Understanding Human B cell Effector Pathways

Implications for Autoimmune, Infectious and Vaccination Responses

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EMORY
UNIVERSITY

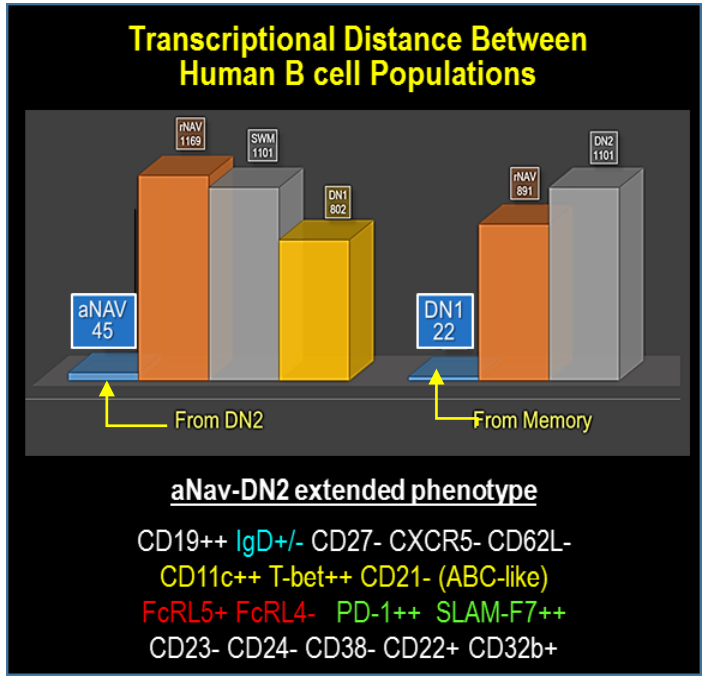
No conflicts

Objectives

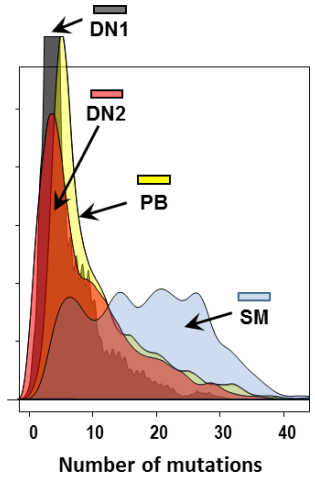
1. Discuss the diversity and characterization of human B cell differentiation pathways. Differentiation between memory and effector responses
2. Characteristics of B cell responses and repertoire features in severe COVID-19 infection
3. Molecular regulation of abnormal B cell responses in SLE

Cellular Components and Phenotypic Definition of the Human Extra-Follicular B cell Pathway

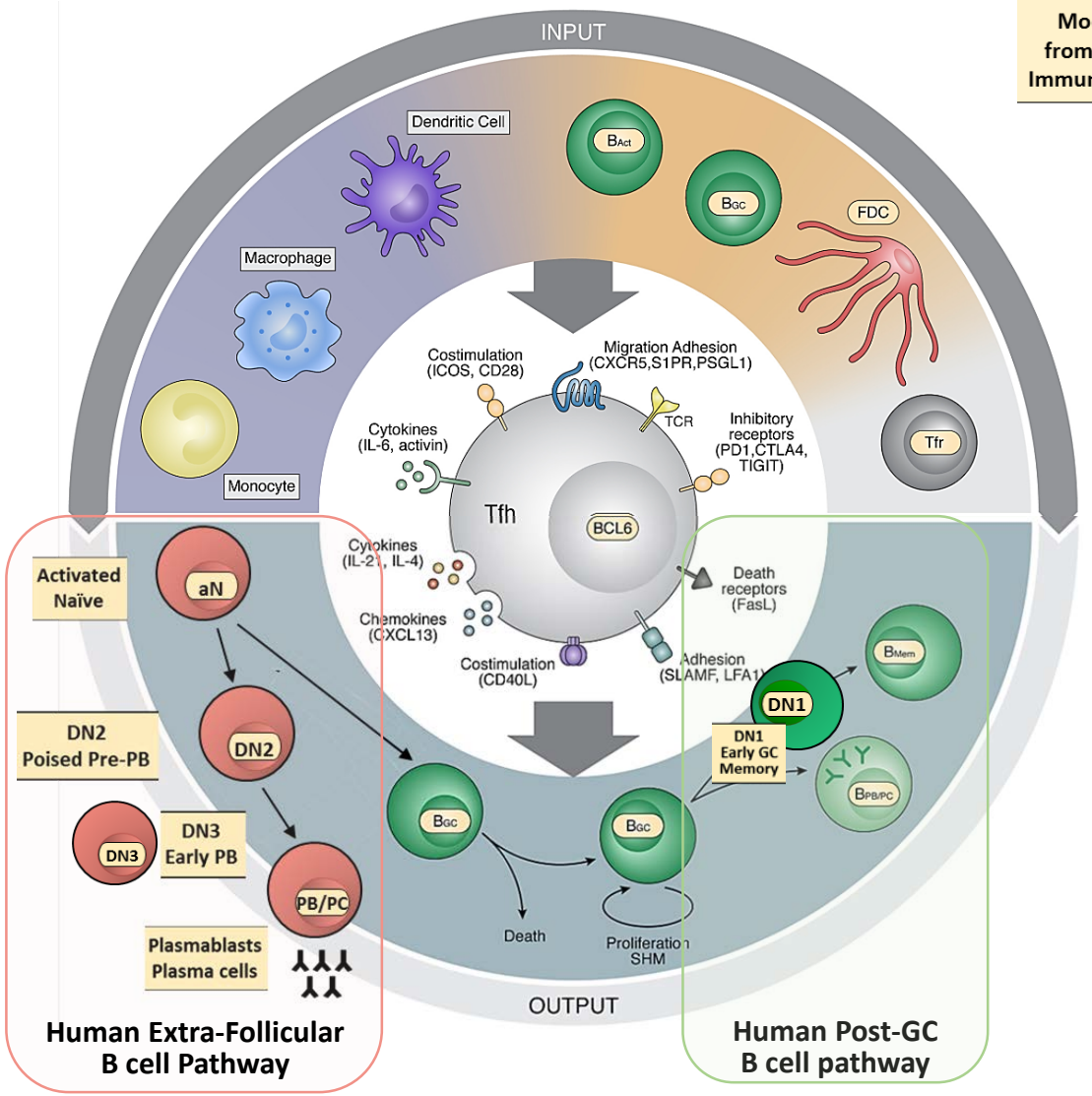
- **Activated naïve B cells (aN) and DN2 cells are very closely related to DN2 cells (45 DEGs)**
- Switched memory and DN1 are almost identical (22 DEGs)



- **DN2 are isotype-switched and somatically mutated**
  - SHM in DN2 << SM
  - SHM in PB << SM (in SLE)
- **Isotype switch and/or SHM should not be used to assign a memory phenotype**
- The EF pathway is major contributor to PB expansions in SLE



Modified from Crotty Immunity 2019

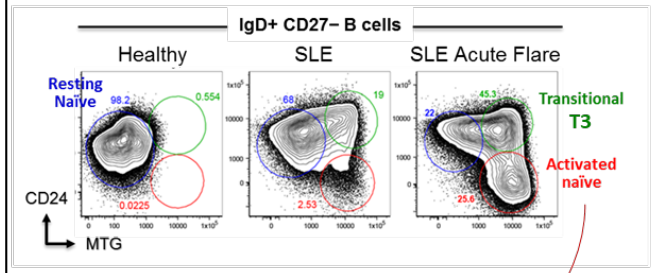


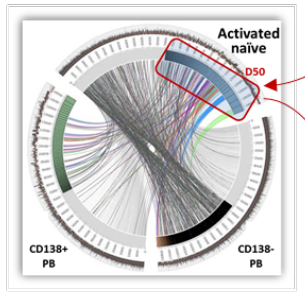
Tipton et al. Nat Immunol 2015; J enks et al. Immunity 2018; Scharer et al. Nat Immunol 2019

The extra-follicular pathway in human SLE

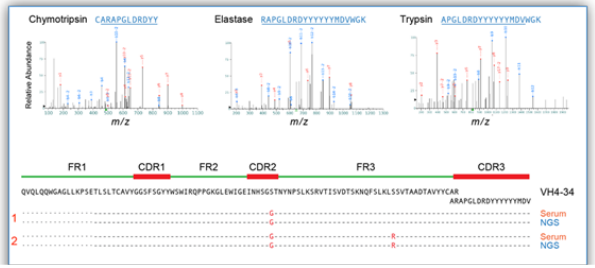
Naïve B cell activation in SLE

Contribute to large ASC expansions





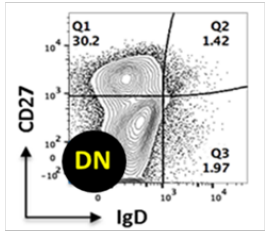
Responsible for dominant serum autoantibodies



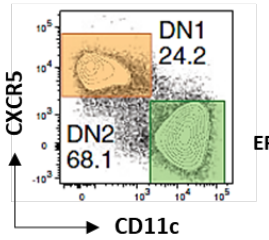
Tipton et al. Nat Immunol. 2015

IgD- CD27- (DN) B cells in SLE

Anolik. A&R 2004 Wei. JI 2007

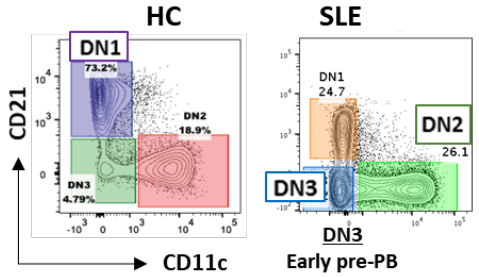


DN2 B cells



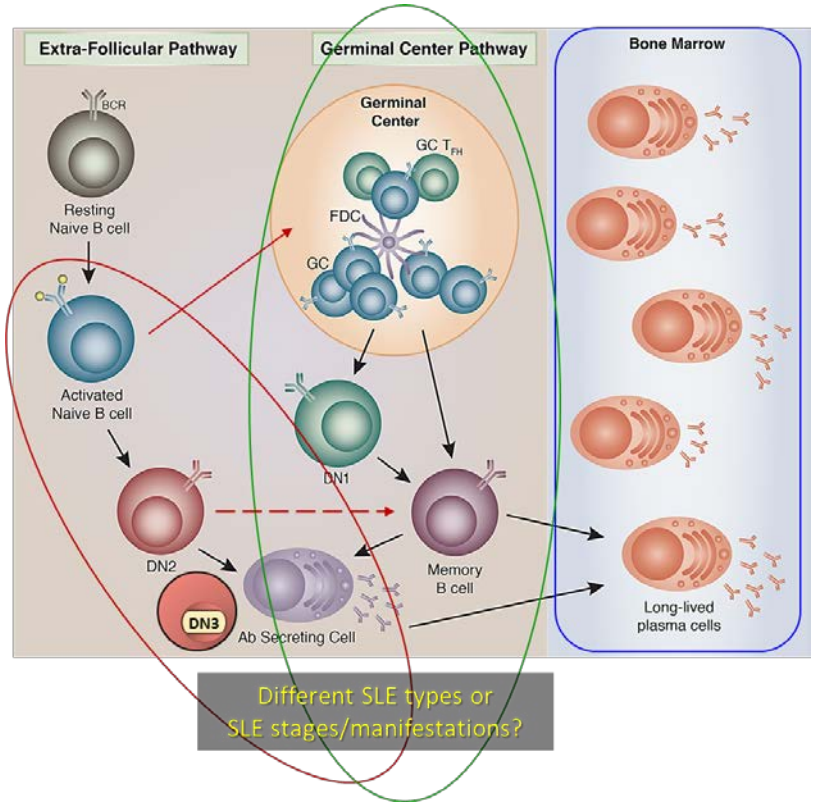
DN2
EF Effectors primed to diff. into PB

Jenks. Immunity 2018; Scharer. Nat Immunol 2019

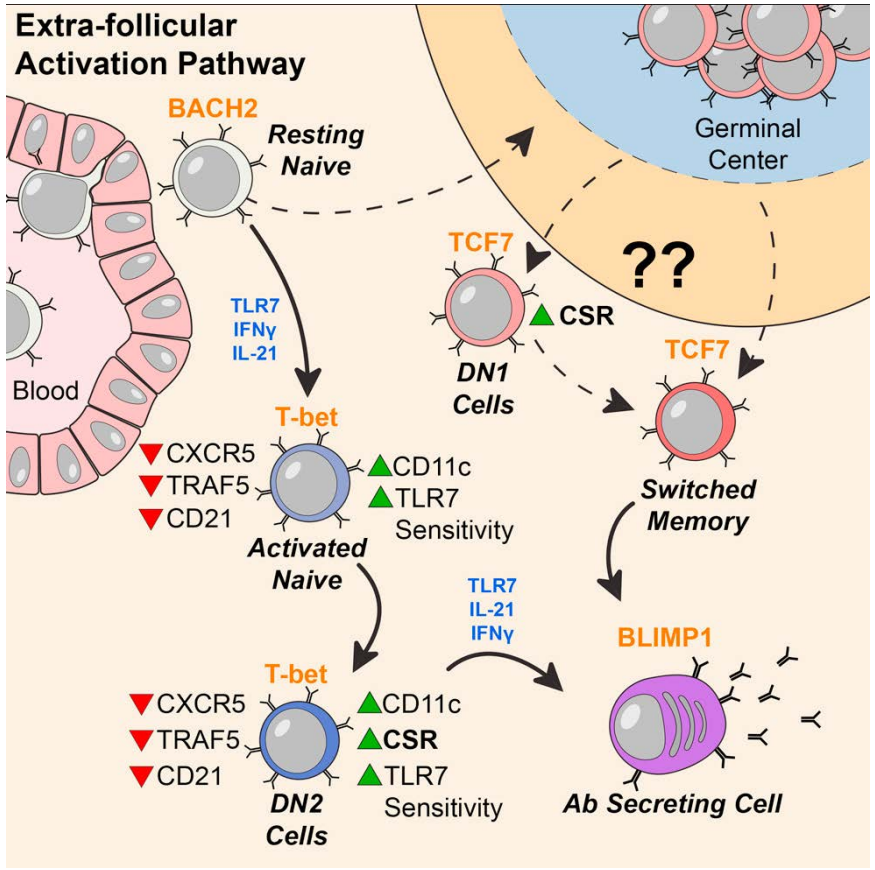


Woodruff, Nat Immunol 2020; Cashman. In preparation

Different pathways contribute to B cell hyperactivity in SLE



The extra-follicular pathway in human SLE




Chris Tipton


Kevin Cashman


Scott Jenks


Esther Zumaquero


Chris Scharer

- Activated naïve B cells → DN2 → Plasmablasts
  - CXCR5- CD11c++ CD21 - T-bet++ FcRL5++ SLAM-F7++ PD1+
  - SLE DN2 cells are deficient in the expression/function of regulatory receptors: A20, TRAF5, CD22 → Ets1
  - Increased viral RNA sensors: TLR7, IFIH1; dsDNA sensors: TRIM56 (STING inducer)
- DN2 cells are epigenetically primed to differentiate into antibody-secreting PB
- Naïve → DN2 cells are responsible for a large autoantibody fraction in flaring SLE
- TLR7-induced, IFNγ and IL-21-dependent
- Hyper-responsive to TLR7 (TRAF5 deficiency), IL-10 and IFNλ but not to CD40L (TRAF5 deficiency)
- Produce anti-RNA and RNA-binding protein antibodies: Ro, Smith/RNP
- Differentiation from naïve B cells in inhibited by IL-4
- Highly enriched in:
  - **African-American**
  - **Active SLE and Lupus Nephritis**
  - **Poor disease outcome**
  - Anti-RNA and RBP serum autoantibodies

Tipton et al. Nat Immunol 2015; Jenks et al. Immunity 2018; Scharer et al. Nat Immunol 2019; Woodruff et at. Nat Immunol 2020

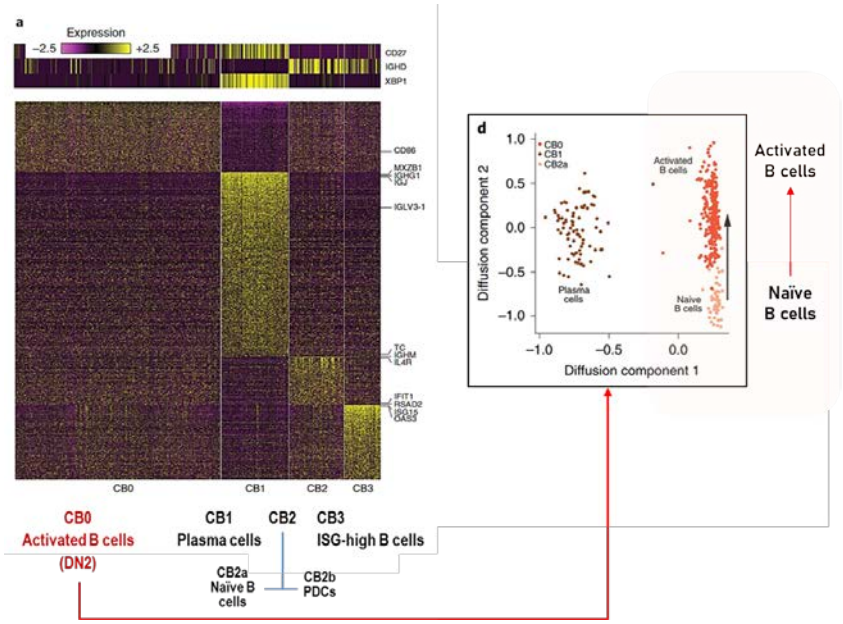
The relevance of extrafollicular naïve B cell differentiation in autoimmunity and infection

nature immunology

Resource | Published: 17 June 2019

The immune cell landscape in kidneys of patients with lupus nephritis

Arnon Arazi, Deepak A. Rao, [...] the Accelerating Medicines Partnership in SLE network



Why are naïve B cells important and competitive with pre-established autoimmune memory cells?

