

In vivo genome editing using *Staphylococcus aureus* Cas9

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Supplementary Discussion on BLESS

The potential for off-target mutagenesis is an important consideration for Cas9-mediated genome editing. Currently, studies on Cas9 specificity have not been able to directly measure genome-wide nuclease activity in an unbiased manner¹⁻⁶. Since BLESS (direct in situ breaks labeling, enrichment on streptavidin and next-generation sequencing)⁷ can capture DNA double-stranded breaks (DSBs) in mammalian cells, we sought to apply it towards evaluating Cas9 specificity.

BLESS captures chromosomal DSB sites via ligation of a biotinylated hairpin adaptor to open ends of genomic DNA (DSB-proximal labeling). Chromosomal DNA with DSBs can subsequently be captured using streptavidin beads and sheared to yield smaller size fragments compatible with next generation sequencing. A distal adaptor carrying the PCR priming site was then ligated on to permit enrichment prior to preparation of sequencing library (**Figure 3a**).

Because DSBs can occur during natural biological processes such as replication, especially around pericentromeric and telomeric regions, as well as sample processing steps due to physical shearing, it is important to accurately identify the DSBs induced by Cas9. To do this, we empirically optimized the parameters for each step of our BLESS analysis, as explained in the following subsections: 1. Determining the clustering window for building regions of DSB enrichment (“DSB clusters”), 2. Defining the distribution of pairwise-distances within each DSB cluster, and 3. Background subtraction using negative controls (**Extended Data Figure 7a**).

1. Determining the clustering window for building regions of DSB enrichment

To build the DSB clusters from the sequencing reads, we took the first 30-bp of sequence reads immediately following the proximal label and mapped them using Bowtie to the hg19 or mm9 reference genome, allowing up to 2 mismatches. Following alignment, the reads were grouped using a nearest-neighbor clustering method, hence referred to as “DSB clusters”: we determined the genomic coordinate of the 5'-most position (first base) for each read, and grouped reads by applying a sliding window of width x , i.e. within each DSB cluster, the first base of any given read will be no more than x bp from its adjacent reads. We empirically determined that 30-bp windows yielded well-defined DSB clusters.

2. Defining the distribution of pairwise-distances within each DSB cluster

The grouping in the previous section using a sliding window identified all DSB regions, but did not distinguish between the ones induced by Cas9 activity and those from background. To determine properties that could be used to separate the two, we compiled a training data set by extracting the reads mapped to the on-target and a subset of known off-target sites. These off-target sites, verified by the presence of indels from sequencing, include those predicted based on similarity to the on-target sequence as well as by dCas9-ChIP (**Supplementary Tables 6 and 7**). We additionally included centromeric regions with DSB signals observed in both experimental (Cas9 and sgRNA co-transfected) and negative control (pUC19 transfected) samples to further refine the specificity of the algorithm.

Since every DSB generates two open chromosomal ends, the sequencing reads from either end of the DSB align to the + and – strands of the reference genome. The pattern and distribution of the forward (+ strand aligned) and reverse (– strand aligned) reads in a given DSB cluster can help

determine whether it is induced by Cas9. Since the DSB site within a centromeric or telomeric region is not consistent from cell to cell, we expect that such a DSB cluster contains forward and reverse reads that are broadly distributed (**Extended Data Figure 7b**)⁷. This contrasts with DSBs induced by Cas9, which typically occur at a well-defined position 3-bp upstream of the PAM⁸⁻¹⁰ (**Extended Data Figure 2**) and result in a characteristic distribution of forward and reverse reads that flank a sharp break site (**Extended Data Figure 7c**). However, due to end-resection during DNA repair, there can be reads aligned away from the cleavage site.

Cas9-induced DSBs can be distinguished from background events by the following analysis: first, we calculated the distance between every possible pair of forward and reverse reads in the DSB cluster by subtracting the chromosomal coordinate of the first base on reverse read from that of the forward read. A distance of 1 thus represents the reverse and forward reads directly abutting and facing away from each other. Distances of >1bp indicate reads that are separated by one or more base pairs, and distances of <1bp indicate reads that overlap. Second, we generated a histogram of these distances for each DSB cluster. Histograms of clusters from centromeric, telomeric, and other background regions had broad distributions of pairwise distances (**Extended Data Figure 7b**), while the histograms from Cas9-induced DSB clusters were sharp and asymmetric (**Extended Data Figure 7c**). Finally, to quantify this difference we calculated the fraction of pairwise reads that overlapped by no more than 7bp (distance ≥ -6 bp) within all possible pairwise distances in each cluster. Based on the training dataset, we found that in the majority of Cas9-induced clusters, this fraction was greater than 0.99. In using this metric to filter out background clusters, we required that a candidate Cas9-induced DSB cluster should have a minimal fraction number of 0.95. This relaxed cut-off value of 0.95 was selected to

increase the sensitivity for detecting bona fide Cas9-induced clusters, particularly for those with fewer read counts where a small number of outlier reads might significantly reduce the fraction value.

3. Background subtraction using negative controls

Finally, we compared the DSB clusters in the experimental versus the negative control group to locate and remove background signals that should be present in both datasets. The DSB score for a given genomic locus was calculated by comparing the count of pass-filter clusters in the experimental samples with the controls using a maximum likelihood estimate (MLE)¹. This score describes the number of expected Cas9-induced DSB clusters per 1×10^5 reads and allowed the final ranking of all candidate DSB sites.

We have taken the above approach to minimize the use of heuristics and limit the introduction of potential biases during the identification of Cas9-induced DSBs. To assess how effectively the candidate DSB sites from BLESS predict levels of indel formation, we performed targeted deep-sequencing on the top ~30 loci that have the highest DSB scores (**Figure 3, Supplementary Data Table 5, and Extended Data Figure 8**). This revealed a significant linear correlation between BLESS DSB scores and rates of indel mutations (**Figure 3c**).

Altogether, these results suggest that BLESS is a sensitive method for detecting Cas9 nuclease activity in an unbiased, genome-wide manner. In particular, BLESS was able to detect off-target sites featuring insertion and deletion mismatches between the RNA guide and the genomic DNA sequence. Furthermore, BLESS is a powerful tool for future studies of designer nuclease

specificity in several ways: first, it is a direct measurement of nuclease activity, unaffected by the efficiency of downstream events. This additionally reduces bias resulting from off-target modification of essential genes, which can affect cell viability. Second, BLESS has the potential to be readily applied *in vivo* without the need to label cleavage events through exogenous markers.

Finally, future implementations of BLESS can be improved by including the use of unique molecular identifiers (UMIs) on the hairpin adaptors to minimize the effects of PCR bias. Additionally, greater sequencing depth can improve the sensitivity for identifying candidate Cas9-induced DSB. Furthermore, taking multiple time points after delivery of Cas9 can shed additional insight on temporal dynamics of its genome-wide activity.

References

1. Hsu, P. D. *et al.* DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat. Biotechnol.* **31**, 827–832 (2013).
2. Mali, P. *et al.* CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nat. Biotechnol.* **31**, 833–838 (2013).
3. Fu, Y. *et al.* High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat. Biotechnol.* **31**, 822–826 (2013).
4. Pattanayak, V. *et al.* High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. *Nat. Biotechnol.* **31**, 839–843 (2013).
5. Lin, Y. *et al.* CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences. *Nucleic Acids Res.* **42**, 7473–7485 (2014).

6. Bae, S., Park, J. & Kim, J.-S. Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinforma. Oxf. Engl.* **30**, 1473–1475 (2014).
7. Crosetto, N. *et al.* Nucleotide-resolution DNA double-strand break mapping by next-generation sequencing. *Nat. Methods* **10**, 361–365 (2013).
8. Garneau, J. E. *et al.* The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* **468**, 67–71 (2010).
9. Jinek, M. *et al.* A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816–821 (2012).
10. Gasiunas, G., Barrangou, R., Horvath, P. & Siksnys, V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **109**, E2579–2586 (2012).

Supplementary Tables

Supplementary Table 1 | List of Cas9 orthologs and predicted RNA components

Cas9	class	direct repeat	tracrRNA	sgRNA scaffold
<i>S. pyogenes</i>	IIA	GUUUUAGAG CUAUGCUGU UUUGAAUGG UCCCAAAC	GUUGGAACCAUUCAAAACA GCAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAGU CGGUGCUUUUU	GUUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGUU AUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUU
<i>S. aureus</i>	IIA	GUUUUAGUA CUCUGUAAU UUUAGGUAU GAGGUAGAC	AUUGUACUUAUACCUAAAA UUACAGAAUCUACUAAAAC AAGGCAAAAUGCCGUGUUU AUCUCGUCAACUUGUUGGC GAGAUUUUU	GUUUUAGUACUCUGGAAACAGA AUCUACUAAAACAAGGCAAAAU GCCGUGUUUAUCUCGUCAACUU GUUGGCGAGAUUUU
<i>S. thermophilus</i>	IIA	GUUUUUGUA CUCUCAAGA UUUAAGUAA CUGUACAAC	CUUACACAGUUAUUAUUUU CUUGCAGAAGCUACAAGA UAAGGCUUCAUGCCGAAAU CAACACCCUGUCAUUUUUAU GGCAGGGUGUUUU	GUUUUUGUACUCGAAAGAAGCU ACAAAGUAAGGCUUCAUGCCG AAAUCAACACCCUGUCAUUUUUA UGGCAGGGUGUUUU
<i>S. pasteurianus</i>	IIA	GUUUUUGUA CUCUCAAGA UUUAAGUAA CCGUAAAAC	CUUGCACGGUUAUUAUUUU CUUGCUGAGCCUACAAGA UAAGGCUUUUAUGCCGAAAU CAAGCACCCCAUGUUUUUGA CAUGAGGUGCUUUUU	GUUUUUGUACUCGAAAGAGCCU ACAAAGUAAGGCUUUUAUGCCG AAUUCAAGCACCCCAUGUUUUUG ACAUGAGGUGCUUUUU
<i>N. cinerea</i>	IIC	GUUGUAGCU CCCAUUCUC AUUUCGCAG UGCUACAAU	AUUGUCGCACUGCGAAAUUG AGAACCUGUUGCUACAUAUA GGCCGUCUGAAAAGAUGUG CCGCAACGCUCUGCCCCUU AAAGCUUCUGCUUUUAAGGG GCAUCGUUUUUUUCGGUUA AAAAUGCCGUCUGAAACCG GUUUUU	GUUGUAGCUCCCAUUCUGGAAA GAGAACCGUUGCUACAUAUAGG CCGUCUGAAAAGAUGUGCCGCA ACGCUCUGCCCCUUAAGCUUC UGCUIUAAGGGGCAUCGUUUUAU UUCGGUUAUUUAUGCCGUCUGA AACCGGUUUUUAGGUUUCAGAC GGCAUUUU
<i>C. lari</i>	IIC	GUUUUAGUC UCUUUUUAA AUUUCUUUA UGAUAAAAU	AAUUCUUGCUAAAGAAAUU UAAAAAGAGACUAAAAUAA GUGGUUUUUGGUCAUCCAC GCAGGGUUAACAUCCCUUU AAAACCAUUAUUUAUCAA UAAACUAGGUUGUAUCAAC UUAGUUUUUU	GUUUUAGUCUCUGAAAAGAGAC UAAAAUAAGUGGUUUUUGGUCA UCCACGCAGGGUUAACAUCCCU UUAAAACCAUUAUUUAUCAAU AAACUAGGUUGUAUCAACUAG UUUU
<i>P. lavamentivorans</i>	IIC	GCUGCGGAU UGCGGCCGU CUCUCGAUU UGCUACUCU	UAGCAAAUCGAGAGGCGGU CGCUUUUCGCAAGCAAUU GACCCCUUGUGCGGGCUCG GCAUCCCAAGGUCAGCUGC CGGUUAUUUAUCGAAAAGGC CCACCGCAAGCAGCGCGUG GGCCUUUUU	GCUGCGGAUUGCGGGAAAUCGC UUUUCGCAAGCAAUUUGACCCC UUGUGCGGGCUCGGCAUCCCAA GGUCAGCUGCCGGUUAUUUCG AAAAGGCCACCGCAAGCAGCG CGUGGGCCUUUU
<i>C. diphtheriae</i>	IIC	ACUGGGGUU CAGUUCUCA AAAACCCUG AUAGACUUC	AGUCACUAAUUAUUUUAAA UAGAACUGAACCUACAGUAA GCAUUGGCUCGUUUCCAAU GUUGAUUGCUCGCCGGUG CUCCUUUUUUUAAGGGCG CCGGCUUUUU	ACUGGGGUUCAGGAAACUGAAC CUCAGUAAGCAUUGGCUCGUUU CCAUGUUGAUUGCUCGCCGG UGCUCUUUUUUUAAGGGCGC CGGCUUUUU

Supplementary Table 2 | Targets used for PAM validation in *in vitro* lysate reaction

