Lessons From Mouse Models of Human Immunodeficiency

John Manis
Division of Laboratory Medicine
Boston Children's Hospital,
Joint Program in Transfusion Medicine
Harvard Medical School

Why mouse models

Mouse immune systems have been well characterized and closely resemble humans

Many patients with Immune Deficiencies suffer from co-morbidities (e.g. infection) that may alter the immune phenotype

Mouse models can be engineered to offer monogenic and polygenic alterations

Mutations can be targeted to specific cell lineages or development

Study tissues beyond peripheral blood

STAT3 and HIES

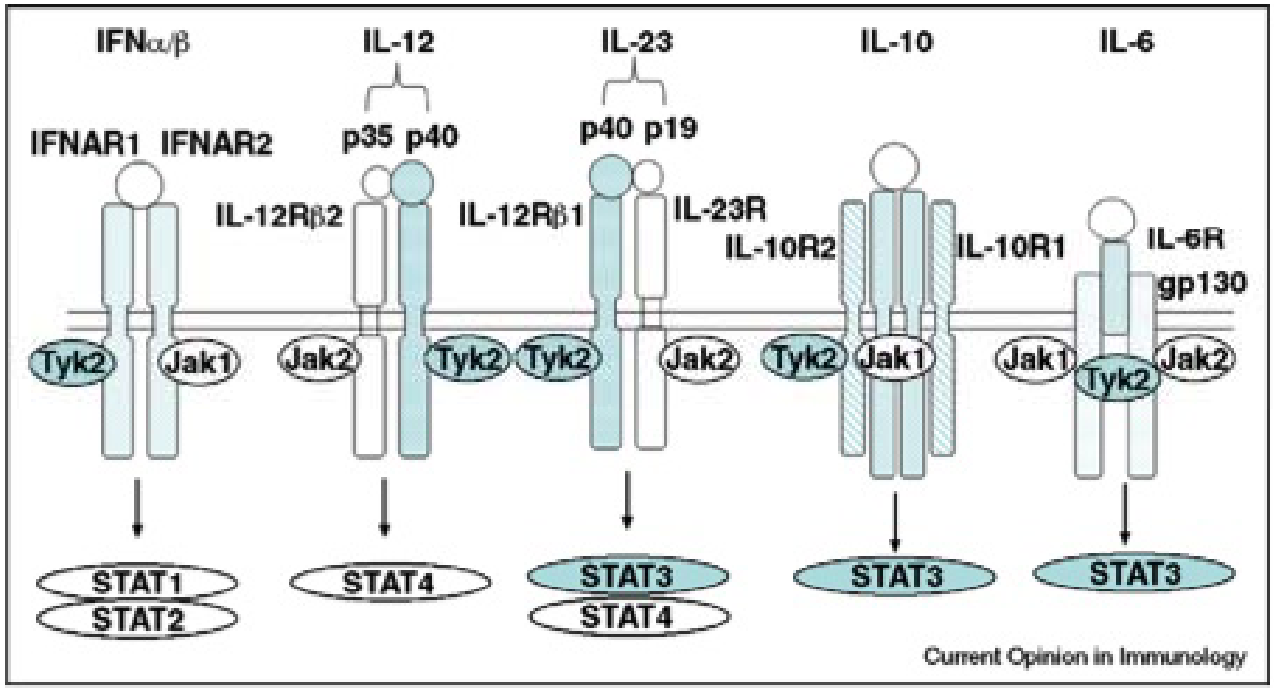
HIES is a rare multisystem disorder clinically characterized by:

Immune manifestations

- elevated serum IgE
- recurrent staphylococcal skin abscesses
- pneumonia with pneumatocele formation
- eczema
- eosinophilia

Non-immune manifestations

- characteristic face
- cathedral palate
- skeletal abnormalities:
  osteoporosis, fractures due to minor trauma, hyperextensive joints, spinal defects
- connective tissue abnormalities:
  delay in dental development and retention of deciduous teeth



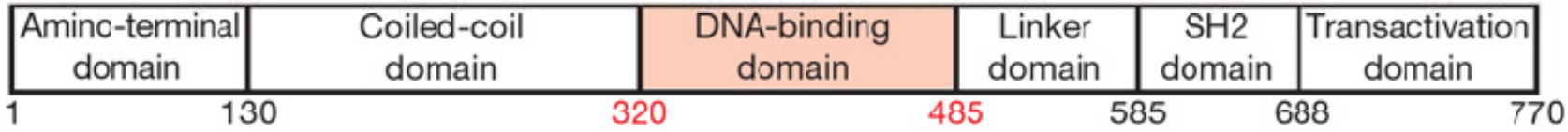
Clinical inflammatory changes and susceptibility to viral infections led work to study IFN and cytokine signaling pathways uncovering null mutations in Tyk2 for autosomal recessive patients

Etiology of Type1 unknown- other pathways were examined…

Heterozygous mutations in the Stat3 gene (predominantly in the DNA binding and in the SH2 domains) have been identified as major molecular cause of AD-HIES

(e.g. Minegishi et al., Nature 2007; Holland et al. N.Engl. J. Med.2007)

STAT3



Clinical Features of AD-HIES

	all HIES		HIES STAT3 wild-type		HIES STAT3 mutated	
	No.	%	No.	%	No.	%
Recurrent pneumonia	85/100	85	24/36	66.7	61/64	95.3
Eczema	90/100	90	32/36	88.9	58/64	90.6
Recurrent skin abscesses	86/100	86	28/36	77.8	58/64	90.6
Characteristic face	82/99	82.8	24/35	68.6	58/64	90.6
Failure to shed deciduous teeth	60/86	68.9	16/31	51.6	44/55	80.0
Lung cyst formation	61/97	62.9	14/34	41.2	47/63	74.6
Eosinophilia	68/94	72.3	27/36	75.0	41/58	70.7
Newborn rash	52/86	60.5	15/29	51.7	37/57	64.9
Other unusual infections	47/94	50	13/34	38.2	34/60	56.7
Increased interalar distance	37/83	44.6	10/31	32.3	27/52	51.9
Cathedral palate	41/84	48.8	12/31	38.7	29/53	54.7
Hyperextensibility	37/87	42.5	8/32	25.0	29/55	52.7
Pathologic bone fractures	32/94	34.0	5/35	14.3	27/59	45.8
Recurrent upper respiratory infections	41/92	44.6	14/33	42.4	27/59	45.8
Candidiasis	37/91	40.6	12/33	36.4	25/58	43.1
Scoliosis	20/83	24.1	7/33	21.2	13/50	26.0
Midline anomaly	12/86	14.0	5/34	14.7	7/52	13.5

Holland et al. N.Engl. J. Med.2007

Spectrum of mutations in Stat3

No. of patients	Protein domain	Site of mutation	DNA sequence change	Predicted amino acid change
1	Coiled-coil	Exon 3	c.172C>T	H58Y
1	DNA-binding	Exon 10	c.982_990dupTGCATGCCC	C328_P330dup
1	DNA-binding	Exon 10	c.1025G>A	G342D
2	DNA-binding	Intron 11	c.1110–2A>G	D371_G380del
1	DNA-binding	Intron 11	c.1110–1G>T	D371_G380del
1	DNA-binding	Intron 12	c.11391 1G>T	D371_G380del
1	DNA-binding	Intron 12	c.11391 2insT	D371_G380del
14	DNA-binding	Exon 13	c.1144C>T	R382W
2	DNA-binding	Exon 13	c.1145G>T	R382L
9	DNA-binding	Exon 13	c.1145G>A	R382Q
2	DNA-binding	Exon 13	c.1150T>C	F384L
1	DNA-binding	Exon 13	c.1166C>T	T389I
1	DNA-binding	Exon 14	c.1268G>A	R423Q
1	DNA-binding	Exon 16	c.1387_1389delGTG	V463del
1	DNA-binding	Exon 16	c. 1396 A>G	N466D
1	DNA-binding	Exon 16	c.1397A>G	N466S
1	DNA-binding	Exon 16	c.1397A>C	N466T
1	DNA-binding	Exon 16	c.1398C>G	N466K
1	DNA-binding	Exon 16	c.1407G>T	Q469H
1	SH2	Exon 20	c.1771A>G	K591E
1	SH2	Exon 20	c.1865C>T	T622I
1	SH2	Exon 21	c.1907C>A	S636Y
10	SH2	Exon 21	c.1909G>A	V637M
1	SH2	Exon 21	c.1910T>C	V637A
1	SH2	Exon 21	c.1915T>C	P639S
1	SH2	Exon 21	c.1970A>G	Y657C
1	SH2	Exon 21	c.2003C>T	S668F
1	Transactivation	Exon 22	c.2124C>G	T708S
1	Transactivation	Exon 22	c.2129T>C	F710C
1	Transactivation	Exon 22	c.2141C>G	T714A
1	Transactivation	Intron 22	c.21441 1G>A	p.?

Generate a mouse model of Job's Syndrome

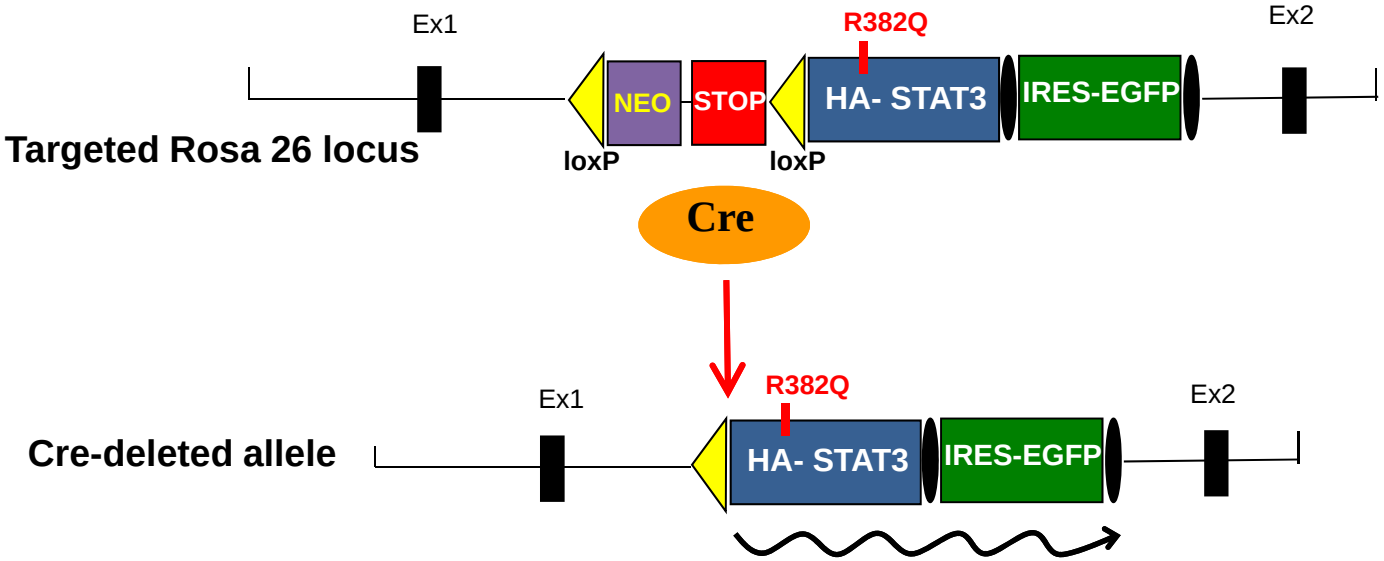
Challenges:

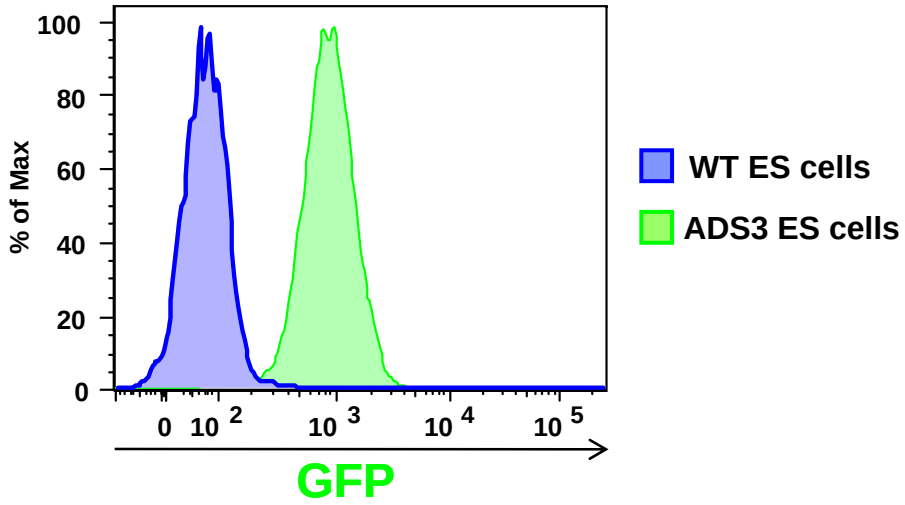
- **Stat 3 is important in maintaining ES cell pluripotency, thus mutant Stat3 cannot be expressed in targeted ES cells for germline expression**

- **Job's Syndrome affects many tissues, it would be useful to express the mutation in a tissue specific fashion**

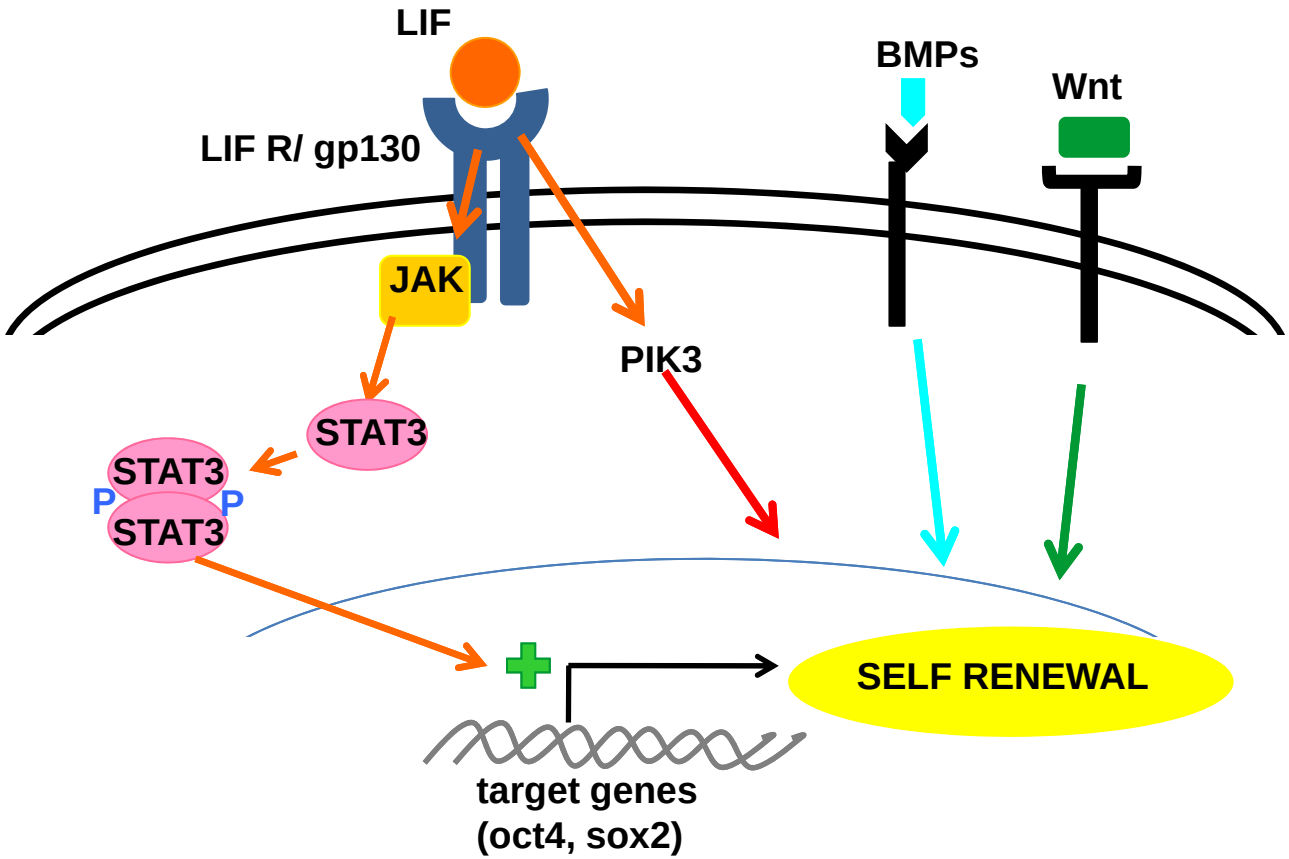
- **Stat3 null mutant mice are embryonic lethal, heterozygous mice have no phenotype c/w Job's Syndrome**

Generation of Stat3 R382Q transgenic ES cells





STAT3 and ES cells

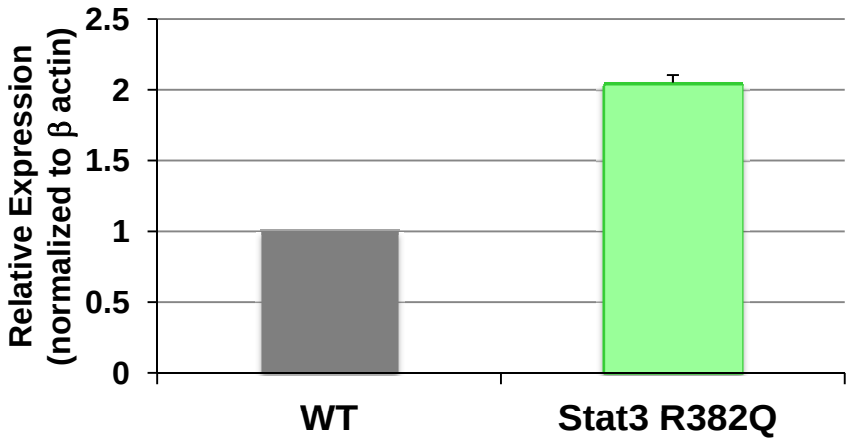


Targeted STAT3 disruption in mice leads to early embryonic lethality due to defects in trophoblast development

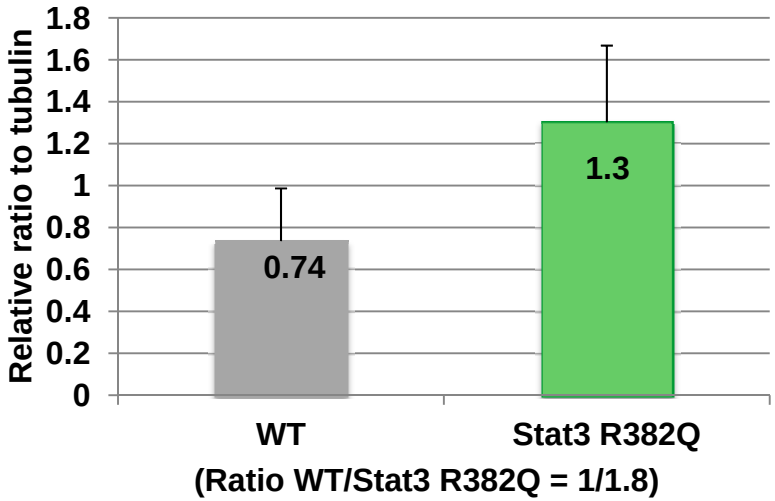
What are the effects of HIES-associated Stat3 mutations on ES cell developmental programs?