Continuous recruitment of naive T cells contributes to heterogeneity of antiviral CD8 T cells during persistent infection. J Exp Med, 2006

Vezys V, Masopust D, Kemball CC, Barber DL, O'Mara LA, Larsen CP, Pearson TC, Ahmed R, Lukacher AE

nature 2015 immunology ARTICLES

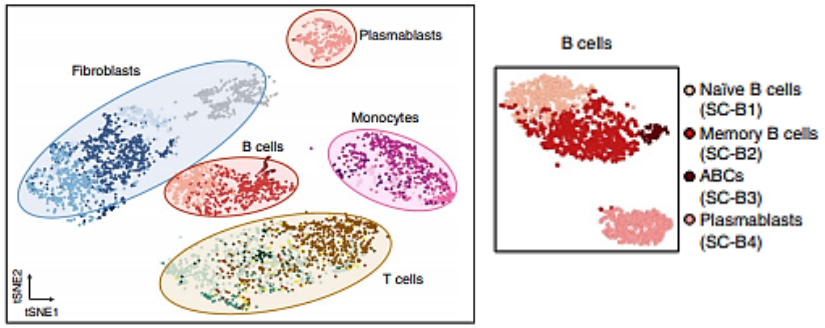
Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus

Christopher M Tipton[1], Christopher F Fucile[2], Jaime Darce[3], Asiya Chida[1], Travis Ichikawa[1], Ivan Gregoretti[3], Sandra Schieferl[3], Jennifer Hom[1], Scott Jenks[1], Ron J Feldman[4], Ramit Mehr[5], Chungwen Wei[1], F Eun-Hyung Lee[6], Wan Cheung Cheung[7,8], Alexander F Rosenberg[2] & Iñaki Sanz[1]

RESOURCE nature immunology

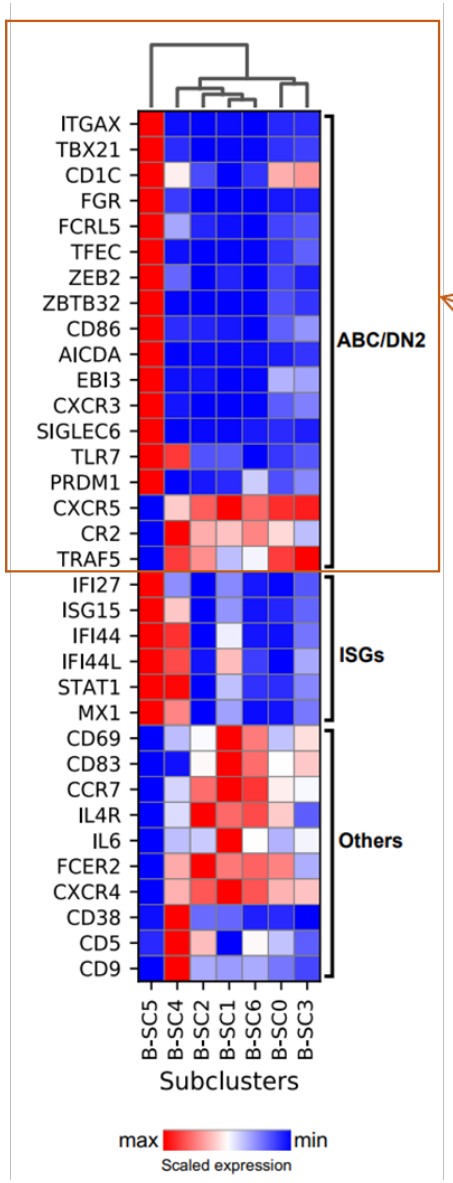
Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry

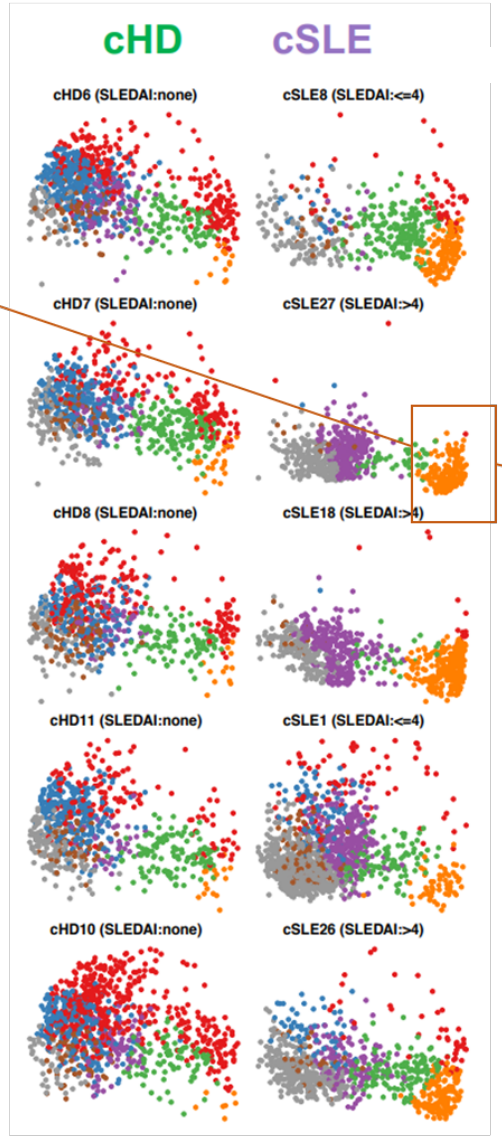
Fan Zhang[1,2,3,4,5,27], Kevin Wei[1,27], Kamil Slowikowski[1,2,3,4,5,27], Chamith Y. Fonseka[1,2,3,4,5,27], Deepak A. Rao[3,27], Stephen Kelly[6], Susan M. Goodman[7,8], Darren Tabechian[9], Laura B. Hughes[10], Karen Salomon-Escoto[10], Gerald F. M. Watts[5], A. Helena Jonsson[5], Javier Rangel-Moreno[9], Nida Meednu[9], Cristina Rozo[11], William Apruzzese[3], Thomas M. Eisenhaure[4], David J. Lieb[4], David L. Boyle[12], Arthur M. Mandelin II[14], Accelerating Medicines Partnership Rheumatoid Arthritis and Systemic Lupus Erythematosus (AMP RA/SLE) Consortium[15], Brendan F. Boyce[16], Edward DiCarlo[17], Ellen M. Gravallese[18], Peter K. Gregersen[19], Larry Moreland[20], Gary S. Firestein[21], Nir Hacohen[4], Chad Nusbaum[4], James A. Lederer[22], Harris Perlman[14], Costantino Pitzalis[23], Andrew Filer[22,23], V. Michael Holers[24], Vivian P. Bykerk[7,8], Laura T. Donlin[8,12,25], Jennifer H. Anolik[9,25,26], Michael B. Brenner[4,28] and Soumya Raychaudhuri[1,2,3,4,5,26,28]*

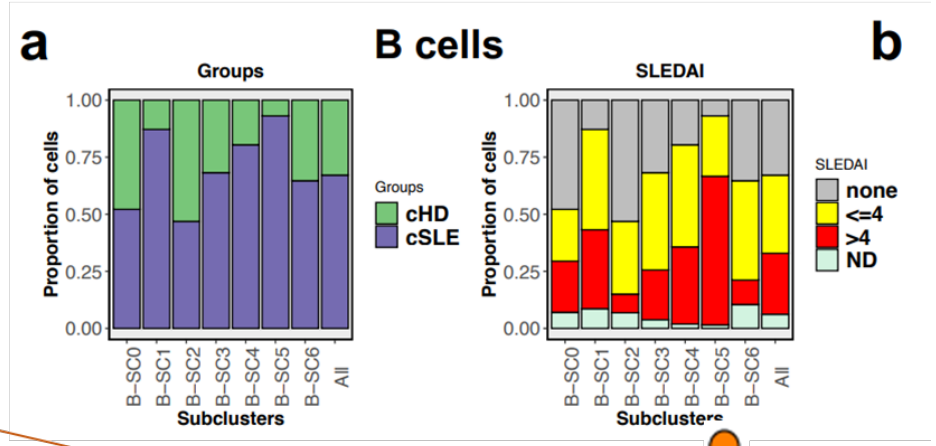


- High % of autoreactive naive B cell (30%)
  ↑↑ total numbers > autoreactive memory cells
- Powerful generation of autoreactive ASCs through an EF pathway devoid of tolerance checkpoints?
- Adept at generating GCs
- May generate long-lived memory and PC

DN2 B cells are uniquely expanded in active childhood SLE







RESOURCE
https://doi.org/10.1038/s41590-020-0743-0
nature immunology

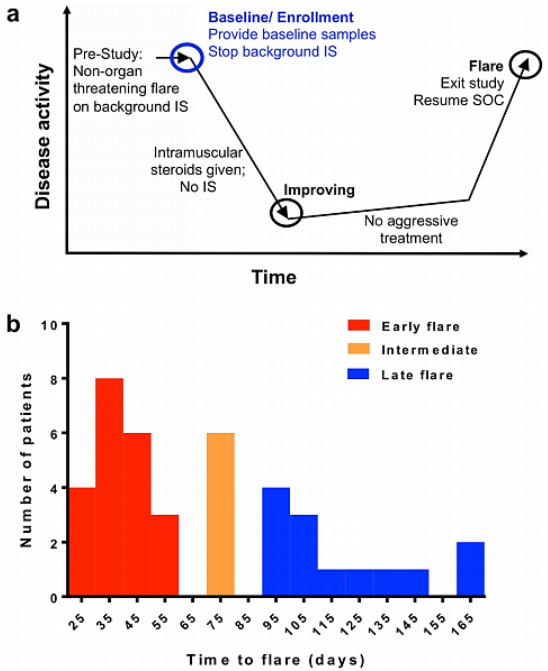
Mapping systemic lupus erythematosus heterogeneity at the single-cell level

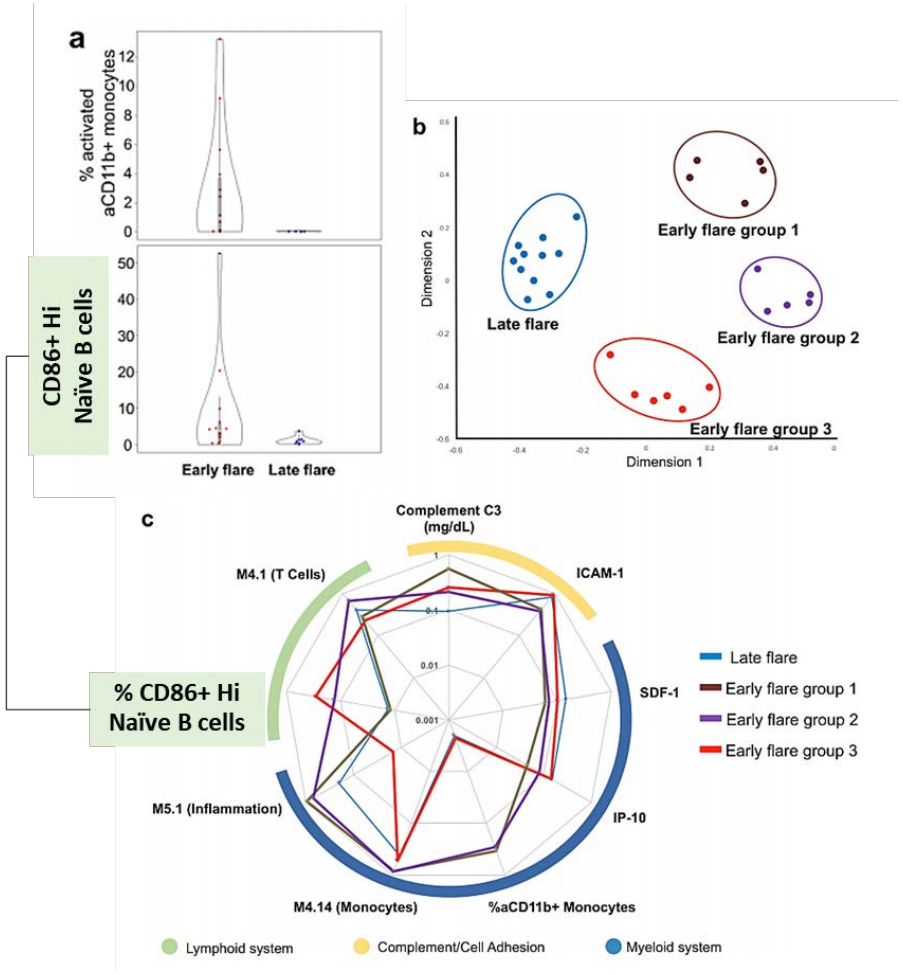
Djamel Nehar-Belaid[1,8], Seunghee Hong[2,8], Radu Marches[1], Guo Chen[1], Mohan Bolisetty[1], Jeanine Baisch[2], Lynnette Walters[3], Marilynn Punaro[3,4], Robert J. Rossi[1], Cheng-Han Chung[1], Richie P. Huynh[1], Prashant Singh[1], William F. Flynn[1], Joy-Ann Tabanor-Gayle[5], Navya Kuchipudi[5], Asuncion Mejias[6], Magalie A. Collet[1], Anna Lisa Lucido[1], Karolina Palucka[1], Paul Robson[1,7], Santhanam Lakshminarayanan[5], Octavio Ramilo[6], Tracey Wright[3,4], Virginia Pascual[2,9] ✉ and Jacques F. Banchereau[1,9] ✉

CD86 high activated naïve B cells and early Lupus flare

Immunologic findings precede rapid lupus flare after transient steroid therapy

Rufei Lu[1,2], Joel M. Guthridge[1,2], Hua Chen[1], Rebecka L. Bourn[1], Stan Kamp[1], Melissa E. Munroe[1], Susan R. Macwana[1], Krista Bean[1], Sudhakar Sridharan[3], Joan T. Merrill[1] & Judith A. James[1,2]

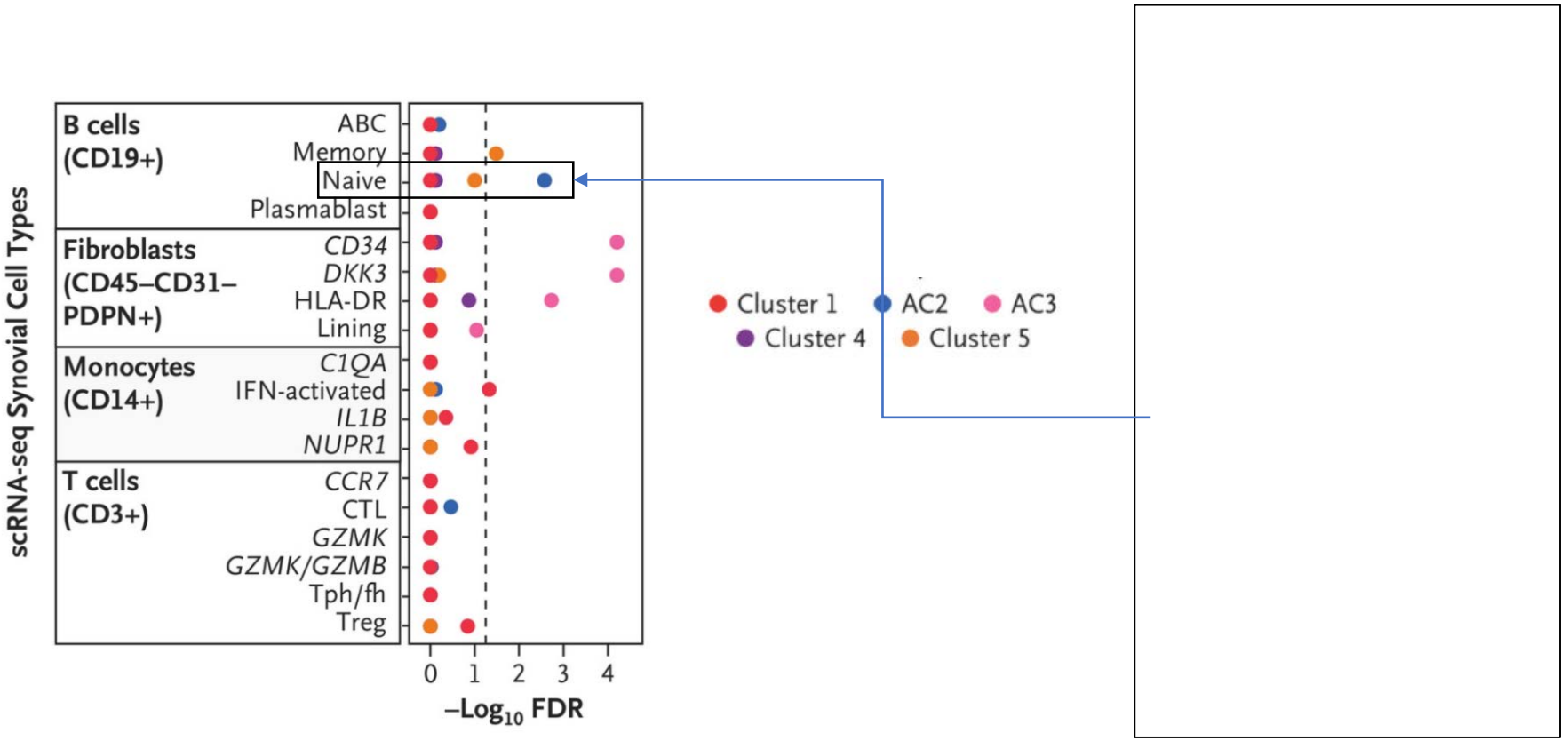




ORIGINAL ARTICLE

RNA Identification of PRIME Cells Predicting Rheumatoid Arthritis Flares

Dana E. Orange, M.D., Vicky Yao, Ph.D., Kirsty Sawicka, Ph.D., John Fak, M.S., Mayu O. Frank, N.P., Ph.D., Salina Parveen, M.A., Nathalie E. Blachere, Ph.D., Caryn Hale, Ph.D., Fan Zhang, Ph.D., Soumya Raychaudhuri, M.D., Ph.D., Olga G. Troyanskaya, Ph.D., and Robert B. Darnell, M.D., Ph.D.



