Supplemental information

Targeted, programmable and precise tandem duplication in mammalian genome

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FANCF TD 50-bp

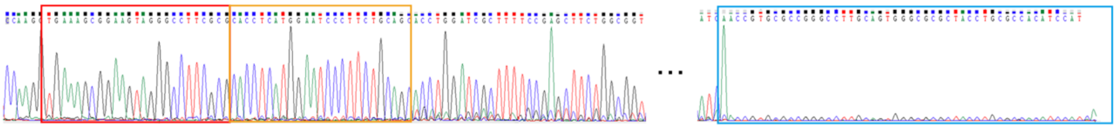
Wild-type sequence:

PAM1 nick nick PAM2

CCAAGGTGAAAGCGGAAGTAGGGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCACC**TGG**ATCGCTTTTC

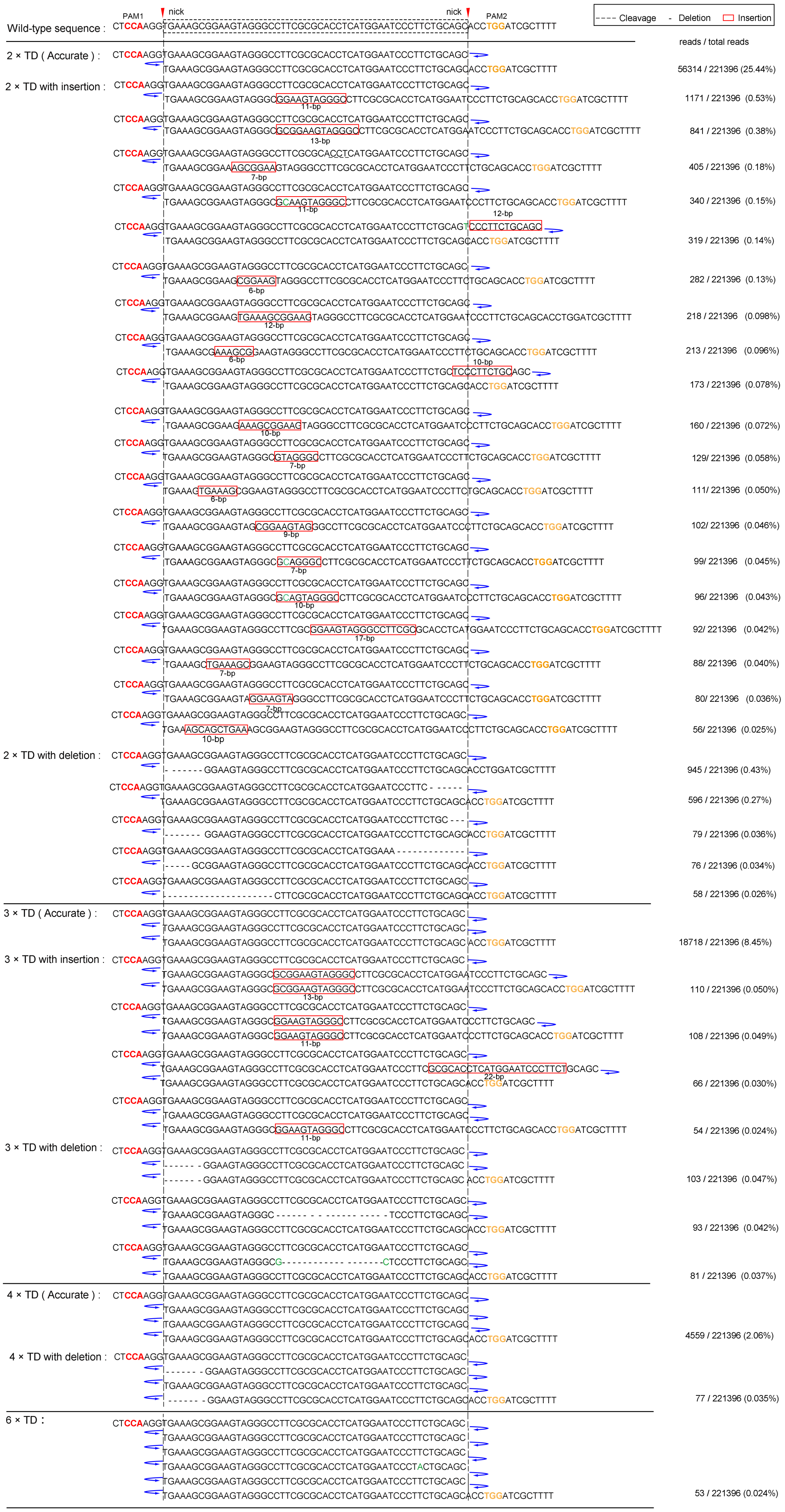
Reference sequence:

CCAAGGTGAAAGCGGAAGTAGGGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCTGAAAGCGGAAGTAGGGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCACC**TGG**ATCGCTTTTC



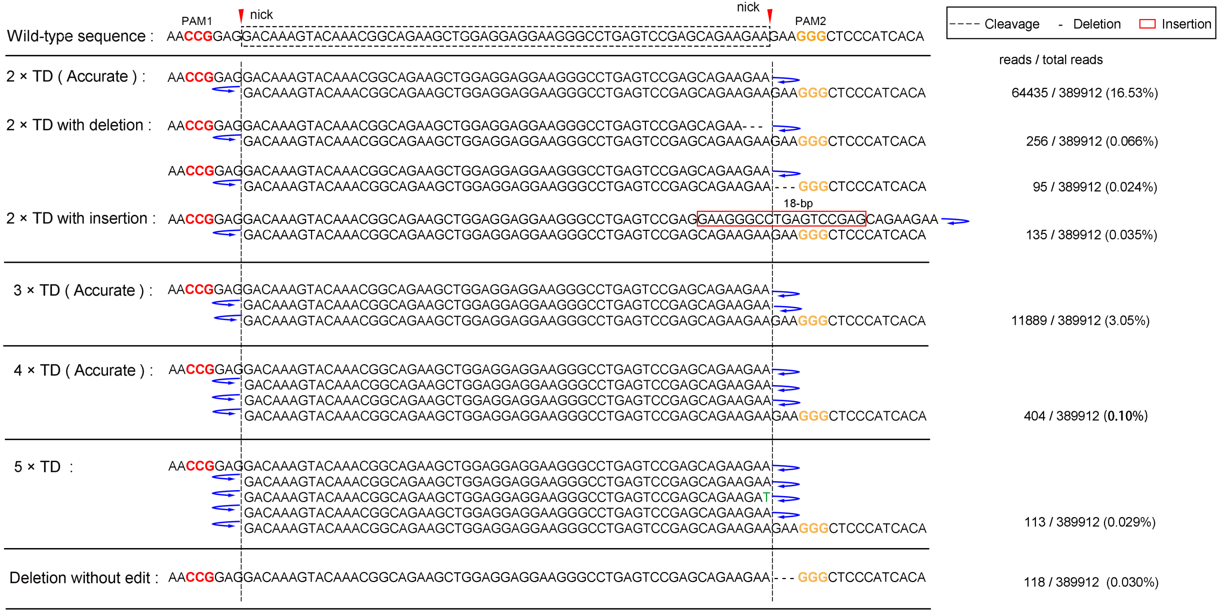
Supplemental Fig. S1 Representative Sanger sequencing results of TD-PE mediated 50-bp tandem duplication at *FANCF* site. PCR amplicons containing targeted tandem duplication was purified and directly subjected to Sanger sequencing. Wild-type sequence and TD reference sequence were shown on top of the sequencing chromatogram. PAM1 and PAM2 of the two pegRNAs were shown in red and yellow respectively. Red arrows indicated the positions of the nicks. The sequences in-between two nicks are the duplicated sequences, with the upstream end shown in red and the downstream end shown in yellow. The peaks in the sequencing chromatogram corresponding to each end were boxed in red and yellow respectively. The blue box marked the fragment that is located at the end of the double peaks. The length of the fragment is the same as that of the duplicated region, and the sequence corresponds to the 3' end of the amplicons that contain TDs.

FANCF TD 50-bp



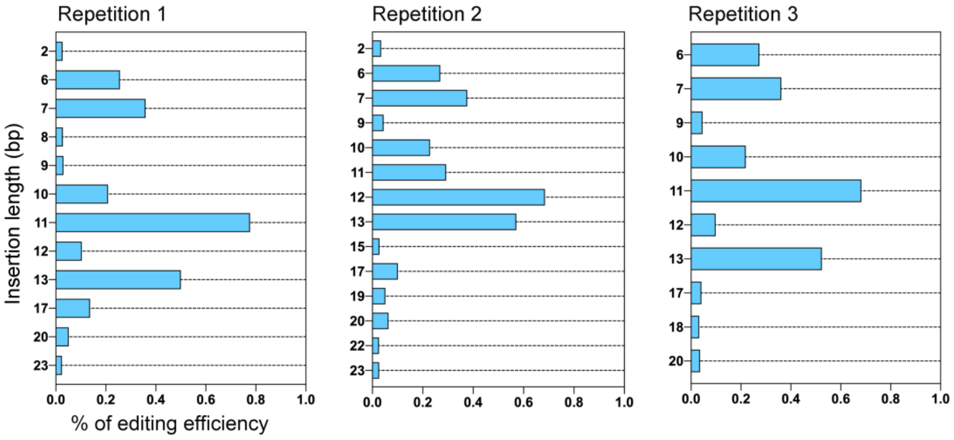
Supplemental Fig. S2 HTS sequences showing the editing result of TD-PE at the *FANCF* site. The fragments containing tandem duplications were amplified by HTS primers and subjected for HTS analysis. Detailed analysis of the undesired TDs revealed that the majority of deletions and insertions occurred at the boundaries of the duplications. The blue curved arrows pointed to the next copy of the tandem duplication. Deletion, insertion and base substitution were indicated by black dash, red box and green letters respectively. The insertion sequence of the red box was consistent with its 5' upstream sequence. Note that to provide an overview of the TDs, we copied the sequences of 2×TD from Fig. 2E to this section.

EMX1 TD 57-bp

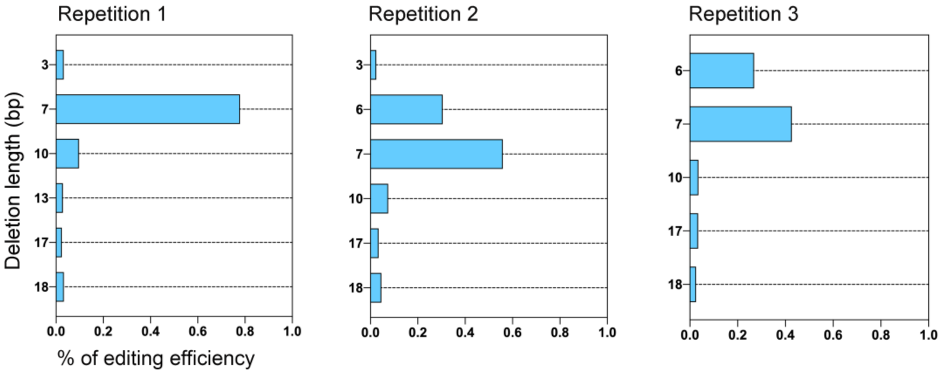

PAM1
nick
nick
PAM2
Wild-type sequence : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACA
- - - - Cleavage
- Deletion
Insertion
reads / total reads
2 × TD (Accurate) : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACA
64435 / 389912 (16.53%)
2 × TD with deletion : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAA- - -
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACA
256 / 389912 (0.066%)
AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA- - - GGGCTCCCATCACA
95 / 389912 (0.024%)
18-bp
2 × TD with insertion : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACA
135 / 389912 (0.035%)
3 × TD (Accurate) : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACA
11889 / 389912 (3.05%)
4 × TD (Accurate) : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACA
404 / 389912 (0.10%)
5 × TD : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAT
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA
GACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACA
113 / 389912 (0.029%)
Deletion without edit : AACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAA- - - GGGCTCCCATCACA
118 / 389912 (0.030%)

Supplemental Fig. S3 HTS sequences showing the editing result of TD-PE at the *EMX1* site. The fragments containing tandem duplication were amplified by HTS primers, purified and then subjected for HTS. The blue curved arrows pointed to the next copy of the tandem duplication. Deletion, insertion and base substitution were indicated by black dash, red box or green letters respectively. The insertion sequence of the red box was consistent with its 5' upstream sequence.

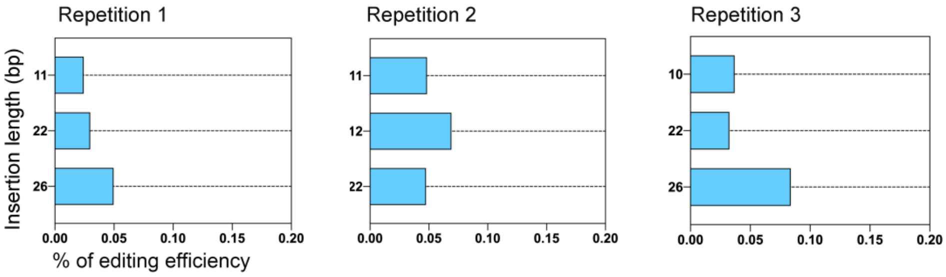
FANCF TD 50-bp 2×TD insertion



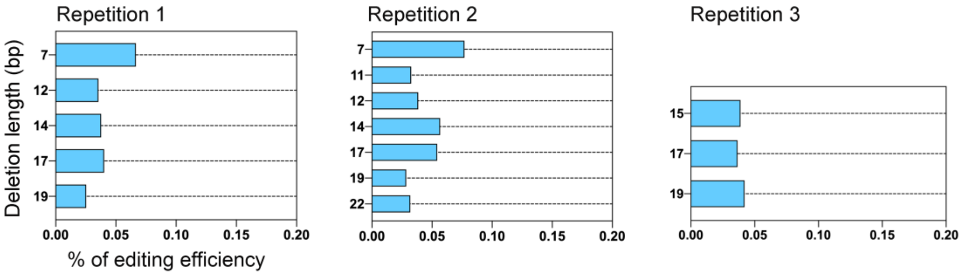
FANCF TD 50-bp 2×TD deletion



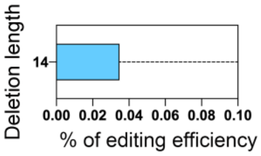
FANCF TD 50-bp 3×TD insertion



FANCF TD 50-bp 3×TD deletion

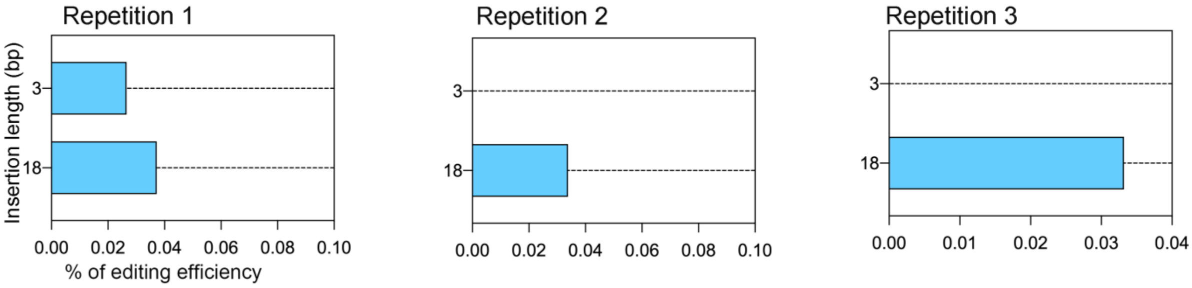


FANCF TD 50-bp 4×TD deletion

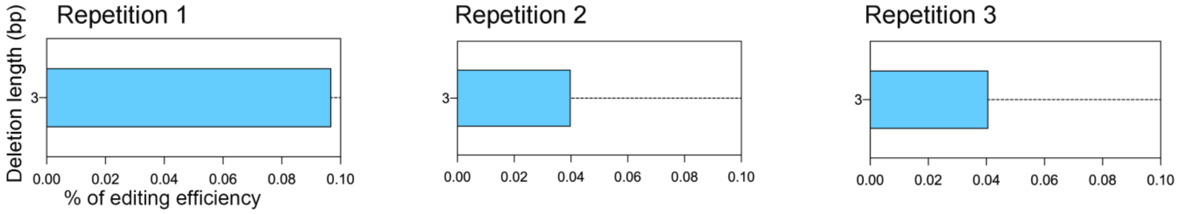


Supplemental Fig. S4 Distribution of the insertion or deletion lengths at *FANCF* site. HTS analysis of the frequencies of undesired edits induced by TD-PE at *FANCF* site (TD 50-bp). The experiment was repeated three times, and the indel data from each repetition were presented separately. Note that the deletion in 4×TD was detectable in only one out of three repetitions.

