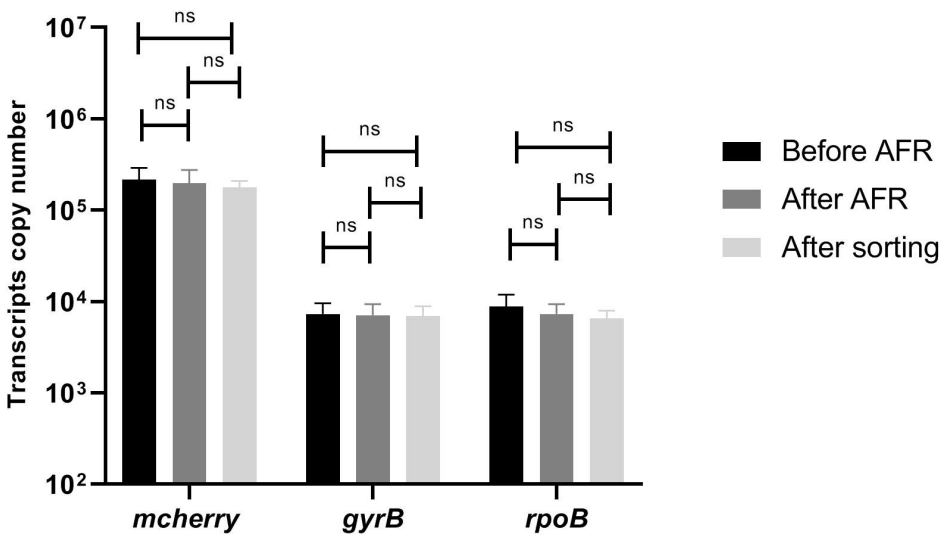

Fig. S1: Stability of RNA

Transcript numbers of *mcherry*, *gyrB* and *rpoB* genes were quantified by RT-qPCR in the initial population (re-suspended 24h biofilm) of MG F'/ pPsfiA*cherry*-PintI1*intI1* strain grown in microfermentor, before AFR (black), after AFR (dark grey) and after sorting (light grey). Data are the average of transcripts levels measured from 6 independent sorting experiments. Errors bars indicate the SD. ns: non-significant (Wilcoxon test). AFR: aerobic fluorescence recovery.

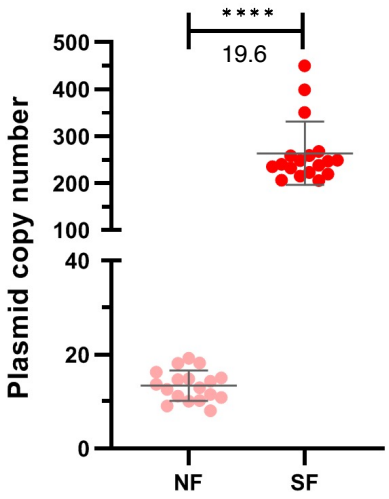

Fig. S2: Plasmid copy number in mutant MGΔ*relA*Δ*spoT*

The plasmid copy number was estimated by calculating the ratio of gene copies number of plasmidic *mcherry* over chromosomal *gyrB* for each sorted sub-populations from a 24h-old biofilm of MGΔ*relA*Δ*spoT* F'/ pPsfiA*cherry*-PintI1*intI1* strain grown in microfermentor.
NF (non fluorescent; light pink), and SF (super-fluorescent; red) sub-populations. Data are the average of 6 independent sorting experiments. Asterisks indicate significant difference: **** $p<0.0001$ (Wilcoxon test). Average and standard deviation are shown as black lines.

