Supplementary Information to "Molecular Mechanisms Controlling Fructose-Specific Memory and Catabolite Repression in Lactose Metabolism by Streptococcus mutans"

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Includes Table S1 and Figures S1 to S9.

Table S1. Primers used in this study.

Primers	Sequence[a]	Purpose
fruI-1	5'- GGC GGT TTT ACT GGT AGG TT -3'	Deleting *fruI* from GS-5
fruI-2GA	5'- GCC ATT TAT TAT TTC CTT CCT CTT TTA GCC TGC AAG TCA AGC AAC A -3'	Deleting *fruI* from GS-5
fruI-3GA	5'- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GCT GTT GGA GCA GTG ATT GCT -3'	Deleting *fruI* from GS-5
fruI-4	5'- AGC TGC TTG GAG ATA ATC TTT ACC T -3'	Deleting *fruI* from GS-5
GAMM-Spc-5'	5'- TAA AAG AGG AAG GAA ATA ATA AAT GGC TGG GAG CTC TCC GGA TCC AA -3'	G.A. Spc marker
GAMM-Spc-3'	5'- TCT ACT AAA ACA ATT CAT CCA GTA AAA TAT ATG CTC CTC TAG ACT CGA GGA A -3'	G.A. Spc marker
pBGE-GA5'	5'- CGC CCG ACA CAA GAA GAT GT -3'	G.A. primer for pBGE cloning
pBGE-GA3'	5'- GTT CAA CCA TAG TCT CTC TCC TAA T -3'	G.A. primer for pBGE cloning
lacR-1	5'- CGT GAA GCA CCC TTT GCT A -3'	Deleting *lacR* from UA159
lacR-2GA	5'- GCC ATT TAT TAT TTC CTT CCT CTT TTA CCT TCC TCA TAA ATA CCT CTT TTC CT -3'	Deleting *lacR* from UA159
lacR-3GA	5'- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GTC AAG ATG ACG ATA AGC TTC A -3'	Deleting *lacR* from UA159
lacR-4	5'- GGC ATC AAT TGC AAT TCC CAA ATT T -3'	Deleting *lacR* from UA159
GS5_lacR-1	5'- GCC ATG ACA CGA TCT GAA AAG AAA CTT -3'	Deleting *lacR* from GS-5
GS5_lacR-4	5'- CAC CGC GAT TCC ACT TTT CTA GA -3'	Deleting *lacR* from GS-5
SK36_lacR-1	5'- CTT TAT CTG ATG GTG ATT TAA AGC ATG A -3'	Deleting *lacR* from SK36

SK36_lacR-2GA	5’- GCC ATT TAT TAT TTC CTT CCT CTT TTA CAT CCT GTC TCT GAT TTT TAC CCA -3’	Deleting *lacR* from SK36
SK36_lacR-3GA	5’- ATA TTT TAC TGG ATG AAT TGT TTT AGT AGA GCT CAG GAA GAA CTG GAA GT -3’	Deleting *lacR* from SK36
SK36_lacR-4	5’- CAG ATG AGG AGC TCA CCA GTA A -3’	Deleting *lacR* from SK36
lacR-5’Bm	5’- ATA ATA GAA GAA AAG AAG GAA AAG AGG GAT CCA TGA -3’	His-LacR
lacR-3’PstI	5’- CTG ATC ACT TTT TGT TAT CTG CAG ATT TAG T -3’	His-LacR
lacR-3'GA	5’- ATG CAG AGT CTC CTG TTT TAC AAC CGG GTG GTC TTC TCT CGC TCT CTT TGA -3’	Complementing *lacR*
lacR-5'GA	5’- TAC AGC CTA TAT TCG AGC AAG GTT TCG ACT GAA GTT GTG GAA ATT TAA TAG CAG ACA -3’	Complementing *lacR*
lacAEMSA-1	5’- GTGATCAGAAAAATAAGATAATCAAAGAGA -3’	EMSA
lacAEMSA-2bio	/5BiosG/GC CAT AGT CAT TCT CCT TGA AAT G -3’	EMSA
lacADFACE-3	5’- CTT CTT CTA ACA TTC TAA AAG CAA CTG CCA TAG TCA TTC TCC TTG AAA TG -3’	DFACE
lacADFACE-5	5’- TTG TCA CTT TTA GGA TTT TGC CGC GAG AGC GAG AGA AGA CC -3’	DFACE

[a] An insertion or mutation is indicated by underlining.

Fig. S1. Schematic diagram depicting the *spp* gene loci in UA159 and GS-5. Each open-reading frame (ORF) is represented by an arrow that is drawn to scale. The red rectangle represents the sequence that is missing in GS-5 in comparison to UA159. This sequence is instead replaced by a 5-ORF locus shown below, which is absent in the genome of UA159.

