Supporting Information

Harnessing human ADAR2 for RNA repair – Recoding a PINK1 mutation rescues mitophagy

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wt hADAR2 protein production from yeast

Gene and protein sequence of the produced wt hADAR2-His6, the expression was controlled by pGal promotor and cyc1 terminator, similar as described before for SNAP-ADAR(1-3). A His_6-tag was directly cloned after ADAR2.

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                10        20        30        40        50        60
1         GAGAAAAAACCCCGGATTCTAGAATGGATATAGAAGATGAAGAAAACATGAGTTCCAGCA
1                         Xba1   M  D  I  E  D  E  E  N  M  S  S  S

                70        80        90       100       110       120
61        GCACTGATGTGAAGGAAAACCGCAATCTGGACAACGTGTCCCCCAAGGATGGCAGCACAC
20        S  T  D  V  K  E  N  R  N  L  D  N  V  S  P  K  D  G  S  T

               130       140       150       160       170       180
121       CTGGGCCTGGCGAGGGCTCTCAGCTCTCCAATGGGGGTGGTGGTGGCCCCGGCAGAAAGC
40        P  G  P  G  E  G  S  Q  L  S  N  G  G  G  G  G  P  G  R  K

               190       200       210       220       230       240
181       GGCCCCTGGAGGAGGGCAGCAATGGCCACTCCAAGTACCGCCTGAAGAAAAGGAGGAAAA
60        R  P  L  E  E  G  S  N  G  H  S  K  Y  R  L  K  K  R  R  K

               250       260       270       280       290       300
241       CACCAGGGCCCGTCCTCCCCAAGAACGCCCTGATGCAGCTGAATGAGATCAAGCCTGGTT
80        T  P  G  P  V  L  P  K  N  A  L  M  Q  L  N  E  I  K  P  G

               310       320       330       340       350       360
301       TGCAGTACACACTCCTGTCCCAGACTGGGCCCGTGCACGCGCCTTTGTTTGTCATGTCTG
100       L  Q  Y  T  L  L  S  Q  T  G  P  V  H  A  P  L  F  V  M  S

               370       380       390       400       410       420
361       TGGAGGTGAATGGCCAGGTTTTTGAGGGCTCTGGTCCCACAAAGAAAAAGGCAAAACTCC
120       V  E  V  N  G  Q  V  F  E  G  S  G  P  T  K  K  K  A  K  L

               430       440       450       460       470       480
421       ATGCTGCTGAGAAGGCCTTGAGGTCTTTCGTTCAGTTTCCTAATGCCTCTGAGGCCCACC
140       H  A  A  E  K  A  L  R  S  F  V  Q  F  P  N  A  S  E  A  H
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                490       500       510       520       530       540
481       TGGCCATGGGGAGGACCCTGTCTGTCAACACGGACTTCACATCTGACCAGGCCGACTTCC
160       L  A  M  G  R  T  L  S  V  N  T  D  F  T  S  D  Q  A  D  F

                550       560       570       580       590       600
541       CTGACACGCTCTTCAATGGTTTTGAAACTCCTGACAAGGCGGAGCCTCCCTTTTACGTGG
180       P  D  T  L  F  N  G  F  E  T  P  D  K  A  E  P  P  F  Y  V

                610       620       630       640       650       660
601       GCTCCAATGGGGATGACTCCTTCAGTTCCAGCGGGGACCTCAGCTTGTCTGCTTCCCCGG
200       G  S  N  G  D  D  S  F  S  S  S  G  D  L  S  L  S  A  S  P

                670       680       690       700       710       720
661       TGCCTGCCAGCCTAGCCCAGCCTCCTCTCCCTGCCTTACCACCATTCCCACCCCCGAGTG
220       V  P  A  S  L  A  Q  P  P  L  P  A  L  P  P  F  P  P  P  S

                730       740       750       760       770       780
721       GGAAGAATCCCGTGATGATCTTGAACGAACTGCGCCCAGGACTCAAGTATGACTTCCTCT
240       G  K  N  P  V  M  I  L  N  E  L  R  P  G  L  K  Y  D  F  L

                790       800       810       820       830       840
781       CCGAGAGCGGGGAGAGCCATGCCAAGAGCTTCGTCATGTCTGTGGTCGTGGATGGTCAGT
260       S  E  S  G  E  S  H  A  K  S  F  V  M  S  V  V  V  D  G  Q

                850       860       870       880       890       900
841       TCTTTGAAGGCTCGGGGAGAAACAAGAAGCTTGCCAAGGCCCGGGCTGCGCAGTCTGCCC
280       F  F  E  G  S  G  R  N  K  K  L  A  K  A  R  A  A  Q  S  A

                910       920       930       940       950       960
901       TGGCCGCCATTTTTAACTTGCACTTGGATCAGACGCCATCTCGCCAGCCTATTCCCAGTG
300       L  A  A  I  F  N  L  H  L  D  Q  T  P  S  R  Q  P  I  P  S

                970       980       990      1000      1010      1020
961       AGGGTCTTCAGCTGCATTTACCGCAGGTTTTAGCTGACGCTGTCTCACGCCTGGTCCTGG
320       E  G  L  Q  L  H  L  P  Q  V  L  A  D  A  V  S  R  L  V  L

               1030      1040      1050      1060      1070      1080
1021      GTAAGTTTGGTGACCTGACCGACAACTTCTCCTCCCCTCACGCTCGCAGAAAAGTGCTGG
340       G  K  F  G  D  L  T  D  N  F  S  S  P  H  A  R  R  K  V  L

               1090      1100      1110      1120      1130      1140
1081      CTGGAGTCGTCATGACAACAGGCACAGATGTTAAAGATGCCAAGGTGATAAGTGTTTCTA
360       A  G  V  V  M  T  T  G  T  D  V  K  D  A  K  V  I  S  V  S

               1150      1160      1170      1180      1190      1200
1141      CAGGAACAAAATGTATTAATGGTGAATACATGAGTGATCGTGGCCTTGCATTAAATGACT
380       T  G  T  K  C  I  N  G  E  Y  M  S  D  R  G  L  A  L  N  D

               1210      1220      1230      1240      1250      1260
1201      GCCATGCAGAAATAATATCTCGGAGATCCTTGCTCAGATTTCTTTATACACAACTTGAGC
400       C  H  A  E  I  I  S  R  R  S  L  L  R  F  L  Y  T  Q  L  E

               1270      1280      1290      1300      1310      1320
1261      TTTACTTAAATAACAAAGATGATCAAAAAAGATCCATCTTTCAGAAATCAGAGCGAGGGG
420       L  Y  L  N  N  K  D  D  Q  K  R  S  I  F  Q  K  S  E  R  G

               1330      1340      1350      1360      1370      1380
1321      GGTTTAGGCTGAAGGAGAATGTCCAGTTTCATCTGTACATCAGCACCTCTCCCTGTGGAG
440       G  F  R  L  K  E  N  V  Q  F  H  L  Y  I  S  T  S  P  C  G

               1390      1400      1410      1420      1430      1440
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1381      ATGCCAGAATCTTCTCACCACATGAGCCAATCCTGGAAGAACCAGCAGATAGACACCCAA
460       D  A  R  I  F  S  P  H  E  P  I  L  E  E  P  A  D  R  H  P

                1450      1460      1470      1480      1490      1500
1441      ATCGTAAAGCAAGAGGACAGCTACGGACCAAAATAGAGTCTGGTGAGGGGACGATTCCAG
480       N  R  K  A  R  G  Q  L  R  T  K  I  E  S  G  E  G  T  I  P

                1510      1520      1530      1540      1550      1560
1501      TGCGCTCCAATGCGAGCATCCAAACGTGGGACGGGGTGCTGCAAGGGGAGCGGCTGCTCA
500       V  R  S  N  A  S  I  Q  T  W  D  G  V  L  Q  G  E  R  L  L

                1570      1580      1590      1600      1610      1620
1561      CCATGTCCTGCAGTGACAAGATTGCACGCTGGAACGTGGTGGGCATCCAGGGATCCCTGC
520       T  M  S  C  S  D  K  I  A  R  W  N  V  V  G  I  Q  G  S  L

                1630      1640      1650      1660      1670      1680
1621      TCAGCATTTTCGTGGAGCCCATTTACTTCTCGAGCATCATCCTGGGCAGCCTTTACCACG
540       L  S  I  F  V  E  P  I  Y  F  S  S  I  I  L  G  S  L  Y  H

                1690      1700      1710      1720      1730      1740
1681      GGGACCACCTTTCCAGGGCCATGTACCAGCGGATCTCCAACATAGAGGACCTGCCACCTC
560       G  D  H  L  S  R  A  M  Y  Q  R  I  S  N  I  E  D  L  P  P

                1750      1760      1770      1780      1790      1800
1741      TCTACACCCTCAACAAGCCTTTGCTCAGTGGCATCAGCAATGCAGAAGCACGGCAGCCAG
580       L  Y  T  L  N  K  P  L  L  S  G  I  S  N  A  E  A  R  Q  P

                1810      1820      1830      1840      1850      1860
1801      GGAAGGCCCCCAACTTCAGTGTCAACTGGACGGTAGGCGACTCCGCTATTGAGGTCATCA
600       G  K  A  P  N  F  S  V  N  W  T  V  G  D  S  A  I  E  V  I

                1870      1880      1890      1900      1910      1920
1861      ACGCCACGACTGGGAAGGATGAGCTGGGCCGCGCGTCCCGCCTGTGTAAGCACGCGTTGT
620       N  A  T  T  G  K  D  E  L  G  R  A  S  R  L  C  K  H  A  L

                1930      1940      1950      1960      1970      1980
1921      ACTGTCGCTGGATGCGTGTGCACGGCAAGGTTCCCTCCCACTTACTACGCTCCAAGATTA
640       Y  C  R  W  M  R  V  H  G  K  V  P  S  H  L  L  R  S  K  I

                1990      2000      2010      2020      2030      2040
1981      CCAAACCCAACGTGTACCATGAGTCCAAGCTGGCGGCAAAGGAGTACCAGGCCGCCAAGG
660       T  K  P  N  V  Y  H  E  S  K  L  A  A  K  E  Y  Q  A  A  K

                2050      2060      2070      2080      2090      2100
2041      CGCGTCTGTTCACAGCCTTCATCAAGGCGGGGCTGGGGGCCTGGGTGGAGAAGCCCACCG
680       A  R  L  F  T  A  F  I  K  A  G  L  G  A  W  V  E  K  P  T

                2110      2120      2130      2140      2150      2160
2101      AGCAGGACCAGTTCTCACTCACGCCCCACCATCACCATCACCAT TAATAG TCGACCTCGA
700       E  Q  D  Q  F  S  L  T  P  H  H  H  H  H  H  *  *  Sal1

                2170      2180
2161      GTCATGTAATTAGTTATGTCACGC
720
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R/G-guideRNA synthesis

Templates for *in vitro* transcription were obtained by Phusion PCR templated with a pMG211 vector that contains the guideRNA downstream of the hammer head cassette. The forward primer (5´-GGT CAGGCCCAGGTTCTCCG) was chosen in a way that additional 280 bp were included before the T7 promotor to improve subsequent agarose gel work-up. The backward primer (5´-ACTCTGTGCTGGGGTGGTGGG) was chosen such that the guideRNA ended cleanly with no additional overhanging nucleotides. Shown is the PCR template from the T7 promotor until its 3´-end:

```
1         GCGAAATTAA TACGACTCAC TATAGGGGAA TTGTGAGCGG ATAACAATTC CCCTCTAGAA
                    T7 promotor

61        ATAATTTTGT TTAACTTTAA GAAGGAGATA TACATATGGC TAGCTATTCC ACCTGATGAG
                                                      HH-casette

121       TTTTTACGAA ACGTTCCCGT GAGGGAACGT C*GTGGAATAG TATAACAATA TGCTAAATGT
                                            *=cut          R/G-guideRNA

181       TGTTATAGTA TCCCACCACC CCAGCACAGA GT
             R/G-guideRNA
```

After urea PAGE-purification the following 61 nt guideRNA results from iv-T7 transcription of the above construct:

```
1         GUGGAAUAGU AUAACAAUAU GCUAAAUGUU GUUAUAGUAU CCCACCACCC
*C* AGCACAGAG U
*C* = counter base
```

Urea/TBE 8%-PAGE-separation of a guideRNA synthesis

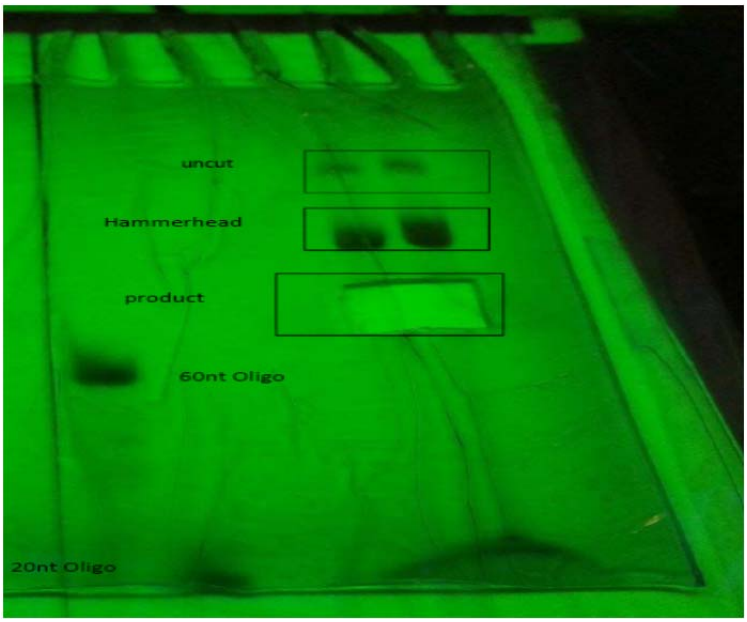


Figure S1. Preparative urea/TBE 8%-PAGE gel for the purification of the *in-vitro* transcribed R/G-guideRNA from the hammer head ribozyme. Indicated are the position of the uncut transcript (156 nt), the hammer head ribozyme (95 nt), the product (61 nt, cut out), and a 60 nt ssDNA and a 20 nt ssDNA as markers. The signal comes from UV shadowing on a TLC plate.

***In-vitro* editing**

RNA sequencing traces of the full eCFP ORF revealing different levels of off-target editing

Figure S2. Full sequencing trace corresponding to the trace shown in Figure 1B, a)

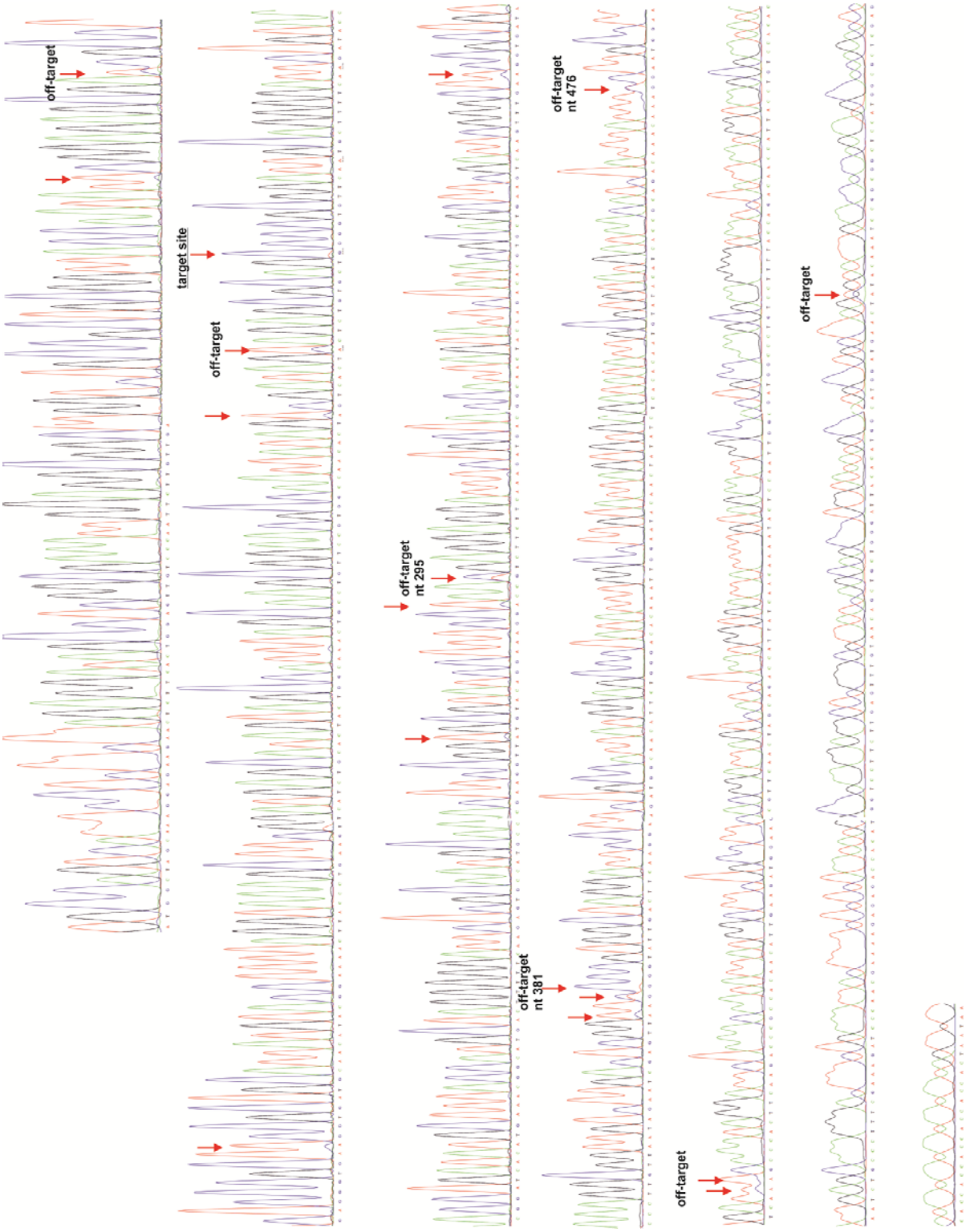


Figure S3. Full sequencing trace corresponding to the trace shown in Figure 1B, c)

Figure S4. Full sequencing trace corresponding to the trace shown in Figure 1C, c)

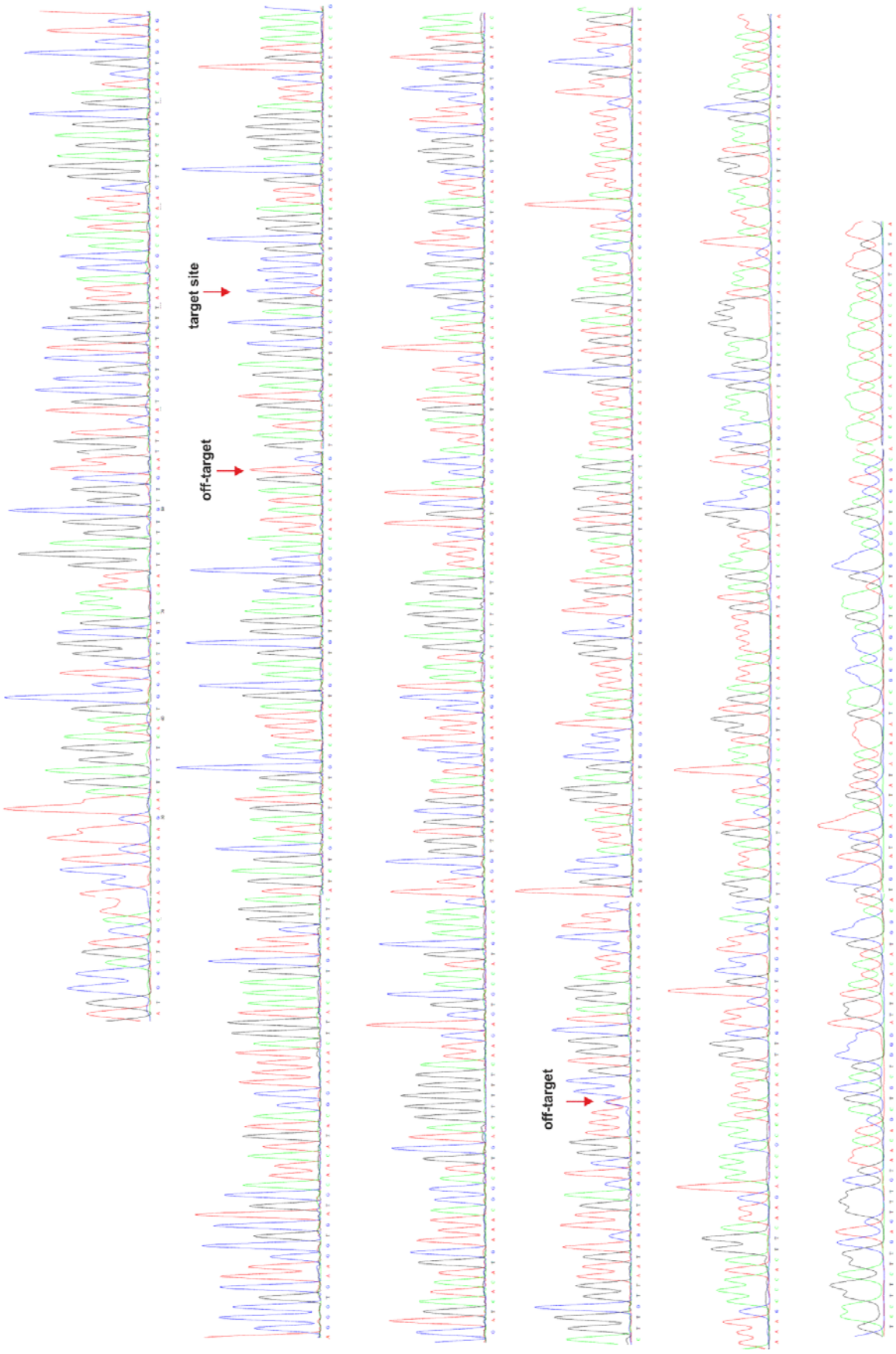
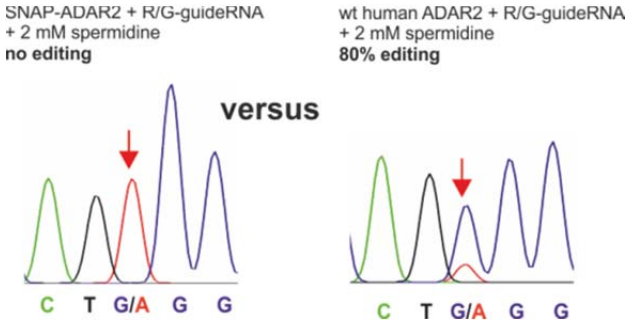


Figure S5. *Control experiment to experiments shown in Figure 1B. The R/G-guideRNA recruits only wt human ADAR2 but not SNAP-ADAR2 for editing. Conditions are identical to Figure 1B.*



