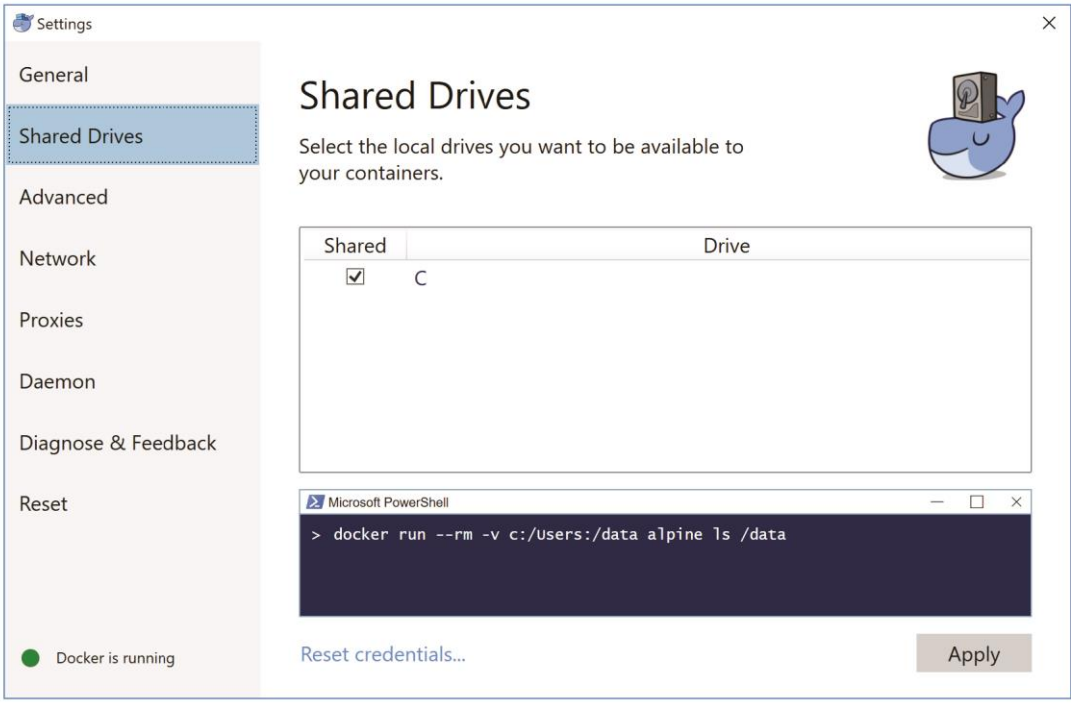
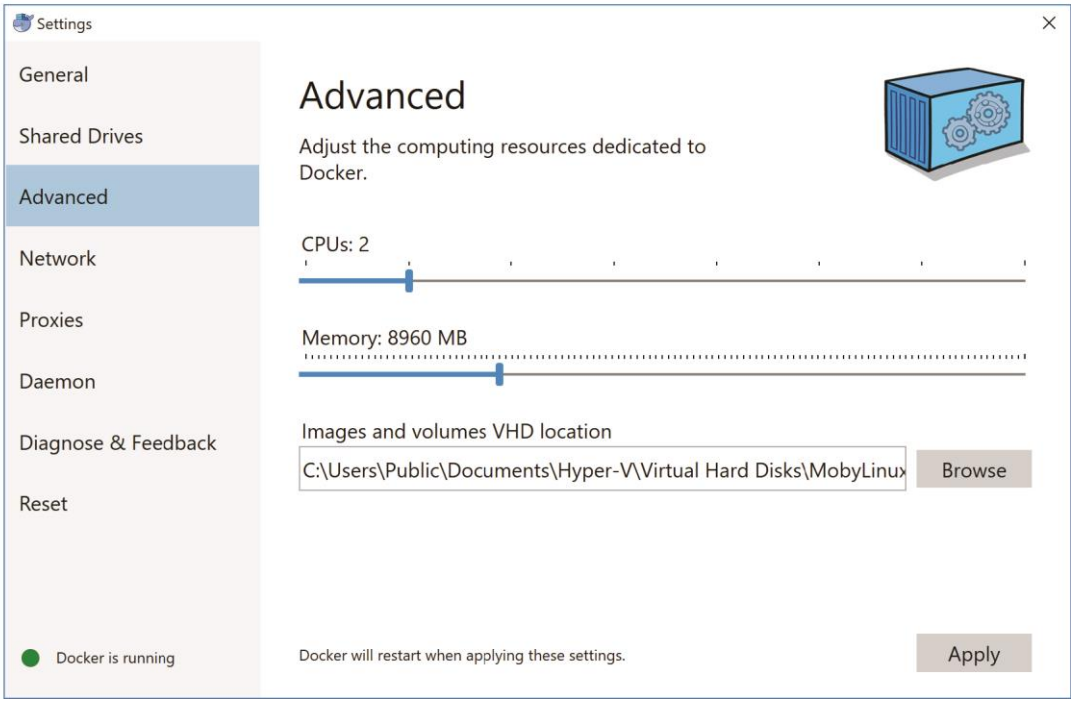
a



