

Original Research Articles

Characterization of ISG56 from grass carp (*Ctenopharyngodon idellus*) and its response to two immune stimuli

Xiaodong Wang^{1,2}, Hong Yuan^{2,3}, Yifang Chen^{2,3}, Dunxue Chen^{2,3a}

¹ Department of Animal Husbandry and Fisheries, Guizhou Vocational College of Agriculture, Guiyang, Guizhou, PR China, ² Guizhou Baiyun Quasipaa spinosa Science and technology small hospital, Guiyang, Guizhou, PR China, ³ College of Animal Science/Key Laboratory of Animal Genetics and Breeding and Reproduction of Plateau and Mountain Animals of Guizhou University, Guiyang, Guizhou, PR China

Keywords: ISG56, TPR, antiviral function, synergistic subtraction effect, *Ctenopharyngodon idellus*

<https://doi.org/10.46989/001c.168765>

Israeli Journal of Aquaculture - Bamidgah

Vol. 78, Issue 3, 2026

Interferon (IFN)-stimulated genes (ISGs) 56 is a prominent antiviral agent and negative regulator of the IFN signaling pathway that specifically binds and discriminates viral RNAs in mammals. However, its role in teleosts is still unknown. In the current study, we successfully identified the ISG56 gene from grass carp (*Ctenopharyngodon idellus*) and investigated its immune function response to poly (I:C) and LPS. In addition, the coordinated pattern of multiple typical ISGs was also determined. The open reading frame (ORF) of the CiISG56 gene was 1299 bp in length and encoded a protein of 432 amino acids. The putative amino acid sequence consisted of 10 complete tetratricopeptide repeat motifs (TPRs). Phylogenetic analysis revealed a relatively distant evolutionary relationship between ISG56 and ISG58 in fish, with ISG56 clearly segregating into two distinct clades among freshwater and marine fish. Furthermore, the CiISG56 gene was found to exhibit constitutive expression across all examined tissues in grass carp, with the spleen displaying the highest level of expression. The expression of the CiISG56 gene was markedly increased in the liver, spleen, and kidney at various time points following stimulation with poly (I:C) or LPS. In addition, upon comparing the RNA expression level of CiISG56 following poly (I:C) stimulation with that after LPS stimulation, ISG56 demonstrated the lowest expression level under Poly (I:C) stimulation. Moreover, ISG56 was included among the genes that displayed high expression levels upon LPS stimulation. This finding implies a refined model in which a small subset of “dominant” ISGs may account for the majority of the inhibitory effects on a given immune response triggered by immune-stimulating substances. Furthermore, following stimulation with poly (I:C) or LPS, a high level of consistency and synergy among multiple ISGs was detected. This discovery indicates that in response to the stimulation imposed by immune stimulants, the eight typical ISGs displayed a coordinated pattern. Taken together, our results will contribute to a better understanding of the immune function of the CiISG56 gene in teleost fish, thereby furnishing valuable insights for disease management in grass carp. These findings may contribute to the development of novel immunostimulants for disease control in aquaculture.

INTRODUCTION

During early developmental stages, fish rely primarily on the innate immune system to resist invasion by endogenous and exogenous pathogens.¹ The interferons (IFNs), as secreted cytokines, have the ability to effectively stimulate the innate immune response and play indispensable roles in antiviral defense, regulation of cell growth, and activation of the immune system.² Upon binding to their respective receptors, IFNs activate the Janus kinase-signal

transducer and activator of transcription signaling pathway (JAK/STAT), thereby inducing the expression of a multitude of interferon-stimulated genes (ISGs) that contribute to the elimination of various pathogens, including viruses.³ In previous investigations, a multitude of ISGs have been identified, and their antiviral functions have been explored.^{3,4} Nevertheless, the focus of researchers has mainly been on identifying individual antiviral ISG effectors and elucidating their mechanisms of action. Several critical aspects have garnered relatively scant attention: the quantity

a Corresponding authors. Dunxue Chen, E-mail: chendunxue@126.com

of ISGs necessary to safeguard cells against a specific virus; whether there exist ISGs with varying levels of antiviral “worth”; and whether ISGs function in concert with one another.⁵ To elucidate these issues, we initially chose a crucial interferon-stimulated gene (*ISG56*) as a representative to dissect its antiviral function. Subsequently, we analyzed the synergistic effects of eight typical ISGs in the context of antiviral activities.

ISG56 has been identified as an effector molecule possessing antiviral properties. It can effectively induce an antiviral state in infected cells and their surrounding cells.⁶ Notably, *ISG56* has been reported in numerous fish species.⁷⁻¹⁰ The *ISG56* demonstrates a high level of conservation across various evolutionary lineages, ranging from mammals to fish.¹¹ The composition of this molecule is distinguished by the presence of multiple tetratricopeptide repeats (TPRs). These TPRs play a pivotal role in mediating protein-protein interactions. For instance, *ISG56* has been shown to have the capacity to bind specific subunits of eukaryotic initiation Factor 3 (eIF3). This binding inhibits the initiation of viral protein translation.¹² Nevertheless, the putative TPR motifs display variability among different species.^{7,13} However, it remained unclear if TPRs demonstrate greater efficacy compared to others, or if there are TPRs that are essential. Generally, in the absence of stimuli, *ISG56* is expressed at low levels. It has been identified in various fish species, including yellow croaker¹⁰ and tongue sole.⁸ Interestingly, LPS can be recognized by TLR4 on the surface of cell membranes and is widely used to model bacterial stimuli,^{14,15} which can induce the expression of members of the IFIT family.¹⁶

Grass carp, *Ctenopharyngodon idellus*, belongs to the family Cyprinidae, order Cypriniformes, and is one of the most important farmed fish in the world due to its rapid growth and high commercial value.¹⁷ Nevertheless, in recent years, plenty of disease outbreaks have occurred in this cultured fish, causing massive economic losses. The present study identified and characterized the *ISG56* gene from grass carp, as well as investigated its mRNA expression patterns in response to Poly (I:C) and LPS stimulation. Additionally, a synergistic effect of multiple typical ISGs was conducted. These findings provide crucial insights for further elucidating the antiviral mechanism of ISGs in fish species.

MATERIALS AND METHODS

EXPERIMENTAL FISH AND SAMPLES COLLECTION

Healthy grass carp with an average weight of approximately 50 ± 5 g were purchased from the experimental farm of Guizhou University, Guizhou, China, and were transported to an indoor culture system (300 L aquaria) for 2 weeks acclimation. The water temperature was maintained at approximately 25 ± 1 °C under natural light and dark conditions. The fish were fed a commercial diet (Haid group Co., Ltd, Guangdong, China. Fully formulated compound pellet feed featuring a protein content of $\geq 30\%$) at approximately 2% of body weight twice daily at 9:00 and 16:30.

After acclimation, the three fish were anesthetized with tricaine methanesulfonate (MS-222, 100 mg/L) for sampling. Gill, liver, spleen, intestine, kidney, muscle, brain, heart, and thymus tissues from three healthy individuals were collected on ice and immediately frozen in liquid nitrogen for ORF clone and tissue-specific expression analysis.

Fish were randomly divided into three groups, namely, the Poly(I:C) group, the LPS group, and the Control group. Each group included three replicates (90 fish in total, 30 fish for each replicate). For the poly (I:C) group, 1 ml of Poly (I:C) solution (Sigma, USA) (0.5 mg/ml) was injected intraperitoneally into the abdomen of the experimental fish. In the LPS group, 1ml LPS solution (0.2 mg/ml) was injected intraperitoneally into the abdomen of the test fish. The Control group was injected with 1ml PBS into the abdomen of the test fish. After injection, three fish of each treatment were anesthetized with MS-222 as mentioned above, and then three selected tissues (liver, spleen, and kidney) were collected from each group at 0 h, 12 h, 24 h, 48 h, 96 h, 120 h and 168 h after post-injection. Throughout the course of the experiment, no abnormal mortalities were detected among the experimental fish. All procedures were carried out on an ice block using sterile scissors. Samples were also immediately stored in liquid nitrogen until further analysis. Fish were not fed during the treatment period. The experimental protocol on fish was conducted according to the guidelines approved by the Animal Ethics Committee of Guizhou University.

GENE CLONING AND SEQUENCE ANALYSIS

Based on the transcriptome sequence of grass carp (Accession number PRJNA759556), specific primers (Table 1) were designed for the amplification of full-length open reading frame (ORF) cDNA sequences corresponding to the *CiISG56* gene. Total RNA was extracted using the Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The integrity and amount of the RNA were checked using an agarose gel (1%) and a spectrophotometer (Nanodrop 2000, Thermo Scientific, USA). First-strand cDNA was synthesized using MonScript RTIII Super Mix with dsD-Nase (Two-step method) (Monad, Suzhou, China), and the products were used as the templates for PCR. The PCR was performed as follows: an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of a denaturing step at 94 °C for 30 s, an annealing step at 57 °C for 30 s, an extension step at 72 °C for 60 s, and a final extension step at 72 °C for 5 min. PCR products were purified using an AxyPrep™ gel extraction kit (Axygen, USA) and cloned into the pMD 19-T vector (Takara, China). Finally, positive cloned vectors were sent to Tsingke (Beijing, China) for sequencing.

