LETTER

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Clonal selection in the germinal centre by regulated proliferation and hypermutation

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RESEARCH ARTICLE

HUMAN IMMUNOLOGY

CD4 T Cells with Effector Memory Phenotype and Function Develop in the Sterile Environment of the Fetus

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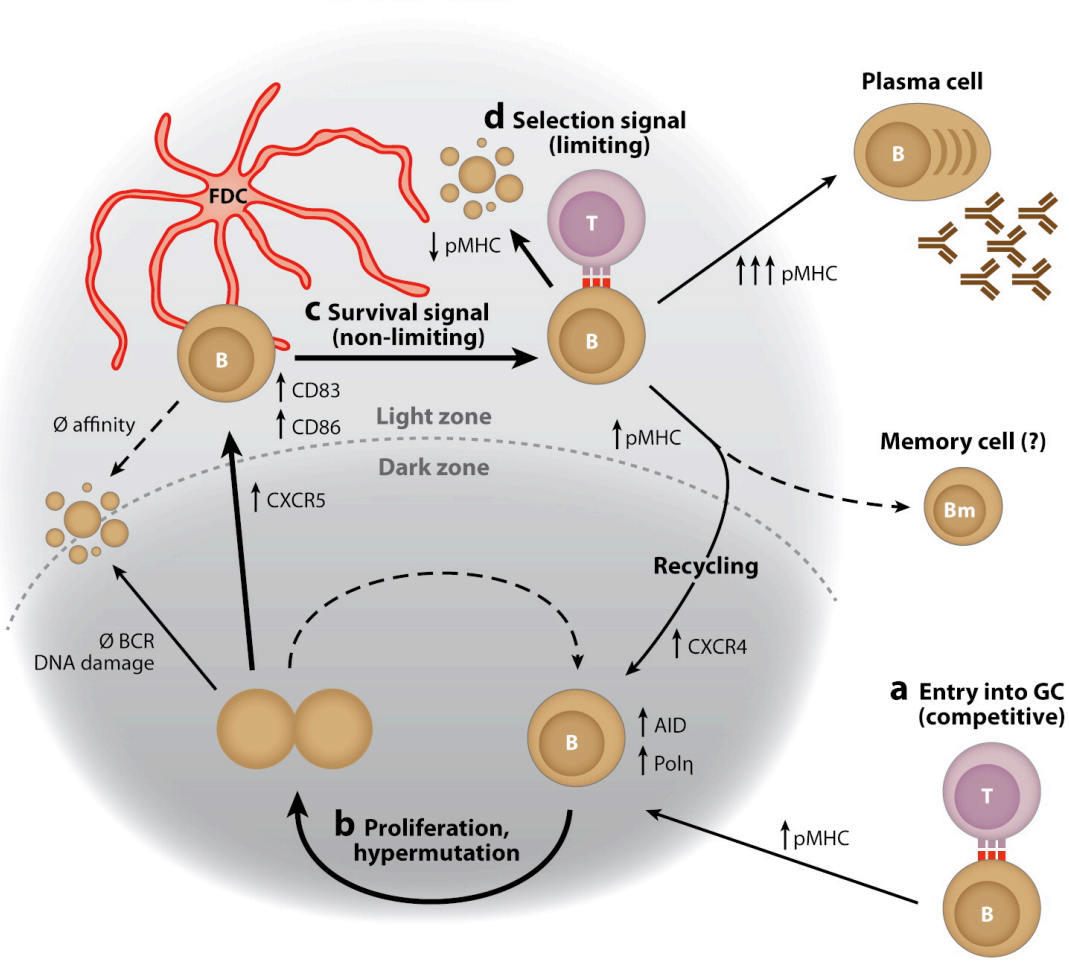
Journal Club

20-June-2014

Background

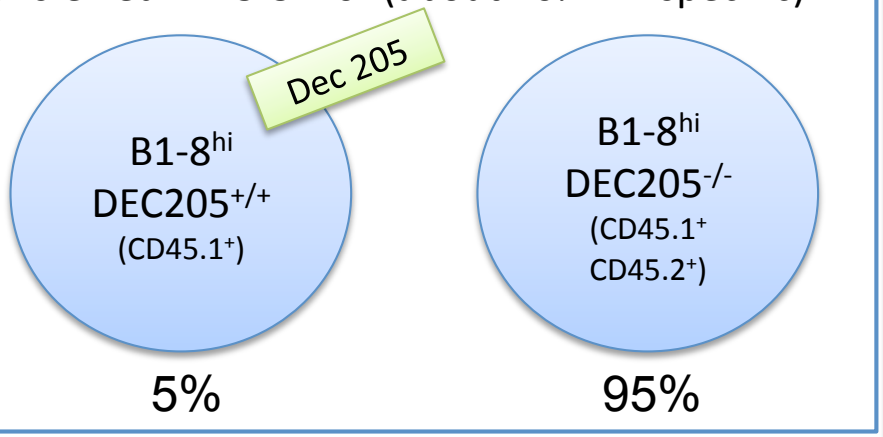


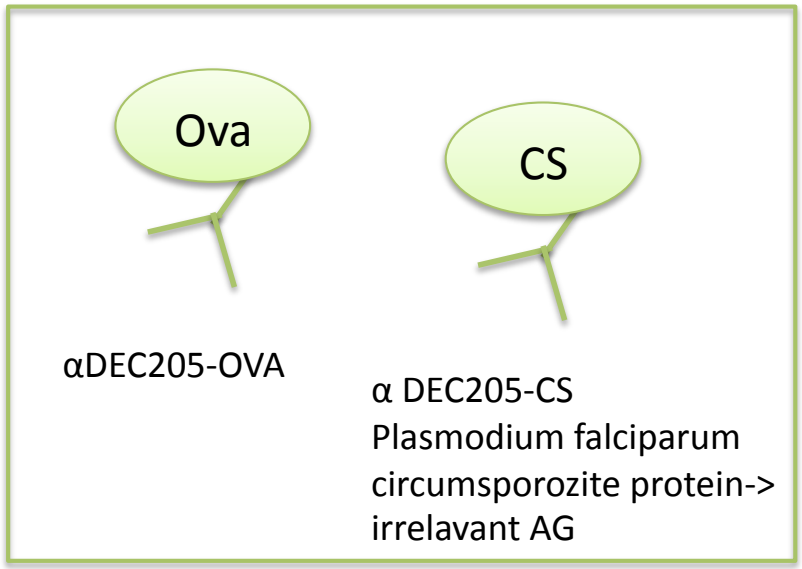
Are the extent of clonal expansion and hypermutation regulated druing interzonal germinal center cycles?

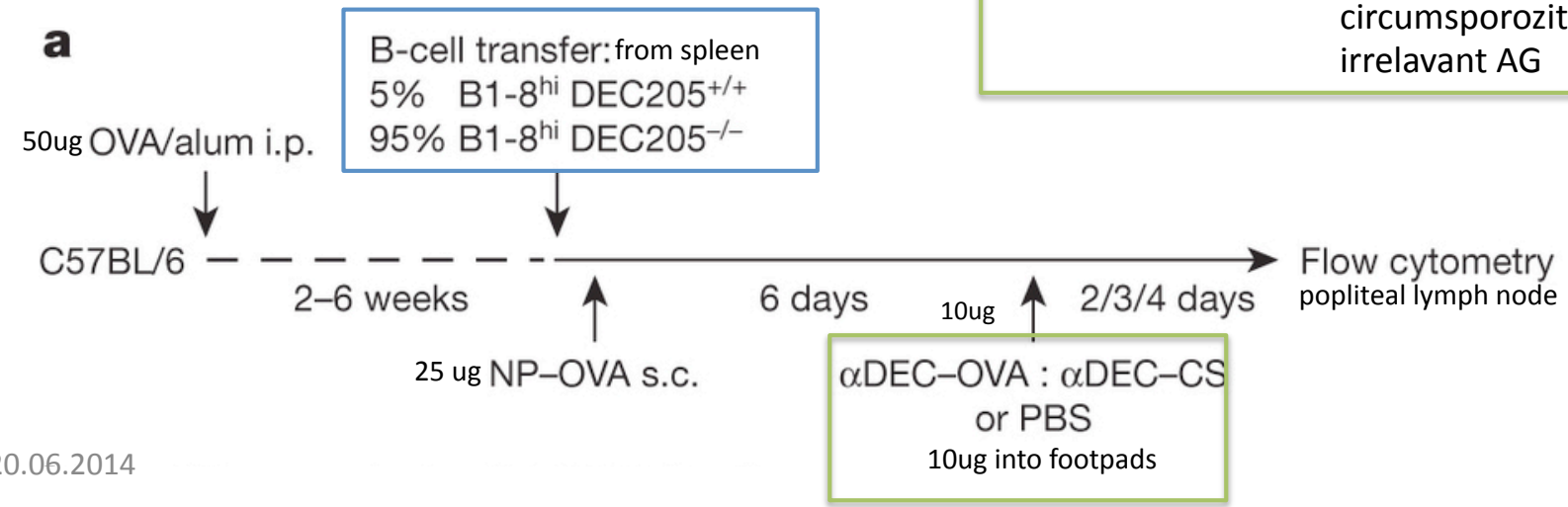
Victora, G. D., & Nussenzweig, M. C. (2012). Germinal Centers. Annu. Rev. Immunol., 30(1), 429–457

Does the amount of AG internalized by GC B cells steer clonal expansion?

