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## Letter to the editor

## Zika virus induces myocardial immune response and myocarditis in mice



## ARTICLE INFO

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## Dear editor,

Many types of viral infections can involve the human cardiovascular, such as Coxsackievirus, parvovirus B19, adenovirus, influenza virus, human herpes virus, Epstein-Barr virus, cytomegalovirus, hepatitis C virus, human immunodeficiency virus (HIV) [1], and even severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is currently circulating worldwide [2]. Zika virus (ZIKV), a type of RNA virus belonging to the family *Flaviviridae* and genus *Flavivirus*, was declared a Public Health Emergency of International Concern in February 2016 by the World Health Organization due to the severe damage it causes to the nervous system. Recently, ZIKV was detected in heart tissue [3], but the pathophysiological processes of ZIKV causing cardiovascular implications are still unclear [4]. Here, we established a mouse model of ZIKV pathogenesis by intraperitoneally (IP) inoculating  $10^4$  plaque-forming units (pfu) of ZIKV into 28-day-old IFN $\alpha/\beta$  receptor knockout (KO) mice, which is an accepted model for high viral loads [6]. All ZIKV-infected mice survived until sacrifice for experiments, but body mass reduction started at 5 days post-infection (DPI), consistent with previous studies [6,7].

ZIKV is transmitted through mosquito bites, sex, blood transfusions, and from a pregnant women to their fetuses [5]. We established a mouse model of ZIKV pathogenesis by intraperitoneally (IP) inoculating  $10^4$  plaque-forming units (pfu) of ZIKV into 28-day-old IFN $\alpha/\beta$  receptor knockout (KO) mice, which is an accepted model for high viral loads [6]. All ZIKV-infected mice survived until sacrifice for experiments, but body mass reduction started at 5 days post-infection (DPI), consistent with previous studies [6,7].

To examine whether direct infection occurred in the mouse myocardium, ZIKV was detected by immunofluorescence using the monoclonal antibody Z6 [7,8]. We found that Z6 was localized in cardiomyocytes ( $\alpha$ -actinin $^+$ ) of ZIKV-IP KO mice at 10 DPI (Fig. 1a) and Z6 was not observed in the heart of ZIKV-IP wild type (WT) mice at 10 DPI (Fig. S1a). Terminal dextrynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assays revealed that cardiomyocyte apoptosis was dramatically increased in ZIKV-infected mice at 10 DPI (Fig. 1b). Masson's staining revealed that myocardial fibrosis was significantly increased at 10 DPI in ZIKV-IP KO mice at 10 DPI (Fig. 1c, d) but myocardial fibrosis was not observed in ZIKV-IP WT mice at 10 DPI (Fig. S1b). Echocardiography demonstrated the deterioration of

fractional shortening and the ejection fraction at 10 DPI and 15 DPI compared to uninfected mice (Fig. 1e). Electro-Chemiluminescence-Assays revealed that the level of CK-MB (creatin kinase-muscle/brain) and cTnT (troponin T) were dramatically elevated in serum at 5, 10, and 15 DPI (Fig. 1f), indicating that cardiac injury occurred. Our results indicated that ZIKV can directly infect the myocardium and lead to cardiomyocyte death, myocardial injury, cardiac fibrosis, and heart dysfunction in ZIKV-infected KO mice but not in ZIKV-IP WT mice.

ZIKV relates to myocarditis and heart failure in patients [4]. We performed hematoxylin-eosin staining of mouse heart sections and found that ZIKV causes massive monocyte infiltration in the myocardium at 10 DPI (Fig. 1g, h). Immunohistochemistry showed that F4/80 $^+$  macrophages and CD3 $^+$  leucocytes were significantly increased in the myocardium at 10 DPI (Fig. 1g). Flow cytometry demonstrated that CD3 $^+$ CD4 $^+$  leucocytes and NK1.1 $^+$  cells were increased in peripheral blood at 10 DPI (Fig. 1i). Simultaneously, the levels of pro-inflammatory cytokines, including CC15, TNF $\alpha$ , IL-6, IFN $\gamma$ , CXCL10, and IFIT3, and the myocarditis-related genes PRF1 [9] and MYH7 [10] were increased in heart tissue as demonstrated by qRT-PCR detection (Fig. 1j). RNA sequencing identified 1794 up-regulated genes and 1730 down-regulated genes with false discovery rate  $< 0.05$  and  $\log_2$  (fold change)  $> 1$  in ZIKV-infected hearts at 10 DPI. Gene ontology analysis revealed that up-regulated genes were enriched for the immune-related pathway, while down-regulated genes were enriched for the pathway related to heart function (Fig. 1k). Anti-virus genes and myocarditis-related genes were up-regulated relative to uninfected mice (Fig. 1l). Comparison of the gene expression profiles with the data available from the ImmGen compendium (<https://www.immgen.org/>) revealed that NKT cells and macrophages were dramatically increased in the ZIKV-infected myocardium (Fig. 1m).