Equivalent expression of mutant and endogenous Stat3 in transgenic Stat3 R382Q ES cells

STAT3 mRNA

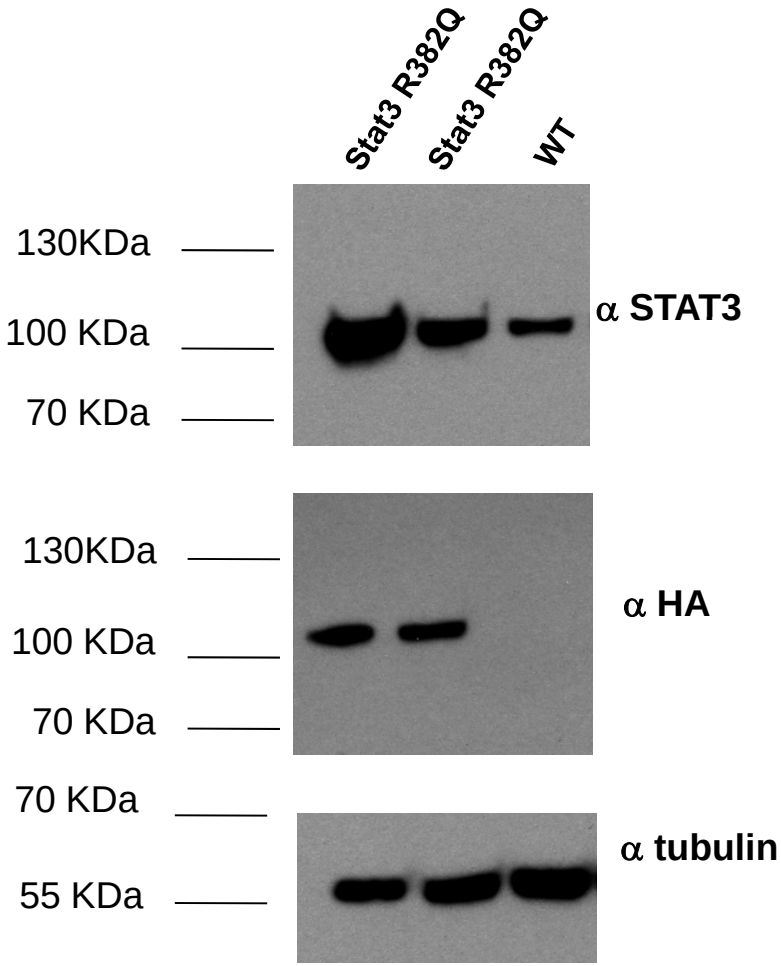


STAT3 protein

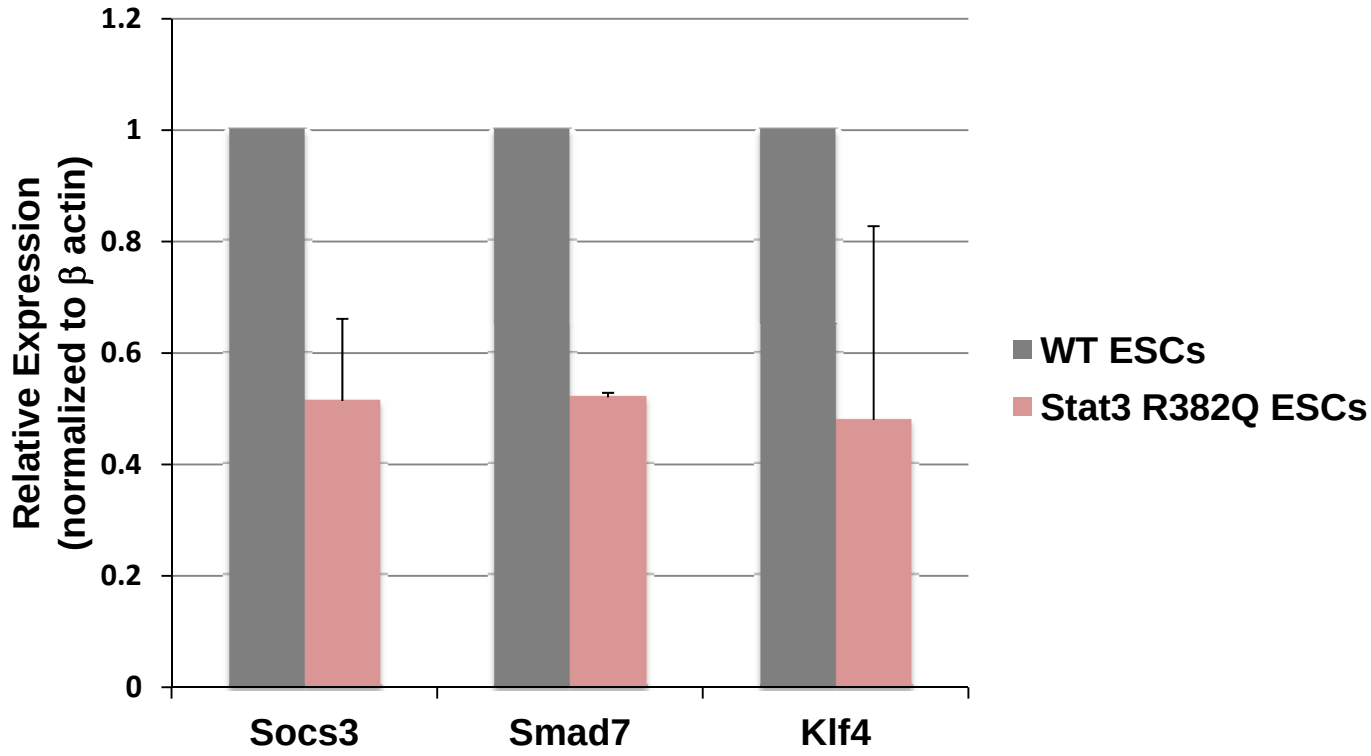


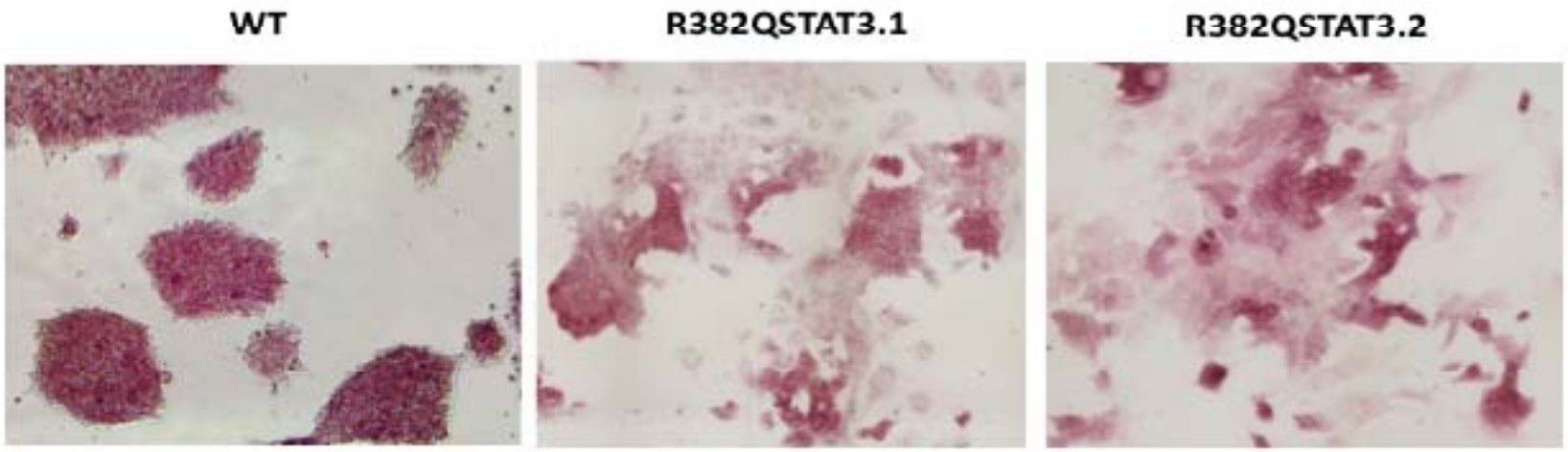
(Ratio WT/Stat3 R382Q = 1/1.8)

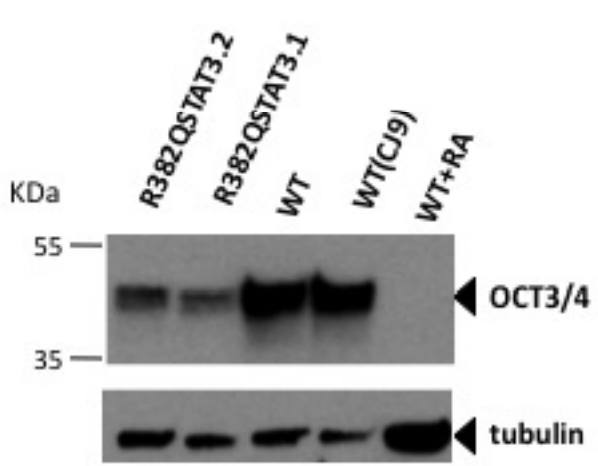
STAT3 protein
(total ES cells extracts)

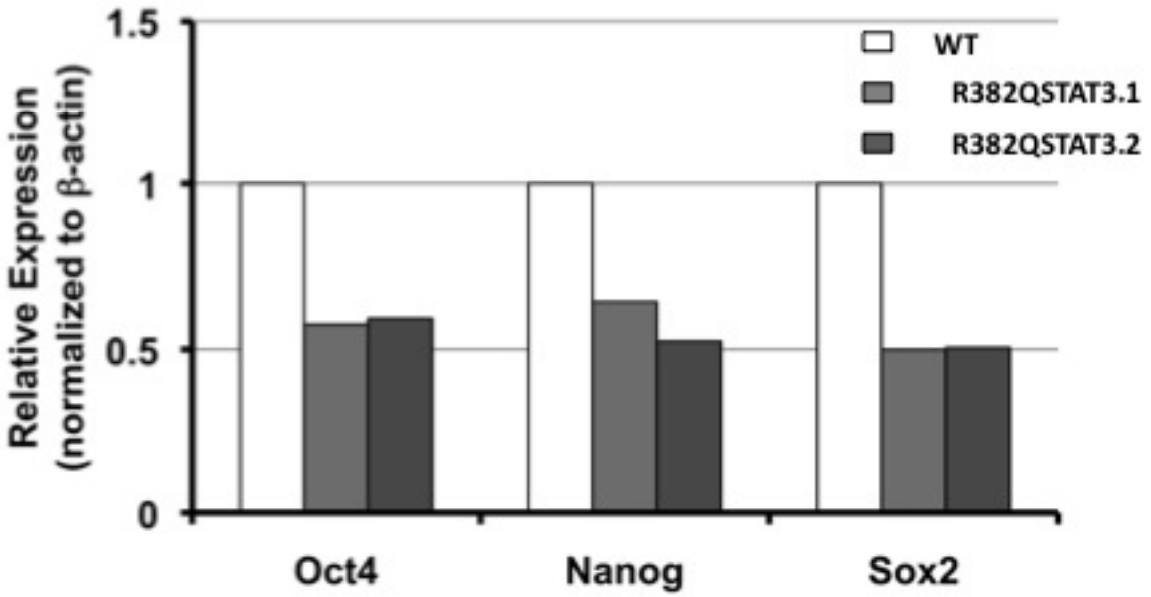


Reduced expression of STAT3 direct targets in R382Q ES cells




WT
R382QSTAT3.1
R382QSTAT3.2


KDa
R382QSTAT3.2
R382QSTAT3.1
WT
WT(CJ9)
WT+RA
55
35
OCT3/4
tubulin


Relative Expression
(normalized to β-actin)
1.5
1
0.5
0
WT
R382QSTAT3.1
R382QSTAT3.2
Oct4
Nanog
Sox2

Summary

R382Q Stat3 expression in murine ES Cells disrupts programs for growth and self renewal

Selection pressures during development may favor the wildtype allele and parallels the sporadic distribution of the disease

STAT3 is involved in many cell signaling pathways, explaining the non-immune phenotypes

Our targeting strategy mirrors the mutations seen in Job's patients

Conditional Expression of R382Q Stat3 in Mice

HIES is a rare multisystem that affects many tissues: it would be useful to express the mutation in a tissue specific fashion

Mice generated from Stat3 R382Q transgenic ES cells

- CD4 Cre: mutant STAT3 expressed specifically in T cells (RS3 CD4)
- Mb1Cre: mutant STAT3 expressed specifically in B cells (RS3 Mb1)
- EIIA Cre: mutant STAT3 expressed in all tissues (ADS3/+)

