CRISPR/Cas9-based genome-wide screening of *Dictyostelium*

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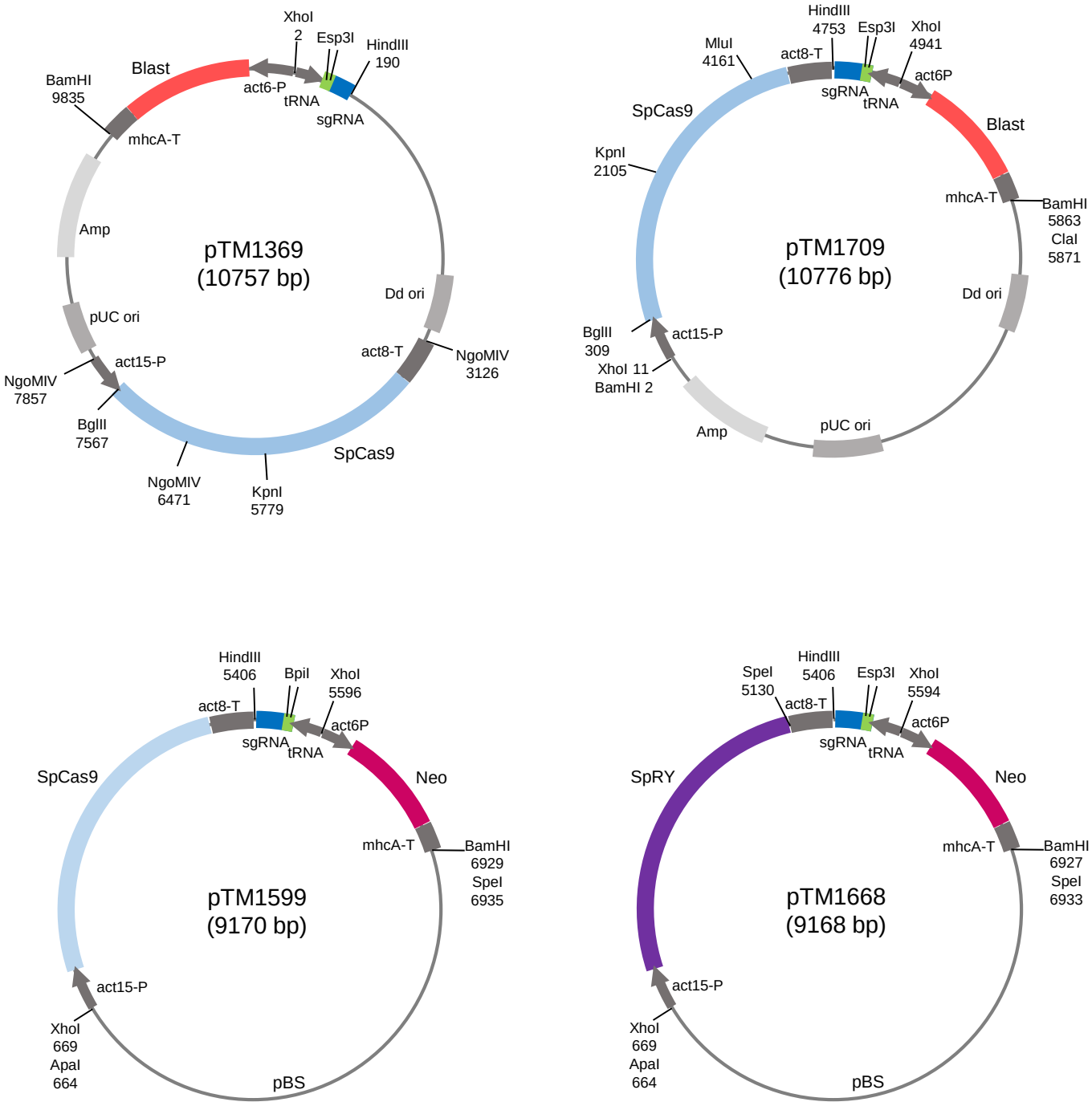
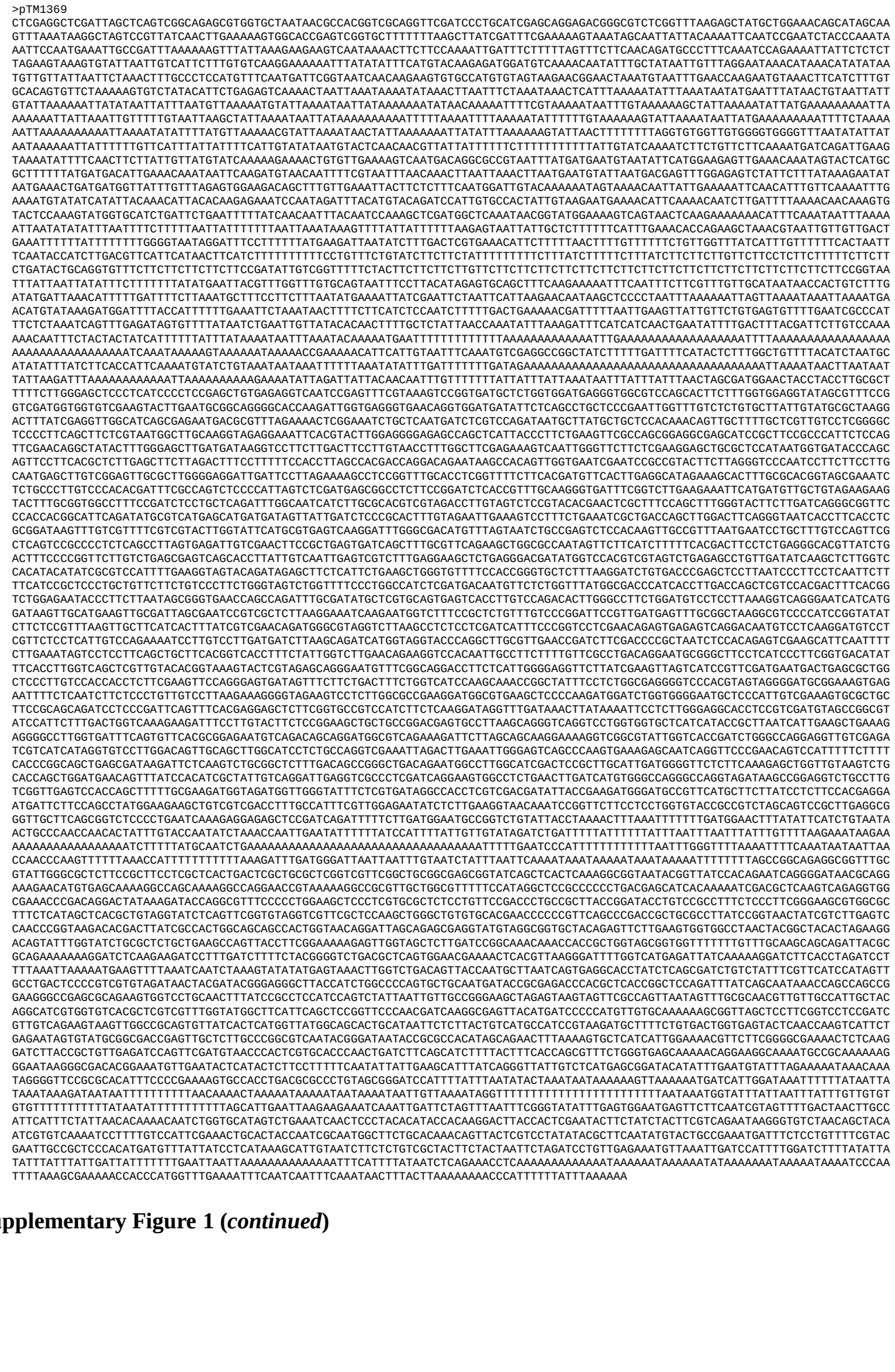


