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**Citation for published version:**

Gervais, O, Papadopoulou, A, Gratacap, R, Hillestad, B, Tinch, AE, Martin, SAM, Houston, R & Robledo, D 2022, 'Transcriptomic response to ISAV infection in the gills, head kidney and spleen of resistant and susceptible Atlantic salmon', *BMC Genomics*, vol. 23, no. 775, pp. 1-13. <https://doi.org/10.1186/s12864-022-09007-4>

**Digital Object Identifier (DOI):**

[10.1186/s12864-022-09007-4](https://doi.org/10.1186/s12864-022-09007-4)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

BMC Genomics

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# Transcriptomic response to ISAV infection in the gills, head kidney and spleen of resistant and susceptible Atlantic salmon

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## Abstract

**Background:** Infectious Salmon Anaemia virus (ISAV) is an orthomyxovirus responsible for large losses in Atlantic salmon (*Salmo salar*) aquaculture. Current available treatments and vaccines are not fully effective, and therefore selective breeding to produce ISAV-resistant strains of Atlantic salmon is a high priority for the industry. Genomic selection and potentially genome editing can be applied to enhance the disease resistance of aquaculture stocks, and both approaches can benefit from increased knowledge on the genomic mechanisms of resistance to ISAV. To improve our understanding of the mechanisms underlying resistance to ISAV in Atlantic salmon we performed a transcriptomic study in ISAV-infected salmon with contrasting levels of resistance to this virus.

**Results:** Three different tissues (gills, head kidney and spleen) were collected on 12 resistant and 12 susceptible fish at three timepoints (pre-challenge, 7 and 14 days post challenge) and RNA sequenced. The transcriptomes of infected and non-infected fish and of resistant and susceptible fish were compared at each timepoint. The results show that the responses to ISAV are organ-specific; an important response to the infection was observed in the head kidney, with up-regulation of immune processes such as interferon and NLR pathways, while in gills and spleen the response was more moderate. In addition to immune related genes, our results suggest that other processes such as ubiquitination and ribosomal processing are important during early infection with ISAV. Moreover, the comparison between resistant and susceptible fish has also highlighted some interesting genes related to ubiquitination, intracellular transport and the inflammasome.

**Conclusions:** Atlantic salmon infection by ISAV revealed an organ-specific response, implying differential function during the infection. An immune response was observed in the head kidney in these early timepoints, while gills and spleen showed modest responses in comparison. Comparison between resistance and susceptible samples have highlighted genes of interest for further studies, for instance those related to ubiquitination or the inflammasome.

**Keywords:** *Salmo salar*, RNA-seq, Aquaculture, Fish, ISAV, Disease resistance

## Background

Atlantic salmon (*Salmo salar*) is a valuable fish species farmed in several countries worldwide, and plays a major role supporting the economies of many rural communities. However, the sustainability of the industry is currently threatened by infectious diseases, which can cause major economic losses. One of the most threatening diseases for salmon farming is infectious salmon anaemia

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(ISA), caused by the infectious salmon anaemia virus (ISAV) [1]. ISAV is listed by the OIE [2] as a notifiable disease and classified as category C+D+E disease by the European Union Reference Laboratories (EURL) [3]. This implies an active surveillance of the presence of the virus in fish farms and culling of stocks upon detection to avoid the transfer of the virus to other farms. Nonetheless, ISAV outbreaks have occurred in many salmon-producing countries, with the 2009 outbreak in Chile being particularly devastating, causing production losses of 75% [4–8]. ISAV belongs to the Orthomyxoviridae family and therefore is related to Influenza viruses [9]. The entry port of ISAV seems to be multiple; the gills are the main tissue of entry, but infection through the skin and pectoral fin is also possible [10, 11]. In Atlantic salmon this virus causes severe anaemia and haemorrhages, result of damage to the endothelial cells in peripheral blood vessels of all organs, which eventually leads to the death of the animal [4].

Nowadays, control of the disease mainly relies on farm surveillance and restriction of fish movements in infected/suspected farms. Some vaccines against ISAV have been developed, and they are extensively used in affected countries, however they do not confer full protection against the disease and therefore affected farms still have to isolate and cull their fish [2, 12]. A potential alternative is to produce stocks that are resistant to ISAV, either through selective breeding or genome engineering. Understanding molecular pathway and discovering functional genes involved in resistance / susceptibility to ISAV can significantly contribute to genomic selection and it is a necessary step to identify suitable targets for genome editing [13].

Previous *in vivo* studies on ISAV infection in Atlantic salmon have identified host genes potentially associated to resistance, such as *hivep2* and *TRIM25* [14, 15]. However, resistance to diseases tends to be multifactorial in nature, involving different biological pathways and complex organism-level responses that determine the balance in the host-pathogen relationship. In a previous study, we studied the response of Atlantic salmon to ISAV infection in the heart of resistant and susceptible fish. The results showed the down-regulation of the complement and coagulation pathway in infected fish compared to non-infected fish, and highlighted *TRIM25* as a potential good candidate for resistance to ISAV [15]. To have a better understanding of the fish systemic response during ISAV infection we have expanded our RNA sequencing study to three additional tissues: gills, head kidney and spleen. These tissues were selected due to their role in ISAV infection; head kidney and spleen are the main fish immune organs, while the gill is a key immune barrier and the main point of entry of ISAV. The transcriptomic

response of these Atlantic salmon tissues to infection was assessed, and genetically resistant and susceptible animals were compared to better understand the genomic basis of resistance to ISAV.

## Results

A total of 24 head kidney, 24 spleen and 24 gill samples were RNA sequenced (3'mRNA tag libraries), producing an average of 13M reads per sample. Principal component analyses showed a clear clustering of the samples of each tissue, but within each tissue no separation was observed between control and infected samples (Fig. 1A).

