# Fasting Reduces Inflammation

Min Dai

Mar. 10th, 2021

daimin@zju.edu.cn

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences

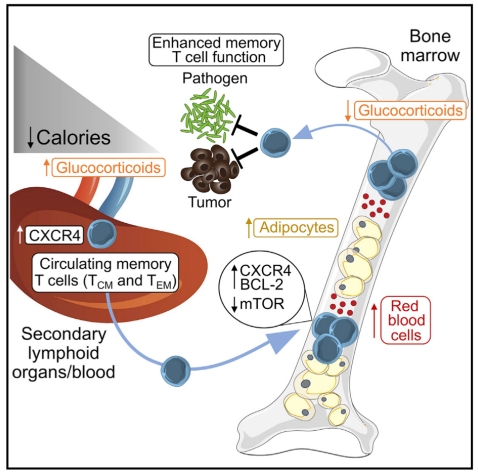
# When fasting gets tough, the tough immune cells get going—or die

Article

Cell

**The Bone Marrow Protects and Optimizes Immunological Memory during Dietary Restriction**

Graphical Abstract





Authors

Nicholas Collins, Seong-Ji Han, Michel Enamorado, ..., Dorian B. McGavern, Pamela L. Schwartzberg, Yasmine Belkaid

Correspondence

ybelkaid@niaid.nih.gov


In Brief

Calorie restriction triggers memory T cell homing to the bone marrow to promote survival and enhanced protective function.

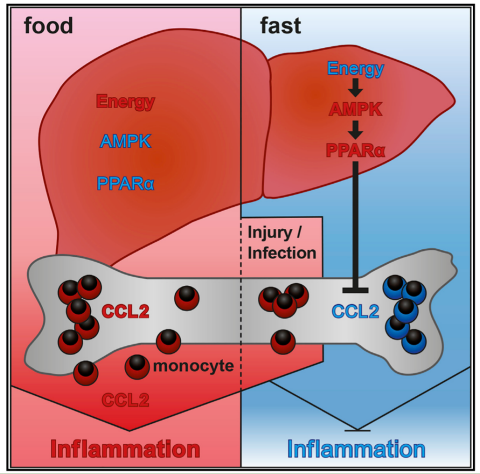
Collins et al., 2019, Cell *178*

Article

Cell

**Dietary Intake Regulates the Circulating Inflammatory Monocyte Pool**

Graphical Abstract





Authors

Stefan Jordan, Navpreet Tung, Maria Casanova-Acebes, ..., Marie-Luise Berres, Emily J. Gallagher, Miriam Merad

Correspondence

miriam.merad@mssm.edu (M.M.), stefan.jordan@mssm.edu (S.J.)


In Brief

Caloric restriction reduces the number of circulating inflammatory monocytes in a CCL2/PPARa-dependent manner, without compromising responses to acute inflammation.

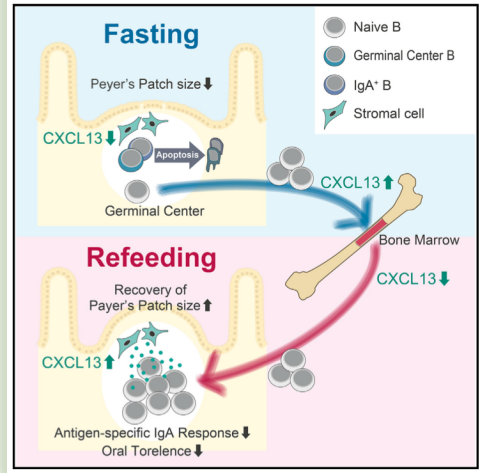
Jordan et al., 2019, Cell *178*

Article

Cell

**Fasting-Refeeding Impacts Immune Cell Dynamics and Mucosal Immune Responses**

Graphical Abstract





Authors

Motoyoshi Nagai, Ryotaro Noguchi, Daisuke Takahashi, ..., Keiyo Takubo, Taeko Dohi, Koji Hase

Correspondence

hase-kj@pha.keio.ac.jp


In Brief

Temporary fasting drastically reduces the levels of B cells in Peyer's patches, with germinal center B cells undergoing apoptosis and naive cells migrating to the bone marrow and only egressing upon refeeding.

Nagai et al., 2019, Cell *178*

# Outline

- Background
- Results
- Summary
- Discussion

# Corresponding author

Icahn School of Medicine at Mount Sinai

**Prof. Miriam Merad, MD/PhD**

- an Algerian professor in cancer immunology
- the director of the Precision Immunology Institute at the Icahn School of Medicine at Mount Sinai in New York City, NY
- a member of the United States National Academy of Sciences

**Research interest:**

- The role dendritic cells and macrophages play within the tumor microenvironment and on how tumors prevent the normal anti-tumor functions of these cells

https://en.wikipedia.org/wiki/Miriam_Merad
https://labs.icahn.mssm.edu/meradlab/miriam-merad/

# Background

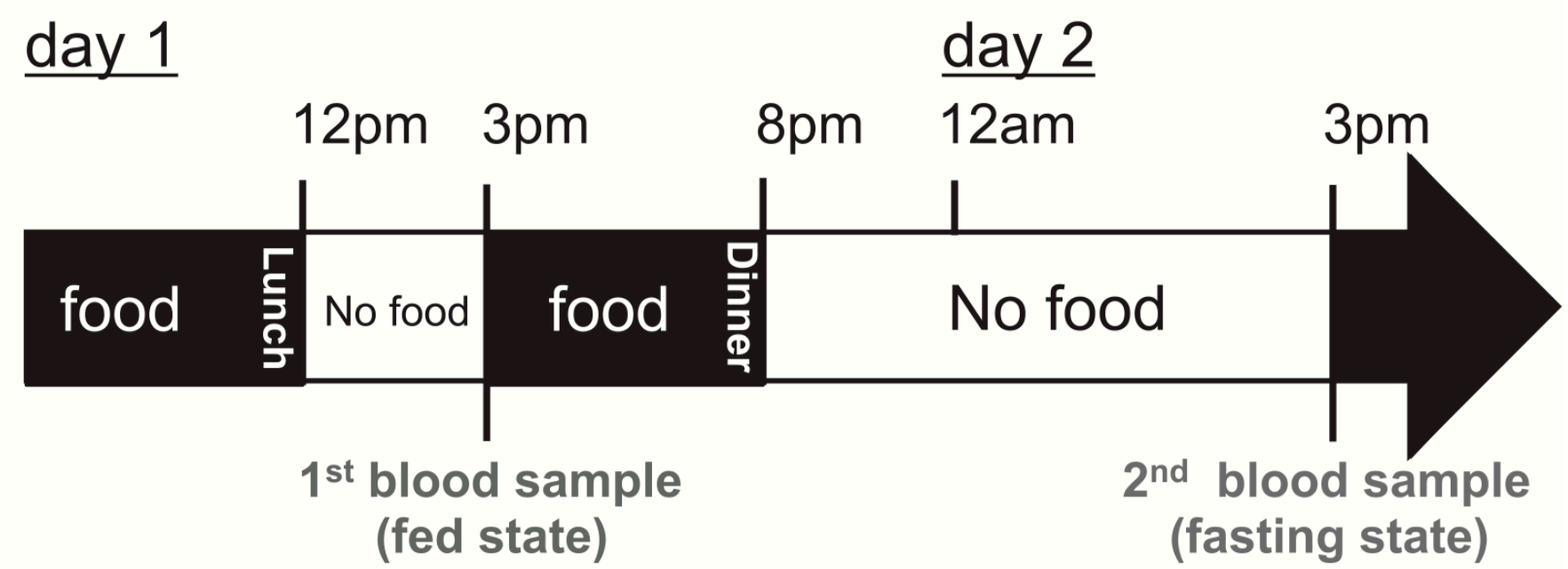
- Hypocaloric diets or fasting are associated with improved outcomes of metabolic, autoimmune, and inflammatory diseases in humans:
  - NAFLD (non-alcoholic fatty liver diseases)
  - T2DM (type 2 diabetes mellitus)
  - CVD (atherosclerosis, cardiovascular disease)
  - multiple sclerosis 多发性硬化症
  - rheumatoid arthritis 风湿性关节炎
  - asthma 哮喘
  - psoriasis 牛皮癣
  - …
- Hypocaloric diets or fasting regimens have been shown to prolong lifespan
- Individuals undergoing intermittent or religious fasting have reduced basal levels of circulating pro-inflammatory cytokines including TNF$\alpha$, IL-6 and IL-1$\beta$
- …

# Scientific question

How fasting modulate systemic inflammation?

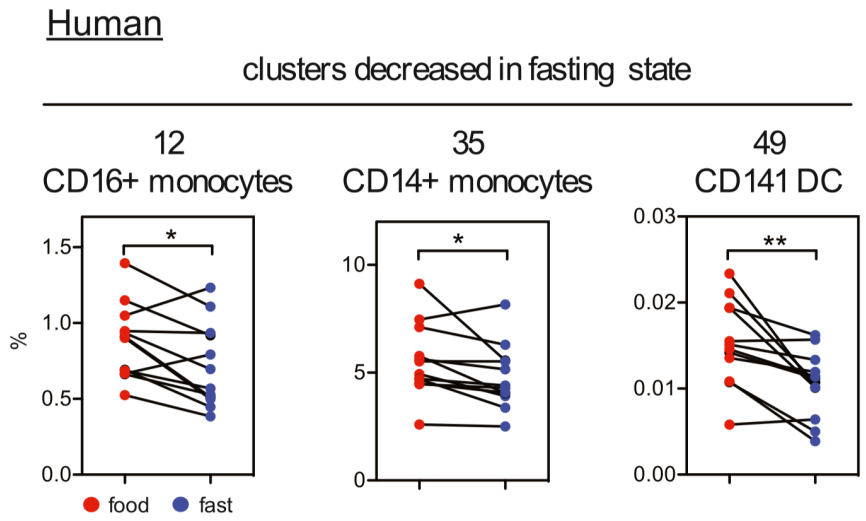
# The design of fasting experiments

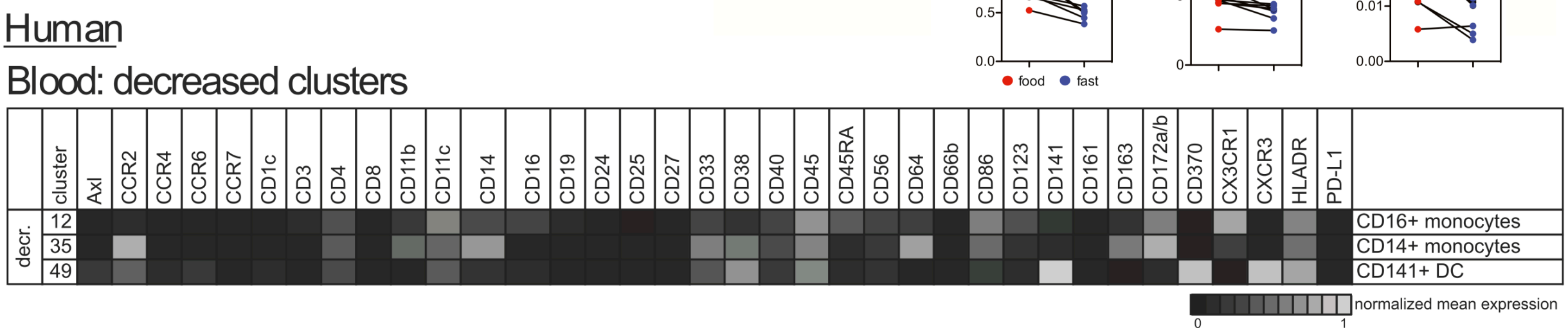
- The authors profiled the composition of blood circulating immune cells of 12 healthy normal weight volunteers (mean age = 30 ± 5 years, BMI = 22 ± 2 kg/m$^2$) 3 h after food intake (fed state) and after 19 h of fasting (fasting state) using cytometry by time-of-flight spectrometry (CyTOF)
- To control for circadian variations, all blood samples were drawn at the same time of the day (3 pm)



