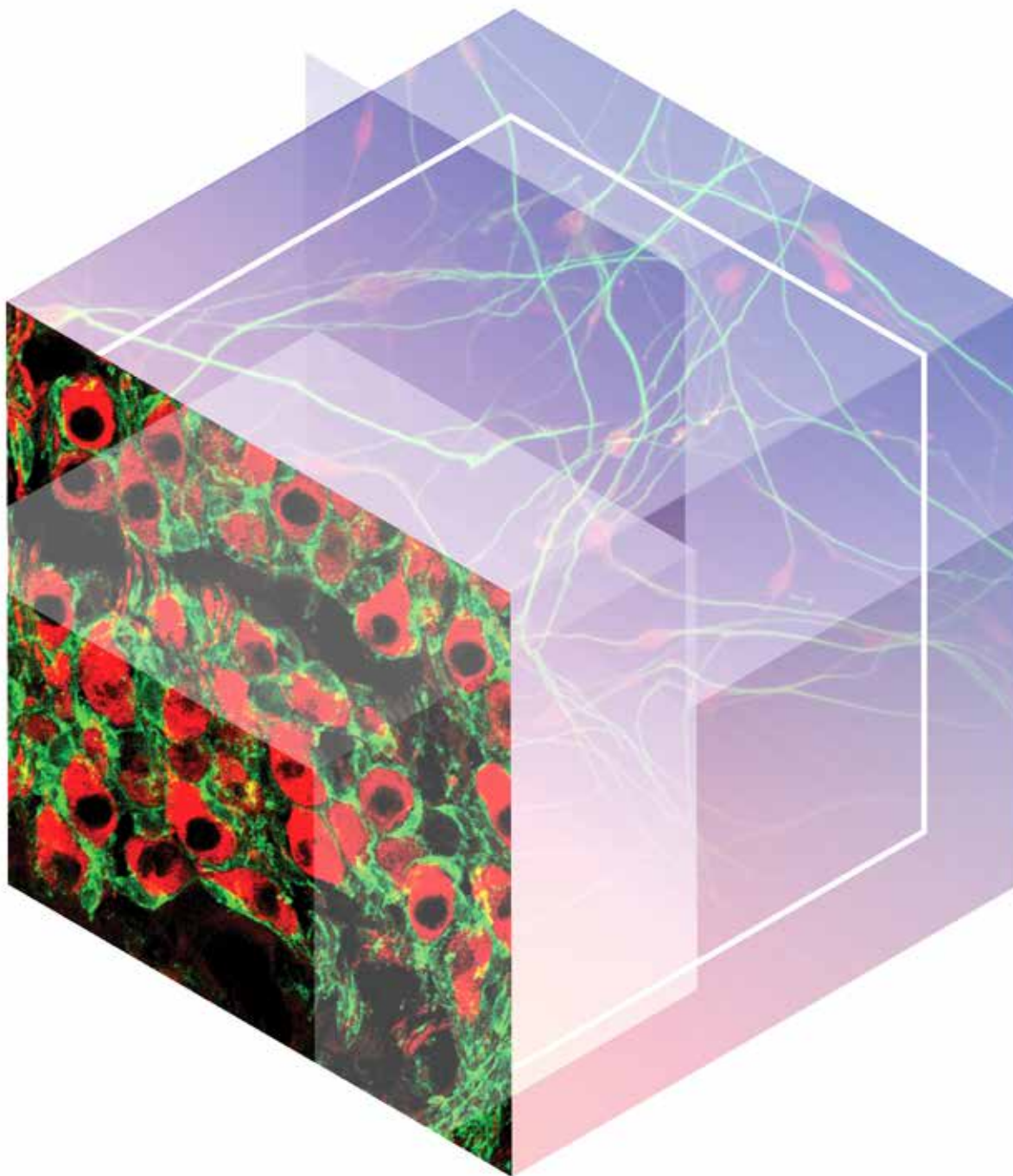





Model Animal Research Center of Nanjing University  
MOE key laboratory of model animal for disease study  
National resource center for mutant mice



# ANNUAL REPORT 2020

## Director's Words

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Year 2020 brought lots of changes to our society, which urge us to rethink of many aspects of our life. However, one thing does not, and also will not, change at MARC is our passion for excellence in science. MARC has been devoted to biomedical research using animal models to make discoveries for a healthy life. Over the last decade, we've become stronger in developing genetically-modified animals for modeling human diseases. Through introducing state-of-art genome editing technologies, we not only accelerate generation of genetically-modified animals, but also start to develop more precise and complex models for studying human diseases. As a member of the International Mouse Phenotyping Consortium (IMPC), we've also established a state-of-art platform for mouse phenomics, which help us to get better understanding of gene functions in a post-genomic era. To improve our abilities for in-depth functional studies, we've expanded other facilities at MARC, including the imaging core, metabolomics core, and flow cytometry core. With these state-of-art core facilities, MARC scientists have tackled some longstanding scientific questions, and made several important discoveries this year. In a study published in the Cell Reports, a joint team led by Dr. Yun Shi and Dr.

Guoqiang Wan identified TMEM63B as an osmosensor that is required for hearing. In another study published in the Nature Communications, Dr. Shuai Chen's group discovered SPEG as a novel insulin-responsive kinase that regulates calcium homeostasis in the heart. In a third study, Dr. Zhenji Gan's team deciphered a new mechanism for a histone methyltransferase MLL4 function in myofiber identity. Looking forward to the year ahead, I believe that more discoveries will be made in our ever-expanding research fields, from genetics and developmental biology to cancer biology, metabolic biology and neurobiology. We will continue to fulfil our mission at MARC pursuing first-class science for improvement of human health with the help of animal models.

My second term as the director of MARC will start from 2021. The privileges working with a great team of devoted professors, talented students and brilliant supporting personnel give me great confidence of a bright future for MARC. The success of MARC is also owed to great supports from friends of ours over years. I wish every MARCER and all friends a happier 2021!



*Shuai Chen*  
*Director*



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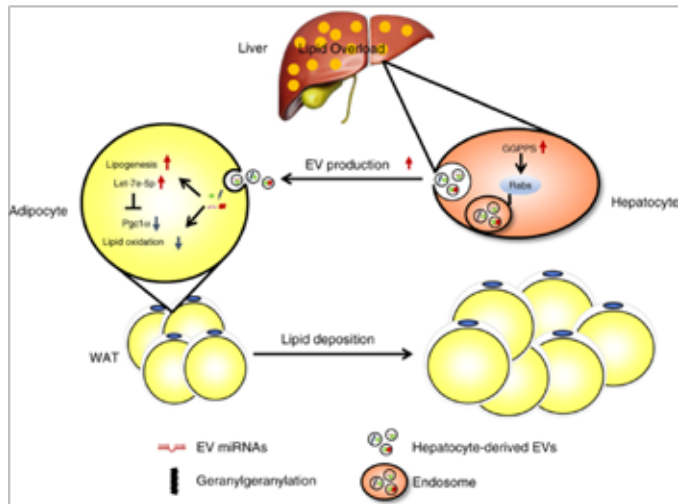
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## Group Chaojun Li

### Liver governs adipose remodelling via extracellular vesicles

Yue Zhao, Meng-Fei Zhao, Shan Jiang, Jing Wu, Jia Liu, Xian-Wen Yuan, Di Shen, Jing-Zi Zhang, Nan Zhou, Jian He, Lei Fang, Xi-Tai Sun, Bin Xue, Chao-Jun Li

Lipid overload results in lipid redistribution among metabolic organs such as liver, adipose, and muscle; therefore, the interplay between liver and other organs is important to maintain lipid homeostasis. Here, we show that liver responds to lipid overload first and sends hepatocyte-derived extracellular vesicles (EVs) targeting adipocytes to regulate adipogenesis and lipogenesis. Geranylgeranyl diphosphate synthase (Ggpps) expression in liver is enhanced by lipid overload and regulates EV secretion through Rab27A geranylgeranylation. Consistently, liver-specific Ggpps deficient mice have reduced fat adipose deposition. The levels of several EV-derived miRNAs in the plasma of non-alcoholic fatty liver disease (NAFLD) patients are positively correlated with body mass index (BMI), and these miRNAs enhance adipocyte lipid accumulation. Thus, we highlight an inter-organ mechanism whereby the liver senses different metabolic states and sends corresponding signals to remodel adipose tissue to adapt to metabolic changes in response to lipid overload.

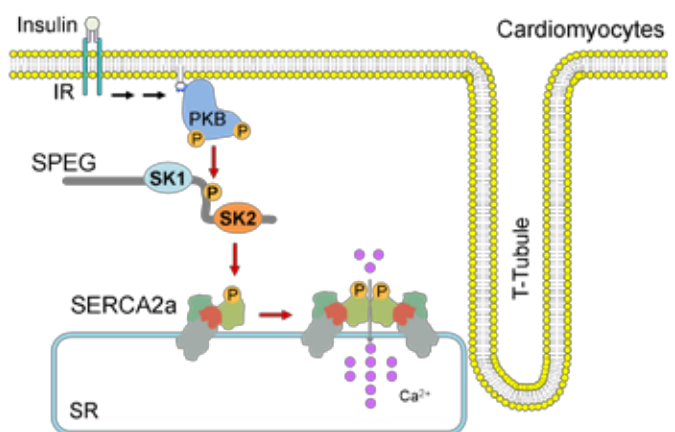


## Group Shuai Chen and Hongyu Wang

### A PKB-SPEG signaling nexus links insulin resistance with diabetic cardiomyopathy by regulating calcium homeostasis

Chao Quan, Qian Du, Min Li, Ruizhen Wang, Qian Ouyang, Shu Su, Sangsang Zhu, Qiaoli Chen, Yang Sheng, Liang Chen, Hong Wang, David G. Campbell, Carol MacKintosh, Zhongzhou Yang, Kunfu Ouyang, Hong Yu Wang\* and Shuai Chen\*

Diabetic cardiomyopathy is a progressive disease in diabetic patients, and myocardial insulin resistance contributes to its pathogenesis. However, the molecular mechanisms linking insulin resistance to diabetic cardiomyopathy are incompletely understood. Striated muscle preferentially expressed protein kinase (SPEG) has two kinase domains (SK1 and SK2) and is a critical regulator of cardiac development and function. Here we show that SPEG is phosphorylated on a small cluster of serine/threonine residues including Ser<sup>2461</sup>/Ser<sup>2462</sup>/Thr<sup>2463</sup> by protein kinase B (PKB) in response to insulin. PKB-mediated phosphorylation of SPEG activates its SK2, which in turn phosphorylates sarcoplasmic/endoplasmic reticulum calcium-ATPase 2a (SERCA2a) and accelerates calcium re-uptake into the sarcoplasmic/endoplasmic reticulum. Deletion of PKB $\alpha/\beta$  in the heart or a high fat diet inhibits insulin-induced phosphorylation of SPEG and SERCA2a, prolongs SR re-uptake of calcium, and impairs cardiac function. A genetically-modified mouse model bearing a Speg<sup>3A</sup> knockin mutation to prevent its phosphorylation by PKB displayed cardiac dysfunction without systemic metabolic changes. Importantly, the Speg<sup>3A</sup> knockin mutation impairs SERCA2a phosphorylation and calcium re-uptake into the SR in cardiomyocytes. Collectively, these data demonstrate that phosphorylation of SPEG by PKB activates its SK2 and the PKB-SPEG signaling nexus is important for maintenance of cardiac function through regulating SERCA2a-mediated calcium re-uptake into the SR in cardiomyocytes. Impairment of this PKB-SPEG signal nexus may contribute to the development of diabetic cardiomyopathy.



Working model of the PKB-SPEG signaling axis in cardiomyocytes.



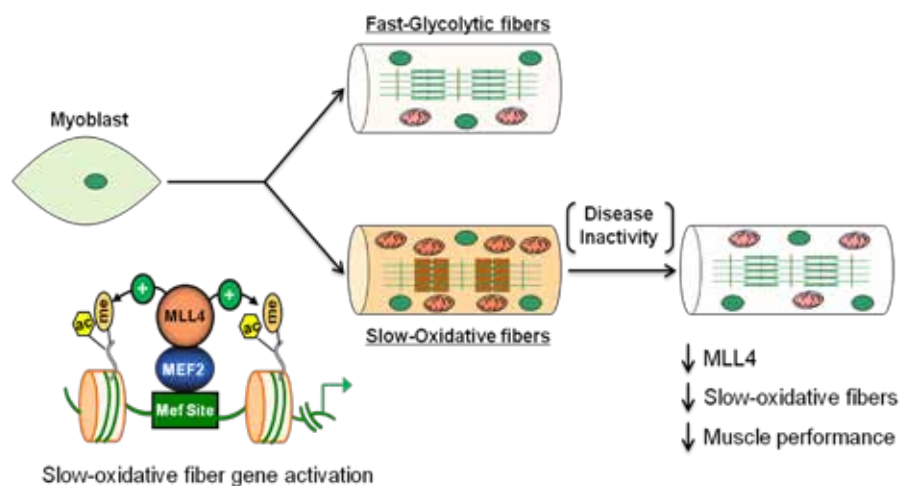
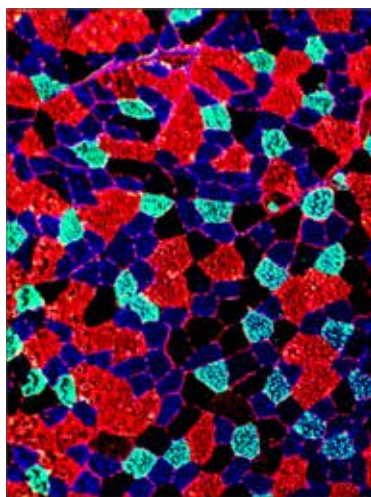
## Group Zhenji Gan

### MLL4 controls myofiber identity and running endurance

Lin Liu, Chenyun Ding, Tingting Fu, Zhenhua Feng, Ji-Eun Lee, Liwei Xiao, Zhisheng Xu, Yujing Yin, Qiqi Guo, Zongchao Sun, Wanping Sun, Yan Mao, Likun Yang, Zheng Zhou, Danxia Zhou, Leilei Xu, Zezhang Zhu, Yong Qiu, Kai Ge, and Zhenji Gan

Skeletal muscle depends on the precise orchestration of contractile and metabolic gene expression programs to direct fiber type specification and to ensure muscle performance. Exactly how such fiber type-specific patterns of gene expression are established and maintained remains unclear, however. Here, we demonstrate that histone monomethyltransferase MLL4, an enhancer regulator enriched in slow myofibers, plays a critical role in controlling muscle fiber identity as well as muscle performance. Skeletal muscle-specific ablation of MLL4 in mice resulted in downregulation of the slow-oxidative myofiber gene program, decreased number of type I myofibers, and diminished mitochondrial respiration, which caused reductions in muscle fat

utilization and endurance capacity during exercise. Genome-wide ChIP-seq and mRNA-seq analyses revealed that MLL4 directly binds to enhancers and functions as a coactivator of the myocyte enhancer factor 2 (MEF2) to activate transcription of slow-oxidative myofiber genes. Importantly, we also found that the MLL4 regulatory circuit is associated with muscle fiber type remodeling in humans. Thus, our results uncover a pivotal role for MLL4 in specifying structural and metabolic identities of myofibers that govern muscle performance. These findings provide new therapeutic opportunities for enhancing muscle fitness to combat a variety of metabolic and muscular diseases.

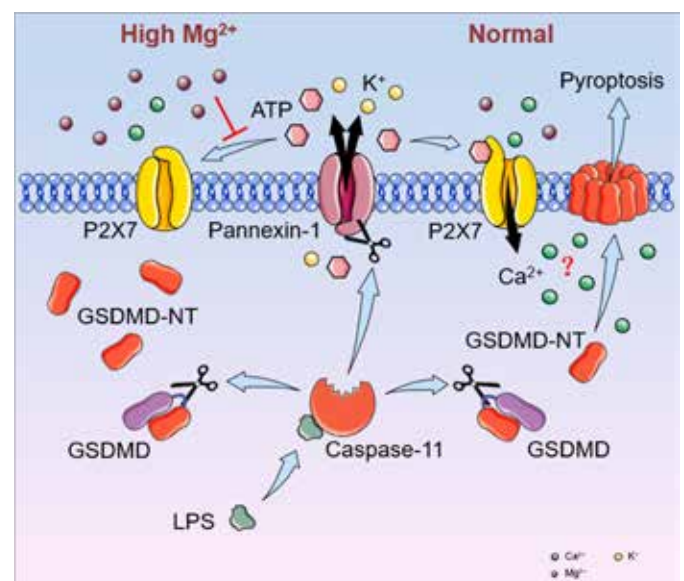


## Group Xiang Gao and Zhaoyu Lin

### Magnesium Protects against Sepsis by Blocking Gasdermin D N-Terminal-Induced Pyroptosis

Dingyu Wang<sup>1,3</sup>, Jiashuo Zheng<sup>2,3</sup>, Qiongyuan Hu<sup>2</sup>, Cheng Zhao<sup>2</sup>, Qianyu Chen<sup>1</sup>, Peiliang Shi<sup>1</sup>, Qin Chen<sup>1</sup>, Yujie Zou<sup>1</sup>, Dayuan Zou<sup>1</sup>, Qiyao Liu<sup>1</sup>, Jingwen Pei<sup>1</sup>, Xiuwen Wu<sup>2</sup>, Xiang Gao<sup>1,\*</sup>, Jianan Ren<sup>2,\*</sup>, Zhaoyu Lin<sup>1,\*</sup>

Hypomagnesemia is a significant risk factor for critically ill patients to develop sepsis, a life-threatening disease with a mortality rate over 25%. Our clinic data analysis showed that hypomagnesemia is associated with a decreased monocyte count in septic patients. At the cellular level, we found that Mg<sup>2+</sup> inhibits pyroptosis. Specifically, Mg<sup>2+</sup> limits the oligomerization and membrane localization of gasdermin D N-terminal (GSDMD-NT) upon the activation of either the canonical or non-canonical pyroptotic pathway. Mechanistically, we demonstrated that Ca<sup>2+</sup> influx is a prerequisite for the function of GSDMD-NT. Mg<sup>2+</sup> blocks Ca<sup>2+</sup> influx by inhibiting the ATP-gated Ca<sup>2+</sup> channel P2X7, thereby impeding the function of GSDMD-NT and inhibiting lipopolysaccharide (LPS)-induced non-canonical pyroptosis. Furthermore, Mg<sup>2+</sup> administration protects mice from LPS-induced lethal septic shock. Together, our data reveal the underlying mechanism of how Mg<sup>2+</sup> inhibits pyroptosis and suggest potential clinic applications of magnesium supplementation for sepsis prevention and treatment.



## The cation channel TMEM63B is an osmosensor required for hearing

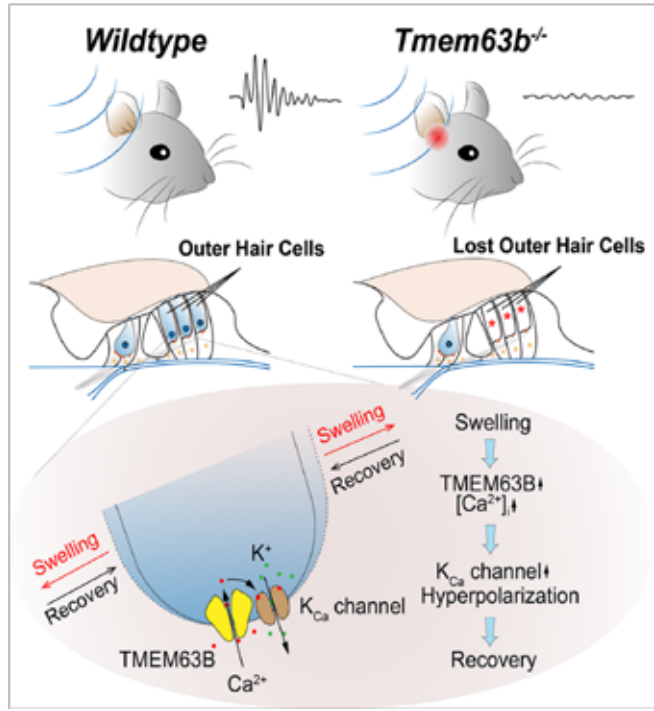
Du H, Ye C, Wu D, Zang YY, Zhang L, Chen C, He XY, Yang JJ, Hu P, Xu Z, Wan G\*, Shi SY\*

### Significance:

Life has evolved appropriate adaptive responses to maintain cell shape and volume when facing osmotic stress. Hypotonic stress causes the activation of swelling-activated nonselective cation channels (NSCCs), which leads to a  $\text{Ca}^{2+}$ -dependent regulatory volume decrease (RVD) and the adaptive maintenance of cell volume; however, the molecular identities of the osmosensitive NSCCs remain unclear. In this study, we identified TMEM63B as an osmosensitive NSCC activated by hypotonic stress. TMEM63B is enriched in the inner ear sensory hair cells. Genetic deletion of TMEM63B results in necroptosis of outer hair cells (OHCs) and progressive hearing loss. Mechanistically, the TMEM63B channel mediates hypoosmolarity-induced  $\text{Ca}^{2+}$  influx, which activates  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels required for the maintenance of OHC morphology. These findings demonstrate that TMEM63B is the osmosensor of mammalian ear and the long-sought cation channel mediating  $\text{Ca}^{2+}$ -dependent RVD.

### Highlights:

1. TMEM63B is a hypoosmolarity-activated cation channel
2. Deficiency of TMEM63B causes hearing loss in mice
3. TMEM63B is localized in hair cells and required for outer hair cell survival
4. TMEM63B mediates  $\text{Ca}^{2+}$ -dependent regulatory volume decrease in outer hair cells



The image features a large, central, glowing blue cell with a prominent, darker blue nucleus. The cell is rendered with a soft, ethereal glow and is set against a background of other similar, but slightly out-of-focus, cells. The overall color palette is dominated by various shades of blue, creating a scientific and futuristic atmosphere. The word "Neurobiology" is superimposed over the central cell in a white, italicized font.

# *Neurobiology*



## Yun Shi , Ph.D

Yun Shi received Ph.D degree in physiology at Georgia State University under the mentoring of Dr. Chun Jiang at Atlanta, USA in 2007. His Ph.D work focus on the function and regulation of vascular KATP channels. He then had postdoctoral training with Dr. Roger Nicoll in UCSF where he worked on synaptic plasticity. In 2013, he joined the Model Animal Research Center, Nanjing University as a professor and principal investigator.

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## The Mechanisms of Neural Plasticity

The central neural system (CNS) is a complex network, in which the appropriate functions depend on the information exchange among neurons through a specified structure named synapse. The neurotransmitters released from presynaptic neurons bind to postsynaptic receptors, enhancing or inhibiting postsynaptic activity. Meanwhile, postsynaptic neurons also release certain regulatory information and modulate presynaptic activity, allowing the feedback to occur. Many of high neuronal functions result from the changes of neurotransmission, or so called plasticity. Dysfunction of synaptic plasticity is one of the major causes of neurodegeneration diseases. Therefore, studying the synaptic transmission not only help unreal human high neural functions but also improve our understanding on the mechanisms of neural diseases and provide cues for cure.

Glutamate is the major excitatory neurotransmitter in CNS. Two groups of glutamate receptors are located on the post-synaptic membrane, i.e., ionotropic and metabotropic glutamate receptors. Ionotropic receptors

include AMPA, NMDA and Kainate receptors; each are composed of different subunits. The normal function of excitatory synapses and the generation of neural plasticity are determined by the composition and expression level of postsynaptic glutamate receptors. However, how the receptors correctly traffic to post-synaptic membrane and how the expression level is appropriately regulated remain unclear.

Hippocampus is a relative simple structure in brain, which is believed to play an essential role in learning and memory. The CA3-CA1 synapses are arguably the best studied synapses in brain. The plasticity of those synapses, including long-term potentiation (LTP) and long-term depression (LTD), are believed to be the fundament of learning and memory mechanisms.

Current research interests in our lab include: 1. The fundament of synaptic plasticity such as LTP and LTD. 2. Diseases associated with glutamate signal pathway. 3. Physiological functions of the mechanosensitive cation channel TMEM63 family.

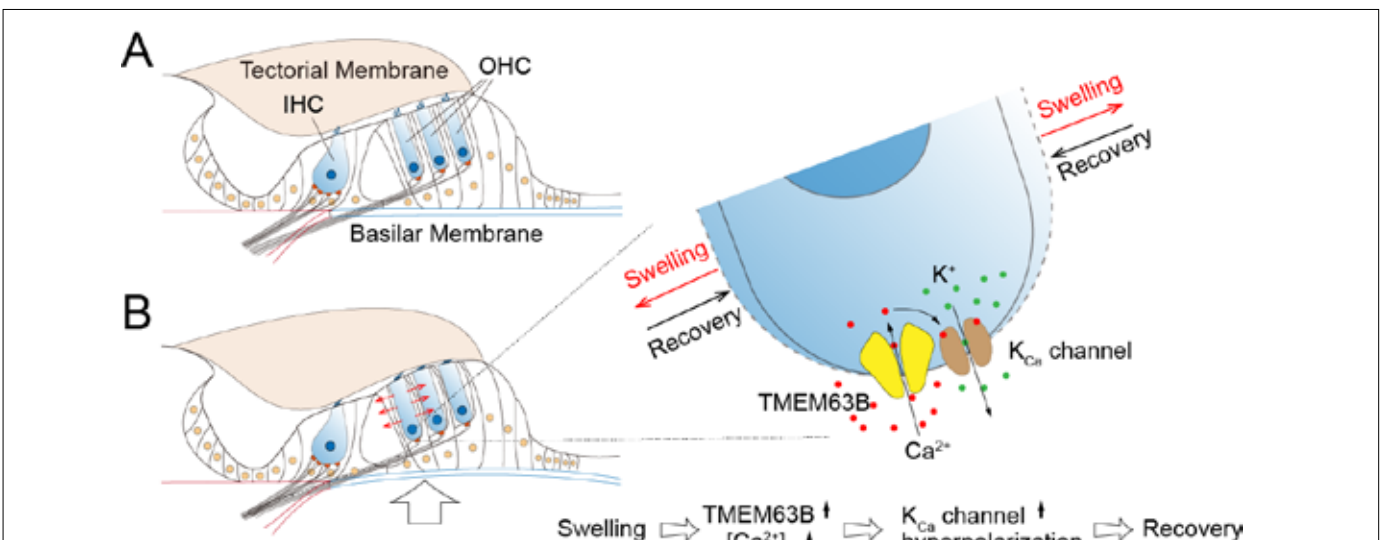


Figure 1. A novel ion channel TMEM63B is an osmosensor required for hearing.

A. The organ of Corti composed of supporting cells and inner and outer hair cells.

B. The basilar membrane transmits vibration to hair cells causing deflection of hair bundles against the tectorial membrane and hair cell depolarization. Outer hair cells are endowed with cell motility. The swelling OHCs induced by the hypoosmotic or other physiological stimuli (such as depolarization) activate the osmosensitive channel TMEM63B, which mediates the  $Ca^{2+}$  influx, leading to activation of the  $Ca^{2+}$ -activated  $K^+$  channel. The efflux of  $K^+$  leads to the hyperpolarization of OHCs, contributing to the shape recovery of OHCs.



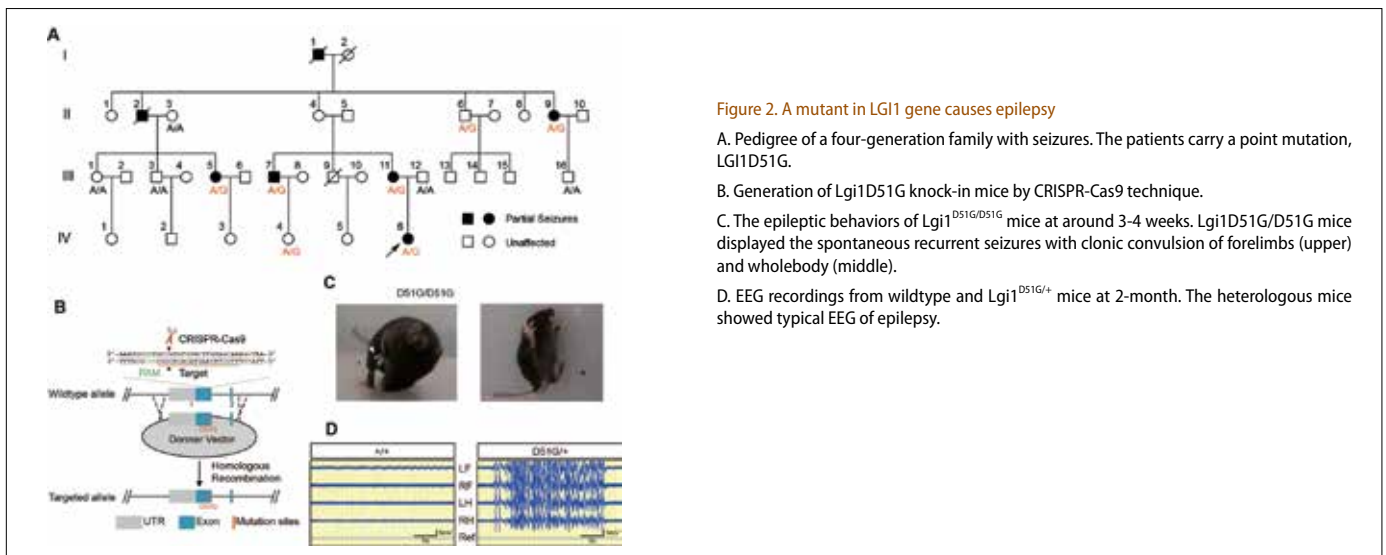


Figure 2. A mutant in *LGI1* gene causes epilepsy

A. Pedigree of a four-generation family with seizures. The patients carry a point mutation, *LGI1*D51G.

B. Generation of *Lgi1*D51G knock-in mice by CRISPR-Cas9 technique.

C. The epileptic behaviors of *Lgi1*<sup>D51G/D51G</sup> mice at around 3-4 weeks. *Lgi1*D51G/D51G mice displayed the spontaneous recurrent seizures with clonic convulsion of forelimbs (upper) and wholebody (middle).

D. EEG recordings from wildtype and *Lgi1*<sup>D51G+</sup> mice at 2-month. The heterologous mice showed typical EEG of epilepsy.

## Selected publications

- Wu D, Zang YY, Shi YY, Ye C, Cai WM, Tang XH, Zhao L, Liu Y, Gan Z, Chen GQ, Xu Y, Yang JJ\*, Shi YS\*. (2020). Distant Coupling between RNA Editing and Alternative Splicing of the Osmosensitive Cation Channel *Tmem63b*. *Journal of Biological Chemistry*. Online
- Wei T, Lu S, Sun J, Xu Z, Yang X, Wang F, Ma Y, Shi YS\* and Chen X\*. (2020) Sanger's Reagent Sensitized Photocleavage of Amide Bond for Constructing Photocages and Regulation of Biological Functions. *Journal of the American Chemical Society*. 142(8):3806-3813.
- Du H, Ye C, Wu D, Zang YY, Zhang L, Chen C, He XY, Yang JJ, Hu P, Xu Z, Wan G\*, Shi SY\*. (2020) The cation channel *TMEM63B* is an osmosensor required for hearing. *Cell Reports*. 31:107596. IF=7.11
- Li YJ, Duan GF, Sun JH, Wu D, Ye C, Zang YY, Chen GQ, Shi YY, Wang J, Zhang W\*, Shi YS\*. (2019) Neto proteins regulate gating of the kainate-type glutamate receptor *GluK2* through two binding sites. *J Biol Chem*. 294(47):17889-17902.
- Duan GF, Xu S, Ye Y, Tao W, Nicoll RA, Shi YS\*, and Sheng N\*. (2018) Signal peptide represses *GluK1* surface and synaptic trafficking through binding to amino-terminal domain. *Nat Commun*. Nov 19;9(1):4879.
- Sheng N, Bembem MA, Díaz-Alonso J, Tao W, Shi YS, Nicoll RA. (2018) LTP requires postsynaptic PDZ-domain interactions with glutamate receptor/auxiliary protein complexes. *PNAS*. 115(15):3948-3953.
- Chen J, Du Y, He X, Huang X\*, Shi YS\*. (2017) A Convenient Cas9-based Conditional Knockout Strategy for Simultaneously Targeting Multiple Genes in Mouse. *Sci Rep*. 7(1):517.
- Niu Y, Dai Z, Liu W, Zhang C, Yang Y, Guo Z, Li X, Xu C, Huang X, Wang Y, Shi YS\*, Liu JJ\*. (2017) Ablation of *SNX6* leads to defects in synaptic function of CA1 pyramidal neurons and spatial memory. *Elife*. 6. pii: e20991.
- Sheng N, Shi YS, Nicoll RA. (2017) The amino-terminal domains of kainate receptors determine the differential dependence on Neto auxiliary subunits for trafficking. *PNAS*. 114(5):1159-1164.



## Group members

### Former graduate students

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Guifang Duan, Peking University;  
Han Du, Getein Biotech;  
Dan Wu, Drum Tower Hospital;  
Chang Ye.

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Shixiao Peng    Wenmin Cai  
Chaohua Jiang   Tianzi Zhang  
Yueying Wang    Yuhan Ge  
Xiaoyu Teng      Yangyang Chen  
Qingqing Li      Jingjing Tu  
Guolin Yang

### Visiting students      Technicians

Xiaohui Tang      Yanyu Zang  
Haoyang Feng  
Xiaoyu Yin



## Guiquan Chen, Ph.D.

Guiquan obtained his PhD in Neuroscience at the University of Edinburgh in Scotland in 2005 and then conducted his postdoctoral research at Harvard Medical School in Boston. He joined the MARC of Nanjing University as a Principle Investigator in December of 2011. His long-term research goal is to understand molecular mechanisms by which the  $\gamma$ -secretase complex regulates neuronal survival and/or death. His lab uses a combination of mouse genetics, molecular biology, cellular and behavioral neuroscience techniques to address this question. Elucidation of molecular mechanisms for age-related neurodegeneration may help identify novel therapeutic targets for the prevention and the treatment of neurodegenerative diseases.

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## Molecular and cellular mechanisms for neurodevelopmental and neurodegenerative diseases

Radial glial progenitors (RGPs) and intermediate progenitors (IPs) are major neural progenitor cells (NPCs) in the developing cortex. Proliferation of RGPs, switch of RGPs to IPs and neuronal differentiation are critical processes during corticogenesis. At early embryonic stages, the proliferation of RGPs and the switch of RGPs to IPs are finely balanced to promote the expansion of neural stem cells (NSCs) and the growth of the cortex.

Presenilin enhancer 2 (Pen-2) is a key component of  $\gamma$ -secretase. It not only contributes to the catalytic property of this enzyme but also acts as a substrate-binding site. Early work reported that knockdown of Pen-2 results in decreased  $\gamma$ -secretase activity. It has been shown that Pen-2 is critical for the endoproteolysis of PS1, and that Pen-2 is sufficient to generate active  $\gamma$ -secretase through the endoproteolysis of presenilin (PS). A recent study revealed an essential role of Pen-2 in development, since germ-line deletion of this gene causes early embryonic lethality

in mice. Abundant evidence has shown that the 19q13 microdeletion syndrome manifests microcephaly and intellectual disability [1-5]. Since Pen-2 is located in the deleted region, it might act as one of the candidate genes to cause this disorder. However, the underlying mechanisms remain unknown.

To address the above question, we generated NPC specific Pen-2 cKO mice. We observed rapid depletion of RGPs but transient increase on IPs in the dorsal telencephalon of Pen-2 cKO mice (Figs.1-2). Molecular analysis reveals decreased  $\gamma$ -secretase activity but increased levels of neurogenic transcription factors such as Ngn2 and NeuroD1 in Pen-2 cKO animals. We show that expression of NICD restores the population of RGPs and IPs in Pen-2 cKO cortices (Fig.3). These findings provide direct evidence indicating that Pen-2 controls the fate of NSCs via Notch-dependent switch of RGPs to IPs.

