

Ovarian Stimulation with Gonadotropins Promotes Unique Dynamics of Ovarian Immune Microenvironment

TIA BRODEUR MD PHD

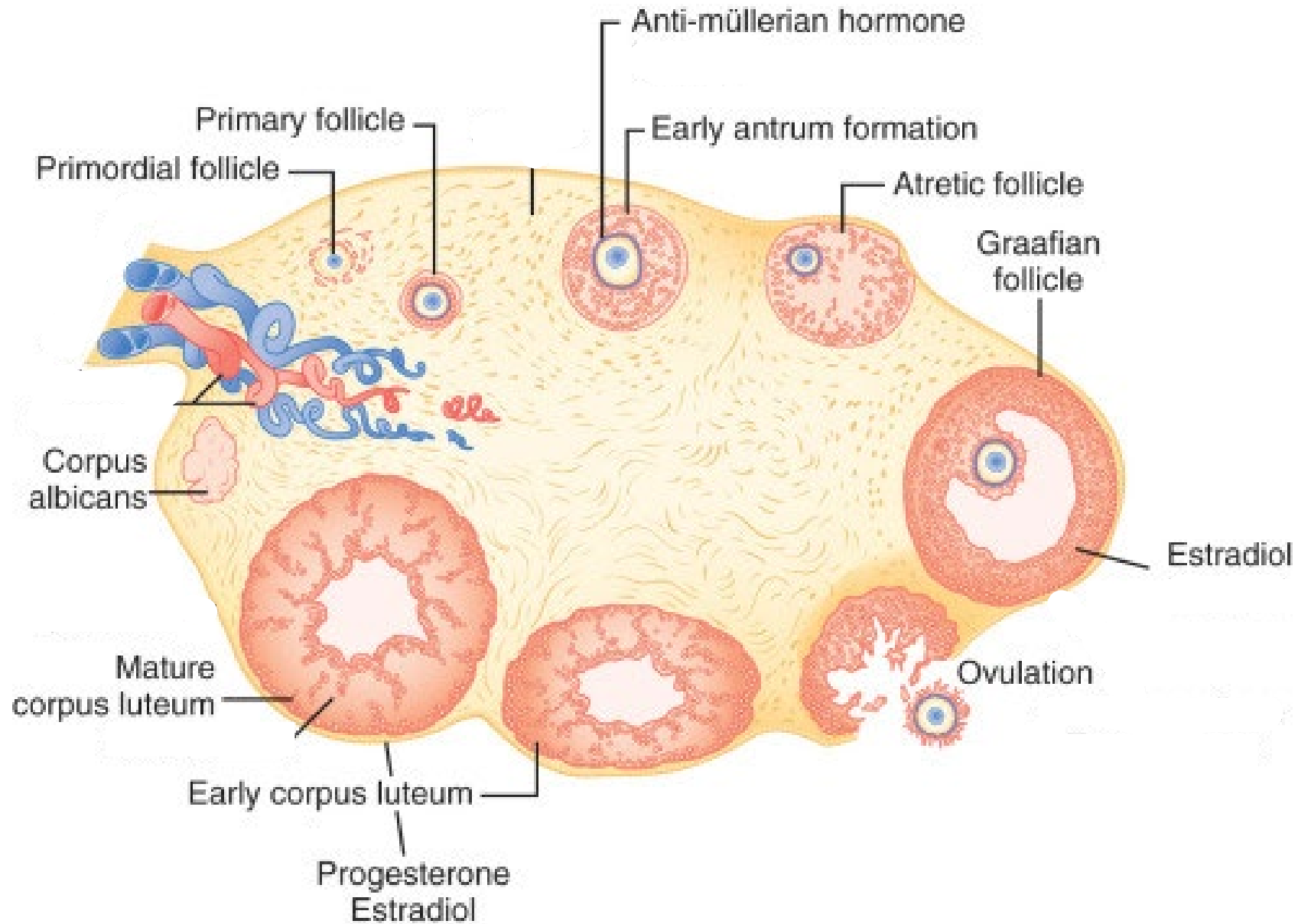
ASSISTANT PROFESSOR

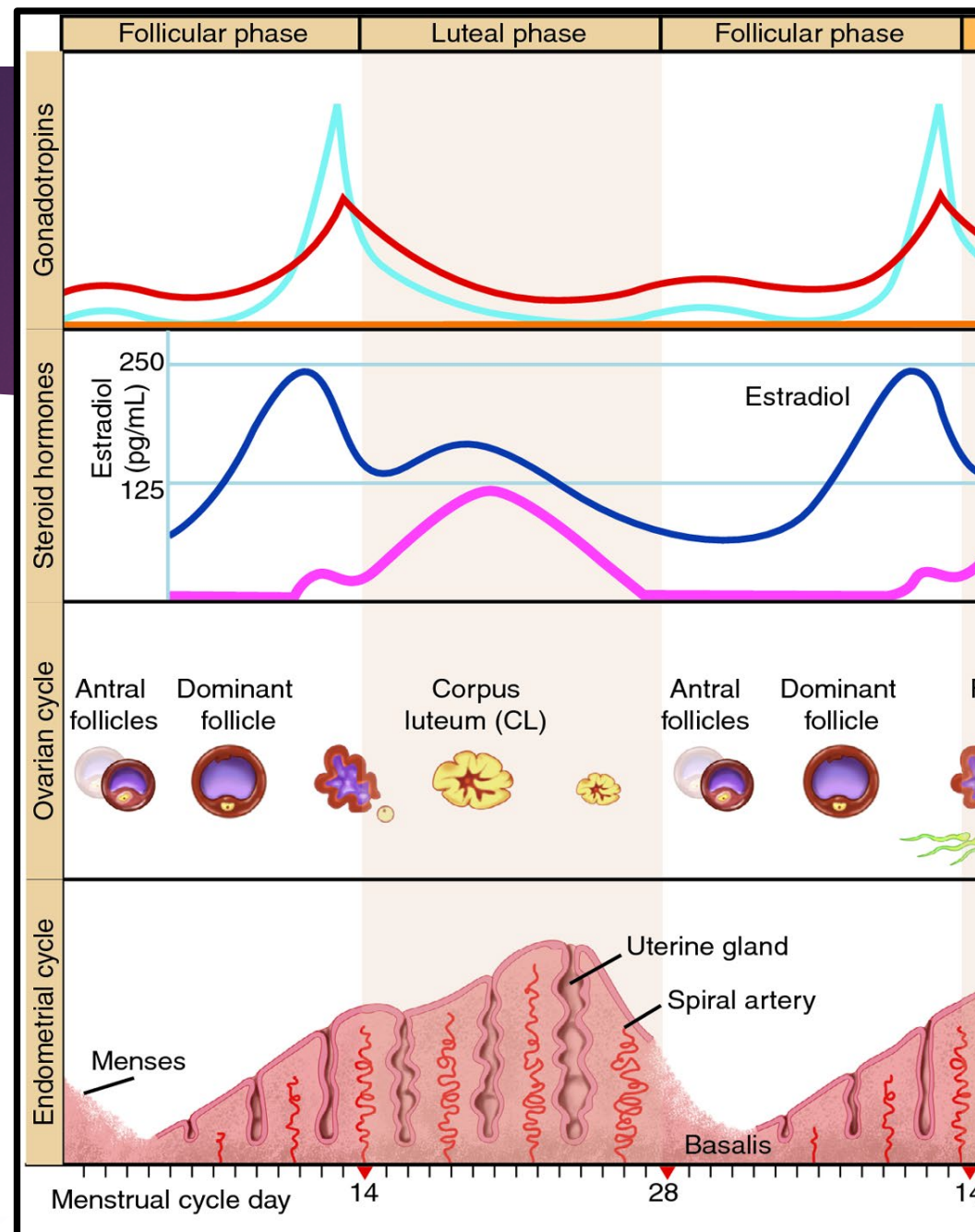
REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

