Table S4 Plasmids generated in this study.

ID	Assay	Features	Selection
			marker
pAS001	In vitro	pUC19-backbone derived plasmids containing	Ampicillin
	plasmids	predicted AGC PAM and a complementary	
	cleavage	protospacer region.	
pAS002	In vitro	pUC19-backbone derived plasmids containing	Ampicillin
	plasmids	predicted CCC PAM and a complementary	
	cleavage	protospacer region.	
pAS003	In vitro	pUC19-backbone derived plasmids containing	Ampicillin
	plasmids	TGCCC PAM and a complementary protospacer	
	cleavage,	region.	
	cleavage sites		
	determination		
pAS004	PAM-	pUC19-backbone derived plasmids containing five	Ampicillin
	depletion	random nucleotides PAM (NNNNN) and a	
		complementary protospacer region.	
pET28a-	Protein	pET28a-backbone derived plasmids containing N-	Kanamycin
His-	purification	terminal hexa-histidine and a SUMO tagged $Cas\pi$ -	
SUMO-		<i>1</i> in MCS (addgene #196029).	
Casπ-1		, j	
pET28a-	Protein	pET28a-backbone derived plasmids containing N-	Kanamycin
His-	purification	terminal hexa-histidine and a SUMO tagged $Cas\pi$ -	-
SUMO-		2 in MCS (addgene #196030).	
Casπ-2			
pBLO62.5-	Cell genome	Plasmid contains Homo sapiens codon optimized	Ampicillin (E.
Casπ1-Dest	editing	$Cas\pi$ -1 driven by CMV promoter. Both N-/ C-	coli)
(p-0380)	_	termini of $Cas\pi$ -1 fused to a SV40 NLS sequences,	Puromycin (H.
		C-terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence. SapI-	
		GG stuffer spacer (addgene #196033).	
pBLO62.5-	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized <i>Casπ</i> -	Ampicillin (E.
Casπ2-Dest	editing	2 driven by CMV promoter. Both N-/ C-termini of	coli)
(p-0381)		Cas $\pi$ -2 fused to a SV40 NLS sequences, C-terminal	Puromycin (H.
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	,
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. SapI-GG stuffer	
		spacer (addgene #196035).	
p-0382	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized	Ampicillin (E.
1		1	• `
	editing	LbCas12a driven by CMV promoter. Both N-/ C-	coli)

		termini of Cas12a fused to a SV40 NLS sequences,	Puromycin (H.
		C-terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	supiens)
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence. SapI-	
0202	G 11	GG stuffer spacer.	A : :11: /E
p-0383	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized	Ampicillin (E.
	editing	SpyCas9 driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas9 fused to a SV40 NLS sequences, C-	Puromycin (H.
		terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence. SapI-	
		GG stuffer spacer.	
p-0384	Cell genome	Plasmid contains $H$ . sapiens codon optimized $Cas\pi$ -	Ampicillin (E.
	editing	<i>1</i> driven by CMV promoter. Both N-/ C-termini of	coli)
		Cas $\pi$ -1 fused to a SV40 NLS sequences, C-terminal	Puromycin (H.
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg1 spacer.	
p-0385	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized <i>Casπ</i> -	Ampicillin (E.
	editing	1 driven by CMV promoter. Both N-/ C-termini of	coli)
		Casπ-1 fused to a SV40 NLS sequences, C-terminal	Puromycin (H.
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg2 spacer.	
p-0386	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized <i>Casπ</i> -	Ampicillin (E.
	editing	<i>I</i> driven by CMV promoter. Both N-/ C-termini of	coli)
		Cas $\pi$ -1 fused to a SV40 NLS sequences, C-terminal	Puromycin ( <i>H</i> .
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg3 spacer.	
	C 11	Plasmid contains <i>H. sapiens</i> codon optimized $Cas\pi$ -	Ampicillin (E.
p-0387	Cell genome	1 lastina contains 11. suprens coach optimizea casa	
p-0387			coli)
p-0387	editing	1 driven by CMV promoter. Both N-/ C-termini of	coli)
p-0387		1 driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal	coli) Puromycin (H.
p-0387		1 driven by CMV promoter. Both N-/ C-termini of	coli)