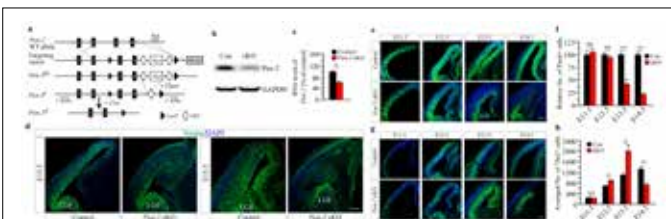


Figure 1. Loss of RGPs and IPs in Pen-2 cKO mice. (a-c) Characterization of NPC specific Pen-2 cKO embryos. (d) IHC for Nestin. (e,f) Loss of Pax6+ cells in Pen-2 cKO mice. (g,h) Changes on Tbr2+ cells in Pen-2 cKO mice.

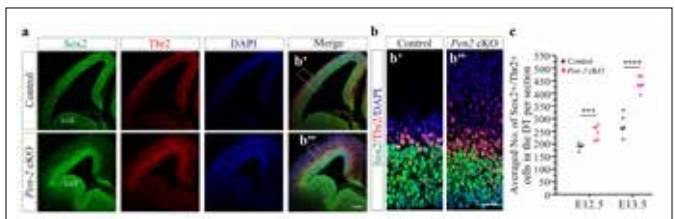


Figure 2. Enhanced cell switch of RGPs to IPs in Pen-2 cKO mice. (a-c) Increased number of Sox2+/Tbr2+ cells in Pen-2 cKO mice.

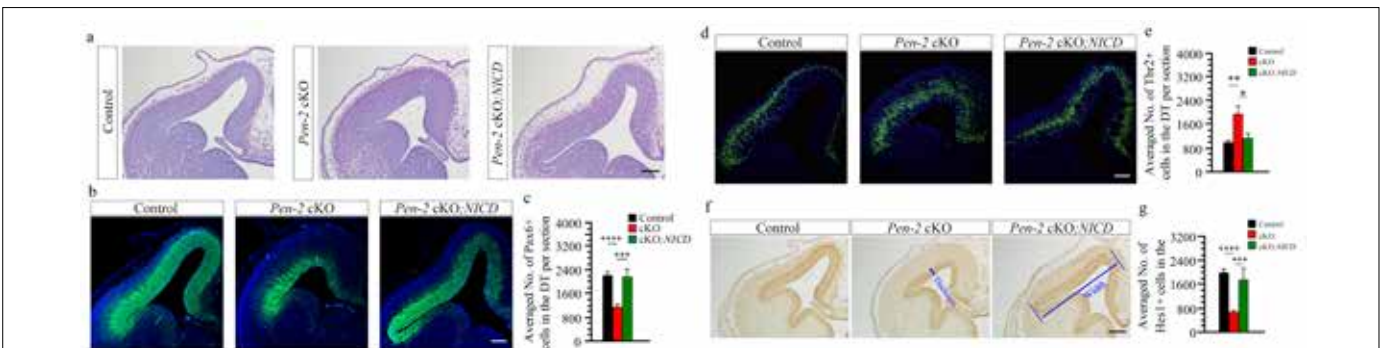


Figure 3. Rescue effects on NPCs in Pen-2 cKO mice by NICD. (a) Nissl staining. (b-c) Comparable number of Pax6+ cells between control and Pen-2 cKO mice expressing NICD. (d,e) Comparable number of Tbr2+ cells between control and Pen-2 cKO mice expressing NICD. (f,g) Comparable number of Hes1+ cells between control and Pen-2 cKO mice expressing NICD.



## Recent publications (\*, Corresponding author)

1. Wu J, Shao C, Ye X, Di X, Li D, Zhao H, Zhang B\*, Chen G\*, Liu H\* and Qian Y\*. In vivo brain imaging amyloid- $\beta$  aggregates in Alzheimer's disease by near-infrared fluorescence probe. *ACS Sensors* 2021, acssensors.0c01914.
2. Wang H, Liu M, Zou G, Wang L, Duan W, He X, Ji M, Zou X, Hu Y\*, Yang J\* and Chen G\*. Deletion of PDK1 in oligodendrocyte lineage cells causes white matter abnormality and myelination defect in the central nervous system. *Neurobiol. Dis.* 2021, 105212.
3. Shao C, Yuan J, Liu Y, Wang X, Gu J, Chen G, Zhang B, Liu H, Zhao J, Zhu H and Qian Y. Epileptic brain fluorescent imaging reveals apigenin can relieve the myeloperoxidase-mediated oxidative stress and inhibit ferroptosis. *Proc. Natl. Acad. Sci. USA* 2020, 117(19): 10155
4. Bi H, Zhou C, Zhang Y, Cai X, Ji M, Yang J, Chen G\*, and Hu Y\*. Neuron-specific deletion of presenilin enhancer2 causes progressive astrogliosis and age-related neurodegeneration in the cortex independent of the Notch signaling. *CNS Neurosci. Ther.* 2020, cns13454.
5. Huang C, Liu T, Wang Q, Hou W, Zhou C, Song Z, Shi SY, Gao X, Chen G\*, Yin Z\* and Hu Y\*. Loss of PP2A disrupts the retention of radial glial progenitors in the telencephalic niche to impair the generation of late-born neurons during cortical development (Cover story). *Cereb. Cortex* 2020, 30(7): 4183-4196.
6. Ma X, Wang Y, Hua J, Xu C, Yang T, Yuan J, Chen G\*, Guo Z\* and Wang X\*. A $\beta$ -sheet-targeted theranostic agent for diagnosing and preventing aggregation of pathogenic peptides in Alzheimer's disease (Cover story). *Sci. China Chem.* 2020, 63(1): 73-82.
7. Cheng S, Liu T, Hu Y, Xia Y, Hou J, Huang C, Zou X, Shi Y, Zheng Y, Lu J\* and Chen G\*. Conditional inactivation of Pen-2 in the developing neocortex leads to rapid switch of apical progenitors to basal progenitors. *J. Neurosci.*, 2019, 39(2): 2195-2207.
8. Xu C, Yu L, Hou J, Jackson RJ, Wang H, Huang C, Liu T, Wang Q, Zou X, Morris RGM, Spire-jones TL, Yang Z, Yin Z, Xu Y and Chen G\*. Conditional deletion of PDK1 in the forebrain causes neuron loss and increased apoptosis during cortical development. *Front. Cell. Neurosci.*, 2017; 00330.



## Group members

### Group leader

Guiquan Chen

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Huiru Bi

Yingqian Xia

Xiaolian Ye

Mengjia Liu

Yizhi Zhang

Xudong Cai

### Former members of the lab

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Long Wang

Congyu Xu

Chen Zhang

Jinxing Hou

Chaoli Huang

Tingting Liu

He Wang



## Huiming Gao M.D., Ph.D.

Huiming Gao received her M.D. in 1993 and Ph.D. in 2003 from Dalian Medial University. Her Ph.D. thesis was carried out at the National Institute of Environmental Health Sciences (NIEHS)/ National Institutes of Health (NIH) in the USA. After her postdoctoral training at University of Pennsylvania School of Medicine and NIEHS/NIH, Dr. Gao joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2013. She is now a professor and a principle investigator in MARC

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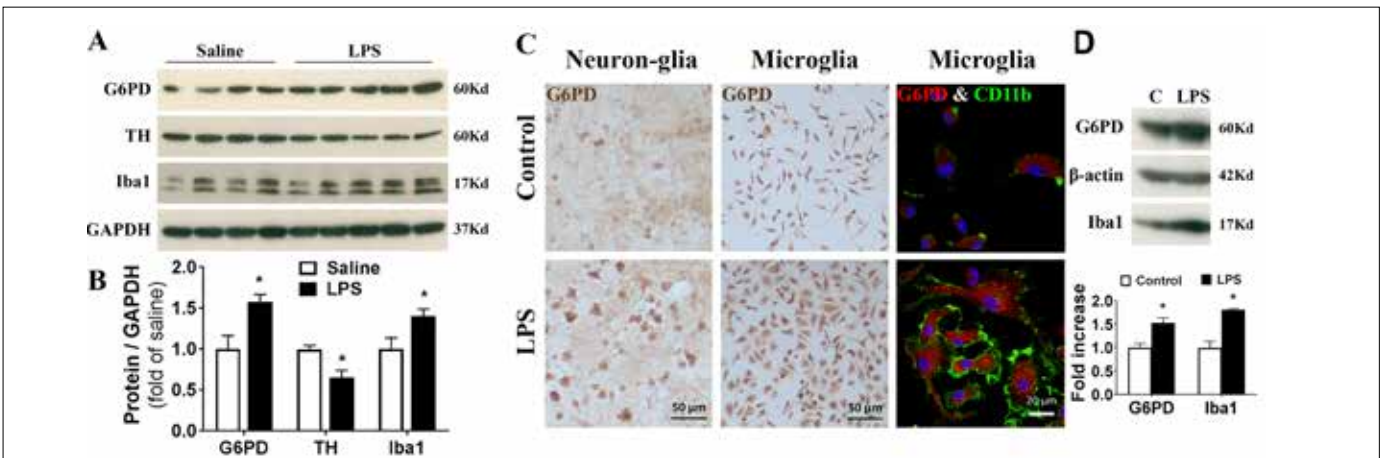
## Neuroinflammation, neurodevelopment, and neurodegeneration

Chronic inflammation contributes to the pathogenesis of both neurodevelopmental diseases such as Autism in early childhood and age-related neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Chronic, irreversible degeneration of brain neurons causes progressive memory loss in AD and movement impairment (e.g. tremor and rigidity) in PD. What drives the decades-long neuronal death and progression of these diseases remains unknown. There is no cure for these devastating diseases. The goal of our research is to investigate a potential driving role for chronic neuroinflammation in progressive neuronal impairment in Autism and neurodegenerative diseases, to identify new therapeutic targets, and to develop novel anti-inflammatory and neuroprotective therapeutics for these diseases.

Metabolic dysfunction and neuroinflammation are increasingly implicated in Parkinson's disease (PD). The pentose phosphate pathway (PPP, a metabolic pathway parallel to glycolysis) converts glucose-6-phosphate into pentoses and generates ribose-5-phosphate and NADPH thereby governing anabolic biosynthesis and redox homeostasis. Brains and immune cells display high activity of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the PPP. Postmortem studies of PD brains reveal perturbation of the PPP and dysregulation of G6PD enzyme. Here, we characterized spatial and temporal changes in activity/expression of G6PD in brains of PD models. We further found that brain inflammation

induced PPP disruption and aberrant metabolic-inflammatory axis in brain microglia mediating chronic dopaminergic neurodegeneration.

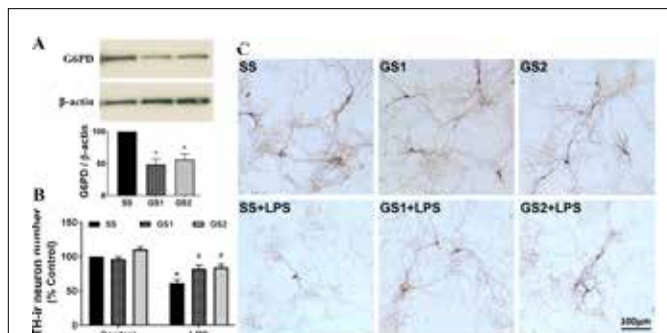
We detected sustained elevation in the expression and activity of G6PD in lipopolysaccharide (LPS)-treated mesencephalic neuron-glia cultures (an in vitro PD model) and in the substantia nigra of mice with an intranigral or intraperitoneal injection of LPS or with daily subcutaneous injection of MPTP for 5 consecutive days (three in vivo PD models). Pharmacological inhibition of G6PD activity by commonly used inhibitors, 6-aminonicotinamide (6-AN) and siRNA-mediated knockdown of microglial G6PD attenuated LPS-elicited chronic dopaminergic neurodegeneration. Moreover, G6PD inhibition by 6-AN injection attenuated LPS-induced oxidative stress, inflammatory response, dopaminergic neurodegeneration, and locomotor impairment. Mechanistically, microglia with elevated G6PD activity produced excessive NADPH and provided abundant substrate to over-activated NADPH oxidase leading to increased production of reactive oxygen species (ROS). Collectively, we demonstrated that PPP-mediated glucose metabolism disruption and neuroinflammation exacerbated each other mediating chronic neurodegeneration. Insight into metabolic-inflammatory interface suggests that manipulation of activity of the PPP and NADPH oxidase is potential therapeutic interventions in PD.



**Figure 1. LPS induced sustained high activity of the pentose phosphate pathway.**

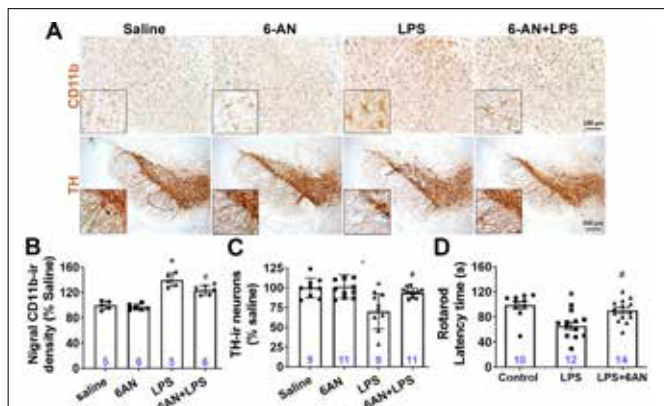
(A, B) Sustained high activity of G6PD after LPS treatment in rodent mesencephalic neuron-glia cultures. (C) Immunocytochemical staining for G6PD on mouse neuron-glia cultures and microglia-enriched cultures, and double-labeled immunofluorescence on microglia. (D) Increased expression of G6PD and Iba1 in microglia-enriched cultures upon treatment with 10 ng/ml LPS for 24 hours.





**Figure 2. Suppression of G6PD activity by siRNA-mediated biological knockdown protected dopamine neurons from LPS-elicited inflammatory insult.**

(A) Both siRNAs of G6PD displayed apparent knockdown of G6PD in primary microglia-enriched cultures transfected with scramble RNA (SS) or siRNAs of G6PD (GS1 and GS2) for 30 hours. (B, C) The reconstituted cultures, which were prepared by adding microglia with 30-h knockdown of G6PD by siRNAs onto mouse neuron-astrocyte layer, were treated with vehicle or LPS for 5–6 days. Knockdown of microglial G6PD by both G6PD siRNAs protected dopamine neurons against LPS-elicited neurodegeneration.



**Figure 3. G6PD inhibition by 6-AN attenuated LPS-elicited microglial activation, dopaminergic neurodegeneration, and locomotor impairment.**

C57BL/6 J mice received an intranigral injection of LPS (2  $\mu$ g) with 6-AN (20 nmol) or saline. Six weeks later, microglial activation (A, B), loss of DA neurons (A, C), and locomotor deficit (D) were determined by immunocytochemistry (A–C) and the rotarod behavior test (D).

### Selected publications (\* Corresponding author)

- Liu D, Zhao Y, Qi Y, Gao Y, Tu DZ, Wang Y, Gao H-M\*, Zhou H\* (2020) Benzo(a)pyrene exposure induced neuronal loss, plaque deposition, and cognitive decline in APP/PS1 mice. *Journal of Neuroinflammation* 17(1):258
- Gao Y, Tu DZ, Yang R, Chu CH, Hong JS and Gao H-M\* (2020) Through Reducing ROS Production, IL-10 Suppresses Caspase-1-Dependent IL-1 Maturation, thereby Preventing Chronic Neuroinflammation and Neurodegeneration. *Int. J. Mol. Sci.*, 21, 465
- Tu DZ, Gao Y, Yang R, Guan T, Hong JS and Gao H-M\* (2019) The pentose phosphate pathway regulates chronic neuroinflammation and dopaminergic neurodegeneration. *Journal of Neuroinflammation* 16:255
- Gao H-M\*, Gao Y, Tu DZ, Liu Y, Zhou H, and Hong JS (2018) Inflammation-mediated Neurodegeneration: Models, Mechanisms, and Therapeutic Interventions for neurodegenerative Diseases. In: *Inflammation—From Molecular and Cellular Mechanisms to the Clinic* (Jean-Marc Cavaillon & Mervyn Singer, ed). pp1255-1278. Wiley-VCH
- Chu CH, Wang S, Chen SH, Wang Q, Lu RB, Gao H-M\*, and Hong JS (2016) Neurons and astroglia govern microglial endotoxin tolerance through macrophage colony-stimulating factor receptor-mediated ERK1/2 signals. *Brain Behavior and Immunity* 55: 260–272
- Chu CH, Chen SH, Wang Q, Langenbach R, Li H, Zeldin D, Chen SL, Wang S, Gao H-M\*, Lu RB\*, Hong JS (2015) PGE2 Inhibits IL-10 Production via EP2-Mediated  $\beta$ -Arrestin Signaling in Neuroinflammatory Condition. *Molecular Neurobiology*. 52(1):587-600.
- Zhou H, Liao J, Aloor J, Nie H, Wilson B, Fessler M, Gao H-M\*, Hong J-S (2013) CD11b/CD18 is a novel receptor mediating extracellular dsRNA-induced immune responses. *Journal of Immunology* 190:115-125
- Gao H-M\*, Zhou H, Hong J-S (2012) NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. *Trends in Pharmacological Sciences* 33(6): 295-303 (SCI citations: 40)



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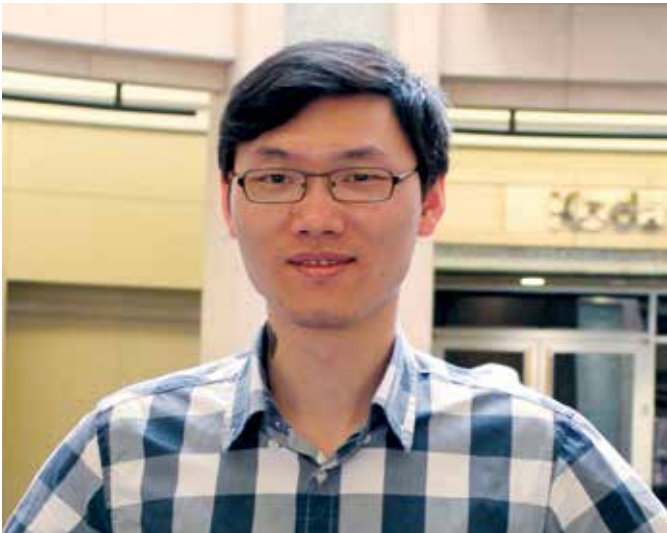
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## Guoqiang Wan, Ph.D.

Guoqiang Wan received both of his BSc in 2004 and PhD in 2011 from the National University of Singapore. He then had postdoctoral training with Dr Gabriel Corfas first at the Harvard Medical School/Boston Children's Hospital from 2011-2014 and then at the University of Michigan from 2014-2016. He joined MARC of Nanjing University as Principal Investigator in July 2016. His long term research goal is to regenerate cochlear sensory cells and synapses for hearing restoration.

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## Development and Regeneration of Auditory Sensory Cells and Synapses

In China, 27.8 million people suffer from disabling hearing loss and this number increases by 300,000 every year. Sensorineural hearing loss (SNHL) accounts for 90% of all hearing loss and in most cases it cannot be medically or surgically treated. Mechanistically, SNHL results from damages to the sensory hair cells that are essential for sound detection and/or the spiral ganglion neurons (SGNs) that are required for transmitting the acoustic signals to the brain. In addition, even with the presence of intact sensory epithelia, hearing problems can also arise from irreversible loss of the synaptic connections between hair cells and SGNs, an auditory pathology termed as cochlear synaptopathy. Therefore, restoration of auditory functions requires not only preservation or regeneration of the sensory hair cells, neurons and non-sensory supporting cells, but also re-establishment of the cochlear synaptic connections (Figure 1). Our lab aims to identify novel molecular targets and pathways for the development and regeneration of cochlear sensory cells and synapses and to explore therapeutic potentials of these targets for treatment of sensorineural hearing loss.

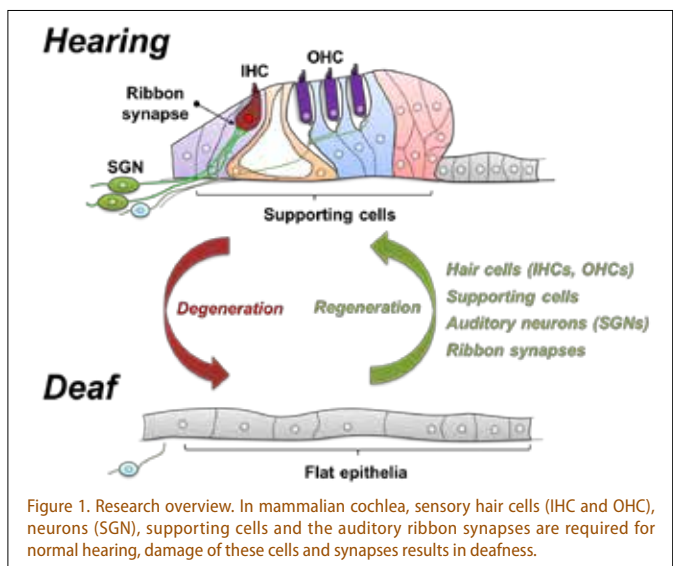


Figure 1. Research overview. In mammalian cochlea, sensory hair cells (IHC and OHC), neurons (SGN), supporting cells and the auditory ribbon synapses are required for normal hearing, damage of these cells and synapses results in deafness.

### (1) Molecular mechanism and treatment of genetic hearing loss in mouse models.

More than 50% of deafness cases are due to genetic defects with no treatment available. DFNA15, caused by mutations of the transcription factor POU4F3, is one of the most common types of autosomal dominant non-syndromic deafness. Here, we established a novel mouse model with the exact Pou4f3 mutation identified in human patients. The mutant mouse display similar auditory pathophysiology as human patients and exhibit multiple hair cell abnormalities. The onset and severity of hearing loss in the mouse model is highly modifiable to environmental factors, such as aging, noise exposure or genetic backgrounds. Using a new knockout mouse model, we found Pou4f3 haploinsufficiency as the underlying mechanism of human DFNA15. Importantly, we identified Aldh inhibitor as a potent small molecule for upregulation of Pou4f3 and treatment of hearing loss in the mutant mouse. The identification of Aldh inhibitor for treatment of DFNA15 deafness represents a major advance in the unmet medical need for this common form of progressive hearing loss. This study (Figure 2) titled "Aldh inhibitor restores auditory function in a mouse model of human deafness" was published in PLOS Genetics.

### (2) Novel regulators of hair cell development and reprogramming using cochlear organoids

Loss of hair cells is the primary cause of sensorineural hearing loss. Unlike fish, birds and amphibians, mammalian hair cells do not regenerate, posing great challenge in restoration of auditory function in deaf humans. Due to the scarcity of cochlear sensory cells and lack of appropriate cell culture models, high throughput screening (HTS) for regulators of hair cells is severely limited. To circumvent this problem, we established a robust, high throughput cochlear organoid platform that facilitates 3D expansion of cochlear progenitor cells and differentiation of hair cells in a temporary-regulated manner. High throughput screening of the FDA-approved drug library identified Regorafenib, a VEGFR inhibitor, as a potent small molecule for hair cell differentiation. Regorafenib also promotes reprogramming and maturation of hair cells in both normal and neomycin-damaged cochlear explants. Mechanistically, inhibition of VEGFR suppresses TGFβ1 expression via MEK pathway and TGFβ1 downregulation directly mediates the effect of Regorafenib on hair cell reprogramming. Our study (Figure 3) not only demonstrates the power of cochlear organoid platform in high throughput analyses of hair cell physiology, but also highlights VEGFR-MEK-TGFβ1 signaling crosstalk as a potential target for hair cell regeneration and hearing restoration.



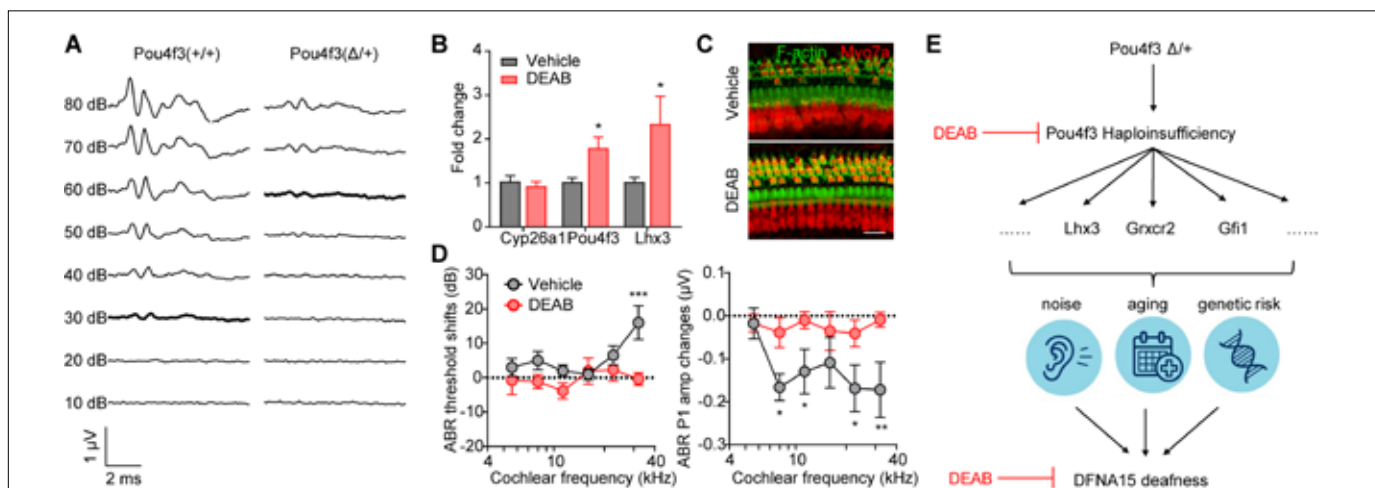


Figure 2. Molecular mechanism and small molecule treatment of human DFNA15 deafness in mouse models. (A) Hearing loss of 3 months old Pou4f3(Δ/+) mutant mice mimics human DFNA15 deafness. (B-D) Aldh inhibitor DEAB promotes expression of Pou4f3 (B), reduces hair cell loss (C) and inhibits hearing loss progression (D). (E) Proposed mechanism of this study (small inserts modified from Mayo Clinic).

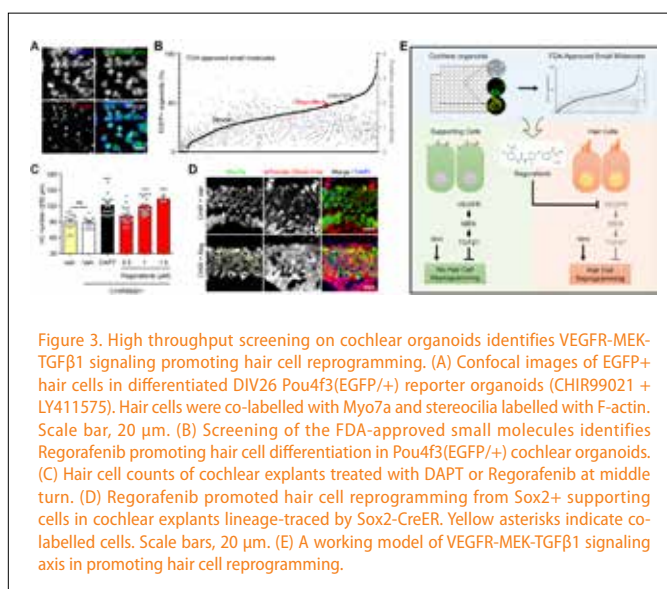


Figure 3. High throughput screening on cochlear organoids identifies VEGFR-MEK-TGFβ1 signaling promoting hair cell reprogramming. (A) Confocal images of EGFP+ hair cells in differentiated DIV26 Pou4f3(EGFP/+) reporter organoids (CHIR99021 + LY411575). Hair cells were co-labelled with Myo7a and stereocilia labelled with F-actin. Scale bar, 20 μm. (B) Screening of the FDA-approved small molecules identifies Regorafenib promoting hair cell differentiation in Pou4f3(EGFP/+) cochlear organoids. (C) Hair cell counts of cochlear explants treated with DAPT or Regorafenib at middle turn. (D) Regorafenib promoted hair cell reprogramming from Sox2+ supporting cells in cochlear explants lineage-traced by Sox2-CreER. Yellow asterisks indicate co-labelled cells. Scale bars, 20 μm. (E) A working model of VEGFR-MEK-TGFβ1 signaling axis in promoting hair cell reprogramming.

### Selected Publications(\*co-first authors, #co-senior authors)

- Zhu, G.J.\*, Gong, S.\*, Ma, D.B.\*, Tao, T.\*, He, W.Q.\*, Zhang, L., Wang, F., Qian, X.Y., Zhou, H., Fan, C., Wang, P., Chen, X., Zhao, W., Sun, J., Chen, H., Wang, Y., Gao, X., Zuo, J., Zhu, M.S.#, Gao, X.#, Wan, G.# (2020). Aldh inhibitor restores auditory function in a mouse model of human deafness. PLOS Genetics, 16(9):e1009040.
- Du, H.\*, Ye, C.\*, Wu, D.\*, Zang, Y.Y., Zhang, L., Chen, C., He, X.Y., Yang, J.J., Hu, P., Xu, Z., Wan, G.# and Shi, Y.S.# (2020). The cation channel TMEM63B is an osmosensor required for hearing. Cell Reports, 31(5):107596.
- Kohrman, D.C.\*, Wan, G.\*, Cassinotti, L.\*, Corfas, G. (2020). Hidden Hearing Loss: A Disorder with Multiple Etiologies and Mechanisms. Cold Spring Harbor Perspectives in Medicine, 10(1):a035493.
- Wan, G.\*, Ji, L.\*, Schrepfer, T., Gong, S., Wang, G.P., Corfas G.# (2019). Synaptopathy as a Mechanism for Age-Related Vestibular Dysfunction in Mice. Frontier in Aging Neuroscience, 11:156.
- Schaefer, S.A., Higashi A.Y., Loomis, B., Schrepfer, T., Wan, G., Corfas, G., Dressler, G.R. and Duncan R.K. (2018). From Otic Induction to Hair Cell Production: Pax2EGFP Cell Line Illuminates Key Stages of Development in Mouse Inner Ear Organoid Model. Stem Cells and Development, 27(4), 237-251.
- Long P.\*, Wan, G.\*, Roberts, M.T., Corfas, G. (2018). Myelin development, plasticity, and pathology in the auditory system. Developmental Neurobiology, 78(2), 80-92.
- Wan, G.# and Corfas, G.# (2017). Transient auditory nerve demyelination as a new mechanism for hidden hearing loss. Nature Communications, 8, 14487.



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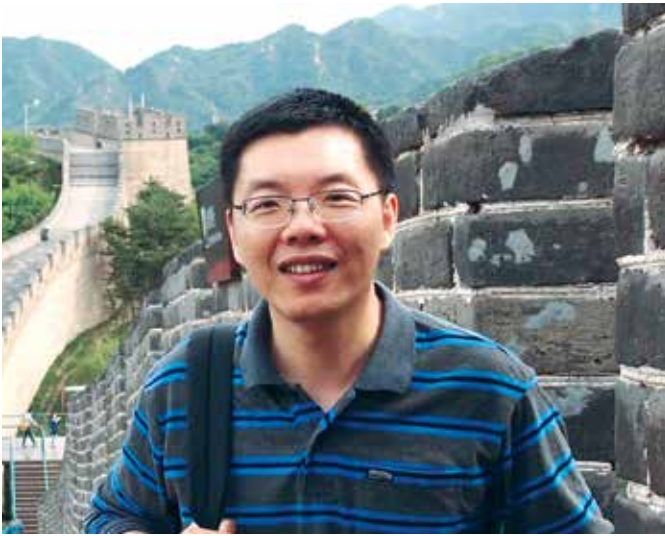
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# *Organogenesis*







## Jiong Chen Ph.D.

Jiong Chen received his Bachelor in Biochemistry (1995) and his Ph.D. in Molecular, Cell and Developmental Biology (2002), both from University of California, Los Angeles (UCLA). His Ph.D. thesis was carried out in Frank Laski's lab and it was focused on the genetics and developmental studies of cell movement processes in the *Drosophila* ovary. From 2002 to 2004, Jiong did his postdoctoral research in *Drosophila* eye development under the guidance of Utpal Banerjee at UCLA, and it was combined with an undergraduate teaching experience that was funded by a HHMI teaching/research grant. He joined the Faculty of Model Animal Research Center (MARC), Nanjing University full time early 2005. He is now a professor of genetics and developmental biology and a principal investigator in MARC.

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## Understanding the driving forces underlying collective cell Migration

Cells do not always migrate individually; they often migrate collectively as a cluster, a sheet, or a strand under physiological, developmental and cancer metastatic conditions. Collective cell migration has recently received much attention from cell and developmental biologists, and it has emerged as an important field of study with many characteristics distinct from those of single cell migration. As a new field, collective migration still has many fundamental questions unresolved. For example, what intrinsic factors or signals pre-determine the migratory fate of a group of cells that will later collectively detach and migrate away from the host tissue (likened to a group of runners pre-selected from a larger group of candidate runners)(Figure 1)? How can the group of cells communicate with each other and collectively know the front vs. back, top vs. bottom and inside vs. outside during migration (Figures 2 and 3)? Finally, what powers the group to migrate collectively (Figure 3)?

A recent and primary focus of my lab has been to address these key questions. We utilize the border cells in *Drosophila* ovary to study collective migration during development, and they are genetically tractable and amenable to live imaging and optogenetic manipulation.

Cell growth regulates fate determination of border cells. Recently, my lab found that the fate determination of border cells was negatively regulated by the growth-promoting InR/Akt/TORC1 signaling pathway (Fig 1; Kang et al., *Dev Cell*, 2018). During development, cell growth and cell differentiation are two distinct yet coupled fundamental processes to give rise to tissues or organs. However, the mechanisms underlying the coordination or coupling

between cell growth and cell differentiation are largely unknown. Our novel finding suggests that specification and differentiation of migratory cells is negatively coupled to cell growth during development.

**Control of front-back polarity.** It is known that the chemotactic migration of border cells is guided by the guidance receptor PVR, in response to extracellular signals secreted from oocyte. But, how guidance signaling sets up the front-back polarity of the entire border cell cluster is not well understood. We've made an interesting discovery that the guidance receptor PVR mediates the asymmetric distribution of exocyst and recycling endosome to set up the front-back polarity. (Wan et al., *Development*, 2013). Furthermore, we find that molecules crucial in apical-basal polarity, including aPKC and Crumbs complex, are required for the establishment of front-back polarity (Fig 3; Wang et al., *Development*, 2018). In addition, we find interesting coordination among the front-back, apical-basal and inside-outside polarities within the border cell cluster.

**Power control of collective migration.** We found that the actin depolymerizing factor Cofilin is required for the formation of actin-based lamellipodia, whose protrusion and adhesion provide force for migration of border cells (Zhang et al., *Development*, 2011). Moreover, Cofilin localization and phosphorylation are regulated by guidance receptor (PVR) signaling in such a way that active and unphosphorylated Cofilin are enriched in the leading border cell, resulting in the predominant protrusion forming only at the front of border cell cluster.