The sequence of *CiISG56* was analyzed using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast>), and the conserved domains were predicted using the SMART program (<http://smart.embl-heidelberg.de/>). The ORF of *CiISG56* was obtained using open reading frame finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). Molecular weight and theoretical isoelectric point (pI) were calculated using the ExPASy compute pI/Mw tool (https://web.expasy.org/compute_pi/). The transmembrane region was

Table 1. Primers used to clone the ORFs of ISG56 and to express the eight typical ISGs of *Ctenopharyngodon idellus*.

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
ISG56-ORF1	5'ATGTCTGAGGACAATCTTAAAC3'	5'GCACTTGAGCCCCAGATACA3'
ISG56-ORF2	5'CTGGAGGGACTGAAGATTGG3'	5'TCAATAATCTGTGCTCTCTGTT3'
ISG58-ORF1	5'ATGAGTATGGAACCTGACAGAA3'	5'ACCCCTTGAATGGTGACTT3'
ISG58-ORF2	5'CAGGAATCAAGGGTCTGTGG3'	5'TTAGTGTAAGGACAGCCTGAG3'
ISG56-qPCR	5'CGTCTGGAGGGACTGAAGATTGG3'	5'TTCGTTTCGTTCTTGTAGCACTT3'
ISG58-qPCR	5'CCTGATGACCTCCTTTATTCTG3'	5'CAGACATTCGGTGTAGTGTTTGAT3'
Mx1-qPCR	5'CAAGACGGCACATACAGCCAGAG3'	5'ATGAGTGCCTGTGCTAACATCTA3'
gig2-qPCR	5'CAAGGGAGGCTGCTCAACAAATC3'	5'TCTGCCGTGGGTGACCTTGATAG3'
viperin-qPCR	5'CGAGCGTCAGCATCGTTAGTA3'	5'AATGGCTCTTCTTGCCCTGA3'
ISG15-1-qPCR	5'GCAAGACGGTGTCCCAAA3'	5'GAATGGTGCTTCCAGATGTGAT3'
ISG15-2-qPCR	5'CCTTTTCAAGTGTTCGTCATTA3'	5'CCAGCCTCCAGTTGTATGCC3'
usp18-qPCR	5'CGTTGCTTGGCTCAGGAAAT3'	5'CACCGTCCACCAGGTTTGA3'

Table 2. Local database for evolutionary analysis of ISG56 and ISG58

Species	GenBank No	Species	GenBank No
<i>Ctenopharyngodon idella</i> ISG56	MW884261	<i>Carassius auratus</i> ISG56	AY267212
<i>Paralichthys olivaceus</i> ISG56	KY399812	<i>Anabarrilius grahami</i> ISG56	ROK15781
<i>Epinephelus coioides</i> ISG56	MN150711	<i>Scophthalmus maximus</i> ISG56	AWP10440
<i>Tetraodon nigroviridis</i> ISG56	CAF89887	<i>Takifugu rubripes</i> ISG56	AY303814
<i>Cynoglossus semilaevis</i> ISG56	AIK29209	<i>Lates calcarifer</i> ISG56	QKV26392
<i>Theropithecus gelada</i> ISG56	XM_025396448	<i>Triplophysa tibetana</i> ISG56	KAA0717763
<i>Hippoglossus stenolepis</i> ISG56	XP_035033096	<i>Sus scrofa</i> ISG56	JN621781
<i>Salvelinus alpinus</i> ISG56	XP_023862797	<i>Rattus norvegicus</i> ISG56	NM020096
<i>Odobenus rosmarus</i> ISG56	XM_004412998	<i>Pseudosciaena crocea</i> ISG56	EU200362
<i>Pan troglodytes</i> ISG56	NM_001205347	<i>Carassius auratus</i> ISG58	AY267211
<i>Ctenopharyngodon idella</i> ISG58	MW884262	<i>Triplophysa tibetana</i> ISG58	KAA0725339
<i>Oncorhynchus tshawytscha</i> ISG58	XP_024242234	<i>Oncorhynchus mykiss</i> ISG58	XP_021420478
<i>Chrysemys picta bellii</i> ISG58	XP_005292572	<i>Alligator sinensis</i> ISG58	XP_006038204
<i>Xenopus tropicalis</i> ISG58	NP_001135660	<i>Ictalurus punctatus</i> ISG58	XP_017338228
<i>Fundulus heteroclitus</i> ISG58	JAR42036	<i>Chelonia mydas</i> ISG58	XP_007071654
<i>Anabarrilius grahami</i> ISG58	ROL48445	<i>Bufo bufo</i> ISG58	XP_040295056
<i>Salvelinus alpinus</i> ISG58	XP_023862656	<i>Salmo salar</i> ISG58	ACI34283

predicted using the online TMHMM tool (<https://services.healthtech.dtu.dk/service.php?TMHMM-2.0>). Additionally, a neighbor-joining (NJ) phylogenetic tree was constructed using MEGA 4.1 software. The inferred phylogeny was validated through bootstrap sampling with 5000 replications. This was based on a local database of protein sequences that were downloaded from NCBI databases (Table 2). A putative TPR motif was identified using TPRpred (<http://toolkit.tuebingen.mpg.de/tpred/>). Finally, Sigmaplot 11 software and Clustal 3.0 software were employed separately for analyzing gene expression in rabbits and generating heat maps.

TISSUE EXPRESSION PROPERTIES OF ISG56 AND ITS RESPONSE TO IMMUNE STIMULI

Total RNA extraction and initial cDNA synthesis were conducted following the aforementioned procedures. Quantitative real-time PCR was used to evaluate the mRNA transcription pattern of *CiISG56* in different tissues (gill, liver, spleen, intestine, kidney, muscle, brain, heart and thymus) and the response of *ISG56* in immune tissues after challenge by Poly(I:C) and LPS. The qPCR was conducted using the MonAmp™ ChemoHS qPCR Mix (Monad, China) in 96-well plates in a LineGene 9600 Plus (Bioer, China), and the final volume of each PCR was 20 µL. The amplification conditions were as follows: initial denaturation at 95°C for 2 min, followed by 40 amplification cycles at 95°C for 5

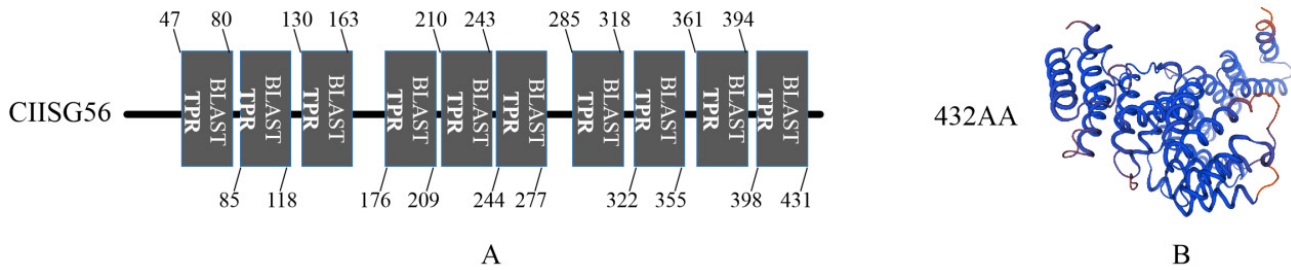


Fig. 1. Structure diagram of grass carp ISG56. A: quantity and position of TPR; B: 3D structural prediction of CiISG56

s and 60°C for 15 s. A non-template control and melting curve were performed to verify that the target PCR product was amplified. The relative expression level of *ISG56* was calculated using the comparative Ct method ($2^{-\Delta\Delta C_t}$). The grass carp β -actin gene was used as an internal reference gene. All reactions were carried out in duplicate.

STATISTICAL ANALYSIS

Data are shown as the mean $\pm$ standard deviation of three replicates in each experimental group. All data were compared using a one-way analysis of variance, followed by Duncan's multiple-range test to determine significant differences between means. $P < 0.05$ was used to indicate a significant difference.

RESULTS

CLONING AND SEQUENCE ANALYSIS OF CiISG56 CDNA

The sequence of *CiISG56* was deposited in GenBank with the accession number MW884261. The cDNA of the *CiISG56* ORF has a length of 1299 bp, ranging from the ATG start codon to the TAG stop codon. The inferred amino acid sequence of *CiISG56* encompasses 432 amino acid residues with a putative molecular weight of 50.2 kDa. The presence of both the signal peptide and the transmembrane domain was not detected in the protein sequence of *CiISG56* using SignalIP 5.0 server and TMHMM prediction software. In silico analysis identified ten TPR motifs formed by residues 47 to 80, 85 to 118, 130 to 163, 176 to 209, 210 to 243, 244 to 277, 285 to 318, 322 to 355, 361 to 394, and 398 to 431 (Fig. 1A). Furthermore, a 3D model prediction of *CiISG56* (with the highest GMQE of 0.9) by SWISS-MODEL is presented, illustrating the helix-turn-helix folds formed by TPRs. The 3D structural prediction reveals that *ISG56* bears the closest structural resemblance to *Sinocyclocheilus anshuiensis* ISG56 (Seq Identity 83.29%) (Fig. 1B).