EMX1 TD 57-bp 2×TD insertion



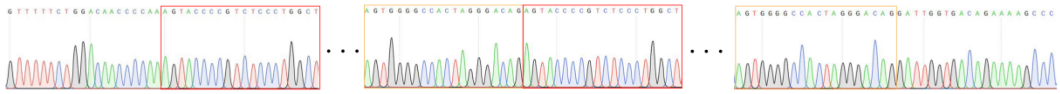
EMX1 TD 57-bp 2×TD deletion



Supplemental Fig. S5 Distribution of the insertion or deletion lengths at *EMX1* site. HTS analysis of the frequencies of undesired edits induced by TD-PE at *EMXI* site (TD 57-bp). The experiment was repeated three times, and the indel data from each repetition were presented separately.

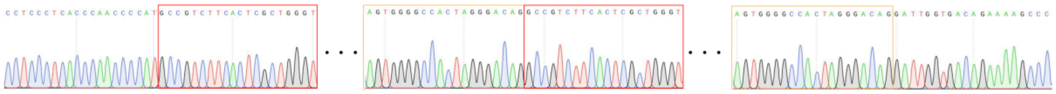
AAVS1 TD 376-bp 2×TD

TD reference sequence: GTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



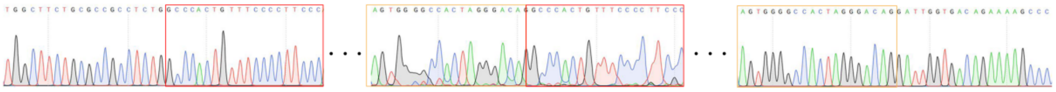
AAVS1 TD 532-bp 2×TD

TD reference sequence: CCTCCCTCACCCAACCCCATGCCGTCTTCACTCGCTGGGT···AGTGGGGCCACTAGGGACAGGCCGTCTTCACTCGCTGGGT···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



AAVS1 TD 846-bp 2×TD

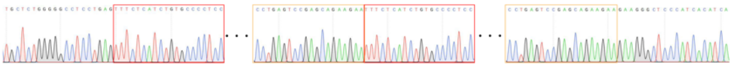
TD reference sequence: TGGCTTCTGCGCCGCCTCTGGCCCACTGTTTCCCCTTCCC···AGTGGGGCCACTAGGGACAGGCCCACTGTTTCCCCTTCCC···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



Supplemental Fig. S6 Representative Sanger sequencing results of large fragment TDs at the *AAVS1* site (related to Fig. 3A). Representative Sanger sequencing results of TD containing amplicons derived from 3 targeted TDs at the *AAVS1* site. The amplicons containing targeted TDs were gel-purified and subjected directly to Sanger sequencing. TD reference sequence was shown on top of each sequencing chromatogram, with sequences corresponding to each end of the TD boxed in red and yellow respectively.

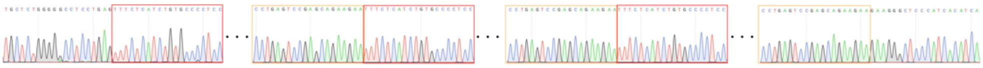
EMX1 TD 116-bp 2×TD

TD reference sequence:TGCTCTGGGGGCCTCCTGAGTTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCA



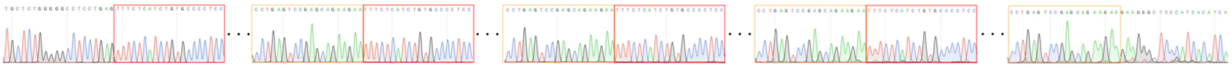
EMX1 TD 116-bp 3×TD

TD reference sequence:TGCTCTGGGGGCCTCCTGAGTTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCA



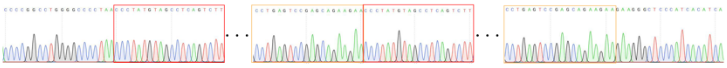
EMX1 TD 116-bp 4×TD

TD reference sequence:TGCTCTGGGGGCCTCCTGAGTTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCC···CCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCA



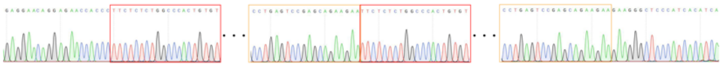
EMX1 TD 201-bp 2×TD

TD reference sequence:CCCCGGCCTGGGGCCCCTAACCCTATGTAGCCTCAGTCTT···CCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTT···CCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCA



EMX1 TD 307-bp 2×TD

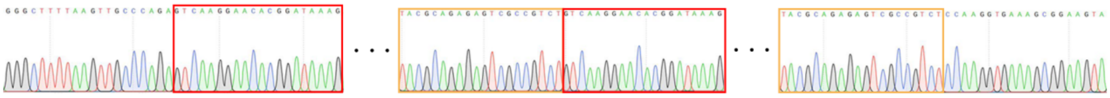
TD reference sequence:GAGGAACAGGAAAACCACCCTTCTCTCTGGCCCACTGT···CCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGT···CCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCA



Supplemental Fig. S7 Representative Sanger sequencing results of large fragment TDs at the *EMX1* site (related to Fig. 3A). Representative Sanger sequencing results of TD containing amplicons derived from 3 targeted TDs at *EMX1* site. The amplicons containing targeted TDs were gel-purified and subjected to Sanger sequencing. TD reference sequence was shown on top of each sequencing chromatogram, with sequences corresponding to each end of the TD boxed in red and yellow respectively. *EMX1* TD 116-bp 3×TD and 4×TD were the sequencing results of the bands marked red stars (*) at the *EMX1* site in Fig. 3A.

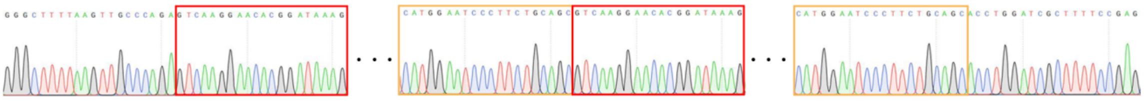
FANCF TD 115-bp 2×TD

TD reference sequence: GGGCTTTTAAGTTGCCCAGAGTCAAGGAACACGGATAAAG···TACGCAGAGAGTCGCCGTCTGTCAAGGAACACGGATAAAG···TACGCAGAGAGTCGCCGTCTCCAAGGTGAAAGCGGAAGTA



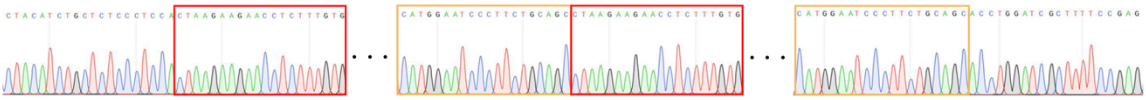
FANCF TD 171-bp 2×TD

TD reference sequence: GGGCTTTTAAGTTGCCCAGAGTCAAGGAACACGGATAAAG···CATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAG···CATGGAATCCCTTCTGCAGCACCTGGATCGCTTTTCCGAG



FANCF TD 231-bp 2×TD

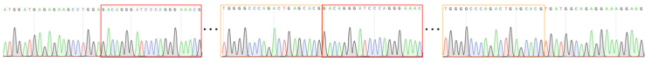
TD reference sequence: CTACATCTGCTCTCCCTCCACTAAGAAGAACCTCTTTGTG···CATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTG···CATGGAATCCCTTCTGCAGCACCTGGATCGCTTTTCCGAG



Supplemental Fig. S8 Representative Sanger sequencing results of large fragment TDs at the *FANCF* site (related to Fig. 3A). Representative Sanger sequencing results of TD containing amplicons derived from 3 targeted TDs at *FANCF* site. The amplicons containing targeted TDs were gel-purified and subjected to Sanger sequencing. TD reference sequence was shown on top of each sequencing chromatogram, with sequences corresponding to each end of the TD boxed in red and yellow.

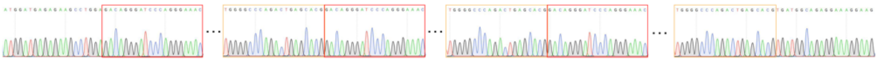
HEK3 TD 136-bp 2×TD

TD reference sequence:ATGGATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



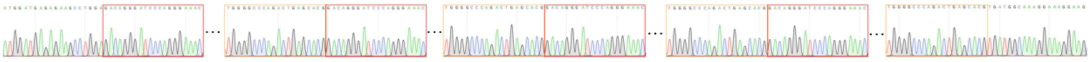
HEK3 TD 136-bp 3×TD

TD reference sequence:ATGGATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



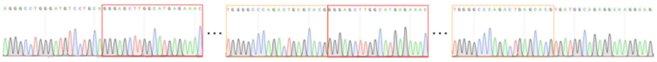
HEK3 TD 136-bp 4×TD

TD reference sequence:ATGGATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



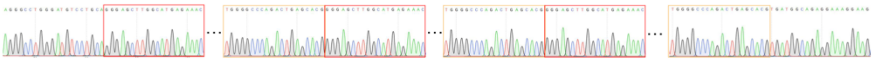
HEK3 TD 222-bp 2×TD

TD reference sequence:AGGGCCTGGGATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



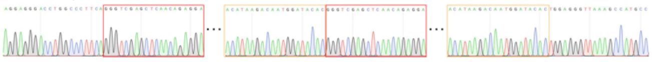
HEK3 TD 222-bp 3×TD

TD reference sequence:AGGGCCTGGGATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



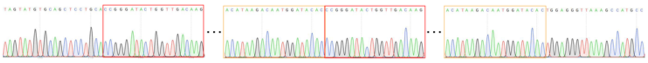
HEK3 TD 397-bp 2×TD

TD reference sequence:AGGAGGGACCTGGCCCTTCAGGGTCGAGCTCAACAGAGGA···ACATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGA···ACATAAGACAATGGATACACTGGAGGGTTAAAGCCATGCC

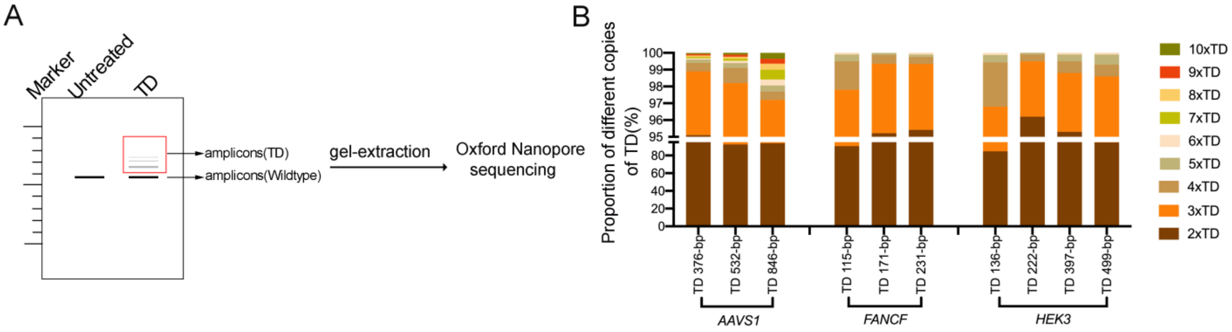


HEK3 TD 499-bp 2×TD

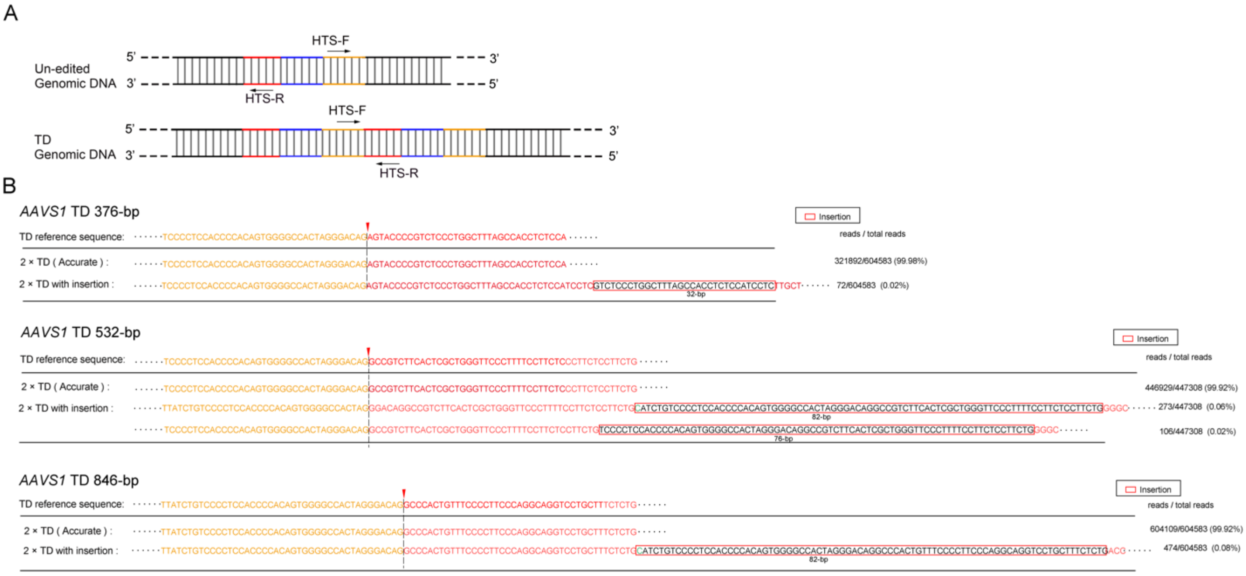
TD reference sequence:TAGTATGTGCAGCTCCTGCACCGGGATACTGGTTGACAAG···ACATAAGACAATGGATACACCCGGGATACTGGTTGACAAG···ACATAAGACAATGGATACACTGGAGGGTTAAAGCCATGCC



Supplemental Fig. S9 Representative Sanger sequencing results of large fragment TDs at the *HEK3* site (related to Fig. 3A). Representative Sanger sequencing results of TD containing amplicons derived from 4 targeted TDs at *HEK3* site. The amplicons containing targeted TDs were gel-purified and subjected to Sanger sequencing. TD reference sequence was shown on top of each sequencing chromatogram, with sequences corresponding to each end of the TD boxed in red and yellow respectively. *HEK3* TD 136-bp 3×TD and 4×TD and *HEK3* TD 222-bp 3×TD were the sequencing results of the bands marked by red stars (*) at the *HEK3* site in Fig. 3A.

