**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 OD600 ~ 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 14,000 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 with 10 mM MgCl₂) 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.
**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.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time of test</th>
<th>ABR threshold (injected vs. uninjected)</th>
<th>DPOAE threshold (injected vs. uninjected)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p Value</td>
<td>F statistic</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>Cas9:Tmc1-mut1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>Cas9:Tmc1-mut2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>Cas9:Tmc1-mut3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>Cas9:Tmc1-mut4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>Cas9:Tmc1-wt3</td>
<td>0.0620</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>Cas9:GFP</td>
<td>0.1187</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>dCas9:Tmc1-mut1</td>
<td>0.9322</td>
</tr>
<tr>
<td>Bth/+</td>
<td>4 weeks</td>
<td>Cas9:LPF2000 (no sgRNA)</td>
<td>0.2039</td>
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<tr>
<td>Bth/+</td>
<td>8 weeks</td>
<td>Cas9:Tmc1-mut1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bth/+</td>
<td>8 weeks</td>
<td>Cas9:Tmc1-mut3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>C3H</td>
<td>4 weeks</td>
<td>Cas9:Tmc1-mut3</td>
<td>&lt; 0.0001</td>
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### Supplementary Table 2. The number of injected and uninjected ears tested for ABRs and DPOAEs at each frequency.

<table>
<thead>
<tr>
<th>Frequencies (kHz)</th>
<th>Number of ears (Bth/+) tested for each frequency</th>
<th>Control injection (4 weeks)</th>
<th>Cas9: Tmc1-mut injection (8 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cas9: Tmc1- mut1</td>
<td>Cas9: Tmc1- mut2</td>
<td>Cas9: Tmc1-mut3</td>
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<tr>
<td>5.66</td>
<td>18</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>11.32</td>
<td>18</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>16</td>
<td>18</td>
<td>6</td>
<td>29</td>
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<tr>
<td>22.64</td>
<td>18</td>
<td>11</td>
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<tr>
<td>32</td>
<td>17</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>45.25</td>
<td>18</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Cas9: Tmc1- mut3</td>
<td>Uninjected</td>
<td></td>
</tr>
<tr>
<td>5.66</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>11.32</td>
<td>12</td>
<td>17</td>
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</tr>
<tr>
<td>16</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>22.64</td>
<td>12</td>
<td>16</td>
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</tr>
<tr>
<td>32</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>45.25</td>
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<td>14</td>
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<tr>
<td></td>
<td>Number of ears (C3H) tested for each frequency</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Cas9: Tmc1- mut3</td>
<td>Uninjected</td>
<td></td>
</tr>
<tr>
<td>5.66</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>11.32</td>
<td>12</td>
<td>17</td>
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</tr>
<tr>
<td>16</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>22.64</td>
<td>12</td>
<td>16</td>
<td></td>
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<tr>
<td>32</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>45.25</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>
### Supplementary Sequences. Oligonucleotides used in this study.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>5’-sequence-3’</th>
<th>Usage</th>
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</thead>
<tbody>
<tr>
<td>GX265</td>
<td>TAAATACGACTCATATAGGGAGAACATCATCTTCAAGGCTACTAGAGAATAG</td>
<td>Forward primer for TMC1-wt1 sgRNA in vitro transcription</td>
</tr>
<tr>
<td>GX266</td>
<td>TAAATACGACTCATATAGGGAGAACATCATCTTCAAGGCTACTAGAGAATAG</td>
<td>Forward primer for TMC1-wt2 sgRNA in vitro transcription</td>
</tr>
<tr>
<td>GX267</td>
<td>TAAATACGACTCATATAGGGAGAACATCATCTTCAAGGCTACTAGAGAATAG</td>
<td>Forward primer for TMC1-wt3 sgRNA in vitro transcription</td>
</tr>
<tr>
<td>GX268</td>
<td>TAAATACGACTCATATAGGGAGAACATCATCTTCAAGGCTACTAGAGAATAG</td>
<td>Forward primer for TMC1-mut1 sgRNA in vitro transcription</td>
</tr>
<tr>
<td>GX269</td>
<td>TAAATACGACTCATATAGGGAGAACATCATCTTCAAGGCTACTAGAGAATAG</td>
<td>Forward primer for TMC1-mut2 sgRNA in vitro transcription</td>
</tr>
<tr>
<td>GX270</td>
<td>TAAATACGACTCATATAGGGAGAACATCATCTTCAAGGCTACTAGAGAATAG</td>
<td>Forward primer for TMC1-mut3 sgRNA in vitro transcription</td>
</tr>
<tr>
<td>GX273</td>
<td>TACCTTCTCCATCAACAGGCTCTTCTCTGCATCTNNNAAAGTTGAGCACTATTACCTTT</td>
<td>HTS for Tmc1 site</td>
</tr>
<tr>
<td>GX274</td>
<td>GCTATCTGAGACTGAGAAGAAC</td>
<td>Cloning for plasmids Tmc1_wild-type and Tmc1_Bth</td>
</tr>
<tr>
<td>Off-T1F</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAAGCCACTGTCTCAGTGTTCC</td>
<td>Tmc1_HTS_F1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T1R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTTCCTCACAAGAGAAACCAGC</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
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<tr>
<td>Off-T2R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGTGAGCTGTTAGCAGAAGCA</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
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<tr>
<td>Off-T3R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGTCAGAGATGTGGAAGAATC</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T4R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTCCCCTTTATTGCTCCATGGCT</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T5R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGACTTTATTGCTCCATGGCT</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T6R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGCAGATGTGGAAGAATC</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T7R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGTCAGAGATGTGGAAGAATC</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T8R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGACTTTATTGCTCCATGGCT</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T9R</td>
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<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
<tr>
<td>Off-T10R</td>
<td>CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGACTTTATTGCTCCATGGCT</td>
<td>Tmc1_HTS_R1 cloning site identified by GUIDE-seq</td>
</tr>
</tbody>
</table>

