

# Multiple Sclerosis (Sclérose en plaque):

Unknown etiology

Inflammatory disease of the CNS, destruction of nerve insulation (myelin sheath), impaired blood-brain-barrier integrity

- First MS cases were reported during the middle-ages
- First case report by Charcot 1868
- Symptoms:
  - Weakness, Paralysis, Spastic
  - Numbness, Pain
  - Visual impairment, Blindness
  - Memory loss, Fatigue, Depressions
- Therapie:  
the No cure, Immuno-modulatory therapy can slow disease progress

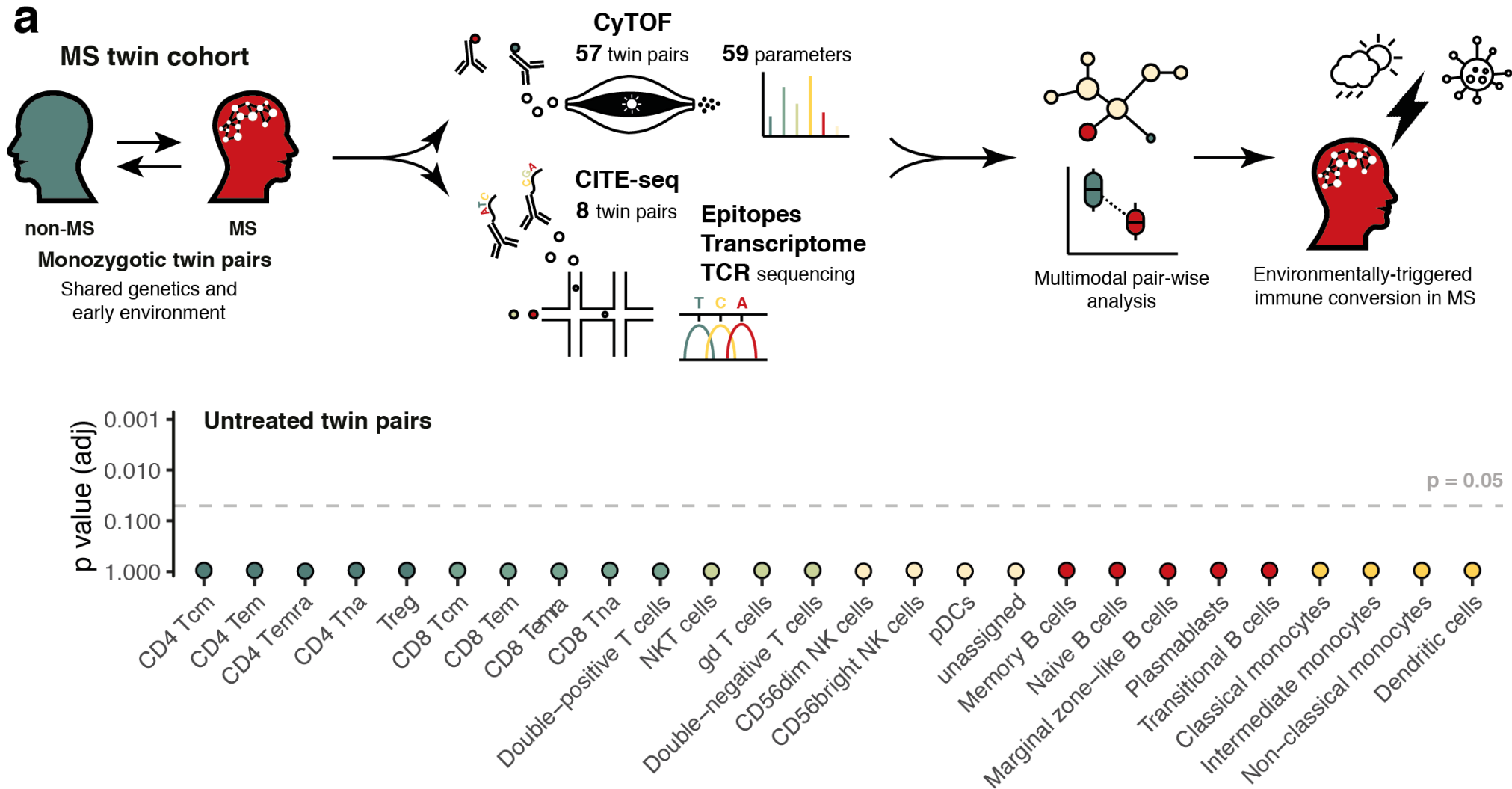
# What (if there is any) is the immune signature of Multiple Sclerosis?

Ingelfinger et al. 2022

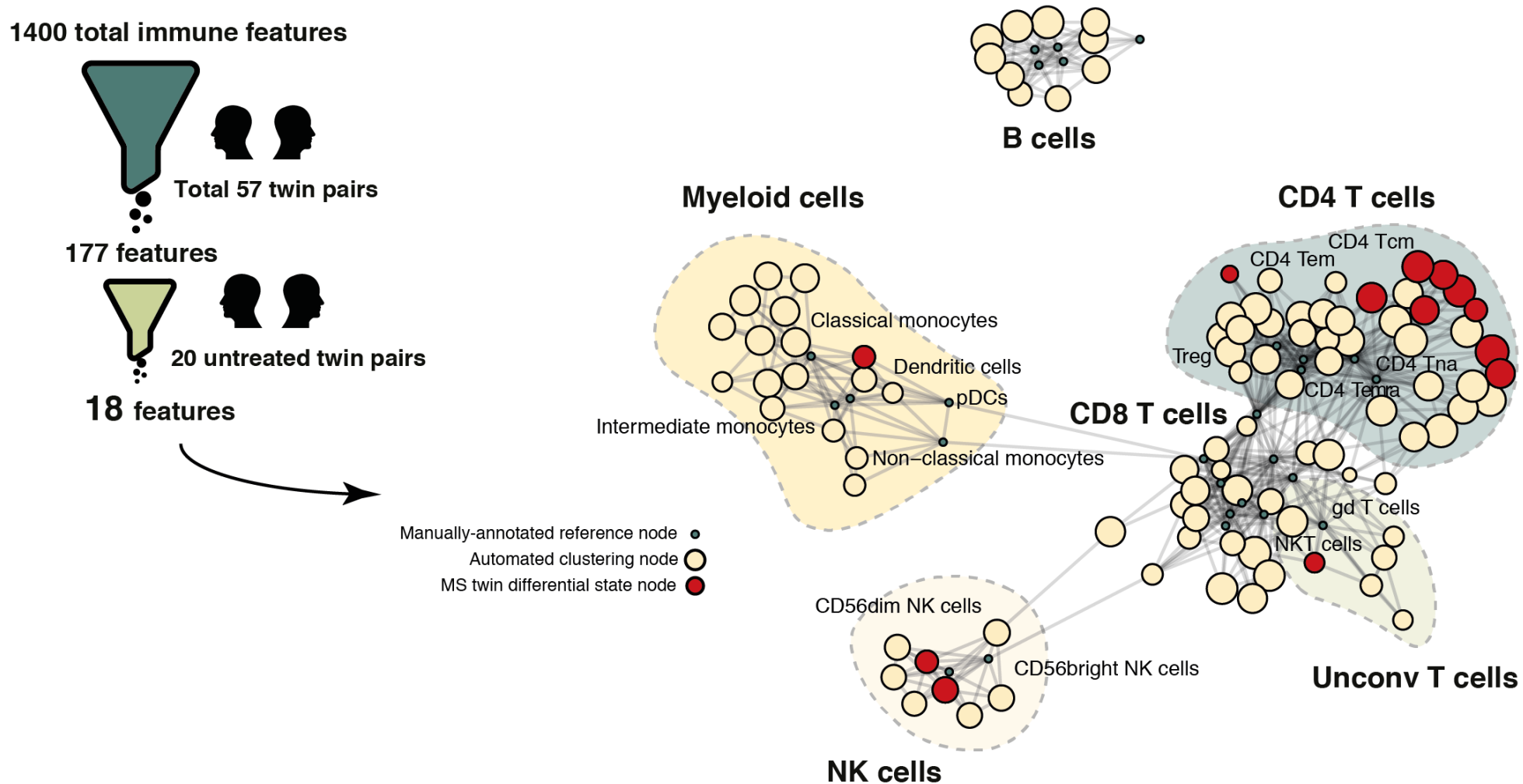
Galli et al. 2019

|               |                                                                                               |
|---------------|-----------------------------------------------------------------------------------------------|
| UZH:          | Felix J. Hartmann, Bettina Schreiner, Sindu Krishnarajah, Pascale Zwicky, Donatella Defeo     |
| Paris:        | Florent Ginhoux, Charles Antoine-Dutertre                                                     |
| ETHZ:         | Eirini Arvaniti, Manfred Claassen                                                             |
| Karolinska:   | Faiez Al Nimer Mohsen Khademi, Fredrik Piehl, Tomas Olsson                                    |
| UniBas:       | Nicholas Sanderson, Martin Diebolt, Tobias Derfuss,                                           |
| LMU:          | Lisa Ann Gerdes, Martin Kerschensteiner, Reinhard Hohlfeld, Eduardo Beltran, Vladyslav Kavaka |
| UniGöttingen: | Christine Stadelman                                                                           |

# Twin study design eliminates genetic predisposition

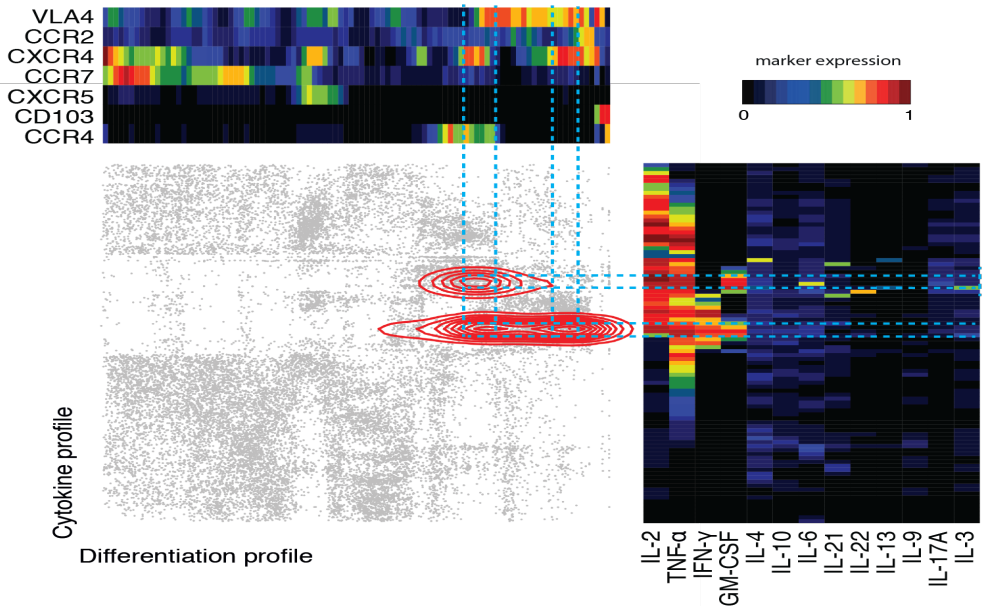
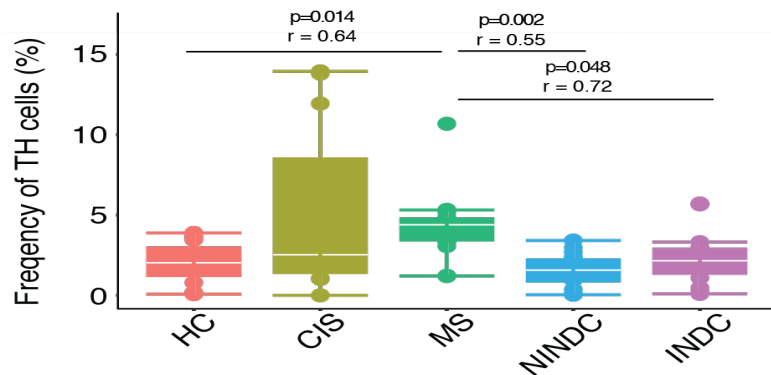
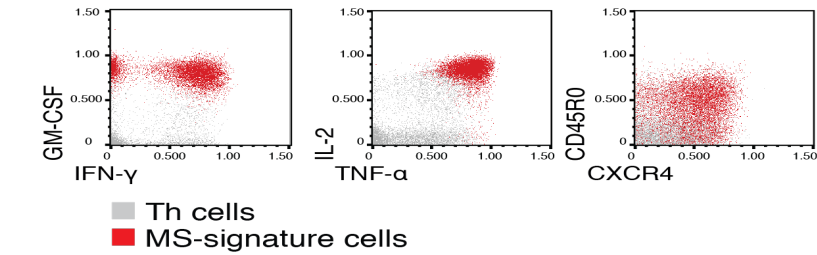
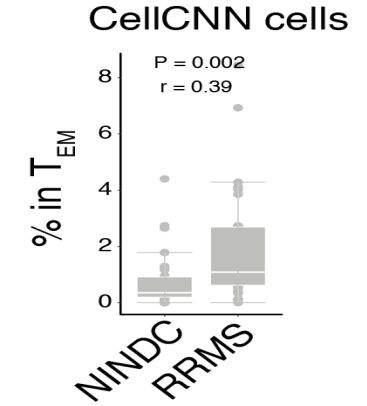
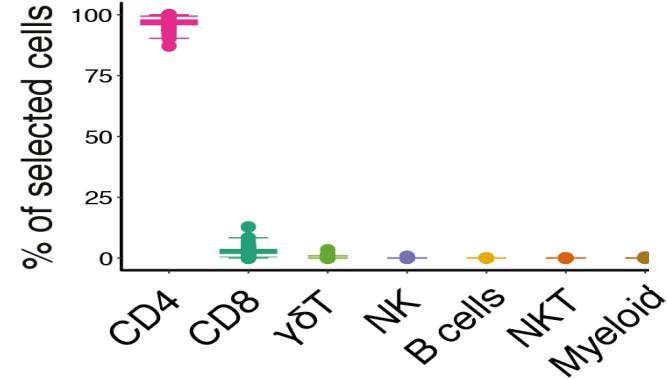
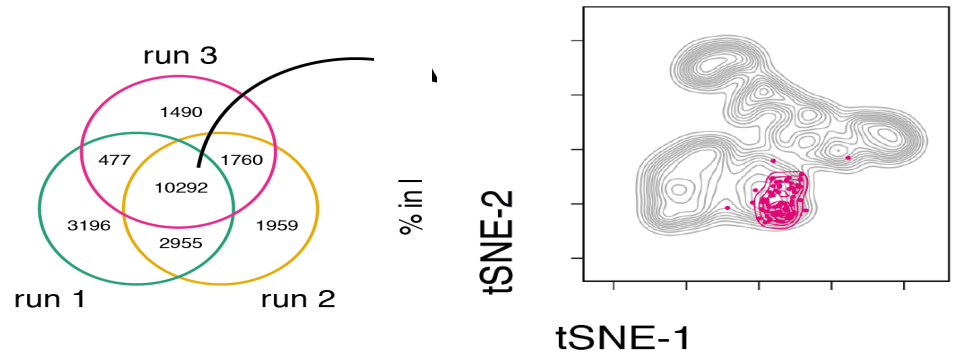


# Manifestation of MS is accompanied by immune conversion in the Th cell and myeloid compartment



# GM-CSF<sup>+</sup>CXCR4/3<sup>+</sup> T-helper cells CellCNN

## identifies an MS-specific signature



Is the GM-CSF T<sub>H</sub> signature  
pathogenically relevant?