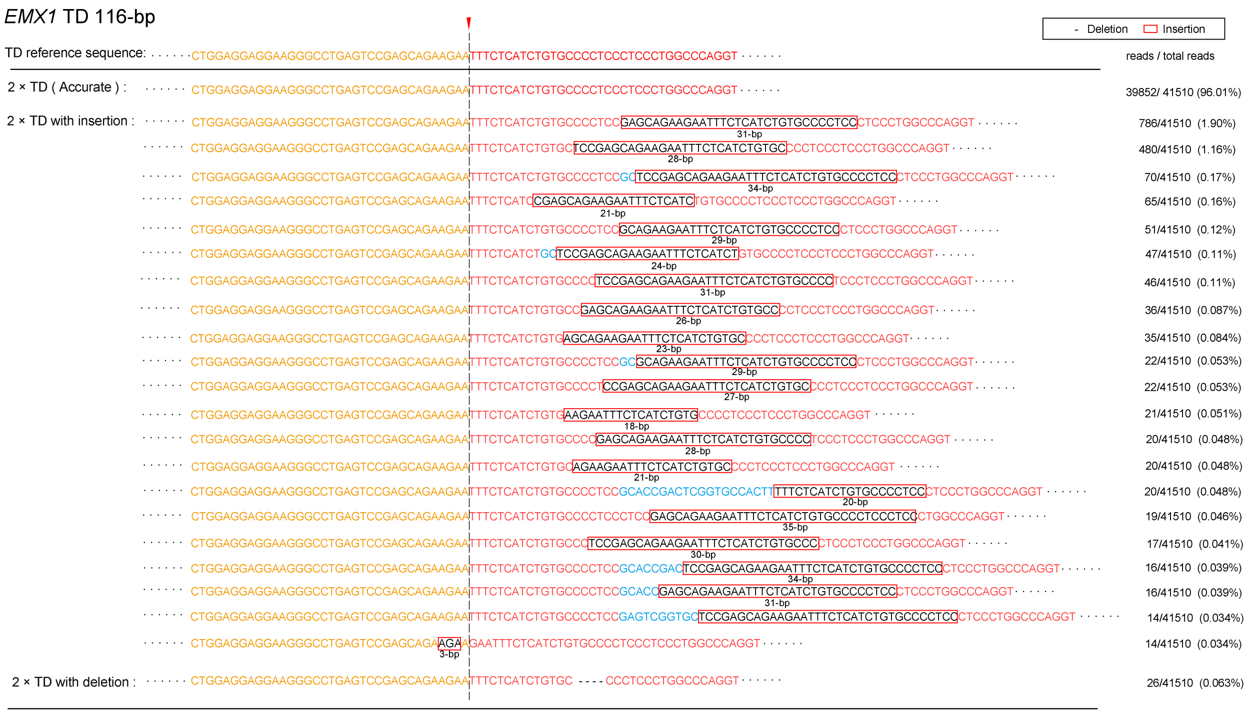

A
Marker
Untreated
TD
amplicons(TD)
amplicons(Wildtype)
gel-extraction
Oxford Nanopore sequencing
B
Proportion of different copies of TD(%)
100
99
98
97
96
95
80
60
40
20
0
TD 376-bp
TD 532-bp
TD 846-bp
TD 115-bp
TD 171-bp
TD 231-bp
TD 136-bp
TD 222-bp
TD 397-bp
TD 499-bp
AAVS1
FANCF
HEK3
10xTD
9xTD
8xTD
7xTD
6xTD
5xTD
4xTD
3xTD
2xTD

Supplemental Fig. S10 Proportions of different copies of TDs were analyzed by Oxford Nanopore sequencing. (A) Diagram showing the procedure to prepare Oxford Nanopore sequencing Samples. The amplicons with size equivalent to or larger than 2×TD were collected and gel extracted for Nanopore sequencing. (B) The proportions of each type of TDs were analyzed.


A
HTS-F
5'
3'
Un-edited
Genomic DNA
3'
5'
HTS-R
HTS-F
5'
3'
TD
Genomic DNA
3'
5'
HTS-R
B
AAVS1 TD 376-bp
Insertion
reads / total reads
TD reference sequence: ······TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCA······
2 × TD (Accurate) : ······TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCA······ 321892/604583 (99.98%)
2 × TD with insertion : ······TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGAGTACCCCGTCTCCCTGGCTTTAGCCACCTCTCCATCCTCGTCTCCCTGGCTTTAGCCACCTCTCCATCCTCTTGCT······ 72/604583 (0.02%)
32-bp
AAVS1 TD 532-bp
Insertion
reads / total reads
TD reference sequence: ······TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCCTTCTCCTTCTG······
2 × TD (Accurate) : ······TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCCTTCTCCTTCTG······ 446929/447308 (99.92%)
2 × TD with insertion : ······TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGCATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGC······ 273/447308 (0.06%)
82-bp
······TCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCGTCTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGC······ 106/447308 (0.02%)
76-bp
AAVS1 TD 846-bp
Insertion
reads / total reads
TD reference sequence: ······TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTG······
2 × TD (Accurate) : ······TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTG······ 604109/604583 (99.92%)
2 × TD with insertion : ······TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGCATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCCACTGTTTCCCCTTCCCAGGCAGGTCCTGCTTTCTCTGACG······ 474/604583 (0.08%)
82-bp

Supplemental Fig.S11 Undesired edits of the *AAVS1* site were determined by HTS. (A) PCR strategy for amplifying the junction of TDs. The primers were designed to specifically amplify the boundary of targeted TDs. The HTS primers were listed in Supplemental Table S5.

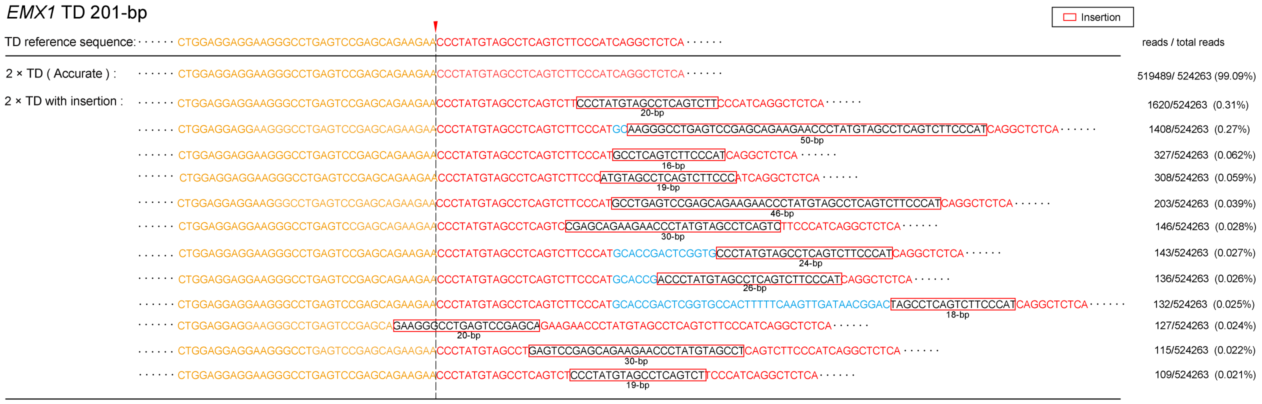
(B) Undesired edits in indicated TDs of the *AAVS1* site. Sequence corresponding to accurate TD allele was shown on top of each analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Insertion and base substitution were indicated by red box and green letters respectively. Note that the inserted sequence was same as its 5' upstream sequence. Sequences with a ratio over 0.02% were shown.



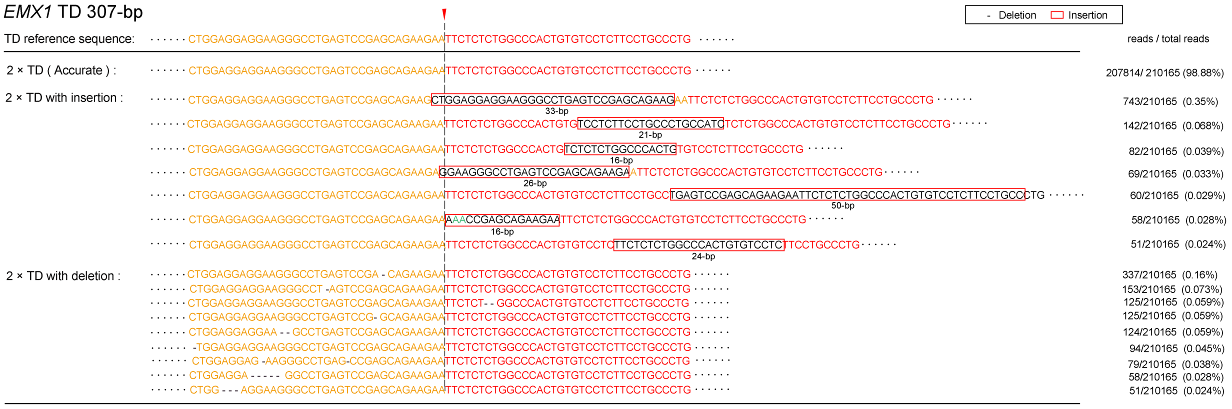
EMX1 TD 116-bp

			reads / total reads
TD reference sequence:	······CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······		
2 × TD (Accurate) :	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······		39852/ 41510 (96.01%)
2 × TD with insertion :	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	31-bp	786/41510 (1.90%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	28-bp	480/41510 (1.16%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	34-bp	70/41510 (0.17%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	21-bp	65/41510 (0.16%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	29-bp	51/41510 (0.12%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	24-bp	47/41510 (0.11%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	31-bp	46/41510 (0.11%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	26-bp	36/41510 (0.087%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	23-bp	35/41510 (0.084%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGCGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	29-bp	22/41510 (0.053%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	27-bp	22/41510 (0.053%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	18-bp	21/41510 (0.051%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	28-bp	20/41510 (0.048%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	21-bp	20/41510 (0.048%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGCACCGACTCGGTGCCACTTTTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	20-bp	20/41510 (0.048%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	35-bp	19/41510 (0.046%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	30-bp	17/41510 (0.041%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGCACCGACTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	34-bp	16/41510 (0.039%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGCACCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	31-bp	16/41510 (0.039%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCGAGTCGGTGCTCCGAGCAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	31-bp	14/41510 (0.034%)
	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAATTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGT ······	3-bp	14/41510 (0.034%)
2 × TD with deletion :	······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTGC ---- CCCTCCCTGGCCCAGGT ······		26/41510 (0.063%)

Supplemental Fig. S12 Undesired edits of *EMX1* TD 116-bp were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion and insertion were indicated by black dash and red box. Note that the inserted sequence was the same as its 5' upstream sequence. The inserted sequences that were likely derived from sgRNA scaffold were highlighted in blue. Sequences with a ratio over 0.02% were shown.


EMX1 TD 201-bp
Insertion
TD reference sequence: · · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · ·
reads / total reads
2 × TD (Accurate) : · · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 519489/ 524263 (99.09%)
2 × TD with insertion : · · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 20-bp 1620/524263 (0.31%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATGCAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 50-bp 1408/524263 (0.27%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 16-bp 327/524263 (0.062%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 19-bp 308/524263 (0.059%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 46-bp 203/524263 (0.039%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 30-bp 146/524263 (0.028%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATGCACCGACTCGGTGCCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 24-bp 143/524263 (0.027%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATGCACCGACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 26-bp 136/524263 (0.026%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 18-bp 132/524263 (0.025%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 20-bp 127/524263 (0.024%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 30-bp 115/524263 (0.022%)
· · · · · · CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCTCAGTCTCCCTATGTAGCCTCAGTCTTCCCATCAGGCTCTCA · · · · · · 19-bp 109/524263 (0.021%))

Supplemental Fig. S13 Undesired edits of *EMX1* TD 201-bp were determined by HTS. Sequences corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Insertions was indicated by red boxes. Note that most inserted sequences were same as their 5' upstream sequences. A small group of insertions that were likely derived from sgRNA scaffold were highlighted in blue. Sequences with a ratio over 0.02% were shown.


EMX1 TD 307-bp
- Deletion
Insertion
TD reference sequence: ······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······
reads / total reads
2 × TD (Accurate) : ······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 207814/ 210165 (98.88%)
2 × TD with insertion : ······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 743/210165 (0.35%)
33-bp
······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTGCCATCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 142/210165 (0.068%)
21-bp
······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 82/210165 (0.039%)
16-bp
······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 69/210165 (0.033%)
26-bp
······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 60/210165 (0.029%)
50-bp
······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAAACCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 58/210165 (0.028%)
16-bp
······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 51/210165 (0.024%)
24-bp
2 × TD with deletion : ······ CTGGAGGAGGAAGGGCCTGAGTCCGA - CAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 337/210165 (0.16%)
······ CTGGAGGAGGAAGGGCCT -AGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 153/210165 (0.073%)
······ CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCT- - GGCCCACTGTGTCCTCTTCCTGCCCTG ······ 125/210165 (0.059%)
······ CTGGAGGAGGAAGGGCCTGAGTCCG- GCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 125/210165 (0.059%)
······ CTGGAGGAGGAA - - GCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 124/210165 (0.059%)
······ -TGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 94/210165 (0.045%)
······ CTGGAGGAG -AAGGGCCTGAG-CCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 79/210165 (0.038%)
······ CTGGAGGA - - - - - GGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 58/210165 (0.028%)
······ CTGG - - - AGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTG ······ 51/210165 (0.024%)

Supplemental Fig. S14 Undesired edits of *EMX1* TD 307-bp were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of each analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion, insertion and base substitution were indicated by black dash, red box and green letters respectively. The inserted sequence in the red box was same as with its 5' upstream sequence. The sequences with a ratio over 0.02% were shown.

FANCF TD 115-bp

Supplemental Fig. S15 Undesired edits of *FANCF* TD 115-bp were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion, insertion and base substitution were indicated by black dash, red box and green letters respectively. Note that the inserted sequence in red box was same as its 5' upstream sequence. A small group of insertions that were likely derived from sgRNA scaffold were highlighted in blue. The sequences with a ratio over 0.02% were shown.

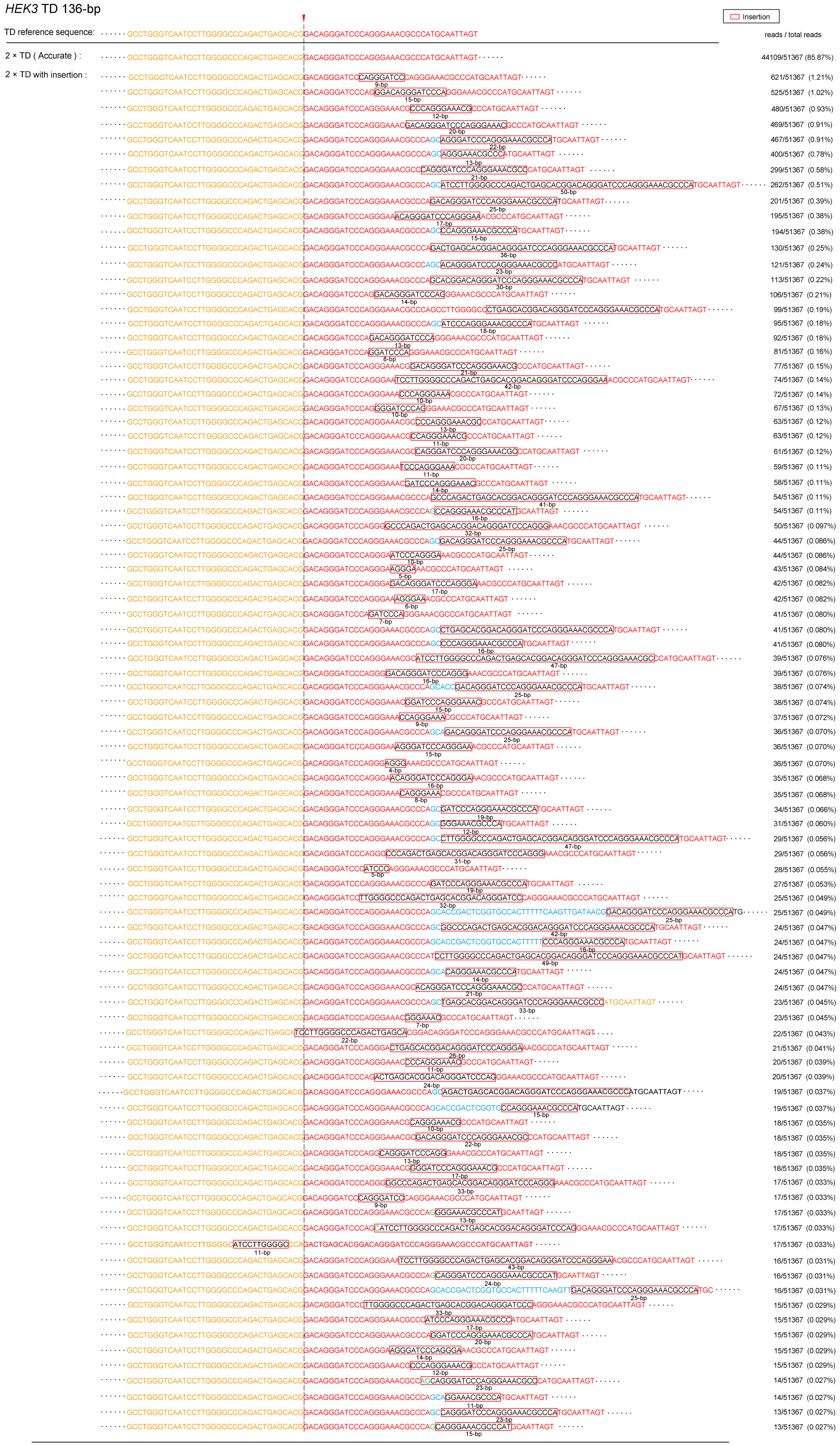

FANCF TD 171-bp
- Deletion
Insertion
TD reference sequence: ......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA......
reads / total reads
2 × TD (Accurate) : ......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 197336/ 208725 (94.54%)
2 × TD with insertion :
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 51-bp 971/208725 (0.47%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 21-bp 829/208725 (0.40%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 21-bp 399/208725 (0.19%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGCACGGATAAAGACGCTGGGAGATTGA...... 17-bp 341/208725 (0.16%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 46-bp 298/208725 (0.14%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 24-bp 233/208725 (0.11%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 48-bp 212/208725 (0.10%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 24-bp 182/208725 (0.087%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACACGGATAAAGACGCTGGGAGATTGA...... 12-bp 129/208725 (0.062%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 19-bp 128/208725 (0.061%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 30-bp 122/208725 (0.058%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 22-bp 112/208725 (0.054%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGCGGATAAAGACGCTGGGAGATTGA...... 15-bp 102/208725 (0.049%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 14-bp 94/208725 (0.045%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 22-bp 86/208725 (0.041%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 16-bp 82/208725 (0.039%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGGATAAAGACGCTGGGAGATTGA...... 10-bp 82/208725 (0.039%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 23-bp 71/208725 (0.034%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGCACACGGATAAAGACGCTGGGAGATTGA...... 19-bp 69/208725 (0.033%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCTTGAAAAAGTGGCACCGAGTCGGTGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 24-bp 67/208725 (0.032%)
......GGCCTTCGCGCACCTCATGGAATCCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 12-bp 66/208725 (0.032%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 50-bp 64/208725 (0.031%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 26-bp 63/208725 (0.030%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 24-bp 62/208725 (0.030%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 45-bp 61/208725 (0.029%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCACCGAGTCGGTGCACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 24-bp 54/208725 (0.026%)
2 × TD with deletion :
......---------------------------------------GTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 5977/208725 (2.86%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCA-- GTCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 154/208725 (0.074%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGC-----------ACGGATAAAGACGCTGGGAGATTGA...... 146/208725 (0.070%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTG------ TCAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 89/208725 (0.043%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGC----------- --GGATAAAGACGCTGGGAGATTGA...... 75/208725 (0.036%)
......GGCCTTCGCGCACCTCAT--------------------------GGAACACGGATAAAGACGCTGGGAGATTGA...... 54/208725 (0.026%)
......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGC-- CAAGGAACACGGATAAAGACGCTGGGAGATTGA...... 51/208725 (0.024%)

Supplemental Fig. S16 Undesired edits of *FANCF* TD 171-bp were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion, insertion and base substitution were indicated by black dash, red box and green letters respectively. Note that most inserted sequences were same as their 5' upstream sequences. A small group of insertions that were likely derived from sgRNA scaffold were highlighted in blue. The sequences with a ratio over 0.02% were shown.

