Table S1. Strains and plasmids used

Name	Characteristics	Source
	Strains	
EAEC 042	Enteroaggregative E. coli strain 042	1
042ΔaarΔaggR	042 derivative carrying aar and aggR deletions	This study
042ΔaarΔaggRΔhns	042 derivative carrying aar, aggR and hns deletions	This study
042ΔaarΔaggR hns repair	042 derivative carrying aar and aggR deletions with repaired hns	This study
042ΔaafA	042 derivative carrying aafA deletion	2
E. coli DH5α	K12 strain	Lab collection
DH5αΔhns	DH5α derivative carrying hns deletion	This Study
	Plasmids	
pBR322	Cloning and expression vector (Amp^R, Tet^R)	Lab collection
pACYC177	Cloning and expression vector (Amp^R, Km^R)	Lab collection
pKNT25	BACTH vector for fusions to the C-terminus of fragment T25	Lab collection
pPrham-aar	pBR322 derivative encoding Aar under the rhamnose promoter	This study
pPlacZ-aggR	pACYC177 derivative encoding AggR under the lacZ promoter	This study
pPlacZ-aggR-D	pACYC177 derivative encoding AggR dimerization domain 69-181 under the lacZ promoter	This study
pKNTHNS	Plasmid encoding orf 1292 fused to the T25 fragment of CyaA	3

1. Nataro JP, Yikang D, Cookson S, Cravioto A, Savarino SJ, Guers LD, Levine MM, Tacket CO. 1995. Heterogeneity of Enteroaggregative “Escherichia coli” virulence demonstrated in volunteers. J Infect Dis 171:465–468.
2. Izquierdo M, Alvestegui A, Nataro JP, Ruiz-Perez F, Farfan MJ. 2014. Participation of integrin α5β1 in the fibronectin-mediated adherence of enteroaggregative Escherichia coli to intestinal cells. Biomed Res Int 2014:781246.
3. Santiago AE, Yan MB, Hazen TH, Sauder B, Meza-Segura M, Rasko DA, Kendall MM, Ruiz-Perez F, Nataro JP. 2017. The AraC Negative Regulator family modulates the activity of histone-like proteins in pathogenic bacteria. PLoS Pathog 13:e1006545.

