Supplementary Materials

Leveraging Endogenous ADAR for Programmable Editing on RNA

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Extended Data Figure Legends

Extended Data Figure 1 | Exploration of an efficient RNA editing platform. a, Schematic of dLbuCas13a-ADAR1$_{DD}$ (E1008Q) fusion protein and the corresponding crRNA. The catalytic inactive LbuCas13a was fused to the deaminase domain of ADAR1 (hyperactive E1008Q variant) using 3× GGGGS linker. The crRNA (crRNA^{Cas13a}) consisted of Lbu-crRNA scaffold and a spacer which was complementary to the targeting RNA with an A-C mismatch as indicated. **b**, Schematic of dual fluorescent reporter system and the Lbu-crRNA with various lengths of spacers as indicated. **c**, Quantification of the EGFP positive (EGFP^{+}) cells. HEK293T cells stably expressing the Repoter-1 were transfected with indicated lengths of crRNA^{Cas13a}, with or without co-expression of the dLbuCas13a-ADAR1$_{DD}$ (E1008Q), followed by FACS analysis. Data are presented as the mean ± s.e.m. (n = 3). **d**, Representative FACS result from the experiment performed with the control (Ctrl crRNA$_{70}$) or the targeting spacer (crRNA$_{70}$).

Extended Data Figure 2 | mRNA expression level of ADAR1/ADAR2 and arRNA-mediated RNA editing. a, Quantitative PCR showing the mRNA levels of *ADAR1* and *ADAR2* in HEK293T cells. Data are presented as the mean ± s.e.m. (n = 3). **b**, Representative FACS results from Fig. 1e.

Extended Data Figure 3 | Quantitative PCR showing the effects of LEAPER on the expression levels of targeted Reporter-1 transcripts by 111-nt arRNA or control RNA in HEK293T cells. Data are presented as the mean ± s.e.m. (n = 3); unpaired two-sided Student's *t*-test, ns, not significant.

Extended Data Figure 4 | Schematic of Reporter-1 (a), -2 (b), and -3 (c), as well as their corresponding arRNAs.

Extended Data Figure 5 | Effects of LEAPER on the expression levels of targeted transcripts and protein products. a, Quantitative PCR showing the expression levels of targeted transcripts from PPIB, KRAS, SMAD4 and FANCC by the corresponding 151-nt arRNA or Control RNA in HEK293T cells. Data are presented as the mean ± s.e.m. (n = 3); unpaired two-sided Student's *t*-test, **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001; ns, not significant. **b**, Western blot results showing the effects on protein products of targeted KRAS gene by 151-nt arRNA in HEK293T cells. β-tubulin was used as a loading control.

Extended Data Figure 6 | Editing endogenous transcripts with LEAPER. a, Schematic of the *KARS* transcript sequence covered by the 151-nt arRNA. The arrow indicates the targeting adenosine. All adenosines were marked in red. **b**, Heatmap of editing rate on adenosines covered by indicated arRNAs in the *KARS* transcript (marked in the bold frame in blue). **c**, Schematic of the *SMAD4* transcript covered by the 151-nt arRNA. **d**, Heatmap of editing rate on adenosines covered by indicated arRNAs in the *SMAD4* transcript. **e**, Schematic of the *FANCC* transcript covered by the 151-nt arRNA. **f**, Heatmap of editing rate on adenosines covered by indicated arRNAs in the *FANCC* transcript. For each arRNA, the region of duplex RNA is highlighted with bold frame in blue. Data (**b**, **d**, and **f**) are presented as the mean (n = 3).

Extended Data Figure 7 | Evaluation of potential off-targets. a, Schematic of the highly complementary region of arRNA$_{111}$-FANCC and the indicated potential off-target sequence, which were predicted by searching homologous sequences through NCBI-BLAST. **b**, Deep sequencing showing the editing rate on the on-target site and all predicted off-target sites of arRNA$_{111}$-FANCC. All data are presented as the mean ± s.e.m. (n = 3).

Extended Data Figure 8 | Editing mutant *TP53*^{W53X} transcripts by LEAPER. Top, schematic of the *TP53* transcript sequence covered by the 111-nt arRNAs. The

arrow indicates the targeted adenosine. All adenosines were marked in red. Bottom, a heatmap of editing rate on adenosines covered by indicated arRNAs in the *TP53* transcript.