# Fasting led to significant reduction of subsets of circulating monocytes and circulating dendritic cells

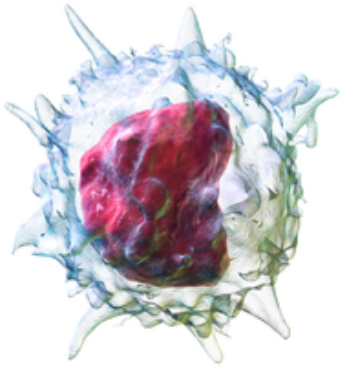
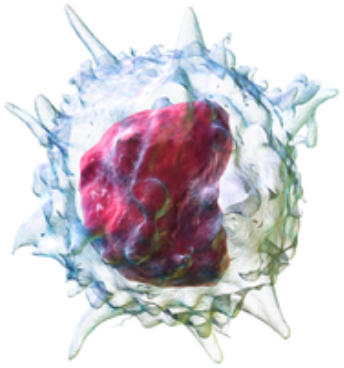
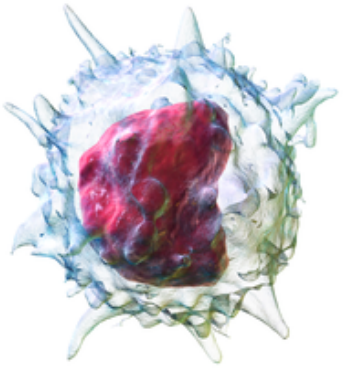
- Cells decreased in fasting state (Human)
  - CD14+ monocytes
  - CD16+ monocytes
  - CD141+ DCs (dendritic cells)



# Monocyte

- Monocytes are a type of leukocyte, or white blood cell
  - the largest type of leukocyte and can differentiate into macrophages and myeloid lineage dendritic cells
  - as a part of the vertebrate innate immune system, monocytes also influence the process of adaptive immunity
- Function:
  - replenishing resident macrophages under normal conditions
  - migration within approximately 8–12 hours in response to inflammation signals from sites of infection in the tissues

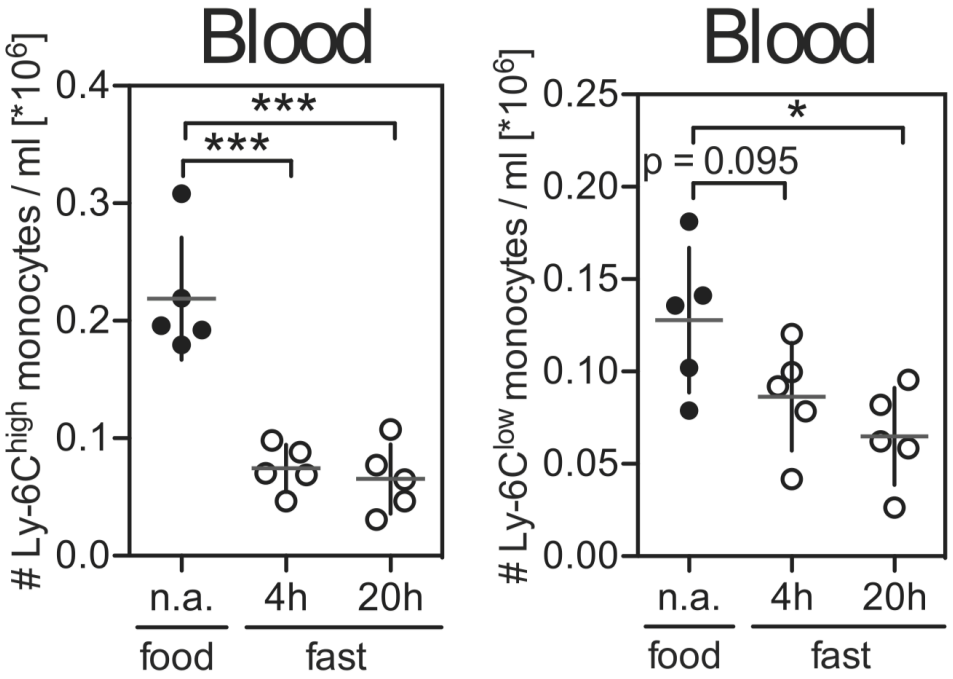




https://en.wikipedia.org/wiki/Monocyte

*How about the effect of fasting in mice?*

# Fasting also reduced circulating pro-inflammatory Ly-6C$^{high}$ monocytes in healthy mice

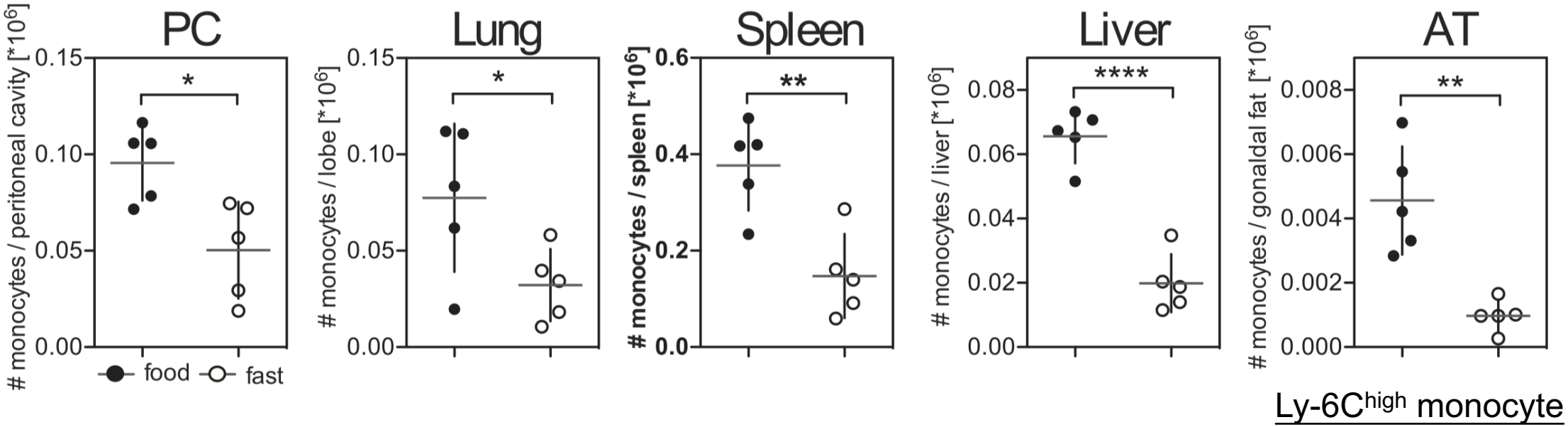
- 4 h short-term fasting protocol during the light period (Zeitgeber [ZT]2-6)
  - ZT: 自然授时光照时间, 是由实验室所设定的环境时间，ZT 0是灯亮的时间点
  - comparable to overnight fasting for humans and is the least stressful fasting strategy in animals




Note: Every dot represents one individual animal. Horizontal bar = mean. Vertical bar = SD. Statistical significance is indicated by *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns = not significant.