Cellular editing

Gene & protein sequence of wt hADAR2 in the context of the pcDNA 3.1 vector, under control of the CMV promotor and BGH terminator:

```
                10        20        30        40        50        60
1          CTCGGATCCACCATGGATATAGAAGATGAAGAAAACATGAGTTCCAGCAGCACTGATGTG
1             BamHI     M  D  I  E  D  E  E  N  M  S  S  S  S  T  D  V

                70        80        90       100       110       120
61         AAGGAAAACCGCAATCTGGACAACGTGTCCCCCAAGGATGGCAGCACACCTGGGCCTGGC
21          K  E  N  R  N  L  D  N  V  S  P  K  D  G  S  T  P  G  P  G

               130       140       150       160       170       180
121        GAGGGCTCTCAGCTCTCCAATGGGGGTGGTGGTGGCCCCGGCAGAAAGCGGCCCCTGGAG
41          E  G  S  Q  L  S  N  G  G  G  G  G  P  G  R  K  R  P  L  E

               190       200       210       220       230       240
181        GAGGGCAGCAATGGCCACTCCAAGTACCGCCTGAAGAAAAGGAGGAAAACACCAGGGCCC
61          E  G  S  N  G  H  S  K  Y  R  L  K  K  R  R  K  T  P  G  P

               250       260       270       280       290       300
241        GTCCTCCCCAAGAACGCCCTGATGCAGCTGAATGAGATCAAGCCTGGTTTGCAGTACACA
81          V  L  P  K  N  A  L  M  Q  L  N  E  I  K  P  G  L  Q  Y  T

               310       320       330       340       350       360
301        CTCCTGTCCCAGACTGGGCCCGTGCACGCGCCTTTGTTTGTCATGTCTGTGGAGGTGAAT
101         L  L  S  Q  T  G  P  V  H  A  P  L  F  V  M  S  V  E  V  N

               370       380       390       400       410       420
361        GGCCAGGTTTTTGAGGGCTCTGGTCCCACAAAGAAAAAGGCAAAACTCCATGCTGCTGAG
121         G  Q  V  F  E  G  S  G  P  T  K  K  K  A  K  L  H  A  A  E

               430       440       450       460       470       480
421        AAGGCCTTGAGGTCTTTCGTTCAGTTTCCTAATGCCTCTGAGGCCCACCTGGCCATGGGG
141         K  A  L  R  S  F  V  Q  F  P  N  A  S  E  A  H  L  A  M  G

               490       500       510       520       530       540
481        AGGACCCTGTCTGTCAACACGGACTTCACATCTGACCAGGCCGACTTCCCTGACACGCTC
161         R  T  L  S  V  N  T  D  F  T  S  D  Q  A  D  F  P  D  T  L

               550       560       570       580       590       600
541        TTCAATGGTTTTGAAACTCCTGACAAGGCGGAGCCTCCCTTTTACGTGGGCTCCAATGGG
181         F  N  G  F  E  T  P  D  K  A  E  P  P  F  Y  V  G  S  N  G

               610       620       630       640       650       660
601        GATGACTCCTTCAGTTCCAGCGGGGACCTCAGCTTGTCTGCTTCCCCGGTGCCTGCCAGC
201         D  D  S  F  S  S  S  G  D  L  S  L  S  A  S  P  V  P  A  S

               670       680       690       700       710       720
661        CTAGCCCAGCCTCCTCTCCCTGCCTTACCACCATTCCCACCCCCGAGTGGGAAGAATCCC
221         L  A  Q  P  P  L  P  A  L  P  P  F  P  P  P  S  G  K  N  P

               730       740       750       760       770       780
721        GTGATGATCTTGAACGAACTGCGCCCAGGACTCAAGTATGACTTCCTCTCCGAGAGCGGG
241         V  M  I  L  N  E  L  R  P  G  L  K  Y  D  F  L  S  E  S  G

               790       800       810       820       830       840
781        GAGAGCCATGCCAAGAGCTTCGTCATGTCTGTGGTCGTGGATGGTCAGTTCTTTGAAGGC
261         E  S  H  A  K  S  F  V  M  S  V  V  V  D  G  Q  F  F  E  G
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                850       860       870       880       890       900
841       TCGGGGAGAAACAAGAAGCTTGCCAAGGCCCGGGCTGCGCAGTCTGCCCTGGCCGCCATT
281        S  G  R  N  K  K  L  A  K  A  R  A  A  Q  S  A  L  A  A  I

                910       920       930       940       950       960
901       TTTAACTTGCACTTGGATCAGACGCCATCTCGCCAGCCTATTCCCAGTGAGGGTCTTCAG
301        F  N  L  H  L  D  Q  T  P  S  R  Q  P  I  P  S  E  G  L  Q

                970       980       990      1000      1010      1020
961       CTGCATTTACCGCAGGTTTTAGCTGACGCTGTCTCACGCCTGGTCCTGGGTAAGTTTGGT
321        L  H  L  P  Q  V  L  A  D  A  V  S  R  L  V  L  G  K  F  G

              1030      1040      1050      1060      1070      1080
1021      GACCTGACCGACAACTTCTCCTCCCCTCACGCTCGCAGAAAAGTGCTGGCTGGAGTCGTC
341        D  L  T  D  N  F  S  S  P  H  A  R  R  K  V  L  A  G  V  V

              1090      1100      1110      1120      1130      1140
1081      ATGACAACAGGCACAGATGTTAAAGATGCCAAGGTGATAAGTGTTTCTACAGGAACAAAA
361        M  T  T  G  T  D  V  K  D  A  K  V  I  S  V  S  T  G  T  K

              1150      1160      1170      1180      1190      1200
1141      TGTATTAATGGTGAATACATGAGTGATCGTGGCCTTGCATTAAATGACTGCCATGCAGAA
381        C  I  N  G  E  Y  M  S  D  R  G  L  A  L  N  D  C  H  A  E

              1210      1220      1230      1240      1250      1260
1201      ATAATATCTCGGAGATCCTTGCTCAGATTTCTTTATACACAACTTGAGCTTTACTTAAAT
401        I  I  S  R  R  S  L  L  R  F  L  Y  T  Q  L  E  L  Y  L  N

              1270      1280      1290      1300      1310      1320
1261      AACAAAGATGATCAAAAAAGATCCATCTTTCAGAAATCAGAGCGAGGGGGGTTTAGGCTG
421        N  K  D  D  Q  K  R  S  I  F  Q  K  S  E  R  G  G  F  R  L

              1330      1340      1350      1360      1370      1380
1321      AAGGAGAATGTCCAGTTTCATCTGTACATCAGCACCTCTCCCTGTGGAGATGCCAGAATC
441        K  E  N  V  Q  F  H  L  Y  I  S  T  S  P  C  G  D  A  R  I

              1390      1400      1410      1420      1430      1440
1381      TTCTCACCACATGAGCCAATCCTGGAAGAACCAGCAGATAGACACCCAAATCGTAAAGCA
461        F  S  P  H  E  P  I  L  E  E  P  A  D  R  H  P  N  R  K  A

              1450      1460      1470      1480      1490      1500
1441      AGAGGACAGCTACGGACCAAAATAGAGTCTGGTGAGGGGACGATTCCAGTGCGCTCCAAT
481        R  G  Q  L  R  T  K  I  E  S  G  E  G  T  I  P  V  R  S  N

              1510      1520      1530      1540      1550      1560
1501      GCGAGCATCCAAACGTGGGACGGGGTGCTGCAAGGGGAGCGGCTGCTCACCATGTCCTGC
501        A  S  I  Q  T  W  D  G  V  L  Q  G  E  R  L  L  T  M  S  C

              1570      1580      1590      1600      1610      1620
1561      AGTGACAAGATTGCACGCTGGAACGTGGTGGGCATCCAGGGTTCCCTGCTCAGCATTTTC
521        S  D  K  I  A  R  W  N  V  V  G  I  Q  G  S  L  L  S  I  F

              1630      1640      1650      1660      1670      1680
1621      GTGGAGCCCATTTACTTCTCGAGCATCATCCTGGGCAGCCTTTACCACGGGGACCACCTT
541        V  E  P  I  Y  F  S  S  I  I  L  G  S  L  Y  H  G  D  H  L

              1690      1700      1710      1720      1730      1740
1681      TCCAGGGCCATGTACCAGCGGATCTCCAACATAGAGGACCTGCCACCTCTCTACACCCTC
561        S  R  A  M  Y  Q  R  I  S  N  I  E  D  L  P  P  L  Y  T  L
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              1750      1760      1770      1780      1790      1800
1741      AACAAGCCTTTGCTCAGTGGCATCAGCAATGCAGAAGCACGGCAGCCAGGGAAGGCCCCC
581        N  K  P  L  L  S  G  I  S  N  A  E  A  R  Q  P  G  K  A  P

              1810      1820      1830      1840      1850      1860
1801      AACTTCAGTGTCAACTGGACGGTAGGCGACTCCGCTATTGAGGTCATCAACGCCACGACT
601        N  F  S  V  N  W  T  V  G  D  S  A  I  E  V  I  N  A  T  T

              1870      1880      1890      1900      1910      1920
1861      GGGAAGGATGAGCTGGGCCGCGCGTCCCGCCTGTGTAAGCACGCGTTGTACTGTCGCTGG
621        G  K  D  E  L  G  R  A  S  R  L  C  K  H  A  L  Y  C  R  W

              1930      1940      1950      1960      1970      1980
1921      ATGCGTGTGCACGGCAAGGTTCCCTCCCACTTACTACGCTCCAAGATTACCAAACCCAAC
641        M  R  V  H  G  K  V  P  S  H  L  L  R  S  K  I  T  K  P  N

              1990      2000      2010      2020      2030      2040
1981      GTGTACCATGAGTCCAAGCTGGCGGCAAAGGAGTACCAGGCCGCCAAGGCGCGTCTGTTC
661        V  Y  H  E  S  K  L  A  A  K  E  Y  Q  A  A  K  A  R  L  F

              2050      2060      2070      2080      2090      2100
2041      ACAGCCTTCATCAAGGCGGGGCTGGGGGCCTGGGTGGAGAAGCCCACCGAGCAGGACCAG
681        T  A  F  I  K  A  G  L  G  A  W  V  E  K  P  T  E  Q  D  Q

              2110      2120      2130      2140      2150
2101      TTCTCACTCACGCCCTCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAG
701        F  S  L  T  P  S  R  G  P  Y  S  I  V  S  P  K  C  *
                        Xba-I
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Gene and protein sequence of W58X eGFP in the context of the pcDNA 3.1 vector

```
               10        20        30        40        50        60
1         CTCGGATCCACCATGGCTAGCAAAGGAGAAGAACTCTTCACTGGAGTTGTCCCAATTCTT
1            BamH1     M  A  S  K  G  E  E  L  F  T  G  V  V  P  I  L

               70        80        90       100       110       120
61        GTTGAATTAGATGGTGATGTTAACGGCCACAAGTTCTCTGTCAGTGGAGAGGGTGAAGGT
21         V  E  L  D  G  D  V  N  G  H  K  F  S  V  S  G  E  G  E  G

              130       140       150       160       170       180
121       GATGCAACATACGGAAAACTTACCCTGAAGTTCATCTGCACTACTGGCAAACTGCCTGTT
41         D  A  T  Y  G  K  L  T  L  K  F  I  C  T  T  G  K  L  P  V

              190       200       210       220       230       240
181       CCGTAGCCGACACTAGTGACGACGCTCTGCTATGGCGTCCAGTGCTTTTCAAGATACCCG
61         P   *  P  T  L  V  T  T  L  C  Y  G  V  Q  C  F  S  R  Y  P
             W58x

              250       260       270       280       290       300
241       GATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAA
81         D  H  M  K  R  H  D  F  F  K  S  A  M  P  E  G  Y  V  Q  E

              310       320       330       340       350       360
301       AGGACCATCTTCTTCAAAGATGACGGCAACTACAAGACACGTGCTGAAGTCAAGTTTGAA
101        R  T  I  F  F  K  D  D  G  N  Y  K  T  R  A  E  V  K  F  E

              370       380       390       400       410       420
361       GGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGACTTCAAGGAAGATGGCAAC
121        G  D  T  L  V  N  R  I  E  L  K  G  I  D  F  K  E  D  G  N
```

```
                430       440       450       460       470       480
421        ATTCTGGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGAC
141         I   L   G   H   K   L   E   Y   N   Y   N   S   H   N   V   Y   I   M   A   D

                490       500       510       520       530       540
481        AAACAAAAGAATGGAATCAAAGTGAACTTCAAGACCCGCCACAACATTGAAGATGGAAGC
161         K   Q   K   N   G   I   K   V   N   F   K   T   R   H   N   I   E   D   G   S

                550       560       570       580       590       600
541        GTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTA
181         V   Q   L   A   D   H   Y   Q   Q   N   T   P   I   G   D   G   P   V   L   L

                610       620       630       640       650       660
601        CCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGA
201         P   D   N   H   Y   L   S   T   Q   S   A   L   S   K   D   P   N   E   K   R

                670       680       690       700       710       720
661        GACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAA
221         D   H   M   V   L   L   E   F   V   T   A   A   G   I   T   H   G   M   D   E

                730       740       750       760       770       780
721        CTATACAAATCCGGCTCTAGAGGGCCCTTCGAACAAAAACTCATCTCAGAAGAGGATCTG
241         L   Y   K   S   G   S   R   G   P   F   E   Q   K   L   I   S   E   E   D   L

                790       800       810       820       830       840
781        AATATGCATACCGGTCATCATCACCATCACCATTGAGTTTAAACCCGCTGATCAGCCTCG
261         N   M   H   T   G   H   H   H   H   H   H   *   V   *
```

Gene sequence of a 16 nt R/G-guideRNA against eGFP W58X in the context of the pSilencer vector

```
1          CGGAAGAGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA
              SapI

61         GCTGGCACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA
121        GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT
181        GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACATG ATTACGAATT
241        GCAACGATTT AGGTGACACT ATAGAAGAGA AGGAATTAAT ACGACTCACT ATAGGGAGAG
301        AGAGAGAATT ACCCTCACTA AAGGGAGGAG AAGCATGAAT TCCCCAGTGG AAAGACGCGC
361        AGGCAAAACG CACCACGTGA CGGAGCGTGA CCGCGCGCCG AGCGCGCGCC AAGGTCGGGC
421        AGGAAGAGGG CCTATTTCCC ATGATTCCTT CATATTTGCA TATACGATAC AAGGCTGTTA
481        GAGAGATAAT TAGAATTAAT TTGACTGTAA ACACAAAGAT ATTAGTACAA AATACGTGAC
541        GTAGAAAGTA ATAATTTCTT GGGTAGTTTG CAGTTTTAAA ATTATGTTTT AAAATGGACT
601        ATCATATGCT TACCGTAACT TGAAAGTATT TCGATTTCTT GGGTTTATAT ATCTTGTGGA
661        AAGGACGCGG GATCC  *GTGGA ATAGTATAAC AATATGCTAA ATGTTGTTAT
AGTATCCCAC
                            * = transcription start   R/G-motif

