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How the epitranscriptome is changing our world

Mary O'Connell



What happened today?

The Nobel Assembly at Karolinska Institutet

has today decided to award the 2022 Nobel Prize in Physiology or Medicine to

Svante Pääbo

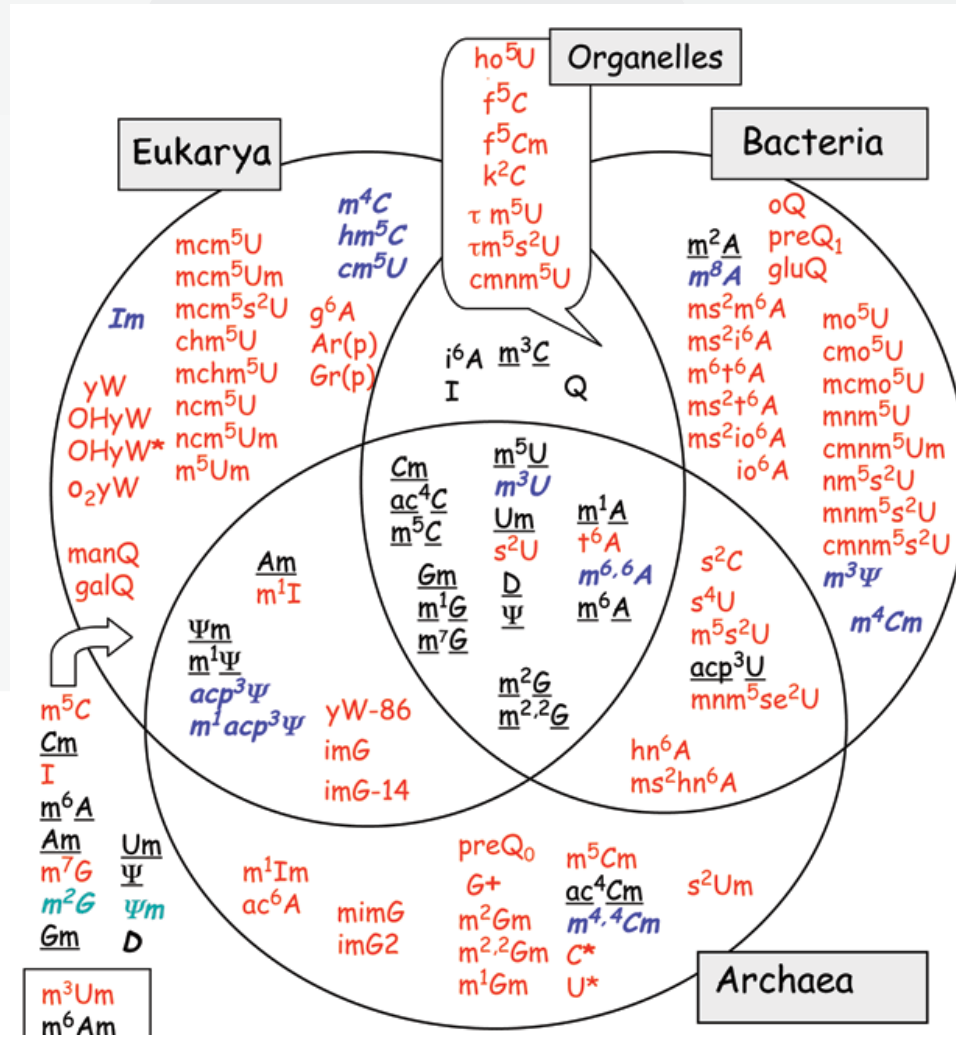
for his discoveries concerning the genomes of extinct hominins and human evolution

What is the epitranscriptome?

Guess!

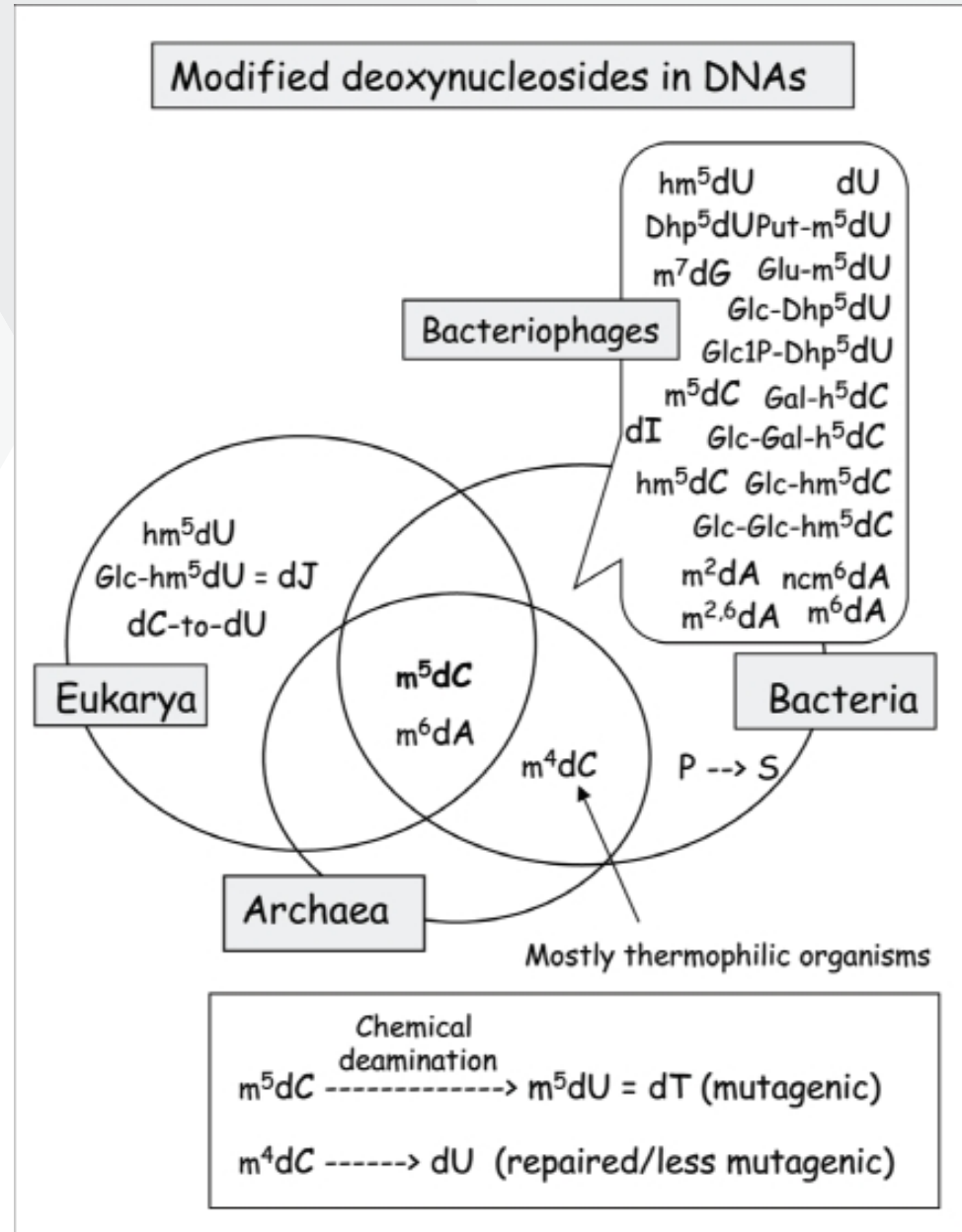
RNA modification

>170 RNA modifications



Henri Grosjean

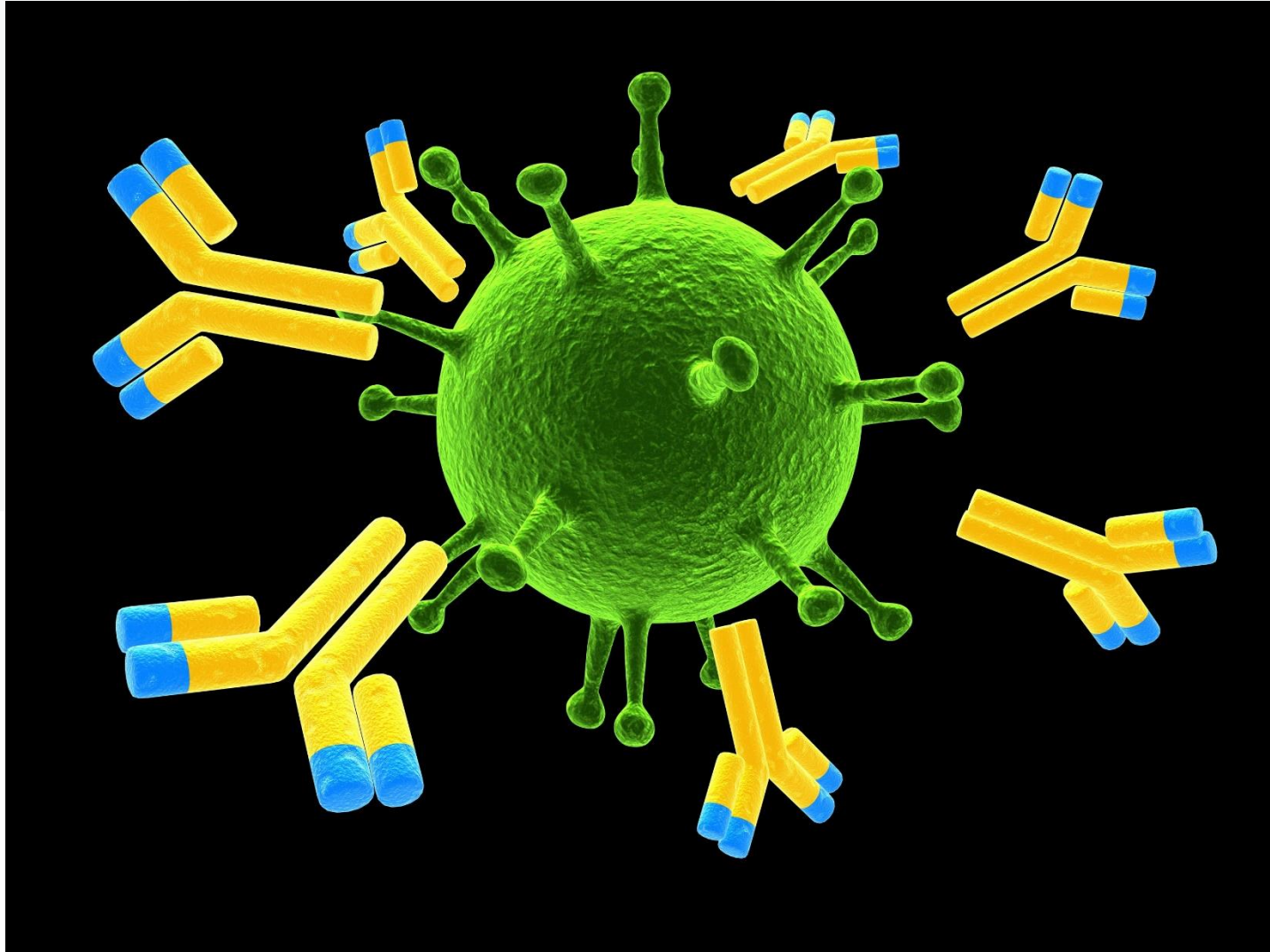
Modifications in DNA



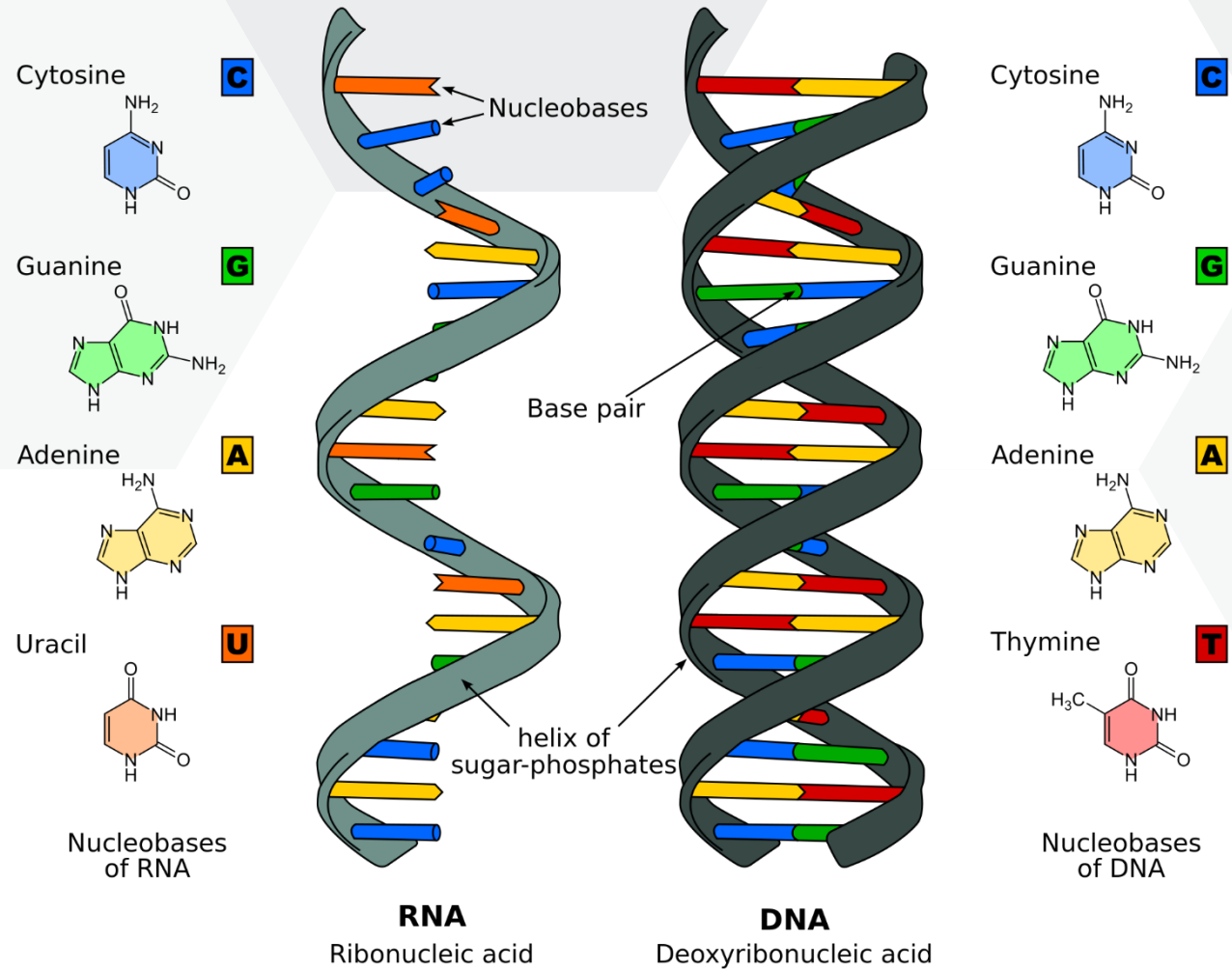
WHY?

