Neutrophils are cells in our immune system that are the first responders to infection. When infection or injury occurs, they are usually the first responders that migrate to inflammation sites. Mitochondria are the powerhouse of cells and provide energy. However, neutrophils do not rely on mitochondria for energy production. The functions of mitochondria in neutrophils biology still remain elusive. We found that the mitochondrial dynamics gene mitofusin 2 (MFN2) is important for neutrophil migration. When the MFN2 gene is depleted in neutrophils in zebrafish, the neutrophils are surprisingly trapped in the vasculature and fail to extravasate. To mimic the blood flow, we observed that mfn2-deficient neutrophils circulate in the bloodstream and flow very fast. It took lots of optimization of live imaging and patience to catch these cells. The second challenge was to switch to cell culture to investigate the molecular mechanisms. We are a zebrafish lab, and cell culture was a new field to us. We started all the cell-line-based assays, including purchasing incubators and other essential equipment. All beginnings are hard. It took us a lot of time and effort to finally become familiar with the cell lines and to establish cell-based assays in the lab. We would like to thank Dr Orion Weiner for sharing the human neutrophil-like cell line, and Dr Anna Huttonlocher and Dr Carole Parent for sharing protocols with us. The mouse work was a challenge for us as well. Our lab had no experience with mouse models before. In order to expand our findings, we initiated the mouse work with tremendous help from our neighboring labs. Another challenge was to build the in vitro flow-adhesion assay. In zebrafish, we observed that mfn2-deficient neutrophils circulate in the vasculature and fail to extravasate. To mimic the blood flow in vivo, we established an in vitro flow-adhesion chamber and we tried very hard to control the cell flow rate, avoid bubbles in the tube, and optimize the live imaging. Besides these, personally, how to balance work and life is a big challenge for me. I had a
several-month-old baby to take care of when I was working on this project. I really appreciate my highly supportive family, whose support meant I could focus on the project when I was in the lab.

A.H.: For me personally, to decipher the role of MFN2 in regulating neutrophil migration, either through physical tethering or mitochondrial dynamics itself, was tricky because genetic ablation would impair both. We tackled this by expressing an artificial tether in our MFN2-knockdown cells to link the mitochondria and ER back together. It was difficult to hit a sweet spot as this ‘forced’ interaction, if not delicately done, would lead to cell death. Thus through multiple rounds of construct design and cloning, we were able to show that a ‘prosthetic’ linker could rescue neutrophil migration, reduce Rac activation levels, and restore mitochondria localization.

Y.W.: The half-life of neutrophil cells is too short to culture in vivo. We used a neutrophil-like cell line, dHL-60 cells. Still, these are pretty fragile and can be activated after exposure to many types of stimuli. It took us some time to complete the gene modifications and verifications in this cell line.

When doing the research, did you have a particular result or ‘eureka’ moment that has stuck with you?

W.Z.: Actually, I think I had lots of ‘eureka’ moments when I was doing this project. I remember the first time I saw the moment of mfn2-deficient neutrophils circulating in vasculature of zebrafish embryos. I could not believe my eyes, because circulating neutrophils had never been reported in zebrafish embryos before. I ran to grab my supervisor from her office to look at those embryos, and we both were so astonished and excited. The first time I saw MFN2-knockdown neutrophils barely moving in the chemotaxis assay, the first time that the in vivo flow-adhesion assay worked, the first time I saw disrupted mitochondrial structure, the first time I saw a hyperactivation of Rac and the first time I saw rescued chemotaxis with Rac inhibitors were all eureka moments for me.

A.H.: Yes, two, in fact. The first one was after my colleague characterized the phenotype of migration in zebrafish and HL-60 cells, and I performed the in vitro recruitment assay in a mice peritonitis model. Since neutrophil recruitment is a complex multi-layered process, I jumped off of my seat when I saw that there was significant impairment in neutrophils recruited to the peritoneal cavity in our conditional knockout mice. The second was the experiment I mentioned above, where we could use artificial bridging to rescue an MFN2 genetic abrogation, thus restoring the migration phenotype.

Y.W.: The phospho-western blot assay for neutrophil samples was a struggle for us due to the high activity of proteases and phosphatases in neutrophils. We tried several different protocols and finally found a good way to perform this experiment. It amazed me how the Rac inhibitors bring down the heightened Rac activation in the MFN2-deficient cells without affecting that in the control cells as much.

Why did you choose Journal of Cell Science for your paper?

