Journal Club

Stress-Induced Metabolic Disorder in Peripheral $CD4^+$ T Cells Leads to Anxiety-like Behavior

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Article

Stress-Induced Metabolic Disorder in Peripheral CD4+ T Cells Leads to Anxiety-like Behavior

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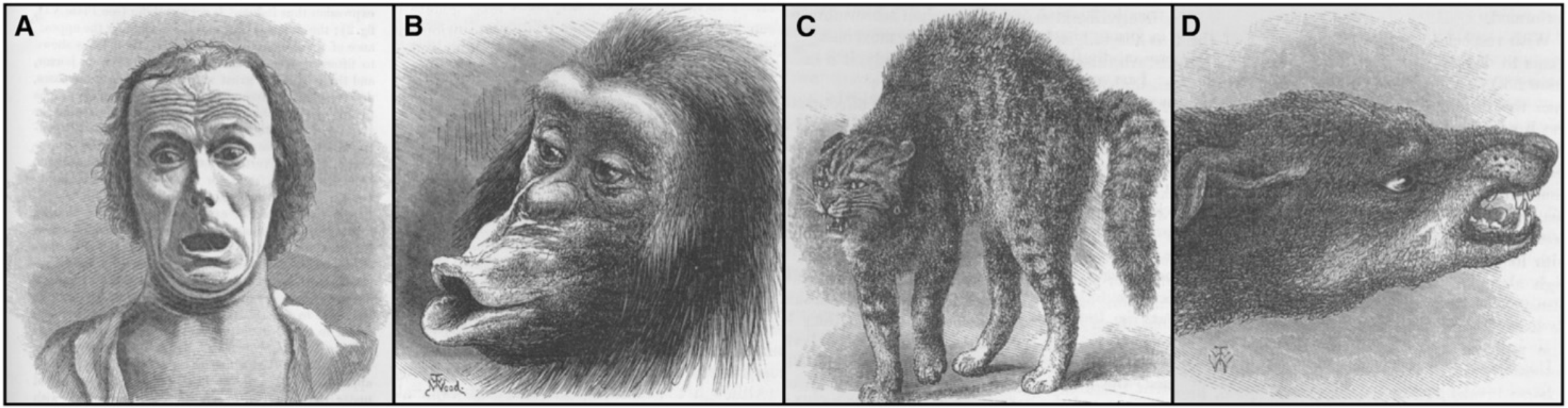
Research Molecular immunology

Innate/adaptive immunity and inflammation

Emotion

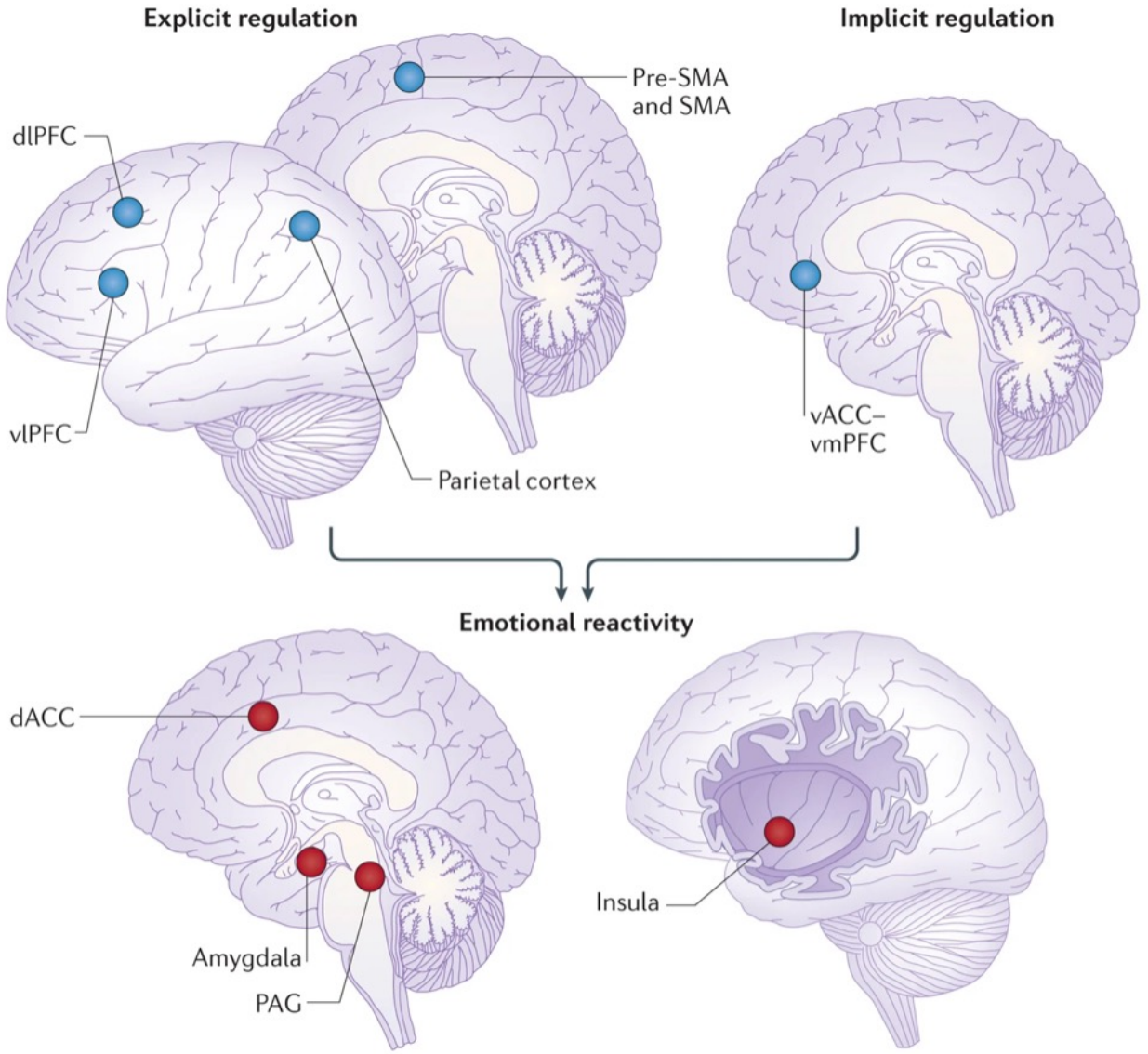


A) Expression of terror in a human. (B) Chimpanzee ‘‘disappointed and sulky.’’ (C and D) hostility in a cat (C) and a dog (D).From Darwin (1872).

Emotions are psychological states brought on by neurophysiological changes, variously associated with thoughts, feelings, behavioural responses, and a degree of pleasure or displeasure. There is currently no scientific consensus on a definition.

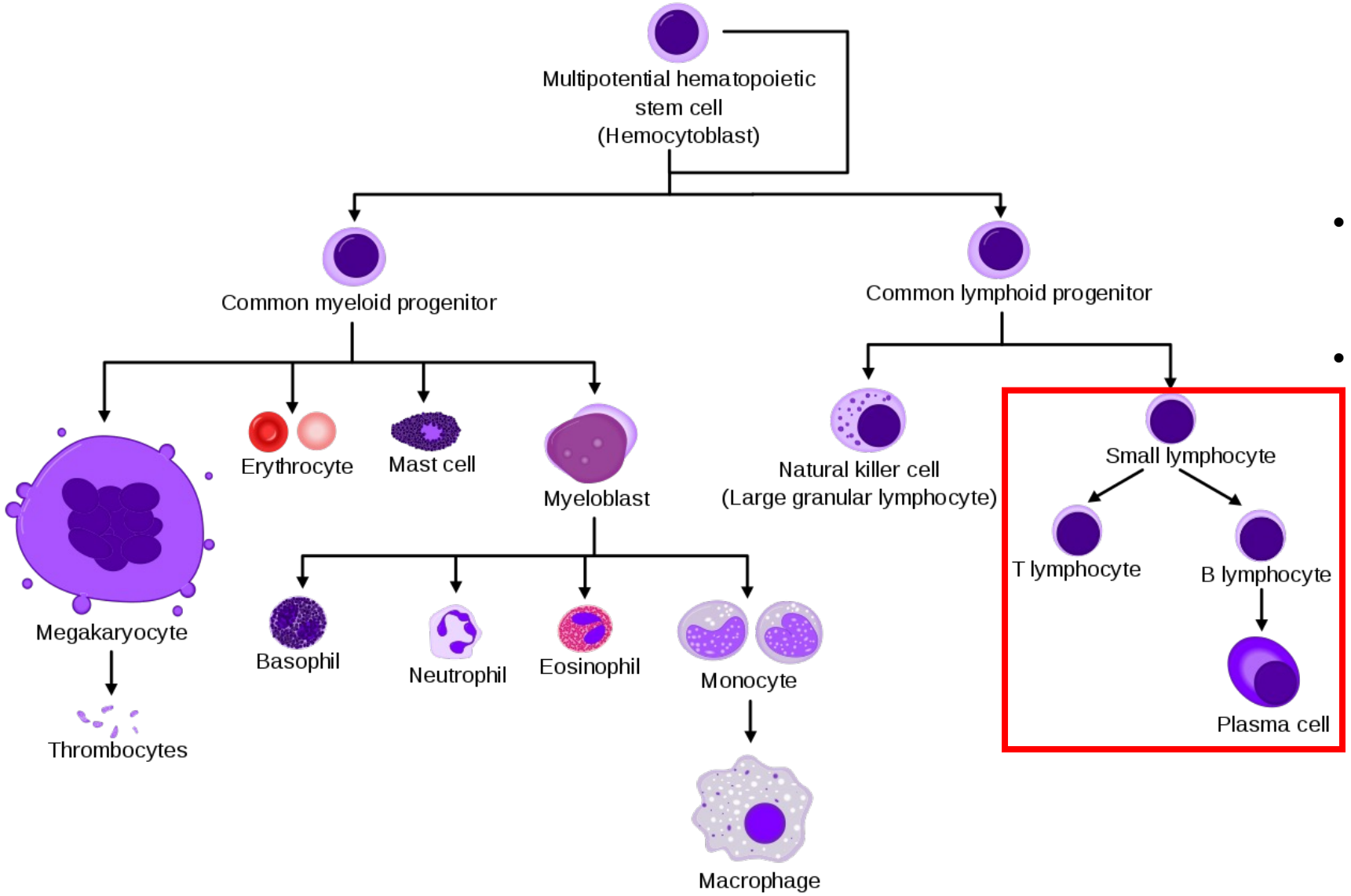
David J. Anderson and Ralph Adolphs, Cell (2014)
Wiki

Brain regions implicated in emotion regulation



Amit Etkin et al., Nature reviews neuroscience (2015)

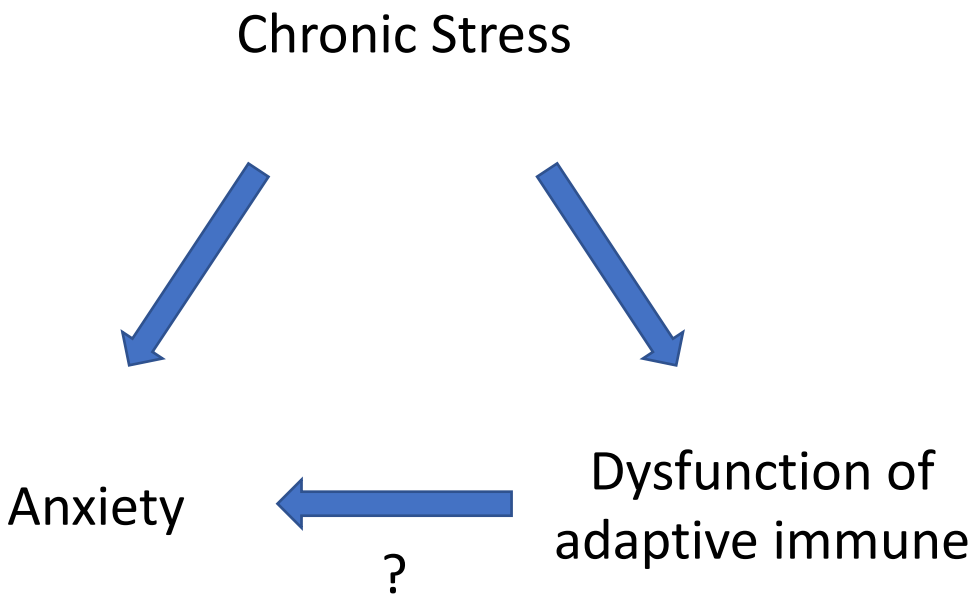
Innate and adaptive immunity



- RAG1 is important for maturing of T cells and B cells.
- $RAG1^{-/-}$ induces immunodeficient (adaptive).

Wiki

Scientific questions



Multiple questions regarding the effect of T cells on the onset of anxiety remain to be answered:

(1) The physiological functions of T lymphocytes in stress-induced anxiety-like behavior

(2) Whether stress-induced anxiety is dependent on the activation of peripheral T cells or not

(3) The imprinting characteristics of pathological T cells in mood disorders

(4) The molecular mechanism by which pathological T cells regulate the activity of neuronal or nonneuronal cells in the CNS.

- Figure 1. $CD4^+$ T Cells Play an Essential Role in Stress-Induced Anxiety-like Behavior
- Figure 2. Stress Causes Metabolic Disorder and Mitochondrial Fission in $CD4^+$ T Cells
- Figure 3. Sustained Mitochondrial Fission in $CD4^+$ T Cells Induces the Anxiety-like Behavior
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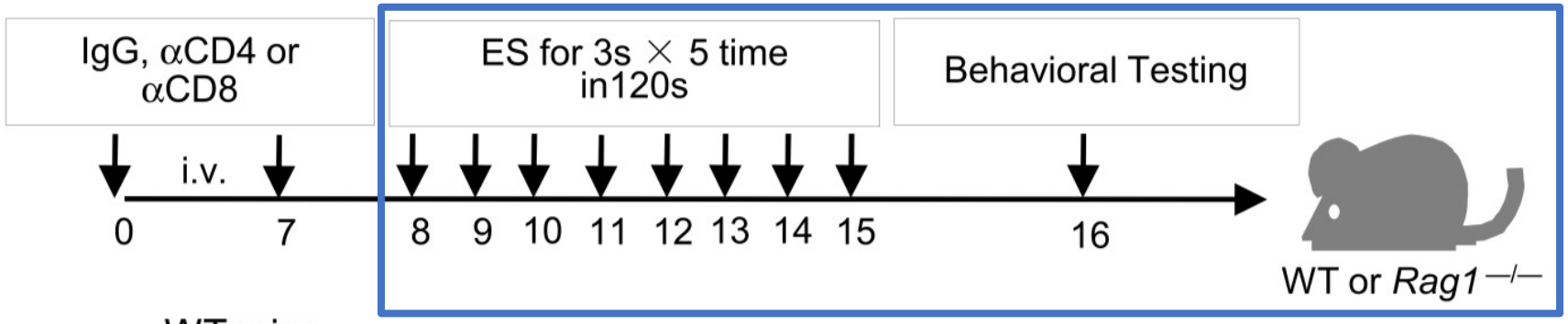
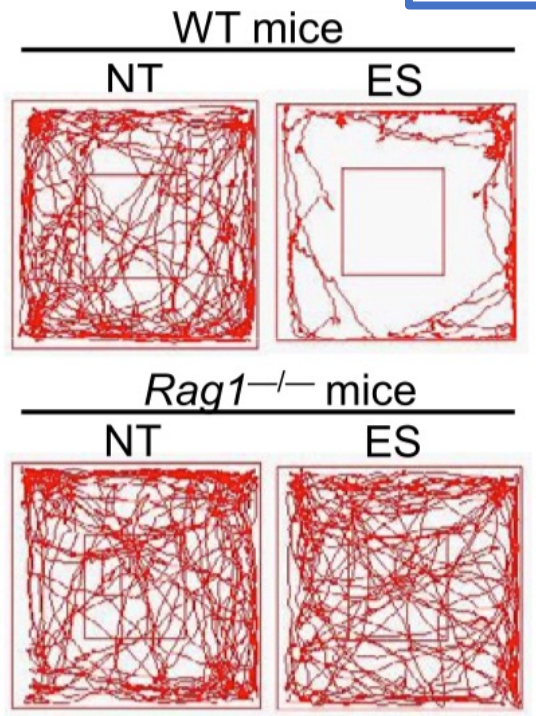
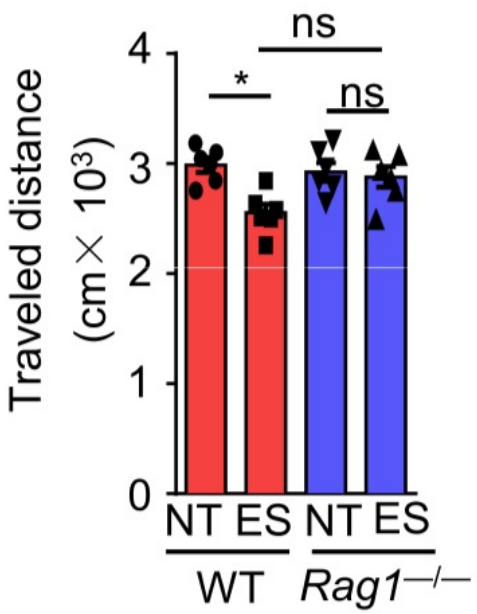
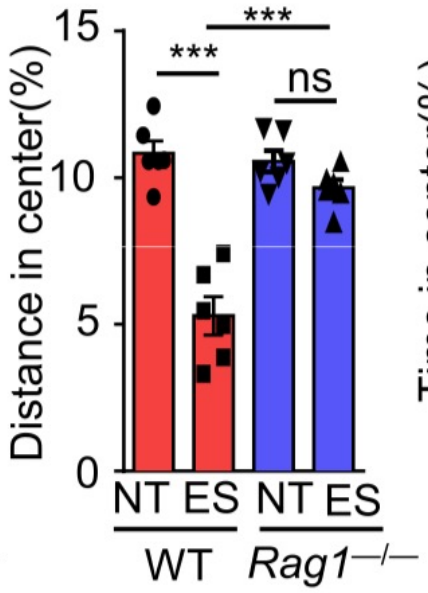
- Figure 5. $CD4^+$ T Cell-Derived Xanthine Acts on the Oligodendrocytes at the Amygdala via AdorA1

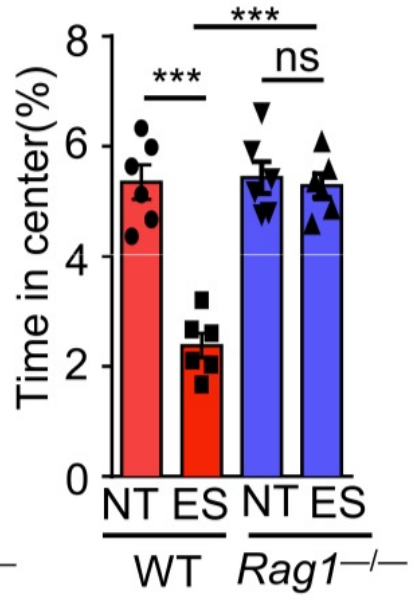
The adaptive immunity is required for the onset of anxiety





