

The background image is a photograph of a modern building with a glass facade and red interior stairs. The building is multi-storied, with several levels visible. The stairs are a vibrant red color, and the interior spaces are brightly lit. A few people can be seen walking on the stairs and on the upper levels. To the right of the modern building is a traditional brick building with many windows. The sky is a clear blue. The overall scene is a mix of modern and traditional architecture.

WILL MY GENETIC VARIANT MAKE ME SICK?

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IRVING MEDICAL CENTER



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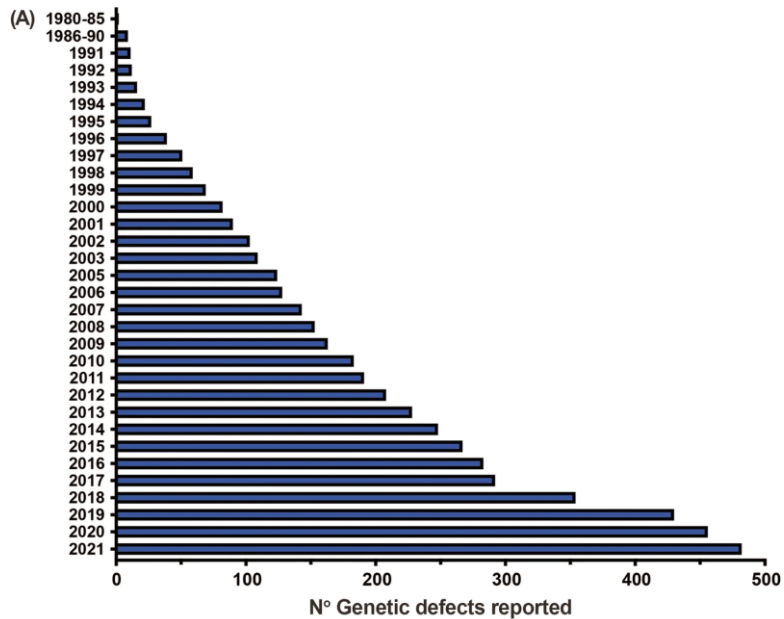
Founder Lab11

Consultant - Volastra

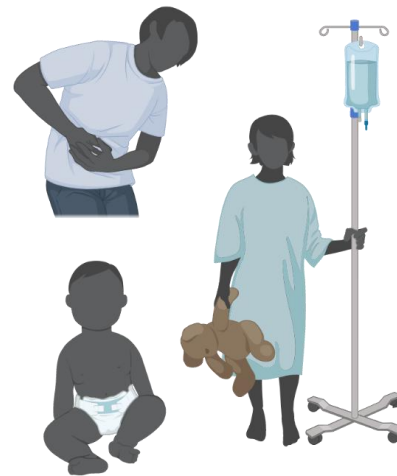
Inborn Errors of Immunity

Inborn errors of immunity (IEI) are genetic disorders that predispose to susceptibility to infections, autoinflammation, autoimmunity, allergy, and malignancy.

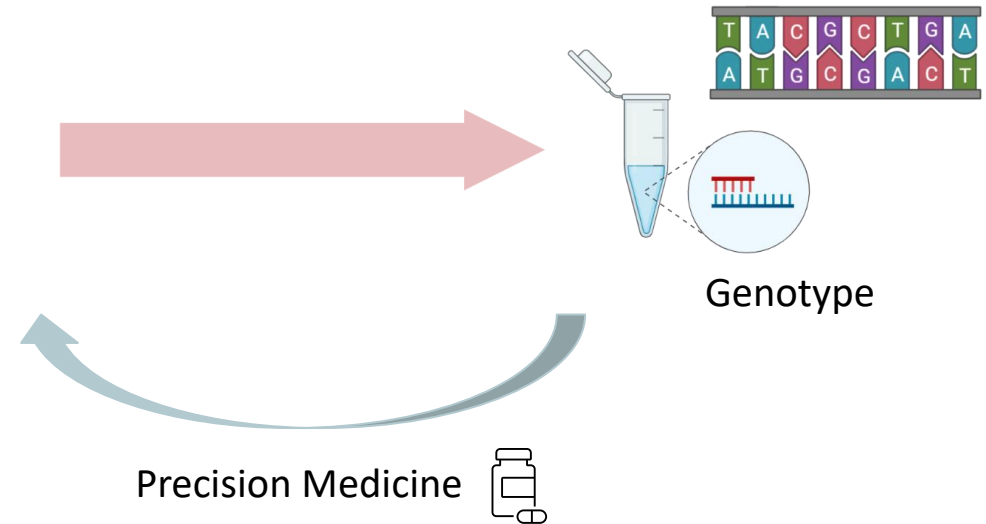
There are over 600 unique IELs, mostly discovered by forward genetics



IELs affect < 1 in 1,000 people

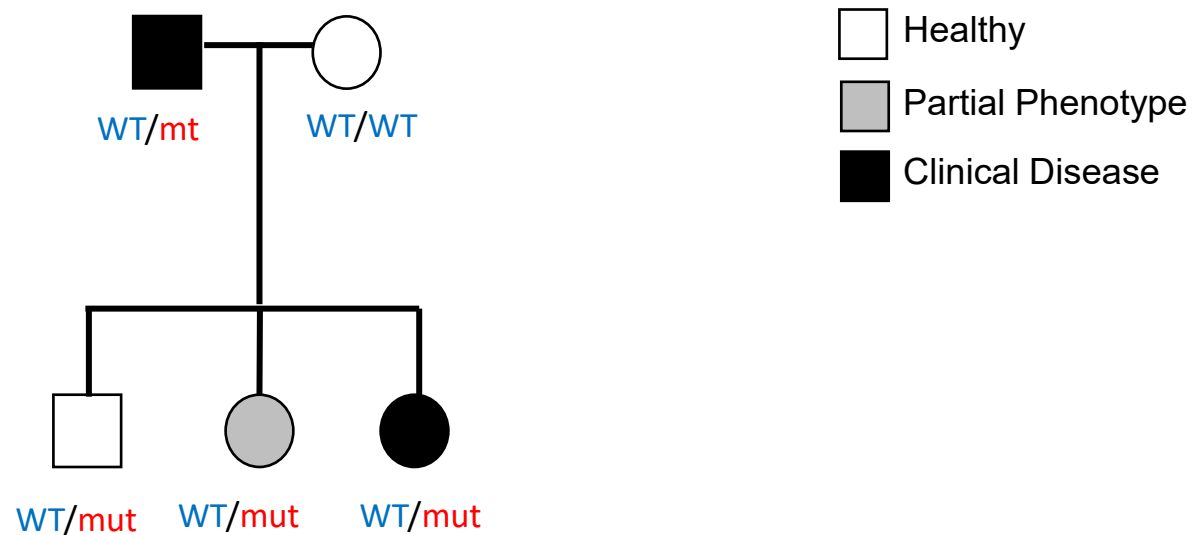


Phenotype



Incomplete penetrance is common ground in genetic disorders

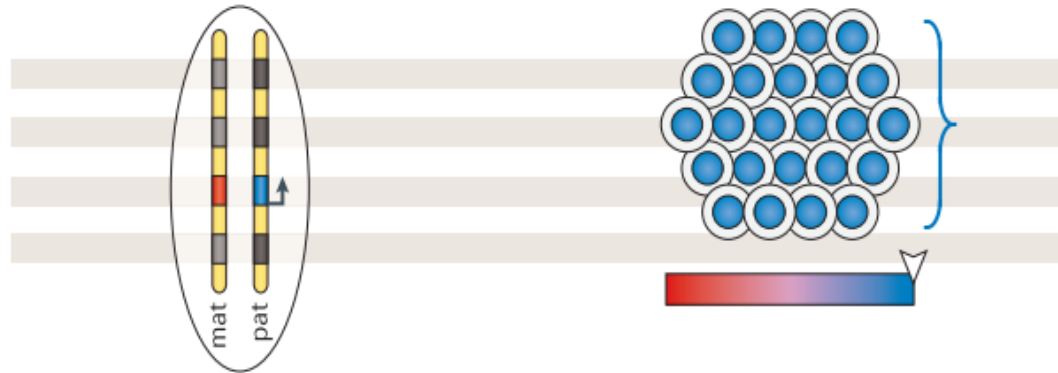
- Families carrying a mutation often have reports of incomplete penetrance.
- This is true in Inborn Errors of Immunity, monogenic disorders of the immune system.



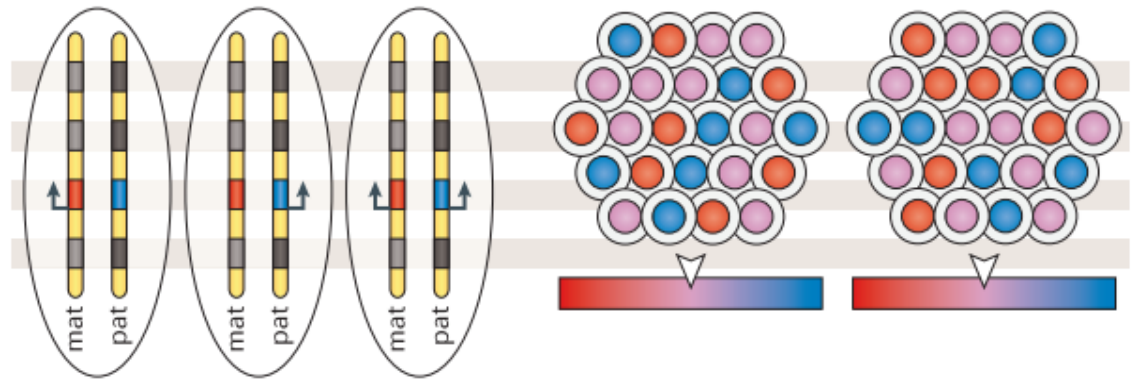
Not all genes are expressed from both alleles