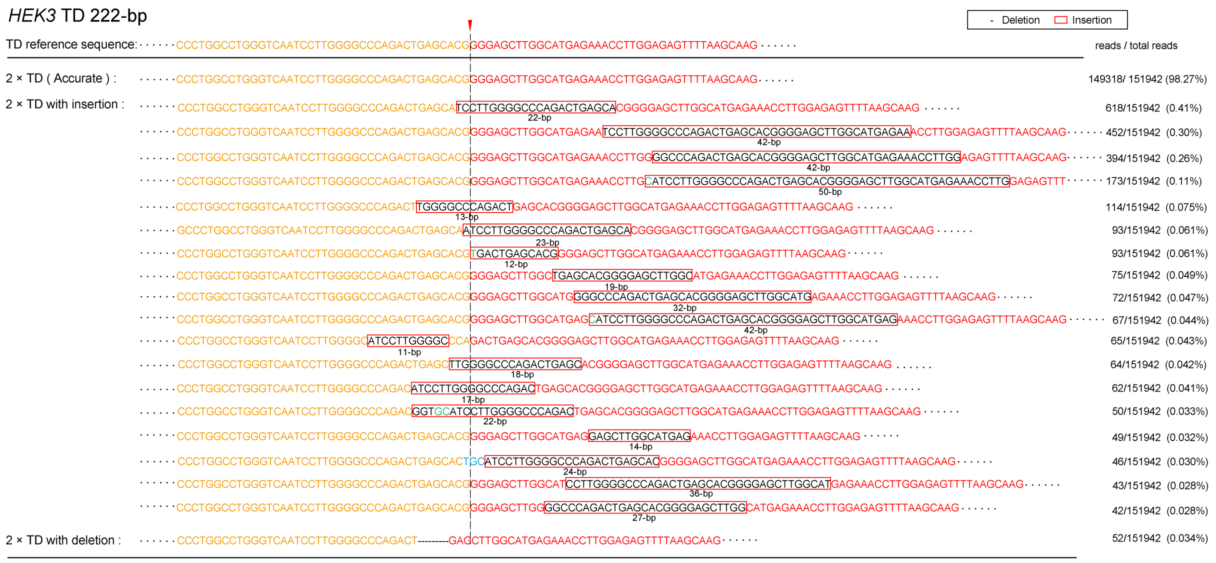
FANCF TD 231-bp

TD reference sequence:	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		reads / total reads
2 × TD (Accurate) :	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		317231/330088 (96.10%)
2 × TD with insertion :	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	13-bp	989/330088 (0.30%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCTCTTTGTGTGGCGAAAGTAAAAG......	17-bp	762/330088 (0.23%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	20-bp	684/330088 (0.21%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	47-bp	677/330088 (0.21%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	46-bp	780/330088 (0.24%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	11-bp	553/330088 (0.17%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	33-bp	358/330088 (0.11%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	19-bp	350/330088 (0.11%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCCTCTTTGTGTGGCGAAAGTAAAAG......	15-bp	319/330088 (0.10%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	23-bp	295/330088 (0.089%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	26-bp	255/330088 (0.077%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCGACTCGGTGCCACTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	24-bp	252/330088 (0.076%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAACCTCTTTGTGTGGCGAAAGTAAAAG......	17-bp	244/330088 (0.074%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	45-bp	223/330088 (0.068%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCTAAGAAGAACCTCTTTGTGTGGCGAAAG......	25-bp	216/330088 (0.065%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGAACCTCTTTGTGTGGCGAAAGTAAAAG......	10-bp	212/330088 (0.064%)
	......GGCCTTCGCGCACCTCATGGAATCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	12-bp	203/330088 (0.061%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCGTGGCGAAAGTAAAAG......	7-bp	198/330088 (0.060%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCTTGAAAAAGTGGCACCGAGTCGGTGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	23-bp	175/330088 (0.053%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	35-bp	172/330088 (0.052%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAGTATTAGGG......	39-bp 39-bp	167/330088 (0.051%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTTGTGTGGCGAAAGTAAAAG......	5-bp	164/330088 (0.050%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	16-bp	161/330088 (0.049%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGAACCTCTTTGTGTGGCGAAAG......	14-bp	157/330088 (0.048%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCCTCTTTGTGTGGCGAAAGTAAAAG......	13-bp	146/330088 (0.044%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTAACCTCTTTGTGTGGCGAAAGTAAAAG......	7-bp	145/330088 (0.044%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCTAAGAAGAACCTCTTTGTGTGGCGAAAG......	23-bp	136/330088 (0.041%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	33-bp	135/330088 (0.041%)
	......GGCCTTCGCGCACCTCATGGAATCCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAG......	13-bp	135/330088 (0.041%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	24-bp	134/330088 (0.041%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCGACTCGGTGTGGCGAAAGTAAAAG......	7-bp	124/330088 (0.038%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	24-bp	123/330088 (0.037%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	45-bp	123/330088 (0.037%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	39-bp	121/330088 (0.037%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCTTTGTGTGGCGAAAGTAAAAG......	10-bp	119/330088 (0.036%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	24-bp	115/330088 (0.035%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCACCGAGTCGGTGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	18-bp	109/330088 (0.033%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCGACCTCTTTGTGTGGCGAAAGTAAAAG......	15-bp	107/330088 (0.032%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCGACTCGGTGCCACTTTTTGTGTGGCGAAAGTAAAAG......	10-bp	106/330088 (0.032%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGAACCTCTTTGTGTGGCGAAAGTAAAAG......	12-bp	104/330088 (0.032%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAG......	24-bp	102/330088 (0.031%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCTCTTTGTGTGGCGAAAG......	14-bp	101/330088 (0.031%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	45-bp	97/330088 (0.029%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	36-bp	95/330088 (0.029%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTAACCTCTTTGTGTGGCGAAAGTAAAAG......	9-bp	93/330088 (0.028%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCCGAGTCGGTGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	17-bp	92/330088 (0.028%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	50-bp	88/330088 (0.027%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	49-bp	87/330088 (0.026%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	25-bp	86/330088 (0.026%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	17-bp	85/330088 (0.026%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCGACTCGGTGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	25-bp	84/330088 (0.025%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	5-bp	83/330088 (0.025%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCGACTCGGTGCCACTTTTTCAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	20-bp	81/330088 (0.025%)
	......GGCCTTCGCGCACCTCATGGAAGCCCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		76/330088 (0.023%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	14-bp	74/330088 (0.022%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGCACCCTCATGGAATCCTTCTGCAGCCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......	50-bp	73/330088 (0.022%)
2 × TD with deletion :	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGC ------------ CTCTTTGTGTGGCGAAAGTAAAAG......		170/330088 (0.052%)
	......GGCCTTCGCGCACCTCATGGAATCCCTT ------- CTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		161/330088 (0.049%)
	......GGCCTTCGCGCACCTCATGGAATCC -----------CTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		148/330088 (0.045%)
	......GGCCTTCGCGCACCTCATG ------------------------- GAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		138/330088 (0.041%)
	......GGCCTTCGCGCACCTCATGGAAT ------------ CCTAAGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		115/330088 (0.035%)
	......GGCCTTCGCGCACCTCATGGAATCCCTTCTGC ------ AGAAGAACCTCTTTGTGTGGCGAAAGTAAAAG......		84/330088 (0.025%)

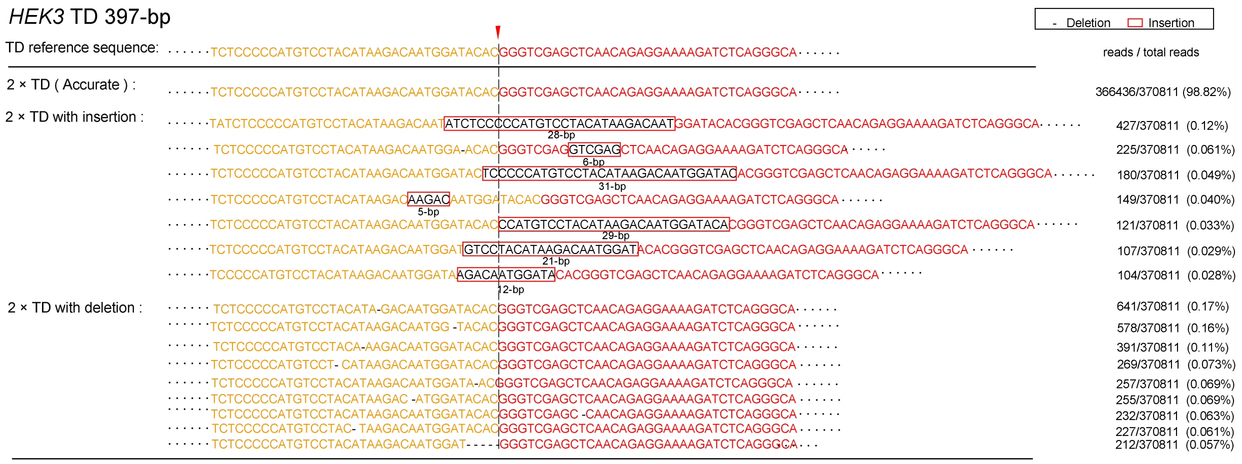
Supplemental Fig. S17 Undesired edits of *FANCF* TD 231-bp were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion, insertion and base substitution were indicated by black dash, red box and green letters respectively. Note that most inserted sequences were same as their 5' upstream sequences. A small group of insertions that were likely derived from sgRNA scaffold were highlighted in blue. The sequences with a ratio over 0.02% were shown.


HEK3 TD 136-bp
Insertion
TD reference sequence:
GCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACGCCCATGCAATTAGT
reads / total reads
2 × TD (Accurate) :
GCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACGCCCATGCAATTAGT
44109/51367 (85.87%)
2 × TD with insertion :
9-bp
621/51367 (1.21%)
15-bp
525/51367 (1.02%)
12-bp
480/51367 (0.93%)
20-bp
469/51367 (0.91%)
22-bp
467/51367 (0.91%)
13-bp
400/51367 (0.78%)

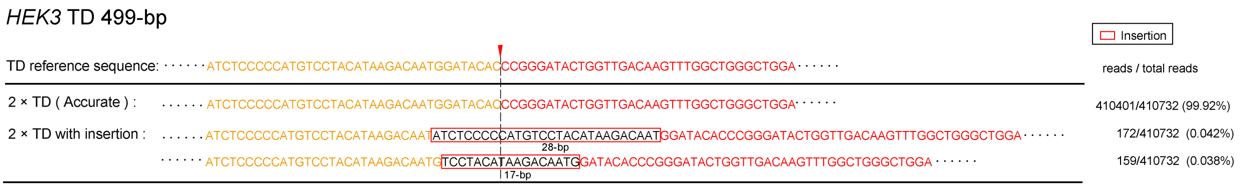
Supplemental Fig. S18 Undesired edits of *HEK3* TD 136-bp were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Insertion and base substitution were indicated by red box and green letters respectively. Note that most inserted sequences were same as their 5' upstream sequences. A small group of insertions that were likely derived from sgRNA scaffold were highlighted in blue. The sequences with a ratio over 0.02% were shown.


HEK3 TD 222-bp
- Deletion
Insertion
reads / total reads
TD reference sequence: ······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······
2 × TD (Accurate) : ······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 149318/ 151942 (98.27%)
2 × TD with insertion : ······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 618/151942 (0.41%)
22-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 452/151942 (0.30%)
42-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 394/151942 (0.26%)
42-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGCATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTT······ 173/151942 (0.11%)
50-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 114/151942 (0.075%)
13-bp
······GCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 93/151942 (0.061%)
23-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 93/151942 (0.061%)
12-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 75/151942 (0.049%)
19-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 72/151942 (0.047%)
32-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGCATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 67/151942 (0.044%)
42-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 65/151942 (0.043%)
11-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 64/151942 (0.042%)
18-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 62/151942 (0.041%)
17-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACGGTGCATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 50/151942 (0.033%)
22-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 49/151942 (0.032%)
14-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACTGCATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 46/151942 (0.030%)
24-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 43/151942 (0.028%)
36-bp
······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 42/151942 (0.028%)
27-bp
2 × TD with deletion : ······CCCTGGCCTGGGTCAATCCTTGGGGCCCAGACT---------GAGCTTGGCATGAGAAACCTTGGAGAGTTTTAAGCAAG······ 52/151942 (0.034%)

Supplemental Fig. S19 Undesired edits of *HEK3* TD 222-bp were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion, insertion and base substitution were indicated by black dash, red box and green letters respectively. Note that most inserted sequences were the same as their 5' upstream sequences. A small group of insertions that were likely derived from sgRNA scaffold were highlighted in blue. The sequences with a ratio over 0.02% were shown.

