

Gene	Function	References	ENSEMBL ID	Forward primer	Reverse primer
<b>Reference genes</b>					
<i>b2m</i>	Beta-2-microglobulin	(Hibbeler et al., 2008)	ENSGACT00000025544	GAAGATGTGTTGAATAGAACAGCTGG	GAAGATGTGTTGAATAGAACAGCTGG
<i>ef1a</i>	Elongation factor 1 $\alpha$	(Hibbeler et al., 2008)	ENSGACT00000002893	CCACCGTTGCCTTGTCC	TGGGACTGTTCATAACCTCC
<i>rpl13a</i>	L13A ribosomal binding protein	(Hibbeler et al., 2008)	ENSGACT00000012319	CACCTTGGTCAACTTGAACAGTG	TCCCTCCGCCCTACGAC
<i>ubc</i>	Ubiquitin	(Hibbeler et al., 2008)	ENSGACT00000010662	AGACGGGCATAGGCCACTTGC	CAGGACAAGGAAGGCATCC
<b>Innate</b>					
<i>cd97</i>	Promotor of granulocyte and neutrophil migration, required for activation of the innate immune response	(Leemans et al., 2004; Rhodes et al., 2009)	ENSGACT00000024871	CTCGTGGCACTCTACGACATGAAAG	CAGCCCTATCTGGTGACCAAGCTTG
<i>csf3r</i>	Granulocyte colony-stimulating factor 3 receptor; role in differentiation and proliferation of granulocytes	(Birrer et al., 2012; Brunner, 2016; Maxson et al., 2013; Tabbara, 1993)	ENSGACT000000018254	TGGGGATTGGTCCCTCTCAG	TGGGTCAAACCTGGTCAAC
<i>il-1<math>\beta</math></i>	Interleukin 1 $\beta$ ; cytokine with function in early response proinflammatory signaling	(Brunner et al., 2017; Zhu et al., 2013)	ENSGACT00000019325	TGACGATGAAGCAGGGTCAAC	ACAGCGTACCGATCTCCTCTTC
<i>marco, RON</i>	Macrophage receptor with collagenous structure; mediates macrophage recognition and clearance of pathogens	(Kissick et al., 2014; Kraai et al., 2000)	ENSGACT00000001965	CCCTTTCGACCTTCACTGCC	TGTTTACCCCCACCCCTCCA

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<i>mif1</i>	Macrophage migration inhibitory factor; stops random macrophage migration through tissue, proinflammatory mediator of the innate immune system	(Brunner et al., 2017; Calandra and Roger, 2003)	ENSGACT00000023656	ATCAGGGAGCTACAAAGC	TCAGGAGAGATGCTCAGGTGTTG
<i>mst1ra</i>	Macrophage stimulating 1 receptor a; plays an important role in macrophage regulation	(Huang et al., 2016; Wang et al., 2002)	ENSGACT00000013997	ATGGCCATCGAAAAGCTTGCA	TGATGTCGTACGGTCCACA
<i>nkef1β, peroxyred oxin 1</i>	Natural killer cell enhancing factor; enhances cytotoxicity of NK cells, also protects against oxidative damage	(Shau et al., 1993; Stutz et al., 2015)	ENSGACT00000021380	ACTTCTCCCACTTGCATGG	CAATGCCCTCATCCCTCCCTTC
<i>p22phox</i>	NADPH oxidase component p22phox; part of the reactive oxygen species production machinery	((Bedard and Krause, 2007; Mayumi et al., 2008)	ENSGACT00000021084	GCCTGGGACTCATTCCTCCT	TGGCCCTCTGCTTCCTGGA
<i>saa1</i>	Serum amyloid A; acute phase protein during inflammation response, mediates release of TNF-α and IL-1β	(Brunner et al., 2017; Haarder et al., 2013; Kovacevic et al., 2015)	ENSGACT00000007599	TCGCAGTGAGGCCAAAGATGAG	AAATCTGCCACCGTGTCCCTGG
<i>sia1</i>	Src-like-adaptor; necessary for maturation and activation of monocytic and dendritic cells, functions in T-cell signaling and B-cell development and function	(Brunner et al., 2017; Marton et al., 2015)	ENSGACT00000007895	ACAGAGTCGGCTCCTTCATGATA	TCACAGAGAGCGAATACAGACCTC