● Maternal ● Paternal ● Biallelic

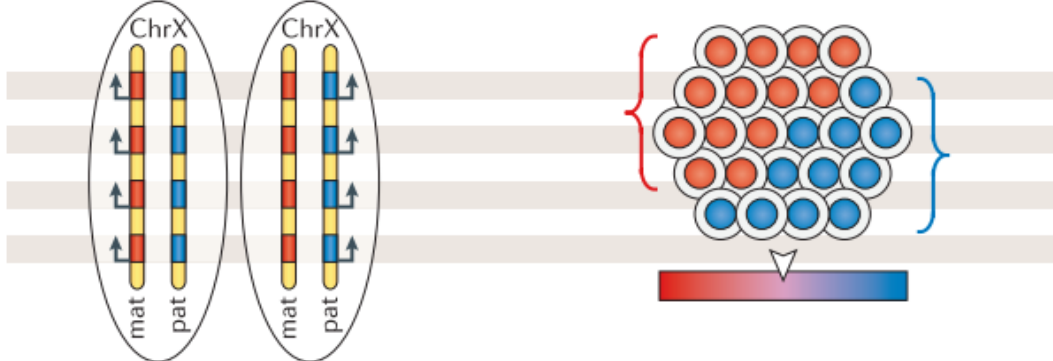
Genomic Imprinting



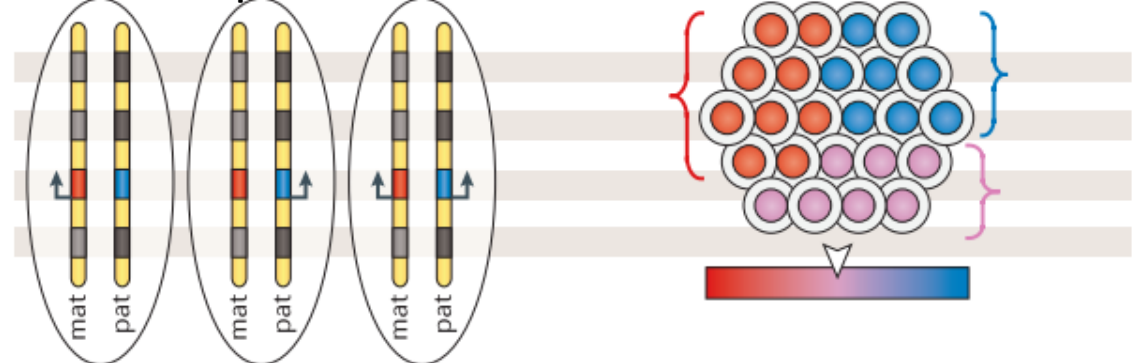
Transcriptional Bursting



X-Inactivation

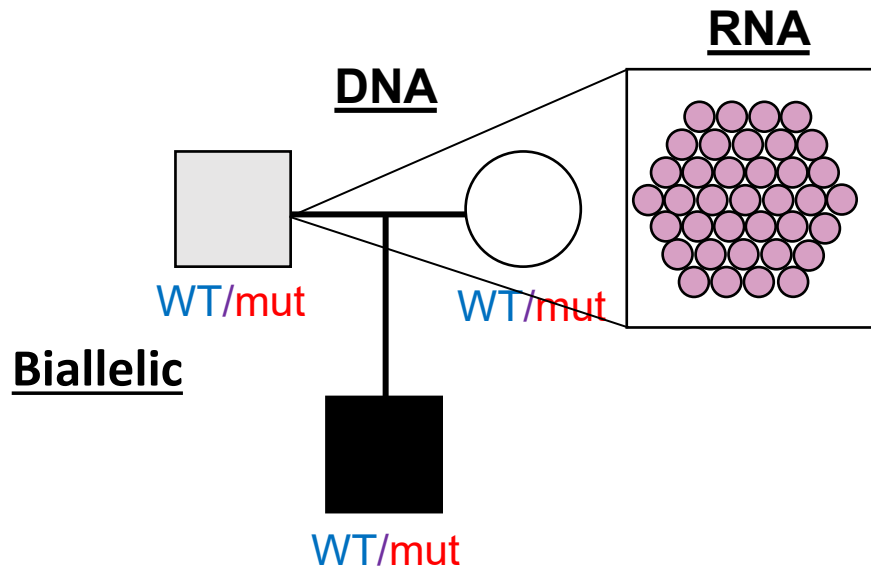


Monoallelic Expression



Biallelic expression vs Monoallelic expression

Maternal Paternal Biallelic



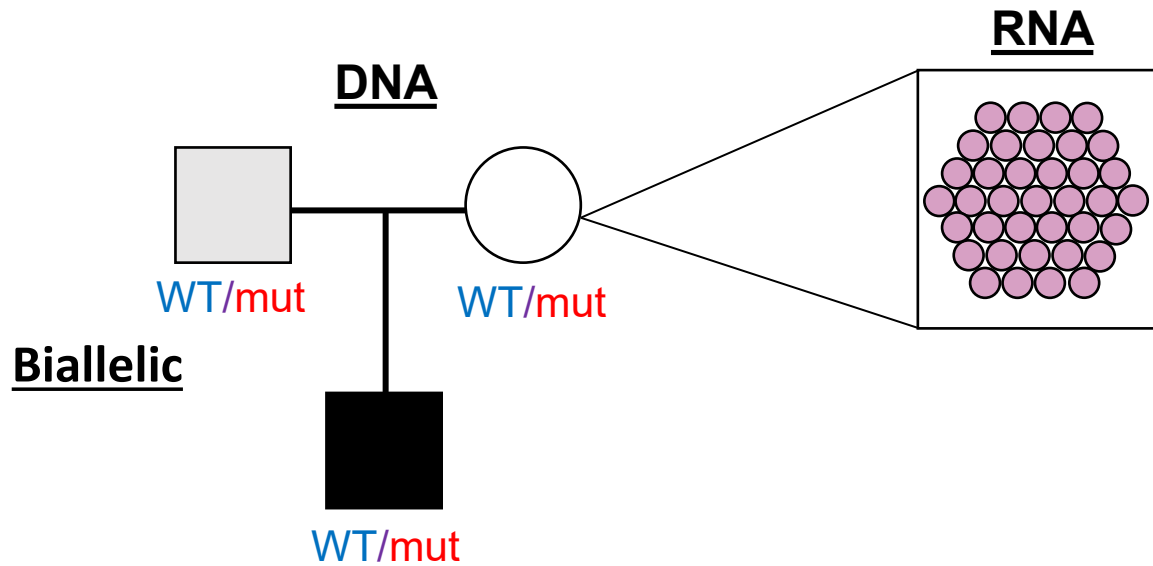
Healthy

Partial Phenotype

Clinical Disease

Biallelic expression vs Monoallelic expression

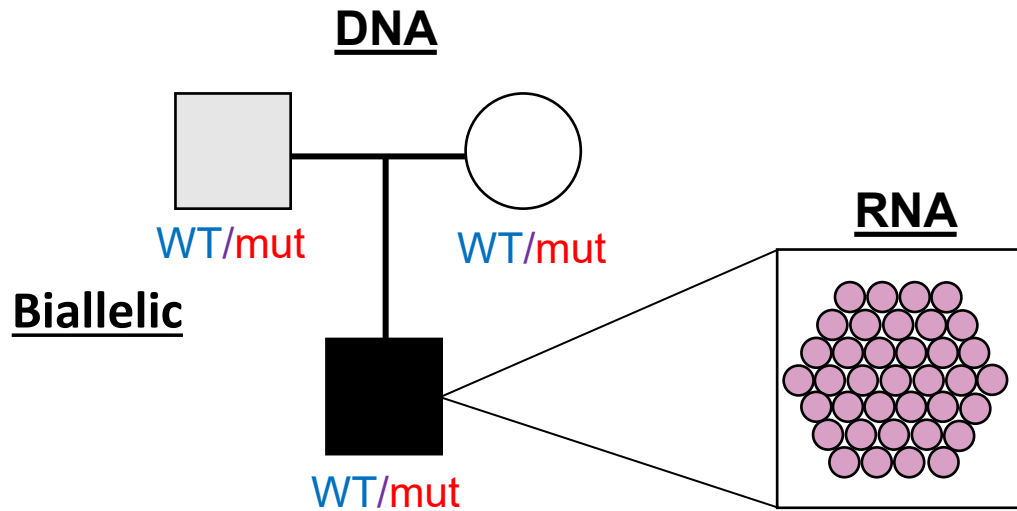
Maternal Paternal Biallelic



Healthy
Partial Phenotype
Clinical Disease

Biallelic expression vs Monoallelic expression

Maternal Paternal Biallelic



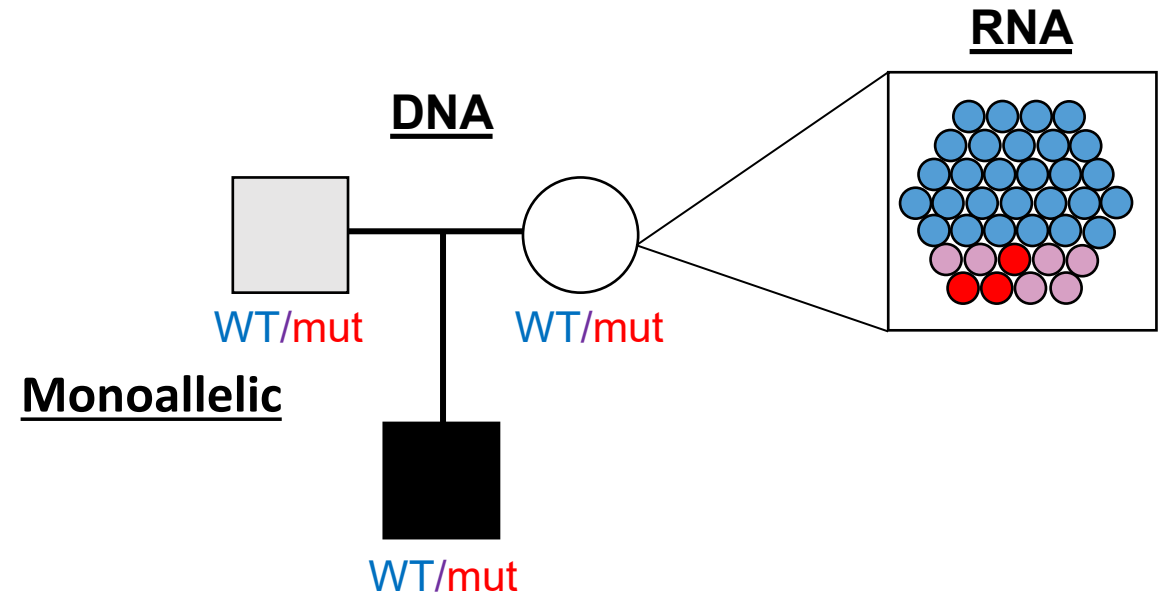
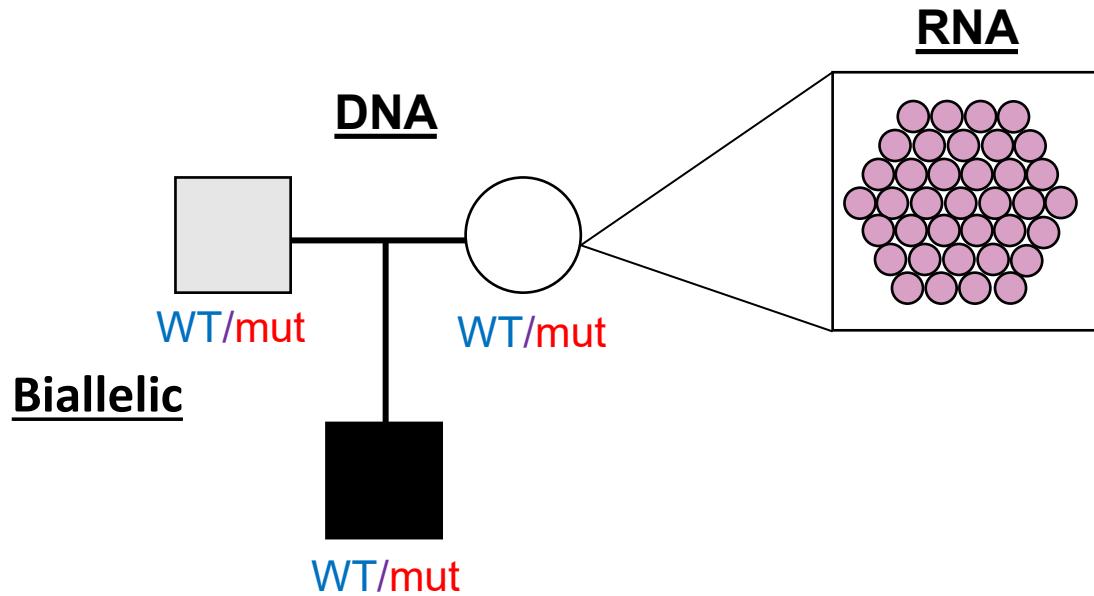
Healthy

Partial Phenotype

Clinical Disease

Biallelic expression vs Monoallelic expression

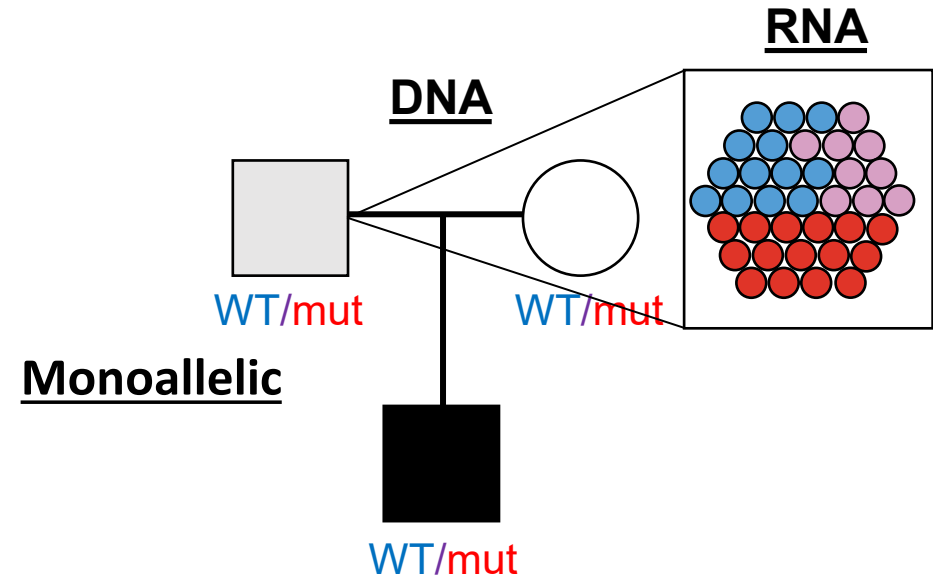
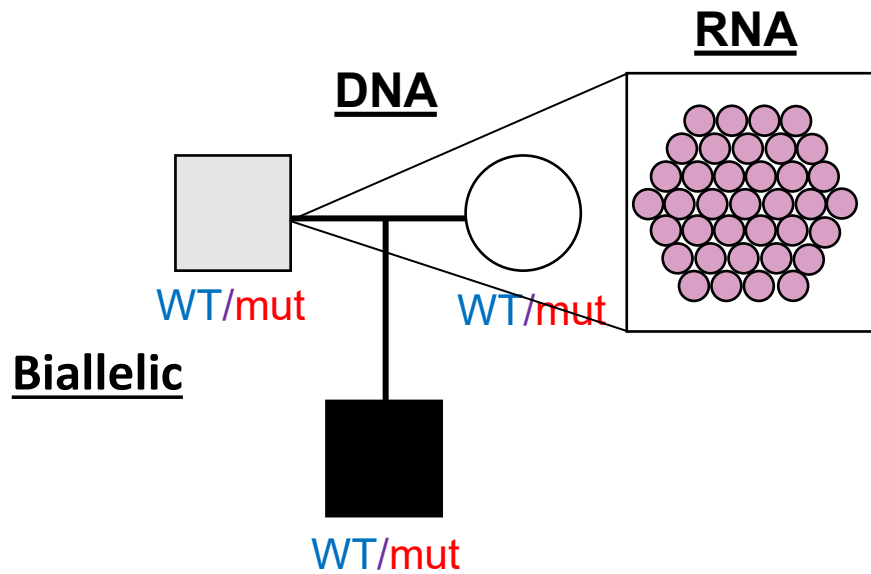
● Maternal ● Paternal ● Biallelic



□ Healthy
■ Partial Phenotype
■ Clinical Disease

Biallelic expression vs Monoallelic expression

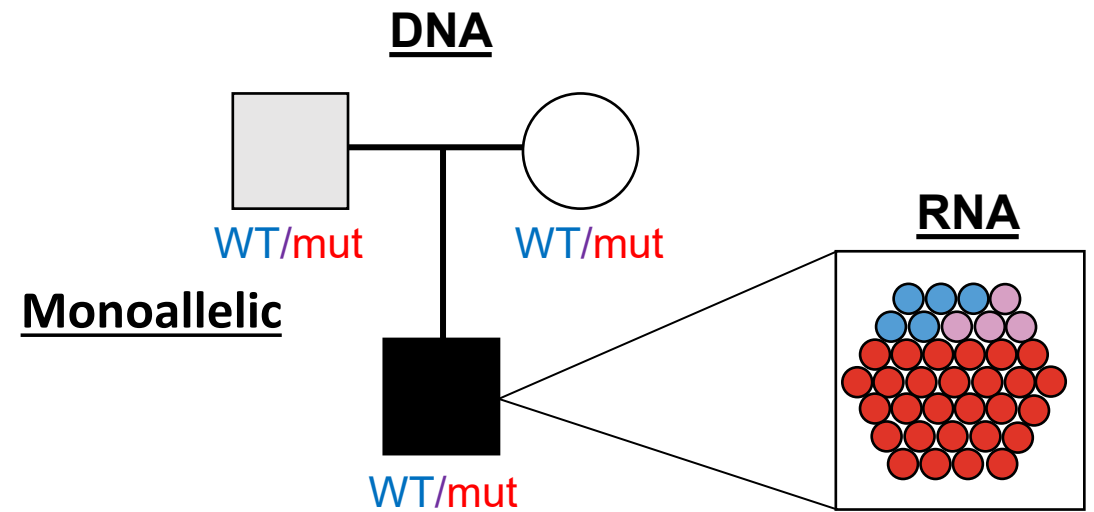
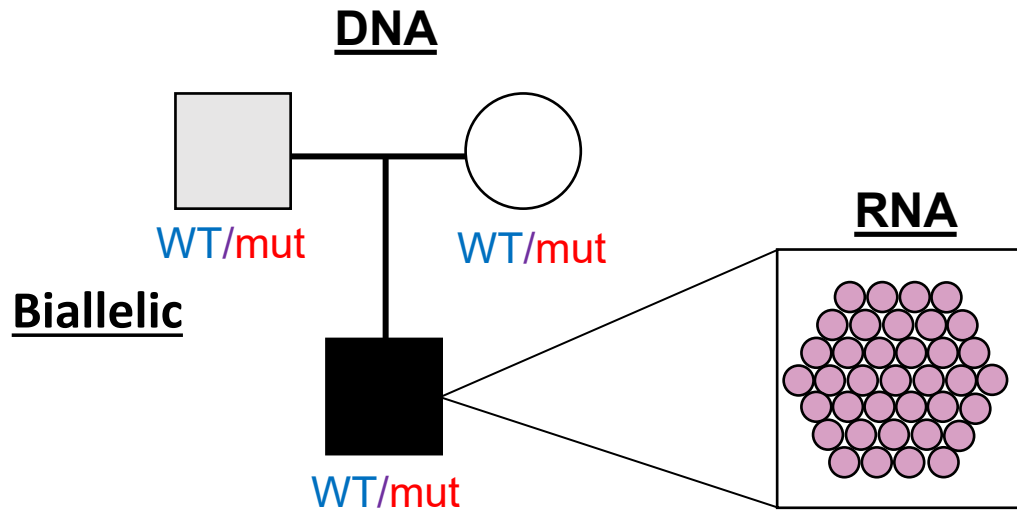
● Maternal ● Paternal ● Biallelic



