FIG S1. *Rickettsia* species import six host metabolites that are required for the synthesis of cell envelope glycoconjugates and glycerophospholipids.

(A) Consensus metazoan pathway for the generation of metabolites (UDP-glucose, *N*-acetyl-alpha-D-glucosamine 1-P (*N*AG-1-P), D-ribose 5-P, dihydroxyacetone P (DHAP), *sn*-glycerol 3-P (G3P), and pyruvate) that *Rickettsia* species must acquire to initiate biosynthesis of peptidoglycan (PGN), lipopolysaccharide (LPS) and glycerophospholipids. Red ellipses, metabolites previously shown to be imported; yellow ellipses, metabolites predicted to be imported based on metabolic network reconstruction. Gray shading depicts each point of the host metabolic network where *Rickettsia* species acquire metabolites, with blue text describing how rickettsiae utilize these metabolites. For biosynthesis of glycerophospholipids, *Rickettsia* species import G3P, but also can convert G3P from DHAP if *gpsA* (encoding glycerol-3-phosphate dehydrogenase) is present (shown in green) (1, 2).

(B) Schema and description of the bifunctional GlmU protein of *E. coli* (WP_000933736) (3).

(C) Eukaryotic (*Homo sapiens*, green) and bacterial (*Escherichia coli*, purple) pathways for conversion of D-glucosamine 6-P to *N*AG-1-P involve different enzymes and intermediates.

(D) Comparison of *E. coli* GlmU with *Rickettsia typhi* GlmU (AAU03917) illustrates that rickettsial GlmU proteins have lost the C-terminal acetyltransferase domain. Sequences were obtained from GenBank and aligned with MUSCLE (default parameters) (4). Domain colors above alignment correspond to description in panel B.

(E) PGN biosynthesis by *Rickettsia* spp. requires import of *N*AG-1-P, L-alanine (L-Ala) and *trans*,*trans*-farnesyl-PP (FPP) from the host. *N*AG-1-P is converted to UDP-*N*-acetyl-alpha-D-glucosamine (UDP-*N*AG) via the uridyltransferase GlmU_N, with the subsequent pathway to Lipid II (green arrows) highly conserved in *Rickettsia* genomes (see Fig. S10). Via alanine racemase (Alr), imported L-Ala is converted to D-Ala, which is incorporated into the stem peptide by D-alanine--D-alanine ligase A (DdlA). Imported FPP is converted via IspU and PgpB in to *di-trans*,*poly-cis*-undecaprenyl-P (orange arrows), which serves as the lipid carrier for Lipid I and

Lipid II (see Fig. 3 of the manuscript for more details). At least nine conserved enzymes participate in incorporation of PGN into the murine sacculus (red). The pathway for PGN recycling (purple arrows) initiates with subunit excision via lytic transglycosylases (Slt and RvhB1). Individual subunits are imported back to the cytoplasm via AmpG transporters, which are present in multiple divergent copies in each *Rickettsia* genome (5). The full Gram-negative bacterial PGN recycling pathway is shown (6), with enzymes lacking in *Rickettsia* genomes marked with red Xs. This minimal set of enzymes generates two recyclable products (purple ellipses), while the fate of the remaining degraded products (light green ellipses) is unknown.

(F) Lipopolysaccharide biosynthesis by *Rickettsia* spp. requires import of *N*AG-1-P, D-ribose 5-P and UDP-glucose from the host. GlmU_N converts imported *N*AG-1-P to UDP-*N*AG, which enters the Raetz pathway (7) for synthesis of Kdo_2-lipid A (light blue arrows). Imported D-ribose 5-P is converted to D-ribulose 5-P via ribose-5-phosphate isomerase B (RpiB), leading to synthesis of CMP-Kdo. A pathway hole (gray circle) indicates the absence of a phosphatase to convert 3-deoxy-D-manno-octulosonate 8-P to 3-deoxy-D-manno-octulosonate (Kdo) (see panels G-I). The incorporation of various fatty acids into the growing lipid A moiety is shown (pink arrows). Acyl chain incorporation into Lipid A follows the structure deduced for *R. typhi* (8). UDP-*N*AG is also ligated to *di-trans*,*poly-cis*-undecaprenyl-P via undecaprenyl-P alpha-*N*-acetylglucosaminyl 1-P transferase (WecA) (orange arrows), creating the lipid carrier for O-antigen (see Fig. 3 of the manuscript for more details). UDP-*N*-acetylglucosamine 2-epimerase (WecB) also utilizes UDP-*N*AG to generate UDP-*N*-acetyl-D-mannosamine (UDP-ManNAc). Imported UDP-glucose is converted to UDP-glucuronate (UDP-GlcA) and UDP-alpha-D-galactose (UDP-Gal) via UDP-glucose 6-dehydrogenase (Udg) and the epimerase/dehydratase FnlA, respectively. UDP-ManNAc, UDP-GlcA and UDP-Gal are predicted to be the main sugars used by peripheral enzymes (e.g., glycosyltransferases, glucosyltransferases, etc.) that polymerize O-antigen and extend the Kdo_2-lipid A acceptor.