A comparison of *CiISG56* with other fish indicated that *CiISG56* shares 99.77%, 65.88%, 38.21%, 38.56% and 37.55% amino acid sequence identities with *ISG56* of *C. auratus* (CaISG56, AY267212), *A. grahami* (AgISG56, ROK15781), *P. crocea* (PcISG56, EU200362), *E. coioides* (EcISG56, MN150711) and *P. olivaceus* (PoISG56, KY399812), respectively (Fig. 2). The results indicate that *CiISG56* is less conservative. A phylogenetic tree was con-

structed to elucidate the evolutionary history of vertebrate *CiISG56*, based on the amino acid sequences of five mammalian *ISG56*, three reptilian *ISG58*, two amphibian *ISG58*, ten freshwater fish *ISG58*, eight freshwater fish *ISG56*, and six marine fish *ISG56* (Table 2). The findings are depicted in four detailed figures (Fig. 3), as follows: (1) Evolutionary separation of *ISG56* and *ISG58* in fish indicates a relatively distant evolutionary relationship. (2) *CiISG56* and *CiISG58* exhibit the closest relationship to crucian carp *ISG56* and *ISG58*, followed by kanglang fish and *Triplophysa tibetana*. (3) Mammalian *ISG56* shows convergence with amphibian and reptilian *ISG58*, suggesting partial functional overlap between *ISG56* and *ISG58*. (4) Freshwater fish *ISG56* and marine fish *ISG56* can be distinctly classified into two clades.

TISSUE DISTRIBUTION AND EXPRESSION PATTERN OF THE ISG56 IN RESPONSE TO POLY I:C CHALLENGE

To investigate the tissue expression profile of the *CiISG56* gene, qPCR was used to analyze its transcriptional levels in various tissues under normal physiological conditions. As depicted in Fig. 4A, *CiISG56* mRNA was constitutively expressed in diverse tissues under examination, including gill, liver, spleen, intestine, kidney, brain, and thymus. Notably, higher mRNA levels were detected in the gill, liver, and spleen, while lower levels were found in the remaining tissues. This indicates that the *CiISG56* gene was widely expressed in all the inspected tissues, although higher expression was observed in immune tissues.

When the fish were stimulated with poly (I:C), *CiISG56* gene expression was significantly up-regulated in the liver, spleen and kidney (Fig. 4B, 4C and 4D). Interestingly, in all three tissues, *CiISG56* demonstrated better agreement at 12 h, reaching a peak and a sharp drop at 24 h ($P < 0.05$) and then remained at low levels, with the exception of *CiISG56* in the liver, which had significantly elevated expression at 120 h. The mRNA expression strength of *CiISG56* showed significant tissue differences. The intensity in increasing order was liver, kidney and spleen (372.6-, 274.1-, and 66.0-fold, respectively) at 12 h post-infection.

INDUCIBLE EXPRESSION OF CiISG56 BY LPS

To assess the sensitivity of *CiISG56* to bacterial infection, we conducted an analysis of its expression in three immune

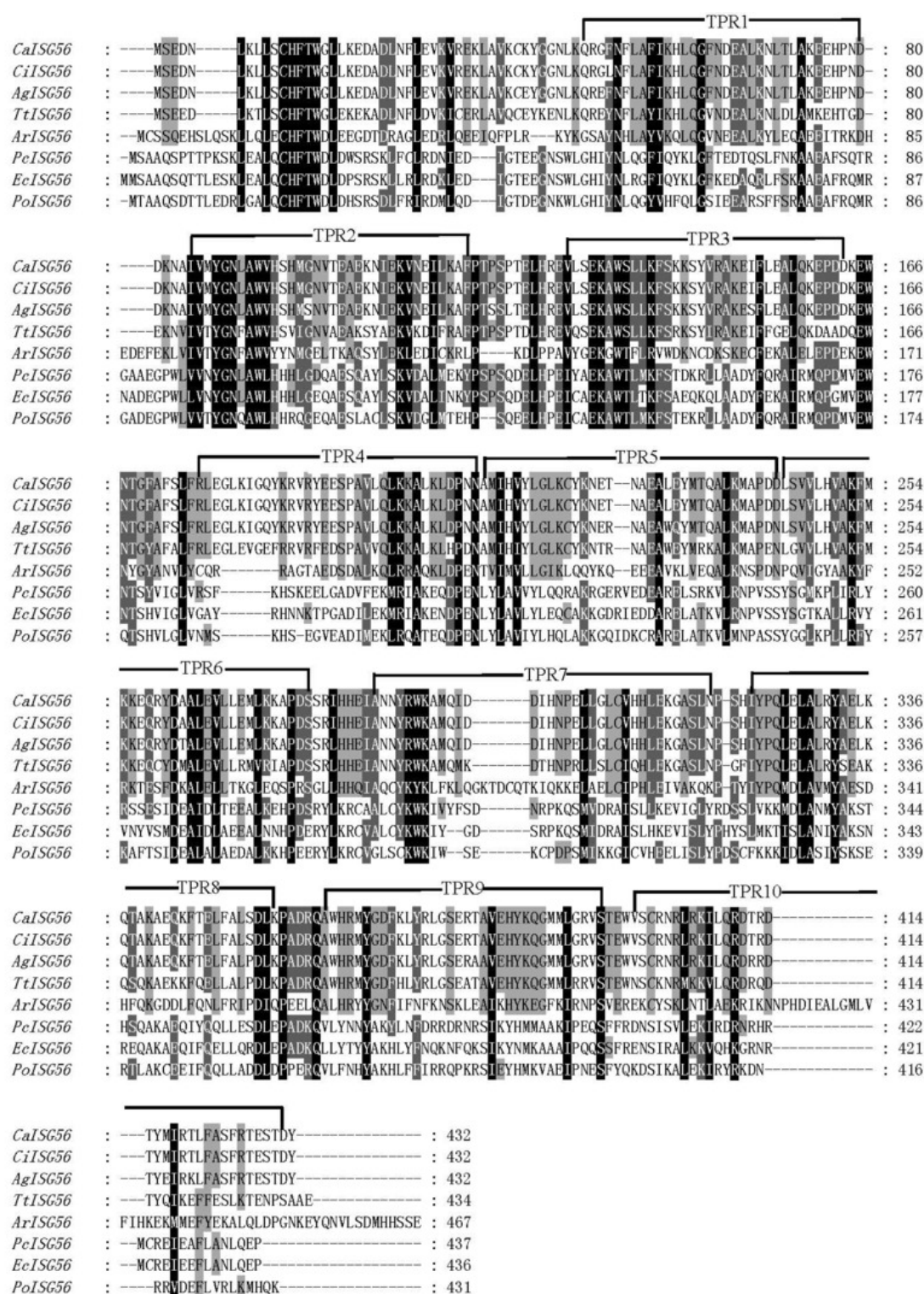


Fig. 2. Alignment of the sequence of *CiISG56* homologs. Dots denote gaps introduced for maximum matching. The number in parentheses indicates the overall sequence identity between *CiISG56* and the compared sequences. Consensus residuals are shown in black, and residues that are 75% identical among aligned sequences are shown in gray. The GenBank accession numbers for the aligned sequences are given in Table 2.

tissues (liver, spleen, and kidney) following stimulation with LPS. The results demonstrated a significant up-regulation in the transcription of *CiISG56* in the three immune tissues following LPS injection, with increased expression levels of *CiISG56* observed at 12-120 h, 24-120 h, and 24-168 h, respectively. The intensity of *CiISG56* expression

in the liver peaked at 49.0-fold at 72 h, while *CiISG56* expression in the spleen ranged from 9.4- to 10.1-fold at 24-48 h and 9.5-fold at 120 h in the kidney (Fig. 5). In addition, two peaks of expression were detected in the kidney, with *ISG56* exhibiting peaks at 48h and 120h, and *ISG58* showing peaks at 48h and 168h respectively. The result sug-