721        TCGGCCACGG AACAGGTTTT TTGGAAAGCT TGG
              mRNA template U6-term.  HindIII
```

Table S1. *List of all R/G-gRNAs used in cell culture. Given is the mRNA binding site and the 3´-terminal hairpin (highlighted in gray) if applicable. The invariant R/G-motif is omitted for clarity.*

Name of R/G-gRNA	Sequence of the mRNA binding site 5'→3'	Experimental number
W58X GFP P6 16nt	UCGG**CCA**CGGAACAGG	Fig. 2B
W58X GFP P6 18nt + boxB	UCGG**CCA**CGGAACAGGCA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2C / Fig. S10
W58X GFP P6 20nt + boxB	UCGG**CCA**CGGAACAGGCAGU*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2C / Fig. S10
W58X GFP P6 25nt + boxB	UCGG**CCA**CGGAACAGGCAGUUUGCC*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2C / Fig. S10
W58X GFP P6 29nt + boxB	UCGG**CCA**CGGAACAGGCAGUUUGCCAGUA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2C / Fig. S10
W58X GFP P3 16 nt + boxB	GG**CCA**CGGAACAGGCA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2D / Fig. S11
W58X GFP P4 16 nt + boxB	CGG**CCA**CGGAACAGGC*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2D / Fig. S11
W58X GFP P5 16 nt + boxB	UCGG**CCA**CGGAACAGG*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2D / Fig. S11
W58X GFP P7 16 nt + boxB	GUCGG**CCA**CGGAACAG*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2D / Fig. S11
W58X GFP P8 16 nt + boxB	UGUCGG**CCA**CGGAACA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2D / Fig. S11
W58X GFP P9 16 nt + boxB	GUGUCGG**CCA**CGGAAC*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2D / Fig. S11
W58X GFP P10 16 nt + boxB	AGUGUCGG**CCA**CGGAA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 2D / Fig. S11
R407Q PINK-1 P4 16 nt + boxB	CC**CCG**AUCCACGUACC*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. S15
R407Q PINK-1 P5 16 nt + boxB	GCC**CCG**AUCCACGUAC*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. S15
R407Q PINK-1 P6 16 nt + boxB	CGCC**CCG**AUCCACGUA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. S15
R407Q PINK-1 P7 16 nt + boxB	CCGCC**CCG**AUCCACGU*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. S15
R407Q PINK-1 P8 16 nt + boxB	UCCGCC**CCG**AUCCACG*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. S15
R407Q PINK-1 P9 16 nt + boxB	UUCCGCC**CCG**AUCCAC*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. S15
R407Q PINK-1 P10 16 nt + boxB	UUUCCGCC**CCG**AUCCA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. S15
W437X Amber PINK-1 P8 16 nt + boxB	CACUGC**CCA**GGCAUCA*GGGCCCUCUUCAGGGCCC*	Fig. 3C / Fig. 3D / Fig. 4
3'UTR TAG#1 Actin P8 16 nt + boxB	ACGCAA**CCA**AGUCAUA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#2 Actin P8 16 nt + boxB	GAAUGA**CCA**UUAAAAA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#3 Actin P8 16 nt + boxB	GCAAUG**CCA**UCACCUC*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#1 GAPDH P8 16 nt + boxB	AGGGGU**CCA**CAUGGCA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#2 GAPDH P8 16 nt	GGCUCC**CCA**GGCCCCU*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B

+ boxB		
V627V TAG#1 GPI P8 16 nt + boxB	UGCCGU**CCA**CCAGGAU*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
L456L TAG#1 GusB P8 16 nt + boxB	CAGAUU**CCA**GGUGGGA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#2 GusB P8 16 nt + boxB	UCCCUG**CCA**GAAUAGA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#1 VCP P8 16 nt + boxB	CUCCGC**CCA**CCAAAUG*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#2 VCP P8 16 nt + boxB	CCCAAA**CCA**CAACAGA*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#3 VCP P8 16 nt + boxB	ACCCAC**CCA**CCCAGGU*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#1 RAB7A P8 16 nt + boxB	CUGCCG**CCA**GCUGGAU*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B
3'UTR TAG#2 RAB7A P8 16 nt + boxB	AGGGAA**CCA**GACAGUU*UCUAGAGGGCCCUGAAGAGGGCCC*	Fig. 3B

RNA sequencing traces of the full eGFP ORF

Figure S6. Full sequencing trace corresponding to the trace shown in Figure 2B, e), first experiment

Figure S7 *Full sequencing trace corresponding to the trace shown in Figure 2E, 50 ng experiment*

Figure S8: The prolongation of the editing time from 24 hours to 48 hours increases the editing yield in cell culture. *All editing samples included the amount of 300 ng W58X eGFP plasmid, 300 ng of ADAR2 plasmid and 1300 ng / 1600 ng of R/G-gRNA plasmid (c)-f)). The positive controls contained 300 ng eGFP plasmid, 300 ng of ADAR2 plasmid and 1300 ng of R/G-gRNA (a), b)). An increment of the fluorescent signal is obtained for the positive controls by prolonging the incubation time up to 48 hours (a-b), as well as for the two editing samples (c->e, d->f). The amount of fluorescing cells and the editing yields were increased for both editing samples. Total magnification: 100x, GFP exposure time: 50 ms*.

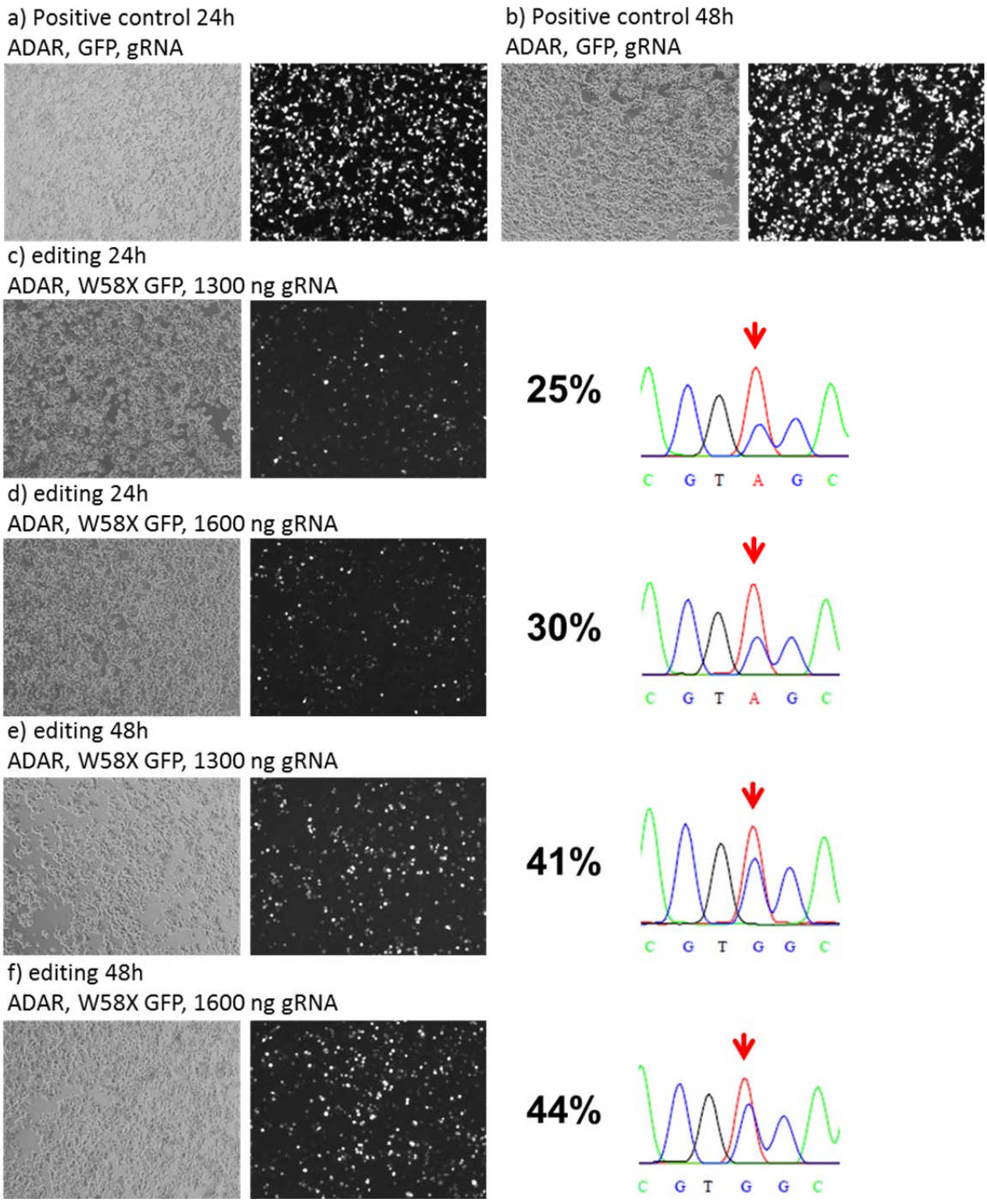
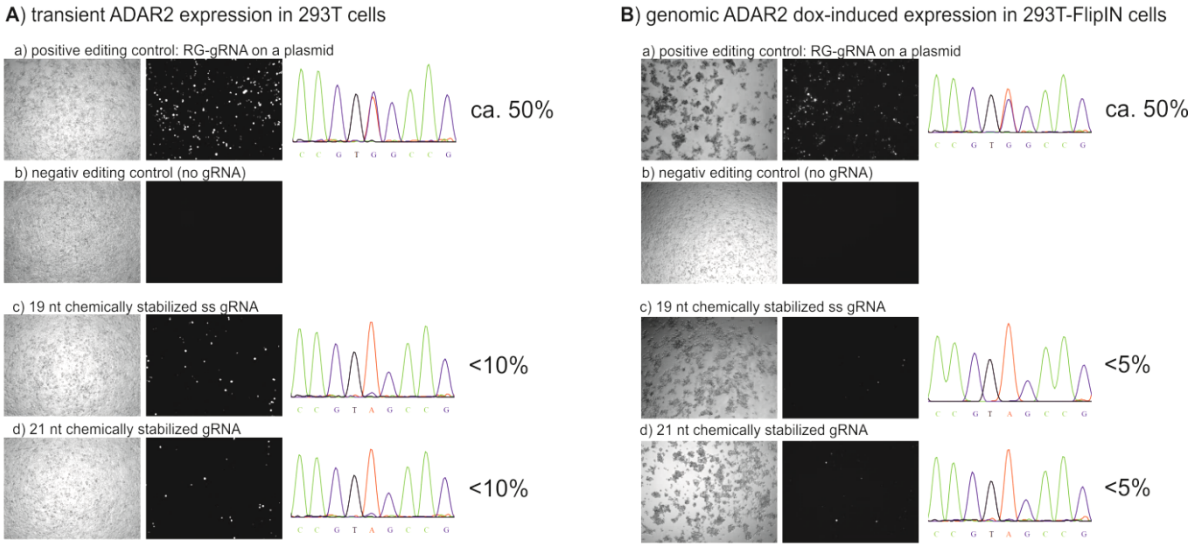


Figure S9: Comparing the editing efficiency using chemically stabilized single-stranded (ss) gRNA and U6-driven unstructured ss guideRNAs, and replacement of ADAR2 by SNAP-ADAR2. A) & **B**) Replacement of the R/G-gRNA plasmid by a ss-gRNA transfection: A) co-transfection of 500 ng W58X eGFP and 100 ng ADAR2 plasmid; B) transfection of 500 ng W58X eGFP plasmid plus induction of ADAR2 with 10 ng/mL doxycycline. 24 hours after plasmid transfection in 24-wells, the cells were detached and reseeded in a 96-well format and directly reverse transfected with 10 pmol (A) or 20 pmol (B) ss-gRNA. After 48h, fluorescence images were taken and RNA was isolated. In the controls a), 260 ng R/G-guideRNA have been transfected. The chemically stabilized guideRNAs contain 2´-O-methyl groups globally apart from a 3 nt gap around the adenosine to be edited, and terminal phosphorothioates. The full guideRNA sequences are given in Hanswillemenke et al., *JACS* **2015**. **C**) & **D**): U6-driven expression of unstructured guideRNAs. Cotransfection in 24-well format: 300 ng W58x eGFP and 1300 ng respective U6-driven guideRNA-vector, in case of C) 100 ng ADAR2 plasmid, in case of D) 10 ng/ml doxycycline. Fluorescence imaging was taken 48 hrs post transfection. The placement of the unstructured guideRNAs (C) and D)) relative to the mRNA is given, 1* stands for the edited adenosine. Negative control z) was like all four positive controls a), but with SNAP-ADAR2 instead of human ADAR2. Total magnification: 100x, GFP exposure time: 50 ms.

Editing with chemically stabilized ss guideRNAs



Editing with U6-driven, unstructured ss guideRNAs

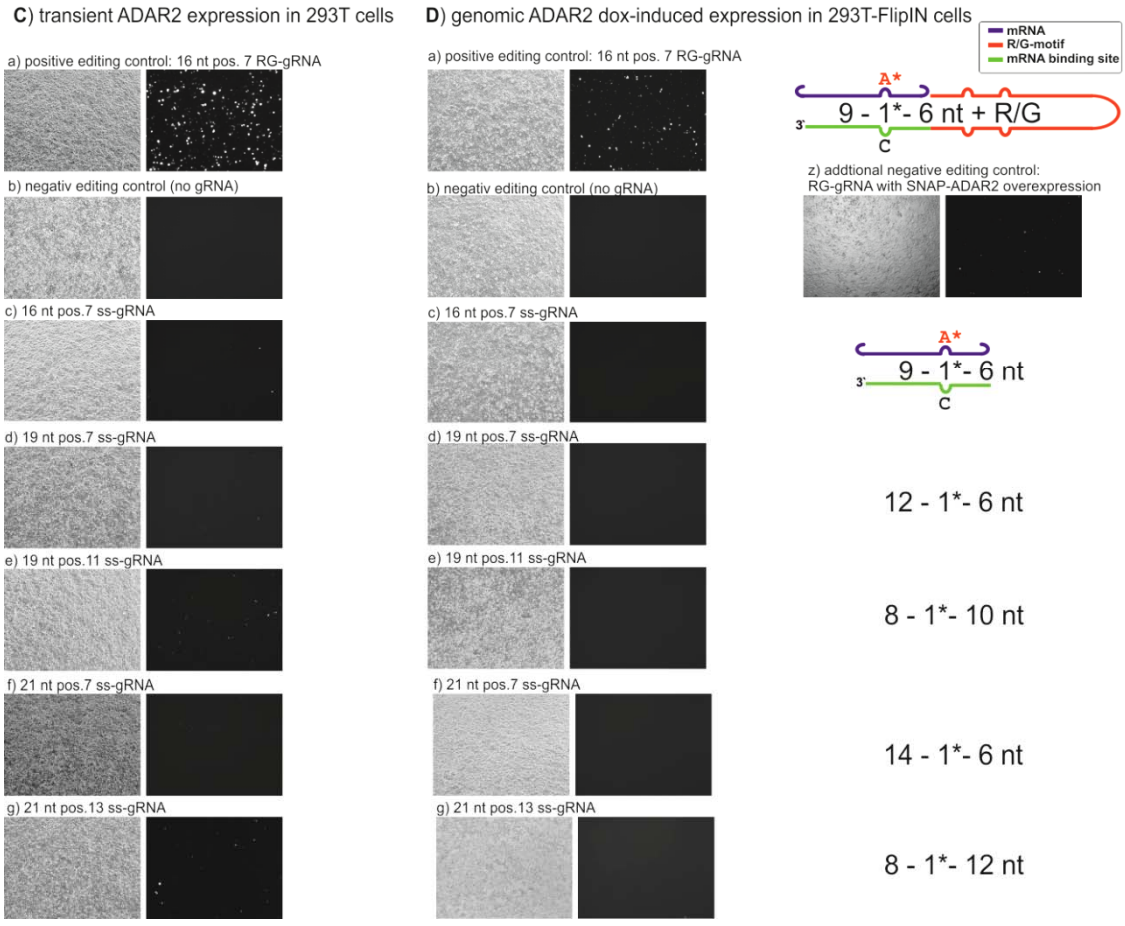


Figure S10: Microscopy analysis and editing yields of R/G-gRNAs with varying length of the flexible part and off-target editing at position 53*. The strongest fluorescent signal and highest editing yield was obtained for a R/G-gRNA with 16 or 18 nt length of the mRNA binding site. With the prolongation of the mRNA binding site of the R/G-gRNA less fluorescent signal and lower editing yields were observed. The off-target adenosine at position 53 is edited up to 10% if a 25 nt and 29 nt long R/G-gRNA is used for the editing reaction. Total magnification: 100x, GFP exposure time: 50 ms.*

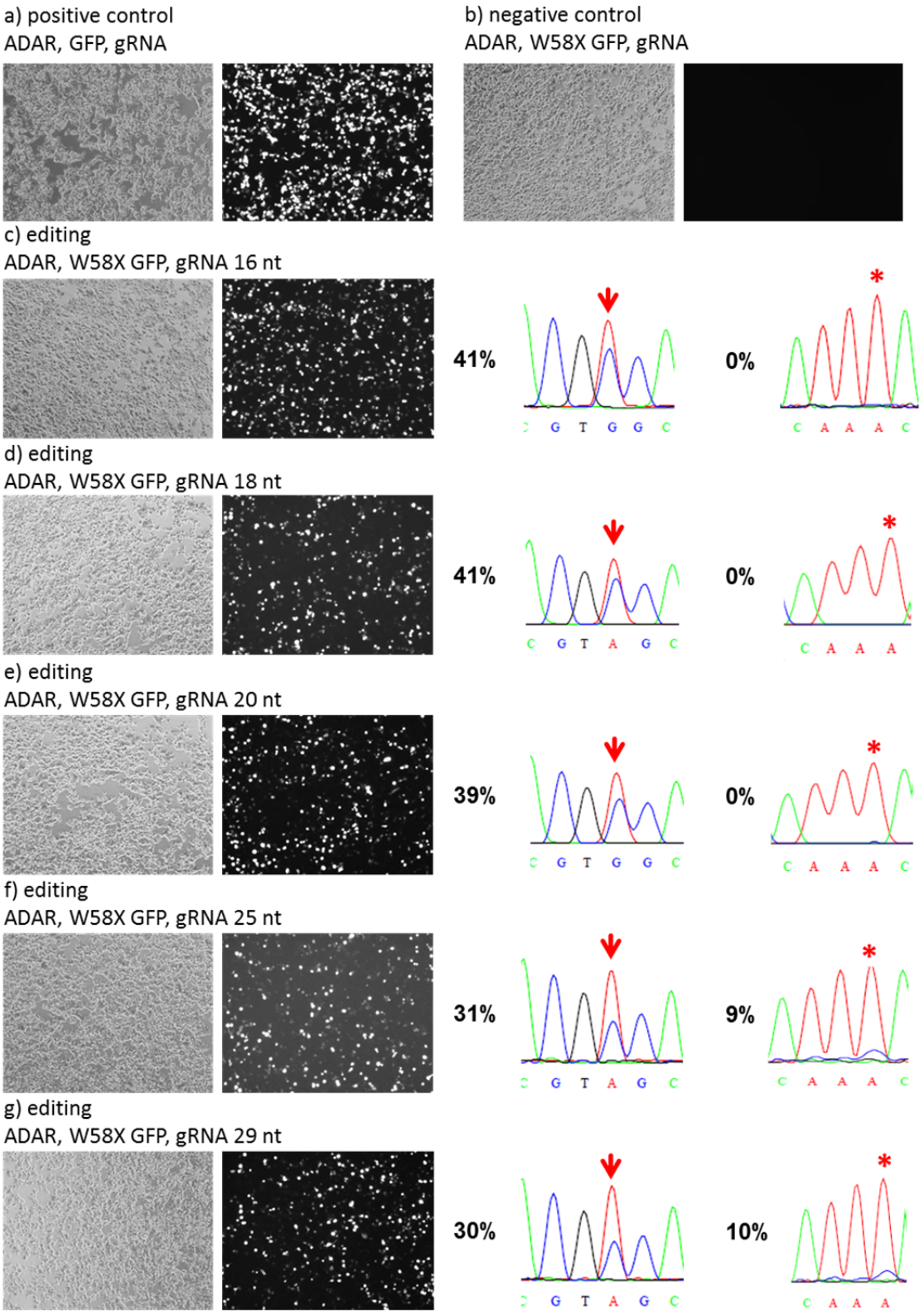
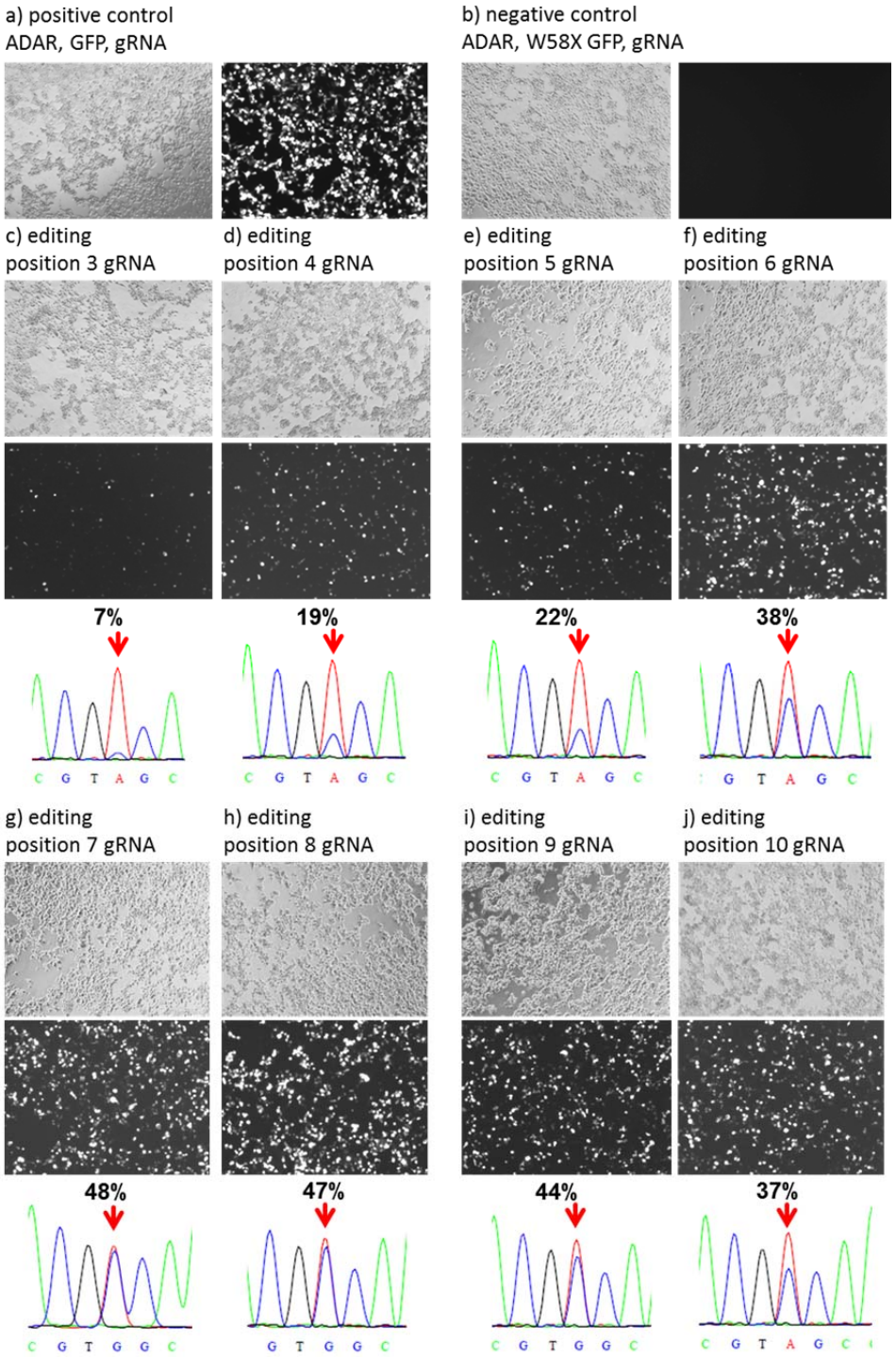


***Figure S11: Effect of variable positions of the W58X eGFP R/G-gRNA towards the target adenosine in cell culture**. Co-transfection of 300 ng W58X eGFP and ADAR2 plasmid together with 1300 ng of the tested position of the R/G-gRNA was performed in a 24-well plate format. The microscopic analysis and RNA isolation for sequence analysis was performed 48 hours post transfection. The R/G-gRNAs positions 3 until 10 are abbreviated by P3 – P10 (this equals 2-9 intervening nucleotides). Starting from the R/G-gRNA position 3 an increasing fluorescent signal and amount is visible until R/G-gRNA position 8. The R/G-gRNA position 9 and 10 showed a dropping fluorescent signal. The microscopic results are confirmed by the sequence analysis and demonstrate that R/G-gRNA position 8 was the most successful guideRNA to achieve maximum editing yields. Total magnification: 100x, GFP exposure time: 50 ms.*

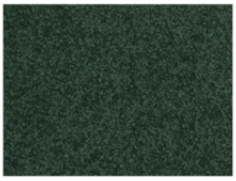


continuation Figure S11, replication of the positional effect.

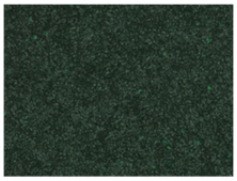
Replication 1: 24-well format, 300 ng ADAR2 plasmid, 300 ng W58X plasmid, 1600 ng R/G-guideRNA plasmid, imaging 48 hrs post transfection, total magnification 100x, 50 ms exposure

a) negative control: no guideRNA

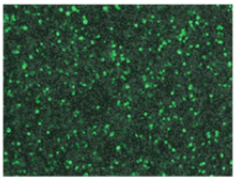
f) negative control: no ADAR



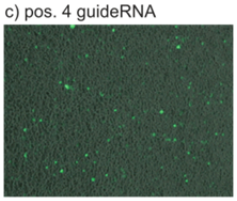
b) pos. 3 guideRNA



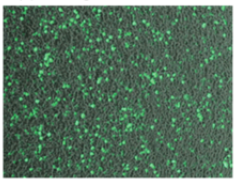
g) pos. 7 guideRNA



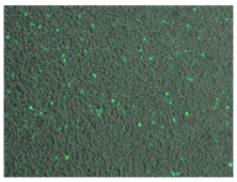
c) pos. 4 guideRNA



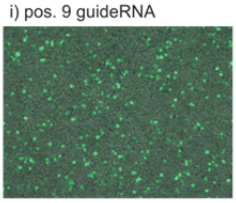
h) pos. 8 guideRNA



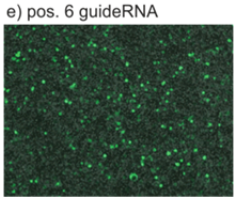
d) pos. 5 guideRNA



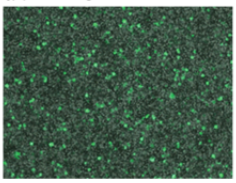
i) pos. 9 guideRNA



e) pos. 6 guideRNA



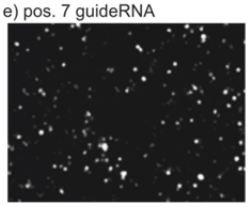
j) pos. 10 guideRNA



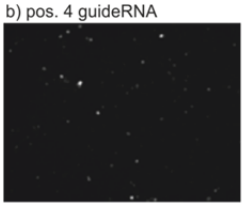
Replication 2: exactly as replication 1, but 1300 ng R/G-guideRNA

a) pos. 3 guideRNA

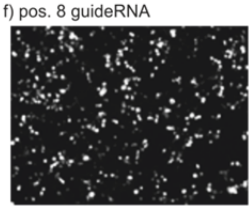
e) pos. 7 guideRNA



b) pos. 4 guideRNA

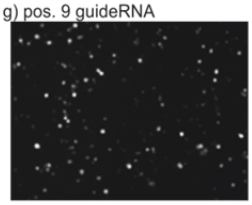


f) pos. 8 guideRNA

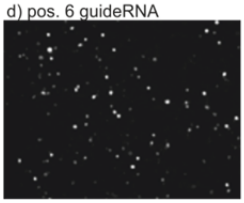


c) pos. 5 guideRNA

g) pos. 9 guideRNA



d) pos. 6 guideRNA



h) pos. 10 guideRNA

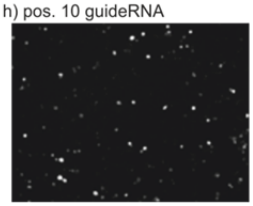


Figure S12: Effect of variable amounts of ADAR2 and R/G-gRNA plasmids in cell culture. Different amounts of R/G-gRNA P8 plasmid were transfected in 24-well plate format together with a constant amount of 300 ng W58X eGFP and ADAR2 plasmid (P8 equals 7 intervening nucleotides). Higher amounts of R/G-gRNA plasmid resulted in more and stronger fluorescence, as well as in higher editing levels. Total magnification: 100x, GFP exposure time: 50 ms.

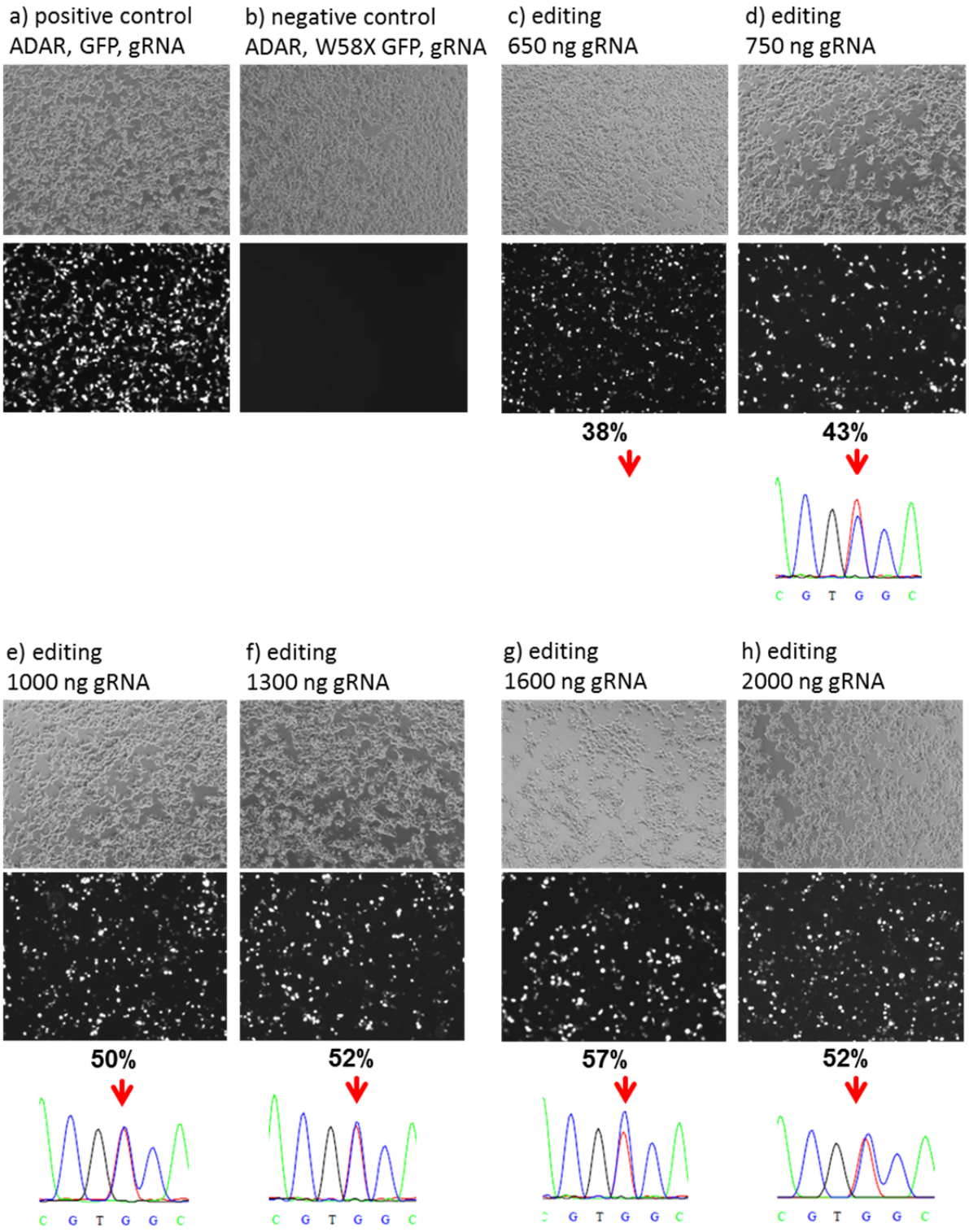
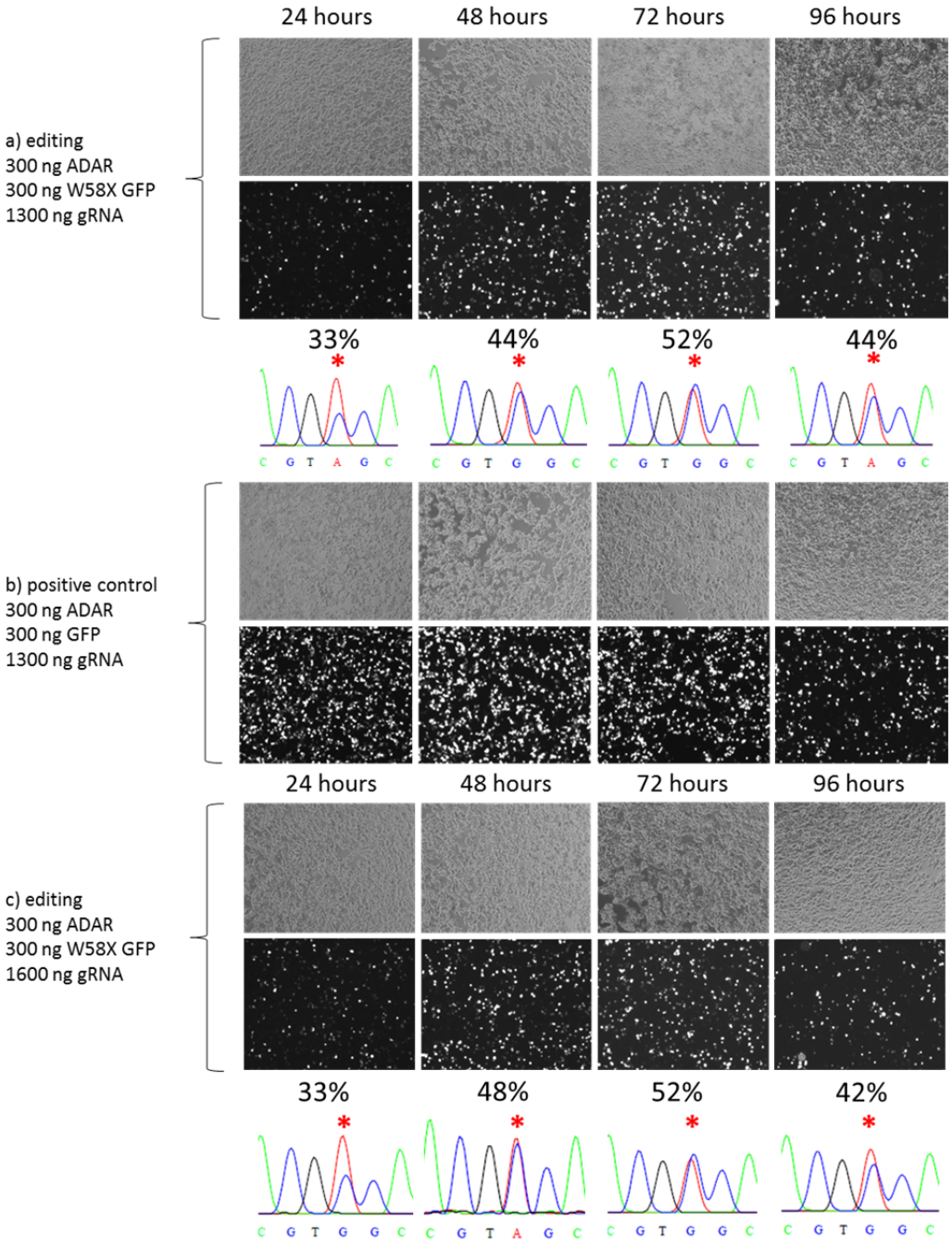

a) positive control
ADAR, GFP, gRNA
b) negative control
ADAR, W58X GFP, gRNA
c) editing
650 ng gRNA
d) editing
750 ng gRNA
38%
43%
C G T G G C
e) editing
1000 ng gRNA
f) editing
1300 ng gRNA
g) editing
1600 ng gRNA
h) editing
2000 ng gRNA
50%
52%
57%
52%
C G T G G C

Figure S13: Prolongation of the editing time for the R/G-gRNA position 8 *(position 8 equals 7 intervening nucleotides). The co-transfection experiment of 300 ng W58X eGFP plasmid, 300 ng of ADAR2 plasmid and 1300 ng or 1600 ng R/G-gRNA P8 plasmid was performed in a 24-well format. The editing efficiency was analyzed 24h, 48h, 72h and 96h post transfection. The positive control showed the strongest fluorescent signal for 48 hours of incubation. Shorter and longer incubation led to a reduced eGFP signal. For both chosen R/G-gRNA P8 plasmid amounts an increasing fluorescent signal and amount until 72h of incubation was visible. The eGFP intensity and amount of cells was declining after 96h of incubation. The sequence analysis confirmed the fluorescent microscopy: the editing yields increased until 72 hours of editing time and decreased after 96h again. Total magnification: 100x, GFP exposure time: 50 ms.*



.

Figure S14: Effect of decreasing amounts of transfected ADAR2 plasmid on the editing yield and the off-target editing at position A381. *In a 24-well format cells were co-transfected with 300 ng W58X eGFP and 1300 ng R/G-gRNA-P8 (P8 equals 7 intervening nucleotides) together with varying amounts of ADAR2 plasmid. The microscopic analysis and RNA isolation was carried out 48 h post transfection. The decrease of the ADAR2 plasmid down to 50 ng reduces the editing yield by 5 % compared to the starting concentration of 300 ng. The usage of 25 ng of ADAR2 plasmid markedly lowers the editing level down to 36% compared to 52% editing yield for 300 ng of ADAR2 plasmid. The reduction of the transfected ADAR2 plasmid amount led to a decrease at the off-target site A381 eGFP. Transfection of 100 ng or lower ADAR2 plasmid amount completely prevents the off-target editing. Total magnification: 100x, GFP exposure time: 50 ms.*

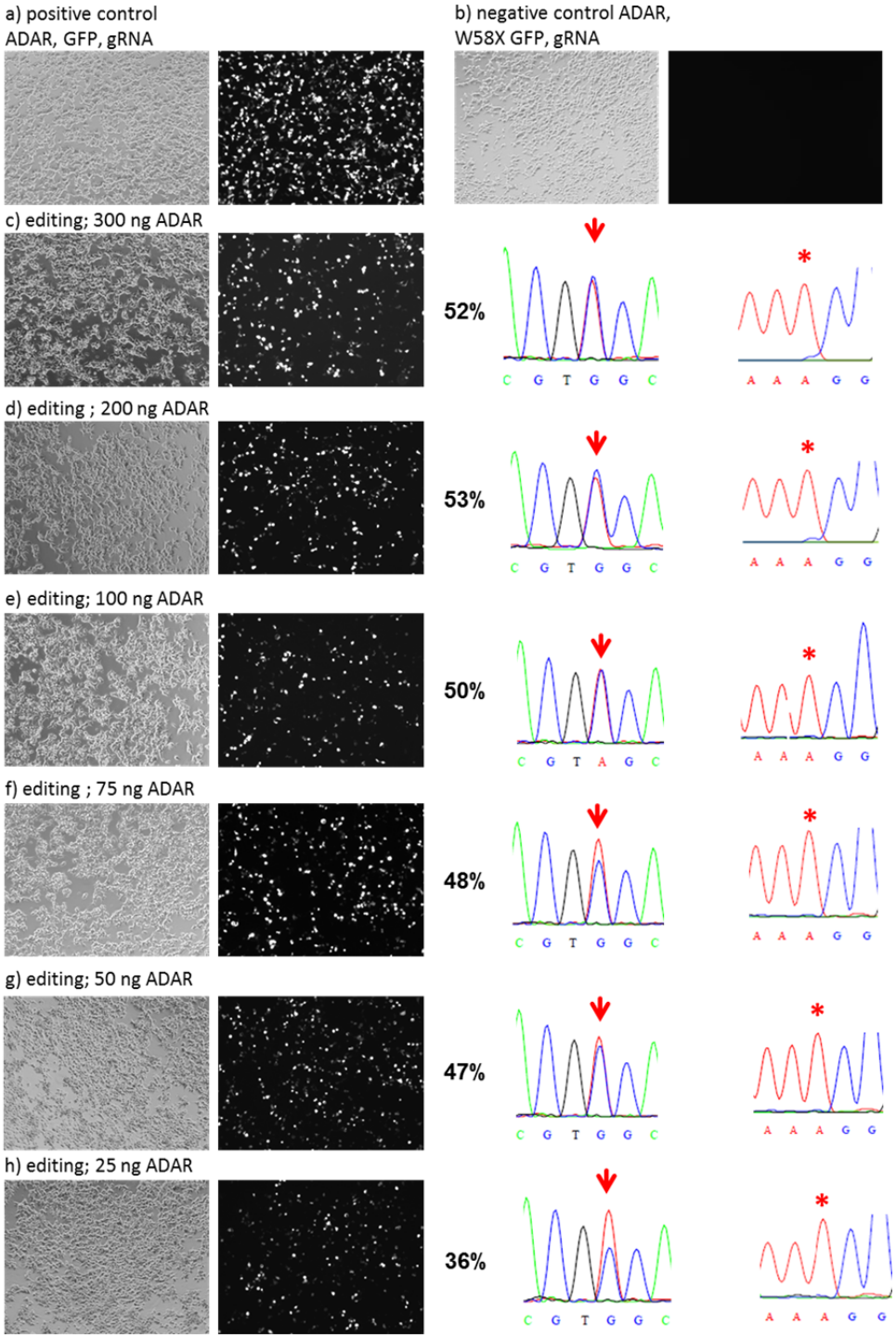


Figure S15 *Editing depends on the position of the targeted adenosine to the R/G-motif. This was also found for editing of the R407Q site in PINK1 in 293 cells analog to the experiments shown in Figure 2D. (n = 3)*

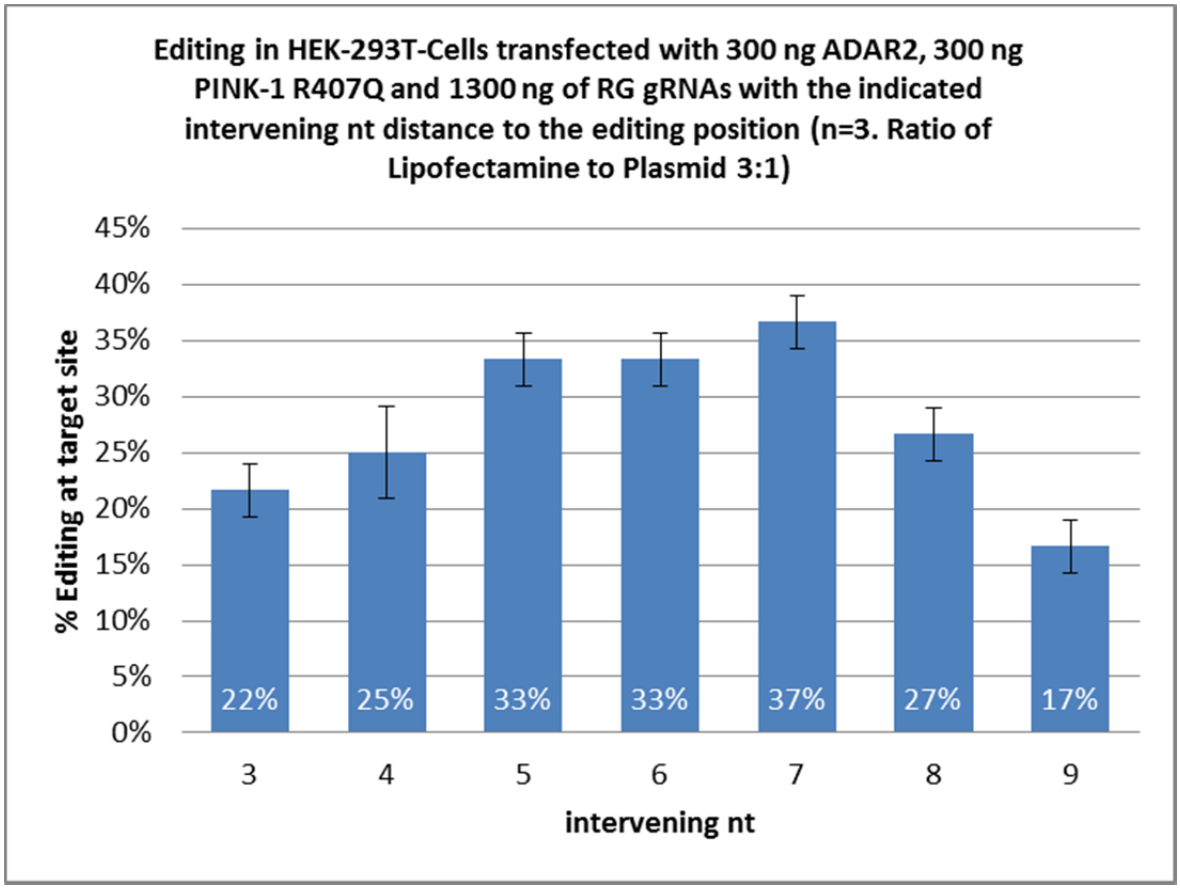
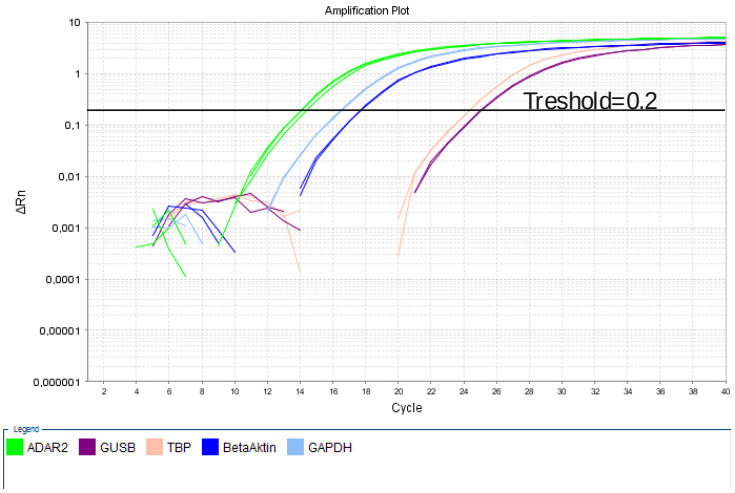


Figure S16. *qPCR analysis of ADAR2 expression. The relative ADAR2 mRNA amount in 293T cells transiently transfected (trans.) with ADAR2 and 293T cells with a genomically integrated copy of ADAR2 controlled by a CMV tet-on promoter (integr.) was determined by quantitative real-time PCR (qPCR) after 24h (doxycycline induced expression of integr. ADAR2) and 48h (expression of trans. and integr. ADAR2). For this, RNA was extracted from cell lysates (RNeasy MinElute Kit, Qiagen). After DNaseI digestion (NEB) and reverse transcription (high capacity cDNA reverse transcription kit, Applied Biosystems), 20 ng cDNA was mixed with Fast SYBR Green Master Mix (Applied Biosystems) and analyzed by the 7500 Fast Real-Time PCR System (Applied Biosystems).* ***(A)*** *For determining gene expression, primers were designed for targeting ADAR2 and the housekeeping genes ß-actin, glyceraldehyde-3-phophate dehydrogenase (GAPDH), ß-glucuronidase (GUSB) and TATA-box binding protein (TBP), (**B**) shows an example of the sybr green traces during qPCR.* ***(C)*** *qPCR of ADAR2 and the housekeeping gene was performed in triplicates and duplicates, respectively. The table displays the mean values of the cycles where the fluorescence crosses the threshold of 0.2 (ct values).* ***(D)*** *Based on these ct values, the expression of ADAR2 compared to housekeeping gene expression was determined in 293T cells after transient ADAR2 transfection or doxycycline induction by the delta ct equation.* ***(E)*** *To compare ADAR2 expression of ADAR2 transiently transfected 293T cells and ADAR2 genomically integrated 293T cells after 48h of expression, two methods were applied. In **method 1**, a calibration curve was generated from 1:5 dilutions of 20 ng cDNA of transiently transfected 293T cells (mean values from triplicates with standard derivation). For normalization, the corresponding ct (ADAR2) values were divided by the ct-value of ß-actin for 20 ng cDNA. In **method 2**, the delta-delta ct method was used to calculate difference in ADAR2 expression.*

(A) *Primers for qPCR*

Gene	***Sequence (5' to 3')***	***Product size***
ADAR2	*fw.: CGGAGATCCTTGCTCAGATT*	*99 bp*
	rev.: CCCTCGCTCTGATTTCTGAA	
ß-actin	*fw.: CGGGACCTGACTGACTAC*	*91 bp*
	rev.: TAATGTCACGCACGATTTCC	
GAPDH	*fw.: CAACAGCCTCAAGATCATCAG*	*96 bp*
	rev.: CCTTCCACGATACCAAAGTTG	
GUSB	*fw.: ACCTGTTCAAGTTGGAAGTG*	*93 bp*
	rev.: CACCTGGCACCTTAAGTTG	
TBP	*fw.: CGGAGAGTTCTGGGATTGTA*	*90 bp*
	rev.: GAAGTGCAATGGTCTTTAGGT	

(B) *An example of the raw data for transient ADAR2 expression (300 ng) in 293T cells.*



(C) *Measured ct-values of all experiments. Values are averaged from three technical replicates for ADAR2 and two technical replicates for the housekeeping genes*

	Sample	ct (ADAR2)	ct (ß-actin)	ct (GAPDH)	ct (GUSB)	ct (TBP)
Tra	293T	25.061	18.089	16.88	24.829	24.174
	293T + 300ng ADAR2, 48h	14.195	17.710	16.532	25.08	24.356
Integr.	293-pcDNA5 + Dox, 24h	23.807	17.143	16.617	23.935	23.086
	293-ADAR2 without Dox	20.916	16.733	16.199	23.696	22.72
	293-ADAR2 + Dox, 24h	18.710	17.483	16.941	24.093	23.546
	293-ADAR2 + Dox, 48h	18.633	17.654	16.766	24.651	23.784

(D) *Calculation of relative expression levels from the Δct values for ADAR2 versus four housekeeping genes*

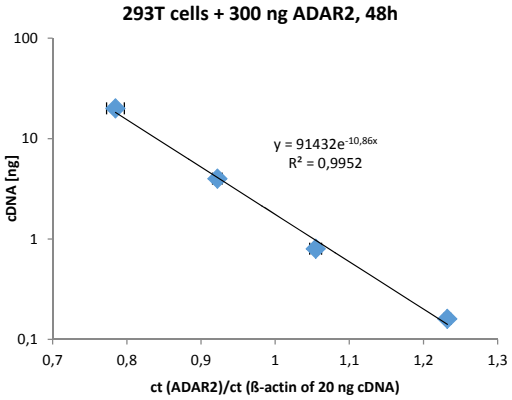
	Sample	ß-actin	GAPDH	GUSB	TPB
Tra	293T	0.008	0.003	0.851	0.541
	293T + 300ng ADAR2, 48h	11.432	5.053	1891.087	1144.895
Integr.	293-pcDNA5	0.009	0.007	1.093	0.607
	293-ADAR2 without Dox	0.055	0.038	6.869	3.492
	293-ADAR2 + 10 ng/ml Dox, 24h	0.427	0.293	41.730	28.562
	293-ADAR2 + 10ng/ml Dox, 48h	0.507	0.274	64.804	35.531

$\text{relative expression} = 2^{-\Delta ct}$, with $\Delta ct = ct(\text{ADAR2}) - ct(\text{housekeeping gene})$

(E) *Comparison of transient versus genomic ADAR2 expression*

Method 1

A calibration curve was taken for four different ADAR2 (transient expression) dilutions (1x, 5x, 25x, 125x). Plotted is the ADAR2 ct values normalized by the ct value for beta-actin of the undiluted sample versus the amount of cDNA.



From the regression curve of the calibration plot, the relative expression of genomically expressed ADAR2 was calculated. For this genomically expressed ADAR2 was normalized to beta-actin.

ct (ADAR2)/ct (ß-actin) = 1.055 [for the experiment with 293-ADAR2 + 10 ng/ml Dox, 48h] (20 ng cDNA)

from x = 1.055 one can calculate y = 0.967, and the genomic expression to ***be 20fold*** *below that of the transient expression*

Method 2

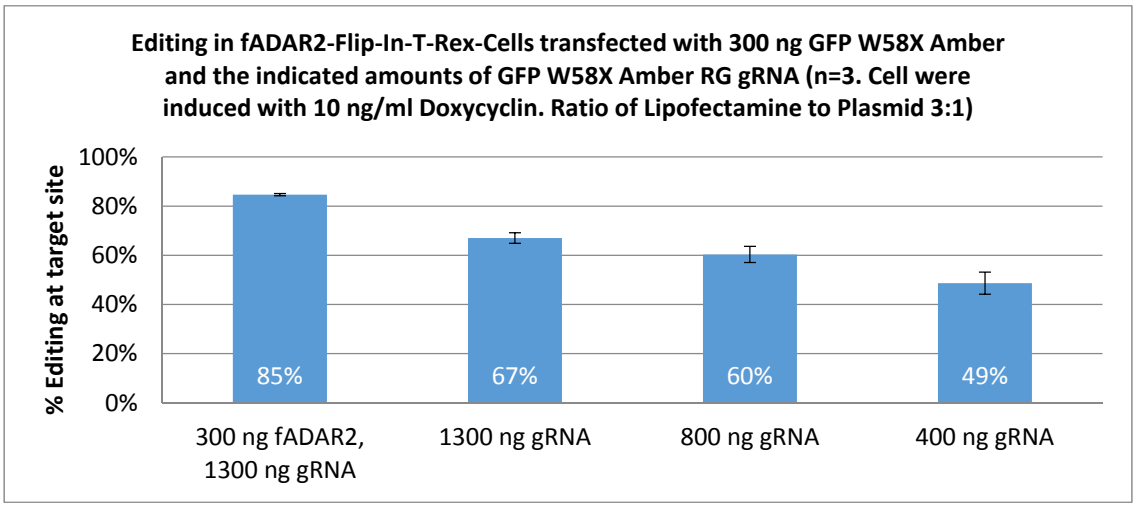
Here we estimated the relative expression level of ADAR2 transient versus genomic by the delta-delta ct method applying the following equation.

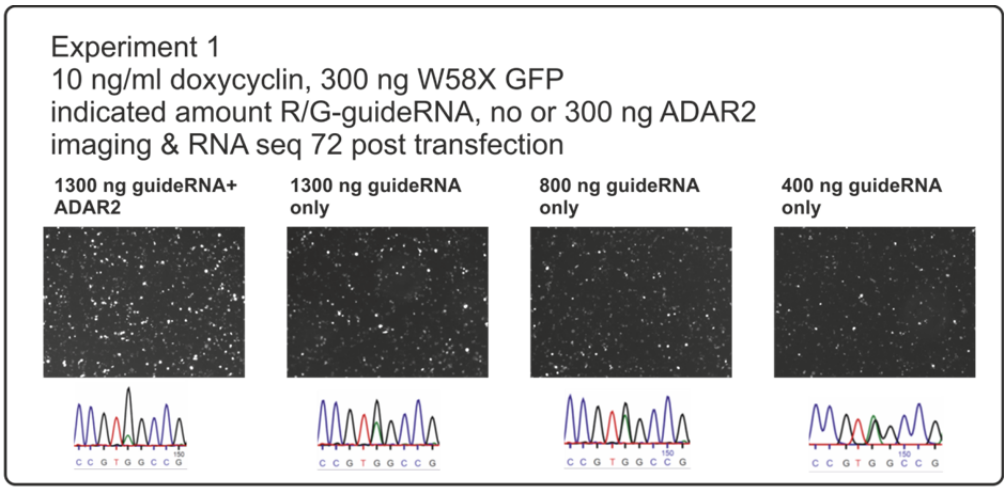
$$\Delta\Delta\mathrm{ct} = (\mathrm{ct}(\mathrm{ADAR2}) - \mathrm{ct}(\text{ßactin}))_{\mathrm{transient}} - ((\mathrm{ct}(\mathrm{ADAR2}) - \mathrm{ct}(\text{ßactin}))_{\mathrm{genomic}}$$

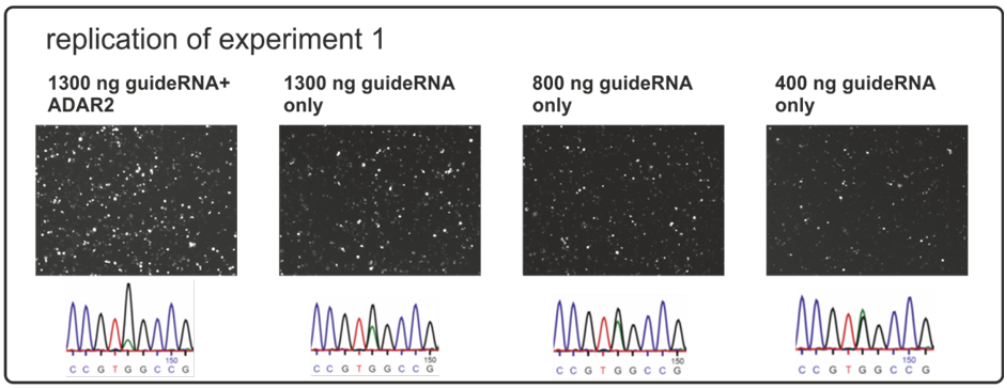
$$\text{relative expression} = 2^{-\Delta\Delta\mathrm{ct}}$$

$\text{relative expression} = 25.795$, meaning genomic expression is approx. **26fold** below transient expression

Figure S17 *Dependency of the editing yield with genomically encoded ADAR2 with varying amounts of guideRNA. Shown are the averaged yields and standard deviation for n=3.*







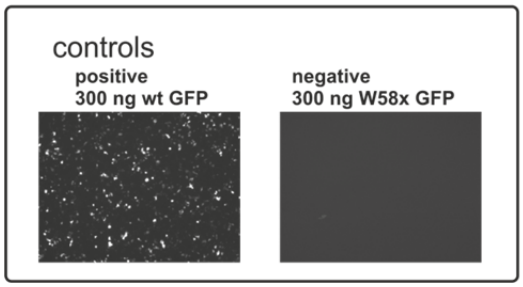


Figure S18. Full sequencing trace corresponding to the trace shown in Figure 3A, b), 1300 ng guideRNA

Editing of endogenous transcripts

Editing experiments have been carried out in duplicates exactly as described before (without further optimization), but without co-transfection of a target or reporter gene. 293 cells have been transfected with 300 ng ADAR2 and of the respective 1300 ng R/G-guideRNA in 24 well format with lipfectamine 2000, and were harvested 48h post transfection. 293-ADAR2 flip-in cells have been induced with doxycycline (10 ng/ml), then 1300 ng of the respective R/G-guideRNA was transfected with lipofectamine 2000. 72 h after transfection of the guideRNA total RNA was isolated, RT-PCR with transcript-specific primers have been done to obtain the RNA sequencing traces. The respective guideRNA sequences are listed in Table S1. Primers for RT-PCR are given in Table S2.

Target sites for site-directed RNA editing on six genes:

cDNA sequence of β-actin

