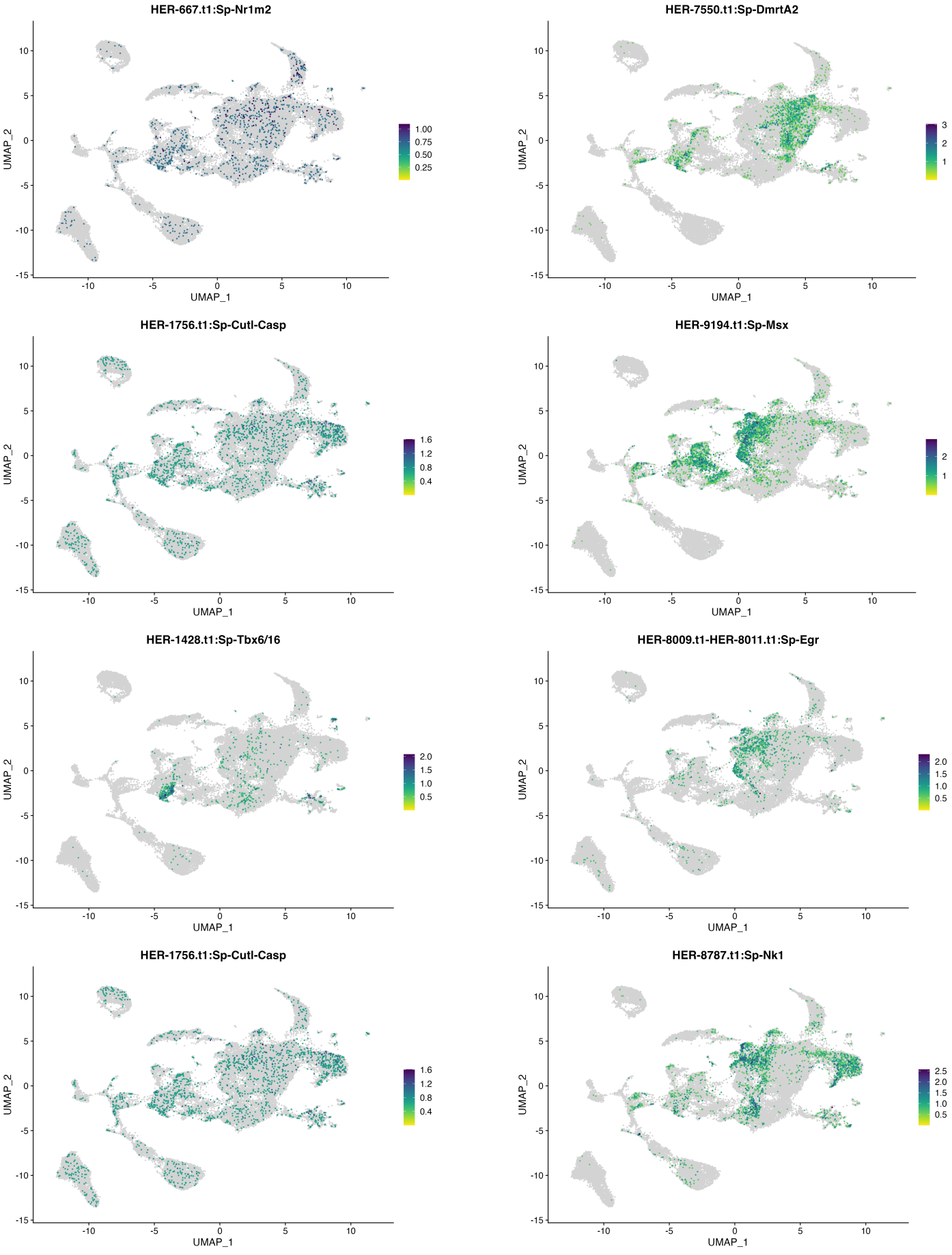


Fig. S5. Dot plot of neural cell type marker gene expression patterns. ~3-6 enriched marker genes (see Table S2) for