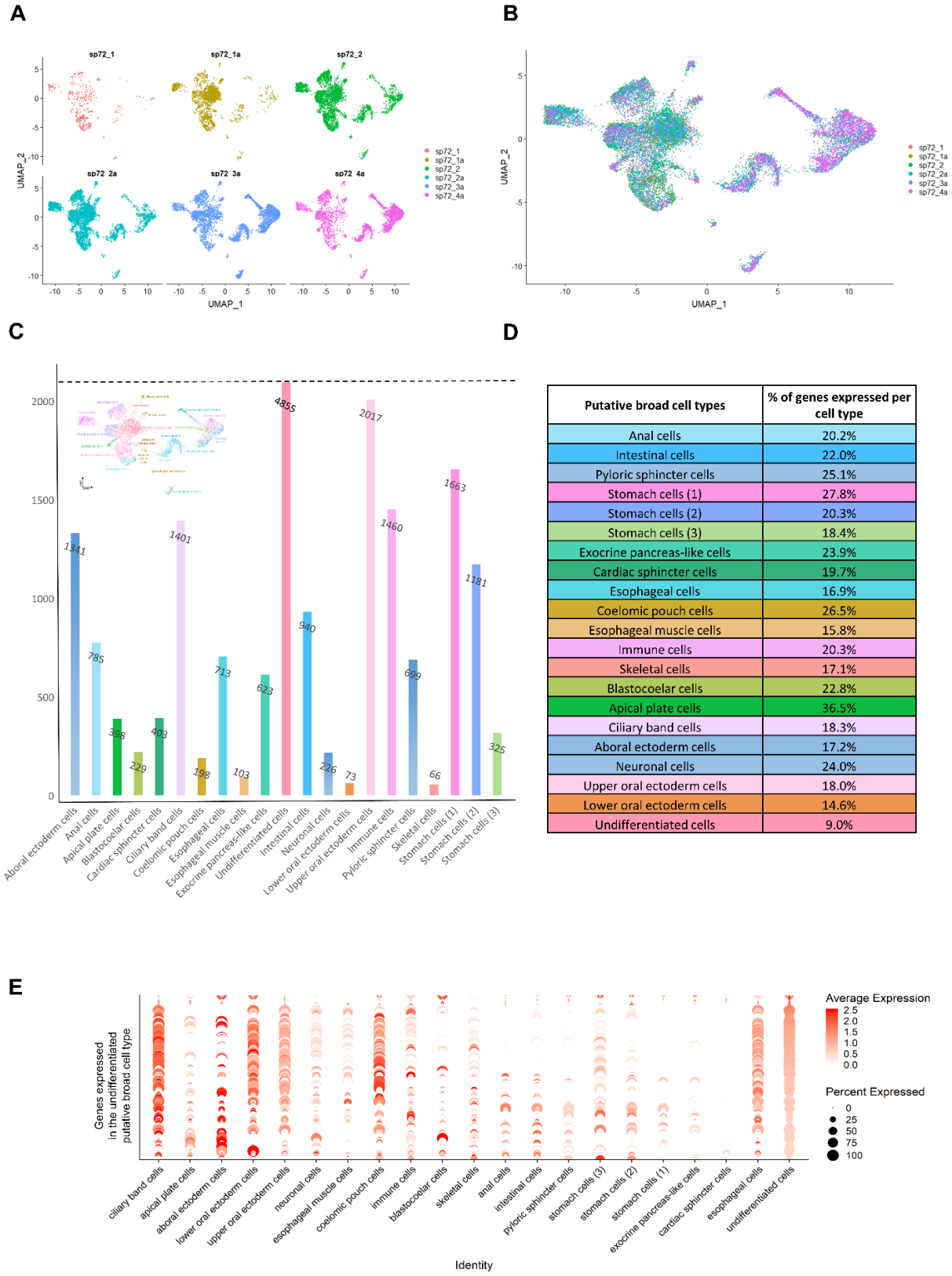


| Putative broad cell types | % of genes expressed per cell type |
|---|---|
| Anal cells | 20.2% |
| Intestinal cells | 22.0% |
| Pyloric sphincter cells | 25.1% |
| Stomach cells (1) | 27.8% |
| Stomach cells (2) | 20.3% |
| Stomach cells (3) | 18.4% |
| Exocrine pancreas-like cells | 23.9% |
| Cardiac sphincter cells | 19.7% |
| Esophageal cells | 16.9% |
| Coelomic pouch cells | 26.5% |
| Esophageal muscle cells | 15.8% |
| Immune cells | 20.3% |
| Skeletal cells | 17.1% |
| Blastocoelar cells | 22.8% |
| Apical plate cells | 36.5% |
| Ciliary band cells | 18.3% |
| Aboral ectoderm cells | 17.2% |
| Neuronal cells | 24.0% |
| Upper oral ectoderm cells | 18.0% |
| Lower oral ectoderm cells | 14.6% |
| Undifferentiated cells | 9.0% |

**Figure 1 – Figure supplement 1. A)** UMAPs split by original identity showing the overlap of the different biological and technical replicates. **B)** Overlay UMAP showing the overlap of the

different biological and technical replicates. **C)** Distribution of cells per putative broad cell type. Color-code is the same as indicated on the UMAP in Figure 1B. **C)** Table showing the percentage of genes expressed per putative broad cell type compared to the total number of genes we identified (15.578). **E)** Dotplot of the total genes expressed in the undifferentiated putative broad cell type, that shows high similarity with ectodermally derived cell types.

