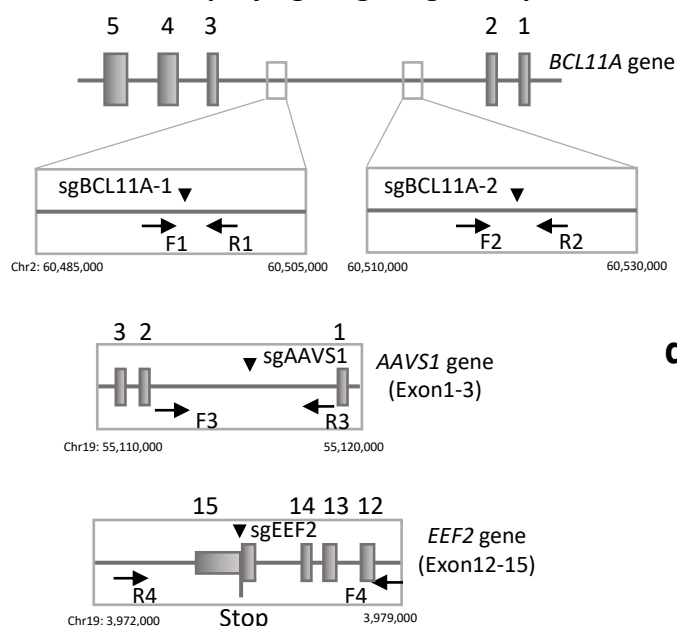


Fig. S1

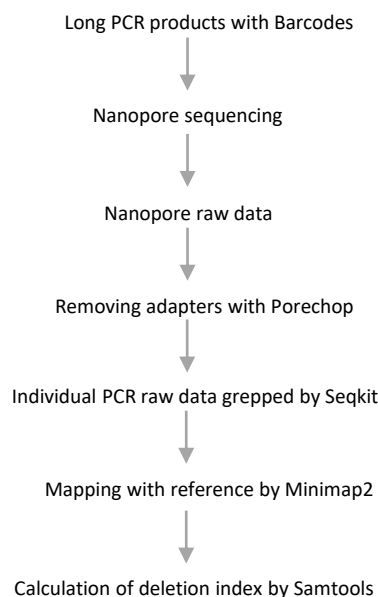
a

Primers for amplifying long-range PCR products



b

Overview for nanopore sequencing data analysis



Command lines for grepping individual PCR sequences:

```

# Extract specific PCR sequences by forward barcodes
seqkit grep -s -f PCR_reads_extraction_forward_barcodes.txt
nanopore_chop.fastq >> PCR-Fwd.temp

# Reverse nanopore chop data
seqkit seq nanopore_chop.fastq -r -p -v -o nanopore_chop_reverse.fastq

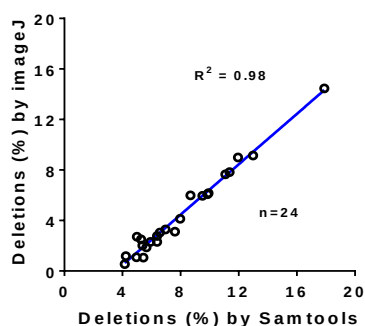
# Extract specific PCR sequences from reversed chop data by forward barcodes
seqkit grep -s -f PCR_reads_extraction_forward_barcodes.txt
nanopore_chop_reverse.fastq >> PCR-Fwd.temp

# Extract specific PCR sequences from forward extracted data by reward
barcodes
seqkit grep -s -f PCR_reads_extraction_reward_barcodes.txt PCR-Fwd.temp >>
PCR.fastq

# Extract PCR reads with barcodes sequences
seqkit grep -R 1:20 -s -f Primer_barcode_file.txt PCR.fastq -o
Primer_barcode_PCR_reads.fastq
  
