

1 **Supplementary Data**

2 **Single-cell transcriptome analysis reveals cell-cell communication and cellular**
3 **diversity in the zebrafish thyroid gland.**

4 Pierre Gillotay, Meghna Shankar, Sema Elif Eski, Susanne Reinhardt, Annekathrin
5 Kränkel, Juliane Bläsche, Andreas Petzold, Gokul Kesavan, Christian Lange, Michael
6 Brand, Vincent Detours, Sabine Costagliola, Sumeet Pal Singh

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8 **Includes**

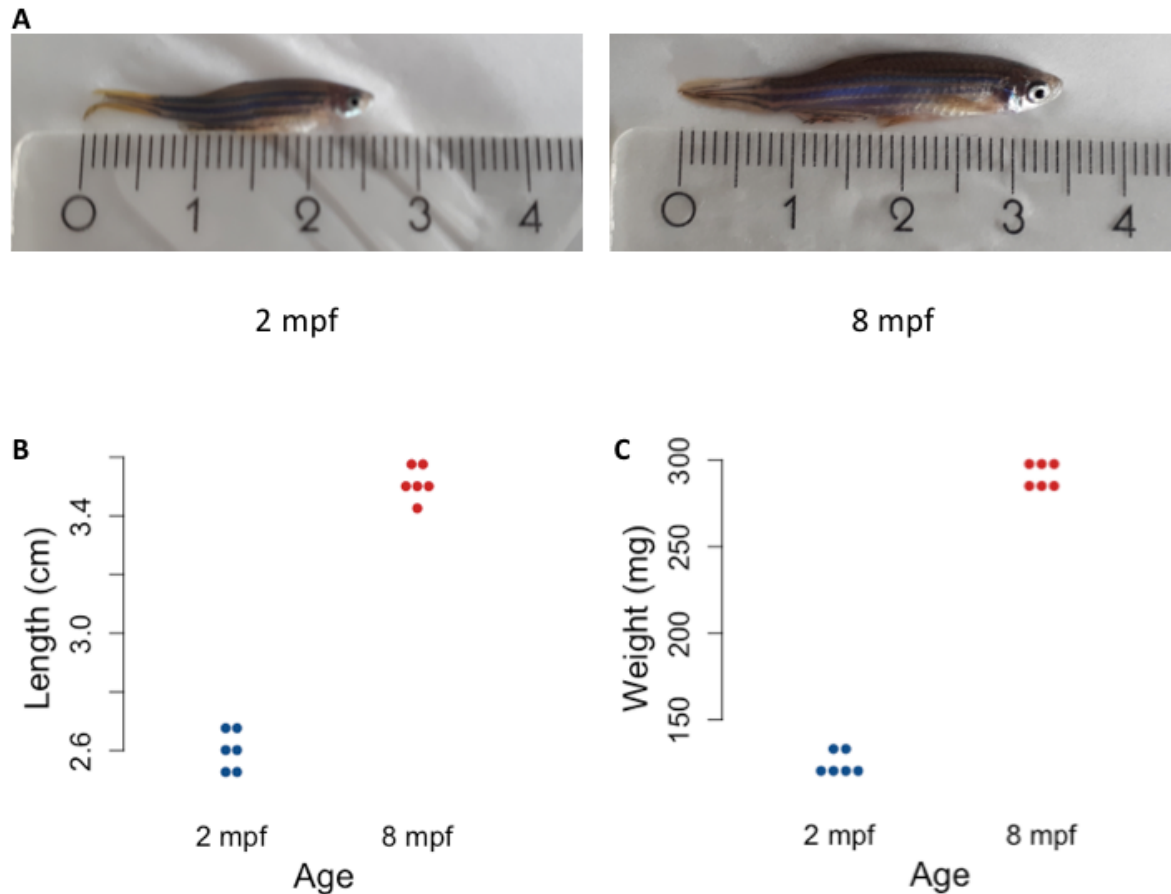
9 Supplementary Figures 1 – 7

10 Supplementary Tables 1 – 3 Legend

11 Supplementary Movie 1 Legend

12 **Supplementary Figures**

13 **Supplementary Figure 1**

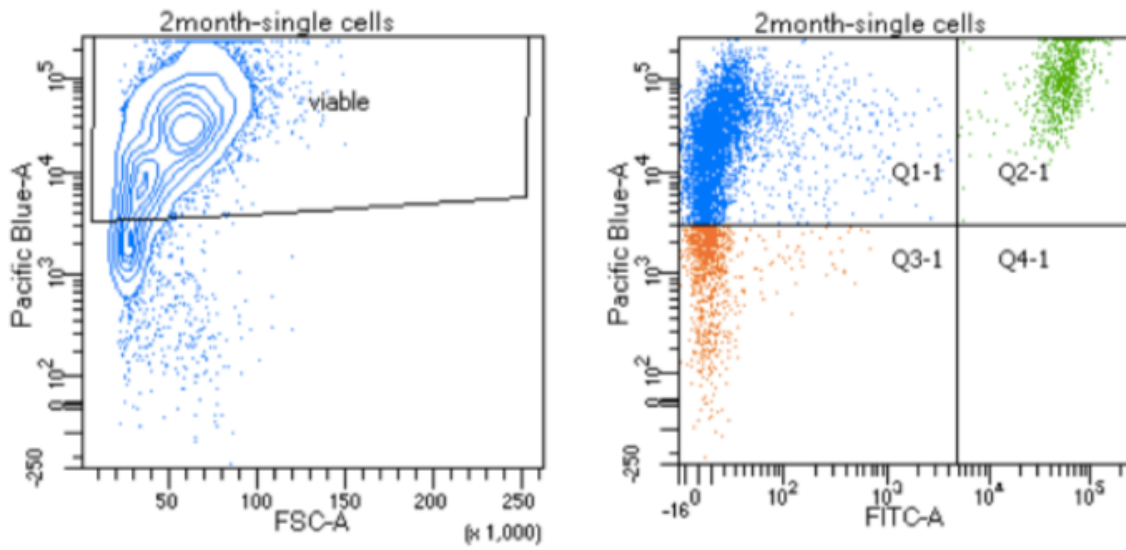