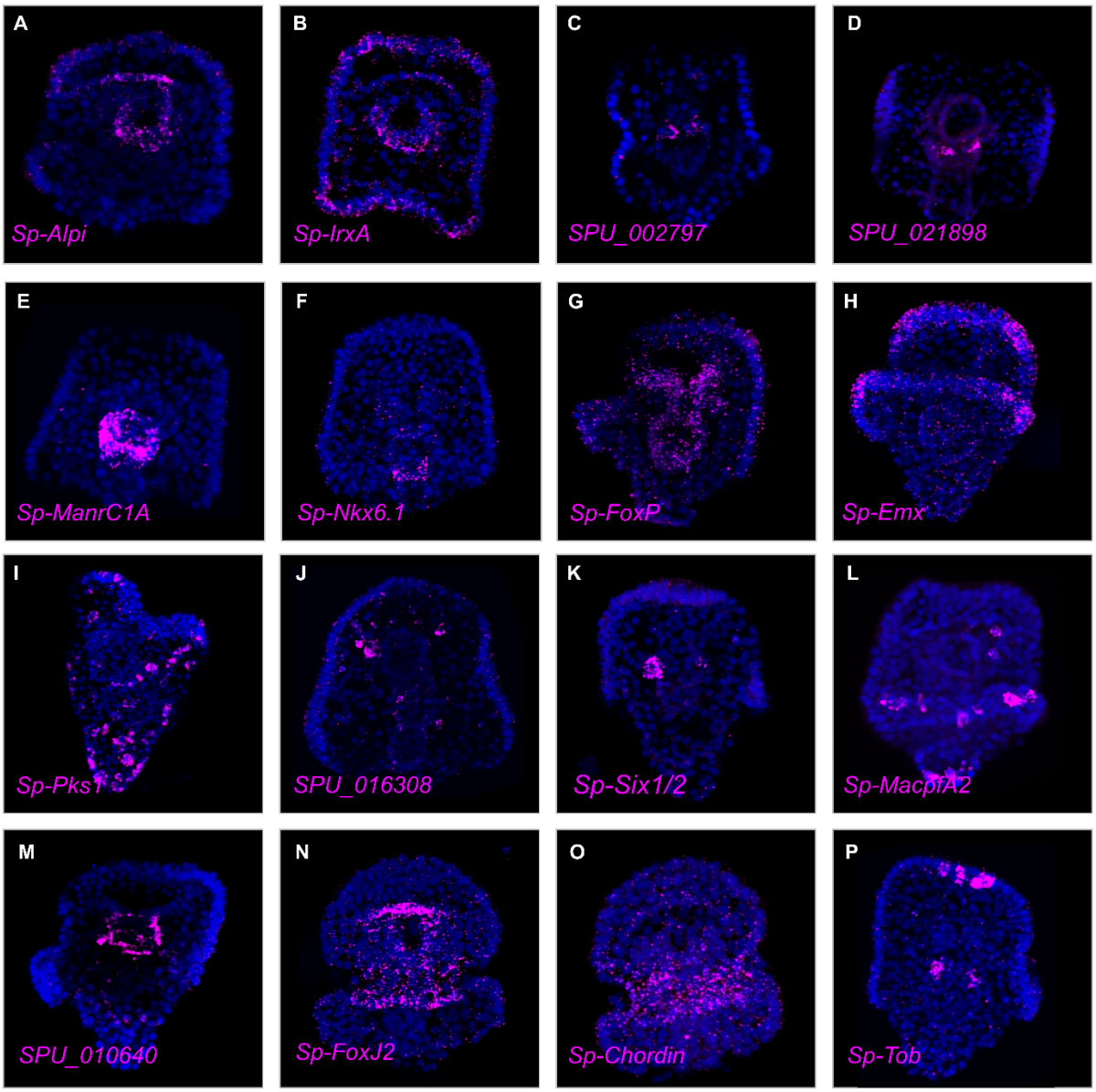


**Figure 2 – Figure supplement 1**. FISH of *S. purpuratus* larvae with specific probes detecting the mRNAs for *Sp-Alpi* (A), *Sp-IrxA* (B), *SPU_002797* (C), *SPU_021898* (D), *Sp-ManrC1A* (E), *Sp-Nkx6.1* (F), *Sp-FoxP* (G), *Sp-Emx* (H), *Sp-Pks1* (I), *SPU_016308* (J), *Sp-Six1/2* (K), *Sp-MacpfA2* (L), *SPU_010640* (M), *Sp-FoxJ2* (N), *Sp-Chordin* (O) and *Sp-Tob* (P). Nuclei are labelled with DAPI (in blue). All images are stacks of merged confocal Z sections.