```

c

Correlation of deletion indexes analyzed by Samtools and ImageJ



d

Background deletion indexes in unedited cells

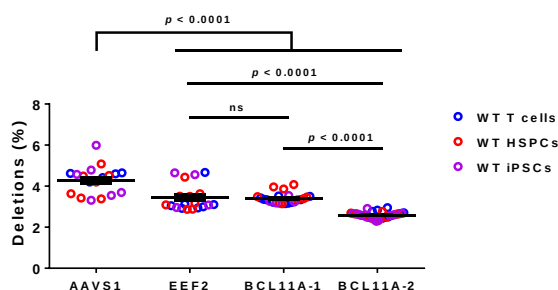


Fig. S2

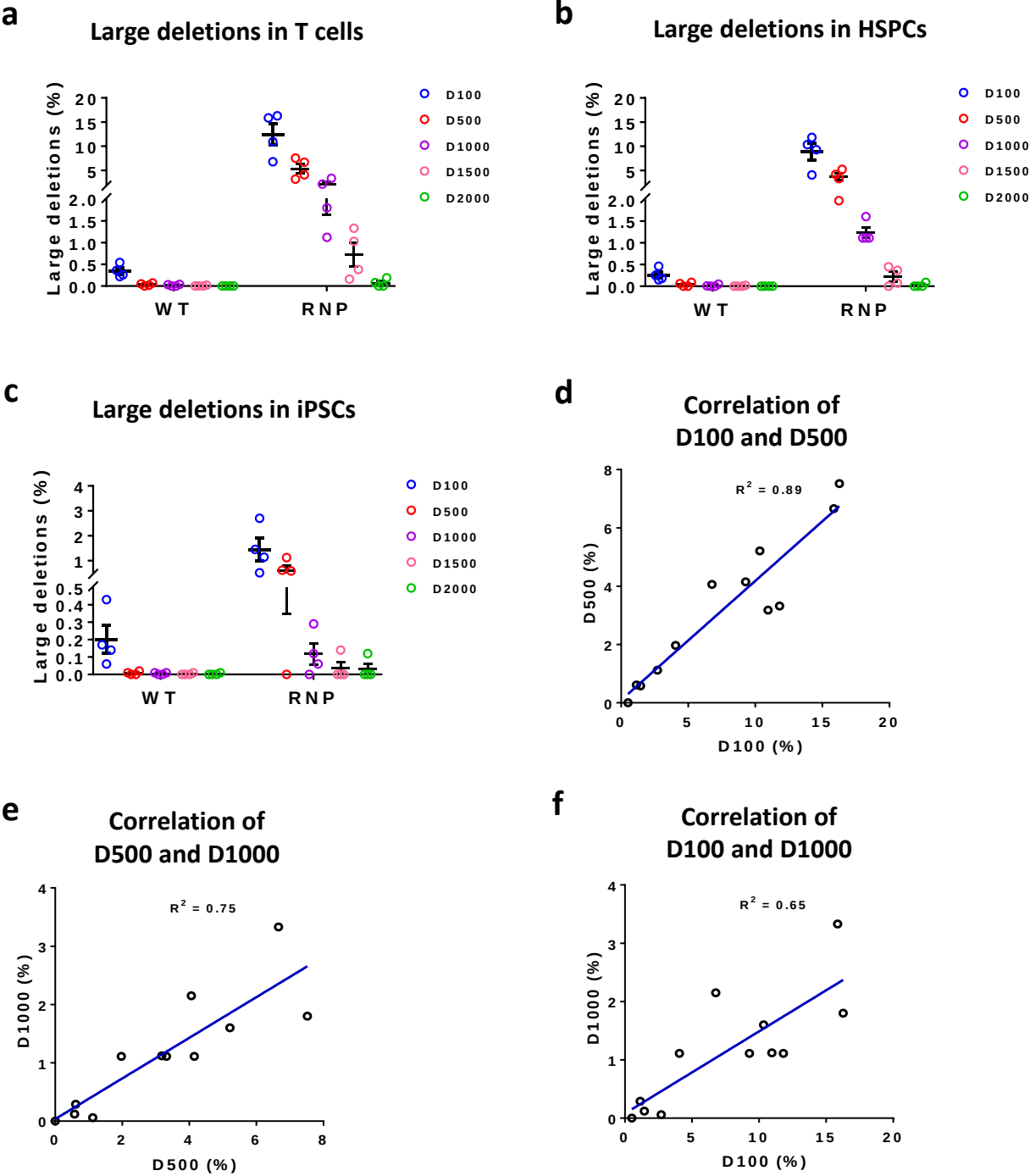


Fig. S3

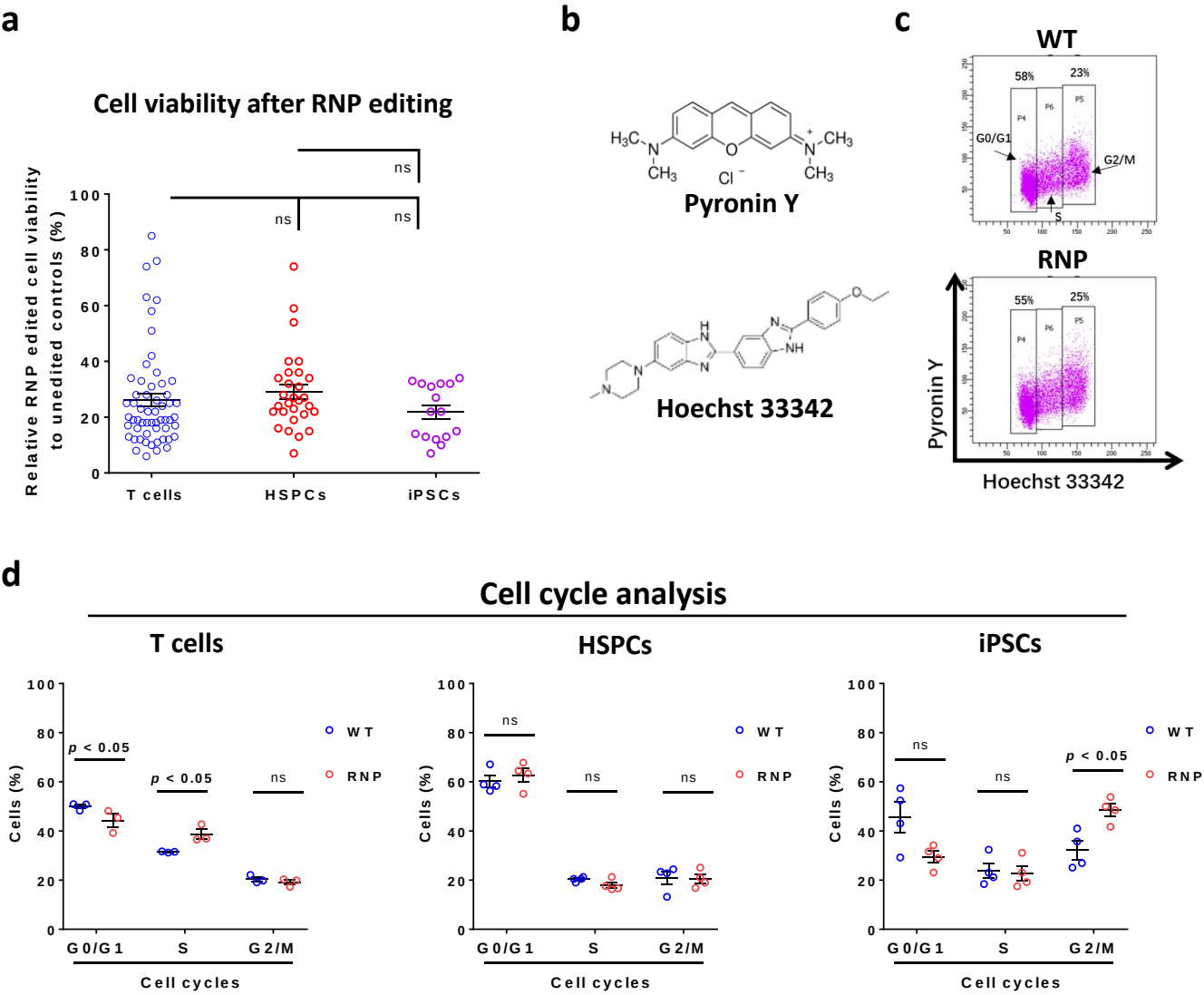
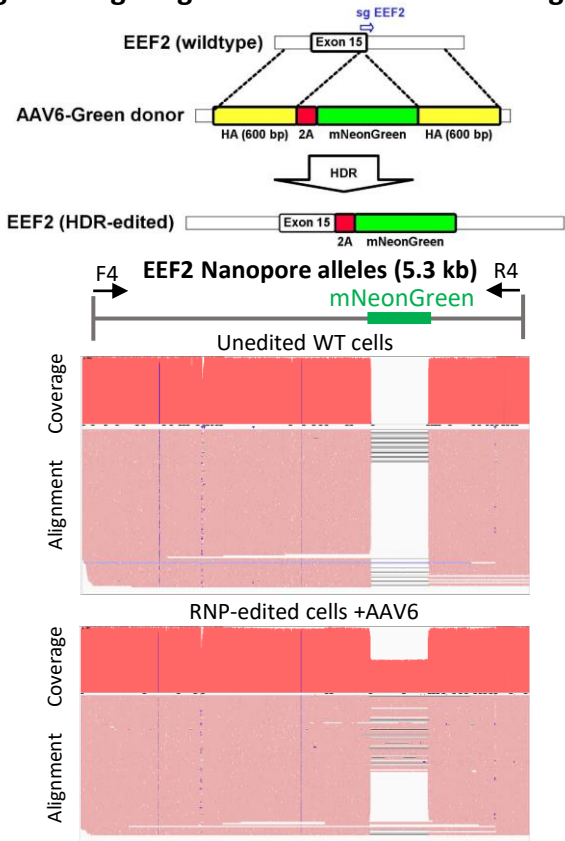
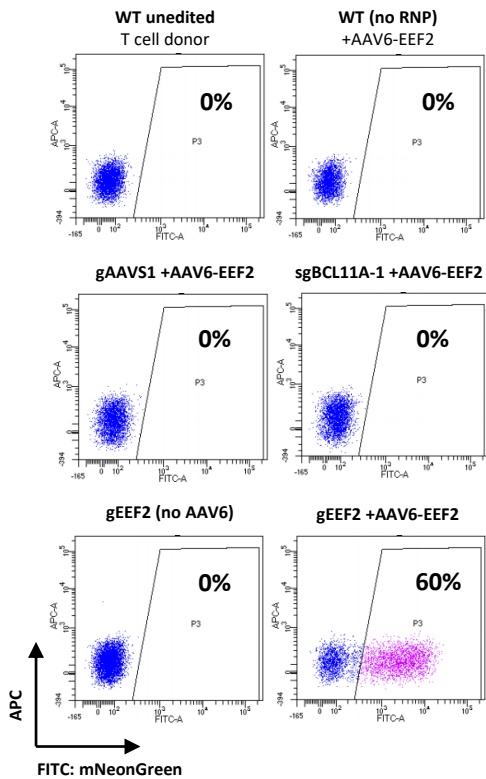


Fig. S4

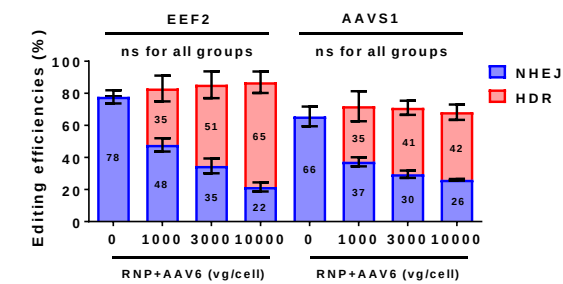
a gRNA targeting *EEF2* and AAV6 donor design



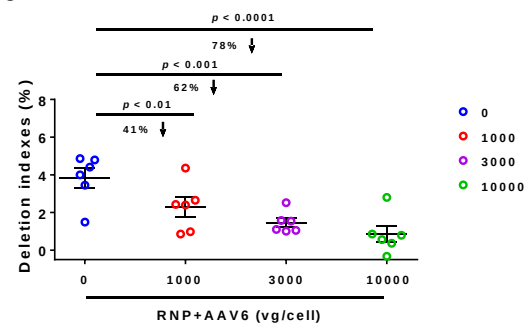
b



c Small indels and HDR



d Deletion indexes



e AAVS1 Nanopore alleles (3.9 kb)

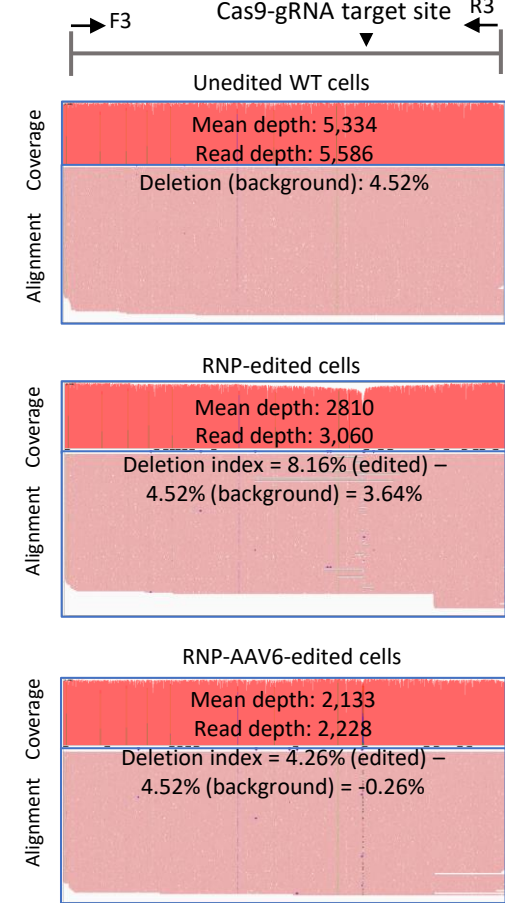
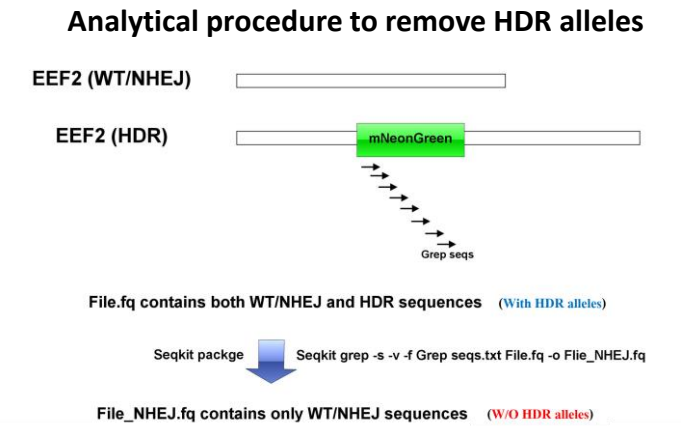
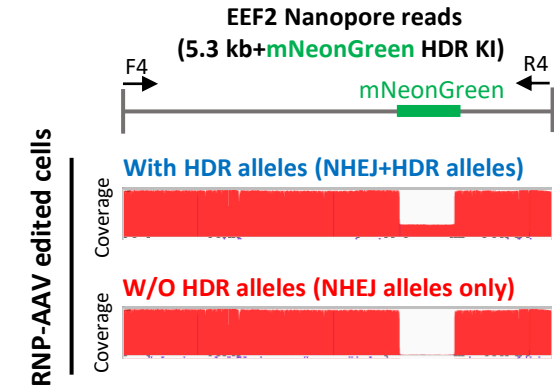