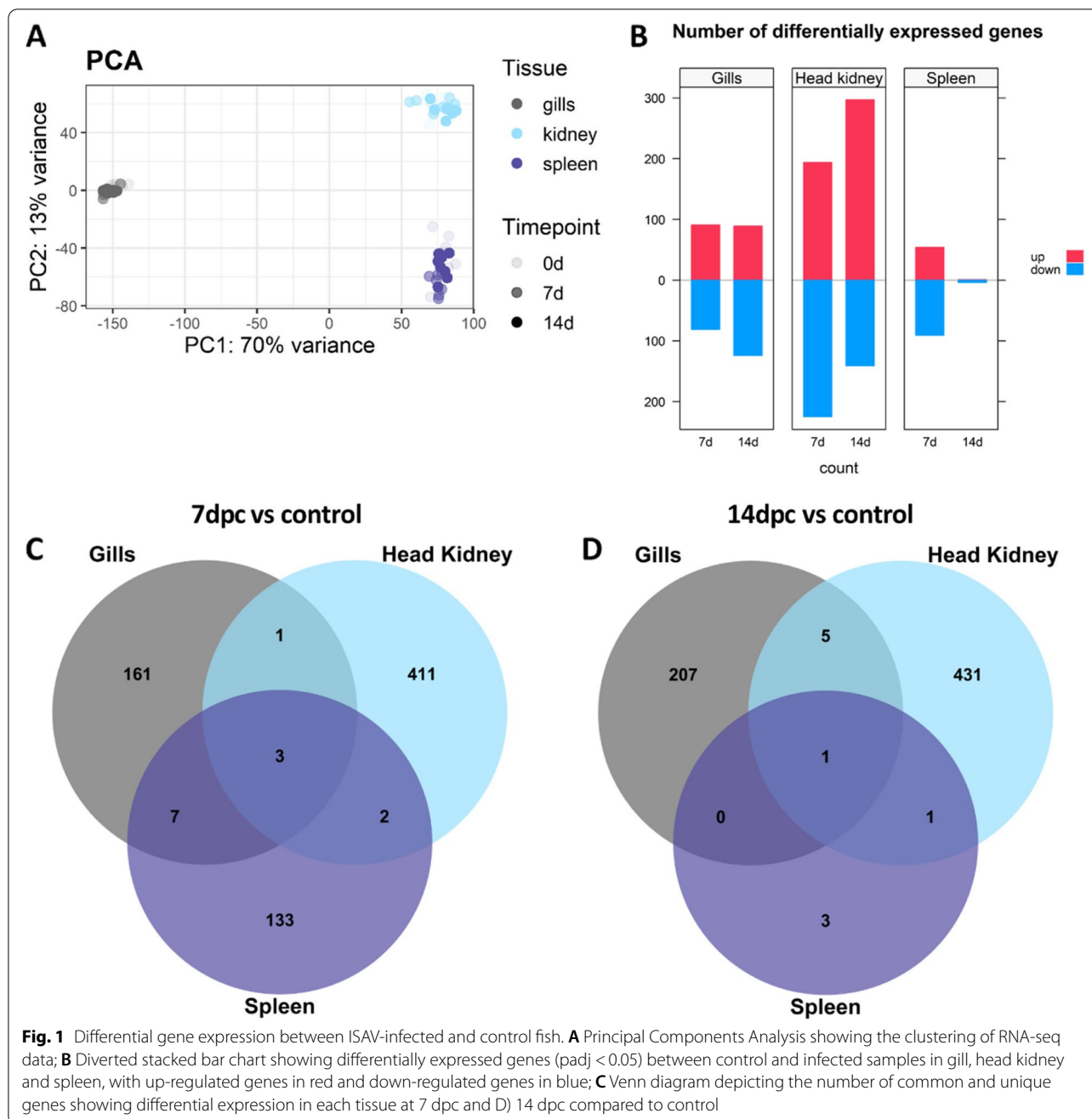
### Differential expression analysis

Differential expression analysis between control and infected samples revealed 172, 417 and 145 genes differentially expressed for gills, head kidney and spleen respectively at 7 dpc (Fig. 1B). At 14 dpc, the number of differentially expressed genes is similar for gills and head kidney with 213 and 438 genes respectively, however in spleen only 4 genes were differentially expressed (Fig. 1B). Generally, a similar number of up- and down-regulated genes were observed in each comparison, except for head kidney 14 days post challenge where a larger number of up-regulated genes were observed (Fig. 1B).

The differentially expressed genes are mostly organ-specific, however a small number of differentially expressed genes are common across the three tissues (Fig. 1C-D). There are 3 common genes at 7 dpc (Peptidyl-prolyl cis-trans isomerase FKBP5, FKBP5; Diencephalon/mesencephalon homeobox protein 1-B, *DMBX1* and Serine protease 23) and just 1 at 14 dpc (Ankyrin repeat domain-containing protein *SOWAHC*, *SOWAHC*). Both *FKBP5*, an immunophilin, and *DMBX1*, a transcriptional repressor, were up-regulated at 7 dpc (Fig. 2A-B), while Serine protease 23 is down-regulated at 7 dpc (Fig. 2C). Finally, *SOWAHC* is part of the ankyrin repeat domain (*ANKRD*) family which mediates protein interactions and is down-regulated 14 dpc (Fig. 2D).

### Response to ISAV in Atlantic salmon gills

In the gills, 172 and 213 differentially expressed genes were observed at 7 and 14 dpc respectively, with 46 of them shared between the two conditions (Fig. 3A). Based on gene ontology (GO) various biological processes (BP) were identified as enriched, with 7 days post challenge showing higher enrichment (Fig. 3B-C). At 7 dpc, processes such as “response to stress”, “protein folding”, “metabolic process”, “immune system process”, “cell cycle” or “autophagy” were enriched amongst down-regulated genes, and at 14 days after the challenge similar processes such as “response to stress” and “immune system process” were still enriched amongst

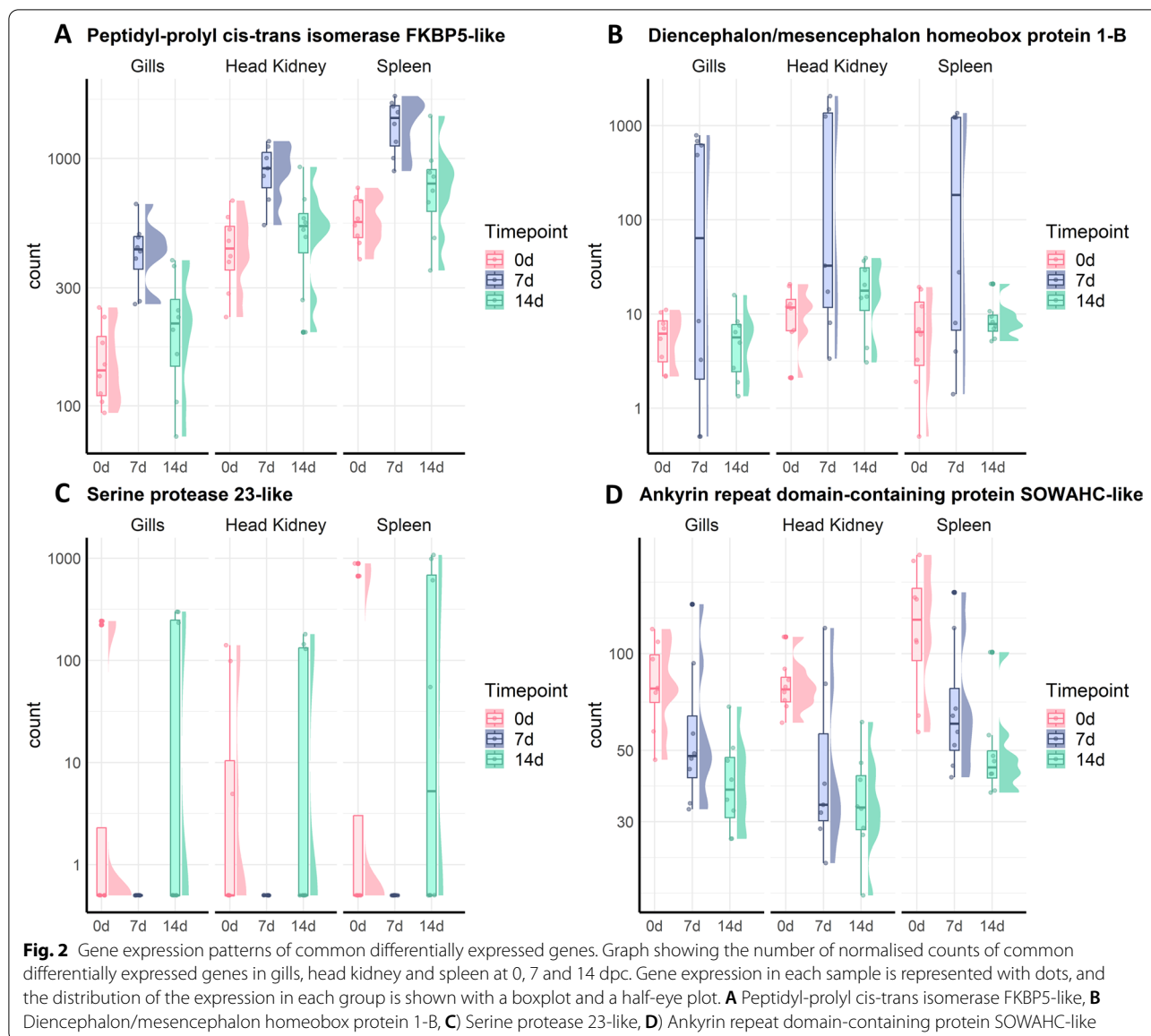


down-regulated genes. This suggests a lack of response to the infection in the gills, although three genes related to major histocompatibility complex II and two C-C motif chemokines were up-regulated at 14 days post challenge (Supplementary file 1).

