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Article | [Published: 17 July 2019](#)

### Incompatible and sterile insect techniques combined eliminate mosquitoes

[Xiaoying Zheng](#), [Dongjing Zhang](#), [Yongjun Li](#), [Cui Yang](#), [Yu Wu](#), [Xiao Liang](#), [Yongkang Liang](#), [Xiaoling Pan](#), [Linchao Hu](#), [Qiang Sun](#), [Xiaohua Wang](#), [Yingyang Wei](#), [Jian Zhu](#), [Wei Qian](#), [Ziqiang Yan](#), [Andrew G. Parker](#), [Jeremie R. L. Gilles](#), [Kostas Bourtzis](#), [Jérémy Bouyer](#), [Moxun Tang](#), [Bo Zheng](#), [Jianshe Yu](#), [Julian Liu](#), [Jiajia Zhuang](#), ... [Zhiyong Xi](#)


[Nature](#) **572**, 56–61 (2019) | [Cite this article](#)

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[Matters Arising](#) to this article was published on 03 February 2021

#### Abstract

The radiation-based sterile insect technique (SIT) has successfully suppressed field populations of several insect pest species, but its effect on mosquito vector control has been limited. The related incompatible insect technique (IIT)—which uses sterilization caused by the maternally inherited endosymbiotic bacteria *Wolbachia*—is a promising alternative, but


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Yongjun Li, Luke Anthony Baton ... Zhiyong Xi  
[Nature](#) | [Matters Arising](#) | 03 Feb 2021

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[Abstract](#)
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If *w*Pip-positive larvae were observed, the sample was further screened by standard PCR using primers specific to the *ribosomal protein S6 (rps6)* gene of *C. quinquefasciatus*, the only possible mosquito species with *w*Pip co-occurring in the field sites, to exclude any false positives resulting from the collection of *Culex* larvae in the ovitraps. The specific-primers used for the assay were designed for *rps6* gene and consisted of: *rps6F*: 5'-TGCCGCGTCGTCTTGAATC-3'; and *rps6R*: 5'-GTATTGACCTCGTCGCGCTT-3'. The 20 µl PCR reaction consisted of 2 µl DNA template, 10 µl PCR Master Mix (Dongsheng), 1 µl of each primer (10 µM) and 6 µl ddH<sub>2</sub>O. The PCR conditions comprised of 5 min at 98 °C, followed by 40 cycles of 30 s at 98 °C, 5 s at 55 °C, 30 s at 72 °C, and then 10 min at 72 °C for the final extension. PCR products were electrophoresed on a 1.5% agarose gel, which contained 1 µg/ml ethidium bromide. If a product size of approximately 350 bp was obtained, the sample was considered to contain *w*Pip derived from *Culex* mosquitoes.

Incompatible and sterile insect techniques combined eliminate mosquitoes.  
Nature. (IF: 69.50)

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The screenshot shows a ScienceDirect article page. The article title is "Narrow mutational signatures drive acquisition of multidrug resistance in the fungal pathogen *Candida glabrata*". The authors listed are Ewa Ksiezopolska, Miquel Àngel Schikora-Tamarit, Reinhard Beyer, Juan Carlos Nunez-Rodriguez, Christoph Schüller, and Toni Gabaldón. The article is from Current Biology, Volume 31, Issue 23, 6 December 2021, Pages 5314-5326.e10. The page includes a sidebar with navigation links like Outline, Highlights, Summary, Keywords, Introduction, Results, Discussion, STAR Methods, Acknowledgments, Supplemental information, Data and code availability, References, and Cited by (26). There are also links for View PDF, Download full issue, and Recommended articles.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Anidulafungin	CYMIT QUIMICA S.L.	Cat# 3D-FA16270-10
Fluconazole	SIGMA-ALDRICH QUIMICA S.L.	Cat# F8929-100MG
Caspofungin diacetate	SIGMA-ALDRICH QUIMICA S.L.	Cat# SML0425-5MG
Voriconazole	SIGMA-ALDRICH QUIMICA S.L.	Cat# PZ0005-5MG
Amphotericin B from Streptomyces sp.	SIGMA-ALDRICH QUIMICA S.L.	Cat# A4888-100MG
Flucytosine	SIGMA ALDRICH	Cat# PHR1659
Chloramphenicol	Merck Life Science S.L.U.	Cat# C1919-25G
Pfu Mix	DongSheng Biotech	Cat# P2022
Taq Mix, 1mix5	DongSheng Biotech	Cat# P2012
Fluorescent Brightener 28 - Calcofluor	SIGMA-ALDRICH QUIMICA S.L.	Cat# F3543-1G

### Target *FKS* and *ERG3* sequencing

All ani-exposed samples (ANI, ANIFLZ and FinA) were examined for mutations in one region of *FKS1* and two regions of *FKS2* encompassing echinocandin resistance mutational HSs.<sup>5</sup> Three samples without mutations in the above-mentioned HSs were also inspected in the HS2 of *FKS1*. All the new *FKS* mutations are in Data S2. We used PCR primers described earlier<sup>26</sup> (Table S5). ANI samples not subjected to WGS were also amplified by two PCRs with two sets of primers (Table S5) to obtain *ERG3* sequences. PCRs were carried out by using Taq DNA polymerase from DongShengBio. The reaction mixture included primers of concentration of 0.4 μM, 20 μL Taq DNA polymerase, 1 μL liquid sample grown for 24-48 h in YPD and water up to a final volume of 40 μL. Optimase ProtocolWriter was used to develop conditions for each primer set.

We tested for the possible trajectories of final *FKS* and *ERG3* mutations in the 10 ANI samples subjected to WGS and presenting *ERG3* alterations to infer which might have appeared first in the evolution. We selected and analyzed single colonies from our glycerol stocks of stored populations after the 2nd passage at 0.032, 0.064, 0.128 and 0.256 μg/ml ani (beginning of the adaptation). PCRs were carried out as described above.

Narrow mutational signatures drive acquisition of multidrug resistance in the fungal pathogen *Candida Glabrata*.

Current Biology. (IF: 10.90)

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The screenshot shows the ScienceDirect interface for the article "Seasonal variation, virulence gene and antibiotic resistance of *Vibrio* in a semi-enclosed bay with mariculture (Dongshan Bay, Southern China)" published in *Marine Pollution Bulletin*, Volume 184, November 2022, 114112. The article is authored by Qiancheng Gao, Xiaowan Ma, Zhichao Wang, Haisheng Chen, Yu Luo, Bi Wu, Shanni Qi, Miaozhen Lin, Jing Tian, Ying Qiao, Hans-Peter Grossart, Wei Xu, and Lixing Huang. The abstract states: "In this study, the virulence genes, antibiotic resistance of culturable *Vibrio* and the environmental factors affecting *Vibrio* abundance were analyzed in four seasons in Dongshan Bay with different intensity of aquaculture practice. A total of 252 bacteria..."

### 2.3. Detection of virulence genes

Nine virulence genes (*vvhA*, *luxR*, *tdh*, *trh*, *toxR<sub>vc</sub>*, *flaC*, *ChiA*, *hlyA* and *vhpA*) of *Vibrio* species were amplified by PCR. The primers are given in Table 1. The final volume of the amplification PCR was 50  $\mu$ L, including 2  $\mu$ L DNA template, 25  $\mu$ L 2  $\times$  pfu Mix (Guangzhou Dongsheng Biotech, Guangzhou, China), 2  $\mu$ L forward and reverse primers, and 19  $\mu$ L ddH<sub>2</sub>O. PCR products were separated on a 2 % agarose gel by using 145 V/360 A, and the bands were recorded by an image analyzer BioDoc-It™ system (UVP Inc., Upland, CA, USA).

Seasonal variation, virulence gene and antibiotic resistance of *Vibrio* in a semi-enclosed bay with mariculture (Dongshan Bay, Southern China). *Marine Pollution Bulletin*. (IF: 7.00)



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### DNA aptamer for use in a fluorescent assay for the shrimp allergen tropomyosin

Short Communication | Published: 21 December 2016  
Volume 184, pages 633–639, (2017) [Cite this article](#)



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Youxiong Zhang, Qingping Wu , Xianhu Wei, Jumei Zhang & Shuping Mo

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#### Abstract

Shrimp allergy is a common cause of allergenic diseases. The authors describe an aptamer-based assay for tropomyosin, one of the major allergens of shrimp. After six rounds of SELEX based on nitrocellulose membrane, several aptamers with high affinity ( $K_d = 109.6$  nM) were identified that can selectively recognize tropomyosin but not other

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the progression of SELEX, the amount of eluted ssDNA was measured by Nanodrop device (Thermo-Scientific, USA). An ssDNA library for the following round was prepared by an asymmetric PCR protocol using **HST<sup>TM</sup> Mix (Dongsheng Biotech, Guangzhou, China, <http://www.dongshengbio.com>)** with a molar ratio of primer-Fw/primer-Rv = 50:1. All the PCR amplifications were performed as follows: 1 cycle at 95 °C for 5 min; 30 cycles at 94 °C for 40 s, 50 °C for 30 s, 72 °C for 30 s; followed by 1 cycle at 72 °C for 10 min. The screening was repeated six times, and the last round eluted ssDNAs were amplified by standard PCR protocol. The

DNA aptamer for use in a fluorescent assay for the shrimp allergen tropomyosin. Microchimica Acta. (IF: 6.41)

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### N-methylimidazolium functionalized magnetic particles as adsorbents for rapid and efficient capture of bacteria

Original Paper | Published: 05 April 2014  
Volume 181, pages 1275–1283, (2014) [Cite this article](#)




**Microchimica Acta**

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Yu Wang, Manchen Deng & Li Jia 

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#### Abstract


Silica coated magnetic particles functionalized with N-methylimidazolium ion (MIm-MPs) were prepared and characterized by transmission electron microscopy, zeta potential, and vibrating sample magnetometry. They were found to enable effective capture of bacteria as confirmed by TEM imaging of the conjugates. The adsorption capacity of the MIm-MPs for

