SUPPLEMENTAL INFORMATION

Contribution of the precursors and interplay of the pathways in the phospholipid metabolism of the malaria parasite

Sharon Wein[1*#], Salma Ghezal[2#], Corinne Buré[3], Marjorie Maynadier[1], Christian Périgaud[2], Henri J. Vial[1], Isabelle Lefebvre-Tournier[2], Kai Wengelnik[1], Rachel Cerdan[1]

[1]Dynamique des Interactions Membranaires Normales et Pathologiques, UMR 5235, CNRS-Université de Montpellier, cc 107, place Eugène Bataillon, 34095 Montpellier Cedex 05, France.

[2]Institut des Biomolécules Max Mousseron, UMR 5247, CNRS- Université de Montpellier, cc 1705, place Eugène Bataillon, 34095 Montpellier Cedex 05, France.

[3]Chimie et Biologie des Membranes et des Nanoobjets, UMR 5248, Centre de Génomique Fonctionnelle, BP 68, Université Bordeaux 2, 146, rue Léo Saignat, 33076 Bordeaux cedex, France.

[#] These authors contributed equally to this work.

Supplemental Table S1. Labeling of PC, PE, PS, their precursors and the reaction intermediates found in *P. falciparum*-iRBC.

	Metabolite labeling (%)			
	UL	**d_3**	**d_4**	**d_9**
PC	89.5 ± 3.0	0.6 ± 0.3	4.5 ± 1.4	5.4 ± 2.1
PE	56.8 ± 12.0	8.5 ± 1.0	34.7 ± 11.0	NA
PS	86 ± 3.4	14.0 ± 3.4	NA	NA
Cho	92.4 ± 2.3	NA	NA	7.6 ± 2.3
P-Cho	89.2 ± 1.3	0.8 ± 0.5	4.6 ± 1.8	5.5 ± 1.5
CDP-Cho	87.7 ± 7.7	0.7 ± 0.7	6.0 ± 0.3	6.9 ± 6.0
P-Etn	42.7 ± 15.5	6.7 ± 4.6	50.7 ± 15.2	NA
CDP-Etn	36.8 ± 23.0	6.3 ±2.0	56.8 ± 21.0	NA
Ser	71.9 ± 17.8	28.1 ± 17.8	NA	NA

Cells were cultured in presence of human serum and 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3.Values are the mean of at least 3 independent experiments ± s.d.

UL: unlabeled; d_9: d_9 labeling (from choline-d_9), d_4 labeling (from ethanolamine-d_4), d_3 labeling (from serine-d_3), NA: not applicable.

Supplemental Table S2. Phosphatidylcholine species in uRBC and *P. falciparum*-iRBC (3D7 and *pfpmtΔ* strains). *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, PLs were extracted, identified and quantified by LC-MS/MS. Gray color indicate the main changes in PC molecular species between RBCs and iRBCs.

