SUPPLEMENTARY INFORMATION

Mosquito metabolomics reveal that dengue virus replication requires phospholipid changes via the remodeling cycle

Contains:

Figures S1-S13

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Legends from Datasets S1-S6

FIGURES

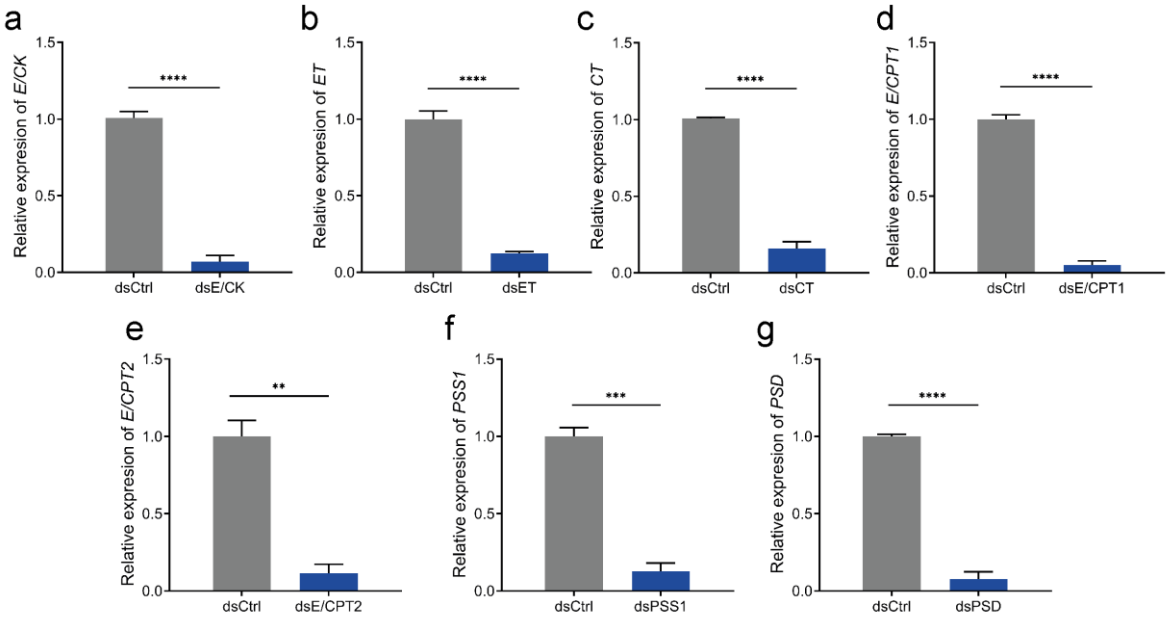


Fig S1. Validation of silencing for *de novo* pathway enzymes. Aag2 cells were transfected with dsRNA against either (a) E/CK, (b) ET, (c) CT, (d) E/CPT1, (e) E/CPT2, (f) PSS1, (g) PSD

www.pnas.org/cgi/doi/10.1073/pnas.2015095117

genes. DsRNA targeting LacZ (Ctrl) was used as control. At 72 h post transfection, mRNA from the targeted genes was quantified by RT-qPCR. *Actin* expression was used for normalization. Lines show mean ± s.e.m. from 6 biological repeats. **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001 as indicated by unpaired t-test. E/CK, ethanolamine/choline kinase; ET, CTP:phosphoethanolamine cytidyltransferase; CT, CTP:phosphocholine cytidyltransferase; E/CPT, DAG:CDP-ethanolamine/choline ethanolamine/cholinephosphotranferase; PSS1, PS synthase; PSD, PS decarboxylase.