Supplementary Figure 1 Restriction maps of all-in-one CRISPR/Cas9 vectors and predicted DNA sequences.
pTM1369, a stable expression vector containing blasticidin-resistance cassette. SpCas9 expression module was inserted in the NgoMIV site. pTM1709, a stable expression vector containing blasticidin-resistance cassette. SpCas9 and tRNA-sgRNA expression cassettes were arranged next to each other. pTM1599, a transient expression vector containing neomycin-resistance cassette. pTM1668, a transient expression vector containing neomycin-resistance cassette. SpCas9 variants SpRY was used to recognise relaxed PAM sequences.



Supplementary Figure 1 (*continued*)

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Supplementary Figure 1 (*continued*)

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Supplementary Figure 1 (*continued*)

>pTM1709
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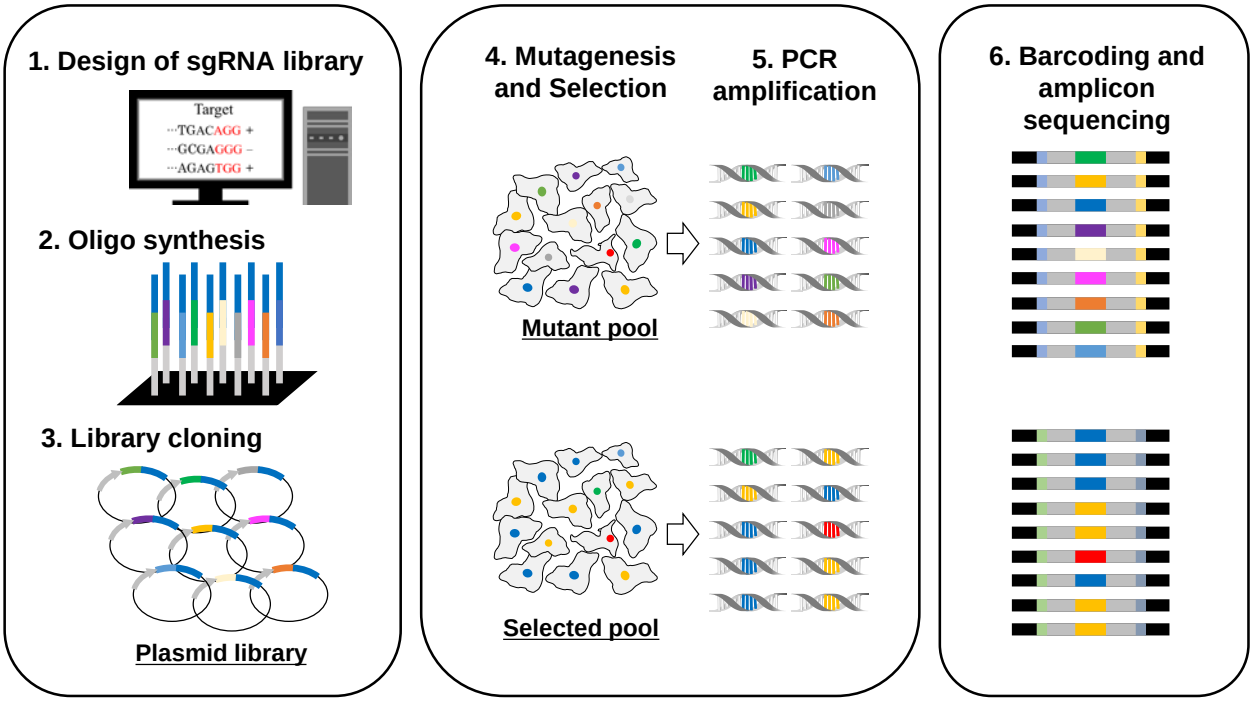
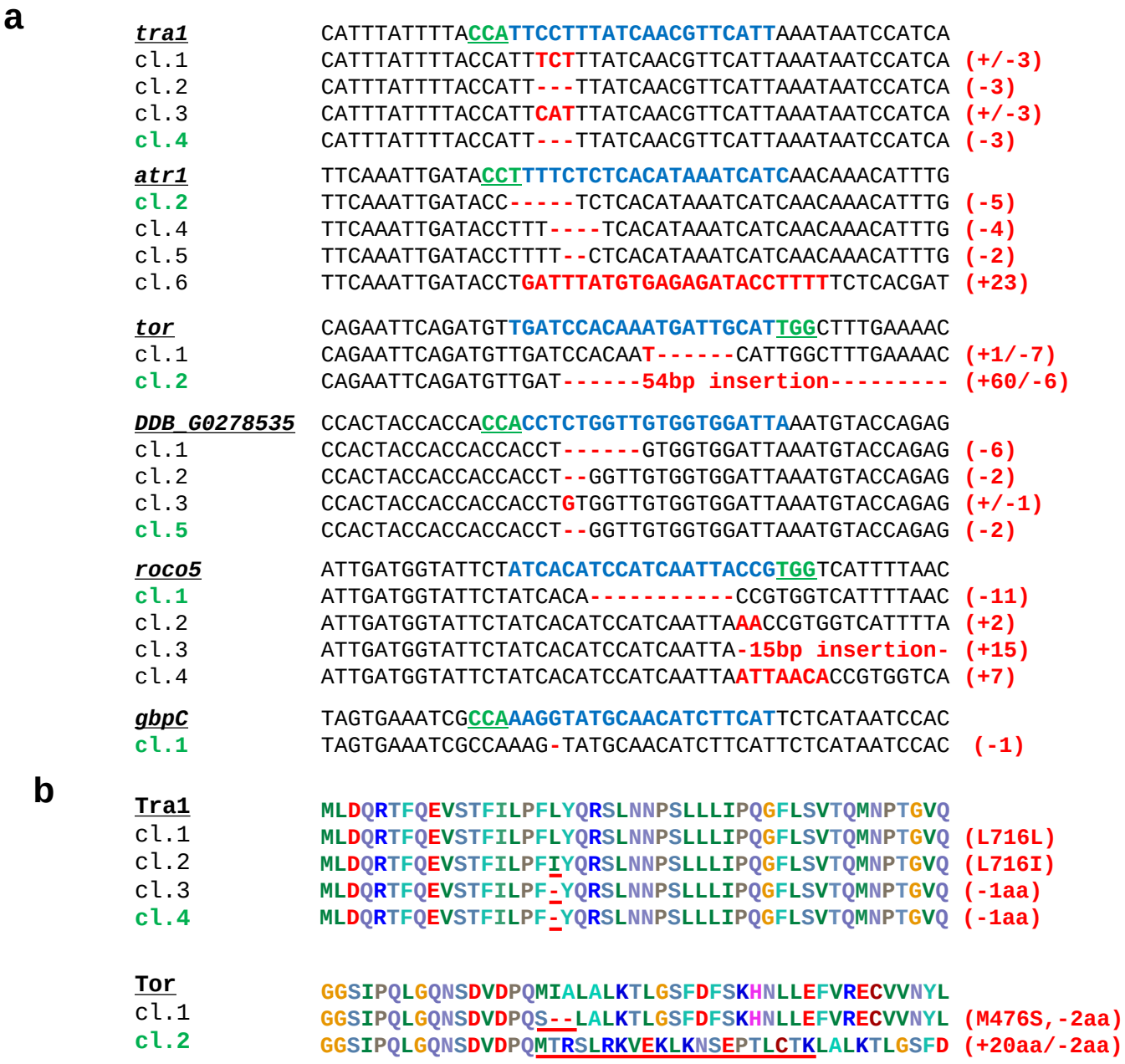
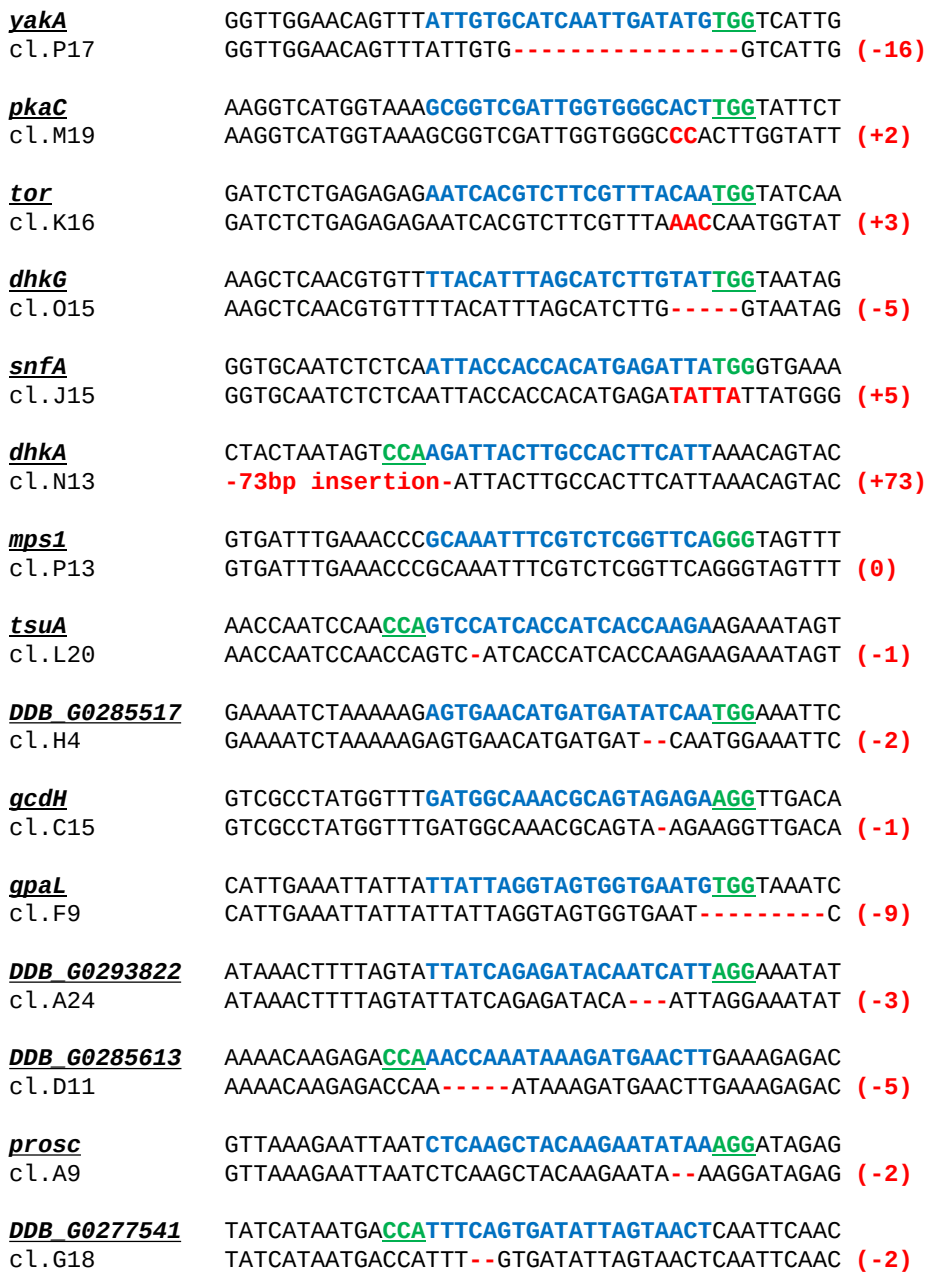
Supplementary Figure 1 (*continued*)