□ Healthy
■ Partial Phenotype
■ Clinical Disease

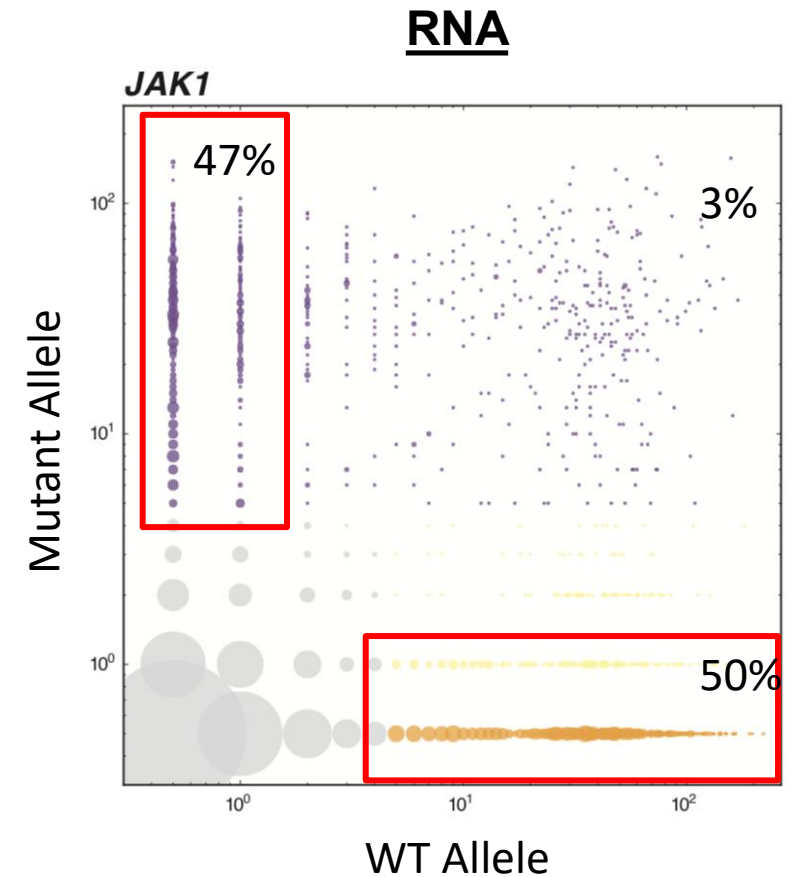
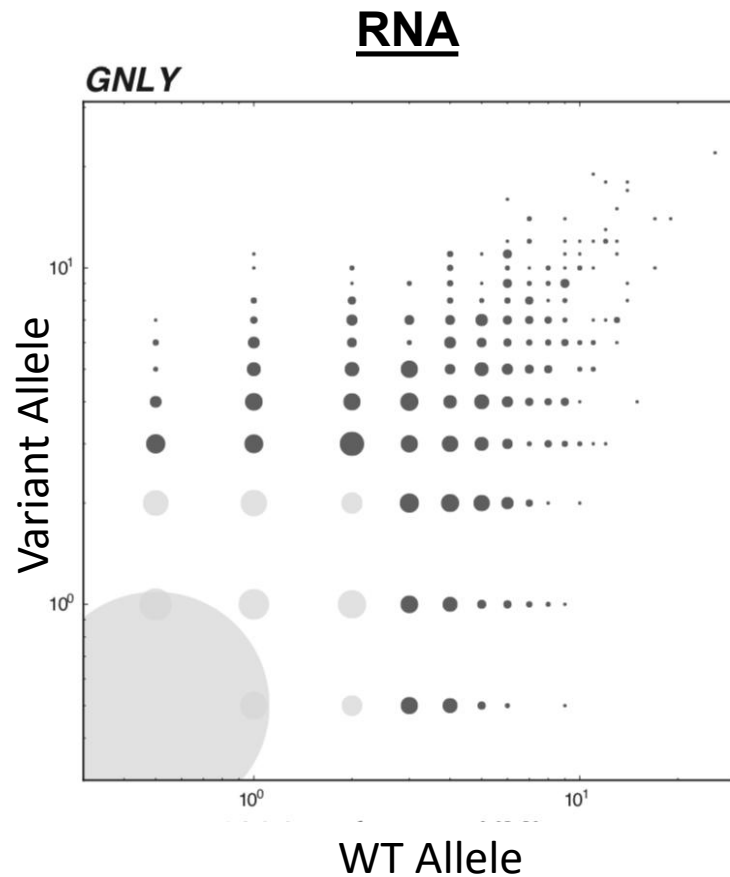
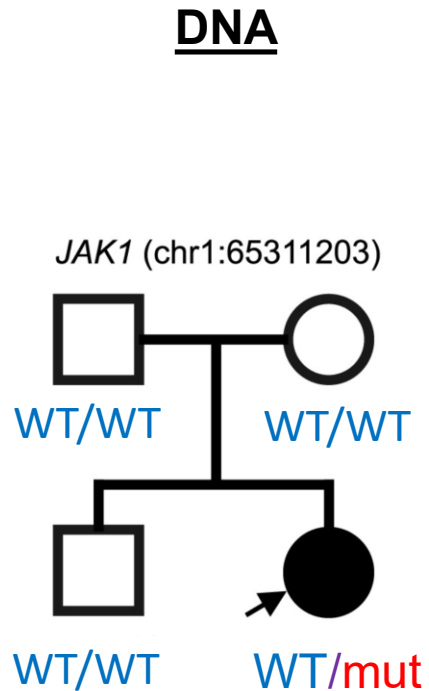
Biallelic expression vs Monoallelic expression

● Maternal ● Paternal ● Biallelic



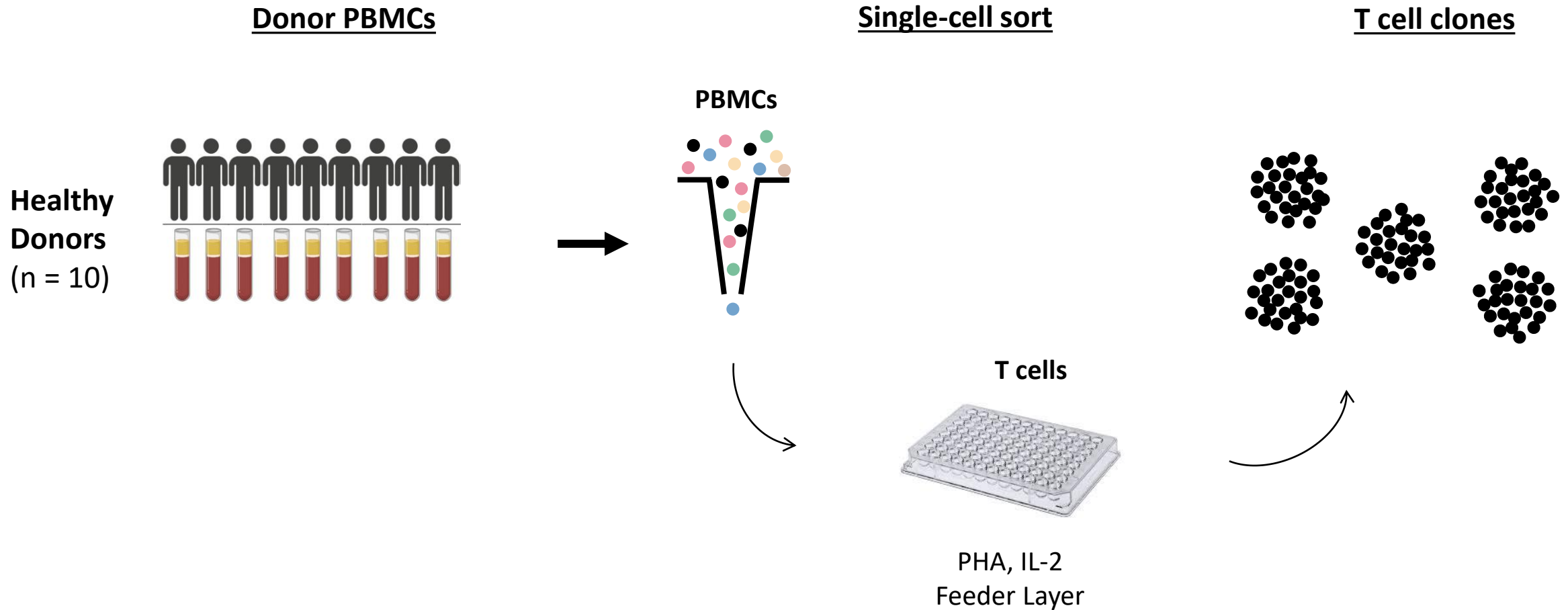
□ Healthy
■ Partial Phenotype
■ Clinical Disease

scRNA-seq identifies *JAK1* allele specific expression



Hypothesis: Monoallelic expression contributes
to phenotypic variability in IEs

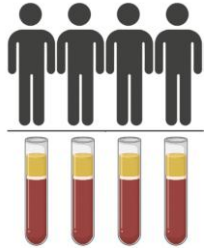
Generating clonal T cell populations



Assessing MAE in genes of interest

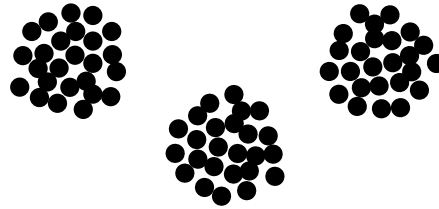
1. Select cell type/system

Primary cells



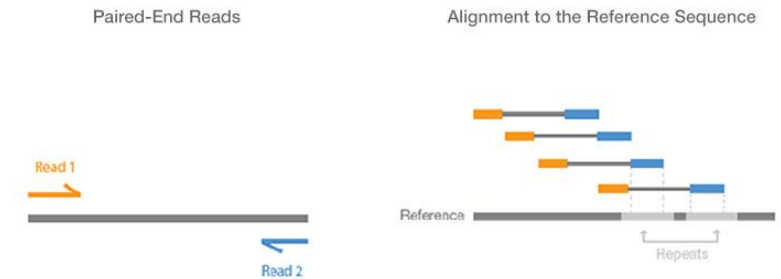
2. Stability of expressed allele

Clonal expansion



3. Differentiate between two alleles

Whole Exome Sequencing
(Heterozygous SNPs)



4. Identify expressed transcripts

Bulk RNA-seq

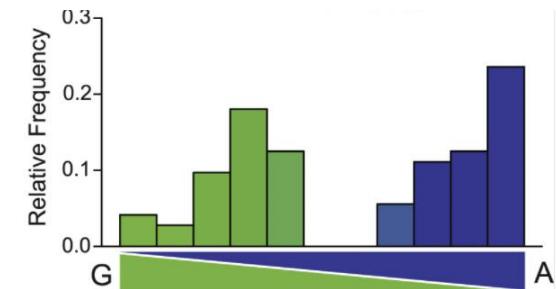


5. Computational pipeline



6. Validation

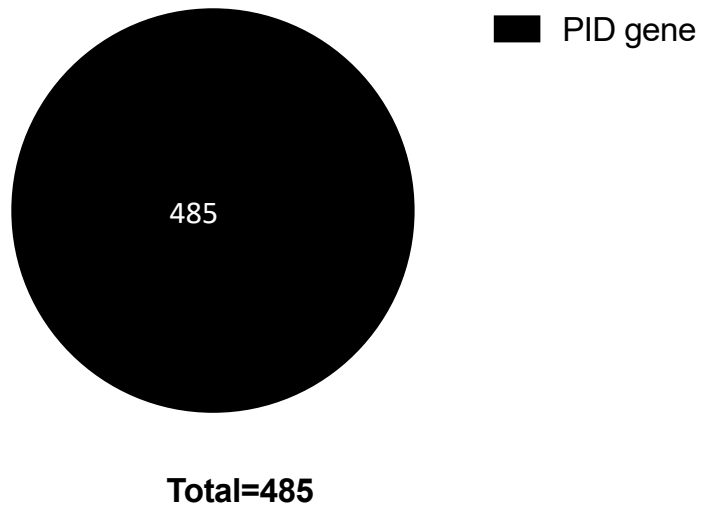
qPCR/ddPCR



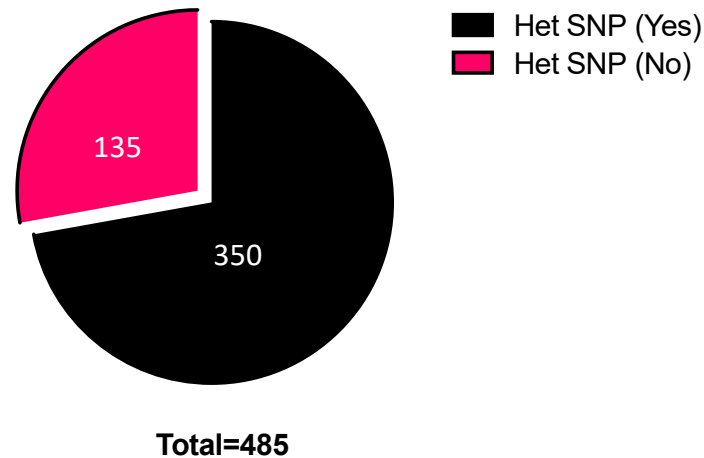
Which PID genes undergo Monoallelic expression?

Half of known IEL genes assessable in T cell system

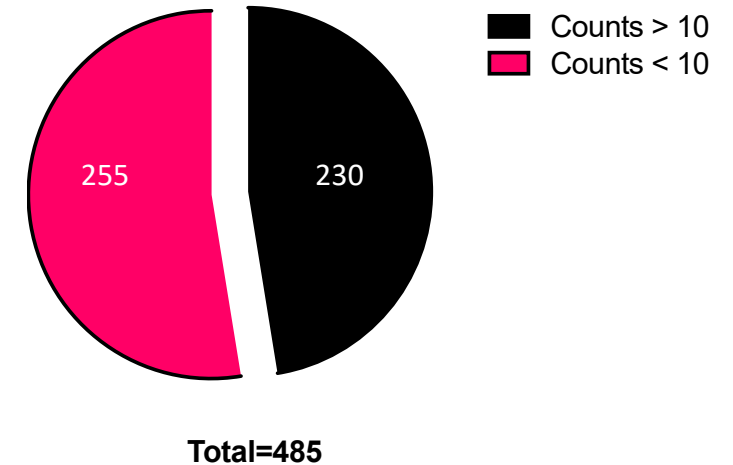
485 PID genes have been reported



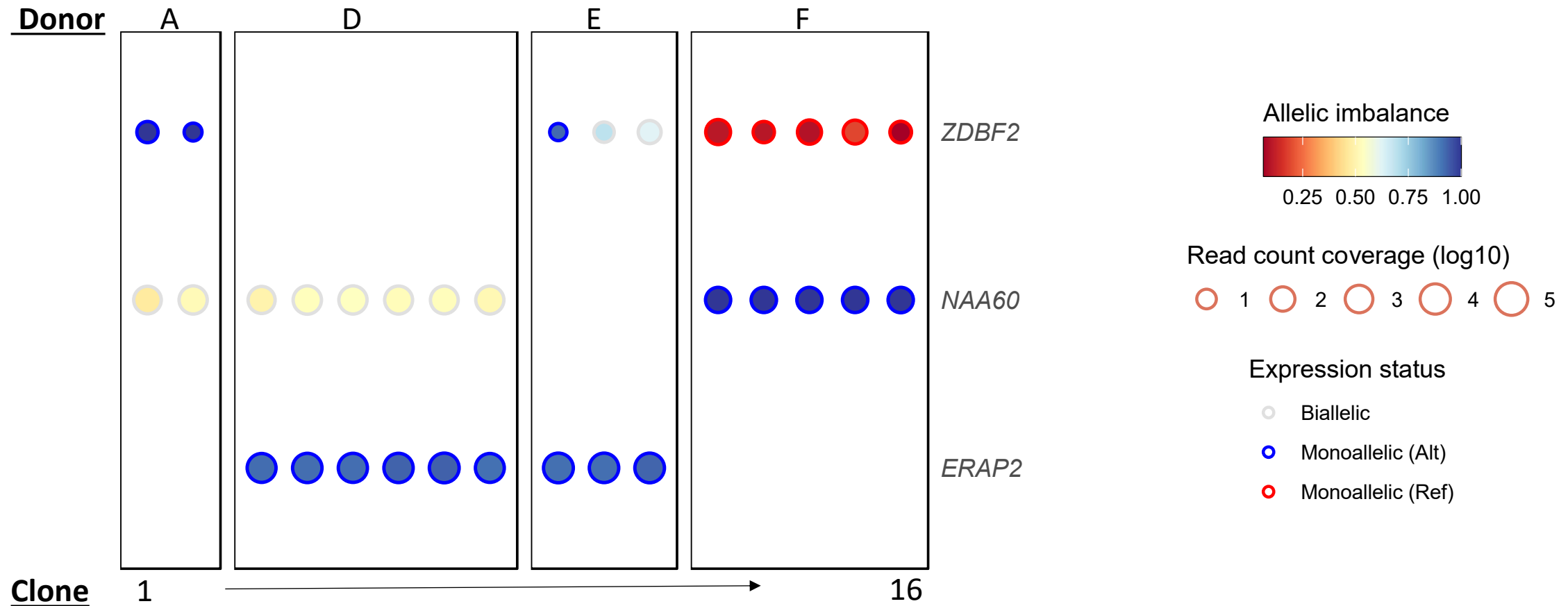
Heterozygous (Het) SNPs present in 350/485 PID genes