Table S2. primers

Name	DNA sequences (5->3)
Primers employed for deletion of aggR (Region 41,080-41,877; GenBank FN554767.1)	
LRAggRFd	TTTTGCCGTTACGCACCACTCCGTCAGTAGCTGAACAGGAG GGACAGCTGATAGAAACAGAAGCCACTGGAGCACCTCAAAA ACACCATCATACACTAAATCAGTAAGTTGGCAGCATCACCAA CTTCAGCCATCTCAATATGTTTATAGCAATCTCAAATAATGATA TGAAACATGTTTGTGTAGGCTGGAGCTGCTTC
LRAggRrev	AAGAATACGATAAATAATTTCCTATTGTAATTATAAGCGTAAAAA TCATATCCCACATGACGATGTGGAAATTAACAAACGTATTTTAT ATGAGTTAAAAATATATCTTTTTATTGATAAGAGTTAGGTCATT CTAACGCAGATTGCCTGATAAAGACATTTTTTTCATGTGAGAA TGATATGGGAATTAGCCATGGTCC
Primers employed for deletion of hns (Region 1,376,831-1,377,244; GenBank FN554767.1)	
hns lambda forward	CGGCGCAAATAGGGCTATATGCCGCGTCTTTTCTGGCTAATT TTATGAAAAGATATTTATTGGCGGCACAAAATAAAGAACAATTT TGAATTCCTTACATTCCTGGCTATTGCACAACTGAATTTAAGG CTCTATTATTACCTCAACAAACCACCCCAATATAAGTTTGAGAT TACTACAGTGTAGGCTGGAGCTGCTTC
hns lambda reverse	AAGTAACATCCGTATCGGTGTTATCCACGAAACGGCGTTGAG TAATCGACGCCGTTTTTTTATAGCTTATTCTTATTAAATTGTCTT AAACCGGACAATAAAAAATCCCGCCGATGGCGGGATTTTTAA GCAAGTGCAATCTACAAAAGATTATTGCTTGATCAGGAAATCG TCGAGGGAATGGGAATTAGCCATGGTCC
Primers employed for screening of 042ΔaarΔaggR and 042ΔaarΔaggRΔhns and DH5αΔhns	
AggRFDLR	TTCAGCCATCTCAATATGTTTATAGCA
AggRrevLR	TGGACTGTTGCGATCGTGAAGCC
AggRFdLR1	TACCGGGTTGAGAAGCGGTGTAA
KanrevLR1	TTGTCCAGATAGCCCAGTAGCTG
Hns sense	ATGAGCGAAGCACTTAAAATTCTGAACAACATCCGTACTCTT CGTGCGC
Hns rev	TTATTGCTTGATCAGGAAATCGTCGAGGGATTTACC
H-NS (upstream) sense	ATCCTTCTGAGCTATCATTACAACTGCC
Primers employed for recombining hns in 042ΔaarΔaggRΔhns and screening of 042ΔaarΔaggR hns repair	
HnsFd	CCCTTACGAAGCCTTGCATAATCCTTCTGAG
HnsRv	GGTGAAAGCGTACCGATGGTTGGC