- B cell activation preceded the expansion of circulating pre-inflammatory mesenchymal (PRIME) cells prior to RA flares (1-2 weeks)
- PRIME cells may be activated by B cells before migrating into the synovium

Immunity

ARTICLE | VOLUME 52, ISSUE 6, P1022-1038.E7, JUNE 16, 2020

Plasmacytoid Dendritic Cells and Type I Interferon Promote Extrafollicular B Cell Responses to Extracellular Self-DNA

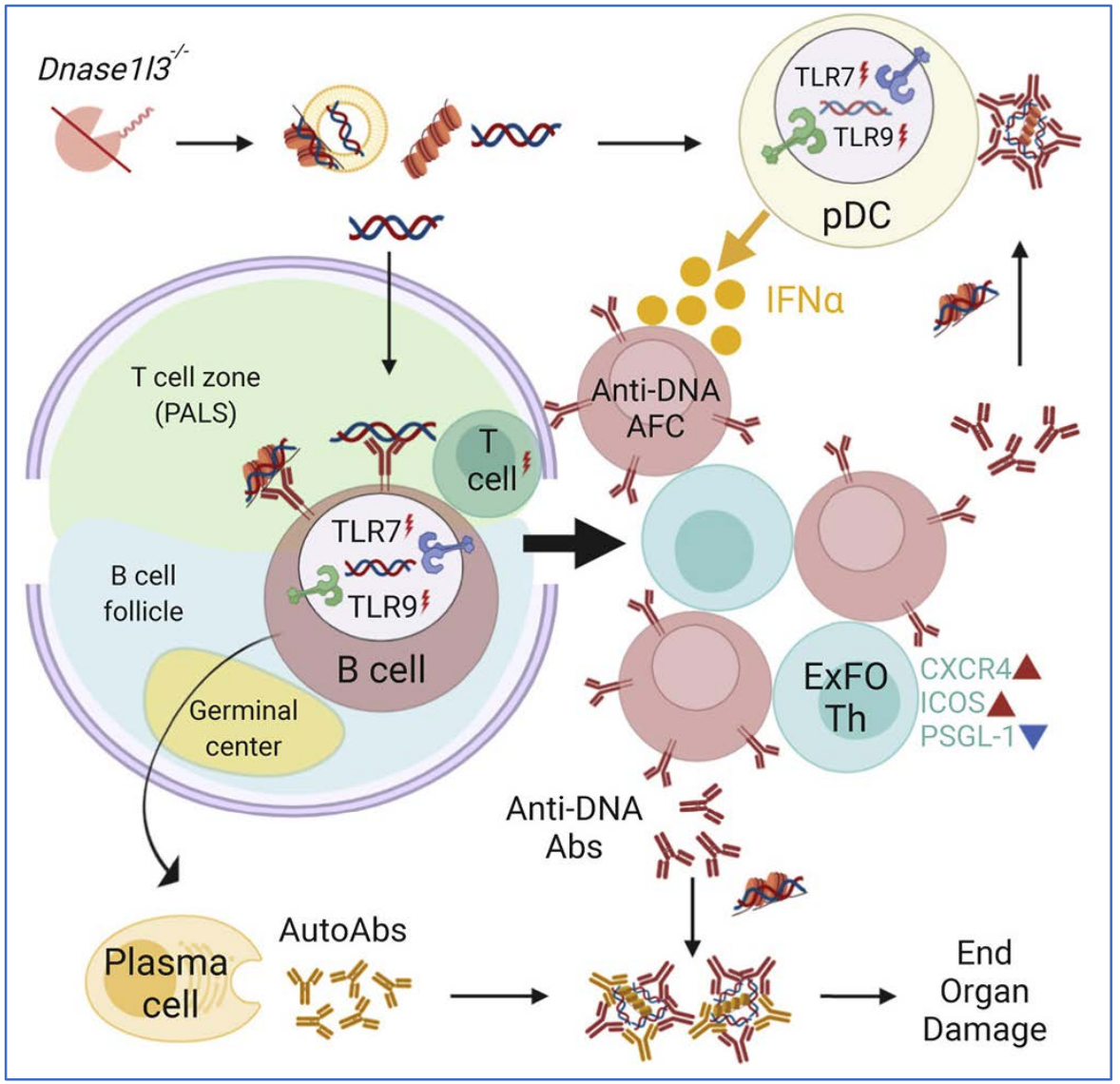
Chetna Soni • Oriana A. Perez • William N. Voss • ... Gregory C. Ippolito • Vanja Sisirak • Boris Reizis [7] • Show all authors • Show footnotes

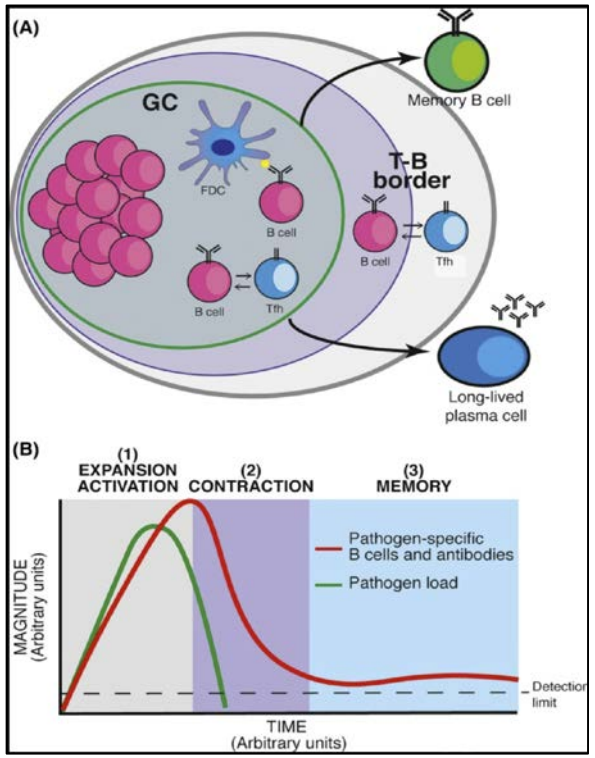
Published: May 25, 2020 • DOI: https://doi.org/10.1016/j.immuni.2020.04.015 • Check for updates

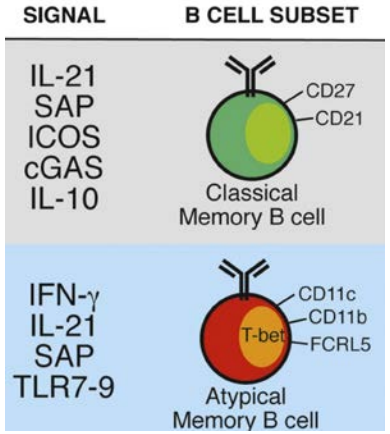
Extrafollicular B cell differentiation into short-lived AFCs as a key mechanism of anti-DNA autoreactivity and reveal a major contribution of pDCs, endosomal Toll-like receptors (TLRs), and IFN-I to this pathway.

Highlights

- Anti-DNA antibody response is driven by T-dependent extrafollicular plasmablasts
- IFN-I signaling propagates anti-DNA responses and SLE-like disease
- IFN-I produced by pDCs promotes plasmablast proliferation and differentiation
- TLR9 drives anti-DNA responses and autoimmunity redundantly with TLR7







Atypical Memory *B cells* are not atypical and mostly non-memory

Can we now move on?

SCIENCE ADVANCES | RESEARCH ARTICLE

IMMUNOLOGY

Expression of inhibitory receptors by B cells in chronic human infectious diseases restricts responses to membrane-associated antigens

Abhijit A. Ambegaonkar, Kihyuck Kwak, Haewon Sohn, Javier Manzella-Lapeira, Joseph Brzostowski, Susan K. Pierce*

Chronic human infectious diseases, including malaria, are associated with a large expansion of a phenotypically and transcriptionally distinct subpopulation of B cells distinguished by their high expression of a variety of inhibitory receptors including FcγRIIB. Because these B cells, termed atypical memory B cells (MBCs), are unable to respond to soluble antigens, it was suggested that they contributed to the poor acquisition of immunity in chronic infections. Here, we show that the high expression of FcγRIIB restricts atypical MBC responses to membrane-associated antigens that function to actively exclude FcγRIIB from the B cell immune synapse and include the co-receptor CD19, allowing B cell antigen receptor signaling and differentiation toward plasma cells. Thus, chronic infectious diseases result in the expansion of B cells that robustly respond to antigens that associate with cell surfaces, such as antigens in immune complexes, but are unable to respond to fully soluble antigens, such as self-antigens.

Review > Immunol Rev. 2019 Nov;292(1):139-148. doi: 10.1111/imr.12809. E

Exhaustion may not be in the human B cell vocabulary, at least not in malaria

Prasida Holla [1], Abhijit Ambegaonkar [1], Haewon Sohn [1], Susan K Pierce [1]

Review > Immunol Rev. 2020 Jan;293(1):57-69. doi: 10.1111/imr.12822. Epub 2019 I

B-cell memory in malaria: Myths and realities

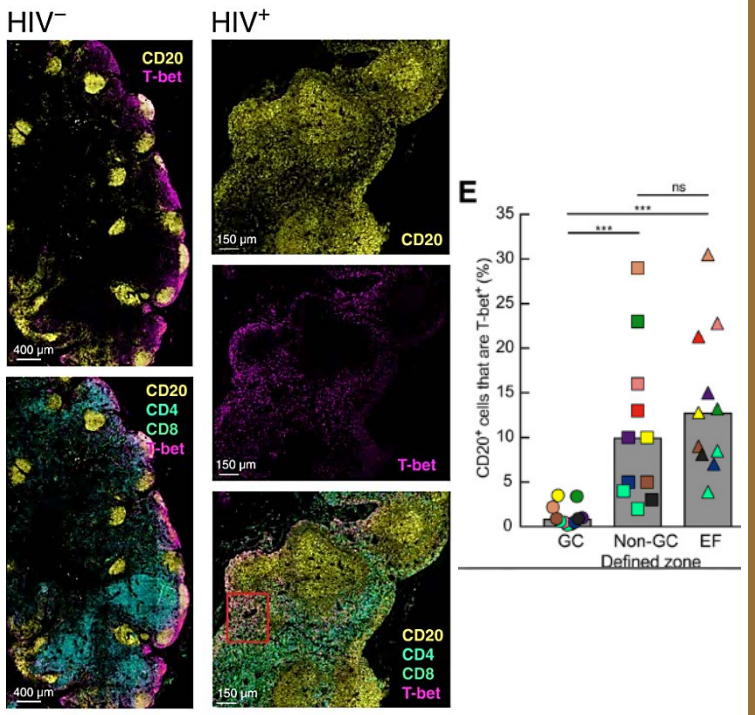
Damián Pérez-Mazliah [1,2], Francis M Ndungu [3], Racheal Aye [4], Jean Langhorne [1]

RESEARCH ARTICLE | HIV

Overexpression of T-bet in HIV infection is associated with accumulation of B cells outside germinal centers and poor affinity maturation

James W. Austin[1,*], Clarisa M. Buckner[1,*], Lela Kardava[1], Wei Wang[1], Xiaozhen Zhang[1], Valerie A. Melson[1], Ryan G. Swanson[1], Andrew J. Martins[2], Julian Q. Zhou[3], Kenneth B. Hoehn[4], J. Nicholas Fisk[3], Yiannis Dimopoulos[5], Alexander Chassiakos[5], Sijy O'Dell[5], Margery G. Smelkinson[6], Catherine A. Seamon[7], Richard W. Kwan[7], Michael C. Sneller[1], Stefania Pittaluga[8], Nicole A. Doria-Rose[5], Adrian McDermott[5], Yuxing Li[9,10], Tae-Wook Chun[1], Steven H. Kleinstein[3,4], John S. Tsang[2,11], Constantinos Petrovas[5] and Susan Moir[1,†]

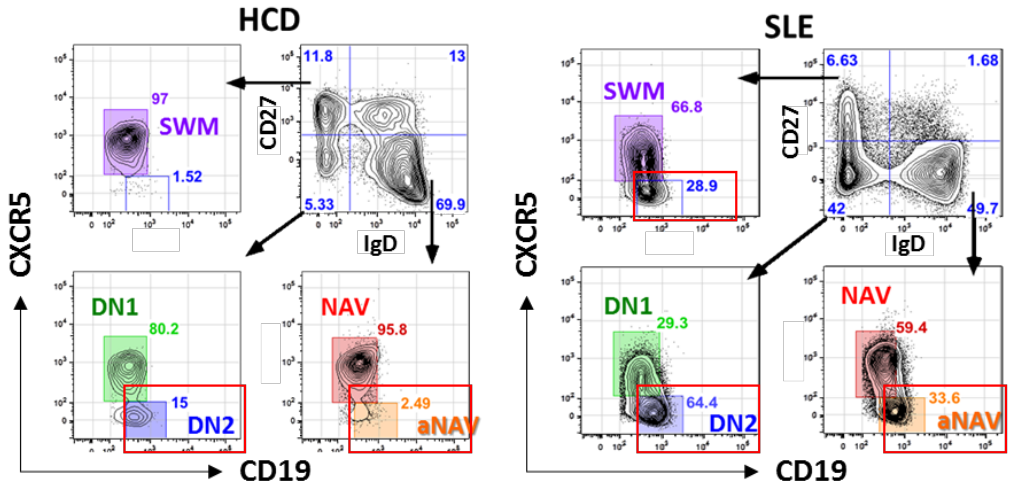
Accumulation of T-bet+ B cells in non-GC areas of LN in HIV infection



James W. Austin et al., Sci Transl Med 2019

Distinct B cell abnormalities in SLE revealed by multidimensional FCM

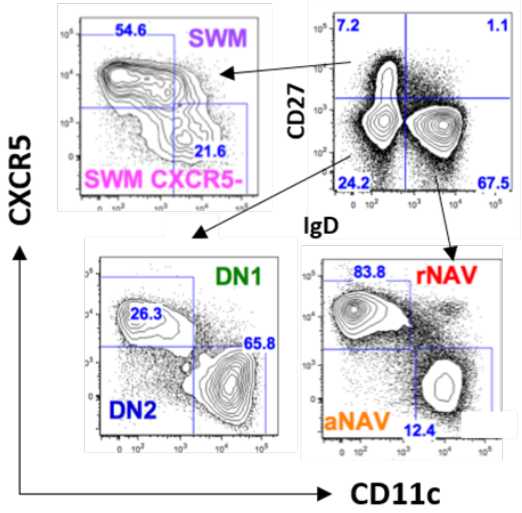
SLE is characterized by expansion of
CXCR5-negative activated naïve (aN) and DN2 cells