INDIANA UNIVERSITY

*SUPPORT FROM RESEARCH ON WOMEN'S HEALTH IN THE IDEA STATES
SUPPORT RECEIVED AS A CLINICAL AND RESEARCH FELLOW*

THE OVARIAN CYCLE

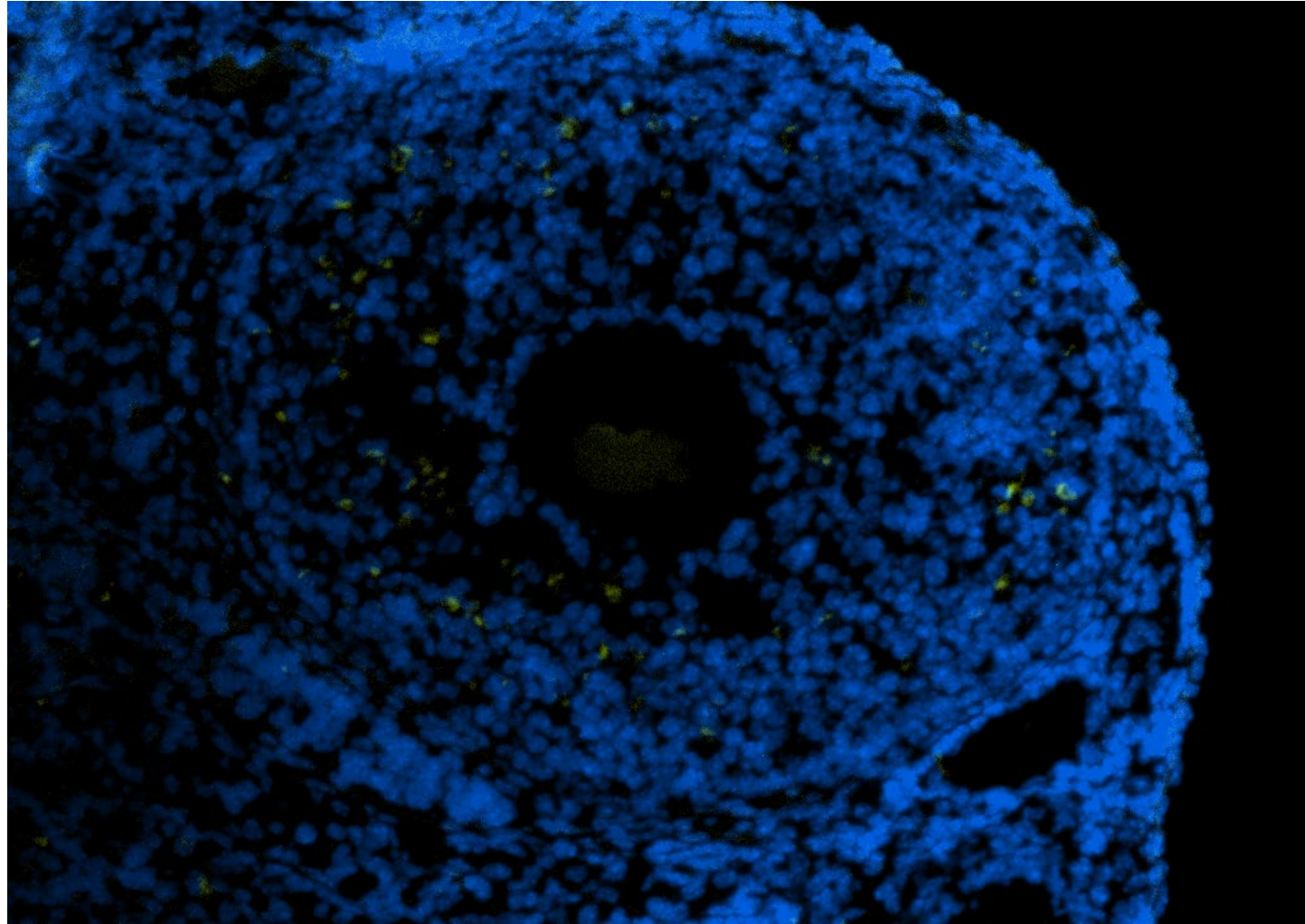




The ovary is a dynamic tissue

- ▶ In ovarian follicles, granulosa cells undergo continuous waves of apoptosis, proliferation, and differentiation
- ▶ This process generates apoptotic debris
- ▶ Depletion of macrophages leads to impaired follicle development and impaired corpus luteum development

Cleaved caspase-3 in ovary of untreated mouse



Why is continual remodeling in the ovary relevant?

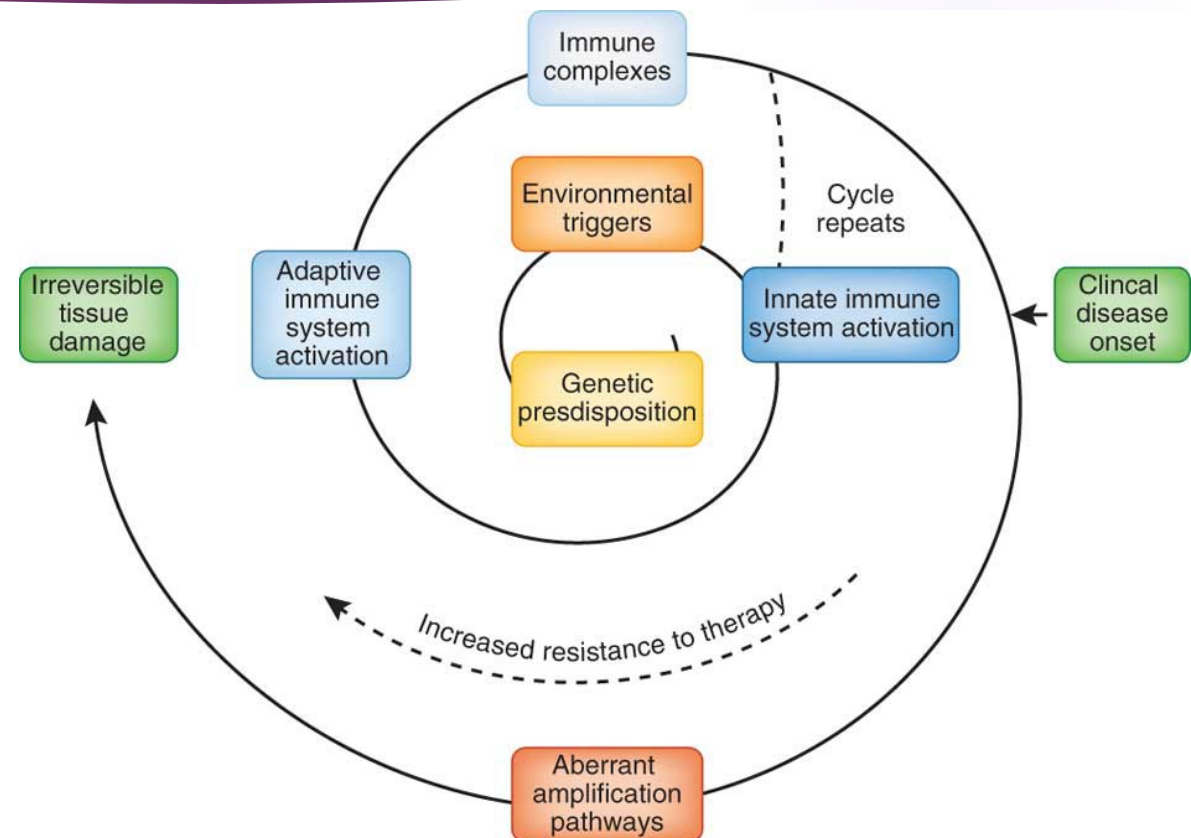
- ▶ Uptake of apoptotic debris promotes a tolerogenic phenotype in phagocytes
- ▶ Inefficient clearance of apoptotic debris may be partially responsible for the initiation/propagation of systemic autoimmunity
- ▶ Inflammation locally could cause impaired ovulation due to oophoritis, and ultimately **primary ovarian insufficiency**

Mahajan, A., et al. (2016). "Clearance Deficiency and Cell Death Pathways: A Model for the Pathogenesis of SLE." *Frontiers in Immunology* **7**(35).

Bakalov, V. K., et al. (2005). "Autoimmune oophoritis as a mechanism of follicular dysfunction in women with 46,XX spontaneous premature ovarian failure." *Fertil Steril* **84**(4): 958-965.

