Autoregulation of *greA* Expression Relies on GraL Rather than on *greA* Promoter Region

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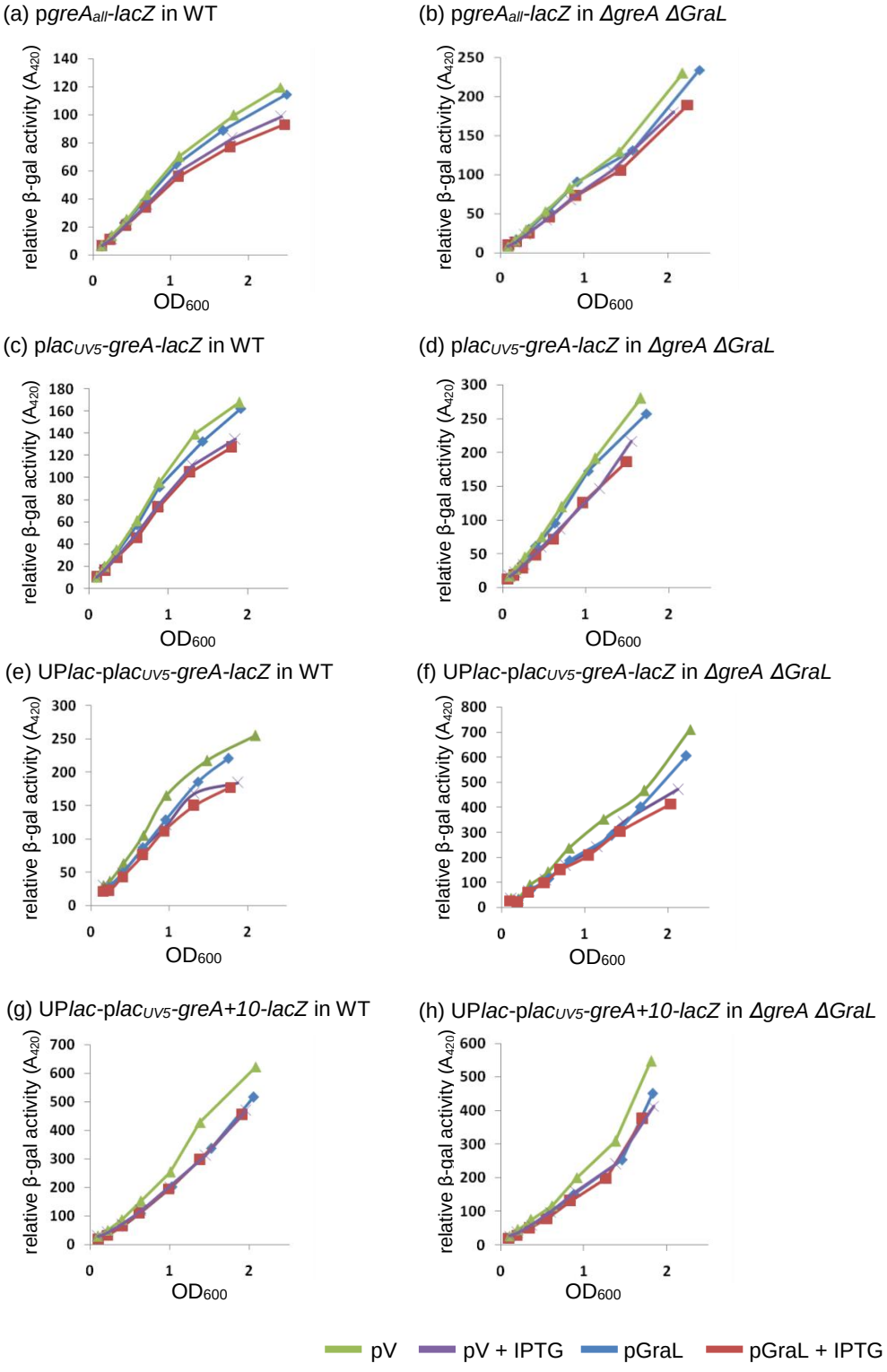

Figure S1. GraL does not affect *greA* expression when supplied *in trans*. Differential plots of beta-galactosidase activity of four *greA*-promoter/leader region fusions with *lacZ* vs OD$_{600}$, in the presence

or absence of GraL overproduced from a multicopy plasmid (pGraL; a pGB2 derivative). When present, IPTG was added to 1 mM at OD_{600}~0.1. (a), (c), (e) and (g) – wt (*ΔlacZ*) (CF15617) strains were used; (b), (d), (f) and (h) - *ΔlacZ ΔgreA ΔGraL* (ECMZ1604) strains were used. pV- vector control (pHM1883). Experiments were done with one biological replicate and 6-10 technical replicates; calculated specific activities are presented in Table S1.

