Supplementary Materials for

Repair of CRISPR-guided RNA breaks enables site-specific RNA editing in human cells.

Anna Nemudraia, Artem Nemudryi, and Blake Wiedenheft
Corresponding authors: artem.nemudryi@gmail.com and bwiedenheft@gmail.com

The PDF file includes:

Figs. S1 to S3
Tables S1 to S6

Other Supplementary Materials for this manuscript include the following:

Data S1 (separate file)

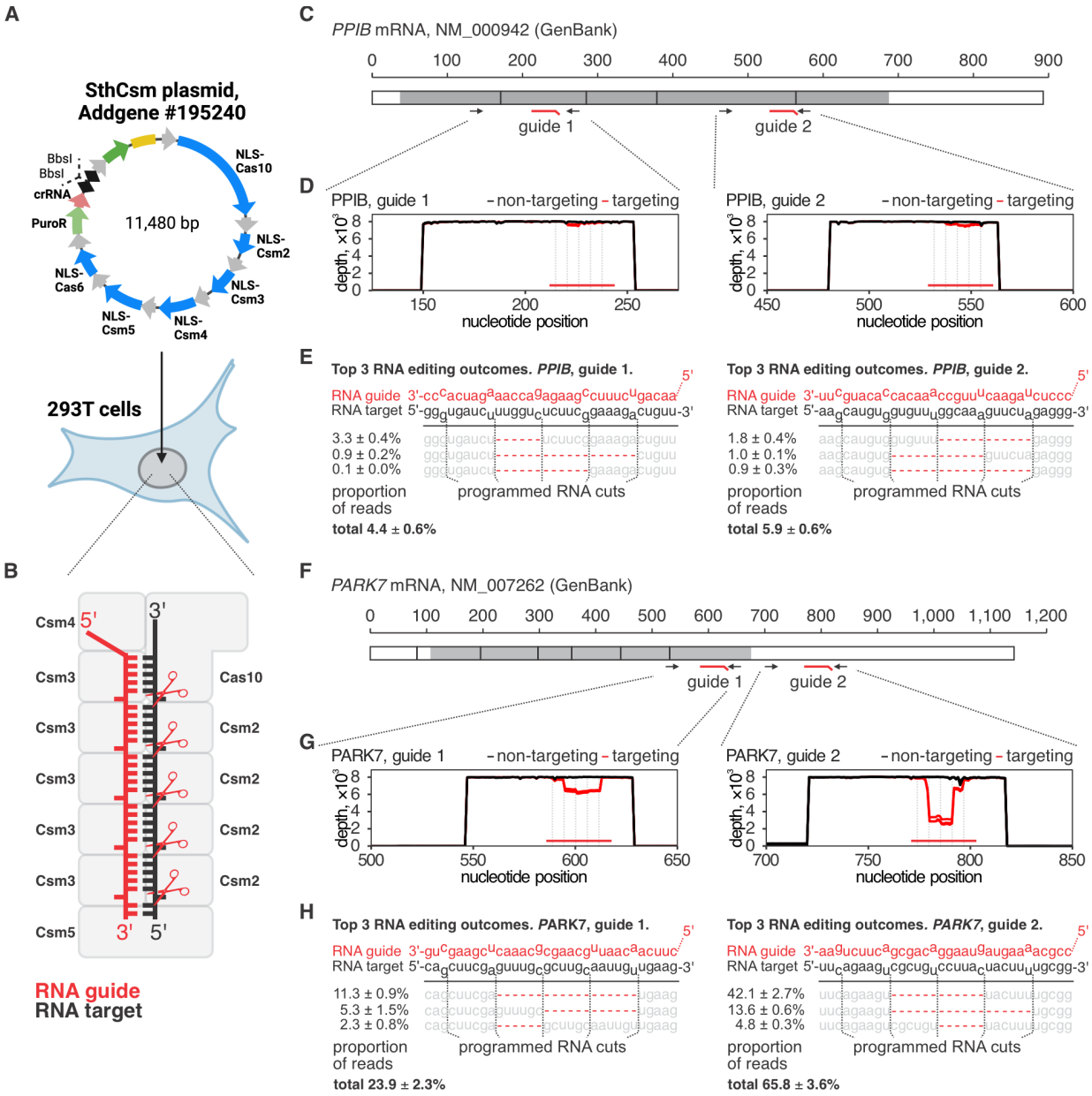

Fig. S1. The distribution and frequency of RNA editing outcomes in *PPIB* and *PARK7* mRNAs. A) Diagram of the plasmid (Addgene #195240) encoding for human codon-optimized and NLS-tagged protein subunits of the type III-A CRISPR-Csm complex from *Streptococcus thermophilus* (SthCsm), CRISPR RNA (crRNA) -processing nuclease Cas6, and single-spacer CRISPR array. CRISPR repeats are shown as black diamonds. The sequence between the repeats has two BbsI restriction sites for cloning spacers for targeting complementary target RNAs. **B)** Diagram of the assembled SthCsm ribonucleoprotein complex bound to the target RNA (black). crRNA is shown with red color. The target RNA is cleaved in six nucleotide intervals by Csm3 nucleases in the backbone of the complex. Cleavage sites are indicated with red scissors. **C)** Diagram of the *PPIB* mRNA (NM_000942, GenBank). The scale shows nucleotide position along the transcript. Blocks show exons in the spliced mRNA, gray color indicates the position of the PPIB open reading frame (ORF). Red lines mark the position of the SthCsm guide RNAs.

Black arrows indicate position of the binding sites for oligonucleotide primers used for RT-qPCR in Fig. 1B. **D)** qPCR amplicons were deep-sequenced, and resulting reads were aligned to the reference sequences. A subset of 8,000 reads was randomly selected from the alignment for the representation. Plots show sequencing coverage (y-axes) along the length of the amplicons (x-axes). Each line represents a single replicate, three replicates total per guide RNA. Vertical dotted gray lines indicate predicted RNA break sites. The horizontal red line shows the position of the guide RNA with respect to the target. **E)** Top three most frequent RNA editing outcomes in PPIB transcript. Dotted lines indicate the positions of RNA breaks introduced with the CRISPR complex. Red dashes depict deletions identified in the sequencing data. Deletion frequency was quantified as the mean ± one standard deviation of three biological replicates. **F)** Same as in (**C**) but for the *PARK7* transcript (NM_007262, GenBank). **G)** Same as in (**D**) but for target sites in the *PARK7* transcript. **H)** Top three most frequent RNA editing outcomes at the target sites in *PARK7*. Data is shown as the mean ± one standard deviation of three biological replicates.

