FIG. S1. Growth curve of ST4/74 WT and mutants in MM with 1% glucose as the sole carbon source. *S.* Typhimurium ST4/74 and mutants, including strains with complementing plasmids, used in this study were grown overnight in LB media. MM containing 1% glucose was inoculated at 1:100 and incubated for 20 h at 37 °C (not shaken). OD_{600} was measured hourly. The experiment was performed three times, and expressed as mean ± standard derivation.

FIG. S2. Binding of GatR to promoter fragments of *gat* genes. (A) Overproduced GatR-His_6 was purified from strain ST4/74 Δ*araA* cultivated in LB medium (left) or in MM with galactitol (right), and increasing amounts of the protein were incubated with a 300 bp fragment representing the promoter of *gatR*. EMSAs were then performed by separation of the DNA/GatR-His_6 mixtures on 12% polyacrylamide gels. Competitor DNA representing the promoter of *argS* (50 ng) served as control. The molar excess of protein to DNA is indicated. The GeneRuler DNA ladder mix was used as a marker. **(B)** As above using promoter fragments of *gatZ* and *gatY*. GatR-His_6 isolated from cultures grown in MM with galactitol was used.

FIG. S3. Growth curves of ST4/74, ST4/74 Δ*crp*, and ST4/74 Δ*crp*/pBAD-*crp*. Growth conditions are described in the legend of **FIG. S1**.