Extended Data Figure 9 | Schematic representation of the selected disease-relevant cDNA containing G to A mutation from ClinVar data and the corresponding 111-nt arRNA.

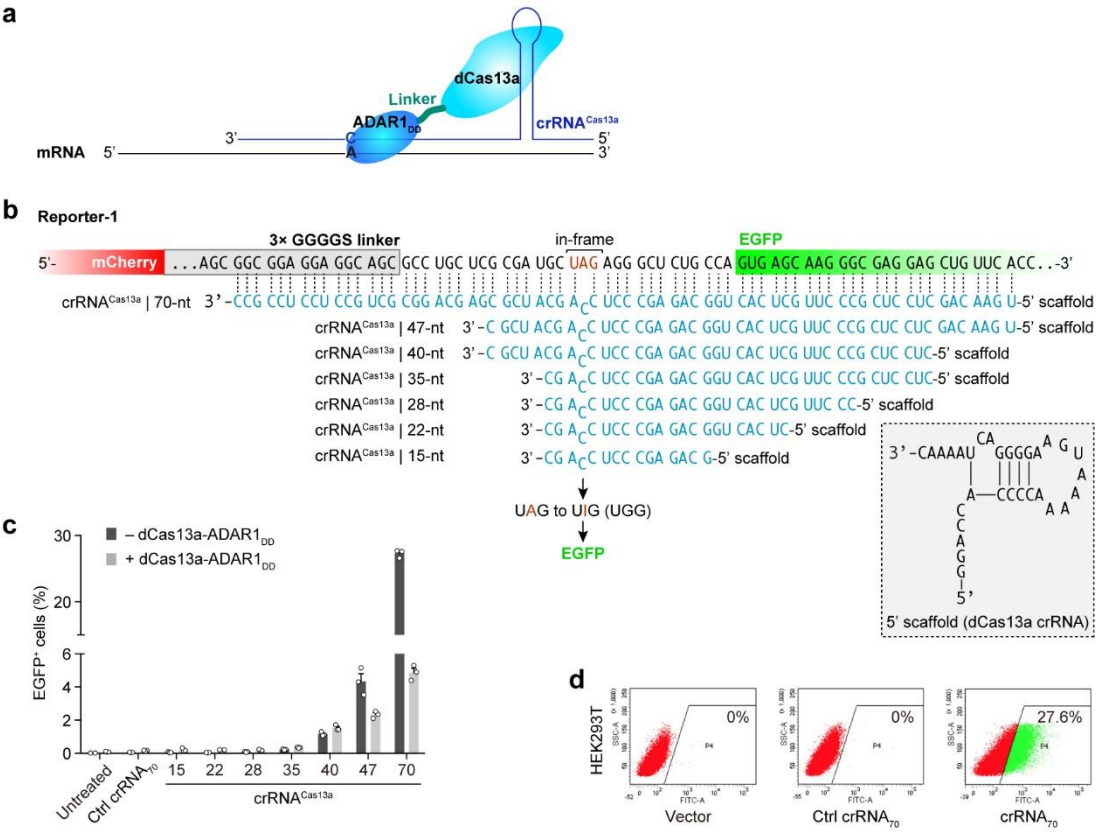
Supplementary Tables

Supplementary Table 1 | LbuCas13 crRNA sequences.

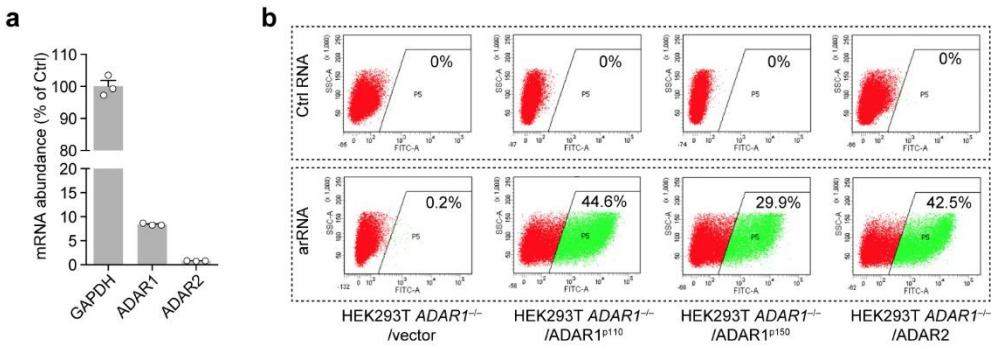
Supplementary Table 2 | Sequences of arRNAs and control RNAs used in this study.

