

Targeting the gene encoding human T-cell leukemia virus type 1 basic zip factor via CRISPR/Cas9 potentially mitigates viral infection

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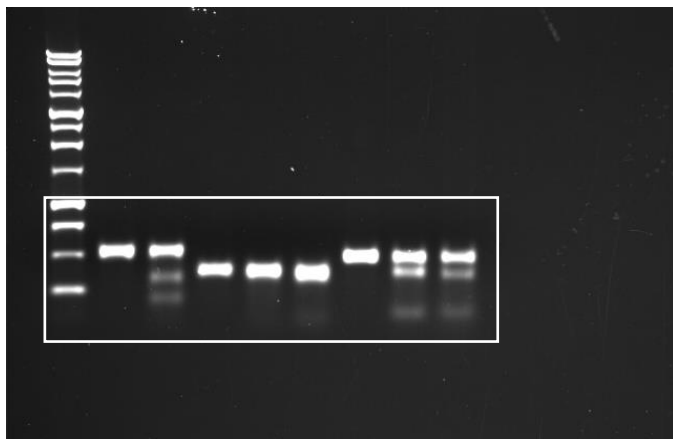
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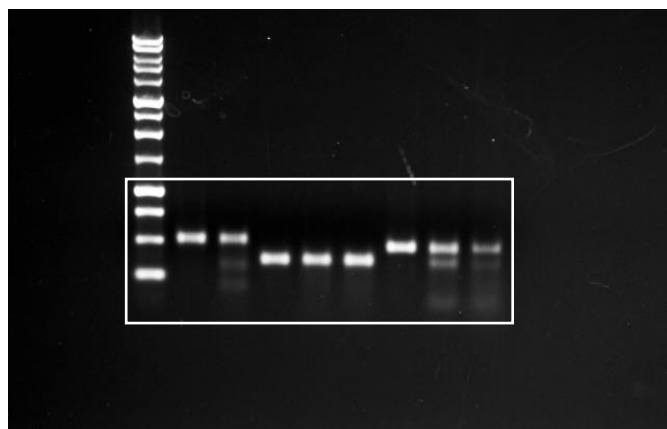
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Supplementary Materials

(a)



(b)



(c)

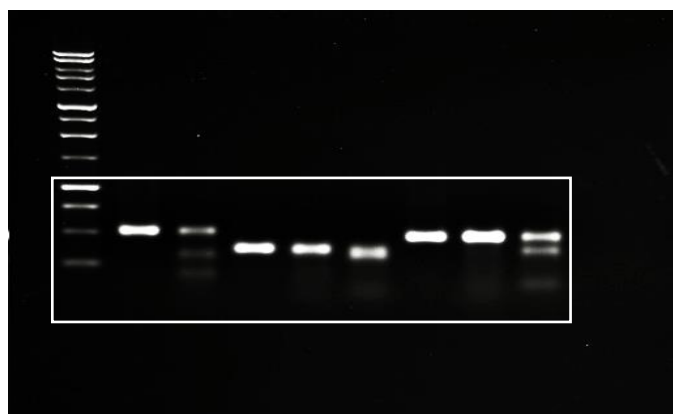


Figure S1. Original photograph of Figure 1.

Entire gel with visible edges. The cleavage assay results are shown for the genomic DNA extracted from (a) MT-1, (b) TL-Om1, and (c) ST-1 cells. The insets are depicted in detail in Figure 1.