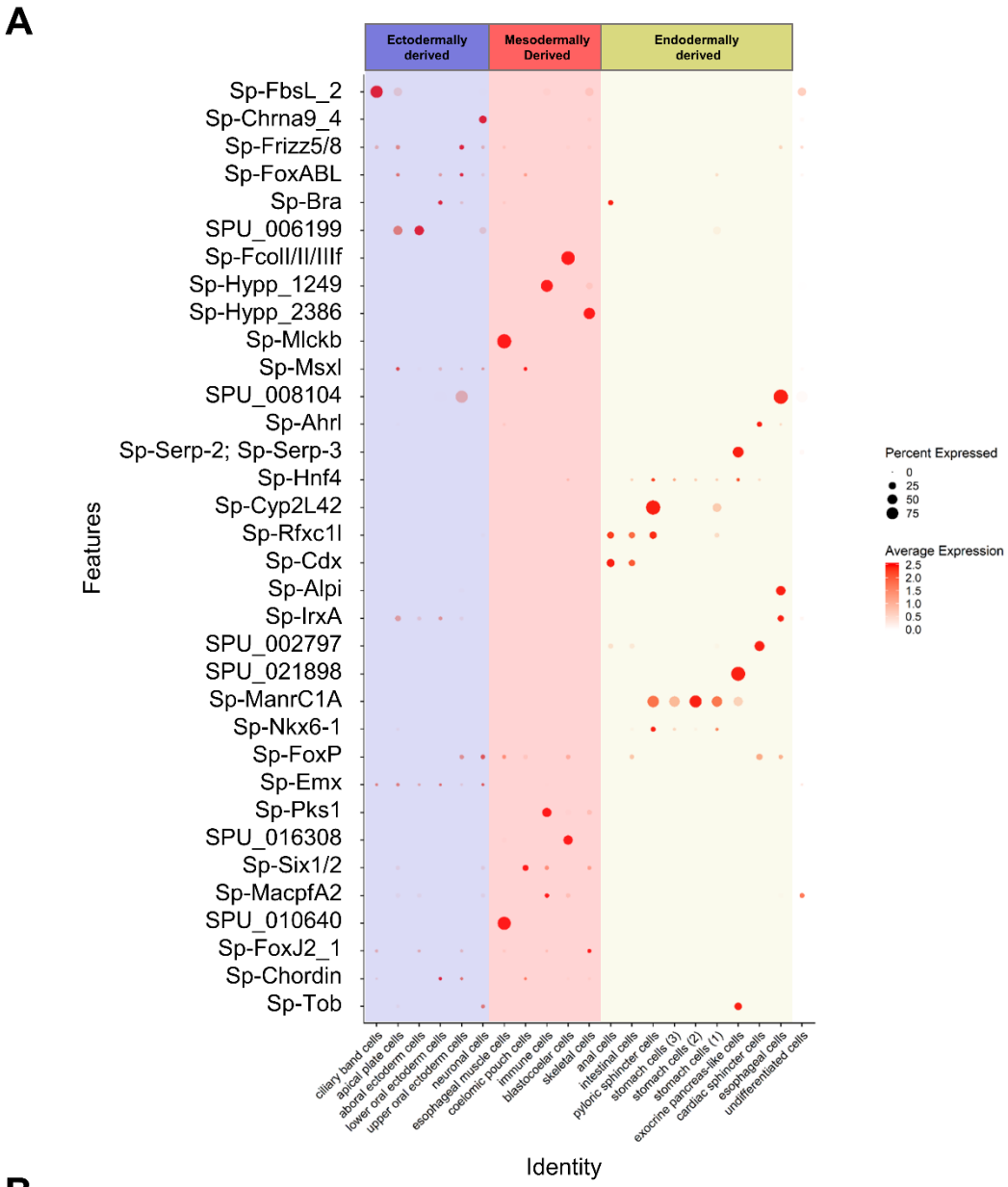


**Figure 2 – Figure supplement 2.** A) Dotplot showing the average expression of genes used as markers to identify the putative broad cell types (Figure 1A and Figure 2- Figure supplement 1).

The putative broad cell types are grouped based on their developmental origins: ectodermally derived, in blue, mesodermally derived, in red, and endodermally derived, in yellow. B) FISH using the antisense probe of Sp-Hbn (B1) paired with immunofluorescent detection of Sp-Msp130 (B2). Merged channels of both signals show localization of Sp-Hbn transcripts in Msp130 positive PMCs (B3). Nuclei are labelled with DAPI (in blue). All images are stacks of merged confocal Z sections. PMCs: Primary mesenchyme cells.