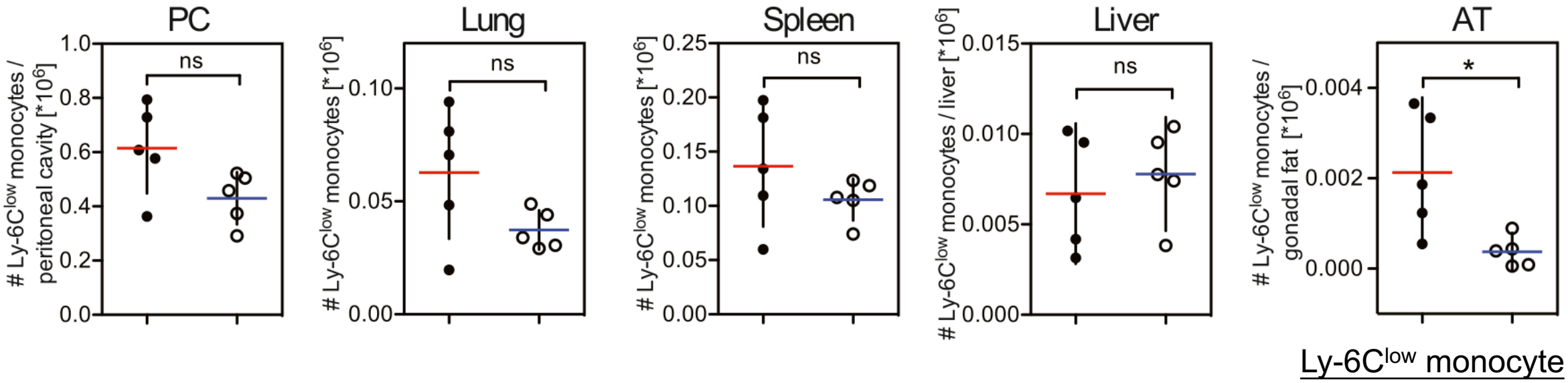
https://pansci.asia/archives/77994

# Fasting led to significant reduction of pro-inflammatory Ly-6C$^{high}$ monocytes in peripheral tissues




- Fast: 20 h
- PC: peritoneal cavity, 腹膜腔
- AT: adipose tissues

# No significant reduction of Ly-6C$^{low}$ monocytes was observed in peripheral tissues




- Fast: 20 h
- PC: peritoneal cavity, 腹膜腔
- AT: adipose tissues

***What regulates the reduction of circulating monocytes in fasting mice?***

# Potential mechanisms for decreased numbers of circulating monocytes

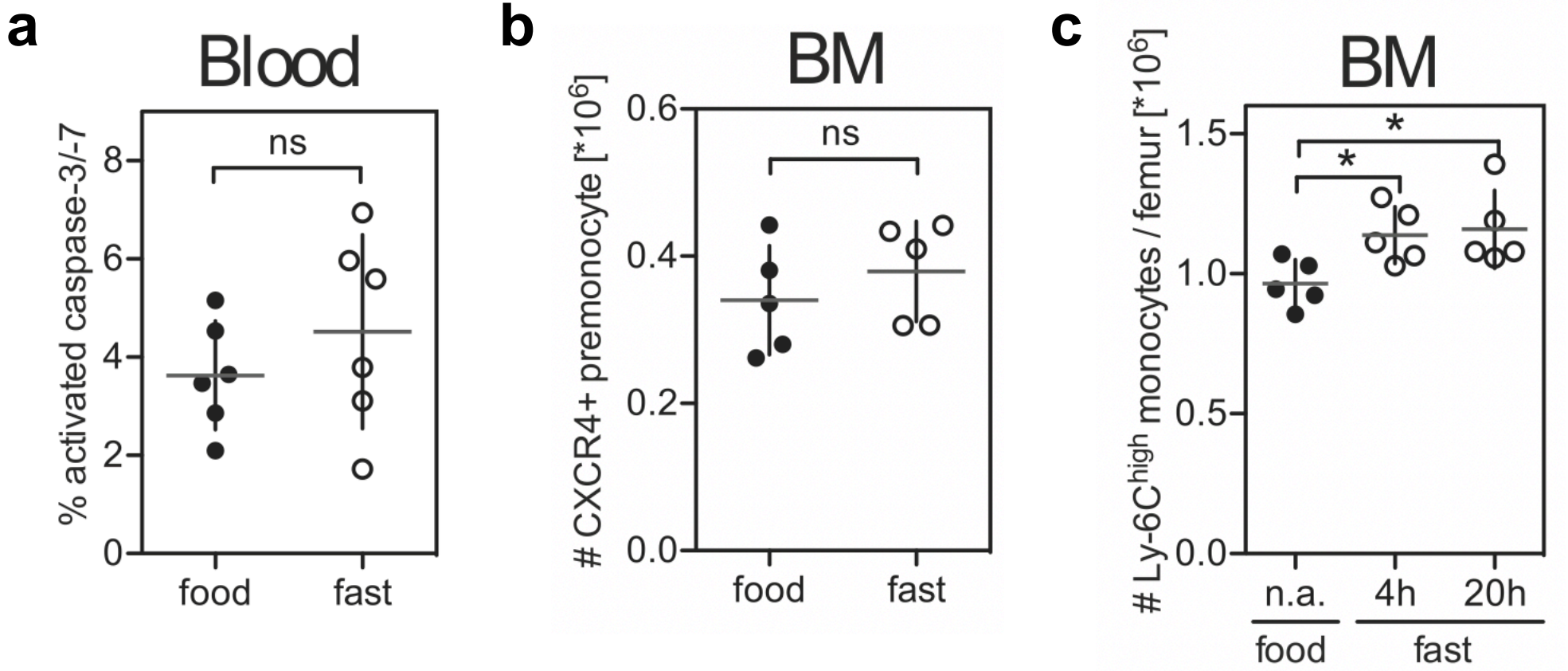
1. increased monocyte cell death
2. reduced bone marrow (BM) myelopoiesis
3. reduced BM egress to the periphery

# Reduction of blood monocytes is due to reduced monocyte egress from the bone marrow to the blood circulation

(a) No increased number of activated caspase-positive monocytes in blood was observed

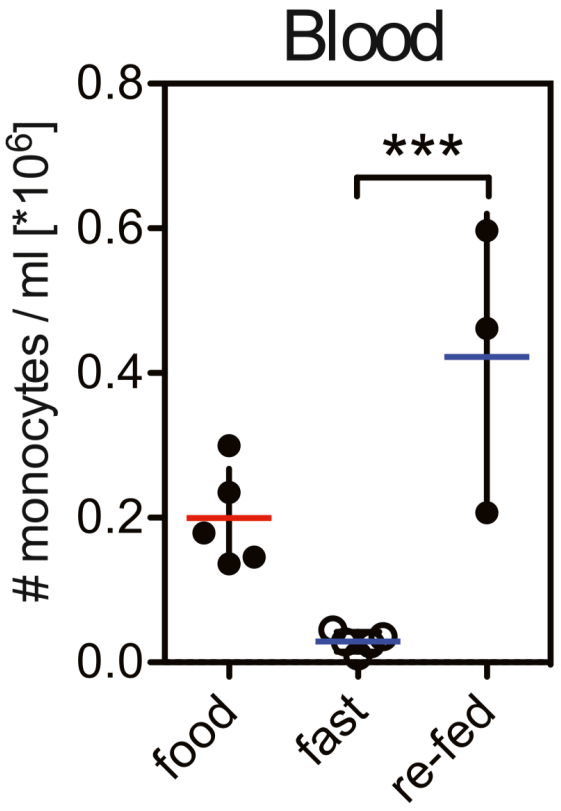
(b) No increased number of CXCR4$^{+}$ monocyte precursors in Bone Marrow (BM) was observed

(c) Accumulation of Ly-6C$^{high}$ monocytes in the BM of fasting mice was observed



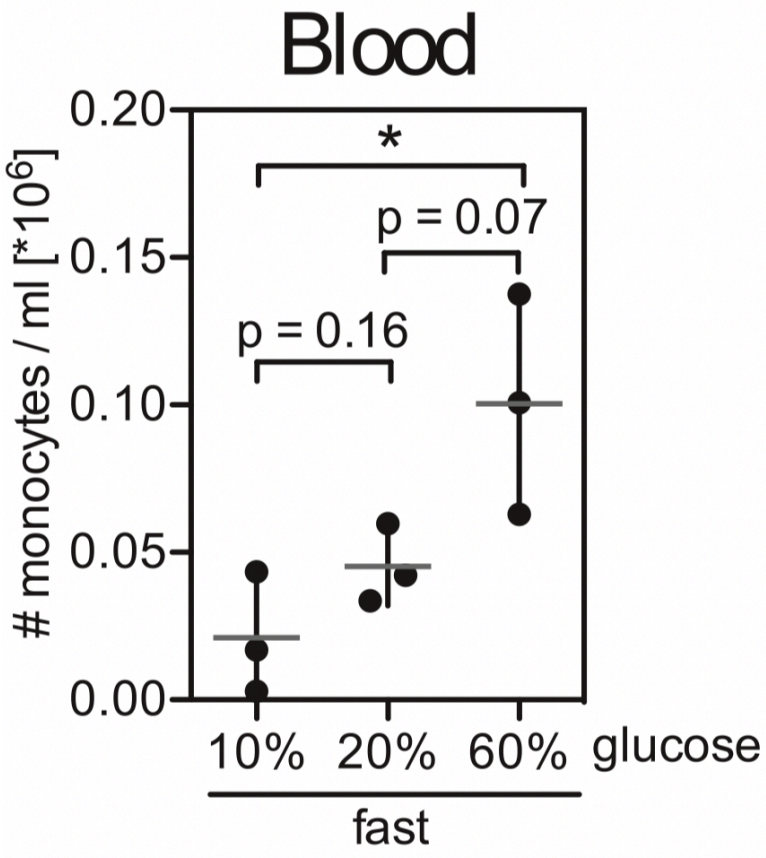
# Fasting-induced inhibition of Bone Marrow egress is revoked upon food intake

- Re-feeding mice for 4 h after an overnight fast restored monocyte numbers in the periphery



# The size of the monocyte pool in the blood circulation depended on the amount of carbohydrate ingested

- Absolute numbers of Ly-6C$^{high}$ monocytes in the blood of mice fasted for 16 h and gavaged with glucose solutions at the indicated concentrations

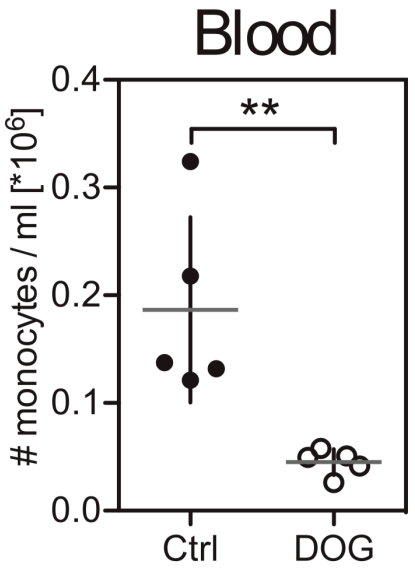


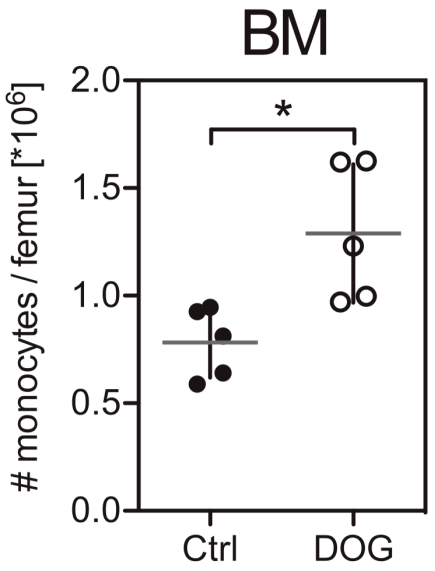
# Hypothesis

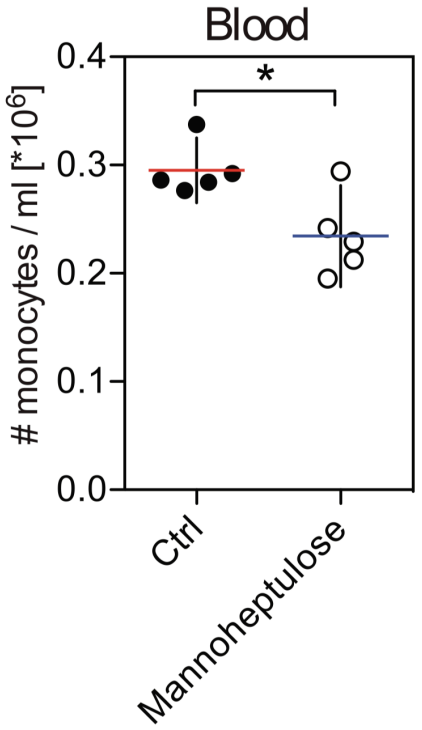
***Carbohydrates might modulate peripheral monocyte numbers by altering cellular energy levels***

