
Maternal gut bacteria drive intestinal inflammation in offspring with neurodevelopmental disorders by altering the chromatin landscape of CD4+ T cells

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Today's Content

01

Fundamentals

02

Introduction

03

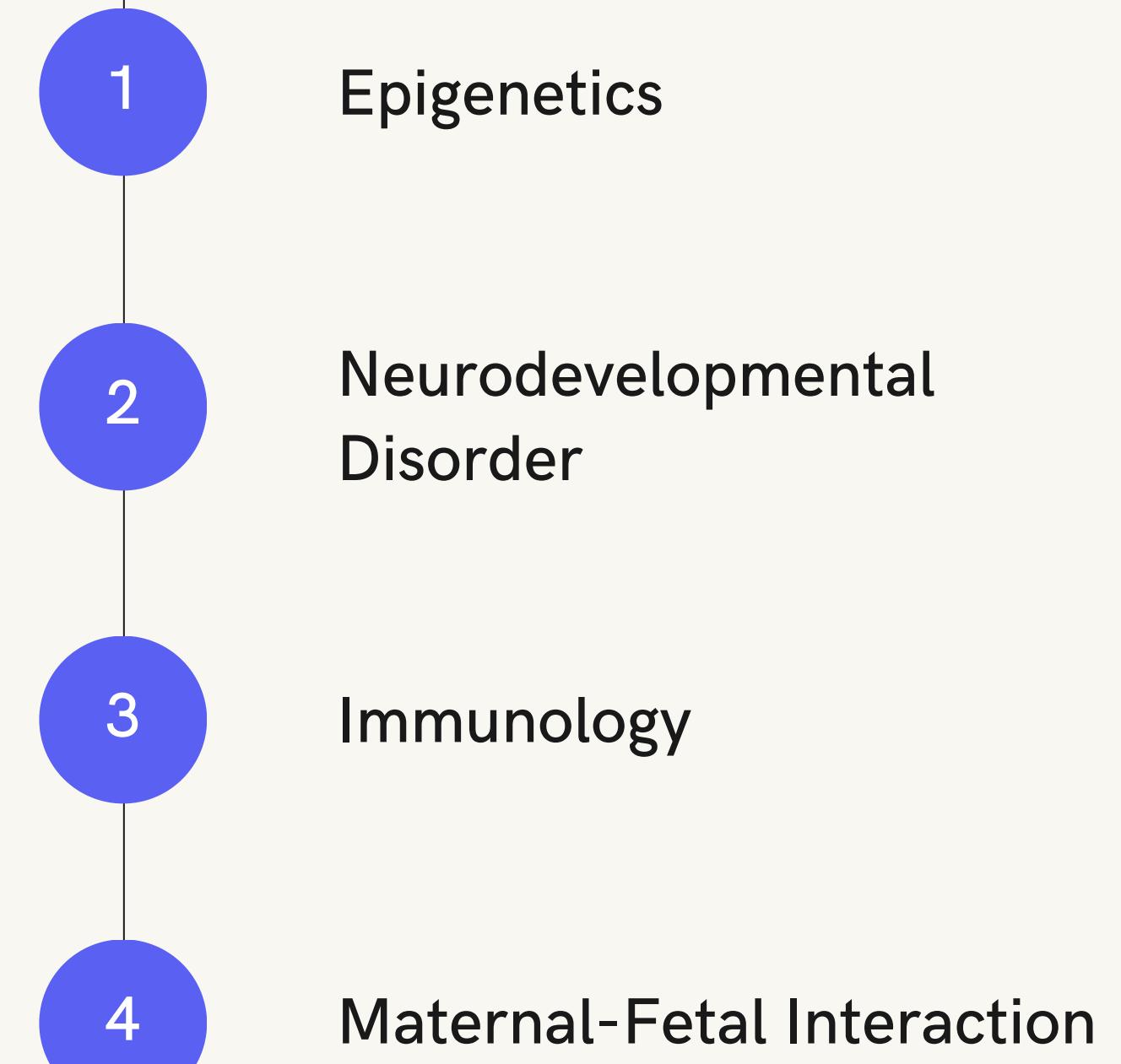
Results

04

Conclusion

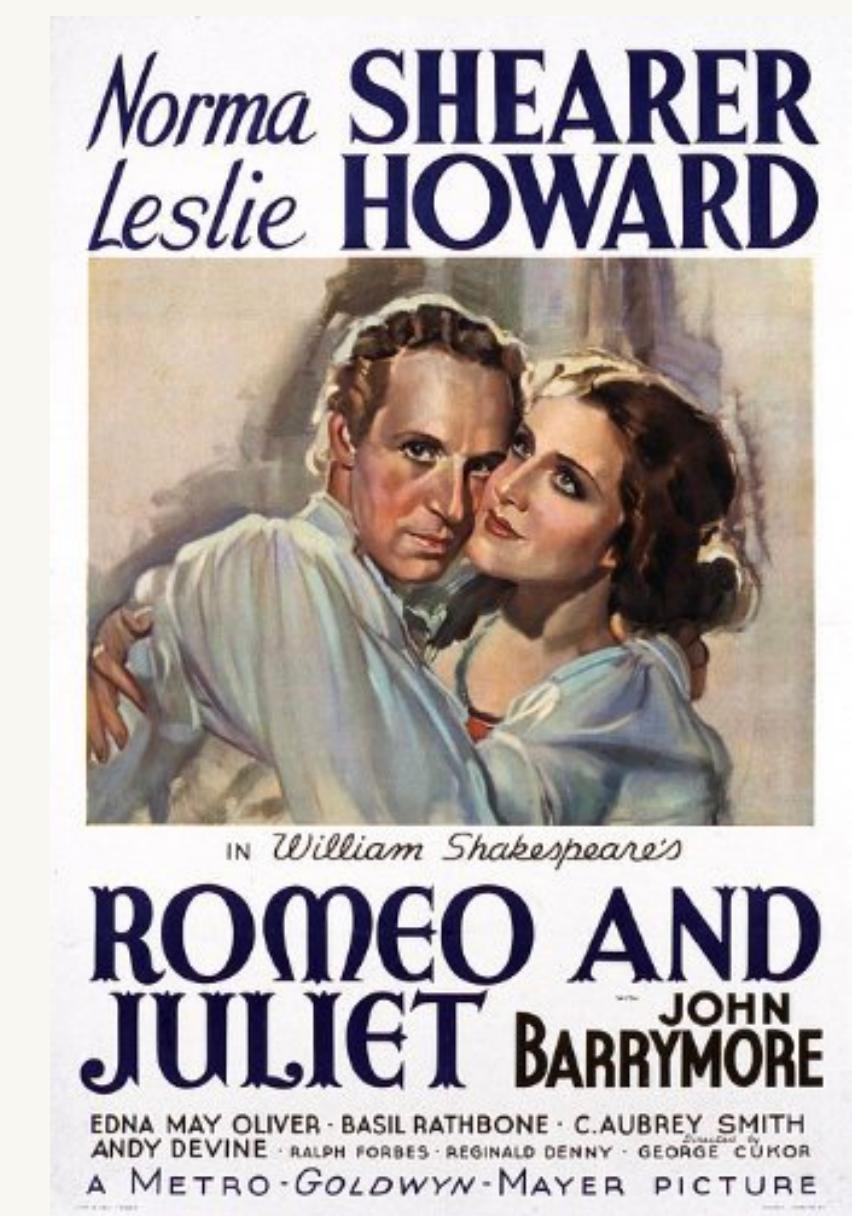
Agenda

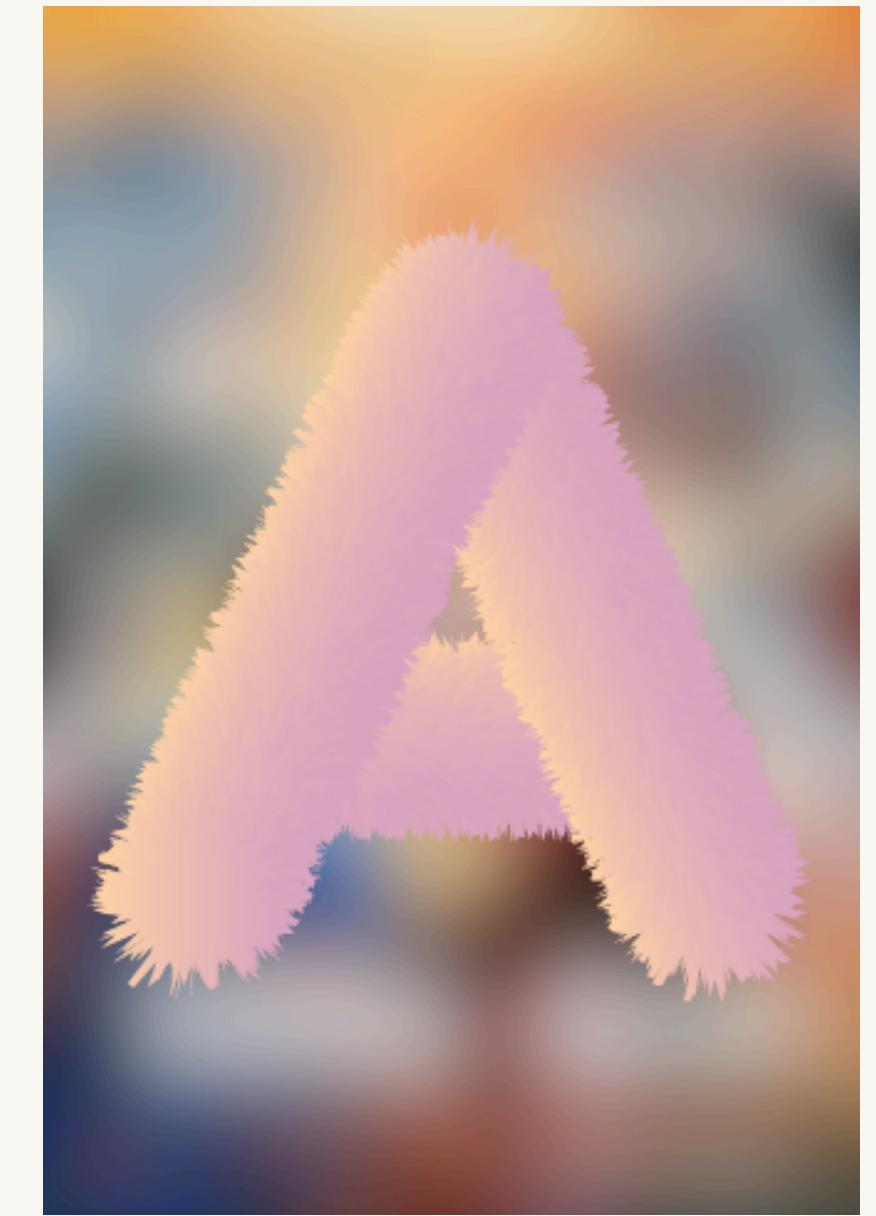
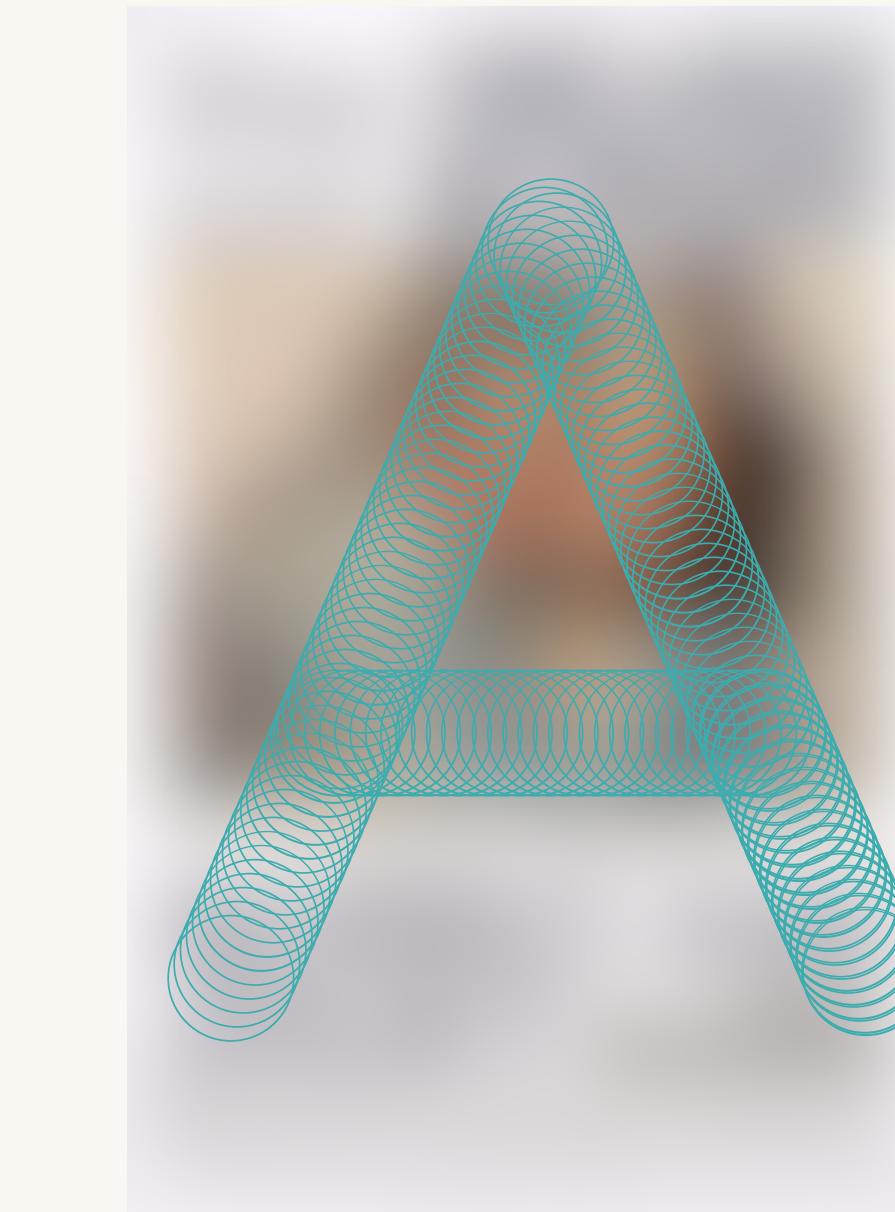
Key points for discussion

- 
- 1 Epigenetics
 - 2 Neurodevelopmental Disorder
 - 3 Immunology
 - 4 Maternal-Fetal Interaction