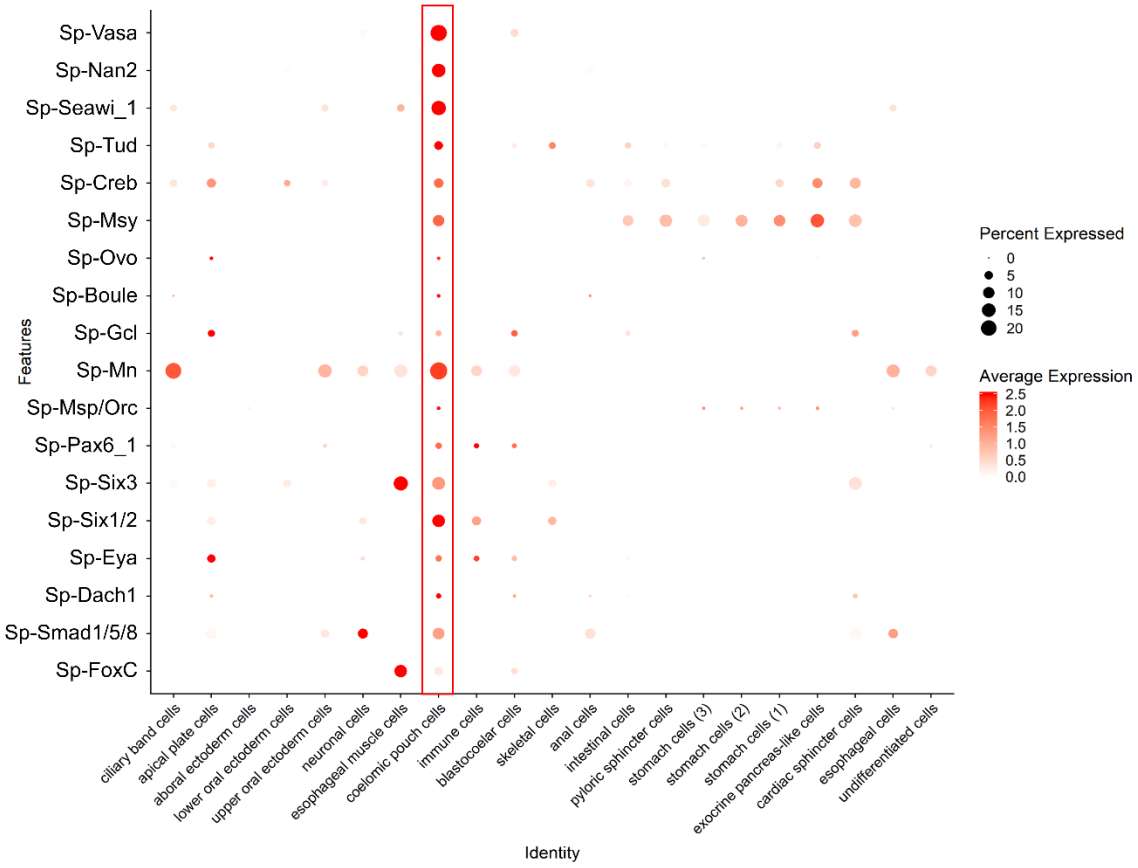


**Figure 2 – Figure supplement 3.** Dotplot of genes known for their involvement in germ line determination and maintenance showing overall enrichment of their average expression in coelomic pouch cells.

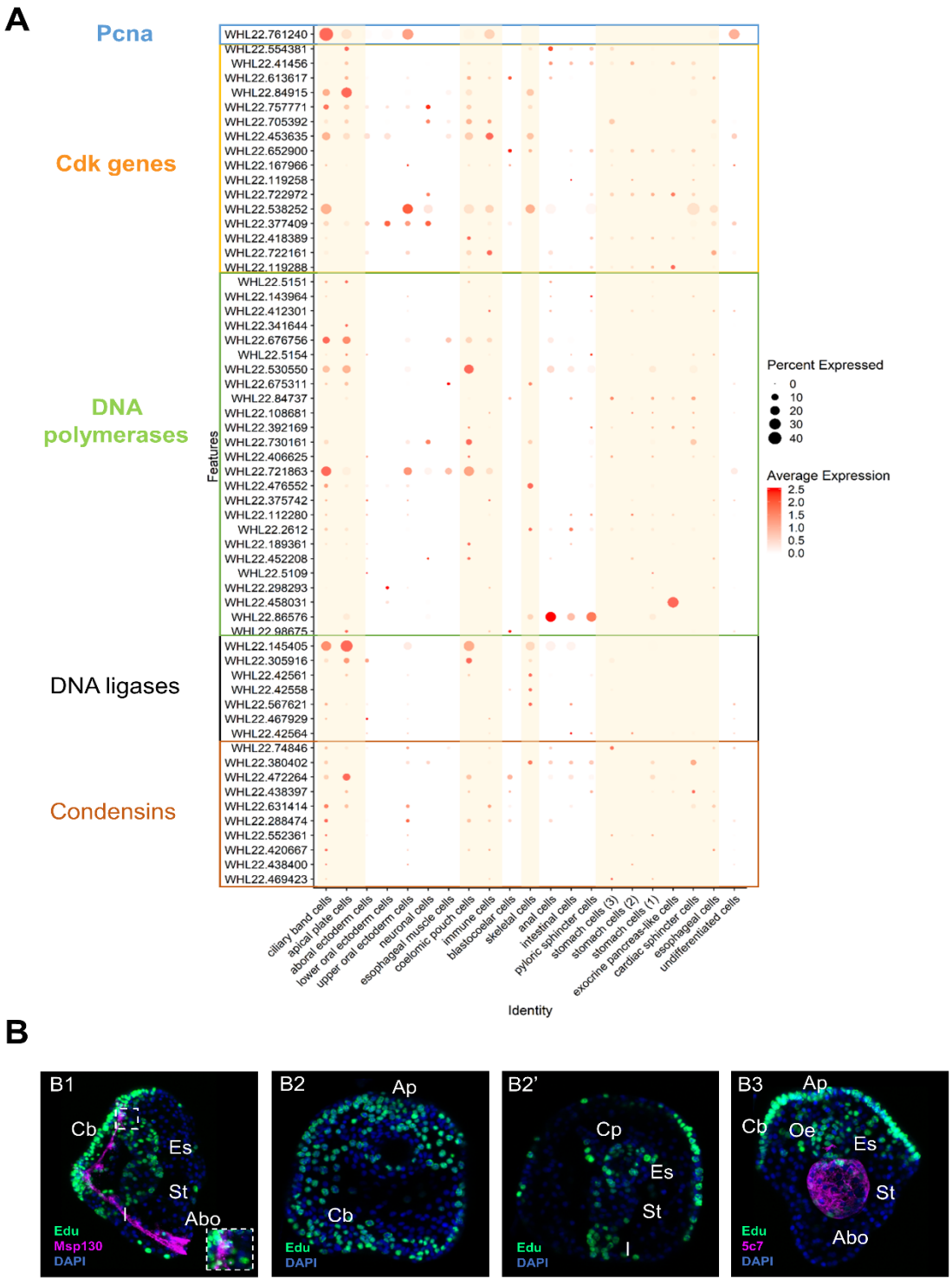


**Figure 2 – Figure supplement 4.** A) Dotplot showing the average expression of genes encoding *cdk1*, *pcna*, DNA polymerases, DNA ligases, condensins, and centromere proteins. The putative broad cell types are grouped based on their developmental origins: ectodermally derived, in blue, mesodermally derived, in red and endodermally derived cell types, in yellow. B) Proliferating cells as indicated by EdU labelling. B1) Larva labelled with EdU and the skeletogenic marker anti-Msp130. B2) Selected confocal sections of larva in oral view labelled

with EdU. B3) Selected confocal sections showing the internal part of the same larva as in B2 in oral view labelled with EdU. B4) Larva labelled with EdU and the endodermal marker 5c7. Abo: Aboral ectoderm; Ap: Apical plate; Cb: Ciliary band; Es: Esophagus; Oe, Oral ectoderm; St: Stomach.

