RNA editing of BFP, a point mutant of GFP, using artificial APOBEC1 deaminase to restore the genetic code

Sonali Bhakta1, Matomo Sakari1, and Toshifumi Tsukahara*1

Supplementary Data 1, S1:

List of candidate genes involved in diseases caused by T-to-C mutations
RNA editing of BFP, a point mutant of GFP, using artificial APOBEC1 deaminase to restore the genetic code

Sonali Bhakta¹, Matomo Sakari¹, and Toshifumi Tsukahara*¹

Supplementary Data 2, S2:

RNA-sequencing (RNA-seq) reads from BFP_1 HEK 293
RNA editing of BFP, a point mutant of GFP, using artificial APOBEC1 deaminase to restore the genetic code

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Supplementary Data 3, S3:

RNA-sequencing (RNA-seq) from experimental HEK 293_1
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Supplementary Data 4, S4:

Calculation of editing efficiency based on peak area and peak height of the Sanger sequencing (N=5)
|       | Height | mean height | Width | Mean Width | Mean area | Mean total | STDv of Height | STDv of width | Ratio of edited and unedited | From the peak height |
|-------|--------|-------------|-------|------------|-----------|------------|----------------|---------------|--------------------------------|---------------------|
| Original C | 431 | 48          |       |            |           |            |                |               |                                |                     |
|       | 384   | 59          |       |            |           |            |                |               |                                |                     |
|       | 410   | 406.2       | 38    | 47.6       | 19335.12  | 17.824     | 8.142          |               |                                |                     |
|       | 395   | 42          |       |            |           |            |                |               |                                |                     |
|       | 411   | 51          |       |            |           |            |                |               | 0.21                            |                     |
|       |       |             |       |            |           |            |                |               | 23490                          | 0.215               |
|       |       |             |       |            |           |            |                |               | 21.5                           | 21%                 |
| Edited T | 120  | 42          |       |            |           |            |                |               |                                |                     |
|       | 110   | 35          |       |            |           |            |                |               | 21.5%                           |                     |
|       | 95    | 108.2       | 38    | 38.4       | 4154.88   | 9.859      | 3.049          |               |                                |                     |
|       | 102   | 36          |       |            |           |            |                |               |                                |                     |
|       | 114   | 41          |       |            |           |            |                |               |                                |                     |
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Supplementary Figure 5, S5:
Comparison of editing efficiency and off target effects when using gRNA of different lengths and different positions of the target in the guide
ADAR1-DD 250 ng
Guide 19 bp
upstream 500 ng

ADAR1-DD 250 ng
Guide 21 bp
upstream 500 ng

ADAR1-DD 250 ng
Guide 23 bp
upstream 500 ng
Editing %

- 5’ position of target in guideRNA
- Middle position of target in guideRNA
- 3’ position of target in guideRNA
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Supplementary Data 6, S6:

Mutation reads at different positions in BFP stably transformed in HEK 293 cells- target/control
| Pattern | Count |
|---------|-------|
| T>G     | 1536  |
| A>C     | 1469  |
| A>G     | 1107  |
| T>C     | 921   |
| G>T     | 634   |
| C>A     | 625   |
| T>A     | 534   |
| A>T     | 481   |
| G>A     | 405   |
| C>T     | 396   |
| G>C     | 361   |
| C>G     | 298   |

| Pattern | Count |
|---------|-------|
| AA>GG   | 69    |
| TT>CC   | 41    |
| GA>TT   | 17    |
| AG>CC   | 15    |
| CT>GG   | 12    |
| AG>TT   | 9     |
| GA>CC   | 9     |
| CC>GG   | 6     |
| TC>AA   | 5     |
| AA>CC   | 5     |
| CT>AA   | 5     |
| TC>GG   | 5     |
| AT>TG   | 4     |
| GG>CC   | 4     |
| GT>CG   | 4     |
| AC>CG   | 4     |
| AAA>GGG | 3     |
| TG>AT   | 3     |
| TT>AA   | 3     |
| GG>AA   | 3     |
| AT>CG   | 3     |
| AT>CC   | 3     |
| TT>CG   | 3     |
| CG>AC   | 2     |
| GC>TA   | 2     |
| CA>AT   | 2     |
| AT>CA   | 2     |
| TA>GG   | 2     |
| CA>TG   | 2     |
| GA>TC   | 2     |
| GG>TT   | 2     |
| CC>TG   | 2     |