Immune responses in Job's patients

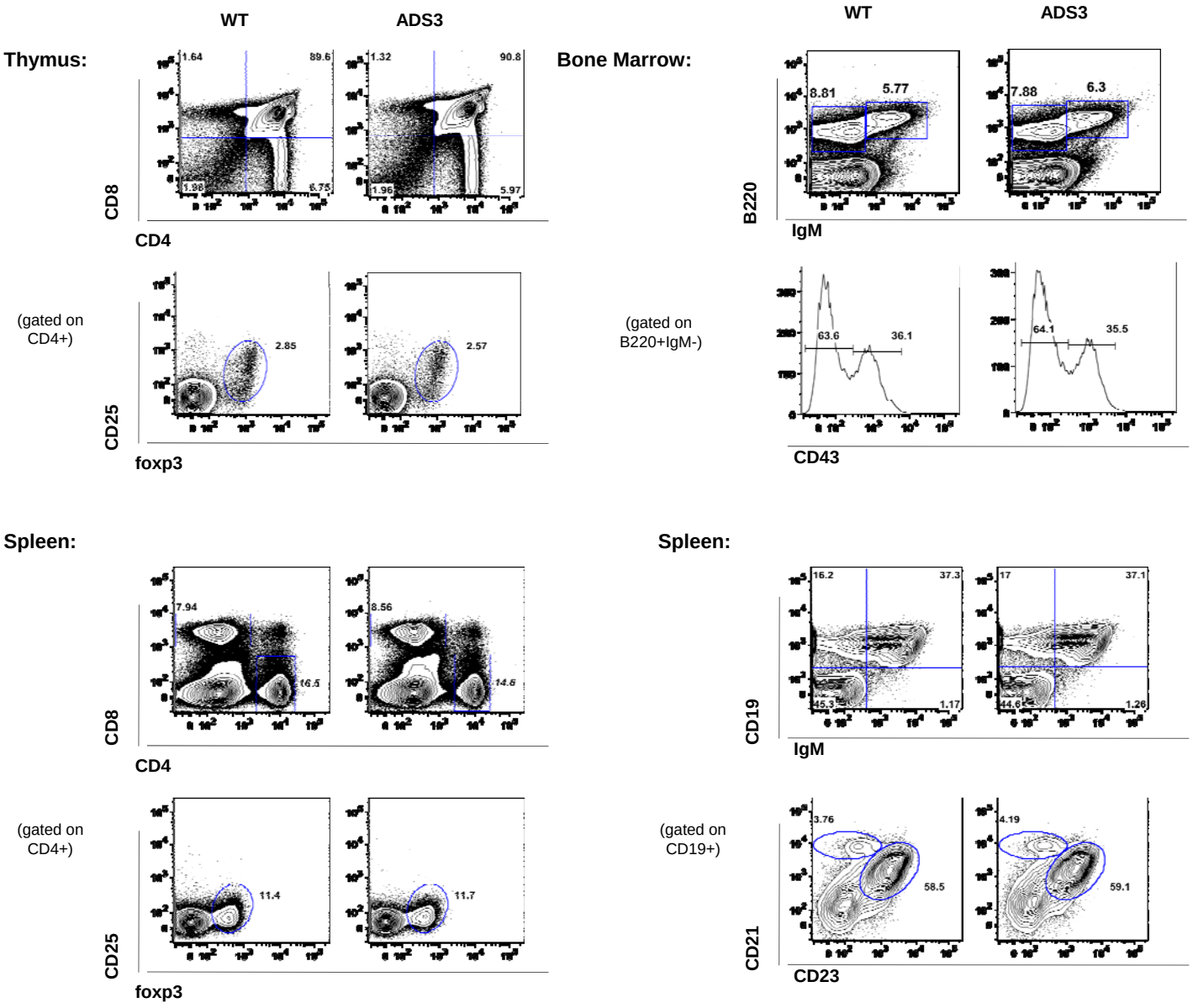
Defective TH17 immunity

Defective TfH cell development

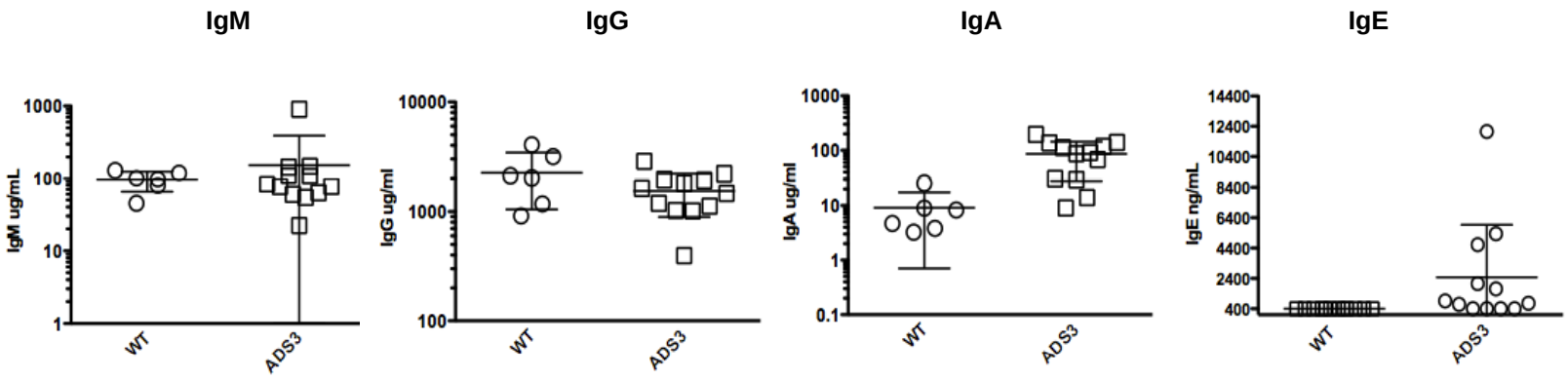
Defective memory cell responses

Elevated serum IgE

Normal B and T cell development in ADS3/+ mice



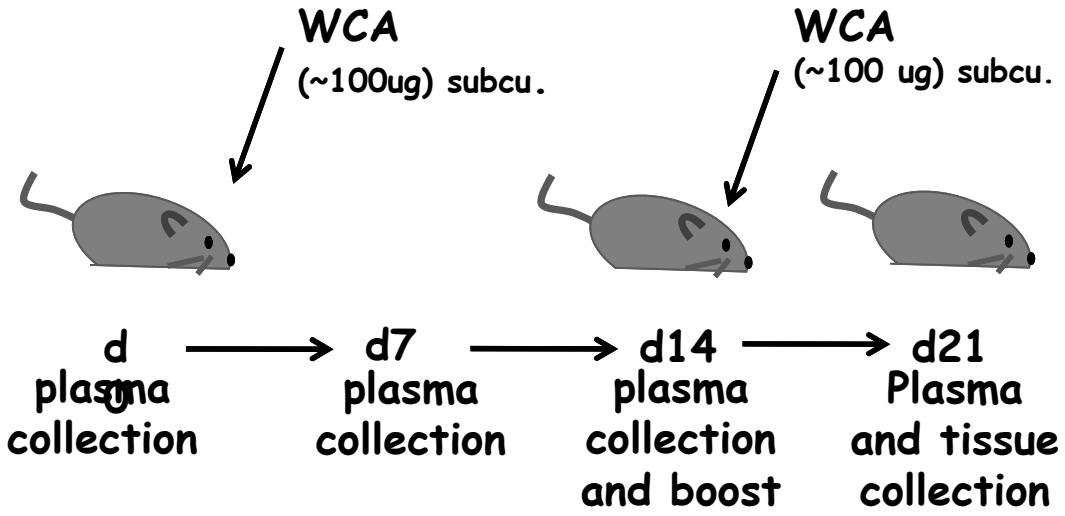
Naïve ADS3/+ mice develop elevated levels of serum IgE



S. pneumoniae whole cell antigen (WCA) immunization

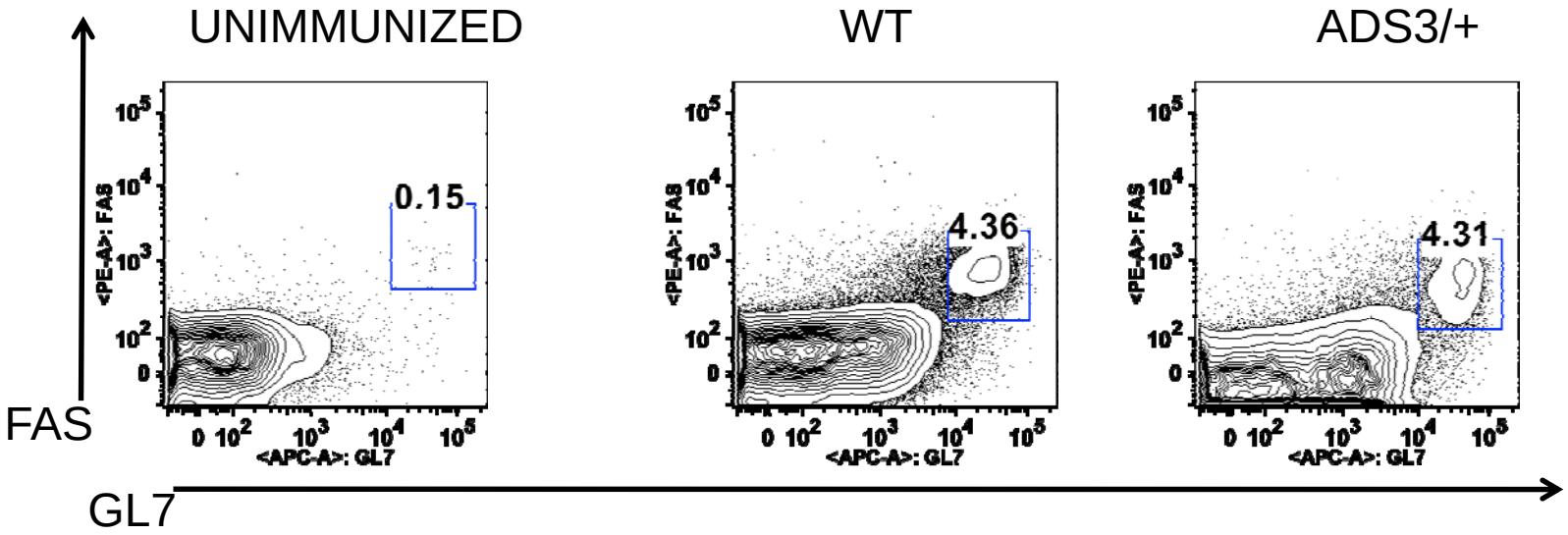
Patients with Job's syndrome have recurrent infections-typically staphylococcal or streptococcal

Immunization to *S Pneumoniae* induces a TH17-dependent immune response (Lu et al, 2008)



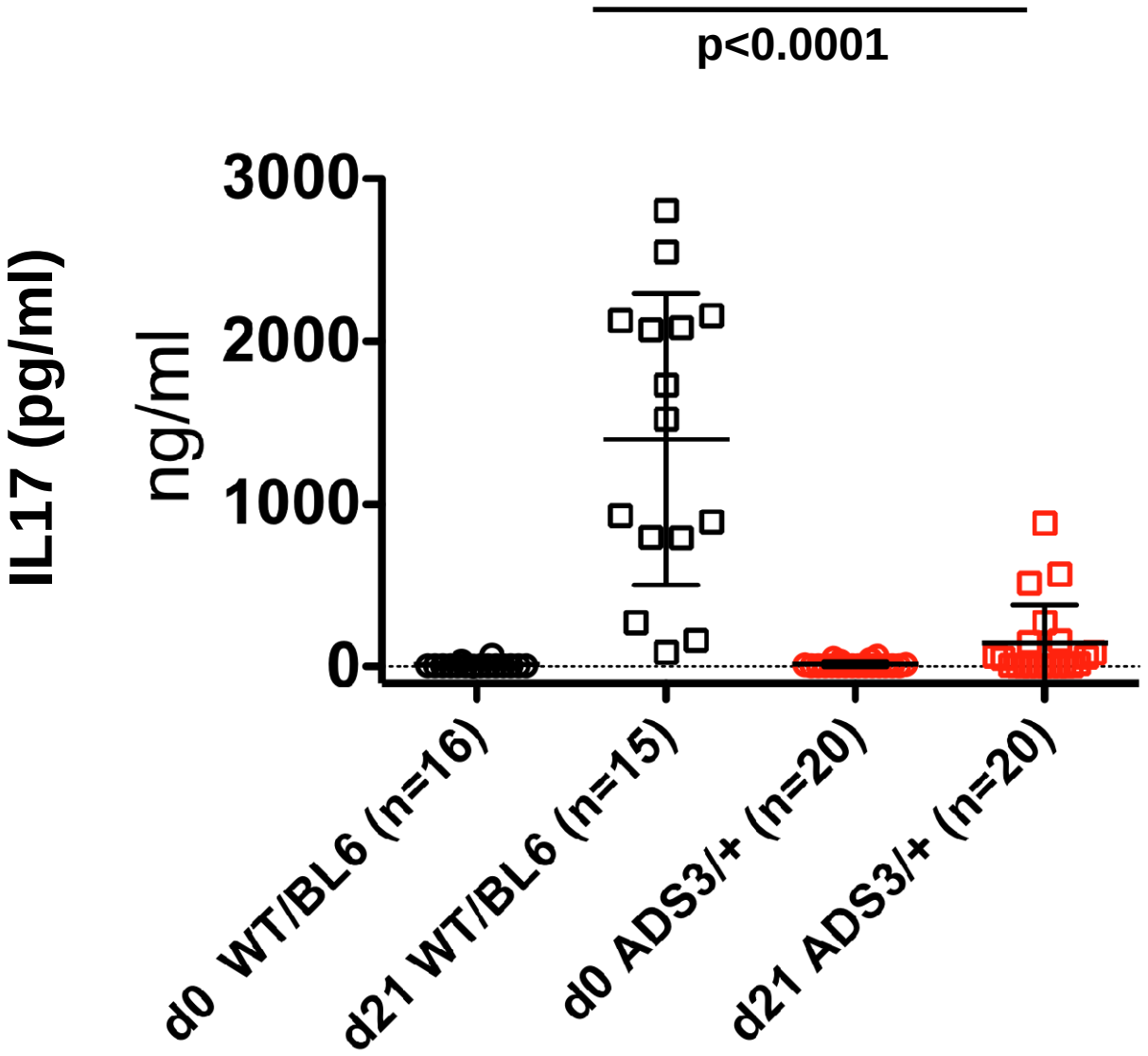
Intact GC formation in ADS3/+ mice

Gate on CD19+ live cells:

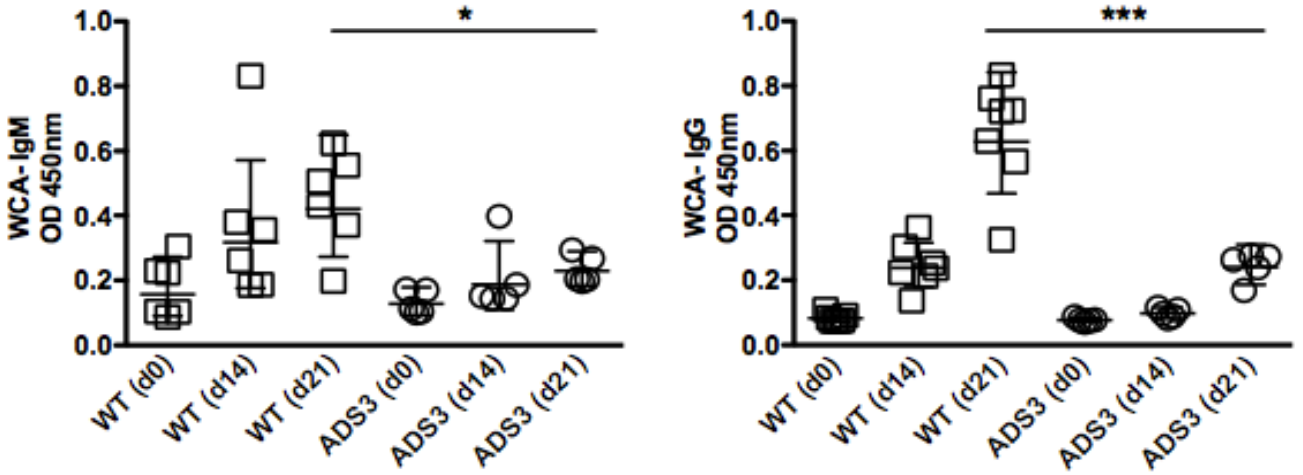


GC B cells (CD19+FAS+GL7+) from draining lymph nodes

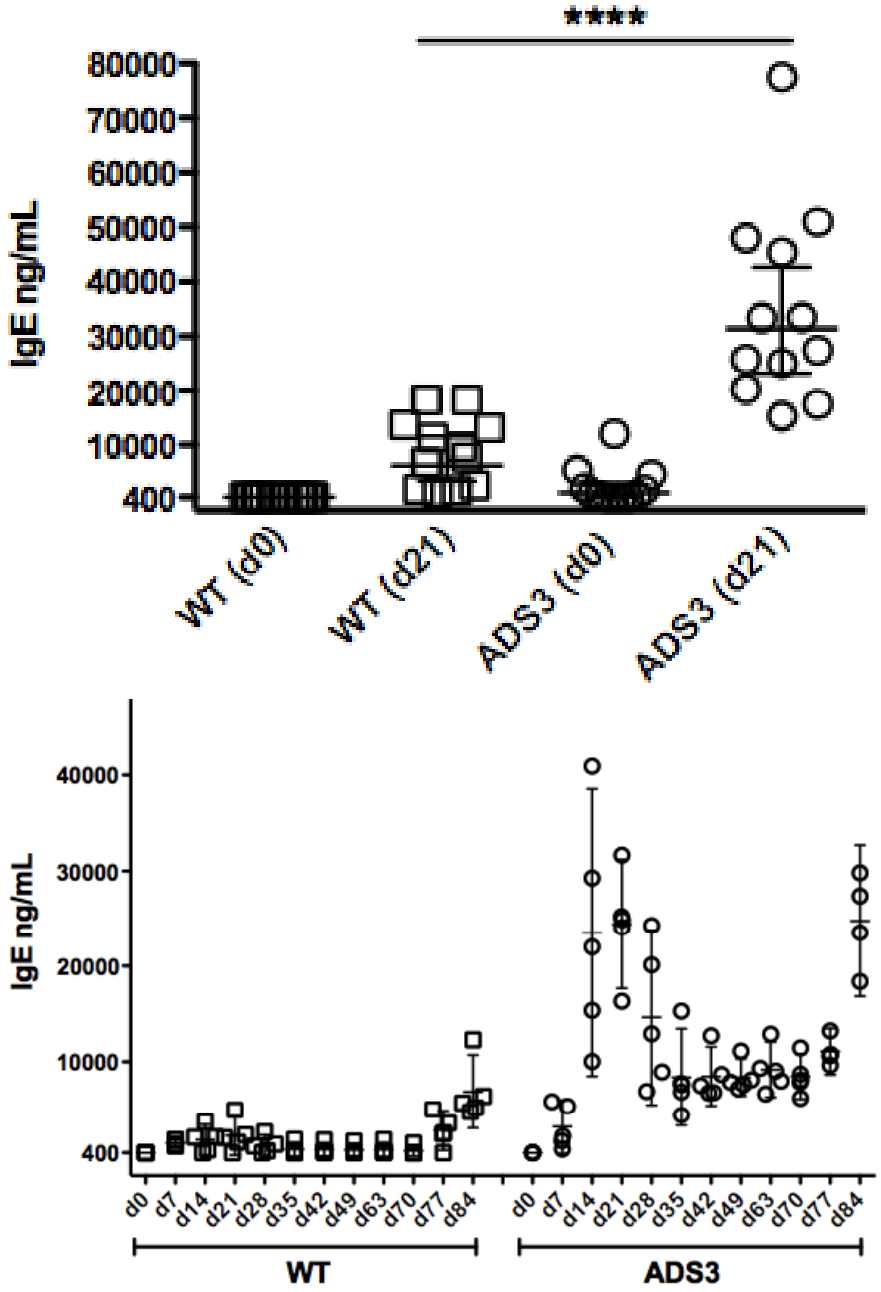
Defective IL17 production in peripheral blood from WCA immunized ADS3/+ mice