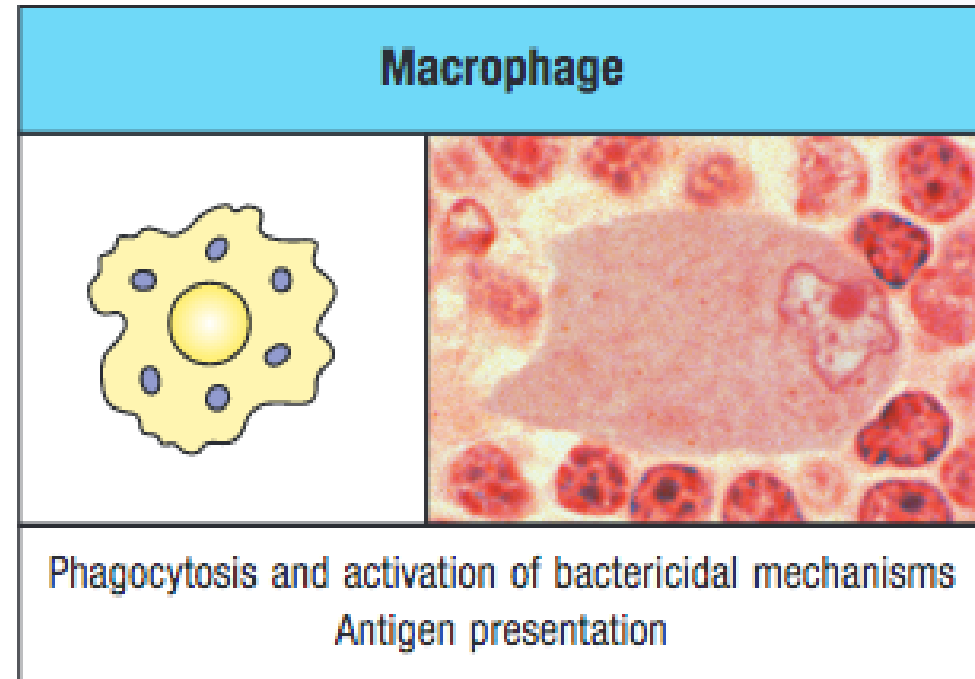
Natural History of Autoimmune Disease

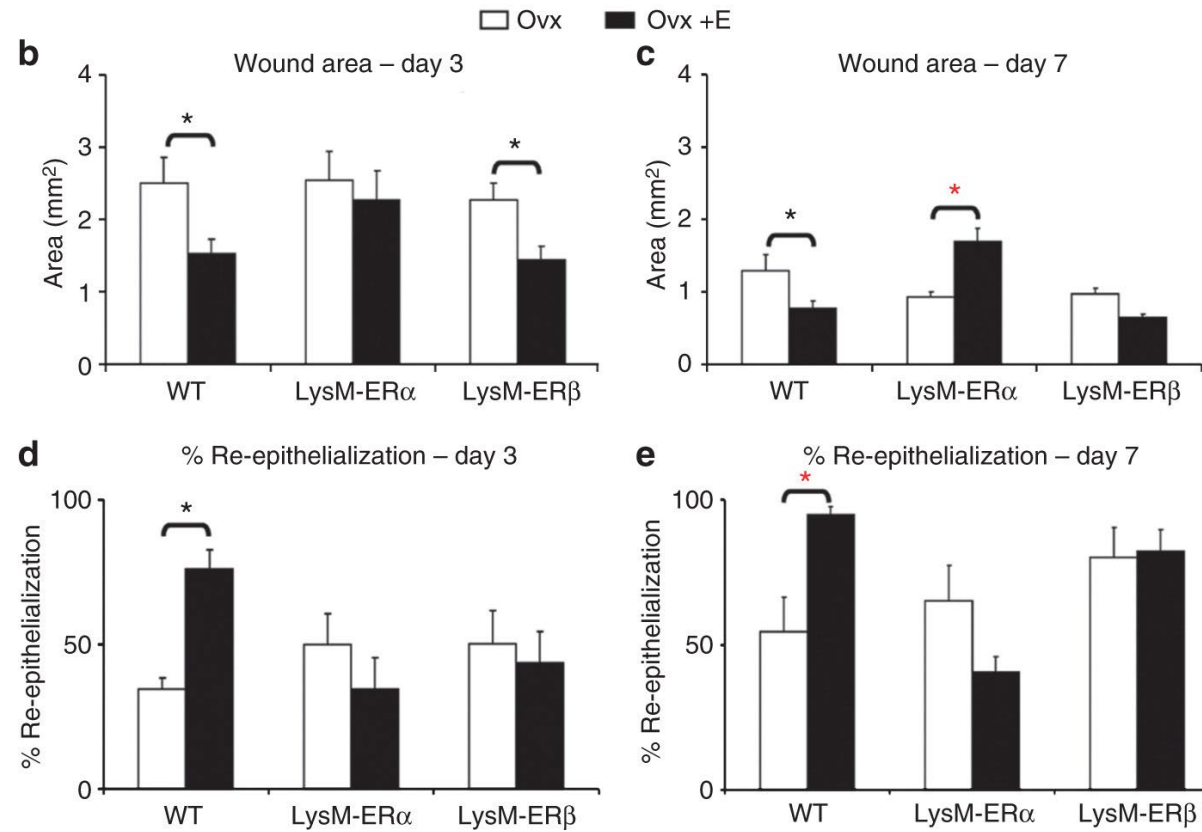
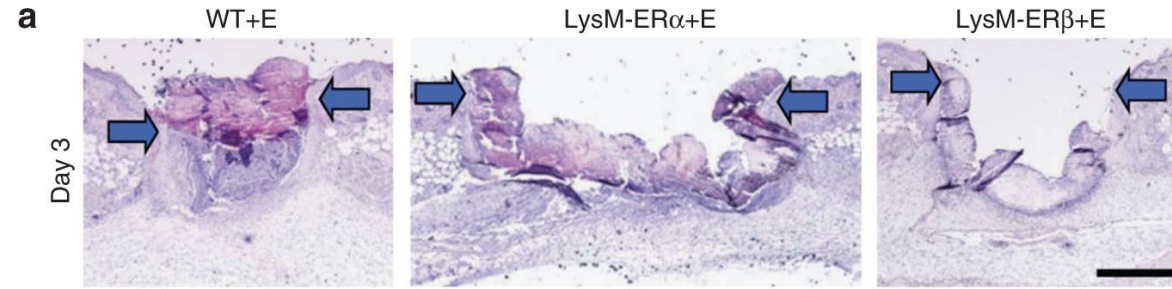
- ▶ Immune system dysfunction leading to a **loss of self-tolerance** and subsequent tissue damage.
- ▶ Persistence of **autoreactive T and B cells**
- ▶ **B cells produce autoantibodies** against cellular antigens



Macrophages

- ▶ Reside in tissues and can be recruited to tissues
- ▶ can present antigen to T cells
- ▶ phagocytose dead cells, **then take on anti-inflammatory phenotype**
- ▶ promote tolerance as well as inflammation
- ▶ **Can mediate tissue repair**



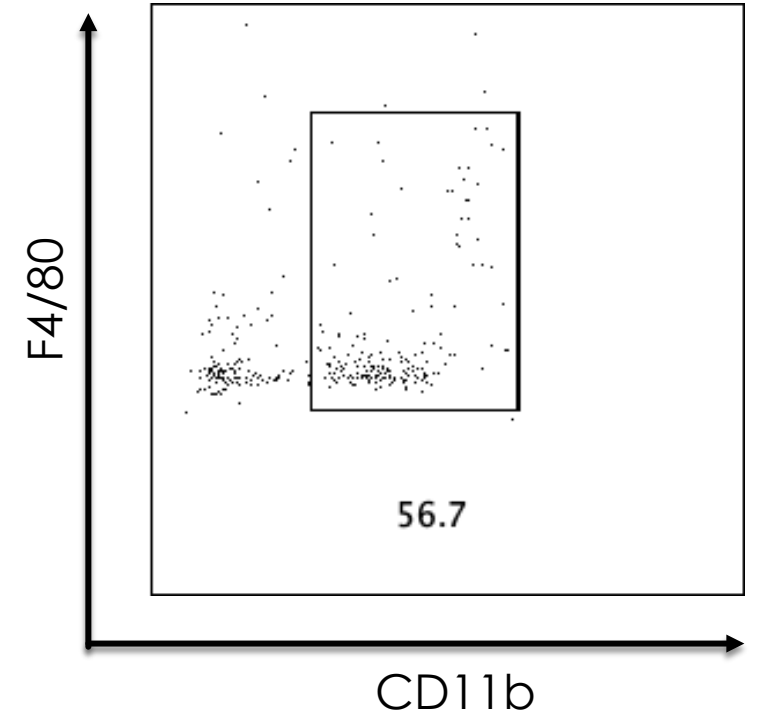
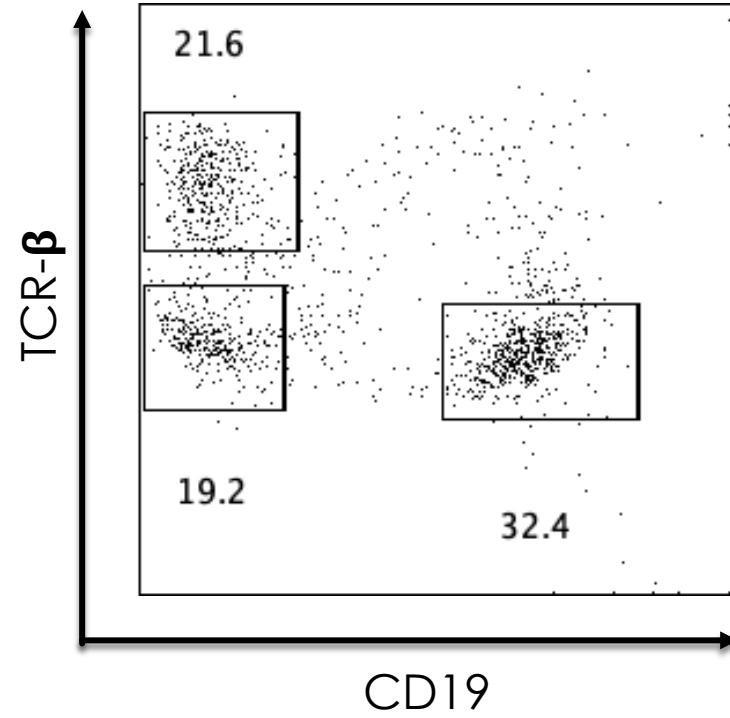
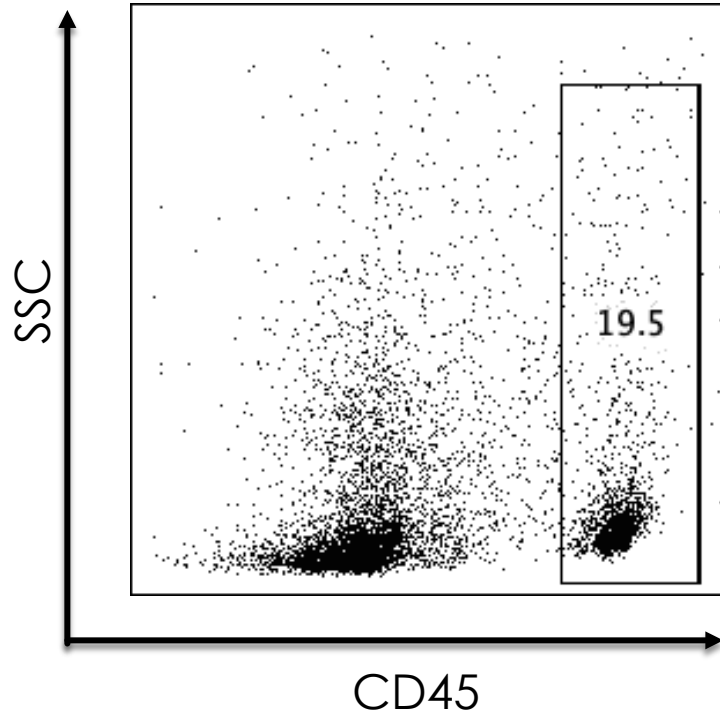


Laura Campbell, et al., Estrogen Receptor-Alpha Promotes Alternative Macrophage Activation during Cutaneous Repair, Journal of Investigative Dermatology, 2014

Hypothesis

We hypothesize that pre-ovulatory peak estradiol levels promote:

