Ovarian Stimulation with Gonadotropins Promotes Unique Dynamics of Ovarian Immune Microenvironment

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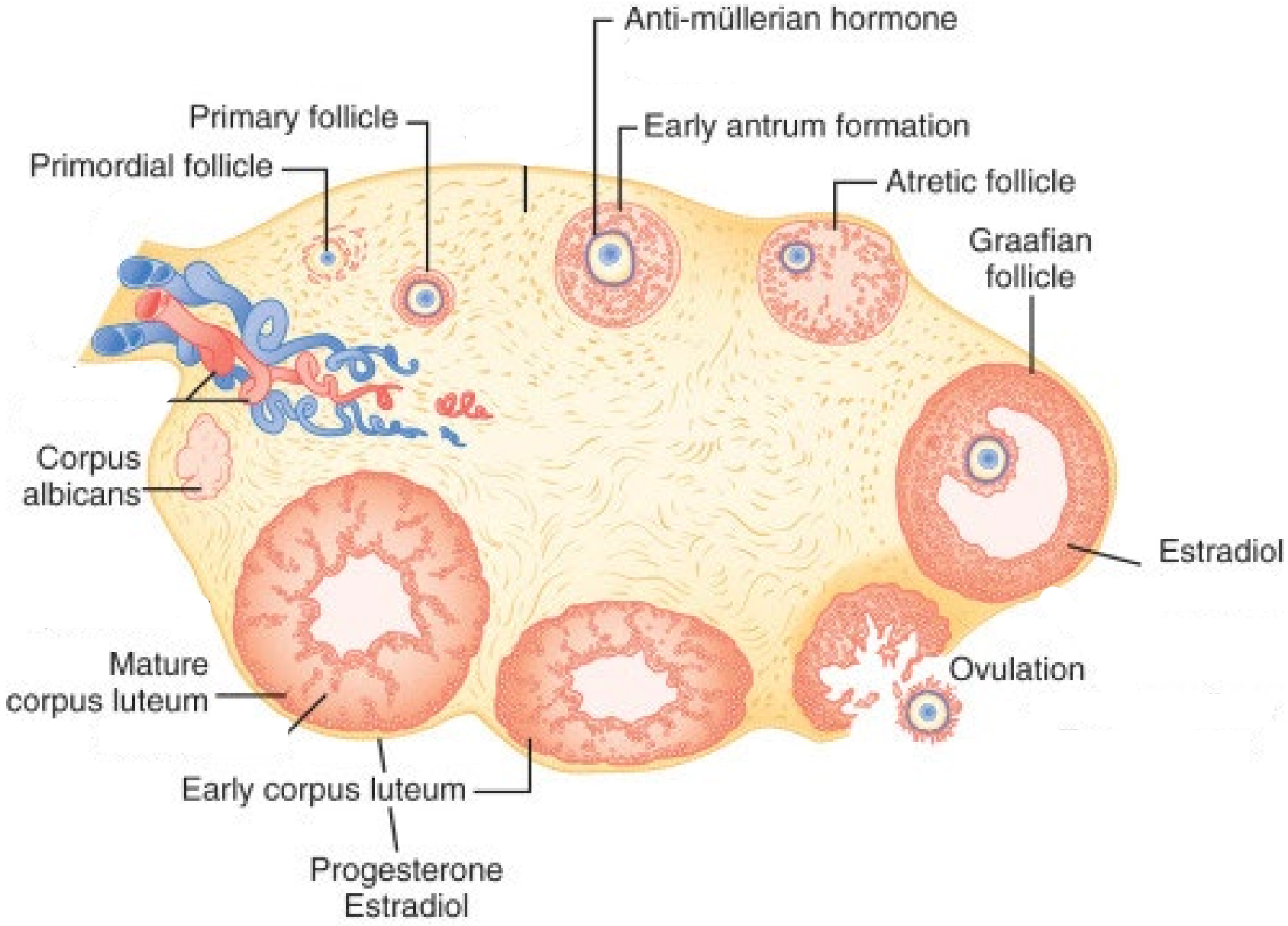
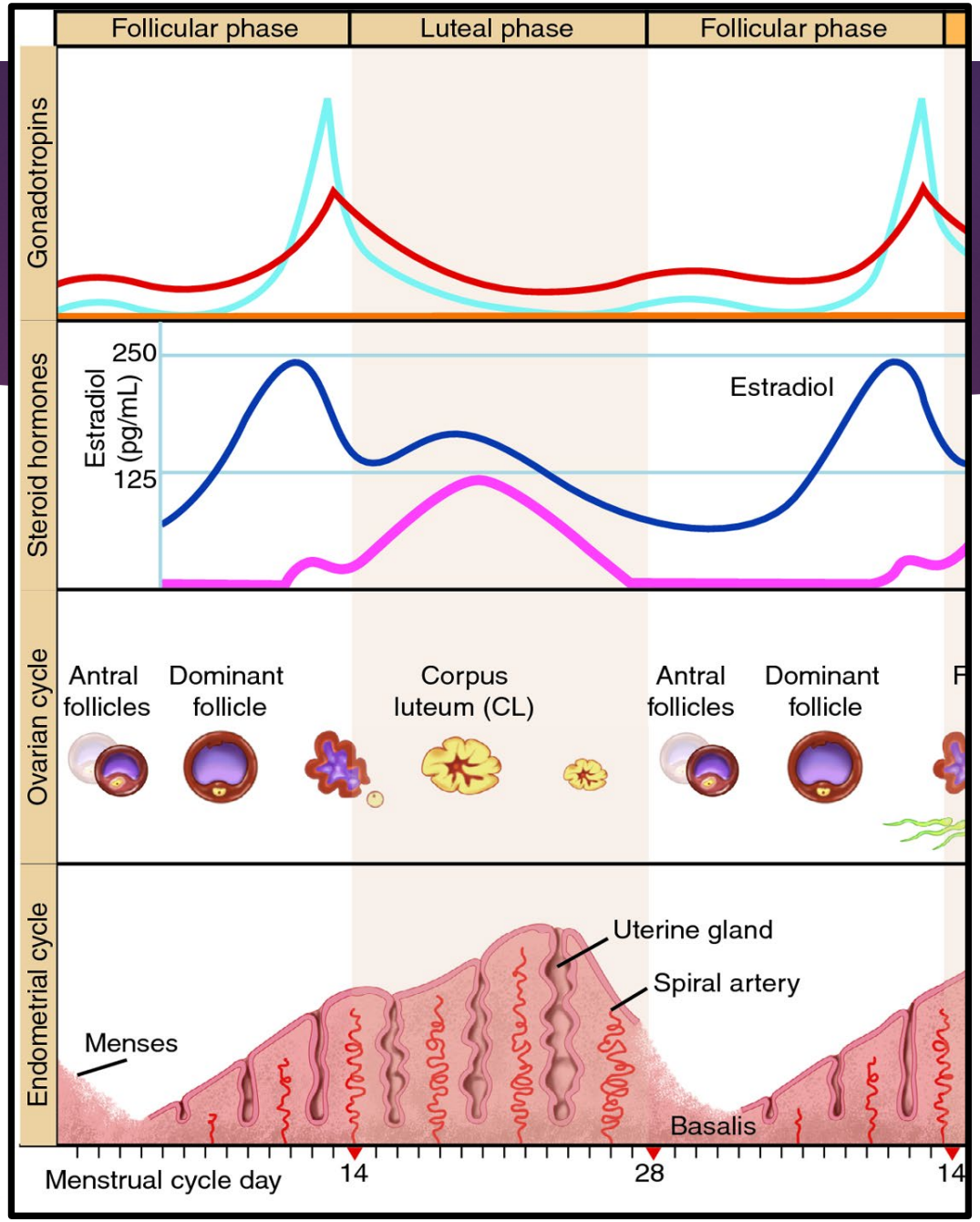
SUPPORT FROM RESEARCH ON WOMEN'S HEALTH IN THE IDEA STATES
SUPPORT RECEIVED AS A CLINICAL AND RESEARCH FELLOW