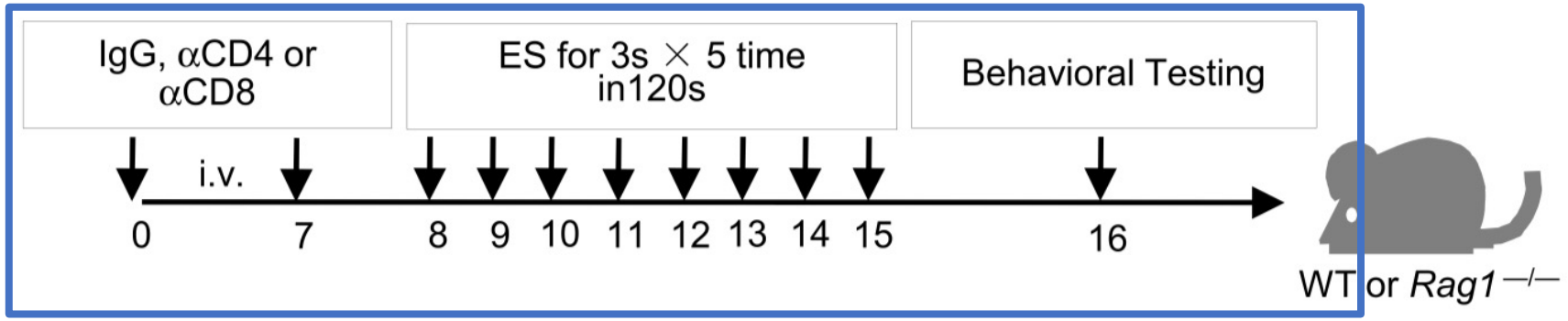


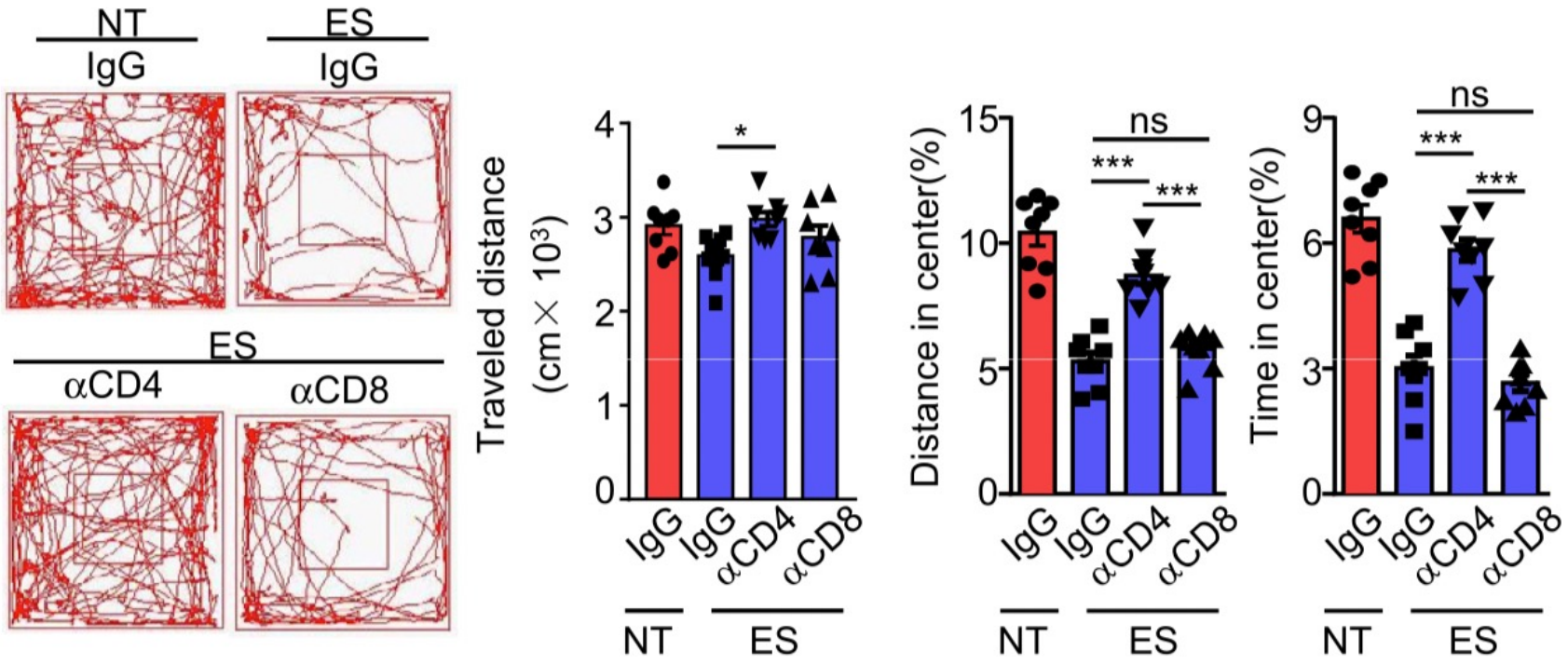




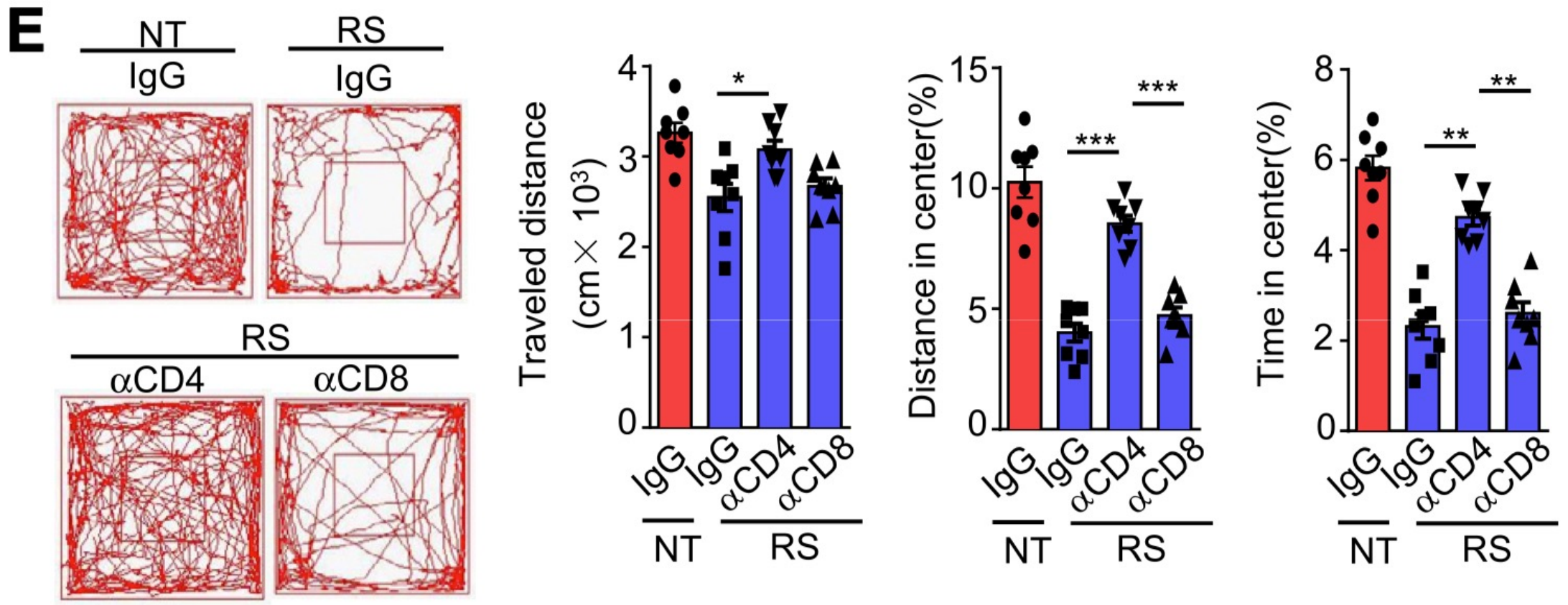
Nontreated (NT), electronic foot shock (ES)

Only CD4⁺ T cell depletion significantly reversed the ES-induced anxiety-like behavior



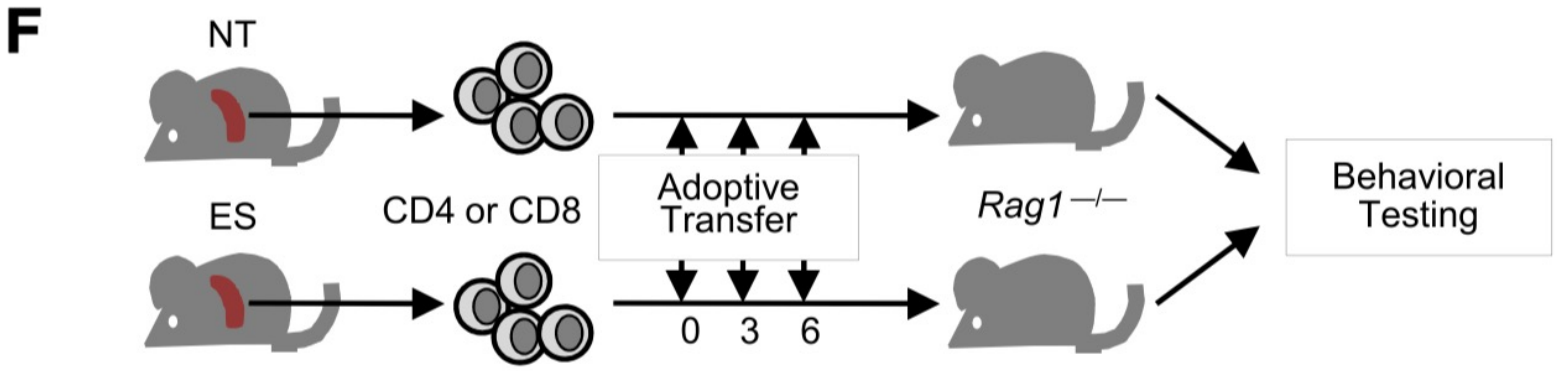


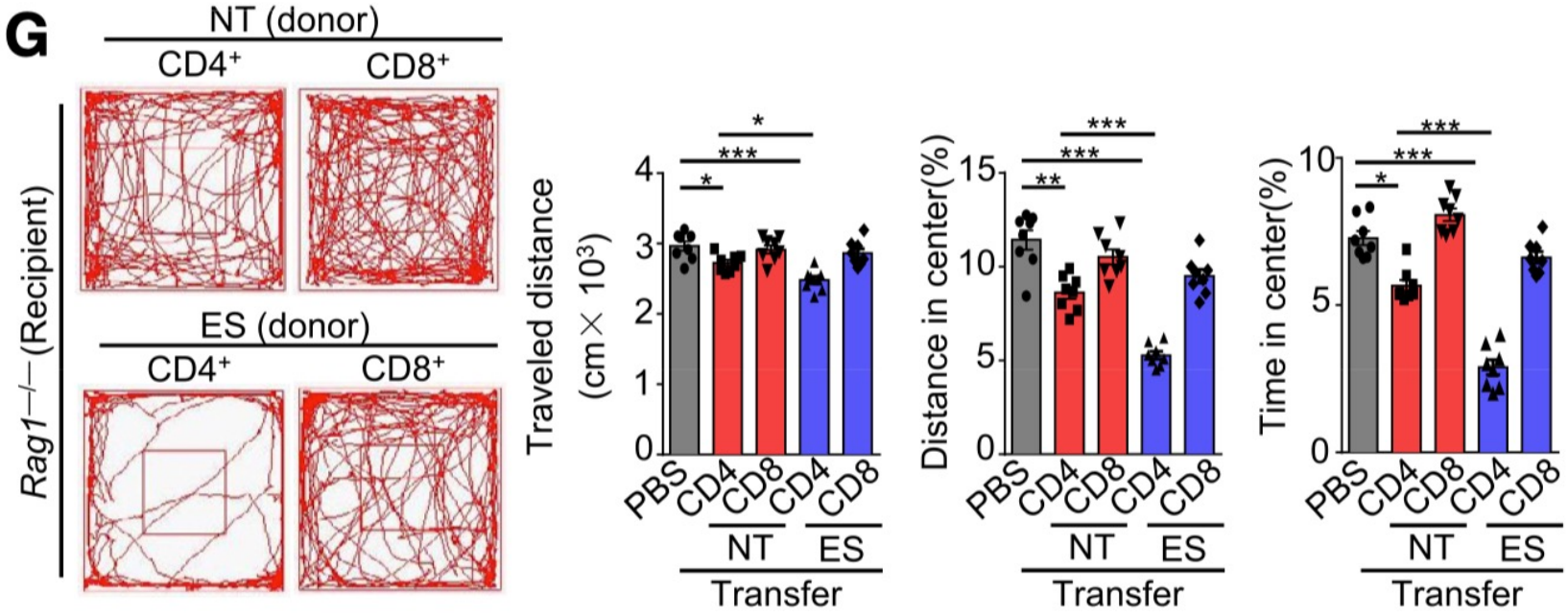
$CD4^+$ T cells have a broad impact on physical stress-induced anxiety-like behavior



restraint stress (RS)

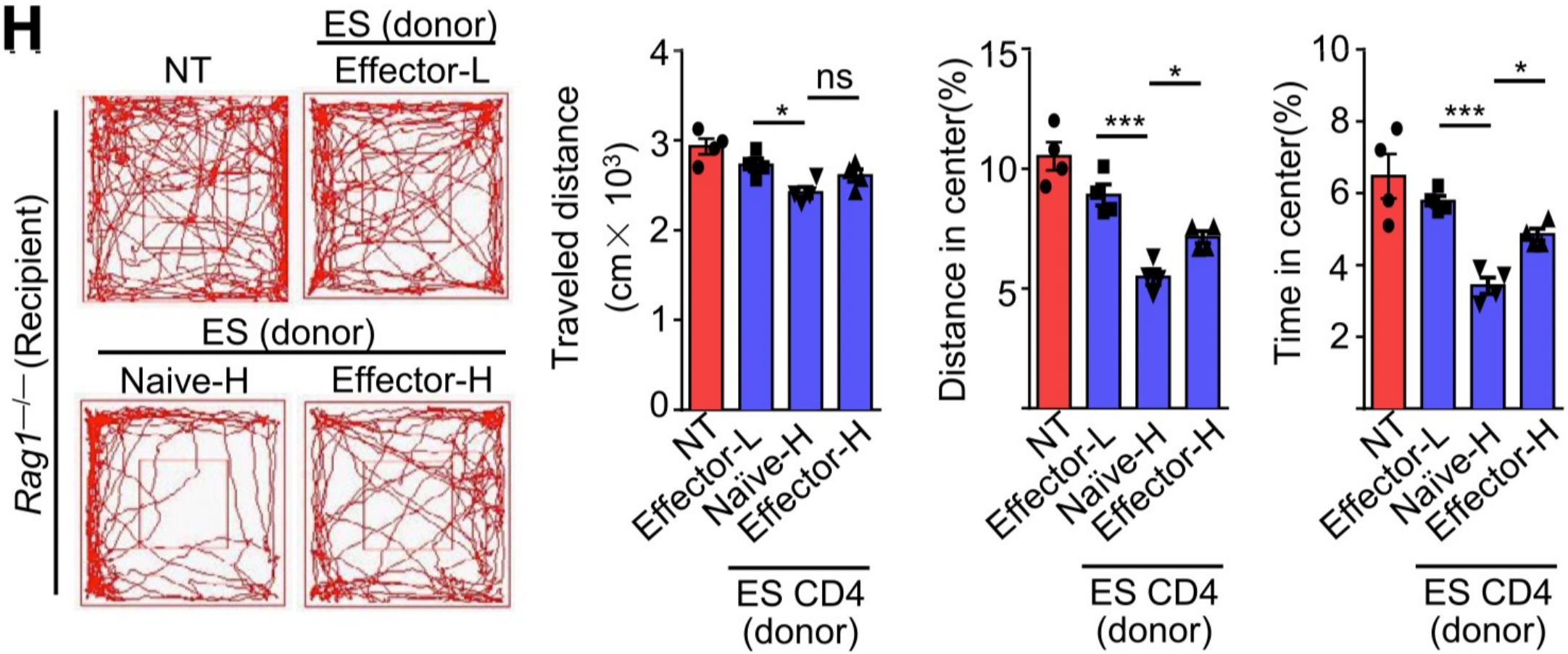
Unstressed mice can develop anxiety-like behavior when receiving stressed CD4⁺ T cells





CD4⁺ T cells can retain anxiety imprints

The pathological CD4^{+} T cells in anxiety exercise their functions mainly by the naïve subtypes



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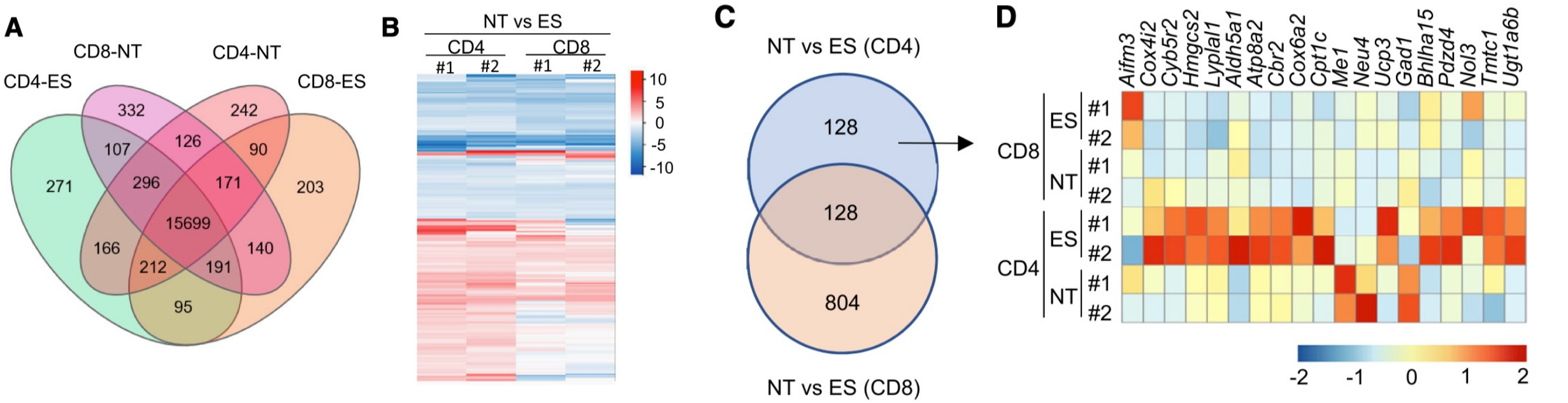
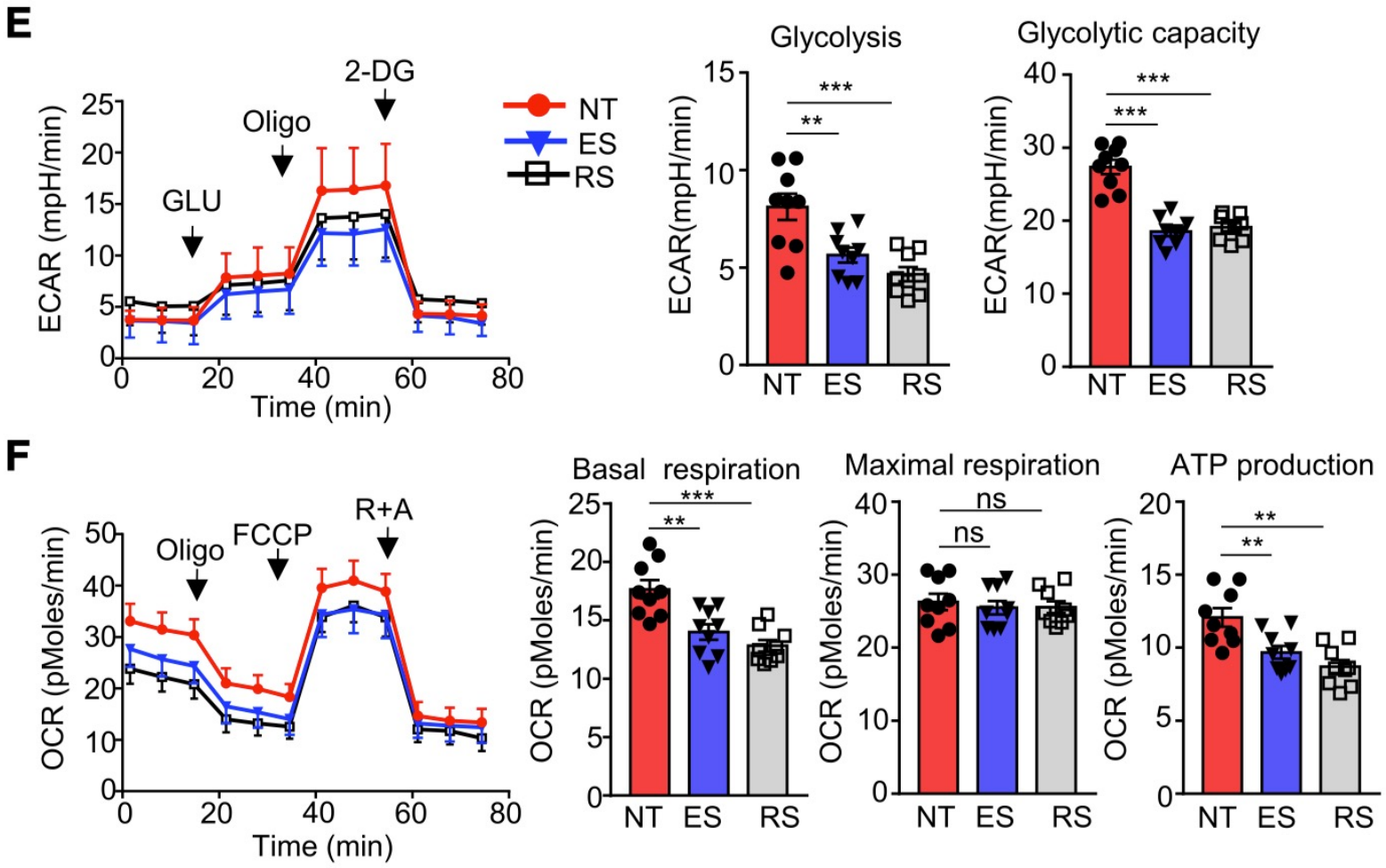
Transcriptomic analysis of naive ES-induced T cells revealed that DEGs encoding mitochondrial proteins were specifically enriched in the CD4 group