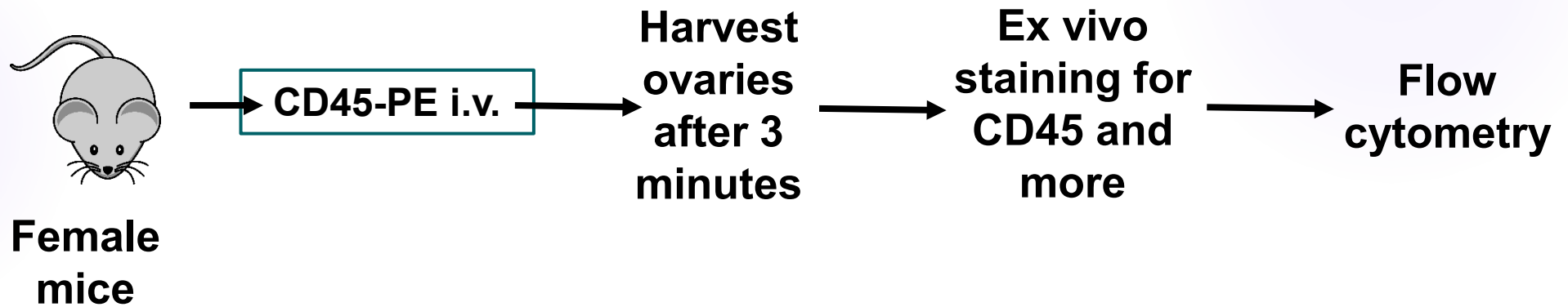
- ▶ increased uptake of apoptotic debris by ovarian resident phagocytes and prevent secondary necrosis that would promote oophoritis
- ▶ Tissue-remodeling phenotype of macrophages in ovary

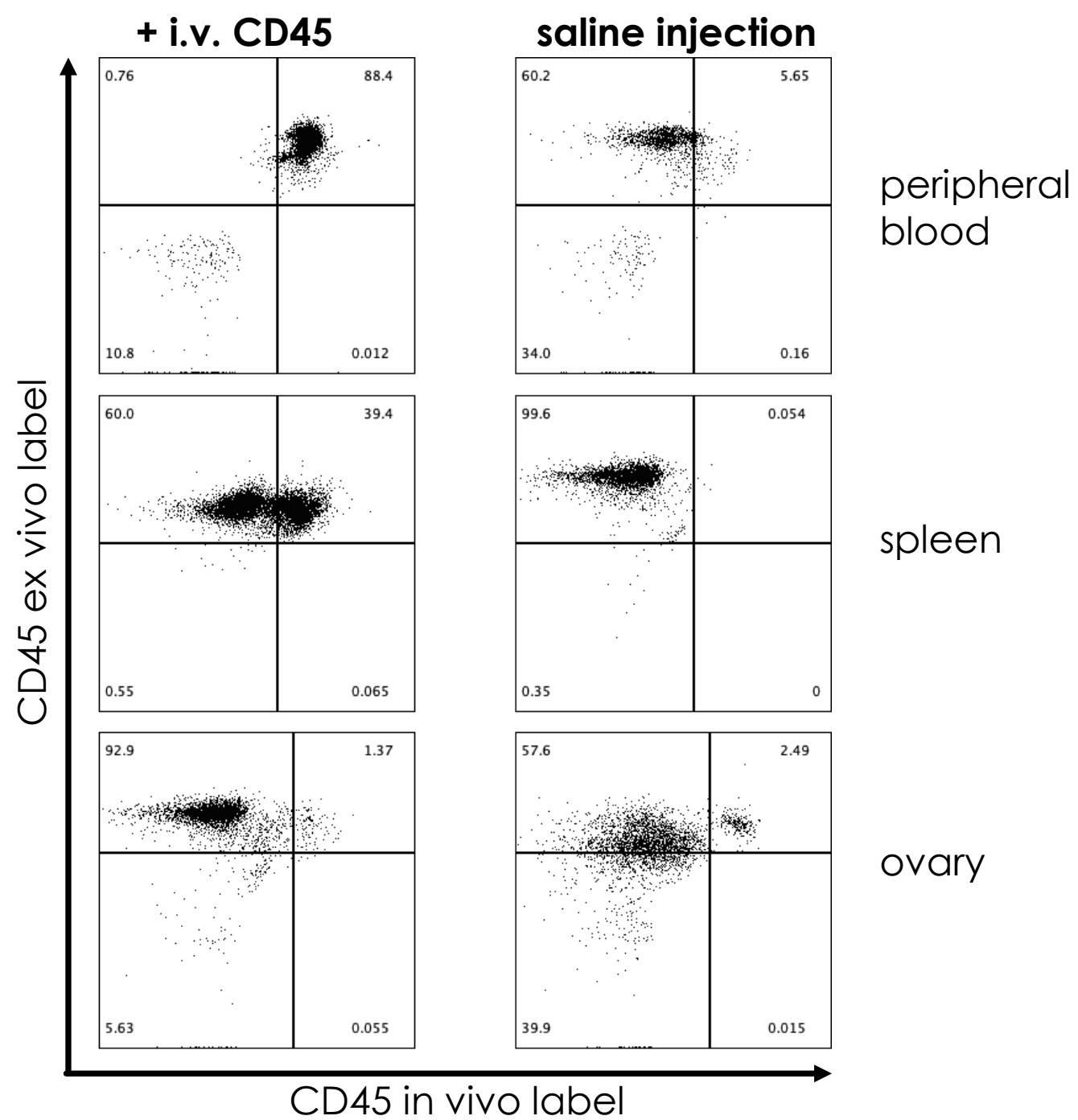


Representative flow cytometry of mouse ovary

Are ovarian leukocytes truly ovary resident?