Table S1. Calculated β -galactosidase specific activity for the four *greA-lacZ* fusions in strains overproducing GraL from a multicopy plasmid (pGraL). pV – vector control. Average values were calculated from plots presented in Figure S1, based on at least 3 points where the β -galactosidase activity values were roughly linear. S.D. is given in parenthesis.

	pgreA$_{all}$-*lacZ*	p*lac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*plac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*Plac*$_{UV5}$-*greA+10- lacZ*
WT strains				
pV	60.25 (+/- 3.33)	103.74 (+/- 3.52)	155.65 (+/- 9.39)	226.39 (+/- 9.31)
pV +IPTG	50.74 (+/- 2.89)	84.38 (+/- 2.38)	125.09 (+/- 3.01)	183.29 (+/- 4.69)
pGraL	57.37 (+/- 2.75)	97.07 (+/- 4.55)	128.89 (+/- 6.75)	164.31 (+/- 8.10)
pGraL+IPTG	49.08 (+/- 3.00)	80.12 (+/- 3.14)	110.20 (+/- 8.80)	160.85 (+/- 14.27)
Δ*graL*Δ*greA* strains				
pV	94.60 (+/- 3.51)	169.54 (+/- 5.16)	275.89 (+/- 17.53)	210.95 (+/- 14.13)
pV +IPTG	77.33 (+/- 3.20)	132.62 (+/- 8.63)	210.64 (+/- 7.65)	170.21 (+/- 6.85)
pGraL	89.49 (+/- 6.33)	158.76 (+/- 8.46)	213.80 (+/- 13.42)	166.75 (+/- 8.16)
pGraL+IPTG	75.82 (+/- 3.19)	126.19 (+/- 6.60)	199.76 (+/- 9.46)	150.89 (+/- 6.93)

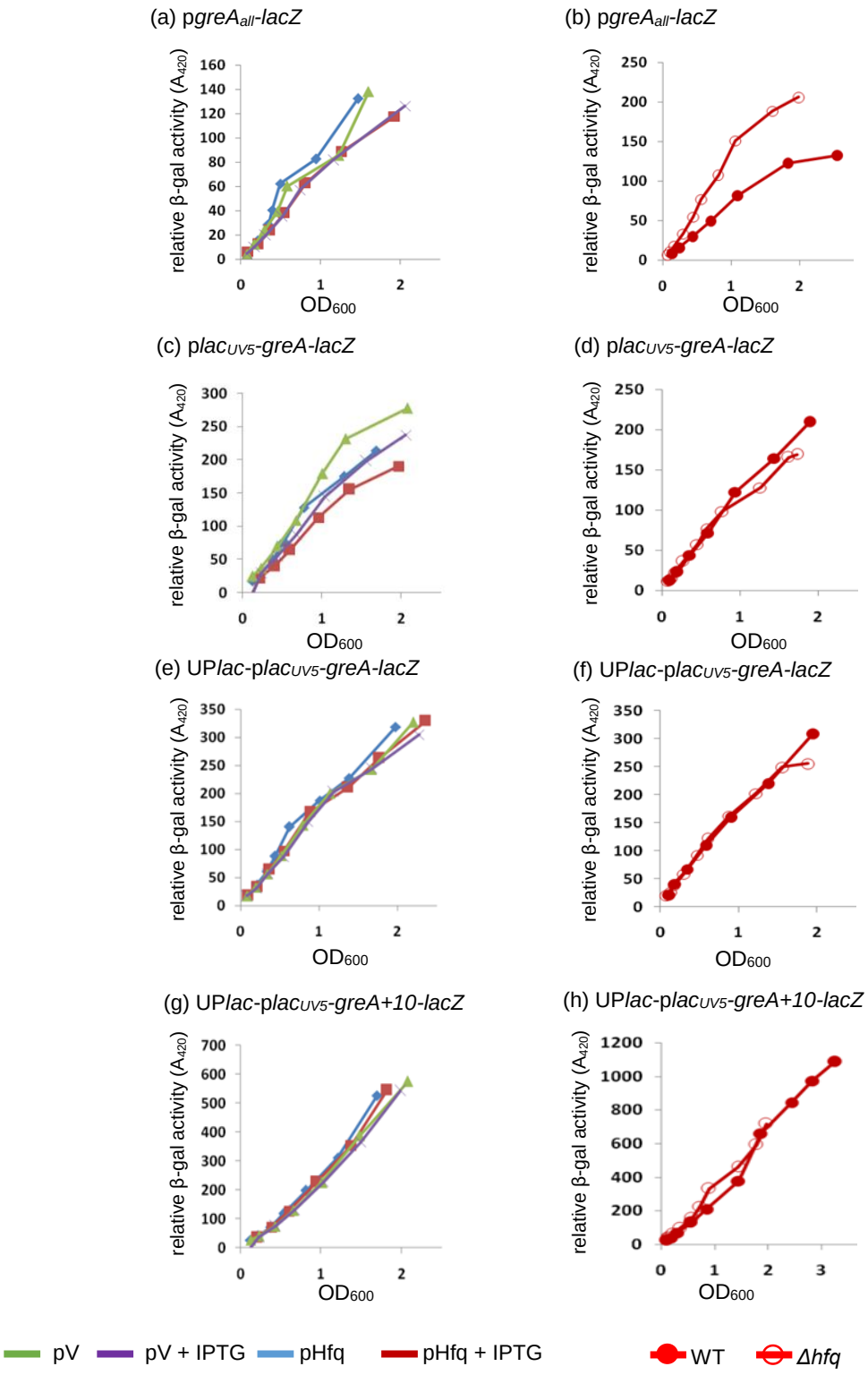

Figure S2. An *hfq* deletion de-represses the p*greA*$_{all}$-*lacZ* fusion's activity, but Hfq has no effect on other fusions where the promoter region has been replaced with p*lac*$_{UV5}$. Differential plots of β-galactosidase activity of four *greA*-promoter/leader region fusions with *lacZ* vs OD$_{600}$. Fusions used are indicated at the top of each panel. (a), (c), (e) and (g) in the presence or absence of Hfq overproduced from a multicopy plasmid (pHfq; an F+ derivative with *hfq* under a p*tac* promoter).