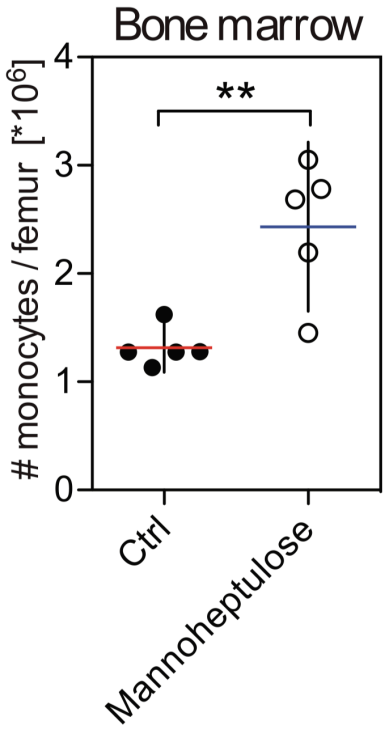
# Cellular energy levels controlled the blood circulating monocyte pool

- Two different inhibitors of hexokinase 己糖激酶, 2-deoxyglucose (DOG), and D-mannoheptulose, in order to block the first step in glycolysis, i.e., cellular energy production



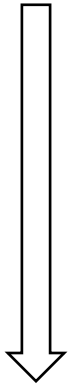



# Clue

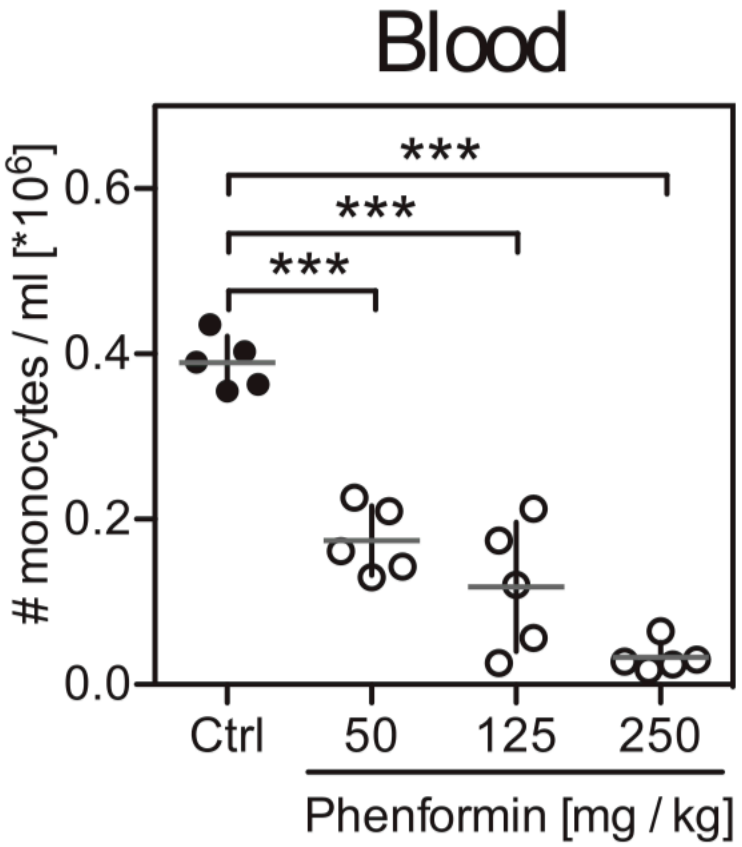
***AMPK is a key cellular energy sensor triggered by an increase in the cellular AMP/ATP ratio that reflects low energy levels***

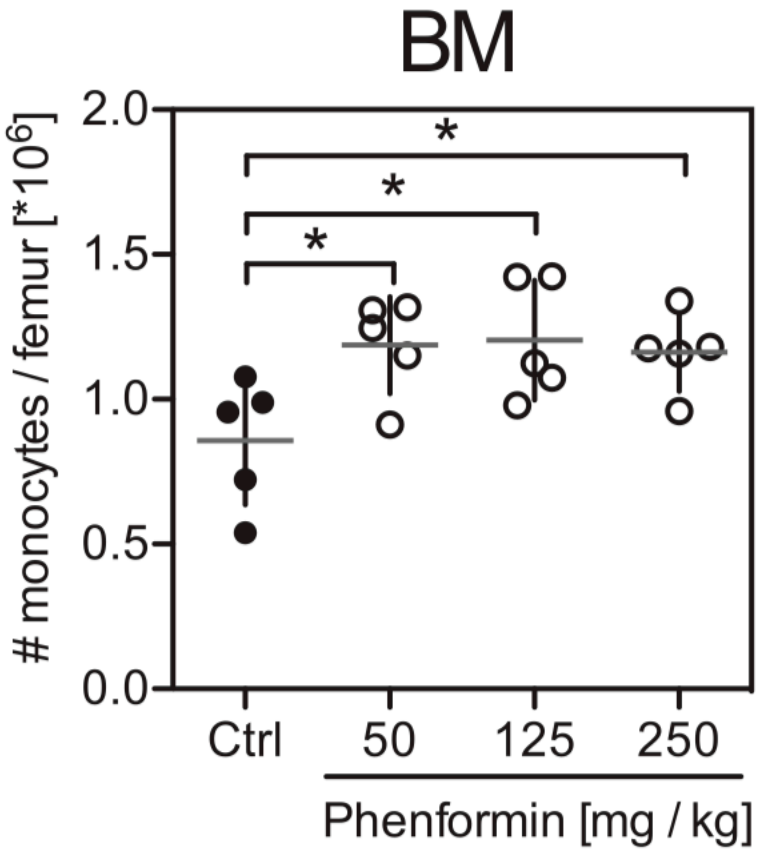


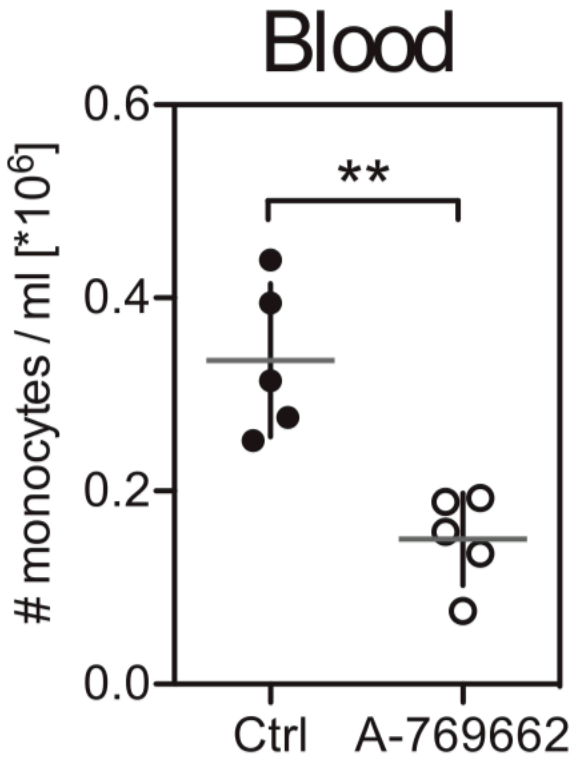
***Next: test whether activation of AMPK is sufficient to inhibit BM monocyte egress to the blood circulation***

# AMPK activation led to reduction of blood peripheral monocytes

- Phenformin 苯乙双胍 is known to elevate the cellular AMP/ATP ratio which results in AMPK activation
- A-769662 is a small molecule activator of AMPK



Reduction of circulating monocytes in fasting mice

Reduced monocyte egress from the bone marrow to the blood circulation

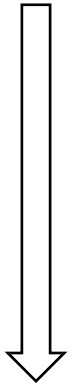
Cellular energy levels controlled the blood circulating monocyte pool

AMPK activation led to reduction of blood peripheral monocytes

***What's next?***

# Clue

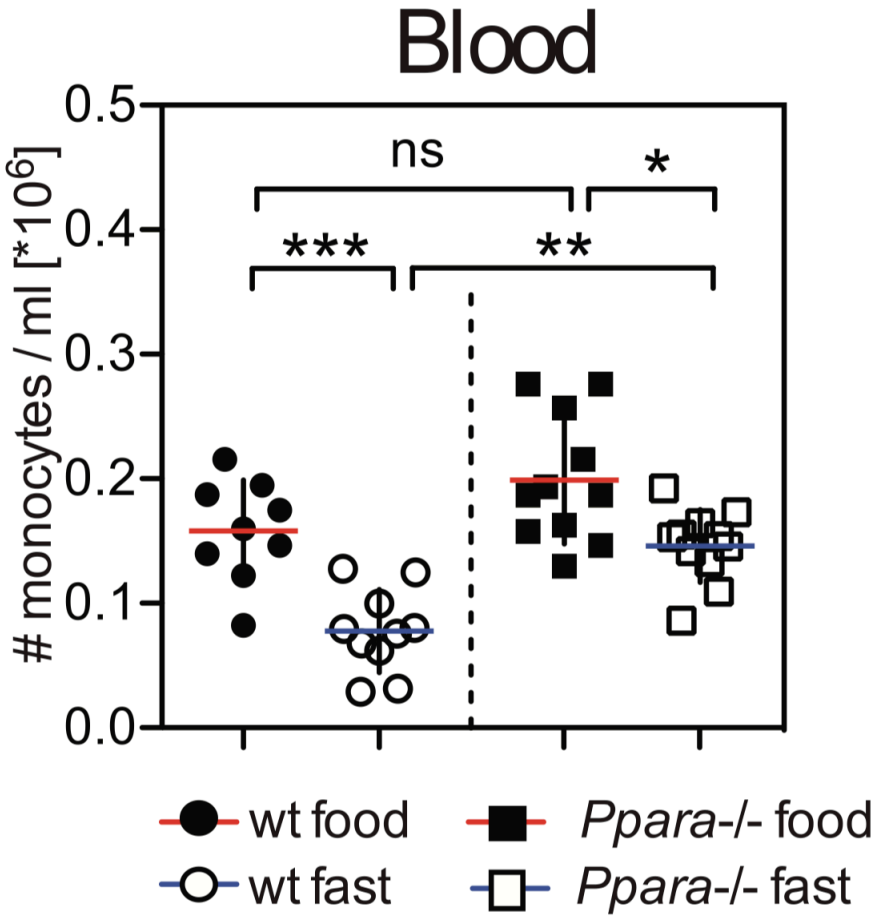
***PPARα is a target of AMPK and is a master transcriptional regulator in the adaptive response to fasting***