# GM-CSF (Csf-2) biology

## Steady State

Produced at low levels under steady state conditions to form and maintain:

- Alveolar Macrophages,
- Minor effect on CD103<sup>+</sup> DCs in epithelial tissues

But NOT

- required for the formation or maintenance of cDCs, Macrophages, Granulocytes

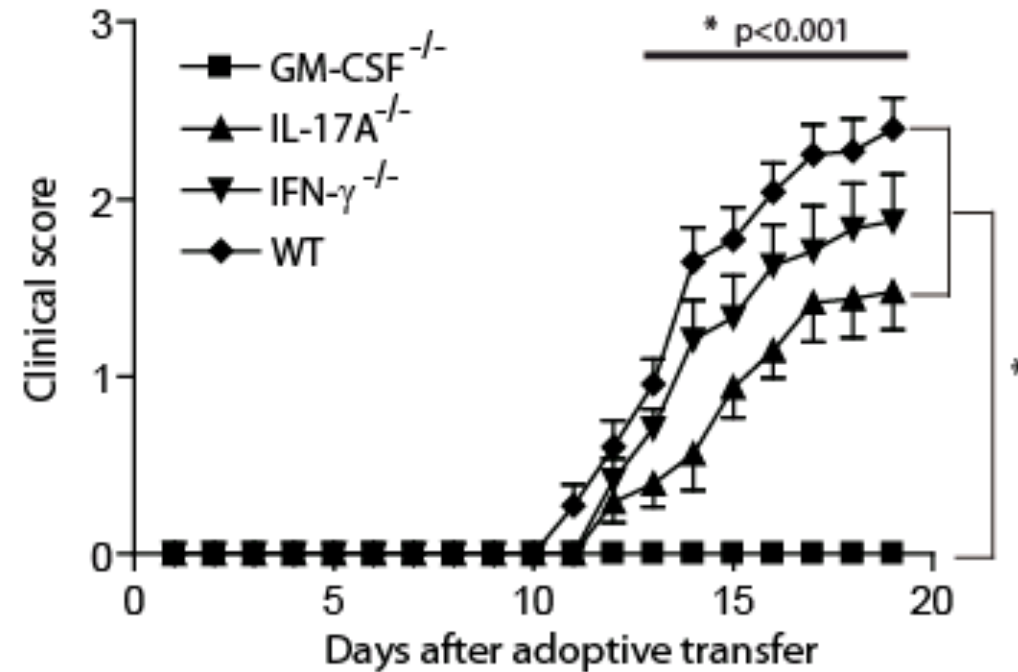
## Inflammation

- GM-CSF activates the transition of monocytes into inflammatory tissue macrophages (*Croxford et al. 2015, Amorim, Defeo, Friebe et al. 2022, Paust et al 2023*)
- GM-CSF expressing T cells are a hallmark of Neuroinflammation (*Galli et al. 2019, Ingelfinger et al. 2022, Codarri et al. 2011*)
- Severe COVID 19 is driven by a GM-CSF dominated cytokine profile (*Kreutmair et al. 2021*)
- Primary source in tissue inflammation are encephalitogenic TH cells (*Komuczki et al. 2019*)
- GM-CSF dysregulation in Th cells triggers CNS-inflammation and monocyte invasion in the CNS (*Spath et al. 2017*)
- GM-CSF mediates GvHD but not GvL (*Tugues, Spath et al 2018*)
- Loss of Treg cells initiates pathogenic GM-CSF production in TH cells (*Pereira et al. 2025*)

# GM-CSF (Csf2)

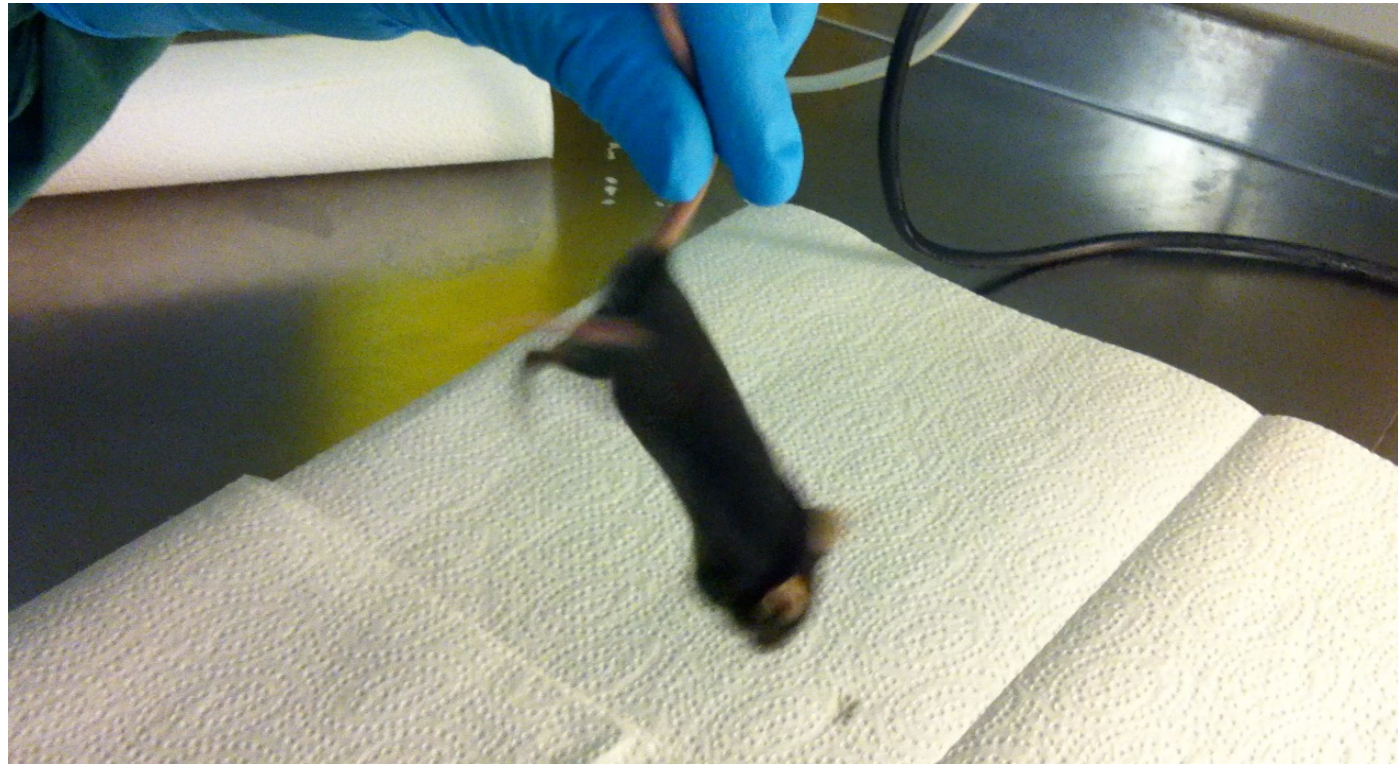
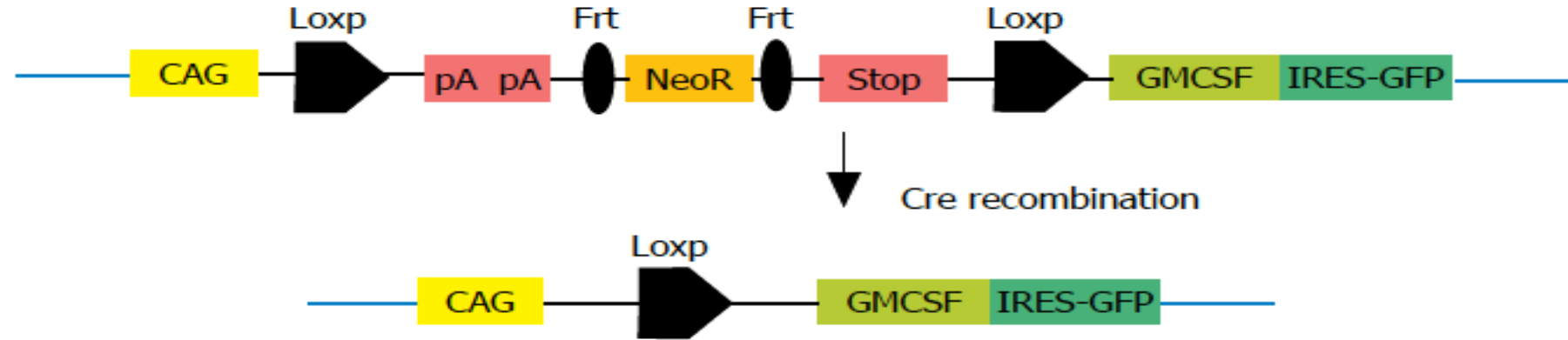
An absolute requirement for T cell pathogenicity

Adoptively transfer  
2d2 wt T cells  
2d2x*Il17a*<sup>-/-</sup> T cells  
2d2x*Ifng*<sup>-/-</sup> T cells  
2d2x*Csf2*<sup>-/-</sup> T cells  
Into wt recipients

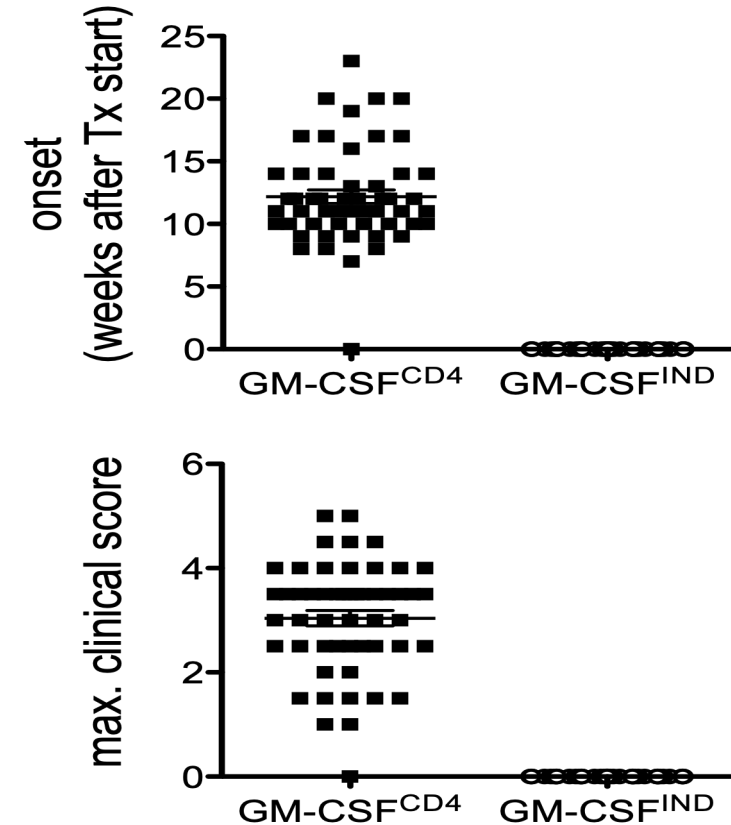
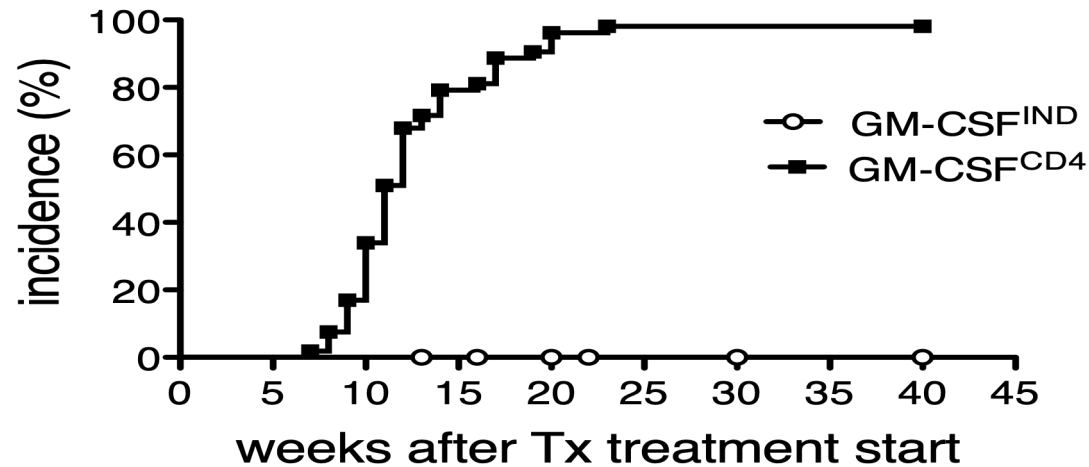


Modeling of dysregulated GM-CSF in mice?

# Inducible expression of GM-CSF in polyclonal (Ag irrelevant) T<sub>H</sub> cells (CD4CreER)

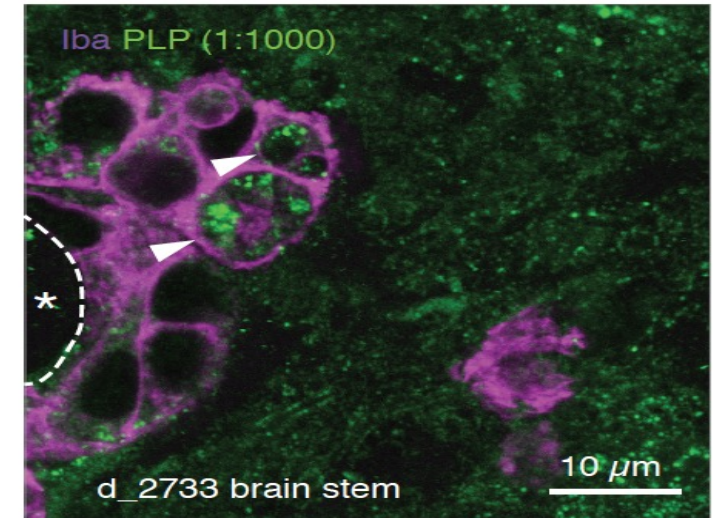
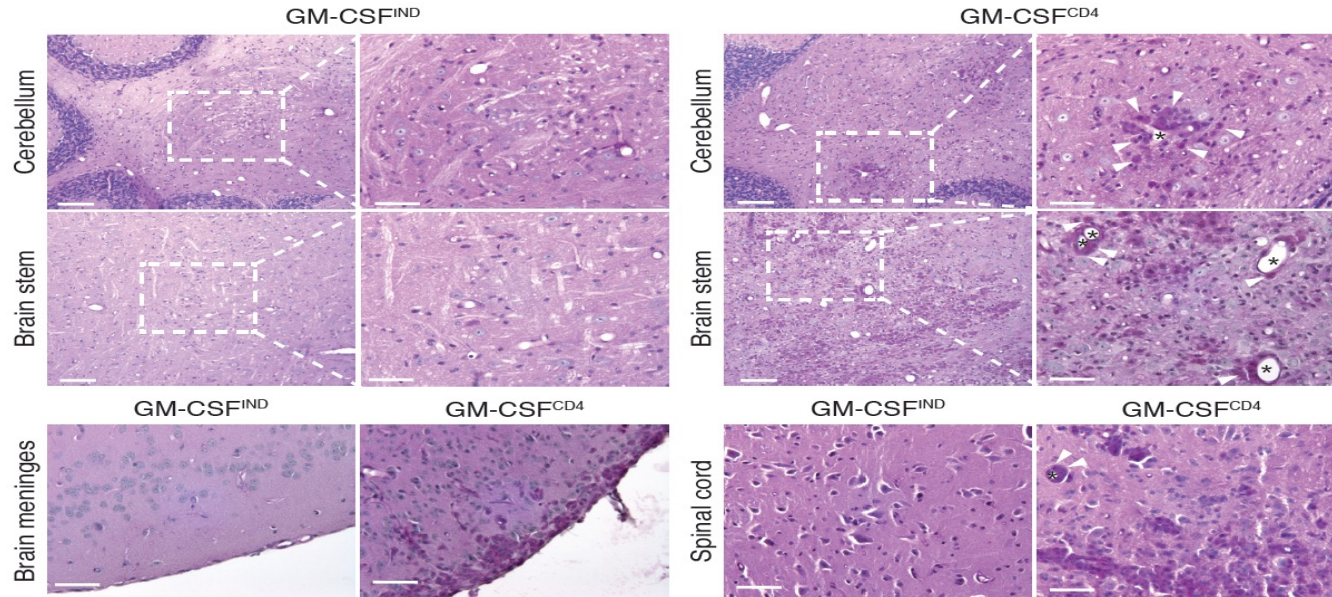


## Incidence of neuroinflammation by elevated GM-CSF levels

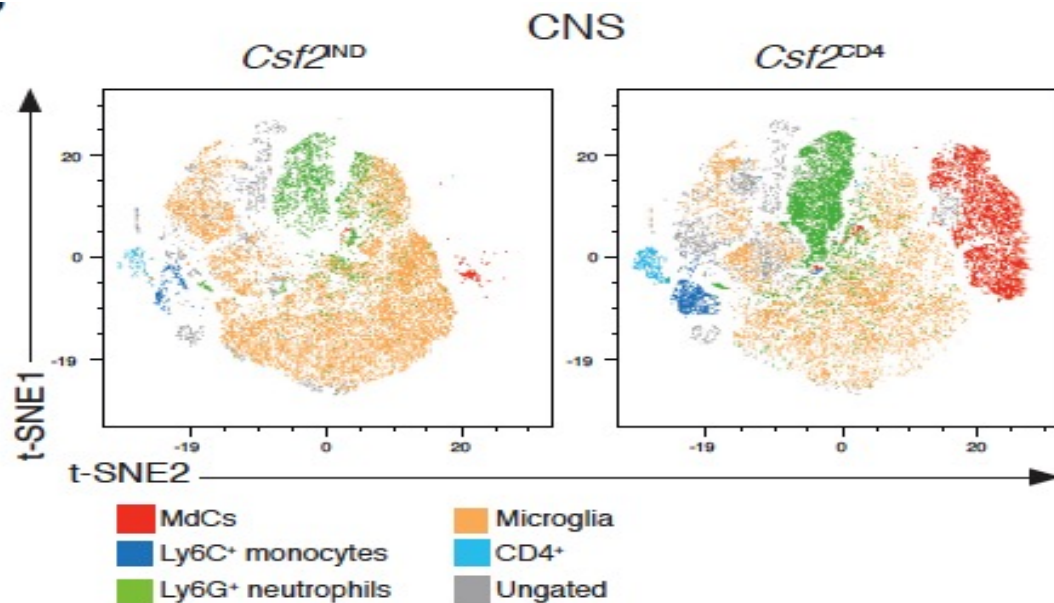


- Mice develop neurological deficits dominated by a rotatory phenotype
- GM-CSF is not only necessary but SUFFICIENT to drive neurological deficits