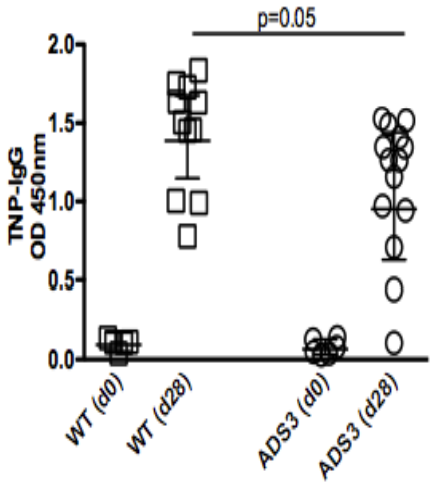
Defective WCA-specific IgM and IgG production in ADS3/+ mice

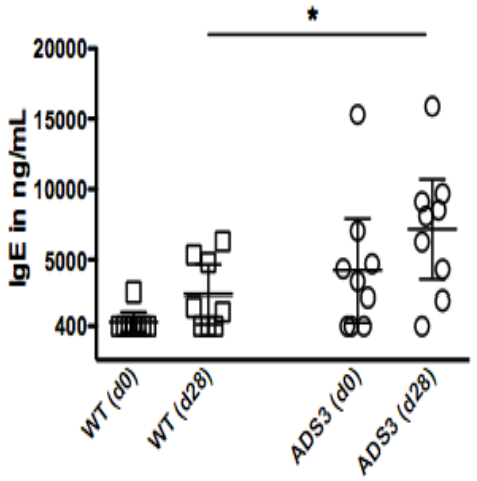


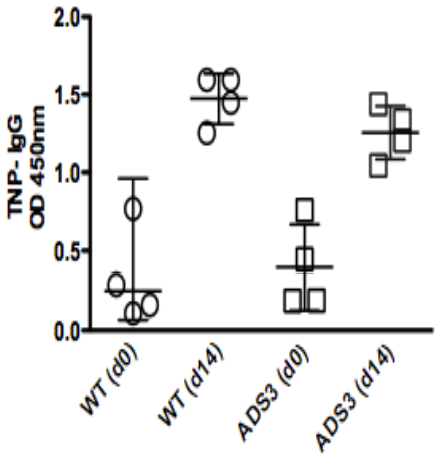
Increased Total IgE in ADS3/+ mice after immunization

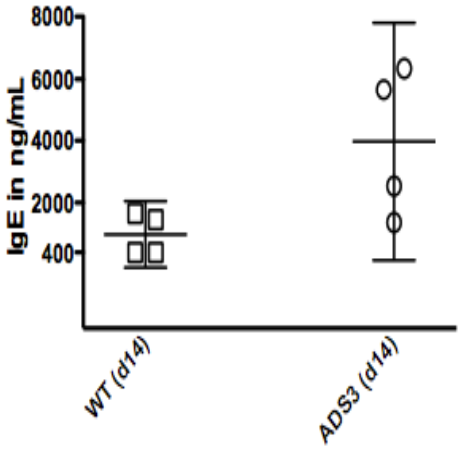


Modest defect in NP specific IgG responses after T-dependent immunization; normal T-Independent responses









Summary

Defective IgG responses in response to TH17 associated antigens

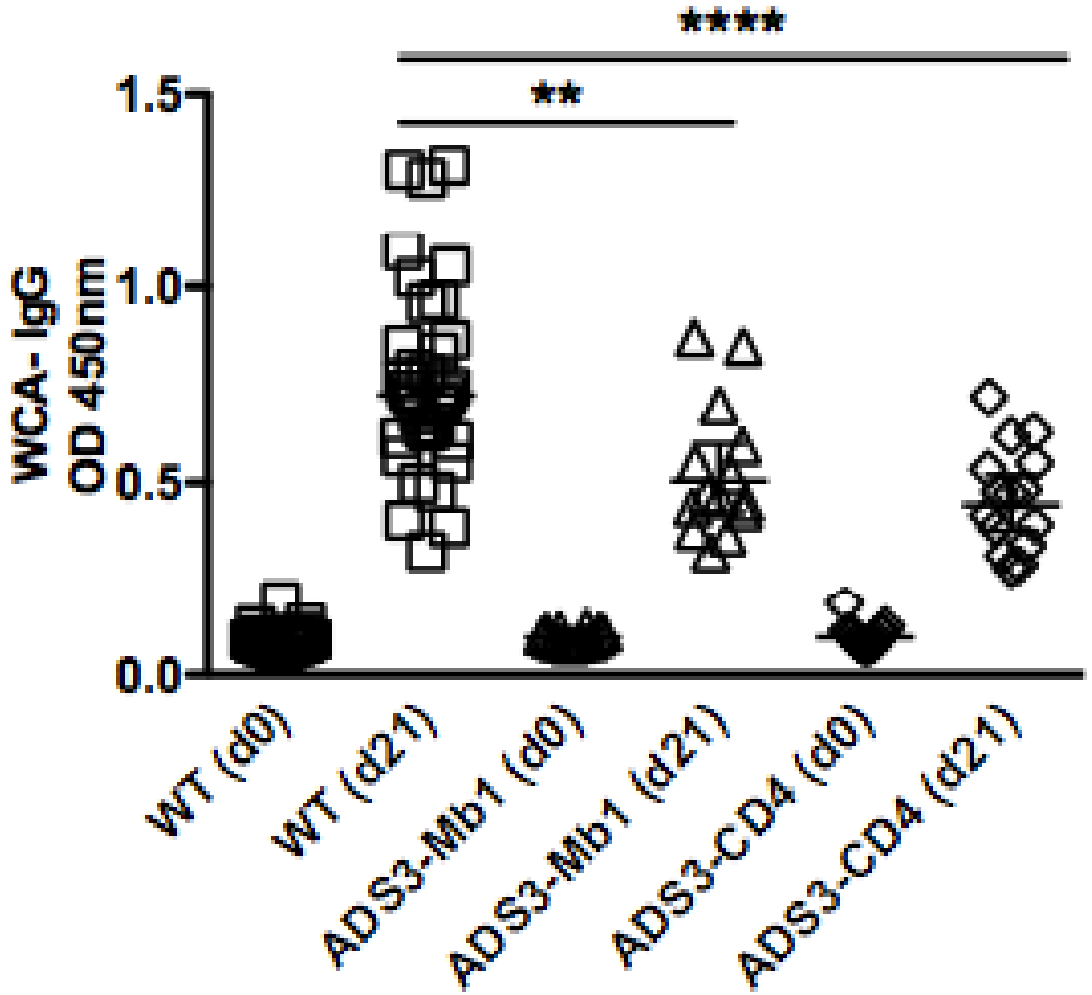
Greatly elevated IgE serum levels in response to antigen

Independent of Alum or adjuvant

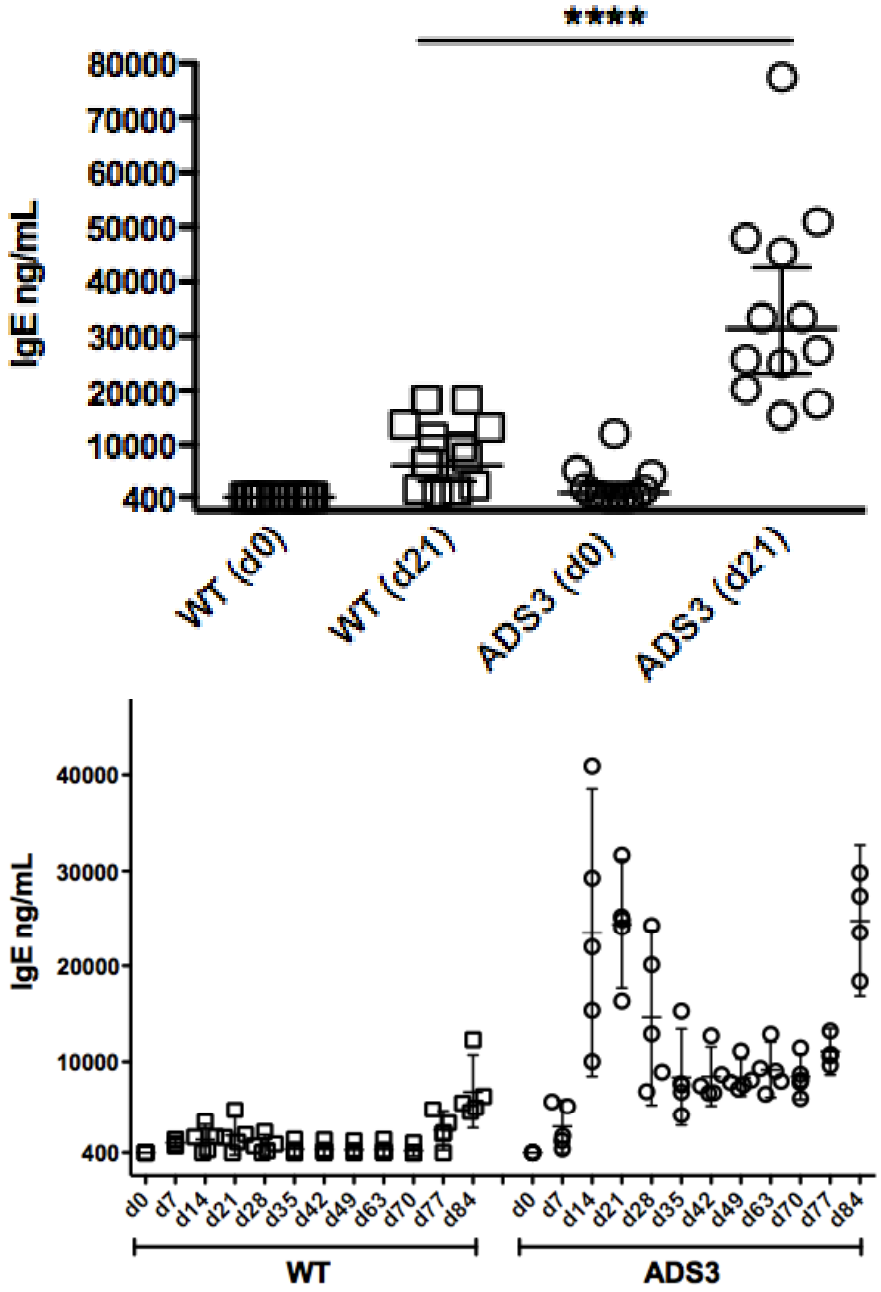
Defective *in vivo* plasma cell differentiation
    Impaired Blimp expression

What is the role of B cells in the immune response?
What mechanisms underlie the elevated serum IgE?

Defective WCA-specific IgG production in B cell or T cell specific ADS3 mice

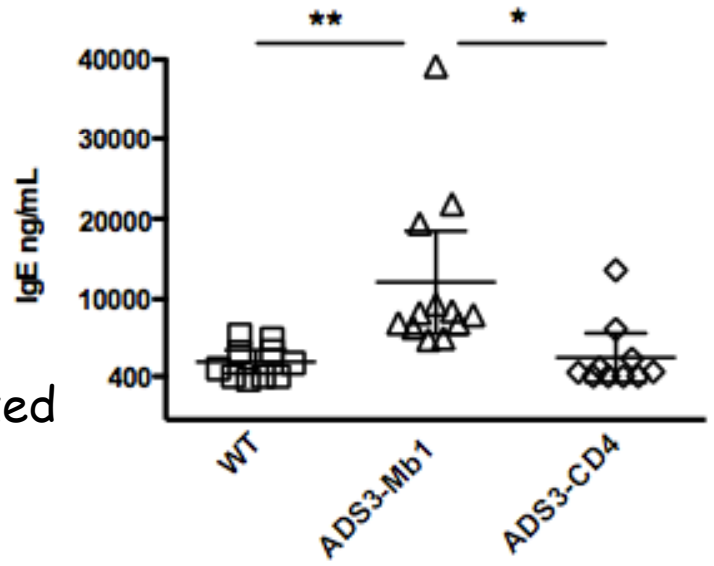


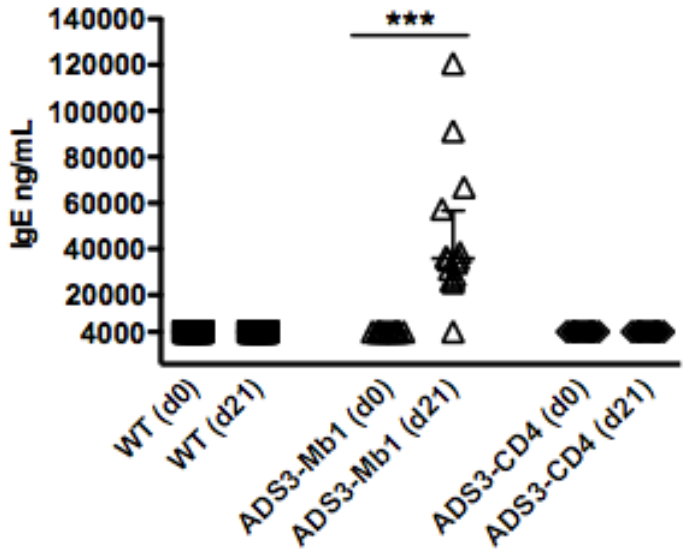
Increased Total IgE in ADS3/+ mice after immunization



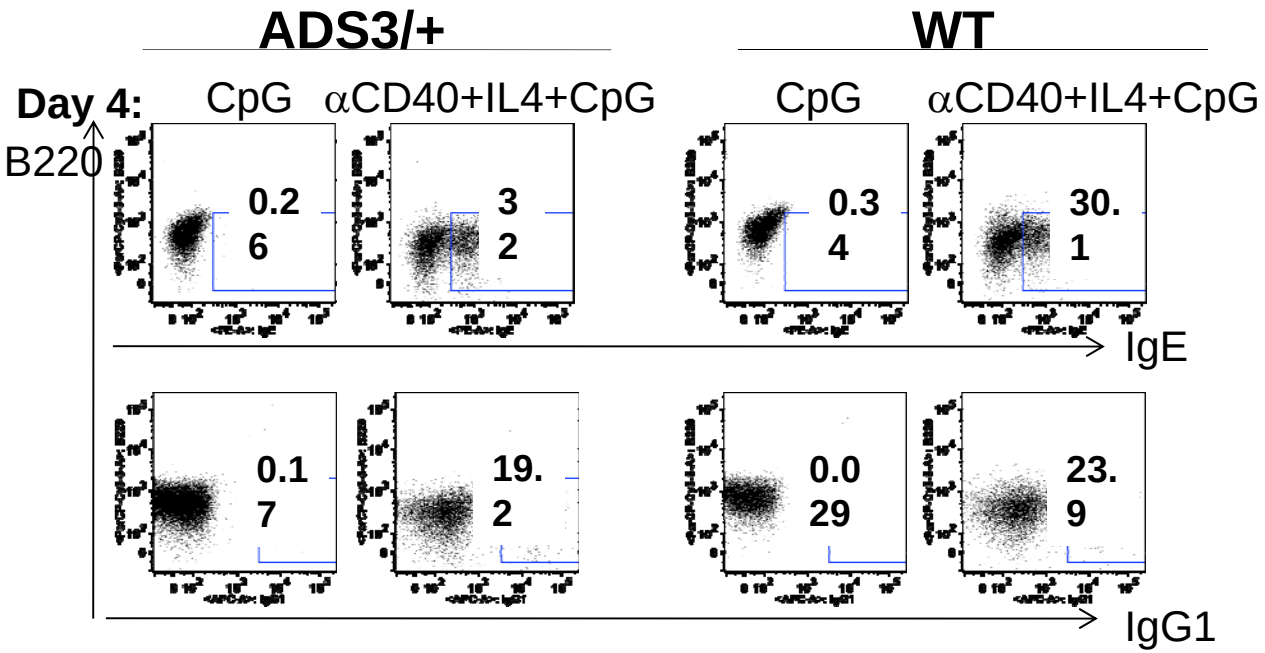
IgE production is seen only in the presence of ADS3/+ B cells

unimmunized

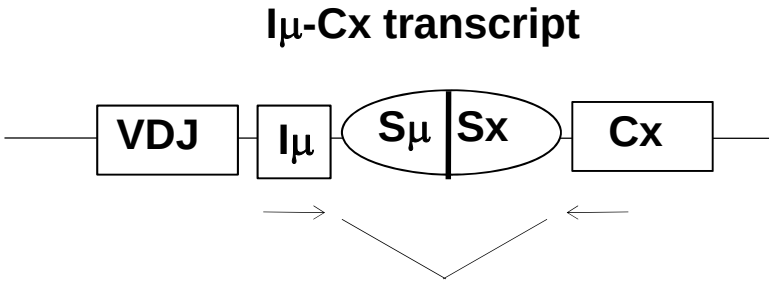


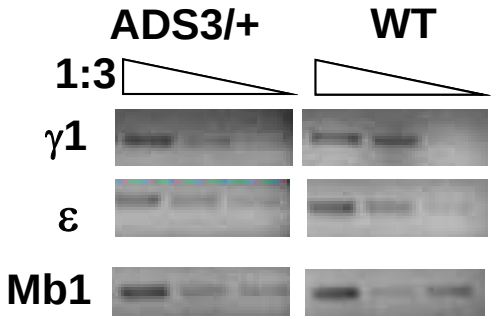


Where is the increased IgE made?
Increased propensity of B cells to undergo switching to IgE?