Supplementary Table 3 | Disease-relevant cDNAs used in this study.

Supplementary Table 4 | Primers used in this study.



Extended Data Figure 1



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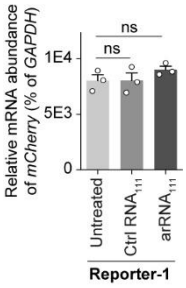

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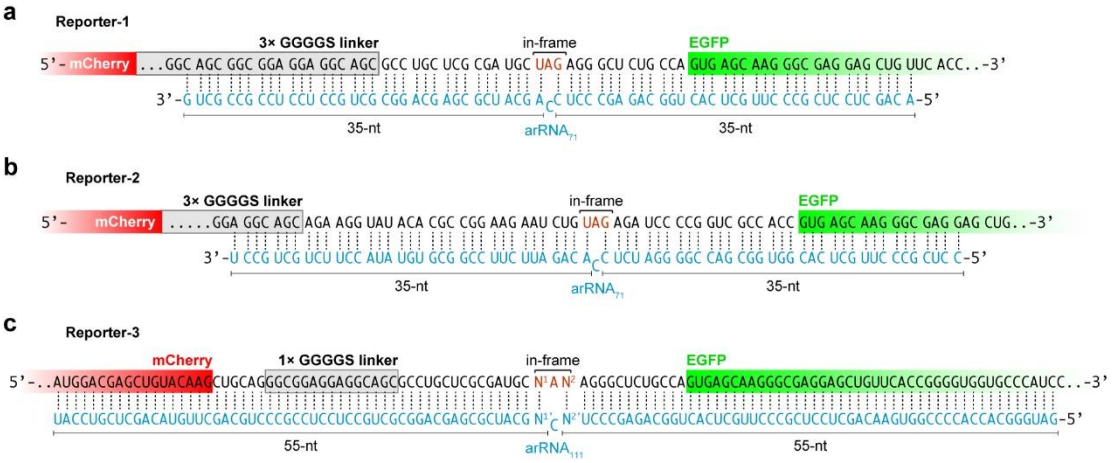

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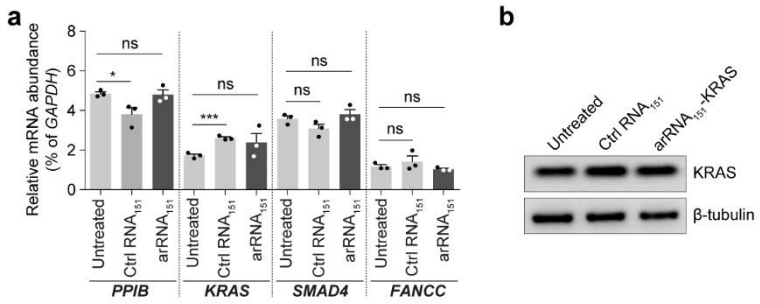

