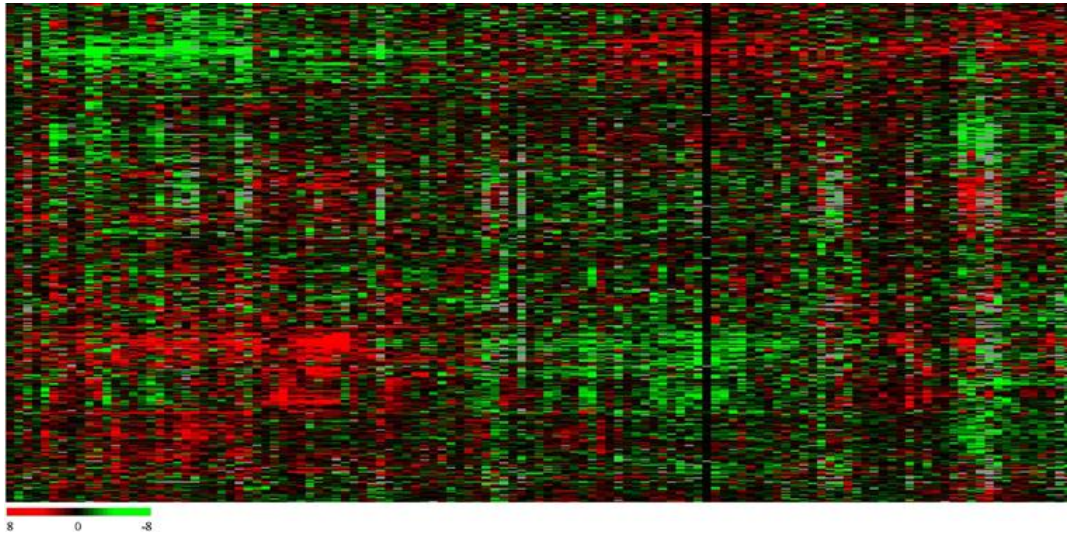
A-to-I RNA editing - immune protector and transcriptome diversifier

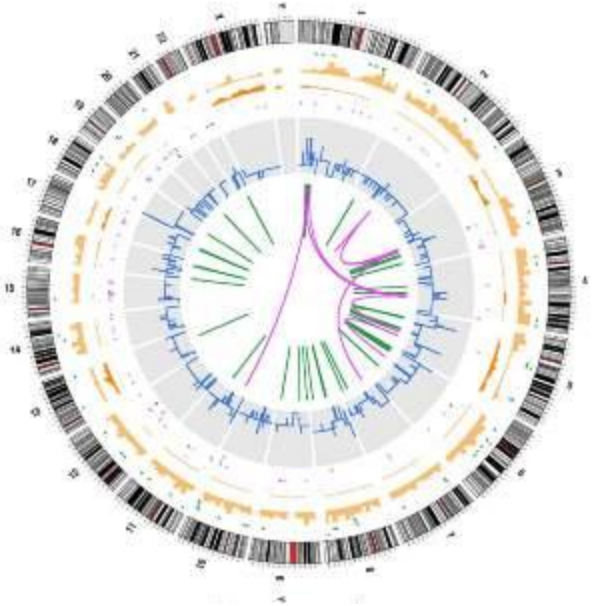
Salzburg 2018

Erez Levanon
Bar-Ilan University
Israel

Layers of Biological variations

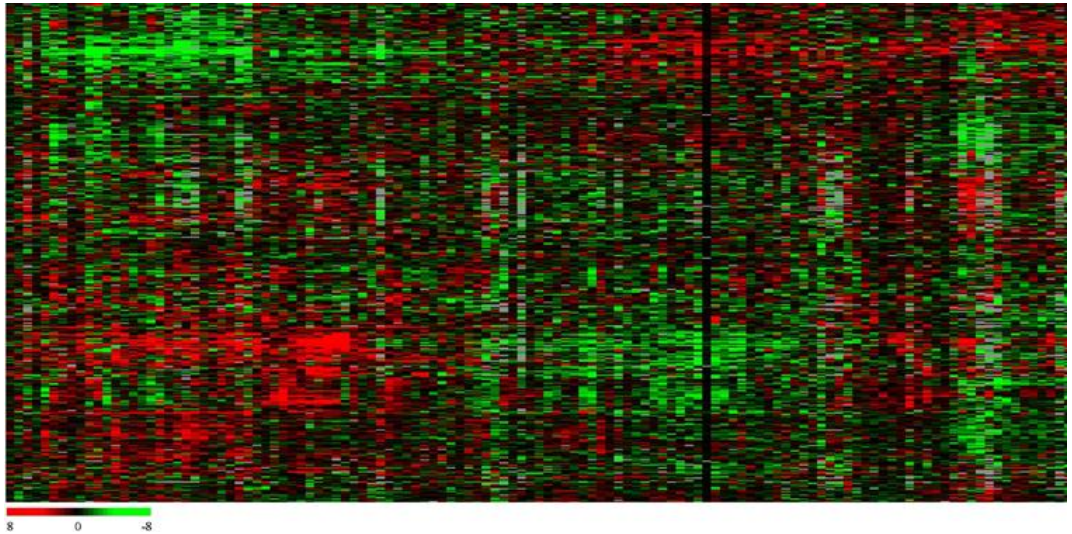


RNA expression

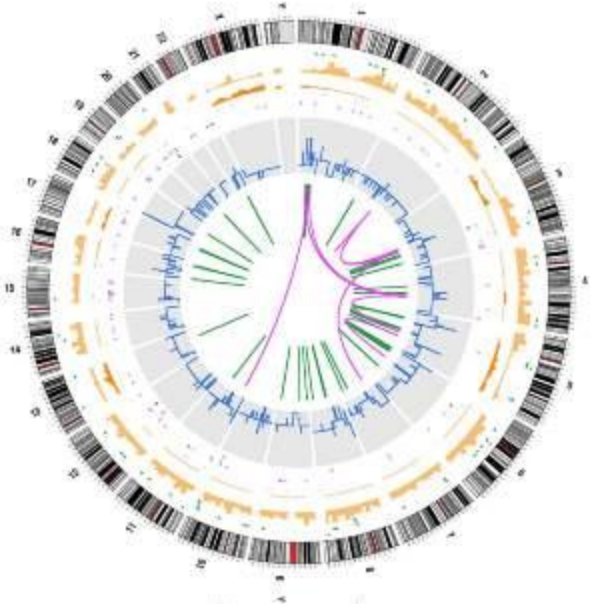


DNA mutations

Layers of Biological variations



RNA expression



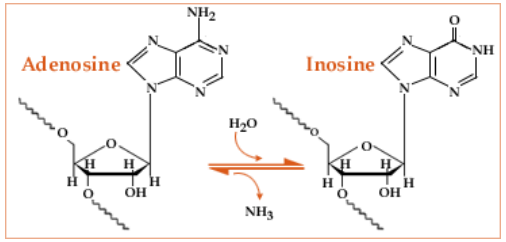
DNA mutations

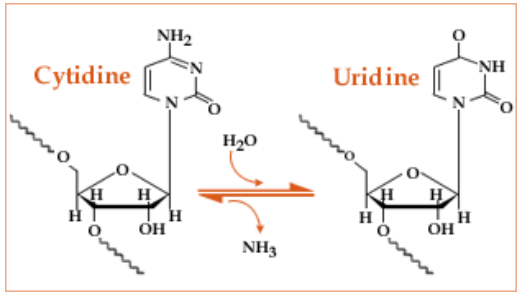
Protein families in human that can alter the original genetic sequence – RNA and DNA editing

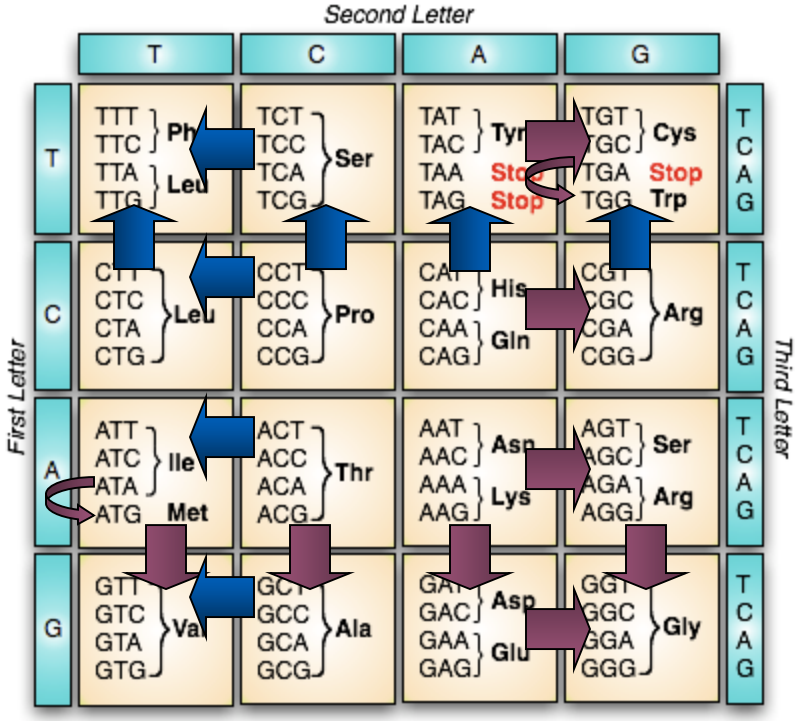
- **ADARs- modify "A" into I**

  I is read as "G"

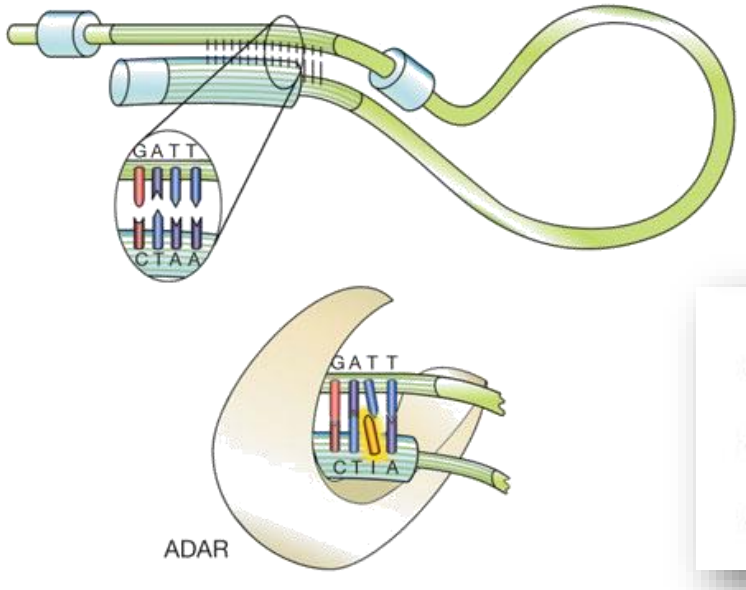
APOBECs - modify "C" into "U"

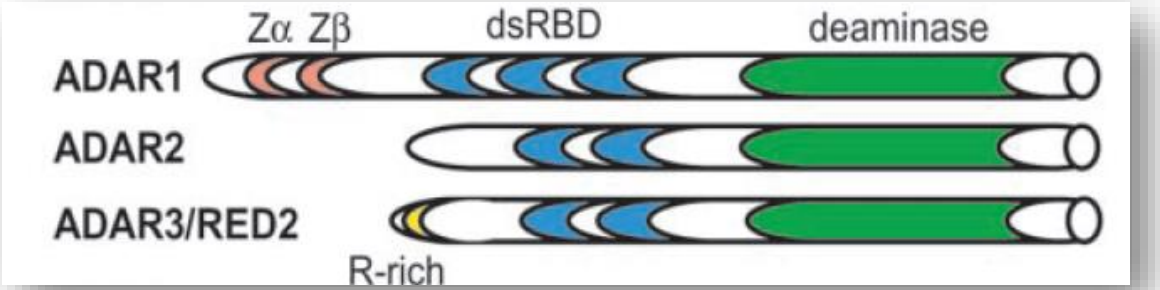






A→I Editing is catalyzed by the dsRNA binding ADARs (adenosine deaminases acting on RNA)





ADAR1 KO are embryonically lethal

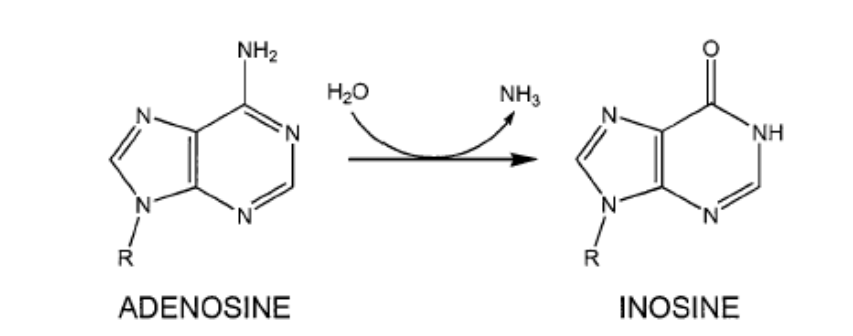
Mutations in ADAR1 cause Aicardi-Goutières syndrome

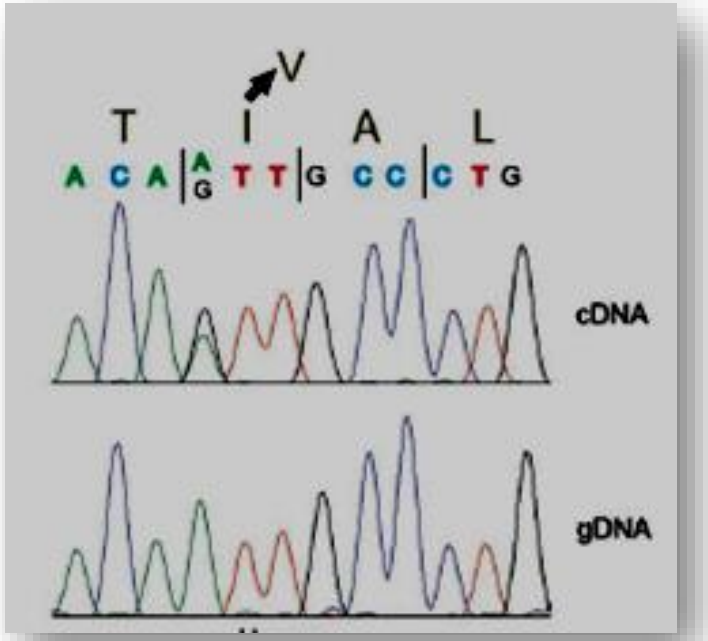
A to I RNA editing

- RNA editing is a post-transcriptional
- Alters the RNA sequence encoded by the DNA in a single-nucleotide, site-specific, manner
- Adenosine can be converted to inosine
- Inosine is read as Guanosine

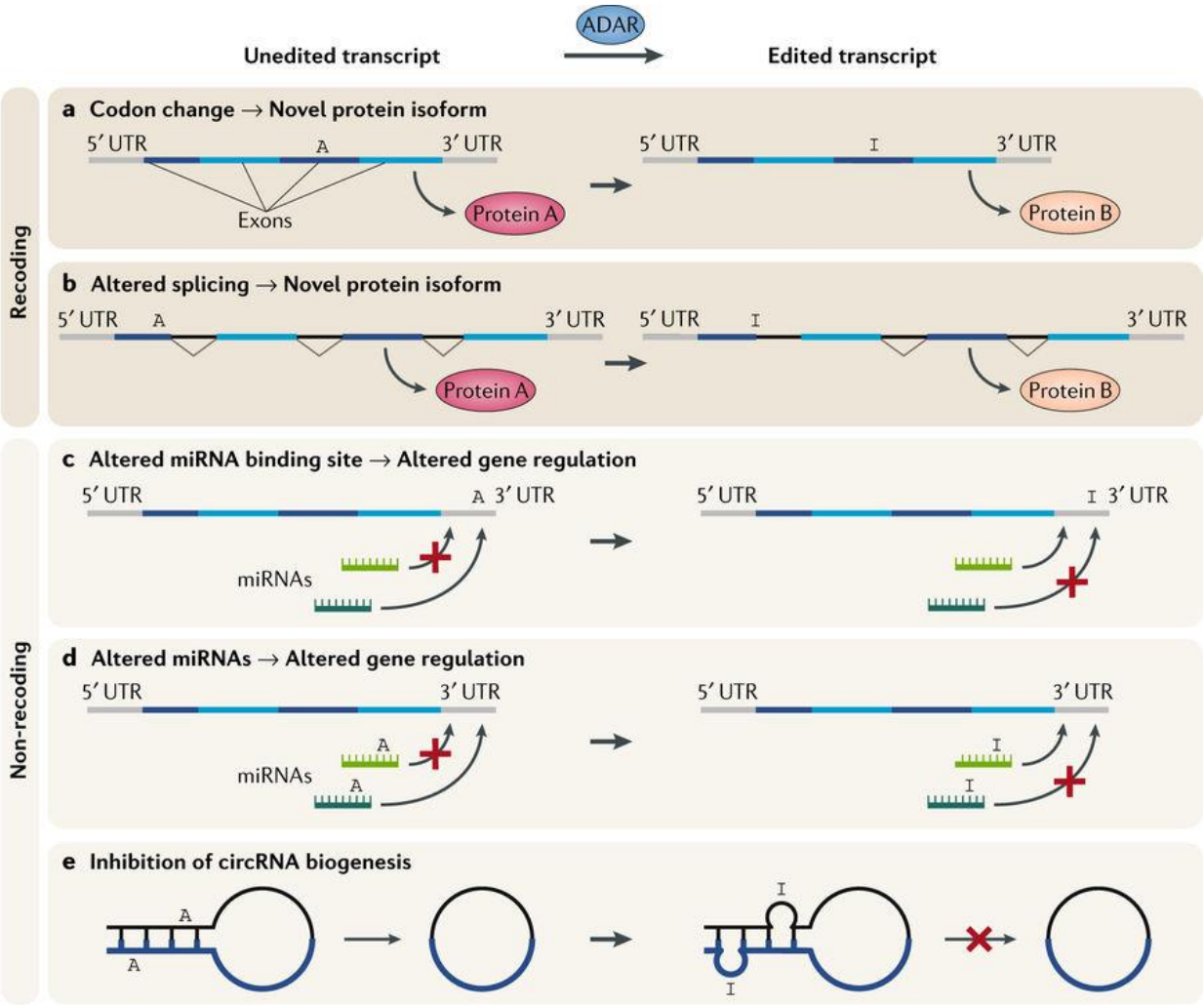
```
CAGATACATTAAGACCAGTGC    Genome
CAGATACATTGAGACCAGTGC    HSM801523
CAGATACATTGAGACCAGTGC    AB032994
CAGATACATTAAGACCAGTGC    BC021008
CAGATACATTAAGACCAGTGC    BC011762
CAGATACATTAAGACCAGTGC    AF160973
CAGATACATTAAGACCAGTGC    BG480006
```