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<i>tnfr1</i>	Tumor necrosis factor receptor 1; functions in regulation of inflammation, mediates cellular apoptosis and differentiation	(Brunner et al., 2017; Zhu et al., 2013)	ENSGACT000000013502	AACTACTACAGAGCCAAGGGCAAG	ACGGCACCTCAGGGTACAAATTCC
Adaptive					
<i>cd83</i>	Marker for mature dendritic cells, expressed on activated B and T cells, costimulatory to activate naive and memory T-cells	(Aerts-Togaert et al., 2007; Stutz et al., 2015)	ENSGACT000000000428	AGGACCCAGCGTATAAATGG	CCCTGGTGATTTCCTCATC
<i>foxp3; forkhead box M2b</i>	Transcription factor; regulates functions important for the establishment of the T-reg lineage, key mediator of T-cell activation	(Kasheta et al., 2017; Rao and Naqvi, 2011; Robertson et al., 2015)	ENSGACT000000007261	GTTGACCCATGCAATTCCGA	CTGCTGTAGTTGGTGCCTG
<i>igm</i>	Immunoglobulin heavy constant mu (IgM); antibody molecule, part unpublished; Rønneseth et al., 2015; Zhu et al., 2013)	(S. Hibbeler, of the humoral immune response et al., 2015; Zhu et al., 2013)	ENSGACT000000016907	AAGGCAGGGAGAAATGAAAACCTTGG	CCGAGTGAGCAGACAGGGACTCTGG
<i>il-16</i>	Interleukin 16; cytokine with function in T-cell migration and expansion, chemoattractant for monocytes and eosinophils	(Brunner et al., 2017; Wen et al., 2006; Zhu et al., 2013)	ENSGACT000000016499	CTGGCTGGGCTTCAGTATTGCC	CTGGAAACACTCTGTGGACTG
<i>mhcII</i>	Major histocompatibility complex class IIb exon 2; pathogen recognizing protein of the adaptive immune response	(Lenz et al., 2009)	ENSGACT000000000425	GTCTTTAACTCCACGGAGCTGAAGG	ACTCACCGGACTTAGTCAG

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<i>stat4</i>	Signal transducer and activator of transcription 4; required for TH1-cell differentiation, opposes TH2 and TH17 like responses	(Kaplan, 2005; Premachandra et al., 2013; Wang and Secombes, 2013)	ENSGACT00000003538	CTCTCAGTTTCGAGGCTTGCTT	GGCAGTTGGCTCACATTGG
<i>stat6</i>	Signal transducer and activator of transcription 6; required for TH2-cell differentiation, regulates expression of TH2 relevant cytokine IL-4	(Robertson et al., 2015; Wang and Secombes, 2013)	ENSGACT00000011232	CTCAGCCACAGTTCCAACCGTTC	GTCGGATGTTCTGGACCTCGAGT
<i>tcr-β</i>	T-cell receptor β-chain; function in binding of MHC-peptide ligands to initiate adaptive immune response	(Smith-Garvin et al., 2009; Stutz et al., 2015; Yanagi et al., 1984)	ENSGACT00000016457	GAGGGCAAAACTTCACACTG	TAGGAGAAATCTGGCCGTTTG
<i>tgf-β</i>	Transforming growth factor β; cytokine with functions in cell growth, migration, differentiation and proliferation of T and B-cells	(Robertson et al., 2015; Zhu et al., 2013)	ENSGACT00000016968	TCCCGCTTCGTCAACCAACCA	ACGTCTGTCGCCACATTAC
Complement					
<i>c7</i>	Complement component 7; initializing function in the membrane attack complex of the complement system	(Brunner et al., 2017; Haase et al., 2014; Zhu et al., 2013)	ENSGACT00000009181	TGGCTCAAGCTCAGGCACAAACAG	AGCGACACGGTTGGTTGATCG
<i>c9</i>	Complement component 9; structural part of the membrane attack complex of the complement system	(Brunner et al., 2017; Haase et al., 2014; Zhu et al., 2013)	ENSGACT00000020968	CCGTGACGAAACAAAGACTCAGTTG	TCTGACCGATGTCAGGCACCTTG