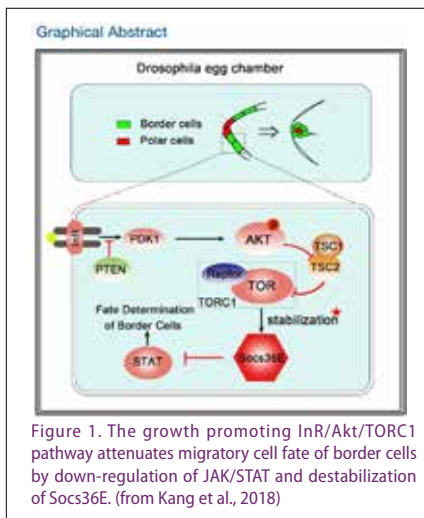


Figure 1. The growth promoting InR/Akt/TORC1 pathway attenuates migratory cell fate of border cells by down-regulation of JAK/STAT and destabilization of Socs36E. (from Kang et al., 2018)

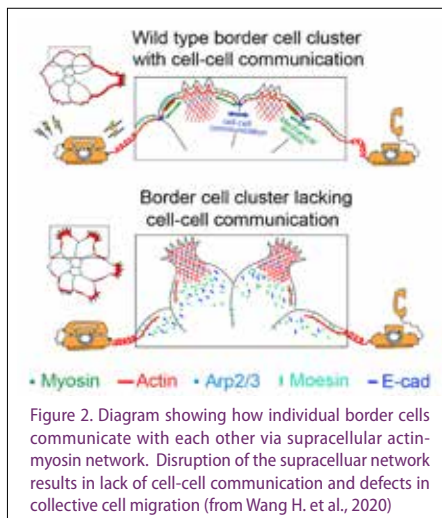


Figure 2. Diagram showing how individual border cells communicate with each other via supracellular actin-myosin network. Disruption of the supracellular network results in lack of cell-cell communication and defects in collective cell migration (from Wang H. et al., 2020)

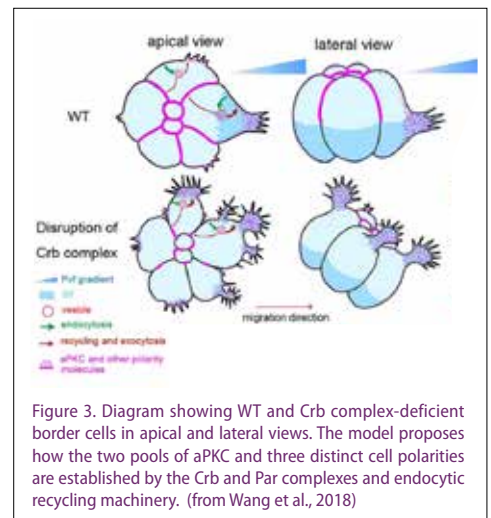
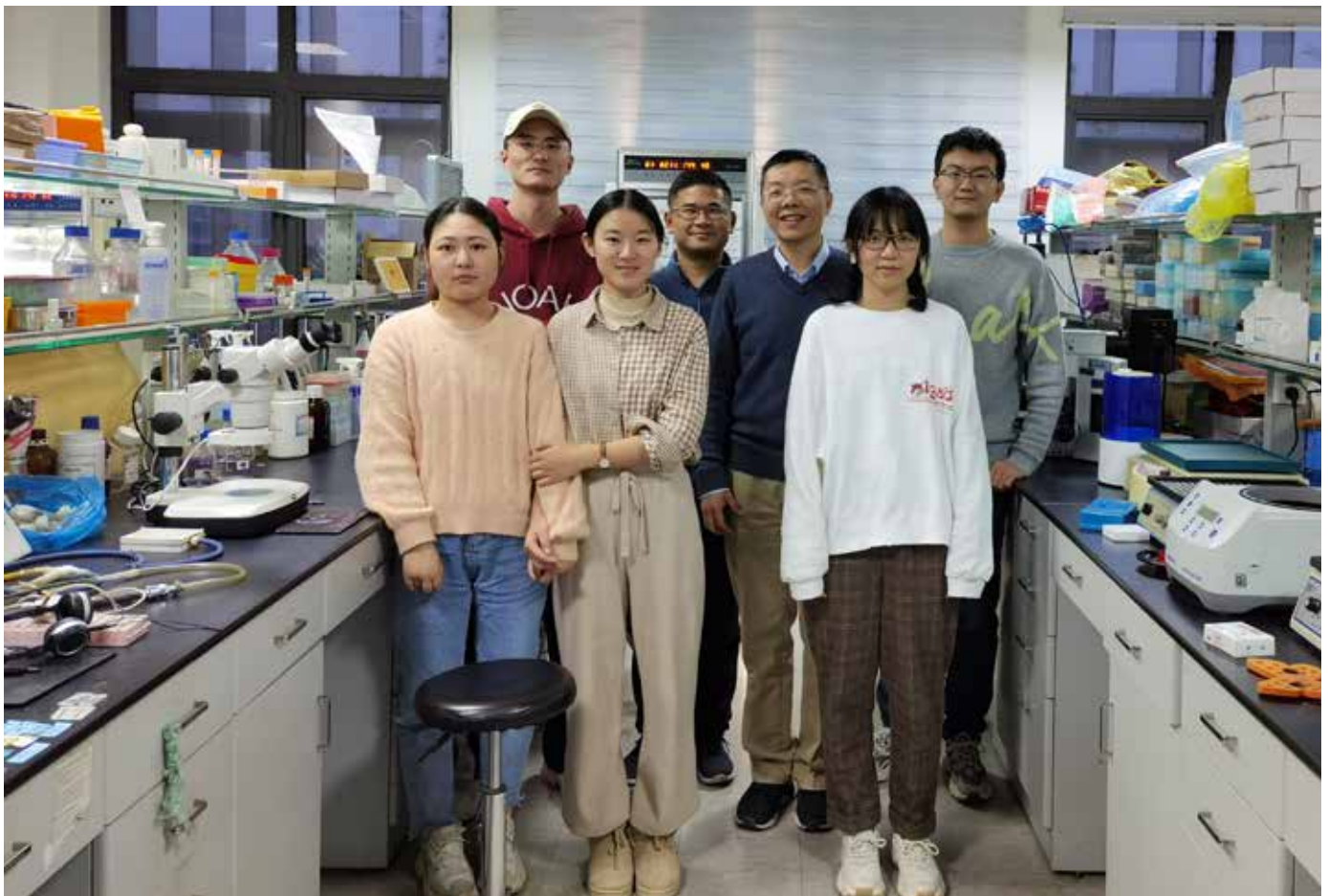


Figure 3. Diagram showing WT and Crb complex-deficient border cells in apical and lateral views. The model proposes how the two pools of aPKC and three distinct cell polarities are established by the Crb and Par complexes and endocytic recycling machinery. (from Wang et al., 2018)

## Selected Publications till 2020

1. Wang, X., Wang, H. \*, Liu, L., Li, S., Emery, G. and Chen, J. \* Temporal coordination of collective migration and lumen formation by antagonism between two nuclear receptors. *Iscience* (2020)
2. Wang, H. \*, Guo, X., Wang, X., Wang, X. and Chen, J. \* Supracellular actomyosin mediates cell-cell communication and shapes collective migratory morphology. *Iscience* (2020)
3. Guo, X., Luo, J., Wang, H. \*, and Chen, J. \*. SERCA regulates collective cell migration by maintaining cytoplasmic Ca<sup>2+</sup> homeostasis, *Journal of Genetics and Genomics* (2019)
4. Luo, J. \*, Zhou P., Guo, X., Wang, D., and Chen, J. \*. The polarity protein Dlg5 regulates collective cell migration during *Drosophila* oogenesis, accepted by *PLoS One* (2019)
5. Luo, J., Shen, P. \* and Chen, J. \*. A modular toolset of phiC31-based fluorescent protein tagging vectors for *Drosophila*. *Fly* (2019)
6. Wang, H., Qiu, Z., Xu, Z., Chen, S., Luo, J., Wang, X. \* and Jiong Chen\*. aPKC is a key polarity molecule coordinating the function of three distinct cell polarities during collective migration, *Development* (2018)
7. Kang, D., Wang, D., Xu, J., Quan, C., Guo1, X., Wang, H., Luo, J., Yang, Z., Chen, S. \*, Chen, J. \*. The InR/Akt/TORC1 growth-promoting signaling negatively regulates JAK/STAT activity and migratory cell fate during morphogenesis, *Developmental Cell* (2018)
8. Wu, J., Wang, H., Xuan, G., Chen, J. \* Cofilin-mediated actin dynamics promotes actin bundle formation during *Drosophila* bristle development *Molecular Biology of the Cell* (2016)
9. Wan, P., Wang, D., Luo, J., Chu, D., Wang, H., Zhang, L., and Chen, J. \* Guidance receptor promotes asymmetric distribution of Exocyst and recycling endosome during collective cell migration *Development* (2013).
10. Chu, D., Pan, H., Wan, P., Wu, J., Luo, J., Zhu, H. and Chen, J. \* AIP1 Acts with Cofilin to Control Actin Dynamics during Epithelial Morphogenesis *Development* 139:3561-3571. (2012)
11. Zhang, L., Luo, J., Wan, P., Wu, J., Laski, F. and Chen, J. \* Regulation of cofilin phosphorylation and asymmetry in collective cell migration during morphogenesis. *Development* 138, 455-64. (2011)



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## Qing Zhang, Ph.D

Qing Zhang received his Ph.D in Microbiology from Fudan University in 2002. Afterwards, he had had his postdoctoral training in Department of Developmental Biology of UT Southwestern Medical Center at Dallas for six years. In 2009, he joined the Model Animal Research Center of Nanjing University as a professor and principle investigator.

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## Regulation of hedgehog signaling

Hedgehog signaling plays critical role in embryonic development and adult tissue homeostasis in species ranging from insects to human. Aberrant Hh signaling activity is associated with many human disorders including birth defects and cancers.

In *Drosophila*, Hh transduces signal through binding its receptor, a 12-transmembrane protein Patched (Ptc), that alleviates suppression of ptc on Smoothed (Smo), a GPCR-like seven-transmembrane protein. Active Smo as the Hh signal transducer triggers a largely conserved signaling cascade that culminates at the activation of latent transcription factors Cubitus interruptus (Ci) which controls Hh targets decapentaplegic (*dpp*), *ptc* and engrailed (*en*) expression.

Based on Hh pathway is conserved among species, we take advantage of *Drosophila* as a model to investigate the mechanism of Hh signaling. Our work mainly focuses on getting the whole picture of around 7000 conserved genes on Hh signaling regulation and trying to answer the long-standing questions, for example, how does Ptc, the membrane receptor of Hh, inhibit Smo activity? how is hyperactive Ci degraded? and so on.

### E3 ligase Herc4 regulates Hedgehog signaling through promoting Smoothed degradation

Hedgehog (Hh) signaling plays conserved roles in controlling embryonic development, its dysregulation causes many diseases including cancers. The G protein-coupled receptor Smoothed (Smo) is the key signal transducer of the Hh pathway, whose posttranslational regulation has been shown to be critical for its accumulation and activation. Ubiquitination has been reported to be an essential posttranslational regulation of Smo. Here, we identify a novel E3 ligase of Smo, Herc4, which binds to Smo and regulates Hh signaling by controlling Smo ubiquitination and degradation. Interestingly, our data suggest that Herc4-mediated Smo degradation is regulated by Hh in PKA-primed phosphorylation dependent and independent manners.

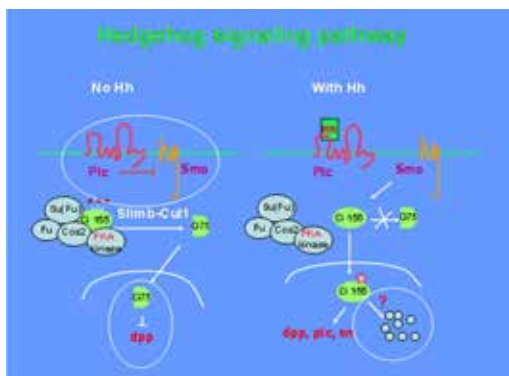


Fig1. Hedgehog signaling pathway in *Drosophila*.

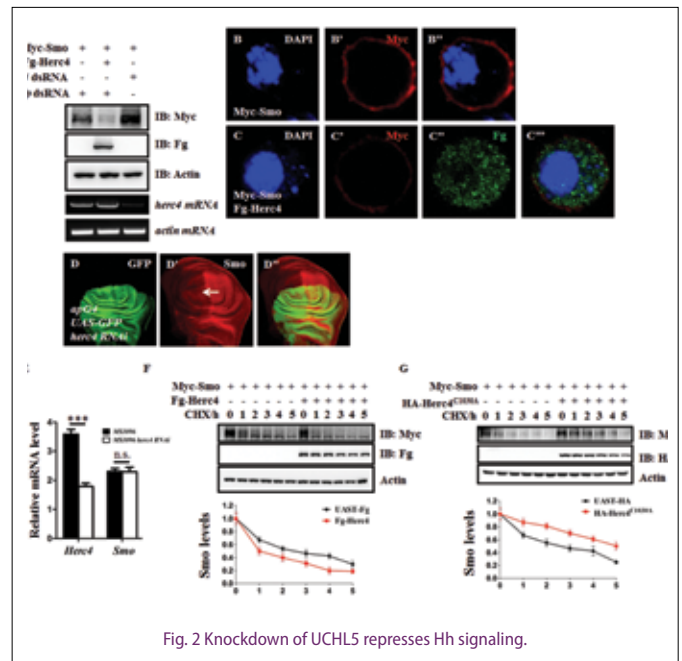
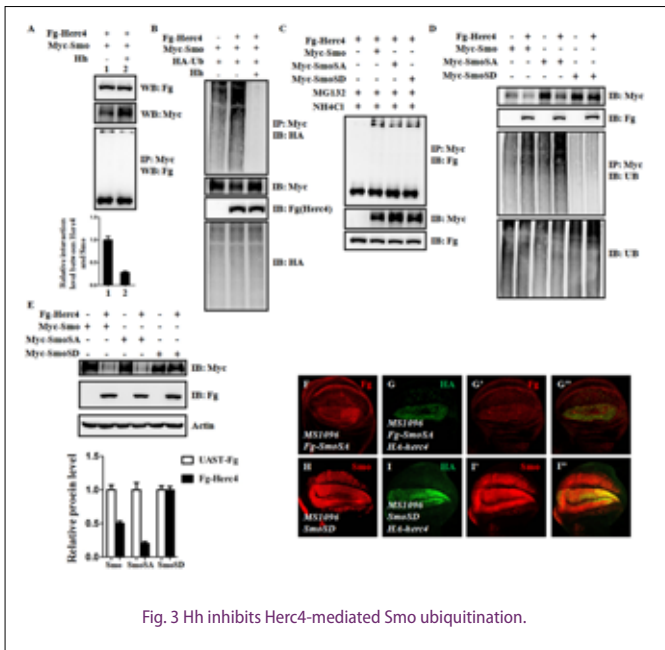


Fig. 2 Knockdown of UCHL5 represses Hh signaling.

(A) Overexpression of Fg-Herc4 downregulated Myc-Smo protein level. Knockdown of *herc4* upregulated Myc-Smo protein. *herc4*-dsRNA could effectively knock down *herc4* mRNA level in S2 cells (bottom two panels). (B-C'') S2 cells transfected with indicated constructs were stained by Myc, Flag antibody and DAPI. Of note, Herc4 inhibited Smo cell membrane accumulation (compare Figures 2C-C'' with Figures 2B-B''). The nuclei were showed by DAPI staining. (D-D'') Knockdown of *herc4* with apG4 increased the anterior compartment Smo protein level of the wing disc. Arrows indicate the increase of Smo. (E) The relative mRNA level of *smo* in wing discs. (F-G) Western blots of lysates from S2 cells expressing indicated proteins and treated with CHX for the indicated time intervals. Quantification analyses were shown below. The results were presented as means $\pm$ s.d. of values from three independent experiments. Of note, Herc4 could promote Smo degradation (F). Herc4C1030A could hamper Smo degradation (G).



(A) Hh treatment inhibited the interaction of Herc4 and Smo. (B) Hh decreased Smo ubiquitination mediated by Herc4. (C) S2 cells were transfected with indicated plasmids and treated with the proteasome/lysosome inhibitors MG132 and NH4Cl. Fg-Herc4 interacts equally with Myc-Smo, Myc-SmoSA and Myc-SmoSD. (D) From cell based ubiquitin assay, Herc4 upregulated the ubiquitination level of Smo and SmoSA, but did not affect the ubiquitination level of SmoSD. (E) Overexpression of Herc4 in S2 cells apparently decreased the protein level of Smo and SmoSA, but did not affect SmoSD protein level. (F-G) Overexpression of Herc4 in wing discs with MS1096-gal4 decrease the expression level of Fg-SmoSA. (compare Figure 3G' with 3F). (H-I) Overexpression of Herc4 in wing discs with MS1096-gal4 has no effect on the protein level of SmoSD. (compare Figure 3I' with 3H).

## Selected Publications

- Jiang W#, Yao X#, Shan Z, Li W, Gao Y, Zhang Q\*. (2019) E3 ligase Herc4 regulates Hedgehog signalling through promoting Smoothed degradation. *J Mol Cell Biol.* 11(9):791-803.
- Zhou Z#, Yao X#, Pang S, Chen P, Jiang W, Shan Z, Zhang Q\*. (2018) The deubiquitinase UCHL5/UCH37 positively regulates Hedgehog signaling by deubiquitinating Smoothed. *J Mol Cell Biol.* 10(3):243-257.
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## Group members

### Group leader

Qing Zhang

### Technical Assistants

Yan Li

### Graduate Students

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Wenting Li            Hui Zuo

Chunhua Jian         Shengnan Li

Xiuxiu Ti              Ying Wang

### Former students

Chen Liu (Ph.D)      Siya Su (Ph.D)

Zizhang Zhou (Ph.D)      Shu Pang (MS)

Ping Chen (Ph.D)      Weirong Jiang (MS)

Xia Yao (Ph.D)





## Ying Cao, Ph.D.

Cao Ying made the PhD study at the University of Essen (now University of Duisburg-Essen), Germany, from 1998 to 2002. During the period he performed a screening and identified a few novel genes that play essential roles in *Xenopus* embryonic development. He received the degree Dr. rer. nat. and graduated summa cum laude in 2002. Afterwards during the years from 2002 to 2008 he joined the Institute of Biochemistry, University of Ulm, Germany, and continued the study on *Xenopus* development, especially on the molecular mechanisms underlying embryonic cell differentiation. In October 2008, he was offered the professor at MARC and set up the laboratory for developmental biology and cancer biology. The results in his group suggest that the property of neural stemness is the key to understand tumorigenicity and differentiation potential. He proposes that "Tumorigenesis represents a process of loss of original cell identity and gain of properties of neural stemness" and "Neural stemness represents the ground or basal state of cell tumorigenicity and differentiation potential."

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## Neural stemness as the ground state of cell tumorigenicity and differentiation potential

Our previous studies demonstrated that cancer cells share characteristics of neural stem/progenitor cells because 1) cancer cells exhibit neuronal differentiation potential and 2) cancer cells share regulatory networks with neural stem/progenitor cells. This led us to the proposal that tumorigenesis may represent a process of loss of original cell identity and gain of neural stemness. However, it remains to be elucidated whether the property of neural stemness is the source of cell tumorigenicity. We show that neural stem cells formed tumors when the cells were transplanted subcutaneously into nude mice (Figure 1A). Immunohistochemical analysis demonstrates the differentiation of tissues derived from all three germ layers in the tumors (Figure 1B). The data the evidence for the link between neural stemness, tumorigenicity and differentiation potential. Moreover, when a somatic cells, the myoblast C2C12 cells, were dedifferentiated by knocking out the key muscle cell differentiation gene, *MyoD1*, the cells gained the property of neural stemness and tumorigenicity. The knockout cells formed tumor in nude mice (Figure 2A), and the tumor showed differentiation of cell types of three germ layers (Figure 2B-D). In agreement, bioinformatic analysis demonstrated that neural genes are mostly associated with cancer and development and have an evolutionary advantage. The

last common unicellular ancestors of protozoa and metazoan are biased towards a neural state. In combination with developmental biology, tumor biology and evolution, we propose a unified model for tumorigenesis and differentiation potential (Figure 3). For detailed information, see our papers listed below.

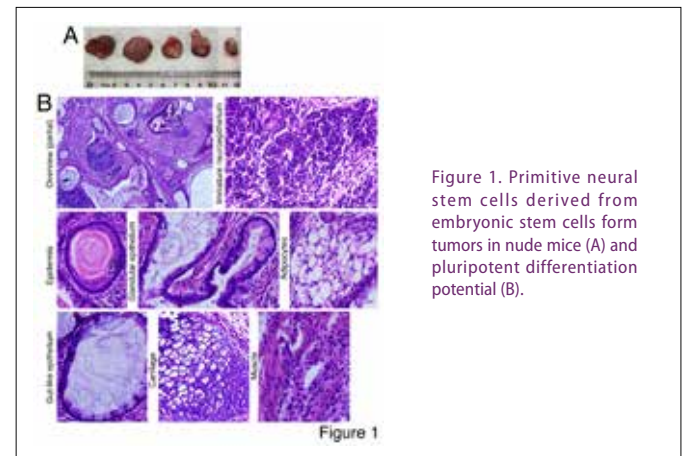


Figure 1. Primitive neural stem cells derived from embryonic stem cells form tumors in nude mice (A) and pluripotent differentiation potential (B).

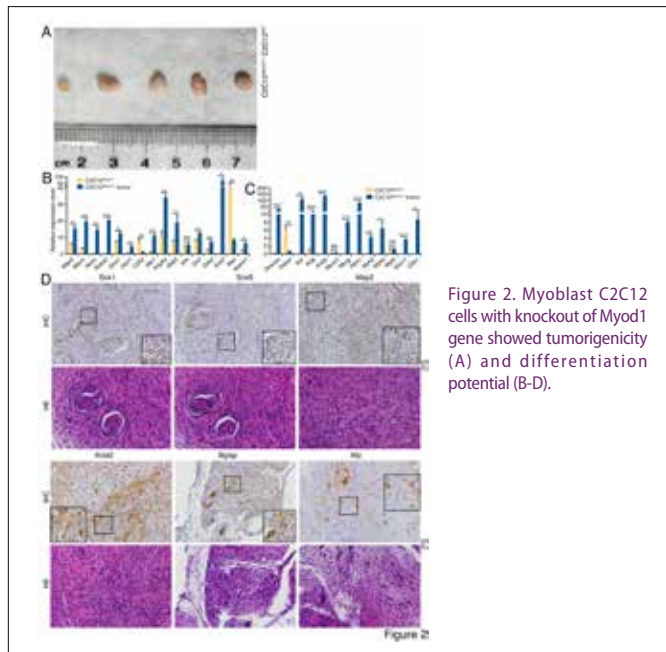


Figure 2. Myoblast C2C12 cells with knockout of *MyoD1* gene showed tumorigenicity (A) and differentiation potential (B-D).

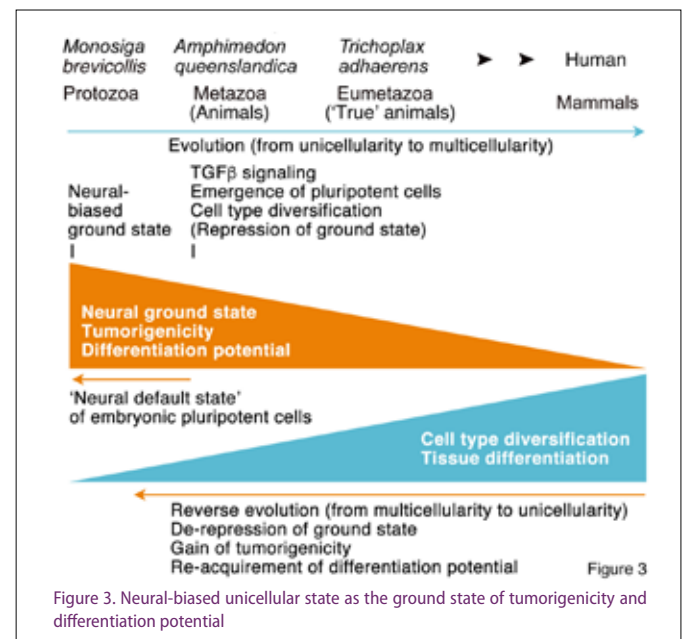
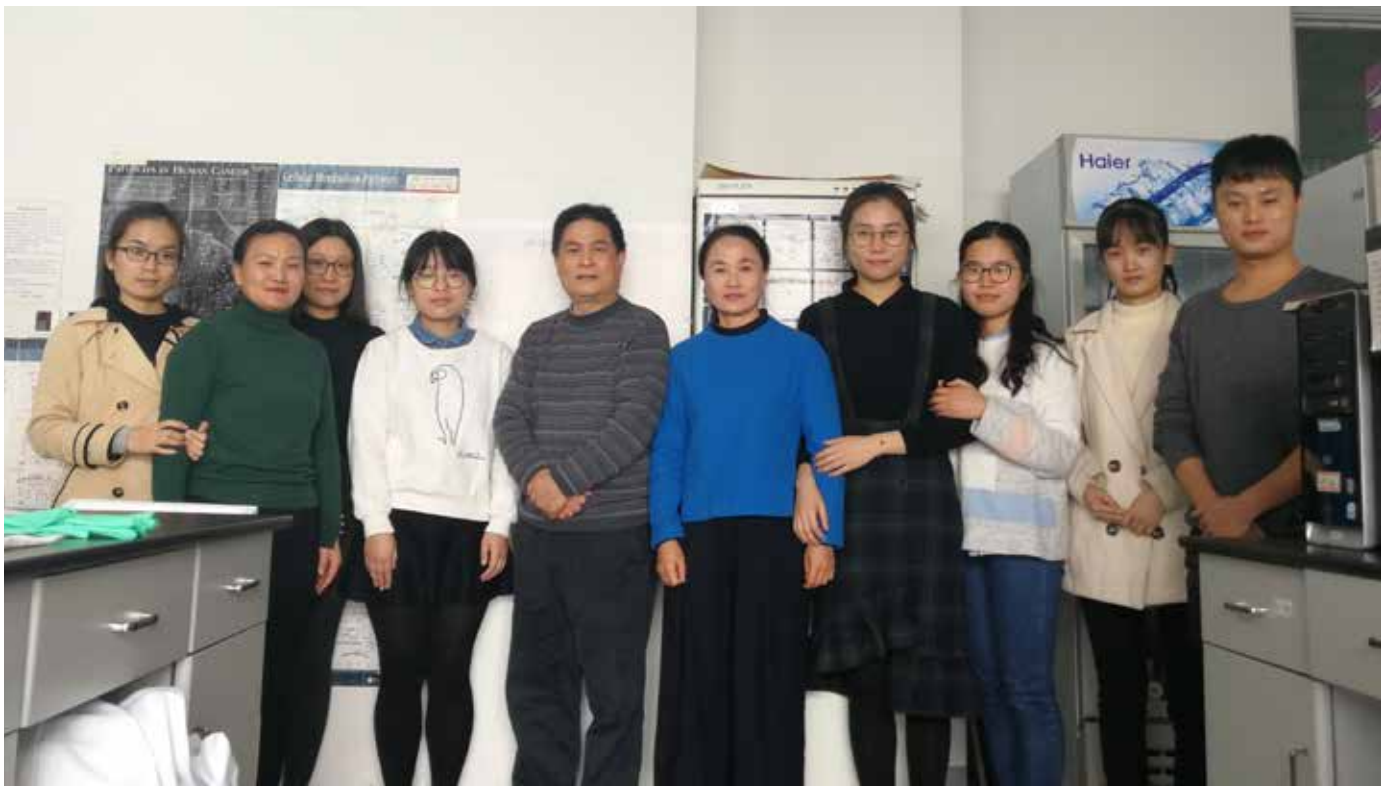


Figure 3. Neural-biased unicellular state as the ground state of tumorigenicity and differentiation potential

### Selected publications (\*Correspondence author)

1. Cao Y\*. (2020). Neural is Fundamental: Neural stemness as the ground state of cell tumorigenicity and differentiation potential. Preprints 2020, 2020120122. DOI: 10.20944/preprints202012.0122.v1.
2. Lei A, Chen L, Zhang M, Yang X, Xu L, Cao N, Zhang Z, Cao Y\*. EZH2 Regulates Protein Stability via Recruiting USP7 to Mediate Neuronal Gene Expression in Cancer Cells. *Front Genet.* 2019 May 3;10:422.
3. Cao Y\*. Tumorigenesis as a process of gradual loss of original cell identity and gain of properties of neural precursor/progenitor cells. *Cell Biosci.* 2017 Nov 7;7:61. (Review)
4. Zhang Z, Lei A, Xu L, Chen L, Chen Y, Zhang X, Gao Y, Yang X, Zhang M, Cao Y\*. 2017. Similarity in gene-regulatory networks suggests that cancer cells share characteristics of embryonic neural cells. *J Biol Chem.* 292(31):12842-12859.
5. Gao Y, Cao Q, Lu L, Zhang X, Zhang Z, Dong X, Jia W, Cao Y\*. 2015. Kruppel-like factor family genes are expressed during *Xenopus* embryogenesis and involved in germ layer formation and body axis patterning. *Dev Dyn.* 244(10):1328-46
6. Zhang X, Gao Y, Lu L, Zhang Z, Gan S, Xu L, Lei A, Cao Y\*. 2015. JmjC Domain-containing Protein 6 (*Jmjd6*) Derepresses the Transcriptional Repressor Transcription Factor 7-like 1 (*Tcf7l1*) and Is Required for Body Axis Patterning during *Xenopus* Embryogenesis. *J Biol Chem.* 290(33):20273-83.
7. Lu L, Gao Y, Zhang Z, Cao Q, Zhang X, Zou J, Cao Y\*. 2015. Kdm2a/b Lysine Demethylases Regulate Canonical Wnt Signaling by Modulating the Stability of Nuclear  $\beta$ -Catenin. *Dev Cell.* 33(6):660-74.



### Group members

#### Principal investigator:

Cao Ying

#### Graduated students:

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Gao Yan, PhD  
Zhang Zan, PhD  
Lei Anhua, PhD

#### Graduate students:

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Chen Lu  
Yang Xiaoli  
Zhang Min  
Shi Lihua  
Cao Ning  
Liu Yang

#### Technicians:

Ma Haihua



## Xin Lou Ph.D.

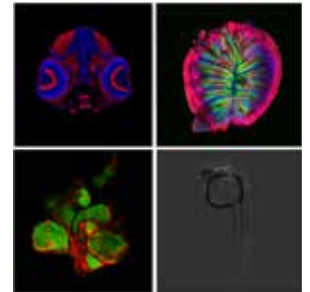
Xin Lou got his Ph.D. in Shanghai Institute of Biochemistry and Cell Biology, CAS in 2008. He was supervised by Prof. Xiaoyan Ding to study body axis patterning in vertebrate. He did post-doctoral training in Dr. Ian Scott's lab at the Hospital for Sick Children, Toronto, where he studied the molecular mechanisms of cardiomyocyte differentiation. He joined the Model Animal Research Center (MARC), Nanjing University as a principle investigator in 2013.

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## Vertebrate Organogenesis and regeneration

Congenital defects and adult-onset cardiovascular disease are among the most critical health problems throughout the world. A greater understanding of the process of cardiogenesis will ultimately be essential for developing new approaches for curing and diagnosing heart defects. Zebrafish is an ideal model to study cardiovascular development and regeneration; researchers are working with this tiny fresh water fish to illustrate the delicate molecular mechanisms regulating these processes. Currently, our research focuses on the following aspects:



## Currently, our research focuses on the following aspects

### 1)THE DYNAMIC CHANGE AND ROLE OF EPIGENETIC REGULATION IN HEART DEVELOPMENT AND REGENERATION

The mammalian heart is incapable of significant regeneration following injury such as an acute myocardial infarction. Unlike the mammalian heart, the injured zebrafish heart normally undergoes minimal scarring and in 30 days the transient fibrin clot is replaced with new contractile muscle. Epigenetic regulation involves all stages of cellular processes in cardiac regeneration: stress-response, re-entry into mitotic cell cycles, "de-differentiation" and re-establishment of mature cell types. We applied transcription array and proteomics approaches on regenerating adult zebrafish heart, characterized the dynamic expression change of epigenetic regulators during heart regeneration. Now we are focusing on a set of chromatin modulators (including components of PRC2 complex and NuRD complex). By using a battery of strategy ranging from experimental molecular genetics to bioinformatics, we are studying the detail function and mechanism of these genes in heart regeneration.

### 2)IDENTIFICATION OF NOVEL REGULATORS OF ORGANOGENESIS.

Zebrafish is widely used model organism for investigating organogenesis. The rapid external development, optical clarity, and large number of embryos

laid allows scientist observe early developmental events lively and applied a wide range of method to understood organ formation. Recently the zebrafish molecular genetic toolbox has expanded to include sophisticated approaches including the Cre-loxP system, transposon-mediated transgenesis and gene modification via use of nucleases. We optimized a "gene-breaker" transposon system, which both recapitulates endogenous gene expression and disrupts gene function to generate a null allele of the trapped gene. By using this system, 35 trapping fish line have been established and we are working on identification of new heart development/regeneration genes and analyzing their biological function.



### Group members

Lab Head	Graduate Students
Xin Lou	Yuxi Yang
	Xue Zhang
	Xiaoxue Pu
	YuanYuan Wei

### Selected Publications

- Zhang L, Yang Y, Li B, Scott IC, Lou X\*. The DEAD-box RNA helicase Ddx39ab is essential for myocyte and lens development in zebrafish. *Development*. 2018 Apr 23;145(8). doi: 10.1242/dev.161018.
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- Hou N, Yang Y, Scott IC, Lou X\*. The Sec domain protein Scfd1 facilitates trafficking of ECM components during chondrogenesis. *Developmental Biology*. 2017 Jan 1;421(1):8-15.
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## Qingshun Zhao, Ph.D

Qingshun obtained his B.S. degree in 1987 and M.S. degree in 1990 from Nanjing University (Nanjing, Jiangsu, China), and received his Ph.D. degree in 2001 from Purdue University (West Lafayette, Indiana, USA). His Ph.D. dissertation was carried out in Dr. Paul Collodi's Lab and it was focused on identification and characterization of fibronectin isoforms during zebrafish development. From 2001 to 2003, Qingshun did his postdoctoral research on roles of retinoid signaling in zebrafish hindbrain development under the guidance of Dr. Elwood Linney in Duke University Medical Center (Durham, North Carolina, USA). In 2003, he became an associate professor and a principal investigator in Model Animal Research Center, Nanjing University. In 2006, Qingshun was promoted full professor of Nanjing University.

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## Zebrafish development

The research interests of the lab focus on investigating the molecular mechanism underlying vertebrate early development using zebrafish as a model animal.

RA (retinoic acid) plays essential roles in vertebrate embryogenesis. Its homeostasis in embryos is determined by the presence of Aldh1A that produces RA and Cyp26 that metabolizes RA into bio-inactive metabolites. Unlike mammals, zebrafish only have *aldh1a2*, *aldh1a3* and *aldh8a1* but not *aldh1a1*. Because both *aldh1a3* and *aldh8a1* are expressed in late organogenesis, *aldh1a2* is the gene that is responsible for RA synthesis in zebrafish early development (Liang et al., 2008). Like mammals, zebrafish possesses a third *cyp26* gene (*cyp26c1*) (Gu et al., 2005) in addition to *cyp26a1* and *cyp26b1*. The Cyp26c1 metabolizes RA but not retinol or retinal in a similar way to Cyp26a1, the major gene that is responsible for RA metabolism in zebrafish early development (Gu et al., 2006). Like *cyp26a1*, proper expression of *cyp26c1* at early developmental stage is essential for the development of anterior–posterior axis and left–right symmetry in zebrafish embryos (Gu et al., 2005; Gu et al., 2006). Analyzing the promoter of *cyp26a1*, we reveal that zebrafish *cyp26a1* possesses three conserved RAREs in response to RA signal and therefore maintains RA homeostasis in a feedback way (Hu et al., 2008; Li et al., 2012). Other than Cyp26s that can limit RA signaling, Ncor1 (nuclear receptor co-repressor) is essential for patterning the anterior–posterior axis of zebrafish hindbrain by actively repressing RA signaling (Xu et al., 2009). Consistent with these results, *znfl1* whose expressions are in response to RA signaling, mediate the roles of RA in patterning zebrafish posterior neuroectoderm by acting upstream of *pou5f3* and *sall4* (Dong et al., 2017). Additionally, *Znfl1*s regulate left-right asymmetry patterning through controlling the expression of *fgfr1a* (Li et al., 2019).