Deamination



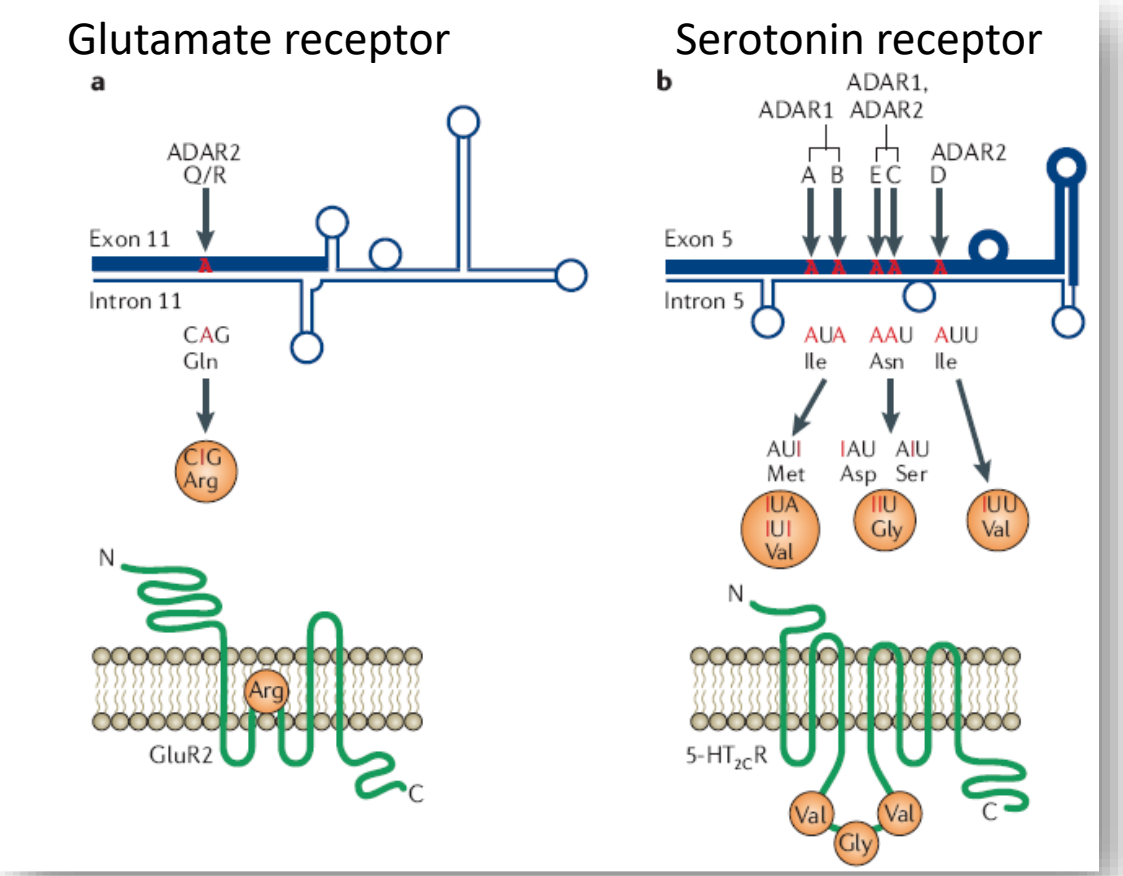


Multiple effects of editing on gene regulation



Eisenberg & Levanon , Nature Reviews Genetics 2018

RNA editing targets that change the CDS are rare in mammals



Nishikura 2006

1st example: GluR-B

Q ⟶ R, controls Ca^{2+} permeability. 100% edited in brain

(Sommer et al, Cell 1991)

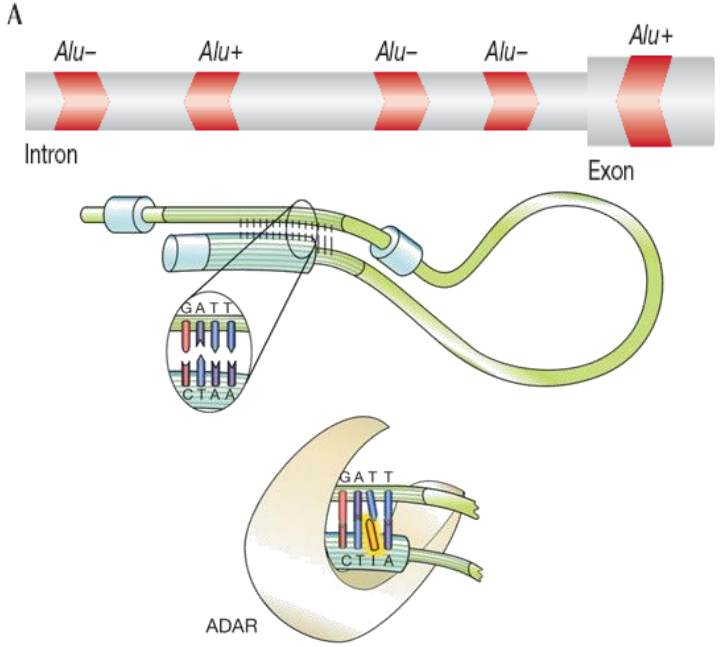
2nd example: 5-HT_{2C}

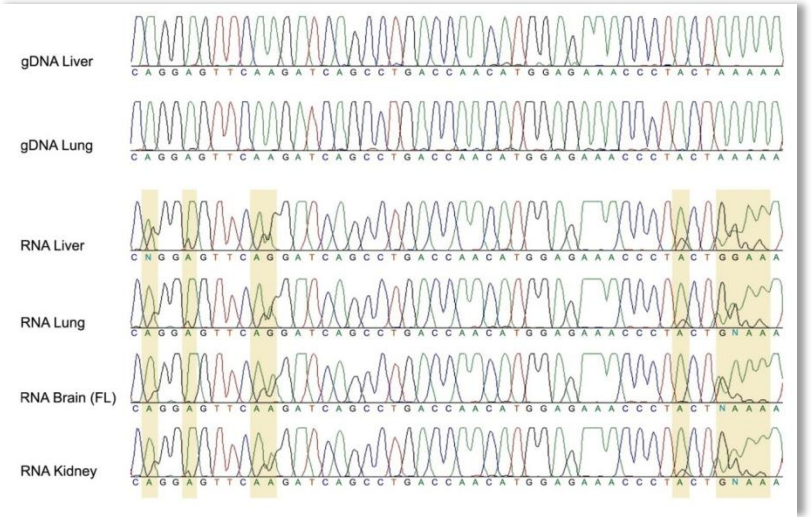
5 sites, >20 combinations, varying functional responses

(Burns et al, Nature 1997)

Editing is common in Alu repeats

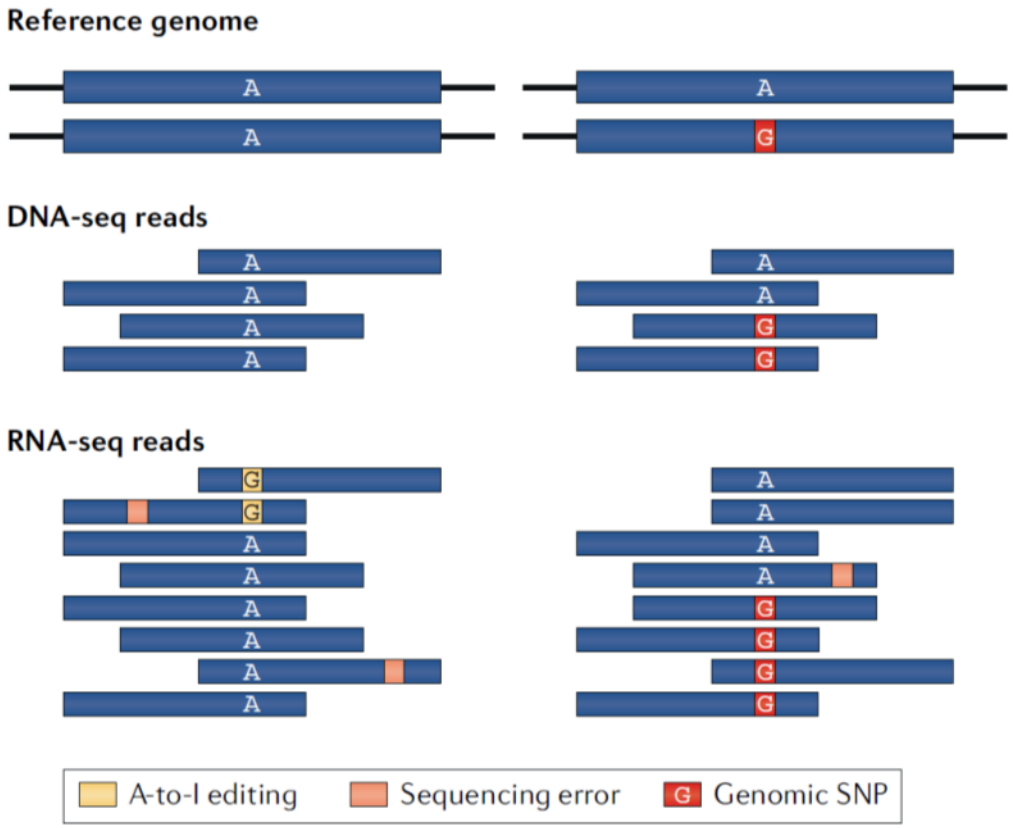
- ~1 million copies of Alu in the human genome (10% of genome)
- About 300 bp long
- Primate specific
- **Form dsRNA structures**

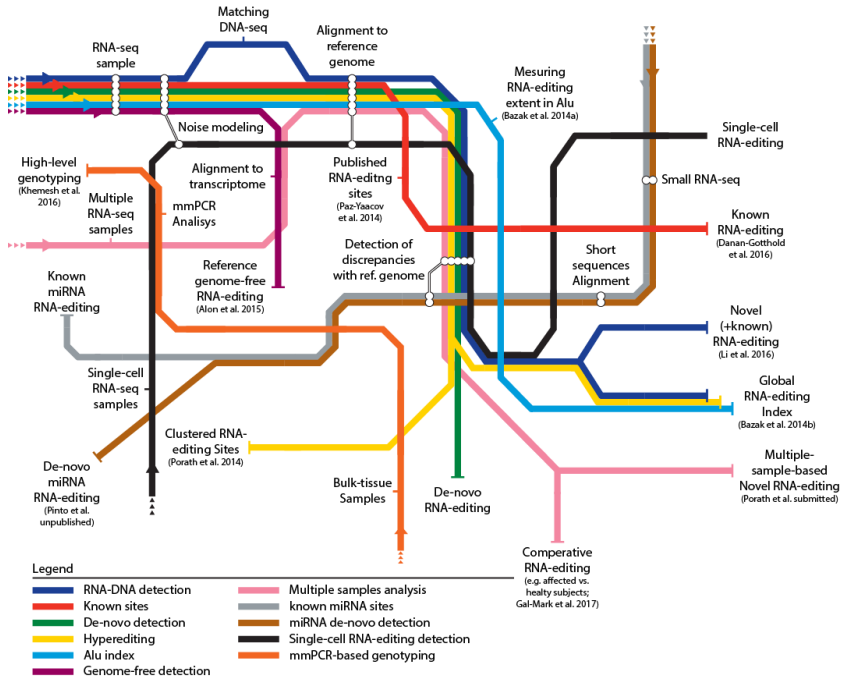


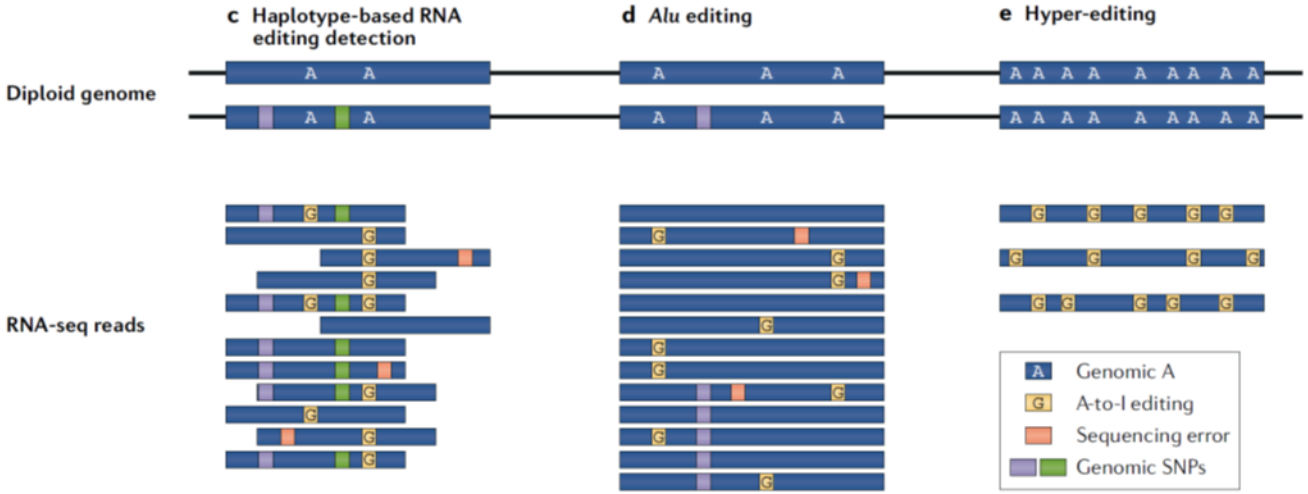


Levanon et al, Nature Biotech. 2004

Systematic detection of RNA editing







Hyper-editing

PAR-SN RNA, Inverted Alus (ambiguous structure)

tgagacAgtAtctcacagtccAggctggAgttcagtggcattaacataactcactgcAgccttgAacacctgagctcAAAcgatcctttcaccttAtcctccagagtAgctgggactAcagtcgcgtgt
caacatgcctggctaattttagttttctaattttttagagttgggatctcActAtgttgcttAgactggtcttgaactcctggcctcatgccatcctcttgcctcagctggtattgtaggtgtgagccactgtgccaggt
actaactttttttatgttagaaaacaagtttttaggtattttatagttacttccttatgacaatatctcatcagaatctaggcctatctgatatctgactccatacgtttataccgtgtagtcagccattcccagatagagg
tcatttgagttacttagtgtttaccagcacttgtttactaccttgcgatgatatttacagtgtatttttgggtatggttttggggttatttggttgttgttaaaatgcctgtaatcccggcactttgggaggttgaggcgggtg
gatcacctgaggtcaggagttcgagcccagcctggccaacatggcgaaaccctacctctactaaaaatacaaaaattAgcctggcgtggtggcaggcacctgtAAAtccaActAcgcaggaggctA
AggcAggagaattgcttgaacccgggaggcggaggttgcagtgagccgagatcgcgccaatggcactccattcagcttgagtgacagaggtgagactcccatctcaaggtaaaaaaaaaaaaaaat
agaaatcagcaactgctatttgagttcacaggagtgagaagtcctctcttgcatggtgagatggggaacatttaattcaatagaaatatttgagaccaggtatggtggctcaAgcctgtaAtcccagcacttt
gggaggctgagttgggtggatcacctgacatcaggAgtttgagactagcctggccaacatAgcAataccctgtctcaaagatacaaaaattacccAAgcttggtggtgtgtgtctgtAAtcccagctAc
tcaggaggctgaggcAggagaatcActtgAAcctgggcggcaaaggttgcagtgagccgagaccatgccactgcactccagcctgggtgacagagtgagactccgtctggaaaaaaaaagaaaaaaa

Detecting hyper-editing

Typical editing is detected by aligning the RNA to the genome and searching for A→G mismatches

```
TCCCCACCCTGAGTAGCTGGGACTACAGGCATGTGCCACCACACCACCATGCTAGGCTAATGGTTTGTATTTGTTTGA : DNA
|||||||||||||*|||||||||||||||*|||||||||||||||||||||||||||||||||||||||*|||||||||
TCCCCACCCTGAGTGGCTGGGACTACAGGCGTGTGCCACCACACCACCATGCTAGGCTAATGGTTTGTGTTTGTTTGA : RNA
```

“Too edited” RNAs will not align to the genome at all!

```
TCCCCACCCTGAGTAGCTGGGACTACAGGCATGTGCCACCACACCACCATGCTAGGCTAATGGTTTGTATTTGTTTGA : DNA
|||||*||||||||*||||||*||||*|||*||||||*||||*||*|||||||*||||**||||||||*||||||||*
TCCCCGCCCTGAGTGGCTGGGACTACGGGCGTGTGCCGCCACGCCGCCATGCTGGGCTGGTGGTTTGTGTTTGTTTGG : RNA
```

How to detect such editing events?