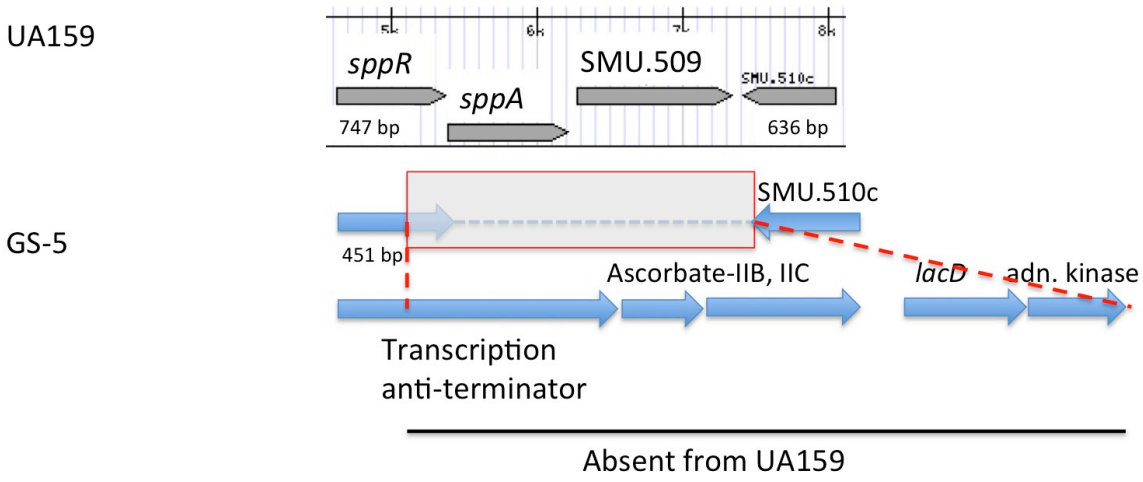


Fig. S2. Growth curves of a *lacR* mutant complemented with a wild-type copy *lacR.* A GS-5 derivative deficient in *lacR* was complemented using an integration vector pBGE that delivers an intact copy of *lacR* to downstream of the *gtfA* promoter. The bacterium was cultured to exponential phase (OD600 ≈ 0.5) in FMC supported by 20 mM of glucose (_G) or fructose (_F), before diluted into FMC supported by 10 mM lactose. Also included was a strain similarly constructed using an empty vector. The results are each the average of three biological repeats.

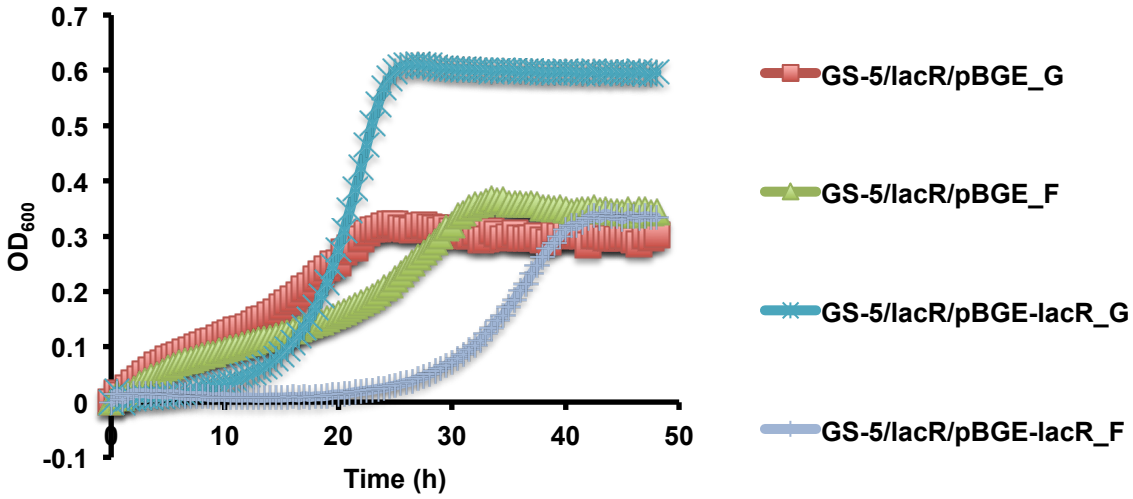
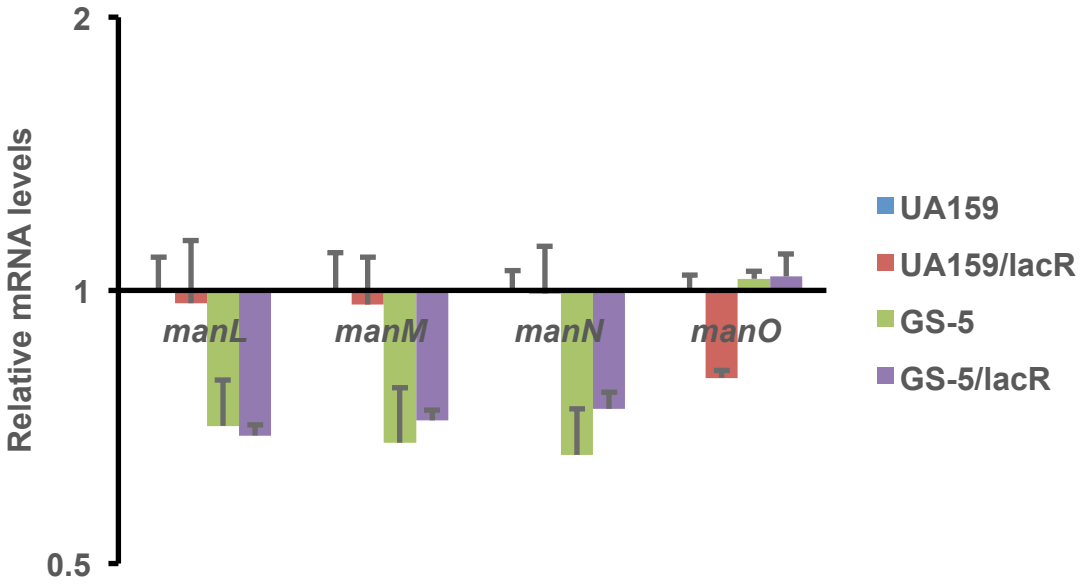


Fig. S3. Results of RT-qPCR measuring transcripts of the *manLMNO* operon (A) and Western blots of ManL (B). Cells were grown in FMC (A) or TV medium (B) supported by 0.5% of glucose. The Western blots were subsequently subjected to densitometry analysis to determine the relative levels of ManL protein.

