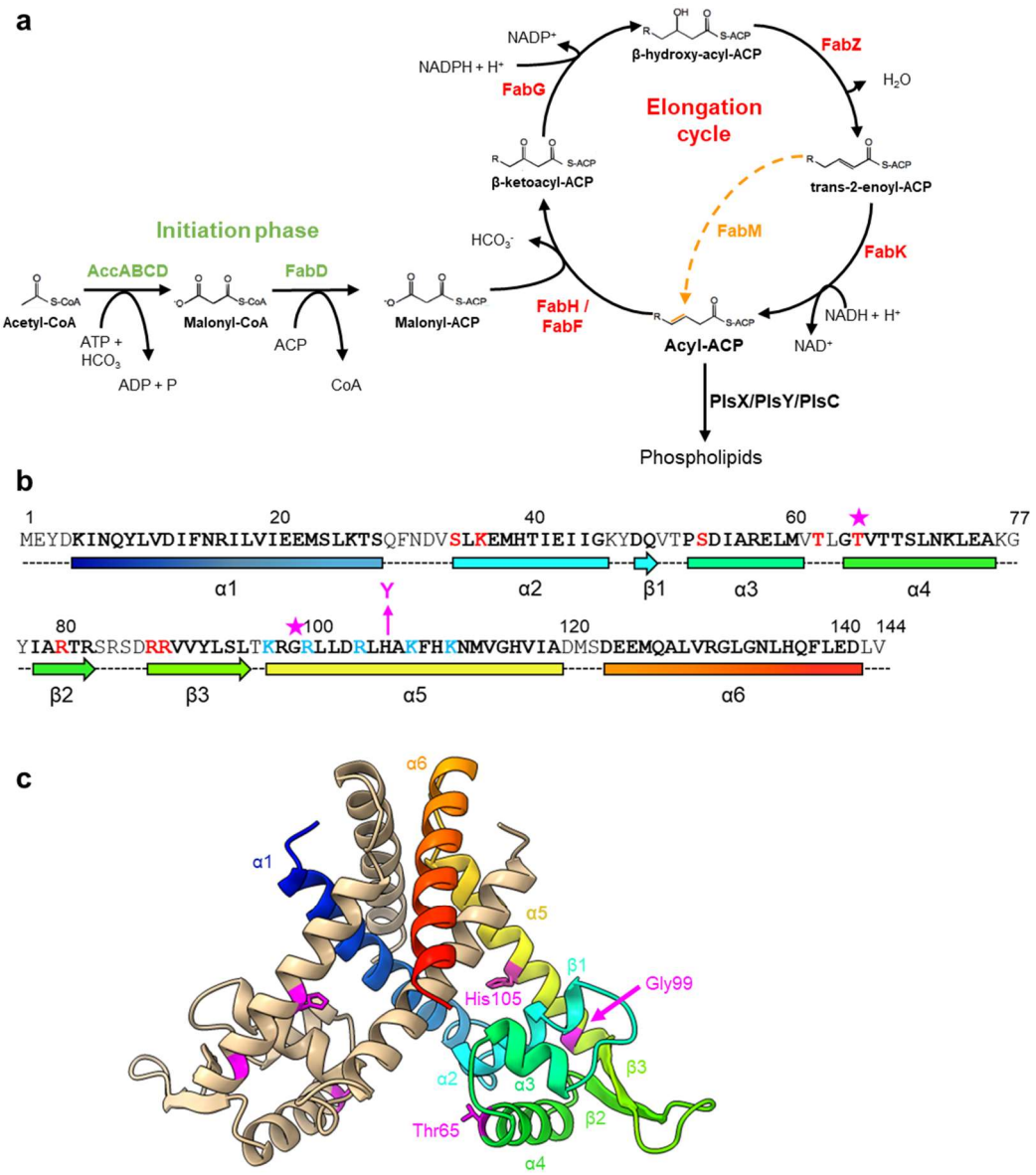
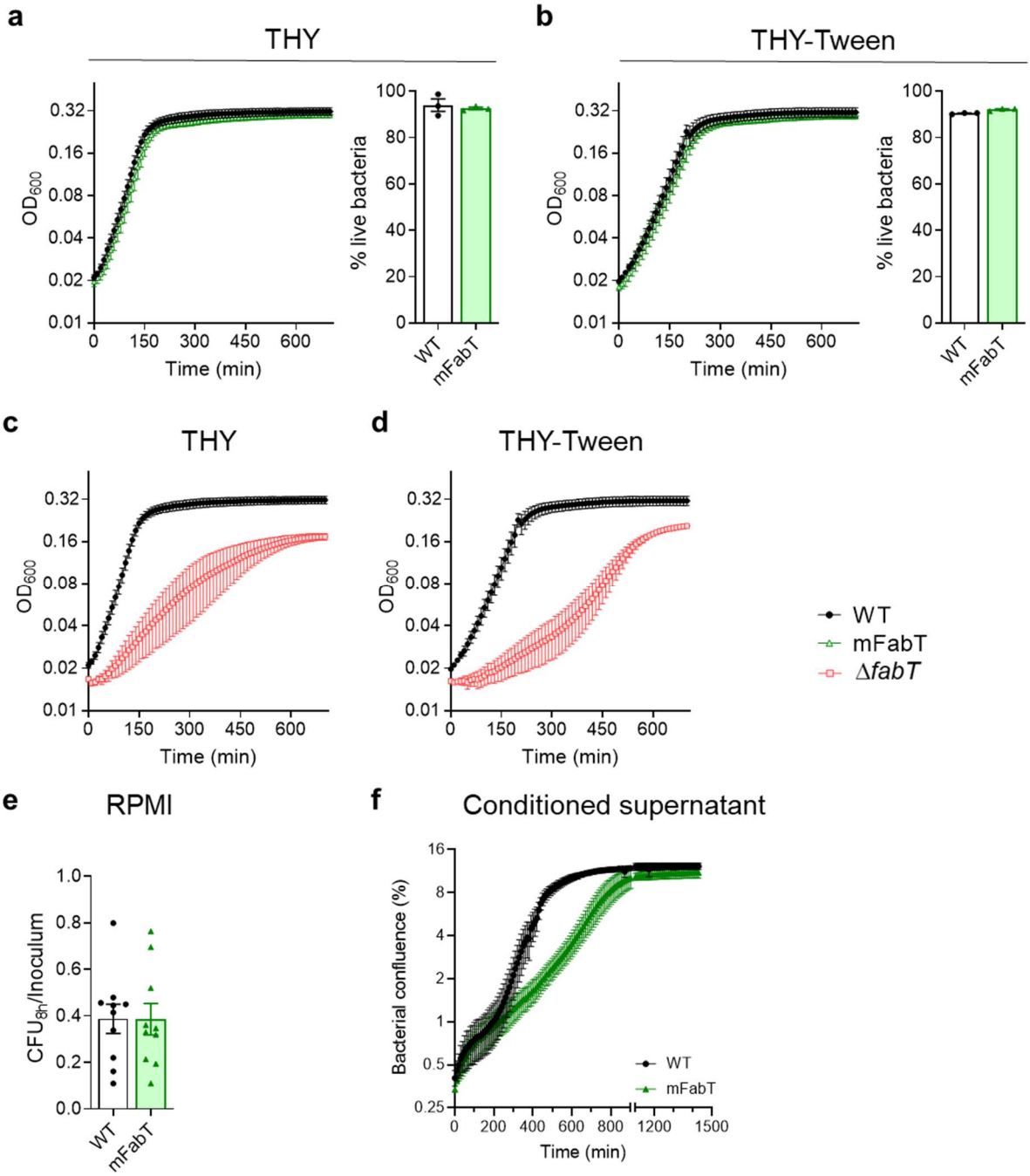