#Epigenetics

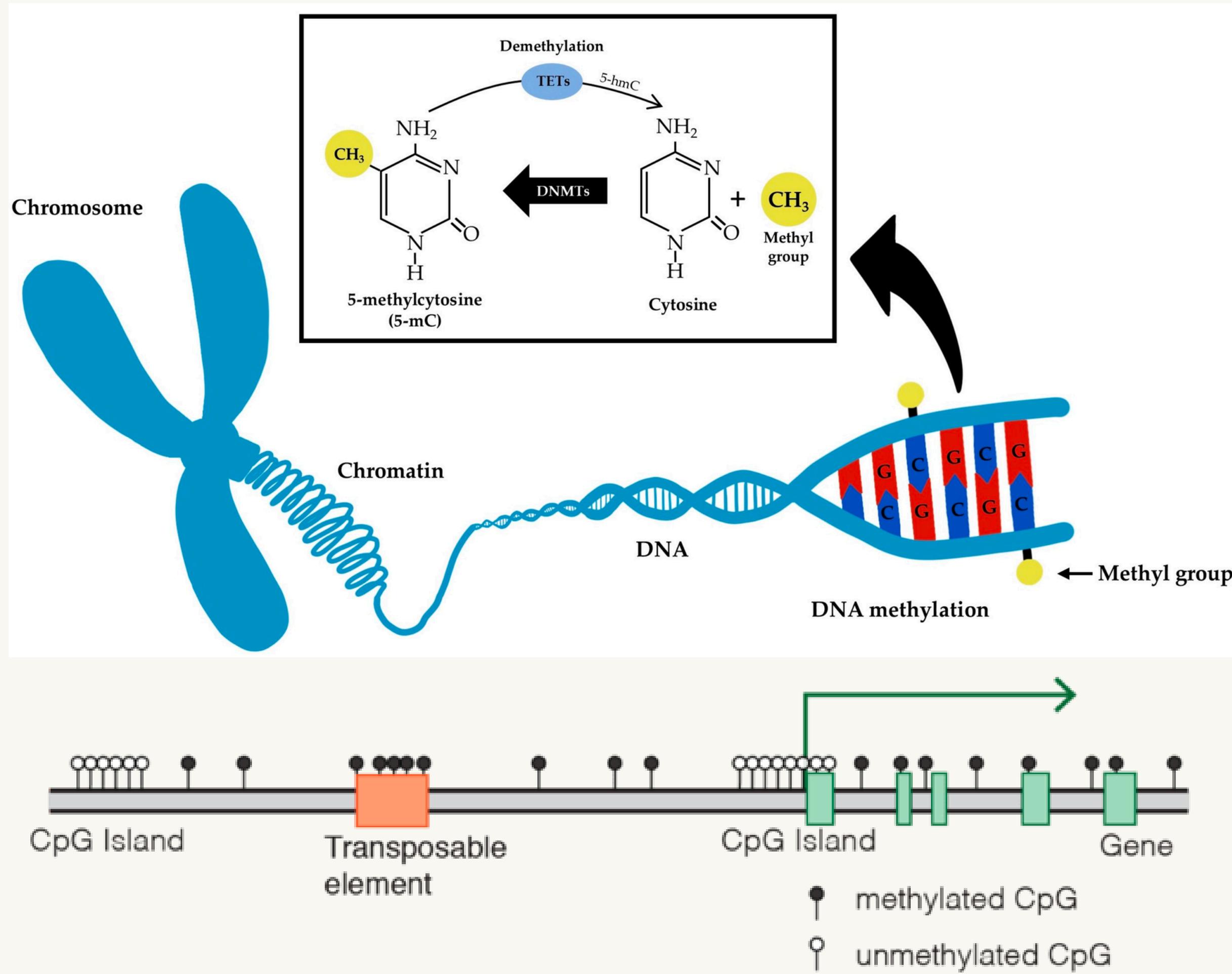
Where we are now

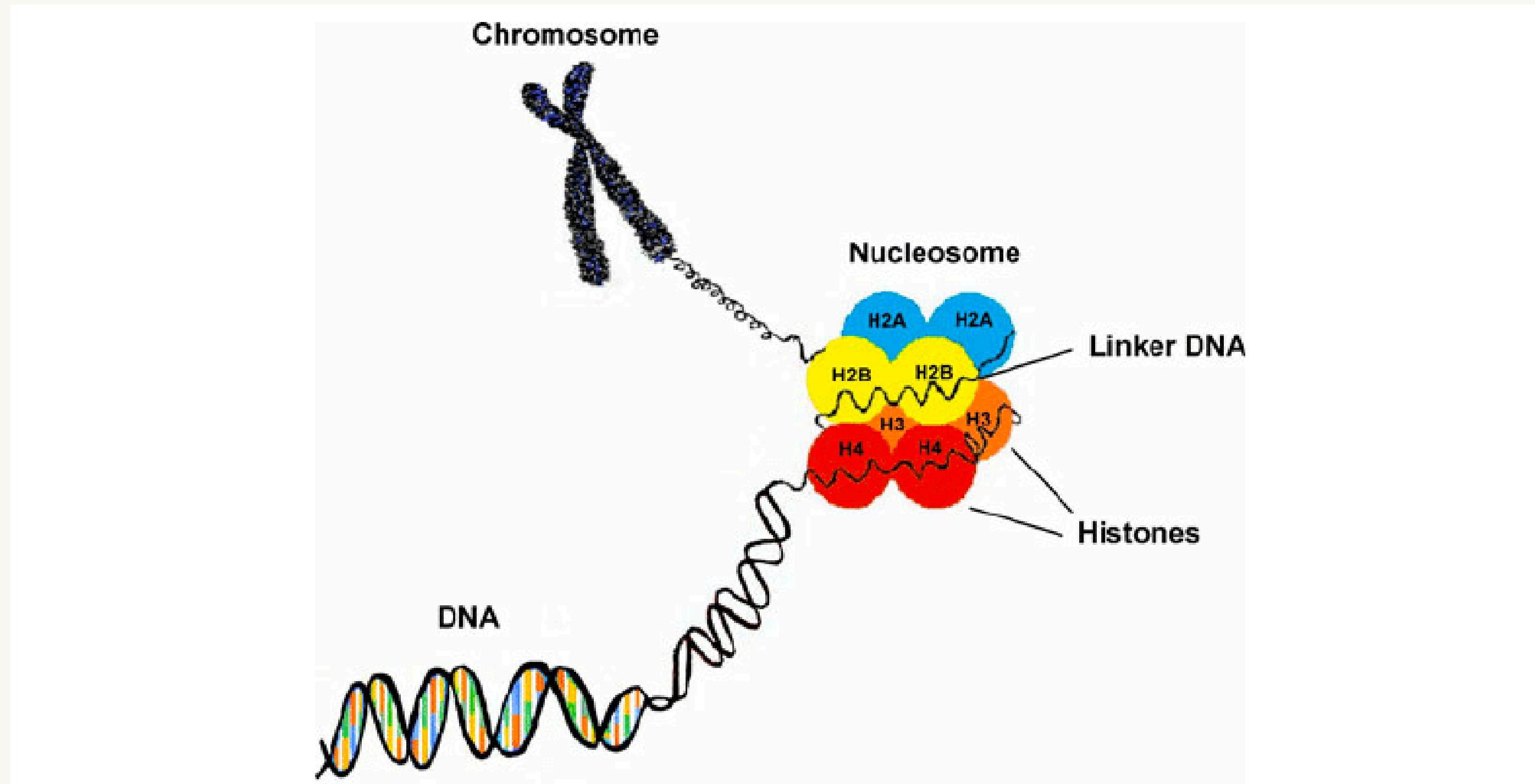


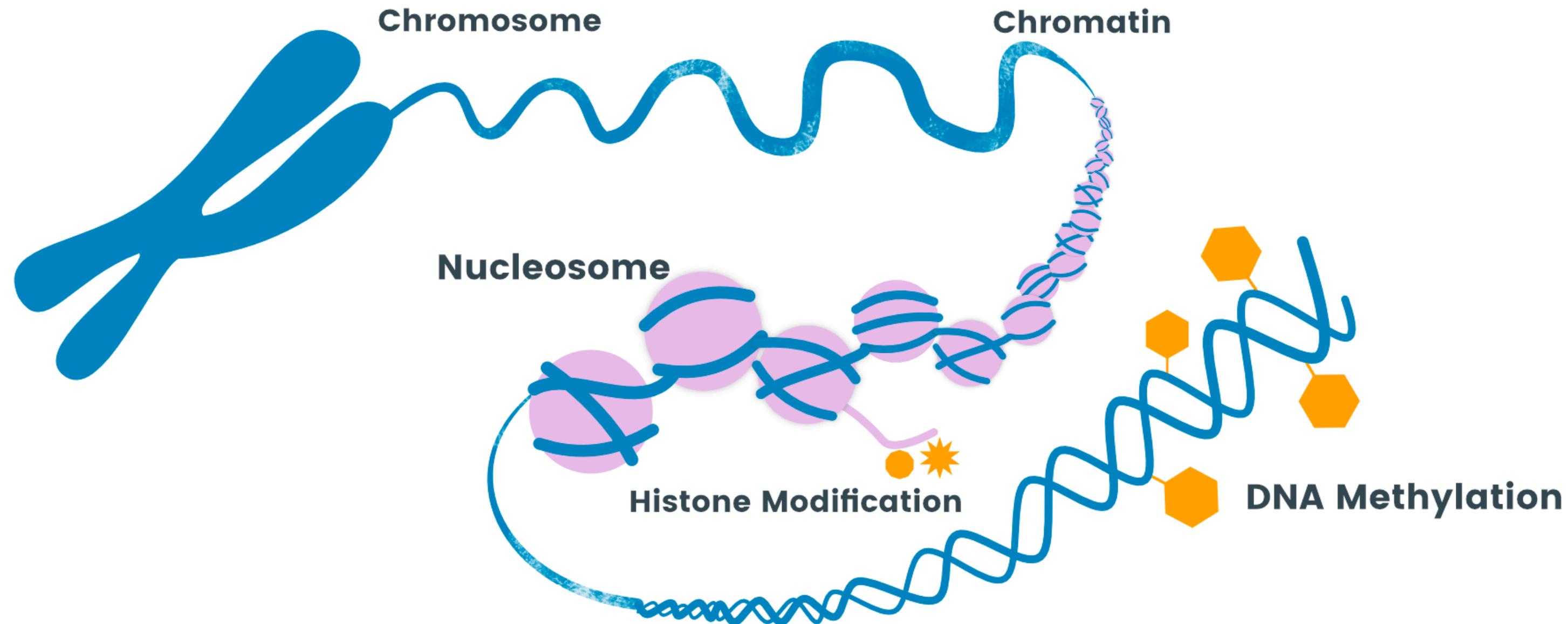


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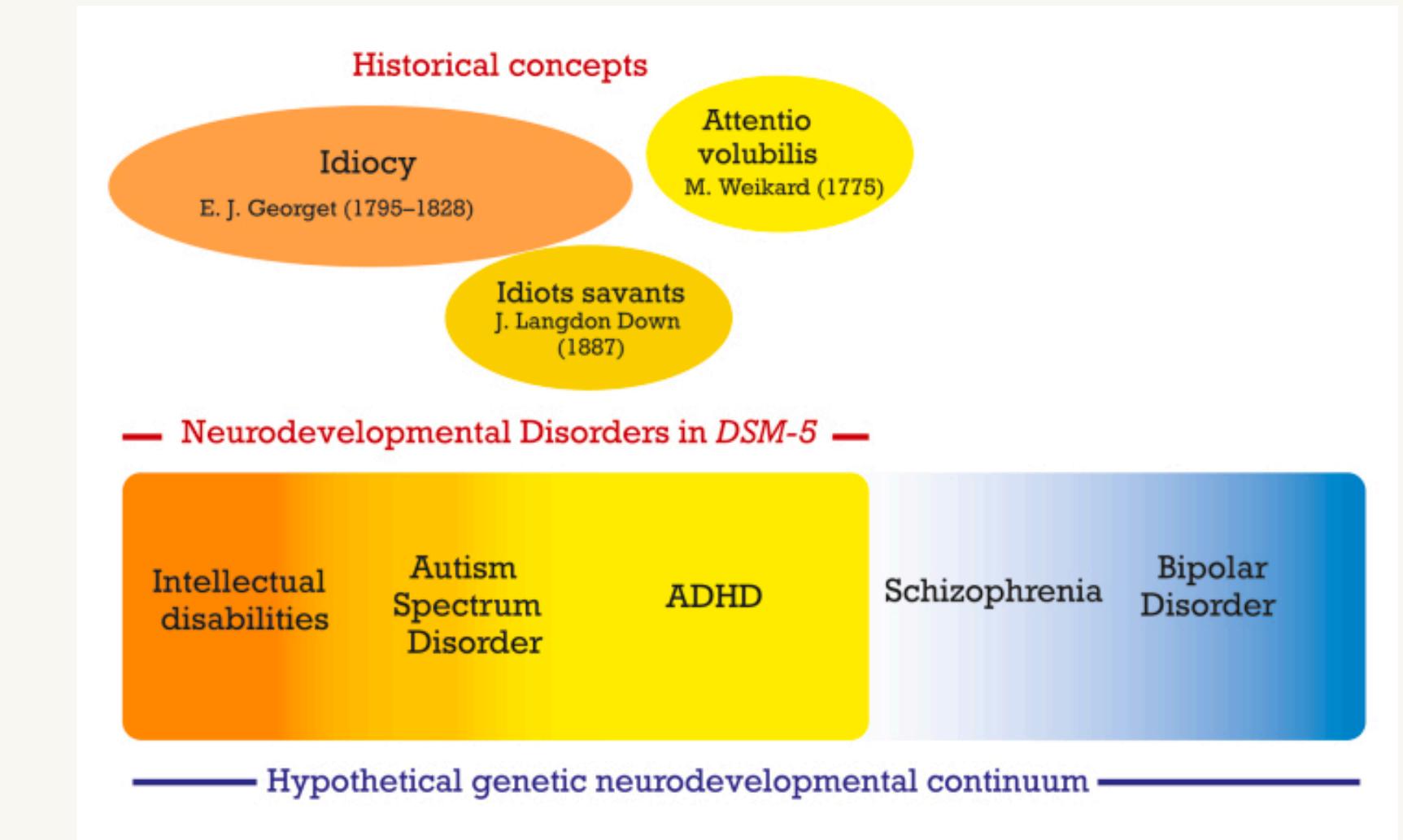






#Neurodevelopmental Disorder

- A group of conditions with onset in the developmental period, inducing deficits that produce impairments of functioning.
- Associated with a known medical or genetic condition or environmental factor.



Morris-Rosendahl, D. J., & Crocq, M. A. (2020). Neurodevelopmental disorders—the history and future of a diagnostic concept . *Dialogues in clinical neuroscience*, 22(1), 65-72.



2023 Fall Seminar 1 Section 3

AUTISM

AUTISM SPECTRUM DISORDER (ASD)

Week 11, 2023 Fall

Is Autism Neurodegenerative?

**Probably Yes.
Because of...**

- Neuronal cell loss
- Activated microglia and astrocytes
- Proinflammatory cytokines
- Oxidative stress
- Elevated 8-oxo-guanosine levels.

Kern, J. K., Geier, D. A., Sykes, L. K., & Geier, M. R. (2013).
Evidence of neurodegeneration in autism spectrum disorder.
Translational neurodegeneration, 2(1), 17.
<https://doi.org/10.1186/2047-9158-2-17>

RESEARCH ARTICLES

NEUROIMMUNOLOGY

The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring

Gloria B. Choi,^{1,*} Yeong S. Yim,^{1,*} Helen Wong,^{2,3*} Sangdoo Kim,⁴ Hyunju Kim,⁴ Sangwon V. Kim,⁵ Charles A. Hoeffer,^{2,3†} Dan R. Littman,^{5,6†} Jun R. Huh^{4,5†}

Viral infection during pregnancy has been correlated with increased frequency of autism spectrum disorder (ASD) in offspring. This observation has been modeled in rodents subjected to maternal immune activation (MIA). The immune cell populations critical in the MIA model have not been identified. Using both genetic mutants and blocking antibodies in mice, we show that retinoic acid receptor–related orphan nuclear receptor gamma t (ROR γ t)–dependent effector T lymphocytes [for example, T helper 17 (T_H17) cells] and the effector cytokine interleukin-17a (IL-17a) are required in mothers for MIA-induced behavioral abnormalities in offspring. We find that MIA induces an abnormal cortical phenotype, which is also dependent on maternal IL-17a, in the fetal brain. Our data suggest that therapeutic targeting of T_H17 cells in susceptible pregnant mothers may reduce the likelihood of bearing children with inflammation-induced ASD-like phenotypes.

Gastrointestinal (GI) issues

- GI problems are reported most commonly as medical comorbidities, especially in ASD.
- The odds of constipation increase with greater social impairment and less verbal ability.
- Increased ASD severity.

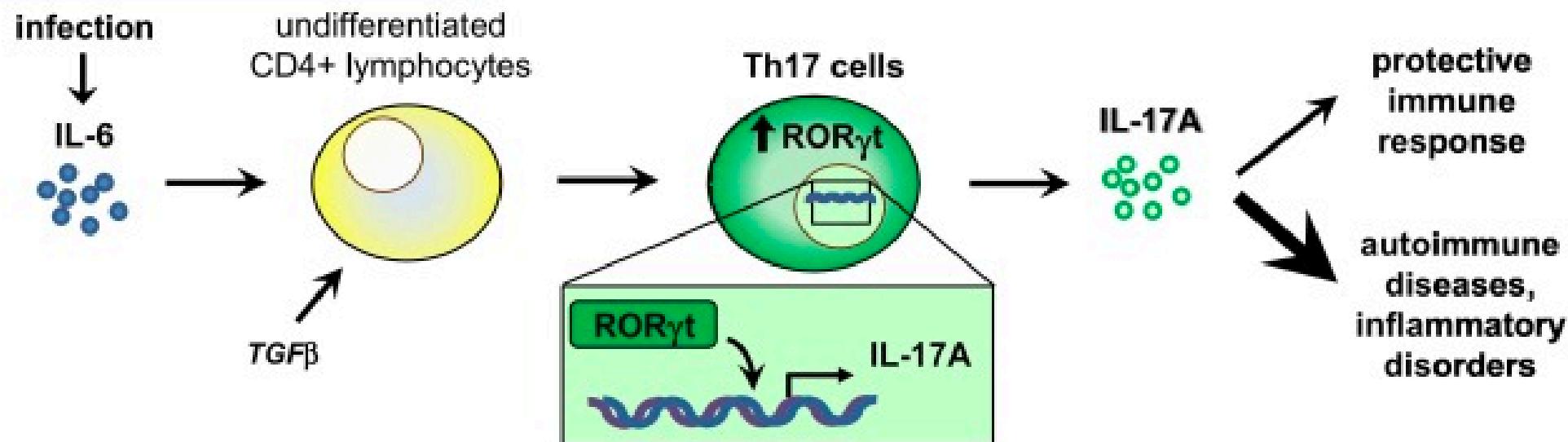
Madra, M., Ringel, R., & Margolis, K. G. (2020). Gastrointestinal Issues and Autism Spectrum Disorder. *Child and adolescent psychiatric clinics of North America*, 29(3), 501-513. <https://doi.org/10.1016/j.chc.2020.02.005>



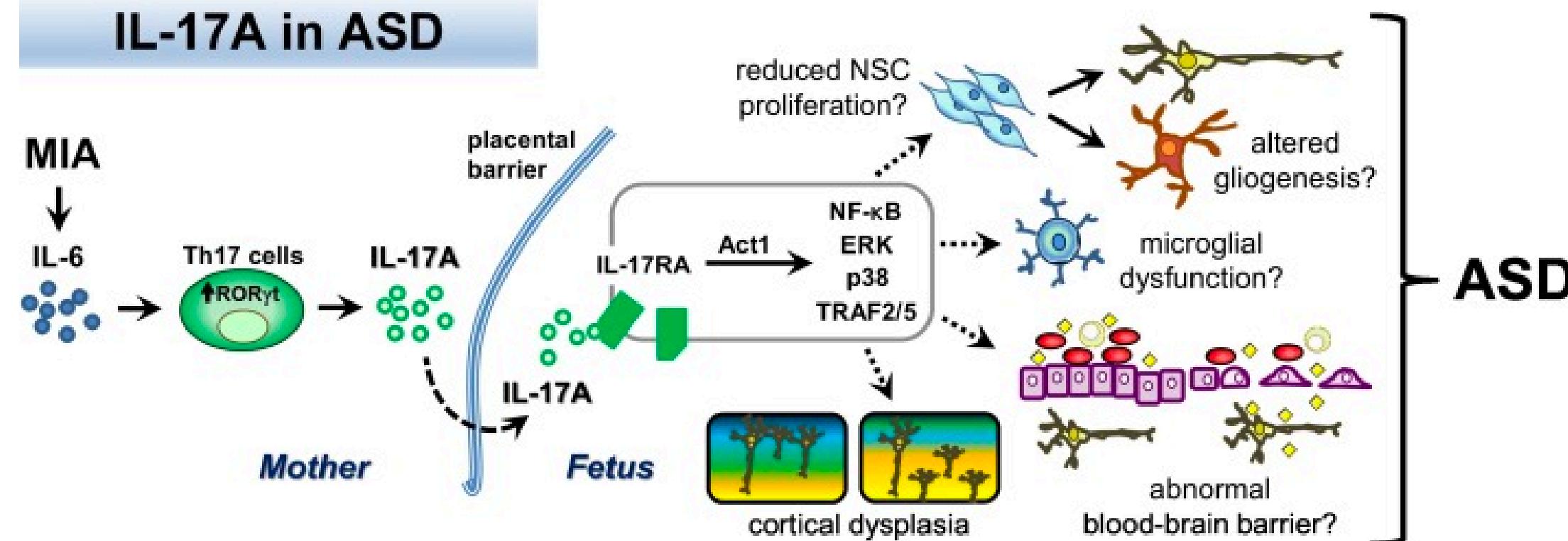
#Immunology

□ Quick review

IL-17A in immunity



IL-17A in ASD



Jash, S., & Sharma, S. (2022). Pathogenic Infections during Pregnancy and the Consequences for Fetal Brain Development. *Pathogens* (Basel, Switzerland), 11(2), 193. <https://doi.org/10.3390/pathogens11020193>

Choi, G. B., Yim, Y. S., Wong, H., Kim, S., Kim, H., Kim, S. V., Hoeffer, C. A., Littman, D. R., & Huh, J. R. (2016). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science*, 351(6276), 933-939. <https://doi.org/10.1126/science.aad0314>

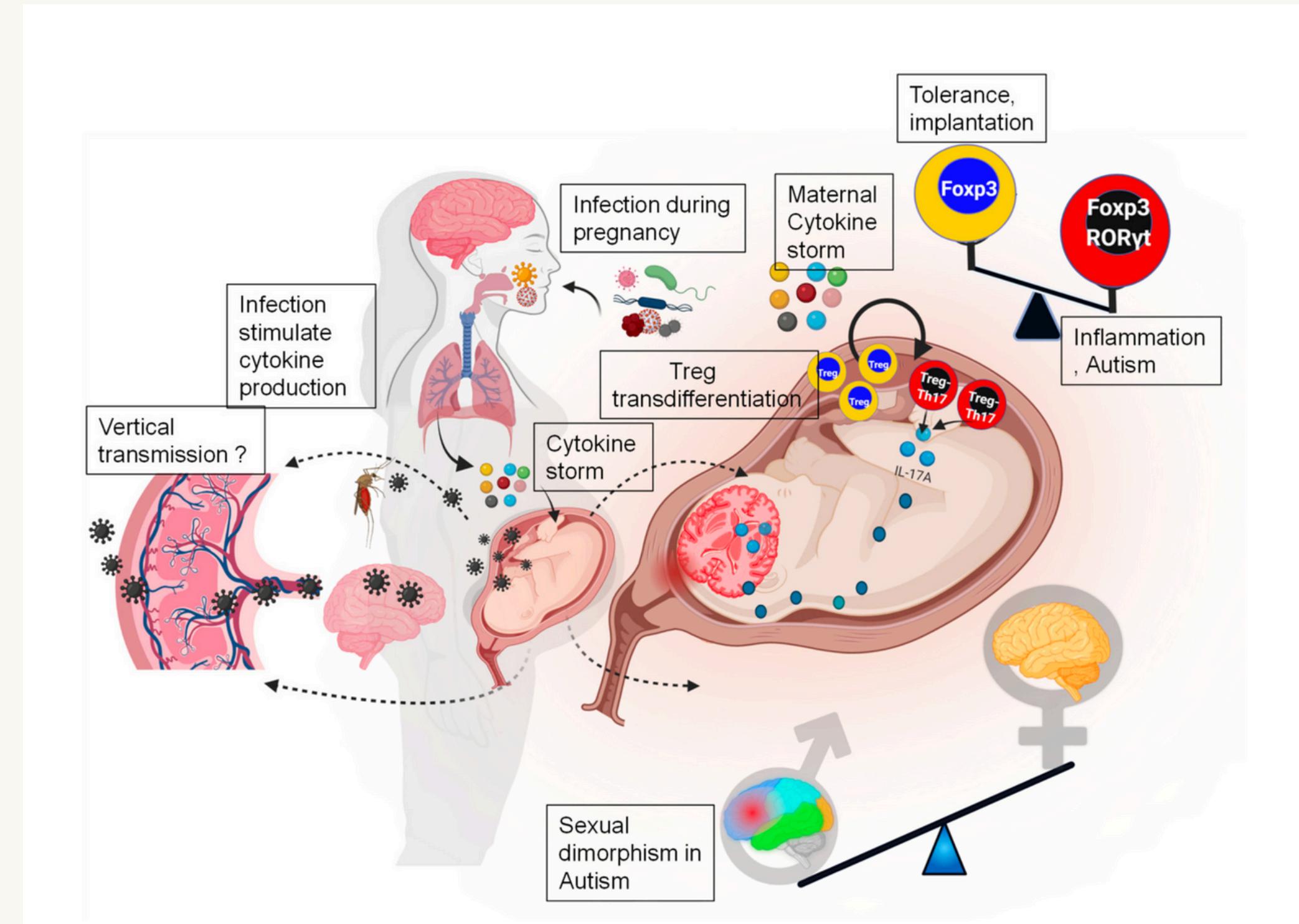
Wong, H., & Hoeffer, C. A. (2018). Maternal IL-17A in autism. *Experimental Neurology*, 299, 228-240. <https://doi.org/10.1016/j.expneurol.2017.04.010>

Wait, how about “sterile womb?”
How does the mother’s condition influence on the baby?

