Supplemental Materials

TABLE S1 The fatty acid profile of phosphatidyl glycerol present in WT *S. aureus* or a *geh lip* mutant supplemented with human LDLs.

	WT cultured in tryptone broth			WT cultured in tryptone broth with human LDLs			*geh lip* cultured in tryptone broth			*geh lip* cultured in tryptone broth with human LDLs		
Phosphatidyl glycerol* (TC:TDB)	Normalized ion abundance / mg of cells	SD	Fatty acids#	Normalized ion abundance / mg of cells	SD	Fatty acids#	Normalized ion abundance / mg of cells	SD	Fatty acids#	Normalized ion abundance / mg of cells	SD	Fatty acids#
PG(28:0)	10.69	0.80	13:0_15:0 14:0_14:0	6.32	0.53	13:0_15:0 12:0_16:0	18.44	1.63	13:0_15:0 16:0_12:0 14:0_14:0	7.09	0.27	13:0_15:0 12:0_16:0
PG(30:0)	726.33	61.40	15:0_15:0 16:0_14:0	491.94	44.97	15:0_15:0 16:0_14:0	859.61	54.09	15:0_15:0 16:0_14:0	453.96	11.47	15:0_15:0
PG(30:1)	0.00	0.00	N.D.	0.11	0.08	N.D.	0.13	0.06	N.D.	0.00	0.00	N.D.
PG(31:0)	337.75	27.78	16:0_15:0 17:0_14:0 13:0_18:0	334.15	33.02	16:0_15:0 17:0_14:0 13:0_18:0	424.11	21.08	16:0_15:0 17:0_14:0 13:0_18:0	318.37	10.16	16:0_15:0 17:0_14:0 13:0_18:0
PG(31:1)	1.96	0.19	16:1_15:0 14:1_17:0 12:1_19:0	7.46	0.38	16:1_15:0 14:1_17:0 12:1_19:0 11:1_20:0	2.64	0.24	16:1_15:0 14:1_17:0 12:1_19:0	3.02	0.18	16:1_15:0 14:1_17:0 12:1_19:0 11:1_20:0
PG(32:0)	2644.01	245.70	17:0_15:0 14:0_18:0	2151.01	183.83	17:0_15:0 14:0_18:0 16:0_16:0	3029.60	83.82	17:0_15:0 14:0_18:0	1981.65	11.24	17:0_15:0
PG(32:1)	1.49	0.07	N.D.	7.79	0.58	17:1_15:0 16:1_16:0 18:1_14:0	1.99	0.29	17:1_15:0 15:1_17:0 13:1_19:0	2.18	0.10	17:1_15:0 16:1_16:0
PG(33:0)	839.55	78.03	18:0_15:0 19:0_14:0 17:0_16:0 20:0_13:0	780.42	67.72	18:0_15:0	956.42	5.25	18:0_15:0 19:0_14:0 17:0_16:0 20:0_13:0	680.65	6.84	18:0_15:0
PG(33:1)	12.76	1.01	18:1_15:0	214.05	19.82	18:1_15:0	14.19	0.59	18:1_15:0	28.02	1.43	18:1_15:0
PG(34:0)	1292.40	122.26	19:0_15:0 17:0_17:0	1100.50	96.94	19:0_15:0 17:0_17:0 18:0_16:0	1491.05	9.92	19:0_15:0 17:0_17:0	968.45	5.89	19:0_15:0 17:0_17:0
PG(34:1)	0.00	0.00	N.D.	16.58	1.53	18:1_16:0 16:1_18:0	0.00	0.00	N.D.	0.00	0.00	N.D.

