Harnessing ADARs for therapeutic RNA editing

Journal Club
26/02/2019

Juliane Bremer

ADARs

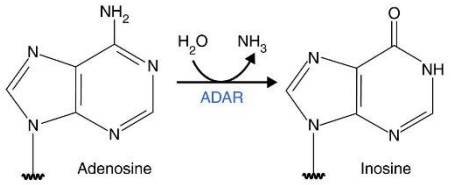
ADAR = Adenosine deaminase acting on RNA

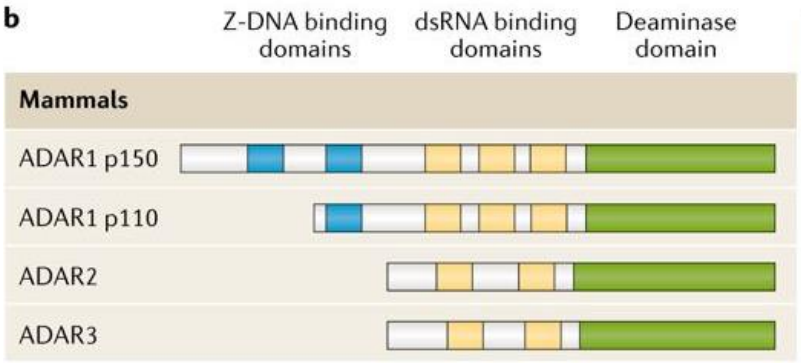
Discovered in 1991

Adenosine-to-inosine (A-to-I) editing

Highly conserved in vertebrates

In mammals 4 different ADARs (3 different genes)



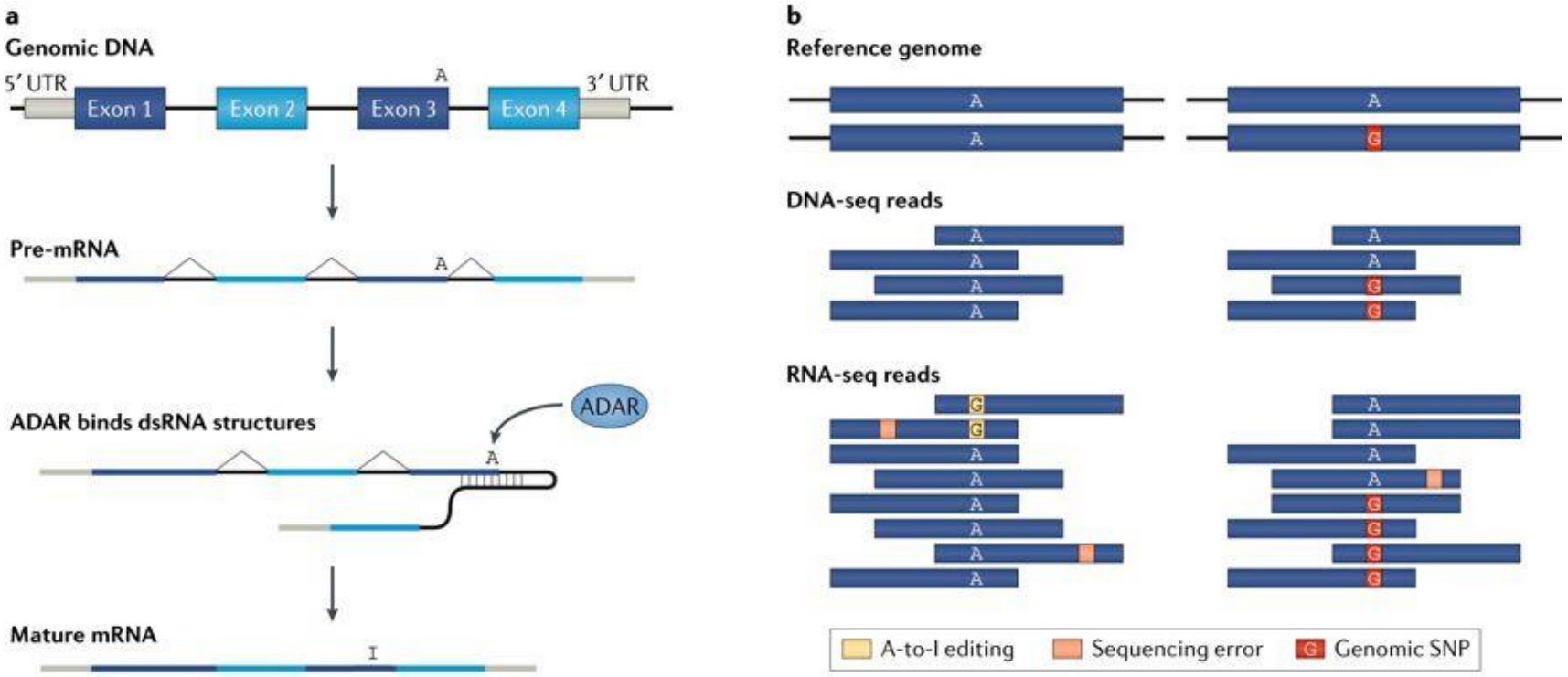


Expression	Function
Ubiquitous Interferon-induced	Non-coding
Ubiquitous	Non-coding
A few select tissues Mainly in neurons	Recoding
Mostly neural tissues	Possibly editing inhibition Catalytically inactive

Slotkin and Nishikura, Genome Medicine 2013; Eisenberg and Levanon, Nature Reviews Genetics 2018

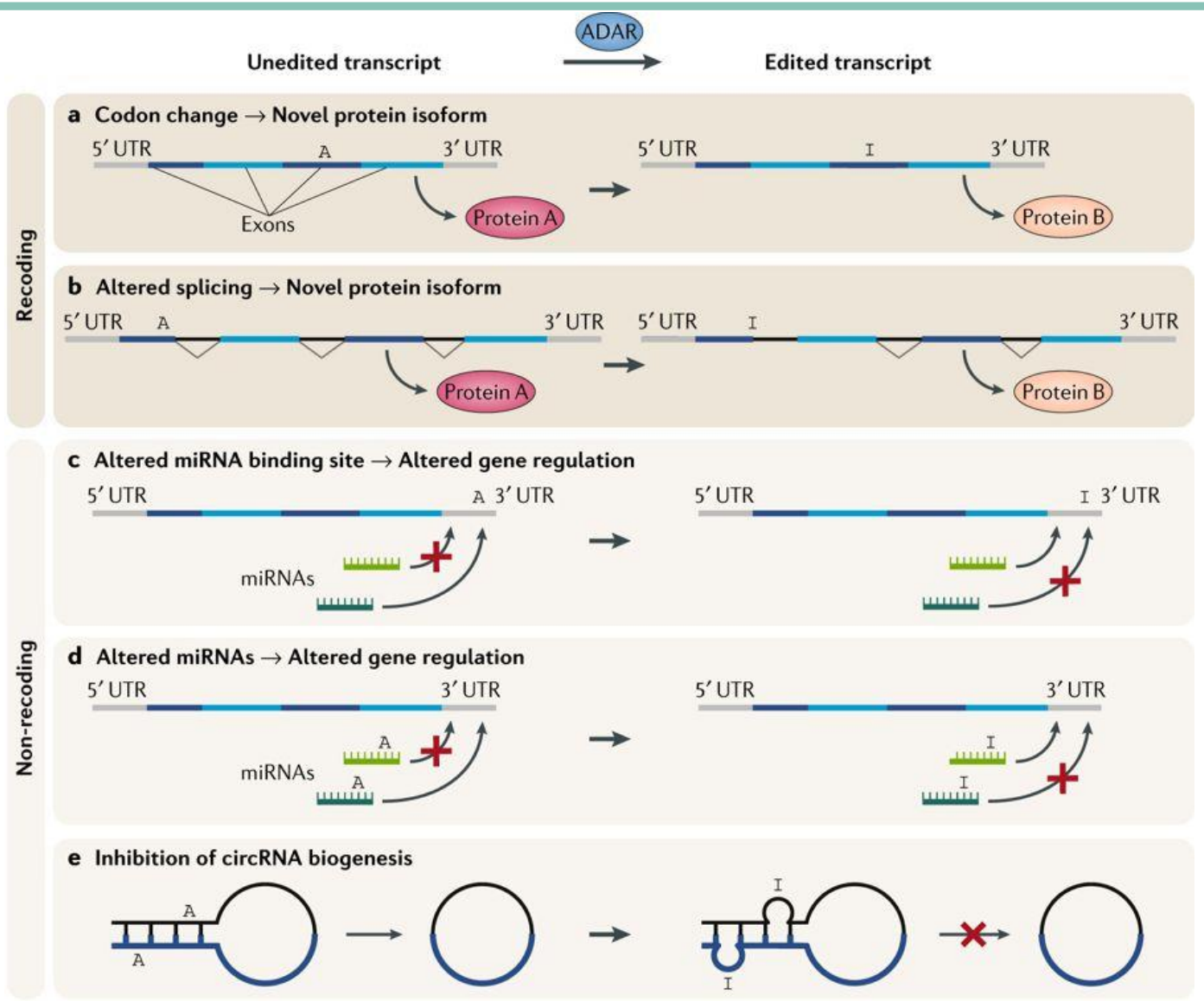
ADARs

Targets dsRNA, incomplete understanding of target recognition
A-to-I editing sites detected as A -> G mismatches in RNAseq data