FIG. S1

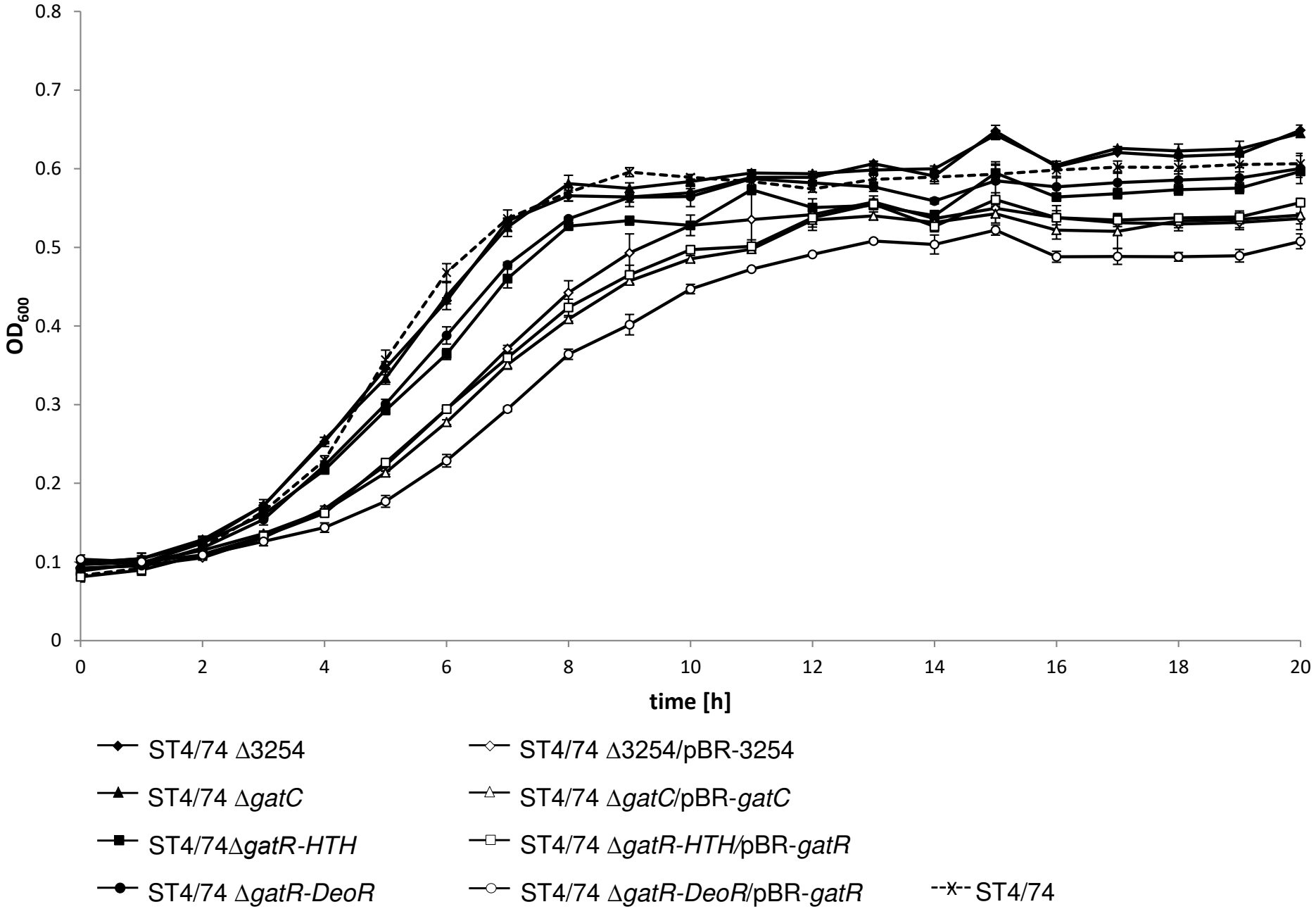
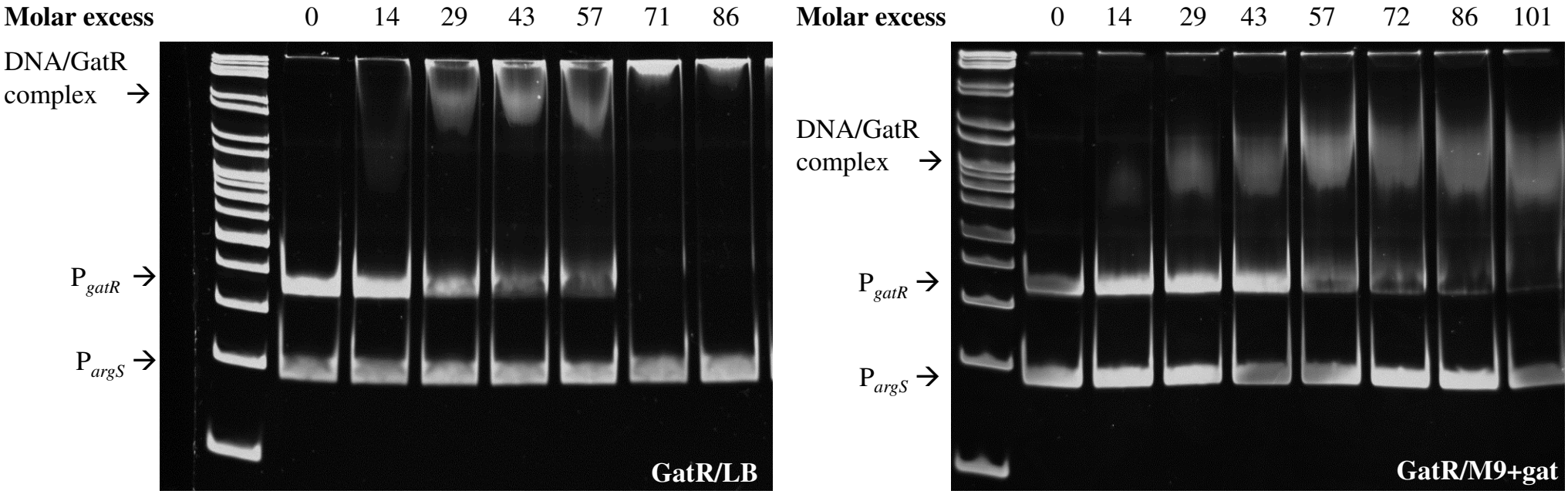

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD600
0
2
4
6
8
10
12
14
16
18
20
time [h]
ST4/74 Δ3254
ST4/74 Δ3254/pBR-3254
ST4/74 ΔgatC
ST4/74 ΔgatC/pBR-gatC
ST4/74ΔgatR-HTH
ST4/74 ΔgatR-HTH/pBR-gatR
ST4/74 ΔgatR-DeoR
ST4/74 ΔgatR-DeoR/pBR-gatR
ST4/74

