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Supplemental information

A pipeline for identifying guide RNA sequences that promote RNA editing of nonsense mutations that cause inherited retinal diseases

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SUPPLEMENTAL METHODS

A. GAPDH 3' UTR insert: The 161 bp insert containing a portion of the 3' UTR of GAPDH, cloned into a reading frame in the pMcherry-EGFP reporter plasmid in which the editing of the target adenosine (blue) prevents translation termination, with a single base change of T>C (yellow) 8 bp downstream of the target adenosine to prevent a second stop codon in the reading frame.

CCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCA
CACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCT
CGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAG

A- Target adenosine

C- T>C modification for prevention of stop codon

B. Chemically modified 73-mer gRNA development by the Stafforst group (1) used to edit a target adenosine in the 3' UTR of GAPDH cloned into the reported plasmid.

Key: (N)=RNA base, [N]=2'-OMe RNA base, *=Phosphorothioate linkage

Sequence for ssRNA (5'→3'):

[G*][G*][U](G)[U][C](GAGAAGAGGAGAA)[C](AA)[U](A)[U](G)[C][U](AAA)[U](G)[UU](G)[UUCUC](G)[UCUCCUC](G A)[C](A) [CCAGGGGU] (CCA) [CAUG][G*][C*][A*][A*][C]

1. Merkle,T., Merz,S., Reautschnig,P., Blaha,A., Li,Q., Vogel,P., Wettengel,J., Li,J.B. and Stafforst,T. (2019) Precise RNA editing by recruiting endogenous ADARs with antisense oligonucleotides. *Nat. Biotechnol.*, **37**, 133–138.

SUPPLEMENTAL TABLES:

Table S1-Yeast strains+plasmids.

YSB ID	Name	BSB ID	Plasmid A	BSB ID	Plasmid B
2907	pYEST-DEST52-hADAR2:LEU2 +FAM161A-R523W-URA3-BamHI:HIS3	641	pYEST-DEST52-hADAR2:LEU2	706	FAM161A-R523W-URA3-BamHI:HIS3
2911	pYEST-DEST52-hADAR2:LEU2 + TRPM1-K294W-URA3-BamHI:HIS3	641	pYEST-DEST52-hADAR2:LEU2	708	TRPM1-K294W-URA3-BamHI:HIS3
2988	pYEST-DEST52-hADAR2:LEU2 +FAM161A-p.R523*- ura3-31nt-GR(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	731	FAM161A-p.R523*- ura3-31nt-GR(tail):HIS3
2989	pRS315::LEU2 +FAM161A-p.R523*- ura3-31nt-GR(tail):HIS3	61	pRS315::LEU2	731	FAM161A-p.R523*- ura3-31nt-GR(tail):HIS3
2990	pYEST-DEST52-hADAR2:LEU2 +TRPM1-p.K294*-ura3-30nt-GR(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	732	TRPM1-p.K294*-ura3-30nt-GR(tail):HIS3
2991	pRS315::LEU2 +TRPM1-p.K294*-ura3-30nt-GR(tail):HIS3	61	pRS315::LEU2	732	TRPM1-p.K294*-ura3-30nt-GR(tail):HIS3
2992	pYEST-DEST52-hADAR2:LEU2 + KIZ-p.R76*-ura3-60nt(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	727	KIZ-p.R76*-ura3-60nt(tail):HIS3
2993	pRS315:LEU2 + KIZ-p.R76*-ura3-60nt(tail):HIS3	61	pRS315:LEU2	727	KIZ-p.R76*-ura3-60nt(tail):HIS3
2994	pYEST-DEST52-hADAR2:LEU2 +TRPM1-p.K294*-ura3 60nt(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	728	TRPM1-p.K294*-ura3-60nt(tail):HIS3
2995	pRS315::LEU2 +TRPM1-p.K294*-ura3-60nt (tail):HIS3	61	pRS315::LEU2	728	TRPM1-p.K294*-ura3-60nt(tail):HIS3
3003	pYEST-DEST52-hADAR2:LEU2 +FAM161A-p.R523*- ura3-60nt(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	736	FAM161A-p.R523*- ura3-60nt(tail):HIS3
3004	pRS315::LEU2 +FAM161A-p.R523*- ura3-60nt(tail):HIS3	61	pRS315::LEU2	736	FAM161A-p.R523*- ura3-60nt(tail):HIS3
3005	pYEST-DEST52-hADAR2:LEU2 + KIZ-R76W-URA3-BamHI:HIS3	641	pYEST-DEST52-hADAR2:LEU2	704	KIZ-R76W-URA3-BamHI:HIS3
3054	pYEST-DEST52-hADAR2:LEU2 + KIZ-p.R76*-ura3-30nt-GR(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	738	KIZ-p.R76*-ura3-30nt-GR(tail):HIS3
3055	pRS315::LEU2 + KIZ-p.R76*-ura3-30nt-GR:HIS3	61	pRS315::LEU2	738	KIZ-p.R76*-ura3-30nt-GR(tail):HIS3
3069	pYEST-DEST52-hADAR2:LEU2 + USH2A-p.W3955*-ura3-30nt-GR(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	744	USH2A-p.W3955*-ura3-30nt-GR(tail):HIS3
3070	pRS315::LEU2 + USH2A-p.W3955*-ura3-30nt-GR(tail):HIS3	61	pRS315::LEU2	744	USH2A-p.W3955*-ura3-30nt-GR(tail):HIS3
3071	pYEST-DEST52-hADAR2:LEU2 + USH2A-p.W3955*-ura3-60nt:HIS3	641	pYEST-DEST52-hADAR2:LEU2	745	USH2A-p.W3955*-ura3-60nt:HIS3
3072	pRS315::LEU2 + USH2A-p.W3955*-ura3-60nt:HIS3	61	pRS315::LEU2	745	USH2A-p.W3955*-ura3-60nt:HIS3
3073	pYEST-DEST52-hADAR2:LEU2 + USH2A-W3955W-URA3:HIS3	641	pYEST-DEST52-hADAR2:LEU2	741	USH2A-W3955W-URA3:HIS3
3097	pYEST-DEST52-hADAR2:LEU2 + KIZ-p.R76*-ura3-60nt mismatches(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	747	KIZ-p.R76*-ura3-60nt mismatches(tail):HIS3
3098	pRS315::LUE2 + KIZ-p.R76*-ura3-60nt mismatches(tail):HIS3	61	pRS315::LUE2	747	KIZ-p.R76*-ura3-60nt mismatches(tail):HIS3