Cas9	Consensus	<i>in vitro</i> lysate targets (Dyrk1a)	PAM	Gene
<i>P. lavamentivorans</i>	NNNCATN	TAATCACTATGGATCTTCTA	TACCATT	<i>DYRK1A</i>
<i>P. lavamentivorans</i>	NNNCATN	TCTTGTAGGAGGAGAGACTT	CAGCATG	<i>DYRK1A</i>
<i>C. diphtheriae</i>	NGGNNNN	GGTGCAAGCCGAACAGATGA	TGGACAG	<i>DYRK1A</i>
<i>C. diphtheriae</i>	NGGNNNN	TATCCTAAAGTTCTTATTTA	AGGTTTG	<i>DYRK1A</i>
<i>S. pasteurianus</i>	NNGTGAN	TTAATTTATGAAAATCTCGT	AGGTGAA	<i>DYRK1A</i>
<i>S. pasteurianus</i>	NNGTGAN	ATGCCCCATTACATCAGTA	CAGTGAC	<i>DYRK1A</i>
<i>N. cinerea</i>	NNNNGAT	GTGTTGAGTAACATATACCT	GTTTGTA	<i>DYRK1A</i>
<i>N. cinerea</i>	NNNNGAT	TAACTAACCAGGTAAGTTCA	TGGAGTA	<i>DYRK1A</i>
<i>S. aureus</i>	NNGRRNN	AATGATACAAACATTAGGAT	ATGAATA	<i>DYRK1A</i>
<i>S. aureus</i>	NNGRRNN	ATGTCAAATGATACAAACAT	TAGGATA	<i>DYRK1A</i>
<i>C. lari</i>	NNGGGNN	GGTCACTGTACTGATGTGAA	TGGGGCA	<i>DYRK1A</i>
<i>C. lari</i>	NNGGGNN	CGGTCACTGTACTGATGTGA	ATGGGGC	<i>DYRK1A</i>
<i>S. pyogenes</i>	NGGNNNN	TGTCAAATGATACAAACATT	AGGATAT	<i>DYRK1A</i>
<i>S. pyogenes</i>	NGGNNNN	AACCTCACTTATCTTCTTGT	AGGAGGA	<i>DYRK1A</i>
<i>S. thermophilus</i>	NNAGAAW	CCAGGTAAGTTCATGGAGTA	TCAGAAA	<i>DYRK1A</i>
<i>S. thermophilus</i>	NNAGAAW	TAACATATACCTGTTTGTAG	TTAGAAA	<i>DYRK1A</i>

Supplementary Table 3 | Targets used for ortholog activity test in cells and *in vivo*

Cas9	Consensus	Targets	PAM	Gene	Cell type	indel (%)
<i>C. diphtheriae</i>	NGGNNNN	TCACCTCCAATGACTAGGGT	GGGCAAC	<i>EMX1</i>	293FT	N.D.
<i>C. diphtheriae</i>	NGGNNNN	TGACGGTGCAAGCCGAACAGAT GA	TGGACAG	<i>DYRK1A</i>	293FT	N.D.
<i>C. diphtheriae</i>	NGGNNNN	ACCTGGTGGGCGACGTGCTG	GGGAGTC	<i>DYRK1A</i>	293FT	N.D.
<i>C. diphtheriae</i>	NGGNNNN	ATGGAGCAGTCTCAGTCTTC	GGGCACC	<i>DYRK1A</i>	293FT	N.D.
<i>N. cinerea</i>	NNNNGAT	GAATGAAAATGACGGTGCAAGC CG	AACAGAT	<i>DYRK1A</i>	293FT	N.D.
<i>N. cinerea</i>	NNNNGAT	TTAATGGTATAGAAGATCCA	TAGTGAT	<i>DYRK1A</i>	293FT	N.D.
<i>C. lari</i>	NNGGGNN	TGTCACCTCCAATGACTAGG	GTGGGCA	<i>EMX1</i>	293FT	N.D.
<i>C. lari</i>	NNGGGNN	CCATGGAGCAGTCTCAGTCT	TCGGGCA	<i>DYRK1A</i>	293FT	N.D.
<i>C. lari</i>	NNGGGNN	GCACCAGCATCGGCACAGTG	GTGGGCA	<i>DYRK1A</i>	293FT	N.D.
<i>C. lari</i>	NNGGGNN	CGACGGTCACTGTACTGATGTG AA	TGGGGCA	<i>DYRK1A</i>	293FT	N.D.
<i>P. lavamentivorans</i>	NNNCATN	CCGAGCAGAAGAAGAAGGGC	TCCCATC	<i>EMX1</i>	293FT	N.D.
<i>P. lavamentivorans</i>	NNNCATN	ATTTTAATCACTATGGATCTTC TA	TACCATT	<i>DYRK1A</i>	293FT	N.D.
<i>P. lavamentivorans</i>	NNNCATN	CCAAAACCTCGAATTC AACCT	GGTCATA	<i>DYRK1A</i>	293FT	N.D.
<i>P. lavamentivorans</i>	NNNCATN	TGCAGCACAGTTTCTTCAAG	GAGCATA	<i>DYRK1A</i>	293FT	N.D.
<i>S. pasteurianus</i>	NNGTGAN	GTTCTTAATTTATGAAAATCTC GT	AGGTGAA	<i>DYRK1A</i>	293FT	N.D.
<i>S. pyogenes</i>	NGGNNNN	GAGTCCGAGCAGAAGAAGAA	GGGCTCC	<i>EMX1</i>	293FT	33.3
<i>S. pyogenes</i>	NGGNNNN	TGACGGTGCAAGCCGAACAGAT GA	TGGACAG	<i>DYRK1A</i>	293FT	3.0
<i>S. thermophilus</i>	NNAGAAW	TGAGTAACATATACCTGTTTGT AG	TTAGAAA	<i>DYRK1A</i>	293FT	5.0
<i>S. aureus</i>	NNGRRNN	CAACCACAAACCCACGAGGG	CAGAGTG	<i>EMX1</i>	293FT	15.9
<i>S. aureus</i>	NNGRRNN	TAGGGTTAGGGGCCCCAGGC	CGGGGTC	<i>EMX1</i>	293FT	13.0
<i>S. aureus</i>	NNGRRNN	CCTCTAACTAACCAGGTAAGTT CA	TGGAGTA	<i>DYRK1A</i>	293FT	6.7
<i>S. aureus</i>	NNGRRNN	TAAGAGAGTAGGCTGGTAGA	TGGAGTT	<i>GRIN2B</i>	293FT	24.2
<i>S. aureus</i>	NNGRRNN	GAGTAGGCTGGTAGATGGAG	TTGGGTT	<i>GRIN2B</i>	293FT	31.7
<i>S. aureus</i>	NNGRRNN	GTTGAAGATGAAGCCCAGAG	CGGAGTG	<i>GRIN2B</i>	293FT	13.4
<i>S. aureus</i>	NNGRRNN	TGGATGCCCAGGATGGGGGT	GAGAGTA	<i>GRIN2B</i>	293FT	18.7
<i>S. aureus</i>	NNGRRNN	AAAGAAAGAGCATGTTAAAA	TAGGATA	<i>GRIN2B</i>	293FT	N.D.
<i>S. aureus</i>	NNGRRNN	TCAGACATGAGATCACAGAT	GCGGGTG	<i>GRIN2B</i>	293FT	29.3
<i>S. aureus</i>	NNGRRNN	GATGCGGGTGATGATGCTCT	TTGGGTC	<i>GRIN2B</i>	293FT	17.6
<i>S. aureus</i>	NNGRRNN	TCATGGCTACCAGTTCCACC	CGGGGTA	<i>GRIN2B</i>	293FT	26.6
<i>S. aureus</i>	NNGRRNN	CCCGGGTGGAACTGGTAGCC	ATGAATG	<i>GRIN2B</i>	293FT	26.2
<i>S. aureus</i>	NNGRRNN	CTTCCGACGAGGTGGCCATC	AAGGATT	<i>GRIN2B</i>	293FT	7.6
<i>S. aureus</i>	NNGRRNN	CACCATCTCTCCGTGGTACC	CCGGGTG	<i>GRIN2B</i>	293FT	18.2
<i>S. aureus</i>	NNGRRNN	CACCGCAGCCACGCAGAGCA	GTGGGTG	<i>Pcsk9 (sg1)</i>	Hepa1-6	4.3
<i>S. aureus</i>	NNGRRNN	CACCGCAGCCACGCAGAGCA	GTGGGTG	<i>Pcsk9 (sg1)</i>	N2a	36.4
<i>S. aureus</i>	NNGRRNN	CCGCTGACCACACCTGCCAG	GTGGGTG	<i>Pcsk9 (sg2)</i>	Hepa1-6	8.3
<i>S. aureus</i>	NNGRRNN	CCGCTGACCACACCTGCCAG	GTGGGTG	<i>Pcsk9</i>	N2a	24.4

<i>S. aureus</i>	NNGRRNN	GCCCATCGGGAGATTGAGGG	CAGGGTC	(sg2) <i>Pcsk9</i>	Hepa1-6	9.7
<i>S. aureus</i>	NNGRRNN	GCAACAACAAGATCTGTGGC	TGGAATT	(sg3) <i>HmgCR</i>	Hepa1-6	2.34
<i>S. aureus</i>	NNGRRNN	CTTTCGTGGGCCCATGGCGG	ATGGATG	<i>ApoB</i> (sg1)	Mouse liver	8.7
<i>S. aureus</i>	NNGRRNN	CAGGCTTTCGTGGGCCCATG	GCGGATG	<i>ApoB</i> (sg2)	Mouse liver	0
<i>S. aureus</i>	NNGRRNN	CACCCCACCATCCATCCGCC	ATGGGCC	<i>ApoB</i> (sg3)	Mouse liver	3.0
<i>S. pasteurianus</i>	NNGTGAN	ATCTAGCCTCTTCTAAGACAGG TT	ACGTGAT	<i>GRIN2B</i>	HEK 239FT	16
<i>S. pasteurianus</i>	NNGTGAN	GGTCAGACATGAGATCACAGAT GC	GGGTGAT	<i>GRIN2B</i>	HEK 239FT	14

N.D.: Not determined.

Supplementary Table 4 | Targets used for SaCas9 PAM determination in 239FT cells

Targets	PAM	Gene	indel (%)
CCTGGACACCCCGTTCTCCT	GTGGAT	<i>AAVSI</i>	5
ACAGCATGTTTGCTGCCTCC	AGGGAT	<i>AAVSI</i>	13
GTGGTCCCAGCTCGGGGACA	CAGGAT	<i>AAVSI</i>	30
CGGTTAATGTGGCTCTGGTT	CTGGGT	<i>AAVSI</i>	35
TGTCCTAGTGGCCCCACTG	TGGGGT	<i>AAVSI</i>	31
TCCTTCCTAGTCTCCTGATA	TTGGGT	<i>AAVSI</i>	34
CCTGAAGTGGACATAGGGGC	CCGGGT	<i>AAVSI</i>	N.D.
GAGAGATGGCTCCAGGAAAT	GGGGT	<i>AAVSI</i>	16
TTGCTTACGATGGAGCCAGA	GAGGAT	<i>AAVSI</i>	N.D.
GAGCCACATTAACCGCCCT	GGGAAT	<i>AAVSI</i>	32
CACAGTGGGGCCACTAGGGA	CAGGAT	<i>AAVSI</i>	27
GACTAGGAAGGAGGAGCCT	AAGGAT	<i>AAVSI</i>	23
GAATCTGCCTAACAGGAGGT	GGGGT	<i>AAVSI</i>	26
TGGGGTGTGTCACCAGATA	AGGAAT	<i>AAVSI</i>	15
CCCTGCCAAGCTCTCCCTCC	CAGGAT	<i>AAVSI</i>	18
CTGGGAGGGAGAGCTTGGCA	GGGGT	<i>AAVSI</i>	N.D.
CAGGGGTGGGAGGGAAGGG	GGGGT	<i>AAVSI</i>	N.D.
GGTGGCTAAAGCCAGGGAGA	CGGGT	<i>AAVSI</i>	N.D.
TAGGGTTAGGGGCCCAGGC	CGGGT	<i>EMXI</i>	N.D.
ATGGGAAGACTGAGGCTACA	TAGGGT	<i>EMXI</i>	N.D.
CATCAGGCTCTCAGCTCAGC	CTGAGT	<i>EMXI</i>	N.D.
GTGGCTGCTCTGGGGCCTC	CTGAGT	<i>EMXI</i>	29
GAAGCTGGAGGAGGAAGGGC	CTGAGT	<i>EMXI</i>	8
TCGATGTCACCTCCAATGAC	TAGGGT	<i>EMXI</i>	15
GCAAGCAGCACTCTGCCCTC	GTGGGT	<i>EMXI</i>	8
CAACCACAAACCACGAGGG	CAGAGT	<i>EMXI</i>	32
AAGCTGGCCAGGGAGTGGC	CAGAGT	<i>EMXI</i>	7
GCCTCCCCAAAGCCTGGCCA	GGGAGT	<i>EMXI</i>	28
GGCCAGGCTTTGGGGAGGCC	TGGAGT	<i>EMXI</i>	24
CAGGCTGAGCTGAGAGCCTG	ATGGGA	<i>EMXI</i>	9
CTCAACACTCAGGCTGAGCT	GAGAGC	<i>EMXI</i>	9
GCCTCAACACTCAGGCTGAG	CTGAGA	<i>EMXI</i>	9
CTGGGGCCTCAACACTCAGG	CTGAGC	<i>EMXI</i>	8
GAGGCCCCCAGAGCAGCCAC	TGGGGC	<i>EMXI</i>	20
GGAGGCCCCCAGAGCAGCCA	CTGGGG	<i>EMXI</i>	21
TGAGAAACTCAGGAGGCCCC	CAGAGC	<i>EMXI</i>	15
GGGGCACAGATGAGAAACTC	AGGAGG	<i>EMXI</i>	10
AGGGGCACAGATGAGAAACT	CAGGAG	<i>EMXI</i>	2
AGGGAGGGAGGGGCACAGAT	GAGAAA	<i>EMXI</i>	5
CCAGGGAGGGAGGGGCACAG	ATGAGA	<i>EMXI</i>	3
TTCACCTGGGCCAGGGAGGG	AGGGGC	<i>EMXI</i>	1
CTTACCTGGGCCAGGGAGG	GAGGGG	<i>EMXI</i>	8
ACCTTACCTGGGCCAGGGA	GGGAGG	<i>EMXI</i>	7
CACCTTACCTGGGCCAGGG	AGGGAG	<i>EMXI</i>	6
ACCACACCTTACCTGGGCC	AGGGAG	<i>EMXI</i>	5
ACACCTTACCTGGGCCAGG	GAGGGA	<i>EMXI</i>	5
CCACACCTTACCTGGGCCA	GGGAGG	<i>EMXI</i>	8
AACCACACCTTACCTGGGC	CAGGGA	<i>EMXI</i>	6
TTCTGGAACCACACCTTAC	CTGGGC	<i>EMXI</i>	7
TGTACTTTGTCTCCGGTTC	TGGAAC	<i>EMXI</i>	2
TTGTACTTTGTCTCCGGTT	CTGGAA	<i>EMXI</i>	2