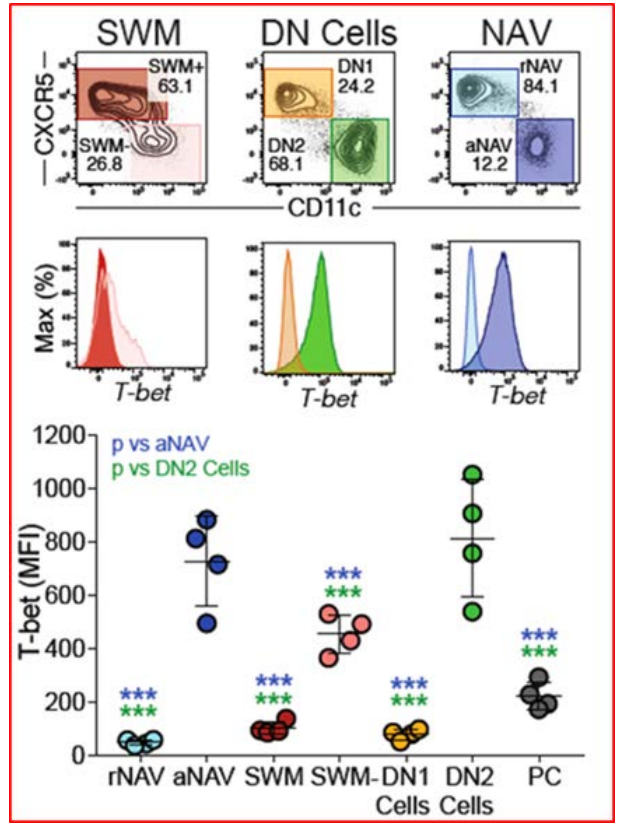


aN and DN2
extended phenotype

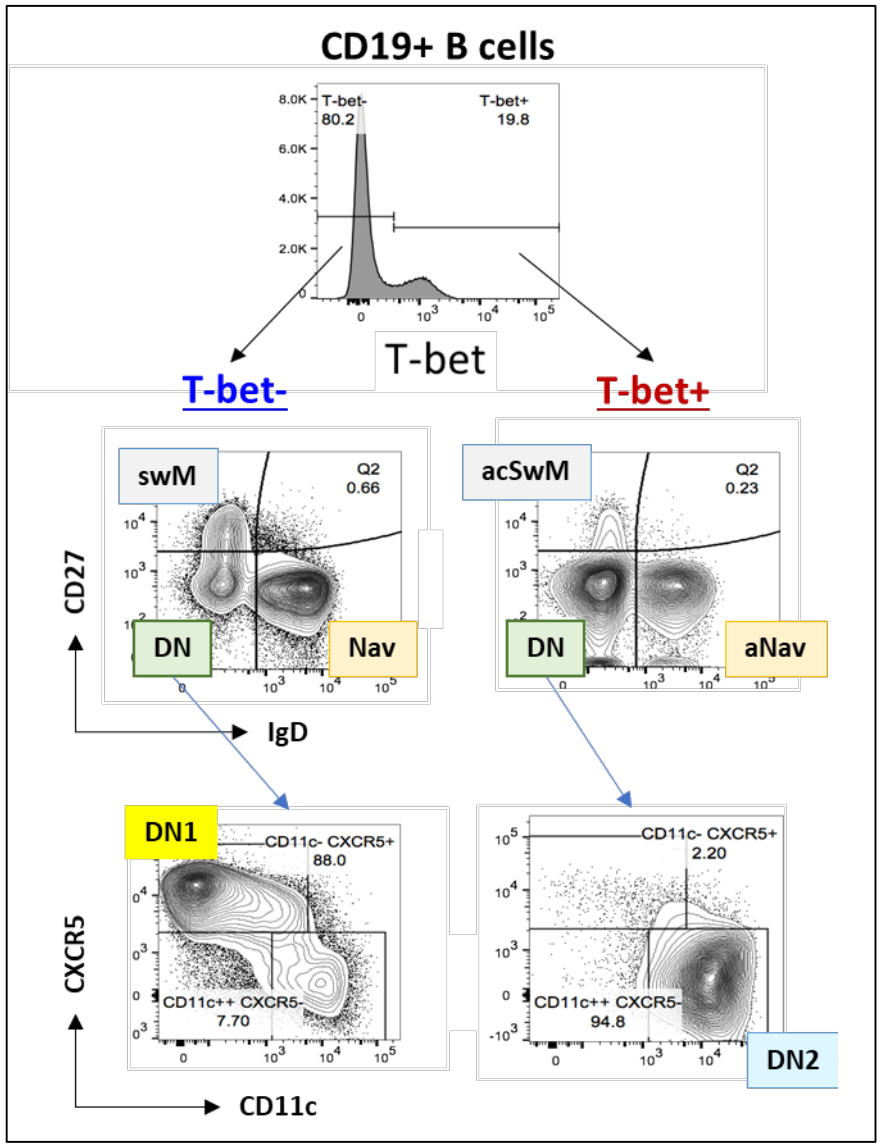
CXCR5- CD62L- CD11c++ CD19++
T-bet++
CD23- CD24- CD38-
PD-1++ CD22+ CD32b+
FcRL4- FcRL5+

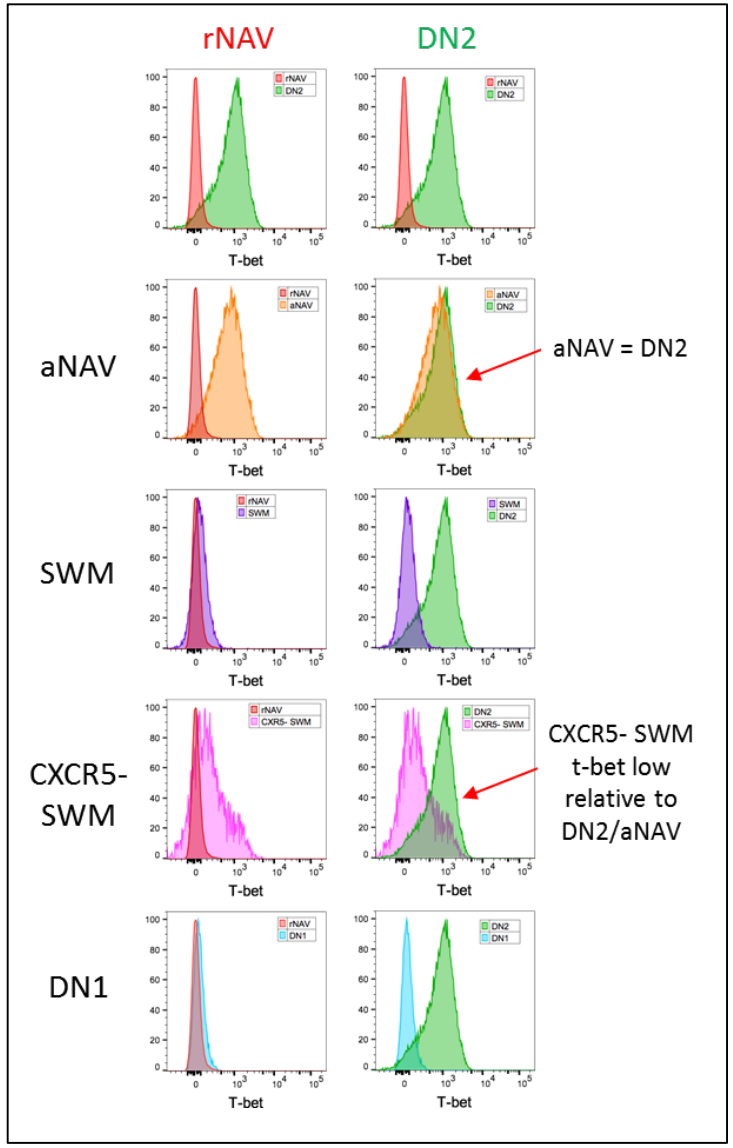
CXCR5- aN and DN2 cells are the dominant
CD11c ^{high} T-bet ^{high} B cells in SLE

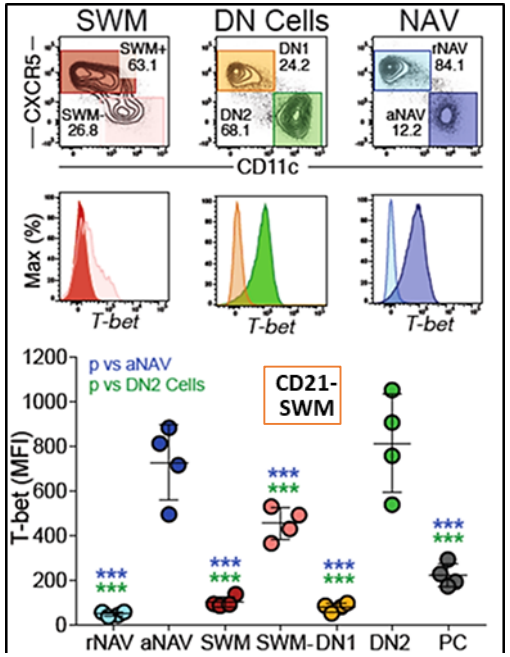




T-bet bright B cells are predominantly CD27-, CXCR5-, CD11c++ -
Only activated naïve cells and DN2 cells are T-bet bright





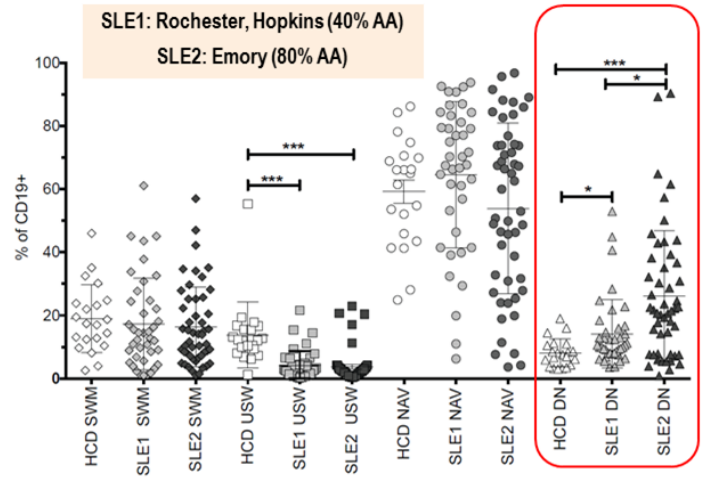


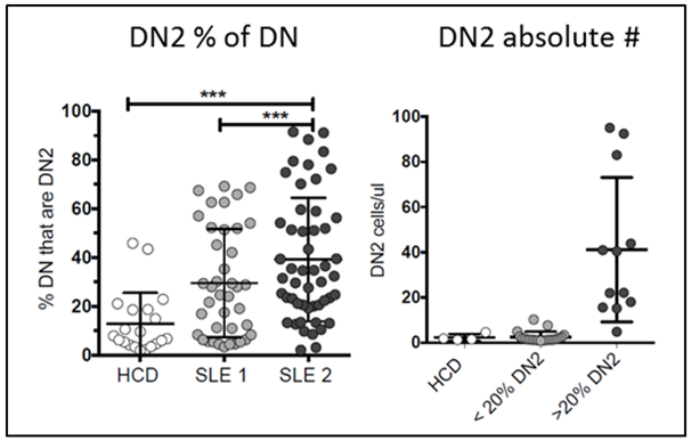
T-bet+/CD21- B cells

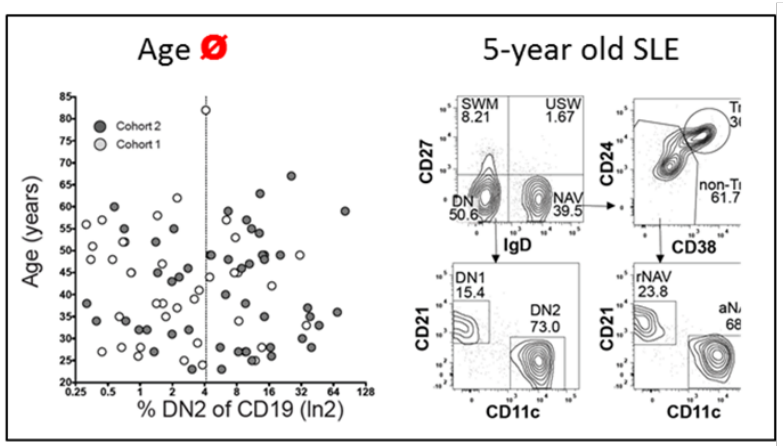
ABC (Age-Associated B cells)
ABC (Activated B cells) –LLPC precursors
CD21- Activated Memory B cells
CD21- Naïve B cells
Exhausted Memory Cells
Atypical Memory Cells
TLR7-activated T1 cells

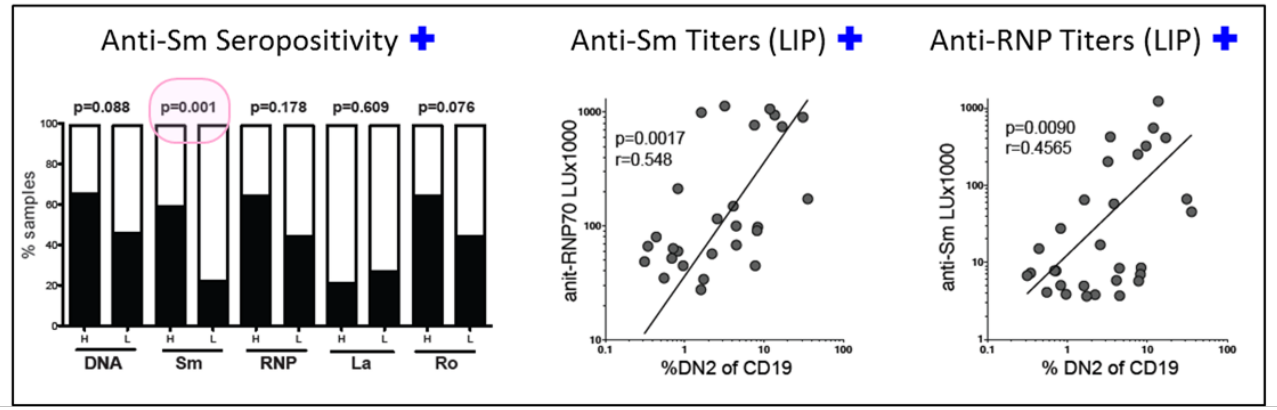
Mice & Humans Humans Mice

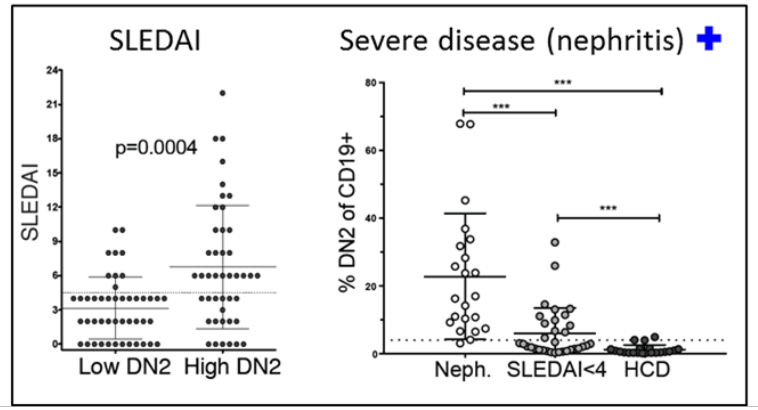
Predominance of DN cells in SLE (African-American) Patients
Clinical Associations