Fig. S5

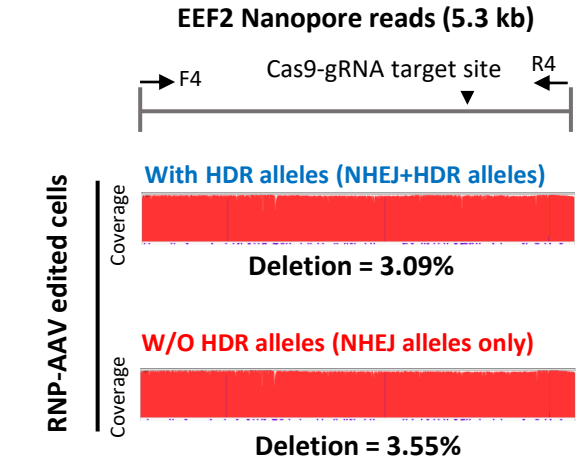
a



b



c



d

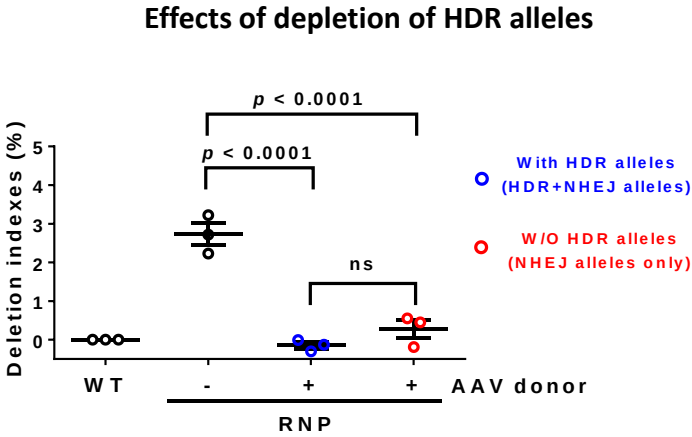


Fig. S6

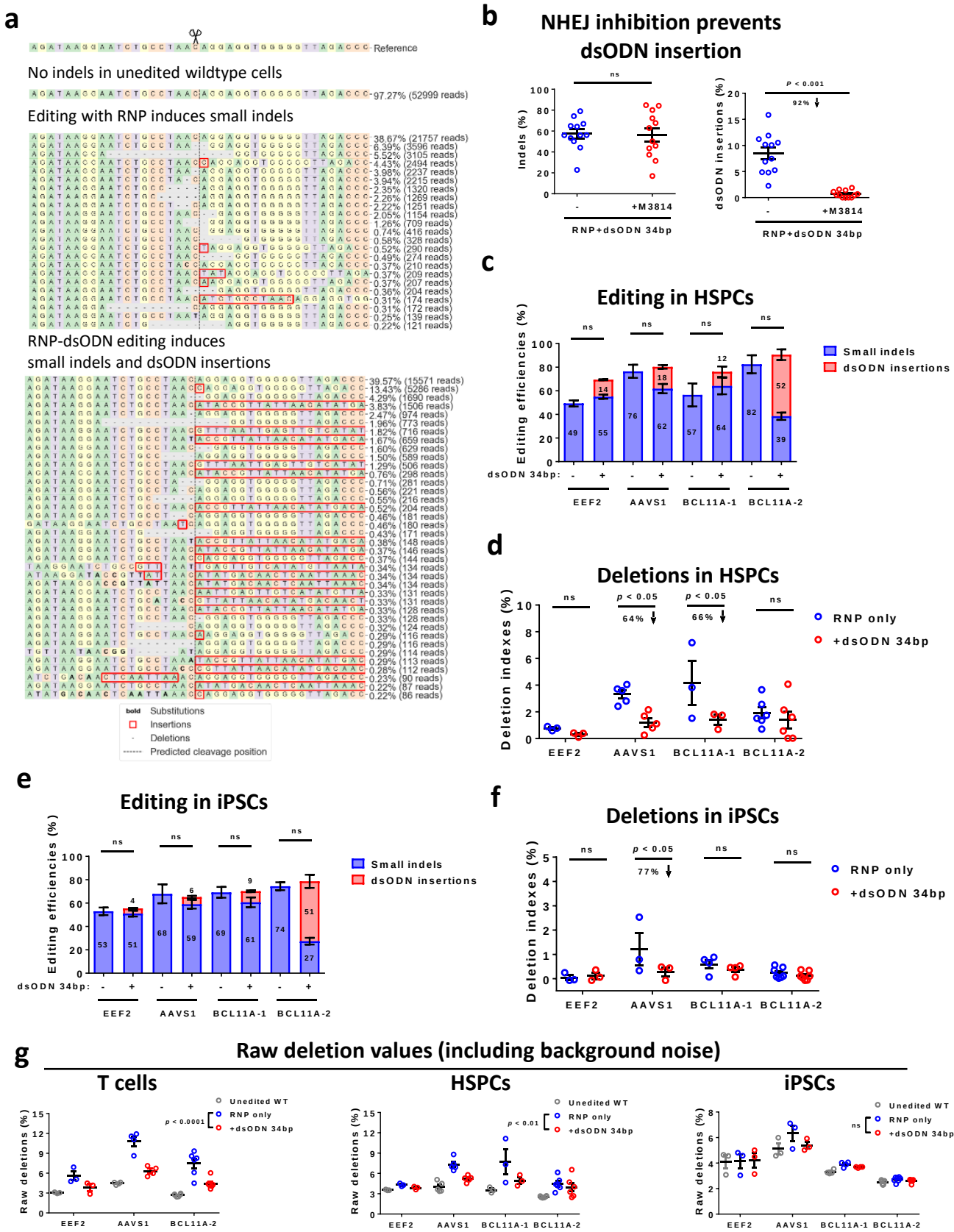
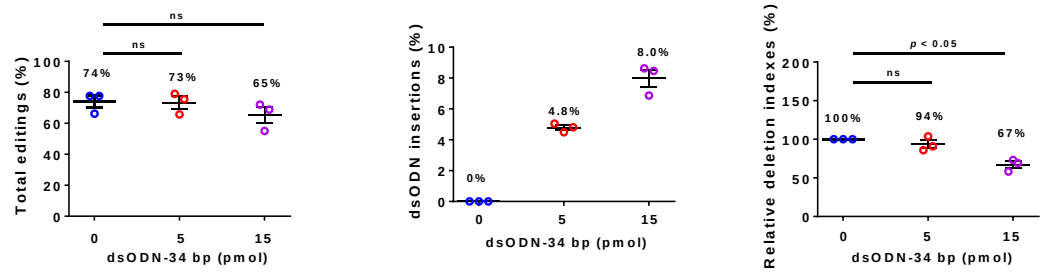


Fig. S7

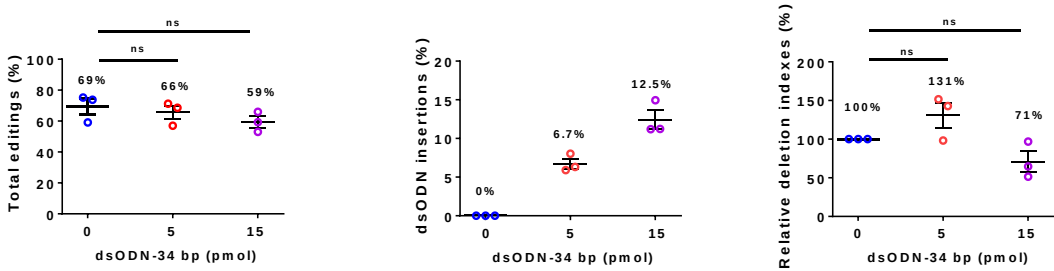
dsODN-mediated editing at *EEF2*

a



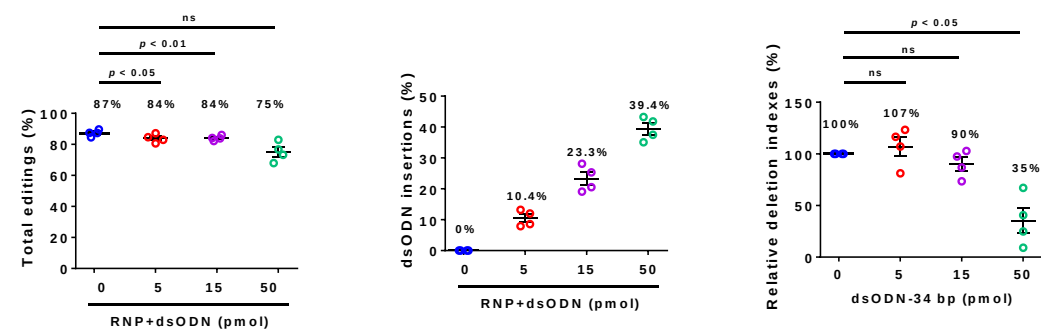
dsODN-mediated editing at *AAVS1*

b



dsODN-mediated editing at *BCL11A-2*

c



d

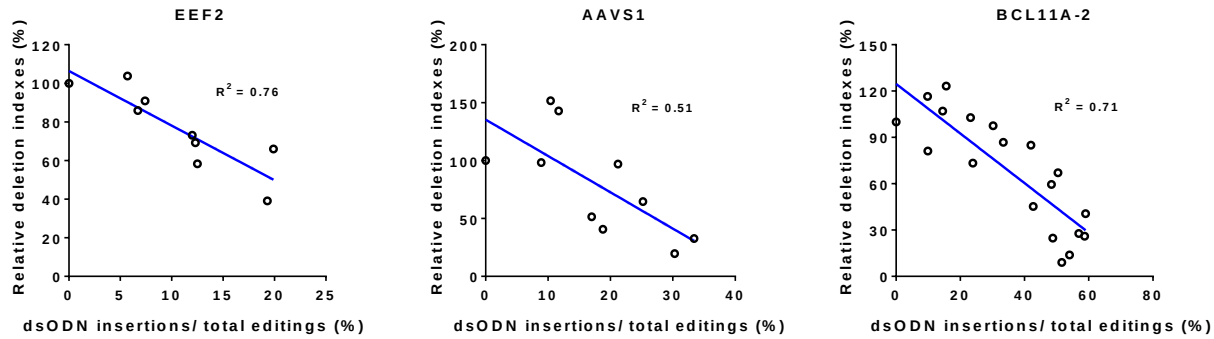
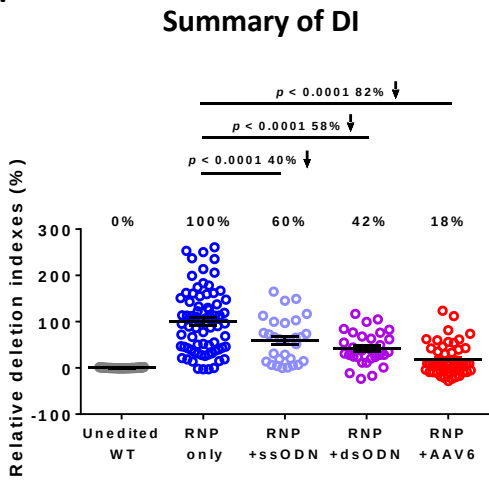
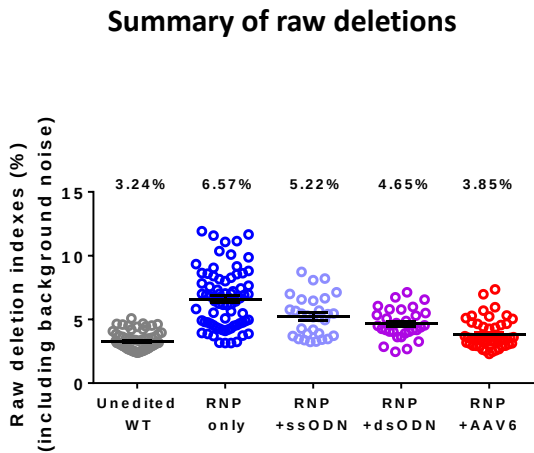


