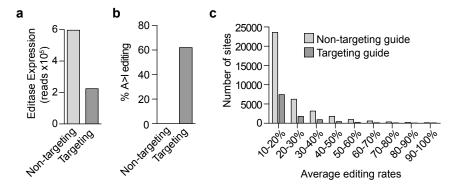
## **Supplemental Information**

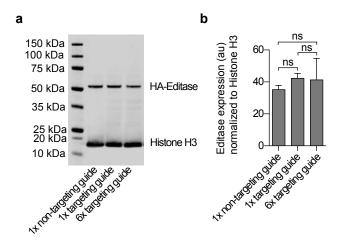
## In Vivo Repair of a Protein Underlying

## a Neurological Disorder by Programmable RNA Editing

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Supplementary Figure 1. Whole transcriptomic RNA-seq analysis of the same RNA used in our published study (Sinnamon et al., 2017) of  $Mecp2^{317G>A}$  hippocampal neurons (DIV14), 7 days following transduction with AAV1/2 virus. Related to Figure 3. Promoters, Editase and guide sequences are identical to that shown in Figure 1a in this study. a) The number of RNA-seq reads that aligned to the Editase coding sequence for each viral condition. b) Histogram showing that on-target editing is guide-dependent. One sample each condition. c) Histogram showing the number of off-target editing sites, binned according to the editing rates.



Supplementary Figure 2. The number of copies of the human U6 promoter driving guide expression does not influence Editase protein expression. Related to Figure 3. a), Representative immunoblot of whole cell lysates prepared from Neuro-2A neuroblastoma cells 72 hours after transfection with plasmids containing the Editase cDNA expressed from the human *Synapsin I* promoter and indicated guides. Blots were probed with anti-HA antibody for Editase detection and anti-Histone H3 for loading control. b) Quantification of Editase expression from immunoblots (mean  $\pm$  SD, n = 3 biological replicates), each condition normalized to Histone H3. au, arbitrary units. ns, not significant by One-way ANOVA and Tukey's multiple comparisons test.

## Supplementary Table 1. Guide and primer sequences. Related to STAR Methods

**Guide sequences** 

mouse Mecp2<sup>317G>A</sup> 2xBoxB Targeting guide

Non-targeting guide

Sequence, 5'→ 3'

gaagagcgagctcttctgttttagagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgc

Amplification of endogenous Mecp2 cDNA

mouse *Mecp2*-14 ATG Fwd

mouse Mecp2-3'UTR+92 Rev

aacccgtccggaaaatggcc

ggaagctttgtcagagccctacccataag

Sequencing primers for *Mecp2* RT-PCR

mouse Mecp2 554 Rev

mouse Mecp2 914 Rev

mouse Mecp2 1122 Rev

mouse Mecp2-3'UTR+92 Rev

ctcctggaggggctccctctc

gaccgtatggaagactccttca

actgctgctgcgcccctt

ggaagctttgtcagagccctacccataag

**Cloning primers** 

Human U6 Ndel Fwd

mouse Mecp2 2xBoxB Targeting guide Rev

gtgtcatatgcttaccgtaacttgaaag

cacagggcccaaaaaagatgaccccaggccct

Target

	М	Υ	D	D	Р	Т	L	Р	Ε	G	W	Т	Q	K	L	K	Q	R	K	S	G	R	S		
5 <b>′</b> -	AUG	UAU	GAU	GAC	CCC	ACC	UUG	CCU	GAA	GGU	UGG	ACA	$C \pmb{A} A$	AAG	CUU	AAA	CAA	AGG	AAG	UCU	GGC	CGA	UCU	G -	·3 <b>'</b>
					$  \cdot  $			$\Box$	$\parallel \parallel \parallel$	$\Box$	$\Box$	X	X		$\Box$	+++			$\Box$	+++					
		3 <b>'-</b>	-CTA	CTG	GGG	T	-AC	GGA	CTT	CCA	ACC	TGG	GCT	TTC	GAA	TTT		TCC	${\tt TTC}$	AGA	C -5	,			
BoxB														BoxE	3										

Mecp2 <sup>317G&gt;A</sup>	Replicate 1 2 3	Injection Condition  6xU6-Mecp2 targeting guide-hSynl Editase 6xU6-Mecp2 targeting guide-hSynl Editase 6xU6-Mecp2 targeting guide-hSynl Editase	6.5% 9.2%	22.6% 31.0%	8.2% 12.7%	6.4 8.6	<b>Target R106Q</b> 39.4% 57.9% 49.7%
Wild-type	Replicate	Injection Condition 6xU6-Mecp2 targeting guide-hSynI Editase	E102G 5.8%	-		T105T 6.7	

Supplementary Table 2. Editing of adenosines within Mecp2 RNA identified in intact hippocampus by Sanger sequencing analysis. Related to Table 1. Top, Mecp2 RNA and deduced primary amino acid sequence (top row) relative to the guide RNA (bottom row). The target adenosine,  $Mecp^{317}$  in the RNA strand (MeCP2 R106Q) is bolded. X, mismatched adenosines. Bottom, Rates of editing at the adenosines located within the guide region from intact hippocampus for each biological replicate. The detection limit for this assay was previously determined to be 5% editing (Sinnamon et al., 2017). Any sites that had  $\leq 5\%$  editing are listed as having no detectable editing (ND). There was no detectable editing in Mecp2 RNA outside of the guide region.