A



B

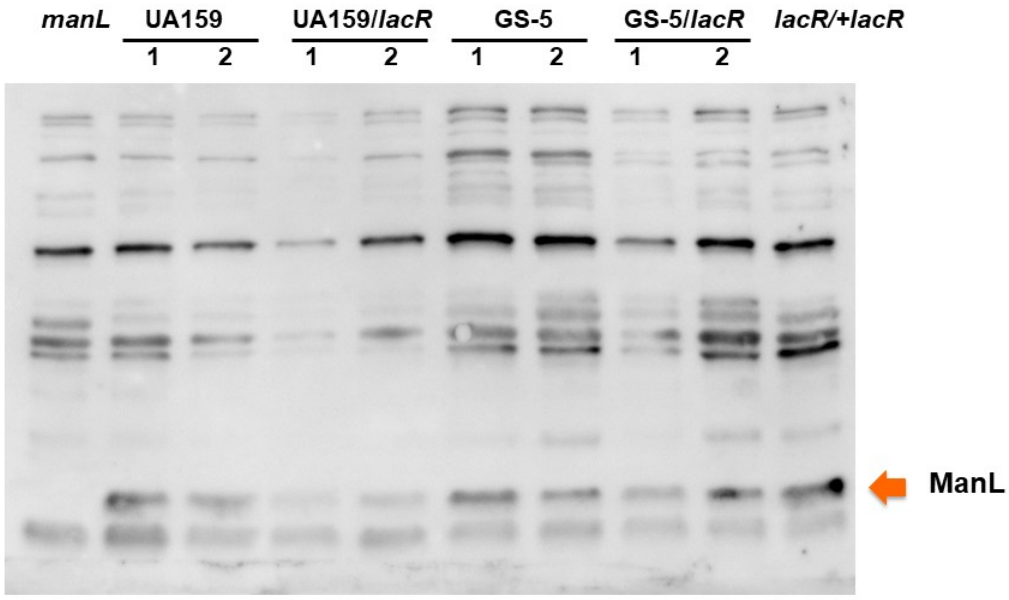
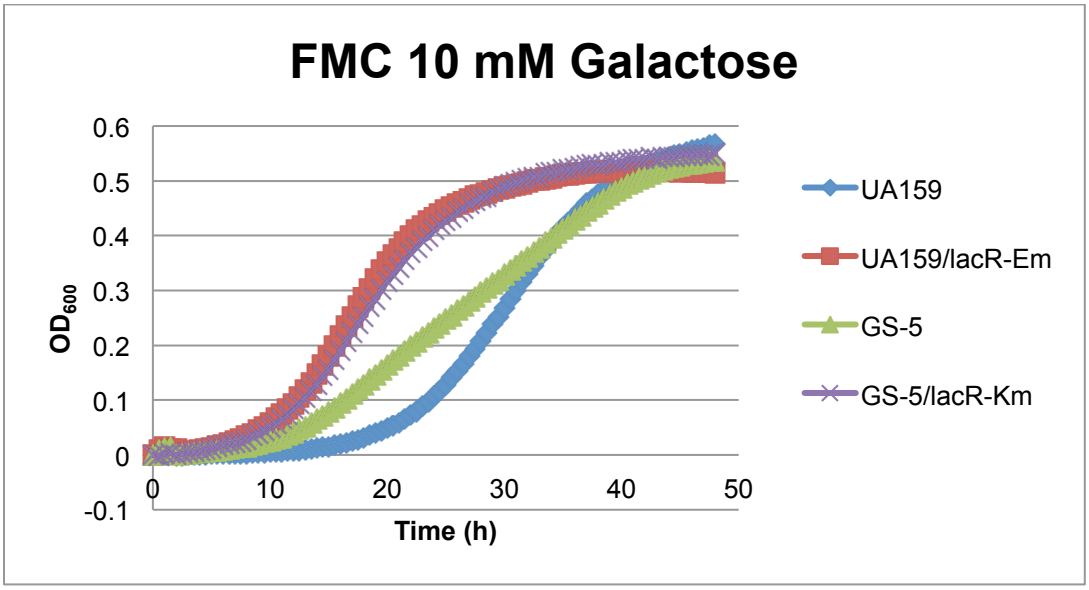