#Maternal-Fetal Interaction

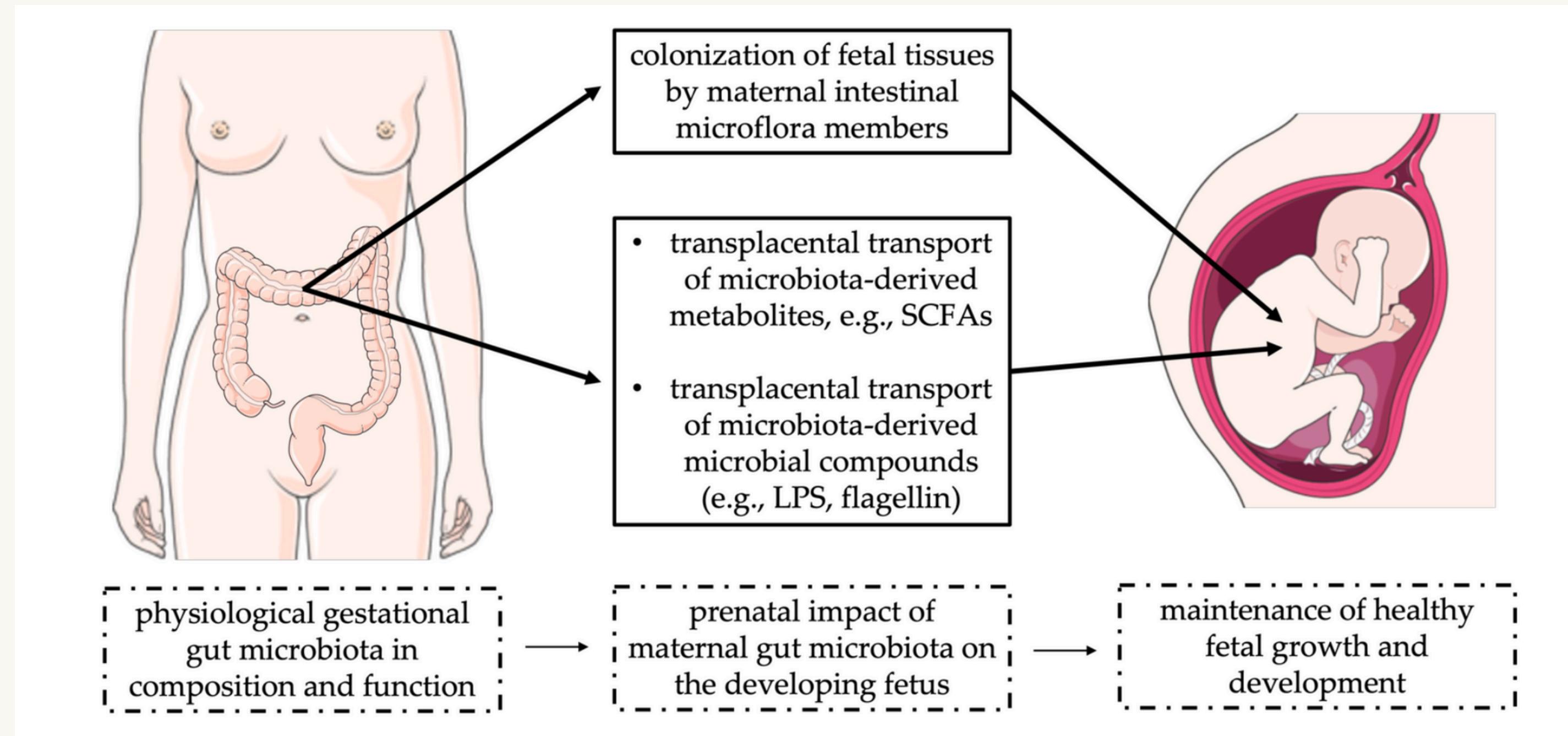
Where we are now

Maternal-Fetal Interface: Placenta(태반)



Jash, S., & Sharma, S. (2022). Pathogenic Infections during Pregnancy and the Consequences for Fetal Brain Development. *Pathogens* (Basel, Switzerland), 11(2), 193.
<https://doi.org/10.3390/pathogens11020193>

Maternal-Fetal Gut Microbiota Axis



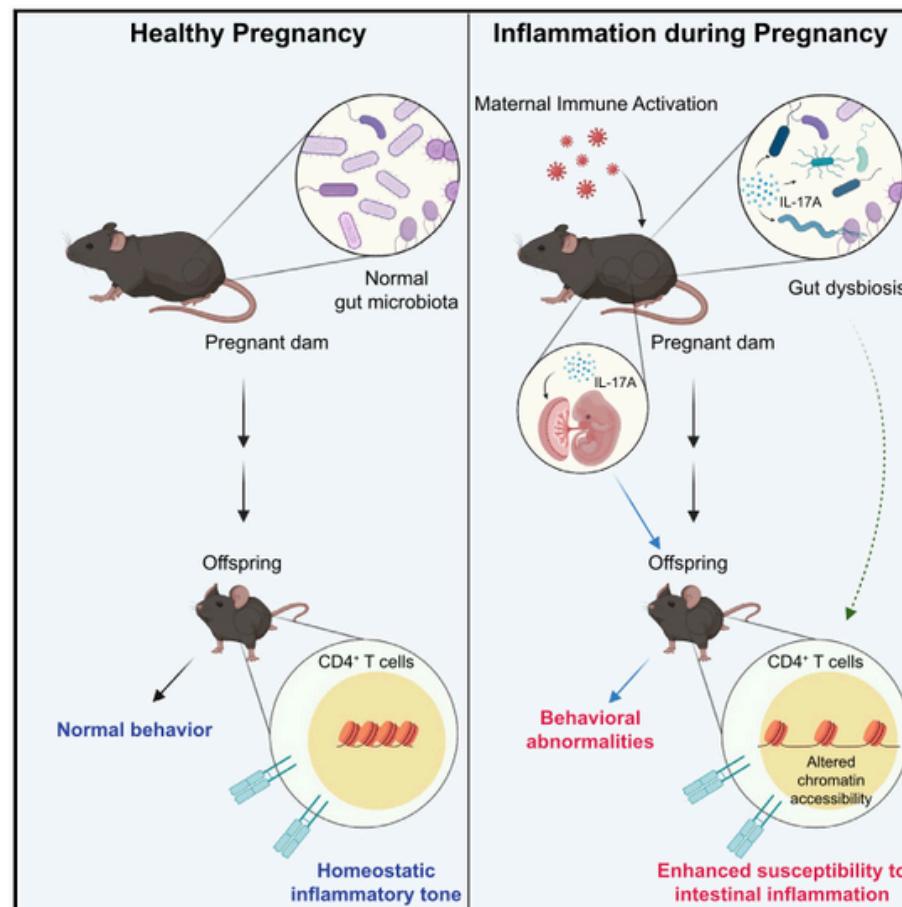
According to actual presumptions, microbes at the maternal site are translocated somehow from the intestinal epithelium into the bloodstream and then delivered to the placenta.

Article

Immunity

Maternal gut bacteria drive intestinal inflammation in offspring with neurodevelopmental disorders by altering the chromatin landscape of CD4⁺ T cells

Graphical abstract



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In brief

Children with autism spectrum disorders often display dysregulated immune responses. Using a preclinical mouse model manifesting neurodevelopmental pathologies following exposure to maternal immune activation, Kim et al. revealed that changes in the gut microbiota of pregnant mice affect chromatin accessibility of naive CD4⁺ T cells in their offspring, leading to immune-primed phenotypes.

Maternal gut bacteria alter the chromatin landscape of CD4+ T cells, which induce intestinal inflammation in offspring with neurodevelopmental disorders.

- 01 **Prenatal inflammation**
raises gut vulnerability.

- 02 MIA offspring's immunity
shaped **postnatally**.

- 03 **Maternal microbiota**
primes offspring's immunity.

- 04 MIA induces **inflammatory**
T cell differentiation.

- 05 MIA **programs**
inflammatory T cells.

- 06 **Maternal IL-17A**
affects offspring's immunity.



Rodent Model

Mice:

- C57BL/6 specific pathogen-free mice
- C57BL/6 Germ-free(GF)
- RAG1-deficient mice
- CD45.1 B6. cogenic mice
- IL-17A-GFP reporter mice

Injection for maternal immune activation (MIA):

- Experimental group: Poly(I:C)
- Control group: Phosphate-buffered saline (PBS)

Infection: *Citrobacter rodentium* (*C. rodentium*)

Result Summary

We are here!



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- 05 MIA programs inflammatory T cells.
- 06 Maternal IL-17A affects offspring's immunity.

Have to know

- 01 IL-6, IFN- γ , TNF, IL-17A
- 02 colon length
- 03 Fox P3 + Treg
- 04 Immune-primed phenotype
- 05 *C. rodentium*

Figure1 MIA vs. PBS

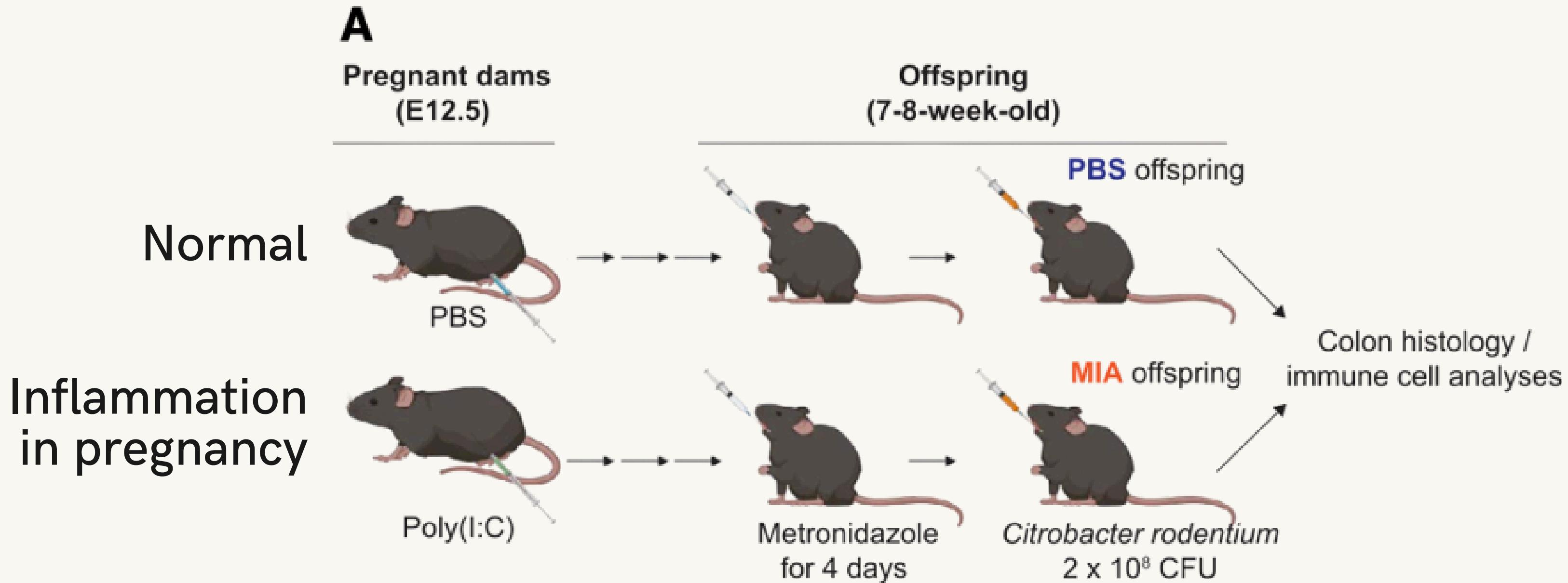
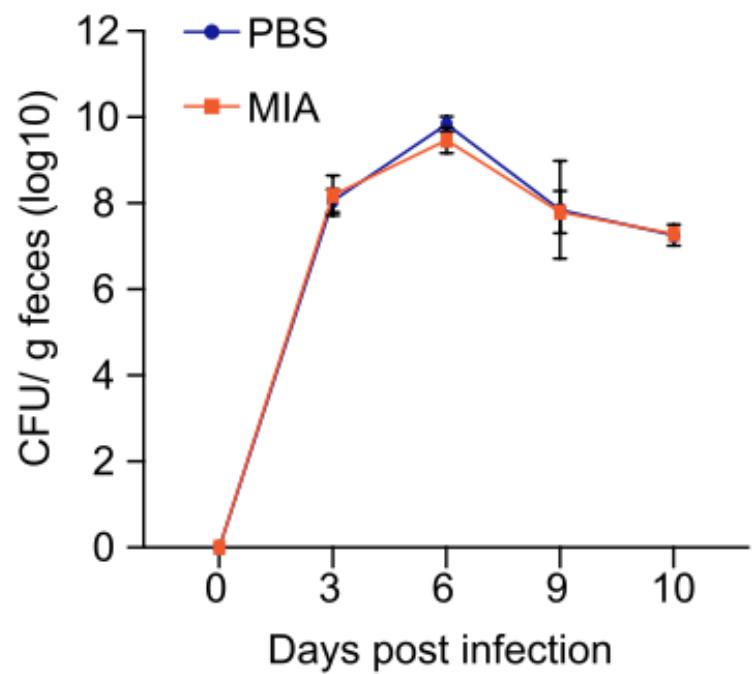
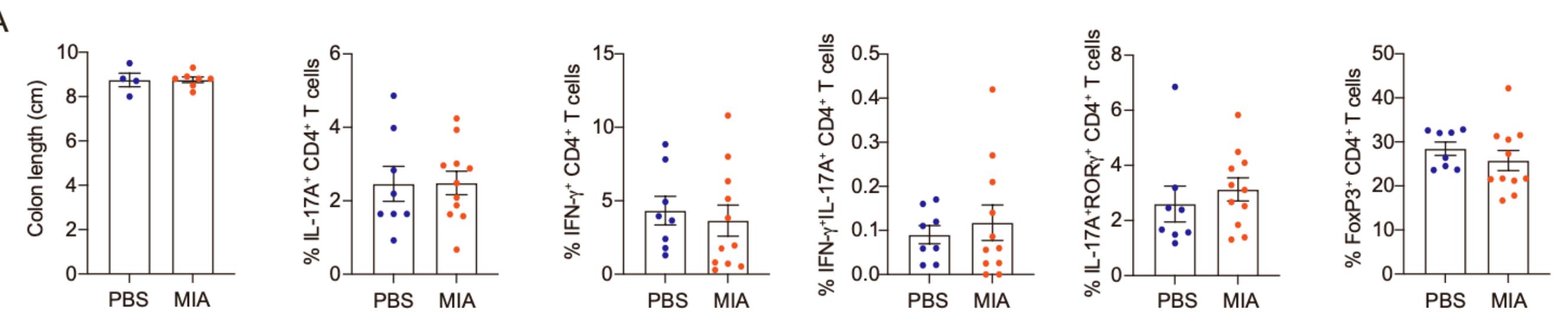


Figure 1. MIA vs. PBS

B

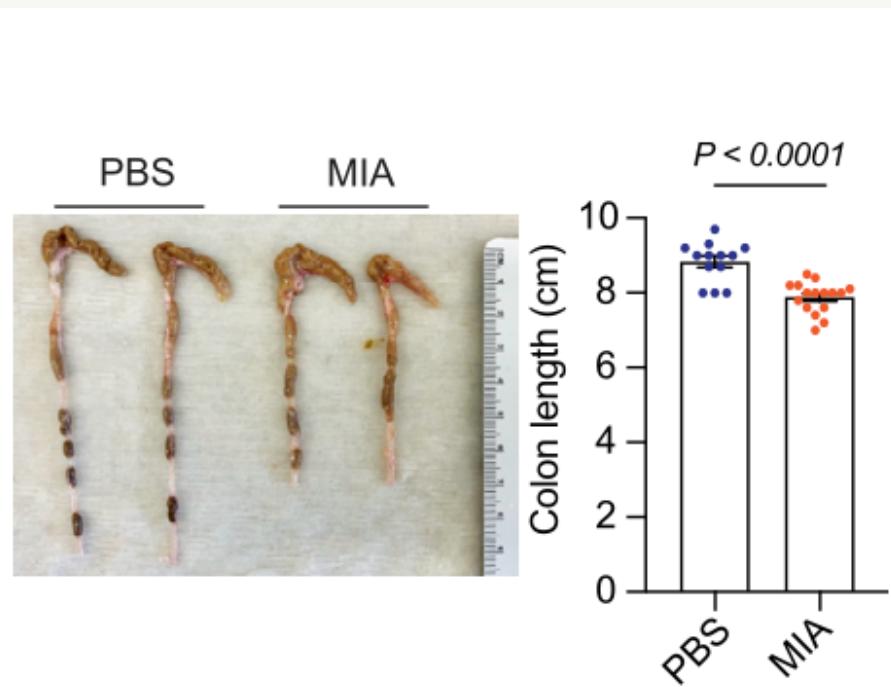


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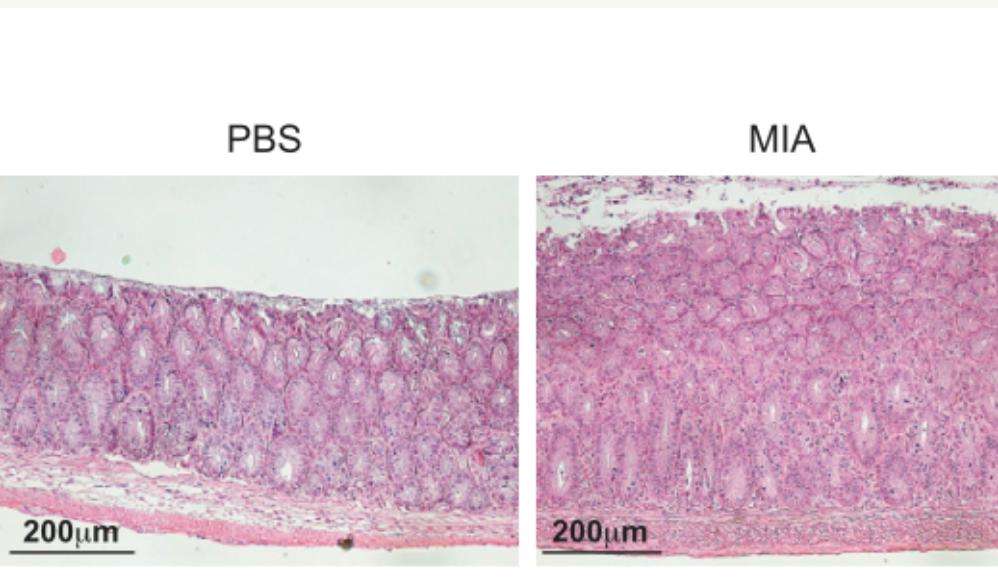