(a) Clones obtained from MT-1 cells 2 days after Cas9/sgRNA 171 transfection

sgRNA171 sequence: GACTCAACCGGCGTGGATGGCGG

LC874449 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAATGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874450 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCG -GGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874451 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAAC - - - - -TGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874452 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCG - - - - -GGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874453 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGA - TCAACCGG - - -GG - TGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874454 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGT - - - - -GCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874455 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACC - - - - -GGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874456 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACT - - - - -CTGCCGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874457 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGT - - - - -AGGGCGGCAGGCGCGTG
LC874458 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCG - - - - -GGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874459 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGC - - - - -GG -CTCAGGTAGGGCGGCAGGCGCGTG
LC874460 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACT - - - - -CAGGTAGGGCGGCAGGCGCGTG
LC874460 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACT - - - - -CAGGTAGGGCGGCAGGCGCGTG
LC874460 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACT - - - - -CAGGTAGGGCGGCAGGCGCGTG
LC874461 TTCAGGAGGCACCACAGGCGGGAGGCGGC - - - - -AGGGCGGCAGGCGCGTG
LC874462 TTCAGGAGGCACCACAGGCGGGAGGCGG - - - - -CTCAGGTAGGGCGGCAGGCGCGTG
LC874463 TTCAGGAGGCACCACAGGCGGGA - - - - -GGCGGGCGCGTG
LC874463 TTCAGGAGGCACCACAGGCGGGA - - - - -GGCGGGCGCGTG
LC874463 TTCAGGAGGCACCACAGGCGGGA - - - - -GGCGGGCGCGTG
LC874464 TTCAGGAGGCACCACAGGC - - - - -GGGCGGCAGGCGCGTG
LC874465 TTCAGGAGGCACCACA - - - - -CGCGTG
LC874466 TTCAGGAGGCACCACAGGCGGGA - - - - -GGCGGTGCTGAGCTG
LC874467 TTCAGGAGGCACCACAGGCGGGAGGCGGCACAAGGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874528 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874528 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874528 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874528 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG
LC874528 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCAGGCGCGTG

(b) Clones obtained from MT-1 cells after 6 days of Cas9/sgRNA 171 transfection

sgRNA171 sequence: GACTCAACCGGCGTGGATGGCGG

[illegible]

(c) Clones obtained from TL-Om1 cells 2 days after Cas9/sgRNA 171 transfection

sgRNA171 sequence: GACTCAACCGGCGTGGATGCGCG

LC874504 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAAC- - - - -GCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874505 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCG- CCTCAGGTAGGGCGGCGGGCGCGTG
LC874506 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGCGGATGCGCG- CCTCAGGTAGGGCGGCGGGCGCGTG
LC874506 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGCGGATGCGCG- CCTCAGGTAGGGCGGCGGGCGCGTG
LC874506 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGCGGATGCGCG- CCTCAGGTAGGGCGGCGGGCGCGTG
LC874507 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874507 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874508 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874509 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874510 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874507 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874511 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874512 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAA- - - - -CGT- - -ATGCGCGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874513 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGC - -CTCAACCGGCGTGG- - -GCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874514 TTCAGGAGGCACCACAGGCGGGAGGCGGCAG- - - - -TGG- TGCGCGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874515 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGC - - - - -GCGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874516 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874517 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874518 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874518 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874511 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874530 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874530 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874530 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874530 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874530 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874530 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874530 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGCGCGGCCTCAGGTAGGGCGGCGGGCGCGTG

(d) Clones obtained from TL-Om1 cells 6 days after Cas9/sgRNA 171 transfection

sgRNA171 sequence: GACTCAACCGGCGTGGATGGCGG

[illegible]

(e) Clones obtained from ST-1 cells 2 days after Cas9/sgRNA 171 transfection

sgRNA171 sequence: GACTCAACCGGCGTGGATGGCGG

LC874473 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGAAATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874473 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGAAATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874474 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAAC-----GGCGGCGGGCGCGTG
LC874475 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGG-TGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874476 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACC-----TGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874477 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAAC-----ATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874477 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAAC-----ATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874478 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACT---CC-AC---GCTGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874479 TTCAGGAGGCACCACAGGCGG-----GATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874480 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGAT-----GGCGGCGCGTG
LC874481 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAA-----GTAGGGCGGCGGGCGCGTG
LC874481 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAA-----GTAGGGCGGCGGGCGCGTG
LC874482 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGAC-----CTCAGGTAGGGCGGCGGGCGCGTG
LC874483 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874484 TTCAGGAGGCACCACAGGCGGGAGGCCGCGAGAGGAGGAGTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874485 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTG---TGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874486 TTCAGGAGGCACCACAGGCGGGAGGCG-----TGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874487 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGC-----GGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874488 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCA-----GCGGGCGCGTG
LC874487 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGC-----GGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874480 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGAT-----GGCGGCGCGTG
LC874529 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874529 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874529 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874529 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874529 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874529 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG
LC874529 TTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTG

[illegible]