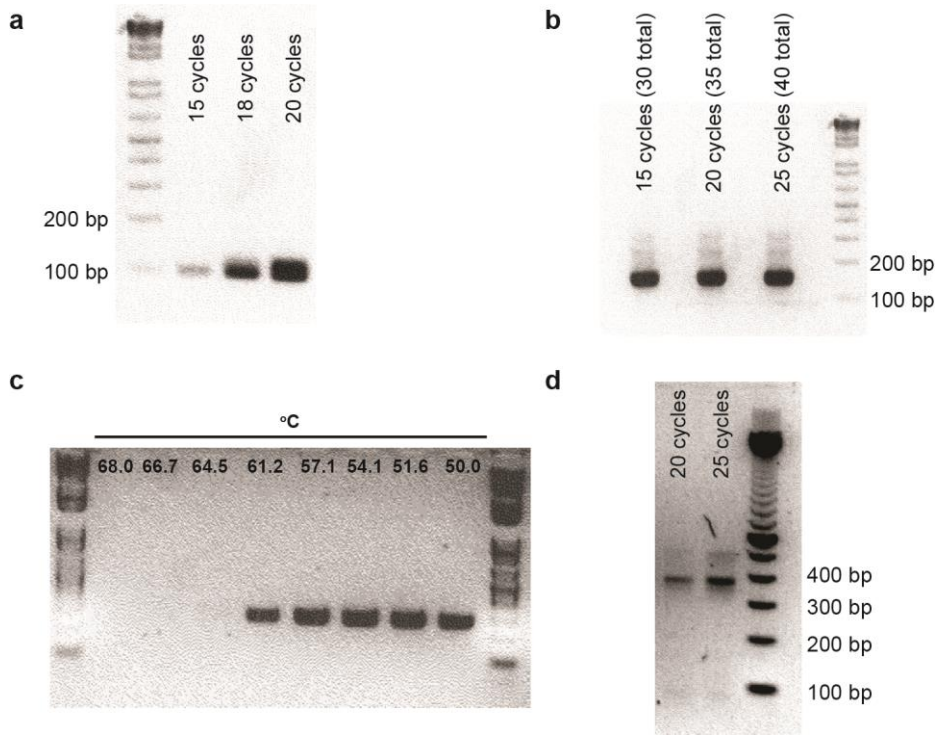
b



Supplementary Figure 1

Screenshot of sharing disk volumes and allocating memory with Docker containers.

a, Screenshot using Docker to ensure that the drive(s) you want to be available to the container is/are checked (under Settings…/ Shared Drives). **b**, Screenshot using Docker to allocate enough memory to the container (under Settings…/ Advanced).



Supplementary Figure 2

Pooled sgRNA library preparation and analysis.

a, Representative results for lsPCR1. **b**, Representative results for lsPCR2. **c**, Gradient PCR for lentiGuide-Puro-specific primers or locus-specific primers for laPCR1. **d**, Representative results for laPCR2.

a

```
Site1 CACACTGTGGCCCCTGTGCCCAGCCCTGGGCTCTCTGTACATGAAGCAAC CCCTGTGCCCAGCCC NA NA
Site2 GTCCTGGTTTTTGGTTTGGGAAATATAGTCATC NA GTCCTGGTTTTTGGTTTAAAAAAATATAGTCATC NA
Site3 TTTCTGGTTTTTGGTTTGGGAAATATAGTCATC NA NA GGAAATATA
```