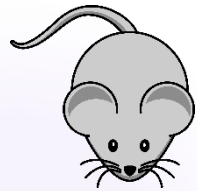
C57BL/6





Experimental Approach

C57BL/6



Female mice

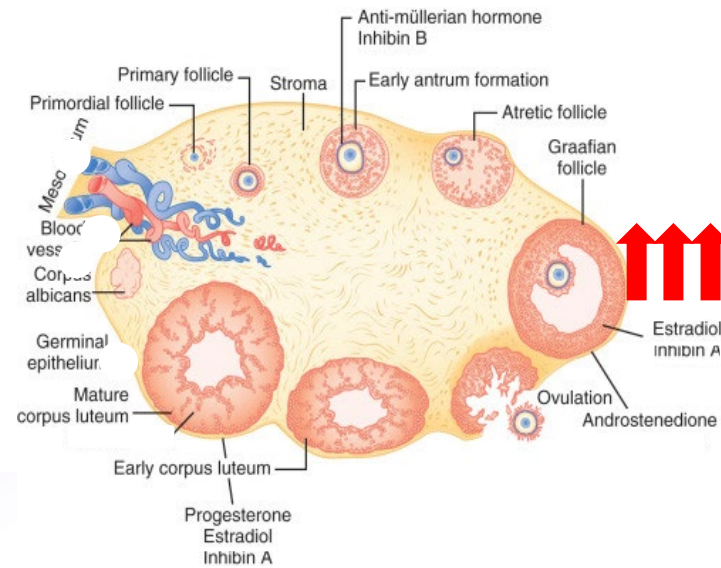
Human menopausal gonadotropin

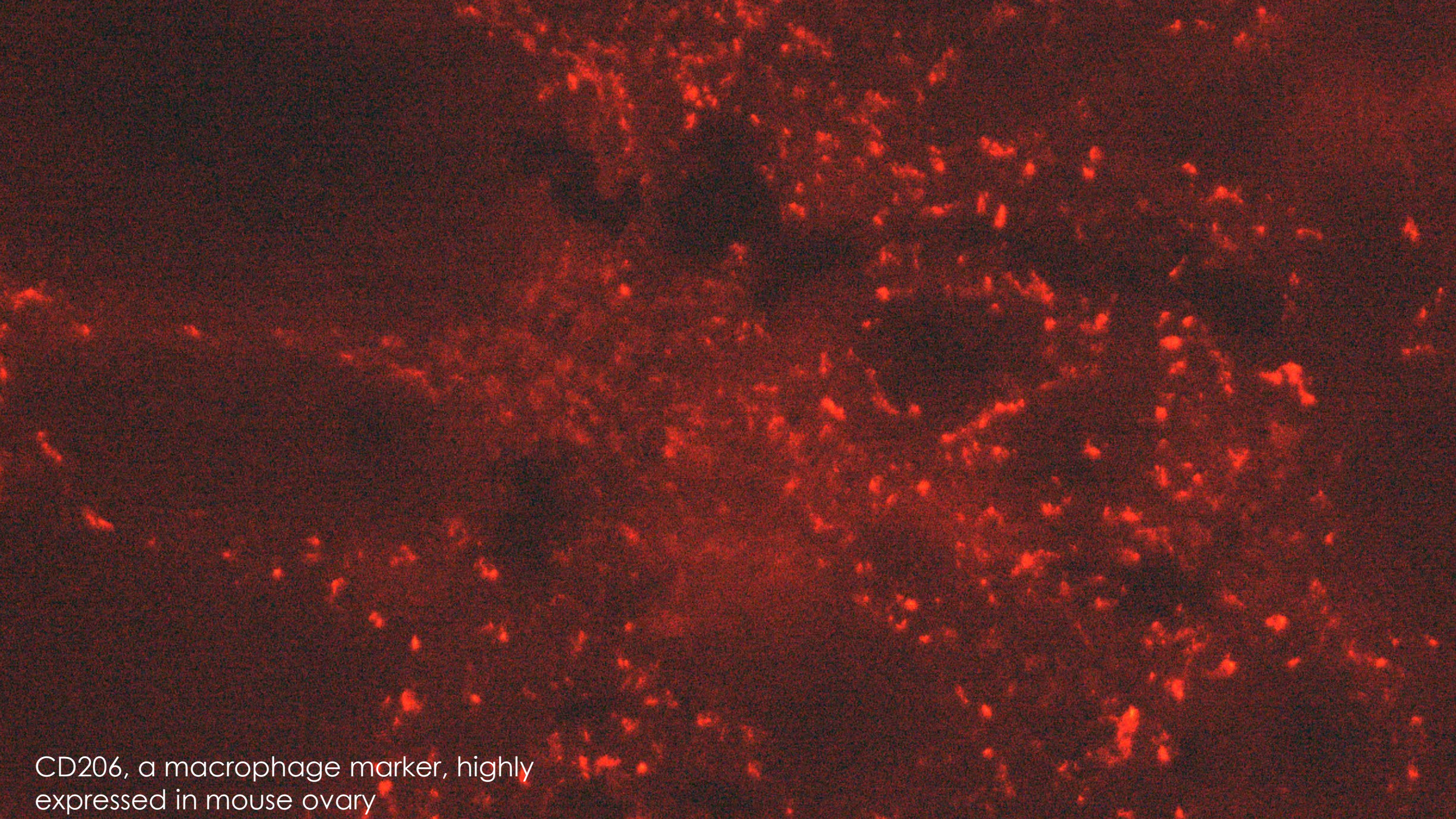
48h, high estrogen, pre-ovulation

Ovary cell isolation

Flow cytometry

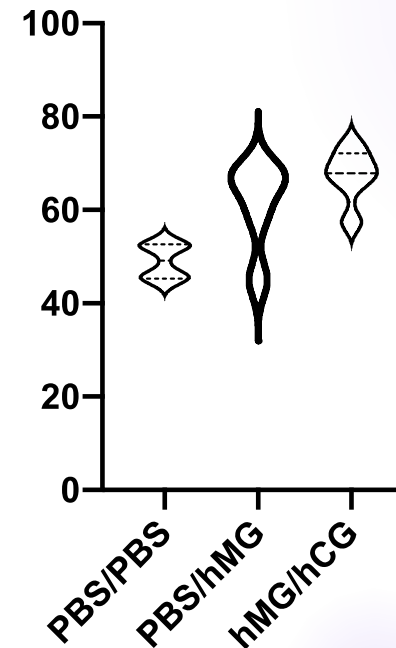
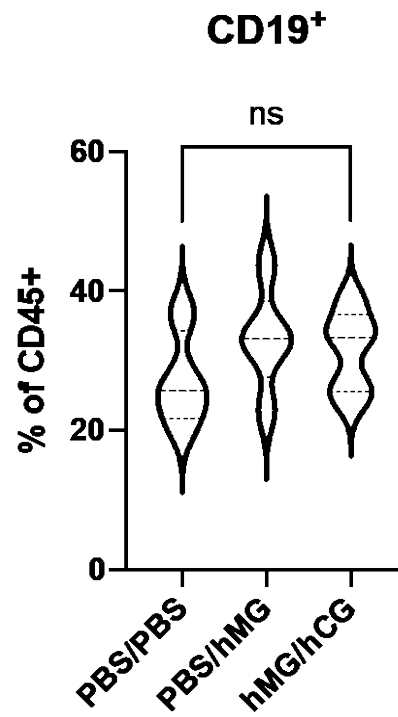
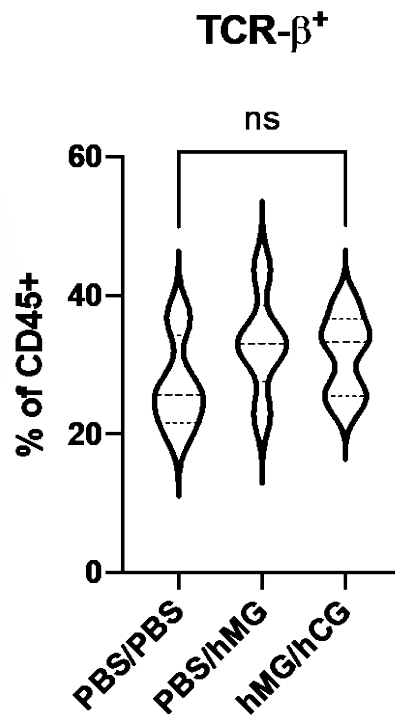
Tissue sections for immunofluorescence



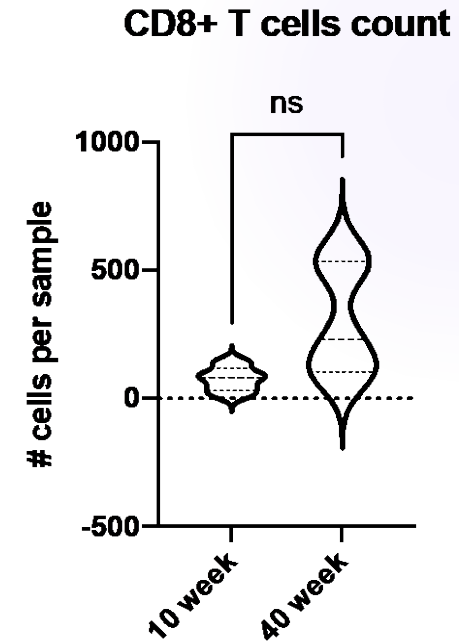
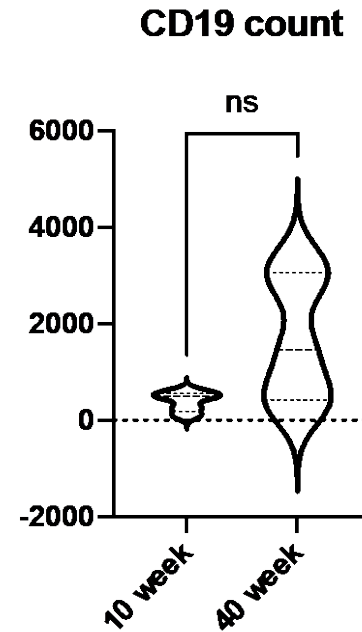
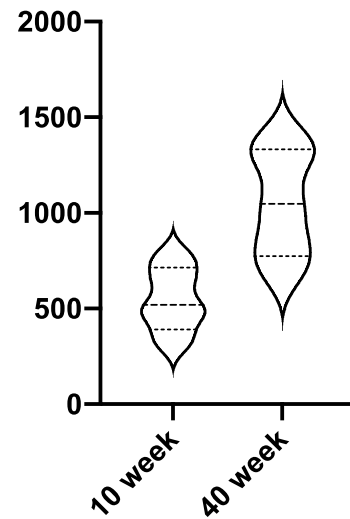