FIG. S2

A


Molar excess
0 14 29 43 57 71 86
DNA/GatR complex
P_{gatR}
P_{argS}
GatR/LB
Molar excess
0 14 29 43 57 72 86 101
DNA/GatR complex
P_{gatR}
P_{argS}
GatR/M9+gat

B

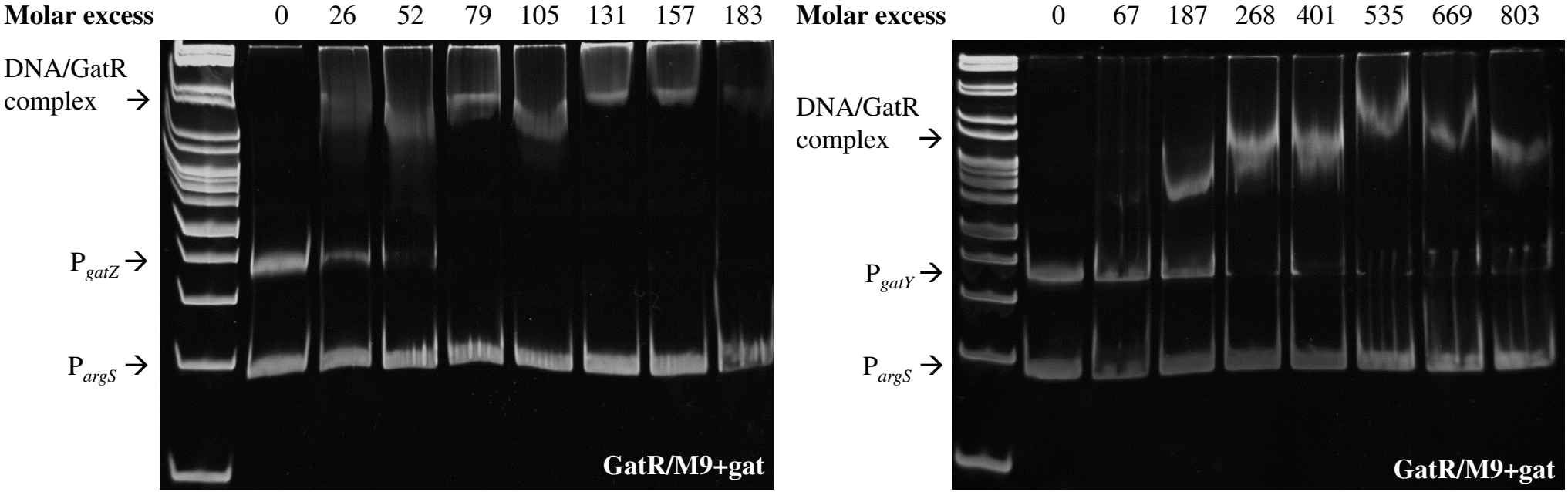

Molar excess
0 26 52 79 105 131 157 183
DNA/GatR complex
P_{gatZ}
P_{argS}
GatR/M9+gat
Molar excess
0 67 187 268 401 535 669 803
DNA/GatR complex
P_{gatY}
P_{argS}
GatR/M9+gat