RA signaling is also essential to vertebrate mesoderm differentiation. It plays a restrictive role in primitive myelopoiesis by inhibiting the specification of ventral mesoderm cells into anterior hemangioblasts through acting downstream of *gata4/5/6* and upstream to *scf* in a dose dependent manner (Liang et al., 2012). Furthermore, zebrafish microRNA miR-210-5p inhibits primitive myelopoiesis by silencing *foxj1b* and *slc3a2a* mRNAs downstream of *gata4/5/6* transcription factor genes (Jia et al., 2019). Moreover, RA is also essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos (Li et al., 2016). Additionally, Ncor1 and Ncor2 play essential but distinct roles in zebrafish primitive myelopoiesis (Li et al., 2014). On the other hand, the differentiation of ventral mesoderm is affected by environmental factors, excessive sodium nitrite affects zebrafish valve leaflet formation by producing too much NO signaling (Li et al., 2014).

RA signaling is genetically controlled by upstream genes. *Foxc1a* is a member

of the forkhead transcription factors. By generating *foxc1a* knockout zebrafish using TALEN (transcription activator-like effector nuclease) technology, we demonstrate that *foxc1a* is essential for somitogenesis by controlling Fgf and Notch signaling through restricting the expression of *aldh1a2* in zebrafish paraxial mesoderm directly (Li et al., 2015) and plays essential roles in zebrafish cardiogenesis by directly activating the expression of *nkx2.5*, encoding a transcriptional regulator of cardiac progenitor cells (Yue et al., 2018), and directly inhibiting the expression of *aldh1a2* in *foxc1a*-expressing cells (Gu et al., Unpublished data). In human cells, we demonstrate that FOXC1 does regulate human NKX2-5 expression in a dose-dependent manner via direct binding to its proximal promoter. A comparison of FOXC1 mutant function in the rat cardiac cell line H9c2 and zebrafish embryos suggested that the zebrafish embryos might serve as a more representative model system than the H9c2 cells. Three of the Axenfeld-Rieger syndrome FOXC1 mutations tested increased whereas a fourth repressed the expression of NKX2-5 implying that mutant FOXC1s might play etiological roles in CHD by abnormally regulating NKX2-5 in the patients. To sum up, zebrafish embryos can serve as a useful in vivo platform for rapidly evaluating disease-causing roles of mutated genes (Zhang et al., 2020).

Engineered endonuclease including ZFN, TALEN and CRISPR/Cas9 are powerful tools to create genome edited animals without species limitation. Employing ZFN and TALEN, we produced heritable targeted inactivation of myostatin genes in yellow catfish, the first endogenous gene knockout in aquaculture fish (Dong et al., 2011, Dong et al., 2014), and the *mstna* null yellow catfish exhibit double muscle phenotype with muscle hyperplasia (Zhang et al., 2019). By co-microinjecting *yfp-nanos3* mRNA with genome editing tools to make founders and then screen them with the help of tentatively fluorescent-labeled PGCs, we invent a new method that significantly increases the ease and speed of generating heritable knockin animals with CRISPR/Cas9 (Dong et al., 2014). Using this method, we develop “two-step strategy” to generate an *aldh1a2* floxed zebrafish line (*aldh1a2<sup>lox/lox</sup>*) by first inserting mloxP sites into its 3rd intron and then into its 4th intron. With the systemic expression of Cre in the eggs of *aldh1a2<sup>lox/lox</sup>* zebrafish, we obtained an *aldh1a2* conventional knockout zebrafish line (*aldh1a2<sup>-/-</sup>*) (Gu et al., Unpublished data). Interestingly, the embryos whose primordial germ cells are eliminated at early development grow up as all-male-like sterile zebrafish (Zhou et al., 2018). Collaborating with the groups of Professors Zhou and Zhu, we developed an alternative novel tool for DNA editing (SGN: structure-guided nuclease) without target sequence limitation (Xu et al., 2016). Unfortunately, our further efforts do not support that the system works in human colorectal carcinoma cell line (HCT116), nor in producing any germline transmission zebrafish mutants (Zhang et al., Unpublished data).



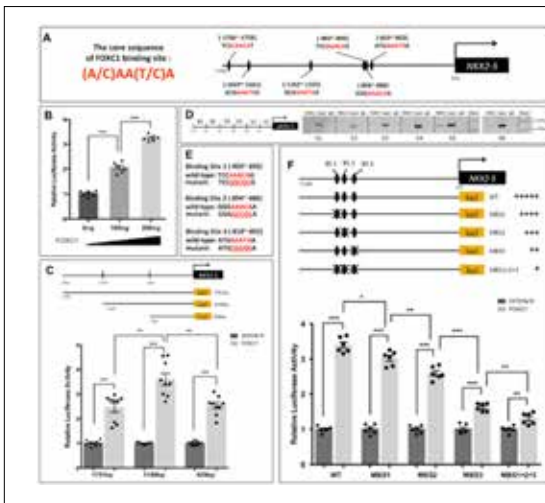


Figure 1 .FOXC1 directly regulates the expression of NKX2-5 by binding to its proximal promoter in H9c2 cells.

A, Schematic showing putative FOXC1 transcription factor binding sites in 1791 bp 5'-flanking sequence upstream of NKX2-5 translation start site (ATG). B, Results of Dual-Luciferase Reporter Assay showing the responses of NKX2-5 promoter to different doses of FOXC1. C, Schematic (top) showing the firefly luciferase reporter expression constructs comprising the different lengths of upstream regulatory sequence of NKX2-5, namely 1791 bp, 1149 bp or 630 bp, and the coding sequences of NKX2-5 or firefly luciferase, and the results (below) of Dual-Luciferase Reporter Assay on the three expression constructs. D, Schematic (left) showing the dissection of the 1149 bp regulatory sequences of NKX2-5 into S1-S6 regions and the results of ChIP-PCR assay (right) indicating that S5 contains FOXC1-binding sites. E, The wild-type sequences and location of FOXC1-binding sites (BS) in S5 of NKX2-5 regulatory sequence (top), and the mutant FOXC1 binding sites (MBS) with changed core sequence. F, Schematic (top) showing the reporter expression constructs carrying wild-type BS or MBS of FOXC1, and the results (below) of Dual-Luciferase Reporter Assay on the five expression constructs. X-axis (B, C, F): The amount of overexpressed FOXC1 (B), the reporter expression constructs with different lengths of regulatory sequences (C), or the reporter expression constructs carrying wild-type BS or MBS of FOXC1 (F). Light grey columns (C, F): transfected with wild-type FOXC1; Dark grey columns (C, F): transfected with the same amount of functional null mutated FOXC1 (p.Q70Hfs\*8) as control. Y-axis (B, C, F): Relative activity of firefly luciferase reporter.

### Selected Publications (\* corresponding author; \*\* co-corresponding author)

- Qinxin Zhang, Dong Liang, Yunyun Yue, Luqingqing He, Nan Li, Dongya Jiang, Ping Hu, Qingshun Zhao\*. 2020. Axenfeld-Rieger syndrome-associated mutants of the transcription factor FOXC1 abnormally regulate NKX2-5 in model zebrafish embryos. *The Journal of Biological Chemistry*, 295(33):11902-11913.
- Wenshuang Jia, Dong Liang; Nan Li, Meijing Liu, Zhangji Dong, Jingyun Li, Xiaohua Dong, Yunyun Yue, Ping Hu, Jihua Yao, Qingshun Zhao\*. 2019. Zebrafish microRNA miR-210-5p inhibits primitive myelopoiesis by silencing *foxj1b* and *slc3a2a* mRNAs downstream of *gata4/5/6* transcription factor genes. *The Journal of Biological Chemistry*, 294(8):2732-2743.
- Yunyun Yue, Mingyang Jiang, Luqingqing He, Zhaojunjie Zhang, Qinxin Zhang, Chun Gu, Meijing Liu, Nan Li, Qingshun Zhao\*. 2018. The transcription factor *Foxc1a* in zebrafish directly regulates expression of *nkx2.5*, encoding a transcriptional regulator of cardiac progenitor cells. *The Journal of Biological Chemistry*, 293(2):638-650.
- Xiaohua Dong, Jingyun Li, Luqingqing He, Chun Gu, Wenshuang Jia, Yunyun Yue, Jun Li, Qinxin Zhang, Lele Chu, Qingshun Zhao\*. 2017. Zebrafish *Znfl1s* control the expression of *hoxb1b* in the posterior neuroectoderm by acting upstream of *pou5f3* and *sal4*. *The Journal of Biological Chemistry*, 292(31):13045-13055.
- Shu Xu, Shasha Cao, Bingjie Zou, Yunyun Yue, Chun Gu, Xin Chen, Pei Wang, Xiaohua Dong, Zheng Xiang, Kai Li, Minsheng Zhu\*\*, Qingshun Zhao\*\*, Guohua Zhou\*. 2016. An alternative novel tool for DNA editing without target sequence limitation: the structure-guided nuclease. *Genome Biology*. 17(1):186.
- Junbo Li, Yunyun Yue, Qingshun Zhao\*. 2016. Retinoic acid signaling is essential for valvulogenesis by affecting endocardial cushions formation in zebrafish embryos. *Zebrafish*, 13(1):9-18. (Cover)
- Jingyun Li, Yunyun Yue, Xiaohua Dong, Wenshuang Jia, Kui Li, Dong Liang, Zhangji Dong, Xiaoxiao Wang, Xiaoxi Nan, Qinxin Zhang, Qingshun Zhao\*. 2015. Zebrafish *foxc1a* plays a crucial role in early somitogenesis by restricting the expression of *aldh1a2* directly. *The Journal of Biological Chemistry*, 290(16):10216-28.
- Zhangji Dong, Xiaohua Dong, Wenshuang Jia, Shasha Cao, Qingshun Zhao\*. 2014. Improving the efficiency for generation of genome-edited zebrafish by labelling primordial germ cells. *The International Journal of Biochemistry & Cell Biology*, 55:329-34.
- Zhangji Dong, Jiachun Ge, Kui Li, Zhiqiang Xu, Dong Liang, Jingyun Li, Junbo Li, Wenshuang Jia, Yuehau Li, Xiaohua Dong, Shasha Cao, Xiaoxiao Wang, Jianlin Pan, Qingshun Zhao\*. 2011. Heritable targeted inactivation of myostatin gene in yellow catfish (*Pelteobagrus fulvidraco*) using engineered zinc finger nucleases. *PLoS ONE*, 6(12):e28897.
- Ping Hu, Miao Tian, Jie Bao, Guangdong Xing, Xingxing Gu, Xiang Gao, Elwood Linney, Qingshun Zhao\*. 2008. Retinoid regulation of the zebrafish *cyp26a1* promoter. *Developmental Dynamics*, 237:3798-3808.



### Group members

#### Graduate Students Old Lab Members

Luqingqing He	Fang Xu, PhD (2007)	Meijing Liu, PhD (2018)
Nan Li	Ping Hu, PhD (2009)	Chun Gu, PhD (2019)
Zhaojunjie Zhang	Jie Bao, PhD (2009)	Qinxin Zhang (2019)
Dongya Jiang	Dong Liang, PhD (2011)	Zhiying Zou, MS (2005)
Yunfeng Zhang	Kui Li, PhD (2011)	Lu Sun, MS (2005)
Shuang Wang	Jingyun Li, PhD (2013)	Wei Song, MS (2006)
Yuxuan Wei	Junbo Li, PhD (2013)	Xiaolin Wang, MS (2006)
	Zhangji Dong, PhD (2014)	Mei Zhang, MS (2008)
	Wenshuang Jia, PhD (2015)	
	Xiaohua Dong, PhD (2015)	
	Shasha Cao, PhD (2016)	
	Yunyun Yue, PhD (2017)	



# ***Metabolism and Immunity***



## Xiang Gao, Ph.D.

Xiang was an alumina of Nanjing University. He received his Ph.D. degree from Thomas Jefferson University in 1994, then did his postdoctoral training at the Jackson Laboratory and University of North Carolina at Chapel Hill. In 2000, Xiang was recruited back to Nanjing University. He later founded both MARC and National Resource Center of Mutant Mice of China. He is also the current director for the State Key Laboratory of Pharmaceutical Biotechnology. Xiang is the recipient for Cheung Kong Scholar from Ministry of Education and Distinguished Young Scholar from National Science Foundation.

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## Physiological regulation and metabolic homeostasis

The advance of modern technologies, especially the NGS and gene editing, transform the biomedical fields. The complicated metabolic regulatory networks crossing the variety of tissues and organs are becoming tangible with these new tools. We are excited to embrace these promising

progresses for identifying the previous unsolvable biological questions. In my laboratory, we are more interested in defining the global regulators for crucial physiological processes. Following are some of our publications:

### 1. Uncovering that magnesium enhances survival of sepsis by blocking pyroptosis (Figure 1)

Hypomagnesemia is a significant risk factor for critically ill patients to develop sepsis, a life-threatening disease with a mortality rate over 25%. Our clinic data analysis showed that hypomagnesemia is associated with a decreased monocyte count in septic patients. At the cellular level, we found that Mg<sup>2+</sup> inhibits pyroptosis. Specifically, Mg<sup>2+</sup> limits the oligomerization and membrane localization of gasdermin D N-terminal (GSDMD-NT) upon the activation of either the canonical or non-canonical pyroptotic pathway. Mechanistically, we demonstrated that Ca<sup>2+</sup> influx is a prerequisite for the function of GSDMD-NT. Mg<sup>2+</sup> blocks Ca<sup>2+</sup> influx by inhibiting the ATP-gated Ca<sup>2+</sup> channel P2X7, thereby impeding the function of GSDMD-NT and inhibiting lipopolysaccharide (LPS)-induced non-canonical pyroptosis. Furthermore, Mg<sup>2+</sup> administration protects mice from LPS-induced lethal septic shock. Together, our data reveal the underlying mechanism of how Mg<sup>2+</sup> inhibits pyroptosis and suggest potential clinic applications of magnesium supplementation for sepsis prevention and treatment. (Wang et al, Cell Death Differ)

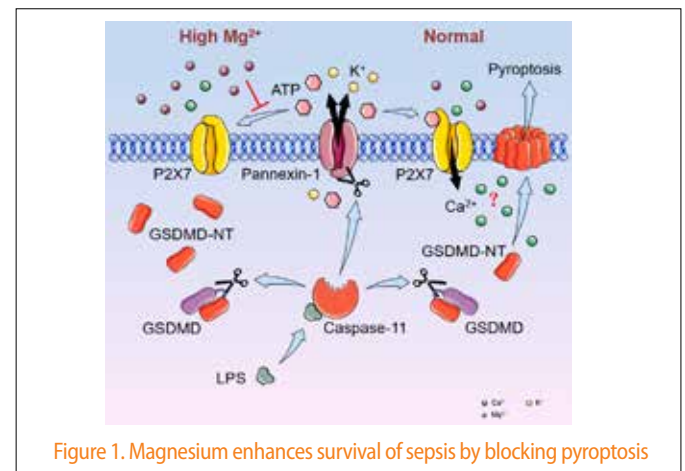


Figure 1. Magnesium enhances survival of sepsis by blocking pyroptosis

### 2. A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption (Figure 2)

Alteration of commensal bacterial composition is associated with many inflammatory diseases. However, few studies pinpointed the specific bacterial genes that may suppress host immune responses against microbes. By screening 3,983 *E. coli* mutants, we discovered that 9 bacterial genes, when deleted, activate innate immunity in the host *Caenorhabditis elegans*. The gene encoding bacterial lipocalin (*blc*), among these 9 genes, shown a distinctive SNP in many clinic pathogenic bacteria. We found bacteria with this SNP, which converts the Blc G84 to Blc E84, are highly enriched in the fecal of inflammatory bowel disease (IBD) patients. Exposure to the BlcE84-encoding bacteria resulted in epithelial barrier disruption and immune activation both in worm and mouse. Detailed analysis indicated the infection of the BlcE84-encoding bacteria causes a significant decrease in lysophosphatidylethanolamine (LPE) levels in the intestine, and subsequently the disruption of gut epithelial integrity in mice. Consistently, the levels of LPE in IBD patients are significantly lower compared to that of health people. Finally, supplement of LPE, which activating the LPA1/PLCβ/PKC signaling, can reverse all the defects induced by the BlcE84-encoding bacteria. Our results identified a novel bacterial gene in *E. coli* that regulates gut integrity and immunity. (Zou et al, Ebiomedicine).

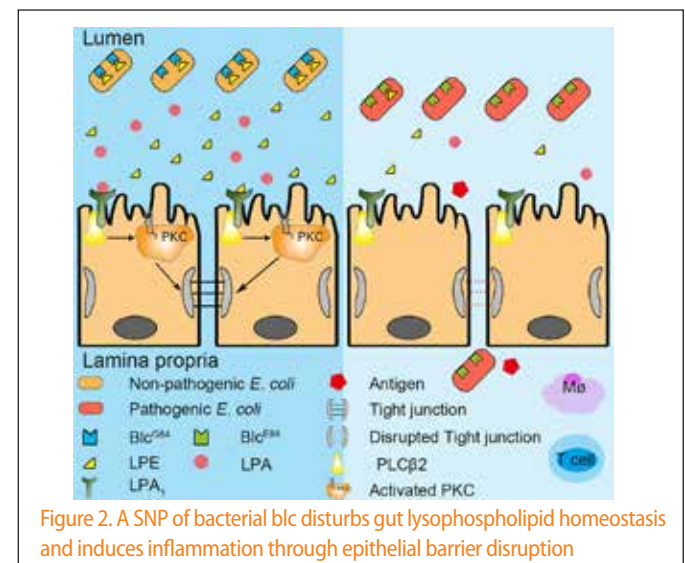


Figure 2. A SNP of bacterial *blc* disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption



## Selected publications

- Zou, D., J. Pei, J. Lan, H. Sang, H. Chen, H. Yuan, D. Wu, Y. Zhang, Y. Wang, D. Wang, Y. Zou, D. Chen, J. Ren\*, X. Gao\*, and Z. Lin\*, A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption. *EBioMedicine*, 2020. 52: p. 102652.
- Wang, D., J. Zheng, Q. Hu, C. Zhao, Q. Chen, P. Shi, Q. Chen, Y. Zou, D. Zou, Q. Liu, J. Pei, X. Wu, X. Gao\*, J. Ren\*, and Z. Lin\*, Magnesium protects against sepsis by blocking gasdermin D N-terminal-induced pyroptosis. *Cell Death Differ*, 2020. 27(2): p. 466-481.
- Zheng, M., D. Li, Z. Zhao, D. Shytikov, Q. Xu, X. Jin, J. Liang, J. Lou, S. Wu, L. Wang, H. Hu, Y. Zhou, X. Gao\*, and L. Lu\*, Protein phosphatase 2A has an essential role in promoting thymocyte survival during selection. *Proc Natl Acad Sci U S A*, 2019. 116(25): p. 12422-12427.
- Xu, Q., X. Jin, M. Zheng, D. Rohila, G. Fu, Z. Wen, J. Lou, S. Wu, R. Sloan, L. Wang, H. Hu, X. Gao\*, and L. Lu\*, Phosphatase PP2A is essential for TH17 differentiation. *Proc Natl Acad Sci U S A*, 2019. 116(3): p. 982-987.
- Wang, Y., P. Shi, Q. Chen, Z. Huang, D. Zou, J. Zhang, X. Gao\*, and Z. Lin\*, Mitochondrial ROS promote macrophage pyroptosis by inducing GSDMD oxidation. *J Mol Cell Biol*, 2019.
- Ma, P., N.N. Song, Y. Li, Q. Zhang, L. Zhang, Q. Kong, L. Ma, X. Yang, B. Ren, C. Li, X. Zhao, Y. Li, Y. Xu, X. Gao\*, Y.Q. Ding\*, and B. Mao\*, Fine-Tuning of Shh/Gli Signaling Gradient by Non-proteolytic Ubiquitination during Neural Patterning. *Cell Rep*, 2019. 28(2): p. 541-553 e4.
- Chen, Q., P. Shi, Y. Wang, D. Zou, X. Wu, D. Wang, Q. Hu, Y. Zou, Z. Huang, J. Ren\*, Z. Lin\*, and X. Gao\*, GSDMB promotes non-canonical pyroptosis by enhancing caspase-4 activity. *J Mol Cell Biol*, 2019. 11(6): p. 496-508.
- Zou, J., B. Lai, M. Zheng, Q. Chen, S. Jiang, A. Song, Z. Huang, P. Shi, X. Tu, D. Wang, L. Lu, Z. Lin\*, and X. Gao\*, CD4+ T cells memorize obesity and promote weight regain. *Cell Mol Immunol*, 2018. 15(6): p. 630-639.



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## Shuai Chen, Ph.D.

Dr. Shuai Chen received his Ph.D. degree in Plant Molecular Physiology from University of Halle-Wittenberg (Germany) in 2005. After his postdoctoral training in the field of cell signaling and molecular physiology at the MRC Protein Phosphorylation Unit, University of Dundee (UK) from 2006 to 2011, Dr. Chen joined MARC as a principle investigator and a professor in Molecular Physiology in 2012.

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## Metabolic Physiology and Diseases

Metabolic diseases including type 2 diabetes mellitus (T2DM), obesity and non-alcoholic fatty liver disease (NAFLD) have become prevalent world-wide in the last few decades, which urges a better understanding of their pathogenesis as well as new therapeutic strategies to combat these diseases. Insulin resistance is a common cause for the pathogenesis of these metabolic diseases, whose underlying mechanism is still not clear. Insulin actions exhibit a tissue/pathway-dependent manner. Therefore, the goal

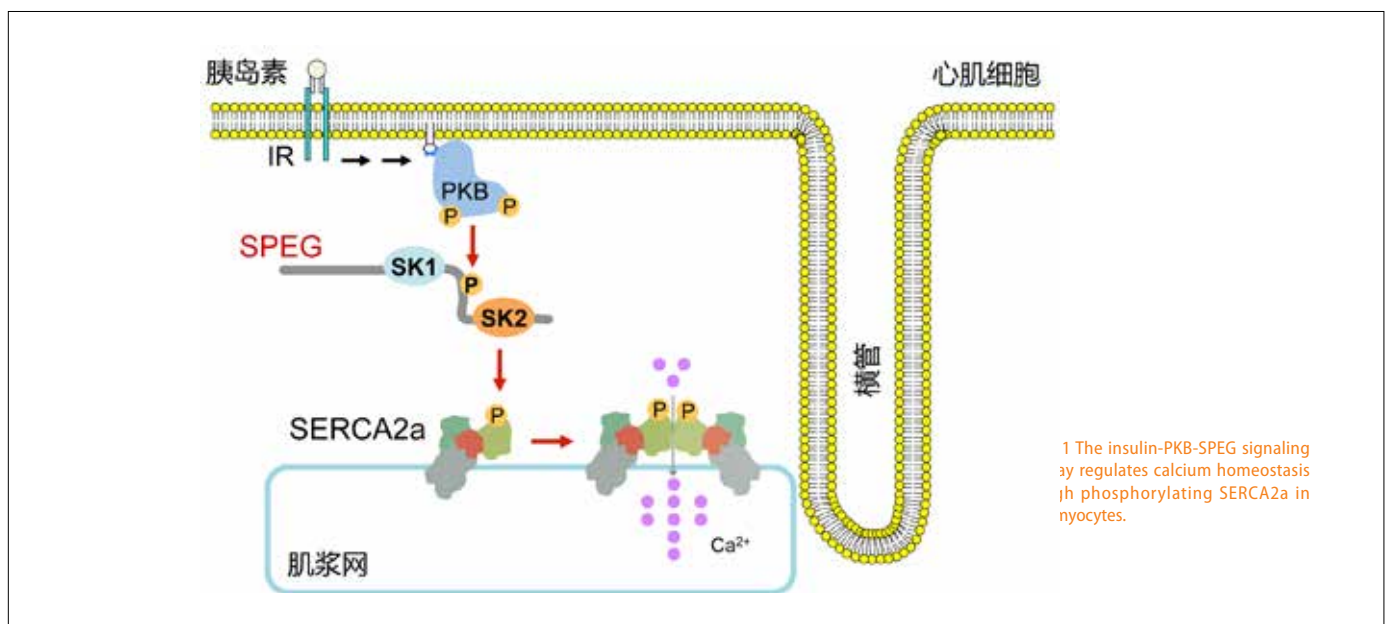
of my laboratory is to understand the molecular basis of tissue/pathway-specific insulin actions, the pathogenic mechanisms of metabolic diseases, and discoveries of leading compounds to combat these diseases. We are currently running three research programmes in the laboratory: (1) protein modifications in mediating insulin actions, (2) tissue/pathway-specific insulin actions and diabetic complications, (3) discoveries of therapeutic targets and agents for metabolic diseases.

### The recent progresses of my lab is as follows:

#### 1. A PKB-SPEG signaling nexus links insulin resistance with diabetic cardiomyopathy by regulating calcium homeostasis

Diabetic cardiomyopathy is a serious complication in type 2 diabetic patients, whose pathogenesis is not fully understood. Metabolic changes such as hyperglycemia are important contributing factors for cardiac dysfunction in type 2 diabetic patients. However, the risk of hospitalization for heart failure remains high in type 2 diabetic patients who have optimal glycemic control via anti-diabetic drugs. We hypothesize that insulin resistance might cause cardiomyocyte

dysfunction independent of metabolic changes. In this study, we identified a novel insulin-stimulated protein kinase SPEG, and show that a PKB-SPEG signaling nexus links insulin resistance with cardiac dysfunction through SERCA2a-mediated calcium re-uptake into the sarcoplasmic reticulum (SR) in cardiomyocytes independent of metabolic assaults. Our findings demonstrate that SPEG might be a promising target for discoveries of drugs for treatment of diabetic cardiomyopathy and heart failure. (Quan C., Du Q., ..., Wang H.Y.\*, Chen S.\* 2020 Nature Communications).





## Selected Publications(\* corresponding author)

1. Quan C, Du Q, Li M, Wang RZ, Su S, Zhu SS, Chen QL, Sheng Y, Chen L, Wang H, Campbell DG, MacKintosh C, Yang ZZ, Ouyang KF, Wang HY\* and Chen S\* (2020) A PKB-SPEG signaling nexus links insulin resistance with diabetic cardiomyopathy by regulating calcium homeostasis. *Nat Commun* 11(1): 2186 doi: 10.1038/s41467-020-16116-9 (\* corresponding author)
2. Chen QL, Rong P, Zhu SS, Yang XY, Ouyang Q, Wang HY and Chen S\* (2019) Targeting RalGAP $\alpha$ 1 in skeletal muscle to simultaneously improve postprandial glucose and lipid control. *Science Advances* 5(4): eaav4116 (\* corresponding author)
3. Quan C, Li M, Du Q, Chen QL, Wang H, Campbell D, Fang L, Xue B, MacKintosh C, Gao X, Ouyang KF, Wang HY and Chen S\* (2019) SPEG controls calcium re-uptake into the sarcoplasmic reticulum through regulating SERCA2a by its second kinase-domain. *Circ Res* 124(5): 712-726 (\* corresponding author)
4. Kang D, Wang D, Xu J, Quan C, Guo X, Wang H, Luo J, Yang Z, Chen S\*, Chen J\*. (2018) The InR/Akt/TORC1 Growth-Promoting Signaling Negatively Regulates JAK/STAT Activity and Migratory Cell Fate during Morphogenesis. *Dev Cell* 44:524-531 e525 (\* corresponding author)
5. Chen Q.L., Rong P., Xu D.J., Zhu S.S., Chen L., Xie B.X., Du Q., Quan C., Sheng Y., Zhao T.J., Li P., Wang H.Y.\*, Chen S.\* (2017) Rab8a deficiency in skeletal muscle causes hyperlipidemia and hepatosteatosis via impairment of muscle lipid uptake and storage. *Diabetes* 66(9): 2387-2399 (\* corresponding author)
6. Chen QL, Xie BX, Zhu SS, Rong P, Sheng Y, Ducommun S, Chen L, Quan C, Li M, Sakamoto K, MacKintosh C, Chen S\* and Wang HY\* (2017) A Tbc1d1Ser231Ala knockin mutation partially impairs AICAR- but not exercise-induced muscle glucose uptake in mice. *Diabetologia* 60(2): 336-345 (\* corresponding author)
7. Xie B.X., Chen Q.L., Chen L., Sheng Y., Wang H.Y.\* and Chen S.\* (2016) The inactivation of RabGAP function of AS160 promotes lysosomal degradation of GLUT4 and causes postprandial hyperglycemia and hyperinsulinemia. *Diabetes* 65(11): 3327-3340 (\* corresponding author)
8. Chen L., Chen Q.L., Xie B.X., Quan C., Sheng Y., Zhu S.S., Rong P., Zhou S.L., Sakamoto K., MacKintosh C., Wang H.Y. \* and Chen S.\* (2016) Disruption of the AMPK-TBC1D1 nexus increases lipogenic gene expression and causes obesity in mice via promoting IGF1 secretion. *PNAS* 113(26): 7219-24 (\* corresponding author)
9. Li M., Quan C., Toth R., Campbell D.G., MacKintosh C.\*, Wang H.Y.\* and Chen S.\* (2015) Fasting and systemic insulin signaling regulate phosphorylation of brain proteins that modulate cell morphology and link to neurological disorders. *J Biol. Chem.* doi:10.1074/jbc.M115.668103 (\* corresponding author)
10. Chen Q.L., Quan C., Xie B.X., Chen L., Zhou S.L., Toth R., Campbell D.G., Lu S.S., Shirakawa R., Horiuchi H., Li C.J., Yang Z.Z., MacKintosh C., Wang H.Y.\* and Chen S.\* (2014) GARNL1, a major RalGAP a subunit in skeletal muscle, regulates insulin-stimulated RalA activation and GLUT4 trafficking via interaction with 14-3-3 proteins. *Cell Signal* 26(8): 1636-1648 (\* corresponding author)
11. Wang H.Y., Ducommun S., Quan C., Xie B.X., Li M., Wasserman D.H., Sakamoto K., MacKintosh C.\* and Chen S.\* (2013) AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues. *Biochem J.* 449 (2): 479-489 (\* corresponding author)
12. Chen S., Synowsky S., Tinti M. and MacKintosh C. (2011) Insulin triggers 14-3-3 capture of many phosphoproteins. *Trends Endocrinol Metab* 22(11): 429-36 (review; Cover story)
13. Chen S.\*, Wasserman D., MacKintosh C. and Sakamoto K. (2011) Mice with AS160/TBC1D4 Thr649Ala knockin mutation are glucose intolerant with reduced insulin sensitivity and altered GLUT4 trafficking. *Cell Metabolism* 13(1): 68-79 (\* corresponding author)



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## Di Chen, Ph.D.

Di Chen got his Ph.D. in Genetics from the University of Missouri-Columbia, USA in 2004. He was supervised by Dr. Donald L. Riddle to study how the nematode *C. elegans* respond to genetic and environmental cues to enter and exit developmental diapause. He did post-doctoral training in Dr. Pankaj Kapahi's lab at the Buck Institute for Research on Aging, USA, where he studied the molecular mechanisms of aging in *C. elegans*. He joined the Model Animal Research Center, Nanjing University as a Principle Investigator in 2013.

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## Basic Biology of Aging

Aging is a process of function decline accompanied with increased mortality rate over time. The evolutionary theory of aging proposed that aging takes place because natural selection favors genes that confer benefit early on life at the cost of deterioration later in life (antagonistic pleiotropy). Using model organisms, researchers have demonstrated that aging can be modulated by highly conserved signaling pathways. Appropriate genetic or environmental modulations not only extend lifespan but also delay age-related pathologies. Many exciting discoveries on the molecular basis of aging were initially made in *C. elegans*, which is a great system for biology research because of the ease of genetics analysis and the conservation with higher species.

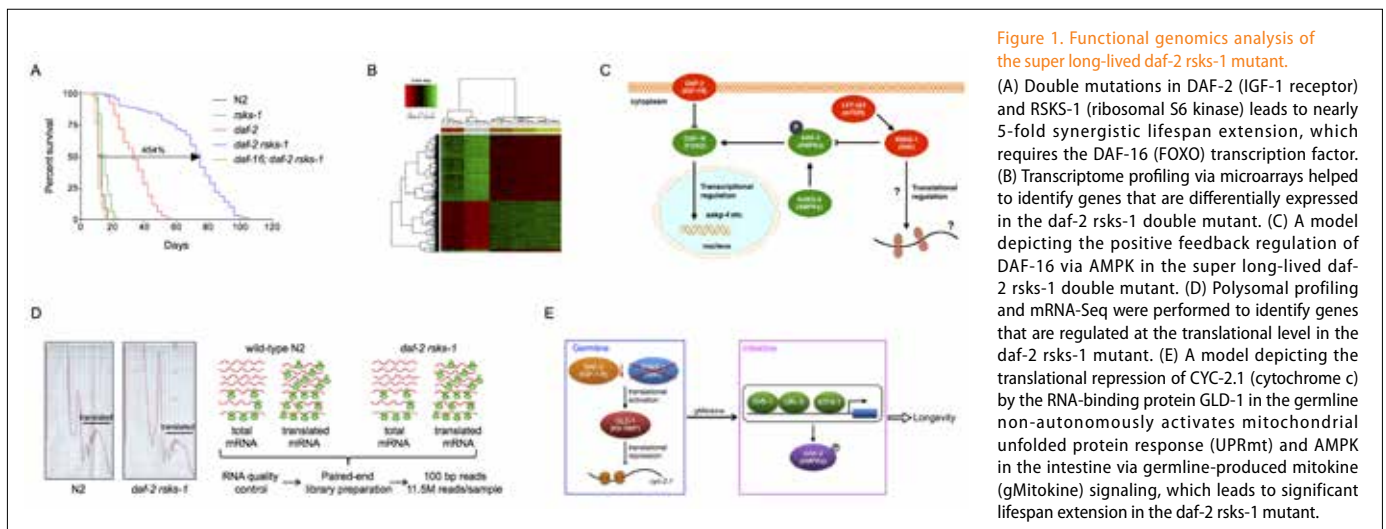
The highly conserved Insulin/IGF-1 signaling (IIS) and Target of Rapamycin (TOR) pathway play an important role in aging in many species. In order to characterize how IIS and TOR pathway interact with each other to modulate aging, we constructed a double mutant in DAF-2 (IGF-1 receptor) and TOR target RSKS-1 (ribosomal S6 kinase). Surprisingly, this *daf-2 rsk-1* mutant shows a nearly 5-fold, synergistic lifespan extension (Figure 1A). Using

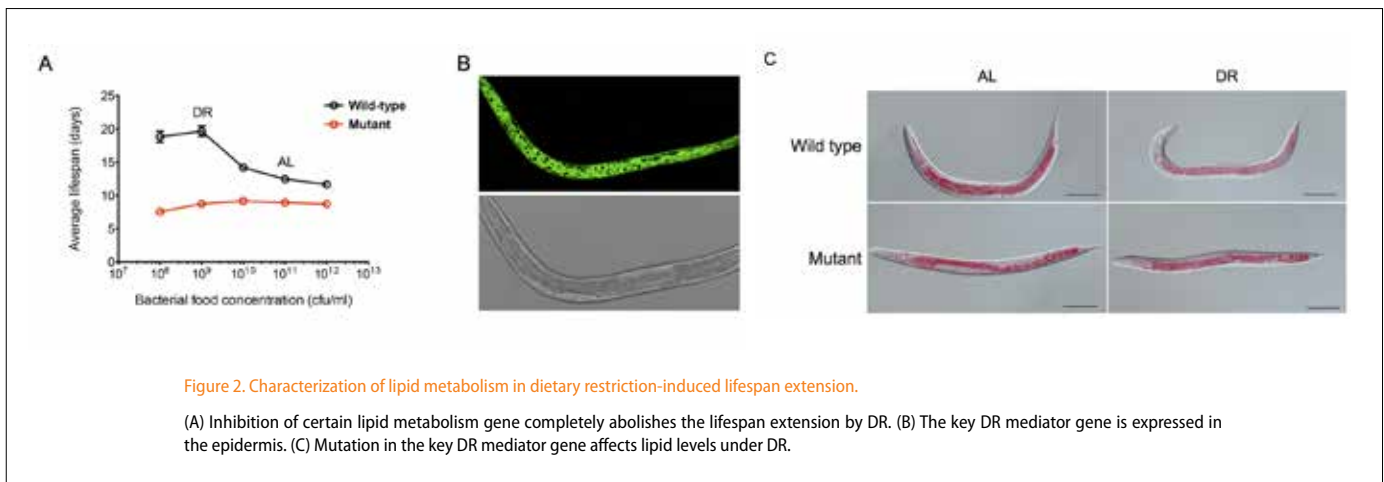
transcriptome profiling, we demonstrated that the underlying mechanisms involve positive feedback regulation of the DAF-16/FOXO transcription factor via the key energy homeostasis regulator AMPK (Figure 1B, C). We then performed polysomal profiling coupled with mRNA-Seq to identify genes that are translationally regulated in the *daf-2 rsk-1* mutant and characterize their roles in aging (Figure 1D). Eventually, we identified a translationally regulated non-autonomous mitochondrial stress response mechanism in the modulation of lifespan by insulin-like signaling and S6K (Figure 1E).