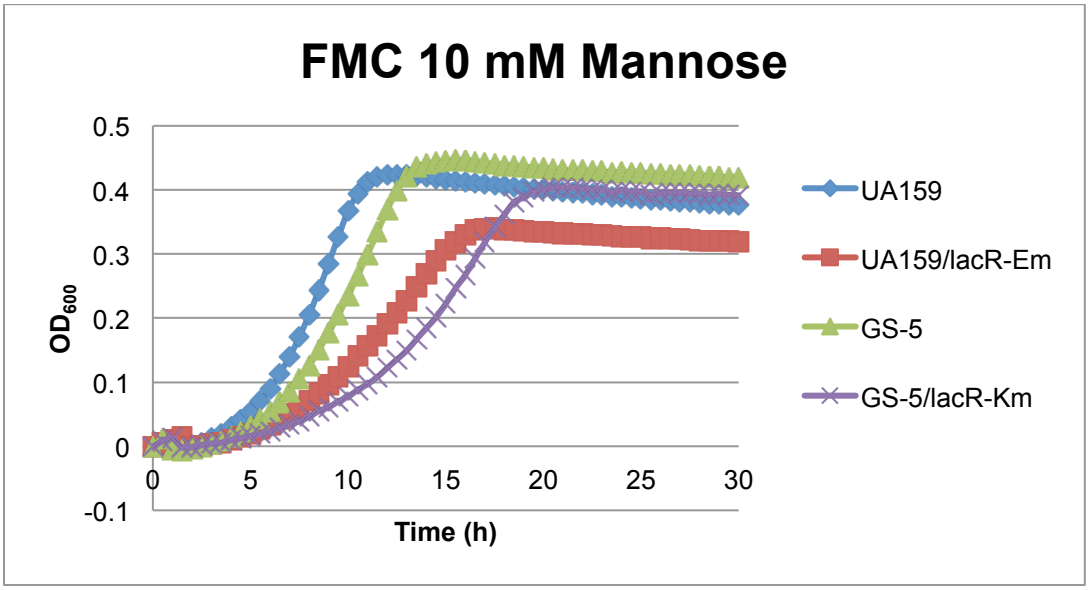
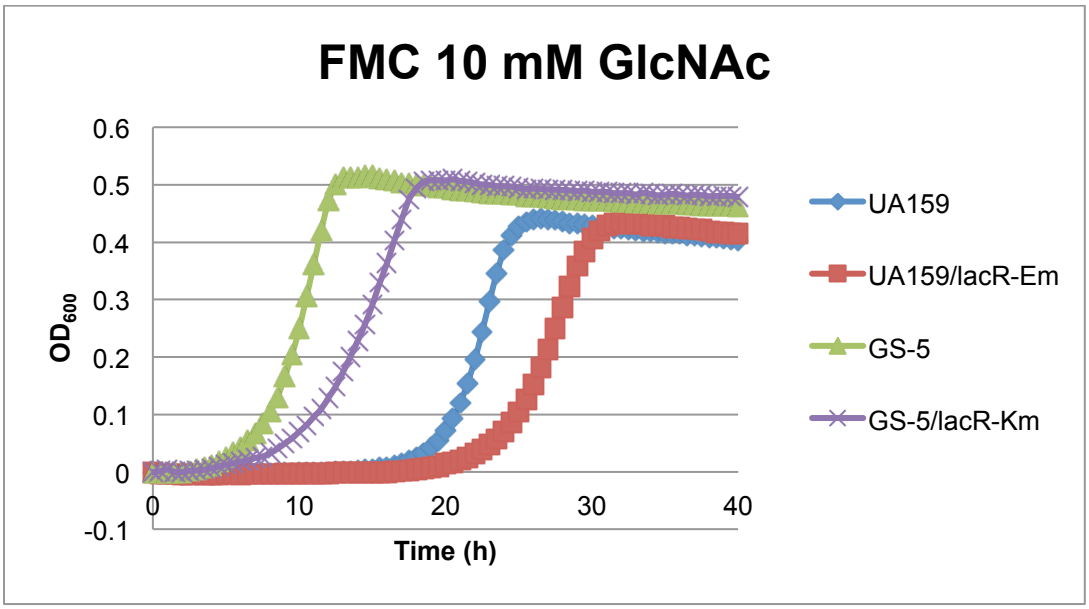
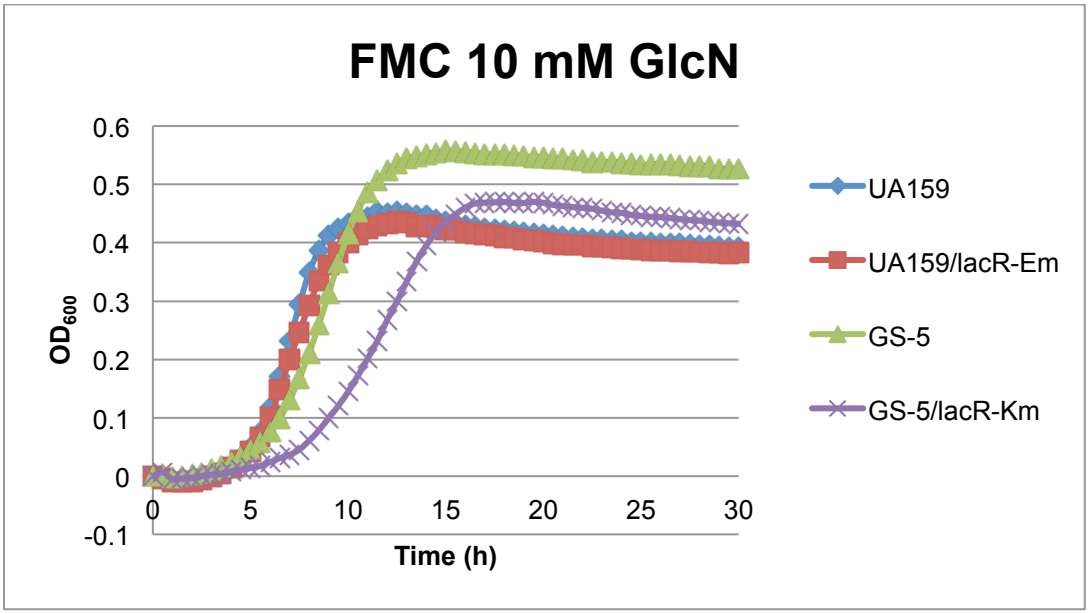