When present, IPTG was added to 1 mM at OD_{600}~0.1; wt *ΔlacZ* (CF15617) strains were used; pV- vector control (F+ *rpoB*). (b), (d), (f) and (h) – either wt *ΔlacZ* or *ΔlacZ Δhfq* strains were used. Experiments were done with one biological replicate and 6-10 technical replicates; calculated specific activities are presented in Table S2.

Table S2. Calculated β -galactosidase specific activity for the four *greA-lacZ* fusions in strains overproducing Hfq from a multicopy plasmid (pHfq) or in a *Δhfq* strain. pV – vector control. Average values were calculated from plots presented in Figure S2, based on at least 3 points where the β -galactosidase activity values were roughly linear. S.D. is given in parenthesis.

	p*greA*$_{all}$-*lacZ*	p*lac*$_{UV5}$-*greA-lacZ*	UP*lac*-*Plac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*Plac*$_{UV5}$-*greA+10- lacZ*
pV	78.29 (+/- 7.49)	166.68 (+/- 10.47)	143.83 (+/- 5.69)	271.20 (+/- 8.58)
pV +IPTG	69.59 (+/- 5.65)	128.88 (+/- 6.01)	129.71 (+/- 3.91)	259.83 (+/- 10.99)
pHfq	83.89 (+/- 9.02)	135.40 (+/- 9.62)	161.21 (+/- 2.65)	240.27 (+/- 18.04)
pHfq+IPTG	70.56 (+/- 5.24)	108.98 (+/- 7.51)	147.43 (+/- 7.17)	257.55 (+/- 5.55)
WT	68.44 (+/- 2.18)	120.93 (+/- 6.92)	186.16 (+/- 9.03)	234.60 (+/- 9.53)
Δhfq	106.69 (+/-5.02)	133.30 (+/- 5.12)	196.73 (+/- 3.44)	283.66 (+/- 14.11)

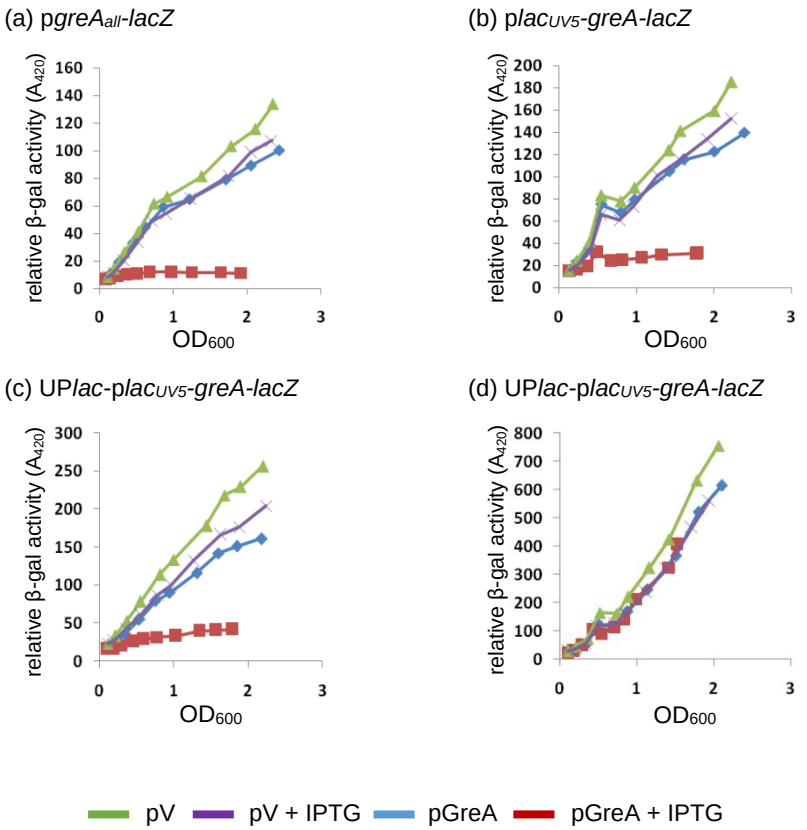

Figure S3. An *hfq* deletion does not alter GreA mediated regulation of *greA* expression. Differential plots of β-galactosidase activity of four *greA*-promoter/leader region fusions with *lacZ* vs OD_{600}, in the presence or absence of GreA overproduced from a multicopy plasmid (pGreA). (a) p*greA*$_{all}$-*lacZ; (b)* p*lac*$_{UV5}$-*greA-lacZ; (c)* UP-p*lac*$_{UV5}$-*greA-lacZ*; (d) UP-p*lac*$_{UV5}$-*greA+10-lacZ* fusions. When present, IPTG was added to 1 mM at OD_{600}~0.1; *ΔlacZ Δhfq* strains were used; pV- vector control. Experiments were done with one biological replicate and 6-10 technical replicates; calculated specific activities are presented in Table S3.

