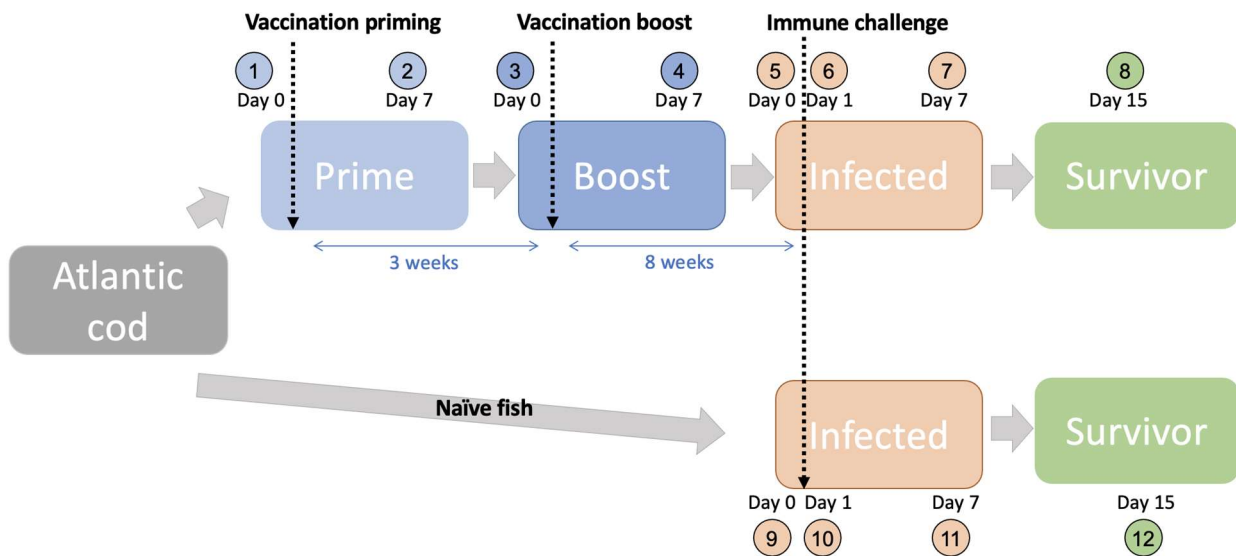
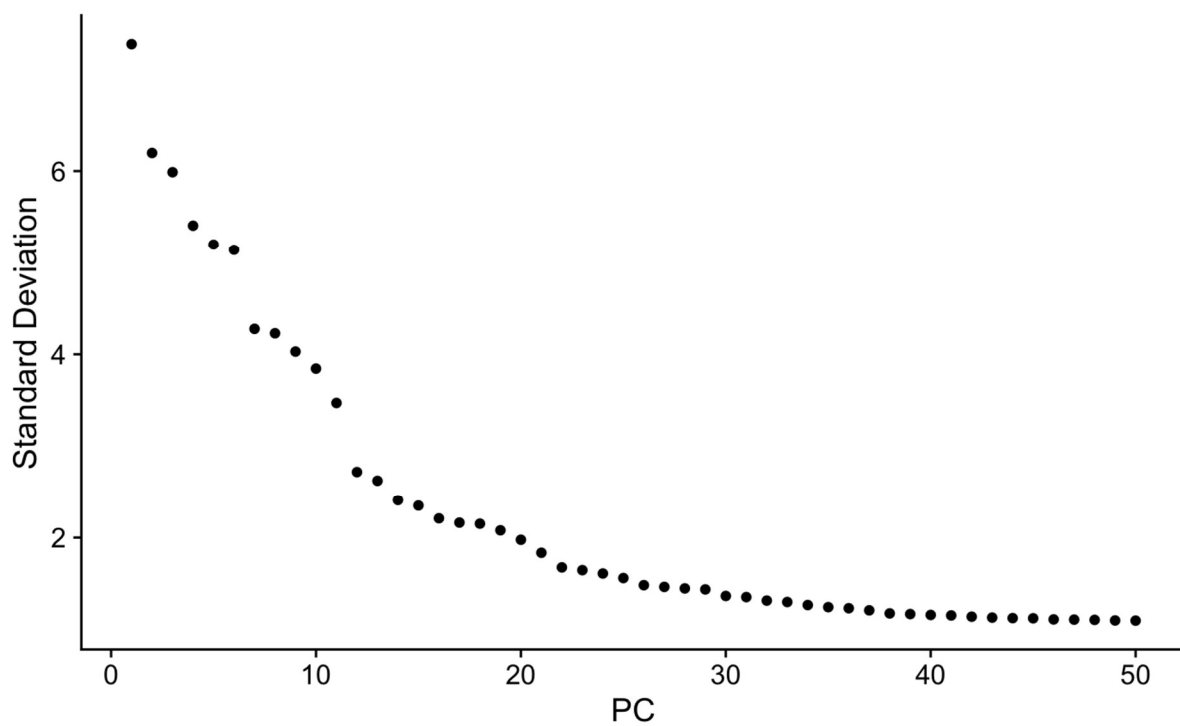