Eisenberg and Levanon, Nature Reviews Genetics 2018

Recoding and non-recoding RNA editing by ADARs



Recoding is enriched in neural tissues, e.g. in ion channels and neuroreceptors (5-HT_{2c})

Eisenberg and Levanon, Nature Reviews Genetics 2018

Physiological function of ADARs

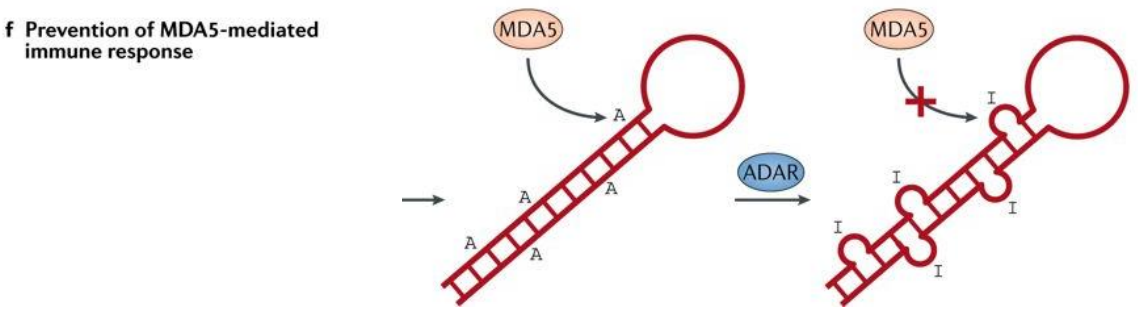
Of >1,000 recoding sites in humans, only a few dozen are conserved across mammals

Function not completely understood

ADAR2 knockout mice: progressive seizure, die within 3 weeks of birth
- rescued by genome alteration in glutamate receptor GRIA2 -> Q/R alteration
- GRIA2 only essential RNA editing target for ADAR2

Majority of RNA editing occurs in non-coding parts of the transcriptome

Main function of ADAR1 is most likely to prevent autoimmunity:
ADAR1-mediated editing to prevent activation of the cytosolic innate immune system
MDA5 knockout rescues embryonic lethal phenotype of ADAR1 knockout mice



«self»dsRNA (resemble viral dsRNA)
Activation of MDA5 (melanoma differentiation-associated protein 5)
-> IFN response which damages host cell

Eisenberg and Levanon, Nature Reviews Genetics 2018

ADAR mediated RNA editing in human diseases

Cancer

- negative correlation between A-to-I editing level and patient survival
- e.g. AZIN1 (antizyme) recoding in hepatocellular carcinoma

-> edited AZIN1 degrades cyclin D1 and ornithine decarboxylase less well

-> increased cell proliferation

Autoimmunity

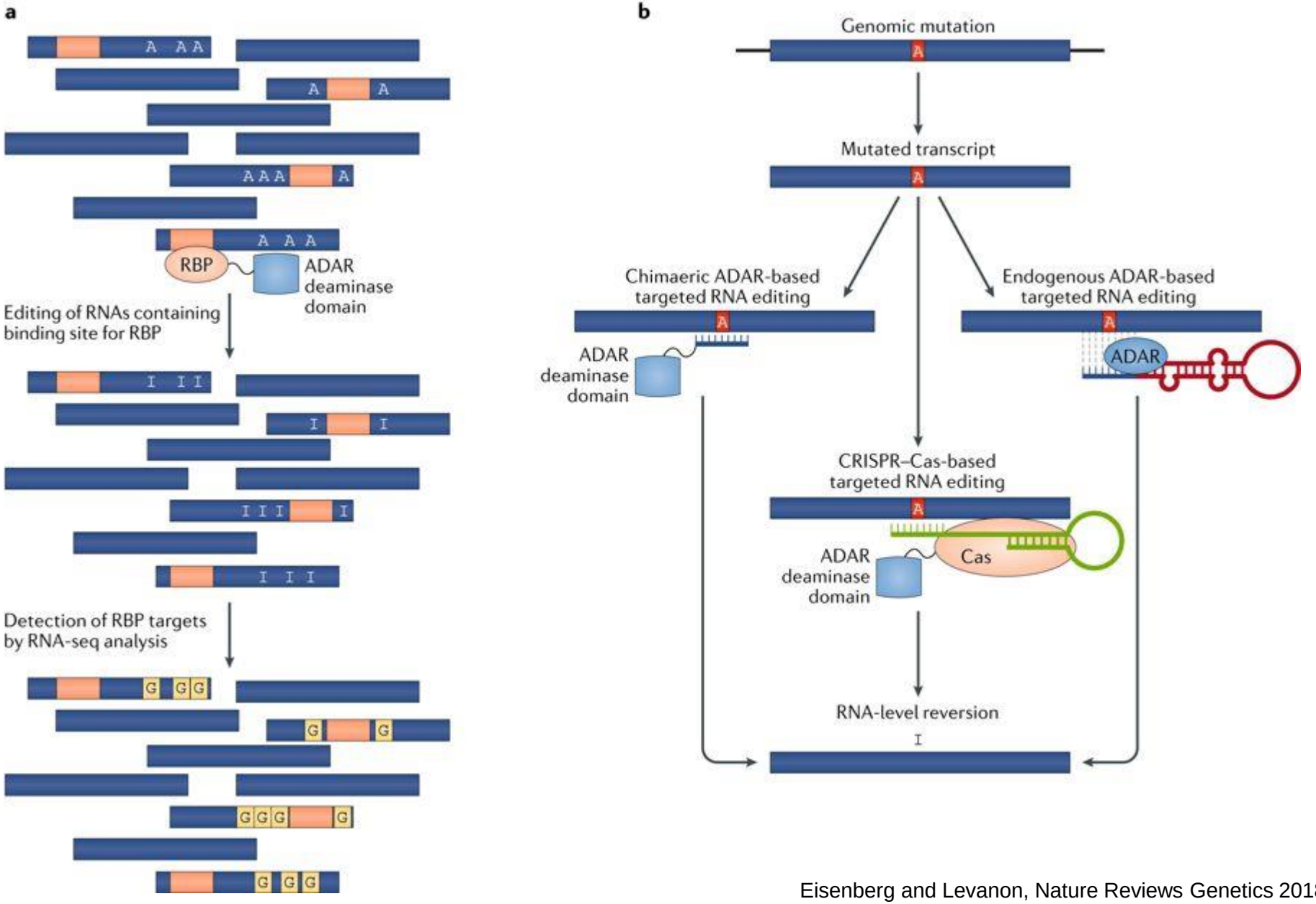
- ADAR1 mutations cause Aicardi-Goutieres syndrome (autoimmune disorder)

Neurological disorders

- altered editing in ALS, Alzheimer, fragile X, epilepsy, bipolar disorder, schizophrenia, autism

