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Supplementary Methods

Supplementary Table 1. Two-way ANOVA analysis of ABR and DPOAE thresholds.

Supplementary Table 2. The number of injected and uninjected ears tested for ABRs and DPOAEs at each frequency.

Supplementary Sequences. Oligonucleotides used in this study.

Supplementary Notes. MATLAB script used to analyze indel percentages.

Supplementary Figure 1: Uncropped gel used in Extended Data Fig. 1 with molecular weight markers.

Supplementary Methods

Construction of sgRNA expression plasmids. DNA primer sequences used in this paper are listed in the Supplementary Sequences. PCR was performed using Phusion DNA Polymerases (Thermo Fisher Scientific). Plasmids expressing sgRNAs were constructed using USER cloning (New England Biolabs). The plasmid pFYF1320 (EGFP sgRNA expression plasmid) was used as a template to generate sgRNA expression plasmids according to the manufacturer's instructions. PCR products were incubated with DpnI (15 U, New England Biolabs) and USER enzyme (0.75 U, New England Biolabs) at 37 °C for 45 min, purified on a QIAprep spin column (Qiagen), and transformed into NEB turbo competent cells (New England Biolabs).

Expression and purification of Cas9 and dCas9 protein. *E. coli* harboring plasmids encoding NLS-Cas9 or NLS-dCas9 were grown overnight in Luria-Bertani (LB) broth containing 50 μg/mL of carbenicillin at 37 °C. The cells were diluted 1:1000 into the same growth medium and grown at 37 °C to OD_{600} 0.6 ~ 1. Isopropyl -β-D-1- thiogalactopyranoside (IPTG) was added at 120 μM to induce protein expression. After ~16 h, the cells were collected by centrifugation at 4,000 *g* and resuspended in lysis buffer (50 mM tris(hydroxymethyl)-aminomethane (Tris)-HCl, pH 8.0, 0.5 M NaCl, 20% glycerol, 2 mM Dithiothreitol (DTT)). The cells were lysed by sonication (10 sec pulse-on, 10 sec pulse-off for 7 min total at 6 W output) and the lysate supernatant was isolated following centrifugation at 14,000 *g* for 30 min. The lysate was incubated with His-Pur nickel-nitriloacetic acid (nickel-NTA) resin (ThermoFisher Scientific) at 4 °C for 4 h to capture the His-tagged protein. The protein-resin mixture was loaded into a gravity flow column and proteins were eluted with an increasing gradient of imidazole in lysis buffer. Cas9 or dCas9 protein was eluted in lysis buffer supplemented with 250 mM imidazole, and concentrated by ultrafiltration (Amicon-Millipore, 100-kDa molecular weight cut-off) to 1 mL total volume. The protein was diluted to 20 mL in low-salt purification buffer containing 50 mM tris(hydroxymethyl)-aminomethane (Tris)-HCl, pH 7.0, 0.1 M NaCl, 20% glycerol, 2 mM DTT and loaded onto SP Sepharose Fast Flow resin (GE Life Sciences). The resin was washed with 40 mL of this low-salt buffer, and the protein eluted with 5 mL of activity buffer containing 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 20% glycerol, 2 mM DTT. The eluted proteins were quantified on a SDS-PAGE gel.

***In vitro* transcription of sgRNAs.** Linear DNA fragments containing the T7 promoter followed by the sgRNA target sequence were transcribed *in vitro* using the primers listed in the Supplementary Sequences with the HiScribe T7 Quick High Yield RNA Synthesis Kit (New England Biolabs) according to the manufacturer's instructions. Guide RNA products were purified using the E.Z.N.A. Total RNA Kit (OMEGA bio-tek) according to the manufacturer's instructions, quantified by UV absorbance, and stored at -80 °C. FitC-Tmc1-mut3 sgRNA was transcribed by adding 10% v/v Fluorescein RNA Labeling Mix® (Sigma Aldrich) to the transcription system, and atto-550®-TracrRNA was purchased from IDT.

