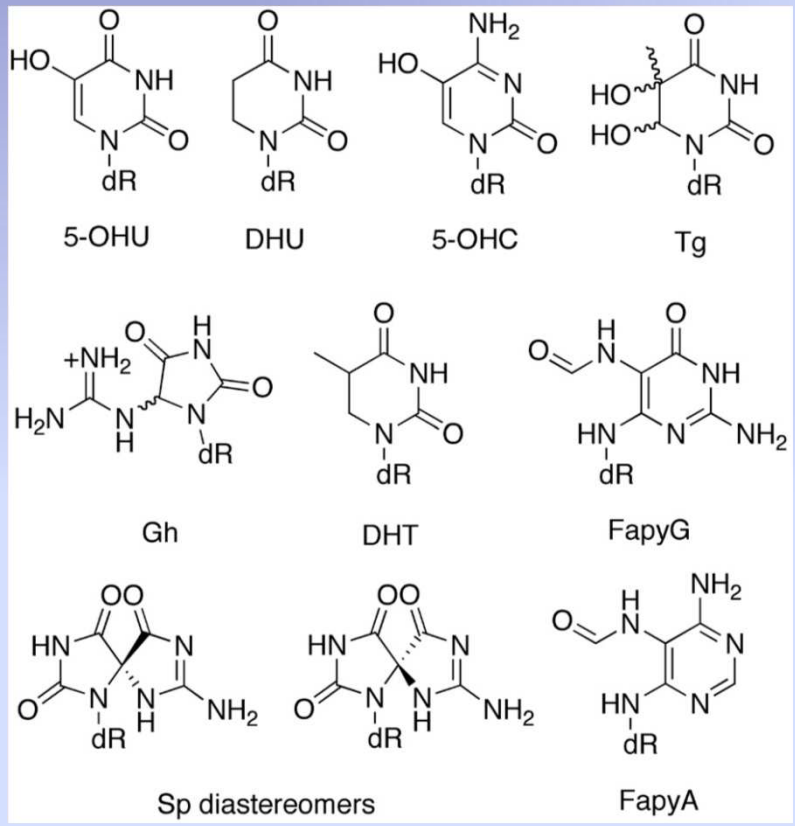
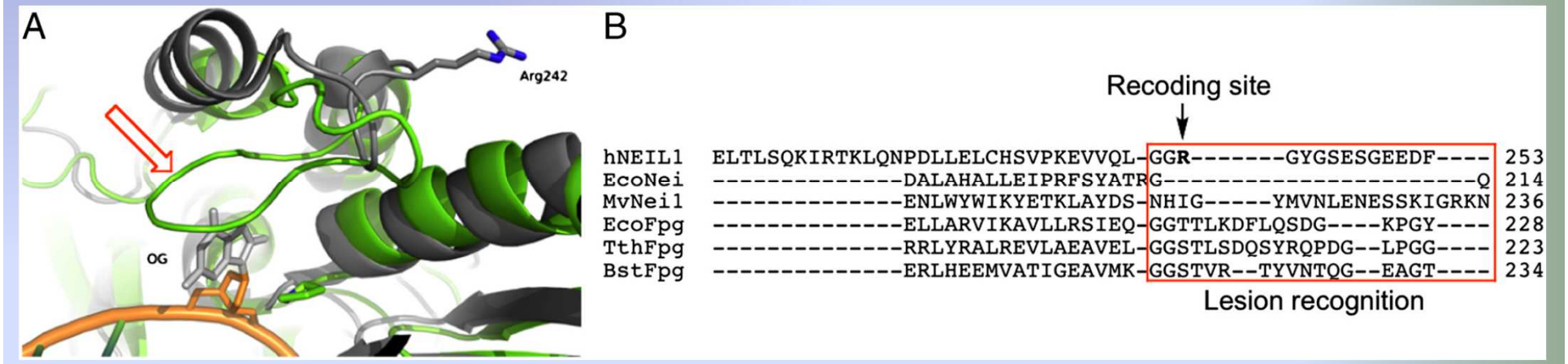
Un caso «speciale» di BER
Neil1

Known substrates for the base excision repair glycosylase NEIL1



NEIL1 plays a key role in the initiation of base excision repair of oxidized base lesions by catalyzing the cleavage of the N-glycosidic linkage to the 2’-deoxyribose

(A) Superposition of human NEIL1 structure (**dark gray**) with that of E. coli Fpg (**green**) bound to 8-oxoguanine-containing DNA. **Red open arrow** indicates lesion recognition loop



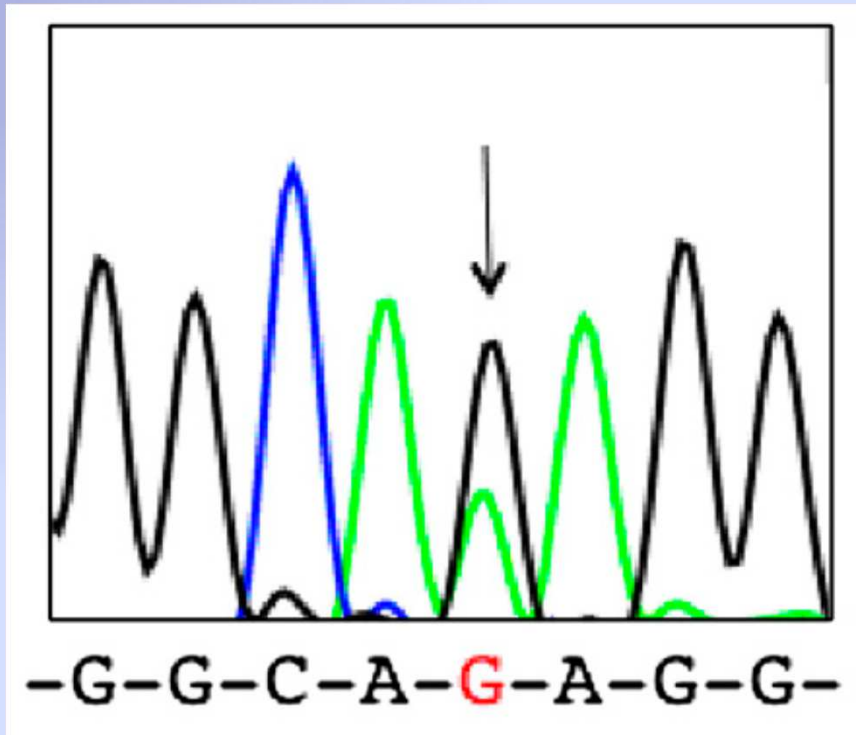
B) Sequence alignment of Fpg/Nei family of DNA repair glycosylases indicating the position of the hNEIL1 lesion recognition loop

- RNA editing changes the lesion specificity for the DNA repair enzyme NEIL1

Whole transcriptome sequence analysis from various human tissues identified over 200 possible A to I editing sites in non repeat sequences,

A site predicted to cause recoding in the mRNA for the DNA repair enzyme NEIL1 (lysine 242 AAA codon edited to AIA codon for arginine)

NEIL1 mRNA sequencing

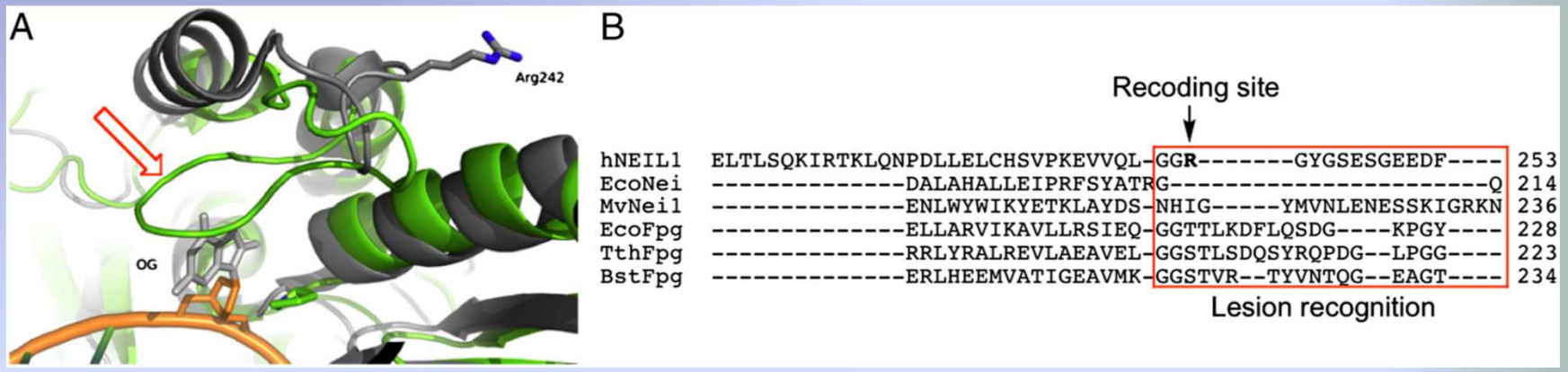


mRNA Editing

A to G

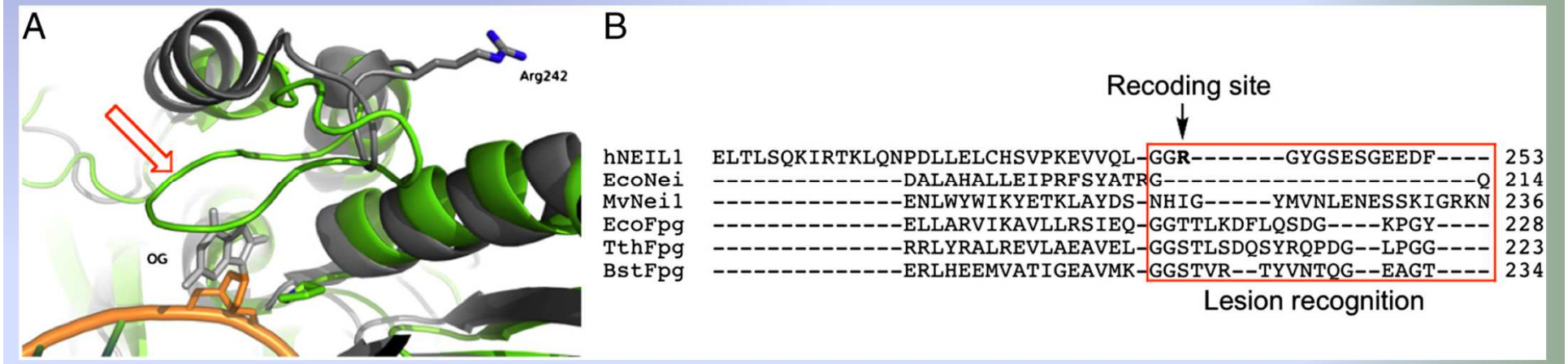
AAA to AIA (AGA)