HC specific for 4-hydroxy-3-nitrophenyl acetyl (NP) with Igλ LC.
Transferred # 1.5-5x10^6 (about 10% NP specific)

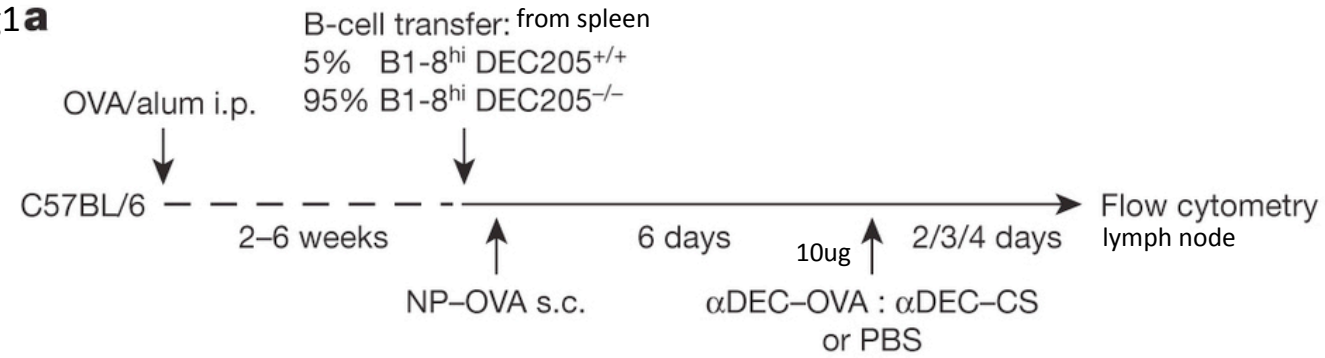






AG amount and capture regulates GC B cell expansion

Fig1**a**



Suppl Fig1**a**

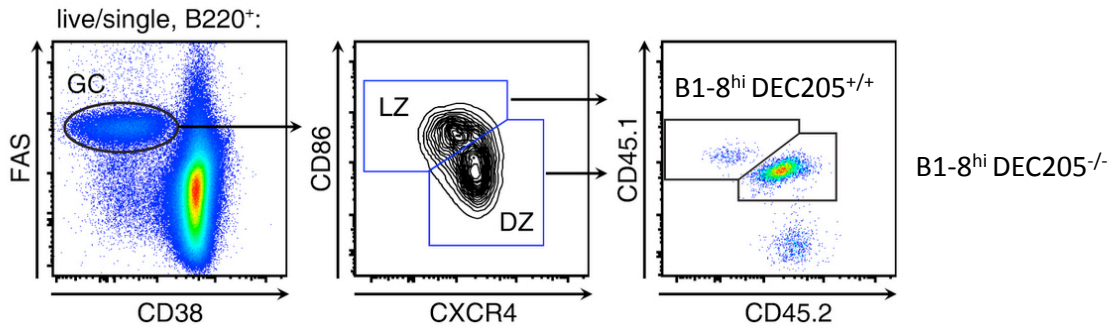
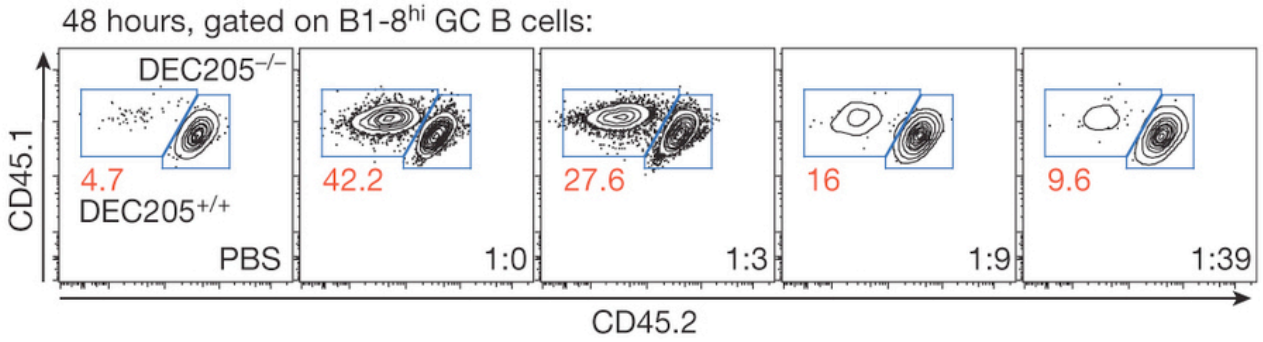
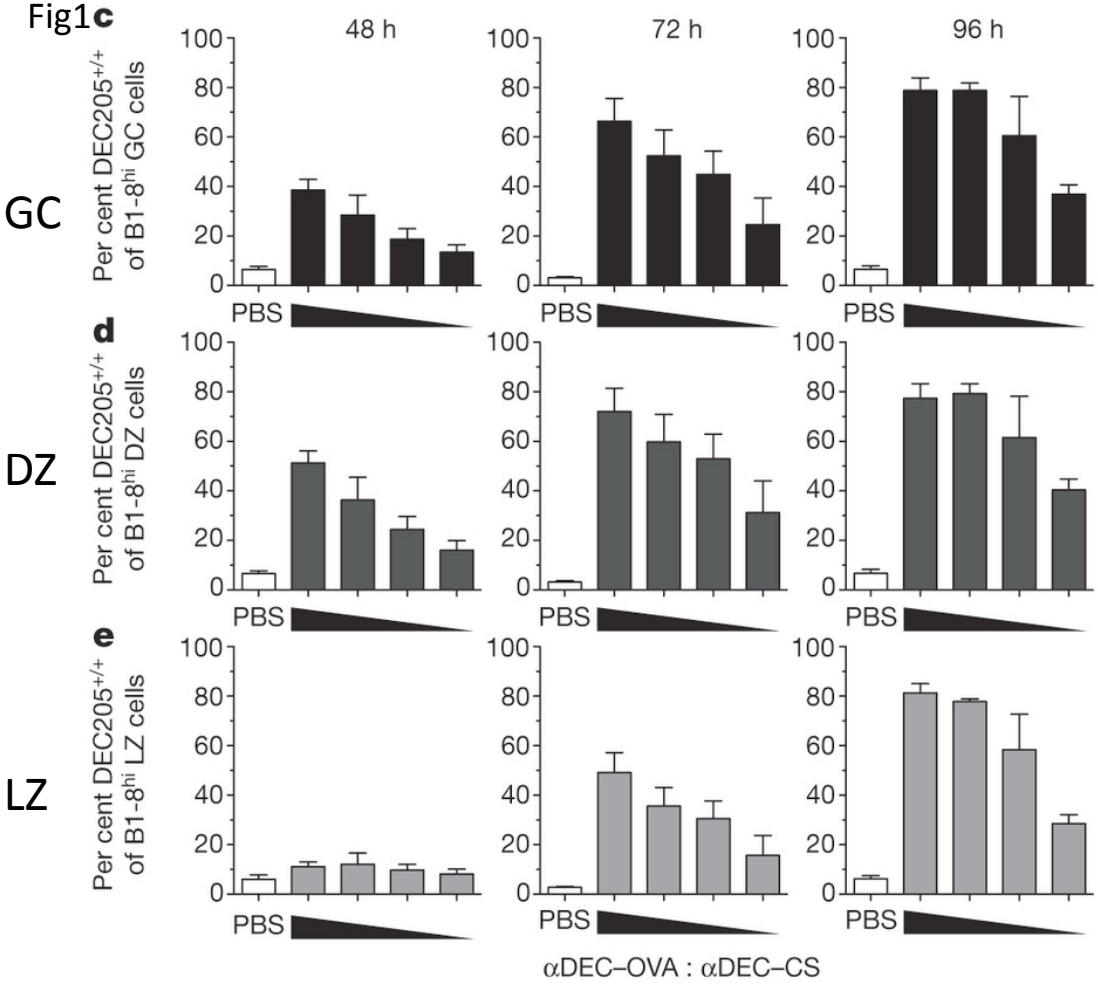