Henri Grosjean

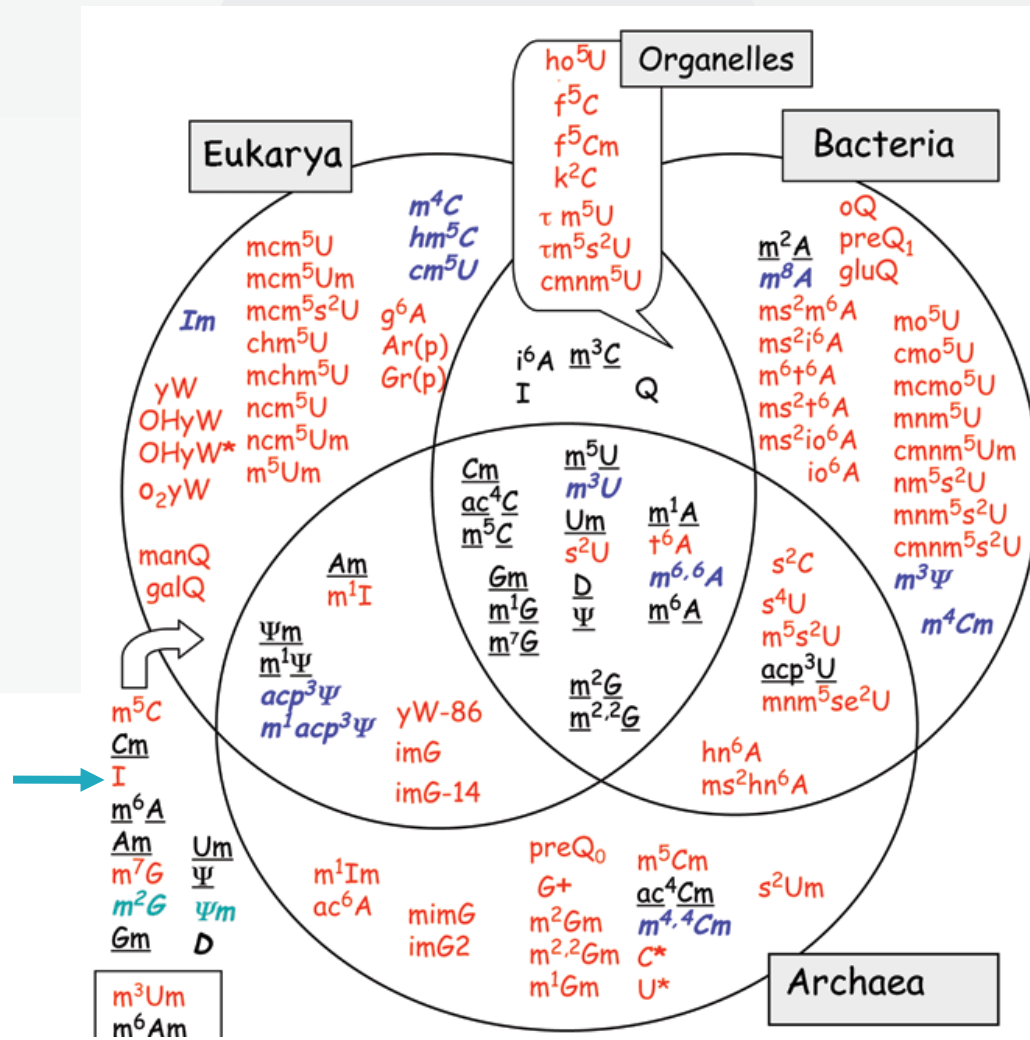
Antibody/Antigen interaction



Problem with nucleic acids



Approximately 170 RNA modifications



Henri Grosjean

RNA vaccines

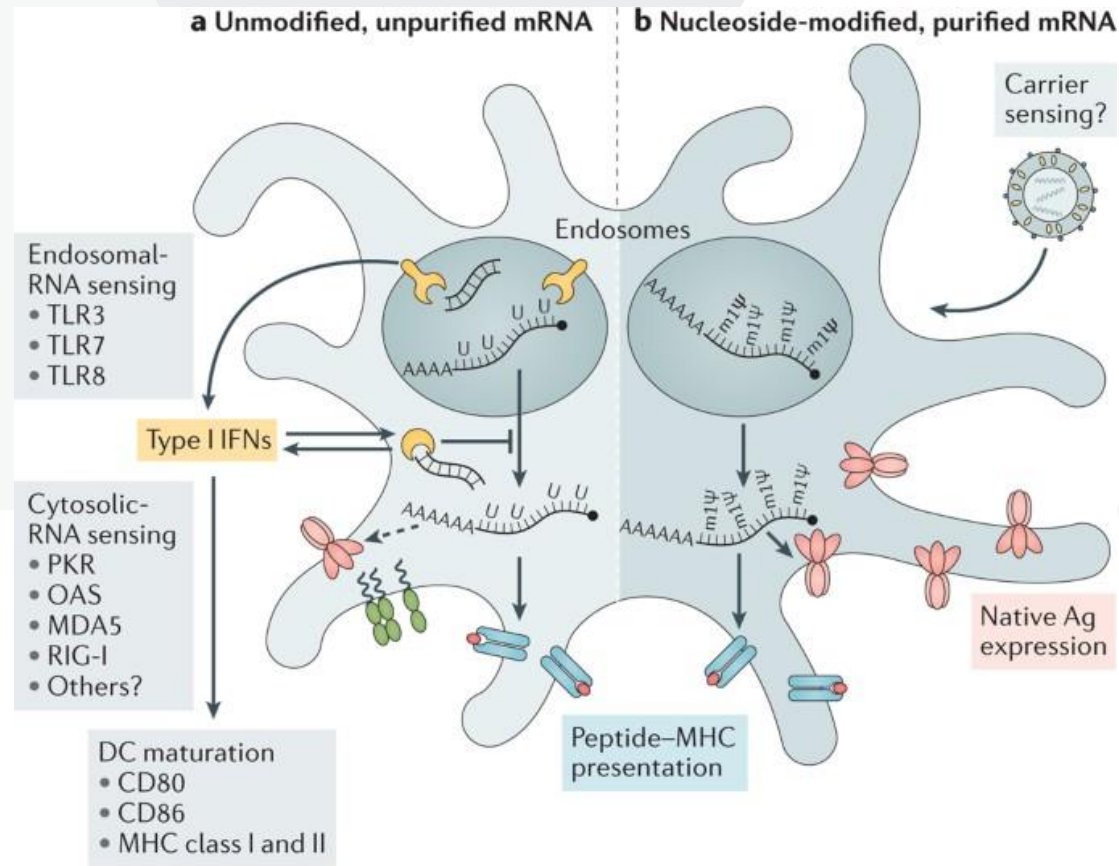


Katalin Kariko



Drew Weissman

Modified versus unmodified RNA



Fast vaccines

NEXT-GENERATION mRNA VACCINES



Computer-designed, a feature that makes them adaptable and rapidly scalable into millions of doses.



Rely on **genetic material called mRNA** to create an immune response against a specific antigen, or toxin—in this case, the coronavirus.



Production for the general population **can take just weeks** rather than the months required for conventional vaccines.

Over 6.5 million people have died worldwide due to Covid 19. WHO

mRNA Vaccine

Components



mRNA (blueprint of protein)

Production



Faster because mRNA molecules are easier to produce

Process

Components are injected into the arm and serve as instructions for the body to make microbial protein

Traditional Vaccine

Components



Microbial protein or inactive microbe

Production



Slower and more difficult to produce the right type of protein

Process

Components are made in a lab and injected into the arm to stimulate immune response

R & D

Antigen determined for immune stimulation

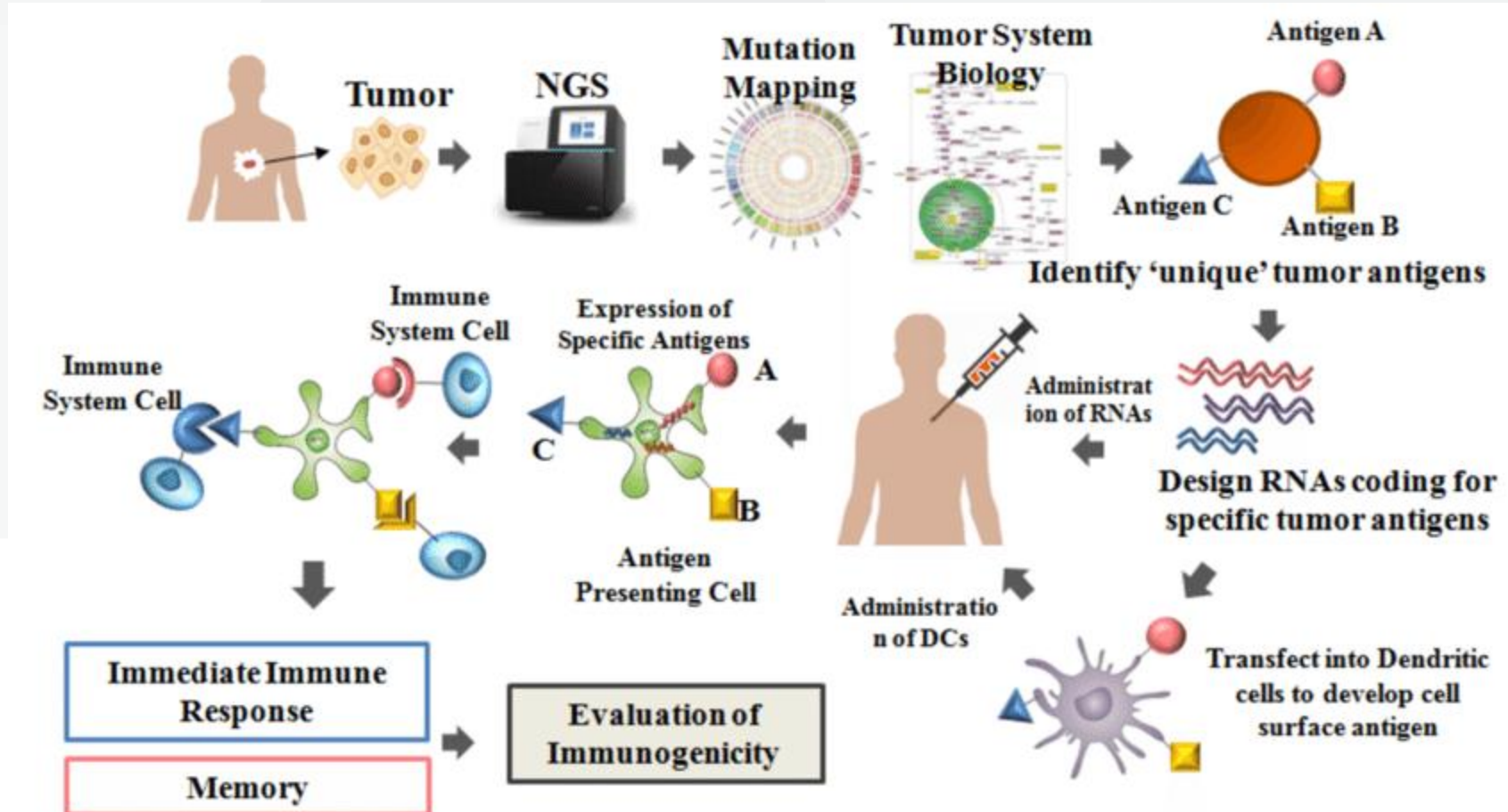


Result

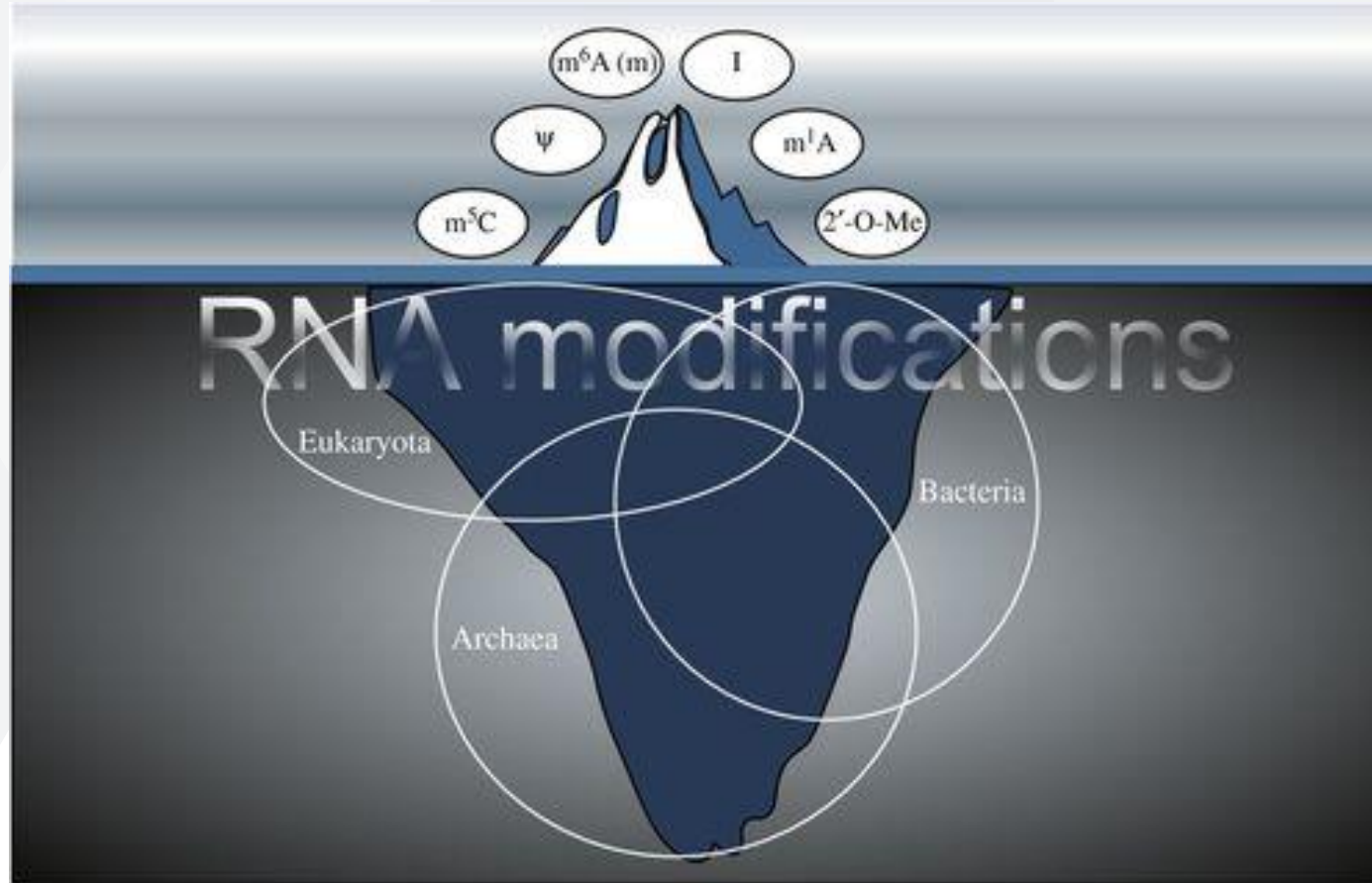
Teaches the body to protect itself against a microbe