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A GeneRuler 100-bp DNA ladder was purchased from Fermentas (Beijing, China, [www.fermentas.com](http://www.fermentas.com)) and consisted of ten fragments ranging from 100 to 1,000 bp with a total concentration of 0.5 mg mL<sup>-1</sup>. Taq DNA polymerase (5 U μL<sup>-1</sup>) was purchased from Dongsheng Biotech (Guangzhou, China, [www.dongshengbio.com](http://www.dongshengbio.com)). 25 mM MgCl<sub>2</sub>, 10×PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl), and deoxynucleotide triphosphate (dNTP) mixture (including dATP, dGTP, dCTP, and dTTP, where the concentration of each dNTP was 2.5 mM) were purchased

N-methylimidazolium functionalized magnetic particles as adsorbents for rapid and efficient capture of bacteria.  
Microchimica Acta. (IF: 6.41)

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The screenshot shows the ScienceDirect interface for an article in the journal *Aquaculture*. The article title is "Screening and characterization of sex-specific markers developed by a simple NGS method in mandarin fish (*Siniperca chuatsi*)". The authors listed are Chong Han, Qiaoying Zhu, Haoming Lu, Chongwei Wang, Xingni Zhou, Cheng Peng, Lin Tang, Lingqiang Han, Jiehu Chen, Shuisheng Li, Guifeng Li, Haoran Lin, and Yong Zhang. The abstract states: "Sex-specific markers are the prerequisite for interpreting the mechanisms of sex determination and sex control breeding in fish. Here, we provided a simple next generation sequencing strategy to identify potential sex-specific sequences in mandarin fish (*Siniperca chuatsi*) that is a greatly important freshwater fish in China. Through". The page also includes a sidebar with article preview options (Abstract, Introduction, Section snippets, References, Cited by) and a recommended articles section.

volume of 10ul, including 5ul **2xTaqMasterMix (DSBIO, China)**, 0.5ul each specific primer (10uM), 0.5ul DNA template and 3.5ul ddH<sub>2</sub>O. The PCR amplification reaction was carried out as following: pre-denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30s; and finally an extension step of 72 °C for 5 min. The amplified products were separated by 1.5% agarose gel. Then, another 16 DNAs (8 females

Screening and characterization of sex-specific markers developed by a simple NGS method in mandarin fish (*Siniperca chuatsi*).  
Aquaculture. (IF: 5.14)



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The screenshot shows the ScienceDirect interface for the article "Screening and characterization of X chromosome-specific markers in mandarin fish (*Siniperca chuatsi*)" published in *Aquaculture*, Volume 562, 15 January 2023, 738833. The article is authored by Shivan Liu, Chang Han, Jingjun Huang, Qiaoying Zhu, Dingrui Liu, Linqiang Han, Shuisheng Li, Guifeng Li, Haoran Lin, and Yong Zhang. The abstract states: "The mandarin fish (*Siniperca chuatsi*) is an important aquaculture fish. All-male mandarin fish culture has the advantages of higher profits, more uniform size and lower energy consumption of gonadal development. However, the lack of accurate sex-specific markers has hampered the all-male breeding of this species. Herein, we successfully adopted a next generation sequencing strategy to identify potential X chromosome-". The page also includes a sidebar with article preview options (Abstract, Introduction, Section snippets, References (66), Cited by (3)) and a recommended articles section.

Based on the filtrated X chromosome-specific sequences, primers were designed by Primer Premier 5.0 software (<http://www.premierbio.com/>). DNAs from XX, XY and YY individuals were used as templates to test the feasibility and universality of X chromosome-specific markers. Each PCR reaction was performed in a total volume of 10  $\mu$ l, including 5  $\mu$ l 2  $\times$  Tap MasterMix (DSBIO, China), 0.5  $\mu$ l each specific primer, 0.5  $\mu$ l DNA template and 3.5  $\mu$ l ddH<sub>2</sub>O. The PCR amplification reaction was carried out as following: pre-denaturation at 95 °C for 5 min; followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30s; and an extension step of 72 °C for 5 min finally. The amplified PCR products were visualized by gel electrophoresis on 1% agarose gels.

Screening and characterization of X chromosome-specific markers in mandarin fish (*Siniperca chuatsi*).  
Aquaculture. (IF: 5.14)

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The screenshot shows the ScienceDirect website interface. The article title is "Screening and characterization of sex-specific markers by NGS sequencing in *Spinibarbus hollandi* with implication of XY sex determination system". The journal is "Aquaculture", Volume 565, 25 February 2023, 739147. The authors listed are Chong Han, Wenwei Huang, Suhan Peng, Jiongwei Zhou, Huawei Zhan, Lin Gui, Wenjun Li, and Qiang Li. The abstract mentions that sex-specific markers are critical for understanding sex determination mechanism and development of unisexual breeding in fish. The article is available for purchase as a PDF.

### 2.4. Development and verification of male-specific sequences

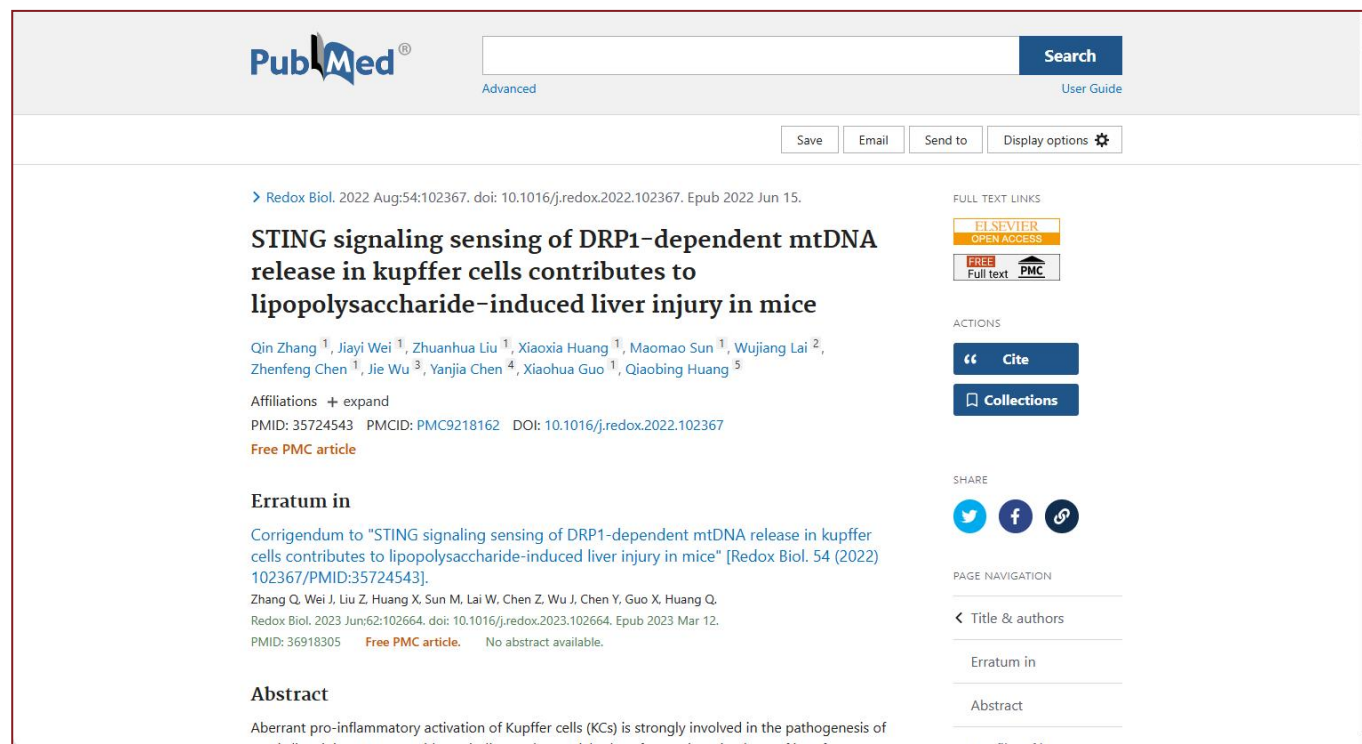
According to the obtained sex-specific sequences, a total of 20 primer pairs were designed using Primer 5.0. These primers were first verified in DNA samples of eight female and eight male *S. hollandi*. Then, the valid primers were further verified in DNA samples of another twenty four females and twenty four males. The PCR amplification reaction was carried out in a total of 10  $\mu$ l reaction system, including 5  $\mu$ l **2 x Taq Master Mix (GDSBIO, China)**, 0.5  $\mu$ l each primer (10  $\mu$ M), 0.5  $\mu$ l DNA template and 3.5  $\mu$ l ddH<sub>2</sub>O. The PCR amplification conditions include 5 min at 95 °C for pre-denaturation, following by 33 cycles 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension for 5 min at 72 °C. Gel electrophoresis was used to detect the PCR products by 1.2% agarose gel.

Screening and characterization of sex-specific markers by NGS sequencing in *Spinibarbus hollandi* with implication of XY sex determination system. Aquaculture. (IF: 5.14)



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The screenshot shows a PubMed article page. The title is "STING signaling sensing of DRP1-dependent mtDNA release in kupffer cells contributes to lipopolysaccharide-induced liver injury in mice". The authors are Qin Zhang, Jiayi Wei, Zhuanhua Liu, Xiaoxia Huang, Maomao Sun, Wujiang Lai, Zhenfeng Chen, Jie Wu, Yanjia Chen, Xiaohua Guo, and Qiaobing Huang. The article is from Redox Biol. 2022 Aug;54:102367. The abstract mentions that aberrant pro-inflammatory activation of Kupffer cells (KCs) is strongly involved in the pathogenesis of liver injury.

