Supporting Information for:

Impact of Disease-Associated Mutations on the Deaminase Activity of ADAR1

Agya Karki[1], Kristen B Campbell[1], Sukanya Mozumder[1,2], Andrew J. Fisher[1,2] & Peter A. Beal*[1]

[1]Department of Chemistry, University of California, Davis, CA, USA 95616.

[2]Department of Molecular and Cellular Biology, University of California, Davis, CA, USA 95616.

*Corresponding author, Email: pabeal@ucdavis.edu

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Table S1. Oligonucleotide sequences used in this work. All bases are ribonucleotides unless specified. **N** is 8-azanebularine.

Oligonucleotide Name	Sequence
32 nt 8-azanebularine top	5'- GCUCGCGAUGCU**N**GAGGGCUCUGAUAGCUACG -3'
32 nt bottom	5'- CGUAGCUAUCAGAGCCCCCCAGCAUCGCGAGC-3'
(3'end Cy5 label) 61 mer 8-azanebularine top	5'- AGCAAGUCCACGUGCAUGGCUCGCGAUGCU**N**GAGGGCUC UGAUAGCGGAUGGACAUCGACG**Cy5** -3'
61 mer bottom	5'- CGUCGAUGUCCAUCCGCUAUCAGAGCCCCCCAGCAUCGC GAGCCAUGCACGUGGACUUGCU -3'
23 nt 8-azanebularine top	5'- GCUCGGAGAAUU**N**GCGGGUCGUG -3'
23 nt bottom	5'- CACGACCCGCCAAUUCUCCGAGC -3'
5HT$_{2C}$R 332 nt	5'- UGGGUACGAAUUCCCACUUACGUACAAGCUUACCUAGAU AUUUGUGCCCCGUCUGGAUUUCUUUAGAUGUUUUAUUUUC AACAGCGUCCAUCAUGCACCUCUGCGCUAUAUCGCUGGAUC GGUAUGUAGCAAU**A**CGUAAUCCUAUUGAGCAUAGCCGUUU CAAUUCGCGGACUAAGGCCAUCAUGAAGAUUGCUAUUGUU UGGGCAAUUUCUAUAGGUAAAUAAAACUUUUUGGCCAUAA GAAUUGCAGCGGCUAUGCUCAAUACUUUCGGAUUAUGUAC UGUGAACAACGUACAGACGUCGACUGGUAACAUUUGCGUU UGAUCGGGUUCU -3'
hGli1 155 nt	5'- CAGAACUUUGAUCCUUACCUCCCAACCUCUGUCUCUCUG UCUACUCACCACAGCCCCCCAGCAUCACUGAGAAUGCUGCC AUGGAUGCU**A**GAGGGCUACAGGAAGAGCCAGAAGUUGGGA CCUCCAUGGUGGGCAGUGGUCUGAACCCCUAUAUG -3'

Table S2. Observed and calculated masses of oligonucleotides.

Oligonucleotide Name	Observed Mass (m/z)	Expected mass (a.m.u.)
32 nt 8-azanebularine top	10329	10328
32 nt bottom	10203	10206
(For 61 bp, top 5'fragment) 38 nt 8-azanebularine	12303	12304
(For 61 bp, top 3'fragment) 23 nt	6994	6991
23 bp 8-azanebularine top	7456	7446
23 bp bottom	7250	7241

Table S3. RT-PCR and sequencing DNA primers used for in vitro deamination in this work.

Oligonucleotide Primers	Sequence
5HT$_{2C}$R RT Forward	5'- TGGGTACGAATTCCCACTTACGTACAAGCTT -3'
5HT$_{2C}$R Reverse	5'- AGAACCCGATCAAACGCAAATGTTAC -3'
hGli1 RT Forward	5'- TAATACGACTCACTATAGGGCAGAACTTTGATCCTTACCTC -3'
hGli1 Reverse	5'- CATATAGGGGTTCAGACCACTG -3'

Table S4. Primers used for site-directed mutagenesis of ADAR1 R3D, ADAR1d, ADAR1 p110.