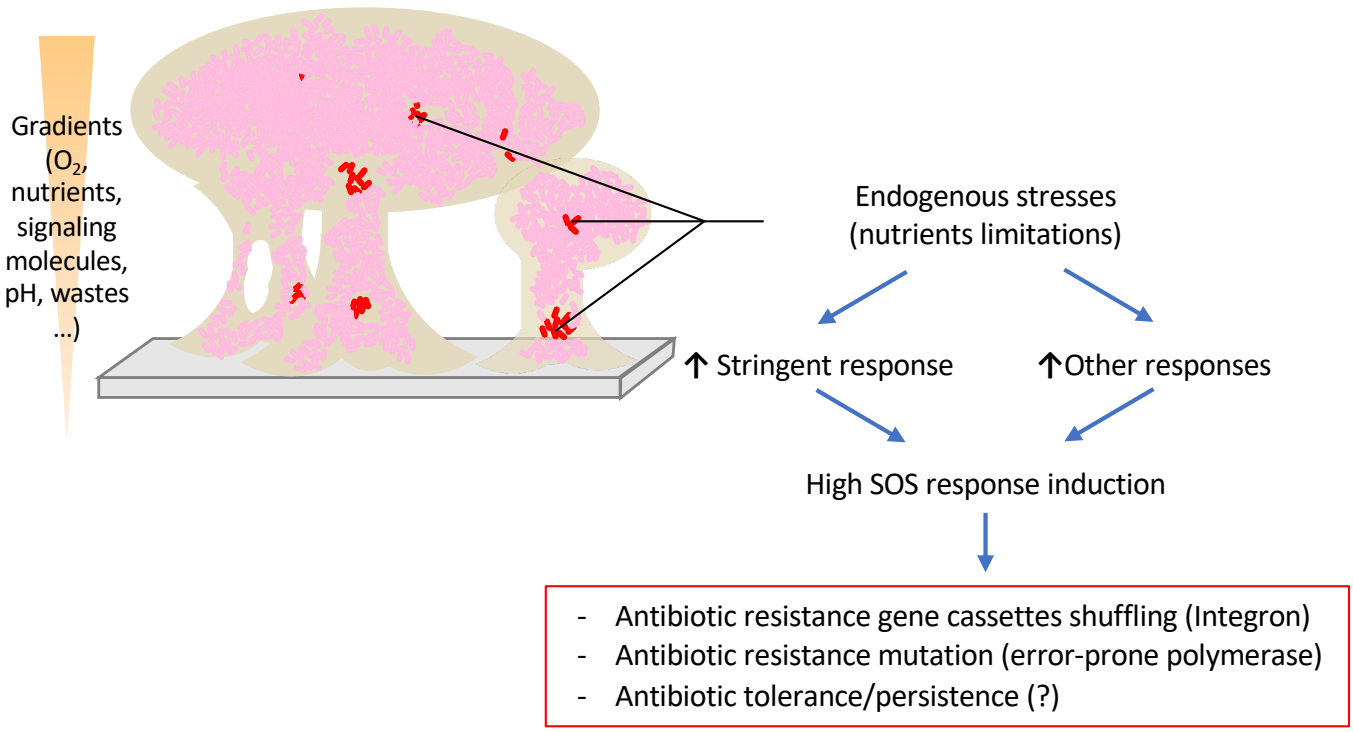

Fig. S3: Heterogeneous expression of *sfiA* and *intI1* in the biofilm

In the biofilm, the vast majority of bacteria (non-fluorescent and intermediate populations, 99% of the bacteria, pink bacteria) express the *sfiA* and *intI1* genes at a low basal level. Only few bacteria in the biofilm (super-fluorescent population, 1%, red bacteria) experience a level of endogenous stress (various nutrients limitations) high enough to induce the SOS response in a stringent response-dependent and stringent response-independent manner. This induction thus allows for high-level expression of genes regulated by the SOS response, leading to various outcomes favoring the acquisition/expression of antibiotic resistance, such as the acquisition/shuffling of resistance gene cassettes via the integron integrase, antibiotic resistance mutation via the error-prone polymerases, and potentially also antibiotic tolerance/persistence.

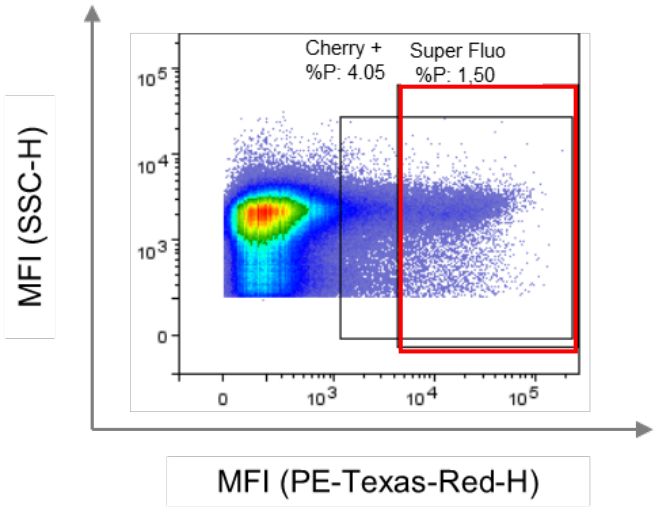

Fig. S4: Quantification of cells expressing *sfiA* in biofilm grown from the sorted super-fluorescent population

Sorted super-fluorescent population of strain MG F'/pPsfiA*cherry*-PintI1*intI1* was used to grow a biofilm in continuous culture in microfermentor for 24h at 37°C. The biofilm was analyzed by flow cytometry. Counting was performed after one hour of aerobic fluorescence recovery at room temperature. The dot-plot analyses is shown. The black square delimits the cherry-positive population (cherry +) based on the non -fluorescent strain MG F'/pSUΔtot and the red rectangle defines the *mcherry*-super-fluorescent population (super Fluo) based on derepressed *mcherry* expression in strain MG F'/ pPsfiA**cherry*-PintI1*intI1*. The data represent one representative experimental replicate.