R to K



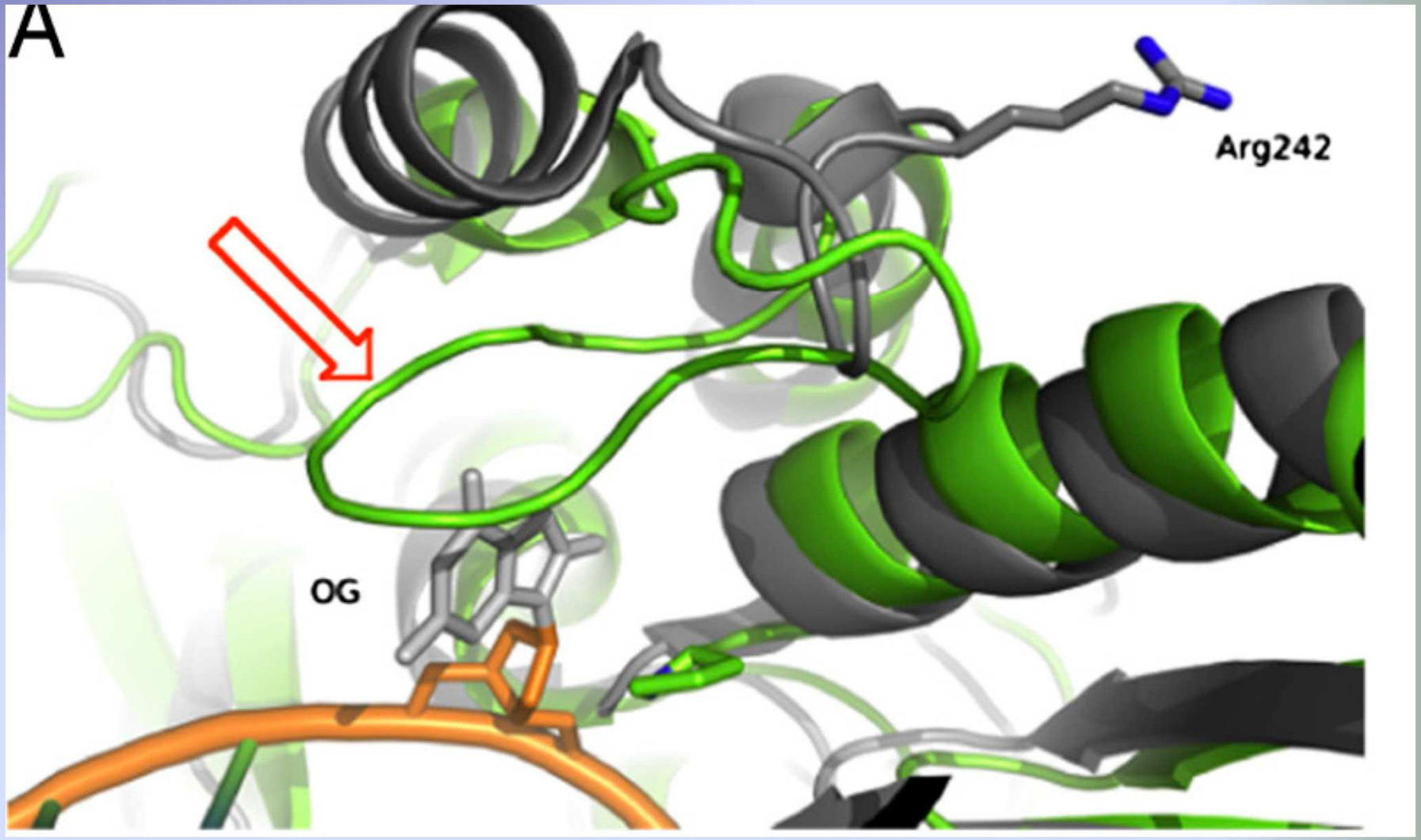
Editing of the pre-mRNA for the DNA repair enzyme NEIL1 causes a lysine to arginine change in the lesion recognition loop

(A) Superposition of human NEIL1 structure (**dark gray**) with that of E. coli Fpg (**green**) bound to 8-oxoguanine-containing DNA. **Red open arrow** indicates lesion recognition loop

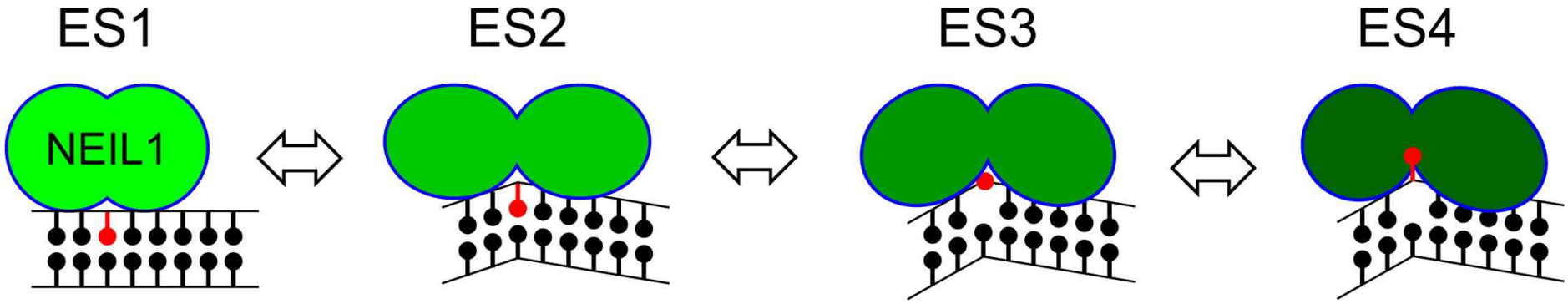


B) Sequence alignment of Fpg/Nei family of DNA repair glycosylases indicating the position of the hNEIL1 lesion recognition loop

and recoding site????

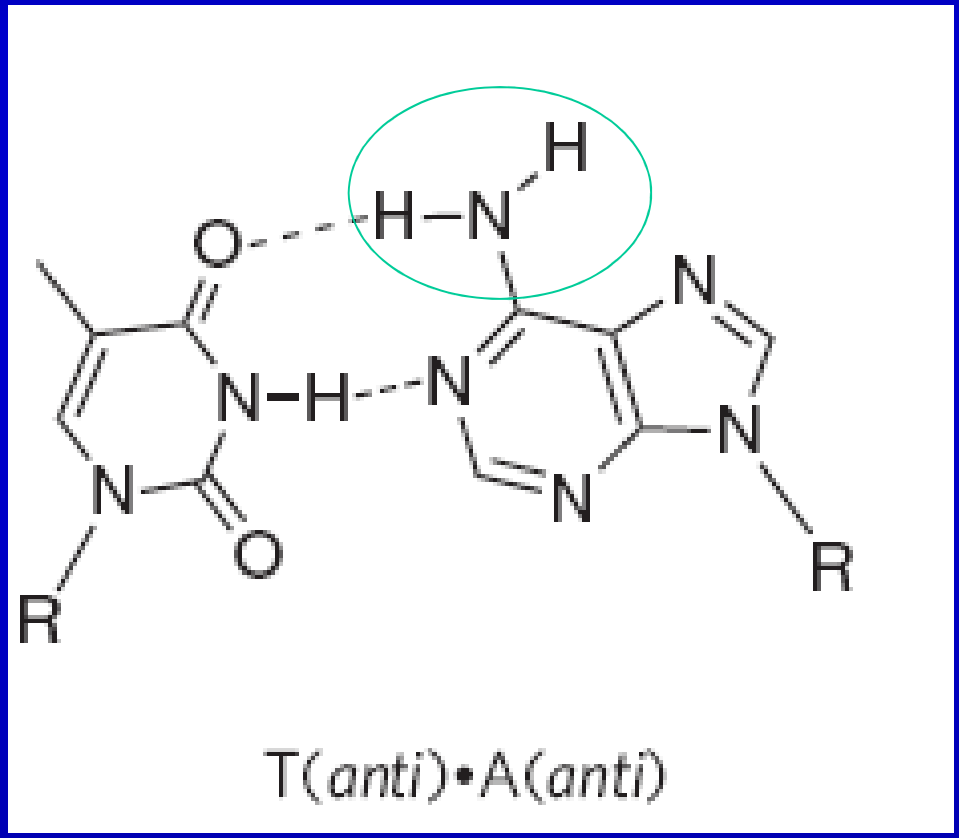


Superposition of human NEIL1 structure (dark gray) with that of E. coli Fpg (OGG1, green) bound to 8-oxoguanine-containing DNA.