THE OVARIAN CYCLE



Yen and Jaffe

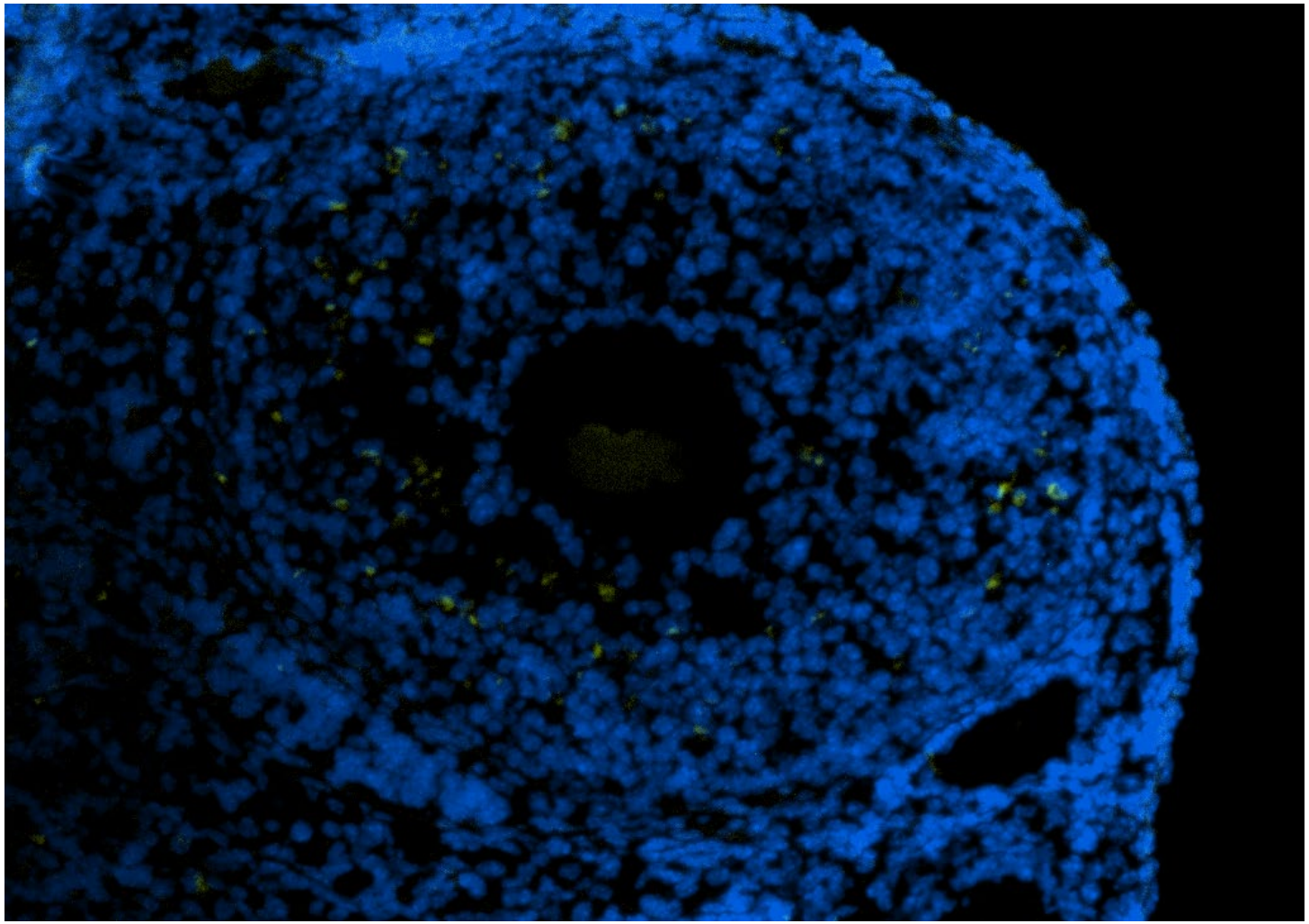


Williams Gynecology
Third Edition

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



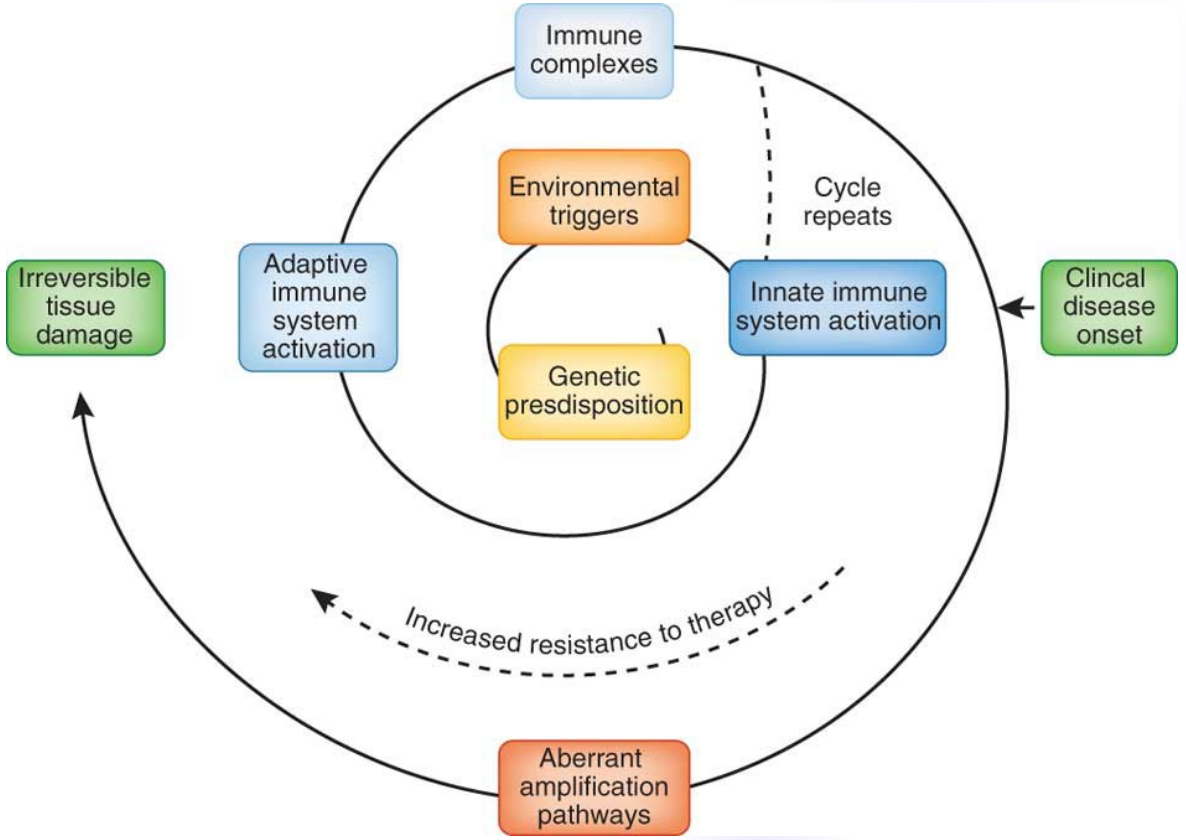
Why is continual remodeling in the ovary relevant?

- 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



Zheng Liu & Anne Davidson
Nature Medicine 18, 871–882 (2012)

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**

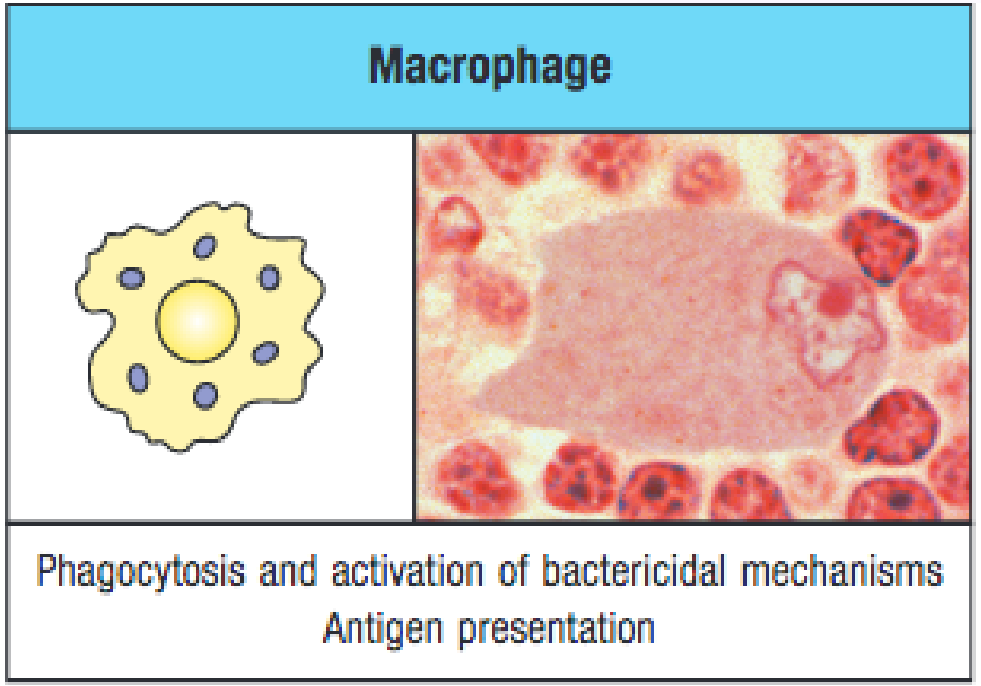
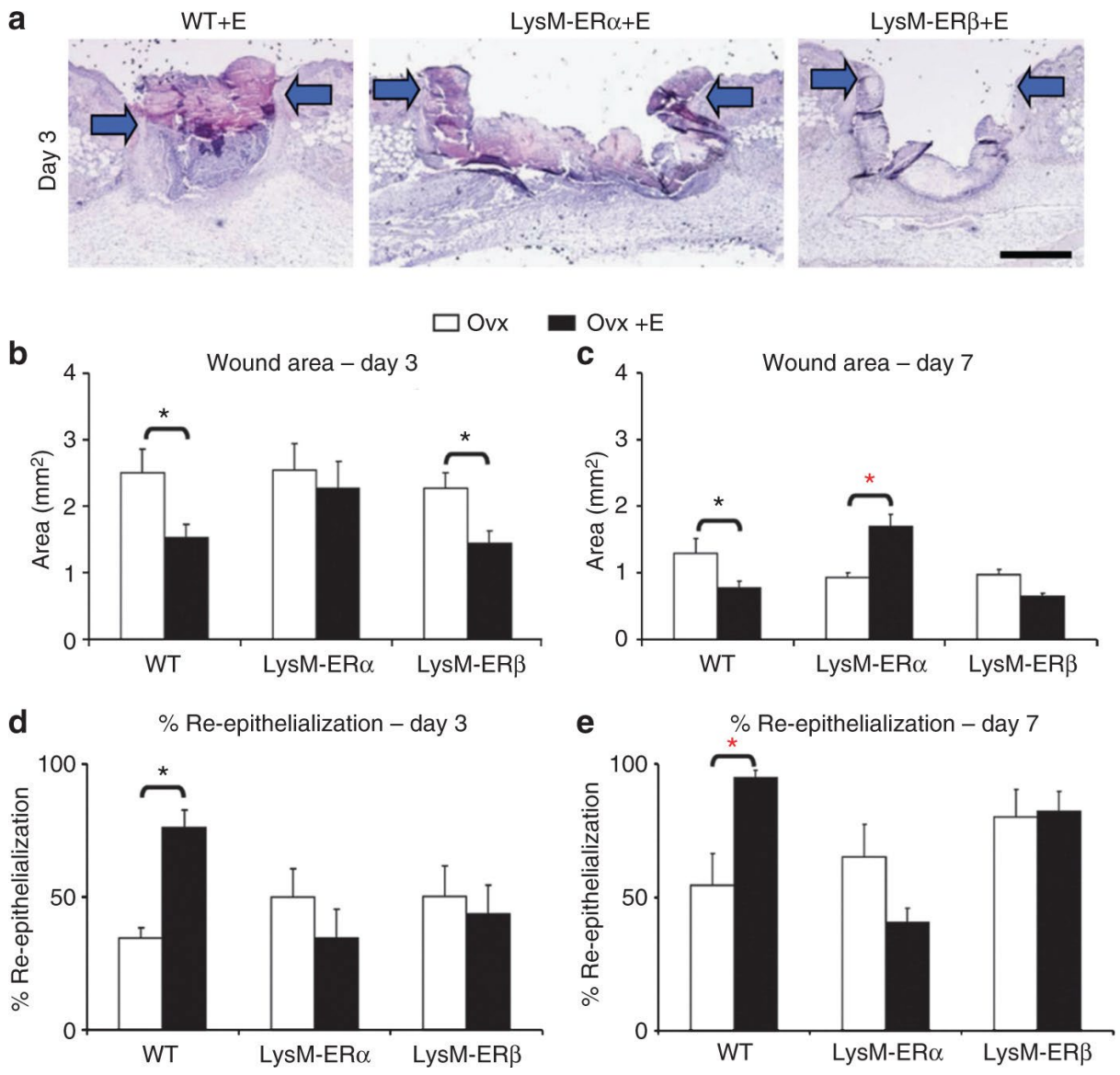