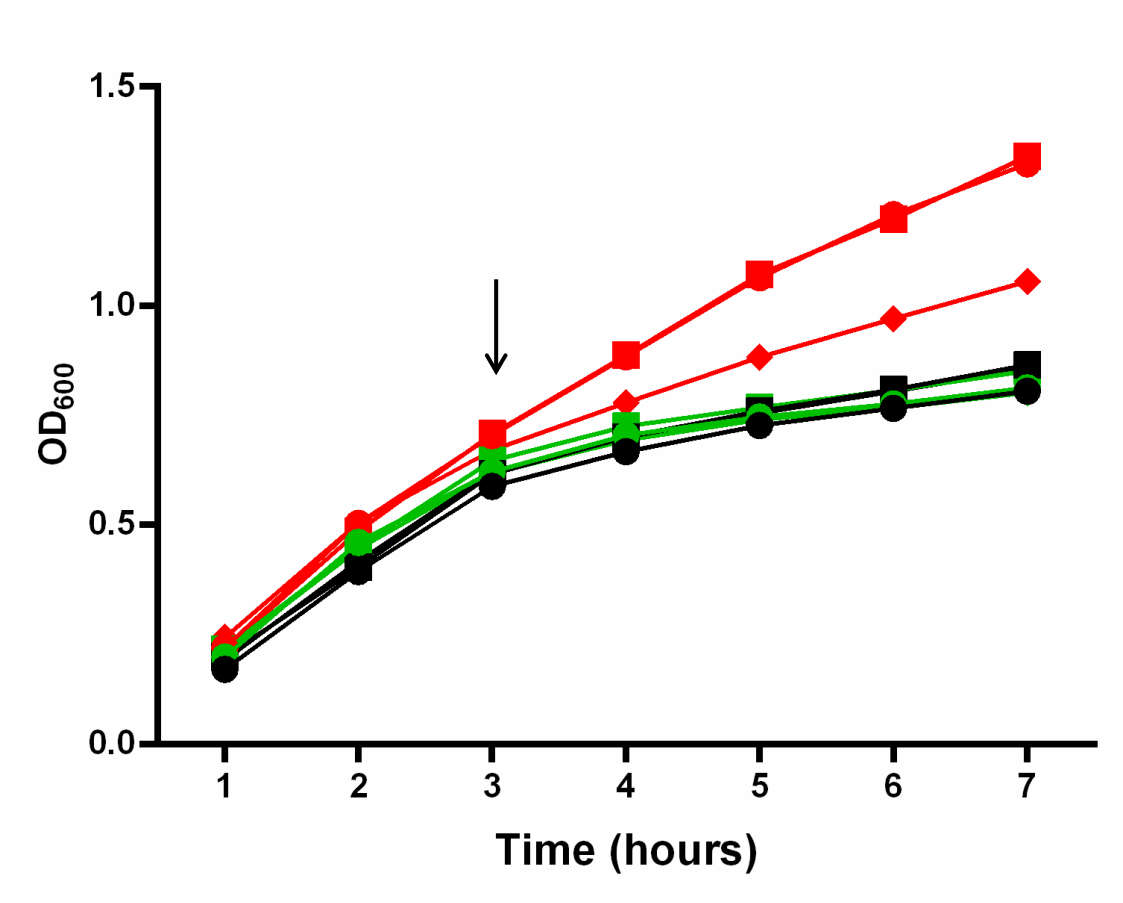

Figure S1. Growth curves in presence and absence of inducer molecules. Growth curves were measured for WT 042 (circles), 042Δ*aar*Δ*aggR* (squares), and 042Δ*aar*Δ*aggR*(p*aar*)(p*aggR*) (diamonds) in LB (black), LB+1mM IPTG (green), or LB+1% rhamnose (red). Growth curve data are representative of at least three independent experiments.