***In vitro* Cas9 DNA cleavage assay.** Plasmids Tmc1_wild-type and Tmc1_*Bth* were constructed by amplifying DNA fragments from wild-type and *Bth* genomic DNA using primers GX273 and GX274, respectively, followed by blunt-ended ligation into PCR-blunt vector (Thermo Fisher Scientific). After DNA sequencing confirmation, 995-bp *Tmc1* DNA fragments were amplified from each plasmid and used as substrates for the *in vitro* cleavage reaction. Typically, the *Tmc1* DNA fragment (100 nM) was incubated for 15 min at 37°C with purified Cas9 protein (300 nM) and sgRNA (300 nM) in Cas9 cleavage buffer (20 mM HEPES pH 7.5, 150 mM KCl, 0.5 mM DTT, 0.1 mM EDTA with10 mM $MgCl_2$) in a total volume of 20 µL in each reaction. Reactions were quenched by adding 500 µL of PB wash buffer (Qiagen), purified on a QIAprep spin column and eluted in 20 µL 1X TE buffer. 10 µL of each reaction were loaded onto a 2% agarose gel and electrophoresed to separate starting DNA and cleaved products.

Lipid nanoparticle formulation. Lipid nanoparticles were prepared as previously reported (*PNAS*, **113**, 2868-2873 (2015)). Briefly, lipid, cholesterol, and DOPE were dissolved in ethanol/sodium acetate buffer (pH = 5.2, v/v = 9/1) at a weight ratio of 16/4/1. The above solution was added dropwise to an aqueous solution of PEG2K-DSPE (PEG2K-DSPE/lipid ratio = 1/16, w/w) under continuous stirring. The solution was dialyzed against DI water for 4 hours in a MWCO 3.5-kDa dialysis cassette (Pierce/Thermo Scientific) and stored at 4 °C for further use.

			ABR threshold (injected vs. uninjected)		DPOAE threshold (injected vs. uninjected)	
Strain	**Time of test**		**p Value**	**F statistic**	**p Value**	**F statistic**
Bth/+	4 weeks	Cas9:Tmc1-mut1	< 0.0001	25.09	< 0.0001	84.66
Bth/+	4 weeks	Cas9:Tmc1-mut2	< 0.0001	84.46	< 0.0001	26.05
Bth/+	4 weeks	Cas9:Tmc1-mut3	< 0.0001	124.4	< 0.0001	39.76
Bth/+	4 weeks	Cas9:Tmc1-mut4	< 0.0001	19.99	< 0.0001	36.33
Bth/+	4 weeks	Cas9:Tmc1-wt3	0.0620	3.526	< 0.0001	41.96
Bth/+	4 weeks	Cas9:GFP	0.1187	2.458	< 0.0001	53.83
Bth/+	4 weeks	dCas9:Tmc1-mut1	0.9322	0.007253	< 0.0001	29.90
Bth/+	4 weeks	Cas9:LPF2000 (no sgRNA)	0.2039	1.626	< 0.0001	62.16
Bth/+	8 weeks	Cas9:Tmc1-mut1	< 0.0001	78.80	< 0.0001	75.96
Bth/+	8 weeks	Cas9:Tmc1-mut3	< 0.0001	51.93	< 0.0001	62.81
C3H	4 weeks	Cas9:Tmc1-mut3	< 0.0001	50.85	< 0.0001	42.95

Supplementary Table 1. Two-way ANOVA analysis of ABR and DPOAE thresholds. Two-way ANOVA analysis of ABR thresholds showed highly significant difference between ears injected with Cas9:Tmc1-mut sgRNAs and control uninjected ears. No significance was detected between various control injections (GFP-targeting sgRNA, catalytically inactivated dCas9, Cas9 + lipid without sgRNA) and uninjected ears. Significant differences were detected in DPOAE thresholds between injected (Tmc1-mut sgRNA and control injections) and uninjected ears.

Frequencies (kHz)	Number of ears (Bth/+) tested for each frequency				
	Cas9:Tmc1-mut1	Cas9:Tmc1-mut2	Cas9:Tmc1-mut3	Cas9:Tmc1-mut4	Uninjected
5.66	18	5	25	7	11
8	16	8	20	6	23
11.32	18	10	26	8	42
16	18	6	29	6	45
22.64	18	11	25	8	56
32	17	13	29	8	66
45.25	18	13	28	8	65

Frequencies (kHz)	Number of ears (C3H) tested for each frequency	
	Cas9:Tmc1-mut3	Uninjected
5.66	12	17
8	12	17
11.32	12	17
16	12	17
22.64	12	16
32	12	16
45.25	12	14

Control injection (4 weeks)

Frequencies (kHz)	Number of ears (Bth/+) tested for each frequency				
	Cas9:Tmc1-wt3	Cas9:LPF2000	Cas9:GFP	dCas9:Tmc1-mut1	Uninjected
5.66	6	5	6	7	14
8	4	5	6	5	15
11.32	5	5	6	6	21
16	7	5	6	8	24
22.64	6	5	6	8	25
32	8	5	6	8	27
45.25	7	5	6	9	28

Cas9:Tmc1-mut injection (8 weeks)

Frequencies (kHz)	Number of ears (Bth/+) tested for each frequency		
	Cas9: Tmc1-mut1, all	Cas9: Tmc1-mut3, all	Uninjected
5.66	21	23	27
8	22	25	32
11.32	25	26	39
16	25	26	39
22.64	24	25	38
32	25	26	39
45.25	24	25	34

Supplementary Table 2. The number of injected and uninjected ears tested for ABRs and DPOAEs at each frequency.