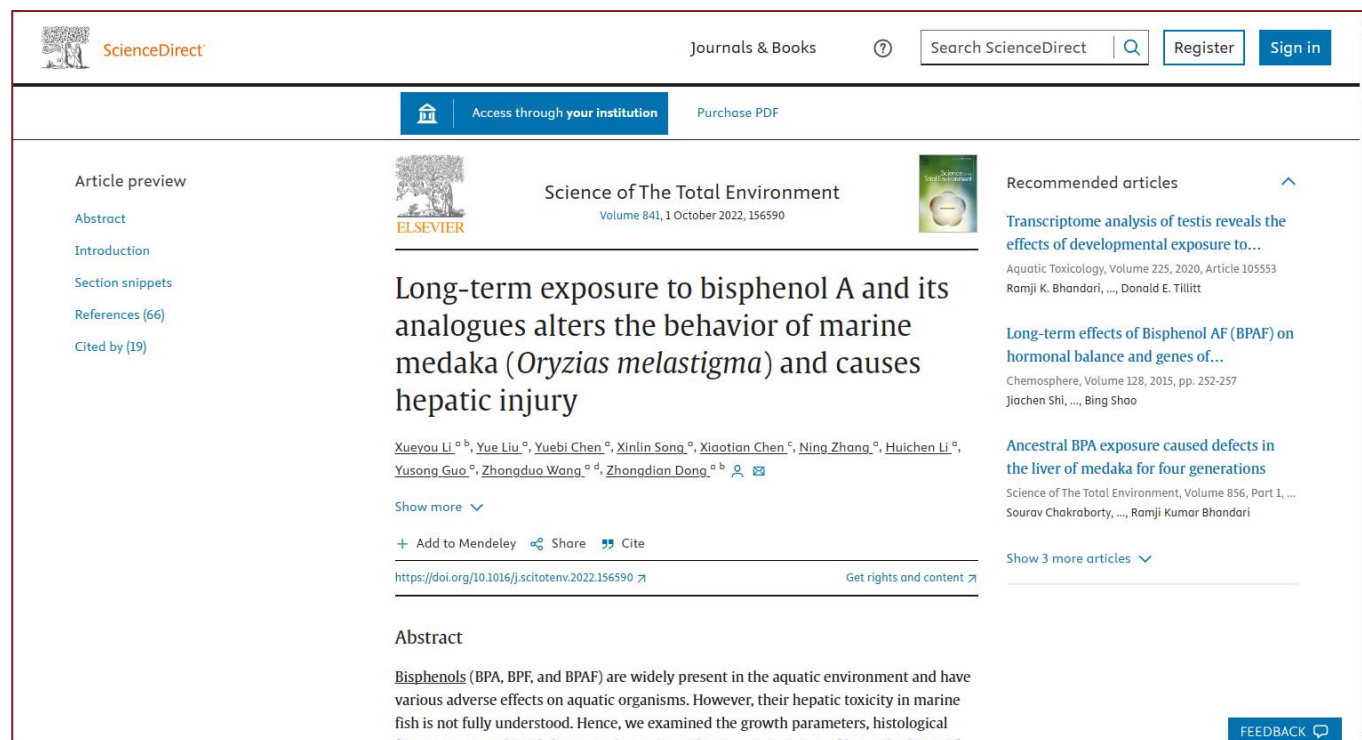
### 2.6. Quantitative real-time PCR

Total RNA from KCs was isolated with Trizol reagent. 500 ng of total RNA was reverse-transcribed into cDNA by using the RT-PCR Mix for qPCR kit (R1031, GDSBio) in which the genomic DNA was removed by DNase digestion. Real-time qPCR was conducted on the 7500 Real-Time PCR System (Applied Biosystems) with a Power Green qPCR Mix (P2102a, GDSBio) according to the manufacturer's procedures. The results of relative expression of mRNA of IFN- $\beta$ , TNF- $\alpha$  and IL-1 $\beta$  were normalized to the Ct value of GAPDH in each sample. The primers for qPCR analysis of sequences were presented in Supplementary Table 1.

STING signaling sensing of DRP1-dependent mtDNA release in kupffer cells contributes to lipopolysaccharide-induced liver injury in mice.  
Redox Biology. (IF: 10.79)

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The screenshot shows the ScienceDirect website interface. The article title is "Long-term exposure to bisphenol A and its analogues alters the behavior of marine medaka (*Oryzias melastigma*) and causes hepatic injury". The authors listed are Xueyou Li, Yue Liu, Yuebi Chen, Xinlin Song, Xiaotian Chen, Ning Zhang, Huichen Li, Yusong Guo, Zhongduo Wang, Zhongdian Dong. The journal is "Science of The Total Environment", Volume 841, 1 October 2022, 156590. The abstract mentions that bisphenols (BPA, BPF, and BPAF) are widely present in the aquatic environment and have various adverse effects on aquatic organisms. The article is available for full-text access through the institution.

### 2.7. Quantitative real-time polymerase chain reaction (qPCR)

Three liver samples were randomly selected from each treatment for total RNA isolation, cDNA synthesis, and quantitative real-time polymerase chain reaction (qPCR). qPCR was performed using a Roche LightCycler 96 (Roche, Basel, Switzerland). The total reaction system was 15  $\mu$ L, including 7.5  $\mu$ L of **2  $\times$  SYBR Premix Ex Taq II (GDSBio, Guangzhou, China)**, 0.3  $\mu$ L of each primer (10  $\mu$ M), 2  $\mu$ L of cDNA, and 4.9  $\mu$ L of ddH<sub>2</sub>O. The reaction program adopted a three-step method: pre-denaturation at 95  $^{\circ}$ C for 180 s, followed by 40 cycles of 10 s at 95  $^{\circ}$ C, 30 s at 60  $^{\circ}$ C, and 30 s at 72  $^{\circ}$ C. A dissociation curve analysis was performed at the end of amplification to determine target specificity. The relative expression levels were measured according to the threshold cycle value and normalized using the  $-\Delta\Delta C_t$  method. *Rps4x* was used as reference gene owing to its stable expression in liver samples under different treatment conditions. All primer sequences used for qPCR are listed in Table S1.

Long-term exposure to bisphenol A and its analogues alters the behavior of marine medaka (*Oryzias melastigma*) and causes hepatic injury.

Science of The Total Environment. (IF: 10.75)

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Advanced
User Guide

[ACS Appl Mater Interfaces](#). 2023 Nov 1. doi: 10.1021/acsami.3c11227. Online ahead of print.

### CpG-Conjugated Silver Nanoparticles as a Multifunctional Nanomedicine to Promote Macrophage Efferocytosis and Repolarization for Atherosclerosis Therapy

Cui Tang <sup>1 2</sup>, Hui Wang <sup>2</sup>, Lina Guo <sup>1</sup>, Chan Zou <sup>2</sup>, Jianming Hu <sup>3</sup>, Hanyong Zhang <sup>4</sup>, Wenhui Zhou <sup>1 4 5</sup>, Guoping Yang <sup>1 2 6 7</sup>

Affiliations [+ expand](#)  
PMID: 37910772 DOI: 10.1021/acsami.3c11227

#### Abstract

Atherosclerosis (AS) is a major contributor to cardiovascular diseases, necessitating the development of novel therapeutic strategies to alleviate plaque burden. Macrophage efferocytosis, the process by which macrophages clear apoptotic and foam cells, plays a crucial role in plaque regression. However, this process is impaired in AS lesions due to the overexpression of CD47, which produces a "do not eat me" signal. In this study, we investigated the potential of CpG, a toll-like receptor 9 agonist, to enhance macrophage efferocytosis for AS therapy. We demonstrated that CpG treatment promoted the engulfment of CD47-positive apoptotic cells and foam cells by macrophages. Mechanistically, CpG induced a metabolic shift in macrophages characterized by enhanced fatty acid oxidation and de novo lipid biosynthesis, contributing to its pro-efferocytic effect. To enable in vivo application, we conjugated CpG on silver nanoparticles (AgNPs) to form CpG-AgNPs, which could protect CpG from

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reaction test (RT-PCR) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). GSH was provided by Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), a cytosolic reactive oxygen species (ROS) probe, was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, USA). SYBR green was purchased from Dongsheng Biotech Co., Ltd. (Guangzhou, China). The RevertAid First Strand cDNA synthesis kit and CellTracker deep red dye were from Thermo Fisher Scientific Co., Ltd. (MA, USA). Cell-tracker green CMFDA was from Maokang

CpG-Conjugated Silver Nanoparticles as a Multifunctional Nanomedicine to Promote Macrophage Efferocytosis and Repolarization for Atherosclerosis Therapy. ACS Applied Materials & Interfaces. (IF: 9.50)



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**KLF4 regulates skeletal muscle development and regeneration by directly targeting P57 and Myomixer**

Shufang Cai, Xiaoyu Wang, Rong Xu, Ziyun Liang, Qi Zhu, Meilin Chen, Zhuhu Lin, Chenggan Li, Tianqi Duo, Xian Tong, Enru Li, Zuyong He, Xiaohong Liu, Yaosheng Chen & Delin Mo

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Materials and methods

Results

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**Abstract**

Krüppel-like factor 4 (KLF4) is an evolutionarily conserved zinc finger-containing transcription factor that regulates diverse cellular processes such as cell proliferation, apoptosis, and differentiation. Our previous study showed that KLF4 expression is upregulated in skeletal muscle ontogeny during embryonic development in pigs, suggesting its importance for skeletal muscle development and muscle function. We revealed here that KLF4 plays a critical role in skeletal muscle development and regeneration. Specific knockout of KLF4 in skeletal muscle impaired muscle formation further affecting physical activity and also defected skeletal muscle regeneration. In vitro, KLF4 was highly expressed in proliferating myoblasts and early differentiated cells. KLF4 knockdown promoted myoblast proliferation and inhibited myoblast

