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Eosinophils are required for the maintenance of plasma cells in the bone marrow

Van Trung Chu[1], Anja Fröhlich[1,2], Gudrun Steinhauser[1], Tobias Scheel[1], Toralf Roch[1,4], Simon Fillatreau[1], James J Lee[3], Max Löhning[1,2] & Claudia Berek[1]

CellPress

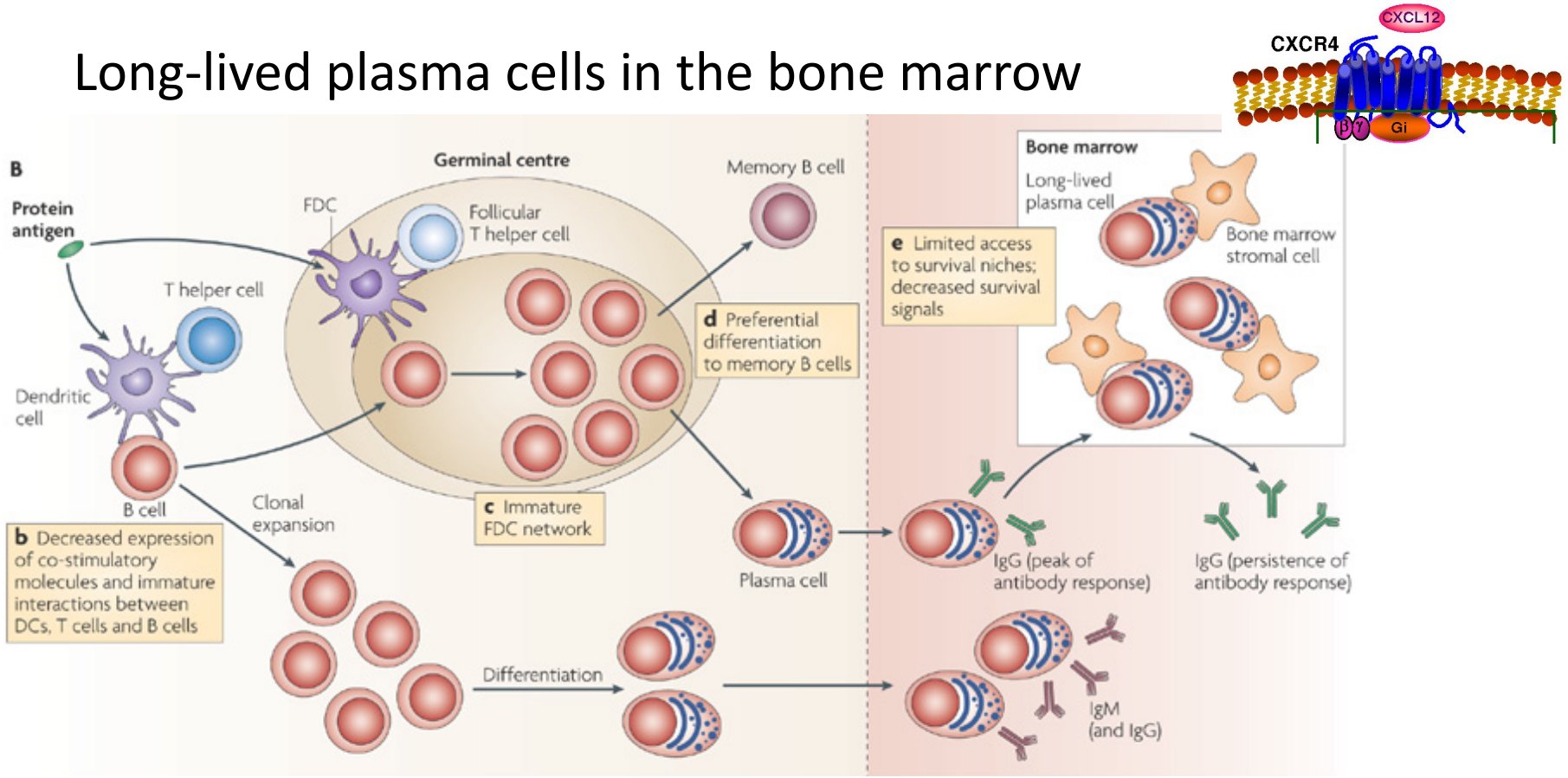
Immunity
Article

2014

Eosinophils Promote Generation and Maintenance of Immunoglobulin-A-Expressing Plasma Cells and Contribute to Gut Immune Homeostasis

Van Trung Chu,[1,5] Alexander Beller,[1] Sebastian Rausch,[2] Julia Strandmark,[2] Michael Zänker,[3] Olga Arbach,[4] Andrey Kruglov,[1] and Claudia Berek[1,*]

Long-lived plasma cells in the bone marrow

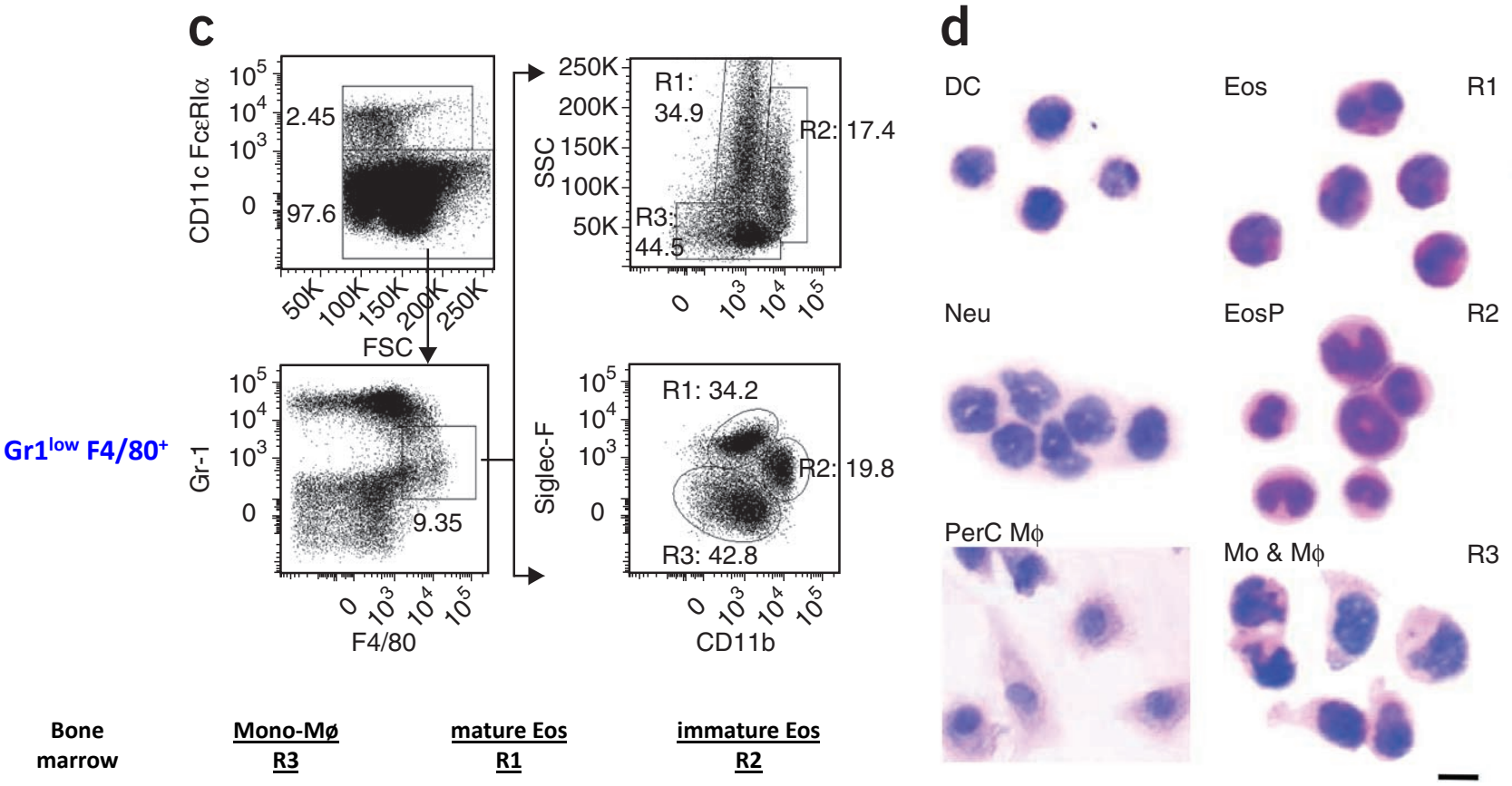


- Long-lived plasma cells are generated in the BM and maintained in niches
- Plasma cell survival
  - *in vitro*: APRIL, IL-6, IL-10, IL-4 and IL-5 support survival
  - *in vitro*: **IL-6** is crucial for PC survival
  - *in vivo*: IL-6-deficient mice show a normal PC comparment in the BM
  - **APRIL**-deficient mice show much reduced PC survival (also BCMA$^{-/-}$ = APRIL receptor)

→ **IL-6 and APRIL** are important to maintain plasma cells in the bone marrow

Aim: To identify the bone marrow population that supplies survival factors for PC

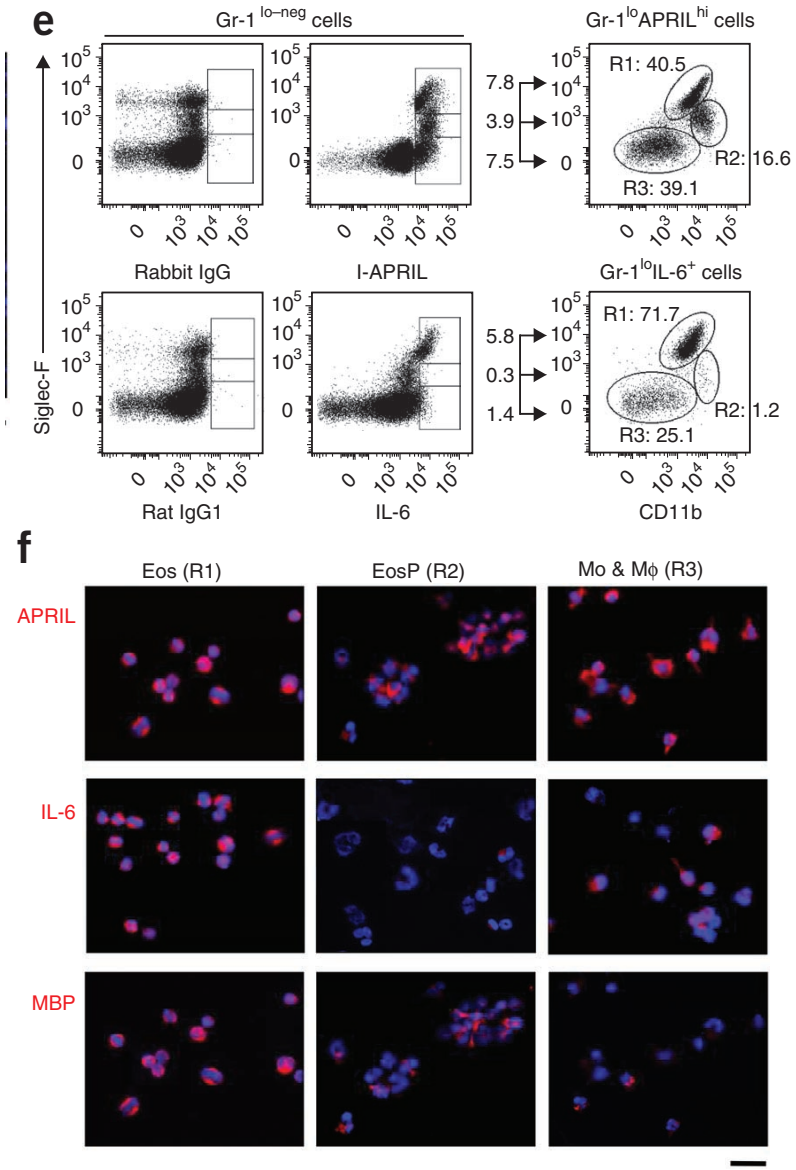
Bone marrow populations



Gr1^{low} F4/80^{+}

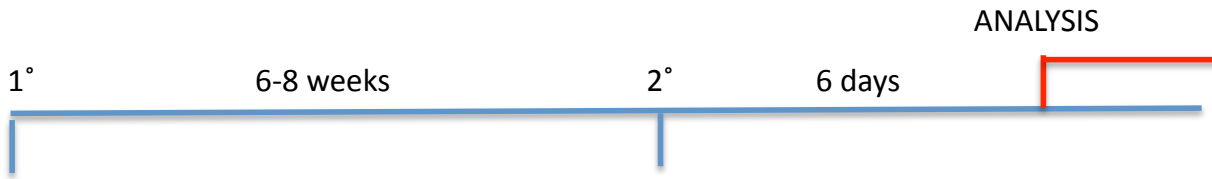
Bone marrow	Mono-Mø R3	mature Eos R1	immature Eos R2
F4/80	+	+	+
Gr-1	low	low	low
CD11b	**int**	**int**	**high**
Siglec-F	**-**	**high**	**+**
SSC	**low**	**high**	**int**