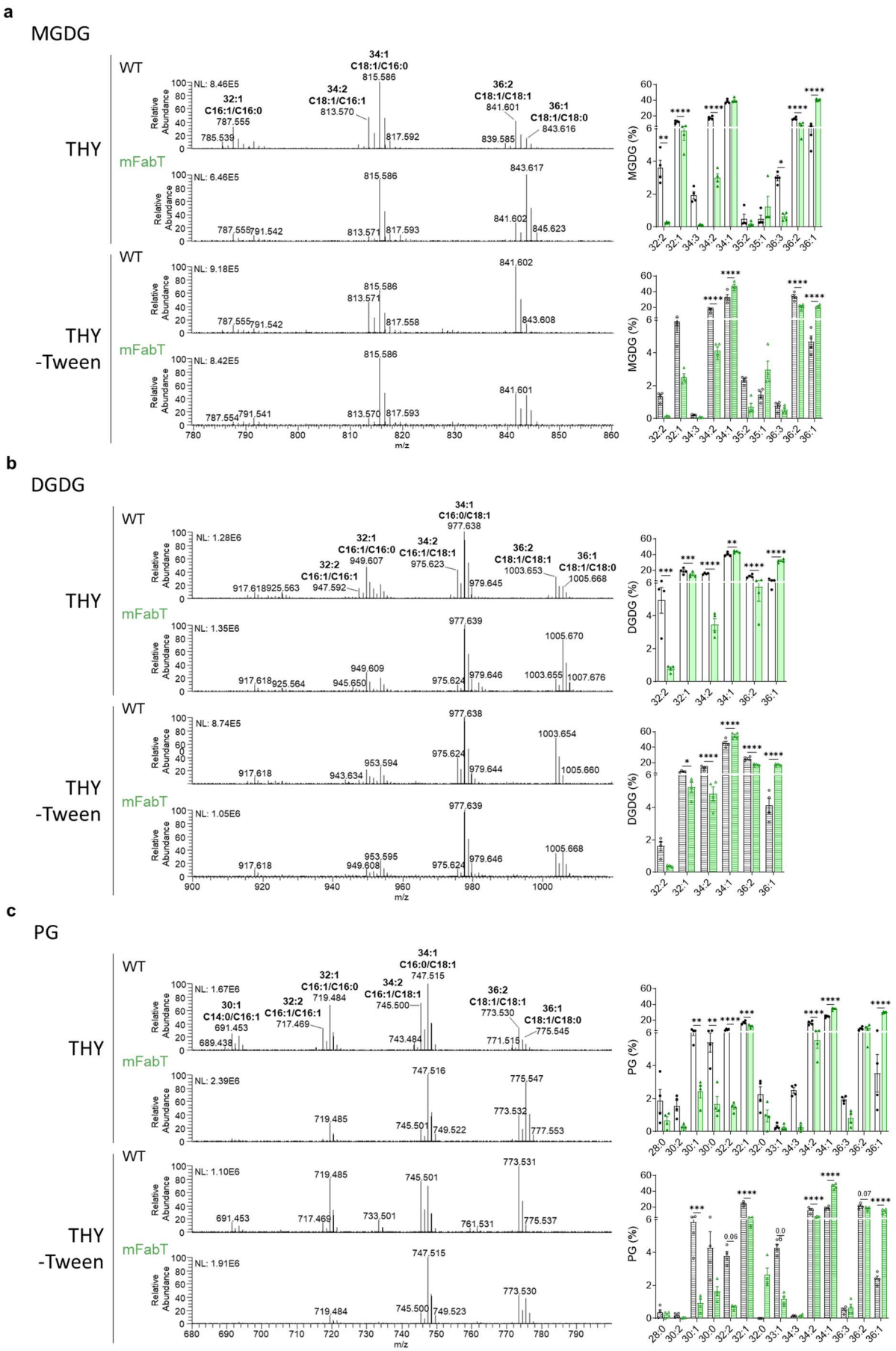
Supplementary Fig. 1 | FabT regulator and FASII pathway in GAS. a, The FASII synthesis pathway comprises a first initiation phase for precursor synthesis, followed by the recursive elongation cycle. The final product, acyl-ACP, supplies FAs for phospholipid synthesis. FabM (orange) leads to unsaturated *cis* FAs; FabK products are saturated. Initiation phase and elongation cycle enzymes are represented in green and red, respectively. From [1]. **b,** FabT sequence; amino acids involved in DNA binding are in red, and those interacting with acyl-Acyl carrier protein are in blue. Arrow indicates the His105Tyr FabT mutation studied in this work. Magenta stars highlight amino acids spontaneously mutated *in vivo* and in a saturated-FA environment (this work). **c,** Overall structure of FabT dimer predicted by Alphafold and adapted with ChimeraX [2,3]; one monomer is represented as multicolored (each color designates a separate domain), and the other is beige. Residues Thr65, Gly99 and His105, in magenta, correspond to mutants isolated in this study.

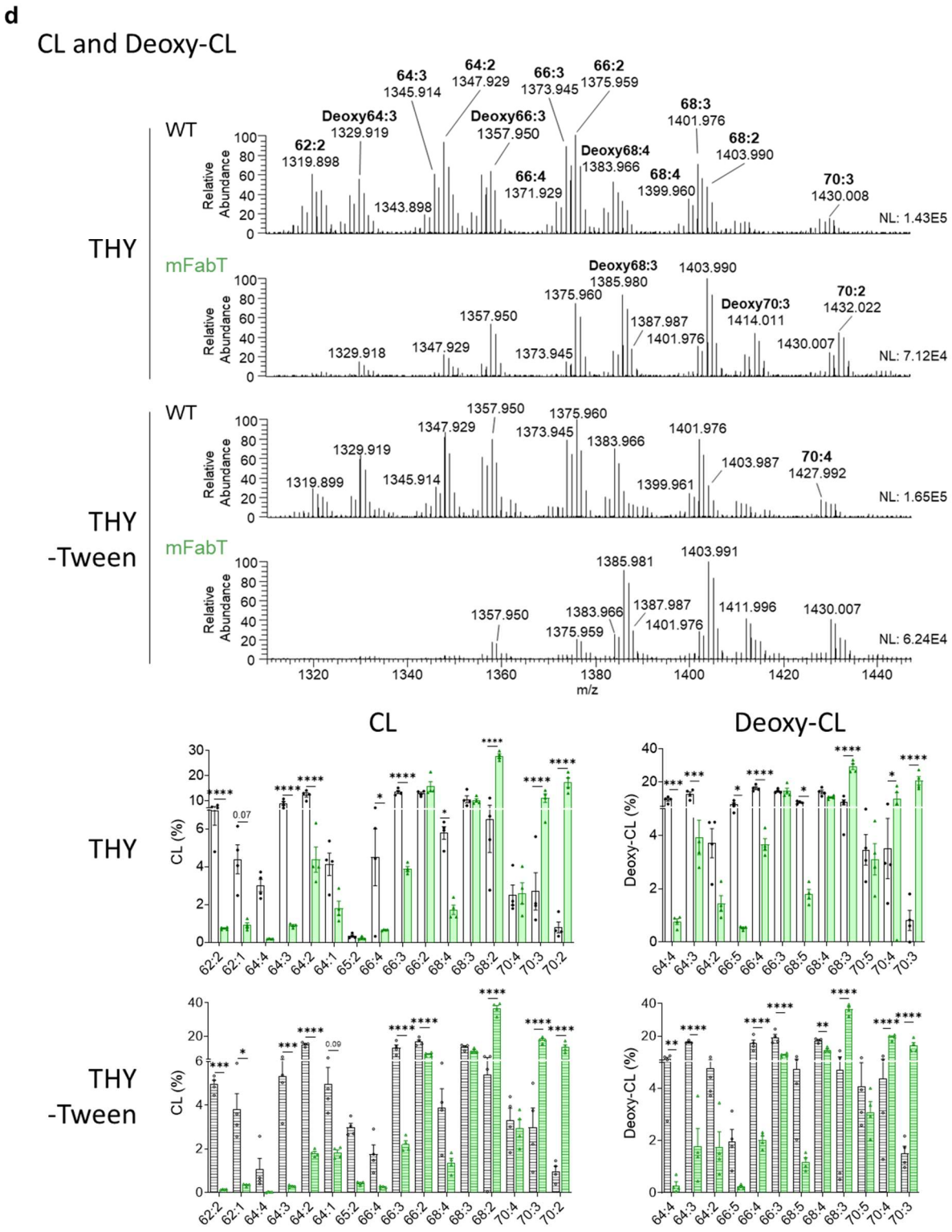
1. Lambert, C., Poyart, C., Gruss, A. & Fouet, A. FabT, a Bacterial Transcriptional Repressor That Limits Futile Fatty Acid Biosynthesis. *Microbiol Mol Biol Rev*, e0002922, doi:10.1128/mmbr.00029-22 (2022).
2. Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583-589, doi:10.1038/s41586-021-03819-2 (2021).
3. Pettersen, E. F. *et al.* UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Sci* **30**, 70-82, doi:10.1002/pro.3943 (2021).