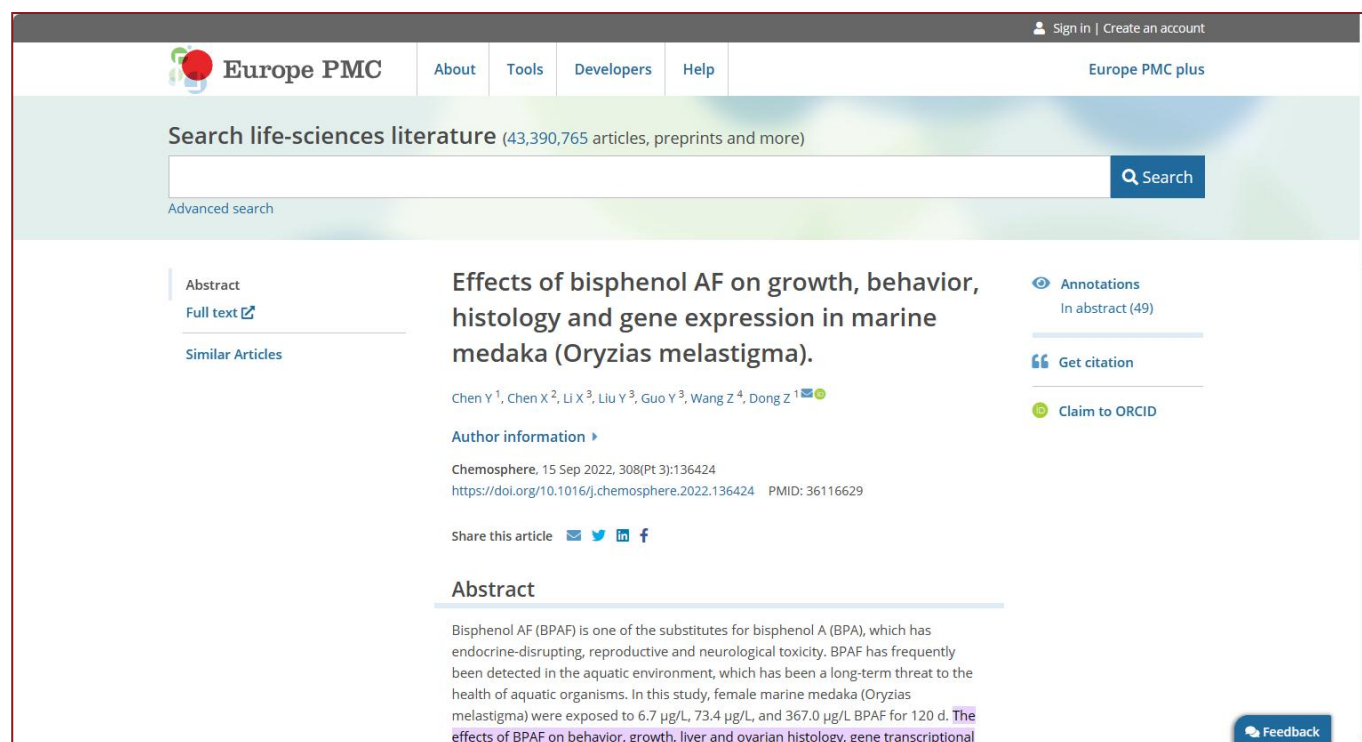
### RNA extraction and Real-time quantitative PCR

Total RNA was extracted from cultured C2C12 cells and regenerating TA muscles using Trizol Reagent (Invitrogen). Then, cDNA was synthesized from 1 µg total RNA using StarScript II First-strand cDNA Synthesis Mix (Genestar, Beijing, China). Real-time quantitative PCR (qPCR) analyses were performed on LightCycler 480 II (Roche, Basel, Switzerland) using SYBR Green qPCR Mix (GDSBio, Guangzhou, China), with GAPDH as an internal control for normalization. Primers are listed in Table S3.

KLF4 regulates skeletal muscle development and regeneration by directly targeting P57 and Myomixer. Cell Death & Disease. (IF: 9.00)

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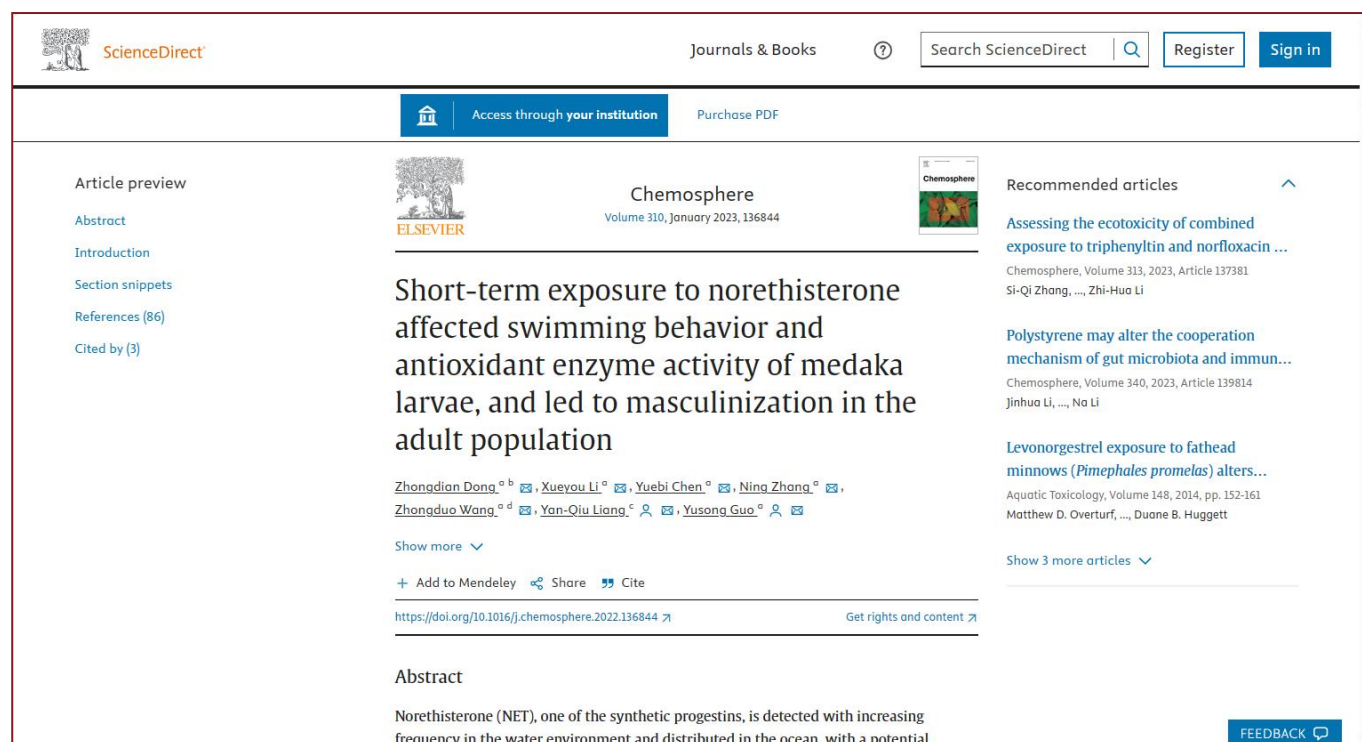
The screenshot shows the Europe PMC website interface. At the top, there's a navigation bar with 'About', 'Tools', 'Developers', and 'Help' links. Below this is a search bar with the text 'Search life-sciences literature (43,390,765 articles, preprints and more)' and a 'Search' button. The main content area displays the article title 'Effects of bisphenol AF on growth, behavior, histology and gene expression in marine medaka (Oryzias melastigma)' by Chen Y<sup>1</sup>, Chen X<sup>2</sup>, Li X<sup>3</sup>, Liu Y<sup>3</sup>, Guo Y<sup>3</sup>, Wang Z<sup>4</sup>, and Dong Z<sup>1</sup>. The article is from 'Chemosphere', 15 Sep 2022, 308(Pt 3):136424. The abstract mentions that Bisphenol AF (BPAF) is one of the substitutes for bisphenol A (BPA), which has endocrine-disrupting, reproductive and neurological toxicity. BPAF has frequently been detected in the aquatic environment, which has been a long-term threat to the health of aquatic organisms. In this study, female marine medaka (Oryzias melastigma) were exposed to 6.7 µg/L, 73.4 µg/L, and 367.0 µg/L BPAF for 120 d. The effects of BPAF on behavior, growth, liver and ovarian histology, gene transcriptional

scriptional expression levels of target genes (Li et al., 2021). Quantitative polymerase chain reaction (qPCR) was performed using the SYBR® Green qPCR Mix (Dongsheng Biotech Co., Ltd., Guangzhou, China). All reactions were performed on the LightCycler® 96 PCR system (Roche, Basel, Switzerland). The list of primers is shown in Table S1. The PCR system (15 µL) contained 7.5 µL of 2 x SYBR® Green qPCR Mix<sup>a</sup>, 0.4 µL of each forward and reverse primers (10 µM), 1.5 µL of cDNA samples, and 5.2 µL of nuclease-free water. The qPCR reaction conditions were as

Effects of bisphenol AF on growth, behavior, histology and gene expression in marine medaka (Oryzias melastigma).  
Chemosphere. (IF: 8.94)

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The screenshot shows the ScienceDirect website interface. The article title is "Short-term exposure to norethisterone affected swimming behavior and antioxidant enzyme activity of medaka larvae, and led to masculinization in the adult population". The journal is "Chemosphere", Volume 310, January 2023, 136844. The authors listed are Zhongdian Dong, Xueyou Li, Yuebi Chen, Ning Zhang, Zhongduo Wang, Yan-Qiu Liang, and Yusong Guo. The abstract mentions that Norethisterone (NET) is detected with increasing frequency in the water environment and distributed in the ocean, with a potential impact on medaka larvae. The article is available for purchase as a PDF.