Supplementary Figure 1.

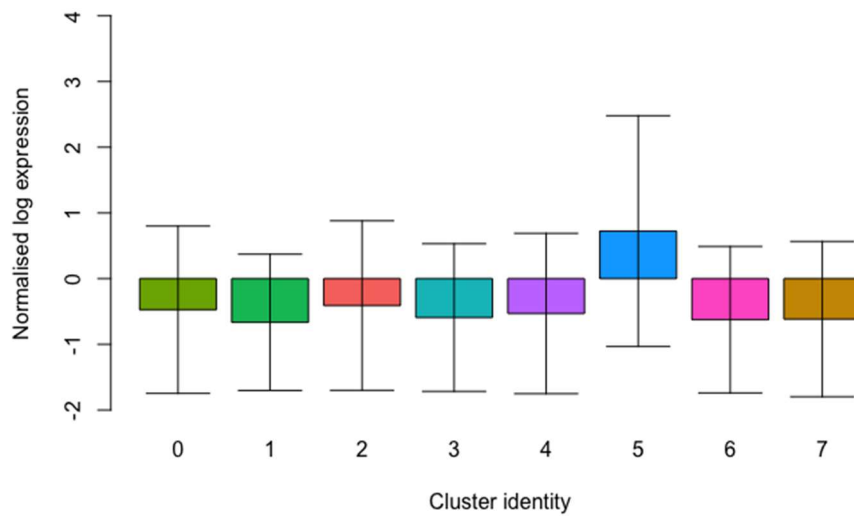
Schematic showing sampling timeline.

Atlantic cod were split into two groups: vaccinated and naïve fish. Following priming and boost immunisations, both groups were challenged by the pathogen *Vibrio anguillarum* and sampled on days 1, 7 and 15 after challenge. Each sampling point, 1-12, represents 2-3 fish. 34 Atlantic cod were sampled in total.