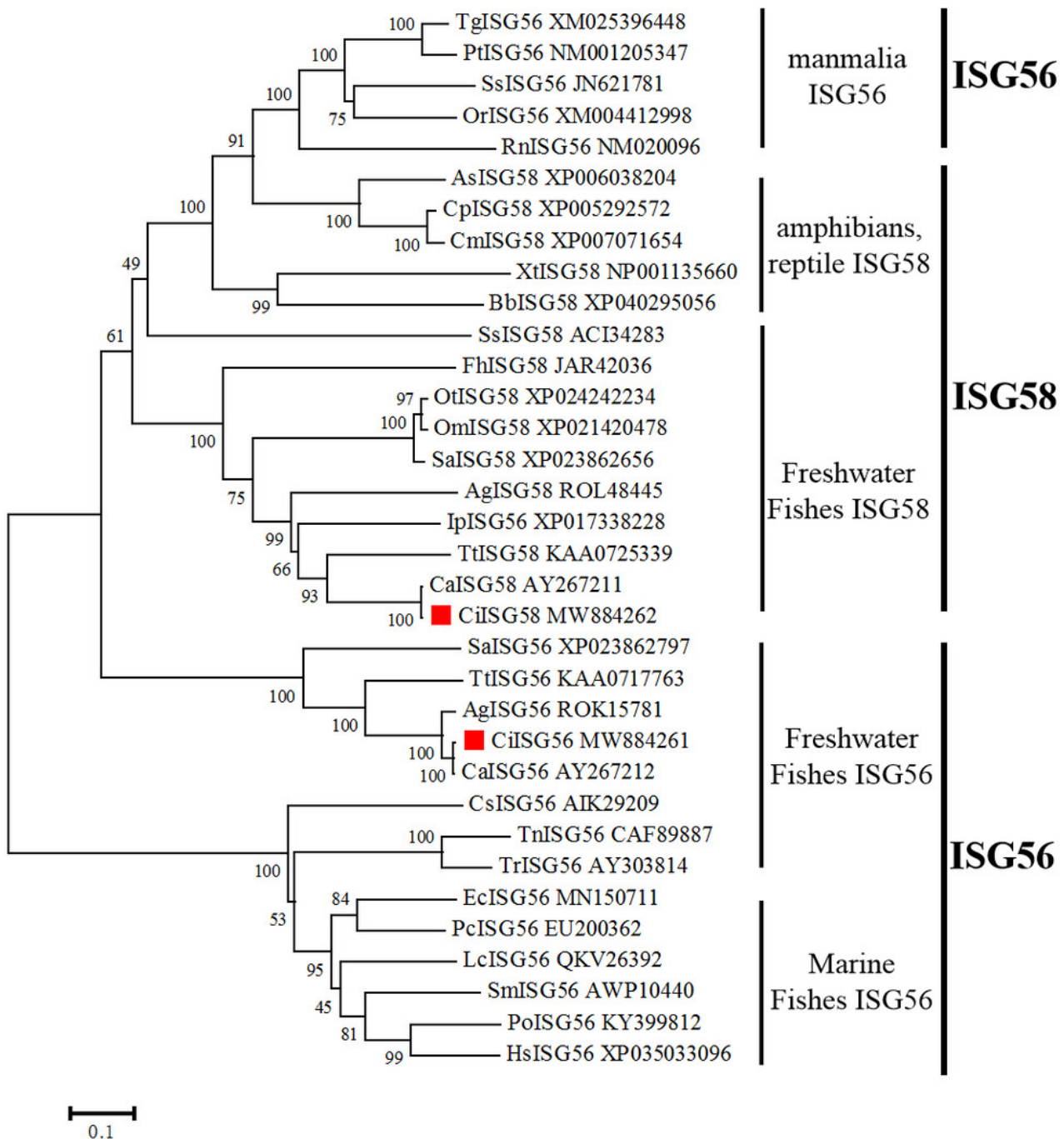


Fig. 3. A neighbor-joining tree was constructed using MEGA 4.0 software based on the protein sequences of ISG56 genes from different species. The numbers at the nodes denote the bootstrap values for 1000 replicates.

gests that *CiISG56* may not only be sensitive to Poly (I:C) but may also occur following treatment by LPS.

SYNERGISTIC SUBTRACTION EFFECTS OF EIGHT TYPICAL ISGS

Eight typical ISGs (*ISG56*, *ISG58*, *gig2* (interferon-induced GTP-binding protein 2), *Mx1*, *ISG15-1*, *ISG15-2*, *viperin*, and *usp18*) were specifically selected for analysis of the synergistic subtraction effect of poly(I:C) and LPS stimulation. The results are succinctly depicted below in six heatmaps illustrating the relative mRNA expression levels (Fig. 6,

Table S1). The co-expression of eight ISGs exhibited a pronounced synergistic effect across all three tissues following poly (I:C) injection, with the simultaneous peak expression of these eight ISGs occurring at 12 hours and subsequently declining rapidly after 48 hours. The IFN response exhibited the highest level of activity in the liver, followed by the kidney and spleen. Among the eight ISGs, *ISG58* demonstrated a significantly high level of gene expression, while *Mx1* and *viperin* showed moderate expression, with the remaining genes being classified as low expressed (*ISG56*, *ISG15-1*, *ISG15-2*, *gig2* and *usp18*). The *ISG58* gene exhibited the highest level of expression at 12 h, with a

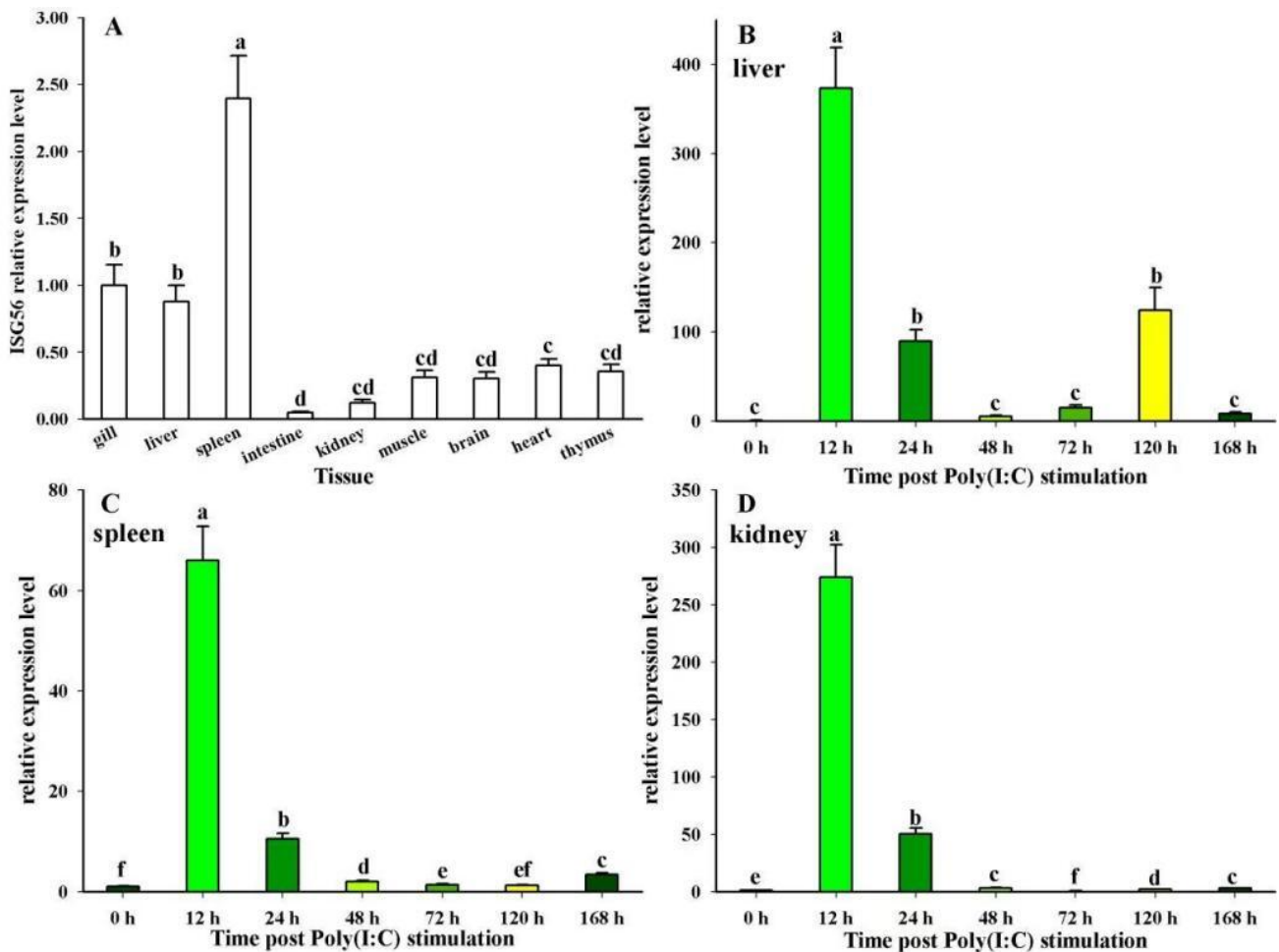


Fig. 4. CiISG56 expression profile. A: The tissue expression difference of CiISG56. B, C, and D: The temporal expression analysis of CiISG56 in the liver, spleen, and kidney post Poly (I:C) stimulation, respectively.

Note: Results were expressed as means $\pm$ SEM. N = 3. P < 0.05.