Fig1**b**



AG amount and capture regulates GC B cell expansion



GC — $B220^+$ FAS^+

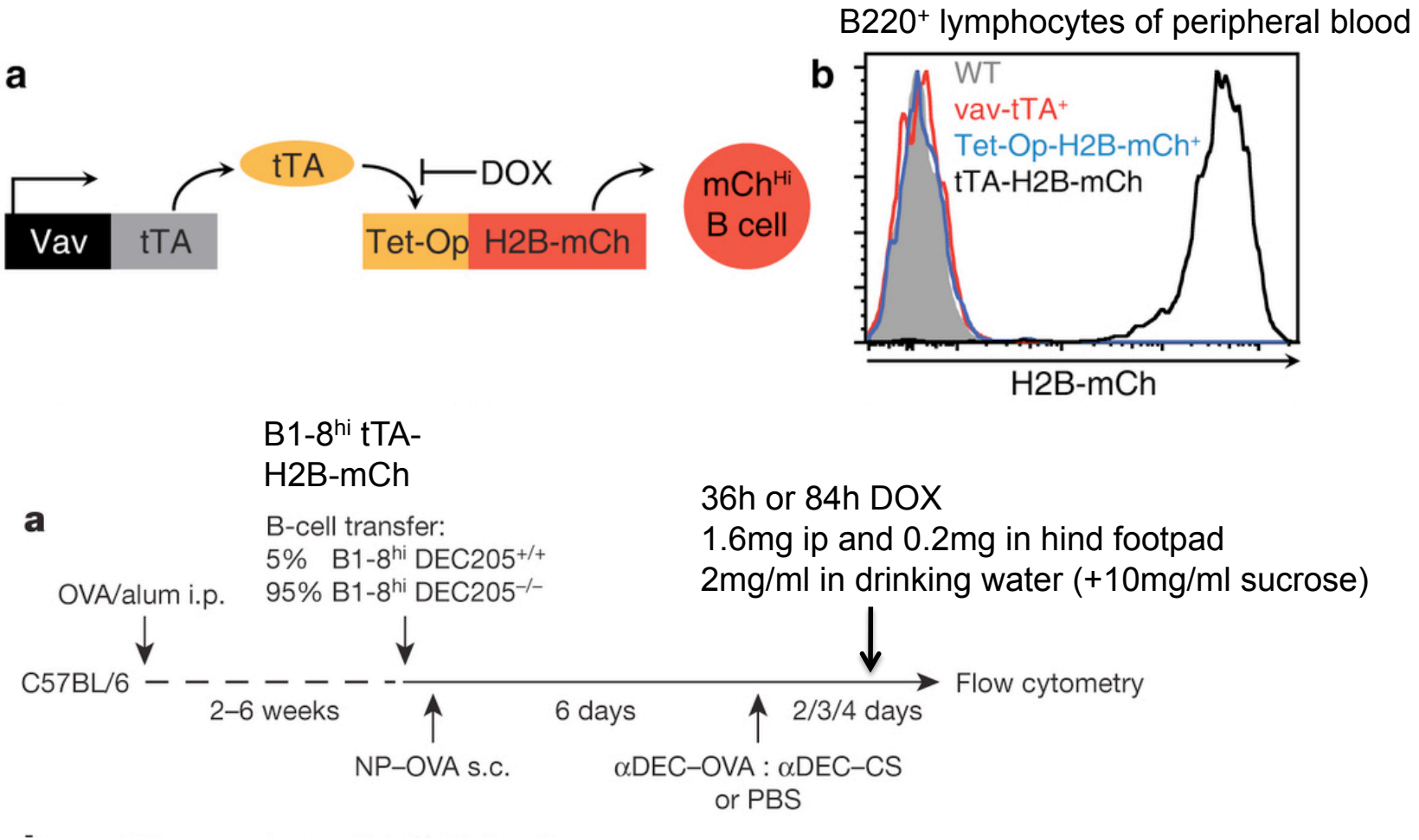
DZ — $B220^+$ FAS^+ $CD38^-$ $CD86^-$ $CXCR4^+$

LZ — $B220^+$ FAS^+ $CD38^-$ $CD86^+$ $CXCR4^-$

Increased amount of cognate AG presented by GC B cell subset to Tfh cells leads to their selective expansion at expense of GC B cells that present less AG.

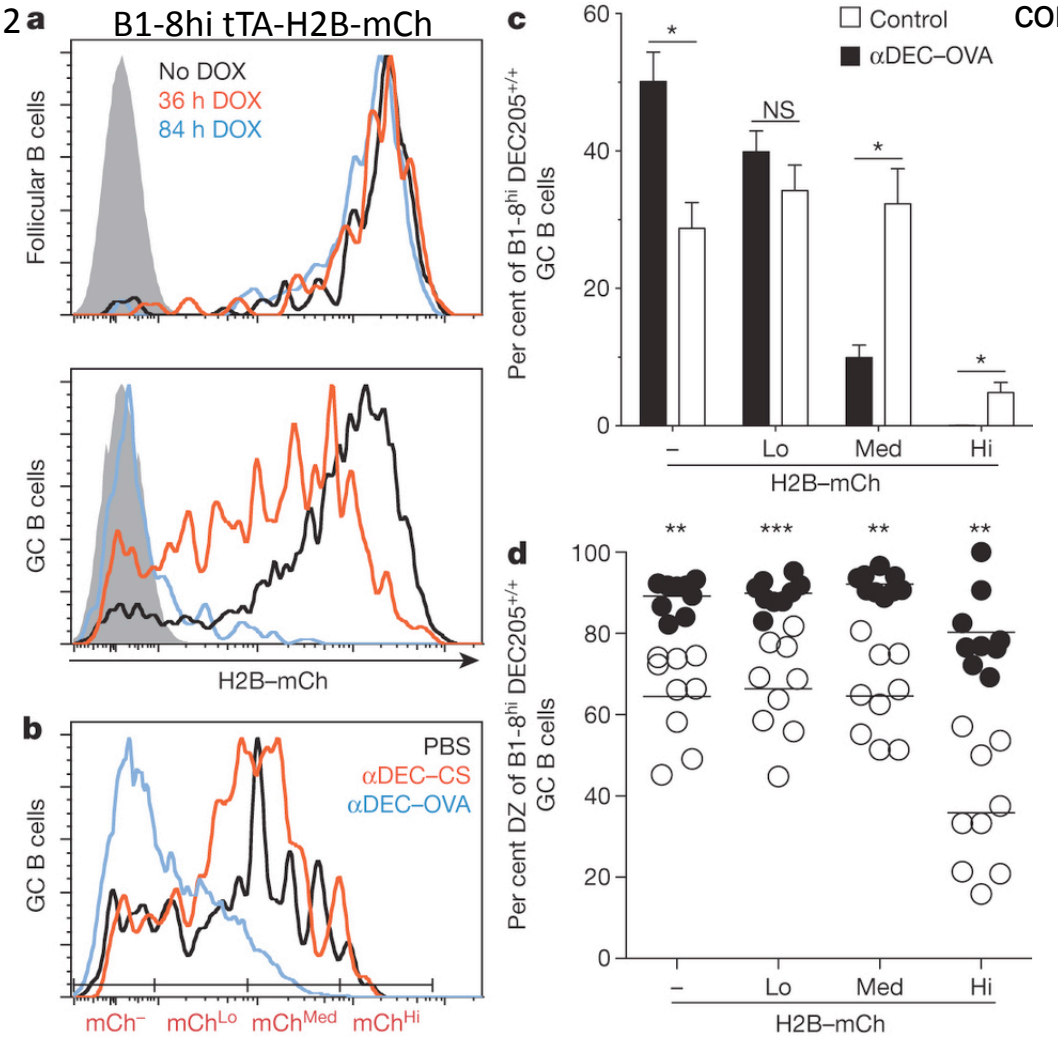
T-cell help regulates the number of GC B-cell divisions

Suppl2



T-cell help regulates the number of GC B-cell divisions

Fig 2



control=αDEC-CS and PBS

tTA-H2B-mCh can be used to monitor cell division in GC (only prime/boost, no αDEC205-OVA)

60h post DOX

72h αDEC205-Ova
36h DOX
Control cells 2:1 in DZ
αDEC205-Ova: 90% DZ

⇧AG capture + presentation➔ ⇧cell division by GC B-cells.
Change in zonal distribution: aDEC-OVA targeted GC B cells almost exclusively in DZ.

Higher AG capture increased S phase initiation in DZ of GC B-cells

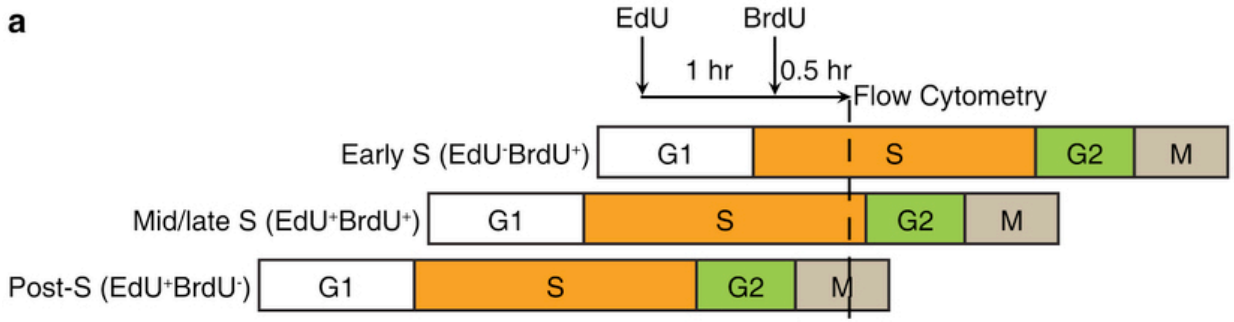
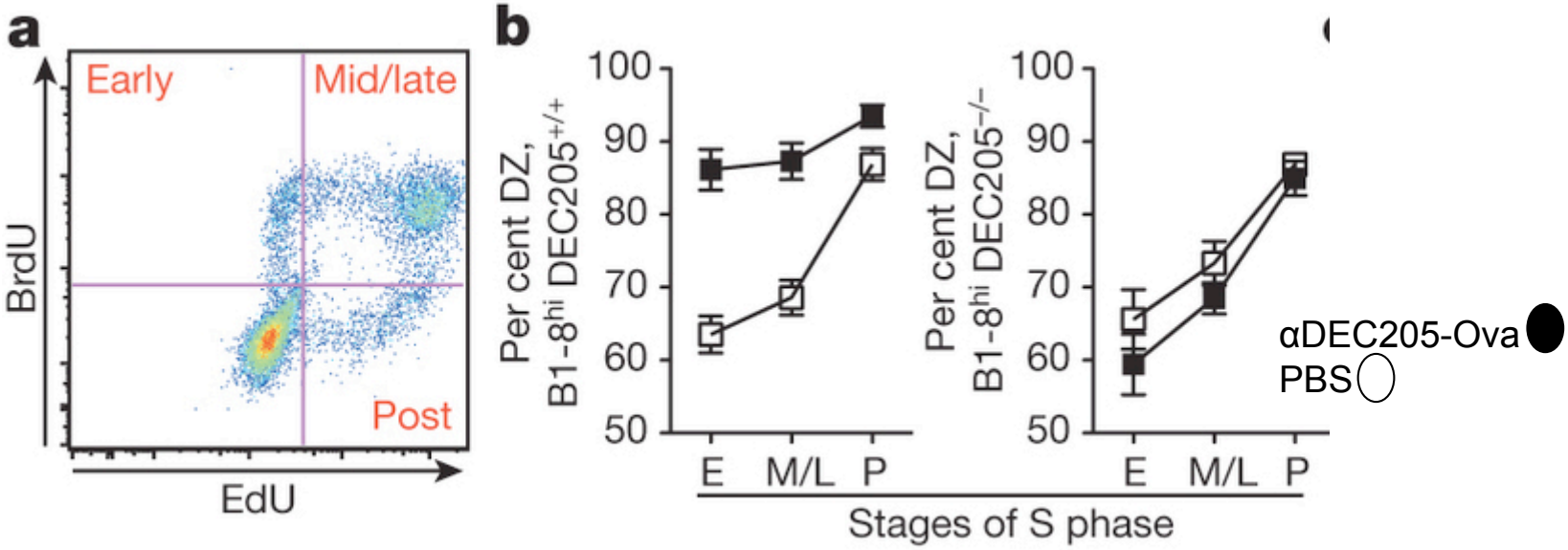


Fig 3



⇧AG captured and presented to T_{FH} ➔ ⇧proportion of cells initiating S phase in DZ.
GC B cells that express ⇧⇧ AG-> initiate additional cell divisions in DZ before returning to LZ.