Supplementary Sequences. Oligonucleotides used in this study.

Primer name	5'-sequence-3'	Usage
GX265	TAATACGACTCACTATAGGGACAGAACATCCCCAGGAGTTTTAGAGCTAGAAATAGC	Forward primer for TMC1-wt1 sgRNA *in vitro transcription*
GX266	TAATACGACTCACTATAGGTGGGACAGAACATCCCCAGGGTTTTAGAGCTAGAAATAGC	Forward primer for TMC1-wt2 sgRNA *in vitro transcription*
GX267	TAATACGACTCACTATAGGGTGGGACAGAACATCCCCGTTTTAGAGCTAGAAATAGC	Forward primer for TMC1-wt3 sgRNA *in vitro transcription*
GX268	TAATACGACTCACTATAGGGACAGAACTTCCCCAGGAGTTTTAGAGCTAGAAATAGC	Forward primer for TMC1-mut1 sgRNA *in vitro transcription*
GX269	TAATACGACTCACTATAGGTGGGACAGAACTTCCCCAGGGTTTTAGAGCTAGAAATAGC	Forward primer for TMC1-mut2 sgRNA *in vitro transcription*
GX270	TAATACGACTCACTATAGGGTGGGACAGAACTTCCCCGTTTTAGAGCTAGAAATAGC	Forward primer for TMC1-mut3 sgRNA *in vitro transcription*
GX283	TAATACGACTCACTATATGGGACAGAACTTCCCCGTTTTAGAGCTAGAAATAGCAAG	Forward primer for TMC1-mut4 sgRNA *iin vitro transcription*
GX84	TAATACGACTCACTATAGGGCACGGGCAGCTTG	Forward primer for GFP sgRNA in vitro transcription
GX85	AAAAAAAGCACCGACTCGGTGCCAC	Universal primer for all *in vitro* transcription
GX273	TGGGCACAGTGAATTCTATGAC	Cloning for plasmids Tmc1_wild-type and Tmc1_Bth
GX274	GGTAATCTGCAGACTGGGAAAC	Cloning for plasmids Tmc1_wild-type and Tmc1_Bth
Tmc1_HTS_F1	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAAGAATGCATGGCTTAATCTTT	HTS for *Tmc1* site
Tmc1_HTS_R1	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNAGAAGAGCAAAAATGCGCC	HTS for *Tmc1* site
Off-T1F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCTCTATGTGTCTAAGTAGGGCA	HTS for *Off-T1* site identified by GUIDE-seq
Off-T1R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNACTGACTTAGTTCATGTGCCA	HTS for *Off-T1* site identified by GUIDE-seq
Off-T2F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGTTCTGTTCCCAACACACTCT	HTS for *Off-T2* site identified by GUIDE-seq
Off-T2R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNACCAGACTGGCAATCCACTG	HTS for *Off-T2* site identified by GUIDE-seq
Off-T3F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGACTTTTGGGATAGCATTGGAAA	HTS for *Off-T3site* identified by GUIDE-seq
Off-T3R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNAGGTACTGGATTGCAGGTGTG	HTS for *Off-T3* site identified by GUIDE-seq
Off-T4F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCTGGTACGCTTTCTGATGG	HTS for *Off-T4* site identified by GUIDE-seq
Off-T4R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNCTCGAGTCCCTCTGGAGTTCTT	HTS for *Off-T4* site identified by GUIDE-seq
Off-T5F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGAGTTTTCTGATTGCCCCA	HTS for *Off-T5* site identified by GUIDE-seq
Off-T5R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGGGAGAGGTAGCTGGAGGTAG	HTS for *Off-T5* site identified by GUIDE-seq
Off-T6F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGTAACCAGCAGTTGATGCCT	HTS for *Off-T6* site identified by GUIDE-seq
Off-T6R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNCTGGCTCCTTAGACAGTGAGAG	HTS for *Off-T6* site identified by GUIDE-seq
Off-T7F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGGGGACACTGCGATGGC	HTS for *Off-T7* site identified by GUIDE-seq
Off-T7R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNTCTAAAGAGTCCCCCAAGG	HTS for *Off-T7* site identified by GUIDE-seq
Off-T8F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCACTTTTCTTACCATCTTCTCG	HTS for *Off-T8* site identified by GUIDE-seq
Off-T8R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGCAAAAACTTATCTCCCAGCCC	HTS for *Off-T8* site identified by GUIDE-seq
Off-T9F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCATGTATCAGAGTTTGGCAGA	HTS for *Off-T9* site identified by GUIDE-seq
Off-T9R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNCAGGGCCATGTTTCCTCTCA	HTS for *Off-T9* site identified by GUIDE-seq
Off-T10F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGGCATGACTCAGCAGAGA	HTS for *Off-T10* site identified by GUIDE-seq
Off-T10R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGCTTGGCTTGCTTGCACAG	HTS for *Off-T10* site identified by GUIDE-seq
Off-T3'F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCCCATTTAGCTGGTAGAGC	HTS for *Off-T3'* site identified by computational prediction
Off-T3'R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNCAGAGTCTTGCCAGAGTCCAA	HTS for *Off-T3'* site identified by computational prediction
Off-T4'F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGAGAATGGGGAATTAACAC	HTS for *Off-T4'* site identified by computational prediction
Off-T4'R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNTAGGTGTCCTCGGGTTTCTCA	HTS for *Off-T4'* site identified by computational prediction
Off-T5'F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGCTGTTCTCAGGATCTCACA	HTS for *Off-T5'* site identified by computational prediction
Off-T5'R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNCTGACTTTATTGCTCCATGGCT	HTS for *Off-T5'* site identified by computational prediction
Off-T6'F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGTCAGAGATGTGGAAGAATC	HTS for *Off-T6'* site identified by computational prediction
Off-T6'R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGGTGAGCTGTTAGCAGAAGCA	HTS for *Off-T6'* site identified by computational prediction
Off-T7'F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTTCCTCACAAGAGAAACCAGC	HTS for *Off-T7'* site identified by computational prediction
Off-T7'R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNACCAAAGTTGCAGGAGAAGAAA	HTS for *Off-T7'* site identified by computational prediction
Off-T8'F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAAGCCACTGTCTCAGTGTTCC	HTS for *Off-T8'* site identified by computational prediction
Off-T8'R	GGAGTTCAGACGTGTGCTCTTCCGATCTNNNNGTTTACCGACTTTGGCCCTG	HTS for *Off-T8'* site identified by computational prediction