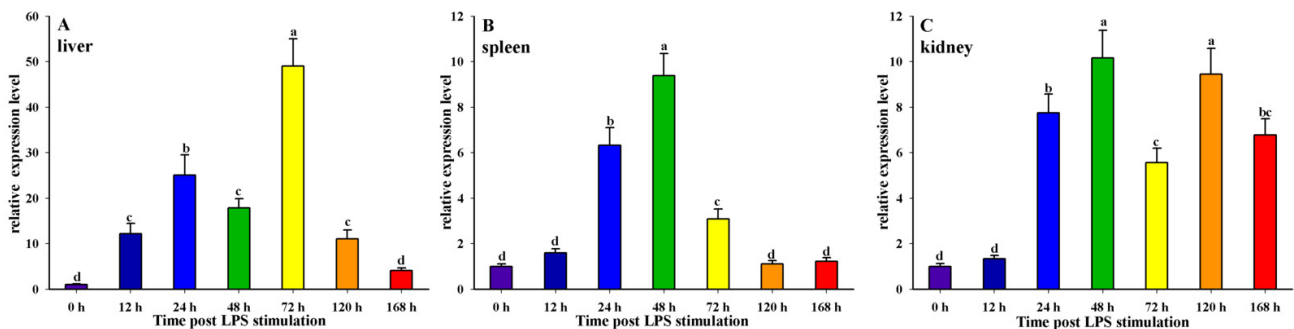


Fig. 5. The temporal expression analysis of CiISG56 in the liver (A), spleen (B), and kidney (C) post LPS stimulation.

Note: Results were expressed as means $\pm$ SEM. N = 3. P < 0.05.

1,942-fold increase, in the liver. Meanwhile, the genes demonstrating the most intense expression at 12 h were *ISG15-2*, showing a 1,610-fold increase in the kidney and a 165-fold increase in the spleen. In addition to the significant down-regulation of the *gig2* gene at 48-168 h post-infection, with expression levels below 1, seven genes exhibited sustained up-regulation. The aforementioned evidence demonstrates that the eight typical ISGs exhibited a robust

response to poly(I:C) stimulation; However, the duration of the antiviral effect was relatively brief, primarily observed within 0-24 hours (Fig. 6A, 6B, and 6C).

During LPS injection, the eight ISGs exhibited unique expression characteristics. In the liver, *ISG56*, *ISG15-1*, *gig2* and *viperin* peaked simultaneously at 24 h and 72 h, respectively, whereas the other four ISGs show only a single expression peak. The antibacterial effects of *ISG56*, *ISG58*,

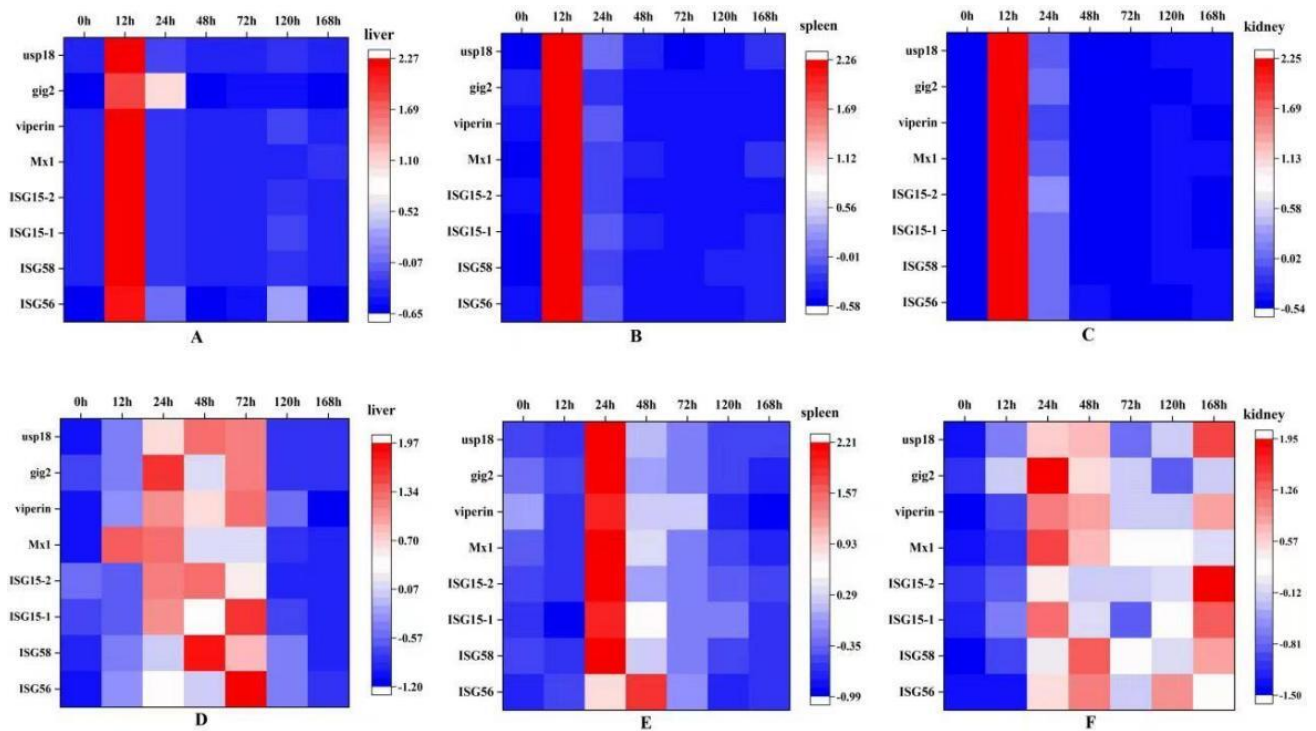


Fig. 6. The expression profiles of eight ISGs in the liver, spleen, and kidney of grass carp post Poly (I:C) and LPS treatment.

viperin and *usp18* were consistently observed throughout the entire duration (0-168 h), as were those of *ISG15-1*, *ISG15-2*, *Mx1* and *gig2* up to 120 h. Among the eight ISGs, *ISG56* exhibited the highest expression levels, followed by *Mx1* and *ISG58*. Conversely, *ISG15-2* was identified as having the lowest expression levels and was predominantly located in the liver. In the spleen, the expression pattern of the seven ISGs showed good agreement, being suppressed at 12 h and then rapidly up-regulated, peaking at 24 h for all except *ISG56*. The expression of the seven ISGs showed continuous down-regulation from 24-168 h, with the exception of *ISG56* which exhibited an initial up-regulation from 0-48 h followed by a subsequent peak. However, later on, *ISG56* also displayed down-regulation similar to the other seven ISGs. The same was true in the liver, where *ISG58* was the most highly expressed, and in the spleen, where the *viperin* expression was the lowest. In the kidney, eight ISGs showed significant up-regulation from 0-48 hours, with *ISG15-1*, *Mx1*, *gig2* and *viperin* reaching their peak expression at 24 hours, while *ISG56* and *ISG58* peaked at 48 hours. Furthermore, *ISG56*, *ISG15-1* and *viperin* demonstrated bimodal expression patterns with peaks observed in both the early (24 hours) and late stages (168 hours) (Table S2). As mentioned above, the expression of eight typical ISGs was upregulated following LPS treatment in the three mainly immune tissues of grass carp. However, there were variations in both the timing and magnitude of these responses among the three tissues. The onset time was dominated by 24-72 h in the liver (Fig. 6D), whereas the ISGs were continuously expressed from start to finish in the kidney (Fig. 6F). In addition, the expression of *usp18*

and 6 ISGs (*ISG58*, *ISG15-1*, *ISG15-2*, *Mx1*, *gig2*, and *viperin*) peaked at the same time (24 h) (Fig. 6E).

DISCUSSION

CHARACTERIZATION OF ISG56 IN GRASS CARP

Multiple studies have demonstrated the antiviral effect of *ISG56* in teleosts, including crucian carp,¹³ yellow croaker,¹⁰ and olive flounder.⁷ In the present study, we have successfully obtained the complete ORF sequence of the *ISG56* gene in grass carp. The *CiISG56* gene encodes a 432-amino acid protein with a predicted molecular weight of 50.2 kDa, which is consistent with that found in other fish species such as olive flounder⁷ and yellow croaker.¹⁰ Meanwhile, the putative *CiISG56* protein contains a typical feature, i.e. TPR motifs,^{11,18} which were highly homologous to those of other bone fishes. The TPR motif composed of 34 amino acid motif folds into a helix-turn-helix, and its function is known to mediate protein-protein interactions and recognize nucleic acids, peptides, and proteins.¹⁹ Interestingly, the number of TPRs domains was variable among the fish species, and the amount of TPRs in freshwater fish is more than that of marine fish. For instance, the representative freshwater fish species such as grass carp, kanglang fish and *Triplophysa tibetana* all contain the 10 TPR motif. Meanwhile, in marine fish, the number of TPR motifs in olive flounder, orange-spotted grouper, yellow croaker, and barramundi is 6, 6, 4, and 4, respectively. It has been reported that freshwater fish species are more exposed to a wide variety of pathogenic microorganisms, compared with marine fish species.²⁰ The freshwater fish