Image Janeway's Immunobiology

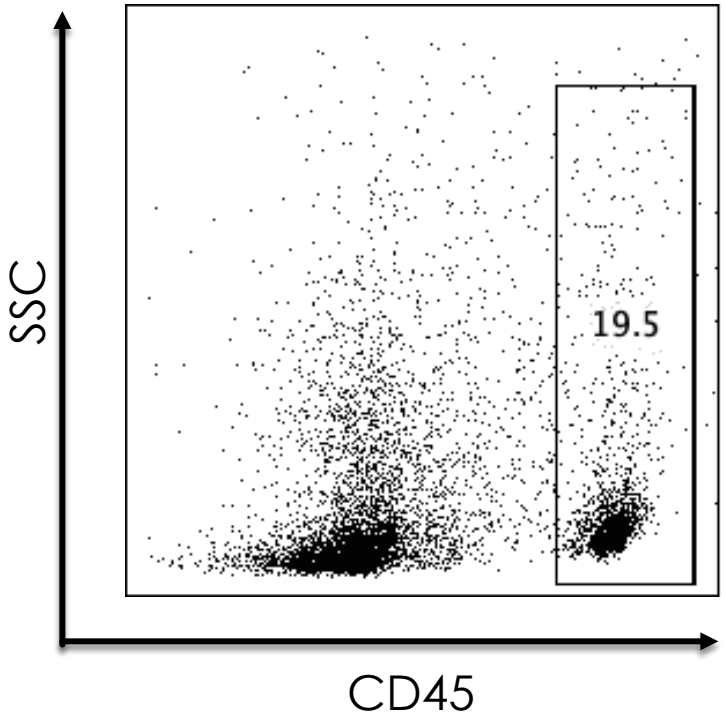


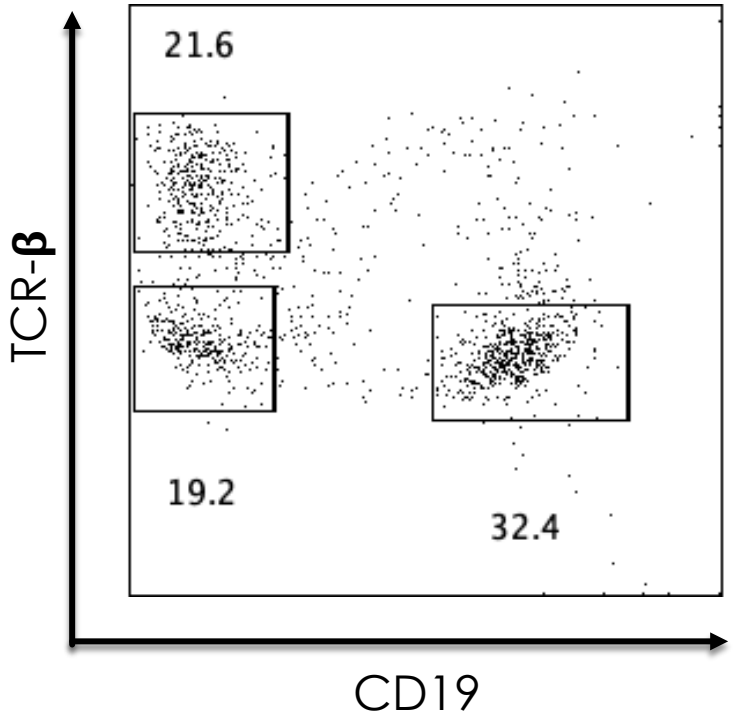
Laura Campbell, et al., Estrogen Receptor-Alpha Promotes Alternative Macrophage Activation during Cutaneous Repair, Journal of Investigative Dermatology, 2014