Before 10 days

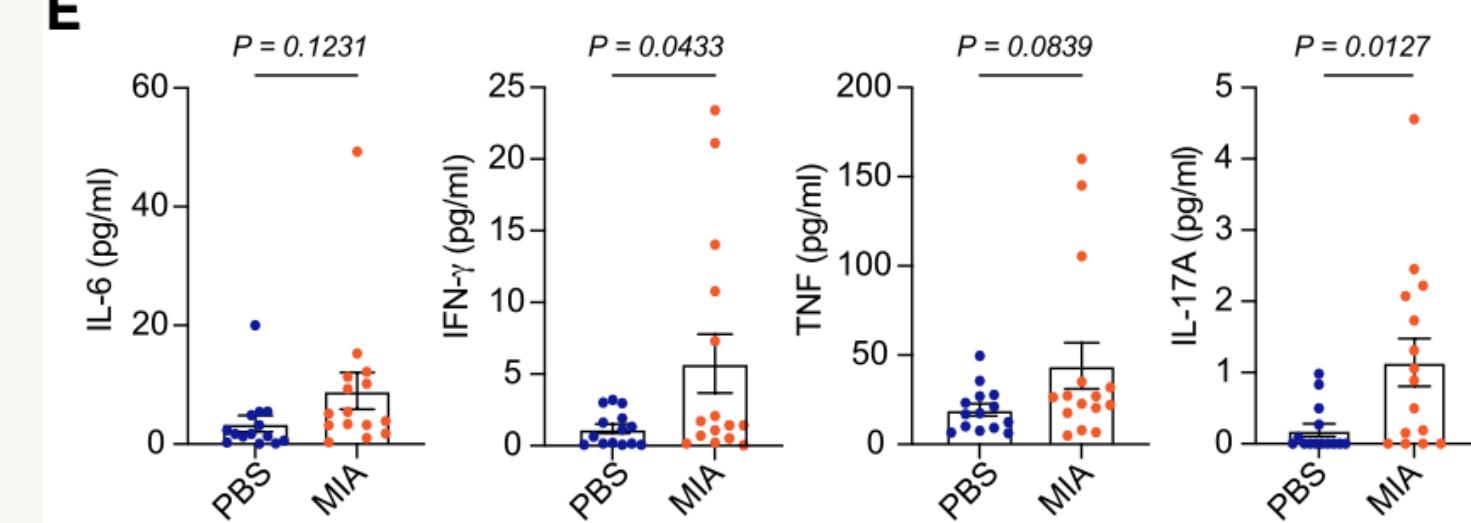
C



D



E



After 10 days

Figure1 MIA vs. PBS

Flow Cytometry

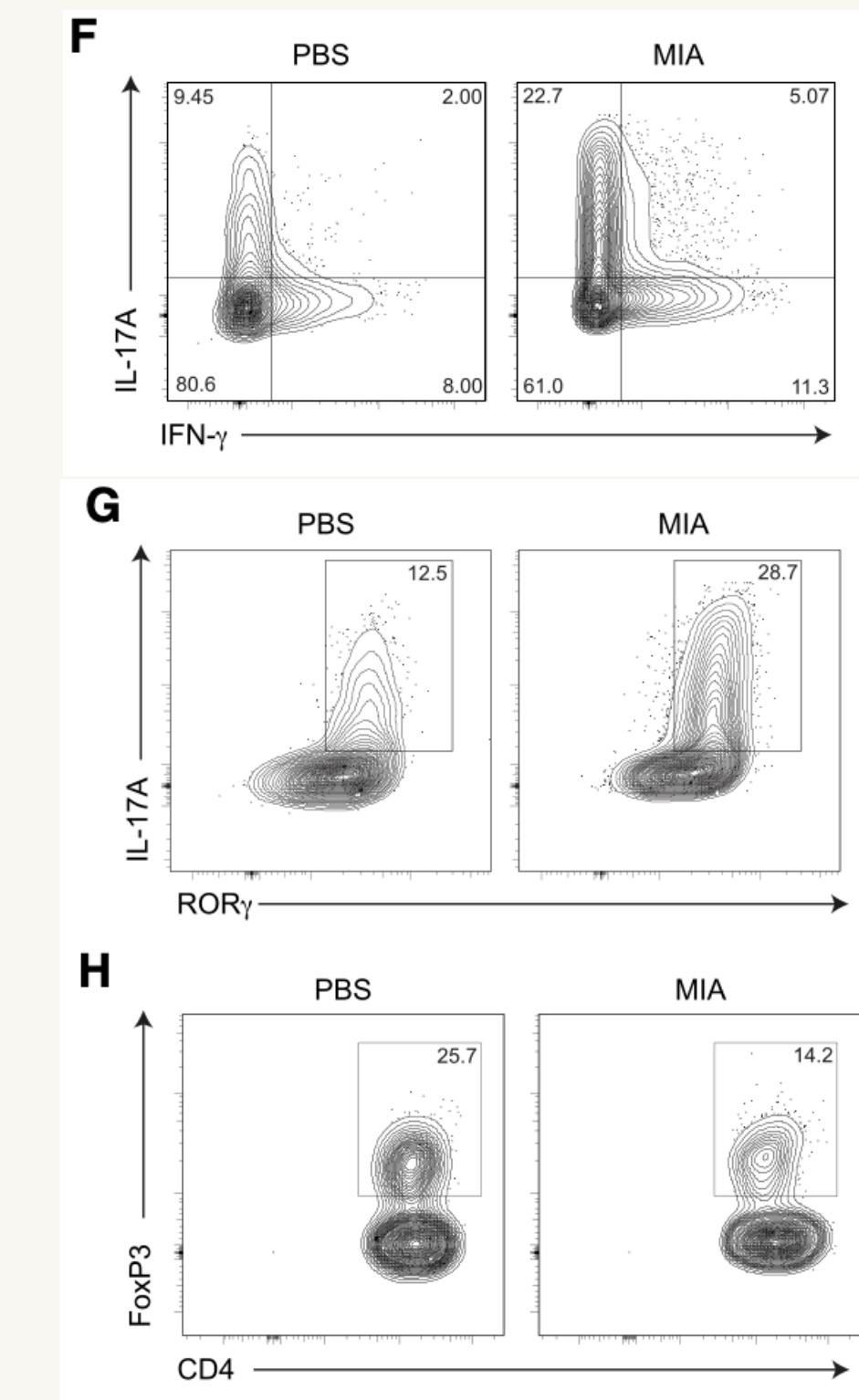
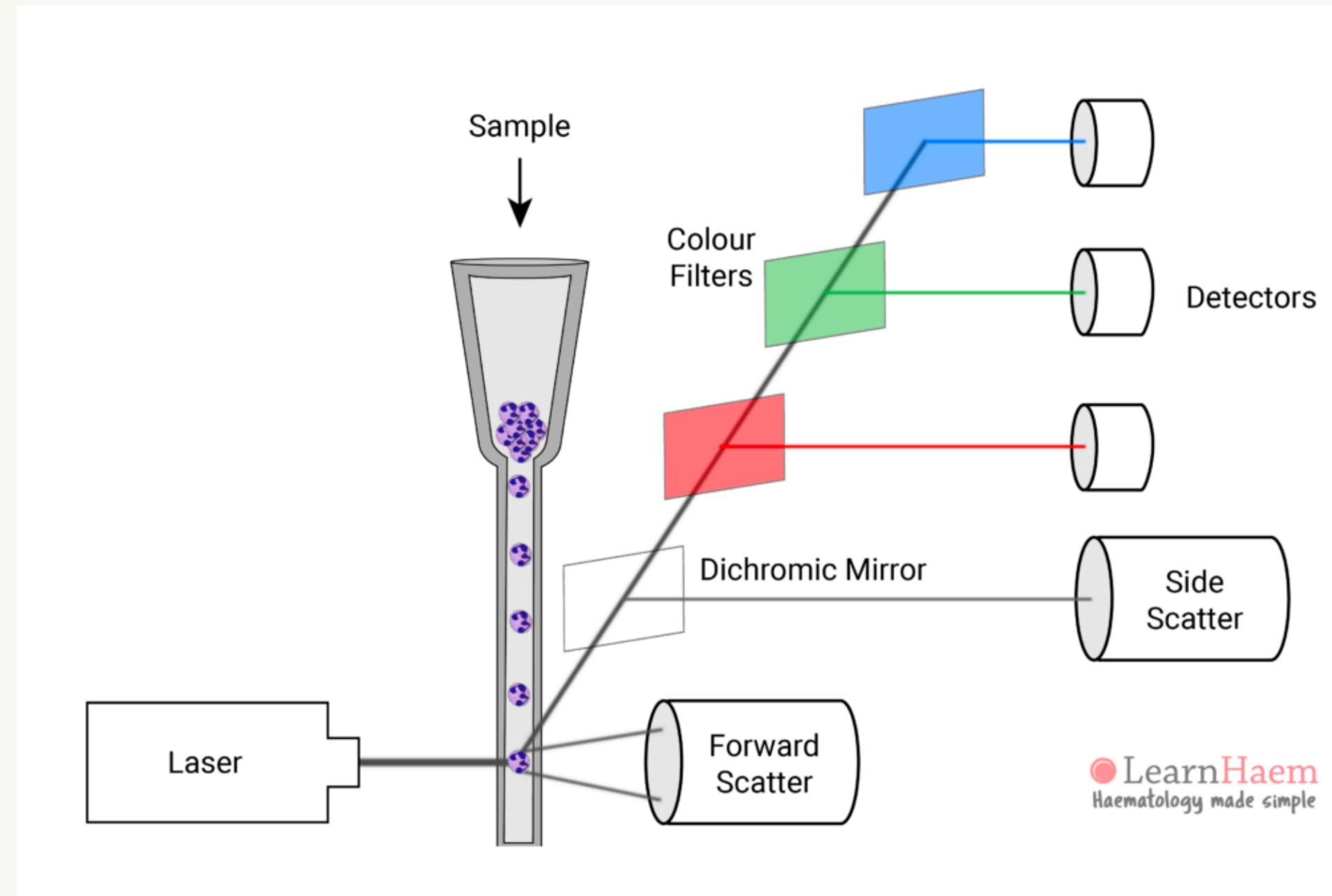
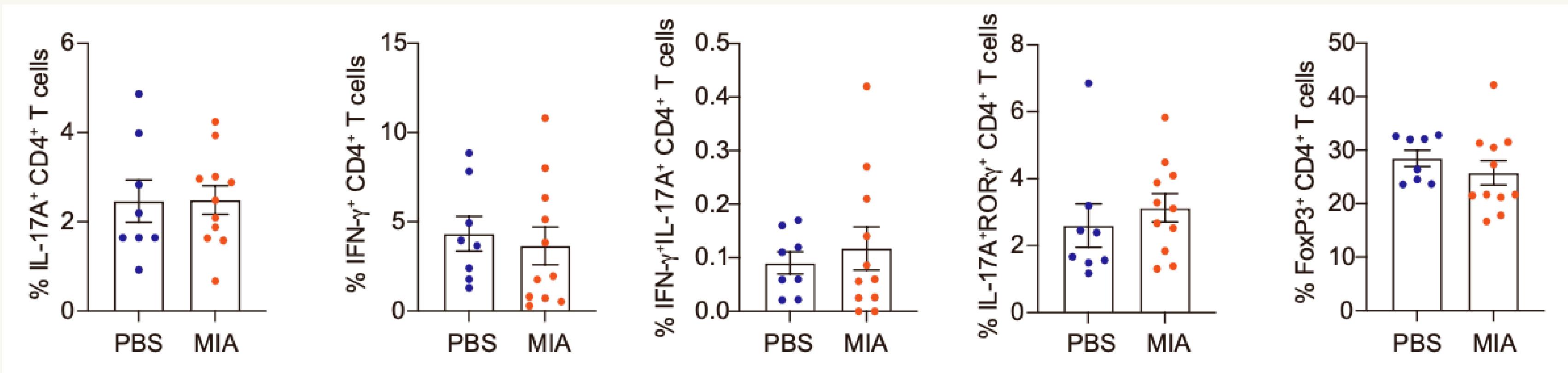
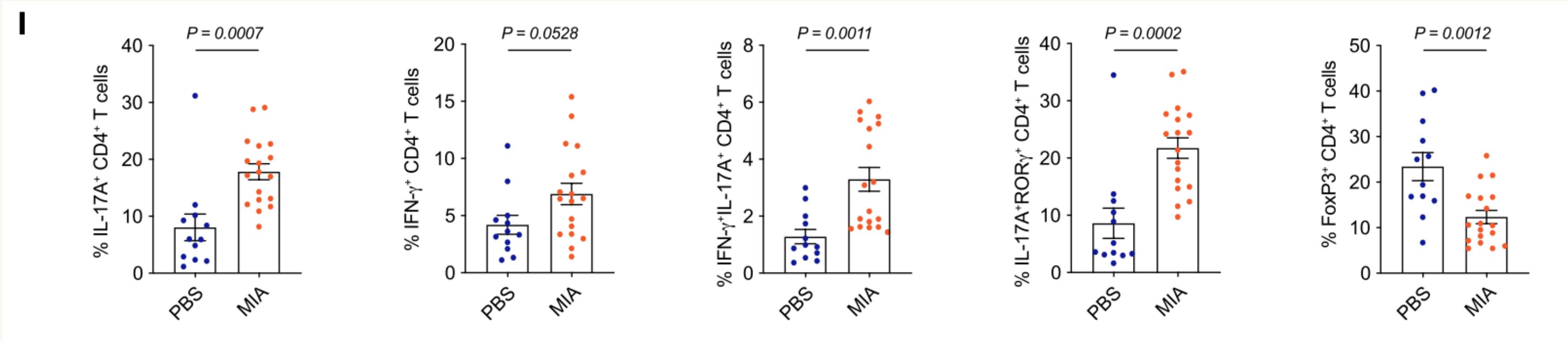
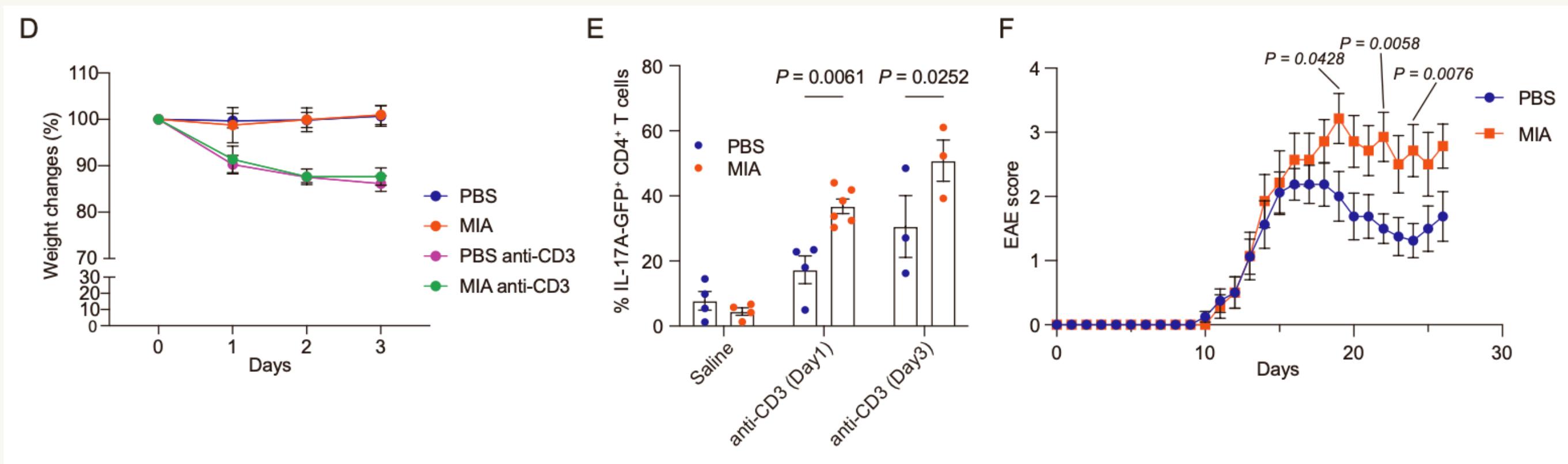


Figure1



FigureS2D-F

Figure1 MIA vs. PBS



FigureS3D-F

Anti-CD3

Result Summary

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Figure2 MIAo-PBSd vs. PBSo-MIAd

A

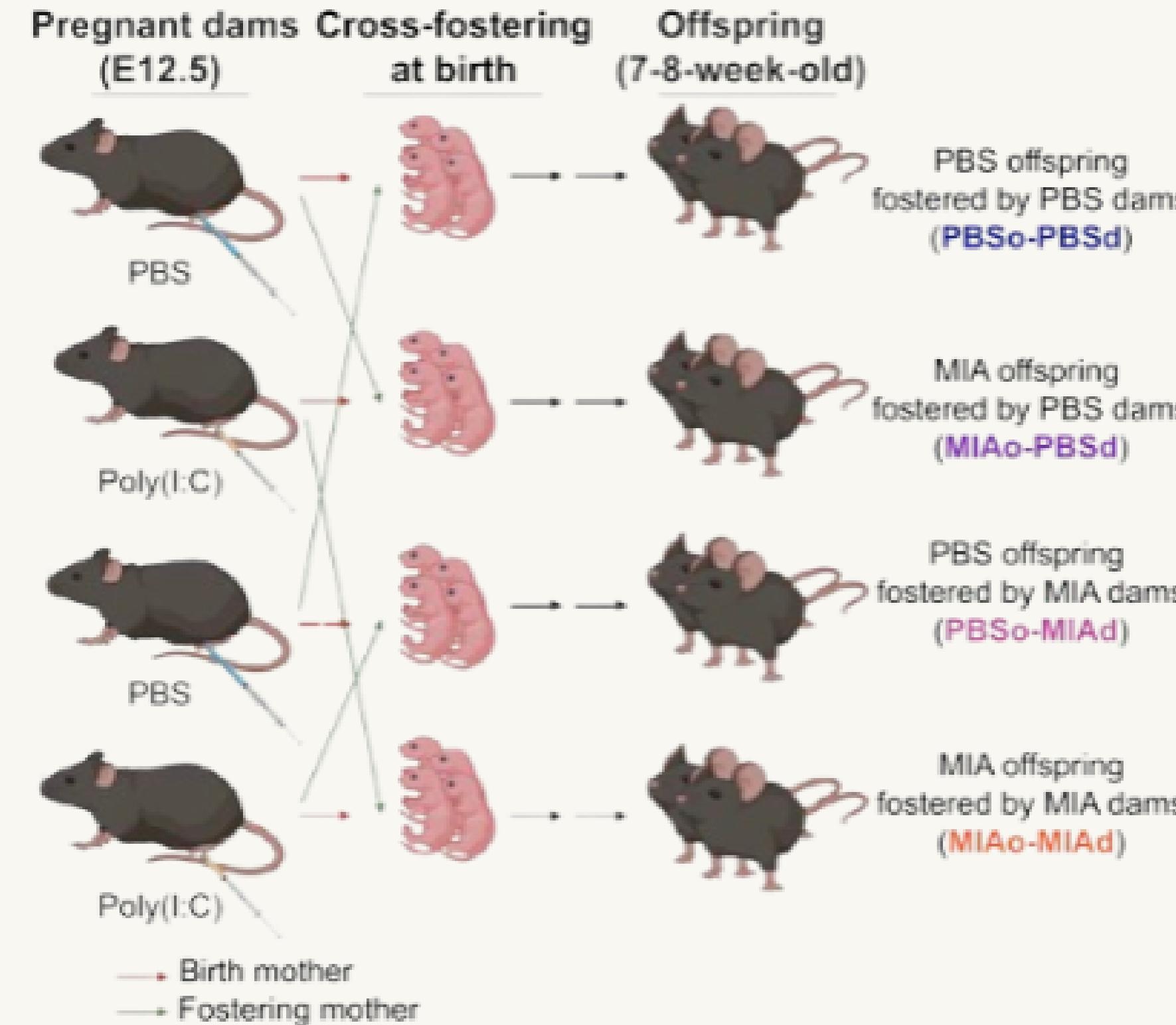
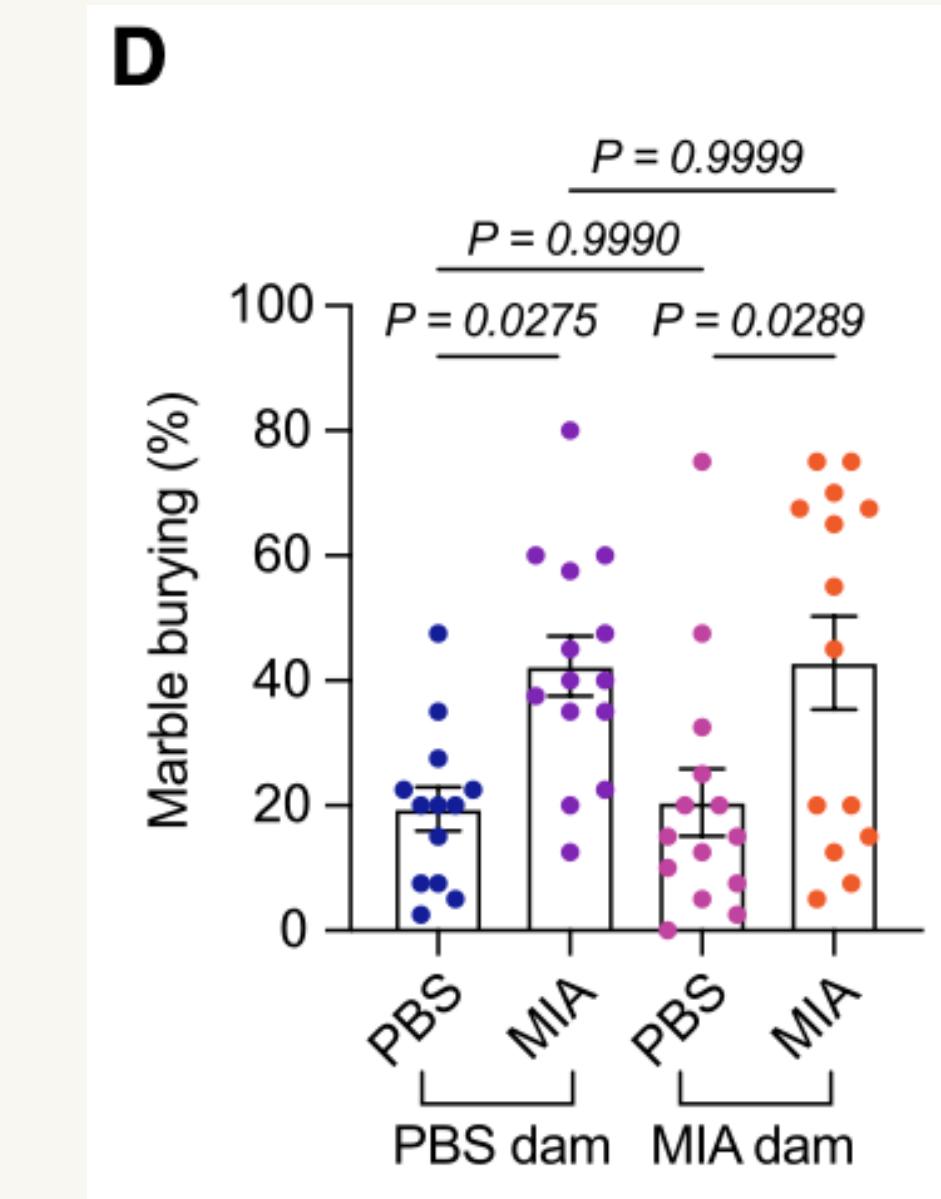
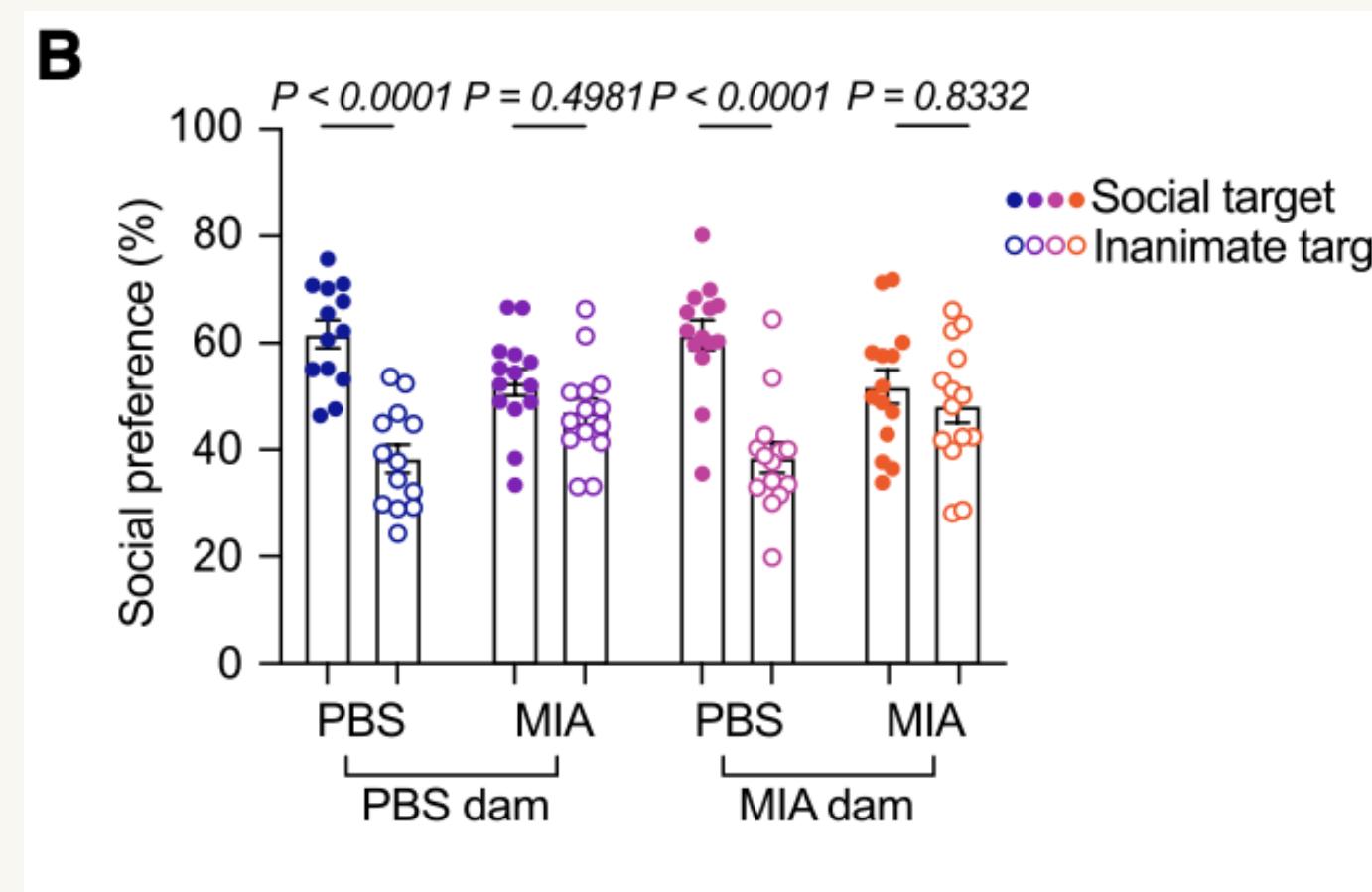


Figure2 MIAo-PBSd vs. PBSo-MIAd

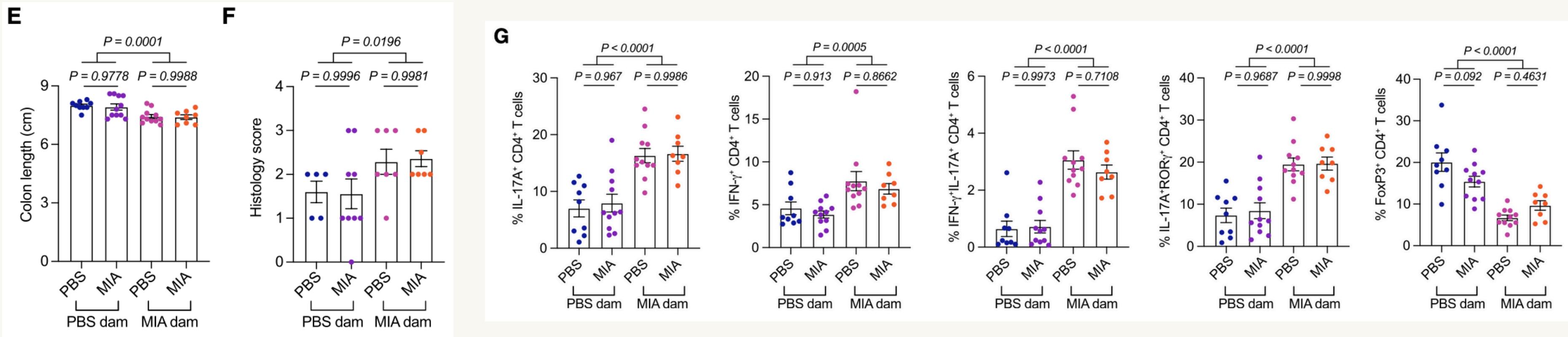
When are behavioral abnormalities determined?



