

## Examination of Factors Affecting Site-Directed RNA Editing by the MS2-ADAR1 Deaminase System

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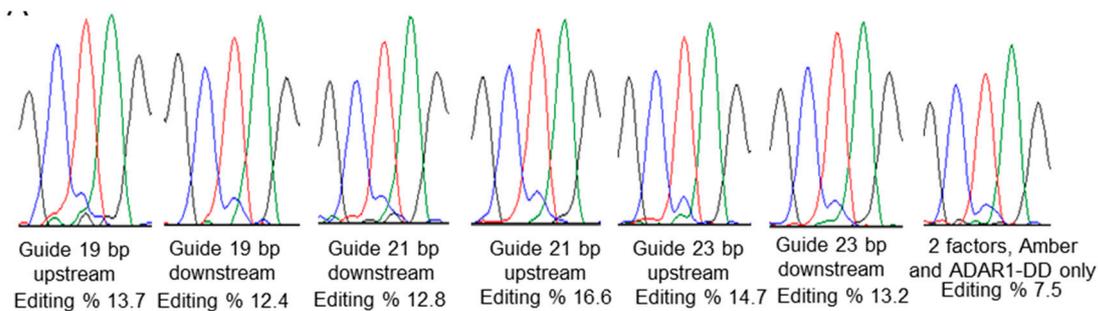
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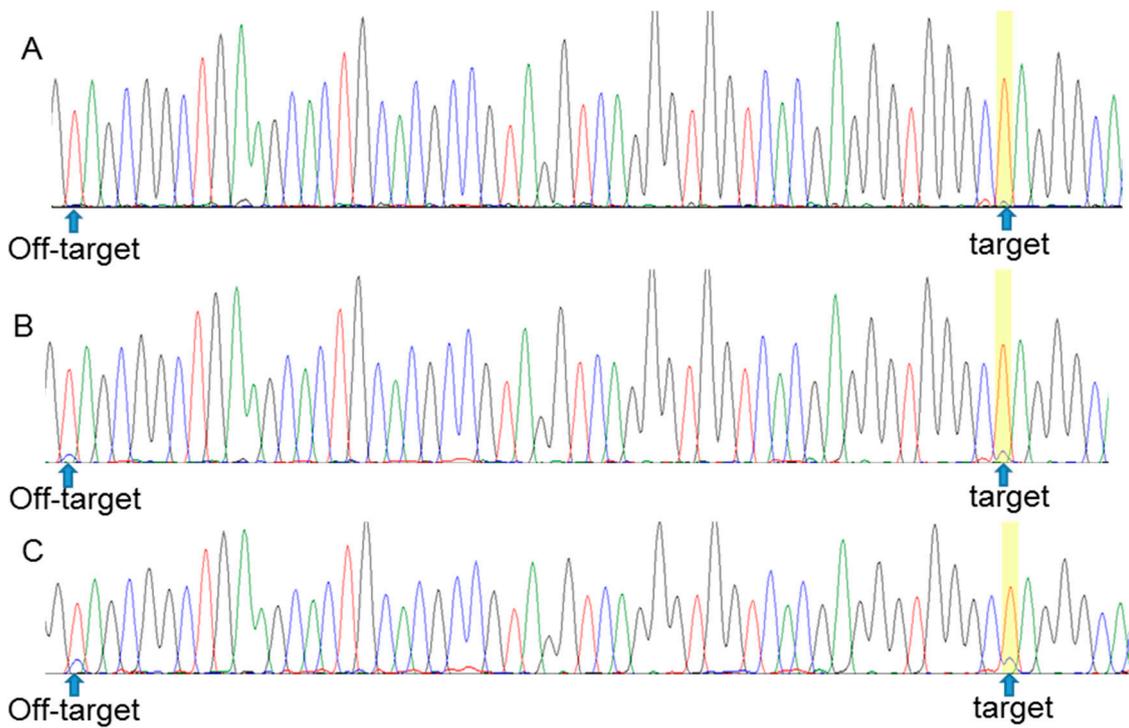
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**Supporting Figure S1.** Sequencing results showing the comparative efficiencies of the six guide RNAs for *EGFP* RNA editing.



**Supporting Figure S2.** Sequencing analysis of *EGFP* for cells transfected with 500 ng of 21 bp upstream guide RNA and 250 ng (A), 500 ng (B), or 1000 ng (C) of MS2-ADADR1-DD.