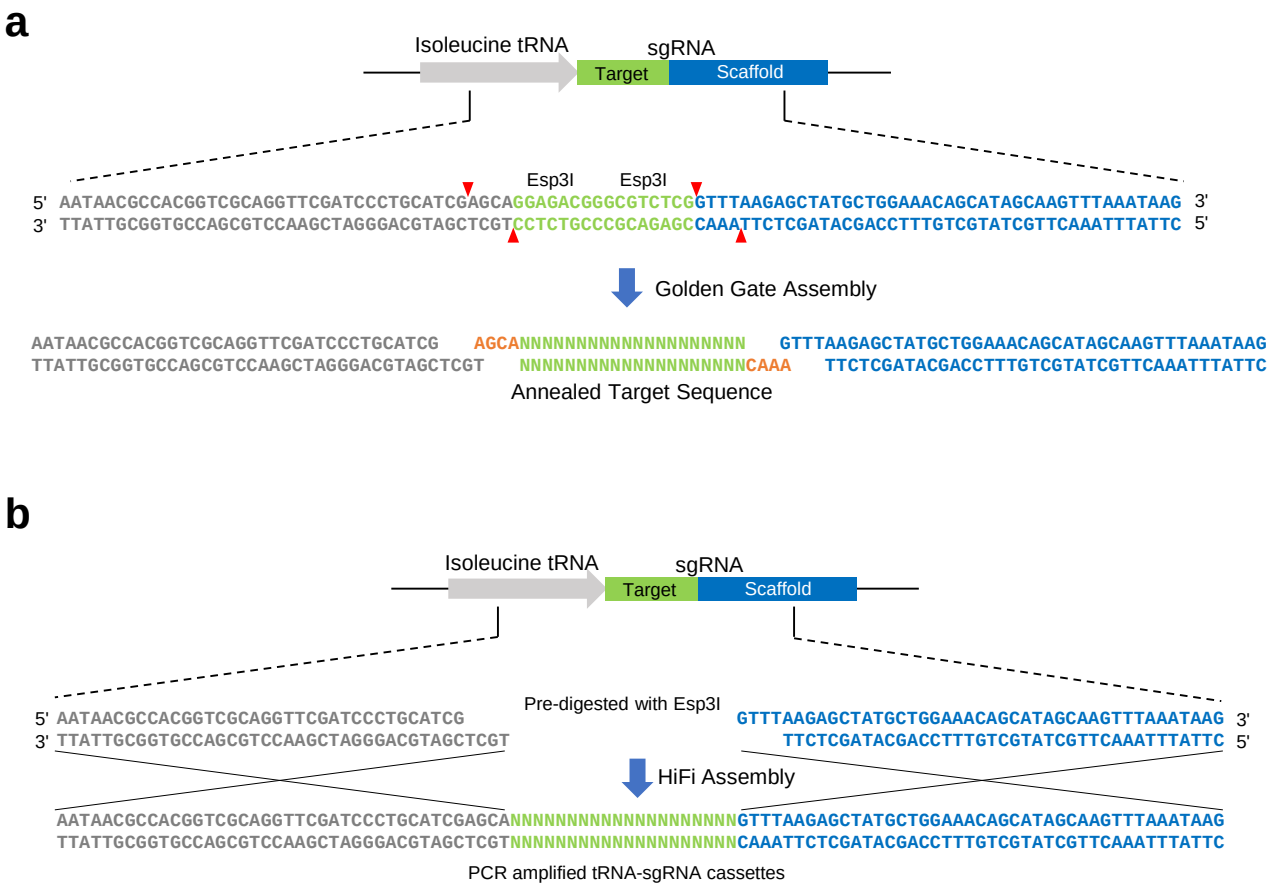
Supplementary Figure 2 General workflow of CRISPR screening.
1) In silico design of target sequences; **2)** Oligo synthesis; **3)** Cloning of plasmid sgRNA library; **4)** mutagenesis of genes in target cells and selection of mutants; **5)** Isolation of nucleic acids and PCR amplification; **6)** Deep sequencing of barcoded sgRNAs.



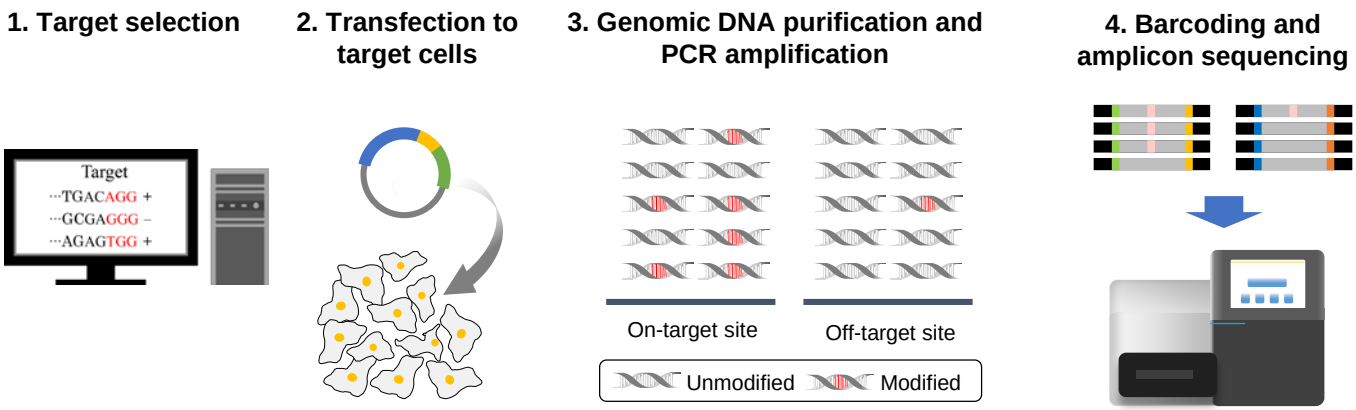
Supplementary Figure 3 Genomic mutations in recreated CRISPR mutants.
a) Sequencing results of recreated CRISPR mutants. The target gRNAs, PAM sequences and mutations are shown in blue, green and red, respectively. Numbers in parentheses indicate the number of modified nucleotides. Clones indicated by green letters were used for further analysis. **b)** Amino acid sequences of Tra1 and Tor. Mutated amino acids are shown in red underlines.