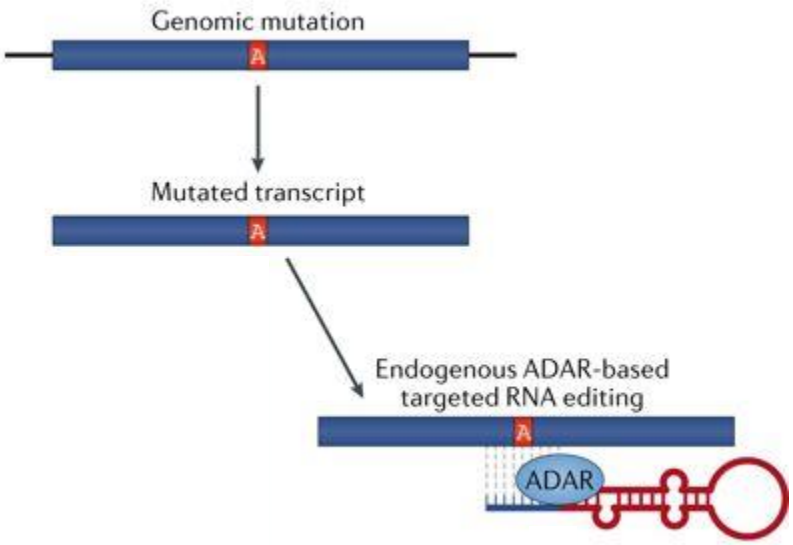
Eisenberg and Levanon, Nature Reviews Genetics 2018

Utilizing ADAR for RNA probing and engineering



Eisenberg and Levanon, Nature Reviews Genetics 2018

Harnessing ADARs for therapeutic RNA editing



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Harnessing human ADAR2 for RNA repair – Recoding a PINK1 mutation rescues mitophagy

Jacqueline Wettengel[1,†], Philipp Reautschnig[1,†], Sven Geisler[2,3], Philipp J. Kahle[2,3] and Thorsten Stafforst[1,*]

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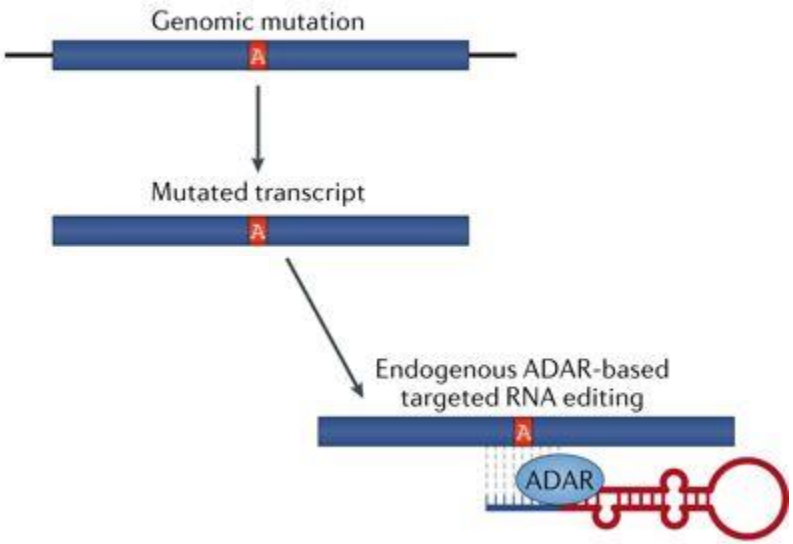

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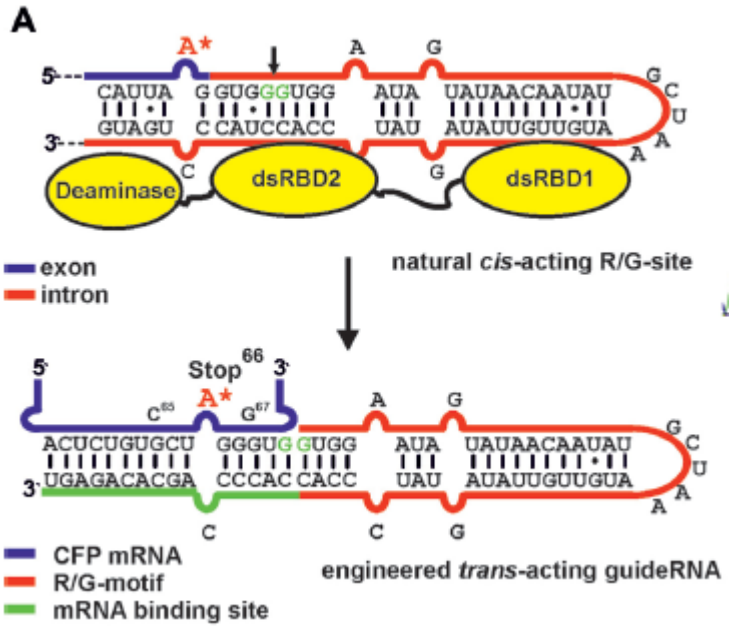
Harnessing ADARs to recode mutant PINK1

Rationale: Advantage of RNA editing: transient and reversible

Aim: site-directed RNA editing at specific sites on user-defined targets
By harnessing endogenous ADARs no need to overexpress a deaminase
To repair a parkinson's disease associated PINK1 mutation

Method: design of guideRNA to harness human ADAR2, composed of
- stem loop structure recruiting ADAR2 via dsRNA binding domains of GluR2
- RNA binding site



Harnessing ADARs to recode mutant PINK1

Results:

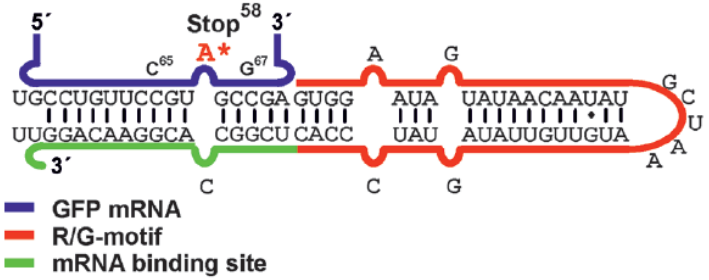
1. Test efficiency in PCR tube with gRNA targeting CFP (T66 stop)
   and recombinant human ADAR2

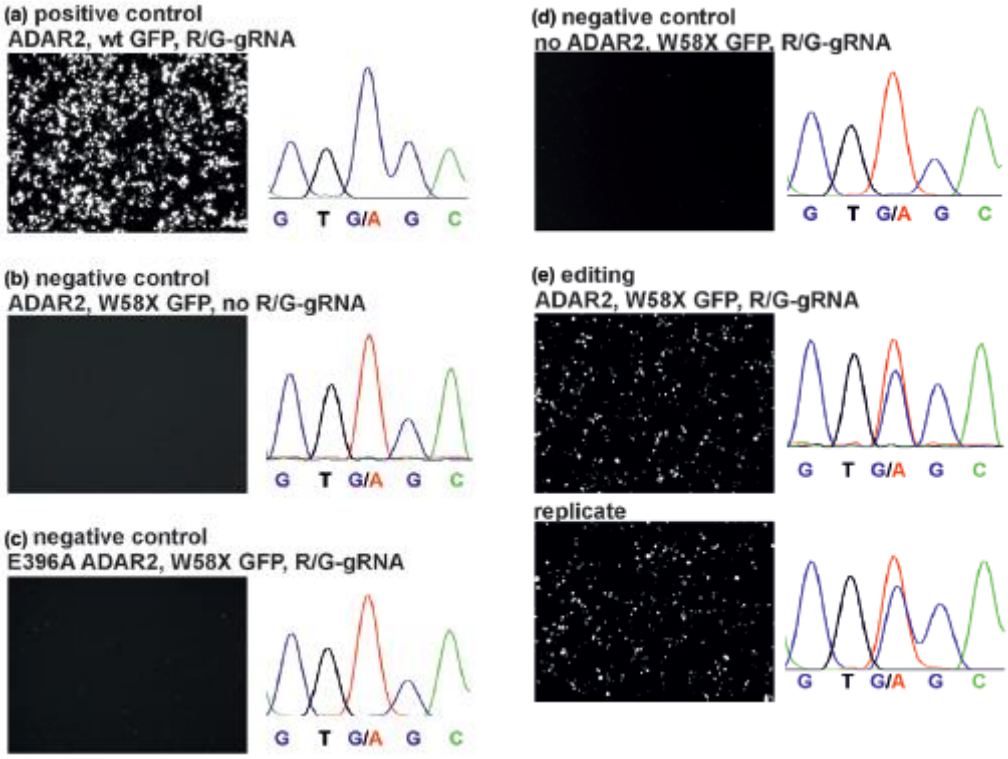
   -> highly efficient editing, but massive off-target editing
   -> off-target editing was reduced by optimizing buffer condition
   (0.5mM spermidine)