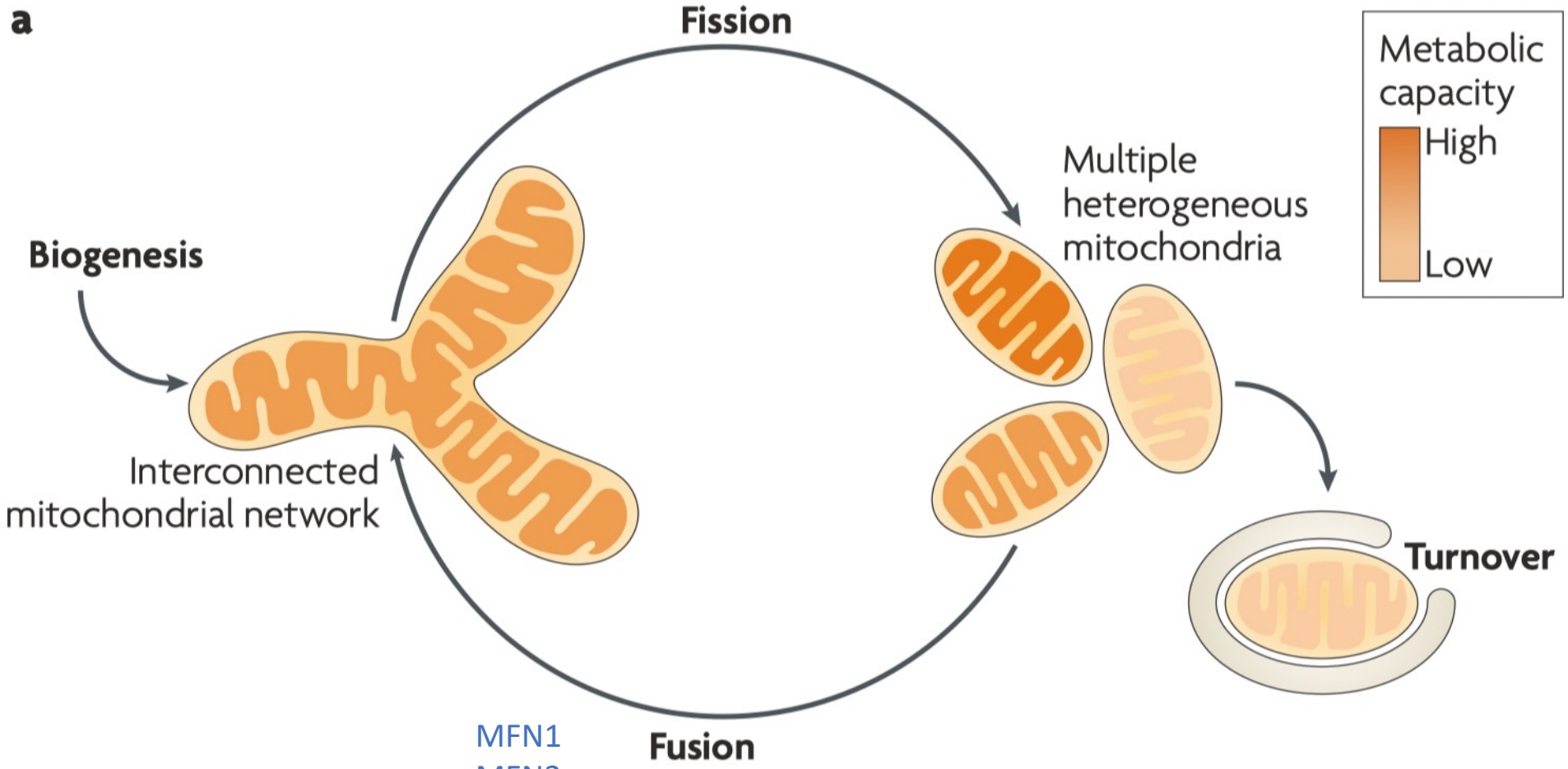


Both ES- and RS-treated naïve CD4$^+$ T cells exhibited severely reduced levels of glycolysis and oxidative phosphorylation



extracellular acidification rate (ECAR)
oxygen consumption rate (OCR)

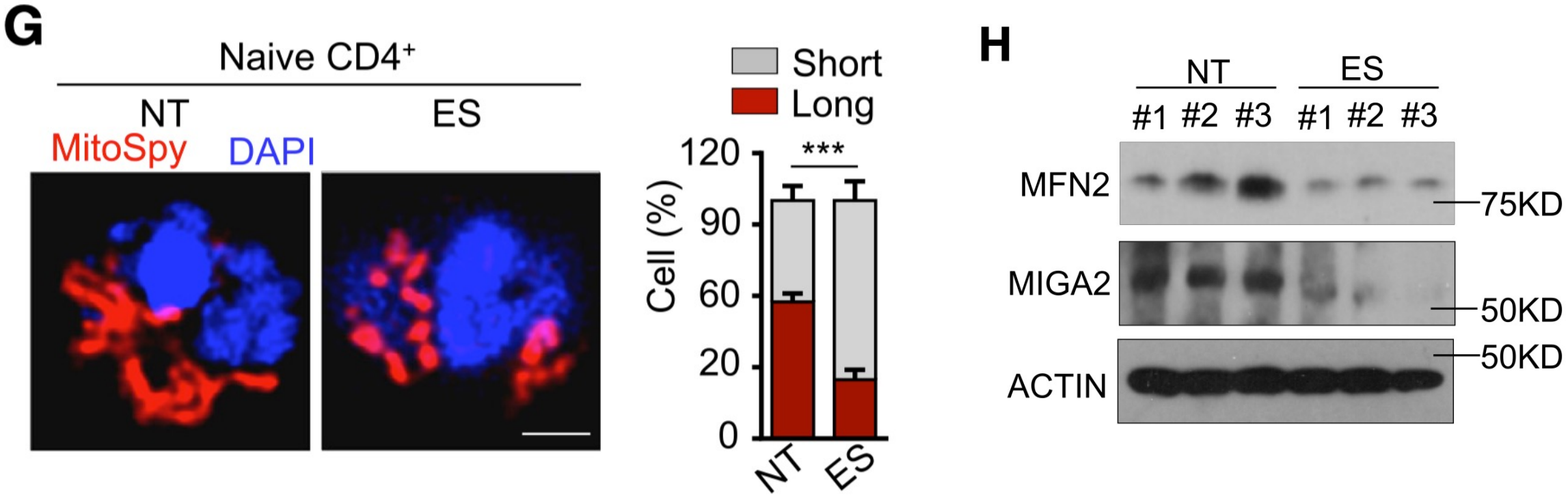
Mitochondrial dynamics: fusion and fission



MFN1
MFN2
MIGA2

Benedikt Westermann, Nature Reviews Molecular Cell Biology (2010)

Stress causes mitochondrial fission in CD4$^+$ T Cells

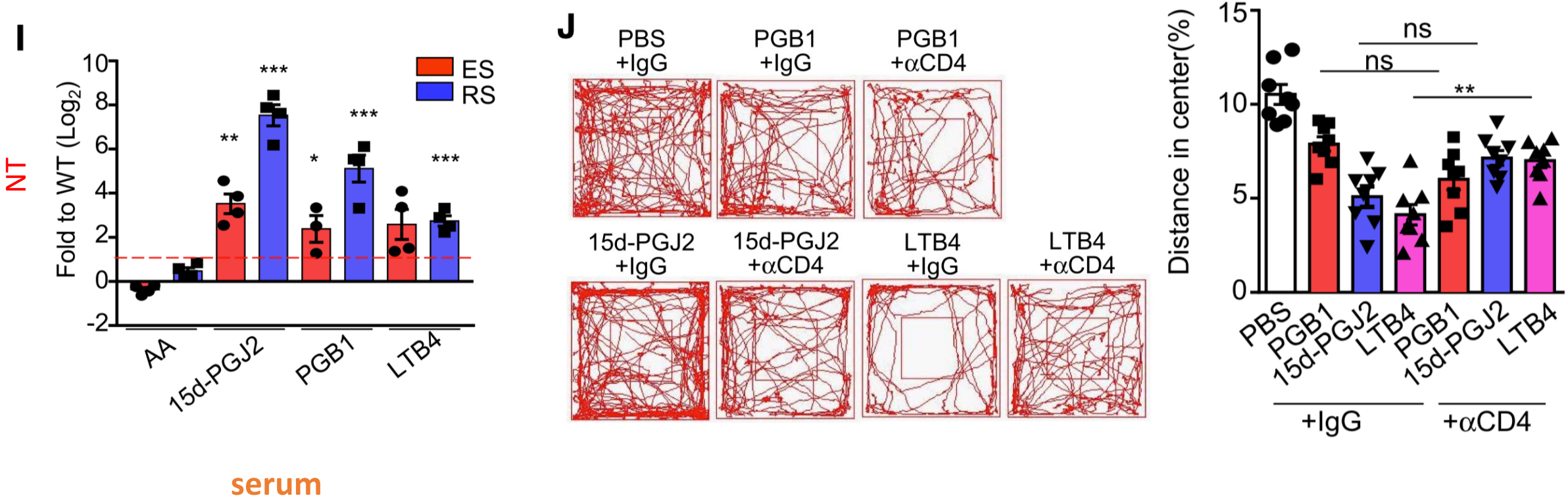


Mitochondrial fusion proteins

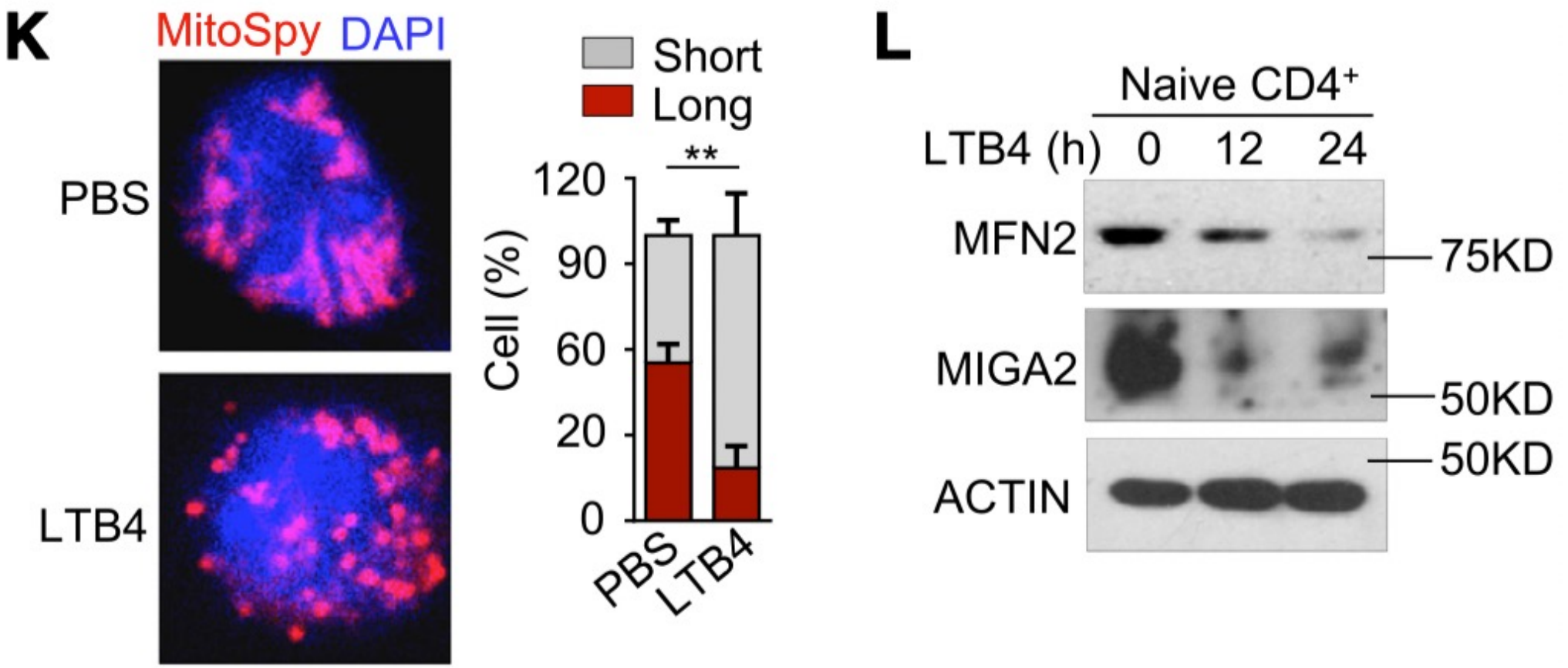
May AA metabolites be the upstream regulator of stressed $CD4^+$ T cells?

- Several findings have demonstrated that depressive animals exhibit increases in omega-6 fatty acids and arachidonic acid (AA) in the brain.
- AA is known to be a critical component of the inflammatory process via metabolism into leukotriene B4 (LTB4) and prostaglandin (PG) E2, which may further act on peripheral lymphocytes.

LTB4 administration caused a severe anxiety-like behavior, which was restored by CD4^{+} T cell removal



LTB4 significantly promoted mitochondrial fission in vitro and reduced MFN2 and MIGA2 expression in naïve CD4⁺ T cells



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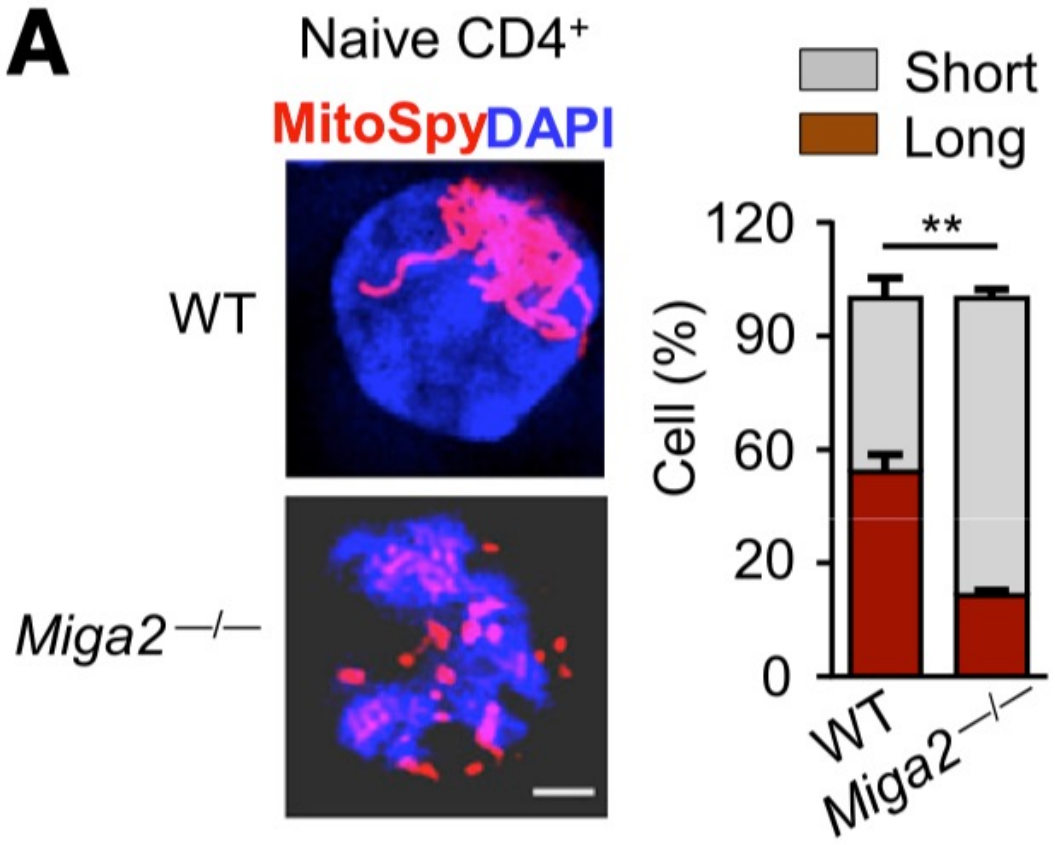
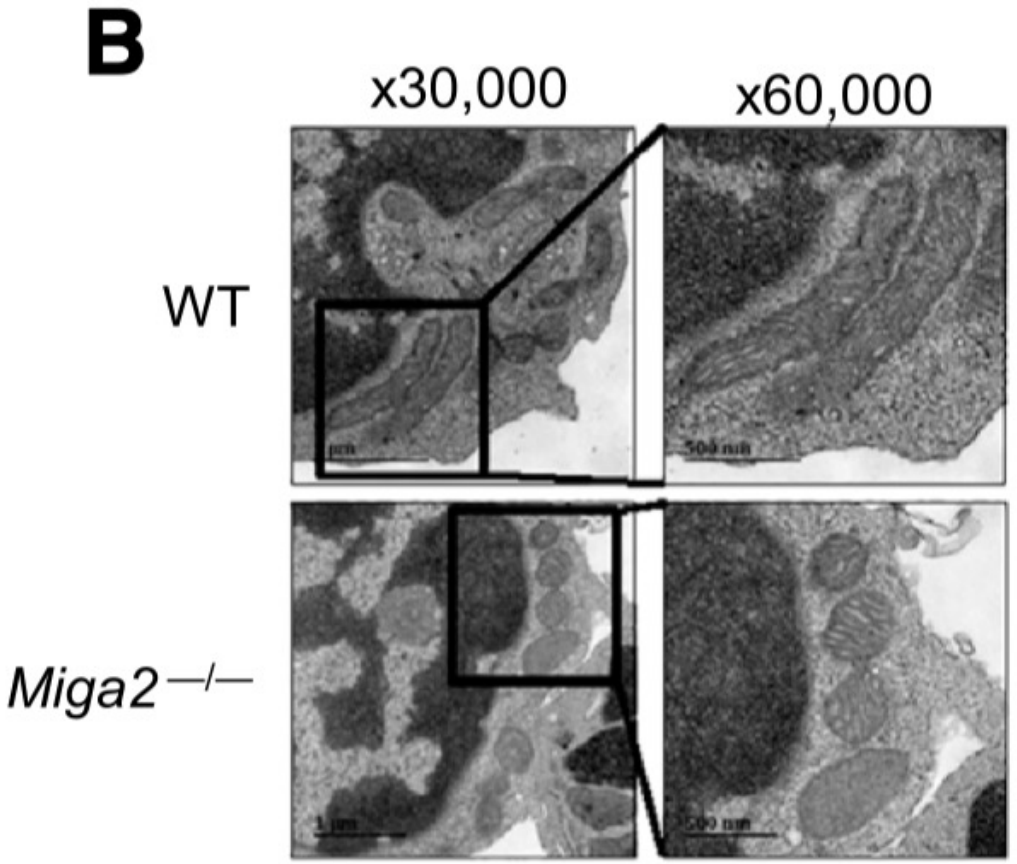
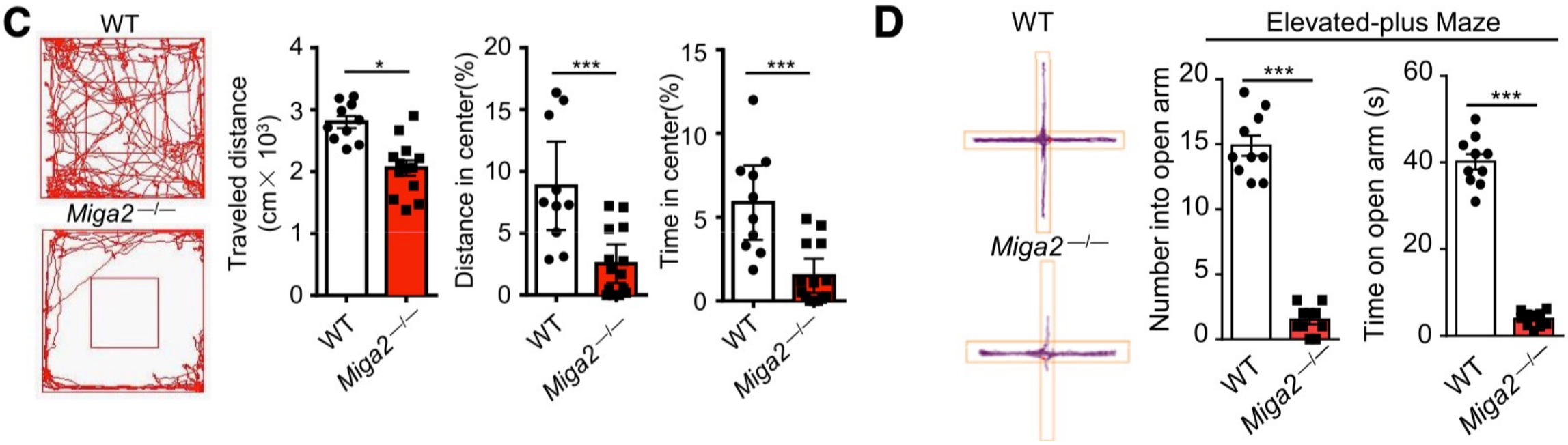
To confirm the relationship between the mitochondrial morphology of T cells and anxious behavior, they generated Miga2 KO mice and observed highly fragmented mitochondria in naive CD4⁺ T cells