Development of personalized RNA-based cancer vaccines

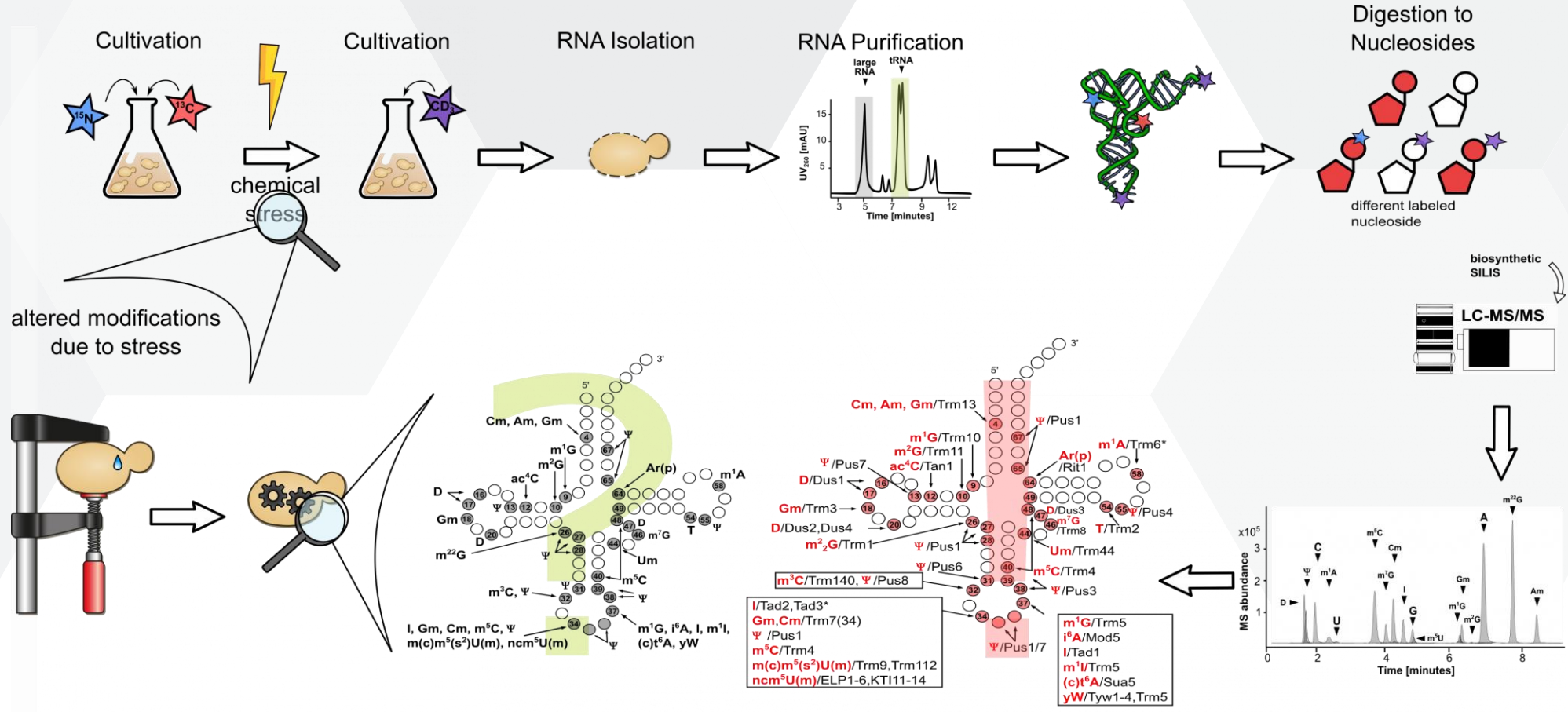


Challenges

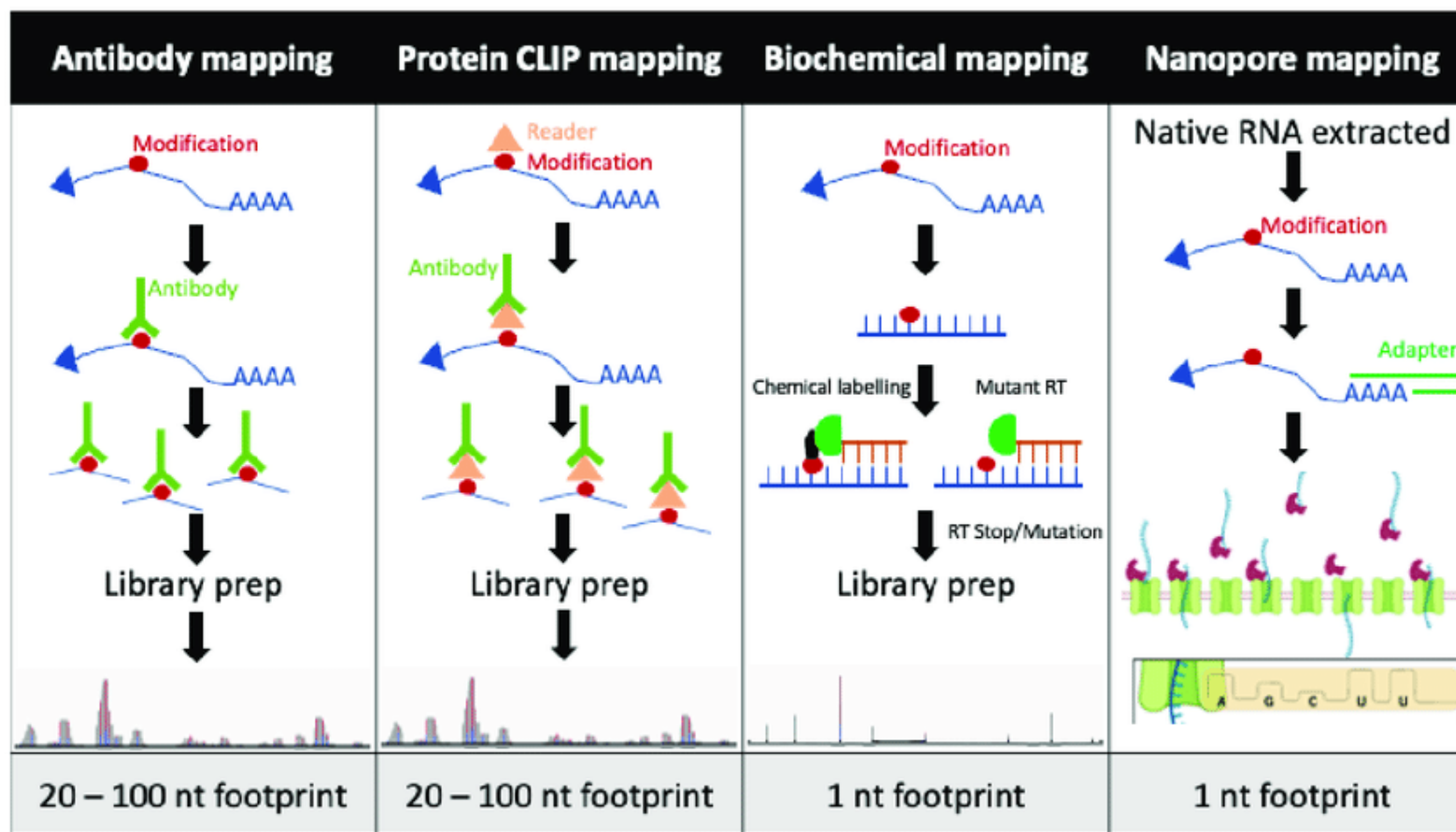


Problems with detection

Amount of starting material



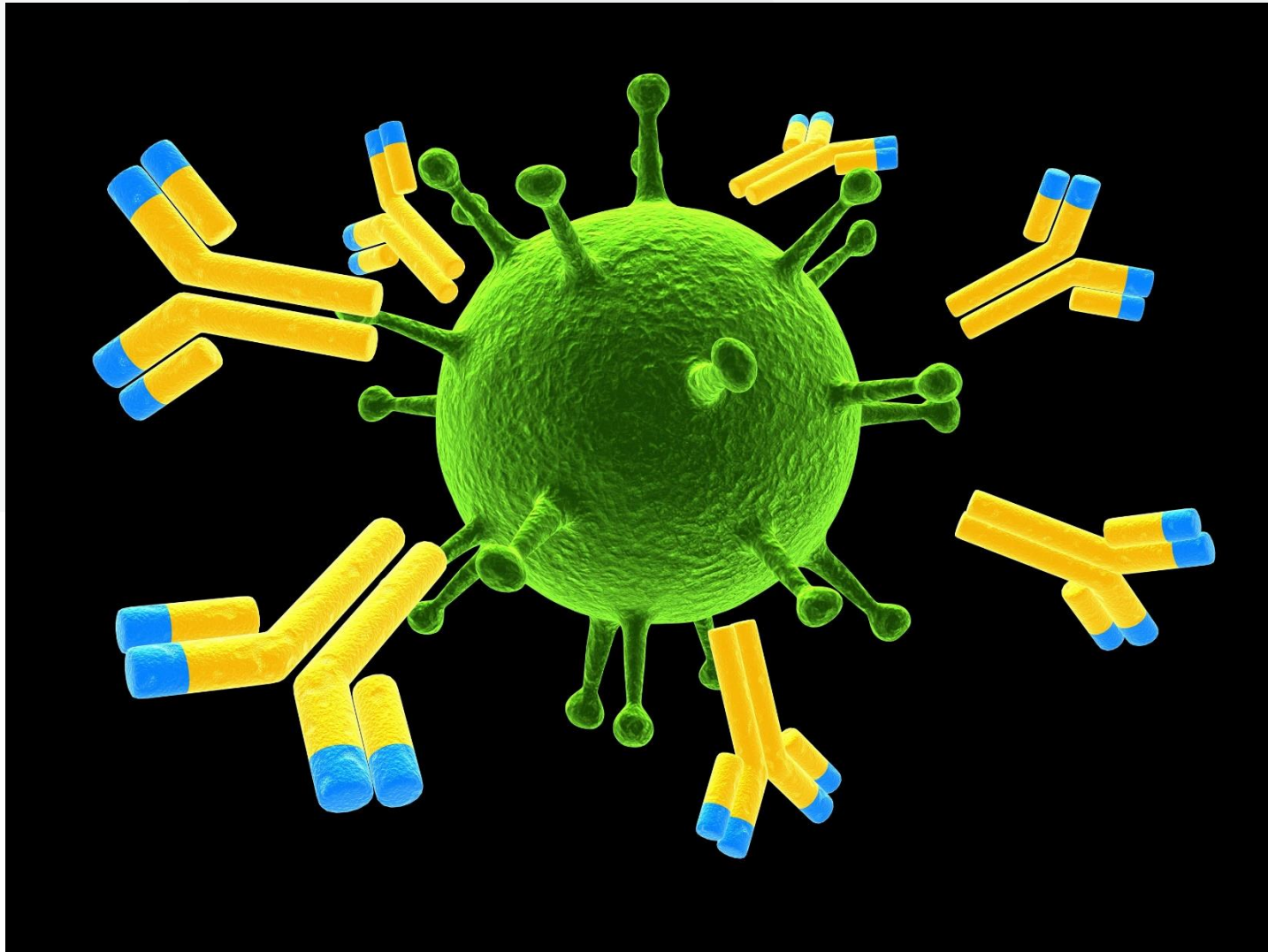
Current techniques



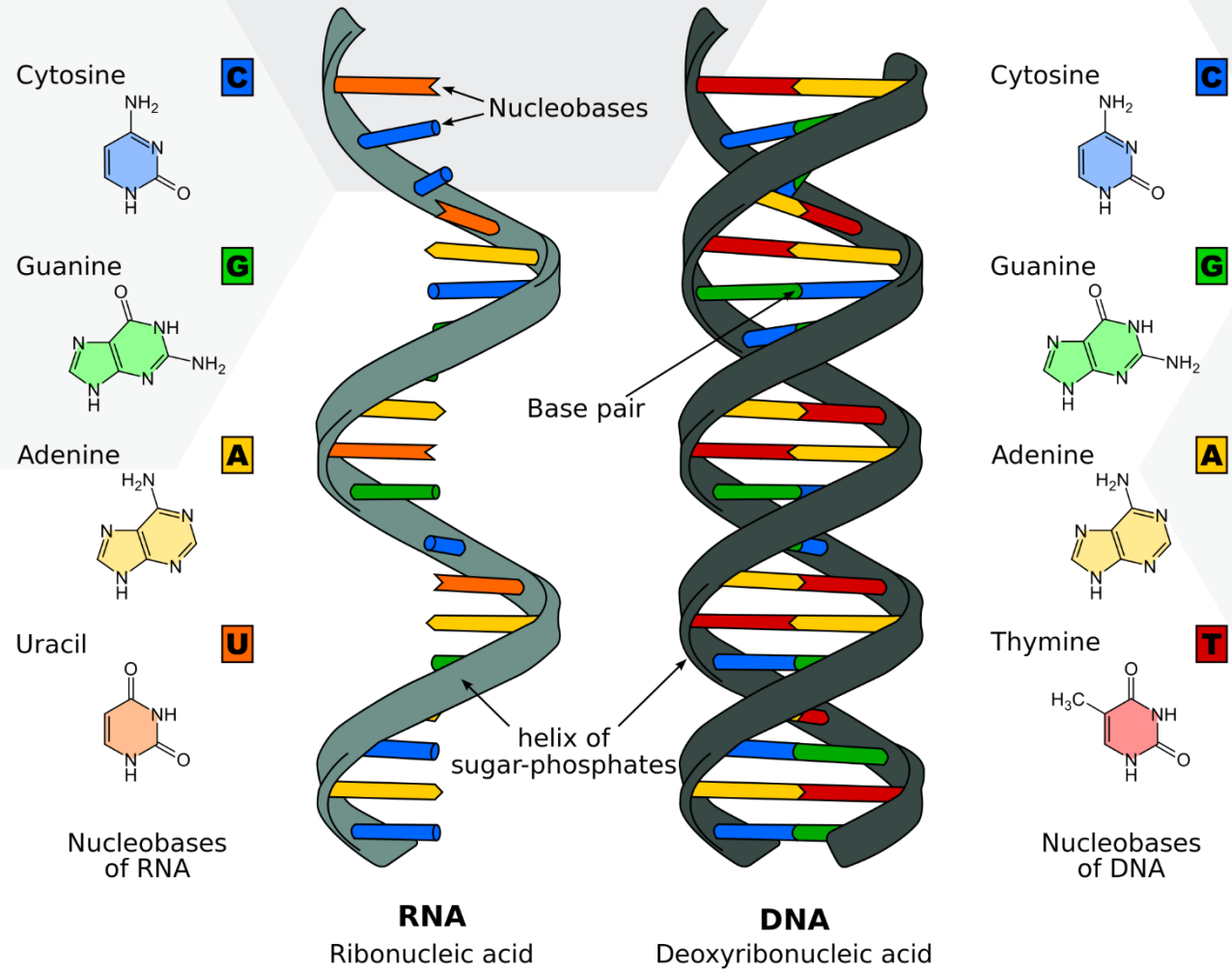
Problem with antibody specificity

What we do

Antibody/Antigen interaction



Problem with nucleic acids



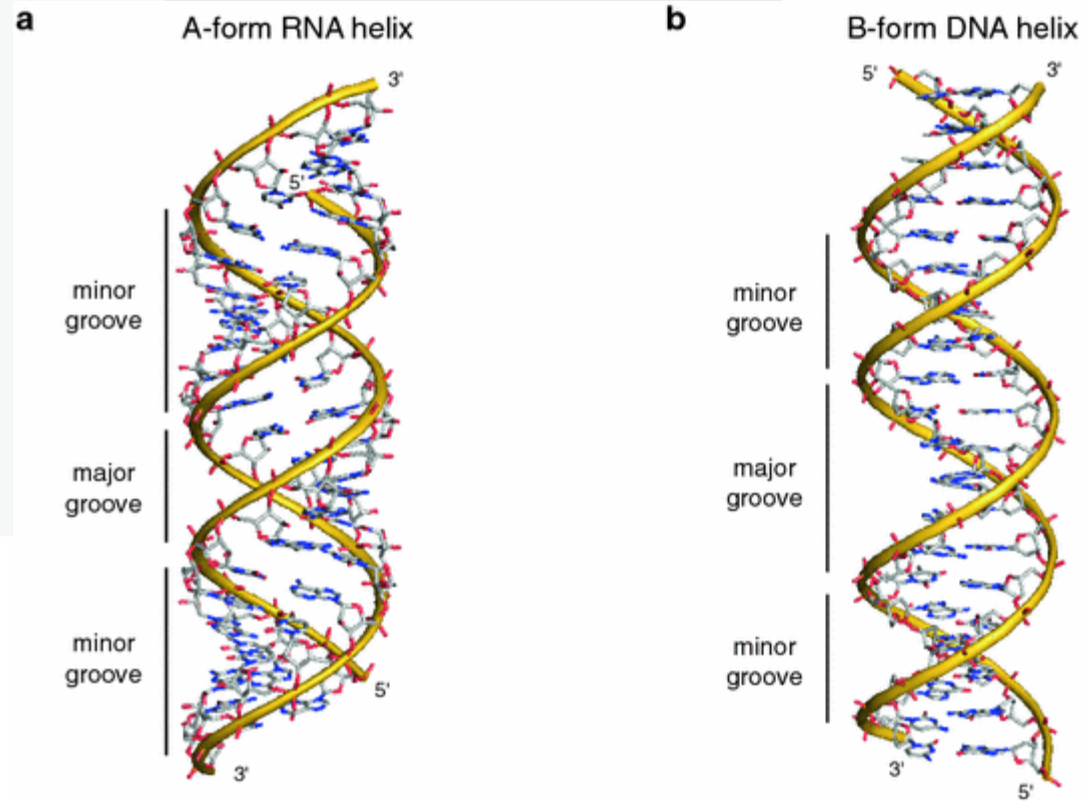
Innate immune pattern recognition receptors (PRRs) discriminate self from non-self



