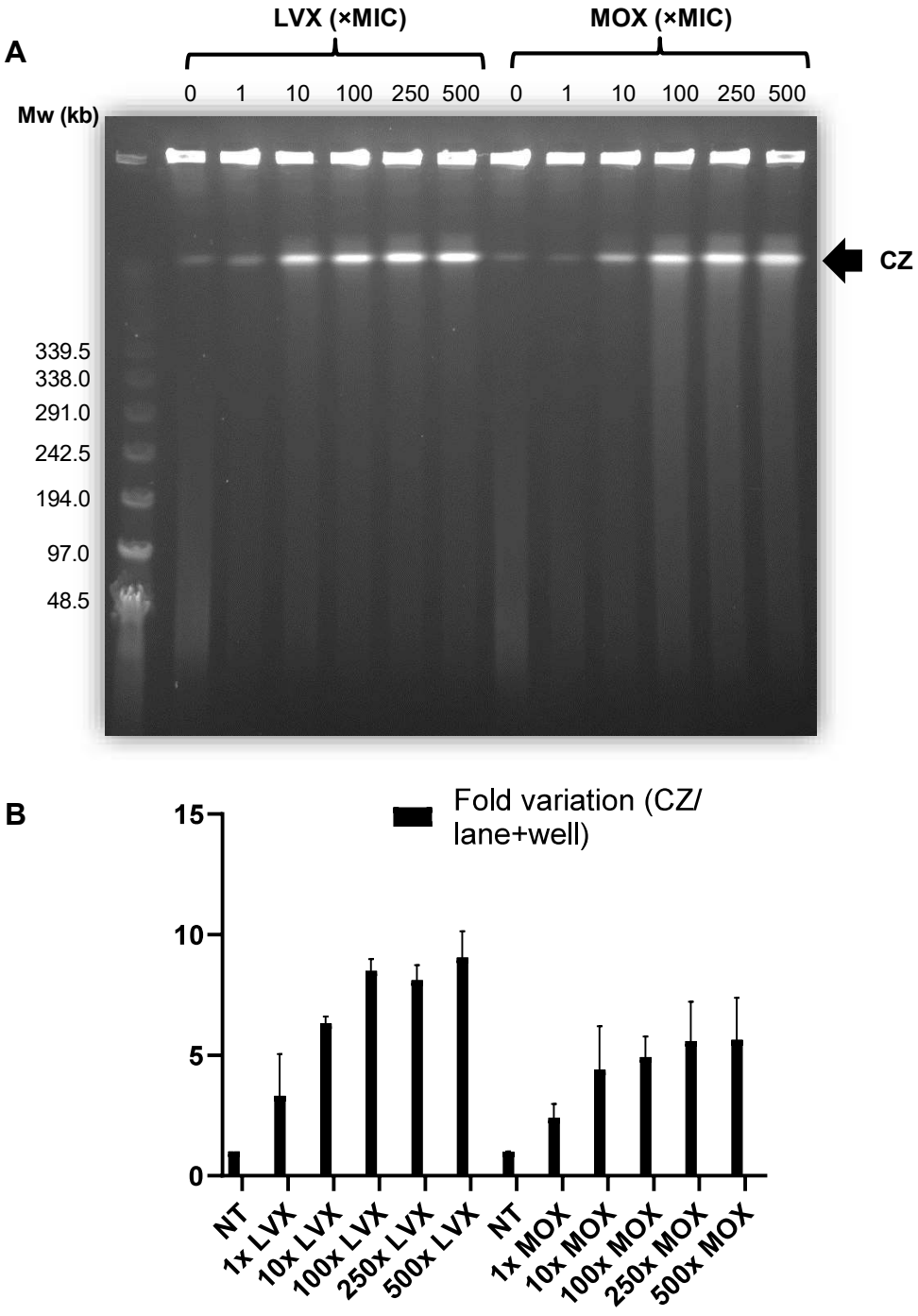
Supplementary Table 1. Oligonucleotides used in this work