PG(34:2)	0.00	0.00	N.D.	5.04	0.37	16:1_18:1 18:2_16:0	0.00	0.00	N.D.	2.00	0.25	16:1_18:1 18:2_16:0
PG(35:0)	668.87	63.04	20:0_15:0	581.48	47.20	20:0_15:0	772.35	19.76	20:0_15:0 18:0_17:0	491.91	6.40	20:0_15:0 18:0_17:0
PG(35:1)	17.07	1.75	20:1_15:0 19:1_16:0	61.86	5.26	20:1_15:0 18:1_17:0 19:1_16:0	19.16	0.22	20:1_15:0 19:1_16:0	14.66	0.46	20:1_15:0 19:1_16:0 18:1_17:0
PG(35:2)	0.00	0.00	N.D.	8.04	0.50	20:2_15:0 18:2_17:0 16:1_19:1	0.03	0.01	N.D.	6.04	0.23	20:2_15:0 18:2_17:0
PG(35:4)	0.00	0.00	N.D.	1.46	0.26	20:4_15:0 17:2_18:2 20:3_15:1	0.00	0.00	N.D.	20.13	0.60	20:4_15:0
PG(35:5)	0.00	0.00	N.D.	0.00	0.00	N.D.	0.00	0.00	N.D.	1.27	0.15	20:5_15:0 20:4_15:1
PG(36:0)	94.41	7.22	21:0_15:0 19:0_17:0 20:0_16:0	74.42	7.10	21:0_15:0 19:0_17:0 20:0_16:0 18:0_18:0	115.92	7.02	21:0_15:0 19:0_17:0 20:0_16:0	67.28	3.08	21:0_15:0 19:0_17:0 18:0_18:0
PG(36:2)	0.00	0.00	N.D.	46.15	4.14	18:1_18:1 18:2_18:0	0.00	0.00	N.D.	0.39	0.16	18:2_18:0 18:1_18:1
PG(36:3)	0.11	0.05	N.D.	17.05	1.56	18:1_18:2 20:3_16:0	0.00	0.00	N.D.	0.20	0.10	18:2_18:1
PG(36:4)	0.00	0.00	N.D.	2.34	0.35	18:2_18:2 16:0_20:4 18:3_18:1	0.00	0.00	N.D.	0.25	0.06	18:2_18:2 20:3_16:1 20:4_16:0
PG(37:0)	11.36	1.17	22:0_15:0 20:0_17:0 18:0_15:0	10.57	0.56	20:0_17:0 18:0_19:0	12.36	0.64	22:0_15:0 20:0_17:0 18:0_15:0	7.73	0.24	22:0_15:0 20:0_17:0 18:0_15:0
PG(37:2)	0.00	0.00	N.D.	1.85	0.11	19:0_18:2	0.00	0.00	N.D.	0.38	0.15	19:0_18:2
PG(37:4)	0.00	0.00	N.D.	0.00	0.00	N.D.	0.00	0.00	N.D.	0.91	0.05	20:4_17:0

Not determined (N.D.)

* Detected as [M-H]- ions

#Fatty acids are listed in order of isomer abundance.

TABLE S2 The fatty acid profile of *S. aureus* grown in tryptic soy broth supplemented with human LDLs.

	Cells cultured in TSB			Cells cultured in TSB with human LDL		
Phosphatidyl glycerol* (TC:TDB)	Normalized ion abundance / mg of cells	Standard deviation	Fatty acids#	Normalized ion abundance / mg of cells	Standard deviation	Fatty acids#
PG(28:0)	12.56	1.51	14:0_14:0	4.76	0.21	14:0_14:0
PG(30:0)	122.24	7.28	15:0_15:0 14:0_16:0	36.57	1.26	15:0_15:0 14:0_16:0
PG(31:1)	3.34	0.27	16:1_15:0	0.00	0.00	N.D.
PG(31:0)	460.63	13.99	16:0_15:0 13:0_18:0 17:0_14:0	118.50	5.24	16:0_15:0 13:0_18:0 17:0_14:0
PG(32:1)	0.00	0.00	N.D.	4.38	0.24	18:1_14:0 16:1_16:0
PG(32:0)	326.07	1.63	17:0_15:0 14:0_18:0 16:0_16:0 19:0_13:0	74.26	2.97	17:0_15:0 14:0_18:0 16:0_16:0
PG(33:1)	15.30	0.38	18:1_15:0 19:1_14:0	21.05	0.71	18:1_15:0
PG(33:0)	1103.22	6.40	18:0_15:0 19:0_14:0 17:0_16:0 20:0_13:0	248.69	13.24	18:0_15:0 19:0_14:0 17:0_16:0 20:0_13:0
PG(34:2)	0.00	0.00	N.D	1.50	0.05	18:2_16:0
PG(34:1)	4.17	0.08	19:1_15:0 20:1_14:0	6.37	0.29	18:1_16:0
PG(34:0)	279.90	2.14	19:0_15:0 20:0_14:0 18:0_16:0 17:0_17:0	47.91	1.95	20:0_14:0 19:0_15:0
PG(35:1)	22.04	0.62	20:1_15:0 21:1_14:0 19:1_16:0	8.74	0.49	20:1_15:0
PG(35:0)	877.73	9.10	20:0_15:0 18:0_17:0 21:0_14:0	150.25	6.87	20:0_15:0
PG(36:3)	0.00	0.00	N.D.	4.56	0.27	18:1_18:2
PG(36:2)	0.00	0.00	N.D.	13.15	0.59	18:1_18:1 18:0_18:2
PG(36:0)	19.83	0.38	21:0_15:0 20:0_16:0 19:0_17:0	2.25	0.03	20:0_16:0 21:0_15:0
PG(37:0)	12.73	0.22	22:0_15:0 20:0_17:0 23:0_14:0	2.15	0.08	22:0_15:0

Not determined (N.D.)

* Detected as $[M-H]^-$ ions

#Fatty acids are listed in order of isomer abundance.