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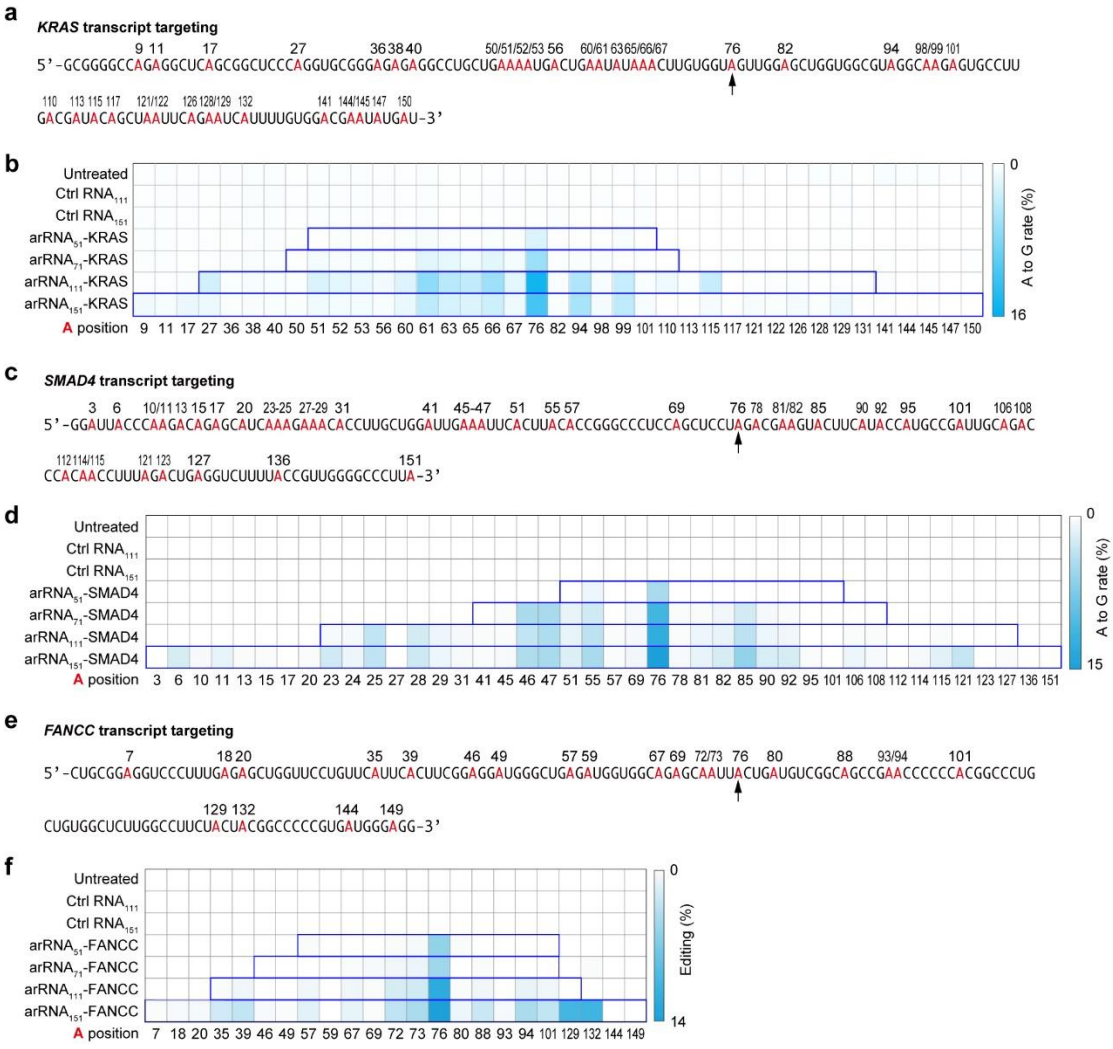

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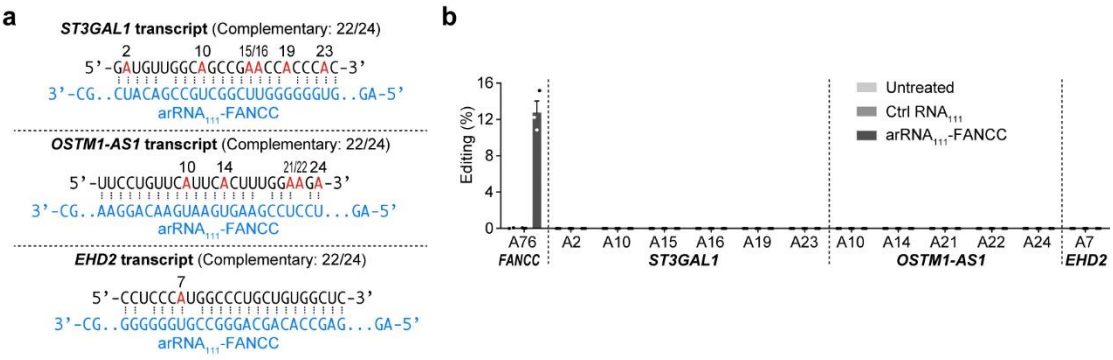