CD206, a macrophage marker, highly
expressed in mouse ovary

High estrogen and postovulatory state drive macrophage accumulation in the ovary

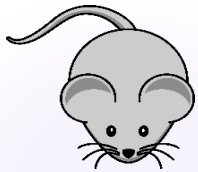


Macrophage cell numbers increase with age



Experimental Approach

C57BL/6



Female mice

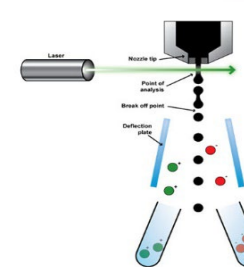
Human
menopausal
gonadotropin

48h, high
estrogen, pre-
ovulation

Ovary cell
isolation

FACS for
CD45+

Individual samples
stained with hashtag
antibodies

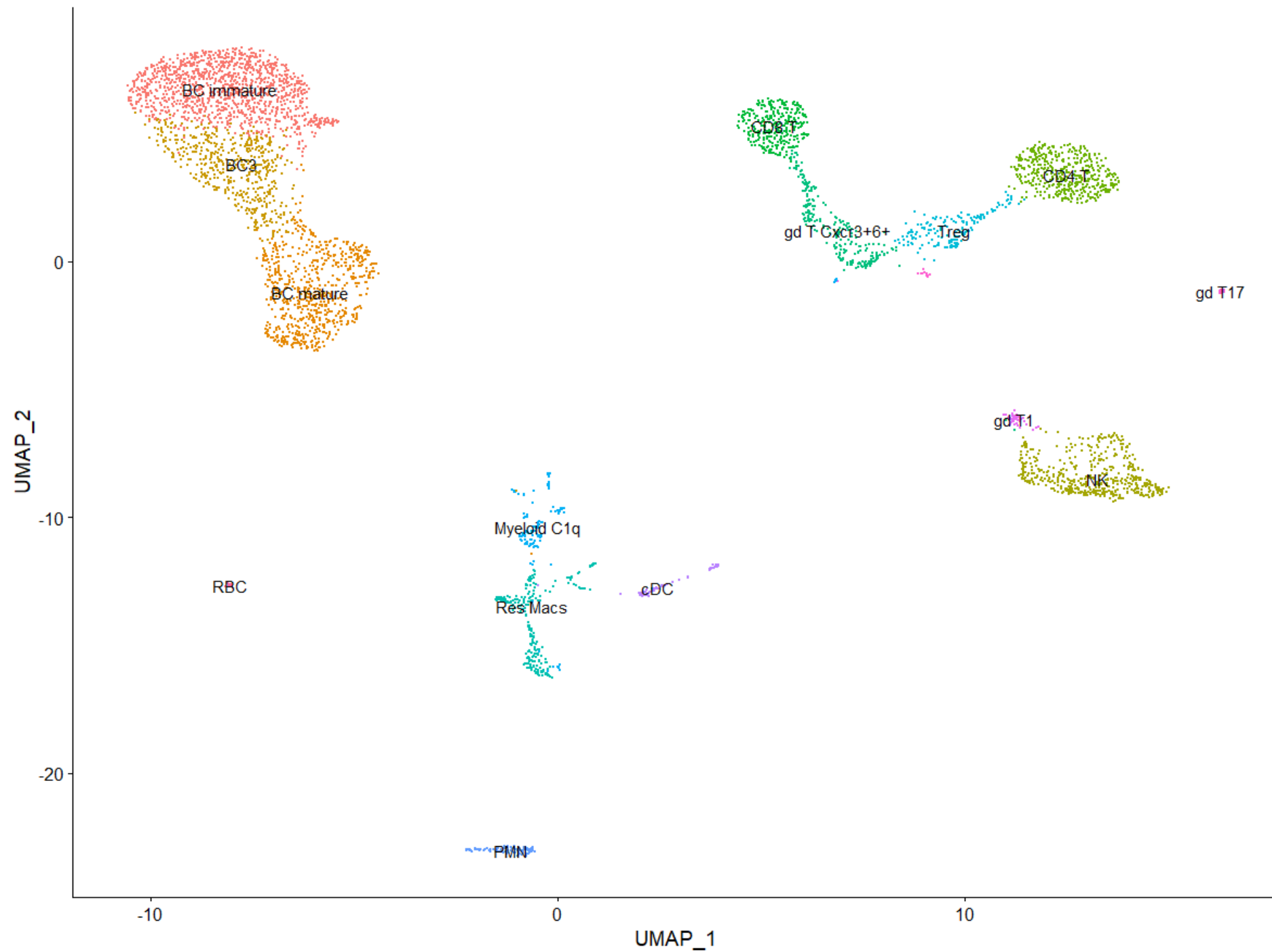


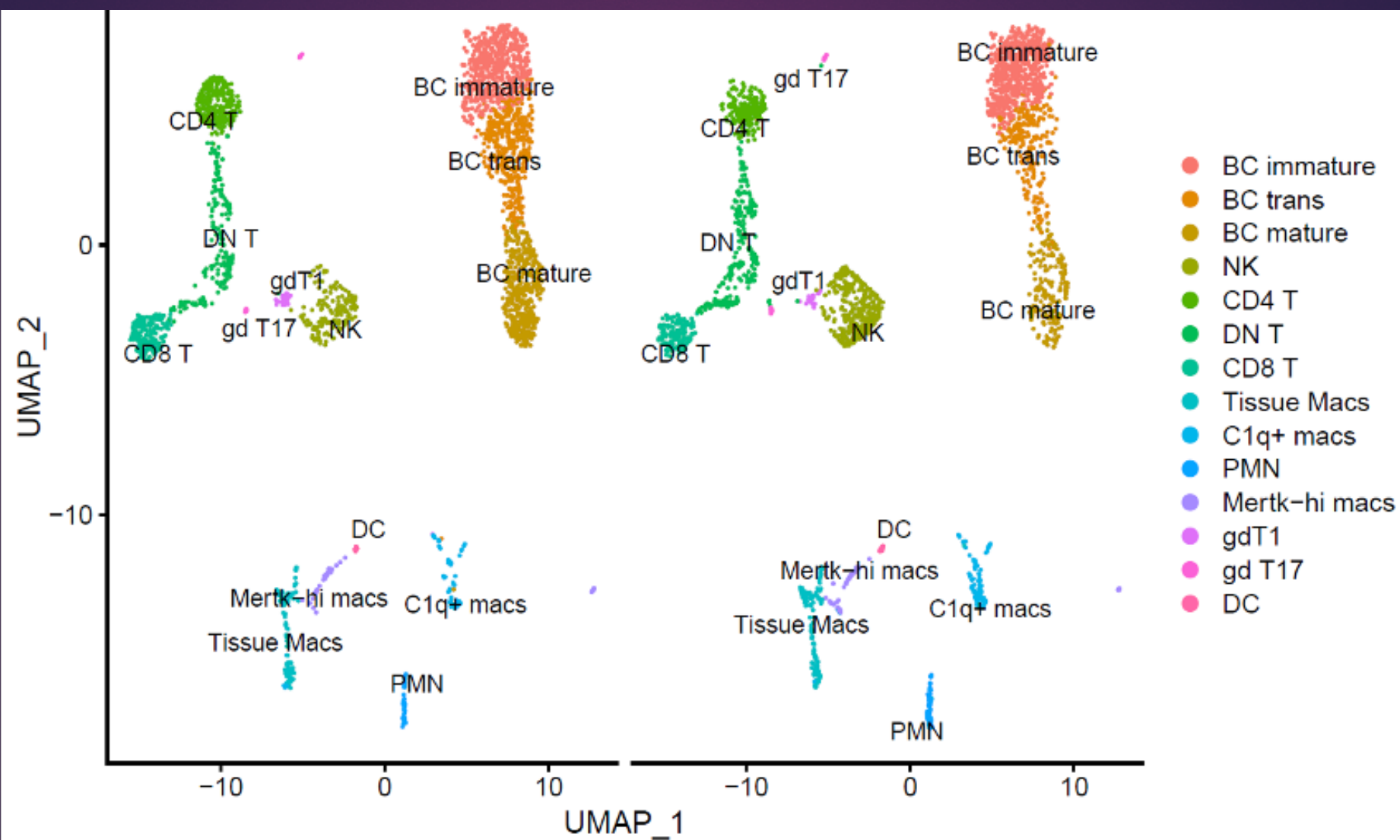
Demultiplex;
Single cell
transcriptional
profiles

Single cell RNAseq
(10xGenomics)

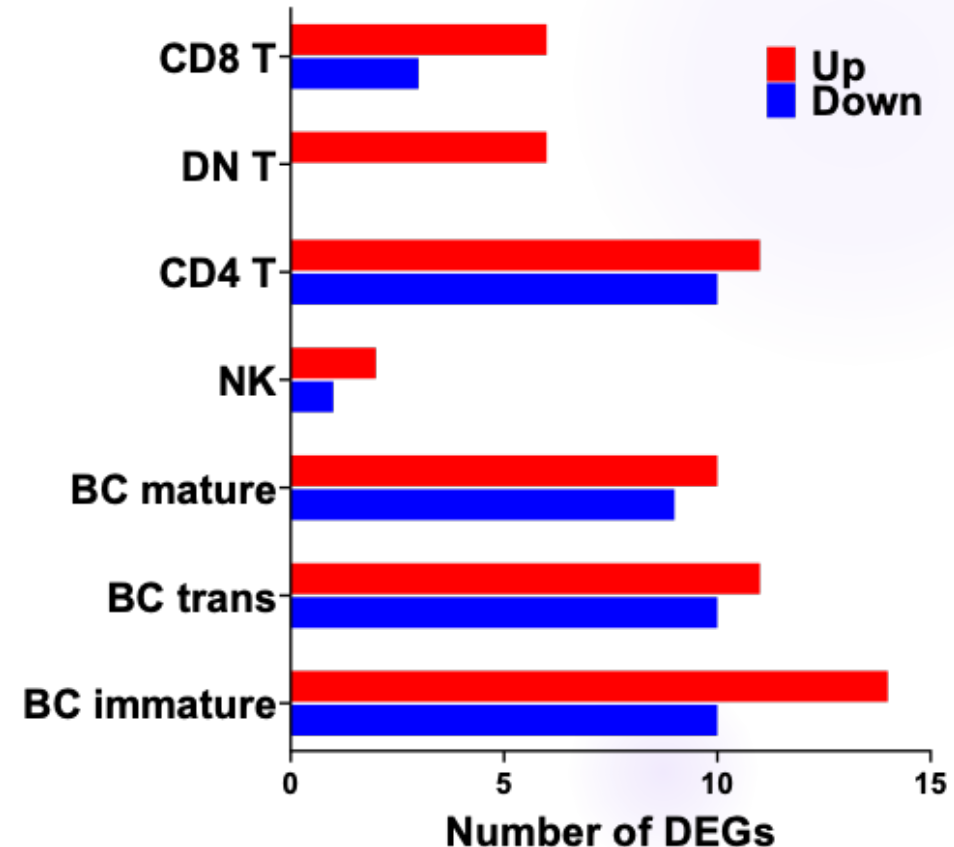
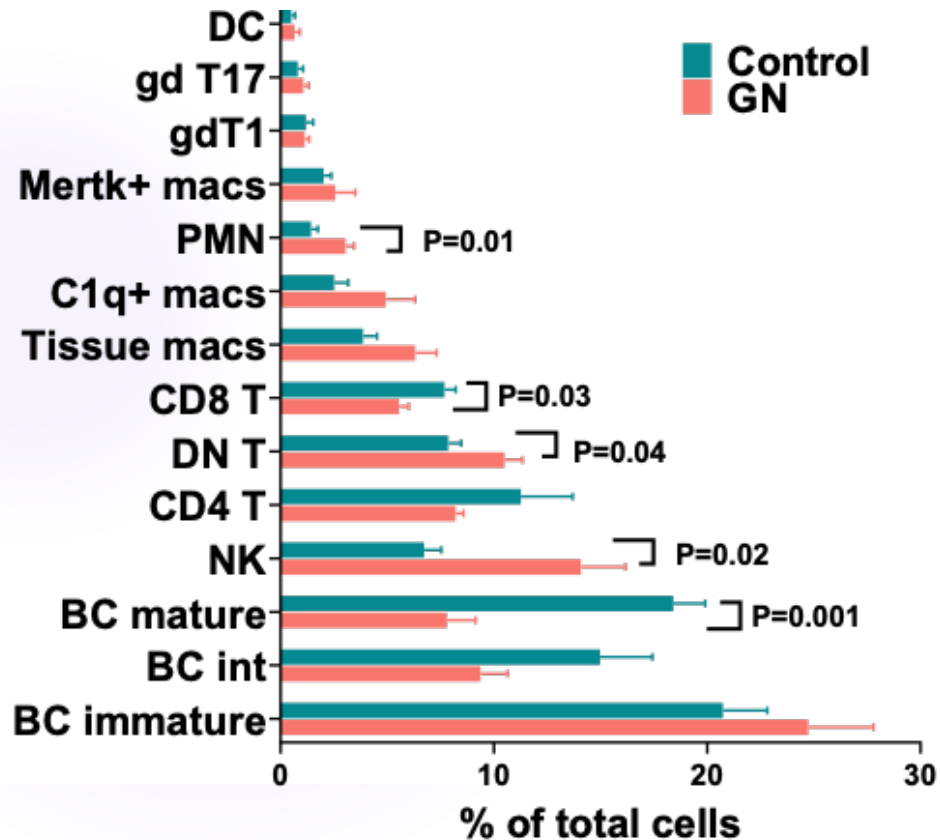
CellRanger