Normal Class Switch Recombination upon *in vitro* stimulation in ADS3/+ B Cells





If there is no evidence for increased IgE production

No increased numbers in LN, BM, peritoneum, spleen

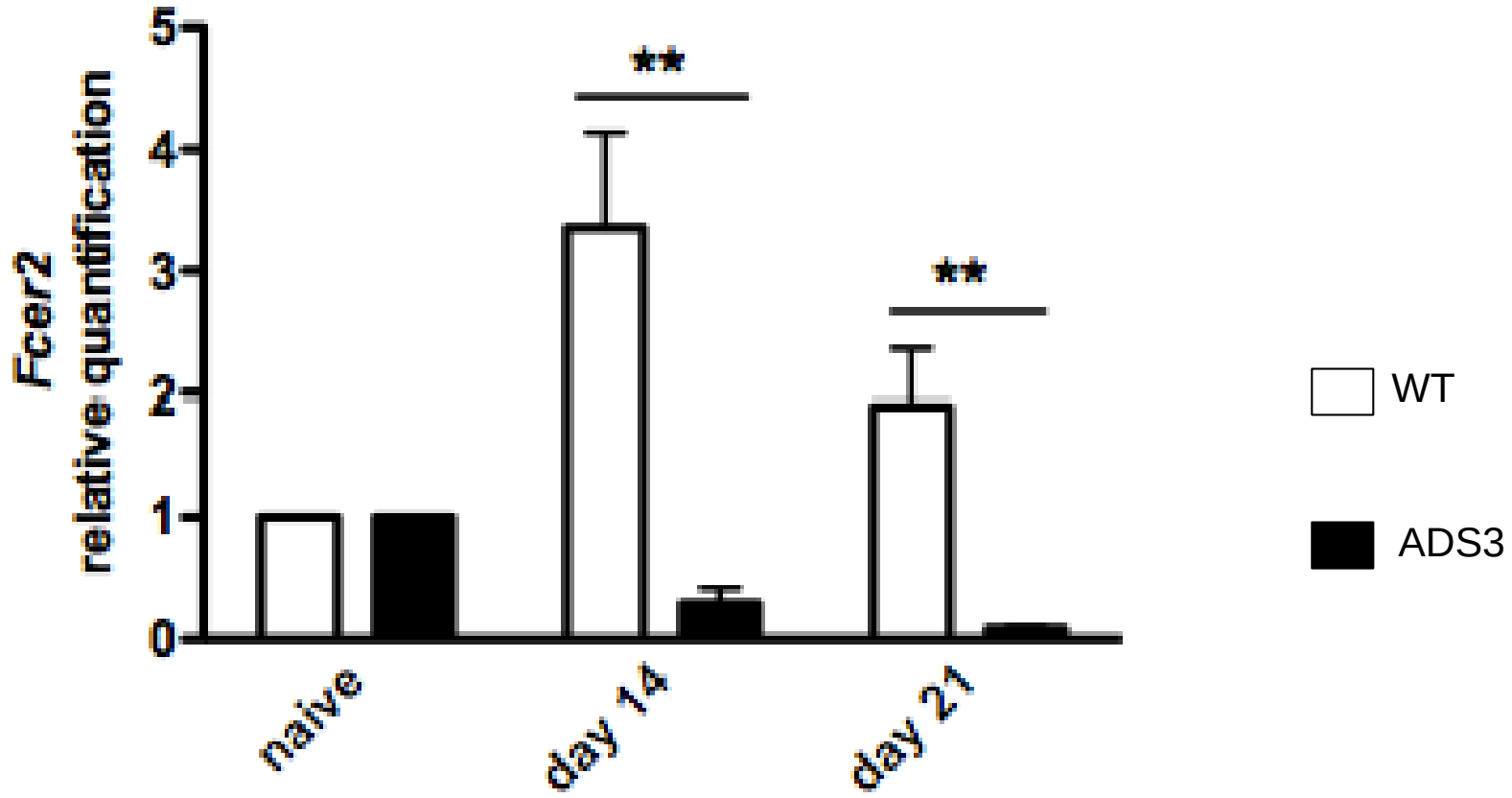
No evidence for more IgE plasma cells

Regulators of serum IgE levels

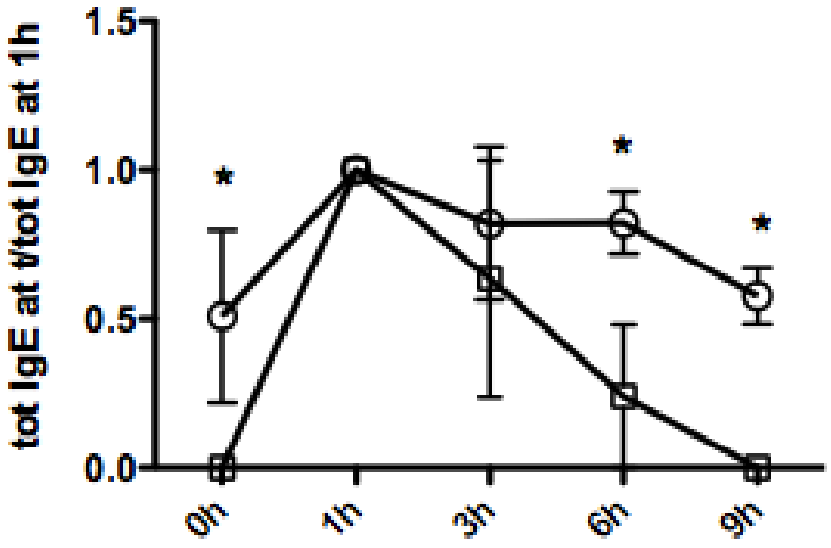
FCER-high affinity expressed on mast cells, basophils

FCER2- low affinity receptor- in mouse expressed on B cells. Mice deficient for FCER2 have elevated IgE

Failure to induce CD23 expression in ADS3 B cells



Altered kinetics of IgE serum clearance in ADS3 mice



Conclusions

Defective response to antigens that function through a TH17-dependent immune response (WCA)

No significant defect in immune responses to other T-dependent antigen (TNP-KLH) or to T independent antigen (TNP Ficoll)

Defective antigen specific IgG responses are due to both T and B cell specific mechanisms

Elevated IgE levels seen in naïve mice, and especially after immunization with specific antigens

Elevated IgE results from mutant B cells

ADS3 B cells intrinsically fail to upregulate FCER2, and contributes to the elevated serum IgE levels

Phenotypic Diversity of RAG1 deficiency

There is increasing recognitions of patients with RAG deficiency presenting:

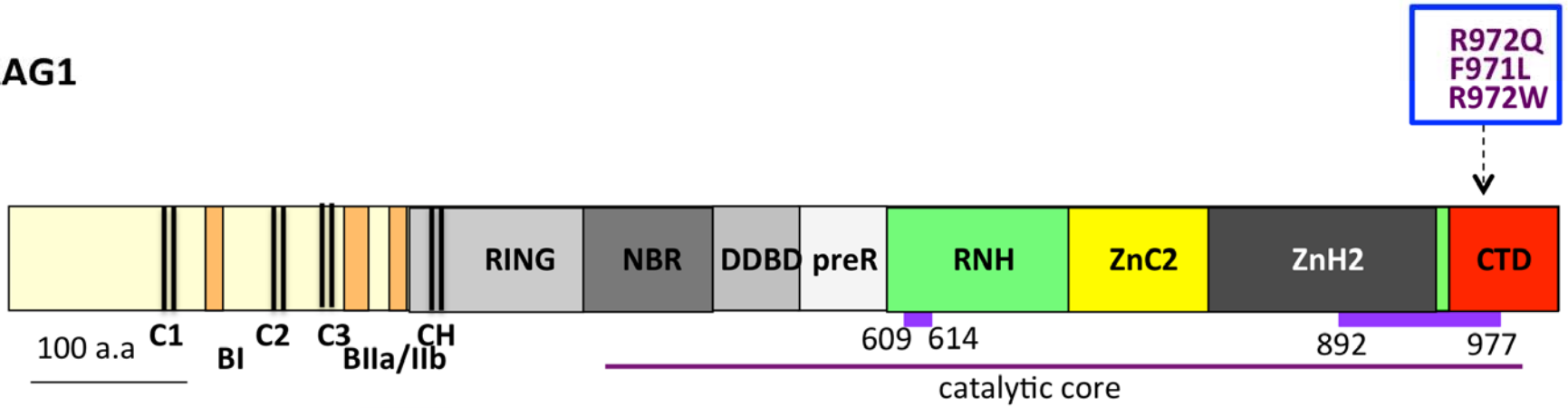
1- At an older age

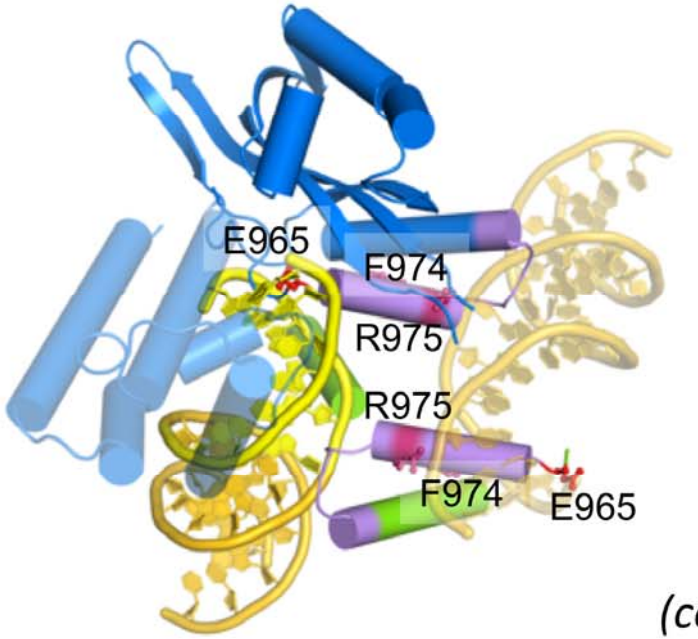
2- Few overwhelming infections during childhood

3- Significant peripheral lymphocyte populations

4- Autoimmune features

RAG1

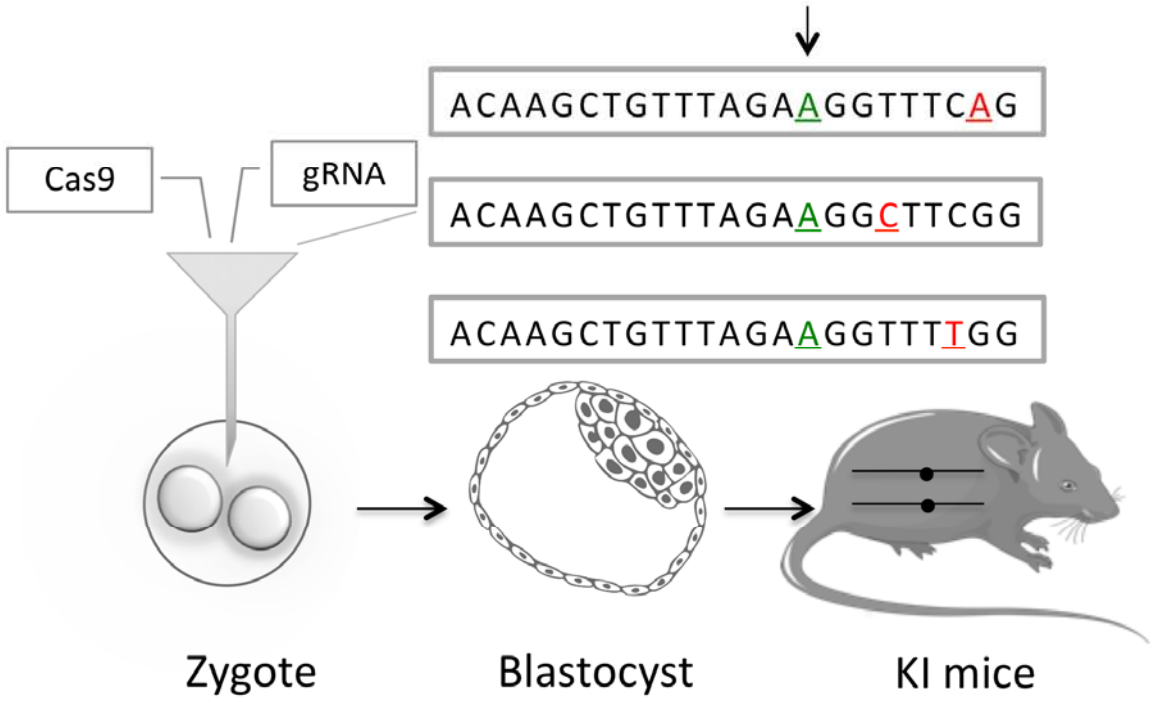




(courtesy: Wei Yang, NIH)

3 models generated with CRISPR/Cas9

- **Silent mutation**
- **ssODN Mix**



R972Q

F971L

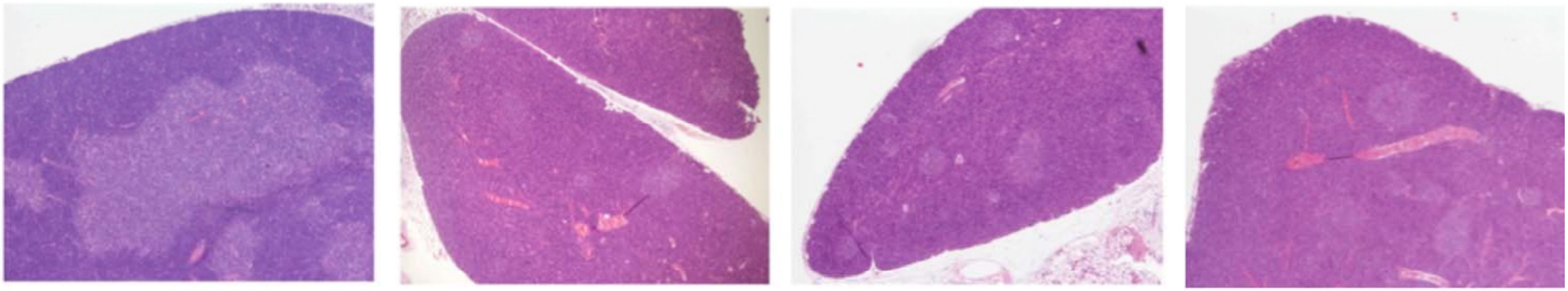
R972W

- 26 pups
- 1 WT/indel
- 25 with mutation

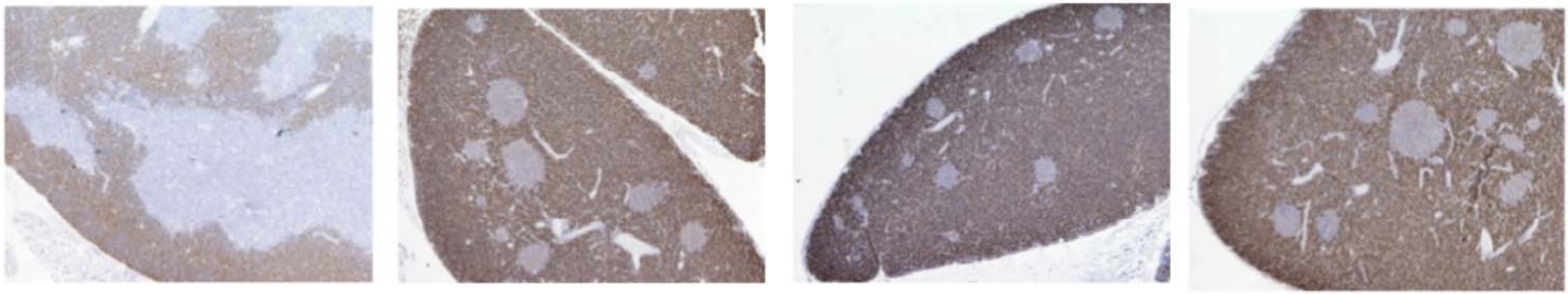
Rapid generation of novel models of RAG1 deficiency by CRISPR/Cas9-induced mutagenesis in murine zygotes. Ott de Bruin et al. Oncotarget. 2016

Thymic architecture

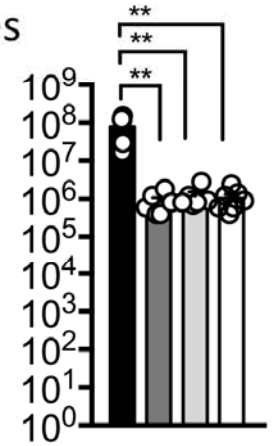
H&E *WT* ■ *R972Q* ■ *F971L* □ *R972W* □