3099	pYEST-DEST52-hADAR2:LEU2 +FAM161A-p.R523*- ura3-60nt mismatches (tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	748	FAM161A-p.R523*- ura3-60nt mismatches (tail):HIS3
3100	pRS315::LEU2 +FAM161A-p.R523*- ura3-60nt mismatches (tail):HIS3	61	pRS315::LEU2	748	FAM161A-p.R523*- ura3-60nt mismatches (tail):HIS3
3101	pYEST-DEST52-hADAR2:LEU2 +TRPM1-p.K294*-ura3 60nt mismatches(tail):HIS3	641	pYEST-DEST52-hADAR2:LEU2	749	TRPM1-p.K294*-ura3 60nt mismatches(tail):HIS3
3102	pRS315::LEU2 +TRPM1-p.K294*-ura3-60nt mismatches(tail):HIS3	61	pRS315::LEU2	749	TRPM1-p.K294*-ura3-60nt mismatches(tail):HIS3

Table S2-Target and Tails' sequence.

Name	Sequence
FAM161A-R523W	ATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgCCTGTGCCTTGTAACTGCAACCCTCCCGTGCCCACGGTATCTTCCAGAGGATGGGAACAAGCCGTAAGGAGATCACTTGAGGAAAAGAAAATGTTGGAAGAAtcgaaagctacatataaggaacgtgctgctactcatcctagtcctgttgctgccaagctatttaata
FAM161A-p.R523*	ATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgCCTGTGCCTTGTAACTGCAACCCTCCCGTGCCCACGGTATCTTCCAGAGGATGAGAACAAGCCGTAAGGAGATCACTTGAGGAAAAGAAAATGTTGGAAGAAtcgaaagctacatataaggaacgtgctgctactcatcctagtcctgttgctgccaagctatttaata
The 31nt+GR gRNA to the target FAM161A-p.R523*	gttacagaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaCTTACGGCTTGTTCcCATCCTCTGGAAGATAGGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCTAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAA
The 60nt gRNA to the target FAM161A-p.R523*	caaagggaagggatgctaaggtagagggtgaacgttacagaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaTCCTCAAGTGATCTCCTTACGGCTTGTTCcCATCCTCTGGAAGATACCGTGGGCACGGGAAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCGGGAATCTCGGTCGTAATGATT
The 60nt gRNA with mismatches to the target FAM161A-p.R523*	gaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaTCCTCAAGTGATCTCCTTACGaCaaGTgCcCAaCgTgTGcAAGATACCGTGGGCACGGGAAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGC
KIZ-R76W	TCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgAAGAATTATCTGAAGGAAATATGTGAATCTGAAAAGAAGGCTCATACTTGGAACCAAGAATATTTAAAGCGATTTGAGCGTGTCCAAGCTCATGTTGTACACtcgaaagctacatataaggaacgtgctgctactcatcctagtcctgttgc
KIZ-p.R76*	TCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgAAGAATTATCTGAAGGAAATATGTGAATCTGAAAAGAAGGCTCATACTTGAAACCAAGAATATTTAAAGCGATTTGAGCGTGTCCAAGCTCATGTTGTACACtcgaaagctacatataaggaacgtgctgctactcatcctagtcctgttgc
The 30nt+GR gRNA to the target KIZ-p.R76*	ttacagaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaAAATATTCTTGGTTcCAAGTATGAGCCTTCGGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCTAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAA
The 60nt gRNA to the target KIZ-p.R76*	caaagggaagggatgctaaggtagagggtgaacgttacagaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaCGCTCAAATCGCTTTAAATATTCTTGGTTcCAAGTATGAGCCTTCTTTTCAGATTCACATAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCGGGAATCTCGGTCGTAATGATT
The 60nt gRNA with mismatches to the target KIZ-p.R76*	gagggtgaacgttacagaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaCGCTCAAATCGCTTTAAATATgCacGccacCAtaccTcAgCCTTCTTTTCAGATTCACATAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAG
TRPM1-K294W	ATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgGTGATTTGTGATGGCAGCGGACGTGCCTCGGACATCCTGTCCTTTGCGCACTGGTACTGTGAAGAAGGCGGAATAATAAATGAGTCCCTCAGGGAGCAGCTTtcgaaagctacatataaggaacgtgctgctactcatcctagtcctgttgctgccaagctatttaata
TRPM1-p.K294*	ATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgGTGATTTGTGATGGCAGCGGACGTGCCTCGGACATCCTGTCCTTTGCGCACTAGTACTGTGAAGAAGGCGGAATAATAAATGAGTCCCTCAGGGAGCAGCTTtcgaaagctacatataaggaacgtgctgctactcatcctagtcctgttgctgccaagctatttaata