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Complement (continued)					
<i>cfb</i>	Complement factor B; activating complement component of the alternative pathway	(Brunner, 2016; Haase et al., 2014; Zhu et al., 2013)	ENSGACT00000027346	GAGCGTGCACAATACTAGGTTG	-TACCAACGGAAAGCGCACAAATC

## References

- Aerts-Toegaert, C., Heirman, C., Tuyaerts, S., Corthals, J., Aerts, J.L., Bonehill, A., Thielemans, K., and Breckpot, K. (2007). CD83 expression on dendritic cells and T cells: Correlation with effective immune responses. *Eur. J. Immunol.* *37*, 686–695.
- Bedard, K., and Krause, K.-H. (2007). The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. *Physiol. Rev.* *87*, 245–313.
- Birrer, S.C., Reusch, T.B.H., and Roth, O. (2012). Salinity change impairs pipefish immune defence. *Fish Shellfish Immunol.* *33*, 1238–1248.
- Brunner, F.S. (2016). Parasitism along the speciation continuum - Variation of parasite effects across speciation stages of *Gasterosteus aculeatus* and their modification by environmental changes. Queen Mary University of London.
- Brunner, F.S., Anaya-Rojas, J.M., Matthews, B., and Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. *Proc. Natl. Acad. Sci.* *201619147*.
- Calandra, T., and Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat. Rev. Immunol.* *3*, 791–800.
- Haarder, S., Kania, P.W., Bahlool, Q.Z.M., and Buchmann, K. (2013). Expression of immune relevant genes in rainbow trout following exposure to live *Anisakis simplex* larvae. *Exp. Parasitol.* *135*, 564–569.
- Haase, D., Rieger, J.K., Witten, A., Stoll, M., Bornberg-Bauer, E., Kalbe, M., and Reusch, T.B.H. (2014). Specific Gene Expression Responses to Parasite Genotypes Reveal Redundancy of Innate Immunity in Vertebrates. *PLoS ONE* *9*, e108001.
- Hibbeler, S., Scharsack, J.P., and Becker, S. (2008). Housekeeping genes for quantitative expression studies in the three-spined stickleback *Gasterosteus aculeatus*. *BMC Mol. Biol.* *9*, 18.
- Huang, Y., Chain, F.J.J., Panchal, M., Eizaguirre, C., Kalbe, M., Lenz, T.L., Samonte, I.E., Stoll, M., Bornberg-Bauer, E., Reusch, T.B.H., et al. (2016). Transcriptome profiling of immune tissues reveals habitat-specific gene expression between lake and river sticklebacks. *Mol. Ecol.* n/a-n/a.
- Kaplan, M.H. (2005). STAT4: A Critical Regulator of Inflammation In Vivo. *Immunol. Res.* *31*, 231–242.
- Kasheta, M., Painter, C.A., Moore, F.E., Lobbardi, R., Bryll, A., Freiman, E., Stachura, D., Rogers, A.B., Houvras, Y., Langenau, D.M., et al. (2017). Identification and characterization of T reg-like cells in zebrafish. *J. Exp. Med.* *214*, 3519–3530.
- Kissick, H.T., Dunn, L.K., Ghosh, S., Nechama, M., Kobzik, L., and Arredouani, M.S. (2014). The Scavenger Receptor MARCO Modulates TLR-Induced Responses in Dendritic Cells. *PLoS ONE* *9*, e104148.
- Kovacevic, N., Hagen, M.O., Xie, J., and Belosevic, M. (2015). The analysis of the acute phase response during the course of *Trypanosoma carassii* infection in the goldfish ( *Carassius auratus* L.). *Dev. Comp. Immunol.* *53*, 112–122.

Kraal, G., van der Laan, L.J.W., Elomaa, O., and Tryggvason, K. (2000). The macrophage receptor MARCO. *Microbes Infect.* 2, 313–316.

Leemans, J.C., te Velde, A.A., Florquin, S., Bennink, R.J., de Bruin, K., van Lier, R.A.W., van der Poll, T., and Hamann, J. (2004). The Epidermal Growth Factor-Seven Transmembrane (EGF-TM7) Receptor CD97 Is Required for Neutrophil Migration and Host Defense. *J. Immunol.* 172, 1125–1131.

Lenz, T.L., Eizaguirre, C., Becker, S., and Reusch, T.B. (2009). RSCA genotyping of MHC for high-throughput evolutionary studies in the model organism three-spined stickleback *Gasterosteus aculeatus*. *BMC Evol. Biol.* 9, 57.