```
                  10        20        30        40        50        60
1         ACCGCCGAGACCGCGTCCGCCCCGCGAGCACAGAGCCTCGCCTTTGCCGATCCGCCGCCC
1

                  70        80        90       100       110       120
61        GTCCACACCCGCCGCCAGCTCACCATGGATGATGATATCGCCGCGCTCGTCGTCGACAAC
21                                M  D  D  D  I  A  A  L  V  V  D  N

                130       140       150       160       170       180
121       GGCTCCGGCATGTGCAAGGCCGGCTTCGCGGGCGACGATGCCCCCCGGGCCGTCTTCCCC
41         G  S  G  M  C  K  A  G  F  A  G  D  D  A  P  R  A  V  F  P

                190       200       210       220       230       240
181       TCCATCGTGGGGCGCCCCAGGCACCAGGGCGTGATGGTGGGCATGGGTCAGAAGGATTCC
61         S  I  V  G  R  P  R  H  Q  G  V  M  V  G  M  G  Q  K  D  S

                250       260       270       280       290       300
241       TATGTGGGCGACGAGGCCCAGAGCAAGAGAGGCATCCTCACCCTGAAGTACCCCATCGAG
81         Y  V  G  D  E  A  Q  S  K  R  G  I  L  T  L  K  Y  P  I  E

                310       320       330       340       350       360
301       CACGGCATCGTCACCAACTGGGACGACATGGAGAAAATCTGGCACCACACCTTCTACAAT
101        H  G  I  V  T  N  W  D  D  M  E  K  I  W  H  H  T  F  Y  N

                370       380       390       400       410       420
361       GAGCTGCGTGTGGCTCCCGAGGAGCACCCCGTGCTGCTGACCGAGGCCCCCCTGAACCCC
121        E  L  R  V  A  P  E  E  H  P  V  L  L  T  E  A  P  L  N  P

                430       440       450       460       470       480
421       AAGGCCAACCGCGAGAAGATGACCCAGATCATGTTTGAGACCTTCAACACCCCAGCCATG
141        K  A  N  R  E  K  M  T  Q  I  M  F  E  T  F  N  T  P  A  M

                490       500       510       520       530       540
481       TACGTTGCTATCCAGGCTGTGCTATCCCTGTACGCCTCTGGCCGTACCACTGGCATCGTG
161        Y  V  A  I  Q  A  V  L  S  L  Y  A  S  G  R  T  T  G  I  V

                550       560       570       580       590       600
541       ATGGACTCCGGTGACGGGGTCACCCACACTGTGCCCATCTACGAGGGGTATGCCCTCCCC
181        M  D  S  G  D  G  V  T  H  T  V  P  I  Y  E  G  Y  A  L  P

                610       620       630       640       650       660
601       CATGCCATCCTGCGTCTGGACCTGGCTGGCCGGGACCTGACTGACTACCTCATGAAGATC
201        H  A  I  L  R  L  D  L  A  G  R  D  L  T  D  Y  L  M  K  I
```

```
                670       680       690       700       710       720
661       CTCACCGAGCGCGGCTACAGCTTCACCACCACGGCCGAGCGGGAAATCGTGCGTGACATT
221        L  T  E  R  G  Y  S  F  T  T  T  A  E  R  E  I  V  R  D  I

                730       740       750       760       770       780
721       AAGGAGAAGCTGTGCTACGTCGCCCTGGACTTCGAGCAAGAGATGGCCACGGCTGCTTCC
241        K  E  K  L  C  Y  V  A  L  D  F  E  Q  E  M  A  T  A  A  S

                790       800       810       820       830       840
781       AGCTCCTCCCTGGAGAAGAGCTACGAGCTGCCTGACGGCCAGGTCATCACCATTGGCAAT
261        S  S  S  L  E  K  S  Y  E  L  P  D  G  Q  V  I  T  I  G  N

                850       860       870       880       890       900
841       GAGCGGTTCCGCTGCCCTGAGGCACTCTTCCAGCCTTCCTTCCTGGGCATGGAGTCCTGT
281        E  R  F  R  C  P  E  A  L  F  Q  P  S  F  L  G  M  E  S  C

                910       920       930       940       950       960
901       GGCATCCACGAAACTACCTTCAACTCCATCATGAAGTGTGACGTGGACATCCGCAAAGAC
301        G  I  H  E  T  T  F  N  S  I  M  K  C  D  V  D  I  R  K  D

                970       980       990      1000      1010      1020
961       CTGTACGCCAACACAGTGCTGTCTGGCGGCACCACCATGTACCCTGGCATTGCCGACAGG
321        L  Y  A  N  T  V  L  S  G  G  T  T  M  Y  P  G  I  A  D  R

               1030      1040      1050      1060      1070      1080
1021      ATGCAGAAGGAGATCACTGCCCTGGCACCCAGCACAATGAAGATCAAGATCATTGCTCCT
341        M  Q  K  E  I  T  A  L  A  P  S  T  M  K  I  K  I  I  A  P

               1090      1100      1110      1120      1130      1140
1081      CCTGAGCGCAAGTACTCCGTGTGGATCGGCGGCTCCATCCTGGCCTCGCTGTCCACCTTC
361        P  E  R  K  Y  S  V  W  I  G  G  S  I  L  A  S  L  S  T  F

               1150      1160      1170      1180      1190      1200
1141      CAGCAGATGTGGATCAGCAAGCAGGAGTATGACGAGTCCGGCCCCTCCATCGTCCACCGC
381        Q  Q  M  W  I  S  K  Q  E  Y  D  E  S  G  P  S  I  V  H  R

               1210      1220      1230      1240      1250      1260
1201      AAATGCTTCTAGGCGGACTATGACTTAGTTGCGTTACACCCTTTCTTGACAAAACCTAAC
401        K  C  F  *            target 1

               1270      1280      1290      1300      1310      1320
1261      TTGCGCAGAAAACAAGATGAGATTGGCATGGCTTTATTTGTTTTTTTTGTTTTGTTTTGG
421

               1330      1340      1350      1360      1370      1380
1321      TTTTTTTTTTTTTTTTGGCTTGACTCAGGATTTAAAAACTGGAACGGTGAAGGTGACAGC
441

               1390      1400      1410      1420      1430      1440
1381      AGTCGGTTGGAGCGAGCATCCCCCAAAGTTCACAATGTGGCCGAGGACTTTGATTGCACA
461

               1450      1460      1470      1480      1490      1500
1441      TTGTTGTTTTTTTAATAGTCATTCCAAATATGAGATGCGTTGTTACAGGAAGTCCCTTGC
481                target 2

               1510      1520      1530      1540      1550      1560
1501      CATCCTAAAAGCCACCCCACTTCTCTCTAAGGAGAATGGCCCAGTCCTCTCCCAAGTCCA
501
```

```
                1570      1580      1590      1600      1610      1620
1561       CACAGGGGAGGTGATAGCATTGCTTTCGTGTAAATTATGTAATGCAAAATTTTTTTAATC
521                     target 3

                1630      1640      1650      1660      1670      1680
1621       TTCGCCTTAATACTTTTTTATTTTGTTTTATTTTGAATGATGAGCCTTCGTGCCCCCCCT
541

                1690      1700      1710      1720      1730      1740
1681       TCCCCCTTTTTTGTCCCCCAACTTGAGATGTATGAAGGCTTTTGGTCTCCCTGGGAGTGG
561

                1750      1760      1770      1780      1790      1800
1741       GTGGAGGCAGCCAGGGCTTACCTGTACACTGACTTGAGACCAGTTGAATAAAAGTGCACA
581

                1810      1820      1830      1840      1850
1801       CCTTAAAAATGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
601
```

cDNA sequence of GAPDH

```
                  10        20        30        40        50        60
1          GCCTCAAGACCTTGGGCTGGGACTGGCTGAGCCTGGCGGGAGGCGGGGTCCGAGTCACCG
1

                  70        80        90       100       110       120
61         CCTGCCGCCGCGCCCCCGGTTTCTATAAATTGAGCCCGCAGCCTCCCGCTTCGCTCTCTG
20