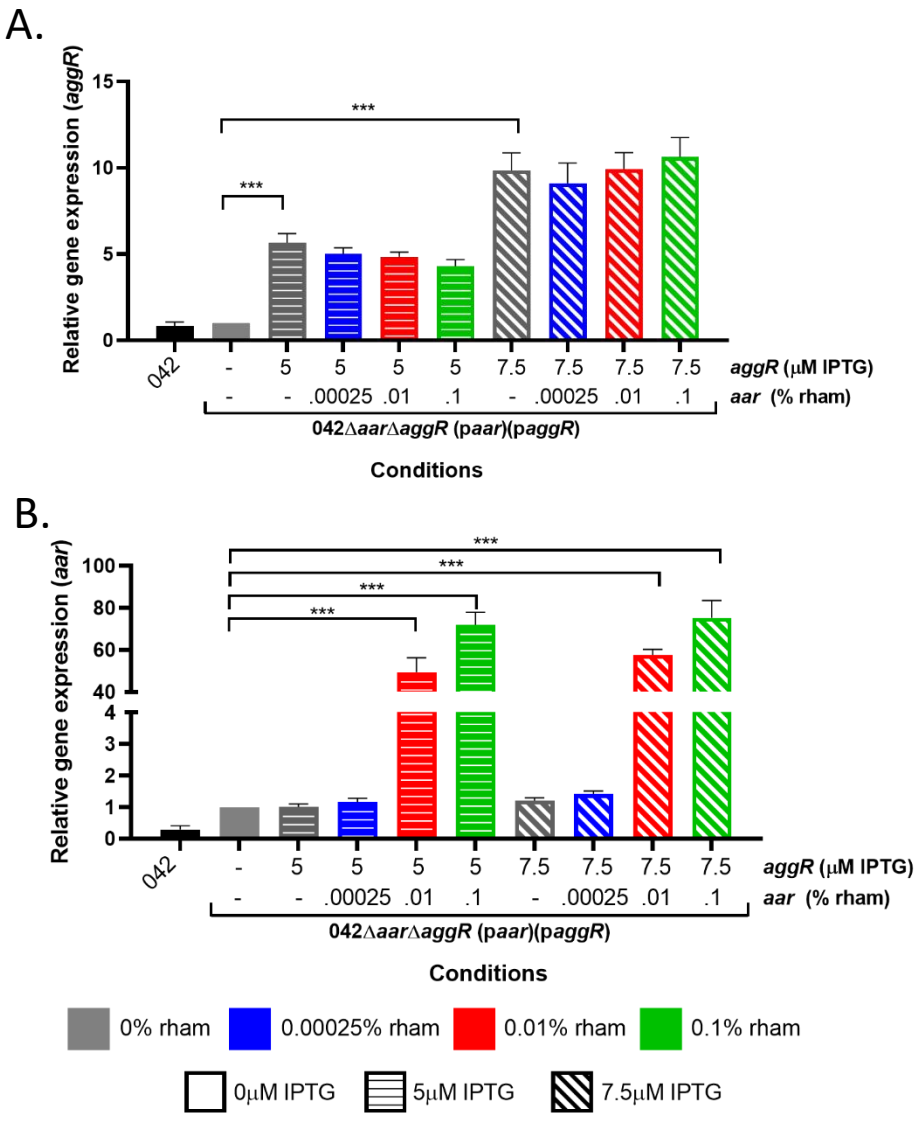

Figure S2. Titration expression of *aar* and *aggR* in 042*ΔaarΔaggR*. (A) qRT-PCR analysis of *aggR* and (B) *aar* using titratable *aar* and *aggR. aggR* expression was induced with either 5μM IPTG (horizontal fill pattern) or 7.5μM IPTG (diagonal fill pattern). *aar* expression was induced with 0.00025% rham (blue), 0.01% rham (red), or 0.1% rham (green). Biofilm data and qRT-PCR data are representative of at least three independent experiments. Asterisks indicate significant differences by ANOVA (*, P<0.05; **, P<0.005; ***, P<0.0005).