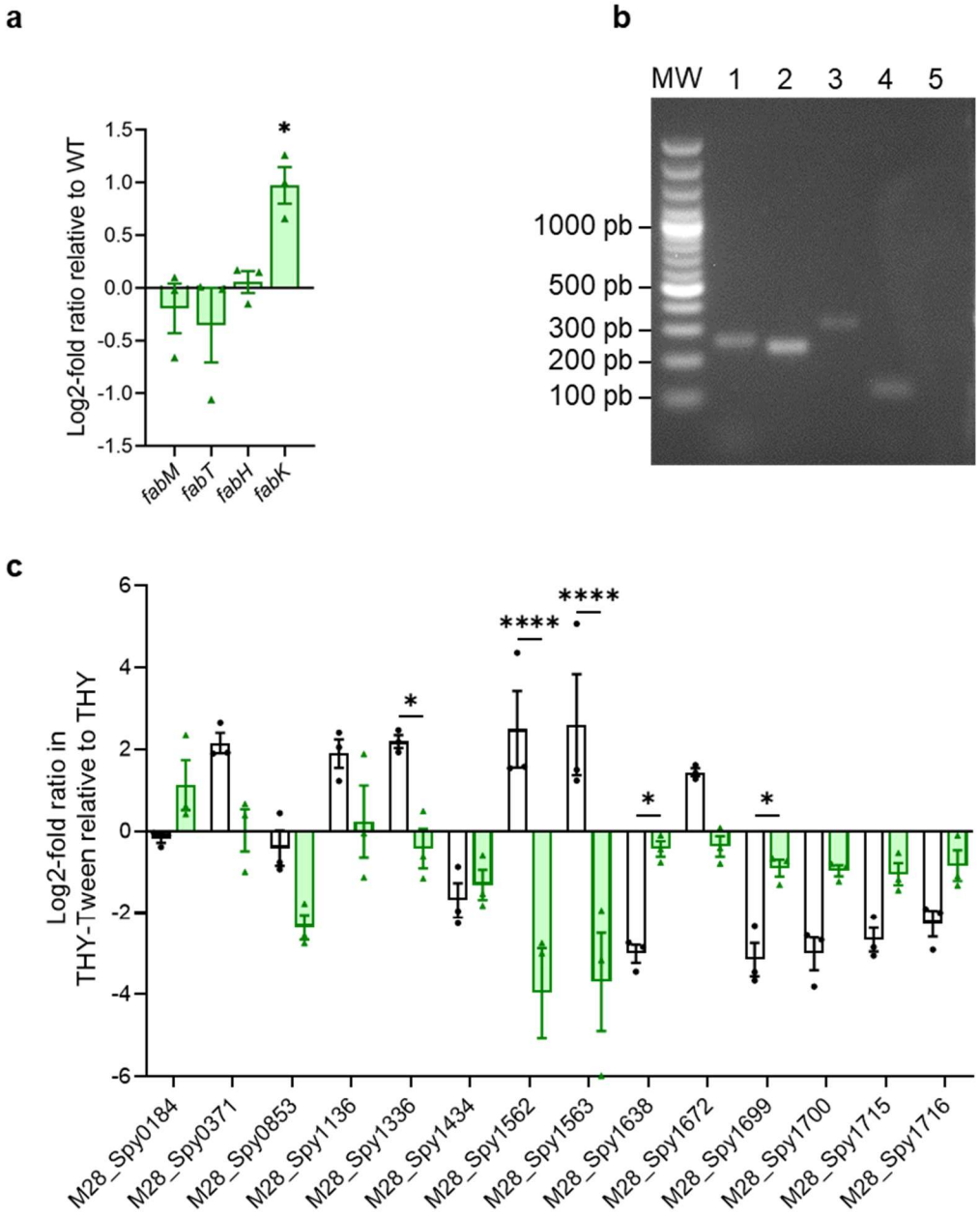
Supplementary Fig. 2 | Impact of FabT mutations on GAS growth. a-d, WT growth was compared to mFabT (**a** and **b** left; adapted from reference 1) or to Δ*fabT* (**c** and **d**) on THY and THY-Tween. Viability tests (**a** and **b,** right), using the LIVE/DEAD® BacLightTM Bacterial Viability Kit, were performed on WT and mFabT cultures after growth to OD_{600} = 0.4 - 0.5. Legend at the right of **d** is for growth curves. **e,** Ratios of CFUs of WT or mFabT strains after 8 h over respective initial inocula (10^3/ml for each) in RPMI medium; ratio below 1 indicates that bacteria die. **f**, Real-time growth of WT and mFabT in endometrial conditioned supernatant followed using Live-Cell Analysis System (IncuCyte®, Sartorius) and the Basic analyzer. **a-d**, **e**, N=3; **f** N=10; differences in **a, b,** and **e** were not statistically significant using T-test. WT, black lines or white bars; mFabT, green lines or bars. **a, b, e**, Outliers were searched using ROUT method (GraphPad), with Q=1 %. WT, black lines or white bars; mFabT, green lines or bars. Source data for **a**, **b**, **c**, **d**, **e** and **f** are provided as a Source Data file.

1. Lambert, C. *et al.* Acyl-AcpB, a FabT corepressor in *Streptococcus pyogenes*. *J Bacteriol* **205**, e0027423, doi:10.1128/jb.00274-23 (2023).


a
MGDG
THY
THY -Tween
WT
mFabT
34:1 C18:1/C16:0
34:2 C18:1/C16:1
32:1 C16:1/C16:0
36:2 C18:1/C18:1
36:1 C18:1/C18:0
MGDG (%)
b
DGDG
32:2 C16:1/C16:1
34:1 C16:0/C18:1
34:2 C16:1/C18:1
DGDG (%)
c
PG
30:1 C14:0/C16:1
PG (%)
m/z
Relative Abundance

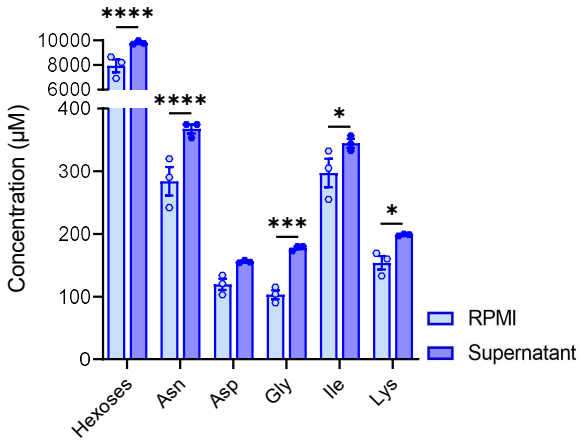


Supplementary Fig. 3 | Phospholipid membrane composition. Identification of **a**, monoglucosyldiacylglycerol (MGDG), **b**, diglucosyldiacylglycerol (DGDG), **c**, phosphatidylglycerol (PG), **d**, cardiolipin (CL) and putative deoxidized cardiolipin (Deoxy-CL). For each class, lipids are presented as the percentage of total lipids, and are quantified in Supplementary Table 2. For **a**, **b**, and **c**, fatty acid assignments are presented directly on mass spectrometry analyses. Cardiolipin assignments were ambiguous and are not shown. Lipids were analyzed on 3 independently prepared culture extracts (N=3). Statistical values were determined using 2-way ANOVA, Bonferroni post-test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Strains were grown in THY (open bars) and THY-Tween (hatched bars). WT, black lines and white bars; mFabT, green lines and bars. Source data are provided as a Source Data file.

