Supplementary Table S1: Method evaluation

True % edited	C and T peak height method *Average ± STD*	C and T peak volume method *Average ± STD*	C peak only normalization method *Average ± STD*
0%	11 ± 3	10 ± 2	10 ± 3
1%	11 ± 1	14 ± 16	10 ± 3
2%	11 ± 2	10 ± 2	11 ± 4
5%	10 ± 2	7 ± 5	8 ± 6
7%	9 ± 4	9 ± 5	10 ± 6
10%	10 ± 5	12 ± 8	13 ±10
15%	14 ± 4	15 ± 6	16 ± 7
20%	19 ± 4	20 ± 6	21 ± 9
30%	28 ± 4	27 ± 7	29 ± 9
40%	36 ± 4	35 ± 5	39 ± 8
50%	46 ± 4	45 ± 6	50 ± 9
60%	57 ± 5	58 ± 11	62 ± 12
70%	67 ± 5	68 ± 10	71 ± 12
80%	78 ± 4	76 ± 13	80 ± 13
85%	82 ± 3	83 ± 6	87 ± 12
90%	87 ± 5	87 ± 7	91 ± 13
93%	90 ± 5	89 ± 6	96 ± 14
95%	91 ± 5	90 ± 6	95 ± 13
98%	94 ± 5	93 ± 6	100 ± 13
99%	94 ± 5	93 ± 6	100 ± 13
100%	95 ± 5	94 ± 5	100 ± 14

Notes: PCR products were sequenced with Primer 32 (Supplementary Table 2), which is outside of the RT-PCR product used for nearest neighbor analysis. Primer 32 allowed nineteen different editing sites to be evaluated, but generated an unacceptably high background in sequencing traces (~10%). In subsequent analyses (Table 1) different primers were used (primer pair 31/32, see Methods). The “C and T peak height method” gave the overall smallest standard deviation (STD) of the three methods tested. The “C and T peak volume method” is calculated as 100% x [C peak volume/(T peak volume + C peak volume)]. Peak volumes were calculated as described[45], and this citation also describes the “C peak only normalization method”, where only the volumes of the C peaks are measured. In brief, the C peak from “editing” is normalized to the surrounding 6 originally coded C peak volumes, three on each side of the “edited” peak. The average value of this method was closer to the true value of the control PCR mixtures, but STD was higher than the other two techniques. For the purposes of comparing nearest neighbor preferences between ADAR enzymes on the same substrate, a decrease in variability was deemed superior to a slight increase in averaged accuracy.

Supplementary Table S2: Primer sequences.