		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg4 spacer.	
p-0388	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized <i>Cas</i> $\pi$ -	Ampicillin (E.
p 0300	editing	<i>I</i> driven by CMV promoter. Both N-/ C-termini of	coli)
	Cutting	Casπ-1 fused to a SV40 NLS sequences, C-terminal	Puromycin ( <i>H</i> .
		containing a 2×FLAG tag and linked to PuroR via	· ·
			sapiens)
		the P2A peptide sequence. U6-promoter activating	
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg5 spacer.	
p-0389	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized $Cas\pi$ -	Ampicillin (E.
	editing	2 driven by CMV promoter. Both N-/ C-termini of	coli)
		Cas $\pi$ -2 fused to a SV40 NLS sequences, C-terminal	Puromycin (H.
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg1 spacer.	
p-0390	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized <i>Casπ</i> -	Ampicillin (E.
	editing	2 driven by CMV promoter. Both N-/ C-termini of	coli)
		$Cas\pi$ -2 fused to a SV40 NLS sequences, C-terminal	Puromycin (H.
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	,
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg2 spacer.	
p-0391	Cell genome	Plasmid contains $H$ . sapiens codon optimized $Cas\pi$ -	Ampicillin (E.
P 0371	editing	2 driven by CMV promoter. Both N-/ C-termini of	coli)
	cutting	Casπ-2 fused to a SV40 NLS sequences, C-terminal	Puromycin ( <i>H</i> .
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	supiens)
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
0202	G II	region targeting guide #π-sg3 spacer.	A
p-0392	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized <i>Cas</i> $\pi$ -	Ampicillin (E.
	editing	2 driven by CMV promoter. Both N-/ C-termini of	coli)
		Casπ-2 fused to a SV40 NLS sequences, C-terminal	Puromycin (H.
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide $\#\pi$ -sg4 spacer.	
p-0393	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized <i>Casπ</i> -	Ampicillin (E.
	editing	2 driven by CMV promoter. Both N-/ C-termini of	coli)

			D : /
		Casπ-2 fused to a SV40 NLS sequences, C-terminal	Puromycin (H.
		containing a 2×FLAG tag and linked to PuroR via	sapiens)
		the P2A peptide sequence. U6-promoter activating	
		the guide transcription and a repeat-spacer unit	
		terminated by a poly-T sequence. MYH8 exon	
		region targeting guide # $\pi$ -sg5 spacer.	
p-0394	Cell genome	Plasmid contains H. sapiens codon optimized	Ampicillin (E.
	editing	LbCas12a driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas12a fused to a SV40 NLS sequences,	Puromycin (H.
		C-terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #12a-sg1	
		spacer.	
p-0395	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized	Ampicillin (E.
p 0373	editing	LbCas12a driven by CMV promoter. Both N-/ C-	coli)
	Carting	termini of Cas12a fused to a SV40 NLS sequences,	Puromycin ( <i>H</i> .
		C-terminal containing a 2×FLAG tag and linked to	,
			sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #12a-sg2	
		spacer.	
p-0396	Cell genome	Plasmid contains H. sapiens codon optimized	Ampicillin (E.
	editing	LbCas12a driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas12a fused to a SV40 NLS sequences,	Puromycin (H.
		C-terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #12a-sg3	
		spacer.	
p-0397	Cell genome	Plasmid contains H. sapiens codon optimized	Ampicillin (E.
	editing	LbCas12a driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas12a fused to a SV40 NLS sequences,	Puromycin ( <i>H</i> .
		C-terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #12a-sg4	
n 0209	Call garage	spacer.	Amminillia (E
p-0398	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized	Ampicillin (E.
	editing	LbCas12a driven by CMV promoter. Both N-/ C-	coli)