The 30nt+GR gRNA to the target TRPM1-p.K294*	gaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaCTTCTTCACAGTACcAGTGCGCAAAGGACAGGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCTAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAA
The 60nt gRNA to the target TRPM1-p.K294*	cgttacagaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaCATTTATTATTCCGCCTTCTTCACAGTACcAGTGCGCAAAGGACAGGATGTCCGAGGCACAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGC
The 60nt gRNA with mismatches to the target TRPM1-p.K294*	gaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaCATTTATTATTCCGCCTTCTTgAaAcTgCcAcTatGCgAAGGACAGGATGTCCGAGGCACAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGC
USH2A-W3955W	ATATATACGCATATGTGGTGTTGAAGAAACATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgGACGAATATCGGGTCAGAGCCTGTAACTCCAAGGGTTCAGTGGAGAGTCTGTGGTCATTAACACAAACTCTGGAAGCTCCACCTCAAGATTTTCCAGCTCCTtcgaaagctacataaggaacgtgctgctactcatcctagtcctgttgctgccaagctatttaatatcatgcacgaaaagcaaacaaacttgtgtgctt
USH2A-p.W3955*	ATATATACGCATATGTGGTGTTGAAGAAACATGAAATTGCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCatgGACGAATATCGGGTCAGAGCCTGTAACTCCAAGGGTTCAGTGGAGAGTCTGTAGTCATTAACACAAACTCTGGAAGCTCCACCTCAAGATTTTCCAGCTCCTtcgaaagctacataaggaacgtgctgctactcatcctagtcctgttgctgccaagctatttaatatcatgcacgaaaagcaaacaaacttgtgtgctt
The 30nt+GR gRNA to the target USH2A-p.W3955*	aaagggaagggatgctaaggtagagggtgaacgttacagaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaGTTTGTGTTAATGACcACAGACTCTCCACTGGGTGGAATAGTATAACAATATGCTAAATGTTGTTATAGTATCCCACCTAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCGGGAATCTCG
The 60nt gRNA to the target USH2A-p.W3955*	gaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaGTGGAGCTTCCAGAGTTTGTGTTAATGACcACAGACTCTCCACTGAACCCTTGGAGTTACAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGC
The 60nt gRNA with mismatches to the target USH2A-p.W3955*	gaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaaGTGGAGCTTCCAGAGTTTGTGTAACGGCCcAGCGTCGCTCCACTGAACCCTTGGAGTTACAAAACTGTATTATAAGTAAATGCATGTATACTAAACTCACAAATTAGAGC

Target gene - blue
Reverse complement “tails” - yellow
Tail with “recruitment element” (GR) - green
The position of targeted A - red
Plasmid homology ends - purple

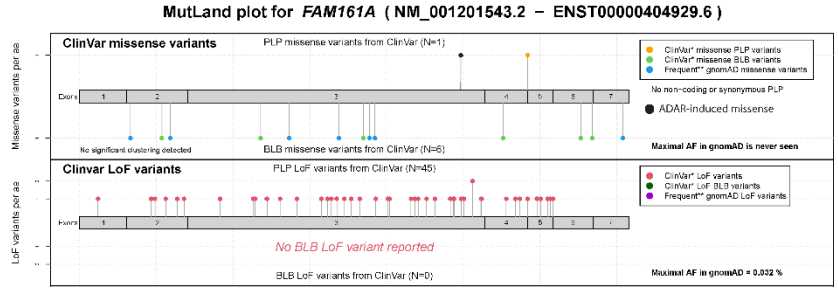
Table S3-NGS primers.

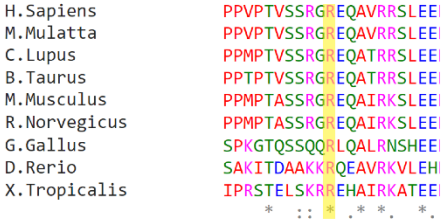
OSB ID	Name	Sequences
2795	Forward primer on the 60nt gRNA to the target FAM161A-p.R523* + sp p5 for deepseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**atgCCTGTGCCTTGTAAC**
2796	Forward primer on the 60nt gRNA to the target TRPM1-p.K294* + sp p5 for deepseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**atgGTGATTTGTGATGGCA**
2797	Forward primer on the 60nt gRNA to the target KIZ-p.R76* + sp p5 for deepseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**atgAAGAATTATCTGAAGGAAATATGT**
2798	Reverse primer (for all the target) on URA3 39bp downstream to start codon + sp p7 for deepseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**atgagtagcagcacgttc**
2840	Forward primer on the 60nt gRNA to the target USH2A-p.W3955* + sp p5 for deepseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**atgGACGAATATCGGGTCAGAG**
1202	Forward primer on URA3 173bp upstream to the A gRNA	GAGACGCATTGGGTCAACAG
502	Reverse primer on URA3 195bp downstream to the gRNA	CGACGGCCAGTGAATTGTAA
2366	Forward primer on URA3 300bp upstream to the target	aatttcacacaggaaacagctatg
207	Reverse primer on URA3 263bp downstream to the target	GTCAGCAAATTTTCTGTCTTCG
2874	Reverse primer for addition tail to the kiz's library with homologous to the 3' of URA3 in the BSB 704 plasmid.	GCTCTAATTTGTGAGTTTAGTATACATGCATTTACTTATAATACAGTTTT
2875	Forward primer for addition tail to the kiz's library with homologous to the 3' of URA3 in the BSB 704 plasmid.	gaaaagcaggctgggaagcatatttgagaagatgcggccagcaaaactaa

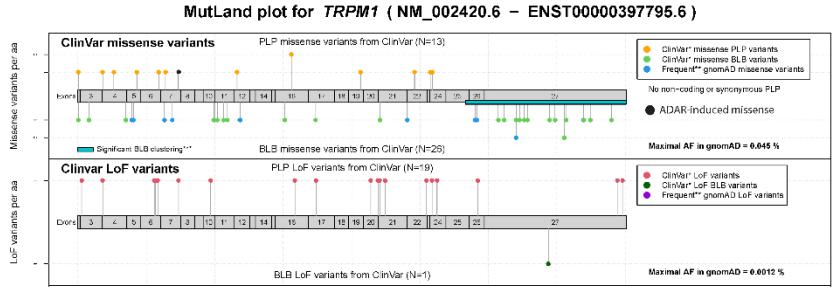
Table S4: Sequences of chemically modified gRNA