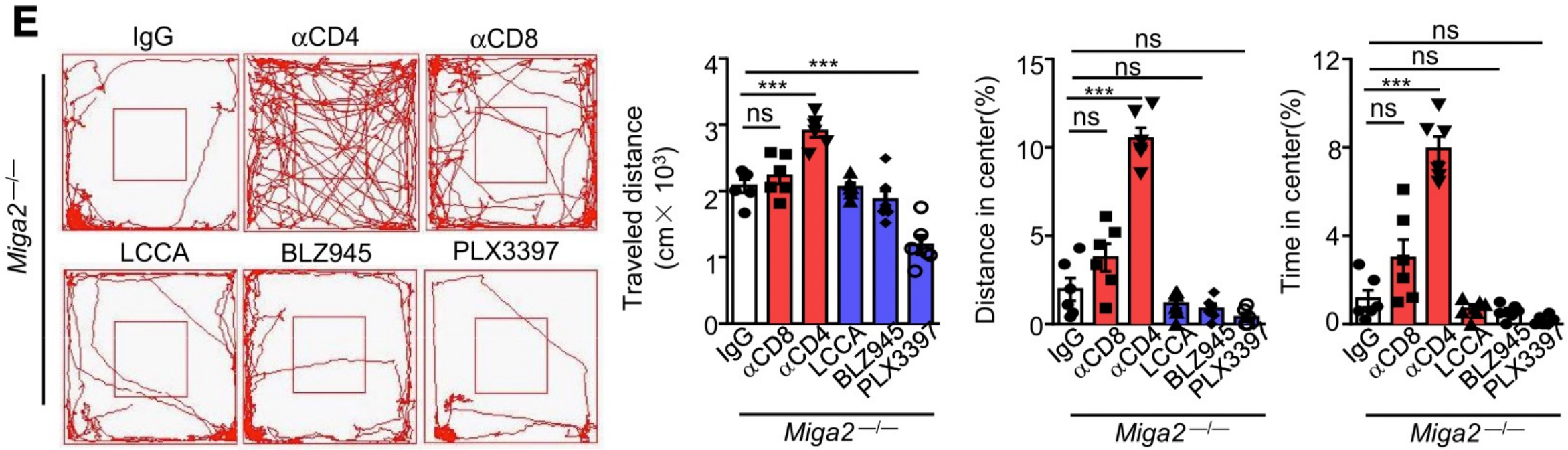


electron microscopy

Miga2 KO mice develop severe anxiety-like symptoms

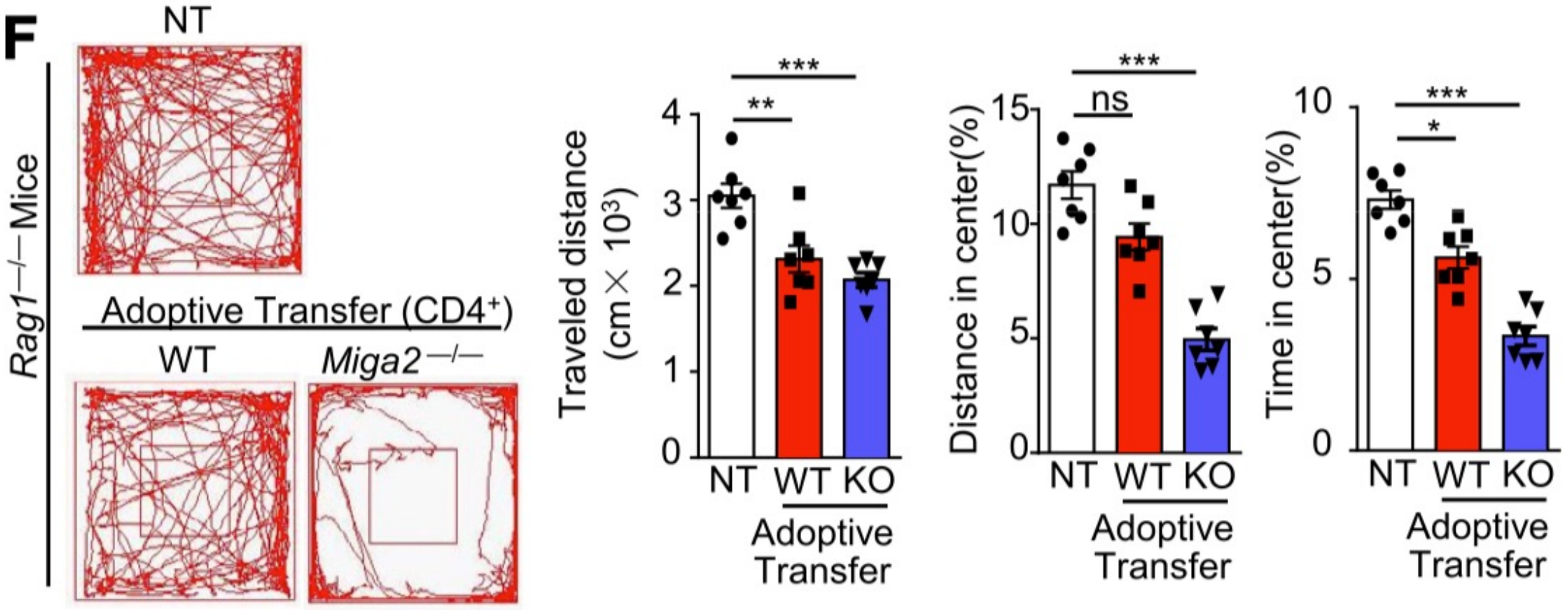


Depletion of CD4⁺, but not CD8⁺, T cells restored the anxiety symptoms caused by continuous mitochondrial division

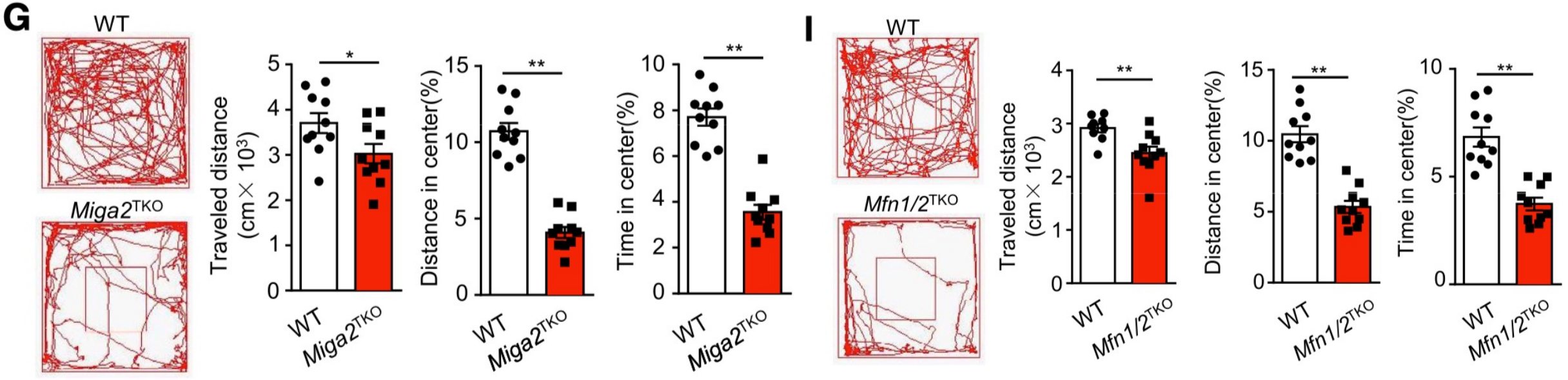


- Selectively eliminating myeloid cells by LCCA, PLX3397, or BLZ945.
- The results indicate that anxiety-like behavior caused by mitochondrial division is dependent on CD4⁺ T cell and independent on myeloid cells

Recipient Rag1$^{-/-}$ mice adoptively transferred with Miga2-deficient naive CD4^{+} T cells further confirmed the essential roles of these cells in anxiety



Miga2-T cell-conditional knockout (KO) (Miga2^{TKO}) mice also develop anxiety-like phenotypes



Mfn1 and Mfn2 T cell-conditional double KO (Mfn1/2TKO) mice also exhibited anxiety-like behavior, which indicates that anxious behavior is promoted by the morphological disorder of mitochondria rather than by a specific function of certain mitochondrial proteins in CD4$^+$ T cells.

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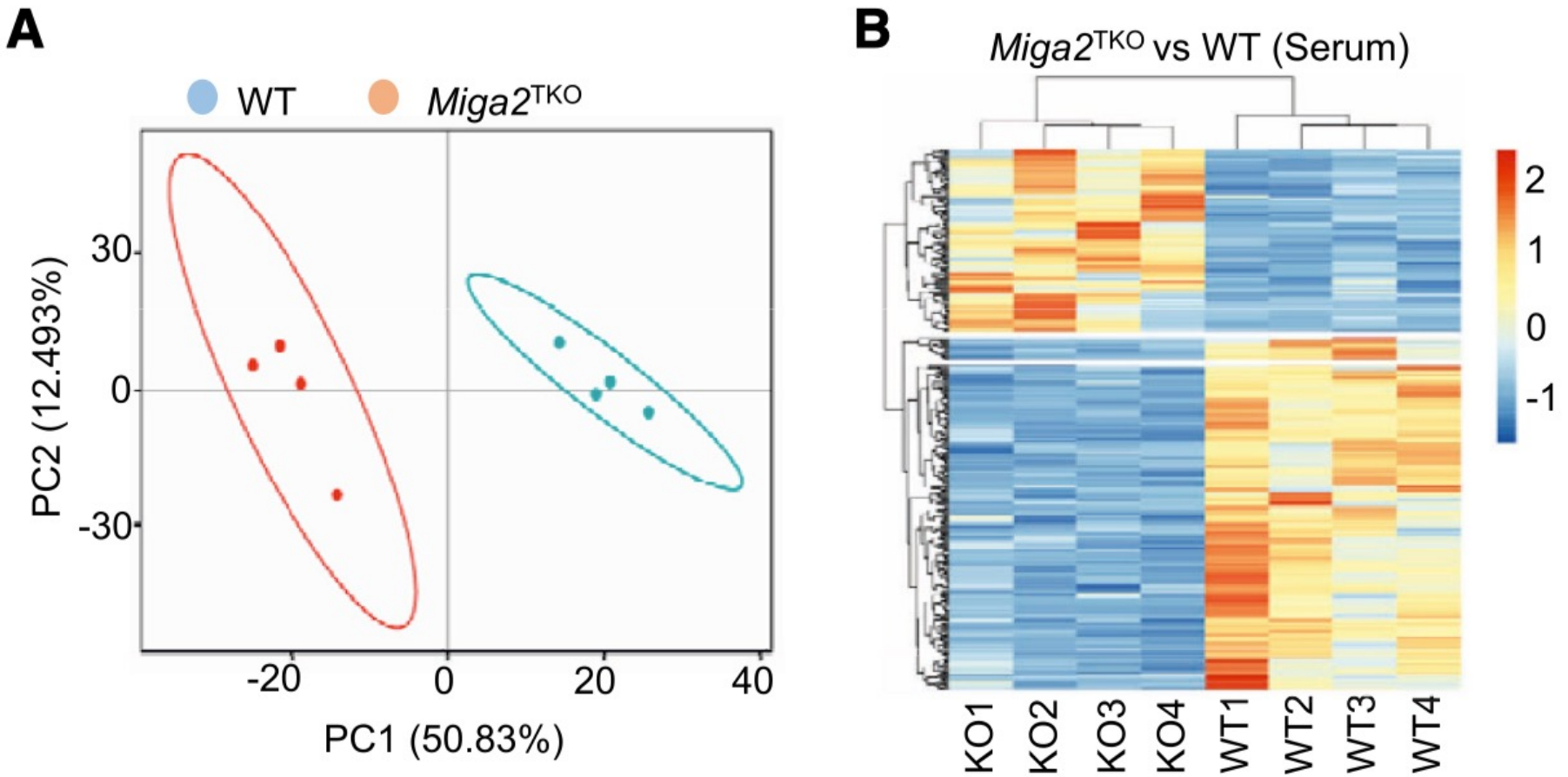
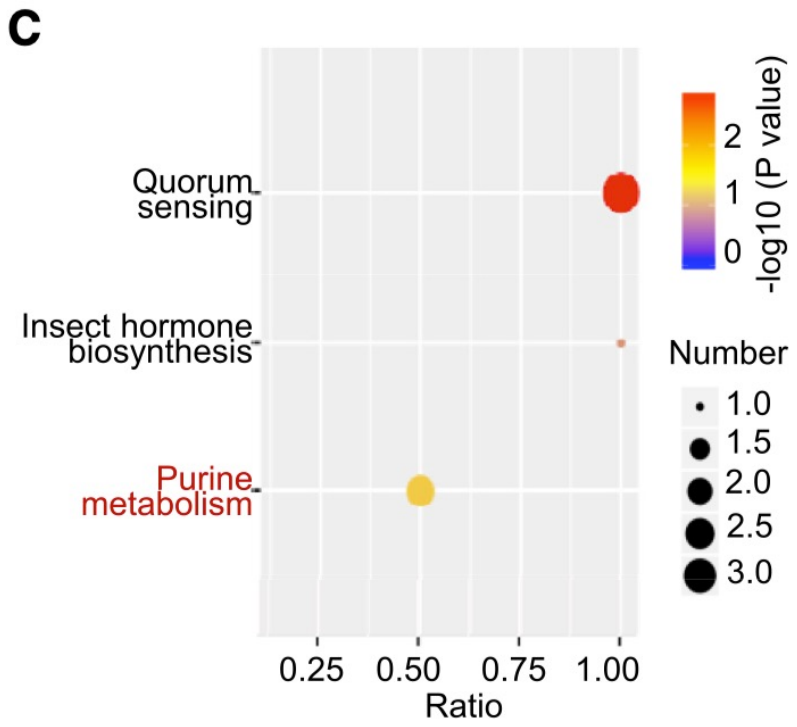
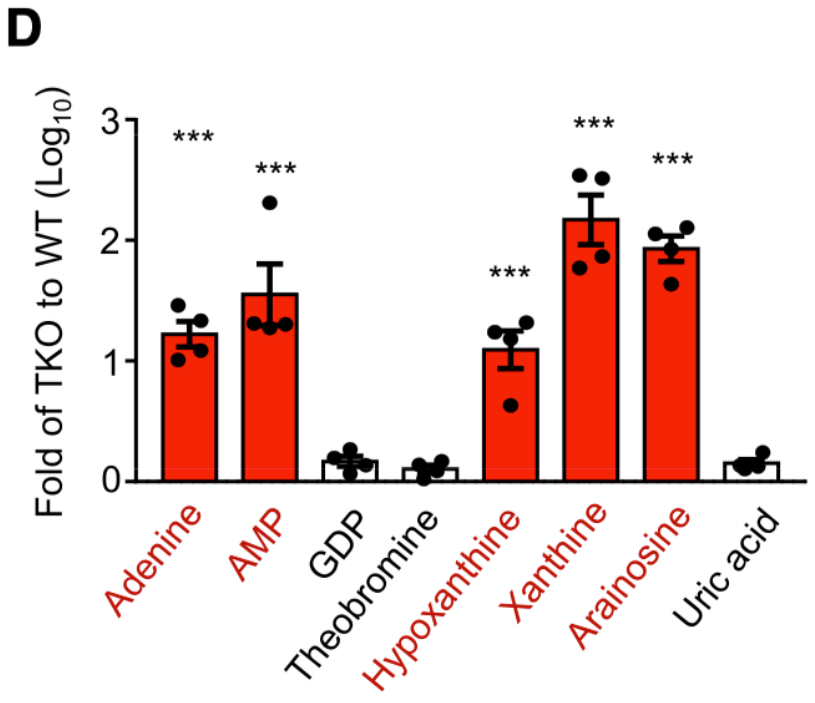
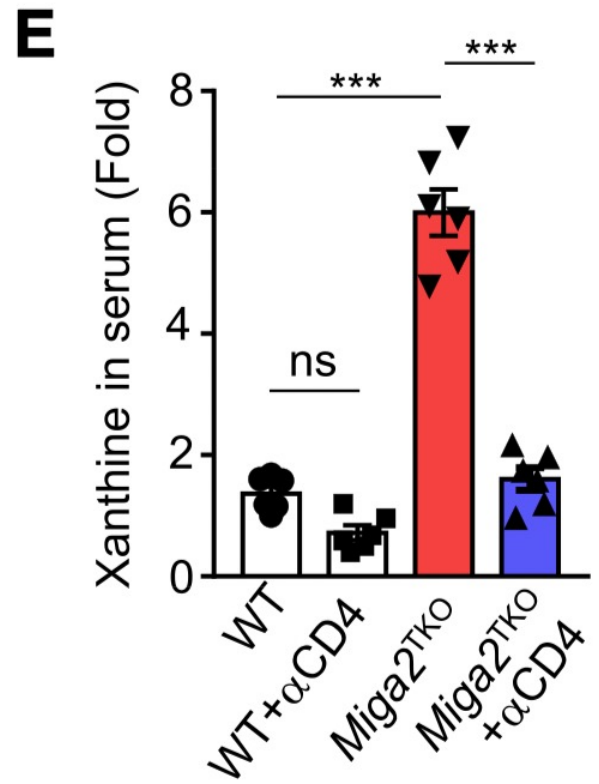
Serum metabolome analysis of Miga2^{TKO} and WT mice



Serum metabolome analysis of Miga2^{TKO} and WT mice indicated that the differential metabolites were mainly enriched in purine metabolism







Serum xanthine was significantly higher in human and mouse with anxiety

Psychoneuroendocrinology
Volume 70, August 2016, Pages 25-32

Purine metabolism is dysregulated in patients with major depressive disorder

Toni Ali-Sisto [a], Tommi Tolmunen [a, b], Elena Toffol [c], Heimo Viinamäki [a, b], Pekka Mäntyselkä [e], Minna Valkonen-Korhonen [a, b], Kirsi Honkalampi [f], Anu Ruusunen [a, b], Vidya Velagapudi [c, d], Soili M. Lehto [a, b]

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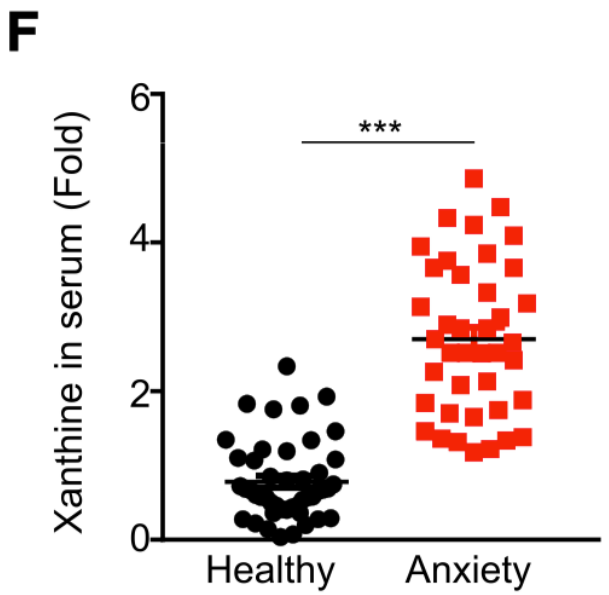
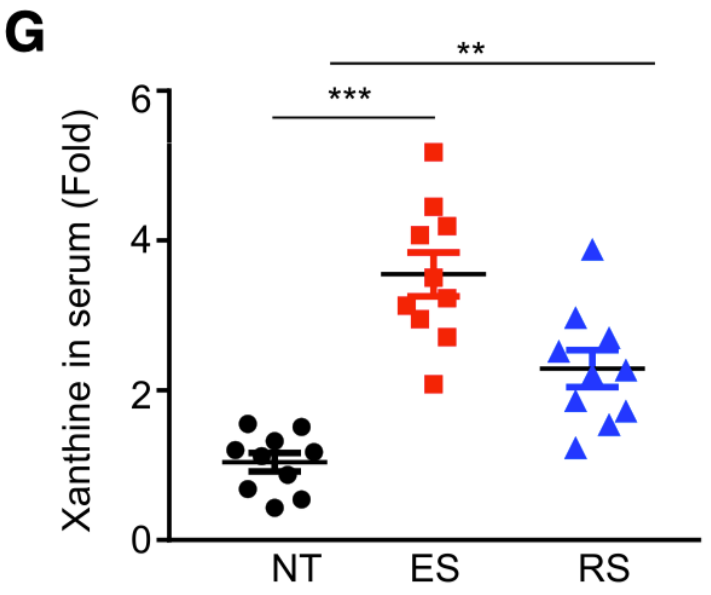
Highlights

- Decreased serum levels of inosine and guanosine were associated with depression.
- Depressed patients also displayed increased serum levels of xanthine
- Purine metabolite alterations did not change during recovery from depression.

