Supporting information:

Nucleoside Analogs in ADAR Guide Strands Targeting 5'-UA sites

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Table S1. Average χ angle of the -1 position across ADAR2 crystal structures (5ED1, 5ED2, 5HP2, 5HP3, 6D06, 6VFF, 7KFN), A-form -1 position, and the average of other adenosines in ADAR2d WT Bdf2-U structure (5HP2).

Measured Adenosine	χ angle
Average -1 position	-90 ± 6
A-Form -1 position	-157
Average of other adenosines	-160 ± 7

Table S2. The effect of -1 gRNA modifications on fitted end points and observed rate constants for ADAR2 deamination reactions with *MECP2.* [a]

-1 modification [a]	Fitted end point (%)[b]	k_{obs} (min^{-1})[b]	k_{rel}[c]
Adenosine	96.2 ± 0.6	0.3 ± 0.1	1
dA	95.2 ± 0.1	0.7 ± 0.3	2.3
2'-O-Me A	93.8 ± 1.0	0.4 ± 0.2	1.3
LNA A	no reaction	no reaction	no reaction
UNA A	87.2 ± 0.7	0.061 ± 0.006	0.2
2'-F A	94 ± 1	0.18 ± 0.02	0.6
FANA A	94.6 ± 0.3	1.0 ± 0.1	3.3
β-L-dA	78.6 ± 0.9	0.031 ± 0.003	0.1
C3 spacer	55.1 ± 0.5	0.17 ± 0.02	0.6
2-aminopurine	90 ± 2	0.071 ± 0.008	0.2
2,6-diaminopurine	90 ± 1	0.076 ± 0.003	0.3
dI	93.0 ± 0.6	0.42 ± 0.04	1.4
dN	94.7 ± 0.6	0.59 ± 0.09	2.0
7-deaza-dA	94.5 ± 0.2	0.68 ± 0.07	2.3
3-deaza-dA	90.2 ± 0.4	0.5 ± 0.1	1.7
dC	76 ± 1	0.0317 ± 0.0008	0.1
Abasic	53.9 ± 1.0	0.051 ± 0.001	0.2
dN -1, LNA -6	92.8 ± 0.2	1.72 ± 0.07	5.7
Orphan dZ	69 ± 2	0.41 ± 0.09	1.4
Orphan dZ, dN -1	74.7 ± 0.6	1.4 ± 0.2	4.7

[a] Reactions were carried out with 5 nM of target RNA and 15 nM ADAR2 WT

[b] Reactions were fitted to the equation $[P]_t = \alpha[1 - \exp(-k_{obs} \cdot t)]$

[c] $k_{rel} = k_{obs}$ for modification/k_{obs} for adenosine

Table S3. The effect of -1 gRNA modifications on observed rate constants for ADAR1 deamination reactions with *MECP2*. [a]

-1 modification [a]	k_{obs} x10^{-2} (min^{-1}) [b]	k_{rel} [c]
Adenosine	0.22 ± 0.02	1
dA	1.2 ± 0.2	5.5
2'-O-Me A	0.17 ± 0.02	0.77
LNA A	no reaction	no reaction
UNA A	no reaction	no reaction
2'-F A	0.11 ± 0.01	0.50
FANA A	0.79 ± 0.04	3.6
dN	4.3 ± 0.7	20
7-deaza-dA	1.05 ± 0.06	4.8
Orphan dZ	0.36 ± 0.01	1.6
Orphan dZ, dN -1	1.89 ± 0.07	8.6

[a] Reactions were carried out with 15 nM of target RNA and 150 nM ADAR1 p110
[b] Reactions were fitted to the equation $[P]_t = \alpha[1 - \exp(-k_{obs} \cdot t)]$. End point was constrained to 65%
[c] $k_{rel} = k_{obs}$ for modification/k_{obs} for adenosine

Table S4. The effect of -1 gRNA modifications on fitted end points and observed rate constants for ADAR2 deamination reactions with *IDUA*. [a]

-1 modification [a]	Fitted end point[b]	k_{obs} (min^{-1})[b]	k_{rel} [c]
Adenosine	94.6 ± 0.4	0.59 ± 0.01	1
dA	95.6 ± 0.1	0.6 ± 0.2	1
dN	92.3 ± 0.7	1.2 ± 0.2	2.0
7-deaza-dA	94.7 ± 0.4	0.59 ± 0.03	1.0