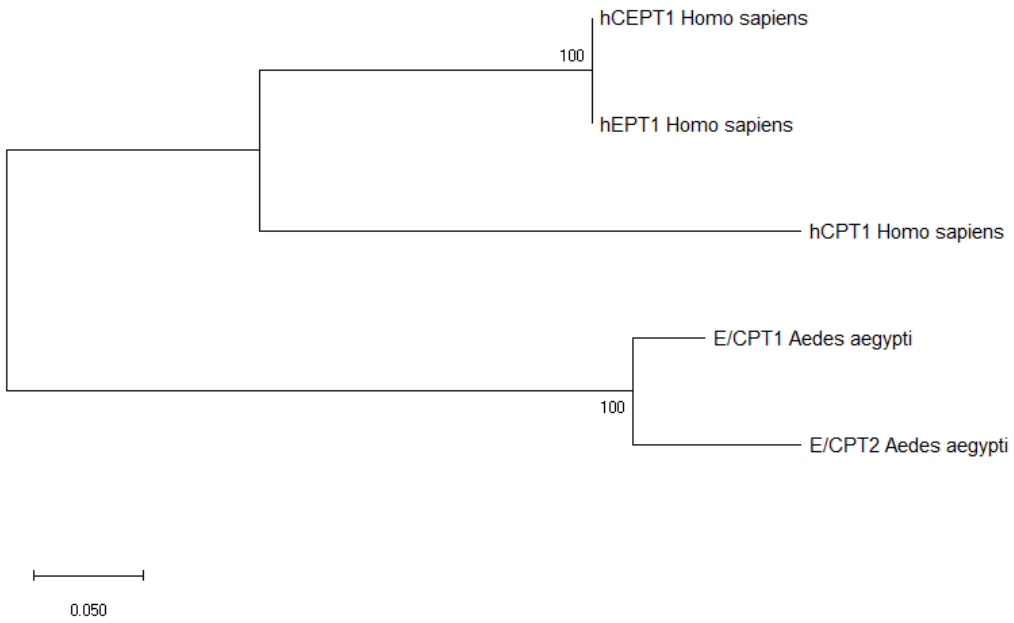
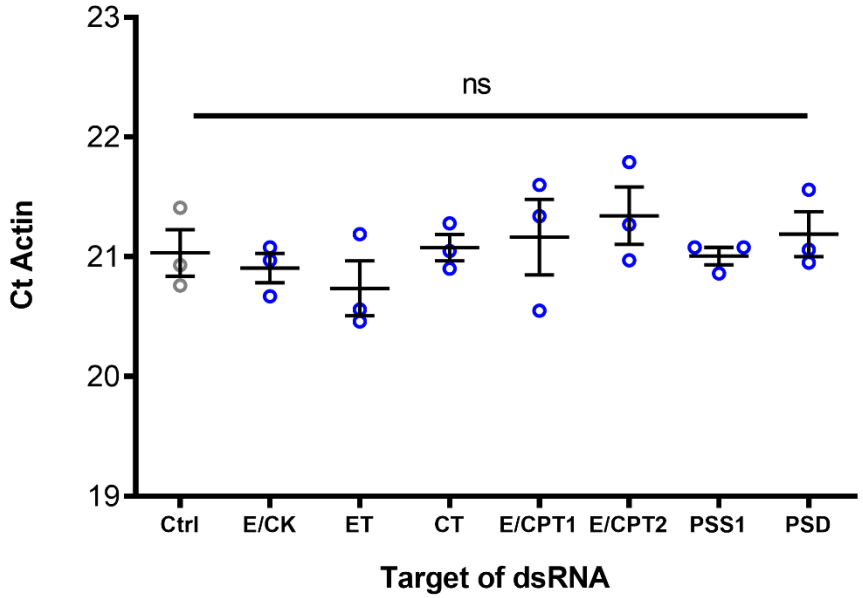


Fig S2. Maximum likelihood tree between CPTs and EPTs from Ae. aegypti and humans.



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29 **Fig S3. Cell survival measured by *actin* gene expression.** Aag2 cells were transfected with
30 dsRNA against either E/CK, ET, CT, E/CPT1, E/CPT2, PSS1, PSD genes. DsRNA targeting
31 LacZ (Ctrl) was used as control. At 72 h post transfection, *Actin* expression was quantified.
32 Lines show mean ± s.e.m. from 3 biological repeats and compared by Dunnett's multiple
33 comparisons test. E/CK, ethanolamine/choline kinase; ET, CTP:phosphoethanolamine
34 cytidyltransferase; CT, CTP:phosphocholine cytidyltransferase; E/CPT, DAG:CDP-
35 ethanolamine/choline ethanolamine/cholinephosphotranferase; PSS1, PS synthase; PSD, PS
36 decarboxylase.

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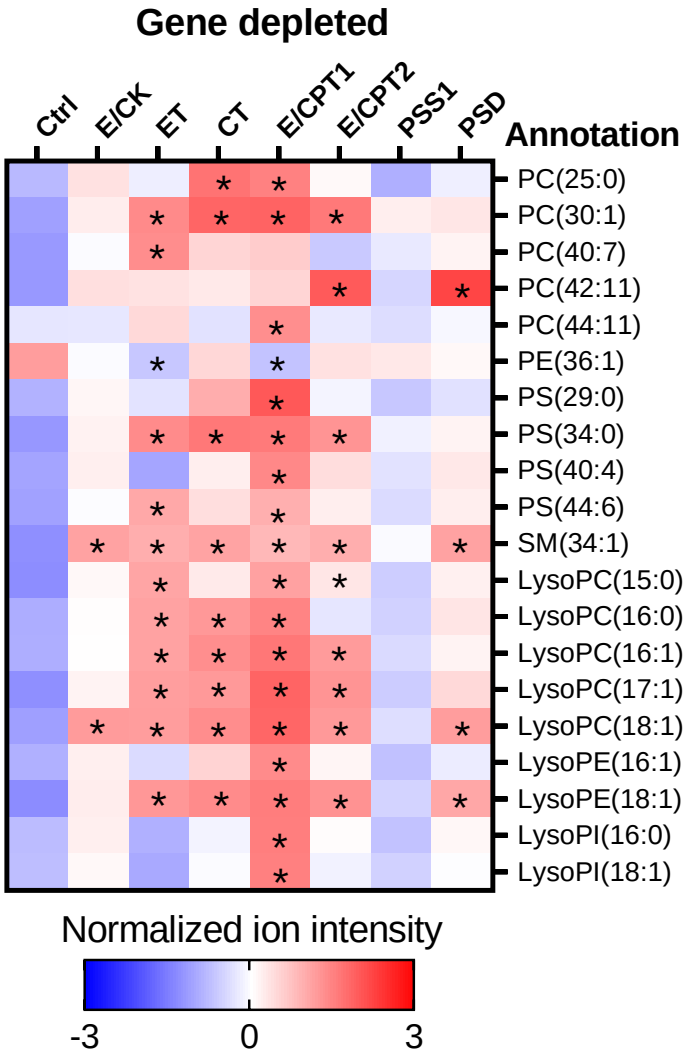

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40 **Fig S4. Ion intensity of regulated metabolites in DENV-infected cells after *de novo***
41 **pathway gene depletion.** Normalized ion intensity was calculated after total ion
42 chromatography normalization and auto scaling from three biological replicates. Conditions
43 with significantly regulated metabolites (p-value <0.05 and |log2 fold change| >1) were
44 indicated with an asterisk. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS,
45 phosphatidylserine; LysoPC, lysophosphatidylcholine; LysoPE, lysophosphatidylethanolamine;
46 LysoPI, lysophosphatidylinositol; SM, Sphingomyelin; Ctrl, Control dsRNA.