***Next: test whether hepatic PPARα controls peripheral blood monocyte numbers***

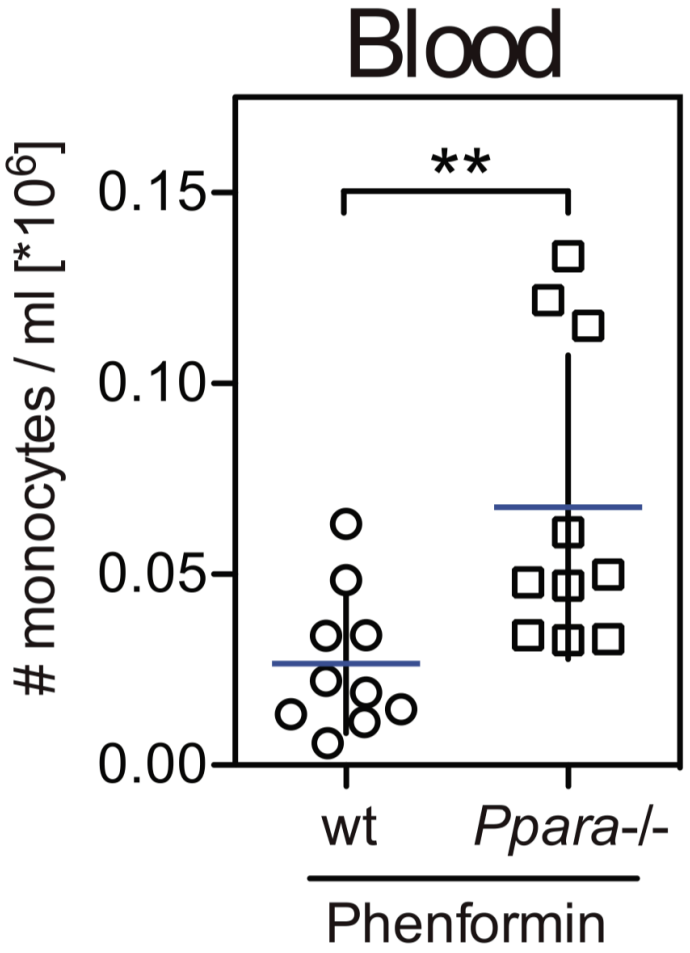
# Activation of PPARα contributed to the regulation of monocyte homeostasis during fasting

- The number of Ly-6C$^{high}$ monocytes in the **wt fast** group was significantly smaller than that in the ***Ppara-/-* fast** group



# AMPK-mediated reduction in peripheral monocyte numbers in fasting mice was in part mediated through PPARα

- Phenformin is known to elevate the cellular AMP/ATP ratio which results in AMPK activation

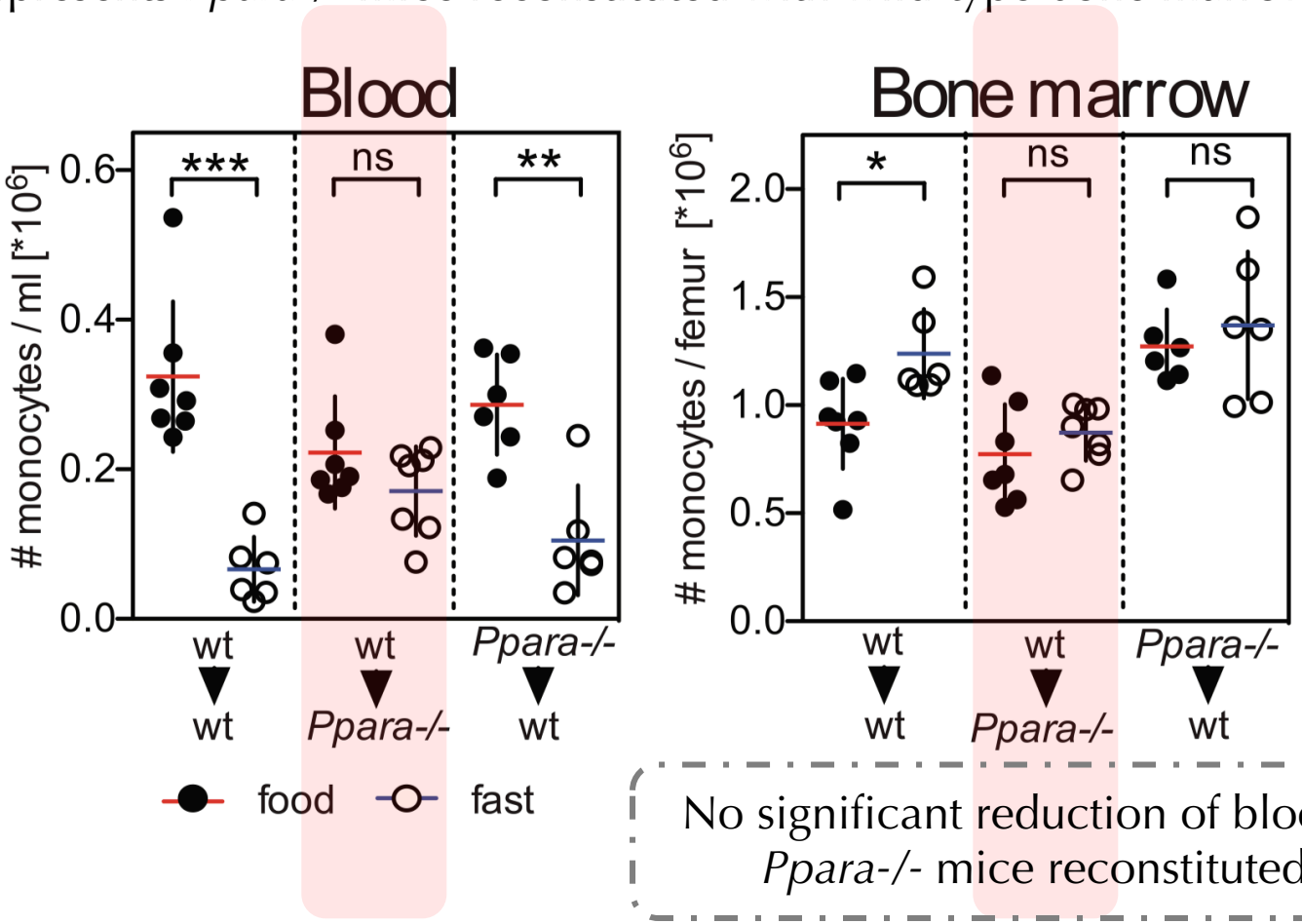


# Question

**Because in the *Ppara-/-* mice, the gene was not uniquely deleted in specific tissue or organ, so we need to investigate whether PPARα functions directly in the monocytes or elsewhere.**

# The control of bone marrow monocyte egress required PPARα expression in cells besides monocytes

- The authors generated bone marrow chimeric animals in which wild-type or *Ppara-/-* bone marrow cells were injected into lethally irradiated hosts
- The mark, wt ▼ *Ppara-/-*, represents *Ppara-/-* mice reconstituted with wild-type bone marrow cells



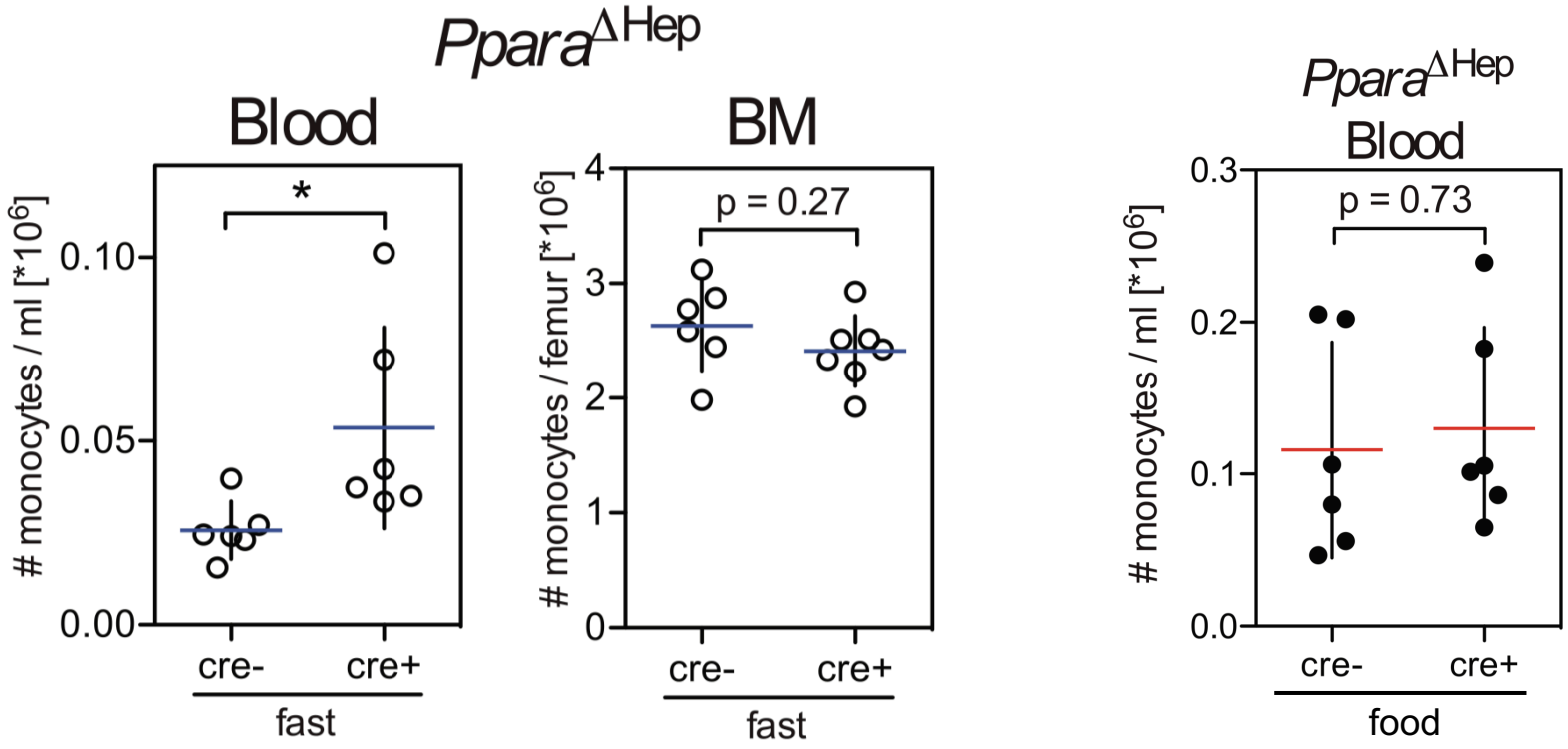

No significant reduction of blood monocytes in fasting *Ppara-/-* mice reconstituted with wild-type BM