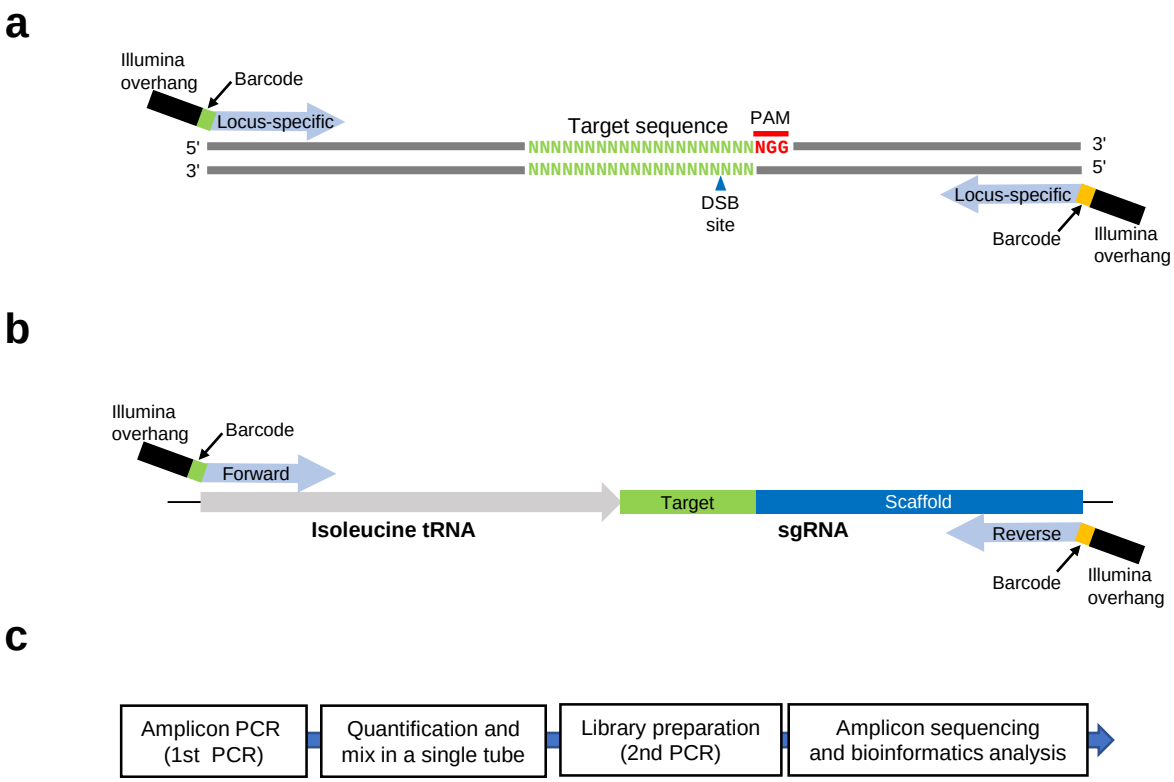
Supplementary Figure 4 Genome sequence analysis of identified gRNA regions.
Sequencing results of isolated CRISPR mutants were shown. The target gRNAs, PAM sequences and mutations are shown in blue, green and red, respectively. Numbers in parentheses indicate the number of modified nucleotides.



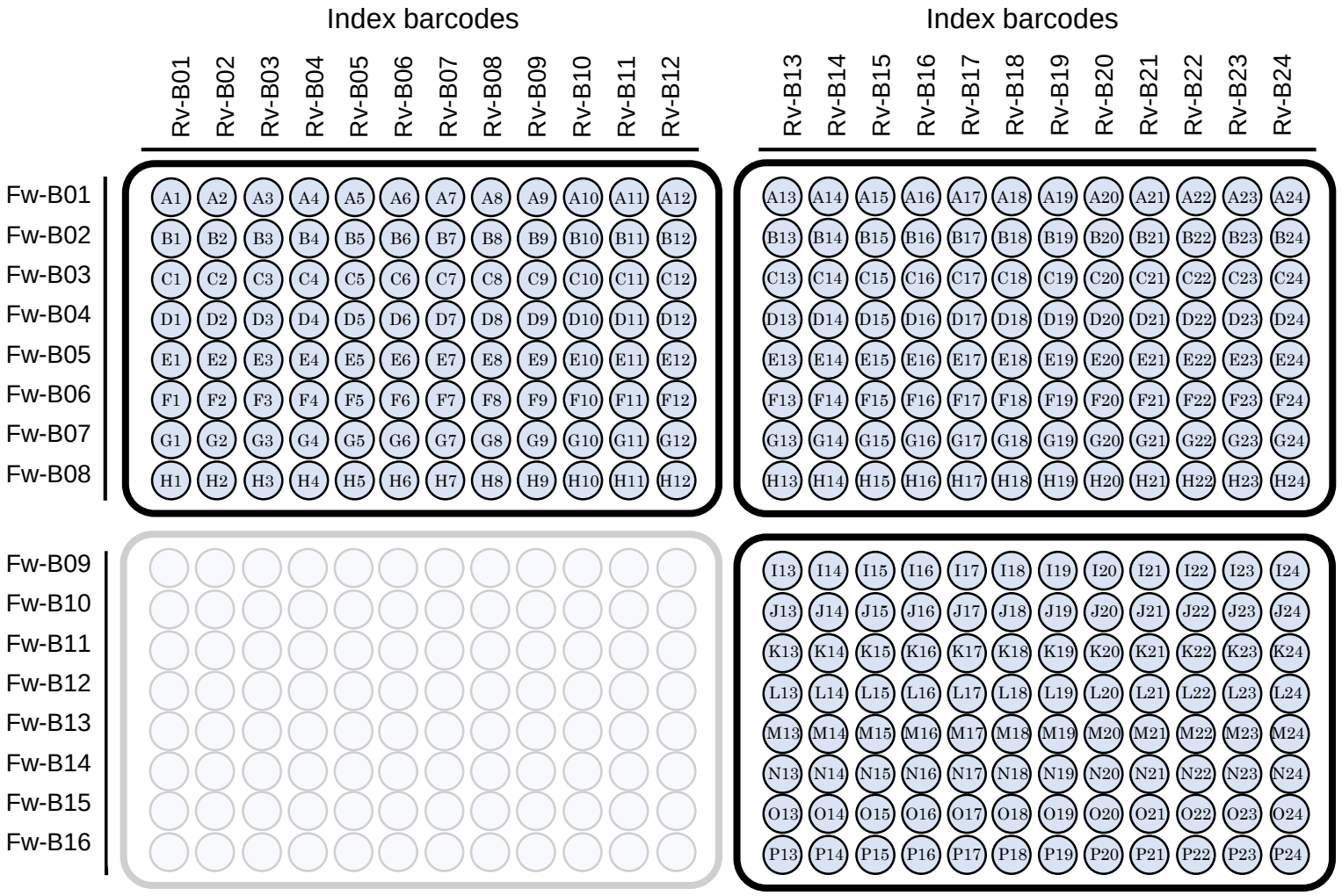
Supplementary Figure 5 Cloning of sgRNA containing target sequence.
a) Methods for cloning of individual target sequences into all-in-one CRISPR/Cas9 vector. Annealed target sequence was ligated to the tRNA-sgRNA junction via Golden Gate reaction. As type IIS restriction enzyme Esp3I site is non-palindromic, re-digestion is not possible. Orange letters show the overhangs for cloning. **b)** Schematic diagram of multi-sgRNA cloning. To clone multiple sgRNAs into all-in-one CRISPR/Cas9 vector, the pTM1709 or pTM1369 were digested with Esp3I. tRNA-sgRNA cassettes containing target sequences were amplified by PCR and the resulting fragments were assembled using a NEBuilder HiFi Assembly.



