

Supplementary Table

Table S1 Summary of all the oligonucleotides designed for this study against various (predicted) B cell or B cell-related markers. Oligonucleotides are divided into two groups: the first five primers target predicted (in common carp) B cell-associated markers whose expression we measured as part of Figure 4 and the Method Details section ‘Quantitative reverse transcription PCR (RT-qPCR) gene expression profiling of B cell activation and differentiation markers’; the second group includes primers targeting 18 *Cyprinus carpio* orthologues of grass carp B cell-associated genes that Pan et al. (1) identified by single-cell RNA sequencing as defining and clustering distinct head kidney IgM⁺ B cell populations. We measured the latter group of genes as part of Figure 5 and the Method Details section ‘Multiplex qPCR gene expression profiling’. These two groups of primers are divided by a dashed line.

| Gene symbol | NCBI or GenBank accession code | Gene product | Forward (F) and reverse (R) primers (5’-3’) | Primer efficiencies (%) | Amplicon length (bp) |
|------------------------|--------------------------------|--|---|-------------------------|----------------------|
| <i>tnfrsf13b</i> | XM_042734762.1 | <i>Cyprinus carpio</i> tumor necrosis factor receptor superfamily member 13B | F: CAGTGCTCCGAGCTGTGT R: AGTACAGCGCTGATCCGGA | 98.68 | 157 |
| <i>xbp1</i> | XM_042753507.1 | <i>Cyprinus carpio</i> X-box binding protein 1 | F: AAAGCACTTCGAAGGAAACTGAAG R: CGAGCTCCA ACTCCAAGACTT | 97.91 | 109 |
| <i>syndecan-3-like</i> | XM_042777052.1 | <i>Cyprinus carpio</i> syndecan-3-like | F: GTCTCTTATGGCCACATTTACTCG R: CCCACAGCAATAATGTCCCAAAA | 96.72 | 122 |

| | | | | | |
|--------------|----------------|---|---|--------|-----|
| <i>pax5</i> | XM_019109396.2 | <i>Cyprinus carpio</i> paired box 5, transcript variants X1, X2, X3, and X4 | F: GGCTATGGCGTCTTTGGCT R: TCGCGACCAGACACAAGTG | 97.06 | 123 |
| | XM_019109382.2 | | | | |
| | XM_019109370.2 | | | | |
| | XM_019109360.2 | | | | |
| <i>cxcr5</i> | XM_042743592.1 | <i>Cyprinus carpio</i> C-X-C chemokine receptor type 5, transcript variants X1 and X2 | F: CTAAACGGCGGAGGAACCT R: GCCCAATAGCTTGCAGAGGA | 100.51 | 150 |
| | XM_042743591.1 | | | | |
| <i>cd22</i> | XM_042754392, | <i>Cyprinus carpio</i> cluster of differentiation 22 | F: CATTCTCTTGCTGATGTCCTTCAT R: ATGAGCGTGTGTTTCAGAGGAGA | 88.48 | 99 |
| | XM_042726754 | | | | |
| <i>cd34</i> | XM_042713470 | <i>Cyprinus carpio</i> cluster of differentiation 34 | F: CTTACAGTTGCTGGGGACAG R: AACAGGTTCAAAAGCAGGTCCAA | 85.39 | 109 |
| <i>cd79b</i> | XM_042767177 | <i>Cyprinus carpio</i> cluster of differentiation 79b | CCTCTCAGCTGAAGTAAATATCC, TGACATCTGATCCATCTTTGACG | 92.74 | 109 |
| <i>cd80</i> | XM_042740109 | <i>Cyprinus carpio</i> cluster of differentiation 80 | CACAGCTGAGTACAGCGTTCC, AGGTGGACGGGGCTTAATCAAA | 95.26 | 143 |
| <i>cd83</i> | XM_019119505 | <i>Cyprinus carpio</i> cluster of differentiation 83 | CCGTCATATGGTACAAGGTTTCT, TGTCTAATGTAAGTGCAGAGGAC | 87.66 | 173 |