Harnessing ADARs to recode mutant PINK1

Results:

2. Test in cell culture (293T cells)
   - cotransfection 3 plasmids encoding gRNA, ADAR2, substrate (eGFP W58X)
   -> single + specific A-to-G conversion at target codon in 25% (24h); 40% (48h)





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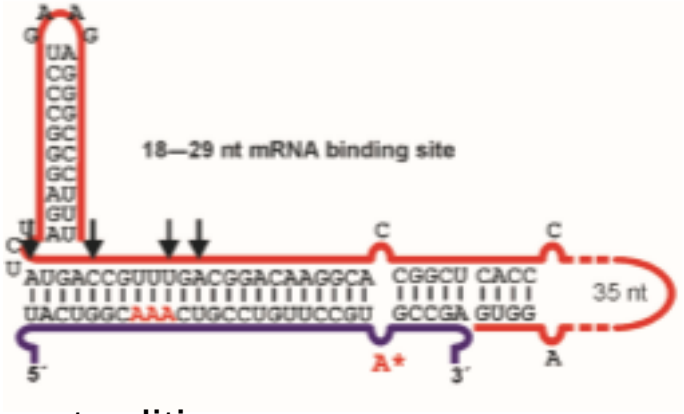
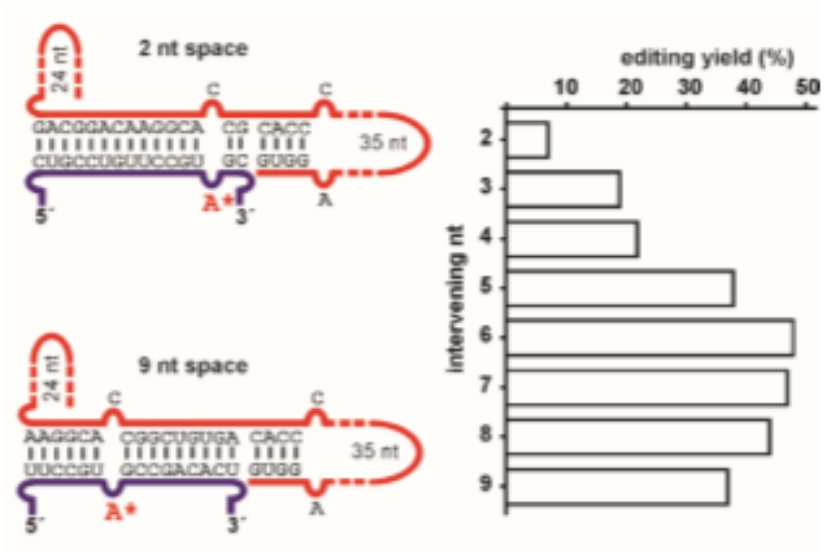
Results:



3. Optimizing guideRNA
   - 3' hairpin facilitated cloning
   - Optimal mRNA binding template length:
     18-20nt: editing ~40%
     25/29nt: editing yield dropped, more off-target editing

   - Optimal distance between 5' of guideRNA and editing site: 6-7nt
     editing yield: 50% (48-72h)



Harnessing ADARs to recode mutant PINK1

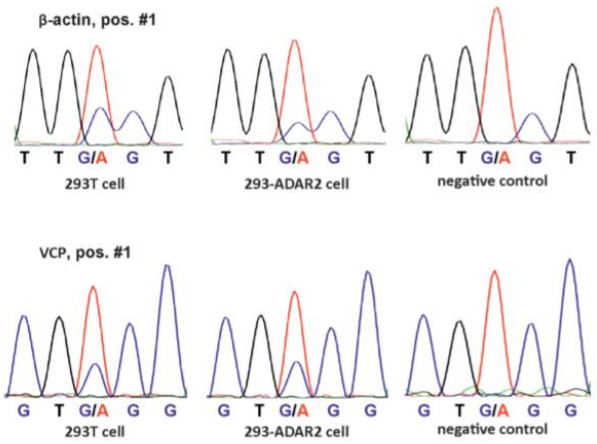
Results:

4. ADAR2 expression from single genomic copy in 293T cells
   - under CMV tet-on promoter
   - sufficed for 45-65% editing
   - no off-target editing

5. Editing of 13 different site in 6 different endogenous transcripts (UAG in 3' UTR)
   - editing yields from 10-35%

B editing of endogenous transcripts

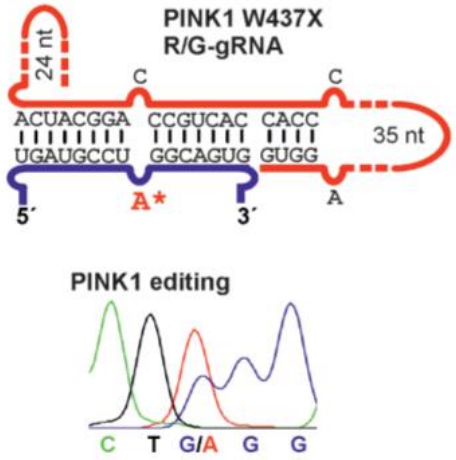
gene	293T cell + ADAR2 + guideRNA	293T-ADAR2 cell + doxycyclin + guideRNA	negative control (no guideRNA)
β-actin			
position #1	27%, 24%	17%, 14%	0%, 0%
position #2	0%, 0%	0%, 0%	0%, 0%
position #3	24%, 23 %	14%	0%, 0%
GAPDH			
position #1	21%, 17%	10%, 10%	0%, 0%
position #2	21%, 19%	12%, 10%	0%, 0%
GPI			
position #1	15%, 16%	12%, 11%	0%, 0%
GUSB			
position #1	11%, 9%	10%, 9%	0%, 0%
position #2	26%, 19%	20%, 16%	0%, 0%
VCP			
position #1	23%	25%, 21%	0%
position #2	16%	15%	0%
position #3	12%, 11%	13%	0%
RAB7A			
position #1	32%, 30%	38%	0%, 0%
position #2	27%, 28%	33%	0%, 0%

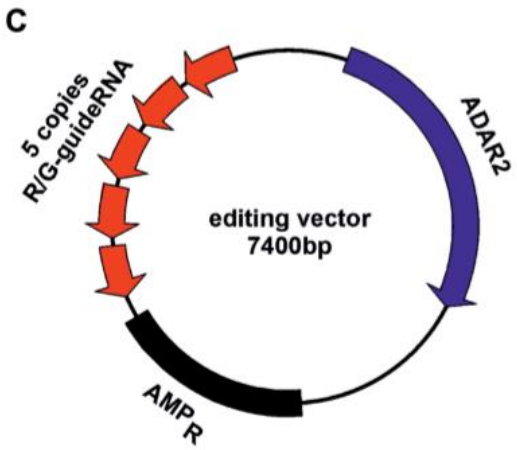


Harnessing ADARs to recode mutant PINK1

Results:

6. RNA editing to repair PINK1 W437X





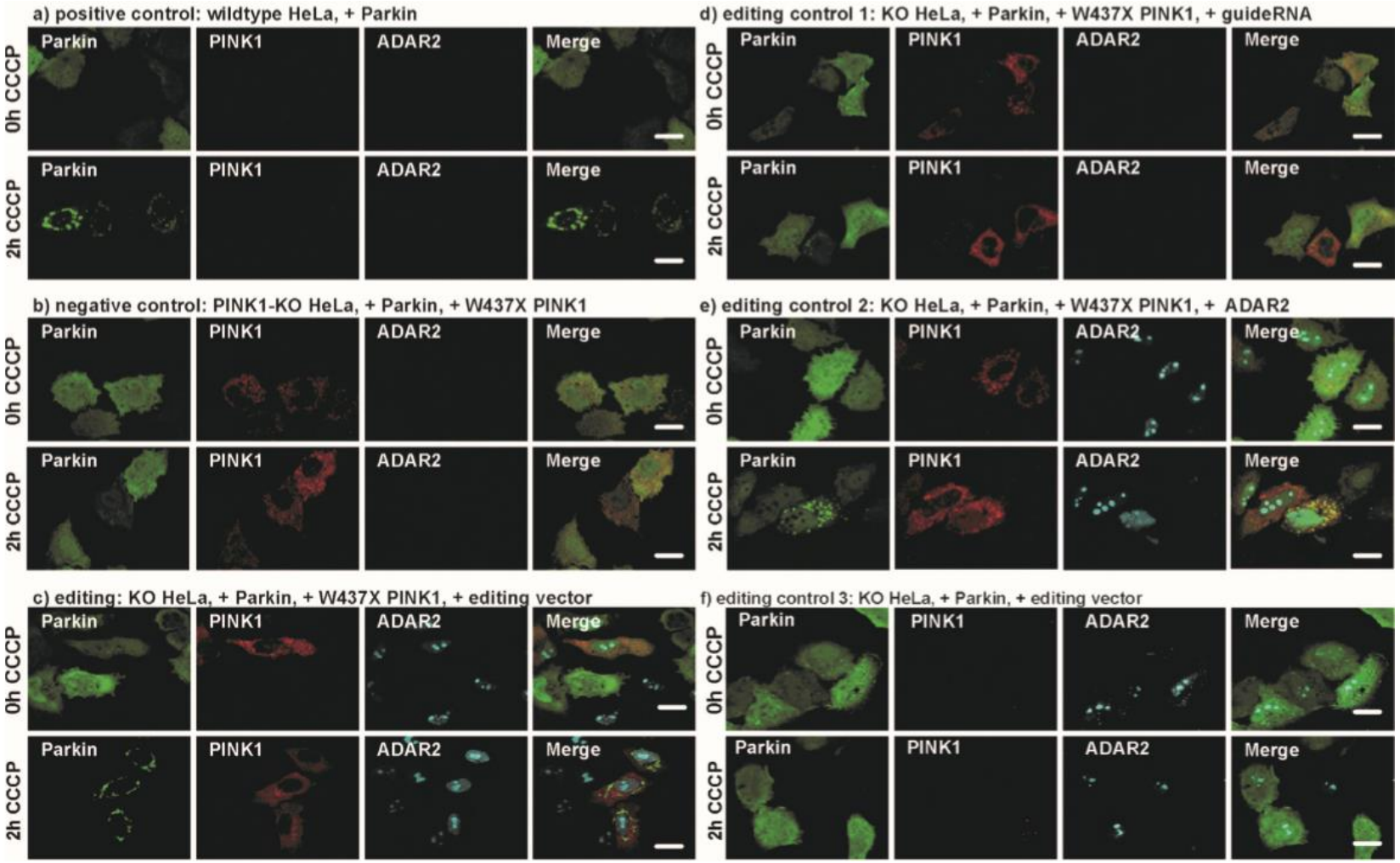
Cotransfection of PINK1 W437X, Parkin-eGFP, editiong vector (gRNA, ADAR2)

Readout: Parkin clustering after CCCP treatment (work only with repaired PINK1)

CCCP (uncoupler) - exposed mitochondria accumulate PINK1
PINK1 recruits parkin, resulting in ubiquitination of mitochondrial proteins
bound by the autophagic proteins p62/SQSTM1 and LC3
resulting in degradation of mitochondria by mitophagy

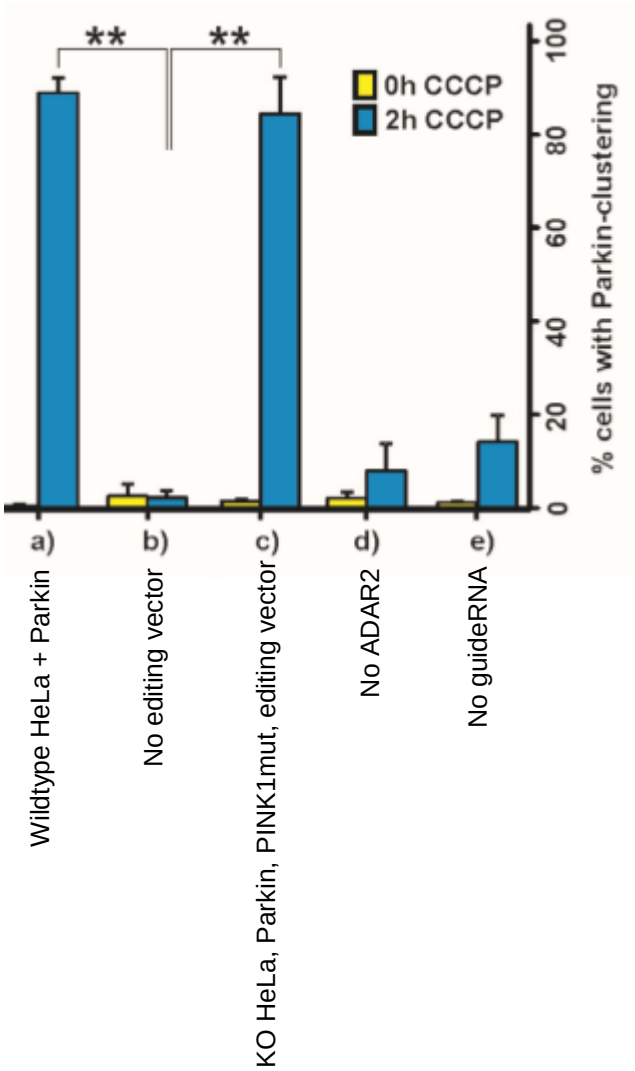
Harnessing ADARs to recode mutant PINK1

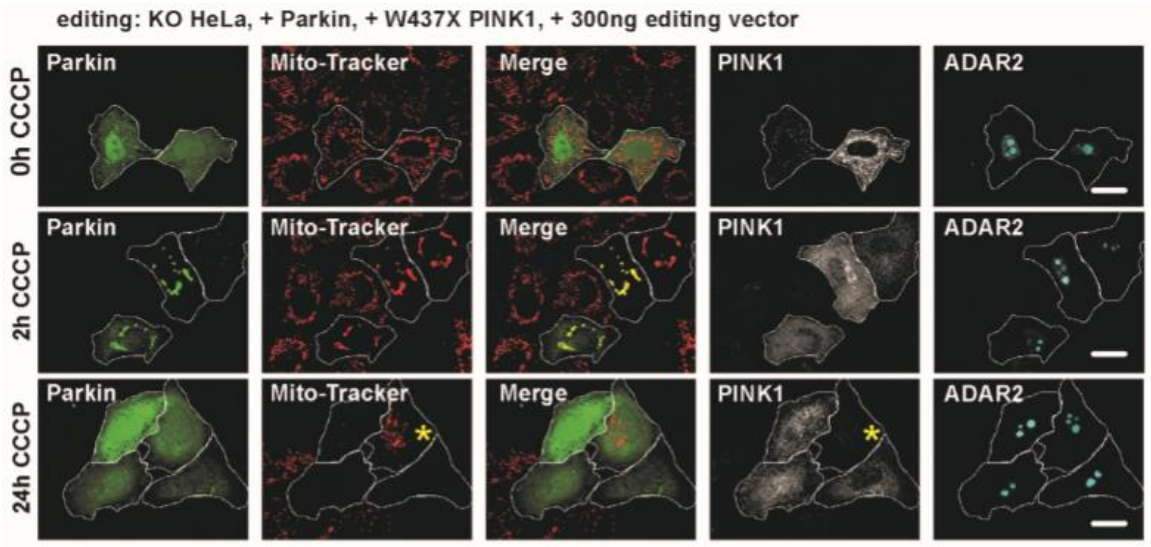
Results: 6. RNA editing to repair PINK1 W437X
(Readout: Parkin clustering after CCCP treatment)



Harnessing ADARs to recode mutant PINK1

Results: 6. RNA editing to repair PINK1 W437stop
(Readout: Parkin clustering after CCCP treatment/ mitophagy)