Conformational Dynamics of Neil1

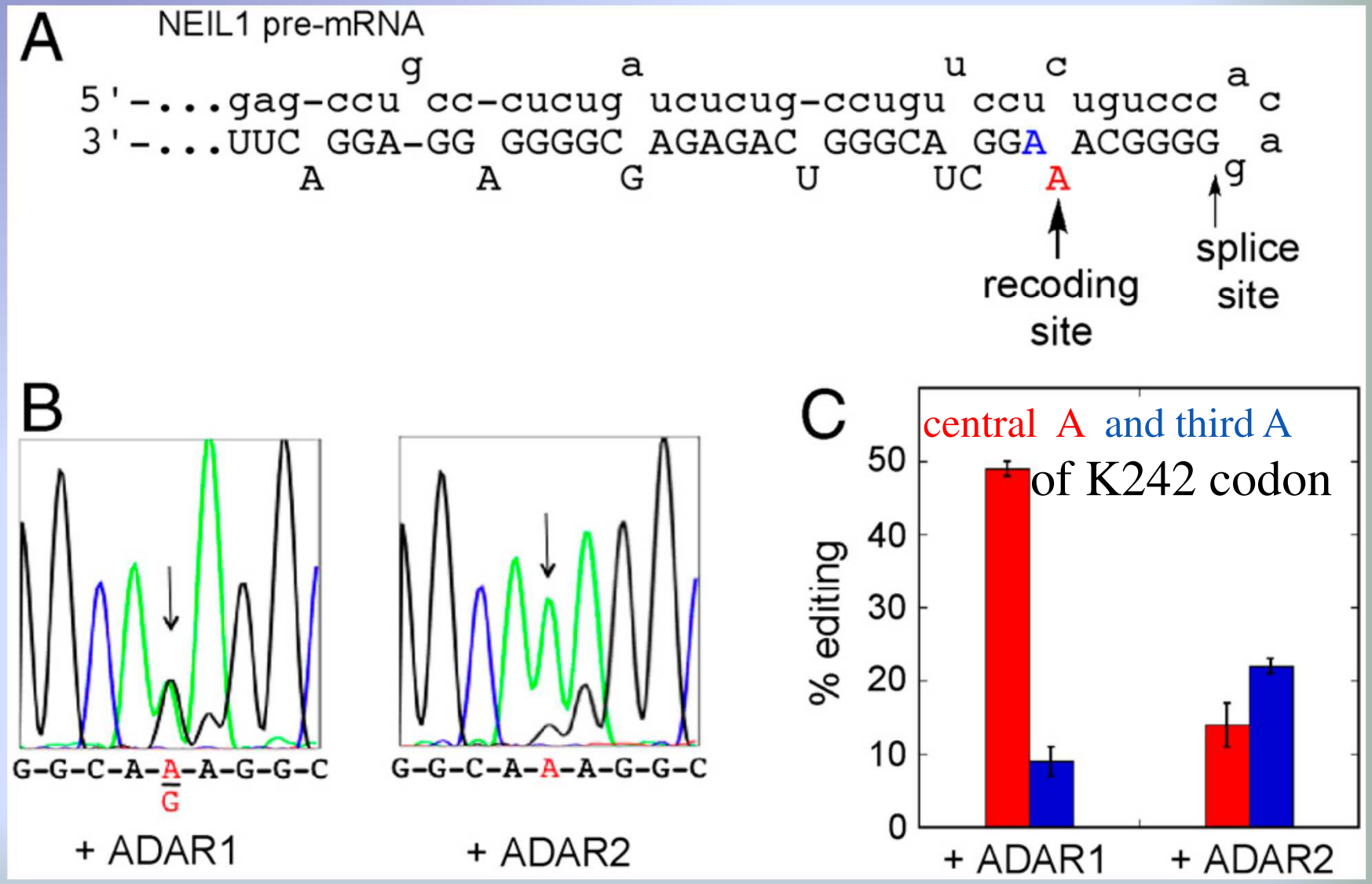
J Mol Biol. 2019 Mar 15;431(6):1098-1112.



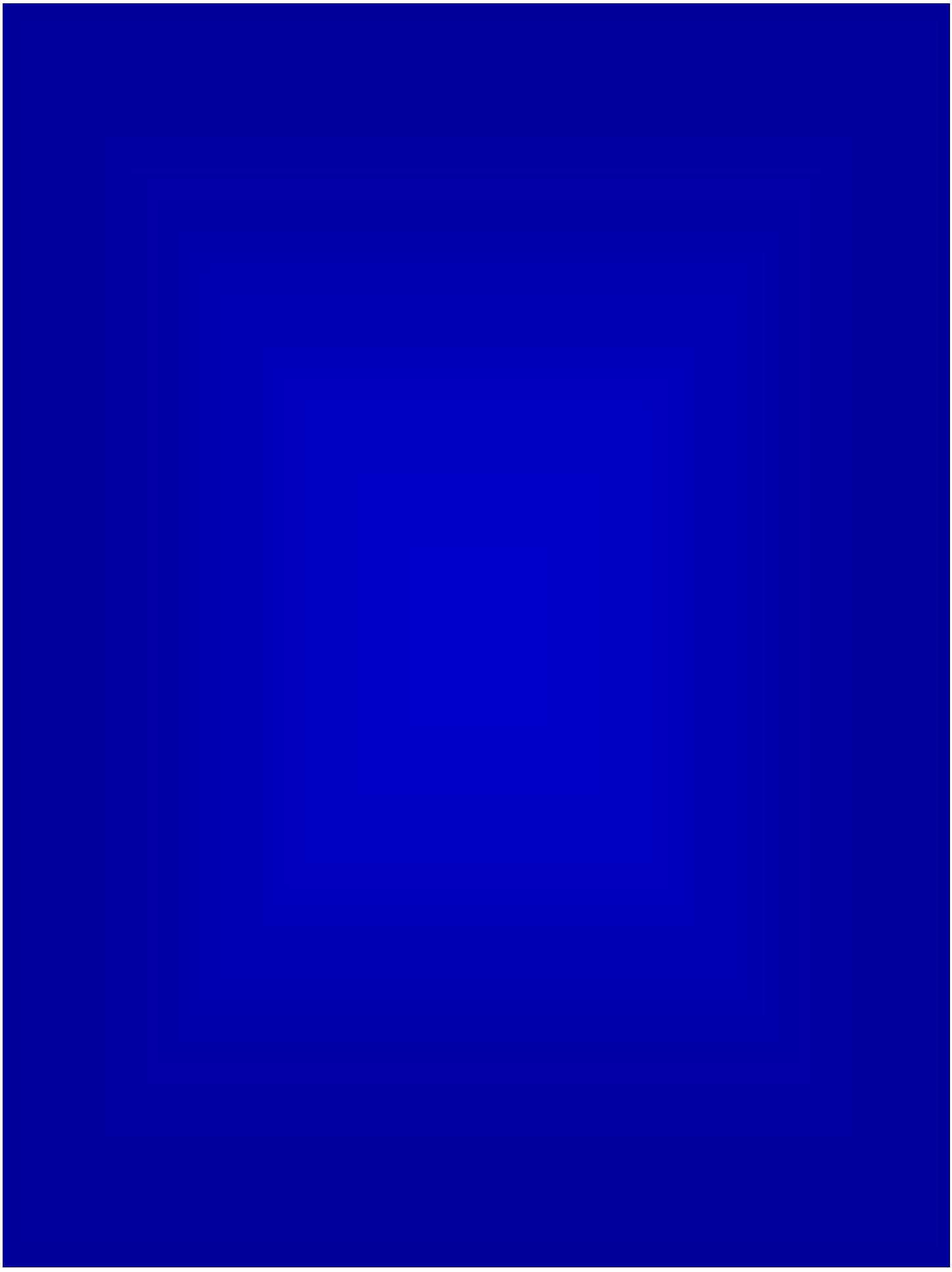
T(*anti*)•A(*anti*)

ADAR catalizza la deaminazione ossidativa di specifiche adenine

In vitro editing: Sequence of products from reaction of 1 μM human ADAR



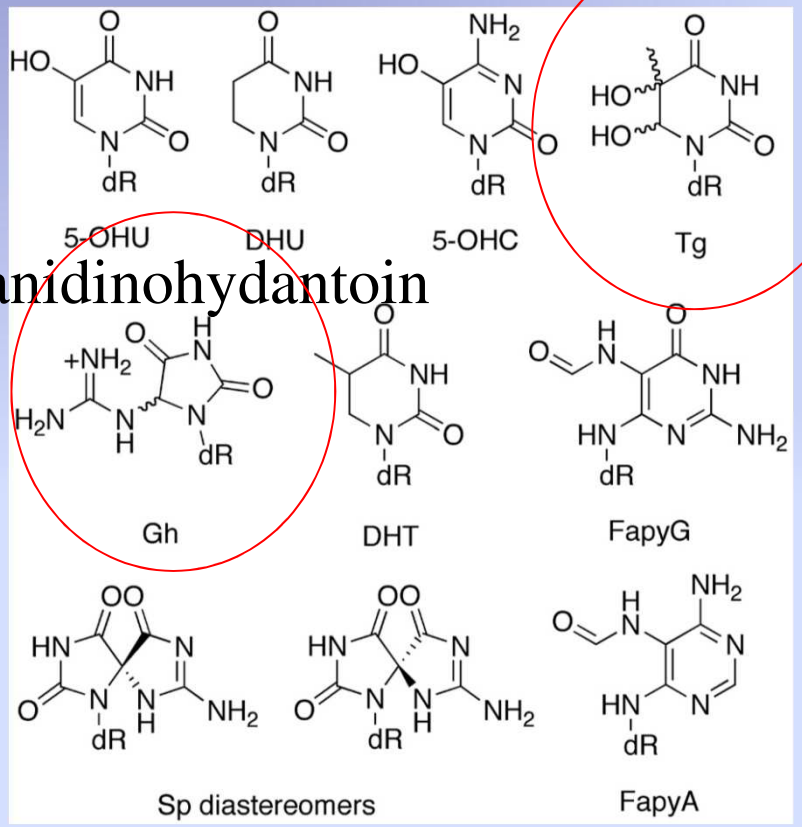
Editing of the pre-mRNA for the DNA repair enzyme NEIL1 causes a lysine to arginine change in the lesion recognition loop of the protein.