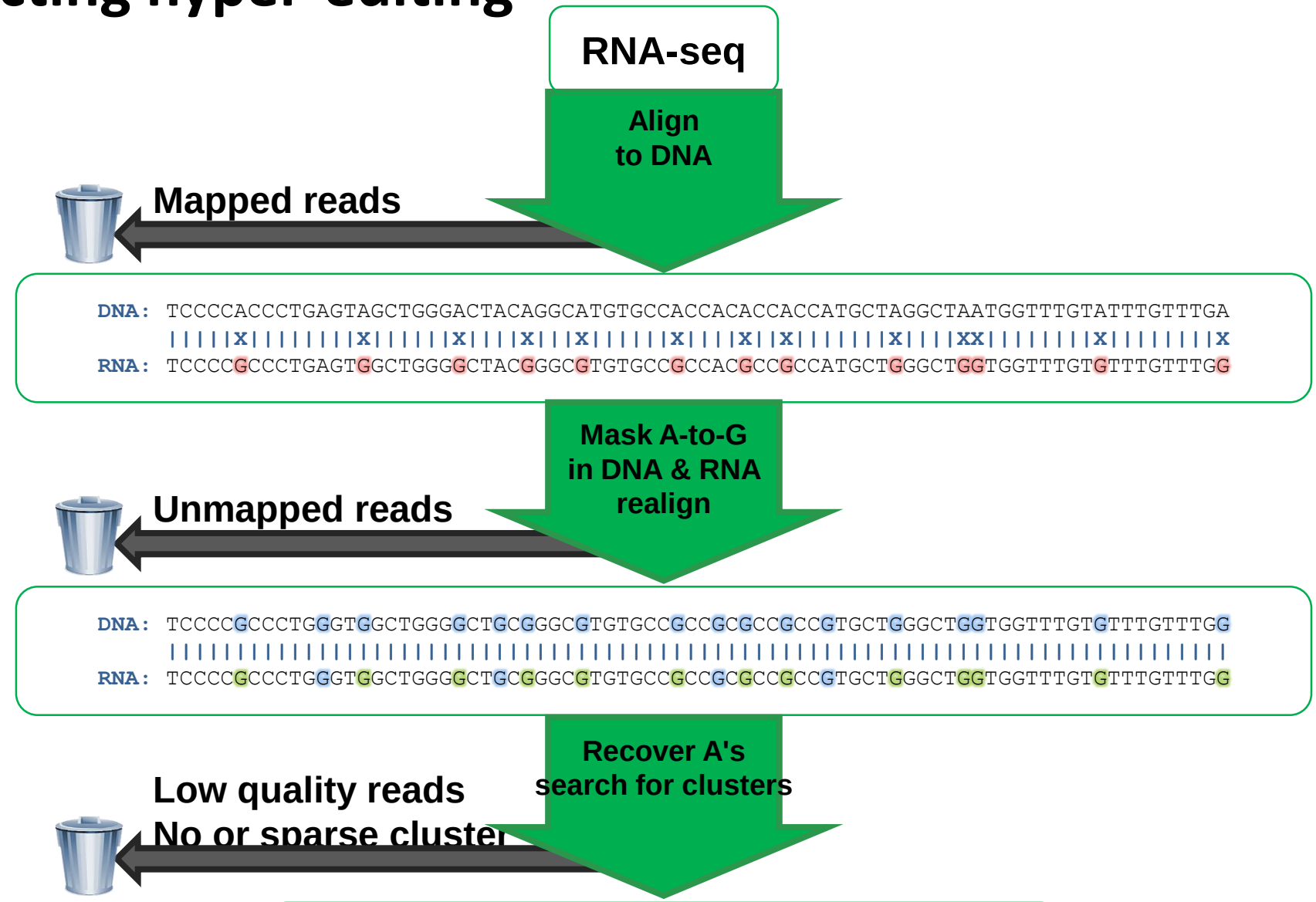
Detecting hyper-editing

```
TCCCCACCCTGAGTAGCTGGGACTACAGGCATGTGCCACCACACCACCATGCTAGGCTAATGGTTTGTATTTGTTTGA : DNA
|||||*||||||||*||||||*||||*|||*||||||*||||*||*||||||||*||||**||||||||*||||||||*
TCCCCGCCCTGAGTGGCTGGGACTACGGGCGTGTGCCGCCACGCCGCCATGCTGGGCTGGTGGTTTGTGTTTGTTTGG : RNA
```

- Collect all sequences that were rejected from alignment to the genome
- Transform the sequences— change every “A” to “G” in the human genome and in the RNA sequences.
- Re-align to the genome.

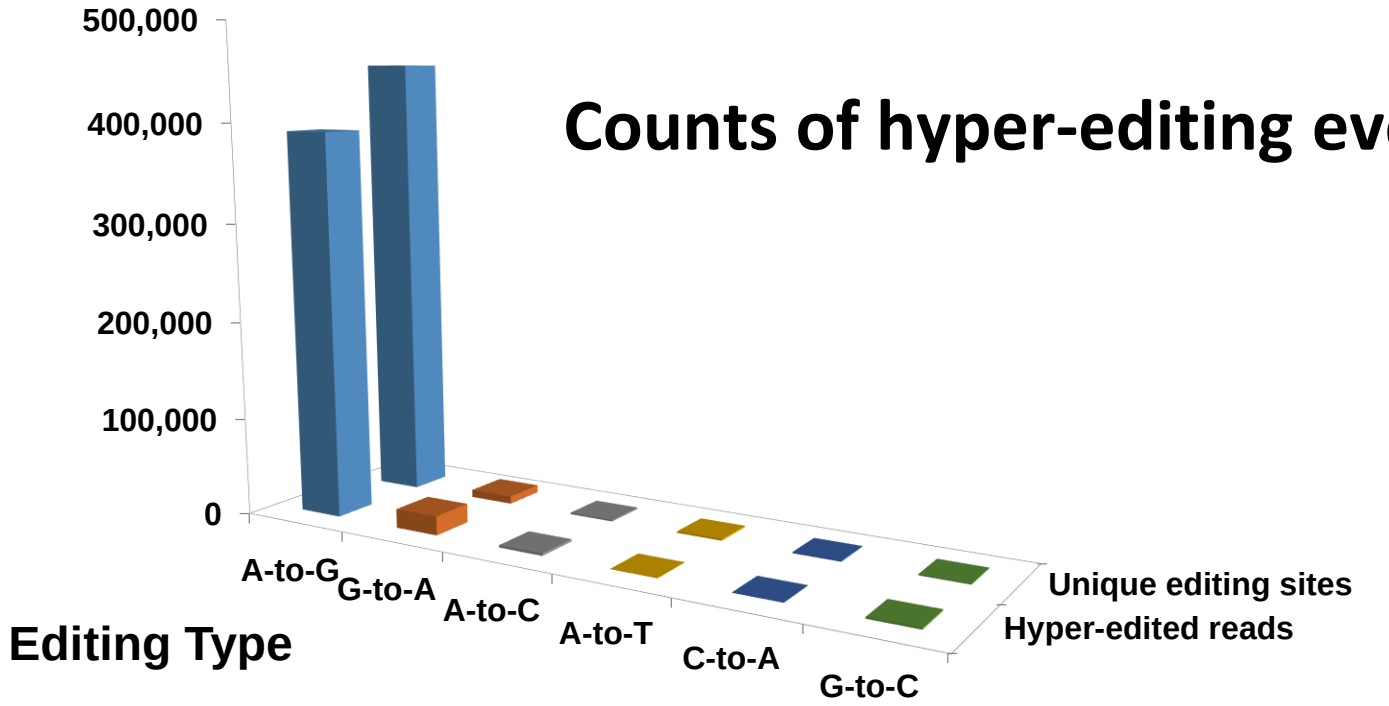
```
TCCCCGCCCTGGGTGGCTGGGGCTGCGGGCGTGTGCCGCCGCGCCGCCGTGCTGGGCTGGTGGTTTGTGTTTGTTTGG : DNA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCCCCGCCCTGGGTGGCTGGGGCTGCGGGCGTGTGCCGCCGCGCCGCCGTGCTGGGCTGGTGGTTTGTGTTTGTTTGG : RNA
```

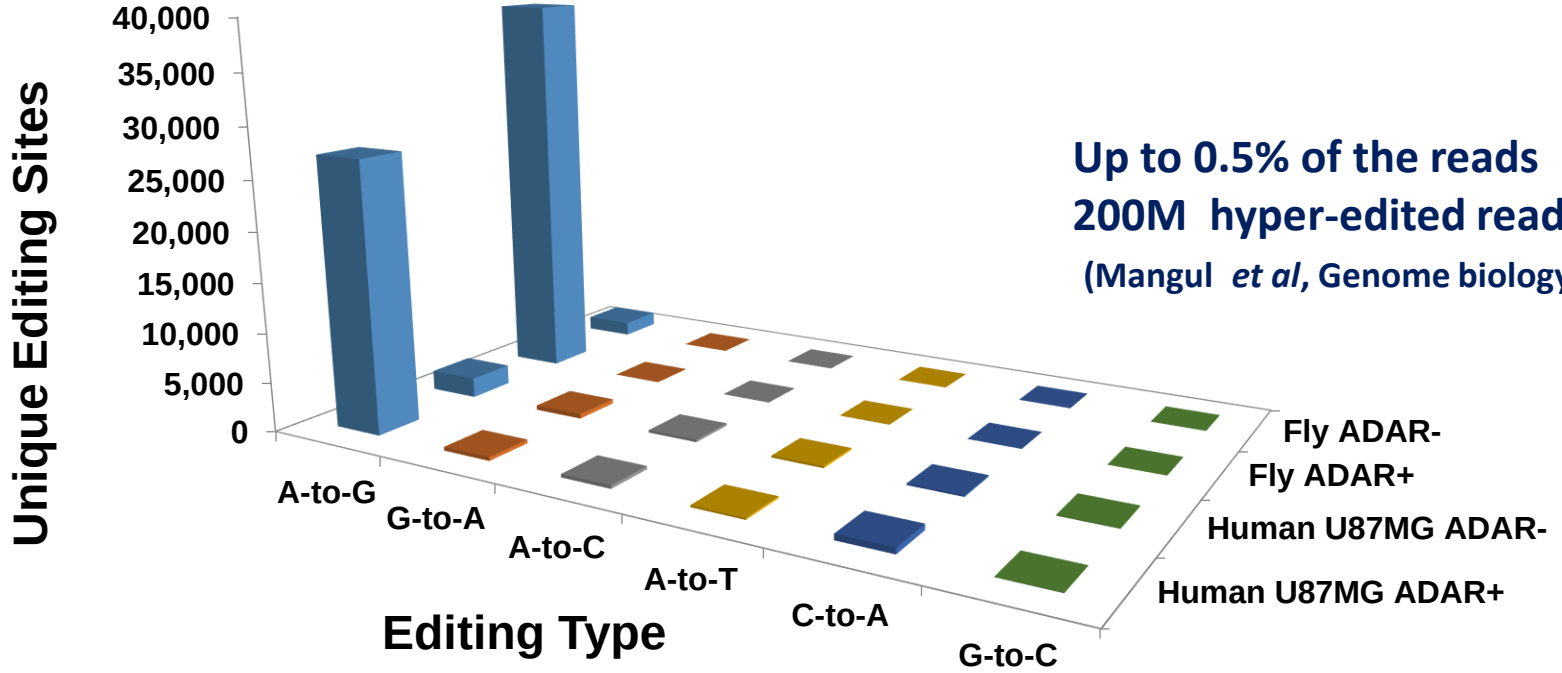
Detecting hyper-editing



Carmi, PLoS Gen. 2011

Counts of hyper-editing events





Up to 0.5% of the reads

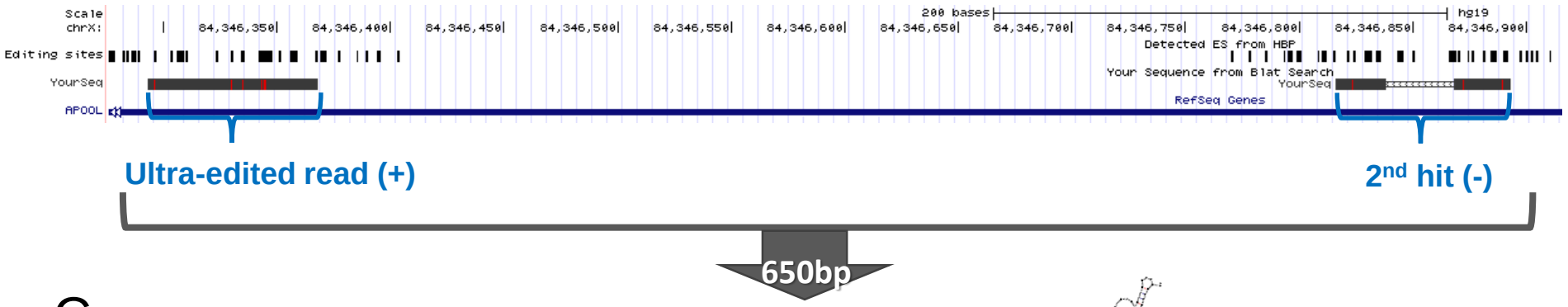
200M hyper-edited reads in GTEx data

(Mangul *et al*, Genome biology 2018)

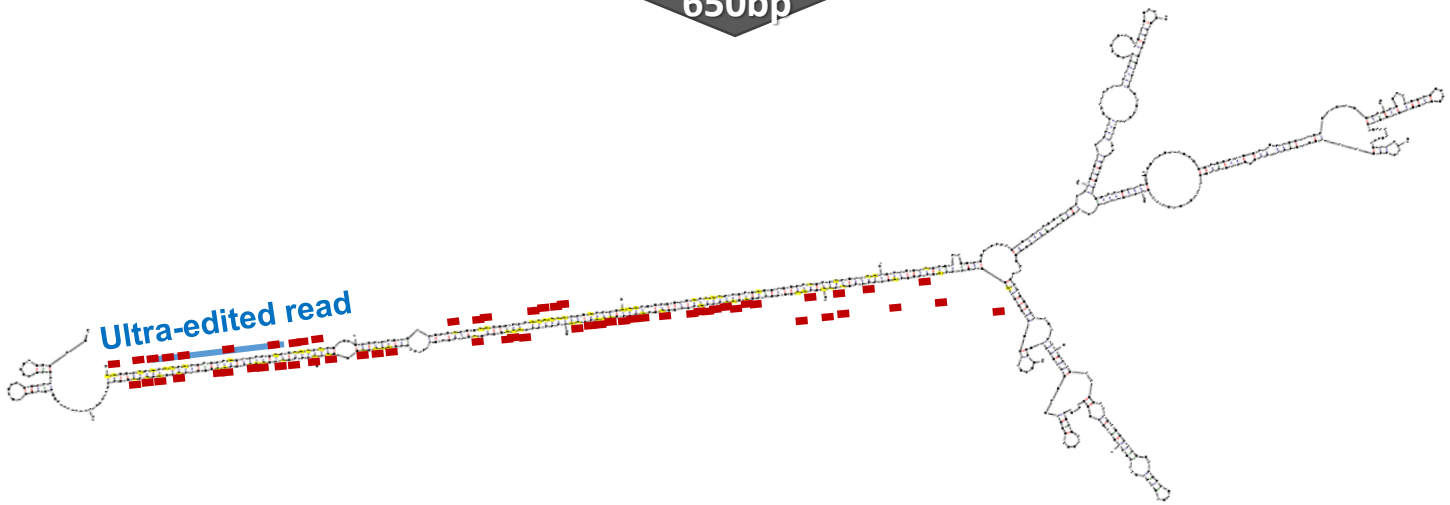
A

```
DNA  chrX:84346294-84346368:  CTTAGATTTGATGAAATATGAGTGTGTTTGAGATCTGATCCAAGGTTTTAAAAAATTCATTTCAAATCCTGGAGA
                              |||*|||||||||||||||||||||||||||||||*||||*|||||||***||||||||||||||||||||||
RNA  1:3:46:17401:113074#0:   CTTGGATTTGATGAAATATGAGTGTGTTTGAGATCTGGTCCAGGGTTTTAGGGAATTCATTTCAAATCCTGGAGA
```