[a] Reactions were carried out with 5 nM of target RNA and 15 nM ADAR2 WT
[b] Reactions were fitted to the equation $[P]_t = \alpha[1 - \exp(-k_{obs} \cdot t)]$
[c] $k_{rel} = k_{obs}$ for modification/k_{obs} for adenosine

Table S5. The effect of -1 gRNA modifications on observed rate constants and fitted end points for ADAR1 deamination reactions with *IDUA*. [a]

-1 modification [a]	Fitted end point[b]	k_{obs} x10^{-2} (min^{-1})[b]	k_{rel} [c]
Adenosine	66 ± 2	4.1 ± 0.2	1
dA	66 ± 2	4.2 ± 0.3	1.0
dN	79 ± 1	17.5 ± 0.4	4.3
7-deaza-dA	73 ± 1	8.0 ± 0.8	2.0

[a] Reactions were carried out with 15 nM of target RNA and 150 nM ADAR1 p110
[b] Reactions were fitted to the equation $[P]_t = \alpha[1 - \exp(-k_{obs} \cdot t)]$
[c] $k_{rel} = k_{obs}$ for modification/k_{obs} for adenosine

Table S6. The effect of -1 gRNA modifications on observed rate constants and fitted end points for ADAR2 deamination reactions with *ACTB*. [a]

-1 modification [a]	Fitted end point[b]	k_{obs} (min^{-1})[b]	k_{rel}[c]
Adenosine	96.9 ± 0.2	0.39 ± 0.03	1
dA	97.5 ± 0.2	0.713 ± 0.001	1.8
dN	94.0 ± 0.1	1.0 ± 0.1	2.6

[a] Reactions were carried out with 5 nM of target RNA and 15 nM ADAR2 WT
[b] Reactions were fitted to the equation $[P]_t = \alpha[1 - \exp(-k_{obs} \cdot t)]$
[c] $k_{rel} = k_{obs}$ for modification/k_{obs} for adenosine

Table S7. The effect of -1 gRNA modifications on observed rate constants and fitted end points for ADAR1 deaminations reactions with *ACTB*. [a]

-1 modification [a]	Fitted end point[b]	k_{obs} x10^{-2} (min^{-1})[b]	k_{rel}[c]
Adenosine	44 ± 4	3.4 ± 0.4	1
dA	65 ± 4	7 ± 1	2
dN	74 ± 4	11 ± 3	3.2

[a] Reactions were carried out with 15 nM of target RNA and 150 nM ADAR1 p110
[b] Reactions were fitted to the equation $[P]_t = \alpha[1 - \exp(-k_{obs} \cdot t)]$
[c] $k_{rel} = k_{obs}$ for modification/k_{obs} for adenosine

Table S8. Sequences of oligonucleotides (nucleotides in brackets are varied). Primers are 2'-deoxynucleotides, all others are ribonucleotides unless otherwise stated.

For cellular guides, unbolded nucleotides indicate 2'-OMe, bolded nucleotides indicate 2'-deoxyribonucleotides, and underlined nucleotides indicate a phosphorothioate backbone.