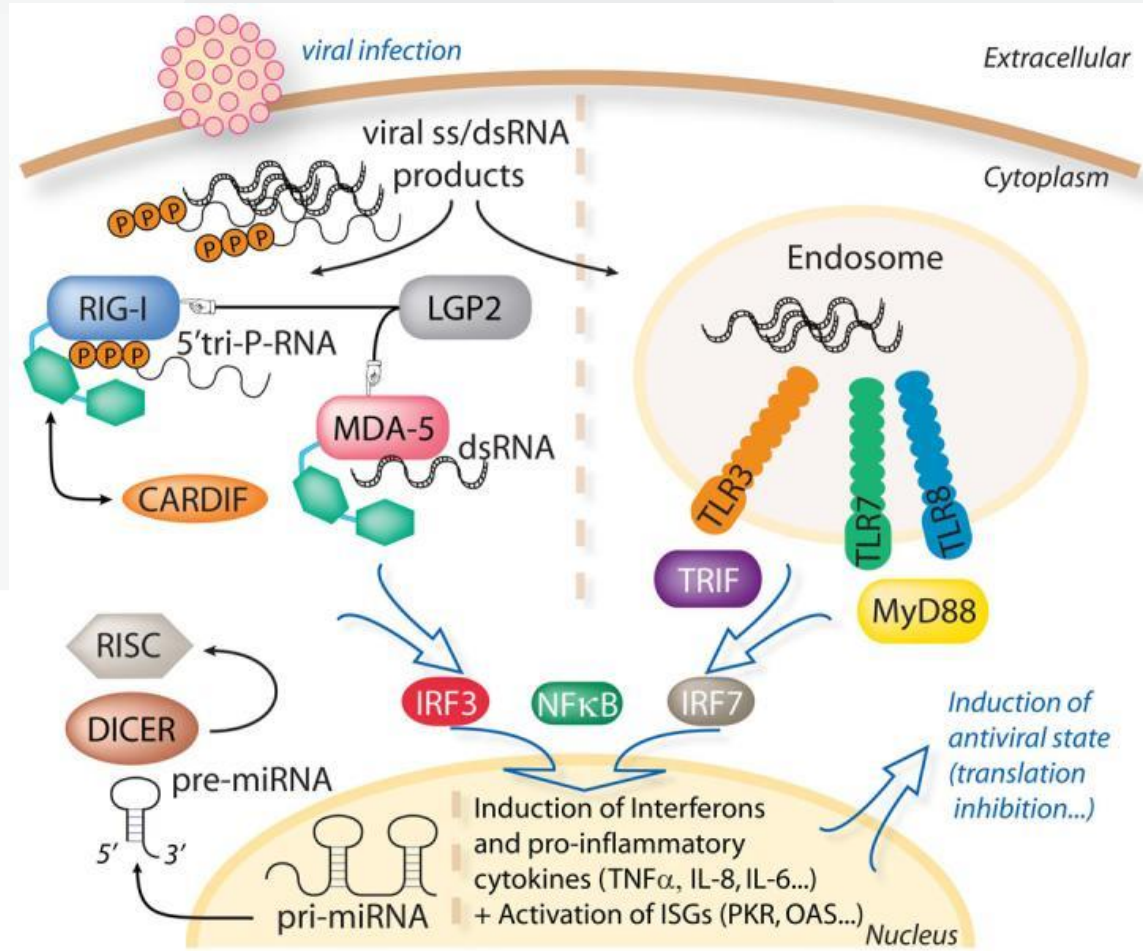
Charles Janeway

Polly Matzinger PRRs also react to damage or danger signals

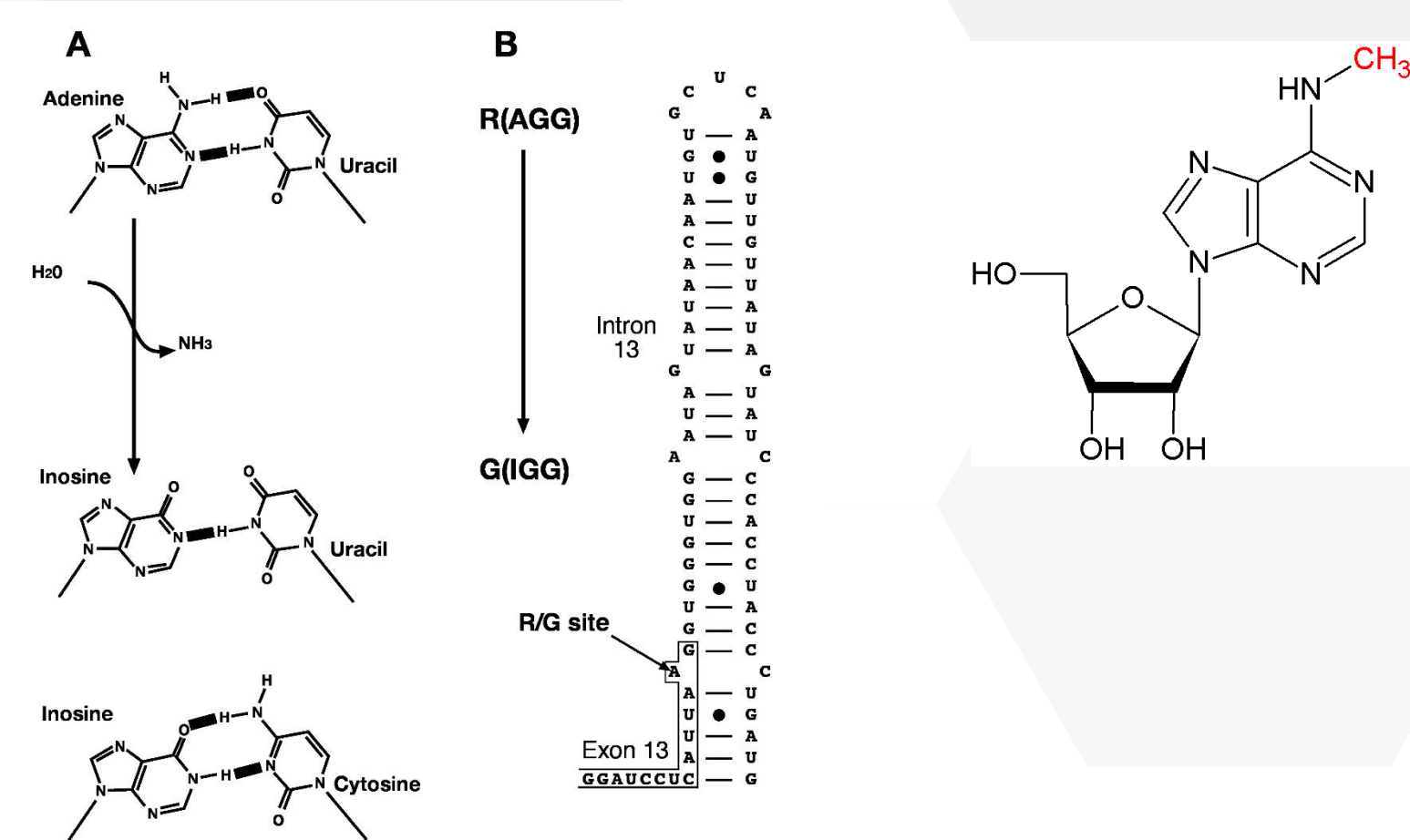
dsRNA versus DNA



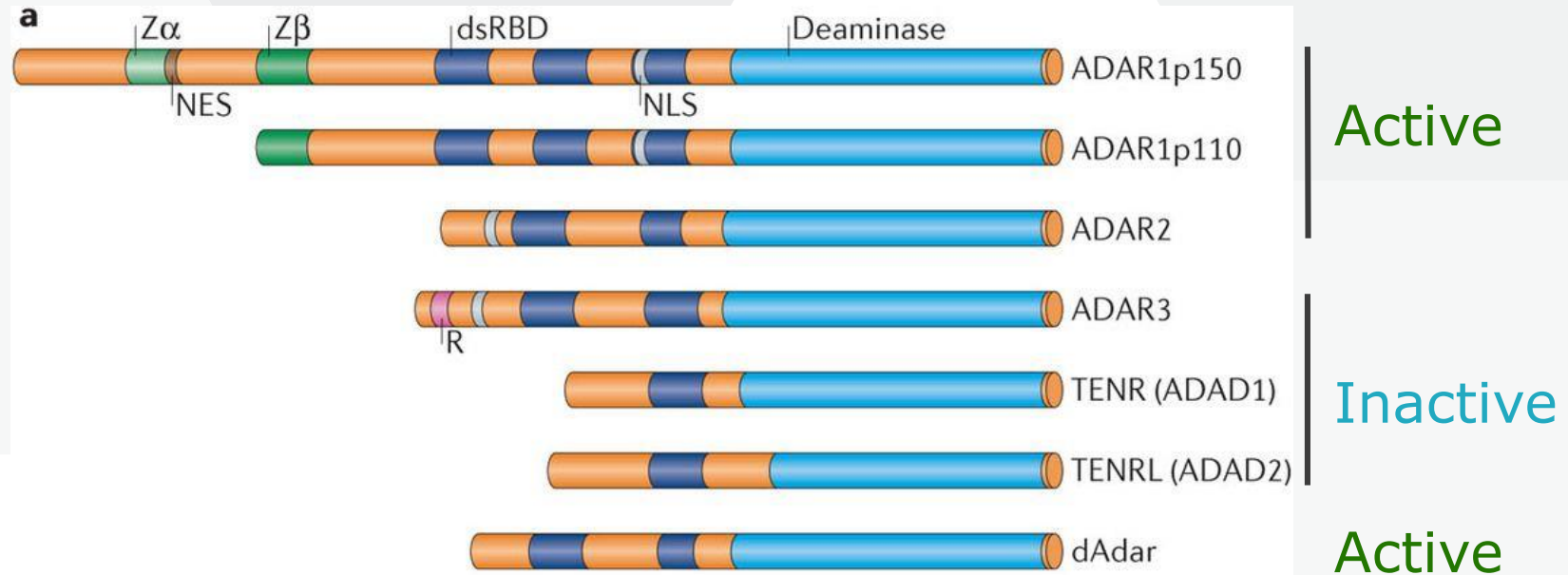
Cellular dsRNA is hazardous. Innate immune and RNAi trigger.



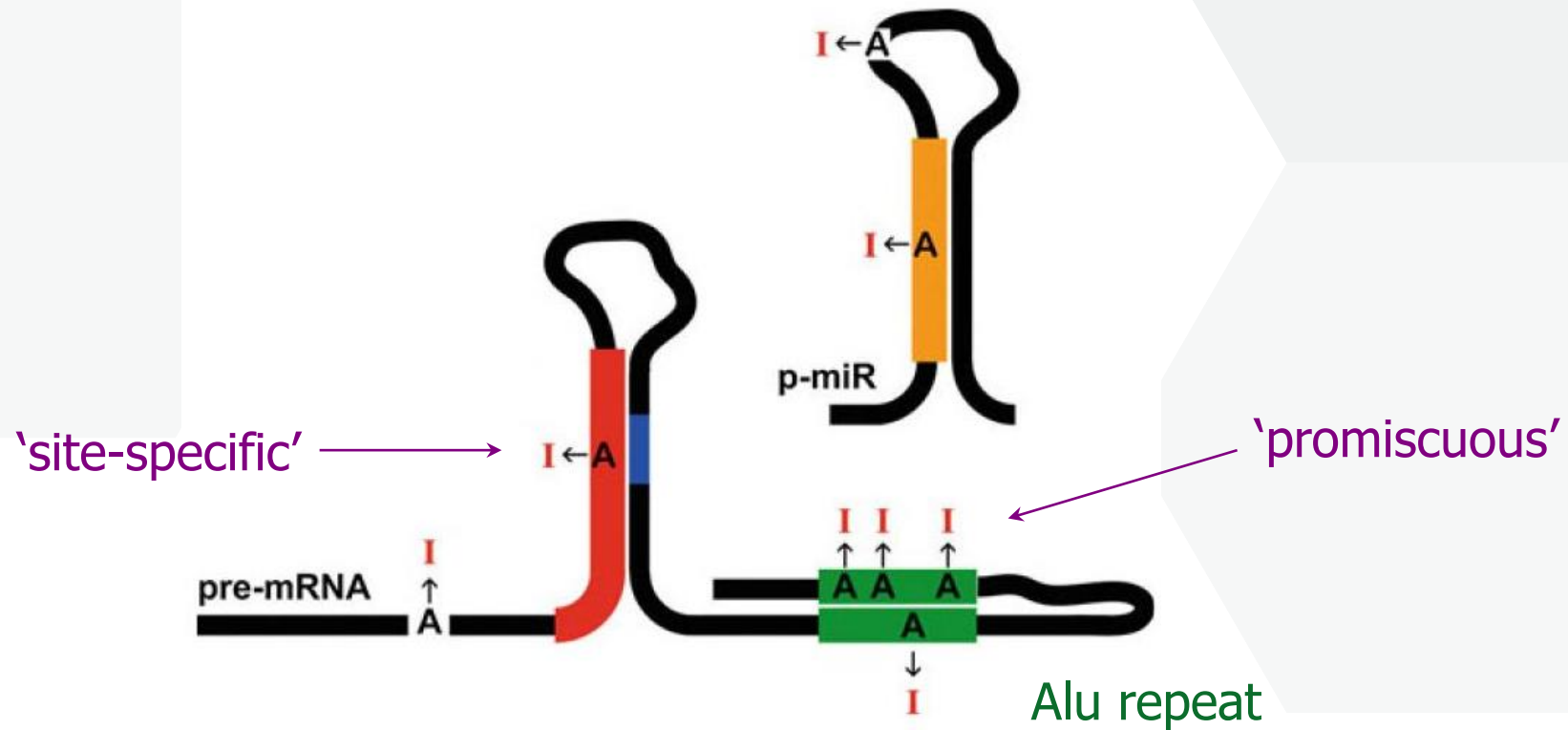
Conversion of adenosine to inosine change the encoded protein



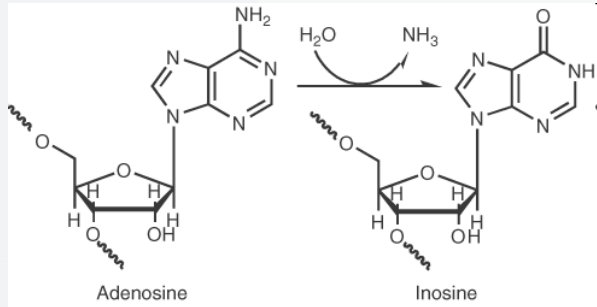
ADAR proteins in vertebrates and in *Drosophila*



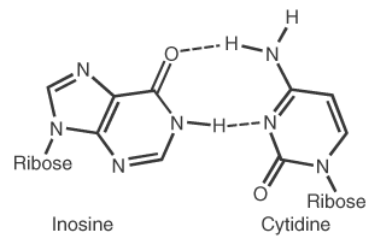
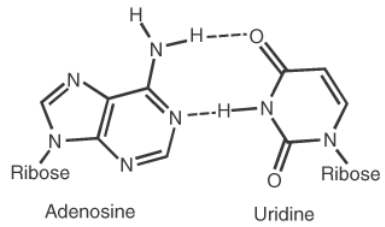
Adenosine Deaminases acting on RNA (ADARs) edit A-to-I in dsRNA



Prevalence of A-to-I editing

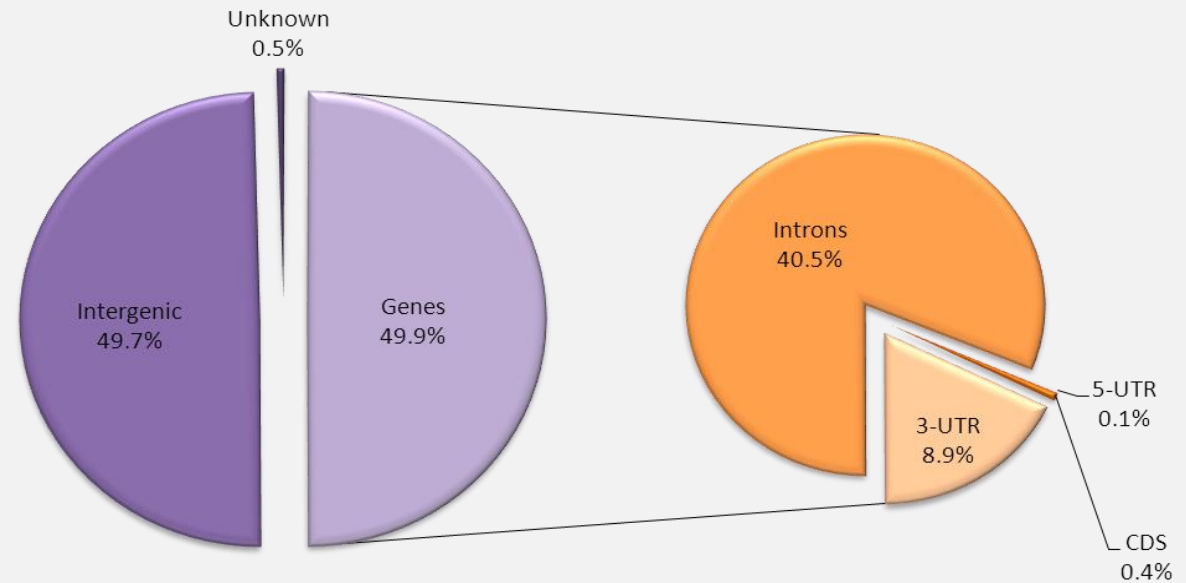


(a)



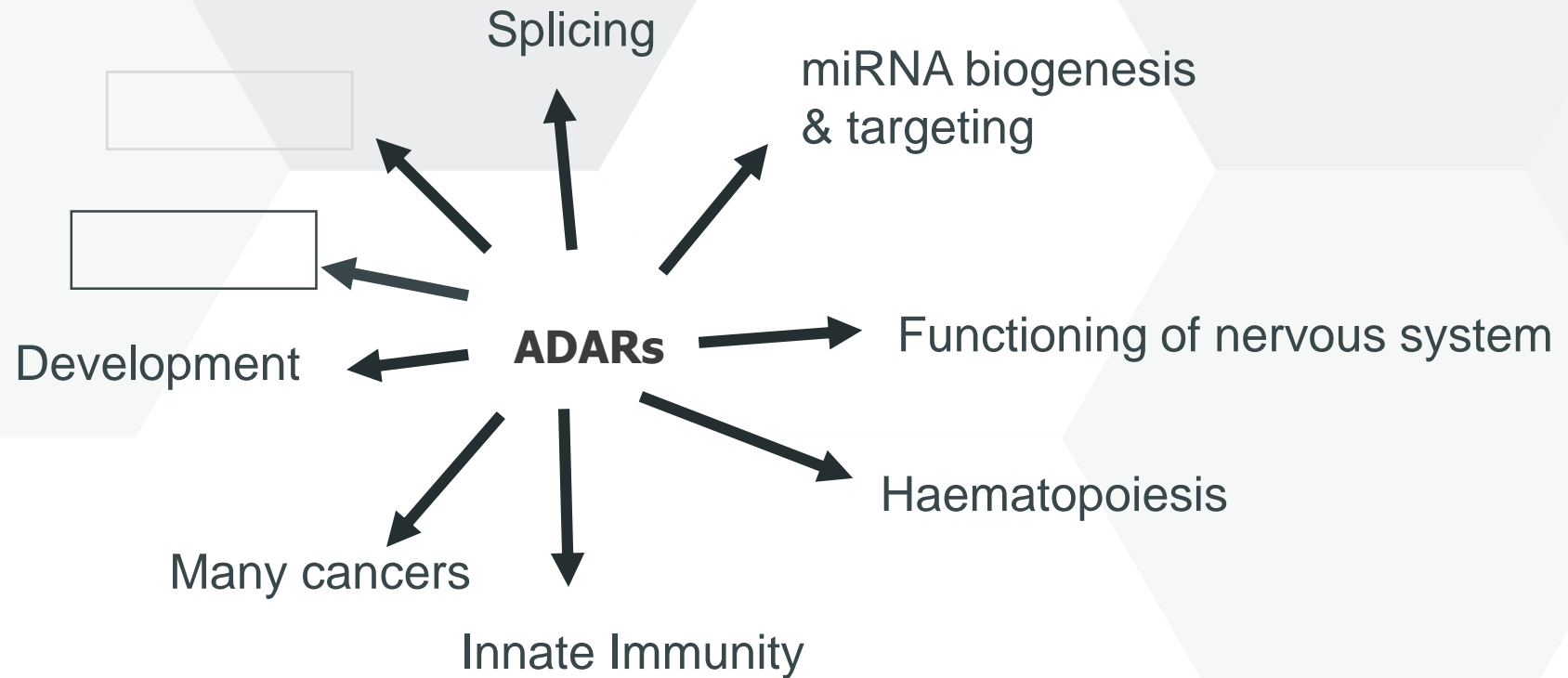
(b)

Human whole transcriptome A→G mismatches (~21,400)