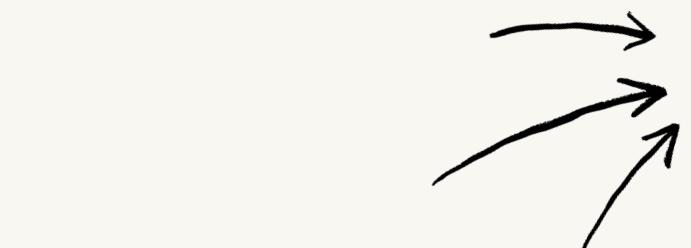
Regardless of their fostering mom,
offspring experienced Poly(I:C) infection shows significant ASD-like behavior

Figure2 MIAo-PBSd vs. PBSo-MIAd

When is the immune-primed phenotype determined?



Result Summary



We are here!

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Result 3

01 Composition of gut bacteria change

MIA induces changes in the composition of gut bacteria in pregnant dams.

02 Immune-primed phenotypes of MIA offsprings

MIA-associated changes contribute to the maternal gut microbiota in producing the immune-primed phenotypes of MIA offspring.

03 Microbiota vs. single species colonization for phenotype change

Single bacterial species are not sufficient to change phenotypes:

Result 3

01 Composition of gut bacteria change

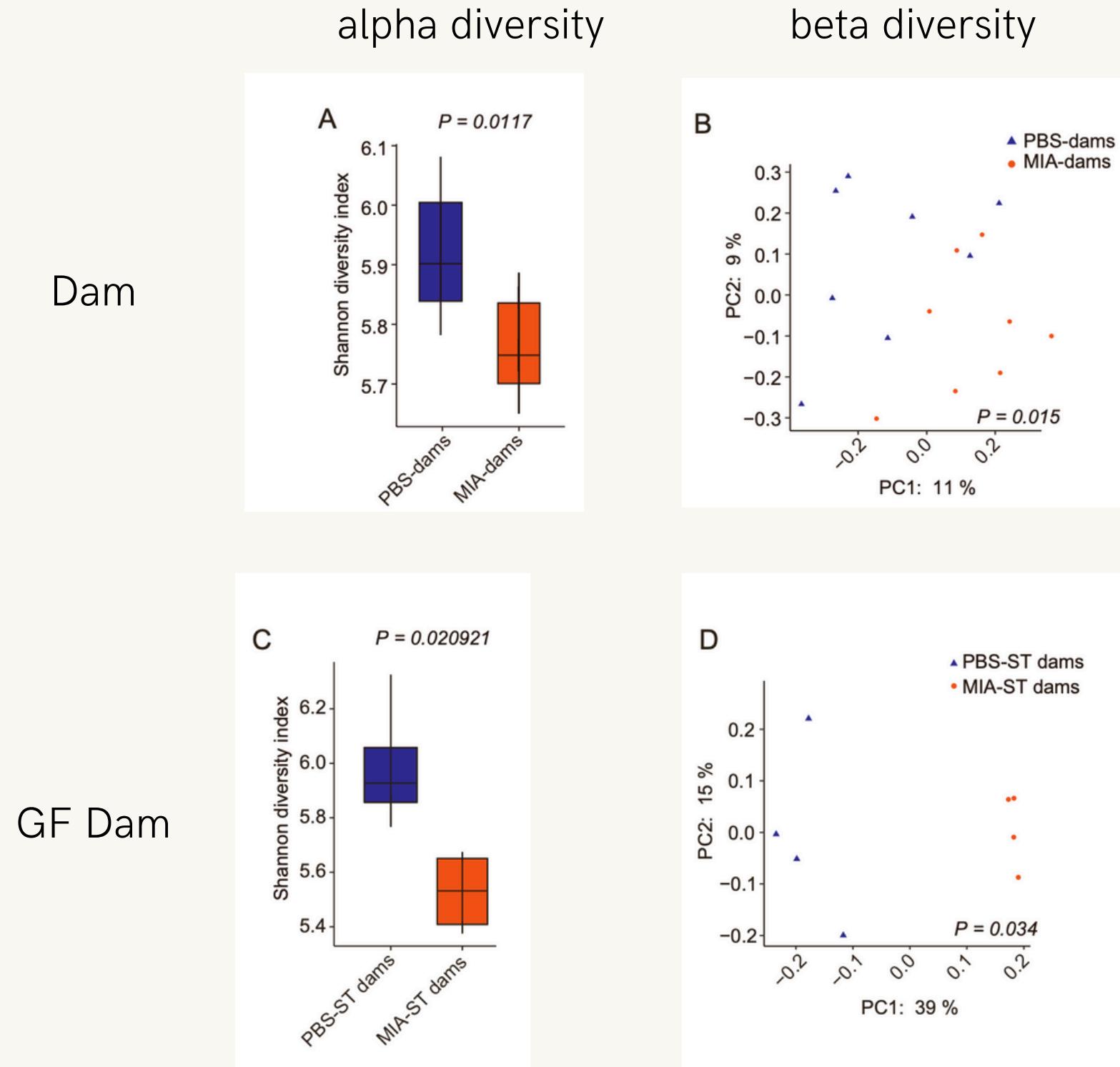
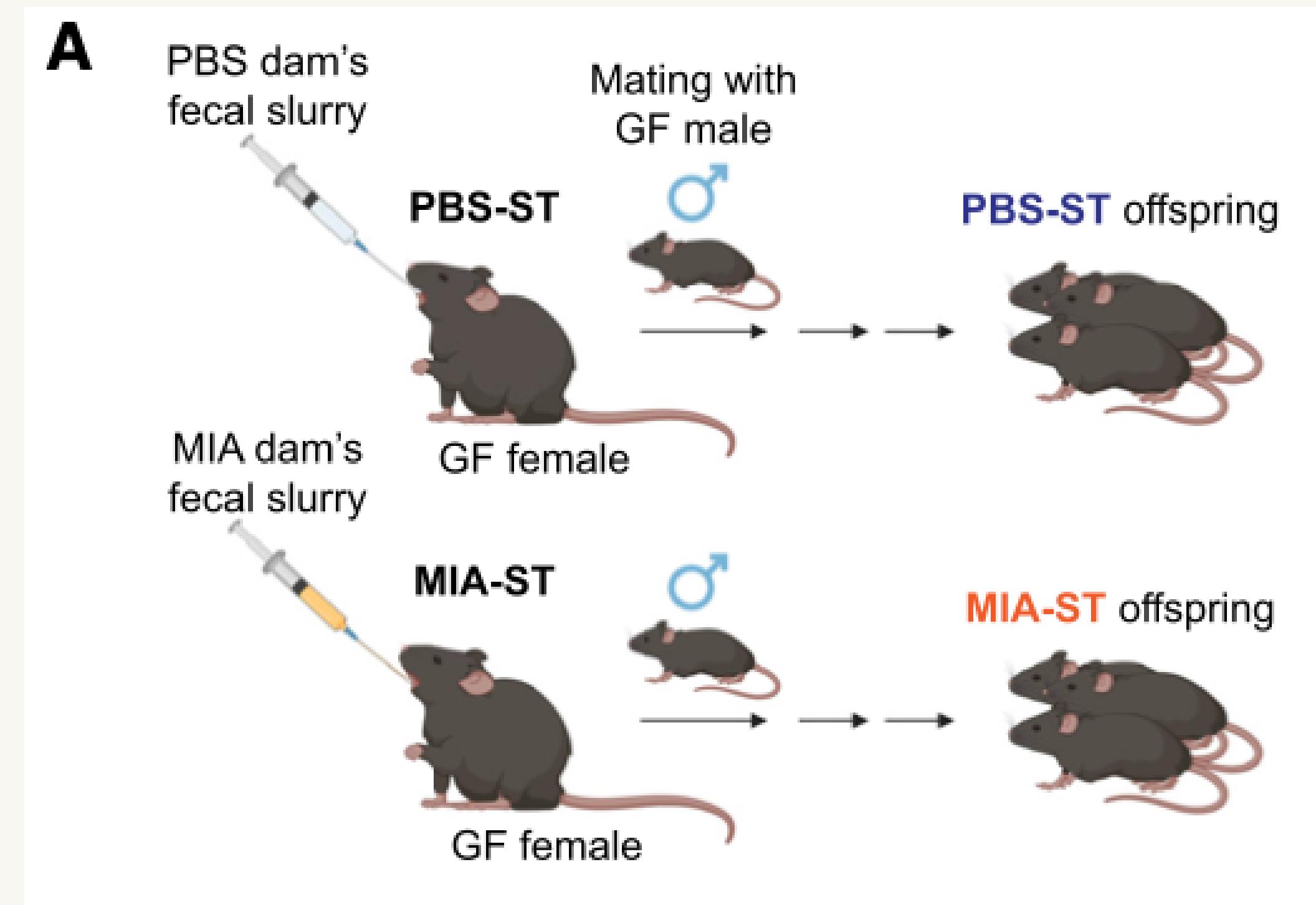


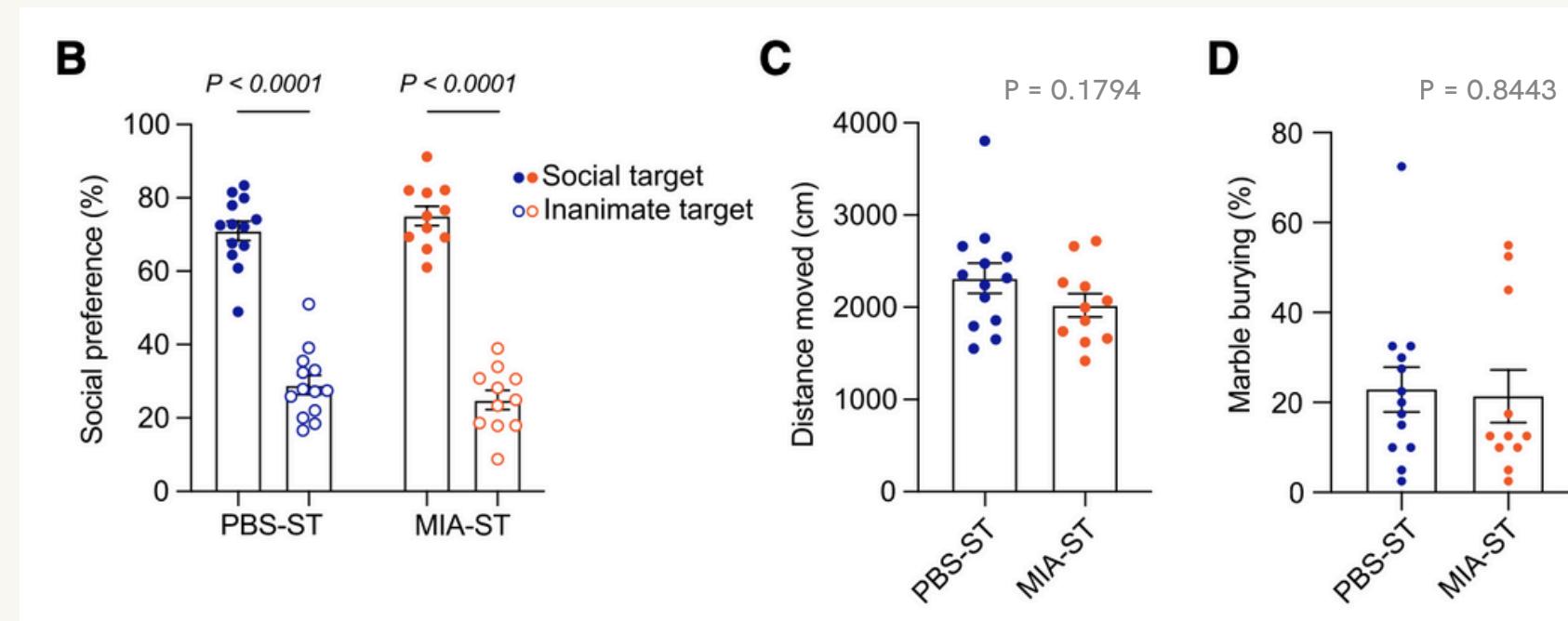
Figure S4A,B,C,D

Gut microbial communities of PBS and of MIA are different.



Result 3

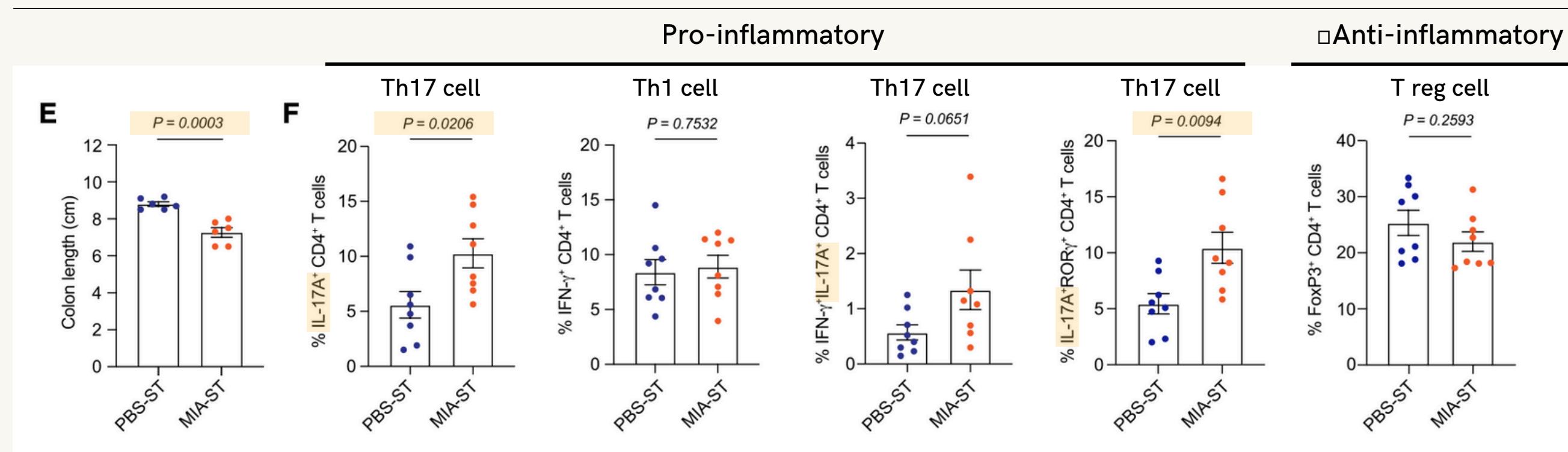
02 Immune-primed phenotypes of MIA offsprings



01

MIA does not make significant behavioral abnormality in GF model

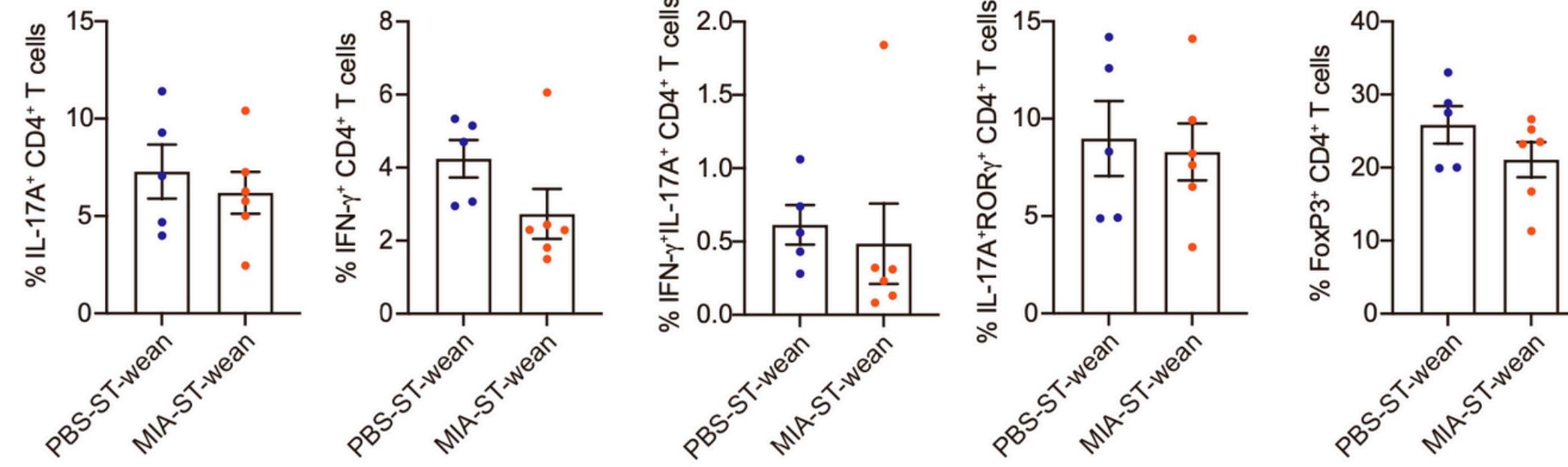
Figure 3B,C,D



02

Similar in Th17 but not in the inflammatory phenotypes, showing by relatively high IL-17A and colon shortening.

Figure 3E,F

G**Figure S4G**

After weaning, no significant difference ($p > 0.05$) in inflammatory phenotypes between PBS-ST and MIA-ST

-> Existence of developmental time



**B6 pregnant dam +
mono-colonized with segmented filamentous bacteria(SFB)**

SFB: promote Th17 cell differentiation and play a crucial role
in the development of behavioral abnormalities in MIA offspring

Result 3

03 Microbiota vs. single species colonization for phenotype change

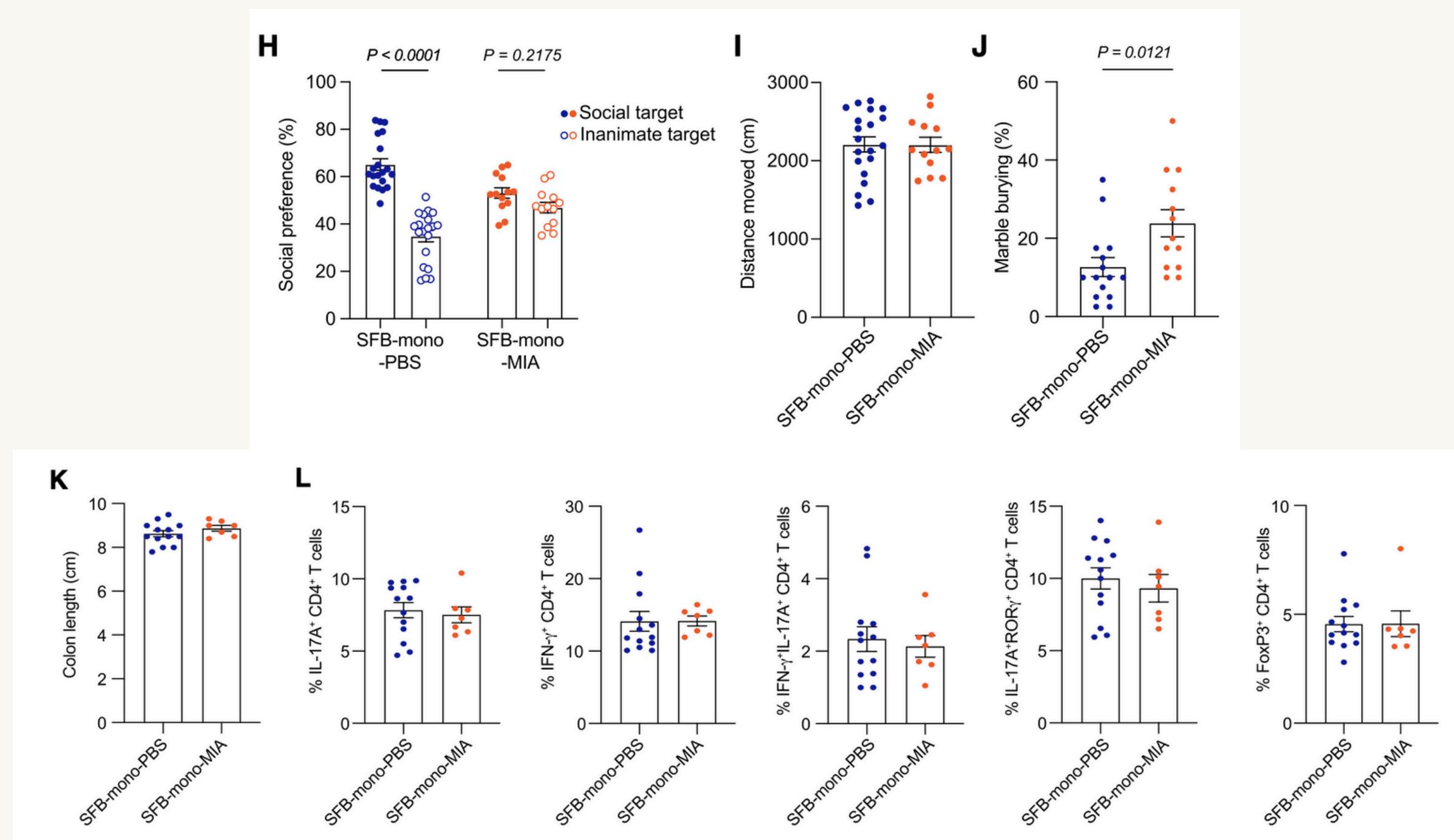


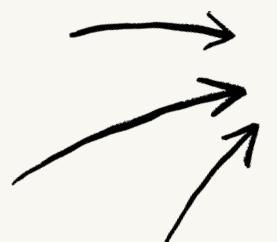
Figure 3H, I, J, K, L

Mono-colonization does not change the immunological phenotypes, although the injected SFB has the ability to increase T cell responses and induce behavioral phenotypes.