**A**

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atgctgascgaagggcagggagctgttcaccggggtggtgcccatcctggtcgagctggac
M V S K G E E L F T G V V P I L V E L D
ggcgacgtaaacggccacaagttcagcgtgtccggcagggcagggcagatgccacctac
G D V N G H K F S V S G E G E G D A T Y
ggcaagctgaccctgaagttcatctgcaccaccgcaagctgcccgtgcccttagccccacc
G K L T L K F I C T T G K L P V P X P T
ctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctgccccgaccacatgaag
L V T T L T Y G V Q C F S R Y P D H M K

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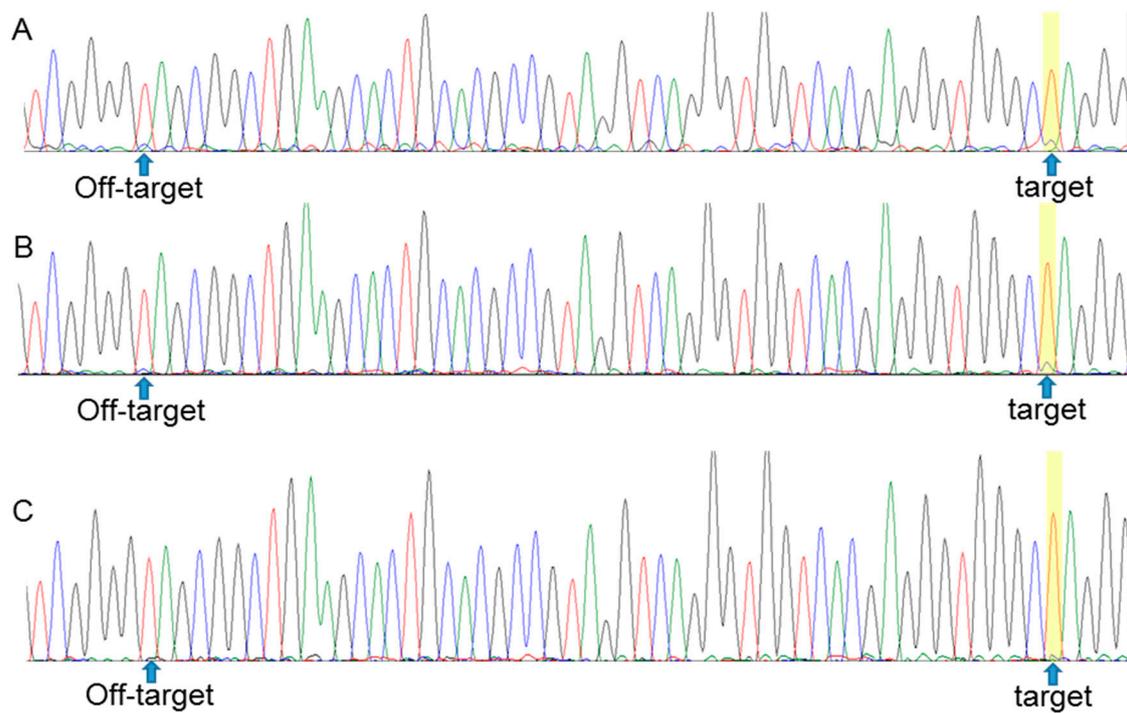
**B**