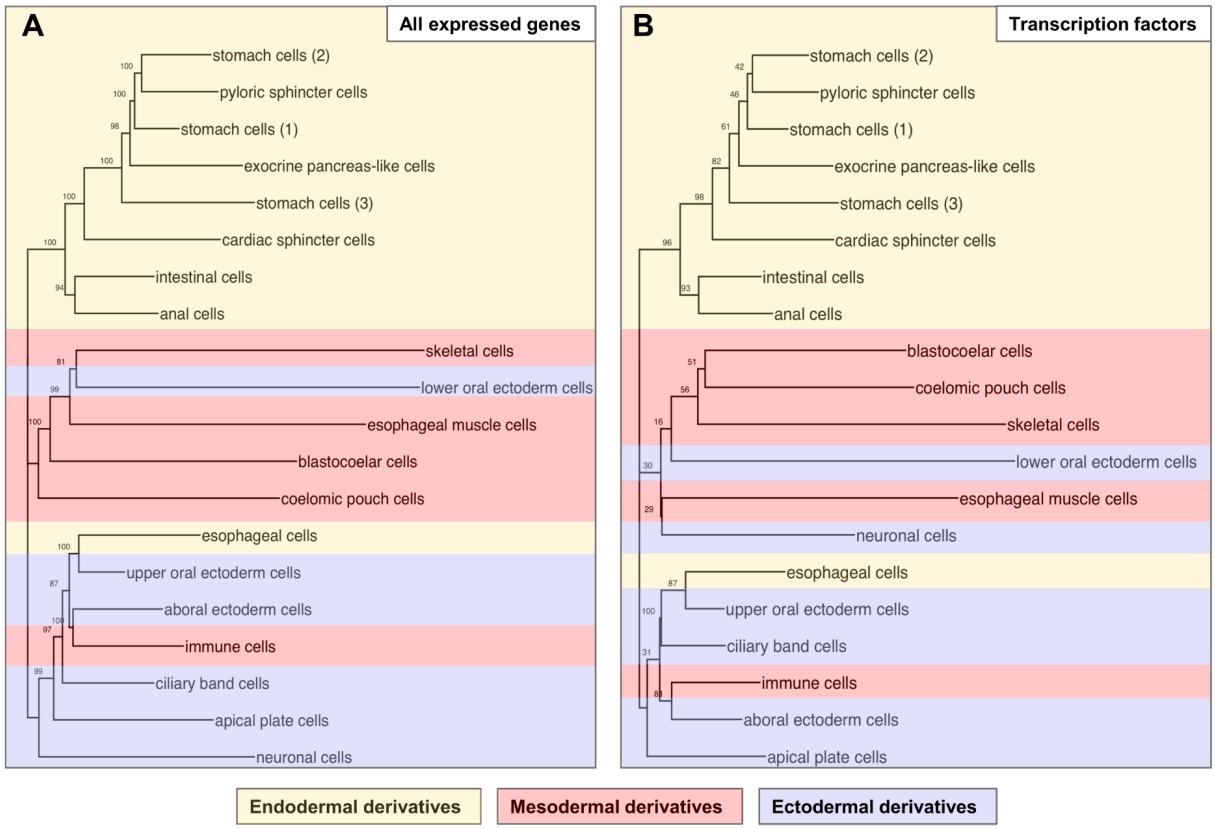


**Figure 3 – Figure supplement 1.** Cell type tree reconstruction using all expressed genes **(A)** and only transcription factors **(B).**