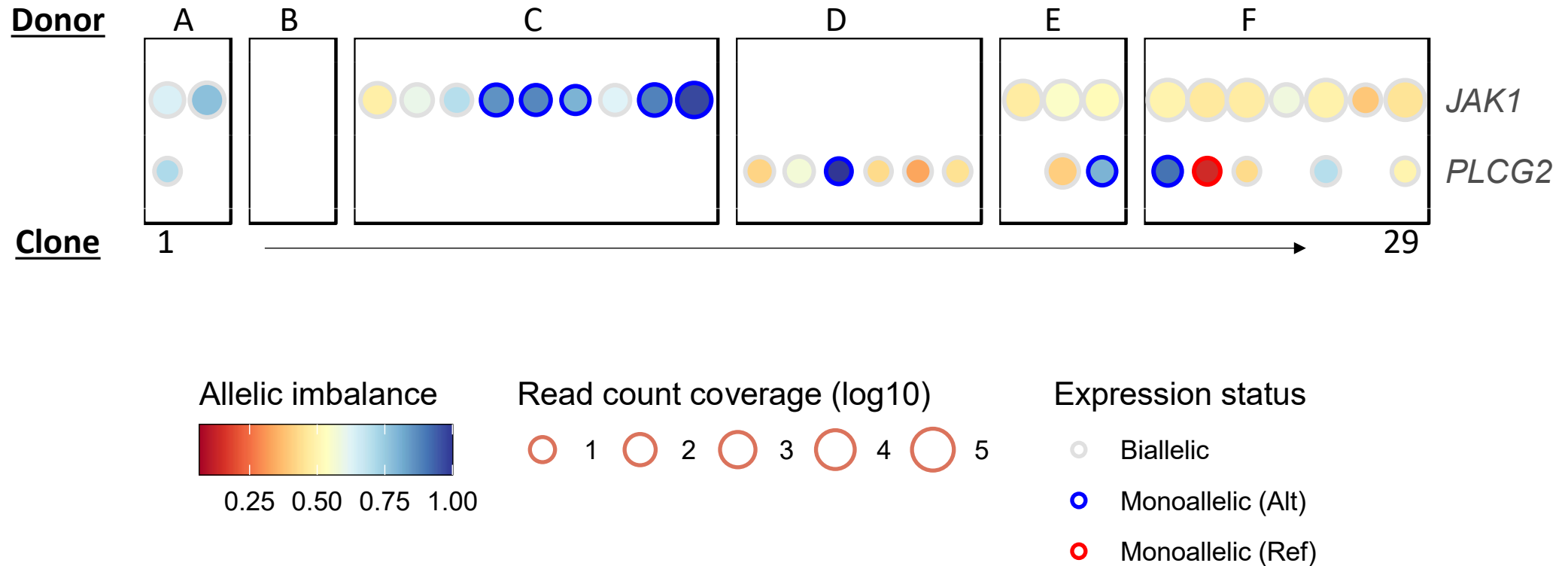
230/485 PID genes have Het SNPs with coverage in T cells



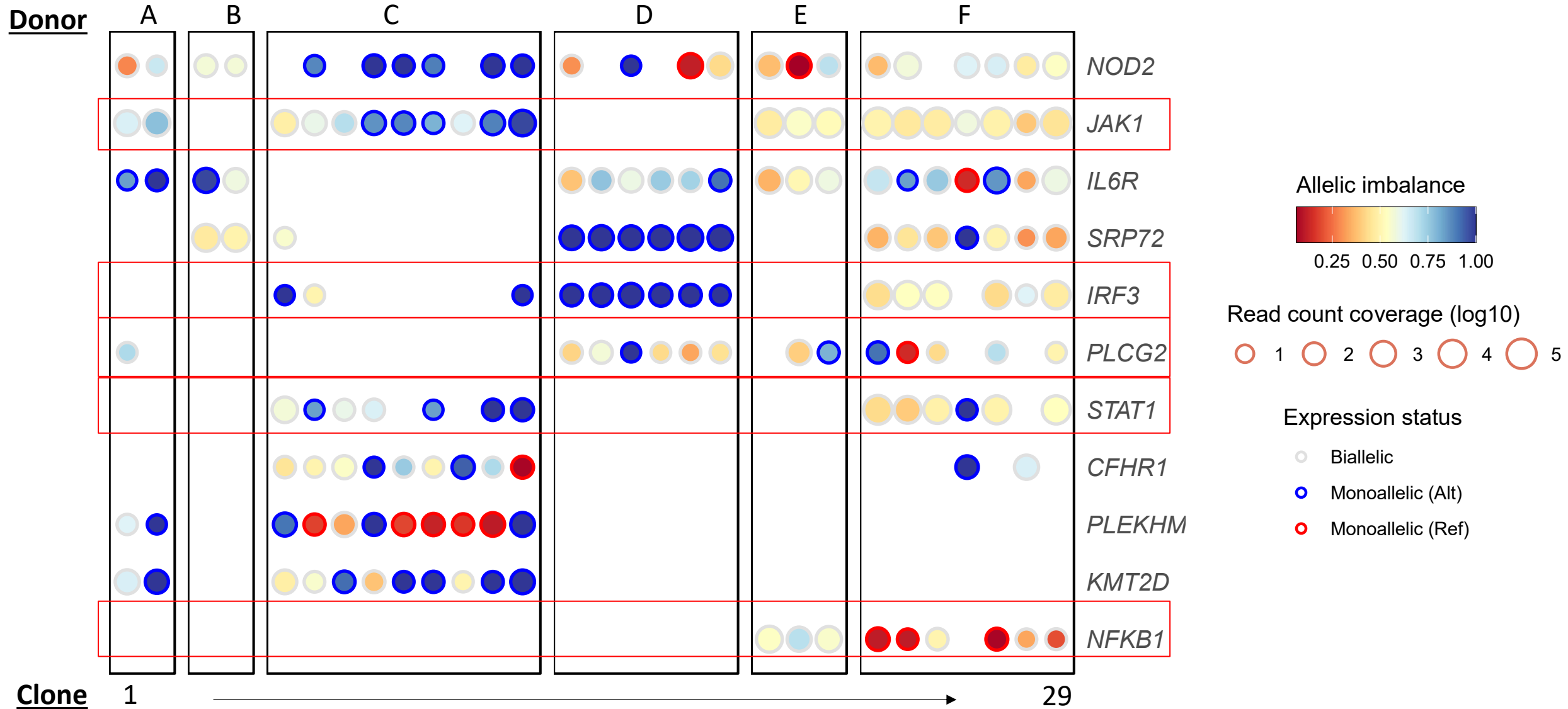
Allele specific expression of imprinted genes



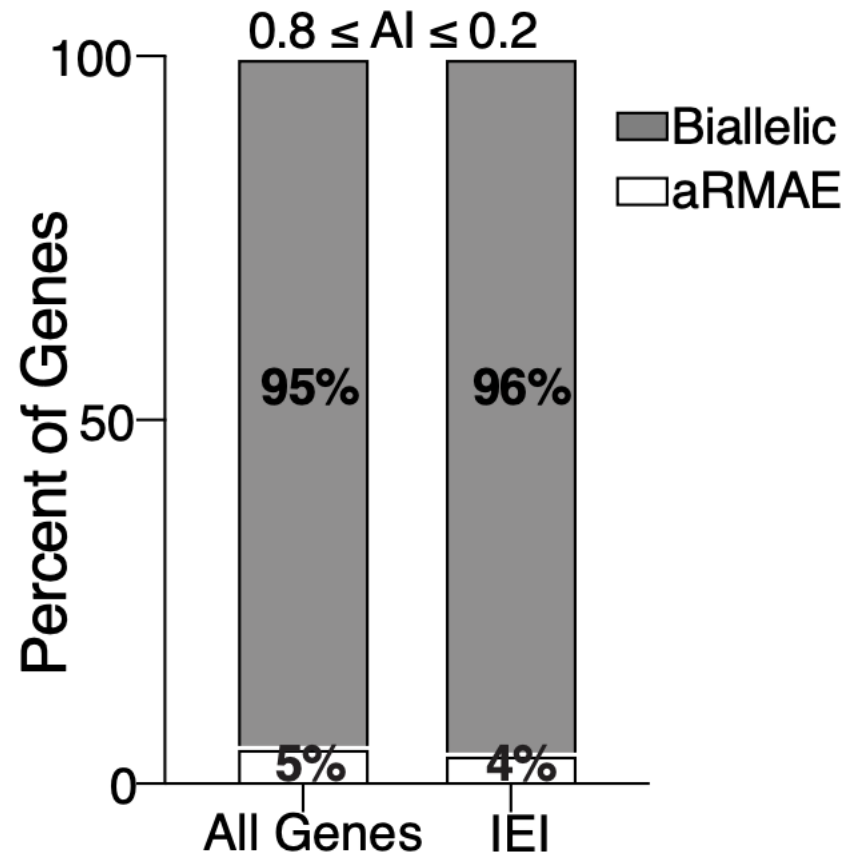
Clonal T cell system reveals MAE of IEI genes



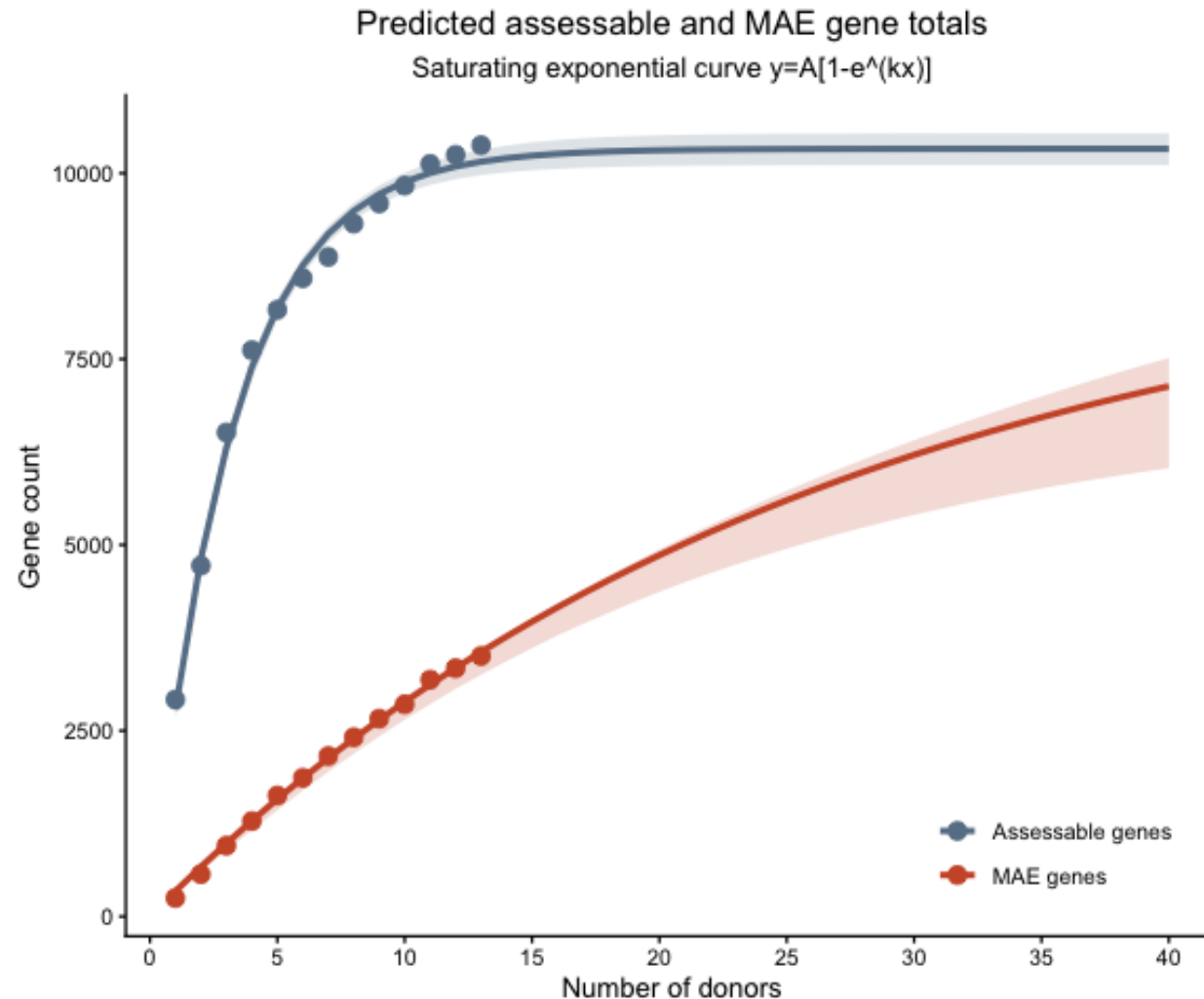
Clonal T cell system reveals MAE of PID genes



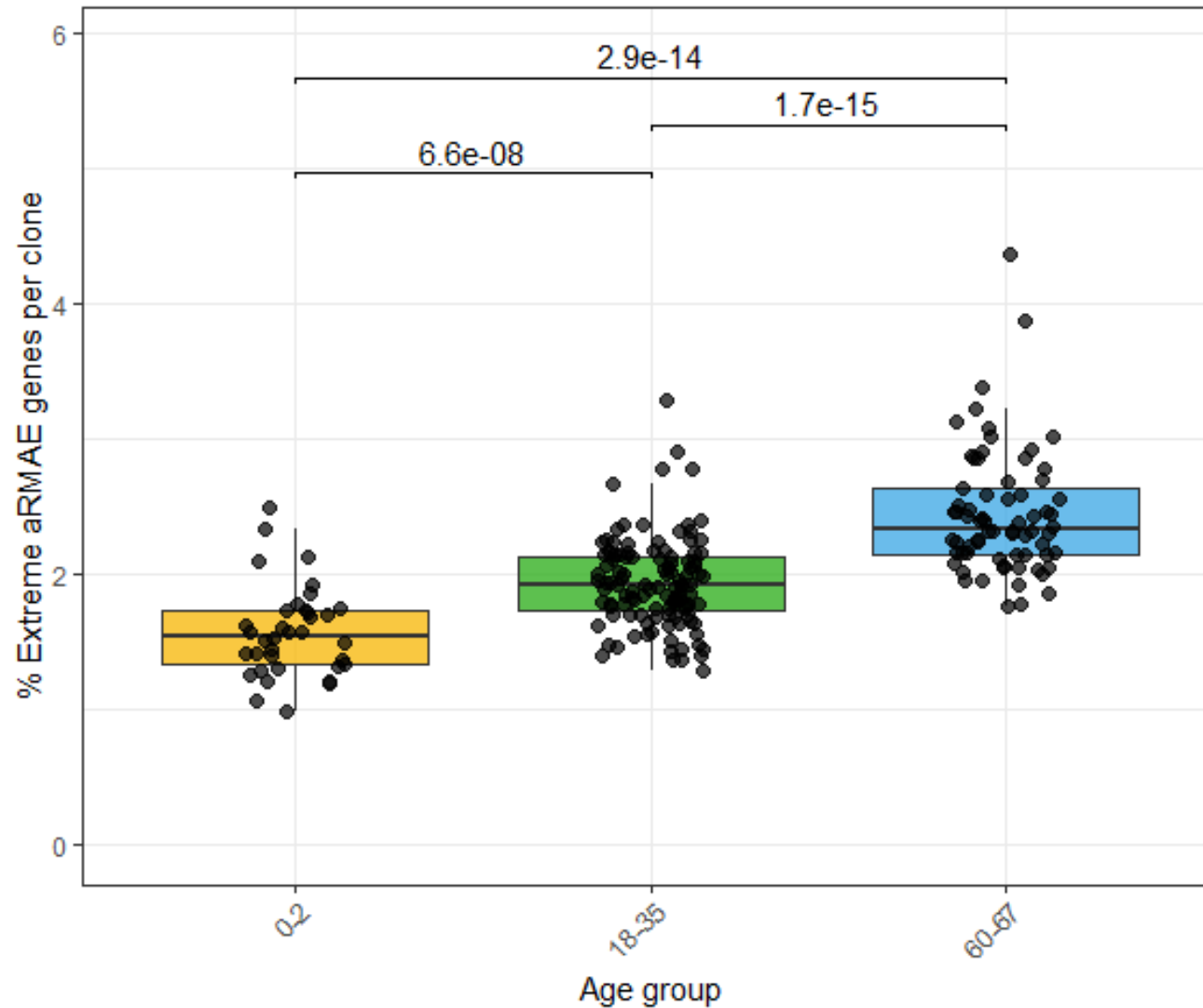
About 5% of all genes can undergo MAE
when assessing ~20 clones



About 50% of all genes (IEI) can undergo MAE when assessing ~200 clones



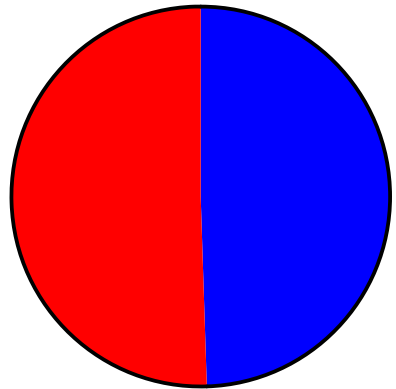
Does MAE increase with age?