Longer DZ residence time during selective expansion of GC B-cells

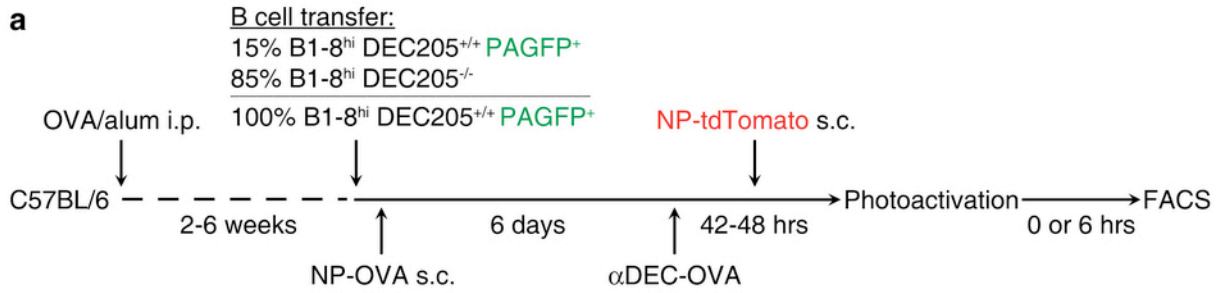
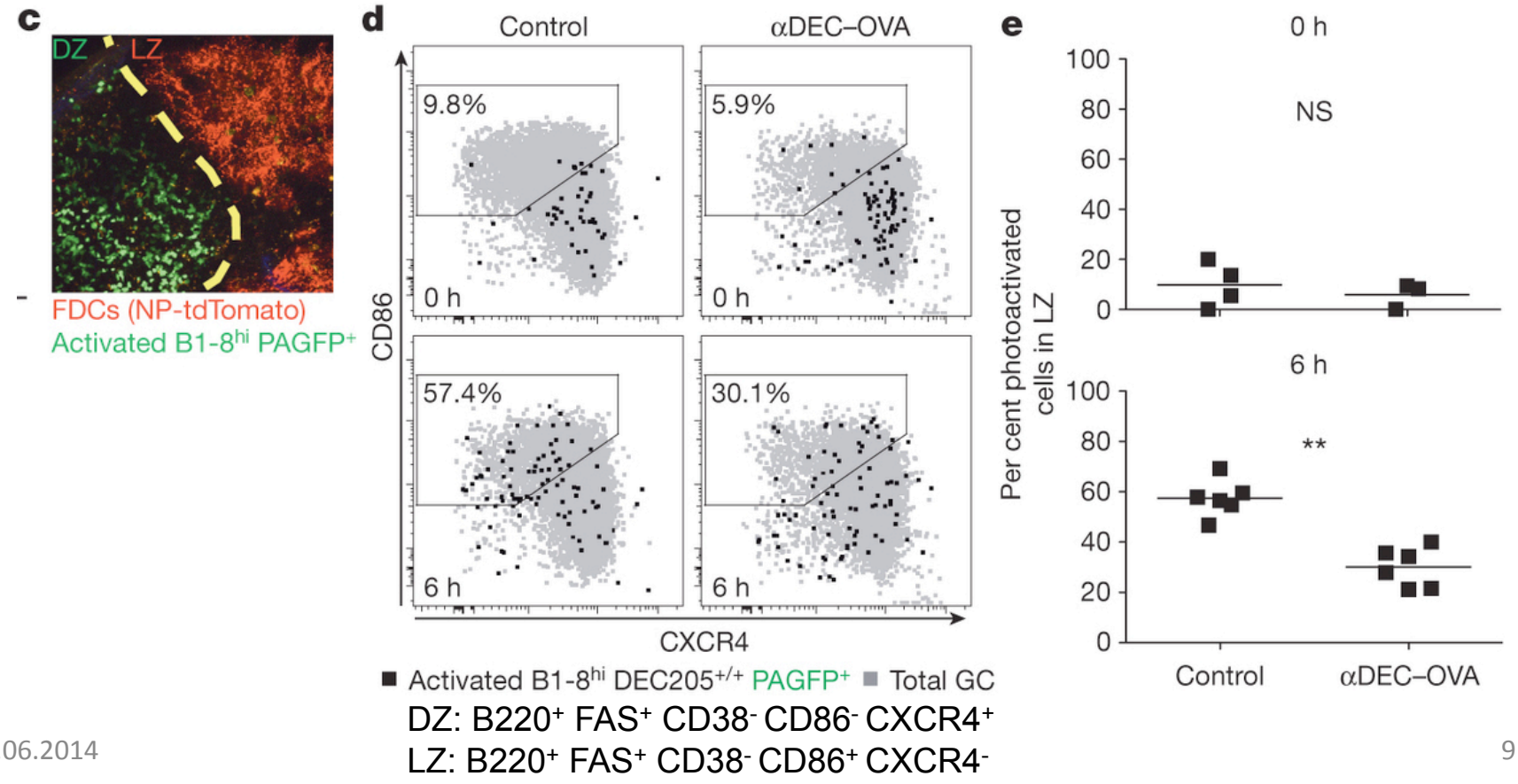


Fig 3



DZ: B220$^+$ FAS$^+$ CD38$^-$ CD86$^-$ CXCR4$^+$

LZ: B220$^+$ FAS$^+$ CD38$^-$ CD86$^+$ CXCR4$^-$

Increased cell division in polyclonal GCs -> ⇧Ig affinity and SHM

tTA-H2B-mCh

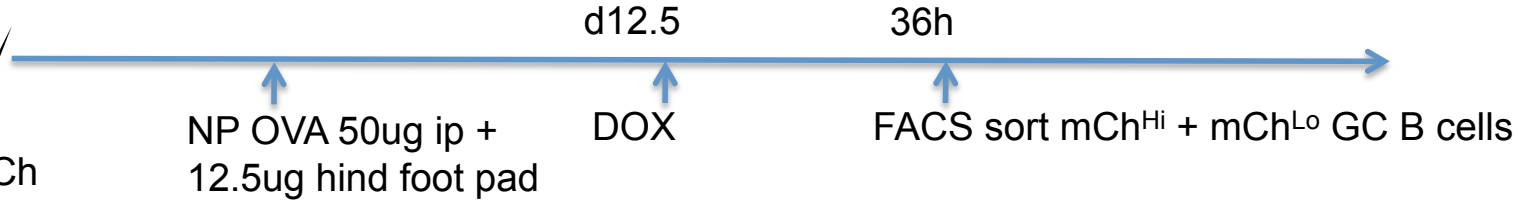
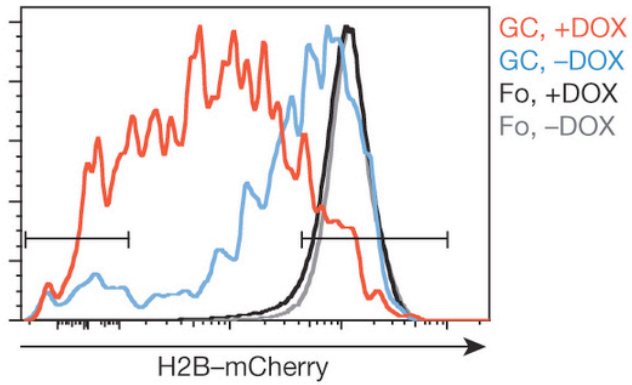
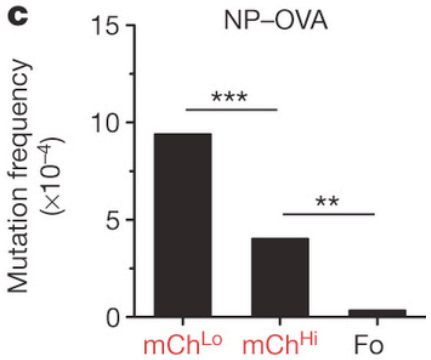
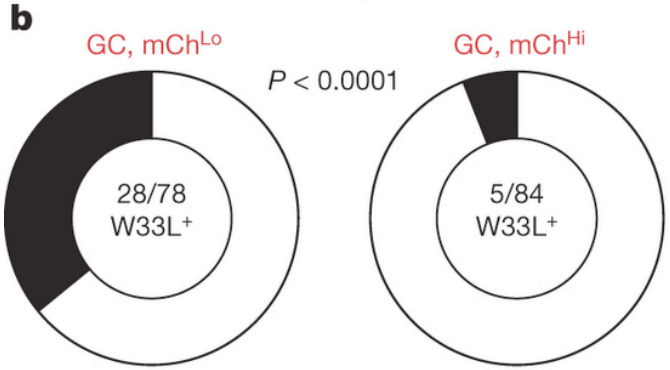