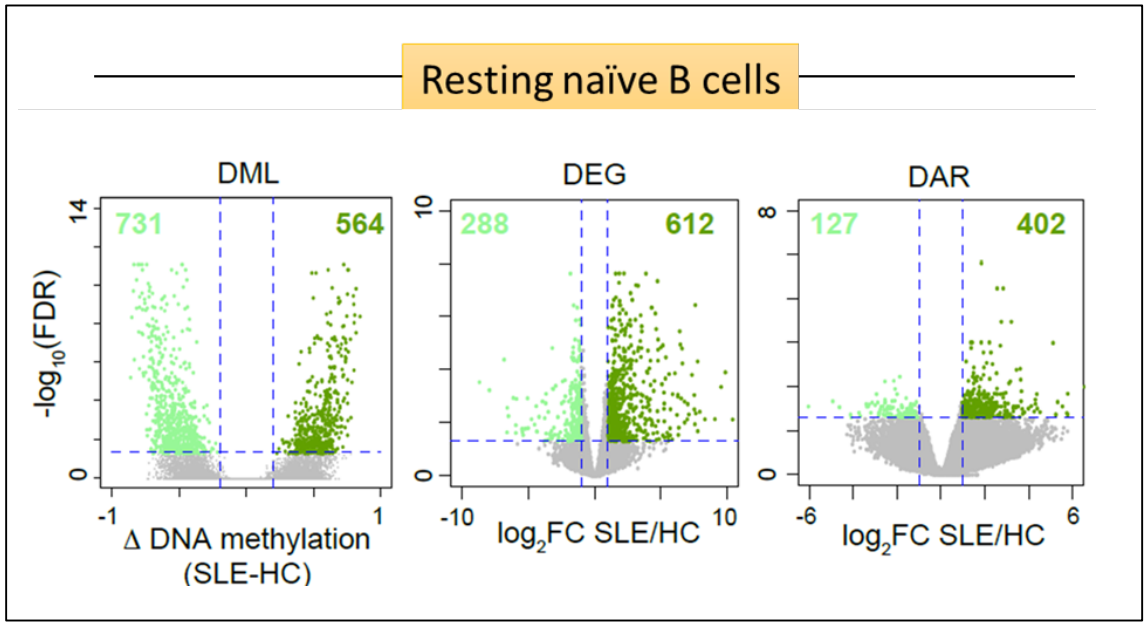




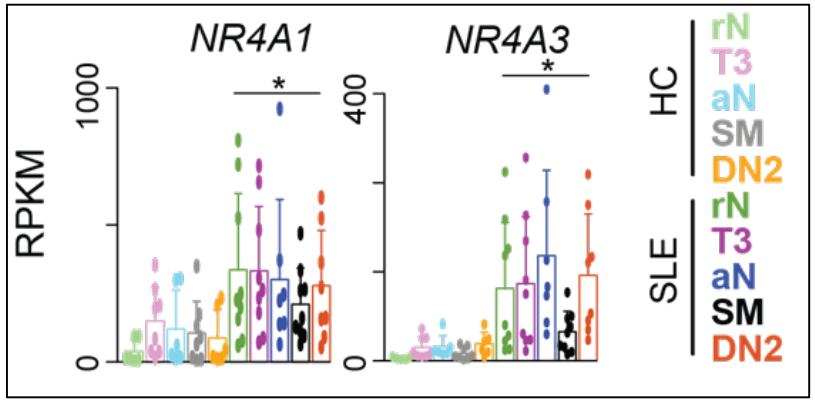


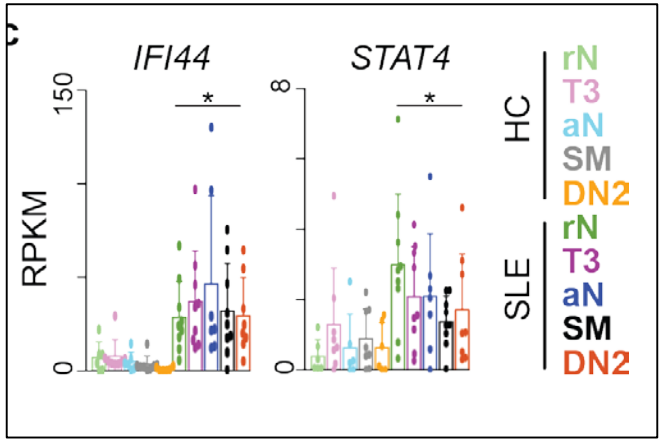
Resting Naïve Cells are epigenetically primed in SLE

The epigenetic Lupus signature is "transmissible" to other B cell subsets with accentuation in the aNAV → D2 pathway



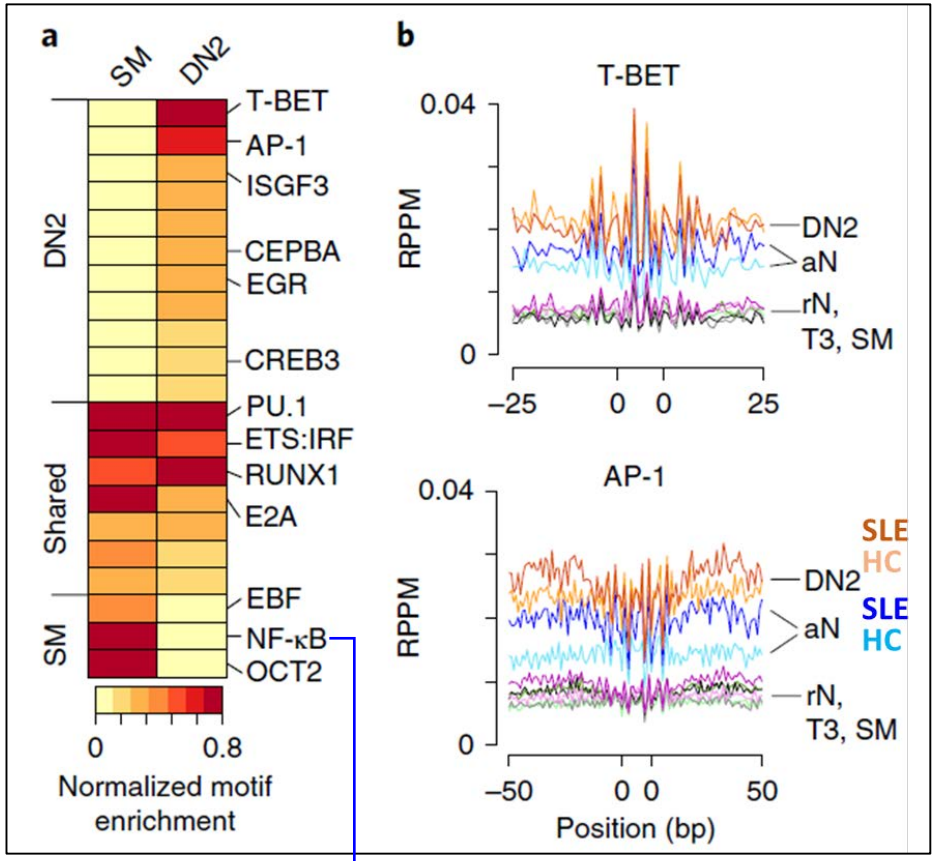
BCR (NR4A1-Nur77) and TLR activated genes (NR4A3) are induced in rN B cells in SLE

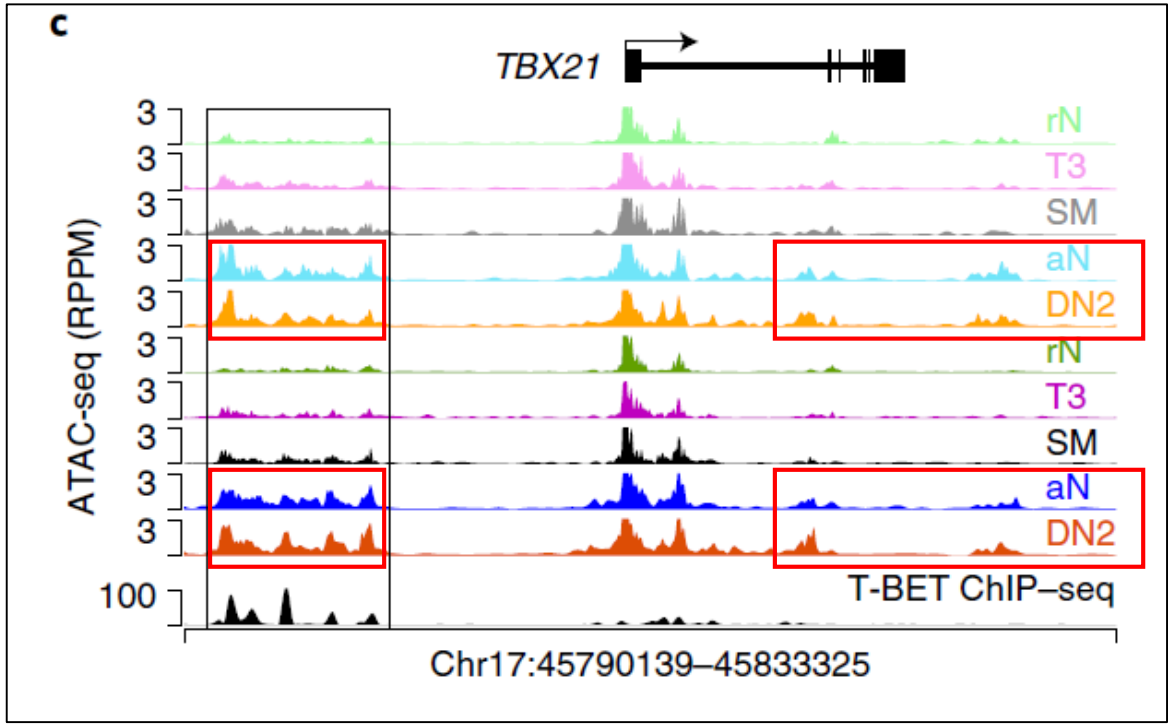




Scharer at el. Nat Immunol 2019

Chromatin accessibility in DN2 B cells is driven by T-BET, AP-1 and EGR transcription factors





- T-BET upregulated and more accessible in aNAV/DN2 B cells irrespective of disease status
- Regulates other characteristic aNAV/DN2 markers including CD11c
- T-BET binds to its own gene, suggesting positive autoregulation in these cells

Sustained NF-κB Signaling in Be1 Cultures Prevents ASC Development. Stone et al (Lund)

Immunity

T-bet Transcription Factor Promotes Antibody-Secreting Cell Differentiation by Limiting the Inflammatory Effects of IFN-γ on B Cells

LAB - Lowance Center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Ankur Saini Matt Woodruff


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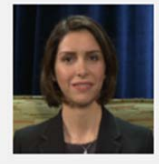
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GEORGIA RESEARCH ALLIANCE

Dominant EF B cell responses correlate with poor clinical outcomes in severe COVID-19 infection despite robust production of neutralizing aantibodies

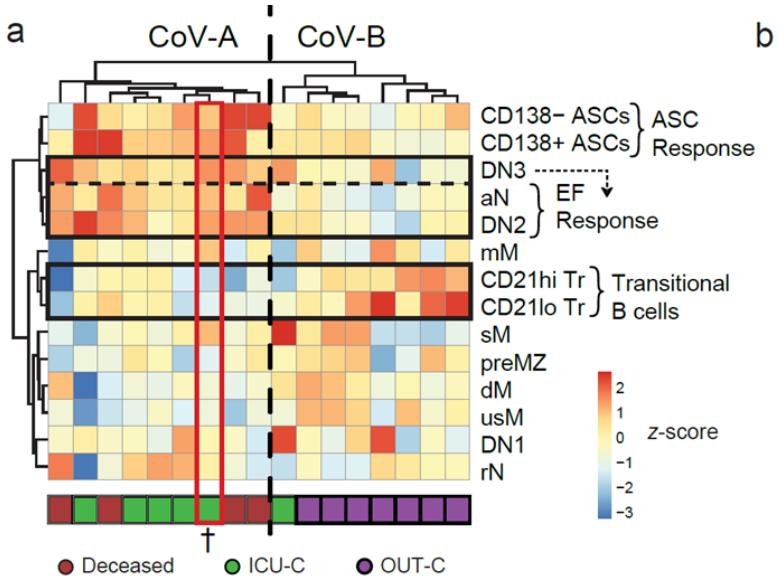
nature immunology
ARTICLES
https://doi.org/10.1038/s41590-020-00814-z
Check for updates

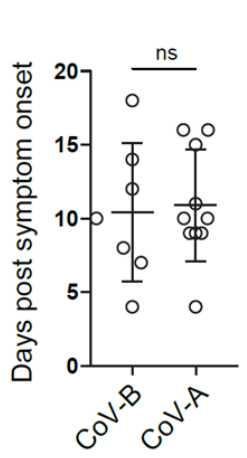
Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19

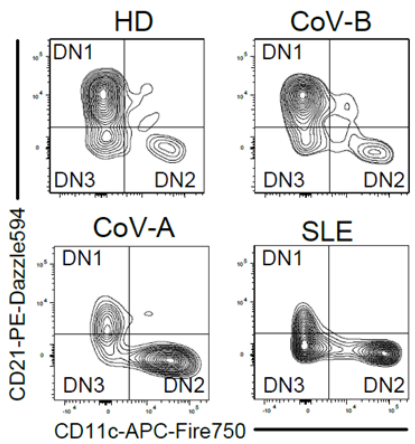
Matthew C. Woodruff[1,2,13], Richard P. Ramonell[3,13], Doan C. Nguyen[3], Kevin S. Cashman[1], Ankur Singh Saini[1], Natalie S. Haddad[3,4], Ariel M. Ley[3], Shuya Kyu[3], J. Christina Howell[5], Tugba Ozturk[5], Saeyun Lee[1,3], Naveenchandra Suryadevara[6], James Brett Case[7], Regina Bugrovsky[1], Weirong Chen[1], Jacob Estrada[1], Andrea Morrison-Porter[3], Andrew Derrico[3], Fabliha A. Anam[1], Monika Sharma[1], Henry M. Wu[8], Sang N. Le[1,3], Scott A. Jenks[1,2], Christopher M. Tipton[1,2], Bashar Staitieh[3], John L. Daiss[4], Eliver Ghosn[1], Michael S. Diamond[7,9,10,11], Robert H. Carnahan[6,12], James E. Crowe Jr.[6,12], William T. Hu[5], F. Eun-Hyung Lee[3] and Ignacio Sanz[1,2]