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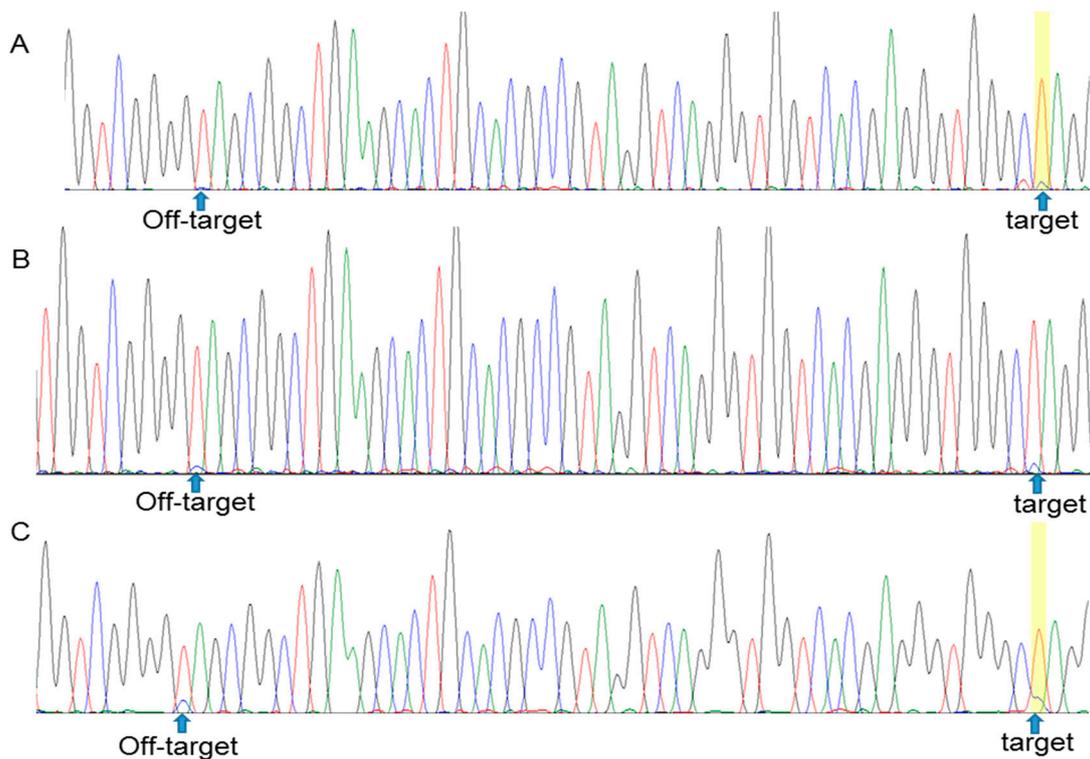
atgctgascgaagggcagggagctgttcaccggggtggtgcccatcctggtcgagctggac
M V S K G E E L F T G V V P I L V E L D
ggcgacgtaaacggccacaagttcagcgtgtccggcagggcagggcagatgccacctac
G D V N G H K F S V S G E G E G D A T Y
ggcaagctgaccctgaagttcatctgcaccaccgcaagctgcccgtgccctggccccacc
G K L T L K F I C T T G K L P V P W P T
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L V T T L T Y G V Q C F S R C P D H M K

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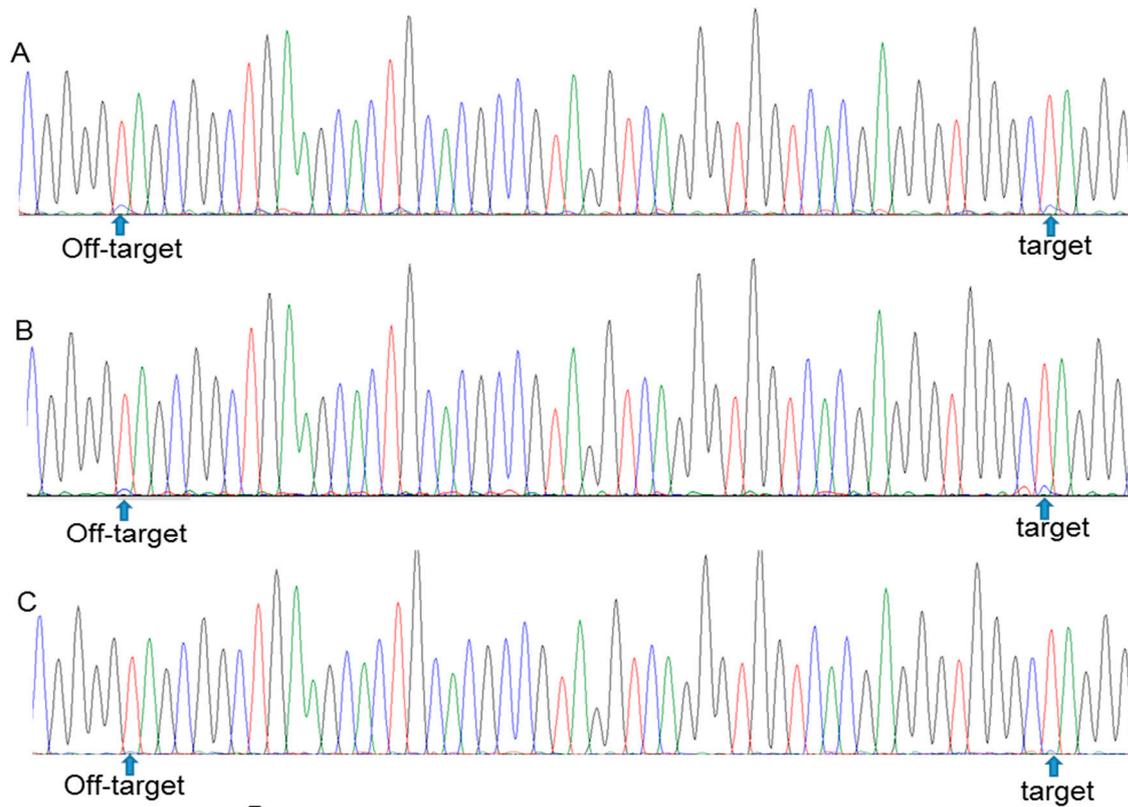
**Supporting Figure S3.** The sequence of the mutated (A) and restored (B) *EGFP* site. The target site (TAG) is indicated. Off-target editing of TAC to TGC is also shown.