# Clue

**PPARα is expressed at higher levels in the liver and acts mainly in hepatocytes.**

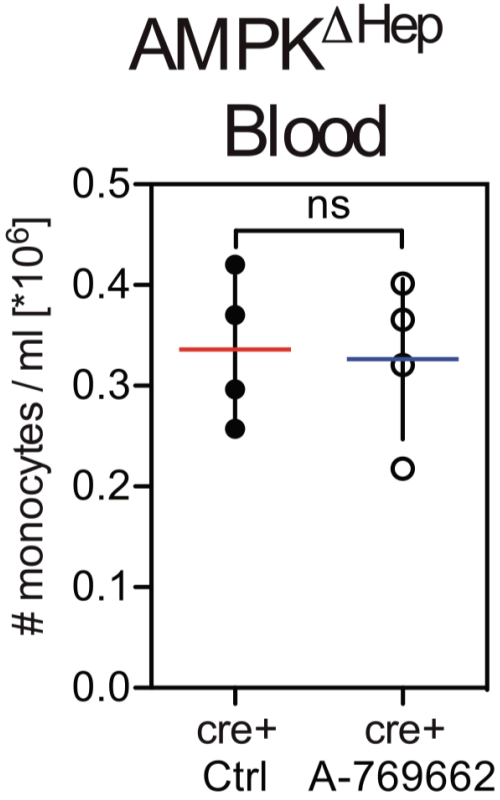
# *Ppara*$^{\Delta Hep}$ mice lost their ability to modulate bone marrow monocyte egress upon fasting

- *Alb*$^{cre/cre}$ mice were crossed to *Ppara*$^{fl/fl}$ mice to delete PPAR$\alpha$ from hepatocytes (*Ppara*$^{\Delta Hep}$) in cre+ mice
- *Ppara*$^{\Delta Hep}$ mice: PPAR$\alpha$ was deleted uniquely in hepatocytes



# Deletion of AMPK specifically in hepatocytes abrogated the reduction of circulating monocytes upon gavage with A-769662

- *Alb*$^{cre/cre}$ mice were crossed to *Prkaa1*$^{fl/fl}$ mice to delete AMPK from hepatocytes (*AMPK*$^{\Delta Hep}$) in cre+ mice
- *AMPK*$^{\Delta Hep}$ mice: AMPK was deleted uniquely in hepatocytes
- A-769662 is a small molecule activator of AMPK
- This result demonstrated the importance of **the liver**



# A brief summary

Energy-sensing by **the liver AMPK-PPAR$\alpha$ pathway** controls the blood monocyte pool in response to caloric intake

Reduction of circulating monocytes in fasting mice

Reduced monocyte egress from the bone marrow to the blood circulation

Cellular energy levels controlled the blood circulating monocyte pool

AMPK activation led to reduction of blood peripheral monocytes

The liver AMPK-PPAR$\alpha$ pathway controlled the blood circulating monocyte pool in response to caloric intake

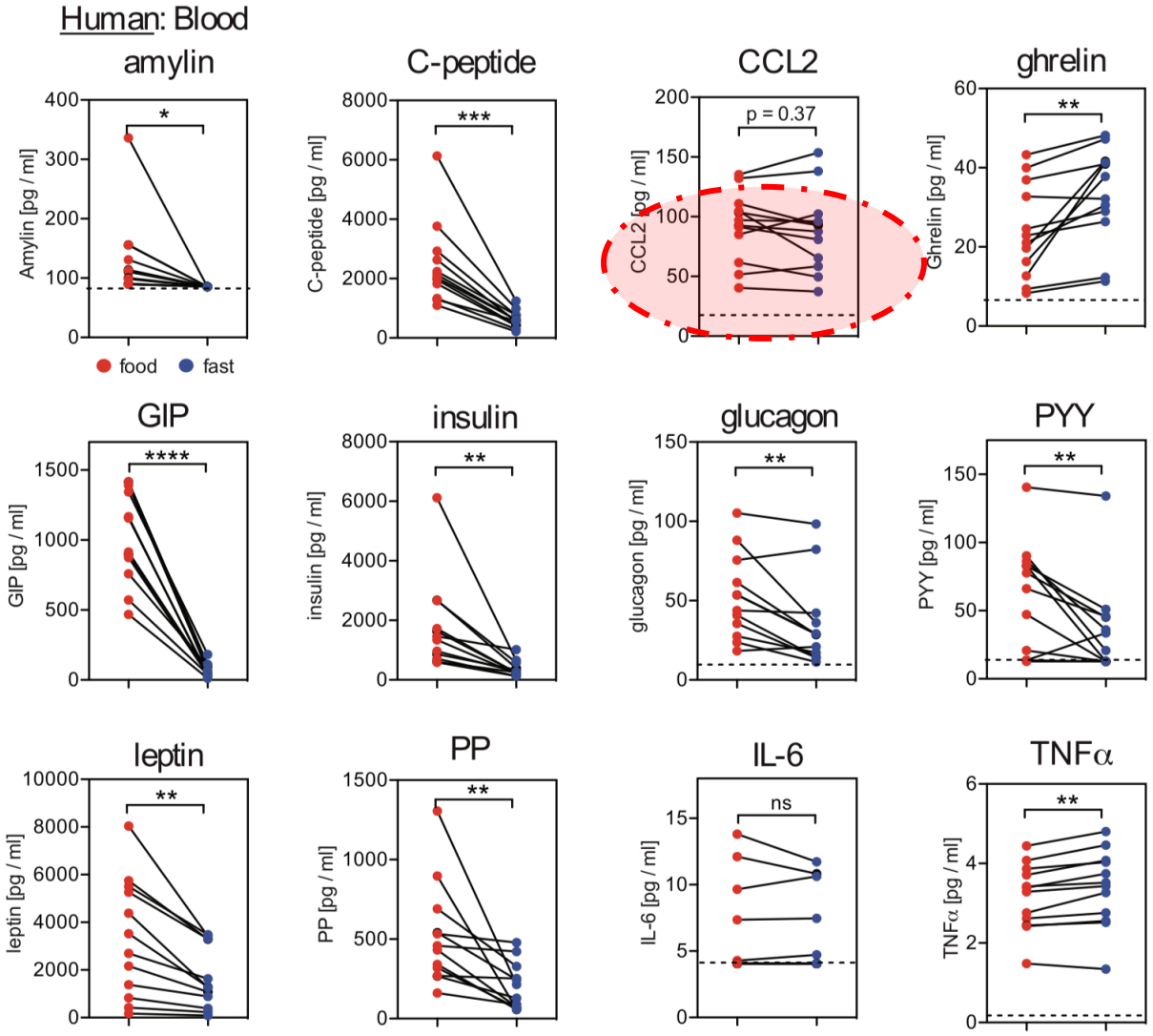
## *What's next?*

the liver AMPK-PPAR$\alpha$ pathway

***How?***

Reduced monocyte egress from the Bone Marrow to the blood circulation

# Multiplex analysis for metabolic hormones in the blood of human and mice




Blood

| | wt | | *Ppara-/-* | |
|---|---|---|---|---|
| | food | fast | food | fast |
| TNFα | | | | |
| CCL2 | | | | |
| Insulin | | | | |
| C.Peptide | | | | |
| Amylin (active) | | | | |
| PP | | | | |
| Leptin | | | | |
| GIP | | | | |
| Ghrelin | | | | |
| Resistin | | | | |
| Glucagon | | | | |
| GLP-1 | | | | |
| IL-6 | | | | |
| PYY | | | | |

# Why CCL2?

CCL2 binds to CCR2, a chemotactic receptor highly expressed on monocytes and shown to mediate monocyte bone marrow egress.

ARTICLES

nature immunology

re.com/natureimmunology

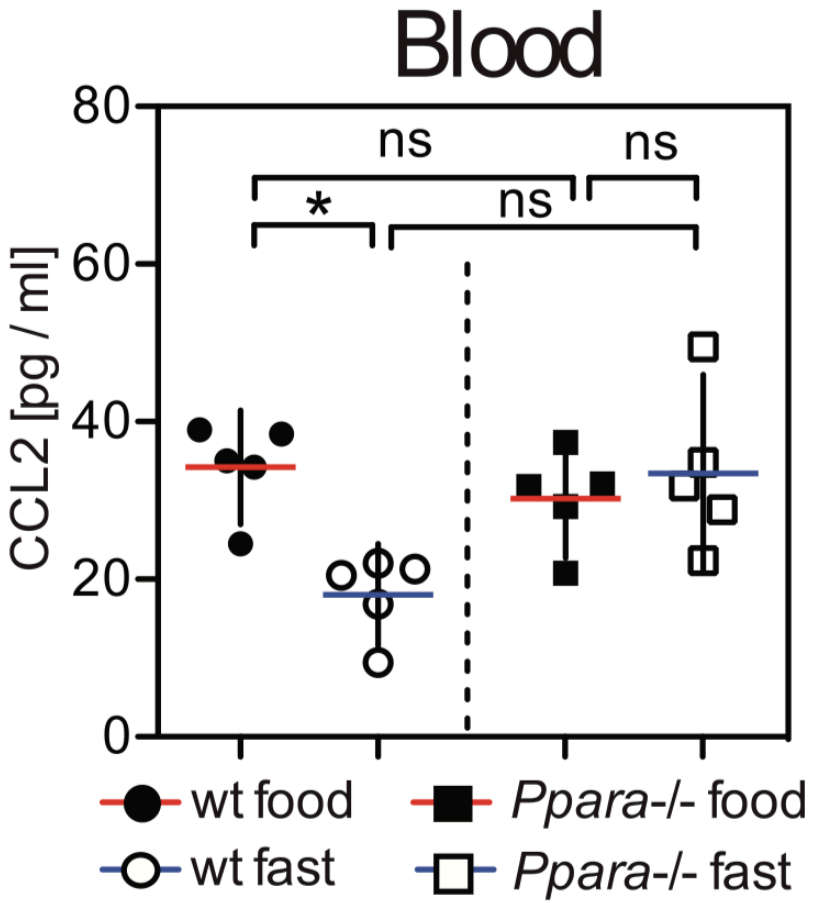
## Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2