human

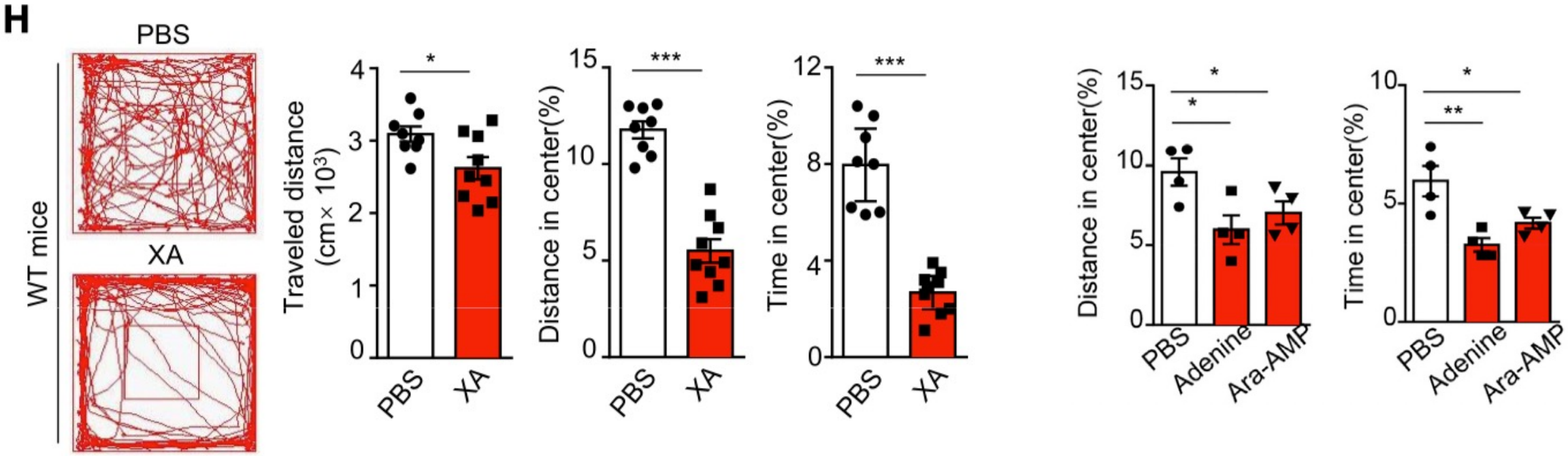


human



mouse

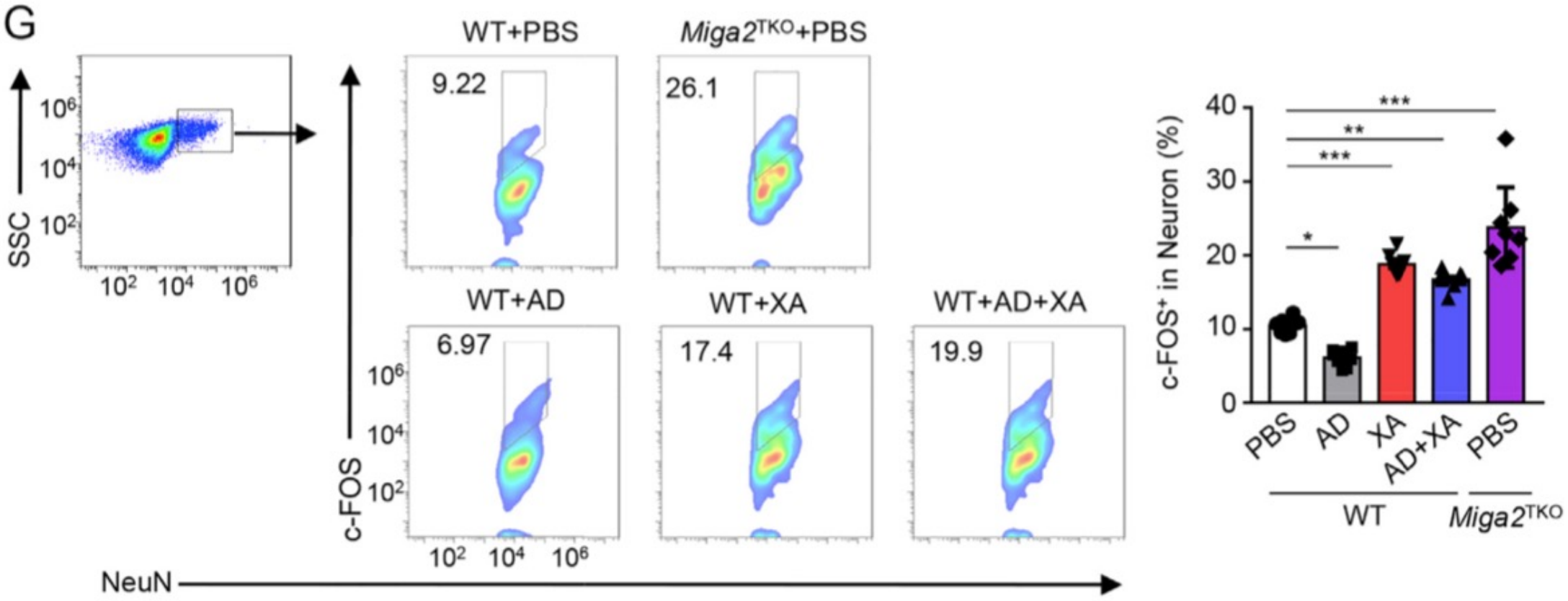
Xanthine, adenine, and adenine arabinoside monophosphate (Ara-AMP) all had the ability to trigger anxiety-like behavior



Other prune, yes

i.p.

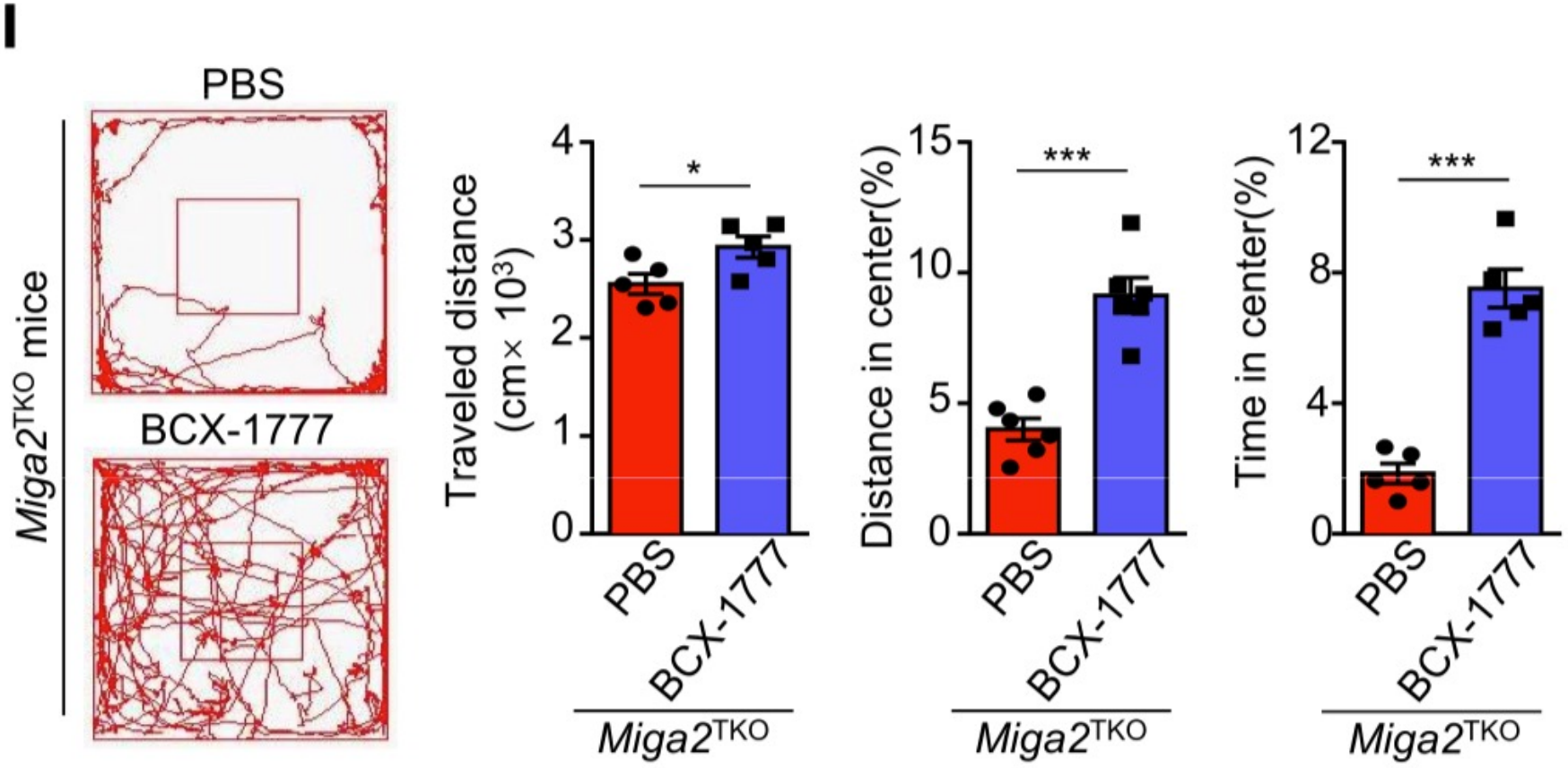
Xanthine plays a more dominant role when the microenvironment contains both of these purines



Expression of c-FOS is an indirect marker of neuronal activity

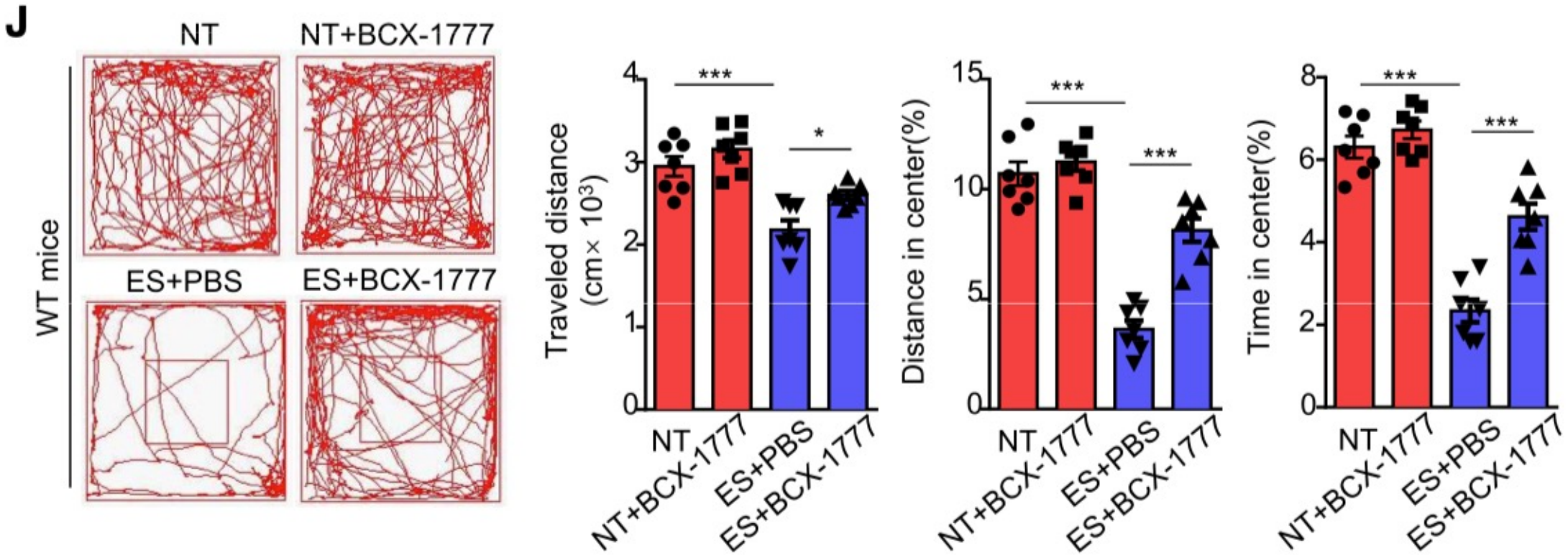
- Mitochondrial division increase c-FOS+ neuron.
- AD and XA have opposite effects on the activity of neurons.
- XA is dominant among purines and is in the same direction with Miga2^{TKO}group.

BCX-1777 treatment significantly reduced the anxiety symptoms in Miga2 TKO and ES mice



BCX-1777 is an inhibitor of purine synthesis

BCX-1777 treatment significantly reduced the anxiety symptoms in Miga2 TKO and ES mice



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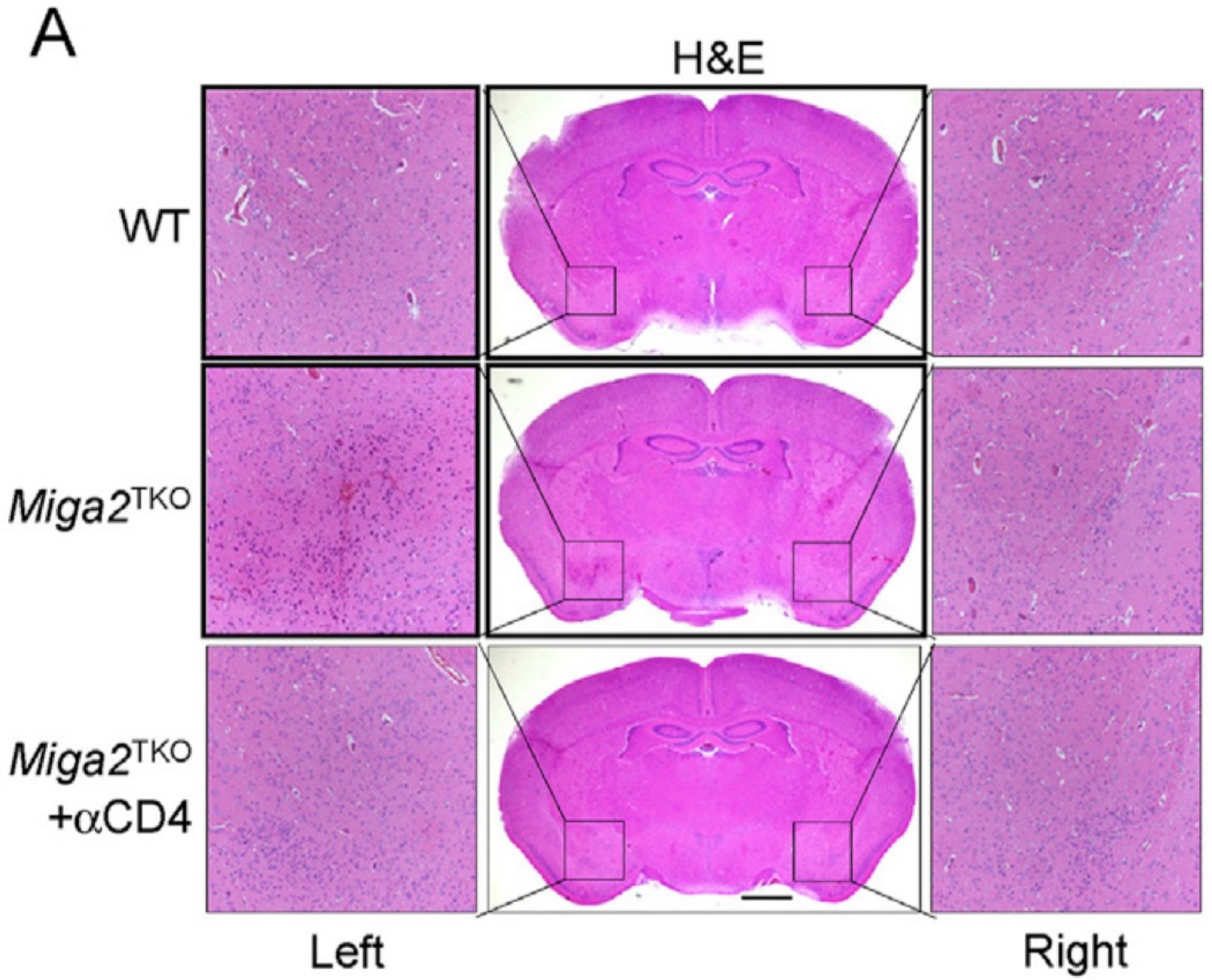
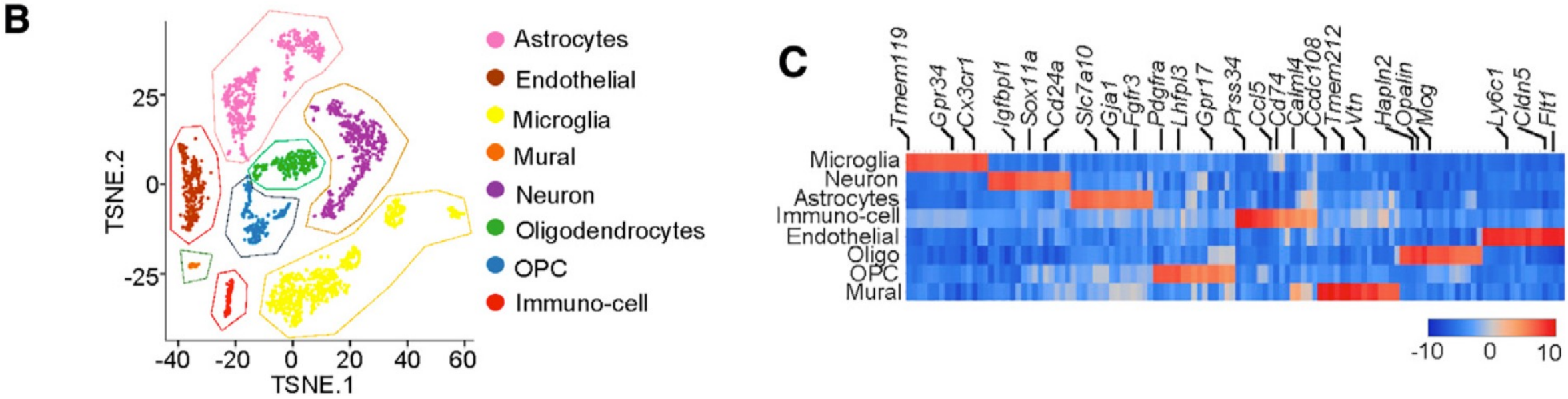
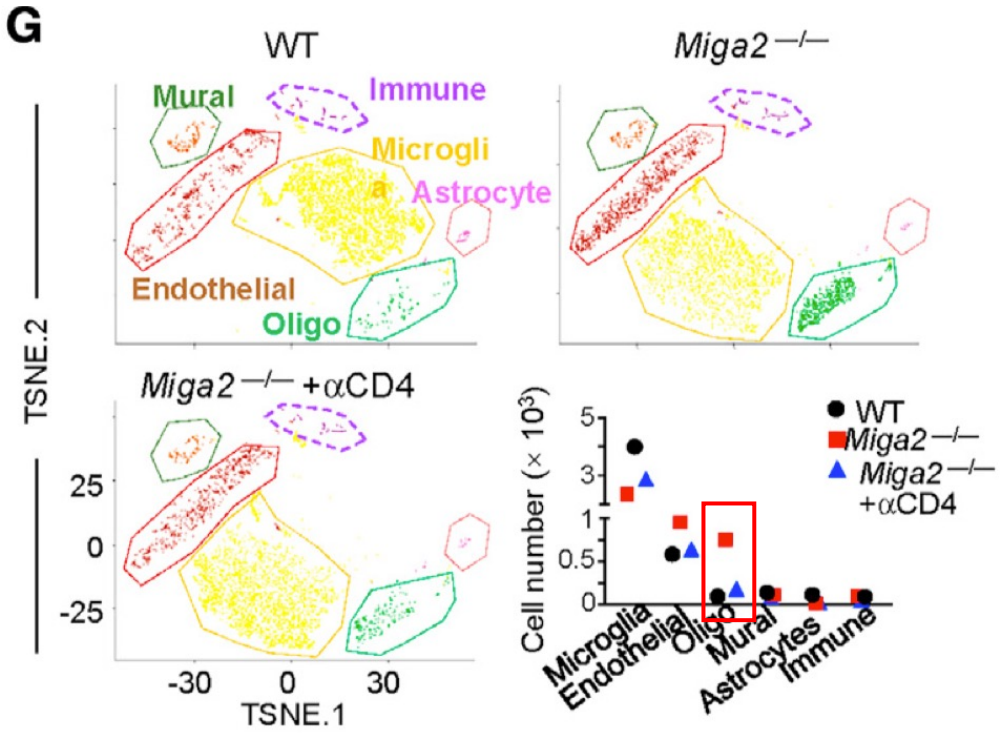
The amygdala of Miga2^{TKO} mice was abnormal in morphology than that of the WT control