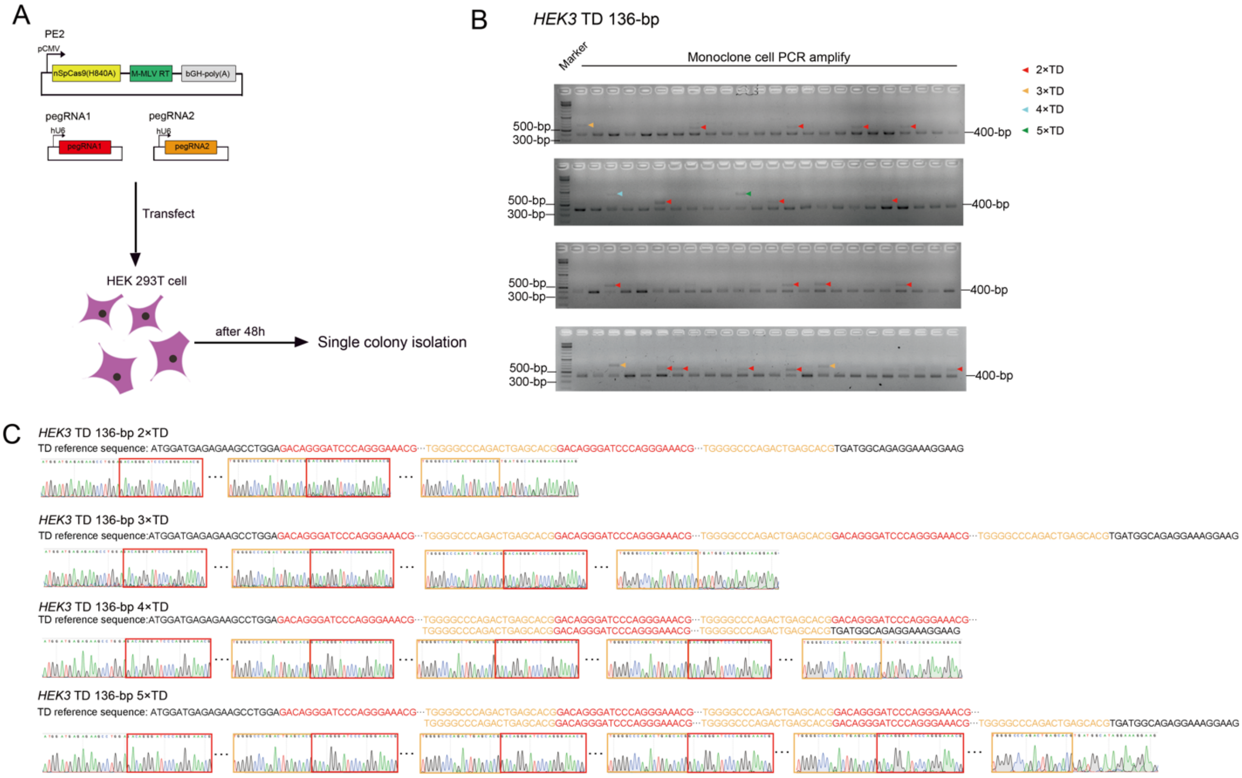

HEK3 TD 397-bp
- Deletion
Insertion
TD reference sequence:
······TCTCCCCCATGTCCTACATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
reads / total reads
2 × TD (Accurate) :
······TCTCCCCCATGTCCTACATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
366436/370811 (98.82%)
2 × TD with insertion :
······TATCTCCCCCATGTCCTACATAAGACAATATCTCCCCCATGTCCTACATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
28-bp
427/370811 (0.12%)
······ TCTCCCCCATGTCCTACATAAGACAATGGA-ACACGGGTCGAGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
6-bp
225/370811 (0.061%)
······TCTCCCCCATGTCCTACATAAGACAATGGATACTCCCCCATGTCCTACATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
31-bp
180/370811 (0.049%)
······TCTCCCCCATGTCCTACATAAGACAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
5-bp
149/370811 (0.040%)
······TCTCCCCCATGTCCTACATAAGACAATGGATACACCCATGTCCTACATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
29-bp
121/370811 (0.033%)
······TCTCCCCCATGTCCTACATAAGACAATGGATGTCCTACATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
21-bp
107/370811 (0.029%)
······TCCCCCATGTCCTACATAAGACAATGGATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
12-bp
104/370811 (0.028%)
2 × TD with deletion :
······ TCTCCCCCATGTCCTACATA-GACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
641/370811 (0.17%)
······TCTCCCCCATGTCCTACATAAGACAATGG -TACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
578/370811 (0.16%)
······ TCTCCCCCATGTCCTACA-AAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
391/370811 (0.11%)
······TCTCCCCCATGTCCT- CATAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
269/370811 (0.073%)
······TCTCCCCCATGTCCTACATAAGACAATGGATA-ACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
257/370811 (0.069%)
······TCTCCCCCATGTCCTACATAAGAC -ATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
255/370811 (0.069%)
······TCTCCCCCATGTCCTACATAAGACAATGGATACACGGGTCGAGC -CAACAGAGGAAAAGATCTCAGGGCA······
232/370811 (0.063%)
······TCTCCCCCATGTCCTAC- TAAGACAATGGATACACGGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA······
227/370811 (0.061%)
······TCTCCCCCATGTCCTACATAAGACAATGGAT----GGGTCGAGCTCAACAGAGGAAAAGATCTCAGGGCA···
212/370811 (0.057%)

Supplemental Fig. S20 Undesired edits of *HEK3* TD 397-*bp* were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion and insertion were indicated by black dash and red box respectively. Note that most inserted sequences were same as their 5' upstream sequences. The sequences with a ratio over 0.02% were shown.

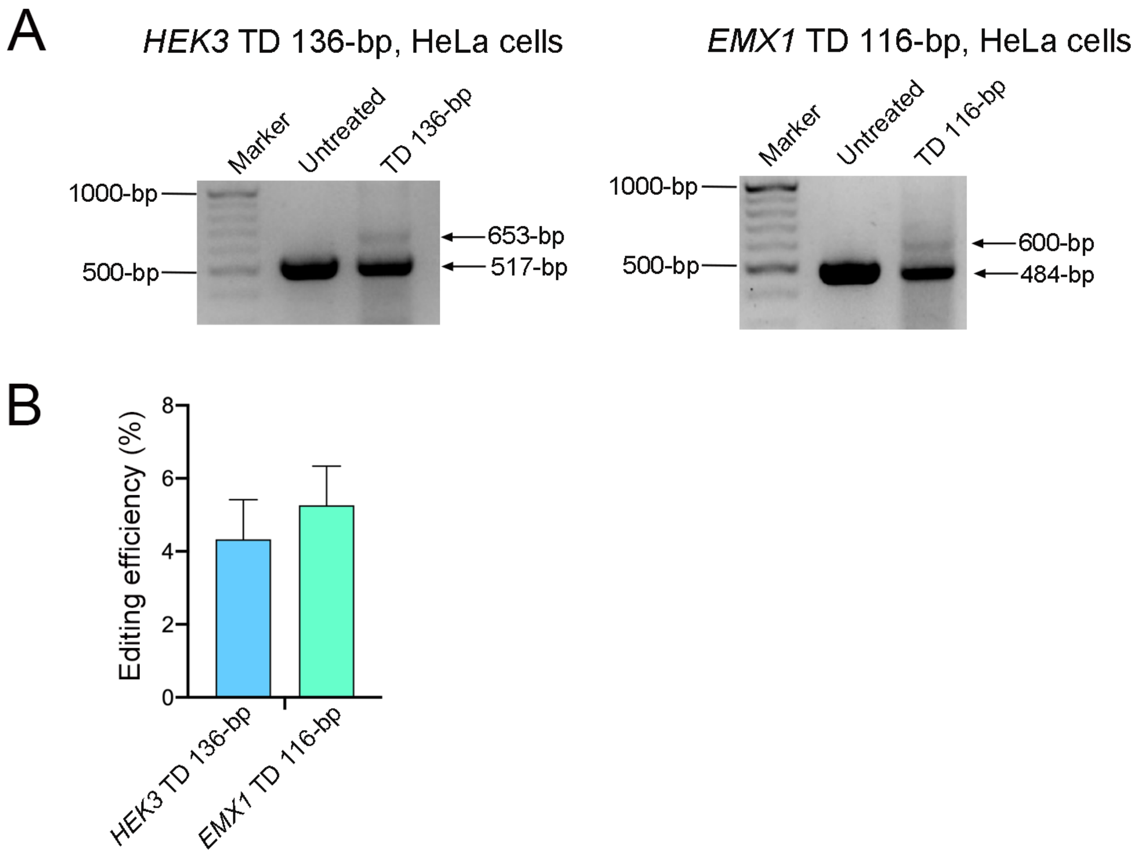


HEK3 TD 499-bp
Insertion
TD reference sequence: ······ATCTCCCCCATGTCCTACATAAGACAATGGATACACCCGGGATACTGGTTGACAAGTTTGGCTGGGCTGGA······
reads / total reads
2 × TD (Accurate) : ······ATCTCCCCCATGTCCTACATAAGACAATGGATACACCCGGGATACTGGTTGACAAGTTTGGCTGGGCTGGA······
410401/410732 (99.92%)
2 × TD with insertion : ······ATCTCCCCCATGTCCTACATAAGACAATATCTCCCCCATGTCCTACATAAGACAATGGATACACCCGGGATACTGGTTGACAAGTTTGGCTGGGCTGGA······
28-bp
172/410732 (0.042%)
······ATCTCCCCCATGTCCTACATAAGACAATGTCCTACATAAGACAATGGATACACCCGGGATACTGGTTGACAAGTTTGGCTGGGCTGGA······
17-bp
159/410732 (0.038%)

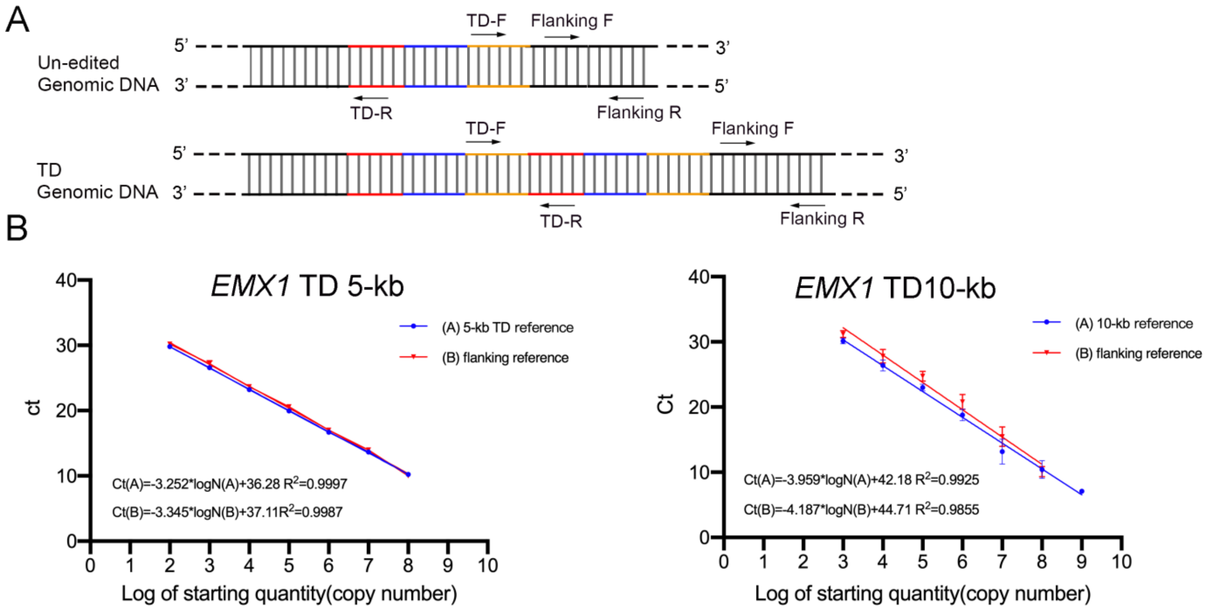
Supplemental Fig. S21 Undesired edits of *HEK3* TD 499-*bp* were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Insertion was indicated by red box. Note that most inserted sequences were same as their 5' upstream sequences. The sequences with a ratio over 0.02% were shown.


A
PE2
pCMV
nSpCas9(H840A)
M-MLV RT
bGH-poly(A)
pegRNA1
hU6
pegRNA1
pegRNA2
hU6
pegRNA2
Transfect
HEK 293T cell
after 48h
Single colony isolation
B
HEK3 TD 136-bp
Marker
Monoclone cell PCR amplify
500-bp
300-bp
400-bp
2×TD
3×TD
4×TD
5×TD
C
HEK3 TD 136-bp 2×TD
TD reference sequence: ATGGATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG
HEK3 TD 136-bp 3×TD
TD reference sequence:ATGGATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG
HEK3 TD 136-bp 4×TD
TD reference sequence:ATGGATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···
TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG
HEK3 TD 136-bp 5×TD
TD reference sequence: ATGGATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···
TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGGACAGGGATCCCAGGGAAACG···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG

Supplemental Fig. S22 Monoclonal analysis of the *HEK3* TD 136-bp. (A) Diagram showing the procedure of monoclonal analysis. HEK293T cells were transfected with TD-PE plasmids, including PE2 and two pegRNA, and 48 hours post the transfection cells were diluted monoclonal isolation. (B) Agarose gel electrophoresis showed the amplicons derived from each monoclonal genome. The colonies containing different copies of TDs were highlighted with triangles of different colors. (C) Sanger sequencing results of representative TDs derived from monoclonal genomes. Reference sequences of each type of TDs were shown on top of the sequence chromatogram, and the duplicated sequences corresponding to each end of the TD were marked by yellow and red boxes respectively.


A
HEK3 TD 136-bp, HeLa cells
Marker
Untreated
TD 136-bp
1000-bp
500-bp
653-bp
517-bp
EMX1 TD 116-bp, HeLa cells
Marker
Untreated
TD 116-bp
1000-bp
500-bp
600-bp
484-bp
B
Editing efficiency (%)
8
6
4
2
0
HEK3 TD 136-bp
EMX1 TD 116-bp

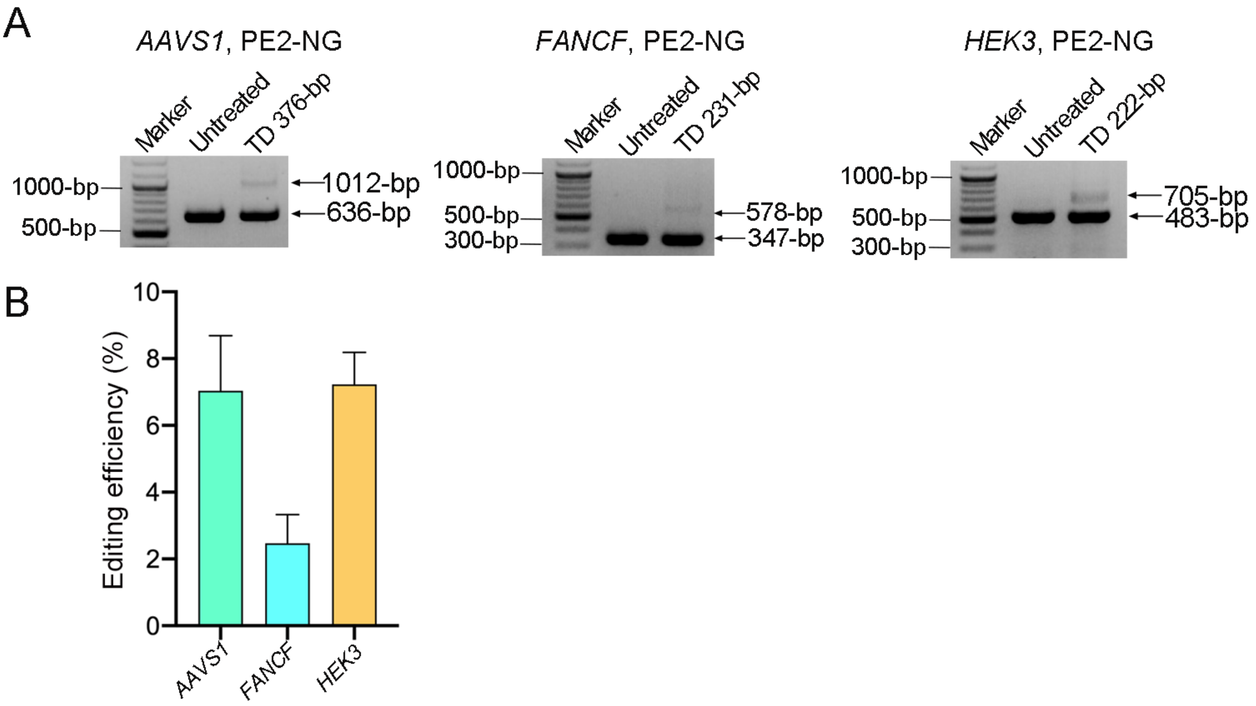
Supplemental Fig. S23 Verification of TD-PE strategy in HeLa cells**.** (A) Agarose gel analysis of the presence of targeted tandem duplication at indicated loci. (B) Quantification of the efficiency of targeted tandem duplication in (A) using Image J. Values and error bars reflect mean± s.d. of n=3 independent biological replicates.