                 130       140       150       160       170       180
121        CTCCTCCTGTTCGACAGTCAGCCGCATCTTCTTTTGCGTCGCCAGCCGAGCCACATCGCT
40

                 190       200       210       220       230       240
181        CAGACACCATGGGGAAGGTGAAGGTCGGAGTCAACGGATTTGGTCGTATTGGGCGCCTGG
60                   M   G   K   V   K   V   G   V   N   G   F   G   R   I   G   R   L

                 250       260       270       280       290       300
241        TCACCAGGGCTGCTTTTAACTCTGGTAAAGTGGATATTGTTGCCATCAATGACCCCTTCA
80         V   T   R   A   A   F   N   S   G   K   V   D   I   V   A   I   N   D   P   F

                 310       320       330       340       350       360
301        TTGACCTCAACTACATGGTTTACATGTTCCAATATGATTCCACCCATGGCAAATTCCATG
100        I   D   L   N   Y   M   V   Y   M   F   Q   Y   D   S   T   H   G   K   F   H

                 370       380       390       400       410       420
361        GCACCGTCAAGGCTGAGAACGGGAAGCTTGTCATCAATGGAAATCCCATCACCATCTTCC
120        G   T   V   K   A   E   N   G   K   L   V   I   N   G   N   P   I   T   I   F

                 430       440       450       460       470       480
421        AGGAGCGAGATCCCTCCAAAATCAAGTGGGGCGATGCTGGCGCTGAGTACGTCGTGGAGT
140        Q   E   R   D   P   S   K   I   K   W   G   D   A   G   A   E   Y   V   V   E

                 490       500       510       520       530       540
481        CCACTGGCGTCTTCACCACCATGGAGAAGGCTGGGGCTCATTTGCAGGGGGGAGCCAAAA
160        S   T   G   V   F   T   T   M   E   K   A   G   A   H   L   Q   G   G   A   K

                 550       560       570       580       590       600
541        GGGTCATCATCTCTGCCCCCTCTGCTGATGCCCCCATGTTCGTCATGGGTGTGAACCATG
180        R   V   I   I   S   A   P   S   A   D   A   P   M   F   V   M   G   V   N   H
```

```
                610       620       630       640       650       660
601       AGAAGTATGACAACAGCCTCAAGATCATCAGCAATGCCTCCTGCACCACCAACTGCTTAG
200       E  K  Y  D  N  S  L  K  I  I  S  N  A  S  C  T  T  N  C  L

                670       680       690       700       710       720
661       CACCCCTGGCCAAGGTCATCCATGACAACTTTGGTATCGTGGAAGGACTCATGACCACAG
220       A  P  L  A  K  V  I  H  D  N  F  G  I  V  E  G  L  M  T  T

                730       740       750       760       770       780
721       TCCATGCCATCACTGCCACCCAGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGGCGTG
240       V  H  A  I  T  A  T  Q  K  T  V  D  G  P  S  G  K  L  W  R

                790       800       810       820       830       840
781       ATGGCCGCGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGG
260       D  G  R  G  A  L  Q  N  I  I  P  A  S  T  G  A  A  K  A  V

                850       860       870       880       890       900
841       GCAAGGTCATCCCTGAGCTGAACGGGAAGCTCACTGGCATGGCCTTCCGTGTCCCCACTG
280       G  K  V  I  P  E  L  N  G  K  L  T  G  M  A  F  R  V  P  T

                910       920       930       940       950       960
901       CCAACGTGTCAGTGGTGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACA
300       A  N  V  S  V  V  D  L  T  C  R  L  E  K  P  A  K  Y  D  D

                970       980       990      1000      1010      1020
961       TCAAGAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTG
320       I  K  K  V  V  K  Q  A  S  E  G  P  L  K  G  I  L  G  Y  T

               1030      1040      1050      1060      1070      1080
1021      AGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACACCCACTCCTCCACCTTTGACGCTG
340       E  H  Q  V  V  S  S  D  F  N  S  D  T  H  S  S  T  F  D  A

               1090      1100      1110      1120      1130      1140
1081      GGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTGGTATGACAACGAAT
360       G  A  G  I  A  L  N  D  H  F  V  K  L  I  S  W  Y  D  N  E

               1150      1160      1170      1180      1190      1200
1141      TTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACC
380       F  G  Y  S  N  R  V  V  D  L  M  A  H  M  A  S  K  E  *

               1210      1220      1230      1240      1250      1260
1201      CCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGA
400

               1270      1280      1290      1300      1310      1320
1261      GTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTA
420                                                             target 1

               1330      1340      1350      1360      1370      1380
1321      GACCCCTTGAAGAGGGGAGGGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAG
440                             target 2

               1390      1400      1410      1420
1381      TACCCTGTGCTCAACCAGTTAAAAAAAAAAAAAAAAAAAA
460
```

cDNA sequence of GPI

```
                 10        20        30        40        50        60
```

```
1          AATAGCCCTTACCACCAGCAGACACACATCATCTGTTGTACTTGCTTATTTGGCACATAT
1

                   70         80         90        100        110        120
61         GTATCCACAGCGCCTAGAACACTGCCTGTAACGTGGAAGGTGTTCGATCTATAGAGTTTT
20

                  130        140        150        160        170        180
121        GTCGAATGAATGAATGAAGCCGACTAGTGCACAGGGAGTGCAGCGGCGCGATGGTAGCTC
40                                                                M  V  A

                  190        200        210        220        230        240
181        TCTGCAGCCTCCAACACCTGGGCTCCAGTGATCCCCGGGCTCTGCCCACCCTCCCCACTG
60         L  C  S  L  Q  H  L  G  S  S  D  P  R  A  L  P  T  L  P  T

                  250        260        270        280        290        300
241        CCACTTCCGGGCAGAGGCCAGCAAAGCGGCGGCGCAAGAGTCCCGCCATGGCCGCTCTCA
80         A  T  S  G  Q  R  P  A  K  R  R  R  K  S  P  A  M  A  A  L

                  310        320        330        340        350        360
301        CCCGGGACCCCCAGTTCCAGAAGCTGCAGCAATGGTACCGCGAGCACCGCTCCGAGCTGA
100        T  R  D  P  Q  F  Q  K  L  Q  Q  W  Y  R  E  H  R  S  E  L

                  370        380        390        400        410        420
361        ACCTGCGCCGCCTCTTCGATGCCAACAAGGACCGCTTCAACCACTTCAGCTTGACCCTCA
120        N  L  R  R  L  F  D  A  N  K  D  R  F  N  H  F  S  L  T  L

                  430        440        450        460        470        480
421        ACACCAACCATGGGCATATCCTGGTGGATTACTCCAAGAACCTGGTGACGGAGGACGTGA
140        N  T  N  H  G  H  I  L  V  D  Y  S  K  N  L  V  T  E  D  V

                  490        500        510        520        530        540
481        TGCGGATGCTGGTGGACTTGGCCAAGTCCAGGGGCGTGGAGGCCGCCCGGGAGCGGATGT
160        M  R  M  L  V  D  L  A  K  S  R  G  V  E  A  A  R  E  R  M

                  550        560        570        580        590        600
541        TCAATGGTGAGAAGATCAACTACACCGAGGGTCGAGCCGTGCTGCACGTGGCTCTGCGGA
180        F  N  G  E  K  I  N  Y  T  E  G  R  A  V  L  H  V  A  L  R

                  610        620        630        640        650        660
601        ACCGGTCAAACACACCCATCCTGGTAGACGGCAAGGATGTGATGCCAGAGGTCAACAAGG
200        N  R  S  N  T  P  I  L  V  D  G  K  D  V  M  P  E  V  N  K
                                    target
                  670        680        690        700        710        720
661        TTCTGGACAAGATGAAGTCTTTCTGCCAGGGACCCCTCATGGTGACTGAAGCCCTTAAGC
220        V  L  D  K  M  K  S  F  C  Q  G  P  L  M  V  T  E  A  L  K

                  730        740        750        760        770        780
721        CATACTCTTCAGGAGGTCCCCGCGTCTGGTATGTCTCCAACATTGATGGAACTCACATTG
240        P  Y  S  S  G  G  P  R  V  W  Y  V  S  N  I  D  G  T  H  I

                  790        800        810        820        830        840
781        CCAAAACCCTGGCCCAGCTGAACCCCGAGTCCTCCCTGTTCATCATTGCCTCCAAGACCT
260        A  K  T  L  A  Q  L  N  P  E  S  S  L  F  I  I  A  S  K  T

                  850        860        870        880        890        900
841        TTACTACCCAGGAGACCATCACGAATGCAGAGACGGCGAAGGAGTGGTTTCTCCAGGCGG
280        F  T  T  Q  E  T  I  T  N  A  E  T  A  K  E  W  F  L  Q  A

                  910        920        930        940        950        960
901        CCAAGGATCCTTCTGCAGTGGCGAAGCACTTTGTTGCCCTGTCTACTAACACAACCAAAG
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300       A  K  D  P  S  A  V  A  K  H  F  V  A  L  S  T  N  T  T  K

                970       980       990      1000      1010      1020
961       TGAAGGAGTTTGGAATTGACCCTCAAAACATGTTCGAGTTCTGGGATTGGGTGGGAGGAC
320       V  K  E  F  G  I  D  P  Q  N  M  F  E  F  W  D  W  V  G  G

               1030      1040      1050      1060      1070      1080
1021      GCTACTCGCTGTGGTCGGCCATCGGACTCTCCATTGCCCTGCACGTGGGTTTTGACAACT
340       R  Y  S  L  W  S  A  I  G  L  S  I  A  L  H  V  G  F  D  N

               1090      1100      1110      1120      1130      1140
1081      TCGAGCAGCTGCTCTCGGGGGCTCACTGGATGGACCAGCACTTCCGCACGACGCCCCTGG
360       F  E  Q  L  L  S  G  A  H  W  M  D  Q  H  F  R  T  T  P  L

               1150      1160      1170      1180      1190      1200
1141      AGAAGAACGCCCCCGTCTTGCTGGCCCTGCTGGGTATCTGGTACATCAACTGCTTTGGGT
380       E  K  N  A  P  V  L  L  A  L  L  G  I  W  Y  I  N  C  F  G

               1210      1220      1230      1240      1250      1260
1201      GTGAGACACACGCCATGCTGCCCTATGACCAGTACCTGCACCGCTTTGCTGCGTACTTCC
400       C  E  T  H  A  M  L  P  Y  D  Q  Y  L  H  R  F  A  A  Y  F

               1270      1280      1290      1300      1310      1320
1261      AGCAGGGCGACATGGAGTCCAATGGGAAATACATCACCAAATCTGGAACCCGTGTGGACC
420       Q  Q  G  D  M  E  S  N  G  K  Y  I  T  K  S  G  T  R  V  D

               1330      1340      1350      1360      1370      1380
1321      ACCAGACAGGCCCCATTGTGTGGGGGGAGCCAGGGACCAATGGCCAGCATGCTTTTTACC
440       H  Q  T  G  P  I  V  W  G  E  P  G  T  N  G  Q  H  A  F  Y

               1390      1400      1410      1420      1430      1440
1381      AGCTCATCCACCAAGGCACCAAGATGATACCCTGTGACTTCCTCATCCCGGTCCAGACCC
460       Q  L  I  H  Q  G  T  K  M  I  P  C  D  F  L  I  P  V  Q  T

               1450      1460      1470      1480      1490      1500
1441      AGCACCCCATACGGAAGGGTCTGCATCACAAGATCCTCCTGGCCAACTTCTTGGCCCAGA
480       Q  H  P  I  R  K  G  L  H  H  K  I  L  L  A  N  F  L  A  Q

               1510      1520      1530      1540      1550      1560
1501      CAGAGGCCCTGATGAGGGGAAAATCGACGGAGGAGGCCCGAAAGGAGCTCCAGGCTGCGG
500       T  E  A  L  M  R  G  K  S  T  E  E  A  R  K  E  L  Q  A  A

               1570      1580      1590      1600      1610      1620
1561      GCAAGAGTCCAGAGGACCTTGAGAGGCTGCTGCCACATAAGGTCTTTGAAGGAAATCGCC
520       G  K  S  P  E  D  L  E  R  L  L  P  H  K  V  F  E  G  N  R

               1630      1640      1650      1660      1670      1680
1621      CAACCAACTCTATTGTGTTCACCAAGCTCACACCATTCATGCTTGGAGCCTTGGTCGCCA
540       P  T  N  S  I  V  F  T  K  L  T  P  F  M  L  G  A  L  V  A

               1690      1700      1710      1720      1730      1740
1681      TGTATGAGCACAAGATCTTCGTTCAGGGCATCATCTGGGACATCAACAGCTTTGACCAGT
560       M  Y  E  H  K  I  F  V  Q  G  I  I  W  D  I  N  S  F  D  Q

               1750      1760      1770      1780      1790      1800
1741      GGGGAGTGGAGCTGGGAAAGCAGCTGGCTAAGAAAATAGAGCCTGAGCTTGATGGCAGTG
580       W  G  V  E  L  G  K  Q  L  A  K  K  I  E  P  E  L  D  G  S

               1810      1820      1830      1840      1850      1860
1801      CTCAAGTGACCTCTCACGACGCTTCTACCAATGGGCTCATCAACTTCATCAAGCAGCAGC
600       A  Q  V  T  S  H  D  A  S  T  N  G  L  I  N  F  I  K  Q  Q
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              1870      1880
1861      GCGAGGCCAGAGTCCAATAA
620       R  E  A  R  V  Q  *
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cDNA sequence of GusB

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                10        20        30        40        50        60
1         GTCCTCAACCAAGATGGCGCGGATGGCTTCAGGCGCATCACGACACCGGCGCGTCACGCG
1

                70        80        90       100       110       120
61        ACCCGCCCTACGGGCACCTCCCGCGCTTTTCTTAGCGCCGCAGACGGTGGCCGAGCGGGG
20

               130       140       150       160       170       180
121       GACCGGGAAGCATGGCCCGGGGGTCGGCGGTTGCCTGGGCGGCGCTCGGGCCGTTGTTGT
40                M  A  R  G  S  A  V  A  W  A  A  L  G  P  L  L

               190       200       210       220       230       240
181       GGGGCTGCGCGCTGGGGCTGCAGGGCGGGATGCTGTACCCCCAGGAGAGCCCGTCGCGGG
60        W  G  C  A  L  G  L  Q  G  G  M  L  Y  P  Q  E  S  P  S  R

               250       260       270       280       290       300
241       AGTGCAAGGAGCTGGACGGCCTCTGGAGCTTCCGCGCCGACTTCTCTGACAACCGACGCC
80        E  C  K  E  L  D  G  L  W  S  F  R  A  D  F  S  D  N  R  R

               310       320       330       340       350       360
301       GGGGCTTCGAGGAGCAGTGGTACCGGCGGCCGCTGTGGGAGTCAGGCCCCACCGTGGACA
100       R  G  F  E  E  Q  W  Y  R  R  P  L  W  E  S  G  P  T  V  D

               370       380       390       400       410       420
361       TGCCAGTTCCCTCCAGCTTCAATGACATCAGCCAGGACTGGCGTCTGCGGCATTTTGTCG
120       M  P  V  P  S  S  F  N  D  I  S  Q  D  W  R  L  R  H  F  V

               430       440       450       460       470       480
421       GCTGGGTGTGGTACGAACGGGAGGTGATCCTGCCGGAGCGATGGACCCAGGACCTGCGCA
140       G  W  V  W  Y  E  R  E  V  I  L  P  E  R  W  T  Q  D  L  R

               490       500       510       520       530       540
481       CAAGAGTGGTGCTGAGGATTGGCAGTGCCCATTCCTATGCCATCGTGTGGGTGAATGGGG
160       T  R  V  V  L  R  I  G  S  A  H  S  Y  A  I  V  W  V  N  G

               550       560       570       580       590       600
541       TCGACACGCTAGAGCATGAGGGGGGCTACCTCCCCTTCGAGGCCGACATCAGCAACCTGG
180       V  D  T  L  E  H  E  G  G  Y  L  P  F  E  A  D  I  S  N  L

               610       620       630       640       650       660
601       TCCAGGTGGGGCCCCTGCCCTCCCGGCTCCGAATCACTATCGCCATCAACAACACACTCA
200       V  Q  V  G  P  L  P  S  R  L  R  I  T  I  A  I  N  N  T  L

               670       680       690       700       710       720
661       CCCCCACCACCCTGCCACCAGGGACCATCCAATACCTGACTGACACCTCCAAGTATCCCA
220       T  P  T  T  L  P  P  G  T  I  Q  Y  L  T  D  T  S  K  Y  P

               730       740       750       760       770       780
721       AGGGTTACTTTGTCCAGAACACATATTTTGACTTTTTCAACTACGCTGGACTGCAGCGGT
240       K  G  Y  F  V  Q  N  T  Y  F  D  F  F  N  Y  A  G  L  Q  R

               790       800       810       820       830       840
781       CTGTACTTCTGTACACGACACCCACCACCTACATCGATGACATCACCGTCACCACCAGCG
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260       S  V  L  L  Y  T  T  P  T  T  Y  I  D  D  I  T  V  T  T  S

                850       860       870       880       890       900
841       TGGAGCAAGACAGTGGGCTGGTGAATTACCAGATCTCTGTCAAGGGCAGTAACCTGTTCA
280       V  E  Q  D  S  G  L  V  N  Y  Q  I  S  V  K  G  S  N  L  F

                910       920       930       940       950       960
901       AGTTGGAAGTGCGTCTTTTGGATGCAGAAAACAAAGTCGTGGCGAATGGGACTGGGACCC
300       K  L  E  V  R  L  L  D  A  E  N  K  V  V  A  N  G  T  G  T

                970       980       990      1000      1010      1020
961       AGGGCCAACTTAAGGTGCCAGGTGTCAGCCTCTGGTGGCCGTACCTGATGCACGAACGCC
320       Q  G  Q  L  K  V  P  G  V  S  L  W  W  P  Y  L  M  H  E  R

              1030      1040      1050      1060      1070      1080
1021      CTGCCTATCTGTATTCATTGGAGGTGCAGCTGACTGCACAGACGTCACTGGGGCCTGTGT
340       P  A  Y  L  Y  S  L  E  V  Q  L  T  A  Q  T  S  L  G  P  V

              1090      1100      1110      1120      1130      1140
1081      CTGACTTCTACACACTCCCTGTGGGGATCCGCACTGTGGCTGTCACCAAGAGCCAGTTCC
360       S  D  F  Y  T  L  P  V  G  I  R  T  V  A  V  T  K  S  Q  F

              1150      1160      1170      1180      1190      1200
1141      TCATCAATGGGAAACCTTTCTATTTCCACGGTGTCAACAAGCATGAGGATGCGGACATCC
380       L  I  N  G  K  P  F  Y  F  H  G  V  N  K  H  E  D  A  D  I

              1210      1220      1230      1240      1250      1260
1201      GAGGGAAGGGCTTCGACTGGCCGCTGCTGGTGAAGGACTTCAACCTGCTTCGCTGGCTTG
400       R  G  K  G  F  D  W  P  L  L  V  K  D  F  N  L  L  R  W  L

              1270      1280      1290      1300      1310      1320
1261      GTGCCAACGCTTTCCGTACCAGCCACTACCCCTATGCAGAGGAAGTGATGCAGATGTGTG
420       G  A  N  A  F  R  T  S  H  Y  P  Y  A  E  E  V  M  Q  M  C

              1330      1340      1350      1360      1370      1380
1321      ACCGCTATGGGATTGTGGTCATCGATGAGTGTCCCGGCGTGGGCCTGGCGCTGCCGCAGT
440       D  R  Y  G  I  V  V  I  D  E  C  P  G  V  G  L  A  L  P  Q

              1390      1400      1410      1420      1430      1440
1381      TCTTCAACAACGTTTCTCTGCATCACCACATGCAGGTGATGGAAGAAGTGGTGCGTAGGG
460       F  F  N  N  V  S  L  H  H  H  M  Q  V  M  E  E  V  V  R  R

              1450      1460      1470      1480      1490      1500
1441      ACAAGAACCACCCCGCGGTCGTGATGTGGTCTGTGGCCAACGAGCCTGCGTCCCACCTAG
480       D  K  N  H  P  A  V  V  M  W  S  V  A  N  E  P  A  S  H  L
                                                              target 1
              1510      1520      1530      1540      1550      1560
1501      AATCTGCTGGCTACTACTTGAAGATGGTGATCGCTCACACCAAATCCTTGGACCCCTCCC
500       E  S  A  G  Y  Y  L  K  M  V  I  A  H  T  K  S  L  D  P  S

              1570      1580      1590      1600      1610      1620
1561      GGCCTGTGACCTTTGTGAGCAACTCTAACTATGCAGCAGACAAGGGGGCTCCGTATGTGG
520       R  P  V  T  F  V  S  N  S  N  Y  A  A  D  K  G  A  P  Y  V

              1630      1640      1650      1660      1670      1680
1621      ATGTGATCTGTTTGAACAGCTACTACTCTTGGTATCACGACTACGGGCACCTGGAGTTGA
540       D  V  I  C  L  N  S  Y  Y  S  W  Y  H  D  Y  G  H  L  E  L

              1690      1700      1710      1720      1730      1740
1681      TTCAGCTGCAGCTGGCCACCCAGTTTGAGAACTGGTATAAGAAGTATCAGAAGCCCATTA
560       I  Q  L  Q  L  A  T  Q  F  E  N  W  Y  K  K  Y  Q  K  P  I
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                1750      1760      1770      1780      1790      1800
1741        TTCAGAGCGAGTATGGAGCAGAAACGATTGCAGGGTTTCACCAGGATCCACCTCTGATGT
580         I  Q  S  E  Y  G  A  E  T  I  A  G  F  H  Q  D  P  P  L  M

                1810      1820      1830      1840      1850      1860
1801        TCACTGAAGAGTACCAGAAAAGTCTGCTAGAGCAGTACCATCTGGGTCTGGATCAAAAAC
600         F  T  E  E  Y  Q  K  S  L  L  E  Q  Y  H  L  G  L  D  Q  K

                1870      1880      1890      1900      1910      1920
1861        GCAGAAAATACGTGGTTGGAGAGCTCATTTGGAATTTTGCCGATTTCATGACTGAACAGT
620         R  R  K  Y  V  V  G  E  L  I  W  N  F  A  D  F  M  T  E  Q

                1930      1940      1950      1960      1970      1980
1921        CACCGACGAGAGTGCTGGGGAATAAAAAGGGGATCTTCACTCGGCAGAGACAACCAAAAA
640         S  P  T  R  V  L  G  N  K  K  G  I  F  T  R  Q  R  Q  P  K

                1990      2000      2010      2020      2030      2040
1981        GTGCAGCGTTCCTTTTGCGAGAGAGATACTGGAAGATTGCCAATGAAACCAGGTATCCCC
660         S  A  A  F  L  L  R  E  R  Y  W  K  I  A  N  E  T  R  Y  P

                2050      2060      2070      2080      2090      2100
2041        ACTCAGTAGCCAAGTCACAATGTTTGGAAAACAGCCTGTTTACTTGAGCAAGACTGATAC
680         H  S  V  A  K  S  Q  C  L  E  N  S  L  F  T  *

                2110      2120      2130      2140      2150      2160
2101        CACCTGCGTGTCCCTTCCTCCCCGAGTCAGGGCGACTTCCACAGCAGCAGAACAAGTGCC
700

                2170      2180      2190      2200      2210      2220
2161        TCCTGGACTGTTCACGGCAGACCAGAACGTTTCTGGCCTGGGTTTTGTGGTCATCTATTC
720

                2230      2240      2250      2260      2270      2280
2221        TAGCAGGGAACACTAAAGGTGGAAATAAAAGATTTTCTATTATGGAAATAAAGAGTTGGC
740
          target 2
                2290      2300      2310      2320
2281        ATGAAAGTGGCTACTGAAAAAAAAAAAAAAAAAAAAAAAAA
760
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cDNA sequence of RAB7A

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                  10        20        30        40        50        60
1           GTCTCGTGACAGGTACTTCCGCTCGGGGCGGCGGCGGTGGCGGAAGTGGGAGCGGGCCTG
1

                  70        80        90       100       110       120
61          GAGTCTTGGCCATAAAGCCTGAGGCGGCGGCAGCGGCGGAGTTGGCGGCTTGGAGAGCTC
21

                 130       140       150       160       170       180
121         GGGAGAGTTCCCTGGAACCAGAACTTGGACCTTCTCGCTTCTGTCCTCCGTTTAGTCTCC
41

                 190       200       210       220       230       240
181         TCCTCGGCGGGAGCCCTCGCGACGCGCCCGGCCCGGAGCCCCCAGCGCAGCGGCCGCGTT
61

                 250       260       270       280       290       300
241         TGAAGGATGACCTCTAGGAAGAAAGTGTTGCTGAAGGTTATCATCCTGGGAGATTCTGGA
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81                M  T  S  R  K  K  V  L  L  K  V  I  I  L  G  D  S  G

                 310        320        330        340        350        360
301        GTCGGGAAGACATCACTCATGAACCAGTATGTGAATAAGAAATTCAGCAATCAGTACAAA
101         V  G  K  T  S  L  M  N  Q  Y  V  N  K  K  F  S  N  Q  Y  K

                 370        380        390        400        410        420
361        GCCACAATAGGAGCTGACTTTCTGACCAAGGAGGTGATGGTGGATGACAGGCTAGTCACA
121         A  T  I  G  A  D  F  L  T  K  E  V  M  V  D  D  R  L  V  T

                 430        440        450        460        470        480
421        ATGCAGATATGGGACACAGCAGGACAGGAACGGTTCCAGTCTCTCGGTGTGGCCTTCTAC
141         M  Q  I  W  D  T  A  G  Q  E  R  F  Q  S  L  G  V  A  F  Y

                 490        500        510        520        530        540
481        AGAGGTGCAGACTGCTGCGTTCTGGTATTTGATGTGACTGCCCCCAACACATTCAAAACC
161         R  G  A  D  C  C  V  L  V  F  D  V  T  A  P  N  T  F  K  T

                 550        560        570        580        590        600
541        CTAGATAGCTGGAGAGATGAGTTTCTCATCCAGGCCAGTCCCCGAGATCCTGAAAACTTC
181         L  D  S  W  R  D  E  F  L  I  Q  A  S  P  R  D  P  E  N  F

                 610        620        630        640        650        660
601        CCATTTGTTGTGTTGGGAAACAAGATTGACCTCGAAAACAGACAAGTGGCCACAAAGCGG
201         P  F  V  V  L  G  N  K  I  D  L  E  N  R  Q  V  A  T  K  R