| Pattern | Count |
|---------|-------|
| TTT>AAA | 1     |
| TA>CC   | 1     |
| TT>GG   | 1     |
| TTT>CCC | 1     |
| TC>GA   | 1     |
| GAA>CGG | 1     |
| AA>TT   | 1     |
| TC>CT   | 1     |
| AT>GG   | 1     |
| TC>GT   | 1     |
| CT>GC   | 1     |
| AAAA>GGCC | 1 |
| CTC>AAA | 1     |
| CT>TA   | 1     |
| AT>GA   | 1     |
| ATC>CAA | 1     |
| CAC>TGG | 1     |
| TG>GC   | 1     |
| TC>CG   | 1     |
| AC>TG   | 1     |
| CG>GC   | 1     |
| AGA>TTT | 1     |
| AC>CA   | 1     |
| GA>AG   | 1     |
| CC>TT   | 1     |
| GAT>TTG | 1     |
| GA>AC   | 1     |
| TG>CC   | 1     |
| GA>AT   | 1     |
| GT>AG   | 1     |
| CA>GG   | 1     |
| GT>CC   | 1     |
| AA>CG   | 1     |
Supplementary Figure, S6: Whole BFP sequencing by placing different primers at different positions and also there were overlapped positions. In total 1109bp were amplified, among these at the position of 200-527 only at this position one off-target event was found, which was located upstream of the targeted C which was to be edited.
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Supplementary Data 7, S7:
Mutation reads at different positions in restored GFP in HEK 293 cells-tested/ experimental
| Pattern   | Count |
|-----------|-------|
| T > G     | 2401  |
| A > C     | 2355  |
| T > C     | 1972  |
| A > G     | 1915  |
| G > T     | 1745  |
| C > A     | 1698  |
| G > A     | 1271  |
| C > T     | 1238  |
| F > A     | 1134  |
| A > T     | 1042  |
| C > G     | 702   |
| G > C     | 648   |

| Pattern   | Count |
|-----------|-------|
| T > G     | 2401  |
| A > C     | 2355  |
| T > C     | 1972  |
| A > G     | 1915  |
| G > T     | 1745  |
| C > A     | 1698  |
| G > A     | 1271  |
| C > T     | 1238  |
| F > A     | 1134  |
| A > T     | 1042  |
| C > G     | 702   |
| G > C     | 648   |

| Pattern   | Count |
|-----------|-------|
| AA > GG   | 58    |
| TT > CC   | 51    |
| GA > TT   | 31    |
| TC > AA   | 23    |
| CT > GG   | 20    |
| AG > CC   | 16    |
| AA > CC   | 14    |
| AG > TT   | 12    |
| AT > CA   | 10    |
| AT > TG   | 10    |
| CT > AA   | 9     |
| TC > GG   | 8     |
| CA > TG   | 8     |
| TG > CA   | 8     |
| GG > CC   | 7     |
| CC > GG   | 6     |
| AC > CG   | 6     |
| GA > CC   | 5     |
| TT > CG   | 5     |
| GT > CG   | 4     |
| CA > AT   | 4     |
| CA > GC   | 3     |
| AC > GT   | 3     |
| CT > TC   | 3     |
| TA > GG   | 3     |
| AAA > GG  | 3     |
| AG > GC   | 3     |
| AA > TT   | 2     |
| AT > CC   | 2     |
| TC > CT   | 2     |
| TA > AC   | 2     |
| AT > GG   | 2     |
| GG > TT   | 2     |

| Pattern   | Count |
|-----------|-------|
| T > G     | 2401  |
| A > C     | 2355  |
| T > C     | 1972  |
| A > G     | 1915  |
| G > T     | 1745  |
| C > A     | 1698  |
| G > A     | 1271  |
| C > T     | 1238  |
| F > A     | 1134  |
| A > T     | 1042  |
| C > G     | 702   |
| G > C     | 648   |

| Pattern   | Count |
|-----------|-------|
| AA > CT   | 1     |
| CTC > AAA | 1     |
| TTT > AA  | 1     |
| GAT > TTG | 1     |
| GC > TG   | 1     |
| CG > AT   | 1     |
| GA > TC   | 1     |
| GTT > CGG | 1     |
| CC > AG   | 1     |
| TT > AG   | 1     |
| TC > GA   | 1     |
| AAG > GTT | 1     |
| GT > CC   | 1     |
| TCG > AAA | 1     |
| TA > CG   | 1     |
| GGG > CCC | 1     |
| AG > GT   | 1     |
| CC > GT   | 1     |
| AGT > CTC | 1     |
| CT > AG   | 1     |
| AGA > CCC | 1     |
| CTC > GGG | 1     |
| AGT > CCG | 1     |
| TCT > AAA | 1     |
| TA > GT   | 1     |
| CA > AC   | 1     |
| AC > TG   | 1     |
| CCCA > GG GT | 1 |
| TT > CA   | 1     |
| GT > TC   | 1     |
| ATC > CA  | 1     |
| TT > GA   | 1     |
| GT > CA   | 1     |
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Supplementary Figure 8, S8

Schematic diagram of the editing by guideRNA (Complementary to the target sequence) and APOBEC 1 deaminase
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Supplementary Data 9, S9:

PCR-RFLP raw gel image without cropping