(G) The pathway from D-Ribulose 5-P to CMP-Kdo is highly conserved across 2227 bacterial genomes (all prokaryotic genomes with an available Kdo_2-lipid A biosynthesis pathway at KEGG). The star depicts the pathway hole (no KdsC) in the *Rickettsia* CMP-Kdo pathway.

(H) Relative to the predominant "*E. coli*-like" pathway, the "*Rickettsia*-like" pathway for CMP-Kdo biosynthesis is far less common, occurring in 442 bacterial genomes (excluding *Rickettsia* genomes). The pathway hole (no KdsC) is noted with a red X.

(I) Taxonomic breakdown of bacterial species containing the "*Rickettsia*-like" CMP-Kdo biosynthesis pathway (442 total genomes). Genomes from intracellular species represent 41% of the total number of "*Rickettsia*-like" pathways.

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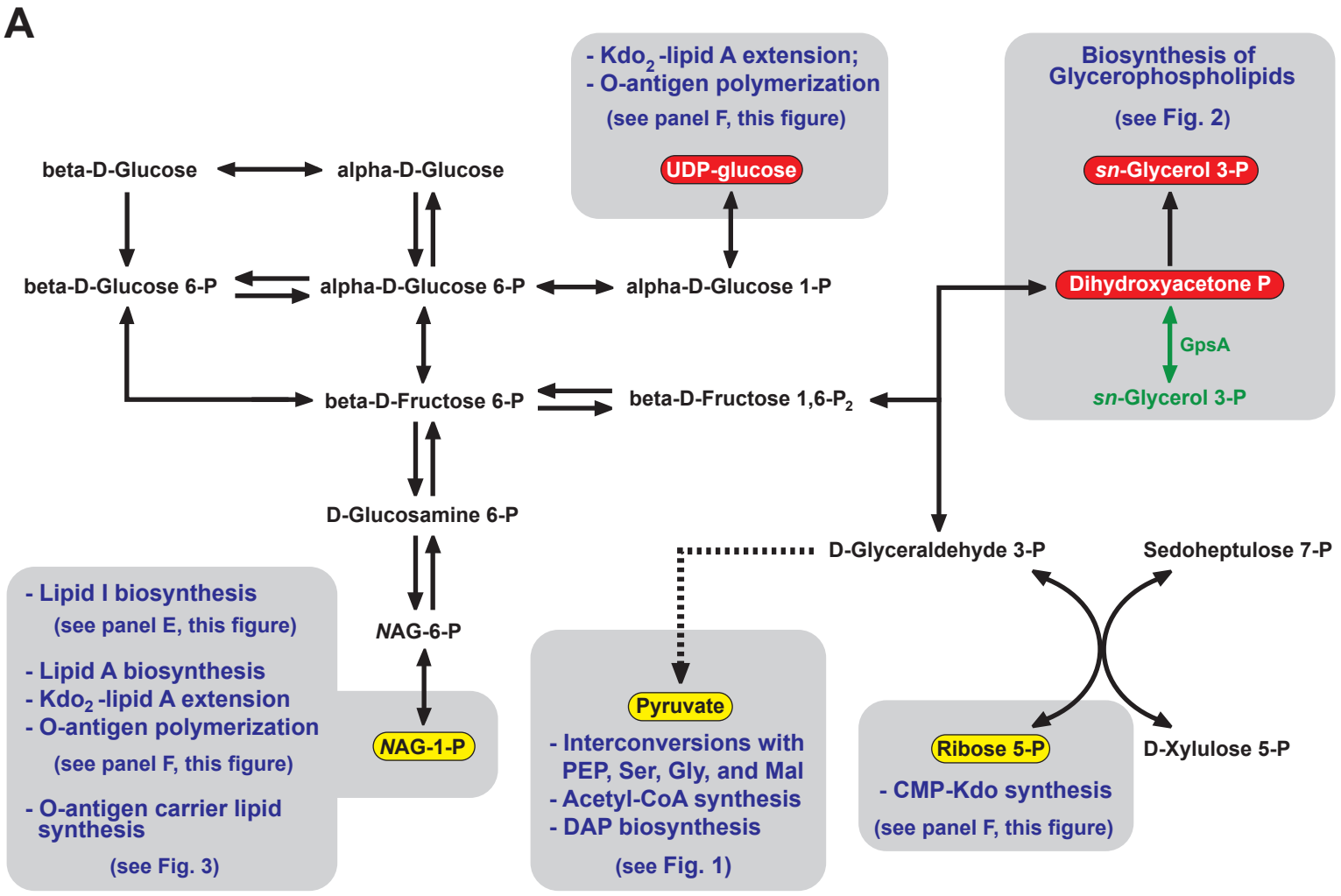
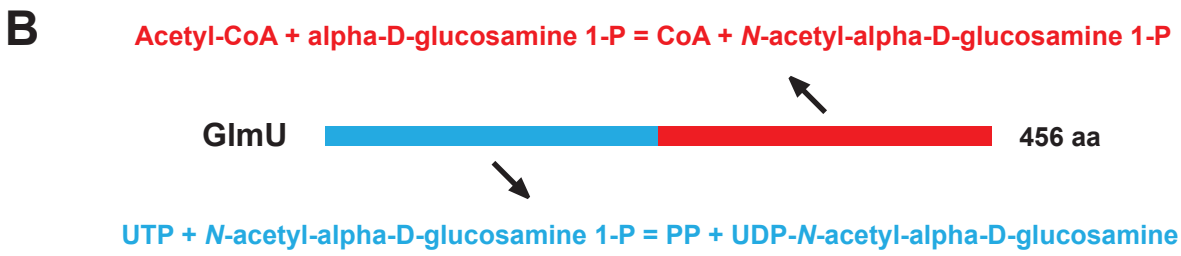