Fig. S8

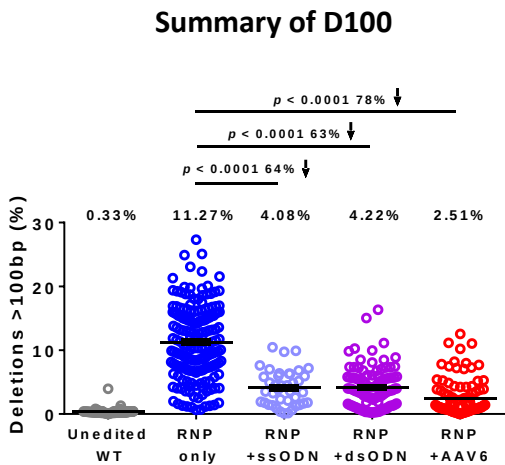
a



b



c



d

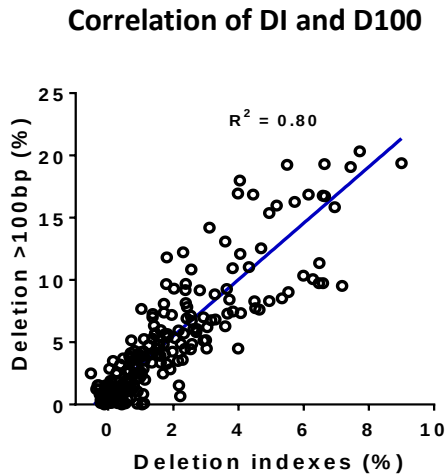


Fig. S9

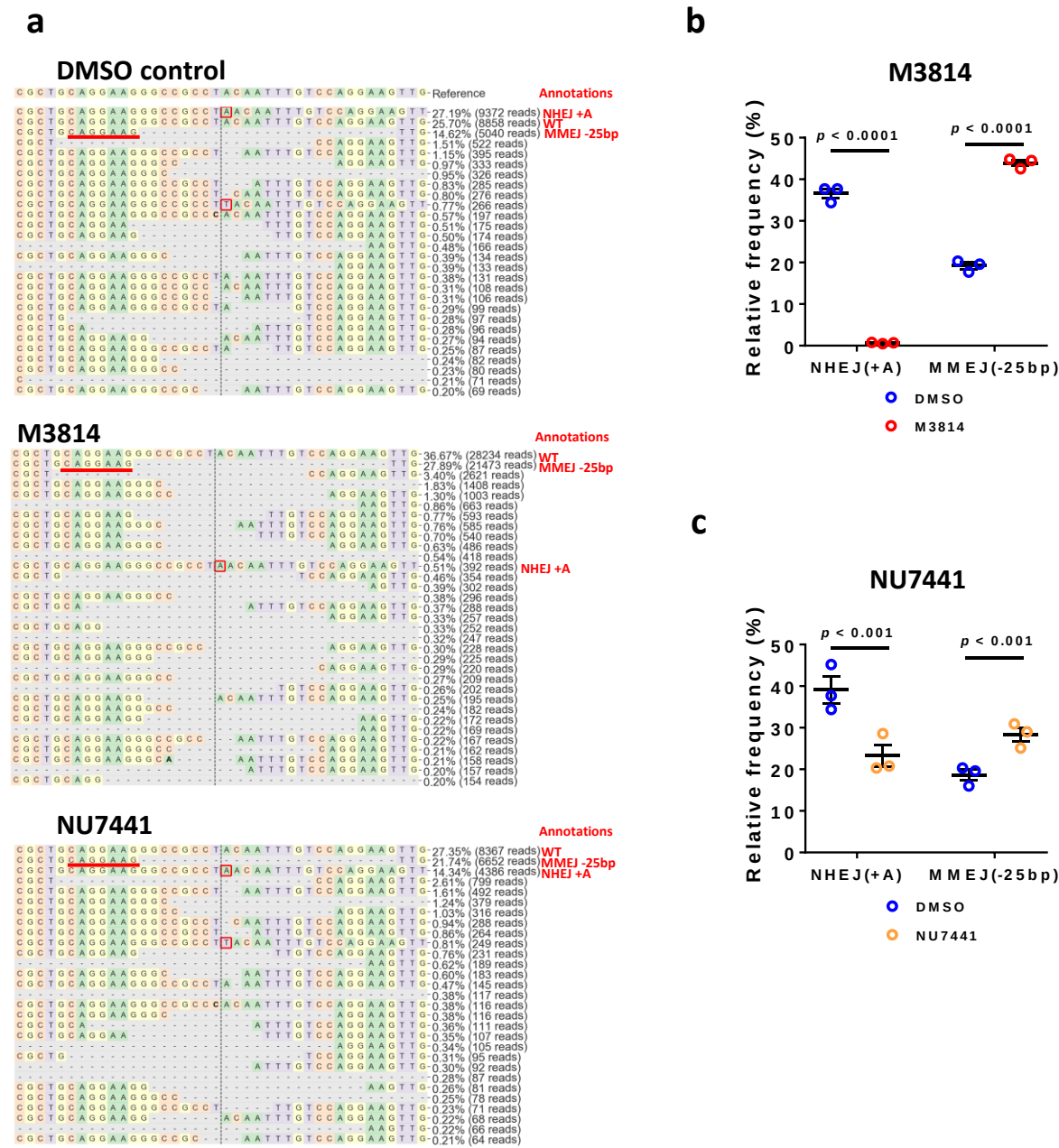
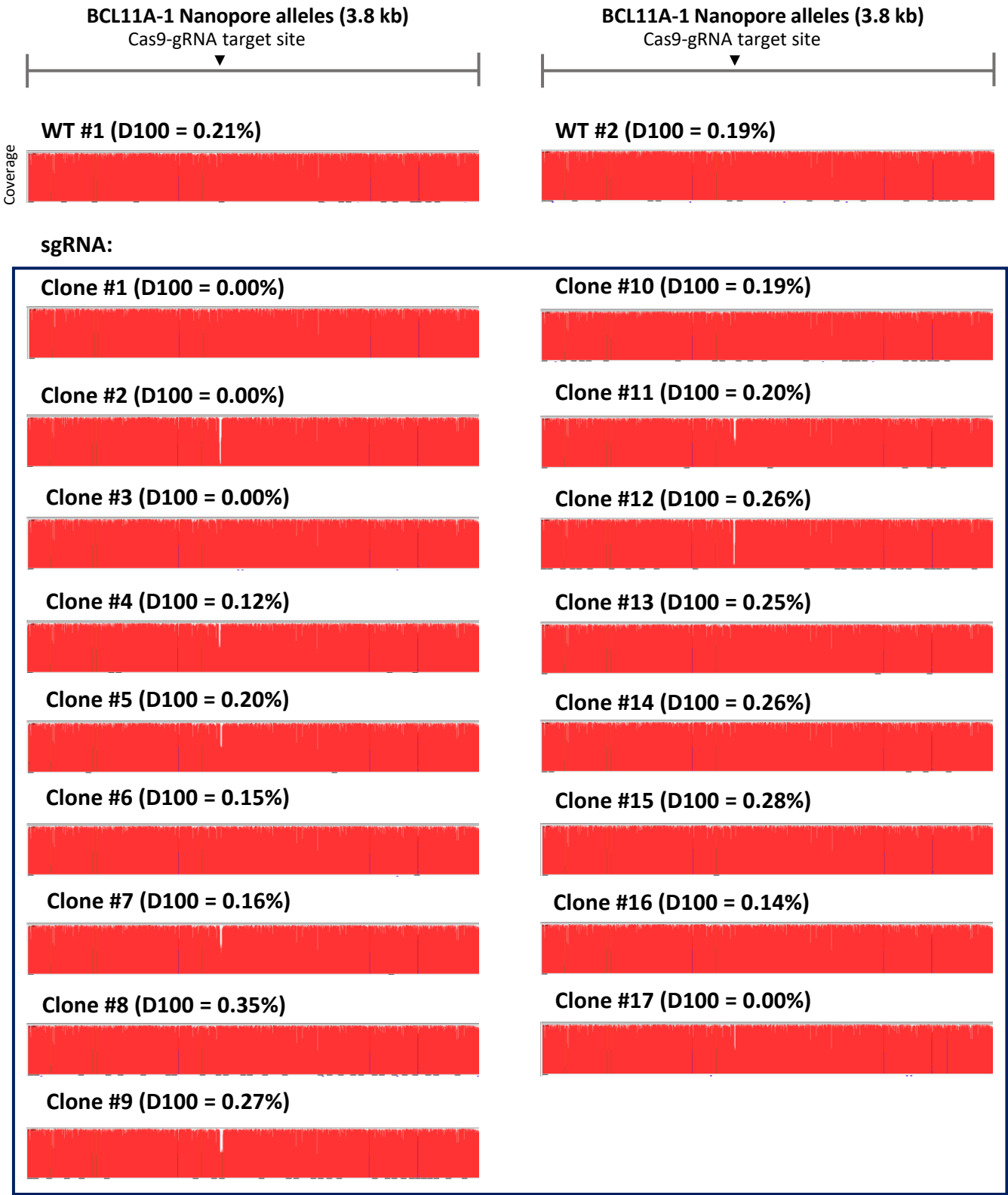


Fig. S10



Continued

BCL11A-1 Nanopore alleles (3.8 kb)
Cas9-gRNA target site



BCL11A-1 Nanopore alleles (3.8 kb)
Cas9-gRNA target site



sgRNA+AAV6:

Clone #18 (D100 = 0.12%)



Clone #19 (D100 = 0.15%)



Clone #20 (D100 = 0.26%)



Clone #21 (D100 = 0.00%)



Clone #22 (D100 = 0.29%)



Clone #23 (D100 = 0.30%)



sgRNA+ssODN:

Clone #24 (D100 = 0.20%)



Clone #25 (D100 = 0.22%)



Clone #26 (D100 = 0.22%)



Clone #27 (D100 = 0.22%)



Clone #28 (D100 = 0.17%)



Clone #29 (D100 = 0.21%)



Clone #30 (D100 = 0.12%)



Clone #31 (D100 = 62.37%: 428-bp deletion)



Clone #32 (D100 = 0.19%)



Clone #33 (D100 = 0.18%)



Clone #34 (D100 = 0.26%)



Clone #35 (D100 = 0.27%)



Clone #36 (D100 = 0.50%)



Clone #37 (D100 = 0.00%)



Clone #38 (D100 = 0.38%)



Continued

BCL11A-1 Nanopore alleles (3.8 kb)
Cas9-gRNA target site

BCL11A-1 Nanopore alleles (3.8 kb)
Cas9-gRNA target site



sgRNA+dsODN:

Clone #39 (D100 = 0.00%)



Clone #40 (D100 = 0.23%)



Clone #41 (D100 = 0.23%)



Clone #42 (D100 = 0.23%)



Clone #43 (D100 = 0.18%)



Clone #44 (D100 = 0.16%)



Clone #45 (D100 = 0.19%)



Clone #46 (D100 = 0.39%)



Clone #47 (D100 = 0.11%)



Clone #48 (D100 = 0.15%)