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15 **Supplementary Figure 1: Physical characteristics of zebrafish at 2 mpf and 8 mpf.**

16 **(A)** Representative images of zebrafish at 2 mpf (left) and 8 mpf (right). **(B)** Dotplot
17 representing the length of individual animals at each stage. Y-axis represents the length
18 of six zebrafish from mouth to end of fin in cm. **(C)** Dotplot representing the weight of
19 individual animals at each stage. Y-axis represents the weight of six zebrafish in mg.

20 **Supplementary Figure 2**



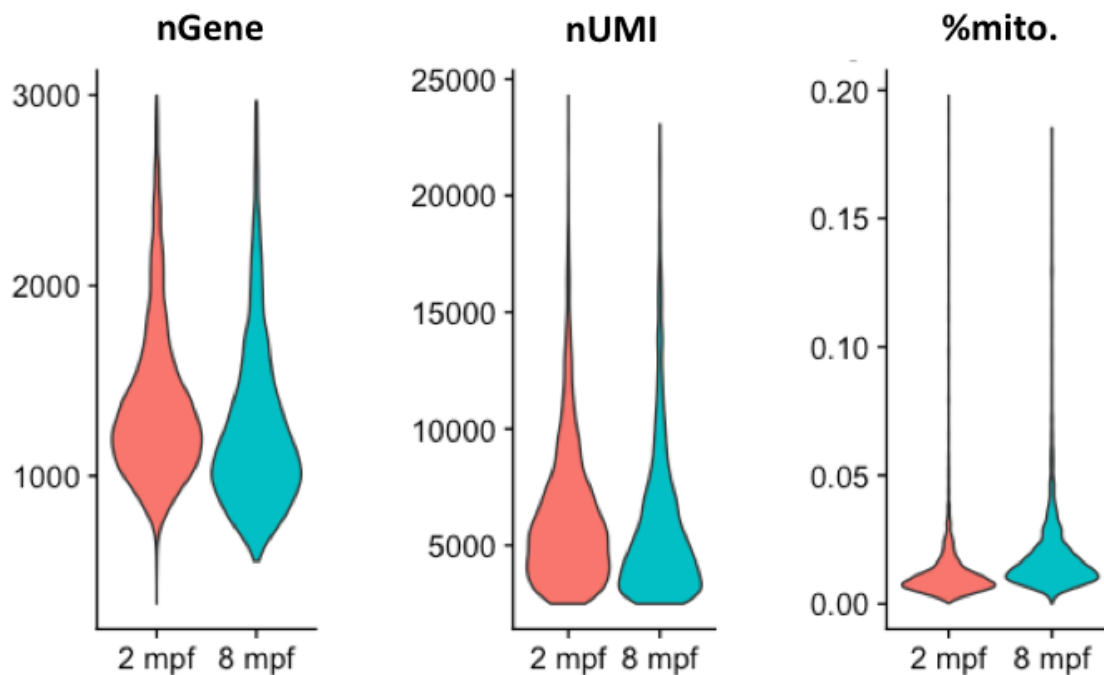
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22 **Supplementary Figure 2: FACS sort of zebrafish thyroid gland**