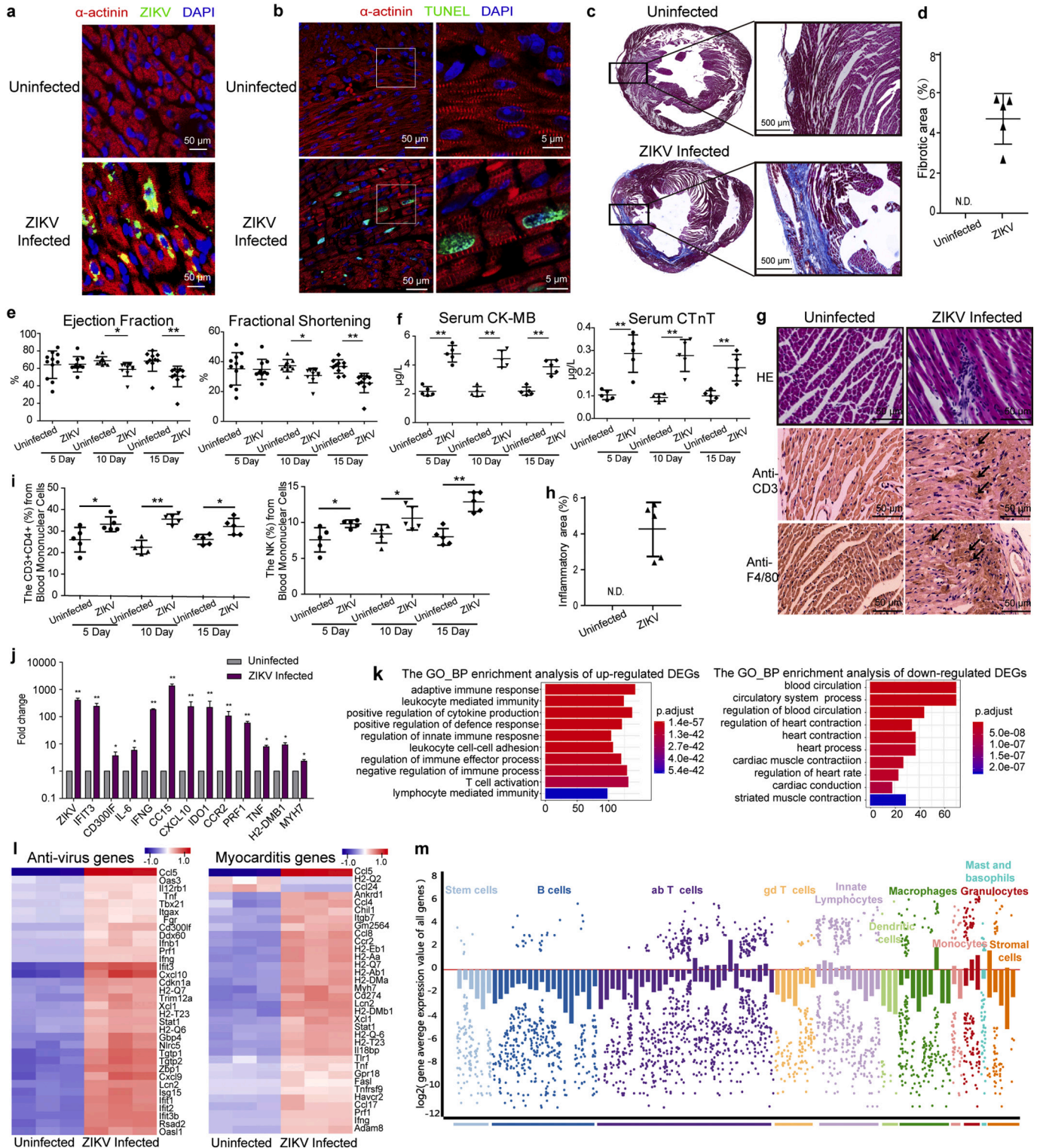
Together, we establish a IFN $\alpha/\beta$  receptor knockout mouse model of ZIKV pathogenesis in heart based on the contemporary strain ZIKV-SMGC-1. IFN $\alpha/\beta$  receptor-deficient mice are an accepted model for studying ZIKV pathogenesis, with high viral loads in organ [6]. However, IFN $\alpha/\beta$  receptor-deficient mice lack of key components of innate antiviral immunity, which only mimics the patients with immune deficiency disorder. Our results show that myocarditis and cardiac dysfunction could be observed in this mouse model, to check the effects of ZIKV on wild type mice, we also processed the study with wild

type mice of ZIKV infection and the results indicate that ZIKV could only induces myocardial immune response and myocarditis in IFN $\alpha$ / $\beta$  receptor knockout mice but not in wild type mice. Similar to ZIKV infected mice, ZIKV infection may causes great harm in persons with low immunity. Our results using the IFN $\alpha$ / $\beta$  receptor knockout mouse model show important implications for explaining heart damage in immunocompromised populations with ZIKV infection, and thus, more

attention should be paid to cardiovascular symptoms in clinical practice.