Harnessing ADARs to recode mutant PINK1

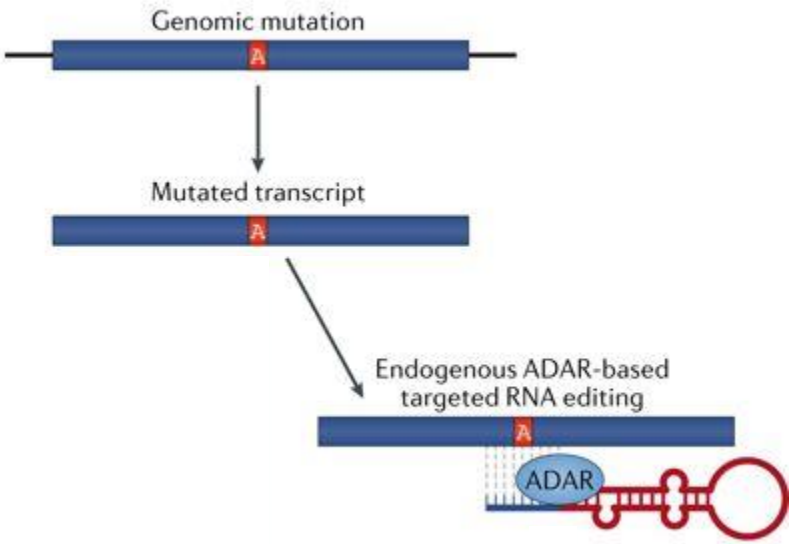
Conclusions:

- First strategy to harness wildtype ADAR2 to stimulate site-selective RNA editing at arbitrary mRNAs

- other RNA-processing enzymes (RNaseH, RNA-induced silencing complex) have been shown to be readdressable toward new targets
- RNAi and RNaseH are limited to up/down-regulation of target transcripts, RNA editing allows recoding

- remains unclear if they are able to recruit endogenously expressed ADAR2 for site-directed RNA editing

Harnessing ADARs for therapeutic RNA editing



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NATURE BIOTECHNOLOGY | VOL 37 | FEBRUARY 2019 | 133–138 | www.nature.com/naturebiotechnology

Recruiting endogenous ADAR for recoding

Aim: Chemically optimizing antisense oligonucleotides that recruit endogenous human ADARs to edit endogenous transcripts

RESTORE = recruting endogenous ADAR to specific transcripts for oligonucleotide-mediated RNA editing

- Without off-target editing
- Without perturbing natural editing homeostasis

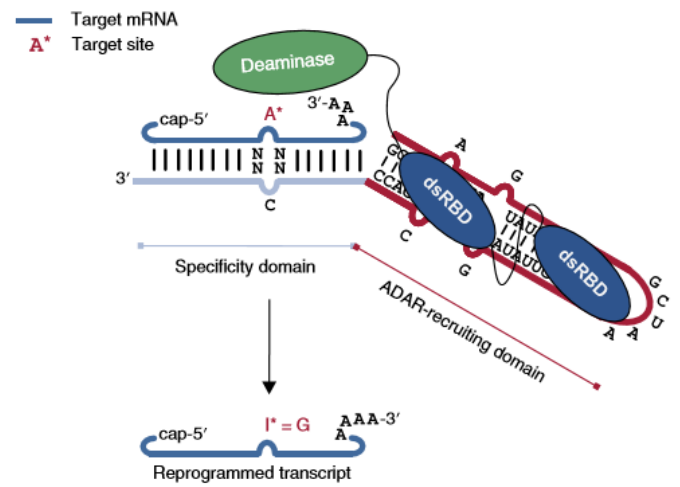
- In human cell lines and human primary cells

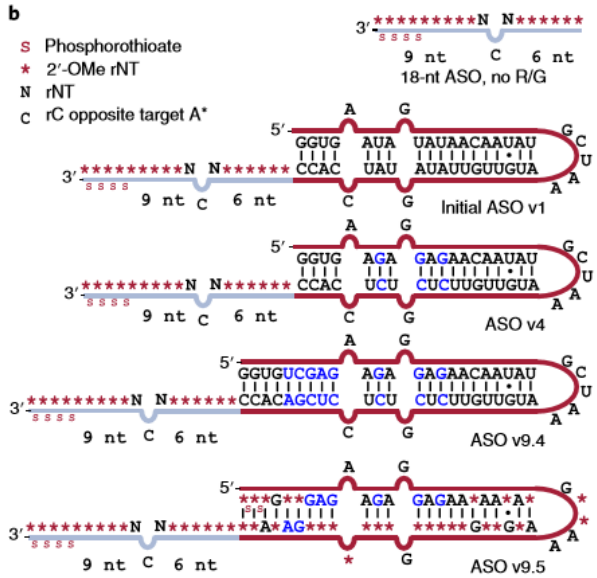
- To repair PiZZ mutation which causes alpha1-antitrypsin deficiency
- To edit phosphotyrosine 701 in STAT1

Method: only apply oligonucleotide (guideRNA) without protein expression

Recruiting endogenous ADAR for recoding

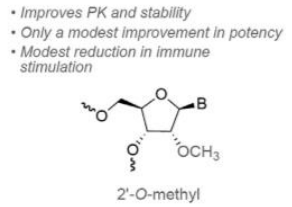
Result 1: Optimization of the ADAR-recruitment domains by chemical modifications



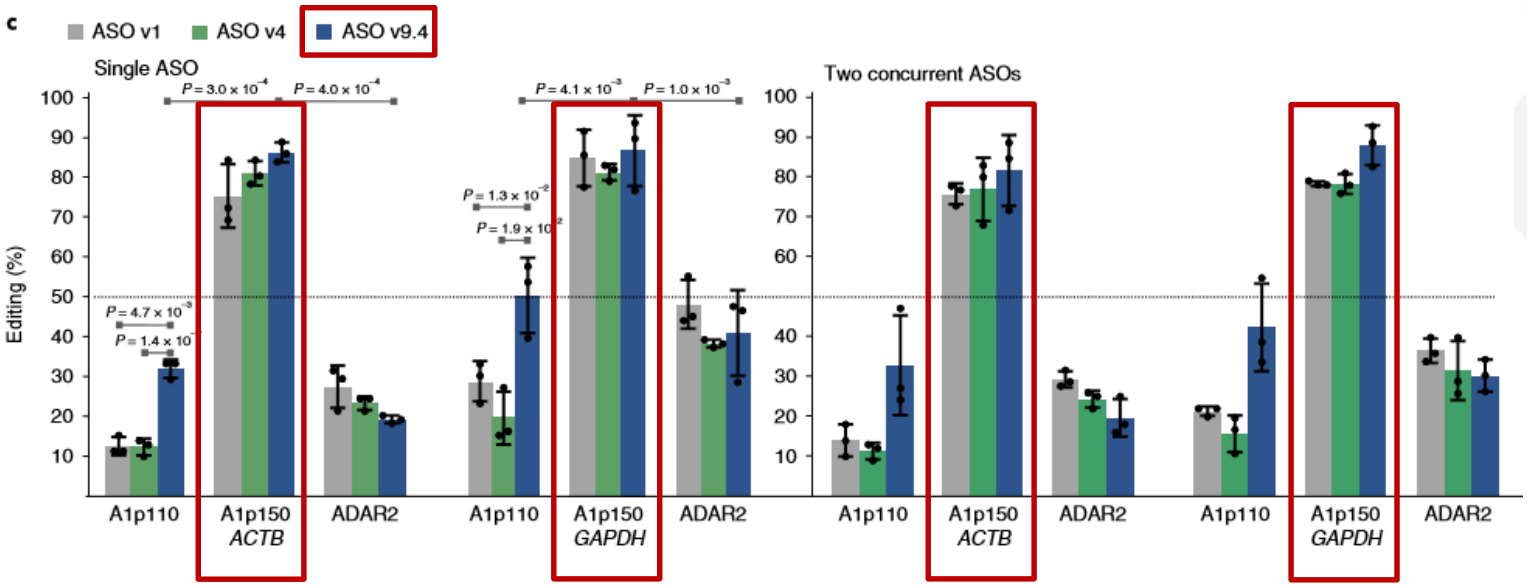


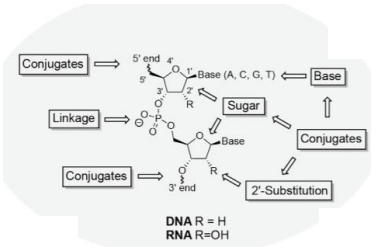
Chemical modifications of specificity domain:

- 2’-O-methylations



- phosphorothioate



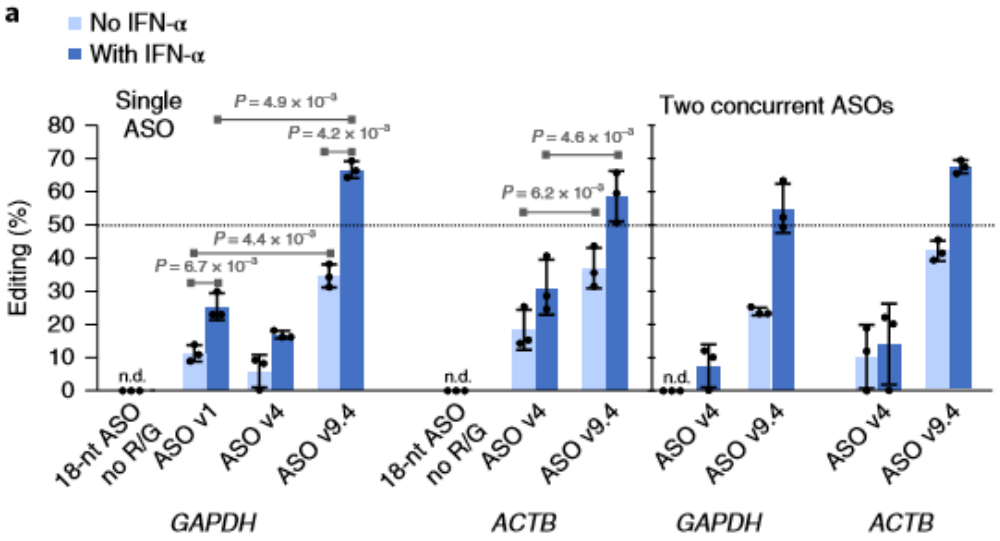


Crooke et al., Cell Metabolism 2018

Highest editing in ADAR1 p150 expressing 293 cells (75-85%)

Recruiting endogenous ADAR for recoding

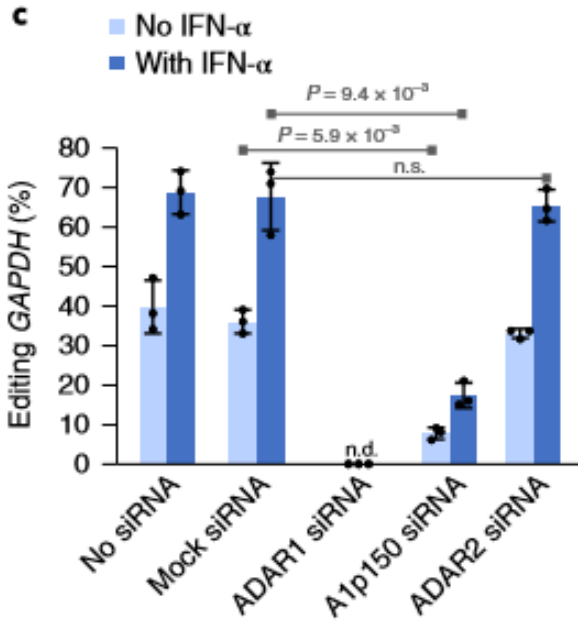
Result 2: AS Oligonucleotide v9.4 -> best editing in HeLa, even more if IFNα was added
- Editing of UAG triplet in the 3' UTR

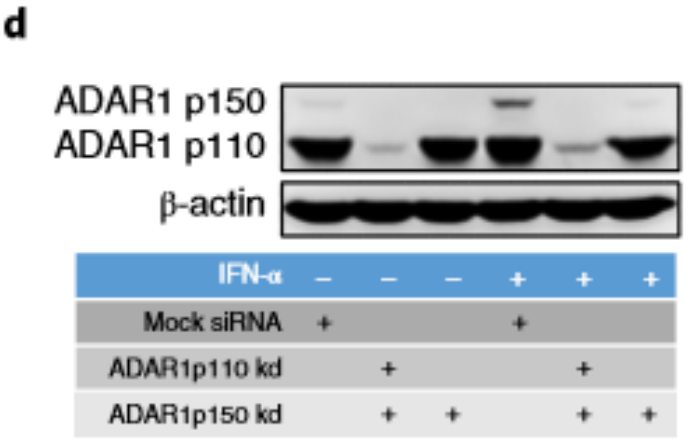


Recruiting endogenous ADAR for recoding

Result 3: Editing depends on ADAR1

- Both isoforms, p110 and p150 contribute to editing
- The weaker expressed p150 isoform contributes more

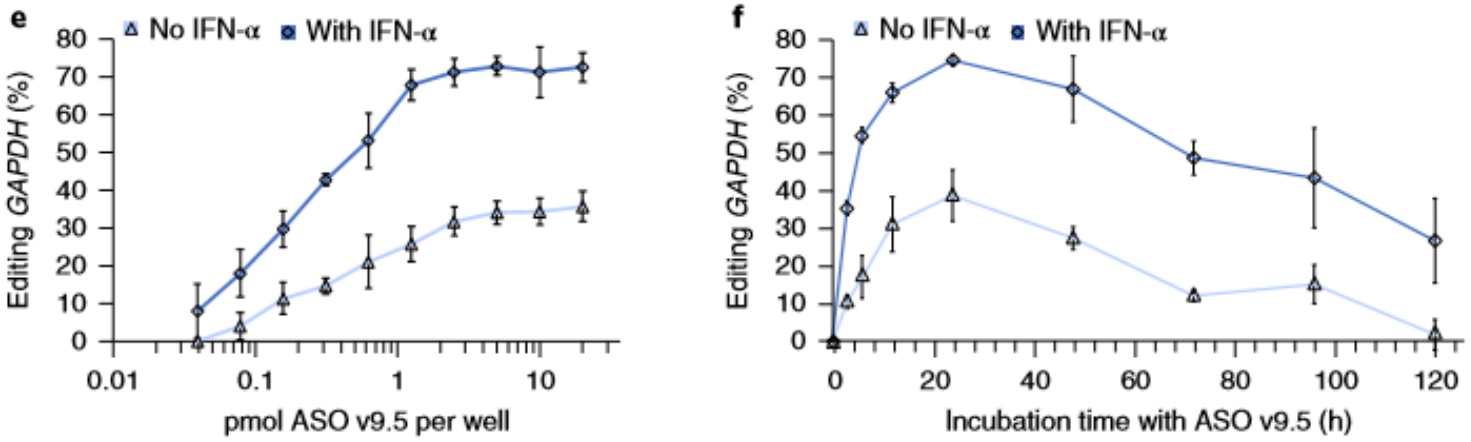




Recruiting endogenous ADAR for recoding

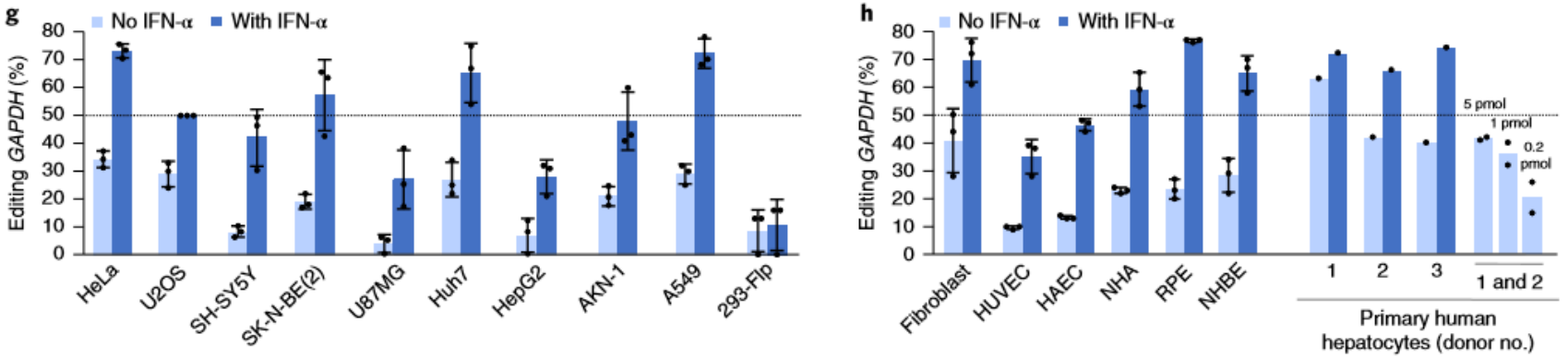
Result 4: Dose and time-dependency of editing