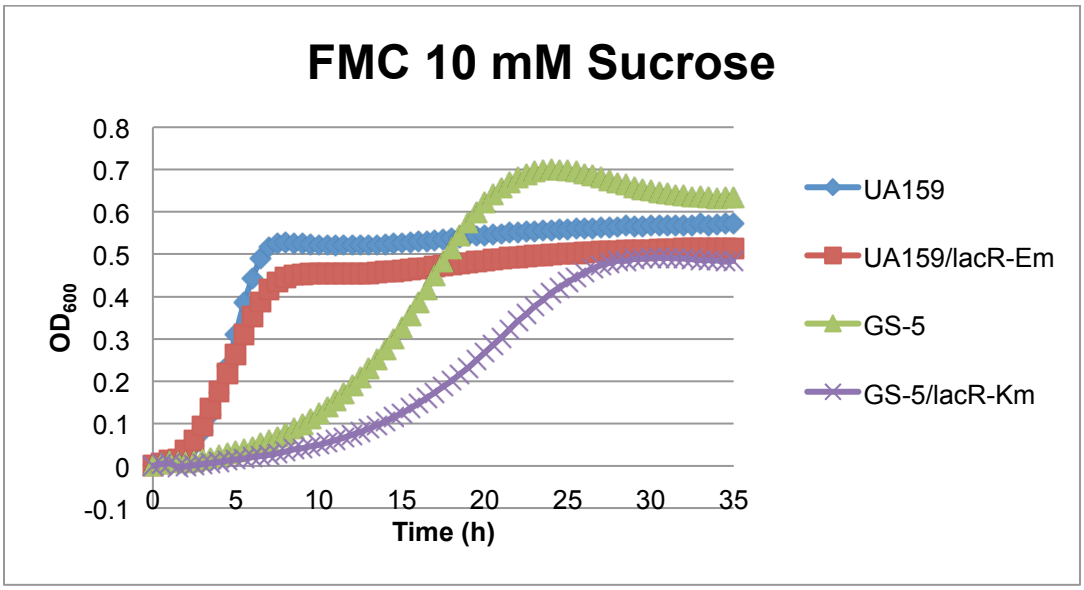
Fig. S4. Growth curves of *lacR* deficient mutants derived from various bacteria. Bacterial strains were grown in BHI to exponential phase (OD600 ≈ 0.5) before diluted at 1:100 ratio into FMC constituted with 10 mM of specified carbohydrates. Growth curves were recorded on a Bioscreen C system at 37°C for up to 2 days.