**Data and materials availability**

ZIKV strain SZ\_SMGC-1: GenBank accession number: KX266255 (<https://www.ncbi.nlm.nih.gov/nuccore/KX266255.1/>).



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**Fig. 1.** ZIKV infects mouse cardiomyocyte directly and causes myocarditis.

a Immunostaining shows that ZIKV (green) is detectable in cardiomyocytes ( $\alpha$ -actinin<sup>+</sup>, red) at 10 DPI.

b TUNEL (green) assay reveals that cardiomyocyte (red) apoptosis is increased in ZIKV-infected hearts at 10 DPI.

c, d Masson's staining displays that myocardial fibrosis is dramatically induced in ZIKV-infected mice at 10 DPI relative to uninfected mice.  $n = 5$  in each group.

e Echocardiography reveals that ejection fraction and fractional shortening is decreased at 10 and 15 DPI.  $n = 10$  in each group.

f ECLA shows that serum CK-MB and cTnT level is evaluated at 5, 10 and 15 DPI.  $n = 5$  in each group.

g, h HE staining displays monocyte infiltration in myocardium. Immunohistochemistry displays that F4/80<sup>+</sup> macrophages and CD3<sup>+</sup> leucocytes are increased significantly in myocardium relative to uninfected mice at 10 DPI.  $n = 5$  in each group.

i Flow cytometry indicates that both CD3<sup>+</sup>CD4<sup>+</sup> leucocytes and NK1.1<sup>+</sup> cells are evaluated in peripheral blood at 5, 10 and 15 DPI in ZIKV-infected mice, respectively.  $n = 5$  in each group.

j qRT-PCR uncovers the upregulation of pro-inflammatory cytokines and the myocarditis-related genes in myocardium at 10 DPI.  $n = 3$  in each group.

k Gene ontology (GO) analysis for up-regulated genes (left) and down-regulated genes (right), basing on RNA-Seq data. Top 10 GO terms is showed.

l Heatmaps show hierarchical clustering of enriched differentially expressed genes between ZIKV-infected and uninfected mouse hearts. Each lane represents result from one mouse.  $n = 3$  in each group.

m Leukocyte subtype analysis using ImmGen database (<https://www.immgen.org/>).

Data are presented as mean  $\pm$  SEM. N.D., not detected. \* $p < 0.05$ ; \*\* $p < 0.01$ . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## Ethics statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Institute of Microbiology, Chinese Academy of Sciences Ethics Committee. The protocols were approved by the Committee on the Ethics of Animal Experiments of Chinese Academy of Sciences. Inoculations were performed under anaesthesia with ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering. ZIKV researches were carried out under biosafety level 2 and animal BSL3 containment.

## Declaration of Competing Interest

Chongzhi Bai, Shihua Li, Shen Song, Qihui Wang, HeeCheol Cho, George Fu Gao, Yu Nie and Pengcheng Han declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmcc.2020.08.014>.

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Chongzhi Bai<sup>a,b,c,1</sup>, Shihua Li<sup>b,1</sup>, Shen Song<sup>d</sup>, Qihui Wang<sup>e</sup>,  
HeeCheol Cho<sup>f</sup>, George Fu Gao<sup>b,c,g</sup>, Yu Nie<sup>d,\*</sup>, Pengcheng Han<sup>f,\*</sup>  
<sup>a</sup> Central Laboratory, Shanxi Province Hospital of Traditional Chinese  
Medicine, Taiyuan 030012, China

<sup>b</sup> CAS Key Laboratory of Pathogenic Microbiology & Immunology, Institute  
of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

<sup>c</sup> Shanxi Academy of Advanced Research and Innovation, Taiyuan 030032,  
China

<sup>d</sup> State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National  
Center for Cardiovascular Disease, Chinese Academy of Medical Sciences  
and Peking Union Medical College, Beijing 100037, China

<sup>e</sup> CAS Key Laboratory of Microbial Physiological and Metabolic Engineering,  
Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101,  
China.

<sup>f</sup> Department of Biomedical Engineering, Emory University, Atlanta, GA  
10033, USA

<sup>g</sup> Research Network of Immunity and Health (RNIH), Beijing Institutes of  
Life Science, Chinese Academy of Sciences, Beijing 100101, China

E-mail addresses: [nieyu@fuwaihospital.org](mailto:nieyu@fuwaihospital.org) (Y. Nie),  
[hpcsoft88@aliyun.com](mailto:hpcsoft88@aliyun.com) (P. Han).

\* Corresponding author.

<sup>1</sup> These authors contributed equally to this work.