Key: (N)=RNA base, [N]=2'-OMe RNA base, *=Phosphorothioate linkage		
gRNA target	**Type**	**gRNA Sequence 5'-3'**
TRPM1 p.K294*	**60 bases**	[C*][A*][U*](UUAUUAUUCCGCCUUCUUCACAGUACCAGUGCGCAAAGGACAGGAUGUCCGAG)(G*)[C*][A*][C]
FAM161A p.R523*	**60 bases**	[T*][C*][C*](TCAAGTGATCTCCTTACGGCTTGTTCCCATCCTCTGGAAGATACCGTGGGCAC)(G*)[G*][G*][A]
KIZ p.R76*	**60 bases**	[C*][G*][C*](UCAAAUCGCUUUAAAUAUUCUUGGUUCCAAGUAUGAGCCUUCUUUUCAGAUUC)(A*)[C*][A*][U]
KIZ p.R76*	**"B4" 60 bases**	[C*][G*][C*](UGAAAUCGCUUUAAAUAUUCUUGGUUCCAAGUAUGUUCCUUCUUUUCAGAUUC)(A*)[C*][A*][U]
KIZ p.R76*	**"B12" 60 bases**	[C*][G*][C*](UCAAAUCGGUUCAAAUAUUCUUGGUUCCAAGUAUGUUCAUUCUUUUCAGAUUC)(A*)[C*][A*][U]
KIZ p.R76*	**"TT" 60 bases**	[C*][G*][C*](UCAAAUCGCUUUAAAUAUUCUUGGUUCCAAGUAUGUUCCUUCUUUUCAGAUUC)(A*)[C*][A*][U]
USH2A p.W3955*	**60 bases**	[G*][U*][G*](GAGCUUCCAGAGUUUGUGUUAAUGACcACAGACUCUCCACUGAACCCUUGGAG)(U*)[U*][A*][C]
USH2A p.W3955*	**30 bases + GR motif**	[G*][U*][U*][U*][GUGUUAAUGA](CCA)[CAGACUCUCCACU](GGG)[U](GGAA)[U](AG)[U](A)[U](AA)[C](AA)[U](A)[U](G)[CU](AAA)[U](G)[UU](G)[UU](A)[U](AG)[U](A)[UCCC](A)[C*][C*][U]

SUPPLEMENTAL FIGURES:







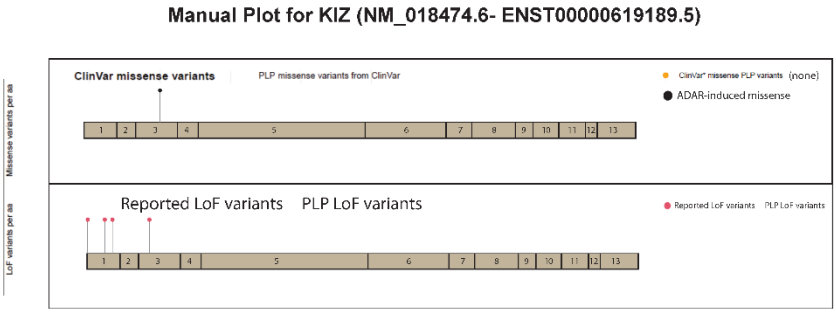


Figure S1. MutLand analysis and conservation of mutations in three genes. Left pane: MutLand plots for *FAM161A*, *TRPM1*, and *KIZ* (created manually due to lack of proper Annovar annotation for the *KIZ* gene) showing exonic location of pathogenic or likely pathogenic missense variants in these genes, as well as Clinvar LoF variants. The black circle was manually added to each plot to show where the ADAR-induced missense mutation would be found in each gene. Right pane: Conservation of amino acids in the regions flanking the ADAR targeted codon for each gene with the ADAR-edited amino acid highlighted in yellow