Supplementary Figure 6 Schematic workflow of the off-target detection method.
1) Target sequences and off-target sites were predicted *in silico*. **2)** All-in-one CRISPR/Cas9 vectors were constructed and transfected into cells to induce specific mutations. **3)** gDNA was extracted from the cells and on-target and predicted off-target sites were amplified by PCR. **4)** The amplified DNA was barcoded and analysed by NGS.



Supplementary Figure 7 Schematic workflow of the amplicon DNA library preparation with NGS index barcodes.
a) Amplicon PCR for off-target assay. In the first PCR, Illumina overhang (black) was added to the tail of the locus-specific primer. An index barcode sequence to distinguish the type of CRISPR vector used and the number of experimental trials was included between the illumine overhang and the locus-specific primer. **b)** Amplicon PCR to analyse sgRNA libraries and mutant pools. **(c)** Dual indexing strategies based on first and second PCR for amplicon sequencing.



Supplementary Figure 8 Gridding of clonal mutants for amplicon sequencing.
Index barcodes in 16 forward and 24 reverse primers were used to distinguish PCR products derived from up to 384 clones. Pooled PCR products were used for addition of Illumina adaptors. Fw; forward primer, Rv; reverse primer.

Supplementary Table 1. GO analysis of the genes under-represented in genome-wide mutant pool.

GO ID	Term	Count	Fold Enrichment	Benjamini *p* value
GO:0005840	ribosome	14	13.03	2.42.E-09
GO:0003735	structural constituent of ribosome	15	11.09	2.73.E-09
GO:0006412	translation	16	8.78	1.85.E-08
GO:0022627	cytosolic small ribosomal subunit	7	23.23	1.90.E-05
GO:0031012	extracellular matrix	12	6.64	4.23.E-05
GO:1902600	hydrogen ion transmembrane transport	5	18.00	9.39.E-03
GO:0046961	proton-transporting ATPase activity, rotational mechanism	4	27.75	1.60.E-02
GO:0045335	phagocytic vesicle	12	3.37	1.66.E-02
GO:0022625	cytosolic large ribosomal subunit	5	10.82	2.14.E-02

Supplementary Table 3. GO analysis of the genes under-represented in growth selection.

# Term ID	Term description	Observed gene count	Background gene count	FDR
GO:0000075	Cell cycle checkpoint	2	31	0.033
GO:0051726	Regulation of cell cycle	3	148	0.023
GO:0048523	Negative regulation of cellular process	4	303	0.008
GO:0048519	Negative regulation of biological process	5	408	0.001
GO:0033554	Cellular response to stress	4	514	0.046
GO:0051716	Cellular response to stimulus	6	1078	0.007
GO:0050789	Regulation of biological process	8	1930	0.001
GO:0050794	Regulation of cellular process	7	1754	0.009

Supplementary Table 4. List of genes for which target gRNA sequences have been detected multiple times from independent mutants.

Gene	Count
yakA	31
pkaC	10
tor	4
dhkG	4
pats1	4
snfA	4
DDB_G0280131	3
dhkA	3
rckA	3
ndrD	3
DDB_G0277541	2
DDB_G0278521	2
DDB_G0279831	2
DDB_G0285517	2
DDB_G0285613	2
DDB_G0292350	2
dhkJ	2
dhkM	2
dnapkcs	2
dokA	2
fnkE	2
gcdh	2
gpaL	2
lvsG	2
mps1	2
mrkC	2
prosc	2
roco11	2
tsuA	2
vps15	2
DDB_G0283065	2
DDB_G0293822	2
mkcB	2

Supplementary Table 5. Recreated CRISPR-mediated mutants and observed phenotype.

No.	Gene	Target gRNA sequence (5′-3′)	Phenotype
1	*yakA*	ATTGTGCATCAATTGATATG	aggregation defect
2	*pkaC*	GCGGTCGATTGGTGGGCACT	aggregation defect
3	*tor*	AATCACGTCTTCGTTTACAA	aggregation defect
4	*dhkG*	TTACATTTAGCATCTTGTAT	delayed growth or development
5	*snfA*	ATTACCACCACATGAGATTA	aggregation defect
6	*dhkA*	AATGAAGTGGCAAGTAATCT	aberrant fruiting body
7	*mps1*	GCAAATTTCGTCTCGGTTCA	
8	*tsuA*	TCTTGGTGATGGTGATGGAC	small fruiting body
9	*mkcB*	CACCATATGCATTGGTGTCG	delayed growth or development
10	*DDB_G0285517*	AGTGAACATGATGATATCAA	
11	*gcdh*	GATGGCAAACGCAGTAGAGA	delayed growth or development
12	*gpaL*	TTATTAGGTAGTGGTGAATG	aggregation defect
13	*DDB_G0293822*	TTATCAGAGATACAATCATT	delayed growth or development
14	*DDB_G0285613*	AAGTTCATCTTTATTTGGTT	
15	*prosc*	CTCAAGCTACAAGAATATAA	
16	*DDB_G0277541*	AGTTACTAATATCACTGAAA	

Supplementary Table 6. List of oligonucleotides used to generate CRISPR/Cas9 all-in-one vectors for off-target assay.