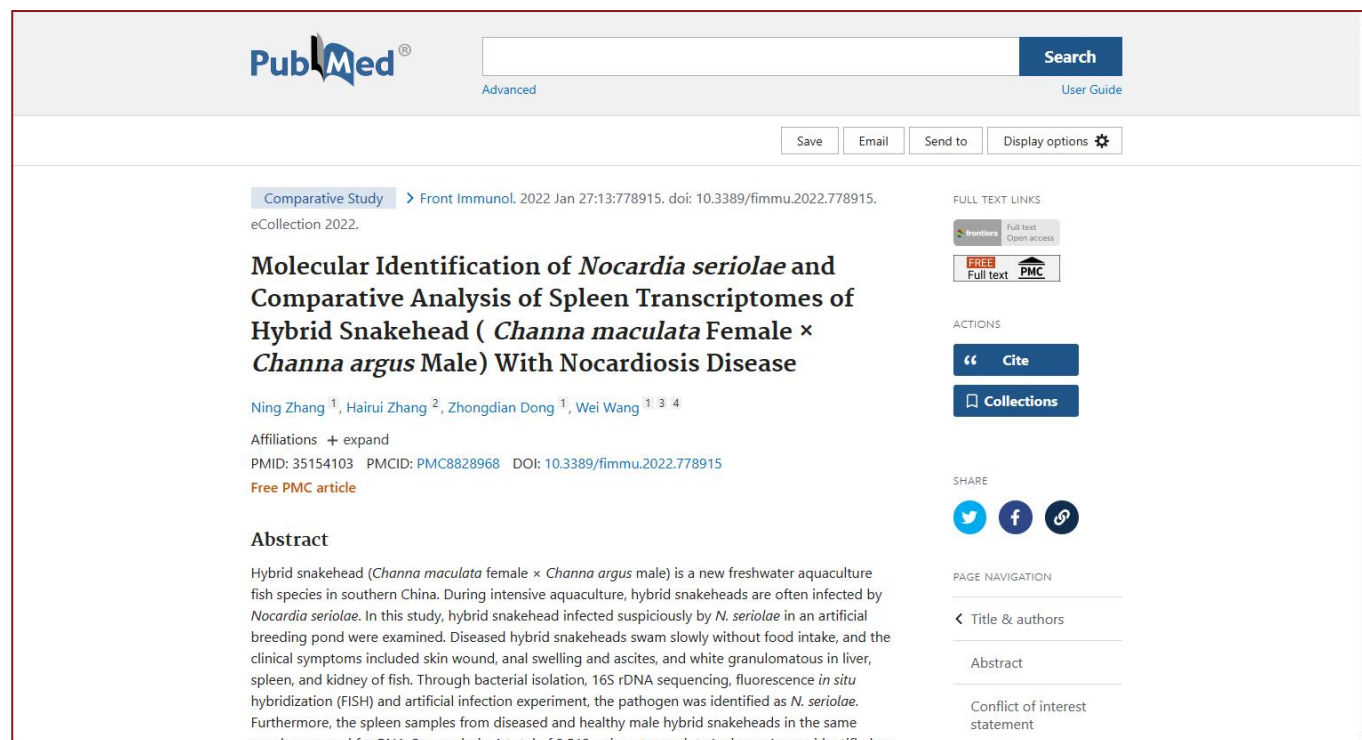
beta-2 (*actb2*) and glyceraldehyde-3-phosphate dehydrogenase (*gadh*) were used as internal reference genes (Dong et al., 2022). Roche LightCycler 96 (Roche, Basel, Switzerland) was used to perform the qPCR reaction, and the qPCR reaction reagent was **Power Green qPCR Mix (GDSBio, Guangzhou, China)**. The system formulation and reaction procedure of qPCR refer to our previous study (Li et al., 2022). The relative mRNA expression level of target gene was transformed as Log<sub>2</sub> according to the 2<sup>-ΔΔCt</sup> method (Livak and Schmittgen, 2001). Graph-Pad Prism 9 (GraphPad Software, Inc., La Jolla, CA, USA) was used to analyze and graph the data (expressed as Log<sub>2</sub> form).

Short-term exposure to norethisterone affected swimming behavior and antioxidant enzyme activity of medaka larvae, and led to masculinization in the adult population.  
Chemosphere. (IF: 8.94)



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The screenshot shows a PubMed article page. The title is "Molecular Identification of *Nocardia seriolae* and Comparative Analysis of Spleen Transcriptomes of Hybrid Snakehead ( *Channa maculata* Female × *Channa argus* Male) With Nocardiosis Disease". The authors are Ning Zhang<sup>1</sup>, Hairui Zhang<sup>2</sup>, Zhongdian Dong<sup>1</sup>, and Wei Wang<sup>1,3,4</sup>. The article is from Front Immunol, 2022 Jan 27;13:778915. doi: 10.3389/fimmu.2022.778915. The abstract mentions that hybrid snakehead (Channa maculata female × Channa argus male) is a new freshwater aquaculture fish species in southern China. During intensive aquaculture, hybrid snakeheads are often infected by Nocardia seriolae. In this study, hybrid snakehead infected suspiciously by N. seriolae in an artificial breeding pond were examined. Diseased hybrid snakeheads swam slowly without food intake, and the clinical symptoms included skin wound, anal swelling and ascites, and white granulomatous in liver, spleen, and kidney of fish. Through bacterial isolation, 16S rDNA sequencing, fluorescence in situ hybridization (FISH) and artificial infection experiment, the pathogen was identified as N. seriolae. Furthermore, the spleen samples from diseased and healthy male hybrid snakeheads in the same pond were used for RNA-Seq analysis. A total of 3,512 unique transcripts (unigenes) were identified as

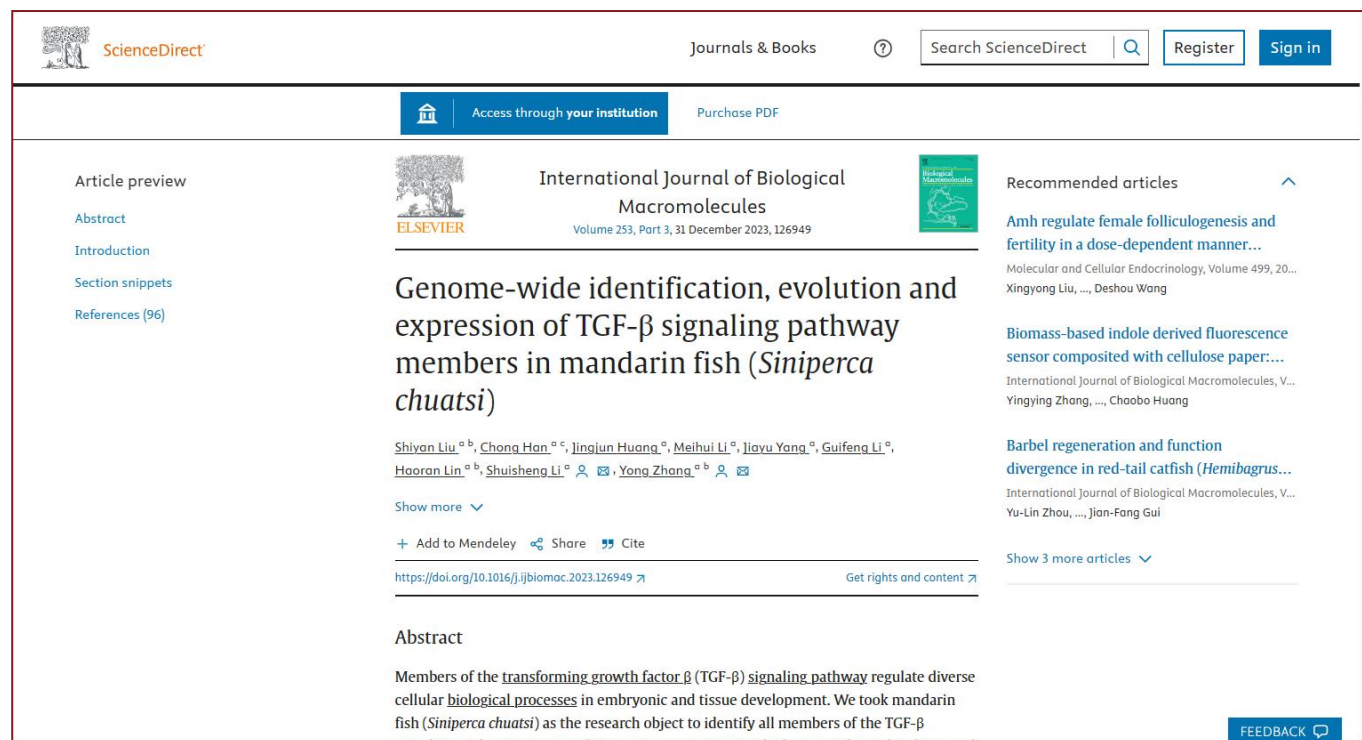
### qPCR Validation

In order to validate the results of the RNA-Seq, twenty DEGs were randomly selected for validation by qPCR. Primers were designed according to the transcriptome sequences (**Supplementary Table 1**). qPCR was performed on Roche LightCycler 96 (Roche, Formentor, Switzerland) using **SYBR<sup>®</sup> Green qPCR Mix (GDSBio, Guangzhou, China)**. The reaction was carried out using a qPCR mixture of 15.0 μL, containing 7.5 μL 2 X SYBR<sup>®</sup> Green qPCR, 0.6 μL of each forward and reverse primer, 1.5 μL cDNA and 4.8 μL ddH<sub>2</sub>O. qPCR amplification was done as follows: 180 s at 95°C for pre-incubation, followed by 40 cycles at 95°C for 15 s, 60°C for 15 s and 72°C for 30 s. Dissociation and melting curves of qPCR products were performed, and results were analyzed. *β-actin* and *ef2b* were used as reference genes to determine relative expression (5). The transcriptional data were

Molecular Identification of *Nocardia seriolae* and Comparative Analysis of Spleen Transcriptomes of Hybrid Snakehead (*Channa maculata* Female × *Channa argus* Male) With Nocardiosis Disease. Frontiers in Immunology. (IF: 8.79)

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The screenshot shows the ScienceDirect interface for the article "Genome-wide identification, evolution and expression of TGF-β signaling pathway members in mandarin fish (*Siniperca chuatsi*)". The article is published in the *International Journal of Biological Macromolecules*, Volume 253, Part 3, 31 December 2023, 126949. The authors listed are Shiyun Liu, Chong Han, Jingjun Huang, Meihui Li, Jiayu Yang, Guifeng Li, Haoran Lin, Shuisheng Li, Yong Zhang, and others. The abstract mentions that members of the transforming growth factor β (TGF-β) signaling pathway regulate diverse cellular biological processes in embryonic and tissue development. The article is available for access through the institution or purchase of the PDF.

According to the genome and transcriptome data of mandarin fish, the open reading frames (ORFs) of sex-associated genes (*amh*, *amhr2*, *gdf9*, *bmp15*, *gsdf*, *gdf3*, *smad1*, *smad5*, *smad9*, *inha*) were predicted, and gene-specific primers were designed using Primer 5.0 software (Table S1). Then, qRT-PCR was conducted using SYBR Green qPCR Mix (GDSBio, China) and performed on a Roche LightCycler 480 real-time PCR system. The qRT-PCR program was as follows: predenaturation at 95 °C for 3 min; followed by 45 cycles of 95 °C for 10 s, 58 °C for 20 s, and 72 °C for 15 s; and a final extension at 72 °C for 5 min, ending with a dissociation curve process. All qRT-PCRs used *β-actin* as the internal control and were carried out in triplicate to confirm the results. The specificity of each primer amplification was conducted with the dissociation curve. Under a fivefold cDNA dilution series, the qPCR

Genome-wide identification, evolution and expression of TGF-β signaling pathway members in mandarin fish (*Siniperca chuatsi*).