Clone #49 (D100 = 0.11%)



Clone #50 (D100 = 0.18%)



Clone #51 (D100 = 0.00%)



Clone #52 (D100 = 0.49%)



Fig. S11

AAV6 HDR donor sequences (Left HA-Insert-Right HA)

EEF2-AAV6
CAGCTTGTGGACCCCTAAATCACTGAATTCACAGGGGAGGGGCTCTCTATCCCCAGTGTGAGAAGGGCTCTGGGCTGGAGCTCTGAAGGCCACGCCCTG
GGCCGGTAGAGCAGCCGAGCTGTAGCACAGGGTTGTCCCAAACGAGCAGCGGCATGAGGCCATGAGTGGCCTGCTAGGCCCTTCTGTGAAGTGTGGGCACC
AGGCCGAGTGTCTGGTCTGCAGGGTGACTCAGGCTGAGGAACTAGCCTGAGCTCCTGACAGGACTTTCCTTCTGCCCTGCCACCTTCTCGATGGCCAGTGAGC
CTCTCGCTTCCCTCTGCAGGCTTACCGCTGACCTGAGGTCCAACACGGGCGGCCAGGCGTTCCCCCAGTGTGTGTTTGACCACTGGCAGATCCTGCCCGGAGA
CCCCCTGCACAACAGCAGCCGCCACGCCAGGTGGTGGCGGAGACCCGCAAGCGCAAGGGCCTGAAAGAAGGCATCCTGCCCTGGACAACCTTCTGGACA
AATTG**CAGTGTACTAATTATGCTCTCTGAAATTTGGCTGGAGATGTTGAGAGCAACCCAGGTCCCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCCTCTCTC**
CCAGCGCACATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAAGGCACCGCAATCCAAATGATGGTTATGAGGAGTTAAAC
CCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGTCGCC
TTTCCAGGCCGCGCATGGTAGATGGCTCCGGATACCAAGTCCATCGCACAAATGCAAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTACGAGG
GAAGCCACATCAAAGGAGAGGGCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCTGTGATGACCAACTCGCTGACCGCTGCGGACTGGTGACGGTCGA
AGAAGACTTACCCCAACGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGCAAGCGCTACCGGAGCACTGCGCGGACCACTACAC
CTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAACAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAG
TGGCAAAGGCCCTTACCGATGTGATGGGCATGGACGAGCTGTACAAGTAACGCGTGCGGCCCTTCTGCAGCGCTGCCGCCCGGGGACTCGCAGCACCC
ACAGCACACGCTCCTCGAATTCTCAGACGACACCTGGAGACTGTCCCGACAGCAGCAGCCTCCCTGAGAGGTTCTGGGGCCCGCTGCGTGCCATCACTCAA
CCATAACACTTGATGCCGTTTCTTTCAATATTTATTTCCAGAGTCCGGAGGCAGCAGACACGCCCTCTTAGTAGGGACTTAATGGGCCGCTGGGGAGGGGGAG
GCGGGATGGGACACCAACTTTTTCCATTTTTCAGAGGGAAACTCAGATGTCCAACTAATTTTAAACAAACGCATTAAAGAGGTTTATTTGGGTACATGGCCCC
GCAGTGGCTTTTGCCCAAGAAAGGGGAAAGAACACGCGGGTAGATGATTTCTAGCAGGCAGGAAGTCTGTGCGGTGTCAACCATGAGCACCTCCAGCTGTAC
TAGTGCCATTGGAATAATAAATTTGATAAGGTGGTGA**CTCTGTTCTGCATTTTTACAGGTGCTTTCGACAGGGGAGCGGGGCTGCCAGTACTGGGCTCCCTGGAG**
CCTAGAAGGGGACCGGGCCCT

AAVS1-AAV6
GGGCATCTCTCTCCCTCACCAACCCCATGCCGTCTTCACTCGCTGGGTTCCCTTTTCTCTCTCTCTGGGGCTGTGCCATCTCTCGTTTCTTAGGATGGCCTT
CTCCGACGGATGTCTCCCTTGGCTGCCGCCCTCCCTCTTCTGTAGGCCTGCATCATCACCGTTTTCTGGACAACCCCAAGTACCCCGTCTCCCTGGCTTTAGCCA
CTCTCCATCCTCTTCTTCTTCTTGGCTGGACACCCCGTTCTCCTGTGGATTGCGGTCACTCTCACTCCTTTCATTTGGGCAGCTCCCTACCCGCCCTTACCTCTCT
AGTCTGTGCTAGCTCTTCCAGCCCCCTGTATGGCATCTTCCAGGGGTCGAGAGCTCAGCTAGTCTTCTTCTCCAACCCGGGCCCTATGTCCACTTCAGGAC
AGCATGTTTGTGCTCCTCCAGGGATCCTGTGTCCCCGAGCTGGGACCACTTATATTTCCAGGGCCGGTTAATGTGGCTTGGTTCTGGGTACTTTTATCTGTGCC
TCCACCCACAGTGGGGCCACTAGGACAGGATTGGTGACAGAAAGCCCATCCTTAGGCCTCCTCCTTAGTCTCTGGTGATTTGGGTCTAACCC**ACGCGT**
AGTTTAAACTTAGGCAGATTCCTTATCTGGTGACACACCCCACTTCTCTGGAGCCATCTCTCTCCTTGCCAGAACCTTAAGGTTTGCTTACGATGGAGCCAGAG
AGGATCCTGGGAGGGAGAGCTTGGCAGGGGGTGGGAGGGGAAGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGCCACCCCTGCGCTACCCCTCTCCAGAA
CCTGAGCTGCTCTGACGCGGCCGTCTGGTGCCTTCTACTGATCCTGGTGCTGCAGCTTCTTACACTTCCCAAGAGGAGAAGCAGTTTGAAAAACAAATCAG
AATAAGTTGGTCTGAGTCTTAACCTTTGGCTCTTACCTTTCTAGTCCCAATTTATATTGTTCTCCGTGCGTCAGTTTACCTGTGAGATAAGGCCAGTAGCCAG
CCCCGTCTGTGACGGGCTGTGGTGAGGAGGGGGGTGTCGGTGTGGAACCTCCCTTGTGAGAATGGTGCCTCCTAGGTGTTACCAGGTGCTGGCCGCTCT
ACTCCCTTCTCTTCTCCATCCTTCTTCTTAAAGAGTCCCCAGTGCTATCTGGGACATATCTCCGCCAGAGCAGGGTCCCGCTTCCCTAAGGCCCTG

BCL11A-1-AAV6
GGGCAATACAGACTGGTCTGTGATGACAAATAACTCCTAGCTATTCTTAATGATTTATCACCAATGTTCTTCTTCTCAGCTGGAATTTAAATATGGACTATC
CGTAAATAGGAATAATAATAGTATATGCTTCATAGGGTTGTATGAAATAAAATGAGTGCCTATTTGTAAGTTCTTAGAGCAGAGTAAGTGTCCGAGCTTG
TGAATAAAATGCTGCCTCCTGGTATTTATGTTACACCTCAGCAGAAACAAAGTTATCAGGCCCTTCCCAATTCCTAGTTTGGGTGAGAAGAAAGGGGAA
AGGGAGAGGAAAAAGGAAAAAGATATGACGTCAGGGGGAGGCAAGTCAAGTTGGGAACACAGATCCTAACACAGTAGCTGGTACCTGATAGGTGCCTATATGT
GATGGATGGGTGGACAGCCGACAGATGAAAAATGGACAATTATGAGGAGGGGAGAGTGCAGACAGGGGAAGCTTCACTCCTTTACAATTTTGGGAGTCCA
CACGGCATGGCATACAAAATTTTTCATTCCATTGAGAAATAAAATCCAATTTCCATCACCAAGAGAGCCTTCCGAAAGAGGCCCCCTGGGCAACGGCCAC
CGATGGAGAGGTCTGCCAGTCTCTTCTACCCCAACCCAGCCCCACCCCTAATCAG**ACGCGTAGTTTAAAC**CTGTGATAAAAGCAACTGTTAGCTTGCCTAG
ACTAGCTTCAAAGTTGTATTGACCCTGGTGTGTATGTCTAAGAGTAGATGCCATATCTCTTTCTGGCCTATGTTATTACCTGTATGGACTTTGCACTGGAATCAG
CTATCTGCTCTTACTTATGCACACCTGGGGCATAGAGCCAGCCCTGTATCGCTTTTTCAGGCATCTCACTACAGATAAAGTCCCAAGTCCGTGTAGCTGCCTTCTT
ATCACAGGAATAGCACCAAGGTCCATCAGTACCTCAGAGTAGAACCCCTATAAACTAGTCTGGTTTGCCCATGGGGCACAGTCAGGCTGTTTTCCAGGGTGG
GGTGACAGACATTCTGCTGCTGTTGTGATGCTTACATATAACGTATAACAGACACACGTATGTGTTGTATCCCTGTGGTTTGAGAGTTTGGAGCTTCCCTAAAAG
TCAAAATATTCTCAATGGGCCCTCAATCAGCACATACACAAAAAGGTACCTGGAAACTGTAATTTCTTCTGCTCAAGACAGGCAATTCAATACCCCTTCC
CCCAACCAAAACCCCT

BCL11A-2-AAV6
GGCTACACTTCTTTTCTTCTCTCCATTTCATCCCTTTCCAAAAGTGTTTAGACAAATAGTTTCCAGACTTGGTTTTATCATGCTGGGTGACAAAGGTTGTG
TACAGAGCTGGAATAATTTTTCTTCTTCTACTGTTGGCAGATCAATATCTTTTTCTGCAAGAGAGGGCTAAGCTTGACAGAAACACCGGTGGGAACCCAC
TGGAGAAGGGCATGGGTGGTTTTGGTTCCGTTGGTGGCTGATCTGATGGGTGGTATAGGCAGAAATGTCTGGCTGCCCTTCTAGATTCCGTAGATTCCGTAGGC
GACCAACATGG**GTTTAAAC**GGTGGGGGTGGGGGACTGTCTGCTTTTTGATGACAAATATCTTCACTCTCCTTGGTGCTTGTGGTTGTGCCTATGAATTTAGGA
CCTTATGCCTCATAGTGTGAGTCAGAGCAAAACAGTTTACATCAAAGGCCACTTATTTAATTTTCTCAGAAAACCTCTGTGAGGATAATTTCTCCTTCTTATAAC
GGAATGCGAATGTGTACACAAATGTGTACATTATCCACCGAAGGGTGATGCTCTTTACTTTTATAATCTAGATTTTAAAAAATTTGGTGCACTGATTTTCAGGT
CCTCAGACTCCAGAAATTTATACTCACTGATTAGTAATGCATTATGCAAAATAATTCAGTTTACATATCAAATACCTGTTTGCATCATTCACACCTCATGTCTTGAAA
AAAAAGTGAATGTTTCTCAAATAAACAAAACCTGCAGAAGAGTTTATTGTAAGGGTGAAAAATTAAGAGGAACCCATTGCTGGGAACCTTTTGAGGAAA
ATTTTAATACTCCTGCATAAATAATTTTGAATTTATTTGATTCTACCTAATCAATATACCTTGCCACGCAAGGTATATTCTATTATCTTTGGCTTTCTGAATTTT
CAATTTTCAGAAAAAGAAGCC