Mutant	Sequence
Y1112F Forward	5'- GGTTGGTAGAGTTTCTATCTTCGACTCTAAGAGACAATCTGG -3'
Y1112F Reverse	5'- CCAGATTGTCTCTTAGAGTCGAAGATAGAAACTCTACCAACC -3'
G1007R Forward	5'- GTTGAGAACTAAGGTTGAAAACAGAGAAGGTACTATCCCAGTTGAATC -3'
G1007R Reverse	5'- GATTCAACTGGGATAGTACCTTCTCTGTTTTCAACCTTAGTTCTCAAC -3'
K999N Forward	5'- CGAAAACCCAAAGCAAGGTAACTTGAGAACTAAGGTTGAAAAC -3'
K999N Reverse	5'- GTTTTCAACCTTAGTTCTCAAGTTACCTTGCTTTGGGTTTTCG -3'
R892H Forward	5'- GTTTCTTTGGGTACTGGTAACCACTGTGTTAAGGGTGACTCTTTGTC -3'
R892H Reverse	5'- GACAAAGAGTCACCCTTAACACAGTGGTTACCAGTACCCAAAGAAAC -3'

Table S5. Sequencing DNA primers for ADAR1 p110, ADAR1 R3D, and ADAR1d constructs.

Oligonucleotide Primers	Sequence
Galpromoterfor	5'- ATGTAAAGAGCCCCATTATCTTAGCC -3'
Zdomainfor	5'- CTACACCACCAATTTGGCATTTGACTG -3'
ADAR1 R3D for	5'- GAGCTATTATGGAAATGCCATCCTTC-3'
ADAR1 R2D for	5'- CAAACTCCAACACCATCTGCTAC -3'
ADAR1 R1D for	5'-CACTAATCCAGTTGGTGGTTTATTGG-3'
ADAR1d for	5'-TTGCCATTGACTGGTTCTACTTTCCAC-3'
ADAR1d for 2	5'- GGGTGAAAGATTGAGAACTATGTC-3'
Intein CBD for	5'- CTTCTACTTGTGTCCAGTTGCAATG -3'
Vector rev	5'- TTTTCTCGGGCAGATCTTTGTC -3'

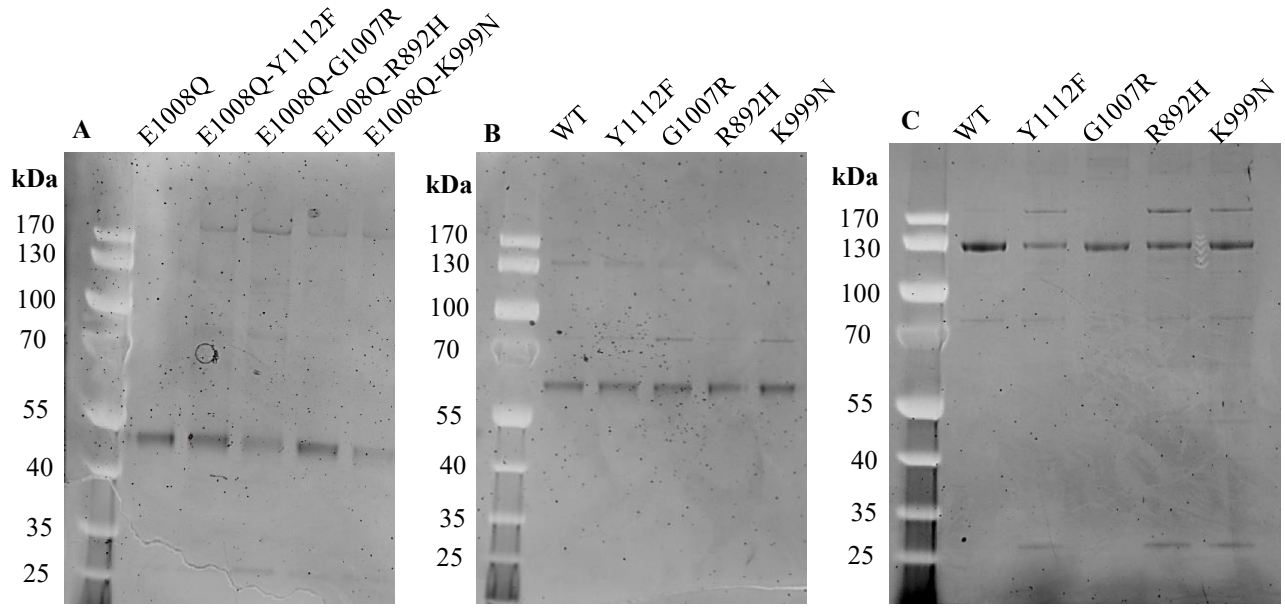

Figure S1. SDS-PAGE gels for three ADAR1 constructs (**A**) ADAR1d = 44 kDa (**B**) ADAR1 R3D = 58 kDa, and (**C**) ADAR1 p110 = 105 kDa overexpression and purification. The first lane of each gel was loaded with 10 uL of PageRuler Prestained Ladder from ThermoFisher and each lane to the right of the ladder was added ~ 0.5 ug of ADAR1 construct in an Invitrogen NuPAGE 4-12% Bolt Bis-Tris Plus gel. Gel components consisted of 50 mM MOPS, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.7 and the gel was run at 200V for 45 minutes.