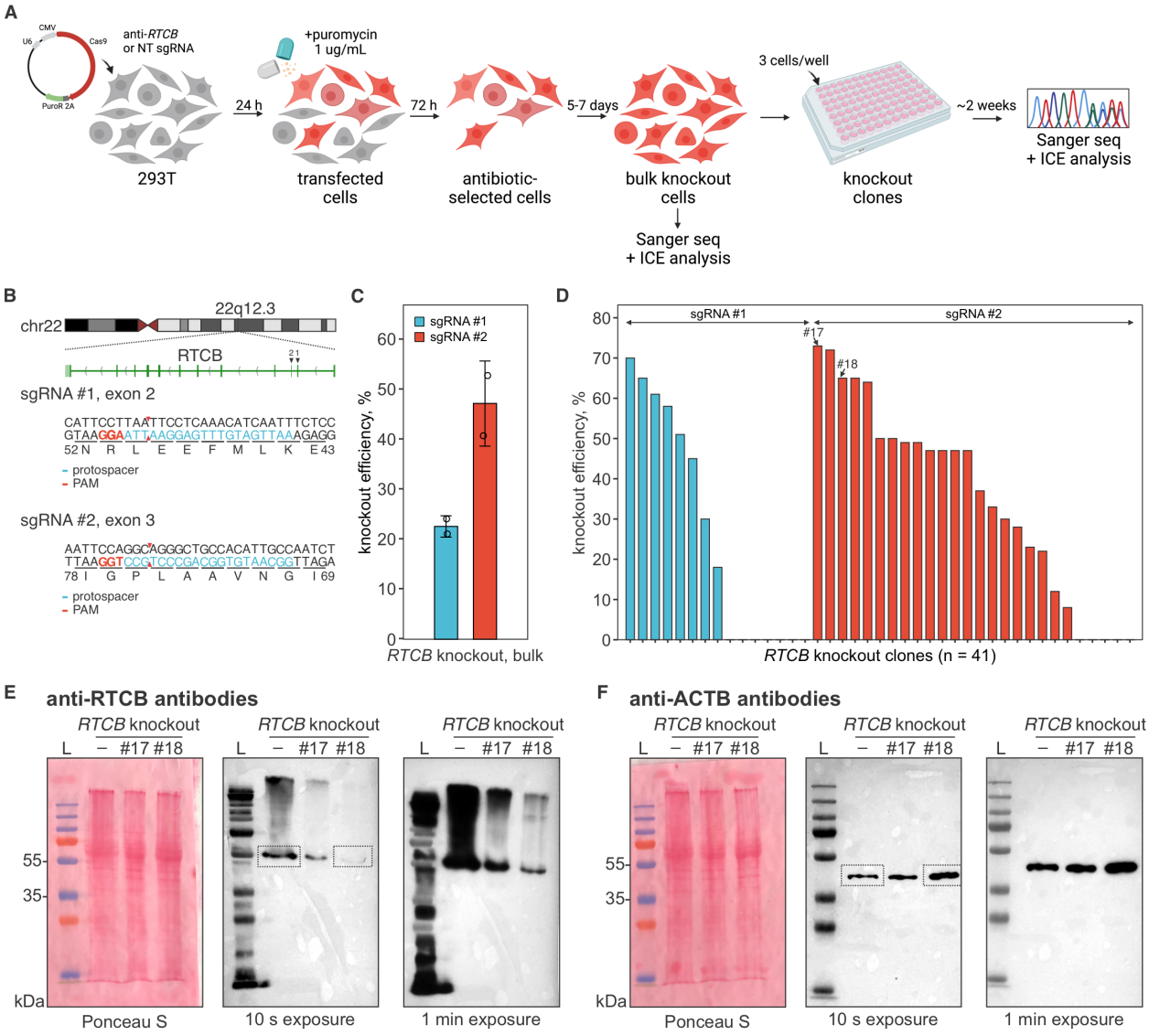

Fig. S2. Knockout of RTCB gene with CRISPR-Cas9. A) Schematics of the knockout strategy. **B)** Diagram showing the exon-intron structure of the *RTCB* gene (top) and sequences targeted with Cas9 (middle and bottom). **C)** *RTCB* knockout efficiency in bulk cells was quantified by analyzing Sanger traces from target site amplicons using the ICE web tool (Synthego). **D)** Quantification of *RTCB* knockout efficiency in 293T cell clones. **E and F)** Clones #17 and #18, indicated with black arrows in (**D**), were lysed, and lysates were probed with anti-RTCB or anti-ACTB antibodies. Dotted boxes show parts of the images that were cropped out for main Fig. 2B.