B

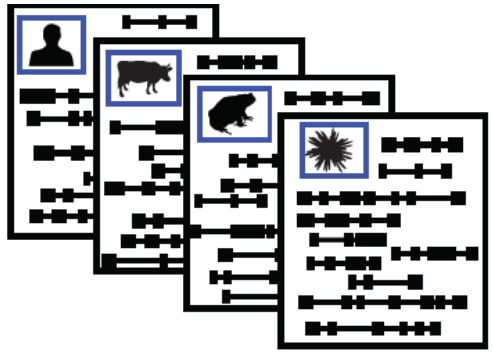


C



Porat, Nat. Comm. 2014

21
RNA-seq datasets
of various species



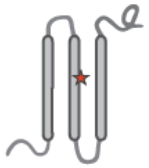
Identify Hyper-Editing

Numerous clusters of editing sites

```
DNA: TCCCCACCCTGAGTAGCTGGGACTACAGGCATGTGCCACCACACCACCATGCTAGGCTAATGGTTTGTATTTGTTTGA
RNA: .....G........G......G........G...............G.......G...............G.........
DNA: GACTTGCCAGACTGATTTGCCACTGGTTCGAACTAGCTGCCCTTAACGTCTTGACGGTCACTGCACGGTTAAACTCAG
RNA: .G........G..........G............G.........G..............G....G......G......
```

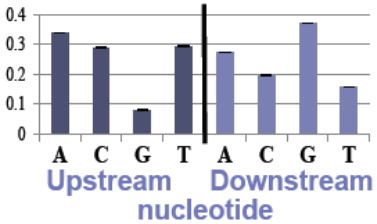
Analyze Hyper-Editing

Extremely rare in coding

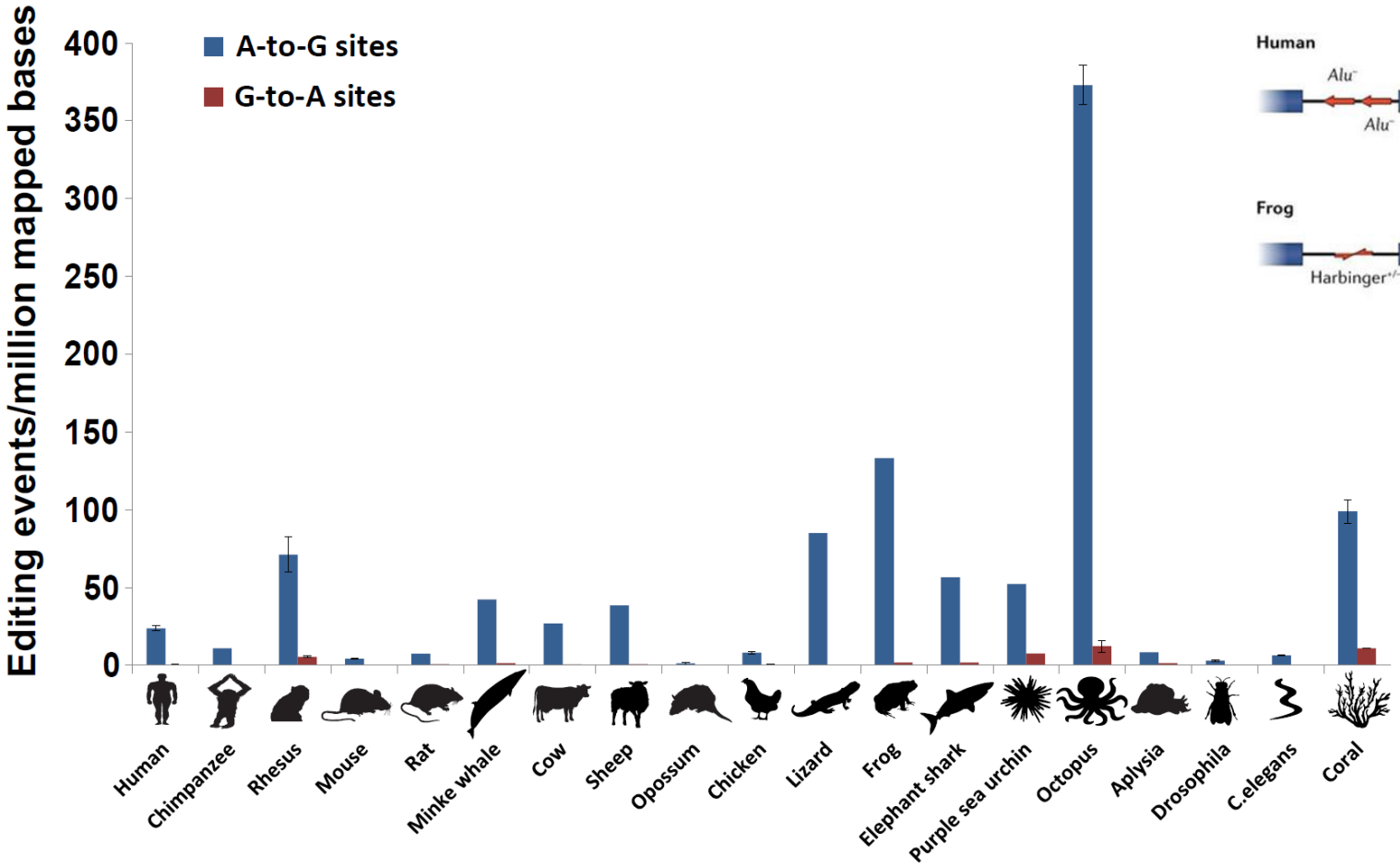


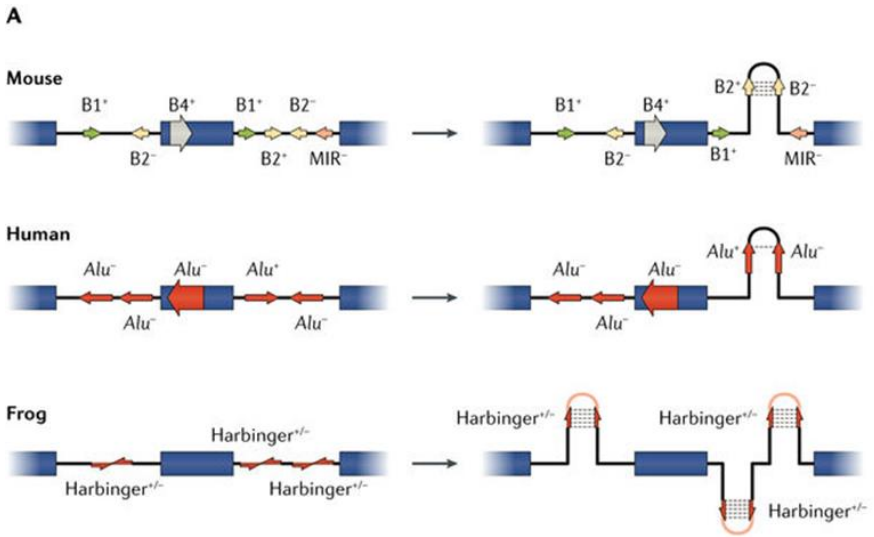
Enriched in dsRNA structures

Evolution of ADAR sequence preference



Editing is everywhere

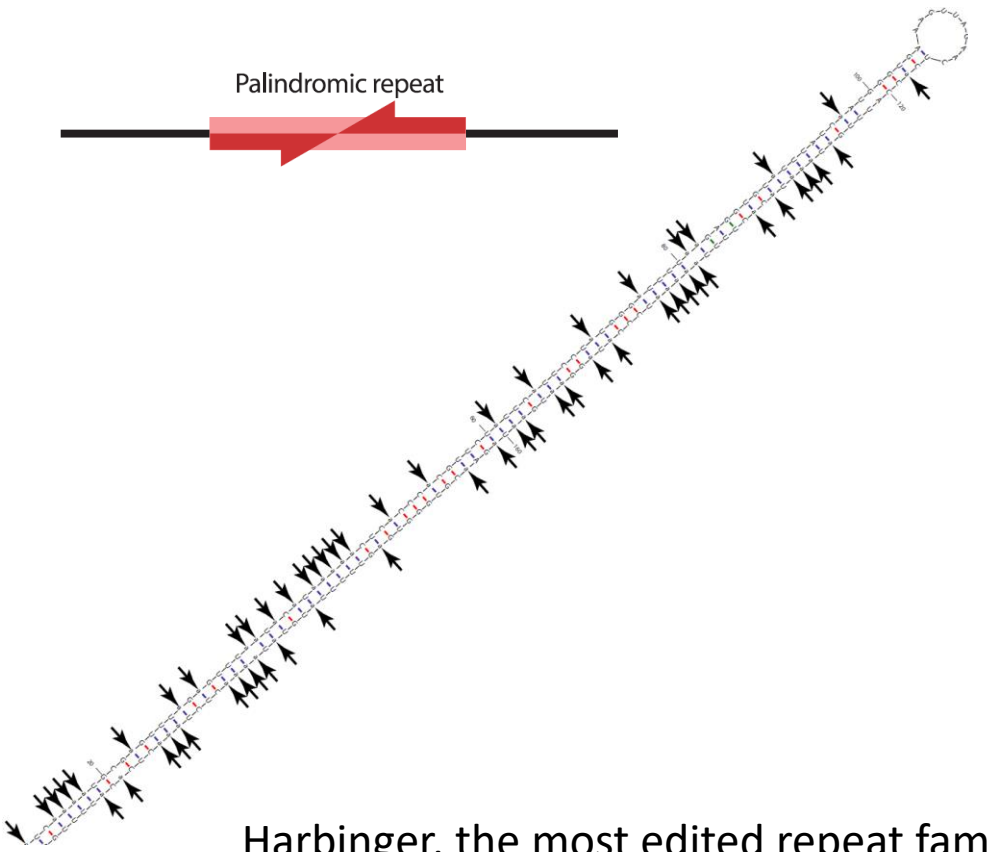


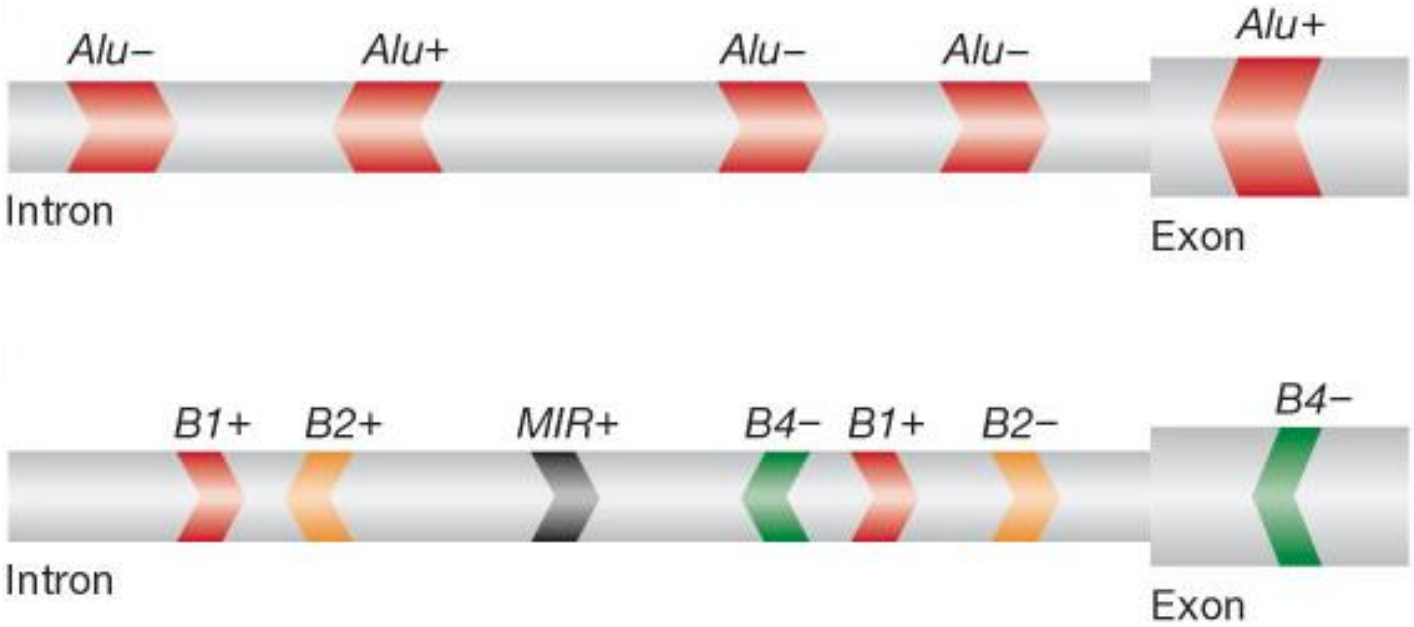


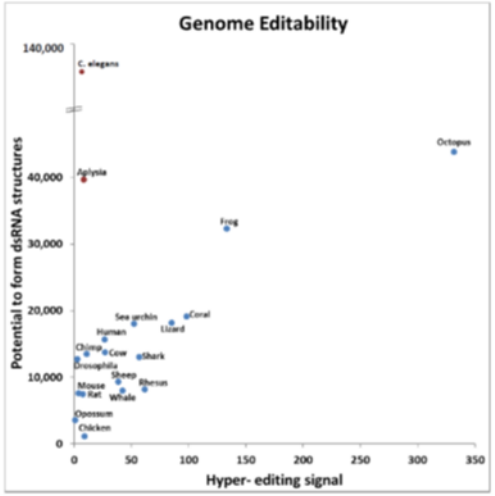
Main edited repeats family	Editing sites found in one brain sample
B1 and B2	13,748
Alu	75,984
Harbinger	147,172