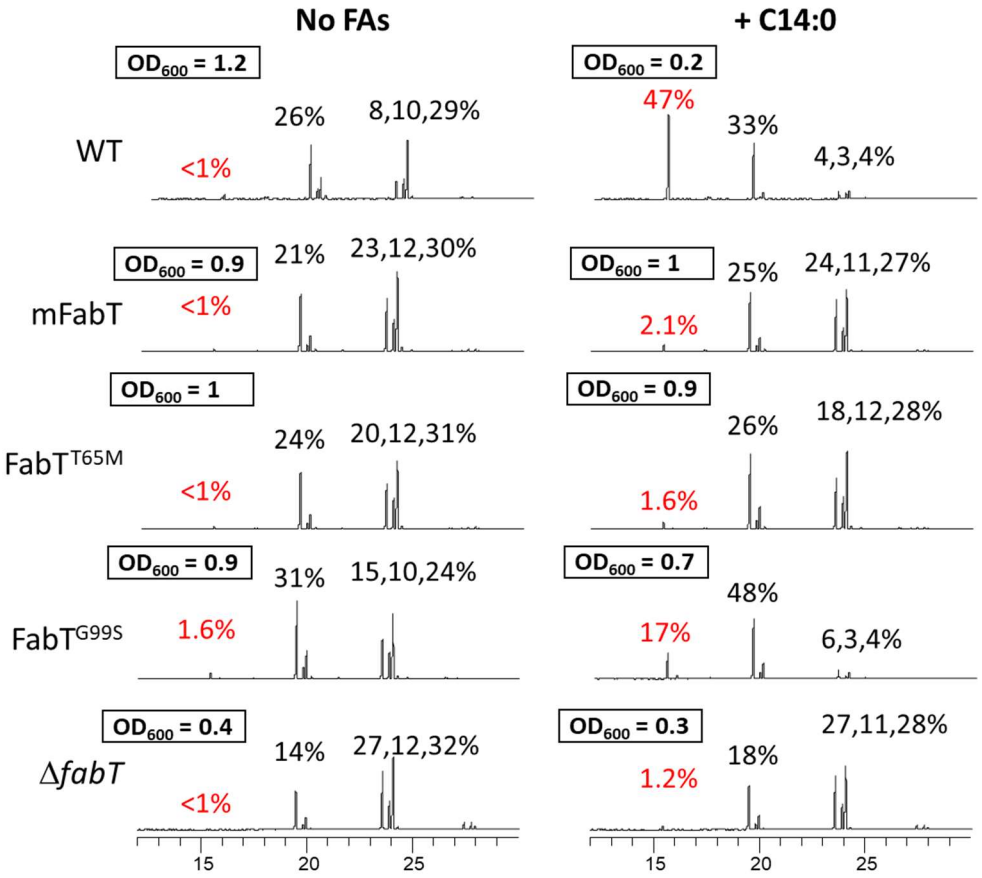


Supplementary Fig. 4 | Impact of mFabT on FASII and non-FASII gene expression in the absence of FA supplementation. **a-c**, WT and mFabT strains were grown in THY medium. **a, c**, RNAs were quantified by qRT-PCR. Expression was normalized to that of *gyrA;* relative gene expression is expressed as the log2-fold ratio. **a,** FASII gene expression of mFabT relative to WT is reproduced from Reference 1**. b**, mRNA transcript analysis of FASII locus genes: agarose gel of PCR amplification products on cDNA using primer pairs from neighboring genes. MW, molecular weight reference (Generuler 100 bp, ThermoFisher Scientific). Lane 1, *fabM-fabT* (234 bp); 2, *fabH-acpA* (221 bp); 3, *acpA-fabK* (302 bp); 4, *fabZ-accC* (105 bp); 5, *accD-serS* (288 bp). Results confirm the FASII transcriptional units indicated in Fig. 2a. **c**, expression of non-FASII genes in WT and mFabT strains **(**see Supplementary Table 1 for gene assignments**)**. The relative gene expression is expressed as the log2-fold ratio in a given strain grown in THY-Tween vs in THY. *, significance of differences in the two media between WT and mFabT. In **a-c,** WT, white bars; mFabT, green bars. **a, b**, N=4; **c**, N=3. **a-c**, 2-way ANOVA, Bonferroni post-test *p<0.05; ****p<0.0001. **c**, expression of non-FASII genes in WT and mFabT strains **(**see Supplementary Table 1 for gene assignments**).** In **a-c,** WT, white bars; mFabT, green bars. **a, b**, N=4; **c**, N=3. **a-c**, 2-way ANOVA, Bonferroni post-test *p<0.05; ****p<0.0001. Source data for **a** and **c** are provided as a Source Data file.

1. Lambert, C. *et al.* Acyl-AcpB, a FabT corepressor in *Streptococcus pyogenes*. *J Bacteriol* **205**, e0027423, doi:10.1128/jb.00274-23 (2023).



Supplementary Fig. 5 | Carbohydrates and amino acid residues produced by human endometrial cells. Metabolomic analysis of RPMI and HEC-1-A conditioned supernatants, as per legend at right. (see Supplementary Table 5 for complete data). N=3, 2-way ANOVA, Bonferroni post-test; *p<0.05; ***p<0.005; ****p<0.001.



Supplementary Fig. 6. FabT mutant growth and FA profiles in the absence and presence of exogenous C14:0. Triplicate cultures of the two isolated *fabT* mutants (FabT^{T65M} and FabT^{G99S}) and controls (WT, mFabT, Δ*fabT*) were prepared in BHI medium with 0.025 % BSA. Medium was without (left column), or with 100 µM C14:0 (right column). OD_{600} is shown for each strain and both conditions after 4 h growth (boxed). For each strain, proportions of C14:0 present in FA profiles of WT and *fabT* mutant strains are in red; proportions, from left to right, of C16:0, C18:0, C18:1Δ9 and C18:1Δ11 are in black.

Supplementary Table 1. Effects of FabT mutation on fatty acid (FA) composition.

	Cultivated in						*p-value*		
	THY			THY-Tween			THY-Tween *vs* THY		
Fatty acids [a]	**WT**[e]	**mFabT**[e]	**Δ*fabT***	**WT**[e]	**mFabT**[e]	**Δ*fabT***	**WT**	**mFabT**	**Δ*fabT***
C14:0	1.9 ± 0.8	0.6 ± 0.1	0.3 ± 0.1	8.1 ± 2.2	0.9 ± 0.1	0.4 ± 0.2	<0.0001	>0.9999	>0.9999
C16:0	29.2 ± 2.3	21.2 ± 2.6	15.2 ± 3.2	21.2 ± 5.8	27.1 ± 2.0	10.5 ± 1.2	<0.0001	0.0001	0.0067
C16:1	17.0 ± 0.5	7.9 ± 1.8	3.6 ± 1.4	8.3 ± 1.6	4.6 ± 0.4	2.0 ± 0.2	<0.0001	0.0694	>0.9999
C18:0	**6.8 ± 0.7**	**26.1 ± 0.7**	**26.1 ± 0.5**	**0.2 ± 0.3**	**12.4 ± 0.8**	**26.3 ± 0.4**	<0.0001	<0.0001	>0.9999
C18:1Δ9	9.1 ± 2.4	10.8 ± 2.0	11.7 ± 0.2	54.8 ± 3.3	30.4 ± 1.5	15.0 ± 0.1	<0.0001	<0.0001	0.1008
C18:1Δ11	29.1 ± 1.3	28.9 ± 1.2	31.6 ± 2.3	3.4 ± 3.0	20.1 ± 1.9	30.5 ± 0.4	<0.0001	<0.0001	>0.9999
others [b]	6.8 ± 1.5	4.5 ± 1.2	11.5 ± 1.7	4.2 ± 2.8	4.5 ± 1.6	15.3 ± 0.9	0.263	>0.9999	0.0394
(C18):(C16) [c]	1.0	2.3	3.7	2.0	2.0	5.8	0.0092	0.5304	0.974
UFA:SFA [d]	1.5	1.0	1.1	2.3	1.4	1.3	0.0577	0.6405	>0.9999

[a] Percent of total FAs. [b] FAs that represent less than 2 % total FAs. [c] Ratio of chain lengths = (C18:0 + C18:1)/(C16:0 + C16:1). [d] Ratio unsaturated/saturated FAs = (C16:1 + C18:1Δ9 + C18:1Δ11)/(C14:0 + C16:0 + C18:0). [e] As reported in reference 1. In bold, percent saturated FA C18:0, a main FA with increased proportions in mFabT. See reference 2 for studies in *Streptococcus pneumoniae* . In blue, differences between mFabT and ΔfabT are highlighted. 2-way ANOVA, Bonferroni post-test.

Source data are provided as a Source Data file.

1. Lambert C, d'Orfani, A, Gaillard M, Zhang Q, Gloux K, Poyart C, Fouet A. Acyl-AcpB, a FabT co-repressor in *Streptococcus pyogenes* . J Bacteriol 205, e00274-23, doi: 10.1128/jb.00274-23 (2023).

2. Lu YJ, and Rock CO. Transcriptional regulation of fatty acid biosynthesis in *Streptococcus pneumoniae* . Mol Microbiol 59:551-66 (2006).

Supplementary Table 2. Membrane lipid composition.