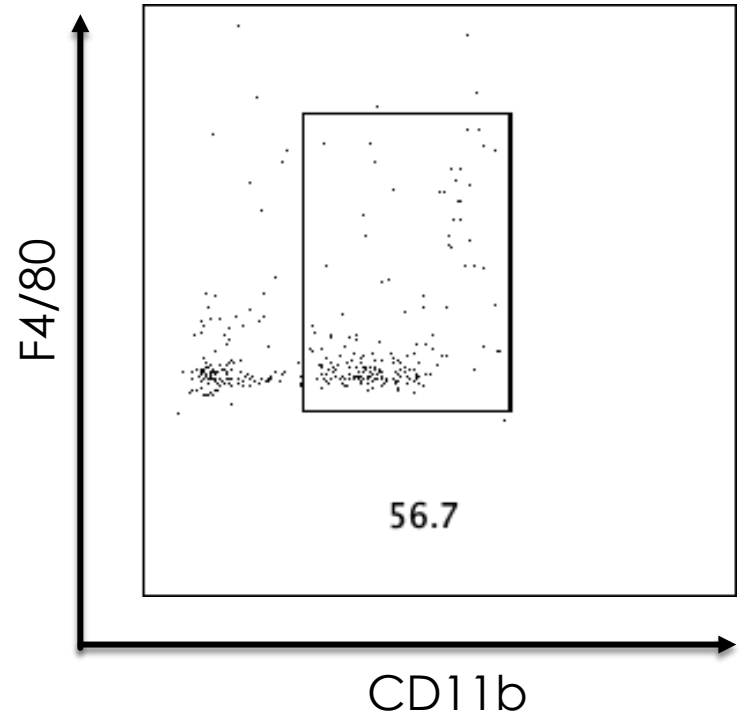
Hypothesis

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



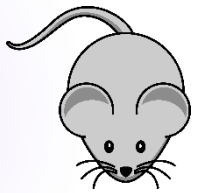




Representative flow cytometry of mouse ovary

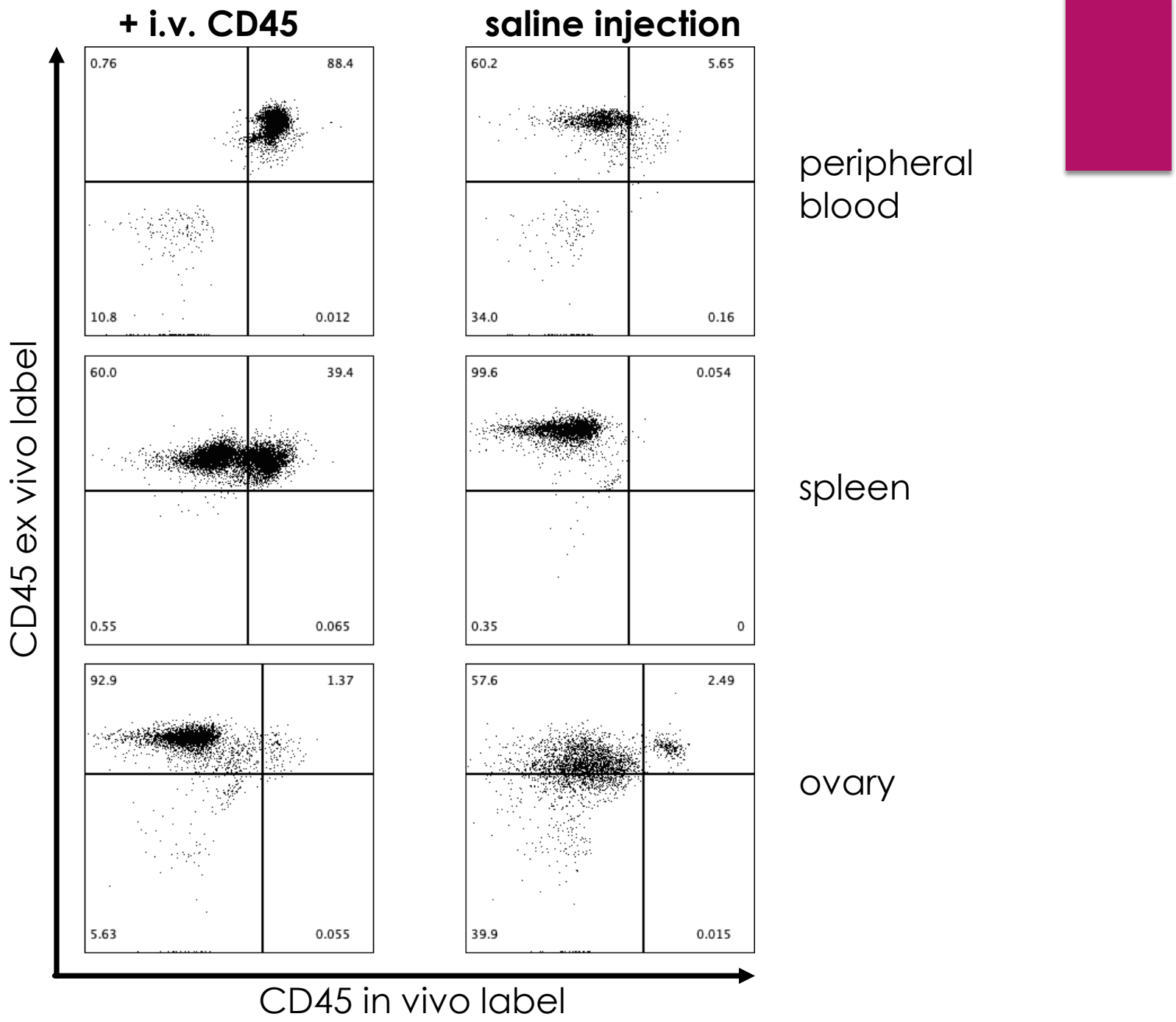
Are ovarian leukocytes truly ovary resident?

C57BL/6



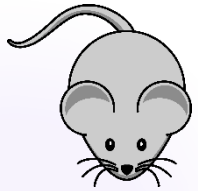
Female mice

→ **CD45-PE i.v.** → **Harvest ovaries after 3 minutes** → **Ex vivo staining for CD45 and more** → **Flow cytometry**


+ i.v. CD45
saline injection
0.76
88.4
10.8
0.012
60.2
5.65
34.0
0.16
60.0
39.4
0.55
0.065
99.6
0.054
0.35
0
92.9
1.37
5.63
0.055
57.6
2.49
39.9
0.015
peripheral blood
spleen
ovary
CD45 ex vivo label
CD45 in vivo label

Experimental Approach

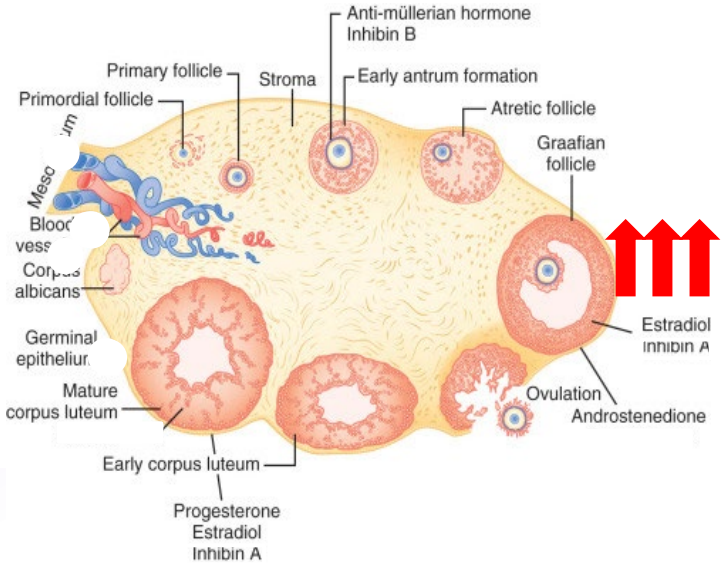
C57BL/6

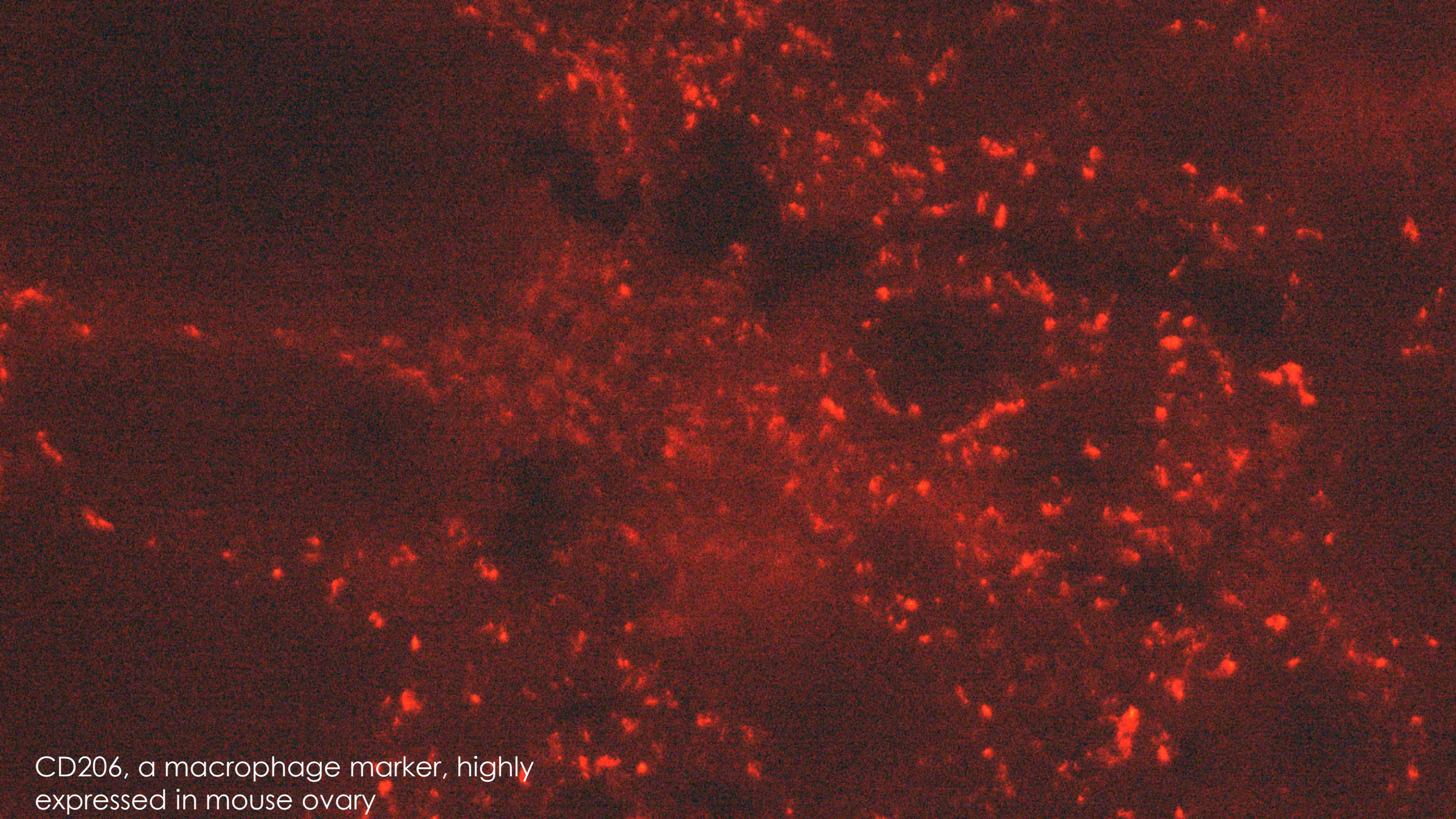


Female mice

→ **Human menopausal gonadotropin** → **48h, high estrogen, pre-ovulation** → **Ovary cell isolation**