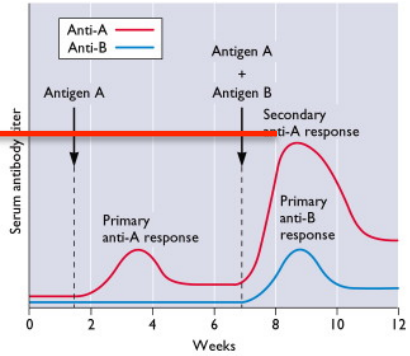
F4/80^{+} Gr-1^{low} subsets in the BM



Bone marrow	Mono-Mø R3	mature Eos R1	immature Eos R2
F4/80	+	+	+
Gr-1	low	low	low
CD11b	**int**	**int**	**high**
Siglec-F	-	**high**	+
SSC	**low**	**high**	**int**
Cytokines	**some April, few IL-6**	**IL-6 April high**	**April high**

2-Phenyloxazolone immunization protocol

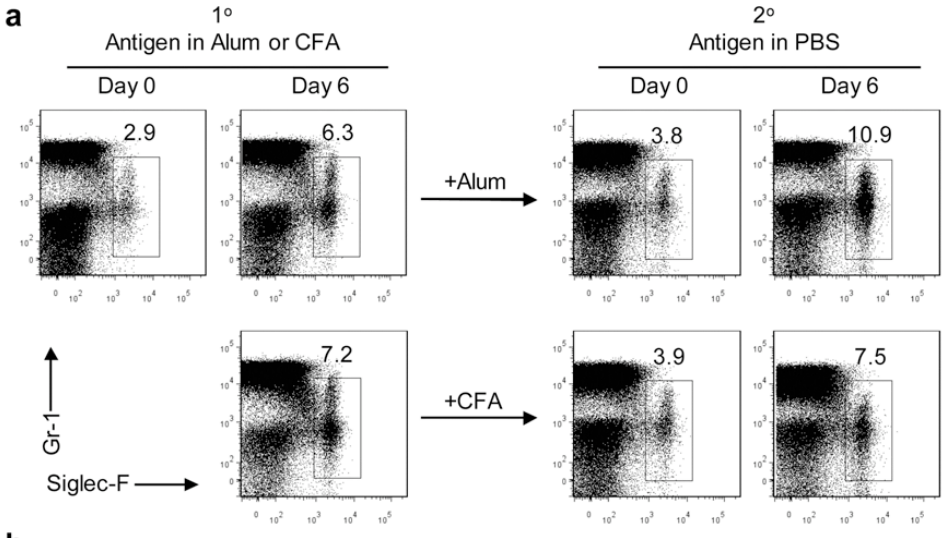




PhOx-Chicken serum albumin
+ AlOH or comp. Freund's adjuvant
ip. 100µg

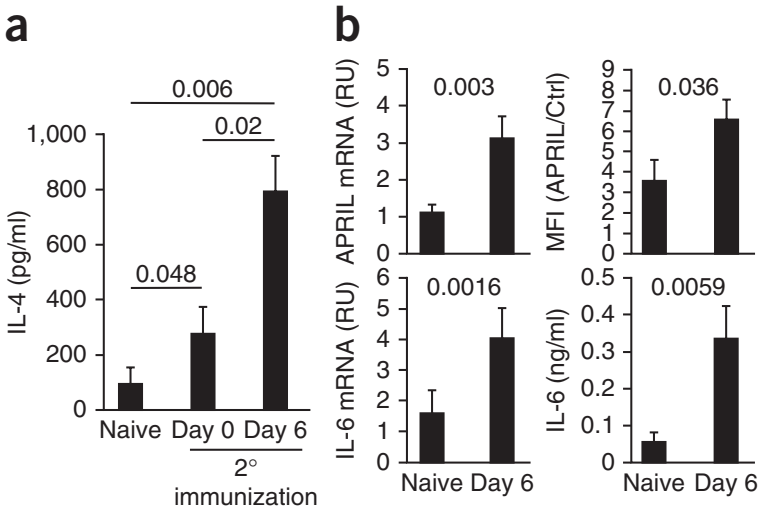
PhOx-Chicken serum albumin
iv. 100µg

- T-dependent immune response
- antigen-independent activation of eosinophils
- those promote expansion/differentiation of antigen-spec. IgM plasma cells