**NOTES:**

- **guide_seq:** Cloning for plasmids Tmc1_wild-type and Tmc1_Bth
- **HTS:** High Throughput Sequencing
- **5’-sequence-3’** refers to the primer sequence in 5’ to 3’ orientation.
- **Usage** indicates the use of primers for different cloning and sequencing purposes.
**Supplementary Notes.** MATLAB script used to analyze indel percentages. Variable parameters are shown in red for different target sites.

\[\text{WTnuc='target genomic site sequence'};\]
\[\%\text{WTnuc='complimentary sequence to the above target site sequence'};\]

\%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:
    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 bases longer 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 filename
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 possess matching flank
% sequences
else
    nSkips=nSkips+1;
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 
 not INDEL %i
 Insertions %i
 Deletions %i
', [nSkips, notINDEL, NumIns, NumDels]);
close(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='AAGAATGACATGGCCTAATCTTAGTTTTATATAATTTAAATATTAAAGGACCAGCTCTGTGAAACCTTTCAACCCGGTCTCCTCCTTCTGAGATGGGTAATCGTCCTCACCCTGGGGATGTTCTGTCCCACCCTGTTTGACTTATTTGCTGAACTGGAAGATTACCATCCTCTCCTGAGGTGGGCTCCTGGGGCGCATTTTTTGTCTTTCTTT';

%WTnuc=AAGAAGCAGGAACATCGGGAGGAGAGGAGGTAATCTTCCAGTGAATTAGGAGAGGTTGAGGGATGTTCTGTCCCACCCTGGGGATGTTCTGTCCCACCCTGTTTGACTTATTTGCTGAACTGGAAGATTACCATCCTCTCCTGAGGTGGGCTCCTGGGGCGCATTTTTTGTCTTTCTTT;

%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=89;
width=40;
flank=10;

**Off-T1:**

WTnuc='TCTCTATGTGCTAAGTAGGCACTGCGAGGCGAGGAATCTGGGCTCCACCAGAGAAACCTTTTGTCTACAAAGGAGACATTTGCTATGCTGTGACACACTCTCTACTACCAGTACACAAAGTTCTTTTGAGCAACTGAATGCT';
%WTnuc='ACTGACTTAGTTCATGTGC CAAAGAACTTTGTGATCGGTAGTGAGATGT GTGTCACAGATGTCTAAGGATTAG GGAGGGACAGAGCTTCCCCAGGAACAAAGGTCTTCCTTTGTAGCAAAGGTTTCCTCTTGGAGCCTGGCAGGTTACCTG CTCTGCAGTGCCTACTTAGACACATAGAGA ';%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=93;
width=40;
flank=10;

Off-T2:
WTnuc='TGTTCTGTTCCCAACACACTCTGAAGATGATTTGCACTGCTGCAGTGTCACCACCACCACACTGAAAAGCAGGC AATACAACGTGGGACTCCAGGGGAAGTTCTCTCCCTCACCAATTATTCTATCGGTAATGAAGCAGCAGAGGAATCAAG AACAACAGGAAACAGTGAGTTGCCAGTCTGTT';%WTnuc='ACCAGACTGGCAATCCACTGTTTC CTGTTGTTCTTGATTCCTCTGCTGCTTCATTACCGCATAGAATAATTGGT GAGGGAGAGAACTCCCTGGAAGCCACCTGTATTTGGCTCTGCTTTTCAAGTGTGCTGTTGGGTGTTGACACTGACAGTAGTGC AAATCATCTTTGAGGTGTGGGAAACAGAACA';%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=96;
width=40;
flank=10;