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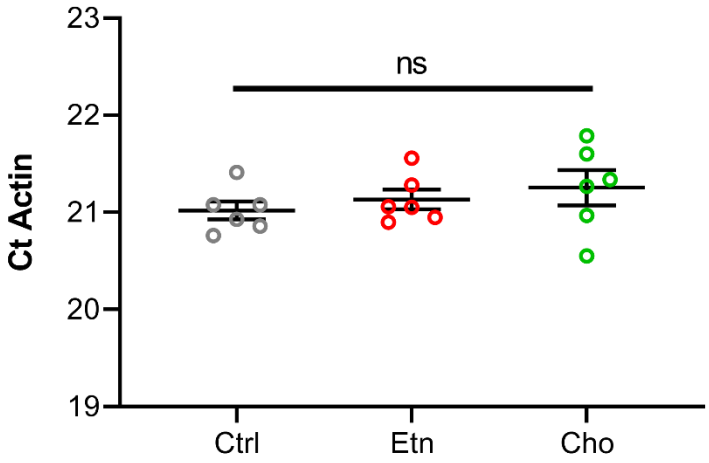

Fig S5. Cell survival measured by *Actin* gene expression in ethanolamine or choline supplemented cells. Aag2 cells were supplemented with either ethanolamine (Etn) or choline (Cho) and compared to standard growth media (Ctrl). At 24h post supplementation, *Actin* expression was quantified. Lines show mean ± s.e.m. from 6 biological repeats and compared by Dunnett's multiple comparison test.

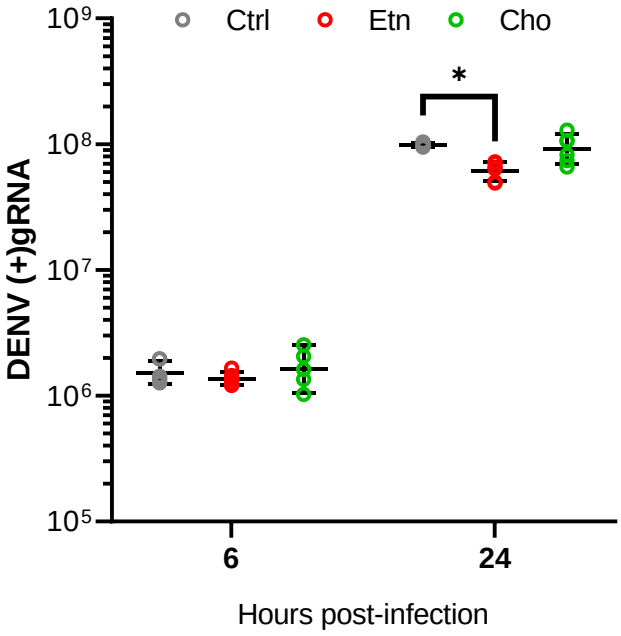

Fig S6. Impact of ethanolamine and choline supplementations on DENV replication after infection at MOI 5. Aag2 cells were supplemented with either ethanolamine (Etn) or choline (Cho) and compared to standard growth media (control). At 24h post supplementation, cells were infected with DENV at MOI 5. Cellular DENV (+)gRNA was quantified at 6 and 24 hpi. Lines show geometric mean ± 95% CI from 6 biological repeats. * p-value < 0.05, as determined by Dunnett's multiple comparisons test.