Table S1: Primers and Probes used in this study

Construction	Primer name	Primer sequence	Reference
MG1656λatt::*cherry*			
	λ*att*.A1.500-5	CGATGGCGATAATATTTCACC	[1]
	λ*att*.B1.500-3	CCCTGATACTCACCAGGCATC	[1]
	λatt.A2.Amp-xfp-3	GCGTTTTTTATTGGTGAGAATTACTAACTTGAGCGAAACG	[1]
	λatt.B2.Amp-xfp-5	TGAGTAGGACAAATCCGCCGCTAAAAAAGCAGGCTTCAACG	[1]
	λATT-ext5	GGCGATAAATTGCCGCATCG	laboratory collection
	λATT-ext3	TGCCACCATCAAGGGAAAGCCC	laboratory collection
	Kmfrt-XhoI-5	AGCCTCGAGATGTGTGTAGGCTGGAGCTGCTTC	[1]
	Kmfrt-SacI-3	CAGGAGCTCCTCATATGAATATCCTCCTTA	[1]
pSU38Psfia*gfp+* and pSU38Psfiagfp+***			
	Psfia-gfp(+)INF-5	CAGGGGCTGGATTGATTATGGATCCCAAAGGAGAAGAACTTTTC	This study
	Psfia-gfp(+)INF-3	GCAAAAGCACCGCCGGACAAGCTTTTATTTGTAGAGCTCATCCATGC	This study
pSU38Psfia*cherry* and pSU38Psfiacherry***			
	hindIII-mcherry	GATAAGCTTGTCGACTTACTTGT	This study
	bamHI-mcherry2	CATGGATCCCAAGGGCGAGGAGGATAA	This study
pSU38Psfia*cherry*-pintI*intI1*$_w$ and pSU38Psfia*cherry-pintI*intI1*$_w$			
	ter-intI-fwd-2	GAGCTCGTAAACTTGTCACCTCGAGGTCGACGG	This study
	inTI-pSU-rev	GGGTGTCAGTGAAGTACGCCTAGGTCTAGGGCGGC	This study
	ter-intI-rev-3	CAAGTTTACGAGCTCGCTTGGAC	This study
	ter-ter-fwd-2	GTAAACTTGGTCTGACGCTCAGTGGAACG	This study
	ter-ter-rev-2	GTCAGACCAAGTTTACGAGCTCG	This study
	pSU-ter-fwd-2	CCTGCCACATGAAGCGTCTGACGCTCAGTGGAACG	This study
Sequencing			
	IntI1 fwd	CGAACGCAGCGGTGGTAA	This study
	pZA for	GTCTTTCGACTGAGCCTTTC	laboratory collection
	MRV-D2	TGCTGACGCACCGGTG	laboratory collection
	pSU38-verif-sq	GCCCACCCCCTGTCCCTC	laboratory collection
qRT-PCR			
	intI1-LC1	GCCTTGATGTTACCCGAGAG	[2]
	intI1-LC5	GATCGGTCGAATGCGTGT	[2]
	sfiA-L1	TTACAGCAACTCGGTCAGCA	laboratory collection
	sfiA-R1	CAGTGTGGCAAGGGGAGA	laboratory collection
	rpoB-R1	GTTTGGTACGCGCAGAGAAG	This study
	rpoB-L2	CCGGTATCGTTTACATTGGTG	This study
	cherry-L2	GTGACCGTGACCCAGGACT	This study
	cherry-R1	TGGTCTTCTTCTGCATTACGG	This study
	gyrB-L3	GCTGCTGTTGACCTTCTTCTA	This study
	gyrB-R3	TGTTCCTGCTTGCCTTTCT	This study
	kana R1	GTAGCCGGATCAAGCGTATG	This study
	kana L1	GAAGGGACTGGCTGCTATTG	This study

sfiA-probe	[6FAM]CTGGGCTACCCTTAACGAAAGTAATGCAGA[TAM]	laboratory collection
rpoB-probe	[6FAM]CTGGTTGGTAAGGTAACGCCGAAAGGT [TAM]	laboratory collection
intI1-probe	[6FAM]ATTCCTGGCCGTGGTTCTGGGTTT [BHQ1]	[2]
gyrB-probe	[6FAM]TCGTCAGATGCCGGAAATCGTTGA [BHQ1]	This study
cherry-probe	[Cy5]CAGGACGGCGAGTTCATCTACAAGGT [BHQ2]	This study
kana-probe	[6FAM]TCCTGTCATCTCACCTTGCTCCTGC[BHQ1]	This study

1. Lacotte, Y., Ploy, M.-C. & Raherison, S. Class 1 integrons are low-cost structures in Escherichia coli. *ISME J.* **11**, 1535–1544 (2017).
2. Barraud, O., Baclet, M. C., Denis, F. & Ploy, M. C. Quantitative multiplex real-time PCR for detecting class 1, 2 and 3 integrons. *J. Antimicrob. Chemother.* **65**, 1642–1645 (2010).