Eos cytokines promote plasma cell survival

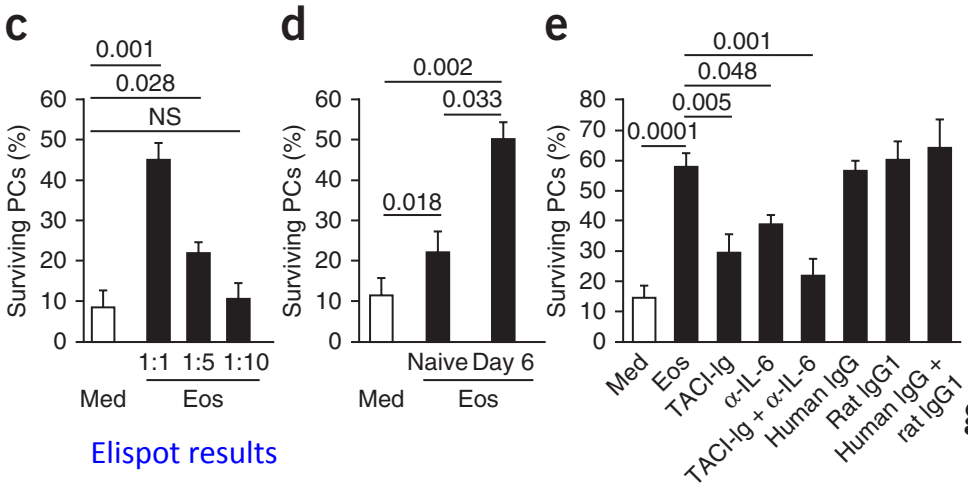
Eos from D6 after 2˚



Immunization with 2-phenyloxazolone and challenge
Day 0 and day 6

→ Eos production of IL-4, IL-6 and APRIL increases

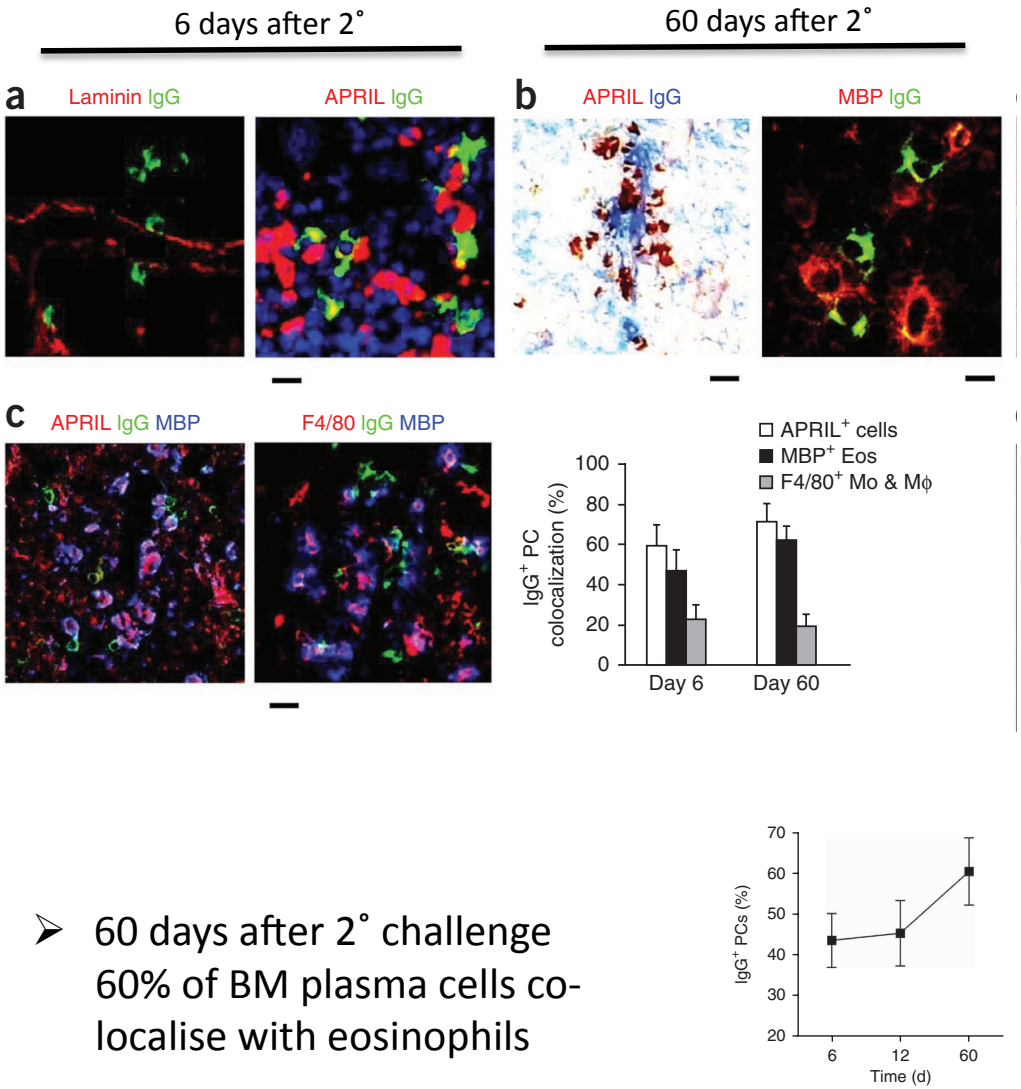
Eos from D6 after 2˚, 2 days culture

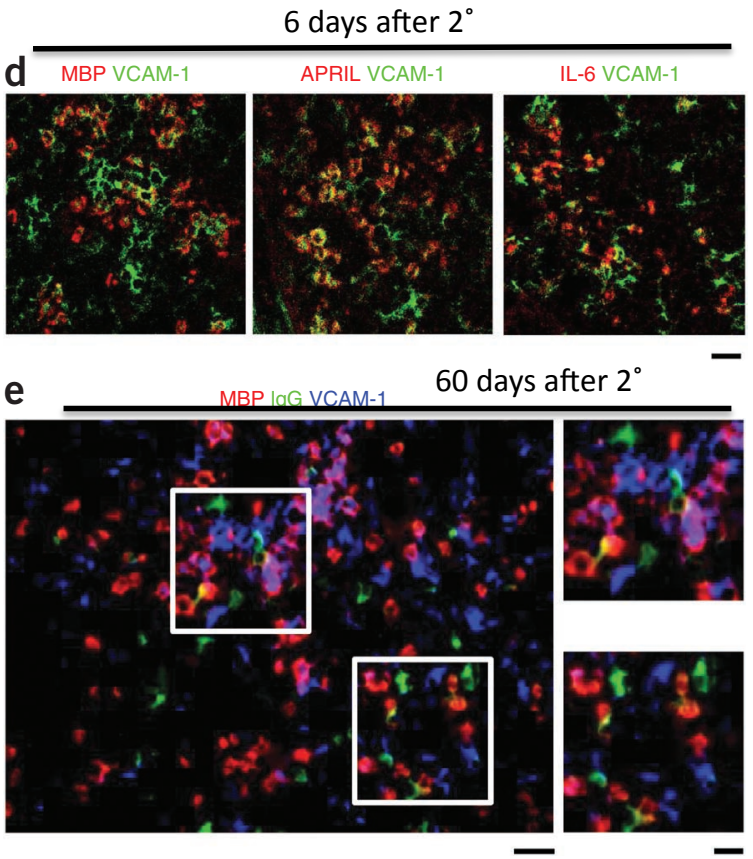


Elispot results

→ Eos promote plasma cell survival via both, IL-6 and APRIL secretion

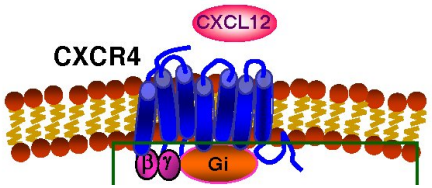
Eosinophils contribute to the survival niche for plasma cells

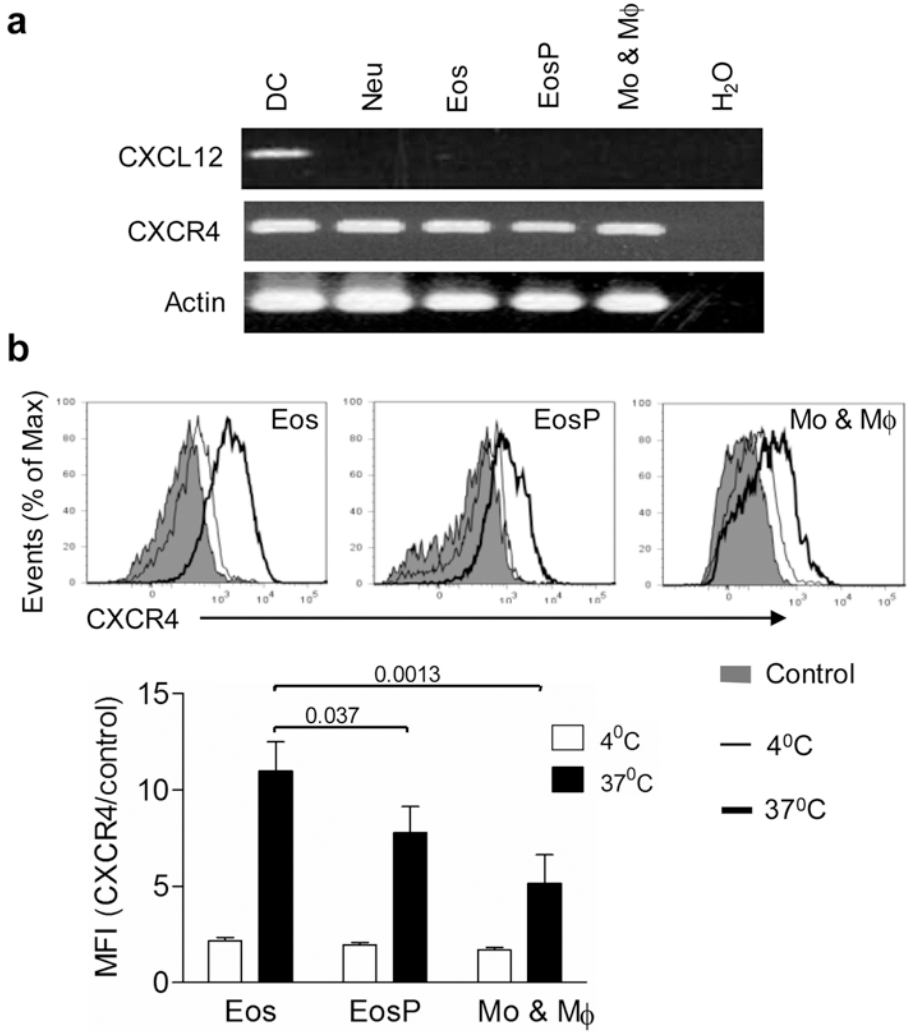




- 60 days after 2˚ challenge 60% of BM plasma cells co-localise with eosinophils

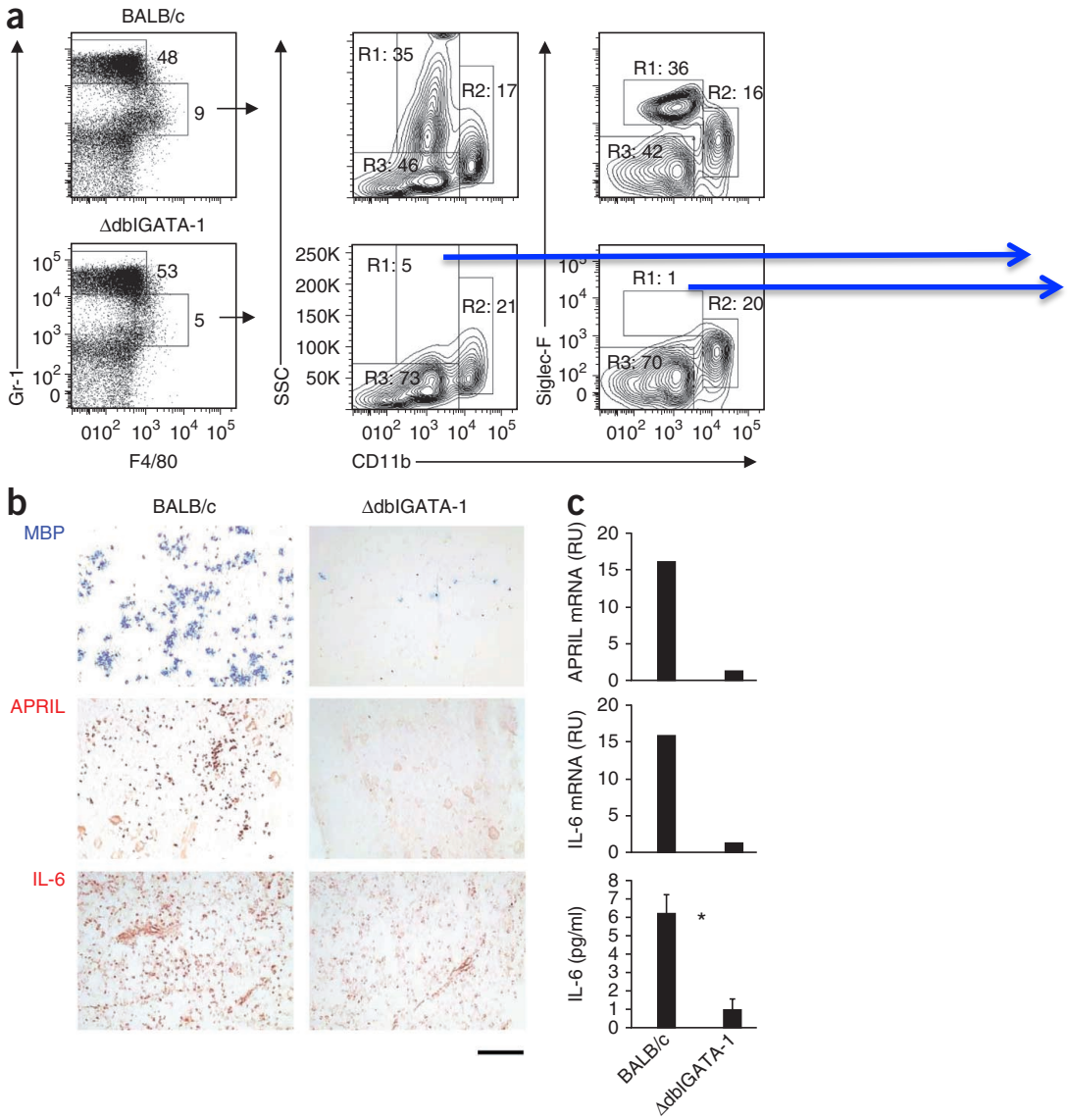
Plasma cell recruitment to the bone marrow





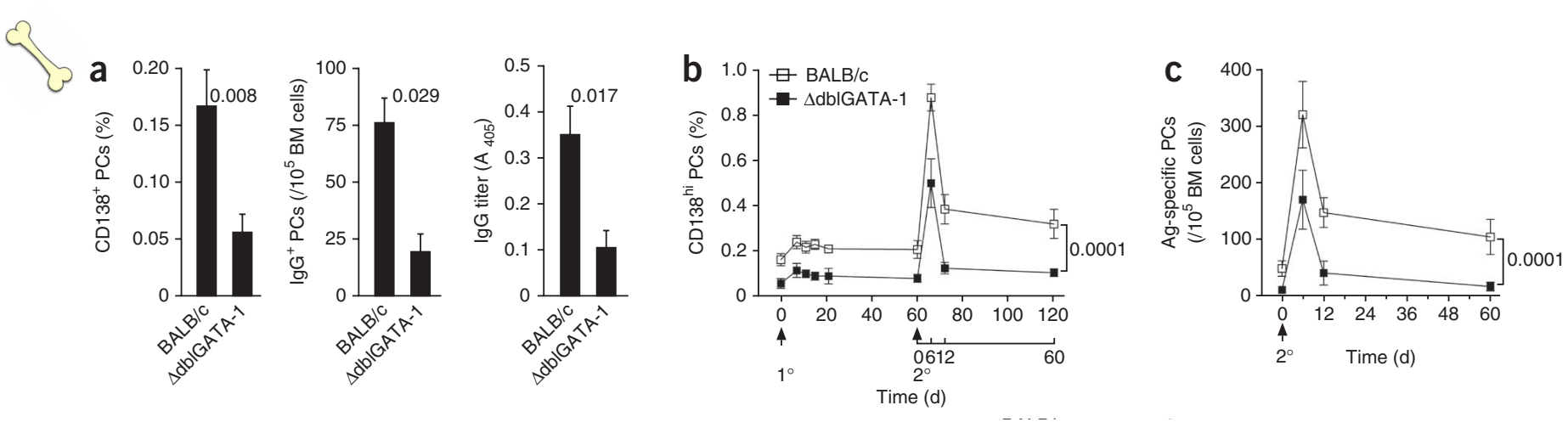
- No CXCL12 expression by eosinophils → eos not involved in recruitment of plasma cells to the BM
- Eos and plasma cells are recruited to their niche by same CXCL12 source

Balb/c and ΔdblGATA-1 (eosinophil knockout)