23 A representative FACS plot of live cells from *Tg(tg:nls-mVenus-T2A-NTR)* animals at 2
24 mpf. Calcein (Pacific Blue) labels live cells, while green fluorescence (FITC) labels
25 thyrocytes.

26

27 **Supplementary Figure 3**

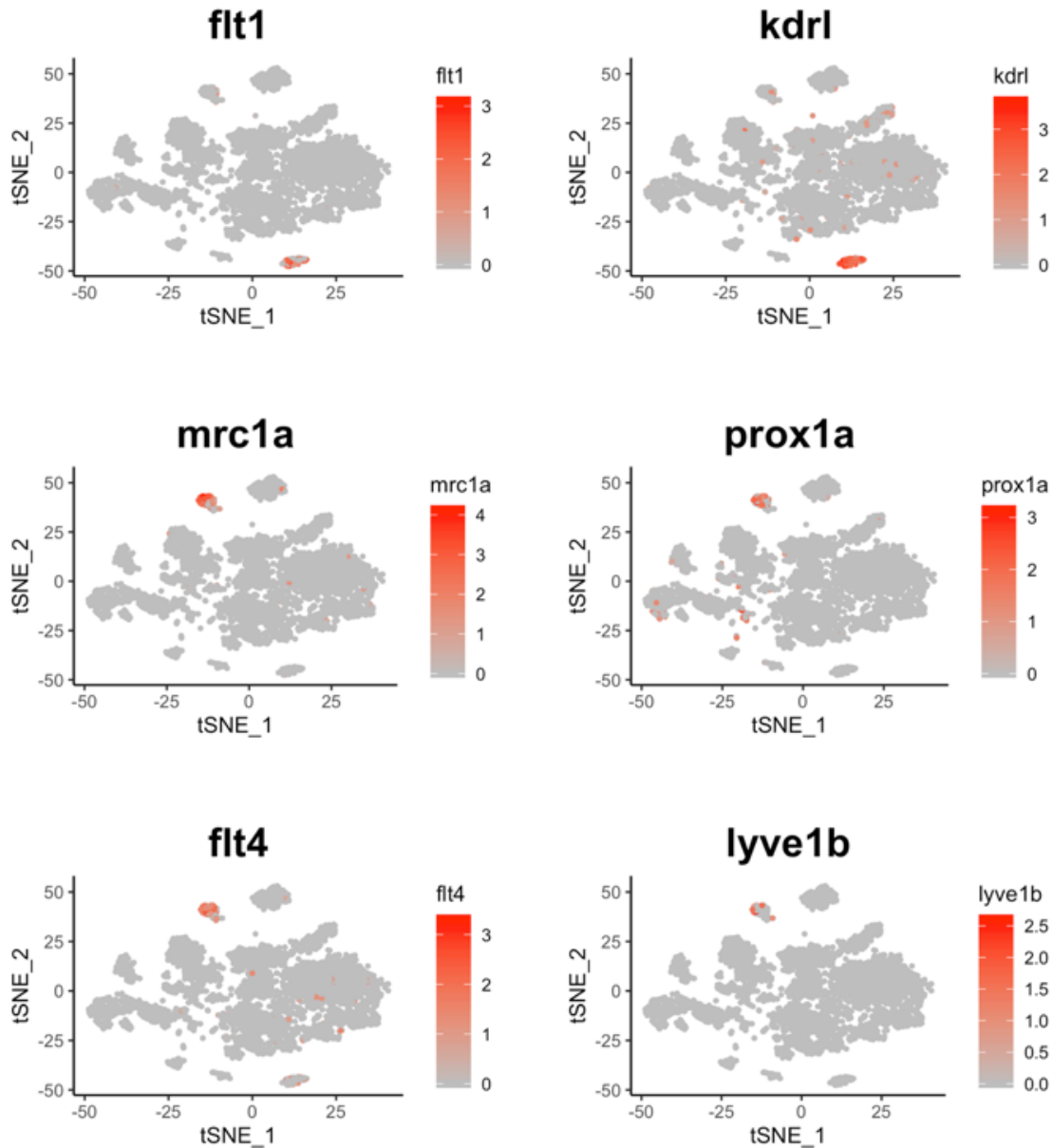


28

29 **Supplementary Figure 3: Quality control parameters for cells present in the**
30 **zebrafish thyroid gland atlas**

31 Violin plots depicting the number of genes (nGene), number of unique RNA molecules
32 detected (nUMI – number of Unique Molecule Identifiers) and the percentage of reads
33 mapped onto the mitochondrial genome for the cells profiled in the zebrafish thyroid
34 gland atlas.