A
- Kdo2-lipid A extension;
- O-antigen polymerization
(see panel F, this figure)
UDP-glucose
Biosynthesis of Glycerophospholipids
(see Fig. 2)
sn-Glycerol 3-P
Dihydroxyacetone P
GpsA
sn-Glycerol 3-P
beta-D-Glucose
alpha-D-Glucose
beta-D-Glucose 6-P
alpha-D-Glucose 6-P
alpha-D-Glucose 1-P
beta-D-Fructose 6-P
beta-D-Fructose 1,6-P2
D-Glucosamine 6-P
D-Glyceraldehyde 3-P
Sedoheptulose 7-P
- Lipid I biosynthesis
(see panel E, this figure)
- Lipid A biosynthesis
- Kdo2-lipid A extension
- O-antigen polymerization
(see panel F, this figure)
- O-antigen carrier lipid synthesis
(see Fig. 3)
NAG-6-P
NAG-1-P
Pyruvate
- Interconversions with PEP, Ser, Gly, and Mal
- Acetyl-CoA synthesis
- DAP biosynthesis
(see Fig. 1)
Ribose 5-P
- CMP-Kdo synthesis
(see panel F, this figure)
D-Xylulose 5-P


B
Acetyl-CoA + alpha-D-glucosamine 1-P = CoA + N-acetyl-alpha-D-glucosamine 1-P
GlmU
456 aa
UTP + N-acetyl-alpha-D-glucosamine 1-P = PP + UDP-N-acetyl-alpha-D-glucosamine