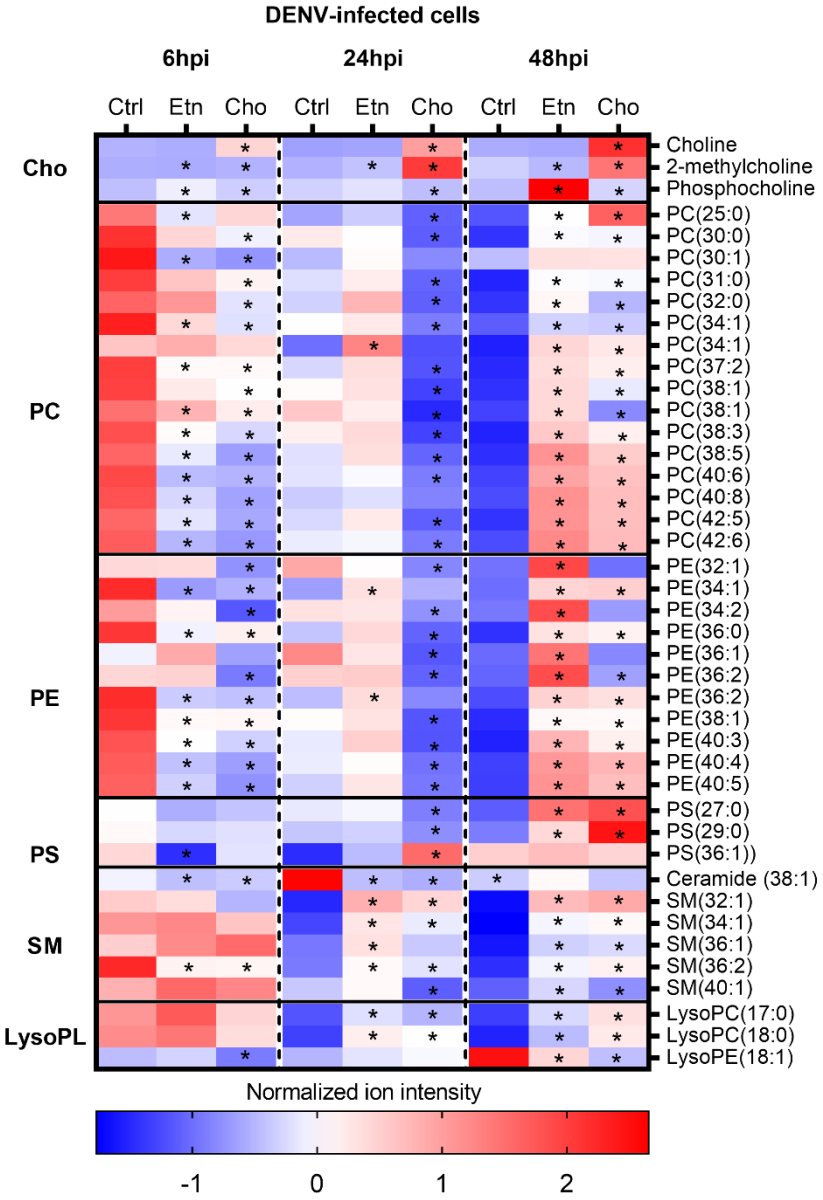

Fig S7. Ion intensity of regulated metabolites in choline or ethanolamine supplemented cells infected with DENV. Normalized ion intensity was calculated after total ion chromatography normalization and auto scaling from three biological replicates. Conditions with significantly regulated metabolites (p-value <0.05 and |log2 fold change| >1) were indicated with an asterisk. Cho, choline; Etn, Ethanolamine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; LysoPL, lysophospholipid LysoPC, lysophosphatidylcholine; LysoPE, lysophosphatidylethanolamine; SM, Sphingomyelin;