35 **Supplementary Figure 4**



36

37 **Supplementary Figure 4: Cells belonging to blood vessels and lymphatic vessels**

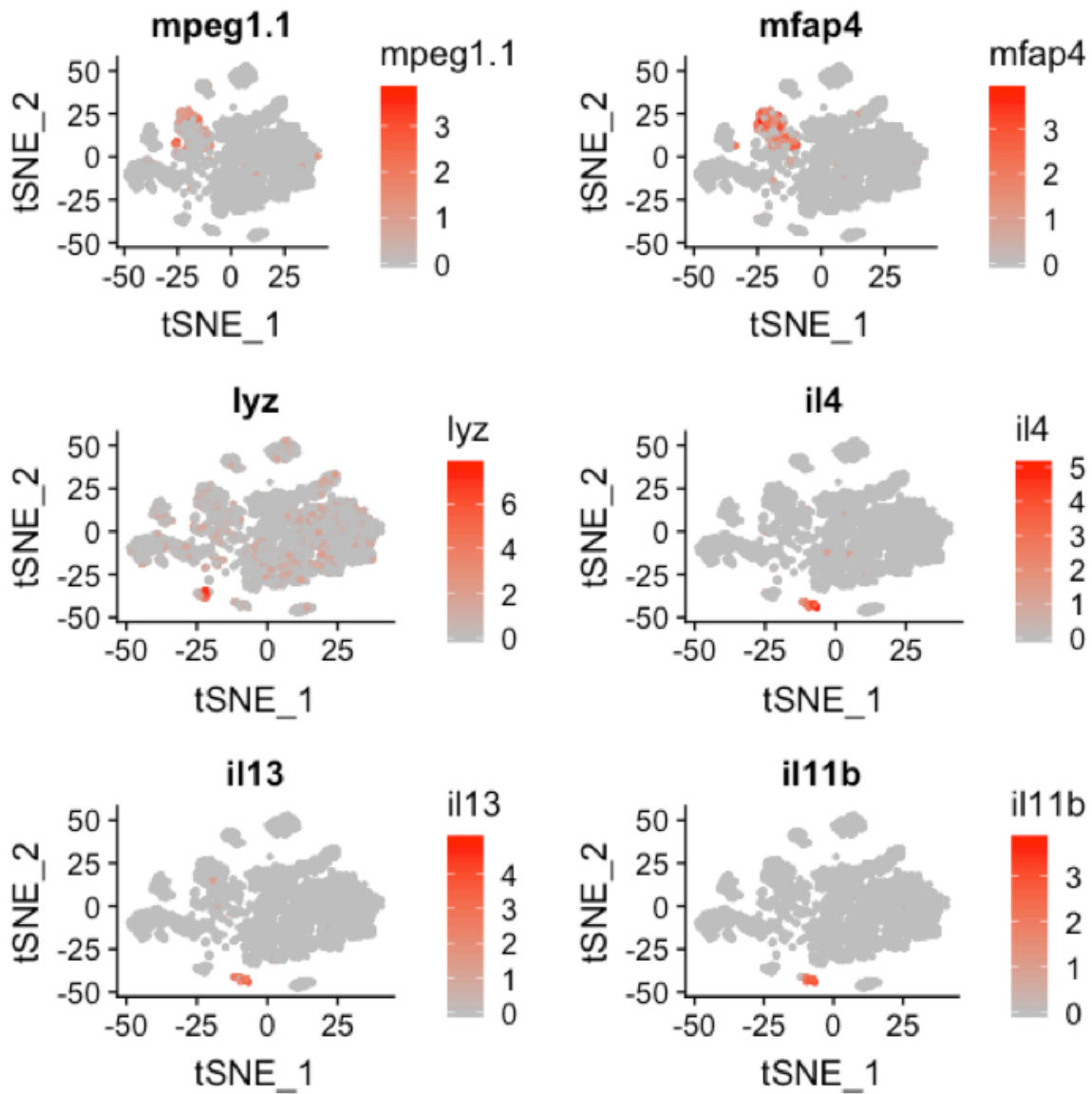
38 **are present the zebrafish thyroid gland atlas**

39 T-SNE plot overlaid with gene expression for genes specific to blood vessels (*flt1* and

40 *kdrl*) and lymphatic vessels (*mrc1a*, *prox1a*, *flt4* and *lyve1b*).