A
Un-edited
Genomic DNA
5’
3’
3’
5’
TD-F
Flanking F
TD-R
Flanking R
TD
Genomic DNA
5’
3’
3’
5’
TD-F
Flanking F
TD-R
Flanking R
B
EMX1 TD 5-kb
ct
Log of starting quantity(copy number)
(A) 5-kb TD reference
(B) flanking reference
Ct(A)=-3.252*logN(A)+36.28 R^2=0.9997
Ct(B)=-3.345*logN(B)+37.11R^2=0.9987
0
10
20
30
40
0 1 2 3 4 5 6 7 8 9 10
EMX1 TD10-kb
Ct
Log of starting quantity(copy number)
(A) 10-kb reference
(B) flanking reference
Ct(A)=-3.959*logN(A)+42.18 R^2=0.9925
Ct(B)=-4.187*logN(B)+44.71 R^2=0.9855
0
10
20
30
40
0 1 2 3 4 5 6 7 8 9 10

Supplemental Fig. S24 Standard curves for absolute quantification by real-time PCR of *EMX1* TD 5-kb and 10-kb. (A) Schematic diagram showing the design of qPCR primers. The flanking fragment of the target site or the one containing tandem duplication obtained by PCR were ligated to the blunt vector to construct reference plasmids. qPCR primers were designed inside the amplified fragment. The primers used to construct the reference plasmids and qPCR primers are listed in Supplemental Table S4. (B) The standard curves for the reference plasmids were determined by CT values against log-transformed concentrations of serial tenfold dilutions (2×10^2, 10^3, 10^4, 10^5, 10^6, 10^7,10^8 and 10^9 copies per 1μl).

EMX1 TD 5-kb
Insertion
TD reference sequence: · · · · · ·AGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAGAGGCATGGAAAGGAGGATTTGGAGGGTCCTTTC· · · · · ·
reads / total reads
2 × TD (Accurate) : · · · · · ·AGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAGAGGCATGGAAAGGAGGATTTGGAGGGTCCTTTC· · · · · ·
100690/100760 (99.931%)
2 × TD with insertion : · · · · · ·AGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAGAGGCATGGAAAGGAGGATTTGGAGGGTCCTTTC · · · · · ·
33-bp
70/100760 (0.069%)
EMX1 TD 10-kb
- Deletion
Insertion
TD reference sequence: · · · · · ·ACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCAACATTTGCTAAGGG · · · · · ·
reads / total reads
2 × TD (Accurate) : · · · · · ·ACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCAACATTTGCTAAGGG · · · · · ·
417717/418256 (99.87%)
2 × TD with insertion : · · · · · ·ACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCAACATTTGCAGAAGCTGGAGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCAACATTTGCTAAGGG · · · · · ·
82-bp
146/418256 (0.035%)
· · · · · ·ACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCAACATTTGCTAAGGG· · · · · ·
33-bp
144/418256 (0.034%)
· · · · · ·ACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCAACATTTGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCAACATTTGCTAAGGG· · · · · ·
73-bp
106/418256 (0.025%)
2 × TD with deletion : · · · · · ·ACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCTGAGTCCCTATGCTGGCCA - - ATTTGCTAAGGG· · · · · ·
143/418256 (0.034%)

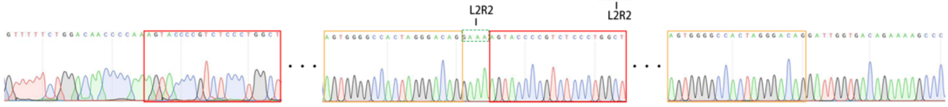
Supplemental Fig. S25 Undesired edits of *EMX1 TD* 5-kb/10-kb were determined by HTS. Sequence corresponding to accurate TD allele was shown on top of the analysis. The sequences corresponding to each end of the TD were showed in red and yellow respectively. The red arrowhead pointed to the boundary of the duplication. Deletion and insertion were indicated by black dash and red box respectively. Note that most inserted sequences were the same as their 5' upstream sequences. The sequences with a ratio over 0.02% were shown.


A
AAVS1, PE2-NG
Marker
Untreated
TD 376-bp
1000-bp
500-bp
1012-bp
636-bp
FANCF, PE2-NG
Marker
Untreated
TD 231-bp
1000-bp
500-bp
300-bp
578-bp
347-bp
HEK3, PE2-NG
Marker
Untreated
TD 222-bp
1000-bp
500-bp
300-bp
705-bp
483-bp
B
Editing efficiency (%)
0
2
4
6
8
10
AAVS1
FANCF
HEK3

Supplemental Fig. S26 Targeted TDs produced by PE2-NG. (A) Targeted TDs produced by PE2-NG. Agarose gel analysis of the presence of targeted tandem duplication at indicated loci. B, Quantification of the efficiency of targeted tandem duplication in (B) using Image J. Values and error bars reflect mean± s.d. of n=3 independent biological replicates.

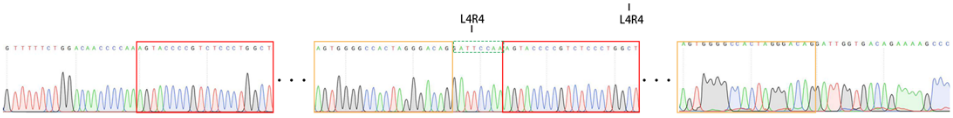
AAVS1 TD 376-bp L2R2

TD reference sequence:GTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGAAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



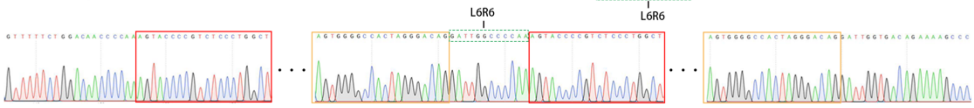
AAVS1 TD 376-bp L4R4

TD reference sequence:GTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



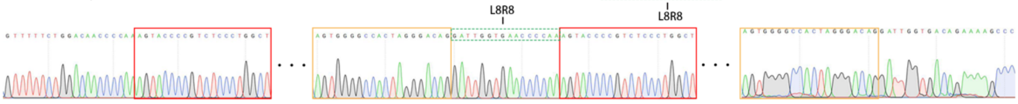
AAVS1 TD 376-bp L6R6

TD reference sequence:GTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGCCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



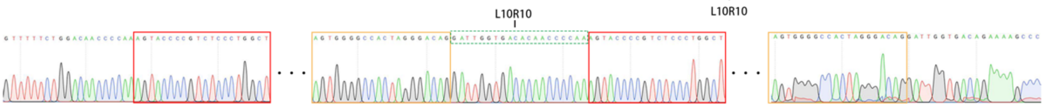
AAVS1 TD 376-bp L8R8

TD reference sequence:GTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



AAVS1 TD 376-bp L10R10

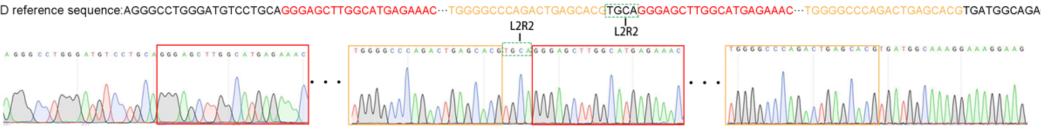
TD reference sequence:GTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGACACAACCCCAAAGTACCCCGTCTCCCTGGCT···AGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCC



Supplemental Fig.S27 Representative Sanger sequencing results of the additive fragment containing TDs at the *AAVS1* site (related to Fig. 4B). The PCR amplicons containing targeted TDs were gel-purified and subjected directly to Sanger sequencing. TD reference sequences were shown on top of each Sequencing chromatogram. The sequences corresponding to each end of the TDs were marked by yellow and red boxes respectively. Sequences in green dashed boxes represent additive fragments.

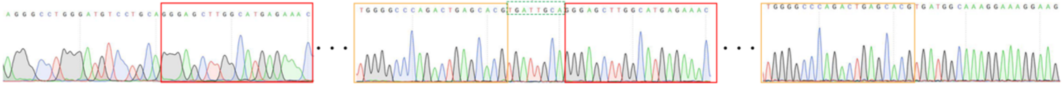
HEK3 TD 222-bp L2R2

TD reference sequence:AGGGCCTGGGATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



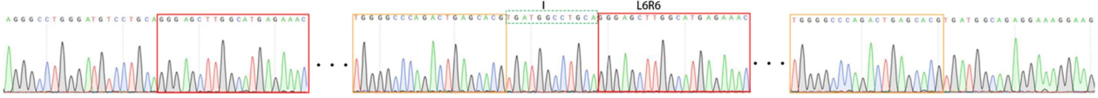
HEK3 TD 222-bp L4R4

TD reference sequence:AGGGCCTGGGATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



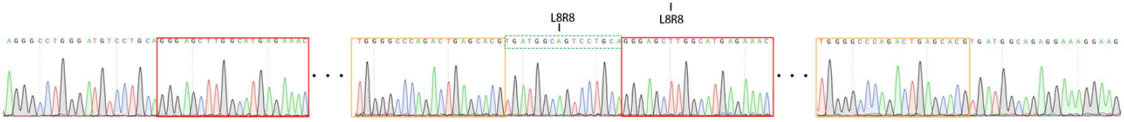
HEK3 TD 222-bp L6R6

TD reference sequence:AGGGCCTGGGATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



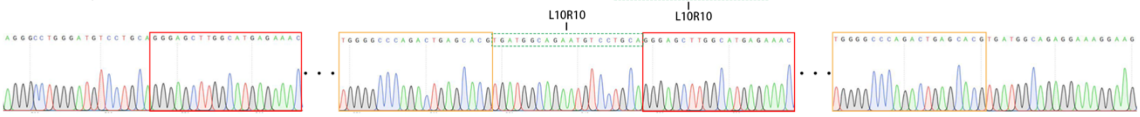
HEK3 TD 222-bp L8R8

TD reference sequence:AGGGCCTGGGATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG



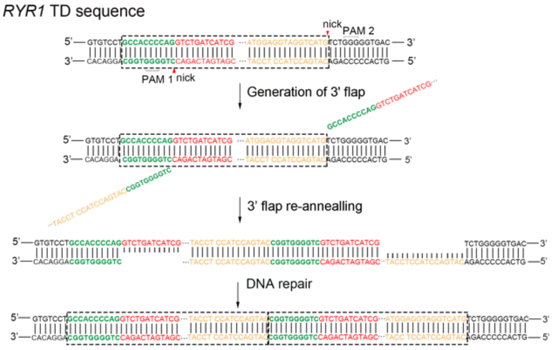
HEK3 TD 222-bp L10R10

TD reference sequence:AGGGCCTGGGATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAATGTCCTGCAGGGAGCTTGGCATGAGAAAC···TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG

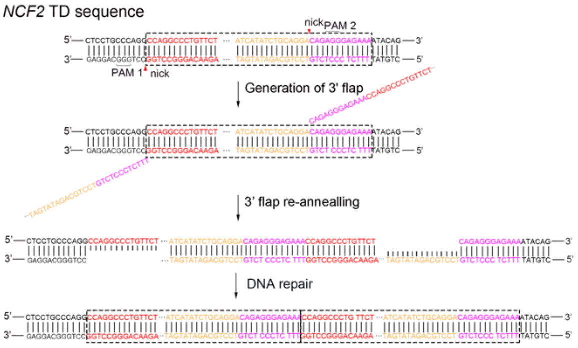


Supplemental Fig.S28 Representative Sanger sequencing results of the additive fragment containing TDs at the *HEK3* site (related to Fig. 4B). The PCR amplicons containing targeted TDs were gel-purified and subjected directly to Sanger sequencing. TD reference sequences were shown on top of each Sequencing chromatogram. The sequences corresponding to each end of the TDs were marked by yellow and red boxes respectively. Sequences in green dashed boxes represent additive fragments.

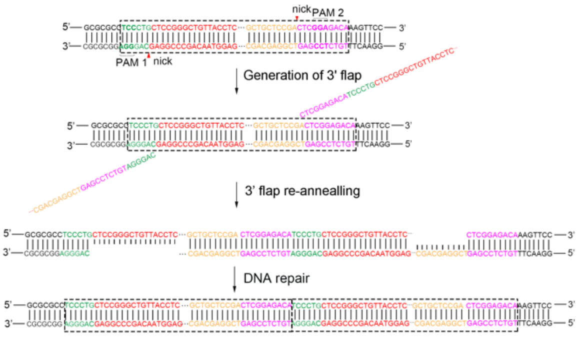
RYR1 TD sequence



NCF2 TD sequence



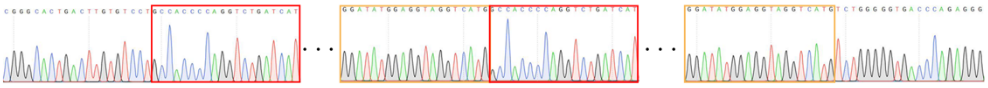
SATB2 TD sequence



Supplemental Fig.S29 PegRNAs design at three disease loci and the putative editing process of TD-PE strategy. PAM1 and PAM2 are the upstream and downstream PAMs respectively, and the red arrows indicate the positions of the nicks. Black dashed box indicates the sequences to be duplicated. Green and pink sequences denote the upstream and downstream addictive fragments respectively.

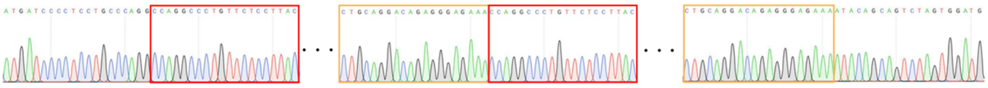
RYR1 TD 85-bp

TD reference sequence:CGGGCACTGACTTGTGTCCTGCCACCCCAGGTCTGATCAT···GGATATGGAGGTAGGTCATGGCCACCCCAGGTCTGATCAT···GGATATGGAGGTAGGTCATGTCTGGGGGTGACCCAGAGGG



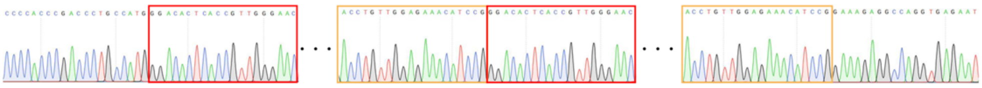
NCF2 TD 149-bp

TD reference sequence:ATGATCCCCTCCTGCCCAGGCCAGGCCCTGTTCTCCTTAC···CTGCAGGACAGAGGGAGAAACCAGGCCCTGTTCTCCTTAC···CTGCAGGACAGAGGGAGAAAATACAGCAGTCTAGTGGATG



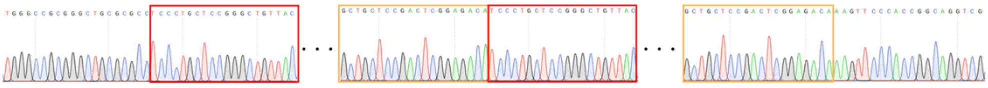
ENG TD 180-bp

TD reference sequence:CCCCACCCGACCCTGCCATGGGACACTCACCGTTGGGAAC···ACCTGTTGGAGAAACATCCGGGACACTCACCGTTGGGAAC···ACCTGTTGGAGAAACATCCGGAAAGAGGCCAGGTGAGAAT



SATB2 TD 209-bp

TD reference sequence:TGGGCCGCGGGCTGCGCGCCTCCCTGCTCCGGGCTGTTAC···GCTGCTCCGACTCGGAGACATCCCTGCTCCGGGCTGTTAC···GCTGCTCCGACTCGGAGACAAAGTTCCCACCGGCAGGTCG



Supplemental Fig.S30 Representative Sanger sequencing results of 4 disease-related TDs at indicated loci (related to Fig. 5B). The PCR amplicons containing targeted tandem duplication were gel-purified and subjected directly to Sanger sequencing. TD reference sequences were shown on top of each Sequencing result. The sequences corresponding to each end of the TDs were marked by red and yellow boxes respectively.