Table S3. Calculated β-galactosidase specific activity for the four *greA-lacZ* fusions in *Δhfq* strains overproducing GreA from a multicopy plasmid (pGreA). pV – vector control. Average values were

calculated from plots presented in Figure S3, based on at least 3 points where the β -galactosidase activity values were roughly linear. S.D. is given in parenthesis.

	p*greA*$_{All}$-l*acZ*	p*lac*$_{UV5}$-*greA-lacZ*	UP*lac-Plac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*Plac*$_{UV5}$-*greA+10- lacZ*
pV	68.41 (+/- 12.24)	92.57 (+/- 4.44)	131.92 (+/- 6.86)	248.16 (+/- 30.41)
pV +IPTG	57.25 (+/- 9.40)	77.15 (+/- 2.52)	105.28 (+/- 4.20)	191.36 (+/- 20.02)
pGreA	59.99 (+/- 12.30)	78.12 (+/- 6.54)	94.43 (+/- 7.62)	190.56 (+/- 25.44)
pGreA+IPTG	12.36*	22.49*	32.38*	164.21 (+/- 5.04)

* β -galactosidase specific activity was calculated at OD_{600}~1.0.

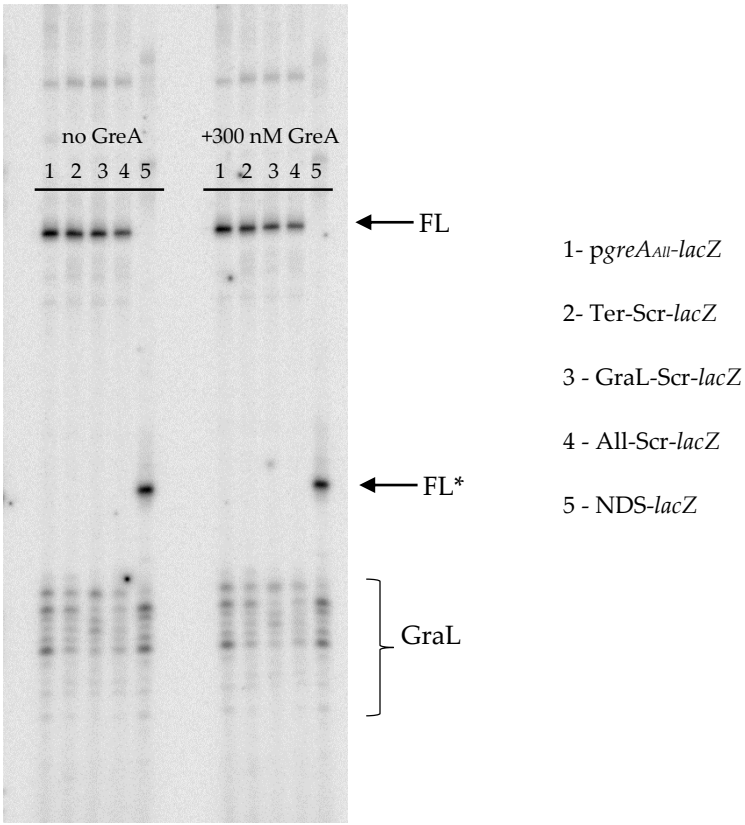

Figure S4. Constructs used for testing GraL leader/terminator region *in vivo* still undergo transcription termination *in vitro* regardless of absence or presence of GreA. Single round *in vitro* transcription was carried out on linear templates as described in the Materials and Methods section, in presence or absence of 300 nM GreA. FL indicates full-length runoff product formed; FL* - full-length runoff product on the shortened (NDS-*lacZ*) template; GraL – short, prematurely terminated transcripts. Templates used are indicated (diagrams of the constructs used are presented in the left panels in Figure 3a and Figure 6a-d). Experiments were done thrice.