Table S1. gRNA sequences

Site	gRNA sequences
EEF2	CTTCCTGGACAAATTGTAGG
AAVS1	TAAGGAATCTGCCTAACAGG
BCL11A-1	CTAACAGTTGCTTTTATCAC
BCL11A-2	GTAGGCGACCAACATGGGGT

Table S2. ssODN sequences for HDR editing

(* indicates phosphorothioate bond)

Site	ssODN sequences
EEF2	G*C*C*TGAAAGAAGGCATCCCTGCCCTGGACAACTTCCTGGACAAATTGT AGTACTAAGCGGCCCTTCCTGCAGCGCCTGCCGCCCCGGGGACTCGCAG CACCCA*C*A*G
AAVS1	C*C*C*ATCCTTAGGCCTCCTCCTTCCTAGTCTCCTGATATTGGGTCTAACC CGAATTCTTAGGCAGATTCCTTATCTGGTGACACACCCCCATTCCTGGAG CCA*T*C*T
BCL11A-1	A*C*G*GCCACCGATGGAGAGGTCTGCCAGTCTTCTACCCACCCACG CCCCCACCCTAATCAGAGGCCAAACCCTTCCTGGAGCCTGTGGGTGTGGC ACAACAGGGTATAAAAGCAACTGTTAGCTTGCACTAGACTAGC*T*T*C
BCL11A-2	G*T*C*TGGCTGCCCTTCAGATTCCTGTAGATTCCGTAGGCGACCAACAT GGGAATTCGGTGGGGGTGGGGGACTGTCTGCTCTTTTGTGCAAATATCT TCAC*T*C*T

Table S3. EEF2 (long-range 5287 bp) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')
EEF2-F-BC1	ATGATAACTAGGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC2	CATCTCATCTCGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC3	CCTGAAGACGTTAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC4	GCAGAAGTCTATAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC5	GTTCAGTAAGACAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC6	TACCAAGTCAATCAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC7	TGCTCATCTGCTAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC8	TGTTGGAAGTCTCAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC9	AGGAGACATCAGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC10	AACGACTATGTCAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC11	GTAGCTGATTCCAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC12	TAAGACACTCGTAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC13	GATACATATCGAAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC14	TTCTGGTGACTGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC15	AGTTGGAGGTTCAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC16	ACTCGTCGTCTAAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC17	AAAGATCCAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC18	AATATGCTAAGTCTTGGGCTCCTCAGTC

EEF2-F-BC19	ACTCGAGTAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC20	AGATGATGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC21	ATTGTACGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC22	CCACTGACAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC23	GATCTTACAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC24	GGAGCGATAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC25	ACGCCACGTTAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC26	CACTCTCAGGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC27	CAGTGACCAGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC28	CCAGGCTCTTAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC29	AGATTACTCTAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC30	CGACTAGACCAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC31	GATCTGACAGAAGTCTTGGGCTCCTCAGTC
EEF2-F-BC32	GCAACTGTCTAAGTCTTGGGCTCCTCAGTC
EEF2-Reverse	GACCAACCAGGCCAAGCAAA

Table S4. AAVS1 (long-range 3928 bp) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')
AAVS1-F-BC1	AAGAGGAGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC2	CTGGAAATTGCAAACAGGAAGTGAACGG
AAVS1-F-BC3	CTTGTTGGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC4	TATGCGTTTGCAAACAGGAAGTGAACGG
AAVS1-F-BC5	TGTTCAAGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC6	AAACTTTGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC7	GGACCGATTGCAAACAGGAAGTGAACGG
AAVS1-F-BC8	AATTATCCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC9	AAACAAACTGCAAACAGGAAGTGAACGG
AAVS1-F-BC10	ACCGCCTATGCAAACAGGAAGTGAACGG
AAVS1-F-BC11	CCAGGTTCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC12	CCTAACGCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC13	CGCTCTTCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC14	GGGTAACGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC15	TATACGACTGCAAACAGGAAGTGAACGG
AAVS1-F-BC16	AACGGACTTGCAAACAGGAAGTGAACGG
AAVS1-F-BC17	GACAACGCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC18	TTAGTCCGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC19	ATTCCGGATGCAAACAGGAAGTGAACGG
AAVS1-F-BC20	CCGACATCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC21	ACCGTTTATGCAAACAGGAAGTGAACGG
AAVS1-F-BC22	GGGTAACGATTGCAAACAGGAAGTGAACGG
AAVS1-F-BC23	TATACGACTATGCAAACAGGAAGTGAACGG
AAVS1-F-BC24	AACGGACTAGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC25	GACAACGCAATGCAAACAGGAAGTGAACGG

AAVS1-F-BC26	ATGATAACTAGGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC27	CATCTCATCTCGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC28	CCTGAAGACGTTTGCAAACAGGAAGTGAACGG
AAVS1-F-BC29	GCAGAAGTCTATTGCAAACAGGAAGTGAACGG
AAVS1-F-BC30	GTTTCAGTAAGACTGCAAACAGGAAGTGAACGG
AAVS1-F-BC31	TACCAGTCAATCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC32	TGCTCATCTGCTTGCAAACAGGAAGTGAACGG
AAVS1-F-BC33	TGTTGGAACCTCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC34	AGGAGACATCAGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC35	AACGACTATGTCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC36	GTAGCTGATTCTTGCAAACAGGAAGTGAACGG
AAVS1-F-BC37	TAAGACACTCGTTGCAAACAGGAAGTGAACGG
AAVS1-F-BC38	GATACATATCGATGCAAACAGGAAGTGAACGG
AAVS1-F-BC39	TTCTGGTGACTGTGCAAACAGGAAGTGAACGG
AAVS1-F-BC40	AGTTGGAGGTTCTGCAAACAGGAAGTGAACGG
AAVS1-F-BC41	ACTCGTCGTCTATGCAAACAGGAAGTGAACGG
AAVS1-Reverse	CGACCTACTCTCTTCCGCAT

Table S5. BCL11A-1 (long-range 3863 bp) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')
BCL11A-1-F-BC1	AAGAGGAGGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC2	CTGGAAATGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC3	CTTGTTGGGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC4	TATGCGTTGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC5	TGTTCAAGGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC6	AAACTTTGGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC7	GGACCGATGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC8	AATTATCCGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC9	AAACAAACGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC10	ACCGCCTAGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC11	CCAGGTTCGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC12	CCTAACGCGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC13	CGCTCTTCGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC14	TTAGTCCGGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC15	GACAACGCGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC16	GGGTAACGGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC17	TATACGACGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC18	AACGGACTGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC19	AGACTCGTGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC20	ATTCCGGAAGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC21	ACTCGAGAAGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC22	TTAGTCCGACGTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC23	GACAACGCTTGTGTGGTGTTCGGAGTCCTA

BCL11A-1-F-BC24	GGGTAACGTA	GTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC25	TATACGACAT	GTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC26	ACGCCACGTT	GTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC27	CACTCTCAGG	GTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC28	CAGTGACCAG	GTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC29	CCAGGCTCTT	GTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC30	AGATTACTCA	GTGTGGTGTTCGGAGTCCTA
BCL11A-1-F-BC31	CGACTAGACC	GTGTGGTGTTCGGAGTCCTA
BCL11A-1- Reverse	AGGAGCGGCAGTTAAGTCT	

Table S6. BCL11A-2 (long-range 5313 bp) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')	
BCL11A-2-F-BC1	AAGAGGAG	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC2	CTGGAAAT	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC3	CTTGTTGGG	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC4	TATGCGTT	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC5	TGTTCAAG	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC6	AAACTTTG	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC7	GGACCGAT	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC8	AATTATCC	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC9	AAACAAAC	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC10	ACCGCCTA	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC11	CCAGGTTT	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC12	CCTAACGC	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC13	CGCTCTTC	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC14	GATCTGACAG	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC15	GCAACTGTCT	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC16	GCTGATCGGA	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC17	GACGACTAAG	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC18	GTCCGGTGAA	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC19	GTGCATATCC	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC20	TAAGGAATCC	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC21	TATGCTATGC	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC22	TCGCGACACT	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-F-BC23	TTCGGATGGT	GCTGTGCTTTCTTCTATGATTCCTC
BCL11A-2-Reverse	TGAAATCTCCCTTCTTTACGGTTCT	

Table S7. EEF2 (PE150) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')	
EEF2-F-BC1	TCACG	CAGTCTCCAGGTGTCGTCTG
EEF2-F-BC2	GATGT	CAGTCTCCAGGTGTCGTCTG
EEF2-F-BC3	TAGGC	CAGTCTCCAGGTGTCGTCTG
EEF2-F-BC4	GACCA	CAGTCTCCAGGTGTCGTCTG

EEF2-F-BC5	CAGTGCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC6	CCAATCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC7	AGATCCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC8	CTTGACAGTCTCCAGGTGTCGTCTG
EEF2-F-BC9	AGTTGCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC10	GCATACAGTCTCCAGGTGTCGTCTG
EEF2-F-BC11	CTATCCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC12	ACTCGCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC13	GGCATCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC14	GTCTTCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC15	TGACTCAGTCTCCAGGTGTCGTCTG
EEF2-F-BC16	TTATTCAGTCTCCAGGTGTCGTCTG
EEF2- Reverse	GTTTGACCACTGGCAGATCC