International Journal of Biological Macromolecules. (IF: 8.20)

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The screenshot shows the ScienceDirect website interface. The article title is "Molecular characterization and expression analysis of nine toll like receptor (TLR) genes in *Scortum barcoo* under *Streptococcus agalactiae* infection". The authors listed are Songze Gao, Wenwei Huang, Suhan Peng, Jiangwei Zhou, Huawei Zhan, Tongfu Lu, Weiqlan Liang, Junwu Li, Yuying Zhang, Wenjun Li, Chang Han, Qiang Li. The journal is "International Journal of Biological Macromolecules", Volume 254, Part 1, January 2024, 127667. The abstract mentions that Toll like receptors (TLRs) are important pattern recognition receptors participating in innate immune system. Up to now, no TLR has been identified in Jade perch (*Scortum barcoo*). In this study, we successfully identified 9 members of TLRs from the Jade perch. Amino acid sequence alignment analysis showed that the whole sequences of these TLRs...

Total RNA was extracted from all obtained tissues using a Trizol RNA extraction kit (Invitrogen, USA) following the manufacturer's instructions. The quantity and quality of RNA were further detected using Nanodrop 2000c (ThermoFisher, USA). Then, all cDNA templates were synthesized using RT-PCR Mix for qPCR (**GDSBIO**, China) and then stored at  $-20^{\circ}\text{C}$ .

To detect expression profiles of SbTLRs, quantitative real time PCR (qRT-PCR) reaction was performed using the SYBR Green qPCR Mix (**GDSBIO**, China) on a LightCycle 480 System (Roche, Germany). All primers used in qRT-PCR reaction were designed by Primer 3.0 (**Table 1**) and further validated using agarose gel electrophoresis and Sanger sequencing. All qRT-PCR reactions were performed in a volume of  $10\ \mu\text{L}$ , containing  $0.5\ \mu\text{L}$  cDNA,  $0.5\ \mu\text{L}$  of each primer ( $10\ \mu\text{M}$ ),  $5\ \mu\text{L}$  SYBR Green qPCR Mix, and  $3.5\ \mu\text{L}$  of  $\text{ddH}_2\text{O}$ . The PCR cycling conditions are pre-

Molecular characterization and expression analysis of nine toll like receptor (TLR) genes in *Scortum barcoo* under *Streptococcus agalactiae* infection.

International Journal of Biological Macromolecules. (IF: 8.20)



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PLOS Pathog. 2021 Feb 26;17(2):e1008690. doi: 10.1371/journal.ppat.1008690. eCollection 2021 Feb.

### Inhibition of anti-viral stress granule formation by coronavirus endoribonuclease nsp15 ensures efficient virus replication

Bo Gao<sup>1</sup>, Xiaoqian Gong<sup>1,2</sup>, Shouguo Fang<sup>3</sup>, Wenlian Weng<sup>1</sup>, Huan Wang<sup>1</sup>, Hongyan Chu<sup>1</sup>, Yingjie Sun<sup>1</sup>, Chunchun Meng<sup>1</sup>, Lei Tan<sup>1</sup>, Cuiping Song<sup>1</sup>, Xusheng Qiu<sup>1</sup>, Weiwei Liu<sup>1</sup>, Maria Forlenza<sup>2</sup>, Chan Ding<sup>1,4</sup>, Ying Liao<sup>1</sup>

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PMID: 33635931 PMCID: PMC7946191 DOI: 10.1371/journal.ppat.1008690  
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#### Abstract

Cytoplasmic stress granules (SGs) are generally triggered by stress-induced translation arrest for storing mRNAs. Recently, it has been shown that SGs exert anti-viral functions due to their involvement in protein synthesis shut off and recruitment of innate immune signaling intermediates. The largest RNA viruses, coronaviruses, impose great threat to public safety and animal health; however, the significance of SGs in coronavirus infection is largely unknown. Infectious Bronchitis Virus (IBV) is the first identified coronavirus in 1930s and has been prevalent in poultry farm for many years. In this study, we provided evidence that IBV overcomes the host antiviral response by inhibiting SGs formation via the virus-encoded endoribonuclease nsp15. By immunofluorescence analysis, we observed that IBV infection not only did not trigger SGs formation in approximately 80% of the

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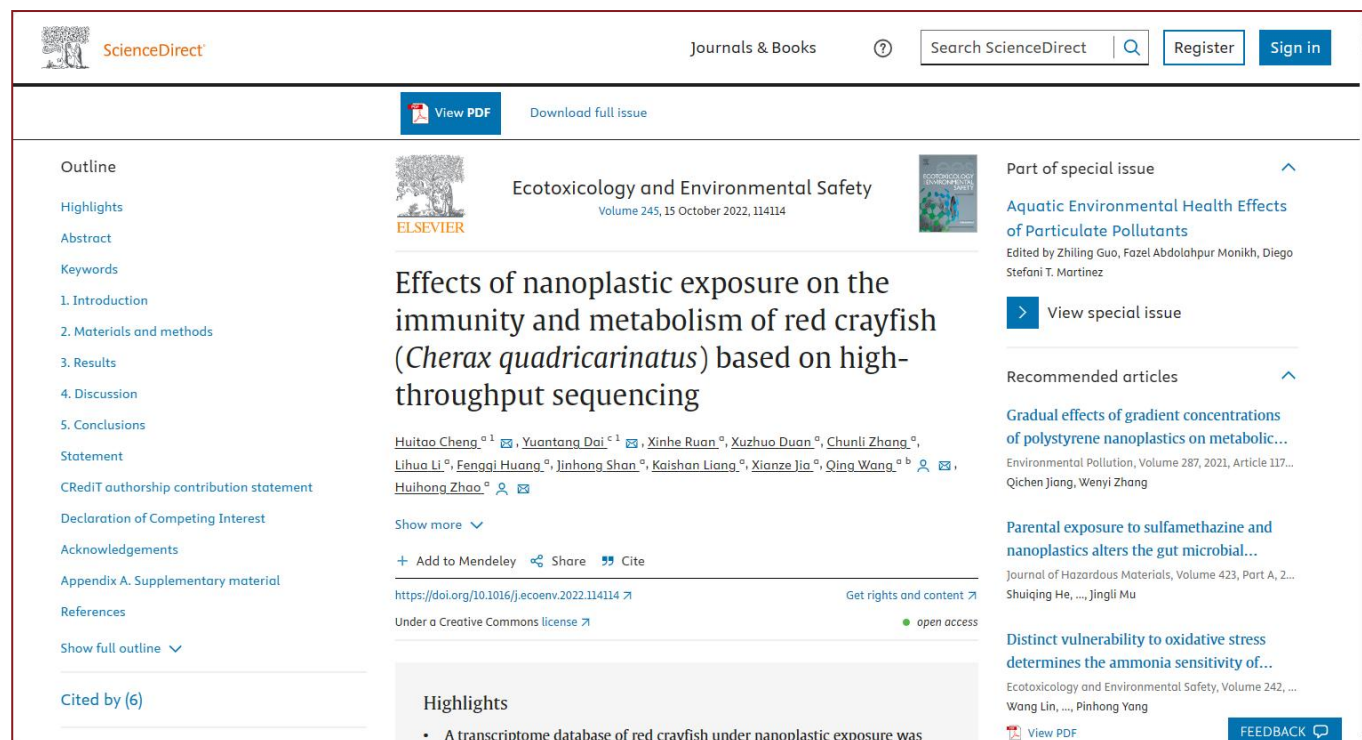
### Quantitative RT-PCR analysis

Total cellular RNAs were extracted using Trizol reagent (Ambion). cDNAs were synthesized from 2µg total RNA using oligo(dT) primers and M-MLV reverse transcriptase system (Promega). cDNA was used as template for quantitative PCR using a Bio-Rad CFX-96 real time PCR apparatus and **SYBR green master mix (Dongsheng Biotech)**. PCR conditions were as follow: an initial denaturation at 94°C for 3 min, 40 cycles of 94°C for 15 s, 60°C for 15 s and 72°C for 20 s. The specificity of the amplified PCR products was confirmed by melting curve analysis after each reaction. The primers used were: for human *IFN-β*, 5'-GCTTGGATTC

Inhibition of anti-viral stress granule formation by coronavirus endoribonuclease nsp15 ensures efficient virus replication. Plos Pathogens. (IF: 7.46)

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The screenshot shows the ScienceDirect article page for the paper: "Effects of nanoplastic exposure on the immunity and metabolism of red crayfish (*Cherax quadricarinatus*) based on high-throughput sequencing". The article is published in *Ecotoxicology and Environmental Safety*, Volume 245, 15 October 2022, 114114. The authors listed are Huitao Cheng, Yuantang Dai, Xinhe Ruan, Xuzhuo Duan, Chunli Zhang, Lihua Li, Fengli Huang, Jinhong Shan, Kaishan Liang, Xianze Jia, Qing Wang, and Huihong Zhao. The article is part of a special issue titled "Aquatic Environmental Health Effects of Particulate Pollutants". The page includes a table of contents on the left, a list of references on the right, and a highlights section at the bottom stating: "A transcriptome database of red crayfish under nanoplastic exposure was".