Immune-primed phenotypes in MIA offsprings
are influenced by **maternal gut microbiota**.

Result Summary

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Observed transcriptomic changes:

Result 4

How maternal immune activation (MIA) impacts the immune-primed nature of CD4+ T cells in offspring

01 Gene expression

increased expression of genes associated with T cell activation

02 Immune signaling pathways

specific immune signaling pathways like MAPK activity, IL6-JAK-STAT3 signaling

03 Th17 cell differentiation

Single bacterial species are not sufficient to change phenotypes
methods:



RAG1 Deficient Mouse

[*#compromised immune system*](#) [*#increased susceptibility to infections*](#)

RAG1 (Recombination Activating Gene 1): a gene that encodes the RAG1 protein, which is responsible for generating the diverse repertoire of antigen receptors on B and T cells.

Mutations or deficiencies in the RAG1 gene can lead to severe combined immunodeficiency (SCID)

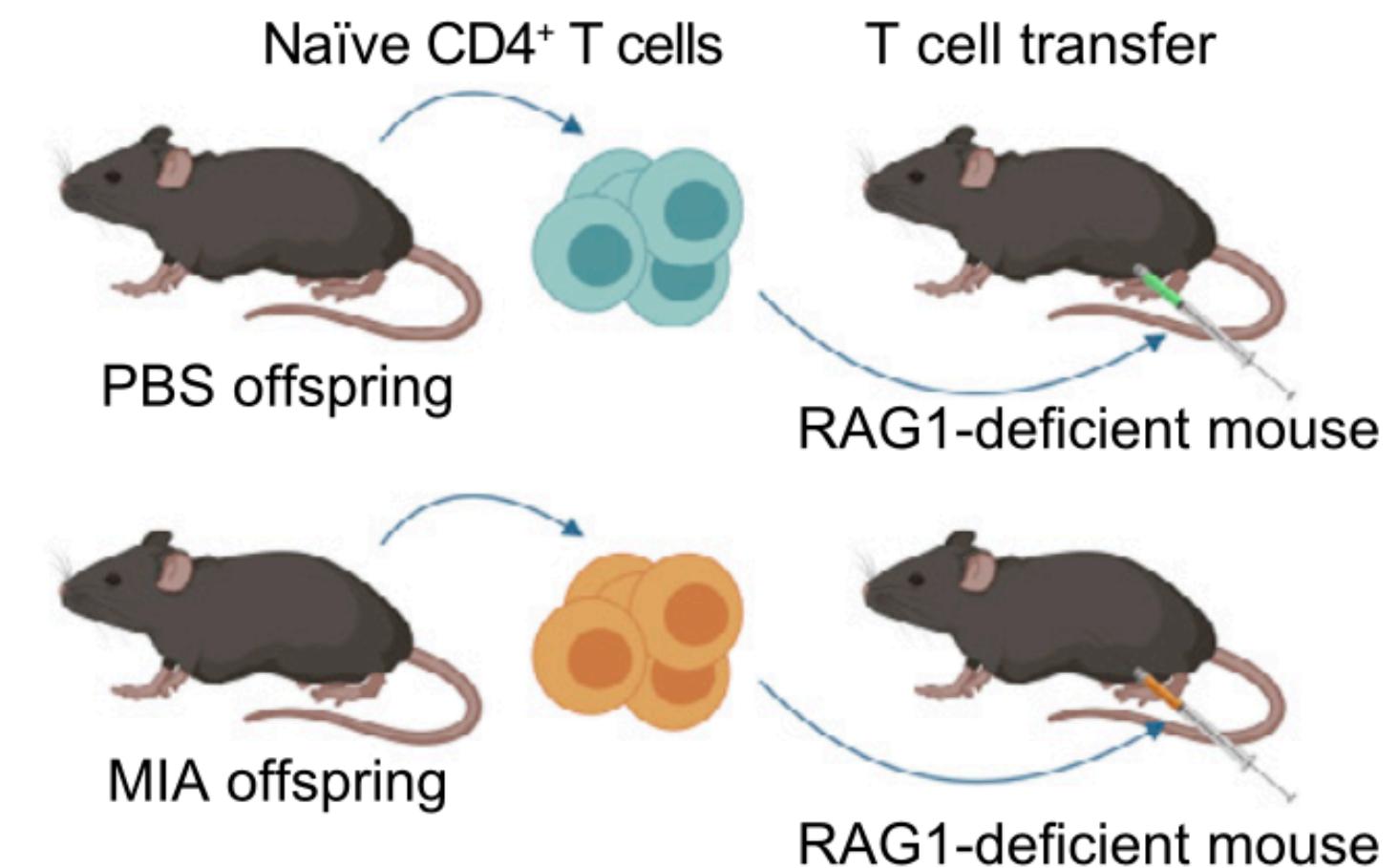
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Figure 4A

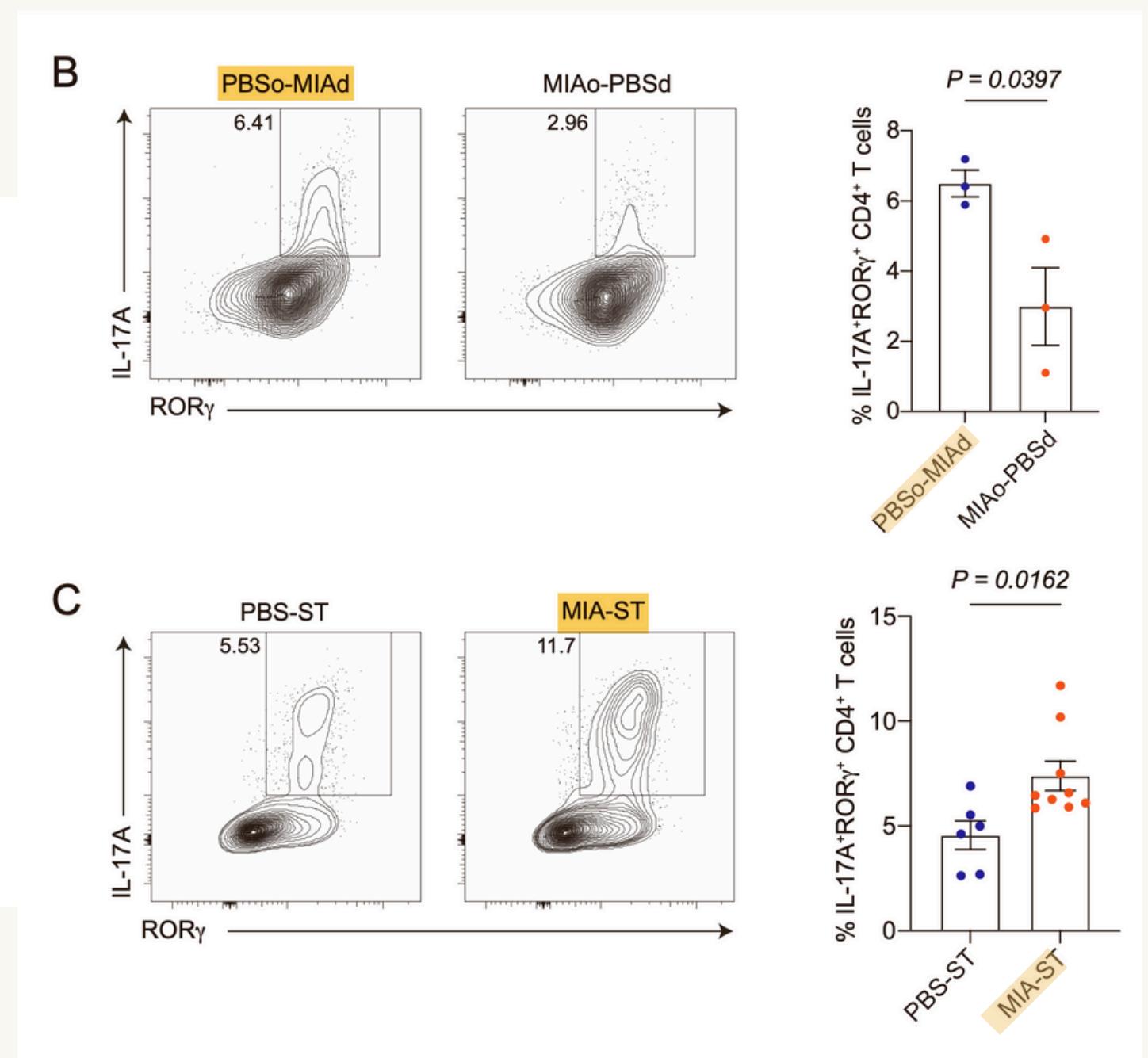
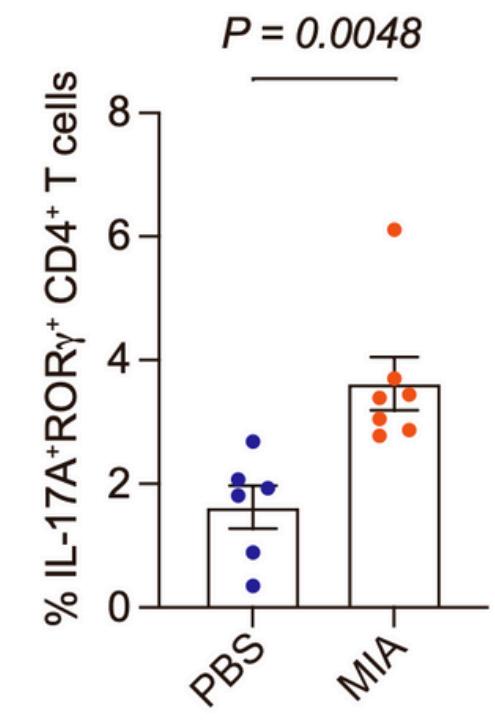
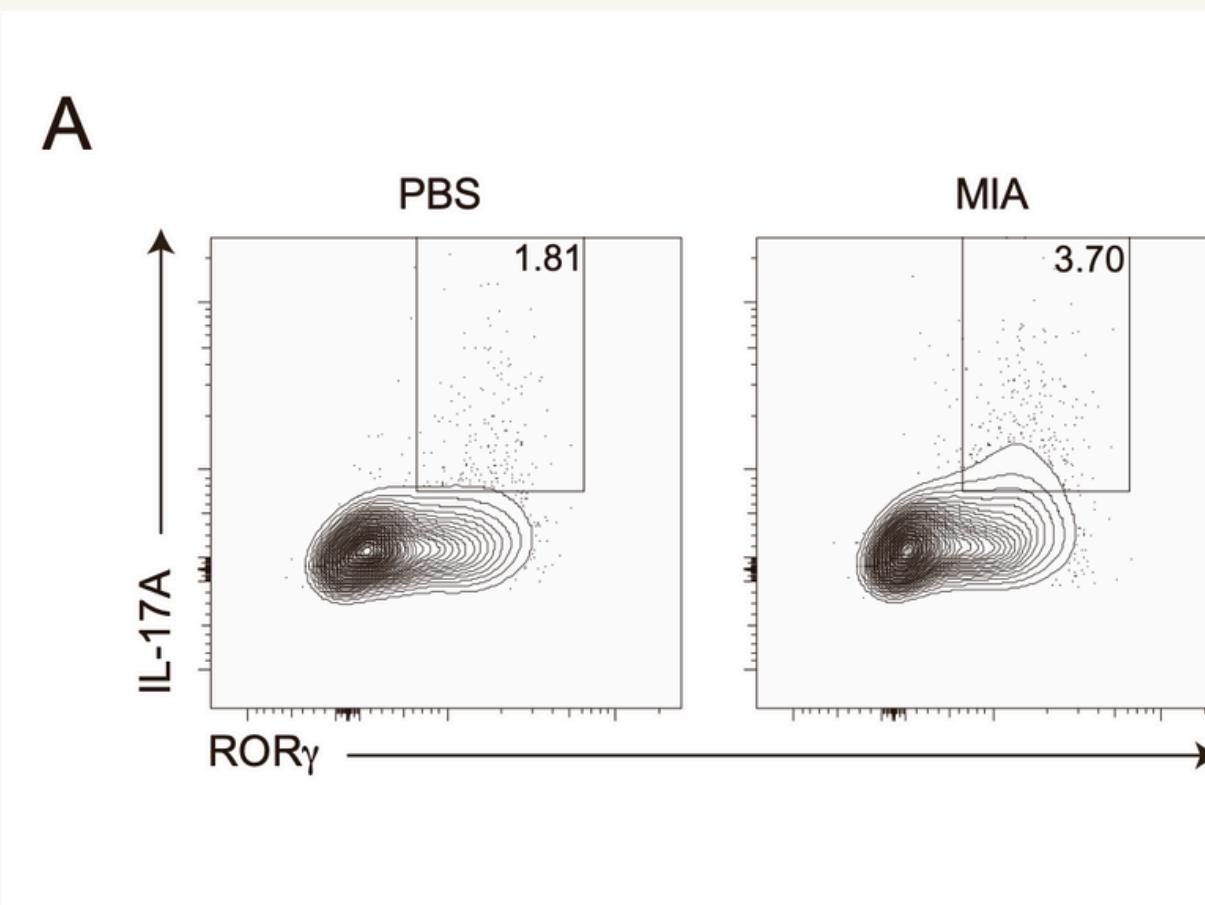
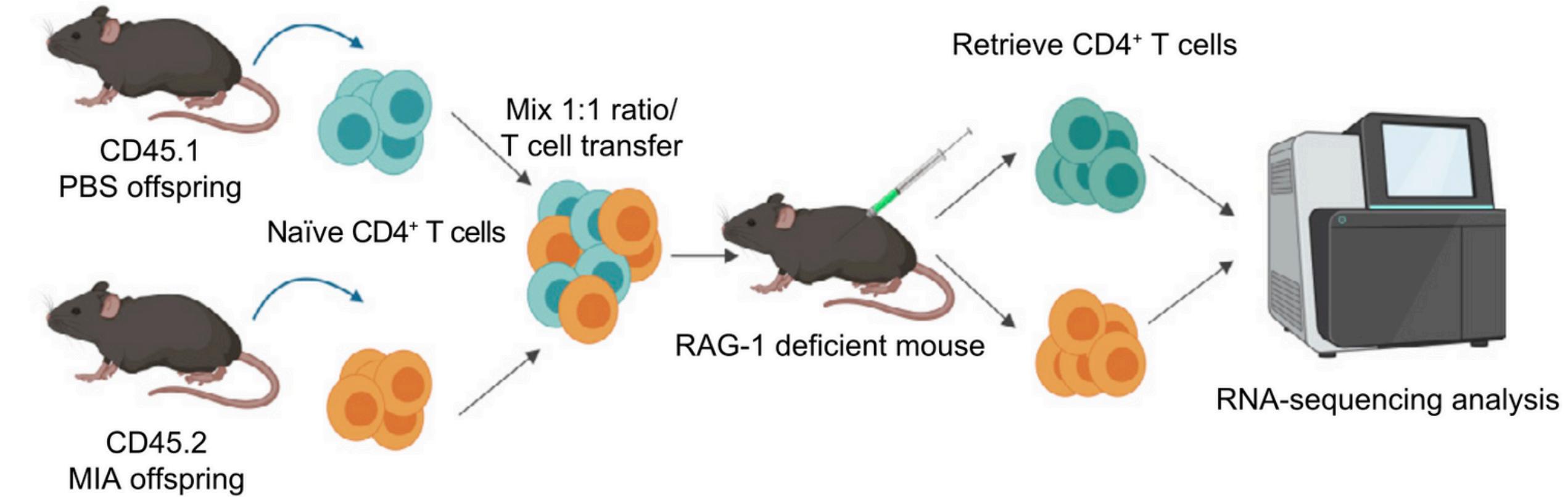
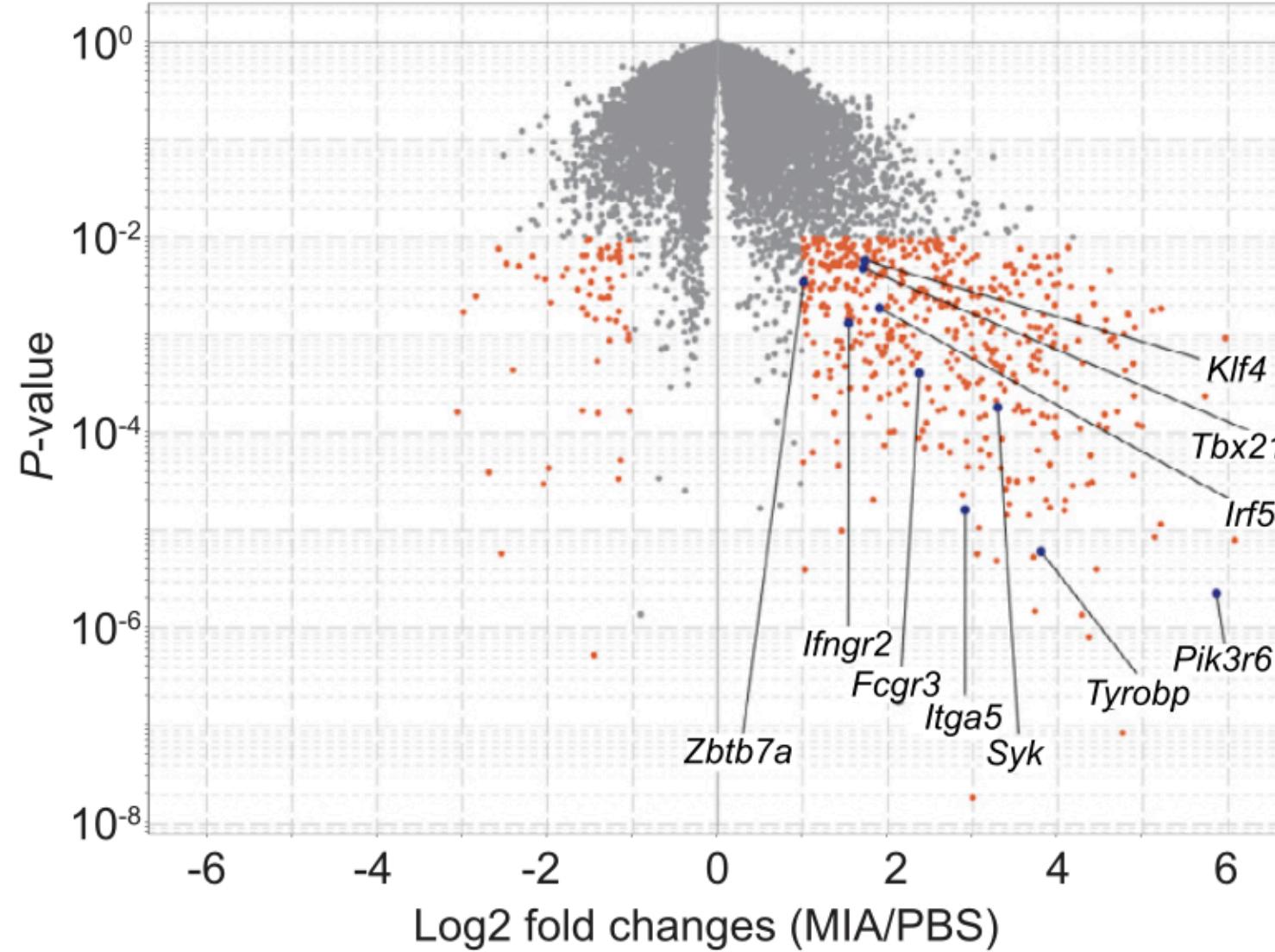


Figure S5A,B,C

E**Figure 4E**

F

- **Immune cell function**

- **Tbx21**: TF
- **Zbtb7a**: TF
- Tyrobp
- **Tbx21**: TF

- **Signaling pathways**

- Fcgr3: Receptor
- Ifngr2: Receptor
- Syk: Kinase
- Pik3r6: Part of a Signaling Pathway

- **Gene regulations**

- **Zbtb7a**: TF
- **Tbx21**: TF
- Ifngr2: Receptor
- Irf5
- Klf4
- (Itga5)

Figure 4F

Fold change(FC) = treatment / control

FC: the direction and strength of the change(regulation)

