Table S4 Plasmids generated in this study.

ID	Assay	Features	Selection marker
pAS001	*In vitro* plasmids cleavage	pUC19-backbone derived plasmids containing predicted AGC PAM and a complementary protospacer region.	Ampicillin
pAS002	*In vitro* plasmids cleavage	pUC19-backbone derived plasmids containing predicted CCC PAM and a complementary protospacer region.	Ampicillin
pAS003	*In vitro* plasmids cleavage, cleavage sites determination	pUC19-backbone derived plasmids containing TGCCC PAM and a complementary protospacer region.	Ampicillin
pAS004	PAM-depletion	pUC19-backbone derived plasmids containing five random nucleotides PAM (NNNNN) and a complementary protospacer region.	Ampicillin
pET28a-His-SUMO-Casπ-1	Protein purification	pET28a-backbone derived plasmids containing N-terminal hexa-histidine and a SUMO tagged *Casπ-1* in MCS (addgene #196029).	Kanamycin
pET28a-His-SUMO-Casπ-2	Protein purification	pET28a-backbone derived plasmids containing N-terminal hexa-histidine and a SUMO tagged *Casπ-2* in MCS (addgene #196030).	Kanamycin
pBLO62.5-Casπ1-Dest (p-0380)	Cell genome editing	Plasmid contains *Homo sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to PuroR via the P2A peptide sequence. U6-promoter activating the guide transcription and a repeat-spacer unit terminated by a poly-T sequence. *Sap*I-GG stuffer spacer (addgene #196033).	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
pBLO62.5-Casπ2-Dest (p-0381)	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to PuroR via the P2A peptide sequence. U6-promoter activating the guide transcription and a repeat-spacer unit terminated by a poly-T sequence. *Sap*I-GG stuffer spacer (addgene #196035).	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0382	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Lb*Cas12a* driven by CMV promoter. Both N-/ C-	Ampicillin (*E. coli*)

		termini of Cas12a fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to PuroR via the P2A peptide sequence. U6-promoter activating the guide transcription and a repeat-spacer unit terminated by a poly-T sequence. *Sap*I-GG stuffer spacer.	Puromycin (*H. sapiens*)
p-0383	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Spy*Cas9* driven by CMV promoter. Both N-/ C-termini of Cas9 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to PuroR via the P2A peptide sequence. U6-promoter activating the guide transcription and a repeat-spacer unit terminated by a poly-T sequence. *Sap*I-GG stuffer spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0384	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg1 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0385	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg2 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0386	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg3 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0387	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to PuroR via the P2A peptide sequence. U6-promoter activating the guide transcription and a repeat-spacer unit	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)

		terminated by a poly-T sequence. MYH8 exon region targeting guide #π-sg4 spacer.	
p-0388	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg5 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0389	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg1 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0390	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg2 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0391	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg3 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0392	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg4 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0393	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of	Ampicillin (*E. coli*)

		Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-sg5 spacer.	Puromycin (*H. sapiens*)
p-0394	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Lb*Cas12a* driven by CMV promoter. Both N-/ C-termini of Cas12a fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0395	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Lb*Cas12a* driven by CMV promoter. Both N-/ C-termini of Cas12a fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0396	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Lb*Cas12a* driven by CMV promoter. Both N-/ C-termini of Cas12a fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0397	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Lb*Cas12a* driven by CMV promoter. Both N-/ C-termini of Cas12a fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0398	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Lb*Cas12a* driven by CMV promoter. Both N-/ C-	Ampicillin (*E. coli*)

		termini of Cas12a fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 spacer.	Puromycin (*H. sapiens*)
p-0399	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Spy*Cas9* driven by CMV promoter. Both N-/ C-termini of Cas9 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0400	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Spy*Cas9* driven by CMV promoter. Both N-/ C-termini of Cas9 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0401	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Spy*Cas9* driven by CMV promoter. Both N-/ C-termini of Cas9 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0402	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Spy*Cas9* driven by CMV promoter. Both N-/ C-termini of Cas9 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)

p-0403	Cell genome editing	Plasmid contains *H. sapiens* codon optimized Spy*Cas9* driven by CMV promoter. Both N-/ C-termini of Cas9 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0404	Cell genome editing	Lentiviral expression MCPyV-backbone plasmid containing the *MYH8* exon (270 bp) from *M. musculus* with 32 bp random flanking sequence linked to the start codon of *EGFP* under the control of CMV promoter.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
pCDFDuet-Casπ1-Dest (p-0405)	Bacteria plasmid interference assay	Plasmid contains *Casπ-1* under the control of Trc promoter. Casπ-1 is N-terminally linked to a 6×His tag. Locus for guide expression is controlled by J23119-promoter and constitutes a repeat-spacer unit terminated by a T7 terminator. *Sap*I-GG stuffer spacer (addgene #196031).	Streptomycin
p-0406	Bacteria plasmid interference assay	Plasmid contains *Casπ-1* under the control of Trc promoter. Casπ-1 is N-terminally linked to a 6×His tag. Locus for guide expression is controlled by J23119-promoter and constitutes a repeat-spacer unit terminated by a T7 terminator. The ccdB gene targeting guide #ccdB-T spacer.	Streptomycin
pCDFDuet-Casπ2-Dest (p-0407)	Bacteria plasmid interference assay	Plasmid contains *Casπ-2* under the control of Trc promoter. Casπ-1 is N-terminally linked to a 6×His tag. Locus for guide expression is controlled by J23119-promoter and constitutes a repeat-spacer unit terminated by a T7 terminator. *Sap*I-GG stuffer spacer (addgene #196032).	Streptomycin
p-0408	Bacteria plasmid interference assay	Plasmid contains *Casπ-2* under the control of Trc promoter. Casπ-1 is N-terminally linked to a 6×His tag. Locus for guide expression is controlled by J23119-promoter and constitutes a repeat-spacer unit terminated by a T7 terminator. The ccdB gene targeting guide #ccdB-T spacer.	Streptomycin
p11-LacY-wtx1	Bacteria plasmid interference assay	Inducible pBAD promoter activating toxin ccdB expression (addgene #69056). Kind gift from Prof. Wei Li group in the institute of zoology, Chinese Academy of Sciences.	Ampicillin
p-0409	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of	Ampicillin (*E. coli*)

		Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-*B2M*-1 spacer.	Puromycin (*H. sapiens*)
p-0410	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-*B2M*-2 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0411	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-1 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-*TP53*-1spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0412	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-*B2M*-1 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0413	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-2* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to 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 #π-*B2M*-2 spacer.	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)
p-0414	Cell genome editing	Plasmid contains *H. sapiens* codon optimized *Casπ-1* driven by CMV promoter. Both N-/ C-termini of Casπ-2 fused to a SV40 NLS sequences, C-terminal containing a 2×FLAG tag and linked to PuroR via the P2A peptide sequence. U6-promoter activating the guide transcription and a repeat-spacer unit	Ampicillin (*E. coli*) Puromycin (*H. sapiens*)

		terminated by a poly-T sequence. MYH8 exon region targeting guide #π-*TP53*-1 spacer.	