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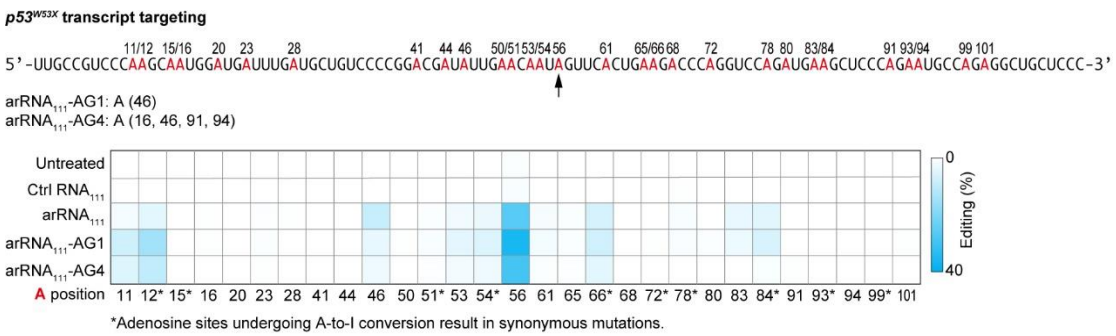
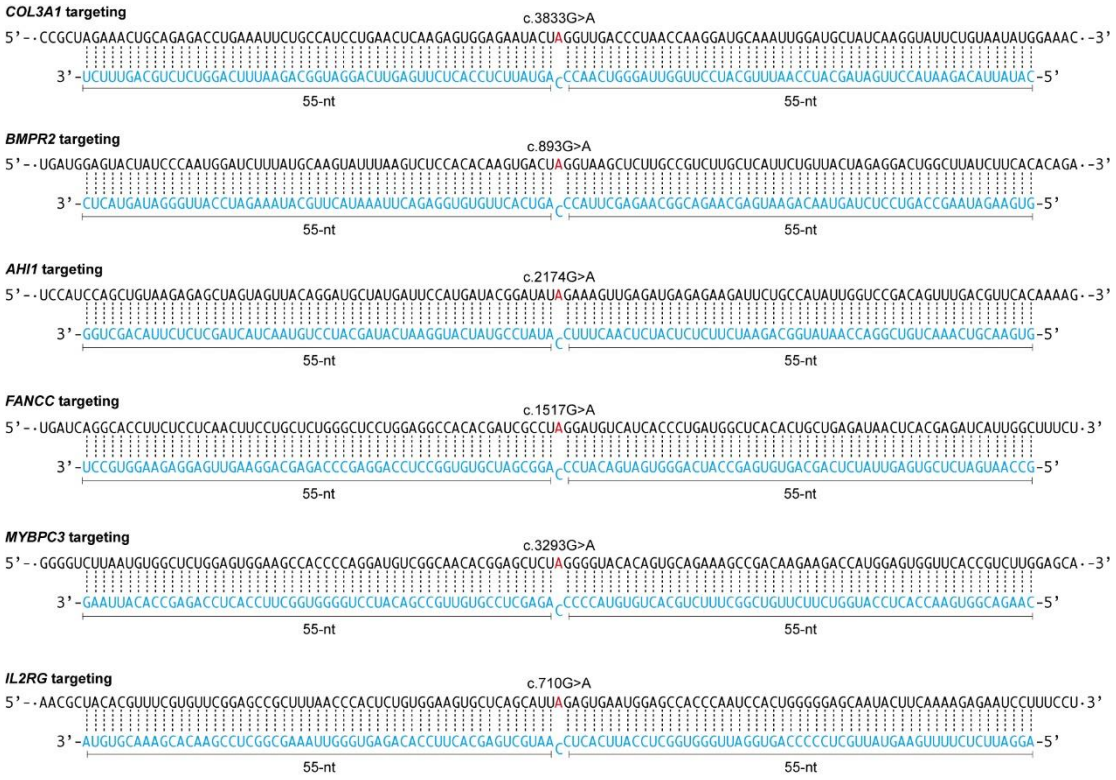


Extended Data Figure 8



Extended Data Figure 9

Supplementary sequences

Reporter-1:

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ADAR1(p150) cDNA

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ADAR2 cDNA

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GATTACAAGGATGACGACGATAAG(Flag tag) TAG-3’

pLenti-MCS-mCherry backbone

5’-

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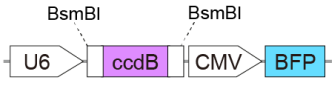
pLenti-arRNA-BFP backbone



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Coding sequence (CDS) of the disease-relevant genes

COL3A1

5’-

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