P-value: how significant the change is

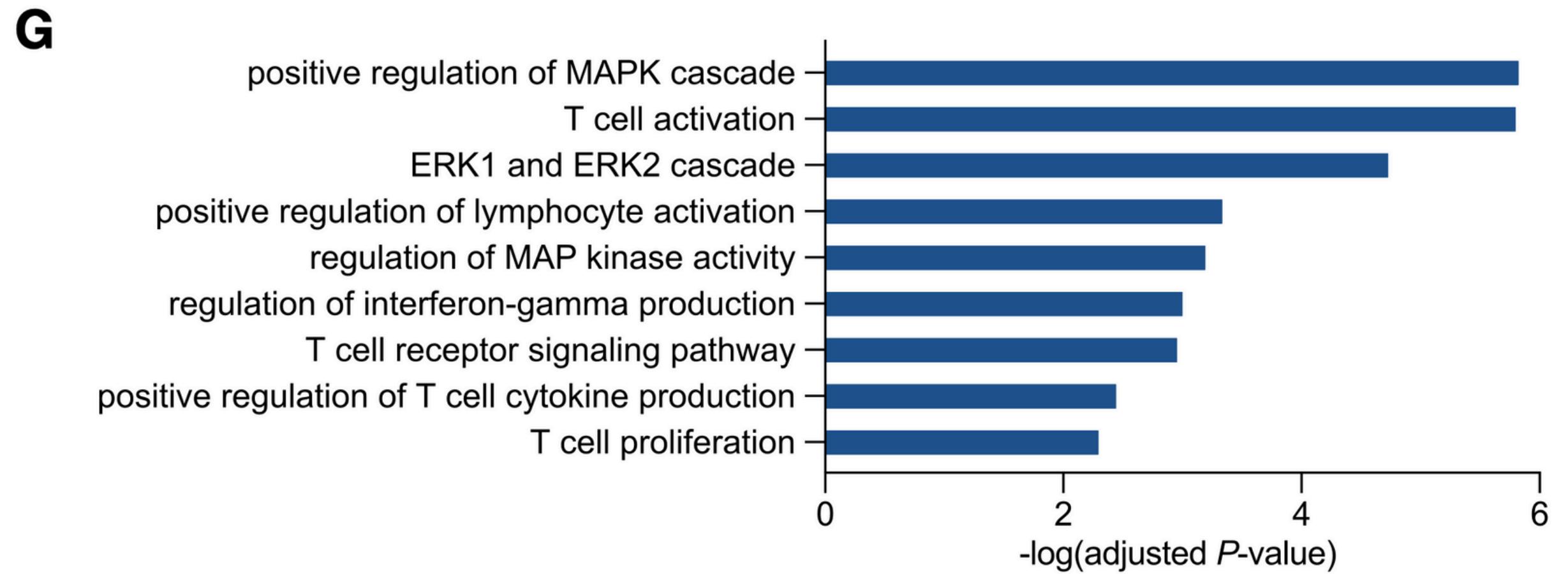


Figure 4-G

GO (gene ontology): a structured model that makes annotation of gene's biological process, molecular functions, and cellular component.

gene set

Enrichment Score

같은 biological pathway/activity를 가진 유전자들 set

up-regulated gene ----- down-regulated gene

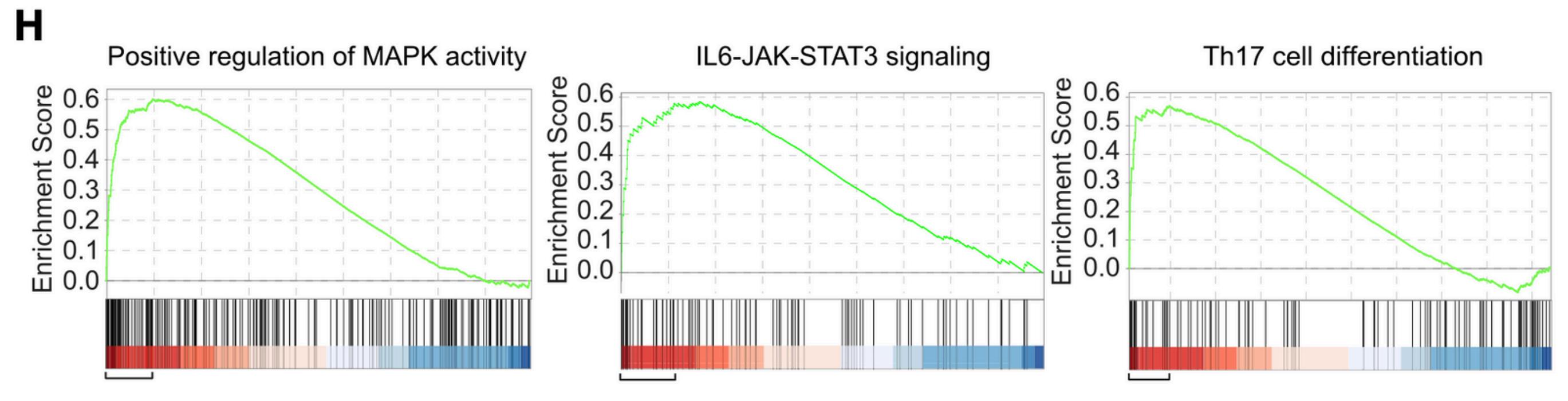


Figure 4H

The genes that induce MAPK activity, IL6-JAK-STAT3 signaling, and Th17 cell differentiation are up-regulated.

Kinase activity

Change in kinase activity allows the primed immune status of CD4+ T cells to respond more effectively when exposed to specific conditions or stimuli.

1. T Cell Activation
2. T Cell Receptor Signaling
3. Inflammatory responses
4. Chromatin Remodeling
5. Immune Priming

IL6-JAK-STAT signaling

The IL-6 (Interleukin-6) signaling pathway, mediated through JAK (Janus kinase) and STAT (Signal Transducer and Activator of Transcription) proteins

- 1. Role in Inflammation**
- 2. Immune System Activation**
- 3. JAK-STAT Signaling**
- 4. Involvement in Disease**
- 5. Th17 Cell Differentiation**

Result 4.

MIA CD4+ T cells tend to be differentiated and activated
into inflammatory effector T cell

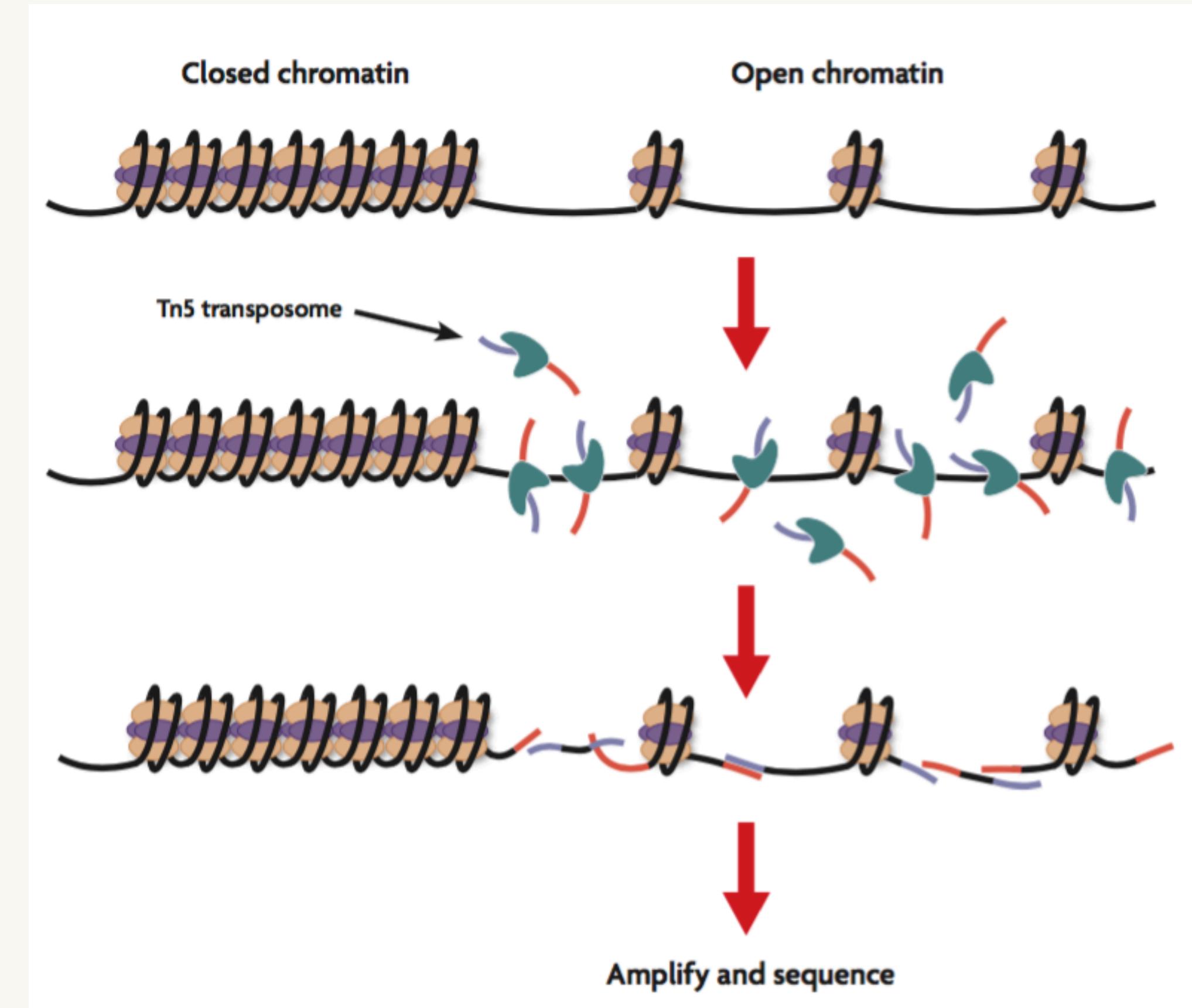
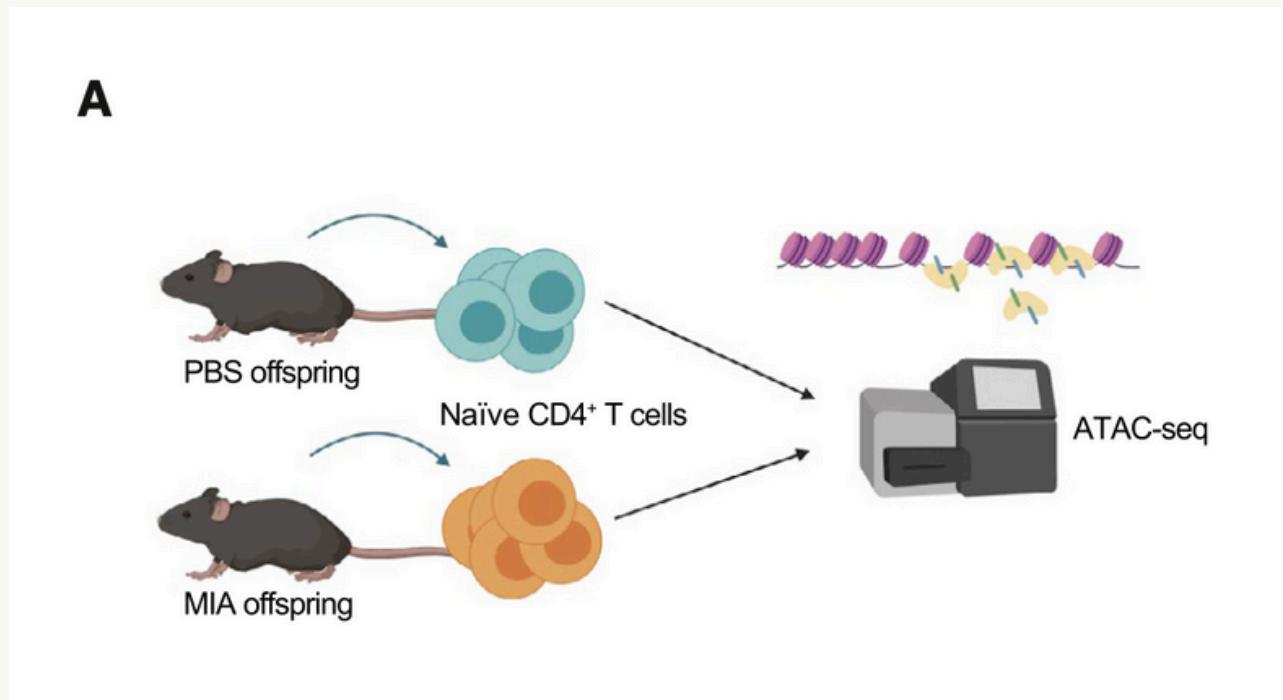
Result Summary

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ATAC-seq

(Assay for transposase-accessible chromatin with high-throughput sequencing)



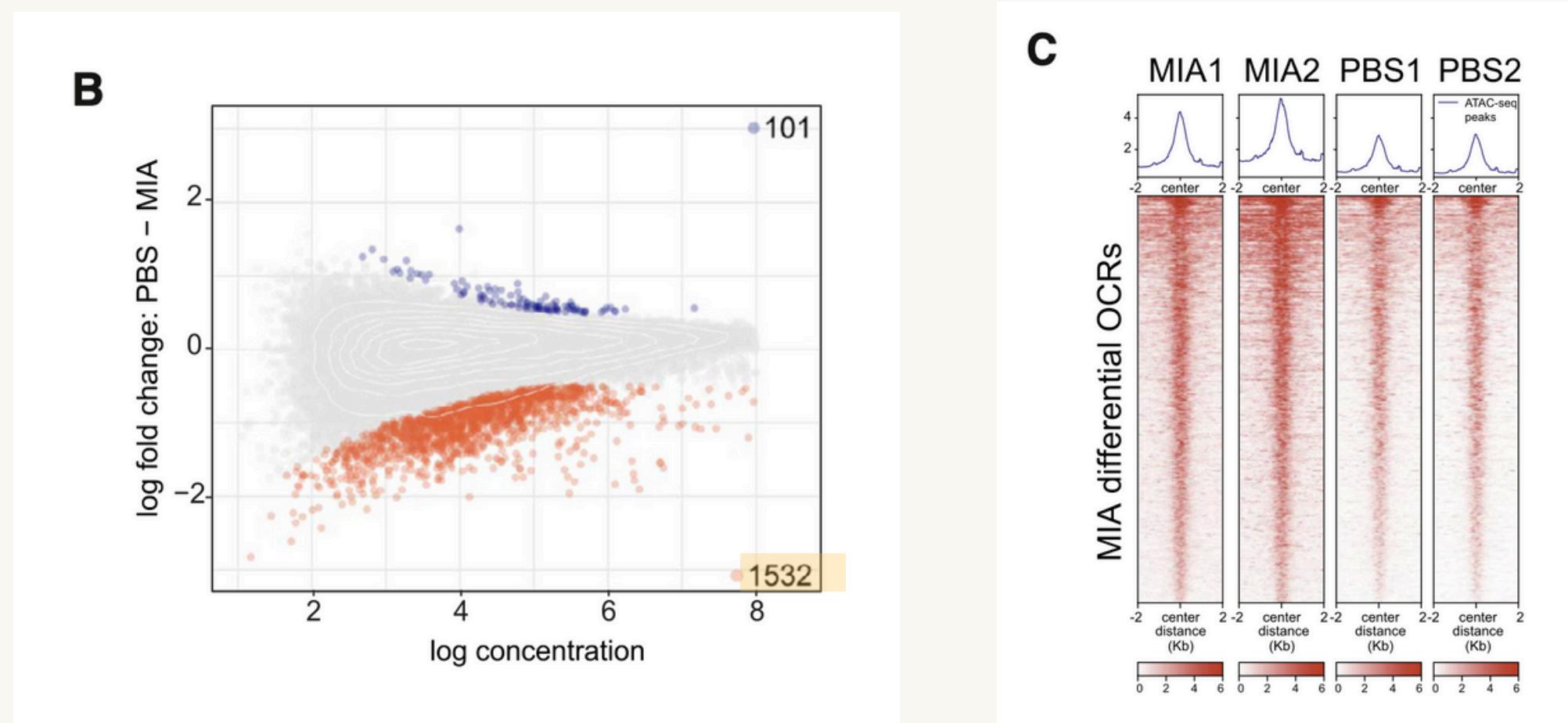


Figure 5B,C

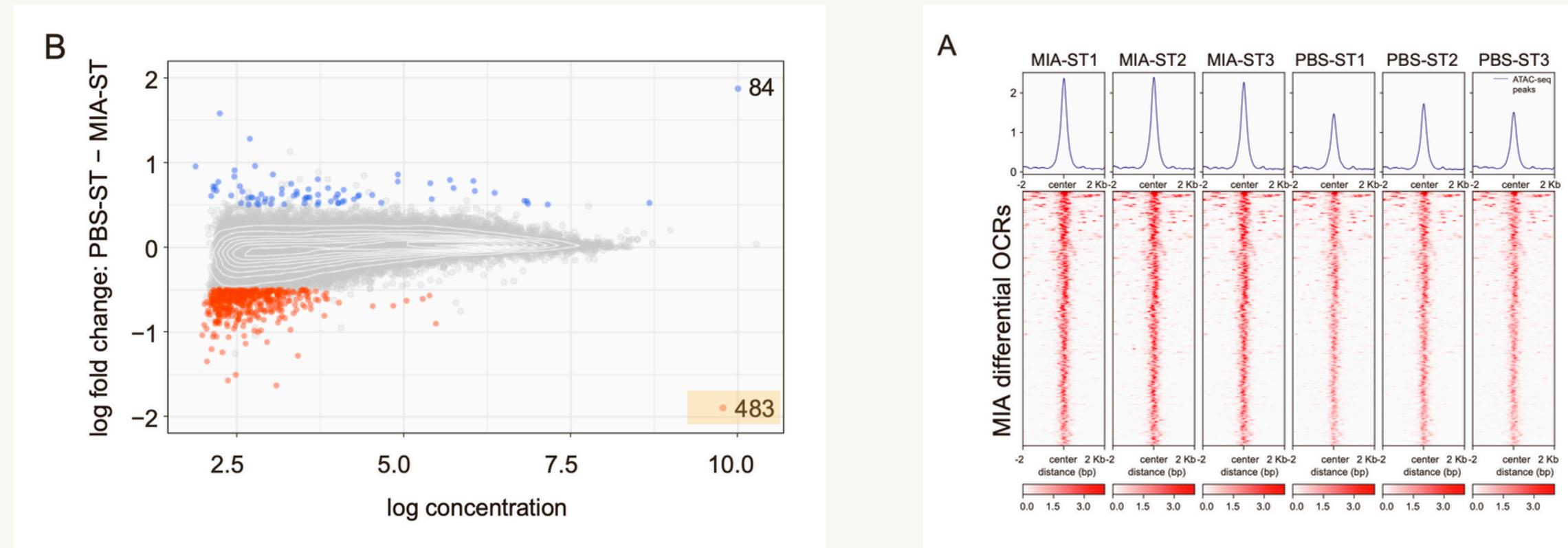


Figure S6A,B

GO terms that were enriched in the differential open chromatin regions(OCRs) of MIA naive CD4+ T cells:

- Epigenetic regulation
- Regulation of adaptive immune responses
- Regulation of kinase activity