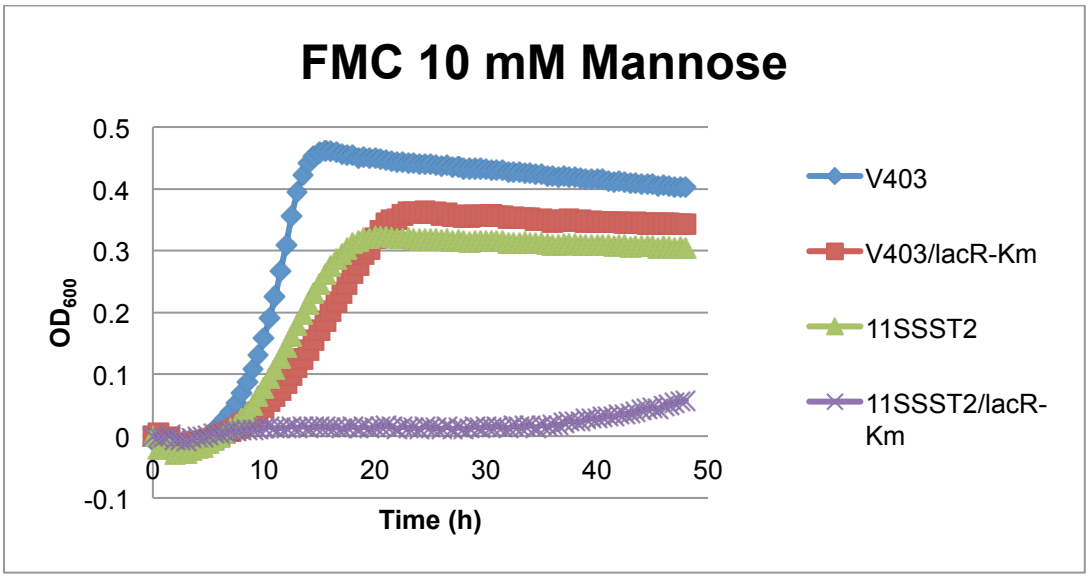


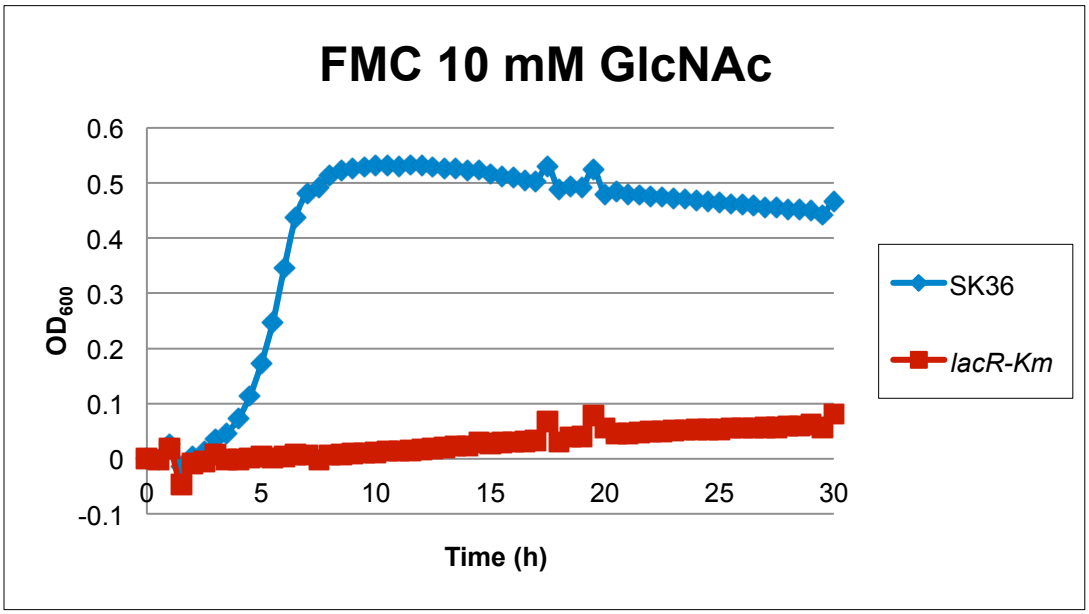



FMC 10 mM GlcNAc
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
OD600
0
10
20
30
40
Time (h)
UA159
UA159/lacR-Em
GS-5
GS-5/lacR-Km


FMC 10 mM GlcN
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
OD600
0
5
10
15
20
25
30
Time (h)
UA159
UA159/lacR-Em
GS-5
GS-5/lacR-Km


FMC 10 mM Sucrose
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
OD600
0
5
10
15
20
25
30
35
Time (h)
UA159
UA159/lacR-Em
GS-5
GS-5/lacR-Km


FMC 10 mM Mannose
0.5
0.4
0.3
0.2
0.1
0
-0.1
OD600
0
10
20
30
40
50
Time (h)
V403
V403/lacR-Km
11SSST2
11SSST2/lacR-Km


FMC 10 mM GlcNAc
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
OD600
0
5
10
15
20
25
30
Time (h)
SK36
lacR-Km

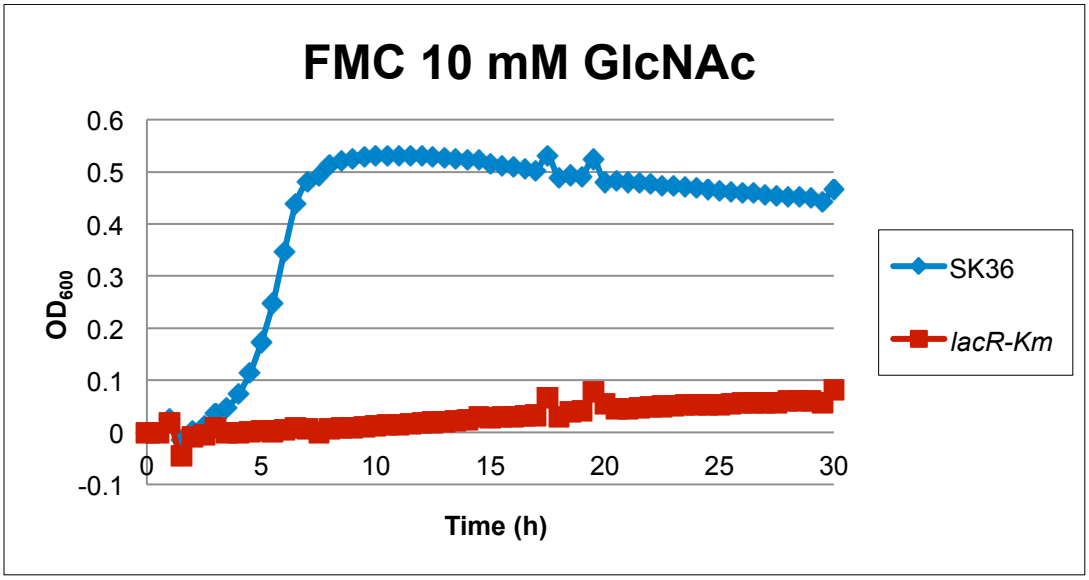

FMC 10 mM GlcNAc
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
OD600
0
5
10
15
20
25
30
Time (h)
SK36
lacR-Km

Fig. S5. SDS-PAGE of purified recombinant LacR proteins used in this study. *lacR* sequences derived from the genomes of GS-5 and UA159 were each cloned into plasmid pQE30 and pMal-p2x for overexpression. Recombinant His-LacR (A) and MBP-LacR (B, C) were then purified according to established protocols from suppliers. Both MBP-LacR proteins were digested with protease Factor Xa according to supplier's protocol (B). When released from MBP-LacR at high concentrations, both rLacR proteins showed a tendency to aggregate and fall out of solution, while MBP remained soluble (C, only showing results from UA159 MBP-LacR). This allowed us to further purify the rLacR protein through centrifugation and redissolving the pellet using a buffer with reduced ionic strength. Subsequently, all protein samples were resolved on an SDS-polyacrylamide gel and the proteins were visualized using a Coomassie Brilliant Blue R-250 stain.