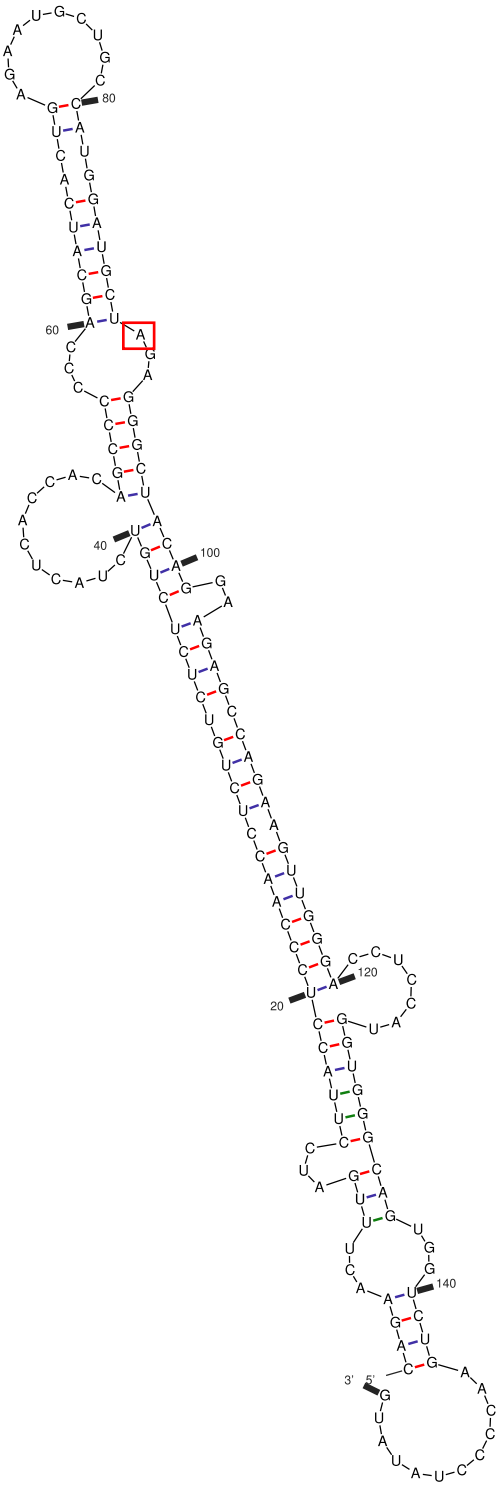

Figure S2. Predicted secondary structure for hGli1-derived substrate RNA. Target adenosine is highlighted with red box.

