

Extended Data Table 1 | Off-target editing after nucleofection of DNA plasmids encoding Cas9 and Tmc1-mut3 sgRNA into primary fibroblasts derived from *Tmc1^{Bth/Bth}* mice**a**

	5'-Sequence-3'	Mismatches (MMs)	NCBI accession	Predicted function	Location	Indels in <i>Bth/Bth</i>
<i>Bth</i>	GGGTGGGACAGAACTTCCCCAGG	0MMs	N/A		chr9	31%
<i>Off-T1</i>	GGGAGGGACAGAGCTTCCCCAGG	2MMs [4:13]	N/A		chr1	8.1%
<i>Off-T2</i>	GTGAGGGAGAGAACTTCCCCTGG	3MMs [2:4:9]	N/A		chr16	4.4%
<i>Off-T3</i>	AGTTGGTACAGAACTTCCCCAGG	3MMs [1:3:7]	NC_000068.7	CD82 antigen	chr2	2.6%
<i>Off-T4</i>	TTGTGGGACAGAAATCCCCAGG	3MMs [1:2:14]	N/A		chr12	3.9%
<i>Off-T5</i>	AGAGGAGACAGAACTCCCCAGG	5MMs [1:3:4:6:16]	N/A		chr13	3.4%
<i>Off-T6</i>	GGGTGGGACAGATCTTCCCCAGG	2MMs [13:20]	NC_000067.6	hemicentin-1 isoform	chr1	0.68%
<i>Off-T7</i>	GTGTAGGACAGAACTTCGCCAGG	3MMs [2:5:18]	XM_006507026.3	inositol 1,4,5-triphosphate receptor 2	chr6	1.5%
<i>Off-T8</i>	GGTGAGACCAGAGCTTCCCCTGG	6MMs [3:4:5:7:8:13]	XR_389309.3	unknown	chr5	1.2%
<i>Off-T9</i>	AGGTGGGAAGAACTTCTCCGGG	3MMs [1:9:18]	NC_000070.6	paralemmin A kinase anchor protein	chr4	1.4%
<i>Off-T10</i>	GGGTGGTAAGAACTTCTCCTGG	3MMs [7:9:18]	N/A		chr10	0.048%

b

	5'-Sequence-3'	Mismatches	NCBI accession	Location	Indels in <i>Bth/Bth</i>
<i>Bth</i>	GGGTGGGACAGAACTTCCCCAGG	0MMs		chr9	31%
<i>Off-T1'</i>	GGGAGGGACAGAGCTTCCCCAGG	2MMs [4:13]		chr1	8.1%
<i>Off-T2'</i>	GTGAGGGAGAGAACTTCCCCTGG	3MMs [2:4:9]		chr16	4.4%
<i>Off-T3'</i>	AGGAAGGCCAGAACTTCCCCTAG	4MMs [1:4:5:8]	NM_001312644.1	chr12	0.037%
<i>Off-T4'</i>	GGAGGGGGCTGAACTTCCCCAGG	4MMs [3:4:8:10]		chr9	0.071%
<i>Off-T5'</i>	GTGTGGAACAGAACTTCCCAGGG	3MMs [2:7:20]		chr1	0.046%
<i>Off-T6'</i>	CCCTGGAAACAGAACTTCCCCAAG	4MMs [1:2:3:7]		chr2	0.097%
<i>Off-T7'</i>	GCGCGGGACAGAACATCCCCTAG	3MMs [2:4:15]		chr5	0.033%
<i>Off-T8'</i>	AGGCAGGACAAAACTTCCCCAAG	4MMs [1:4:5:11]		chr1	0.091%

a, Off-target sites identified by GUIDE-seq²³. Mismatch positions are indicated, counting the PAM as positions 21–23. *Off-T3*, *Off-T6*, *Off-T7*, *Off-T8* and *Off-T9* are located within predicted gene regions, while the rest are intergenic. One thousand nanograms Cas9 plasmid and 300 ng Tmc1-mut3 sgRNA plasmid were nucleofected into *Tmc1^{Bth/Bth}* fibroblasts using a LONZA 4D-Nucleofector and indels were detected by HTS at Tm *Tmc1^{Bth}* on-target and each off-target site. Mismatches compared to the on-target sequence are shown in red and PAMs are in blue. **b**, Off-target sites identified by computational prediction using the CRISPR Design Tool²⁴. Among the top eight computationally predicted off-target sites, only two (*off-T1'* and *off-T2'* with two and three mismatches, respectively) were identified as bona fide off-targets in cells by GUIDE-seq.