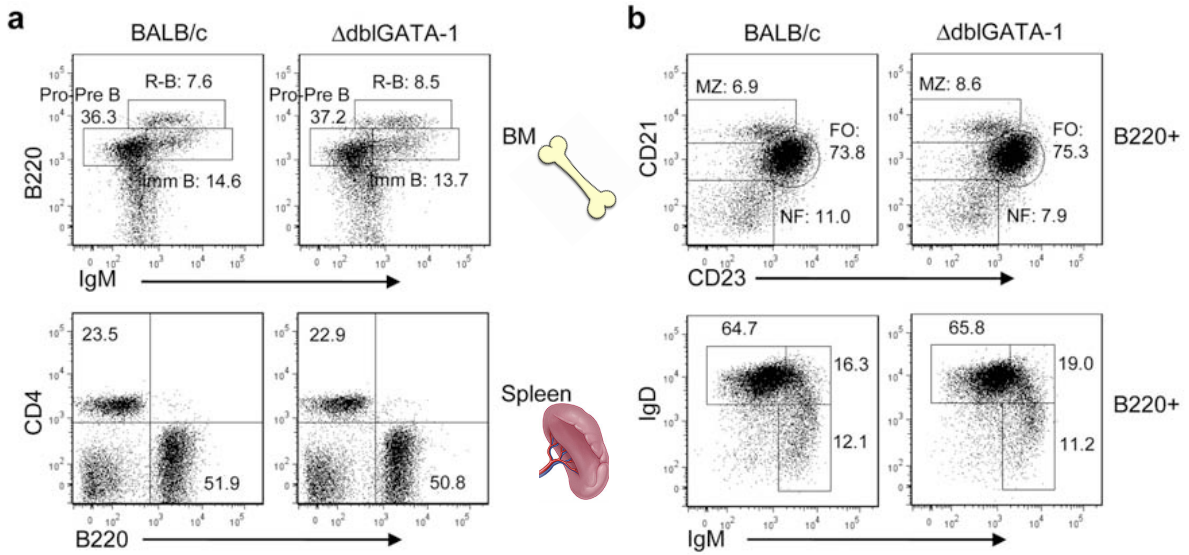


- no mature eosinophils
- residual MBP-staining presumably from EoP (eosinophil precursor)
- MUCH reduced April and IL-6 in the bone marrow

Balb/c and ΔdblGATA-1 (eosinophil knockout)



- **Plasma cell** numbers are reduced by 30% in the absence of eosinophils

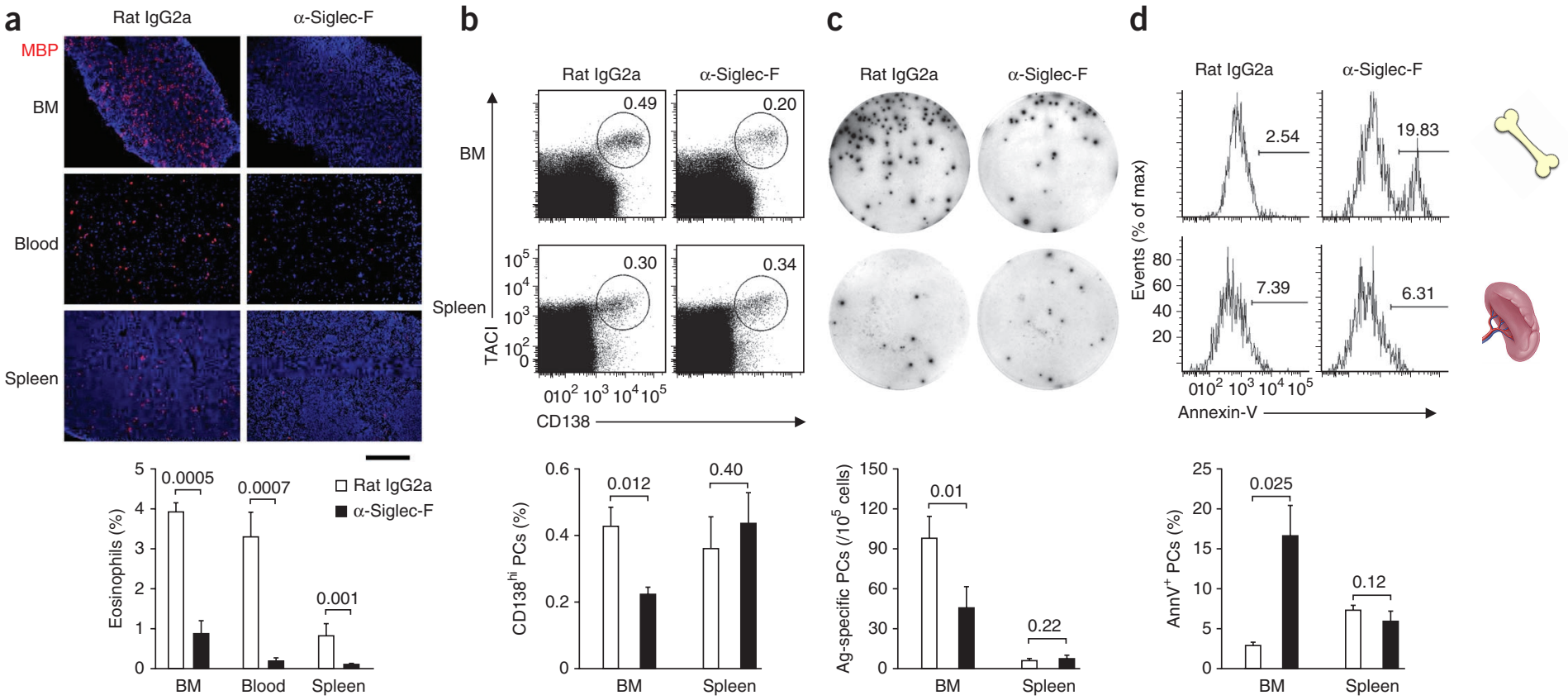


- B cell development and maturation seems normal in eos-deficient mice

Figure 5 (top) and Suppl. 8 (bottom)

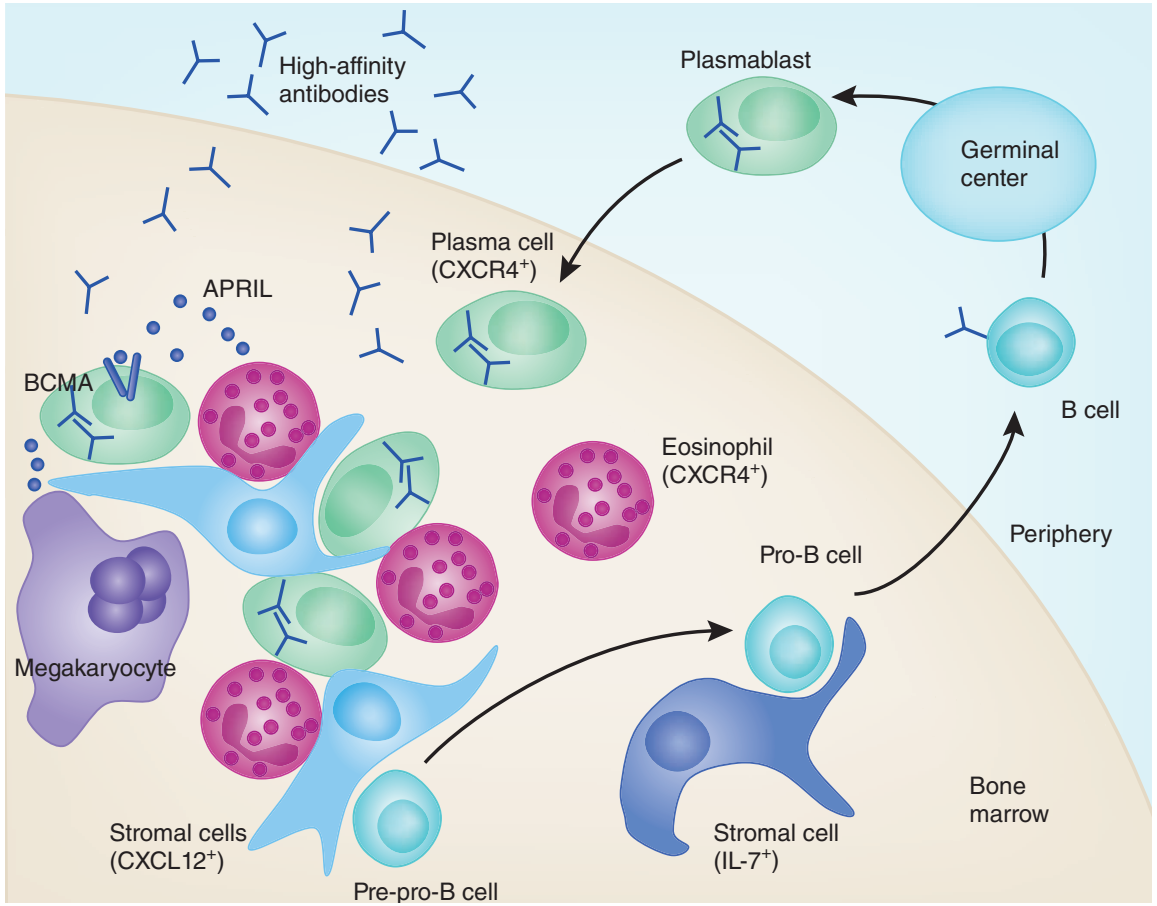
Long-term survival of plasma cells is dependent on eosinophils

- Protocol: 3x 20µg α-Siglec-F ip



Conclusions and summary

- F4/80^{+} Gr-1^{low} population in the BM produces April and IL-6
- the major source are eosinophils
- Eosinophils provide survival factors (APRIL and IL-6) for BM plasma cells
- Eosinophils co-localise with plasma cells in their niche (CXCR4 expression)