Table S3 Characterization of FASII initiation genes of the fatty acid auxotrophs**.**

Isolate	*fabD* variation (SAUSA300_1123)	*accA* variation (SAUSA300_1646)	*accB* variation (SAUSA300_1476)	*accC* variation (SAUSA300_1475)	*accD* variation (SAUSA300_1647)
1A	WT	WT	NtΔ 1,644,142 – 1,644,251	WT	WT
1B	WT	WT	WT	WT	WT
1C	WT	WT	WT	NtΔ 1,643,889 – 1,643,971	WT
2A	WT	NtΔ 1,808,761 - 1,808,943	WT	WT	WT
2B	WT	WT	WT	WT	WT
2C	WT	WT	WT	WT	Nt 1,809,715 C→A
3A	WT	WT	WT	WT	WT
3B	WT	WT	WT	WT	WT
3C	NtΔ 1,243,511 – 1,243,626; Nt 1,243,630 C→A	WT	WT	NtΔ 1,642,929 T	WT

Nt - Nucleotide (Nt)

NtΔ nucleotide(s) deletion

Table S4 Phosphatidyl glycerol species from cells cultured with human LDLs that contain exogenous, unsaturated fatty acids in both the sn1 and sn2 position.

Phosphatidyl glycerol	Fatty acids
PG(34:2)	16:1_18:1
PG(35:2)	16:1_19:1
PG(35:4)	17:2_18:2 20:3_15:1
PG(36:2)	18:1_18:1
PG(36:3)	18:2_18:1
PG(36:4)	18:2_18:2 18:3_18:1

Table S5 Hypothetical lipases of *S. aureus* strain USA300

Locus tag	Description
SAUSA300_0070	putative lysophospholipase
SAUSA300_0641	putative lipase/esterase
SAUSA300_1710	putative lysophospholipase
SAUSA300_2457	phospholipase/carboxylesterase

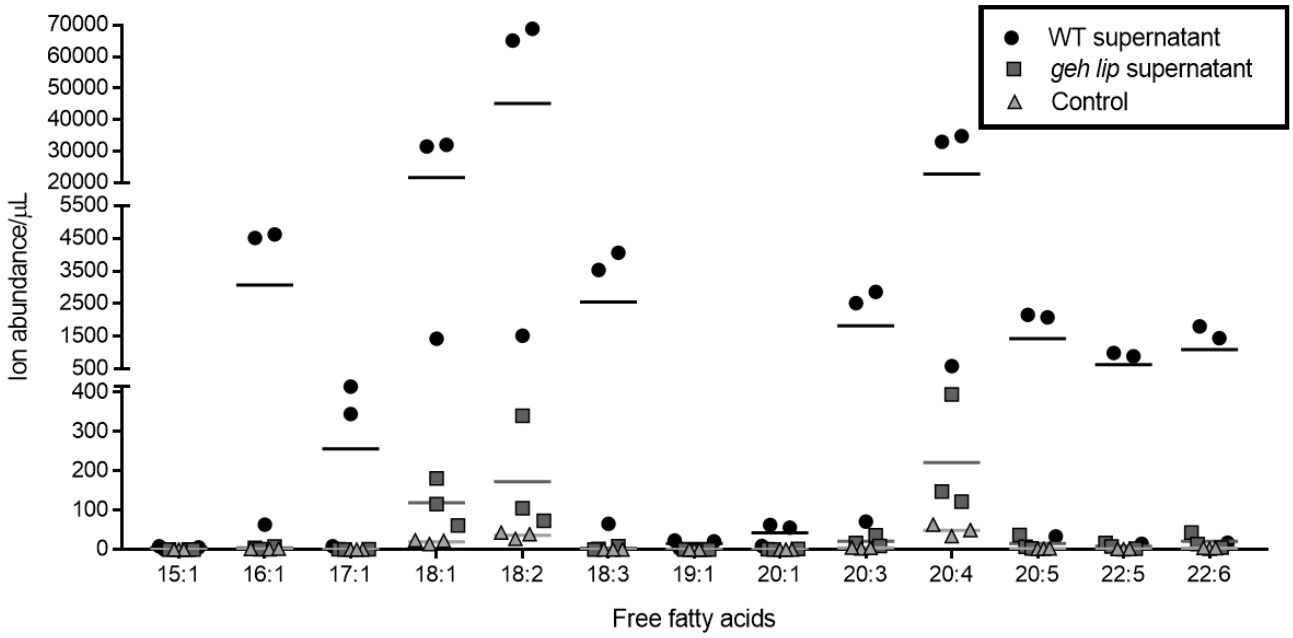

FIG S1 Incubation of human LDLs with *S. aureus* supernatants results in the liberation of free fatty acids that is dependent on secreted lipases. WT and the *geh lip* double mutant were grown overnight in tryptone broth. Cultures were pelleted and the supernatants collected and chemically sterilized. The supernatants or a broth only control (control) were incubated with 5% human LDLs at 37°C for two hours in triplicate. Free fatty acids were detected by direct infusion high resolution/accurate mass spectrometry and tandem mass spectrometry.