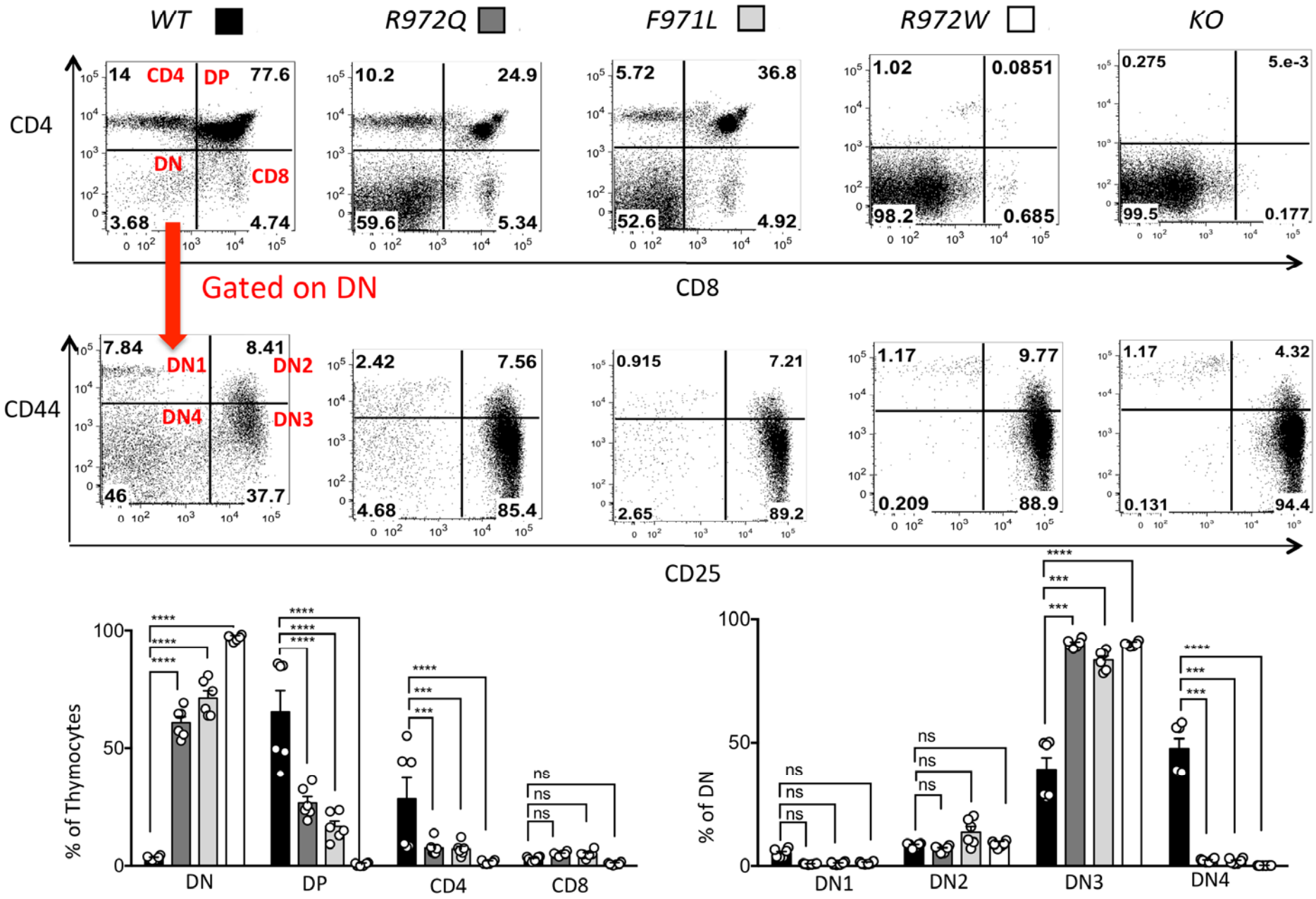
TdT



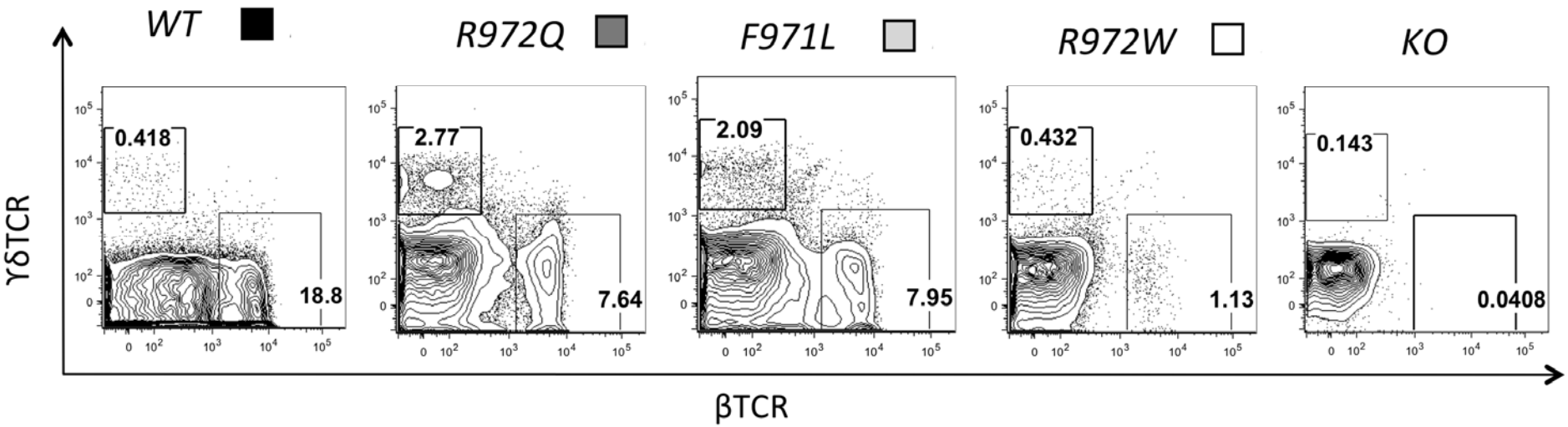
Total Thymocytes

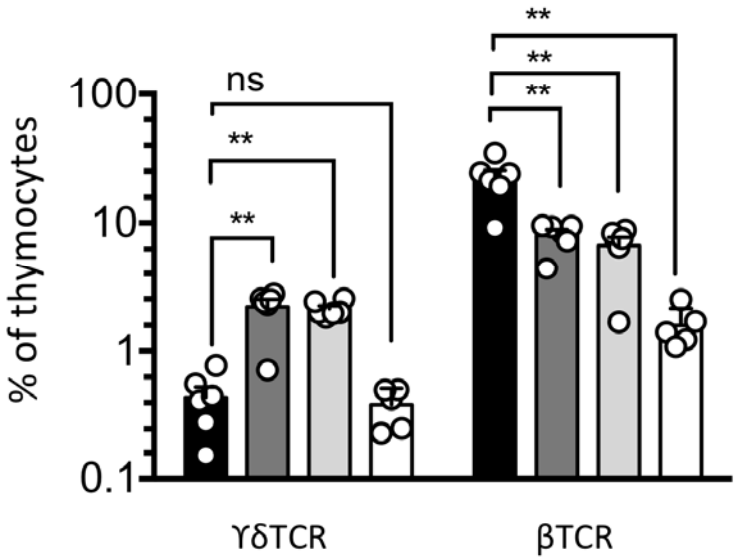


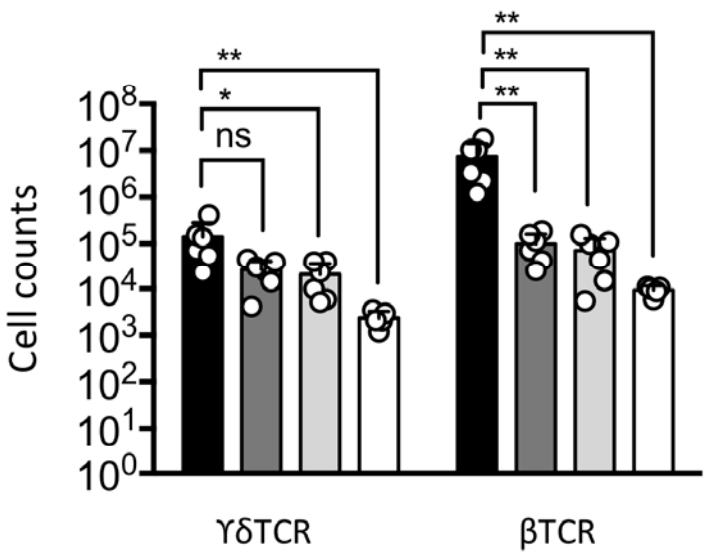
Thymus


WT
R972Q
F971L
R972W
KO
CD4
CD8
14
CD4
DP
77.6
DN
CD8
3.68
4.74
10.2
24.9
59.6
5.34
5.72
36.8
52.6
4.92
1.02
0.0851
98.2
0.685
0.275
5.e-3
99.5
0.177
Gated on DN
CD44
CD25
7.84
DN1
8.41
DN2
DN4
DN3
46
37.7
2.42
7.56
4.68
85.4
0.915
7.21
2.65
89.2
1.17
9.77
0.209
88.9
1.17
4.32
0.131
94.4
% of Thymocytes
DN
DP
CD4
CD8
% of DN
DN1
DN2
DN3
DN4
ns
****
***

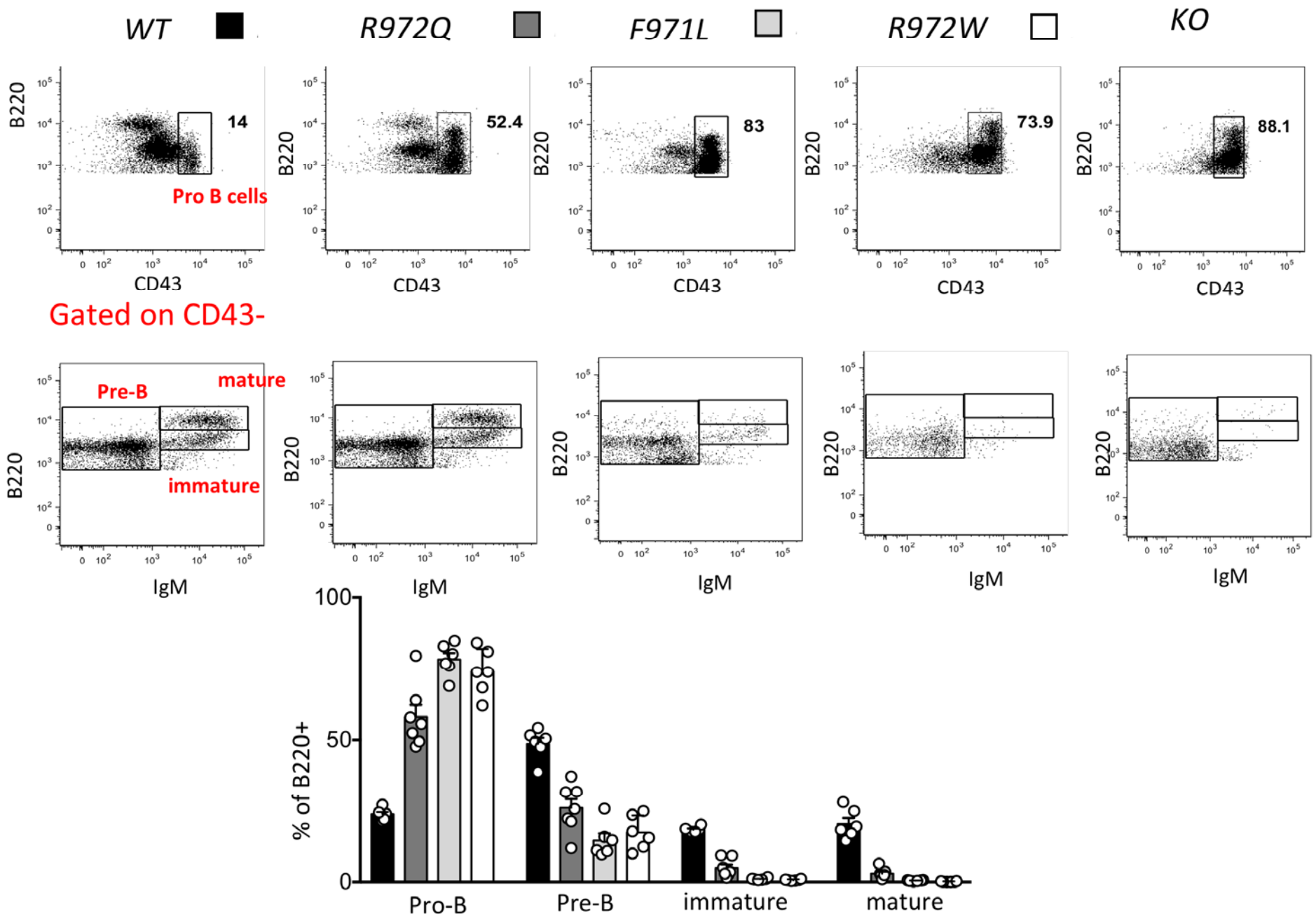
γδ TCR proportion increased in Thymus







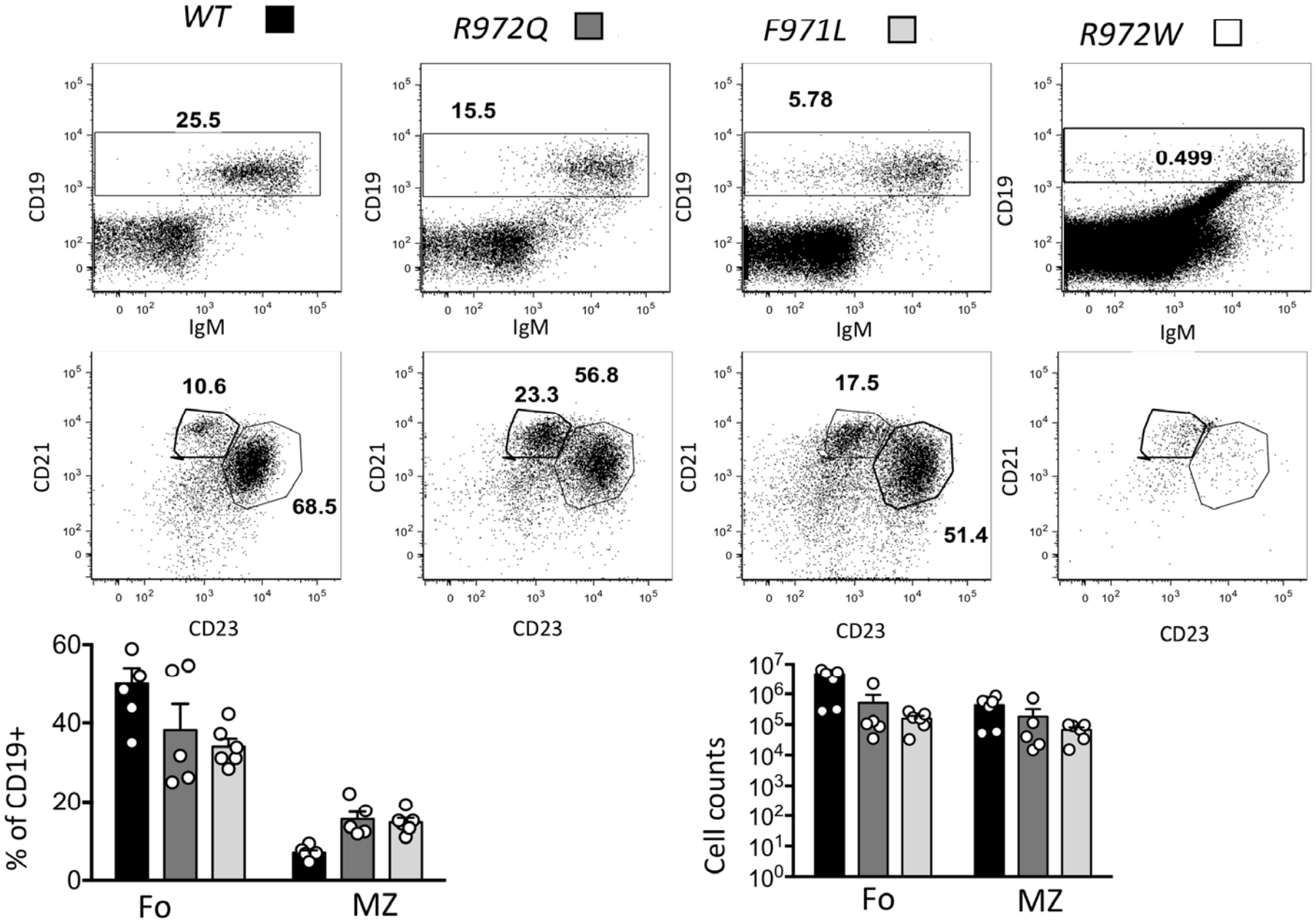
B cell development in Bone Marrow



Summary 1: T and B cell development

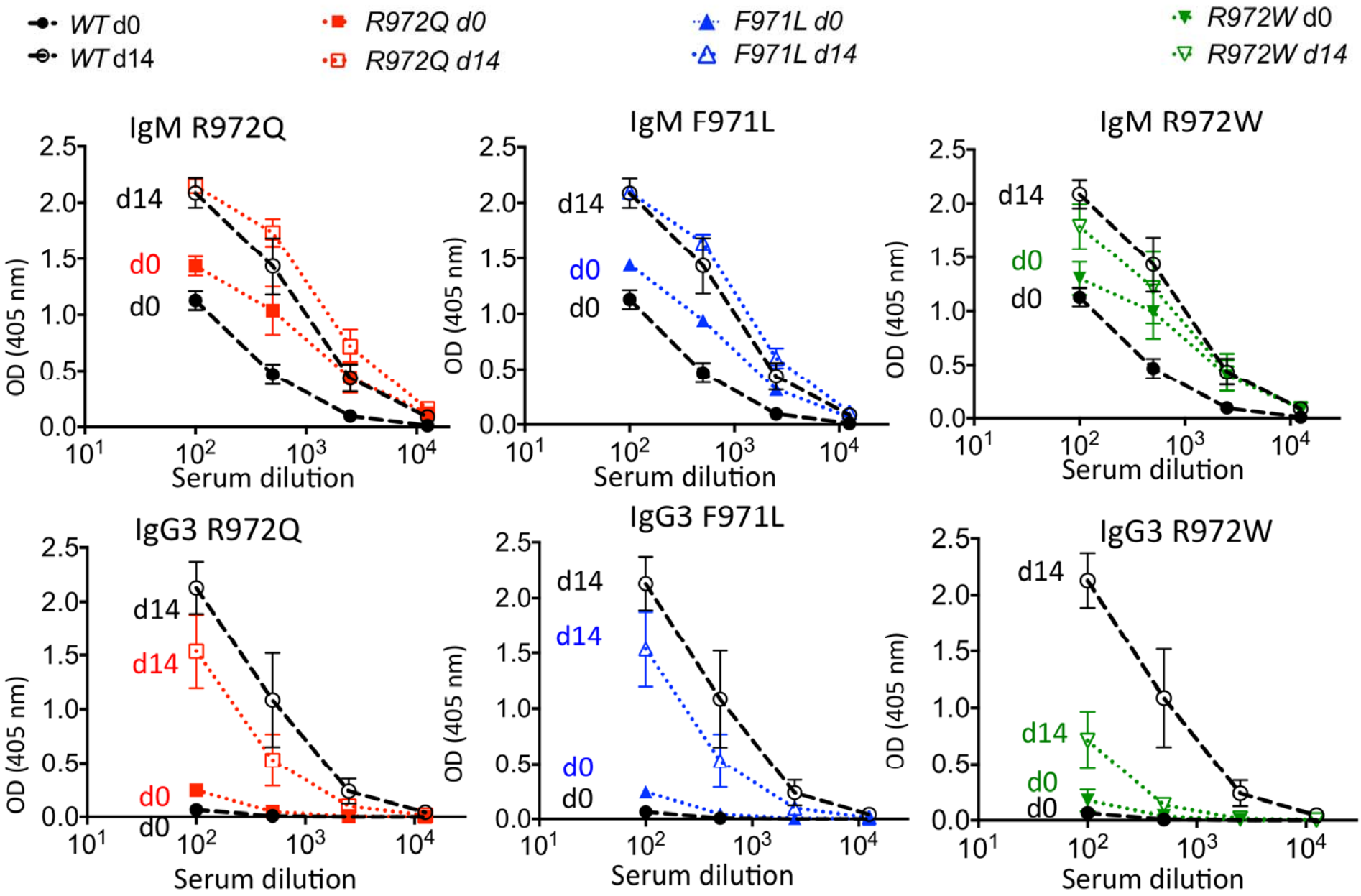
- Thymus shows partial block at DN3
- Bone Marrow shows partial block at pro-B
- Variable Leakiness (R972Q > F971L >R972W)