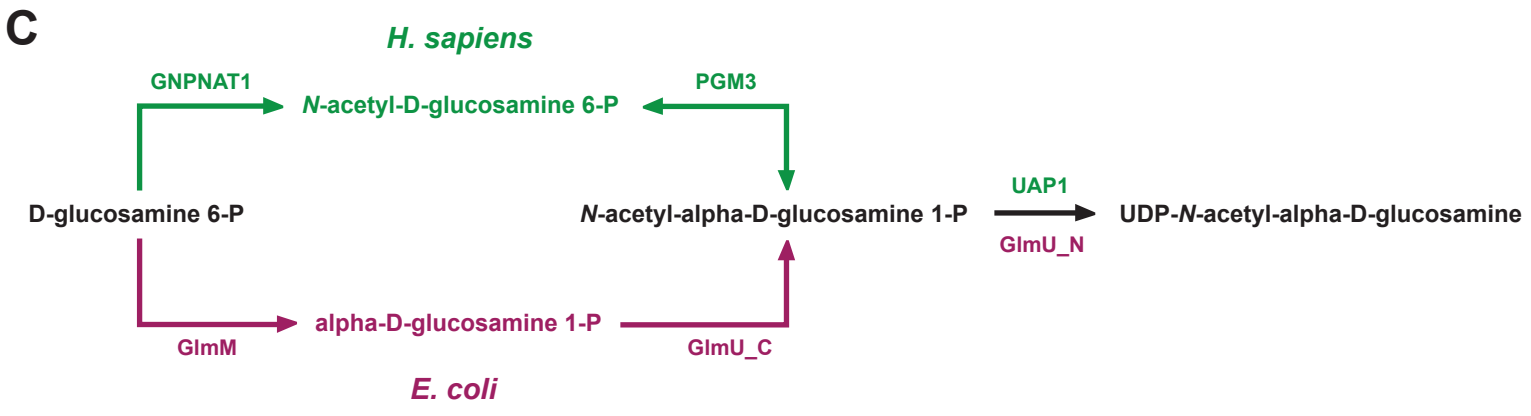

C
H. sapiens
GNPNAT1
N-acetyl-D-glucosamine 6-P
PGM3
D-glucosamine 6-P
N-acetyl-alpha-D-glucosamine 1-P
UAP1
UDP-N-acetyl-alpha-D-glucosamine
GlmU_N
alpha-D-glucosamine 1-P
GlmM
GlmU_C
E. coli