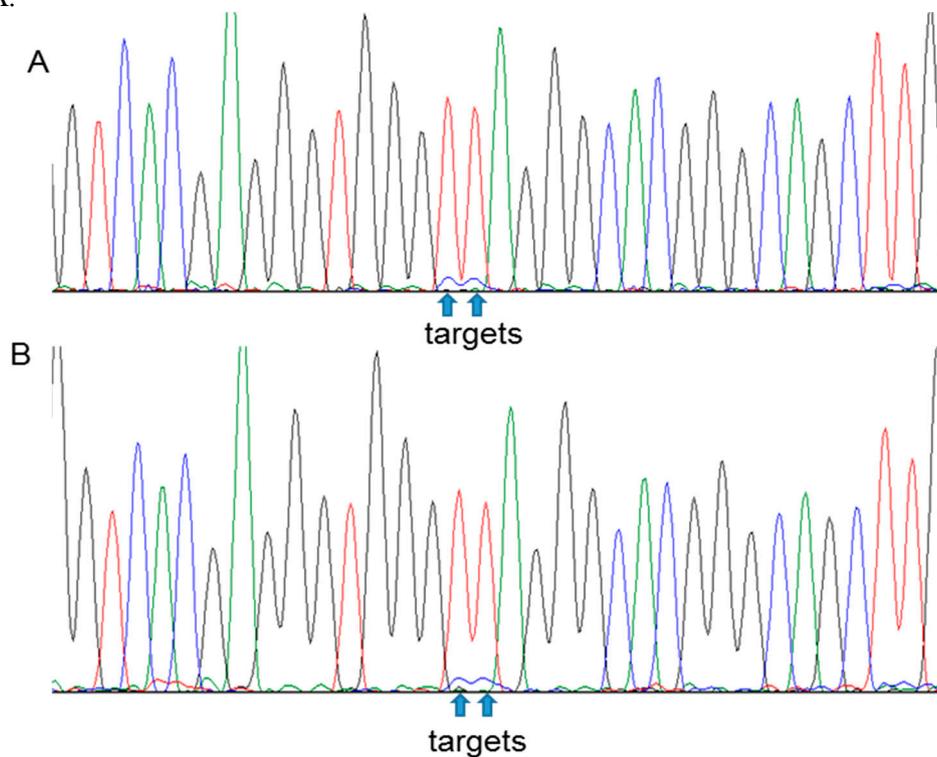
**Supporting Figure S4.** Sequencing analysis of *EGFP* in cells transfected with 500 ng of MS2-ADADR1-DD and 250 ng (A), 500 ng (B), or 1000 ng (C) of 21 nt upstream guide RNA.



**Supporting Figure S5.** Sanger sequencing analysis of *EGFP* in cells transfected with 500 ng of double repeated 19 nt 2× upstream guide RNA and 250 ng (A), 500 ng (B), or 1000 ng (C) of MS2-ADADR1-DD.



**Supporting Figure S6.** Sequencing analysis of *EGFP* in cells transfected with 500 ng of MS2-ADADR1-DD and 250 ng (A), 500 ng (B), or 1000 ng (C) of double repeated 19 nt 2 $\times$  upstream guide RNA.



**Supporting Figure S7.** Sequencing analysis of the conversion of the ochre stop codon (TAA) to the tryptophan codon (TGG). (A) and (B) are replicates of the experiment. HEK-293 cells were transfected with 500 ng of MS2-ADADR1-DD and 250 ng of the 21 nt upstream guide RNA.