Dietary restriction (DR) is one of most robust environmental manipulations that slow down aging in various species. However, the molecular mechanisms of DR remain largely unknown. Previously, we demonstrated that the hypoxia inducible factor-1 (HIF-1) plays an important role in DR-induced lifespan extension by regulating the IRE-1 ER stress pathway. To gain better insights on the relationship between nutrients and aging, we performed an RNAi-based genetic screen and identified a key mediator of DR. Mutations in this gene affect DR-induced lifespan extension and lipid metabolism in a tissue-specific manner (Figure 2).

### Currently, our research focuses on the following aspects:

- 1) Cell non-autonomous regulation of mitochondrial stress response.
- 2) Lipid metabolism in dietary restriction-induced lifespan extension.
- 3) RNA metabolism in aging and age-related diseases.





### Recent publications (\*, corresponding authors):

1. Di Wu#, Waijiao Cai#, Xuan Zhang, Jianfeng Lan, Lina Zou, Samuel J. Chen, Zixing Wu, Di Chen\*, Inhibition of PAR-1 Delays Aging via Activating AMPK in *C. elegans*. *Aging*, 2020, 12(24): 25700-25717. (#, co-first authors)
2. Jianfeng Lan#, Jarod A. Rollins#, Xiao Zang#, Di Wu#, Lina Zou, Zi Wang, Chang Ye, Zixing Wu, Pankaj Kapahi\*, Aric N. Rogers\*, Di Chen\*, Translational Regulation of Non-autonomous Mitochondrial Stress Response Promotes Longevity. *Cell Reports*, 2019, 28(4): 1050-1062.e6. (#, co-first authors)
3. Lina Zou, Di Wu, Xiao Zang, Zi Wang, Zixing Wu, Di Chen\*, Construction of a Germline-specific RNAi Tool in *C. elegans*. *Scientific Reports*, 2019, 9(1): 2354.
4. Lei Gao, Rujun Zhang, Jianfeng Lan, Ruonan Ning, Di Wu, Di Chen\*, Weimin Zhao\*,  $\beta$ -Dihydroagarofuran-type Sesquiterpenes from the Seeds of *Celastrus monospermus* and Their Lifespan-extending Effects on the Nematode *Caenorhabditis elegans*. *Journal of Natural Products*, 2016, 79(12): 3039-3046.
5. Lei Hou#, Dan Wang#, Di Chen#, Yi Liu, Yue Zhang, Hao Cheng, Chi Xu, Na Sun, Joseph McDermott, William B. Mair, Jing-Dong J. Han, A Systems Approach to Reverse Engineer Lifespan Extension by Dietary Restriction. *Cell Metabolism*, 2016, 23(3): 529-540. (#, co-first authors)
6. Jianfeng Lan, Xuan Zhang, Di Chen\*, Molecular Mechanisms of Dietary Restriction in Aging - Insights from *Caenorhabditis elegans* Research. *Science China Life Sciences*, 2015, 58(4): 352-358.
7. Di Chen\*, Jiuli Zhang, Justin Minnerly, Tiffany Kaul, Donald L. Riddle, Kailiang Jia\*, *daf-31* Encodes the Catalytic Subunit of N alpha-Acetyl Transferase that Regulates *Caenorhabditis elegans* Development, Metabolism and Adult Lifespan. *PLoS Genetics*, 2014, 10(10): e1004699.
8. Di Chen\*, Patrick Wai-Lun Li, Benjamin A. Goldstein, Waijiao Cai, Emma Lynn Thomas, Fen Chen, Alan E. Hubbard, Simon Melov, Pankaj Kapahi\*, Germline Signaling Mediates the Synergistically Prolonged Longevity Produced by Double Mutations in *daf-2* and *rsk-1* in *C. elegans*. *Cell Reports*, 2013, 5(6): 1600-1610.



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## Chao-Jun Li, Ph.D

Chao-Jun Li received his Ph. D in Physiology from Nanjing University in 1994. He did his postdoctoral training at the Hong Kong University of Science and Technology from 1996-1998 and the Medical School of Yale University from 1999-2000. He worked as an extinguishing professor in Nanjing Normal University from 1994-2008 before he joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2008. He is now a professor of Cell Biology and a principal investigator in MARC and the Medical School of Nanjing University. He was elected as the vice-president of Chinese Society for Cell Biology since 2014.

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## Metabolic reprogramming and protein prenylation balance

Protein prenylation is a critical process for the membrane association of plenty of signaling proteins, including small G proteins. Geranylgeranyl diphosphate synthase (Gggs1) catalyzes the formation of geranylgeranyl diphosphate (GGPP) from farnesyl diphosphate (FPP), both of which are used to prenylate proteins with CaaX motif in their carboxyl termini, called farnesylation and geranylgeranylation respectively. The prenylated proteins then are able to associate with membrane to initiate their function. We first identified Gggs1 as a directly target gene of Egr-1, which can positively feedback to increase Egr-1 accumulation during chronic stress stimulation through enhance Ras prenylation and its membrane association (Am J Path, 2011a, 2011b; J Biol Chem 2011; EMBO J, 2011). Our hypothesis is that the

balance of protein farnesylation and geranylgeranylation or FPP and GGPP inside the cell is critical to cell homeostasis by affecting signal transduction and protein functions (J Biol Chem 2020). We have found that Gggs1 regulated protein prenylation balance is involved in spermatogenesis and infertility (J Exp Med, 2013; Sci Rep, 2016; PLoS Genetics, 2017; CDDis 2019); hypertrophy and heart failure (J Path, 2015; Cardiovasc Res. 2018); lipid-induced muscle insulin resistance (J Biol Chem, 2015; FASEB J); intracellular vesicle formation (Nat Comms, 2020; J Path, 2016); pulmonary development (Am J Path, 2016) and NAFLD/HCC progression (J Path, 2018). Right now, we are exploring protein prenylation balance and the metabolic reprogramming like glucose/lipid shift during pathological and physiological processes.

### 1. GGPP allosterically activates FBP1 homotetramer to link cholesterol synthesis with glucose metabolism (Lei Fang; Chao-Jun Li)

In previous research, GGPP and its precursor FPP are mainly noticed as substrates of protein prenylation. Nevertheless, metabolite-protein interactions (MPIs) not just include covalent co- or post-translational modification but also exist non-covalent effects like co-factors, ligand-receptor, substrate-enzyme, and client-carrier relationships. And many of which represent key nodes in biochemical networks that regulate physiological processes and diseases. Herein, we have generated an extensive interaction network between GGPP and proteins in primary hepatocytes using biotin-streptavidin system (BAS)-based affinity purification combined with label-free quantification mass spectrometry. Further bioinformatics analysis revealed novel interactions between GGPP and several key enzymes in glucose and lipid metabolism. Driven by further validation experiments, we demonstrated that hepatic GGPP directly binds to a gluconeogenesis rate-limiting enzyme FBP1. GGPP binding extremely enhanced its gluconeogenic activity. Further X-ray crystalline experiments were performed to describe the structure of binding between GGPP and FBP1 and explore the mechanism how GGPP coupled cholesterol synthesis with glucose metabolism, which is critical to coordinate the metabolic programs to fit the demand of cells under stress.

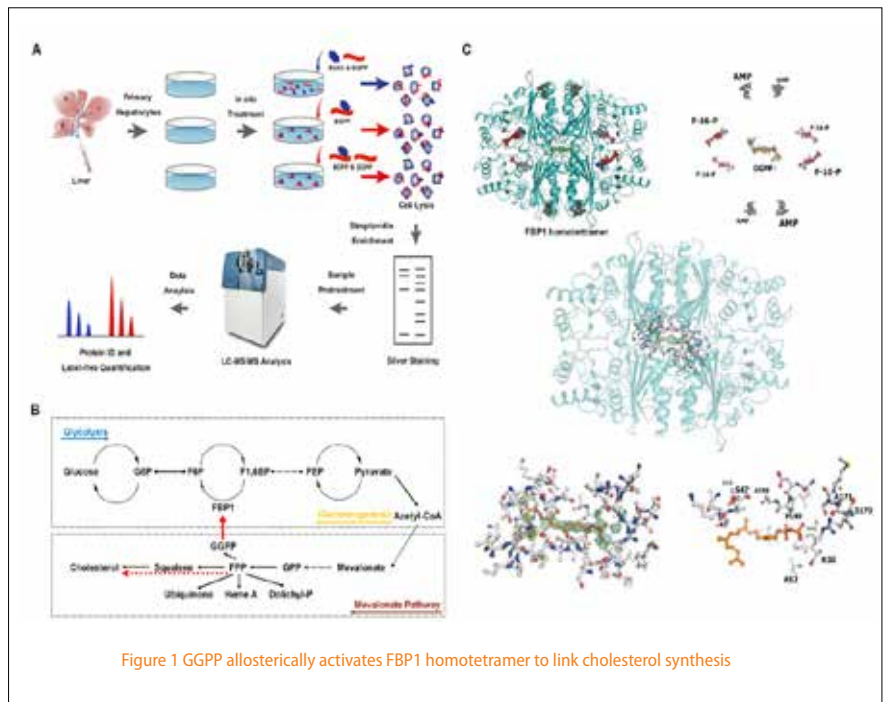
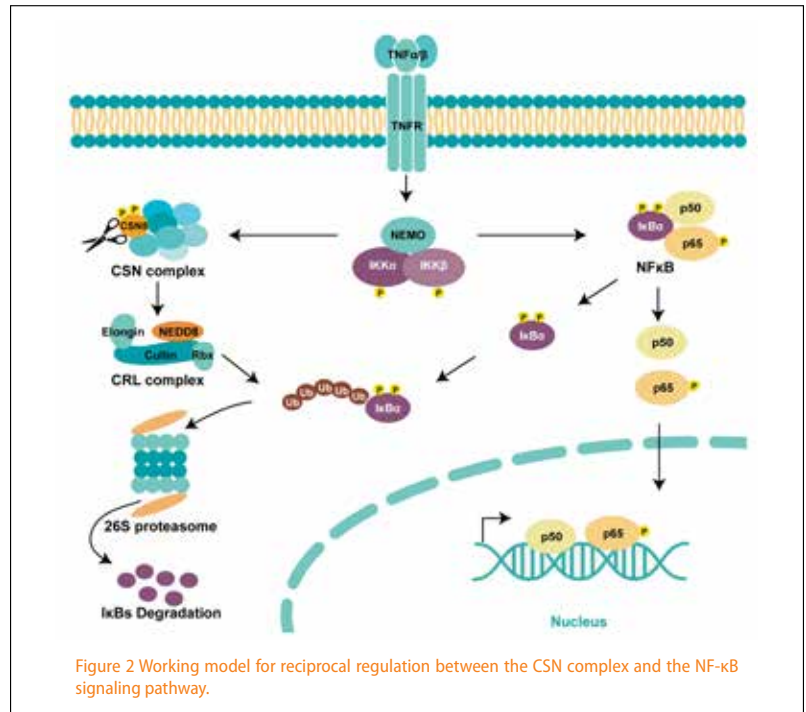


Figure 1 GGPP allosterically activates FBP1 homotetramer to link cholesterol synthesis



## 2. IKK-mediated regulation of the COP9 Signalosome via phosphorylation of CSN5 (Lei Fang)

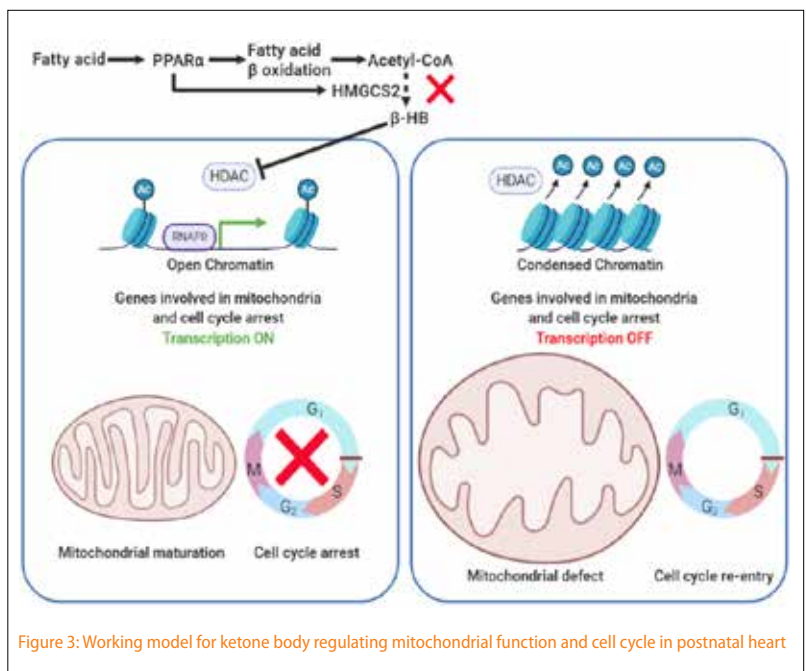
The COP9 signalosome (CSN) is an evolutionarily conserved multi-subunit protein complex, which controls protein degradation through deneddylation and inactivation of cullin-RING ubiquitin E3 ligases (CRLs). Recently, the CSN complex has been linked to the NF- $\kappa$ B signaling pathway due to its association with the IKK complex. However, how the CSN complex is regulated in this signaling pathway remains unclear. Here, we have carried out biochemical experiments and confirmed the interaction between the CSN and IKK complexes. In addition, we have determined that overexpression of IKK $\alpha$  or IKK $\beta$  leads to enhanced phosphorylation of CSN5, the catalytic subunit for CSN deneddylation activity. Mutational analyses have revealed that phosphorylation at serine 201 and threonine 205 of CSN5 impairs CSN-mediated deneddylation activity in vitro. Interestingly, TNF- $\alpha$  treatment not only enhances the interaction between CSN and IKK but also induces an IKK-dependent phosphorylation of CSN5 at serine 201, linking CSN to TNF- $\alpha$  signaling through IKK. Moreover, TNF- $\alpha$  treatment affects the CSN interaction network globally, especially the associations of CSN with the proteasome complex, eukaryotic translation initiation factor complex, and CRL components. Collectively, our results provide new insights into IKK-mediated regulation of CSN associated with the NF- $\kappa$ B signaling pathway.



## 3. Ketone body regulates mitochondrial maturation and cell cycle arrest in neonatal heart stimulated by colostrum fatty acids (Chao-Jun Li)

Mitochondrial oxidative phosphorylation dominates the generation of ATP supplying for myocardial contraction and pump function in adult heart. While glycolysis is preferred in fetal heart, fetal cardiomyocytes' mitochondria undergo perinatal replacement by mature mitochondria. Meanwhile, heart regeneration capacity decreases dramatically after birth and vanishes in 1 week. Here we showed that the expression of HMGCS2, the rate-limiting enzyme in ketogenesis, and its downstream ketone body appeared transiently in postnatal heart. Fatty acid in maternal milk induced HMGCS2 expression in neonatal cardiomyocytes. The functional study showed that ketone body was essential for mitochondrial improvement induced by fatty acid in neonatal cardiomyocytes. Moreover, ketone body deficiency decreased mitochondrial respiration in neonatal heart. TEM analysis revealed that mitochondrial ultrastructure was disrupted and mitochondria were swelled in ketone body-deficient heart. Cardiac function was also impaired in postnatal heart. Simultaneously, cardiomyocyte proliferation was increased in ketone body-deficient heart. Further studies showed that histone acetylation was significantly reduced in ketone body-deficient mouse hearts. Transcriptomics and subsequent GSEA analysis showed that the expression of a large number of genes in the hearts of in ketone body-deficient mice was decreased, while the genes with increased expression were relatively few. The genes with decreased expression were mainly involved in mitochondrial oxidative phosphorylation and electron transport chain. Genes related to the cell cycle were mainly negative regulators of cell cycle. Besides, histone deacetylase (HDAC) inhibitor could also promote cardiomyocyte

mitochondrial respiration and heart function but block cell proliferation. Our findings establish that the ketone body which appears transiently after birth regulates the expression of mitochondrial oxidative phosphorylation and cell cycle arrest-related genes by maintaining histone acetylation, and further promotes the perfection of cardiac mitochondrial function and cardiomyocyte cell cycle arrest in postnatal mouse.



## Selected Publications (#:Co-first authors; \*:Co-corresponding authors)

- Zhao Y, et al., Fang L\*, Sun XT\*, Xue B\*, Li CJ\*. Liver Governs Adipose Remodeling via Extracellular Vesicles in Response to Lipid Overload. *Nat Comms*. 2020; 11(1):719. doi: 10.1038/s41467-020-14450-6.
- Zhao Y, et al., Li CJ\*. The balance of protein farnesylation and geranylgeranylation during the progression of nonalcoholic fatty liver disease. *J Biol Chem*. 2020 pii: jbc.REV119.008897.
- Chen WW, et al., Li CJ\*, and Sun LY\*. Lipocalin-2 Exacerbates Lupus Nephritis by Promoting Th1 Cell Differentiation. *JASN* 31; 2020. doi: ASN.2019090937
- Zhang JZ, et al., Li CJ, Huang L\*, Fang L\*. IKK-mediated regulation of the COP9 signalosome via phosphorylation of CSN5. *J Proteome Res*. 2020 Jan 17. doi: 10.1021/acs.jproteome.9b00626.
- Zhang JZ et al, Li CJ, Qiu YD\*, Xue B\*, Fang Li\*. Global Phosphoproteomic Analysis Reveals Significant Metabolic Reprogramming in the Termination of Liver Regeneration in Mice. *J Proteome Res*. 2020 Feb 27. doi: 10.1021/acs.jproteome.0c00028.
- Liu J#, Jiang S#, Zhao Y#, et al., Xue B\*, Li C\*. Geranylgeranyl diphosphate synthase (Ggps1) regulates non-alcoholic fatty liver disease (NAFLD)-fibrosis progression by determining hepatic glucose/fatty acid preference under high-fat diet conditions. *J Pathol*. 2018 Nov;246(3):277-288. (Commentary by Fullerton MD. Does prenylation predict progression in NAFLD? *J Pathol*. 2018; Oct 30)
- Chen Z, et al., Li C\*. Geranylgeranyl pyrophosphate synthase facilitates the organization of cardiomyocytes during mid-gestation through modulating protein geranylgeranylation in mouse heart. *Cardiovasc Res*. 2018 Jun 1;114(7):965-978. (Commentary by Helen M. Phillips. Protein geranylgeranylation: a possible new player in congenital heart defects. *Cardiovasc Res*. 2018;114:922-924)
- Jiang C#, Diao F#, et al., Li CJ\*. GGPP-mediated protein geranylgeranylation in oocyte is essential for the establishment of oocyte-granulosa cell communication and primary-secondary follicle transition in mouse ovary. *PLoS Genetics*, 2017, 13(1): e1006535.
- Lai,SS et al., Gao X\*, Li CJ\*, Xue B\* PP2A $\alpha$  Positively Regulates Mice Liver Regeneration Termination through AKT/GSK3 $\beta$ /Cyclin D1 Pathway. *J Hepatology* 2016, 64(2):352-360
- Jiang S#, Shen D#, et al., Xue B\*, and Li CJ\*. Ggps1 mediated Rab27A geranylgeranylation regulates  $\beta$ -cell dysfunction during type 2 diabetes development via affecting insulin granule docked pool formation. *J Pathol*, 2016; 238: 109-119. (Commentary by Kowluru A. A lack of "glue" misplaces Rab27A to cause islet dysfunction in diabetes. *J Pathol*. 2016; 238: 375-377)



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Meng-Fei Zhao (Ph.D.)

Dan-Dan Bu (Ph.D.)

Yang-Qing Li (Ph.D.)

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Bing-Hao Wang (M.Phil.)

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Chao-Fan Yang (M.Phil.)

Yan Guo (M.Phil.)

Xin-Yu Li (M.Phil.)

Xiao-Chen Wang (M.Phil.)



## Zhenji Gan, Ph.D.

Zhenji received his Ph.D. degree in Biochemistry and Molecular Biology (2003 - 2008) from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. His Ph.D. work was carried out in Dr. Yong Liu's lab focused on metabolic diseases. From 2008 to 2013, Zhenji pursued his post-doctoral training in the areas of nuclear receptor signaling and energy metabolism under the guidance of Dr. Daniel Kelly at Sanford-Burnham Medical Research Institute. In 2013, he started a Principal Investigator position in the Model Animal Research Center (MARC) of Nanjing University.

### Contact Information

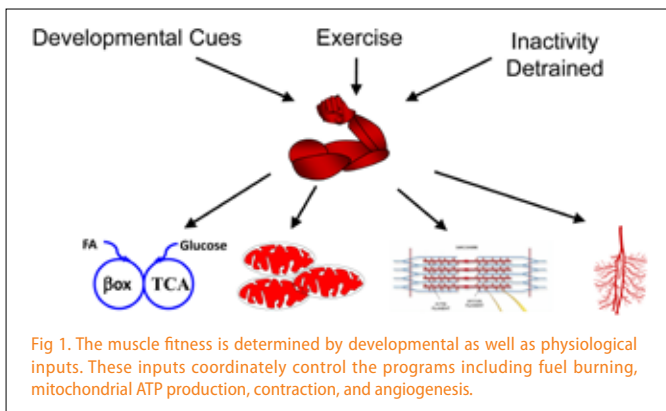
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## Energy metabolism and muscle fitness

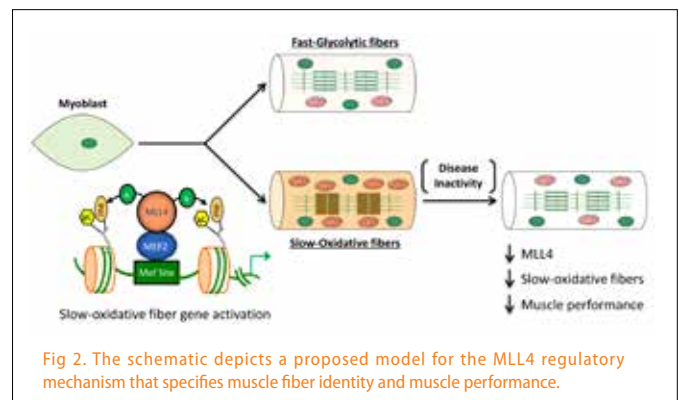
Muscle fitness is an important determinant of health and disease. Exercise training enhances muscle endurance and performance by augmenting the capacity of mitochondria to burn fuels and by increasing the proportion of slow oxidative fibers and blood supply. Conversely, reduced physical activity such as occurs with chronic illness, obesity, and aging results in de-trained muscle (Fig. 1). Our lab focuses on fundamental mechanisms that control muscle energy metabolism in physiological and disease states. We are particularly interested in exploring the regulatory networks involved in the coordinate control of energy metabolic and structural programs that define muscle fitness and their potential for therapeutic development.



### Histone methyltransferase MLL4 controls myofiber identity and running endurance

Skeletal muscle fitness is an important determinant of health and disease and muscle depends critically on the precise orchestration of contractile and metabolic gene expression programs to direct fiber type specification and to ensure muscle performance. However, exactly how such fiber type-specific patterns of gene expression are established and maintained remains unclear. Myofibers are generally classified as slow-twitch (type I) and fast-twitch (type II) with different contractile and energy metabolism functions. Type I myofibers are rich in mitochondria, relying largely on mitochondrial oxidative metabolism and resistant to fatigue, whereas type II myofibers generally contain fewer mitochondria, have lower oxidative capacity and are fatigue sensitive, and can be sub-classified as either type IIa, IIx, or IIb in rodents based on the type of myosin heavy chain (MHC) isoform expressed. Recently, we investigated the role of histone methyltransferase MLL4, an enhancer regulator enriched in slow myofibers, in controlling muscle fiber identity as well as muscle performance. We show that MLL4 is required for establishing and maintaining slow type I fiber program to ensure running endurance. MLL4 directly binds to enhancers and functions as a coactivator of MEF2

to drive the slow-oxidative type I fiber gene program. In addition, the MLL4 regulatory circuit is associated with muscle fiber type remodeling in humans. These results uncover a pivotal role for MLL4 in specifying structural and metabolic identities of myofibers that govern muscle performance (Fig. 2).



### Delineate a nutrient-sensing regulatory nexus that integrates nutritional states and the COPII vesicle trafficking.

As a metabolically active tissue, liver possesses a remarkable adaptive capacity to secrete lipids and proteins according to physiological fluctuations of nutrient availability. The cytoplasmic coat protein complex-II (COPII) is an evolutionarily conserved secretory machinery that is essential for cellular protein and lipid trafficking. One-third of the mammalian proteome are transported by the COPII secretory vesicles, and COPII vesicle trafficking in the liver is of particular importance in systemic metabolic homeostasis. Changes in nutrient availability elicit metabolic adaptations that require coordinated alterations in the profile of COPII cargos in the liver. Intracellular nutrient-sensing and COPII-mediated trafficking machinery need to be precisely coupled for maintaining hepatic metabolic homeostasis. However, it remains largely unexplored how the COPII machinery is regulated to meet the cellular secretory demand in response to various physiological stimuli. Recently, we show that COPII vesicle trafficking is highly dynamic and responsive to nutrient availability fluctuations. We also uncover that nutrient-sensing IRE1 $\alpha$ -XBP1s axis links COPII-mediated trafficking to nutrient availability. Furthermore, restoration of XBP1s in mice lacking hepatic IRE1 $\alpha$  activates COPII-dependent lipoprotein traffic and reverses hepatosteatosis and hypolipidemia. Hence, we reveal a mechanism for the orchestration of COPII vesicle trafficking in response to nutrient availability (Fig. 3).



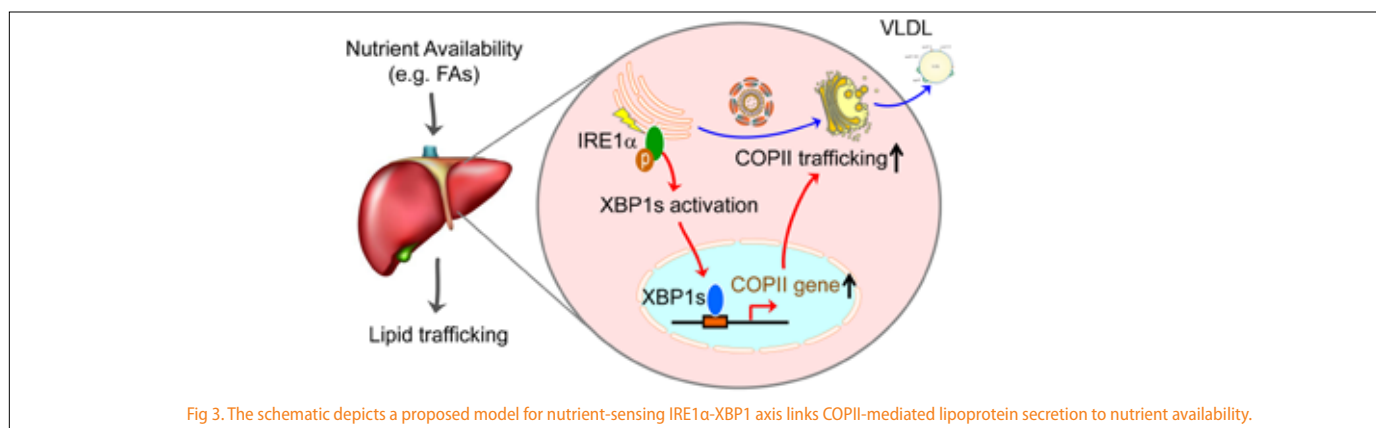


Fig 3. The schematic depicts a proposed model for nutrient-sensing IRE1 $\alpha$ -XBP1 axis links COPII-mediated lipoprotein secretion to nutrient availability.

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- Liu L, Cai J, Wang H, Liang X, Zhou Q, Ding C, Zhu Y, Fu T, Guo Q, Xu Z, Xiao L, Liu J, Yin Y, Fang L, Xue B, Wang Y, Meng ZX, He A, Li JL, Liu Y, Chen XW\*, Gan Z\*. Coupling of COPII vesicle trafficking to nutrient availability by the IRE1 $\alpha$ -XBP1s axis. *Proc Natl Acad Sci USA.* 2019;116(24):11776-11785.
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- Gan Z, Rumsey J, Hazen BC, Lai L, Leone TC, Vega RB, Xie H, Conley KE, Auwerx J, Smith SR, Olson EN, Kralli A, Kelly DP. Nuclear receptor-microRNA circuitry links muscle fiber type to energy metabolism. *J Clin Invest.* 2013;123(6):2564-75. Press Release at EurekaAlert: Differences between 'marathon mice' and 'couch potato mice' reveal key to muscle fitness. [http://www.eurekaalert.org/pub\\_releases/2013-05/smri-db043013.php](http://www.eurekaalert.org/pub_releases/2013-05/smri-db043013.php)
- Gan Z, Burkart-Hartman EM, Han DH, Finck B, Leone TC, Smith EY, Ayala JE, Holloszy J, Kelly DP. The nuclear receptor PPAR $\beta$ /d programs muscle glucose metabolism in cooperation with AMPK and MEF2. *Genes & Development.* 2011;25(24):2619-30. Press Release at EurekaAlert: Super athletic mice are fit because their muscles burn more sugar. [http://www.eurekaalert.org/pub\\_releases/2011-11/smri-sam112811.php](http://www.eurekaalert.org/pub_releases/2011-11/smri-sam112811.php)



## Group members

### Group Leader

Zhenji Gan

### Graduate students

Zhisheng Xu    Qiqi Guo    Zheng Zhou  
Yujing Yin    Wanping Sun    Yuhuan Jia  
Tingting Fu    Zongchao Sun    Xinyi Chen  
Dengqiu Xu    Chenyun Ding    Likun Yang

### Former Graduate Students

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Xijun Liang (Ph.D.)  
Tingting Fu (Ph.D.)  
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### Technical Assistant

Danxia Zhou

### Research Associates

Tingting Fu  
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## Hong-Yu Wang, Ph.D.

Dr. Hong-Yu Wang gained a PhD in Plant Molecular Genetics from Saarland University in 2006 and following Postdoctoral Research posts at University of Dundee and the University of Oxford, joined Model Animal Research Center in 2012.

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The liver is a key organ in vertebrates, which has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of chemicals for digestion. Nonalcoholic fatty liver disease (NAFLD) is a range of condition caused by the hepatic fat accumulation, which is also considered the hepatic manifestation of metabolic syndrome affecting about one-third of the population worldwide. Up to 25% of NAFLD patients develop a progressive inflammatory and damaged liver disease termed non-alcoholic steatohepatitis (NASH) that may progress towards cirrhosis, hepatic carcinoma, and the need for liver transplantation. Yet, the pathogenesis of NAFLD/NASH has not been completely elucidated. However, insulin resistance, inflammatory cytokines, and oxidative stress are thought to be important in the development and/or progression of the disease. Lifestyle modification with exercise and diet has been the first step in NAFLD/NASH treatment.

Our laboratory aims to understand the molecular mechanisms of the development and progression of NAFLD/NASH. Lipidomics, biochemistry, cell biology and transgenics approaches are applied to identify novel components for diagnosis and intervention of NAFLD/NASH progressions.

14-3-3 as an evolutionarily-conserved proteins regulate many cellular processes through binding to various phosphorylated targets in eukaryotes, which first appears in Dictyostelium. In our recent study, we show that 14-3-3 interacting with its targets is up-regulated in response to developmental events including starvation, osmotic stress and cAMP. As important role in cyclic AMP (cAMP) signaling pathway, 14-3-3 can regulate chemotaxis and tip formation in Dictyostelium, strengthen our knowledge about the evolution of 14-3-3 and its interactomes in eukaryotes



### Group members

#### PI

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#### Graduate students

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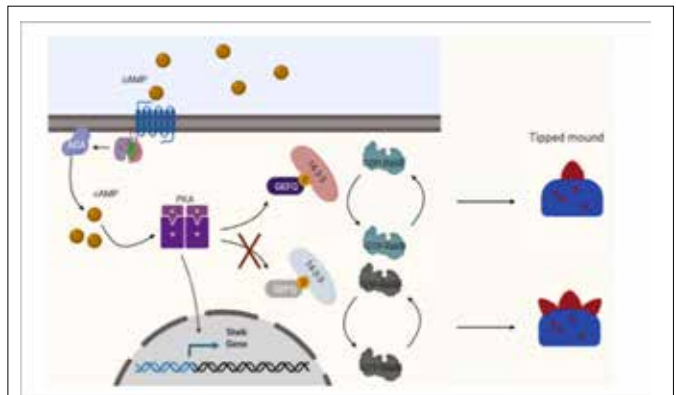


Fig. 1 Graphic illustration of the 14-3-3 and its interactors in regulation of tip formation

### Selected publications:

- Li M; Quan C, Chen S; Wang HY ; \* The 14-3-3 protein is an essential component of cyclic AMP signaling for regulation of chemotaxis and development in Dictyostelium, Cellular Signalling, 2020, 75 109739
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- Chen QL, Rong P, Zhu SS, Yang XY, Ouyang Q, Wang HY and Chen S\* (2019) Targeting RalGAPa1 in skeletal muscle to simultaneously improve postprandial glucose and lipid control. Science Advances 5(4): eaav4116
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## Yan LI Ph.D.