Human *MECP2* W104X target sequence (target A indicated in red)	5′-GGGAGCCCGCAGAGGCAGGCAAAGCAGAGACAUCAGAAGGGUCAGGC UCCGCCCCGGCUGUGCCGGAAGCUUCUGCCUCCCCCAAACAGCGGCGCUC CAUCAUCCGUGACCGGGGACCCAUGUAUGAUGACCCCACCCUGCCUGAAG GCUAGACACGGAAGCUUAAGCAAAGGAAAUCUGGCCGCUCUGCUGGGAAG UAUGAUGUGUAUUUGAUCAAUCCCCAGGGAAAAGCCUUUCGCUCUAAAGU GGAGUUGAUUGCGUACUUCGAAAAGGUAGGCGACACAUCCCUGGACCCUA AUGA-3′
Mouse *IDUA* W392X target sequence (target A indicated in red)	5′-GGGCUCCUCCCAUCCUGUGGGCUGAACAGUAUAACAGACUCCCAGUA UACAAAUGGUGGGAGCUAGAUAUUAGGGUAGGAAGCCAGAUGCUAGGUAU GAGAGAGCCAACAGCCUCAGCCCUCUGCUUGGCUUAUAGAUGGAGAACAA CUCUAGGCAGAGGUCUCAAAGGCUGGGGCUGUGUUGGACAGCAAUCAUAC AGUGGGUGUCCUGGCCAGCACCCAUCACCCUGAAGGCUCCGCAGCGGCCU GGAGUACCACAGUCCUCAUCUACACUAGUGAUGACACCCACGCACACCCC GGAUCC-3′
Human *ACTB* target sequence (target A indicated in red)	5′-GGGUCCUCCUGAGCGCAAGUACUCCGUGUGGAUCGGCGGCUCCAUCC UGGCCUCGCUGUCCACCUUCCAGCAGAUGUGGAUCAGCAAGCAGGAGUAU GACGAGUCCGGCCCCUCCAUCGUCCACCGCAAAUGCUUCUAGGCGGACUA UGACUUAGUUGCGUUACACCCUUUCUUGACAAAACCUAACUUGCGCAGAA AACAAGAUGAGAUUGGCAUGGCUUUAUUUGUUUUUUUUGUUUUGUUUUGG UUUUUUUUUUUUUUUUGGCUUGACUCAGGAUUUAAAAACUGGAACGGUGA AGGUGAC-3′
Human *MECP2* W104X gRNAs	5′-UAAGCUUCCGUGUC[C][A]GCCU[U]CAGGCAGG-3′

Mouse *IDUA* W392X gRNAs	5′-UUGAGACCUCUGCCC[A]GAGUUGUUCUCCA-3′
Human *ACTB* gRNAs	5′-GGGUGUAACGCAACC[A]AGUCAUAGUCCGC-3′
Human *MECP2* W104X cellular guides	5′-UUUCCUUUGCUUAAGCUUCCGUGUC**C**[A]**G**CCUUCAGGC-3′
Standard DNA oligo sequence for MALDI	5′-TTACGCCAGAATGCGTTCGCACAGCCGCCA-3′
MECP2 RT-PCR and sequencing primer forward	5′-AGAGGCAGGCAAAGCAGA-3′
MECP2 amplification and RT-PCR primer reverse	5′-TCATTAGGGTCCAGGGAT-3′
MECP2 T7 DNA amplification forward	5′-TAATACGACTCACTATAGGGAGC-3′
IDUA RT-PCR primer forward	5′-CTCCCATCCTGTGGGCT-3′
IDUA amplification, RT-PCR and sequencing primer reverse	5′-GGATCCGGGGTGTGC-3′
IDUA T7 DNA amplification forward	5′-TAATACGACTCACTATAGGGCTC-3′
ACTB RT-PCR and sequencing primer forward	5′-GTCACCTTCACCGTTCCA-3′
ACTB amplification and RT-PCR primer reverse	5′-TCCTCCTGAGCGCAAG-3′
ACTB T7 DNA amplification forward	5′-TAATACGACTCACTATAGGGTCCT-3′
MECP2 W104X sequence for dual luciferase assay reporter plasmid (target A indicated in red)	5′-CGCTCCATCATCCGTGACCGGGGACCCATGTATGATGACCCCACCCT GCCTGAAGGCTAGACACGGAAGCTTAAGCAAAGGAAATCTGGC-3′
RT-PCR forward primer for sequencing of cellular editing	5′-CTGCTTACTGGCTTATCGAAATTAATACGACT-3′
RT-PCR reverse primer for sequencing of cellular editing	5′-TTACGCCAGAATGCGTTCGCACAGCCGCCA-3′
Nested PCR and sequencing forward primer for sequencing of cellular editing	5′-CACTATAGGGAGACCCAAGCTTGCCAC-3′
Nested PCR reverse primer for sequencing of cellular editing	5′-GCCGGTCACTCCGTTGATGGTTACT-3′

Table S9. Masses of gRNAs and guide oligonucleotides (cellular).