Genomic DNA from Cas9/sgrNA171-transfected MT-1, TL-OM1, and ST-1 cells were amplified by PCR, subcloned, and sequenced. Each cell was sequenced 2 and 6 days after transfection. **The accession numbers of the subclone sequences are shown at the left end of each line.** The double-underlined areas indicate the start of translation. The PAM site (NGG) is shown in gray. Deletions, substitutions, and insertions are indicated by the dotted lines, underlines, and dashed lines, respectively. Nucleotide sequence results showed that the LTR containing *HBZ* mutations remained in the cells up to 6 days after transfection. The sequence of the clones obtained from MT-1 cells **(a)** 2 and **(b)** 6 days after Cas9/sgrNA 171 transfection; TL-Oml cells **(c)** 2 and **(d)** 6 days after Cas9/sgrNA 171 transfection; and ST-1 cells **(e)** 2 and **(f)** 6 days after Cas9/sgrNA 171 transfection.

(a) Off-target A (MT-1 Chromosome 8 q21.13)

sgRNA 171 sequence: GACTCAACCGGCGTGGATGCGGG

Clone.1	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.2	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.3	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.4	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.5	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.6	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.7	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.8	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.9	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.10	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.11	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.12	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.13	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.14	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.15	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.16	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG

(b) Off-target B (MT-1 Chromosome 16 p12.3)

sgRNA 171 sequence : GACTCAACCGGCGTGGATGCGGG

Clone.1	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.2	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.3	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.4	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.5	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.6	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.7	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.8	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.9	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.10	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.11	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.12	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.13	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.14	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.15	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.16	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC

(c) Off-target C (MT-1 Chromosome 19 p13.3)

sgRNA 171 sequence: GACTCAACCGGCGTGGATGGCGG

Clone.1	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.2	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.3	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.4	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.5	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.6	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.7	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.8	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.9	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.10	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.11	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.12	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.13	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.14	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.15	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.16	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA

(d) Off-target D (MT-1 Chromosome 19 p13.2)

sgRNA171 sequence: GACTCAACCGGCGTGGATGGCGG

Clone.1	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.2	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.3	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.4	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.5	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.6	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.7	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.8	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.9	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.10	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.11	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.12	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.13	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.14	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.15	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.16	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT

(e) Off-target A (TL-Oml Chromosome 8 q21.13)

sgRNA 171 sequence: GACTCAACCGGCGTGGATGGCGG

Clone.1	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.2	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.3	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.4	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.5	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.6	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.7	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.8	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.9	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.10	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.11	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.12	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.13	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.14	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.15	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.16	CAGGGGAGGAGCTGGGGCAGGAAGAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG

(f) Off-target B (TL-Oml Chromosome 16 p12.3)

sgRNA 171 sequence: GACTCAACCGGCGTGGATGGCGG

Clone.1	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.2	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.3	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.4	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.5	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.6	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.7	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.8	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.9	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.10	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.11	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.12	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.13	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.14	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.15	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.16	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC

(g) Off-target C (TL-Oml Chromosome 19 p13.3)

sgRNA 171 sequence: GACTCAACCGGCGTGGATGGCGG

Clone.1	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.2	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.3	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.4	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.5	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.6	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.7	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.8	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.9	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.10	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.11	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.12	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.13	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.14	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.15	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.16	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA

(h) Off-target D (TL-Oml Chromosome 19 p13.2)

sgRNA171 sequence: GACTCAACCGGCGTGGATGGCGG

Clone.1	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.2	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.3	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.4	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.5	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.6	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.7	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.8	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.9	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.10	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.11	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.12	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.13	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.14	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.15	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.16	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT

(i) Off-target A (ST-1 Chromosome 8 q21.13)

sgRNA 171 sequence: GACTCAACCGGCGTGGATGCGGG

Clone.1	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.2	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.3	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.4	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.5	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.6	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.7	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.8	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.9	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.10	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.11	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.12	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.13	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.14	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.15	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG
Clone.16	CAGGGGAGGAGCTGGGGCAGGAAGAAAAGACCGGGGGCGGCGGCGTGGATGGGGGGCAGGG

(j) Off-target B (ST-1 Chromosome 16 p12.3)

sgRNA 171 sequence: GACTCAACCGGCGTGGATGCGGG

Clone.1	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.2	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.3	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.4	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.5	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.6	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.7	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.8	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.9	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.10	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.11	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.12	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.13	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.14	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.15	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC
Clone.16	CCGAGCCCGCCGACGGCGTGGATGGCGGCTTCCCGTTGCTTAAGGTGGCCGTCCTGCTCC