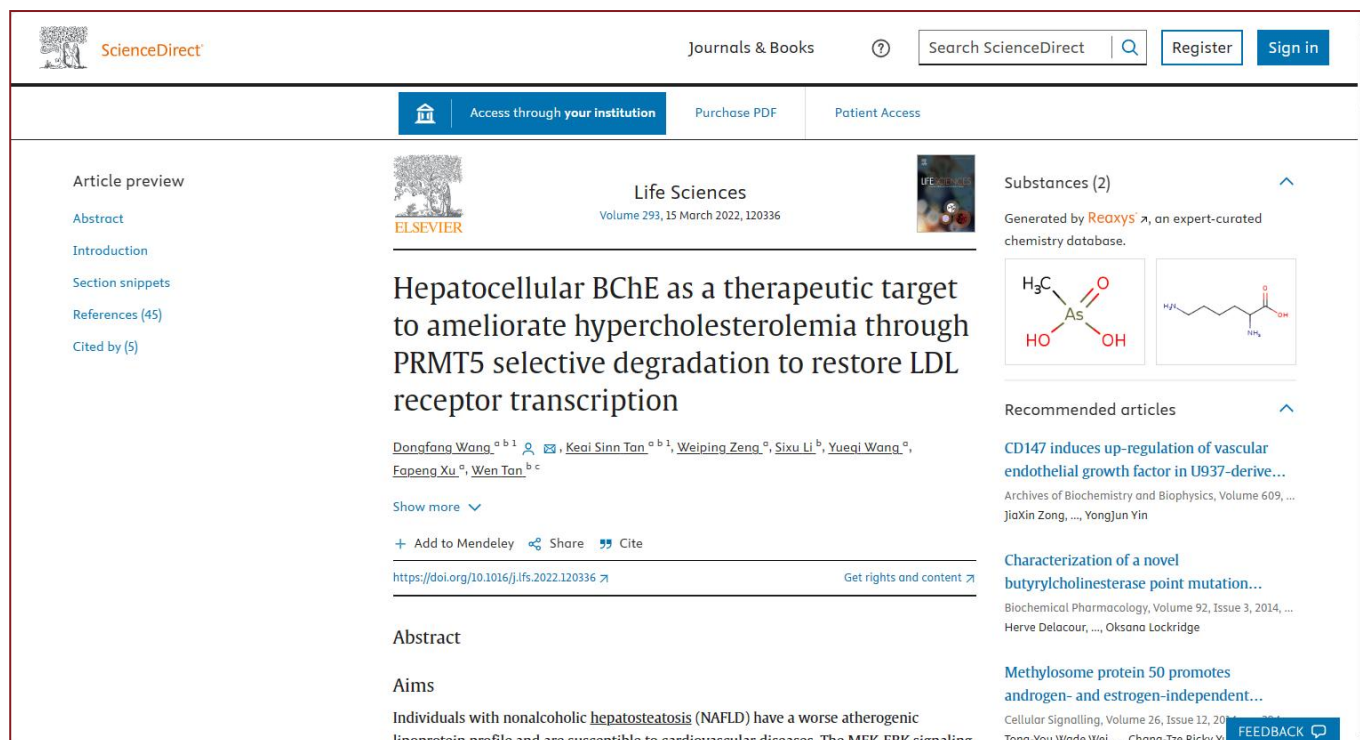
### 2.3.4. Verification of DEGs by quantitative real-time PCR (qRT-PCR)

To verify the reliability of the transcriptome data, 12 differentially expressed genes were selected for qRT-PCR validation. Based on the 12 genes and  $\beta$ -actin sequences, Premier6 software were used to design specific primers for qRT-PCR analysis (Table S1). The PCR reaction system was 10  $\mu$ L consisting of 5  $\mu$ L 2  $\times$  SYBR Ex Taq (Guangzhou Dongsheng Biotechnology Co., Ltd.), 0.2  $\mu$ L of each gene-specific primer (10 nmol), 1  $\mu$ L cDNA and 3.6  $\mu$ L ribonuclease-free water composition. Cycling conditions were 3 min at 94  $^{\circ}$ C; 40 cycles of 94  $^{\circ}$ C for 15 s, 60  $^{\circ}$ C for 15 s, and 72  $^{\circ}$ C for 20 s. The relative expression was calculated by the  $2^{-\Delta\Delta CT}$  method with  $\beta$ -actin gene as the internal reference gene.

Effects of nanoplastic exposure on the immunity and metabolism of red crayfish (*Cherax quadricarinatus*) based on high-throughput sequencing. *Ecotoxicology and Environmental Safety*. (IF: 7.13)

# Collection of Papers on PCR Products

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The screenshot shows a ScienceDirect article page. The article title is "Hepatocellular BChE as a therapeutic target to ameliorate hypercholesterolemia through PRMT5 selective degradation to restore LDL receptor transcription". The authors listed are Dongfang Wang, Keai Sinn Tan, Weiping Zeng, Sixu Li, Yueqi Wang, Fapeng Xu, and Wen Tan. The article is published in Life Sciences, Volume 293, 15 March 2022, 120336. The abstract mentions that individuals with nonalcoholic hepatosteatosis (NAFLD) have a worse atherogenic lipoprotein profile and are susceptible to cardiovascular diseases. The article also includes a chemical structure diagram of a molecule, likely related to the study.

Total RNA was extracted from Aml-12 cells using TRIzol Reagent (Invitrogen, 15596026) as described by the manufacturer. RNA integrity was determined by agarose electrophoresis, and RNA concentration was measured by NanoDrop 2000. Reverse transcription was performed to synthesize single-stranded cDNA using 5 µg RNA as the template according to the protocol of the manufacturer (Hifair® III 1st Strand cDNA Synthesis Kit (gDNA digester plus), 11139ES60, Yeasen Biotechnology). Real-time PCR was performed with **Power Green qPCR Mix No ROX (P2104, GDSbio, China)** on the Roche LightCycler® 96 Instrument. β-actin was used as the internal control to normalize the data. The amplification program was as follows, 95 °C for 3 min, and 40 cycles at 95 °C for 15 s, 60 °C for 15 s, 72 °C for 20 s. Dissociation curves were analyzed at the end of the amplification. The fold change of gene

Hepatocellular BChE as a therapeutic target to ameliorate hypercholesterolemia through PRMT5 selective degradation. Life Sciences. (IF: 6.78)



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The screenshot shows a PubMed article page. The title is "Bacterial cellulose membranes used as artificial substitutes for dural deflection in rabbits". The authors are Chen Xu<sup>1</sup>, Xia Ma<sup>2</sup>, Shiwen Chen<sup>3</sup>, Meifeng Tao<sup>4</sup>, Lutao Yuan<sup>5</sup>, and Yao Jing<sup>6</sup>. The article is from the International Journal of Molecular Sciences (Int J Mol Sci), 2014 Jun 16;15(6):10855-67. The PMID is 24937688, PMCID is PMC4100185, and the DOI is 10.3390/ijms150610855. The abstract describes the evaluation of bacterial cellulose (BC) membranes as artificial dura mater substitutes in a rabbit model. The BC membranes were prepared from Acetobacter xylinum and used to repair dural defects. The results showed that BC membranes could repair dural defects and have a decreased inflammatory response compared to traditional materials.

The PCRs were performed in 20  $\mu$ L reaction volumes containing 2 $\times$  SYBR Green Mix 10  $\mu$ L, Primer Mix 1  $\mu$ L 9 (DongshengBio, Guangzhou, China), DNA template 1  $\mu$ L, ddH<sub>2</sub>O 8  $\mu$ L. Denaturation program (95  $^{\circ}$ C for 2 min), amplification and quantification program repeated 40 times (95  $^{\circ}$ C for 15 s, 59  $^{\circ}$ C for 20 s, 72  $^{\circ}$ C for 20 s with a single fluorescence measurement); Melting curve program (60–95  $^{\circ}$ C

Bacterial Cellulose Membranes Used as Artificial Substitutes for Dural Deflection in Rabbits.

International Journal of Molecular Sciences. (IF: 6.21)

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The screenshot shows the ScienceDirect website interface. The article title is "Newcastle-disease-virus-induced ferroptosis through nutrient deprivation and ferritinophagy in tumor cells". The authors listed are Xianjin Kan, Yuncong Yin, Cuiqing Song, Lei Tan, Xusheng Qiu, Ying Liao, Weiwei Liu, Songshu Meng, Yingjie Sun, and Chan Ding. The article is from iScience, Volume 24, Issue 8, 20 August 2021, 102837. The page includes a sidebar with navigation links like Outline, Highlights, Summary, etc., and a right sidebar with recommended articles and a feedback button.

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Newcastle-disease-virus-induced ferroptosis through nutrient deprivation and ferritinophagy in tumor cells. iScience. (IF: 6.11)

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Front Oncol. 2022 May 26;12:866289. doi: 10.3389/fonc.2022.866289. eCollection 2022.

### Identification and Validation of Novel Immune-Related Alternative Splicing Signatures as a Prognostic Model for Colon Cancer

Yunze Liu <sup>1, 2</sup>, Lei Xu <sup>3, 4</sup>, Chuanchuan Hao <sup>1</sup>, Jin Wu <sup>3</sup>, Xianhong Jia <sup>1</sup>, Xia Ding <sup>2</sup>, Changwei Lin <sup>5</sup>, Hongmei Zhu <sup>1</sup>, Yi Zhang <sup>3, 4</sup>

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PMID: 35692800 PMCID: PMC9178000 DOI: 10.3389/fonc.2022.866289  
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#### Abstract

**Background:** Individual immune-related alternative splicing (AS) events have been found to be significant in immune regulation and cancer prognosis. However, a comprehensive analysis of AS events in cancer cells based on immune-related genes (IRGs) has not been performed, and its clinical value is unknown.

**Methods:** Colon cancer cases with AS data were obtained from TCGA, and then, we identified overall survival-related AS events (OS-ASEs) based on IRGs by univariate analyses. Using Lasso regression, multivariate Cox regression, Kaplan-Meier analysis and nomograms, we constructed an AS risk model based on the calculated risk score. Furthermore, associations of the risk score with clinical and immune features were confirmed through the Wilcoxon rank sum test, association analysis, etc. Finally, by qRT-PCR, cell coculture and CCK-8 analyses, we validated the significance of OS-ASEs in

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### RNA Extraction and qRT-PCR