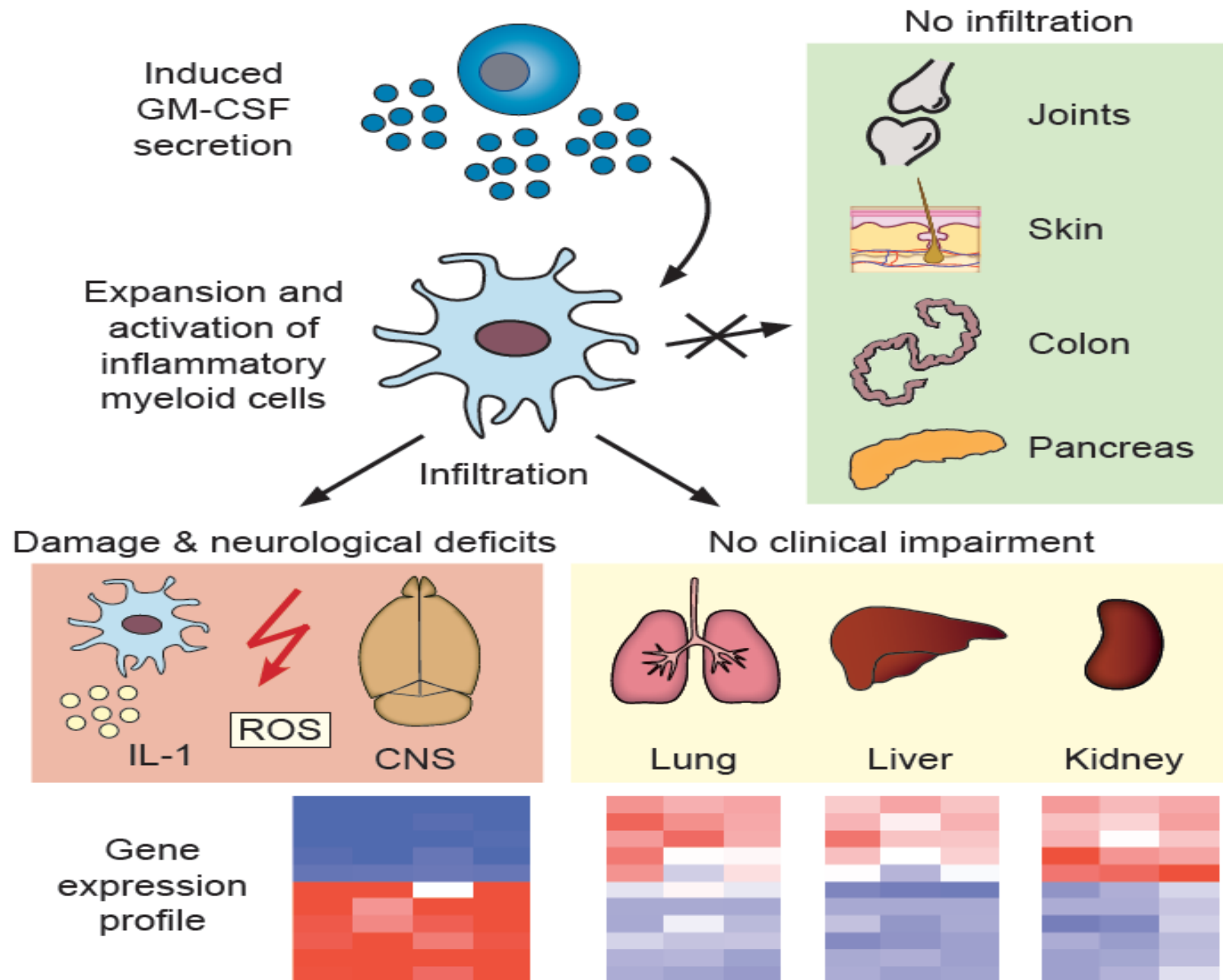
# Invasion of inflammatory monocyte-derived cells into the CNS



Scale bars: 100  $\mu\text{m}$  (brain stem/cerebellum) and 50  $\mu\text{m}$  (insets, brain meninges/spinal cord)



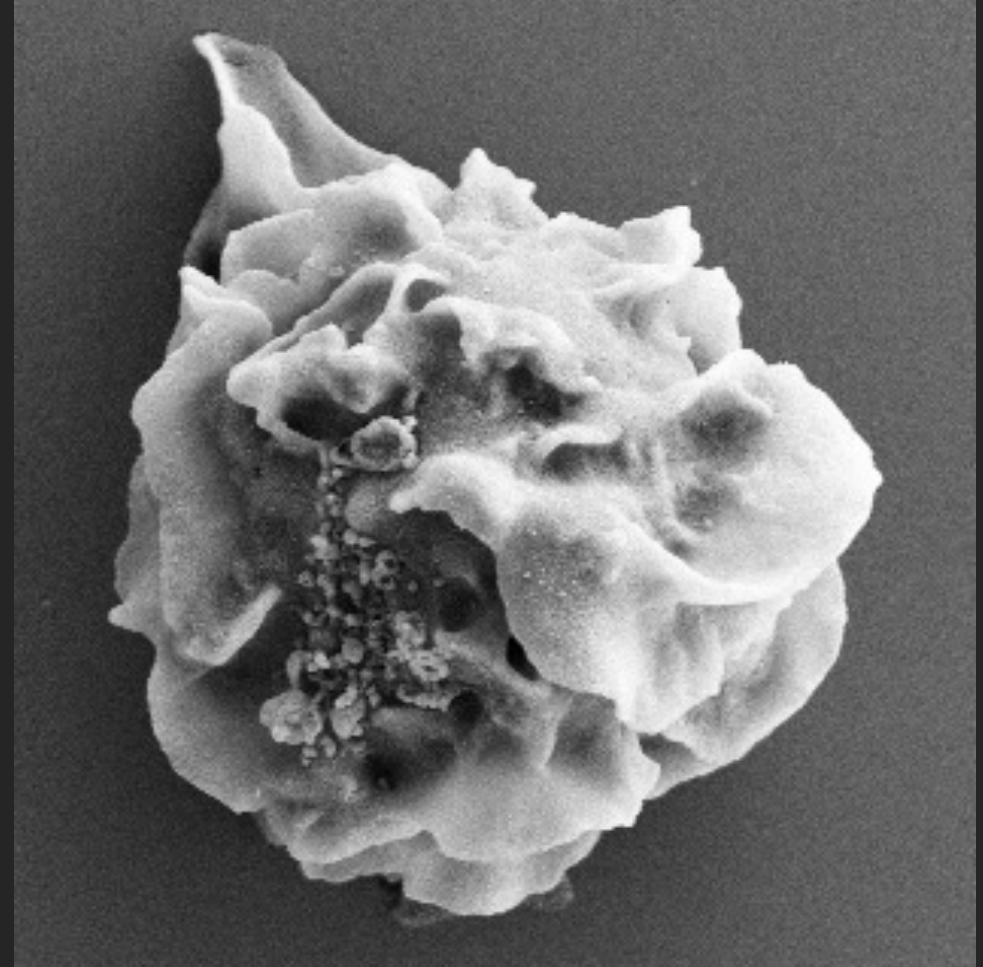
- Infiltrates are located in the brain stem, meninges and the cerebellum
- Myeloid infiltrates phagocytose myelin
- Most prominent infiltrates in the CNS are inflammatory MdCs



# Adenophages

**an atypical  
macrophage  
population in exocrine  
glands sustained by  
ILC2-derived GM-CSF**

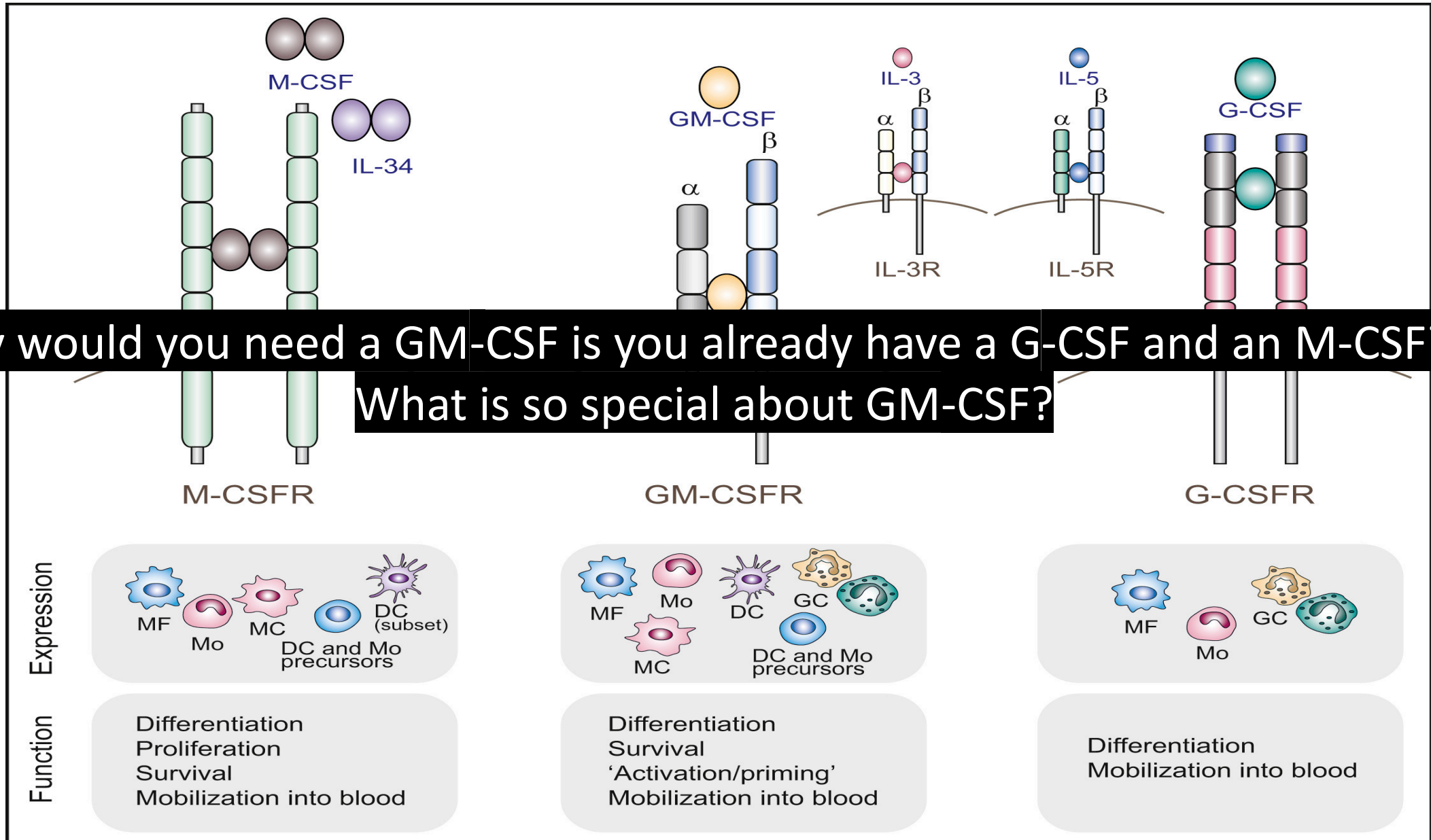
Westerman, Tuzlak et al. 2026



## What we know to date (..about GM-CSF)

- In tissue inflammation, myeloid cells represent the predominant infiltrate (not lymphocytes) and are best equipped to cause tissue damage
- **in tissue inflammation/immunopathology:** GM-CSF is a central communication conduit between effector lymphocytes and monocyte-derived Phagocytes GM-CSF licenses inflammatory monocytes to cause tissue damage
- Steady-state (physiology) function of GM-CSF **limited to airway macrophages where epithelial cells supply GM-CSF**

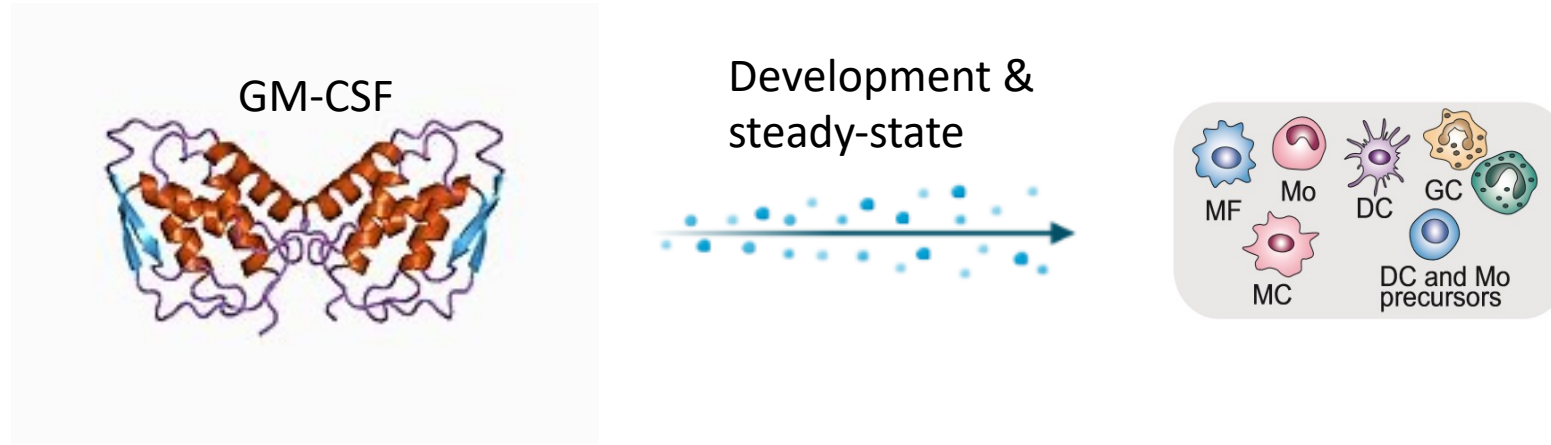
# Colony stimulating factors and why GM-CSF is weird



Why would you need a GM-CSF if you already have a G-CSF and an M-CSF???

What is so special about GM-CSF?

# A terrible “A waste of Biology” or is there more?



Does GM-CSF have really only one cellular target (AMs) in mammalian myelopoiesis during development and in steady-state adulthood?



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Frederike Westermann



Selma Tuzlak

**Collaborators:**

Quantitative Systems Biology - LIMES-Institute, University of Bonn: Prof. Andreas Schlitzer, Dr. David Bejanero

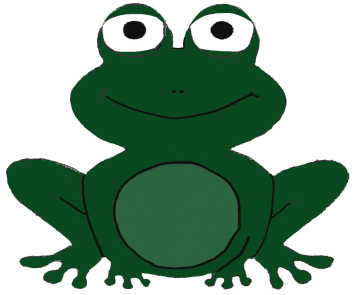
Gustave Roussy Cancer Campus, Villejuif, France: Prof. Florent Ginhoux

Institute for Regeneration and Repair, The University of Edinburgh: Prof. Elaine Emmerson

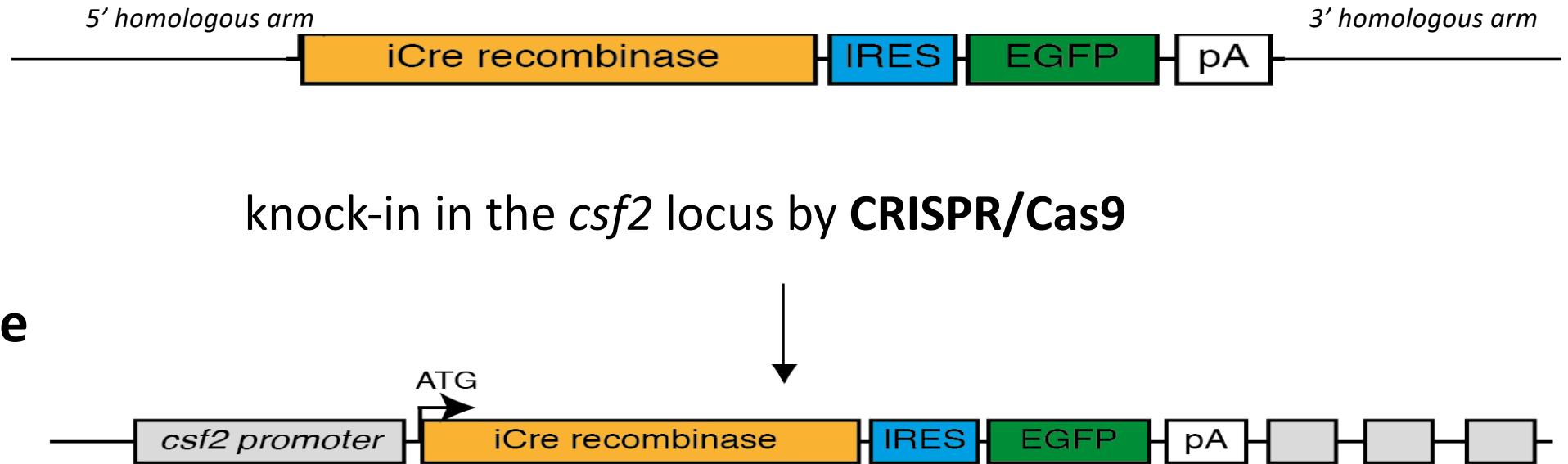
University of Calgary: Prof. Kathy McCoy, Dr. Aline Ignacio