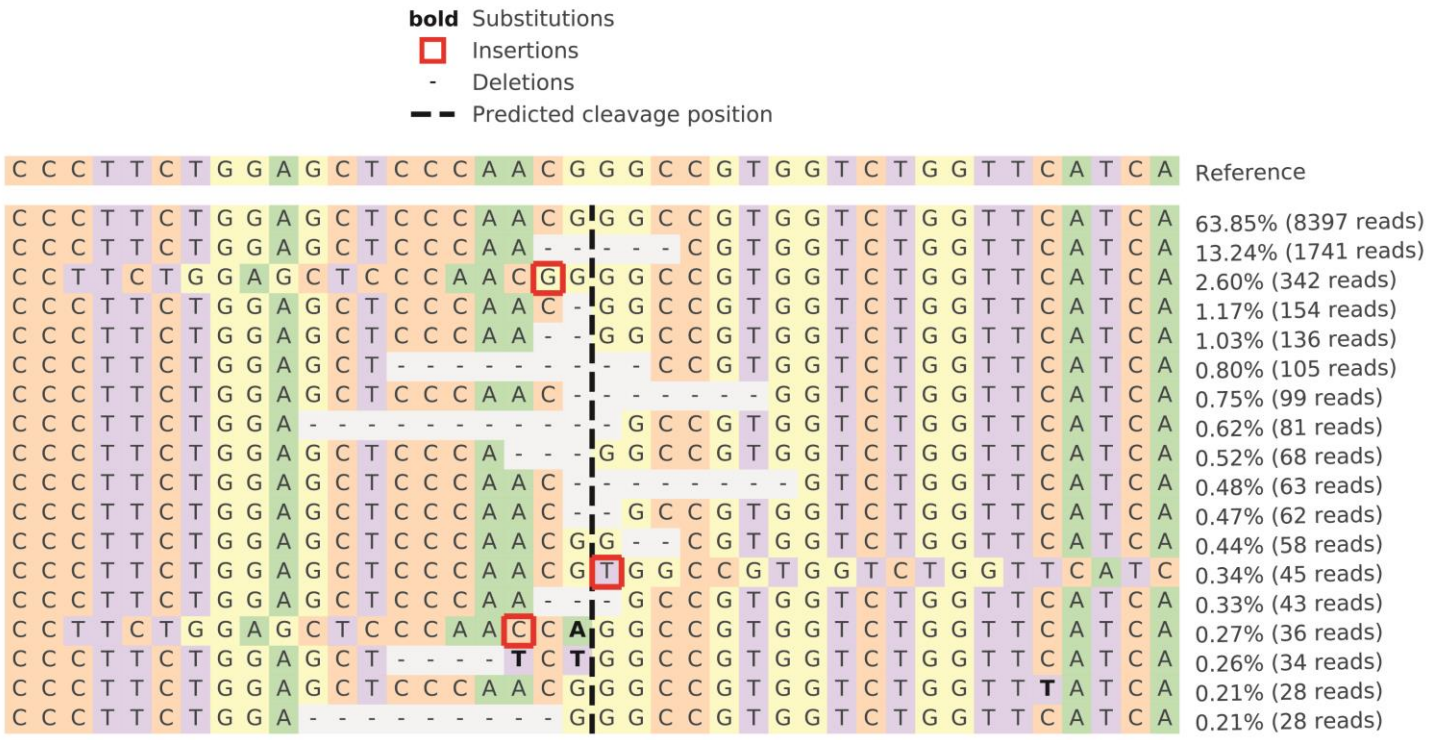
b

```
chr1 65118211 65118261 R1 CTACAGAGCCCCAGTCCTGG NA NA
chr6 51002798 51002820 R2 NA NA NA
```

Supplementary Figure 3

Example amplicon and regions description files.