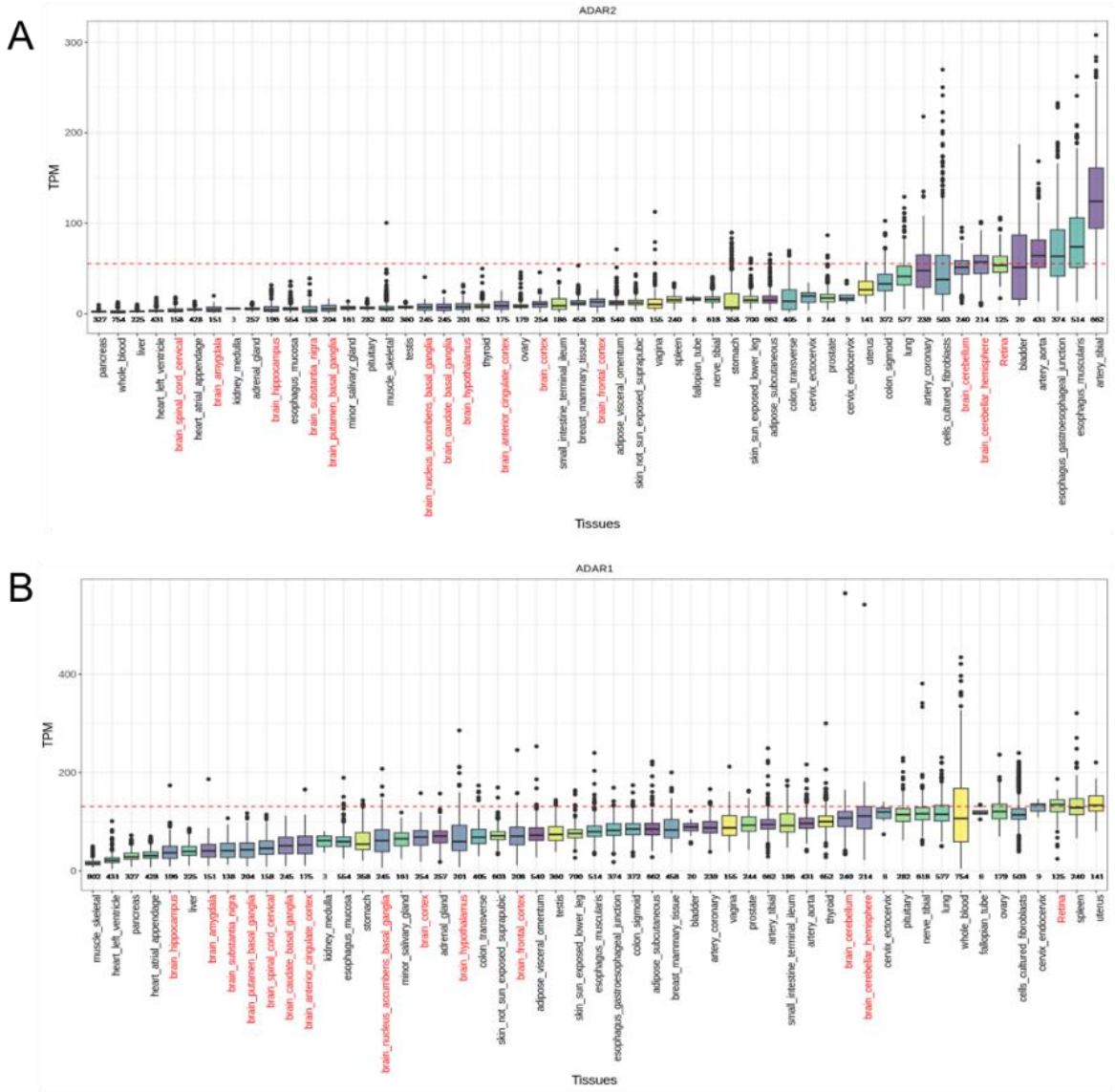
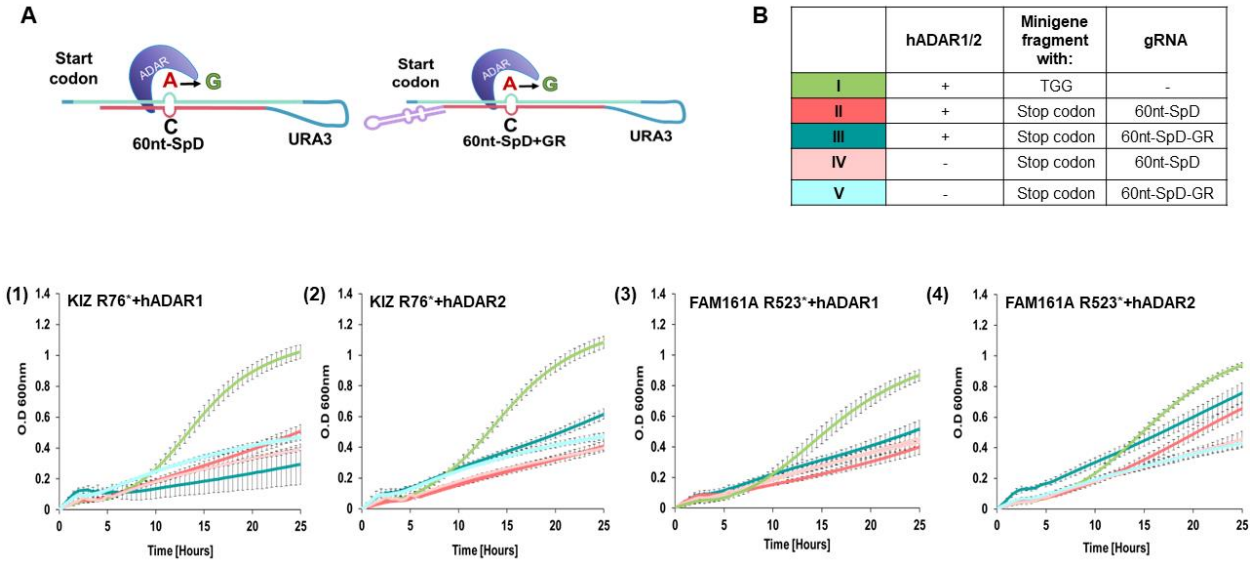

A
ADAR2
TPM
Tissues
B
ADAR1
TPM
Tissues
C
Editing Level
Neuronal tissues
Other tissues
Retina
AZIN1 chr6:102829408
AZIN1 chr8:102829409
BLCAP chr20:37519131
BLCAP chr20:37519161
BLCAP chr20:37519170
CACNA1D chr3:53786865
CADPS chr3:62438132
COG3 chr13:45516236
COPA chr1:160332454
CYFIP2 chr5:157309800
FLNA chrX:154351582
FLNB chr3:58156074
GABRA3 chrX:152189847
GRIA2 chr4:157336723
GRIA2 chr4:157336727
GRIA2 chr4:157360142
GRIA3 chrX:123465111
GRIA4 chr11:105933968
GRIK1 chr21:29581430
GRIK2 chr6:101889814
GRIK2 chr6:101889827
GRIK2 chr6:101924714
NOVA1 chr14:26448324
SON chr21:33550495
SON chr21:33551013
TMEM63B chr6:44152612
UNC80 chr2:209970889

Figure S2. Expression and editing levels of ADAR in the retina compared to various tissues. The presented analysis is based on data retrieved from the GTEx project [1] and a downloaded dataset (Bio Project: PRJNA476171, only samples exhibiting MGS level of zero or one were included) used for the retinal-related data. A&B). Expression levels of hADAR2 **(A)** and hADAR1 **(B)** across different tissues. Expression levels are depicted as transcript per million (TPM). The number of samples included for each tissue is indicated at the bottom of each graph. The dashed red line represents the mean expression levels in the retina. Neuronal tissues are highlighted in red. **(C)** Editing levels of conserved editing sites across 48 tissues. Editing levels for the retina were calculated based on the above-mentioned dataset, and editing levels for the other tissues were retrieved from [2]. Retinal and neuronal tissue-related data are marked in red and yellow, respectively. Each dot represents the combined data from all samples derived from a given tissue.

1. GTEx Consortium (2013). The Genotype-Tissue Expression (GTEx) project. Nat. Genet. *45*, 580–585. 10.1038/ng.2653.

2. Gabay, O., Shoshan, Y., Kopel, E., Ben-Zvi, U., Mann, T.D., Bressler, N., Cohen-Fultheim, R., Schaffer, A.A., Roth, S.H., Tzur, Z., et al. (2022). Landscape of adenosine-to-inosine RNA recoding across human tissues. Nat. Commun. *13*, 1184. 10.1038/s41467-022-28841-4.



	hADAR1/2	Minigene fragment with:	gRNA
I	+	TGG	-
II	+	Stop codon	60nt-SpD
III	+	Stop codon	60nt-SpD-GR
IV	-	Stop codon	60nt-SpD
V	-	Stop codon	60nt-SpD-GR

Figure S3. No improvement in the growth of *KIZ*-p.R76* and *FAM161A* p.R523* mutants upon elongation of the 30nt specificity domain to 60nt. The experiments presented here are similar to those presented in Figure 3, however in the current experiments a longer specificity domain of 60nt (60nt-SpD-GR) was used instead of 30nt-SpD-GR gRNA .