Dr. Yan Li acquired his Ph.D in 2012 from Singapore-MIT alliance program under the supervision of MIT Professor Jianzhu CHEN. From 2012 to 2016, he completed post-doctoral training with Prof. James DI SANTO in Institut Pasteur, Paris, and was promoted as an assistant professor in 2016. In 2018, he was hired as a professor in Nanjing University and a principal investigator at the State Key Laboratory of Pharmaceutical Biotechnology. In 2019, he was qualified as a doctoral supervisor, a principal investigator at the Innovation Institute of Nanjing University, awarded as Jiangsu Province "Innovative and Entrepreneurial Talent" and "Distinguished Professor", and received the grant support from the National Key R&D Program Youth Program (formerly the Youth 973 Program). In 2020, he was awarded the leading talent of Jiangsu Innovative and Entrepreneurial Team Program. His main research results have been published in prestigious journals such as Cell, Nature Methods, Nature Communications, etc., and have been invited for presentation at international conferences 9 times, and filed one international patent. Dr. Yan Li is currently a member of the Immune Cell Branch of the Chinese Society of Cell Biology, a member of the Chinese Anti-Cancer Association, a board member of the Jiangsu Society of Cell and Developmental Biology, an associate editor of the Frontiers in Immunology humanized mouse topic, and a member of the Scientific Committee of the International Workshops on Humanized Mice. In addition, he has reviewed manuscripts for journals such as PNAS, European Journal of Immunology, and Frontiers in Immunology.

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## Next-generation humanized mouse models for translational medicine

In order to accelerate the translation from basic biological discoveries into clinical treatments, our team has developed a series of mouse strains and experimental methods, which chimerized the human

immune system and a variety of human tissues into mice, and solve scientific questions that are difficult to answer by traditional mouse models and clinical trials.

### The current research fields of our team include (Figure 1) :

- (1) Development of humanized mouse models for fully human antibody discovery and vaccine evaluation.
- (2) HLA fully matched immune system + liver/lung doubly humanized mouse model.
- (3) Immunotherapy for tumors and autoimmune diseases.
- (4) Regulation of human immune development and function via gut microbiota derived metabolites.

At present, the laboratory is supported by the National Natural Science Foundation of China/Ministry of Science and Technology, Jiangsu Provincial Department of Science and Technology/Education and

Nanjing University. We have undertaken some projects including the National Key Research Plan, Jiangsu Innovative and Entrepreneurial Team, and the Fundamental Research Funds for the Central Universities.

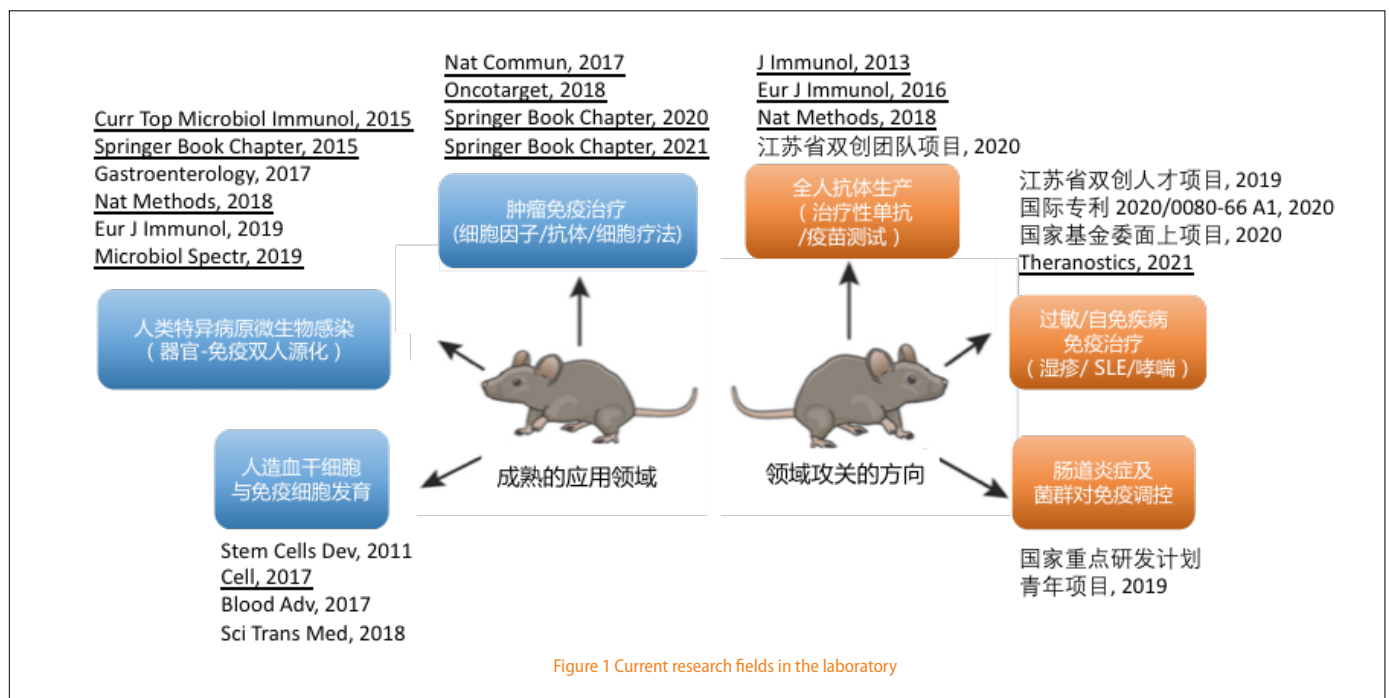


Figure 1 Current research fields in the laboratory



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4. Yan Li\* and James P. Di Santo (2019). Modeling infectious diseases in mice with a 'humanized' immune system. *Bacteria and Intracellular Microbiol Spectrum* 7(2):BAI-0019-2019. doi:10.1128/microbiolspec.BAI-0019-2019.
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## Group members

### Group leader

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### Postdoctoral Fellow

Deshan Ren

### Graduate student

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Chunyu Cheng

Shuang Liu

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Xiang Duan

Zijian Zhang

### Trainees in collaboration

Rujie Zhu

### Technical Assistant

Wei Zhao



## Zhaoyu Lin, Ph.D.

Zhaoyu Lin received his Ph.D degree in 2012 from Nanjing University under the mentoring of Dr.Gao Xiang. He has been a visiting scholar in Medical School of Washington University in St. Louis for three years. In 2014, he joined the Model Animal Research Center (MARC) of Nanjing University as research associated professor. In 2019, he became associated professor and a principle investigator in MARC.

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## Immune and metabolic regulation of physical homeostasis

Immune and metabolism is the key factors to maintain the physical homeostasis. The disruption of immune or metabolic regulation of physical homeostasis will lead to the occurrence of complex diseases, like autoimmune disease, obesity, cancer, cardiovascular disease and Alzheimer's disease. In our laboratory, we are interest in analysis of functions and the underlying molecular mechanisms of the disease related genes in immune or metabolic homeostasis.

Recently, we focus on a new discovered immunoregulatory protein family-Gasdermin. Our lab analyzed the roles of Gasdermin family in physical status and autoimmune diseases. Gsdmd and Gsdme are demonstrated to be the executors of pyroptosis, which is a type of pro-inflammatory programmed cell death. We discovered that Gasdermin directly trigger cell death and inflammation firstly in 2015, which was selected as "Breakthrough of the year" by Science. Our recent works are mainly about the regulation of Gsdmd in pyroptosis (Figure 1). We found that inhibition of ROS reduces the cleavage of Gsdmd in canonical pyroptosis and inhibition of GSDMB reduces the cleavage of GSDMD in non-canonical pyroptosis. We developed several methods to block pyroptosis in autoimmune diseases. One of the methods is using magnesium to block the membrane translocation of Gsdmd.

Furthermore, supplement of magnesium could greatly enhance the survival rate of sepsis mice model.

We are also interesting with the relationship between obesogenic memory and immunity. Obesity, as a rapidly emerging public health problem, are associated with many severe diseases/complications, resulting in significantly compromised life quality of the patients. These patients can be greatly benefited by weight management. However, weight is very often regained during and after the treatments for obesity. This phenomenon is named obesogenic memory, leading to the failure of weight management and more importantly, of controlling the obesity-associated health problems including diabetes. Therefore, understanding the mechanisms regulating obesogenic memory, is especially beneficial for the patients with obesity. In the previous work, we has demonstrated that among immune cells, CD4+ T cells are the direct carrier, which is necessary and sufficient to induce and maintain obesogenic memory in mice. Recently, we found that obesogenic memory related CD4+ T cells are a subpopulation of central memory T cells with high expression of CD300C, which is a receptor of phosphatidylethanolamine (PE), an essential group of phospholipids in the cell membrane (Figure 2).

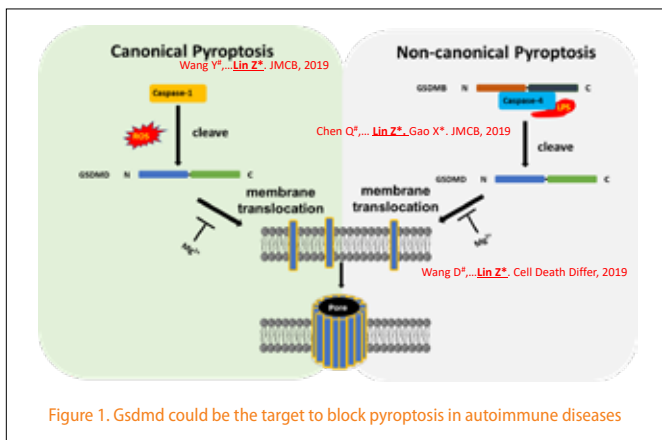


Figure 1. Gsdmd could be the target to block pyroptosis in autoimmune diseases

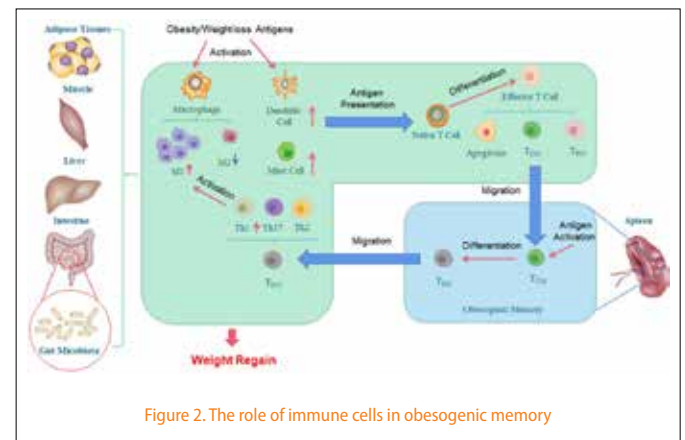


Figure 2. The role of immune cells in obesogenic memory

## Selected Publications

1. Zou, D., J. Pei, J. Lan, H. Sang, H. Chen, H. Yuan, D. Wu, Y. Zhang, Y. Wang, D. Wang, Y. Zou, D. Chen, J. Ren\*, X. Gao\*, and Z. Lin\*, A SNP of bacterial blc disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption. *EBioMedicine*, 2020. 52: p. 102652.
2. Wang, D., J. Zheng, Q. Hu, C. Zhao, Q. Chen, P. Shi, Q. Chen, Y. Zou, D. Zou, Q. Liu, J. Pei, X. Wu, X. Gao\*, J. Ren\*, and Z. Lin\*, Magnesium protects against sepsis by blocking gasdermin D N-terminal-induced pyroptosis. *Cell Death Differ*, 2020. 27(2): p. 466-481.
3. Sun, M., S. Zheng, X. Gao\*, and Z. Lin\*, The role of immune cells in obesogenic memory. *Cell Mol Immunol*, 2020.
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5. Chen, Q., P. Shi, Y. Wang, D. Zou, X. Wu, D. Wang, Q. Hu, Y. Zou, Z. Huang, J. Ren\*, Z. Lin\*, and X. Gao\*, GSDMB promotes non-canonical pyroptosis by enhancing caspase-4 activity. *J Mol Cell Biol*, 2019. 11(6): p. 496-508.
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7. Zou, J., B. Lai, M. Zheng, Q. Chen, S. Jiang, A. Song, Z. Huang, P. Shi, X. Tu, D. Wang, L. Lu, Z. Lin\*, and X. Gao\*, CD4+ T cells memorize obesity and promote weight regain. *Cell Mol Immunol*, 2018. 15(6): p. 630-639.
8. Lin, Z. \*, F. Liu, P. Shi, A. Song, Z. Huang, D. Zou, Q. Chen, J. Li, and X. Gao, Fatty acid oxidation promotes reprogramming by enhancing oxidative phosphorylation and inhibiting protein kinase C. *Stem Cell Res Ther*, 2018. 9(1): p. 47.



## Group members

Group Leader	Technicians	Graduate Student
Zhaoyu Lin	Jiafeng Zou Jiaxiang Zou	Ruyue Xin





# *Cancer and Stem Cell Biology*



## Geng Liu, Ph.D.

Geng Liu received his B.S. degree in Biochemistry from Wuhan University, China and his Ph.D. degree in Gene & Development from University of Texas Graduate School of Biomedical Sciences at Houston in 1999. After his postdoctoral training at University of Texas M.D. Anderson Cancer Center, Dr. Geng Liu joined the Model Animal Research Center of Nanjing University as a principal investigator and professor of Genetics in 2006.

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## Cellular stress response and metabolism on cell behavior and function

Integral to their functions, various cell behaviors are dictated by extrinsic and intrinsic stimuli through a network of signaling mechanisms. Our laboratory is interested in studying the determinants of cell behaviors and their close connections with stress responses and cellular metabolism in the contexts of tissue homeostasis as well as cancer. We investigated how stress response as mediated by the p53 signaling pathway regulated cell behaviors including cell proliferation, cell competition, inflammatory response and Epithelial-Mesenchymal

transition. While cellular metabolisms are required for the execution of proper cell functions, they could also serve as a signaling module in adapting the cells to certain behaviors. In addition, cell metabolisms are intrinsically connected to cellular redox state and stress response. Therefore, dissecting the intricate interplay between cell behaviors, stress responses and metabolism may allow us to fully understand the complex cell behaviors in many fundamental processes including development, ageing and tumorigenesis.

### p53 stress response pathway influences cell behaviors in distinctive manners.

p53 is extremely important for stress response and tumor suppression as exemplified by its mutations found in over 50% of human cancers. p53 protein is undetectable in normal tissues. With the BAC transgenic p53 reporter mice, we revealed a regulatory mechanism controlling p53 expression and activity selectively in the proliferating cellular compartments during mouse development, postnatal growth, tissue homeostasis, regeneration and tumorigenesis (Chen, et al., 2015). The close monitoring of cellular proliferation state by p53 also serves as a base to generate genetic tools in studying the cardiomyocyte proliferation during heart regeneration (Xiao, et al., 2017).

In the present of stress, p53 is activated to exert its role in influencing the cell fate. Various degree of stresses result in different level of p53 activation. Instead of directing the classic pathways of cell cycle arrest, senescence or apoptosis, we demonstrated that low dose X-ray induced mild p53 activation affected the EMT process during valvuloseptal morphogenesis of mouse cardiac development and resulted in congenital heart defects in mice (Zhang, et al., 2012). p53 also play a crucial role in macrophage polarization in the tumor microenvironment to affect tumorigenesis in a non-cell autonomous manner (He, et al., 2015). Our recent study found that mild p53 activation in cells renders them less competitive in multi-cellular context during mouse embryogenesis, possibly contributing to the control of tissue fitness (Zhang, et al, 2017). These results indicate that p53 signaling pathway critically and delicately influence cell behaviors and functions in distinctive manners. We are currently addressing the link between stress signaling and tumor cell plasticity.

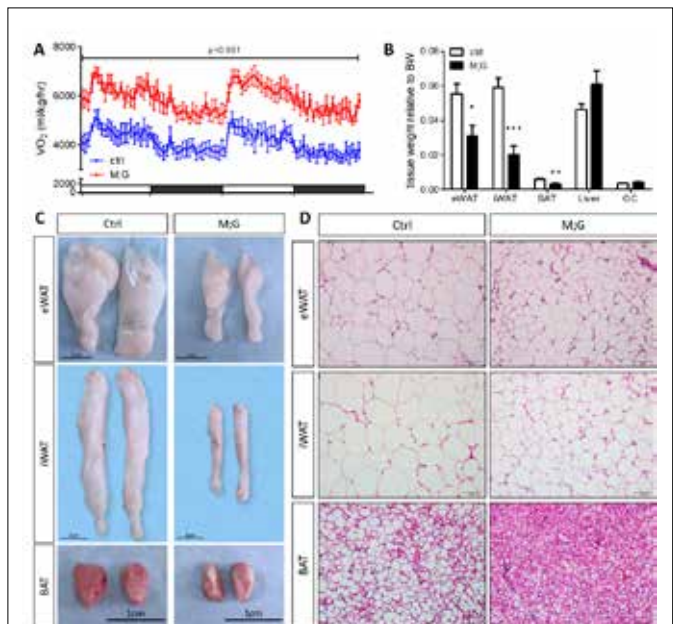


Figure 1. Enhanced glucose metabolism in skeletal muscle of M;G mice coordinates with increased lipid metabolism in adipose tissue for systemic homeostasis under high-fat-diet condition.

(A) Increased energy expenditure in the M;G mice; (B) Reduced weight of adipose tissue in the M;G mice; (C,D) Reduced sizes of both fat tissue (C) and adipocytes (D) in M;G mice.



## Manipulating and probing cellular metabolism in vivo.

To study the influence of cellular metabolism on cell behaviors and function in a multitude of in vivo contexts, we have established a series of mouse models involved in promoting specific metabolic pathways in a controlled manner. Our results showed that cellular metabolisms could be manipulated in vivo and may have great impact on either cell behavior or systemic homeostasis. By genetically manipulating glucose metabolism in the mouse skeletal muscle, lipid metabolism in the adipose tissue was dramatically elevated resulting

from crosstalk of the two tissues (Fig.1). We also found that the alteration of T cell metabolism could potentially stimulate the anti-tumor immune response. In addition, as cell metabolic state is tightly linked to the intrinsic regulatory mechanisms, we have established models in probing the metabolic heterogeneity within the tissues to study diverse cellular functional and regulatory attributes in physiological and pathological contexts.

### Publications

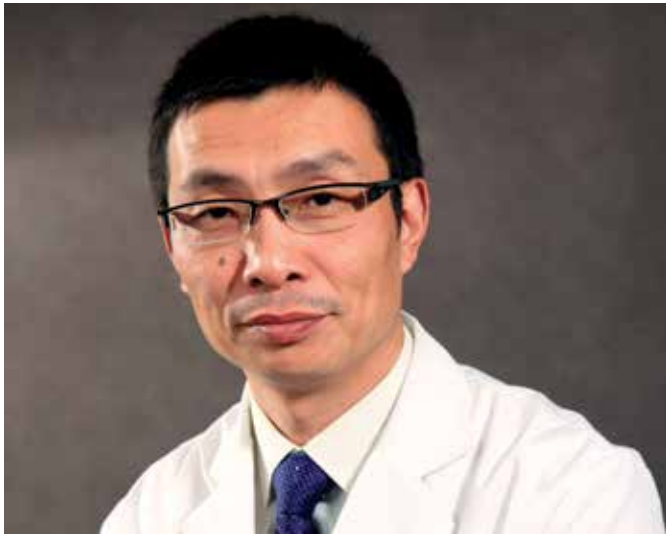
1. Xiang C, Zhang YN, Chen QL, Sun AN, Peng YM, Zhang GX, Zhou DX, Xie YY, Hou XS, Zheng FF, Gan ZJ, Chen S\*, and Liu G\* Increased anaerobic glycolysis in skeletal muscle coordinates with adipose tissue in systemic metabolic homeostasis. In submission.
2. Zhang GX; Xie YY; Zhou Y; Xiang C; Chen L; Zhang CX; Hou XS; Chen J; Zong H; Liu G\* (2017) p53 pathway is involved in cell competition during mouse embryogenesis. *Proc Natl Acad Sci U S A*. 2017 Jan 17; 114(3):498-503.
3. Xiao Q, Zhang GX, Wang HJ, Chen L, Lu SS, Pan DJ, Liu G\* and Yang ZZ\* (2017) A p53 based genetic tracing system to follow postnatal cardiomyocyte expansion in heart regeneration. *Development*. 2017 Feb 15;144(4):580-589.
4. Wang J, Ye H, Zhang D, Cheng K, Hu Y, Yu X, Lu L, Hu J, Zuo C, Qian B, Yu Y, Liu S, Liu G, Mao C, Liu S (2017) Cancer-derived circulating MicroRNAs promote tumor angiogenesis by entering dendritic cells to degrade highly complementary MicroRNAs. *Theranostics*. 2017 Mar 24;7(6):1407-1421.
5. Zhang CX, Zhang Q, Xie YY, He XY, Xiang C, Hou XS, Zhou Y, Chen L, Zhang GX and Liu G\* (2016) MDM 2 actively suppresses p53 activity in oocytes during mouse folliculogenesis. *Am J Pathol*. 2017 Feb;187(2):339-351.
6. He XY, Xiang C, Zhang CX, Xie YY, Chen L, Zhang GX and Liu G\* (2015) p53 in myeloid lineage modulates an inflammatory microenvironment limiting initiation and invasion of intestinal tumors. *Cell Rep*. 2015 Nov 3; 13(5):888-97.
7. Chen L, Zhang GX, He XY, Zhang CX, Xie YY and Liu G\* (2015) BAC transgenic mice provide evidence that p53 expression is highly regulated in vivo. *Cell Death Dis*. 2015 Sep 17;6:e1878.



### Group members

Group Leader	Graduate students	Former lab members	Technical staff
Geng Liu	Xiaoshuang Hou	Lai Chen (Ph.D.)	Cong Xiang (Ph.D.)
Postdoctoral fellow Xie Yinyin	Yamei Peng	Xueyan He (Ph.D.)	Ying Zhou (MS)
	Yannan Zhang	Chenxi Zhang(Ph.D.)	Weikang Hou (MS)
	Fangfang Zheng	Guoxin Zhang (Ph.D.)	Aina Sun (M.S.)
	Fan Wang	Yinyin Xie (Ph.D.)	





## Qing Jiang Ph.D., M.D.

Chief physician / Professor / Doctoral supervisor; The head of the department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital/ The vice president of school of medicine, Nanjing University/ Director, institute of medical 3D printing, Nanjing University. Prof. Jiang, the first sports medicine clinical doctor cultivated by China, had been engaged in orthopedic and sports medicine clinical and basic research since 1989 and got the PhD degree in Beijing Medical University in 1999. In 2008, he was appointed professor in Nanjing University and Adjunct Professor in Model Animal Research Center (MARC). Prof. Jiang won the National Science Fund for Distinguished Young Scholars in 2011. The department of Sports Medicine and Adult Reconstructive Surgery is the only joint disease treatment center identified by Jiangsu Provincial Health Department, and also the training base of artificial joint and arthroscopic techniques in Jiangsu province. Jiang's team has established human gene bank of bone and joint disease including osteoarthritis (OA), developmental dysplasia of the hip (DDH), deep venous thrombosis (DVT), ankylosing spondylitis (AS) and osteoporosis (OP), and published 232 Chinese core articles, 138 SCI articles, included Nature Medicine, Nature Genetic, ACS NANO, Advanced Functional Materials, Annals of the Rheumatic Diseases, Biomaterials, Small. Prof. Jiang is the first domestic scholars who hold the post of committee member of the OARSI, who also is vice chair of China branch of ICRS, vice chairman of sports medicine branch of Chinese medical association, chairman of sports medicine branch of Jiangsu medical association, vice chairman of orthopedics branch, vice chairman of trauma branch, and next chairman orthopaedic branch of Jiangsu medical association, etc.

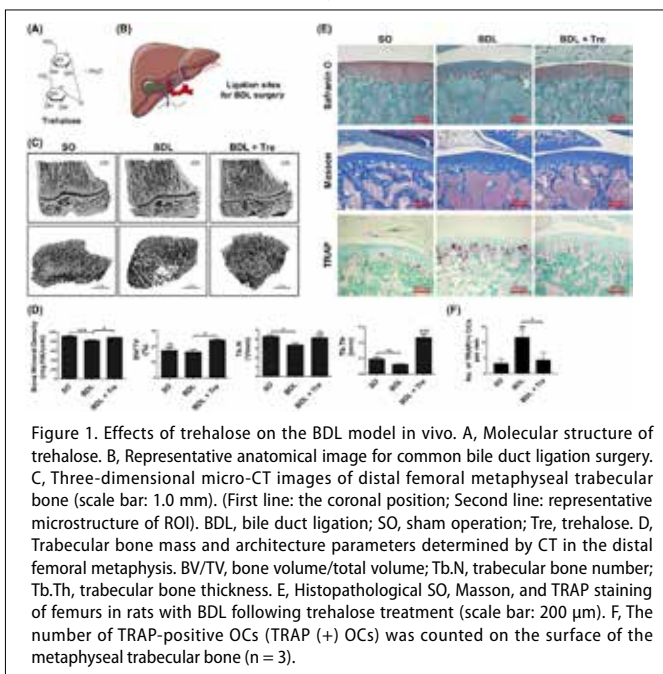
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## Skeletal System Disease

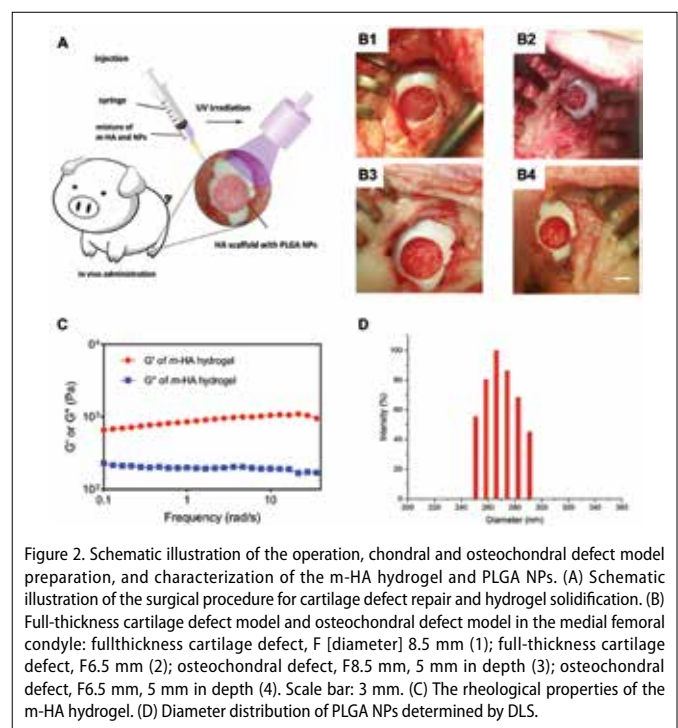
**Cartilage regeneration scaffold.** We evaluated whether the co-culture of human umbilical cord Wharton's jelly-derived MSCs (hWJMSCs) and primary cartilage cells (pACs) in a double biomimetic acellular cartilage extracellular matrix (ACECM)-oriented scaffold could achieve a joint surface that mimics the native state as closely as possible and return the articular cartilage to its natural biomechanics and compositions (Zhang, 2020). An *in vivo* study was conducted on knee joint of a rabbit to verify the practicability of the proposed approach in repair of cartilage. Our approach may enhance *in situ* 3D bio-printing in clinical treatment (Ma, 2020).

**Mechanism and treatment study of Orthopedics.** A bile duct-ligated (BDL) male rat model was used to establish the features of biliary cirrhosis and the characteristics of osteoporosis. The effect of intraperitoneal injection of trehalose on bone mass in BDL male rats and the related mechanisms were investigated (Figure 1) (Xu, 2020). Treatment of cartilage lesions is clinically challenging. According to our study, (1) m-HA 1 KGN treatment facilitated hyaline cartilage and subchondral bone tissue repair in a porcine model at the 12-month follow-up. (2) m-HA 1 KGN treatment demonstrated better therapeutic efficacy in defects with a diameter of 6.5 mm or full-thickness chondral-type defects. (Figure 2) (Yan, 2020).



**Identification and functional characterization of novel inheritable disease-causing genes.** We have performed GWAS study of Developmental dysplasia of the hip (DDH), and detected novel genes and signaling pathways. A total of 406 DDH-associated genes ( $P < 0.001$ ) were identified. An intronic SNP, the minor allele (rs61930502-A), tended to prevent DDH showed a dominant effect. In previous association studies, genes such as GDF5, TBX4, and ASPN was detected associate with DDH by case-control studies. We focused on and expertise in searching molecular mechanisms in human genome for genetic skeletal diseases. The DNA bank for skeletal diseases is still enlarging.

**Deep vein thrombosis (DVT).** Lots of retrospective and prospective experiments had conducted to study how to prevent DVT in our group. Total joint arthroplasty (TJA) is a form of high-risk postoperative venous thromboembolism (VTE). We determined the relationship between preoperative Soleal veins (SVs) diameter and postoperative DVT after TJA. A large SV diameter was significantly associated with postoperative total DVT after TJA, and also symptomatic DVT after TJA. The bank for DVT patients is still enlarging.



### Below is a brief list of main research projects currently going on in the lab.

1. (Key projects of NSFC 8173000209) The mechanism study of the cartilage and subchondral bone defect reconstruction using a hydrogel with sustained release of small molecule kartogenin.
2. (Major Projects of NSFC) Study on the role and regulatory mechanisms of bone-derived factors in maintaining homeostasis of the body--Manipulating bone derived factors to develop therapeutic strategies for extra-bone diseases
3. (Excellent Young Scholars NSFC 81622033) The study of repair cartilage defect by using hyaline hydrogel with sustained small molecule BIO.
4. (NSFC 81702151) Tendons outside source of stem cells secrete body by passing mirnas injured tendon repair.
5. (SBK2017040751) 3D printing more peptide base the numerical modeling and optimization of the subchondral bone and animal studies for the treatment of net focal cartilage injury Natural Science Foundation of Jiangsu Province, China
6. (NSFC 81871832) Effect and mechanism of exosomes in circulating blood on venous thrombosis during perioperative orthopedics.
7. (NSFC 81702151) Exosomes derived from tendon stem cells facilitate repair of damaged tendons by delivering microRNA.
8. (NSFC 8180090535) A study on the application of hollow porous magnetic nanoparticles to target aggregation and control of the release of icariin to promote fracture healing in mice
9. (NSFC 81972124) The Effect and Mechanism of Low Magnitude High Frequency Mechanical Stimulation on Promoting the Transformation of Tissue Engineering Polylactic-hydroxyapatite Scaffold into Normal Tissue.
10. (NSFC 81902174) The study of LRP1 regulating the pathogenesis of developmental hip dysplasia (DDH) through the Wnt/  $\beta$ -catenin signaling pathway.
11. (NSFC 82002370) Mechanism of effective therapy for Rheumatoid arthritis by Engineered neutrophil-derived exosomes
12. (Six Talent Peaks Project of Jiangsu Province WSW-061) Peptide hydrogel repair of sustained-release small molecule organic compound BIO
13. (Natural Science Foundation of Jiangsu Province, China BK2017040751) Three Digital modeling and optimization of polypeptide subchondral bone and animal study on the treatment of focal cartilage injury
14. (Natural Science Foundation of Jiangsu Province, China BK20180127) Study on the role of natural carbohydrate trehalose in maintaining cartilage homeostasis by regulating autophagy rhythm through cartilage cell clock gene Bmal1
15. (Natural Science Foundation of Jiangsu Province, China BK2020040424) Study on the mechanism of Engineered neutrophil-derived exosomes in effective therapy for Rheumatoid arthritis

### Selected publications

1. Yan W, Xu X, Xu Q, et al. (2020) An Injectable Hydrogel Scaffold With Kartogenin-Encapsulated Nanoparticles for Porcine Cartilage Regeneration: A 12-Month Follow-up Study. *American Journal of Sports Medicine*. 2020 Nov;48(13):3233-3244. (IF=5.810)
2. Xu X, Wang R, Wu R, et al. (2020) Trehalose reduces bone loss in experimental biliary cirrhosis rats via ERK phosphorylation regulation by enhancing autophagosome formation. *FASEB J*. 34(6):8402-8415. (IF= 5.595)
3. Zhang Y, Hao C, Guo W, et al. (2020). Co-culture of hWJMSCs and pACs in double biomimetic ACECM oriented scaffold enhances mechanical properties and accelerates articular cartilage regeneration in a caprine model. *Stem Cell Research & Therapy*. 19;11(1):180. (IF= 5.116)
4. Kaiwei Ma, Tianzheng Zhao, Longfei Yang, Peng Wang, Jing Jin, Huajian Teng, Dan Xia, Liya Zhu, Lan Li\*, Qing Jiang\*, Xingsong Wang\*. Application of robotic-assisted in situ 3D printing in cartilage regeneration with HAMA hydrogel: An in vivo study. *Journal of Advanced Research*. 23 (2020) 123–132. (IF= 6.992)
5. Zhu X, Chen F, Lu K, et al. (2019) PPAR $\gamma$  preservation via promoter demethylation alleviates osteoarthritis in mice. (2019) *Annals of the Rheumatic Diseases*. 78(10): 1420-1429. (IF= 14.299).
6. Li Y, Zhao S, Li S, et al. (2019) Surface engineering of biodegradable magnesium alloys for enhanced orthopedic implants. *Small*. 2019, 15, 1904486 (IF= 10.856)
7. Zhang L, Shan X, Meng X, et al. (2019) The first integrin  $\beta$ 3-mediated cellular and nuclear targeting therapeutics for prostate cancer. *Biomaterials*. 2019,223: 119471. (IF=10.273)
8. Zhou S, Li Y, Qiao L, et al. (2019) Inactivation of Lkb1 in postnatal chondrocytes leads to epiphyseal growth plate abnormalities and promotes enchondroma-like formation. *FASEB J*. 33(8): 9476-9488. (IF= 5.595)

### Group members

#### Graduated Doctors

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#### Graduated Masters

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Zixu Wang

Jianxiang Wu

Ping Pan

#### Technicians

Sheng Shen

Peng Wang

Lan Li

Qiting Ge

Wenjin Yan

Jing Jin

#### Postdoctoral

Lei Zhang

Yixuan Li

Bin Wang

Jinjing Hou

Huixin Liang





## Jianghuai Liu, Ph.D.

Jiang-huai Liu received his Ph.D. degree in Biochemistry from Boston University School of Medicine in 2005. Upon completion of his postdoctoral fellowship at University of Pennsylvania in 2009, he joined MARC as a principle investigator and professor of genetics.

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## Macrophage biology and cellular plasticity

Our immune system is programmed to exert very diverse functions that range, for instance, from defending against foreign pathogens to regulation of metabolic homeostasis. Such diversity is mediated, at least in part, by the functional and populational plasticity of macrophages. These are the innate immune cells, as suggested by the great Ilya Metchnikoff (1845-1916) from an evolutionary perspective, that help to maintain the organismal “harmony”. Dysregulation within the macrophage compartment is known to underlie many disease states. However, therapeutic targeting of macrophages has not become a common practice, likely reflecting a lack of understandings to macrophage heterogeneity in disease. A major line of research in our lab is to use mouse models to investigate the regulation of macrophages in inflammation and cancer.

From a broader perspective, the complexities in macrophage biology reflect the fundamental principle of cellular plasticity and its regulation in health and disease. To study cellular plasticity in a more straightforward manner, novel tools to characterize and target cells in pathway- or subset-specific manner are highly desirable. We therefore are also dedicated to developing genetic tools that reveal and track cell fate.

## The ongoing projects in the lab are outlined in the following section:

### 1. A monocyte-intrinsic type I IFN-IL-4 cytokine cascade drives an M2-skewed phenotype in tumor-associated macrophages (TAMs):

Harnessing the nucleic acid-engaged innate immunity has strong implications for cancer therapies. Various signaling pathways activated by nucleic acids converge on the induction of type I IFNs (IFN-I), which often exert immune-stimulatory anti-tumor functions. On the other hand, our previous work showed an opposite action by IFN-I to potentially promote an arginase (ARG1)-dependent immunosuppressive axis in tumor-associated monocytes/macrophages (Tong Y et al., 2019). Such an unexpected pathway may operate as a “checkpoint” under various IFN-engaging treatment strategies.

