R258C	Fold-change	P279S	G324D	N340S
tyrS	4.43			
rydC	4.25			
ydcH	3.88	+		
gapC	3.74			
aldA	3.12		++	
ydcP	3			
mokA	2.98			
yfhR	2.87	++	++	++
fimC	2.84	++	++	+
ydcY	2.79			
efeO	2.7			
borD	2.68			
hicB	2.67			
fimD	2.63	++	++	++
ydcD	2.63	++		
rcbA	2.56			
ydcZ	2.53			
insP	2.44			
racR	2.43			
ydcA	2.41			
ydaW	2.39			
cybB	2.38		++	
rzpD	2.35			
shoB	2.35			
yjeV	2.33			
insQ	2.31			
mgtA	**2.31**	**++**	**++**	**++**
uspE	2.29			
fimI	2.29	+	++	+
ydcX	2.27			
rbsR	2.21			
rlmN	2.2			
nrdG	2.2		+	
yigE	2.11			
yliF	2.11	++	++	+
Rho	2.1			
dgcZ	2.09			
ykfH	2.06	++	++	++
fimF	2.05	++	++	++
insC1	2.04	++	++	++
pinQ	2	++		
mngR	2			
ilvX	1.99		+	
rbsK	1.98			
efeB	1.98			
fimA	1.98	+	+	+
ydcR	1.96			
nmpC	1.95			
yghF	1.93			
suhB	1.92			
dusC	1.9			
rsmG	1.9			
zraP	1.88			
nrdD	1.86		+	
ykfG	1.86	++	++	++
ttcA	1.85			
wecF	1.85	++	++	++
proW	1.84			
cfa	1.84			
hsrA	1.84			
yafW	1.83	++	++	++
fimG	1.83	++	++	++
ydhB	1.82			
efeU	1.82			
insZ	1.8			
tehB	1.77			
rlmC	1.77			
ydiE	1.77	++	++	
rrrD	1.77			
truD	1.76		++	
gntP	1.76			

R258C	Fold-change	P279S	G324D	N340S
dnaE	1.74			
yncJ	1.74			
ridA	1.71			
yhiI	1.71	+	+	+
insH1	1.71			
insJ	1.71	++	+	++
nuoC	1.71			
nuoL	1.7			
yafX	1.7	++	++	++
sokB	1.7			+
ydhI	1.69	++	++	+
ybaA	1.69	++		
fabF	1.68		++	
ymjD	1.67	+	+	+
ymdE	1.67	+	+	+
ymfD	1.67	++	++	
hybG	1.67			
pyrE	1.66			
truB	1.65			
oppC	1.65			
queA	1.64			
truA	1.64			
tdrA	1.64			
ychQ	1.64			
rdlC	1.64			
yahA	1.63	++	++	++
yhjV	1.63			
chaA	1.63			
proS	1.63			
yceF	1.63			
glyS	1.62			
mnmG	1.62			
yidD	1.62			
ydcL	1.61			
thiI	1.6			
yahB	1.6			
cmoB	1.57			
gltI	1.57		+	+
malK	1.56			
abrB	1.56			
yegZ	1.55	+	+	+
wzyE	1.55	++	++	++
ykgH	1.55		++	
rlmL	1.55		++	
rsxC	1.55		++	++
rnhB	1.55			
gfcE	1.54	+	++	++
yajL	1.54		+	
rfaH	1.53			
sodA	1.52			
ykfI	1.52	++	++	++
yjfZ	1.52	++	++	++
ycfZ	1.52	++		
proX	1.52			
prfC	1.51		++	
tdrB	1.51			
trmH	1.51			
tcdA	1.51			
nuoM	1.51			
yebZ	1.51			
wecD	1.51	+	+	+
slyA	1.5			
rlmM	1.5			
yfcJ	1.5	++	++	++
rlmN	1.49			
mnmA	1.49			
yceI	1.49	+		
ybcI	1.49			
ydcI	1.49			