D MIA offspring's

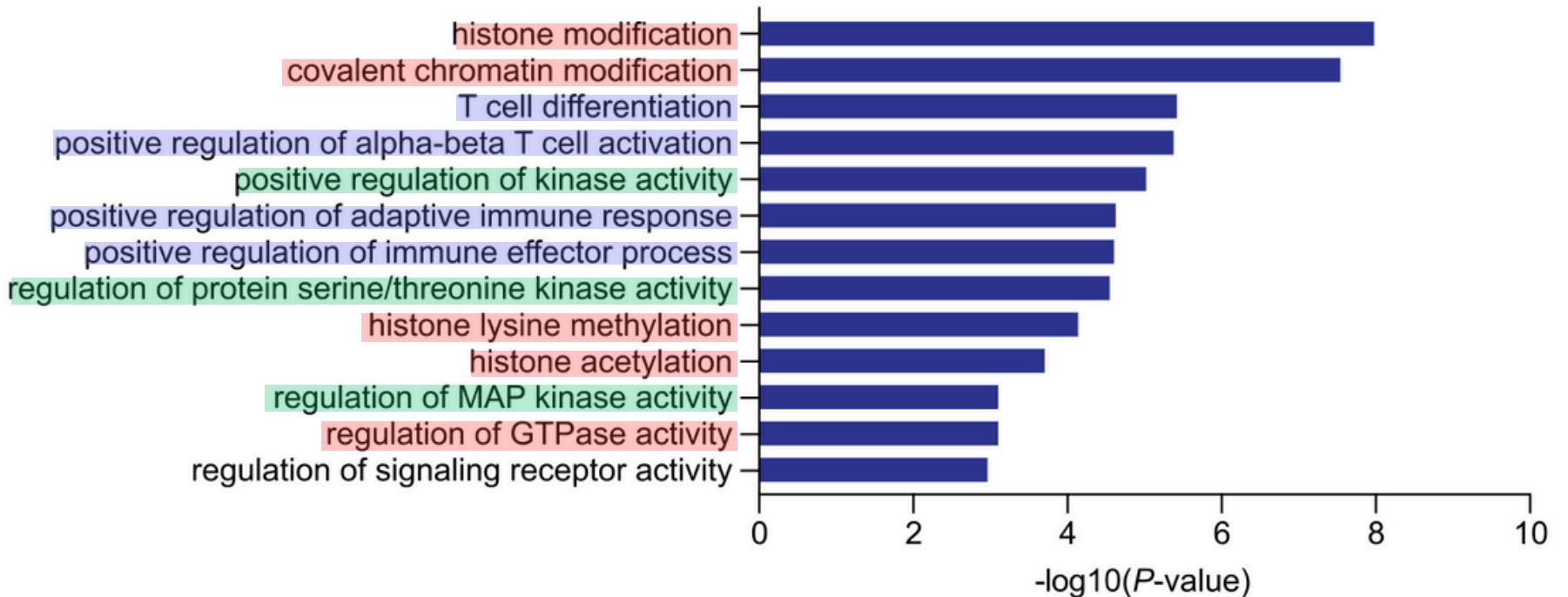


Figure 5D

C MIA-ST offspring's

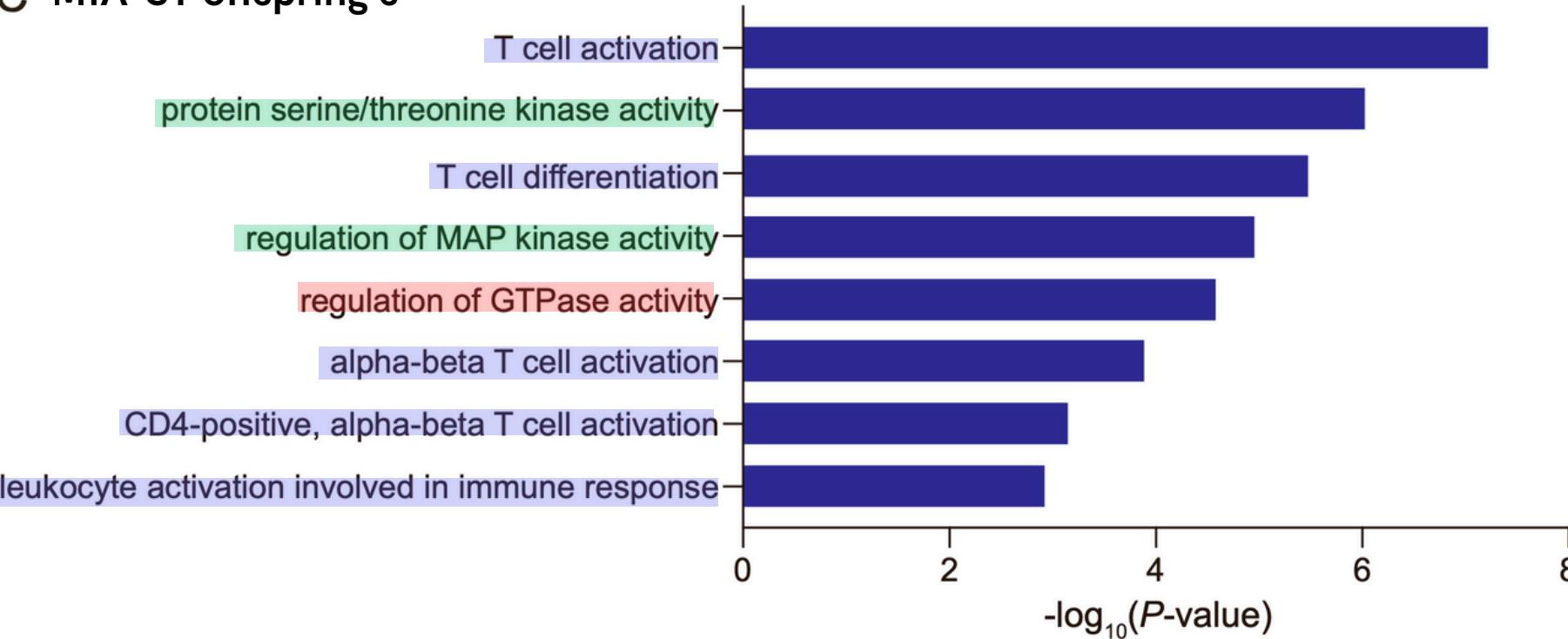
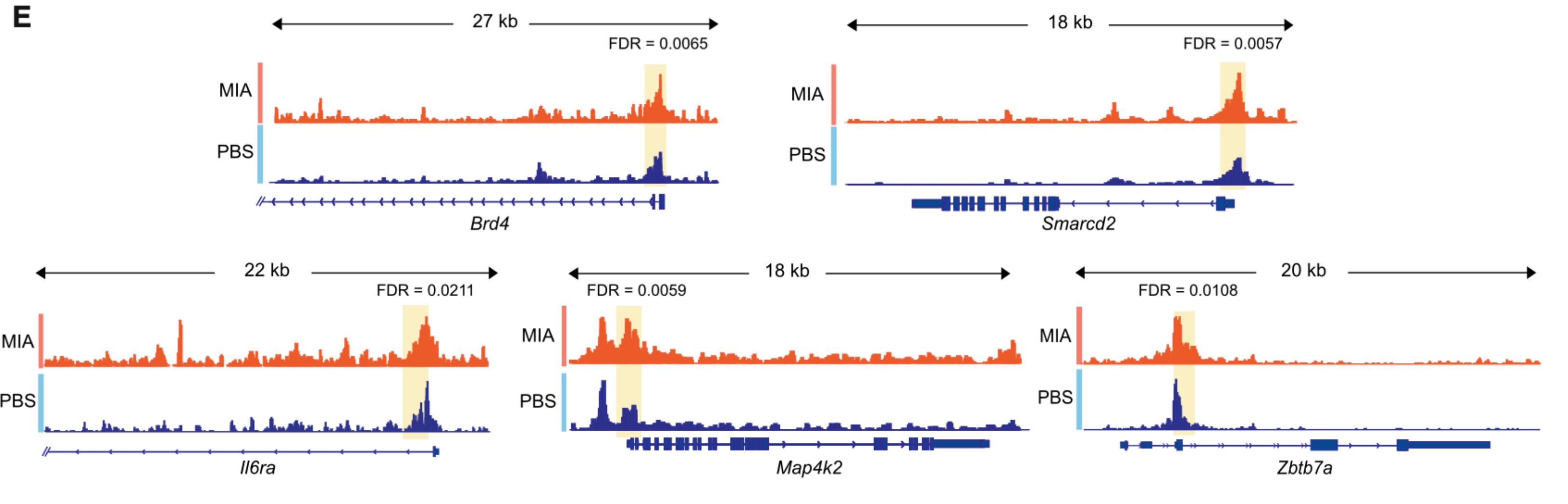


Figure S6C

Figure 5E



Chromatin remodeling

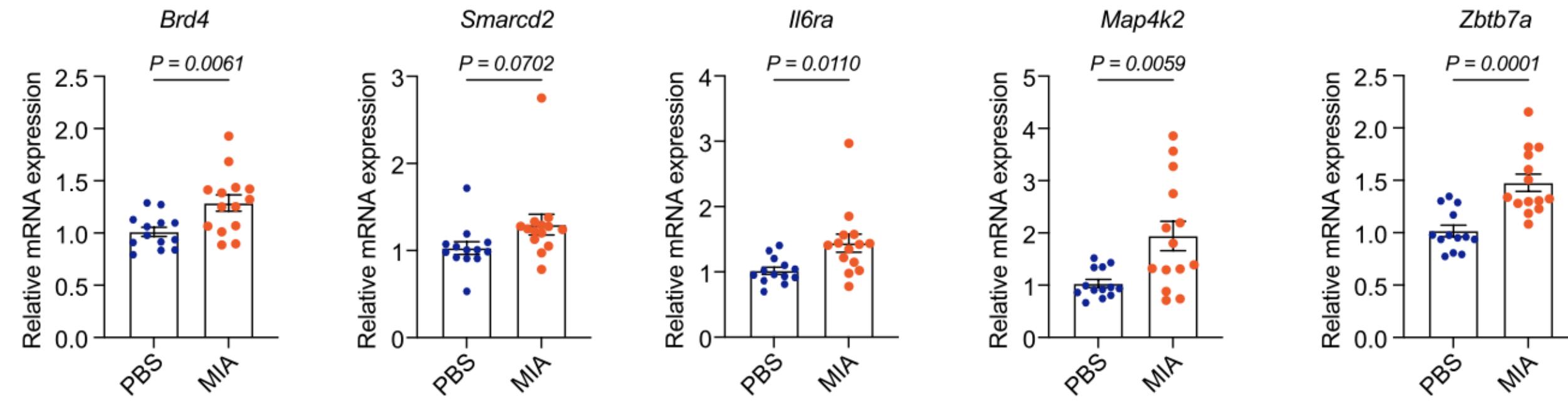
- **Brd4:** A protein that recognizes and binds to acetylated histones and controls gene transcription and cell processes.
- **Smarcd2:** Part of the SWI/SNF chromatin remodeling complex, which shapes chromatin structure and gene expression.

T effector cell differentiation and activation

- **Il6ra:** A receptor for the inflammatory cytokine, IL-6.
- **Map4k2:** is a member of the MAP4 kinase family associated with the regulation of T cell activity
- **Zbtb7a:** A transcription factor that influences gene expression by binding to DNA sequences.

Figure 5F

F



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Result Summary

- 01 Prenatal inflammation raises gut vulnerability.
- 02 MIA offspring's immunity shaped **postnatally**.
- 03 Maternal **microbiota** primes offspring's immunity.
- 04 MIA induces **inflammatory T cell differentiation**.
- 05 MIA **programs** inflammatory T cells.
- 06 **Maternal IL-17A** affects offspring's immunity.

We are here!

Figure6 MIA isotype-ST vs. MIA anti-IL-17A-ST

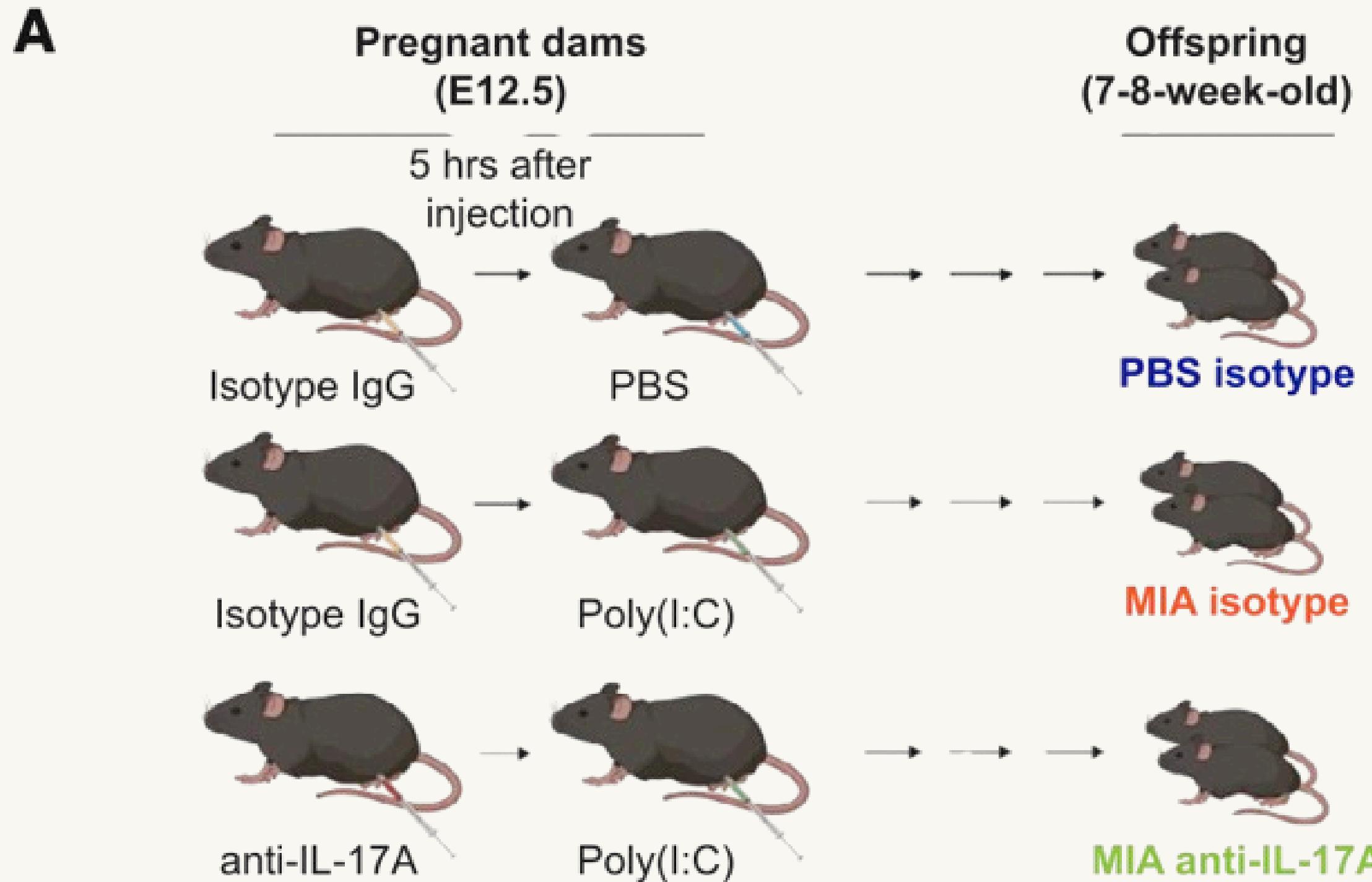


Figure6 MIA isotype-ST vs. MIA anti-IL-17A-ST

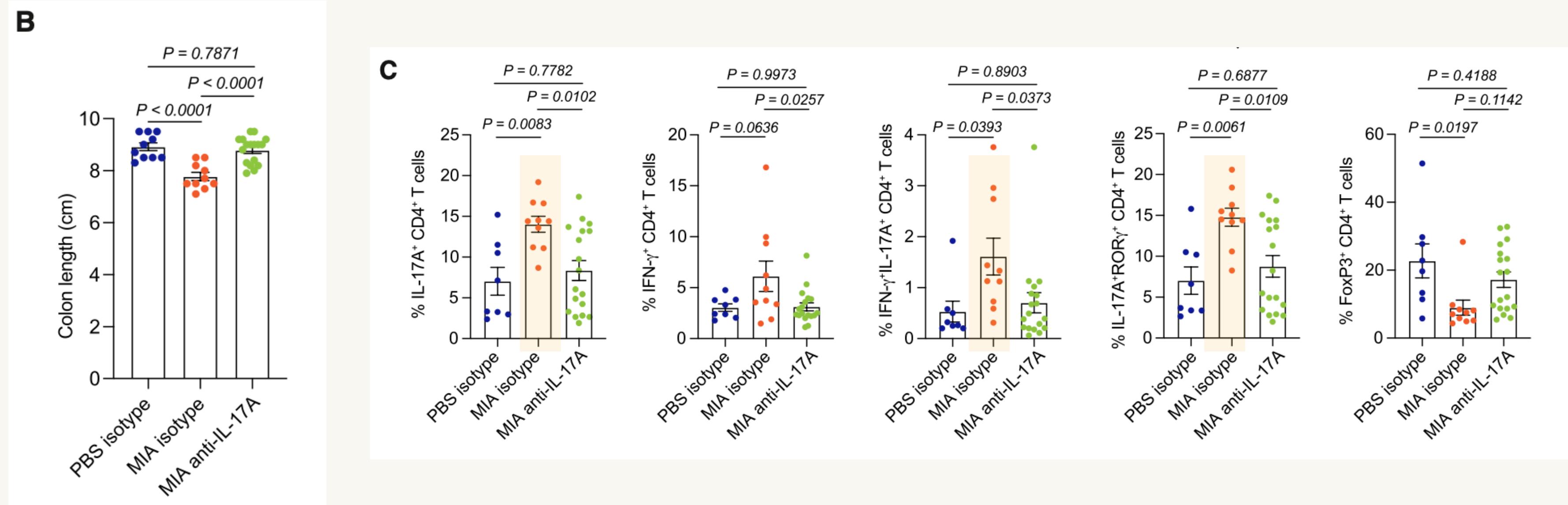


Figure6 MIA isotype-ST vs. MIA anti-IL-17A-ST

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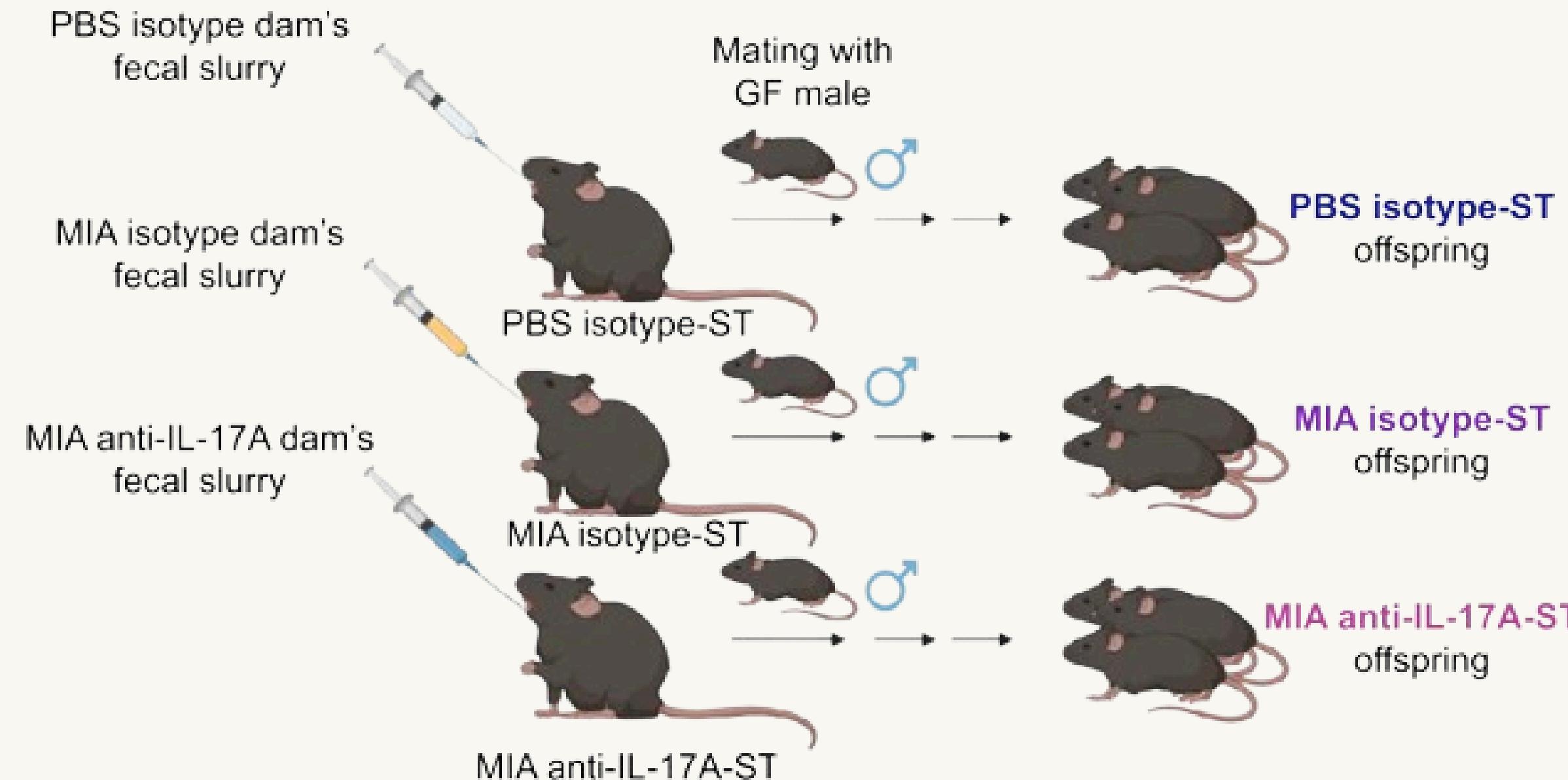
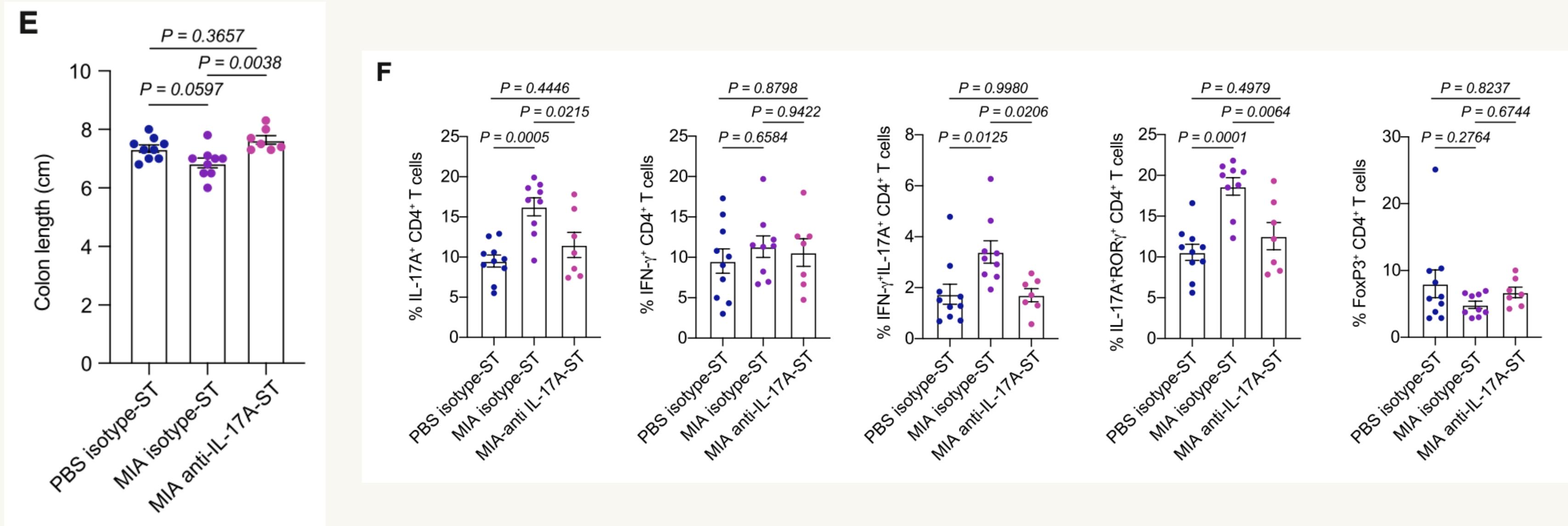


Figure6 MIA isotype-ST vs. MIA anti-IL-17A-ST



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- 01 **Prenatal inflammation**
raises gut vulnerability. MIA vs. PBS
-
- 02 MIA offspring's immunity
shaped **postnatally**. MIAo-PBSd vs. PBSo-MIAd
-
- 03 **Maternal microbiota**
primes offspring's immunity. MIA-ST vs. SFB-Mono-MIA
-
- 04 MIA induces **inflammatory**
T cell differentiation. RNA-seq
-
- 05 MIA **programs**
inflammatory T cells. ATAC-seq
-
- 06 **Maternal IL-17A**
affects offspring's immunity. MIA isotype-ST vs. MIA anti-IL-17A-ST
-

Conclusion

- 01 Prenatal inflammation raises gut vulnerability.
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Thank you!

Feel free to call or message for
any questions or clarifications.