	RBC (% of total PC)		**3D7 *P. f.*-iRBC (% of total PC)**		***pfpmtΔ P. f.*-iRBC (% of total PC)**	
	Mean	**s.e.m. (n=3)**	**Mean**	**s.e.m. (n=7)**	Mean	**s.e.m. (n=3)**
PC 14:0-16:1	**3.67**	0.56	**0.92**	0.16	**0.66**	0.11
PC 16:0/16:0	**4.28**	0.11	**13.01**	1.29	**15.39**	1.27
PC 14:0-18:1	**1.11**	0.24	**1.96**	0.48	**1.25**	0.05
PC 14:0-18:2	**0.16**	0.01	**0.28**	0.05	**0.17**	0.01
PC 16:0-18:0	**5.78**	2.31	**11.70**	3.22	**9.12**	1.40
PC 16:0-18:1	**22.57**	1.21	**32.42**	1.94	**29.60**	0.81
PC 16:0-18:2	**22.91**	0.49	**14.99**	0.89	**13.95**	0.79
PC 18:0-18:1	**5.46**	0.55	**6.12**	0.33	**6.52**	0.54
PC 18:0-18:2 + PC 18:1/18:1	**10.75**	1.02	**5.89**	0.33	**5.71**	0.39
PC 16:0-20:3 + PC 18:1-18:2	**6.46**	0.37	**3.21**	0.19	**3.71**	0.36
PC 16:1-20:3 + PC 16:0-20:4 + PC 18:2/18:2	**7.41**	0.73	**4.28**	0.30	**6.73**	1.12
PC 38:3	**1.17**	0.16	**0.69**	0.06	**0.97**	0.01
PC 18:0-20:4 + PC 18:1-20:3	**3.69**	0.08	**1.73**	0.15	**2.51**	0.23
PC 38:5	**1.91**	0.27	**1.15**	0.10	**1.61**	0.15
PC 16:0-22:6	**2.15**	0.24	**1.30**	0.10	**1.78**	0.19
PC 40:4	**0.15**	0.01	**0.07**	0.01	**0.10**	0.01
PC 40:5	**0.34**	0.07	**0.17**	0.02	**0.24**	0.01

PC: phosphatidylcholine

Supplemental Table S3. Phosphatidylethanolamine species in uninfected RBC and *P. falciparum*-iRBC (3D7 and *pfpmtΔ* strains). *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, PLs were extracted, identified and quantified by LC-MS/MS. Gray color indicate the main change in PE molecular species between RBCs and iRBCs.

	RBC (% of total PE)		**3D7 *P. f.*-iRBC (% of total PE)**		***pfpmtΔ P. f.*-iRBC (% of total PE)**	
Species	**Mean**	**s.e.m. (n=3)**	**Mean**	**s.e.m. (n=3)**	**Mean**	**s.e.m. (n=3)**
PE 14:0/18:1	**0.22**	0.03	**0.55**	0.12	**0.47**	0.05
PE 14:0/18:2	**0.09**	0.01	**0.50**	0.14	**0.47**	0.08
PE 16:0/16:0	**0.77**	0.12	**2.39**	0.48	**2.24**	0.83
PE 16:0/18:0	**0.24**	0.01	**0.43**	0.14	**1.05**	0.34
PE 16:0/18:1	**23.49**	0.68	**29.14**	1.04	**27.46**	0.80
PE 16:0/18:2	**8.17**	0.38	**26.90**	0.91	**24.60**	1.49
PE 16:0/20:1	**0.23**	0.01	**0.14**	0.06	**0.21**	0.03
PE 16:0/20:3	**0.39**	0.20	**0.42**	0.12	**0.00**	0.00
PE 16:0/20:4	**12.54**	1.16	**9.92**	0.90	**13.35**	0.83
PE 16:0/22:4	**3.84**	0.12	**0.83**	0.09	**0.64**	0.06
PE 16:0/22:5	**2.24**	0.21	**0.69**	0.16	**1.17**	0.02
PE 16:0/22:6	**2.58**	0.39	**1.12**	0.12	**1.13**	0.06
PE 18:0/18:1	**6.85**	0.27	**4.82**	0.64	**5.88**	0.40
PE 18:0/18:2	**2.25**	0.15	**3.32**	0.25	**4.89**	0.43
PE 18:0/20:3	**0.59**	0.30	**0.78**	0.18	**1.24**	0.27
PE 18:0/20:4	**9.02**	0.38	**4.11**	0.38	**4.72**	0.71
PE 18:0/22:4	**1.30**	0.09	**0.27**	0.07	**0.24**	0.01
PE 18:1/18:1	**7.57**	0.55	**3.23**	0.46	**2.87**	0.34
PE 18:1/18:2	**7.30**	0.38	**2.92**	0.68	**4.06**	0.26
PE 18:1/20:3	**0.93**	0.07	**0.12**	0.06	**0.00**	0.00
PE 18:1/20:4	**7.66**	0.40	**1.60**	0.12	**1.61**	0.11
PE 18:1/22:4	**1.08**	0.09	**0.10**	0.04	**0.12**	0.01
PE 18:2/18:2	**0.66**	0.02	**0.61**	0.30	**1.57**	0.13

PE: phosphatidylethanolamine

Supplemental Table S4. Phosphatidylserine species in uninfected RBC and *P. falciparum*-iRBC (3D7 and *pfpmtΔ* strains). *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, PLs were extracted, identified and quantified by LC-MS/MS. Gray color indicate the main changes in PS molecular species between RBCs and iRBCs.