G181D	Fold-change	P279S	G324D	N340S
pyrB	4.66			
pyrI	4.48			
ydcH	5.61	+		
rydC	4.57			
ydcD	3.85	++		
dmsA	3.66	++		
nrfA	3.6	+		+
bssR	3.59	++		
tdcD	3.57			**+**
dmsB	3.56	++		
pinQ	3.54	++		
hicB	3.39			
yciE	3.39	++	+	
dmsC	3.15	+		
nrfC	3.13	+		+
carA	3.12		++	++
rimL	3.06			
grpE	3.04			
ompW	2.98			++
carB	2.91		**++**	**+**
racR	2.9			
iraM	2.88			
ycaC	2.87			
ydaW	2.85			
ansB	2.81			
insP	2.78			
nrfD	2.77	+		+
ydcY	2.77			
uspE	2.72			
ynfE	2.69	++		++
ydcA	2.68			
cysQ	2.64	+	+	+
insQ	2.62			
ttcA	2.56			
ybbD	2.55	+	+	+
nrfB	2.54	+		+
ydcZ	2.53			
cfa	2.52			
tomB	2.45			
yjhQ	2.43	**+**	**++**	**++**
ydcX	2.41			
gapA	2.41			
ldhA	2.36			
ridA	2.36			
rimP	2.34			
hypC	2.32			
yncI	2.32			
gapC	2.32			
maa	2.3			
ynfH	2.29	+	+	++
cybB	2.27		++	
ycbC	2.26	+		
ynfF	2.25	++		++
yegZ	2.25	+	+	+
ybeZ	2.22	**+**	**++**	**+**
ynfG	2.22	++		++
yfcE	2.21	**+**	**+**	**+**
curA	2.21			
tdcE	2.2			**+**
hypB	2.19			
ylcI	2.17			
aldA	2.17		++	
pptA	2.15	++	++	++
rbfA	2.14			
preA	2.13			
tehB	2.08			
nrfE	2.07	+		++
marA	2.06			
ymjD	2.05	+	+	+
ydcL	2.04			
ydcP	2.04			
ydaF	2.04			

G181D	Fold-change	P279S	G324D	N340S
truB	2.04			
dusC	2.02		++	
hfq	2.02			
infB	2.02			
ilvM	2.02	++	++	+
hybC	2			
ybcW	1.99			
hypD	1.97			
ydcS	1.95		++	
ybbC	1.95	++	+	++
hokB	1.92			
mnaT	1.92			
csgG	1.88	+	++	++
ynaJ	1.87			
ydhY	1.86	++		++
rzpD	1.85			
yqeK	1.85			
hypE	1.85			
ycaD	1.83			++
yhbU	1.83			
yjeV	1.82			
ariR	1.81		+	
ymgI	1.81			
mcbR	1.8			
chaA	1.8			
ymgA	1.8		++	
ydcK	1.77			
ribC	1.77			
ycgZ	1.77		+	
yibW	1.74			
ydhI	1.73	++	++	+
ydaS	1.72			
pabC	1.71	++	++	+
rhsH	1.7			
mnmH	1.7			
ynfK	1.7			
truA	1.69			
yciF	1.69	+	++	
proS	1.68			
cbl	1.68		++	
ygdI	1.67			
fimC	1.67	++	++	+
ampC	1.67			
rlmI	1.66			
yccT	1.66	++	++	
dgcZ	1.65			
dcuC	1.65			
tehA	1.65			
ttcC	1.64			
ydaG	1.64			
nirD	1.63			
ogrK	1.61	++	++	++
nikA	1.6			
yibV	1.59	++	++	++
ylbG	1.58			
pinR	1.58	+		
rrrD	1.57			
csgF	1.57	++	++	++
nirB	1.57			
ybaK	1.56			
yihA	1.55			
ydcR	1.55			
chiX	1.55			
ralR	1.55			
rsuA	1.54			
ymgC	1.53		++	
ydaT	1.53			
yccF	1.53			
yceO	1.53			
kch	1.52			
yibL	1.52			
ytfI	1.5	++	++	++

G181D	Fold-change	P279S	G324D	N340S
citD	1.5			++
ydaU	1.5			
nrfF	1.5	+		++
ymdE	1.49	+	+	+

Genes up-regulated in *nusA* mutants:

🟦 **R258C**
🟩 **G181D**

☐ **Fold-change in mRNA level with respect to WT *nusA***

🟨 ***rho* SBS mutants**

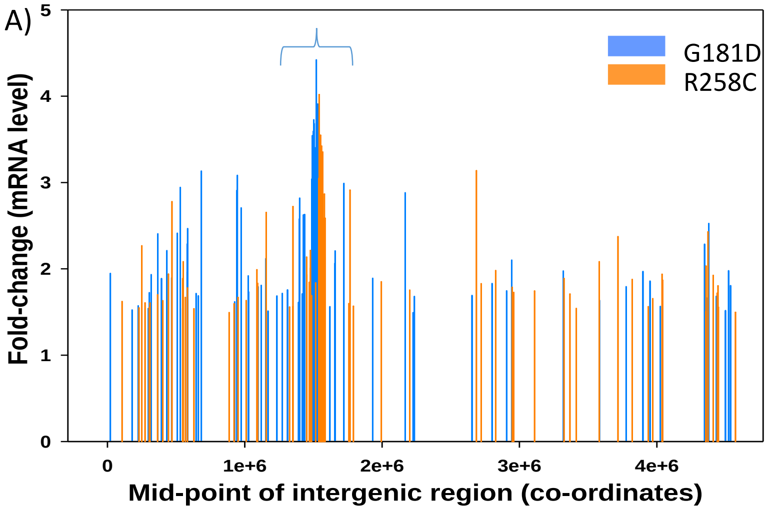
++ **Same gene up-regulated in *rho* mutant**

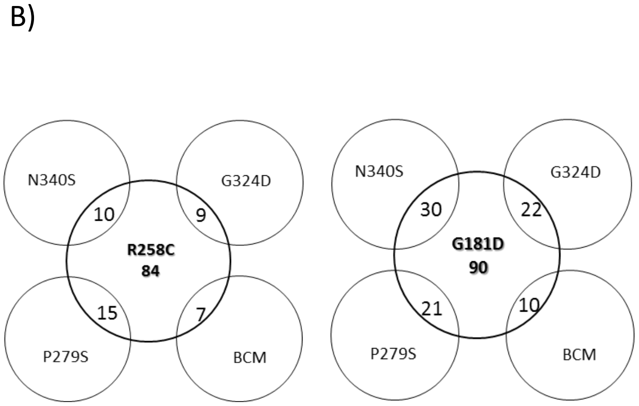
+ **Some other gene from the operon up-regulated in *rho* mutant**

Upregulated genes common between the two nusA mutants are shown in pink.

🟩 **mgtA** Riboswitch controlled

Figure S1





C)

	nusA G181D				
	nusA	***rho***			
Prophage	**G181D**	**P279S**	**G324D**	**N340S**	**BCM**
Rac	**racR**				
	ydaW				
	ydaF				
	lomR				
	rzoR				
	ybcW				
	ydaS				
	ydaG				
	pinR	++			++
	ydaT				
	ydaU				
	rzpR				
DLP12	**rrrD**				
	ybcW				
	rzoD				
	insE1	+	+	+	++
Qin	**pinQ**	++			++
CP4-6	**yafY**	+	+	+	+
	yafX	++	++	++	
P2 remnant	ogrK	++	++	++	++

	nusA R258C				
	nusA	***rho***			
Prophage	**R258C**	**P279S**	**G324D**	**N340S**	**BCM**
Rac	**ydaF**				
	ydaS				
	racR				
	ydaW				
	kilR				
	rzoR				
	ydaY				
DLP12	**insE1**	+	+	+	++
	rzpD				
	rzoD				
	rrrD				
	essD				
Qin	**pinQ**	++			++
CP4-6	**yafY**	+	+	+	++
	ykfG	++	++	++	+
	yafX	++	++	++	+
	insH1				
	ykfI	++	++	++	+
CP4-44	insC1	++	++	++	++
Qsr	nmpC				
e14	ymfD	++	++		

++ identical gene up-regulated

\+ Some other gene from same operon up-regulated.

Genes in bold are shared between the two *nusA* mutants.