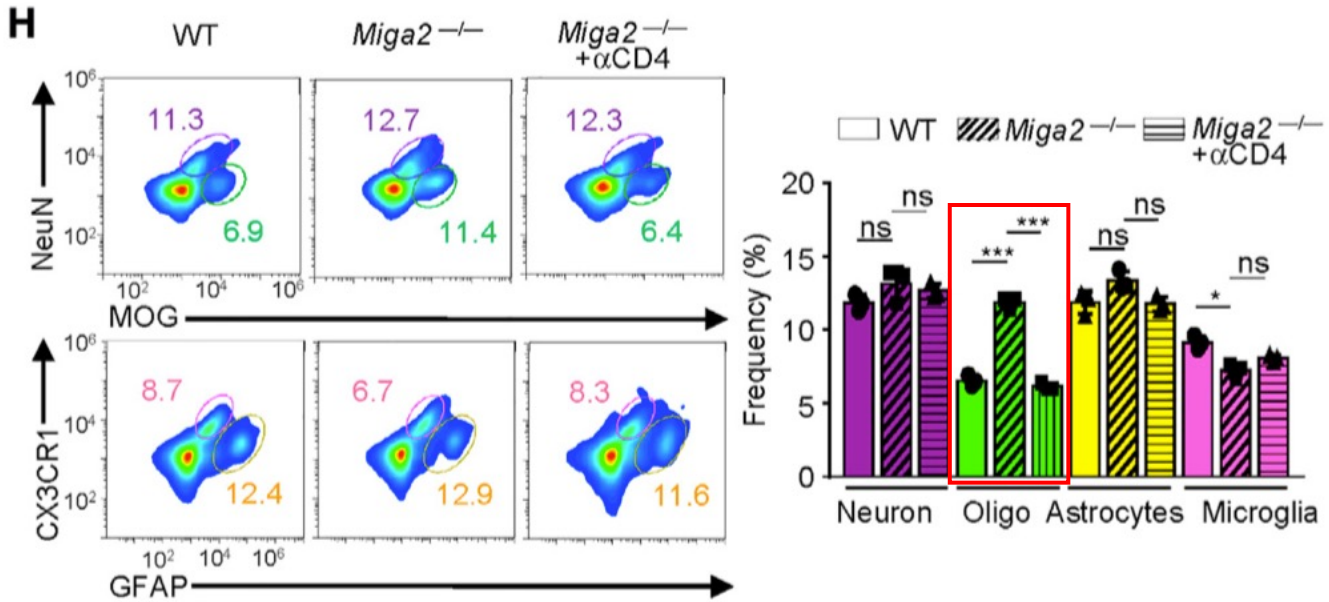


Single-cell RNA sequencing of amygdala



Both scRNA-seq and FACS analysis indicated a significantly increased percentage of oligodendrocytes in Miga2$^{-/-}$ mice, which could be reversed by depleting CD4^{+} T cells

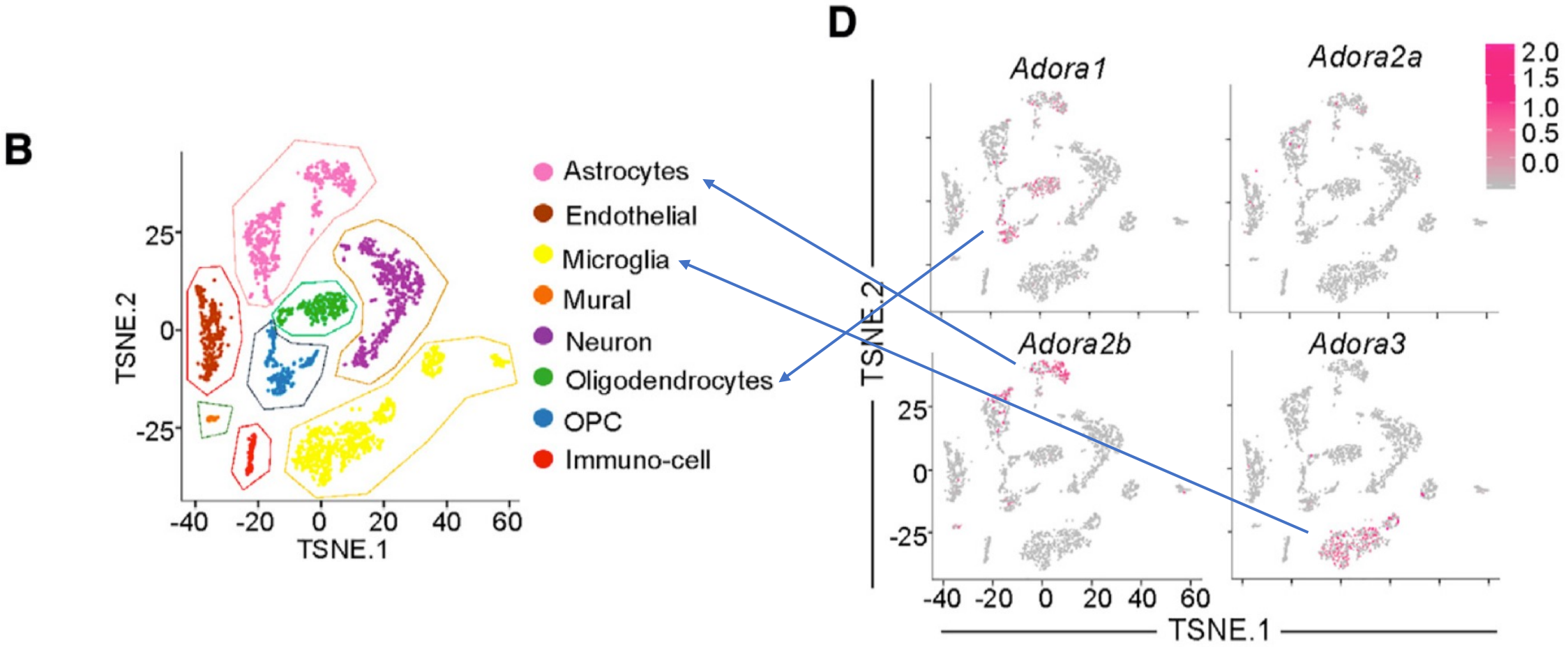




scRNA

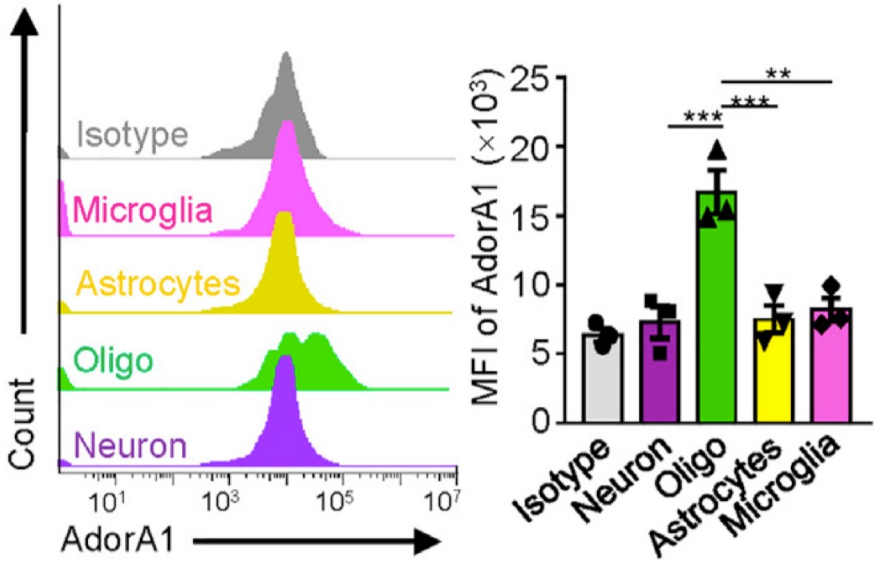
FACS

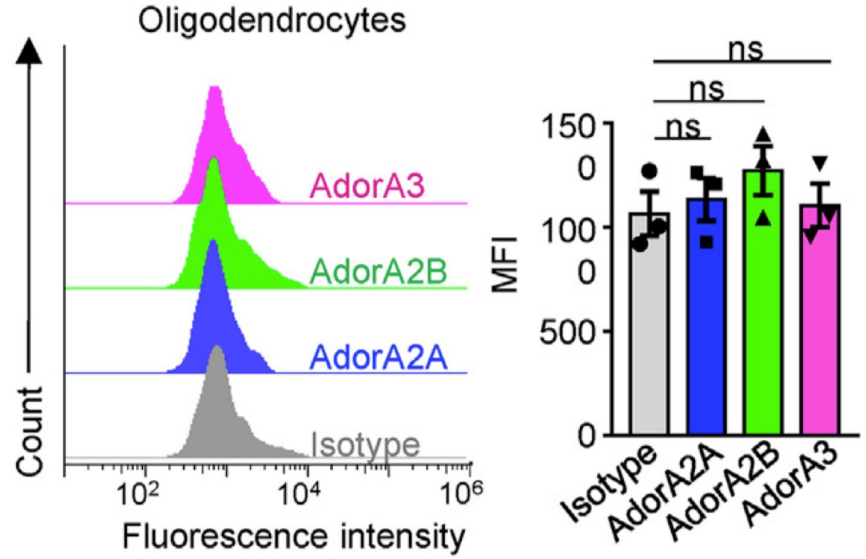
Distribution of purine receptors in amygdala

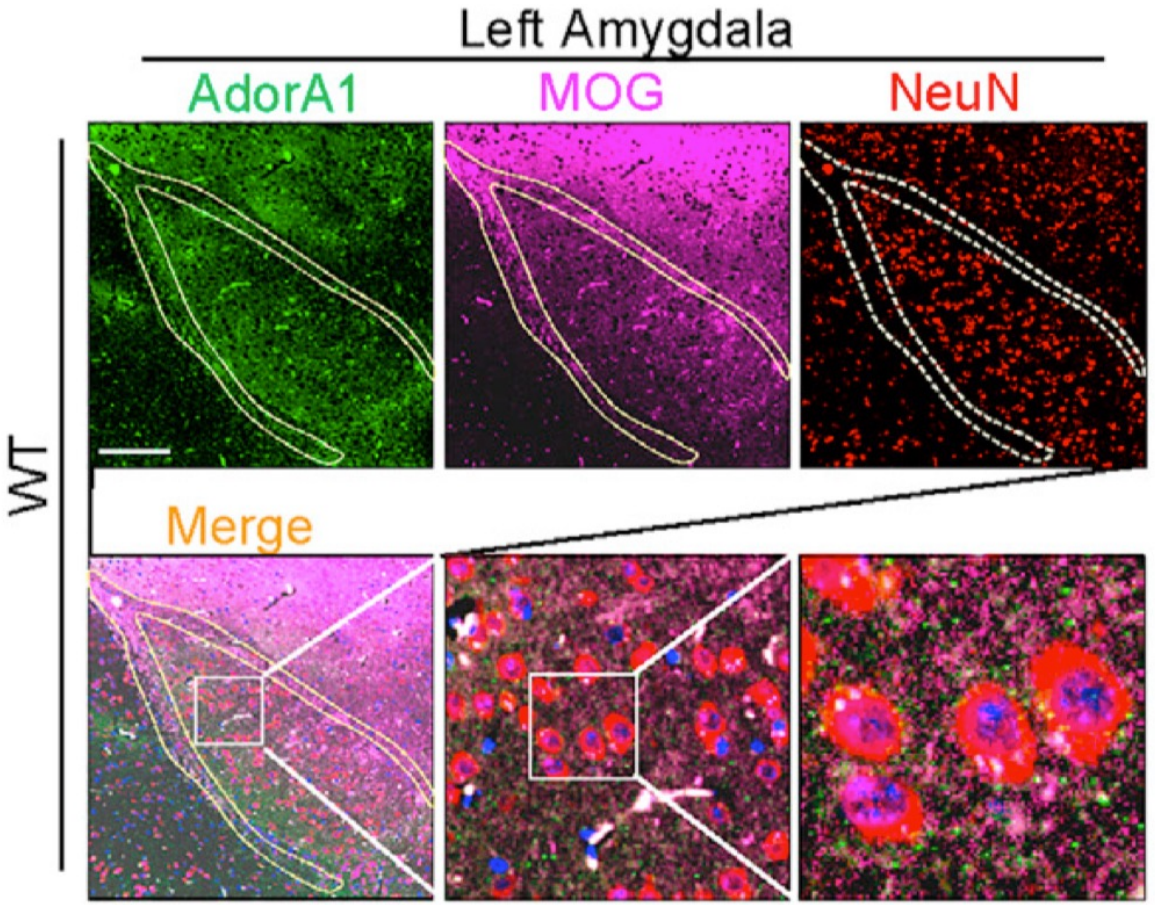


purine receptors: A1, A2A, A2B, and A3

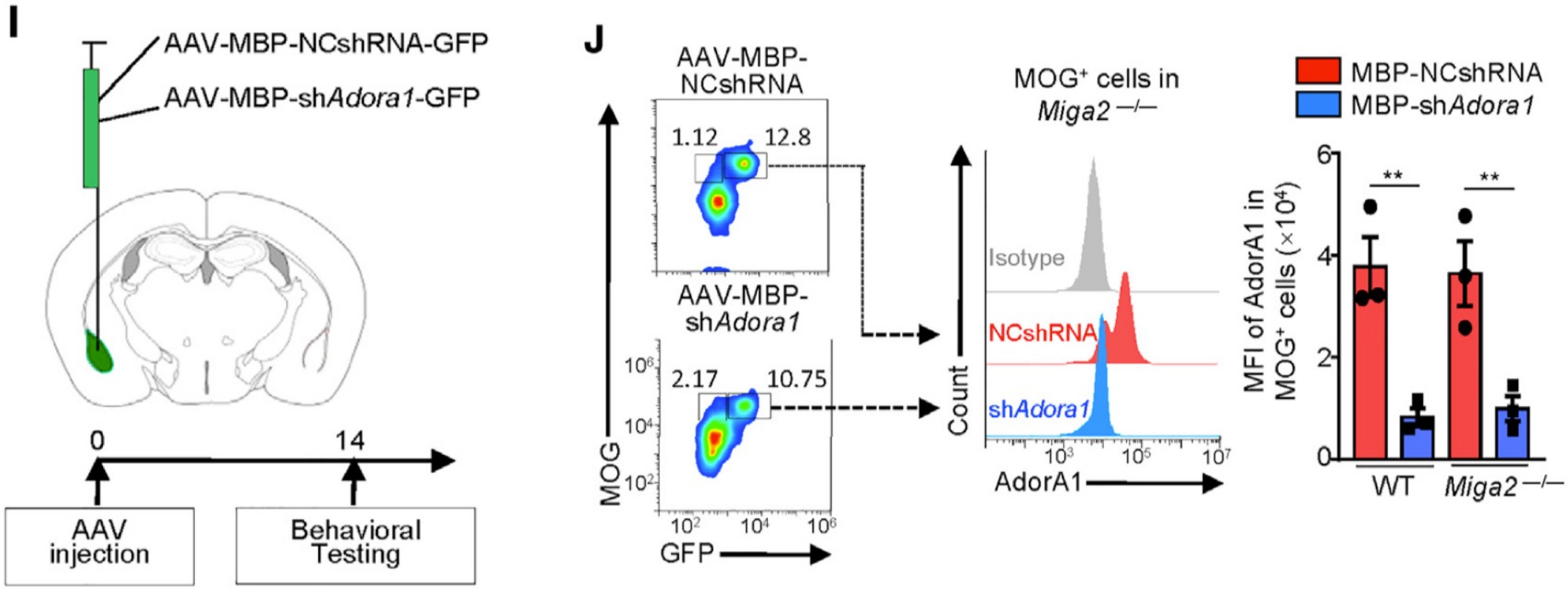
FACS and IF analysis further confirmed that A1 expression was largely distributed in oligodendrocytes, but no expression of A2A, A2B, or A3 was detected



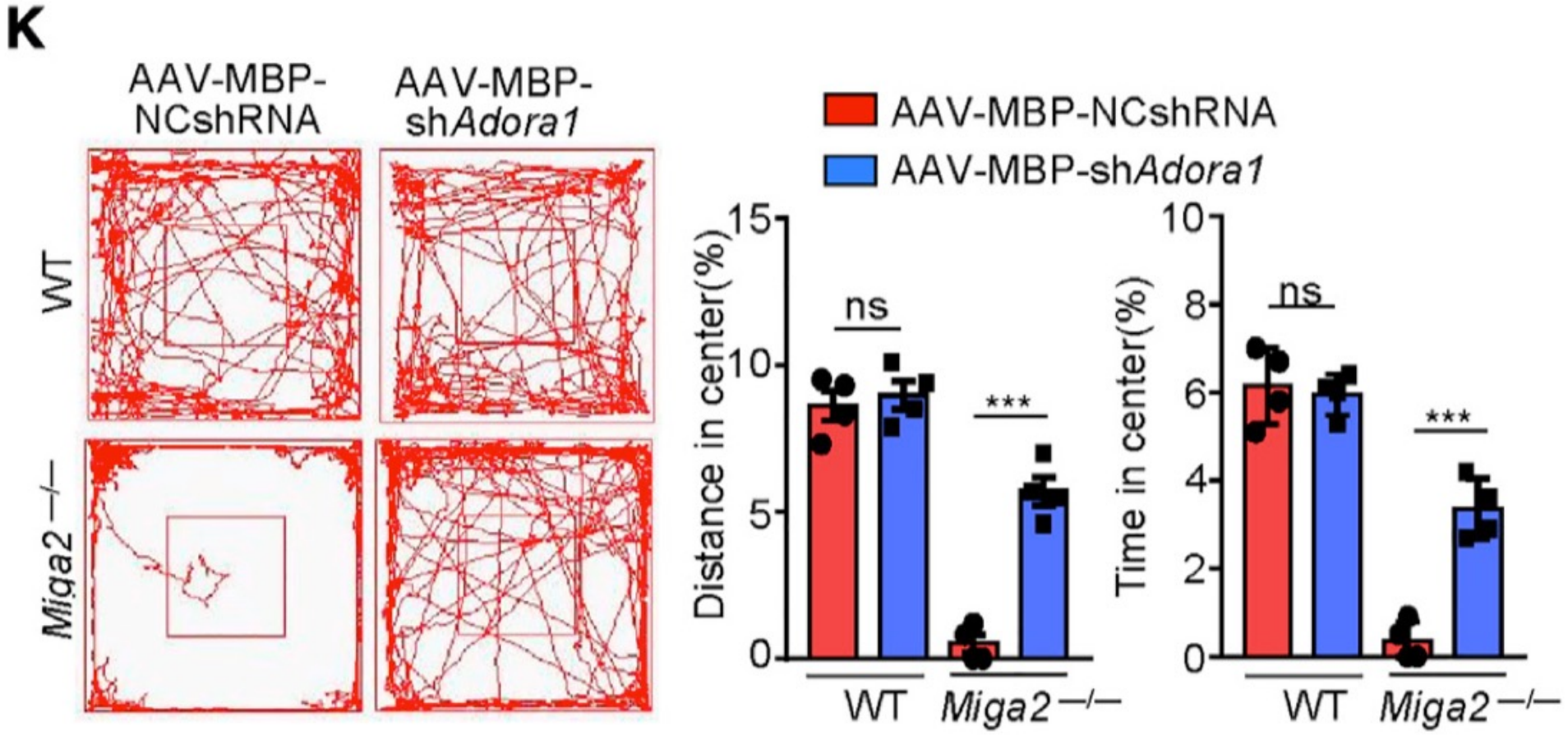




Knockdown of Adora1 in left amygdala oligodendrocytes

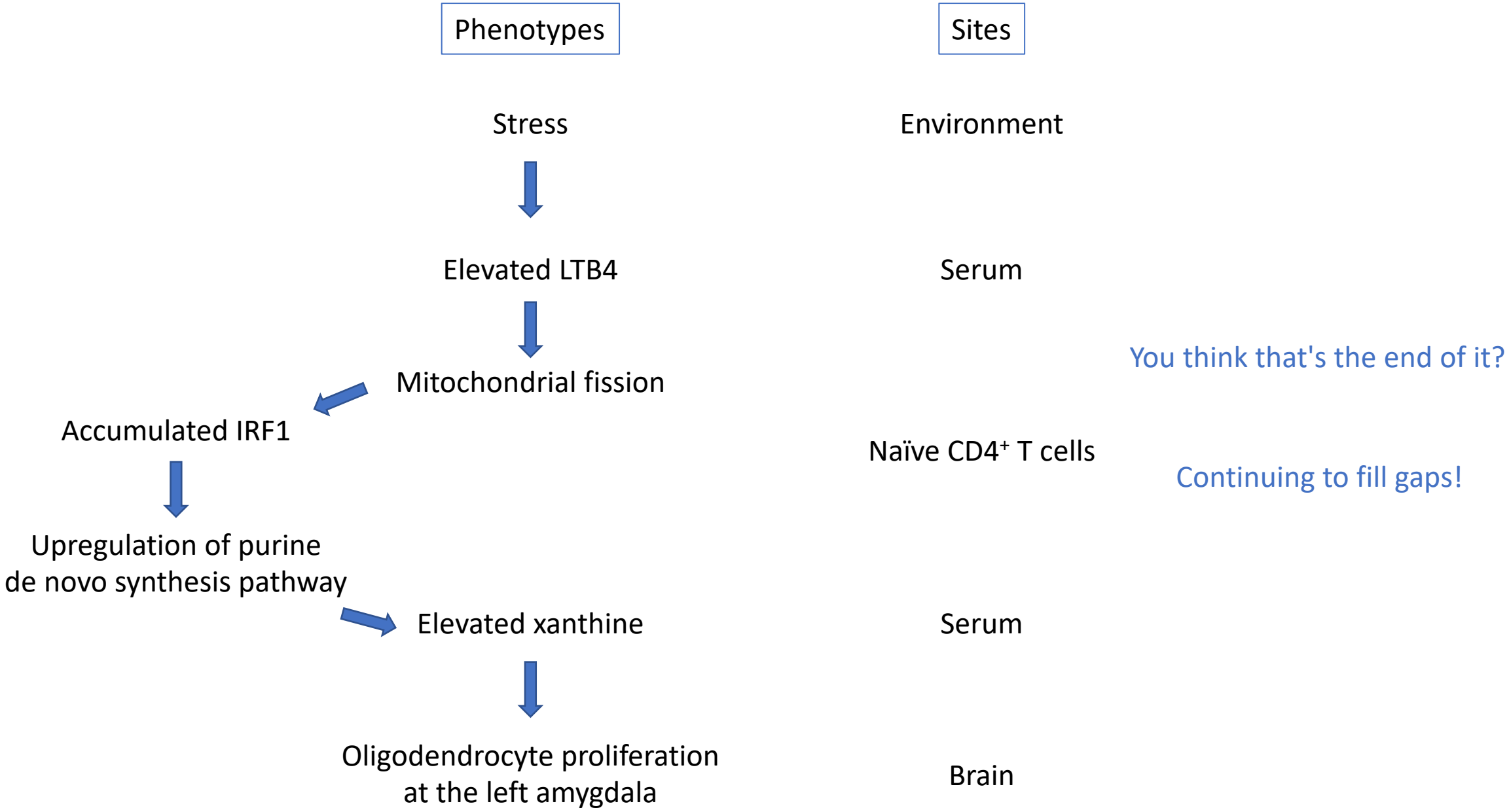


Without the A1 receptor in oligodendrocytes, Miga2-deficient mice no longer displayed anxiety-like symptoms