# Fate-Map and Reporter of GM-CSF - FROG mouse line



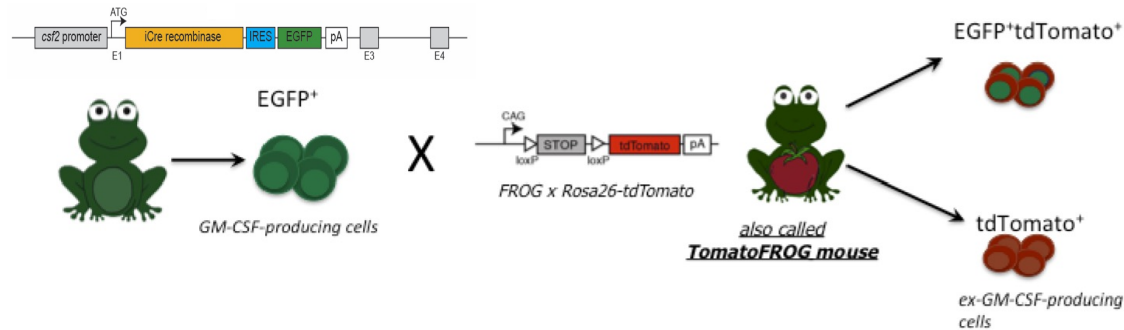
FROG mouse line



# Steady-state GM-CSF production

## Flow Cytometry analysis of Fate-Map and Reporter for GM-CSF mice

### tomatoFROG mouse

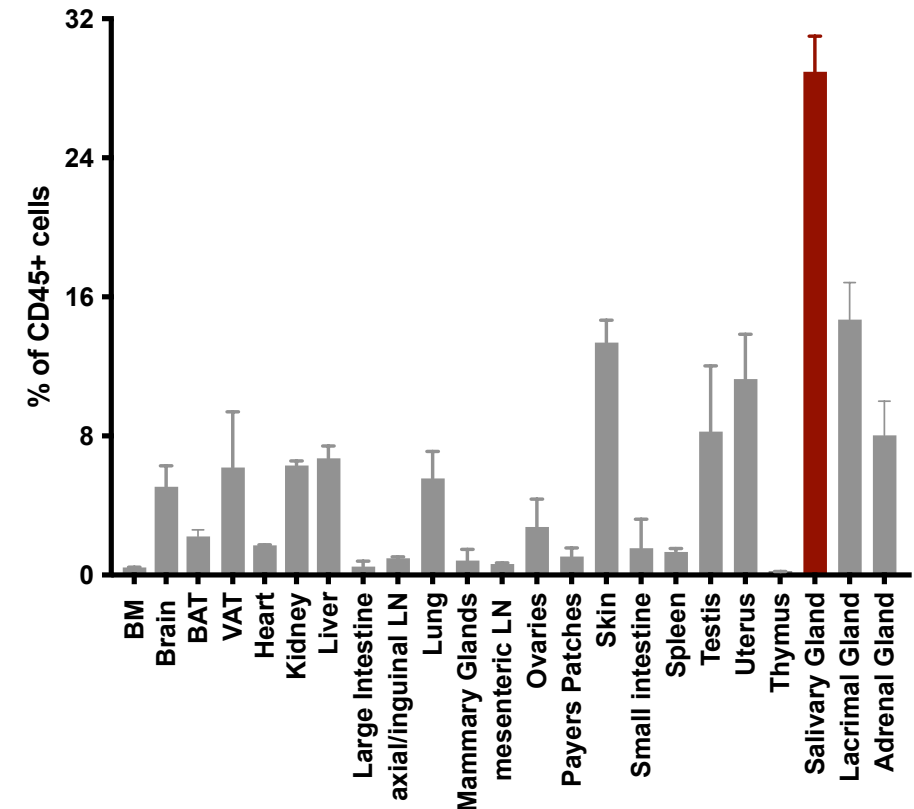


Komuzcki et al., Immunity (2019)

## Flow Cytometry analysis of Fate-Map and Reporter for GM-CSF mice

Total tdTomato<sup>+</sup> cells in the CD45<sup>+</sup> compartment

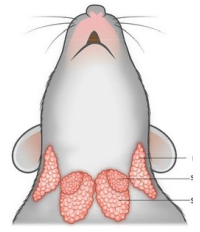
→ Total active and exGM-CSF producing cells



Adults, data pooled from 2-3 independent experiments

Westermann, Tuzlak et al., in press

# Introduction

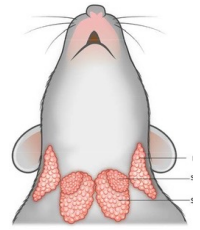


Maruyama CL et al.,  
Oral Diseases, 2019

## Salivary Gland (SG)

- Composed of three main glands: sublingual, submandibular and parotid
- SG are critical for immunity in the oral cavity - IgA secretion, saliva production
- Mucosal effector site – immune landscape:
  - Contains ILCs as crucial cells in barrier immunity, tissue homeostasis and immune regulation
  - Macrophages make up around 40-60% of total CD45+ cells

# Active GM-CSF production in SG during postnatal development



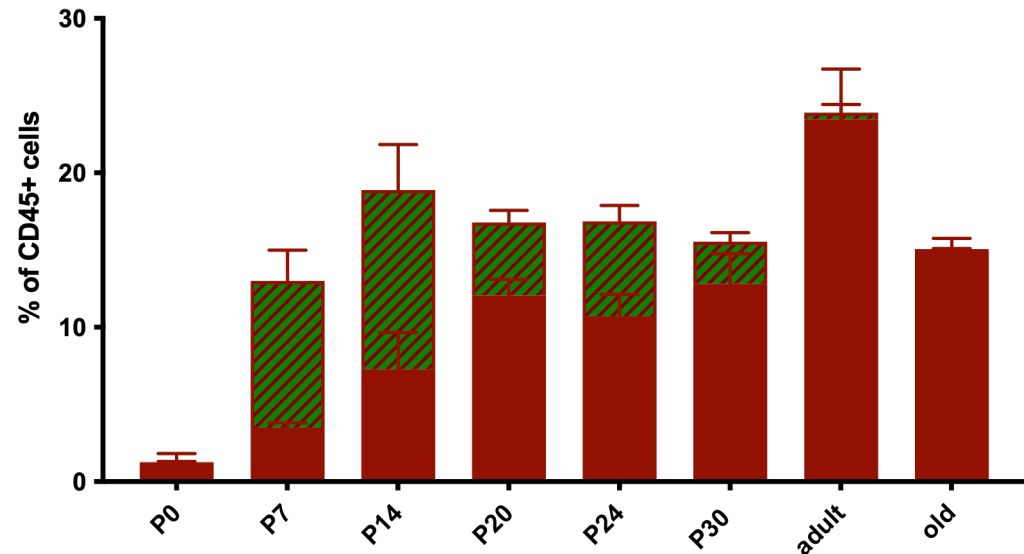
## Salivary Gland (SG)

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## Flow Cytometry analysis of Fate-Map and Reporter for GM-CSF SG during postnatal development

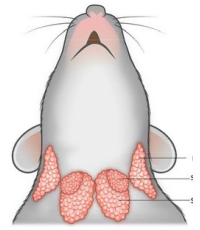
exGM-CSF producing cells →  tdTom+

Active GM-CSF producing cells →  tdTom+GFP+

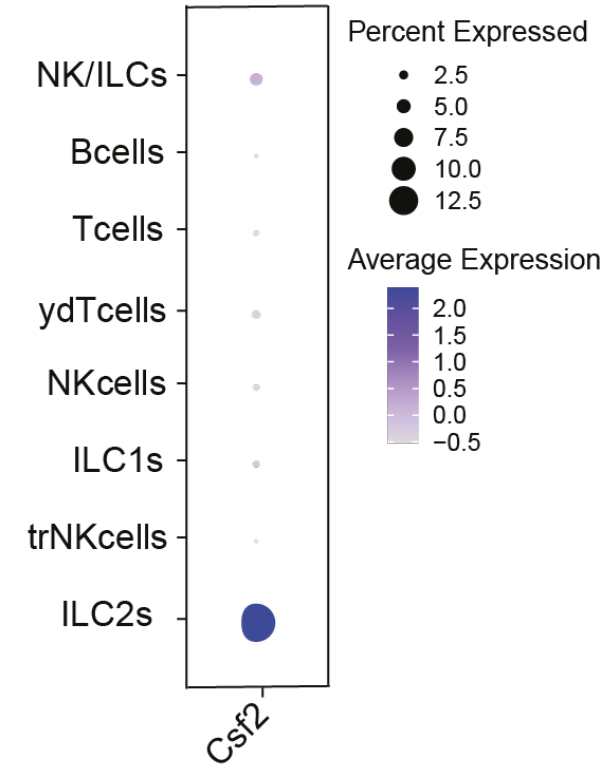
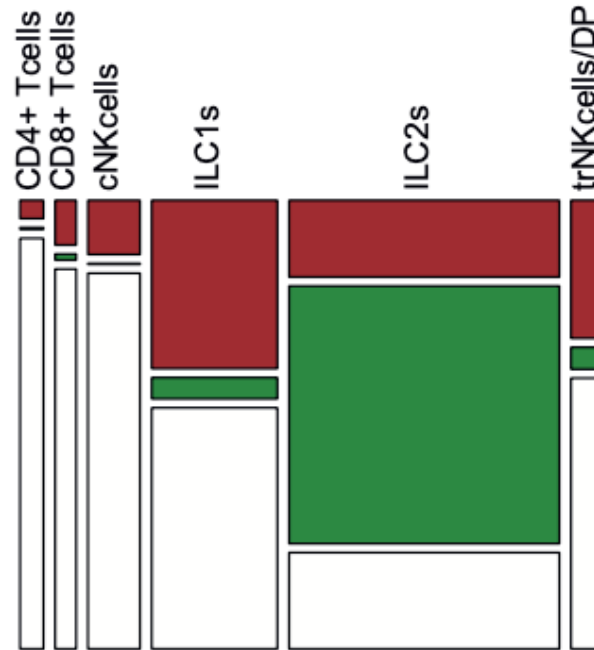
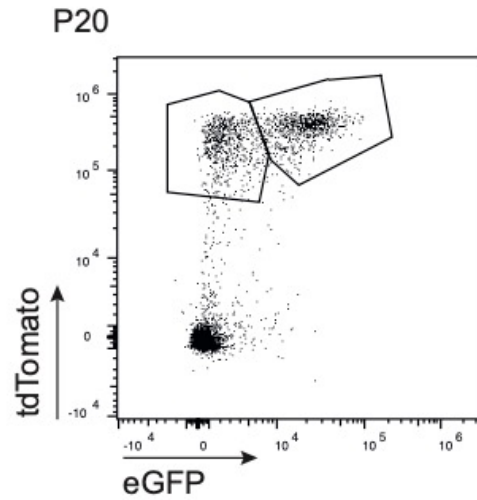


Data pooled from 2-3 independent experiments per time point

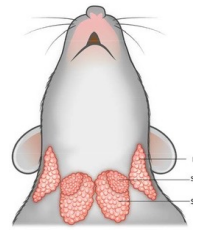
# GM-CSF is produced by ILCs in the SG during postnatal development



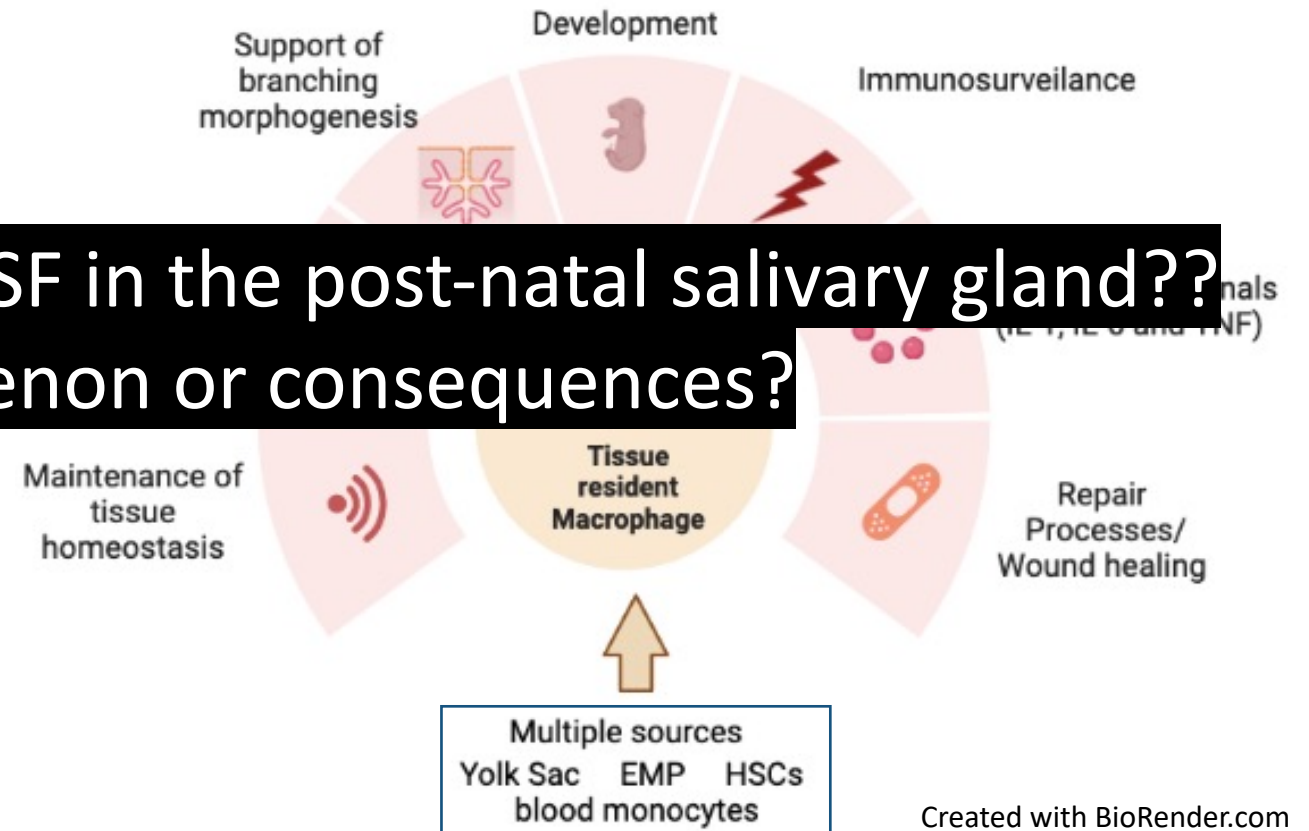
Pregated on live CD45+



scRNAseq data –  
P20 *Csf2*<sup>+/-</sup>



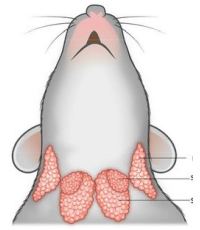
Why do ILC's produce GM-CSF in the post-natal salivary gland??  
40-60% of Immune cells in Salivary Gland (SG),  
An epiphenomenon or consequences?



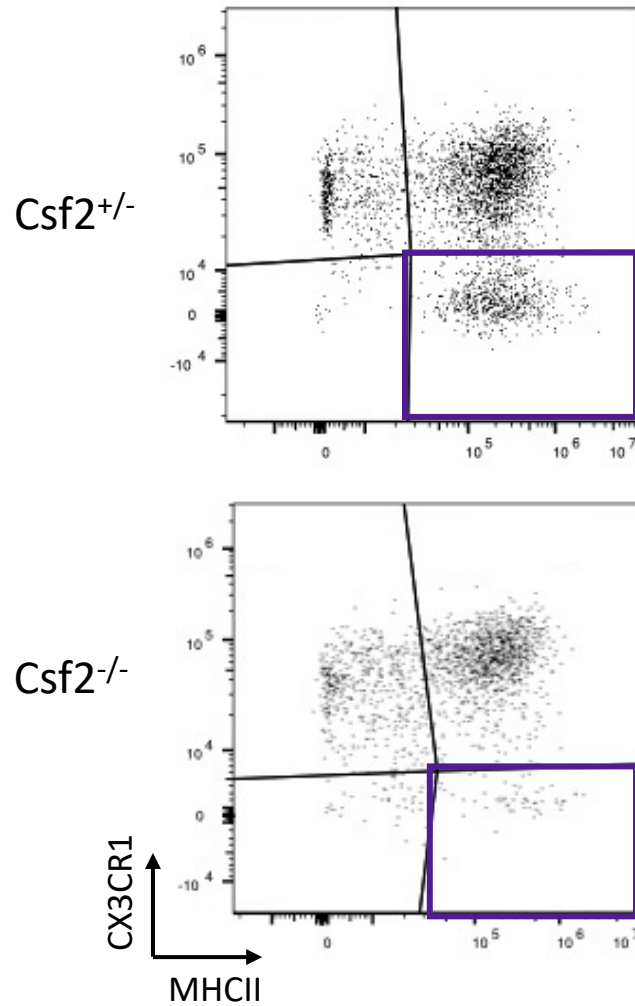
Created with BioRender.com

Lee et al., Development (2022); Pollard, Nature Reviews Immunology (2009); Sehgal, Seminars in Immunology (2021); Becher et al., Immunity (2016); Chakarov et al., Science (2019)