each neural cell type in the scRNA-seq atlas were selected, and the average expression of the genes in each cell type was plotted.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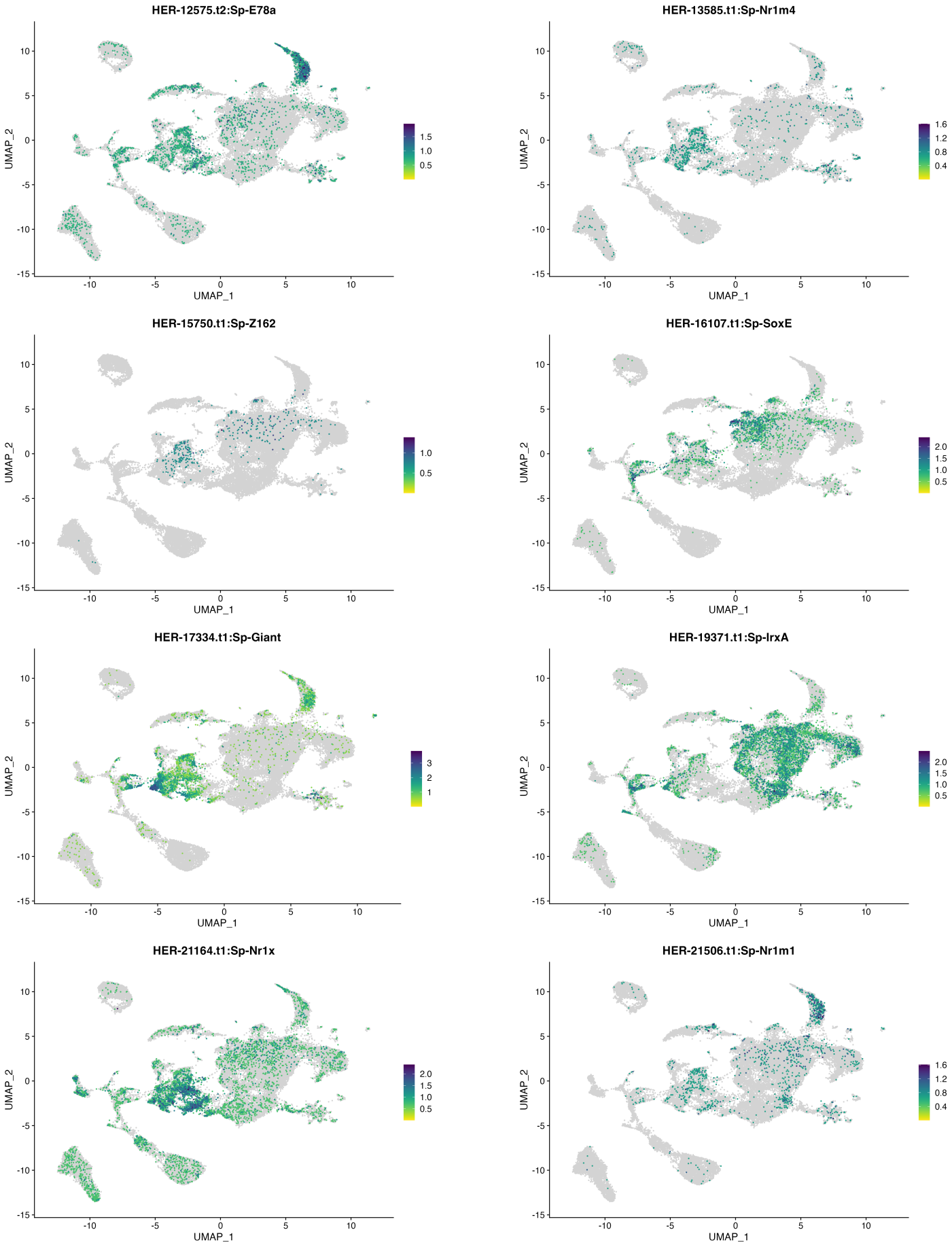
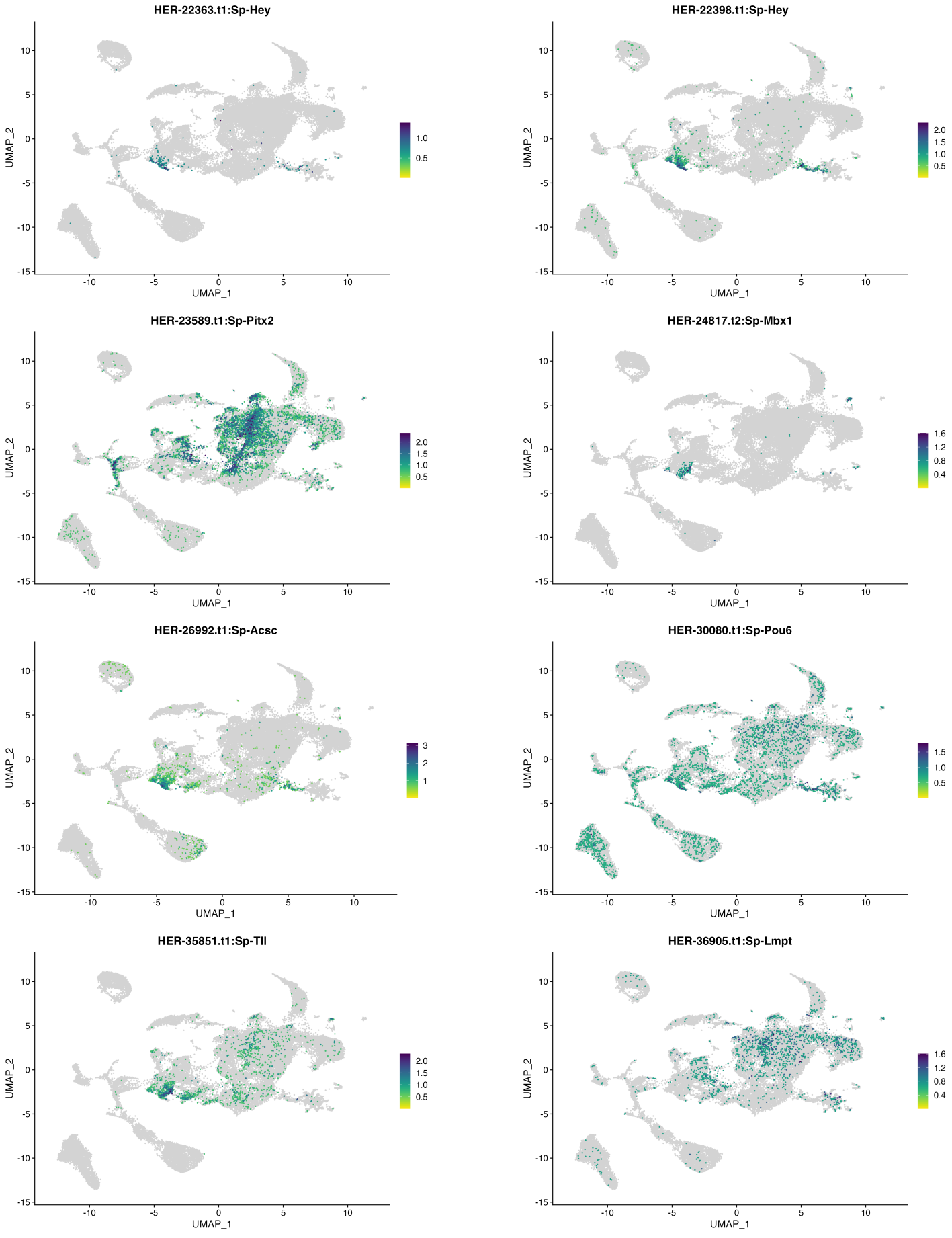
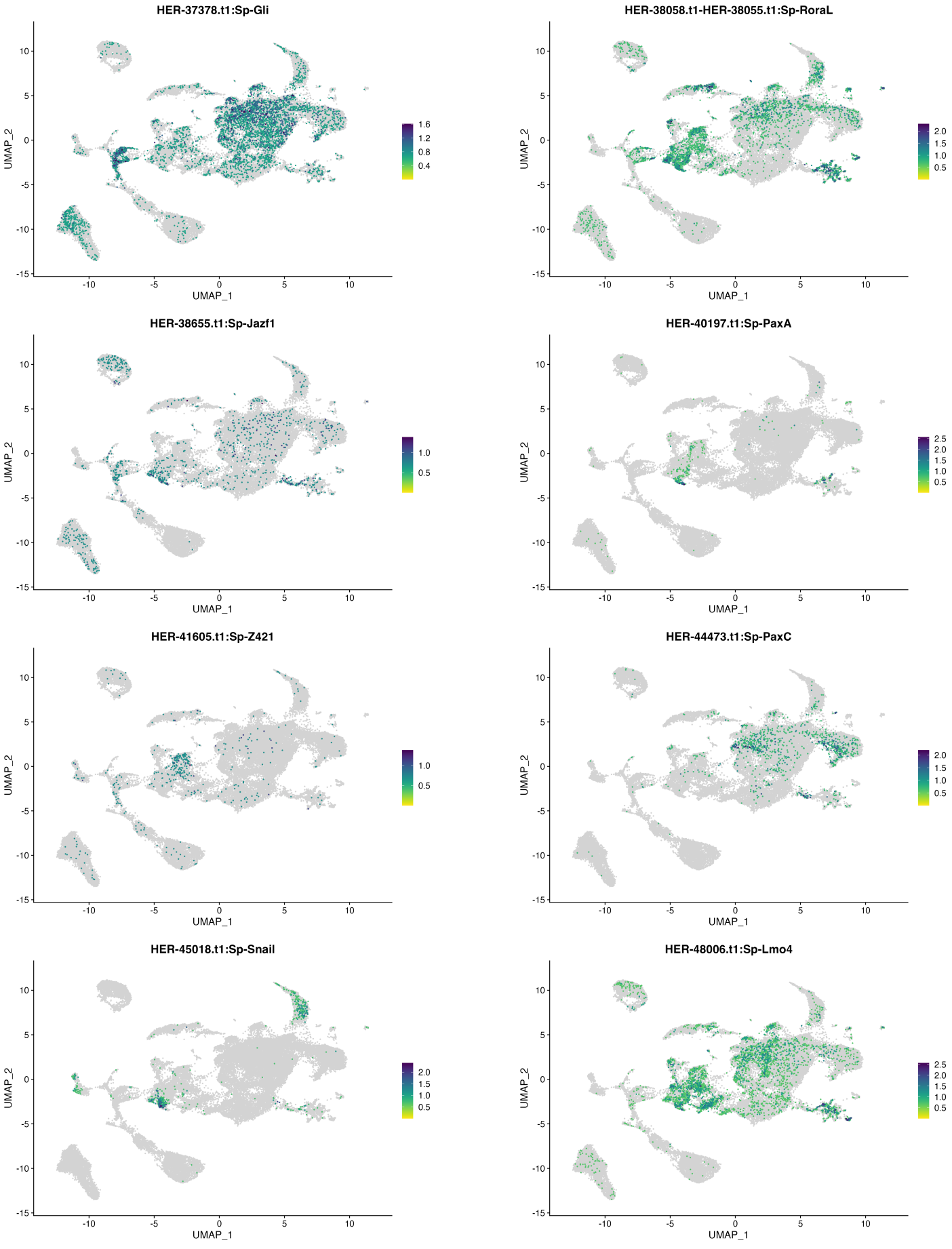
HER-7550.t1:Sp-DmrtA2
HER-1756.t1:Sp-Cutl-Casp
HER-9194.t1:Sp-Msx
HER-1428.t1:Sp-Tbx6/16
HER-8009.t1-HER-8011.t1:Sp-Egr
HER-1756.t1:Sp-Cutl-Casp
HER-8787.t1:Sp-Nk1
UMAP_1
UMAP_2