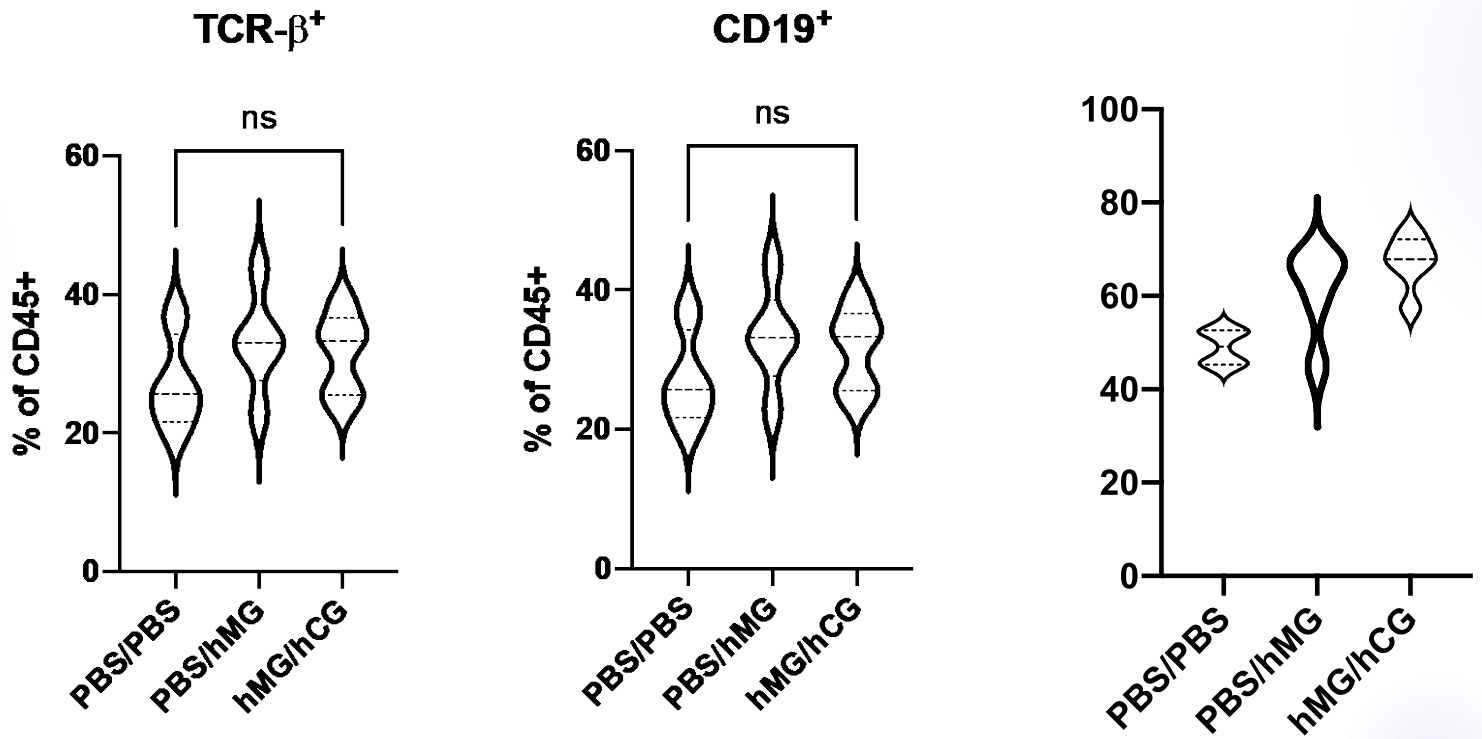
- Flow cytometry
- Tissue sections for immunofluorescence



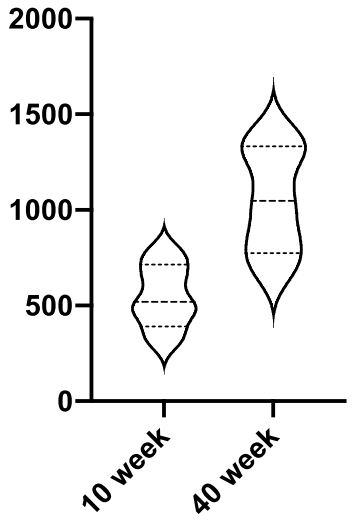


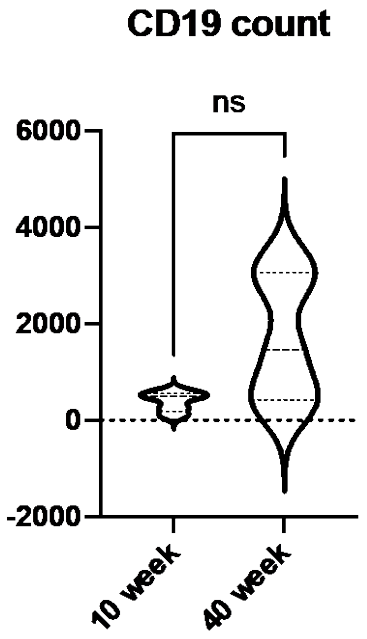
CD206, a macrophage marker, highly expressed in mouse ovary

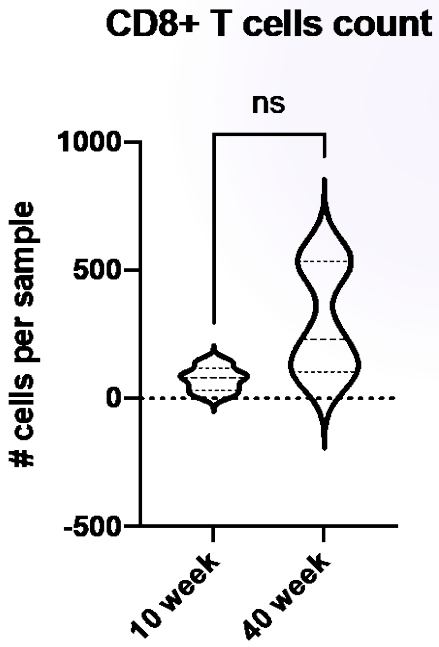
High estrogen and postovulatory state drive macrophage accumulation in the ovary