**Response to ISAV in Atlantic salmon head kidney**

In head kidney, 417 and 438 differentially expressed genes were found at 7 and 14 dpc respectively compared to control fish, with 126 in common between both

timepoints (Fig. 4A). Many biological processes were enriched in the head kidney at both 7 (20 for up- and 24 for down-regulated genes) and 14 (14 for up- and 6 for down-regulated genes) days after the infection (Fig. 4B-C). At 7 dpc the most enriched processes for up-regulated genes include “translation”, “ribosome biogenesis”, “protein targeting” and “catabolic process”, while other interesting terms such as “ribonucleoprotein complex assembly”, “cell death” and “cell cycle” show more moderate enrichment. Similar to the gill results, processes the



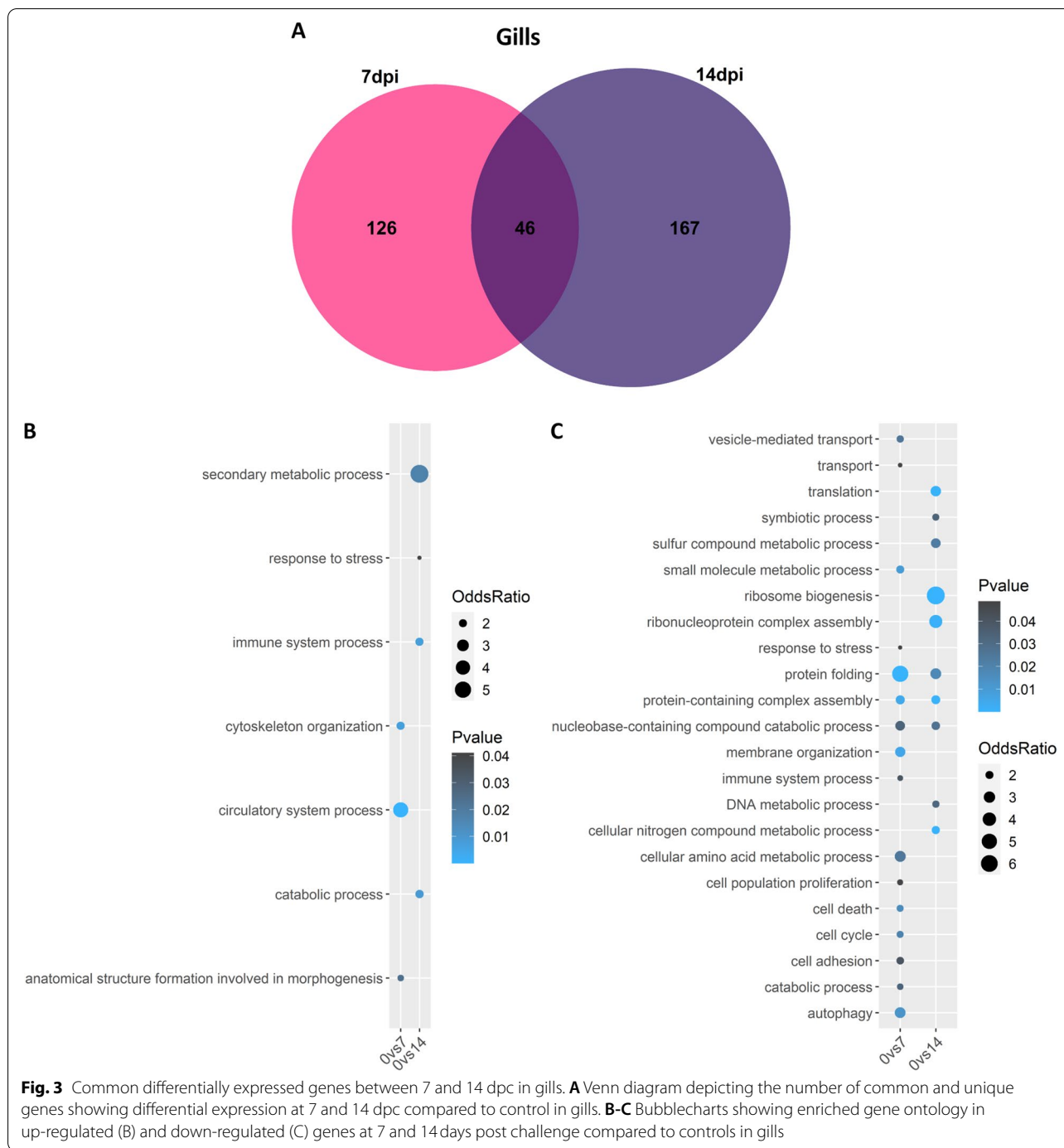
most enriched for down-regulated genes are “response to stress”, “cell division” and “cell cycle”. At 14 days post challenge there were less enriched terms, but for example “ribosome biogenesis” and “ribonucleoprotein complex assembly” were more enriched. Curiously, at both timepoints the cellular component term “ribosome” was the most enriched.

While we did not observe an enrichment of biological process related to immunity, we did observe up-regulation of multiple interferon related genes at both 7 (interferon alpha/beta receptor 1a,  $\log_{2}FC = 0.99$ ) and 14 days post challenge (interferon-induced GTP-binding protein Mx,  $\log_{2}FC = 3.59$ ; interferon-induced protein 44,  $\log_{2}FC = 2.01$ ; interferon regulatory factor 7,  $\log_{2}FC = 1.63$ )

(Supplementary file 2). Genes related to the NLR pathway such as proteins NLRC5 ( $\log_{2}FC = 1.46$ ) or protein NLRC3 ( $\log_{2}FC = 0.95$ ) were also up-regulated at 14 dpc.

#### Response to ISAV in Atlantic salmon spleen

In the spleen only 145 and 5 genes were differentially expressed at 7dpc and 14dpc respectively, with no common genes between both conditions. Enriched biological processes in the spleen do not show an obvious connection to viral infection (e.g. “nitrogen cycle metabolic process” or “cell adhesion”; Fig. 5A-B). On the other hand, the enriched terms for down-regulated genes at 7 dpc include “response to stress”, “protein folding”, “cell death” and “cell cycle” (Fig. 5C; Supplementary file 3).