GGGAGCCCTTCTTCTTCTGC	TCGGAC	<i>EMXI</i>	N.D.
GCGCCACCGTTGATGTGAT	GGGAGC	<i>EMXI</i>	2
TGCGCCACCGTTGATGTGA	TGGGAG	<i>EMXI</i>	7
ATGCGCCACCGTTGATGTG	ATGGGA	<i>EMXI</i>	N.D.
CTCTCAGCTCAGCCTGAGTG	TTGAGG	<i>EMXI</i>	11
TTGAGGCCCCAGTGGCTGCT	CTGGGG	<i>EMXI</i>	N.D.
TGAGGCCCCAGTGGCTGCTC	TGGGGG	<i>EMXI</i>	N.D.
GAGGCCCCAGTGGCTGCTCT	GGGGGC	<i>EMXI</i>	N.D.
CCCCTCCCTCCCTGGCCAG	GTGAAG	<i>EMXI</i>	4
CCCAGGTGAAGGTGTGGTTC	CAGAAC	<i>EMXI</i>	4
GTGAAGGTGTGGTTCAGAA	CCGGAG	<i>EMXI</i>	N.D.
TGAAGGTGTGGTTCAGAAC	CGGAGG	<i>EMXI</i>	12
AAGGTGTGGTTCAGAACCG	GAGGAC	<i>EMXI</i>	10
GGAGGACAAAGTACAAACGG	CAGAAG	<i>EMXI</i>	3
CAAAGTACAAACGGCAGAAG	CTGGAG	<i>EMXI</i>	2
AAAGTACAAACGGCAGAAGC	TGGAGG	<i>EMXI</i>	3
AGTACAAACGGCAGAAGCTG	GAGGAG	<i>EMXI</i>	3
GTACAAACGGCAGAAGCTGG	AGGAGG	<i>EMXI</i>	8
ACAAACGGCAGAAGCTGGAG	GAGGAA	<i>EMXI</i>	3
CAAACGGCAGAAGCTGGAGG	AGGAAG	<i>EMXI</i>	4
ACGGCAGAAGCTGGAGGAGG	AAGGGC	<i>EMXI</i>	26
GGAGGAGGAAGGGCCTGAGT	CCGAGC	<i>EMXI</i>	5
AGGAAGGGCCTGAGTCCGAG	CAGAAG	<i>EMXI</i>	13
AAGGGCCTGAGTCCGAGCAG	AAGAAG	<i>EMXI</i>	8
GGCCTGAGTCCGAGCAGAAG	AAGAAG	<i>EMXI</i>	1
CTGAGTCCGAGCAGAAGAAG	AAGGGC	<i>EMXI</i>	1
TCAACCGGTGGCGCATTGCC	ACGAAG	<i>EMXI</i>	7
GGCCACTCCCTGGCCAGGCT	TTGGGG	<i>EMXI</i>	N.D.
GCCACTCCCTGGCCAGGCTT	TGGGGA	<i>EMXI</i>	N.D.
CCACTCCCTGGCCAGGCTTT	GGGGAG	<i>EMXI</i>	5
CACTCCCTGGCCAGGCTTTG	GGGAGG	<i>EMXI</i>	7
TGGCCAGGCTTTGGGGAGGC	CTGGAG	<i>EMXI</i>	N.D.
GGCCTCCCCAAAGCCTGGCC	AGGGAG	<i>EMXI</i>	5
AGGCCTCCCCAAAGCCTGGC	CAGGGA	<i>EMXI</i>	9
TGTCACCTCCAATGACTAGG	GTGGGC	<i>EMXI</i>	1
GTGGGCAACCACAAACCCAC	GAGGGC	<i>EMXI</i>	5
TGGTTGCCACCCTAGTCAT	TGGAGG	<i>EMXI</i>	1
GTGGTTGCCACCCTAGTCA	TTGGAG	<i>EMXI</i>	1
GGCCTGGAGTCATGGCCCCA	CAGGGC	<i>EMXI</i>	5
GAGTCATGGCCCCACAGGGC	TTGAAG	<i>EMXI</i>	7
GCCCCGGGCTTCAAGCCCTG	TGGGGC	<i>EMXI</i>	N.D.
GGCCCCGGGCTTCAAGCCCT	GTGGGG	<i>EMXI</i>	3
CATTGCCACGAAGCAGGCCA	ATGGGG	<i>EMXI</i>	16
ATTGCCACGAAGCAGGCCAA	TGGGGA	<i>EMXI</i>	10
TTGCCACGAAGCAGGCCAAT	GGGGAG	<i>EMXI</i>	N.D.
TGCCACGAAGCAGGCCAATG	GGGAGG	<i>EMXI</i>	15
CCACGAAGCAGGCCAATGGG	GAGGAC	<i>EMXI</i>	30
GGGTGGGCAACCACAAACCC	ACGAGG	<i>EMXI</i>	6
GCTGCTGGCCAGGCCCTGC	GTGGGC	<i>EMXI</i>	3
GAGTCCAGCTTGGGCCACG	CAGGGG	<i>EMXI</i>	6

N.D.: not determined.

Supplementary Table 5 | Top genomic DSB peaks identified by BLESS in 293FT cells

Site	Fwd Priming Site	Rev Priming Site	Chromosome	DSBs frequency	indel %
EMX1-sg1-SaCas9 (on target)	AACCCACGAGGGC AGAGT	GAGGAGAAGGCCA AGTGGT	chr2:73161112- 73161204	1.345E-04	19.806
EMX1-sg1-SaCas9-BLESS-1	GAAGCTTTGAGGG GAATGTG	GTTGTTGCGATTG GTGGATT	chr11:44986379- 44986518	7.840E-05	15.139
EMX1-sg1-SaCas9-BLESS-2	GATGCTGCCCTTC TGACCTC	CTGGAAGAAGCTG GGGAAGA	chr11:118533529- 118533597	1.170E-05	5.695
EMX1-sg1-SaCas9-BLESS-3	GGCCCTTGCTCTT CAGATTT	ATTAGCATGGGGA CGCTTTT	chr15:59537262- 59537279	2.820E-06	1.991
EMX1-sg1-SaCas9-BLESS-4	TCCTCATTAGGAA ATTTAGGATACAA	TCAAATCATTTC AGCTAATGC	chr13:66937528- 66937545	2.400E-06	0.015
EMX1-sg1-SaCas9-BLESS-5	AATGCATGATGCT AATGTCAGG	AGCTTGATGAATT TCCACATCC	chr10:92612638- 92612643	2.400E-06	0.012
EMX1-sg1-SaCas9-BLESS-6	AATTCAGTCTTGC CCAAAATTC	GCATGATATTTCC AACTTCGTTT	chr23:63868424- 63868483	2.340E-06	0.007
EMX1-sg1-SaCas9-BLESS-7	TTAGGGTGTGGG AGAGAGTTG	CCCCTGTCTGTCA CTCAGC	chr17:61699672- 61699691	2.280E-06	0.029
EMX1-sg1-SaCas9-BLESS-8	CCCAGCAGTCTCC AGTTATCTT	GCTGCTCTTCCAT TAGAAAGGT	chr7:10341511- 10341552	2.280E-06	0.078
EMX1-sg1-SaCas9-BLESS-9	TCAAAAGATAAAC CCCAAATGA	TCATATTTTGCCA ATTTTTCTATGA	chr15:98906148- 98906184	2.220E-06	0.056
EMX1-sg1-SaCas9-BLESS-10	ACGATTACTTTTG CACCAACCT	GGCTGTTTCTTCT TCTGTGGAC	chr8:100942881- 100942887	2.220E-06	0.036
EMX1-sg1-SaCas9-BLESS-11	TTGTTTCATGATTT TCACAACAGC	ACAGGACTGAAAC ACAGGATGA	chr1:73353979- 73353988	2.100E-06	0.014
EMX1-sg1-SaCas9-BLESS-12	TCATGAACAATTC CTTTATGCAA	TATAGTGGATCCC AATGATTTTATG	chr13:63377894- 63377894	2.100E-06	0.052
EMX1-sg1-SaCas9-BLESS-13	AGCCTGGGTGACA GAGCAAG	TTCTCCCTGAGAT CAAGACAAGAA	chr13:66444613- 66444619	2.100E-06	N.D.
EMX1-sg1-SaCas9-BLESS-14	CAAAACAAACTTT AAAGCAACAGC	TTTGGGAGCTCTA GTGTTAGGTG	chr5:67863420- 67863424	2.100E-06	0.389
EMX1-sg1-SaCas9-BLESS-15	CATCAGCCAGAGA TTGTAACCA	CACTGCCAGAGGA GAGTGTCTA	chr7:53360032- 53360051	2.100E-06	0.011
EMX1-sg1-SaCas9-BLESS-16	TTCTCCTCCCTC TTTTCTGC	GCATAAAAGCAAG CTTGCCAAC	chr12:69985597- 69985651	2.100E-06	0.003
EMX1-sg1-SaCas9-BLESS-17	TGAGAAAAGCAGC ATGAAATGT	ATCTGTAATGGC AGAGCTGGT	chr12:26995768- 26995780	2.040E-06	0.005
EMX1-sg1-SaCas9-BLESS-18	TCCATTGGTGTAC TGGAATGA	ATCCACCATAACC GATTTGAAG	chr14:30465710- 30465757	2.040E-06	0.012
EMX1-sg1-SaCas9-BLESS-19	ATGACCTGTCCAG TGCTGTCA	AGGGCTTGTTC CAGGAAAA	chr7:91451893- 91451918	2.040E-06	0.014
EMX1-sg1-SaCas9-BLESS-20	CAGCCTGACAAAG ATGCATATAA	AGGACTAAAATTG CTGGTACG	chr13:62964045- 62964093	1.980E-06	0.023
EMX1-sg1-SaCas9-BLESS-21	CCATCTTGTGGAG TAAAGTTTGG	AAAGTATGGCTCA TCCTGTGGA	chr23:42178420- 42178425	1.980E-06	0.045
EMX1-sg1-SaCas9-BLESS-22	TCAAACAGAAAAG TCATAAGAATAAA AA	ATACATTACTCTC ATACAGTTCAGGA AT	chr23:119901238- 119901264	1.980E-06	0.018
EMX1-sg1-SaCas9-BLESS-23	GCAGTGTGAGGCC TTTCG	GTGCACGCCATGC TTTCT	chr10:3148315- 3148317	1.980E-06	0.006
EMX1-sg1-	TCTTAGGACATAG	TTTGAAACTTTG	chr10:114021252-	1.980E-06	0.011