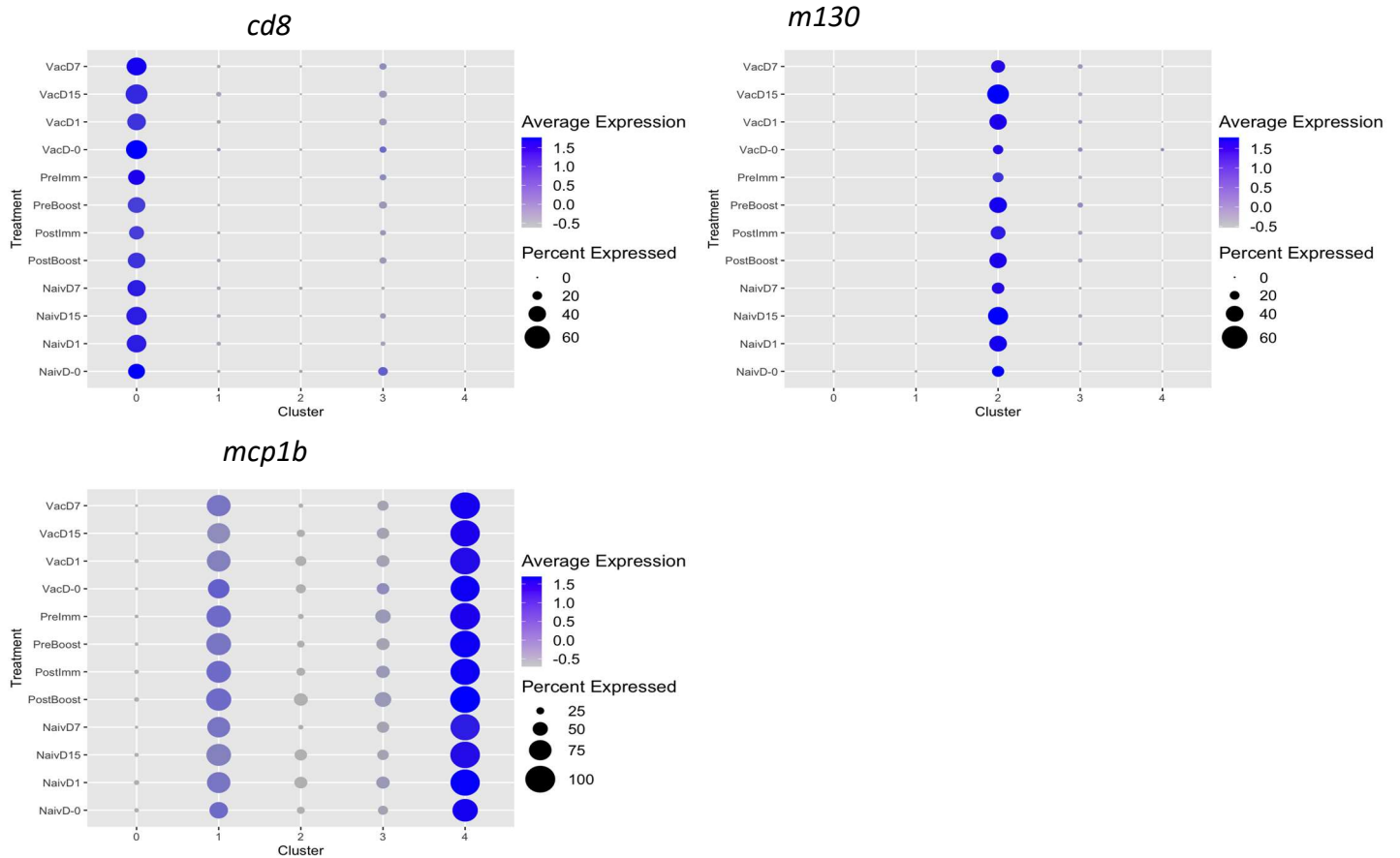
Supplementary Figure 2.

Elbow plot showing a ranking of principal components based upon the percentage of variance explained by each. Here we observe an ‘elbow’ around principal components 30-40, suggesting that the majority of true signal is captured in the first 40 principal components.