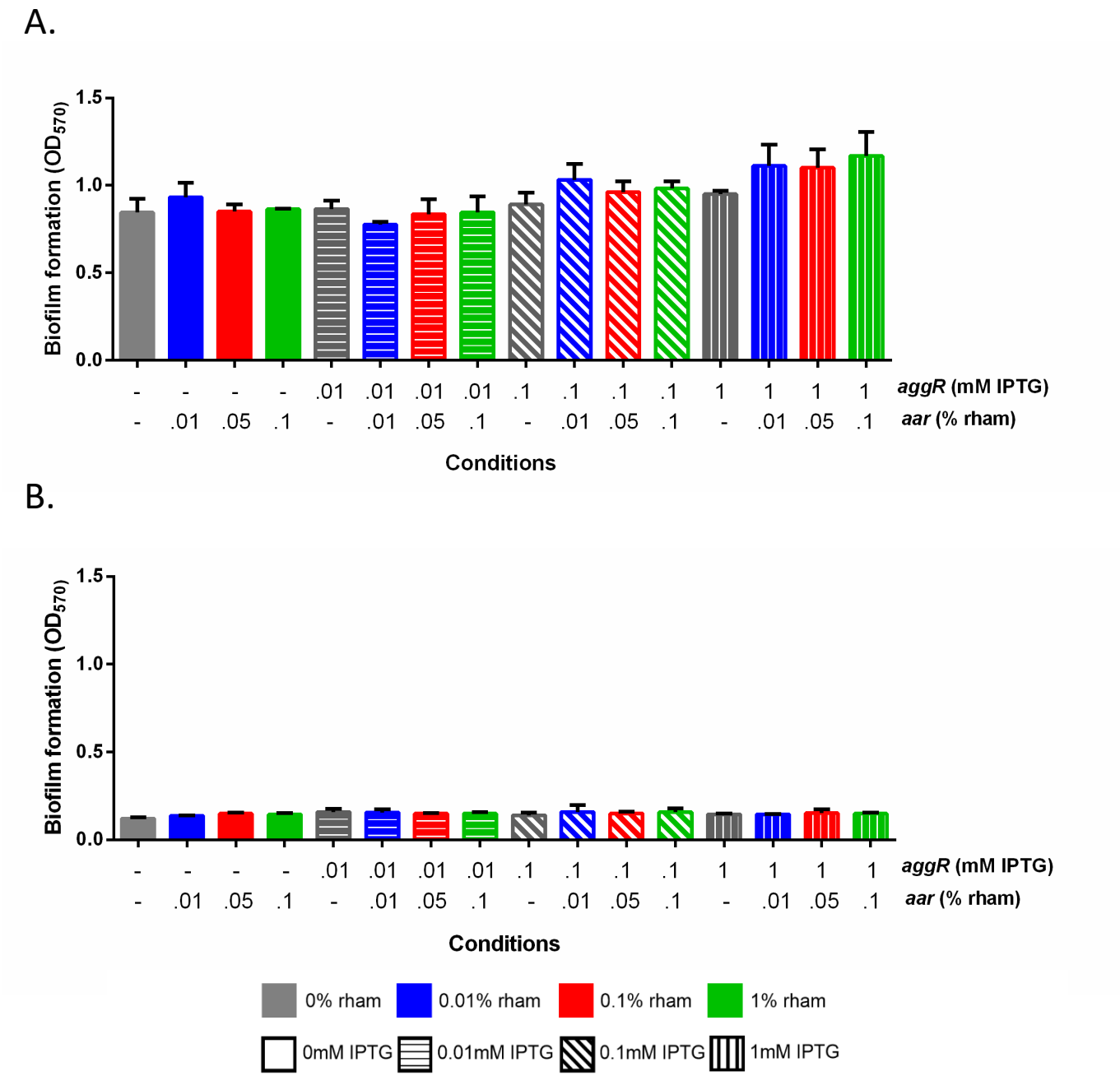

Figure S3. Inducer effects in titration constructs. (A) Biofilm growth of 042*ΔaarΔaggR*(p*aggR*) at 3 hours post induction with increasing concentration of IPTG and rhamnose. (B) Biofilm growth of 042*ΔaarΔaggR*(p*aar*) at 3 hours post induction with increasing concentration of IPTG and rhamnose. *aggR* expression was induced with 0.01mM IPTG (horizontal fill pattern), 0.1mM IPTG (diagonal fill pattern), or 1mM IPTG (vertical fill pattern). *aar* expression was induced with 0.01% rham (blue), 0.05% rham (red), or 0.1% rham (green). Biofilm data are representative of at least three independent experiments.