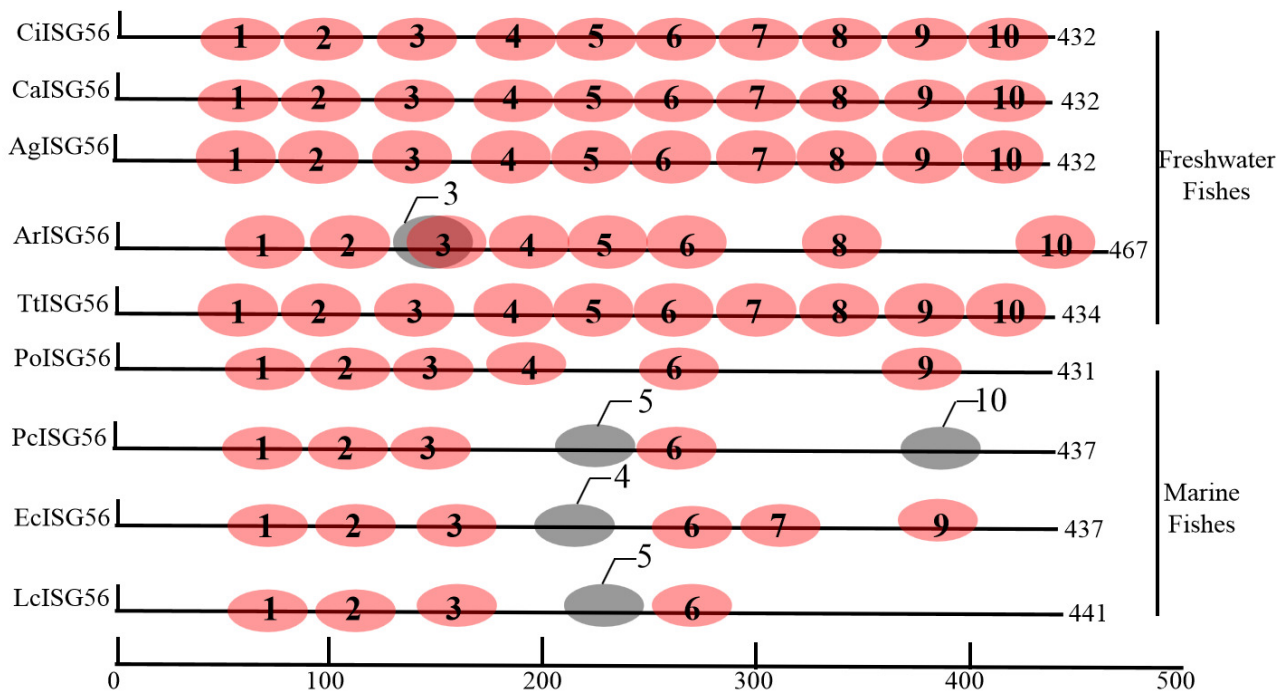


Fig. 7. Tetratricopeptide repeat (TPR) motifs on the ISG56 proteins from nine fish.

Note: CiISG56: *C. idella* ISG56; CaISG56: *C. auratus* ISG56; AgISG56: *A. grahami* ISG56; ArISG56: *A. ruthenus* ISG56; TtISG56: *T. tibetana* ISG56; PoISG56: *P. olivaceus* ISG56; PcISG56: *P. crocea* ISG56; EcISG56: *E. coiooides*; ISG56 LcISG56:

contain more TPR domains, which may aim to better respond to pathogen invasion.⁷

Furthermore, the positions of the TPR motifs were meticulously analyzed across nine representative fish species, encompassing five freshwater fish species and four marine fish species. The results indicated that TPR1, 2, 3, and 6 were detected in both freshwater and marine fish. This finding suggests that these four TPR domains could be the core sequence responsible for the antiviral function in fish. The research findings indicate that there are still certain disparities in the antiviral effect of the same ISG among different species. Nevertheless, additional in-depth investigations are necessary to verify this hypothesis. Whereas TPR5, 8, and 10 are only absent in marine fish species, suggesting that they are optional in marine fish.¹⁰ To verify this prediction, the incomplete TPR domains (SELs) were predicted by TPRpred software. We also found that two predicted SELs in PcISG56 are located at residues 213-248 and 336-401 of the amino acid sequence, corresponding to TPR5 and 10 in grass carp, respectively. A similar phenomenon was observed in other teleosts, for example, an SEL (196-231 AA) in orange-spotted grouper corresponded to TPR4, and an SEL (214-249 AA) in *Lates Calcarifer* corresponded to TPR5 was predicted, according to our hypothesis²¹ (Fig. 7).

PHYLOGENETIC ANALYSIS OF CIISG56 AND CIISG58

To elucidate the evolutionary relationship of vertebrate ISG56, a phylogenetic tree was generated using protein sequences retrieved from local databases (Table 2). Previous studies have reported that fish ISG56 and ISG58 exhibit a relatively distant genetic relationship and that fish ISG56

is distant from mammalian ISG56.^{6,7} In the current study, mammalian ISG56 is clustered with amphibians and reptiles ISG58, suggesting a partial functional overlap between ISG56 and ISG58. However, in the study of CaISG56 and CaISG58, the ITIF family forms a distinct clade to the exclusion of mammalian IFIT proteins,¹³ which was different from our results. This may be due to the small number of ITIFs selected, such as crucian carp ISG56 and ISG58, rainbow trout ISG58, and puffer fish (*Takifugu rubripes*) ISG56. Of the 19 ISG56 and 15 ISG58, CiISG56 shows the closest relationship to CaISG56, followed by AgISG56 and TtISG56, as well as CiISG58.

ISG56 OF GRASS CARP RESPONSE TO TWO IMMUNE STIMULI

It has been reported that ISG56 participates in the antiviral effects of IFNs against numerous viruses in mammals.⁶ In the current study, the expression of CiISG56 mRNA was constitutively expressed in all tissues collected, which is similar to the results in yellow croaker¹⁰ and orange spotted grouper.⁷ Differently, Zhang and Gui¹³ found that the transcript level of crucian carp ISG56 was not detected in crucian carp blastulae embryonic cells,¹³ which may be due to species and/or tissue-specific. The higher basal expression of CiISG56 in immune-related tissues (gill, liver, and spleen) (Fig. 4A) may be related to their roles as primary barriers against pathogens. The gill, as a major site of pathogen entry, showed high expression, suggesting its importance in the first line of defense. Interestingly, lower mRNA levels of CiISG56 were observed in the muscle, as well as in the tongue sole,⁸ yellow croaker¹⁰ and olive flounder,⁷ suggesting that ISG56 may not be involved in

the protection of muscles against viral infection, which requires further investigation.

In a previous study, GCRV infection was shown to induce the expression of *ISG56* in grass carp.⁴ In this study, we identified different expression patterns and showed that *CiISG56* expression was obviously up-regulated after treatment with poly (I:C) and LPS, which is different from the study results of Hwang.⁷ In Hwang's study, *ISG56* of olive flounder could not be induced by LPS stimulation. After stimulation with poly (I:C), *CiISG56* gene expression was obviously up-regulated in the liver, spleen, and kidney, and peaked at 12 h. Notably, the induction time of poly(I:C) is more rapid compared to that of other immune stimuli. Upon infection of the fish with GCRV, the expression of the *CiISG56* gene reached its peak in the liver, spleen, and kidney at 48 h, 96 h, and 12 h, respectively. This peak expression pattern is relatively slow when compared to the response under poly(I:C) stimulation.⁴ Likewise, in CAB cells, Poly(I:C) (48 h) triggered the expression of viperin more promptly compared to GCRV (72 h).²² This is consistent with those of Chen et al.²³ who reported that the expression of *usp18* was more rapid by Poly (I:C) than by GCRV infection.²³ Furthermore, the expression intensity, which was induced by Poly(I:C) was also higher than that induced by GCRV.⁴ Bacterial LPS is an important inducer of gene expression in members of the IFIT family.¹⁶ LPS-induced *IFIT2* expression is dependent on the endogenous secretion of type I IFNs.²⁴ After LPS infection, zebrafish type I IFN (IFN1) and type II IFN (IFN2 and IFN3) showed strong antiviral activity but only type I IFN was able to protect the fish against bacterial infection.²⁵ Transfection of *IRF9* up-regulated the expression of *ISG15* in epithelioma papulosum cyprini cells (EPCs) upon LPS stimulation.²⁶ In our study, the expression of *ISG56* was significantly increased in the liver, spleen, and kidney after LPS stimulation. Similarly, the viruses induced stronger expression of *ISG56* than bacteria in olive flounder.⁷ However, the expression intensity of *ISG56* was significantly weaker in response to LPS than to Poly(I:C) induction. In addition, fibroblasts treated with poly(I:C) have much higher anti-HIV activity than those treated with LPS stimulation.²⁷