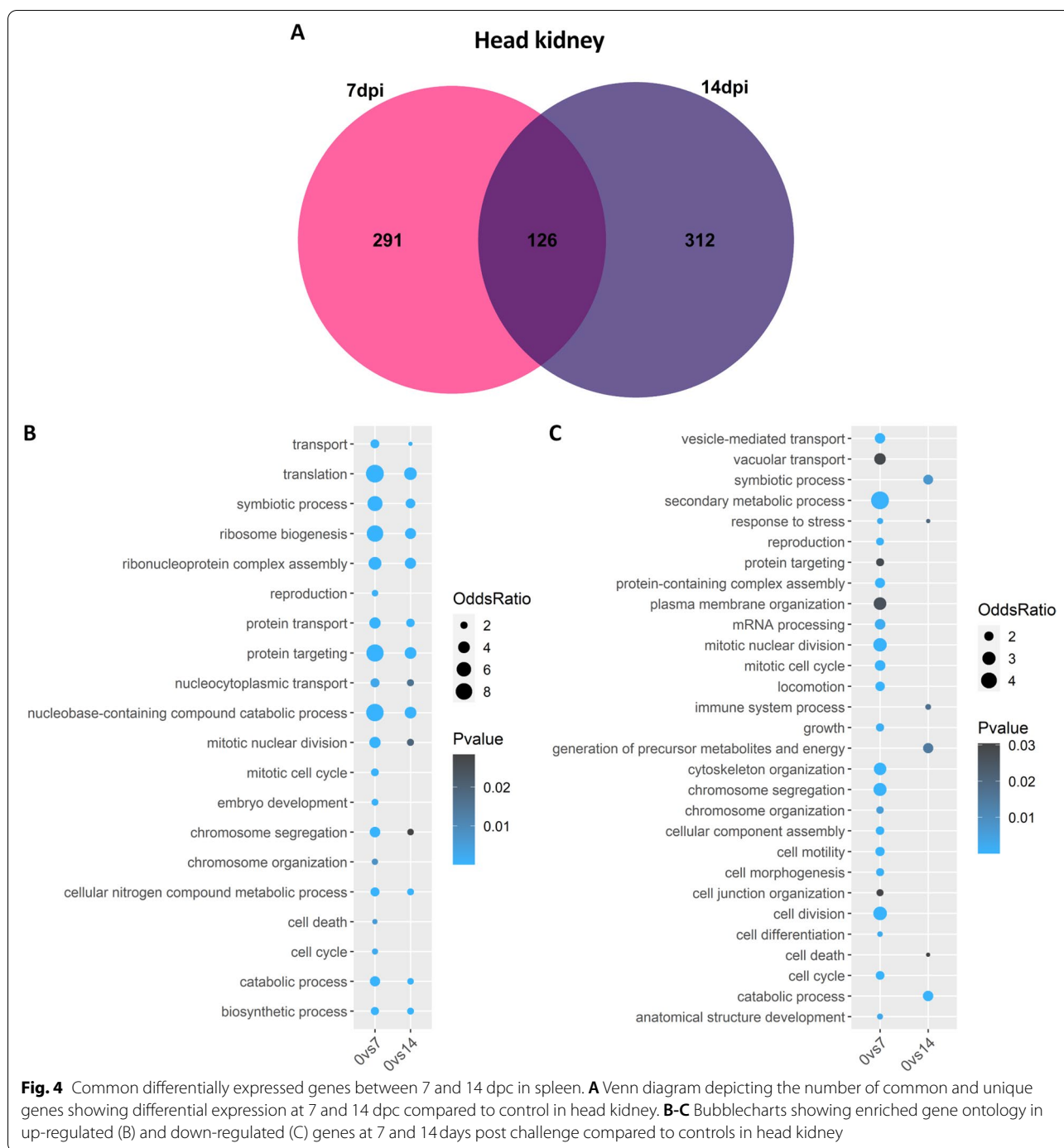


**Genomic signatures of resistance to ISAV in gill, head kidney and spleen**

At each time point, four fish were classified as resistant and four as susceptible based on their individual EBVs and family mortalities. The average GEBVs of resistance to ISAV for the resistant and susceptible groups were 0.05 (range -0.01 to 0.13) and 0.41 (range 0.28 to 0.54, respectively, with average family survival rates of 64 and

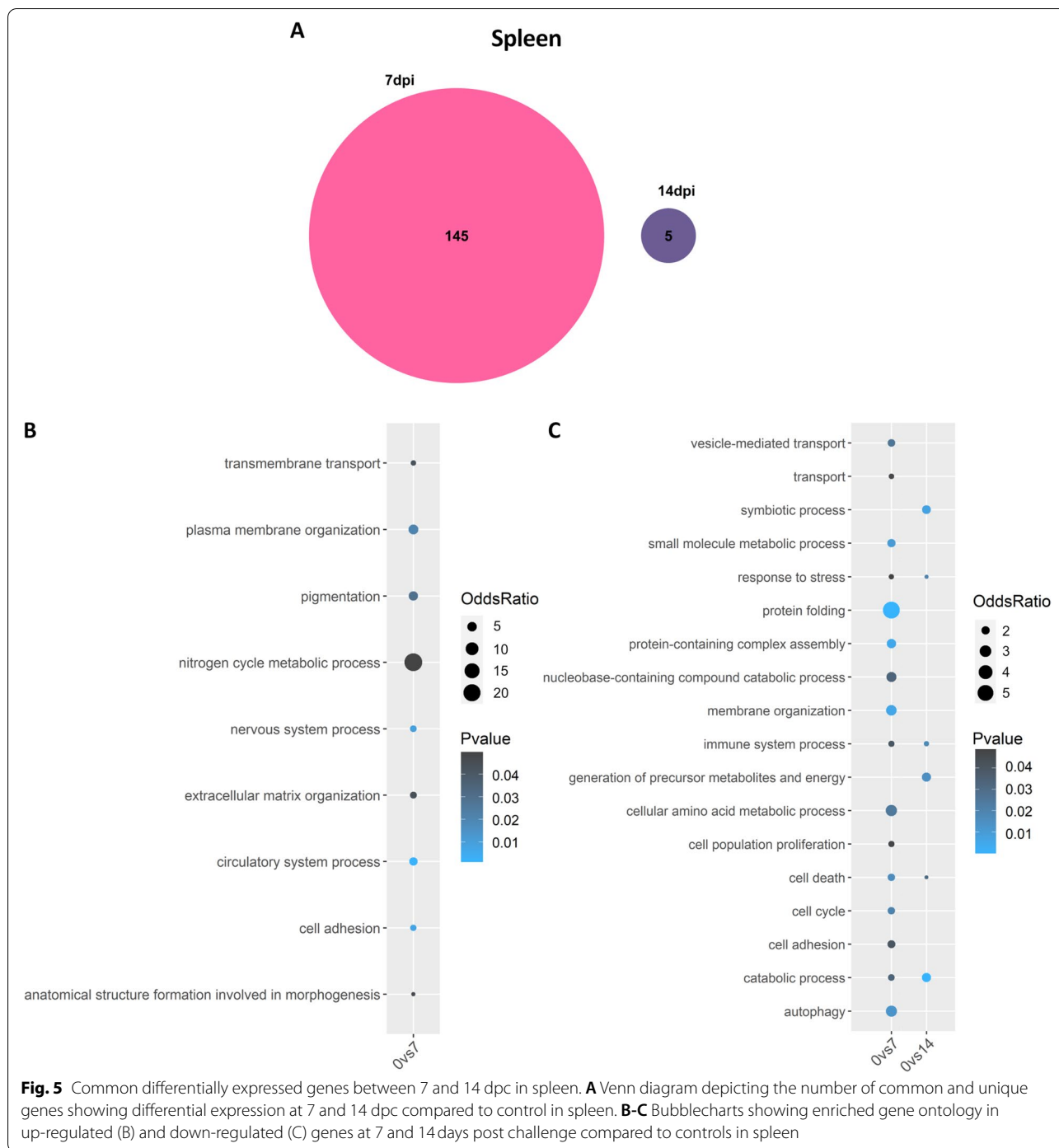
17% for each group (full description and methods in [15]). The transcriptomes of resistant and susceptible fish were compared for each tissue and timepoint (4 resistant vs 4 susceptible fish).

A small number of differentially expressed genes between resistant and susceptible samples were found in the gills (8-17 DEG per timepoint, Fig. 6 and Supplementary file 4). Some of those genes are related to



the immune response. For instance, NACHT, LRR and PYD domains-containing protein 1-like (NLRP1), a key component of the inflammasome, is more expressed in susceptible samples at 7 dpc ( $\log_{2}FC = -2.8$ ). Also at 7 dpc, phospholipase A1 member A-like isoform X3 (PLA1A), involved in type I IFN production [16], is more expressed in resistant samples ( $\log_{2}FC = 5.7$ ). At