Off-T3:
WTnuc='AGGTACTGGATTGCAGGTGTGCTCACTGCTAGTGTATGCAGTGCTAGAGACTGACCCCAAGTTGACCTG GGGAAGTTCTGTACCAACTGAGCCACACCCTAGCTATGGGATCTTTTTTTTTAAAAATATTTTTATTACATATTTCCTC AATTACATTTCACCAGTATTTCCAAAAGTC';%WTnuc='GACTTTTTGGGATAGCATTGGAAATGTAATTGAGGAAAATATGTAATAAAAAATATTTTTAAAAAAGAATCCTC ATAGGC TAGGGTGTGGCTACAGAAGAACTCTCCCCAGTTGGTCACTTACAGCAGCATACACTCGACGATGG TGGACACACCATGGAATCAGTACCT';%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=75;
width=40;
flank=10;

Off-T4:
WTnuc='CCTGGTACGCTTTCTGATGGAGCCTGGGATGTCCCCAGCAAAAGCTGTCAGCTGTTCTTTGTGGGACAGAAAT TCCCCAGTCTCGGAGGATTTTGGTGAGGACGAAGATAT TCCCCAGTCTCAGGCAACCTGGTAGTTTGGGATTTTTACCTATTGTTAAAATATTTCTATATTGAGAAAGAAAAC AAAAAACCCGAAAGAATCCAGGAGCGACTGAGG';%WTnuc='CCTGAGTGCCCTCTGGAGTTCTTCGGTATTTTTTTTTTTTTTTTCTTTTCTACATTAGATAAAAGATTTAT TAAAGTGACCCAGGAAAAGGACACGCTGACACGACTCTGCTGG GGCATCCCAGARTCCATAGAAGCGTACCAGG ';
%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=75;
width=40;
flank=10;

Off-T5:
WTnuc='TTGAGTTTTCTGATTGCCCCAGCTCTTAACCTGCAAAGGTTAGGGTGCTCTTTGAAGCCCAGCCAACAGACTTGTGCTTTTTGTAGCCTGGGGGAGTTCTGTCTCCTCTAGTACAAGAGTCATTTGTAGGAGGGTTCCAGTGACTTACTACCTCCAGCTACCTCTCCC';

%WTnuc='GGGAGAGGTAGCTGGAGGTAGTAAGTCACTGGAACCTCCTACAAAATGACTCTTTGACTAGAGAGGAGACAGA
ACTCCCCAGGCTTTACAACACAAAACCAAGTCAGTGTGAGGTGGCCTGCTCAAAGAGCACACACTTTTGCAGTTAGAGACT
GGGGCAATCAGAAAATCAA ';
%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=94;
width=40;
flank=10;

Off-T6:
WTnuc='TGTAACCAGCAGTTGATGCCTGGAGGCCAGGATGCCAAGCACCCTGCAGTGCCTGGAAGAGCCCTTCCCTGG
GAAGATCTGTCCCTACCCTCTACAGATGCTTAACTCCTACACCTGTGGGAGCCAG';

%WTnuc='CTGGCTCCTTAGACAGTGAGAGAAGAAACAAGAGGAAGTGTTTGATGCCACATGGCCTAGAGCAGTGCTTTTC
CAATGGGGGCTTTGAGTACAGGGGTGGGACAGATCTTCCCAGGGAAGGGCTCTTCCAGGCATGCCAGGTGCTTGG
CATCCTGCGCTCCAGGACTCAACTGCTGGTTACA ';%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=72;
width=40;
flank=10;

Off-T7:
WTnuc='TGGGGACACTGCGATGGCCCCGCCGCCGCGGCTGCACCCTGCCCCACAGAAGTGGTTTCTGCTTTGCTGGCGA
AGTTCTGCTACACCTCCTACCCCTCTGCCGCGGCCGCCGCCGCGTCCGCTCCCAGAGTCGCTCCGCTCCAGGCCTGAGC
GGAGCTCTAGGTGTCTCCCAGGGGACCTTTAGA';

%WTnuc='TCTAAAGAGTCCCCCAAGGGGACCTAGAGGCTCCGCTACGGGCAGGGAGCAACTGTTGGGGAGACAGGAGG
CTCGCCGGCCAGGGGAGGAGAGAGGAGAGAGAGGGGTAGGAGACAGAACTCGCCAGGACAGGAAAACACTTTCTGCGGAGG
GCGGACGGCGCGCGCCGCGCCCTCAGTGCTCCCA ';%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=69;
Off-T8:

WTnuc='GCACTTTTCTTACCATCTTCTCAGGAAATGTTGAGGTTGAGACAGAGA
GCTCCCCTGTAGTTTATAATAATAGAATGTAACATTTCCAGAGACAGCTCCCTAAACGGCTGGAGGGTGGG
CTGGGAGATAAAGTTTGC';

%WTnuc='GCAAAAACTTATCTCCCAGCCCCCACCCTGTCACAGCCGGGTTTAGAGGGAGCTTCCTCTGTGAATGTTACATT
CTATTTTATAACTGACCAGGGGAAGCTCTGGTCTCACCCACACCATTTCCCTTACAAACATACTGAGACTGGGAAGTTCGA
GAAGATGTAAGAAAAGTGC '

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=79;

width=40;

flank=10;

Off-T9:

WTnuc='TCCATGTATCAGGTTTGGCAGAAGAGACCAAATGTGAGGATTCAGGAAAAGGAAACAAACATGGAGTAGGTG
GGAAGAAGTCTCTCGGGGAAAGTAGGAGAAACCCTGGAGAGAAAGCAAAAGAAAGGCAAGCATGGATCCAGGCTT
GAGAGAAGAACTTCCTGCCCACTGATACATGGGAAGTGC'

%WTnuc='CAGGGCCATTTTCTCTCAAGAGCTTGACATGCTTTGCTTTTCTGCTCTCAGAGCTTCTCTACCT
CTGTTGCCAGGAAGTCTTTTCTCACCCTACTCTCATATTGTCTGCTCTTCTGCAAACTCTGATACTAGGAA'

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=85;

width=40;

flank=10;

Off-T10:

WTnuc='GTGGCATGACTCAGCAGAGACAGCCAGGCCCCAGCTGAATTGTCATGAGTCAGCAGGAGGGACCAGGACCAC
CAGGGATGTTGGCCAGGAAGTCTTTTACACCCTCTCTCAAGGAAGATGTAAGACAGGAAAGTTGCTGCAAGACAGAC
AGCTGAGCTGGCAAGCAAGCCAAGC';

%WTnuc='GCTGGCTTGCTTGCAACACTTCTTCCAGTATGCTTTGCTCTTGACTTTTGGAGAGAGGGGT
GGTAAGAAGTCTTCTCTTGCCACCATCCCTGCTGGTGGCTCTGCTCTGCTGACTTGATGAAATTCAGCTGGGCTC
GGCTGCTCTGCTAGCTGCACCT';

%cycle through fastq files for different samples

files=dir('*.fastq');

indelstart=93;

width=40;

flank=10;
Off-T3'

WTnuc='TCCCCATTTAGGTAGATGGCTGTGCTTTGGCTGTTGCTTGGTCTGCTGCTTTATCTGCTGCTTCTCTATC
ATCAAGCTAGGGGAAGTTCTGGCCTCTGAACCAGCAGCTGAGATGAGGCTGAGGTCAGTAATTCTCTTT
GGACTCTGGCAAGACTCTG';

%WTnuc='CAGAGTCTTGCCAGAGTCCAAAGGAATTTACTGACACCTGCTCCACAGCTTCTGCTTACCTGGCTGGTTCAGG
AAAGGCCAGAACTTCCCCTAGCTGATGATGAAGGACCAGATAACCCAGAGACTGCCAACACCCAAGGAGGCCCAGGCA
GCTCTACGACGTTAATGGGGA';

%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=85;
width=40;
flank=10;

Off-T4'

WTnuc='TAGGTGTCTGCTGGTTTCTCATAATCTGGACCTACAACTCCAGCAAAGAGCCTAGAAAAGCATCAGCTTCAACT
CACCTGGGGAAGTTCAAGCCCCCTCCCATCTCGTGGAGAGAAGCTTGAAAGGGTGAGCAGCCATAGGGACCTTGCT
AGGTCTTATGTGTTAATTCCCCATTCTCCTCCT';

%WTnuc='AGGGAGAATGGGGAATTAACACATAAGACCTAGACCAAGGTCCCTATGGCTGCTCAAGCCTTTCAAGCTTCT
CTCCACAGGATGGGGAAGCTGCTTGACTCTCTTTCTGCTCAGCTTTTGCTGTTGATGCTCTTCTTCTGCTTTGCT
AGTCCAGATATGAGAAACCCGAGCACCTA';

%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=82;
width=40;
flank=10;

Off-T5'