(k) Off-target C (ST-1 Chromosome 19 p13.3)

	sgRNA 171 sequence: GACTCAACCGGCGTGGAT <u>GGCGG</u>
Clone.1	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.2	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.3	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.4	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.5	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.6	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.7	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.8	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.9	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.10	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.11	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.12	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.13	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.14	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.15	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA
Clone.16	CCTCAGTTTCCCCCGGCCACCCCGTGGTCCCTCTAGGCCTCGGCGTGGATGGGGGATGGA

(l) Off-target D (ST-1 Chromosome 19 p13.2)

	sgRNA171 sequence: GACTCAACCGGCGTGGAT <u>GGCGG</u>
Clone.1	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.2	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.3	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.4	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.5	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.6	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.7	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.8	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.9	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.10	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.11	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.12	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.13	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.14	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.15	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT
Clone.16	GCTCCACGGCGGCGTGGATGGCGGCGGGAACGCGCGGTGCGCCTCCGACACGCGCTCTT

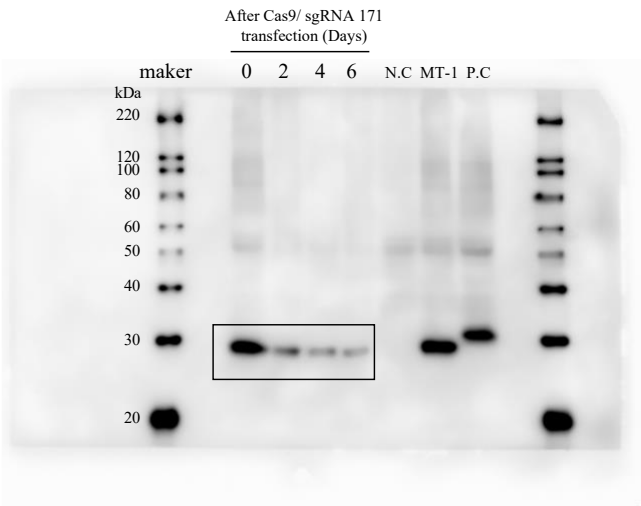
Figure S3. Nucleotide sequence analysis of off-target regions in the CRISPR/Cas9-transfected cell line

Human genome sequences homologous to the sgRNA 171 sequence were identified using BLATsearch (<https://genome.ucsc.edu/cgi-bin/hgBlat>). The off-target effects were analyzed for the four regions with partial homology among them. After culturing Cas9/sgRNA-transfected cells for 48 h, the cell pellets were collected by centrifugation at 1,500 rpm for 5 minutes. Moreover, 50 μ L of cell lysis buffer containing 4% protein degrader was added to the cell pellets and heated at 68°C for 15 min and then at 95°C for 10 min. This solution was used as a template for PCR. The PCR primers for the chromosome 8 q21.13 target region were as follows: forward primer—8F: 5'-CTCCATAGCAACAGCGTGGG-3' and reverse primer—8R: 5'-CCCTCATCCCCGCTCCTAC-3'. The PCR primers for the chromosome 16 q12.3 target region were as follows: forward primer—16F: 5'-TGTCGGTGCCTACACCCCTC-3' and reverse primer—16R: 5'-CTCACGTGCGCCTTGCTGTG-3'. The PCR primers for the chromosome 19 q13.3 target region were as follows: forward primer—19F-1: 5'-GCAGCCGTCTGAACATGTAC-3' and reverse primer—19R-1: 5'-CCAGTGTCAATTACCACCTGC-3'. The PCR primers for the chromosome 19 q13.2 target region were as follows: forward primer—19F-2: 5'-AGTCGCGGTCTGAAGATGAGG-3' and reverse primer—19R-2: 5'-CTTCTCGCTGGGGCCTGACC-3'. PCR was performed using 2 μ L of cell lysates prepared as described above under the following conditions: one cycle of 95°C for 10 min, 40 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 20 s, and extension at 72°C for 30 s. The resultant PCR products were subjected to a sequencing assay using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, USA) and ABI Prism 3500 Genetic Analyzer (Applied Biosystems Thermo Fisher, California, United States), according to the manufacturer's instructions. (a)-(l) The double-underlined area indicates the start of translation, and the PAM site (NGG) is shown in gray. The accession numbers of the nucleotide sequences amplified to investigate off-target are as follows: Off-target A (MT-1), LC874625; Off-target A (TL-Om1), LC874633; Off-target A (ST-1), LC874629; Off-target B (MT-1), LC874626; Off-target B (TL-Om1), LC874634; Off-target B (ST-1), LC874630; Off-target C (MT-1), LC874627; Off-target C (TL-Om1), LC874635; Off-target C (ST-1), LC874631; Off-target D (MT-1), LC874628; Off-target D (TL-Om1), LC874636; Off-target D (ST-1), LC874632. The nucleotide sequence number of the chromosome searched for at BLAT