Porath et. al, Genome Biology, *2017*

Editing in repeats strongly depends on the repeats repertoire



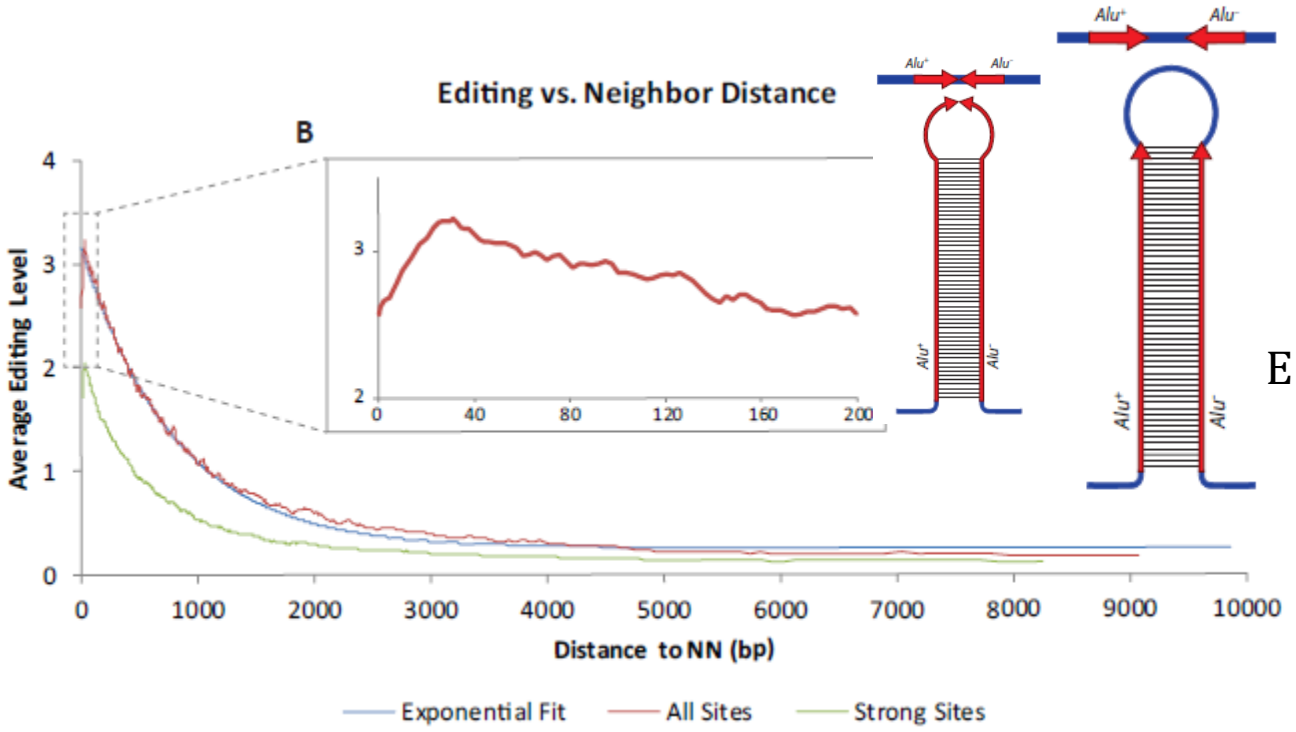




Harbinger, the most edited repeat family in Xenopus tropicalis is a palindrome

Alu Editability

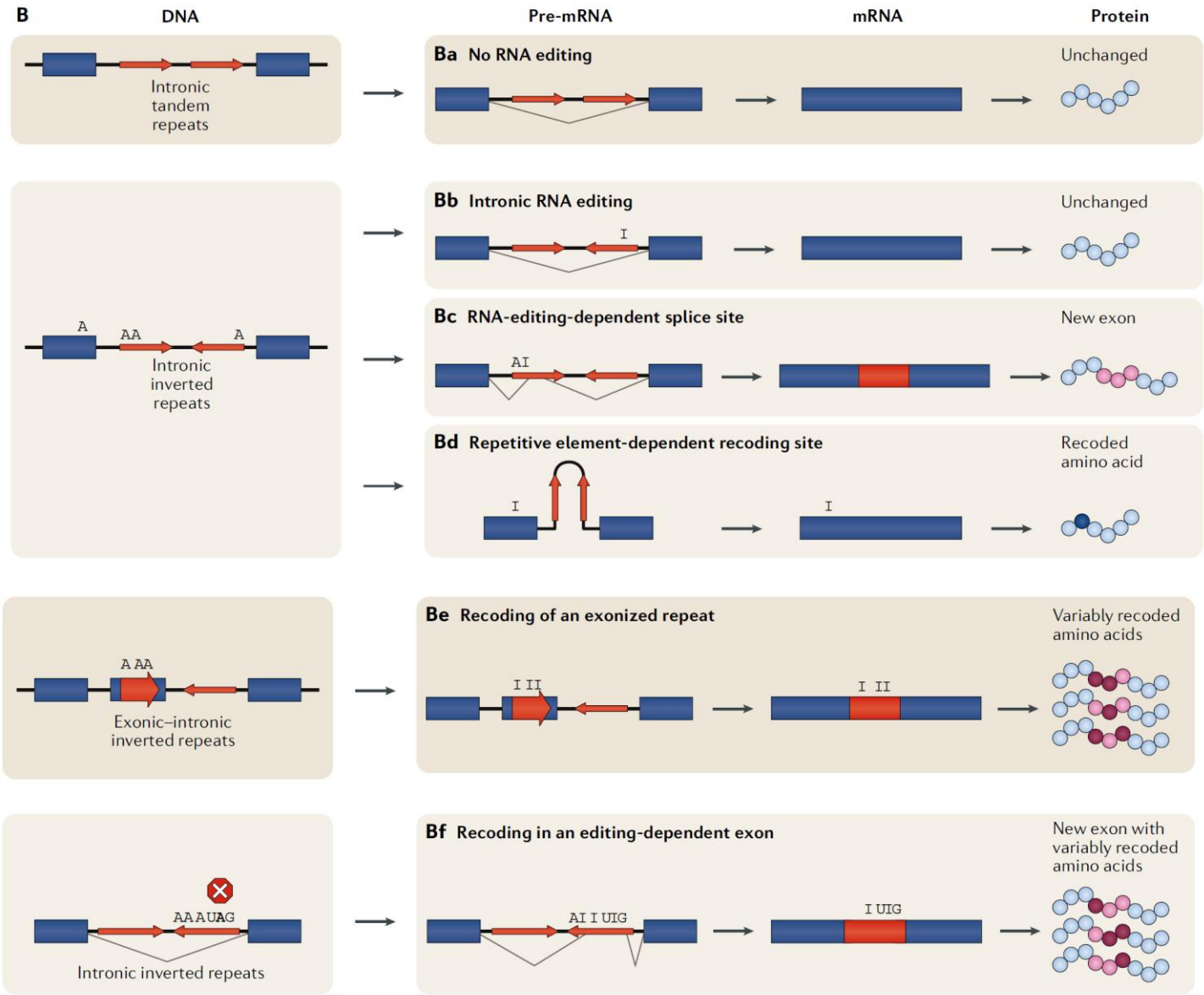
- Distance to the closest reversely-oriented neighbor
- Number of *Alu* repeats of the same and reverse strand in the genomic vicinity
- Expressed strand of the *Alu*



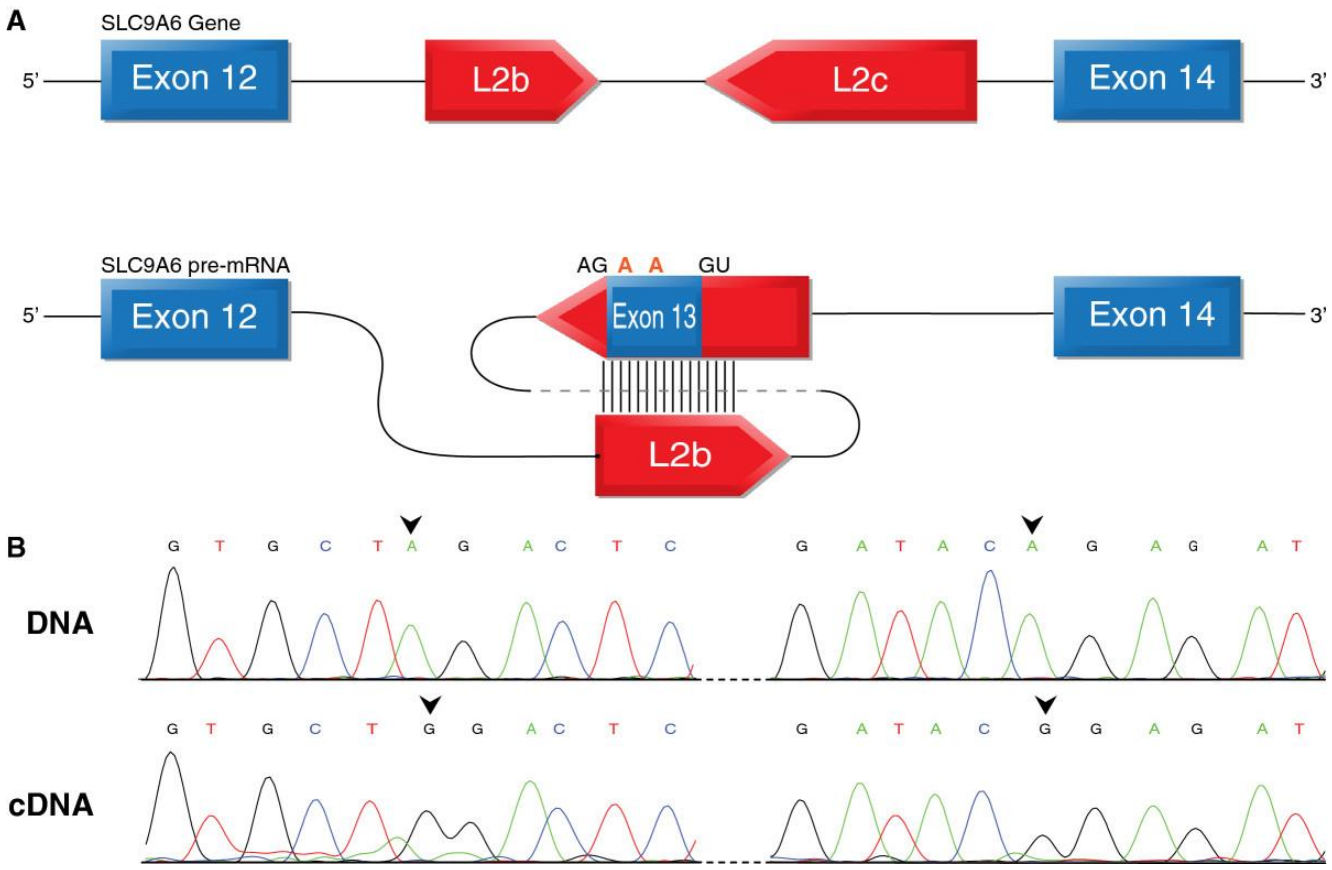
$$E = 2.9 \cdot e^{-d/800} + 0.25$$

Bazak, NAR 2014

Consequences of editing in repetitive elements

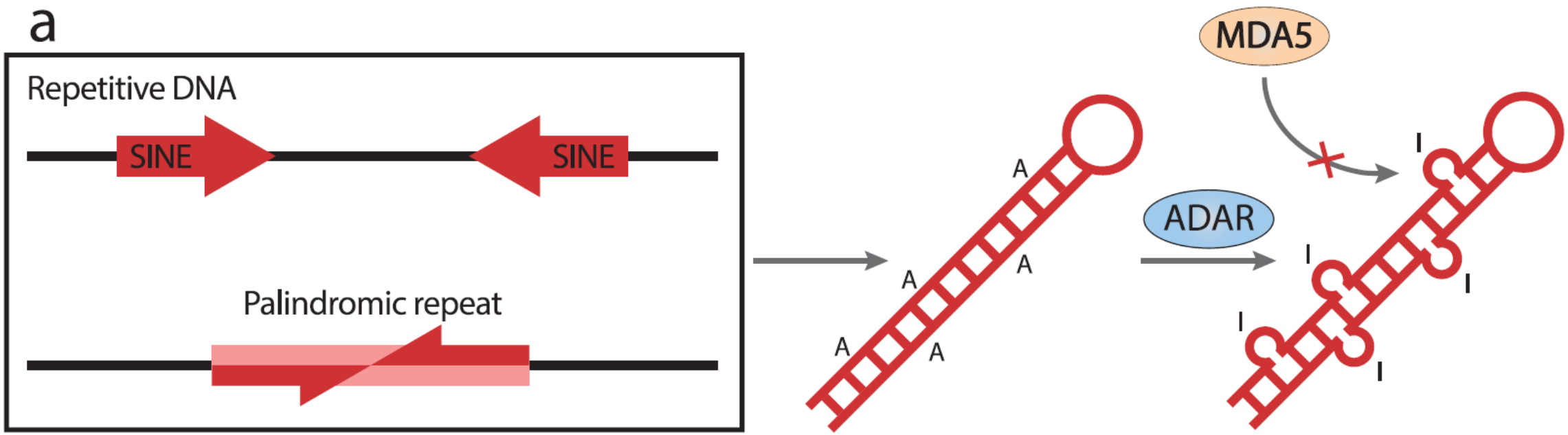


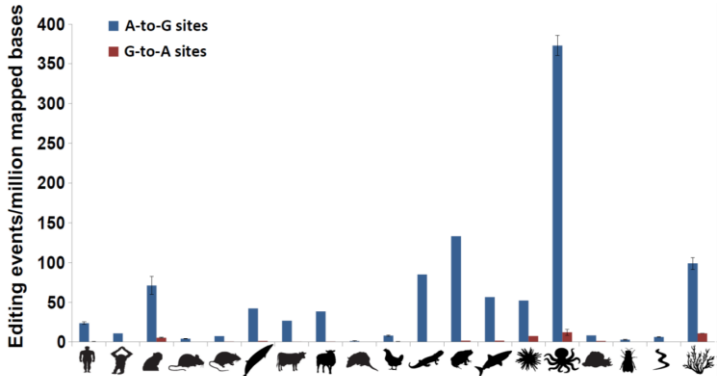
Editing and exonization of L2 element (SLC9A6 gene)