		Relative abundance (%)				P-value	
		Cultivated in THY		Cultivated in THY-Tween		THY-Tween *vs* THY	
Lipids [a]	**m/z**	**WT**	**mFabT**	**WT**	**mFabT**	**WT**	**mFabT**
MGDG species							
32:2	785.54	3.6 ± 1.0	0.3 ± 0.1	1.3 ± 0.3	0.1 ± 0.0	>0.9999	>0.9999
32:1	787.56	12.1 ± 1.8	5.9 ± 1.2	5.9 ± 1.4	2.5 ± 0.4	0.0136	<0.0001
34:3	811.56	1.9 ± 0.4	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	>0.9999	>0.9999
34:2	813.57	17.2 ± 2.2	3.0 ± 0.5	17.0 ± 2.6	4.1 ± 0.5	>0.9999	0.8522
34:1	815.59	38.2 ± 2.5	39.7 ± 3.0	32.6 ± 7.4	46.9 ± 4.6	0.0138	<0.0001
35:2	827.59	0.5 ± 0.6	0.2 ± 0.2	2.4 ± 0.2	0.7 ± 0.5	>0.9999	>0.9999
35:1	829.60	0.5 ± 0.4	1.3 ± 1.3	1.4 ± 0.4	3.0 ± 1.1	>0.9999	0.105
36:3	839.59	3.0 ± 0.3	0.6 ± 0.3	0.8 ± 0.3	0.5 ± 0.2	>0.9999	>0.9999
36:2	841.60	16.3 ± 1.7	8.7 ± 2.7	33.7 ± 5.0	20.7 ± 3.1	<0.0001	<0.0001
36:1	843.62	6.7 ± 2.2	40.3 ± 1.4	4.7 ± 0.7	21.2 ± 1.8	>0.9999	<0.0001
DGDG species							
32:2	947.59	4.9 ± 1.6	0.8 ± 0.2	1.6 ± 0.6	0.4 ± 0.1	0.1369	>0.9999
32:1	949.61	18.8 ± 3.6	14.3 ± 3.1	8.4 ± 1.0	5.3 ± 0.6	<0.0001	<0.0001
34:2	975.62	15.5 ± 1.0	3.5 ± 0.7	13.3 ± 2.7	4.8 ± 0.9	0.7991	>0.9999
34:1	977.64	40.1 ± 2.5	43.3 ± 1.5	45.1 ± 5.3	54.9 ± 3.9	0.0043	<0.0001
36:2	1003.65	12.3 ± 2.1	5.8 ± 1.8	25.1 ± 2.5	17.1 ± 1.5	<0.0001	<0.0001
36:1	1005.67	7.1 ± 1.4	31.8 ± 2.2	4.1 ± 0.9	17.2 ± 1.5	0.2587	<0.0001
PG species							
28:0	665.44	1.9 ± 1.4	0.7 ± 0.5	0.4 ± 0.3	0.2 ± 0.2	>0.9999	>0.9999
30:2	689.44	1.6 ± 0.6	0.3 ± 0.2	0.2 ± 0.1	0.0 ± 0.0	>0.9999	>0.9999
30:1	691.45	6.4 ± 1.1	2.4 ± 0.8	5.8 ± 1.8	0.9 ± 0.5	>0.9999	0.1678
30:0	693.47	5.4 ± 1.2	1.7 ± 0.9	4.3 ± 2.0	1.6 ± 0.6	>0.9999	>0.9999
32:2	717.47	7.8 ± 1.0	1.5 ± 0.3	3.8 ± 0.5	0.7 ± 0.1	0.0153	>0.9999
32:1	719.48	16.7 ± 2.0	12.1 ± 2.1	23.5 ± 2.7	6.2 ± 0.7	<0.0001	<0.0001
32:0	721.50	2.3 ± 0.9	0.9 ± 0.7	0.0	2.6 ± 0.8	0.8064	0.0995
33:1	733.50	0.3 ± 0.2	0.2 ± 0.2	4.3 ± 0.4	1.2 ± 0.4	0.0232	>0.9999
34:3	743.48	2.5 ± 0.3	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.6926	>0.9999
34:2	745.50	16.2 ± 2.6	5.6 ± 1.0	15.8 ± 3.6	7.4 ± 0.6	>0.9999	0.0983
34:1	747.51	24.4 ± 1.3	34.1 ± 1.5	18.2 ± 2.3	46.1 ± 3.5	<0.0001	<0.0001
36:3	771.51	2.0 ± 0.2	0.8 ± 0.5	0.5 ± 0.2	0.7 ± 0.4	>0.9999	>0.9999
36:2	773.53	9.0 ± 2.2	9.6 ± 3.8	20.7 ± 3.3	17.6 ± 2.5	<0.0001	<0.0001
36:1	775.55	3.5 ± 2.3	29.8 ± 1.1	2.4 ± 0.4	14.6 ± 2.6	>0.9999	<0.0001
CL species							
62:2	1319.90	4.8 ± 1.2	0.5 ± 0.1	3.3 ± 0.2	0.1 ± 0.0	>0.9999	>0.9999
62:1	1321.91	3.0 ± 1.2	0.6 ± 0.2	2.5 ± 0.9	0.2 ± 0.1	>0.9999	>0.9999
64:4	1343.90	2.1 ± 0.4	0.1 ± 0.0	0.7 ± 0.6	0.0 ± 0.0	>0.9999	>0.9999
64:3	1345.91	6.1 ± 1.1	0.6 ± 0.1	3.5 ± 1.2	0.2 ± 0.0	0.1157	>0.9999
64:2	1347.93	8.5 ± 1.4	2.8 ± 0.9	10.1 ± 0.2	1.1 ± 0.1	0.8048	0.1591
64:1	1349.95	2.9 ± 0.9	1.1 ± 0.5	3.3 ± 1.0	1.1 ± 0.2	>0.9999	>0.9999
65:2	1361.95	0.2 ± 0.1	0.1 ± 0.1	2.0 ± 0.2	0.3 ± 0.1	0.7997	>0.9999
66:4	1371.93	3.1 ± 2.0	0.4 ± 0.0	1.2 ± 0.5	0.2 ± 0.0	0.6299	>0.9999
66:3	1373.94	9.3 ± 0.7	2.5 ± 0.2	8.7 ± 2.3	1.4 ± 0.2	>0.9999	>0.9999
66:2	1375.96	8.9 ± 0.9	9.9 ± 2.5	11.1 ± 1.3	5.7 ± 0.3	0.1184	<0.0001
68:4	1399.96	4.0 ± 0.3	1.1 ± 0.3	2.6 ± 1.1	0.8 ± 0.3	>0.9999	>0.9999
68:3	1401.98	7.3 ± 1.8	6.4 ± 0.8	9.1 ± 1.4	7.0 ± 0.6	0.454	>0.9999
68:2	1403.99	4.5 ± 2.3	17.7 ± 2.4	3.7 ± 2.6	22.6 ± 2.6	>0.9999	<0.0001
70:4	1427.99	1.7 ± 0.7	1.6 ± 0.7	2.2 ± 0.9	1.8 ± 0.5	>0.9999	>0.9999
70:3	1430.01	1.8 ± 1.3	7.0 ± 2.1	2.0 ± 1.3	11.1 ± 1.7	>0.9999	<0.0001
70:2	1432.02	0.5 ± 0.4	11.0 ± 3.1	0.7 ± 0.3	8.4 ± 2.3	>0.9999	0.002
Deoxy-CL species							
64:4	1327.90	9.3 ± 1.9	0.8 ± 0.2	5.8 ± 2.3	0.3 ± 0.3	0.2081	>0.9999
64:3	1329.92	12.7 ± 2.8	3.9 ± 1.3	16.3 ± 0.8	1.8 ± 1.4	0.1888	>0.9999
64:2	1331.93	3.7 ± 1.1	1.4 ± 0.6	4.8 ± 1.6	1.7 ± 1.2	>0.9999	>0.9999
66:5	1353.92	6.6 ± 1.8	0.5 ± 0.1	2.0 ± 1.0	0.2 ± 0.1	0.0299	>0.9999
66:4	1355.93	16.2 ± 1.8	3.7 ± 0.4	15.4 ± 4.3	2.0 ± 0.3	>0.9999	>0.9999
66:3	1357.95	14.4 ± 1.2	14.5 ± 3.5	19.2 ± 3.8	8.3 ± 1.1	0.0236	0.005
68:5	1381.95	7.6 ± 0.8	1.8 ± 0.4	4.7 ± 2.2	1.2 ± 0.3	0.561	>0.9999
68:4	1383.97	13.9 ± 2.3	10.7 ± 1.1	17.3 ± 1.3	11.4 ± 1.4	0.2831	>0.9999
68:3	1385.98	7.7 ± 3.1	29.3 ± 3.5	4.7 ± 5.0	36.6 ± 3.2	0.502	0.0005
70:5	1409.98	3.5 ± 1.1	3.1 ± 1.2	4.1 ± 1.8	3.1 ± 0.8	>0.9999	>0.9999
70:4	1412.00	3.5 ± 2.3	9.7 ± 7.8	4.4 ± 2.8	19.3 ± 1.7	>0.9999	<0.0001
70:3	1414.01	0.8 ± 0.8	20.7 ± 5.2	1.5 ± 0.6	14.1 ± 3.9	>0.9999	0.0023

[a] Percent lipid species. Lipids were analyzed on 3 independently prepared culture extracts (N=3).

MGDG, monoglucosyldiacylglycerol; DGDG, diglucosyldiacylglycerol; PG, phosphatidylglycerol; CL, cardiolipin; Deoxy-CL, putative deoxidised cardiolipin species.