FIG. S3

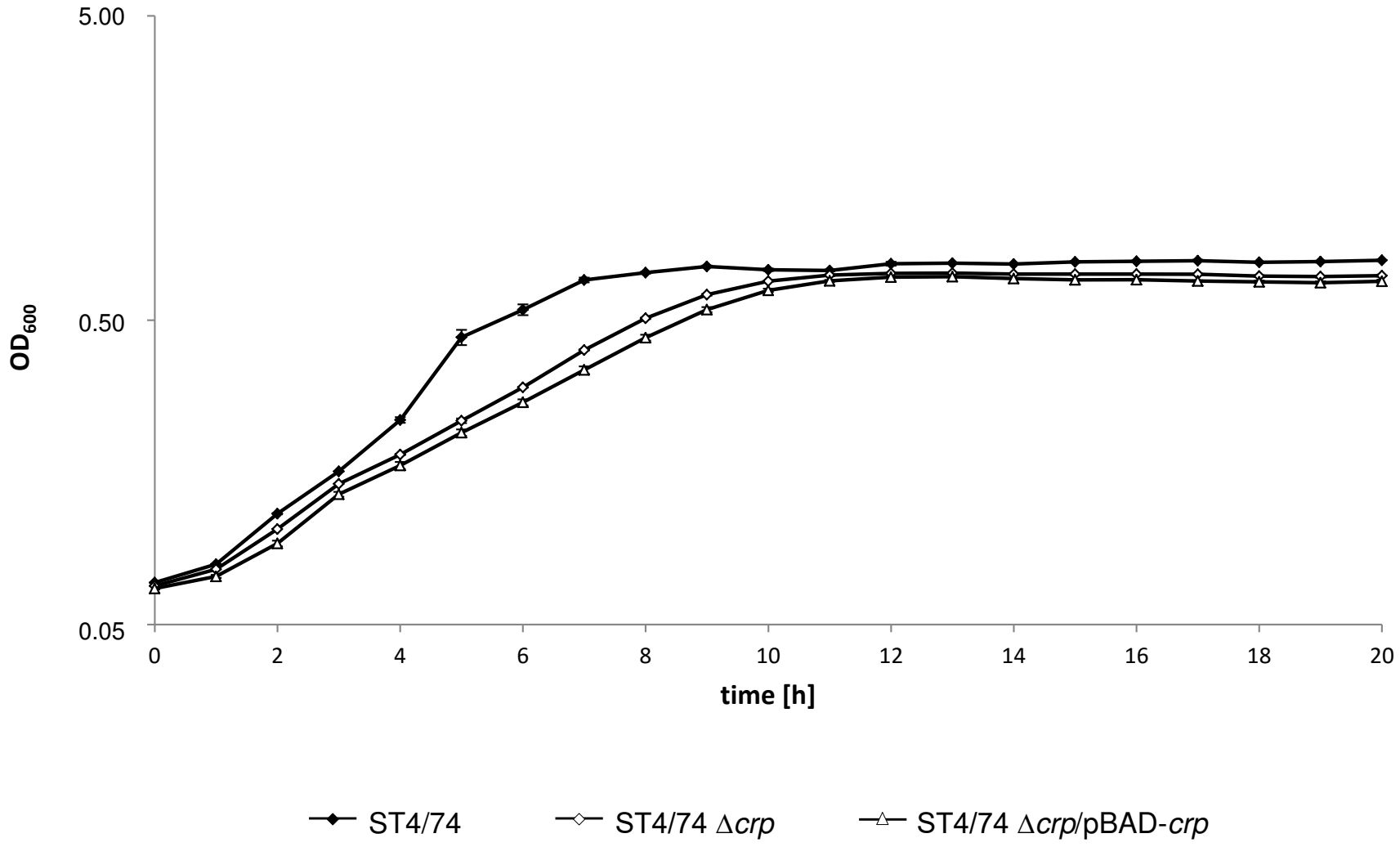

5.00
0.50
0.05
OD600
0
2
4
6
8
10
12
14
16
18
20
time [h]
ST4/74
ST4/74 Δcrp
ST4/74 Δcrp/pBAD-crp

TABLE S1. Primers used in this study.