HER-12575.t2:Sp-E78a
HER-13585.t1:Sp-Nr1m4
HER-15750.t1:Sp-Z162
HER-16107.t1:Sp-SoxE
HER-17334.t1:Sp-Giant
HER-19371.t1:Sp-IrxA
HER-21164.t1:Sp-Nr1x
HER-21506.t1:Sp-Nr1m1
UMAP_1
UMAP_2


HER-22363.t1:Sp-Hey
HER-22398.t1:Sp-Hey
HER-23589.t1:Sp-Pitx2
HER-24817.t2:Sp-Mbx1
HER-26992.t1:Sp-Acsc
HER-30080.t1:Sp-Pou6
HER-35851.t1:Sp-Tll
HER-36905.t1:Sp-Lmpt
UMAP_1
UMAP_2


HER-37378.t1:Sp-Gli
HER-38058.t1-HER-38055.t1:Sp-RoraL
HER-38655.t1:Sp-Jazf1
HER-40197.t1:Sp-PaxA
HER-41605.t1:Sp-Z421
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HER-48006.t1:Sp-Lmo4
UMAP_1
UMAP_2

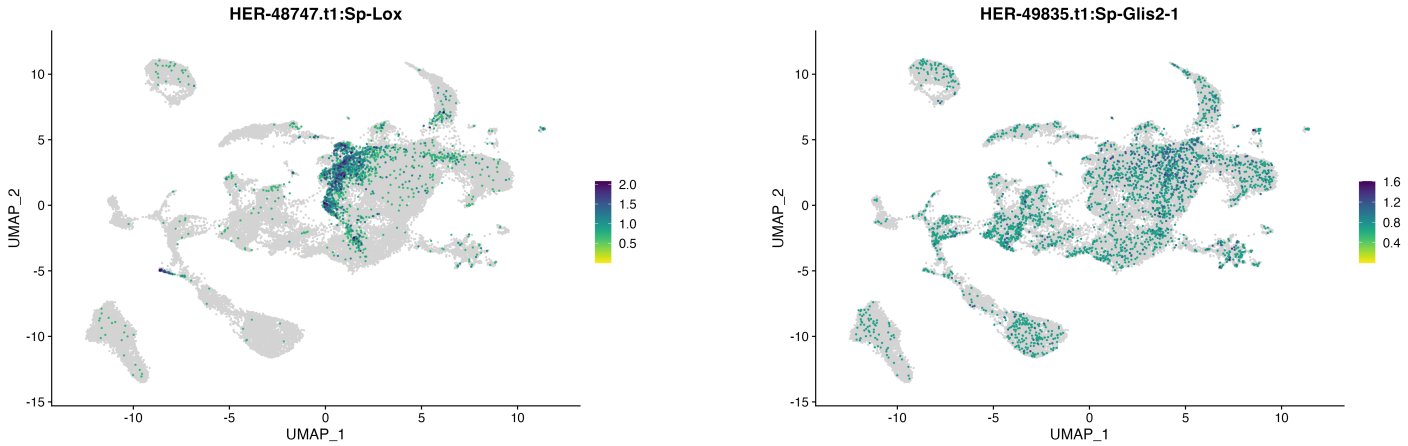

Fig. S6. Expression patterns of the candidate transcription factors for adult rudiment development. Each UMAP shows the scRNA-seq expression pattern of one of the 33 candidate transcription factors that was shown to be enriched in adult rudiment tissues (rudiment ectoderm and posterior ectoderm).