PLCG2 MAE is stable for weeks in culture

DNA

DONOR E

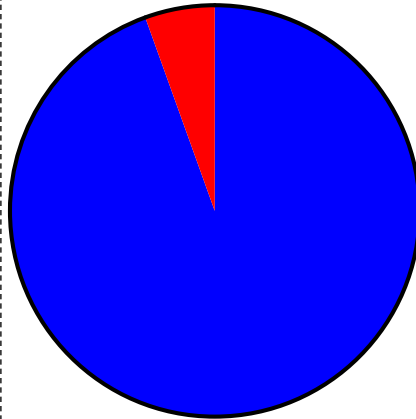


49.49% REF
50.51% ALT

RNA

WEEK 1

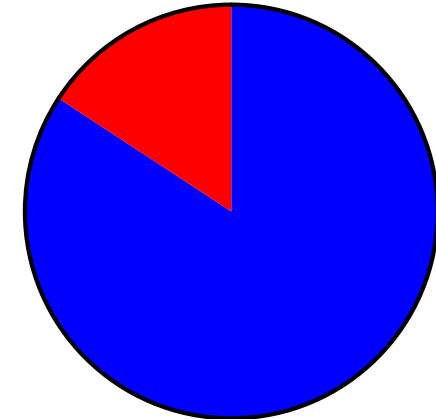
CLONE 1



94.47% REF
5.53% ALT

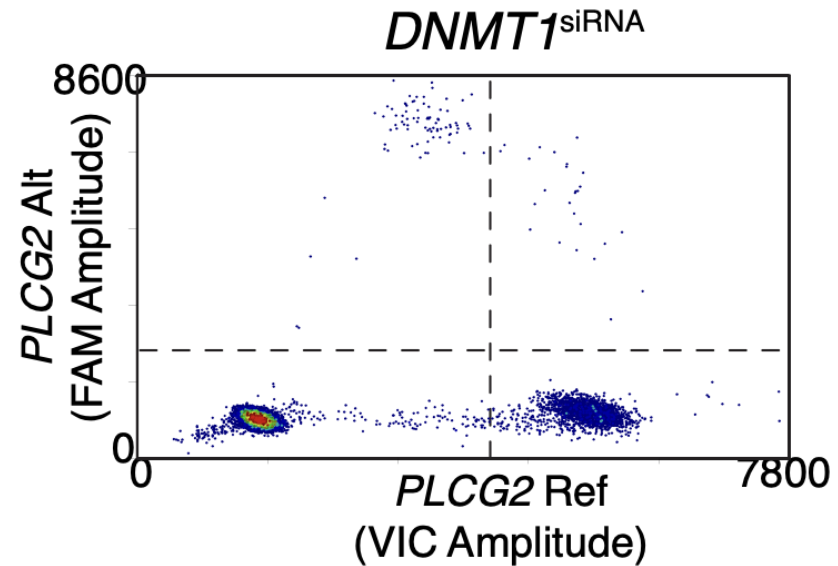
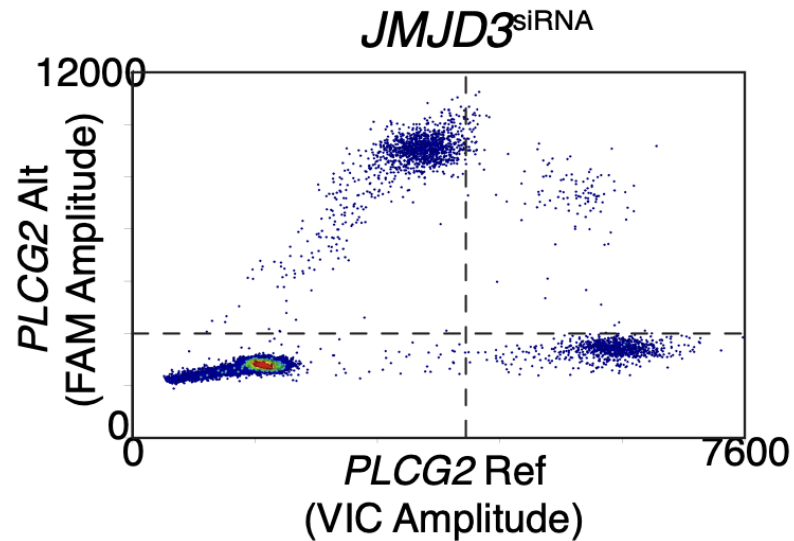
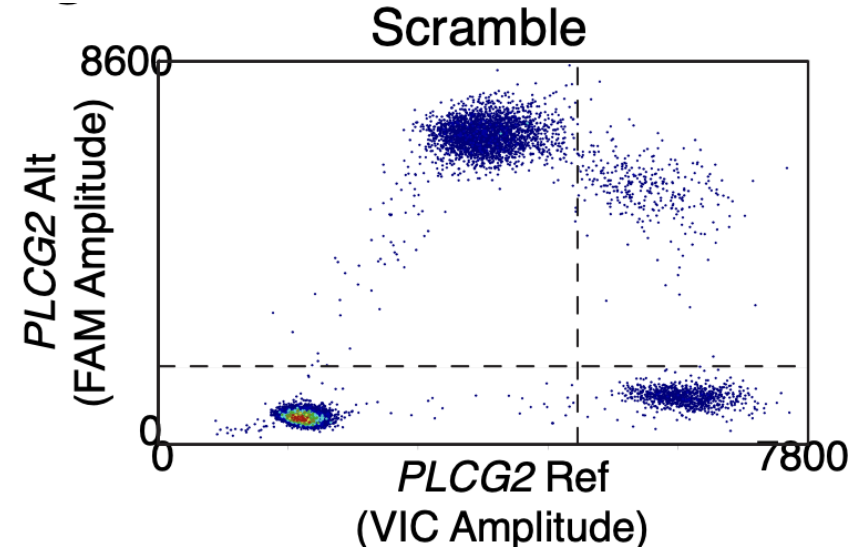
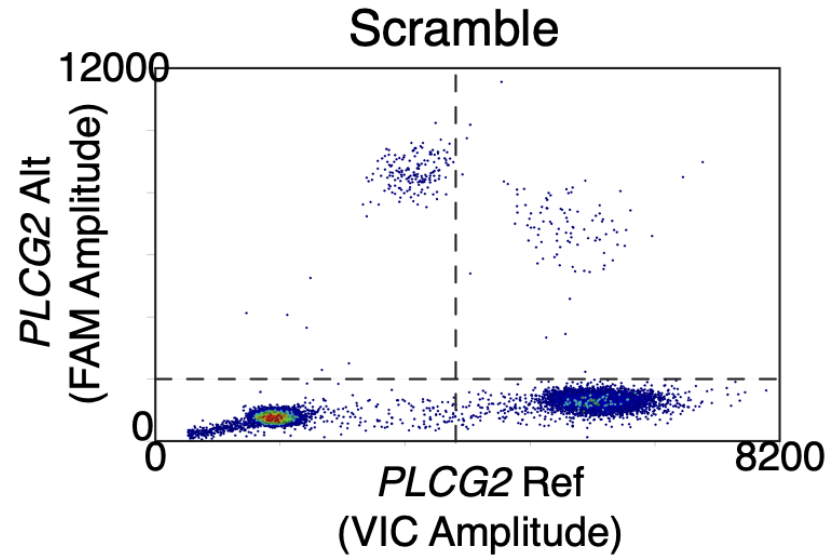
WEEK 4