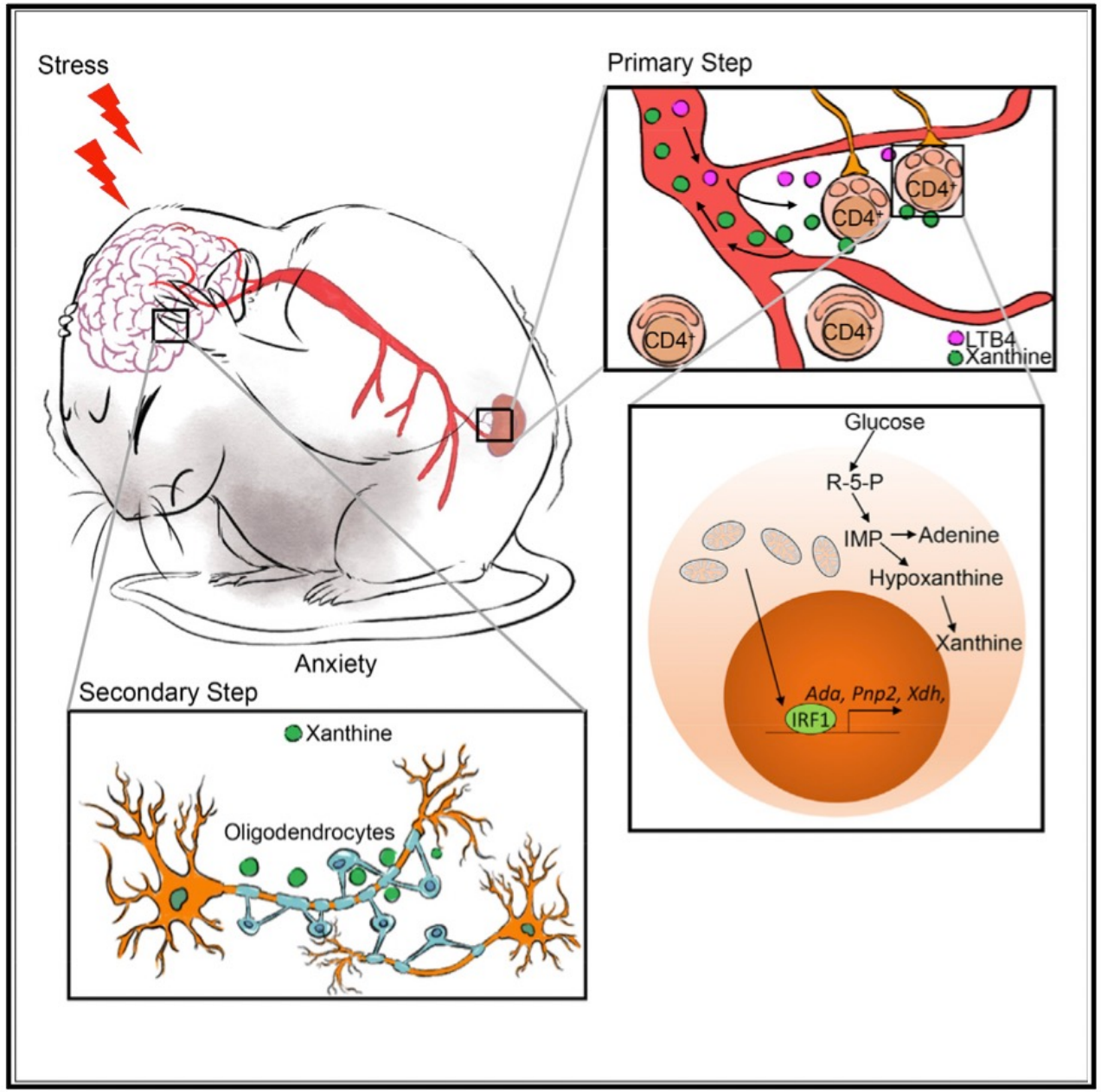


Phenotypes	Sites
Stress	Environment
↓	
Elevated LTB4	Serum
↓	
Mitochondrial fission	
↓	
Metabolic disorder	Naïve $CD4^+$ T cells
↓	
Elevated xanthine	Serum
↓	
Oligodendrocyte proliferation at the left amygdala	Brain

You think that's the end of it?


Phenotypes
Sites
Stress
Environment
Elevated LTB4
Serum
Mitochondrial fission
You think that's the end of it?
Accumulated IRF1
Naïve CD4+ T cells
Continuing to fill gaps!
Upregulation of purine
de novo synthesis pathway
Elevated xanthine
Serum
Oligodendrocyte proliferation
at the left amygdala
Brain

Graphical Abstract



Discussion

- Very thoughtful
- Learning materials of experiments and analysis
- Learning materials of project design

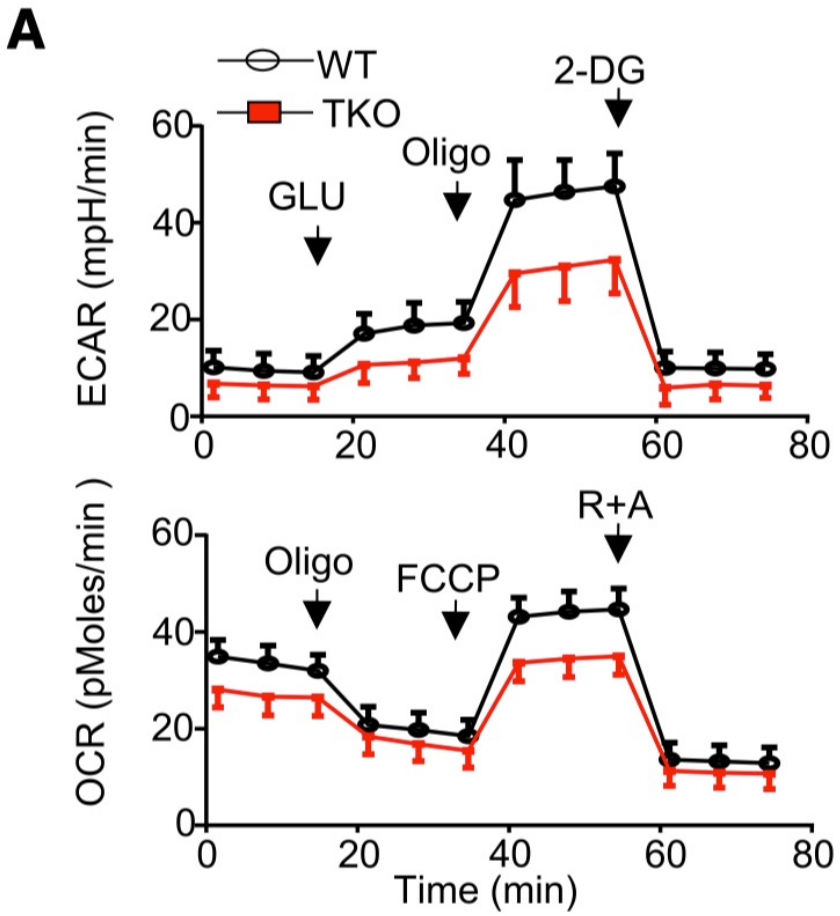
- How oligodendrocytes regulate neuronal activity? By its canonical myelin sheath formation function? No direct evidence

Thanks for your attention!

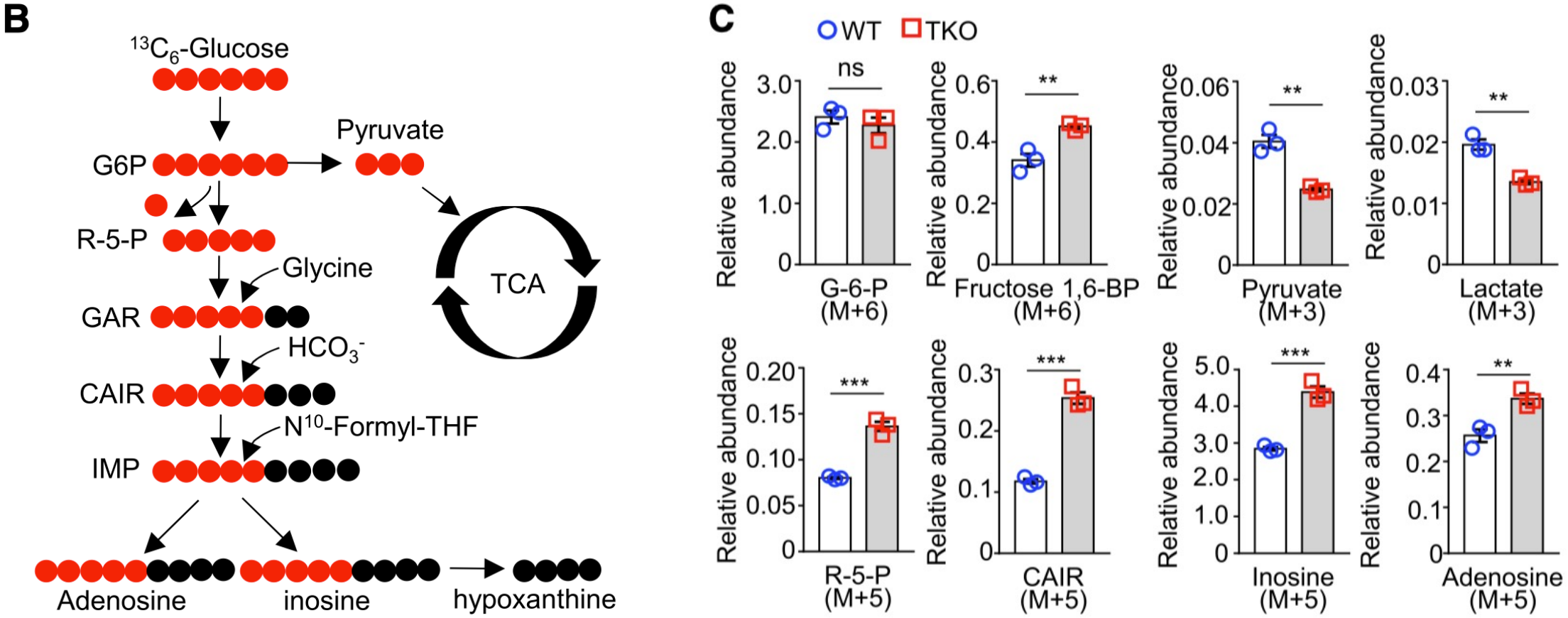
- Figure 1. $CD4^+$ T Cells Play an Essential Role in Stress-Induced Anxiety-like Behavior
- Figure 2. Stress Causes Metabolic Disorder and Mitochondrial Fission in $CD4^+$ T Cells
- Figure 3. Sustained Mitochondrial Fission in $CD4^+$ T Cells Induces the Anxiety-like Behavior
- Figure 4. Mitochondrial Fission in $CD4^+$ T Cells Leads to a Systemic Increase in Serum Purines
- Figure 5. $CD4^+$ T Cell-Derived Xanthine Acts on the Oligodendrocytes at the Amygdala via AdorA1
- Figure 6. Mitochondrial Fission Promotes Purine de novo Synthesis Pathway in $CD4^+$ T Cells
- Figure 7. Accumulated IRF-1 Controls Purine Synthesis in $CD4^+$ T Cells and Anxiety-like Behavior

- Purine can be synthesized via two distinct pathways: the de novo and salvage pathways. In the de novo synthesis pathway, the glucose metabolic product 5-phosphoribosyl-1-pyrophosphate (PRPP) provides a backbone to form the purine ring.

Similar to ES-treated T cells, Miga2-deficient CD4 + T cells exhibited markedly reduced activities of OXPHOS and glycolysis



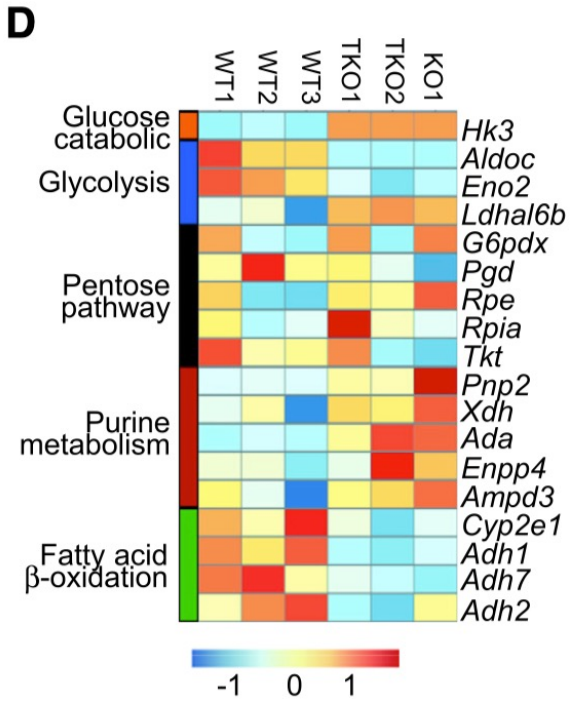
Miga2-deficient naive CD4⁺ T cells had lower glycolysis levels but produced more purines than the WT naive CD4⁺ T cells

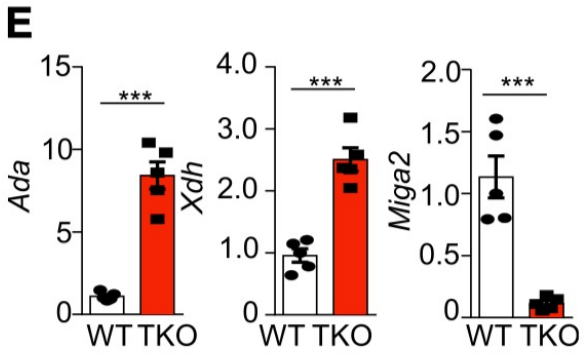


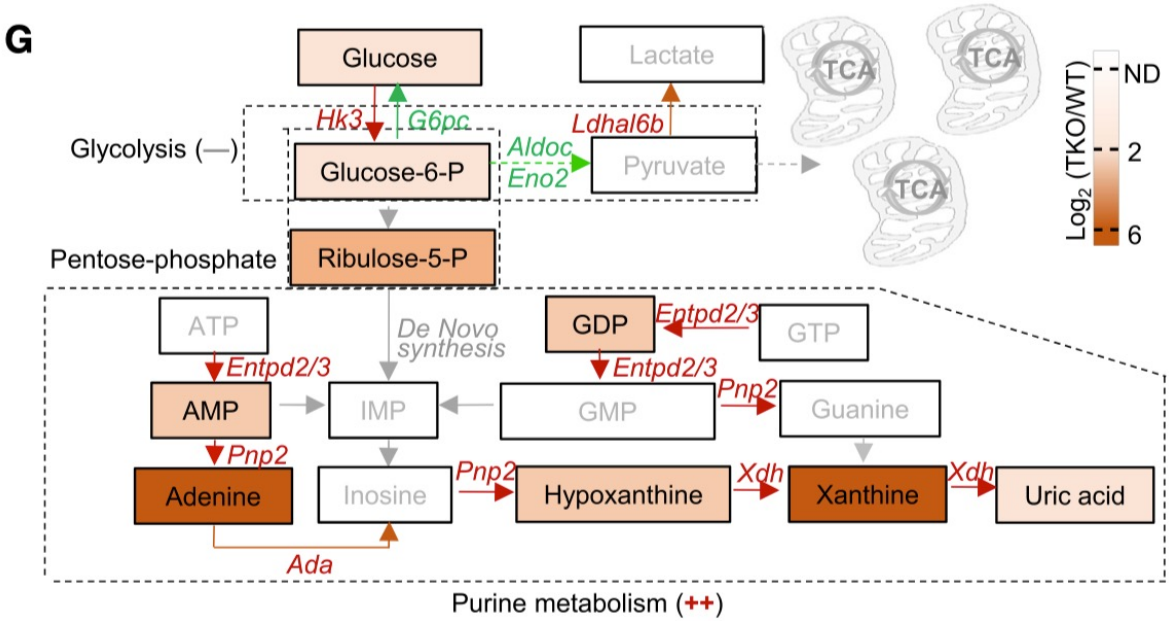
Schematic diagram of the conversion of13C–glucose into purine metabolism and the tricarboxylic acid (TCA) cycle.

LC-MS was performed for M+3-, M+5-, and M+6-labeled metabolic productions.