2-way ANOVA, Bonferroni post-test; *p<0.05; ** p<0.01; *** p<0.001; ****p<0.0001

Primary lipid data are available at http://dx.doi.org/10.21228/M88C05 .

Supplementary Table 3. Membrane lipid species.

	Cultivated in				*p-value*	
	THY		THY-Tween		THY-Tween vs THY	
Lipids [a]	**WT**	**mFabT**	**WT**	**mFabT**	**WT**	**mFabT**
MGDG	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	>0.9999	>0.9999
DGDG	1.1 ± 0.4	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.4	0.0803	>0.9999
PG	0.7 ± 0.3	0.7 ± 0.2	0.5 ± 0.1	0.7 ± 0.4	0.9259	>0.9999
CL	1.0 ± 0.6	0.4 ± 0.2	0.7 ± 0.2	0.5 ± 0.3	0.3312	>0.9999
Total	3.0	1.9	2.1	2.1	0.3126	>0.9999

[a] Milligrams lipids extracted from the equivalent of OD_{600} = 100 (N=4).

MGDG, monoglucosyldiacylglycerol; DGDG, diglucosyldiacylglycerol; PG, phosphatidylglycerol; CL, cardiolipin.

2-way ANOVA, Bonferroni post-test; *p<0.05.

Source data are provided as a Source Data file.

Supplementary Table 4. Modification of gene expression under all tested conditions.

Locus	Gene	Gene product	Fold-change				Function	Adjusted P-values			
			THY mFabT *vs* WT	THY-Tween mFabT *vs* WT	WT THY-Tween *vs* THY	mFabT THY-Tween *vs* THY		THY mFabT *vs* WT	THY-Tween mFabT *vs* WT	WT THY-Tween *vs* THY	mFabT THY-Tween *vs* THY
M28_Spy0022	***purC***	Phosphoribosylaminoimidazole-succinocarboxamide synthase, PurC	1.327				Purine synthesis	6.63E-03			
M28_Spy0023	***purL***	Phosphoribosylformylglycinamidine synthase, PurL	1.245				Purine synthesis	2.31E-03			
M28_Spy0024	***purF***	Amidophosphoribosyltransferase, PurF	1.102				Purine synthesis	5.22E-03			
M28_Spy0025	***purM***	Phosphoribosylformylglycinamidine cyclo-ligase, PurM	1.313				Purine synthesis	5.22E-03			
M28_Spy0026	***purN***	Phosphoribosylglycinamide formyltransferase, PurN	1.469				Purine synthesis	6.10E-03			
M28_Spy0107	***cpa***	Collagen-binding protein, Cpa	1.937				Adhesin	1.80E-09			
M28_Spy0108	***sipA***	Signal peptidase I, SipA	2.081				Signal peptidase	3.44E-08			
M28_Spy0109	***M28_Spy0109***	T28 T-antigen	2.195				Adhesin	1.36E-09			
M28_Spy0110	***srtB***	Class B sortase protein, SrtB	1.734				Sortase	3.37E-05			
M28_Spy0111	***fctB***	Pilin minor structural protein, FctB	1.949				Adhesin	2.53E-08			
M28_Spy0137*	***nga***	NAD glycohydrolase, Nga	-1.350				Virulence factor	2.53E-06			
M28_Spy0138*	***ifs***	Immunity factor for SPN/NAD glycohydrolase protein, Ifs	-1.540				Resistance factor	2.48E-04			
M28_Spy0139*	***slo***	Thiol-activated cytolysin, SLO	-1.357				Virulence factor	1.68E-03			
M28_Spy0140	*M28_Spy0140*	Uncharacterized protein	-1.578		-1.688		Unknown function	7.30E-05		1.41E-04	
M28_Spy0184*	*M28_Spy0184*	RofA-related transcriptional regulator			-1.082		Transcriptional regulator			2.09E-06	
M28_Spy0249	*M28_Spy0249*	Transposase *	-14.443				Transposition	3.36E-02			
M28_Spy0338	*M28_Spy0338*	Uncharacterized protein	-1.613	*-0.836*	-1.207		Unknown function	9.48E-14	4.11E-02	2.42E-06	
M28_Spy0341	*M28_Spy0341*	Uncharacterized protein	-1.689		*-0.895*		Unknown function	2.20E-09		3.76E-02	
M28_Spy0346	*M28_Spy0346*	Uncharacterized protein	-1.475		-1.011		Unknown function	1.66E-10		4.75E-04	
M28_Spy0371**	*M28_Spy0371*	67 kDa myosin-crossreactive antigen MCRA; fatty acid double bond hydratase	1.534		1.941	*0.588*	Fatty acid degradation	1.67E-19		4.26E-26	2.62E-02
M28_Spy0658	*M28_Spy0658*	5'-nucleotidase	1.187				DNA regulation	1.16E-03			
M28_Spy0757	*M28_Spy0757*	PTS system, mannose/fructose family IIA component	-0.996				Phosphotransferase system	4.15E-02			
M28_Spy0853**	*M28_Spy0853*	Diacylglycerol kinase family lipid kinase	*0.877*			-1.102	Lipid metabolism	2.22E-07			3.34E-12
M28_Spy0855**	*M28_Spy0855*	Uncharacterized protein	1.609			-1.969	Unknown function	6.63E-03			2.32E-08
M28_Spy0977	*phage protein*	Hyaluronoglucosaminidase			1.594		Phage	1.73E-05		7.54E-04	
M28_Spy1014	*M28_Spy1014*	Phage DNA/RNA helicase	1.028				Phage	3.33E-02			
M28_Spy1098**	*grab*	Protein G-related alpha 2M-binding protein, Grab	1.647				Virulence factor	5.01E-03			
M28_Spy1112**	*gapN*	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, GapN	-1.220		*-0.740*		Glycolysis	2.53E-08			
M28_Spy1136	*M28_Spy1136*	D-alanyl-D-alanine carboxypeptidase	1.633		*0.917*		Peptidoglycan biosynthesis	1.89E-17		1.89E-04	
M28_Spy1137	*M28_Spy1137*	Uncharacterized protein	1.596				Unknown function	8.69E-03			
M28_Spy1138	*M28_Spy1138*	Uncharacterized protein	1.767				Unknown function	5.64E-03			
M28_Spy1147*	*kup*	Probable potassium transport system protein, Kup	2.234			*-0.854*	Transporter	6.46E-17			6.13E-02
M28_Spy1228	*M28_Spy1228*	Phage protein			1.361		Phage			4.08E-03	
M28_Spy1229	*M28_Spy1229*	Phage protein			1.061		Phage			9.75E-03	
M28_Spy1234	*M28_Spy1234*	Phage endopeptidase			1.082		Phage			4.28E-02	
M28_Spy1236	*M28_Spy1236*	Phage protein			1.223		Phage			1.10E-02	
M28_Spy1237	*M28_Spy1237*	Phage protein			1.160		Phage			1.80E-02	
M28_Spy1238	*M28_Spy1238*	Phage protein			1.240		Phage			2.53E-02	
M28_Spy1239	*M28_Spy1239*	Major tail shaft protein			1.302		Phage			4.08E-03	
M28_Spy1244	*M28_Spy1244*	Major head protein			1.258		Phage			8.34E-03	
M28_Spy1245	*M28_Spy1245*	Phage protein			1.199		Phage			1.22E-02	
M28_Spy1249	*M28_Spy1249*	Minor capsid protein			1.126		Phage			7.22E-03	
M28_Spy1251	*M28_Spy1251*	Phage protein			1.225		Phage			2.42E-02	
M28_Spy1255	*M28_Spy1255*	ParB-like protein			1.219		Phage			8.34E-03	
M28_Spy1336	*M28_Spy1336*	R28 protein GAS-specific adhesin	1.041		1.412		Adhesin	5.22E-03		1.42E-04	
M28_Spy1434	*M28_Spy1434*	DegV family protein, FakB3, fatty acid metabolism			-1.491	-1.409	Lipid metabolism			3.12E-07	4.29E-08
M28_Spy1473**	***accD***	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha, AccD		2.567	-2.343		Fatty acid biosynthesis		6.07E-15	7.81E-15	
M28_Spy1474**	***accA***	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta, AccA		2.589	-2.357		Fatty acid biosynthesis		9.75E-12	1.07E-11	
M28_Spy1475**	***accC***	Biotin carboxylase, AccC		2.666	-2.431		Fatty acid biosynthesis		2.61E-11	3.01E-11	
M28_Spy1476**	***fabZ***	3-hydroxyacyl-[acyl-carrier-protein] dehydratase, FabZ		2.559	-2.335		Fatty acid biosynthesis		1.65E-09	2.29E-09	
M28_Spy1477**	***accB***	Biotin carboxyl carrier protein of acetyl-CoA carboxylase, AccB		2.534	-2.535		Fatty acid biosynthesis		3.23E-11	2.47E-13	
M28_Spy1478**	***fabF***	3-oxoacyl-[acyl-carrier-protein] synthase 2, FabF		2.601	-2.524		Fatty acid biosynthesis		3.81E-32	2.09E-36	
M28_Spy1479**	***fabG***	3-oxoacyl-[acyl-carrier-protein] reductase, FabG		2.676	-2.486		Fatty acid biosynthesis		1.78E-24	2.96E-25	
M28_Spy1480**	***fabD***	Malonyl CoA-acyl carrier protein transacylase, FabD		2.434	-2.430		Fatty acid biosynthesis		3.83E-21	2.96E-25	
M28_Spy1481**	***fabK*** [a]	Enoyl-[acyl-carrier protein] reductase (NADH), FabK		2.351	-2.397		Fatty acid biosynthesis		7.62E-10	3.35E-12	
M28_Spy1483	***fabH***	3-oxoacyl-[acyl-carrier-protein] synthase 3, FabH		*0.679*	-1.176		Fatty acid biosynthesis		9.91E-02	3.40E-07	
M28_Spy1484	***fabT***	Transcriptional regulator, FabT			*-0.921*		Fatty acid biosynthesis			4.22E-02	
M28_Spy1485*	***fabM*** [a]	Enoyl-CoA hydratase, FabM			-1.160		Fatty acid biosynthesis			2.67E-02	
M28_Spy1562**	***uspA***	Universal stress protein family, UspA	-1.740	-4.066	1.843		Stress response	3.88E-03	1.13E-11	4.08E-03	
M28_Spy1563**	*norA*	Quinolone resistance protein, NorA	-1.288	-3.229	1.529		Antibiotic resistance	6.14E-06	2.22E-26	4.97E-07	
M28_Spy1638**	*fakB4*	DegV family protein, FakB4		*0.835*	-1.083		Fatty acid storage-catabolism		8.71E-03	2.09E-06	
M28_Spy1672*	*ska*	Streptokinase, Ska	*0.929*		1.069		Tissue invasion	9.29E-03		6.66E-03	
M28_Spy1675	***sclA/scl1***	Collagen-like surface protein A, SclA/Scl1	-1.823		-0.996		Adhesin	9.80E-09		3.86E-02	
M28_Spy1699**	***M28_Spy1699***	Cell surface protein	*-0.923*		-1.822		Adhesin	3.36E-02		4.97E-07	
M28_Spy1700**	***scpA***	C5a peptidase, ScpA	-1.162		-2.073		Adhesin	2.92E-03		7.84E-09	
M28_Spy1701	***M28_Spy1701***	Enn protein	*-0.871*		-1.026		Adhesin	4.49E-02		2.55E-02	
M28_Spy1702	***M28_Spy1702***	M28 protein	-1.985				Adhesin	1.22E-07			
M28_Spy1715	***sfbX***	Fibronectin-binding protein, SfbX	*-0.889*		-1.359		Adhesin	5.22E-03		5.78E-06	
M28_Spy1716	***sof***	Serum opacity factor	-1.093		-1.375		Adhesin	1.93E-05		4.29E-07	
M28_Spy1813	*M28_Spy1813*	Phage protein	-1.491				Phage	1.98E-04			
M28_Spy1838**	*M28_Spy1838*	Phage protein	2.661	1.618	3.203	2.160	Phage	1.77E-31	6.00E-08	1.24E-37	2.28E-22