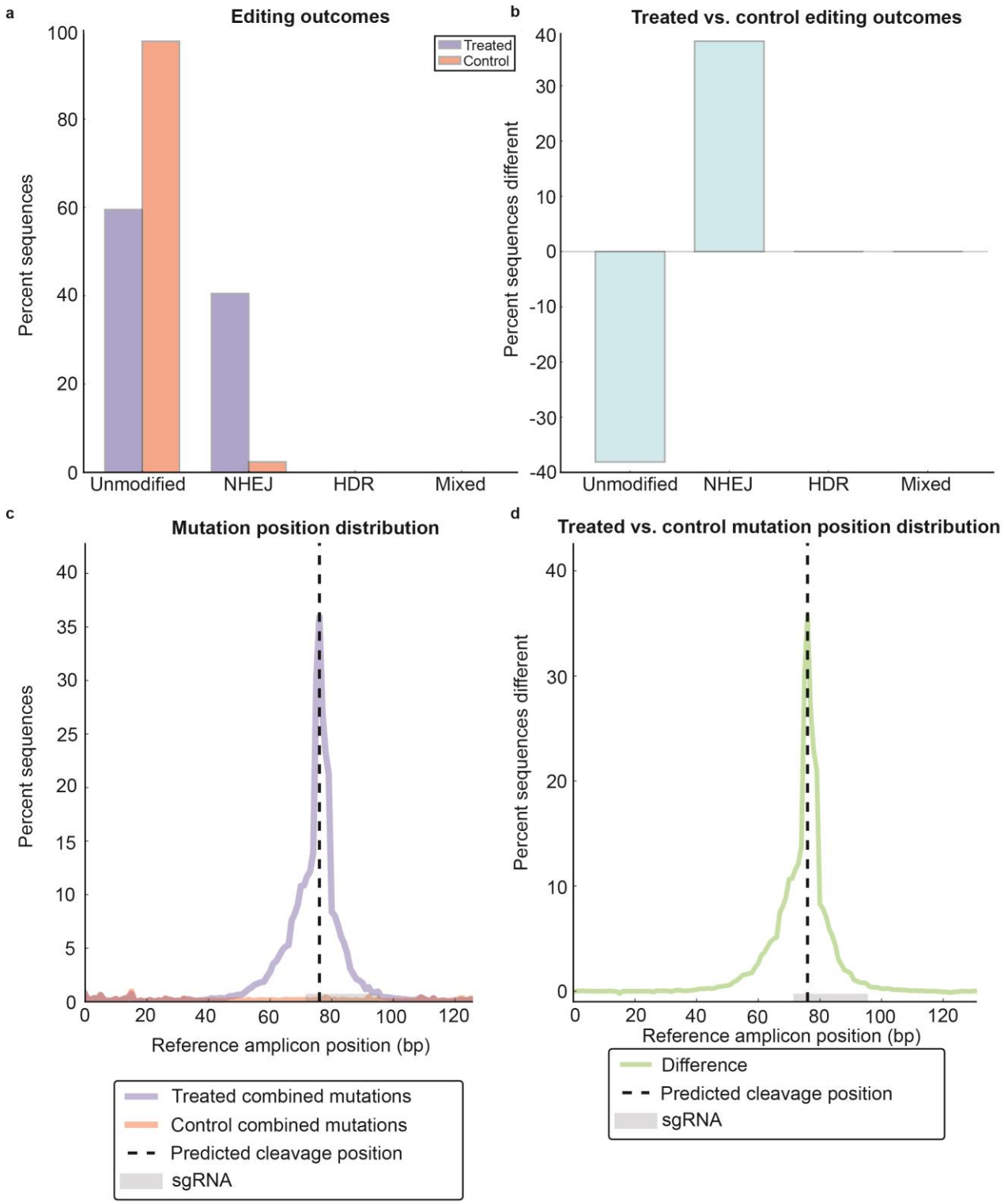
a, Example of a properly formatted amplicon description file. This file is a tab delimited text file with up to 5 columns (first 2 columns required). No column heading is required. **b**, Example of a properly formatted regions description file. This file is a tab delimited text file with up to 7 columns (4 required) and contains the coordinates of the regions to analyze and some additional information. No column heading is required.



Supplementary Figure 4

Visualization of the distribution of identified alleles generated from targeting *BCL11A* exon 2.

Nucleotides are indicated by unique colors (A = green; C = red; G = yellow; T = purple). Substitutions are shown in bold font. Red rectangles highlight inserted sequences. Horizontal dashed lines indicate deleted sequences. The vertical dashed line indicates the predicted double-strand break position.



Supplementary Figure 5

Direct comparison of *BCL11A* exon 2 sequence between a *BCL11A* exon 2 targeted sgRNA sample ("edited") and a non-edited control sample ("non-edited").

a, Distribution of editing outcomes (unmodified, NHEJ, HDR, and mixed alleles) for treated (edited) and control (non-edited) samples. **b**, Comparison of the percent different editing outcomes (unmodified, NHEJ, HDR, and mixed alleles)

between the treated (edited) and control (non-edited) samples. **c**, Combined (substitutions/deletions/insertions) mutation position distribution for treated (edited) and control (non- edited) samples. The vertical dashed line indicates the position of predicted Cas9 cleavage. The position of the sgRNA is shown in gray. **d**, Comparison of the percent different combined mutations (substitutions/deletions/insertions) between the treated (edited) and control (non-edited) samples. The vertical dashed line indicates the position of predicted Cas9 cleavage. The position of the sgRNA is shown in gray.