Fig 4







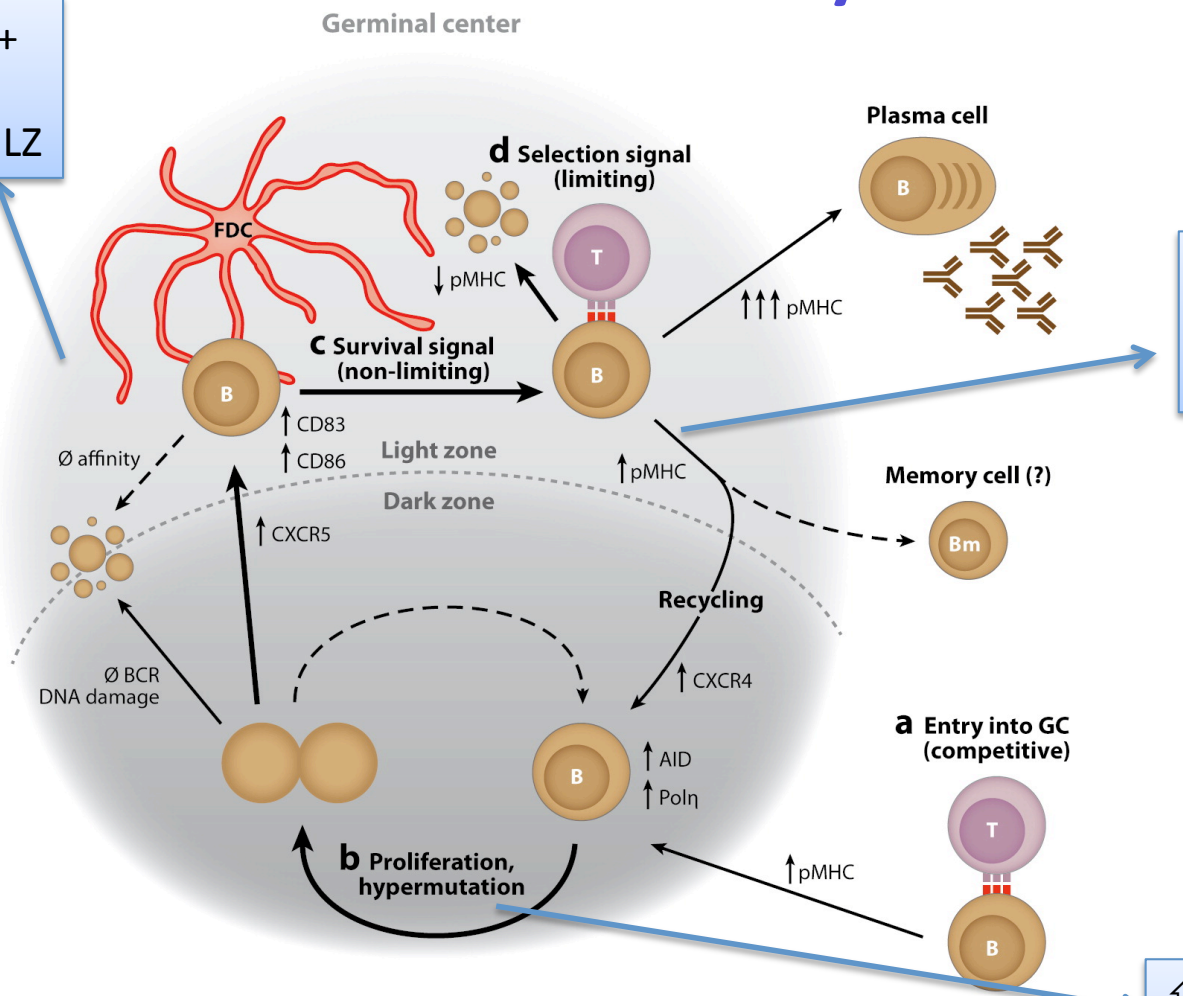
VH 186.2 family genes analysed for high affinity anti-NP W33L mutation

Analyze intron downstream of JH4 for SHM (region is target for SHM but not subject to selection)

⇧ rates of proliferation and ⇧mutation rate of high-affinity GC B cells in a polyclonal response.

Summary

least proliferative + lowest affinity B cells are mainly in LZ



⇧AG captured -> ⇧ T cell help to return to DZ

⇧AG captured -> ⇧cell division-> ⇧SHM (⇧AG affinity)

variable GC B cell divisions per DZ cycle (1-6) -> regulated by amount of AG captured in LZ (high affinity clones outcompete low affinity clones)

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Background

- At 20 weeks of gestation T cells in fetus begin to colonize the periphery (blood, secondary lymphoid organs.)
- So far the neonatal T cell compartment was supposed to only contain naive T cells (T_N) and T_{reg} for feto-maternal tolerance (tolerant to noninherited maternal AG ->NIMA).
- Fetal T cell compartmet was thought to be devoid of memory T cells T_{EM} (adults: 50% of T cells in blood are T_{EM})
- The placenta harbors a nonpathogenic microbiota and is not sterile as previously thought. *Aagaard, K., et al Science Translational Medicine, 6(237), 237ra65–237ra65*