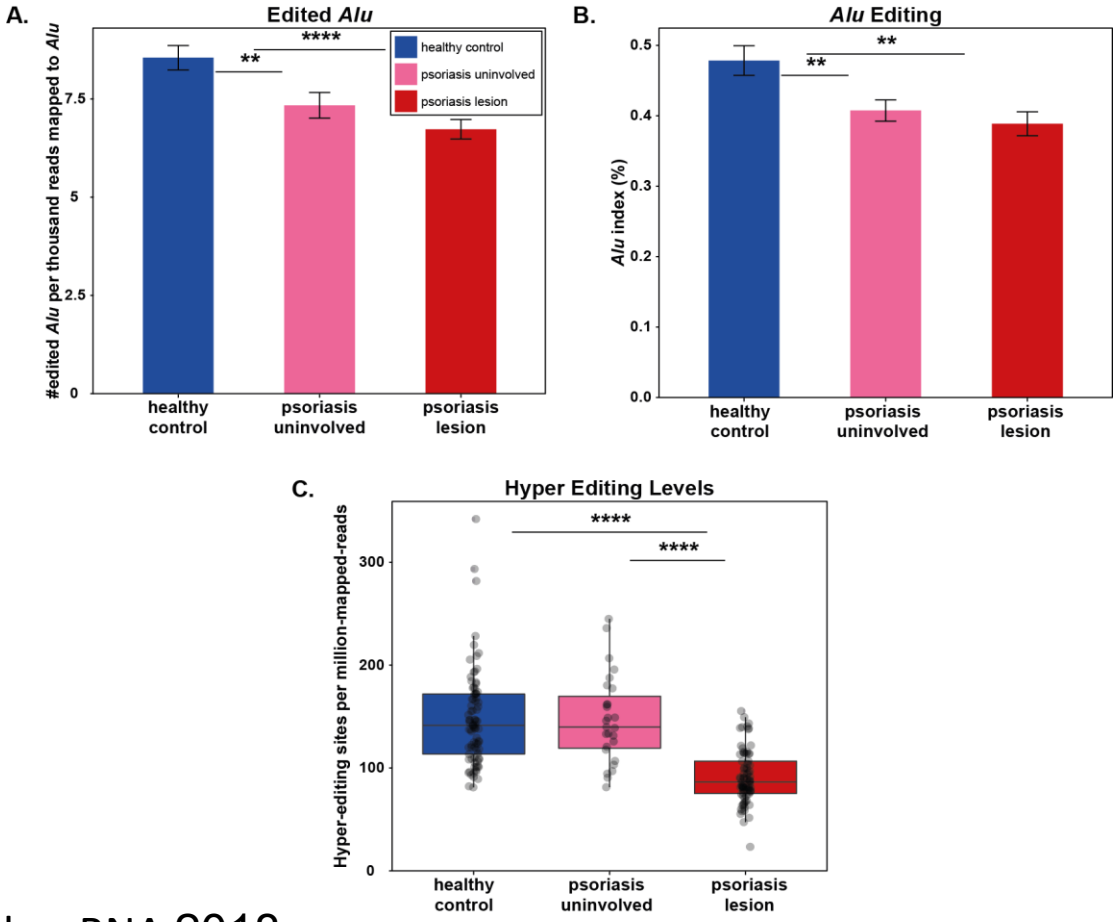
Pinto, Genome Biology 2014

Editing inhibit recognition of cytoplasmic dsRNA by the innate immune system



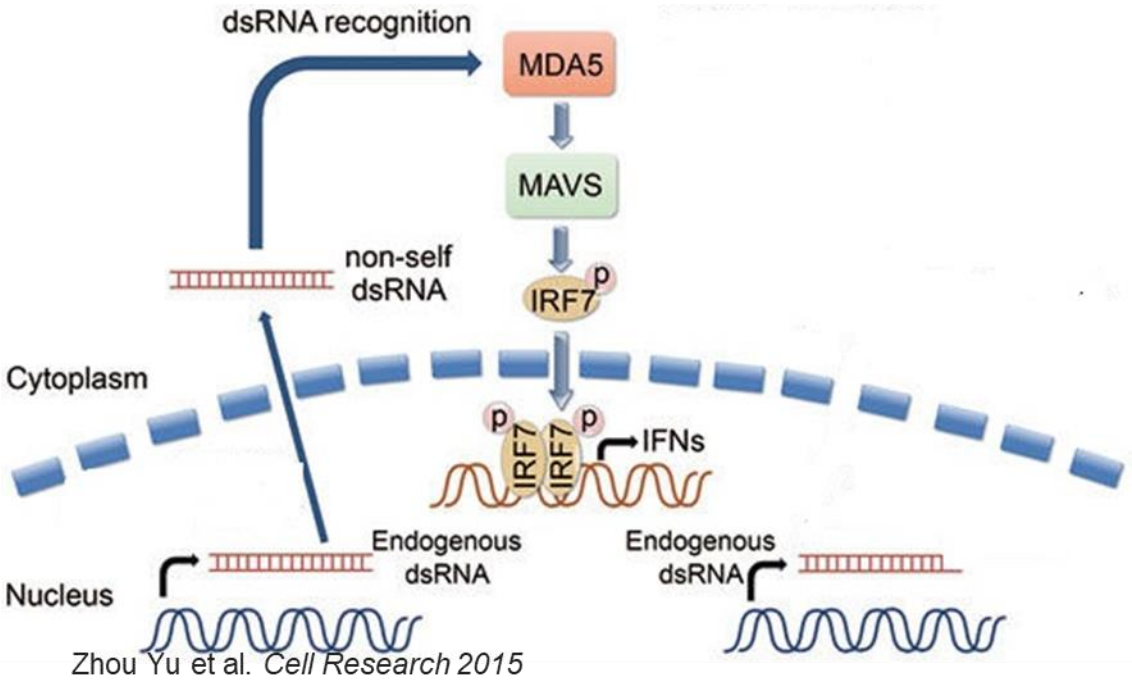


Reduce A-to-I RNA Editing in Psoriasis



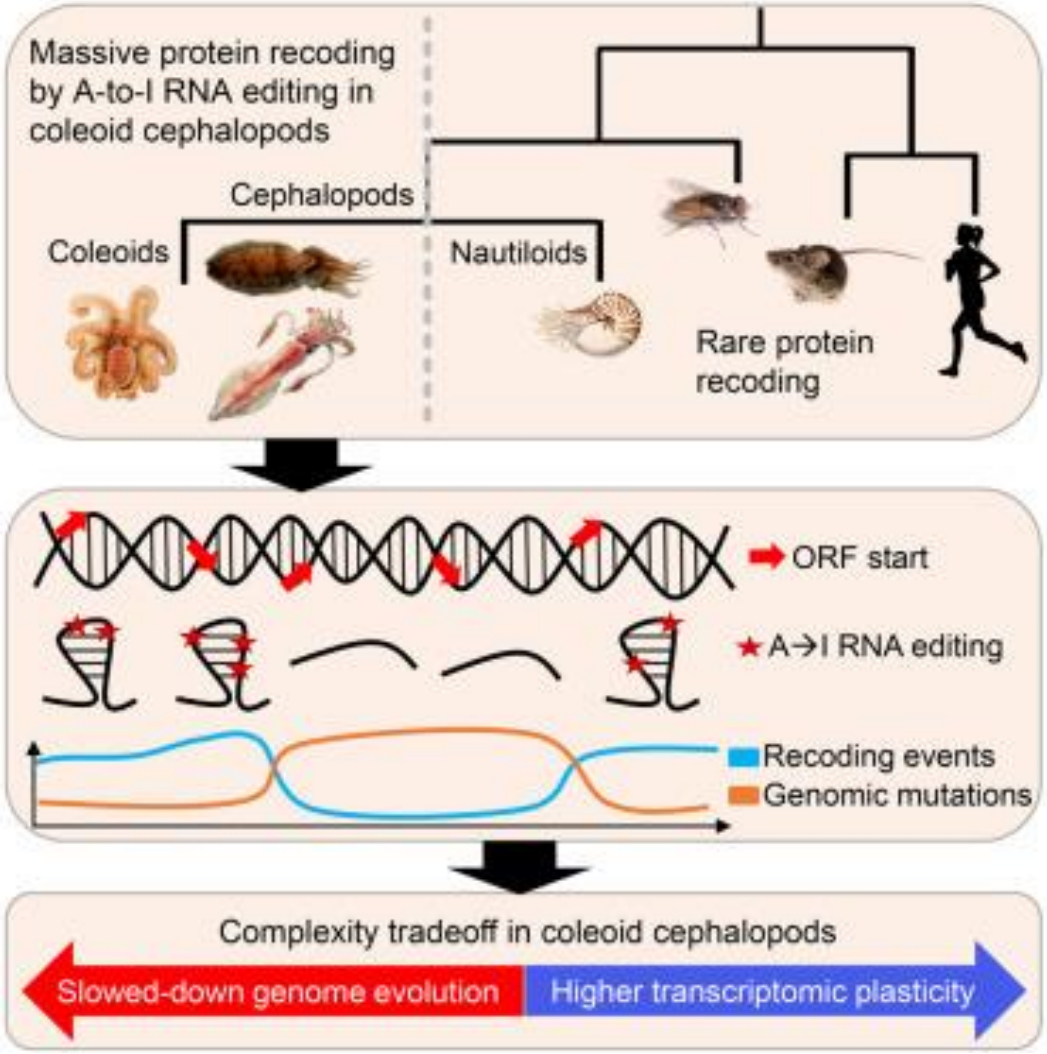
Shalev, RNA 2018

dsRNA triggers the innate immune system, leading to an interferon response

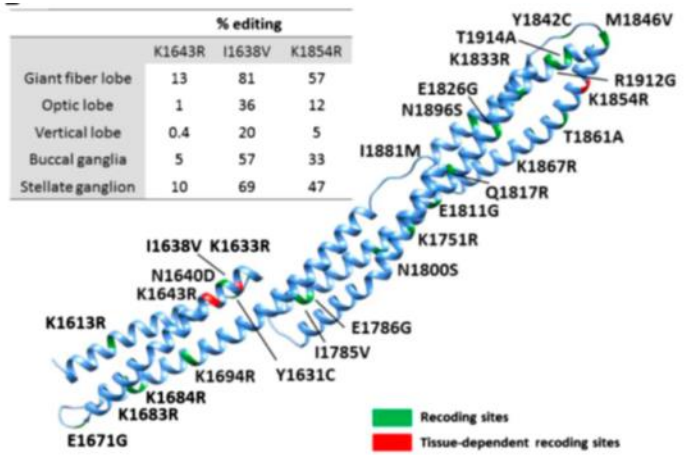


Zhou Yu et al. *Cell Research* 2015

Trade-off between Transcriptome Plasticity and Genome Evolution in Cephalopods

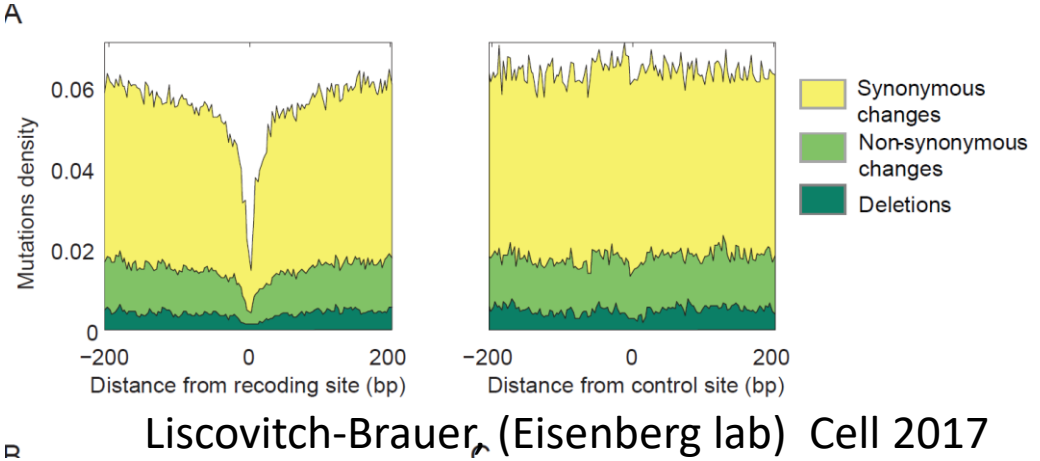


α Spectrin



	% editing		
	K1643R	I1638V	K1854R
Giant fiber lobe	13	81	57
Optic lobe	1	36	12
Vertical lobe	0.4	20	5
Buccal ganglia	5	57	33
Stellate ganglion	10	69	47

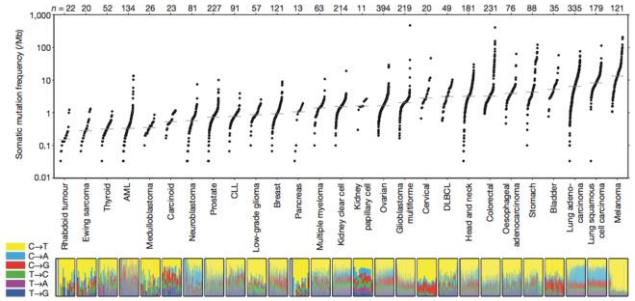
Alon et al elife 2015



Liscovitch-Brauer (Eisenberg lab) Cell 2017

In search for “mutations” at the RNA level

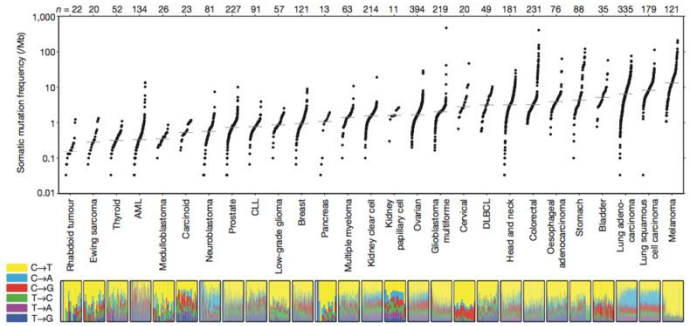
TCGA data



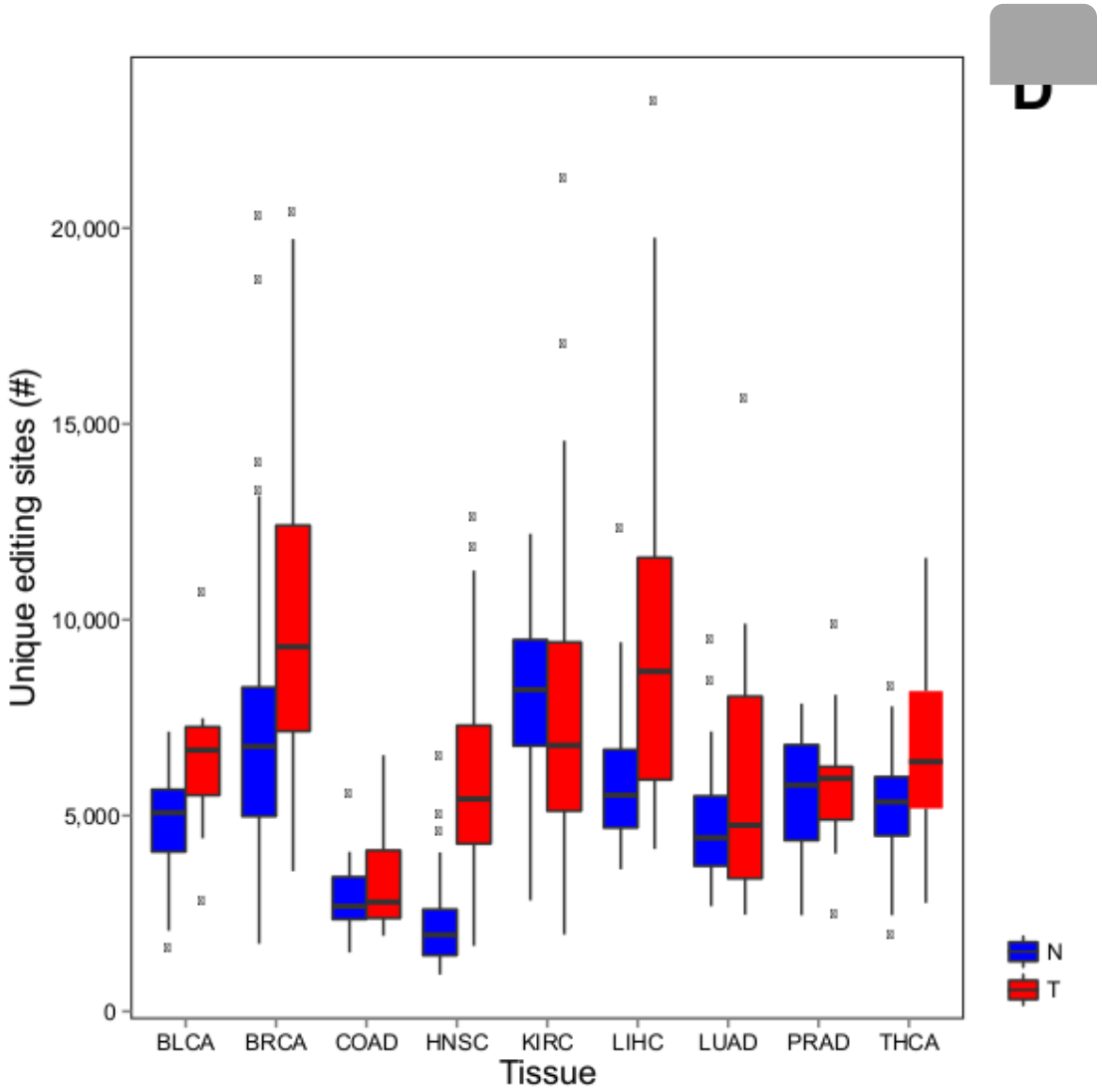
Cancer type		# tumors samples	# normal samples	# matched samples
BLCA	Bladder Urothelial Carcinoma	14	13	13
BRCA	Breast invasive carcinoma	95	96	95
COAD	Colon adenocarcinoma	137	18	18
HNSC	Head and Neck squamous cell carcinoma	33	30	29
KIRC	Kidney renal clear cell carcinoma	65	68	62
LIHC	Liver hepatocellular carcinoma	30	33	30
LUAD	Lung adenocarcinoma	266	38	36
PRAD	Prostate adenocarcinoma	37	35	31
THCA	Thyroid carcinoma	48	48	42
PAAD	Pancreatic adenocarcinoma	32	1	1
GBM	Glioblastomamultiforme	163	-	
Total matched		356 patients / 712 RNA-Seqs		

A-to-I RNA editing alteration in cancer

More editing sites in most cancer types tested (TCGA data, 712 matched samples)

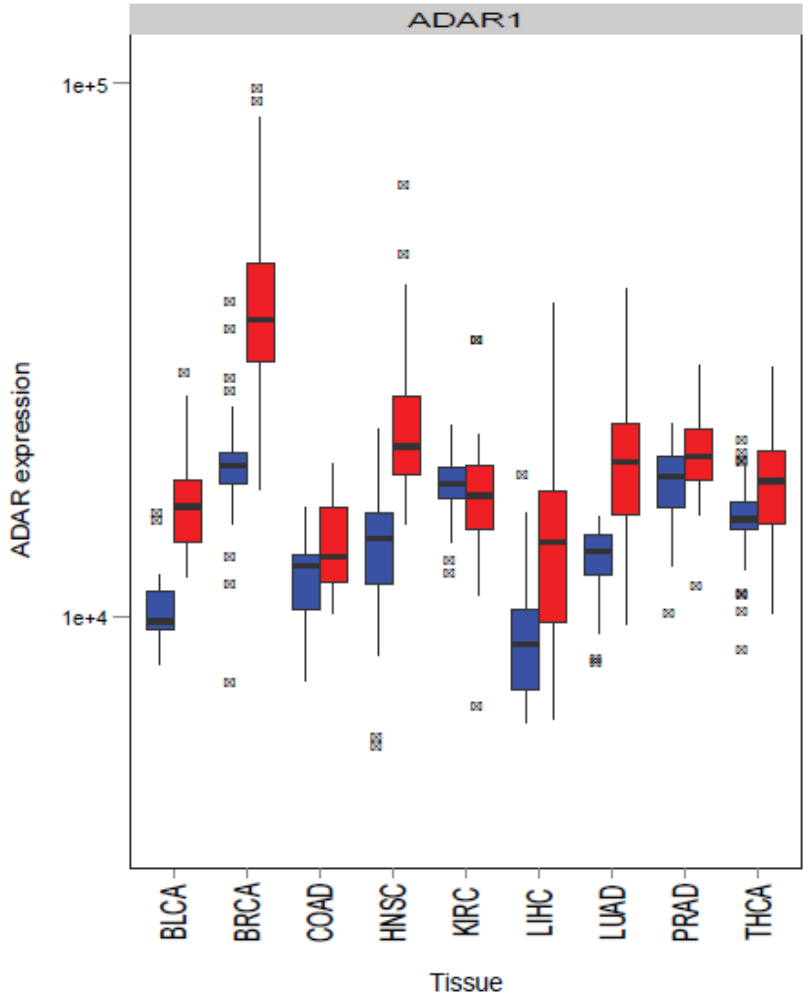


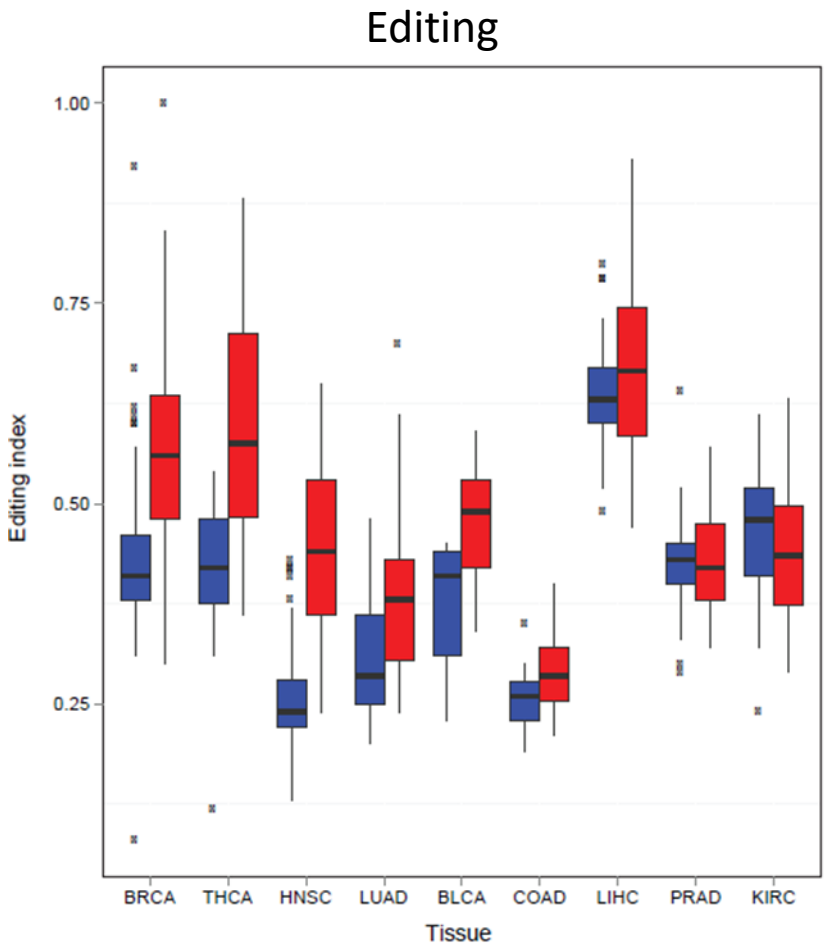
Same order of magnitude as somatic mutations