On-target gene	Sequence (5'- to -3') Forward	Sequence (5'- to -3') Reverse
abcA6	agcaGCTTACAAACTGATATCATT	aaacAATGATATCAGTTTGTAAGC
grlJ	agcaAGAAACGTGCAATTACCAAT	aaacATTGGTAATTGCACGTTTCT
omt11	agcaGAAACAGTGGTTGATATTGG	aaacCCAATATCAACCACTGTTTC
DDB_G0285837	agcaTCCTCTTCAATTTGATCCCA	aaacTGGGATCAAATTGAAGAGGA

Supplementary Table 7. CRISPR/Cas9 all-in-one vectors for off-target assay.

Plasmid	On-target gene	Backbone vector	Cas9 type	Drug resistance
pTM1768	*abcA6*	pTM1709	Stable SpCas9	Blasticidin S
pTM1910	*grlJ*	pTM1709	Stable SpCas9	Blasticidin S
pTM1929	*omt11*	pTM1709	Stable SpCas9	Blasticidin S
pTM1950	*DDB_G0285837*	pTM1709	Stable SpCas9	Blasticidin S
pTM1954	*abcA6*	pTM1599	Transient SpCas9	G418
pTM1955	*grlJ*	pTM1599	Transient SpCas9	G418
pTM1956	*omt11*	pTM1599	Transient SpCas9	G418
pTM1957	*DDB_G0285837*	pTM1599	Transient SpCas9	G418
pTM1958	*abcA6*	pTM1668	Transient SpRY	G418
pTM1959	*grlJ*	pTM1668	Transient SpRY	G418
pTM1960	*omt11*	pTM1668	Transient SpRY	G418
pTM1961	*DDB_G0285837*	pTM1668	Transient SpRY	G418

Supplementary Table 8. List of primers used for off-target assay.

Gene	Forward / Reverse	Barcode index	Sequence (5'- to -3')
abcA5	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGTTACCAAC**AATCTCAATGTTAACTGGACT
abcA5	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGCGCAGTAC**AATCTCAATGTTAACTGGACT
abcA5	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCGTCAGTAC**AATCTCAATGTTAACTGGACT
abcA5	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGGTAACGAC**AATCTCAATGTTAACTGGACT
abcA5	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ACTGCGCAAC**AATCTCAATGTTAACTGGACT
abcA5	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ACTGACGGAC**AATCTCAATGTTAACTGGACT
abcA5	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**TCAATCTCATTTGCCATTTTCAA
abcA6	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TAAGTAGAAC**GATTGGTATGTTGACAGGT
abcA6	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGATAGCAC**GATTGGTATGTTGACAGGT
abcA6	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ACGATCGAAC**GATTGGTATGTTGACAGGT
abcA6	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCTACTTAAC**GATTGGTATGTTGACAGGT
abcA6	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GCTATCGAAC**GATTGGTATGTTGACAGGT
abcA6	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGATCGTAC**GATTGGTATGTTGACAGGT
abcA6	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**TGTTGCCATTCTTTCAGCCT
DDB_G0285837	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CAGGACGGCG**TTGCATCTGAACCAAAACC
DDB_G0285837	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATCGATCCG**TTGCATCTGAACCAAAACC
DDB_G0285837	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GAAGAAGTCG**TTGCATCTGAACCAAAACC
DDB_G0285837	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCGTCCTGCG**TTGCATCTGAACCAAAACC
DDB_G0285837	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GTCTGAGCCG**TTGCATCTGAACCAAAACC
DDB_G0285837	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ACTTCTTCCG**TTGCATCTGAACCAAAACC
DDB_G0285837	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**TCATCATCATCCTGTTGTTGT
forE	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATGCACAGA**GCAAAGAAACCACCAGCA
forE	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGCTTGCCGA**GCAAAGAAACCACCAGCA
forE	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GCAATCGAGA**GCAAAGAAACCACCAGCA
forE	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGTGCATCGA**GCAAAGAAACCACCAGCA
forE	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGCAAGCTGA**GCAAAGAAACCACCAGCA
forE	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGATTGCGA**GCAAAGAAACCACCAGCA
forE	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**ACTTGAGTTGGCCCCTTTG
grlF	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGAGCATGCG**ATGGTAGTTGTCGTAGTAGAG
grlF	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CAGATTGCCG**ATGGTAGTTGTCGTAGTAGAG
grlF	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GTCATCAGCG**ATGGTAGTTGTCGTAGTAGAG
grlF	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CATGCTCACG**ATGGTAGTTGTCGTAGTAGAG

grlF	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GCAATCTGCG**ATGGTAGTTGTCGTAGTAGAG
grlF	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TAGCCGTACG**ATGGTAGTTGTCGTAGTAGAG
grlF	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**TCAGCTACAATATCACCCAATGC
grlJ	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCATGCTAT**TTGCACAATCACCAACCAA
grlJ	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCGATAGAT**TTGCACAATCACCAACCAA
grlJ	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TACGATCGAT**TTGCACAATCACCAACCAA
grlJ	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGCATGATAT**TTGCACAATCACCAACCAA
grlJ	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CTATCGATAT**TTGCACAATCACCAACCAA
grlJ	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGATCGTAAT**TTGCACAATCACCAACCAA
grlJ	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**CAAACCTTGCCAAATAGCCAA
omt11	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGCCTTGTG**GTGAAAATCAAGGACCTTCA
omt11	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGATTGCTTG**GTGAAAATCAAGGACCTTCA
omt11	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGATCGATTG**GTGAAAATCAAGGACCTTCA
omt11	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CAAGGCGATG**GTGAAAATCAAGGACCTTCA
omt11	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGCAATCGTG**GTGAAAATCAAGGACCTTCA
omt11	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCGATCGTG**GTGAAAATCAAGGACCTTCA
omt11	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**AGAAATCACCAGCAACATGTTT
omt9	Fw	index1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATTCTAGGTA**CACTGAAGCCGCCATATC
omt9	Fw	index2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATGACTGCTA**CACTGAAGCCGCCATATC
omt9	Fw	index3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGCTAGATA**CACTGAAGCCGCCATATC
omt9	Fw	index4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCTAGAATTA**CACTGAAGCCGCCATATC
omt9	Fw	index5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GCAGTCATTA**CACTGAAGCCGCCATATC
omt9	Fw	index6	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCTAGCGATA**CACTGAAGCCGCCATATC
omt9	Rv	index1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**TGAGTTGGCCAATCATGAAGG

Index barcodes are shown in red letters.