Macrophage cell numbers increase with age







Experimental Approach

C57BL/6

Female mice

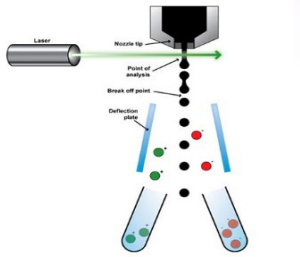
Human menopausal gonadotropin

48h, high estrogen, pre-ovulation

Ovary cell isolation

Individual samples stained with hashtag antibodies

FACS for CD45+

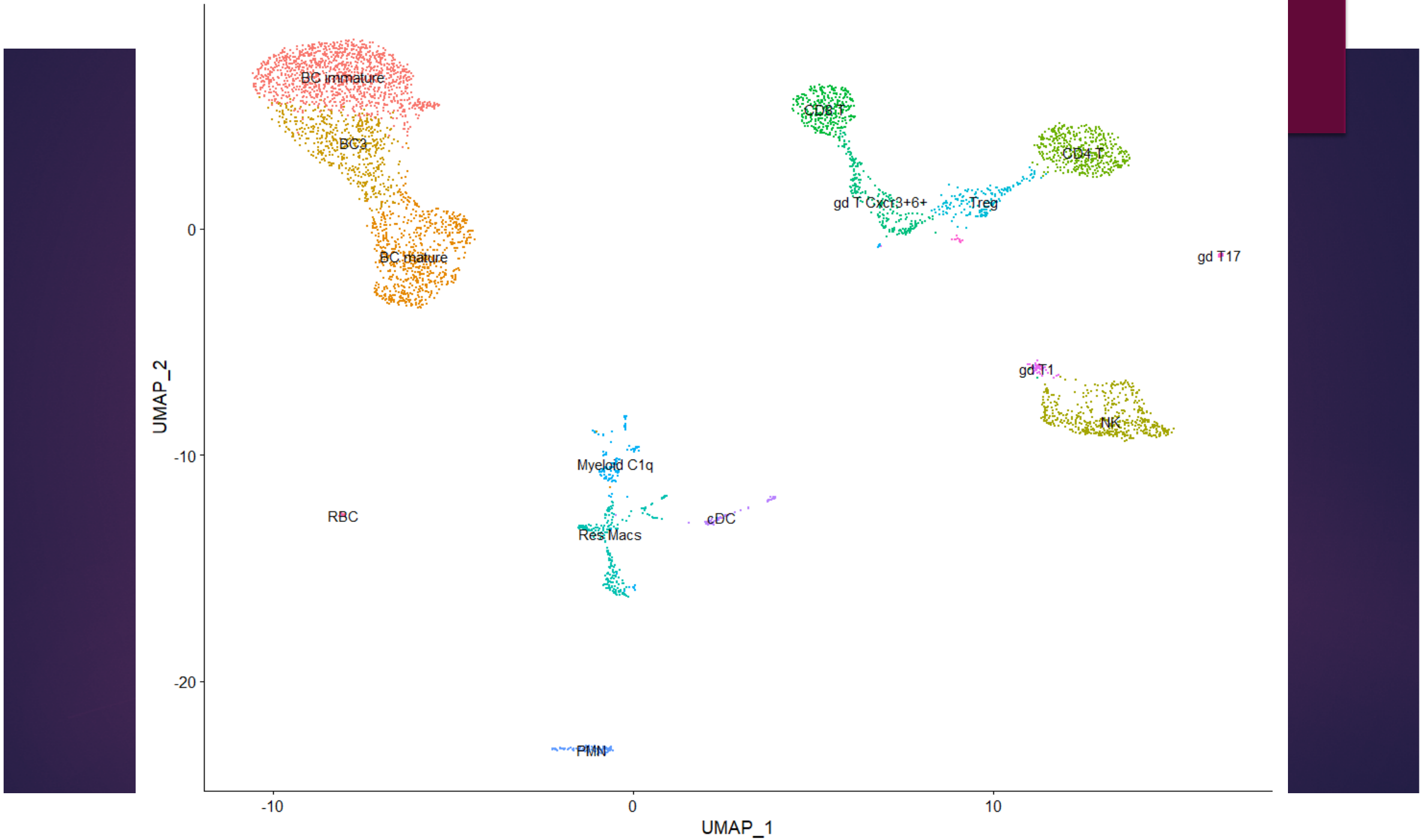


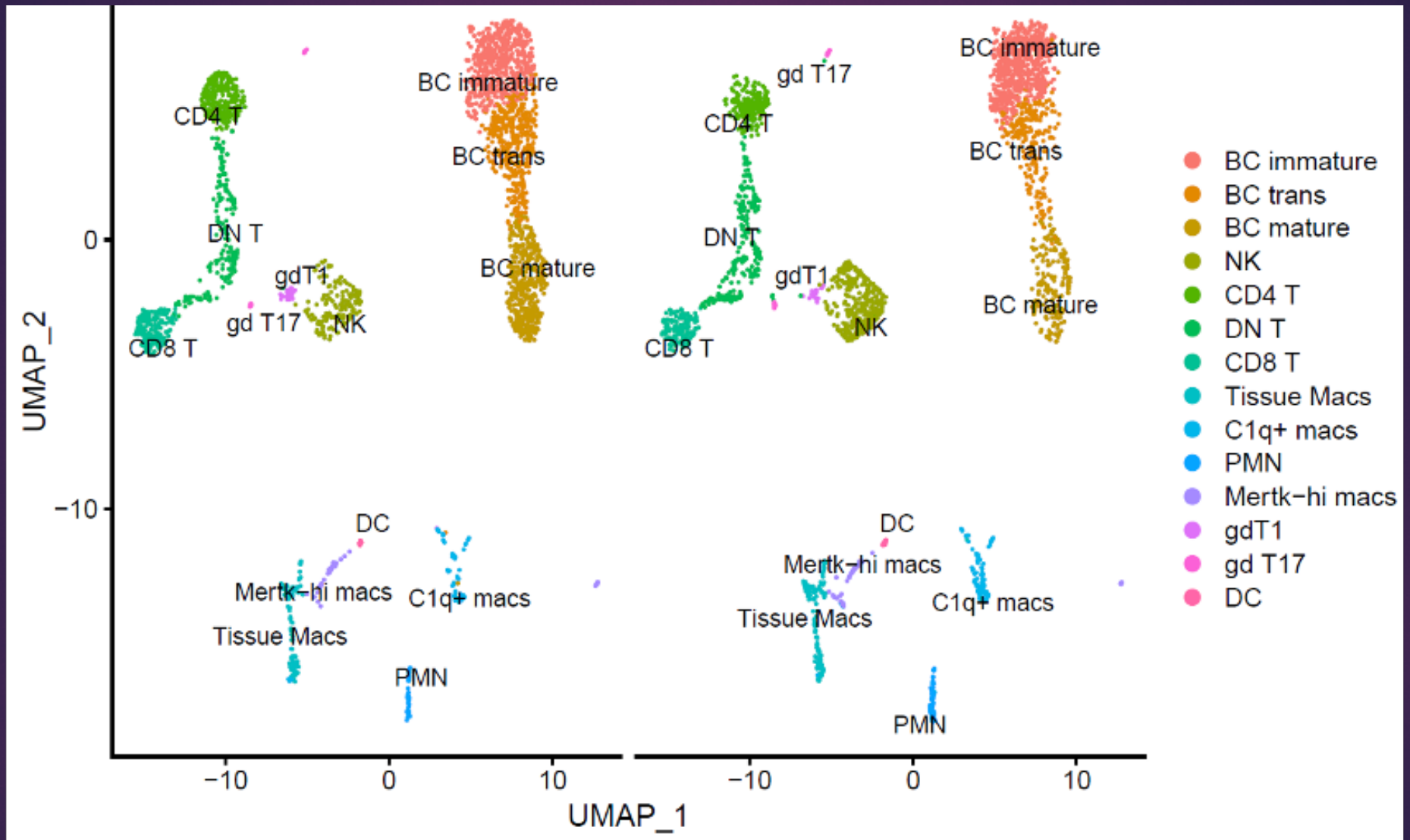
Demultiplex; Single cell transcriptional profiles

Single cell RNAseq (10xGenomics)