Table S8. AAVS1 (PE150) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')
AAVS1-F-BC1	TCACGGGCAAGGAGAGAGATGGC
AAVS1-F-BC2	GATGTGGCAAGGAGAGAGATGGC
AAVS1-F-BC3	TAGGCGGCAAGGAGAGAGATGGC
AAVS1-F-BC4	GACCAGGCAAGGAGAGAGATGGC
AAVS1-F-BC5	CAGTGGGCAAGGAGAGAGATGGC
AAVS1-F-BC6	CCAATGGCAAGGAGAGAGATGGC
AAVS1-F-BC7	AGATCGGCAAGGAGAGAGATGGC
AAVS1-F-BC8	CTTGAGGCAAGGAGAGAGATGGC
AAVS1-F-BC9	AGTTGGGCAAGGAGAGAGATGGC
AAVS1-F-BC10	GCATATGGCAAGGAGAGAGATGGC
AAVS1-F-BC11	CTATCAGGCAAGGAGAGAGATGGC
AAVS1-F-BC12	ACTCGTGGCAAGGAGAGAGATGGC
AAVS1-F-BC13	GGCATTGGCAAGGAGAGAGATGGC
AAVS1-F-BC14	GTCTTGGGCAAGGAGAGAGATGGC
AAVS1-F-BC15	TGACTGGGCAAGGAGAGAGATGGC
AAVS1-F-BC16	TTATTTGGCAAGGAGAGAGATGGC
AAVS1-Reverse	GGCTCTGGTTCTGGGTACTT

Table S9. BCL11A-1 (PE150) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')
BCL11A-1-F-BC1	TCACGTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC2	GATGTTCATCACCAAGAGAGCCT
BCL11A-1-F-BC3	TAGGCTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC4	GACCATCCATCACCAAGAGAGCCT
BCL11A-1-F-BC5	CAGTGTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC6	CCAATTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC7	AGATCTCCATCACCAAGAGAGCCT

BCL11A-1-F-BC8	CTTGATCCATCACCAAGAGAGCCT
BCL11A-1-F-BC9	AGTTGGTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC10	GCATATTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC11	CTATCATCCATCACCAAGAGAGCCT
BCL11A-1-F-BC12	ACTCGTTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC13	GGCATTTCATCACCAAGAGAGCCT
BCL11A-1-F-BC14	GTCTTGTCATCACCAAGAGAGCCT
BCL11A-1-F-BC15	TGACTGTCCATCACCAAGAGAGCCT
BCL11A-1-F-BC16	TTATTTTCATCACCAAGAGAGCCT
BCL11A-1- Reverse	AGACATAACACACCAGGGTCA

Table S10. BCL11A-2 (PE150) forward primers with barcodes (BC, in red) and reverse primer

ID	Primer sequences (5'-3')
BCL11A-2-F-BC17	AAACAGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC18	ATTCCGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC19	CCGACACACAACCACAGAAGCACCAA
BCL11A-2-F-BC20	AGCTTGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC21	ATGAAGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC22	CACCTCCACAACCACAGAAGCACCAA
BCL11A-2-F-BC23	GACAACCACAACCACAGAAGCACCAA
BCL11A-2-F-BC24	CGAGGTCACAACCACAGAAGCACCAA
BCL11A-2-F-BC25	GTCTGACACAACCACAGAAGCACCAA
BCL11A-2-F-BC26	GAAGGCCACAACCACAGAAGCACCAA
BCL11A-2-F-BC27	GGGATTCACAACCACAGAAGCACCAA
BCL11A-2-F-BC28	GGTTCTCACAACCACAGAAGCACCAA
BCL11A-2-F-BC29	TCCTTGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC30	AAAGTTCACAACCACAGAAGCACCAA
BCL11A-2-F-BC31	AAGCATCACAACCACAGAAGCACCAA
BCL11A-2-F-BC32	TTCTATCACAACCACAGAAGCACCAA
BCL11A-2-F-BC33	TCACGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC34	GATGTCACAACCACAGAAGCACCAA
BCL11A-2-F-BC35	TAGGCCACAACCACAGAAGCACCAA
BCL11A-2-F-BC36	GACCACACAACCACAGAAGCACCAA
BCL11A-2-F-BC37	CAGTGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC38	CCAATCACAACCACAGAAGCACCAA
BCL11A-2-F-BC39	AGATCCACAACCACAGAAGCACCAA
BCL11A-2-F-BC40	CTTGACACAACCACAGAAGCACCAA
BCL11A-2-F-BC41	AGTTGGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC42	GCATATCACAACCACAGAAGCACCAA
BCL11A-2-F-BC43	CTATCACACAACCACAGAAGCACCAA
BCL11A-2-F-BC44	ACTCGTCACAACCACAGAAGCACCAA
BCL11A-2-F-BC45	GGCATTTCACAACCACAGAAGCACCAA
BCL11A-2-F-BC46	GTCTTGTCACAACCACAGAAGCACCAA

BCL11A-2-F-BC47	TGACTGCACAACCACAGAAGCACCAA
BCL11A-2-F-BC48	TTATTTACAACCACAGAAGCACCAA
BCL11A-2-Reverse	GCTTGACGAAAACACCGGT

Table S11. Primers for qPCR analysis to detect mega-deletions (up to 160 kbp) after cleavage at BCL11A-1

ID	Primer sequences (5'-3')
BCL11A-1-q-160-F	GTCATAGAACTTCCTGTGC
BCL11A-1-q-160-R	CCACAAAGTTAGAGCTAGTG
BCL11A-1-q-80-F	TCACAATCAGGGAAAGTTGC
BCL11A-1-q-80-R	ACCTCAGTCTTTCCAGTTCT
BCL11A-1-q-40-F	GCATCAGTTCAGGTATGTAG
BCL11A-1-q-40-R	CATGAGAACCTAGCCAACAT
BCL11A-1-q+40-F	CAAGGTCTGGTTTACTTCTG
BCL11A-1-q+40-R	AATCCAGGTTCCACGATAC
BCL11A-1-q+80-F	TGAACACCTATGGTCTGACT
BCL11A-1-q+80-R	GTGAAGGCTGATGTTTCGAG
BCL11A-1-q+160-F	CTAACTCCTGGCTTGTGCA
BCL11A-1-q+160-R	CATTCACAGAAGGTGAGGGT
AAVS1-F	GGCAAGGAGAGAGATGGC
AAVS1-R	GGCTCTGGTTCTGGGTAATT
β -ACTIN-F	TCGTGCGTGACATTAAGGAG
β -ACTIN-R	GGCAGCTCGTAGCTCTTCTC

Reference sequences for nanopore sequencing data alignment

EEF2:

AAGTCTTGGGCTCCTCAGTCGGGAGCTAGAAGTAAGGAGCATAGAAGGAATATAGGGTGA
TACTTGTGCCAGCCACAACCTTTGTTCTTAGCCAGTGAGGGCATTCTGGAGAAGCTCC
AAGCCCCAGGGCACAGCACACCCCTGCTCATCTGTGTCCCTGGGCACAGGTGGGTGGCGG
CCGTGACCCATACCCAGGGAGGAGGGCAGTGATTCTGAAGAGGATGTTTCCTGACAGCC
CCGACGCAGCAGTCAGGGCCTTGTGGAAGCCACCAGTCTGCCCTGGGACCTGGCAGGAGG
GCCACCAACTCCGGGAGGGACCTGCCCTGGCCCTCCAGGGTCCAGCACAAACATCCCTGC
GGCCTGCTGCCGCCTTCTGCTTCTCCAGAACAATCTTGATGATGGGCCGCTACGTGGAG
CCCATCGAGGATGTGCCTTGTGGGAACATTGTGGGCCTCGTGGGCGTGGACCAGTTCCTG
GTGAAGACGGGCACCATCACACCTTCGAGCACGCGCACAAACATGCGGGTGATGAAGTTC
AGCGTCAGCCCTGTTGTGAGAGTGGCCGTGGAGGCCAAGAACCCGGCTGACCTGCCCAAG
CTGGTGGAGGGGCTGAAGCGGCTGGCCAAGTCCGACCCCATGGTGCAGGTGGGCACGGAG
TGCACCTGCTGGGAGCAGACACCCTGAGGGGGGACGGCTTGTGTCCTGAGTTGTGGCTGT
GTGGGTTGCTGTGGTTGGTGGATTGGGGTGAAGTGGTGCATGAAAGGAGGGGTTCTTGGCTG
TGGCTGTAAATGGAGACTCCTGCCAGGCAGCCACCACCTCTCCTAGGCTGCTCTGCGC
GTGTTCACTGTAGCTTGTGGACTTAACATTTCTTCAGGAGTTTGTGCTGGTCCCAAGTT
CCCAGCACCTTCCCTGAAATCTTGTCTGGCGGGTCCCTAGCTAAAGGGAAGCTGGGAG
CCTGGTCCCGCCTGTTCCGAGCCGCCTACCCCTTGGTGCTGACCCGCATCCCTTTCAGT
GCATCATCGAGGAGTCGGGAGAGCATATCATCGGGGCGCCGGCGAGCTGCACCTGGAGA

TCTGCCTGAAGGACCTGGAGGAGGACCACGCCTGCATCCCCATCAAGGTGAGGCGCCAGT
GACCAGCCTTCCCCACGCCCCACCCCGGACACCTGCCCTCTGCTTTAAAGCTGAGCTGAG
CTAGGCTCTGCAGACGCCTAGACTTGATCTCGGTCTTGGTCTCAGCTTCTTAAGTTCCTC
AAGTTTCTTCTTTGCAGTCTCCACAGGCATGTGGGGCTGTTTTTGTGTTGTTGTTGTT
TTTTTTGAGATAGTCTCGCTGTGTCATCCAGGCTGGAGTGCAGTGGCGTGATCTTGGCTC
GCTAGAACCTCCACCTCCCGGGTTCAAGCGATTCTTGCCTCACTCAGCTTCCTGAGTA
ACTGGGACTACAGGCGCGTGCTACCACGCCCCGGCTAATTTTTTTTTGTATTTTTAGTAGA
GGCGGGGTTTCACTGTGTAGTAAGTCAAGATGGTCTCGATCTCCTGACCTCGTGATCCC
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CTGACCTCGTGATCCACCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCA
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CCTGTCTTGTCTGGAGTGAGATGCGCTTGGCACTGCAACACTGACTCCGCTTCGTTCT
GATTGACGTGGCTCTACCAGGCTGTATGAGGTCCGCCTTGCTAGGAAAAGCTTTTAAGGA
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CGGGCGCGCTACCTGGCCGAGAAGTACGAGTGGGACGTGGCTGAGGCCCCGAAGATCTGG
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CTTCGACGTCCACGACGTACCCTGCACGCCGACGCCATCCACCGCGGAGGGGGCCAGAT
CATCCCCACAGCACGGCGCTGCCTCTATGCCAGTGTGCTGACCGCCCAGCCACGCCTCAT
GGAGCCCATCTACCTTGTGGAGATCCAGGTGAGGTCTACCGCCCCACCGCTGACCCTGCC
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CCAGTTAAGCTTAGCAAGGTGTTAAAGGAGGCGTCTGATGGGAGCAGGTGATGGATGGA
GCAGGTGGTCCAGTTTCTGACAGCTTGTGGACCCCTAAATCACTGAATTCCCAGGGGAG
GGGCTCTCCTATCCCCAGTGTGAGAAGGGCTCTGGGCCTGGAGCTCTGAAGGCCTACGCC
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GGCCCATGAGTGGCCTGCTAGGCCCTTCGTGAAGTGCTGGGCACCAGGCCGAGTGTCTGG
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ACCTGAGGTCCAACACGGGCGGCCAGGCGTTCCCCAGTGTGTGTTTGACCACTGGCAGA
TCCTGCCCCGAGACCCCTTCGACAACAGCAGCCGCCCCAGCCAGGTGGTGCGGAGACCC
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TCCACCCGCTTGGCCTCCCAAAGCGTTGGGATTGTACCACTGTGCCCCGGCCTTGTAAC
ACTTTTTTAGAGGCAGAGTCTTGCTGTCTCCAGATGAGTATAGTGGTGCAGTCAGCTCA
CTGCATCCTCCACCTCCTGGACTCTCCTGCCTCAGCCTGCCAGGTAGCTGGGACTCCAGG
CATGTGCCACCATGCCAGCTAATTTTTATTTATTTGAGACAGAATTTTGCTTGGCCTG
GTTGGTC