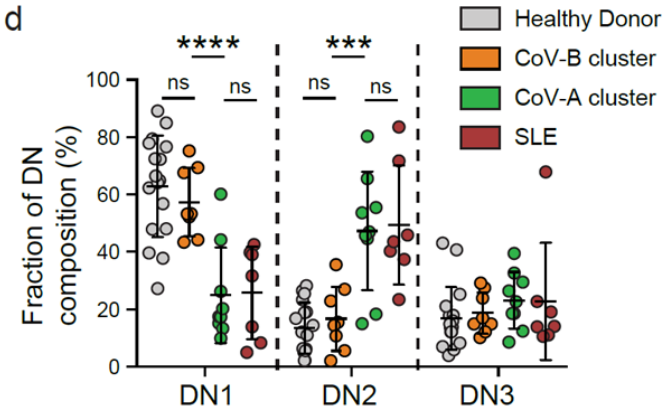
Matt Woodruff

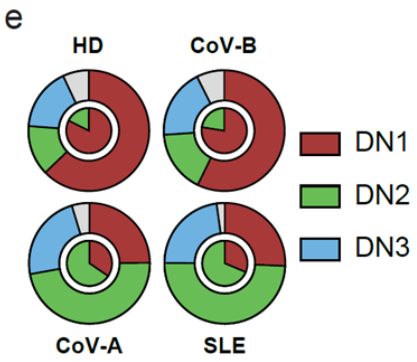
Rich Ramonell

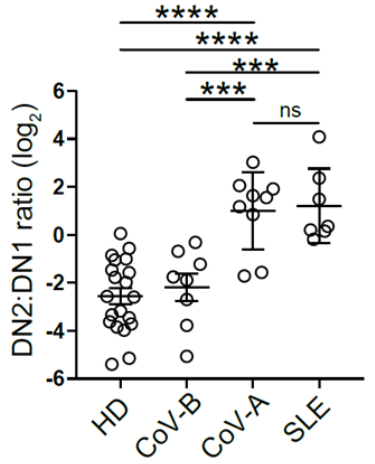








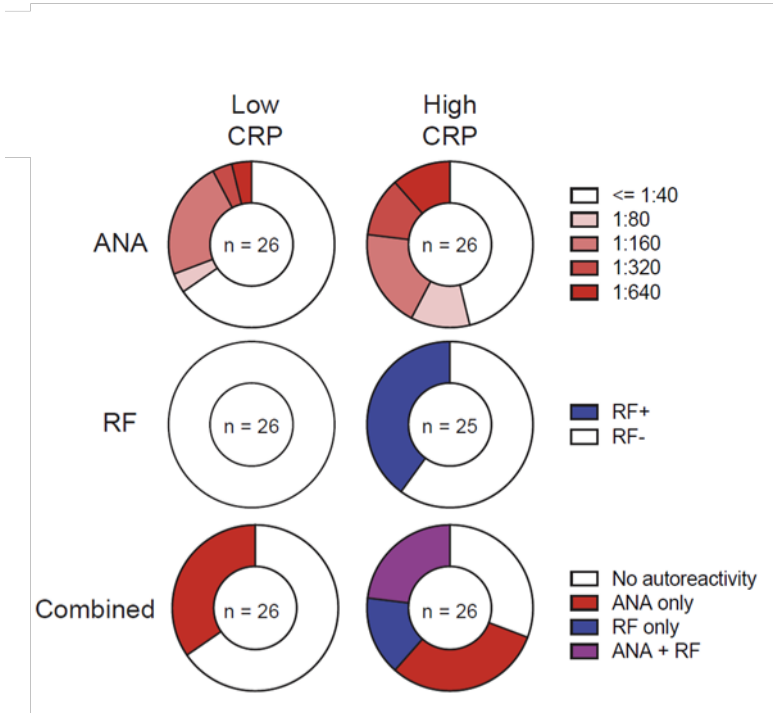


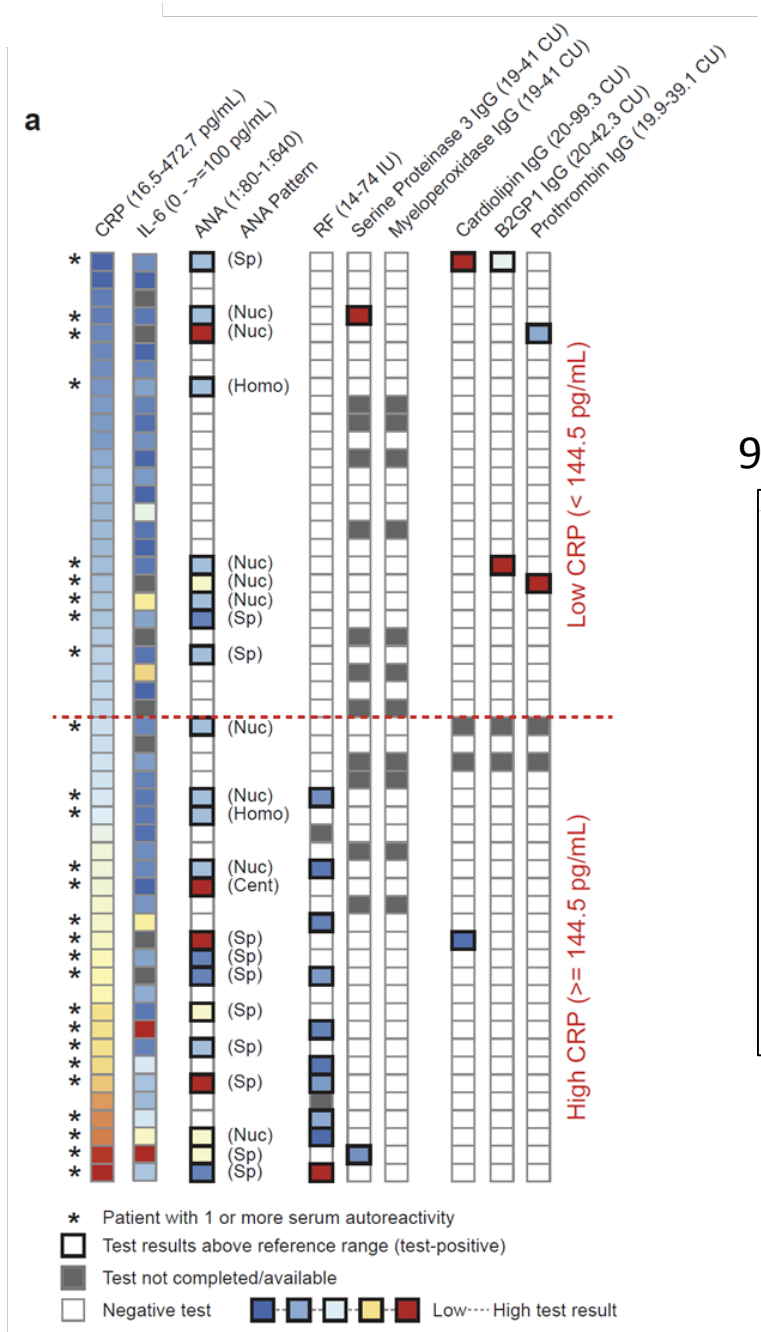


medRxiv. 2020 Oct 23;2020.10.21.20216192. doi: 10.1101/2020.10.21.20216192. Preprint

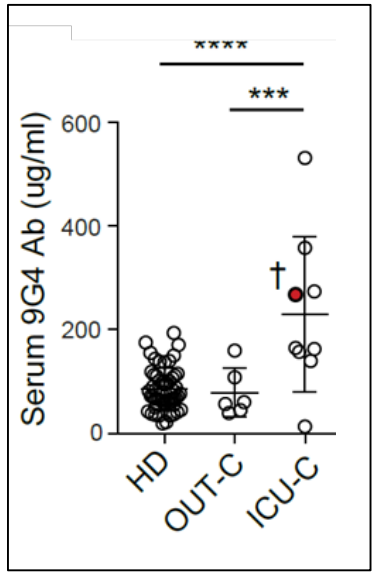
Broadly-targeted autoreactivity is common in severe SARS-CoV-2 Infection

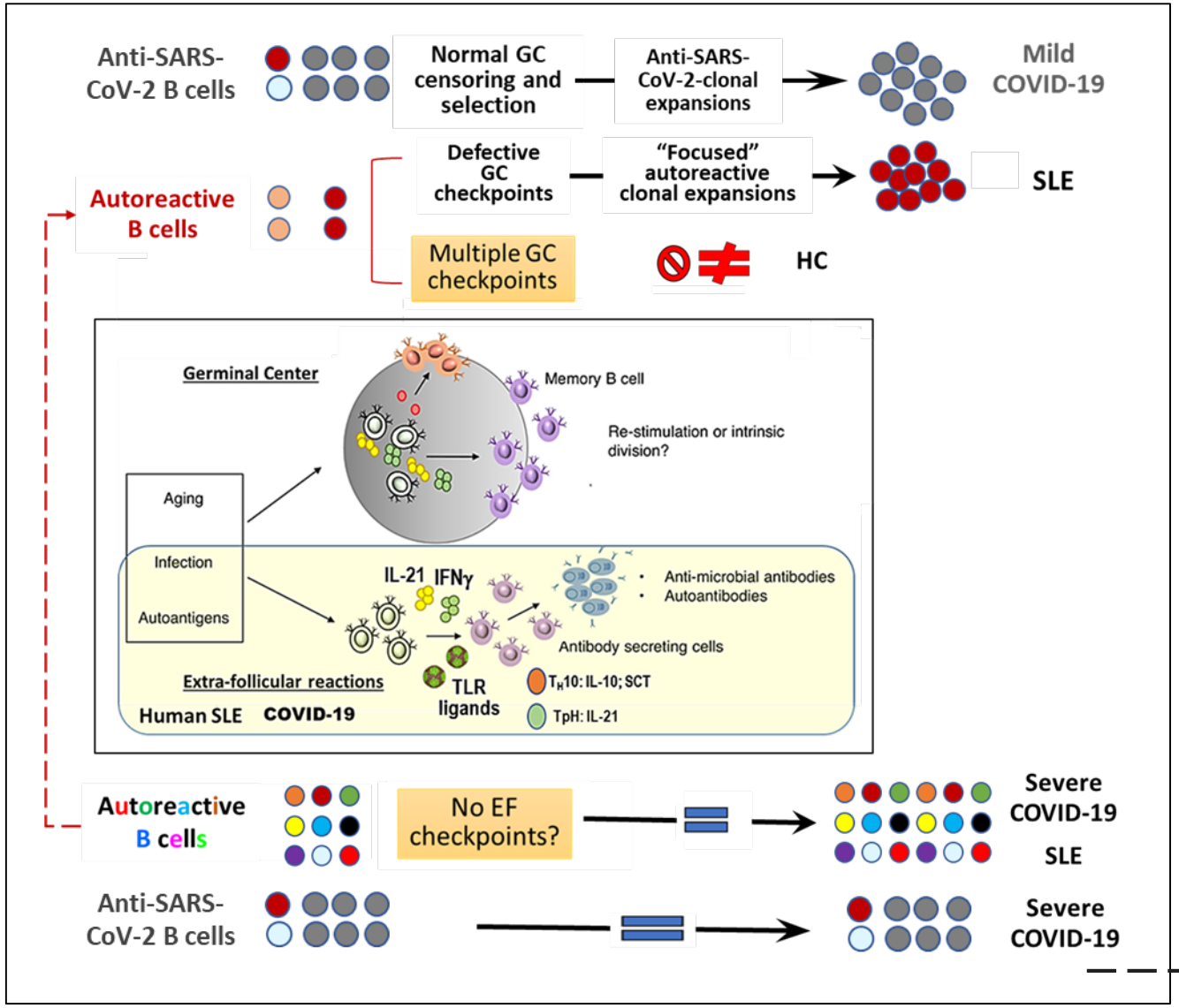
Matthew C Woodruff, Richard P Ramonell, F Eun-Hyung Lee, Ignacio Sanz



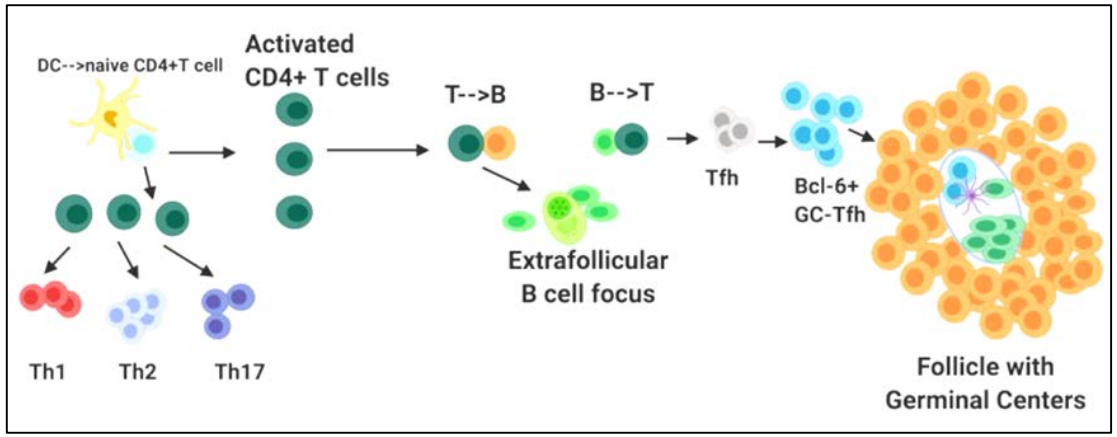


9G4 Autoantibodies

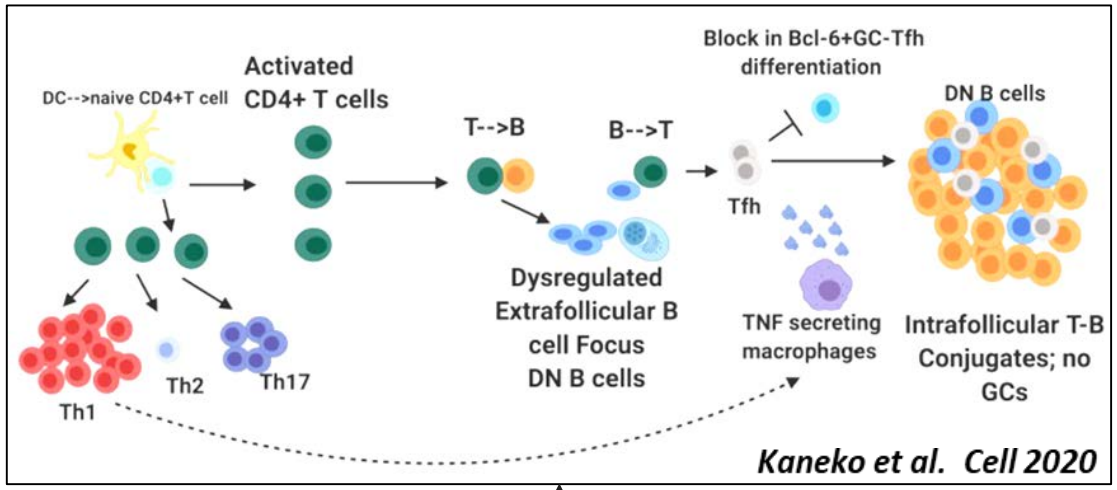




Mild COVID-19

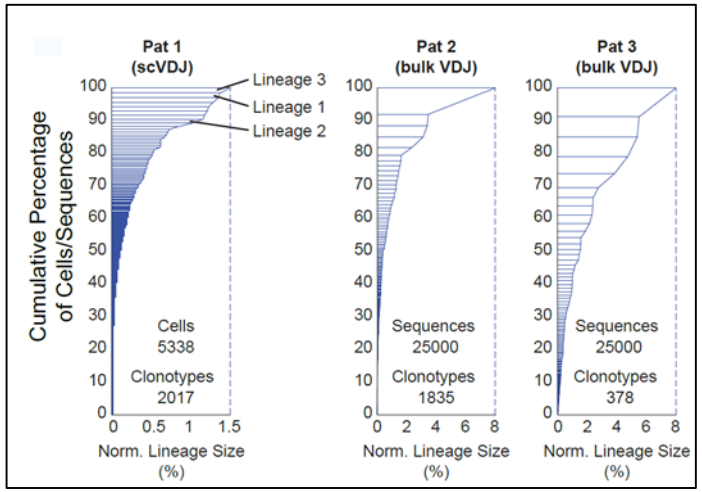


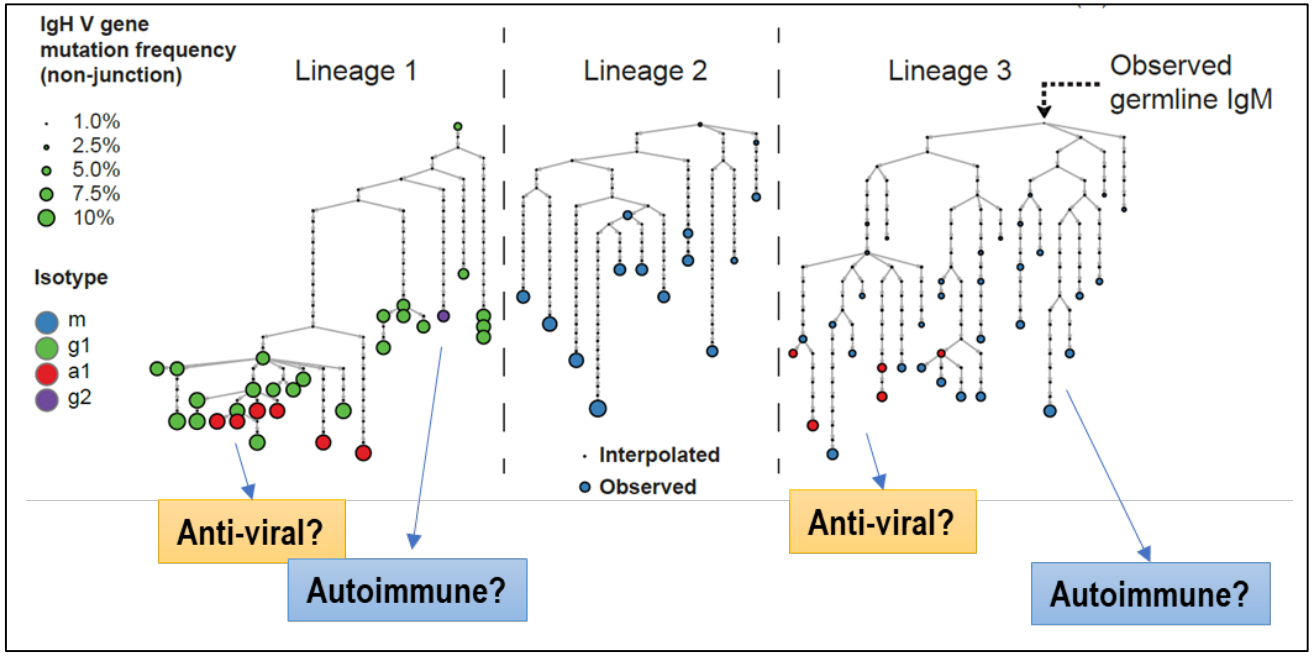
Severe COVID-19

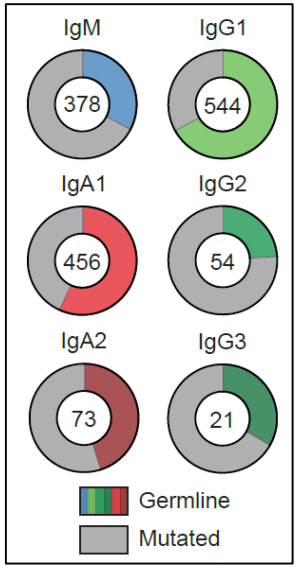


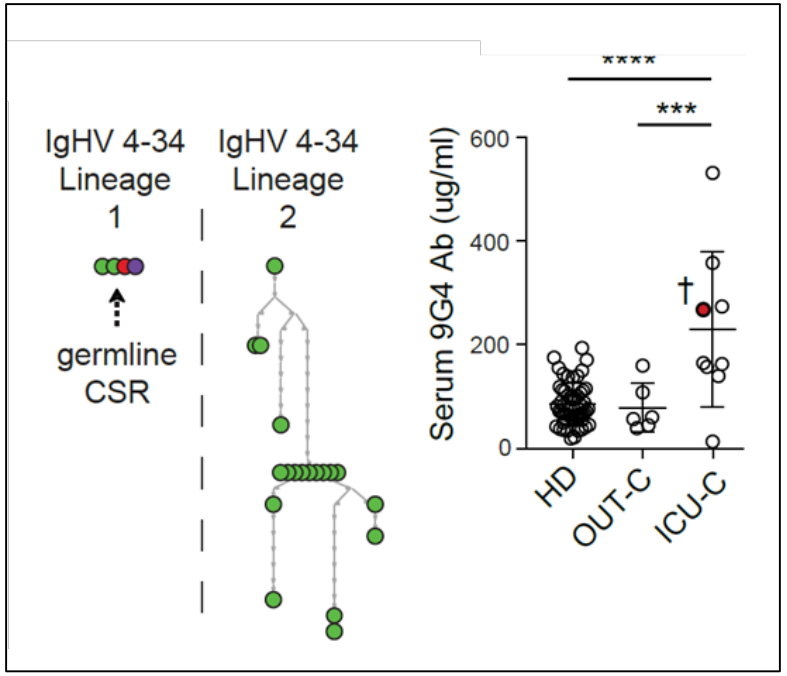
Woodruff et al. Nat Immunol 2020
Woodruff et al. MedRxiv 2020
Wang et al. MedRxiv 2020
Chen et al. Cell 2020