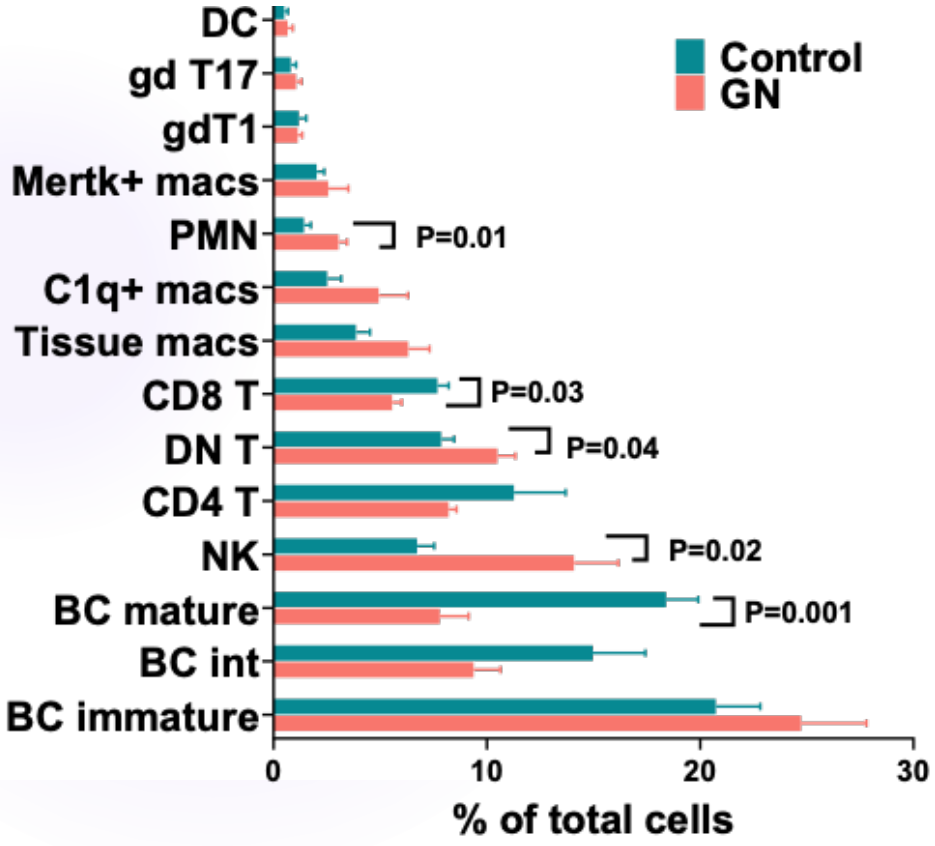
CellRanger

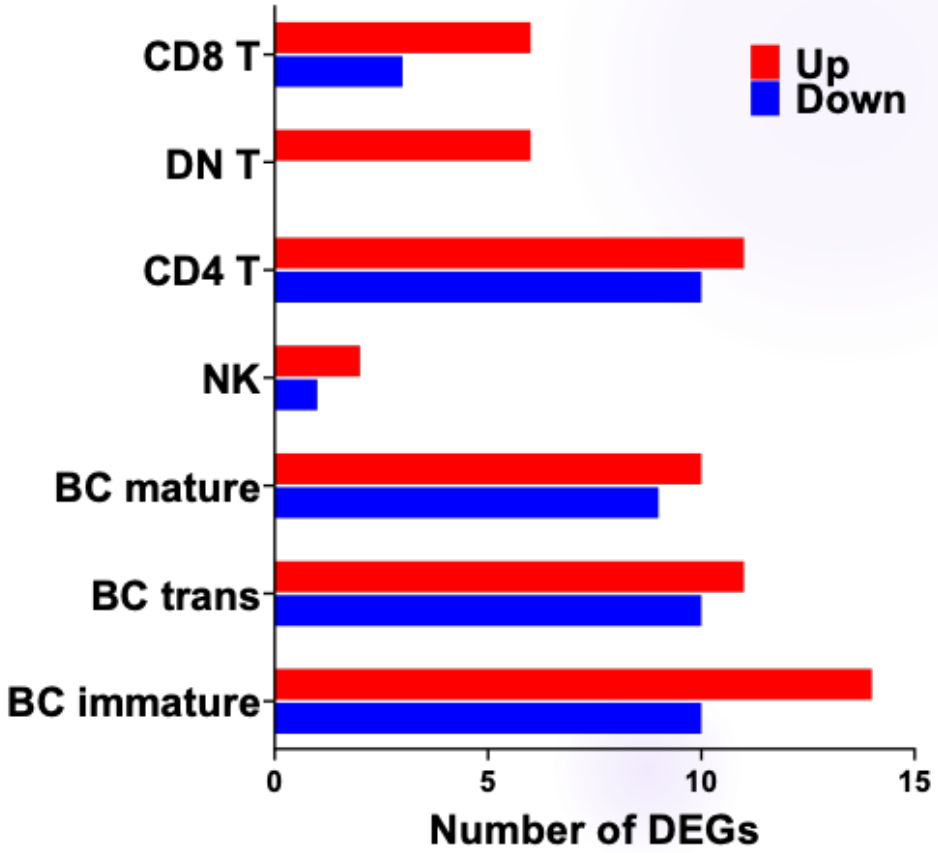
Seurat v3

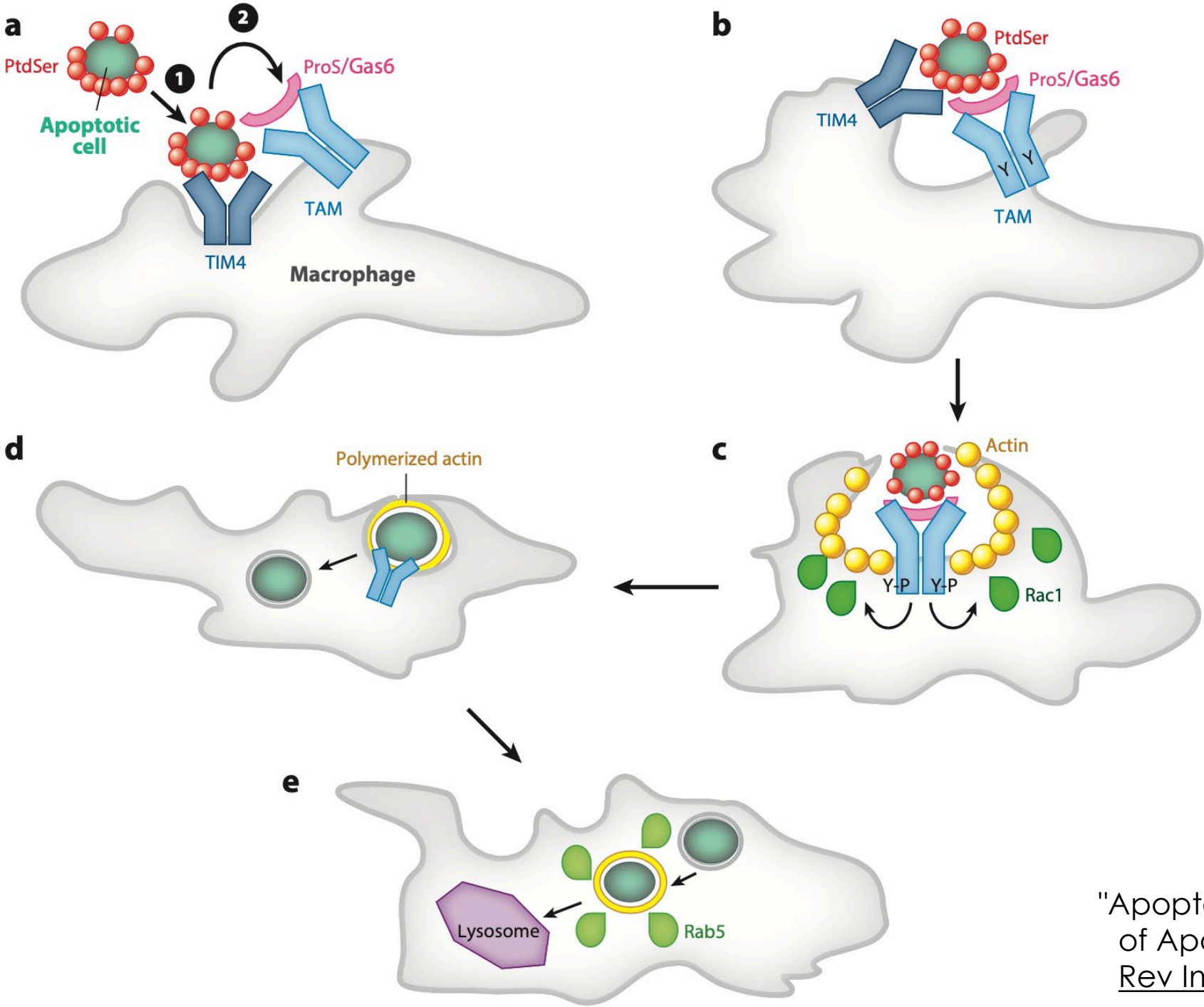

BC immature
BC1
BC mature
CD8 T
CD4 T
gd T Cxcr3+6+
Treg
gd T17
gd T1
NK
Myeloid C1q
RBC
cDC
Res Macs
PMN
UMAP_2
UMAP_1
0
-10
-20
-10
0
10


BC immature
BC trans
BC mature
NK
CD4 T
DN T
CD8 T
Tissue Macs
C1q+ macs
PMN
Mertk−hi macs
gdT1
gd T17
DC
UMAP_1
UMAP_2
−10
0
10