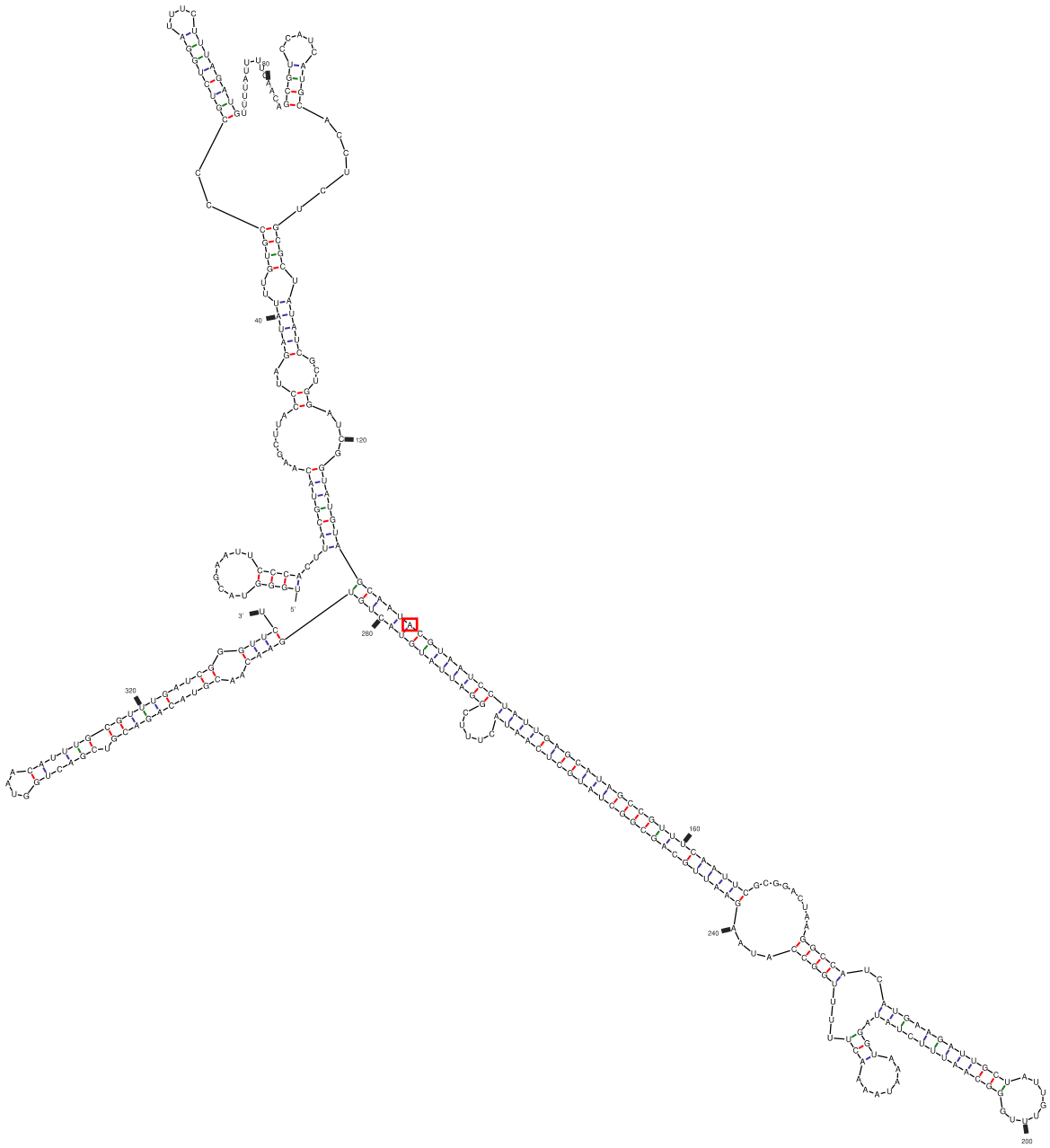

Figure S3. Predicted secondary structure for 5-HT_{2c}R-derived substrate RNA. Target adenosine is highlighted with red box.