Miga2-deficient CD4 + T cells showed reduced transcription of several critical enzymes related to the glycolytic and fatty acid b-oxidation pathway, but increases in the molecules required for purine synthesis



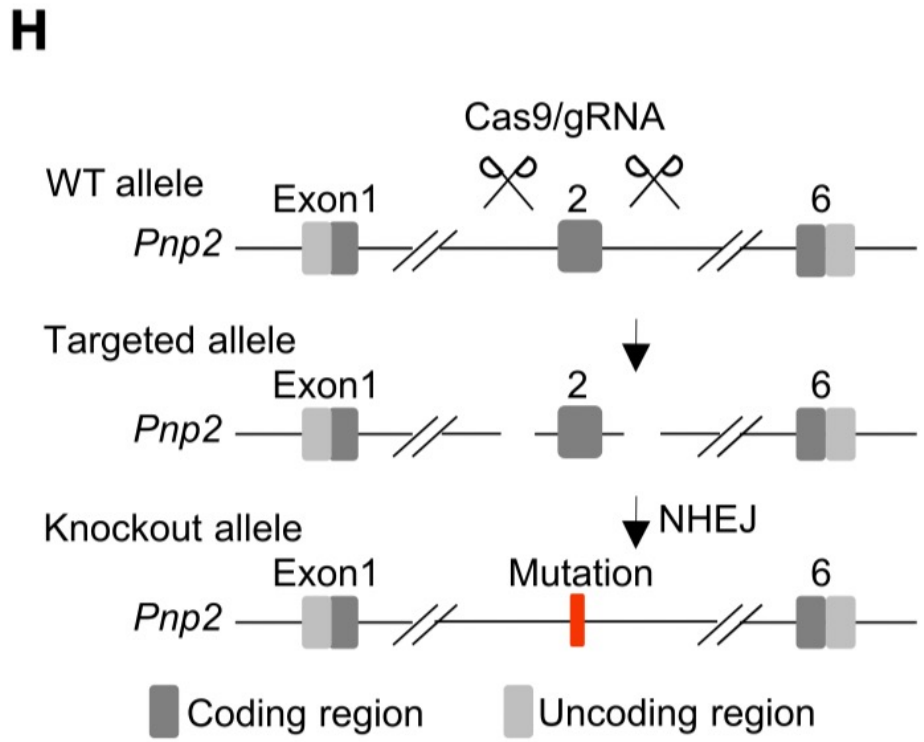


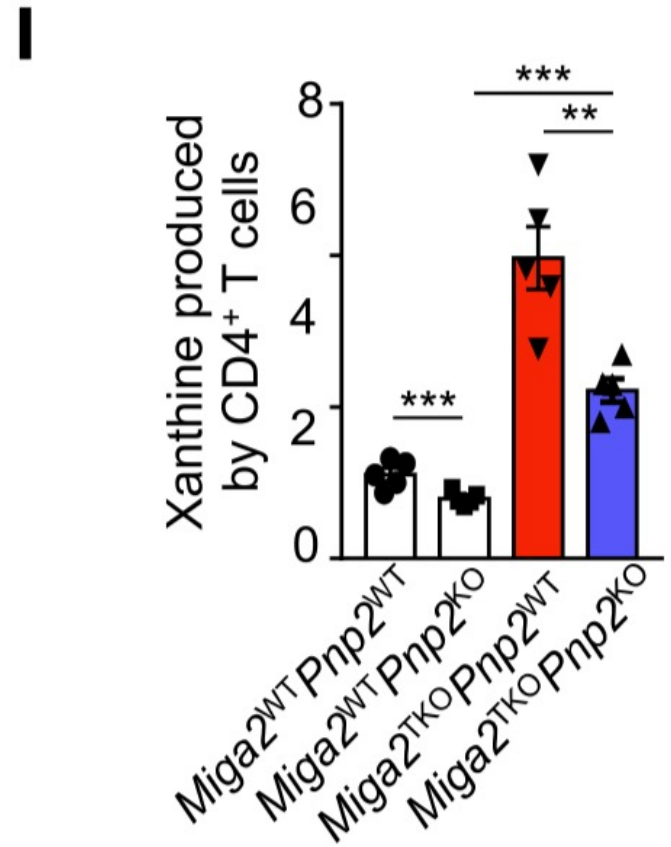


D: RNAseq
E: qPCR
F: Western blot

purine metabolic pathways

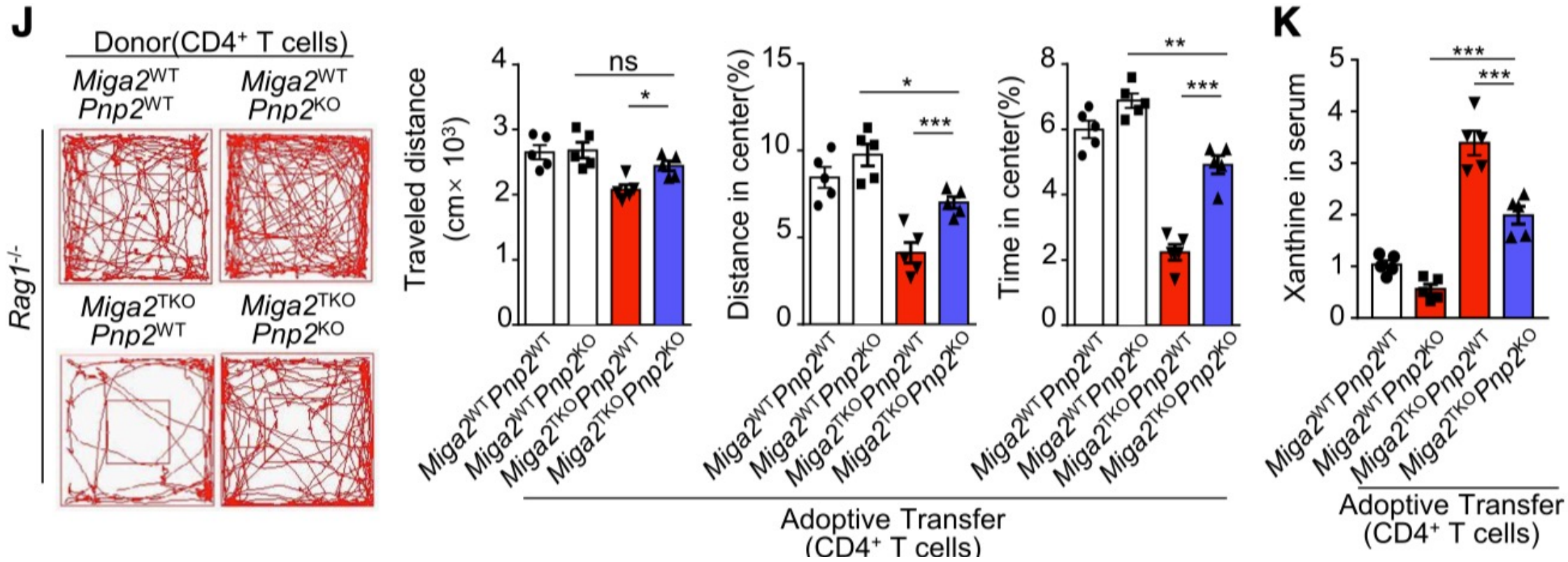
Pnp2-deficient mice were thus further crossed with Miga2 À/À mice to generate Pnp2À/À Miga2À/À mice





我感觉他这个项目得用一屋子老鼠

After adoptive transfer into Rag1$^{-/-}$ mice, Pnp2$^{-/-}$ Miga2$^{-/-}$ CD4^{+} T cells did not induce anxiety symptoms as strong as those induced by Miga2$^{-/-}$ CD4^{+} T cells



思考下为什么直接用双敲的小鼠做行为，而是用Rag1-/-小鼠作为recipient，双敲小鼠作为CD4^{+} T cell的donor?

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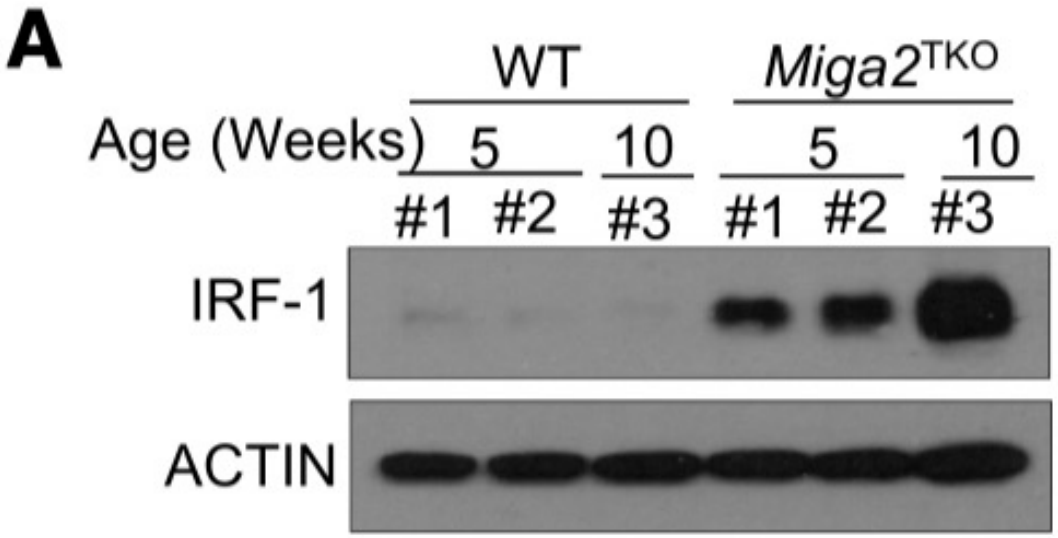
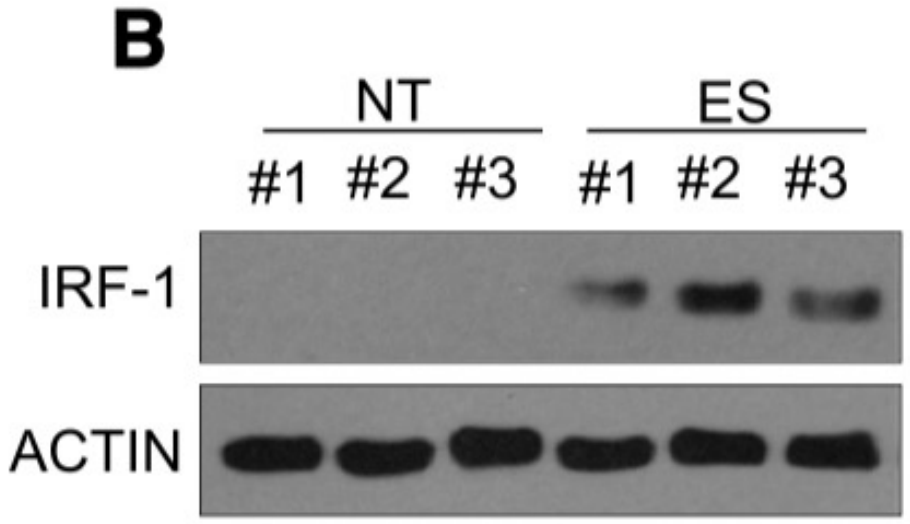
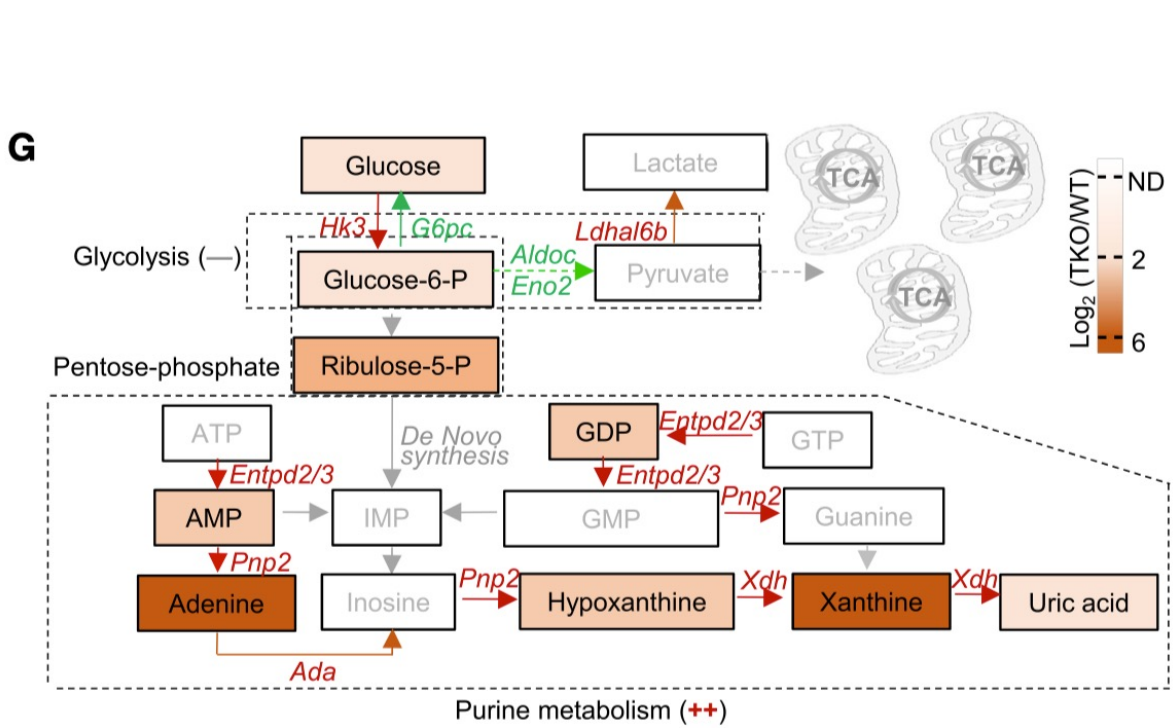
- Interferon regulatory factor-1 (IRF-1), a transcription factor, participates in various cellular processes, including cell proliferation, differentiation, apoptosis, and immunological regulation. Our previous study revealed that constitutive mitochondrial fission promoted the accumulation of IRF-1 in innate immune cells

Miga2 deficiency and ES triggered significant aggregation of IRF-1 in $CD4^+$ T cells

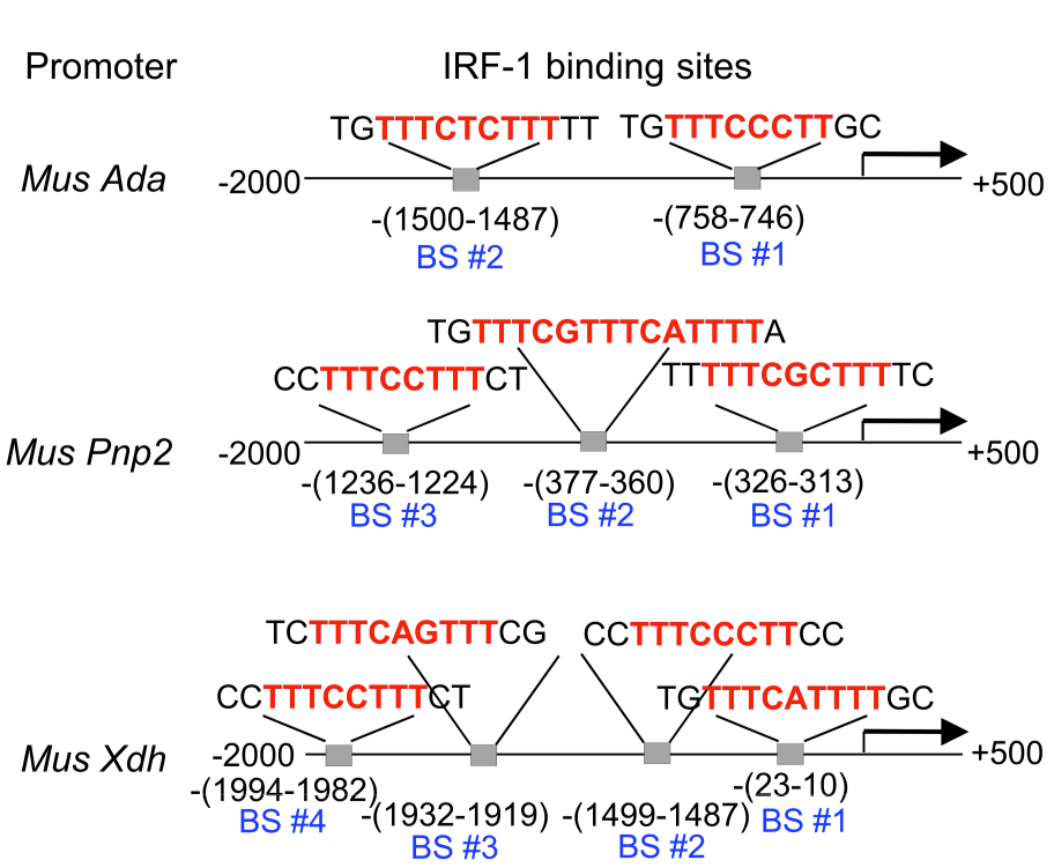




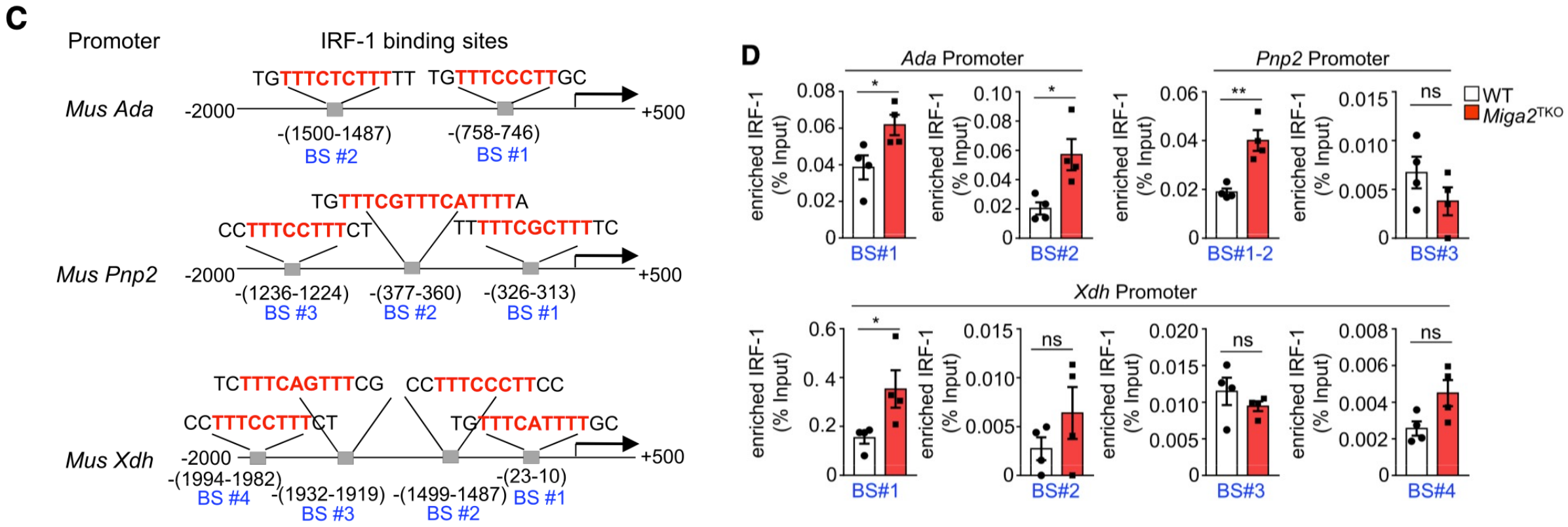
Identification of several potential IRF-1 binding motifs near the transcription start site of genes encoding purine synthesis enzymes



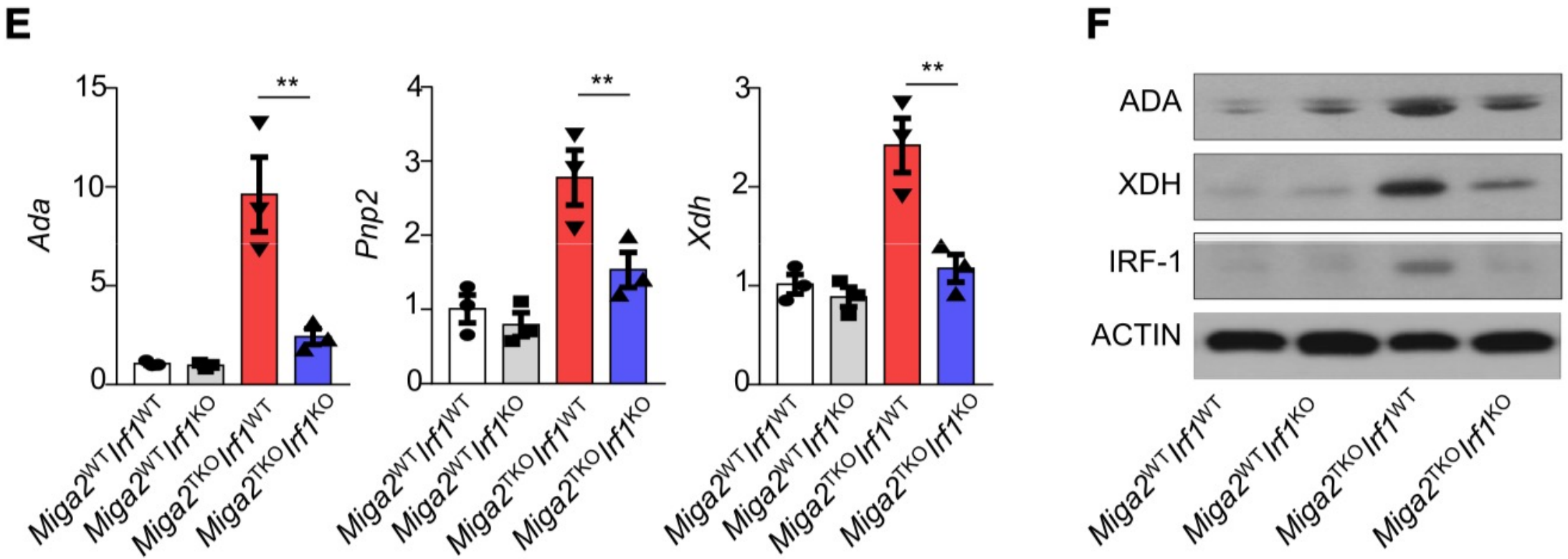
Previous result



ChIP-qPCR assays further revealed that the accumulated IRF-1 in Miga2-deficient CD4 + T cells was significantly enriched at certain binding sites in the TSSs of Ada, Pnp2, and Xdh compared to those in WT controls



IRF-1 deficiency clearly normalized both the mRNA and protein levels of Ada and Xdh in Miga2-deficient CD4^{+} T cells



The lack of IRF-1 restored most of the anxiety-like phenotypes in Miga2^{TKO} mice