A

B

	hADAR1/2	Minigene fragment with:	gRNA	Mismatches
I	+	Stop codon	60nt-SpD	-
II	+	Stop codon	60nt-SpD	+

C

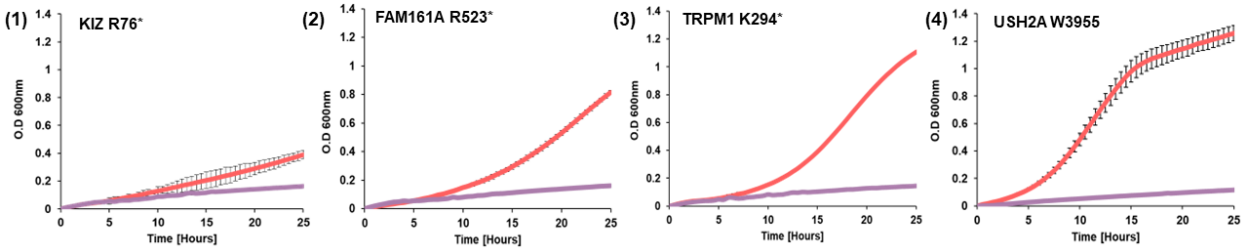


Figure S4. hADAR2-mediated editing of selected IRD mutations depends on the dsRNA **structure formed by the gRNA. (A)** Schematic representation of the dsRNA structure formed when the "tail" containing the selected gRNA sequences folds back at the RNA level on the target to form the dsRNA structure for ADAR recruitment, and of the control experiment that in addition to the A-C mismatch, carried additional mismatches around the target A. (B) A table describing the contents of the strains (denoted as I-II) tested in panels C-F. **(C)** Growth curves of the indicated strains were produced as described in Figure 2D.

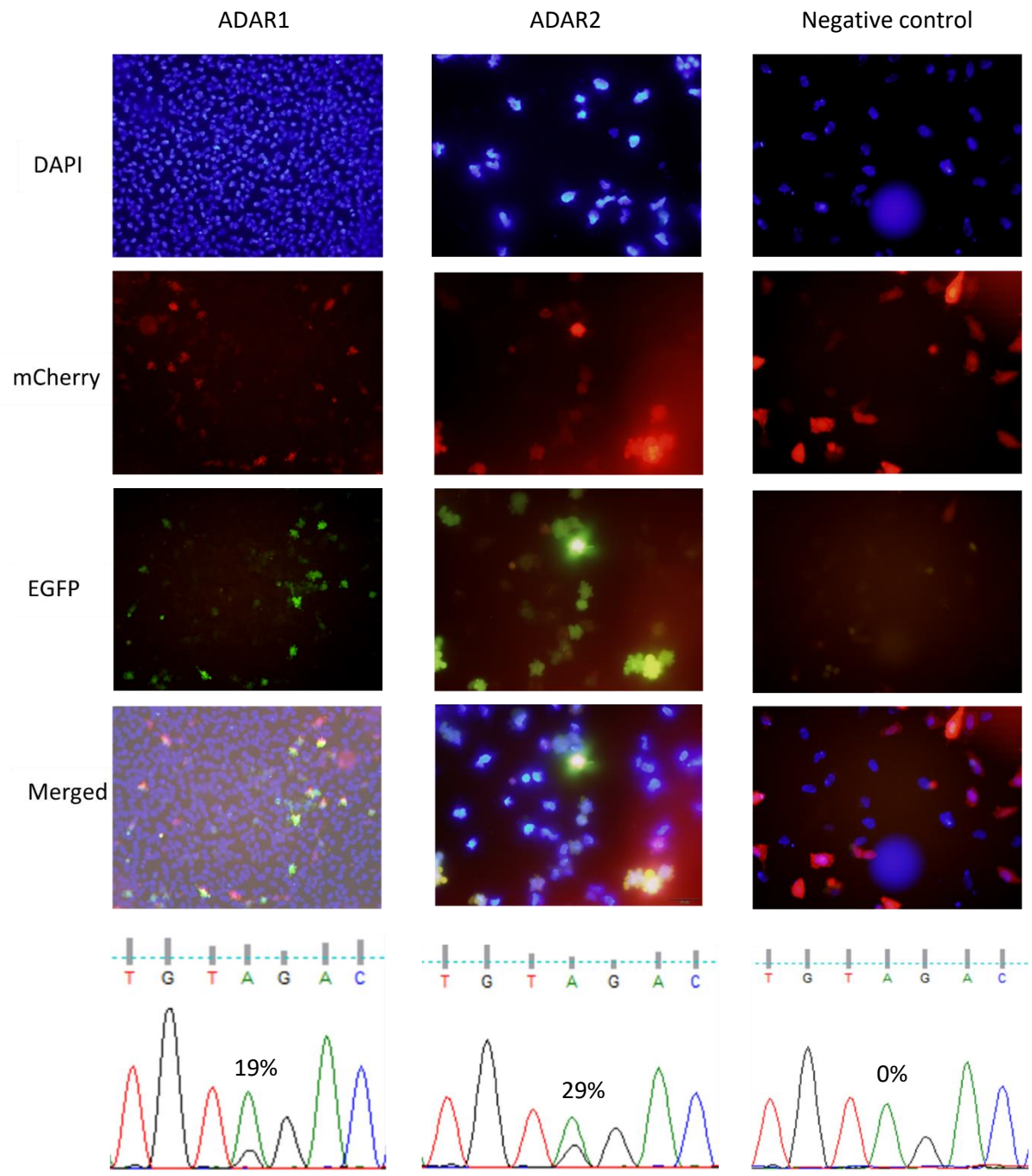


Figure S5: Confirmation of the reporter system. Representative images from samples of ADAR1 p.110 (ADAR1) and ADAR2 overexpressing HeLa cells transfected with both the 3'-UTR *GAPDH* nonsense mutation reporter plasmid and chemically modified gRNA (18 bp long complementarity region and a 55 bp GR motif tail) or no gRNA (negative control) at 96 hours post-seeding using fluorescent microscopy as well as representative Sanger sequences.

