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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No custom-made code was required to collect data. The Western blot pictures were taken with the FusionCapt Advance SL4 (16.09b) software installed on a Fusion SL Vilber Lourmat (Vilber) western blot analyzer. No further image processing was done with respect to brightness or contrast. Next-generation sequencing of Poly(A)+ mRNA was done by CeGaT (Germany). The library was prepared with the TruSeq Stranded mRNA Library Prep Kit (Illumina, USA), and sequenced with the NovaSeq 6000 (50 M reads, 2 × 100 bp paired end, Illumina, USA).
Data analysis	As outlined in full detail in the online methods all software tools used for NGS are publically available: Mapping of RNA-seq and reads: BWA (version 0.7.10) was used to align the reads to a combination of the reference genome sequences (hg19) and exonic sequences surrounding known splicing junctions from known gene models, obtained through the UCSC Genome Browser for Gencode, RefSeq, Ensembl, and UCSC Genes. Unique and non-duplicate reads were subjected to local realignment and base score recalibration using the IndelRealigner and TableRecalibration from the Genome Analysis Toolkit (GATK, version 3.6). Identification of editing sites from RNA-seq data: We used the UnifiedGenotyper from GATK27 to call variants from the mapped RNA-seq reads. In contrast to the usual practice of variant calling, we identified the variants with relatively loose criteria by using the UnifiedGenotyper tool. We first removed all known human SNPs present in dbSNP, build 137 (except SNPs of molecular type "cDNA"; database version 135; http://www.ncbi.nlm.nih.gov/SNP/), the 1000 Genomes Project, and the University of Washington Exome Sequencing Project (http://evs.gs.washington.edu/EVS/). Finally, variants were annotated using ANNOVAR (version 11122013) based on gene models from Gencode, RefSeq, Ensembl, and UCSC. The resulting sets of sites identified from RNA-seq data were compared with all sites available in the RADAR database and were subsequently referred to as 'known' sites if also found in RADAR, or 'novel' sites if not found. Code can also be downloaded at: http://lilab.stanford.edu/SNPiR/

Data was analyzed using Excel 2016 and GraphPad Prism 7, Figures were created with CorelDraw 2017, the manuscript was written with Word 2016

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

This manuscript provides additional Supplementary Information on primary data and further controls (Supplementary Figures 1-15), it contains a Table of all oligonucleotides used (Supplementary Table 1) and a list of all target sequences (Supplementary Note 1). Furthermore it contains 3 excel spread sheets with the NGS off-target analysis. The accession code of the NGS raw data is not yet applicable but will be available before publication.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Experiments for evaluating editing yields of endogenous targets via Sanger sequencing were mostly done in triplicate (independent biological experiments) in rare cases in duplicate to validate reprodicibilty and to provide appropriate standard deviations. Single data points are always given. NGS analysis was performed with two independent replicates per sample; the required sequencing depth was determined in a previous study (Vogel et al. Nature Methods 2018) and saturated with 50 Mio 100 bp paired-end reads at 25 000 detected transcripts. This sequencing depths was also similar to other very recent papers on global off-target effects of site-directed RNA editing (Cox et al. Science 2017, Rosenthal et al. RNA Biol. 2018)
Data exclusions	no data was excluded
Replication	all experiments could be reproduced, as shown in the manuscript, the number of replications and nature of replicates is always given in the figure caption
Randomization	no randomization was performed, samples were treated according to the same protocols side-by-side with the respective controls
Blinding	no blinding was performed, editing experiments were allocated to several experimentators

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All commercial primary cells were obtained from Lonza. Three cell lines, which are not standard cell lines, were donations from other labs, primary fibroblasts (Dr. Enza Maria Valente, Università degli Studi di Salerno, Fisciano, Italy), U2OS Flp In cells (Dr. Elmar Schiebel, ZMBH, Heidelberg, Germany), and AKN-1 cells (Dr. Andreas Nüssler, UKT, Tübingen, Germany), as indicated below. Please ask the respective lab to obtain the respective cell line from us or them.