SaCas9-BLESS-24	CTCCAGTTGC	ATGTTACAG	114021278		
EMX1-sg1-SaCas9-BLESS-25	CACTGTCTTCGAC CTCCAATC	CCCTCTGATTGAT CGCTGTT	chr8:42010337- 42010447	1.980E-06	0.068
EMX1-sg1-SaCas9-BLESS-26	TTCCTCCTATCAT ACTCTTGCTCA	CCTTGGCATCTTC TAACACTTTG	chr13:83934142- 83934184	1.980E-06	0.012
EMX1-sg1-SaCas9-BLESS-27	CTAAGAAGGCCAT CATCAGGAC	AAGTATAGCCCAT GGTGAGCTG	chr23:115497088- 115497106	1.980E-06	0.012
EMX1-sg1-SaCas9-BLESS-28	GAGCCGGGAATAA AAACAATTT	CCTACAGTCAGGA GGAAACCTG	chr1:196377000- 196377015	1.920E-06	0.012
EMX1-sg1-SaCas9-BLESS-29	TCCTCCTAATTTT TACCCAAA	CTTTGGCCATGGA TGATTTTTT	chr12:63463688- 63463692	1.920E-06	0.030
EMX1-sg1-SpCas9 (on target)	AACCCACGAGGGC AGAGT	GAGGAGAAGGCCA AGTGGT	chr2:73161140- 73161200	1.231E-04	19.274
EMX1-sg1-SpCas9-BLESS-1	AATGTGGGAGGGG GAGAG	TGCGATTGGTGGA TTCTGT	chr11:44986405- 44986503	1.215E-04	19.001
EMX1-sg1-SpCas9-BLESS-2	AATCAGCAGTGAT TTGACTAGGG	AATTCCAGCAGGG AAGAACC	chr9:89337340- 89337467	9.060E-05	11.564
EMX1-sg1-SpCas9-BLESS-3	ATGGGGTCTGTTT GATCAGC	GACTGCCTTGCTC TCACCA	chr22:46315601- 46315691	4.670E-05	6.971
EMX1-sg1-SpCas9-BLESS-4	AGGCCATGGTCCT TAGGATG	CAGGACAGCCCTA ACCTGAA	chr2:135317592- 135317674	2.700E-05	4.074
EMX1-sg1-SpCas9-BLESS-5	TCCTCATTAGGAA ATTTAGGATACAA	TCAAATCATTTCC AGCTAATGC	chr1:222769727- 222769752	2.580E-05	N.D.
EMX1-sg1-SpCas9-BLESS-6	TCTTGTGAGGACT GTGAGTTCC	AAGATGTAGTGCT CTGGGAAGG	chr1:24580823- 24580840	2.560E-05	0.006
EMX1-sg1-SpCas9-BLESS-7	CAGATGCGCATTG ACTTTCT	CTGCTTTTCCAAG GATTTGA	chr3:139977190- 139977250	2.490E-05	1.304
EMX1-sg1-SpCas9-BLESS-8	AAAAAGTGAAAAA TGGGGATGA	GCCAAGAATATGT TTTTAGCATC	chr1:196230679- 196230698	2.380E-05	0.011
EMX1-sg1-SpCas9-BLESS-9	CACCTTGAATTTT GTTGGTCCT	CCTTCATCCAAAA AGTATCATGG	chr10:11953662- 11953664	2.380E-05	0.012
EMX1-sg1-SpCas9-BLESS-10	CTGTCCTGGAAAG CTCCTCA	GCTTCCTTTTTCC AGGTGCT	chr2:173222794- 173222833	2.280E-05	N.D.
EMX1-sg1-SpCas9-BLESS-11	AAGCCAGTCATTG CTCTCTGT	TTGAGGAGGATAA CTGTGCCTA	chr3:24838294- 24838296	2.150E-05	0.016
EMX1-sg1-SpCas9-BLESS-12	TGTTTTGACAGTAT GTA CTGAAGTTGA A	GCTTCCAGTTTTG GGACAAT	chr12:49867338- 49867341	2.130E-05	0.003
EMX1-sg1-SpCas9-BLESS-13	TGGTCATAGTTTG CATTTTCT	AATGGGCAGAGGA CATGAATAG	chr23:82219224- 82219226	1.920E-05	0.207
EMX1-sg1-SpCas9-BLESS-14	TTTACATGGTCTC ACCCAGATG	GCAAAAAGAAAAA TGCAGTGAA	chr23:147401787- 147401798	1.910E-05	0.015
EMX1-sg1-SpCas9-BLESS-15	ATGAAAAGCTGAA GGAAAAGCA	TTTGATGAAGTGT TCTGTCTGGA	chr1:233770599- 233770612	1.860E-05	0.017
EMX1-sg1-SpCas9-BLESS-16	TGCACATTTAAGG GTTTTTACCA	AGCATCAGGTATT TTCGTGGAT	chr2:52411969- 52411986	1.850E-05	0.118
EMX1-sg1-SpCas9-BLESS-17	AACCAGACAAA TTCGTGAGC	AATGCTTCCAGCT GCAGTTTAT	chr4:29481950- 29481970	1.850E-05	0.008
EMX1-sg1-SpCas9-BLESS-18	GGTTTAGGAGAAG CCCTCAGA	TTCTGAGGTGGCA TAAAATTGA	chr1:236441905- 236441922	1.830E-05	0.020
EMX1-sg1-SpCas9-BLESS-19	CACATTGTTATGA ACCCATTCAA	CTAACCCCATTCG GACAAATAC	chr12:93528803- 93528825	1.820E-05	0.009
EMX1-sg1-SpCas9-BLESS-20	CCCTCAATTAGCT GTGTTTCT	ACCAGGCGTACAC ATTTGAAG	chr4:188092874- 188092901	1.790E-05	0.008

EMX1-sg2-SaCas9 (on target)	AACCCACGAGGGC AGAGT	GAGGAGAAGGCCA AGTGGT	chr2:73161165- 73161252	2.364E-04	16.004
EMX1-sg2- SaCas9-BLESS-1	AAGACAGCCCTGA CGGAAAA	CCTTGCTTTCAGC TGCTCTG	chr9:97853295- 97853347	1.116E-05	2.052
EMX1-sg2- SaCas9-BLESS-2	AATCTGTGGGCAG CAGAAGA	TGGAAGCTGAGCT AGGACCA	chr5:153672156- 153672181	9.009E-06	0.592
EMX1-sg2- SaCas9-BLESS-3	GGAGAGACCCAGG TGTGAAT	ACTTCTCCCTTT GGCTACC	chr11:44986458- 44986473	3.967E-06	0.175
EMX1-sg2- SaCas9-BLESS-4	CCACCTATGCCCT CTACTGC	CCATATCGGGACA GGCATT	chr11:869038- 869055	3.885E-06	1.547
EMX1-sg2- SaCas9-BLESS-5	CCAAAAGTTGATA TCATGCTTAGTGA T	ATGATGATAATTT TGCTTCTTCATT C	chr9:114789234- 114789291	2.397E-06	N.D.
EMX1-sg2- SaCas9-BLESS-6	AGCCCCGTTGATA TCACTTC	CACAGAAAATGAC AAACCCAGA	chr9:128168612- 128168629	2.314E-06	0.106
EMX1-sg2- SaCas9-BLESS-7	ATGAAAAGGGTCG ATTCATCAG	GGGAGTATTAAC CCTCCAACC	chr23:78380434- 78380467	2.232E-06	0.033
EMX1-sg2- SaCas9-BLESS-8	GAGATGTTCTACT CCAAAGGGACT	AGCAAATTGTAC TCCTGCTTT	chr5:112797561- 112797576	2.232E-06	0.013
EMX1-sg2- SaCas9-BLESS-9	CCCCAGAAGCAAT TATTTTCAA	GGAATTAATCAT TTGTATAGTAGGA AGTC	chr1:225712809- 225712830	2.149E-06	0.134
EMX1-sg2- SaCas9-BLESS-10	TCCTACCTGTGGT CTCCAAA	GCTGTTCCCCAAA GACAAGA	chr1:182944884- 182944888	2.066E-06	0.016
EMX1-sg2- SaCas9-BLESS-11	TCATTGGCTGCCG GAGT	CCTTCTCCTCCC AGTCTCC	chr3:66551623- 66551687	1.984E-06	0.019
EMX1-sg2- SaCas9-BLESS-12	TGCATTTTCTTTC ACTTTTGGGA	AAAACCTGAGCAT TTCTTCCAC	chr3:68174160- 68174181	1.984E-06	0.015
EMX1-sg2- SaCas9-BLESS-13	GCTGGCTGAATCC AGTTTGT	GTGCAATTGGGAA CGTCAT	chr3:138080529- 138080557	1.984E-06	0.268
EMX1-sg2- SaCas9-BLESS-14	CATGGCATTGGA CTCCTCA	TGACCCCTCCCCT CATTATT	chr4:101440760- 101440765	1.984E-06	N.D.
EMX1-sg2- SaCas9-BLESS-15	GGGACATTAAGTG GTAATGGACA	TTCATCACTCAGA CTTCCTTGG	chr6:65726838- 65726850	1.984E-06	0.055
EMX1-sg2- SaCas9-BLESS-16	TTCAGGTATCCCA CCTCTGAGT	GCCAACAAGATTT GGTCTTTGT	chr8:136640477- 136640503	1.984E-06	0.022
EMX1-sg2- SaCas9-BLESS-17	CAGGCTTCTGAGG ATGGCTA	CACCAGCTCCCAT CCTAGTT	chr1:165717581- 165717582	1.901E-06	0.014
EMX1-sg2- SaCas9-BLESS-18	GGCCAAGGAAGTA GACTCCA	TGCGCTAGTGTC TTCATTTT	chr13:23854595- 23854595	1.901E-06	0.031
EMX1-sg2- SaCas9-BLESS-19	ACTCCAGCCTGGG TGACAGA	GGATGTTTTCTCC CTGAGATCAA	chr13:98618410- 98618422	1.901E-06	N.D.
EMX1-sg2- SaCas9-BLESS-20	ATGGGTGAATTTT AATCGCAGT	GCTGGTAGTGCTA ATCCAATC	chr2:214716358- 214716369	1.901E-06	0.013
EMX1-sg2- SaCas9-BLESS-21	TCTTTCACTAAAG GTAAGGAAATGAA	ACTTCCATCTTGC CTGCTGT	chr2:218400210- 218400211	1.901E-06	0.019
EMX1-sg2- SaCas9-BLESS-22	CTGCTGCCACGTC TTCACT	TGAAATTTCCAAG CAGCTAAA	chr4:171182176- 171182188	1.901E-06	0.022
EMX1-sg2- SaCas9-BLESS-23	CAGAGTACATGTC TAACGCCTCTT	TTTTGCCTAGCAA ACTTCACAA	chr5:36911739- 36911742	1.901E-06	0.014
EMX1-sg2- SaCas9-BLESS-24	GTCAGCTCCTGTG TCCCTTT	GGACTTTGCAGGA AACATACG	chr6:23894524- 23894553	1.901E-06	0.033
EMX1-sg2-SpCas9 (on target)	AACCCACGAGGGC AGAGT	GAGGAGAAGGCCA AGTGGT	chr2:73161146- 73161225	2.997E-04	11.981