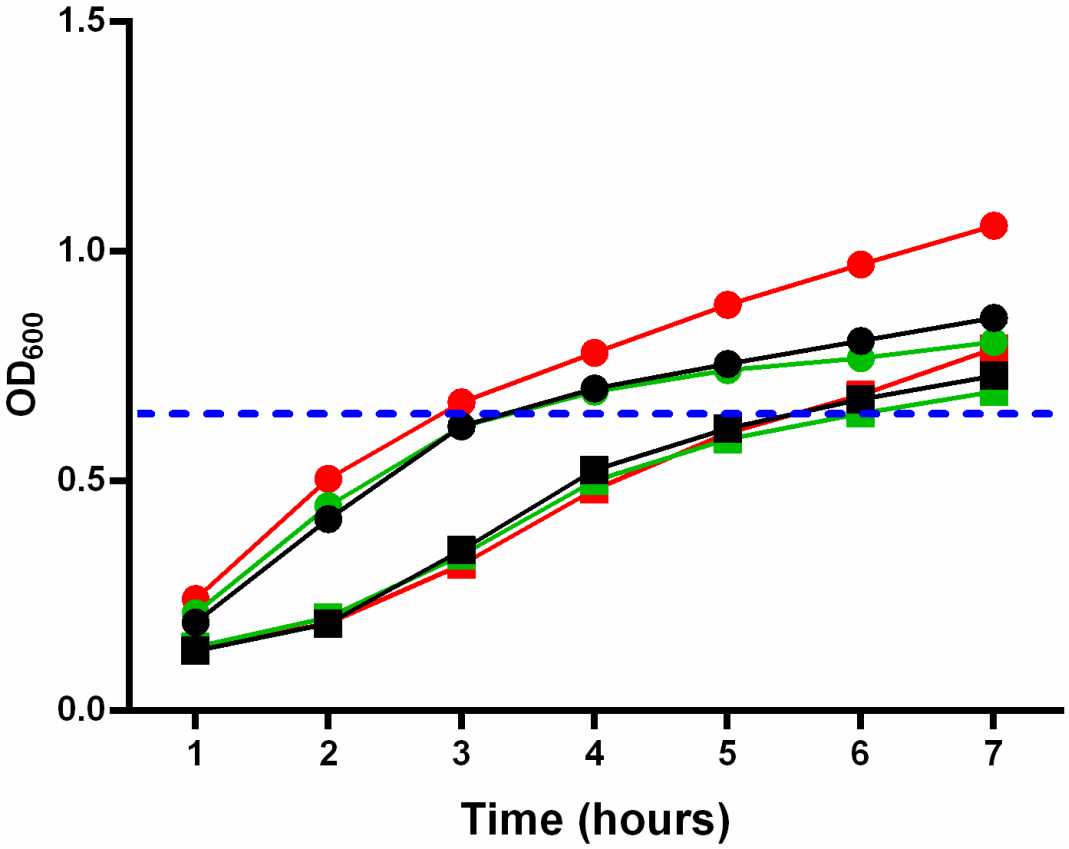

Figure S4. Growth curve of 042Δ*aar*Δ*aggR* and 042Δ*aar*Δ*aggR*Δ*hns* in different conditions. Growth curves were measured for 042Δ*aar*Δ*aggR*(p*aar*)(p*aggR*) (circles) and 042Δ*aar*Δ*aggR*Δ*hns*(p*aar*)(p*aggR*) (squares) in LB (black), LB+1mM IPTG (green), or LB+1% rhamnose (red). Growth curve data are representative of at least three independent experiments.