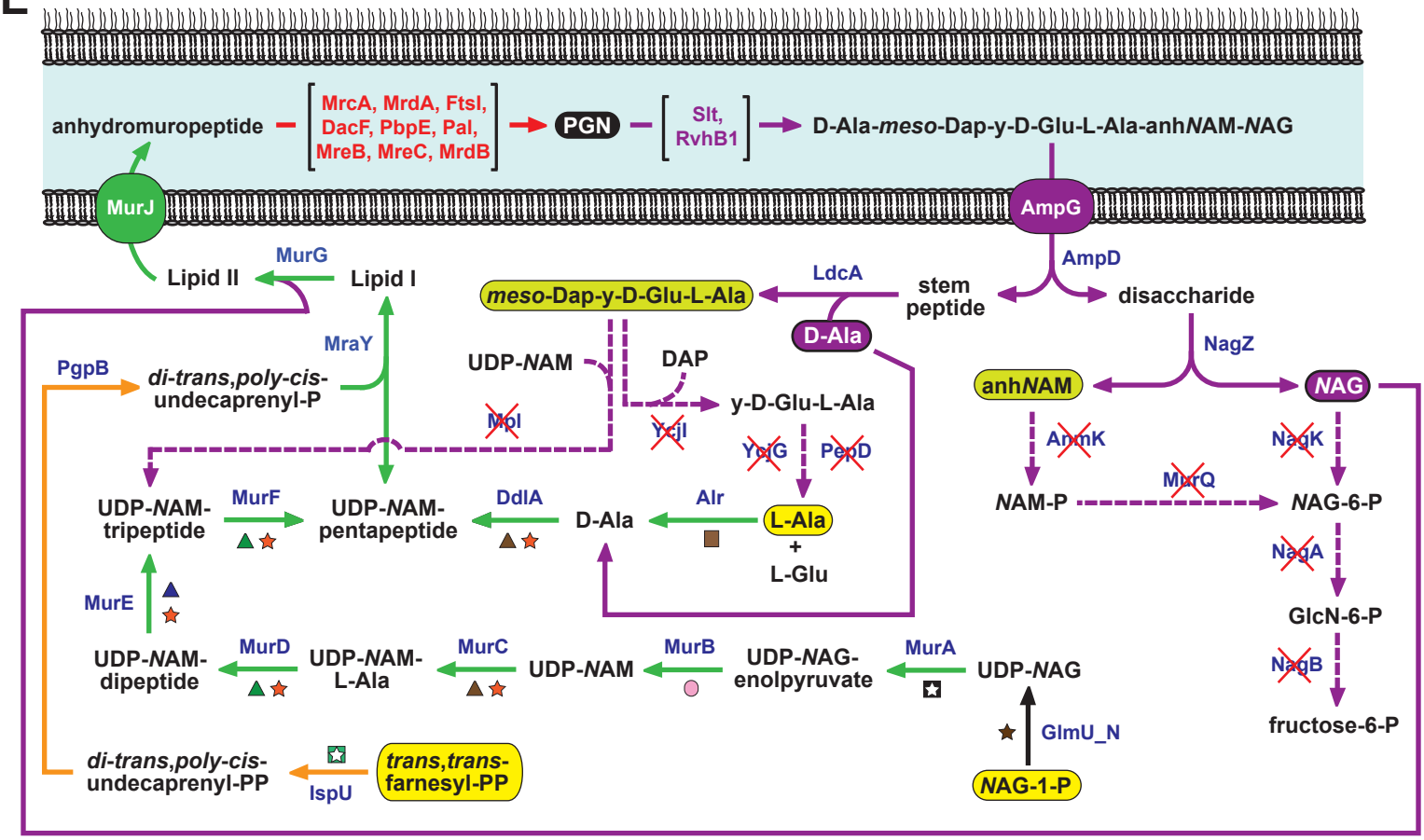
FIG. S1

D

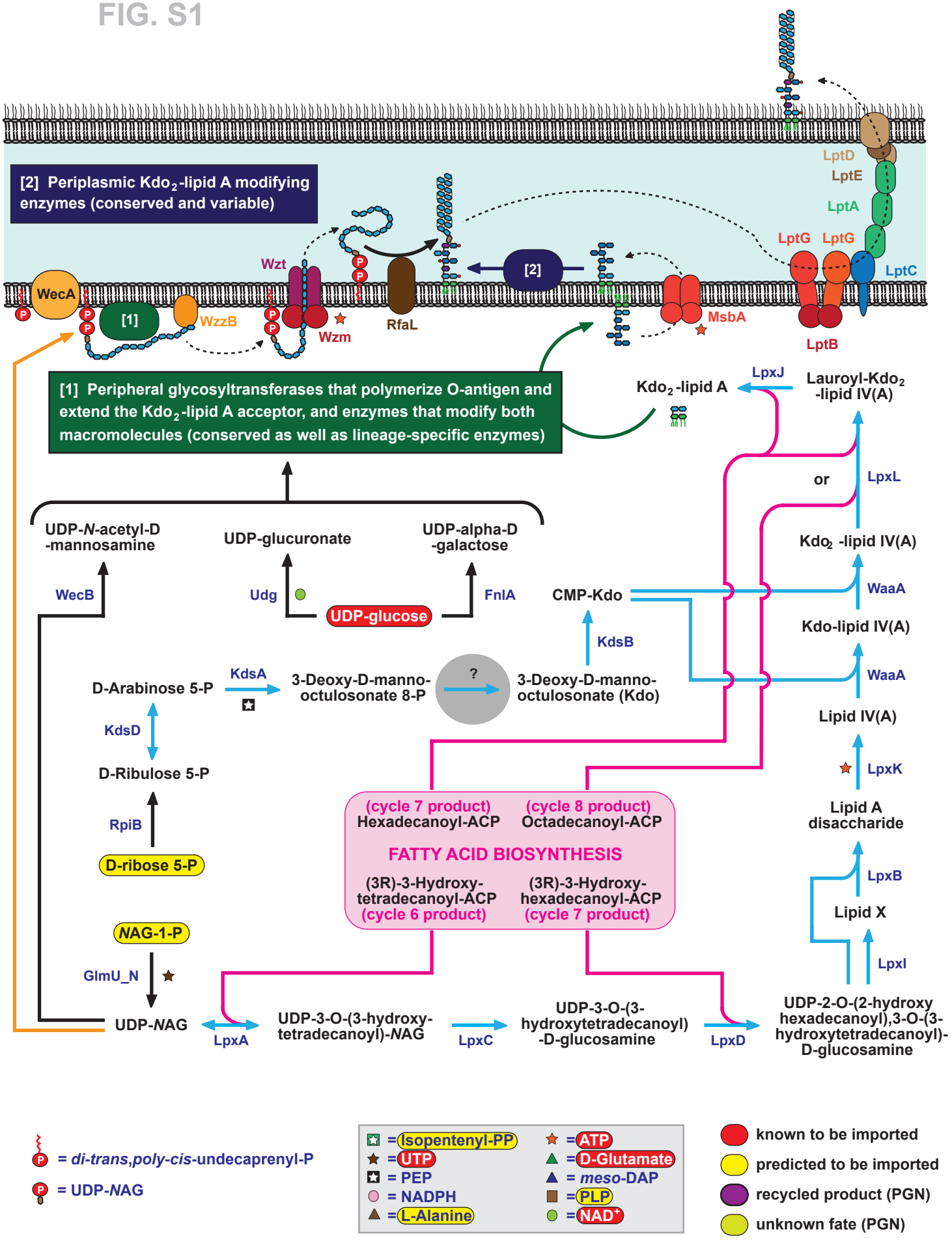
```
E. coli    MLNNA-MSVVILAAGKGTRMYSDLPKVLHTLAGKAMVQHVIDAANELGAAHVHLVYGHGG    59
R. typhi   MLHNENYQIIILAAGKGTRMESDLPKVMHEVGGVPMLETVLKNALKI-THDVIIVY---S    56
           **:*   .::*********** ******:* :.* .*:: *:. * :: :  * :**   .

E. coli    DLLKQALK--DDNLNWVLQAEQLGTGHAMQQAAPFFADDEDILMLYGDVPLISVETLQRL    117
R. typhi   EALKKYLTPYENMCRFVLQEEPKGTAHATHAAIDLIDQNKIILVLYGDHPLITPTLMYEL    116
           : **: *.  ::  .:*** *  **.** : *  :: ::: **:**** ***:   :  *

E. coli    RDAKPQGGIGLLTVKLD--DPTGYGRITRE-NGKVTGIVEHKDATDEQRQIQEINTGILI    174
R. typhi   IDYLSIINAALVTLSFERANPAQYGRIAIDKHGNFLEIIEYKNASEEEKKIKLCNSGIMA    176
           *  .  . .*:*:.::  :*: ****: : :*:.  *:*:*:*::*:.:*:  *:**:

E. coli    ANGADMKRWLAKLTNN--NAQGEYYITDIIALAYQEGREIVAVHPQRLSEVEGVNNRLQL    232
R. typhi   FSSGILNKYLPLFANNNTNCNQEIYLTEIVKICKNHGEKVSYLLSTDHDLIVGINTQSEL    236
           ... ::.:*. ::**  *.: * *:*:*: :. : * ::  : .   . : *:*.. :*

E. coli    SRLERVYQSEQAEKLLLAGVMLRDPARFDLRGTLTHGRDVEIDTNVIIEGNVTLGHRVKI    292
R. typhi   KEANNIFFQNKS----------------------------------------------    248
           .  :.:: .:::

E. coli    GTGCVIKNSVIGDDCEISPYTVVEDANLAAACTIGPFARLRPGAELLEGAHVGNFVEMKK    352
R. typhi   ------------------------------------------------------------    248

E. coli    ARLGKGSKAGHLTYLGDAEIGDNVNIGAGTITCNYDGANKFKTIIGDDVFVGSDTQLVAP    412
R. typhi   ------------------------------------------------------------    248

E. coli    VTVGKGATIAAGTTVTRNVGENALAISRVPQTQKEGWRRPVKKK    456
R. typhi   ---------------------------------------------    248
```