41

42 **Supplementary Figure 5**

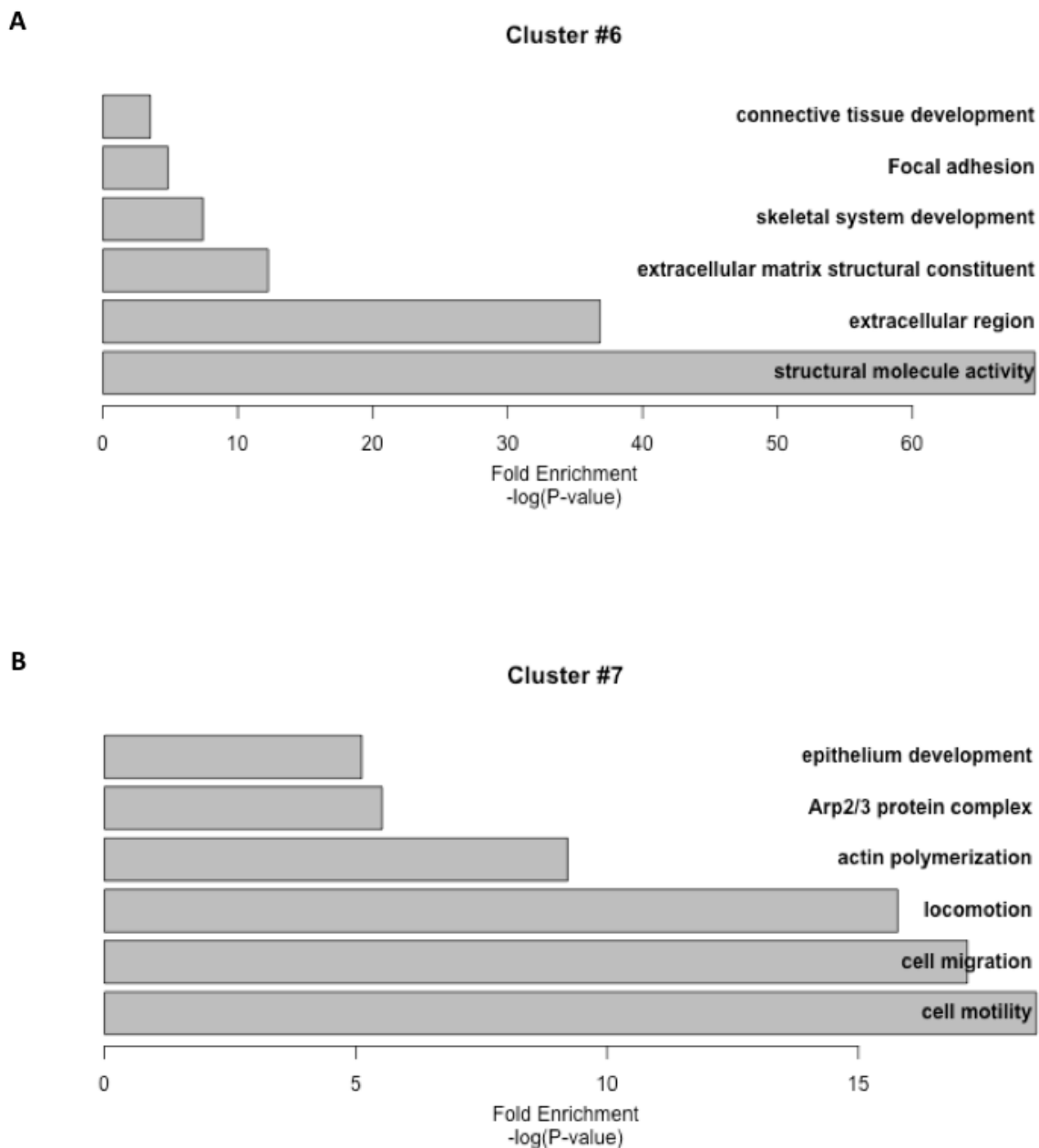


43

44 **Supplementary Figure 5: Cells belonging to diverse hematopoietic lineage are**
45 **present in the zebrafish thyroid gland atlas**

46 T-SNE plot overlaid with gene expression for genes specific to macrophages (*mpeg1.1*
47 and *mfap4*), neutrophils (*lyz*) and lymphocytes (*il4*, *il13* and *il11b*).