Spleen B cells

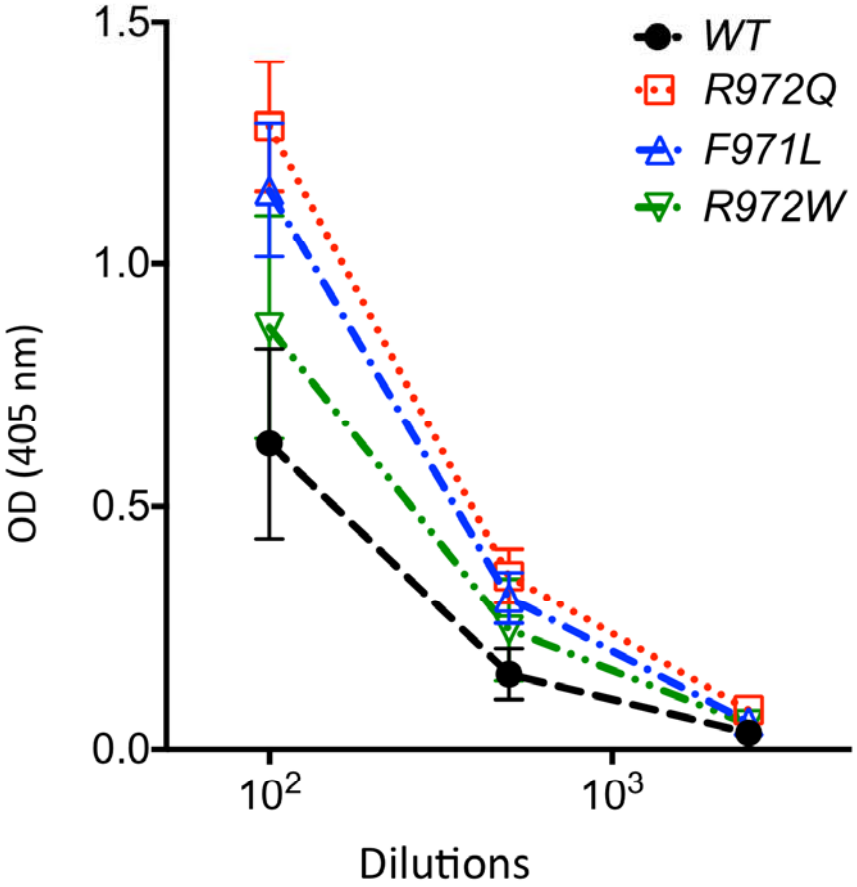


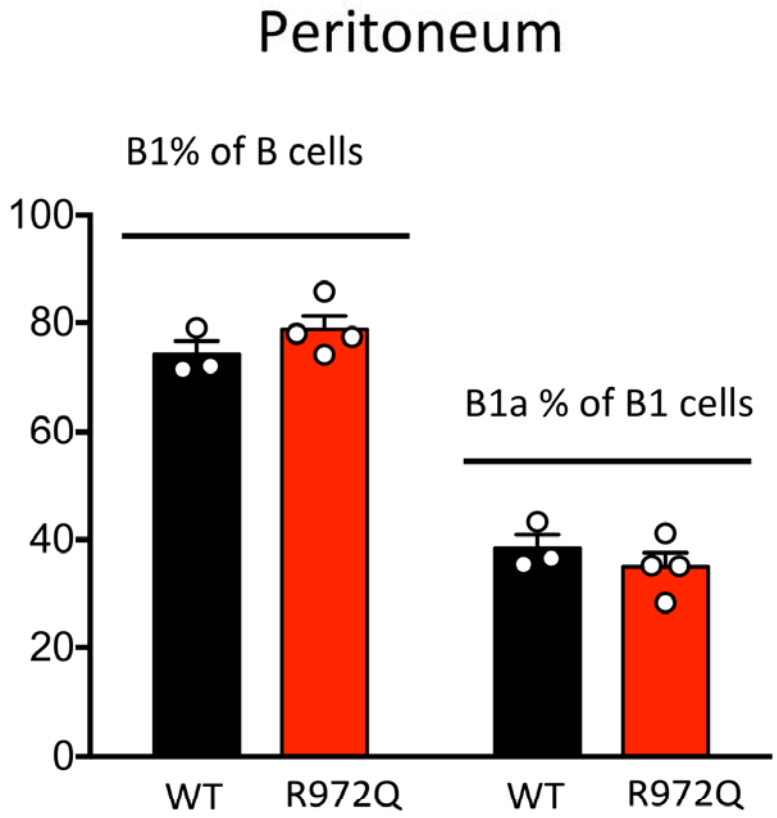
T-independent immune response

TNP-Ficoll

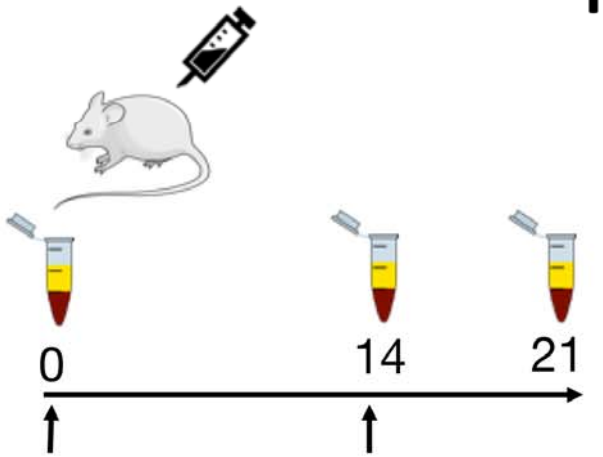


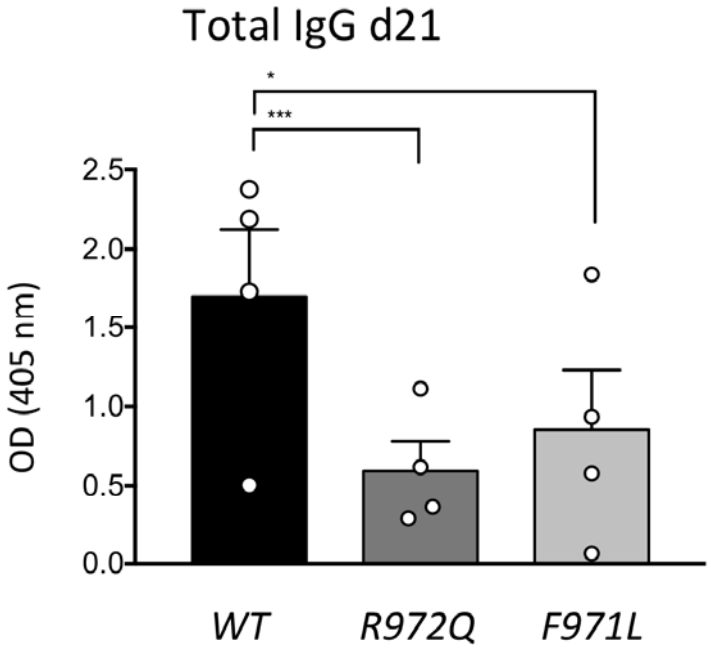
Poly reactive IgM anti-Phosphocholine

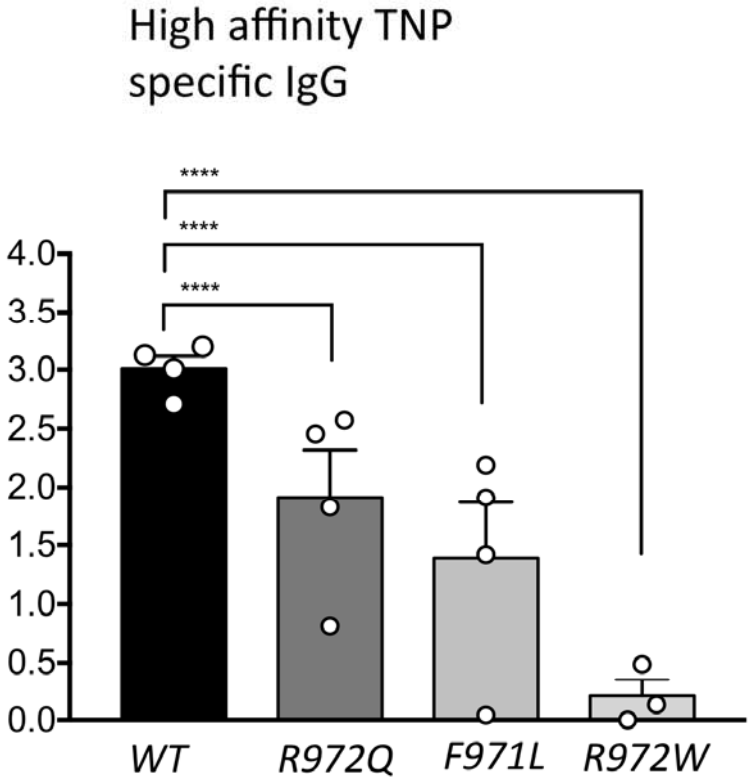




T-dependent immune response
TNP-KLH

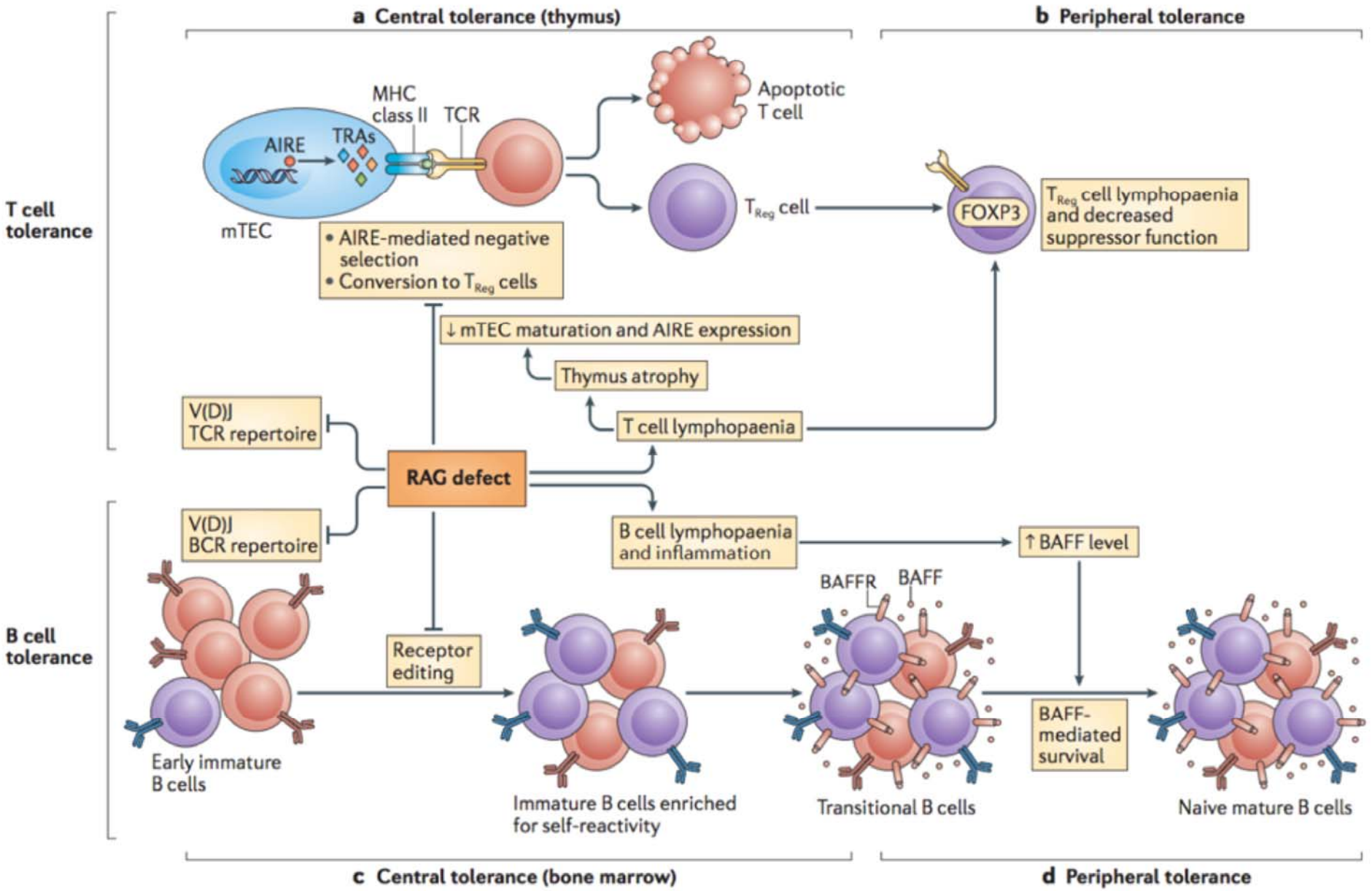






Summary 2

- T independent response normal for R972Q and F971L but not for R972W
- T dependent response defective
  - T cells?
  - B cells? (MZ relatively intact compared to Fo)
- Polyreactive IgM in all mutants



Notarangelo 2016

Is it the Primary Repertoire?