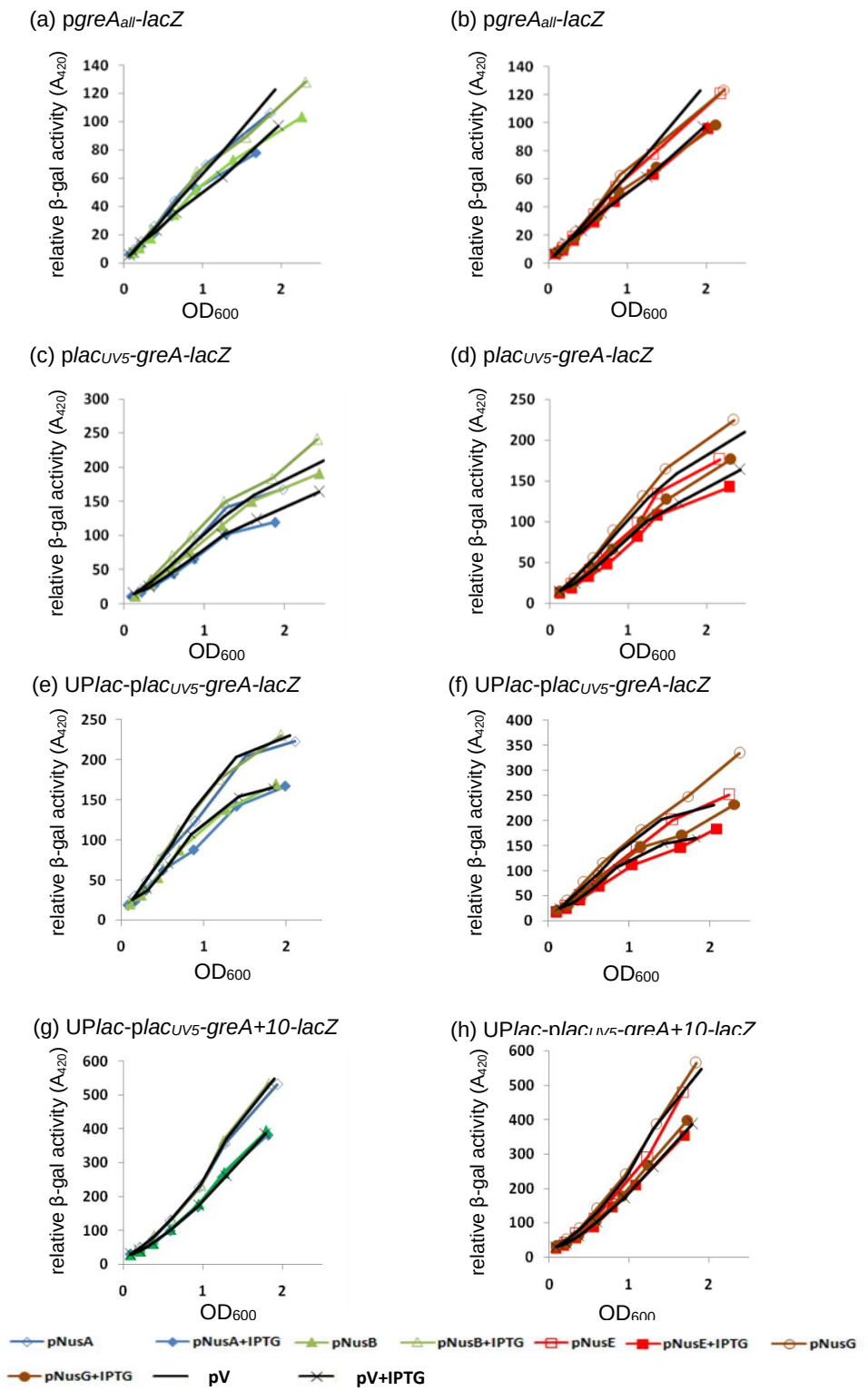

Figure S5. Nus factors do not alter *greA* expression. Differential plots of β-galactosidase activity of four *greA*-promoter/leader region fusions with *lacZ* vs OD_{600}, in the presence or absence of plasmids overproducing Nus factors. (a), (c), (e) and (g) – the effect of NusA or NusB was assessed; (b), (d), (f) and (h) – the effect of NusE or NusF was assessed. When present, IPTG was added to 1 mM at OD_{600}~0.1; wt *ΔlacZ* (CF15617) strains were used; pV- vector control. Fusions used are indicated at the top of each panel. Experiments were done with one biological replicate and 6-10 technical replicates; calculated specific activities are presented in Table S4.

Table S4. Calculated β -galactosidase specific activity for the four *greA-lacZ* fusions in strains overproducing Nus factors (NusA, NusB, NusG or NusE) from a multicopy plasmid (pNusA, pNusB, pNusG or pNusE; pGB2 derivatives). pV – vector control (pHM1883). Average values were calculated from plots presented in Figure S5, based on at least 3 points where the β -galactosidase activity values were roughly linear. S.D. is given in parenthesis.

	greA$_{all}$-*lacZ*	*Plac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*Plac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*Plac*$_{UV5}$-*greA+10- lacZ*
pV	65.70 (+/- 4.54)	99.47 (+/- 2.71)	146.45 (+/- 20.09)	238.53 (+/- 28.90)
pV+IPTG	57.71 (+/- 7.30)	77.78 (+/- 2.71)	111.80 (+/- 13.58)	183.73 (+/- 14.04)
pNusA	69.23 (+/- 3.41)	98.08 (+/- 8.20)	153.29 (+/- 18.14)	234.34 (+/- 27.57)
pNusA+IPTG	57.93 (+/- 3.26)	74.91 (+/- 4.10)	118.05 (+/- 17.40)	182.31 (+/- 17.11)
pNusB	65.34 (+/- 4.90)	112.09 (+/- 8.33)	150.08 (+/- 18.51)	239.36 (+/- 29.81)
pNusB+IPTG	54.59 (+/- 1.89)	91.34 (+/- 2.90)	113.81 (+/- 15.03)	184.55 (+/- 17.96)
pNusE	60.78 (+/- 1.92)	87.41 (+/- 6.96)	136.56 (+/- 5.68)	219.10 (+/- 14.86)
pNusE+IPTG	51.30 (+/- 1.97)	70.76 (+/- 5.26)	104.88 (+/- 8.98)	176.34 (+/- 14.02)
pNusG	66.18 (+/- 3.54)	106.43 (+/- 5.62)	164.05 (+/- 13.54)	247.76 (+/- 25.71)
pNusG+IPTG	54.91 (+/- 3.16)	83.01 (+/- 2.51)	124.96 (+/- 12.55)	190.84 (+/- 16.21)