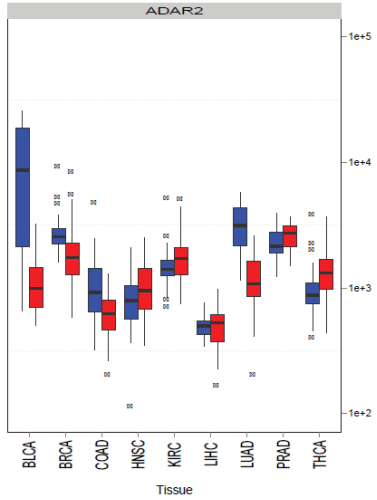


Paz-Yaakov, Cell Reports 2015

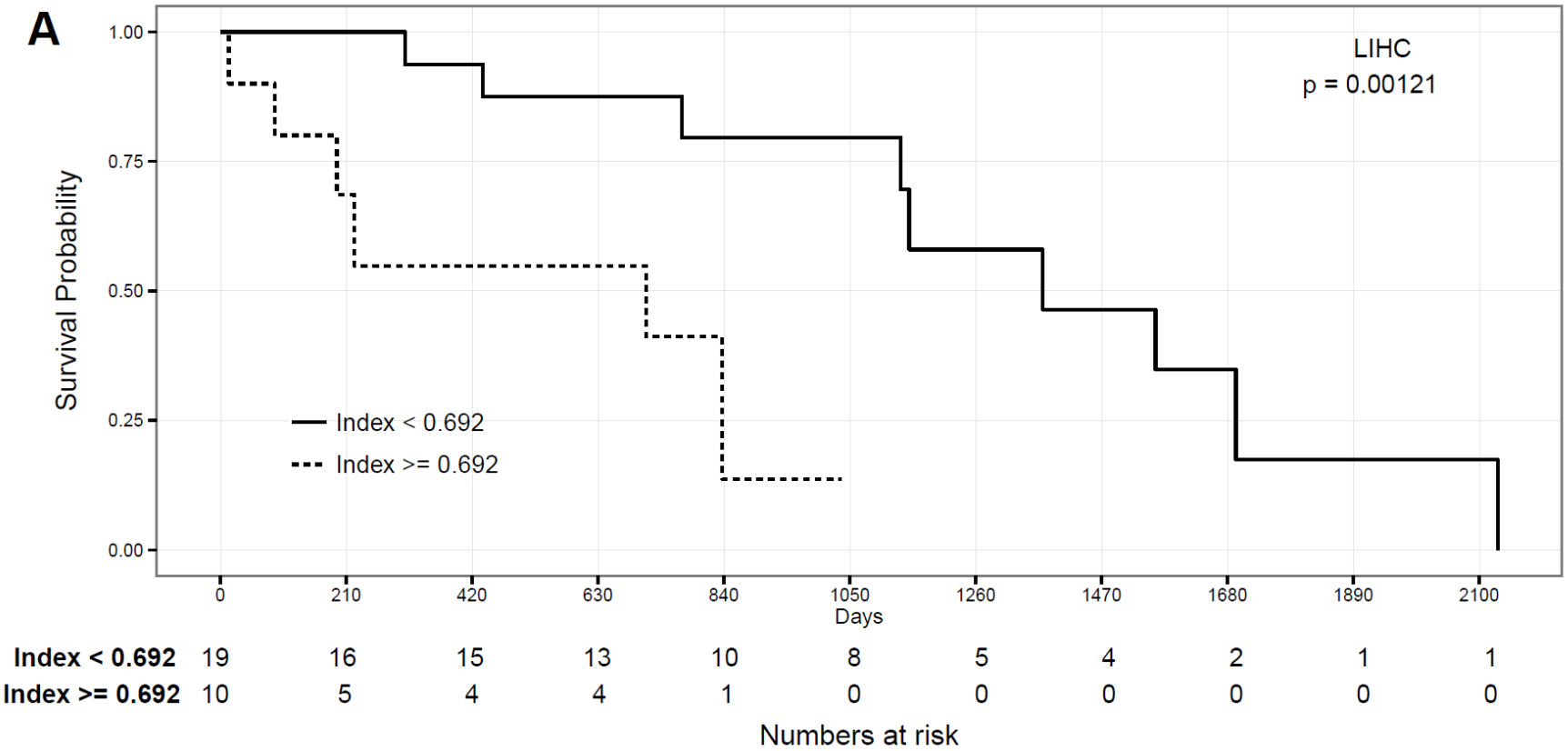
ADAR1 expression levels are elevated in cancer



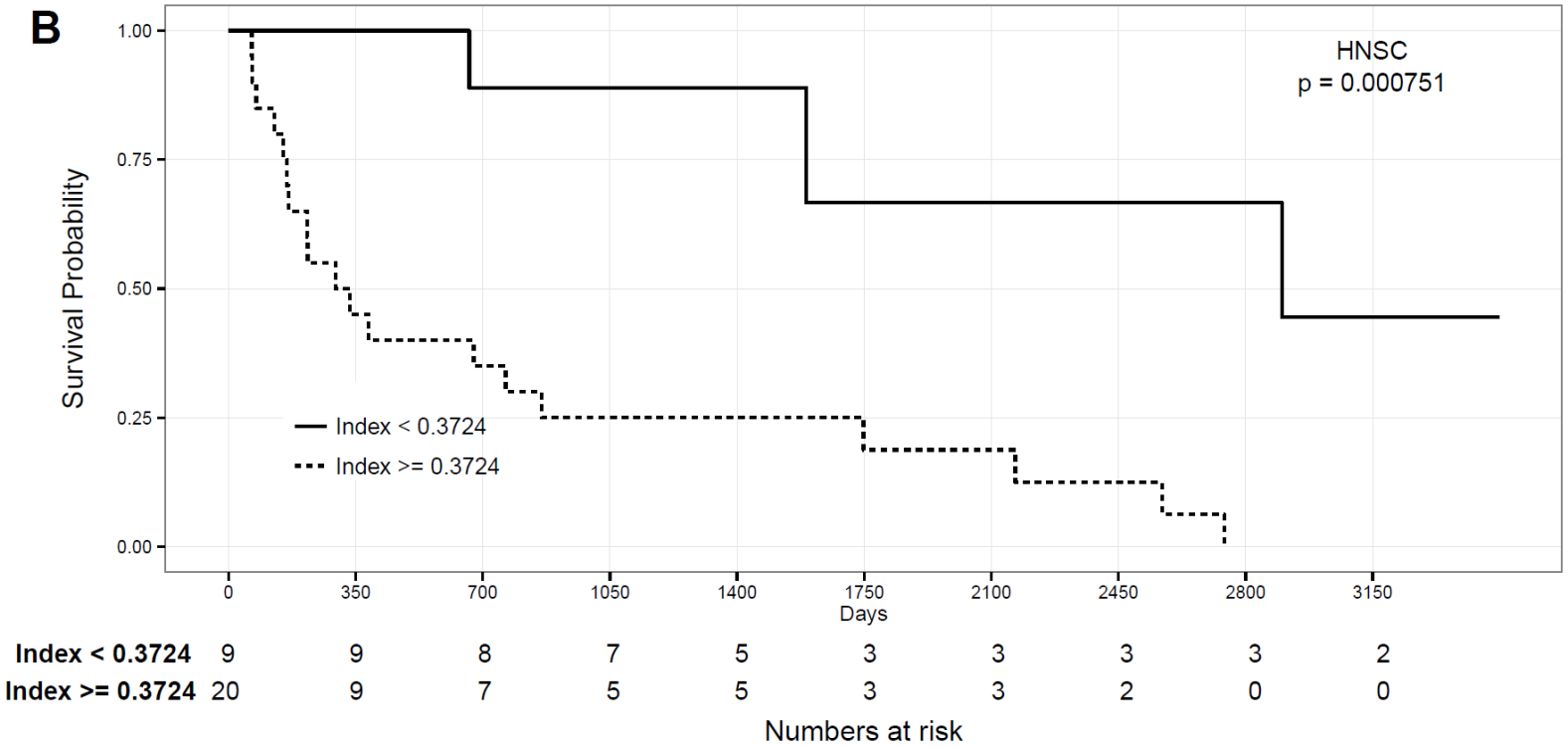




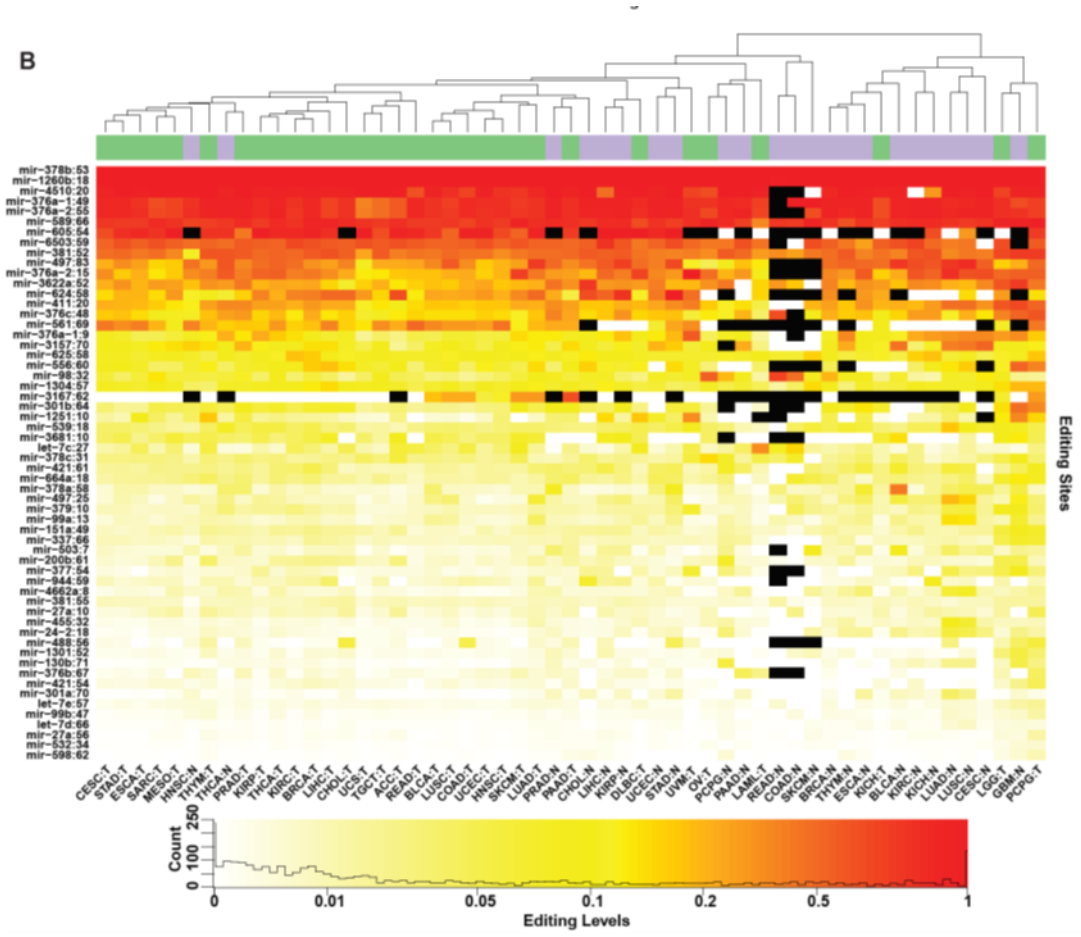
Editing is a potential prognostic predictor in liver hepatocellular carcinoma