- Sigmoidal dose dependency for ASO v9.5
- Time profile with maximum editing at 12-48 h



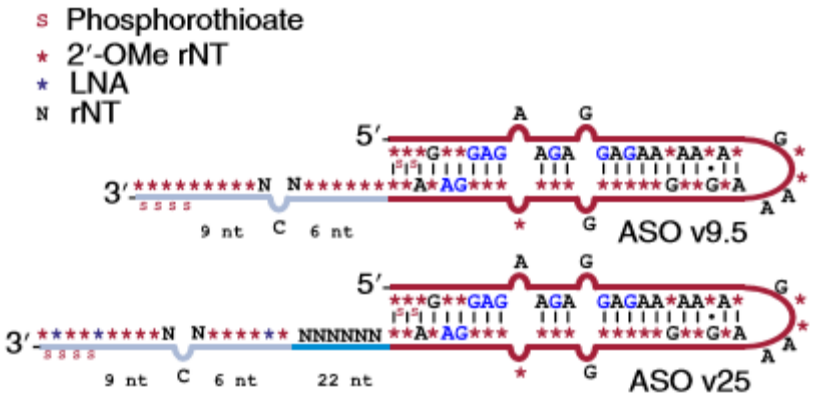
Recruiting endogenous ADAR for recoding

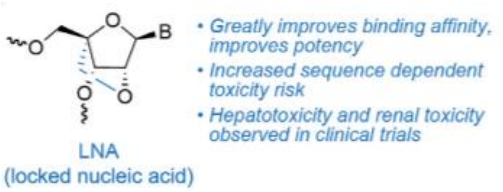
Result 5: Good editing yield in 10 immortilized human cell lines and 7 primary cells



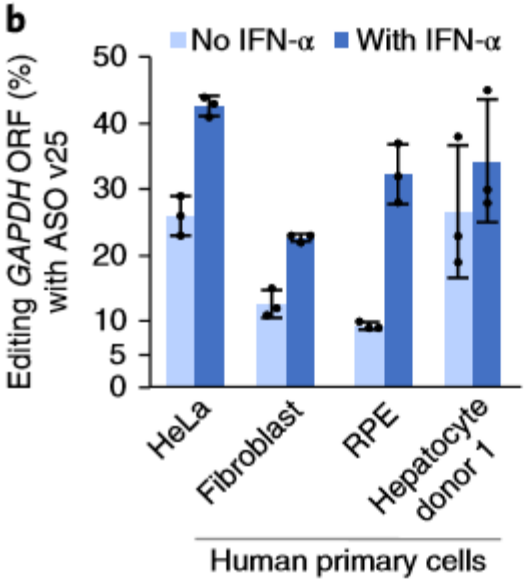
Recruiting endogenous ADAR for recoding

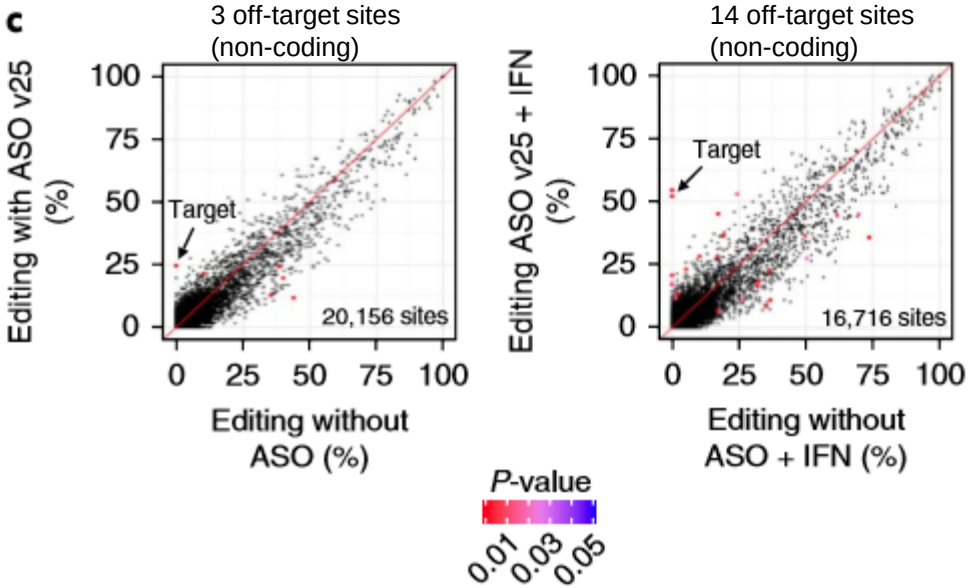
Result 6: Editing the ORF was limited by translation
- required further ASO modifications (longer specificity domain = 40nt, LNA)





- good editing yields in HeLa cell line and human primary cells ,little off-target editing

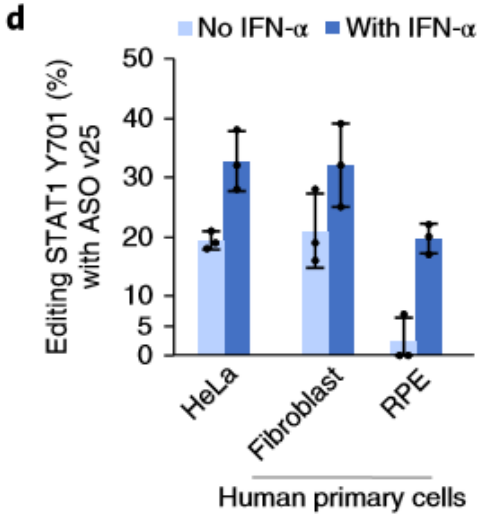




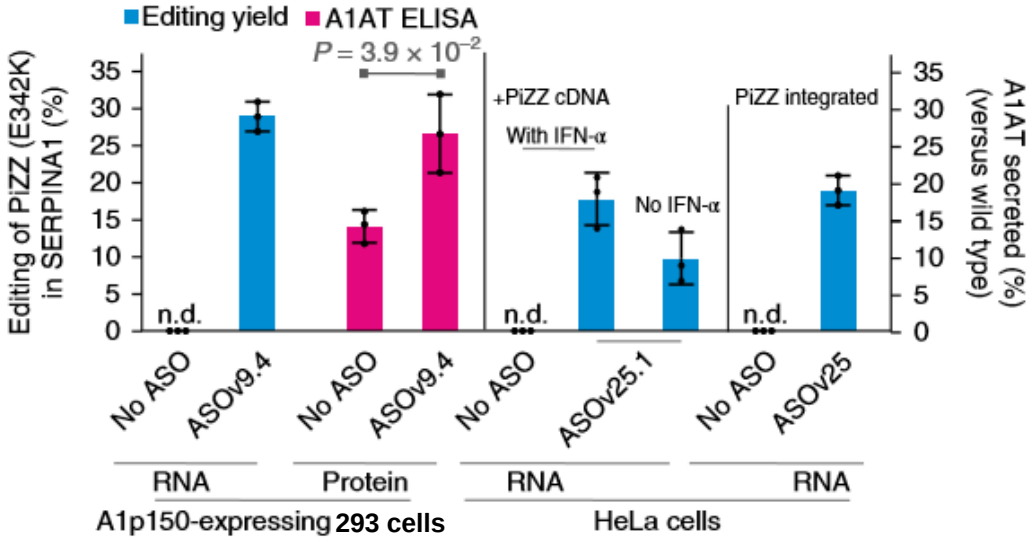
Recruiting endogenous ADAR for recoding

Result 7: Therapeutic potential of RESTORE

- Targeting functionally important phophotyrosine 701 in STAT1



- Editing of PiZZ mutation (E342K) in SERPINA1 (serpin family A member 1) most common cause of α1-antitrypsin (A1AT) deficiency



Recruiting endogenous ADAR for recoding

Conclusions

- most previous systems require codelivery of an artificial deaminase, with massive off-target editing

- RESTORE simplifies delivery (ASO only)

- RESTORE allows editing with minimal off-target effects without perturbing the natural editing homeostasis

- Codon scope is limited by the codon preference of natural ADARs

- future modifications of ASOs may improve pharmacological properties