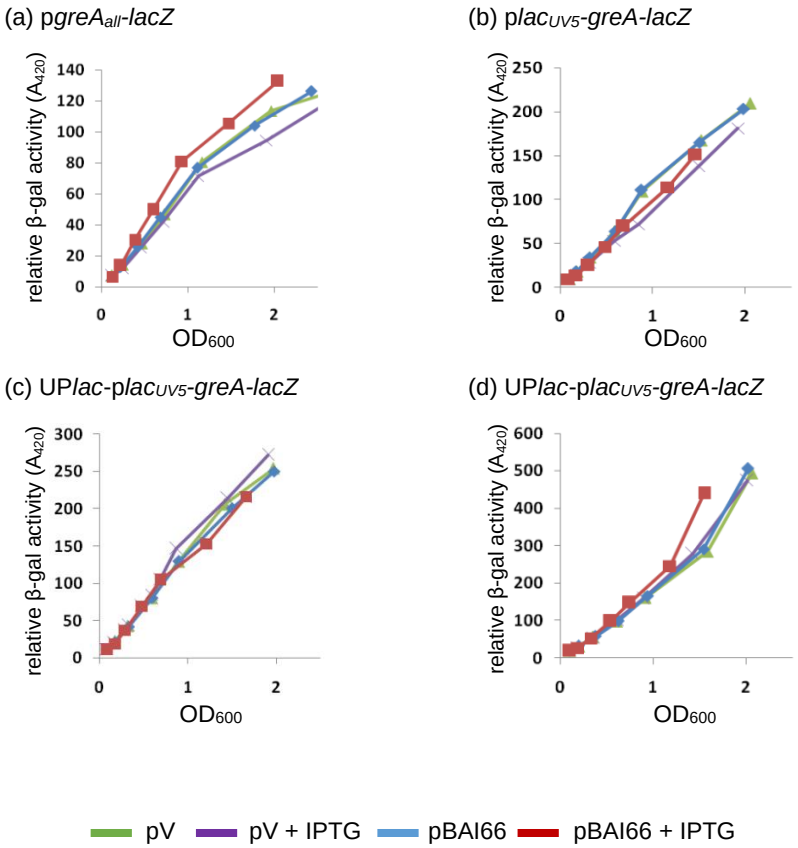

Figure S6. σ^E induction has a moderate effect on the *greA* P2 promoter but has no effect on transcription proceeding through the *greA* leader region. Differential plots of β-galactosidase activity of four *greA*-promoter/leader region fusions with *lacZ* vs OD$_{600}$, in the presence or absence of a misfolded peptide accumulation; the YYF peptide was overproduced from a multicopy plasmid (pBAI66). (a) p*greA*$_{all}$-*lacZ; (b)* p*lac*$_{UV5}$-*greA-lacZ; (c)* UP-p*lac*$_{UV5}$-*greA-lacZ;* (d) UP-p*lac*$_{UV5}$-*greA+10-lacZ* fusions. When present, IPTG was added to 1 mM at OD$_{600}$~0.1; wt *ΔlacZ* (CF15617) strains were used; pV- vector control (pTrc99). Experiments were done with one biological replicate and 6-10 technical replicates; calculated specific activities are presented in Table S5.

Table S5. Calculated β -galactosidase specific activity for the four *greA-lacZ* fusions in strains overproducing a misfolded YYF peptide, whose accumulation induces σ^E activity (pBAI66 plasmid, a pTrc99 derivative). pV – vector control (pTrc99). Average values were calculated from plots presented in Figure S6, based on at least 3 points where the β -galactosidase activity values were roughly linear. S.D. is given in parenthesis.

	greA$_{all}$-*lacZ*	P*lac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*Plac*$_{UV5}$-*greA-lacZ*	UP$_{lac}$-*Plac*$_{UV5}$-*greA+10- lacZ*
pV	60.51 (+/- 2.85)	106.51 (+/- 3.96)	131.69 (+/- 2.55)	162.29 (+/- 0.87)
pV+IPTG	54.01 (+/- 5.36)	89.80 (+/- 2.11)	131.53 (+/- 11.07)	158.19 (+/- 6.89)
pBAI66	61.46 (+/- 4.51)	108.03 (+/- 1.05)	128.56 (+/- 5.90)	161.60 (+/- 6.25)
pBAI66+IPTG	74.36 (+/- 11.14)	89.04 (+/- 6.89)	128.11 (+/- 18.15)	166.08 (+/- 18.54)

Table S6. Strains used in this work.