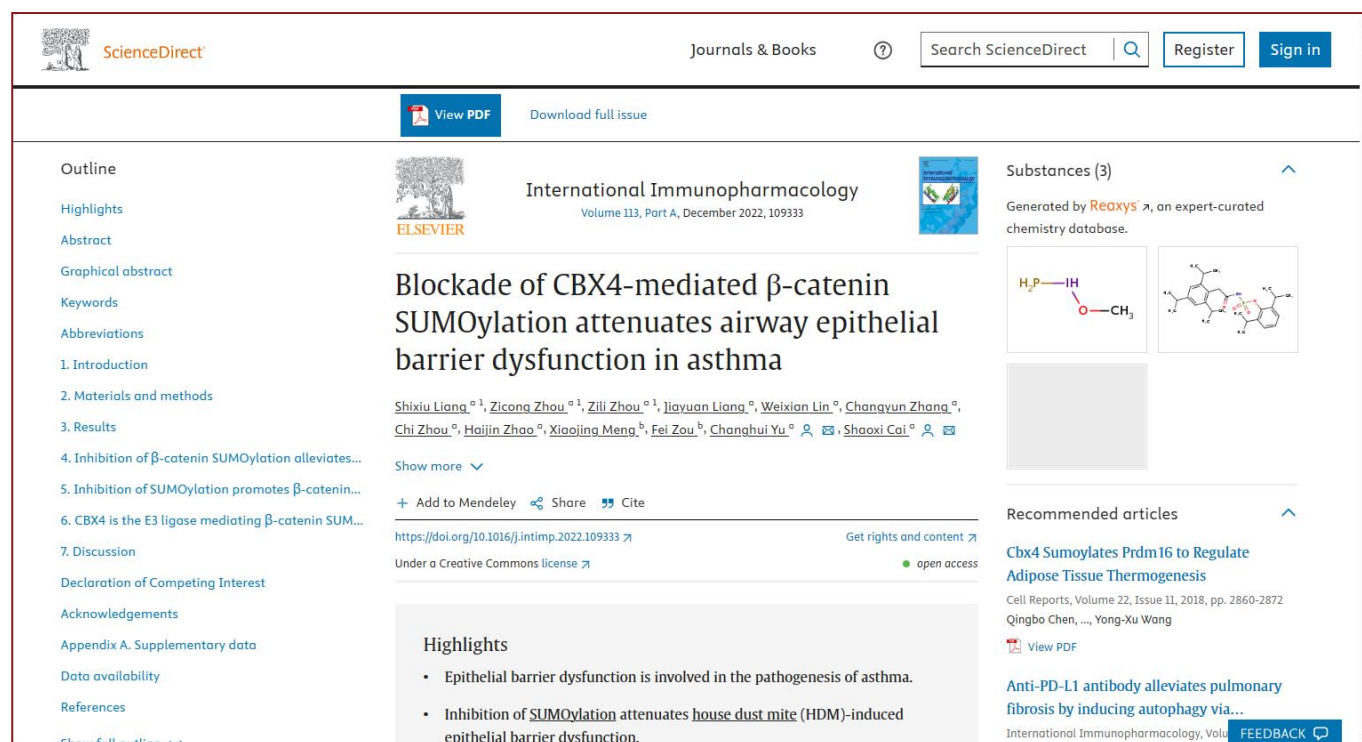
Total RNA was extracted from cells and tissues using the **TRIzol method (Dongsheng Bio. #R1022)** following the protocol. Then, the obtained RNAs were processed for cDNA synthesis. qRT-PCR was then performed using **SYBR Green qPCR Mix (Dongsheng Bio. #P2092)** and analysed on a Roche LightCycler system. The expression levels of the target genes were normalized based on the expression level of GAPDH. The primer sequences used for amplification and siRNA sequences are listed in the **Supplemental Material**.

Identification and Validation of Novel Immune-Related Alternative Splicing Signatures as a Prognostic Model for Colon Cancer.  
Frontiers in Oncology. (IF: 5.74)



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The screenshot shows the ScienceDirect interface for the article "Blockade of CBX4-mediated  $\beta$ -catenin SUMOylation attenuates airway epithelial barrier dysfunction in asthma" from the journal *International Immunopharmacology*, Volume 113, Part A, December 2022, 109333. The article is authored by Shixiu Liang, Zicong Zhou, Zili Zhou, Jiayuan Liang, Weixian Lin, Changyun Zhang, Chi Zhou, Haijin Zhao, Xiaojing Meng, Fei Zou, Changhui Yu, and Shaoli Cai. The article is available under a Creative Commons license. The page includes a sidebar with navigation links (Outline, Highlights, Abstract, Graphical abstract, Keywords, Abbreviations, 1. Introduction, 2. Materials and methods, 3. Results, 4. Inhibition of  $\beta$ -catenin SUMOylation alleviates..., 5. Inhibition of SUMOylation promotes  $\beta$ -catenin..., 6. CBX4 is the E3 ligase mediating  $\beta$ -catenin SUM..., 7. Discussion, Declaration of Competing Interest, Acknowledgements, Appendix A. Supplementary data, Data availability, References, Show full outline), a main content area with the title, authors, abstract, and highlights, and a right sidebar with substances (3) and recommended articles.


### 2.7. Quantitative PCR (qPCR)

RNA was isolated by a TRIzol reagent, following the manufacturer's instructions. The cDNA was synthesized via the reverse transcription (Accurate Biology, AG11706). **SYBR Green (GDSBio, P2105)** was used to perform qPCR using the Real-Time PCR instrument (Bio-Rad). The data were calculated through  $2^{-\Delta\Delta C_t}$  method to compare the difference. The following primers were used to amplify the target genes — MMP-9 forward: 5'-CGCCTCTGGAGGTTTCGAC-3', reverse: 5'-AACT-CACGCGCCAGTAGAAG-3'; VEGF forward: 5'-ATCTTCAAGC-CATCCTGTGTGC-3', reverse: 5'-CAAGGCCACAGGGATTTTC-3'; TGF- $\beta$ 1 forward: 5'-CGACTCGCCAGAGTGGTTAT-3', reverse: 5'-CGGTAGT-GAACCCGTTGATGT-3'; GAPDH forward: 5'-CCTCCTTCTGCACA-CATTTGAA-3', reverse: 5'-GAAGATGGTGATGGGATTTTC-3'.

Blockade of CBX4-mediated  $\beta$ -catenin SUMOylation attenuates airway epithelial barrier dysfunction in asthma. *International Immunopharmacology*. (IF: 5.71)

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*Foods*. 2022 Aug; 11(15): 2322.  
Published online 2022 Aug 3. doi: [10.3390/foods11152322](#)

PMCID: PMC9368296  
PMID: [35954087](#)

**Biochemical Traits, <sup>1</sup>H NMR Profile and Residual DNA Content of 'Asprinio', White Wine from Campania Region (Southern Italy)**

[Nicola Landi](#), [Monica Scognamiglio](#), [Pasqualina Woodrow](#), [Loredana F. Ciarmiello](#), [Sara Ragucci](#), [Angela Clemente](#), [Hafiza Z. F. Hussain](#), [Antonio Fiorentino](#), and [Antimo Di Maro](#)\*

Nuno Mateus, Academic Editor

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### 2.7.2. DNA Grapevine and Yeast Quantification

DNA quantification was carried out by quantitative PCR analyses using **SYBR Green qPCR Mix (high ROX; GDSBio)** by Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific, Rodano (MI), Italy). The reaction mix (20 µL) consisted of: 2× SYBR Green qPCR Mix, Forward 10 µM for each primer, and DNA template 50 ng. The amplification protocol was: 95 °C initial denaturation step for 3 min, followed by amplification cycles (40×) of denaturing at 95 °C for 10 s, annealing at 60 °C for 10 s and extension at 72 °C for 20 s. Amplification products were visualized on 1.5% (*w/v*) agarose gel, using a UV light. Primers used in this study are listed in Table S2.

Biochemical Traits, <sup>1</sup>H NMR Profile and Residual DNA Content of 'Asprinio', White Wine from Campania Region (Southern Italy). *Foods*. (IF: 5.56)

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The screenshot shows the ScienceDirect article page for the paper "Vvrr2: A new *Vibrio* ncRNA involved in dynamic synthesis of multiple biofilm matrix exopolysaccharides, biofilm structuring and virulence" published in *Aquaculture*, Volume 563, Part 2, 30 January 2023, Article 738925. The authors listed are Hongyan Cai, Ying Ma, Yingxue Qin, Lingmin Zhao, Qingpi Yan, and Lixing Huang. The abstract states that *Vibrio alginolyticus* is a widely distributed aquatic pathogen and that the study found Vvrr2 to be involved in virulence. The page also includes a sidebar with navigation links (Article preview, Abstract, Introduction, Section snippets, References (44), Cited by (4)) and a recommended articles section.

### 2.18. Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was performed using QuantStudio 6 Flex real-time PCR system (Life Technologies, Carlsbad, CA, USA) together with the **Power Green qPCR Mix** (Guangzhou Dongsheng Biotech Co., Ltd., China). Primers synthesized by the Xiamen Borui Biotechnology co., LTD (China) were listed in Supplementary Material 1. *gyrB* was used to normalize the gene expression levels. The relative levels of gene expression were calculated by  $2^{-\Delta\Delta Ct}$  method (Luo et al., 2016).

Vvrr2: A new *Vibrio* ncRNA involved in dynamic synthesis of multiple biofilm matrix exopolysaccharides, biofilm structuring and virulence.

*Aquaculture*. (IF: 5.14)



# Collection of Papers on PCR Products

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The screenshot shows a PubMed article page. The title is "Newcastle Disease Virus Manipulates Mitochondrial MTHFD2-Mediated Nucleotide Metabolism for Virus Replication". The authors are Ning Tang, Pingyi Chen, Changrun Zhao, Panrao Liu, Lei Tan, Cuiping Song, Xusheng Qiu, Ying Liao, Xiufan Liu, Tingrong Luo, Yingjie Sun, and Chan Ding. The article is from J Virol, 2023 Mar 30;97(3):e0001623. The abstract discusses the mechanism by which the Newcastle disease virus (NDV) remodels nucleotide metabolism to support self-replication. The article is available for free on the PMC (Public Medical Center) platform.

**RT-qPCR.** Total RNA was extracted from the cell samples using traditional extraction methods and reverse transcribed using M-MLV reverse transcriptase (Promega; M1705). The RT-qPCR probes were obtained using SYBR green qPCR mix (GDSBio, Guangzhou Gongsheng Biotech Co., Ltd.; P2093). The primers targeting different genes were as follows: c-Myc (35), SHMT1 (44), MTHFD1L (45), and MTHFD2 (46). The NDV NP primer sequences were as follows: 5'-CAACAATAGGAGTGGAGTGTCTGA-3' and downstream primer, 5'-CAGGGTATCG GTGATGTCTTCT-3'. All primer pairs were synthesized by Shanghai Sangon Biotech (Shanghai, China). All samples were standardized according to the mRNA level of  $\beta$ -actin.

Newcastle Disease Virus Manipulates Mitochondrial MTHFD2-Mediated Nucleotide Metabolism for Virus Replication.  
Journal of Virology. (IF: 5.10)