                 670        680        690        700        710        720
661        GCACAGGCCTGGTGCTACAGCAAAAACAACATTCCCTACTTTGAGACCAGTGCCAAGGAG
221         A  Q  A  W  C  Y  S  K  N  N  I  P  Y  F  E  T  S  A  K  E

                 730        740        750        760        770        780
721        GCCATCAACGTGGAGCAGGCGTTCCAGACGATTGCACGGAATGCACTTAAGCAGGAAACG
241         A  I  N  V  E  Q  A  F  Q  T  I  A  R  N  A  L  K  Q  E  T

                 790        800        810        820        830        840
781        GAGGTGGAGCTGTACAACGAATTTCCTGAACCTATCAAACTGGACAAGAATGACCGGGCC
261         E  V  E  L  Y  N  E  F  P  E  P  I  K  L  D  K  N  D  R  A

                 850        860        870        880        890        900
841        AAGGCCTCGGCAGAAAGCTGCAGTTGCTGAGGGGGCAGTGAGAGTTGAGCACAGAGTCCT
281         K  A  S  A  E  S  C  S  C  *

                 910        920        930        940        950        960
901        TCACAAACCAAGAACACACGTAGGCCTTCAACACAATTCCCCTCTCCTCTTCCAAACAAA
301

                 970        980        990       1000       1010       1020
961        ACATACATTGATCTCTCACATCCAGCTGCCAAAAGAAAACCCCATCAAACACAGTTACAC
321

               1030       1040       1050       1060       1070       1080
1021       CCCACATATCTCTCACACACACACACACACGCACACACACACACACAGATCTGACGTAAT
341

               1090       1100       1110       1120       1130       1140
1081       CAAACTCCAGCCCTTGCCCGTGATGGCTCCTTGGGGTCTGCCTGCCCACCCACATGAGCC
361

               1150       1160       1170       1180       1190       1200
1141       CGCGAGTATGGCAGCAGGACAAGCCAGCGGTGGAAGTCATTCTGATATGGAGTTGGCATT
381
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                1210      1220      1230      1240      1250      1260
1201      GGAAGCTTATTCTTTTTGTTCACTGGAGAGAGAGAGAACTGTTTACAGTTAATCTGTGTC
401

                1270      1280      1290      1300      1310      1320
1261      TAATTATCTGATTTTTTTTATTGGTCTTGTGGTCTTTTTACCCCCCCTTTCCCCTCCCTC
421

                1330      1340      1350      1360      1370      1380
1321      CTTGAAGGCTACCCCTTGGGAAGGCTGGTGCCCCATGCCCCATTACAGGCTCACACCCAG
441

                1390      1400      1410      1420      1430      1440
1381      TCTGATCAGGCTGAGTTTTGTATGTATCTATCTGTTAATGCTTGTTACTTTTAACTAATC
461

                1450      1460      1470      1480      1490      1500
1441      AGATCTTTTTACAGTATCCATTTATTATGTAATGCTTCTTAGAAAAGAATCTTATAGTAC
481

                1510      1520      1530      1540      1550      1560
1501      ATGTTAATATATGCAACCAATTAAAATGTATAAATTAGTGTAAGAAATTCTTGGATTATG
501

                1570      1580      1590      1600      1610      1620
1561      TGTTTAAGTCCTGTAATGCAGGCCTGTAAGGTGGAGGGTTGAACCCTGTTTGGATTGCAG
521

                1630      1640      1650      1660      1670      1680
1621      AGTGTTACTCAGAATTGGGAAATCCAGCTAGCGGCAGTATTCTGTACAGTAGACACAAGA
541                               target 1

                1690      1700      1710      1720      1730      1740
1681      ATTATGTACGCCTTTTATCAAAGACTTAAGAGCCAAAAAGCTTTTCATCTCTCCAGGGGG
561

                1750      1760      1770      1780      1790      1800
1741      AAAACTGTCTAGTTCCCTTCTGTGTCTAAATTTTCCAAAACGTTGATTTGCATAATACAG
581         target 2

                1810      1820      1830      1840      1850      1860
1801      TGGTATGTGCAATGGATAAATTGCCGTTATTTCAAAAATTAAAATTCTCATTTTCTTTCT
601

                1870      1880      1890      1900      1910      1920
1861      TTTTTTTCCCCCCTGCTCCACACTTCAAAACTCCCGTTAGATCAGCATTCTACTACAAGA
621

                1930      1940      1950      1960      1970      1980
1921      GTGAAAGGAAAACCCTAACAGATCTGTCCTAGTGATTTTACCTTTGTTCTAGAAGGCGCT
641                                 target 3

                1990      2000      2010      2020      2030      2040
1981      CCTTTCAGGGTTGTGGTATTCTTAGGTTAGCGGAGCTTTTTCCTCTTTTCCCCACCCATC
661

                2050      2060      2070      2080      2090      2100
2041      TCCCCAATATTGCCCATTATTAATTAACCTCTTTCTTTGGTTGGAACCCTGGCAGTTCTG
681
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                2110       2120       2130       2140       2150       2160
2101        CTCCCTTCCTAGGATCTGCCCCTGCATTGTAGCTTGCTTAACGGAGCACTTCTCCTTTTT
701

                2170       2180       2190       2200       2210       2220
2161        CCAAAGGTCTACATTCTAGGGTGTGGGCTGAGTTCTTCTGTAAAGAGATGAACGCAATGC
721

                2230       2240
2221        CAATAAAATTGAACAAGAACAATG
741
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Only 3’ UTR sequence of VCP (very long transcript, thus only this part is shown)

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                  10         20         30         40         50         60
1           …ATTCCCTTCAGGGAACCAGGGTGGAGCTGGCCCCAGTCAGGGCAGTGGAGGCGGCACAGG
1
                  70         80         90        100        110        120
61          TGGCAGTGTATACACAGAAGACAATGATGATGACCTGTATGGCTAAGTGGTGGTGGCCAG
21
                 130        140        150        160        170        180
121         CGTGCAGTGAGCTGGCCTGCCTGGACCTTGTTCCCTGGGGGTGGGGGCGCTTGCCCAGGA
41
                 190        200        210        220        230        240
181         GAGGGACCAGGGGTGCGCCCACAGCCTGCTCCATTCTCCAGTCTGAACAGTTCAGCTACA
61
                 250        260        270        280        290        300
241         GTCTGACTCTGGACAGGGGGTTTCTGTTGCAAAAATACAAAACAAAAGCGATAAAATAAA
81
                 310        320        330        340        350        360
301         AGCGATTTTCATTTGGTAGGCGGAGAGTGAATTACCAACAGGGAATTGGGCCTTGGGCCT
101                    target 1
                 370        380        390        400        410        420
361         ATGCCATTTCTGTTGTAGTTTGGGGCAGTGCAGGGGACCTGTGTGGGGTGTGAACCAAGG
121                    target 2
                 430        440        450        460        470        480
421         CACTACTGCCACCTGCCACAGTAAAGCATCTGCACTTGACTCAATGCTGCCCGAGCCCTC
141
                 490        500        510        520        530        540
481         CCTTCCCCCTATCCAACCTGGGTAGGTGGGTAGGGGCCACAGTTGCTGGATGTTTATATA
161                          target 3
                 550        560        570        580        590        600
541         GAGAGTAGGTTGATTTATTTTACATGCTTTTGAGTTAATGTTGGAAAACTAATCACAAGC
181
                 610        620        630        640        650        660
601         AGTTTCTAAACCAAAAAATGACATGTTGTAAAAGGACAATAAACGTTGGGTCAAAATGGA
201
                 670        680        690        700        710        720
661         GCCTGAGTCCTGGGCCCTGTGCCTGCTTCTTTTCCTGGGAACAGCCTTGGGCTACCCACC
221
                 730        740        750        760        770        780
721         ACTCCCAAGGCATTCTTCCAAATGTGAAATCCTGGAAGTAAGATTGCACCTTCTTCCTCT
241
                 790        800        810        820        830        840
781         CCTGATCAACATCGGTATGATGTCTCCTGTTGCCTCACCCTTTGTCTGCAGTATCACTGG
261
                 850        860        870        880        890        900
841         ATAGGACTGGTGGAAAGGGAGCAGCCTGACAGAGCTCCAAATGTGGAGAATATGGCATCC
281
                 910        920        930        940        950        960
901         CTCCACCTATATTTGATGTGGACGGTAAGGCTAGGCCTGCAGGATCCCTTATCCTGACCA
```

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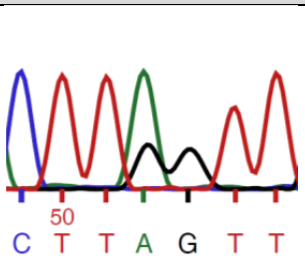
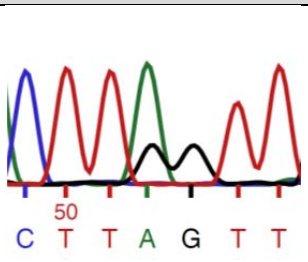
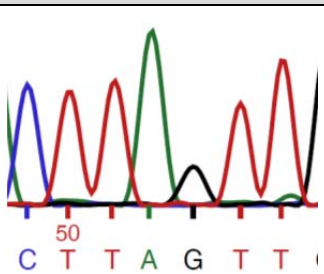
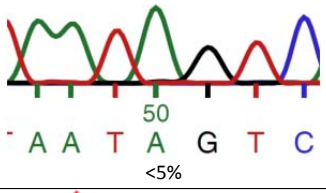
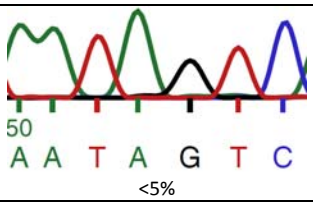
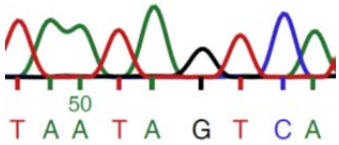
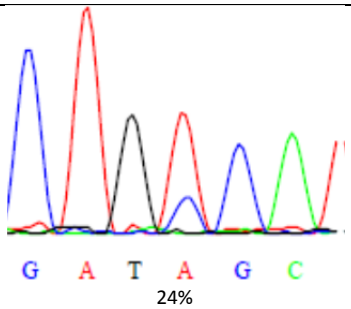
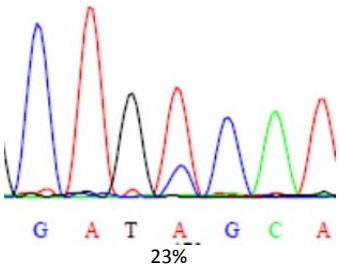
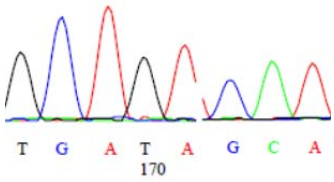
301
                970        980        990       1000       1010       1020
961         AAGACTGTGTTGGGGTGCCATTTGAAAATCGCAGGGTTGCAAAAGAATACAATCTTACTT
321
               1030       1040       1050       1060       1070       1080
1021        GCAGGTGGATATTCTCTATACTCTCTTTTAATGCATCTAAAAATCCCAAACATCCCCTGG
341
               1090       1100       1110       1120       1130       1140
1081        TTGGTGATCACTTACAGTTGTGTCCACCTTTATTTTATGTACTTTGATTAAAAAAAAAAA
361
               1150
1141        ACTTTTTGTTAATATAAAA
381
```

Figure S19. Sequencing results. *Not all RT-PCR reactions were successful or gave sufficient sequencing quality. Those are indicated here with n.d. Primers used in RT-PCR are given on Table S2.*

a) Editing in 293T-Cells with ADAR2 overexpression

settings	experiment A:	experiment B:	negative control:
General Settings: 300 ng pTS57 (ADAR2), 1300 ng gRNA, DNA to Lipofectamine Ratio 1:3, negative control just without guideRNA the rest is treated equally			
B-actin			
TAG#1			
TAG#2			
TAG#3			
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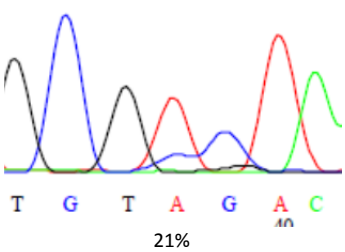
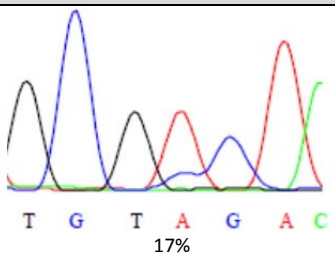
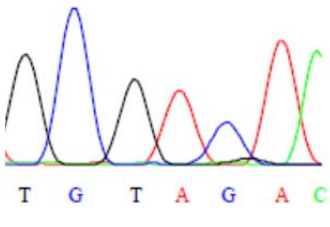
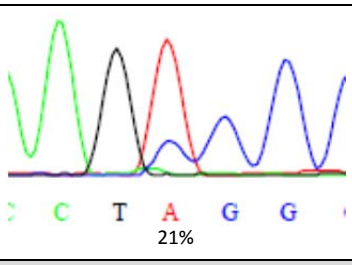
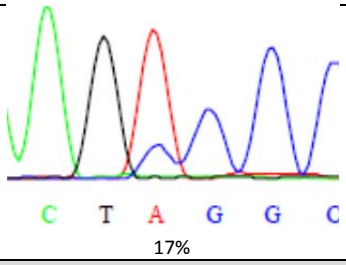
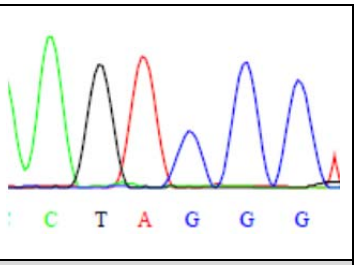
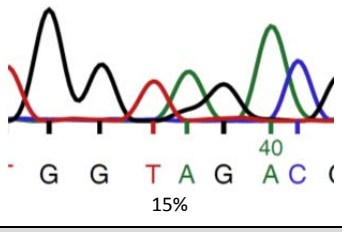
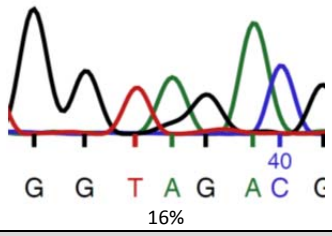
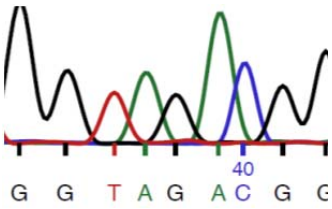
TAG#1			

TAG#2			
GPI			
TAG#1			
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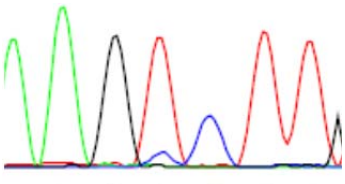
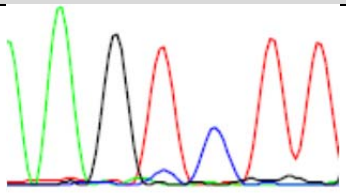
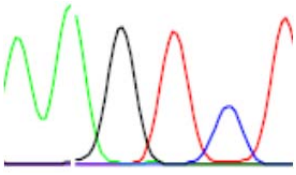
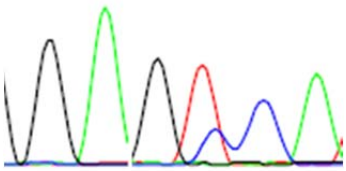
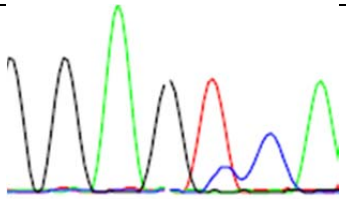
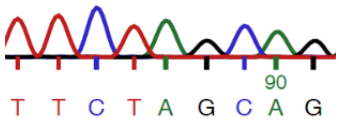
TAG#1			
TAG#2			
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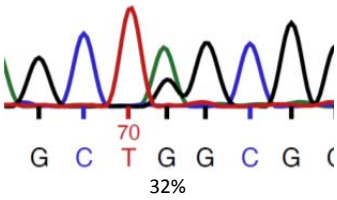
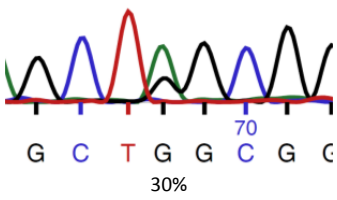
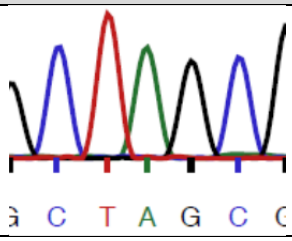
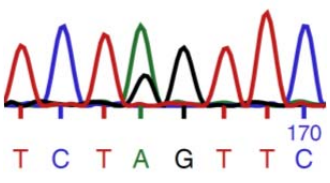
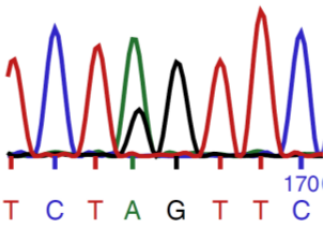
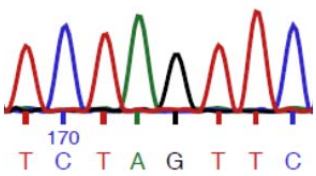
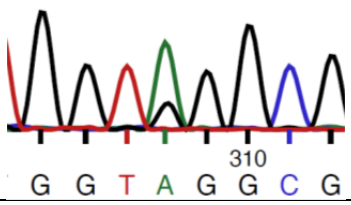
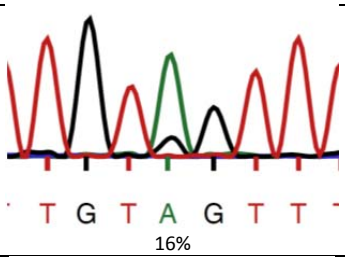
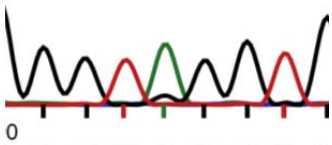
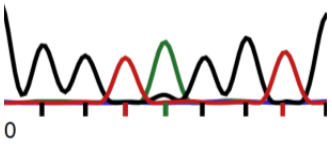
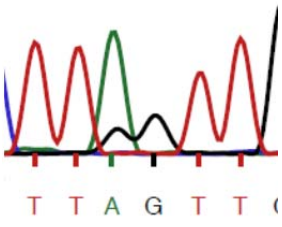
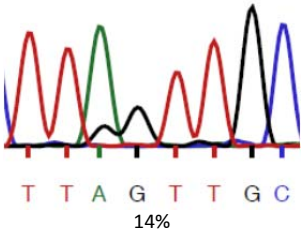
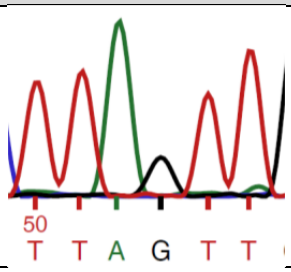
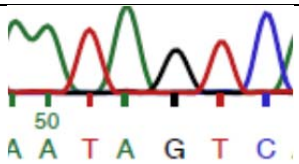
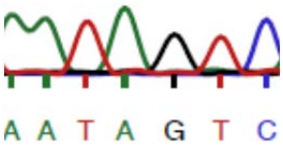
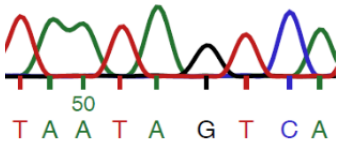
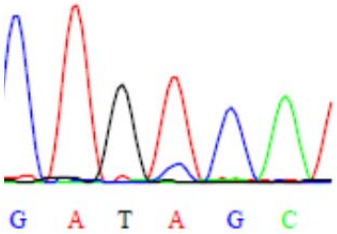
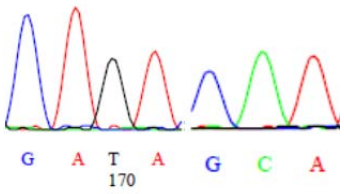
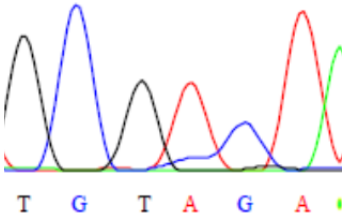
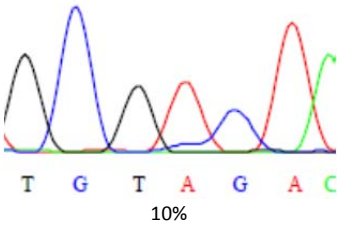
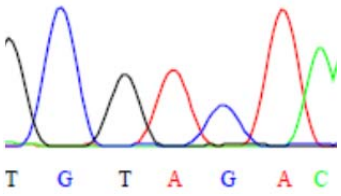
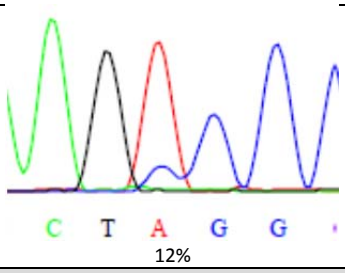
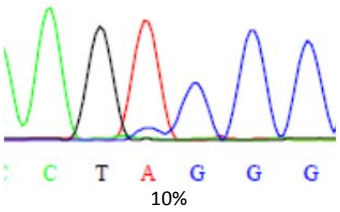
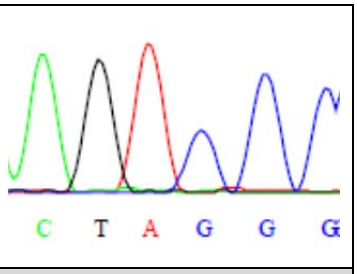
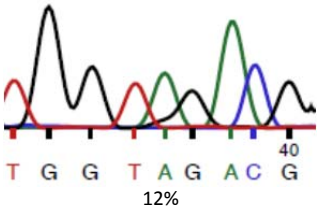
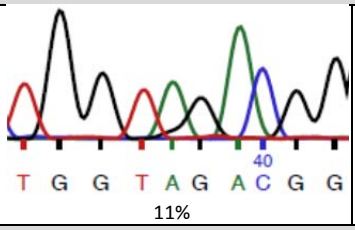
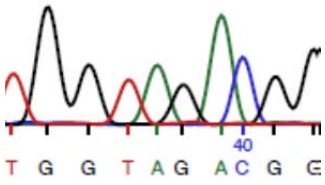
TAG#1			
TAG#2			
VCP			
TAG#1	n.d.		n.d.

		25%	
TAG#2	 16%	n.d.	n.d.
TAG#3	 12%	 11%	n.d.

cont. Figure S19 b) Editing in 293-ADAR2-Flip-In-Cells

settings	experiment A:	experiment B:	negative control:
General Settings: 1300 ng gRNA, DNA to Lipofectamine Ratio 1:3; negative control just empty transfection			
B-actin			
TAG#1	 17%	 14%	
TAG#2	 <5%	 <5%	
TAG#3	 14%	n.d.	
GAPDH			
TAG#1	 10%	 10%	

TAG#2			
GPI			
TAG#1			
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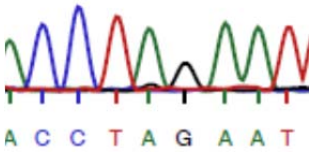
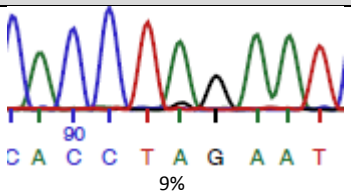
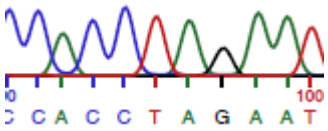
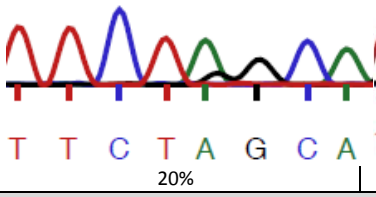
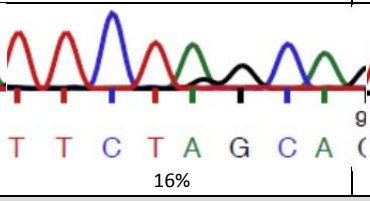
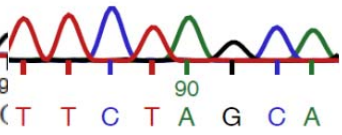
TAG#1			
TAG#2			
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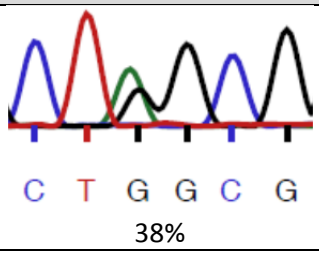
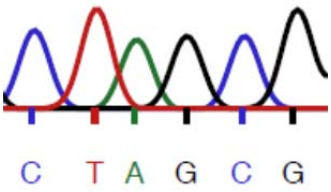
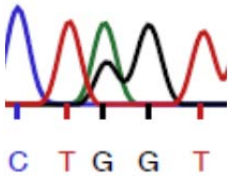
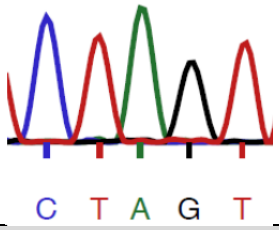
TAG#1		n.d.	
TAG#2		n.d.	
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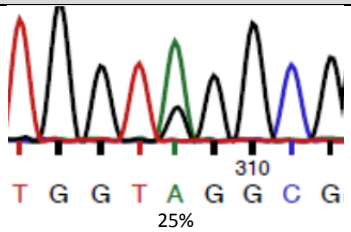
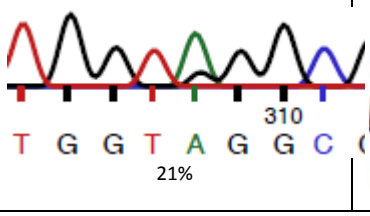
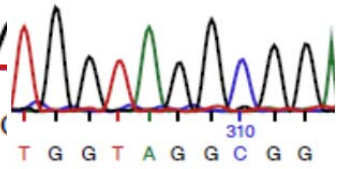
TAG#1			

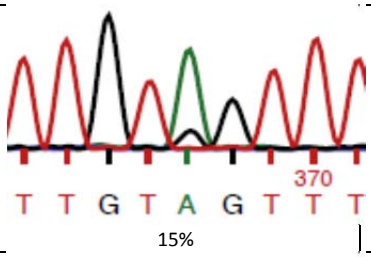
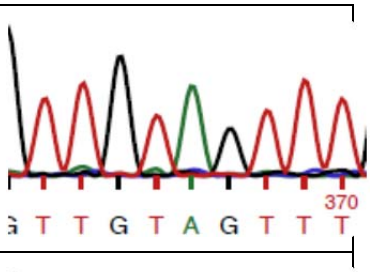
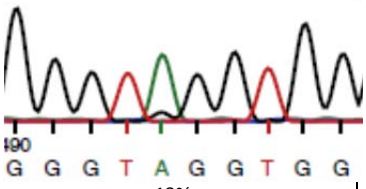
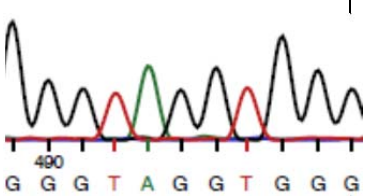
TAG#2	 15%	n.d.	
TAG#3	 13%	n.d	

Table S2.