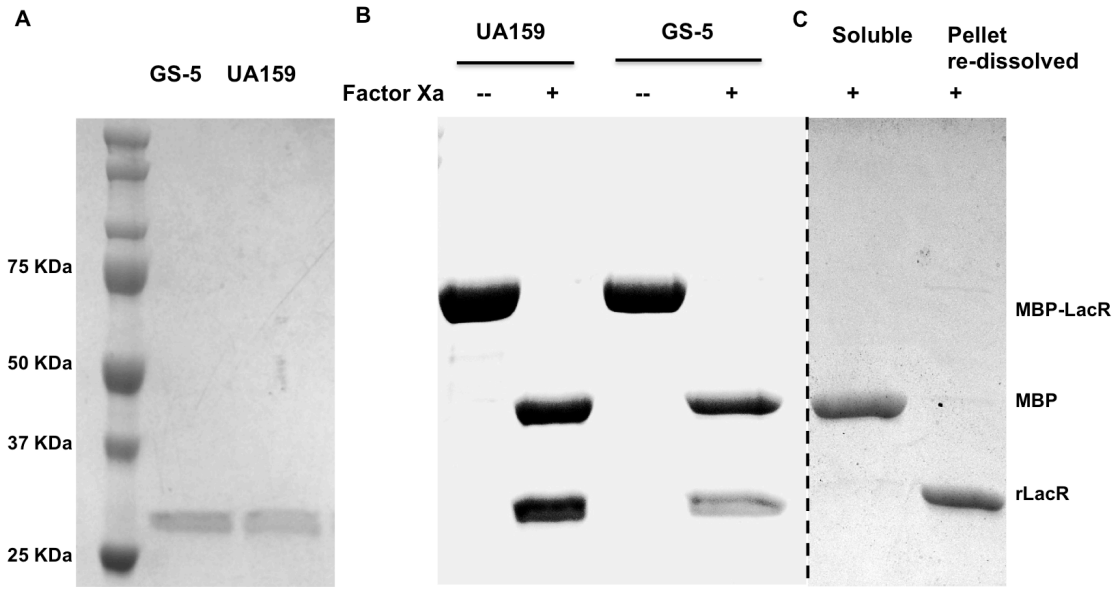
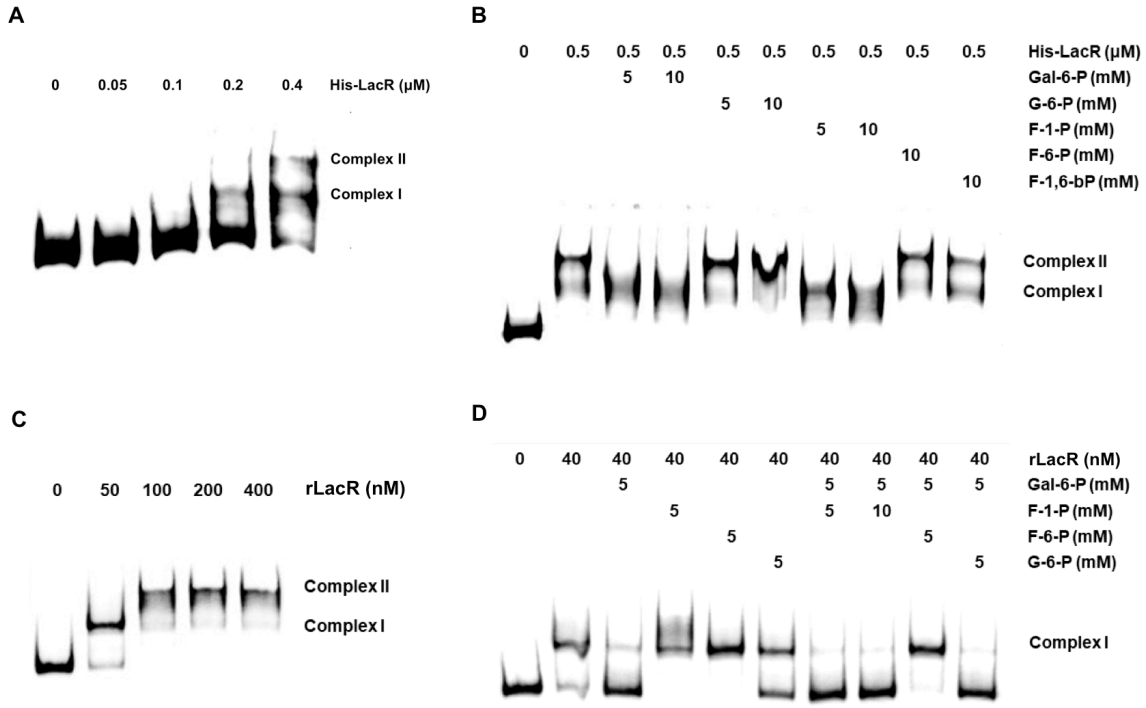


Fig. S6. Electrophoretic mobility shift assay (EMSA) performed with recombinant LacR and IGR of *lacA*, both derived from strain UA159. Reactions were performed at room temperature using varying amounts of His-LacR protein (A, B) or rLacR released from MBP-LacR fusion protein (C, D, E), in the presence (B, D, E) or absence (A, C) of various metabolic intermediates. Panel (D) indicates that there might be some minor effects by F-1-P and F-6-P when used alone, however this was disproven when repeated (E).



E


0 5 10 20 40 40 40 40 40 40
rLacR (nM)
5
Gal-6-P (mM)
5
F-1-P (mM)
5
F-6-P (mM)
5
F-1,6-bP (mM)
5
G-6-P (mM)
UA159
Complex I

Fig. S7. DNase I footprinting assay (DFACE) between rLacR and IGR of *lacA* conducted in GS-5 background. The fluorescently-labeled *lacA* probe was allowed to interact with 0 or 2 μM rLacR protein, and 0 or 5 mM of sugar phosphate (CHO-P). Boxes denote two sites (A, B) that are protected by rLacR, and one showing hypersensitivity in the presence of Gal-6-P.