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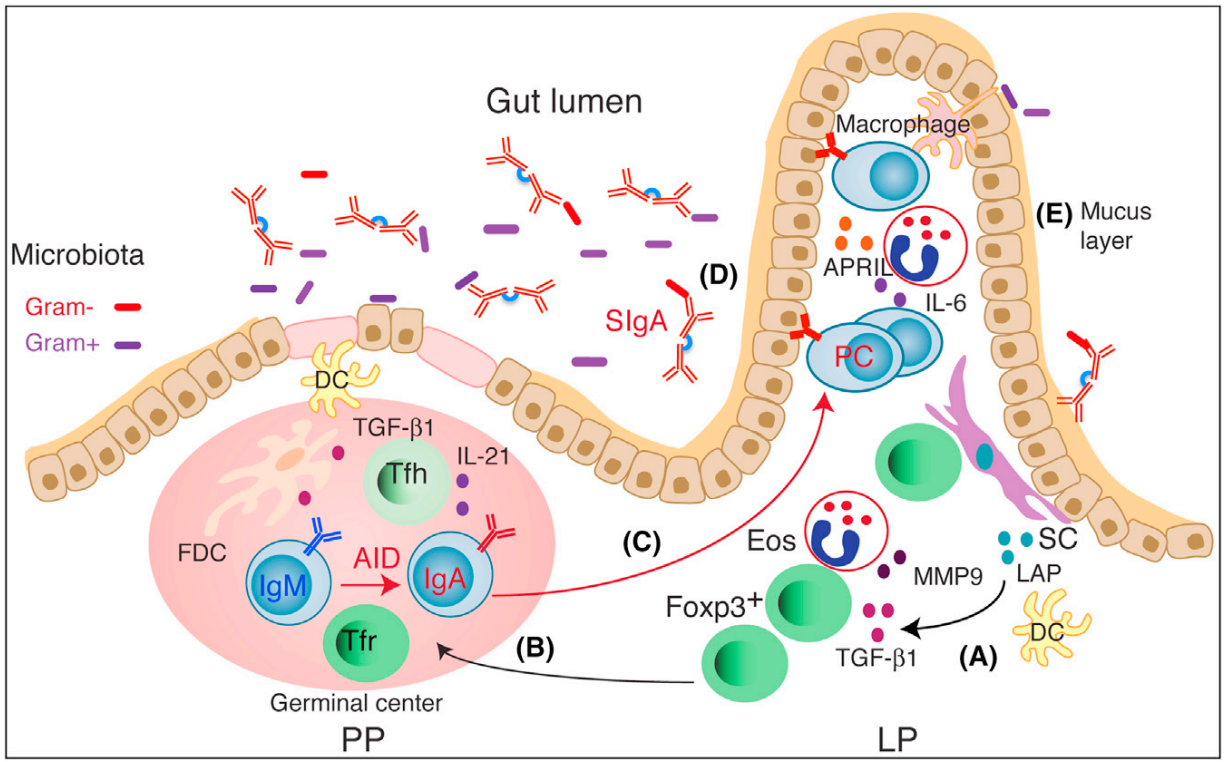
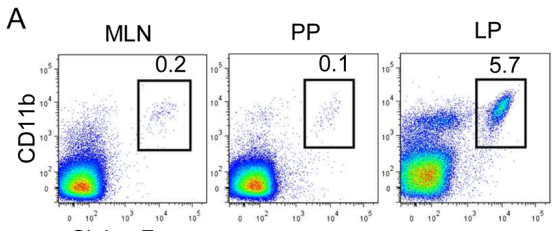
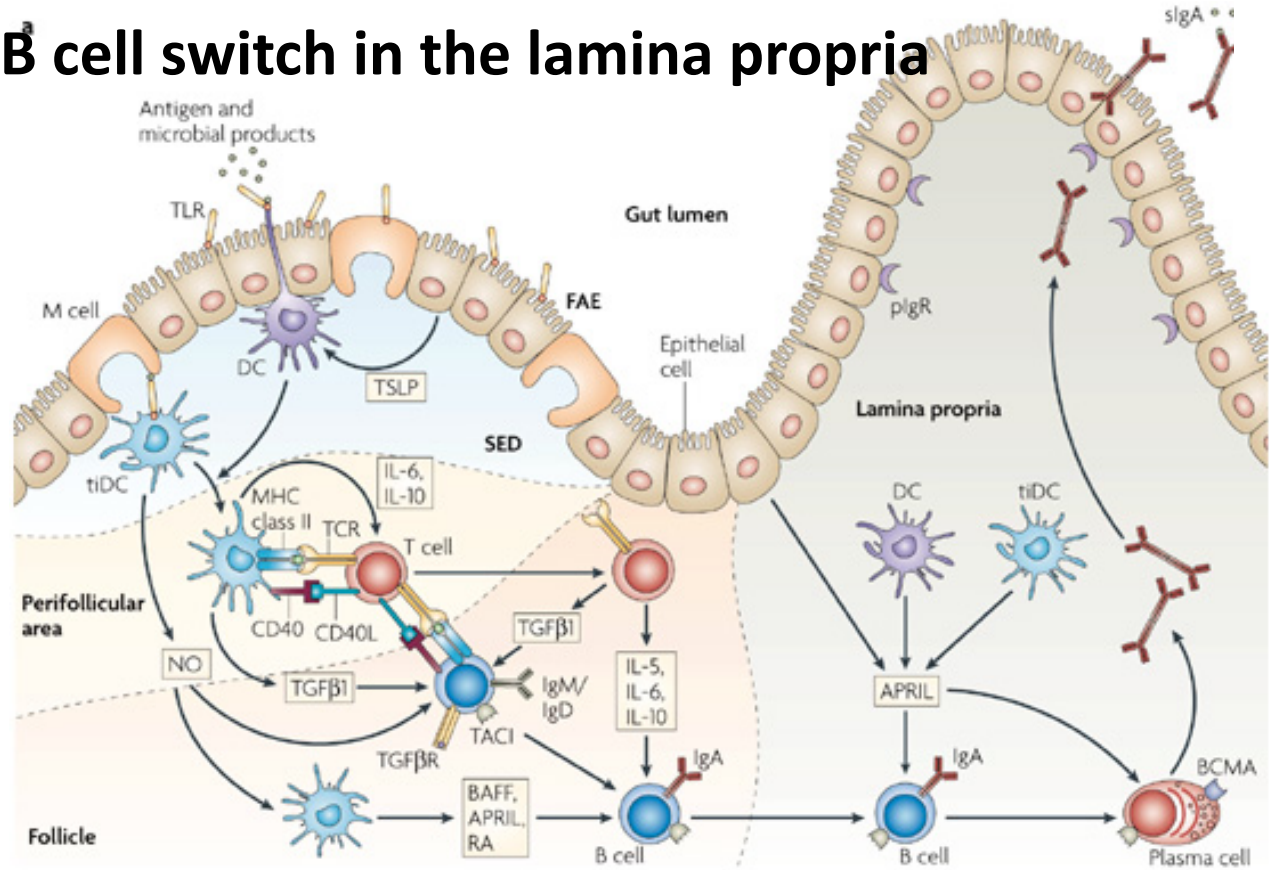


Small intestinal lamina propria eosinophils

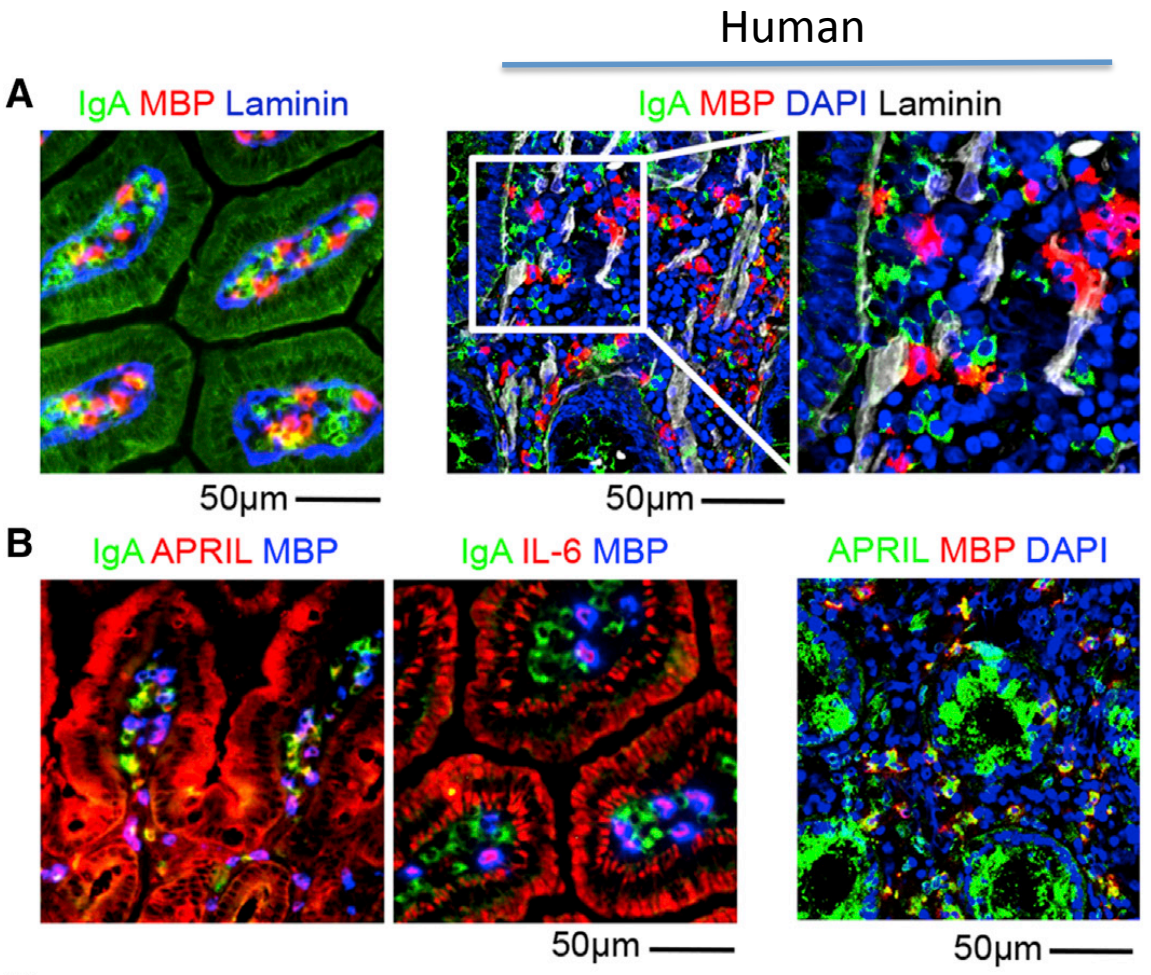
- 5% of small intestinal lamina propria immune cells are eosinophils
- → function under *homeostasis* is unclear



B cell switch in the lamina propria

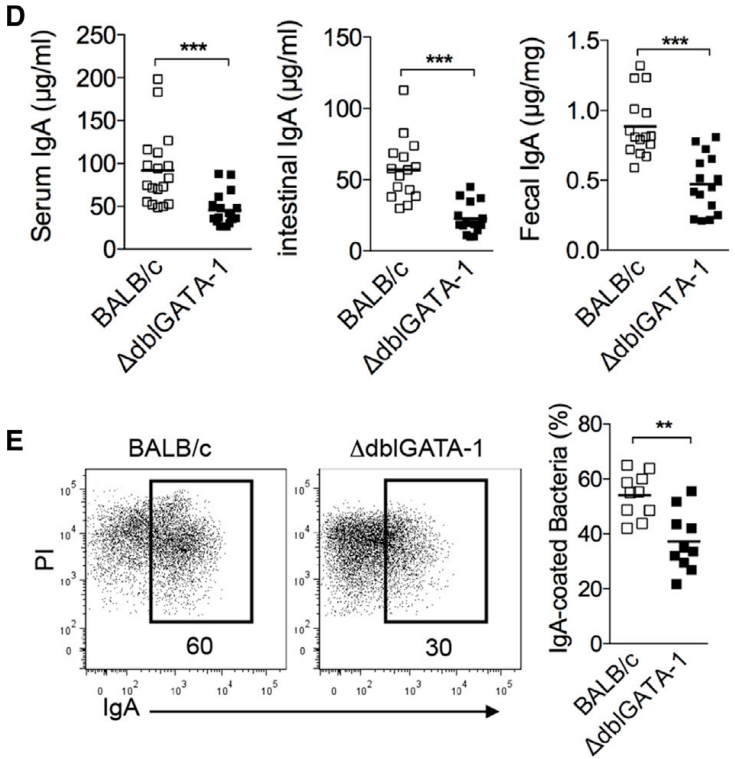


Eosinophils in the small intestine produce April and IL-6 as do eosinophils in the bone marrow



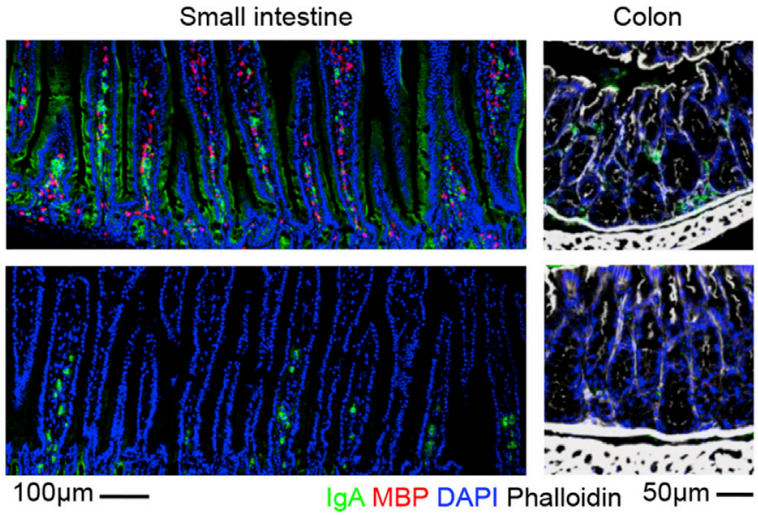
- Eos co-localise with IgA+ B cells
- All eos produce APRIL, most of them IL-6