Strain	**Genotype**	**Source**
MG1655	F-, *ilvG*, *rph1*	Jin and Gross, 1988
AAG1	MG1655 Δ*lacZ*	Aberg et al. 2008
CF15617	MG1655 Δ*lacZ*	Vinella et al., 2012
ECMZ1601	CF15617 Δ*greA::cat*	Dylewski et al., 2018
ECMZ1604	CF15617 Δ*GraL* Δ*greA::cat*	Dylewski et al., 2018
JW4130-1	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *λ*$^-$, *rph-1*, *Δ(rhaD-rhaB)568*, *Δhfq-722::kan*, *hsdR514*	Baba et al., 2006
KP517	CF15617 (λ *greA*$_{all}$-*lacZ*)	This work
KP623	CF15617(λ p*lac*$_{UV5}$-*greA-lacZ*)	This work
KP625	CF15617 (λ UP$_{lac}$-p*lac*$_{UV5}$-*greA-lacZ*)	This work
LFC3	AAG1 (λ greA -1030 to +175-lacZ)	This study
LFC4	AAG1 greA::Cm (λ greA -1030 to +175-lacZ)	This study
MD324	CF15617 (λ UP$_{lac}$-*Plac*$_{UV5}$-*greA+10- lacZ*)	This work
MD404	KP517 *Δhfq-722::kan*	This work
MD405	KP625 *Δhfq-722::kan*	This work
MD406	KP623*Δhfq-722::kan*	This work
MD407	MD324 *Δhfq-722::kan*	This work
MD441	CF15617 (λ NDS-*lacZ*)	This work
MD442	CF15617 (λ GraL-Scr-*lacZ*)	This work
MD443	CF15617 (λ Ter-Scr-*lacZ*)	This work
MD444	CF15617 (λ All-Scr-*lacZ*)	This work
MD474	KP517 Δ*graL*Δ*greA::cat*	This work
MD475	KP623 Δ*graL*Δ*greA::cat*	This work
MD476	MD324 Δ*graL*Δ*greA::cat*	This work
TP1204	MG1655 *greA* D41A	Poteete, 2011

Table S7. Plasmids used in this work.

Plasmid	**Description**	**Source**
pBR322	*ori* pMB1, Ap^R, Tc^R	Bolivar et al., 1977
pBR-greA	*greA* with native promoter cloned into pBR322	This work
pBR-greA D41A	*greA* D41A with native promoter cloned into pBR322	This Work
pHM1786	pGB2 derivative, *ori* pSC101, *lacI*^{q}, Spc^R	[Vinella et al., 2012]
pGraL	GraL under p*tac* promoter cloned into pHM1786	This work
pGreA (pHM1873)	*greA* under p*tac* promoter cloned into pHM1786	[Vinella et al., 2012]
pV (pHM1883)	Vector control for pGreA and pGraL	[Vinella et al., 2012]

pRS415	For construction of transcriptional fusions with *lacZ* and subsequent transfer to λ and recombination on the chromosome; ApR	[Simons et al., 1987]
pRS551	pRS415 derivative, Ap^R Kan^R	[Simons *et al.*, 1987]
pKP-pAall	Region spanning -100 to +136 (relative to +1 of p*greA* P1 promoter) fused to *lacZ* in pRS415 at the *BamH*I/*Hind*III sites	[Potrykus et al., 2010]
pKP- $plac_{UV5}$-greA-lacZ	The same as pKP-pAall but promoter region replaced by $plac_{UV5}$	This work
pKP- UP_{lac}-$plac_{UV5}$-greA-lacZ	The same as pKP- $plac_{UV5}$-greA-lacZ but UP region replaced by region upstream of the native plac promoter; CRP binding site mutated (G->A at position -66, and C->T at position -55)	This work
pMD- UP_{lac}-*Plac*$_{UV5}$-*greA+10-lacZ*	The same as pKP- UP_{lac}-$plac_{UV5}$-greA-lacZ, but the region from +10 to +136 deleted	This work
pMD-GraL_Scr-*lacZ*	The same as pKP-pAall but the sequence from +1 to +18 randomly scrambled	This work
pMD- Ter_Scr-*lacZ*	The same as pKP-pAall but the sequence from +19 to +48 scrambled so as to switch the terminator hairpin arms with each other	This work
pMD- pAll_Scr-*lacZ*	The same as pMD- Ter_Scr but also with the sequence from +1 to +18 scrambled	This work
pMD-NDS-*lacZ*	The same as pKP-pAall but the region from +60 to +134 deleted	This work
pTrc99A	Vector, pBR322 ori, Ap^R	[Amann et al., 1988]
pBAI66	Plasmid for overproduction of a misfolded peptide (YYF) to induce σ^E; pTrc99A derivative	[Walsh et al., 2003]
pNusA	*nusA* cloned into pGB2	[Friedman et al., 1990]
pNusB	*nusB* cloned into pGB2	[Friedman et al., 1990]
pNusE	*nusE* cloned into pGB2	[Friedman et al., 1990]
pNusG	*nusG* cloned into pGB2	[Friedman et al., 1990]
F+rpoB	*rpoB* under p*tac* promoter in an F+ plasmid	[Saka et al., 2005]
F+hfq	*hfq* under p*tac* promoter in an F+ plasmid	[Saka et al., 2005]

Table S8. Oligonucleotides and synthetic DNA fragments used in this work.