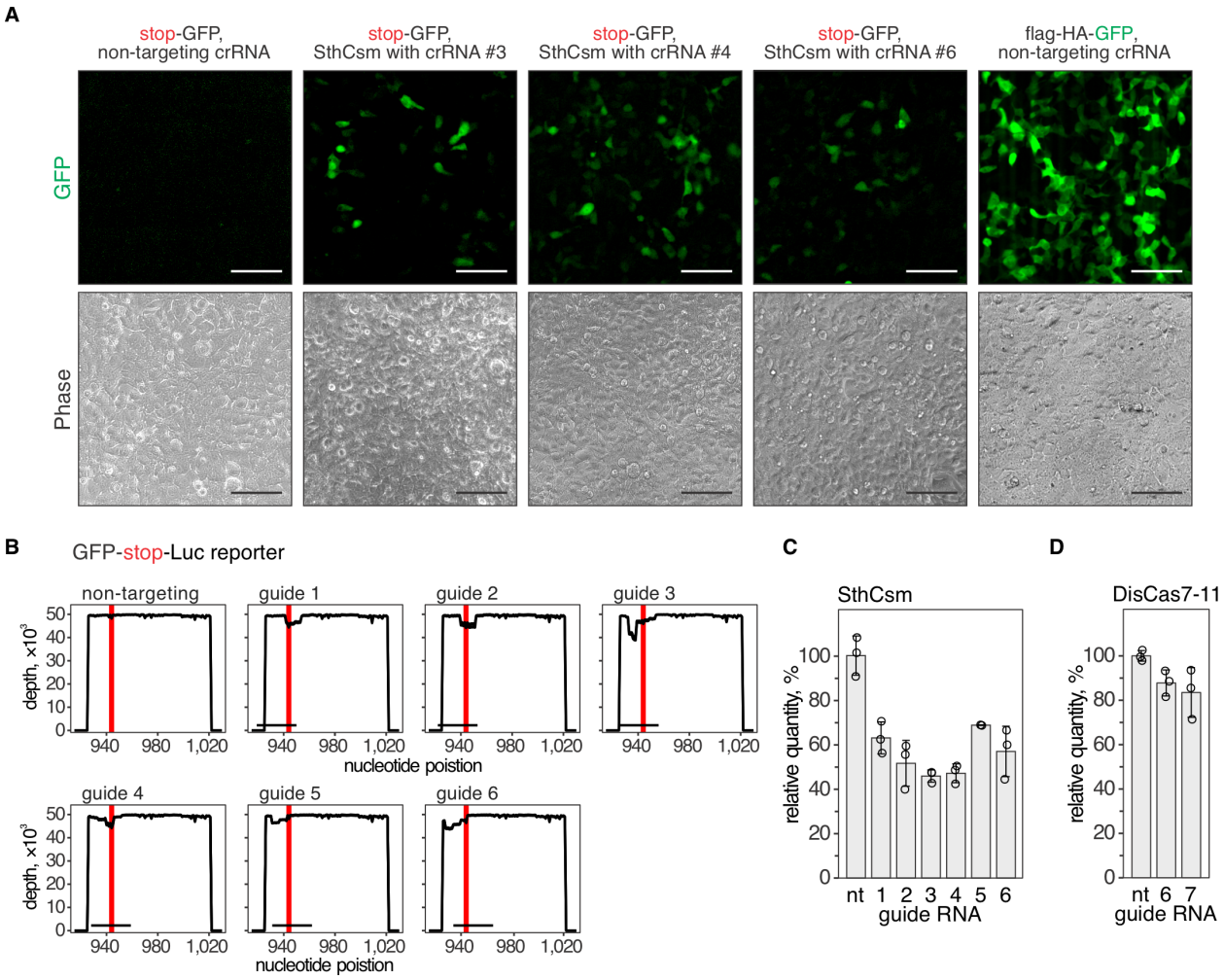

Fig. S3. Programmable deletion of stop codons in RNA restores protein expression.

A) Fluorescent imaging of cells transfected with plasmids for the stop-GFP reporter and SthCsm with a non-targeting guide (left), stop-GFP reporter and SthCsm with targeting guides, or GFP reporter and SthCsm with the non-targeting guide (right). **B)** Deep-sequencing of qPCR amplicons in (C). Reads were aligned to the reference sequence of the GFP-stop-Luc reporter. Graphs show sequencing depth (y-axes) at the amplified region of the transcript (x-axes). Every line shows a biological replicate (n = 3). The horizontal black bar indicates a region complementary to the guide RNA of the SthCsm complex. Vertical red line marks the postion of the stop codon targeted with SthCsm. **C)** RNA was extracted from the same lysates that were used in luciferase assays shown in Fig. 3E. RT-qPCR was performed with oligonucleotide primers that flank the region targeted with SthCsm in the GFP-stop-Luc reporter transcript. Transcript quantities are normalized to *ACTB* and non-targeting guide RNA control using the ΔΔCt method. The mean ± one standard deviation of three biological replicates is shown. nt – non-targeting guide RNA. **D)** Same as in (C), but for the experiment with DisCas7-11 in Fig. 3H.

Table S1.