W.Z.: We chose Journal of Cell Science because it has a broad audience of cell biologists. According to the description of the journal, we believe it is appropriate to report our findings involving mitochondria, immune cell chemotaxis and intracellular signaling here.

A.H.: We felt that JCS provides a good group of readers and a broad scope of audience to share our work with. I personally feel that our work, though cool and exciting, raises more possibilities and work to do. But we needed to wrap up somewhere to deliver a straight and strong point and keep our story concise. The way the JCS editorial board and journal are laid out was perfect for what we wanted to share.

Y.W.: I am very happy that our paper was accepted by Journal of Cell Science.

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

W.Z.: Dr Qing Deng, my PhD supervisor, has been highly supportive in my research and life. Besides providing insightful advice on projects, she often shares her experience with me about work–life balance as a female scientist. She always encourages me to pursue what I want to do in research and also in life. I am very grateful for her trust and confidence in me.

A.H.: Dr Stan Gelvin at Purdue University, to whom I owe deep gratitude for filling me in, because at the time I was lacking background in molecular biology and current biological methods. Furthermore, Dr Gelvin would often speak to me about the pros and cons of academia and steer me toward a correct mindset to approach science – with rigor and unrelenting passion.

Y.W.: I want to thank my partner Alan Hsu. Wenqing started the project, and Alan and I had a good time working together to finish the project after Wenqing’s graduation. He inspired me and gave me new perspectives about immunology, neutrophil biology and scientific careers.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

W.Z.: The exciting moments when assays are worked out, the thrilling moments when a hypothesis being tested is correct, and the proud moments when my work is reported motivate and intrigue me to pursue a career in science. At the time when I read the email about acceptance of my first manuscript six years ago, I was so thrilled and I called my best friend immediately to tell her my exciting news, then I realized that this is what I want to do in my life.

A.H.: My first curious question came from my time in clinic, when I wondered how dengue virus causes bone marrow suppression. Can dengue virus directly infect bone marrow cells? If so, which lineage? After getting a feel for testing hypotheses and designing experiments to address my curiosity during my early years in
science, I decided to further my training by pursuing a PhD. The next big moment came when I discovered that terminally differentiated neutrophils required cell cycle proteins for their function, which seemed counterintuitive at the time, but the pleasure and joy of setting out to prove this and to find it is true was unparalleled. I carry the same curiosity to this day, and seek to understand biology and immunology. I look forward hopefully to my next chapter and ‘a-ha’ moment, as they are what fuels me.

**Who are your role models in science? Why?**

**W.Z.:** My PhD supervisor Dr Qing Deng and my current mentor Dr Gregory Sonnenberg are my role models in science, as both of them are intelligent, diligent and passionate about science. Importantly, they are highly supportive, training their trainees as independent scientists, and caring about not only their research projects but also their careers and lives.

**A.H.:** Trained as an infectious disease specialist, then moving on to study neutrophils in the context of cell migration and now shifting to neutrophil immunology, I especially look up to Dr Michael Diamond, as his interdisciplinary training and his scientific reflex and thinking are all I hope to be, and more. But on a more heartfelt note, I would say I look up to my past and current mentors Dr Guey-Chuen Perng, Dr Qing Deng, and Dr Hongbo Luo, as they all share qualities of diligence, intelligence, scientific logic, and most of all patience and passion to mentor a young and junior scientist-to-be such as myself.

**What’s next for you?**

**W.Z.:** Currently, I am a postdoctoral associate at Weill Cornell Medicine, pursuing my interests in immune homeostasis and regulation at mucosal sites. I hope to pursue a career in academia as an independent investigator.

**A.H.:** I have recently started my postdoc training in Dr Hongbo Luo’s lab at Harvard Medical School, where I am continuing to learn about the more immunological and developmental aspects of neutrophils. After my training, I hope to continue my passion and pursue a career in academia and hopefully pass on the torch, as so many have done for me.

**Tell us something interesting about yourself that wouldn’t be on your CV**

**W.Z.:** I am a big fan of suspense movies. I enjoy thinking and figuring out puzzles and mysteries as the movie goes, just like I really enjoy asking questions, developing a hypothesis, testing the hypothesis and addressing questions during research.

**A.H.:** Besides trying my best and being as detailed and rigorous I can be in science, I carry the same attitude to other aspects in life, including a long-time leisure activity of mine – gaming. So much so that I achieved number one in the world rankings for a game I play – Starcraft. Playing video games not only helps me to relax but also to learn collaboration and teamwork. And just like in my science, I could not do it without the help of great mentoring, great coaching and an exceptional team!

**Reference**