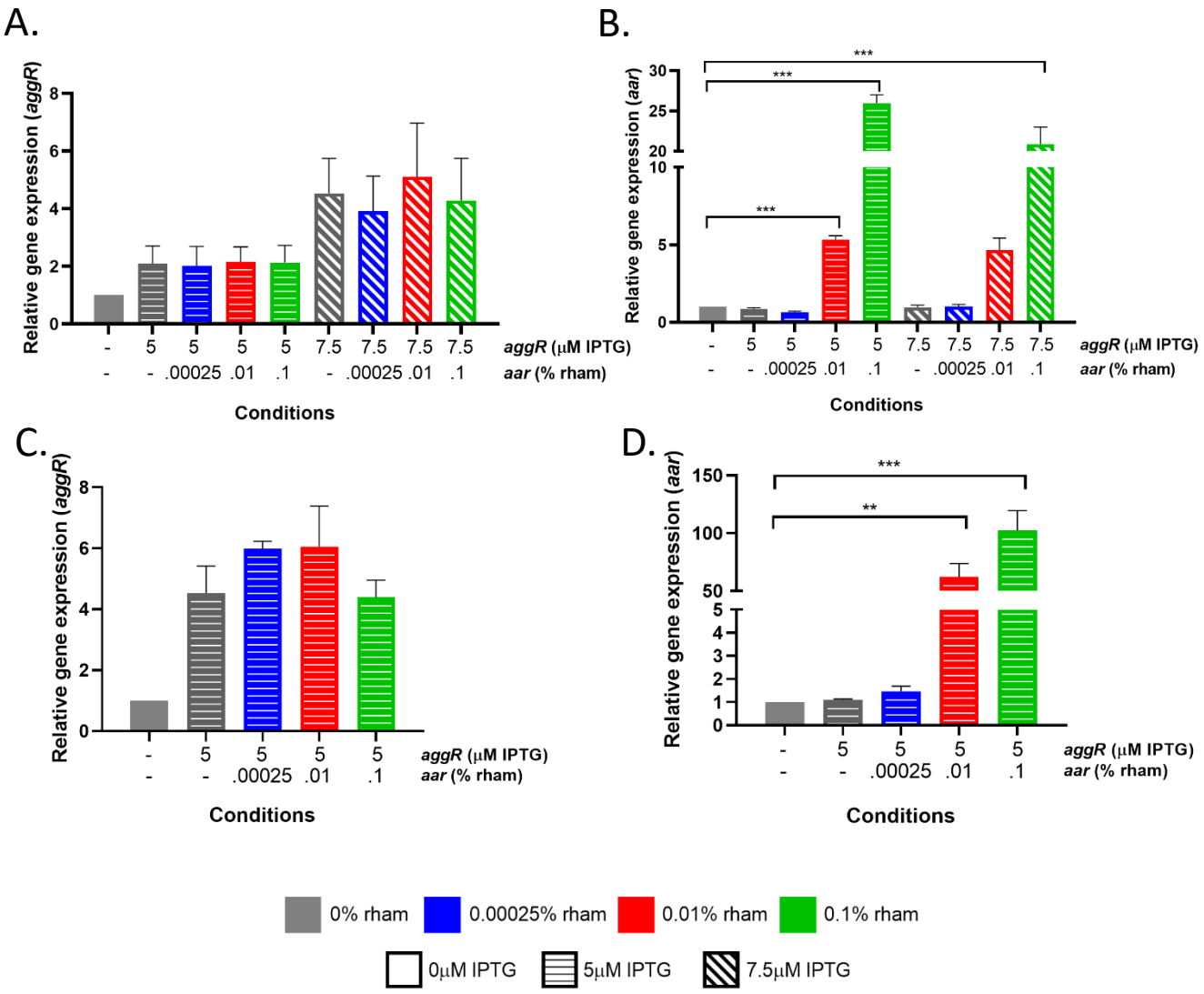

Figure S5. Titration expression of *aar* and *aggR* in 042Δ*aar*Δ*aggR*Δ*hns* and *hns* repair. (A) qRT-PCR analysis of *aggR* and (B) *aar* using titratable *aar* and *aggR* in 042Δ*aar*Δ*aggR*Δ*hns* after 5h. (C) qRT-PCR analysis of *aggR* and (D) *aar* using titratable *aar* and *aggR* in the *hns* repaired 042Δ*aar*Δ*aggR* after 3h. *aggR* expression was induced with either 5μM IPTG (horizontal fill pattern) or 7.5μM IPTG (diagonal fill pattern). *aar* expression was induced with 0.00025% rham (blue), 0.01% rham (red), or 0.1% rham (green). qRT-PCR data are representative of at least three independent experiments. Asterisks indicate significant differences by ANOVA (*, P<0.05; **, P<0.005; ***, P<0.0005).