Search Genome correspond to the accession numbers as follow. Off-target A, chr8:80578536-80578653; Off-target B, chr16:19114547-19114666; Off-target C, chr19:3456983-3457102; Off-target D, chr19:7501011-7501130. The figures show the regions of homology to the sgRNA of the registered sequence.

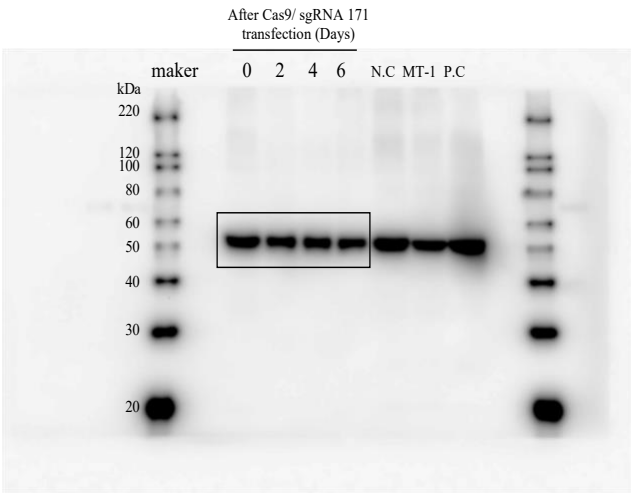
(a)

MT-1 HBZ



(b)

MT-1 Tubulin (reprobe)



(c)

MT-1 Tax

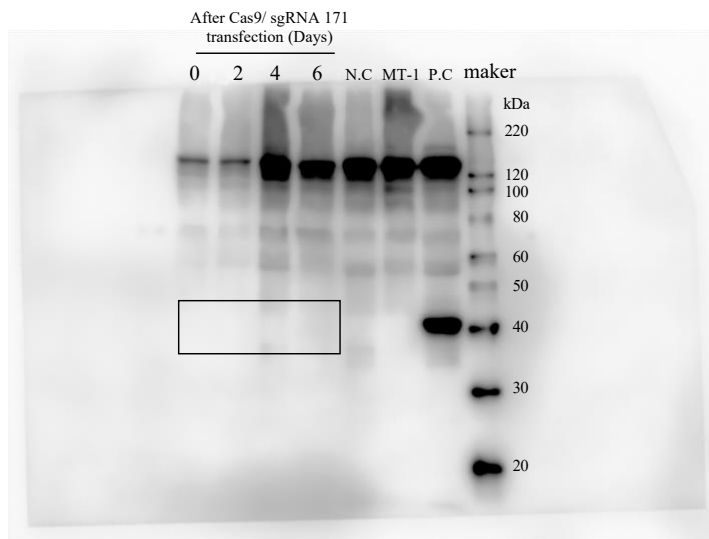
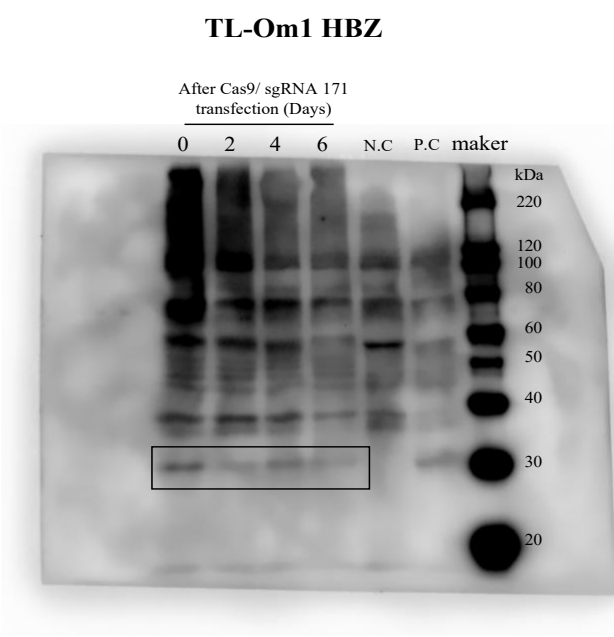