Germline clonotypes dominate the COVID-19 ASC repertoire yet, also evolve by isotype switch and SHM

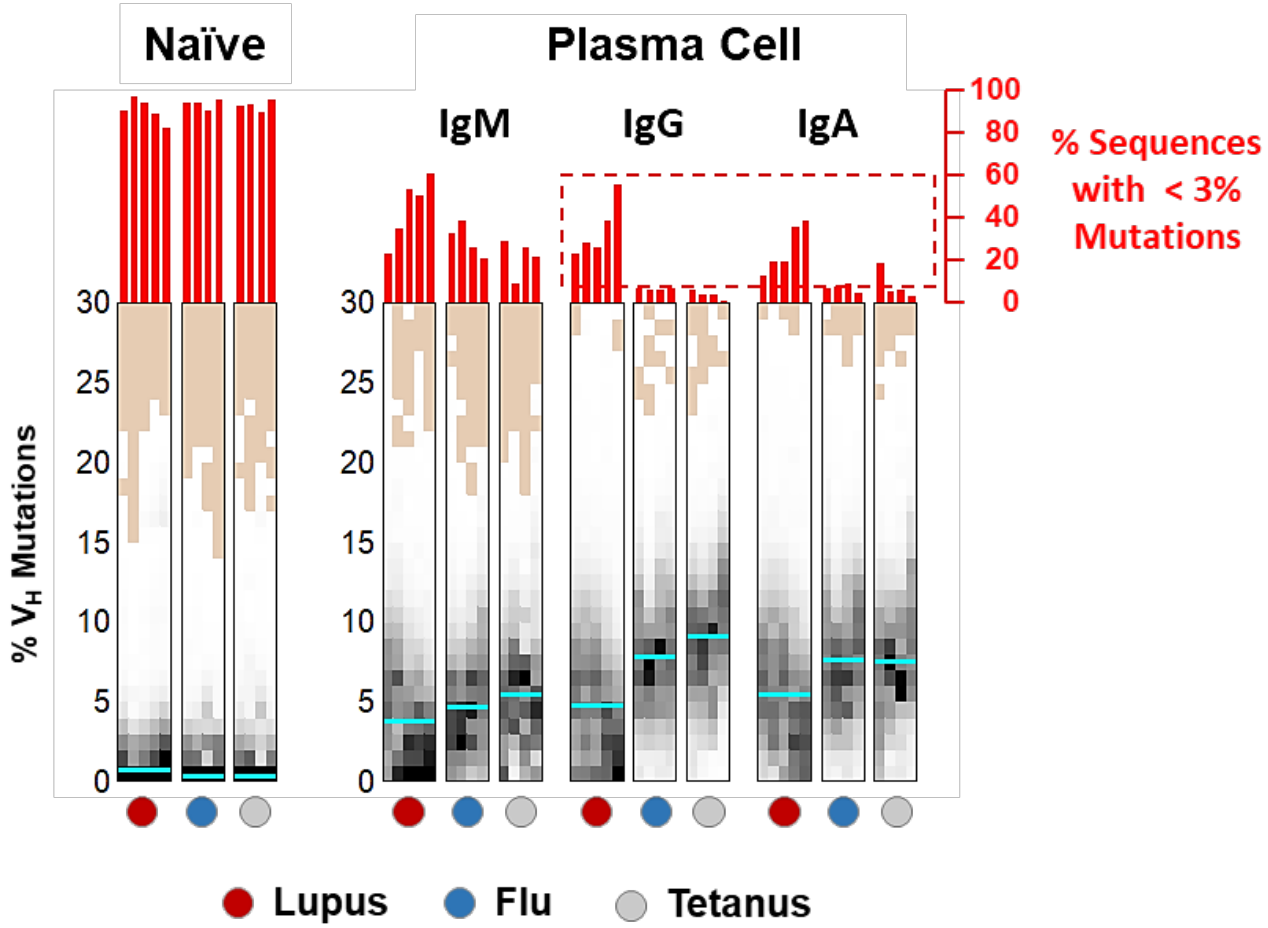








Substantial naïve B cell contribution to circulating ASC is also supported by much lower levels of SHM

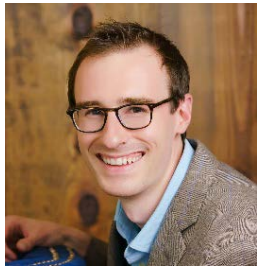


Tipton et al.
Nat Immunol 2015

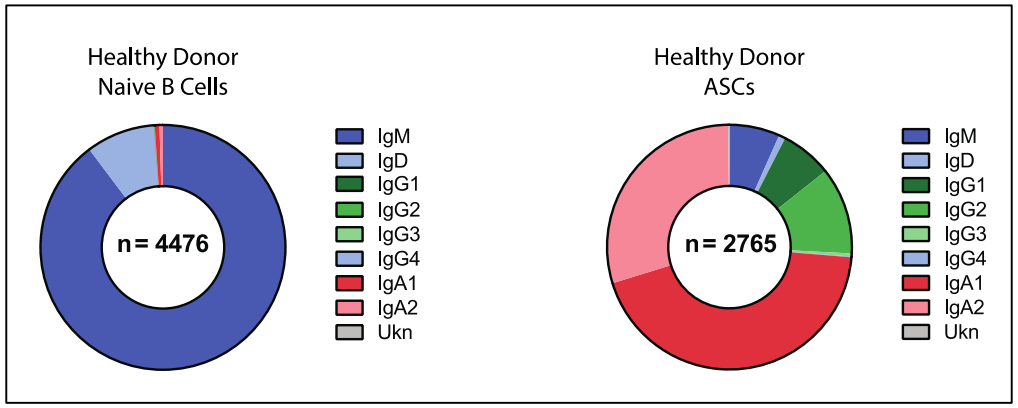
ASC in Severe COVID-19 are enriched in IgM and IgG1

Chris Tipton

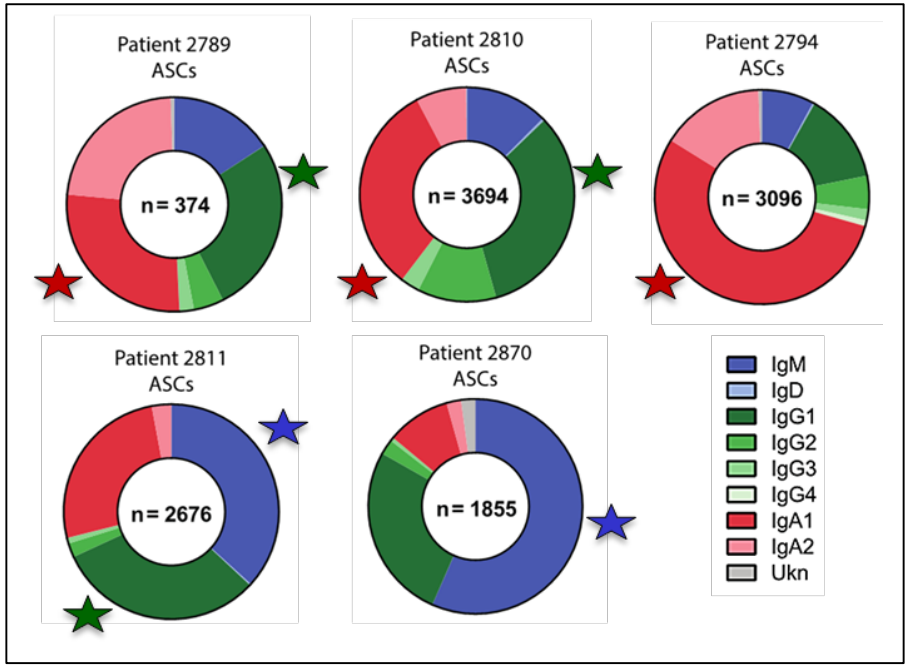
Matt Woodruff



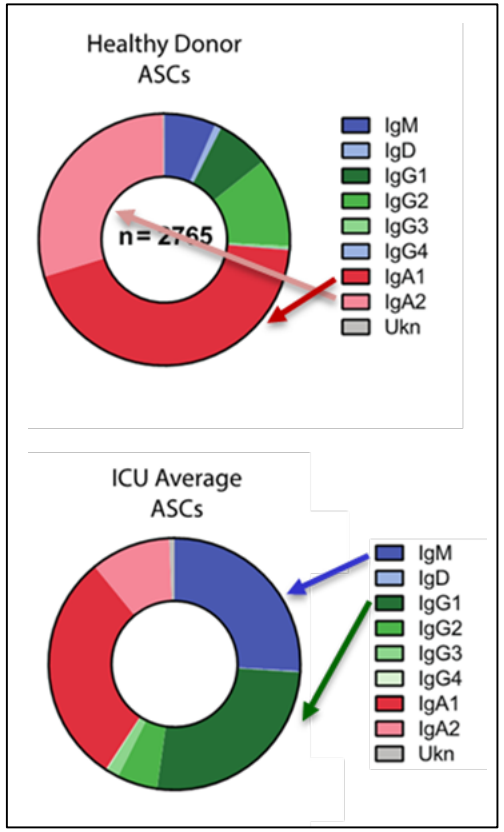
Isotype Expression in Resting HC ASC



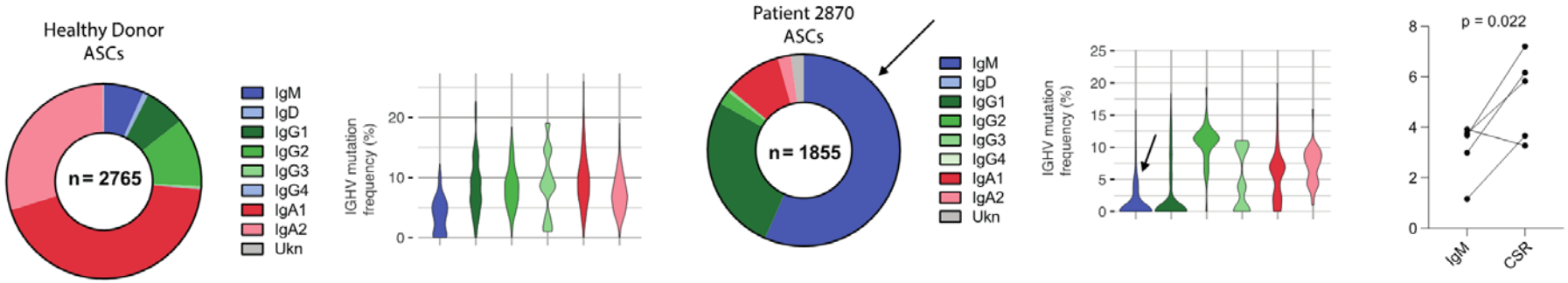
Isotype Expression in COVID-19 ICU ASC



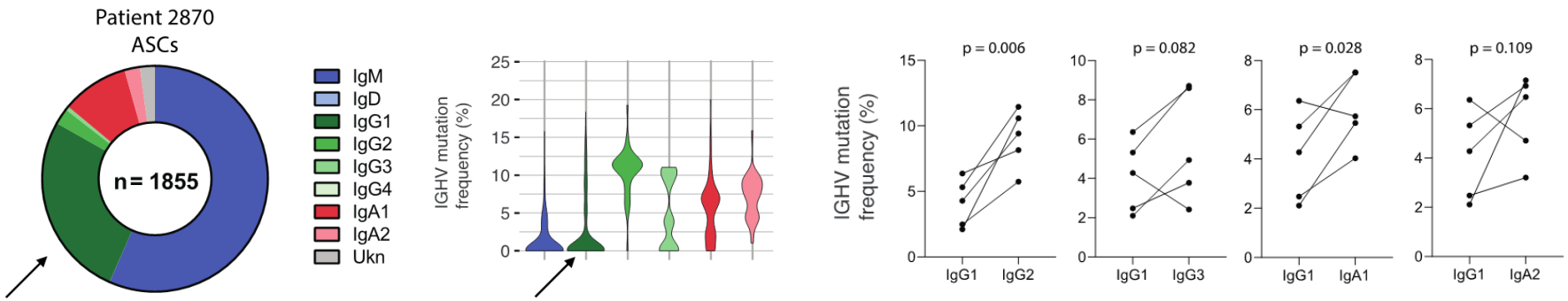
- 3 died, 2 survived
- All ICU
- AA steady state control



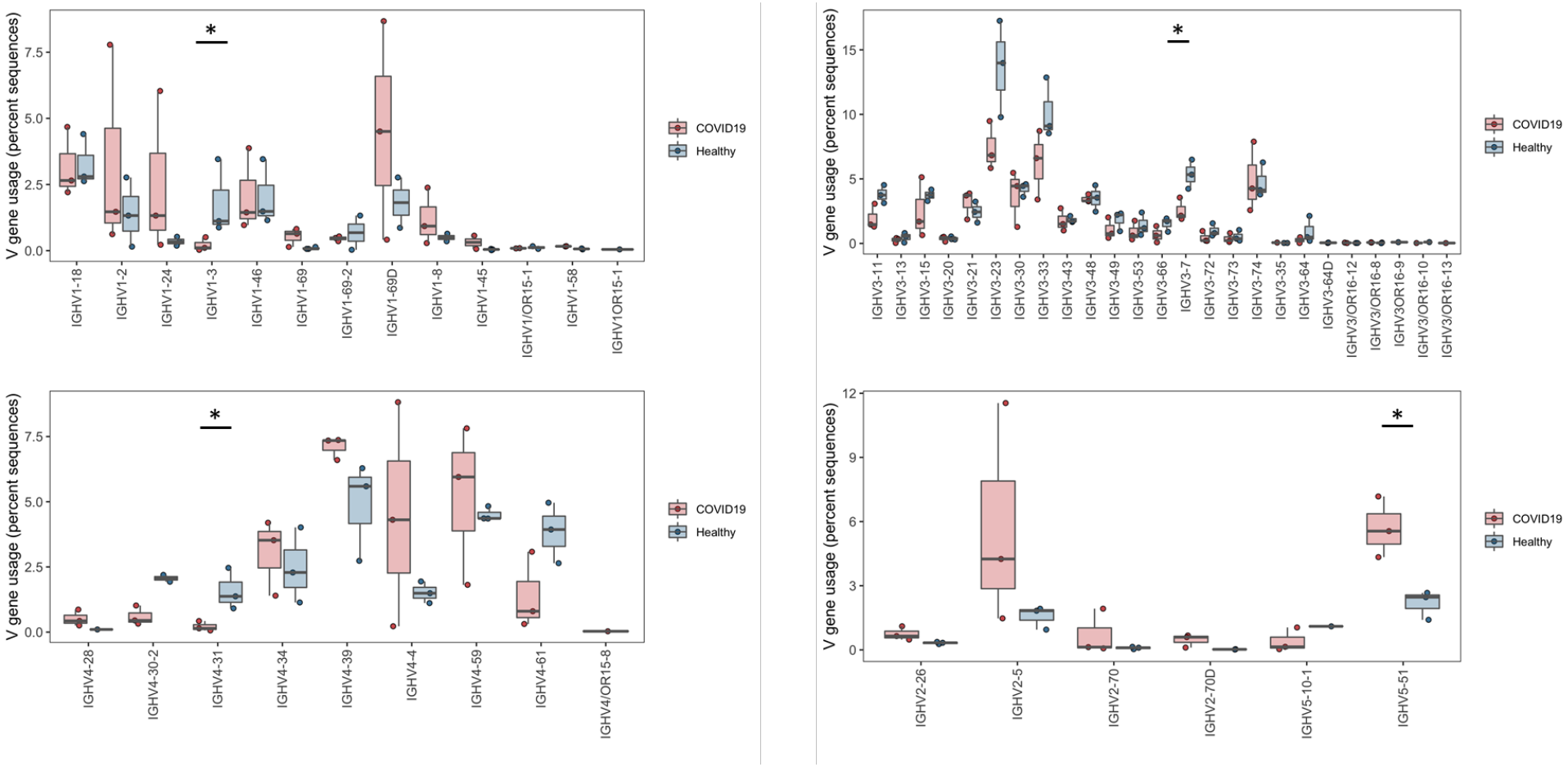
IgM ASC express very low mutation rate in severe COVID-19