CLONE 1



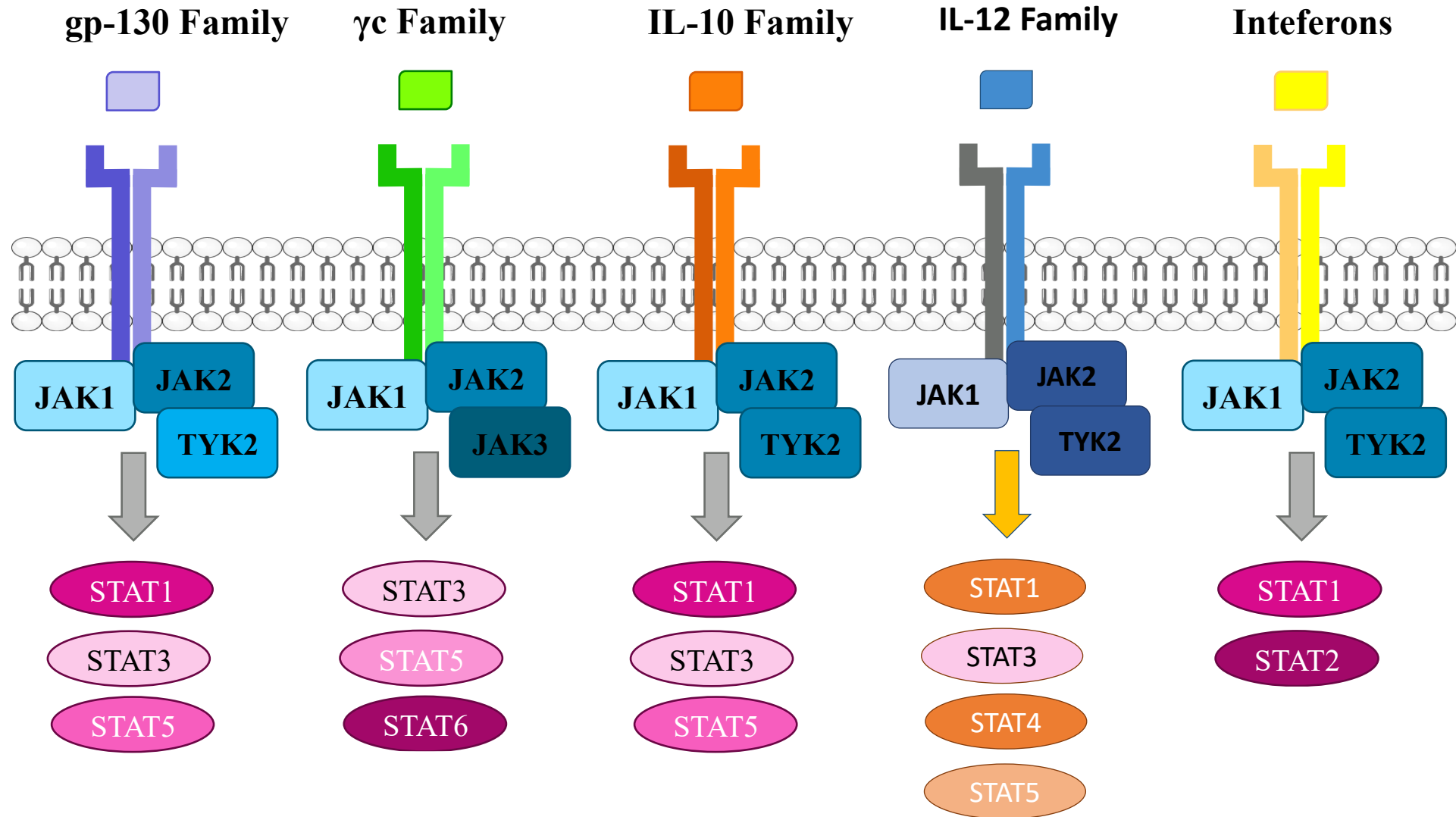
84.21% REF
15.79% ALT

H3K27me3 and DNA methylation regulate MAE

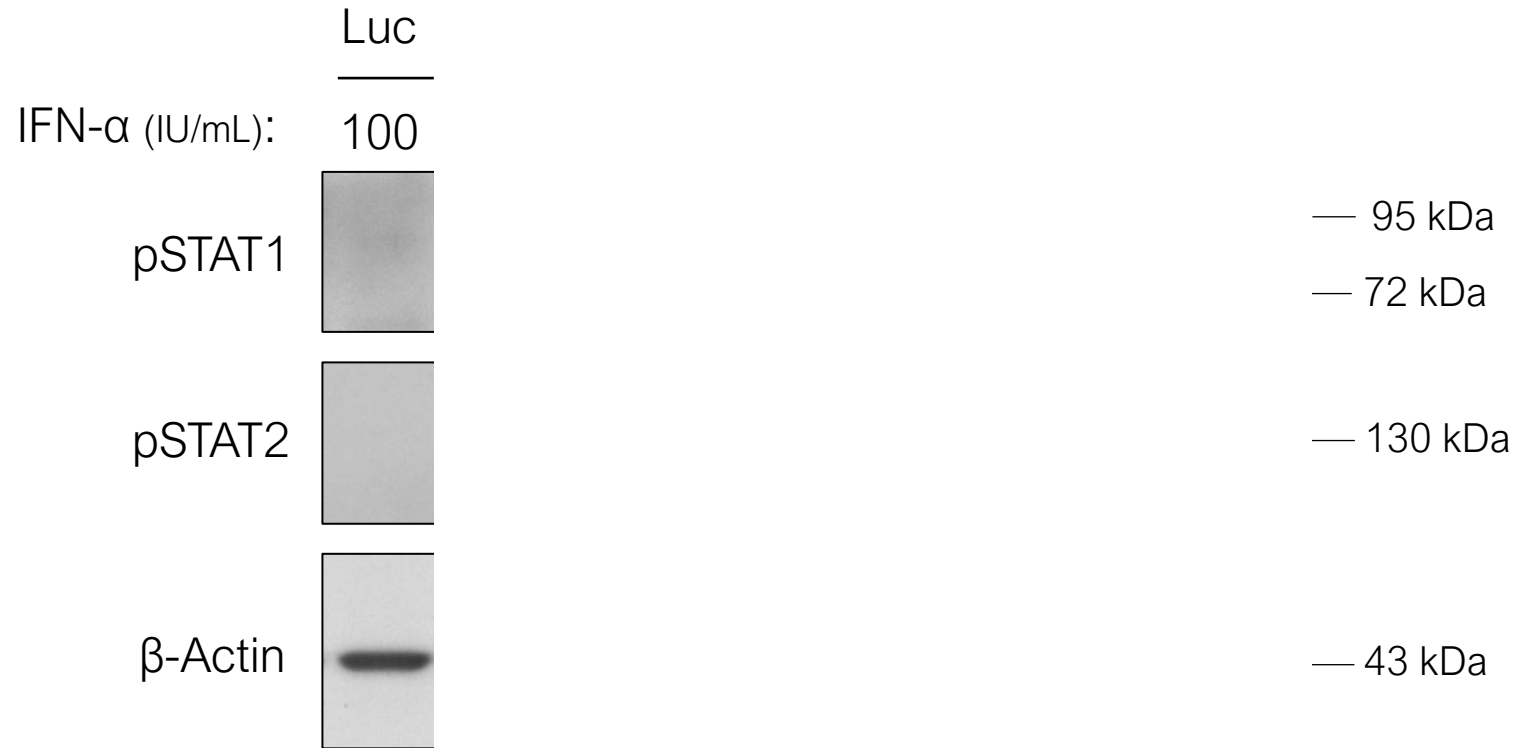


JAK1

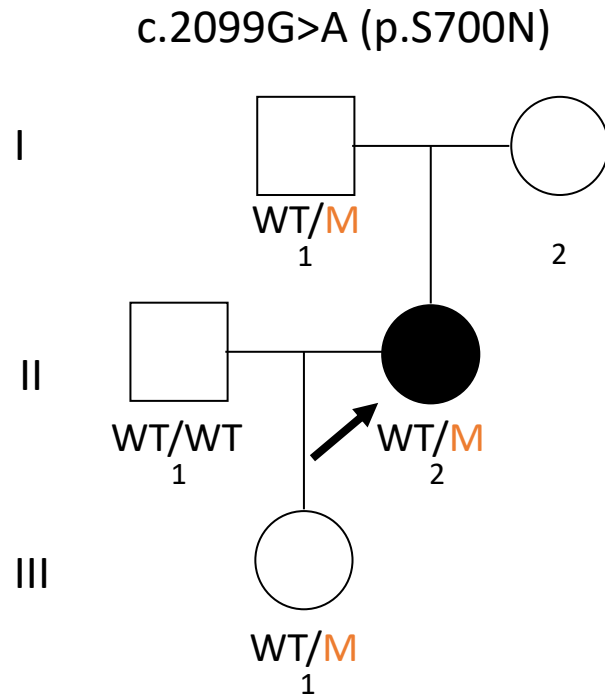
JAK1 mediates response to 25+ cytokines



In vitro analysis of S703I — gain-of-function

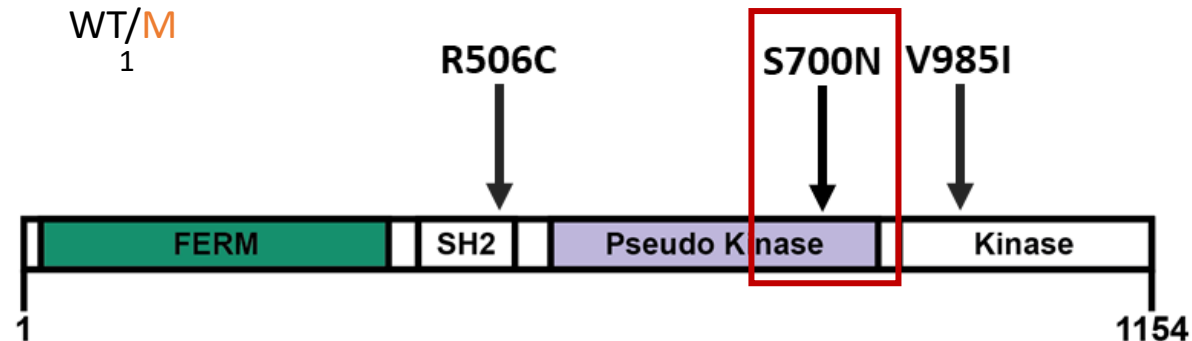


Incomplete Penetrance in family with JAK1 mutation

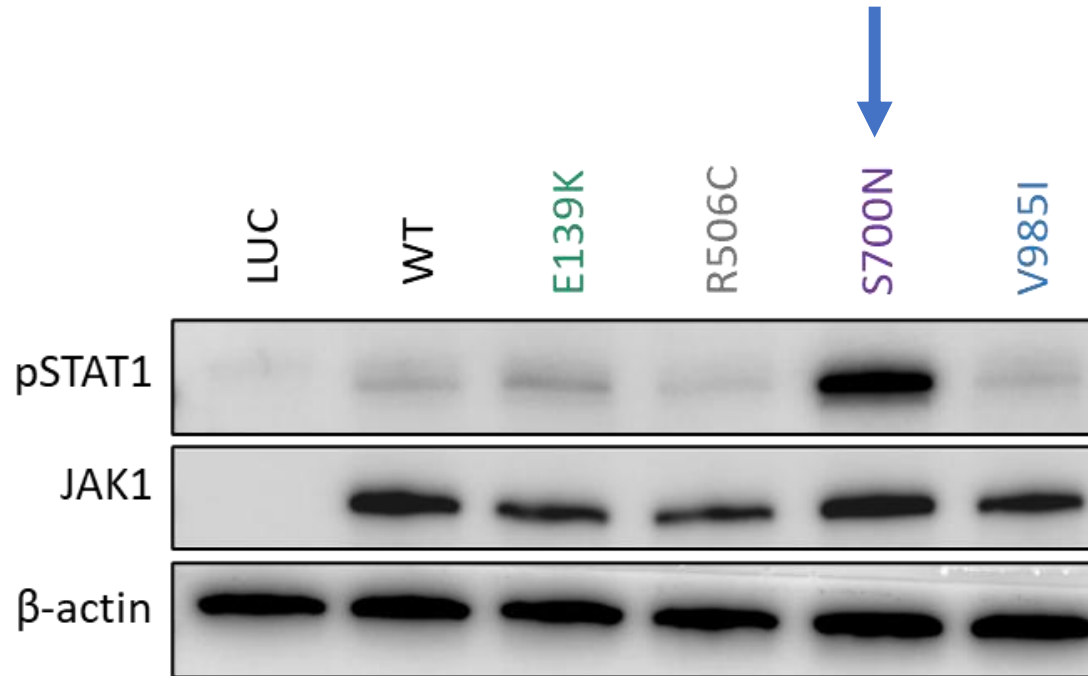


II-2

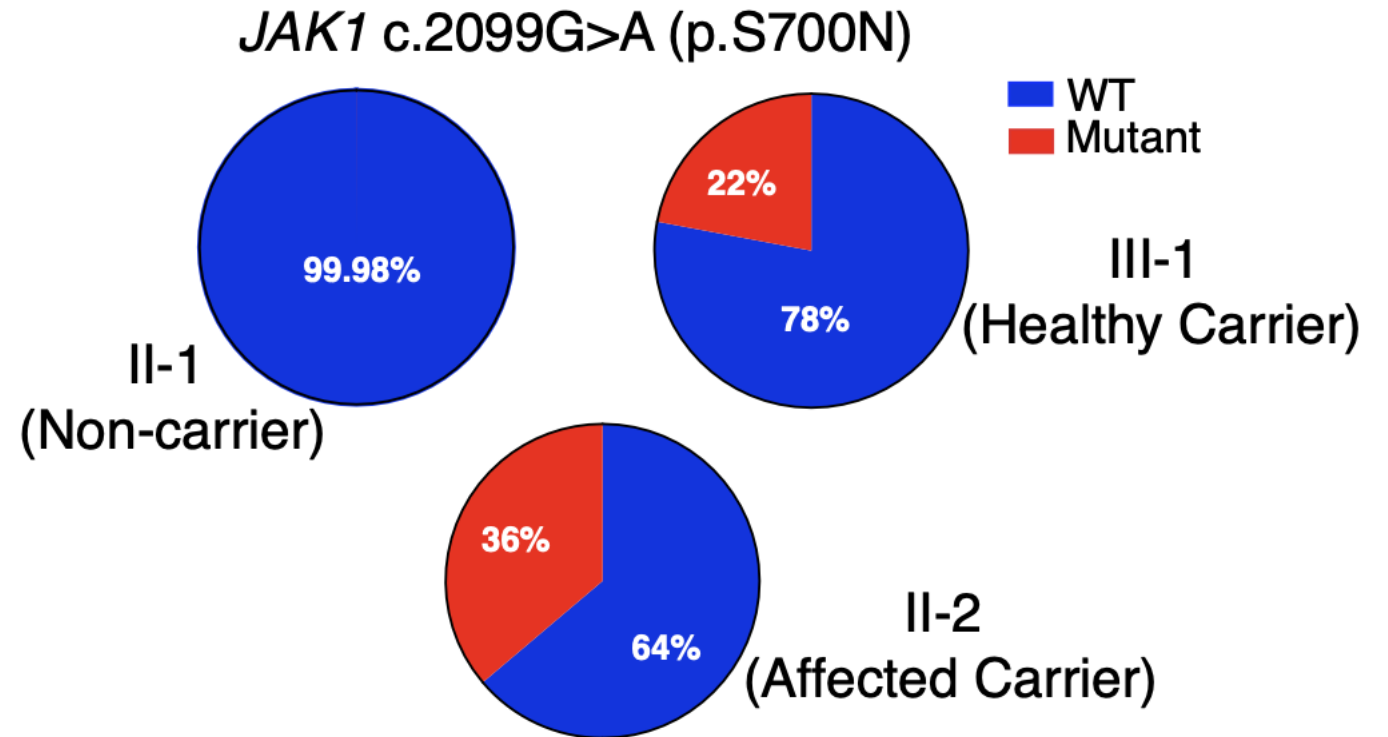
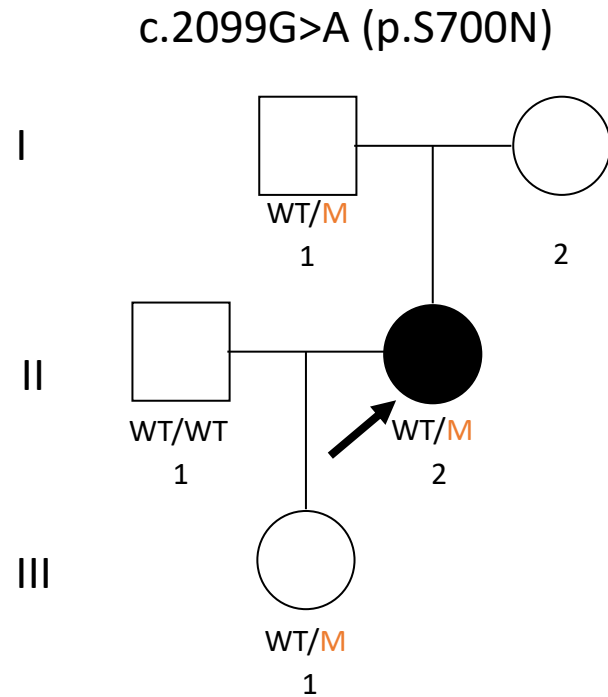
- 34 yo Female
- Healthy until toxoplasmosis at 28yo
- Recurrent infections, CVID -> hyper-IgM
- Splenomegaly -> severe pancytopenia
- Thrombocytopenia requiring transfusion



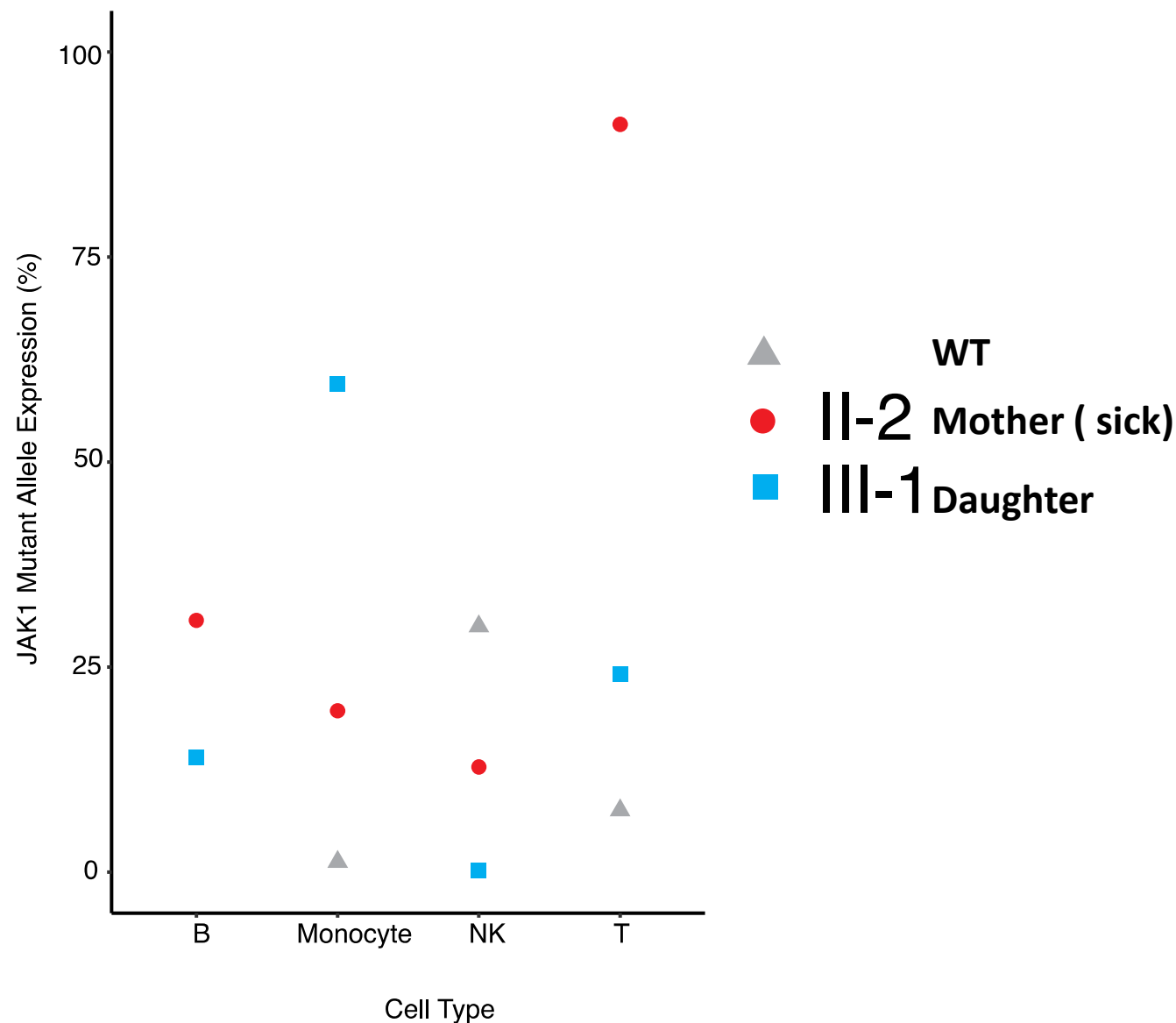
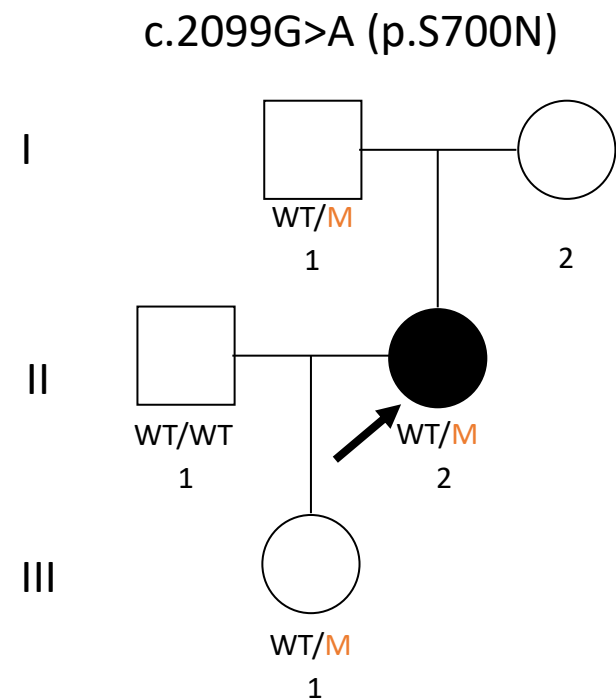
S700N variation in JAK1 leads to GOF