# We found a GM-CSF dependent phagocyte subset in the SG

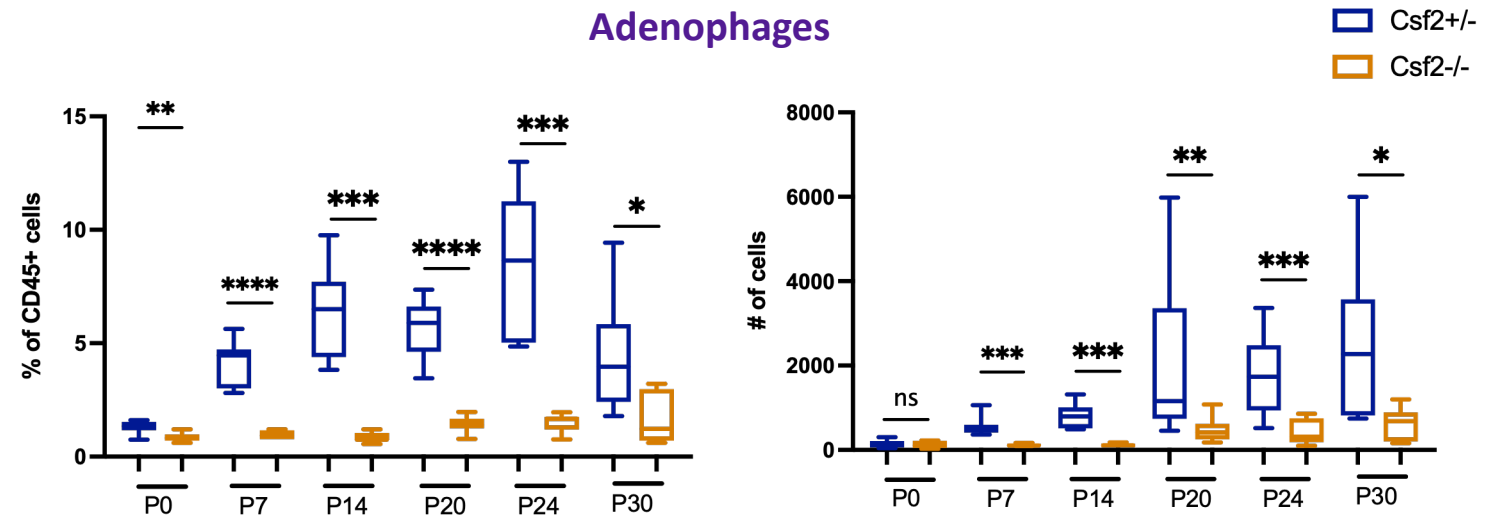


P20 – total Macrophages



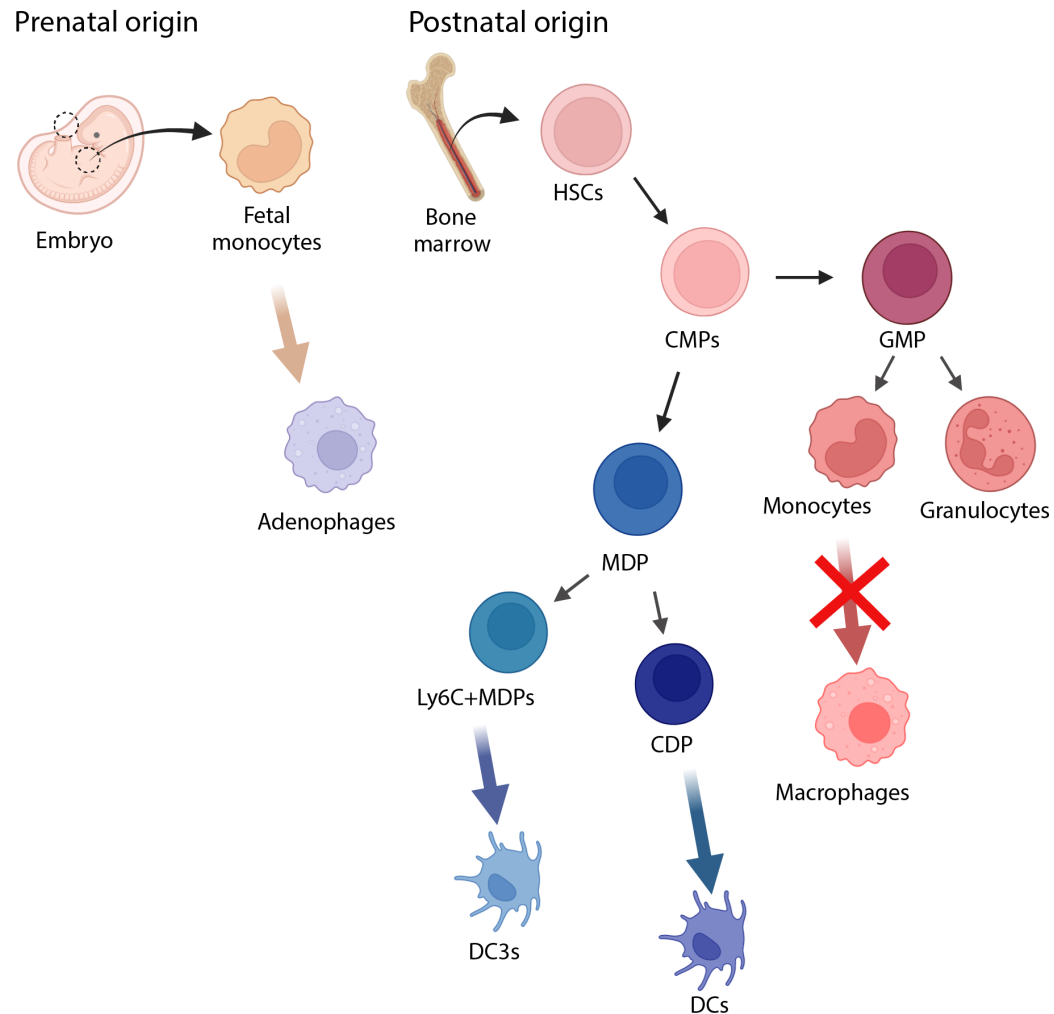
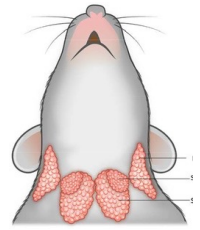
Pregated on live CD45+Lin-Ly6G-SiglecF-Ly6C-CD11b+F4/80+

Adenophages



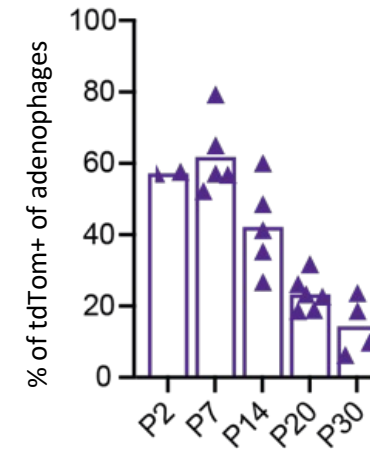
GM-CSF dependent phagocyte subset in the salivary gland that we termed:  
**adenophages [ greek: gland eater ]**

# Adenophages arise from fetal monocytes

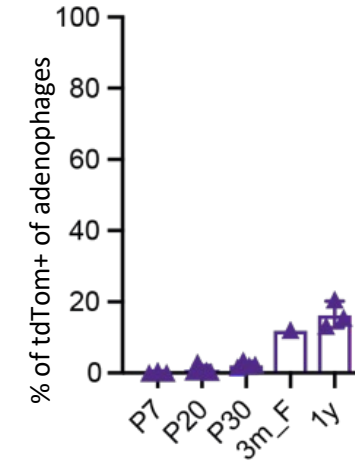


*CCr2*<sup>CreERT2</sup>x*Ai14*

P0/P1

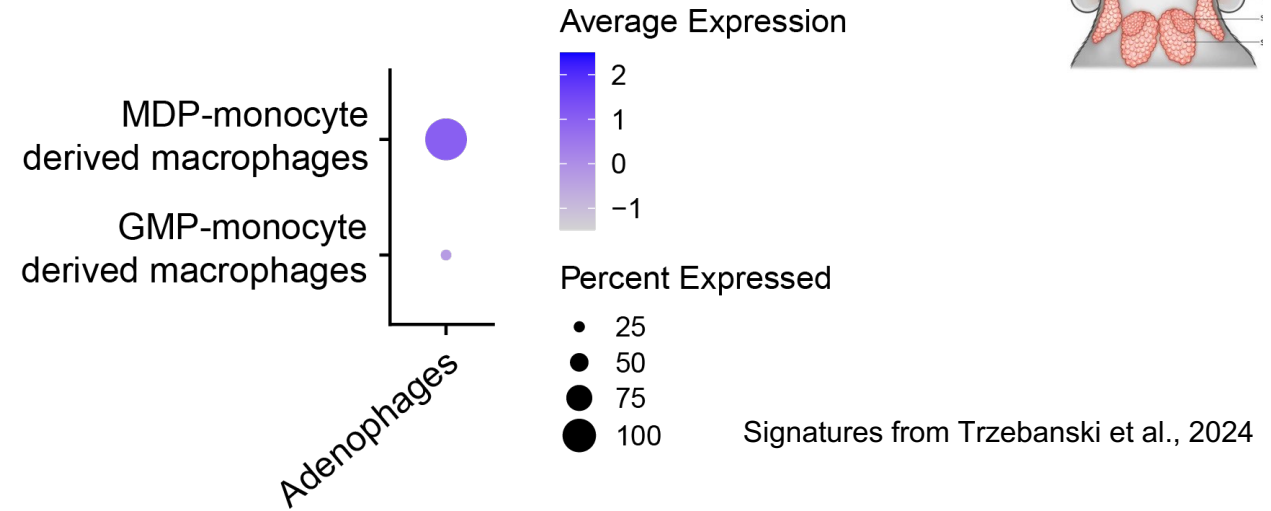
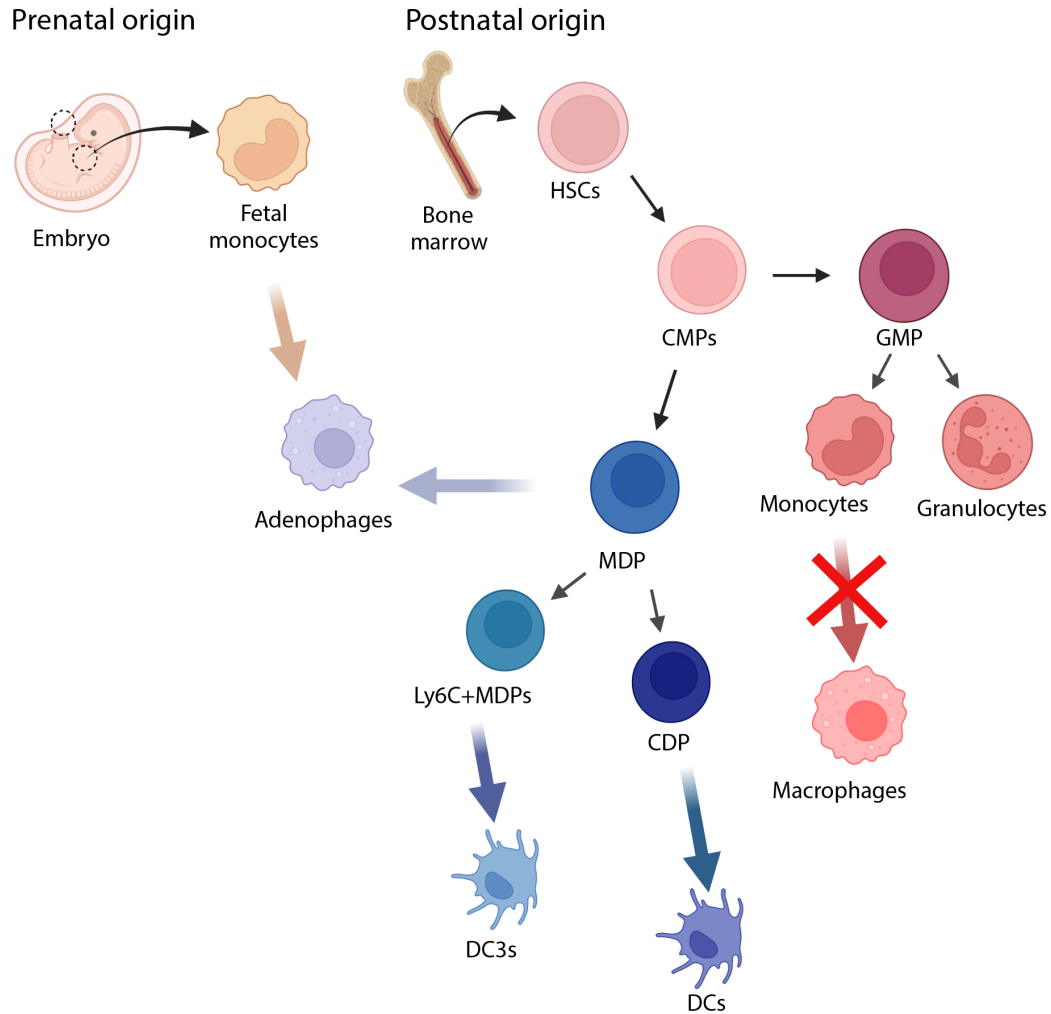
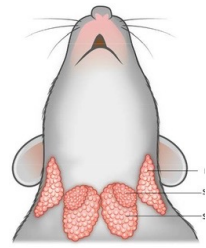


*Ms4a3*<sup>Cre</sup>x*Ai14*

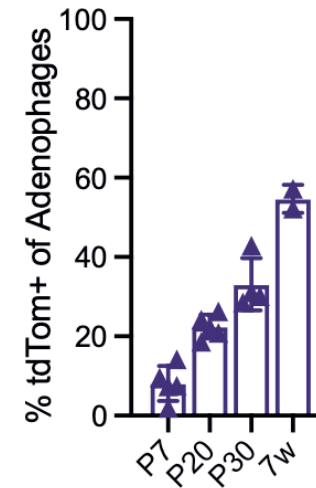


Adenophages arise from fetal monocytes but the population is not self-maintained (or replenished by GMP-monocytes)

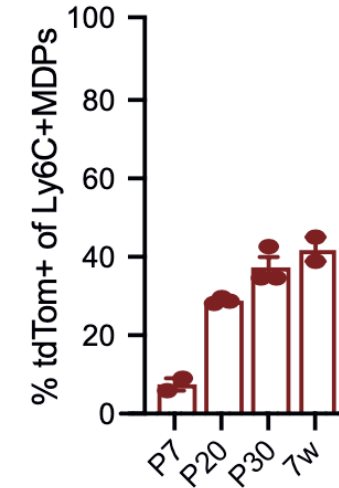
# Postnatal adenophages are replenished through the MDP lineage