IgG1 ASC express very low mutation rate in severe COVID-19

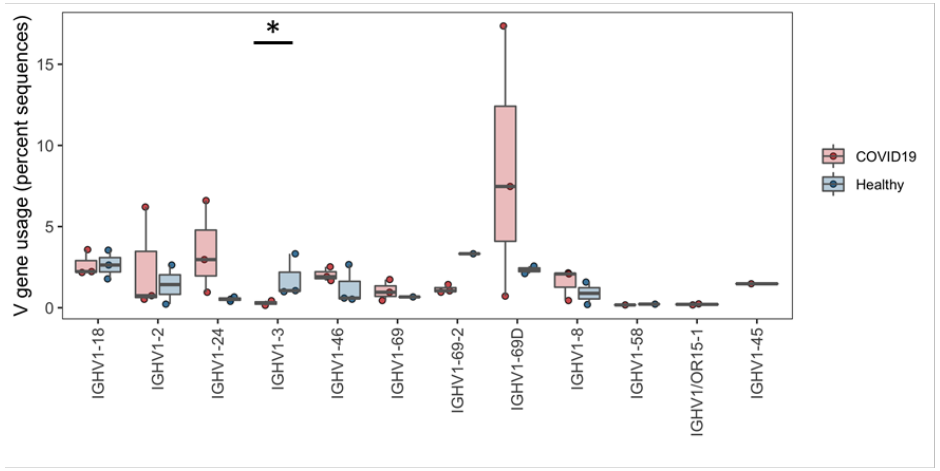


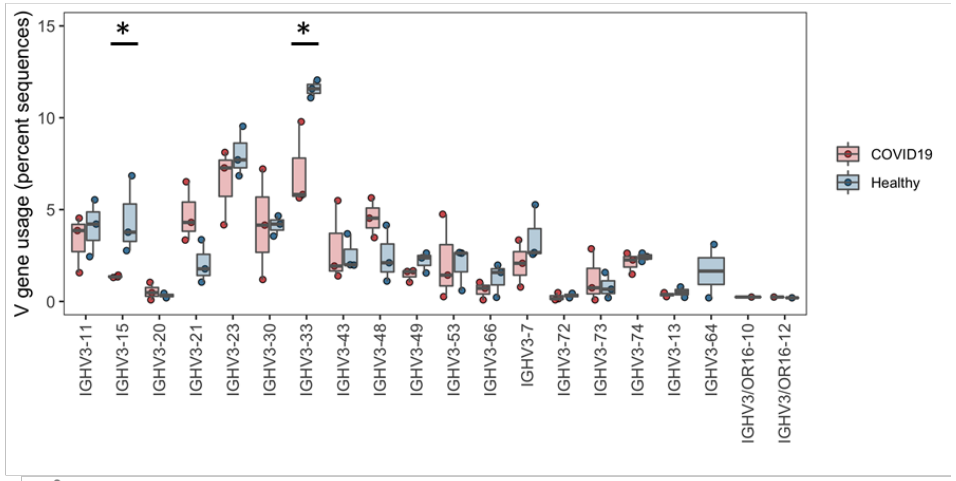
ASC - V gene usage (All Isotypes)

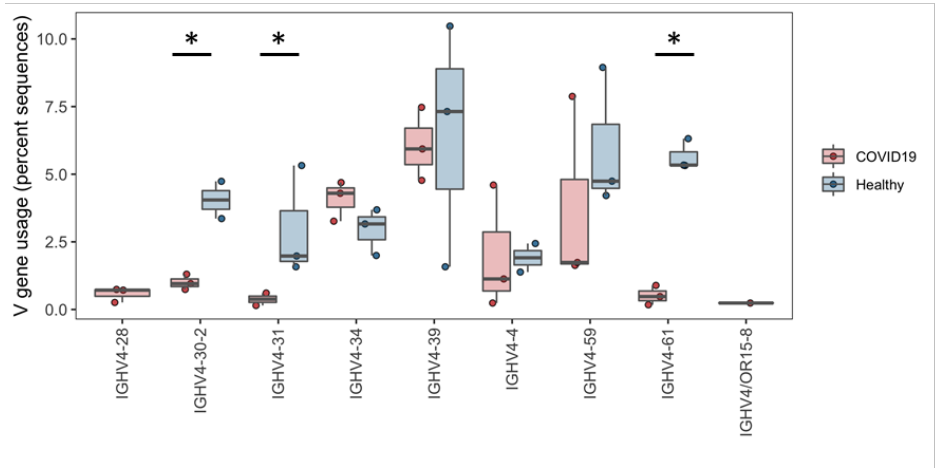


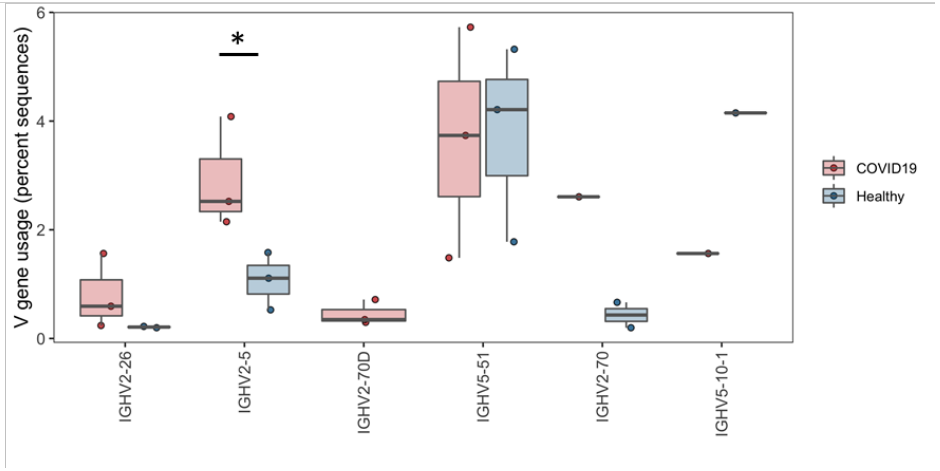
- *ALL ISOTYPES*
  - V genes significantly increased in COVID-19 ASC: VH5-51
  - V genes significantly increased in HC: VH1-3, VH3-7, VH4-31

ASC - V gene usage (IgG1)







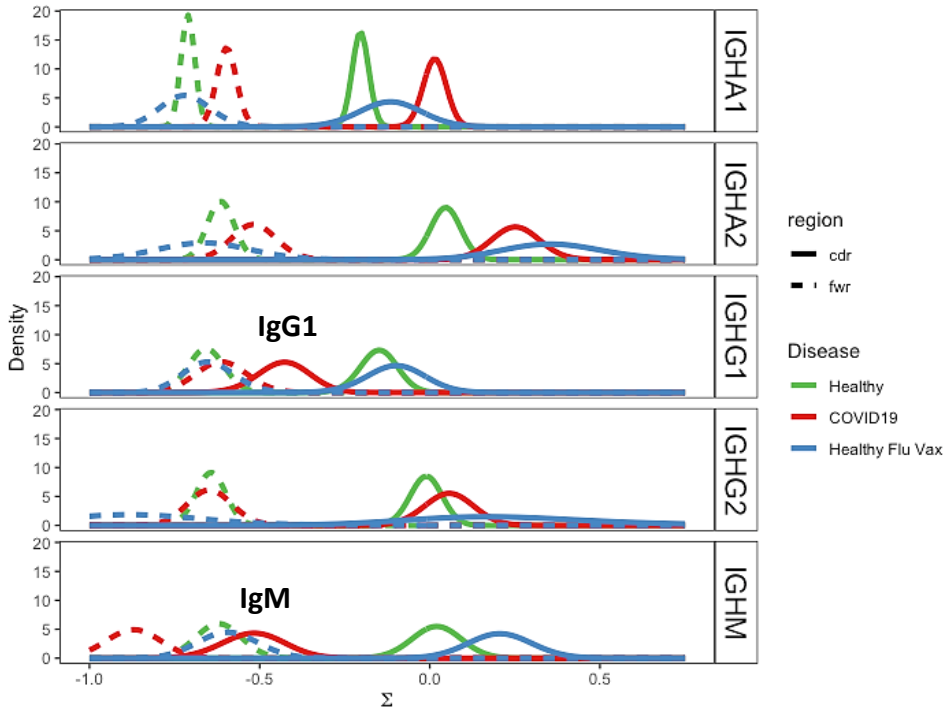


IgG1

- V genes significantly increased in COVID-19 ASC: VH2-5
- V genes significantly increased in HC: VH1.3, VH3.15, VH3.33, VH4.30.2, VH4-31, VH4-61

IgG1 and IgM in COVID19 samples show less selection pressure than healthy and healthy vaccinated samples

(COVID19 n = 4, healthy n = 2, health vax n = 1)



BASELINe

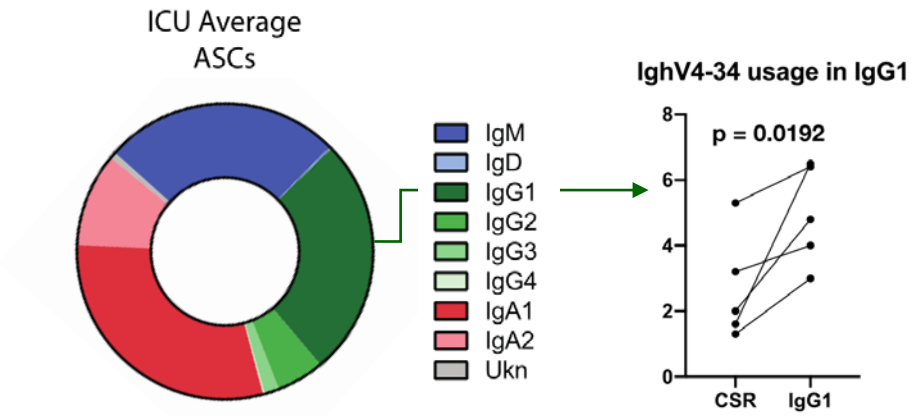
Bayesian Estimation of Antigen-Driven Selection in Immunoglobulin Sequences

Yaari et al. 2012; 2013

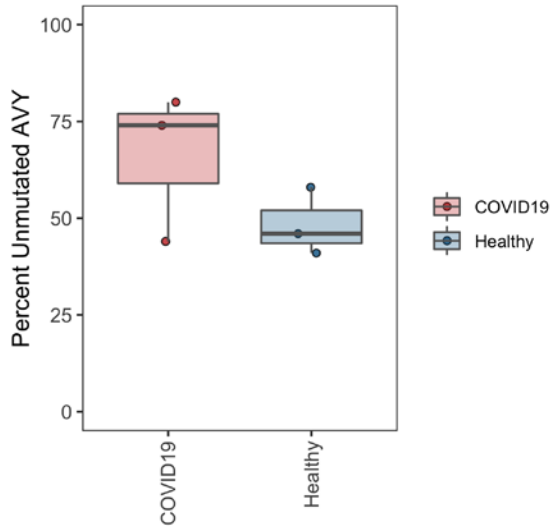
BASELINe quantifies selection pressure by calculating the posterior probability density function (PDF) based on observed mutations compared to expected mutation rates derived from an underlying SHM targeting model.

Selection is quantified via the following steps:
1. Calculate the selection scores for individual sequences.
2. Group by relevant fields for comparison and convolve individual selection PDFs.
3. Plot and compare selection scores of different groups of sequences.

VH4-34 expression is over-represented in IgG1 over other switched isotypes

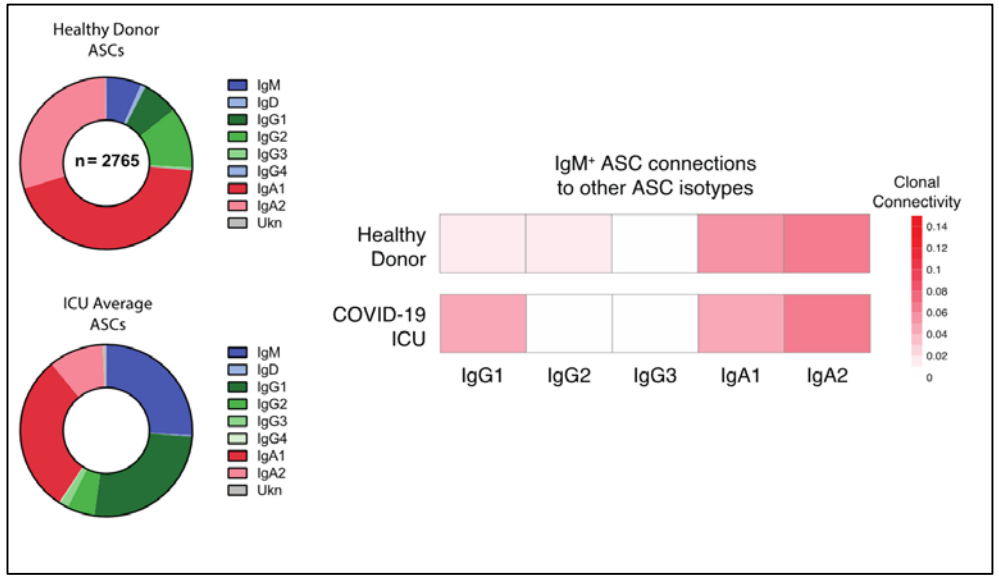


AVY unmutated VH4-34 is over-represented in COVID vs HC ASC

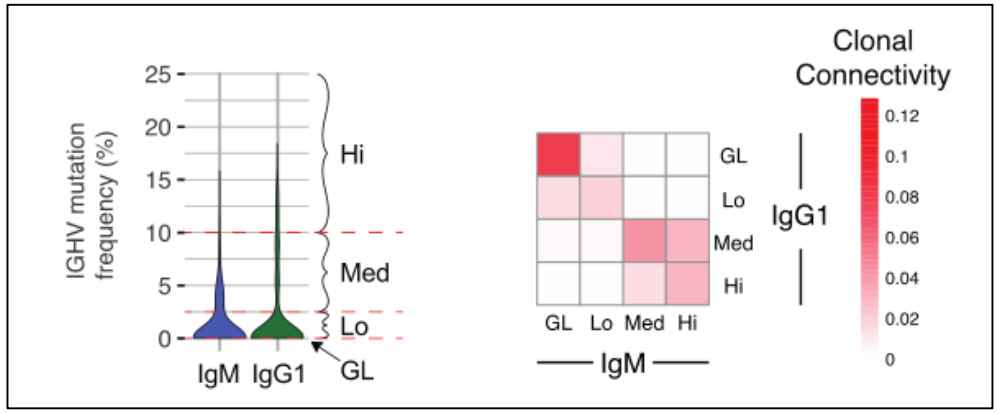


- Decreased “redemption” is similar to SLE
- SLE ASC have higher overall FR1 mutation
  - -Increased + selection of GL autoreactivity in SLE
  - -Lack of selection in COVID-19?

IgM and IgG1 compartments display significant clonal connectivity in COVID-19 ASC



IgM → IgG1 connectivity is disproportionally contributed by low mutation clones



Connectivity metric:
Simpson index subtracted from 1 (x = 1 - Simpson Index)

Summary-2

- In COVID-19 ICU patients, there is an expansion of IgM+ and IgG1+ ASCs
  - IgM and IgG1 ASCs display significantly decreased SHM
  - The IgG1 compartment shows increased usage of IGHV4-34
  - The IgM and IgG1 compartments are clonally connected, with the highest connectivity between germline clones
  - Naive B cells showed significant clonal expansions, as large in some patients as 4-5% of the entire population
  - DN and Switched memory B cells had very similar clonality between COVID and healthy, flu vaccinated samples
  - There was unusually high connectivity between Naive B cells and ASC in COVID-19 patients.
    - ❖ High naïve → ASC connectivity also characteristic of active SLE but not of healthy, vaccinated subjects

- BASELINe analysis
  - selection pressure in COVID-19 IgG1 and IgM CDR << HC and vaccinated HC
  - COVID-19 samples had slightly less hotspot mutability relative to HC

- VH4-34 FR1 GL AVY was retained in higher frequency in 2/3 COVID-19 subjects examined (the subjects with extremely low SHM). The other subject contributed to lack of significance when comparing the groups.
  - ❖ Is the frequency/pattern of VH4-34 redemption a cause and/or marker of different autoreactivity in COVID-19?