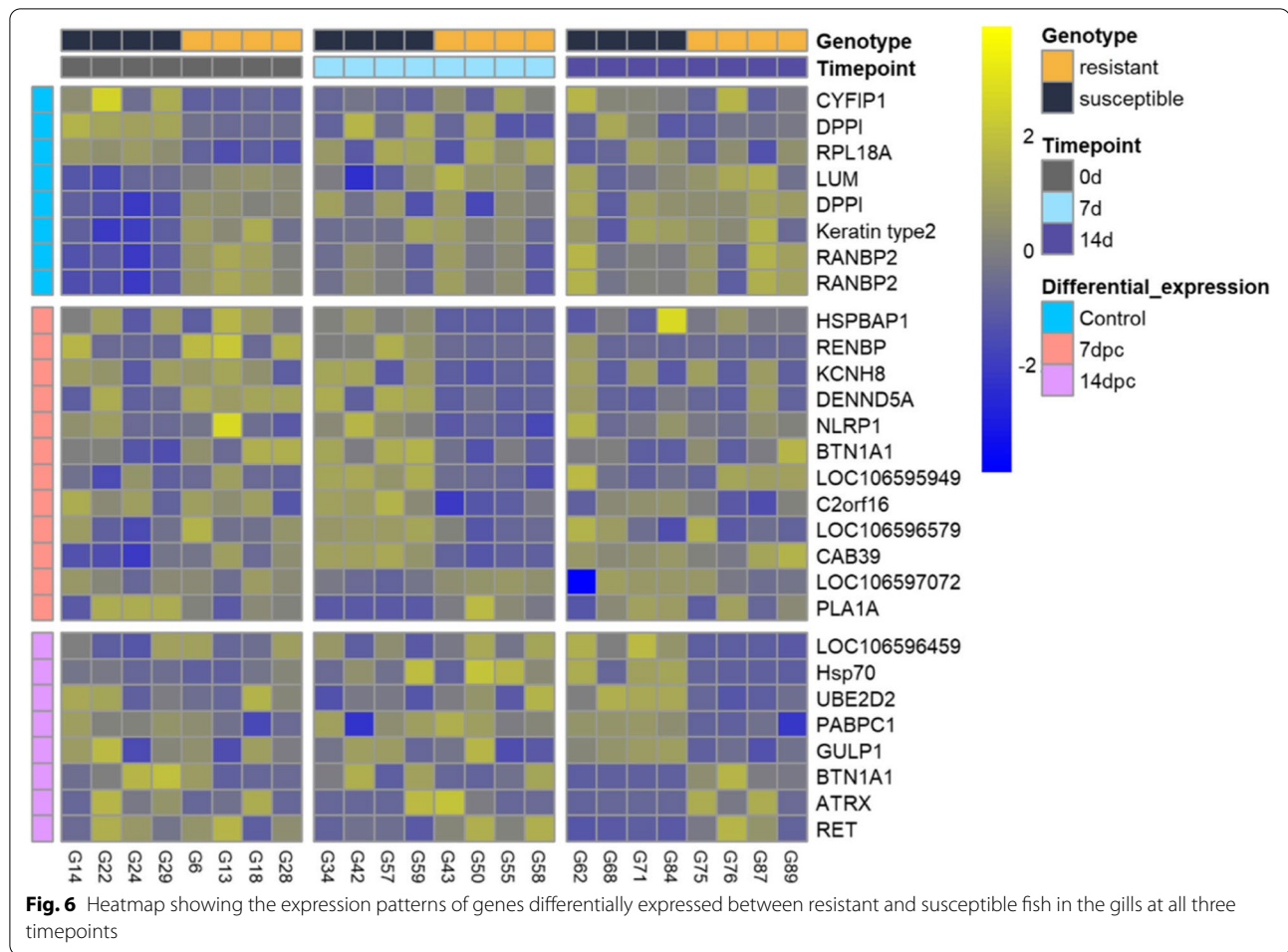
14 dpc, some differentially expressed genes are involved in response infections, such as transcriptional regulator ATRX-like (ATRX,  $\log_{2}FC = 6.4$ ), which plays a role in the maintenance of herpes simplex virus heterochromatin [17, 18]; the viral heterochromatin is formed during the lytic infection, where nucleosomes are assembled on the viral DNA and act as an epigenetic barrier to viral



gene expression [19–21]. Polyadenylate-binding protein 1-like (PABC1) is less expressed in resistant fish at 14 dpc ( $\log_{FC} = -0.9$ ), the cellular distribution of this gene is altered in various viral infections [22]. A gene involved in ubiquitination, ubiquitin-conjugating enzyme E2 D2 (UBE2D2), is also less expressed in resistant fish ( $\log_{FC} = -0.95$ ).

In head kidney, only 4 and 1 genes were differentially expressed between resistant and susceptible samples at 0 and 14 dpc, respectively. However, at 7 dpc a total of 152 genes were differentially expressed (Supplementary file 5). Interestingly, of those 152 genes only three genes were more expressed in resistant samples, with one of them being particularly interesting, nedd4-binding





protein 2-like 1 a protein (N4BP2L1), which is involved in ubiquitination/neddylation [23, 24]. Most of the genes less expressed in resistant samples were involved in pathways related to the cytoskeleton or endosome, both important cellular machineries used by virus for its intracellular transport.

Finally, in spleen a large number of genes were differentially expressed between resistant and susceptible samples at 0 dpc (264 genes), but not at 7 and 14 dpc (11–8 DEG) (Supplementary file 6). Those genes were mostly related to hemoglobin and ribosomes. At 7 dpc, terminal uridylyltransferase 7-like (TUT7), known to reduce Influenza A virus replication in early stages by inducing uridylation of mRNA and lead to his degradation [25], was more expressed in resistant samples ( $\log_{2}FC = 1.2$ ). An inhibitor of phospholipase A2 (phospholipase A2 inhibitor 31 kDa subunit), a gene that can act as a regulator of the inflammation process [26–28], was significantly up-regulated in resistant fish at 14 dpc ( $\log_{2}FC = 4.4$ ).

## Discussion

We have performed a multi-tissue RNA sequencing experiment to complement our previous work on the heart response to ISAV and gain a systemic view of the response of Atlantic salmon to this viral infection. ISA is a disease affecting the whole organism, where the virus circulates through the body of the fish using the blood vessels, multiplying in the epidermis of multiple tissues [29, 30]. In addition to the systemic immune response, each tissue can respond differently to the virus, and therefore a multi-tissue approach is important to understand this host-pathogen interaction. The three tissues studied in this experiment, gills, head kidney and spleen, were selected due to their involvement in the immune response and / or the entry of the virus into the fish.

The transcriptomic changes observed in these three tissues were milder than those observed in the heart in our previous study [15]. This is consistent with the pathogenesis of the virus, with an incubation period of 10–20 days [29]. Our results suggest that by day 15 ISAV has not spread throughout the whole fish, and that it has

managed to limit a system immune response. In fact, the virus was only detected in the gills and spleen of 2 fish and in the head kidney of one fish at 14dpi. Nonetheless, the observed response to ISAV was markedly different in the three selected tissues, with a small number of common differentially expressed genes. Two of those genes could have an important role in the response to ISAV: FKBP5 and serine protease 23. Immunophilins such as FKBP5 have been previously reported to participate in viral replication during some infections such as HIV-1 [31]. Regarding serine protease 23, ISAV possesses a fusion glycoprotein (F) and an hemagglutinin esterase (HE), both necessary for viral attachment and internalization into salmon cells [32, 33]. Influenza A has to be cleaved for the virus to enter the cell, and that is done by a serine protease secreted by the host cell [33].

Our results show that head kidney displays a higher number of differentially expressed genes than gills and spleen. Head kidney is involved in haematopoiesis and cells found in this organ are capable of various immune functions, such as phagocytosis and antigen processing [34]. The head kidney showed up-regulation of immune genes at both 7 and 14 dpc, particularly of interferon genes (*irf2*, *irf4* and *irf7*) and the antiviral response triggered by the NLR pathway, highlighting the key immune function of this organ. These results contrast with those found in the heart, where despite the larger number of up-regulated genes, up-regulation of the interferon pathway was not observed until 14 dpc [15]. A previous study showed similar results during early infection, with a more important up-regulation of interferon genes in the head kidney than in other tissues [35]. However, the interferon response was previously shown to be up-regulated also in other tissues, for instance in liver [35].

The difference observed during the infection between head kidney and the other two tissues (and the heart) is probably linked to their distinct immune function [36]. The spleen is the secondary lymphoid organ in teleost fish with an abundance of macrophages, responsible of erythrophagocytosis in early infection with ISAV [36, 37]. The increase of cell adhesion in spleen can potentially be related to the phagocytosis activity of macrophages as previously reported [37]. In gills, genes related to the major histocompatibility complex II (MHC II) and chemokine signalling were up-regulated. Previous studies during early ISAV infection have not shown an induction of MHC II, but up-regulation of MHC I has been reported [35]. Our results suggest that the gills not only act as the first barrier against ISAV, but that they are also capable of initiating specific immune responses against this pathogen.