Supplementary Notes. MATLAB script used to analyze indel percentages. Variable parameters are shown in red for different target sites.

```
WTnuc='target genomic site sequence';
%WTnuc='complimentary sequence to the above target site sequence';
%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=69;
width=40;
flank=10;
SUMMARY={};
SUMMARY{1,1}='Filename';
SUMMARY{1,2}='Skipped reads';
SUMMARY{1,3}='not INDEL';
SUMMARY{1,4}='Insertions';
SUMMARY{1,5}='Deletions';
SUMMARY{1,6}='INDEL rate';
foldername=strcat(num2str(width),'_',num2str(indelstart),'summary.csv');

for d=1:total sample number
    filename=files(d).name;
    %read fastq file
    [header,seqs,qscore] = fastqread(filename);
    seqsLength = length(seqs);          % number of sequences
    seqsFile = strcat(strrep(filename,'.fastq',''),'_INDELS');      % trims off .fastq
    %create a directory with the same name as fastq file+_INDELS
    if exist(seqsFile,'dir');
        error('Directory already exists. Please rename or move it before moving on.');
    end
    mkdir(seqsFile);                    % make directory
    wtLength = length(WTnuc);           % length of wildtype sequence
    sBLength = length(seqs);            % number of sequences

    % initialize counters and cell arrays
    nSkips=0;
    notINDEL=0;
    ins={};
```

```
dels={};
NumIns=0;
NumDels=0;
% iterate through each sequencing read
for i = 1:sBLength
%search for 10BP sequences that should flank both sides of the "INDEL WINDOW"
   windowstart=strfind(seqs{i},WTnuc(indelstart-flank:indelstart));
   windowend=strfind(seqs{i},WTnuc(indelstart+width:indelstart+width+flank));
   %if these flanks are found proceed\
   if length(windowstart)==1 && length(windowend)==1
      %if the sequence length matches the INDEL window length save as
      %not INDEL
      if windowend-windowstart==width+flank
         notINDEL=notINDEL+1;
      %if the sequence is two or more baseslonger than the INDEL
      %window length save as an Insertion
      elseif windowend-windowstart>=width+flank+1
         NumIns=NumIns+1;
         ins{NumIns,2}=seqs{i};
         ins{NumIns,1}=filename(1:2);
      %if the sequence is two or more bases shorter than the INDEL
      %window length save as a Deletion and name the second column
      %with the fileame
      elseif windowend-windowstart<=width+flank-1
         NumDels=NumDels+1;
         dels{NumDels,2}=seqs{i};
         dels{NumDels,1}=filename(1:2);
      %keep track of skipped sequences that are either one base
      %shorter or longer than the INDEL window width
      else
         nSkips=nSkips+1;
      end
   %keep track of skipped sequences that do not posess matching flank
   %sequences
   else
      nSkips=nSkips+1;
   end
```

```
    end
    SUMMARY{d+1,1}=seqsFile;
    SUMMARY{d+1,2}=nSkips;
    SUMMARY{d+1,3}=notINDEL;
    SUMMARY{d+1,4}=NumIns;
    SUMMARY{d+1,5}=NumDels;
    SUMMARY{d+1,6}=(NumIns+NumDels)/(NumIns+NumDels+notINDEL);
    fid=fopen(strcat(seqsFile, '/summary.txt'), 'wt');
    fprintf(fid, 'Skipped reads %i\n not INDEL %i\n Insertions %i\n Deletions %i\n', [nSkips, notINDEL, NumIns, NumDels]);
    fclose(fid);
    save(strcat(seqsFile, '/nSkips'), 'nSkips');
    save(strcat(seqsFile, '/notINDEL'), 'notINDEL');
    save(strcat(seqsFile, '/NumIns'), 'NumIns');
    save(strcat(seqsFile, '/NumDels'), 'NumDels');
    save(strcat(seqsFile, '/dels'), 'dels');
    dlmcell(strcat(seqsFile, strcat('/dels_', filename(1:2), '.csv')), dels, ',');
    save(strcat(seqsFile, '/ins'), 'ins');
    dlmcell(strcat(seqsFile, strcat('/ins_', filename(1:2), '.csv')), ins, ',');
end
dlmcell(strcat(foldername), SUMMARY, ',')
```