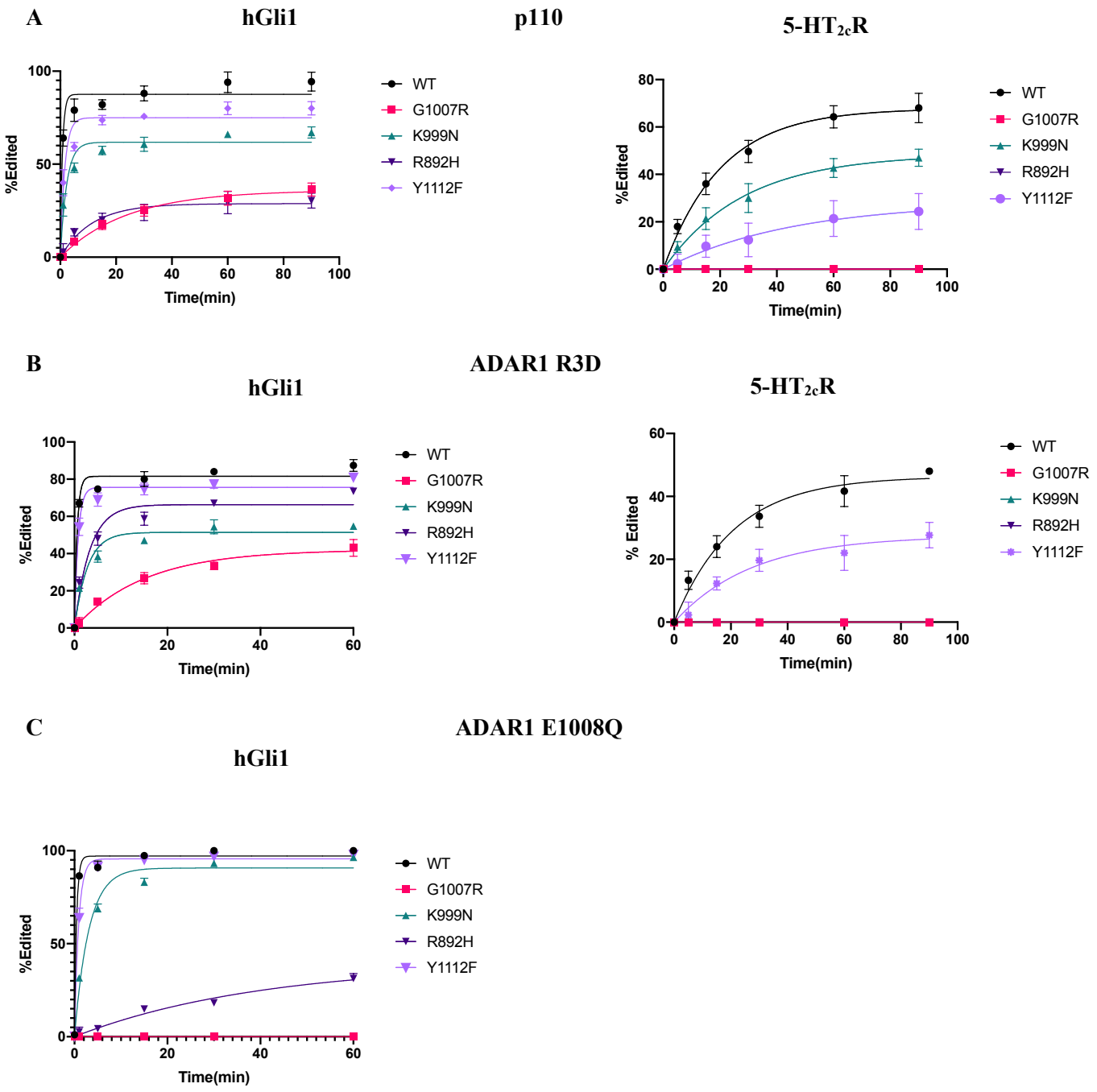

Figure S4. Progress curves for reactions of **(A)** full-length p110, **(B)** ADAR1 R3D, and **(C)** ADAR1d E1008Q with hGli1 and 5HT$_{2c}$R substrate, B site. Reactions were performed under single turn over conditions with 100 nM [enzyme] and 10 nM [substrate]. Plotted values are the means of three technical replicates ± standard deviation for each time point.

A

5'-AGCAAGUCCACGUGCAUGGCUCGCGAUGCU**N**GAGGGCUCUGAUAGCGGAUGGACAUCGACGCy5-**3'**
3'-UCGUUCAGGUGCACGUACCGAGCGCUACGACCCCCCGAGACUAUCGCCUACCUGUAGCUGC-**5'**