Name	**Sequence (5'-3')**	**Description**
Oligonucleotides		
pBplasdw	CTTTCATCGGTTGTCGGATCC	For *in vitro* transcription template preparation, based on [Potrykus et al., 2010]
G1	GAGAATTCGCGATCATGTTGTCCGAC	For construction of pBR-GreA and pBR-GreA D41A plasmids, and construction of λ *greA -1030 to +175-lacZ* fusion

G7	CAGGATCCCGTAAGGTCATCGGAATAGC	For construction of λ *greA -1030 to +175-lacZ* fusion
G11	CACTGCAGCAACATCTTGAGTATTGGG	For construction of pBR-GreA and pBR-GreA D41A plasmids
HMPr368	CAGGAATTGGGGATCGGAATTC	For *in vitro* transcription template preparation, based on [Potrykus et al., 2010]
KPr77	TATGAATTCGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTAAGTTAGCTCATTCATTAGGCACCCCAGGCTTTACACTTTATGCTT	Forward primer to make the UP_{lac}-$Plac_{UV5}$-*greA+10- lacZ* fusion (with pKP- UP_{lac}-$plac_{UV5}$-greA-lacZ as template in the PCR reaction)
KPr78	TCTGGATCCACATTTTGATCCACACATTATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAATGAGCTAACTTACAT	Reverse primer to make the UP_{lac}-$Plac_{UV5}$-*greA+10- lacZ* fusion (with pKP- UP_{lac}-$plac_{UV5}$-greA-lacZ as template in the PCR reaction)
MDGLDWN	ATCAAGCTTAAGCAAAAAAATACCGACCCGGGTACAAGTCCCAGGTCAG	Reversed primer for GraL cloning (anneals to MDGLUP)
MDGLUP	ATGAATTCATCAAAATGTGAATTGTAGCTGACCTGGGACTTGTACCCG	Forward primer for GraL cloning (anneals to MDGLDWN)
pAp2sRNA	AAAATACCGACCGGGTACAAGTCCCAGGTCAGCTACAAT	Probe for P^{32} labeling; complementary to GraL (based on [Potrykus et al., 2010])
5SProbe	Cy3-CATGGGGTCAGGTGGGACCACCGCGCTACGGCCGCCAGGC	Cy3-labeled probe to 5S RNA (based on [Moll et al., 2003])
Synthetic DNA fragments [obtained through GeneStrings (GeneArt) service (Thermo)] *Bam*HI/ *Eco*RI sites are indicated		
$plac_{UV5}$-greA	TATGAATTCATAATCTCGCGCTAACAACCTGGAATCGAGCCGTCATACTACGGCGCAACGCCCTATAAAGTAAACGTTTACACTTTATGCTTCCGGCTCGTATAATGGTGGATCAAAATGTGAATTGTAGCTGACCTGGGACTTGTACCCGGGTCGGTATTTTTTTGCTTCTGGTCCCGGTAAGGAGTTATGCCGGGCAGGCCGAACAGCCGGGGTGGGTGAAGACTTGCCCTATCAGGAATATTCAGGATCC AGA	
UP_{lac}-$plac_{UV5}$-greA	TATGAATTCGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTAAGTTAGCTCATTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATCAAAATGTGAATTGTAGCTGACCTGGGACTTGTACCCGGGTCGGTATTTTTTTGCTTCTGGTCCCGGTAAGGAGTTATGCCGGGCAGGCCGAACAGCCGGGGGGGTGAAGACTTGCCCTATCAGGAATATTCAGGATCCAGA	
NDS_	TATGAATTCCATAATCTCGCGCTAACAACCTGGAATCGAGCCGTCATACTACGGCGCAACGCCCTATAAAGTAAACGATGACCCTTCGGGAACTTCAGGGTAAAATGACTATCAAAATGTGAATTGTAG	

	CTGACCTGGGACTTGTACCCGGGTCGGTATTTTTTTGCTTGGATCCAGA
GraL_Scr	TATGAATTCCATAATCTCGCGCTAACAACCTGGAATCGAGCCGTCATACTACGGCGCAACGCCCTATAAAGTAAACGATGACCCTTCGGGAACTTCAGGGTAAAATGACTATAAGATATATGTACTAGGCTGACCTGGGACTTGTACCCGGGTCGGTATTTTTTTGCTTCTGGTCCCGGTAAGGAGTTATGCCGGGCAGGCCGAACAGCCGGGGTGGGTGAAGACTTGCCCTATCAGGAATATTCAGGATCCAGA
Ter_Scr	TATGAATTCCATAATCTCGCGCTAACAACCTGGAATCGAGCCGTCATACTACGGCGCAACGCCCTATAAAGTAAACGATGACCCTTCGGGAACTTCAGGGTAAAATGACTATCAAAATGTGAATTGTATGGCTGGGCCCATGTTCAGGGTCCAGTCGATTTTTTTGCTTCTGGTCCCGGTAAGGAGTTATGCCGGGCAGGCCGAACAGCCGGGGTGGGTGAAGACTTGCCCTATCAGGAATATTCAGGATCCAGA
All_Scr	TATGAATTCCATAATCTCGCGCTAACAACCTGGAATCGAGCCGTCATACTACGGCGCAACGCCCTATAAAGTAAACGATGACCCTTCGGGAACTTCAGGGTAAAATGACTATAAGATATATGTACTAGTGGCTGGGCCCATGTTCAGGGTCCAGTCGATTTTTTTGCTTCTGGTCCCGGTAAGGAGTTATGCCGGGCAGGCCGAACAGCCGGGGTGGGTGAAGACTTGCCCTATCAGGAATATTCAGGATCCAGA