EMX1-sg2- SpCas9-BLESS-1	GCAACGCTAGGAG ACTGGAA	AACCATCACCCCA AACACAG	chr5:34026343- 34026377	8.099E-05	4.919
EMX1-sg2- SpCas9-BLESS-2	ACTGTGGGGAGCA CAGACAG	CTTGCTCCGCTC AGTGTT	chr6:168705429- 168705482	7.038E-05	4.877
EMX1-sg2- SpCas9-BLESS-3	TGCCAGTTGTGGT AGAGACC	GTCTTCAGGGGCT TGGTGT	chr10:44277653- 44277701	5.639E-05	7.813
EMX1-sg2- SpCas9-BLESS-4	AGACAAACGGAGC TCAGAGG	GAGGGCATGTGTG AGCTGT	chr17:76992455- 76992533	3.531E-05	6.055
EMX1-sg2- SpCas9-BLESS-5	GTCTTTTGTGGGG CGAGTC	ACTGAAGGAGCTT CACGAAC	chr5:174178968- 174179012	2.619E-05	1.608
EMX1-sg2- SpCas9-BLESS-6	CAAGGTCCCTTTG GTCAGTG	GTTTGGTTTTTGG GTCCTCA	chr1:22446341- 22446395	1.895E-05	1.518
EMX1-sg2- SpCas9-BLESS-7	CGGAATCCGCATA TCACCTA	CGTCCTTGCAATC TCCACTC	chr16:56715741- 56715800	1.661E-05	0.760
EMX1-sg2- SpCas9-BLESS-8	TGGAGAGAGGCAA TGAGAGG	CTGCCTCTGGGAA TGATTTG	chr2:121153585- 121153651	1.230E-05	1.516
EMX1-sg2- SpCas9-BLESS-9	CCACAGATGCCTT GAACTTG	TCGTGTGCTTCTT ACACATCA	chr16:73470885- 73470941	3.819E-06	0.125
EMX1-sg2- SpCas9-BLESS-10	GTCAACCTCCCTT CCTCCAT	ACCTCCCCAACCT CAGTCTT	chr11:67410073- 67410091	3.075E-06	0.182
EMX1-sg2- SpCas9-BLESS-11	TTGATAACAGAGC TTCTATGGACA	CATGTGTTACGGT CTAGCATTTT	chr8:115470881- 115470913	2.976E-06	0.002
EMX1-sg2- SpCas9-BLESS-12	CGTCCCTGATTGG ATAATGTTT	GCAGCTTGATTT CACTCTTTC	chr7:64023552- 64023569	2.926E-06	0.028
EMX1-sg2- SpCas9-BLESS-13	CTTTTTCTGTTGC CACAACG	TCTGTTATGTGCG TGGTTCC	chr3:23919932- 23919933	2.877E-06	0.068
EMX1-sg2- SpCas9-BLESS-14	GCCCCTGTCCTAA TAAATGC	TGTGTGTGTGTAT AGGTATGGAAGG	chr2:221448894- 221448898	2.728E-06	0.034
EMX1-sg2- SpCas9-BLESS-15	CCCACAGTCCTTA TGATTACTGAA	AAAGAGGATAGGG AAAGGGAAA	chr2:188791974- 188791979	2.678E-06	0.050
EMX1-sg2- SpCas9-BLESS-16	CCCCAACCTGCAA TCTTACATTA	TCTGTAGACCAA GCCAAATTA	chr15:89843910- 89843932	2.579E-06	0.011
EMX1-sg2- SpCas9-BLESS-17	TGTGATGACTGAT GTCTTGCTG	AAGGAAGGACTTT TGGTAAGTGC	chr6:164141026- 164141065	2.579E-06	0.046
EMX1-sg2- SpCas9-BLESS-18	GCTTCTCTTTTCT TCCCTACCT	ATCACAGCCAAGT GAAAATGTG	chr9:115570151- 115570181	2.529E-06	0.003
EMX1-sg2- SpCas9-BLESS-19	CTCCCCGGTTTCCAG GAAATC	CCCTGGAGTTCCA GACCAAC	chr11:17251613- 17251629	2.430E-06	0.057
EMX1-sg2- SpCas9-BLESS-20	ATACTGTGCGGGA TGAGACTTT	TTCTTCTTGAACA CCCATAGCA	chr17:57233732- 57233777	2.430E-06	2.181
EMX1-sg2- SpCas9-BLESS-21	AATCAAAATTCTC TGGCAGTGG	CATCAAGTGCACA AAAAGCTTAC	chr2:99940670- 99940683	2.430E-06	0.003
EMX1-sg2- SpCas9-BLESS-22	TAAGCAAAAAGCA CCCACAGTA	AACAGCATTGTGA TACGGGTTT	chr6:136475185- 136475200	2.430E-06	0.003
EMX1-sg2- SpCas9-BLESS-23	AAATAGGCATTTT CAGGGAAGG	CCAAAACTATCC TATGAGCTACCA	chr13:58589424- 58589450	2.331E-06	0.003
EMX1-sg2- SpCas9-BLESS-24	CAACCTGAGGTGT GTTTATTTTT	TCAAGGCTATTGG AGTGGAGA	chr13:71915182- 71915184	2.331E-06	0.007
EMX1-sg2- SpCas9-BLESS-25	CGCATTTGGGTTT GACTGAT	AGCAAAGGGCACA CTGACAT	chr7:73517789- 73517793	2.331E-06	0.004
EMX1-sg2- SpCas9-BLESS-26	CAGGGAATTCGAG CTTAGACA	AAGGCTGCCCTC TCTATTC	chr18:75311780- 75311797	2.281E-06	0.281
EMX1-sg2- SpCas9-BLESS-27	TCCTAGCATGCCC ATTATCC	TGTGAAGGGATTC TCTGAGG	chr3:9429210- 9429213	2.281E-06	0.015

EMX1-sg2- SpCas9-BLESS-28	CAAAGGGAAACAA TACAAACAAA	CAGCATTTCCAAA TGTTATCCA	chr6:122022169- 122022208	2.281E-06	0.024
EMX1-sg2- SpCas9-BLESS-29	CCCAAATCACTTC TTTCTCTCG	TTAGCTGCTTTCA CCATCACC	chr1:226521173- 226521200	2.232E-06	0.002
EMX1-sg2- SpCas9-BLESS-30	CGGCGACAAGAGC AAGACT	AGATGGGGTTTCG CCATGTT	chr15:90488808- 90488838	2.232E-06	N.D.
EMX1-sg2- SpCas9-BLESS-31	GAAGGATCCTGCA AGAGAACAT	TACAACAAATGTG TGTGCGTGT	chr7:69405987- 69406009	2.232E-06	0.003
EMX1-sg2- SpCas9-BLESS-32	TTCCATCAAGTGG TGAGGTCTA	CAACAGGGTACAA ACCAAAACC	chr3:479351- 479365	2.182E-06	0.003
EMX1-sg2- SpCas9-BLESS-33	TTTGAAGTTTCA GATGAAGGAA	GTTGAATCAATTT GGGGATCTG	chr4:165374754- 165374774	2.182E-06	0.003
EMX1-sg2- SpCas9-BLESS-34	CTGCAACATCAAC CCAGAAATA	ACTTAGCCATGTG GCTGTGTTT	chr5:122768218- 122768269	2.182E-06	0.003
EMX1-sg2- SpCas9-BLESS-35	TGGAATTCCTCCA TCACATGAC	ATATTTCACTGGG GTGGAGTTG	chr9:30815886- 30815893	2.182E-06	0.004
EMX1-sg2- SpCas9-BLESS-36	TGATGTTCAGGTG ATAGAGTCTTGA	TTCCAAGAACACA CAGGAAATG	chr13:74839817- 74839846	2.133E-06	0.003
EMX1-sg2- SpCas9-BLESS-37	AATGTTTACAGTT TCCTGCCTGA	TGCCACAGACTGA CAAATTACC	chr7:135932093- 135932107	2.133E-06	0.043
EMX1-sg2- SpCas9-BLESS-38	CCTCCCAAGGTTT GAGTATTGT	TTTTGTGGCATGC AGTAACTT	chr1:82257080- 82257095	2.083E-06	0.032
EMX1-sg2- SpCas9-BLESS-39	TCTCTGAACCTCA GTCTCCTCA	CTCCCCATTCCAC AGTAATGAT	chr1:180540263- 180540277	2.083E-06	0.025
EMX1-sg2- SpCas9-BLESS-40	CTCCCTTCAAGAC TGAACCACT	GAGCACAGCTTGA ACCAGATAA	chr11:100072559- 100072561	2.083E-06	0.006

N.D.: Not determined.

Supplementary Table 6 | Top genomic peaks identified by ChIP in 293FT cells

Site	Target	Fwd Priming Site	Rev Priming Site	Locus	MACS Score	Motif Score	Indel (%)
Sa_EMX1_target1	GCCTCCCCAA AGCCTGGCCA	AACCCACGAGGG CAGAGT	GAGGAGAAGG CCAAGTGGT	chr2:73161121 -73161222	1666	26	19.806
Sa_ChIP_OT1.1	ACCTCCCCAT AGCCTGGCCA	GAAGCTTTGAGG GGAATGTG	GTTGTTGCGA TTGGTGGATT	chr11:4498641 2-44986513	982	18	15.139
Sa_ChIP_OT1.2	TCCTCCCCAC AGCCTGGCCA	GGGAAATAAAG CCTGTGGT	GAAGAACCCC TGAGGCATCT	chr9:89337324 -89337425	83	17	0.045
Sa_ChIP_OT1.3	TTCTCTCCAA AGCCTGGCCA	TGGCTACAACCT CGGCAAT	CAGGCCTTTC AGGTTCAAGG	chr20:5614052 1-56140622	259	16	0.008
Sa_ChIP_OT1.4	TCCTCACCAC AGCCTGGCCA	CACAGCAGAGCT TCATTTGC	CACTCCTCTG GGAAGGTGAG	chr10:1259660 91-125966192	80	16	0.002
Sa_ChIP_OT1.5	GGCTCCCCAA AGCTTGGCCA	TGCCAGAGCCTG AGGATAAT	AGGGTTGCCC TGTGGAAT	chr3:13206742 6-132067527	68	15	0.002
Sa_ChIP_OT1.6	GCCTCTGCAC AGCCTGGCCA	AGGAGAGGCACC TGCAGTC	CTCCCCTACT TTTGTCTGTC	chr9:13909758 6-139097687	64	14	0.025
Sa_ChIP_OT1.7	GTCCTCTGAA AGCCTGGCCA	GAAGGGACTGGA TGTTACATAA	CCTCAATCCT GCTCCCTCTA	chr9:13133079 2-131330893	116	14	0.027
Sa_ChIP_OT1.8	CACTCCCCAC ACCCTGGCCA	GGTTGTAGGAGT GGGACAGG	AATCAGGAGC TGGAATGTG	chr9:12942054 4-129420645	114	14	0.007
Sa_ChIP_OT1.9	AACACCCCCA AGCCTGGCCA	TATGCTGGGATT CTGGGAAG	GGAGGTAAGA GGGGGACAGT	chr22:2001705 4-20017155	288	14	0.017
Sa_ChIP_OT1.10	TGACCCCCGA AGCCTGGCCA	ATATCACACCGG CCTCCATA	CTGCAGTTAG GTGCCATTCA	chr16:1308253 -1308354	264	14	0.015
Sa_ChIP_OT1.11	GCCTGCACAA TGCCTGGCCA	TAAAGTGCATGC AAACCACCT	ATGAGTCCTG CCATCAAACC	chr15:8875181 2-88751913	1232	14	0.002
Sa_ChIP_OT1.12	GACCCCTCAA AGCCTGGCCA	CTGGTGGTTCTG GTTGTCCT	TTGCTGCTCT GAAAAAGCTG	chr13:2482223 4-24822335	233	14	0.002
Sa_ChIP_OT1.13	TCCTCATCAC AGCCTGGCCA	CGCATCCTGACC TCAATACA	TTGGGAGTCA GTGGGAGTGT	chr10:1038764 43-103876544	479	14	0.022
Sa_ChIP_OT1.14	AAATGCCAAA AGCCTGGCCA	GAGTCGGCCAAG GAATGTT	CCACGTCTCC AGCATACAAA	chrX:7334803 2-73348133	79	13	0.053
Sa_ChIP_OT1.15	ACCTGACCCA AGCCTGGCCA	TGGGTGATGGAG GAGTAAGG	CTCCAGCTCT CTTCCCCTTC	chrX:7043653 2-70436633	729	13	0.006
Sa_ChIP_OT1.16	ACCTCACCCA TGCCTGGCCA	CACTTGCTGAGG ACCCAGAT	TCTCACACGC TGCTCACAG	chrX:1325673 0-13256831	265	13	0.011
Sa_ChIP_OT1.17	AGAGCCTGGA AGCCTGGCCA	TCTGGGTCCCTT TGTGACTC	GGAGGTGGGG ATAAGGTCAG	chr6:31839680 -31839781	1877	11	0.002
Sa_ChIP_OT1.18	CCTGCCTCTG AGCCTGGCCA	GGTGTGCGCCAG AATAGTTT	ATTTGTGGGG TTGTTGAAA	chr7:14868571 8-148685819	813	10	0.027
Sa_ChIP_OT1.19	ATTGGCCCCA AGCCTGGCCA	GGAAGGAAGGAA AGAAAGAGAGA	CGTGAGAGCA GAGCAGTGTC	chr22:3879892 5-38799026	1636	13	0.003
Sa_ChIP_OT1.20	TGCAACACAC AGCCTGGCCA	GCCTGCTCTAGA TCCAGCAGT	TGGGATCTTC CAGAGGACAG	chr8:12665533 4-126655435	1016	11	0.026
Sa_EMX1_target2	GGCAGGCTT TGGGGAGGCC	AACCCACGAGGG CAGAGT	GAGGAGAAGG CCAAGTGGT	chr2:73161138 -73161239	1815	25	16.004
Sa_ChIP_OT2.1	TGCCAGGCTC TGGGGAGGCC	GCTGGCTGAATC CAGTTTGT	TGCAGCCTTC CATAGGAATC	chr3:13808051 4-138080615	92	17	0.268
Sa_ChIP_OT2.2	GTCCAGGCTC TGGGGAGGCC	CCACCTATGCCC TCTACTGC	CCATATCGGG ACAGGCATT	chr11:868990- 869091	73	17	0.856
Sa_ChIP_OT2.3	TCCCAGGCTT GGGGGAGGCC	CCCCACCTGGAA TCAAGTC	CAATAGGGAG CCAAAGCAAG	chr5:17767974 5-177679846	306	16	0.031