WTnuc='CTGACTTTATGGCTCATGCTGCTTGGCTCCTTTGCTTAAAACTTGGGCACTGATCGATGCTTGAATTCTCTCC
TGGGAAGTTCAAGCCCCCTCCCATCTCGTGGAGAGAAGCTTGAAAGGCTGCTGCTGCTTTTGCTGTTGATGCTCTT
CTCAGAGTCTTGGAAGCTGCTTGACTCTTCTTCTGCTCAGCTTTTGCTGTTGATGCTCTTCTTCTGCTTTGCT
AGTCCAGATATGAGAAACCCGAGCACCTA';

%WTnuc='AGCTGTTCTCAGGATCTCACATTCAAATACCCAGCTCTTTTCTGCTGCTTAACCTACGAAATGTGATATCAGA
GATAGAGATGCTGTGGAGGAAGAAACTTCCCAGGGAATTAAGACATCGTCTTTTATTGAAATTAGTGAAACCA
CTGAGCCATGAGCAATAAAGTCA';

%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=79;
width=40;
flank=10;

Off-T6'

WTnuc='TGAGTCAGAGATGTGGAAGAATCTTAAAATCACCATAAGAAAAATAGCATGATGCTTAATTCTTTCC
TGGGAAGTTCAAGCCCCCTCCCATCTCGTGGAGAGAAGCTTGAAAGGCTGCTGCTGCTTTTGCTGTTGATGCTCTT
CTCAGAGTCTTGGAAGCTGCTTGACTCTTCTTCTGCTCAGCTTTTGCTGTTGATGCTCTTCTTCTGCTTTGCT
AGTCCAGATATGAGAAACCCGAGCACCTA';

%WTnuc='CTGACTTTATGGCTCATGCTGCTTGGCTCCTTTGCTTAAAACTTGGGCACTGATCGATGCTTGAATTCTCTCC
TGGGAAGTTCAAGCCCCCTCCCATCTCGTGGAGAGAAGCTTGAAAGGCTGCTGCTGCTTTTGCTGTTGATGCTCTT
CTCAGAGTCTTGGAAGCTGCTTGACTCTTCTTCTGCTCAGCTTTTGCTGTTGATGCTCTTCTTCTGCTTTGCT
AGTCCAGATATGAGAAACCCGAGCACCTA';

%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=79;
width=40;
flank=10;
%WTnuc='GGTGAGCTGTTAGCAGAAGCAAGTGTCAACCACGAAAGAGGAGAAAATCTGTAAGGAAGGAGCAG ATGGAA
GCTCTTGGGGAAGTTCTGTTCCAGGGGGTGAGGGATAGCATTTTTCTTCATCTTTTGCTATTTCTTATGGGATTTTAAGA
TTCTTCCACATCTCTGACTCA ';
%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=93;
width=40;
flank=10;

Off-T7'
WTnuc='TTTCCTCACAAGAGAAAAACACGAGGTTGTTTTAGCTAATGTTGAACGGGATGGGTTGAAAAACTAGGGGATGT
TCTGCTCCGCGCATCACAATTCTCAATGGAAGACGTCACTTTACGTGTTGTTGCTTGGAGACGTGTCACACAAACAT
TTCTTCTCTTCTGAGAAAA ';
%WTnuc='ACCAAAGTTGCAGGAGAAGAAAATGTTGTTTACGTGACGTCTCCAGCAAGCA CAACCACGTGAAACTGAGCCTTC
TTCCATTGTAAATTGATGCGCGGGACAGAACATCCCCTAGGTTTTTTCAACCATCCGTTCAACATTTAGCTAAAAACACCT
GGCTGGTTTCTCTTGTGAGGAAA ';
%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=70;
width=40;
flank=10;

Off-T8'
WTnuc='AAGCCAACGTCTCAGTCCGTCCACAGGAGATTTGTTTTTTCAGCAAGAACATGCCACTCAACTCCAAGGCAGG
ACAAAACTTCCCAAGGCTTGTAGCTAGCATTATTTAACTCTCTTGACGTACATCACTTTACCTTGTCAGG
GCCAAAAGTCCTAAACCTTAACCTAAACCTTGCTTATAGACCTCCTCCAGC ';
%WTnuc='CTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCCAGCTGACTGAGATGGGACCTACCCCAG
GTGAACAGCTCCTCCTGCCCTTGCCTACCACAGGCGTGGCGTCTCCTTAAAGGTGAGTCCTGATTAGCGCCCGCCGATCTCTCA
GCGGAATCGACTGGTTCACTAAAACCAGCTCGTCTATAGACCTCCTCCACC ';
%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.