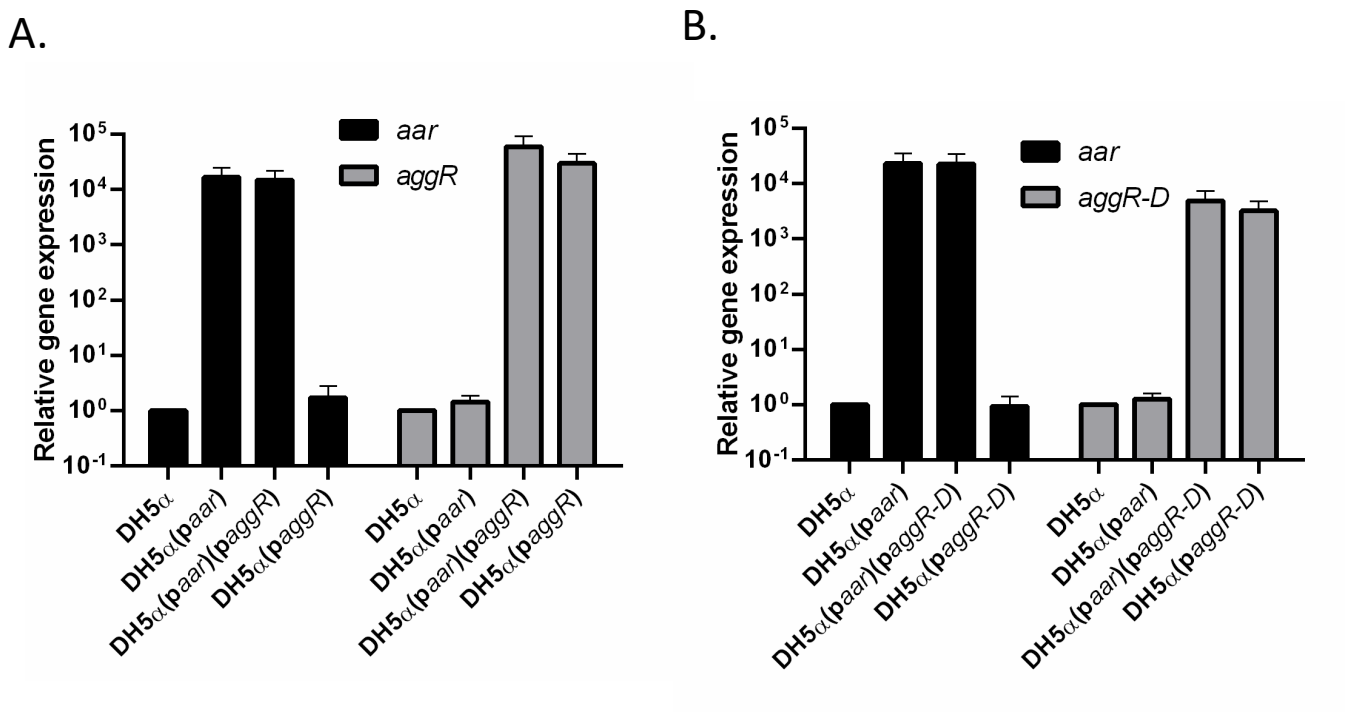

Figure S6. Expression levels of *aar*, *aggR*, and *aggR-D* in DH5α transformed with p*aar* and/or p*aggR*/p*aggR-D*. (A) DH5α was transformed with p*aar* and p*aggR* expressing full length *aggR* or their corresponding empty vectors pBR322 and pACYC177, respectively. Transcriptional levels of *aar* and *aggR* were analyzed by qRT-PCR. (B) DH5α was transformed with p*aar* and p*aggR-D* expressing the AggR dimerization domain or their corresponding empty vectors. Transcriptional levels of *aar* and *aggR-D* were analyzed by qRT-PCR. RT-PCR data are representative of at least three independent experiments .

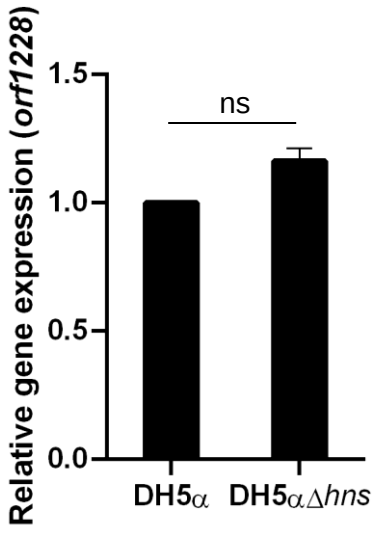

Figure S7. Expression of *orf1228* in DH5α and DH5αΔ*hns*. Transcriptional levels of *orf1228* in DH5α and DH5αΔ*hns* were analyzed by qRT-PCR. RT-PCR data are representative of at least three independent experiments .