Known substrates for the base excision repair glycosylase NEIL1.

thymine glycol

guanidinohydantoin



Duplex	5′ – TGT TCA TCA TGG GTC XTC GGT ATA TCC CAT – 3′ 3′ – ACA AGT AGT ACC CAG GAG CCA TAT AGG GTA – 5′
Single-Strand	5′ – TGT TCA TCA TGG GTC XTC GGT ATA TCC CAT – 3′
Bubble	TCXT G C 5′ – TGT TCA TCA TGC GGT ATA TCC CAT – 3′ 3′ – ACA AGT AGT ACG CCA TAT AGG GTA – 5′ A T TTCT
Bulge	X 5′ – TGT TCA TCA TGC GTC TC GGT ATA TCC CAT – 3′ 3′ – ACA AGT AGT ACG CAG – AG CCA TAT AGG GTA – 5′

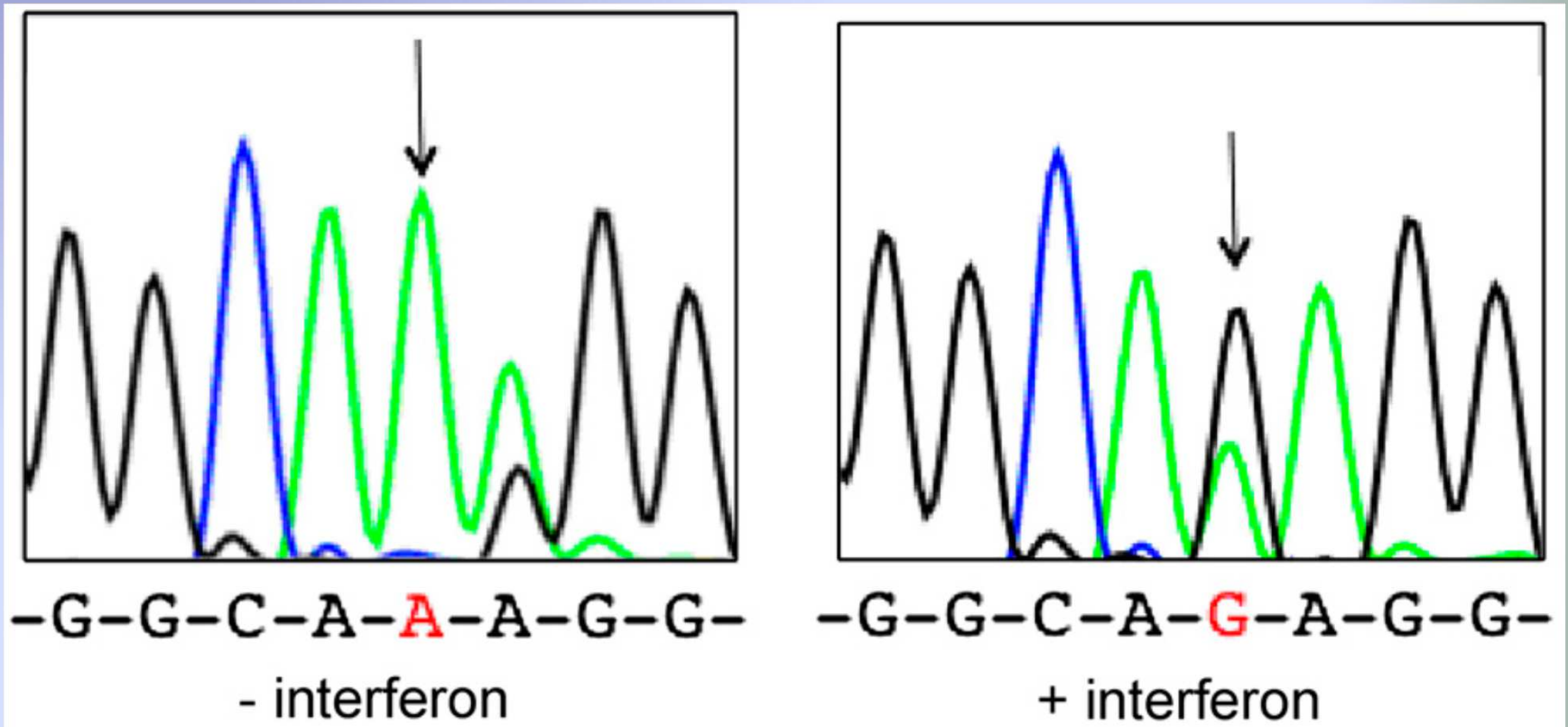
X = Gh, Sp, or Tg

Table 1. Rate constants (k_g)* of base removal by edited versus unedited NEIL1

	Tg†			Gh‡		
Context§	Unedited	Edited	Ratio¶	Unedited	Edited	Ratio
Duplex (X: G)	76 ± 10	2.5 ± 0.1	30	130 ± 20	370 ± 40	0.4
Single strand	0.6 ± 0.1	0.02 ± 0.01	30	1.2 ± 0.1	2.4 ± 0.6	0.5
Bulge	1.4 ± 0.1	0.04 ± 0.02	35	5.0 ± 0.6	13 ± 1	0.4
Bubble	1.2 ± 0.1	0.06 ± 0.02	20	30 ± 6	94 ± 8	0.3

*Rate constants in min^{-1} measured under single-turnover conditions (20 nM substrate, 200 nM enzyme) at 37 °C. Reactions wi
go to completion; slow reactions rates were determined based on initial rate rather than complete fitting of the progres
†Tg paired with G. Rate constants in the same duplex paired with A for edited and unedited NEIL1 are 1.3 ± 0.1 min^{-1}, and 53
ratio is 40.

- The two forms of NEIL1 have distinct enzymatic properties.
- The edited form removes thymine glycol Tg from duplex DNA 30 times more slowly than the form encoded in the genome,
- whereas editing enhances repair of the guanidinohydantoin Gh lesion by NEIL1.



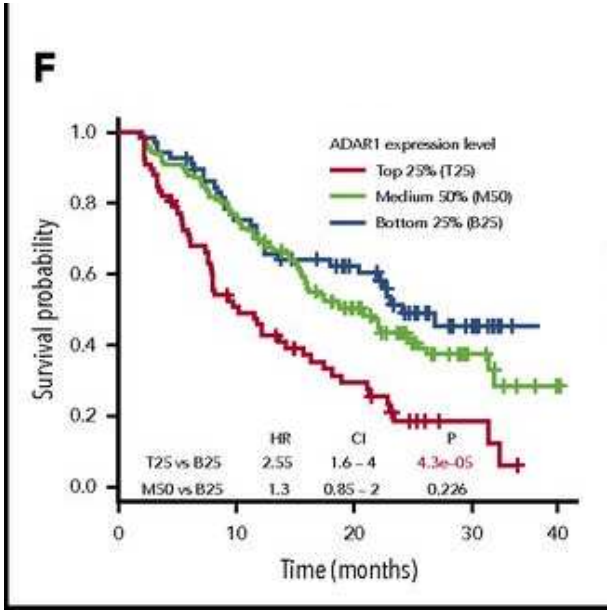
NEIL1 editing in response to IFN-α.
(Left) Sequence at the recoding site in NEIL1 cDNA from U87 human glioblastoma cells cultured in the absence of IFN-α.
(Right) NEIL1 cDNA sequence from U87 cells treated with IFN-α.

Sommario Neil 1

- ADAR1-catalyzed editing of the NEIL1 mRNA causes the genomically encoded AAA lysine codon, corresponding to amino acid position 242 in the lesion recognition loop of the protein, to be converted to a codon for Arg.
- The two forms of the NEIL1 protein (edited and unedited) have distinct enzymatic properties with changes observed for both glycosylase activity and lesion specificity.
- Editing occurs in a hairpin duplex structure formed near the intron 5/exon 6 boundary in the NEIL1pre-mRNA.

- Furthermore, NEIL1 mRNA recoding is regulated extracellularly by interferon, as predicted for an ADAR1-catalyzed reaction.
- These results suggest a regulatory mechanism for DNA repair based on RNA editing.