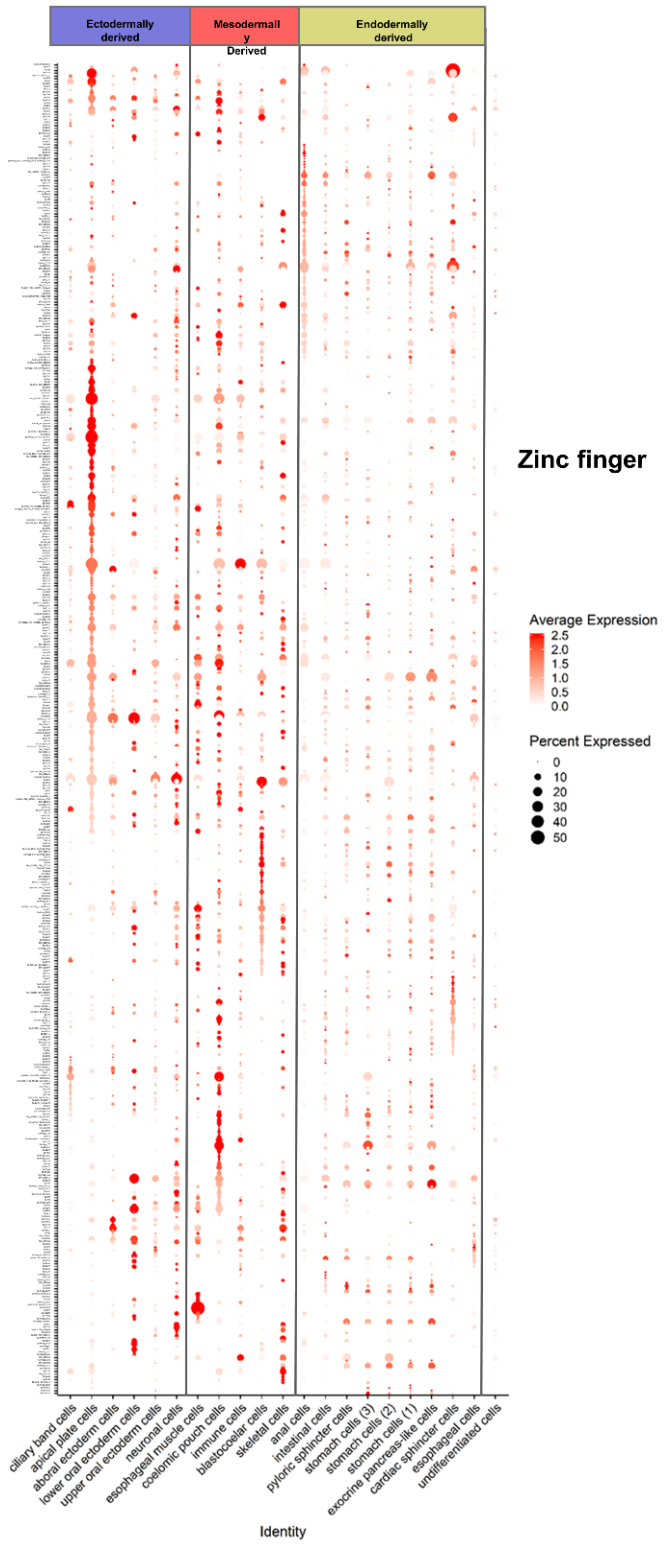


**Figure 4– Figure supplement 1.** Dotplot showing the mRNA average expression of the zinc finger transcription family members. The putative broad cell types are grouped based on their developmental origins: ectodermally derived, in blue, mesodermally derived, in red and endodermally derived, in yellow.

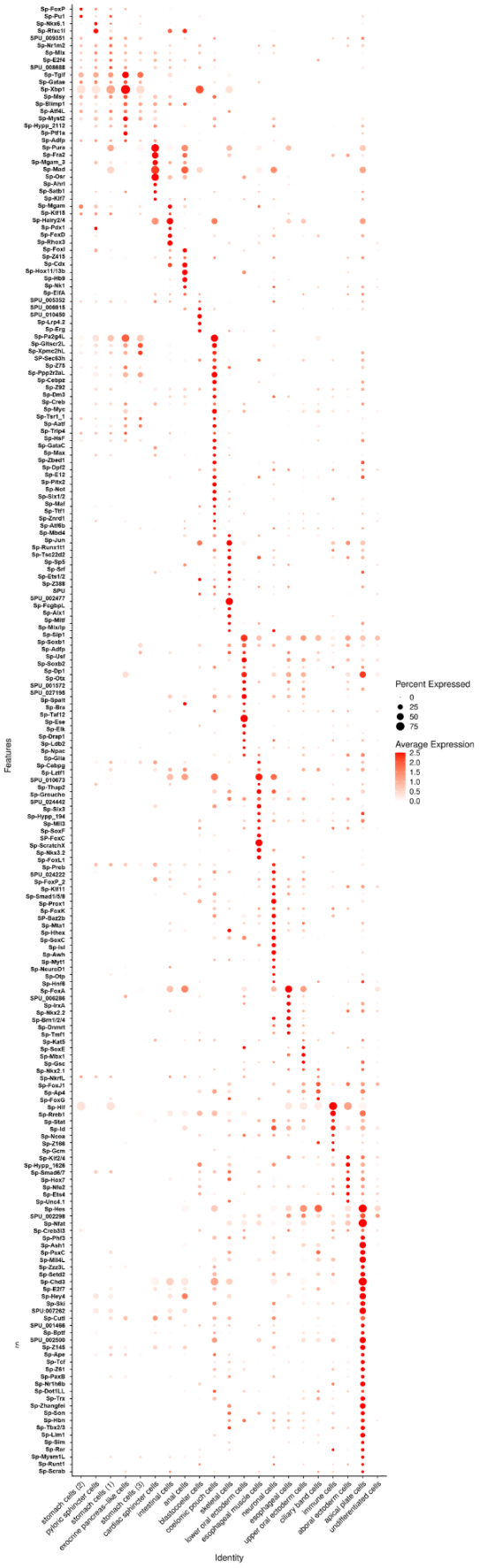
Percent Expressed
0
25
50
75
Average Expression
2.5
2.0
1.5
1.0
0.5
0.0
Features
Identity
stomach cells (2)
pyloric sphincter cells
stomach cells (1)
endocrine pancreas-like cells
stomach cells (3)
cardiac sphincter cells
intestinal cells
anal cells
blastocoelar cells
coelomic pouch cells
skeletal cells
lower oral ectoderm cells
esophageal muscle cells
neuronal cells
esophageal cells
upper oral ectoderm cells
ciliary band cells
immune cells
aboral ectoderm cells
apical plate cells
undifferentiated cells

**Figure 4– Figure supplement 2.** Dotplot showing the average scaled expression of differentially expressed TFs with p-value less than 0.5 among the 21 clusters. The cluster order is according to the transcription factor based tree as depicted in Figure 3-Figure supplement 1B.