B

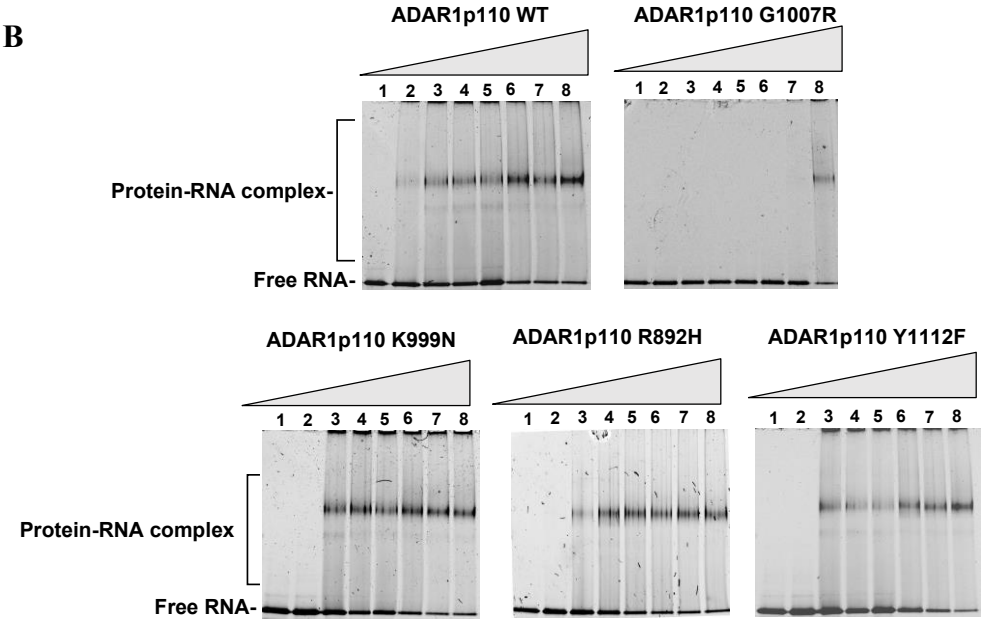

Figure S5. Binding of AGS mutants of ADAR1 p110 to a 61 bp duplex bearing 8-azanebularine (N) analyzed by EMSA. **(A)** Sequence of 61 bp RNA duplex with the adenosine analog 8-azanebularine (N). N allows for trapping of the protein-RNA complex in the base-flipped conformation [28,29]. The sequence is labeled with Cy5 at the 3' end. **(B)** Representative EMSA gels of p110 WT and AGS mutants (G1007R, K999N, R892H, and Y1112F) with the 61 bp duplex.

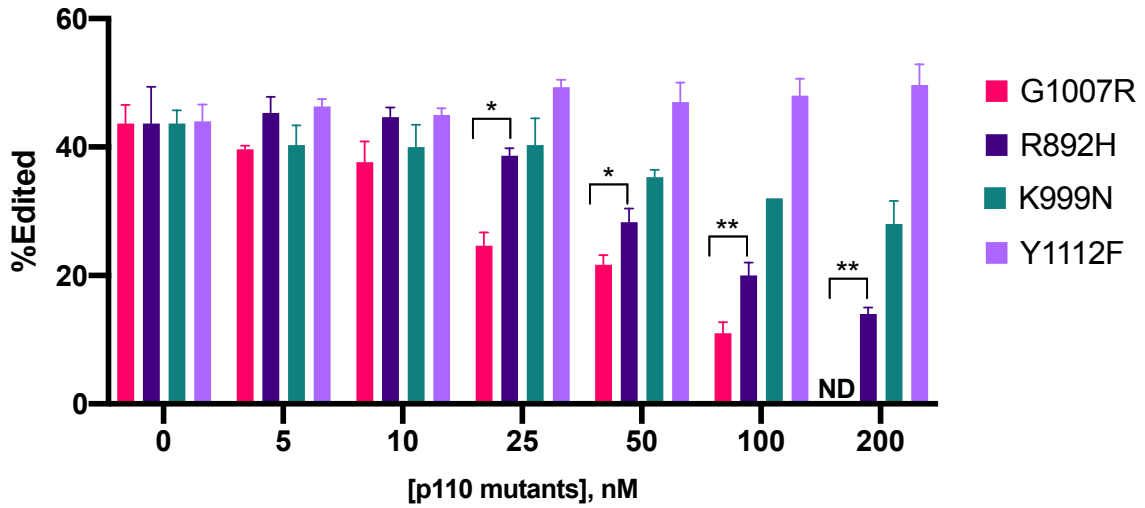

Figure S6. G1007R is a competitive inhibitor of ADAR1 p110 wild-type. Inhibition of ADAR1 p110 with select AGS mutants (G1007R, R892H, K999N and Y1112F) tested on the 5-HT_{2c}R substrate. In vitro deamination was performed with 10nM substrate, 50 nM wild-type and 0, 5, 10, 25, 50, 100 and 200 nM p110 AGS mutants at 30°C. Reaction was initiated by the addition of substrate and quenched after 30 min. Error bars represent n ≥ 3 technical replicates.