Supplemental Table 1. List of the targets tested in this study.

sgRNA	Target sequence	Oligo-F	Oligo-R	Reference
FANCF	GGAATCCCTTCTGCAGCACC **TGG**	CACCGGAATCCCTTCTGCAGCACC	AAACGGTGCTGCAGAAGGGATTCC	(Anzalone et al. 2019)
FANCF -50	GCCCTACTTCCGCTTTCACCT **TGG**	CACCGCCCTACTTCCGCTTTCACCT	AAACAGGTGAAAGCGGAAGTAGGG	(Anzalone et al. 2019)
EMX1	AGTCCGAGCAGAAGAAGAA **GGG**	CACCGAGTCCGAGCAGAAGAAGAA	AAACTTCTTCTTCTGCTCGGACTC	(Anzalone et al. 2019)
EMX1-57	CCGTTTGTACTTTGTCCTC **CGG**	CACCGCCGTTTGTACTTTGTCCTC	AAACGAGGACAAAGTACAAACGGC	(Anzalone et al. 2019)
FANCF+115	GCAGAGAGTCGCCGTCTCCA **AGG**	CACCGCAGAGAGTCGCCGTCTCCA	AAACTGGAGACGGCGACTCTCTGC	This study
FANCF-115	TATCCGTGTTCCTTGACTCT **GGG**	CACCGTATCCGTGTTCCTTGACTCT	AAACAGAGTCAAGGAACACGGATAC	This study
FANCF-171	TATCCGTGTTCCTTGACTCT **GGG**	CACCGTATCCGTGTTCCTTGACTCT	AAACAGAGTCAAGGAACACGGATAC	This study
FANCF-231	AAAGAGGTTCTTCTTAGTGG **AGG**	CACCGAAAGAGGTTCTTCTTAGTGG	AAACCCACTAAGAAGAACCTCTTTC	This study
EMX1-116	GGGGCACAGATGAGAAACTC **AGG**	CACCGGGGCACAGATGAGAAACTC	AAACGAGTTTCTCATCTGTGCCCC	(Anzalone et al. 2019)
EMX1-201	ACTGAGGCTACATAGGGTTA **GGG**	CACCGACTGAGGCTACATAGGGTTA	AAACTAACCCTATGTAGCCTCAGTC	This study
EMX1-307	CAGTGGGCCAGAGAGAAGGG **TGG**	CACCGCAGTGGGCCAGAGAGAAGGG	AAACCCCTTCTCTCTGGCCCACTGC	This study
AAVS1	GGGGCCACTAGGGACAGGAT **TGG**	CACCGGGGCCACTAGGGACAGGAT	AAACATCCTGTCCCTAGTGGCCCC	This study
AAVS1-376	CAGGGAGACGGGGTACTTTG **GGG**	CACCGCAGGGAGACGGGGTACTTTG	AAACCAAAGTACCCCGTCTCCCTGC	This study
AAVS1-532	CAGCGAGTGAAGACGGCATG **GGG**	CACCGCAGCGAGTGAAGACGGCATG	AAACCATGCCGTCTTCACTCGCTGC	This study
AAVS1-846	AAGGGGAAACAGTGGGCCAG **AGG**	CACCGAAGGGGAAACAGTGGGCCAG	AAACCTGGCCCACTGTTTCCCCTTC	This study
HEK3	GGCCCAGACTGAGCACGTGA **TGG**	CACCGGCCCAGACTGAGCACGTGA	AAACTCACGTGCTCAGTCTGGGCC	(Anzalone et al. 2019)
HEK3-136	TCCCTGGGATCCCTGTCTCC **AGG**	CACCGTCCCTGGGATCCCTGTCTCC	AAACGGAGACAGGGATCCCAGGGAC	This study
HEK3-222	TCTCATGCCAAGCTCCCTGC **AGG**	CACCGTCTCATGCCAAGCTCCCTGC	AAACGCAGGGAGCTTGGCATGAGAC	This study

HEK3+1	TAAGACAATGGATACACTGG **AGG**	CACCGTAAGACAATGGATACACTGG	AAACCCAGTGTATCCATTGTCTTAC	This study
HEK3-397	TCTGTTGAGCTCGACCCTGA **AGG**	CACCGTCTGTTGAGCTCGACCCTGA	AAACTCAGGGTCGAGCTCAACAGAC	This study
HEK3-499	GTCAACCAGTATCCCGGTGC **AGG**	CACCGTCAACCAGTATCCCGGTGC	AAACGCACCGGGATACTGGTTGAC	This study
ENG+1	GTTTCTGCAAGACCTGTTGG **AGA**	CACCGTTTCTGCAAGACCTGTTGG	AAACCCAACAGGTCTTGCAGAAAC	This study
ENG-180	CTTCCTGGAGTTCCCAACGG **TGA**	CACCGCTTCCTGGAGTTCCCAACGG	AAACCCGTTGGGAACTCCAGGAAG C	This study
SATB2+1	GCTCCATGCTGCTCCGACTC **GGA**	CACCGCTCCATGCTGCTCCGACTC	AAACGAGTCGGAGCAGCATGGAGC	This study
SATB2-209	GAGGTAACAGCCCGGAGCAG **GGA**	CACCGAGGTAACAGCCCGGAGCAG	AAACCTGCTCCGGGCTGTTACCTC	This study
NCF2+1	AAATCATATCTGCAGGACAG **AGG**	CACCGAAATCATATCTGCAGGACAG	AAACCTGTCCTGCAGATATGATTTC	This study
NCF2-149	AGGAGAACAGGGCCTGGCCT **GGG**	CACCGAGGAGAACAGGGCCTGGCCT	AAACAGGCCAGGCCCTGTTCTCCTC	This study
RYR1+1	TATGGAGGTAGGTCATGTCT **GGG**	CACCGTATGGAGGTAGGTCATGTCT	AAACAGACATGACCTACCTCCATAC	This study
RYR1-85	AGCGTCGATGATCAGACCTG **GGG**	CACCGAGCGTCGATGATCAGACCTG	AAACCAGGTCTGATCATCGACGCTC	This study

Supplemental Table 2. Sequences of pegRNAs used in mammalian cell experiments.

pegRNA	spacer	3' extension (5' to 3')	PBS length (nt)	HA length (nt)
FANCF+TD50 pegRNA	GGAATCCCTTCTGCAGCACC	CGCGAAGGCCCTACTTCCGCTTTCAGCTGCAGAAGGGA	13	25
FANCF-TD50 pegRNA	GCCCTACTTCCGCTTTCACCT	CACCTCATGGAATCCCTTCTGCAGCTGAAAGCGGAAGT	13	25
EMX1+TD57 pegRNA	GAGTCCGAGCAGAAGAAGAA	CTCCAGCTTCTGCCGTTTGTACTTTGTCTTCTTCTGCTCGG	13	28
EMX1-TD57 pegRNA	CCGTTTGTACTTTGTCCTC	GAGGAAGGGCCTGAGTCCGAGCAGAAGAAGACAAAGTACAAA	13	29
FANCF+TD115 pegRNA	GCAGAGAGTCGCCGTCTCCA	AGCGTCTTTATCCGTGTTCCTTGACAGACGGCGACTC	12	25
FANCF-TD115 pegRNA	TATCCGTGTTCCTTGACTCT	ATCAGTACGCAGAGAGTCGCCGTCTGTCAAGGAACAC	12	25
FANCF+TD171 pegRNA	GGAATCCCTTCTGCAGCACC	AGCGTCTTTATCCGTGTTCCTTGACGCTGCAGAAGGGA	13	25
FANCF-TD171 pegRNA	TATCCGTGTTCCTTGACTCT	ACCTCATGGAATCCCTTCTGCAGCGTCAAGGAACACG	13	24
FANCF+TD231 pegRNA	GGAATCCCTTCTGCAGCACC	GCCACACAAAGAGGTTCTTCTTAGGCTGCAGAAGGGA	13	24
FANCF-TD231 pegRNA	AAAGAGGTTCTTCTTAGTGG	ACCTCATGGAATCCCTTCTGCAGCCTAAGAAGAACCT	13	24
EMX1+TD116 pegRNA	GAGTCCGAGCAGAAGAAGAA	GGGAGGGAGGGGCACAGATGAGAAATTCTTCTGCTCGG	13	25
EMX1-TD116 pegRNA	GGGGCACAGATGAGAAACTC	AAGGGCCTGAGTCCGAGCAGAAGAATTTCTCATCTGTG	13	25
EMX1+TD201 pegRNA	GAGTCCGAGCAGAAGAAGAA	ATGGGAAGACTGAGGCTACATAGGGTTCTTCTGCTCGG	13	25
EMX1-TD201 pegRNA	ACTGAGGCTACATAGGGTTA	AAGGGCCTGAGTCCGAGCAGAAGAACCCTATGTAGCCT	13	25
EMX1+TD307 pegRNA	GAGTCCGAGCAGAAGAAGAA	GATGGCAGGGCAGGAAGAGGACACAGTGGGCCAGAGAGAATTCTTCTGCTCGG	13	40
EMX1-TD307 pegRNA	CAGTGGGCCAGAGAGAAGGG	AGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAATTCTCTCTGGCCC	13	40
AAVS1+TD376 pegRNA	GGGGCCACTAGGGACAGGAT	GAGGATGGAGAGGTGGCTAAAGCCAGGGAGACGGGGTACTCTGTCCCTAGTGG	13	40
AAVS1-TD376 pegRNA	CAGGGAGACGGGGTACTTTG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGAGTACCCCGTCTC	13	40
AAVS1+TD532 pegRNA	GGGGCCACTAGGGACAGGAT	AGAAGGAGAAGGAAAAGGGAACCCAGCGAGTGAAGACGGCCTGTCCCTAGTGG	13	40
AAVS1-TD532 pegRNA	CAGCGAGTGAAGACGGCATG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAG GCCGTCTTCACTC	13	40

AAVS1+TD846 pegRNA	GGGGCCACTAGGGACAGGAT	AGAGAAAGCAGGACCTGCCTGGGAAGGGGAAACAGTGGGCCTGTCCCTAGTGG	13	40
AAVS1-TD846 pegRNA	AAGGGGAAACAGTGGGCCAG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGCCCACTGTTTCC	13	40
HEK3+TD136 pegRNA	GGCCCAGACTGAGCACGTGA	TGGGCGTTTCCCTGGGATCCCTGTCCGTGCTCAGTCTG	13	25
HEK3-TD136 pegRNA	GTCCCTGGGATCCCTGTCTCC	ATCCTTGGGGCCCAGACTGAGCACGGACAGGGATCCCA	13	23
HEK3+TD222 pegRNA	GGCCCAGACTGAGCACGTGA	AAGGTTTCTCATGCCAAGCTCCCCGTGCTCAGTCTG	13	23
HEK3-TD222 pegRNA	GTCTCATGCCAAGCTCCCTGC	ATCCTTGGGGCCCAGACTGAGCACGGGGAGCTTGGCAT	13	25
HEK3+TD397 pegRNA	TAAGACAATGGATACACTGG	TGGGTGCCCTGAGATCTTTTCCTCTGTTGAGCTCGACCCGTGTATCCATTGT	13	39
HEK3-TD397 pegRNA	TCTGTTGAGCTCGACCCTGA	TAATATCTCCCCCATGTCCTACATAAGACAATGGATACACGGGTCGAGCTCAA	13	40
HEK3+TD499 pegRNA	GTCAACCAGTATCCCGGTGC	TAATATCTCCCCCATGTCCTACATAAGACAATGGATACACCCGGGATACTGGT	13	40
HEK3-TD499 pegRNA	TAAGACAATGGATACACTGG	TGGCTTCCAGCCCAGCCAAACTTGTCAACCAGTATCCCGGGTGTATCCATTGT	13	40
EMX1+TD5K pegRNA	GAGTCCGAGCAGAAGAAGAA	AGCAGGAAAGGACCCTCCAAATCCTCCTTTCCATGCCTCTTTCTTCTGCTCGG	13	40
EMX1-TD5K pegRNA	CTCCTTTCCATGCCTCTGAG	AGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAGAGGCATGGAAA	13	40
EMX1+TD10K pegRNA	GAGTCCGAGCAGAAGAAGAA	AGCAAATGTTGGCCAGCATAGGGACTCAGCTCCCACATGCTTCTTCTGCTCGG	13	40
EMX1-TD10K pegRNA	ACTCAGCTCCCACATGCAGG	AGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGCATGTGGGAGCT	13	40
AAVS1+TD L2R2 pegRNA	GGGGCCACTAGGGACAGGAT	GAGGATGGAGAGGTGGCTAAAGCCAGGGAGACGGGGTACTTTTCCTGTCCCTAGTGG	13	40
AAVS1-TD L2R2 pegRNA	CAGGGAGACGGGGTACTTTG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGAAAAGTACCCCGTCTC	13	40
AAVS1+TD L4R4 pegRNA	GGGGCCACTAGGGACAGGAT	GAGGATGGAGAGGTGGCTAAAGCCAGGGAGACGGGGTACTTTGGAATCCTGTCCCTAGTGG	13	40
AAVS1-TD L4R4 pegRNA	CAGGGAGACGGGGTACTTTG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTCCAAAGTACCCCGTCTC	13	40
AAVS1+TD L6R6 pegRNA	GGGGCCACTAGGGACAGGAT	GAGGATGGAGAGGTGGCTAAAGCCAGGGAGACGGGGTACTTTGGGGCCAATCCTGTCCCTAGTGG	13	40

AAVS1-TD L6R6 pegRNA	CAGGGAGACGGGGTACTTTG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGCCCCAAAGTACCCCGTCTC	13	40
AAVS1+TD L8R8 pegRNA	GGGGCCACTAGGGACAGGAT	GAGGATGGAGAGGTGGCTAAAGCCAGGGAGACGGGGTACTTTGGGGTTCACCAATCCTGTCCCTAGTGG	13	40
AAVS1-TD L8R8 pegRNA	CAGGGAGACGGGGTACTTTG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGAACCCCAAAGTACCCCGTCTC	13	40
AAVS1+TD L10R10 pegRNA	GGGGCCACTAGGGACAGGAT	GAGGATGGAGAGGTGGCTAAAGCCAGGGAGACGGGGTACTTTGGGGTTGTGTCACCAATCCTGTCCCTAGTGG	13	40
AAVS1-TD L10R10 pegRNA	CAGGGAGACGGGGTACTTTG	ATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACACAACCCCAAAGTACCCCGTCTC	13	40
HEK3+TD L2R2 pegRNA	GGCCCAGACTGAGCACGTGA	AAGGTTTCTCATGCCAAGCTCCCTGCACGTGCTCAGTCTG	13	23
HEK3-TD L2R2 pegRNA	GTCTCATGCCAAGCTCCCTGC	ATCCTTGGGGCCCAGACTGAGCACGTGCAGGGAGCTTGGCAT	13	25
HEK3+TD L4R4 pegRNA	GGCCCAGACTGAGCACGTGA	AAGGTTTCTCATGCCAAGCTCCCTGCAATCACGTGCTCAGTCTG	13	23
HEK3-TD L4R4 pegRNA	GTCTCATGCCAAGCTCCCTGC	ATCCTTGGGGCCCAGACTGAGCACGTGATTGCAGGGAGCTTGGCAT	13	25
HEK3+TD L6R6 pegRNA	GGCCCAGACTGAGCACGTGA	AAGGTTTCTCATGCCAAGCTCCCTGCAGGCCATCACGTGCTCAGTCTG	13	23
HEK3-TD L6R6 pegRNA	GTCTCATGCCAAGCTCCCTGC	ATCCTTGGGGCCCAGACTGAGCACGTGATGGCCTGCAGGGAGCTTGGCAT	13	25
HEK3+TD L8R8 pegRNA	GGCCCAGACTGAGCACGTGA	AAGGTTTCTCATGCCAAGCTCCCTGCAGGAGCCATCACGTGCTCAGTCTG	13	23
HEK3-TD L8R8 pegRNA	GTCTCATGCCAAGCTCCCTGC	ATCCTTGGGGCCCAGACTGAGCACGTGATGGCAGTCCTGCAGGGAGCTTGGCAT	13	25
HEK3+TD L10R10 pegRNA	GGCCCAGACTGAGCACGTGA	AAGGTTTCTCATGCCAAGCTCCCTGCAGGACATTCTGCCATCACGTGCTCAGTCTG	13	23
HEK3-TD L10R10 pegRNA	GTCTCATGCCAAGCTCCCTGC	ATCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAATGTCCTGCAGGGAGCTTGGCAT	13	25
ENG+180 pegRNA	GTTTCTGCAAGACCTGTTGG	CATGTCCTCTTCCTGGAGTTCCCAACGGTGAGTGTCCCGGATGTTTCTCCAACAGGTCTTGCAG	13	25
ENG-180 pegRNA	CTTCCTGGAGTTCCCAACGG	AATGGACTGTTTCTGCAAGACCTGTTGGAGAAACATCCGGGACACTCACCGTTGGGAACTCCAG	13	25
SATB2+209 pegRNA	GCTCCATGCTGCTCCGACTC	GGCCGTGGGAGGTAACAGCCCGGAGCAGGGATGTCTCCGAGTCGGAGCAGCATG	13	25

SATB2-209 pegRNA	GAGGTAACAGCCCGGAGCAG	GCTCCGCCGCTCCATGCTGCTCCGACTCGGAGACATCCCTGCTCCGGGCTGTTA	13	25
NCF2+149 pegRNA	AGGAGAACAGGGCCTGGCCT	GTGAGGTAAGGAGAACAGGGCCTGGTTTCTCCCTCTGTCCTGCAGATATG	13	25
NCF2-149 pegRNA	AAATCATATCTGCAGGACAG	TGATAGCCAAATCATATCTGCAGGACAGAGGGAGAAACCAGGCCCTGTTC	13	25
RYR1+85 pegRNA	TATGGAGGTAGGTCATGTCT	TCACCAAAAGCGTCGATGATCAGACCTGGGGTGGCCATGACCTACCTC	13	25
RYR1-85 pegRNA	AGCGTCGATGATCAGACCTG	AAGGAGGATATGGAGGTAGGTCATGGCCACCCCAGGTCTGATCATCGA	13	25

Supplemental Table 3. Summary of primers for amplification of each target sites.