| | | | | | |
|---------------|---|--|--|--------|-----|
| <i>cd86</i> | XM_042778525 | <i>Cyprinus carpio</i> cluster of differentiation 86 | GTGCGCCATTCTTCATTAAGGG, CTACATGAGGACGTCAGACTAG | 92.14 | 148 |
| <i>cxcr4a</i> | XM_042725575 | <i>Cyprinus carpio</i> C-X-C chemokine receptor type 4 alpha | TACGAACACATCGTCTTTGAAGAT, GAGCCAACTTTGAGGTTCCGTG | 94.77 | 92 |
| <i>cxcr4b</i> | XM_042763477 | <i>Cyprinus carpio</i> C-X-C chemokine receptor type 4 beta | CTTCTTCATCTGTTGGTTGCCTTA, TGCTGTCTCAACCCCATCCTTA | 93.73 | 165 |
| <i>egr1</i> | XM_019064348 | <i>Cyprinus carpio</i> early growth response protein 1 | ACTGGAGACACGCTTTCAGAAAT, TCTTACACGGGCCGTTTCACC | 97.00 | 102 |
| <i>ier2</i> | XM_019067280 | <i>Cyprinus carpio</i> immediate early response protein 2 | CTCTAGAAAGCGACGGAGCAAA, CGTGCCTATGCCAAGAACAATTG | 94.13 | 173 |
| <i>ighm</i> | AB004106, AB004107, MH352354, MH352353 | <i>Cyprinus carpio</i> immunoglobulin M heavy chain membrane-bound and secretory protein | CGAATATGCAGTTCCTATTCAAGA T AACATGTGGAACCTTGATGCCCC | 97.55 | 216 |
| <i>irf4</i> | XM_019126566 | <i>Cyprinus carpio</i> interferon regulatory factor 4 | GGCCCTCTCAGATTACCGCTTA, TAGTCCAGAGGGCTGTCAGCT | 103.31 | 93 |
| <i>irf8</i> | XM_042754085, XM_042743728 | <i>Cyprinus carpio</i> interferon regulatory factor 8 | ATCTTCAAAGCGTGGGCGATATT, ATTTTGAGGAAGTTACTGACCGAT | 91.43 | 130 |

| | | | | | |
|--------------|--------------|--|---|--------|-----|
| <i>klf2</i> | XM_019065449 | <i>Cyprinus carpio</i> Krüppel like factor 2 alpha | GAAAACAGGTGGAAGGAGGAAC, TTATTCTGGCCAACACTGTGGG | 86.07 | 146 |
| <i>mki67</i> | XM_042735109 | <i>Cyprinus carpio</i> marker of proliferation Ki-67 | GTTCGGAAGGAAGCTGGACTG, AGAACAAGGAGCTCATTTTGACC | 100.37 | 103 |
| <i>pax5</i> | XM_019109360 | <i>Cyprinus carpio</i> paired box 5 | CAACAGGATCATTCGCACTAAAG, GTGACCCAGGTATCTGCAGTAA | 87.60 | 107 |
| <i>top2a</i> | XM_042735154 | <i>Cyprinus carpio</i> DNA topoisomerase II alpha | ACTCAGCAAATGTGGGTGTTTGAT, GGTTAACATTGACTCGGAGAATAA | 99.96 | 170 |
| <i>xbp1</i> | XM_042753507 | <i>Cyprinus carpio</i> X-box binding protein 1 | TTGGAGTTGGAGCTCGAGAATC, GACTGGGGTTAGATACCCTGG | 93.73 | 121 |

(1) Pan Y, Wu C, Zhong Y, Zhang Y, Zhang X. An Atlas of Grass Carp IgM+ B Cells in Homeostasis and Bacterial Infection Helps to Reveal the Unique Heterogeneity of B Cells in Early Vertebrates. *J Immunol* (2023) **211**:964–980. doi: 10.4049/jimmunol.2300052.