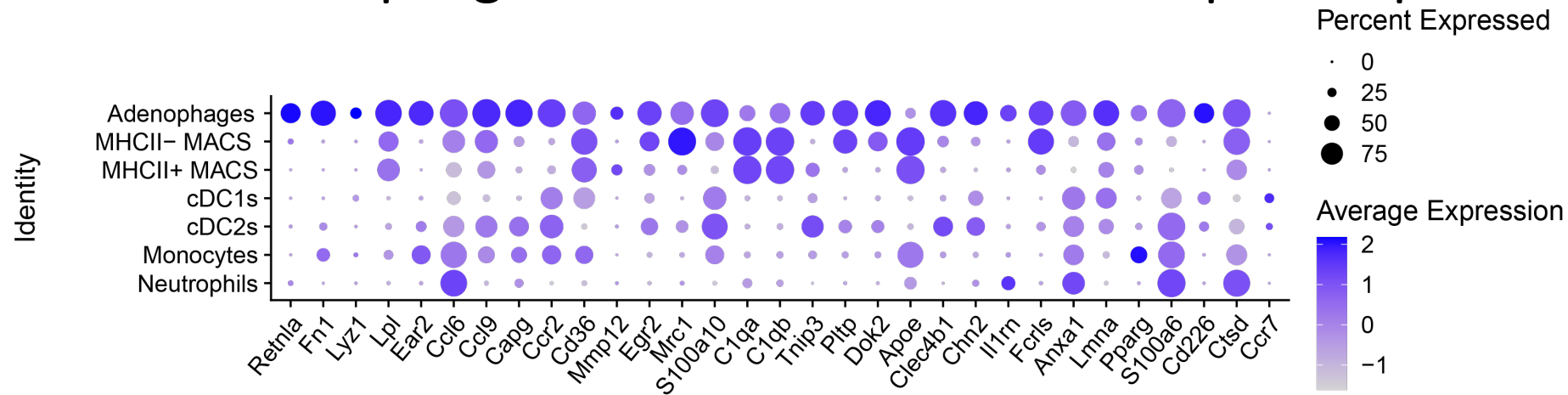
**G** *Clec9a*<sup>Cre</sup>xAi14



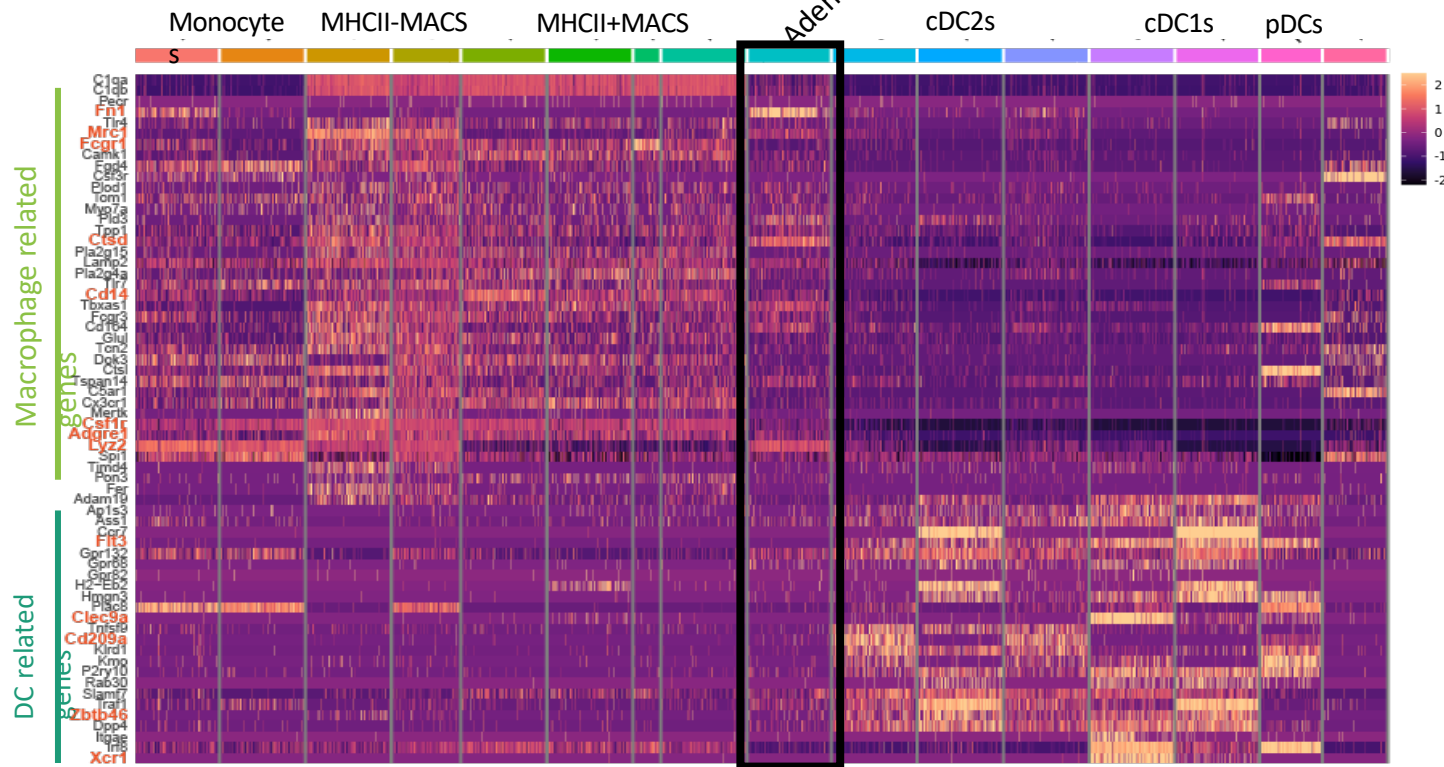
**H** *Clec9a*<sup>Cre</sup>xAi14



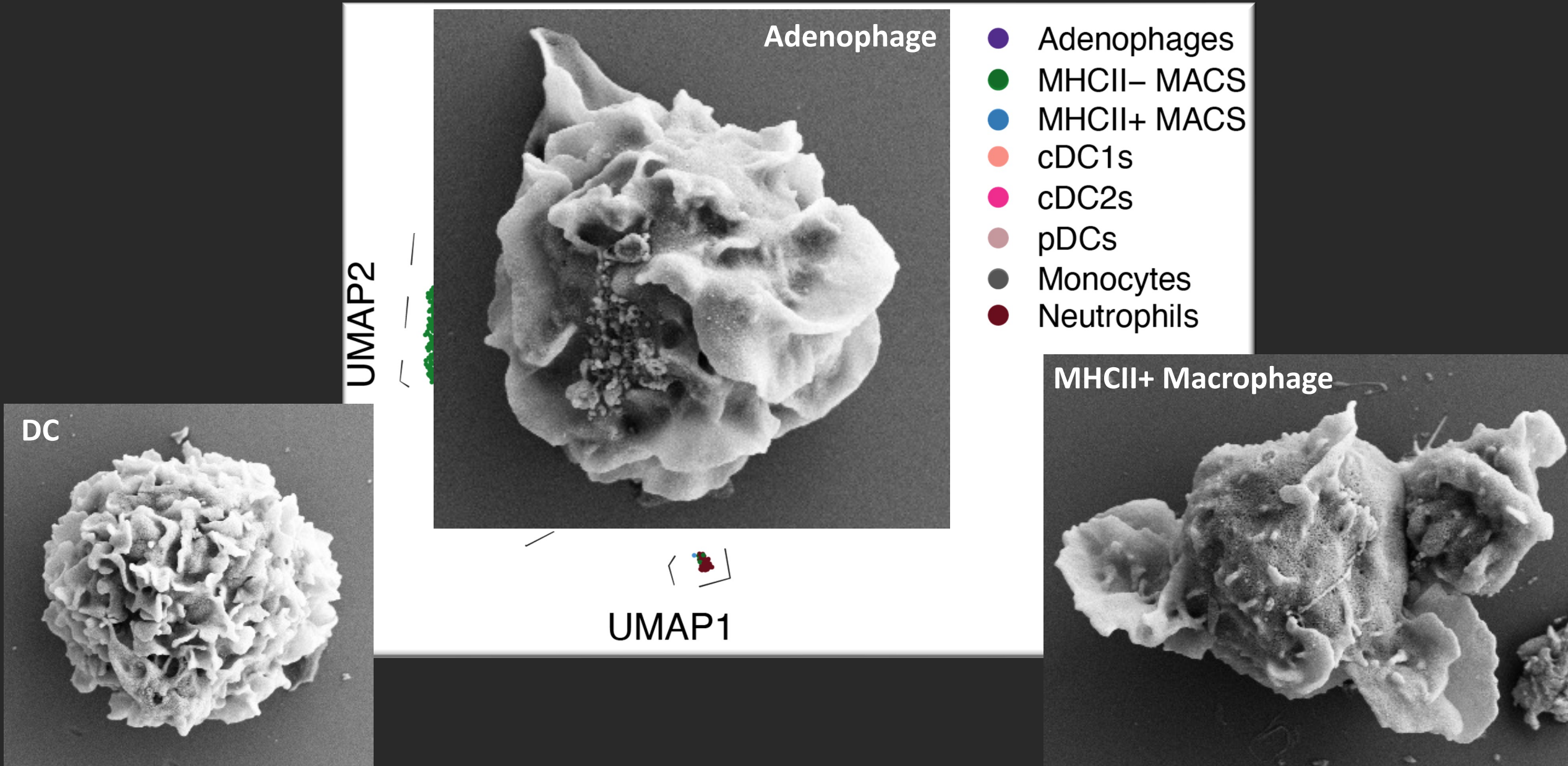
# Adenophages have demarcated transcriptional profile



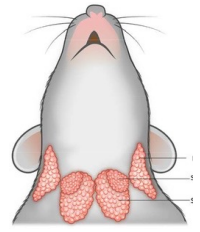
## Macrophage and DC signature genes



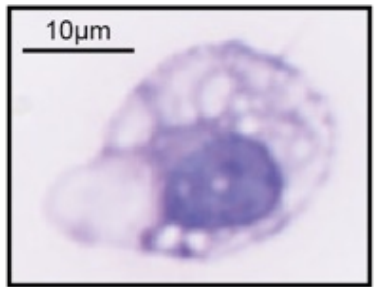
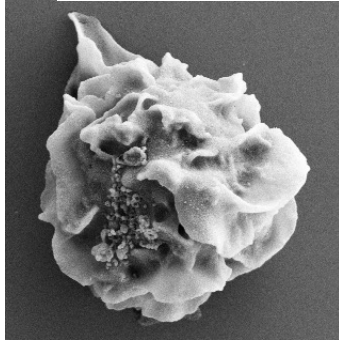
# Adenophages are hybrid cells with macrophage and DC features



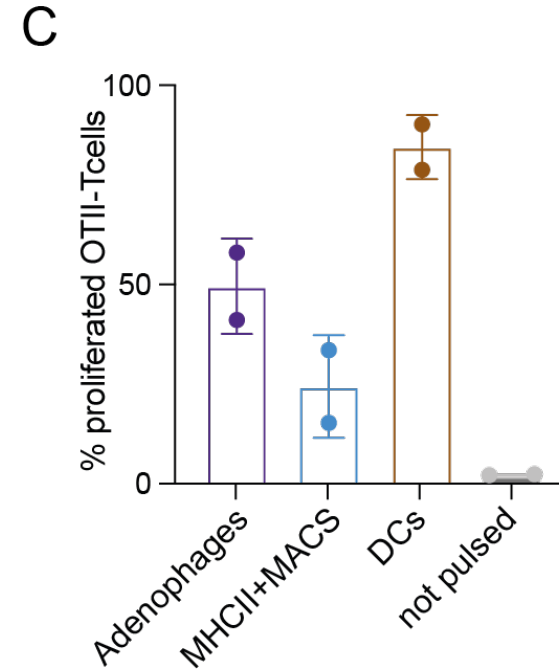
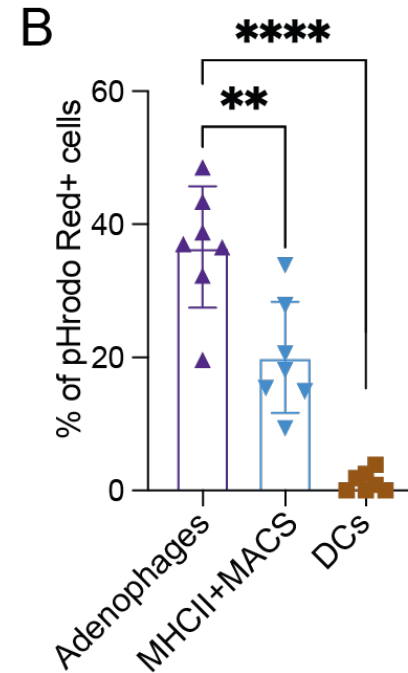
# Adenophages in the SG phagocytose and are strong APCs



Adenophages



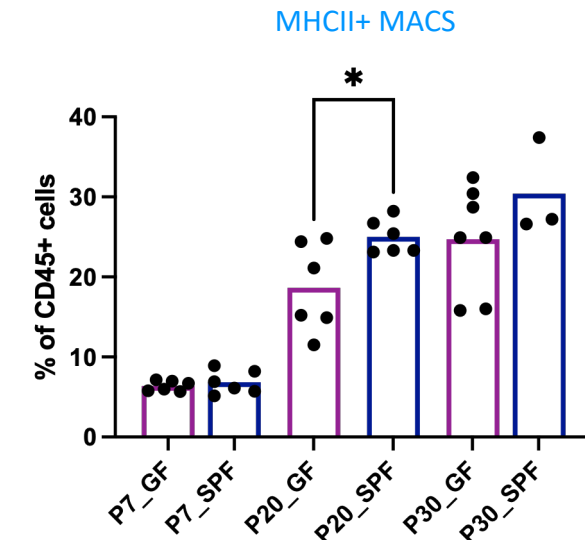
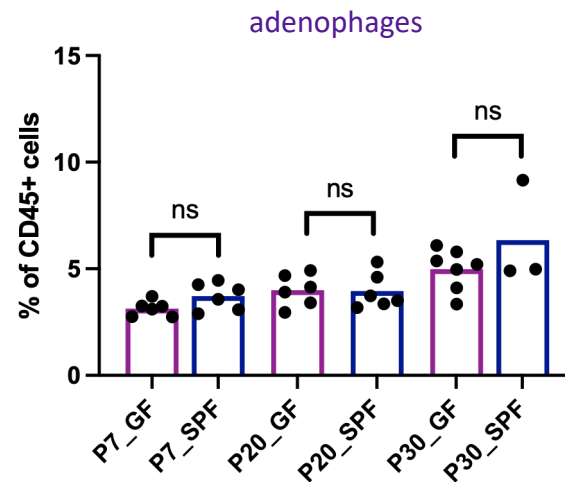
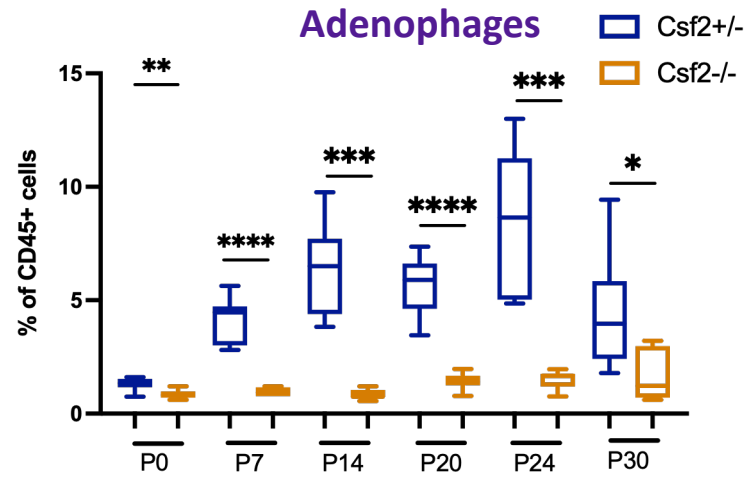
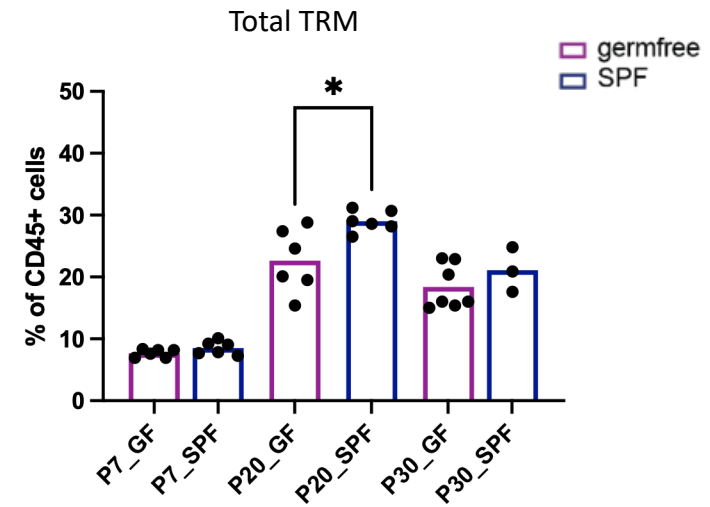
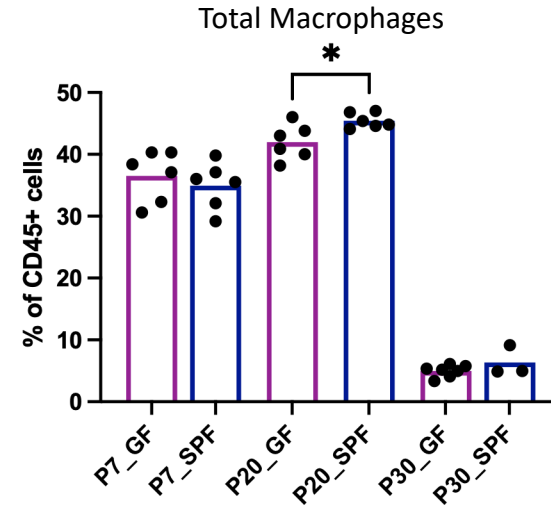
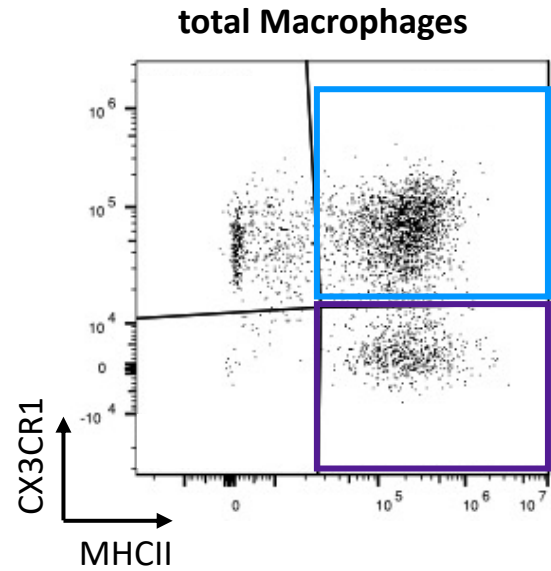
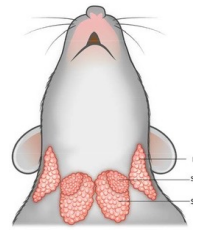
*In Vitro:*



Not only morphological but also Functionally similarities to macrophages and DCs

Timing of Adenophages emerging correlates with changes in the microbiome, so we wondered if they also emerge in germ-free mice.

# Adenophage development is hard-wired and not influenced by the microbiome

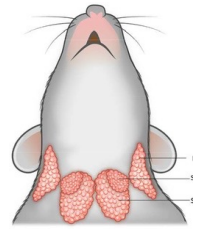


AMs depend 100% on lung epithelial cells to produce GM-CSF (Gschwend et al. 2021)

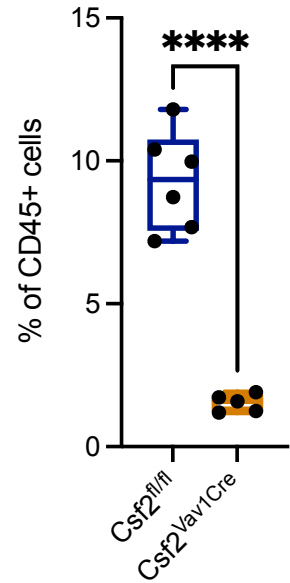
Do you really need ILC2 for Adenophage development and maintenance?