EEF2 with mNeonGreen insertion:

AAGTCTTGGGCTCCTCAGTCGGGAGCTAGAAGTAAGGAGCATAGAAGGAATATAGGGTGA
TACTTGTGCCAGCCACAACCTTTGTTCTTAGCCAGTGAGGGCATTTCTGGAGAAGCTCC
AAGCCCCAGGGCACAGCACACCCCTGCTCATCTGTGTCCCTGGGCACAGGTGGGTGGCGG
CCGTCGACCCATACCCAGGGAGGAGGGCAGTGATTCTGAAGAGGATGTTTCCTGACAGCC
CCGACGCAGCAGTCAGGGCCTTGTTGAAGCCACCAGTCTGCCCTGGGACCTGGCAGGAGG
GCCACCAACTCCGGGAGGGACCTGCCCTGGCCCTCCAGGGTCCAGCACAAACCATCCCTGC
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GCATCATCGAGGAGTCGGGAGAGCATATCATCGCGGGCGCCGGCGAGCTGCACCTGGAGA
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GACCAGCCTTCCCCACGCCCCACCCGGACACCTGCCCTCTGCTTTAAAGCTGAGCTGAG
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TTTTTGTAGATAGTCTCGCTGTGTCATCCAGGCTGGAGTGCAGTGGCGTGATCTTGGCTC
GCTAGAACCTCCACCTCCCGGGTTCAAGCGATTCTTGCCTCACTCAGCTTCCTGAGTA
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GGCGGGGTTTCACTGTGTAGTAAGTCAAGATGGTCTCGATCTCCTGACCTCGTGATCCC
CCTGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACCATGCCTGGCCCAGG
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CTGACCTCGTGATCCACCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCA
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TGCCTCTCCAAGTCCCCCAACAAGCACAAACCGGCTGTACATGAAGGCGCGGCCCTTCCCC
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 CTCATGGCAGAACCGTAAAGAAGGGAGATTTC

Supplementary Figure Legends

Fig. S1 Procedures for determining deletion indexes after RNP editing. **a** Primers for long-range PCR. The barcoded primers were used to amplify 4-6 kb DNA flanking the RNP target sites. Eight to 98 amplicons were combined for nanopore sequencing. **b** Schematic overview for nanopore sequencing data analysis. The demultiplexed fastq data were mapped to reference sequences using Minimap2. The deletion indexes were calculated using Samtools. **c** Deletion indexes analyzed by Samtools and ImageJ are highly correlated. Subtracting background noise, two methods gave rise to identical deletion indexes. **d** Raw data of background deletions. 2-6% background noise was detected in unedited cells, reflecting the ONT sequencing errors. The levels of background deletions are amplicon-dependent. The data in **d** were shown as mean $\pm$ SEM, and the significance was determined by the two-way ANOVA test. Adjusted *p* values were indicated. "ns" means no significance (*p* > 0.05).

Fig. S2 Analysis of deletions of different sizes. **a-c** Analysis of large deletions in T cells (**a**), HSPCs (**b**) and iPSCs (**c**). The percentages of deletions larger than 100-bp (D100), 500-bp (D500), 1000-bp (D1000), 1500-bp (D1500), and 2000-bp (D2000) were shown. **d-f** Linear correlation of alleles of D100 vs. D500 (**d**), D500 vs. D1000 (**e**), D100 vs. D1000 (**f**).

Fig. S3 Cell viability and cell cycle profiles in T cells, HSPCs, and iPSCs after RNP editing. **a** Cell viability after editing in T cells, HSPCs, and iPSCs was comparable. Alive cells were enumerated 3 days after RNP transfection. The cell numbers of edited cells were normalized to those of unedited cells. **b** Chemical structure of Pyronin Y and Hoechst 33342 used in cell cycle analysis. **c** Representative FACS data of HSPC cell cycle profiles of unmanipulated cells (WT) and RNP edited cells (RNP). The G0/G1, S, and G2/M phases were gated and depicted with arrows in the FACS plot. **d** Cell cycle profiles of WT control and edited T cells, HSPCs, and iPSCs one day after RNP nucleofection. The data in **a** were examined by the one-way ANOVA test. The data in **d** were analyzed by the two-way ANOVA test. Adjusted *p* values were indicated. "ns" means no significance (*p* > 0.05).

Fig. S4 AAV6 donor-mediated HDR reduces large deletions. **a** Schematic of HDR editing with Cas9-sgRNA RNPs and promoter-less mNeonGreen AAV6 donor templates. Representative IGV presentations of PCR amplicons before and after mNeonGreen knock-in were shown. **b** Representative FACS plots. APC was an irrelevant channel. As controls, omitting gRNA or AAV donors or using mismatched gRNA and AAV donors led to 0% HDR editing. **c, d** Effects of MOI of AAV6 donors on the frequencies of NHEJ and HDR (**c**) and deletion index (**d**). Data are shown as mean $\pm$ SEM of *n* = 6 independent experiments from 2 editing loci. **e** Visualization of representative deletion data of the AAVS1 locus in HSPCs. Unedited wild-type cells showed a background deletion. This noise was subtracted from deletions in edited cells to obtain deletion indexes. The data in **c** and

d were statistically analyzed by the two-way ANOVA test. Adjusted p values were indicated. "ns" means no significance ($p > 0.05$).

Fig. S5 Removal of HDR alleles does not affect analytical results. **a** Removal of HDR alleles with mNeonGreen knock-in at *EEF2* by Seqkit tools. **b** Representative IGV display nanopore reads mapped to the HDR sequence before and after removal of HDR reads. **c** Illustration of nanopore reads mapped to the wild-type sequence. **d** Deletion indexes analyzed with or without the HDR alleles. The data in **d** was statistically analyzed by the one-way ANOVA test. Adjusted p values were indicated. "ns" means no significance ($p > 0.05$).

Fig. S6 Insertion of double-stranded oligonucleotides reduces large deletions. **a** Examples of small indels and dsODN insertions analyzed by amplicon sequencing and CRISPResso2. The long red boxes indicate the insertion of perfect or partially deleted 34bp-dsODN in the forward or reverse orientation. **b** The insertion of dsODN depends on the NHEJ repair pathway. The NHEJ inhibitor M3814 (2 μ M) barely affects total indels but almost completely blocked dsODN insertion. **c, e** Frequencies of small indels (NHEJ) and dsODN insertions (treated as HDR by CRISPResso2 analysis) in HSPCs (**c**) and iPSCs (**e**). **d, f** dsDNA insertions decrease deletion indexes of HSPCs (**d**) and iPSCs (**f**). **g** Raw data of deletion analysis by Samtools. Error bars represent mean $\pm$ SEM of $n = 3-6$ independent experiments. Data in **b** were statistically analyzed by Student's T-test. The data in **c-g** were statistically analyzed by the two-way ANOVA test. Adjusted p values were indicated. "ns" means no significance ($p > 0.05$).

Fig. S7 Insertion of double-stranded oligonucleotides reduces large deletions in a dosage-dependent manner. **a-c** Analysis of total editings, dsODN insertions, and relative large deletions in T cells with RNP and indicated amounts of 34bp dsODN at *EEF2* (**a**), *AAVS1* (**b**), and *BCL11A-2* (**c**) target sites. **d** Correlation analysis of relative dsODN insertions (insertion frequencies/total editings) and relative deletions (normalized deletion indexes to editing without dsODNs) in edited T cells at *EEF2*, *AAVS1*, and *BCL11A-2* target sites, respectively. Raw data were from Additional file 1: Fig. S7, b for *EEF2* and *AAVS1* analysis, respectively. The data from Fig. 4b and Additional file 1: Fig. S7c were combined for *BCL11A-2* analysis. For **a-c**, error bars represent mean $\pm$ SEM of $n = 3-4$ independent experiments. The data in **a-c** were statistically analyzed by the one-way ANOVA test. Adjusted p values were indicated. "ns" means no significance ($p > 0.05$).

Fig. S8 Summary of large deletions in T cells and HSPCs by different gene-editing approaches. **a-b** Summary of relative deletion indexes (**a**) and raw deletions (**b**) of edited human cells. All the results of edited T cells and HSPCs presented in Fig. 2-4 were summarized ($n = 20-50$). **c** Summary of D100 data in T cells and HSPCs. **d** Correlation analysis of deletion indexes and D100 (percentage of alleles with deletion larger than 100-bp). The data in **a** and **c** were statistically analyzed by the one-way ANOVA test. Adjusted p values were indicated.

Fig. S9 Effects of NHEJ inhibitors on editing outcomes at the *EEF2* locus. **a** Representative editing outcomes analyzed by amplicon sequencing and CRISPResso2. WT indicates the unedited allele. Predominant NHEJ +A and MMEJ -25bp alleles were indicated. The microhomology of MMEJ was underlined in red. **b** and **c** Relative frequencies of +A NHEJ and -25bp MMEJ editings in the absence

and presence of the NHEJ inhibitors M3814 (**b**) or NU7441 (**c**). The data in **b** and **c** were statistically analyzed by the two-way ANOVA test. Adjusted *p* values were indicated. "ns" means no significance ($p > 0.05$).

Fig. S10 Analysis of large deletions in iPSC single-cell clones. Coverage of nanopore reads of WT cells (WT #1 and WT #2) and 52 edited iPSC single-cell clones shown in Fig. 6. The percentages of reads with deletions larger than 100 bp (D100) were indicated.

Fig. S11 AAV6 HDR donor sequences used in this study. Blue indicates the left homology arm; green denotes the right homology arm; the inserted sequences are shown in red.