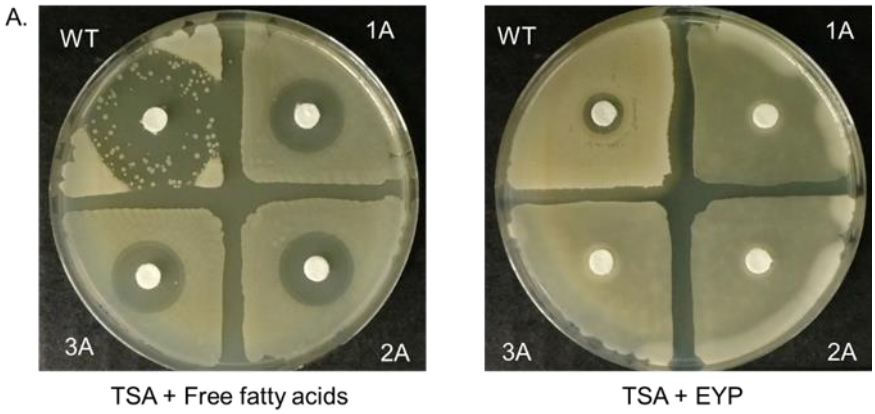

FIG S2 The growth of triclosan-resistant, fatty acid auxotrophs is supported by lipoprotein particles. (A) Triclosan-resistant fatty acid auxotrophs were plated as a lawn on TSA with the following supplement: free fatty acid mixture or 1% EYP. A 6mm filter disk containing with 10µL of 1.7mM triclosan was placed on top of the agar. Plates were incubated for ~36 hours at 37°C.

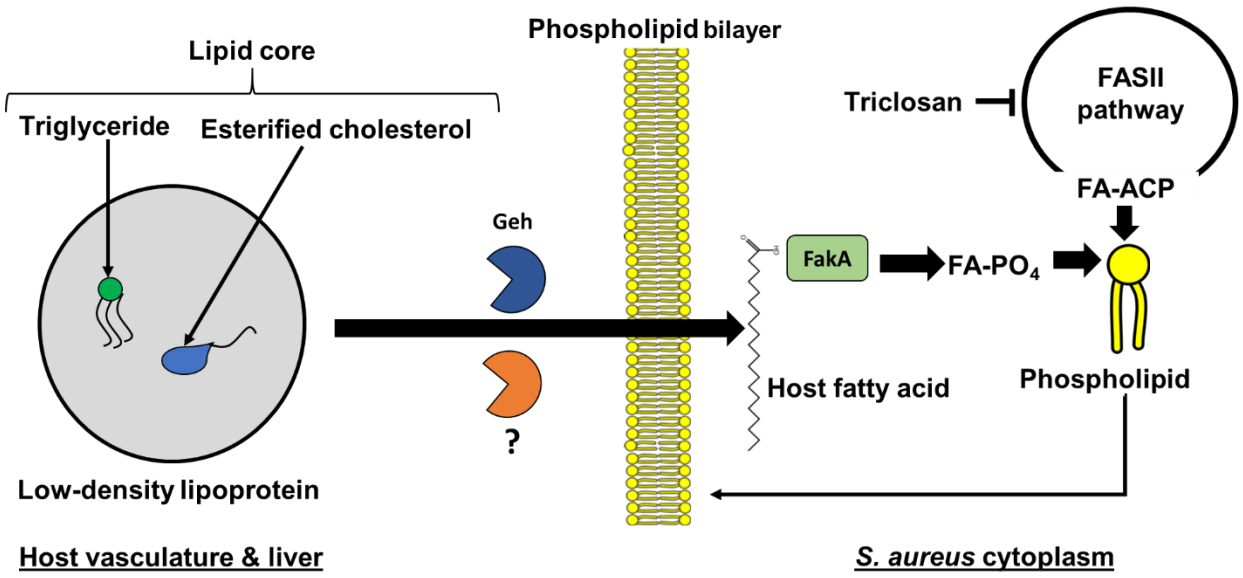

FIG S3 A model for the incorporation of fatty acids present in host LDLs into the *S. aureus* membrane. Lipases secreted by *S. aureus* liberate fatty acids from the triglycerides, esterified cholesterol, and phospholipids of human LDL. The fatty acids are then incorporated into the staphylococcal membrane via the activity of FakA. This process allows *S. aureus* to bypass FASII inhibition by triclosan.