	RBC (% of total PS)		**3D7 *P. f.*-iRBC (% of total PS)**		***pfpmtΔ P. f.*-iRBC (% of total PS)**	
Species	**Mean**	**s.e.m. (n=3)**	**Mean**	**s.e.m. (n=7)**	**Mean**	**s.e.m. (n=3)**
PS 16:0-18:0	**3.85**	0.77	**3.54**	0.63	**4.25**	0.53
PS 16:0-18:1	**0.81**	0.16	**10.62**	1.41	**12.85**	1.42
PS 16:0-18:2	**0.00**	0.00	**4.56**	0.89	**5.90**	0.94
PS 16:0-20:1	**0.00**	0.00	**0.19**	0.16	**0.00**	0.00
PS 16:0-22:4	**0.15**	0.10	**0.00**	0.00	**0.00**	0.00
PS 16:0-22:5	**0.00**	0.00	**0.03**	0.03	**0.00**	0.00
PS 16:0-22:6	**0.00**	0.00	**0.39**	0.30	**0.00**	0.00
PS 18:0-18:1	**9.44**	0.69	**14.96**	0.93	**16.36**	1.46
PS 18:0-18:2	**2.85**	0.12	**9.26**	0.51	**11.28**	0.20
PS 18:0-20:3	**6.60**	0.37	**4.83**	0.69	**5.33**	0.26
PS 18:0-20:4	**63.11**	1.97	**32.72**	2.45	**29.04**	1.61
PS 18:0-22:4	**8.58**	0.76	**4.66**	0.86	**3.17**	0.39
PS 18:1/18:1	**0.79**	0.07	**5.83**	1.02	**3.91**	0.15
PS 18:1-18:2	**0.16**	0.03	**3.41**	0.63	**3.47**	0.12
PS 18:1-20:3	**0.40**	0.11	**0.50**	0.19	**0.00**	0.00
PS 18:1-20:4	**3.86**	1.02	**3.80**	0.39	**4.44**	0.43
PS 18:1-22:4	**0.29**	0.15	**0.00**	0.00	**0.00**	0.00
PS 18:2/18:2	**0.00**	0.00	**0.24**	0.12	**0.00**	0.00

PS: phosphatidylserine

Supplemental Table S5. Molecular species of PC in the 3D7 strain according to the precursor of PLs. *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, PLs were extracted, identified and quantified by LC-MS/MS. Values are the mean ± s.e.m. (n=4). Gray color indicate the main changes in PC molecular species between unlabeled and labeled PC.