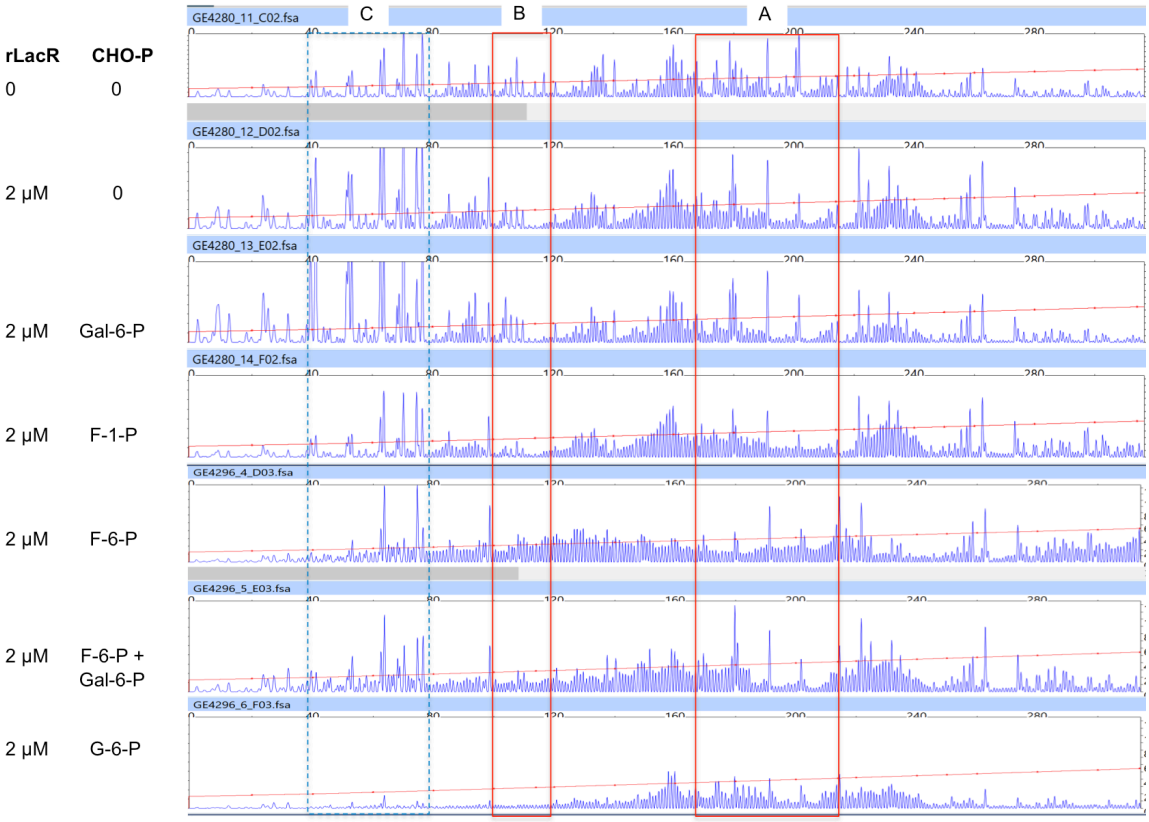


Fig. S8. Growth curves of the *lacR* mutant of GS-5 on galactose. GS-5 wild type and its otherwise-isogenic mutant *lacR* were cultured to exponential phase in FMC supported by 20 mM of glucose (_G) or fructose (_F), before diluted into FMC supported with 20 mM galactose for growth monitoring. The results are each the average of at least three biological repeats.

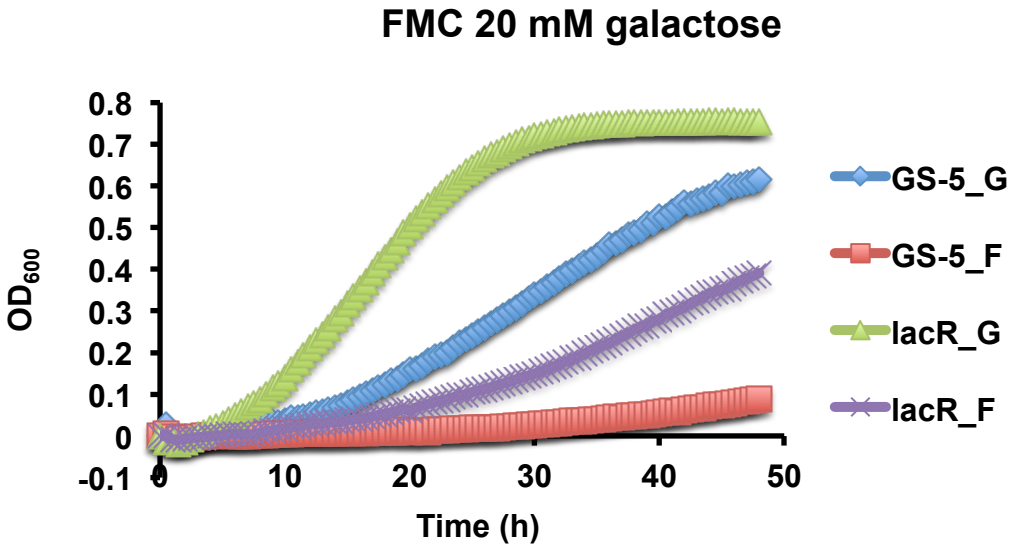


Fig. S9. EMSA using rLacR derived from MBP-LacR and His-CcpA in interaction with biotin-labeled *lacA* probe derived from both UA159 and GS-5 backgrounds.