Oligonucleotides used for cloning SthCsm guide RNAs

Name	Sequence 5`-3`
Guides targeting endogenous transcripts	
PPIB_g1F	AAACAACAGTCTTTCCGAAGAGACCAAAGATCACCC
PPIB_g1R	TATCGGGTGATCTTTGGTCTCTTCGGAAAGACTGTT
PPIB_g2F	AAACCCCTCTAGAACTTTGCCAAACACCACATGCTT
PPIB_g2R	TATCAAGCATGTGGTGTTTGGCAAAGTTCTAGAGGG
PARK7_g1F	AAACCTTCAACAATTGCAAGCGCAAACTCGAAGCTG
PARK7_g1R	TATCCAGCTTCGAGTTTGCGCTTGCAATTGTTGAAG
PARK7_g2F	AAACCCGCAAAAGTAGTAAGGACAGCGACTTCTGAA
PARK7_g2R	TATCTTCAGAAGTCGCTGTCCTTACTACTTTTGCGG
Guides targeting stop-GFP reporter	
sthcsm_stop_gfp_g1F	AAACGCGGCGGGGGCAGAACCTCCTTATCCACCAGC
sthcsm_stop_gfp_g1R	TATCGCTGGTGGATAAGGAGGTTCTGCCCCCGCCGC
sthcsm_stop_gfp_g2F	AAACGCGGGGGCAGAACCTCCTTATCCACCAGCGTA
sthcsm_stop_gfp_g2R	TATCTACGCTGGTGGATAAGGAGGTTCTGCCCCCGC
sthcsm_stop_gfp_g3F	AAACGGGGCAGAACCTCCTTATCCACCAGCGTAATC
sthcsm_stop_gfp_g3R	TATCGATTACGCTGGTGGATAAGGAGGTTCTGCCCC
sthcsm_stop_gfp_g4F	AAACGCAGAACCTCCTTATCCACCAGCGTAATCTGG
sthcsm_stop_gfp_g4R	TATCCCAGATTACGCTGGTGGATAAGGAGGTTCTGC
sthcsm_stop_gfp_g5F	AAACGAACCTCCTTATCCACCAGCGTAATCTGGAAC
sthcsm_stop_gfp_g5R	TATCGTTCCAGATTACGCTGGTGGATAAGGAGGTTC
sthcsm_stop_gfp_g6F	AAACCCTCCTTATCCACCAGCGTAATCTGGAACATC
sthcsm_stop_gfp_g6R	TATCGATGTTCCAGATTACGCTGGTGGATAAGGAGG
Guides targeting GFP-stop-Luc reporter	
csm_gfp_stop_luc_g1F	AAACTCACCGGTCACATTGATCCTTTAAGCAGAAGC
csm_gfp_stop_luc_g1R	TATCGCTTCTGCTTAAAGGATCAATGTGACCGGTGA
csm_gfp_stop_luc_g2F	AAACCCGGTCACATTGATCCTTTAAGCAGAAGCACA
csm_gfp_stop_luc_g2R	TATCTGTGCTTCTGCTTAAAGGATCAATGTGACCGG
csm_gfp_stop_luc_g3F	AAACGTCACATTGATCCTTTAAGCAGAAGCACAGGC
csm_gfp_stop_luc_g3R	TATCGCCTGTGCTTCTGCTTAAAGGATCAATGTGAC
csm_gfp_stop_luc_g4F	AAACACATTGATCCTTTAAGCAGAAGCACAGGCTGC
csm_gfp_stop_luc_g4R	TATCGCAGCCTGTGCTTCTGCTTAAAGGATCAATGT
csm_gfp_stop_luc_g5F	AAACTTGATCCTTTAAGCAGAAGCACAGGCTGCAGG
csm_gfp_stop_luc_g5R	TATCCCTGCAGCCTGTGCTTCTGCTTAAAGGATCAA
csm_gfp_stop_luc_g6F	AAACATCCTTTAAGCAGAAGCACAGGCTGCAGGGTG
csm_gfp_stop_luc_g6R	TATCCACCCTGCAGCCTGTGCTTCTGCTTAAAGGAT

Table S2.