In addition to immune related genes, other interesting regulatory pathways seem to play a role during

early ISAV infection. Two of them are ubiquitination and neddylation, posttranslational modifications that modulate most cellular processes [38–40]. Ubiquitination-related genes were especially up-regulated in the head kidney of infected fish (7 at 7dpc and 15 at 14dpc). Moreover, two genes involved in this process were differentially expressed between resistant and susceptible fish; UBE2D2 was down-regulated in the gill of resistant fish, while N4BP2L1 was up-regulated in the head kidney of resistant fish. N4BP2L1 is involved in neddylation, a process that has been connected to resistance to infectious pancreatic necrosis virus (IPNV) in Atlantic salmon [41]. Nedd4 was also found to promote Influenza virus infection [42]. Additionally, some viruses need to hijack the host ubiquitination process for their own advantage [43, 44], and in fact the infection cycle of the Influenza A virus requires ubiquitination for both cellular entry and replication [45]. Moreover, a previous study has highlighted the interaction of the sORF2 protein of ISAV with ubiquitin and interferon stimulated gene 15 (an ubiquitin-like protein) in cell culture using Atlantic salmon kidney (ASK) cells [46]. However, the molecular mechanisms underlying these interactions are still not known. In head kidney, two copies of the E3 ubiquitin-protein ligase HERC3 were up-regulated in response to the virus at 14 dpc. In our previous study with the same population of fish and in the heart, a gene of the same family (HERC4) co-located with a putative QTL for resistance to ISAV [15]. Additionally, another gene of this family, HERC5, was previously described as an antiviral protein in Influenza virus infection, catalysing ISGylation of NS1 and avoiding its interaction with the antiviral protein kinase R (PKR), which reduces viral propagation [47]. Our previous study in heart also highlighted the E3 ubiquitin ligase TRIM25, up-regulated in resistant fish, as a potential key target for functional studies aiming to develop ISAV-resistant fish. Posttranscriptional modifications seem to play an important role during ISAV infection and it would be interesting to further investigate their role.

Our results also show an up-regulation of ribosomal protein genes in head kidney at both 7 and 14 dpc (e.g. RPS10, RPLP0, RPL15, RPL17 or RPL7). The role of ribosomal proteins (RPs) during viral infection has been investigated in multiple viruses, and interactions between RPs and viral proteins have been described in connection with viral protein biosynthesis as part of the normal replication cycle of the virus [48]. Different viruses prioritise certain RPs to complete their viral cycle. For example, in HIV-1 and white spot syndrome virus (WSSV) viral proteins interact with the ribosomal protein RPL7, while for RPS27a an interaction with a protein of Epstein-Barr viruses (EBV) has been described

[48]. Additionally, many host proteins also interact with the viral ribonucleoprotein complex (RNP) of influenza virus, responsible for viral transcription and replication, and are fundamental for its transport and assembly [49, 50]. In our study, the ribonucleoprotein assembly complex process was up-regulated in head kidney in response to infection at both timepoints, potentially reflecting the hijack of the host machinery by ISAV as part of its infective process.

The comparison of susceptible and resistant fish highlighted certain genes of potential interest for further investigation, in addition to the previously mentioned genes involved in ubiquitination. The largest differences were observed in the head kidney, where interestingly the difference between resistant and susceptible fish does not stem from differences in immune pathways, but mostly a down-regulation of various pathways involved in intracellular transport: cytoskeleton, microtubules and endosomes. Many viruses exploit these cellular processes for cell entry and intracellular transport [51, 52], and endosomes and lysosomes have been previously reported to be the entry way of ISAV into the cell [53]. Their down-regulation in resistant animals may affect viral replication by reducing viral entry and trafficking on infected cells, but it is also possible that susceptible animals simply have a higher expression of these pathways as a consequence of a more severe viral infection. These processes and associated genes require more investigation to validate their role in resistance / susceptibility to ISAV.

In the gills of resistant fish, we observed an increase of the expression of NLRP1 at 7 dpc, a core protein of the inflammasome. Moreover, two other genes involved in the inflammasome were modulated in response to ISAV. Interleukin 1 was down-regulated in gills and caspase 1 up-regulated in head kidney at 7 and 14 dpc respectively when compared to controls. The inflammasome is a key regulator of the host response against pathogens, which can promote cell death to clear infected cells [54, 55]. There are multiple types of inflammasomes (NLRP3, NLRP1, AIM2, NAIP-NLRC4, etc.) which are activated via different pathways, for example the NLRP3 inflammasome is activated by several viral viroporins [56]. Inflammasomes are highly regulated since inappropriate or excessive activation can lead to significant pathology [57]. Further, some viruses such as orthopoxvirus and Influenza virus can inhibit inflammasome signalling [55]. Inflammasomes are understudied in fish and it would be interesting to investigate their role during ISAV infection.

In the spleen, two genes up-regulated in resistant fish seem to be interesting for ISAV resistance. The first one is TUT7, a potent antiviral factor during early stages of RNA virus infection, and its deletion leads to increased IAV and orsay virus mRNA [25]. The other

one is Phospholipase A2 inhibitor (PLA2); two inhibitors of phospholipase A2 were previously found to be up-regulated in fish infected with ISAV showing delayed mortality, but not in early mortalities [27]. Additionally, flavivirus West Nile virus was found to manipulate lipid homeostasis using PLA2 to facilitate its replication [28].

## Conclusions

The transcriptomic analysis of ISAV-infected Atlantic salmon has revealed a complex tissue-specific response. Each tissue responds differently to the infection with the head kidney presenting a high number of immune response related genes compared to gills and spleen. Comparison of genetically resistant and susceptible animals suggests there is not a single clear resistance mechanism, which is consistent with the polygenic nature of ISAV resistance in Atlantic salmon. Our results also reveal that resistance to ISAV may not only be dependant purely on immune pathways and cellular mechanisms, as posttranslational modification or various intracellular transport pathways may also contribute to ISAV resistance. Further validation through functional studies are necessary to explore the importance of these genes and pathways, and reveal the cellular mechanisms underlying resistance to ISAV in Atlantic salmon.

## Methods

### Disease challenge and sampling

The population used for the ISAV cohabitation challenge experiment comprised 2833 parr Atlantic salmon (mean weight  $37.5 \pm 9.2$  g) from 194 nuclear families originating from Benchmark Genetics breeding programme. The challenge experiment and sampling were conducted in the facilities of VESO Vikan (Norway). The disease challenge and sampling protocols were previously described in detail in [15]. Briefly, after acclimation of the fish during 3 week, 300 carrier fish (Atlantic salmon from the same population) were intraperitoneally injected with 0.1 mL of ISAV (Glaesvær, 080411, grown in ASK-cells, 2 passage, estimated titre  $10^6$  PFU / mL [58]) and introduced to the challenge tank with naïve fish. Fish and tanks were monitored on daily basis, mortalities were registered and sampled, environmental parameters were also recorded. The trials ended when the mortality reached the levels near zero. In addition, gills, head kidney and spleen of 30 cohabitation-challenged fish were collected for three timepoint (pre-infection, 7 dpc and 14 dpc – 10 fish per time point) into TRI Reagent (Sigma, UK) and stored at  $-80^{\circ}\text{C}$  until RNA extraction.

### RNA extraction and RNA sequencing

For each timepoint (control, 7 dpc and 14 dpc), 4 resistant and 4 susceptible fish, representing 8 different

families, were selected; fish were classified in resistant / susceptible based on their individual EBVs and family mortalities, as previously described [15]. Gills, head kidney and spleen RNA samples from the same fish were extracted from preserved tissue samples in TRI reagent (Sigma, UK) and RNA extracted following the manufacturer's instructions ( $n = 24$  per tissue; control = 8; 7 dpc = 8; 14 dpc = 8). The RNA pellet was eluted in 15  $\mu$ L of nuclease-free water and quantified on a Nanodrop 1000 spectrophotometer (NanoDrop Technologies) prior to DNase treatment with QuantiTect<sup>®</sup> Reverse Transcription kit (Qiagen). The quality of the RNA was examined by electrophoresis on a 1% agarose gel (Sigma Aldrich), prepared in Tris-Acetate-EDTA (TAE) buffer, stained with 1% SYBR Safe (Sigma Aldrich) and run at 80 V for 30 min. Sample concentration was measured with Invitrogen Qubit 3.0 Fluorometer using the Qubit RNA HS Assay Kit (ThermoFisher Scientific). The 3'mRNA tag-seq libraries were prepared by Oxford Genomic Centre using the poly-A tail as an adapter, incorporating priming site for 1st strand synthesis, followed by RNA template removal. The libraries were sequenced on a Illumina Novaseq6000 with an average of 13.1 M reads (minimum 9.3 M).