Gene expression was considered modified in a given condition when the absolute value of log2-Foldchange (FC) was greater than or equal to 1, with an adjusted p-value ≤ 0.05. * Genetic loci in red were validated by qRT-PCR (Supplementary Figure 4c). [a] Differences in *fabK* and *fabM* expression in THY were below the significance threshold by transcriptomics, and were therefore examined by qRT-PCR (Supplementary Fig. 4a). Asterisks highlight genes for which expression changes match those from a previous transcriptomic analysis of a GAS *fabT* deleted mutant *vs* WT strain cultivated at different temperatures: 35 °C (blue asterisk) and 40 °C (red asterisk) in exponential phase. In italics, log2-fold changes that are less than our log2-fold threshold but have a p-value below 0.05 and are significantly modified in published comparisons [1]. In bold are the genes referred to in the text. Source data are provided as a Source Data file (also for Fig 2b-e).

1. Eraso, J. M. et al. Genomic Landscape of Intrahost Variation in Group A Streptococcus: Repeated and Abundant Mutational Inactivation of the fabT Gene Encoding a Regulator of Fatty Acid Synthesis. Infect Immun 84, 3268-3281, doi:10.1128/IAI.00608-16 (2016).

Supplementary Table 5. Metabolites present in the different media tested.

Metabolites	LOD* [µM]	Infection medium (RPMI)			Conditioned supernatants (SN)			SN inoculated with											
								WT strain 8 h			mFabT strain 8 h			WT strain 16 h			mFabT strain 16 h		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Amino acids																			
Asn	102	290	242	320	**375**	**375**	**353**	392	328	381	394	378	352	371	351	381	341	301	304
Asp	153	121	103	134	155	158	154	162	165	168	170	175	164	211	163	183	174	182	189
Gly	103	104	90	115	**180**	**180**	**173**	185	164	180	185	186	175	190	167	181	172	160	165
His	20.1	66.9	56.6	73.3	85.5	86.4	82.5	93.6	83.5	89.6	91.8	91.3	87.5	92.1	82.4	89.9	83.4	80	82.1
Ile	205	305	255	332	**344**	**357**	**333**	369	328	365	357	367	348	374	330	371	332	307	328
Lys	104	160	133	169	**200**	**199**	**197**	213	188	210	211	218	200	216	195	214	190	183	187
Met	44.6	45.9	39.5	51.1	60.2	59.5	58.6	63.6	55.7	63.9	63.8	64.7	61.4	66.6	57.5	63.5	59.2	56.4	58.7
Pro	93.8	127	107	136	159	161	154	169	152	165	165	173	163	169	155	173	155	148	153
Ser	156	215	183	228	198	210	200	226	196	217	224	229	215	223	206	220	202	192	198
Thr	116	130	109	140	164	167	158	172	156	173	170	172	169	177	155	173	158	151	153
Tyr	42.2	77.2	66.1	83.4	97.8	98.9	95.6	103	92.4	103	105	105	99.8	108	94	105	93.8	90.6	93.8
Kynurenine	0.285	1.1	0.87	1.21	1.4	1.5	1.44	1.53	1.44	1.52	1.55	1.63	1.42	1.59	1.45	1.61	1.36	1.43	1.39
Met-SO	27.9	29	24.4	31	34.1	35.2	33.7	37	34.4	36.9	36.5	37.4	36	36.8	34.4	37.4	33.4	32.5	32.9
Sugar																			
Hexoses	529	8212	6915	8642	**9672**	**9746**	**9962**	10393	9172	10093	10243	9859	10215	10244	9217	10213	9411	8581	8967

* LOD: limit of detection Results of independent triplicates (1, 2, and 3) are shown. Values are all in µM. In bold, metabolites produced by uninfected cells. In red, products that were consumed more in mFabT- than in WT- depleted conditioned supernatants. Source data are available at http://dx.doi.org/10.21228/M88C05 .

Supplementary Table 6. Bacterial growth in the presence of platensimycin (1 µg/mL)[a]

	OD_{600} at 4 h	
	WT	**mFabT**
BHI+BSA	1.05 ± 0.13	1.03 ± 0.22
BHI+BSA+Platensimycin	0.34 ± 0.05	0.91 ± 0.18
BHI+BSA+C17:1	1.38 ± 0.19	1.28 ± 0.21
BHI+BSA+C17:1+Platensimycin	1.37 ± 0.19	1.23 ± 0.19

[a] Bacteria were grown in indicated conditions starting from an overnight BHI culture and then diluted to an initial OD_{600} = 0.05 (N=3, corresponding to 3 independent culture samples). Medium was BHI supplemented with FA-free BSA (0.025 %), to which Platensimycin (1 µg/mL) and C17:1 100 µM were added when indicated.

Source data are provided as a Source Data file.

Supplementary Table 7. Strains and plasmids used in this study.