Marton, N., Baricza, E., &xc9;, Rsek, B., Buz&#xe1;, S., E.I., Nagy, G., and rgy (2015). The Emerging and Diverse Roles of Src-Like Adaptor Proteins in Health and Disease. *Mediators Inflamm.* 2015, e952536.

Maxson, J.E., Gotlib, J., Polleyea, D.A., Fleischman, A.G., Agarwal, A., Eide, C.A., Bottomly, D., Wilmot, B., McWeeney, S.K., Tognon, C.E., et al. (2013). Oncogenic CSF3R Mutations in Chronic Neutrophilic Leukemia and Atypical CML. *N. Engl. J. Med.* 368, 1781–1790.

Mayumi, M., Takeda, Y., Hoshiko, M., Serada, K., Murata, M., Moritomo, T., Takizawa, F., Kobayashi, I., Araki, K., Nakanishi, T., et al. (2008). Characterization of teleost phagocyte NADPH oxidase: Molecular cloning and expression analysis of carp (*Cyprinus carpio*) phagocyte NADPH oxidase. *Mol. Immunol.* 45, 1720–1731.

Premachandra, H.K.A., Elvitigala, D.A.S., Bathige, S.D.N.K., Whang, I., Lee, Y., De Zoysa, M., and Lee, J. (2013). Genomic structure and immunological response of an STAT4 family member from rock bream (*Oplegnathus fasciatus*). *Fish Shellfish Immunol.* 35, 1829–1837.

Rao, D.N., and Naqvi, R.A. (2011). FoxP3: A Key Player in T Regulatory Biology. *Indian J. Clin. Biochem.* 26, 1–2.

Rhodes, L.D., Wallis, S., and Demlow, S.E. (2009). Genes associated with an effective host response by Chinook salmon to *Renibacterium salmoninarum*. *Dev. Comp. Immunol.* 33, 176–186.

Robertson, S., Bradley, J.E., and MacColl, A.D.C. (2015). Measuring the immune system of the three-spined stickleback – investigating natural variation by quantifying immune expression in the laboratory and the wild. *Mol. Ecol. Resour.* n/a-n/a.

Rønneseth, A., Ghebretnsae, D.B., Wergeland, H.I., and Haugland, G.T. (2015). Functional characterization of IgM+ B cells and adaptive immunity in lumpfish (*Cyclopterus lumpus* L.). *Dev. Comp. Immunol.* 52, 132–143.

Shau, H., Gupta, R.K., and Golub, S.H. (1993). Identification of a Natural Killer Enhancing Factor (NKEF) from Human Erythroid Cells. *Cell. Immunol.* 147, 1–11.

Smith-Garvin, J.E., Koretzky, G.A., and Jordan, M.S. (2009). T Cell Activation. *Annu. Rev. Immunol.* 27, 591–619.

Stutz, W.E., Schmerer, M., Coates, J.L., and Bolnick, D.I. (2015). Among-lake reciprocal transplants induce convergent expression of immune genes in threespine stickleback. *Mol. Ecol.* 24, 4629–4646.

Tabbara, I.A. (1993). Granulocyte colony-stimulating factor. *South. Med. J.* *86*, 350–355.

Wang, T., and Secombes, C.J. (2013). The cytokine networks of adaptive immunity in fish. *Fish Shellfish Immunol.* *35*, 1703–1718.

Wang, M.-H., Zhou, Y.-Q., and Chen, Y.-Q. (2002). Macrophage-Stimulating Protein and RON Receptor Tyrosine Kinase: Potential Regulators of Macrophage Inflammatory Activities. *Scand. J. Immunol.* *56*, 545–553.

Wen, Y., Shao, J.-Z., Xiang, L.-X., and Fang, W. (2006). Cloning, characterization and expression analysis of two *Tetraodon nigroviridis* interleukin-16 isoform genes. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* *144*, 159–166.

Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S.P., Aleksander, I., and Mak, T.W. (1984). A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature* *308*, 145–149.

Zhu, L., Nie, L., Zhu, G., Xiang, L., and Shao, J. (2013). Advances in research of fish immune-relevant genes: A comparative overview of innate and adaptive immunity in teleosts. *Dev. Comp. Immunol.* *39*, 39–62.