Below is the variable portion of the script used for each target site analysis:

Tmc1 on target site:

```
WTnuc='AAGAATGCATGGCTTAATCTTTAGTTTTATAAAATATTAAAGGGACCGCTCTGAAAACCTTTCCAACCGTGTCTC
CTTGTAGATGAACATGGTAATGTCCCTCCTGGGGATGTTCTGTCCCACCCTGTTTGACTTATTTGCTGAACTGGAAGATT
ACCATCCTCTCATTGCTCTGAAGTGGCTCCTGGGGCGCATTTTTGCTCTTCT';

%WTnuc=AGAAGAGCAAAAATGCGCCCCAGGAGCCACTTCAGAGCAATGAGAGGATGGTAATCTTCCAGTTCAGCAAAT
AAGTCAAACAGGGTGGGACAGAACATCCCCAGGAGGGACATTACCATGTTCATCTACAAGGAGACACGGTTGGAAAGG
TTTTCAGAGCGGTCCCTTTAATATTTTATAAAACTAAAGATTAAGCCATGCATTCTT';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=89;

width=40;

flank=10;
```

Off-T1:

```
WTnuc='TCTCTATGTGTCTAAGTAGGGCACTGCAGAGCAGGTAACCTGCCAGGCTCCAAGAGGAAACCTTTGCTACAAA
GGAAGACCTTTGTTCCTGGGGAAGCTCTGTCCCTCCCTAATCCTTAGACATCTGTGACACACATCTCACTACCGATCACA
AAGTTCTTTGGCACATGAACTAAGTCAGT';
```

%WTnuc='ACTGACTTAGTTCATGTGCCAAAGAACTTTGTGATCGGTAGTGAGATGTGTGTCACAGATGTCTAAGGATTAG
GGAGGGACAGAGCTTCCCCAGGAACAAAGGTCTTCCTTTGTAGCAAAGGTTTCCTCTTGGAGCCTGGCAGGTTACCTG
CTCTGCAGTGCCCTACTTAGACACATAGAGA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=93;

width=40;

flank=10;

Off-T2:

WTnuc='TGTTCTGTTCCCAACACACTCTGAAGATGATTTGCACTGCTGCAGTGTCACCACCACCACACTGAAAAGCAGGC
AATACAACGTGGGACTCCAGGGGAAGTTCTCTCCCTCACCAATTATTCTATGCGGTAATGAAGCAGCAGAGGAATCAAG
AACAACAGGAAACAGTGGATTGCCAGTCTGGT';