ADAR1 overexpression is an independent marker of death

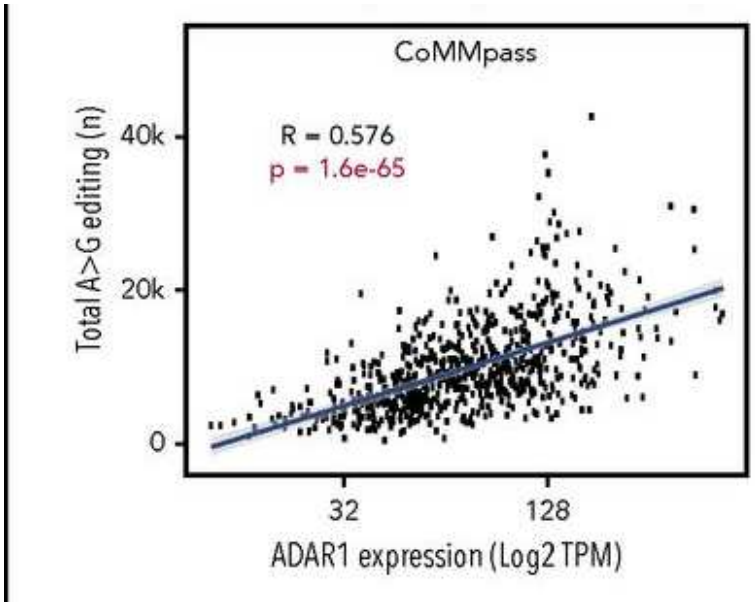


Kaplan-Meier curve - overall survival of MM, multiple myeloma patients

Phaik Ju Teoh et al. Blood 2018;132:1304-1317

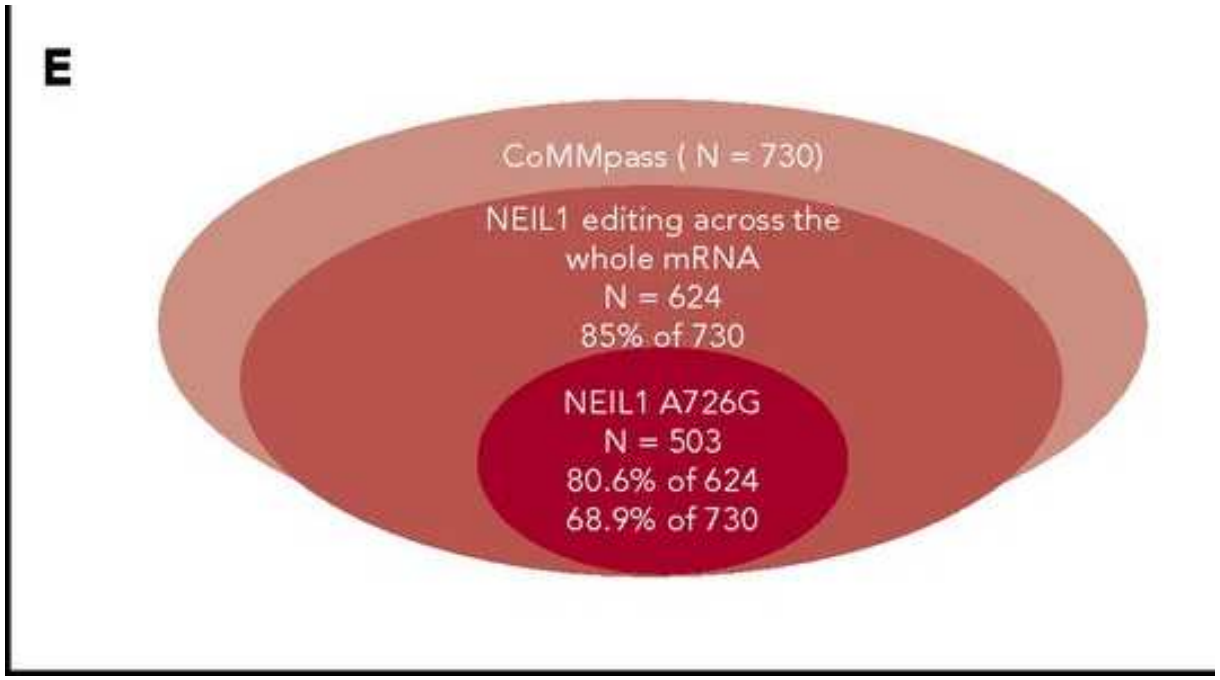
Multiple Mieloma transcriptome is hyperedited and directly regulated by ADAR1

Correlation of global A-to-G editing events with ADAR1 expression



Phaik Ju Teoh et al. Blood 2018;132:1304-1317

NEIL1 is an important editing target of ADAR1 in MM.

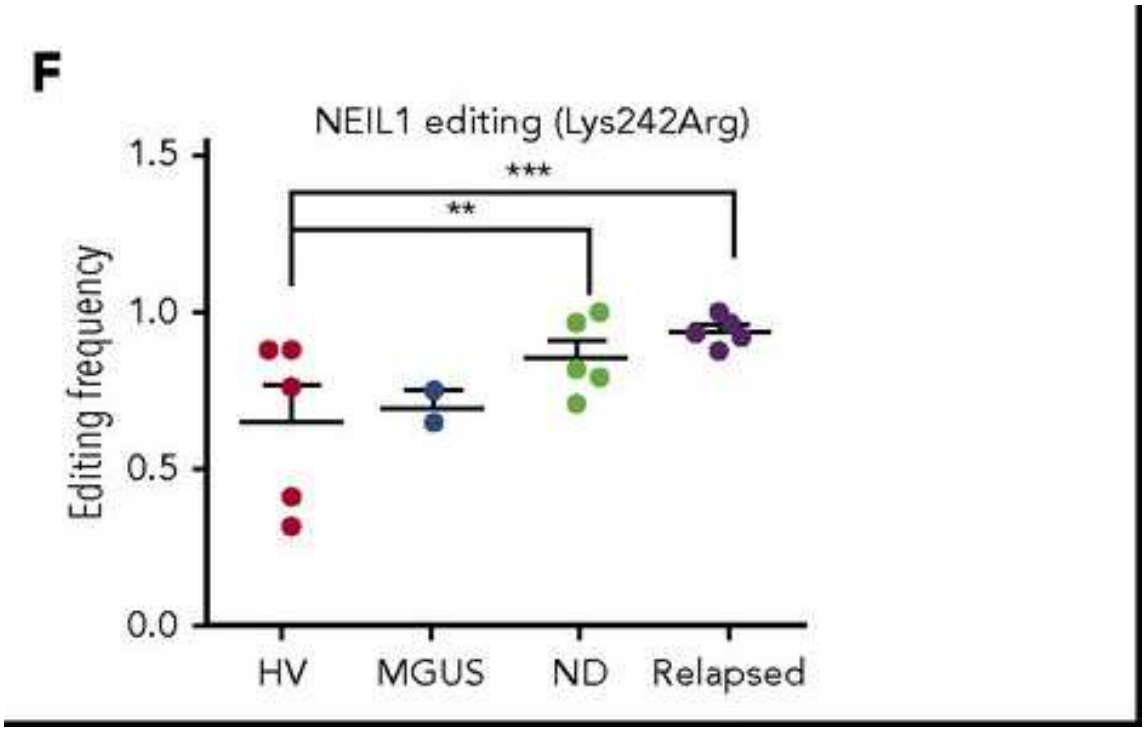


NEIL1 editing across the whole mRNA and at the specific recoding site (A726G)

Phaik Ju Teoh et al. Blood 2018;132:1304-1317

NEIL1 is an important editing target of ADAR1 in MM

The frequency of NEIL1 editing (A726G) in the transcripts of patients from different disease stages. **P < .05; ***P < .001.

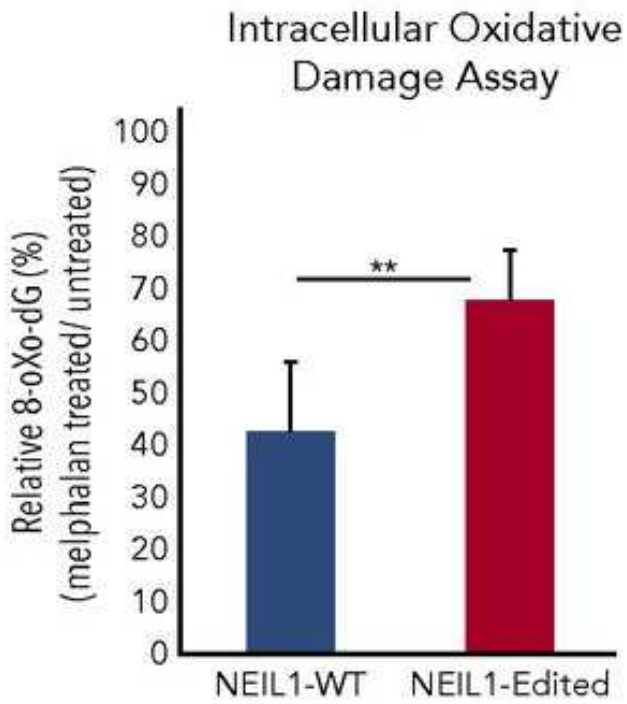


HV, healthy volunteer;
MGUS, monoclonal gammopathy of undetermined significance
ND, newly diagnosed;;

Phaik Ju Teoh et al. Blood 2018;132:1304-1317

Edited-NEIL1 has reduced ability to repair DNA damage after Malfalan Treatment

ELISA quantifying oxidative damage (8-hydroxy-2-deoxyguanosine) in the untreated and melphalan-treated NEIL1-WT and NEIL1-edited cells



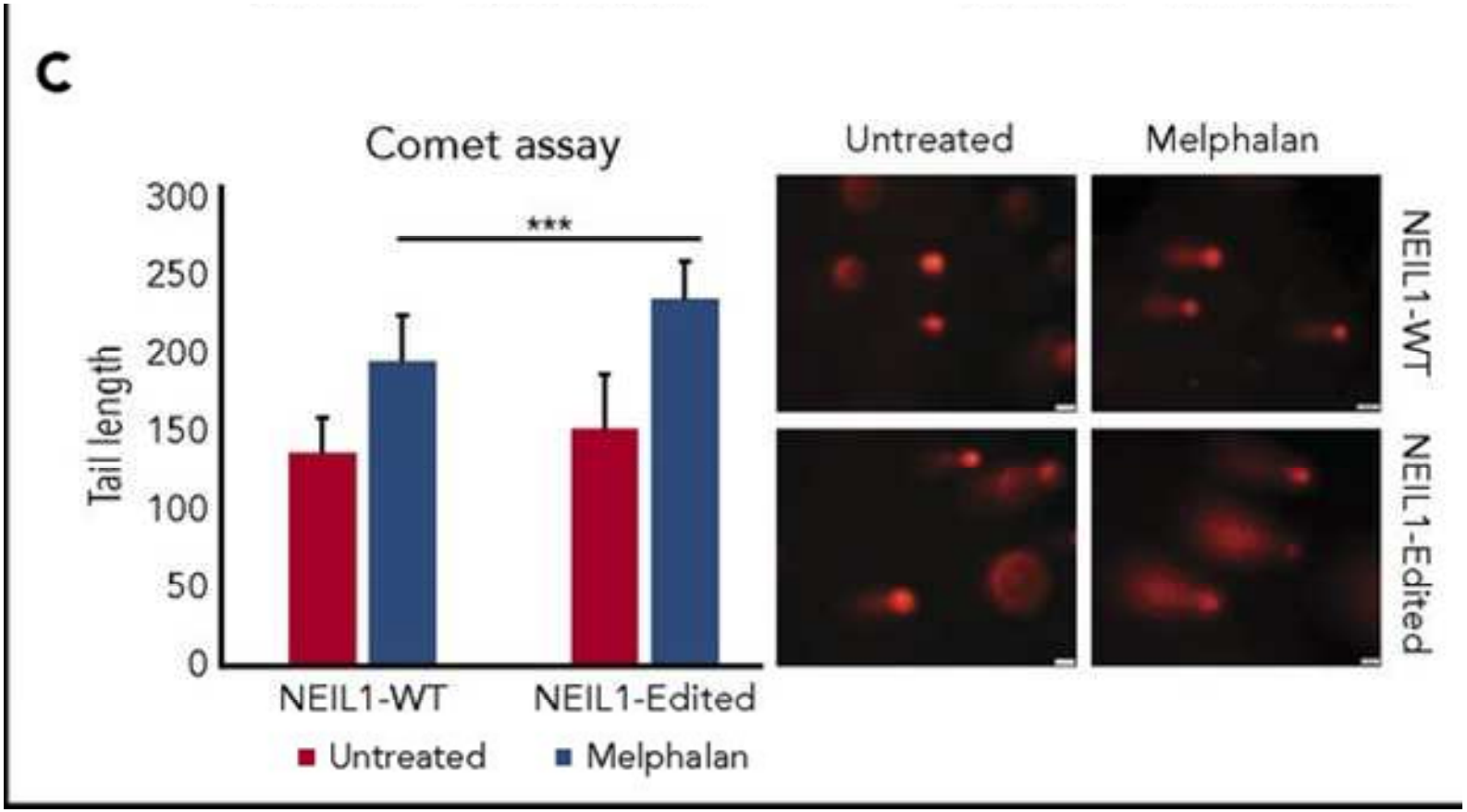
Phaik Ju Teoh et al. Blood 2018;132:1304-1317

Melfalan

phenylalanine derivative with antineoplastic activity.
Melphalan alkylates DNA at the N7 position of guanine and induces DNA inter-strand cross-linkages, resulting in the inhibition of DNA and RNA synthesis and cytotoxicity against both dividing and non-dividing tumor cells.