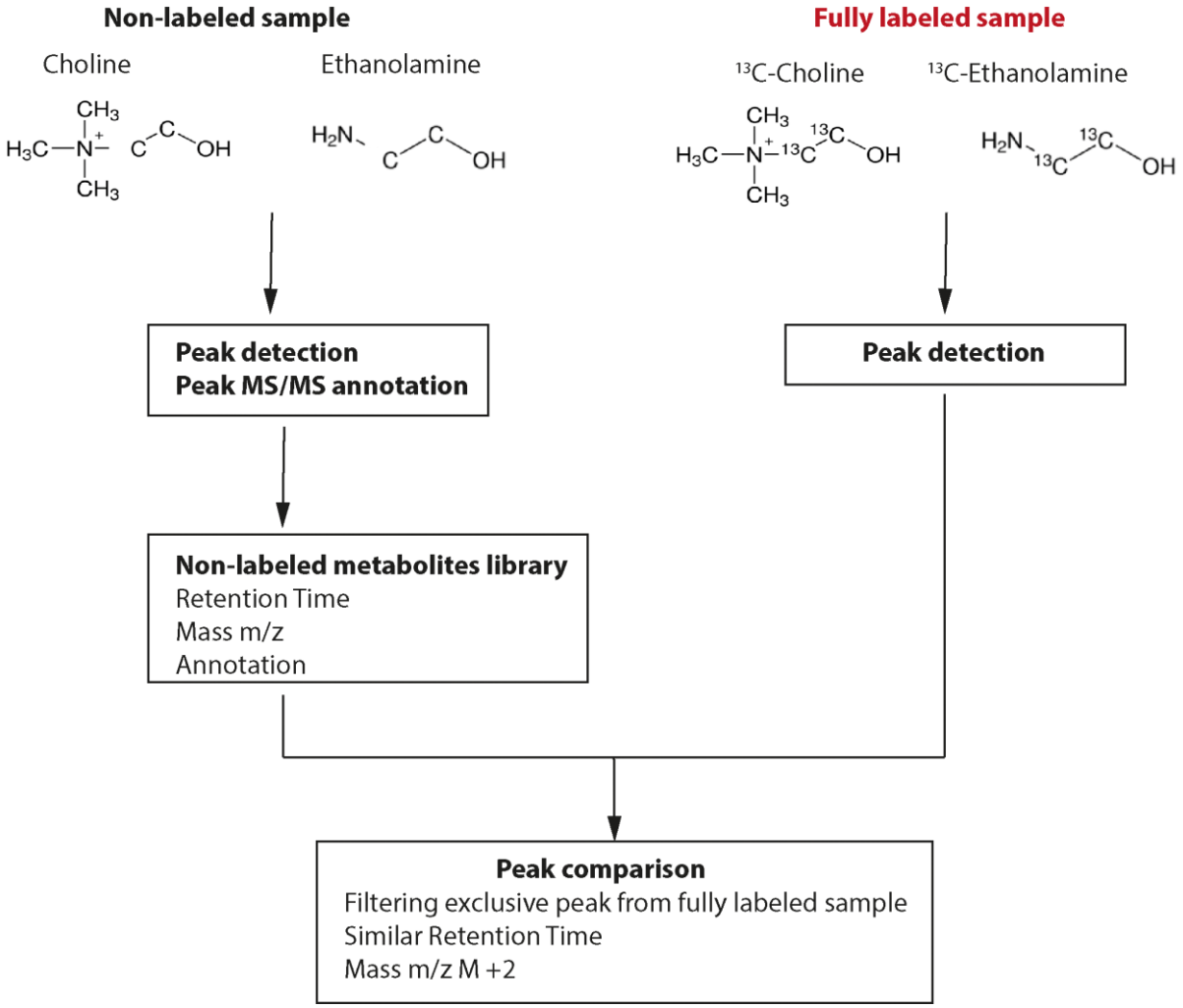

Fig S8. Scheme to identify isotope labeled ^{13}C ethanolamine or choline incorporation in phospholipids.

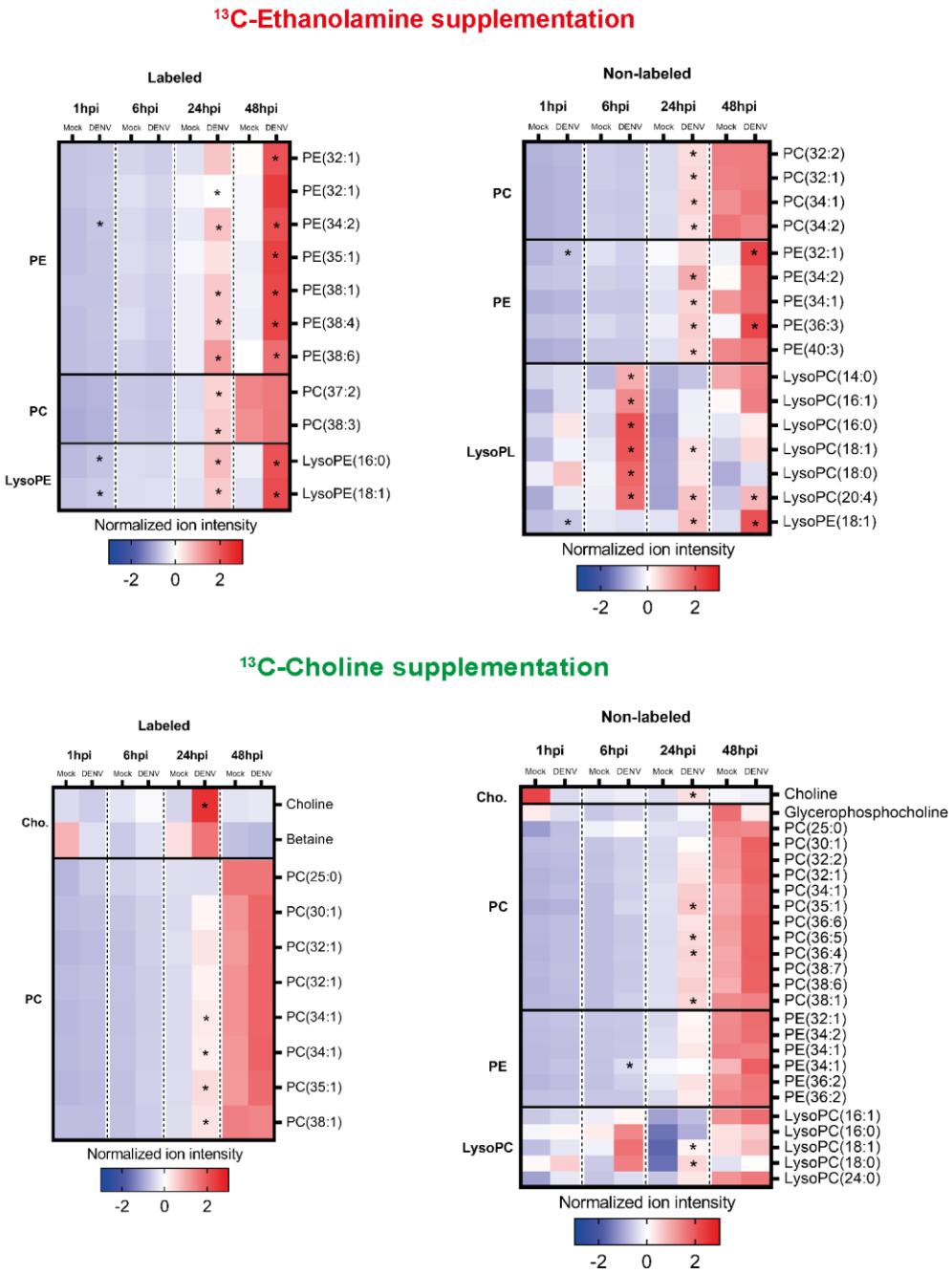

Fig S9. Ion intensity of regulated metabolites in choline or ethanolamine supplemented cells infected with DENV. Normalized ion intensity was calculated after total ion chromatography normalization and auto scaling from three biological replicates. Conditions with significantly regulated metabolites (p-value <0.05 and |log2 fold change| >1) were indicated with an asterisk. Cho, choline; Etn, Ethanolamine; PE,

phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; LysoPL, lysophospholipid LysoPC, lysophosphatidylcholine; LysoPE, lysophosphatidylethanolamine; SM, Sphingomyelin;