Sa_ChIP OT2.4	CGCCAGGCTT TGTGGAGTCC	GGCAGAAGTGCC AGGAGTTA	GACCATGAGC TTGGATGTTG	chr9:92448781 -92448882	289	15	0.002
Sa_ChIP OT2.5	TGCCAGGCC TGGGGAGGCC	AGCCTCAGGACA AGACATGC	GGCCAGCCAC ACTGAGTAG	chr9:13177409 3-131774194	125	15	0.007
Sa_ChIP OT2.6	AGGTGGGCTT TGGGGAGGCC	CACACATAGGCC CCGTCTC	GACACAAGAG GGGACAGGAG	chr2:24260725 0-242607351	507	15	0.006
Sa_ChIP OT2.7	TGCCAGGGTT GGGGGAGGCC	TGACGCAGCTTC TTTGTCTAG	AAAGCATGAG CTTGGTCACA	chr20:3487394 7-34874048	511	15	0.043
Sa_ChIP OT2.8	TGCCAGGCTC TGGGGAGGCC	ATACACTTGGGC AGCAATGA	CCTGCTCAGG AAATCCACAT	chr10:3451897 5-34519076	113	15	0.005
Sa_ChIP OT2.9	TGCCAGGGCT TGGGGAGGCC	ATCACCGTTGAA CTCAGAGC	GGAAGCTGAG GAGGAGACG	chr10:1121260 34-112126135	199	15	0.207
Sa_ChIP OT2.10	CCCCAGGCTC TAGGGAGGCC	TCTCTCTCTGCA GCTGTGGT	ATACCCACAG GGCTCACTCA	chrX:4704086 3-47040964	94	14	0.005
Sa_ChIP OT2.11	GGCCTGGCTC CTGGGAGGCC	CTGGGCAATCAT ACCCACTT	CCTGGAGACT GGGTCCCTA	chr8:18770971 -18771072	248	14	0.003
Sa_ChIP OT2.12	GGCCAGTCTC AGGGGAGGCC	TCAGGGAAGGTG TTTTGGAG	AGGTAGGACC TGCTGCTTCA	chr8:14469369 7-144693798	190	14	0.016
Sa_ChIP OT2.13	GGGCAGACCT TGGGGAGTCC	CCTCACACTCCA ACCCTCTC	GCATATGTGT CTCCCACACG	chr6:39135451 -39135552	162	14	0.002
Sa_ChIP OT2.14	TTAGAGGCTA TGGGGAGGCC	ATGAATGGGCTC AGACACCT	TCCACAGGCT TCAAAAGGAC	chr6:16412563 4-164125735	253	14	0.006
Sa_ChIP OT2.15	TACCAGGTGT TGGGGAGGCC	AAGTCCTCCAGC TGTGCATT	TCATTTGCAA CAACATAGAT GG	chr5:7760955- 7761056	265	14	0.024
Sa_ChIP OT2.16	CTGATACTTC TGGGGAGGCC	GCCACAAAAAG CCTTACAC	GGCACAGG GAAAGCTG TACT	chr18:3604248 9-36042590	1246	10	0.049
Sa_ChIP OT2.17	GTGGGGAGGA AGAGGAGGCC	AACGGATACAGG GAGGAAGG	GCTCTGGA GGGAGAAG TGG	chr22:2410521 3-24105314	844	7	0.002
Sp_EMX 1_target1	GCCTCCCCAA AGCCTGGCCA	GGAAGGAAGGAA AGAAAGAGAGA	GAGGAGAAGG CCAAGTGGT	chr2:73161120 -73161221	385	26	19.274
Sp_ChIP OT1.1	ACCTCCCCAT AGCCTGGCCA	GAAGCTTTGAGG GGAATGTG	GTTGTTGCGA TTGGTGGATT	chr11:4498641 3-44986514	855	18	19.001
Sp_ChIP OT1.2	TCCTCCCCAG AGCCTGGCCA	ATGGGGTCTGTT TGATCAGC	GACTGCCTTG CTCTCACCA	chr22:4631559 2-46315693	370	17	6.971
Sp_ChIP OT1.3	AGTGATCCAA AGCCTGGCCA	AACGGATTTGAG GGGAGAAT	GTCTCCCACG TCTTTCTGGA	chr6:85481642 -85481743	104	14	0.002
Sp_ChIP OT1.4	GTACAGCAAA AGCCTGGCCA	ATCTGGCTCAGC AAAGAAGG	CGGGAGGGGA ATTAGAACAT	chr22:2524141 9-25241520	110	12	0.018
Sp_ChIP OT1.5	AAAACACAAA AGCCTGGCCA	AAAGTTGATCTG GCCGTGAG	CAGAAAGCAG CATGAAACCA	chr11:2549865 -2549966	55	12	0.042
Sp_ChIP OT1.6	TTGTGGTCAC AGCCTGGCCA	AATTGCTGAGCA GGCATAAT	GGCAGTTTGT CATTCCAGGT	chr9:94668357 -94668458	66	11	0.002
Sp_ChIP OT1.7	AGCCTCCCCA AACCTGGCCA	ACCTTCTGAAAC AGCCTCCA	TTTTCTGAG GGTTCCTGG	chr7:10199955 9-101999660	135	11	0.024
Sp_ChIP OT1.8	AGAGCCTGGA AGCCTGGCCA	TCTGGGTCCCTT TGTGACTC	GGAGGTGGGG ATAAGTTCAG	chr6:31839698 -31839799	79	11	0.023
Sp_ChIP OT1.9	CATCTACGGA AGCCTGGCCA	ATGCACCCTCCA TACTCCAC	CTCCCACAAT TTACCCATGA A	chr4:55480130 -55480231	78	11	0.002
Sp_ChIP OT1.10	CTTGCCTGGA AGCCTGGCCA	TTCTCCATCACT GGCTCTCC	GCCAGTCTCC CTTCTGTTTG	chr17:7693029 6-76930397	158	11	0.003
Sp_ChIP	TGAGTGCACA	CTCCTCTCAGCC	TGACCACAGA	chr17:5601356	91	11	0.026

_OT1.11	AGCCTGGCCA	TCAACCTG	GCAATTGGAG	8-56013669			
Sp_ChIP OT1.12	AATTGTCTGA AGCCTGGCCA	CGGGAGAAATTC ACAGATGA	CACAGCCACC CTTTCTCTTC	chr1:22031827 -22031928	75	11	0.002
Sp_ChIP OT1.13	TTCCCAGTGG AGCCTGGCCA	GAAGCTCAAGCC TGAAGCAG	TGACTGCCCC TTCTGTTCTC	chr8:49730969 -49731070	95	10	0.005
Sp_EMX 1_target2	GGCCAGGCTT TGGGGAGGCC	AACCCACGAGGG CAGAGT	GAGGAGAAGG CCAAGTGGT	chr2:73161130 -73161231	224	25	11.981
Sp_ChIP OT2.1	GGCCAGGCTC TGAGGAGGCC	GCAACGCTAGGA GACTGGAA	AACCATCACC CCAAACACAG	chr5:34026317 -34026418	56	16	4.919
Sp_ChIP OT2.2	AGACAGGCTC TGGGGAGGCC	TTTCTTCCCTCC ACAGATGC	CGGTTTTTGC CTTTGAAAAT	chr16:7347084 9-73470950	66	16	0.061
Sp_ChIP OT2.3	AGGTGGGCTT TGGGGAGGCC	CACACATAGGCC CCGTCTC	GACACAAGAG GGGACAGGAG	chr2:24260726 1-242607362	113	15	0.003
Sp_ChIP OT2.4	GACCAGACTT CCGTGAGGCC	GGCAGGGAGATA AGGGTCTC	CTTTTTCCCT CCAAGGCAGT	chr9:13587983 6-135879937	162	14	0.020
Sp_ChIP OT2.5	TACCAGGTGT TGGGGAGGCC	AAGTCCTCCAGC TGTGCATT	TCATTTGCAA CAACATAGAT GG	chr5:7760993- 7761094	66	14	0.002
Sp_ChIP OT2.6	TGGGGAGCTT TGGGGAGGCC	GGTGACACAGCA CCACAGTT	CAAGCCACTT GCCTAAGCTC	chr5:17230058 3-172300684	72	14	0.010
Sp_ChIP OT2.7	GTTGAGGTTT TGGGGAGGCC	CTAGGACTCCGC CTGAGATG	TGAGAGGGGA GTGTGTAGGG	chr22:3806919 4-38069295	170	14	0.014
Sp_ChIP OT2.8	GGACAGACTG TGGGGAGGCC	TGATCAAACAGC CAGACACC	ACATGCCATA CAAGCACCAG	chr20:3768122 4-37681325	128	14	0.031
Sp_ChIP OT2.9	CCTGAGGCTG TGGGGAGGCC	GCACCTTAGTGT GGTGTGGA	ACACCCGGCC TAAAGTTTGT	chr16:8881841 0-88818511	156	14	0.002
Sp_ChIP OT2.10	TGTAGTGCTT TGGGGAGGCC	CAGTGGGCTTGA CCAGAAAC	ACGAGTACGA GGAAGCAAGC	chr10:1205769 88-120577089	51	14	0.036
Sp_ChIP OT2.11	AGCTAGGCGT AGGGGAGGCC	CCCCAGTAGGCT TTCACTTTT	TGGAACAAAA GCCTCACTC	chr8:81806113 -81806214	217	13	0.006
Sp_ChIP OT2.12	TGCCAGGAAG TGGGGAGGCC	ATGGGTCATCCT GTGGCTAA	GTTTGCCCCG GTCATAGACT	chr7:4077865- 4077966	216	13	0.041
Sp_ChIP OT2.13	GGCAGTGGTT TGGGGAGGCC	TGAAGGTGTTTG AAAGAAACCA	CGGCTGAATG AAACCAGAAT	chr5:105618- 105719	101	13	0.024
Sp_ChIP OT2.14	GGAGAGGCGG TGGGGAGGCC	GGCACTTTGGAA ACAAGGAA	TGAGTCACTG TGCTTGTTCCA	chr3:72204651 -72204752	138	13	0.037
Sp_ChIP OT2.15	GAGCCAGGGC TGGGGAGGCC	CCACGTGCATGT TTTCTCAT	AAAGAGTTTG CCGGTTTGG	chr3:12587076 -12587177	473	10	0.023
Sp_ChIP OT2.16	AAATGAGGAG TGGGGAGGCC	TAGCCTGGGAGA TGGAACAG	CCGTGTCCAT GTTGTCTTGT	chr15:9060591 4-90606015	395	10	0.002
Sp_ChIP OT2.17	GTGTGCCTCT TGGGGAGGCC	CGAGTGCTCTGC TGGAGAAT	AACGTGTTCC CTGTCAGCTT	chr19:6092559 -6092660	310	11	0.073

Supplementary Table 7 | Top off-targets predicted by motif-mismatch

Site	Target	PAM	Forward priming site	Reverse priming site	Sp indel (%)	Sa indel (%)
EMX1 target1	GCCTCCCCAA AGCCTGGCCA	GGGAGT	AACCCACGAGGGCA GAGT	GAGGAGAAGGCCA AGTGGT	19.27	19.81
OT1.1	ACCTCCCCAT AGCCTGGCCA	GGGAGT	AATGTGGGAGGGGG AGAG	TGCGATTGGTGGA TTCTGT	17.14	13.88
OT1.2	TCCTCCCCAC AGCCTGGCCA	GGGAGG	AATCAGCAGTGATT TGACTAGGG	AATTCCAGCAGGG AAGAACC	11.56	0.02
OT1.3	TCCTTCCCAA AGCCTGGCCA	GGGGAA	GTCGTGGAAGGGAT CCTGTA	CACACACAGGGCA GACTGG	0.06	0.01
OT1.4	TTCTCCCCAA AGCCTGGCCA	CAGAAA	ACAACCTCGGGCAC TACCTG	GATCCAAGGCTCC TTCCTTT	0.10	0.02
OT1.5	GCGTGCCAG AGCCTGGCCA	CAGGAA	TGAGCACGTTCTTC CTTCCT	TGGAGCATTTTTTC CCTACAG	0.01	0.04
OT1.6	GACCCCTCAA AGCCTGGCCA	GAGAAC	CCTGACTGACAGCC CTTTTT	GCAGGAGTTCTTT GCTGCTC	0.04	0.07
OT1.7	TTCTCTCAA AGCCTGGCCA	GAGAGG	TGGTACAACCTCG GCAAAT	ATCCTCCTTGGGG ATGTAGC	0.08	N.D.
OT1.8	TCCTACCAC AGCCTGGCCA	GTGAGA	CATCTCAAAGTCCC ACAGCA	GGCTGAAGGGAAT GAATGAC	N.D.	0.04
OT1.9	GCCTGCCGAA GGCCTGGCCA	GGGGAG	AACAGTTAGGGGCA CAGAGG	AGGCCCTGATACC ATTAGCC	0.00	0.01
OT1.10	CCCTCCCCAC CGCCTGGCCA	CCGAAG	TGACTTCCCTATCT GGCTGAA	GACAACCTTGGGT CTTTCCA	N.D.	0.00
OT1.11	ACCAACCCAG AGCCTGGCCA	GGGAGC	AACACACGGGAAA CCAAC	TGCCAGGCACAGA GGTAAAT	0.02	0.00
OT1.12	GGCCGCCCAG AGCCTGGCCA	GAGAGG	TCTGTGGGTGAAGA AAAGCA	CTCCCTCTGAGCC AGTGAGT	0.03	0.00
OT1.13	GCTGCCCAG AGCCTGGCCA	TGGGAG	GCCTGAGCTCAAAG GAGAGA	CAAAAGCGCCTCA TTAAAGG	0.00	0.02
OT1.14	ACCTCCCCAC AGCCTGCCCA	CCGGGA	TGTAGGGGGTCTCA GTTTGG	AAAAGTCCCTCCC CGAATAA	N.D.	0.06
OT1.15	GCCTCCCTAG AGCCTGACCA	GTGGAT	GGGACACATCTGGA TCTCTTG	AGGTTTCTCCCAC ACTCACAG	N.D.	0.01
OT1.16	GCTGTCGCAA AGCCTGGCCA	AAGGAA	TATGCAGATGCGGT AAGCAG	ACCCGAGCTTCTT TTCTTCC	N.D.	0.15
OT1.17	AACACCCCA AGCCTGGCCA	GAGGAT	TATGCTGGGATTCT GGGAAG	AGCTCACCATGTG ACTGCAA	N.D.	0.04
OT1.18	GAGGCCCGA AGCCTGGCCA	CTGGGA	GCAGTGTGAGGCCT TTCG	GTGCACGCCATGC TTTCT	N.D.	0.02