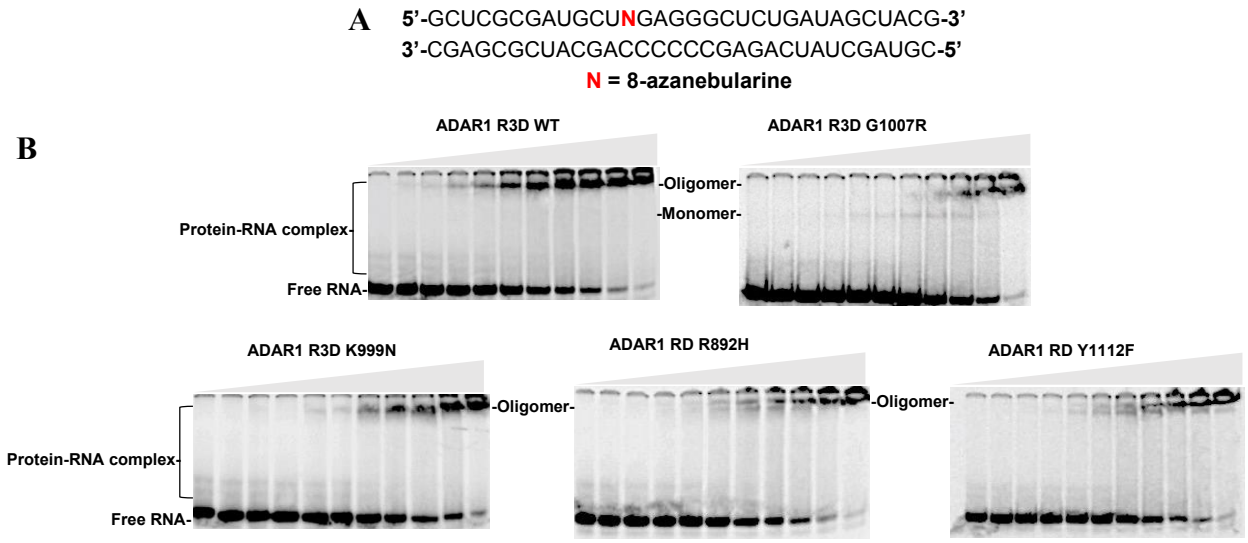

Figure S7. Binding of AGS mutants of ADAR1 R3D to a 32 bp duplex bearing 8-azanebularine (N) analyzed by quantitative EMSA. **(A)** Sequence of 32 bp duplex bearing 8-azanebularine (N). **(B)** Representative EMSA gels for ADAR1 R3D WT and AGS mutants (G1007R, K999N, R892H, and Y1112F). Reaction was performed at 1.1 nM 32 bp duplex and protein concentration was varied from 0 to 128 nM.

A 23 bp duplex

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5′- GCUCGGAGAAUANGCGGGUCGUG -3′
3′- CGAGCCUCUUAUCCGCCCAGCAC -5′
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B

N = 8-azaN

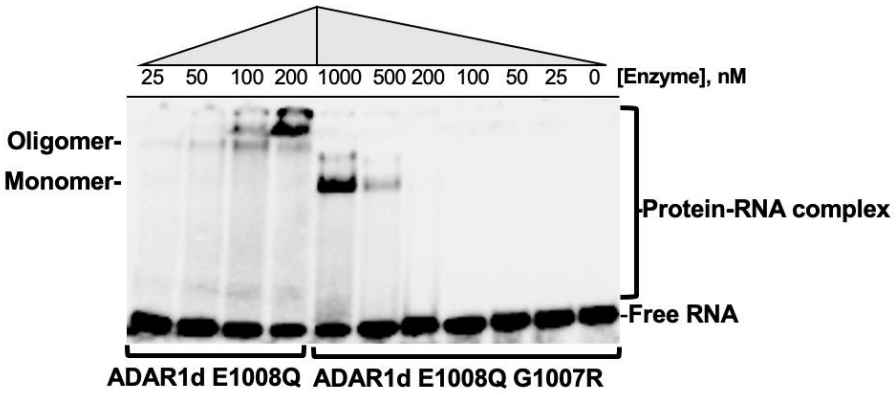

Figure S8. EMSA of ADAR1d E1008Q and ADAR1d E1008Q G1007R with a 23 bp duplex bearing 8-azaN. **A**) Sequence of 23 bp duplex used. **B**) EMSA gel with ADAR1d E1008Q and ADAR1d E1008Q G1007R. Reaction was performed at 5 nM 8-azaN-containing 23 bp duplex and protein concentrations [ADAR1d E1008Q]: 25 nM, 50 nM, 100 nM, 200 nM and [ADAR1d E1008Q G1007R]: 1000 nM, 500nM, 200 nM, 100nM, 50 nM and 25 nM.