48 **Supplementary Figure 6**



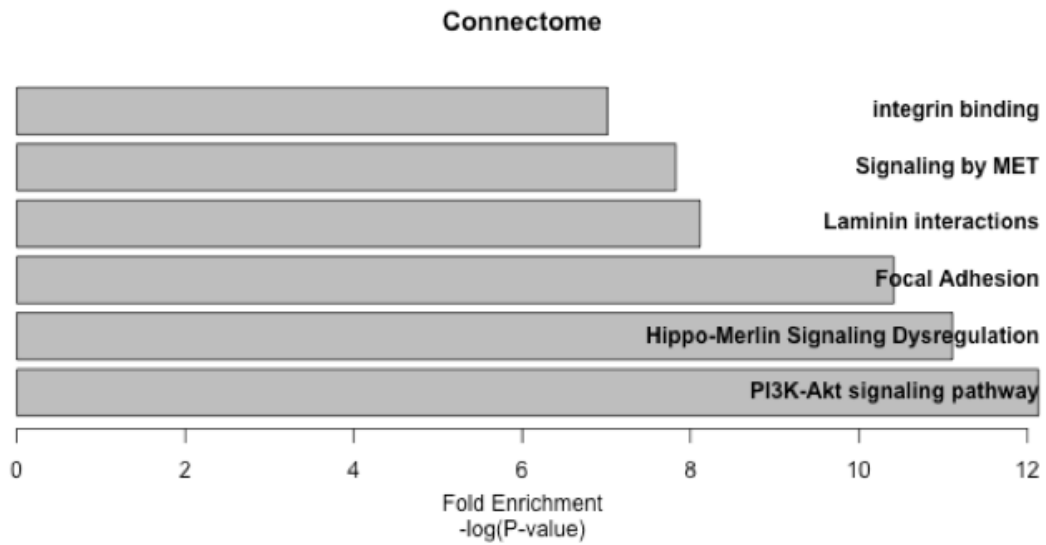
49

50 **Supplementary Figure 6: GO analysis for Cluster #6 and #7 marker genes**

51 Barplot depicting the GO categories identified for marker genes enriched in Cluster #6

52 **(A)** and Cluster #7**(B)**. X-axis represents a negative log of p-value.

53 **Supplementary Figure 7**



54

55 **Supplementary Figure 7: GO analysis for zebrafish thyroid gland connectome**

56 Barplot depicting the GO categories for ligands expressed in different cell-types present
57 in the thyroid gland atlas and their respective receptors in thyrocytes. X-axis represents
58 a negative log of p-value.

59

60 **Supplementary Table Legends**

61 **Table 1: Marker genes for each cluster of the zebrafish thyroid gland atlas (.xls)**

62 A table listing the marker genes for the seven clusters identified in the zebrafish thyroid
63 gland atlas. The tables also lists the fold-change and adjusted p-value for each gene.

64 **Table 2: Connectome for the zebrafish thyroid gland atlas (.xls)**

65 A table listing the ligands expressed in different cell-types of the thyroid gland and their
66 corresponding receptors expressed on the thyrocytes.

67 **Table 3: Differential gene expression analysis between the two sub-populations of the** 68 **thyrocytes (.xls)**

69 A table listing the differentially expressed genes between the 'Cluster-Blue' and
70 'Cluster-Red' subpopulations of the thyrocytes. The table lists fold-change and p-value
71 for each gene. Genes showing a significant difference (p-value < 0.05) in gene
72 expression are listed.

73 **Supplementary Movie Legend**

74 **Movie 1: Time-lapse of *pax2a*^{mKO2} expression during embryonic development**

75 A time-lapse video from confocal imaging of the *pax2a*^{mKO2}; *Tg(tg:nls-EGFP)* zebrafish
76 embryo from 36 hpf to 55 hpf. Live imaging reveals expression of mKO2 in anatomical
77 structures known to express *pax2a*. Moreover, co-expression of mKO2 and GFP can be
78 observed in the developing thyroid gland.