%WTnuc='ACCAGACTGGCAATCCACTGTTTCCTGTTGTTCTTGATTCCTCTGCTGCTTCATTACCGCATAGAATAATTGGT
GAGGGAGAGAACTTCCCCTGGAGTCCCACGTTGTATTGCCTGCTTTTCAGTGTGGTGGTGGTGACACTGCAGCAGTGC
AAATCATCTTCAGAGTGTGTTGGGAACAGAACA';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=96;

width=40;

flank=10;

Off-T3:

WTnuc='AGGTACTGGATTGCAGGTGTGTGCCACCATGCCTAGTGTATGCAGTGCTAGAGACTGACCCCAAGTTGACCTG
GGGAAGTTCTGTACCAACTGAGCCACACCCTAGCCTATGAGGATTCTTTTTTTTAAAATATTTTTTATTACATATTTTCCTC
AATTACATTTCCAATGCTATCCCAAAAGTC';

%WTnuc='GACTTTTGGGATAGCATTGGAAATGTAATTGAGGAAAATATGTAATAAAAAATATTTTAAAAAAAAGAATCCTC
ATAGGC

TAGGGTGTGGCTCAGTTGGTACAGAACTTCCCCAGGTCAACTTGGGGTCAGTCTCTAGCACTGCATACACTAGGCATGG
TGGCACACACCTGCAATCCAGTACCT';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=75;

width=40;

flank=10;

Off-T4:

WTnuc='CCTGGTACGCTTTCTGATGGAGCCTGGGATGTCCCCAGCAAAAGCTGTCAGCTGTTCTTTGTGGGACAGAAAT
TCCCCAGGTCTCTGAACACGTTGGATCTTTGCTACTTTAAATAAATCTTTATCTAATGTAGAAAAGAAAAAAACAAAAAAC
AAAAAAACCCGAAGAACTCCAGAGGGACTCGAGG';

%WTnuc='CCTCGAGTCCCTCTGGAGTTCTTCGGGTTTTTTTGTTTTTTGTTTTTTTCTTTTCTACATTAGATAAAGATTTAT
TTAAAGTAGCAAAGATCCAACGTGTTCAGAGACCTGGGGAATTTCTGTCCCACAAAGAACAGCTGACAGCTTTTGCTGG
GGACATCCCAGGCTCCATCAGAAAGCGTACCAGG ';

```
%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=75;

width=40;

flank=10;
```

Off-T5:

```
WTnuc='TTGAGTTTTCTGATTGCCCCAGTCTCTAACCTGCAAAGGTTAGGGTGCTCTTTGAAGCCCAGCCAACAGACTTG
TGTGGTTTTGTAAGCCTGGGGGAGTTCTGTCTCCTCTAGTACAAGAGTCATTTGTAGGAGGGTTCCAGTGACTTACTAC
CTCCAGCTACCTCTCCC';

%WTnuc='GGGAGAGGTAGCTGGAGGTAGTAAGTCACTGGAACCCTCCTACAAATGACTCTTGTACTAGAGGAGACAGA
ACTCCCCCAGGCTTACAAAACCACACAAGTCTGTTGGCTGGGCTTCAAAGAGCACCCTAACCTTTGCAGGTTAGAGACT
GGGGCAATCAGAAAACTCAA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=94;

width=40;

flank=10;
```

Off-T6:

```
WTnuc='TGTAACCAGCAGTTGATGCCTGGAGGCCAGGATGCCAAGCACCCTGCAGTGCCTGGAAGAGCCCTTCCCTGG
GAAGATCTGTCCCACCCCTGTACTAACAAGGCCCCATTGAAAAGCACTGCTCTAGGCCATGTGGCATCAAACACTTCCT
CTTGTTTCTTCTCTCACTGTCTAAGGAGCCAG';

%WTnuc='CTGGCTCCTTAGACAGTGAGAGAAGAAACAAGAGGAAGTGTTTGATGCCACATGGCCTAGAGCAGTGCTTTT
CAATGGGGCCTTGTTAGTACAGGGGTGGGACAGATCTTCCCAGGGAAGGGCTCTTCCAGGCACTGCAGGGTGCTTGG
CATCCTGGCCTCCAGGCATCAACTGCTGGTTACA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=72;

width=40;

flank=10;
```