primer name	target gene	modification	5'-3' sequence
*construction of non-polar deletion mutants**			
GatR_Del_fwd	*gatR*		AACTAGTTACAGTTAATTATCAGGCAAATCAATATGAACTCATTTGAGCGGTGTAGGCTGGAGCTGCTTC
Gat_op_Del_rev	*gatY - gatR/ gatR*		TAACCGACTCCTTTTTAGACGATCATTAATTCCACGCCTGTTTTCGCTAACATATGAATATCCTCCTTA
STM3254_Del_fwd	*STM3254*		GCGGCTGTGAAGGGCAACTGTGAGGAATCGCCTGTGATCTACACGCTAACGTGTAGGCTGGAGCTGCTTC
STM3254_Del_rev	*STM3254*		TATTGTAATTTAACCTACAGCTTTCTGACGTGGATTTGCGAAAGTAAGGTCATATGAATATCCTCCTTA
GatC_Del_fwd	*gatC*		AAATTCTGTCCATTCTCATGGGGTAACGTATGTTTAGCGAAATAATGCGGTGTAGGCTGGAGCTGCTTC
GatC_Del_rev	*gatC*		TTGCTCCCCTAAAATTAAGAGGCGATTTGACTTTGCTGTAGCGCCGTGGCCATATGAATATCCTCCTTA
gatR_Del-DR_fwd	*gatR-DeoR*		GGAAGTCATCTGGCGGAAGGCGACAATCAGGAAGTCATTCTGGAAGATCGGTGTAGGCTGGAGCTGCTTC
gatR_Del_HTH_rev	*gatR-HTH*		GCAGCCGCCTGGGCAATCCGCTTTTTCGGATCGCTGGCGAGTTGATACCGCATATGAATATCCTCCTTA
AraA_Del_fwd	*araA*		GCAATCCTGACTCATTAAGGACACGACAATGACGATTTTTGATAATTATGGTGTAGGCTGGAGCTGCTTC
AraA_Del_rev	*araA*		CAATCCGTTTCACCAATTAACGTTTGAACCCGTAATACACCTCGTTCCAGCATATGAATATCCTCCTTA
Crp_Del_fwd	*crp*		CTGGAGACAGCTTATAACAGAGGATAACCGCGCATGGTGCTTGGCAAACCGTGTAGGCTGGAGCTGCTTC
Crp_Del_rev	*crp*		CCATTCTGACGGAATTAACGGGTGCCGTAGACGACGATGGTCTTGCCATGCATATGAATATCCTCCTTA
*construction of pBR322-complementation vectors** and test of insertion of kanR and gene deletion*			
GatR_ScaI_fwd	*gatR*	*Sca*I	GATC**AGTACT**CTGATGGTACGAGCCAGCAAC
Gat_Op_PstI_rev	*gatY - gatR/ gatR*	*Pst*I	GATC**CTGCAG**CTTTCCTTCGGGCTTACTCTC
STM3254_PvuI_fwd	*STM3254*	*Pvu*I	GATC**CGATCG**CCCGCCACTATATGCAACC
STM3254_PstI_rev	*STM3254*	*Pst*I	GATC**CTGCAG**GTATGCGCAATACCGGTAG
GatC_PvuI_fwd	*gatC*	*Pvu*I	GATC**CGATCG**CGTGGGTATTGAAGCATTAC
GatC_PstI_rev	*gatC*	*PstI*	GATC**CTGCAG**CATAACATTCTCCAGGAAGG
AraA_fwd	*araA*		GATGGCGCTTCGCTTATC
AraA_rev	*araA*		AGCTTTACCGCCGCTACCAGCAG
Crp_fwd	*crp*		GCATGTATGCAGAGGACATC
Crp_rev	*crp*		ACCAGCGTTTGCCGTAGTGC
pBR322_PstI_seq***	verify complementation		GAGTAAGTAGTTCGCCAG
pBR322_ScaI_seq***	verify complementation		GGCGCGGTATTATCCCG
*construction of recombinant pBADMyc/HisC plasmids**			
GatR_C_fwd_XhoI	*gatR*	*Xho*I	GATCCTCGAGTATGAACTCATTTGAGCGAAG
GatR_C_rev_HindIII	*gatR*	*Hind*III	GATCAAGCTTGACGATCATTAATTCCACGC
*cloning, testing and sequencing of reporter fusions pUTs-luxCDABE(Cm^R) and pUTs-gfp(Cm^R)**			
PgatY_NotI_fwd	promoter *gatY*	*Not*I	GATC**GCGGCCGC**AAGTTATCCCGTGCTTTAAC
PgatY_KpnI_rev	promoter *gatY*	*Kpn*I	GATC**GGTACC**GGATGTTAAAGGCTGGCACG
PgatY_KpnI_fwd	promoter *gatY*	*Kpn*I	GATC**GGTACC**TTCCGCTTCGTCGGGAAG
PgatY_XmaI_rev	promoter *gatY*	*Xma*I	GATC**CCCGGG**TAAAGGCTGGCACGGCATAG
P3257_NotI_fwd	promoter *STM3257*	*Not*I	GATC**GCGGCCGC**AAGATGCACAGCAGGCACTAC
P3257_KpnI_rev	promoter *STM3257*	*Kpn*I	GATC**GGTACC**TCTCCTGCTTTATGACGAGC
P3257_KpnI_fwd	promoter *STM3257*	*Kpn*I	GATC**GGTACC**CGAAGATGCACAGCAGGCAC
P3257_XmaI_rev	promoter *STM3257*	*Xma*I	GATC**CCCGGG**TCTCCTGCTTTATGACGAGC
PgatR_NotI_fwd	promoter *gatR*	*Not*I	GATC**GCGGCCGC**AGTTGGATGAACTACTCCG
PgatR_KpnI_rev	promoter *gatR*	*Kpn*I	GATC**GGTACC**GAAAGGTCCATCACCAGCAC
PgatR_KpnI_fwd	promoter *gatR*	*KpnI*	GATC**GGTACC**TCCTCGGCAGTTGGATGAAC
PgatR_XmaI_rev	promoter *gatR*	*Xma*I	GATC**CCCGGG**GAAAGGTCCATCACCAGCAC