### RNA-Seq analyses

Raw reads were quality trimmed using Trimalore v0.6.3. Briefly, adapter sequences were removed, low quality bases were filtered (Phred score < 20) and reads with less than 20 bp were discarded. Trimmed reads were pseudo aligned against the Atlantic salmon reference transcriptome (ICSASG\_v2 Annotation Release 100 [59]) using kallisto v0.44.0 [60]. Transcript level expression was imported into R v4.0.2 [61] and summarised to the gene level using the R/tximport v1.10.1 [62]. Differential expression analysis was performed using R/DESeq2 v1.28.1 [63], and genes with False Discovery Rate adjusted  $p$ -values < 0.05 were considered to be differentially expressed. Gene Ontology (GO) enrichment analyses were performed in R v.3.5.2 using Bioconductor packages GOstats v.2.54.0 [64] and GSEABase v.1.50.1 [65]. GO term annotation for the Atlantic salmon transcriptome was obtained using the R package Ssa.RefSeq.db v1.3 (<https://gitlab.com/cigene/R/Ssa.RefSeq.db>). The over-representation of GO terms in differentially expressed gene lists compared to the corresponding transcriptomes (gills, head kidney or spleen) was assessed with a hypergeometric test. A GO terms was considered enriched if it showed  $\geq 5$  DE genes assigned and a  $p$ -value < 0.05.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-022-09007-4>.

**Additional file 1: Supplementary file 1.** Differential expression between infected and control samples in the gill.

**Additional file 2: Supplementary file 2.** Differential expression between infected and control samples in the head kidney.

**Additional file 3: Supplementary file 3.** Differential expression between infected and control samples in the spleen.

**Additional file 4: Supplementary file 4.** Differential expression between resistant and susceptible samples in the gills.

**Additional file 5: Supplementary file 5.** Differential expression between resistant and susceptible samples in the head kidney.

**Additional file 6: Supplementary file 6.** Differential expression between resistant and susceptible samples in the spleen.

**Additional file 7: Supplementary file 7.** Sample information, including sequencing and alignment statistics.

### Acknowledgements

We thank the Oxford Genomics Centre at the Wellcome Centre for Human Genetics (funded by Wellcome Trust grant reference 203141/Z/16/Z) for the generation and initial processing of sequencing data.

### Authors' contributions

RH, SAM and DR were responsible for the concept and design of this work. BH and AE were responsible for the disease challenge. OG, AB, AP and RG analysed the data. OG, DR and RH drafted the manuscript. All authors reviewed and approved the manuscript.

### Funding

The authors gratefully acknowledge funding from BBSRC (BB/R008612/1, BB/R008973/1), in addition to BBSRC Institute Strategic Programme Grants to the Roslin Institute (BB/P013759/1 and BB/P013740/1).

### Availability of data and materials

RNA sequencing raw reads have been deposited in the NCBI's Short Read Archive (SRS) repository with accession number PRJNA780199. Sample information, including sequencing and pseudoalignment statistics, can be found in Supplementary File 7.

### Declarations

#### Ethics approval and consent to participate

The challenge experiment was performed at VESO Viken with approval from the Norwegian Food Safety Authority, National Assignments Department, approval no. 16421, in accordance with the Norwegian Animal Welfare Act. All methods were carried out in accordance with relevant guidelines and regulations and are reported in accordance with the ARRIVE guidelines.

#### Consent for publication

Not applicable.

#### Competing interests

A commercial organisation (*Benchmark Holdings plc*) was involved in the development of this study. BH and AET work for Benchmark at the time. The remaining authors declare that they have no competing interests.

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Received: 24 March 2022 Accepted: 11 November 2022  
Published online: 28 November 2022