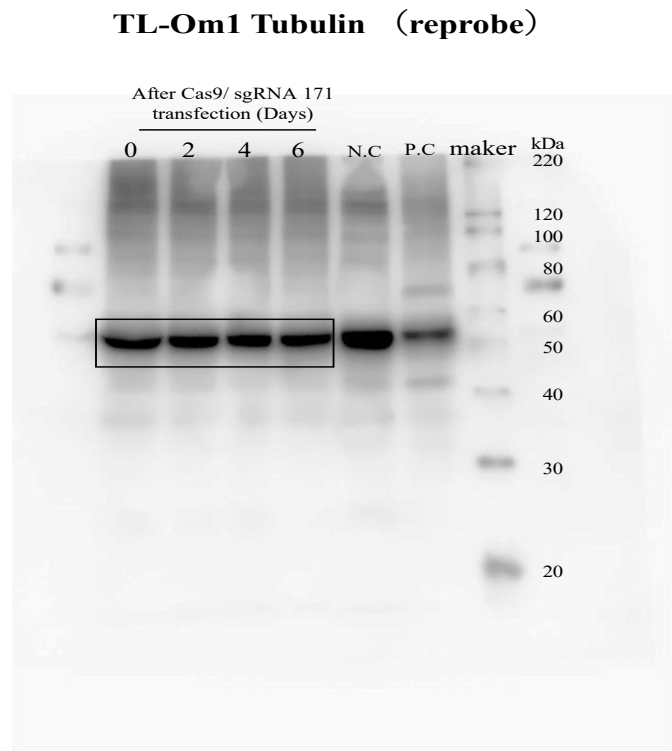
Figure S4. Original Western blot image of the MT-1 cells.

Western blot images show the expression of (a) HBZ, (b) tubulin, and (c) Tax in MT-1 cells. The entire membrane is shown with the edges visible. The insets depict the results in Figure 4a, where tubulin was detected by reprobing after HBZ detection.

(a)



(b)



(c)

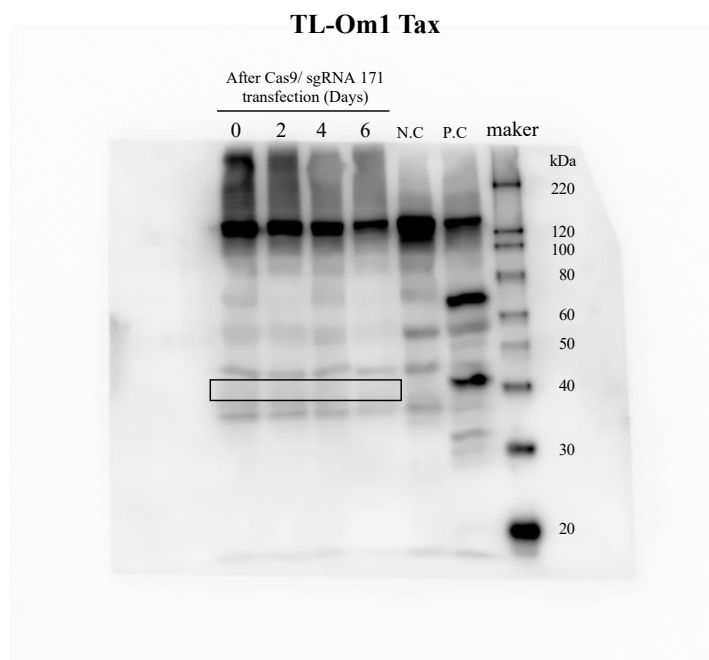
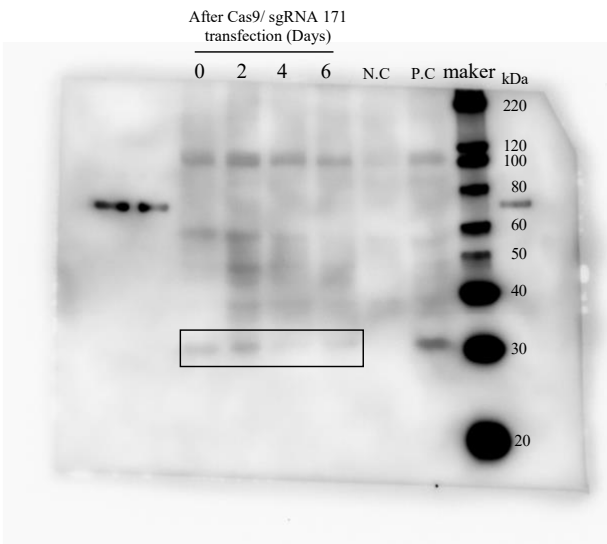