Off-T7:

```
WTnuc='TGGGGACACTGCGATGGCCCCGCGCCGCCGTCGCCGCCCCTGCCCAGAAGTAGGTTTCCTGTTCCTGGCGA
AGTTCTGTCCTACACCTTCGCTCCTCCTCCCTGGCCCGCGAGCCCTCTGCGTCCCCACAGTTCGCTCCCGGCCTGAGC
GGAGCCTCTAGGTCCCCTTGGGGGACTCTTTAGA';

%WTnuc='TCTAAAGAGTCCCCCAAGGGGACCTAGAGGCTCCGCTCAGGCCGGGAGCGAACTGTGGGGACGCAGAGGG
CTCGCGGGCCAGGGAGGAGGAGCGAAGGTGTAGGACAGAACTTCGCCAGGAACAGGAAACCTACTTCTGGGCAGGG
GCGGCGACGGCGGCGCGGGGCCATCGCAGTGTCCCCA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=69;
```

```
width=40;

flank=10;
```

Off-T8:

```
WTnuc='GCACTTTTCTTACCATCTTCTCGAACTTCCCAGTCCAGTGTATGTTGTAGGGAAATGGTGTGGGTGAGACCAGA
GCTTCCCCTGGTCAGTTATAAATAGAATGTGAACATTTCACAGAGGAAGCTCCCTCTAAACGGCTGGACAGGGGTGGGG
CTGGGAGATAAGTTTTTGC';

%WTnuc='GCAAAAACTTATCTCCCAGCCCCACCCCTGTCCAGCCGTTTAGAGGGAGCTTCCTCTGTGAAATGTTCACAT
TCTATTTATAACTGACCAGGGGAAGCTCTGGTCTCACCCACACCATTTCCCTACAACATACACTGGACTGGGAAGTTCGA
GAAGATGGTAAGAAAAGTGC ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=79;

width=40;

flank=10;
```

Off-T9:

```
WTnuc='TCCATGTATCAGAGTTTGGCAGAAGAGACCAAATGTGAGGATTCAGGAAAAGGAAACAAACATGGAGTAGGTG
GGAAAGAACTTCTCCGGGCACAGGAAGTGAGAAACCCTGGAGAGAAAGCAAAAGAAAGGCAAGCATGGATCCAGGCTT
GAGAGGAAACATGGCCCTG';

%WTnuc='CAGGGCCATGTTTCCTCTCAAGCCTGGATCCATGCTTGCCTTTCTTTTGCTTTCTCTCCAGGGTTTCTCACTT
CCTGTGCCCGGAGAAGTTCTTTCCCACCTACTCCATGTTTGTTTCCTTTTCCTGAATCCTCACATTTGGTCTCTTCTGCCA
AACTCTGATACATGGA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=85;

width=40;

flank=10;
```

Off-T10:

```
WTnuc='GTGGCATGACTCAGCAGAGACAGCCAGGCCCCAGCTGAATTGTCATGAGTCAGCAGGAGGGACCAGGACCAC
CAGGGATGGTGGCCAGGAGAAGTTCTTTACCACCCCTCTCTCAAGGAAGCAAAGATGTGAGACCAGGAAGTGTTGCGA
AGCTAGCTGTGCAAGCAAGCCAAGC';

%WTnuc='GCTTGGCTTGCTTGCACAGCTAGCTTCGCAACACTTCCTGGTCTCACATCTTTGCTTCCTTGAGAGAGGGGT
GGTAAAGAACTTCTCCTGGCCACCATCCCTGGTGGTCCTGGTCCCTCCTGCTGACTCATGACAATTCAGCTGGGGCCT
GGCTGTCTCTGCTGAGTCATGCCAC ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=93;

width=40;

flank=10;
```

Off-T3'

```
WTnuc='TCCCCATTTAGCTGGTAGAGCTGCCTGGGCCTCCTTGGGTGTTGGCAGTCTCTGGGTTATCTGGTCCTTCATC
ATCAGCTAGGGGAAGTTCTGGCCTTCCTGAACCAGCCAGGTAAGCAGAAGCTGTGGACAGGGTGCAGTAAATTCCTTT
GGACTCTGGCAAGACTCTG';

%WTnuc='CAGAGTCTTGCCAGAGTCCAAAGGAATTTACTGCACCCTGTCCACAGCTTCTGCTTACCTGGCTGGTTCAGG
AAGGCCAGAACTTCCCCTAGCTGATGATGAAGGACCAGATAACCCAGAGACTGCCAACACCCAAGGAGGCCCAGGCAG
CTCTACCAGCTAAATGGGGA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=85;

width=40;

flank=10;
```