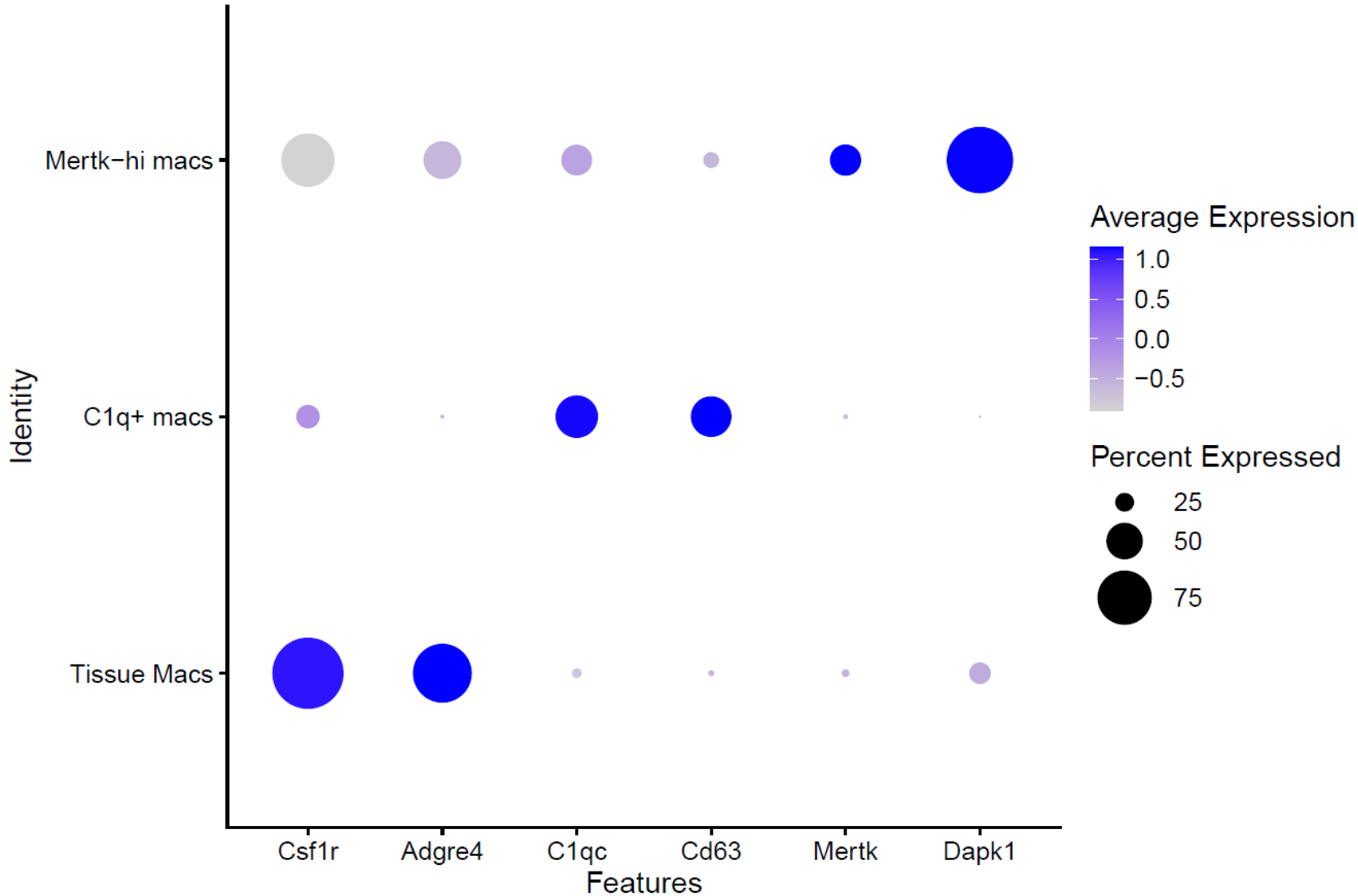
Marker analysis and DEG



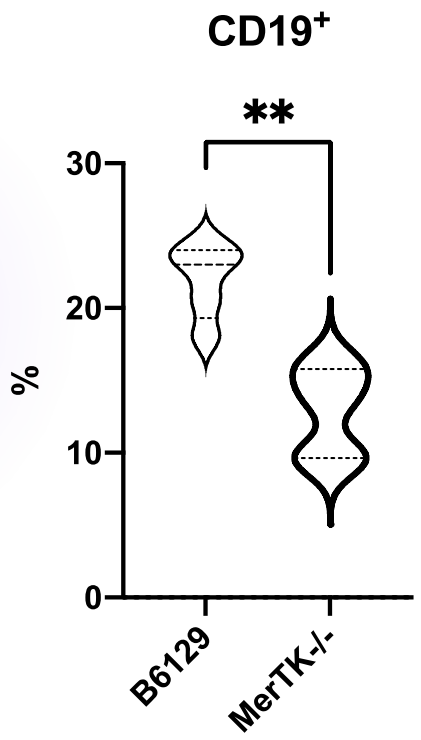


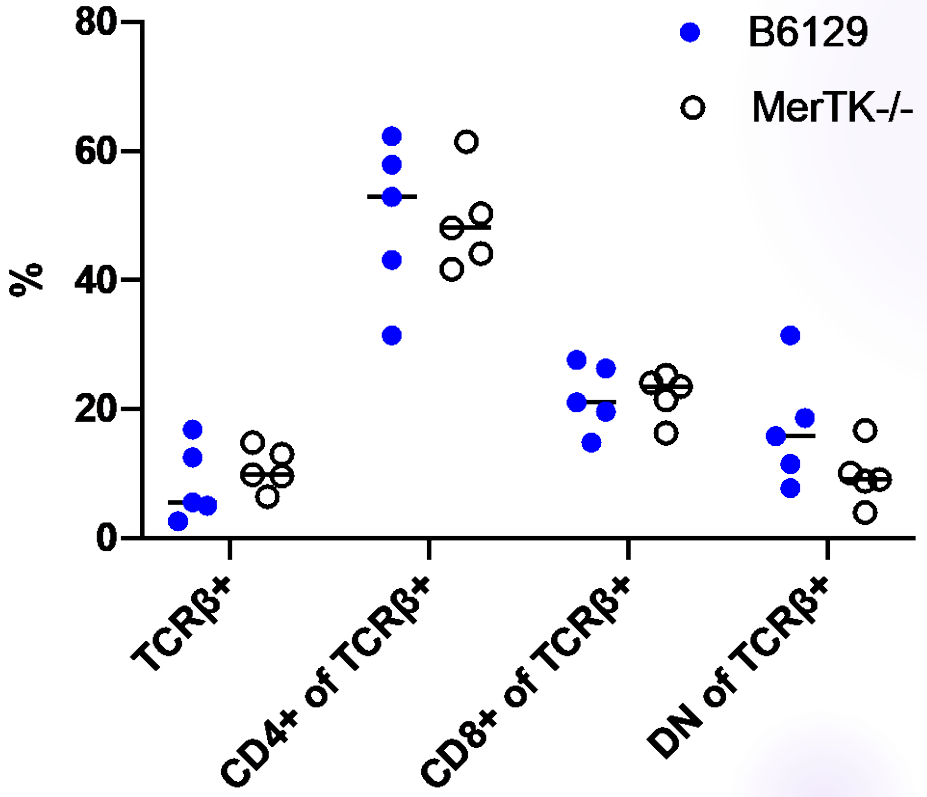


Nagata, S. (2018). "Apoptosis and Clearance of Apoptotic Cells." Annu Rev Immunol **36**: 489-517.


Mertk−hi macs
C1q+ macs
Tissue Macs
Identity
Csf1r
Adgre4
C1qc
Cd63
Mertk
Dapk1
Features
Average Expression
1.0
0.5
0.0
−0.5
Percent Expressed
25
50
75

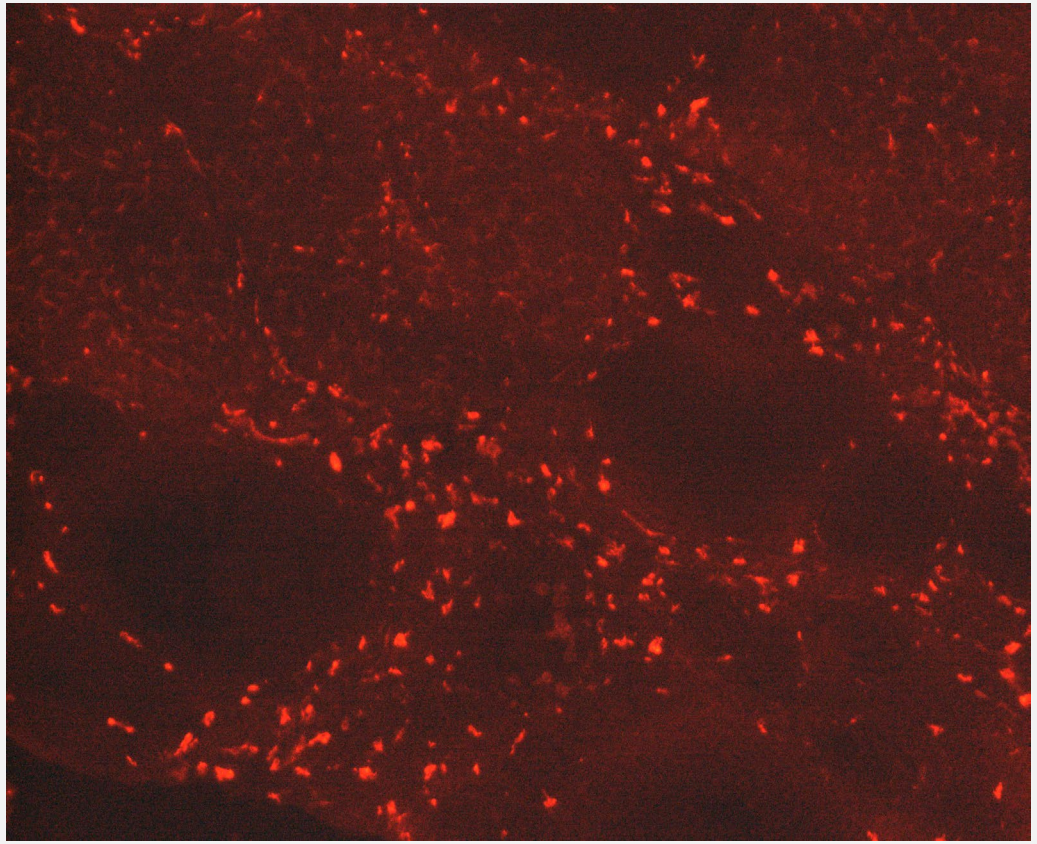
Decreased B cell frequency in ovaries of MerTK deficient mice

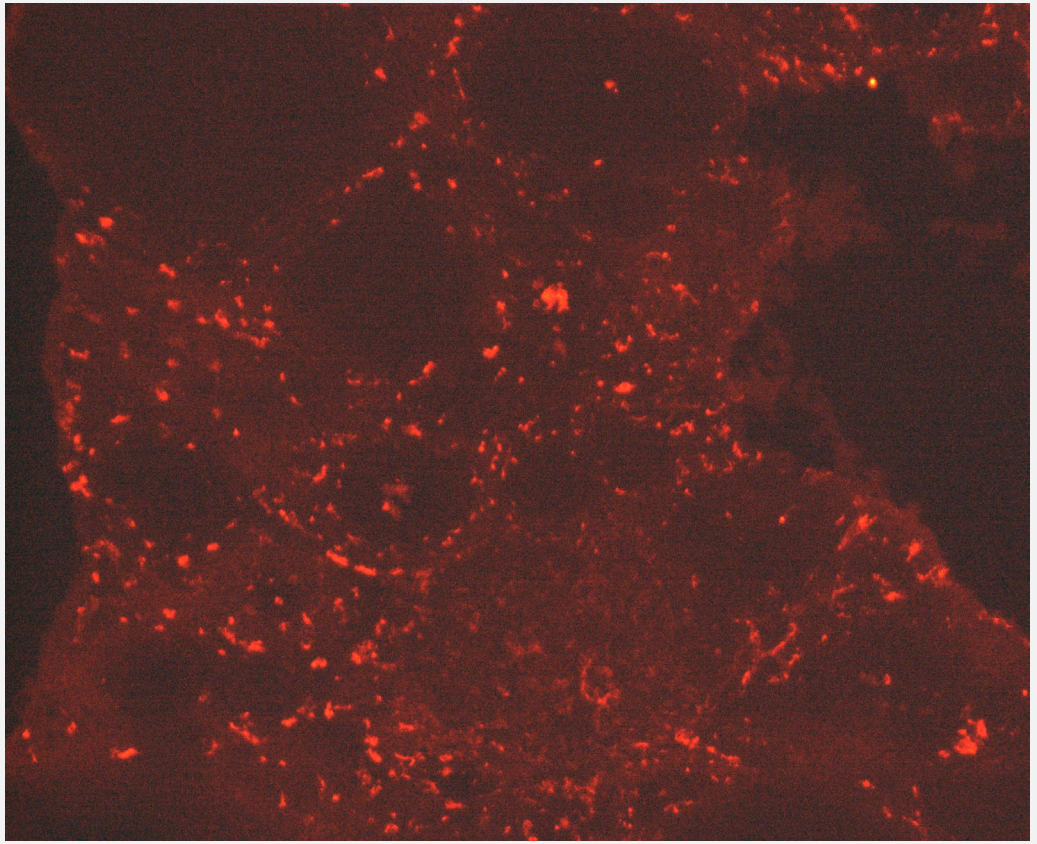




B6129 control

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)

Acknowledgements

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