Strains or plasmids	Relevant properties	Source or reference
Streptococcus pyogenes		
WT	Wild-type representative *emm28* clinical isolate, M28PF1	1
CCH1718	M28PF1 derivative harboring multiple mutations including the replacement in *fabT* of C313 by T encoding the FabT^{H105Y} polymorphism	Laboratory collection
Δ*fabT*	M28PF1 deleted for *fabT*	This study
mFabT	M28PF1 mutated in *fabT* C313T (FabT^{H105Y})	2
WT$^{eryR\text{-}igfp}$	M28PF1 with the integrated pG1-lacA-PTetO-gfp containing an erythromycin resistance cassette	3
mFabT$^{eryR\text{-}igfp}$	mFabT with the integrated pG1-lacA-PTetO-gfp containing an erythromycin resistance cassette	This study
Escherichia coli		
Stellar^{TM}	F-, *endA1,* supE44, *thi-1, recA1, gyrA96, phoA, Φ80d lacZ* Δ M15, Δ(*lacZYA – argF*) U169, Δ(*mrr – hsdRMS –mcrBC*), Δ*mcrA, λ-*	Clontech
Plasmids		
pG1	thermosensitive broad-host-range vector	4
pG1-Δ*fabT*	pG1-Δ*fabT* construct for *fabT* deletion in *S. pyogenes*	This study
pG1-lacAPTetO-gfp	pG1-lacA-Perm-gfp with the Erm promoter replaced by the tetO tetR Pxyl promoter of pTCV_TetO. Can be integrated in *S. pyogenes* genome for anhydrotetracycline inducible *gfp* expression	3

1. Longo, M. et al. Complete Genome Sequence of Streptococcus pyogenes emm28 Clinical Isolate M28PF1, Responsible for a Puerperal Fever. Genome Announc 3, doi:10.1128/genomeA.00750-15 (2015).
2. Lambert, C. et al. A *Streptococcus pyogenes* DegV protein regulates the membrane lipid content and limits the formation of extracellular vesicles. PLoS One 18, e0284402, doi:10.1371/journal.pone.0284402 (2023).
3. Weckel, A. et al. Streptococcus pyogenes infects human endometrium by limiting the innate immune response. J Clin Invest 131, doi:10.1172/JCI130746 (2021).
4. Biswas, I., Gruss, A., Ehrlich, S. D. & Maguin, E. High-efficiency gene inactivation and replacement system for gram-positive bacteria. J Bacteriol 175, 3628-3635, doi:10.1128/jb.175.11.3628-3635.1993 (1993).

Supplementary Table 8. Primers used in this study for cloning, clone verifications, PCR, and qRT-PCR experiments.

Primer name	Sequence	Used for
P47	CGCCAGGGTTTTCCCAGTCACGAC	Plasmid construction for Δ*fabT* strain
RP48	AGCGGATAACAATTTCACACAGGA	Plasmid construction for Δ*fabT* strain
ermB-F	GAGTGTGTTGATAGTGCAGT	Plasmid construction for Δ*fabT* strain
ermB-R	TAGGCGCTAGGGACCTCTTTA	Plasmid construction for Δ*fabT* strain
FabTavF	GATATTTTCAACCGGGCGTGGACTAGGGAATCTAC	Plasmid construction for Δ*fabT* strain
FabTavR2	CCATGATTACGAATTCGCACCATCTGTGTGTAAT	Plasmid construction for Δ*fabT* strain
FabTamR	CCGGTTGAAAATATCAACTAAGTATT	Plasmid construction for Δ*fabT* strain
FabTamF2	CGACTCTAGAGGATCCGCCAAAACCAGTCATCCTT	Plasmid construction for Δ*fabT* strain
PFabTamEco	ATATAGAATTCGGCAGAGCTTGCTATTCAAGA	PCR overlapping *fabM* and *fabT*
PFabTavBam	ATAGGATCCGCAAGATCATCTACAACATCA	PCR overlapping *fabM* and *fabT*
fabH_acpP_Am	GGGACAGATTAGGTTAGATGGT	PCR overlapping *fabH* and *acpA*
fabH_acpP_Av	GCGTTACTTCTTCTGTCTCCTT	PCR overlapping *fabH* and *acpA*
acpP_fabK_Am	GGTGACCTTGTTGCTTA	PCR overlapping *acpA* and *fabK*
acpP_fabK_Av	CCATTCCTCCTTGAAAA	PCR overlapping *acpA* and *fabK*
fabZ_accC_Am	GCAGCAAGTGGAACATTAA	PCR overlapping *fabZ* and *accC*
fabZ_accC_Av	CACCACGATTAGCAATTAA	PCR overlapping *fabZ* and *accC*
accD-end	GCGTTACCAACGCTTTCGTAAA	PCR overlapping *accD* and *serS*
serS-end	GGCGAAACCGTCATTTCACCT	PCR overlapping *accD* and *serS*
FabT-222	GGTCTGGCAAAGCTTTTTCA	Identification of spontaneous *fabT* mutants
FabTavComp	GGCGTTCTGCTATTCCTGTT	Identification of spontaneous *fabT* mutants
rofA-Am	GGCGTTAAATTGTCGAAGATACTGC	qRT-PCR of M28_*Spy0184* coding for RofA
rofA-Av	CCTACTGGCTATTTTGTTGACAGAA	qRT-PCR of M28_*Spy0184* coding for RofA
0371-Am	GCCGATTTCTGATCTACCCCTT	qRT-PCR of M28_*Spy0371*
0371-Av	GCCCTTACCTTTGGAGTGATGA	qRT-PCR of M28_*Spy0371*
0853_Am	GCCAACATGGTTTTTCCGAA	qRT-PCR of M28_*Spy0853*
0853_Av	GGGATAACCAAGTCAATCTT	qRT-PCR of M28_*Spy0853*
1136-Am	CCGGATATGCAAGAATATTCCTT	qRT-PCR of M28_*Spy1136*
1136-Av	CCCGTAGCGGTCAAATAATTGGT	qRT-PCR of M28_*Spy1136*
R28-Am	GGGCGCAATTACCTTTACTGCAAA	qRT-PCR of M28_*Spy1336* coding for R28
R28-Av	CCGTTTGGTCCTTACCTTTTGGTT	qRT-PCR of M28_*Spy1336* coding for R28
1434-Am	CCCTACAGATTGGCACAGAAAT	qRT-PCR of M28_*Spy1434* coding for FakB3
1434-Av	GGGCTTGGACAGCTAGATGT	qRT-PCR of M28_*Spy1434* coding for FakB3
1562-Am	GCACGTTTAGATACATGACAATTGG	qRT-PCR of M28_*Spy1562* coding for UspA
1562-Av	GCTCTCACGATCAACAGATCAAT	qRT-PCR of M28_*Spy1562* coding for UspA
norA-Am	GTGGCCATCACAGTAATAAACTTT	qRT-PCR of M28_*Spy1563* coding for NorA
norA-Av	CCCTGTTGCCAATCCTGCCT	qRT-PCR of M28_*Spy1563* coding for NorA
1638-Am	GCCAGCTTTCATCTTTTTCAAAAGG	qRT-PCR of M28_*Spy1638* coding for FakB4
1638-Av	GGGCAGAAGATCATGATATTGTC	qRT-PCR of M28_*Spy1638* coding for FakB4
ska-Am	CCGTCCATCTGTCAACAACAGCCA	qRT-PCR of M28_*Spy1672* coding for Ska
ska-Av	CCTCCATGAGCAGGTTGTGATGTT	qRT-PCR of M28_*Spy1672* coding for Ska
csp-Am	GGCTGCATGCTATCTACGGCA	qRT-PCR of M28_*Spy1699* coding for Cell Surface protein
csp-Av	GCCGCTATTCCATTCGCAAACT	qRT-PCR of M28_*Spy1699* coding for Cell Surface protein
scpA-Am	GGGGGTTCTACGGCTTGTTC	qRT-PCR of M28_*Spy1700* coding for ScpA
scpA-Av	GCGCTCATGTCTACGAGCAT	qRT-PCR of M28_*Spy1700* coding for ScpA
sfbX-Am	GGGGCTTGAAACTCGGTGTT	qRT-PCR of M28_*Spy1715* coding for SfbX
sfbX-Av	GGCCAAGTCTGGGACCCACT	qRT-PCR of M28_*Spy1715* coding for SfbX
sof-Am	GGGGTGAGGCTGGAGTAGTG	qRT-PCR of M28_*Spy1716* coding for SOF
sof-Av	GCAGCTGGGAGCGGAGCTA	qRT-PCR of M28_*Spy1716* coding for SOF
gyrA1	GCCATGAGTGTCATTGTGGC	qRT-PCR of *gyrA*
gyrA2	GGCGATAACTCCACCACTGA	qRT-PCR of *gyrA*