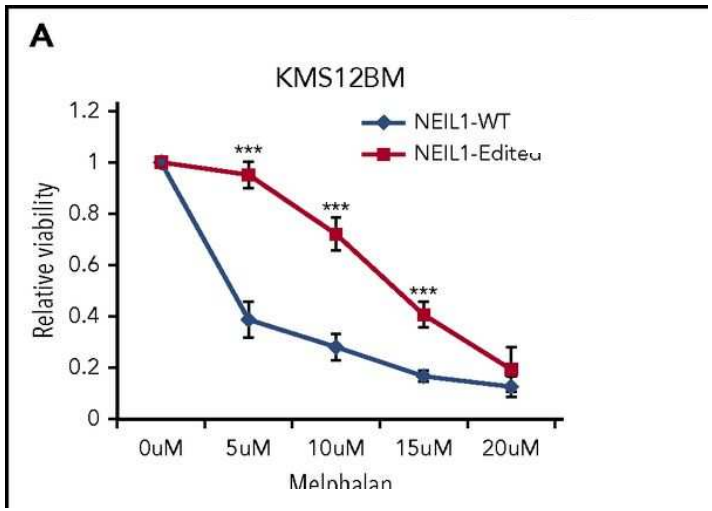
Il melfalan è un agente chemioterapico appartenente alla classe dei farmaci cosiddetti alchilanti, sostanze che esercitano un'azione tossica a livello cellulare (per questo sono definite citotossiche), provocando in tal modo la morte delle cellule neoplastiche

Edited-NEIL1 compromised DNA-damage repair capability.



Comet assay of untreated and melphalan-treated cells.
Assay was performed in an alkaline condition for specific detection of single-stranded DNA breaks.

Phaik Ju Teoh et al. Blood 2018;132:1304-1317



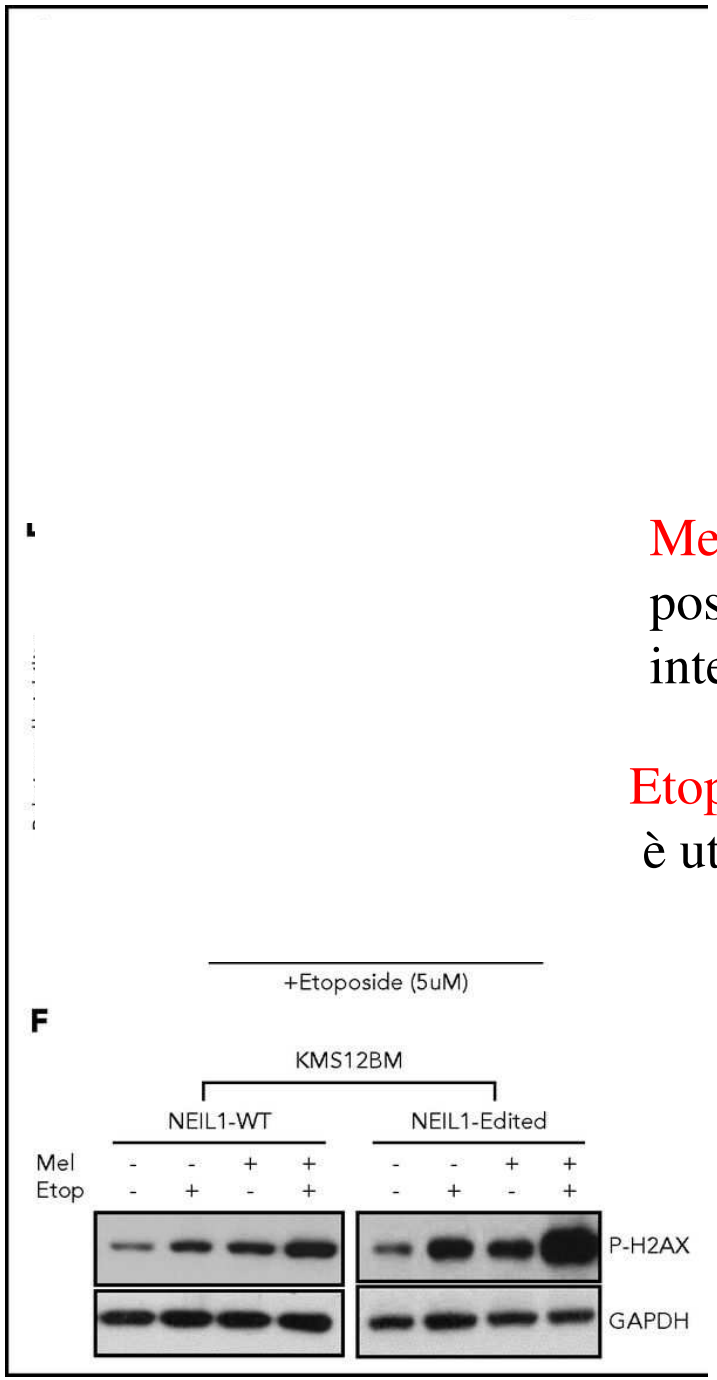
Cell viability of NEIL1-edited cells is higher when treated with increased doses of melphalan for 48 hours

KMS12BM=Linea cellulare Mieloma Multiplo

Melphalan= alkylates DNA at the N7 position of guanine and induces DNA inter-strand cross-linkages

Phaik Ju Teoh et al. Blood 2018;132:1304-1317

NEIL1-editing enhances double-stranded DNA damage repair responses.



Melphalan= alkylates DNA at the N7 position of guanine and induces DNA inter-strand cross-linkages

Etoposide= inibitore della Topoisomerasi II è utilizzato come antineoplastico

Western blot analysis of phospho-H2AX expression after the cells were subjected to either etoposide, melphalan or a combination of both **P < .05; ***P < .001.

Phaik Ju Teoh et al. Blood 2018;132:1304-1317

PARADOSSO

With a compromised DNA damage repair function one would expect NEIL1-edited cells to be more sensitive to DNA-damaging drugs (Melfalan)

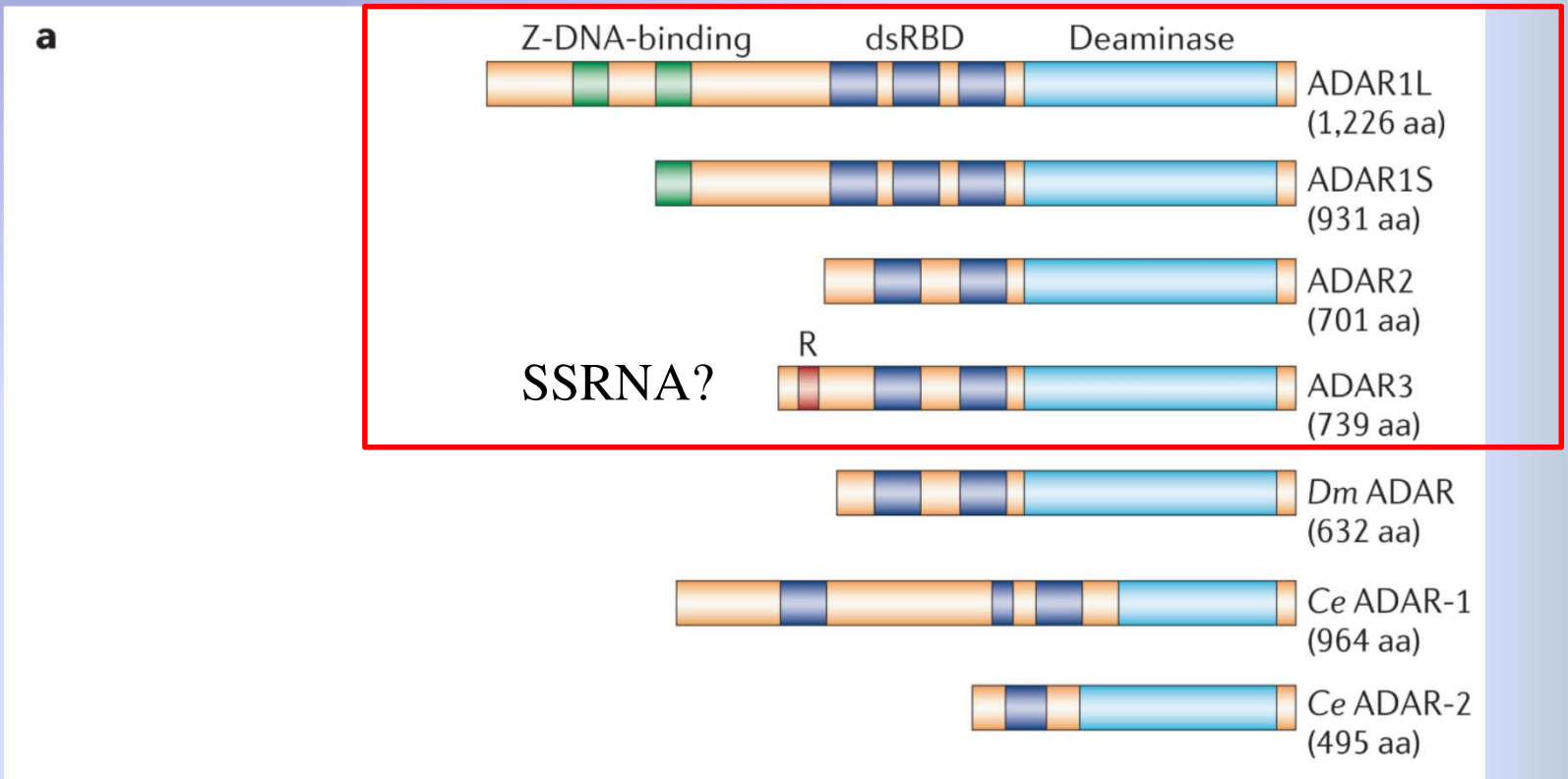
Ironically,we repeatedly saw them to be moreresistant to melphalan than the NEIL1-WT cells!!