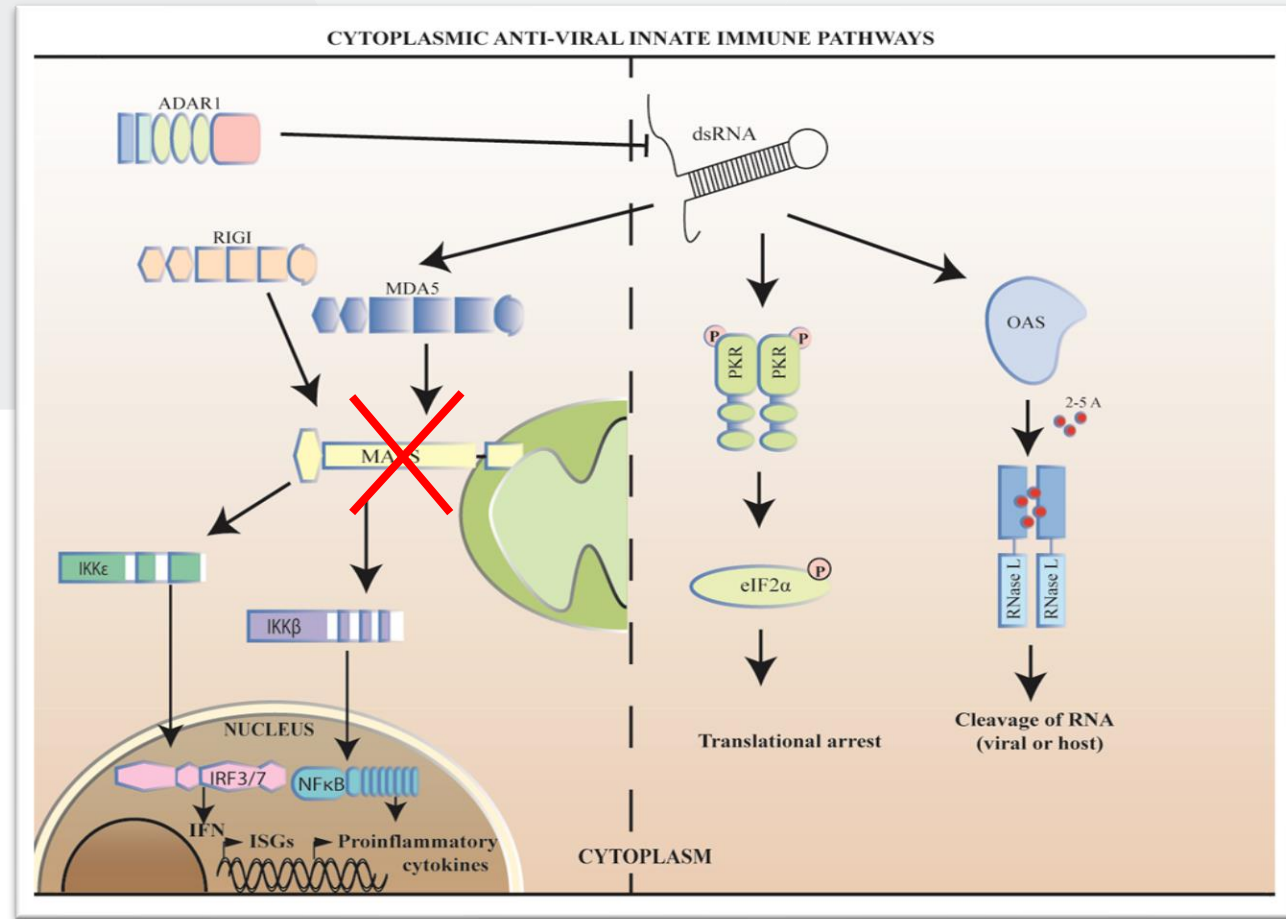
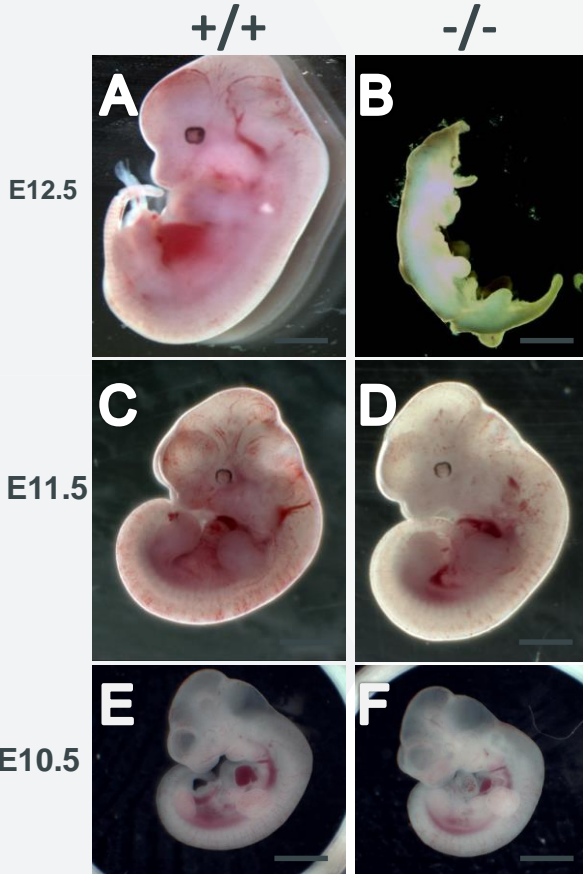
Compiled from Peng et al. (2012) Nat Biotechnol 30, 253-60

Biological roles of ADARs



Editing dependent & independent roles of ADAR

Rescue of *Adar* lethality by preventing innate immune sensing of intracellular dsRNA

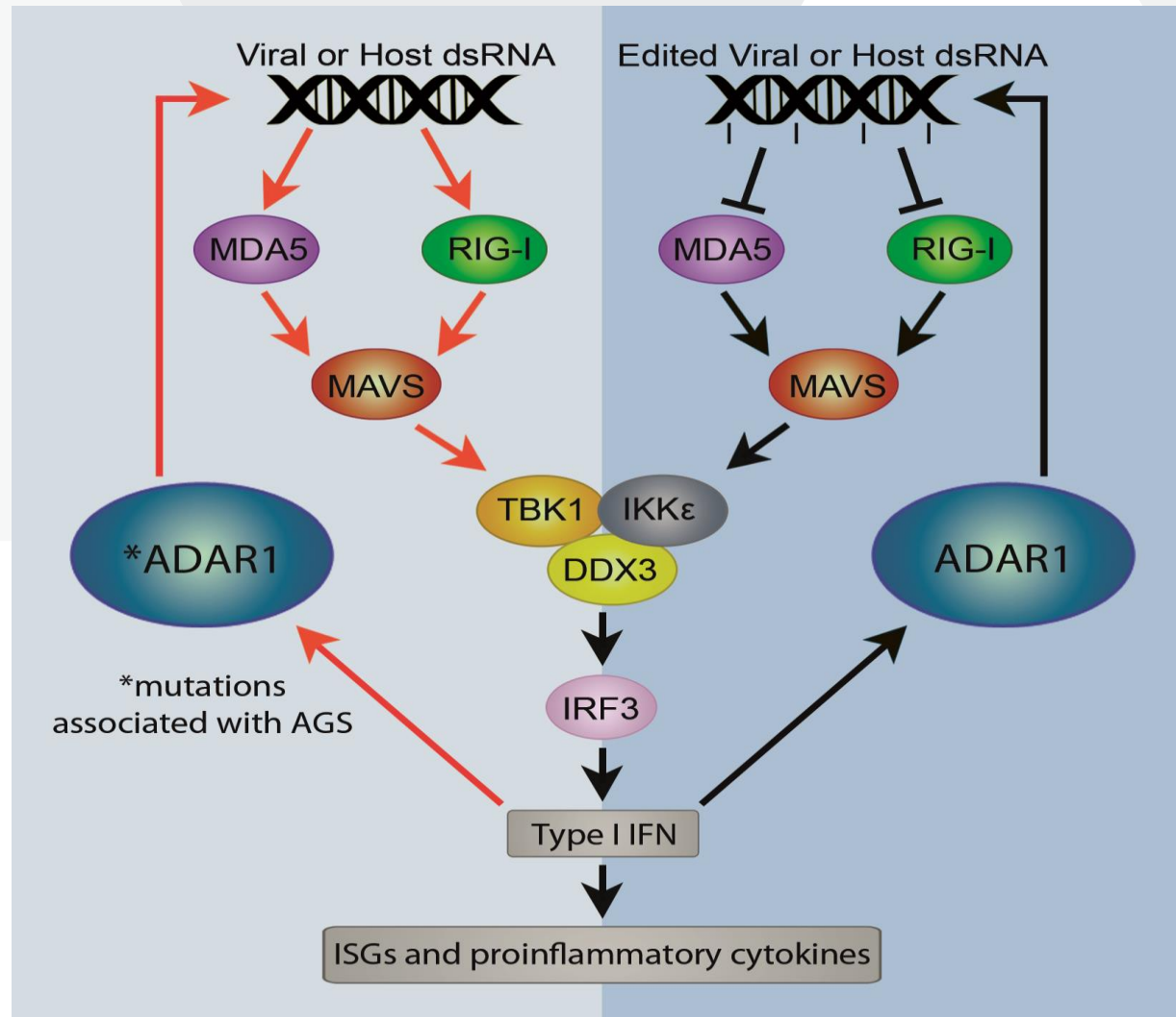


Mavs knockout prevents all known signalling in the RLR pathway.

Mannion, 2014; complete null, death E12.5. Double mutant with *Mavs* dies at birth.

Liddicot 2015; inactive mutant *Adar*^{E/A}, dies by E14.5. Double mutant with *Mda5* has *no phenotype*.

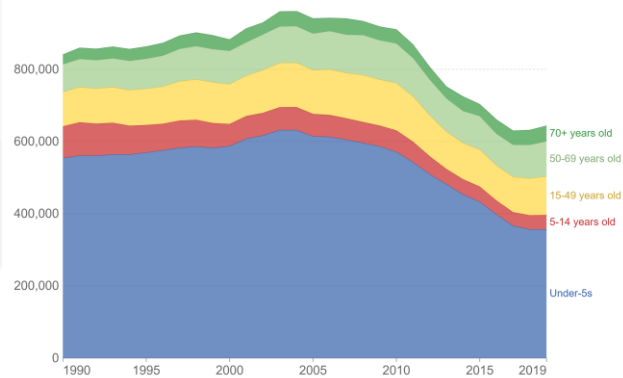
Inosine in RNA helps discriminate self from non-self



The impact of malaria

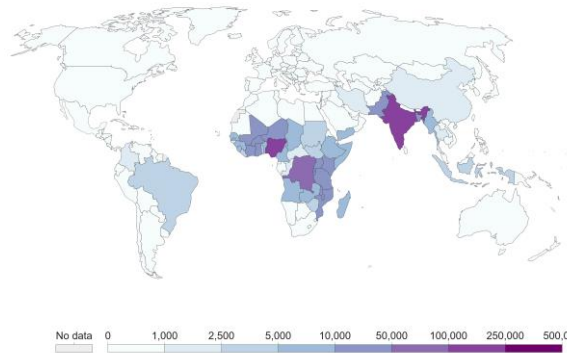
WHO World Malaria Report. “After an unprecedented period of success in global malaria control, progress has stalled. Data from 2015 ... highlight that no significant progress in reducing global malaria cases was made in this period.”

Deaths from malaria, by age, World, 1990 to 2019
Annual number of deaths from malaria, differentiated by age category.



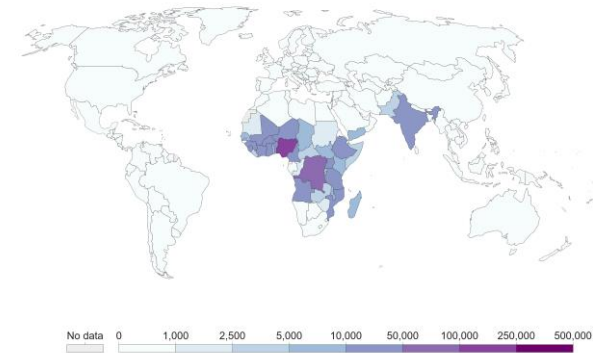
Source: IHME, Global Burden of Disease

Number of deaths from malaria, 1990



Source: Institute for Health Metrics and Evaluation (IHME)

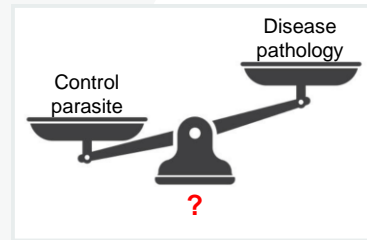
Number of deaths from malaria, 2019



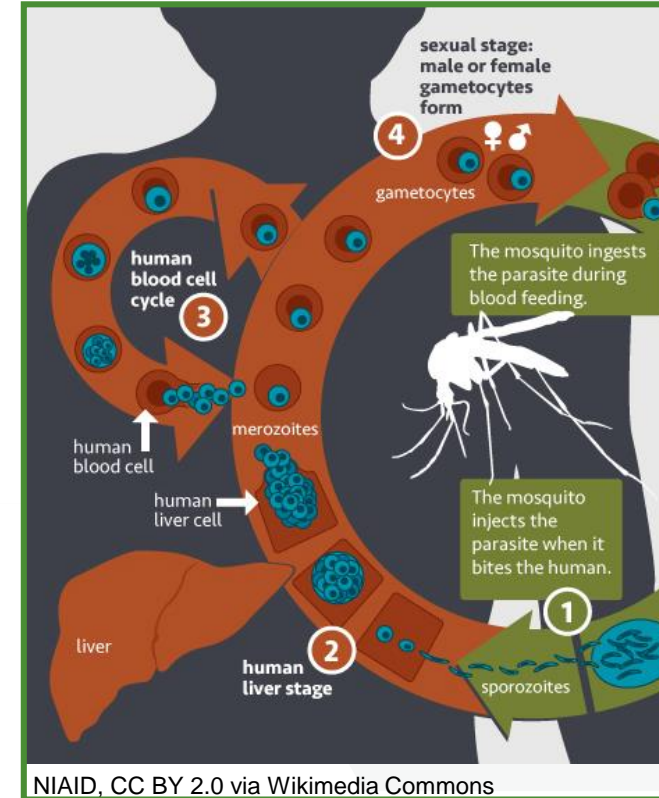
OurWorldInData.org/malaria • CC BY

Malaria

- Malaria is caused by infection with *Plasmodium* protozoan parasite.
- *P. falciparum* infections can range from asymptomatic to life threatening.



- Protection from malaria only after years of repeated infections.
- *P.falciparum* has strategies to 'hide' from the immune system.
- Immune responses to *P.falciparum* may contribute to the pathology of the disease.

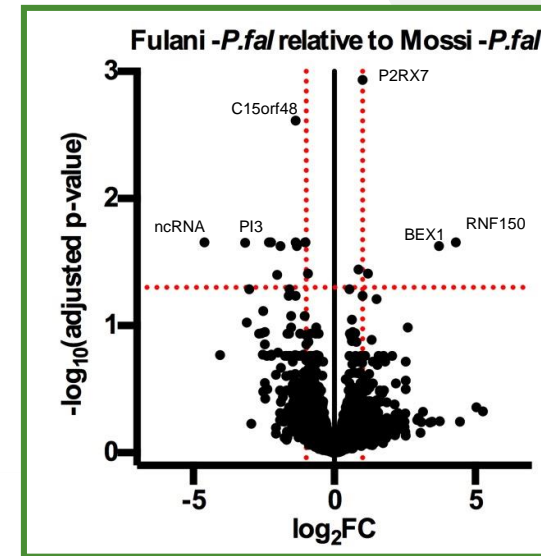
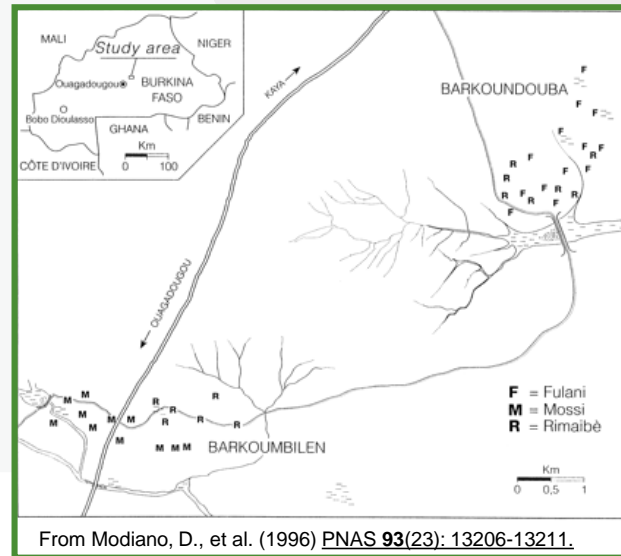


The Fulani ethnic group resistant to malaria.

- Ethnic group in the Sahel in Africa.
- Approximately 30 million people.
- Traditionally nomadic pastoralists.
- Predominantly Islamic.
- Distinct genetic heritage
- **Protected from malaria**
 - Fewer individuals with *P.falciparum*.
 - Lower levels of parasite in blood.
 - Fewer cases of symptomatic malaria.