Bright image, left panel

Fluorescence image, Right panel

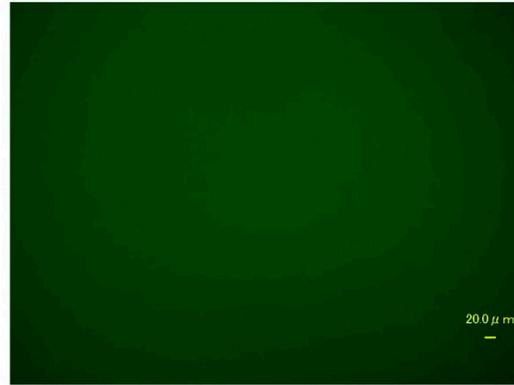
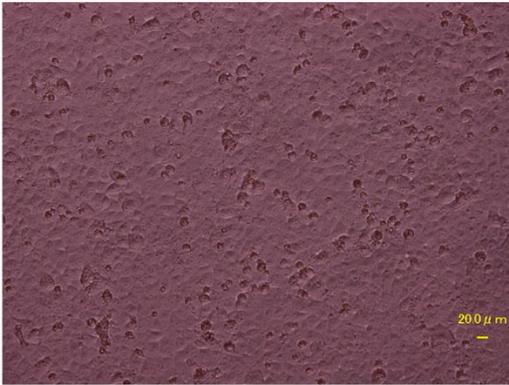


Fig. Negative Control

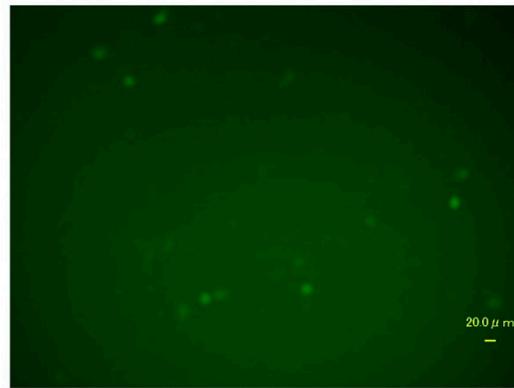
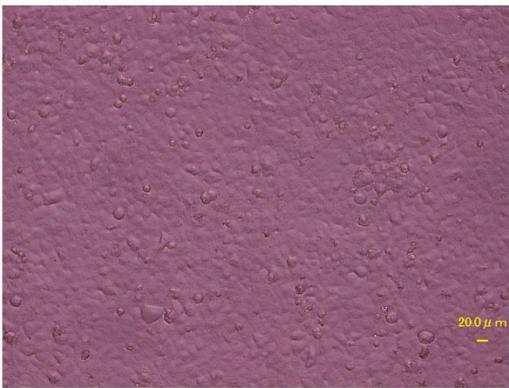


Fig. Experimental, MS2-ADAR1-DD, mutated EGFP and Guide RNA

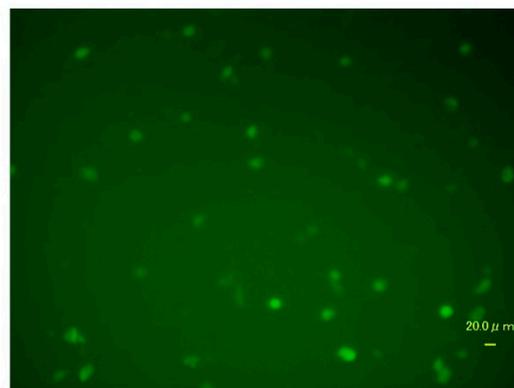
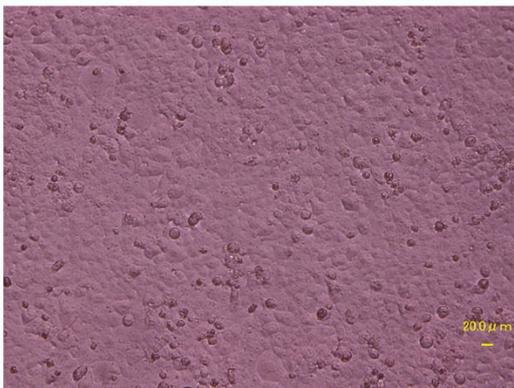


Fig. Positive Control, EGFP

**Supporting Figure S8.** Transfection experiment in HeLa cells. Bright image in left panel and fluorescence image in right panel of same focus. Figures were taken by Keyence Biozero fluorescence microscope, BZ-X800. Except the green fluorescence signal, the microscopic images show no remarkable observable differences among the treatment and control groups of cells