luxC1R***	Test of insertion (pUTs-*lux*)		CATAAGGCAATATTTGCTCA
pUTs-TestF***	Test of insertion (pUTs-*lux* or pUTs-*gfp*)		TGGAATTCTGACTCTTATAC
GFPtestR	Test of insertion (pUTs-*gfp*)		CCTTCACCCTCTCCAC
*construction of fragments used in GMS assay***			
PgatY_bs_fwd	promoter *gatY*		CACCGAATGCTCAATGG
PgatY_bs_rev	promoter *gatY*		TAAAGGCTGGCACGGCATAG
PgatY_1_1_fwd	promoter *gatY* part I		CACTTTTTCTTTCATTTAAG
PgatY_SPR_1_rev	promoter *gatY* part I		ACATGATTGTGATCATCAAC
PgatY_SPR_2_fwd	promoter *gatY* part II		ATTATCCTGCTTTTGTGTTG
PgatY_2_2_rev	promoter *gatY* part II		AGTAATGTAACGATCATTGC
PgatY_SPR_3_fwd	promoter *gatY* part III		ATGATCGTTACATTACTTTC
PgatY_SPR_2_rev	promoter *gatY* part III		TTTACTGGAAATAATGAAC
GatY-54_fwd	region upstream von STM3254		GAGCGACTGGCAGAAATACG
P54_bs_rev	region upstream von STM3254		CGCGCTATTAAGTGTTAGCGTG
3254-55_fwd	region upstream von STM3255		CGCCAACGGTTGATTTGGTG
P55_bs_rev	region upstream von STM3255		GGCTTCTTCAGCCATAAATGTATGC
3255-56_fwd	region upstream von STM3256		GATCCTTTGCGTGTCATCCC
P56_bs_rev	region upstream von STM3256		TATCGAGGCGCTCATCGCTG
P57_bs_fwd	promoter *gatZ*		CCACCAGAAGTCATGCAGCCAAAG
P57_bs_rev	promoter *gatZ*		CAGATGCCCAGATGTTCTCC
P57_SPR_2_fwd	promoter *gatZ* part I		ATTACACACCATTTCGTTAC
PgatZ_2_1_rev	promoter *gatZ* part I		AAATGAAAGTTTCGAAAGG
P57_SPR_3_fwd	promoter *gatZ* part II		TTTCGAAACTTTCATTTATC
PgatZ_3_2_rev	promoter *gatZ* part II		ATAGGCTTTCGTAATGTTG
PgatZ_2_3_fwd	promoter *gatZ* part III		CAACATTACGAAAGCC
P57_SPR_2_rev	promoter *gatZ* part III		CGAGCAATTATTTCTTTCAC
3257-gatA_fwd	region upstream von *gatA*		CACCCGCGTATTCGTCAAAG
PgatA_bs_rev	region upstream von *gatA*		TCTCTTTGCCAATGTGCGCC
gatA-B_fwd	region upstream von *gatB*		CCGCCTGTTTAGTGAGCTAC
PgatB_bs_rev	region upstream von *gatB*		CGCCGTCCATATAGGTTTCG
gatB-C_fwd	region upstream von *gatC*		GAGCTCGACTTAGTGCAATG
PgatC_bs_rev	region upstream von *gatC*		CCAAGCTTCATTCCCAGCAG
gatC-D_fwd	region upstream von *gatD*		ACCCAACTGGCTGCCAATGC
PgatD_bs-rev	region upstream von *gatD*		TCTCCCTCAGCGTGAATAAC
gatD-R_fwd	promoter *gatR*		CCAGGAGAAGAATGGGAAAC
PgatR_bs_rev	promoter *gatR*		CGCTGCCTTGCGTATTAATC
PgatR_1_1_fwd	promoter *gatR* part I		TCAACTTTCCTGATGGTACG
PgatR_SPR_1_rev	promoter *gatR* part I		GGAAATGTGAGCCAGCGCAG
PgatR_SPR-2_fwd	promoter *gatR* part II		CATGCAGGGCAAAATTTTAC
PgatR_SPR-2_rev	promoter *gatR* part II		AGTTCATATTGATTTGCCTG
SPR			
PgY_SPR-2_fwd_bt	promoter *gatY*	biotinylated	ATTATCCTGCTTTTGTGTTG
PgatY_SPR-2_rev	promoter *gatY*		TTTACTGGAAATAATGAAC
P57_SPR-2_fwd_bt	promoter *gatZ*	biotinylated	ATTACACACCATTTCGTTAC
P57_SPR-2_rev	promoter *gatZ*		CGAGCAATTATTTCTTTCAC
PgR_SPR-2_fwd_bt	promoter *gatR*	biotinylated	BIO-CATGCAGGGCAAAATTTTAC