CAT Sense related primers

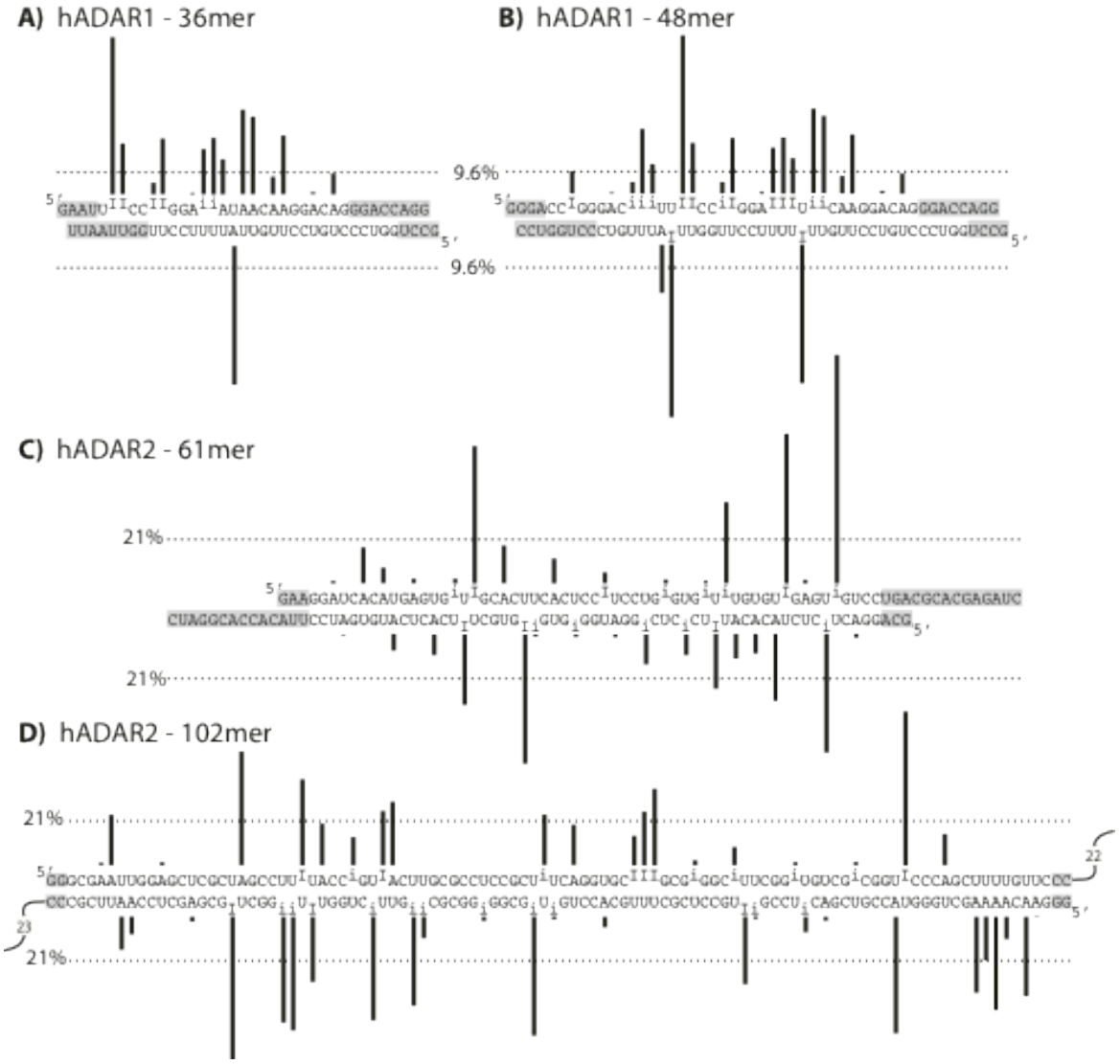
52: GATCCCCGA(T/C)CCGGCGAA(T/C)(T/C)(T/C)C(T/C)GCCA(T/C)(T/C)CA(T/C)CCG
54: GAATACACGGAATTCGAGCTCGCCC(A/G)GCTTGGCG
56: GG(T/C)GAAAACGGGGGCGAAGAAG
58: CGG(T/C)G(T/C)AACAAGGG(T/C)GAACAC
64: GGAAGCCA(T/C)CACAGACGGC
66: GAA(T/C)AAAGGCCGGA(T/C)AAAAC
68: ACGCCCCGCCC(T/C)GCCAC(T/C)CA(T/C)CGCAG

CAT Antisense related primers

31: TGTCGTTAGAACGCGGCTAC
32: CACAGGAAACAGCTATGACC
51: GATCCCCAGC(T/C)(T/C)GGCGAGA(T/C)(T/C)(T/C)(T/C)CAGGAGC(T/C)AAGG
53: GAATACACGGAATTCGAGCTCGCCCG(A/G)TCCGGCG
55: GGCAA(T/C)GAAAGACGG(T/C)GAGC
57: ACG(T/C)GGCCAA(T/C)A(T/C)GGACAAC
63: G(T/C)ACC(T/C)A(T/C)AACCAGACCG
65: ACGCAAGGCGACAAGG(T/C)GC
67: ACGG(T/C)GAAAACC(T/C)GGCC(T/C)A
69: GGAG(T/C)GAA(T/C)ACCACGACGA
73: GA(T/C)GAG(T/C)GGCAGGGCGGGGC

5HT2c related primers

76: CAGGAAACAGCTATGACGCTTGGCG
80: GCGGAAGCTTTAATACGACTCACTATAGGATATTTGTGCC
81: CCGCGCTGCAGATTTAAATAAAAGAACCCGATCAAACGC
82: TCACTATAGGATATTTGTGCCCCGTCTGGATTTCTTTAGATGTTTTATTTTCAACAGCGTCCAT CATGCACCTCTGCGCTATATCGCTGGATCGGTATGTAGC
83: AGCAATCTTCATGATGGCCTTAGTCCGCGAATTGAAACGGCTATGCTCAATAGGATTACGTAT TGCTACATACCGATCCAGCGATATAGCGCAGAGG
84: TAAGGCCATCATGAAGATTGCTATTGTTTGGGCAATTTCTATAGGTAAATAAAACTTTTTGGCC ATAAGAATTGCAGCGGCTATGCTCAATACTTTCGG
85: CCGCGCTGCAGATTTAAATAAAAGAACCCGATCAAACGCAAATGTTACCAGTCGACGTCTGT ACGTTGTTCACAGTACATAATCCGAAAGTATTGAGCATAGCCGCTGCAATTCTTATGGCC
86: GCGAAAGGGGGATGTGCTGC
87: GTTGTGTGGAATTGTGAGCG
90: AAA(T/C)AAAAGAACCCGA(T/C)CAAACGC
91: GG(A/G)T(A/G)TTTGTGCCCCGTCTGG
92: CAGGAAACAGCTATGACGCTTGGCGAAA(T/C)AAAAGAACCCGA(T/C)CAAACGC