Immunity & Central tolerance

Defective RAG:

- Less overall activity (cutting/ rearrangements)?
- Specific skewing:
  - Coding flank sensitive region
  - Roth et al 2008

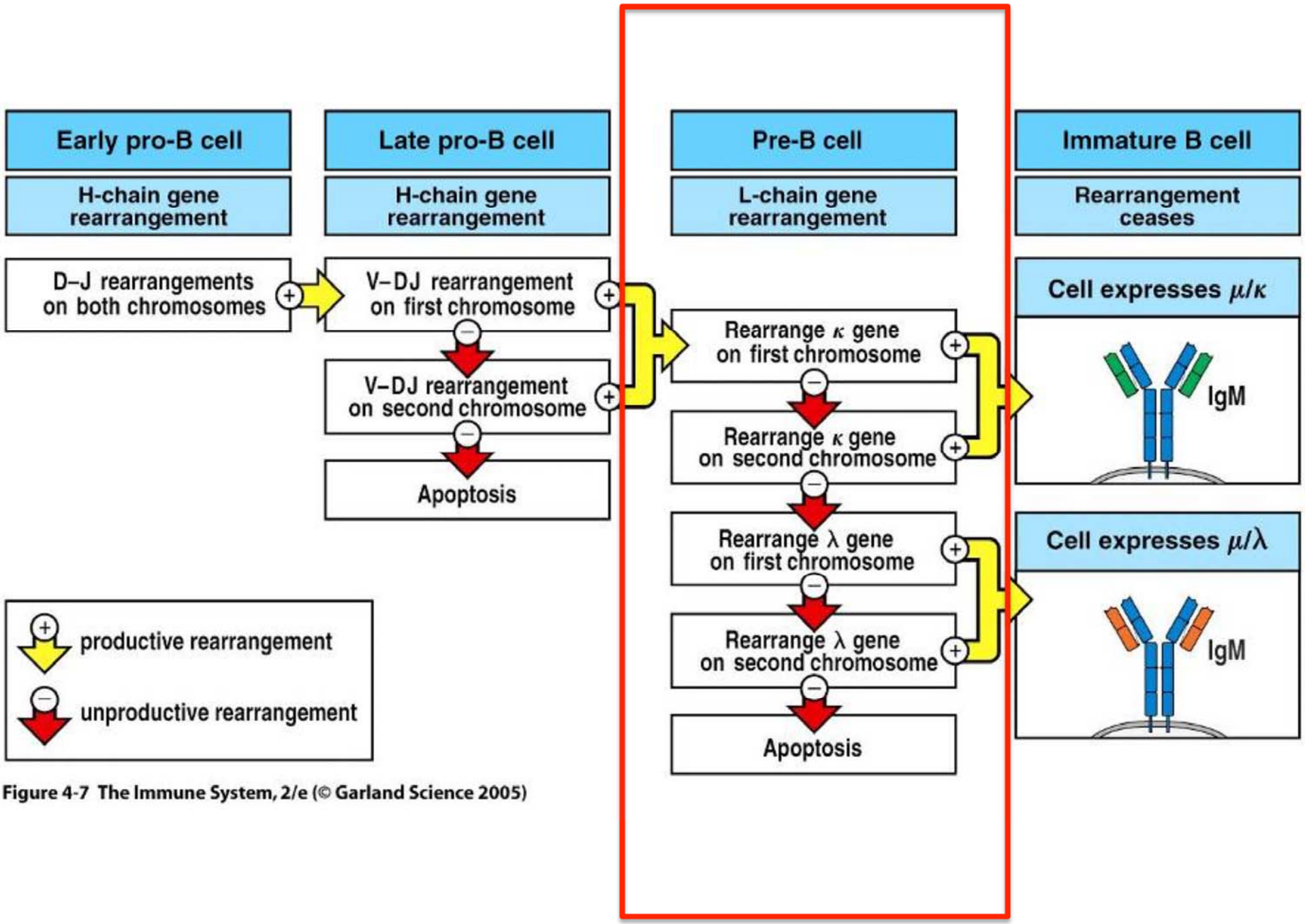
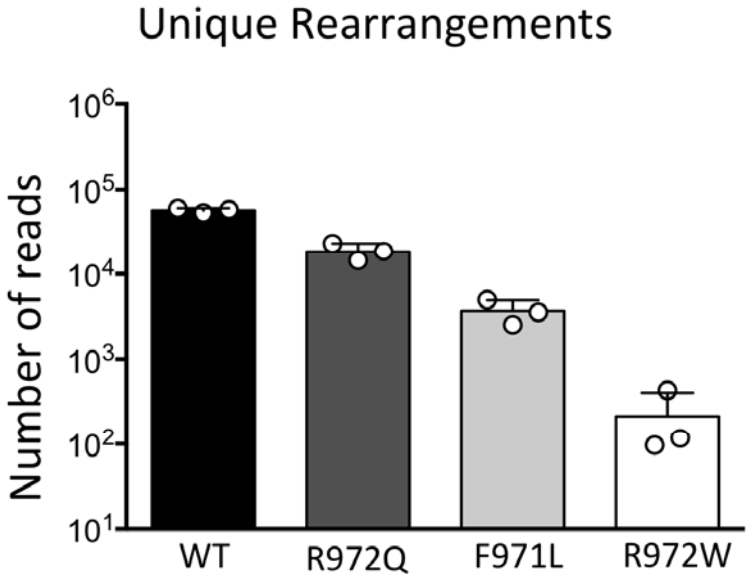
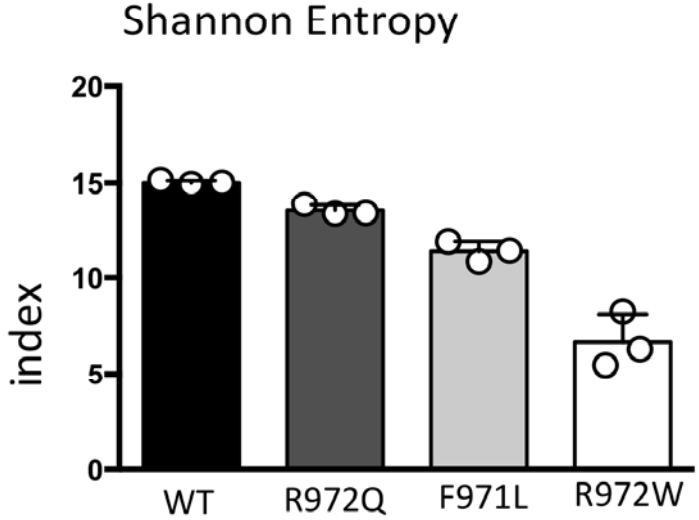


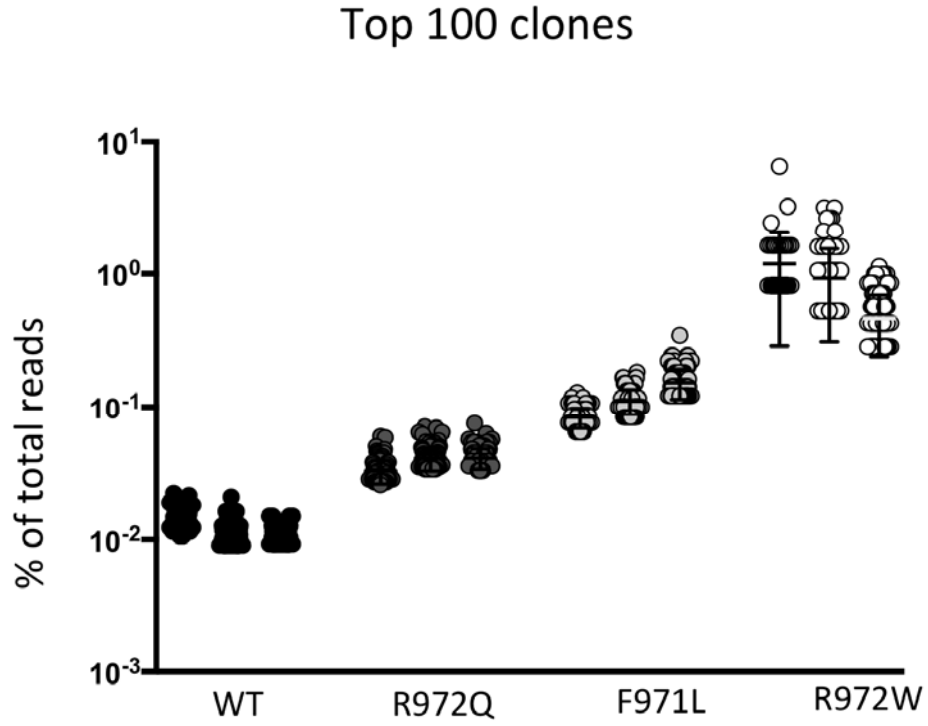
Figure 4-7 The Immune System, 2/e (© Garland Science 2005)

Igh repertoire

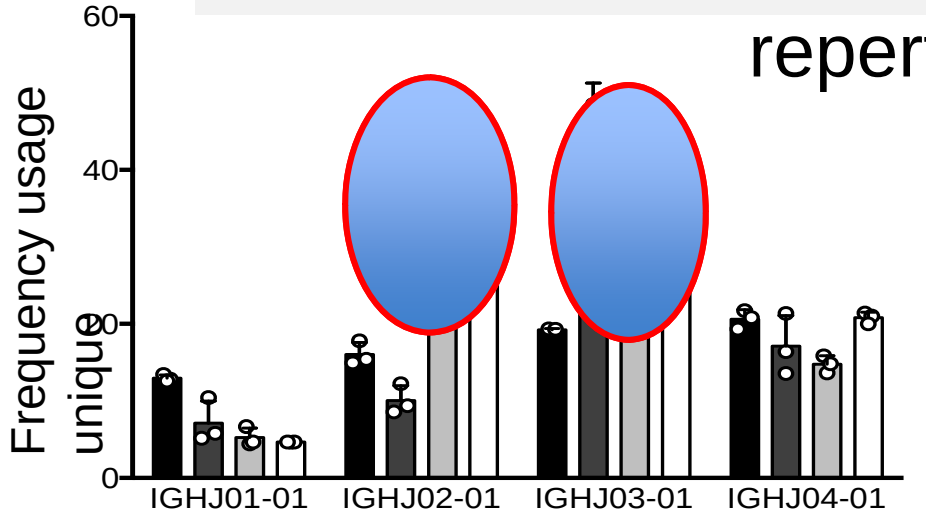
sorted pre-B cells: B220+ IgM- CD43-



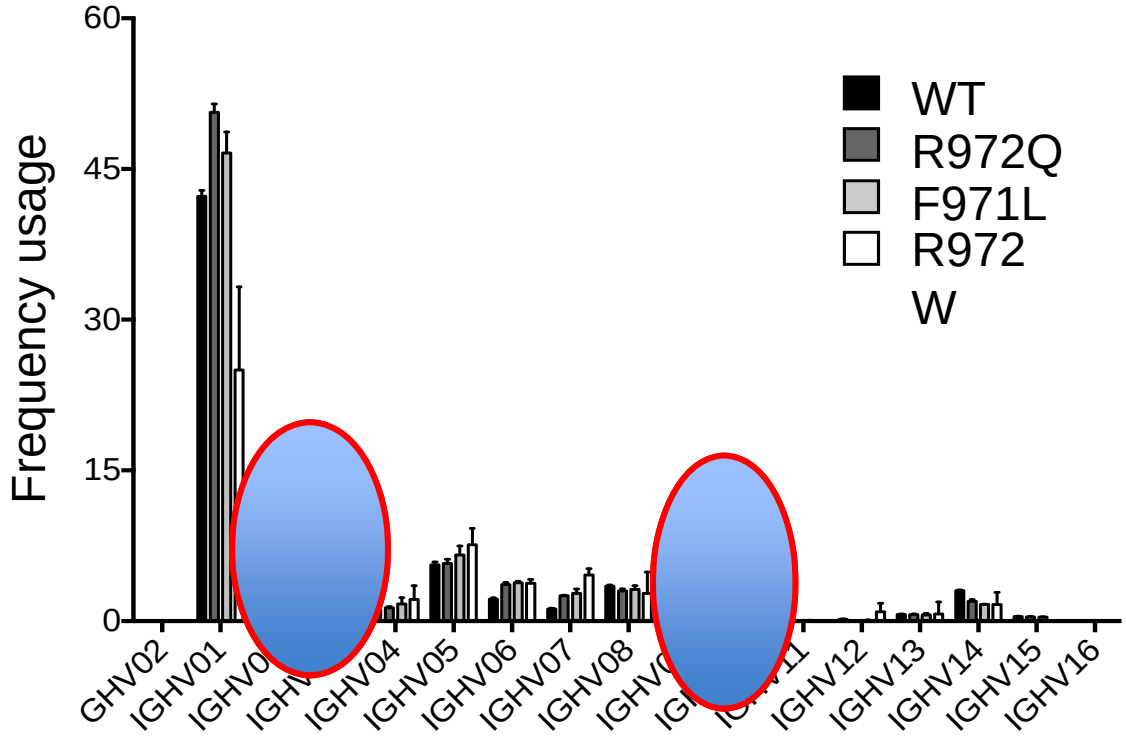




Pre-B cells bone marrow: primary repertoire

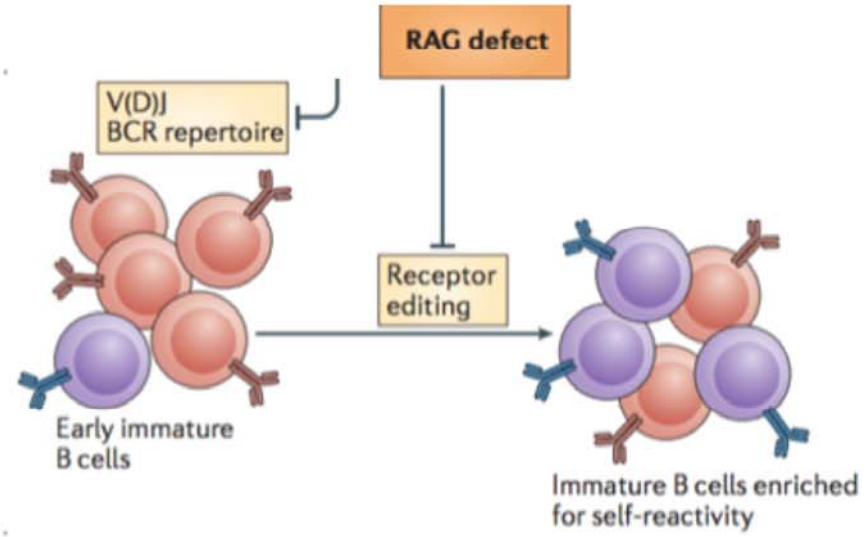


J gene



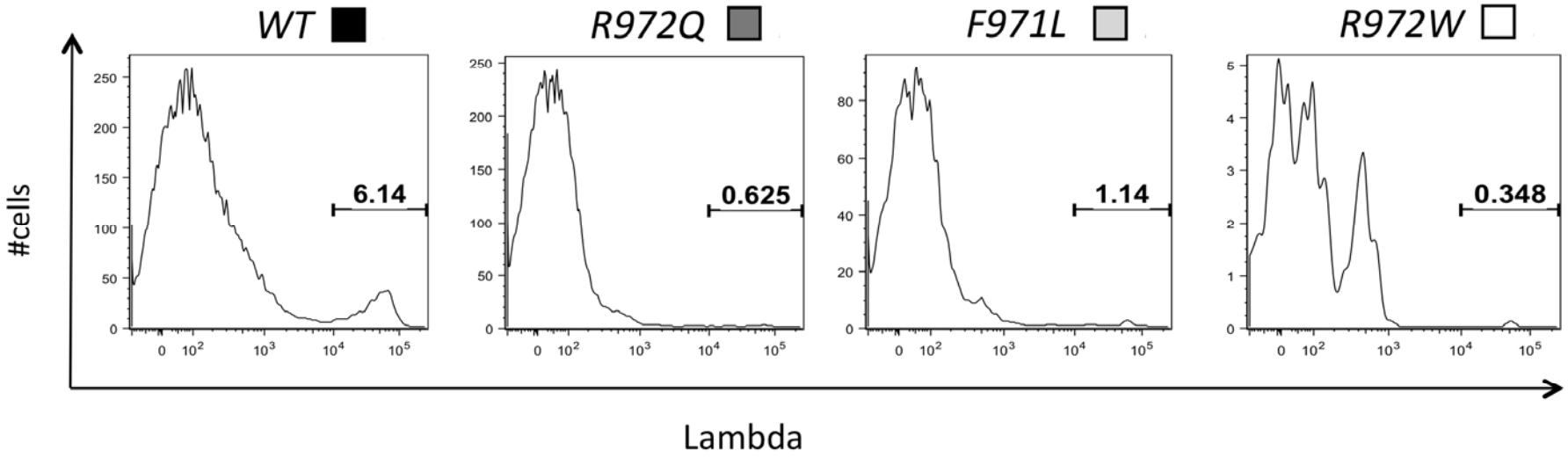
V gene

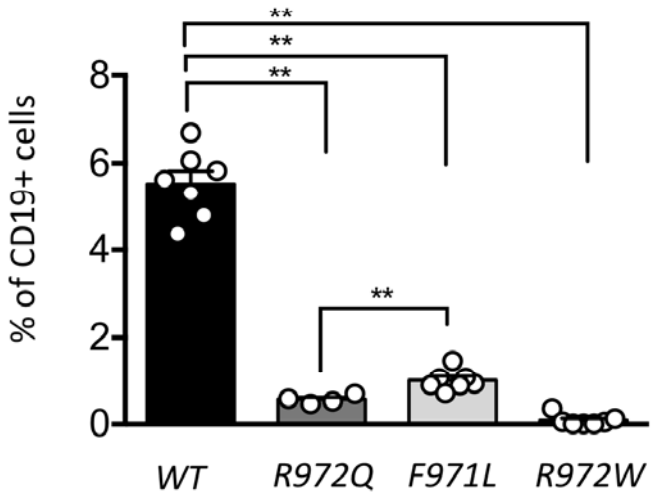
Secondary rearrangements

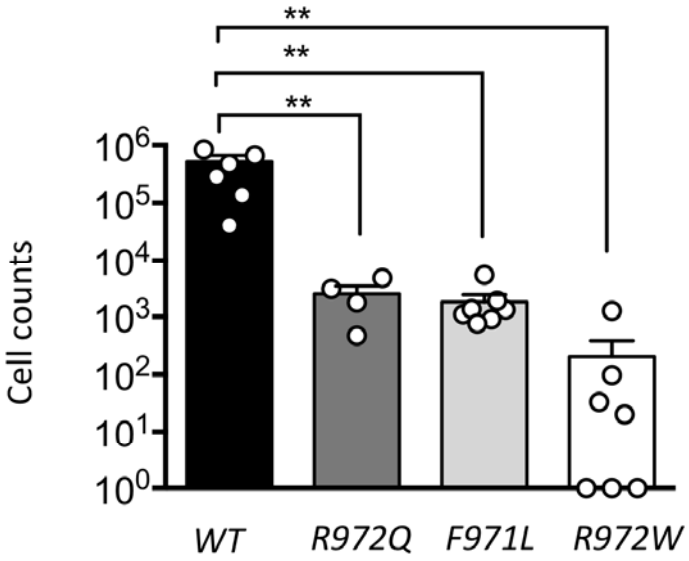


Receptor editing

B cells spleen







Summary

The IgH B cell repertoire is potentially skewed early in development, altering the landscape for subsequent rearrangements and IgH gene modifications

Rag activity may correlate with the ability to develop infections versus autoimmunity

Not all lymphocyte compartments are affected to the same degree

Some immune responses are preserved

Acknowledgments

Boston Children's Hospital
Harvard Medical Scool

Francesca Rucci
Susan Blasi
Katherine Graves
Kim Ching
Sabrina Volpi
Alex Chen

Rick Malley
Kristen Moffitt

Fred Alt
Sherry Lin

NIH
Luigi Notarangelo
Stefano Volpi
Lisa Ott de Bruin