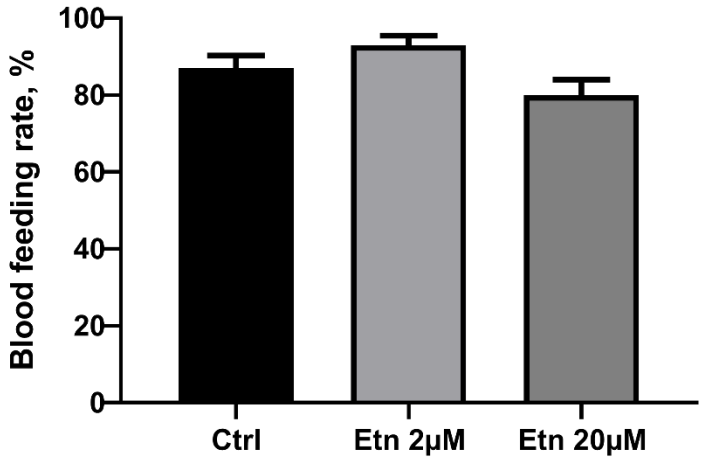

Fig S10. Impact of Ethanolamine supplementation on mosquito blood feeding. Blood feeding rate was calculated as the percentage of mosquitoes that imbibed blood over the total number of mosquitoes that were offered the infectious blood meal. Lines represent percentage + s.e.

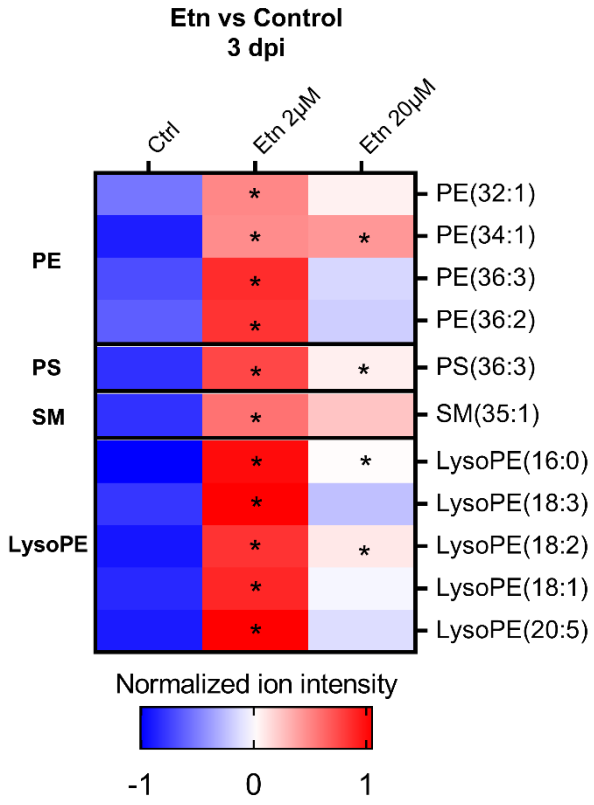

Fig S11. Ion intensity of regulated metabolites at 3 dpi on ethanolamine-supplemented DENV-infectious blood. Normalized ion intensity was calculated after total ion chromatography normalization and auto scaling from four biological replicates, each containing 10 mosquitoes. Conditions with significantly regulated metabolites (p-value <0.05 and |log2 fold change| >1) were indicated with an asterisk. Etn, Ethanolamine; PE, phosphatidylethanolamine; PS, phosphatidylserine; LysoPE, lysophosphatidylethanolamine; SM, Sphingomyelin;

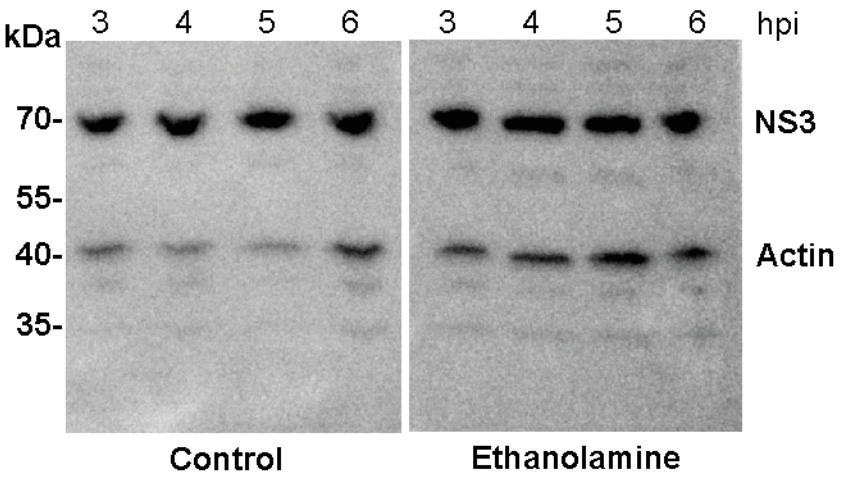

Fig S12. Immunoblot of DENV NS3 expressed in DENV-infected Aag2 cells at 3, 4, 5 and 6 hpi. 30µg of protein extracted from mosquito cells lysates (Aag2) were electrophoresed and western blotted with anti-NS3 polyclonal antibody and detected by chemiluminescence. Anti-beta actin was used as loading control.