Plasma cell numbers and secreted IgA are reduced in the LP of eosinophil-deficient ΔdblGATA-1 mice



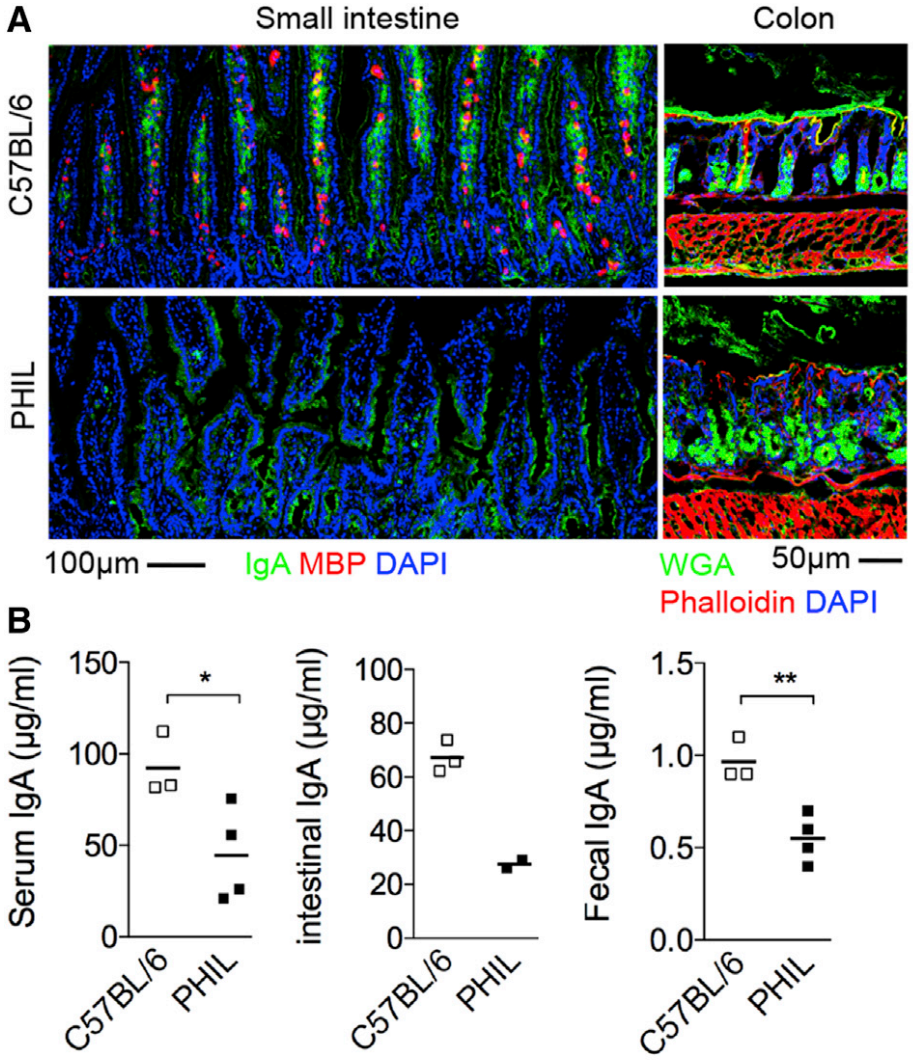
- reduced intestinal IgA
- less IgA-coated bacteria
- disrupted mucus layer, but normal numbers of goblet cells

- reduced IgA+ cells in the siLP



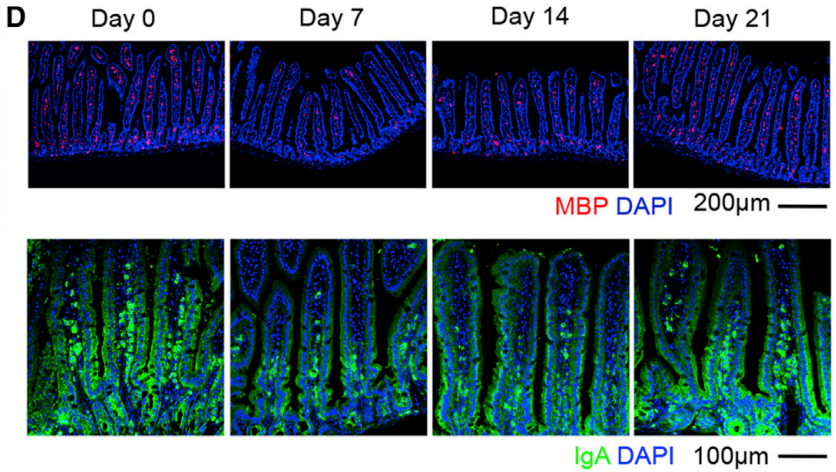
Eosinophils are essential for the maintenance of IgA+ plasma cells

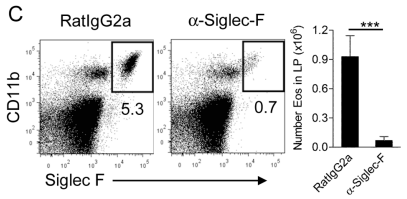
PHIL mice



Eosinophils are essential for the maintenance of IgA+ B cells

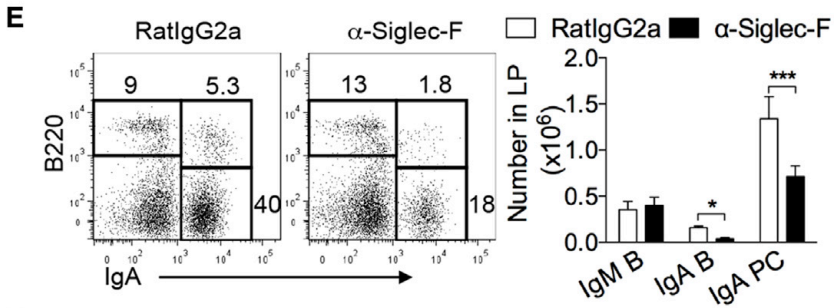
IgA+ B cells





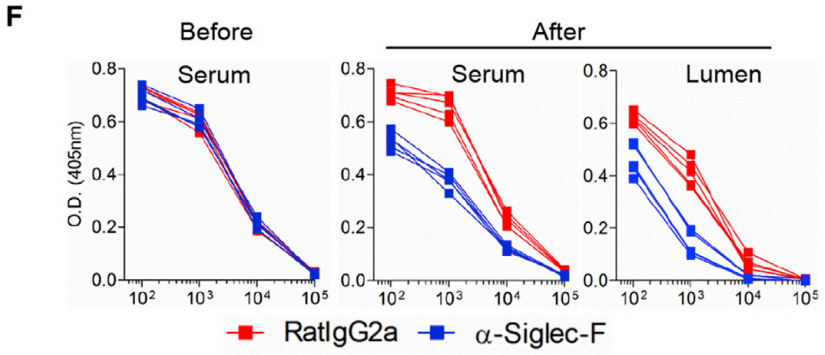
Eos depletion for 1 week with α-Siglec-F antibody
3x20µg ip.

IgA+ B cells



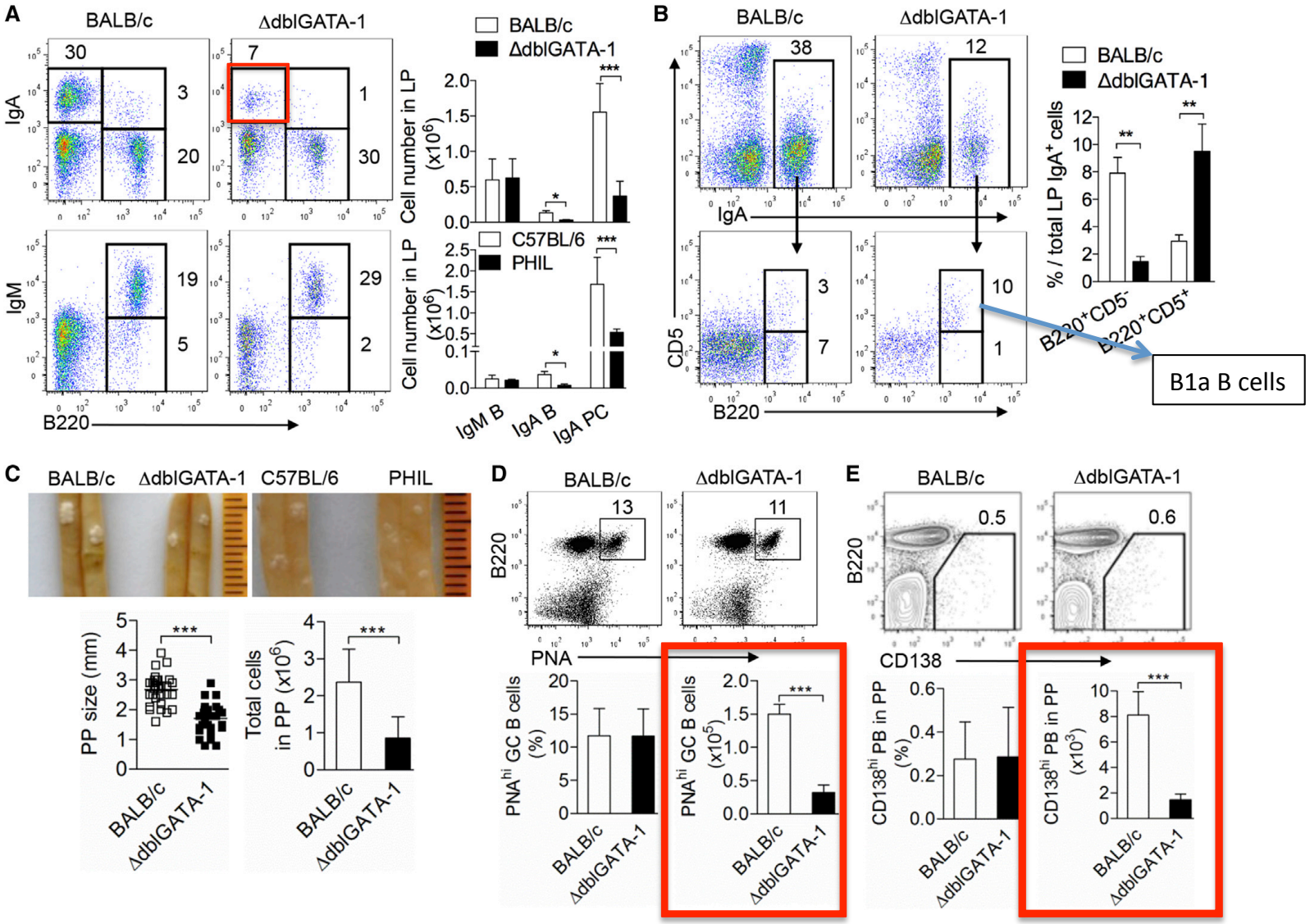
Eos depletion for 2 weeks with α-Siglec-F antibody

IgA titers



Eos depletion for 2 weeks with α-Siglec-F antibody

IgA+ B and plasma cells is reduced in the LP of eosinophil-deficient mice

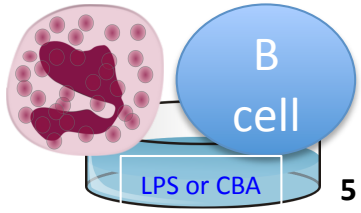


Smaller PP, but normal architecture

In vitro: Eosinophils support both plasma cell survival and B cell differentiation

