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Supplemental Material to:

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Cas3 stimulates runaway replication of a ColE1 plasmid in Escherichia coli and antagonises RNaseHI

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Table S1 Primers used in this study.

Primer	Sequence (all 5' to 3')	
ygcB-1B	TGCCATTACTGGGGAAAATCCTCAA	Deletion of *cas3* (*ygcB*).
	AAAGCTCATGTGCAGCTCCATCAGCA	Sequence for aprR is underlined.
ygcB-2B	TCTGGTCATCCCTTCATCCCCTGTATAG	Deletion of *cas3* (Δ*ygcB*).
	GTCCGCCCAGATACAGAAAAGCC	
ygcB-3	GCAGCATCGAAAAATAGC	Verification PCR of Δ*ygcB*
ygcB-4	CGTTTTATTATTAAAGAA	Verification PCR of Δ*ygcB*
ygcB-bad2	CCGGGTACCATAGAACCTTTTAAATAT	Cloning *cas3* into pUC19 *via* KpnI (underlined).
ygcB-Y	GGAATTCTTATTTGGGATTTGCAGG	Cloning *cas3* into pUC19 *via* EcoRI (underlined).
Cascade-F	GCATATGAAGG*TCTAGA*AATGAATTT	Cloning (*casA-casE)* into pUC19.
cascade-R	CATTTTAT*GGTACC*TCAATCACAGTGG	
casC-F	GTAAGGAAA*TCTAGA*TATGTCTAAC	Cloning *casC* into pUC19 *via* XbaI and KpnI.
casC-R	CAAATAA*GGTACC*ATGTTCACGCC	

Table S2. Plasmid yields in *E. coli* MG1655 Δ*pcnB*.

strain (genotype)[a)]	Plasmid	Plasmid concentration (ng/µL)[b)]	OD_{600}	Viability cells per ml ($x10^7$)	Amp^r cells (% total)[c)]

[a] Δ*pcnB* was generated by P1 transduction into *E. coli* K12 MG1655.

[b] Plasmid concentrations were measured in a Nanovue spectrophotometer (GE Healthcare). Values are means of three independent experiments with standard deviations given in parentheses.

[c] The percentage cells retaining plasmid was measured by comparing viable colony counts after plating on agar with, or without ampicillin. [c)] Viable cell number was from counting colonies after plating 100 µl of serially diluted bacteria on LB agar plates from at least two independent experiments.

	pUC19	53 (± 0.71)	1,67(± 0.02)	40(±1)	100.0
IIB955 (Δ*pcnB*)	pCas3^{+}	228.7 (± 51.17)	1.42(± 0.17)	118(±21)	25.1
	pCas3^{K320L}	65.25 (± 3.18)	1.67(± 0.02)	97.5(± 3)	100.0
	pCas3^{K78L}	165 (± 12.73)	0.99(± 0.19)	12.5(±3)	100.0