Table S4 Plasmids used in this study.

Strains	Relevant characteristic(s)	Source and or reference
pJV53-Cas12a	Codon optimized Cas12a under control of the P*myc1tetO* promoter inserted in pJV53 (*oriM*; *oriE*; Km^r; Che9c; Cas12a)	reference (1)
pCR-Hyg	Shuttle vector containing crRNA cassette under contro of *hsp60* promoter (pBR322 ori; pAL5000ts; Hyg^r)	reference (1)
pCR-Zeo	Shuttle vector containing crRNA cassette under control of *hsp60* promoter (pBR322 ori; pAL5000ts; Zeo^r)	reference (1)
pBp4	Shuttle vector containing a replicon of mycobacterial self-replicative plasmid pMF1 (pBR322 ori; pMF1 replicon; Km^r)	reference (2)
pNHEJ	*Mycobacterium smegmatis ku* (*MSMEG_5580*) and *ligaseD* (*MSMEG_5570*) gene under control of arabinose promoter from pKD46 (*oriE*; Cm^r; *ku*;*ligD*)	reference (3)
pZCas9	A plasmid carrying *Cas9* gene (p15A ori) (Kan^r) (p15A ori; Km^r; *Cas9*;)	reference (3)
psgRNA-lacZ	A plasmid with a sgRNA (*lacZ* spacer) targetting *lacZ* gene (pBR322-ori; Cm^r)	reference (3)
pLJR965	CRISPRi integrating plasmid expressing $dCas9_{sth1}$ and the cognate sgRNA (L5; *oriE*; Km^r; $dCas9_{sth1}$;)	reference (4)
pSL003	Shuttle vector expressing site-specific recombinase. (oriM; oriE; Zeo^r; Cre)	reference (5)
pMV261-Cas12a	Optimized Cas12a under control of the P_{myc1}*tetO* promoter inserted in pMV261 (*oriM*; *oriE*; Kn^r; Cas12a)	reference (1)
pYC1103	*whiB6* crRNA inserted in pCR-Hyg digested with *Bpm*I and *Hin*dIII (pBR322 ori; pAL5000ts;gfp crRNA cassette; Hyg^r)	This study
pYC1178	*recD* crRNA inserted in pCR-Hyg digested with *Bpm*I and *Hin*dIII. (pBR322 ori; pAL5000ts;gfp crRNA cassette; Hyg^r)	This study
pYC1521	*nrgA* crRNA inserted in pCR-Hyg digested with *Bpm*I and *Hin*dIII (pBR322 ori; pAL5000ts;gfp crRNA cassette; Hyg^r)	This study
pNHEJ-Cas12a	NHEJ machinery (Ku-NrgA-LigD) of *M. marinum* inserted into KpnI and NheI site of pJV53-Cas12a (*oriE*; *oriM*; Km^r; MmNHEJ; Cas12a)	This study
pNHEJ-Cas12a-*recX*	The *recX* from the *M. smegmatis* under control of P*myc* inserted into *Hin*d III and Sal I site of pNHEJ-Cas12a (*oriE*; *oriM*; Km^r; MmNHEJ; *recX*)	This study
pNHEJ-Cas12a-$recA_{mu}$	The *recA R60C* from the *M. smegmatis* under control of native promoter inserted into *Hin*d III and *Sal* I site of pNHEJ-Cas12a	This study

	(*oriE*; *oriM*; Km^r; MmNHEJ; *recA$_{mu}$*)	
pYC1376	NHEJ machinery without NrgA of *M. marinum* inserted into KpnI and NheI site of pNHEJ-Cas12a-*recX* (*oriE*; *oriM*; Km^r; *ku*; *ligD*; *recX*)	This study
pYC1655	The sgRNA under the control of an optimized TetR-regulated promoter inserted into pSL003 (pBR322 ori; pAL5000ts; Zeo^r)	This study
pYC1286	dCas9$_{sth1}$ reverted to nuclease-active Cas9 in pLJR965 (L5; *oriE*; Km^r; Cas9$_{sth1}$;)	This study
pNHEJ	pNHEJ-Cas12a digested with SpeI and religated to delete Cas12a (*oriE*; *oriM*; km^r; MmNHEJ;)	This study
pNHEJ-Cas9$_{sth1}$	Cas9$_{sth1}$ under control of the P$_{myc1}$*tetO* promoter replace Cas12a (*oriE*; *oriM*; Km^r; MmNHEJ; Cas9$_{sth1}$)	This study
pNHEJ-*recX*	The *recX* from the *M. tuberculosis* under control of P*myc* inserted into KpnI site of pNHEJ (*oriE*; *oriM*; Km^r; MmNHEJ; *recX*)	This study
pNHEJ-*recA$_{mu}$*	The *recA R61C* from the *M. tuberculosis* under control of native promoter inserted into *Kpn* I site of pNHEJ (*oriE*; *oriM*; Km^r; MmNHEJ; *recA$_{mu}$*)	This study
pYC1654	pYC1376 digested with SpeI and religated to delete Cas12a (*oriE*; *oriM*; Km^r; *ku*; *ligD*; *recX*)	This study
pYC1446	Integrating plasmid expressing Cas9$_{sth1}$ and the cognate sgRNA (L5; *oriE*; Cas9$_{sth1}$; Zeo^r)	This study
pNHEJ-*recX-sacB*	*sacB* cloned into pNHEJ -recX$_{mtb}$ (*oriE*; *oriM*; Km^r; MmNHEJ; *recX*; *sacB*)	This study
pNHEJ- *recA$_{mu}$-sacB*	*sacB* cloned into pNHEJ -recA$_{mu}$ (*oriE*; *oriM*; Km^r; MmNHEJ; *recX*; *sacB*)	This study
pYC1640	Shuttle vector containing cas9 $_{sth1}$,sgRNA cassette and the pMF1 replicon. (*oriE*; pMF1 replicon; Zeo^r)	This study
pYC2085	Shuttle vector containing cas9 $_{sth1}$, sgRNA cassette and the pMF1 replicon. (*oriE*; pMF1 replicon; Hyg^r)	This study
pZCas9-*recX*	The *recX* from the *E.coli* under control of native promoter inserted into pZCas9 (p15A ori; Km^r; *Cas9*; *recX*)	This study

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