	Unlabeled PC		**PC-d_3**		**PC-d_4**		**PC-d_9**	
	mean (%)	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**
PC 14:0-16:1	**0.64**	0.19	**0**	0	**0.06**	0.04	**0.05**	0.03
PC 16:0/16:0	**12.05**	1.46	**13.01**	0.99	**12.78**	1.72	**12.79**	1.83
PC 14:0-18:1	**2.26**	0.67	**1.15**	0.39	**2.53**	0.62	**2.57**	0.53
PC 14:0-18:2	**0.32**	0.07	**0.07**	0.07	**0.24**	0.08	**0.26**	0.09
PC 16:0-18:0	**13.04**	3.29	**4.87**	2.14	**3.70**	0.98	**3.63**	0.82
PC 16:0-18:1	**32.67**	3.07	**43.45**	5.35	**40.22**	1.22	**40.63**	1.63
PC 16:0-18:2	**14.76**	1.12	**17.59**	2.21	**16.76**	1.36	**15.83**	0.89
PC 18:0-18:1	**5.87**	0.29	**5.89**	0.41	**5.81**	0.71	**6.31**	0.32
PC 18:0-18:2 + PC 18:1/18:1	**5.82**	0.54	**4.91**	1.86	**5.76**	0.16	**5.82**	0.21
PC 16:0-20:3 + PC 18:1-18:2	**3.27**	0.43	**2.58**	0.89	**3.29**	0.16	**3.43**	0.16
PC 16:1-20:3 + PC 16:0-20:4 + PC 18:2/18:2	**4.15**	0.44	**3.04**	1.25	**4.51**	0.18	**4.36**	0.13
PC 36:8	**0.18**	0.00	**0**	0	**0.19**	0.00	**0.20**	0.00
PC 38:3	**0.65**	0.08	**0.14**	0.14	**0.40**	0.15	**0.38**	0.13
PC 18:0-20:4 + PC 18:1-20:3	**1.66**	0.29	**1.31**	0.58	**1.39**	0.18	**1.23**	0.42
PC 38:5	**1.23**	0.23	**0.56**	0.42	**0.92**	0.33	**1.00**	0.35
PC 16:0-22:6	**1.35**	0.22	**0.84**	0.50	**1.38**	0.26	**1.44**	0.14
PC 40:4	**0.06**	0.02	**0**	0	**0.05**	0.02	**0.06**	0.03
PC 40:5	**0.16**	0.04	**0**	0	**0.14**	0.06	**0.17**	0.06
Total	100%		100%		100%		100%	

Supplemental Table S6. Molecular species of PC in *pfpmtΔ* strain according to the source of PLs. *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, phospholipids were extracted, identified and quantified by LC-MS/MS. Results are mean ± s.e.m. (n=3).

	Unlabeled PC		**PC-d_9**	
	mean (%)	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**
PC 14:0-16:1	**0.67**	0.11	**0.00**	0.00
PC 16:0/16:0	**15.47**	1.27	**11.15**	1.53
PC 14:0-18:1	**1.24**	0.04	**1.96**	0.75
PC 14:0-18:2	**0.17**	0.01	**0.00**	0.00
PC 16:0-18:0	**9.16**	1.42	**7.54**	0.99
PC 16:0-18:1	**29.62**	0.77	**28.18**	3.90
PC 16:0-18:2	**13.95**	0.79	**13.87**	0.79
PC 18:0-18:1	**6.54**	0.54	**5.66**	0.73
PC 18:0-18:2 + PC 18:1/18:1	**5.69**	0.41	**6.79**	0.58
PC 16:0-20:3 + PC 18:1-18:2	**3.69**	0.37	**4.58**	0.36
PC 16:1-20:3 + PC 16:0-20:4 + PC 18:2/18:2	**6.68**	1.12	**9.24**	1.36
PC 38:3	**0.93**	0.03	**2.84**	0.73
PC 18:0-20:4 + PC 18:1-20:3	**2.51**	0.23	**2.58**	0.12
PC 38:5	**1.58**	0.16	**2.83**	0.45
PC 16:0-22:6	**1.76**	0.20	**2.55**	0.09
PC 40:4	**0.11**	0.01	**0.00**	0.00
PC 40:5	**0.24**	0.01	**0.22**	0.22
Total	100%		100%	

Supplemental Table S7. Molecular species of PE in 3D7 strain according to the source of PLs. *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, phospholipids were extracted, identified and quantified by LC-MS/MS. Results are mean ± s.e.m. (n=4). Gray color indicate the main change in PE molecular species between unlabeled and labeled PE.