SUPPLEMENTARY TABLES

Supplementary Table 1 | List of available PAM sequences for CRISPOR analysis. R = A or G; Y = C or T; V = A, C, or G; N = A, C, G or T

Nuclease	Species	PAM	PAM position	References
Cas9	*Streptococcus pyogenes*	NGG	3’	1,2
Cas9	*Streptococcus pyogenes* HF1/eSpCas9/HypaCas9	NGG	3’	81-83
Cas9	*Streptococcus pyogenes VQR* variant	NGA	3’	97
Cas9	*Streptococcus pyogenes VRER* variant	NGCG	3’	97
Cas9	*Staphylococcus aureus*	NNGRRT	3’	98
Cas9	*Staphylococcus aureus KKH* variant	NNNRRT	3’	99
Cas9	*Streptococcus thermophilus ST1*	NNAGAA	3’	100
Cas9	*Streptococcus thermophilus A*	NGGNG	3’	101
Cas9	*Neisseria meningitidis*	NNNNGATT	3’	100
Cas9	*Campylobacter jejuni*	NNNNACA	3’	101,102
Cpf1	*Acidaminococcus sp. BV3L6; Lachnospiraceae bacterium ND2006*	TTTV	5’	4
Cpf1	*Acidaminococcus sp. BV3L6; Lachnospiraceae bacterium ND2006* variant (S542R/K607R)	TYCV	5’	103
Cpf1	*Acidaminococcus sp. BV3L6; Lachnospiraceae bacterium ND2006* variant (S542R/K548V/N552R)	TATV	5’	103

Supplementary Table 2 | Barcoding strategy for oligonucleotide pool synthesis. The sgRNA sequence is denoted with N's

Barcode Number	Forward barcode (bold)	Reverse barcode (dotted underline)	Homologous sequence upstream of sgRNA (italics)	Homologous sequence downstream of sgRNA (underline)	Full oligonucleotide sequence
1	**CGGGTTCCGT**	GCTTAGAATAGAA	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**CGGGTTCCGT***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCGCTTAGAATAGAA
2	**GTTTATCGGGC**	ACTTACTGTACC	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**GTTTATCGGGC***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCACTTACTGTACC
3	**ACCGATGTTGAC**	CTCGTAATAGC	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**ACCGATGTTGAC***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCCTCGTAATAGC
4	**GAGGTCTTTCATGC**	CACAACATA	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**GAGGTCTTTCATGC***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCCACAACATA
5	**TATCCCGTGAAGCT**	TTCGGTTAA	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**TATCCCGTGAAGCT***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTTCGGTTAA
6	**TAGTAGTTCAGACGC**	ATGTACCC	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**TAGTAGTTCAGACGC***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCATGTACCC
7	**GGATGCATGATCTAG**	CATCAAGC	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**GGATGCATGATCTAG***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCCATCAAGC
8	**ATGAGGACGAATCT**	CACCTAAAG	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**ATGAGGACGAATCT***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCCACCTAAAG
9	**GGTAGGCACG**	TAAACTTAGAACC	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**GGTAGGCACG***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAAACTTAGAACC
10	**AGTCATGATTCAG**	GTTGCAAGTCTAG	*GGAAAGGACGAAACACCG*	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC	**AGTCATGATTCAG***GGAAAGGACGAAACACCG*NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCGTTGCAAGTCTAG

Supplementary Table 3 | Primers for lsPCR1 library preparation

Primer	Sequence (5’ to 3’)
Barcode1F	CGGGTTCCGTGGAAAGG
Barcode1R	TTCTATTCTAAGCGCCTTATTTTAACTTGC
Barcode2F	GTTTATCGGGCGGAAAGG
Barcode2R	GGTACAGTAAGTGCCTTATTTTAACTTGC
Barcode3F	ACCGATGTTGACGGAAAGG
Barcode3R	GCTATTACGAGGCCTTATTTTAACTTGC
Barcode4F	GAGGTCTTTCATGCGGAAAGG
Barcode4R	TATGTTGTGGCCTTATTTTAACTTGC
Barcode5F	TATCCCGTGAAGCTGGAAAGG
Barcode5R	TTAACCGAAGCCTTATTTTAACTTGC
Barcode6F	TAGTAGTTCAGACGCGGAAAGG
Barcode6R	GGGTACATGCCTTATTTTAACTTGC
Barcode7F	GGATGCATGATCTAGGGAAAGG
Barcode7R	GCTTGATGGCCTTATTTTAACTTGC
Barcode8F	ATGAGGACGAATCTGGAAAGG
Barcode8R	CTTTAGGTGGCCTTATTTTAACTTGC
Barcode9F	GGTAGGCACGGGAAAGG
Barcode9R	GGTTCTAAGTTTAGCCTTATTTTAACTTGC
Barcode10F	AGTCATGATTCAGGGAAAGG
Barcode10R	CTAGACTTGCAACGCCTTATTTTAACTTGC