IPOTESI

This led us to hypothesize that the suboptimal single-stranded DNA breaks repair in the NEIL1-edited cells predisposed them to double-stranded DNA breaks (DSB)

Activation of the DSB repair pathway would promote cell growth and survival.

Three human ADAR
(adenosine deaminase acting on RNA)-family members

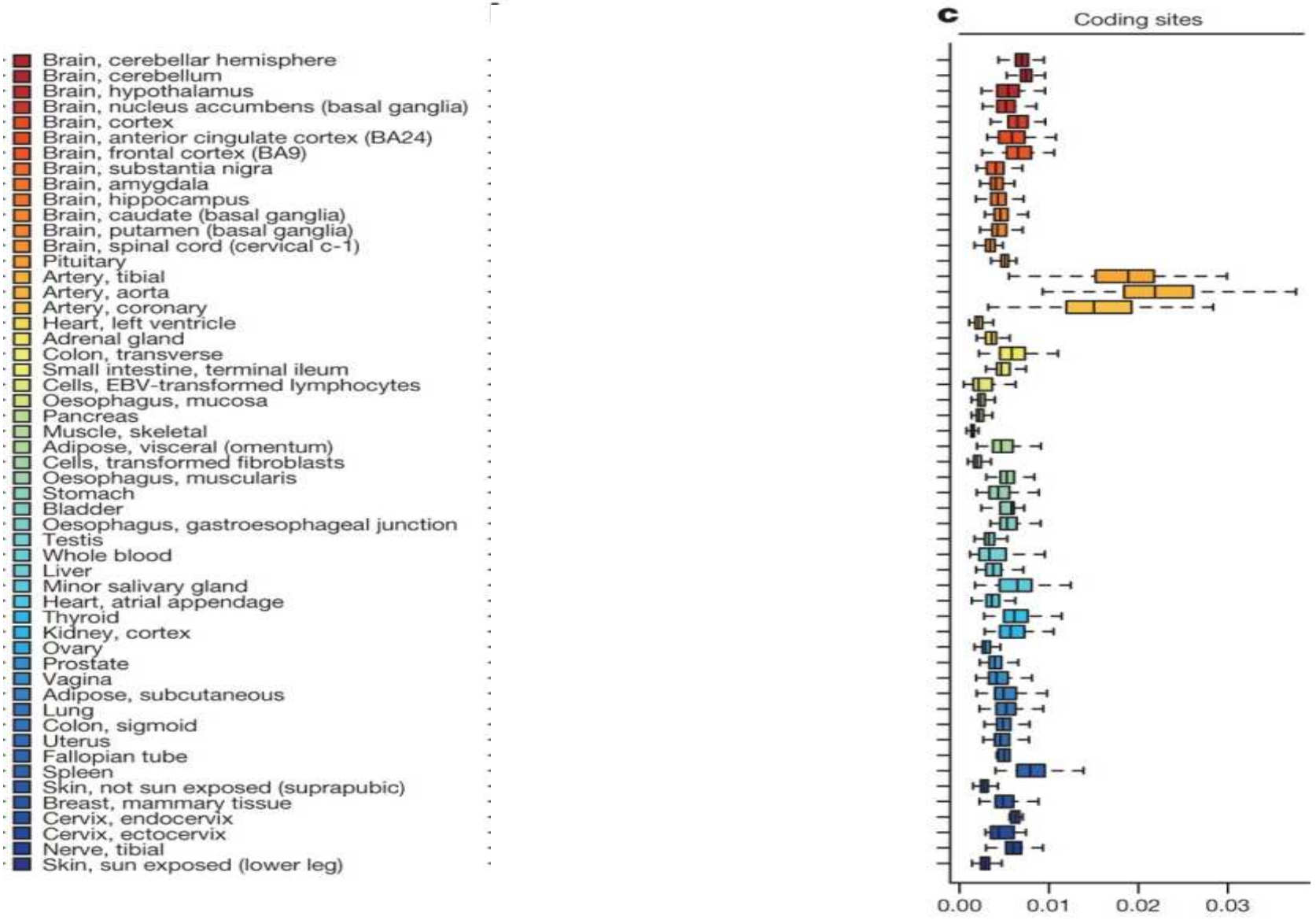


ADAR1L is detected mainly in the cytoplasm, whereas ADAR1S localizes in the nucleoplasmand nucleolus

ADAR2 localizes predominantly in the nucleolus

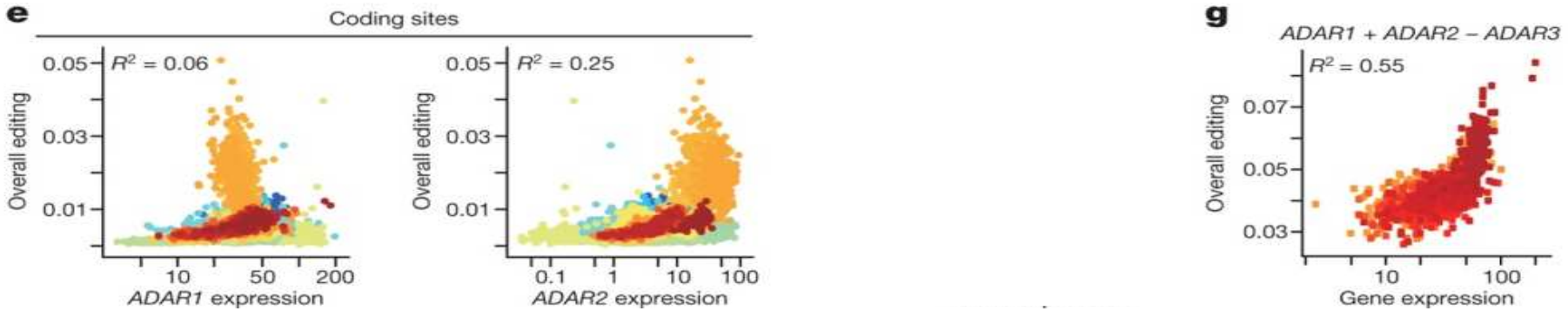
The Genotype-Tissue Expression Consortium multi-tissue RNA editome

editing levels of non-repetitive (c) coding sites in various human tissues tissues



M H Tan *et al. Nature* **550,** 249–254 (2017) doi:10.1038/nature24041

nature



Correlations between expression levels of ADAR1/2 and overall editing levels of non-repetitive (e) coding sites
g, Correlation of ADAR1 and ADAR2 expression with overall editing of all sites in the brain tissues