D)

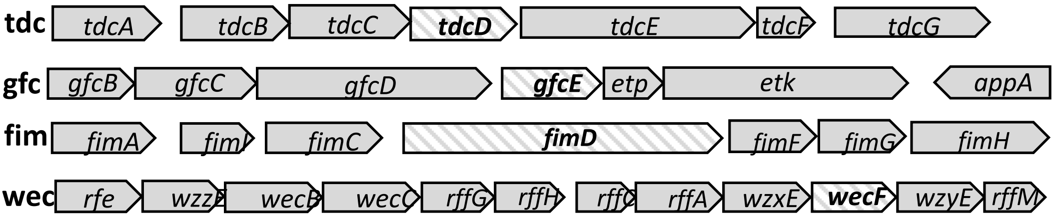
nusA G181D

Small RNA	Fold-change
chiX	1.55
rydC	4.57

nusA R258C

Small RNA	Fold-change
rydC	4.25
sokB	1.70
rdlC	1.64
rttR	1.42
rdlB	1.40
tRNA^{thrV}	1.59

E)



wec operon

Genes	R258C	P279S	N340S	G324D
rfe				
wzzE				
wecB			1.59	1.54
wecC				
rffG		1.65		
rffH		1.68		
rffC	1.51	2.04	1.79	1.48
rffA		2.00	2.21	1.91
wzxE		2.02		
wecF		1.95	2.5	2.06
wzyE	1.55	1.84	2.31	1.79
rffM		1.69	1.84	

gfc operon

Genes	G181D	P279S	N340S	G324D
appA				
etk		2.9	2.3	4.1
etp		5.8	4.7	5.6
gfcE	1.54	4.29	2.92	4.0
gfcD		6.9	4.87	5.6
gfcC		6.5	5.8	5.9
gfcB		7.8	3.7	3.3

fim operon

Genes	R258C	P279S	N340S	G324D
fimA	1.98	1.64		1.77
fimI	2.29	1.9		
fimC		2.9		2.8
fimD	2.63	1.8	1.89	3.7
fimF	2.05	2.8	1.7	3.7
fimG	1.83	2.3	2.1	3.9
fimH		1.87		2.58

tdc operon

Genes	G181D	P279S	N340S
tdcG		2.01	2.9
tdcF			
tdcE	2.20	2.14	2.75
tdcD	3.57	2.0	1.9
tdcC			
tdcB			
tdcA			

Figure S2

Figure S1: Tables showing the complete lists of the up-regulated genes (>1.5 fold with p value <0.2) from the Agilent tiling microarray profiles obtained in the presence of the NusA mutants, R258C (left panels) and G181D (right panels). Fold changes (2^{nd} column) in the hybridization intensities were obtained from the ratios of mutants and the WT NusA. Common up-regulated genes between the two NusA mutants are indicated in pink shades. Same genes or those from the same operons those were upregulated in three Rho mutants, N340S, G324D and P279S, are indicated as "++" or "+", respectively, against the gene names. The micro-array data obtained for Rho mutants were originally reported in Ref. 1. This overlap between the Rho and NusA mutants could be under-estimated as the Rho data were obtained from a low density micro-array having fewer probes. Meaning of different notations are described in the figure. *mgtA*, expression which is under riboswitch control is indicated in green shade.

Figure S2. A) Plots showing the fold change in the mRNA level of the NusA mutants *w.r.t* the WT strain from the intergenic regions that do not code any gene. The co-ordinate of the intergenic region in the X-axis is expressed as the mid-point of each of the intergenic stretch. The fold change is the average fold change calculated from the fold changes of all the probes corresponding to each intergenic region. **B)** Venn diagrams showing the pattern of overlap of these up-regulated intergenic regions among the NusA and Rho mutants and also the pattern obtained in the presence of bicyclomycin (BCM), antibiotic that binds to Rho. In these analyses, we included the same and the neighboring (± 300nt) intergenic regions. **C)** List of prophage genes that are upregulated both in the NusA (G181D, R258C) and Rho mutants (P279S, G324D, N340S) and also upon addition of BCM. Meaning of "+" and "++" are indicated below the tables. **D)** The list of small RNA genes that are up-regulated in the NusA mutants. **E)** Description of some of the long operons, *tds, gfc, fim* and *wec,* of which at least one of the genes (*tdcD, gfcE, fimD* and *wecf*; indicated by shades) were upregulated in the NusA and Rho mutants as validated by q-PCR assays (figure 8). Different tables show the fold changes in gene expression levels of different genes in these long operons in the presence of different NusA and Rho mutants. The fold changes *w.r.t.* the WT strain for the Rho mutants were obtained from ref. 1.

Reference:

1. Shashni, R., S. Mishra, B.S. Kalayani & R. Sen, (2012) Suppression of in vivo Rho-dependent transcription termination defects: evidence for kinetically controlled steps. *Microbiology* **158**: 1468-1481.