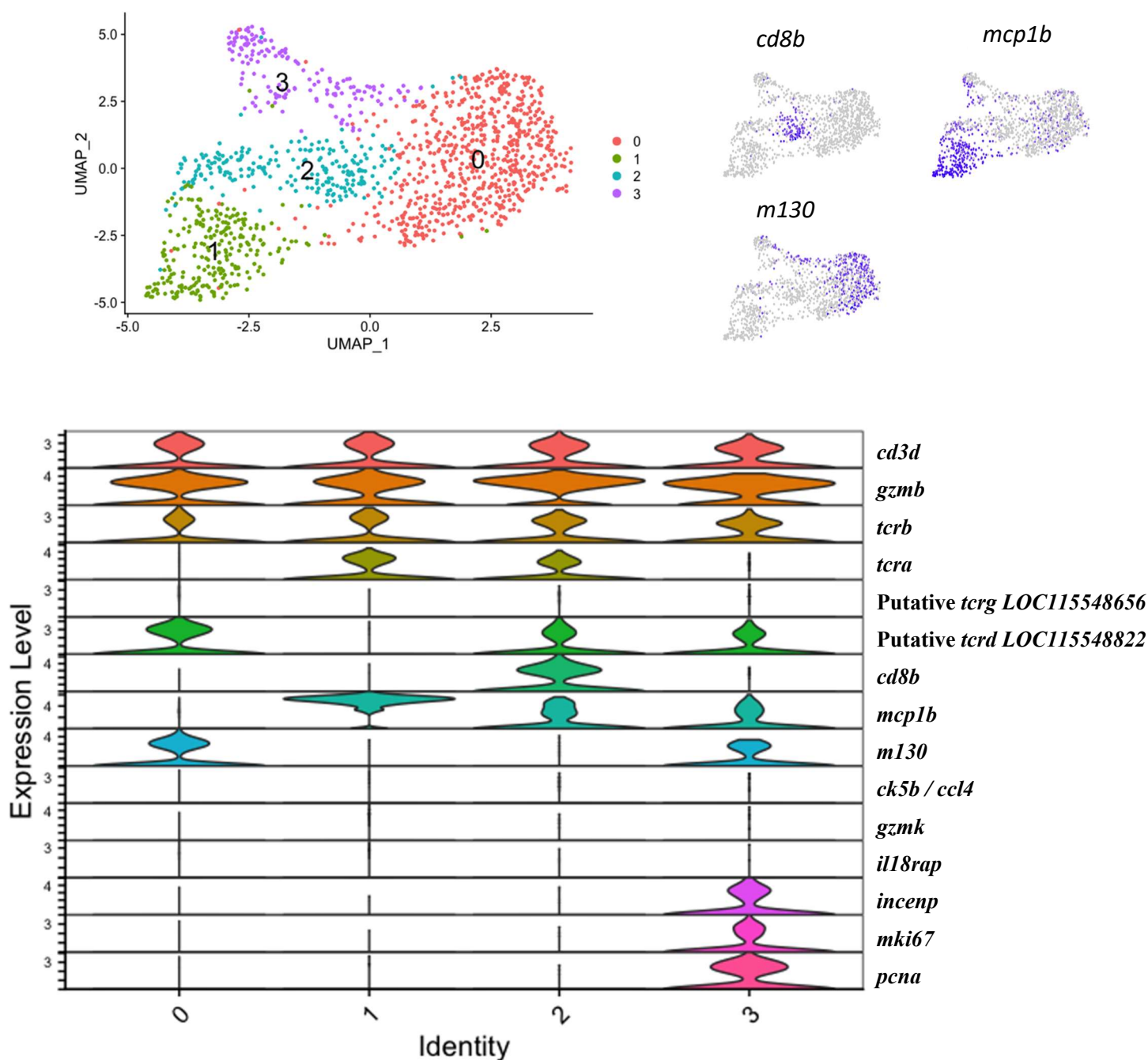
Supplementary Figure 3.

A bar chart showing the normalised log-transformed expression of immunoglobulin genes in each of the B cell sub-clusters. The error bars show the standard deviation.



Supplementary Figure 4

Dot plot showing expression of *cd8*, *mcp1b* and *m130* genes in T-cell subclusters across vaccination and immune challenge study timeline. The size of the dot encodes the percentage of cells within a subcluster expressing the gene, while the colour intensity encodes the average expression level of 'expressing' cells.