RNA-sequencing study in Fulani



- We performed genome-wide DNA-methylation and RNA-sequencing analysis of CD14+ monocytes in uninfected vs infected Fulani, and a sympatric ethnic group (Mossi)

Pilot RNA-sequencing study in Fulani



SHORT REPORT

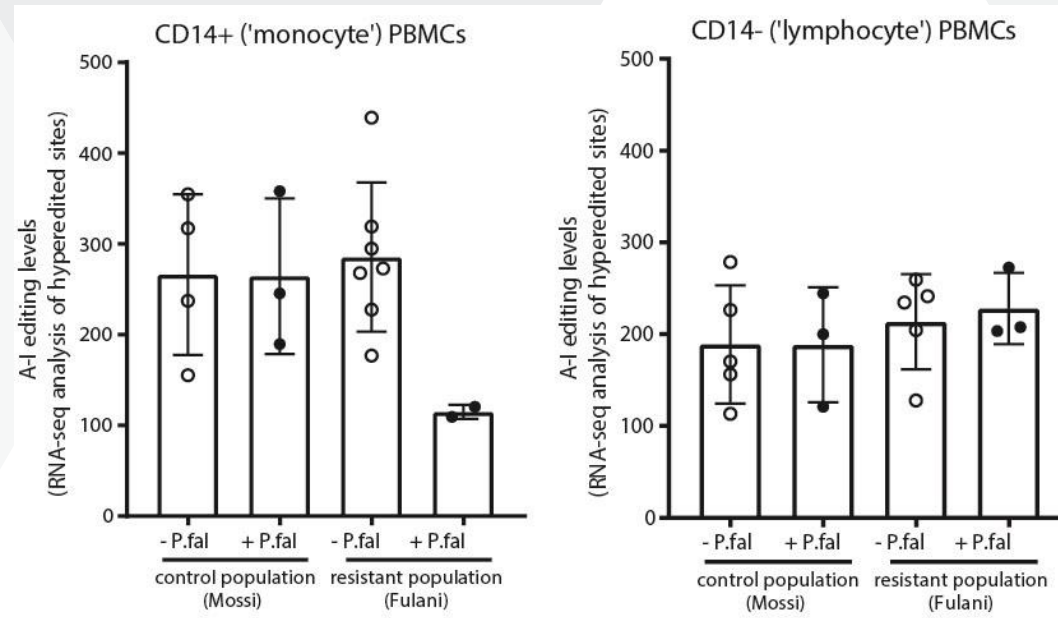


Major transcriptional changes observed in the Fulani, an ethnic group less susceptible to malaria

Jaclyn E Quin^{1‡}, Ioana Bujila^{1‡}, Mariama Chérif^{2,3‡}, Guillaume S Sanou^{2‡}, Ying Qu⁴, Manijeh Vafa Homann^{5†}, Anna Rolicka^{1§}, Sodiomon B Sirima², Mary A O'Connell⁶, Andreas Lennartsson⁴, Marita Troye-Blomberg¹, Issa Nebie², Ann-Kristin Östlund Farrants^{1*}

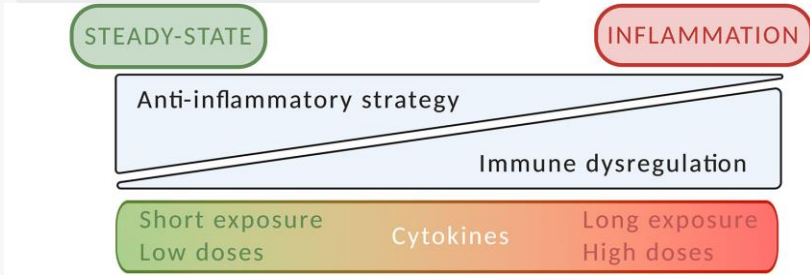
¹Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden; ²Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso; ³Université Polytechnique de Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso; ⁴Department of Biosciences and Nutrition, Karolinska Institute, Stockholm, Sweden; ⁵Unit of Infectious Diseases, Department of Medicine, Karolinska Institute, Stockholm, Sweden; ⁶Central European Institute of Technology, Brno, Czech Republic

Reduced A-to-I editing is associated with protection from malaria



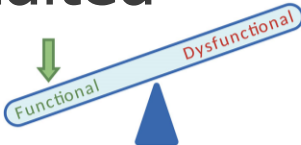
- Fulani – an ethnic group resistant to malaria. Have reduced A-I editing specifically in APCs, following infection with *P. falciparum* malaria

ADAR1 is essential for homeostasis and thresholding

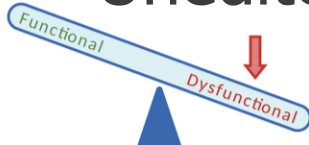


dsRNA

Edited

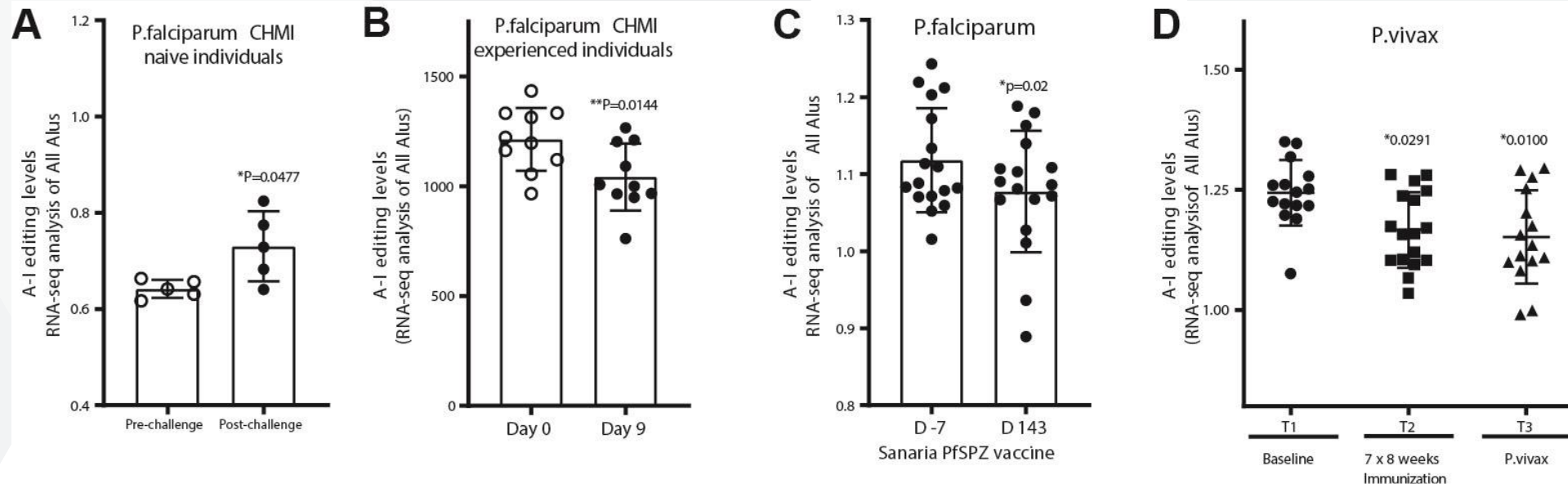


Unedited



ADAR1

Reduced A-to-I editing is associated with protection from malaria

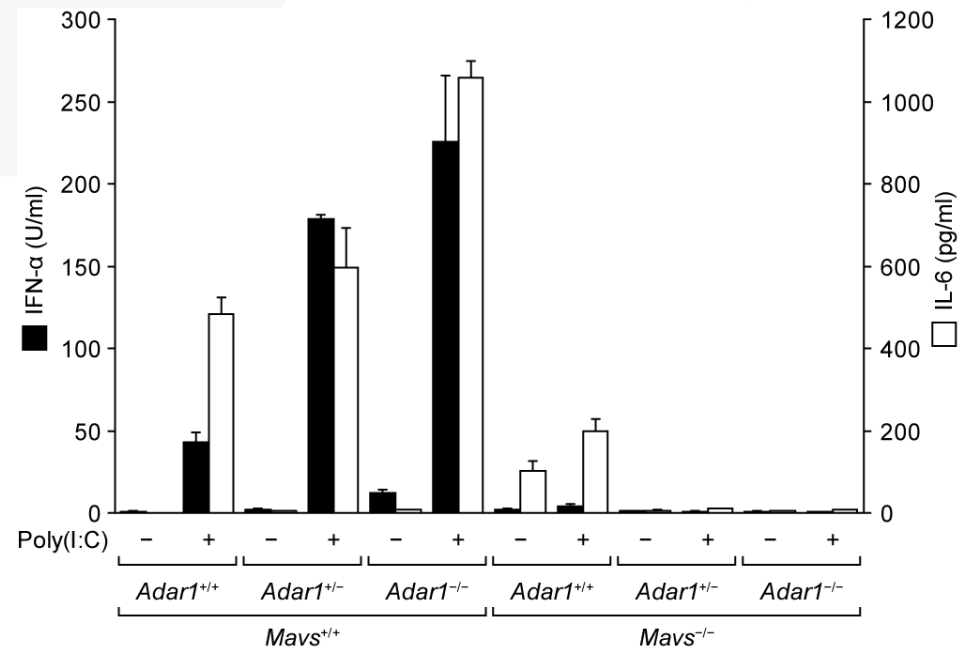


Malaria naïve individuals display a strong but opposite reaction to malaria experienced individuals.

- Naïve: Increased editing levels.
- Protected (experienced or vaccinated): Decreased editing levels.

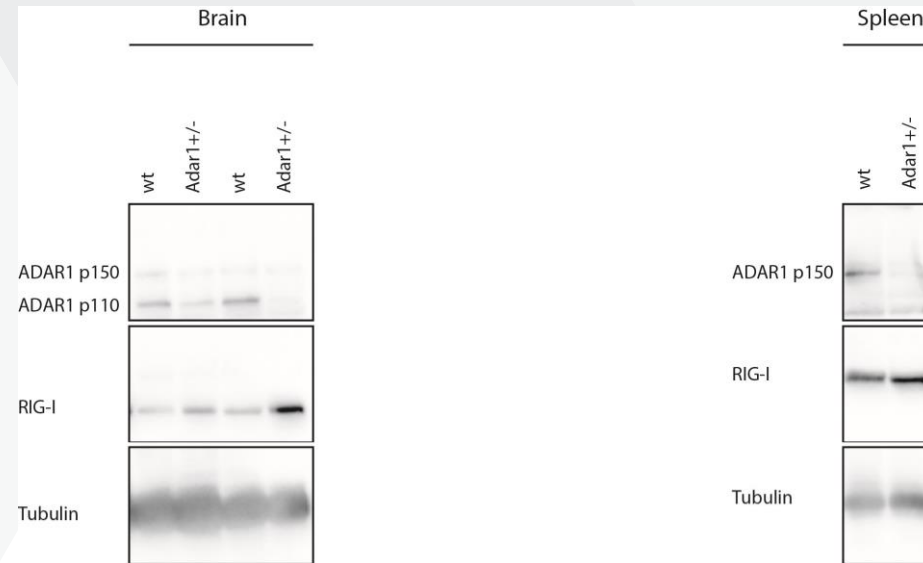
Mouse infection Experimental Plan

- A) Tail vein injection of *P.yoelii* from passage mouse, monitor parasitemia daily until self resolution in wildtype versus *Adar1*^{-/+}
- B) Then for day of peak parasitemia, collect blood and plasma for analysis



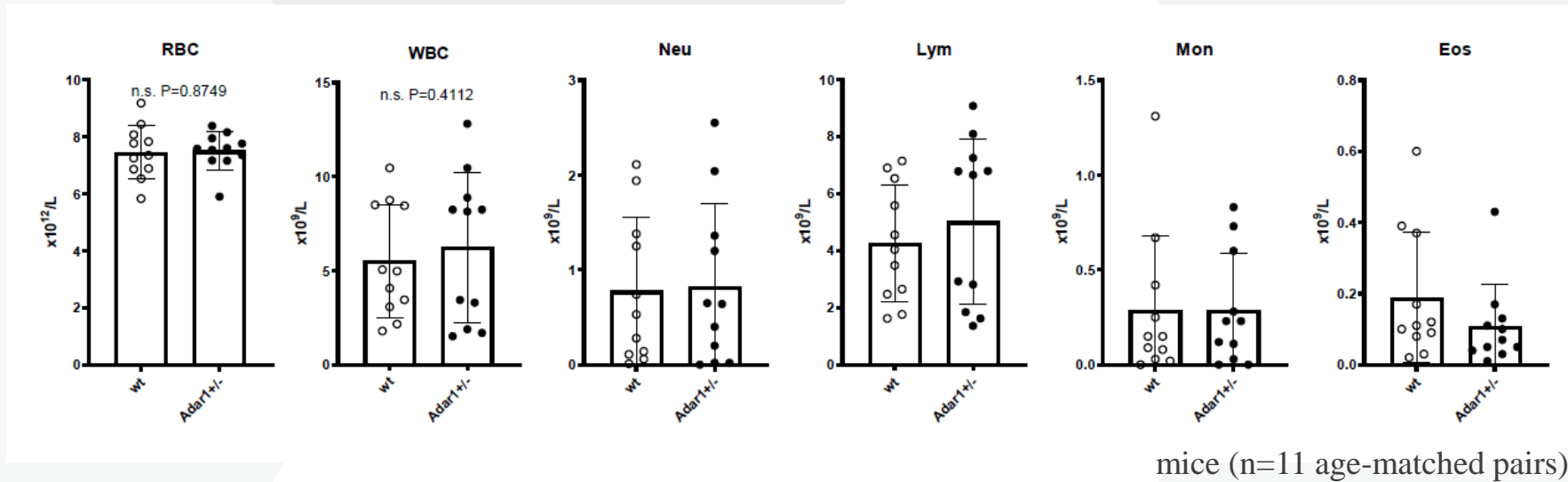
Mannion *et al.* Cell Reports 2014

Levels of Adar1 protein in *Adar1*^{+/-} mutant mice



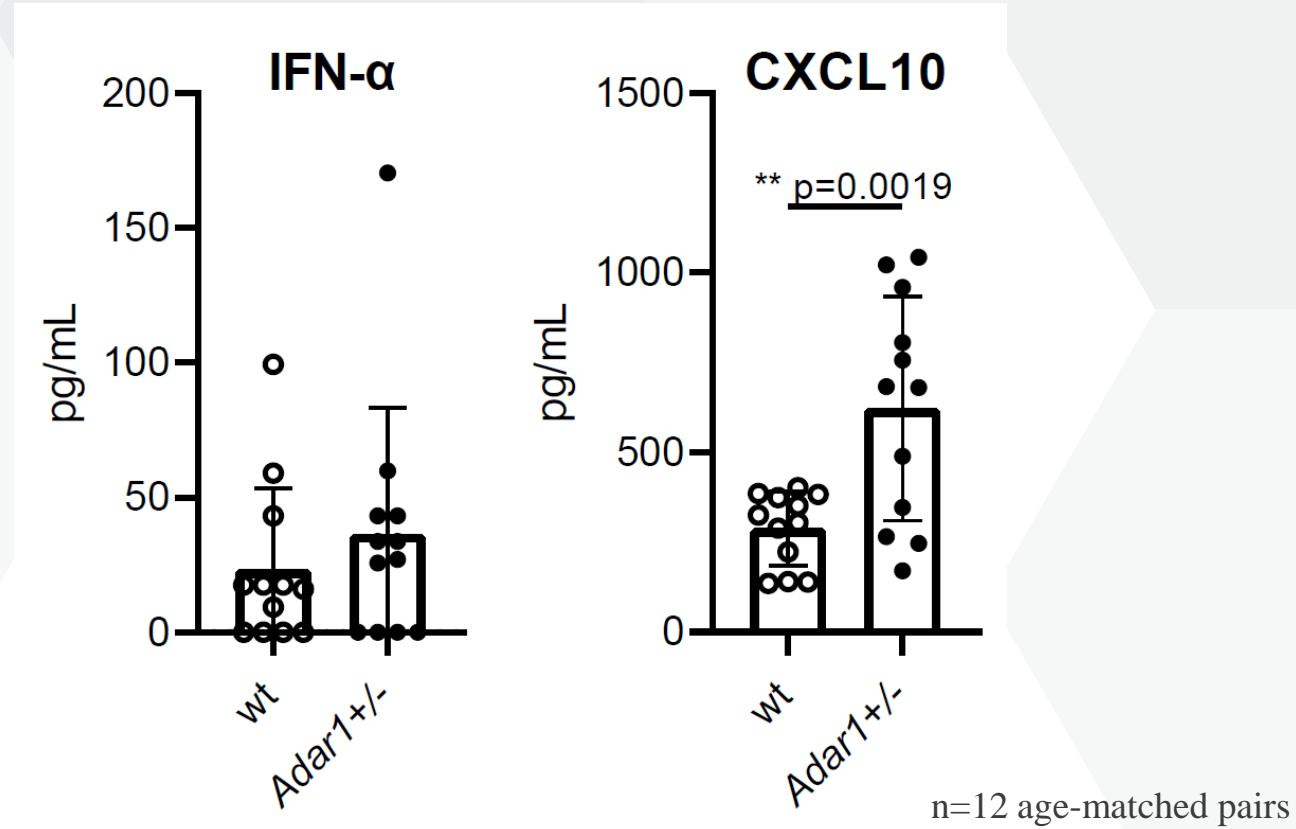
- Uninfected *Adar1*^{+/-} mice may have lower levels of ADAR1 protein

Hematology analysis of wild-type and *Adar*^{+/-}

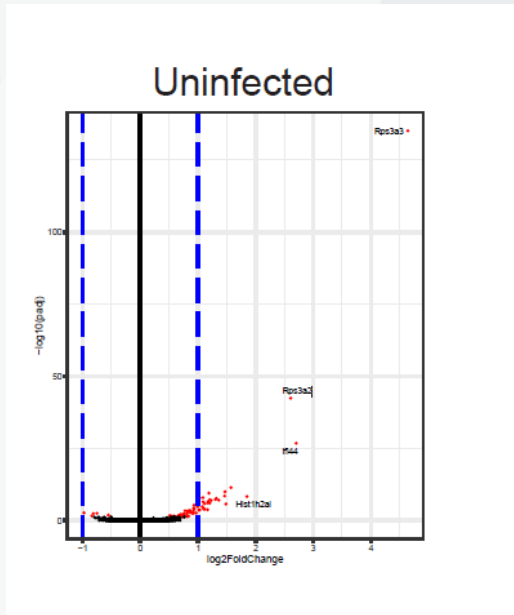


Peripheral blood cell numbers of erythrocytes (RBC), total white blood cells (WBC), neutrophils (Neu), lymphocytes, (Lymph), monocytes (Mon) and eosinophils (Eos)