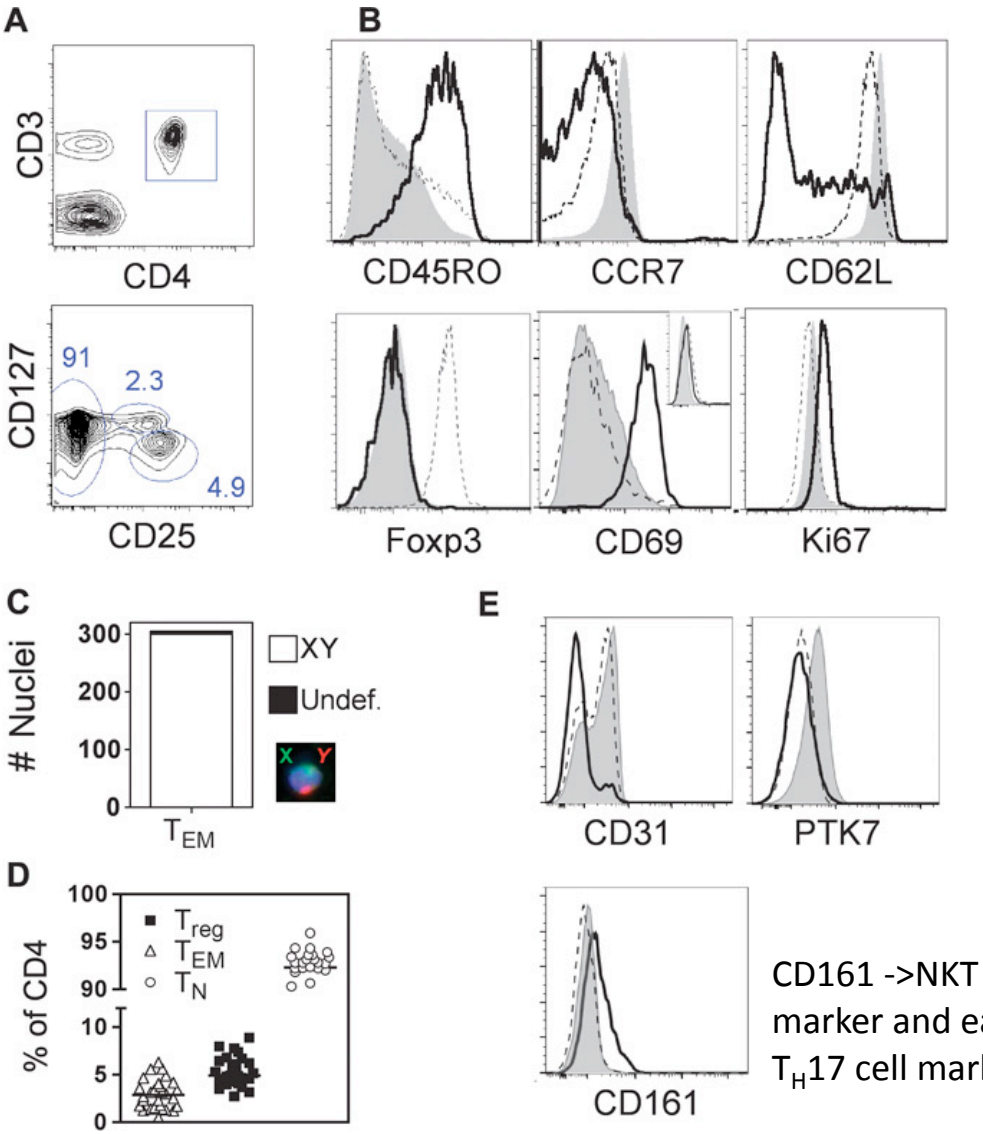
Cord blood contains CD4 T_{EM} cells

T_n $CD25^{-}CD127^{hi}$

T_{reg} $CD25^{+}CD127^{lo}$

activated Tcells $CD25^{lo}CD127^{lo}$

$CD25^{lo}CD127^{hi}CD3^{+}CD4^{+}$
-> no maternal oringin



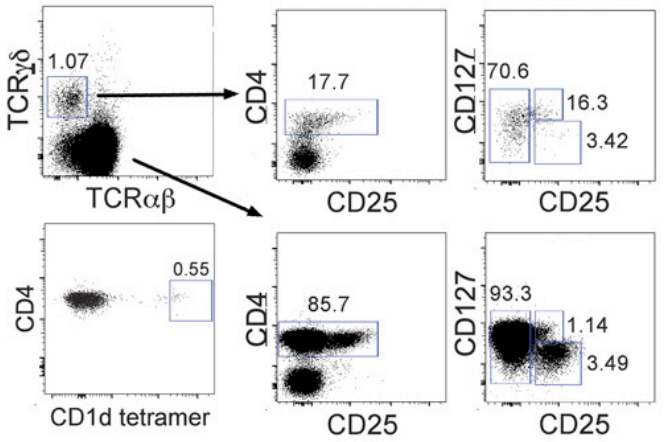
maturation associated molecules

no recent thymic emigrants

CD161 ->NKT cell marker and early T_H17 cell marker

Neonatal T_{EM} are TCRαβ T cells with a polyclonal TCR repertoire

Fig2 **A**



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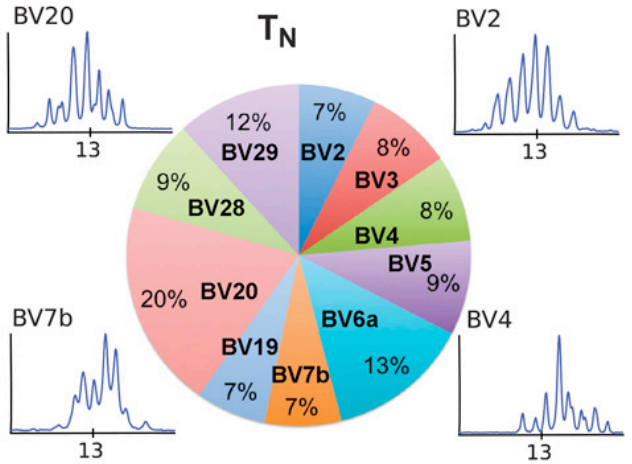

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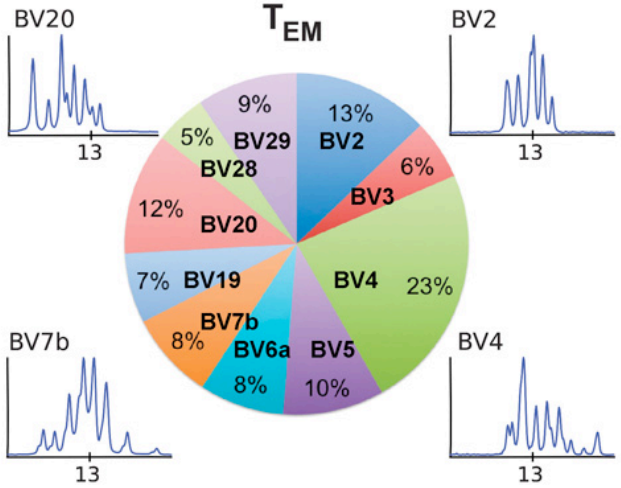

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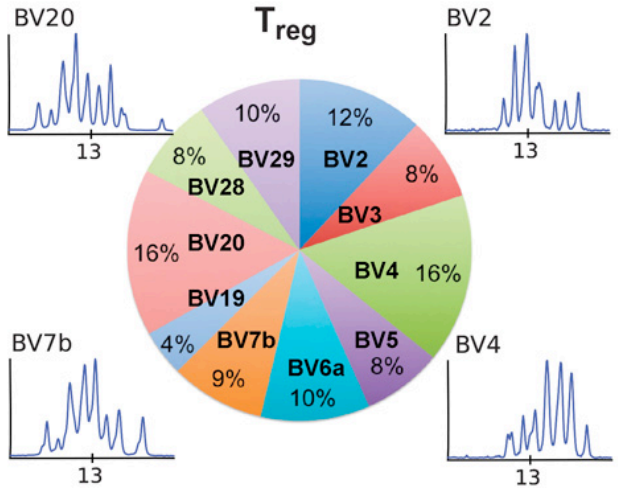
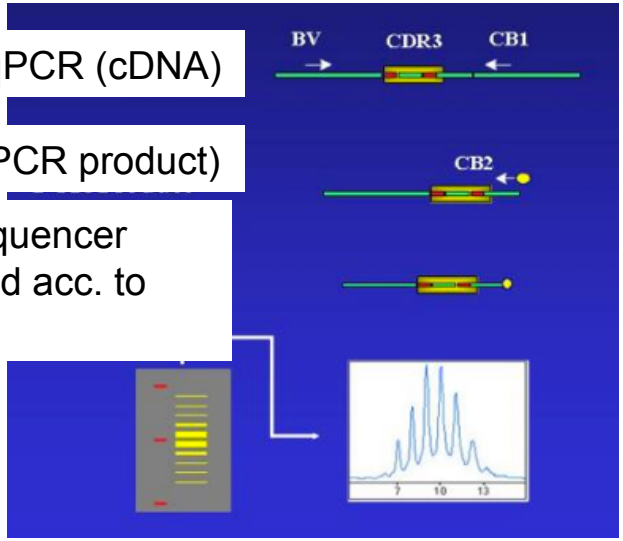


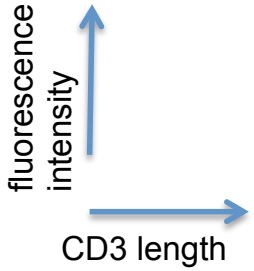
Neonatal T_{EM} are TCRαβ T cells with a polyclonal TCR repertoire

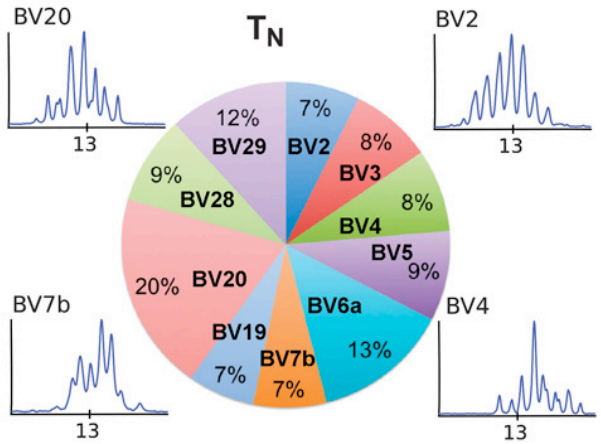
qPCR (cDNA)

Run Off (PCR product)

DNA Sequencer separated acc. to length







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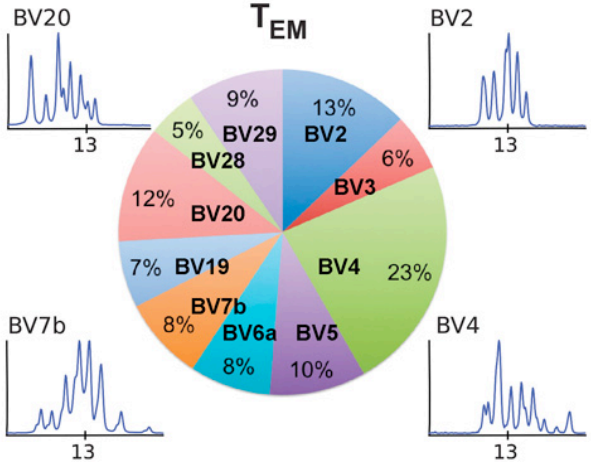

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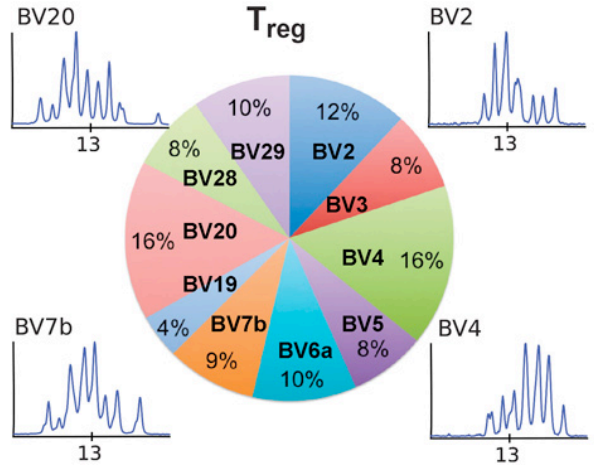