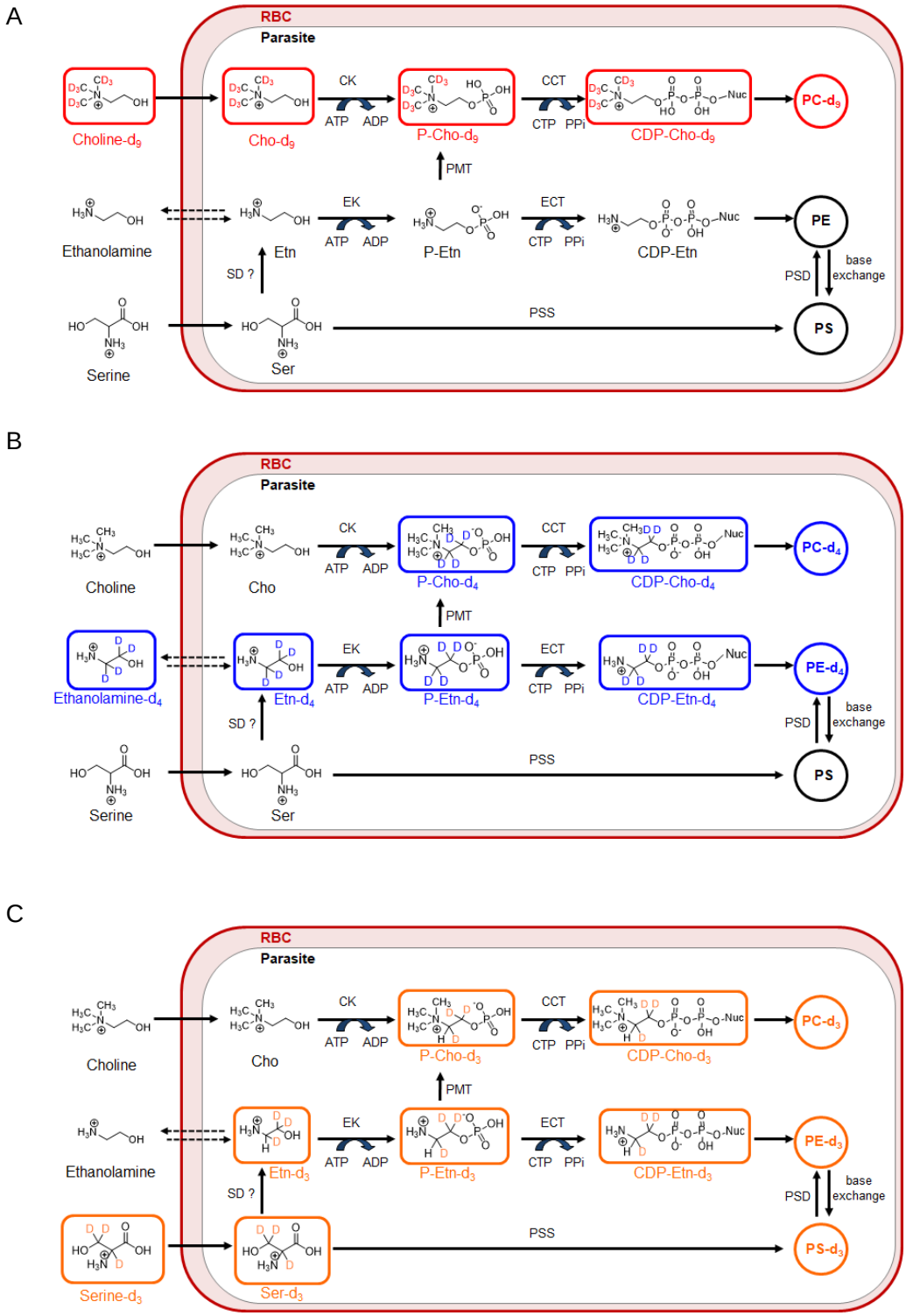
	Unlabeled PE		**PE-d_3**		**PE-d_4**	
	mean (%)	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**
PE 14:0-18:1	**0.64**	0.10	**0.47**	0.24	**0.60**	0.17
PE 14:0-18:2	**0.48**	0.09	**0.50**	0.32	**0.51**	0.21
PE 16:0/16:0	**1.97**	0.52	**2.17**	0.61	**2.03**	0.54
PE 16:0-18:0	**0.59**	0.19	**0.07**	0.07	**0.48**	0.14
PE 16:0-18:1	**30.25**	0.94	**31.01**	2.12	**31.80**	1.51
PE 16:0-18:2	**24.51**	1.47	**24.00**	1.41	**31.43**	1.98
PE 16:0-20:1	**0.18**	0.08	**0.05**	0.05	**0.12**	0.07
PE 16:0-20:3	**0.55**	0.19	**0.73**	0.73	**0.40**	0.23
PE 16:0-20:4	**9.38**	0.79	**10.55**	2.02	**9.50**	1.09
PE 16:0-22:4	**1.30**	0.08	**0.43**	0.16	**0.77**	0.20