Natalya V Serbina & Eric G Pamer

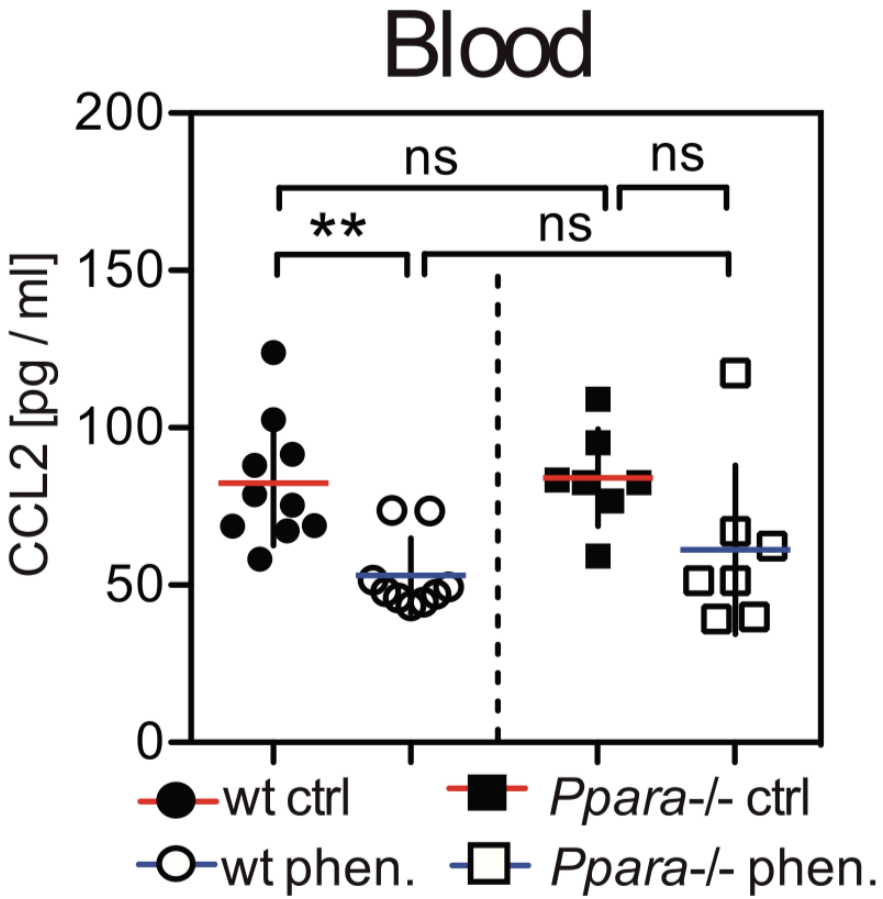
Serbina and Pamer, Nat. Immunol. (2006)

# Strong reductions of CCL2 were observed in fasting mice as well as upon AMPK activation

- **phen** (phenformin) is known to elevate the cellular AMP/ATP ratio which results in AMPK activation




**(fasting)**




**(AMPK activation)**

# How to validate whether CCL2 is important or not?

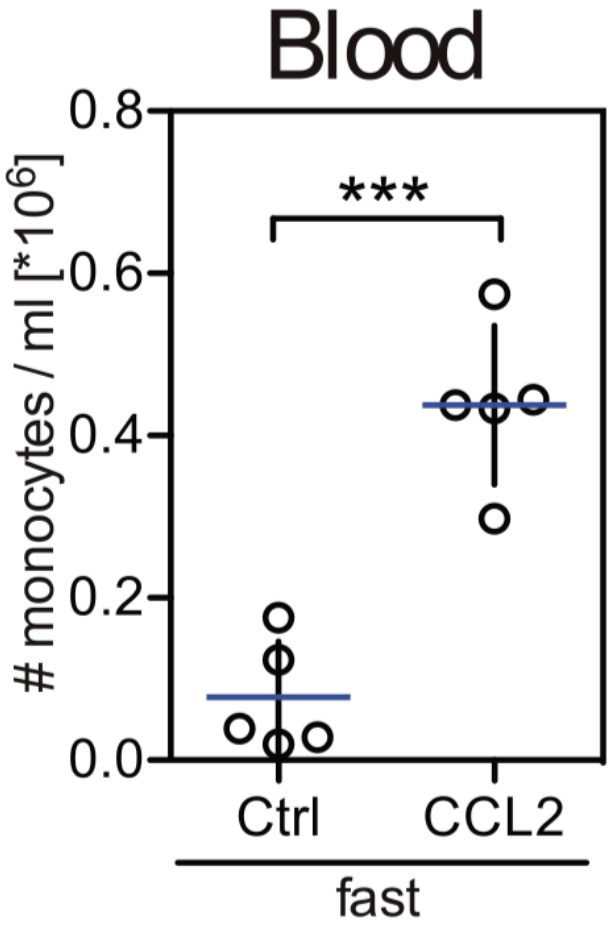
# What will happen

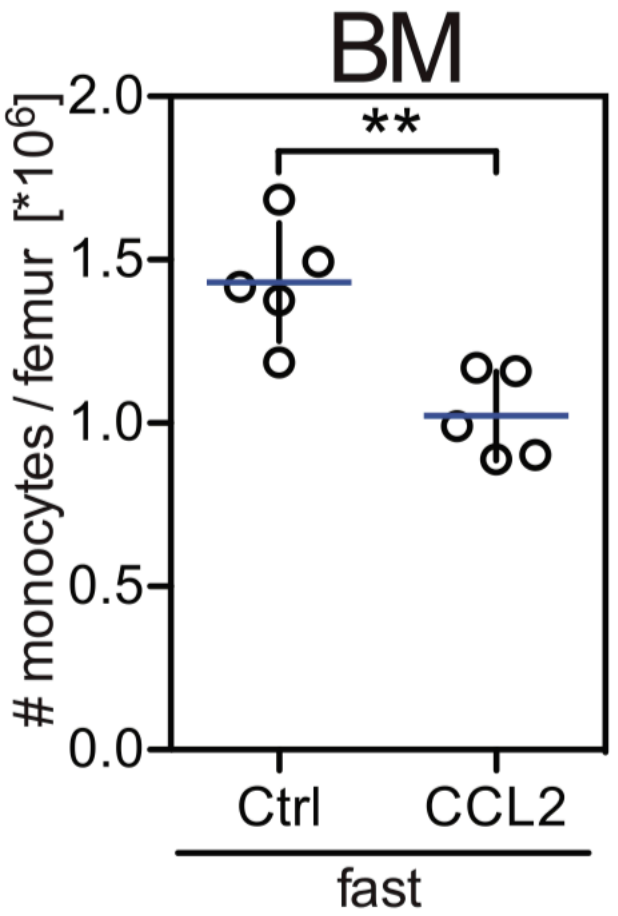
# if

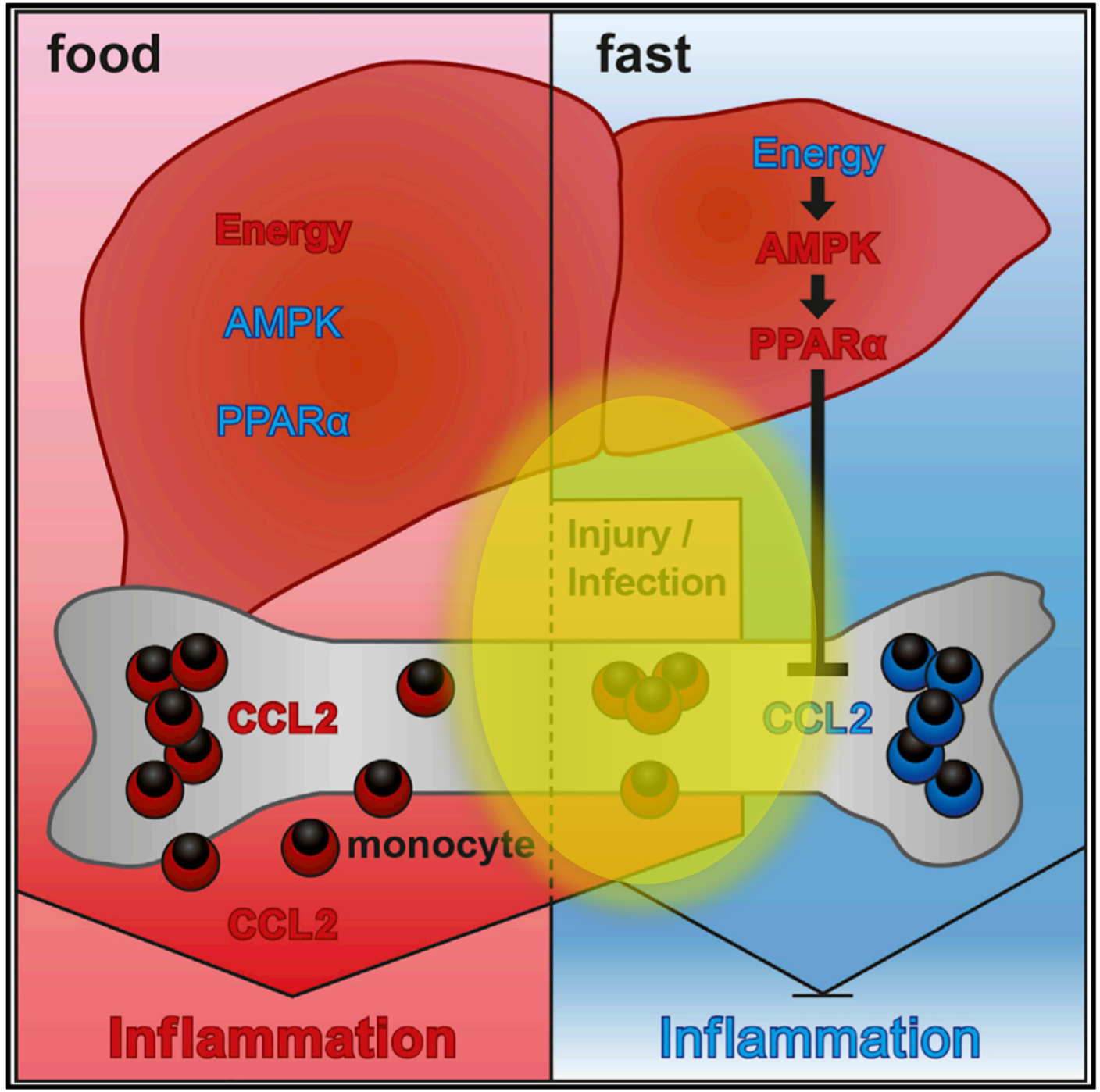
# we restore plasma CCL2 levels in fasting mice?