Cytokine levels of wild-type and *Adar*^{+/-} adult mice



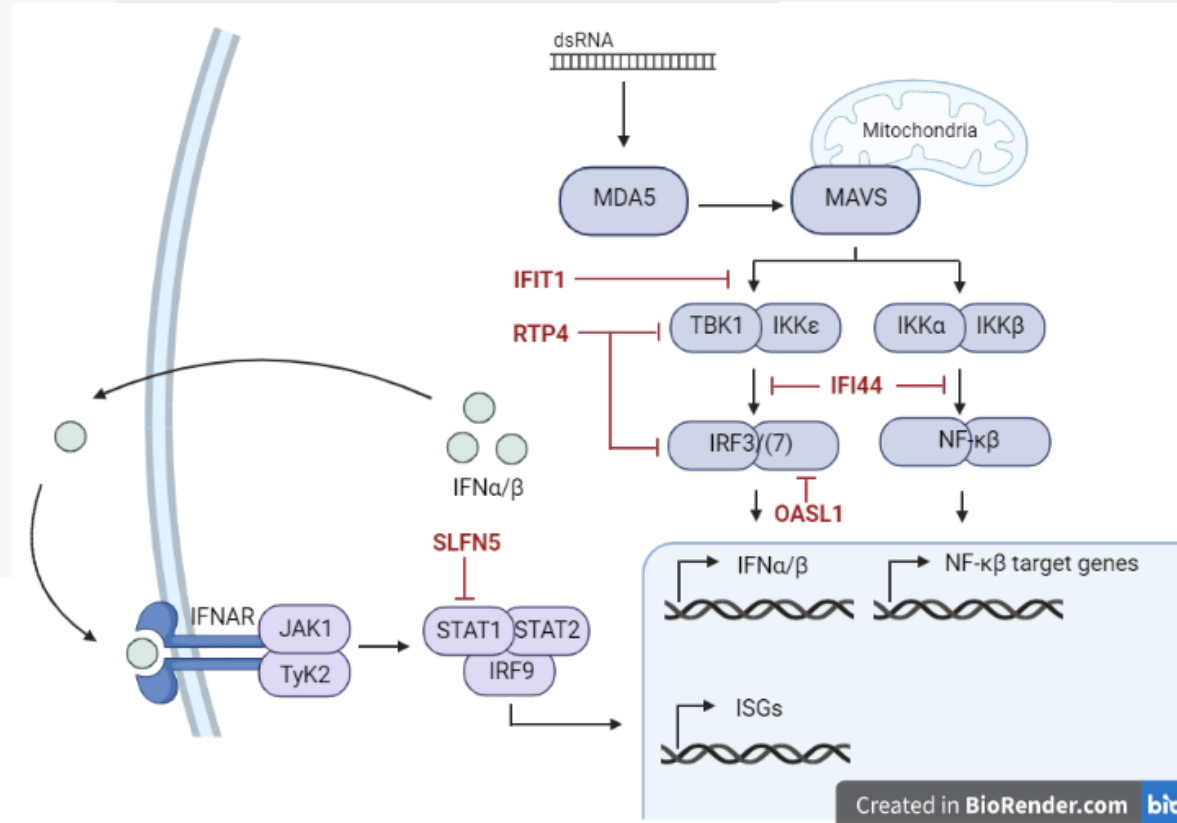
RNA Seq analysis of uninfected *Adar*^{+/-} versus wildtype



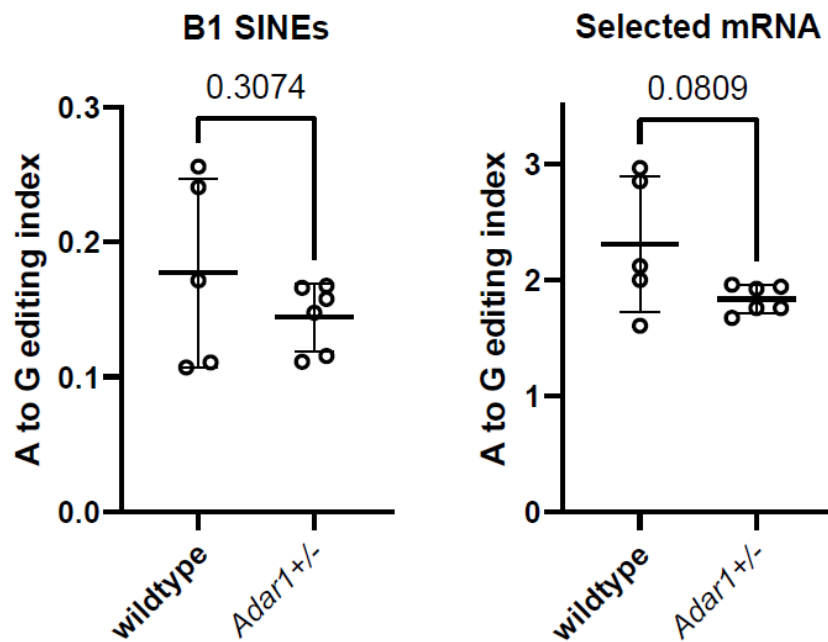
<i>Adar</i> ^{+/-} _ wildtype		
DE genes (Log2FC>1 and Adj.pval<0.05)		
Gene	Log2 Fold Change	Adjusted p-value
Rps3a3	4.6413	6.5945 e-136
Rps3a2	2.6078	4.0160 e-43
Ifi44	2.7033	1.8075 e-27
Oas1g	1.5725	3.9719 e-12
Iigp1	1.4696	1.2109 e-10
Ly6a	1.1902	3.6617 e-10
Ifit3	1.4621	3.5491 6e-09
Hist1h2a1	1.8484	5.2539 e-09
Ifi213	1.0865	1.3113 e-08
Ifit1b1	1.0324	1.7035 e-08
Oas12	1.3196	2.3903 e-08
Oas11	1.3027	5.5773 e-08
Sdc3	1.2023	7.4443 e-08
Ifit3b	1.3609	9.3261 e-08
Rtp4	1.2308	1.2286 e-07
Gm6545	1.1782	2.9906 e-07
Sifn5	1.0888	3.9181 e-07
Gbp6	1.2045	9.2690 e-07
Ifi2712a	1.1706	9.2690 e-07
Oas1a	1.1156	1.5637 e-06
Gm42743	1.4863	2.1166 e-06
Oas2	1.0761	1.9805 e-05
Ifit1	1.1021	0.0001
Ly6i	1.1159	0.0001
Hbb-y	1.1672	0.0001
Mx1	1.0317	0.0002
Fcgr1	1.0139	0.0002

Gene Ontology Enrichment Analysis		
Functional Annotation		
UP_KW_BIOLOGICAL_PROCESS		
Name	p-value	Adjusted p-value
Antiviral defense	5.9E-12	4.7E-11
Innate immunity	2.1E-11	8.5E-11
Immunity	2.0E-10	5.2E-10
Gene Ontology		
GOTERM_BP_DIRECT		
Name	p-value	Adjusted p-value
response to virus	3.3E-16	2.7E-14
defense response to virus	1.4E-14	5.5E-13
cellular response to interferon-beta	3.2E-11	8.7E-10
regulation of ribonuclease activity	4.3E-10	8.9E-9
cellular response to interferon-alpha	5.2E-9	8.6E-8
response to bacterium	1.2E-8	1.6E-7
innate immune response	1.6E-8	1.9E-7
negative regulation of viral genome replication	2.0E-7	2.0E-6
immune system process	6.8E-7	6.2E-6

Some of the ISG genes that are upregulated are negative regulators of IFN response



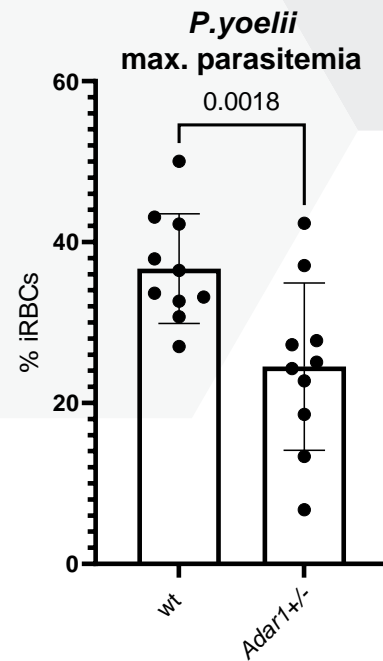
A-to-I RNA editing analysis of wild-type and *Adar*^{+/-} adult mice from RNA sequencing



n=6 age-matched pairs of mice

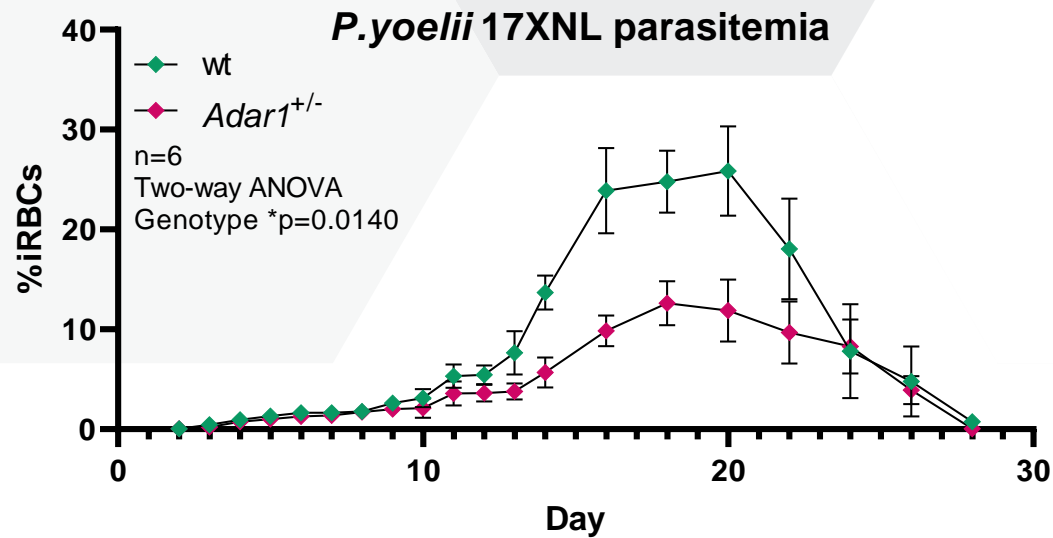
Infection

Adar^{+/-} mutant mice are protected from *P.yoelii*



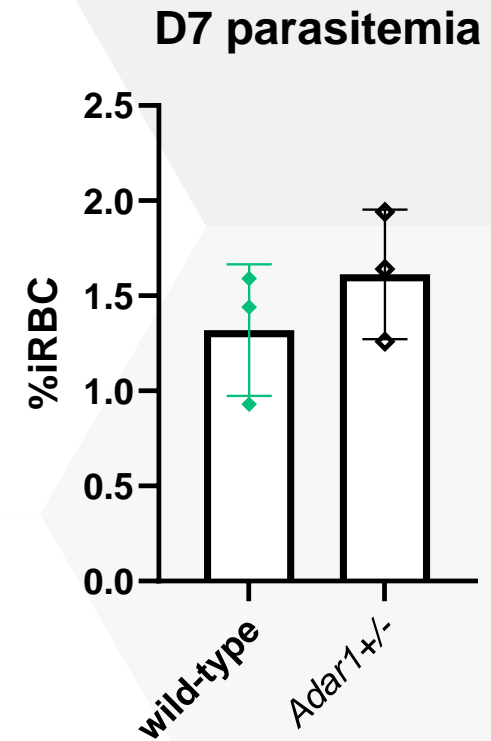
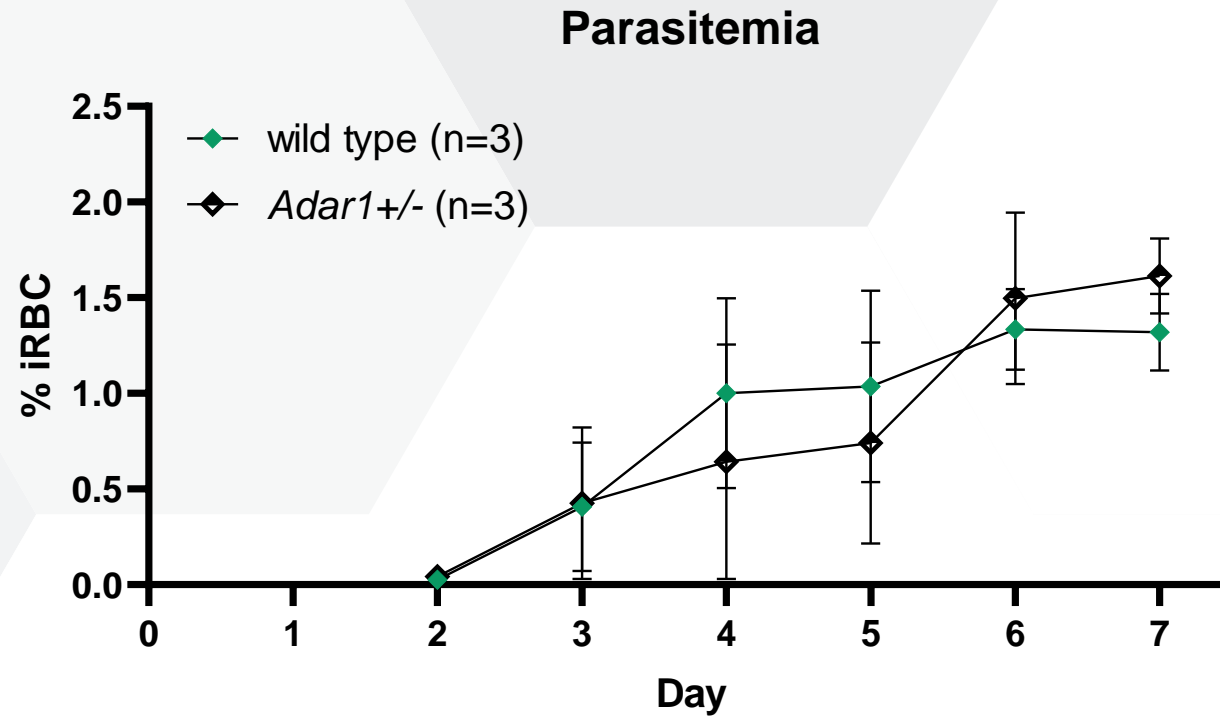
- *Adar1*^{+/-} heterozygous mutant mice.
- *P.yoelii* 17XNL self-resolving rodent malaria (10⁵ iRBCs by I.V.)

Adar^{+/-} mutant mice are protected from *P.yoelii*

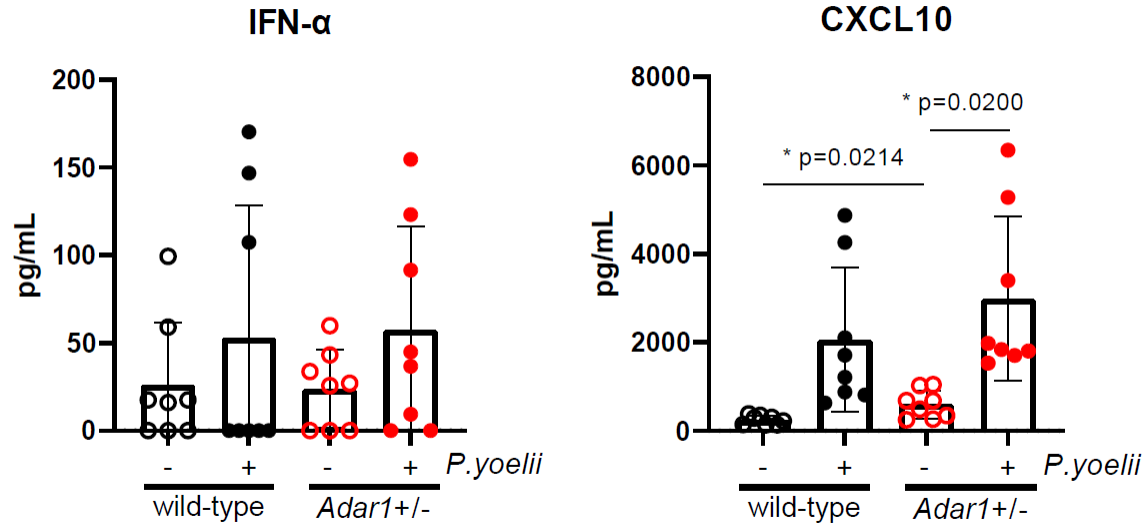


- *Adar*^{+/-} mice are protected from malaria, with reduced levels of *P.yoelii* parasitemia across the course of infection.