PE 16:0-22:5	**0.76**	0.27	**0.25**	0.14	**0.44**	0.17
PE 16:0-22:6	**1.25**	0.16	**0.76**	0.57	**1.19**	0.35
PE 18:0-18:1	**6.57**	0.81	**7.26**	0.16	**4.57**	0.41
PE 18:0-18:2	**4.01**	0.64	**4.24**	1.03	**2.92**	0.53
PE 18:0-20:3	**0.91**	0.09	**0.22**	0.13	**0.84**	0.30
PE 18:0-20:4	**4.97**	0.33	**4.56**	2.53	**3.00**	0.48
PE 18:0-22:4	**0.53**	0.05	**0.05**	0.05	**0.19**	0.04
PE 18:1/18:1	**4.83**	1.01	**5.86**	1.24	**2.52**	0.40
PE 18:1-18:2	**3.18**	0.14	**4.32**	0.31	**4.13**	2.57
PE 18:1-20:3	**0.33**	0.11	**0.08**	0.05	**0.20**	0.11
PE 18:1-20:4	**2.07**	0.16	**1.73**	0.32	**1.03**	0.25
PE 18:1-22:4	**0.25**	0.05	**0.01**	0.01	**0.00**	0.00
PE 18:2/18:2	**0.52**	0.05	**0.71**	0.29	**1.35**	1.22
Total	100%		100%		100%	