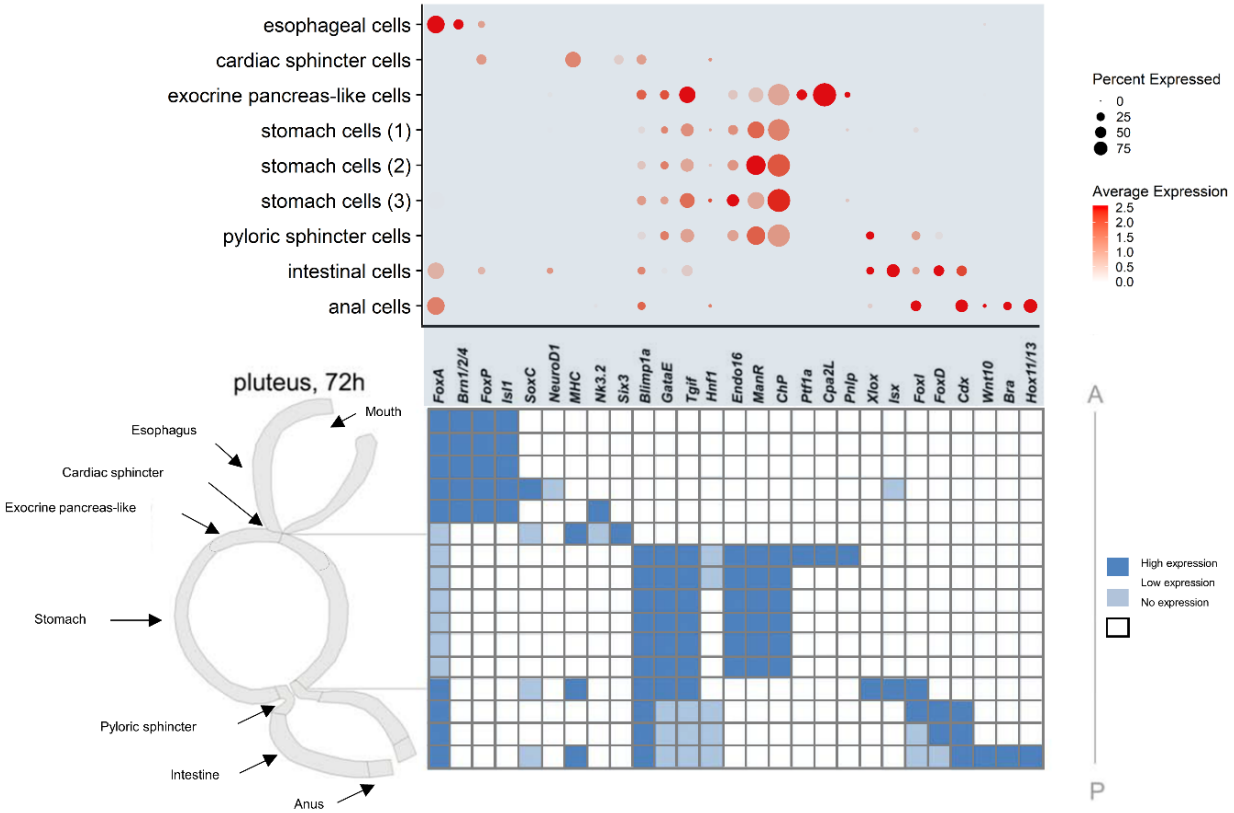


**Figure 5– Figure supplement 1.** Dotplot showing the regionalized average expression of marker genes labeling specific endodermal domains. The scRNA-seq prediction recapitulates the previously described compartmentalization of gene expression along the digestive tract of the 3 dpf sea urchin larva.