OT1.1 9	GCCGCCACG AGCCTGGCCA	GAGGAG	GCCCAGGAAGGGTC CTCT	CGCTCAGTGAACA TTTACAGC	N.D.	0.04
OT1.2 0	TGCTCCCCAG TGCCTGGCCA	TAGGAG	TTCTTTTGGAGGGAG GACAGG	GTGACACCCAGCC TAGCTCT	N.D.	0.03
OT1.2 1	CACTCCCCAC ACCCTGGCCA	AAGGGA	GTGGGGGCTGGGTT GTAG	GAATTTGAGGCTG GCTTGCT	N.D.	0.02
EMX1 target2	GGCCAGGCTT TGGGGAGGCC	TGGAGT	AACCCACGAGGGCA GAGT	GAGGAGAAGGCCA AGTGGT	11.98	16.00
OT2.1	TGCCAGGCTC TGGGGAGGCC	AAGGGC	GCTGGCTGAATCCA GTTTGT	GTGCAATTGGGAA CGTCAT	0.006	0.21
OT2.2	GTCCAGGCTC TGGGGAGGCC	CTGGGG	GTGGGACCCAGTGG TGAG	CCATATCGGGACA GGCATT	N.D.	1.09
OT2.3	GGCTGGGGTT TGGGGAGGCC	CAGAGC	CCGAGGTGGCTGGA GATG	AGGGCTCACCTGG CTTCT	0.012	0.04
OT2.4	AGACAGGCTC TGGGGAGGCC	TGGAGC	CAAGACCCTCCAGT GACACA	TGTAAGAAGTCGT GTGCTTCT	0.004	0.02
OT2.5	GGCCTGGCTT TGGGCAGGCC	CAGAAA	GGGCTGTGTGTGAC AGATTG	CCTAAACGAAGCA AGCTACCC	0.005	0.01
OT2.6	CGCCAGACTG TGGGGAGGCC	TTGGGA	GTGACAACCTGGGG GTCCTA	ACCCCTCCAGAAC ACAAGAG	N.D.	0.05
OT2.7	TGCCAGGGCT TGGGGAGGCC	TTGGAT	TGAACTCAGAGCCT GAAGGAA	AGGAGACGGAGGT GTGAGAA	N.D.	0.11
OT2.8	TGCCAGGGTT GGGGGAGGCC	CTGGAT	GTTTCCCCTCTTC CCTGAC	GGCGAGATCTAAA TCAGGGTCT	N.D.	0.26
OT2.9	TGCCAGGCC TGGGGAGGCC	CTGAGG	GCCTTAAGCACAGC CTCAG	GGCCAGCCACACT GAGTAG	N.D.	0.05
OT2.1 0	GGTCTGGCTT AGGGGAGGCC	CTGGGA	GTGGTTCTGGGTCT CAGAGG	AAGCTGCAGGTGG ACTGG	N.D.	0.09
OT2.1 1	CGCCAGGCTC AGGGGAGGCC	CGGGAG	GAGAAACGTGGCTC TGTTTC	GCAGCTGCTCCTG CTAACTC	0.005	0.01
OT2.1 2	GTTGAGGTTT TGGGGAGGCC	CGGAGG	CTAGGACTCCGCCT GAGATG	GGACCCAGAGAGG GGATG	0.006	0.01
OT2.1 3	AGGTGGGCTT TGGGGAGGCC	GGGAGT	CACACATAGGCCCC GTCT	GGGACATGGTACC AAAGAGC	0.098	0.02
OT2.1 4	GTGAGGGCTT TGGGGAGGCC	CTGGGA	GGAAGTTGGGATGA GGCTCT	ACCCTTATTCCCC TCCCATC	N.D.	0.07
OT2.1 5	GACTAGGGTC TGGGGAGGCC	AGGAAG	GCCATAAGCCTCAA ATGCTG	AATCCAGGCATCA GGAGGTA	N.D.	0.14
OT2.1 6	GGTCAGGCTC CGGGGAGGCC	GAGAGC	TTCTGGACACCGCT CACC	ACCTCAGGGTGCC CACTAC	0.006	0.11
OT2.1 7	GCACAGGCTT TGGGGAAGCC	CTGGAG	CTCTGGGGTAAGGT GCACAG	CCTGCAGACCACT CTGACAA	N.D.	0.01
OT2.1 8	GGTCAGGATT TGGGGAGGGC	GAGGGG	TTGATTTAGTTGCA CTCCATC	TTGCTCACTGACC ATCTTGC	N.D.	0.07
OT2.1 9	AGCCTGGGGT TGGGGAGGCC	AGGAAA	CTGGATCCTGGGGC TGTAG	GCATCCCAGACCT GACTA	N.D.	0.01

N.D.: could not be determined

Supplementary Table 8 | Top DSB peaks identified by BLESS in N2a cells

Site	Fwd Priming Site	Rev Priming Site	Chromosome	DSBs frequency (N2A)	indel % (N2A)	indel % (liver)
Pcsk9-sg1 (on target)	CAGGCGTCCA TGTCTTC	GCGAGCATCA GCTCTTCATA	chr4:106136424 -106136576	3.340E-05	34.4111	36.1298
Pcsk9-sg1-BLESS-1	CGTAAAATGG TCGCTATGAC AA	ATGAGTGGAG GGCTATCGTT T	chr11:29147310 -29147353	1.450E-06	0.0170	0.0427
Pcsk9-sg1-BLESS-2	TTCACCACAG AGGCTTAGAA TG	CTAGGAAGGA AGAAAGCTGC AA	chr6:50138067- 50138068	1.450E-06	0.0420	0.0939
Pcsk9-sg1-BLESS-3	ATCTATGCAA TGTGCTGTCTG TT	CCCTCCCTCA CTCTCTCTTT CT	chr6:110500737 -110500752	1.450E-06	0.0603	0.0729
Pcsk9-sg1-BLESS-4	CAGGAGAGGC CTGTGAAATA AT	AATCCCACGT CACATTGTTA AA	chr8:130023648 -130023687	1.450E-06	0.1210	0.0675
Pcsk9-sg1-BLESS-5	GCATGTCTGT GAACCGTGTA GT	GGTATGGGCT TTGAGAAACA AT	chr17:35979985 -35980029	1.370E-06	0.0927	0.0533
Pcsk9-sg1-BLESS-6	GACAGTGACG TGGATGTGCT	CTGCTTGGGT GCTGGAG	chr6:82724511- 82724632	1.370E-06	0.0135	0.0266
Pcsk9-sg1-BLESS-7	GACAGCGAGT GAGCAGTGT	TCGCTGTCTT CTCACACACA	chr11:10160145 0-101601538	1.290E-06	0.0285	0.0396
Pcsk9-sg1-BLESS-8	TCAGCACTTT AGTGTCAAGAA ATAATTG	TGTAGGGTTC CTGCCCATTT	chr8:19800400- 19800550	1.290E-06	N.D.	N.D.
Pcsk9-sg1-BLESS-9	CCCTCAGGAG CTATTAACAC CA	GGTCTCACAA CACGGTCCTC	chr10:77950398 -77950454	1.210E-06	0.0705	0.0418
Pcsk9-sg1-BLESS-10	ATTTAGTGGA CAAGGCCAGA GA	TTCTGACTAA TGTCACCCGA GA	chr11:69044356 -69044444	1.210E-06	0.1200	0.0687
Pcsk9-sg1-BLESS-11	TCAGAGCTAC AGAAAGCCCA GT	CAGGCAGATC TCTGAGTTGG AG	chr16:50527704 -50527732	1.210E-06	0.1136	0.1222
Pcsk9-sg1-BLESS-12	ACCCTCTCCT CAGTAGGGAA AC	GAAGTGAAGA CCGCTTAAAG GA	chr16:77090401 -77090424	1.210E-06	0.0487	0.0567
Pcsk9-sg1-BLESS-13	TTTAAACAGAA ATGGAGGGGA AA	GTGGGGTTTTG TTTTTATTTT GC	chr1:4795600- 4795635	1.210E-06	N.D.	N.D.
Pcsk9-sg1-BLESS-14	GACTATACGT GCTTGTGCAT GG	GCTTCAAAGA AATGGGAAGA GA	chr1:164227264 -164227271	1.210E-06	0.0845	0.2051
Pcsk9-sg1-BLESS-15	TAGATTGATG GGCTTTTCTC CT	CACAGTTAGG AGGCTGGTTA TC	chr3:14116391- 14116424	1.210E-06	0.0400	0.0568
Pcsk9-sg1-BLESS-16	CTTCAGGGTG AGTAGAGGGA GA	GGGGTTTCTT GTCTGGTTTT AG	chr5:15113799- 15113890	1.210E-06	0.0829	0.2481
Pcsk9-sg1-BLESS-17	AATCCACACT TTGCAGGGTA AG	ATCACAGCTG CCATTGTTAC AG	chr6:3009780- 3009786	1.210E-06	N.D.	N.D.

Pcsk9-sg1-BLESS-18	AGACGCATCT GAAAGAATCC AC	ATTTCAAAT CCAATCCATG CT	chr8:15008971- 15009007	1.210E-06	0.0819	0.0627
Pcsk9-sg1-BLESS-19	GGTGGTTTGG CTGTAGACAC TT	TCATTGTTCC ATCTCTGAGT GC	chr8:19952249- 19952298	1.210E-06	0.0731	0.0434
Pcsk9-sg1-BLESS-20	GCAGTCTGCA TCAGCATCAG	CAGGAAAGCT TGGGTCTCTT T	chr2:60067768- 60067817	1.130E-06	0.0190	0.0348
Pcsk9-sg1-BLESS-21	ACTGAAGCAT CCACTCTTTG GT	TACCTTCTCC CAAATTGACC AT	chr11:27713705 -27713726	1.130E-06	0.0871	0.1182
Pcsk9-sg1-BLESS-22	TGTGGTTACT TTCCCTACC TG	AGATGAGTGG GATCTCAAGT GG	chr11:92229536 -92229574	1.130E-06	0.1466	0.1089
Pcsk9-sg1-BLESS-23	TTTTCTATTT TGCCAAGCCA CT	CTAATTGAGG CAGGATGACA AA	chr16:26910810 -26910841	1.130E-06	0.1237	0.0602
Pcsk9-sg1-BLESS-24	TTTCTGAAA TGGATCCAAC AT	TCATCTTCCA CTTTCTGCAT TC	chr17:41354776 -41354804	1.130E-06	0.1253	0.0786
Pcsk9-sg1-BLESS-25	TGTGTTGCTG AGTTCCATTT TT	CACCACGTGA GTCTGTCTTA GC	chr18:10599312 -10599324	1.130E-06	0.0715	0.1658
Pcsk9-sg2 (on target)	GATTGATCAG GCGAGCAAGT	TGGGTAGAAC CCAGAGAGTC A	chr4:106126838 -106127032	2.560E-05	24.4127	48.2720
Pcsk9-sg2-BLESS-1	AGACAAGGCA GACCTCAAAC TC	ATGCAGTCAA AACACCAATG AG	chr11:3058818- 3058885	1.900E-06	0.0778	0.1019
Pcsk9-sg2-BLESS-2	AAAAACCCCA AAGGACTTGC	GTGCTCCCTT CGTAAAGTGA AG	chr8:20011416- 20011519	1.720E-06	0.0999	0.0512
Pcsk9-sg2-BLESS-3	GCATTTTGGGA CCTAACAGT CC	AAACCAAAAC CAAAACCAAA AA	chr8:19161731- 19161795	1.630E-06	0.0778	0.0612
Pcsk9-sg2-BLESS-4	TCTTATTCCC TGCTGTTTGT CC	GGGAGAGGGA AGCTAGAACA TT	chr6:89113301- 89113327	1.450E-06	0.0499	0.0321
Pcsk9-sg2-BLESS-5	ATCTTTATCT CCCCTCCTGT CG	GCCACTCAGC ATCATTACAC AT	chr10:33895821 -33895833	1.360E-06	0.1318	0.0770
Pcsk9-sg2-BLESS-6	TAGATTTCTA GGCCGTGTGT CA	CTCAGAATGC TATGGTCTGG TG	chr10:67135151 -67135239	1.360E-06	0.0436	0.0273
Pcsk9-sg2-BLESS-7	TAGCTACATG GGGAGGGAAG TA	GGATCCGTA AAGTGTCTCT CC	chr10:85780322 -85780348	1.360E-06	0.1441	0.0430
Pcsk9-sg2-BLESS-8	ACTGGGTGTA TCTTGAGCAC TG	TCAGGAAAAC AAACTGGAAT GA	chr15:10972737 -10972754	1.360E-06	0.0565	0.0289
Pcsk9-sg2-BLESS-9	TAGAAGCAGG AACATGGAGT GA	CAAGGCAACA CTTTTCTGAT TG	chr19:41910319 -41910330	1.360E-06	0.0976	0.0523
Pcsk9-sg2-BLESS-10	CTGGGGCCTA AAGTCTCTCA	ATCTCTCAGA CACTGAGCCA	chr1:45707849- 45707893	1.360E-06	0.0000	0.0000