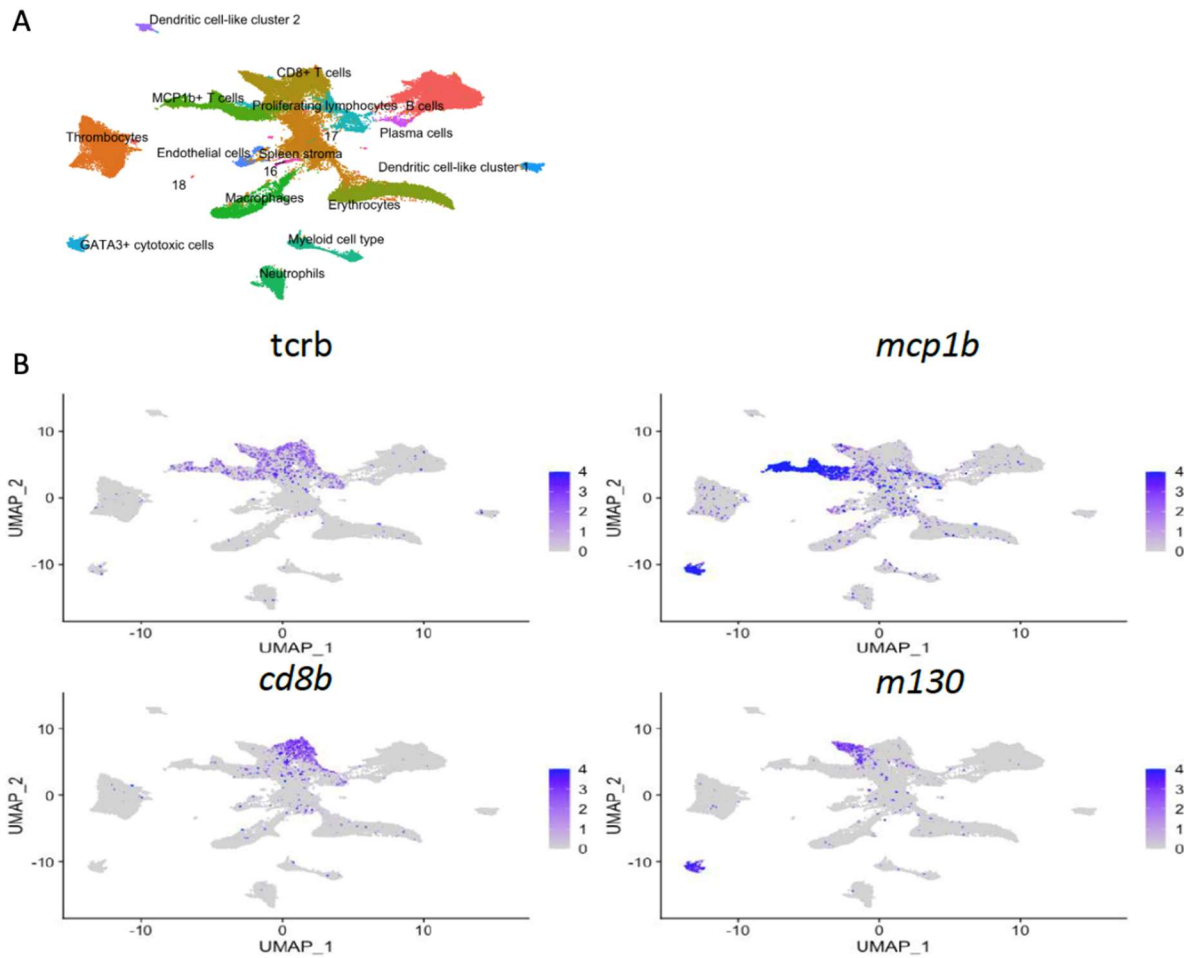
Supplementary figure 5.

Atlantic cod T lymphocytes sub-clusters from previous work. (Guslund et al., 2020)

A) 1,304 T lymphocyte cells clustered at a resolution of 0.3 using UMAP reveals 4 sub-clusters.

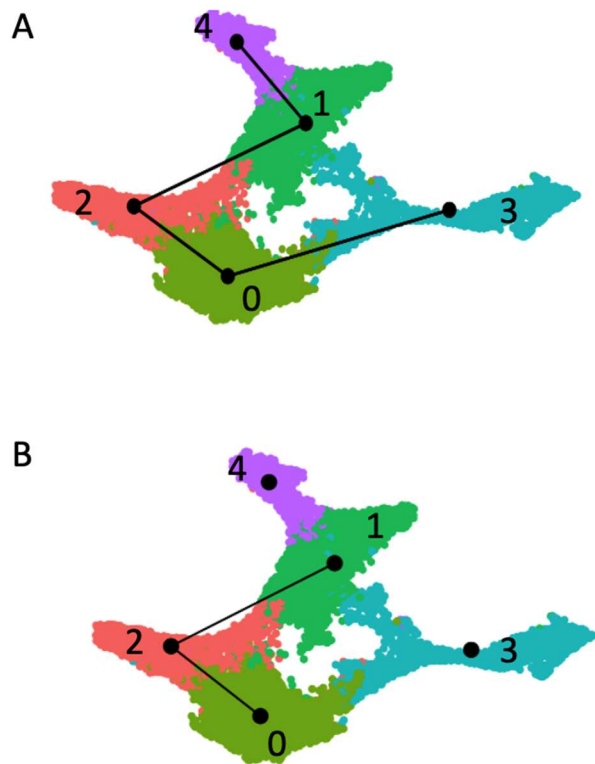
B) Violin plots showing expression of selected genes identified by differential expressed gene analysis. The *y-axis* indicates normalized and log-transformed average expression of the selected genes. The *x-axis* indicates the T cell cluster identity shown in the UMAP.