Figure S5. Original Western blot image of TL-Om1 cells.

Western blot images show the expression of (a) HBZ, (b) tubulin, and (c) Tax in TL-Om1 cells. The entire membrane is shown with the edges visible. The insets depict the results in Figure 4a, where tubulin was detected by reprobing after HBZ detection.

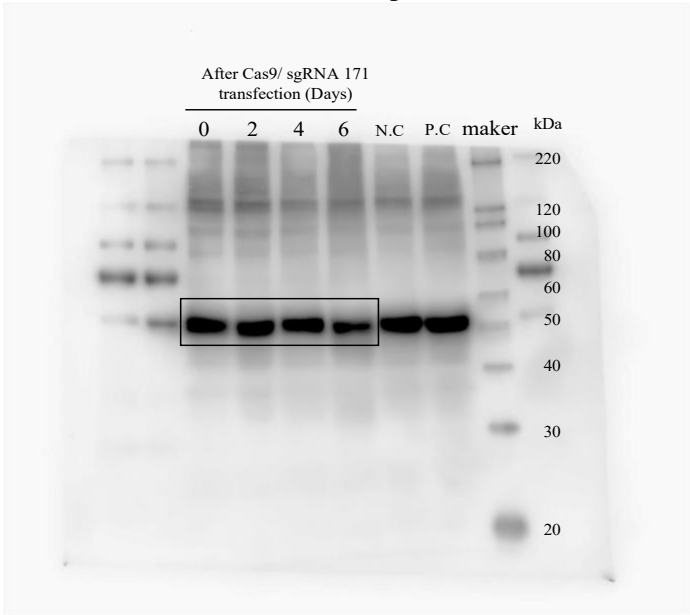
(a)

ST-1 HBZ



(b)

ST-1 Tubulin (reprobe)



(c)

ST-1 Tax

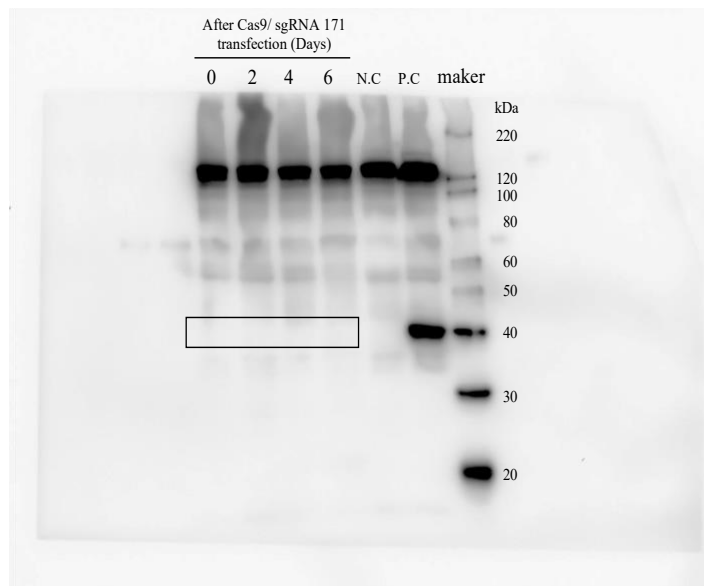


Figure S6. Original Western blot image of ST-1 cells.

Western blot images show the expression of (a) HBZ, (b) tubulin, and (c) Tax in ST-1 cells. The entire membrane is shown with the edges visible. The insets depict the results in Figure 4a, where tubulin was detected by reprobing after HBZ detection.