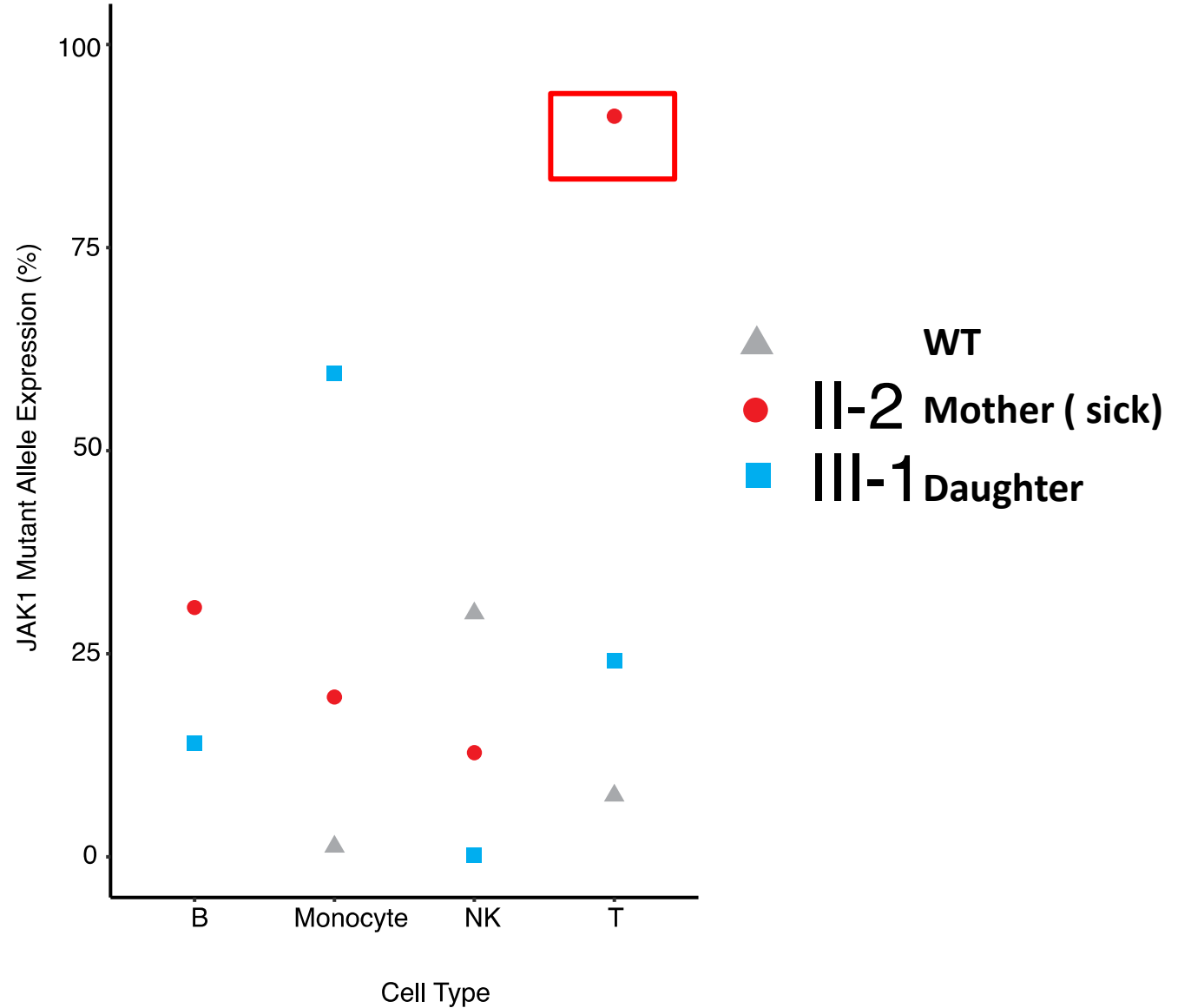
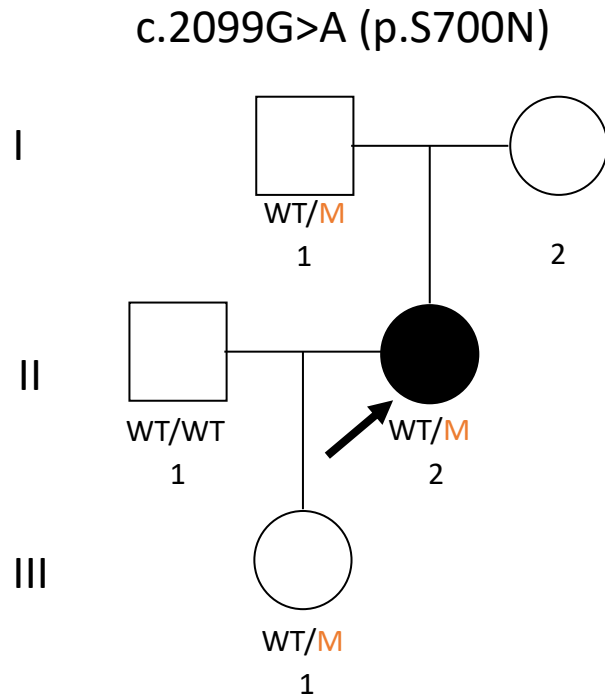
Monoallelic expression in *JAK1*^{WT/S700N} Patient cells



Monoallelic expression in *JAK1*^{WT/S700N} Patient cells



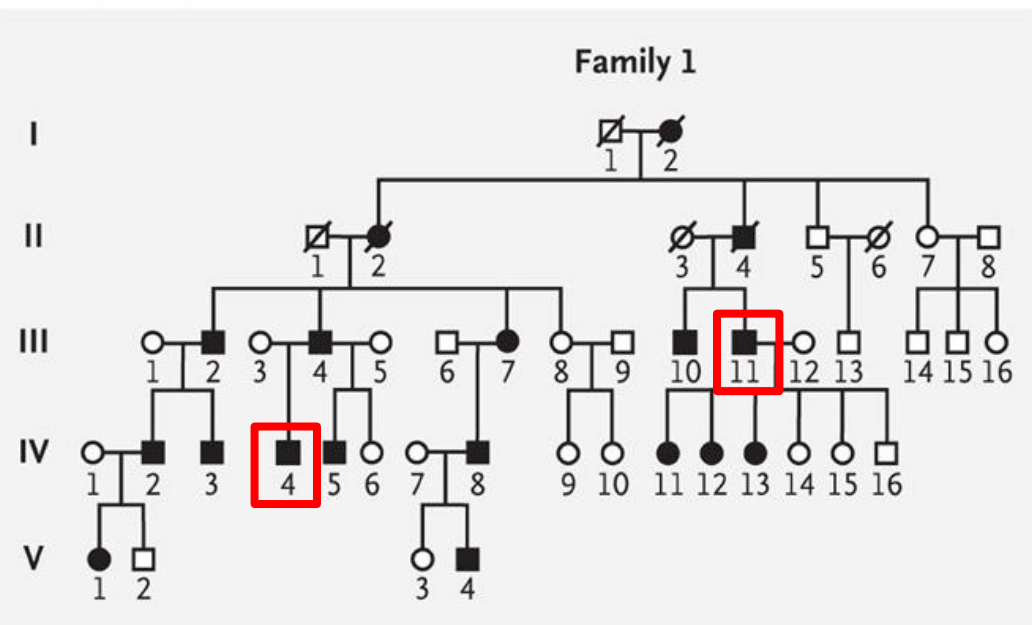
Monoallelic expression in JAK1^{WT/S700N} Patient cells



PLCG2

Discordant Class-Switched antibody production in PLAID patients

Family Pedigrees



Patient A (III-11)

- Asthma
- Skin granuloma of nose (resolved by Age 1)
- Vitiligo
- Anti Nuclear Antibody - Positive
- **IgG normal (1480) IgM normal (44), IgA normal (283), IgE normal (54)**

Patient B (IV-4)

- Onchomycosis
- Mild Disease
- **IgG low (325) IgM low (<21), IgA low (45), IgE normal (18)**

Discordant Class-Switched antibody production in PLAID patients

Patient A (III-11)

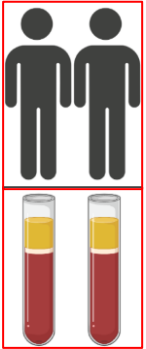
- Asthma
- Skin granuloma of nose (resolved by Age 1)
- Vitiligo
- Anti Nuclear Antibody - Positive
- **IgG normal (1480) IgM normal (44), IgA normal (283), IgE normal (54)**

Patient B (IV-4)

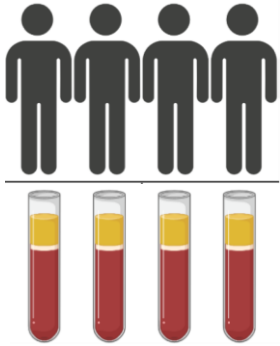
- Onchomycosis
- Mild Disease
- **IgG low (325) IgM low (<21), IgA low (45), IgE normal (18)**

Experiment Design

PLCG2^{wt/mut}



Healthy Controls



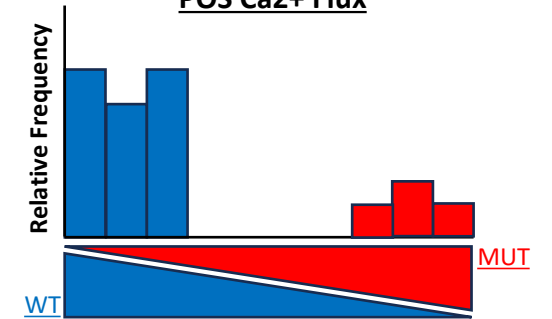
Sort B cells based on calcium flux

FLUO-4
(labeled calcium indicator)

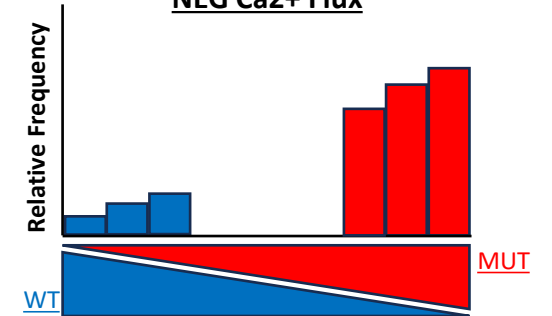


qPCR/ddPCR

POS Ca²⁺ Flux



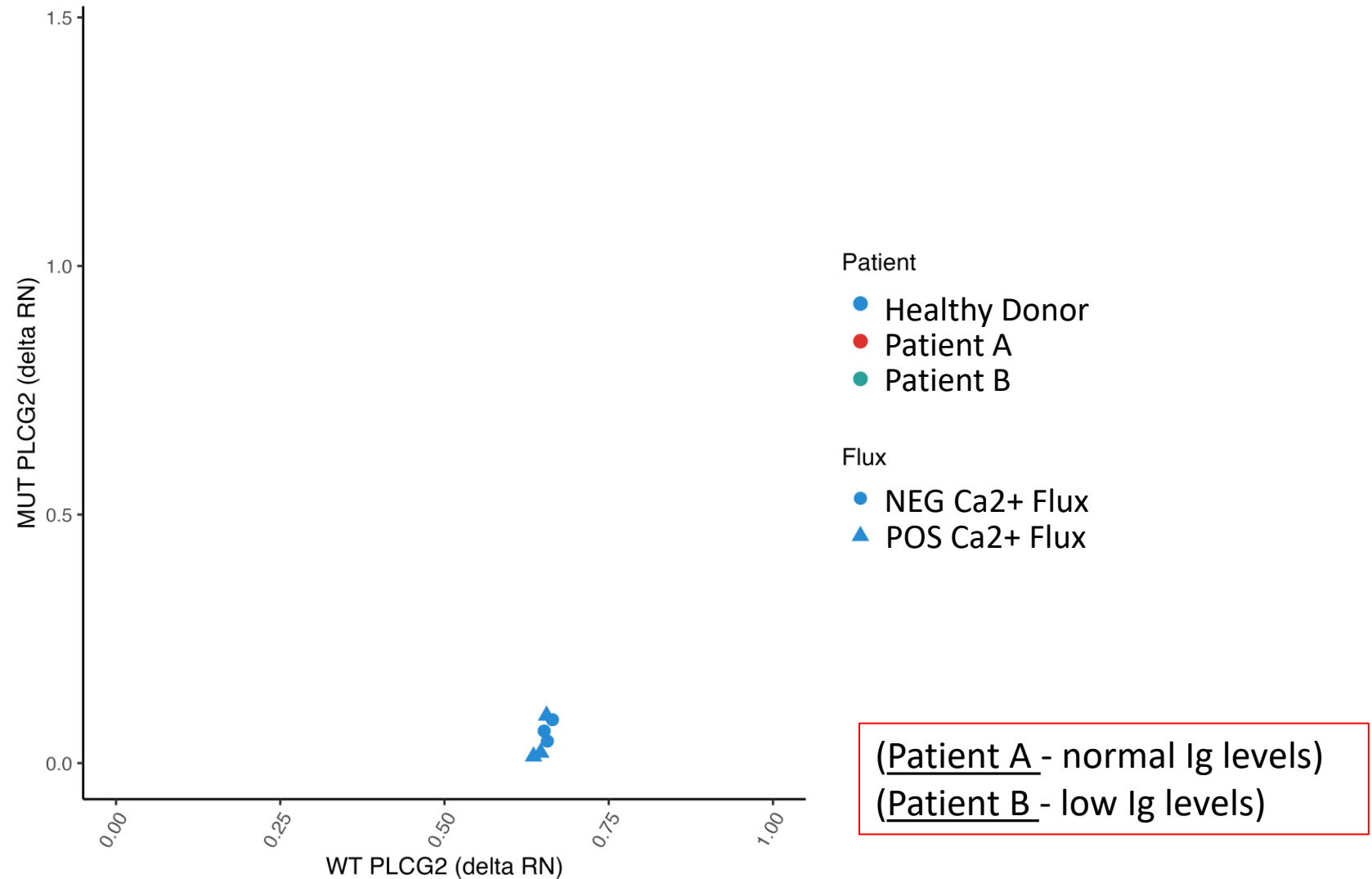
NEG Ca²⁺ Flux



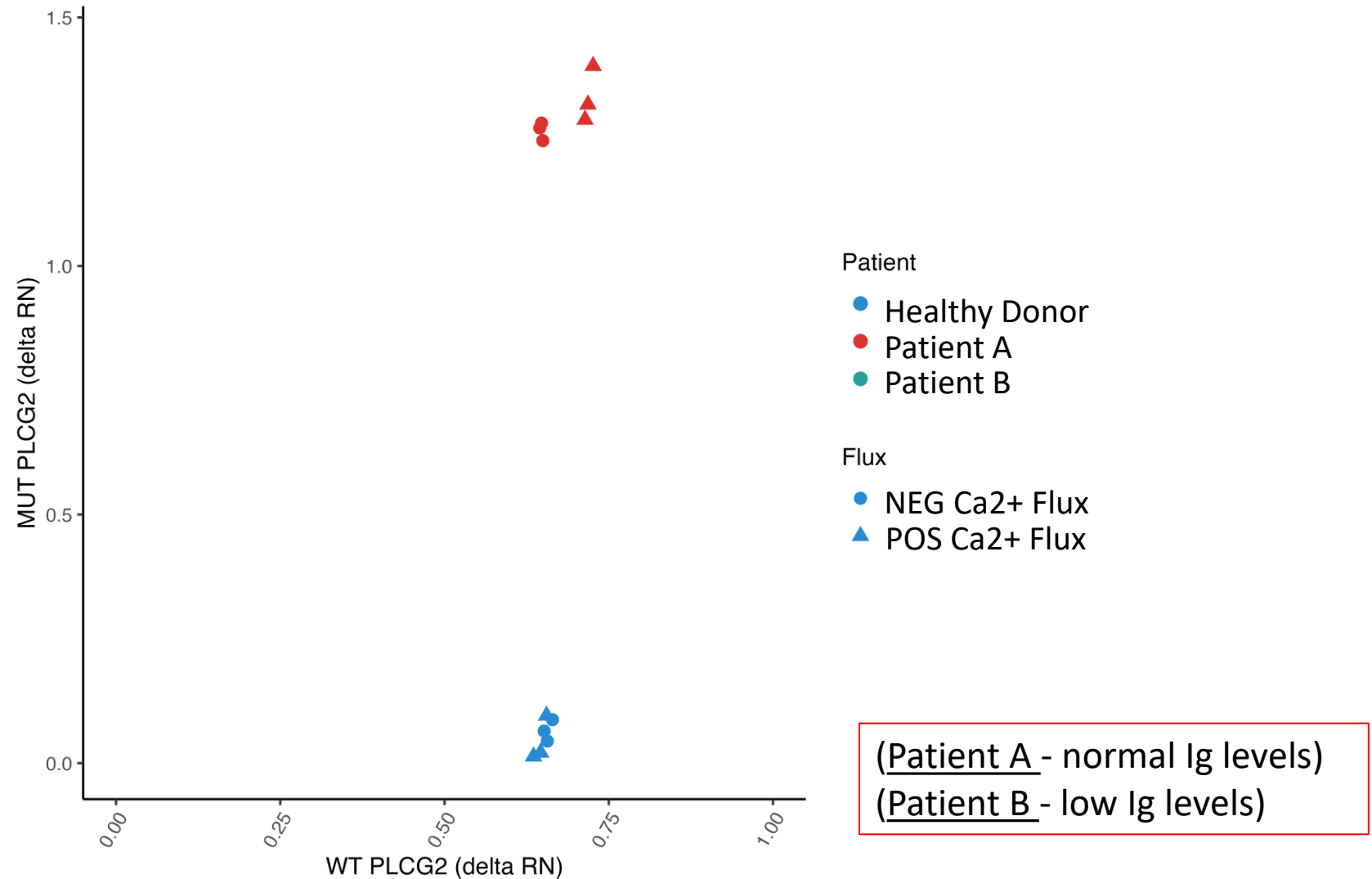
(Patient A - normal Ig levels)