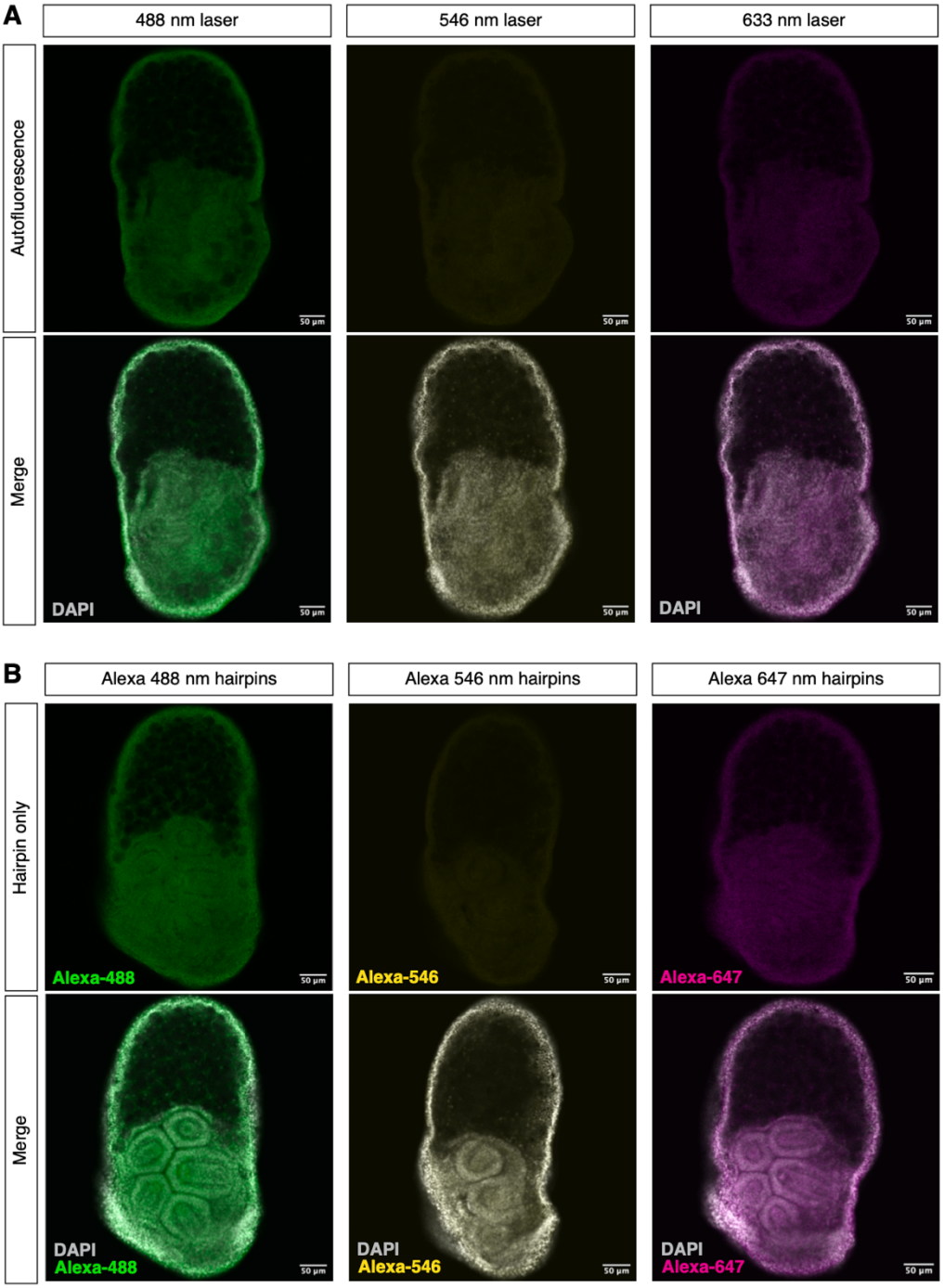

Fig. S7. Control experiments to verify HCR specificity. (A) HCR micrographs of 53 hpf *He* larvae that were incubated with no probes and no hairpins. (B) HCR micrographs of 53 hpf *He* larvae that were incubated with no probes. Neither condition resulted in localized fluorescent patterns, confirming the specificity of the probes used in this study.

Table S1. Cell type marker genes and references for the full scRNA-seq atlas

Available for download at
https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.203015#supplementary-data

Table S2. Neural cell type marker genes and references for the neural-only scRNA-seq atlas

Available for download at
https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.203015#supplementary-data

Table S3. List of putative transcriptional regulators of adult rudiment development

Available for download at
https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.203015#supplementary-data

Table S4. Probe sequences used in HCR experiments

Available for download at
https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.203015#supplementary-data

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