Primers used for reverse-transcription and PCR:	Sequence 5'->3':
Beta-Aktin_fw	CAGCAGATGTGGATCAGCAAGCAGGAG
Beta-Aktin_bw	GGAAGGGGGGGCACGAAGGCTCATC
Beta-Aktin_Seq_fw	GGTGACAGCAGTCGGTTGGAGCGAGC
GAPDH_fw	CTCAAGATCATCAGCAATGCCTCCTGC
GAPDH_bw	GAGCACAGGGTACTTTATTGATGGTACATGACAAGG
GAPDH_Seq_fw	CACTGCTGGGGAGTCCCTGCCAC
GPI_fw_1	CTACACCGAGGGTCGAGCCGTGCTG
GPI_fw_2	CCTGGACACCACCCAGAGCACCCTC
GPI_fw_3	GGGAGTACAGGCACCTGCCACCATG
GPI_bw_1	CAGGGCAACAAAGTGCTTCGCCACTGC
GPI_bw_2	CAGAGGTGAGGAGTGGAAAACAGTCTTGGG
GPI_bw_3	CAGCTATGATTGTATCACTGCAGTCCAGCCTG
GUSB_fw_1	CAACCAAGATGGCGCGGATGGCTTCAGG
GUSB_fw_2	CGCTGCCGCAGTTCTTCAACAACGTTTCTCTG
GUSB_bw_1	GTTGATGGCGATAGTGATTCGGAGCCGGG
GUSB_bw_2	CAGTAGCCACTTTCATGCCAACTCTTTATTTCCATAATAG
GUSB_seq_fw1	CCTGCGTGTCCCTTCCTCCCCG
GUSB_seq_fw2	CCGGCCTGTGACCTTTGTGAGCAACTC
RAB7A_fw	CTTGGATTATGTGTTTAAGTCCTGTAATGCAGGCC
RAB7A_bw	GGAGCAGAACTGCCAGGGTTCCAACC
RAB7A_Seq_fw	CAGTGGTATGTGCAATGGATAAATTGCCG
VCP_3'UTR_Exon-Junction_fw	GTCGGGGCTTTGGCAGCTTCAGATTCC
VCP_3'UTR_bw	CCTACTCTCTATATAAACATCCAGCAACTGTGGCC

PINK1 Editing

Gene sequence of **wt PINK1** with a C-terminal V5 and His_6-tag in the context of the pcDNA3.1 vector. PINK1 is controlled by the CMV promoter and the BGH terminator:

```
                10        20        30        40        50        60
1          CTG**GCTAGCATG**GCGGTGCGACAGGCGCTGGGCCGCGGCCTGCAGCTGGGTCGAGCGCTG
1              Nhe-I M  A  V  R  Q  A  L  G  R  G  L  Q  L  G  R  A  L

                70        80        90       100       110       120
61         CTGCTGCGCTTCACGGGCAAGCCCGGCCGGGCCTACGGCTTGGGGCGGCCGGGCCCGGCG
21          L  L  R  F  T  G  K  P  G  R  A  Y  G  L  G  R  P  G  P  A

               130       140       150       160       170       180
121        GCGGGCTGTGTCCGCGGGGAGCGTCCAGGCTGGGCCGCAGGACCGGGCGCGGAGCCTCGC
41          A  G  C  V  R  G  E  R  P  G  W  A  A  G  P  G  A  E  P  R

               190       200       210       220       230       240
181        AGGGTCGGGCTCGGGCTCCCTAACCGTCTCCGCTTCTTCCGCCAGTCGGTGGCCGGGCTG
61          R  V  G  L  G  L  P  N  R  L  R  F  F  R  Q  S  V  A  G  L

               250       260       270       280       290       300
241        GCGGCGCGGTTGCAGCGGCAGTTCGTGGTGCGGGCCTGGGGCTGCGCGGGCCCTTGCGGC
81          A  A  R  L  Q  R  Q  F  V  V  R  A  W  G  C  A  G  P  C  G

               310       320       330       340       350       360
301        CGGGCAGTCTTTCTGGCCTTCGGGCTAGGGCTGGGCCTCATCGAGGAAAAACAGGCGGAG
101         R  A  V  F  L  A  F  G  L  G  L  G  L  I  E  E  K  Q  A  E

               370       380       390       400       410       420
361        AGCCGGCGGGCGGTCTCGGCCTGTCAGGAGATCCAGGCAATTTTTACCCAGAAAAGCAAG
121         S  R  R  A  V  S  A  C  Q  E  I  Q  A  I  F  T  Q  K  S  K

               430       440       450       460       470       480
421        CCGGGGCCTGACCCGTTGGACACGAGACGCTTGCAGGGCTTTCGGCTGGAGGAGTATCTG
141         P  G  P  D  P  L  D  T  R  R  L  Q  G  F  R  L  E  E  Y  L

               490       500       510       520       530       540
481        ATAGGGCAGTCCATTGGTAAGGGCTGCAGTGCTGCTGTGTATGAAGCCACCATGCCTACA
161         I  G  Q  S  I  G  K  G  C  S  A  A  V  Y  E  A  T  M  P  T

               550       560       570       580       590       600
541        TTGCCCCAGAACCTGGAGGTGACAAAGAGCACCGGGTTGCTTCCAGGGAGAGGCCCAGGT
181         L  P  Q  N  L  E  V  T  K  S  T  G  L  L  P  G  R  G  P  G

               610       620       630       640       650       660
601        ACCAGTGCACCAGGAGAAGGGCAGGAGCGAGCTCCGGGGGCCCCTGCCTTCCCCTTGGCC
201         T  S  A  P  G  E  G  Q  E  R  A  P  G  A  P  A  F  P  L  A

               670       680       690       700       710       720
661        ATCAAGATGATGTGGAACATCTCGGCAGGTTCCTCCAGCGAAGCCATCTTGAACACAATG
221         I  K  M  M  W  N  I  S  A  G  S  S  S  E  A  I  L  N  T  M

               730       740       750       760       770       780
721        AGCCAGGAGCTGGTCCCAGCGAGCCGAGTGGCCTTGGCTGGGGAGTATGGAGCAGTCACT
241         S  Q  E  L  V  P  A  S  R  V  A  L  A  G  E  Y  G  A  V  T

               790       800       810       820       830       840
781        TACAGAAAATCCAAGAGAGGTCCCAAGCAACTAGCCCCTCACCCCAACATCATCCGGGTT
```

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261        Y  R  K  S  K  R  G  P  K  Q  L  A  P  H  P  N  I  I  R  V

                850        860        870        880        890        900
841        CTCCGCGCCTTCACCTCTTCCGTGCCGCTGCTGCCAGGGGCCCTGGTCGACTACCCTGAT
281         L  R  A  F  T  S  S  V  P  L  L  P  G  A  L  V  D  Y  P  D

                910        920        930        940        950        960
901        GTGCTGCCCTCACGCCTCCACCCTGAAGGCCTGGGCCATGGCCGGACGCTCTTTCTAGTC
301         V  L  P  S  R  L  H  P  E  G  L  G  H  G  R  T  L  F  L  V

                970        980        990       1000       1010       1020
961        ATGAAGAACTATCCCTGTACCCTGCGCCAGTACCTTTGTGTGAACACACCCAGCCCCCGC
321         M  K  N  Y  P  C  T  L  R  Q  Y  L  C  V  N  T  P  S  P  R

              1030       1040       1050       1060       1070       1080
1021       CTCGCCGCCATGATGCTGCTGCAGCTGCTGGAAGGCGTGGACCATCTGGTTCAACAGGGC
341         L  A  A  M  M  L  L  Q  L  L  E  G  V  D  H  L  V  Q  Q  G

              1090       1100       1110       1120       1130       1140
1081       ATCGCGCACAGAGACCTGAAATCCGACAACATCCTTGTGGAGCTGGACCCAGACGGCTGC
361         I  A  H  R  D  L  K  S  D  N  I  L  V  E  L  D  P  D  G  C

              1150       1160       1170       1180       1190       1200
1141       CCCTGGCTGGTGATCGCAGATTTTGGCTGCTGCCTGGCTGATGAGAGCATCGGCCTGCAG
381         P  W  L  V  I  A  D  F  G  C  C  L  A  D  E  S  I  G  L  Q

              1210       1220       1230       1240       1250       1260
1201       TTGCCCTTCAGCAGCTGGTACGTGGATCGGGGCGGAAACGGCTGTCTGATGGCCCCAGAG
401         L  P  F  S  S  W  Y  V  D  R  G  G  N  G  C  L  M  A  P  E

              1270       1280       1290       1300       1310       1320
1261       GTGTCCACGGCCCGTCCTGGCCCCAGGGCAGTGATTGACTACAGCAAGGCTGATGCCTGG
421         V  S  T  A  R  P  G  P  R  A  V  I  D  Y  S  K  A  D  A  W

              1330       1340       1350       1360       1370       1380
1321       GCAGTGGGAGCCATCGCCTATGAAATCTTCGGGCTTGTCAATCCCTTCTACGGCCAGGGC
441         A  V  G  A  I  A  Y  E  I  F  G  L  V  N  P  F  Y  G  Q  G

              1390       1400       1410       1420       1430       1440
1381       AAGGCCCACCTTGAAAGCCGCAGCTACCAAGAGGCTCAGCTACCTGCACTGCCCGAGTCA
461         K  A  H  L  E  S  R  S  Y  Q  E  A  Q  L  P  A  L  P  E  S

              1450       1460       1470       1480       1490       1500
1441       GTGCCTCCAGACGTGAGACAGTTGGTGAGGGCACTGCTCCAGCGAGAGGCCAGCAAGAGA
481         V  P  P  D  V  R  Q  L  V  R  A  L  L  Q  R  E  A  S  K  R

              1510       1520       1530       1540       1550       1560
1501       CCATCTGCCCGAGTAGCCGCAAATGTGCTTCATCTAAGCCTCTGGGGTGAACATATTCTA
501         P  S  A  R  V  A  A  N  V  L  H  L  S  L  W  G  E  H  I  L

              1570       1580       1590       1600       1610       1620
1561       GCCCTGAAGAATCTGAAGTTAGACAAGATGGTTGGCTGGCTCCTCCAACAATCGGCCGCC
521         A  L  K  N  L  K  L  D  K  M  V  G  W  L  L  Q  Q  S  A  A

              1630       1640       1650       1660       1670       1680
1621       ACTTTGTTGGCCAACAGGCTCACAGAGAAGTGTTGTGTGGAAACAAAAATGAAGATGCTC
541         T  L  L  A  N  R  L  T  E  K  C  C  V  E  T  K  M  K  M  L

              1690       1700       1710       1720       1730       1740
1681       TTTCTGGCTAACCTGGAGTGTGAAACGCTCTGCCAGGCAGCCCTCCTCCTCTGCTCATGG
561         F  L  A  N  L  E  C  E  T  L  C  Q  A  A  L  L  L  C  S  W
```

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                1750      1760      1770      1780      1790      1800
1741       AGGGCAGCCCTGCTCGAGTCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCCTCTC
581         R  A  A  L  L  E  S  R  G  P  F  E  G  K  P  I  P  N  P  L

                1810      1820      1830      1840      1850
1801       CTCGGTCTCGATTCTACGCGTACCGGTCATCATCACCATCACCATTGAGTTTAAACCCG
601         L  G  L  D  S  T  R  T  G  H  H  H  H  H  H  *    Pme-I
```

Gene & protein sequence of PINK1 W437X amber with a C-terminal V5- and His_6-tag in the context of the pcDNA 3.1 vector, under control of the the CMV promoter and the BGH terminator. The amber Stop codon is highlighted in yellow.:

```
                  10        20        30        40        50        60
1          CTGGCTAGCATGGCGGTGCGACAGGCGCTGGGCCGCGGCCTGCAGCTGGGTCGAGCGCTG
1             Nhe-I  M  A  V  R  Q  A  L  G  R  G  L  Q  L  G  R  A  L

                  70        80        90       100       110       120
61         CTGCTGCGCTTCACGGGCAAGCCCGGCCGGGCCTACGGCTTGGGGCGGCCGGGCCCGGCG
21          L  L  R  F  T  G  K  P  G  R  A  Y  G  L  G  R  P  G  P  A

                 130       140       150       160       170       180
121        GCGGGCTGTGTCCGCGGGGAGCGTCCAGGCTGGGCCGCAGGACCGGGCGCGGAGCCTCGC
41          A  G  C  V  R  G  E  R  P  G  W  A  A  G  P  G  A  E  P  R

                 190       200       210       220       230       240
181        AGGGTCGGGCTCGGGCTCCCTAACCGTCTCCGCTTCTTCCGCCAGTCGGTGGCCGGGCTG
61          R  V  G  L  G  L  P  N  R  L  R  F  F  R  Q  S  V  A  G  L

                 250       260       270       280       290       300
241        GCGGCGCGGTTGCAGCGGCAGTTCGTGGTGCGGGCCTGGGGCTGCGCGGGCCCTTGCGGC
81          A  A  R  L  Q  R  Q  F  V  V  R  A  W  G  C  A  G  P  C  G

                 310       320       330       340       350       360
301        CGGGCAGTCTTTCTGGCCTTCGGGCTAGGGCTGGGCCTCATCGAGGAAAAACAGGCGGAG
101         R  A  V  F  L  A  F  G  L  G  L  G  L  I  E  E  K  Q  A  E

                 370       380       390       400       410       420
361        AGCCGGCGGGCGGTCTCGGCCTGTCAGGAGATCCAGGCAATTTTTACCCAGAAAAGCAAG
121         S  R  R  A  V  S  A  C  Q  E  I  Q  A  I  F  T  Q  K  S  K

                 430       440       450       460       470       480
421        CCGGGGCCTGACCCGTTGGACACGAGACGCTTGCAGGGCTTTCGGCTGGAGGAGTATCTG
141         P  G  P  D  P  L  D  T  R  R  L  Q  G  F  R  L  E  E  Y  L

                 490       500       510       520       530       540
481        ATAGGGCAGTCCATTGGTAAGGGCTGCAGTGCTGCTGTGTATGAAGCCACCATGCCTACA
161         I  G  Q  S  I  G  K  G  C  S  A  A  V  Y  E  A  T  M  P  T

                 550       560       570       580       590       600
541        TTGCCCCAGAACCTGGAGGTGACAAAGAGCACCGGGTTGCTTCCAGGGAGAGGCCCAGGT
181         L  P  Q  N  L  E  V  T  K  S  T  G  L  L  P  G  R  G  P  G

                 610       620       630       640       650       660
601        ACCAGTGCACCAGGAGAAGGGCAGGAGCGAGCTCCGGGGGCCCCTGCCTTCCCCTTGGCC
201         T  S  A  P  G  E  G  Q  E  R  A  P  G  A  P  A  F  P  L  A

                 670       680       690       700       710       720
```

```
661       ATCAAGATGATGTGGAACATCTCGGCAGGTTCCTCCAGCGAAGCCATCTTGAACACAATG
221        I  K  M  M  W  N  I  S  A  G  S  S  S  E  A  I  L  N  T  M

                730       740       750       760       770       780
721       AGCCAGGAGCTGGTCCCAGCGAGCCGAGTGGCCTTGGCTGGGGAGTATGGAGCAGTCACT
241        S  Q  E  L  V  P  A  S  R  V  A  L  A  G  E  Y  G  A  V  T

                790       800       810       820       830       840
781       TACAGAAAATCCAAGAGAGGTCCCAAGCAACTAGCCCCTCACCCCAACATCATCCGGGTT
261        Y  R  K  S  K  R  G  P  K  Q  L  A  P  H  P  N  I  I  R  V

                850       860       870       880       890       900
841       CTCCGCGCCTTCACCTCTTCCGTGCCGCTGCTGCCAGGGGCCCTGGTCGACTACCCTGAT
281        L  R  A  F  T  S  S  V  P  L  L  P  G  A  L  V  D  Y  P  D

                910       920       930       940       950       960
901       GTGCTGCCCTCACGCCTCCACCCTGAAGGCCTGGGCCATGGCCGGACGCTCTTTCTAGTC
301        V  L  P  S  R  L  H  P  E  G  L  G  H  G  R  T  L  F  L  V

                970       980       990      1000      1010      1020
961       ATGAAGAACTATCCCTGTACCCTGCGCCAGTACCTTTGTGTGAACACACCCAGCCCCCGC
321        M  K  N  Y  P  C  T  L  R  Q  Y  L  C  V  N  T  P  S  P  R

               1030      1040      1050      1060      1070      1080
1021      CTCGCCGCCATGATGCTGCTGCAGCTGCTGGAAGGCGTGGACCATCTGGTTCAACAGGGC
341        L  A  A  M  M  L  L  Q  L  L  E  G  V  D  H  L  V  Q  Q  G

               1090      1100      1110      1120      1130      1140
1081      ATCGCGCACAGAGACCTGAAATCCGACAACATCCTTGTGGAGCTGGACCCAGACGGCTGC
361        I  A  H  R  D  L  K  S  D  N  I  L  V  E  L  D  P  D  G  C

               1150      1160      1170      1180      1190      1200
1141      CCCTGGCTGGTGATCGCAGATTTTGGCTGCTGCCTGGCTGATGAGAGCATCGGCCTGCAG
381        P  W  L  V  I  A  D  F  G  C  C  L  A  D  E  S  I  G  L  Q

               1210      1220      1230      1240      1250      1260
1201      TTGCCCTTCAGCAGCTGGTACGTGGATCGGGGCGGAAACGGCTGTCTGATGGCCCCAGAG
401        L  P  F  S  S  W  Y  V  D  R  G  G  N  G  C  L  M  A  P  E

               1270      1280      1290      1300      1310      1320
1261      GTGTCCACGGCCCGTCCTGGCCCCAGGGCAGTGATTGACTACAGCAAGGCTGATGCCTAG
421        V  S  T  A  R  P  G  P  R  A  V  I  D  Y  S  K  A  D  A  *

               1330      1340      1350      1360      1370      1380
1321      GCAGTGGGAGCCATCGCCTATGAAATCTTCGGGCTTGTCAATCCCTTCTACGGCCAGGGC
441        A  V  G  A  I  A  Y  E  I  F  G  L  V  N  P  F  Y  G  Q  G

               1390      1400      1410      1420      1430      1440
1381      AAGGCCCACCTTGAAAGCCGCAGCTACCAAGAGGCTCAGCTACCTGCACTGCCCGAGTCA
461        K  A  H  L  E  S  R  S  Y  Q  E  A  Q  L  P  A  L  P  E  S

               1450      1460      1470      1480      1490      1500
1441      GTGCCTCCAGACGTGAGACAGTTGGTGAGGGCACTGCTCCAGCGAGAGGCCAGCAAGAGA
481        V  P  P  D  V  R  Q  L  V  R  A  L  L  Q  R  E  A  S  K  R

               1510      1520      1530      1540      1550      1560
1501      CCATCTGCCCGAGTAGCCGCAAATGTGCTTCATCTAAGCCTCTGGGGTGAACATATTCTA
501        P  S  A  R  V  A  A  N  V  L  H  L  S  L  W  G  E  H  I  L

               1570      1580      1590      1600      1610      1620
1561      GCCCTGAAGAATCTGAAGTTAGACAAGATGGTTGGCTGGCTCCTCCAACAATCGGCCGCC
```

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521        A  L  K  N  L  K  L  D  K  M  V  G  W  L  L  Q  Q  S  A  A

                1630       1640       1650       1660       1670       1680
1621       ACTTTGTTGGCCAACAGGCTCACAGAGAAGTGTTGTGTGGAAACAAAAATGAAGATGCTC
541         T  L  L  A  N  R  L  T  E  K  C  C  V  E  T  K  M  K  M  L

                1690       1700       1710       1720       1730       1740
1681       TTTCTGGCTAACCTGGAGTGTGAAACGCTCTGCCAGGCAGCCCTCCTCCTCTGCTCATGG
561         F  L  A  N  L  E  C  E  T  L  C  Q  A  A  L  L  L  C  S  W

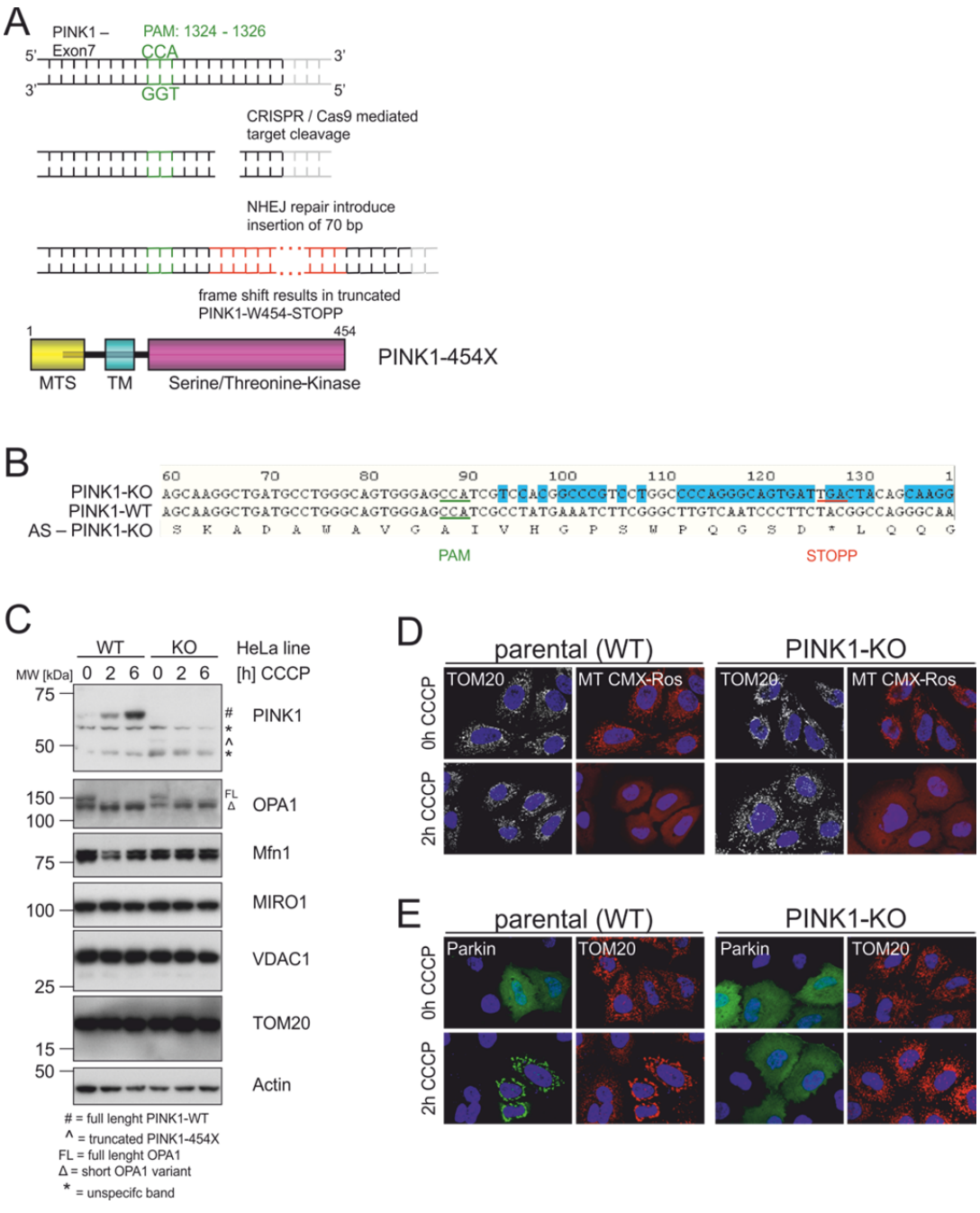
                1750       1760       1770       1780       1790       1800
1741       AGGGCAGCCCTGCTCGAGTCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCCTCTC
581         R  A  A  L  L  E  S  R  G  P  F  E  G  K  P  I  P  N  P  L