Oligonucleotides used for cloning Cas9 guides targeting RTCB gene

Name	Sequence 5`-3`
RTCB_sg1F	CACCGAATTGATGTTTGAGGAATTA
RTCB_sg1R	CAAATAATTCCTCAAACATCAATTC
RTCB_sg2F	CACCGGCAATGTGGCAGCCCTGCC
RTCB_sg2R	CAAAGGCAGGGCTGCCACATTGCC

Table S3.

Oligonucleotide primers used for Q5 SDM

Name	Sequence 5`-3`
NLS-DisCas711_F	AAGCGGAAGGTCGGCGGTAGCACTACTATGAAGATTTCAATTGAATTC
NLS-DisCas711_R	CTTCTTTGGGGCCATGGTGGCGGCTCTCCCTATAGTG

Table S4.

Oligonucleotides used for cloning DisCas7-11 guide RNAs

Name	Sequence 5`-3`
discas711_gfp_stop_luc_g1F	GAACTTGATCCTTTAAGCAGAAGCACAGGCTGCAGG
discas711_gfp_stop_luc_g1R	AAAACCTGCAGCCTGTGCTTCTGCTTAAAGGATCAA
discas711_gfp_stop_luc_g2F	GAACTGATCCTTTAAGCAGAAGCACAGGCTGCAGGG
discas711_gfp_stop_luc_g2R	AAAACCCTGCAGCCTGTGCTTCTGCTTAAAGGATCA
discas711_gfp_stop_luc_g3F	GAACGATCCTTTAAGCAGAAGCACAGGCTGCAGGGT
discas711_gfp_stop_luc_g3R	AAAAACCCTGCAGCCTGTGCTTCTGCTTAAAGGATC
discas711_gfp_stop_luc_g4F	GAACATCCTTTAAGCAGAAGCACAGGCTGCAGGGTG
discas711_gfp_stop_luc_g4R	AAAACACCCTGCAGCCTGTGCTTCTGCTTAAAGGAT
discas711_gfp_stop_luc_g5F	GAACTCCTTTAAGCAGAAGCACAGGCTGCAGGGTGA
discas711_gfp_stop_luc_g5R	AAAATCACCCTGCAGCCTGTGCTTCTGCTTAAAGGA
discas711_gfp_stop_luc_g6F	GAACCCTTTAAGCAGAAGCACAGGCTGCAGGGTGAC
discas711_gfp_stop_luc_g6R	AAAAGTCACCCTGCAGCCTGTGCTTCTGCTTAAAGG
discas711_gfp_stop_luc_g7F	GAACCTTTAAGCAGAAGCACAGGCTGCAGGGTGACG
discas711_gfp_stop_luc_g7R	AAAACGTCACCCTGCAGCCTGTGCTTCTGCTTAAAG

Table S5.

Oligos used to amplify Cas9-targeted regions in the *RTCB* gene

Name	Sequence 5`-3`
RTCB_seq_F1	AGATGGAGCTTCAAATCCGTT
RTCB_seq_R1	TGTGGTTTTGAGGGTATGAGAAT
RTCB_seq_F2	GGTTATGTCTGGCTGTCCAAAG
RTCB_seq_R2	ATCTGGAATTTGAAGCTGGGTAG

Table S6.

Primers used for RT-qPCR

Name	Sequence 5`-3`
ACTB_F	AGAGCCTCGCCTTTGCC
ACTB_R	ATGCCGGAGCCGTTGTC
PPIB_cr1_qF1	GCGGCCGATGAGAAGAAGAA
PPIB_cr1_qR1	AGCTAAGGCCACAAAATTATCCAC
PPIB_cr2_qF1	CGCAGGCAAAGACACCAAC
PPIB_cr2_qR1	TTCCGCACCACCTCCA
PARK7_cr1_qF1	CCTACTCTGAGAATCGTGTGGA
PARK7_cr1_qR1	GAGCCGCCACCTCCTTG
PARK7_cr2_qF1	AGAGAAACAGGCCGTTAGGA
PARK7_cr2_qR1	GGCTGAGAAATCTCTGTGTAGTTG
gfp_stop_luc_qF1	GGATGGACCGTCACCCTG
gfp_stop_luc_qR1	TGTTTTTGGCGTCTTCCATACC

Data S1. (separate file)
Analysis of the sequencing data in Figs. 1-3, fig. S1 and fig. S3