Aided by a newly generated Arg1-YFP reporter mice, we established that IFN-I-induced arginase expression was localized in TAMs (Fig. 1A, B). We further demonstrated a surprising monocyte-specific “cytokine cascade” induced by IFN-I, leading to the release of IL-4, which in turn switches the co-existing, mature macrophages towards an immunosuppressive, pro-tumoral phenotype (Fig. 1C, D, E, F, G). Our work highlights an under-appreciated role by the monocytes in coordination of inflammation and repair which warrants future investigations.

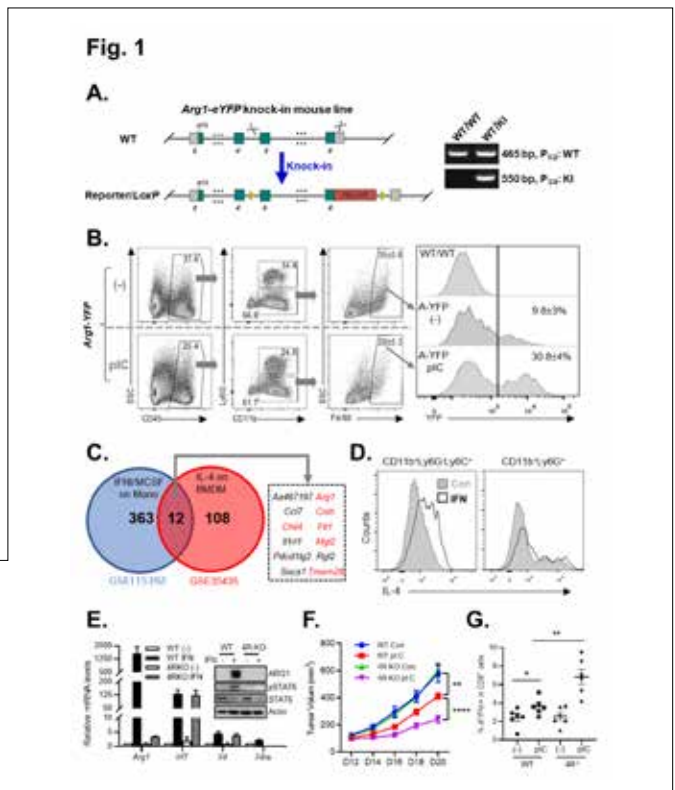


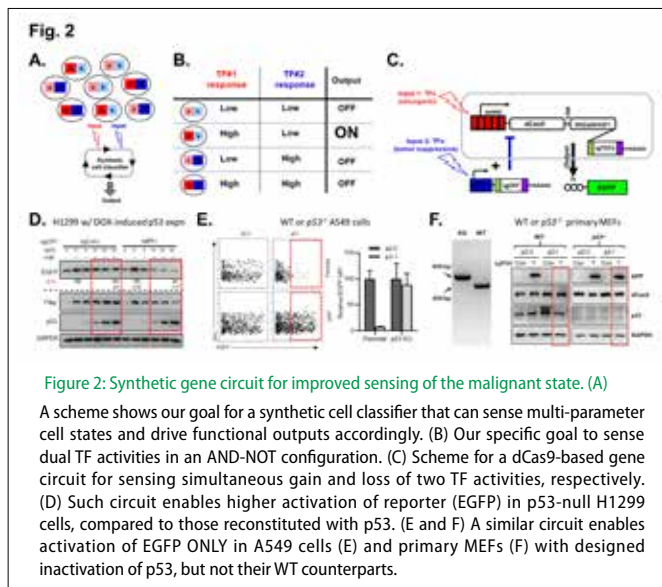
Figure 1: Mechanistic characterization of the IFN-I-ARG1 axis in TAMs. (A)

Construction of an Arg1-YFP mouse line. (B) Poly(I:C)/IFN-mediated induction of Arg1 is restricted in TAMs. (C) IFN treatment of M-CSF-influenced monocytes leads to induction of genes known to be downstream of IL-4. (D) IFN treatment of BM mononuclear cells leads to induction of IL-4 selectively in the monocyte compartment. (E) Inactivation of IL-4 receptor abrogates IFN-ARG1 axis in differentiating monocytes. (F and G) IL-4 receptor deficiency in mice led to improved anti-tumor effects by poly(I:C) (F), which is associated with higher abundance of IFN +CD8+ T cells (G).



## 2. Development of synthetic genetic circuits to sense cell states with better accuracy:

Various cell states are defined by their characteristic transcriptional programs. However, the activity of a single transcription factor (TF) is not a precise indication for a given cell state. Taking advantage of the synthetic biology discipline, we set out to build a synthetic cell classifier that can precisely report a cell state based on multiple transcription factor activities (Fig. 2A). Particularly, we focused on the simultaneous gain and loss of two respective TFs that may be suitable to define the malignant cells (Fig. 2B, C). We have provided evidence that this synthetic gene circuit enabled specific activation of a reporter gene ONLY in p53-deficient, but not -sufficient, tumor cells and primary MEFs (Fig. 2D, E, F). Further modifications of this cell classifier include additional empowerment with anti-tumor activities. We envision that genetic tools based on such synthetic biology design shall allow more profound understandings to regulation of cellular plasticity.

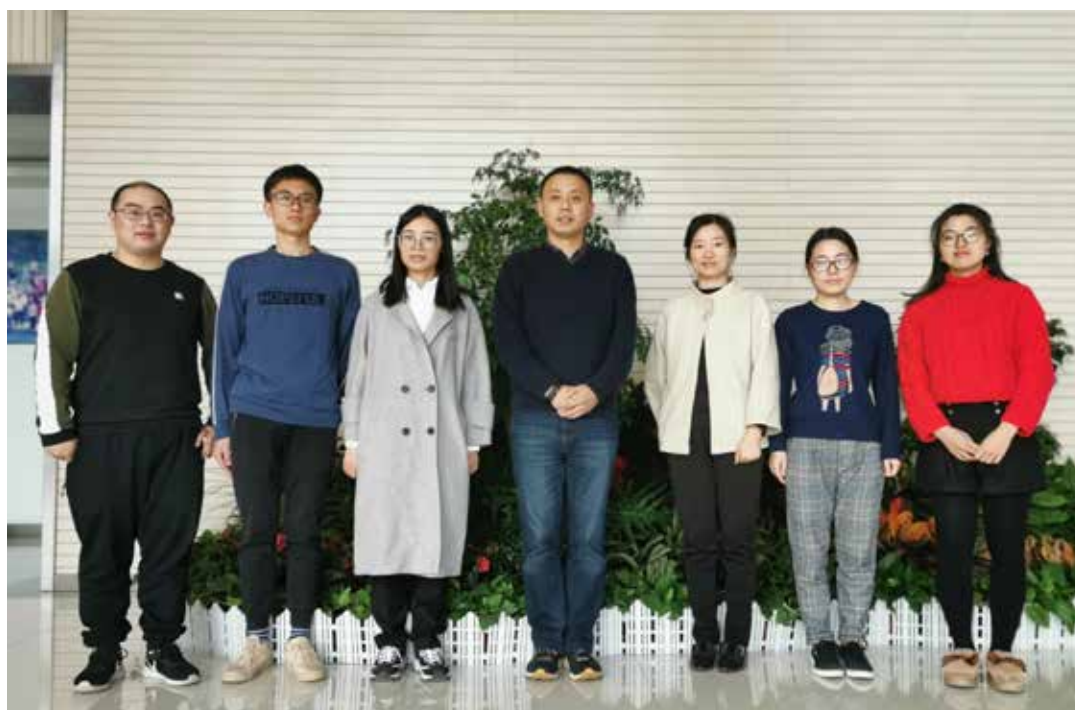


**Figure 2: Synthetic gene circuit for improved sensing of the malignant state. (A)**

A scheme shows our goal for a synthetic cell classifier that can sense multi-parameter cell states and drive functional outputs accordingly. (B) Our specific goal to sense dual TF activities in an AND-NOT configuration. (C) Scheme for a dCas9-based gene circuit for sensing simultaneous gain and loss of two TF activities, respectively. (D) Such circuit enables higher activation of reporter (EGFP) in p53-null H1299 cells, compared to those reconstituted with p53. (E and F) A similar circuit enables activation of EGFP ONLY in A549 cells (E) and primary MEFs (F) with designed inactivation of p53, but not their WT counterparts.

### Selected publications: (\*corresponding author)

- Gup P, Yang L, Zhang M, Tong Y, Cao Y and Liu J\*. A monocyte-orchestrated IFN-I-to-IL-4 cytokine axis instigates pro-tumoral macrophages and thwarts poly(I:C) therapy. In submission.
- Tong Y, Zhou L, Yang L, Cao Y, Qin FX and Liu J\*. Concomitant Type I IFN and M-CSF signaling reprograms monocyte differentiation to drive pro-tumoral arginase production. EBioMedicine 2019, 39: 132-44.
- Tao T, Sun J, Peng Y, Li Y, Wang P, Chen X, Zhao W, Zheng YY, Wei L, Wang W, Zhou Y, Liu J, Shi YS, Zhu MS. Golgi-resident TRIO regulates membrane trafficking during neurite outgrowth. J Biol Chem 2019, 294(28):10954-10968.
- Lu Y, Dai J, Kong N, Liu J, Gong J, Yao Y. Internalization Characterization of Si Nanorod with Camouflaged Cell Membrane Proteins Reveals ATXN2 as a Negative Regulator. Cells 2019, 8(8). pii: E931. doi: 10.3390/cells8080931.
- Jiang H, Shi H, Sun M, Wang Y, Meng Q, Guo P, Cao Y, Chen J, Gao X, Li E\* and Liu J\*. PFKFB3-driven macrophage glycolytic metabolism is a crucial component of innate antiviral defense. J Immunol (2016), 197(7), 2080-90.
- Dong Z, Huang M, Liu Z, Xie P, Dong Y, Wu X, Qu Z, Shen B, Huang X, Zhang T, Li J, Liu J, Yanase T, Zhou C and Xu Y\*. Focused screening of mitochondrial metabolism reveals a crucial role for a tumor suppressor Hbp1 in ovarian reserve. Cell Death Differ (2016), 23(10):1602-14.
- Zhang Y, Wang Y, Zhang C, Wang J, Pan D, Liu J\* and Feng F\*. Targeted Gene Delivery to Macrophages by Biodegradable Star-Shaped Polymers. ACS Appl Mater Inter (2016), 8(6):3719-24.
- Du Y, Meng Q, Zhang J, Sun M, Shen B, Jiang H, Kang Y, Gao J, Huang X\* and Liu J\*. Functional annotation of cis-regulatory elements in human cells by dCas9/sgRNA. Cell Res (2015), 25(7):877-80.



### Group members

#### Technical assistant:

Yan-lan Cao

#### Graduate students:

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Ya-feng Wang

Li-min Yang

Meng-fan Zhang

Gui-quan Zhang

Yu-yan Zhang

Dao-lin Cheng

#### Lab alumni:

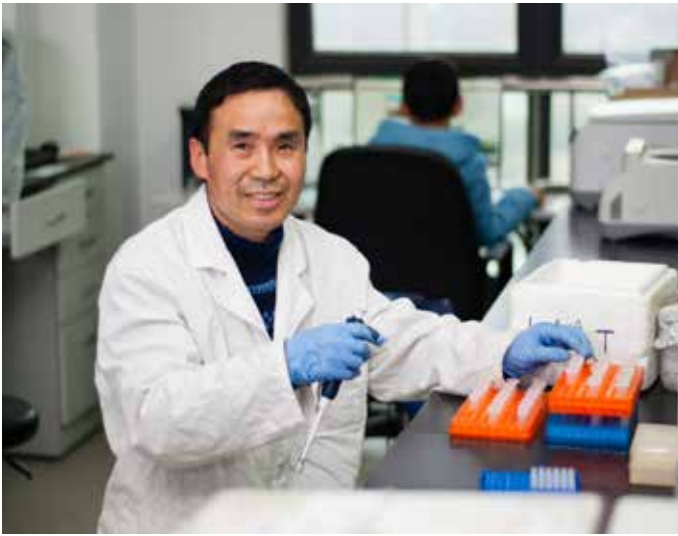
Dr. Hui Jiang (Molecular Infection Medicine Sweden)

Dr. Yi Lu (Abcam, Hangzhou)

Dr. Yuan-yuan Tong (China Pharmaceutical University)

Dr. Qingzhou Meng (ShanghaiTech University)

Man Sun, M.S. (Abcam, Hangzhou)



## Jinzhong Qin , Ph.D.

Jinzhong Qin received his Ph.D. from Cleveland State University (Ohio, USA) in 2004 after completing a research project at Department of Immunology, Cleveland Clinic Foundation. His research at Cleveland Clinic was focused on the regulation of Innate Immune signaling pathways. From 2005 to 2008, Jinzhong did his postdoctoral fellowship at the Massachusetts General Hospital Cancer Center, Harvard Medical School in Boston, USA, and he was promoted to Assistant in Genetics within the same Institution in 2008. Using murine genetics, he described an essential role of L3mbtl2-containing atypical Polycomb Repressive Complex 1 (PRC1) in embryonic stem cells (ESCs) proliferation and early embryonic development. He joined the Faculty of Model Animal Research Center (MARC), Nanjing University in 2013. He is now a Professor of genetics and developmental biology and a Principal Investigator in MARC.

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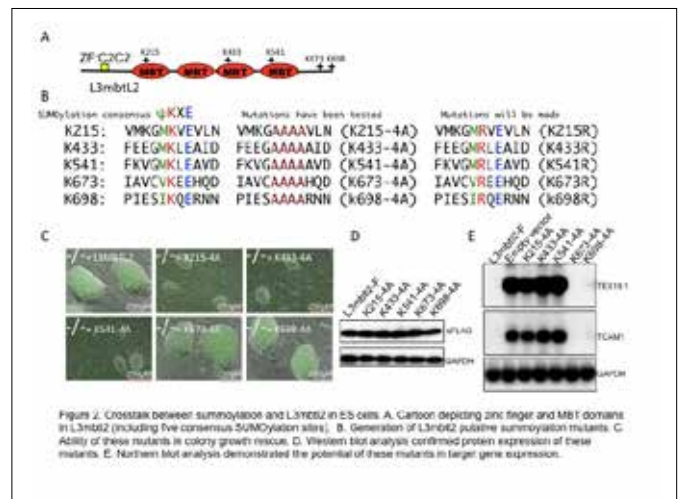
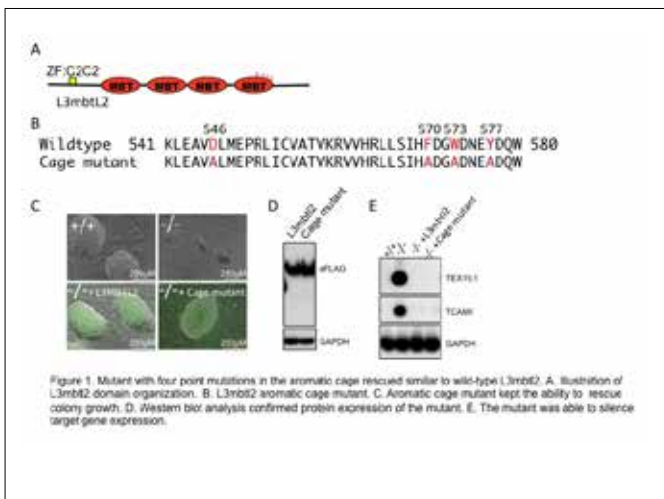
## Roles of the polycomb group proteins in stem cells & early development

Pluripotent stem cells are capable of differentiating into any cell type in the body and therefore hold tremendous promise for the future of regenerative medicine. However, a detailed understanding of the underlying molecular mechanisms that regulate the pluripotent state is still elusive. Our previous studies demonstrated that L3mbtl2, an mbt family member, is critical for early embryo development as well as pluripotency maintenance in embryonic stem (ES) cells. Deletion of L3mbtl2 results in embryonic lethality with failure of gastrulation and accordingly this correlates with compromised proliferation and abnormal differentiation of L3mbtl2-deficient ES cells. In ESCs, L3mbtl2 establishes an atypical PRC1 complex that includes Oct4, G9A and several components of the E2F6 and NuRD repressor complexes. Accordingly, the majority of genes bound and repressed by L3mbtl2 in ESCs are not occupied by canonical PRC1 and PRC2, although a small set of lineage commitment genes are co-occupied by all three complexes.

The central goal of our group is to comprehensively establish the role of L3mbtl2 containing atypical PRC1 in stem cells, embryonic development,

and cancer and to characterize its function at a molecular, mechanistic level. The success of our study will not only contribute to uncovering novel and essential molecular mechanism for governing stem cell pluripotency but also provide basic knowledge that in the long term is required for realizing the therapeutic potential of stem cells. Our ongoing studies address the following specific aims:

1. Elucidate the precise molecular mechanisms of L3mbtl2-mediated transcriptional repressive complex. We have generated different L3mbtl2 mutants (see figures below) and we are currently investigating the role of posttranslational modifications such as SUMOylation in L3mbtl2-mediated maintenance of self-renewal of ES cells.
2. Defines the roles of other components of L3mbtl2-containing repressive complex in ESC self-renewal by genetic approaches.
3. Identify functions of L3mbtl2-mediated complex in cancer and other diseases.



**Selected publications:**

1. Zhao W., Liu M., Ji H., Zhu Y., Wang C., Huang Y., Ma X., Xing G., Xia Y., Jiang Q., Qin J\*. 2018. The polycomb group protein Yaf2 regulates the pluripotency of embryonic stem cells in a phosphorylation-dependent manner. *J Biol Chem.*293(33):12793-12804.
2. Huang, Y., Zhao, W., Wang, C., Zhu, Y., Liu, M., Tong, H., Xia, Y., Jiang, Q., and Qin, J\*. (2018) Combinatorial Control of Recruitment of a Variant PRC1.6 Complex in Embryonic Stem Cells. *Cell reports* 22, 3032 -3043.
3. Liu W., Chen B., Wang Y., Meng C., Huang H., Huang XR., Qin J., Mulay SR., Anders HJ., Qiu A., Yang B., Freeman GJ., Lu HJ., Lin HY., Zheng ZH., Lan HY., Huang Y., Xia Y\*. 2018. RGMB protects against acute kidney injury by inhibiting tubular cell necroptosis via an MLKL-dependent mechanism. *Proc Natl Acad Sci U S A* 115(7):E1475-E1484.
4. Huang H., Xu C, Wang Y., Meng C., Liu W., Zhao Y., Huang XR., You W., Feng B., Zheng ZH., Huang Y., Lan HY., Qin J\*, Xia Y\*. Lethal (3) malignant brain tumor-like 2 (L3MBTL2) protein protects against kidney injury by inhibiting the DNA damage-p53-apoptosis pathway in renal tubular cells. *Kidney Int.* 2018 Apr;93(4):855-870.
5. Zhao, W., Huang, Y., Zhang, J., Liu, M., Ji, H., Wang, C., Cao, N., Li, C., Xia, Y., Jiang, Q., and Qin, J\*. (2017) Polycomb group RING finger proteins 3/5 activate transcription via an interaction with the pluripotency factor Tex10 in embryonic stem cells. *J Biol Chem* 292, 21527 -21537.
6. Yan, Y., Zhao, W., Huang, Y., Tong, H., Xia, Y., Jiang, Q., and Qin, J\*. (2017) Loss of Polycomb Group Protein Pcgf1 Severely Compromises Proper Differentiation of Embryonic Stem Cells. *Sci Rep* 7, 46276.
7. Zhao, W., Tong, H., Huang, Y., Yan, Y., Teng, H., Xia, Y., Jiang, Q., and Qin, J\*. (2017) Essential Role for Polycomb Group Protein Pcgf6 in Embryonic Stem Cell Maintenance and a Noncanonical Polycomb Repressive Complex 1 (PRC1) Integrity. *J Biol Chem* 292, 2773 -2784.
8. Qin, J., Whyte, W. A., Anderssen, E., Apostolou, E., Chen, H. H., Akbarian, S., Bronson, R. T., Hochedlinger, K., Ramaswamy, S., Young, R. A., and Hock, H\*. (2012) The polycomb group protein L3mbtl2 assembles an atypical PRC1 -family complex that is essential in pluripotent stem cells and early development. *Cell Stem Cell* 11, 319 -332.

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**Graduate Students**

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Mengjie Liu

Congcong Wang

Yaru Zhu

Lixia Dong

**Former Graduate Students**

Wukui Zhao (Ph.D.)

Yun Yan (MS)

**Technical Assistants**

Lijun Xu





## Pingping Shen , Ph.D.

Pingping Shen received her PhD degree at Nanjing University in 2000. From 2002 to 2003, she studied at University of California at San Diego as a visiting scholar. In 2004, she was appointed as a professor in Nanjing University and moved to Model Animal Research Center (MARC) as Adjunct Professor for research on inflammation and related diseases. Research in Pingping Shen's Lab is mainly focused on two fields: regulation of macrophage functions in inflammation and development of new clinical immunoassay techniques.

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# CDK5 Inhibition Abrogates TNBC Stem cell Property and Enhances Anti-PD-1 Therapy

**T**riple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, in which the higher frequency of cancer stem cells (CSCs) correlates with poor clinical outcome. Aberrant activation of CDK5 was found to associate with TNBC progression closely. CDK5 mediates PPAR $\gamma$  phosphorylation at its Ser 273, which induces CD44 isoform switching from CD44s to CD44v, increasing the stemness of TNBC cells. Blocking CDK5/pho-PPAR $\gamma$  significantly reduced CD44v<sup>+</sup> BCSCs population in tumor tissues, thus abrogated metastatic progression in the TNBC mouse model. Strikingly, diminishing stemness transformation reversed immunosuppressive microenvironment and enhanced anti-PD-1 therapeutic efficacy on TNBC. Mechanistically, CDK5 switches the E3 ubiquitin ligase activity of PPAR $\gamma$  and directly protected ESRP1 from ubiquitin-dependent proteolysis. This finding firstly indicates that CDK5 blockade could be a potent strategy to diminish stemness transformation and to increase the response to PD-1 blockade in TNBC therapy.

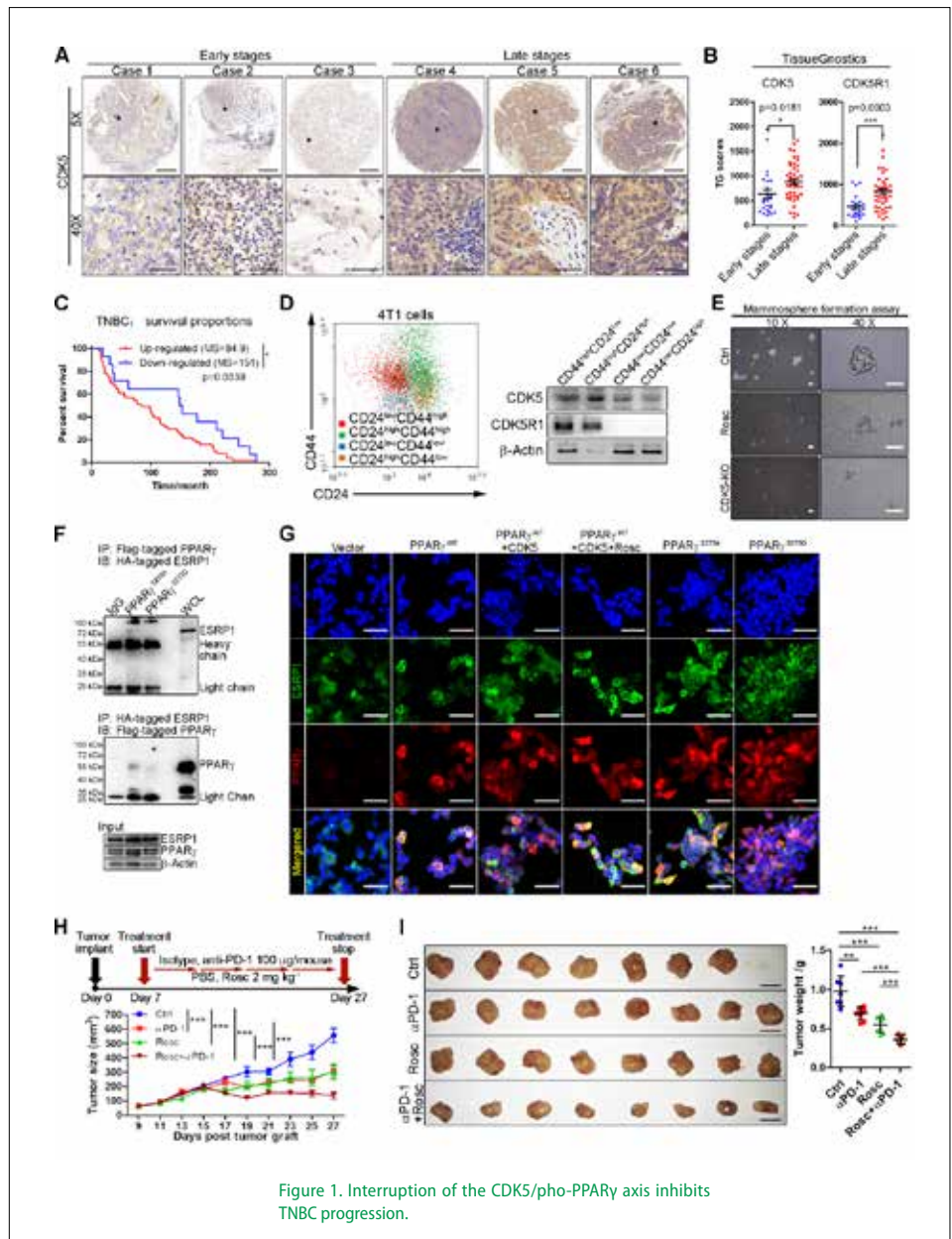


Figure 1. Interruption of the CDK5/pho-PPAR $\gamma$  axis inhibits TNBC progression.

## Intervening PCB stunts tumor growth by strengthening anti-tumor actions of TAMs

Shaping the actions of tumor-associated macrophages (TAMs) is one of the current goals in cancer immunotherapy, but the underline mechanisms that control the anti and pro-tumor activity of TAMs remain largely unknown. Here, we show that pyruvate carboxylase (PCB), a gluconeogenic enzyme, is suppressed in TAMs by the hypoxic tumor microenvironment, but upregulated by tumor-derived versican through TLR2-MyD88-RelB axis when hypoxia is relieved. Elevating PCB level significantly modifies the action of TAMs, meanwhile reverses the immune-suppressive microenvironment, which coupled with tumor progression stalling. Mechanistically, PCB translocates from the mitochondria to the cytosol and interacts with myosin heavy chain 6 (MYH6), which in turn reinforces phagocytosis of tumor cells by TAMs. Meanwhile, PCB inhibits PD-L1 expression on TAMs in an activity-dependent manner, which influences the T-cell suppressive capacity of TAMs. Accordingly, mitigating tumor hypoxia and activating TAMs-intrinsic PCB by administrating dual-functional Perfluorodecalin-Glyceryl triacetate @ HSA (FDC-GTA@HSA) nanoparticles restore anti-tumor immunity and stunt tumor growth. Collectively, our findings unveil an unexpected role of PCB in featuring TAMs and that restoring PCB in TAMs may offer a unique approach to enhance the specific cancer immunotherapy.

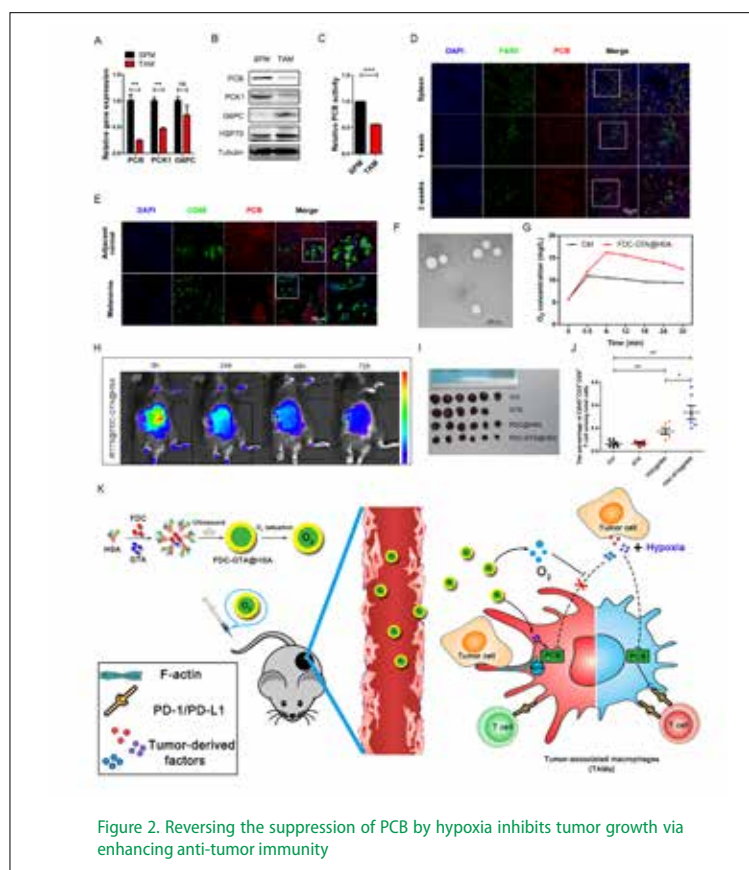


Figure 2. Reversing the suppression of PCB by hypoxia inhibits tumor growth via enhancing anti-tumor immunity

## Engineered immune cells in cancer therapy

Considering the capacity of immune cells to modulate the TME, we modified cells with engineered ligands and/or receptors. The set of techniques have showed significant tumor control by promoting killing cells to penetrate into solid tumors.

### Selected publications

1. Bei Y.C., Cheng N., Chen T., Shu Y.X., Yang Y., Yang N.F., Zhou X.Y., Liu B.R., Wei J., Liu Q., Zheng W., Zhang W.L., Su H.F., Zhu W.G., Ji J.G., Shen P.P.\*. CDK5 Inhibition Abrogates TNBC Stem cell Property and Enhances Anti-PD-1 Therapy. *Advanced Science*, 2020, 7, 2001417.
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3. Yang N.F., Tang Q., Qin W.T., Li Z.Y., Wang D.D., Zhang W.J., Cao X.C., Lu Y., Ge X.Y., Sun H.B.\*. Treatment of obesity-related inflammation with a novel synthetic pentacyclic oleanane triterpenoids via modulation of macrophage polarization. *EBioMedicine*, 45 (2019) :473-486.
4. Liu P., Li C., Zhang R.X., Tang Q., Wei J., Lu Y., Shen P.P.\*. An ultrasensitive electrochemical immunosensor for procalcitonin detection based on the gold nanoparticles-enhanced tyramine signal amplification strategy. *Biosensors and Bioelectronics*. 126(2019):543-550.
5. Zhang W.L., Liu L., Su H.F., Liu Q., Shen J., Dai H.R., Zheng W., Lu Y., Zhang W.J., Bei Y.C., Shen P.P.\*. Chimeric antigen receptor macrophage therapy for breast tumours mediated by targeting the tumour extracellular matrix. *British Journal of Cancer*, 2019,121(10):837-845.
6. Dai H.R., Zhou Y., Tong C., Guo Y.L., Shi F.X., Wang Y., Shen P.P.\*. Restoration of CD3+CD56+ cell level improves skin lesions in severe psoriasis: A pilot clinical study of adoptive immunotherapy for patients with psoriasis using autologous cytokine-induced killer cells. *Cytotherapy*, 2018:1-9.
7. Niu Z.Y., Shi Q., Zhang W.L., Chen B., Wang Q.S., Zhao X.Y., Chen J.J., Shu Y.X., Cheng N., Feng X.J., Ji J.G., Shen P.P.\*. Caspase-1 cleaves PPAR $\gamma$  for potentiating the pro-tumor action of TAMs. *Nature Communications*, 2017,8:766.
8. Feng X.J., Yu W., Li X.D., Zhou F.F., Zhang W.L., Shen Q., Li J.X., Zhang C., Shen P.P.\*. Apigenin, a modulator of PPAR $\gamma$ , attenuates HFD-induced NAFLD by regulating hepatocyte lipid metabolism and oxidative stress via Nrf2 activation. *Biochemical Pharmacology*, 2017, 136:136-149.
9. Feng X.J., Weng D., Zhou F.F., Young D. Owen, Qin H.H., Zhao J.F., Yu W., Huang Y.H., Chen J.J., Fu H.J., Yang N.F., Chen D.H., Li J.X., Tan R.X., Shen P.P.\*. Activation of PPAR $\gamma$  by a natural flavonoid modulator, apigenin ameliorates obesity-related inflammation via regulation of macrophage polarization. *EBioMedicine* 2016(9): 61-76.
10. Yao Y.F., Shi Q., Chen B., Wang Q.S., Li X.D., Li L., Huang Y.H., Ji J.G., Shen P.P.\*. Identification of Caspase-6 as a New Regulator of Alternatively Activated Macrophages. *Journal of Biological Chemistry*, 2016, 291(33):17450-17466.



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## Yohei Niikura, Ph.D.

Yohei obtained his PhD in Chemistry at the University of Florence, Italy in 2000 and then conducted his postdoctoral research at Switzerland (Friedrich Miescher Institute), Japan (National Center for Geriatrics and Gerontology), and US (St. Jude Children's Research Hospital; Nationwide Children's Hospital; Greehey Children's Cancer Research Institute). He joined the MARC of Nanjing University as a Principle Investigator in September of 2018. His current research interest is molecular mechanism of cell division in differentiation and human diseases.

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## Mitotic regulators in diseases

Our lab is interested in the molecular mechanism involved in both cell division and human diseases, currently focusing on cancer and nervous system disorder.

During cell division, proper chromosomes segregation must be achieved otherwise it can result in unequal distribution of chromosomes to daughter cells. Spindle microtubules must attach to a single region of each chromosome, termed the "centromere," in most eukaryotes. The kinetochore is a complex of proteins that is located at the centromere (Figure 1). Defects in the centromere-kinetochore function leads to chromosome instability (CIN). CIN, unequal distribution of chromosomes to daughter cells results in aneuploidy (i.e., an incorrect number of chromosomes) – the consequences of aneuploidy are usually profound and may include cancer, birth defects, and developmental disorders such as Down syndrome. CIN is a hallmark of cancers, and is often associated with a poor prognosis. Therefore, it is highly important to study the temporal-special regulation and the structure of centromere and kinetochore protein(s) to understand CIN and cancer progression.

However, the relationship between centromere-kinetochore components and tumor regulating components and its precise mechanism during mitosis remain unclear, and many mutational studies of tumor suppressors and oncoproteins in past were limited in non-mitotic stage. Mitotic regulators of our interests include but not limited to centromere-kinetochore proteins, microtubule binding proteins, mitotic enzymes (e.g., kinases, phosphatases, ubiquitin ligases, deubiquitinases etc.), and chaperons and co-chaperons that facilitate proper chromosome segregation. Importantly, the functions of mitotic regulators are neither limited to the roles of cell division, and often extended to the roles of other cell cycle stages such as G1(G0)/S phases. Moreover, numerous mitotic regulators are often widely expressed in different tissues, it is very interesting to investigate how these regulators change their functions in different tissues and organs. In tissues such as the brains, hearts, and muscles where mitosis is not frequent and have lost the ability of spontaneous regeneration, error of the G1(G0)/S functions (e.g., protein quality control such as ubiquitin-proteasome machinery) would be a serious problem. While such as the blood, skin, bone, and gut where mitosis is frequent and have high turnover rates of the regeneration, error of (G2)/M functions (e.g., chromosome segregation) would be a serious problem. We note that the mechanism of the chromosome segregation is also highly regulated by the ubiquitin-proteasome machinery. Therefore, studying the cell cycle-specific functions of mitotic regulators involved in different type of cancer and other diseases in different organs would be expected for future research. Our lab is currently focusing on the function of the mitotic regulators in cancer and nervous system disorder.