                1810       1820       1830       1840       1850
1801       CTCGGTCTCGATTCTACGCGTACCGGTCATCATCACCATCACCATTGA<u>**GTTTAAACCCG**</u>
601         L  G  L  D  S  T  R  T  G  H  H  H  H  H  H  *     Pme-I
```

Generation and Characterization of the PINK1-Knock out HeLa cell line

To genomically engineer a PINK1-knock out HeLa cell line, the CRISPR/Cas9 system was used. Therefore, the guideRNA sequence 5'-**CCA**TCGCCTATGAAATC-3' (with the PAM (protospacer adjacent motif) in bold) within exon 7 of PINK1, was inserted into the Cas9 expression plasmid pSpCas9(BB)-2A-Puro (F. A. Ran et al. Genome engineering using the CRISPR-Cas9 system. Nat. Protocols, 2013, doi:10.1038/nprot.2013.143) to yield pSpCas9(PINK1-KO)-2A-PURO. The parental HeLa cells were transfected with 2 µg of the pSpCas9(PINK1-KO)-2A-PURO plasmid using the Human Stem Cell Nucleofector Kit (Lonza) and a nucleofector device (Amaxa). 48h after transfection, the cells were selected using 2 µg/ml puromycin in DMEM+10% FCS for 48h. The genomic DNA from clones was isolated using the the ReliaPrep gDNA Tissue Miniprep System (Promega). The PINK1 exon 7 was amplified by flanking intronic primers (forward: 5' TGGATCAGGTGATGTGCAGGA 3' and reverse: 5' AGGATCTGTCACTGTG GCTCT 3'). The CRISPR/Cas9 genomic editing resulted in an introduction of a 70 bp DNA fragment into PINK1 exon 7, 4 bp upstream of the PAM sequence. This disrupts the functional PINK1 allele by introducing a premature Stop codon at position 454 inside the kinase domain (A, B). Due to the premature Stop codon, the resulting PINK1 protein is non-functional. While in parental wt Hela cells a stabilization of full length PINK1 is seen after CCCP treatment (#, Fig. S21 C), no PINK1 protein expression in mutant HeLa cells was detected (Fig. S21 C). Indeed, only faint amounts of the unstable truncated PINK1-454X were detected (^, Fig. S21 C). We thus call this cell line "PINK1 knock-out (KO)" even though a truncated PINK1 protein of similar length like the pathogenic W437X mutant could potentially be expressed. The characterization of the PINK1-KO cells highlights no differences in mitochondrial protein levels compared to wt HeLa cells (Fig. S21 C). In addition, PINK1 functional null did not interfere with the disruption of the mitochondrial membrane potential by CCCP, since the loss of the long OPA1 isoform (Fig. S21 C) as well as the loss of mitochondrial staining, with the membrane potential sensitive MitoTracker Red CMX-ROS was observed (Fig. S21 D). The mutated PINK1 is in addition also unable to induce perinuclear co-localization of Parkin and mitochondria (see microscopy pictures, Fig. S21 D and E). Thus, we considered that the PINK1-KO Hela cells are functional null regarding PINK1. Important, due to the insertion mutation this non-functional transcript is not repaired by site-directed RNA editing.

Figure S20. Generation and chracterization of the PINK1-KO HeLa cell line
Besides the antibodies described on page 61-62, the following additional antibodies were used:
rabbit anti-OPA1 (BD Transduction Laboratories, #612606)
mouse anti-Mfn1 (Abnova, H00055669-M04)
rabbit anti-VDAC1 (Millipore, Ab10527)
mouse anti-MIRO1 (Sigma, WH0055288M1).



PINK1 Functional Assay

HeLa cells (PINK1 wt or KO) were cultured under standard conditions (DMEM + 10% FBS, 37°C, 5% CO_2). The mitophagy assay was performed in 24-wells. Each well contained a cover-slip coated with poly-D-lysine (Sigma Aldrich). The cells were seeded at $2.5x10^4$/well. After 24h the cells were transfected with the indicated plasmids using FuGene6 (Promega). If the editing vector was transfected a 1-to-6 ratio was used. If ADAR2 or guideRNA alone were transfected, a 1-to-3 ratio was applied. If not indicated, the plasmid amounts/well has been 300 ng for EGFP-Parkin, 300 ng for PINK1 wt or PINK1 W437amber, 300 ng editing vector. In control experiment d) 1300 ng of a guideRNA plasmid based on pSilencer lacking ADAR2 was co-transfected instead of the editing vector. In control e) 200 ng of an editing vector lacking any guideRNAs but containing ADAR2 was co-transfected instead of the original editing vector. Treatment with 10 µM CCCP (in DMEM + 10% FBS) was either performed 46h after transfection for 2h or 24h after transfection for 24 h. The depolarization of the mitochondrial membrane potential with 10 µM CCCP (in DMEM + 10% FBS) was either performed 46h after transfection for 2h or 24h after transfection for 24 h. To visualize the mitochondria with a membrane potential sensitive dye, like MitoTracker Red CMXROS, a CCCP wash out was performed. For this, the depolarizing agent CCCP was washed out by changing the media twice every 15 min. Then the cells were incubated with 100 nM MitoTracker Red CMXROS (Invitrogen, M7512) in DMEM for 30 min at 37°C directly prior fixation or harvesting. Then 48 h after transfection, the cells were washed once with PBS and then either fixated for immunocytochemical staining (A) or harvested for RNA isolation (B).

(A) After fixation with 4% PFA/PBS for 20 minutes at RT and three wash steps with PBS, the cells were permeabilized with 1% Triton X-100/PBS for 5 minutes at RT followed by three wash steps with PBS. Then, the cells were blocked with 10% FCS/PBS for 1h at RT and stained with following antibodies diluted in 5% FCS/PBS for 2h at RT: mouse anti-ADAR2 (Santa Cruz, SC-73409, 1:1000), and rabbit anti-PINK1 (Novus Biologicals, BC 100-949, 1:750). The cells were then washed three times with PBS and incubated with the following secondary antibodies diluted in 10% FCS/PBS: goat anti-mouse or rabbit Alexa Fluor-488, 568 or 647 (Invitrogen, 1:1000). After two washing steps with PBS the nuclei of the cells where stained with Hoechst33342 (Thermo Fisher, 1:5000) for 5 minutes at RT. The cover-slips where mounted onto glass-slides using the Dako fluorescent mounting medium. Confocal images with a slice thickness of 0,7 µm were obtained with an AxioImager microscope equipped with an ApoTome imaging system (Carl Zeiss) using a 63x objective. The images were processed using the AxioVision software 4.8.1. For the quantification of Parkin clustering, double- (EGFP-Parkin and PINK1) and triple- (EGFP-Parkin, PINK1 and hADAR2) positive cells were evaluated. More than 100 cells per cover slip and condition were analyzed for quantification.

(B) The cells were harvested by trypsination using 60 µl Trypsin-EDTA. After inactivation with 440 µl DMEM+10%FBS the cells were peletted at 300 g for 5 minutes at 4°C. After removing the supernatant the cells where washed once in 500 µl ice-cold PBS and centrifuged again at 300 g for 5 minutes at 4°C. The cell pellet was snap frozen in liquid nitrogen prior RNA isolation using the RNeasy Mini Kit (Qiagen). Remaining Plasmid DNA that could interfere with following PCRs was removed by DNase-I (NEB) digestion for 10 min at 37°C. Then, the RNA was reverse transcribed using 1 µl M-MuLV reverse transcriptase (NEB), 0,25 µl murine RNase inhibitor (NEB) and a BHG backward primer (5´-CTAGAAGGCACAGTCGAGGC). The cDNA was isolated using the nucleospin gel and PCR-cleanup kit (Machery-Nagel). The following Phusion-PCR was performed with 4% DMSO, 0.8 M betaine and a PINK1 specific primer pair 5´-CTAGA AGGCACAGTCGAGGC and 5´-GAGCCAGGAGCTGGTCCCAGCGAGCCGAG". The 1167 bp

PINK1 DNA fragment was isolated from the 1,4% agarose gel using the nucleospin gel and PCR-cleanup kit (Machery-Nagel). The sequencing was performed by the company Eurofins using the sequencing primer 5´-GGTACGTGGATCAGGGCG GAAACGGC.

Figure S21. Western Blot analysis *of PINK1-W437X-V5 editing. HeLa WT or PINK1-KO cells were transfected with plasmids for wt PINK1-V5 or PINK1-W437X-V5, and with or without the editing vector for 48h as indicated. The cells were treated with 10 µM CCCP 6h prior lysis with 8M Urea buffer (10 mM Tris (pH 8.0), 100 mM NaH_2PO_4, 8M urea). 10 µg of total lysates were separated by SDS-PAGE and transferred onto PVDF membrane using the wet blotting method. The following primary antibodies were used: mouse anti-ADAR2 (Santa Cruz, sc-73409), rabbit anti-PINK1 (Novus Biologicals, BC 100-494), mouse anti-V5 (Invitrogen, R960-25, rabbit anti-TOM20 (Santa Cruz, sc-11415), and mouse anti beta-actin (Sigma, clone AC-15). In panel* ***A****, the potential PINK1 variants are shown.In panel* ***B****, the PINK1 antibody stains all processed and truncated versions of the wt and the overexpressed PINK1 variants. Upon editing a small fraction of fulllength PINK1 (FL & ΔFL) appears beside an excess of truncated PINK1 (X & ΔX) which is in accordance with an editing yield of 10%. The V5 antibody detects fulllength V5-tagged PINK1 (FL & ΔFL) only in presence of the editing vector, which again indicates the successful editing of the transfected PINK1-W437X-V5. After editing and CCCP treatment TOM20 ubiquitinylation (Ub-1, Ub-2, Ub-3) is visible similar to wt PINK1.*

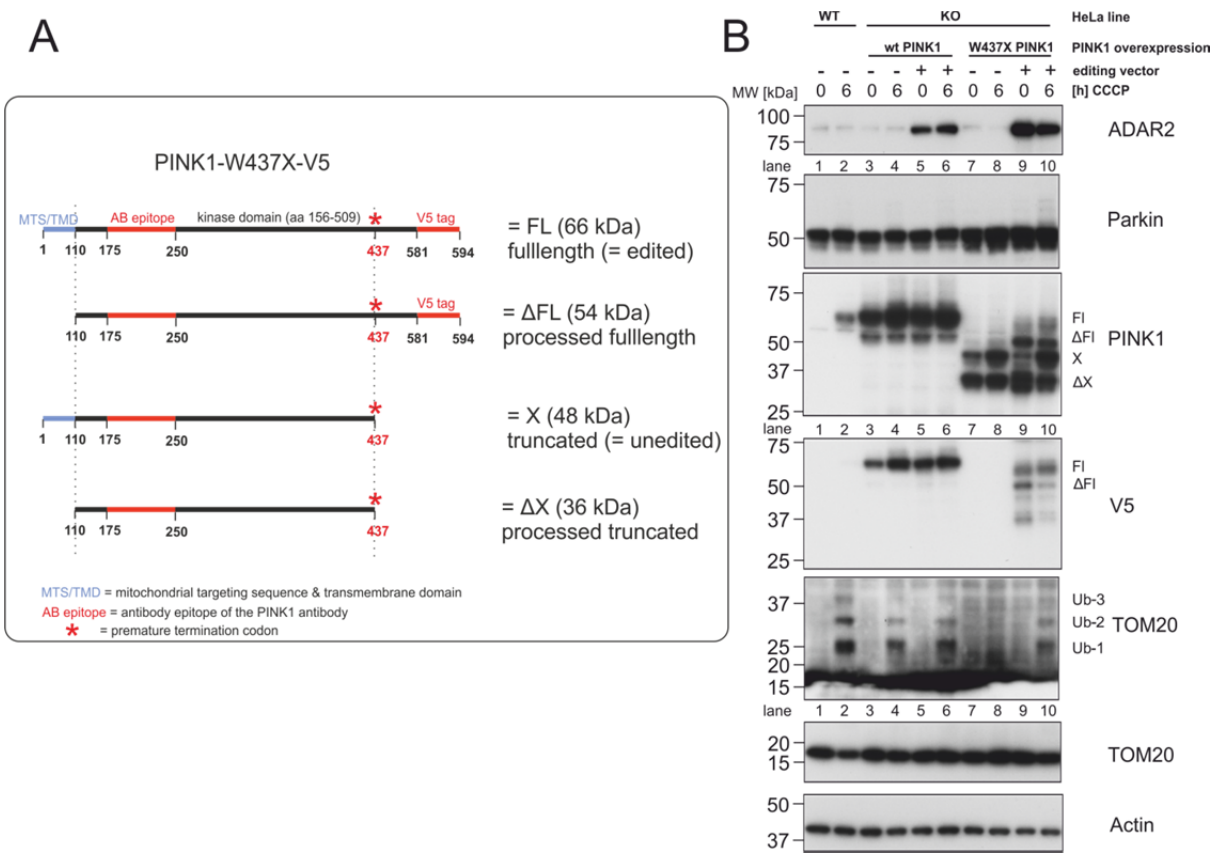


Figure S22. *Full sequencing trace of the PINK1 W437amber editing experiment in HeLa cells, corresponding to the experiment shown in Figure 4A, 4B c).*

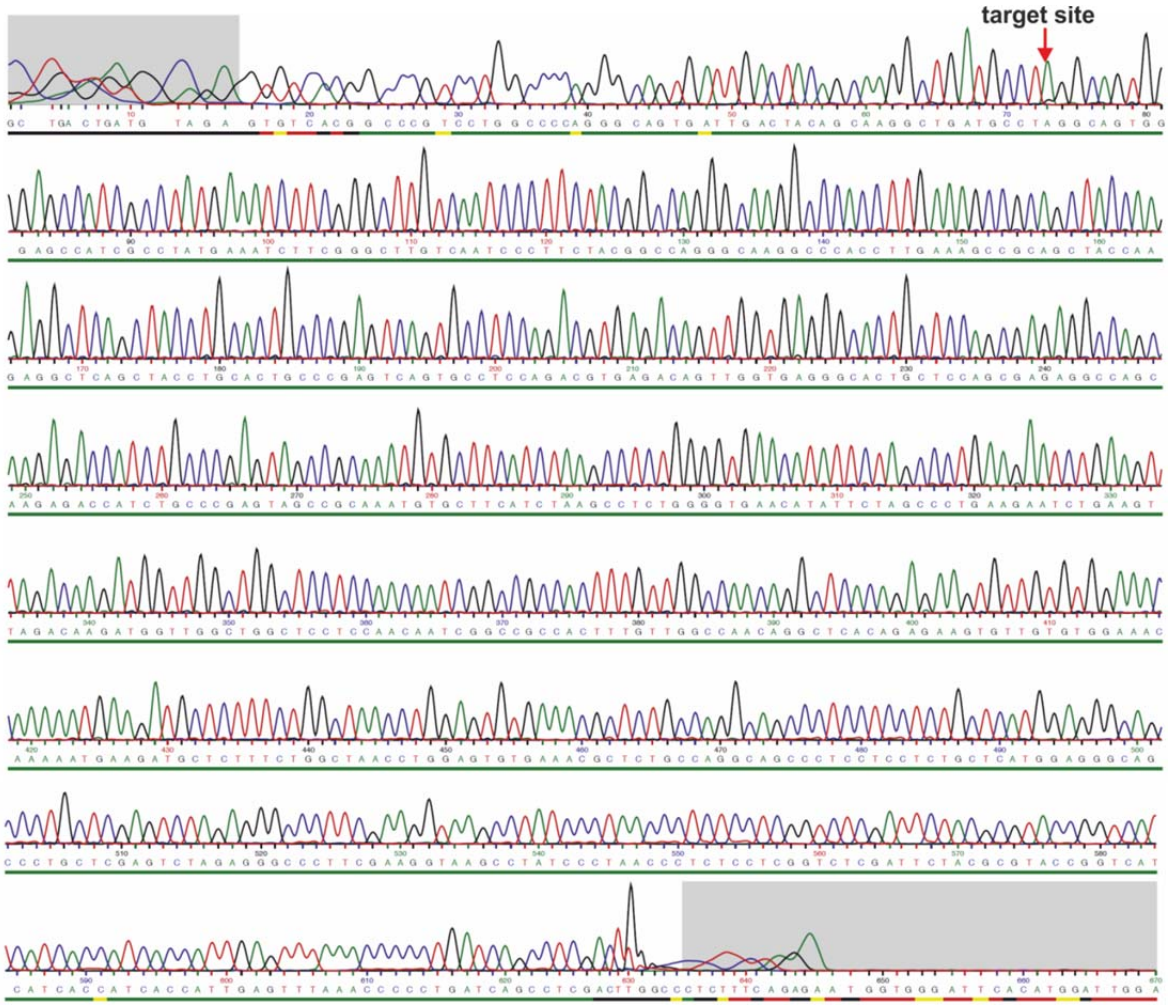
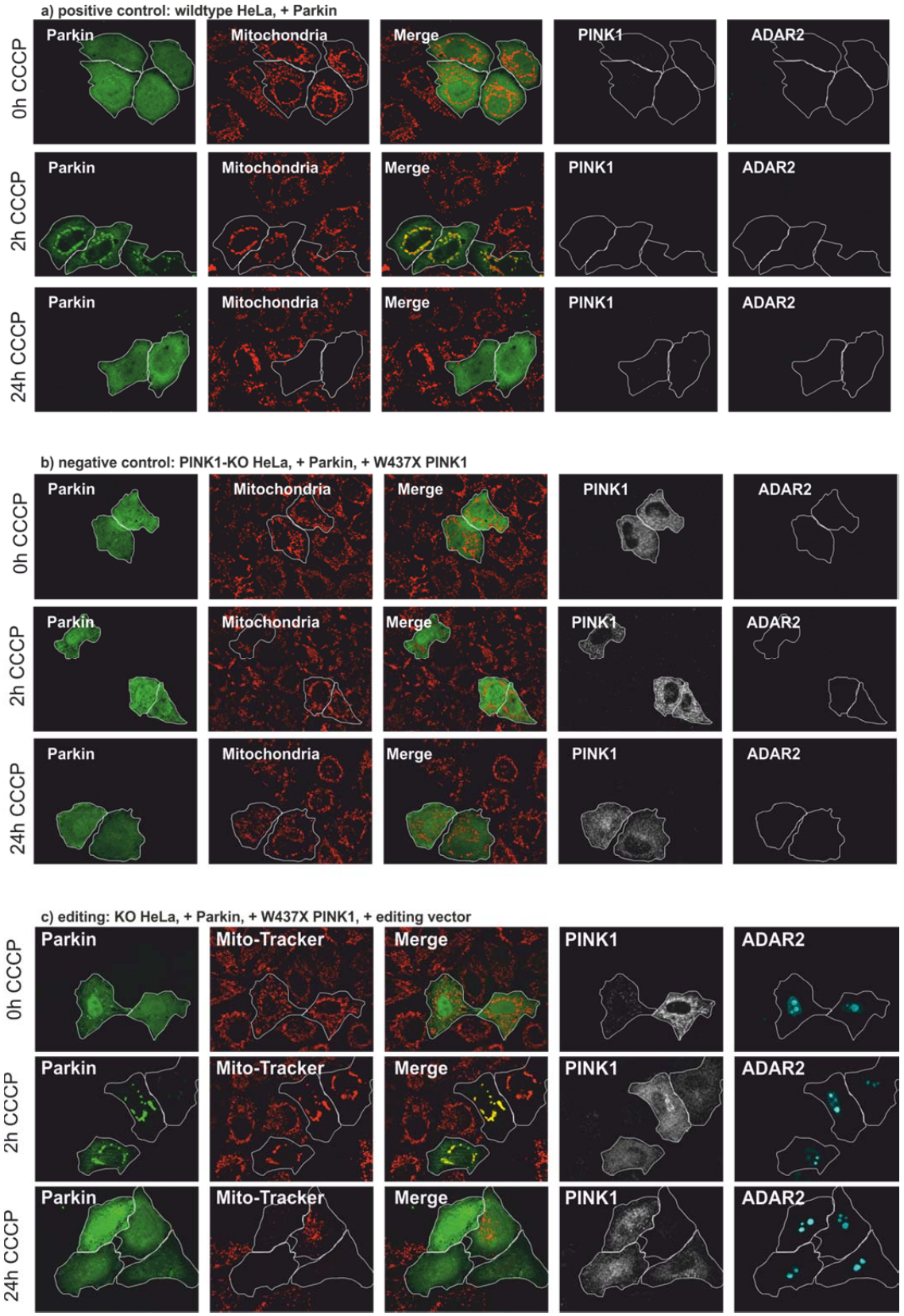
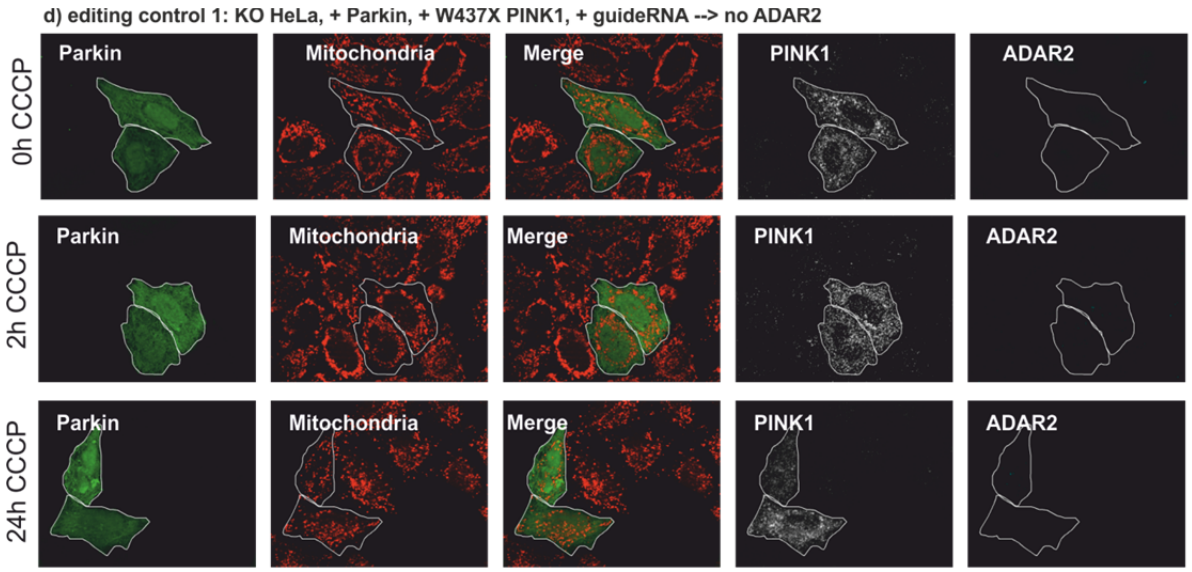
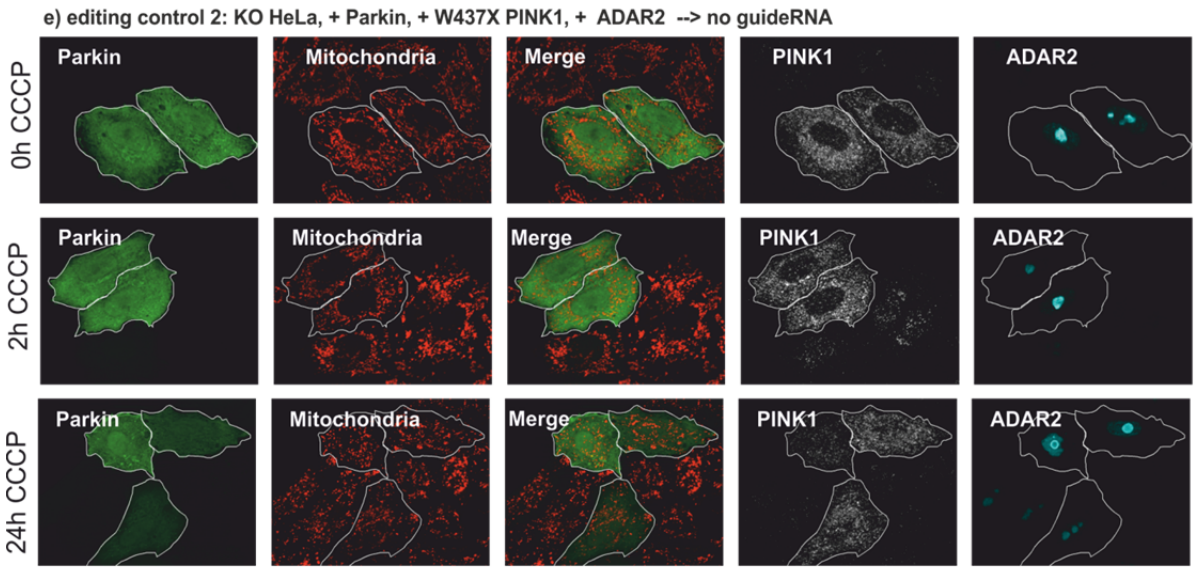
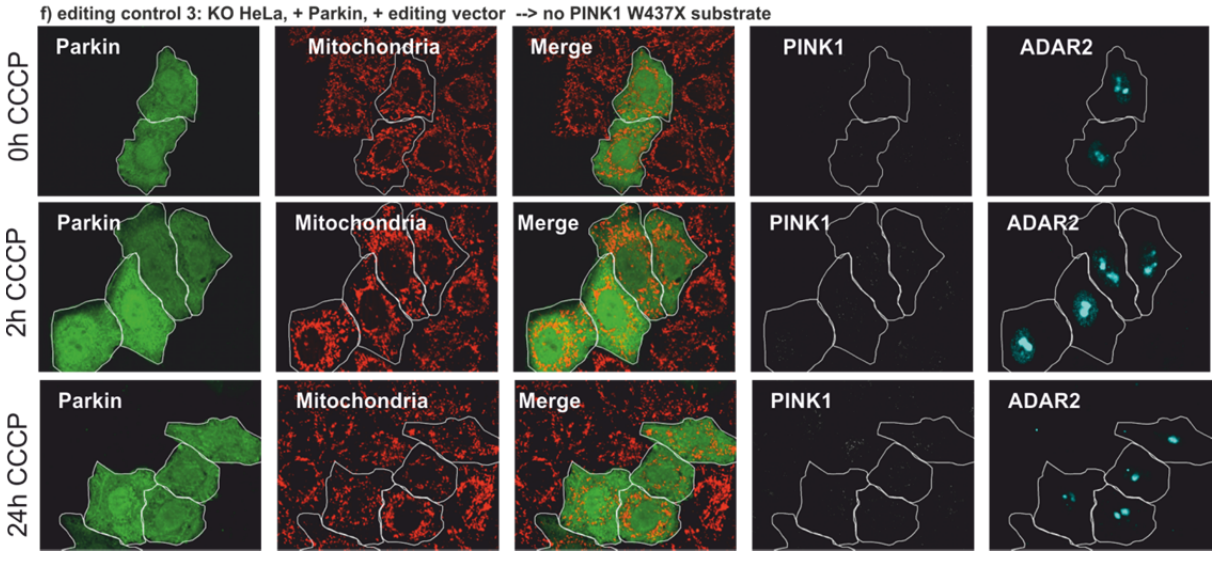


Figure S23. Mitophagy assay. Shown are the complete mitophagy assays that relate to the Parkin-clustering experiments a) – f) shown on Figure 4A in the main text. The mitophagy assay shown in c) is the same as shown in the main text Figure 4C. For better visualization of mitophagy, the Parkin-positive cells were encircled.




d) editing control 1: KO HeLa, + Parkin, + W437X PINK1, + guideRNA --> no ADAR2
0h CCCP
2h CCCP
24h CCCP
Parkin
Mitochondria
Merge
PINK1
ADAR2


e) editing control 2: KO HeLa, + Parkin, + W437X PINK1, + ADAR2 --> no guideRNA
0h CCCP
2h CCCP
24h CCCP
Parkin
Mitochondria
Merge
PINK1
ADAR2


f) editing control 3: KO HeLa, + Parkin, + editing vector --> no PINK1 W437X substrate
0h CCCP
2h CCCP
24h CCCP
Parkin
Mitochondria
Merge
PINK1
ADAR2