Primer name	Sequence (5′→ 3′)	Nucleotide positions[b]
CATDOWNSALTER	ctctcgtcgacGATATGGATCTGGAGCTGTAA	Complementary to 735 to 755 of *cat*
GYRAPROM2	GAGGTGCACCTGCAGTGATC	–316 to –294 of *gyrA*
GYRA3FLAGXBA	gcgcgtctagatcacttgtcgtcgtcgtccttgtagtcgatgtcgtggtccttgtagtcaccgtcgtggtccttgtagtcTGCTTCACCTTCTGTTTCG	Complementary to 2448 to 2466 of *gyrA*
UPTETXBA	cgcgcgaattcGACCGATGATGAAGAAAAGAAT	–
SRTA184SAL	gcgcggtcgacCGTGTGACACCAGATCGTGTTG	550 to 571 of *srtA*
SPR1095R1	GGGCAGCTGTTGTAGGATTGG	35 to 55 of *spr1095*
GYRAPROM1	ATGAACCTACAGCACCATCA	–254 to –234 of *gyrA*
SPR1095R2	CCATGCCCACCGATAAACTTAG	455 to 476 of *spr1095*
GYRA44	CCGTCGCATTCTCTACGGAATGAATGAATT	129 to 158 of *gyrA*
GYRA8	GGTGCTCTTGTCATGGGGAAAT	652 to 673 of *gyrA*
GYRA430	GACAGGTTTGGAACGCGA	1287 to 1304 of *gyrA*
CAT9	CTCTTTTCTCTTCCAATTGTC	25 to 45 of *cat*
ERMECO	cgcgcgaattcTGGCGGAAACGTAAAAGAA	–146 to –127 of *ermB*
ERMHIN	gcgcgaagcttAGCTCCTTGGAAGCTGTCAGT	
GALTF1	GGCATCTATCATCCCCACAAGGA	1126 to 1148 of *galT*
SPR1666R1ECO	cgcgcgaattcCCCACTCACCTTTCTTTATATAC	Complementary to 419 to 441 of *spr1666*
PBUXF1HIN	gcgcgaagcttGGCTCGCACCTCTTTTTCTTA	1261 to 1281 of pbuX
PBUXR1	GCATCTGGGATTTACGTGGTTC	Complementary to 310 to 331 of *pbuX*
GALTF2	GTGGGGGGCTCTATTCTAACTCA	766 to 788 of *galT*
PBUXR2	CCCATCATGATTGCGACAGCCC	Complementary to 85 to 106 of *pbuX*
KANRSAC	cgcgcgagctcGGGCCCGTTTGATTTTTAATGG	–
SPR1863F	CCACTCCCAACCGGACCAGCA	389 to 410 of *spr1863*
DPNUPSAC	gcgcggagctcCCTTTACCATGTTTGTCACAGGCC	–215 to –192 of *dpn* cassette
DPNR6DOWNSAL	gcgcggtcgacCTACTCGCTATTGTCCTCAATGCCG	Complementary to 1216 to 1240 of *pbuX*
DPN23FDOWNSAL	gcgcggtcgacCTACTCACTATTGTCCTCAATGCCG	Complementary to 1216 to 1240 to *SPN23F_18630*

DPNHBDOWNSAL	gcgcggtcgacCTACTCGCCATTGTCCTCAATGCCG	Complementary to 413 to 439 of *orfR*
TERSAL	cgcgcgtcgacTATAAGAAAAAATGA	–
SPR1866R	GTAGACCTAGACGATAACCGC	Complementary to 591 to 612 of *spr1866*
SPR1863F2	GATTAATCTCTGGTAGCAGACT	20 to 41 of *spr1863*
SPR1866R2	TGGATTTTCGGTCACTTGATTG	Complementary to 328 to 349 of *spr1866*
pLS1F	GTGCCGAGTGCCAAAATCAA	
pLS1R	TTTCAAGTACCGATTCACTTAATG	

[a] Lower case indicates bases added to the annealing sequence. Underlined sequences correspond to restriction targets. The double underlined sequence corresponds to 3×Flag nucleotide sequence. [b] Nucleotide numbers refer to the genes of the pneumococcal strains R6, HB264 (DPNHBDOWNSAL) or ATCC70669 (DPN23FDOWNSAL) sequences, except *cat*, *ermB*, and *kan*. The first nucleotide of the gene is considered nt 1.



Supplementary Figure 1. Cleavage by gyrase is saturated at high concentrations of LVX or MOX. (**A**) Cleavage in the presence of the indicated drug concentrations. PFGE gel of R6GA3F strain.Cultures grown to OD_{620nm} = 0.4 were treated for 15 min with or without the indicated drug concentrations. (**B**) The Y axis represents the signal of the compression zone (CZ) divided by the combined signal of the lane plus well, calculated relative to that of untreated cultures. The results are presented as the mean ± SD of three independent replicates.