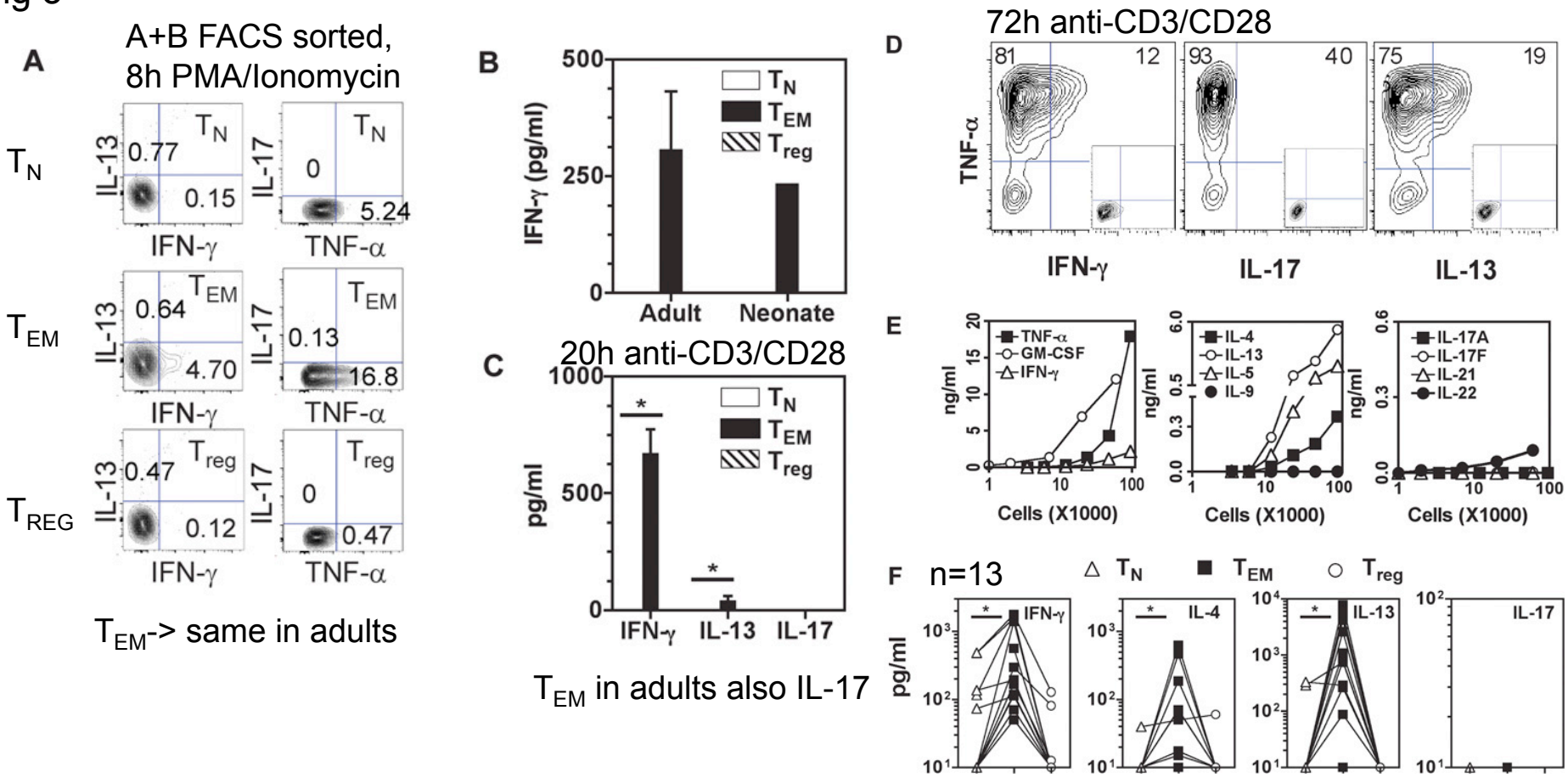
TCRBVb repertoire analysis

+Immunoscope technology for CD3 length

TCRBV germline genes cluster in 24 families according to level of homology-> they selected 10 for analysis (ca 70% of adult T cell repertoire). Wide expression of 10 Vb genes and variable CDR3 region-> polylonal TCR repertoire.

Neonatal T_{EM} display T_H1 and T_H2 functions

Fig 3



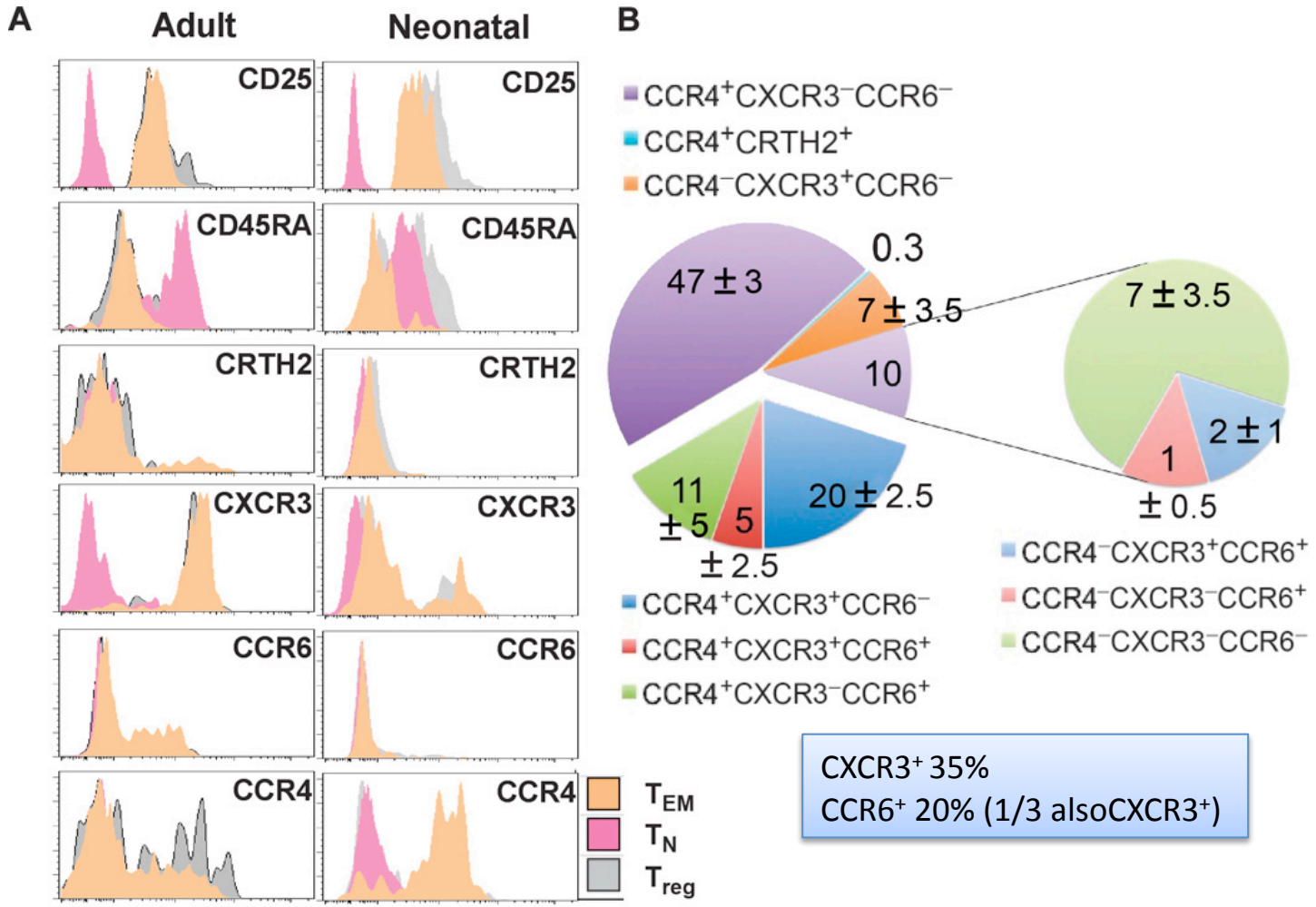
T_{EM}-> same in adults

T_{EM} in adults also IL-17

T_{EM} cells can readily secrete T_H1/T_H2 cytokines. Under the same conditions this was not true for T_{reg} and T_N cells

Chemokine receptor analysis shows a large variety of neonatal CD4 T cells

Fig4



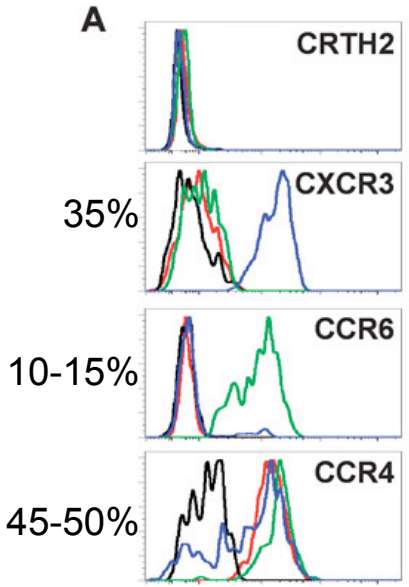
- CXCR3 on T_H1 cells
- CRTH2 onT_H2 cells
- CCR6 on T_H17 cells
- CCR4 not specific for T_H2 but associated with CRTH2 in adults

CXCR3⁺ 35%
CCR6⁺ 20% (1/3 alsoCXCR3⁺)

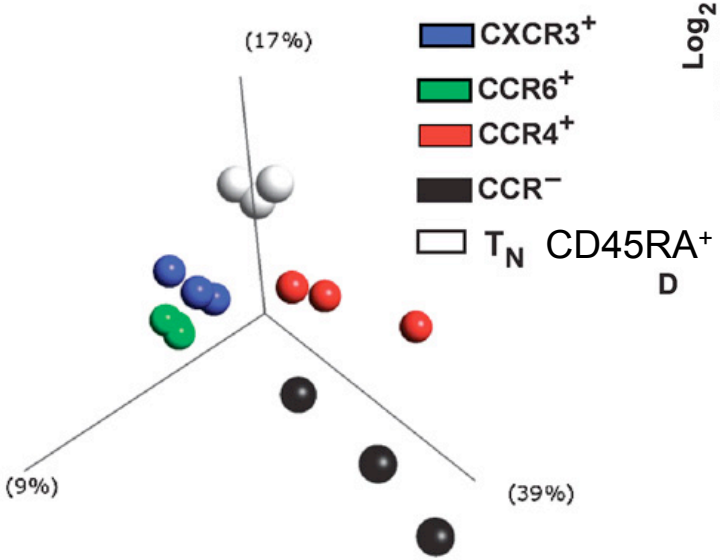
9 combinatorial phenotypes of chemokine receptors in neonates. Chemokine rec. expression confirms T_H1 population but does not discriminate other T_H functions.