Parasitemia on Day 7

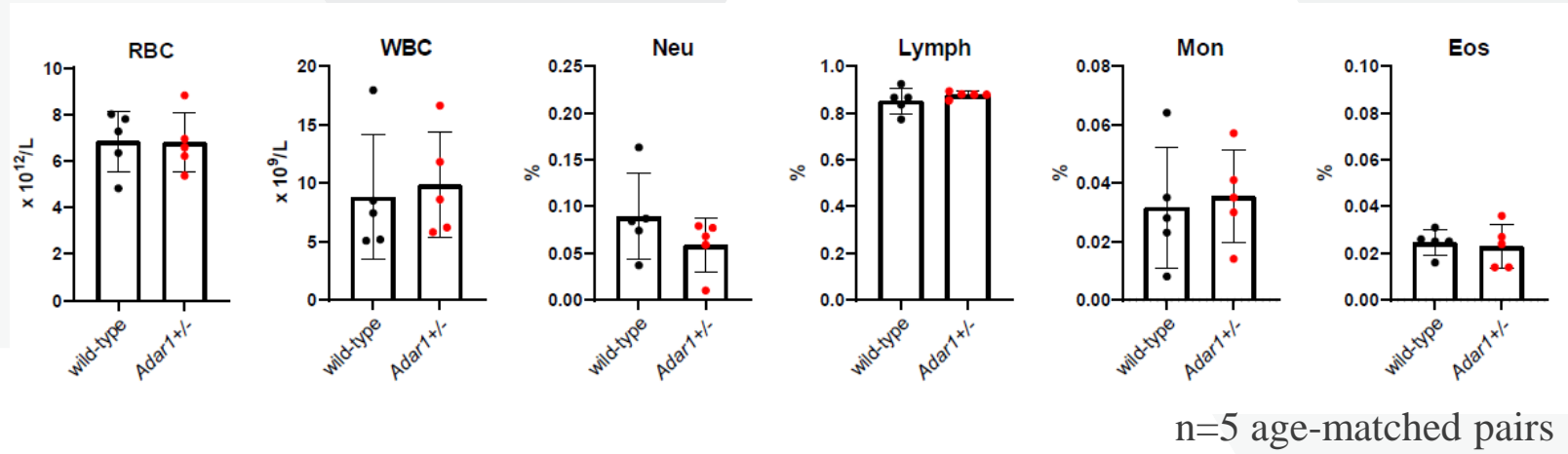


Cytokine levels of wild-type and *Adar*^{+/-} adult mice following *P.yoelii* infection on day 7



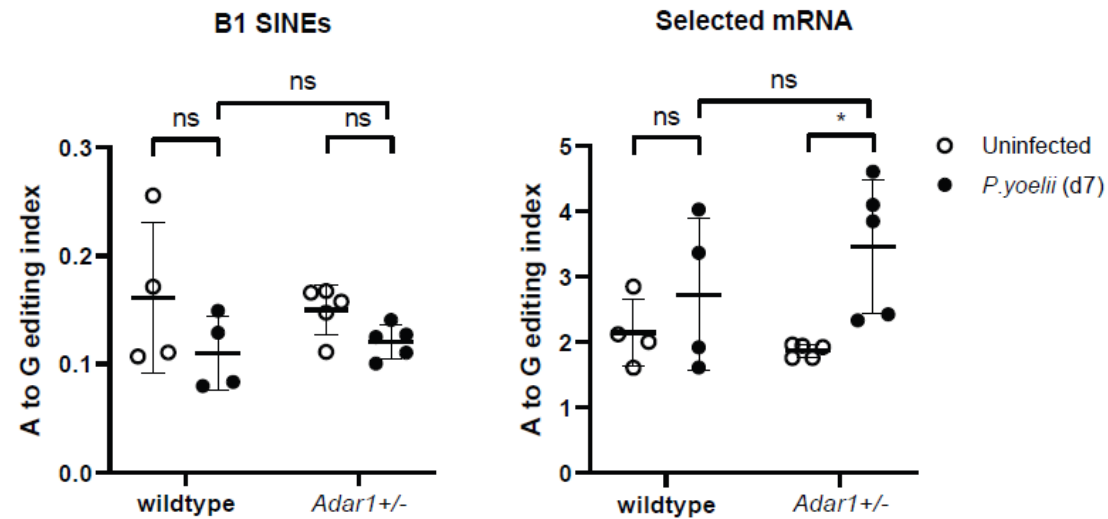
n=8 age-matched pairs

Hematology analysis of wild-type and *Adar1*^{+/-} adult mice following *P.yoelii* infection on day 7

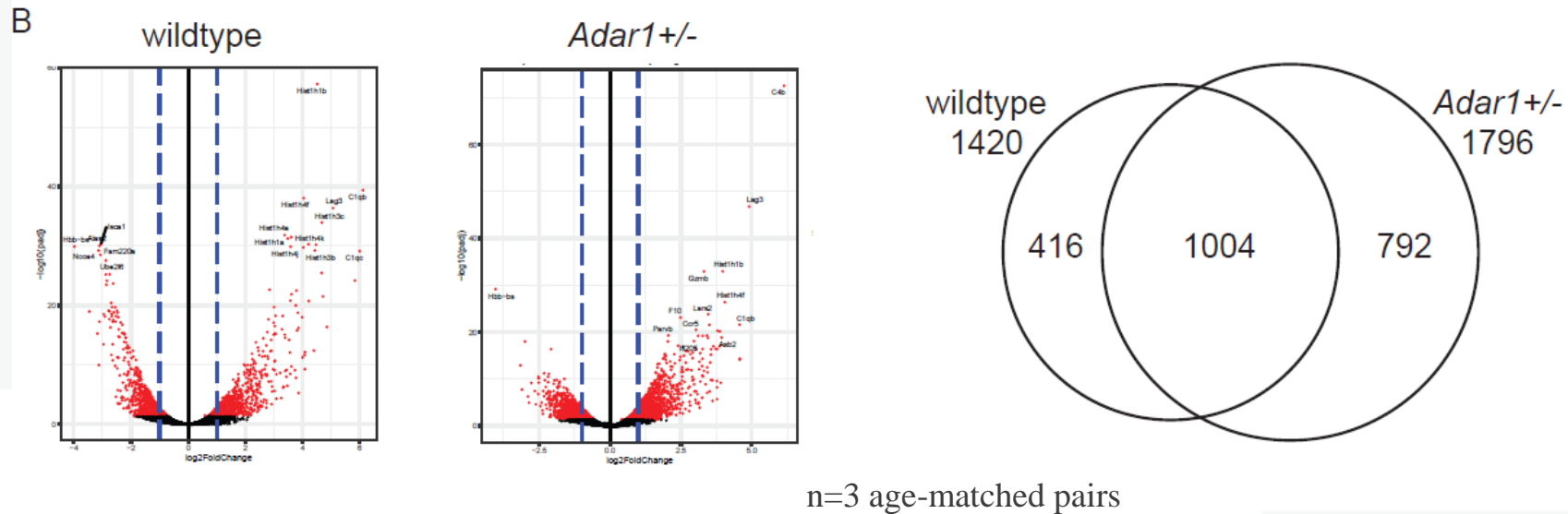


A-to-I RNA editing analysis of wild-type and *Adar*^{+/-} adult mice from RNA sequencing

D



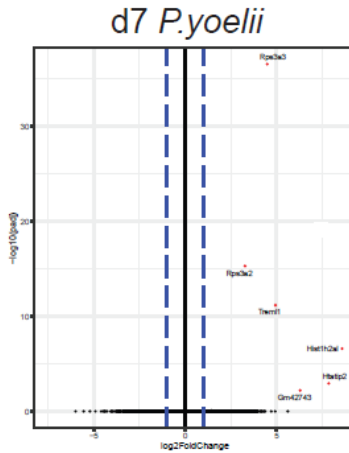
RNA sequencing analysis of wild-type and *Adar1*^{+/-} mice



Volcano plot of differentially expressed genes comparing d7 *P.yoelii* wildtype with uninfected wildtype mice, and d7 *P.yoelii* *Adar1*^{+/-} mice with uninfected *Adar1*^{+/-} mice.

Differentially expressed genes on day 7 of infection between *Adar*^{+/-} and wildtype mice.

C



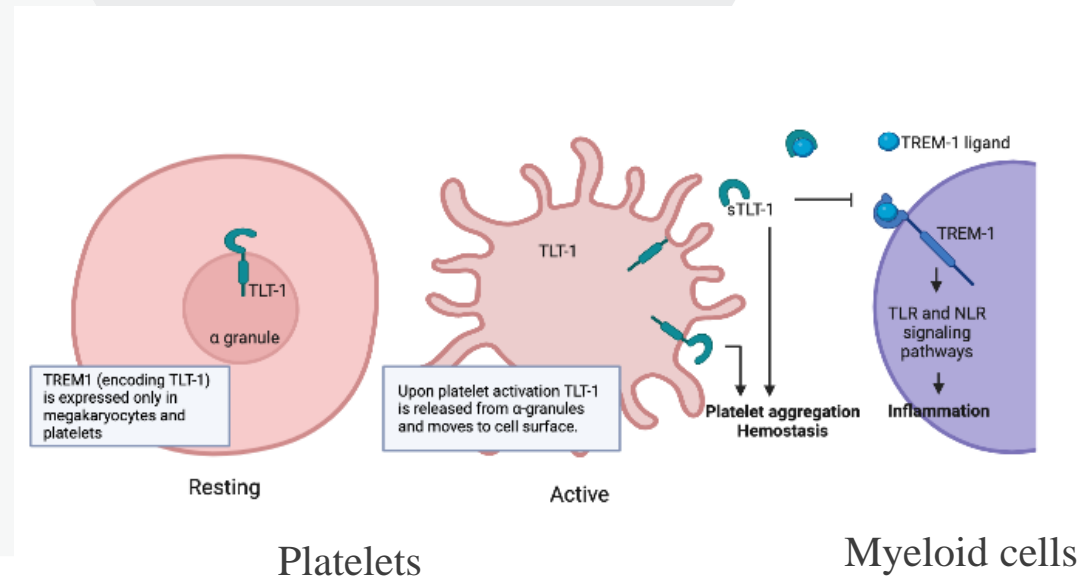
<i>Adar</i> ^{+/-} <i>P.yoelii</i> d7 _ wildtype <i>P.yoelii</i> d7		
DE genes (Log2FC>1 and Adj.pval<0.05)		
Gene	Log2 Fold Change	Adjusted p-value
Rps3a3	4.4902	2.93 e-37
Rps3a2	3.2762	5.15 e-16
Trem1	4.9406	6.77 e-12
Hist1h2al	8.5847	2.53 e-07
Htatip2	7.8542	0.0011
Gm42743	6.2906	0.0060

Volcano plot of differentially expressed genes comparing d7 *P.yoelii* *Adar*^{+/-} mice with d7 *P.yoelii* wildtype mice.

Triggering Receptor Expressed On Myeloid Cells 1 (TREM-1) concentration is significantly increased throughout the infection periods and TREM-1 is positively correlated with malaria parasitemia development. This suggests a positive involvement of TREM-1 in severe malaria development.

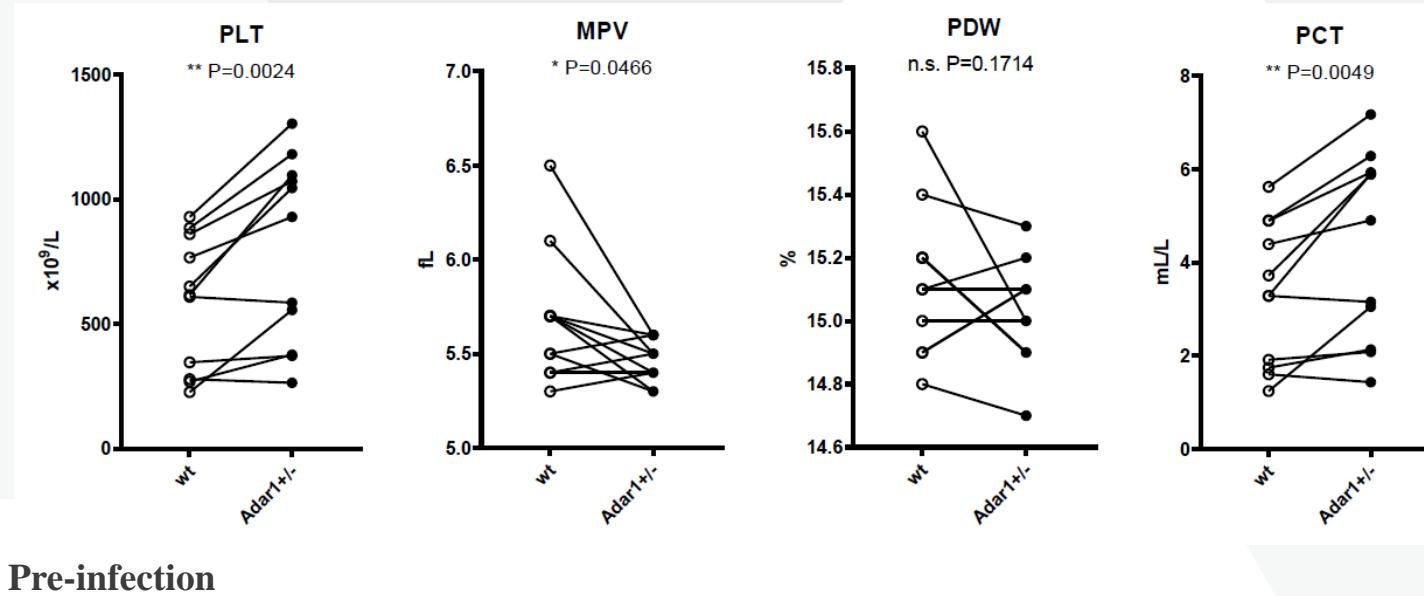
Triggering Receptor Expressed On Myeloid Cells Like 1 (TREML-1, TLT-1) promotes anti-inflammatory responses by binding to TREM-1 ligands and competing with TREM-1, thus antagonizing TREM-1 activation to reduce inflammation.

TLT-1 and platelets

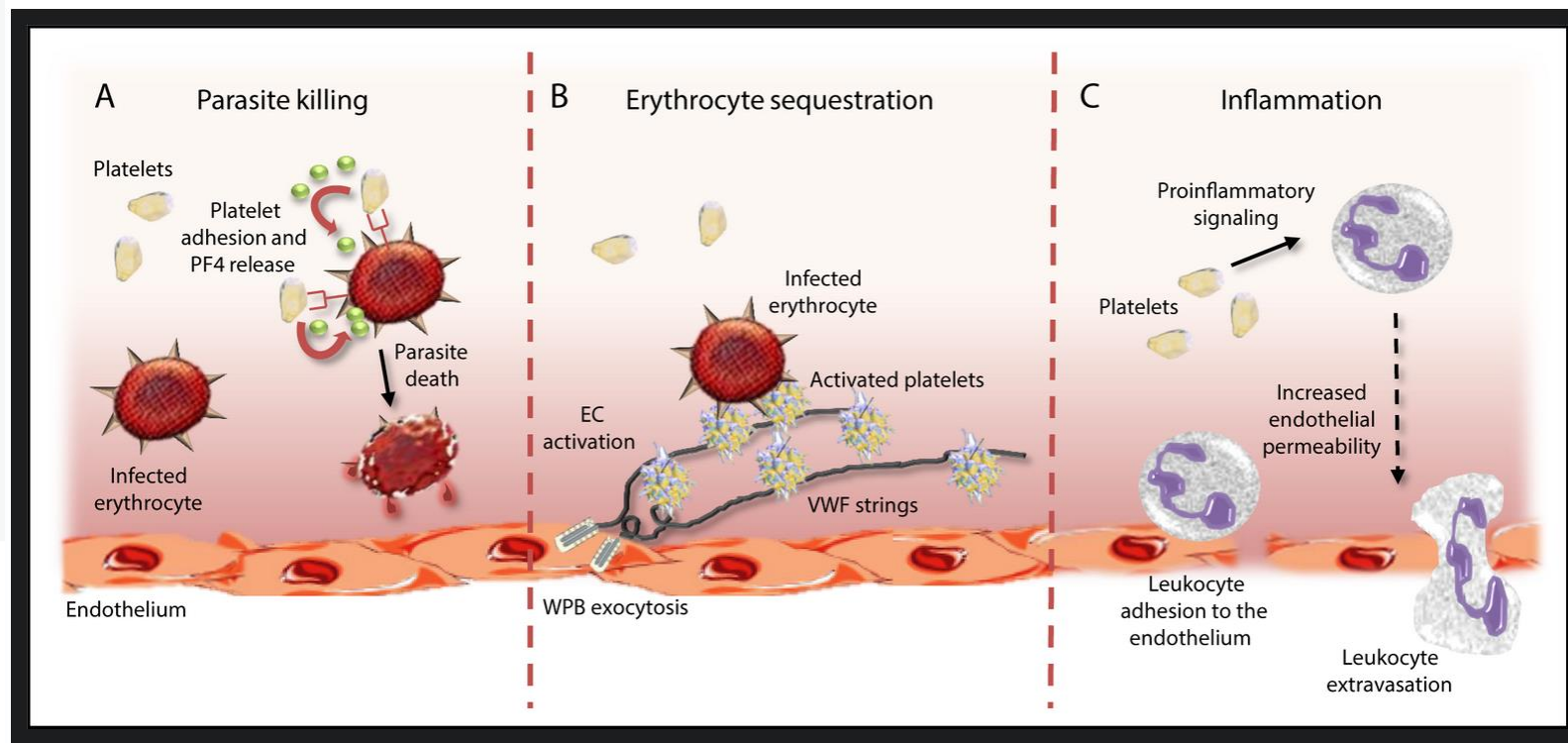


Created in BioRender.com 

Significant change in blood platelets in *Adar*^{-/+} mice



Platelets in malaria



Jamie M. O'Sullivan, James S. O'Donnell, Platelets in malaria pathogenesis, *Blood*, 2018,

Summary

- The Fulani ethnic group have reduced levels of A-to-I RNA editing when infected with *P.falciparum*, compared to sympatric ethnic groups.
- There are transient and significant changes in A-to-I RNA editing levels during *P.falciparum* infection,
- Reduced level of A-to-I RNA editing following *P.falciparum* infection is associated with protection from malaria.
- Reduced ADAR1 activity contributes to protection from parasitemia during rodent malaria.
- TREML-1 has been identified as a candidate gene that may be involved in protection against malaria in *Adar*^{-/+} mice.
- *We have identified ADAR1 as a novel target to combat malaria.*

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