	AT	CA				
Pcsk9-sg2-BLESS-11	ACATGGGAGG TAGACGTTGA GT	CGTGCCATAT CTGTCTGTTT GT	chr4:140700963 -140701050	1.360E-06	0.0979	0.1175
Pcsk9-sg2-BLESS-12	GAGGAAGAGA ACCAGCATTT TG	TGTAGTGATG ATGCAGTGGA GA	chr6:4492821- 4492826	1.360E-06	0.0469	0.0768
Pcsk9-sg2-BLESS-13	TCAATCAAGA AAATGCTCCG TA	GACAGAAAAT AGCTGGGCTT TG	chr6:58581527- 58581544	1.360E-06	0.1178	0.1101
Pcsk9-sg2-BLESS-14	GCAGGACATG GTGAACCTTA TT	ACTCCAAGAA TCCTGATAAC ATTT	chr6:79172116- 79172155	1.360E-06	0.2116	0.1669
Pcsk9-sg2-BLESS-15	CAAAACAAAA CAAAACAAAT TGAA	ATCTAATGCC GATATGCAGA CA	chr8:53816263- 53816300	1.360E-06	0.1761	0.1272
Pcsk9-sg2-BLESS-16	GATTTCCAGA GAGGGCAAGA T	GCTTGCTGAT CAGACCTTCA C	chr2:73215157- 73215162	1.360E-06	0.0151	0.0092
Pcsk9-sg2-BLESS-17	GTGGACAGTG GCAATTC AAT AA	GAGAATGCCA GGACATCAAT TA	chr10:64706424 -64706426	1.260E-06	0.0829	0.0753
Pcsk9-sg2-BLESS-18	ATGCAAATCG CAATTACATC CT	AAGTCCAGTG AGCTGGAGAG AC	chr10:10975938 7-109759435	1.260E-06	0.1821	0.0409
Pcsk9-sg2-BLESS-19	GTCGGGGAAG CTGTAGAGAA AT	GCAGCTTAGC ATCTTATGAG CA	chr13:9092702- 9092759	1.260E-06	0.0323	0.0073
Pcsk9-sg2-BLESS-20	TATAAAAACA TGGCGGACAG TG	GCCTGAAATT CCATAACAAC AC	chr15:84196279 -84196349	1.260E-06	0.0678	0.0358
Pcsk9-sg2-BLESS-21	AGTTGAGGTG AGGTAGGGTG AC	ATCCAAATCC TACAGAGCCT GA	chr15:97783672 -97783735	1.260E-06	0.0806	0.0301
Pcsk9-sg2-BLESS-22	CTTAAGGCAA AGAACCCAAA TG	TAAGAACAGG CCATAAGGAA GC	chr16:67968637 -67968661	1.260E-06	0.0784	0.0706
Pcsk9-sg2-BLESS-23	TCTTATTGTT GCTTGTTTCT GG	TTTGGAAATG AAATTATAGG GATGA	chr16:77485308 -77485324	1.260E-06	0.0000	0.4695
Pcsk9-sg2-BLESS-24	CTTACGGTCT TGAGGGGTCT CT	GACGACAGAC AGGCTCTGC	chr17:39980913 -39980976	1.260E-06	0.0849	0.0117
Pcsk9-sg2-BLESS-25	GATTTCTTTT ATCCCTCGT CT	GGCTTGTTTG CTATACATGT GG	chr18:20718137 -20718170	1.260E-06	10.0000	0.0459

N.D.: Not determined.

Supplementary Table 9 | Top off-targets for *Pcsk9* identified by motif-mismatch prediction

Site	Target	Fwd Priming Site	Rev Priming Site	Motif Score	indel % (N2a)	Indel% (Liver)
Pcsk9.sg1 (on target)	CACCGCAGCCA CGCAGAGCA	CAGGCGTCCATGTC CTTC	GCGAGCATCAGCTCT TCATA	100.00	34.411	36.130
Pcsk9.OT 1.1	CCCATCAGCAA CGCAGAGCA	ACGGAGGCTCATTT GCACGTGT	ACACTGAAGTTGGTG TAGCTAGTGTAGCG	1.40	0.002	0.033
Pcsk9.OT 1.2	GAAAGCAGCAA CGCAGAGCA	TGCTGTGGGAAATG TCGCTTCA	AGCTGTGTATGTGGG CAGTGGA	1.41	0.100	0.027
Pcsk9.OT 1.4	GGCCACTGCCA CGCAGAGCA	TGATGACTCTCTCA TTGTAGGAGGTGA	GTGCTCCTGAGGACT GAGTGAAA	1.00	0.012	0.018
Pcsk9.OT 1.5	TGCCTCAGCCA AGCAGAGCA	AGGCTTGTCTCTCT GCATGAGGC	GCCGTCTTCATGGCC AAAGTTACA	0.82	0.002	0.442
Pcsk9.OT 1.6	AGCTGCAGCCA CGCAGAGCC	ACGGAGCCCCATAA GACCTTCA	AAGAATGCCTAACTG CGGGCTC	0.00	0.010	0.116
Pcsk9.OT 1.7	CAGCTCAGCAA GGCAGAGCA	AGGAGGGGTGTCTT CTAGACCGT	AAGACAGGACGGATC TGGCTCC	0.72	0.043	0.023
Pcsk9.OT 1.8	CATGTCAGCCA CCCAGAGCA	AGAGAGGCCCTTAGT GTGCTGCA	CAACAGAGGCCTTGT GACTGGC	0.00	0.002	0.093
Pcsk9.OT 1.9	CACAGCAGCCA GCCAGAGCA	CTCACAGCCCTCGA GAGTCTGG	GCCTGGGATAGGAAG CTCACGT	0.57	0.031	0.029
Pcsk9.OT 1.10	CCTCGCAGGCT CGCAGAGCA	GATTCCCCAGACTC CCTTCCGG	GGCCAGATGCTGAAA TAGGTGGC	0.54	0.002	0.074
Pcsk9.OT 1.11	CTCCTAAGCCC CGCAGAGCA	TCCAGACTCACGGC AATCTTGA	GAGCCTGCAAGCGAG TCCTTC	0.52	0.009	0.006
Pcsk9.OT 1.12	AACAGCAGGCA GGCAGAGCA	TCCGGCAACAGCTA AGATAGGCT	CCCAGGCAGAGCAAC AGTGAGA	0.51	0.002	0.055
Pcsk9.OT 1.13	CGCAGTAGCCA GGCAGAGCA	ACGTGAGGAGGCTT CCTTGAGA	AATGTTCCCCTCCAC TGGAGCC	0.48	0.026	0.102
Pcsk9.OT 1.14	CCTCGGAGCCA AGCAGAGCA	ACCTGTCAAGATCC CAGCTGGAG	AGCATGGGGCCTTGA AGCTCTA	0.48	0.037	0.212
Pcsk9.OT 1.15	CACAGCACTCA GGCAGAGCA	TGGCAGGCAGTTTT CTTGGTCA	GCAGGTGTGAATGTA CCCACCA	0.46	0.029	0.014
Pcsk9.sg2 (on target)	CCGCTGACCAC ACCTGCCAG	GATTGATCAGGCGA GCAAGT	TGGGTAGAACCCAGA GAGTCA	100.00	24.413	47.348
Pcsk9.OT 2.1	CACCTGAGCAC ACCTGCCAG	TACTCAGAGACCCA GCCCTCA	CCAGCATCTTGGCTT CCAGCAA	2.63	0.014	0.028
Pcsk9.OT 2.2	CCGCTGCCAC AGCTGCCAG	TGAGCGCAGACTGA CATGGGAA	GCTGCGTGCTTTCAT AGGGTGA	1.77	0.002	0.012
Pcsk9.OT 2.3	CCCATGACCAC ACCTGCCAT	TCCACCCCATCTCT GCCTTCTGA	GGGTAGGTCTCACAC AAGGGCC	1.75	0.009	0.031
Pcsk9.OT 2.4	ACTCTGTCCCC ACCTGCCAG	CGCCAACGTCCATC TGAAACCA	GATGTGAGGCGAGGC TAGGGAG	0.99	0.017	0.322
Pcsk9.OT 2.5	CCACAGCCCCC ACCTGCCAG	TTCCCTCCCTTGGG TGTTCCAC	AACAGCATCCTTCGC TGGAAGC	0.92	0.002	0.056
Pcsk9.OT 2.6	CCCCGGCCCCG ACCTGCCAG	CACCGATACCCACA ATGGCCGG	GCTGTGGGAGACTGA AGGGCCA	0.92	0.043	0.061
Pcsk9.OT 2.7	CAGCTGATGTC ACCTGCCAG	GACAGGTCTTTGCC TTGCTTTAATATCT GA	TGCTGCTATCCTCTA ATTCAGCTGCT	0.85	0.002	0.018
Pcsk9.OT 2.8	ACTCTAACCTC ACCTGCCAG	TCCCTCTTACCAC TTGCCAGT	GCAGGTTCACTGCTG AGCAGAG	0.87	0.005	0.586
Pcsk9.OT 2.9	CTGCTGGCCAC ACCTGTCAG	CGAGGCAAGAAAGA GGCTGATAGAG	TGCAGCACTCTCGGA TTCATGG	1.01	0.026	0.026

Pesk9.OT 2.10	CTGCTGCCAC AGCTGCCAG	GGGGGAAGAACCTG GAAGGGAG	GGGTGAATTACACAG TGTGGATCGT	0.85	0.031	0.010
Pesk9.OT 2.11	CCGCGCACCAC AACTGCCAG	CCATCCGCCTAGTT CAAGTGCC	GGCGCTCTGGTTCCT GGTTTTA	0.67	0.007	0.023
Pesk9.OT 2.12	GTGATGACCAC ACCTGGCAG	TGAGCTCCTAGCCT CCTCCCTT	GCAACACCTGGCATC CACATCC	0.74	0.020	0.017
Pesk9.OT 2.13	ATGATGACCAC ACCTGACAG	AGGTTGACAGTCCT GCTCAGGG	GGAAAGACTCCATCA GCTGCTCC	0.74	0.015	0.025
Pesk9.OT 2.14	CAGGTGCCAAC ACCTGCCAG	GGAAGAAGGATGGG CGAGATGC	TAACCACCAGCCAGG GCCTG	0.63	0.002	0.018
Pesk9.OT 2.15	ATGATGACCAC AGCTGCCAG	GGTTAAGGCAGTAT GTGGTTTATCTCGG	CCAGCCCTTCTCCAT TTGAGAGGA	0.66	0.079	0.195
Pesk9.OT 2.16	GCTATGACCAC ATCTGCCAG	TGAAGTGACAGTAA GAAACTAAGTTGGC ATTTTG	TGCACCAATGTTAGA CAAACATGATTATTG ACTG	0.64	0.002	0.047
Pesk9.OT 2.17	CAGCAGACCCC AGCTGCCAG	TCACTTGGTGGGTT AGCAATTATATCTT GGTT	TGAATCTGTTTGGTC CATGTCTGCCT	0.61	0.015	0.042
Pesk9.OT 2.18	CCGCTGCCGAC TCCTGCCAG	GGGGGTCAGCAGGA GTAGGT	CCGAGGCTGAGCTGC ACTAAC	0.53	0.030	0.056

Supplementary Sequences

Italic: HA-tag

Underlined: NLS sequences

Parvibaculum lavamentivorans Cas9

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Corynebacter diphtheria Cas9

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GCAGtaa

Streptococcus pasteurianus Cas9

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SURVEYOR assay primers

<u>Gene</u>	<u>Surveyor primer F</u>	<u>Surveyor primer R</u>
<i>DYRK1A</i>	GGAGCTGGTCTGTTGGAGAA	TCCCAATCCATAATCCCACGTT
<i>GRIN2B</i>	GCATACTCGCATGGCTACCT	CTCCCTGCAGCCCCTTTTTTA
<i>EMX1</i>	CCATCCCCTTCTGTGAATGT	GGAGATTGGAGACACGGAGA
<i>Apob</i>	GGGCATGCTGACGGGATAAT	GGGAGCCCTCACAACTAAA
<i>Pcsk9 (for sg1)</i>	ATGAGCCGTCTAATGCGTGG	AGTACTCACCCACAGACCCG
<i>Pcsk9 (for sg2 and sg3)</i>	CAGGCGTCCAGTACCCACAC	ATCACCCCAACCCCAAAGCA
<i>AAVS1</i>	CCCCTTACCTCTCTAGTCTGTGC	CTCAGGTTCTGGGAGAGGGTAG
<i>Rosa26</i>	CTTGCTCTCCCAAAGTCGCT	CCAATGCTCTGTCTAGGGGT