C) Feature plot showing the expression of T cell co-receptor *cd8b*, cytokine *mcp1b* and *m130*.



Supplementary Figure 6

Figure showing the expression of key T cell marker genes. (A) Global UMAP containing all splenic cells showing identity of cell clusters. **(B)** Feature plots showing the expression of the T cell receptor *tcrb*, T cell co-receptor *cd8b*, cytokine *mcp1b*, and *m130*, on the UMAP from the sampled Atlantic cod.



Supplementary Figure 7

Pseudotime analysis of T lymphocytes using Slingshot. (A) T cell sub-clusters depicting one Slingshot-predicted lineage trajectory, starting at lineage 3 and ending at lineage 4. (B) Selecting multiple trajectories suggests sub-clusters 3 and 4 are separate lineages, while sub-clusters 0, 1 and 2 are connected.



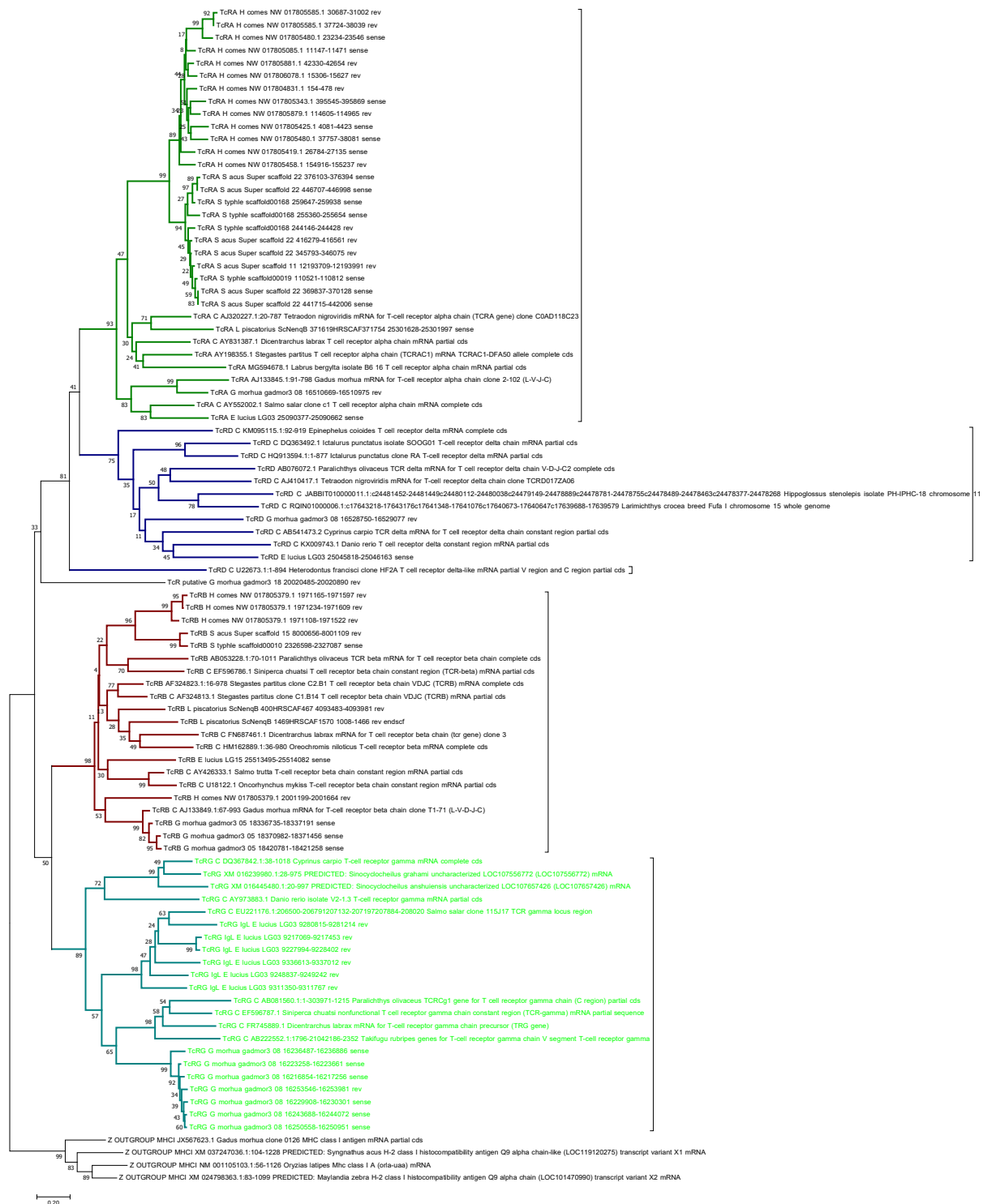
Supplementary Figure 8A

Neighbour joining phylogram of CD163/Scavenger receptor M160/M130 from Atlantic cod (*Gadus morhua*) and selected other species. Scavenger Receptor Cysteine-Rich Type 1 Protein M130 (CD163) references were collected from the groups Mammalia, Aves, Reptilia, Amphibia and Teleostei at Uniprot.org where available. The predicted gene models of the scRNA-reported annotations were then subjected to a blastp at NCBI towards mammalian and teleostei databases using default parameters. Here, hits annotated as M130/M160/CD163 were randomly selected to be included in the multiple sequence alignment. Members of the group B SRCR family were included (CD5, CD5L and CD6) together with two other scavenger receptors: scavenger receptor cysteine rich family member with 4 domains (SSC4D) and Scavenger Receptor Class B Member 1 (SCARB1). A multiple sequence alignment was generated using MEGA7 and its MUSCLE alignment program using default setting. A neighbour joining tree using Poisson distribution, pairwise deletion and 300 bootstrap replicates was made using MEGA7. The resulting tree was imported into FigTree for annotations and presentation. Root placed on the SCARB1 clade is coloured green. Members of the superfamily beyond CD163 and CD163L colored magenta (SSC4D), red (CD5 and CD5L) and orange (CD6). The *mcb1b* gene model from scRNA analyses is coloured blue.



Supplementary Figure 8B

