Supporting Information

Optimal guideRNAs for Re-directing Deaminase Activity of hADAR1 and hADAR2 in trans

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Sequencing results



Figure S1. Effect of the counter base in our standard Stop66 CFP transcript. The adenosine base (A*) of the Stop66 codon (UA*G) was either paired with uridine or mismatched with cytosine, guanosine or adenosine by incubating the mRNA with one of the four BG-modified guideRNAs 5'-BG-r(UCG GAA CAC CC<u>X</u> AGC ACA GA), with <u>X</u> = C, U, G, or A. The standard editing conditions (concentrations, editing times, etc.) including 0.75 mM magnesium have applied as described in the method part of the manuscript.

Figure S2.1 Sequencing traces for the editing of the **UAG codon** under standard conditions including 0.75 mM magnesium as described in the method part of the manuscript.







































UAG codon under standard conditions Figure S2.2 Replicate of experiments shown in Figure S2.1, editing of the including 0.75 mM magnesium.















ADAR1, A/C









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ADAR1, C/U
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ADAR1, A/U



T G C T GIA G G G T

ADAR2, C/C

ADAR2, U/C



ADAR2, G/U



ADAR2, C/U T G C T G/A G G G T ADAR2, U/U

T G C T GIA G G G T

T G C T GIA G G G T ADAR2, A/U















































Figure S3.2. Replicate of the editing of the AAG codon with 0.75 mM magnesium as in Figure S3.



ADAR1. G/C

G C A G/A G т G



ADAR1, U/C



ADAR1, A/C



ADAR1, U/C

ADAR1, U/C

т G A G/A G G







ADAR1, A/U т G C A G/A G G G





т G С Α G/A G G G



ADAR2, G/U G С A G/A G G т G С A G/A G G ADAR2, U/U





T G C A G/A G G G T

Figure S4.1. Sequencing traces for the mM magnesium as described in the standard conditions including 0.75 editing of the CAG codon under method part of the manuscript.



ADAR1, G/C

C C C T

ADAR1, C/C

ADAR1, U/C

G C C GIA G G G T

ADAR1, A/C

T G C C GIA G G G T

6

ц Б ADAR1, G/U

ADAR1, C/U

ADAR1, U/U

L D ADAR1, A/U

T G C C GIA G G G T ADAR2, G/C

T G C C GIA G G G T

ADAR<mark>2</mark>, G/U

ADAR2, C/C

ADAR2, C/U

ADAR2, U/C

ADAR2, U/U

T G C C GA G G G T

ADAR2, A/C

ADAR2, A/U













T G C C GIA G G G T ADAR1, G/U

ADAR1, C/U

ADAR1, U/U T G C C GIA G G G T

ADAR1, A/U

MMMM GCCGIAGGGT

ADAR2, G/C

ADAR<mark>2</mark>, C/U

ADAR2, G/U

ADAR2, C/C

T G C C GIA G G G T

ADAR2, U/C

ADAR2, U/U

ADAR2, A/C

ADAR2, A/U

Figure S5.1. Sequencing traces for the mM magnesium as described in the standard conditions including 0.75 editing of the **GAG codon** under method part of the manuscript.









ADAR1, C/C

ADAR1, C/U

r c c c dA c c c T

ADAR1, U/U A

ADAR1, U/C

- ADAR1, A/U

- T G C G GIA G G G T DAR2, G/C
 - ADAR2, C/C
- - ADAR2, U/C
- ADAR2, A/C

ADAR2, G/U

- T G C G GA G G G T
- T G C G GA G G G T ADAR2, C/U
- ADAR2, A/U

8

ADAR1, A/C





























T & C & G GIA & C & C T ADAR2, C/U

ADAR2, U/U T G C G GIA G G G T





Figure S6. Sequencing traces for the editing of all four **XAG codons** (X = U, A, C, G) with SNAP-**ADAR3** in presence of one of the four guideRNAs putting the targeted adenosine into A/C mismatch. Editing was done under standard conditions but in the absence of magnesium as described in the method part of the manuscript.

Figure S7. Sequencing traces for the editing of all four XAG codons (X = U, A, G, C) with SNAP-**ADAR3** and a few selected guideRNAs under standard conditions including 0.75 mM magnesium as described in the method part of the manuscript.



F

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ADAR3

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CAA G IIIII II GUU CAA ADAR3

conditions were kept constant compared to the Figure S8. Sequencing traces for the editing of the AAG codon under standard conditions in absence of magnesium as described in the method part of the manuscript. All other experiments shown in Figure S3.













ADAR1, U/C



c

0 0

ADAR1, A/C

G C A GIA G







ADAR2, G/U













н 9

G C A GIA G G





	0
\sim	U
\geq	U
\leq	GIA
<	۷
\leq	U
\leq	U
\leq	⊢

C

Figure S9.1. Sequencing traces for the editing of the **CAG codon** under standard conditions in **absence** of manuscript. All other conditions were kept constant magnesium as described in the method part of the compared to the experiments shown in Figure S4.















ADAR1, A/C





ADAR2, G/U





c















0

C C GIA G G

G

under standard conditions in absence of magnesium Figure S9.2. Replicate of editing of the CAG codon as described Figure S9.1





TGCCGIAGGGT VINAVIN

ц Б ADAR1, U/C

C C GIA G G G ADAR1, A/C

GGGT ADAR1, G/U

ADAR1, C/U

U ADAR1, U/U

C C G/A G G G T ADAR1, A/U c

MMMM r g c c gia g g g T ADAR2, G/C

ADAR2, G/U

ADAR2, C/C

ц Б

L D ADAR2, U/C

VN/VV/V T G C C GIA G G ADAR2. A/C

ADAR2, C/U

T G C C GIA G G G T ADAR<mark>2, U/U</mark> A

VWWWV ц Б G C C GIA G G ADAR2, A/U

G T

14

GAG codon under standard conditions in **absence** of manuscript. All other conditions were kept constant Figure S10. Sequencing traces for the editing of the magnesium as described in the method part of the compared to the experiments shown in Figure S5.



2,-

	-1-		
	0		20
6	U		0
	0		0
	G		Se
Q	\triangleleft°	U	\triangleleft°
G	\triangleleft°	Ö	\sim
AR1	- 0	AR1	
AD/	\triangleleft	AD/	\leq

G Ċ G G C G G/A

ADAR1, G/U

ADAR1, C/U

G|**A** G

ADAR1, U/U

ADAR1, U/C

U c G C G G/A

ADAR1, A/U

U

c

c

GIA ø

> C , U

ADAR1, A/C

U G G/A G c υ Ċ

ADAR2, G/C

ADAR2, C/C

U U G C G GIA G ADAR2, U/C

c c G GIA ADAR2, A/C υ c

G U , U GIA G 0

c U c G C G G/A ADAR2, G/U ADAR2, C/U

G

U

G GIA

ບ ຫ

H

0

ADAR2, U/U

ADAR2, A/U

G C G G/A

Ċ 0

Ċ G/A Ċ υ 0

15

H U

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C G G/A G

C