Antibodies

Antibodies used	ADAR1 antibody (α-ADAR1, mouse monoclonal IgG, Santa Cruz cat. no.: sc-73408, clone no. 15.8.6, lot no. C2514, used in 1:1000 dilution) against amino acids 440-826 corresponding to the middle region of ADAR1 of human origin, ADAR2 antibody(α-ADAR2, mouse monoclonal IgG, Santa Cruz cat. no.: sc-73409, clone no. 1.3.1, lot no. G1613, used in 1:1000 dilution) against N-terminal region corresponding to amino acids 2-179 of ADAR2 of human origin, Clone AC-15 (α-ACTB, mouse monoclonal IgG, Sigma Aldrich cat. No.: A5441) against Actin N-terminal peptide, Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Ile-Asp-Asn-Gly-Ser-Gly-Lys.
Validation	ADAR1 antibody: Validated in our lab via siRNA KO and Western Blot (in several cell lines), and by overexpression / Western blot PMID: # 28669490 PMID: # 28278381 PMID: # 27573237 PMID: # 27907896 ADAR2 antibody: Validated in our lab via overexpression and Western Blot PMID: # 26601943 PMID: # 24345557 PMID: # 27907896 Clone AC-15 antibody: PMID: # 15809369 PMID: # 15048076 PMID: # 21217779

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HeLa: ATCC (Cat.No.: ATCC CCL-2), U2OS-Flp-In T-Rex: kind donation from Prof. Elmar Schiebel, SK-N-BE(2) ATCC: (Cat.No.: ATCC CRL-2271), SK-N-BE(2): ATCC (Cat.No.: ATCC CRL-2271), U87MG: ATCC (Cat.No.: ATCC HTB-14), Huh7:CLS (CLS GmbH, Heidelberg, Cat.No.: 300156), HepG2 DSMZ (DSMZ, Braunschweig, Germany Cat.No.: ACC180), AKN-1: kind donation from the Nüssler lab, A549: ECACC (European Collection of Authenticated Cell Cultures ECACC 86012804),SH-SY5Y: ATCC (Cat.No.: ATCC CRL-2266), HEK-Flp-In T-Rex-A1p110 (R78007, Thermo Fisher scientific, stably transfected with ADAR1 p110 vector in our lab), HEK-Flp-In T-Rex-A1p150 (R78007, Thermo Fisher scientific, stably transfected with ADAR1p150 vector in our lab), HEK-Flp-In T-Rex-ADAR2 (R78007, Thermo Fisher scientific, stably transfected with ADAR2 vector in our lab), empty HEK-Flp-In T-Rex (R78007, Thermo Fisher scientific, stably transfected with empty pcDNA5 vector), primary fibroblasts: kind gift from the Valente lab. Human Umbilical Vein Endothelial Cells: Lonza (HUVEC, Cat.No.:CC-2517),Human Aortic Endothelial Cells: Lonza (HAEC, Cat.No.:CC-2535), Normal Human Astrocytes: Lonza (NHA, Cat.No.: CC-2565), Human Retinal Pigment Epithelial Cells: Lonza (H-RPE, Cat.No.: 194987), Normal Human Bronchial Epithelial Cells: Lonza (NHBE, Cat.No.: CC-2540) and Primary Human Hepatocytes: Lonza (PHH,Cat.No.: HUCPI)
Authentication	HUVEC, HAEC, NHA, RPE, NHBE, and primary hepatocytes were authenticated by the supplier. Commercial standard cell lines like HeLa, SK-N-BE, U87MG, Huh7, SH-SY5Y, empty HEK-293-Flp-In T-Rex were authentificated by the respective suppliers. Cell lines were not additionally authenticated by us.
Mycoplasma contamination	HUVEC, HAEC, NHA, RPE, and NHBE were certified as mycoplasma-free by the supplier. Flp-In T-REx 293 ADAR1p110, Flp-In T-REx 293 ADAR1p150, Flp-In T-REx 293 ADAR2, HeLa, SK-N-BE(2), Huh7, A549 have been tested as mycoplasma-free in house.
Commonly misidentified lines (See ICLAC register)	none were used