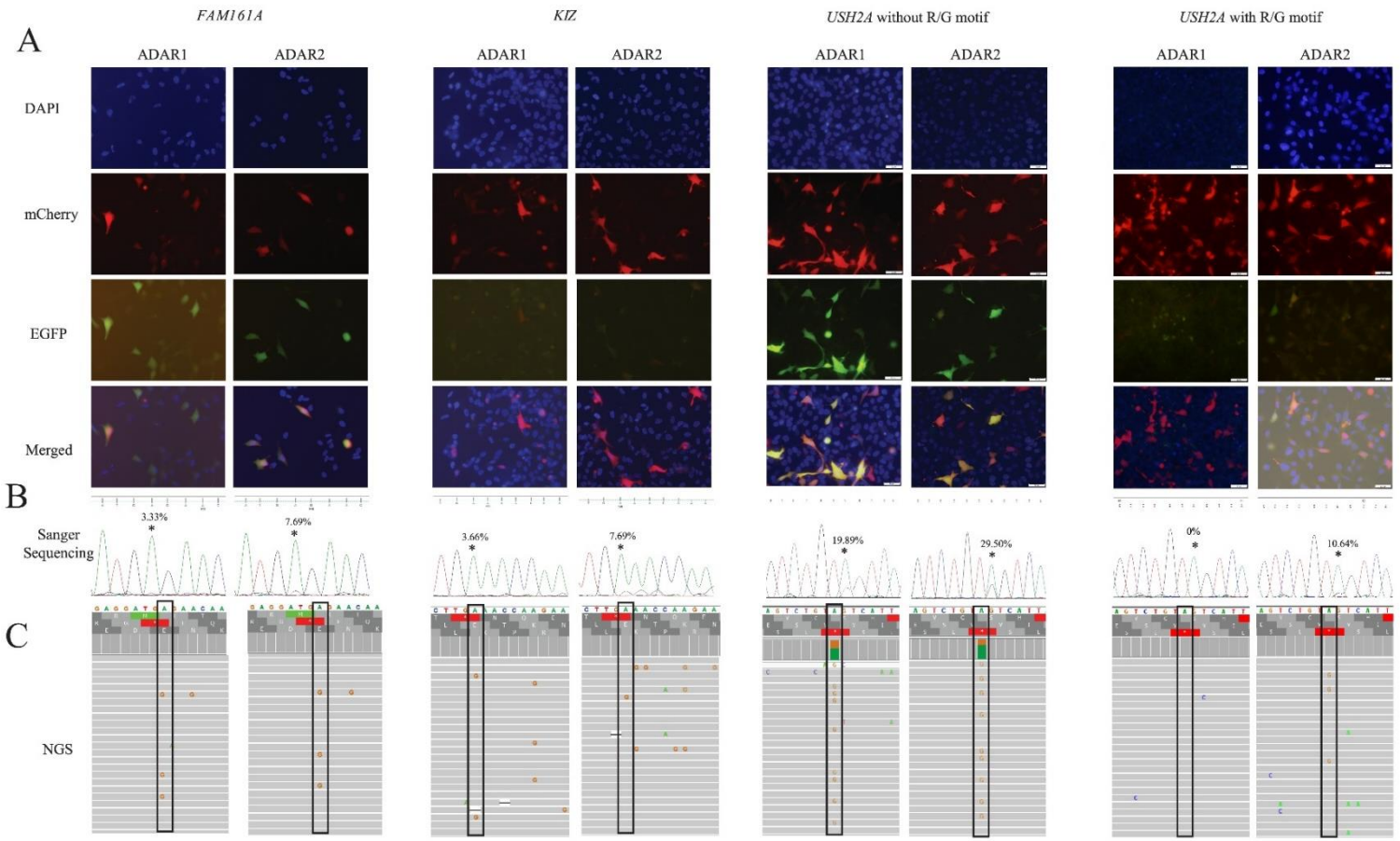


Figure S6: RNA editing of three IRD-causing mutations. Representative images from samples of ADAR1 p.110 (ADAR1) and ADAR2 overexpressing HeLa cells transfected with either the *FAM161A*, *KIZ*, or *USH2A* (with and without GR motif) nonsense mutation reporter plasmid and 60-mer chemically modified gRNA at 96 hours post-seeding. **(A)** Fluorescent microscopy **(B)** Sanger sequence **(C)** Next generation sequencing reads in IGV viewer