*Deletion of GM-CSF in lymphocytes and ILC2s*

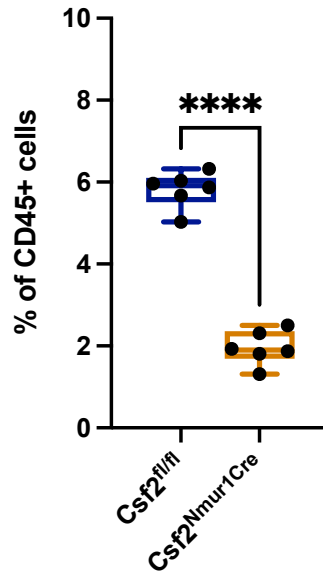
# Adenophages specifically depend on GM-CSF from ILC2s in the SG



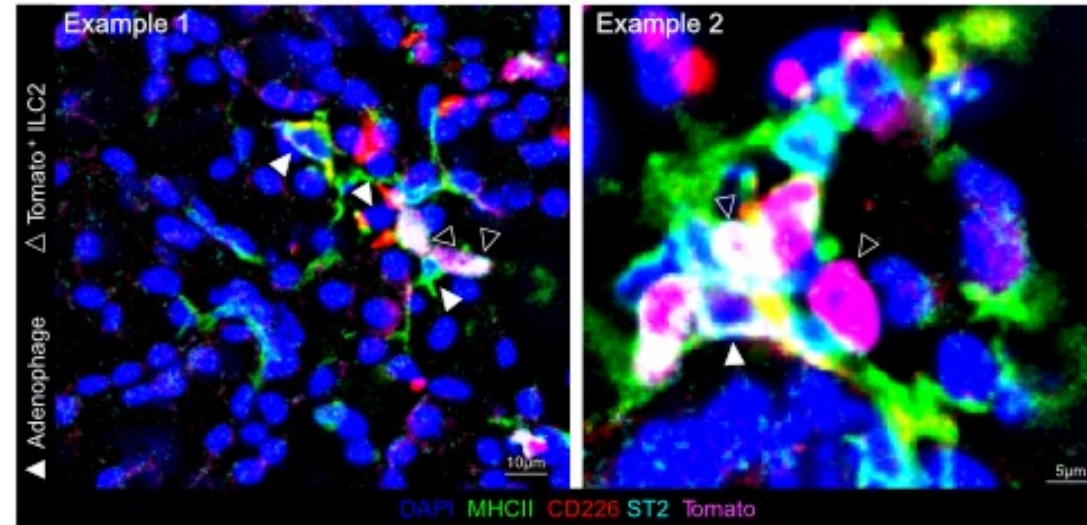
P20 adenophages



adenophages

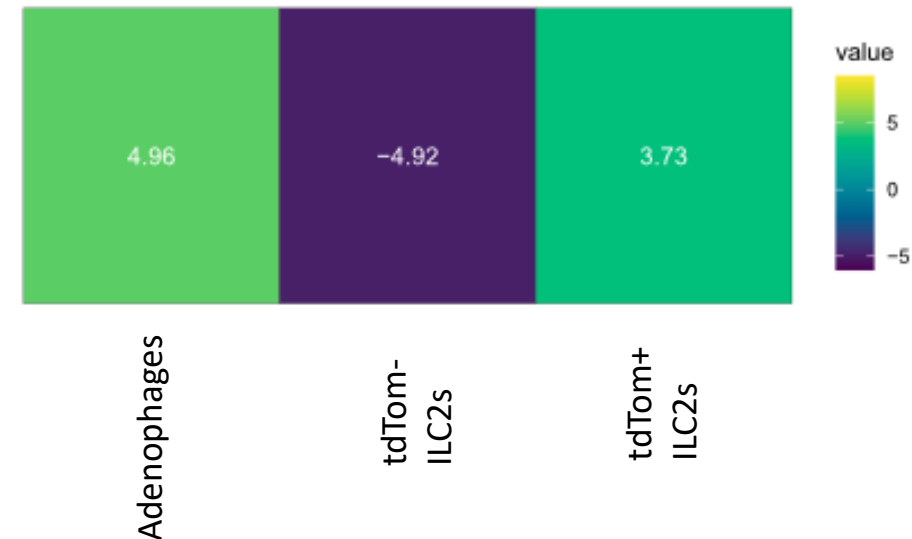


CODEX

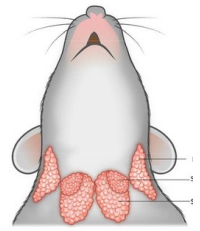


Adenophages specifically depend on GM-CSF from ILC2s and are found strongly associated with those GM-CSF producing ILC2s

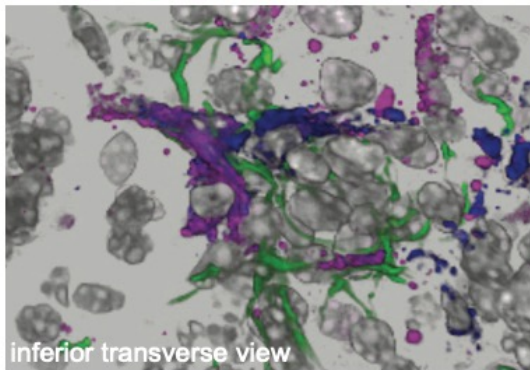
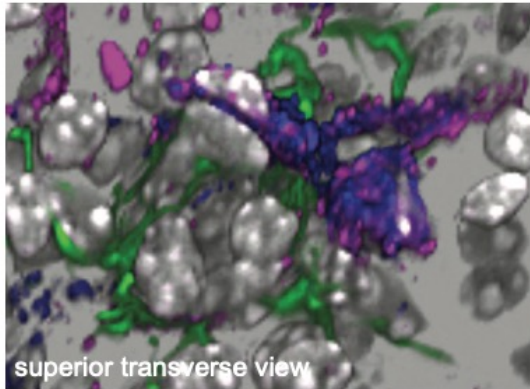
Adenophages



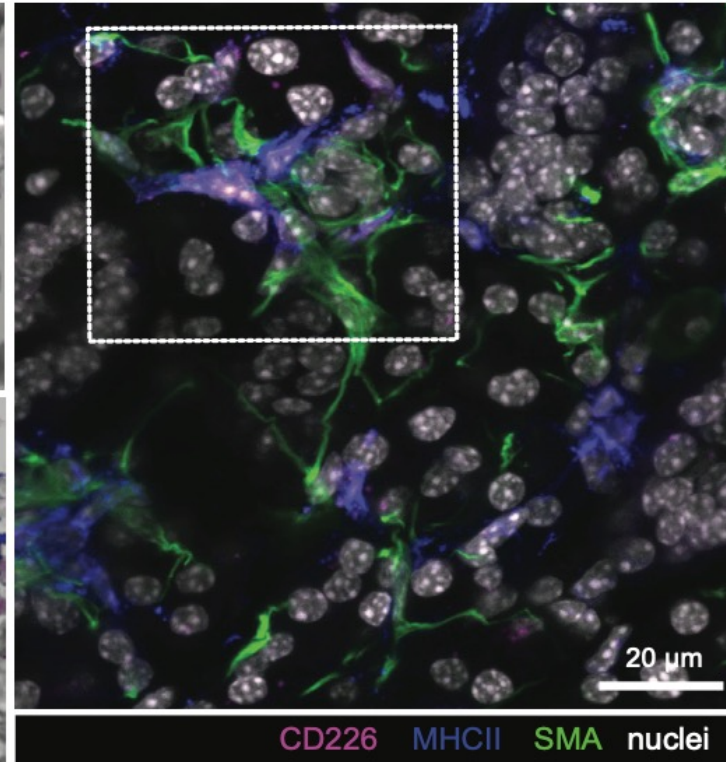
# Myoepithelial cells are part of the adenophage niche in the SG



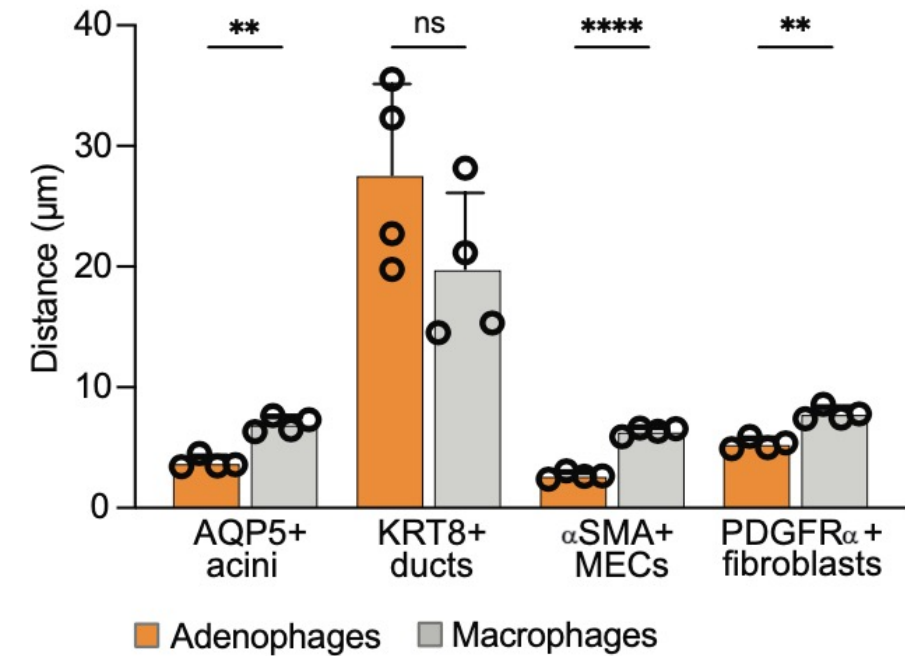
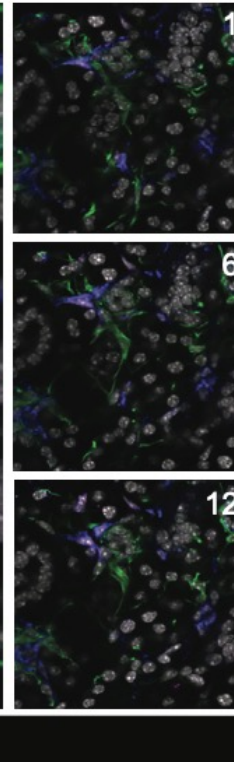
3D projection (100  $\mu$ m)



Maximum projection (20  $\mu$ m)

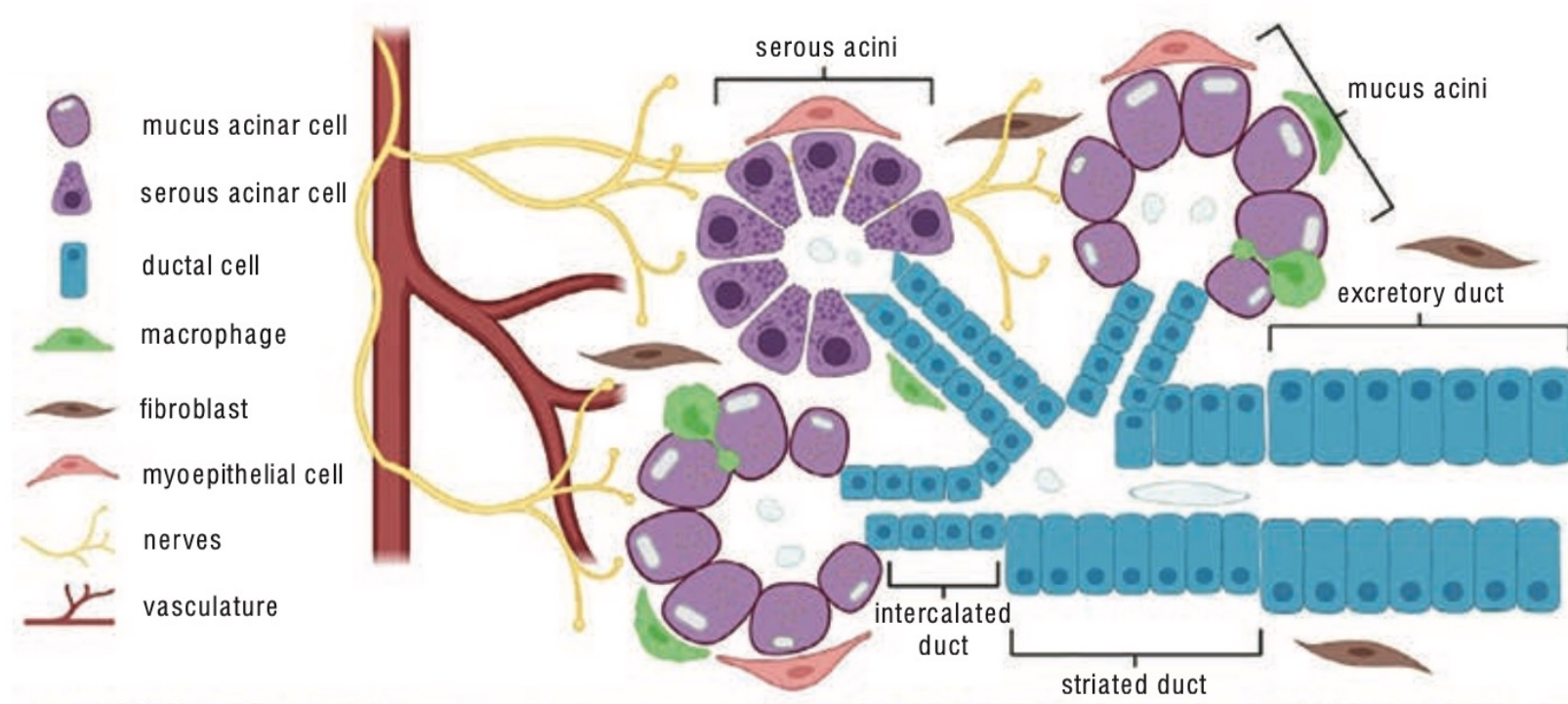
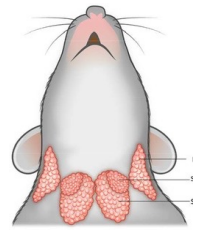


1  $\mu$ m planes



Adenophages can be found in close proximity to MECs

# Myoepithelial cells are located around the acini

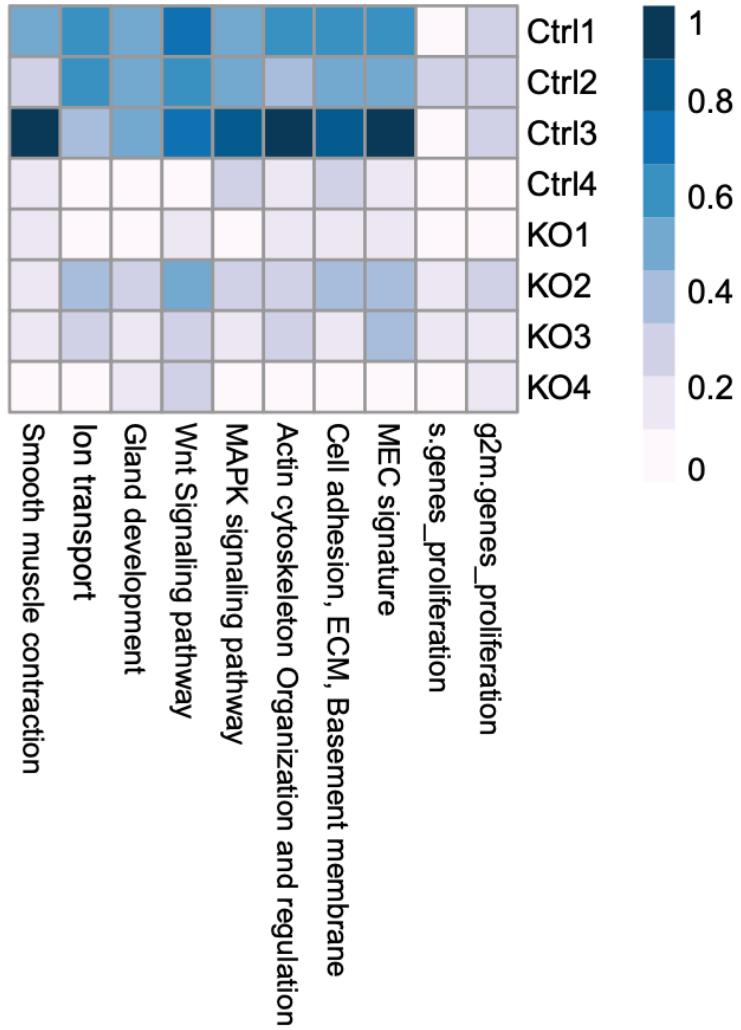


International Review of Cell and Molecular Biology, 2022, Volume 368, Chapter One

Myoepithelial cells are important for excretory function – by contraction they “squeeze” saliva from the acini into the ducts

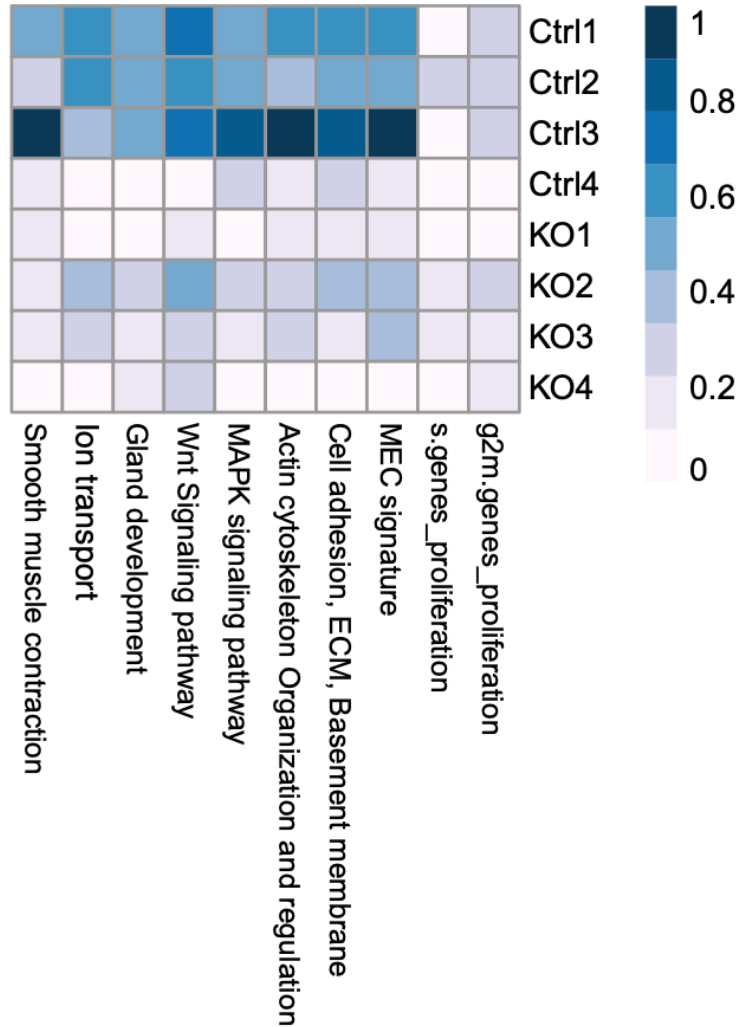
## Loss of Adenophages alters MEC phenotype