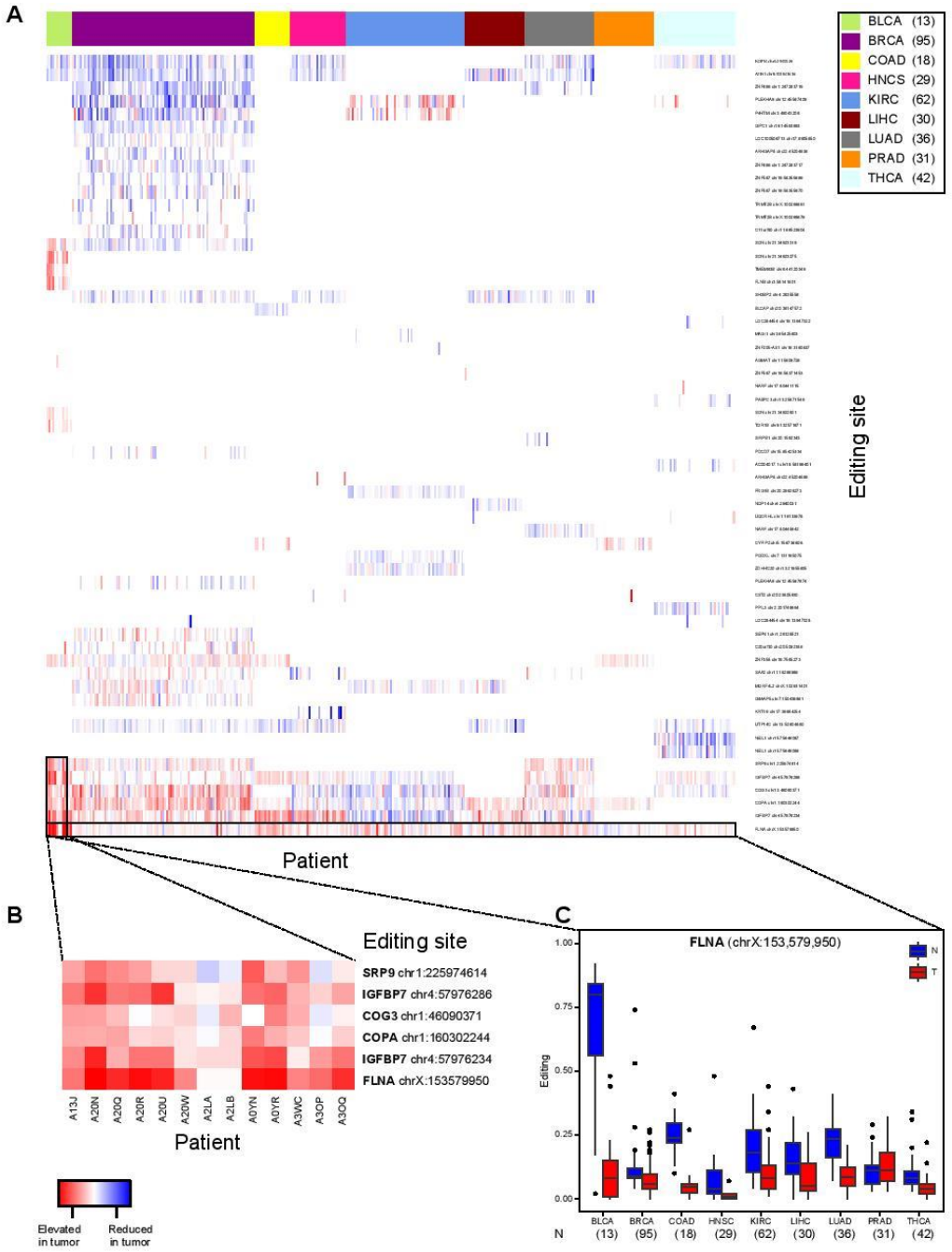


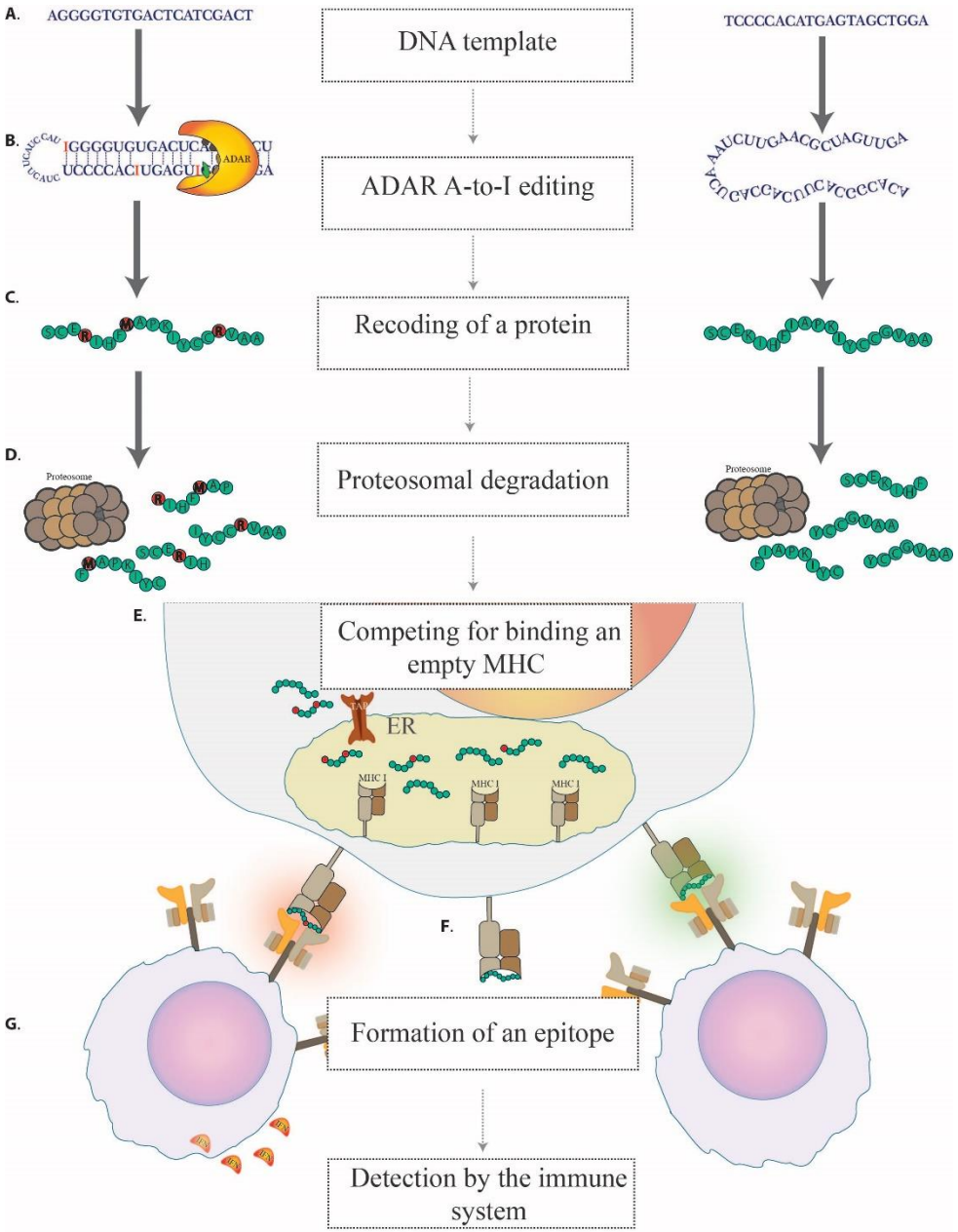
Prognostic predictor in head and neck tumors



Pan-cancer editing alteration (CDS and miRNA sites)



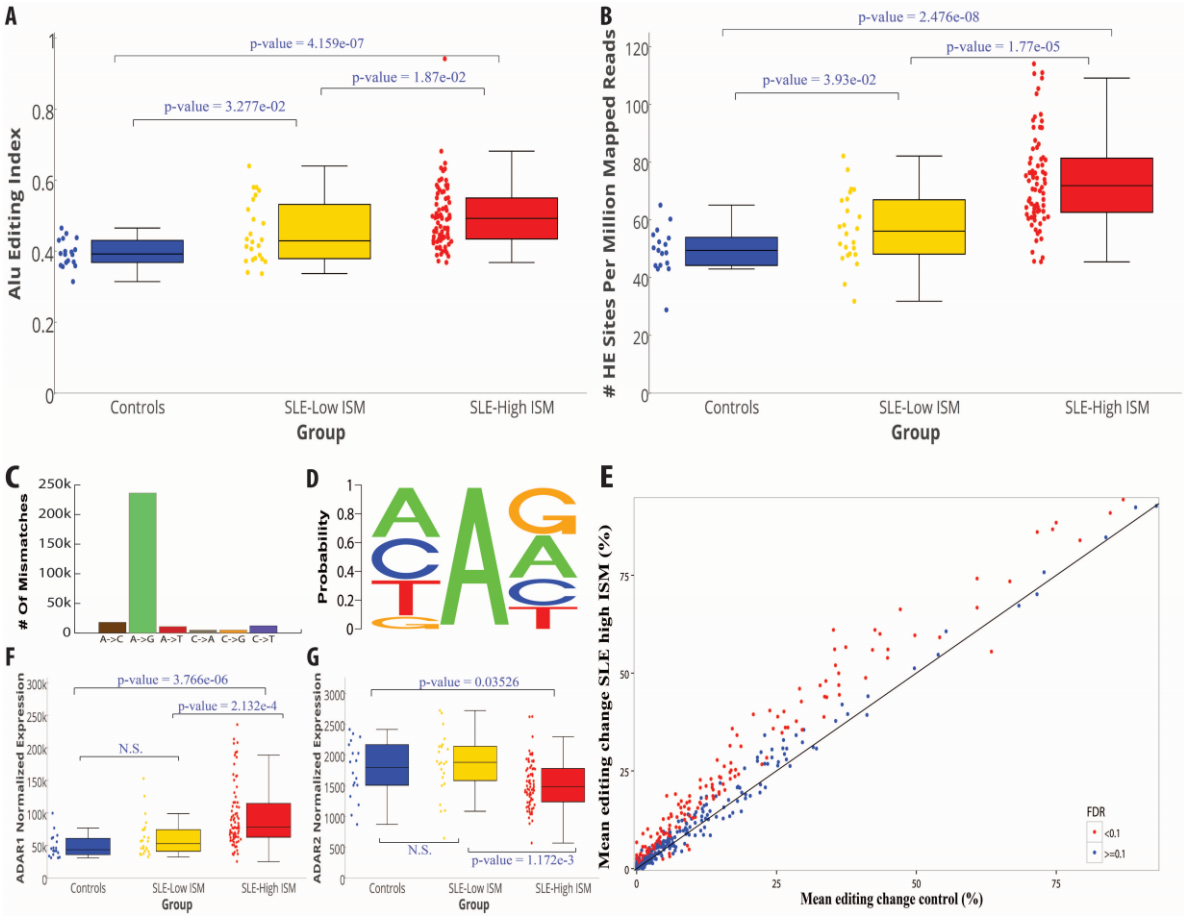




Extensive RNA editing increase immune self-representation diversity in medullary thymic epithelial cells

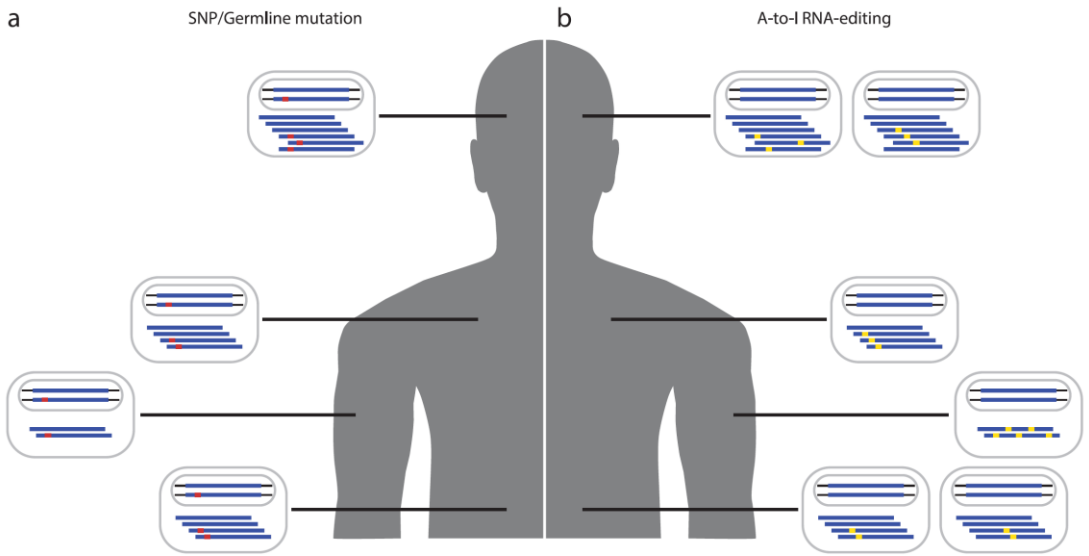
Danan-Gotthold *et al* Genome Biology 2016

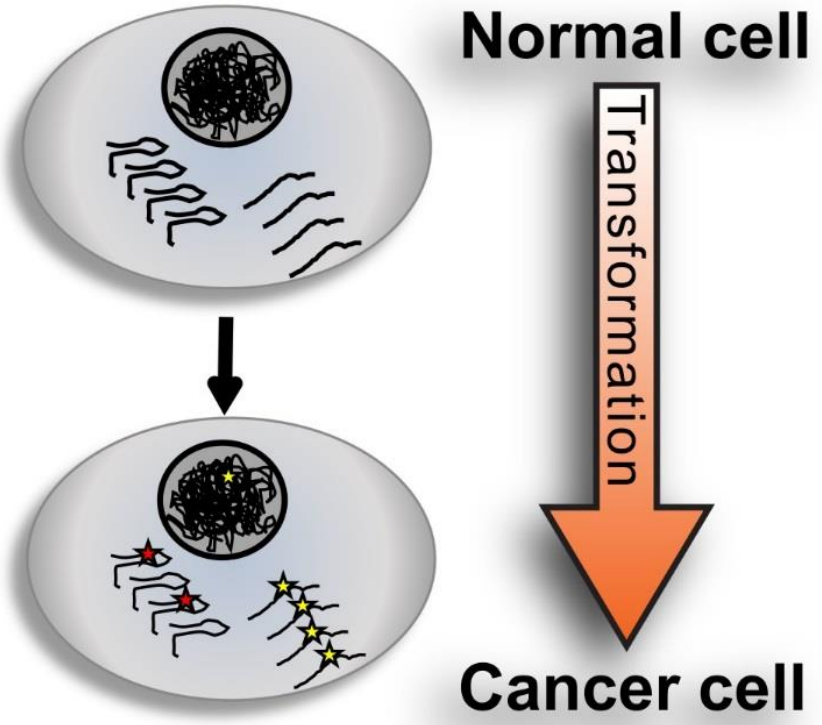
Increased A-to-I RNA editing provide a possible source for autoantigens in Systemic lupus erythematosus



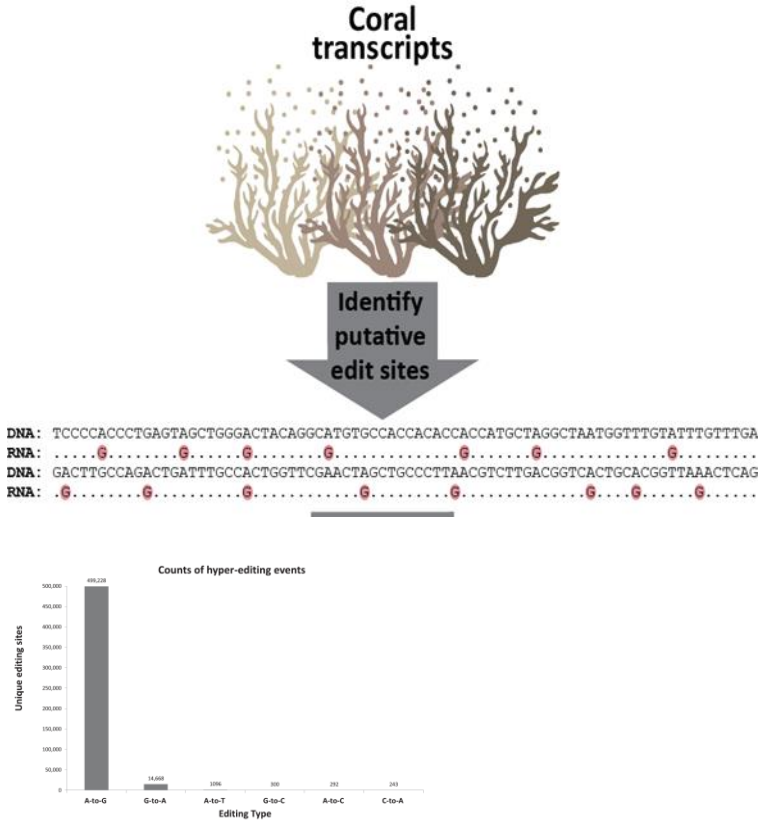
Roth, Cell Reports 2018

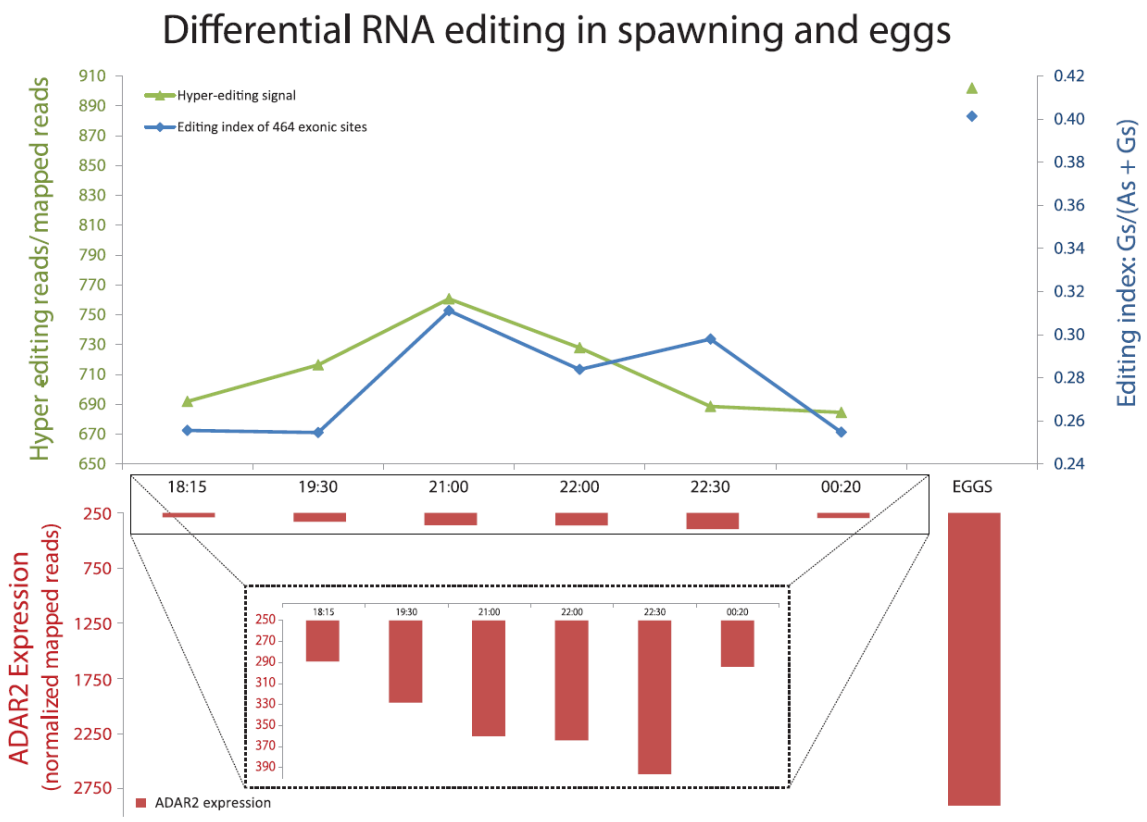
RNA editing vs Mutations





Editing in Corals





Porath et al MBE 2017

Summary

- A-to-I editing of repetitive elements is abundant in metazoans
- Editing in repeats strongly depends on the repeats repertoire
- Editing as a new type of “RNA mutations” in cancer
- Alterations of editing activity can be involved with autoimmune diseases

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