Target site	Forward primer	Reverse primer	Figures
FANCF	AAGACGCTGGGAGATTGACA	CAAAGCGCCGATGGATGTG	Fig. 1B
EMX1	TGGGGGCCTCCTGAGTTT	CTCTGCCCTCGTGGGTTTGT	Fig. 1B
FANCF	CATCATCTCGCACGTGGTTC	TGCTGACGTAGGTAGTGCTT	Fig. 2B
EMX1	GCTTCCAGAGCCTGCACTCC	TCGATGTCCTCCCCATTGGC	Fig. 2B
AAVS1	GAACTCTGCCCTCTAACGCT	AGAGGTTCTGGCAAGGAGAG	Fig. 2B
HEK3	GTGAGCTGCTGTCCCAGAGG	ATGCAGGTGCTGAAAGCCAC	Fig. 2B
HEK3	CACGTGATGGCAGAGGAAAG	GGCATTGTTATTTCCCTCCCA	Fig. 2B
HEK3 TD136-bp	TTGATGAATCAGTGCTGGAG	GAGCTGCACATACTAGCC	Supplemental Fig. S22B
EMX1 TD 5-kb	TGGGGGCCTCCTGAGTTT	AGCTCCTCACAGAACCACTC	Fig. 2D
EMX1 TD 10-kb	TGGGGGCCTCCTGAGTTT	CAACACACACACTGGAGACT	Fig. 2D
AAVS1	CCTGTGCCATCTCTCGTTTC	AGAGGTTCTGGCAAGGAGAG	Fig. 3B
ENG	CTAACGAGGACTCAGCCACTG	AGTCCCTAGGAGACGTTTGGA	Fig. 3C
SATB2	TAATCCTACACCGCGACAGC	TGGCCTGAACGTCTATCACG	Fig. 3C
NCF2	CATGGTCCTTTGCGCTTCAC	GGGAAGGGAGAAGCAGACTC	Fig. 3C
RYR1	GCAAGCCCTGGAGGTAGGTA	CACCCCCTTTCCTAACTGGC	Fig. 3C

Supplemental Table S4. Primers used for qPCR to detect the copy number of target DNA fragments.

Target site	Forward primer	Reverse primer
EMX1 TD 5-kb/10-kb flanking (plasmid)	AGGGCTCCCATCACATCAA	ACGCGTTTGCTCTACCAGCC
EMX1 TD 5-kb (plasmid)	CTGTGACCCTTTGTTTGAGA	CACCGTGAGCTAGGAACAAA
EMX1 TD 10-kb (plasmid)	CTGCCATCCCCTTCTGTGAA	CTTCCCCTTTCTCCACCTCT
EMX1 TD 5-kb/10-kb flanking	GGCCAATGGGGAGGACATC	TTGTCCCTCTGTCAATGGCG
EMX1 TD 5-kb	GGCCTGAGTCCGAGCAGAA	TCCCCCTCTTATCCATCCCTC
EMX1 TD 10-kb	CGAGCAGAAGAAGCATGTGGG	GTTTCTGCATGGGCGTGTG

Supplemental Table S5. Sequences of primers used for HTS.

sample name	Primer Name	Sequence
FANCF TD 50-bp	*FANCF*-HTS-1-forward	acacagtAGGCGTATCATTTCGCGGAT
	FANCF-HTS-reverse	AAAAGCGATCCAGGTGCTGC
FANCF TD 50-bp	*FANCF*-HTS-2-forward	acagtcaAGGCGTATCATTTCGCGGAT
	FANCF-HTS-reverse	AAAAGCGATCCAGGTGCTGC
FANCF TD 50-bp	*FANCF*-HTS-3-forward	actctgaAGGCGTATCATTTCGCGGAT
	FANCF-HTS-reverse	AAAAGCGATCCAGGTGCTGC
FANCF-CTR	*FANCF*-HTS-4-forward	gtagtctAGGCGTATCATTTCGCGGAT
	FANCF-HTS-reverse	AGGTAGCGCGCCCACTGCAA
FANCF-CTR	*FANCF*-HTS-5-forward	gtcacatAGGCGTATCATTTCGCGGAT
	FANCF-HTS-reverse	AGGTAGCGCGCCCACTGCAA
FANCF-CTR	*FANCF*-HTS-6-forward	gtctagtAGGCGTATCATTTCGCGGAT
	FANCF-HTS-reverse	AGGTAGCGCGCCCACTGCAA
EMX1 TD 57-bp	*EMX1*-HTS-1-forward	catcagaTGGGGGCCTCCTGAGTTT
	EMX1-HTS-reverse	TGATGTGATGGGAGCCCTTC
EMX1 TD 57-bp	*EMX1*-HTS-2-forward	catgtctTGGGGGCCTCCTGAGTTT
	EMX1-HTS-reverse	TGATGTGATGGGAGCCCTTC
EMX1 TD 57-bp	*EMX1*-HTS-3-forward	cacaagtTGGGGGCCTCCTGAGTTT
	EMX1-HTS-reverse	TGATGTGATGGGAGCCCTTC
EMX1-CTR	*EMX1*-HTS-4-forward	acacgtaTGGGGGCCTCCTGAGTTT
	EMX1-HTS-reverse	TGATGTGATGGGAGCCCTTC
EMX1-CTR	*EMX1*-HTS-5-forward	acagatcTGGGGGCCTCCTGAGTTT
	EMX1-HTS-reverse	TGATGTGATGGGAGCCCTTC
EMX1-CTR	*EMX1*-HTS-6-forward	acagcatTGGGGGCCTCCTGAGTTT
	EMX1-HTS-reverse	TGATGTGATGGGAGCCCTTC
AAVS1 TD 376-bp	*AAVS1* TD-376 -HTS -1-forward	cttgagtATTCCCAGGGCCGGTTAATG
	AAVS1 TD-376 -reverse	GTGACCCGAATCCACAGGAG
AAVS1 TD 376-bp	*AAVS1* TD-376 -HTS -2-forward	ctcacgaATTCCCAGGGCCGGTTAATG
	AAVS1 TD-376 -reverse	GTGACCCGAATCCACAGGAG
AAVS1 TD 376-bp	*AAVS1* TD-376 -HTS -3-forward	ctcagacATTCCCAGGGCCGGTTAATG
	AAVS1 TD-376 -reverse	GTGACCCGAATCCACAGGAG
AAVS1 TD 532-bp	*AAVS1* TD-532 -HTS -1-forward	ctctcagATTCCCAGGGCCGGTTAATG
	AAVS1 TD-532 -reverse	GAGACATCCGTCGGAGAAGG
AAVS1 TD 532-bp	*AAVS1* TD-532 -HTS -2-forward	ctctgtaATTCCCAGGGCCGGTTAATG
	AAVS1 TD-532 -reverse	GAGACATCCGTCGGAGAAGG
AAVS1 TD 532-bp	*AAVS1* TD-532 -HTS -3-forward	ctgatgtATTCCCAGGGCCGGTTAATG
	AAVS1 TD-532 -reverse	GAGACATCCGTCGGAGAAGG
AAVS1 TD 846-bp	*AAVS1* TD-846-HTS -1-forward	ctgtagaATTCCCAGGGCCGGTTAATG
	AAVS1 TD-846 -reverse	CAAGCTGCAGACAGAAAGCG
AAVS1 TD 846-bp	*AAVS1* TD-846 -HTS -2-forward	gaacactATTCCCAGGGCCGGTTAATG
	AAVS1 TD-846 -reverse	CAAGCTGCAGACAGAAAGCG
AAVS1 TD 846-bp	*AAVS1* TD-846 -HTS -3-forward	gatctcaATTCCCAGGGCCGGTTAATG
	AAVS1 TD-846-reverse	CAAGCTGCAGACAGAAAGCG

EMX1 TD 116-bp	*EMX1* TD-116-HTS -1-forward	cacactgTCCAGAACCGGAGGACAAAG
	EMX1 TD-116-reverse	ACCACACCTTCACCTGGG
EMX1 TD 116-bp	*EMX1* TD-116-HTS -2-forward	cacttgaTCCAGAACCGGAGGACAAAG
	EMX1 TD-116-reverse	ACCACACCTTCACCTGGG
EMX1 TD 116-bp	*EMX1* TD-116-HTS -3-forward	cactgatTCCAGAACCGGAGGACAAAG
	EMX1 TD-116-reverse	ACCACACCTTCACCTGGG
EMX1 TD 201-bp	*EMX1* TD-201-HTS -1-forward	cagatcaTGGGGGCCTCCTGAGTTT
	EMX1 TD-201-reverse	CCTCAACACTCAGGCTGAGC
EMX1 TD 201-bp	*EMX1* TD-201-HTS -2-forward	cagacatTGGGGGCCTCCTGAGTTT
	EMX1 TD-201-reverse	CCTCAACACTCAGGCTGAGC
EMX1 TD 201-bp	*EMX1* TD-201-HTS -3-forward	cagagtcTGGGGGCCTCCTGAGTTT
	EMX1 TD-201-reverse	CCTCAACACTCAGGCTGAGC
EMX1 TD 307-bp	*EMX1* TD-307-HTS -1-forward	cagtactGTTTCTCATCTGTGCCCCTCC
	EMX1 TD-307-reverse	GCTCCCATGGGTCTAACATTC
EMX1 TD 307-bp	*EMX1* TD-307-HTS -2-forward	cagtctaGTTTCTCATCTGTGCCCCTCC
	EMX1 TD-307-reverse	GCTCCCATGGGTCTAACATTC
EMX1 TD 307-bp	*EMX1* TD-307-HTS -3-forward	ctagacaGTTTCTCATCTGTGCCCCTCC
	EMX1 TD-307-reverse	GCTCCCATGGGTCTAACATTC
FANCF TD 115-bp	*FANCF* TD-115-HTS -1-forward	cagtactATAGCATTGCAGAGAGGCGT
	FANCF TD-115-reverse	GGTCGAAATGCATGTCAATC
FANCF TD 115-bp	*FANCF* TD-115-HTS -1-forward	cagtactATAGCATTGCAGAGAGGCGT
	FANCF TD-115-reverse	GGTCGAAATGCATGTCAATC
FANCF TD 115-bp	*FANCF* TD-115-HTS -1-forward	cagtactATAGCATTGCAGAGAGGCGT
	FANCF TD-115-reverse	GGTCGAAATGCATGTCAATC
FANCF TD 171-bp	*FANCF* TD-171-HTS -1-forward	tgtcagtATAGCATTGCAGAGAGGCGT
	FANCF TD-171-reverse	GGTCGAAATGCATGTCAATC
FANCF TD 171-bp	*FANCF* TD-171-HTS -1-forward	tgtgtcaATAGCATTGCAGAGAGGCGT
	FANCF TD-171-reverse	GGTCGAAATGCATGTCAATC
FANCF TD 171-bp	*FANCF* TD-171-HTS -1-forward	caactgtATAGCATTGCAGAGAGGCGT
	FANCF TD-171-reverse	GGTCGAAATGCATGTCAATC
FANCF TD 231-bp	*FANCF* TD-231-HTS -1-forward	catcagaCGCTGGGAGATTGACATGCA
	FANCF TD-231-reverse	CCGTGTTCCTTGACTCTGGG
FANCF TD 231-bp	*FANCF* TD-231-HTS -1-forward	catgtctCGCTGGGAGATTGACATGCA
	FANCF TD-231-reverse	CCGTGTTCCTTGACTCTGGG
FANCF TD 231-bp	*FANCF* TD-231-HTS -1-forward	cacaagtCGCTGGGAGATTGACATGCA
	FANCF TD-231-reverse	CCGTGTTCCTTGACTCTGGG
HEK3 TD 136-bp	*HEK3* TD-136-HTS -1-forward	gacatctGCATTTGTAGGCTTGATGCT
	HEK3 TD-136-reverse	ATGCTTACTTGCAGCAGAAA
HEK3 TD 136-bp	*HEK3* TD-136-HTS -2-forward	gacagtaGCATTTGTAGGCTTGATGCT
	HEK3 TD-136-reverse	ATGCTTACTTGCAGCAGAAA
HEK3 TD 136-bp	*HEK3* TD-136-HTS -3-forward	gactacaGCATTTGTAGGCTTGATGCT
	HEK3 TD-136-reverse	ATGCTTACTTGCAGCAGAAA
HEK3 TD 222-bp	*HEK3* TD-222-HTS -1-forward	gactcacGCATTTGTAGGCTTGATGCT
	HEK3 TD-222-reverse	ATGCTTACTTGCAGCAGAAA
HEK3 TD 222-bp	*HEK3* TD-222-HTS -2-forward	gagtcgtGCATTTGTAGGCTTGATGCT

	HEK3 TD-222-reverse	ATGCTTACTTGCAGCAGAAA
HEK3 TD 222-bp	*HEK3* TD-222-HTS -3-forward	gtacagaGCATTTGTAGGCTTGATGCT
	HEK3 TD-222-reverse	ATGCTTACTTGCAGCAGAAA
HEK3 TD 397-bp	*HEK3* TD-397-HTS -1-forward	gtagtctTGAGCACTTGTTACACGCAG
	HEK3 TD-397-reverse	CTGATTTTCATGCAGGTGCT
HEK3 TD 397-bp	*HEK3* TD-397-HTS -2-forward	gtcacatTGAGCACTTGTTACACGCAG
	HEK3 TD-397-reverse	CTGATTTTCATGCAGGTGCT
HEK3 TD 397-bp	*HEK3* TD-397-HTS -3-forward	gtctagtTGAGCACTTGTTACACGCAG
	HEK3 TD-397-reverse	CTGATTTTCATGCAGGTGCT
HEK3 TD 499-bp	*HEK3* TD-499-HTS -1-forward	gtcgtgaGTTACACGCAGGGCACTATG
	HEK3 TD-499-reverse	TGTTGAGCTCGACCCTGAAG
HEK3 TD 499-bp	*HEK3* TD-499-HTS -2-forward	gtgaacaGTTACACGCAGGGCACTATG
	HEK3 TD-499-reverse	TGTTGAGCTCGACCCTGAAG
HEK3 TD 499-bp	*HEK3* TD-499-HTS -3-forward	gtgactcGTTACACGCAGGGCACTATG
	HEK3 TD-499-reverse	TGTTGAGCTCGACCCTGAAG
EMX1 TD 5-kb	*EMX1* TD 5kb-HTS-1-forward	atctgctTCCAGAACCGGAGGACAAAG
	EMX1 TD 5kb-reverse	TCCCCCTCTTATCCATCCCTC
EMX1 TD 5-kb	*EMX1* TD 5kb-HTS-2-forward	atgtgacTCCAGAACCGGAGGACAAAG
	EMX1 TD 5kb-reverse	TCCCCCTCTTATCCATCCCTC
EMX1 TD 5-kb	*EMX1* TD 5kb-HTS-3-forward	atgcactTCCAGAACCGGAGGACAAAG
	EMX1 TD 5kb-reverse	TCCCCCTCTTATCCATCCCTC
EMX1 TD 10-kb	*EMX1* TD 10kb-HTS-1-forward	acaactgGTTCCAGAACCGGAGGACAAA
	EMX1 TD 10kb-reverse	CCAGCCTCCCTACTGTCTTTTT
EMX1 TD 10-kb	*EMX1* TD 10kb-HTS-2-forward	acatcgaGTTCCAGAACCGGAGGACAAA
	EMX1 TD 10kb-reverse	CCAGCCTCCCTACTGTCTTTTT
EMX1 TD 10-kb	*EMX1* TD 10kb-HTS-3-forward	acatgagGTTCCAGAACCGGAGGACAAA
	EMX1 TD 10kb-reverse	CCAGCCTCCCTACTGTCTTTTT

Supplemental Reference

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