## Characteristic MEC gene signatures reduced in MECs isolated from adenophage-devoid SGs

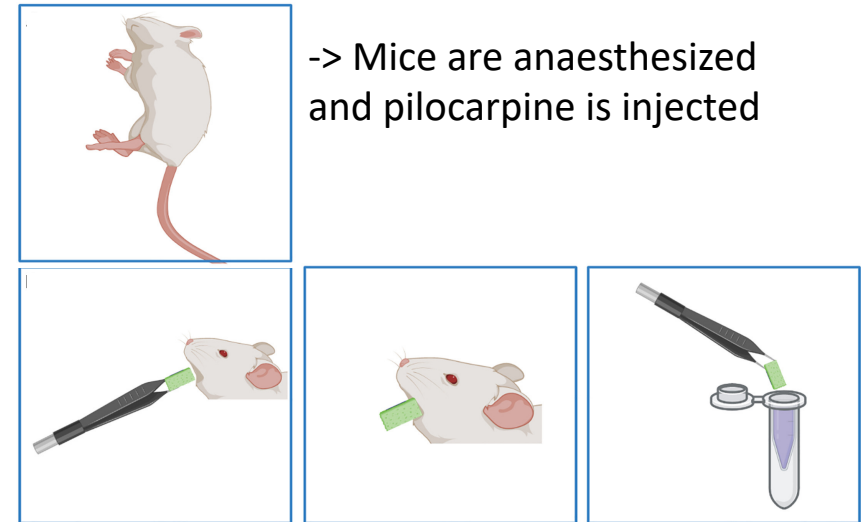


# Loss of Adenophages alters MEC phenotype

Characteristic MEC gene signatures reduced in MECs isolated from adenophage-devoid SGs



Pilocarpine-induced saliva collection

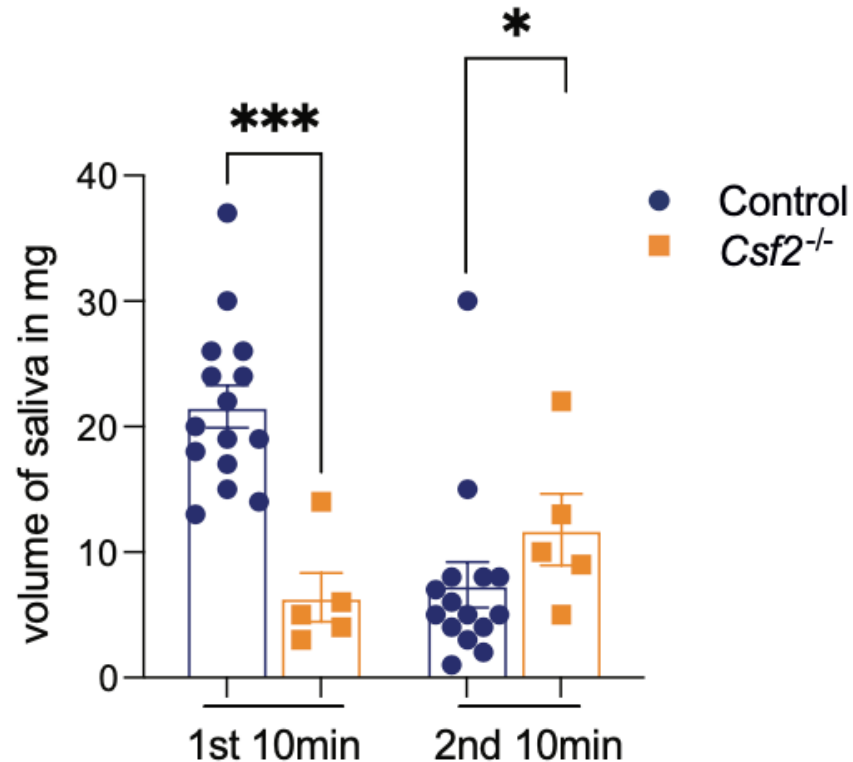


-> Saliva is collected for 2x 10min

Zubeidat et al., 2022; STAR Protocols

# Adenophages support the functional properties of myoepithelial cells

Pilocarpine-induced saliva output is delayed in mice lacking adenophages

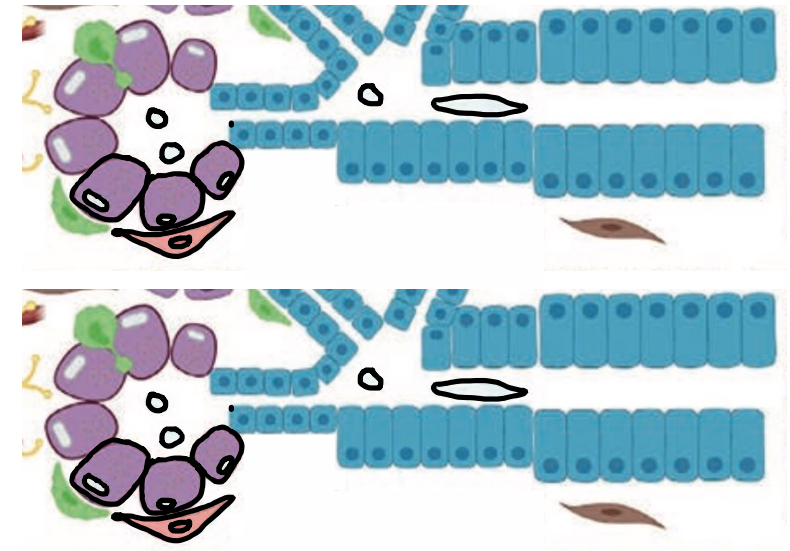


Data from 3 independent experiments, P20

Change in MEC phenotype upon loss of adenophages results in slower saliva output

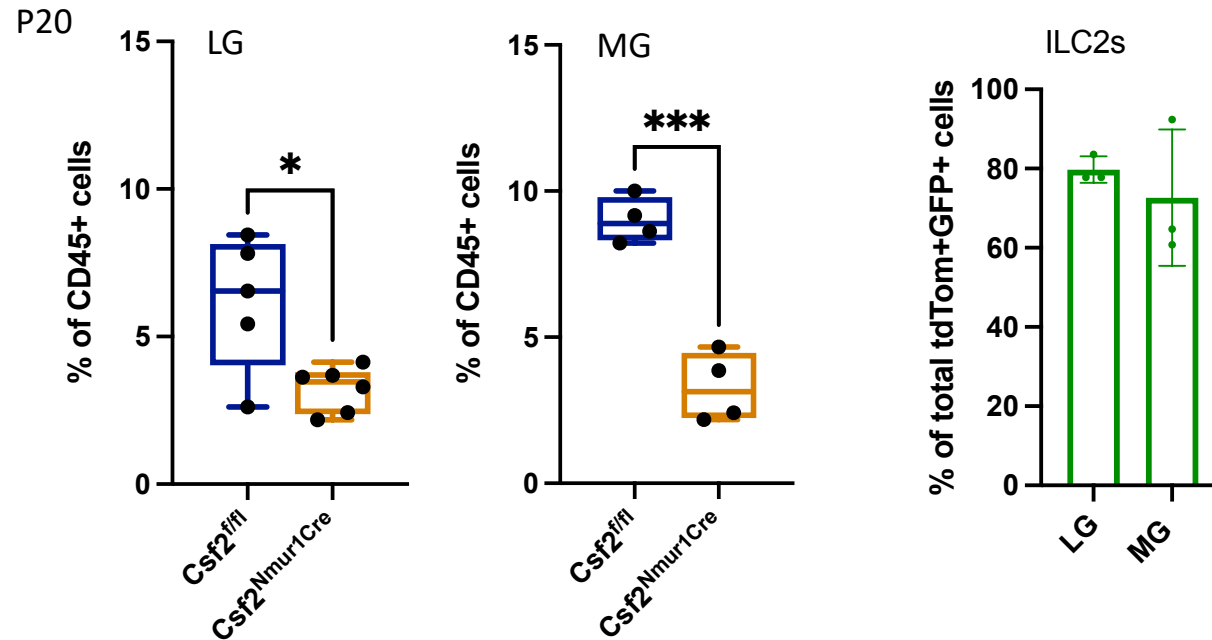
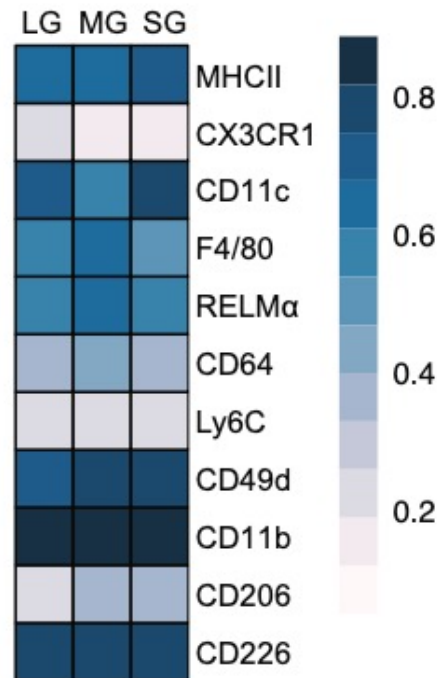
Normal saliva output

Slower saliva output



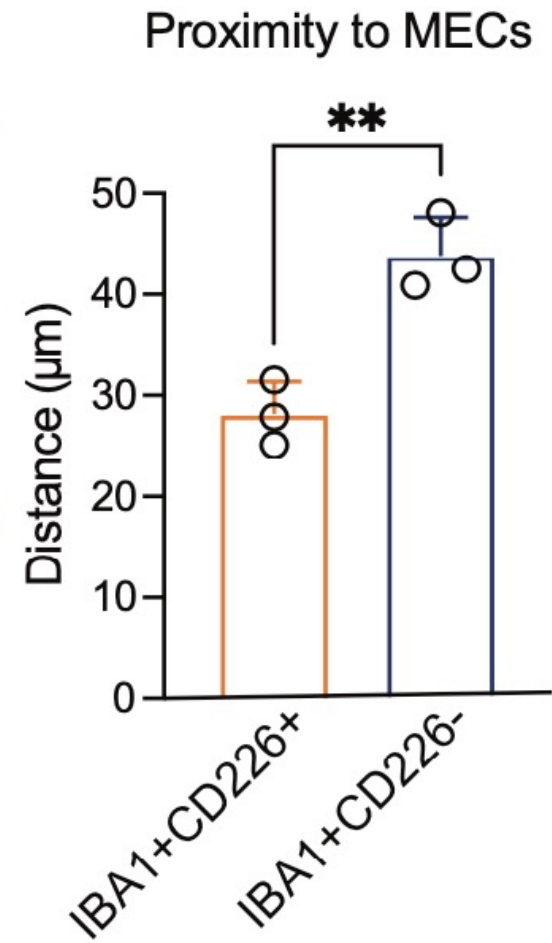
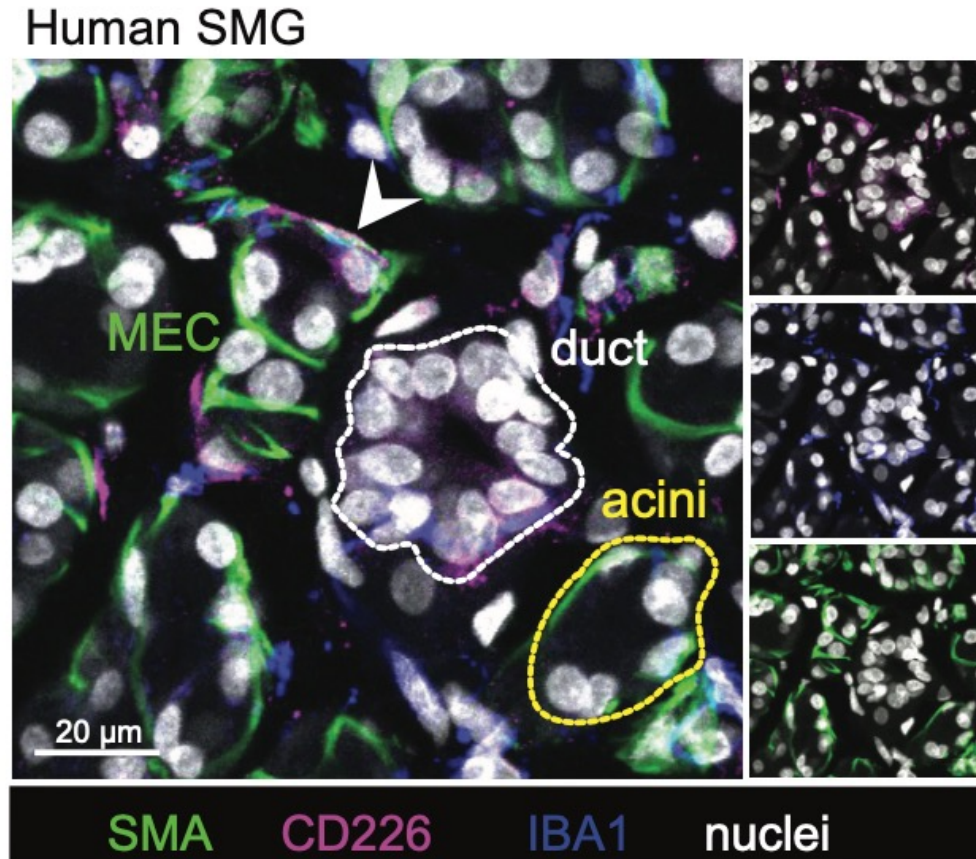
# Adenophages are a phenomenon across multiple exocrine glands in the mouse

## Adenophages

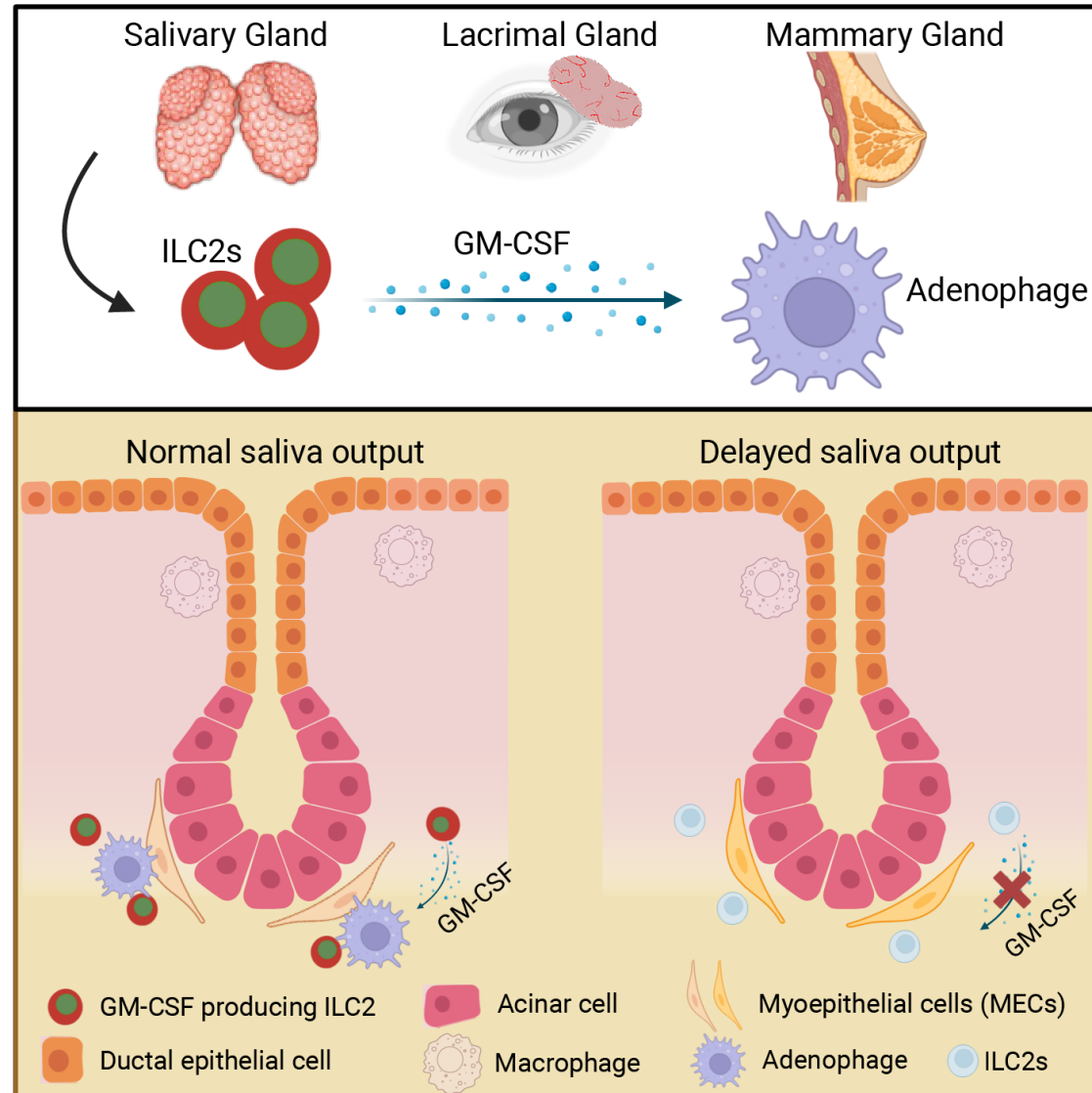


Adenophages can be found in other exocrine glands, such as lacrimal gland and mammary gland and are again dependent on ILC2-derived GM-CSF

# CD226 and IBA1 identify MEC-associated macrophages in human SG



# Summary



# Thank you for your attention



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