The Genotype-Tissue Expression Consortium multi-tissue RNA editome

M H Tan *et al. Nature* **550,** 249–254 (2017) doi:10.1038/nature24041

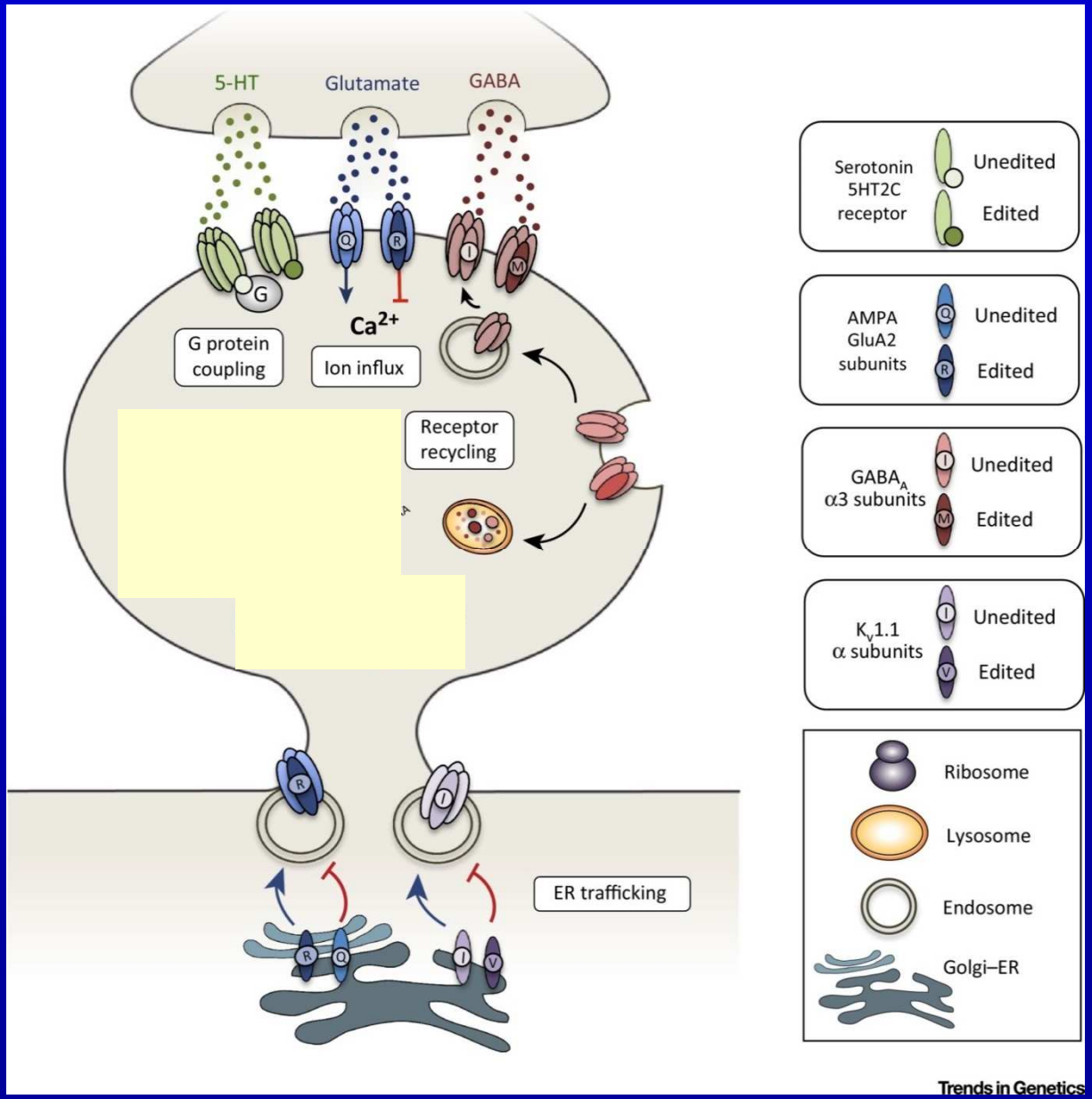
nature

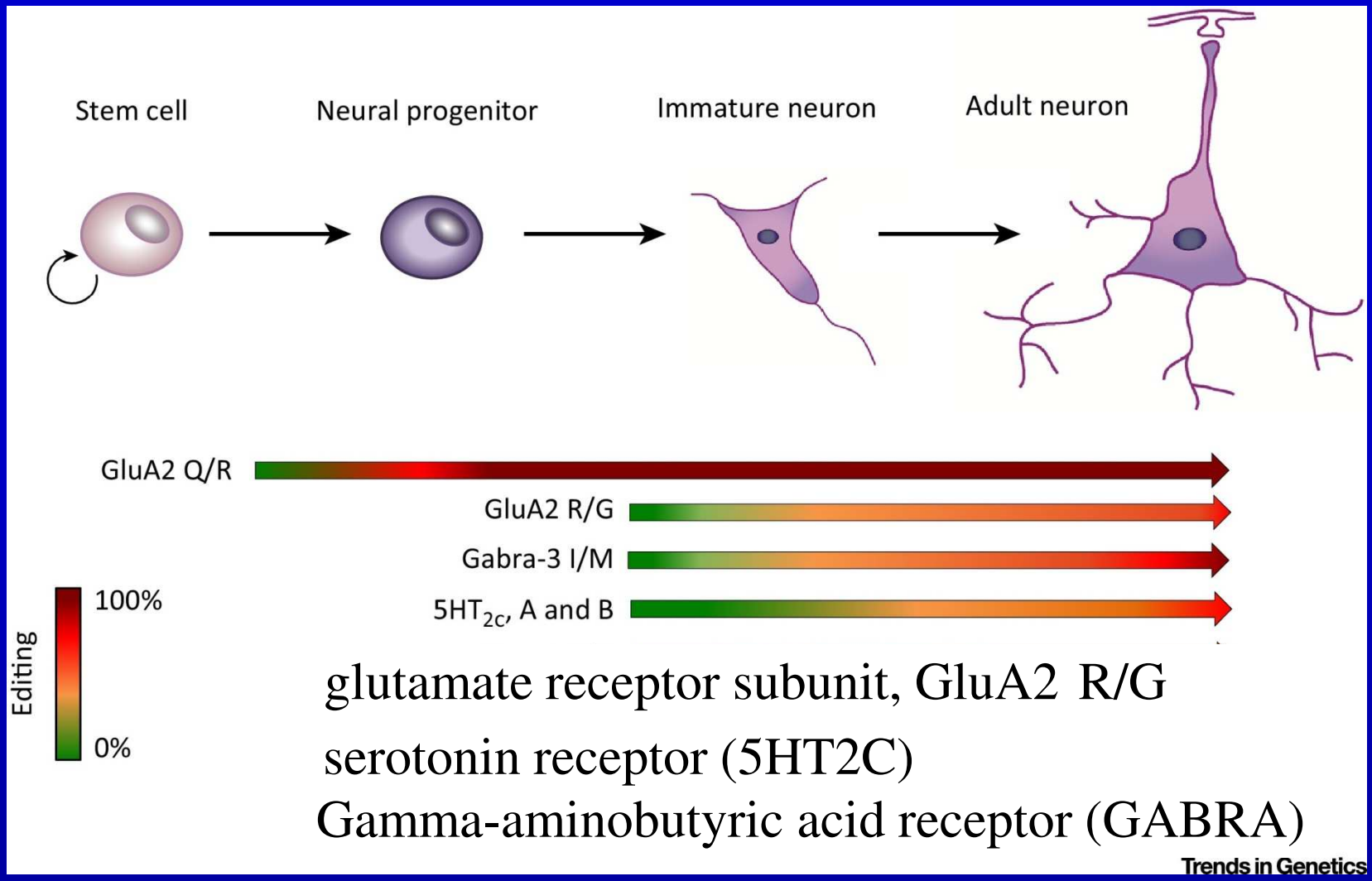
Deciphering the functions and regulation of brain-enriched A-to-I RNA editing Nat Neurosci. 2013.

- Adenosine-to-inosine (A-to-I) RNA editing, in which genomically encoded adenosine is changed to inosine in RNA, is catalyzed by adenosine deaminase acting on RNA (ADAR).
-
- This fine-tuning mechanism is critical during normal development and diseases, particularly in relation to brain functions.

- A large number of RNA editing sites have recently been identified as a result of the development of deep sequencing and bioinformatic analyses.

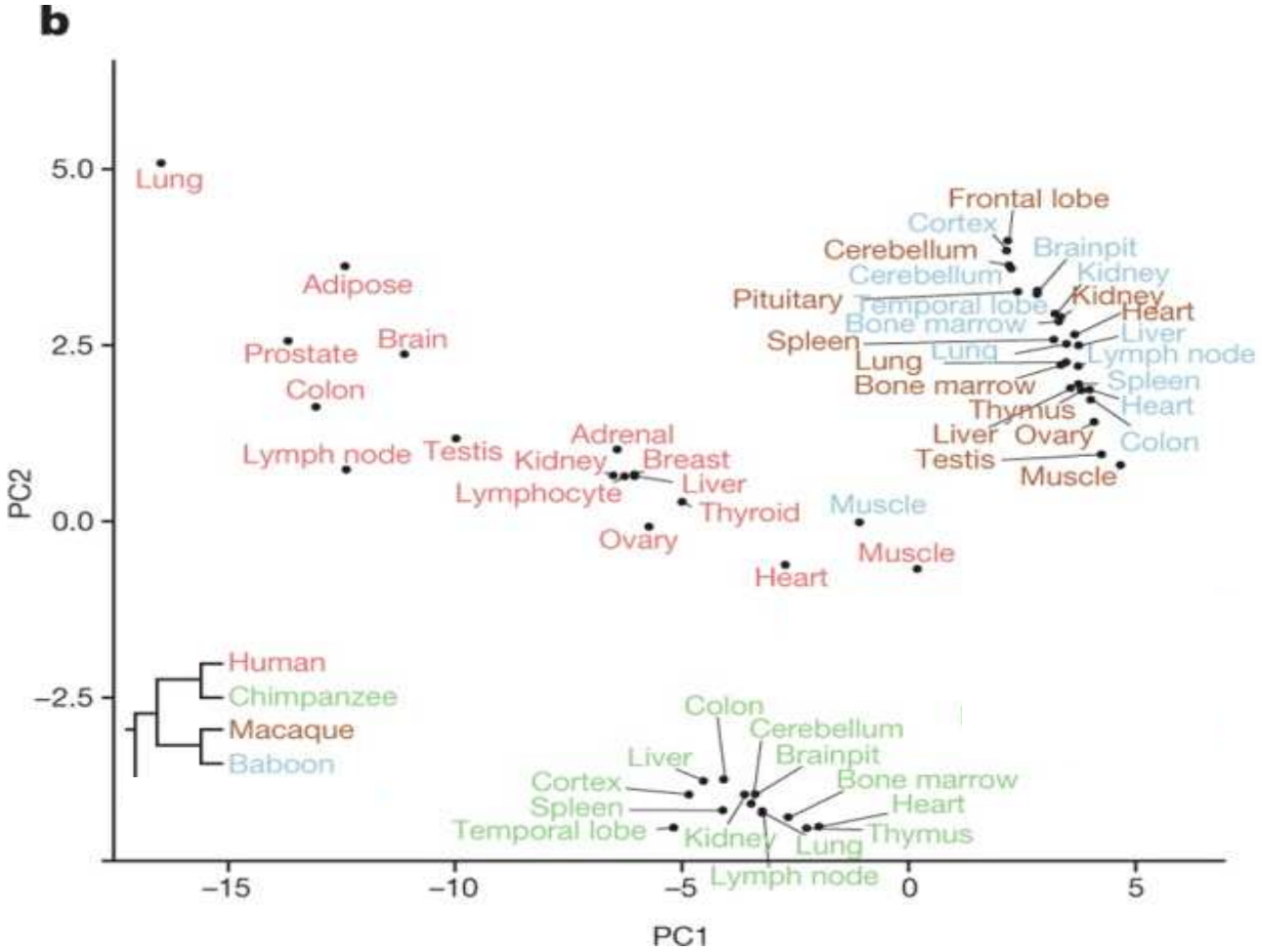
RNA Editing Events Contribute to Synaptic Plasticity





RNA Editing Is Differentially Regulated During Neuronal Differentiation and Maturation

Comparison of A-to-I editing between human and non-human primate tissues



M H Tan *et al. Nature* **550,** 249–254 (2017) doi:10.1038/nature24041