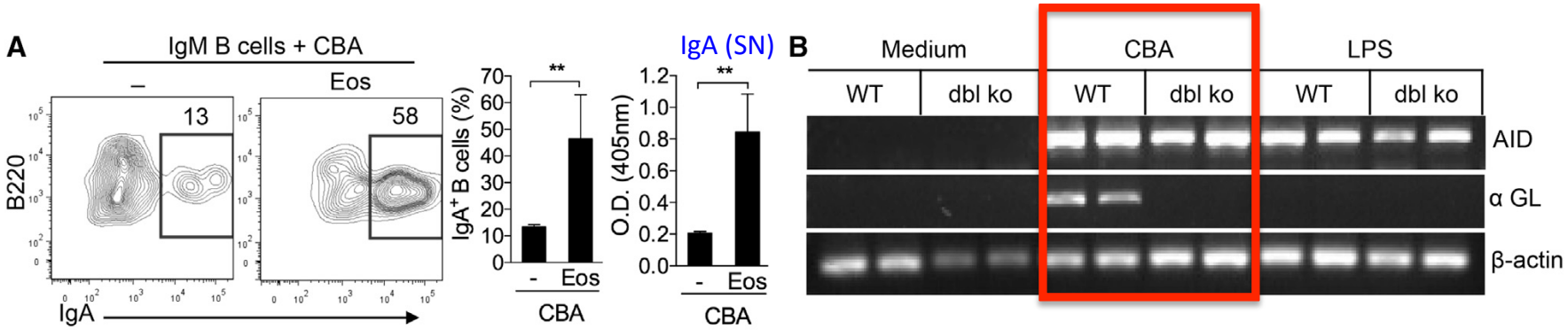
A./B. coculture eos-B cells

small intestinal LP
CD11b+MHCII-Gr1-
WT or MT$^{-/-}$



5 days

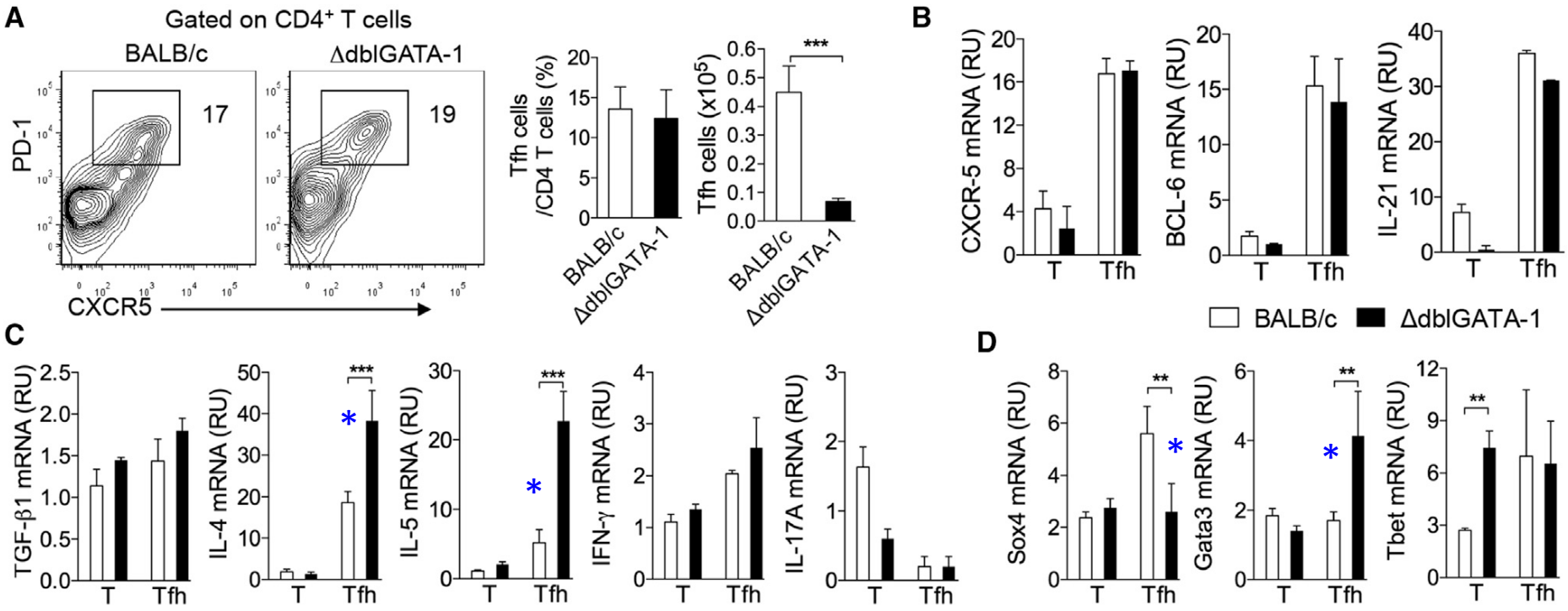
spleen: IgD+B220+ (A)
LP: IgM+B220+ (B)



T follicular helper cells
&
IgG1

In the PP of ΔdblGATA-1 mice Tfh cell-derived cytokines favour class switch to IgG1

Tfh: CD4+ PD-1+ CXCR5+ in PP

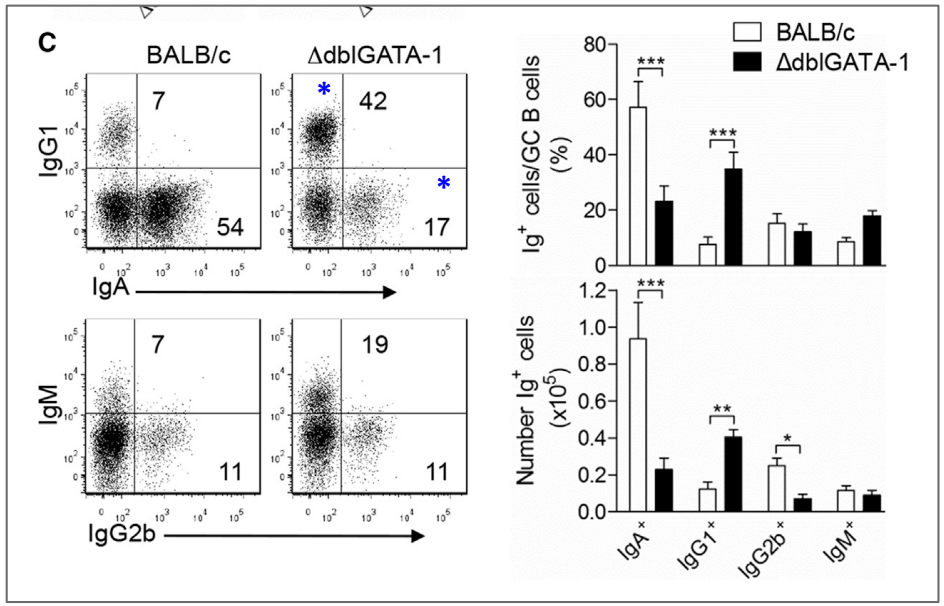


- normal frequencies, but reduced total numbers (smaller PP)
- less IL-5 and IL-4, lower Sox4, higher GATA-3 → Th2 phenotype

GC B cells in PP of eosinophil-deficient mice preferentially switch to IgG1

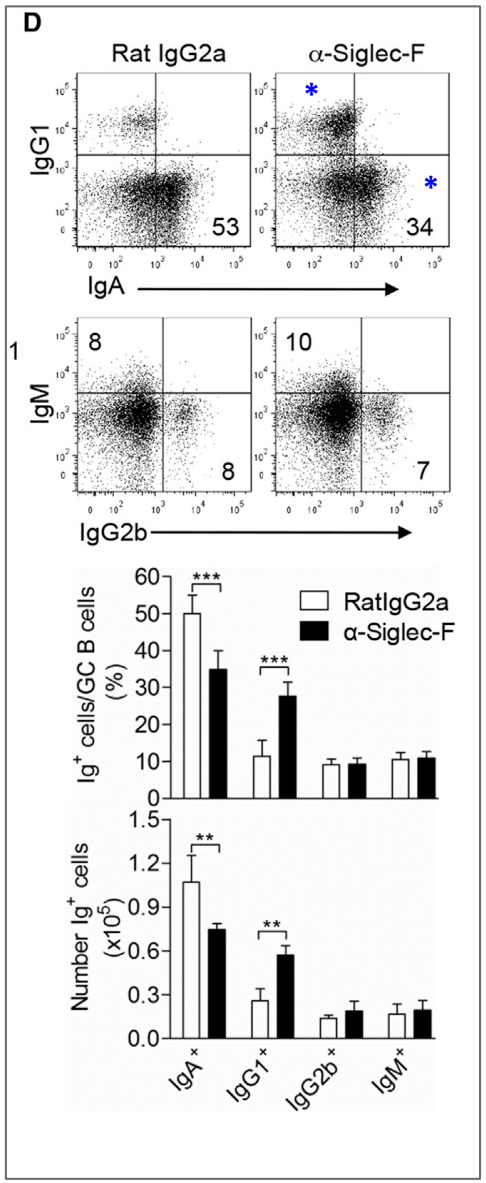
PP

Depletion for 2 weeks



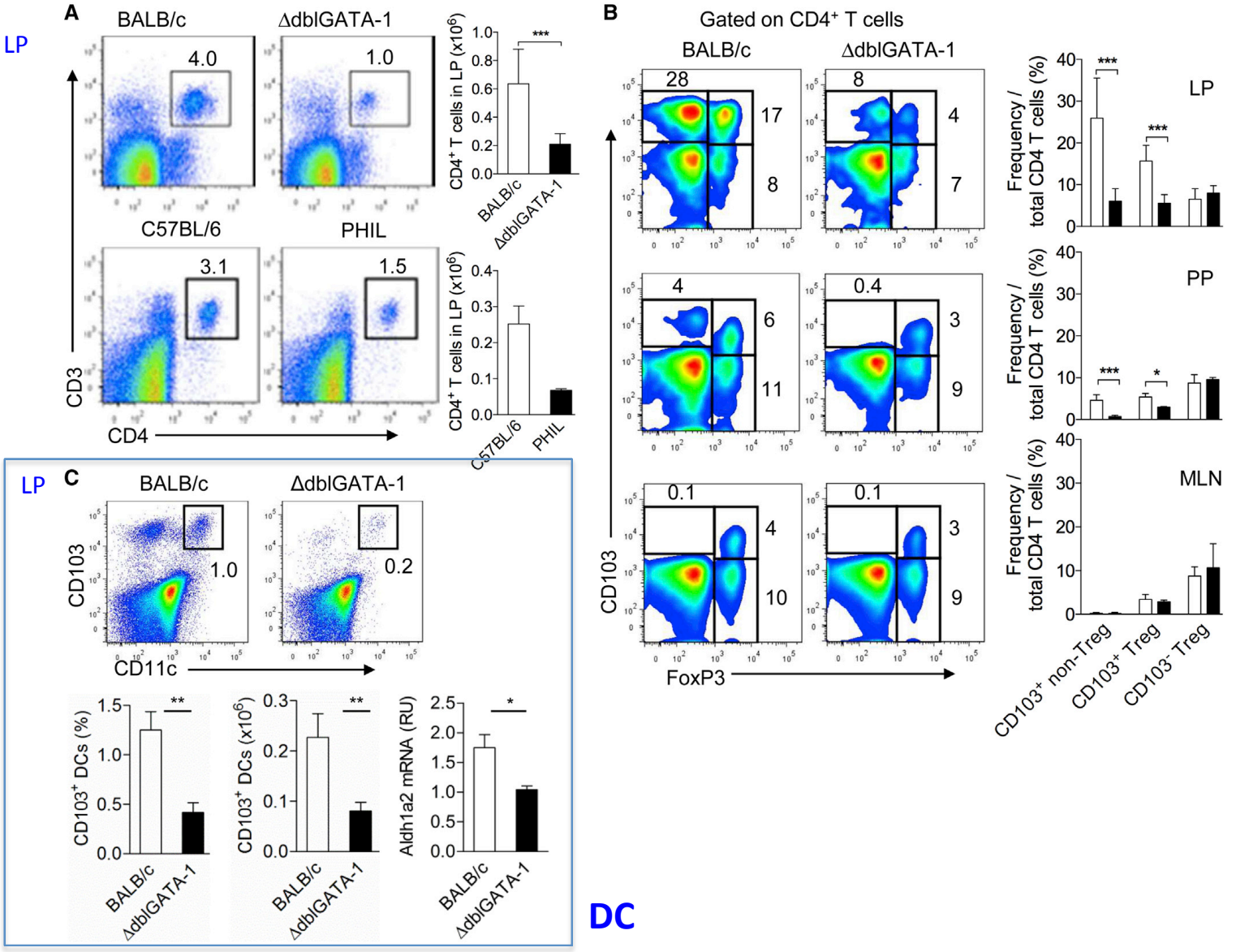
PP

Depletion for 2 weeks



CD103+
T cells and DC

CD103+ DC and CD103+ T cells including FoxP3+ Treg cells are reduced in eosinophil-deficient mice