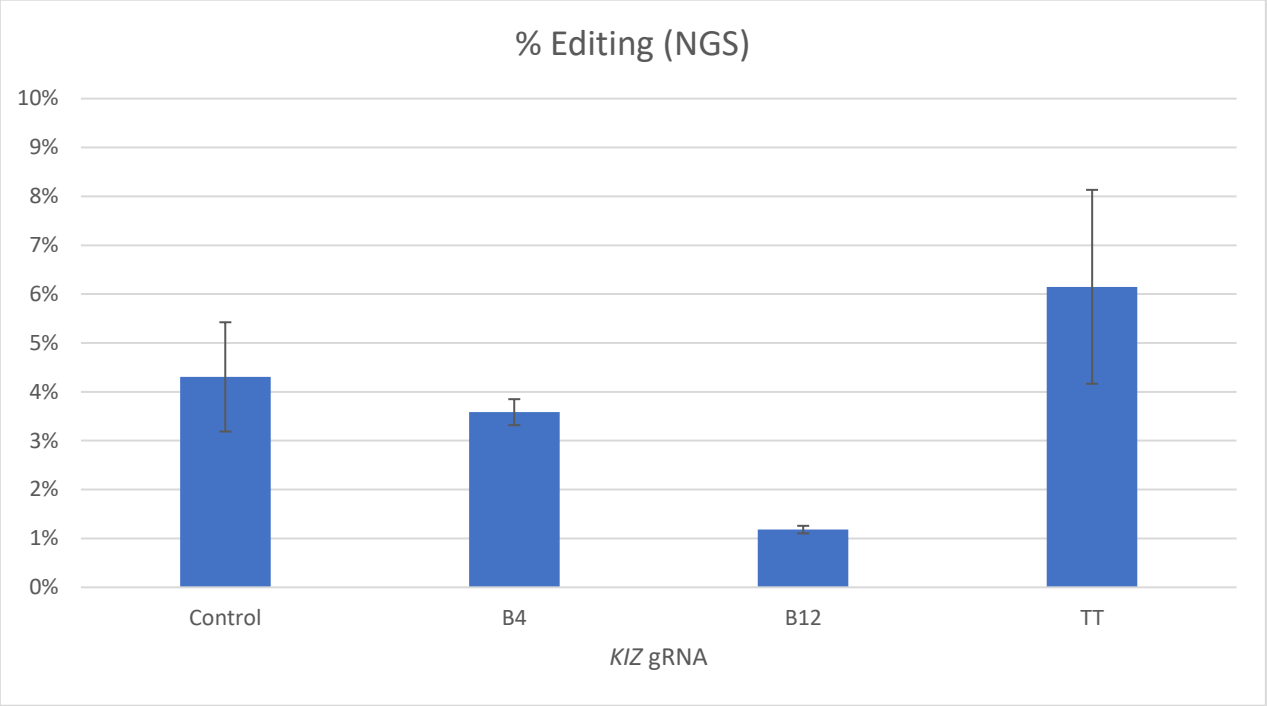


Figure S7: Editing levels of selected gRNAs. Editing levels of adenosine target in the *KIZ* minigene in ADAR2-expressing HeLa cells after introduction of B4, B12, or TT gRNA with multiple mismatches, corrected for background. Results are mean ± standard error of the mean (SEM n = 3).

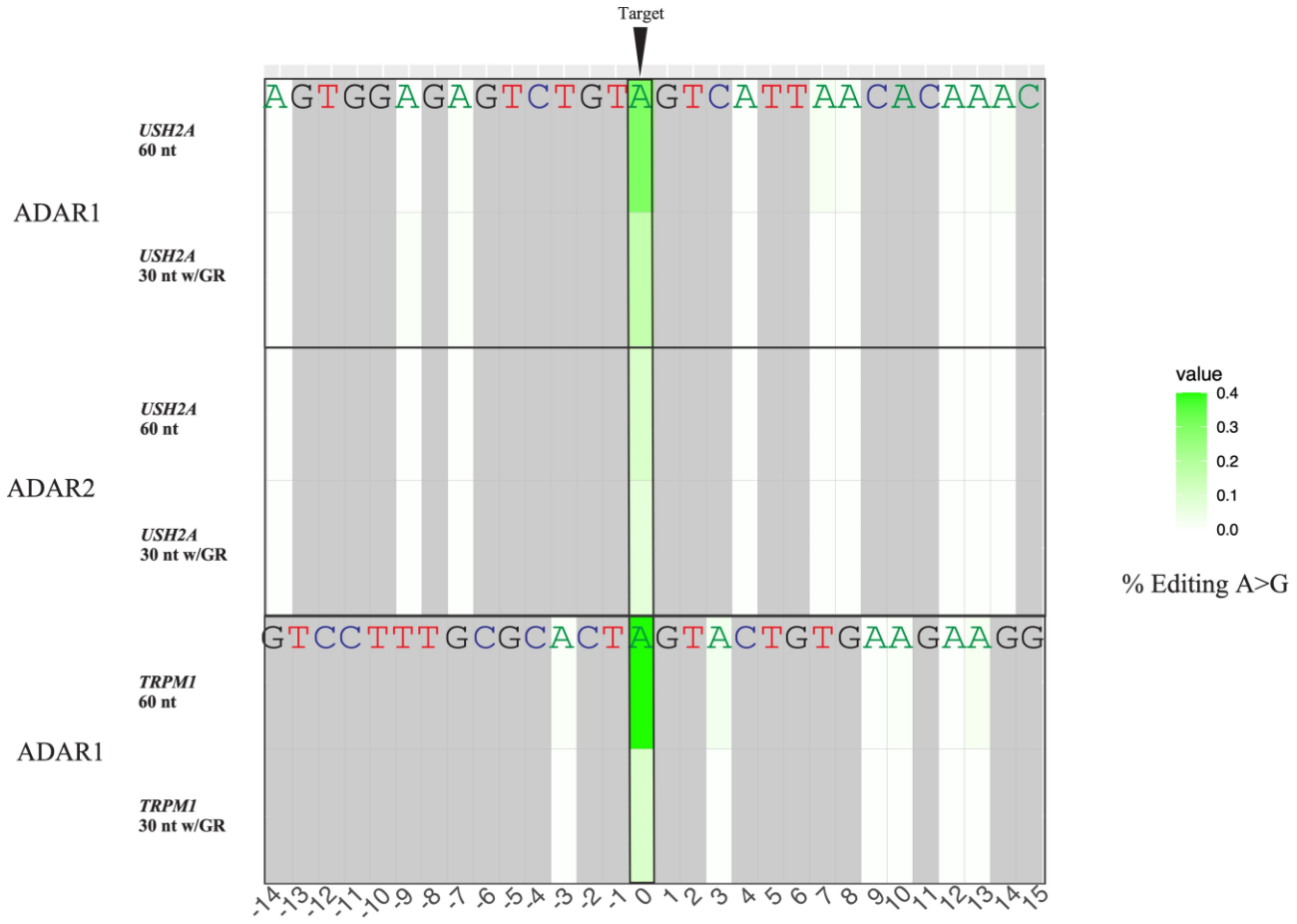


Figure S8: Heatmap off-target analysis of three IRD-causing mutations. Using shorter gRNAs can reduce bystander editing, but at the cost of potentially negatively impacting the target adenosine. Heatmap of average off-target and on-target editing rate for adenosines in the indicated strains. Average editing levels are shown are the result of NGS analysis (n=3). Positions below the heatmap are relative to the target adenosine (0), bases in grey are non-adenosine bases while adenosines with 0% editing are white gradually increasing to deep green in correlation with increasing editing levels.