### TSG101-DAXX in cancer

Mitotic arrest deficiency 2 (Mad2), a critical component of the spindle checkpoint, is overexpressed in many cancer cells. Thus, we hypothesized that Mad2 overexpression could specifically make cancer cells

susceptible to death by inducing a synthetic dosage lethality defect. We performed a synthetic genetic array analysis in yeast and revealed that Mad2 overexpression induced lethality in 13 gene deletions. Yeast STP22 (homolog of mammalian TSG101) is among 13 genes whose deletion caused synthetic dosage lethality in Mad2- overexpressing yeast cells. TSG101 is commonly known as a component of the ESCRT-I complex, a regulator of vesicular trafficking process, and is required for the sorting of endocytic ubiquitylated cargos into multivesicular bodies (MVBs). While initially discovered as negative regulator for tumorigenesis, accumulating evidence now describes TSG101 as a positive modulator of cancer progression. Consistent with this notion, overexpression of TSG101 has been reported in various cancer types. The challenge will be to define precisely how TSG101 exerts its oncogenic properties in cancer development.

TSG101 physically interacts with the H3.3 chaperone DAXX, and they cooperatively repress glucocorticoid receptor (GR)-mediated transcriptional activity. DAXX also protects protein degradation of DNA methyltransferase 1 (DNMT1)-associated protein (DMAP1) in vivo. Ectopic localization of the histone variant CENP-A in human cells depends on DAXX, and this aberrant nucleosome occludes CTCF binding, forming a heterotypic particle with H3.3, and has a minor effect on gene expression. Cells overexpressing CENP-A are more tolerant of DNA damage, and both the survival advantage and CTCF occlusion in these cells are dependent on DAXX. DAXX is a death domain-binding protein implicated in Fas-mediated cell death and physically interacts with CENP-C mediated by the amino-terminal 315 amino acids of CENP-C and the carboxyl-terminal 104 amino acids of DAXX. In normal conditions DAXX is mainly accumulated at Promyelocytic Leukemia Nuclear Bodies (PML NBs), and has a minor association with centromeres and pericentromeres (CEN/ pericEN). Application of physiological Heat Shock (HS) changes this balance forcing very robust and reversible accumulation of DAXX on CEN/pericEN heterochromatin. Depletion of DAXX leads to HS-induced changes in the balance of epigenetic modifications at heterochromatin, most dramatically elevating levels of active H3K4Me2 modification at pericEN, suggesting dualistic function of DAXX-containing complexes at CEN/pericEN: (1) regulation of H3.3 loading in normal conditions and (2) protection of epigenetic status upon stress-induced accumulation, thus collectively guarding epigenetic identity of CEN/pericEN heterochromatin. DAXX-USP7 (ubiquitin-specific-processing protease 7) regulates Aurora A stability, and DAXX-RSSF1 (RAS-association domain family protein 1; a mitotic checkpoint protein) regulates taxane response during mitosis. Interestingly, PTEN regulates glioblastoma oncogenesis through chromatin-associated complexes of DAXX and histone H3.3. Many PTM sites of DAXX are reported (data not shown), but their functional roles and their mechanisms to cause/avoid the genomic instabilities and cancer are poorly understood.

**TSG101-DAXX in cancer (preliminary Investigation)**

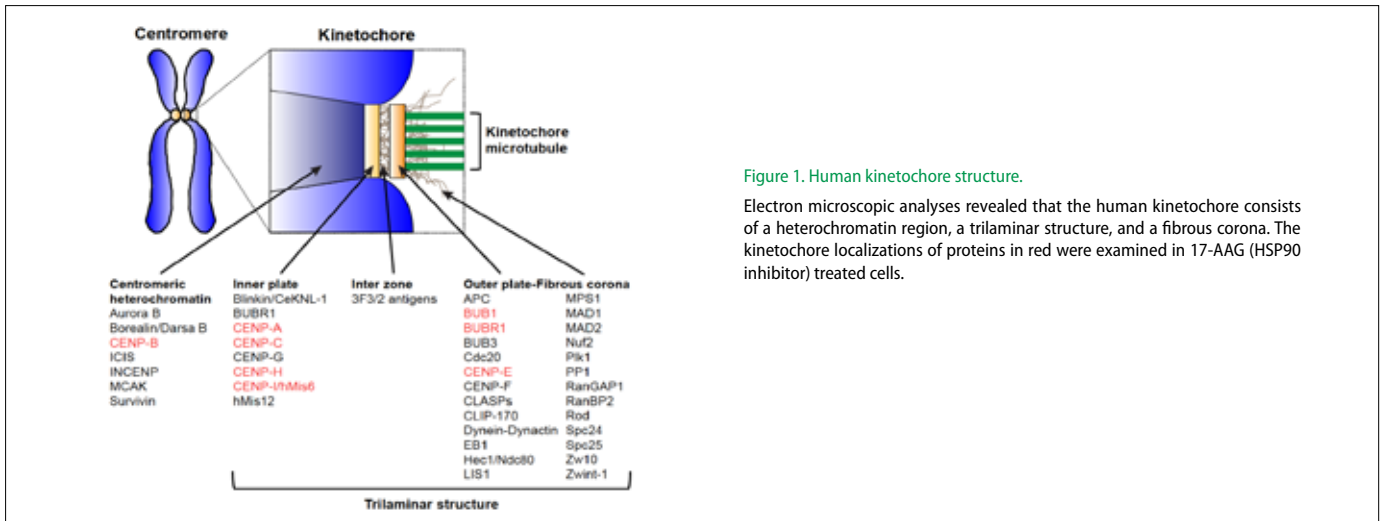
In our preliminary study, we detected aberrant mitotic progression in HeLa cells depleted of TSG101, which is reminiscent of kinetochore-defective cellular phenotype (Figure 2) which is consistent with the previous report. These cells also demonstrated significant increase of number of metaphase cells with misaligned chromosome and abnormal anaphase B or telophase cells with lagging chromosome. These morphological abnormalities were similar to those observed in cells which are defective of other centromere/kinetochore components or spindle checkpoint proteins. Interestingly, we detected that ectopic localization of CENP-C and CENP-H on chromosome arm are increased in TSG101-depleted HeLa cells (data not shown). In addition, we also found multiple consensus sites for mitotic kinases (e.g., PLK1, CDK1/ Polo kinases, Mps1, Aurora-A, etc.) in TSG101 (Table 1). We are going to construct site-specific mutants and apply them for further analyses.

Further study will address the signaling pathway involving PTM of TSG101. We will confirm synthetic lethality of MAD2 overexpressing cancer cells with TSG101 depletion in human cancer cell lines and model animal xenografts.

**Table 1: Consensus sites found in TSG101**

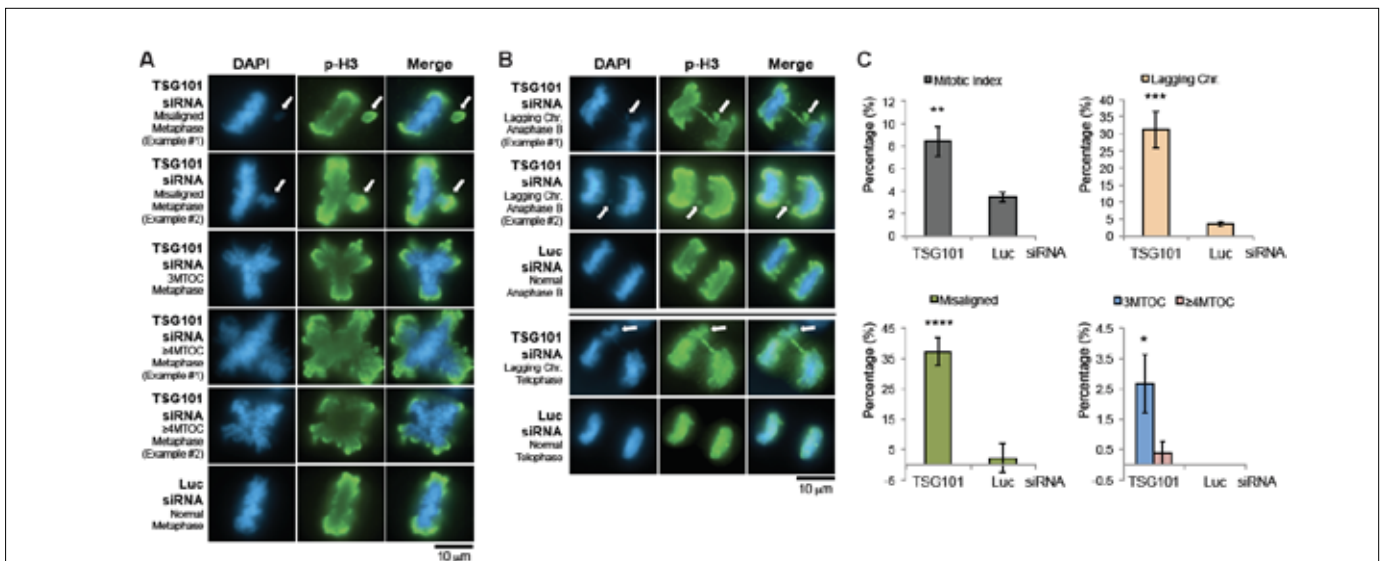
consensus seq.	consensus for...	TSG101 peptide sequence
S(S/T)(P/X)	CDK1/some Polo	SSR, SSM, SSQ, SSA
Similar to PLK1 consensus.	Mps1	DLTVRET, DGSRSRE, DGTISE
K(L/D/N)XXX(F/L/I)XXLK	Bubs binding	(K)YLPYLHEWK
RX(S/T)(L/V)	Aur-A	RETV, RASL
(S/T)XX(S/T)	CK-I	SSMT, SEDT, SLIS, SAVS, TIKT, TTSS
SXX(E/D/Yp/Sp or any acidic)	CK-II	SSRE
Psi KX(E/D)	SUMOylation	MIKEE, QKLE
P(S/T)AP	Late budding motif	P TAP

**Figure Legends**



**Figure 1. Human kinetochore structure.**

Electron microscopic analyses revealed that the human kinetochore consists of a heterochromatin region, a trilaminar structure, and a fibrous corona. The kinetochore localizations of proteins in red were examined in 17-AAG (HSP90 inhibitor) treated cells.



**Figure 2. Abnormal mitotic progression in TSG101-depleted HeLa cells.**

(A) Abnormal metaphase was observed by DAPI stain in TSG101 siRNA-treated HeLa cells. Arrows indicate misaligned metaphase chromosomes. (B) Abnormal anaphase B or telophase was observed by DAPI stain in TSG101 siRNA-treated HeLa cells. Arrows indicate lagging chromosomes. (C) Histogram summarizing abnormal metaphase, and anaphase B or telophase cells shown in (A) and (B). Mitotic index was shown. Misaligned, misaligned metaphase cell [arrows in (A)]; Lagging Chr., anaphase B or telophase cell with lagging chromosome [arrows in (B)]; 3 MTOC, cell with 3 microtubule-organizing center (MTOC); ≥4 MTOC, cell with four or more microtubule-organizing center (MTOC).

**Selected publications (\*Co-corresponding author)**

1. Niikura Y\*, Kitagawa R, Kitagawa K\*. CENP-A ubiquitylation is indispensable to cell viability. *Dev Cell*. 2019.
2. Niikura Y\*, Kitagawa K\*. The function of SUGT1 (the human homolog of SGT1), a co-chaperon. *Heat Shock Protein 90 in Human Diseases and Disorders*. Dordrecht, Netherlands: Springer Nature Publishers [a book chapter]; 2019.
3. Niikura Y\*, Kitagawa R, Kitagawa K\*. CENP-A Ubiquitylation Contributes to Maintaining the Chromosomal Location of the Centromere. *Molecules*. 2019;24(3). Epub 2019/01/27. doi: 10.3390/molecules24030402. PubMed PMID: 30678315.
4. Niikura Y, Kitagawa R, Ogi H, Kitagawa K. SGT1-HSP90 complex is required for CENP-A deposition at centromeres. *Cell Cycle*. 2017;1-12. doi: 10.1080/15384101.2017.1325039. PubMed PMID: 28816574.
5. Niikura Y, Kitagawa R, Kitagawa K. CENP-A Ubiquitylation Is Required for CENP-A Deposition at the Centromere. *Dev Cell*. 2017;40(1):7-8. doi: 10.1016/j.devcel.2016.12.020. PubMed PMID: 28073011.
6. Niikura Y, Kitagawa R, Kitagawa K. CENP-A Ubiquitylation Is Inherited through Dimerization between Cell Divisions. *Cell Rep*. 2016;15(1):61-76. doi: 10.1016/j.celrep.2016.03.010. PubMed PMID: 27052173; PMCID: PMC4826857.
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**Group members****Graduate students**

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# NJU-MARC Core Facilities

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After four years in operation, the Core Facilities of MARC have begun to take shape. We have been equipped with more than 24 state of the art instruments and provide over 19000 hours service within or outside MARC research community in 2020.

So far, we have set up Microscopy and Imaging Core, Flow Cytometry Core, Molecular and Metabolomics Core, providing a diverse range of resources and services, including high resolution imaging, flow cytometry, protein and gene expression profiling, and metabolic analysis. The featured instruments are listed below and more resources could be found on our website. <http://core.nicemice.cn:8081/>.

## Imaging

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### ► Services

- Live cell imaging
- Optical sectioning of thick biological samples
- 3D reconstruction of images
- 3-D mosaic imaging
- Multi-area time-laps and spectral scanning
- Super-resolution imaging

### ► Equipment

- Olympus FluoView 1000 confocal
- Zeiss LSM880 with Airyscan
- Leica TCS II sp5 confocal
- GE Healthcare DeltaVision Imaging System
- GE Healthcare DeltaVision OMX 3D-SIM

## Mass Spectrometry

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### ► Services

- Quantitative analysis of small molecules
- Identification of unknown metabolites
- Able to analyze various kinds of samples
- Metabolomics study

### ► Equipment

- Agilent 6550 iFunnel Q-TOF LC/MS System

## High Resolution Ultrasound

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### ► Services

- Cardiovascular research
- Oncology study
- Drug metabolism study
- .....

### ► Equipment

- FUJIFILM Vevo® 3100 LAZR-X system
- FUJIFILM Vevo® 770

## Flow Cytometry

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### ► Services

- Cell sorting
- Able to analyze multiple fluorescent probes simultaneously

### ► Equipment

- BD LSRFortessa™ Flow Cytometer
- BD FACSCalibur Flow Cytometer
- BD FACSAria™ III Cell Sorter

## Cellular Metabolism

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### ▶ Services

- Live cell energy metabolism

### ▶ Equipment

- Agilent Seahorse Xfe24 Extracellular Flux Analyzer

## Biomolecule Analysis

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### ▶ Services

- Fast and reliable protein purification
- SPR based molecular interaction

### ▶ Equipment

- GE ÄKTA pure protein purification system
- GE Biacore T200 SPR system

## Real time qPCR

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### ▶ Services

- Gene expression detection

### ▶ Equipment

- ABI StepOne Plus
- Roche LightCycler 96

## Others

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### ▶ Equipment

- BioTek synergy H1 plate reader
- Beckman OPTIMA XPN-100 centrifuge

# National Resource Center for Mutant Mice



With support of the National Key Technology R&D Program, Nanjing University started the construction of the National Resource Center for Mutant Mice (NRCMM), and established the Nanjing University Model Animal Research Institute accordingly. The co-construction unit is GemPharmatech, Co., Ltd. The Center has 33 staff members, including 14 professors/associate professors engaged in genetically engineered mice research. There are 1 Cheung Kong Scholar Professor of the Ministry of Education, 2 winners of The National Science Fund for Distinguished Young Scholars, 2 winners of the 1000 talents plan for young talents , 1 chief of the 973 project, 2 winners of Education Ministry's New Century Excellent Talents Supporting Plan, The core team members are all recruited abroad by NJU.

The National Resource Center for Mutant Mice is a national science and technology resource sharing service platform integrating resource preservation and supply, disease model creation and development, laboratory animal talent training and international exchanges. It aims to provide a complete set of national biomedical innovation and development needs, including the development of mouse model resources, breeding and conservation, service consulting, talent training and international exchanges and other services. In 2019, it became one of 30 National germplasm resource platforms. NRCMM is the initiator and core member of the International Mouse Phenotyping Consortium (IMPC), and one of the founders of the Asian Mouse Mutagenesis Resource Association (AMMRA) and the Asian Mouse Phenotyping Consortium (AMPC). It has established cooperation and exchange relationships with 27 resource centers in 11 countries, including KOMP, Eucomm, MMRRC, RikenBRC, JAX, Taconic, etc.

In 2020, NRCMM established the first expert committee and held its first meeting on December 9; on July 18, the "2020 Symposium on Collaboration and Sharing of Laboratory Animal Facilities Management" was held in Changzhou; on December 10, the "Second Symposium on Disease Animal Models and Biomedicine" was held in Nanjing.

In 2020, NRCMM launched the "Mouse Collection" project for the whole country and developed 81 new mouse disease models in collaboration with the co-construction unit GemPharmatech, Co., Ltd. By the end of 2020, 20,771 mouse strains have been created, collected and agency served. It have served domestic universities, hospitals, research institutes, national scientific research centers, pharmaceutical companies, and CRO companies at home and abroad, and promoted the development of life sciences, medicine, pharmacy and other related disciplines in China.

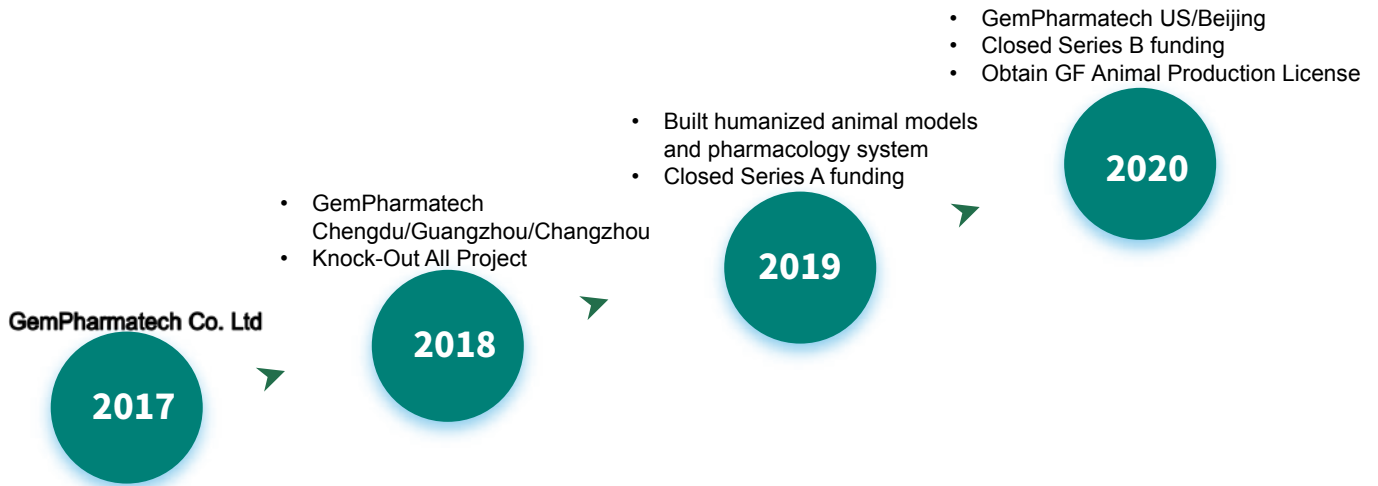
By 2025, NRCMM will strive to become an international gathering place for genetically engineered mouse resources and a leader in genetically engineered mouse innovation. It will also strive to become the No.1 in mouse strain resources all over the world. It will provide resource sharing and related technical services for basic research, drug development, disease diagnosis, treatment and evaluation in biomedicine field, especially to promote the development of antibody drugs, the use of cell therapy technology and the research of symbiotic microorganisms.





## Co-construction unit of NRCMM

GemPharmatech is a global biotech company dedicated to providing a one-stop-shop solution for in vivo biomedical research using genetically engineered mouse models, it is also a co-construction unit of the National Resource Center for Mutant Mice of China (NRCMM). The GPT team has 20 years of experience in generating and breeding disease models and conducting preclinical efficacy studies for both academic and industrial clients.

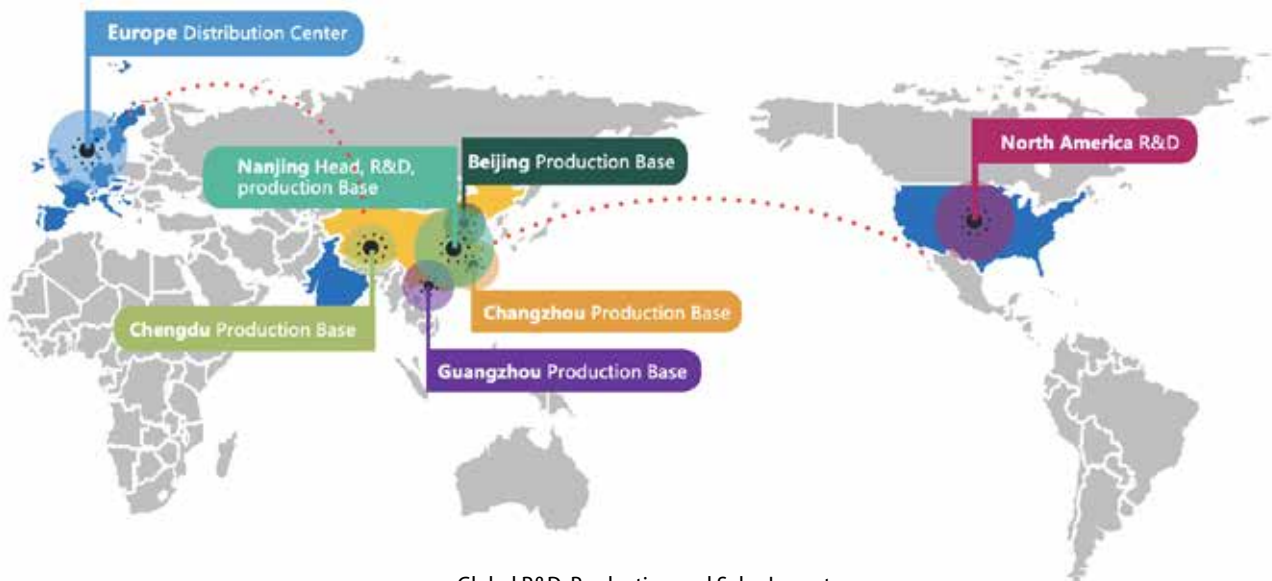


## OUR VISION

We are dedicated to our long-term mission of providing world class mouse models and pre-clinical services to facilitate an efficient and rapid advancement of science to treat human diseases. We believe that the next 20 years will bring forth amazing medical breakthroughs.

## SERVICES

The company has a comprehensive service platform which includes humanized mouse models, customized model generation, germ-free mice service, breeding and husbandry and numerous disease models. Our ambitious knockout all platform (KOAP) is currently generating knockout and conditional knockout mice for all protein coding genes. In addition, we can perform phenotyping, drug development and efficacy testing.



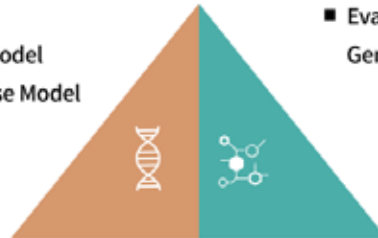


◎ Humanized Mouse Model R&D Platform

- Target Gene Humanized Mouse Model
- CDX, PDX
- Immune System Humanized Mouse Model
- Intestinal Microflora Humanized Mouse Model
- Hepar humanized Mouse Model

◎ New Drug R&D Platform

- Efficiency and Safety Evaluation
- Evaluation of New Therapy(Cell Therapy, Gene Therapy, Combination Therapy)

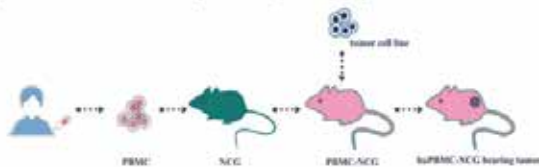


◎ Microbiomics Research and Transformation Platform

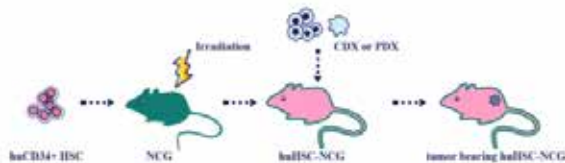
- Germfree Gene Editing Mouse Model
- Research of Microbiomics
- Evaluation of Biopharmaceuticals

## Technology Innovation Platform

▶ Human T cell ( CD4+, CD8+)



▶ Human T cell ( CD4+, CD8+, Treg+)



▶ NCG+Humanized cytokine



- Inhibit cytokine storm
- Promote myeloid immune cell reconstruction
- Increase the colonization rate of human immune cells

## Technology Platform of Immune System Humanized Mouse Model



# Publications in 2020

1.	Wei, T. W., Lu, S., Sun, J. H., Xu, Z. J., Yang, X., Wang, F., Ma, Y., Shi, Y. S., and Chen, X. Q. (2020) Sanger's Reagent Sensitized Photocleavage of Amide Bond for Constructing Photocages and Regulation of Biological Functions, <i>J Am Chem Soc.</i> 142:3806-3813.
2.	Zhao, Y., Zhao, M. F., Jiang, S., Wu, J., Liu, J., Yuan, X. W., Shen, D., Zhang, J. Z., Zhou, N., He, J., Fang, L., Sun, X. T., Xue, B., and Li, C. J. (2020) Liver governs adipose remodelling via extracellular vesicles in response to lipid overload, <i>Nature Communications.</i> 11(1):719
3.	Zhang, Z., Zong, C., Jiang, M., Hu, H., Cheng, X., Ni, J., Yi, X., Jiang, B., Tian, F., Chang, M. W., Su, W., Zhu, L., Li, J., Xiang, X., Miao, C., Gorospe, M., de Cabo, R., Dou, Y., Ju, Z., Yang, J., Jiang, C., Yang, Z., and Wang, W. (2020) Hepatic HuR modulates lipid homeostasis in response to high-fat diet, <i>Nature Communications.</i> 11:(1) 3067.
4.	Quan, C., Du, Q., Li, M., Wang, R., Ouyang, Q., Su, S., Zhu, S., Chen, Q., Sheng, Y., Chen, L., Wang, H., Campbell, D. G., MacKintosh, C., Yang, Z., Ouyang, K., Wang, H. Y., and Chen, S. (2020) A PKB-SPEG signaling nexus links insulin resistance with diabetic cardiomyopathy by regulating calcium homeostasis, <i>Nature Communications.</i> 11(1): 2186.
5.	Liu, L., Ding, C., Fu, T., Feng, Z., Lee, J. E., Xiao, L., Xu, Z., Yin, Y., Guo, Q., Sun, Z., Sun, W., Mao, Y., Yang, L., Zhou, Z., Zhou, D., Xu, L., Zhu, Z., Qiu, Y., Ge, K., and Gan, Z. (2020) Histone methyltransferase MLL4 controls myofiber identity and muscle performance through MEF2 interaction, <i>The Journal of Clinical Investigation.</i> 130(9):4710-4725.
6.	Wang, D. Y., Zheng, J. S., Hu, Q. Y., Zhao, C., Chen, Q. Y., Shi, P. L., Chen, Q., Zou, Y. J., Zou, D. Y., Liu, Q. Y., Pei, J. W., Wu, X. W., Gao, X., Ren, J. N., and Lin, Z. Y. (2020) Magnesium protects against sepsis by blocking gasdermin D N-terminal-induced pyroptosis, <i>Cell Death and Differentiation.</i> 27: 466-481.
7.	Dalangood, S., Zhu, Z., Ma, Z. H., Li, J. X., Zeng, Q. H., Yan, Y. L., Shen, B., Yan, J., and Huang, R. M. (2020) Identification of glycogene-type and validation of ST3GAL6 as a biomarker predicts clinical outcome and cancer cell invasion in urinary bladder cancer, <i>Theranostics.</i> 10:10078-10091.
8.	Du, H., Ye, C., Wu, D., Zang, Y. Y., Zhang, L., Chen, C., He, X. Y., Yang, J. J., Hu, P., Xu, Z., Wan, G., and Shi, Y. S. (2020) The Cation Channel TMEM63B Is an Osmosensor Required for Hearing, <i>Cell Rep.</i> 31(5):107596.
9.	Chen, W., Li, W., Zhang, Z., Tang, X., Wu, S., Yao, G., Li, K., Wang, D., Xu, Y., Feng, R., Duan, X., Fan, X., Lu, L., Chen, W., Li, C., and Sun, L. (2020) Lipocalin-2 Exacerbates Lupus Nephritis by Promoting Th1 Cell Differentiation, <i>J Am Soc Nephrol.</i> 31(10):2263-2277.
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## Seminar in 2020

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	Date	Speaker	Title	Unit
1	2020/1/8	Chenglin Miao Ph.D.	Neural network for spatial location	Peking University
2	2020/1/8	Liangyi Chen Ph.D.	Hypersensitive hyperresolution imaging of living cells	Peking University
3	2020/6/18	Qian Bian Ph.D.	Regulation of 3D Genome Organization via Chromatin Modifications	Shanghai Jiao Tong University
4	2020/9/25	Lei Chen Ph.D.	Regulatory Mechanisms of Intestinal Homeostasis	Human Genetics Institute of New Jersey, Rutgers University
5	2020/11/9	Tongjin Zhao Ph.D.	Dynamic palmitoylation regulates CD36 - mediated fatty acid absorption	Fudan university



# Courses and Teachers

The MARC, as an institute of the University of Nanjing, is home to approximately 102 PhD students. They carry out their dissertation studies under the supervision of MARC group leaders. In addition, MARC group leaders present lectures and laboratory courses at universities in China, in particular, at Nanjing University, and in other countries. In 2020, the following lecture series and courses were given by MARC staff at Nanjing University (unless indicated otherwise).

## Progress in Life Sciences

All PIs in MARC

## Cell Biology and Molecular Biology

Guoqiang Wan

Hongyu Wang

## Genetics

Qing Zhang

Jinzhong Qin

Di Chen

Xin Lou

## Mechanism of Development

Jiong Chen

Ying Cao

Zhongzhou Yang

Qingshun Zhao

## Information Genomics:

Zhenji Gan

Minsheng Zhu

Yohei Niikura

## Topics in Genetics I

All PIs in MARC

## Topics in Developmental Biology I

All PIs in MARC

## Frontier of Cell Biology

Qian Bian (Shanghai Jiao Tong University)

## Basic Concepts and Frontiers in Immunology

Jianghuai Liu

Yan Li

Huiming Gao

Zhaoyu Lin

## Doctoral qualification exam I&II

All PI in MARC

## MARC seminar in Genetics

All PIs in MARC

## MARC seminar in Developmental Biology

All PIs in MARC

## Physiology

Yun Shi

Shuai Chen

Chaojun Li

Guiquan Chen

# PhD Theses

## MARC students successfully defended the following PhD theses in 2020

### PhD Theses:

#### Group Xiang Gao

##### Yufang Wang

Regulation of Pyroptosis mediated by GSDMD and GSDME in Inflammatory Diseases

#### Group Yun Shi

##### Chang Ye

The Cation Channel TMEM63B Mediates Noxious Heat Sensing in Primary Sensory Neuron

#### Shixiao Peng

AMPA receptors regulate emotions

#### Group Guiquan Chen

##### He Wang

Essential role of PDK1/Akt signaling in oligodendrocyte development

#### Tingting Liu

Role of gamma-secretase in cortical development and the transition of neural progenitors

#### Group Zhenji Gan

##### Lin Liu

The mechanistic actions of MLL4 and XBP1 in skeletal muscle and liver metabolism

#### Liwei Xiao

The function and mechanistic actions of FNIP1 in skeletal muscle mitochondrial metabolism

#### Group Jianghuai Liu

##### Yafeng Wang

Synthetic gene circuits to selectively rewire tumor cells with compound gain- and loss-of-transcription factor activities

#### Group Di Chen

##### Lina Zou

Molecular Mechanisms of Aging in *C. elegans*

#### Group Minsheng Zhu

##### Yanyan Zheng

Thymus Regulates Skeletal Muscle Regeneration through Amplification of Satellite Cells Pool

#### Lisha Wei

The Mechanism of MVA pathway Trigger Metaflammation in Vascular Smooth Muscle

#### Ye Qiong Li

LIMK2 Is Required for F-actin Cytoskeleton Organization and Contributes to Mechanical Property of Contracting Airway Smooth Muscle

#### Group Jun Yan

##### Jiakuan Liu

The metastasis suppressor role of ubiquitously transcribed tetratricopeptide repeat on chromosome X in prostate cancer

#### Group Chaojun Li

##### Yongjuan Sang

The function and mechanistic actions of protein prenylation in oocyte maturation

#### Danyang Chong

Remodeling of organ function in postnatal mouse: Study on the mechanism of ketone body regulating cardiometabolic conversion and loss of heart regeneration

#### Weiwei Chen

The role and mechanism of LCN2 in lupus nephritis and diffuse alveolar hemorrhage

As the primary task for MARC is to excel in scientific research and education, graduate students are the most valuable assets of our institute. In order to attract more outstanding students to MARC, we held the 11th Summer Camp on July 24 this summer. Twelve excellent undergraduates were selected from a pool of 101 applicants.

This summer camp was broadcast live at Station B, 936 candidates participated online, allowing productive two-way communication of enrollment and queries.

A set of wonderful programs have been organized in order to better facilitate the interactions between undergraduate students and our faculty members/graduate students. Five faculty members introduced the development of Model Animal Research Center and the current progress in biomedical researches. Moreover, we have specially invited "MARC STAR" graduates to share experiences with students about the connection between scientific research and campus life.

The purpose of the Summer Camp is to train and attract students for future biomedical researches involving model animals both at MARC and at other institutes in China. So we invited all the PIs to participate in this activity, PIs and the summer camp students who share same passion continued to change ideas and communicate after the broadcast. Overall, the online Summer Camp allowed the participants to experience the strong atmosphere of academic research at MARC and stimulate their enthusiasm for scientific research.

## MARC 专场宣讲



石云 教授

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南京大学  
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课题组组长代表



王鼎玉 博士

南京大学  
模式动物研究所  
优秀毕业生代表



## 2020 Students Union

The student activities are always rich and colorful in MARC. Besides studying and researching, students of MARC may also find themselves cultivated by a culture promoting humanity, critical thinking, and social well-being here. Thanks to the generous financial support guaranteed by both the MARC and the government, we have adequate resources to enrich our lives here.

We rented three badminton courts, all members of MARC can enjoy the happiness of playing badminton every Friday afternoon. On the badminton fields, we all felt the charm of this sport activity, relaxed ourselves and strengthened our own bodies a lot.

In September, we organized an online sharing session about civil servant selection policy with another three students unions of Nanjing University. By this sharing session, our students knew more about what is civil servant selection and how to prepare for it. This activity not only broadened horizon of MARCers, but also promoted the communications between MARC and other colleges of Nanjing University. Besides, we introduced many information and experiences about civil servant examination to our students this term, which are useful for our career pursuing.

In the end of 2020, we held a party to celebrate the coming of 2021 new year. Freshman of grade 2020 gave a series of fascinating performances, which is a feast for the eyes. We all buried ourselves in the beautiful voice of Doctor Yohei. We together, overcome the tough days of 2020, and welcomed a promising 2021.







**MODEL ANIMAL RESEARCH CENTER OF NANJING UNIVERSITY**  
**MOE KEY LABORATORY OF MODEL ANIMAL FOR DISEASE STUDY**  
**NATIONAL RESOURCE CENTER FOR MUTANT MICE**