## References

- Thorud K, Djupvik HO. Infectious anaemia in Atlantic salmon (*Salmo salar* L.). *Bull Eur Assoc Fish Pathol.* 1988;8:109–11.
- OIE-Listed diseases 2020: OIE - World Organisation for Animal Health. 2020. <https://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2020/>. Accessed 17 Nov 2020.
- EUR-Lex - 32018R1882 - EN - EUR-Lex. [https://eur-lex.europa.eu/eli/reg\\_impl/2018/1882/oj](https://eur-lex.europa.eu/eli/reg_impl/2018/1882/oj). Accessed 31 Aug 2022.
- Aamelfot M, Dale OB, Falk K. Infectious salmon anaemia – pathogenesis and tropism. *J Fish Dis.* 2014;37:291–307.
- Bouchard D, Brockway K, Giray C, Keleher W. First report of infectious salmon anemia (ISA) in the United States. *Bull Eur Assoc Fish Pathol.* 2001;21:86.
- Bouchard D, Keleher W, Opitz HM, Blake S, Edwards KC, Nicholson BL. Isolation of infectious salmon anemia virus (ISAV) from Atlantic salmon in New Brunswick, Canada. *Dis Aquat Org.* 1999;35:131–7.
- Godoy MG, Aedo A, Kibenge MJT, Groman DB, Yason CV, Grothusen H, et al. First detection, isolation and molecular characterization of infectious salmon anaemia virus associated with clinical disease in farmed Atlantic salmon (*Salmo salar*) in Chile. *BMC Vet Res.* 2008;4:28.
- Rodger HD, Richards RH. Haemorrhagic smolt syndrome: a severe anaemic condition in farmed salmon in Scotland. *Vet Rec.* 1998;142:538–41.
- NJ ML, Dubovi EJ. Chapter 21 - Orthomyxoviridae. In: Fenner's veterinary virology, 5th ed. Boston: Academic Press; 2017. p. 389–410.
- Aamelfot M, McBeath A, Christiansen DH, Matejusova I, Falk K. Infectious salmon anaemia virus (ISAV) mucosal infection in Atlantic salmon. *Vet Res.* 2015;46:120.
- Weli SC, Aamelfot M, Dale OB, Koppang EO, Falk K. Infectious salmon anaemia virus infection of Atlantic salmon gill epithelial cells. *Virology.* 2013;10:5.
- Falk K. Vaccination against infectious Salmon Anemia. In: Fish vaccination: John Wiley & Sons, Ltd; 2014. p. 313–20.
- Houston RD, Bean TP, Macqueen DJ, Gundappa MK, Jin YH, Jenkins TL, et al. Harnessing genomics to fast-track genetic improvement in aquaculture. *Nat Rev Genet.* 2020;21:389–409.
- Dettleff P, Moen T, Santi N, Martinez V. Transcriptomic analysis of spleen infected with infectious salmon anemia virus reveals distinct pattern of viral replication on resistant and susceptible Atlantic salmon (*Salmo salar*). *Fish Shellfish Immunol.* 2017;61:187–93.
- Gervais O, Barria A, Papadopoulou A, Gratacap RL, Hillestad B, Tinch AE, et al. Exploring genetic resistance to infectious salmon anaemia virus in Atlantic salmon by genome-wide association and RNA sequencing. *BMC Genomics.* 2021;22:345.
- Gao X, Chen D, Hu X, Zhou Y, Wang Y, Wu C, et al. PLA1A participates in the antiviral innate immune response by facilitating the recruitment of TANK-binding kinase 1 to mitochondria. *J Innate Immun.* 2018;10:315–27.
- Cabral JM, Cushman CH, Sodroski CN, Knipe DM. ATRX limits the accessibility of histone H3-occupied HSV genomes during lytic infection. *PLoS Pathog.* 2021;17:e1009567.
- Cabral JM, Oh HS, Knipe DM. ATRX promotes maintenance of herpes simplex virus heterochromatin during chromatin stress. *eLife.* 2018;7:e40228.
- Cliffe AR, Knipe DM. Herpes simplex virus ICP0 promotes both histone removal and acetylation on viral DNA during lytic infection. *J Virol.* 2008;82:12030–8.
- Lee JS, Raja P, Knipe DM. Herpesviral ICP0 protein promotes two waves of heterochromatin removal on an early viral promoter during lytic infection. *mBio.* 2016;7:e02007–15.
- Oh J, Fraser NW. Temporal Association of the Herpes Simplex Virus Genome with histone proteins during a lytic infection. *J Virol.* 2008;82:3530–7.
- Massimelli MJ, Majerciak V, Kruhlak M, Zheng Z-M. Interplay between Polyadenylate-binding protein 1 and Kaposi's sarcoma-associated herpesvirus ORF57 in accumulation of Polyadenylated nuclear RNA, a viral long noncoding RNA. *J Virol.* 2013;87:243–56.
- Oberst A, Malatesta M, Aqeilan RI, Rossi M, Salomoni P, Murillas R, et al. The Nedd4-binding partner 1 (N4BP1) protein is an inhibitor of the E3 ligase itch. *Proc Natl Acad Sci.* 2007;104:11280–5.
- Nepravishta R, Ferrentino F, Mandaliti W, Mattioni A, Castagnoli L, Cesareni G, et al. The ubiquitin versus NEDD8 binding preference of NEDD4 binding protein 1 (N4BP1) is based on a mutual conformational perturbation; 2019. <https://doi.org/10.20944/preprints201902.0213.v1>.
- Le Pen J, Jiang H, Di Domenico T, Kneuss E, Kosalka J, Leung C, et al. Terminal uridylyltransferases target RNA viruses as part of the innate immune system. *Nat Struct Mol Biol.* 2018;25:778–86.
- Fenard D, Lambeau G, Valentin E, Lefebvre J-C, Lazdunski M, Doglio A. Secreted phospholipases A2, a new class of HIV inhibitors that block virus entry into host cells. *J Clin Invest.* 1999;104:611–8.
- Jørgensen SM, Afanasyev S, Krasnov A. Gene expression analyses in Atlantic salmon challenged with infectious salmon anemia virus reveal differences between individuals with early, intermediate and late mortality. *BMC Genomics.* 2008;9:179.
- Liebscher S, Ambrose RL, Aktepe TE, Mikulasova A, Prier JE, Gillespie LK, et al. Phospholipase A2 activity during the replication cycle of the flavivirus West Nile virus. *PLoS Pathog.* 2018;14:e1007029.
- Dannevig BH, Mjaaland S, Rimstad E. Infectious Salmon Anemia virus. In: Mahy BWJ, Van Regenmortel MHV, editors. *Encyclopedia of virology.* 3rd ed. Oxford: Academic Press; 2008. p. 89–95.
- Falk K, Gonzalez M. Infection with HPR-deleted or HPRO infectious salmon anaemia virus. In: *Manual of Diagnostic Test for Aquatic Animals 2021;* 2021.
- Hopkins S, Gallay PA. The role of immunophilins in viral infection. *Biochim Biophys Acta Gen Subj.* 2015;1850:2103–10.
- Cook JD, Sultana A, Lee JE. Structure of the infectious salmon anemia virus receptor complex illustrates a unique binding strategy for attachment. *Proc Natl Acad Sci.* 2017;114:E2929–36.
- Ojeda N, Cárdenas C, Marshall S. Interaction of the amino-terminal domain of the ISAV fusion protein with a cognate cell receptor. *Pathogens.* 2020;9:416.
- Fish HB, System I. In: Assenmacher M, Avraham HK, Avraham S, Bala S, Barnett J, Basketter D, et al., editors. *Encyclopedic reference of Immunotoxicology.* Heidelberg: Springer; 2005. p. 242–5.
- Jørgensen SM, Hetland DL, Press CMCL, Grimholt U, Gjøn T. Effect of early infectious salmon anaemia virus (ISAV) infection on expression of MHC pathway genes and type I and II interferon in Atlantic salmon (*Salmo salar* L.) tissues. *Fish Shellfish Immunol.* 2007;23:576–88.
- Björge H, Koppang EO. Anatomy of teleost fish immune structures and organs. *Immunogenetics.* 2021;73:53–63.
- Falk K, Press CMCL, Landsverk T, Dannevig BH. Spleen and kidney of Atlantic salmon (*Salmo salar* L.) show histochemical changes early in the course of experimentally induced infectious salmon anaemia (ISA). *Vet Immunol Immunopathol.* 1995;49:115–26.
- Swatek KN, Komander D. Ubiquitin modifications. *Cell Res.* 2016;26:399–422.
- Yau R, Rape M. The increasing complexity of the ubiquitin code. *Nat Cell Biol.* 2016;18:579–86.
- Zou T, Zhang J. Diverse and pivotal roles of neddylation in metabolism and immunity. *FEBS J.* 2021;288:3884–912.
- Pavelin J, Jin YH, Gratacap RL, Taggart JB, Hamilton A, Verner-Jeffreys DW, et al. The nedd-8 activating enzyme gene underlies genetic resistance to infectious pancreatic necrosis virus in Atlantic salmon. *Genomics.* 2021;113:3842–50.
- Chesarino NM, McMichael TM, Yount JS. E3 ubiquitin ligase NEDD4 promotes influenza virus infection by decreasing levels of the antiviral protein IFITM3. *PLoS Pathog.* 2015;11:e1005095.
- Gu H, Jan FB. Specificity in ubiquitination triggered by virus infection. *Int J Mol Sci.* 2020;21:4088.
- Huang X, Wei S, Ni S, Huang Y, Qin Q. Ubiquitin–proteasome system is required for efficient replication of singapore grouper iridovirus. *Front Microbiol.* 2018;9:2798.
- Rudnicka A, Yamauchi Y. Ubiquitin in influenza virus entry and innate immunity. *Viruses.* 2016;8:293.
- Olsen CM, Markussen T, Thiede B, Rimstad E. Infectious Salmon Anaemia virus (ISAV) RNA binding protein encoded by segment 8 ORF2 and its interaction with ISAV and intracellular proteins. *Viruses.* 2016;8:52.

47. Tang Y, Zhong G, Zhu L, Liu X, Shan Y, Feng H, et al. Herc5 attenuates influenza A virus by catalyzing ISGylation of viral NS1 protein. *J Immunol.* 2010;184:5777–90.
48. Li S. Regulation of ribosomal proteins on viral infection. *Cells.* 2019;8:508.
49. Mayer D, Molawi K, Martínez-Sobrido L, Ghanem A, Thomas S, Baginsky S, et al. Identification of cellular interaction partners of the influenza virus ribonucleoprotein complex and polymerase complex using proteomic-based approaches. *J Proteome Res.* 2007;6:672–82.
50. Yang C, Liu X, Gao Q, Cheng T, Xiao R, Ming F, et al. The nucleolar protein LYAR facilitates ribonucleoprotein assembly of influenza A virus. *J Virol.* 2018;92:e01042–18.
51. Döhner K, Sodeik B. The role of the cytoskeleton during viral infection. In: Marsh M, editor. *Membrane trafficking in viral replication.* Heidelberg: Springer; 2005. p. 67–108.
52. Simpson C, Yamauchi Y. Microtubules in influenza virus entry and egress. *Viruses.* 2020;12:E117.
53. Eliassen TM, Frøystad MK, Dannevig BH, Jankowska M, Brech A, Falk K, et al. Initial events in infectious Salmon Anemia virus infection: evidence for the requirement of a low-pH step. *J Virol.* 2000;74:218–27.
54. Chen I-Y, Ichinohe T. Response of host inflammasomes to viral infection. *Trends Microbiol.* 2015;23:55–63.
55. Lamkanfi M, Dixit VM. Modulation of Inflammasome pathways by bacterial and viral pathogens. *J Immunol.* 2011;187:597–602.
56. Zheng D, Liwinski T, Elinav E. Inflammasome activation and regulation: toward a better understanding of complex mechanisms. *Cell Discov.* 2020;6:1–22.
57. Chavarría-Smith J, Vance RE. The NLRP1 inflammasomes. *Immunol Rev.* 2015;265:22–34.
58. Mjaaland S, Rimstad E, Falk K, Dannevig BH. Genomic characterization of the virus causing infectious salmon anemia in Atlantic salmon (*Salmo salar* L.): an orthomyxo-like virus in a teleost. *J Virol.* 1997;71:7681–6.
59. Lien S, Koop BF, Sandve SR, Miller JR, Kent MP, Nome T, et al. The Atlantic salmon genome provides insights into rediploidization. *Nature.* 2016;533:200–5.
60. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol.* 2016;34:525–7.
61. R Core Team. *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing; 2020.
62. Sonesson C, Love MI, Robinson MD. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research.* 2016;4:1521.
63. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15:550.
64. Falcon S, Gentleman R. Using GOSTats to test gene lists for GO term association. *Bioinforma Oxf Engl.* 2007;23:257–8.
65. Morgan M, Falcon S, Gentlemen R. SEABase: gene set enrichment data structures and methods; 2020.

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