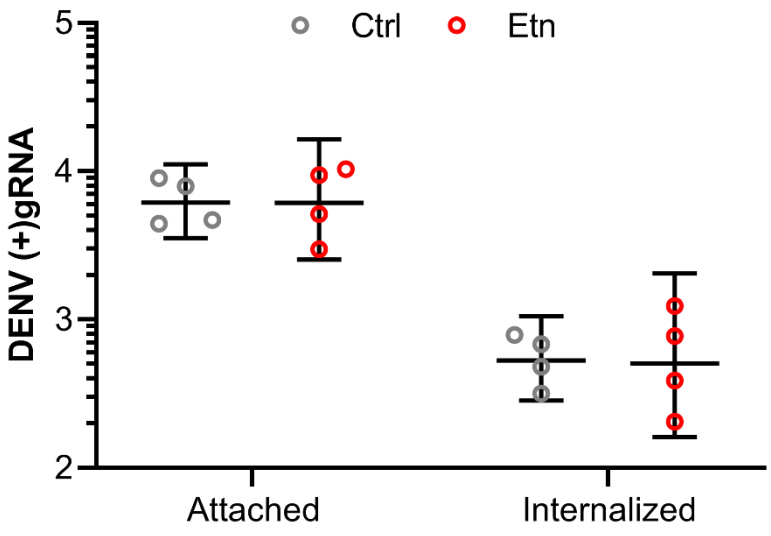

Fig S13. Attachment and internalization for DENV propagated in ethanolamine-supplemented cells. Aag2 cells were supplemented with either ethanolamine (Etn) or control media (Ctrl) for 24 h before infection with DENV. Viruses grown in Etn- or Ctrl-supplemented media were used to estimate attached and internalized (+)gRNA in Aag2 cells. Lines show geometric mean ± 95% CI from 4 biological repeats. Not significant as determined by unpaired t-test.

TABLES

Table S1. CDP-aminoalcohol motif comparison between human and *Ae. Aegypti* CPT and EPT homologues. Human choline/ethanolaminephosphotransferase (hCEPT1), cholinephosphotransferase (hCPT1) and ethanolaminephosphotransferase (hEPT1) are involved in the synthesize of PC and PE through the *de novo* PL pathway[42,43]. hCEPT1 has the dual ability to synthesize choline- and ethanolamine-containing phospholipids, while hCPT1 exclusively synthesizes PC and hEPT1 exclusively synthesizes PE. hCEPT1 and hEPT1 contain an identical CDP-alcohol phosphotransferase motif spanning residues 136-158 of hCEPT1[26,42]. hCPT1 differs in position 146 by a Cysteine instead of Serine, suggesting that this difference is responsible for CDP-Choline specificity. *Ae. aegypti* contains phosphotransferases (E/CPT1 and 2). Red indicates amino acids shared between E/CPT1-2 and hEPT1 and hCEPT1. Green indicates amino acids specific to hCPT1. Yellow indicates amino acids specific to E/CPT1-2,

Species	Protein	RefSeq	Amino acids	CDP-alcohol phosphotransferase motif
Homo sapiens	hCEPT1	NP_001317672.1	416	D G K Q A R R T N S S S P L G E L F D H G C D
Homo sapiens	hEPT1	NP_277040.1	406	D G K Q A R R T N S S S P L G E L F D H G C D
Homo sapiens	hCPT1	NP_064629.2	397	D G K Q A R R T N S C S P L G E L F D H G C D
Ae. aegypti	E/CPT1	AAEL014395	378	D G K Q A R R T N S S T P L G E L F D H G C D
Ae. aegypti	E/CPT2	AAEL011841	367	D G K Q A R R T N S S T P L G E L F D H G C D

Table S2. **^{13}C isotope-labeled phospholipids after 24h supplementation of ^{13}C-ethanolamine or ^{13}C-choline in mock-infected Aag2 cells.**

	^{13}C fully labeled		Non-labeled		
Supplementation	**RT**	**Mass m/z**	**Annotation**	**RT**	**Mass m/z**
Ethanolamine	2.976	692.5139	PE(18:1(9Z)/14:0)	2.939	690.5084
Ethanolamine	2.842	718.5323	PE(16:0/18:2(9Z,12Z))	2.895	716.5224
Ethanolamine	3.155	720.5444	PE(18:1(9Z)/16:0)	3.229	718.5375
Ethanolamine	2.897	720.5446	PE(18:1(9Z)/16:0)	2.944	718.5377
Ethanolamine	6.446	744.5445	PE(16:0/20:3(8Z,11Z,14Z))	6.499	742.5374
Choline	11.184	106.1078	Choline	11.167	104.1067
Choline	7.634	668.4445	PC(16:0/9:0(COOH))	7.635	666.4341
Choline	6.527	706.5334	PC(14:0/16:1(9Z))	6.514	704.5219
Choline	6.503	734.5647	PC(14:0/18:1(11Z))	6.501	732.5535

Table S3. Primers for dsRNA synthesis.