Supplementary Table 4 | Primers for lsPCR2 library preparation

Primer	Sequence (5' to 3')
lsPCR2_forward	TAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG
lsPCR2_reverse	ACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

Supplementary Table 5 | Primers for laPCR1 for pLentiGuide-specific deep sequencing for sgRNA enumeration. Bold sequence is Illumina Nextera handle sequence. Underlined sequence is specific to the lentiGuide-Puro plasmid.

Primer	Sequence (5' to 3')
lentiGuide_forward	**TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAGAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCG
lentiGuide_reverse	**GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAGCTTTAGTTTGTATGTCTGTTGCTATTATGTCTACTATTCTTTCCC

Supplementary Table 6 | Primers for laPCR1 for locus-specific deep sequencing. Bold sequence is Illumina Nextera handle sequence. Recommend 20 bp of locus-specific sequence.

Primer	Sequence (5' to 3')
Locus_forward	**TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAG-Locus-Specific-Sequence
Locus_reverse	**GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG-Locus-Specific-Sequence

Supplementary Table 7 | Illumina forward sequencing primers for laPCR2 (**i5**-Index-Handle)

Primer	Sequence (5' to 3')
F501	**AATGATACGGCGACCACCGAGATCTACAC**TAGATCGCTCGTCGGCAGCGTC
F502	**AATGATACGGCGACCACCGAGATCTACAC**CTCTCTATTCGTCGGCAGCGTC
F503	**AATGATACGGCGACCACCGAGATCTACAC**TATCCTCTTCGTCGGCAGCGTC
F504	**AATGATACGGCGACCACCGAGATCTACAC**AGAGTAGATCGTCGGCAGCGTC
F505	**AATGATACGGCGACCACCGAGATCTACAC**GTAAGGAGTCGTCGGCAGCGTC
F506	**AATGATACGGCGACCACCGAGATCTACAC**ACTGCATATCGTCGGCAGCGTC
F507	**AATGATACGGCGACCACCGAGATCTACAC**AAGGAGTATCGTCGGCAGCGTC
F508	**AATGATACGGCGACCACCGAGATCTACAC**CTAAGCCTTCGTCGGCAGCGTC
F517	**AATGATACGGCGACCACCGAGATCTACAC**GCGTAAGATCGTCGGCAGCGTC

Supplementary Table 8 | Illumina reverse sequencing primers for laPCR2 (**i7**-Index-Handle)

Primer	Sequence (5' to 3')
R701	**CAAGCAGAAGACGGCATACGAGAT**TCGCCTTAGTCTCGTGGGCTCGG
R702	**CAAGCAGAAGACGGCATACGAGAT**CTAGTACGGTCTCGTGGGCTCGG
R703	**CAAGCAGAAGACGGCATACGAGAT**TTCTGCCTGTCTCGTGGGCTCGG
R704	**CAAGCAGAAGACGGCATACGAGAT**GCTCAGGAGTCTCGTGGGCTCGG
R705	**CAAGCAGAAGACGGCATACGAGAT**AGGAGTCCGTCTCGTGGGCTCGG
R706	**CAAGCAGAAGACGGCATACGAGAT**CATGCCTAGTCTCGTGGGCTCGG
R707	**CAAGCAGAAGACGGCATACGAGAT**GTAGAGAGGTCTCGTGGGCTCGG
R708	**CAAGCAGAAGACGGCATACGAGAT**CCTCTCTGGTCTCGTGGGCTCGG
R709	**CAAGCAGAAGACGGCATACGAGAT**AGCGTAGCGTCTCGTGGGCTCGG
R710	**CAAGCAGAAGACGGCATACGAGAT**CAGCCTCGGTCTCGTGGGCTCGG
R711	**CAAGCAGAAGACGGCATACGAGAT**TGCCTCTTGTCTCGTGGGCTCGG
R712	**CAAGCAGAAGACGGCATACGAGAT**TCCTCTACGTCTCGTGGGCTCGG

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