Supplementary Figure S1: The hADAR1 and hADAR2 eight-term nearest neighbor regression models as predictive tools on short RNA duplexes previously reported[18]. Extent of editing at each site in the RNA population for these duplexes was determined by nuclease mapping (hADAR1 substrates) or primer extension (hADAR2 assays). (a) The 36mer edited by hADAR1. (b) The 48mer edited by hADAR1. (c) The 61mer edited by hADAR2. (d) The 102mer edited by hADAR2. As previously illustrated[18], major editing sites are shown by "I" (>50% deamination), minor editing sites by "i" (<50% deamination), and below-detection/no editing sites ("A"), with vertical lines illustrating the magnitude of editing predicted by the eight-term model described in this report. The dotted horizontal lines at 9.6% for hADAR1 and 21% for hADAR2 relate to Figure 4 in the main text, and represent predicted editing percentages that best fit the measured data for the boundary between sites that are edited (I+i) or unedited (A). The gray highlighted ends of the duplexes denote regions where hADAR1 or hADAR2 are unable to edit due to proximity to the 5' or 3' ends of the duplex[18]. Unlike the RNA duplex used to derive the predictive model, editing data for these duplexes was derived from reactions performed to completion[18].

Supplementary Methods

Protein purification: Proteins were expressed in *Saccharomyces cerevisiae* and purified as described[36], except the NaCl gradient used to bind and elute hADAR1-D from the heparin ion exchange column started at 50 mM NaCl. In all buffers prior to the final Ni-NTA column wash of the bound His-tagged protein, Roche Complete EDTA-free Protease Inhibitor Cocktail Tablets were used at half the manufacturer's recommended concentration. hADAR2, hADAR2-D and hADAR1-D were dialyzed into Storage Buffer A (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM 2-mercaptoethanol, 15% glycerol). These proteins were purified to >98% as estimated by SYPRO Red staining of SDS-polyacrylamide gels with BSA standards[18]. hADAR2 (76.6 kDa) was concentrated to 174 nM, hADAR2-D (45.0 kDa), 19.4 μM, and hADAR1-D (45.5 kDa), 6.8 μM.

The optimized hADAR1 protocol differed in that the heparin ion exchange and gel filtration chromatography columns were excluded, and NaCl concentration was never allowed to go below 350 mM. The first Ni-NTA wash buffers were 750 mM NaCl, followed by 2 washes at 350 mM NaCl, and higher imidazole elution also at 350 nM NaCl; other buffer components were as described[14,36,40]. Following the Ni-NTA column, protein was immediately treated with TEV and dialyzed, again passed over an Ni-NTA column[14,36,40], the flow through collected, concentrated and the buffer exchanged into Storage Buffer B with Millipore Centricons. Storage Buffer B is 50 mM Tris-HCl (pH 8.0), 200 mM KCl, 5 mM EDTA (pH 8.0), 0.01% NP-40, 10% glycerol and 1 mM DTT[35]. Using this protocol, hADAR1 (103.6 kDa) was purified to 80%, twice the purity previously achieved for hADAR1[18]. The final purified and stored concentration of hADAR1 (103.6 kDa) was 1870 nM.

5-HT_{2C} RNA sequence: The 5' G (italics) in the RNA is a remnant of transcription and its sequence (NCBI Reference Sequence NT_028405.12), is: 5'-
*G*GAUAUUUGUGCCCCGUCUGGAUUUCUUUAGAUGUUUUAUUUUCAACAGCGUCCAUCAU
GCACCUCUGCGCUAUAUCGCUGGAUCGGUAUGUAGCAAUACGUAAUCCUAUUGAGCAUA
GCCGUUUCAAUUCGCGGACUAAGGCCAUCAUGAAGAUUGCUAUUGUUUGGGCAAUUUCU
AUAGGUAAAUAAAACUUUUUGGCCAUAAGAAUUGCAGCGGCUAUGCUCAAUACUUUCGG
AUUAUGUACUGUGAACAACGUACAGACGUCGACUGGUAACAUUUGCGUUUGAUCGGGUU
CUUUUAUUU -3'.

Supplementary Reference

45. Leakey, T.I. et al. A simple algorithm for quantifying DNA methylation levels on multiple independent CpG sites in bisulfite genomic sequencing electropherograms. *Nucleic Acids Res* **36**, e64 (2008).