gRNA	Calculated Mass (Da)	Observed Mass (Da)
MECP2 rA -1	9231	9240
MECP2 dA -1	9215	9224
MECP2 2'-O-Me -1	9245	9248
MECP2 LNA A -1	9243	9236
MECP2 UNA A -1	9238	9238
MECP2 2'-F A -1	9233	9230
MECP2 2'-F-arabino A -1	9233	9224
MECP2 β-L-dA -1	9215	9221
MECP2 C3 spacer -1	9040	9035
MECP2 2-aminopurine -1	9231	9229
MECP2 2,6-diaminopurine -1	9246	9240
MECP2 dI -1	9216	9215
MECP2 dN -1	9200	9205
MECP2 7-deaza-dA -1	9214	9225
MECP2 3-deaza-dA -1	9214	9225
MECP2 dC -1	9191	9198
MECP2 abasic -1	9082	9097
MECP2 LNA -6, dN -1	9212	9228
MECP2 orphan dZ	9259	9242
MECP2 orphan dZ, dN -1	9228	9227
IDUA rA -1	9153	9144
IDUA dA -1	9137	9137
IDUA dN -1	9122	9123
IDUA 7-deaza-dA -1	9136	9141
ACTB rA -1	9301	9297
ACTB dA -1	9285	9280
ACTB dN -1	9270	9272
MECP2 LNA A -1 (cellular)	12350	12350
MECP2 dN -1 (cellular)	12307	12307
MECP2 2'-F A -1 (cellular)	12340	12340
MECP2 FANA A -1 (cellular)	12340	12340

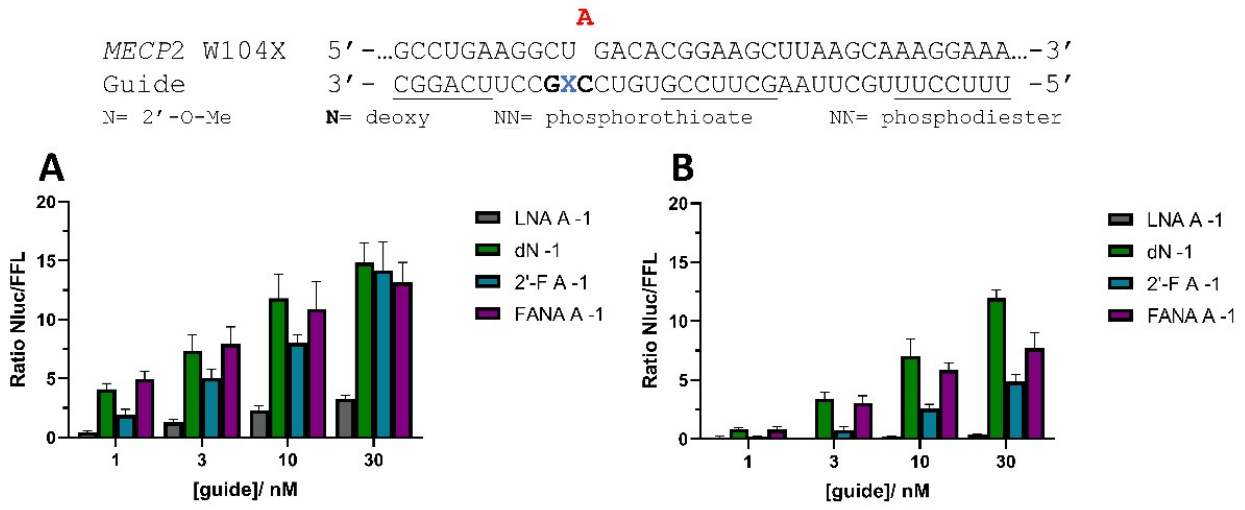

Figure S1. Concentration dependence of effect of -1 nucleoside analogs on cellular editing as measured by dual luciferase assay. Relative ratio of Nluc/FFL was measured in HEK293T cells with varying concentrations of guide oligonucleotides with four different -1 analogs with overexpressed (**A**) ADAR2 or (**B**) ADAR1. Error bars, s.d. (n ≥ 5 biological replicates). A Grubbs test for outliers was conducted at p = 0.05 significance level.

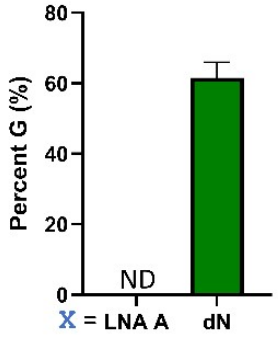

Figure S2. Effect of -1 nucleoside analogs on cellular editing as measured by Sanger sequencing. The percent G at the *MECP2* W104X target site was measured in HEK293T cells after transfection of 30 nM of -1 nucleoside analog containing guide oligonucleotide with overexpressed ADAR1. Error bars, s.d. (n = 3 biological replicates). ND = no detected editing.