# The critical role of CCL2 in monocyte homeostasis

- Use administration of recombinant protein to restore plasma CCL2 levels

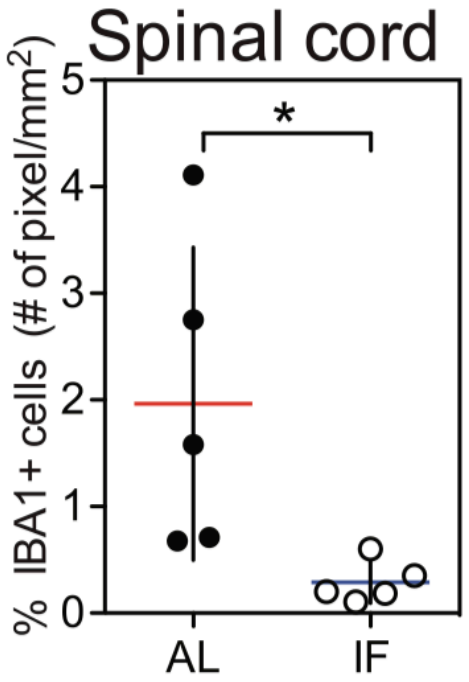


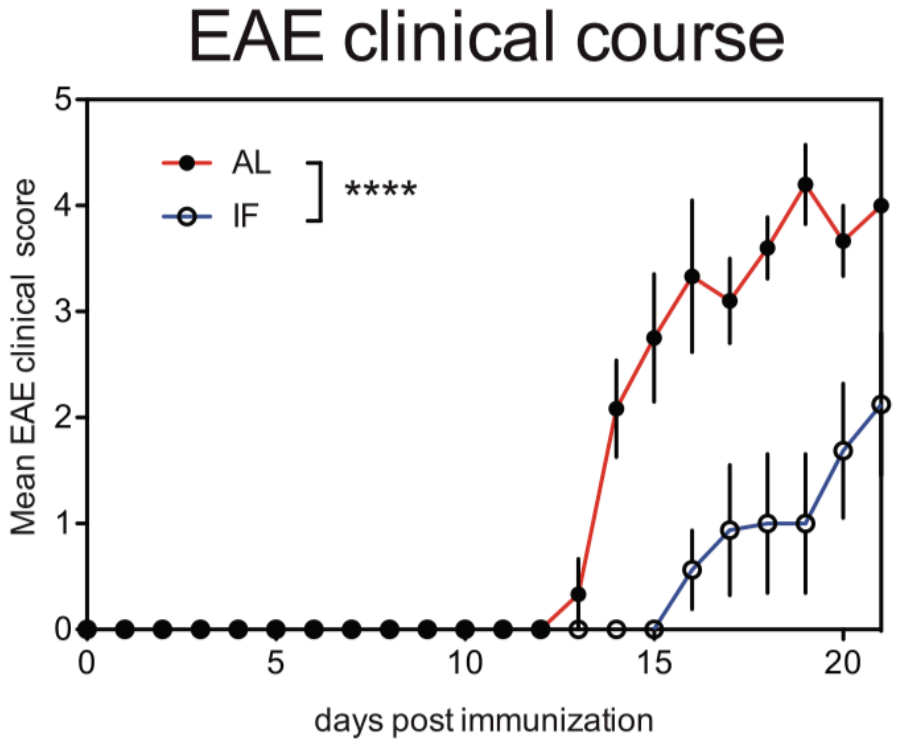




food
fast
Energy
AMPK
PPARα
Energy
AMPK
PPARα
Injury /
Infection
CCL2
CCL2
monocyte
CCL2
Inflammation
Inflammation

# Intermittent fasting led to a strong reduction of monocyte accumulation in the EAE mice

- AL: mice were fed *ad libitum* 随意 ; IF: mice were subjected to intermittent fasting
- EAE: experimental autoimmune encephalomyelitis 实验性自体免疫型脑炎, the main preclinical model for multiple sclerosis 多发性硬化症

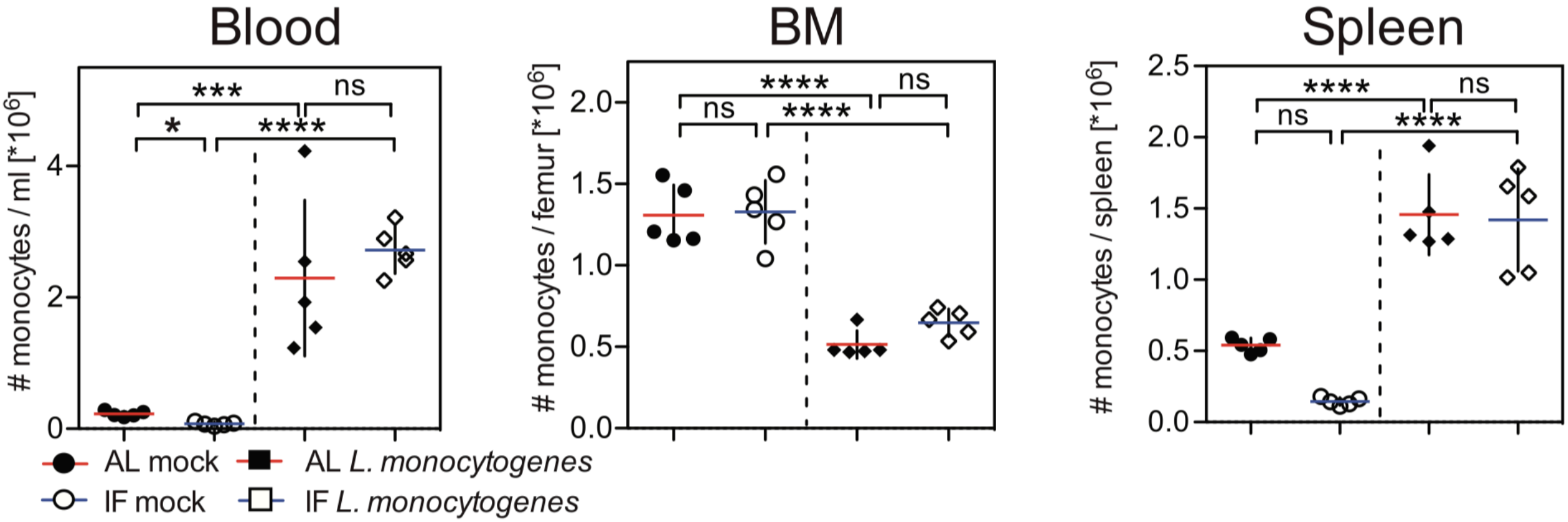



Proportion of IBA1+ myeloid cells in spinal cords

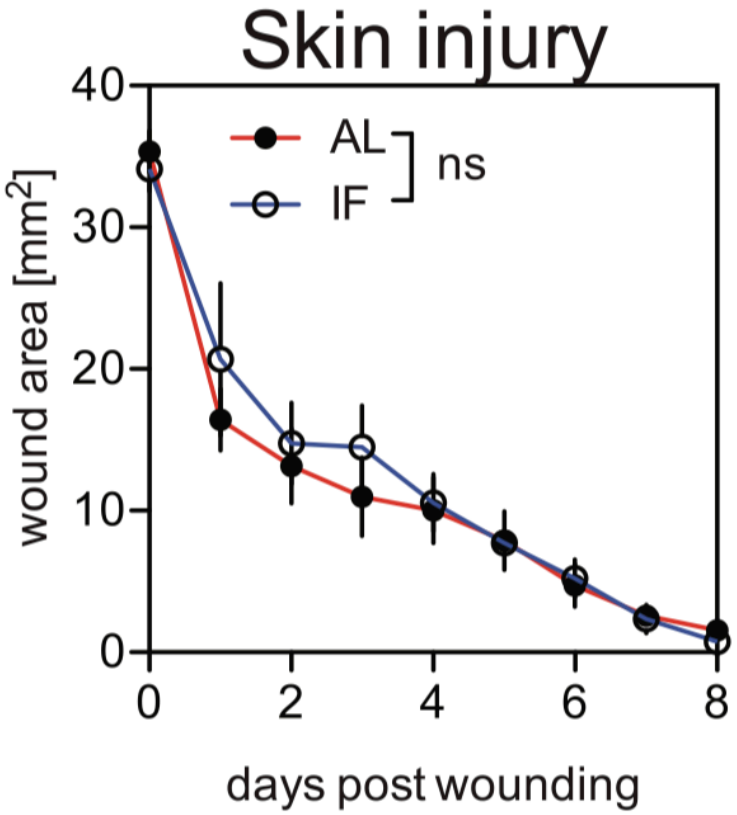
# Intermittent fasting did not affect monocyte emergency mobilization upon *Listeria monocytogenes* infection

- Clue: tissue restoration after injury and therapeutic immunity against *Listeria monocytogenes* critically depend on monocytes
- AL: mice were fed *ad libitum* 随意 ; IF: mice were subjected to intermittent fasting



# Intermittent fasting also did not affect wound repair potential compared to *ad libitum* fed mice

- Clue: mobilization of monocytes to the site of injury is critical for wound repair
- AL: mice were fed *ad libitum* 隨意 ; IF: mice were subjected to intermittent fasting




Note: Every dot represents one individual animal. Horizontal bar = mean. Vertical bar = SD.

# Summary

Reduction of circulating monocytes in fasting mice ⇨ Egress from the bone marrow to the blood circulation ⇨ Cellular energy levels controlled the blood circulating monocyte pool

---

⇨ AMPK activation led to reduction of blood peripheral monocytes ⇨ The AMPK-PPAR$\alpha$-CCL2 axis

---

⇨ Fasting improves chronic inflammation without compromising monocyte emergency mobilization during acute inflammation

***What's next?***

# Discussion

- **The Authors selected AMPK** to study because AMPK is a key cellular energy sensor triggered by an increase in the cellular AMP/ATP ratio that reflects low energy levels
  - How about **SIRT1 and other sirtuins**?
    - "SIRT1 is activated in response to changes in the energy status to promote transcription of genes that mediate the metabolic response to stress, starvation or calorie restriction."
  - What are the effects of fasting on ageing?
  - Other nutrient sensors, e.g., insulin, mTOR, …
-
- It is about cellular energy, so what's **the role of mitochondria in fasting**?

Fulco, M., and Sartorelli, V. (2009). Comparing and Contrasting the Roles of AMPK and SIRT1 in Metabolic Tissues. Cell Cycle *7*, 3669–3679.

# Discussion

- **Metformin activates AMPK pathway**, so will the use of metformin also lead to reduced monocyte egress from the bone marrow to the blood circulation?
  - the authors used phenformin in this study

- The influence of fasting on the **microbiome** as well as the interaction between microbiome and immunity

- Is there any "**epigenetic memory**" after fasting, e.g., regulation of CCL2's expression?

# Thank you!