Neighbour joining phylogram of CCL2/MCP1b from Atlantic cod (*Gadus morhua*), Channel catfish and selected species. Monocyte chemotactic protein 1 (MCP1/CCL2) references were collected from the groups Mammalia, Aves, Reptilia, Amphibia and Teleostei at Uniprot.org where available. The predicted gene models of the scRNA-reported annotations were then subjected to a blastp at NCBI towards mammalian and teleostei databases using default parameters. A set of references for the most common CC chemokines in humans and fish were downloaded from genbank and added to the alignment. Some CXC chemokines were added to function as a collective outgroup. Finally, complete CC chemokine characterizations from Channel catfish and Atlantic cod were added, together with all predicted gene models from gadMor3 reported from a tblastn search using the CCL2 references, default parameters and e-value cutoff at $1e-1$. A multiple sequence alignment was generated using MEGA7 and its MUSCLE alignment program using default setting. A neighbour joining tree using Poisson distribution, pairwise deletion and 300 bootstrap replicates was made using MEGA7. The resulting tree was imported into FigTree for annotations and presentation. Root placed on the CXC chemokines clade is coloured green. CC chemokines characterized in Atlantic cod are coloured teal. CC chemokines characterized in Channel catfish are coloured orange. Genes annotated as CCL2 from databases are coloured red. Gene model from scRNA analyses is coloured blue.



Supplementary Figure 8C

A neighbour joining phylogram of TCR genes in Atlantic cod (*Gadus morhua*) and selected other species. Annotated *tcrb* (LOC115544273) and *tcrd* (LOC115548821) genes in gadMor3, as well as putative TCR genes (LOC115548656 and LOC115548822) identified by blasting gadMor3 with TCR genes from different teleosts, reptiles and birds were compared. All genes with hits above 1e-100 were collected and added to the overall sequence alignment. A multiple sequence alignment was generated using MEGA7 and its MUSCLE alignment program using default setting. From here a neighbor joining tree using Poisson distribution, pairwise deletion and 200 bootstrap replicates was made again using MEGA7. The resulting tree was imported into FigTree for annotations and presentation purposes. LOC115548656 clustered with known *tcrg* genes and LOC115548822 with known *tcrd* genes suggesting that these two genes represent Atlantic cod *tcrg* and *tcrd*, respectively. Blue text: TCR gene loci mentioned in the manuscript; Teal text: genome constants; Green: TcRA; Mustard: TcRD; Red: TcRB; Blue TcRG