SYNERGISTIC EFFECTS OF EIGHT ISGS ON THE ESTABLISHMENT OF ANTIVIRAL STATUS

"How many ISGs are necessary to inhibit a specific virus? Do ISGs function in a coordinated manner? This is an issue that merits significant attention". In recent years, a growing number of interferon-stimulated genes (ISGs) have been identified.²⁸ Nevertheless, researchers have primarily focused on identifying individual antiviral ISG effectors and elucidating their mechanisms of action. To preserve the organism's homeostasis, it is advantageous for the host to trigger the production of several relatively less potent effector molecules. This approach potentially fosters a mild intracellular milieu, as opposed to up-regulating a group of highly potent interferon-stimulated genes (ISGs).²⁹ Generally, the additive effect of two interferon-stimulated genes (ISGs) on antiviral efficacy is more potent than that of a single ISG, regardless of cell type and virus type. Con-

versely, the simultaneous knockout of *ISG20* and *ZAP* proves to be more effective than the single knockout of either *ZAP* or *ISG20* in facilitating the replication of sindbis virus.³⁰

To validate this hypothesis, eight ISGs were selected for investigating their responses to two immune stimuli. The expression levels of eight ISGs all reached their peaks at 12 hours post poly (I:C) injection and subsequently declined rapidly, demonstrating a high degree of consistency and synergy. In the identical case, following LPS treatment, the expression levels of eight typical ISGs were upregulated in the three major immune tissues of grass carp. This phenomenon was likewise noted during GCRV infection.⁴ Similarly, a collectively formidable antiviral defense was identified at 24 hours post-IAV infection in humans. This defense is based on a set of genes including *IFIT2*, *IFIT1*, *ISG15*, *MX1*, and so on.³¹ Furthermore, upon the hosts' infection with African swine fever virus (ASFV), a series of ISGs, such as *IFIT1*, *IFITM1*, *MX1*, *OASL*, *ISG15*, etc., were significantly enriched within the immune system.³² Rezapour et al.³³ demonstrated a consistent activation of ISGs (such as *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1*) across all viruses by utilizing the MAS and RMAS algorithms. This finding proved that the ISGs function in a coordinated manner.³³ Above evidence presented herein indicates that in response to the stimulation of immune stimulants, the eight typical ISGs exhibit a coordinated pattern. On the other hand, the expression intensity in the poly(I:C) treatment was significantly higher than that observed in GCRV infection⁴ and LPS stimulation (in the present study). This phenomenon may be caused by plenty of synthetic dsRNAs from Poly(I:C) compared with GCRV.³⁴ Indeed, the innate immune responses to Poly(I:C) and viruses differ. IFN- α is produced by virus-produced dsRNA stimulation in human plasmacytoid dendritic cells but not by poly (I:C).³⁵

When comparing the expression levels of interferon-stimulated genes (ISGs) in the three immune tissues, *ISG56* was among the highly expressed genes upon lipopolysaccharide (LPS) stimulation. Conversely, it exhibited the lowest expression level under Poly (I:C) stimulation. Our data proposes a refined model of the antiviral interferon response. In this model, a small subset of "dominant" interferon-stimulated genes (ISGs) may account for the majority of the inhibition of a given immune stimulus.⁵ However, these ISGs display varying response intensities and strategies when confronted with different immune stimuli. Likewise, the expression of the same interferon-stimulated gene (ISG) exhibits notable disparities across different tissues. For instance, upon stimulation with LPS, the expression level of *Mx1* is relatively low in the kidneys and spleen, yet it is highly expressed in the liver. Similarly, when stimulated with Poly (I:C), *Mx1* demonstrates a comparable expression pattern. Upon comprehensive analysis, a high-intensity expression of ISGs was detected in the liver. Analogous phenomena have also been observed in mice injected with IFN- β via the caudal intravenous (IV) route,³⁶ and in juvenile zebrafish following injection with IFN- $\alpha 1$.³⁷ Pathogens are known to accumulate in the liver preferentially, and the antiviral response induced by interferon con-

tributes to the control of large-scale infections, suggesting that maintaining a mildly antiviral state in the liver may be beneficial to the host.⁴

CONCLUSION

In summary, we obtained the ORF sequence of *CiISG56* with 10 TPR motifs. The number of TPRs in freshwater fish is greater than that in marine fish. Based on the position of the TPR motifs in grass carp, TPR1, 2, 3, and 6 are the core sequences for antiviral function in fish. The expression of *CiISG56* was found to be constitutive in various tissues examined, with higher levels in immune tissues. Upon stimulation with poly (I:C) or LPS, a high degree of consistency and synergy of expression of eight ISGs was observed with poly (I:C) or LPS stimulation. The results indicated that in response to the stimulation by immune stimulants, the eight typical interferon-stimulated genes (ISGs) displayed a coordinated pattern. *ISG56* was among the genes that exhibited high expression levels upon LPS stimulation. Additionally, *ISG56* showed the lowest expression level under Poly (I:C) stimulation. These data suggest a refined model in which a small subset of “dominant” interferon-stimulated genes (ISGs) may account for the majority of the inhibitory effects on a given immune response triggered by immune-stimulating substances. These findings may contribute to the development of novel immunostimulants for disease control in aquaculture.

ACKNOWLEDGMENTS

This study was supported by the Project of the Basic Research Plan (Natural Science) of Guizhou (MS[2025]009)

AUTHORS' CONTRIBUTION

Formal Analysis: Xiaodong Wang (Lead). Funding acquisition: Xiaodong Wang (Lead). Investigation: Xiaodong Wang (Lead). Supervision: Xiaodong Wang (Lead). Writing – original draft: Xiaodong Wang (Lead). Software: Hong Yuan (Lead). Visualization: Hong Yuan (Lead). Data curation: Yifang Chen (Lead). Methodology: Yifang Chen (Lead). Conceptualization: Dunxue Chen (Lead). Project administration: Dunxue Chen (Lead). Resources: Dunxue Chen (Lead). Validation: Dunxue Chen (Lead). Writing – review & editing: Dunxue Chen (Lead).

COMPETING OF INTEREST – COPE

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL CONDUCT APPROVAL – IACUC

The experimental protocol on fish was conducted according to the guidelines approved by the Animal Ethics Committee of Guizhou University (No:EAE-GZU-2024-T130)

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

Submitted: July 15, 2026 CDT. Accepted: August 17, 2025 CDT. Published: September 24, 2026 CDT.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