FIG. S1

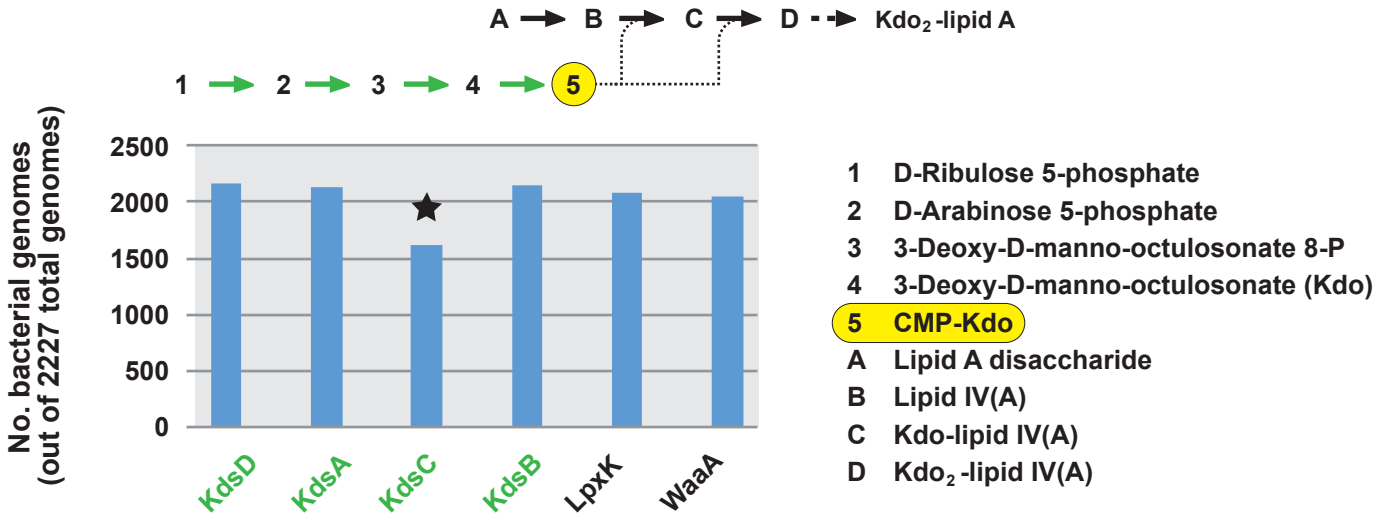
E



F FIG. S1


[2] Periplasmic Kdo2-lipid A modifying enzymes (conserved and variable)
[1] Peripheral glycosyltransferases that polymerize O-antigen and extend the Kdo2-lipid A acceptor, and enzymes that modify both macromolecules (conserved as well as lineage-specific enzymes)
WecA
[1]
WzzB
Wzt
Wzm
RfaL
[2]
MsbA
LptB
LptC
LptG
LptG
LptE
LptD
LptA
Kdo2-lipid A
LpxJ
Lauroyl-Kdo2-lipid IV(A)
or
LpxL
Kdo2-lipid IV(A)
WaaA
Kdo-lipid IV(A)
WaaA
Lipid IV(A)
LpxK
Lipid A disaccharide
LpxB
Lipid X
LpxI
UDP-2-O-(2-hydroxy hexadecanoyl),3-O-(3-hydroxytetradecanoyl)-D-glucosamine
LpxD
UDP-3-O-(3-hydroxytetradecanoyl) -D-glucosamine
LpxC
UDP-3-O-(3-hydroxy-tetradecanoyl)-NAG
LpxA
UDP-NAG
GlmU_N
NAG-1-P
D-ribose 5-P
RpiB
D-Ribulose 5-P
KdsD
D-Arabinose 5-P
KdsA
3-Deoxy-D-manno-octulosonate 8-P
?
3-Deoxy-D-manno-octulosonate (Kdo)
KdsB
CMP-Kdo
UDP-glucose
Udg
WecB
FnlA
UDP-N-acetyl-D-mannosamine
UDP-glucuronate
UDP-alpha-D-galactose
(cycle 7 product) Hexadecanoyl-ACP
(cycle 8 product) Octadecanoyl-ACP
FATTY ACID BIOSYNTHESIS
(3R)-3-Hydroxy-tetradecanoyl-ACP (cycle 6 product)
(3R)-3-Hydroxy-hexadecanoyl-ACP (cycle 7 product)
= di-trans,poly-cis-undecaprenyl-P
= UDP-NAG
= Isopentenyl-PP
= UTP
= PEP
= NADPH
= L-Alanine
= ATP
= D-Glutamate
= meso-DAP
= PLP
= NAD+
known to be imported
predicted to be imported
recycled product (PGN)
unknown fate (PGN)

G



H

Pathway	Composition	No. genomes
E. coli-like	1 → 2 → 3 → 4 → 5	1505
Rickettsia-like	1 → 2 → 3 ✗ 4 → 5	442

FIG. S1

I