		termini of Cas12a fused to a SV40 NLS sequences,	Puromycin ( <i>H</i> .
		C-terminal containing a 2×FLAG tag and linked to	sapiens)
			sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #12a-sg5	
	2.44	spacer.	
p-0399	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized	Ampicillin (E.
	editing	SpyCas9 driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas9 fused to a SV40 NLS sequences, C-	Puromycin (H.
		terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #Cas9-sg1	
		spacer.	
p-0400	Cell genome	Plasmid contains H. sapiens codon optimized	Ampicillin (E.
	editing	SpyCas9 driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas9 fused to a SV40 NLS sequences, C-	Puromycin (H.
		terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #Cas9-sg2	
		spacer.	
p-0401	Cell genome	Plasmid contains H. sapiens codon optimized	Ampicillin (E.
	editing	SpyCas9 driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas9 fused to a SV40 NLS sequences, C-	Puromycin (H.
		terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #Cas9-sg3	
		spacer.	
p-0402	Cell genome	Plasmid contains H. sapiens codon optimized	Ampicillin (E.
	editing	SpyCas9 driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas9 fused to a SV40 NLS sequences, C-	Puromycin (H.
		terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #Cas9-sg4	
		spacer.	
		<del>'</del>	

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p-0403	Cell genome	Plasmid contains <i>H. sapiens</i> codon optimized	Ampicillin (E.
	editing	SpyCas9 driven by CMV promoter. Both N-/ C-	coli)
		termini of Cas9 fused to a SV40 NLS sequences, C-	Puromycin ( <i>H</i> .
		terminal containing a 2×FLAG tag and linked to	sapiens)
		PuroR via the P2A peptide sequence. U6-promoter	
		activating the guide transcription and a repeat-	
		spacer unit terminated by a poly-T sequence.	
		MYH8 exon region targeting guide #Cas9-sg5	
		spacer.	
p-0404	Cell genome	Lentiviral expression MCPyV-backbone plasmid	Ampicillin (E.
	editing	containing the MYH8 exon (270 bp) from M.	coli)
		musculus with 32 bp random flanking sequence	Puromycin (H.
		linked to the start codon of <i>EGFP</i> under the control	sapiens)
		of CMV promoter.	
pCDFDuet-	Bacteria	Plasmid contains $Cas\pi-1$ under the control of Trc	Streptomycin
Casπ1-Dest	plasmid	promoter. Casπ-1 is N-terminally linked to a 6×His	- •
(p-0405)	interference	tag. Locus for guide expression is controlled by	
<b>u</b> /	assay	J23119-promoter and constitutes a repeat-spacer	
		unit terminated by a T7 terminator. SapI-GG stuffer	
		spacer (addgene #196031).	
p-0406	Bacteria	Plasmid contains $Cas\pi-1$ under the control of Trc	Streptomycin
P * * * *	plasmid	promoter. Cas $\pi$ -1 is N-terminally linked to a 6×His	~ · · · · · · · · · · · · · · · · ·
	interference	tag. Locus for guide expression is controlled by	
	assay	J23119-promoter and constitutes a repeat-spacer	
	ussuy	unit terminated by a T7 terminator. The ccdB gene	
		targeting guide #ccdB-T spacer.	
pCDFDuet-	Bacteria	Plasmid contains $Cas\pi$ -2 under the control of Trc	Streptomycin
Casπ2-Dest	plasmid	promoter. Cas $\pi$ -1 is N-terminally linked to a 6×His	Sucptomyem
(p-0407)	interference	tag. Locus for guide expression is controlled by	
(p-0407)		J23119-promoter and constitutes a repeat-spacer	
	assay		
		unit terminated by a T7 terminator. SapI-GG stuffer	
0400	Destanta	spacer (addgene #196032).	Ctti.
p-0408	Bacteria	Plasmid contains <i>Casπ-2</i> under the control of Tro	Streptomycin
	plasmid	promoter. Casπ-1 is N-terminally linked to a 6×His	
	interference	tag. Locus for guide expression is controlled by	
	assay	J23119-promoter and constitutes a repeat-spacer	
		unit terminated by a T7 terminator. The ccdB gene	
11.7.77	D	targeting guide #ccdB-T spacer.	
p11-LacY-	Bacteria	Inducible pBAD promoter activating toxin ccdB	Ampicillin
wtx1	plasmid	expression (addgene #69056). Kind gift from Prof.	
	interference	Wei Li group in the institute of zoology, Chinese	
	assay	Academy of Sciences.	
p-0409	Cell genome	Plasmid contains $H$ . sapiens codon optimized $Cas\pi$ -	Ampicillin (E.
	editing	1 driven by CMV promoter. Both N-/ C-termini of	coli)

	terminated by a poly-T sequence. MYH8 exon	
	region targeting guide $\#\pi$ -TP53-1 spacer.	