Eosinophil-deficient mice have an altered microbial composition

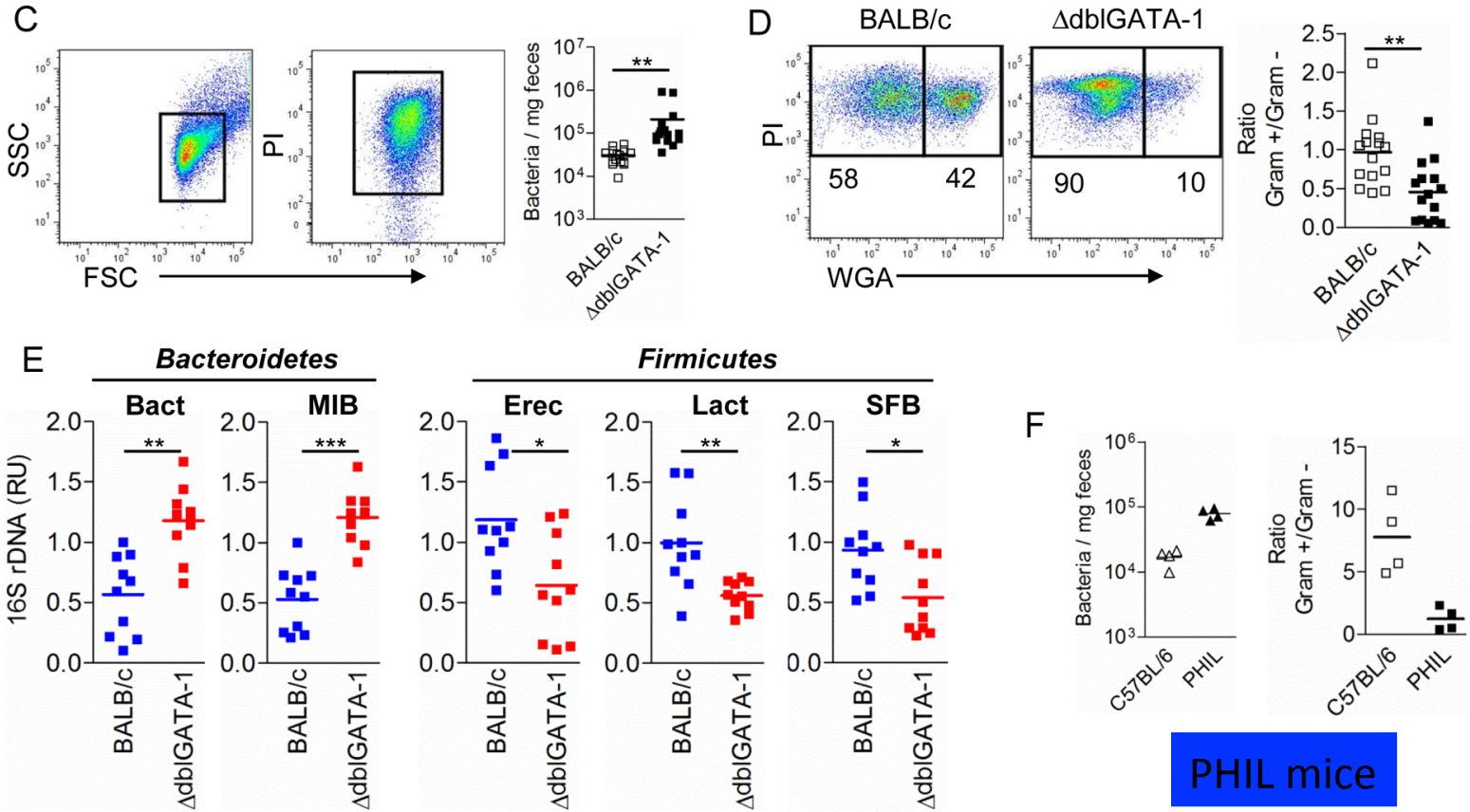
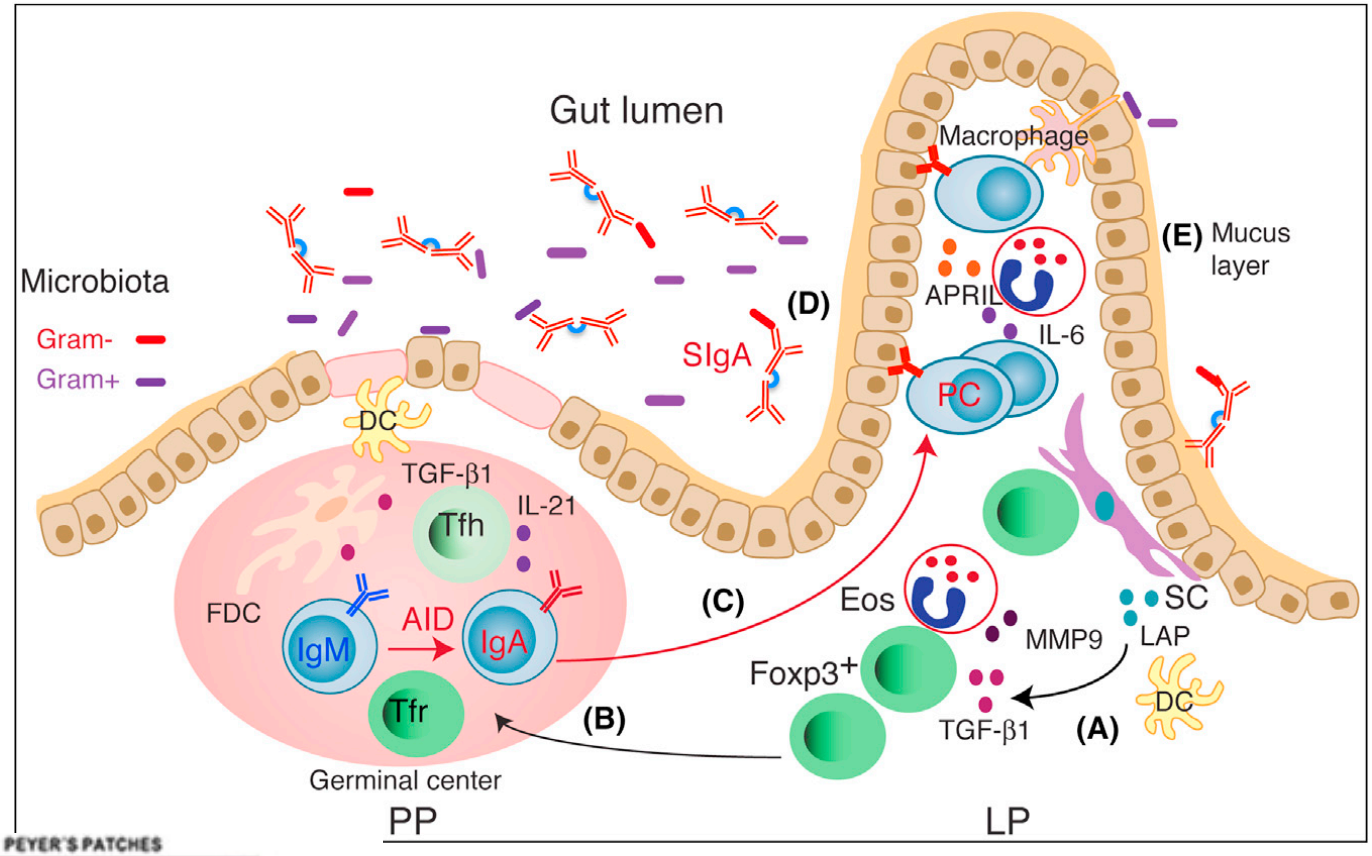
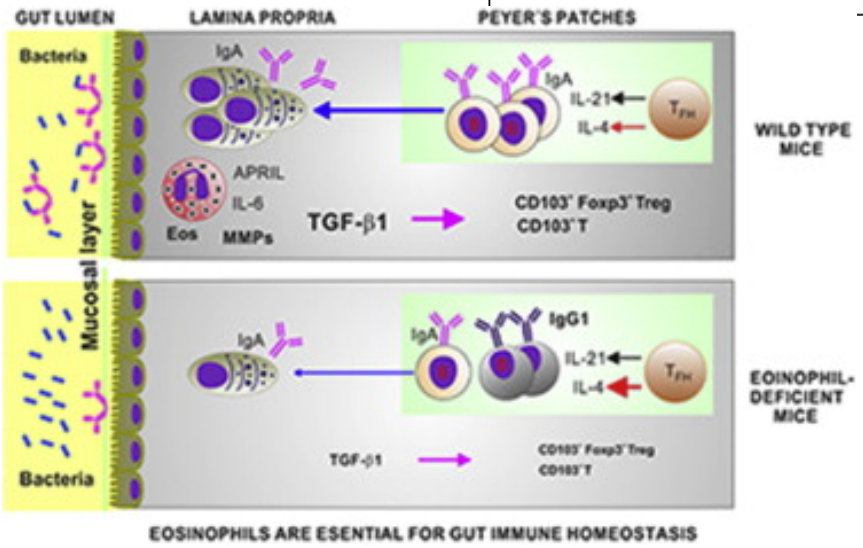


Figure S1, related to Figure 1. Changes in the enteric microbiota in eosinophil-deficient

Summary


Gut lumen
Macrophage
(E) Mucus layer
Microbiota
Gram-
Gram+
(D)
SIgA
APRIL
IL-6
PC
DC
TGF-β1
IL-21
Tfh
FDC
IgM
AID
IgA
(C)
Eos
SC
LAP
MMP9
Foxp3+
DC
TGF-β1
(A)
Tfr
(B)
Germinal center
PP
LP


GUT LUMEN
LAMINA PROPRIA
PEYER'S PATCHES
Bacteria
IgA
IL-21
IL-4
APRIL
IL-6
Eos
MMPs
TGF-β1
CD103+ Foxp3+ Treg
CD103+ T
WILD TYPE MICE
Mucosal layer
IgG1
EOSINOPHIL-DEFICIENT MICE
Bacteria
EOSINOPHILS ARE ESENTIAL FOR GUT IMMUNE HOMEOSTASIS