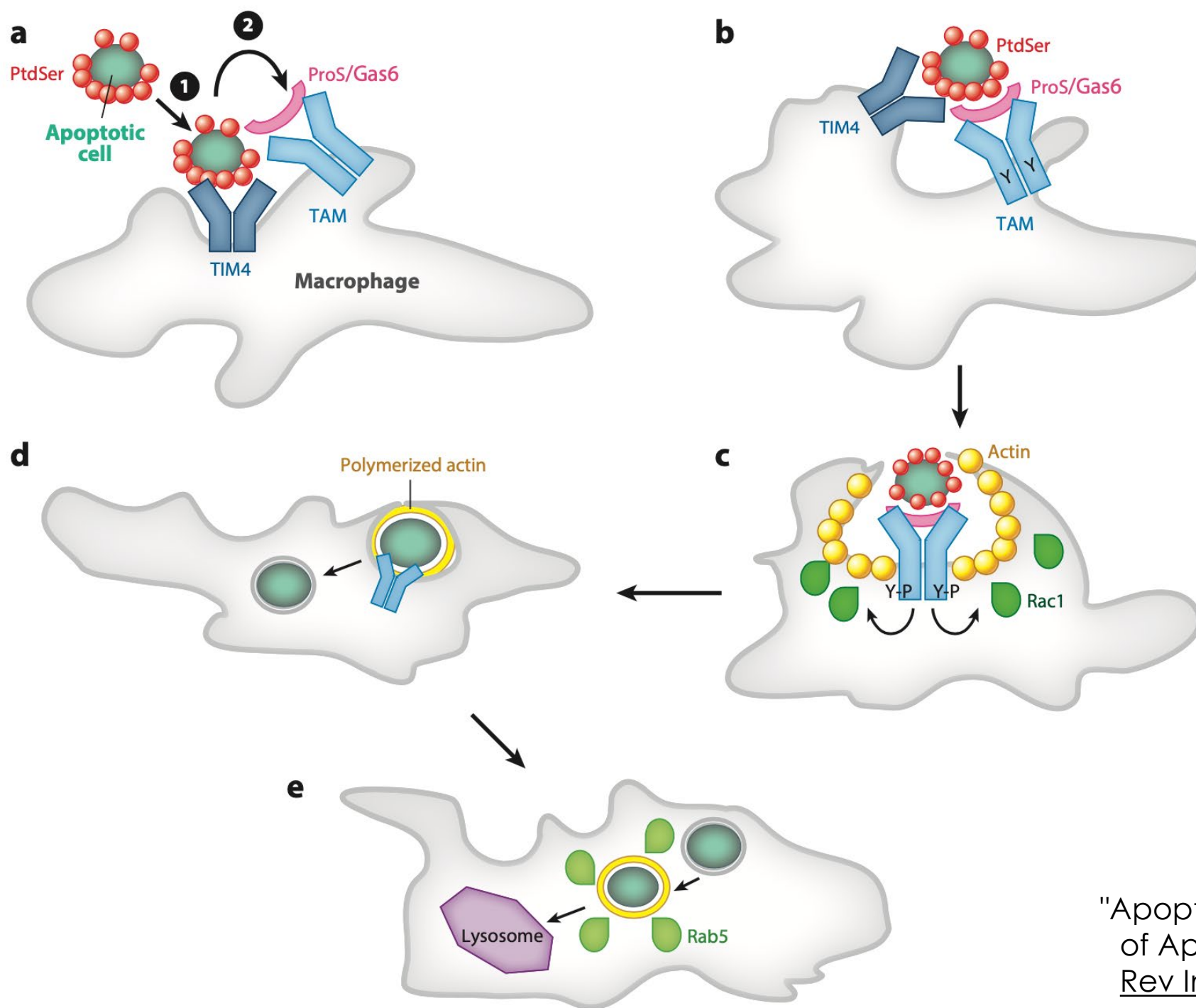
Seurat v3



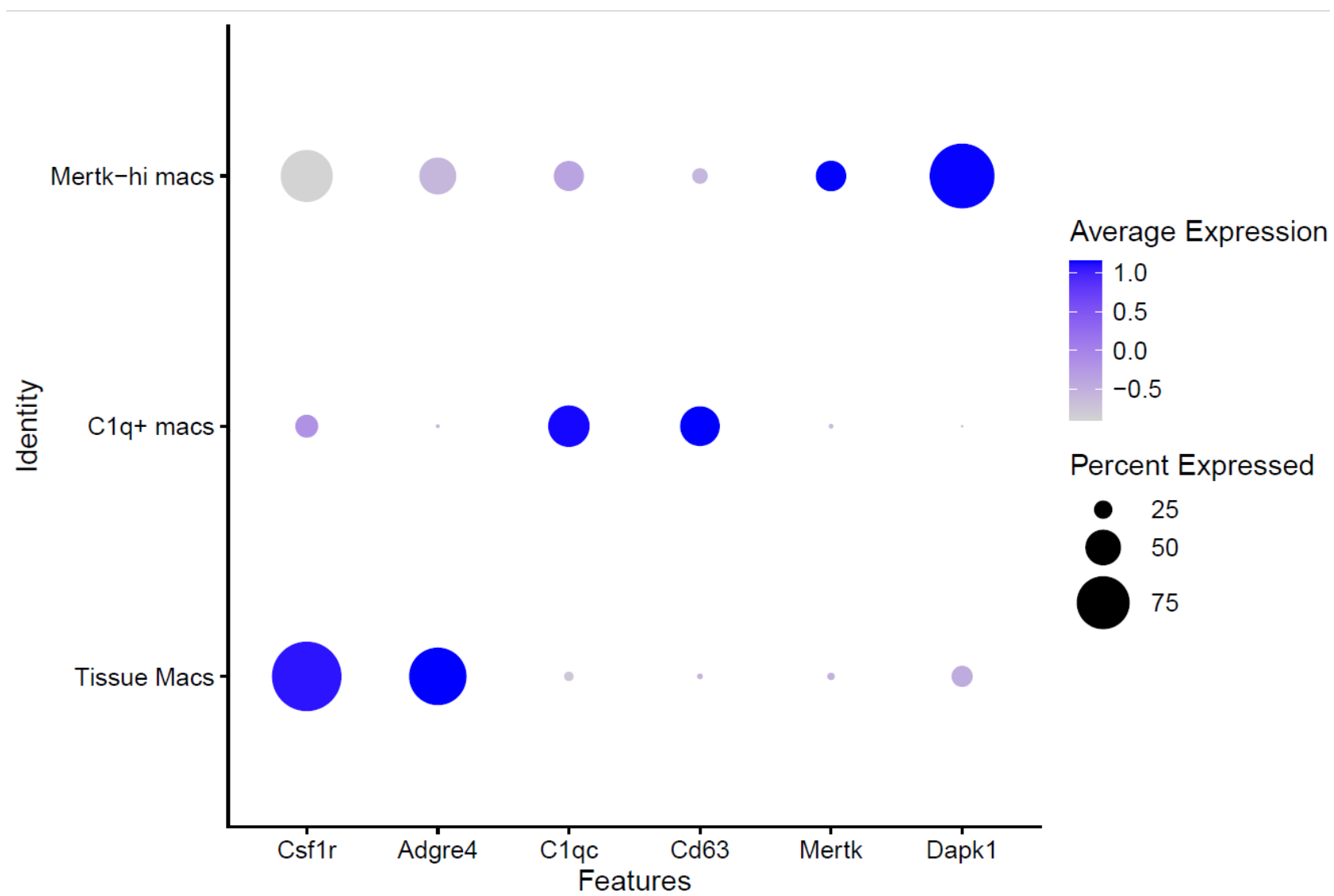


Marker analysis and DEG

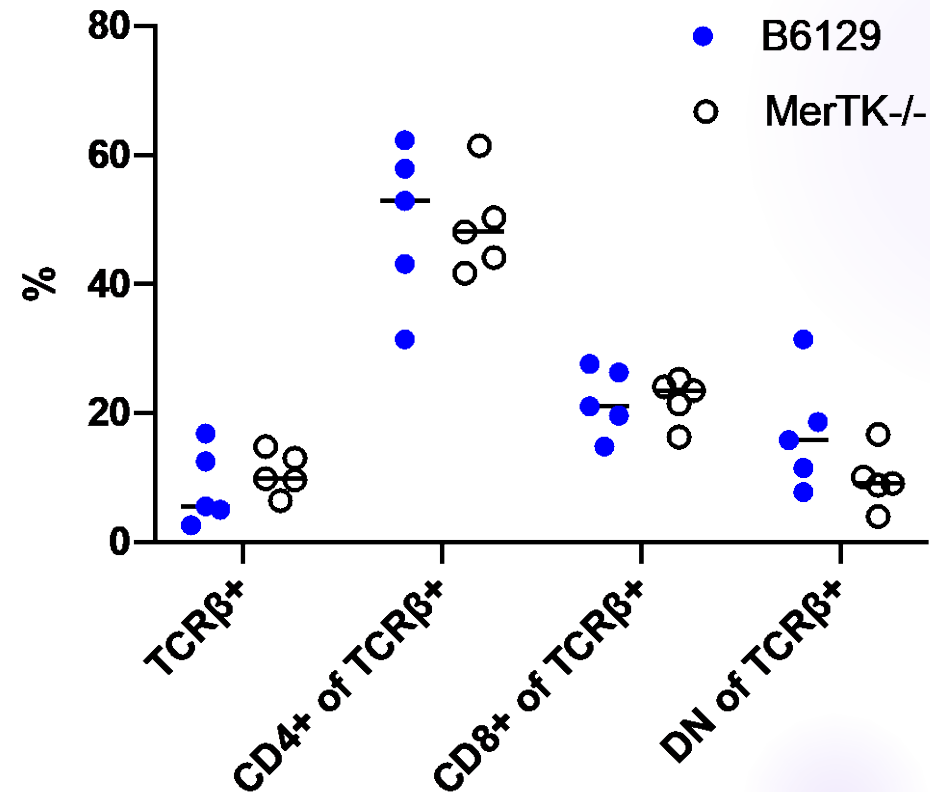
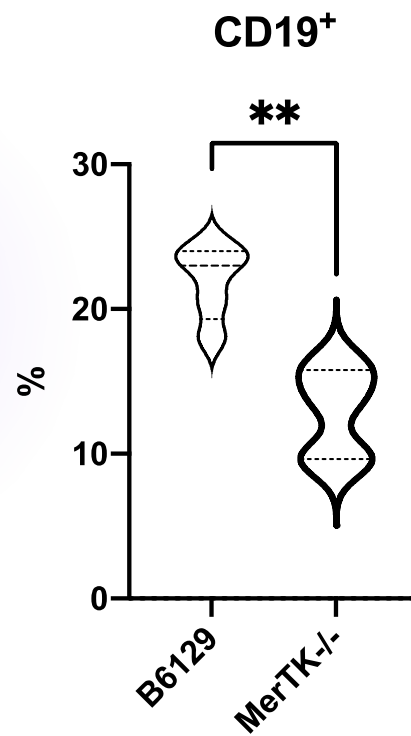




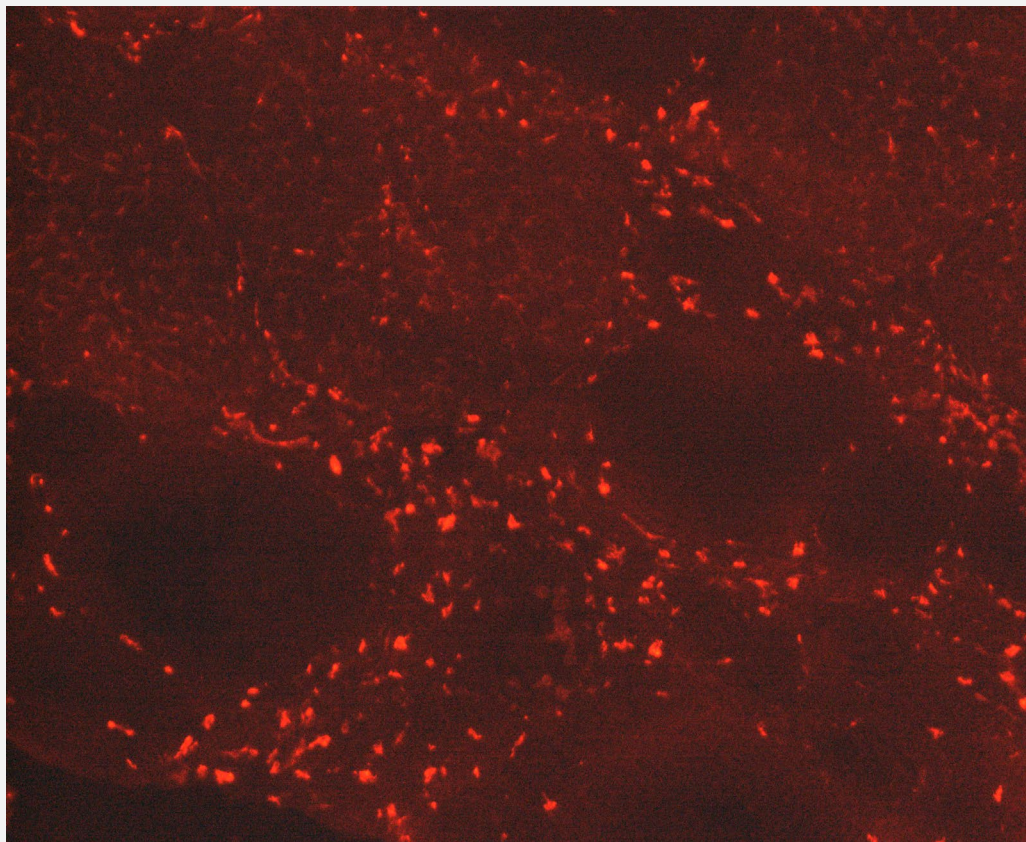
Nagata, S. (2018). "Apoptosis and Clearance of Apoptotic Cells." Annu Rev Immunol **36**: 489-517.



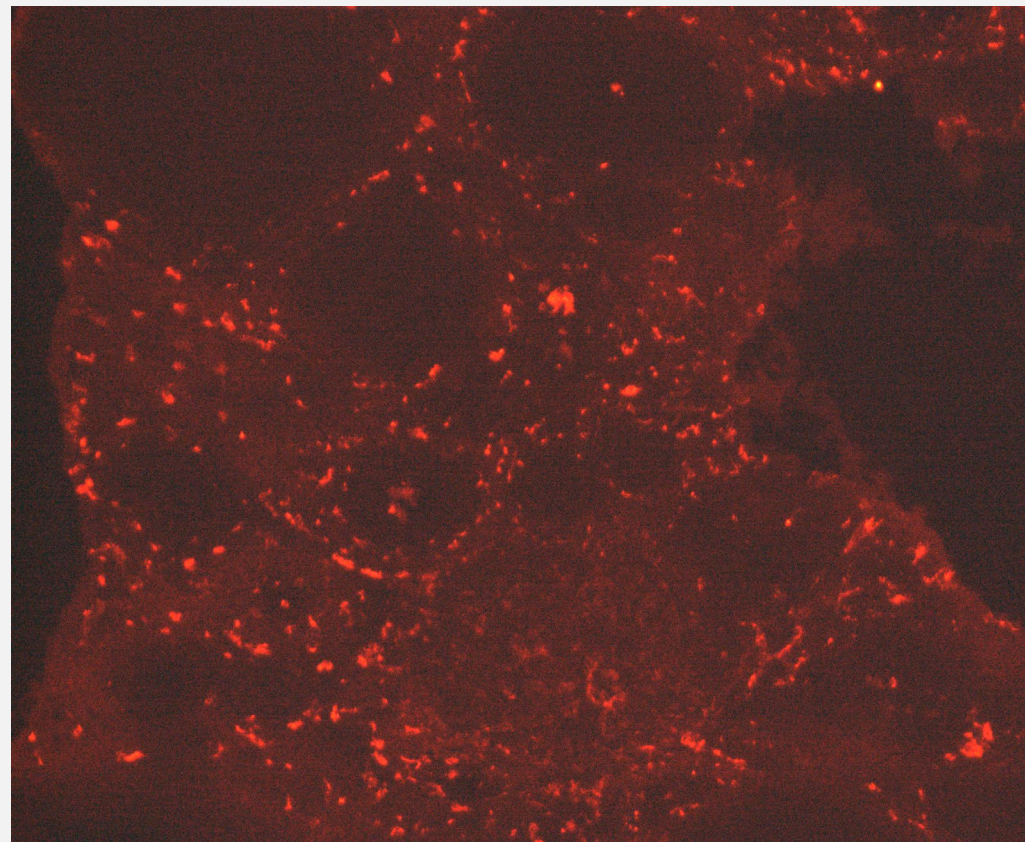
Decreased B cell frequency in ovaries of MerTK deficient mice



B6129 control



MerTK KO



CD206

Summary

- ▶ CD206+ macrophages are abundant in ovary, but expression does not appear to be significantly impacted by high estrogen state
- ▶ MerTK function in maintaining fertility in the testis is likely dispensable in the ovary
- ▶ Despite possible association with male factor infertility, MerTK does not appear to be a crucial target in ovarian remodeling (in young mice)
- ▶ B cells and NK cells unexpectedly respond to “estrous” in superovulated mice

Future Directions

Further investigation of macrophages as potential mediators of “inflammaging”

Investigating phenotype of mice with estrogen receptor deficient macrophages

- ▶ Cytokine and metabolic profile
- ▶ Accumulation of apoptotic debris
- ▶ Fecundity
- ▶ Ovarian lifespan (oocyte loss, fibrosis)

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