Off-T4'

```
WTnuc='TAGGTGTCCTCGGGTTTCTCATAATCTGGACCTACAACTCCAGCAAAGAGCCTAGAAAAGCATCAGCTTCAACT
CACCTGGGGAAGTTCAGCCCCCTCCCATCCTGTGGAGAGAAGCTTGAAAGGCTTGAGCAGCCATAGGGACCTTGGTCT
AGGTCTTATGTGTTAATTCCCCATTCTCCCT';

%WTnuc='AGGGAGAATGGGGAATTAACACATAAGACCTAGACCAAGGTCCCTATGGCTGCTCAAGCCTTTCAAGCTTCT
CTCCACAGGATGGGAGGGGGCTGAACTTCCCCAGGTGAGTTGAAGCTGATGCTTTTCTAGGCTCTTTGCTGGAGTTGTA
GGTCCAGATTATGAGAAACCCGAGGACACCTA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=82;

width=40;

flank=10;
```

Off-T5'

```
WTnuc='CTGACTTTATTGCTCCATGGCTCAGTGGTTTCCACTTAAATTTCCAATAAAATAAGCATGATGTCTAATTTCTCCC
TGGGAAGTTCTGTTCCACACATCTCTATCTCTGATATAACATTCGTGAGGTTAGCCTACAGAAAGAGCTGGTTGATTTGA
ATGTGAGATCCTGAGAACAGCT';

%WTnuc='AGCTGTTCTCAGGATCTCACATTCAAATCAACCAGCTCTTTCTGTAGGCTAACCTCACGAATGTTATATCAGA
GATAGAGATGTGTGGAACAGAACTTCCCAGGGAGAAATTAGACATCATGCTTATTTTATTGGAAATTTAAGTGGAAACCA
CTGAGCCATGGAGCAATAAAGTCAG ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=79;

width=40;

flank=10;
```

Off-T6'

```
WTnuc='TGAGTCAGAGATGTGGAAGAATCTTAAAATCACCATAAGAAATAGCAAAAGATGAAGAAAAATGCTATCCCTCA
CCCCCTGGAACAGAACTTCCCCAAGAGCTTCCATCTGCTCCTTCCTTACAGATTTTCTCCTCTTTCGTGGTTGACACTTG
CTTCTGCTAACAGCTCACC';
```

```
%WTnuc='GGTGAGCTGTTAGCAGAAGCAAGTGTCAACCACGAAAGAGGAGAAAATCTGTAAGGAAGGAGCAGATGGAA
GCTCTTGGGGAAGTTCTGTTCCAGGGGGTGAGGGATAGCATTTTTCTTCATCTTTTGCTATTTCTTATGGTGATTTTAAGA
TTCTTCCACATCTCTGACTCA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=93;

width=40;

flank=10;
```

Off-T7'

```
WTnuc='TTTCCTCACAAGAGAAACCAGCCAGGTTGTTTTAGCTAATGTTGAACGGGATGGGTTGAAAAACTAGGGGATGT
TCTGTCCCGCGCATCAATTTACAATGGAAGAAGGCTCAGTTTCACGTGGTTGTGCTTGCTGGAGACGTGTCAACAACAT
TTTCTTCTCCTGCAACTTTGGT';

%WTnuc='ACCAAAGTTGCAGGAGAAGAAAATGTTGTTGACACGTCTCCAGCAAGCACAACCACGTGAAACTGAGCCTTC
TTCCATTGTAAATTGATGCGCGGGACAGAACATCCCCTAGTTTTTCAACCCATCCCGTTCAACATTAGCTAAAACAACCT
GGCTGGTTTCTCTTGTGAGGAAA ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=70;

width=40;

flank=10;
```

Off-T8'

```
WTnuc='AAGCCACTGTCTCAGTGTTCCACGCTAGGAGATTGTTTTCAGCAGAACAATGCCCACCTCACTCCAAGGCAGG
ACAAAACTTCCCCAAGGCTTTGTTAGCCATTAACCCTATTTATAACTCTCCTGACGTCATACGACCTTATTCCTGGTCAGG
GCCAAAGTCGGTAAAC';

%WTnuc='CTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCG
GTGAACAGCTCCTCGCCCTTGCTCACCACGGTGGCGGCTCTCCCTAGGGTGAGTCGTATTAGCGGCCGCGGATCTCTA
GCGGATCTGACGGTTCACTAAACCAGCTCTGCTTATATAGACCTCCCACCG ';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=83;

width=40;

flank=10;
```

Supplementary Figure 1. Uncropped gel used in Extended Data Fig. 1 with molecular weight markers.