Gene name	Gene code	Fragment size	Forward primer	Reverse primer
E/CK	AAEL009765	301	GGCTTAGGGGATCGAGAGAC	GTCATCGTTGGCGTTATTGTT
CT	AAEL011564	305	CCGGTACGGTTGTACGGA	CGCCTCAAGGTTTCGATTTA
E/CPT1	AAEL014395	395	ATCATCGCGAATGCAATTTT	CAGCTGTAGGGCATGGACTT

E/CPT2	AAEL011841	312	GACCCTGTTCTACTGTGCCC	AACAGGAACGGTATGATGGG
ET	AAEL005651	313	ACGGAGCTCGGAGGCTTACT	TCGTCAACCCATTTAATGCC
PSD	AAEL010223	314	GGTCTGTACTCGACCGCTTT	GCATTGTCGGGTGATTTCTT
PSS	AAEL008393	337	GTGGACGATATTTCGCTGGA	TAAAATTCCGTAACGTGGGG
LacZ	/	370	TACCCGTAGGTAGTCACGCA	TACGATGCGCCCATCTACAC

Table S4. Primers for qPCR.

Gene name	Gene code	Forward primer	Reverse primer
E/CK	AAEL009765	GGCTTAGGGGATCGAGAGAC	GTCATCGTTGGCGTTATTGTT
CT	AAEL011564	CCGGTACGGTTGTACGGA	CGCCTCAAGGTTTCGATTTA
E/CPT1	AAEL014395	ATCATCGCGAATGCAATTTT	CAGCTGTAGGGCATGGACTT
E/CPT2	AAEL011841	GACCCTGTTCTACTGTGCCC	AACAGGAACGGTATGATGGG
ET	AAEL005651	ACGGAGCTCGGAGGCTTACT	TCGTCAACCCATTTAATGCC
PSD	AAEL010223	GGTCTGTACTCGACCGCTTT	GCATTGTCGGGTGATTTCTT
PSS	AAEL008393	GTGGACGATATTTCGCTGGA	TAAAATTCCGTAACGTGGGG
Actin	AAEL011197	GAACACCCAGTCCTGCTGACA	TGCGTCATCTTCTCACGGTTAG

DATASETS

Dataset S1. Metabolites detected upon gene depletion of de novo PL pathways in DENV-infected cells at 48 hpi. Details of the detected compounds: m/z, retention time (Rt in min), MS/MS spectrum fragmentation and intensity, adducts ion name, regulation of compounds (p-value < 0.05; |log2 fold change| ≥ 1) with dsRNA as compared to dsControl and annotation classes (3 rank) with MS-Finder.

Dataset S2. Metabolites detected upon ethanolamine or choline supplementation before infection. Details of the detected compounds: m/z, retention time (Rt in min), MS/MS spectrum fragmentation and intensity, adducts ion name, regulation of compounds (p-value < 0.05; |log2 fold change| ≥ 1) with Etn or Cho supplementation as compared to Control and annotation classes (3 rank) with MS-Finder.

Dataset S3. Metabolites detected upon ethanolamine and choline supplementations in DENV-infected cells at 6, 24 and 48 hpi. Details of the detected compounds: m/z, retention time (Rt in min), MS/MS spectrum fragmentation and intensity, adducts ion name, regulation of compounds (p-value < 0.05; |log2 fold change| ≥ 1) with Etn or Cho supplementation as compared to Control at 6, 24 and 48 hpi and annotation classes (3 rank) with MS-Finder.

Dataset S4. **Non-labeled phospholipids after 24h supplementation of labeled ethanolamine or choline in mock-infected cells.** The two tabs contains details of the detected compounds in Etn or Cho supplemented cells: m/z, retention time (Rt in min), MS/MS spectrum fragmentation and intensity, adducts ion name, average ion intensity (TIC normalization) at 24h post-supplementation and annotation classes (3 rank) with MS-Finder.

Dataset S5. Labeled and non-labeled metabolites detected upon ^{13}C-ethanolamine or ^{13}C-choline supplementations in DENV-infected cells at 1, 6, 24 and 48 hpi. The four tabs contains details of the detected compounds in ^{13}C-Etn or ^{13}C-Cho supplemented cells and in ^{12}C-Etn or ^{12}C-Cho supplemented cells for the generation of the library: m/z, retention time (Rt in min), MS/MS spectrum fragmentation and intensity, adducts ion name, regulation of compounds (p-value < 0.05; |log2 fold change| ≥ 1) with ^{13}C-Etn or ^{13}C-Cho supplementation

as compared to Control at 1, 6, 24 and 48 hpi, average ion intensity (TIC normalization) in Mock or DENV condition at 1, 6, 24 or 48 hpi for ^{12}C-Etn or ^{12}C-Cho supplemented cells and annotation classes (3 rank) with MS-Finder.

Dataset S6. Metabolites detected following ethanolamine blood supplementation in DENV-infected mosquitoes at 3 dpi. Details of the detected compounds: m/z, retention time (Rt in min), MS/MS spectrum fragmentation and intensity, adducts ion name, regulation of compounds (p-value < 0.05; |log2 fold change| ≥ 1) with 2µM or 20µM Etn supplementation as compared to Control at 3 dpi and annotation classes (3 rank) with MS-Finder.