Supplementary Table 9. List of primers for NGS analysis of kinase and genome-wide sgRNA plasmid libraries.

Name	Amplified plasmid	Sequence (5'- to -3')
sgRNAlibraryIndex1_Fw	pTM1376	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCGCCTTGCGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex1_Rv	pTM1376	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTCTAGGAGTTGATAACGGACTAGCCTTA
sgRNAlibraryIndex2_Fw	pTM1810	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATAGCGTCCGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex2_Rv	pTM1810	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGTTACCAAGTTGATAACGGACTAGCCTTA

Index barcodes are shown in red letters.

Supplementary Table 10. List of primers for NGS analysis of kinase and genome-wide mutant pools.

Name	Mutant pool	Sequence (5'- to -3')
sgRNAlibraryIndex3_Fw	Kinase	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCATGCTTA**CGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex4_Fw	Kinase	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATGCACATCT**CGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex5_Fw	Kinase	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGATTGCTCGAC**CGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex3_Rv	Kinase	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GAAGAAGT**AGTTGATAACGGACTAGCCTTA
sgRNAlibraryIndex4_Rv	Kinase	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATGACTGC**AGTTGATAACGGACTAGCCTTA
sgRNAlibraryIndex5_Rv	Kinase	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TGCACAGT**AGTTGATAACGGACTAGCCTTA
sgRNAlibraryIndex6_Fw	Genome-wide	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGATAGCAATTC**CGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex7_Fw	Genome-wide	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCGATAGTTGCTT**CGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex8_Fw	Genome-wide	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATCGATCCAGTTAG**CGATTAGCTCAGTCGGCAGAGCG
sgRNAlibraryIndex6_Rv	Genome-wide	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CGAATTGC**AGTTGATAACGGACTAGCCTTA
sgRNAlibraryIndex7_Rv	Genome-wide	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GAGCACTT**AGTTGATAACGGACTAGCCTTA
sgRNAlibraryIndex8_Rv	Genome-wide	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AGCTTGCC**AGTTGATAACGGACTAGCCTTA

Index barcodes are shown in red letters.

Supplementary Table 11. List of primer sets used for amplicon PCR of 384 samples.

Name	Sequence (5'- to -3')
Fw-B01	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TAAGTAGA**CGATTAGCTCAGTCGGCAGAGCG
Fw-B02	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCATGCTTA**CGATTAGCTCAGTCGGCAGAGCG
Fw-B03	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGCCTTG**CGATTAGCTCAGTCGGCAGAGCG
Fw-B04	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATAGCGTC**CGATTAGCTCAGTCGGCAGAGCG
Fw-B05	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GTCTGATG**CGATTAGCTCAGTCGGCAGAGCG
Fw-B06	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTACGCAC**CGATTAGCTCAGTCGGCAGAGCG
Fw-B07	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTGAATAG**CGATTAGCTCAGTCGGCAGAGCG
Fw-B08	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGCTTCAG**CGATTAGCTCAGTCGGCAGAGCG
Fw-B09	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATGCACATCT**CGATTAGCTCAGTCGGCAGAGCG
Fw-B10	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGATTGCTCGAC**CGATTAGCTCAGTCGGCAGAGCG
Fw-B11	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TCGATAGCAATTC**CGATTAGCTCAGTCGGCAGAGCG
Fw-B12	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCGATAGTTGCTT**CGATTAGCTCAGTCGGCAGAGCG
Fw-B13	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATCGATCCAGTTAG**CGATTAGCTCAGTCGGCAGAGCG
Fw-B14	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGATCGATTTGAGCCT**CGATTAGCTCAGTCGGCAGAGCG
Fw-B15	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ACGATCGATACACGATC**CGATTAGCTCAGTCGGCAGAGCG
Fw-B16	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TACGATCGATGGTCCAGA**CGATTAGCTCAGTCGGCAGAGCG
Rv-B01	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCCTTG**AGTTGATAACGGACTAGCCTTA
Rv-B02	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GAAGAAGT**AGTTGATAACGGACTAGCCTTA
Rv-B03	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATTCTAGG**AGTTGATAACGGACTAGCCTTA
Rv-B04	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CGTTACCA**AGTTGATAACGGACTAGCCTTA
Rv-B05	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAGGACGT**AGTTGATAACGGACTAGCCTTA
Rv-B06	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATGACTGC**AGTTGATAACGGACTAGCCTTA
Rv-B07	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TGCACAGT**AGTTGATAACGGACTAGCCTTA
Rv-B08	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CGAATTGC**AGTTGATAACGGACTAGCCTTA
Rv-B09	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GAGCACTT**AGTTGATAACGGACTAGCCTTA
Rv-B10	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AGCTTGCC**AGTTGATAACGGACTAGCCTTA
Rv-B11	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CATTGCGA**AGTTGATAACGGACTAGCCTTA
Rv-B12	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ACGTACGT**AGTTGATAACGGACTAGCCTTA
Rv-B13	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CGGCTACA**AGTTGATAACGGACTAGCCTTA
Rv-B14	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GTACATCT**AGTTGATAACGGACTAGCCTTA
Rv-B15	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCAGCTGC**AGTTGATAACGGACTAGCCTTA
Rv-B16	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCGTCAGT**AGTTGATAACGGACTAGCCTTA

Rv-B17	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GACTGGAT**AGTTGATAACGGACTAGCCTTA
Rv-B18	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TGTACCAG**AGTTGATAACGGACTAGCCTTA
Rv-B19	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TACGGCTA**AGTTGATAACGGACTAGCCTTA
Rv-B20	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GATCAGTC**AGTTGATAACGGACTAGCCTTA
Rv-B21	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATCGGCAT**AGTTGATAACGGACTAGCCTTA
Rv-B22	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ACTCGGTA**AGTTGATAACGGACTAGCCTTA
Rv-B23	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GTCATCAG**AGTTGATAACGGACTAGCCTTA
Rv-B24	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCGCTAGA**AGTTGATAACGGACTAGCCTTA

Index barcodes are shown in red letters.

Supplementary Data 1.

List of genes and gRNA target sequences used in genome-wide sgRNA library.

Supplementary Data 2.

List of gRNAs designed to increase the number of target genes.

Supplementary Data 3.

List of genes and gRNA target sequences used in kinase sgRNA library.

Supplementary Data 4.

Identified gRNA target sequences from individual developmental defective mutants.

Supplementary Table 2.

List of under- or over-represented genes by CRISPR screens for cell growth.