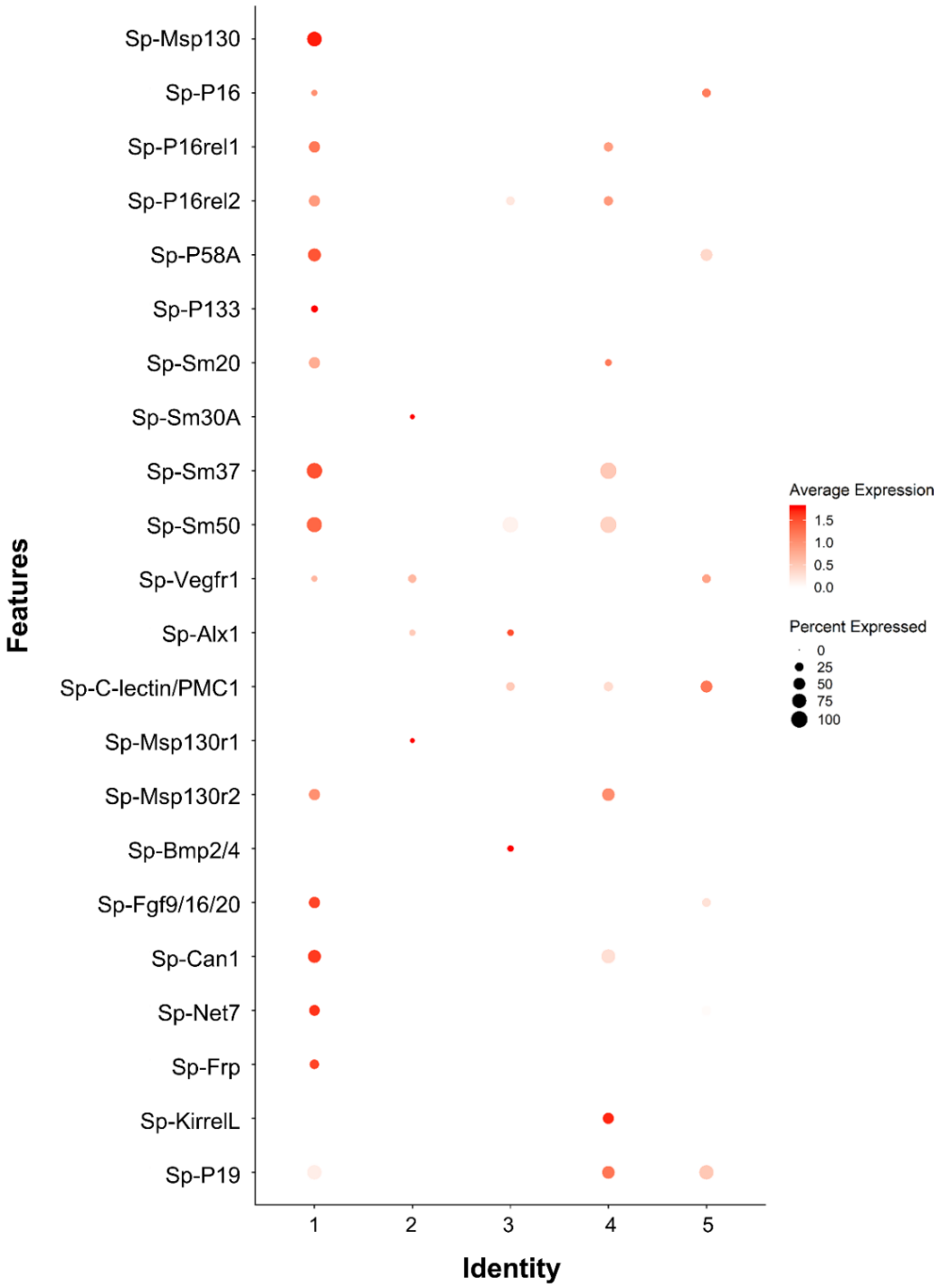


**Figure 6- Figure supplement 1.** Dotplot showing the mRNA average expression of 22 skeletal gene markers and their distribution among 5 skeletal subtypes.

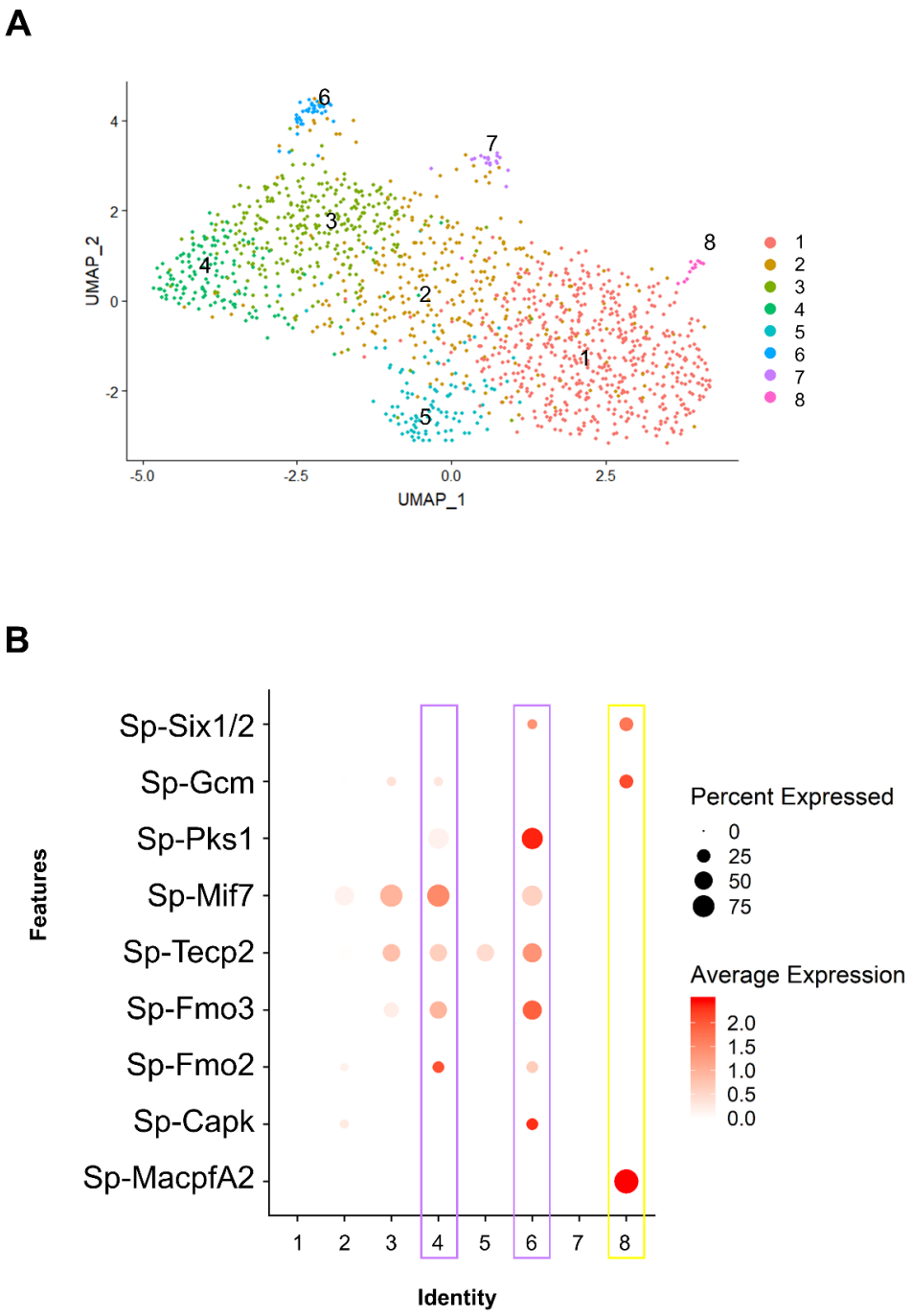


**Figure 6- Figure supplement 2. A)** UMAP showing 8 distinct immune cells populations as revealed by our subclustering analysis. **B)** Dotplot showing gene markers expressed in the different immune cell population. Pigment cells gene markers are highlighted in purple (box), while non pigmented globular cells are evident in yellow (box).