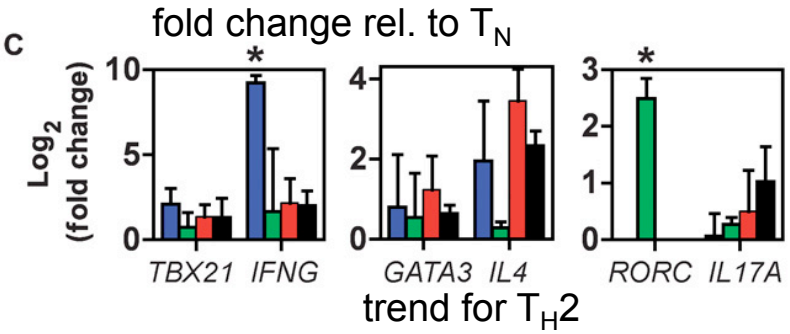
Chemokine receptor expression pattern defines molecularly different cord blood T_{EM} subsets

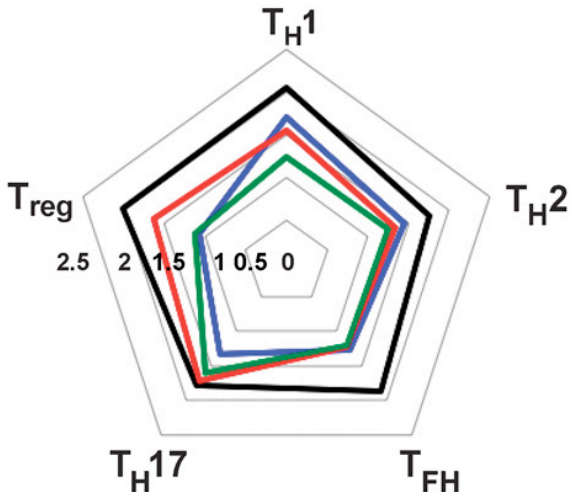
Fig 5 n=3 male donors



sequential gating strategy for different chemokine rec.
Rare CRTH2 population was not included





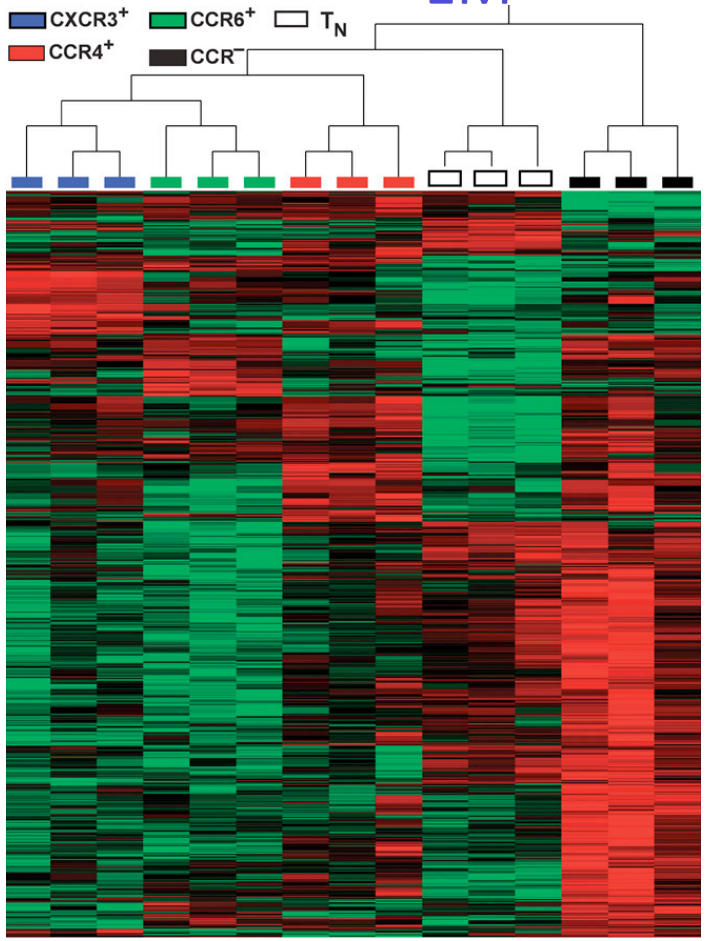


Functional studies (before) show Th1 and Th2 functions of T_{EM}.
Microarray analysis of four T_{EM} phenotypes, show close affiliation of $CXCR3^+$ T_{EM} to T_H1 and $CCR6^+$ to T_H17.

Microarray analysis discriminates between different T_{EM} subsets

24h anti-CD3/CD28

Fig 6

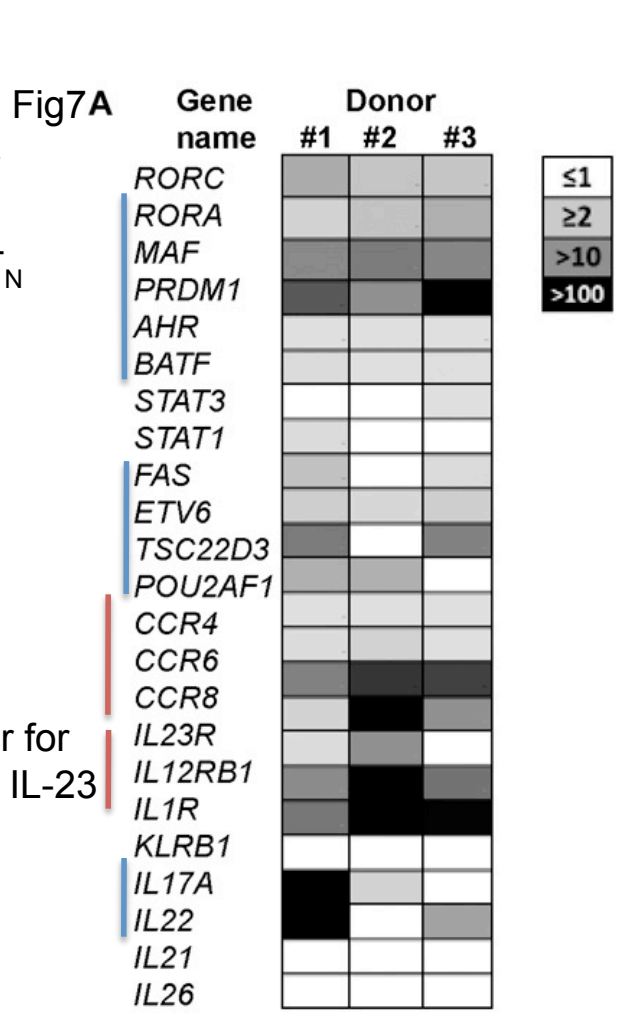


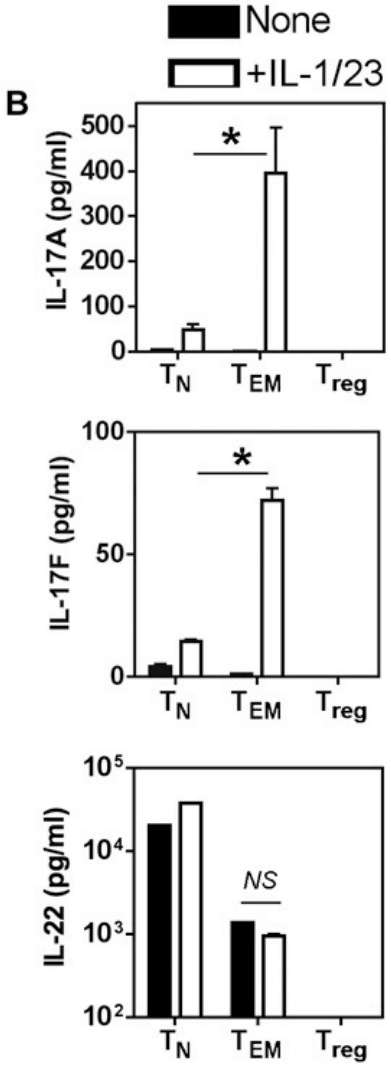
Distinct gene clusters between T_{EM} subgroups. CCR^- uplregulate several genes-> potentially still capable of acquiring different phenotypes (intermediate between T_N and T_{EM}?).

Assessment of T_H17 potential for CCR6$^+$ T_{EM} cells

fold change of CCR6$^+$ T_{EM} compared to T_N

Receptor for IL-1 and IL-23





4-6d
anti-CD3/CD28 +/- IL-1 and IL-23

IL-22 secretion indep. of IL-1 and IL-23-> indicates no association with T_H17 response.

Neonatal T_{EM} cells can develop into T_H17 cells.

Discussion

- Identification of memory type CD4 T cells in neonatal cord blood ($CD25^{lo}CD127^{hi}$), 1-3% of total CD4 T cells.
- Functional studies: Upon activation T_H1 and T_H2 like functions and also potential for T_H17 when stimulated with IL-1 and IL-23.
- Microarray: CXCR3+ T_{EM} cells express IFNγ transcripts-> inflammatory T_H1 cells early in life w/o infection at steady state.
- $CCR6^+$ T_{EM} cells express T_H17 related genes w/o the secretion of IL-17
- Neonatal T_{EM} are highly diverse.
- Do T_{EM} develop in response to maternal AG? -> no response to NIMA (non inherited maternal AG) in vitro.
- Do T_{EM} develop in response to mild/asympomatic infection or vaccination of mother?
- Signal for immune maturation from commensal bacteria in placenta?
- Antigen Specificity/Self-reactivity?-> origin and role of those cells? Role in Vaccines and infection?