1. Mutwiri G, Gerdt V, Lopez M, Babiuk LA. Innate immunity and new adjuvants. *Rev Sci Tech*. 2007;26(1):147-156. doi:[10.20506/rst.26.1.1731](https://doi.org/10.20506/rst.26.1.1731)
2. Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev*. 2001;14(4):778-809. doi:[10.1128/CMR.14.4.778-809.2001](https://doi.org/10.1128/CMR.14.4.778-809.2001)
3. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol*. 2014;32(1):513-545. doi:[10.1146/annurev-immunol-032713-120231](https://doi.org/10.1146/annurev-immunol-032713-120231)
4. Wang X, Chen D, Lv Z, et al. Transcriptomics analysis provides new insights into the fish antiviral mechanism and identification of interferon-stimulated genes in grass carp (*Ctenopharyngodon idella*). *Mol Immunol*. 2022;148:81-90. doi:[10.1016/j.molimm.2022.05.120](https://doi.org/10.1016/j.molimm.2022.05.120)
5. McDougal MB, De Maria AM, Ohlson MB, Kumar A, Xing C, Schoggins JW. Interferon inhibits a model RNA virus via a limited set of inducible effector genes. *EMBO Rep*. 2023;24(9):e56901. doi:[10.15252/embr.202356901](https://doi.org/10.15252/embr.202356901)
6. Zhang Y, Wang Y, Liu Z, et al. Grouper IFIT1 inhibits iridovirus and nodavirus infection by positively regulating interferon response. *Fish Shellfish Immunol*. 2019;94:81-89. doi:[10.1016/j.fsi.2019.08.075](https://doi.org/10.1016/j.fsi.2019.08.075)
7. Hwang JY, Ahn SJ, Kwon MG, Seo JS, Hwang SD, Son MH. Interferon-induced protein 56 (IFI56) is induced by VHSV infection but not by bacterial infection in olive flounder (*Paralichthys olivaceus*). *Fish Shellfish Immunol*. 2017;66:382-389. doi:[10.1016/j.fsi.2017.05.027](https://doi.org/10.1016/j.fsi.2017.05.027)
8. Long H, Sun L. CsIFIT1, an interferon-induced protein with tetratricopeptide repeat, inhibits viral infection in tongue sole (*Cynoglossus semilaevis*). *Fish Shellfish Immunol*. 2014;41(2):231-237. doi:[10.1016/j.fsi.2014.09.006](https://doi.org/10.1016/j.fsi.2014.09.006)
9. Varela M, Diaz-Rosales P, Pereiro P, et al. Interferon-induced genes of the expanded IFIT family show conserved antiviral activities in non-mammalian species. *PLoS One*. 2014;9(6):e100015. doi:[10.1371/journal.pone.0100015](https://doi.org/10.1371/journal.pone.0100015)
10. Wan X, Chen XH. Molecular characterization and expression analysis of interferon-inducible protein 56 gene in large yellow croaker *Pseudosciaena crocea*. *J Exp Mar Biol Ecol*. 2008;364:91-98. doi:[10.1016/j.jembe.2008.07.027](https://doi.org/10.1016/j.jembe.2008.07.027)
11. Fensterl V, Sen GC. The ISG56/IFIT1 gene family. *J Interferon Cytokine Res*. 2011;31(1):71-78. doi:[10.1089/jir.2010.0101](https://doi.org/10.1089/jir.2010.0101)
12. Hui DJ, Bhasker CR, Merrick WC, Sen GC. Viral stress-inducible protein p56 inhibits translation by blocking the interaction of eIF3 with the ternary complex eIF2.GTP. Met-tRNAi. *J Biol Chem*. 2003;278(41):39477-39482. doi:[10.1074/jbc.M305038200](https://doi.org/10.1074/jbc.M305038200)
13. Zhang YB, Gui JF. Identification and expression analysis of two IFN-inducible genes in crucian carp (*Carassius auratus* L.). *Gene*. 2004;325:43-51. doi:[10.1016/j.gene.2003.09.039](https://doi.org/10.1016/j.gene.2003.09.039)
14. Voogdt CGP, Wagenaar JA, van Putten JPM. Duplicated TLR5 of zebrafish functions as a heterodimeric receptor. *Proc Natl Acad Sci USA*. 2018;115(14):3221-3229. doi:[10.1073/pnas.1719245115](https://doi.org/10.1073/pnas.1719245115)
15. Hwang SD, Asahi T, Kondo H, Hirono I, Aoki T. Molecular cloning and expression study on Toll-like receptor 5 paralogs in Japanese flounder, *Paralichthys olivaceus*. *Fish Shellfish Immunol*. 2010;29(4):630-638. doi:[10.1016/j.fsi.2010.06.011](https://doi.org/10.1016/j.fsi.2010.06.011)
16. Fitzgerald KA. The interferon inducible gene: Viperin. *J Interferon Cytokine Res*. 2011;31(1):131-135. doi:[10.1089/jir.2010.0127](https://doi.org/10.1089/jir.2010.0127)
17. Liang X, Zhu D, Li Y, et al. Molecular characterization and functional analysis of peroxiredoxin 4 in grass carp (*Ctenopharyngodon idella*). *Dev Comp Immunol*. 2021;125:104213. doi:[10.1016/j.dci.2021.104213](https://doi.org/10.1016/j.dci.2021.104213)
18. Sen GC, Fensterl V. Crystal structure of IFIT2 (ISG54) predicts functional properties of IFITs. *Cell Res*. 2012;22(10):1407-1409. doi:[10.1038/cr.2012.130](https://doi.org/10.1038/cr.2012.130)
19. Perez-Riba A, Itzhaki LS. The tetratricopeptide-repeat motif is a versatile platform that enables diverse modes of molecular recognition. *Current Opinion in Structural Biology*. 2019;54:43-49. doi:[10.1016/j.sbi.2018.12.004](https://doi.org/10.1016/j.sbi.2018.12.004)
20. Buchmann K. Evolution of Innate Immunity: Clues from Invertebrates via Fish to Mammals. *Front Immunol*. 2014;5:459. doi:[10.3389/fimmu.2014.00459](https://doi.org/10.3389/fimmu.2014.00459)
21. Ma T. *Molecular Characterization and Expression Analyses of Interferon Stimulated Gene 56 (ISG56) and Interferon Stimulated Gene 15 (ISG15) in Epinephelus lanceolatus*. Shanghai Ocean University; 2020.

22. Wang B, Zhang YB, Liu TK, Shi J, Sun F, Gui JF. Fish viperin exerts a conserved antiviral function through RLR-triggered IFN signaling pathway. *Dev Comp Immunol*. 2014;47(1):140-149. doi:[10.1016/j.dci.2014.07.006](https://doi.org/10.1016/j.dci.2014.07.006)
23. Chen C, Zhang YB, Gui JF. Expression characterization, genomic structure and function analysis of fish ubiquitin-specific protease 18 (USP18) genes. *Dev Comp Immunol*. 2015;52(2):112-122. doi:[10.1016/j.dci.2015.05.003](https://doi.org/10.1016/j.dci.2015.05.003)
24. Terenzi F, Saikia P, Sen GC. Interferon-inducible protein, P56, inhibits HPV DNA replication by binding to the viral protein E1. *EMBO J*. 2008;27(24):3311-3321. doi:[10.1038/emboj.2008.241](https://doi.org/10.1038/emboj.2008.241)
25. López-Muñoz A, Roca FJ, Meseguer J, Mulero V. New insights into the evolution of IFNs: zebrafish group II IFNs induce a rapid and transient expression of IFN-dependent genes and display powerful antiviral activities. *J Immunol*. 2009;182(6):3440-3449. doi:[10.4049/jimmunol.0802528](https://doi.org/10.4049/jimmunol.0802528)
26. Zhu Y, Shan S, Feng H, et al. Molecular characterization and functional analysis of interferon regulatory factor 9 (irf9) in common carp *Cyprinus carpio*: a pivotal molecule in the Ifn response against pathogens. *J Fish Biol*. 2019;95(2):510-519. doi:[10.1111/jfb.14000](https://doi.org/10.1111/jfb.14000)
27. Patel MV, Shen Z, Wira CR. Poly (I:C) and LPS induce distinct immune responses by ovarian stromal fibroblasts. *J Reprod Immunol*. 2018;127:36-42. doi:[10.1016/j.jri.2018.05.001](https://doi.org/10.1016/j.jri.2018.05.001)
28. Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol*. 2011;1(6):519-525. doi:[10.1016/j.coviro.2011.10.008](https://doi.org/10.1016/j.coviro.2011.10.008)
29. Randall RE, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol*. 2008;89(1):1-47. doi:[10.1099/vir.0.83391-0](https://doi.org/10.1099/vir.0.83391-0)
30. Zhang Y, Burke CW, Ryman KD, Klimstra WB. Identification and characterization of interferon-induced proteins that inhibit alphavirus replication. *J Virol*. 2007;81(20):11246-11255. doi:[10.1128/JVI.01282-07](https://doi.org/10.1128/JVI.01282-07)
31. Rezapour M, Walker SJ, Ornelles DA, et al. Exploring the host response in infected lung organoids using NanoString technology: A statistical analysis of gene expression data. *PLoS One*. 2024;19(11):e0308849. doi:[10.1371/journal.pone.0308849](https://doi.org/10.1371/journal.pone.0308849)
32. Feng W, Zhou L, Du H, et al. Transcriptome analysis reveals gene expression changes of pigs infected with non-lethal African swine fever virus. *Genet Mol Biol*. 2023;46(3):e20230037. doi:[10.1590/1678-4685-GMB-2023-0037](https://doi.org/10.1590/1678-4685-GMB-2023-0037)
33. Rezapour M, Walker SJ, Ornelles DA, McNutt PM, Atala A, Gurcan MN. Analysis of gene expression dynamics and differential expression in viral infections using generalized linear models and quasi-likelihood methods. *Front Microbiol*. 2024;15:1342328. doi:[10.3389/fmicb](https://doi.org/10.3389/fmicb)
34. Wu L, Zhou Y, Abbas MN, et al. Molecular structure and functional characterization of the peroxiredoxin 5 in *Procambarus clarkii* following LPS and Poly I:C challenge. *Fish Shellfish Immunol*. 2017;71:28-34. doi:[10.1016/j.fsi.2017.09.072](https://doi.org/10.1016/j.fsi.2017.09.072)
35. Löseke S, Grage-Griebenow E, Heine H, et al. In vitro-generated viral double-stranded RNA in contrast to polyinosinic: polycytidylic acid induces interferon-alpha in human plasmacytoid dendritic cells. *Scand J Immunol*. 2006;63(4):264-274. doi:[10.1111/j.1365-3083.2006.01736.x](https://doi.org/10.1111/j.1365-3083.2006.01736.x)
36. Pulverer JE, Rand U, Lienenklaus S, et al. Temporal and spatial resolution of type I and III interferon responses in vivo. *J Virol*. 2010;84(17):8626-8638. doi:[10.1128/JVI.00303-10](https://doi.org/10.1128/JVI.00303-10)
37. Briolat V, Jouneau L, Carvalho R, et al. Contrasted innate responses to two viruses in zebrafish: insights into the ancestral repertoire of vertebrate IFN-stimulated genes. *J Immunol*. 2014;192(9):4328-4341. doi:[10.4049/jimmunol.1302611](https://doi.org/10.4049/jimmunol.1302611)

SUPPLEMENTARY MATERIALS

Supplemental Tables: Characterization of ISG56 from grass carp (Ctenopharyngodon idellus) and its response to two immune stimuli

Table S1 The temporal expression analysis of eight ISGs in three tissues post Poly(I:C) stimulation

Table S2 The temporal expression analysis of eight ISGs in three tissues post LPS stimulation

Download: https://ija.scholasticahq.com/article/168765-characterization-of-isg56-from-grass-carp-ctenopharyngodon-idellus_-and-its-response-to-two-immune-stimuli/attachment/362302.pdf