(Patient B - low Ig levels)

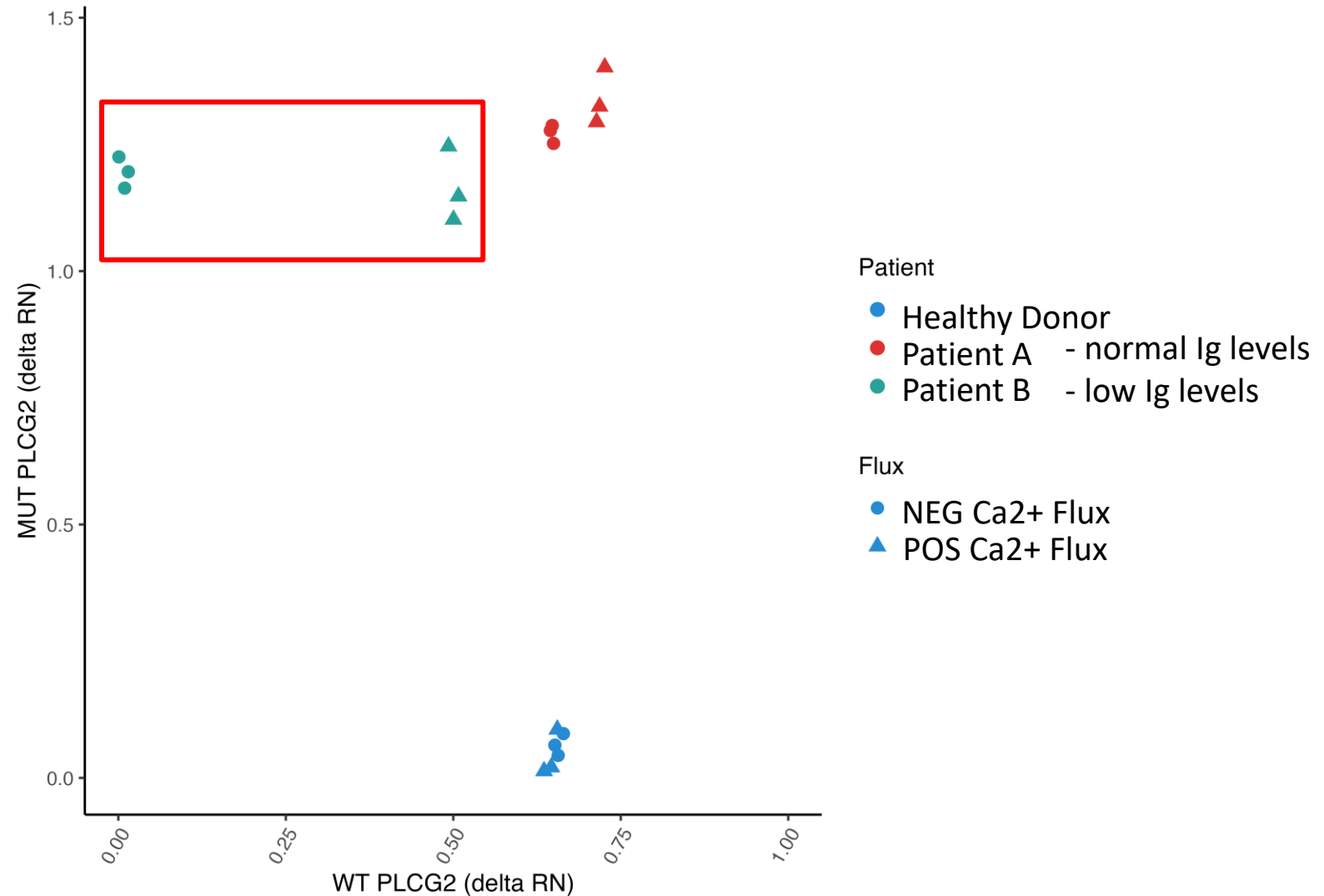
Monoallelic expression in *PLCG2*^{WT/delEX19} *B* cells



Monoallelic expression in *PLCG2*^{WT/delEX19} *B* cells

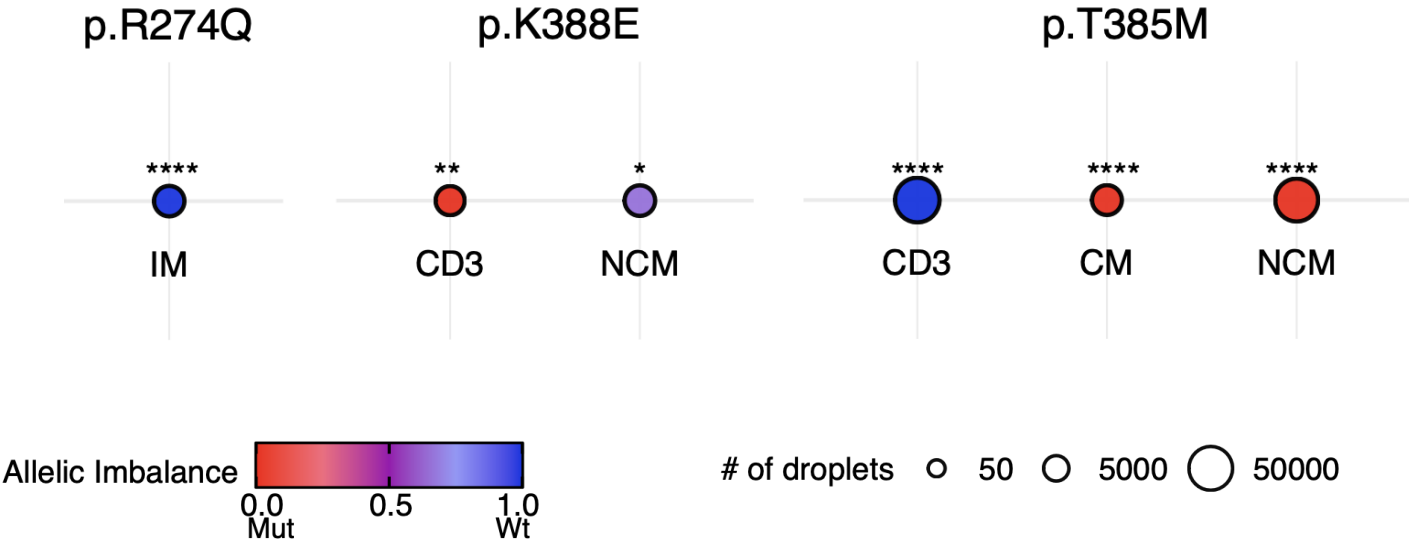
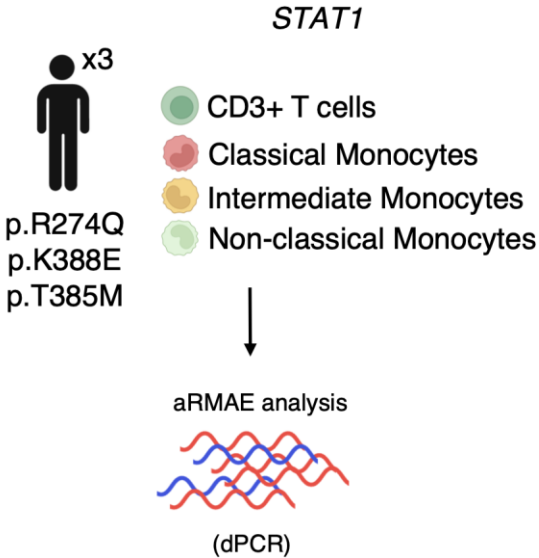


Monoallelic expression in *PLCG2*^{WT/delEX19} *B* cells

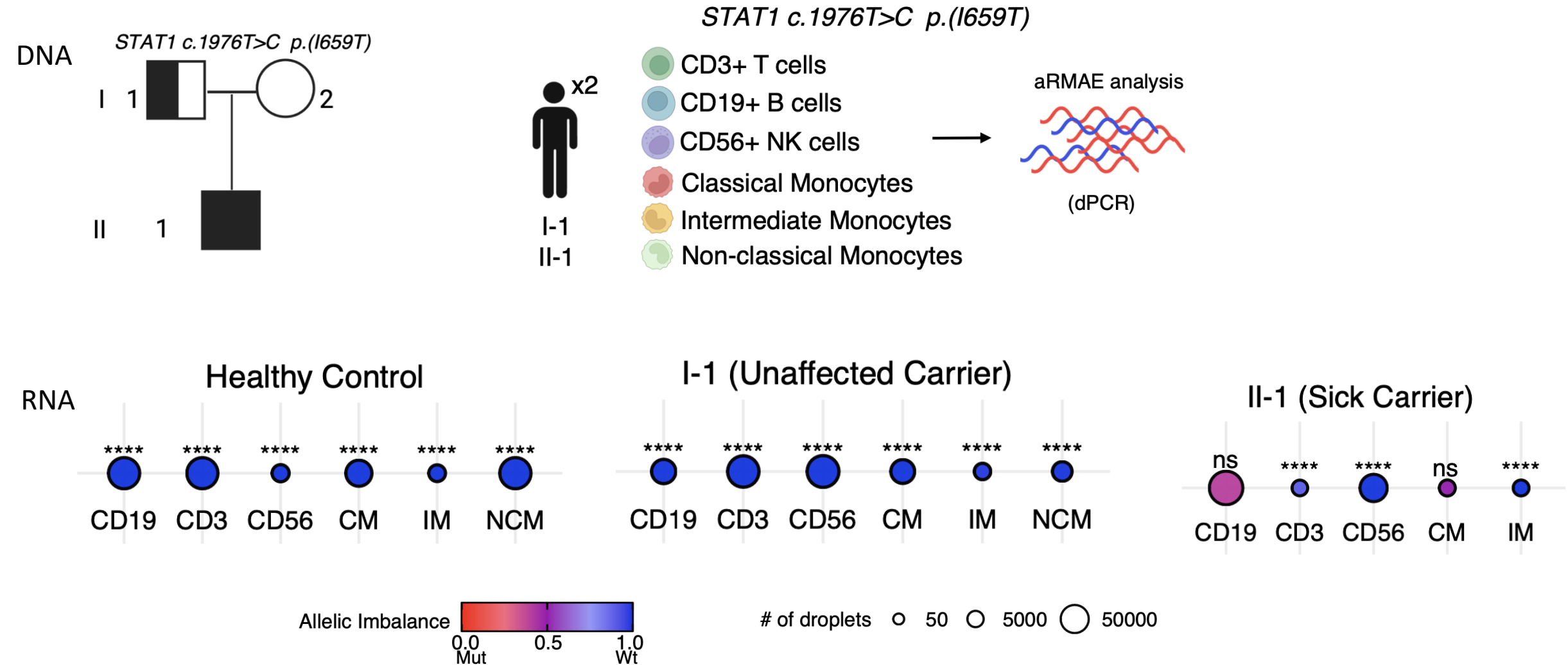


Monoallelic expression in *STAT1* GOF

	<i>STAT1</i>		
	p.R274Q	p.K388E	p.T385M
			
Chronic Mucocutaneous Candidasis	+	+	+
Bacterial Infections	+	+	+
Viral Infections	-	-	+
Thyroid Dysfunction	-	+	-
Other autoimmune symptoms	-	+	+
Atopy	-	-	+
Lymphangioma	-	-	+
Bronchiectasis	-	-	+



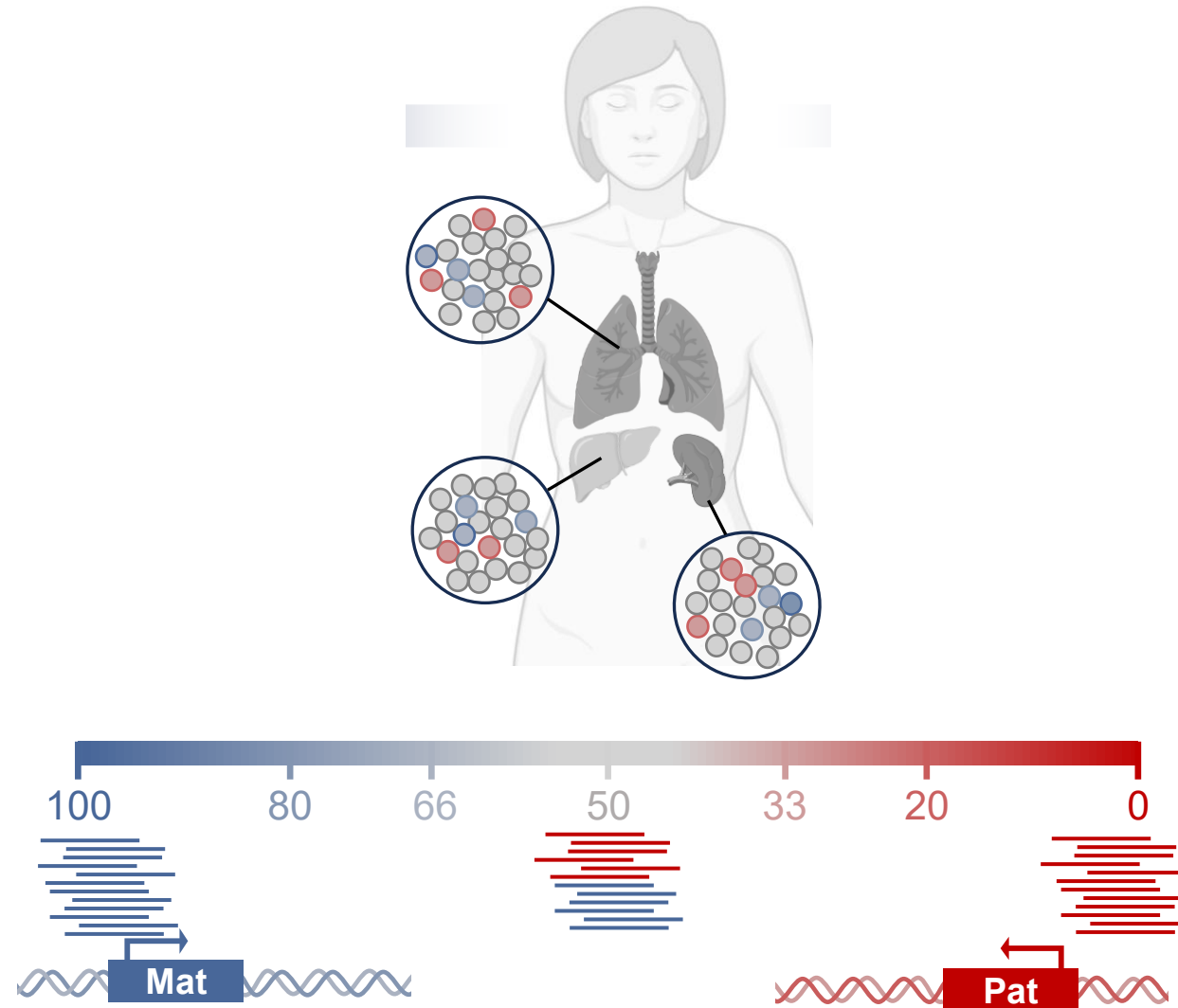
Monoallelic expression in *STAT1* DN



Conclusions

- MAE occurs naturally in all of us. Why?

Personal Evolution Theory



Conclusions

MAE renders explanation to some incomplete penetrance cases.

In a way, a family member that has a variant may not get sick, but this is not for sure. We need to monitor it.