PgatR_SPR-2_rev	promoter *gatR*		AGTTCATATTGATTTGCCTG
argS_SPR_fwd_bt	promoter *argS*	biotinylated	CAACCTTTGATTTGATTG
argS-conbs_rev	promoter *argS*		AAGAGCCTGAATATTCAC
gatC_SPR_fwd_bt	upstream region of *gatC*	biotinylated	GGCTGGTGATTGGTGTACTG
gatC_SPR_rev	upstream region of *gatC*		CAGTAGCGCTGGATCAAGGC
agaR_SPR_fwd_bt	promoter *agaR*	biotinylated	CCATTGAGCATTCGGTG
agaR_SPR_rev	promoter *agaR*		CAACAGGCTGGTAGATGGTT

* template DNA: pKD4
** template DNA: chromosomal DNA of *S*. Typhimurium ST4/74
***template DNA: plasmid in mutants (complementation, promoterfusions)

Table S2. Growth attenuation of mutants during colonization of animals.

ST4/74	Mouse FC	Chicken FS	Pig FS	Calf FS
gatY	-0,15	-0,01		-0,34
	-0,07	1,04		1,2
	0,07	0,75		0,41
	0,49	-5,05	-15	
STM3254	0,97	-1,36	-0,37	-0,5
	0,43	-4,26	-15	-15
	0,42	-2,78	-15	-15
	0,67			
	-1,59			
	0,062			
STM3255	0,10	-6,22	-15	-15
	-0,02	-2,79	-15	-7,61
	-0,5	-0,28	-0,02	0,03
	0,19	0,57	-0,002	1,75
	-0,54			
gatR	-1,38	-8,82	-4,87	-5,25
		-5,51	-1,60	
		-8,52	-1,18	

Supplementary Material:

The first *gat* gene cluster represented by that of strain ST4/74 is present in the genomes of *S. enterica* serovars Aqua, Abony, Bareilly, Bovismorbificans, Braenderup, Cerro, var. Copenhagen, Give, Hadar, Hartford, Hvittingfoss, Inverness, Javiani, LT2, Manhattan, Mississippi, Montevideo, Muenster, Muenchen, Namur, Norwich, Paratyphi A, Paratyphi B, Pomona, Poona, Ohio, Rubislaw, Saintpaul, Thompson, Typhi, Uganda, and Wandsworth.

The second type *gat* gene cluster is found in *S. enterica* serovars Agona, Alachua, Albany, Baildon, Bredeney, Chester, Choleraesuis, Cubana, Derby, Dublin, Enteritidis, Gallinarum, Gallinarum/Pullorum, Gaminara, Havana, Heidelberg, Indiana, Infantis, Kentucky, Mbandaka, Meleagridis, Nchanga, Newport, Rissen, Schwarzengrund, Senftenberg, Tennessee, Virchow, Weltevreden, and Worthington as well as in the species *S. bongori*.

The genes of the galactitol operon are completely absent in the genomes of *S. enterica* subspecies *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* (serovar Bornheim).