Supplemental Table S8. Molecular species of PS in 3D7 strain according to the source of PLs. *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, phospholipids were extracted, identified and quantified by LC-MS/MS. Results are mean ± s.e.m. (n=4). Significant differences are shown ns: not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

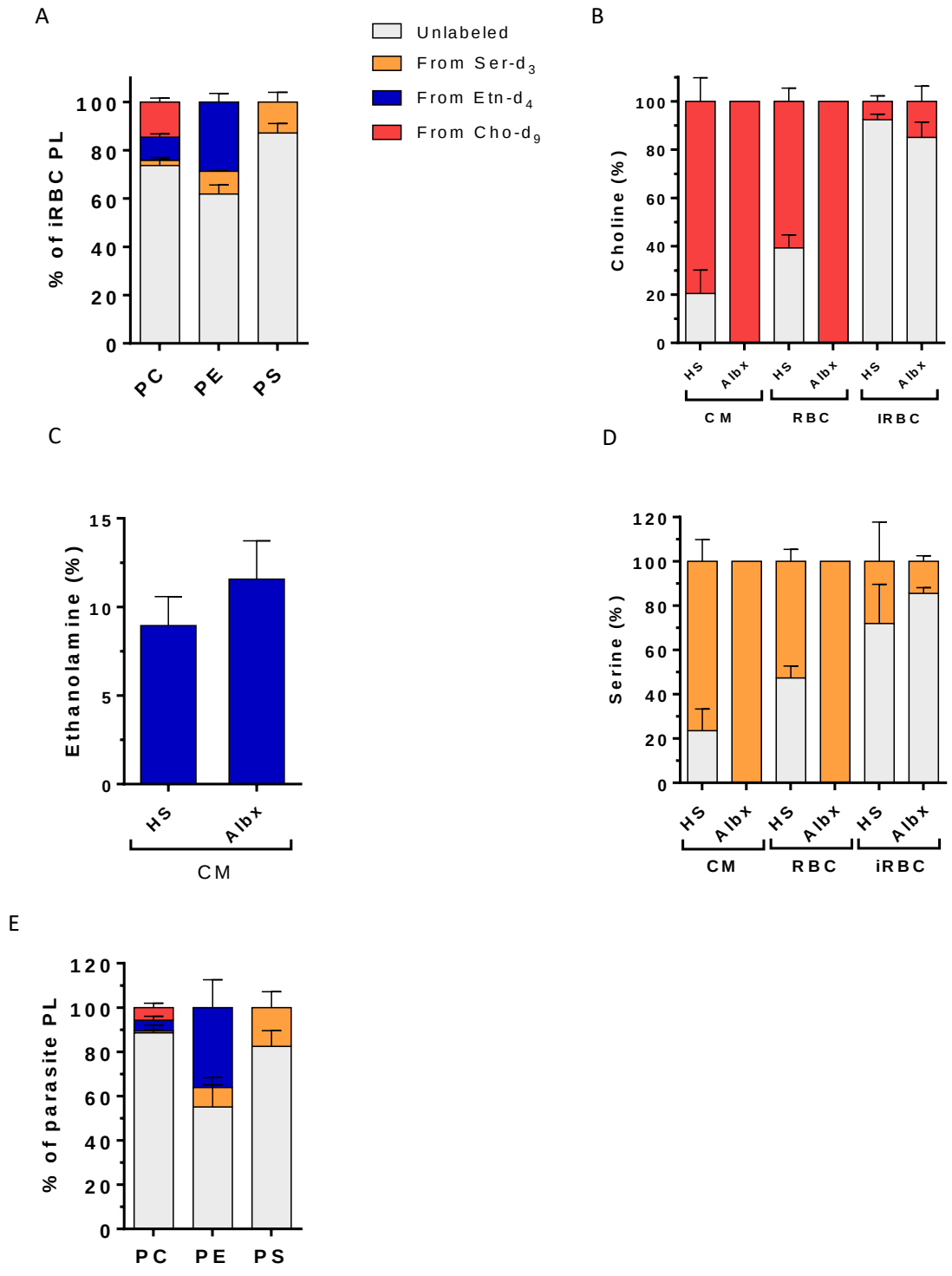
	Unlabeled PS		**PS-d_3**		**Significant difference**
	mean (%)	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**	
PS 16:0-18:0	**2.91**	0.75	**0.00**	0.00	**
PS 16:0-18:1	**11.23**	0.92	**20.45**	0.99	**
PS 16:0-18:2	**3.74**	0.18	**5.28**	1.21	ns
PS 16:0-20:1	**1.95**	0.85	**1.29**	0.71	ns
PS 18:0-18:1	**15.58**	0.68	**20.92**	3.89	ns
PS 18:0-18:2	**8.65**	0.30	**15.11**	0.20	****
PS 18:0-20:3	**4.50**	0.26	**0.11**	0.11	****
PS 18:0-20:4	**31.79**	0.76	**10.80**	5.40	**
PS 18:0-22:4	**4.13**	0.65	**0.00**	0.00	**
PS 18:1/18:1	**6.98**	1.46	**14.05**	1.18	*
PS 18:1-18:2	**3.24**	0.20	**7.21**	1.11	**
PS 18:1-20:3	**0.90**	0.18	**0.00**	0.00	**
PS 18:1-20:4	**3.74**	0.41	**4.78**	1.01	ns
Total	100%		100%		

Supplemental Table 9. Molecular species of PS in *pfpmtΔ* strain according to the source of PLs. *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 10% human serum in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, phospholipids were extracted, identified and quantified by LC-MS/MS. Results are mean ± s.e.m. (n=3).

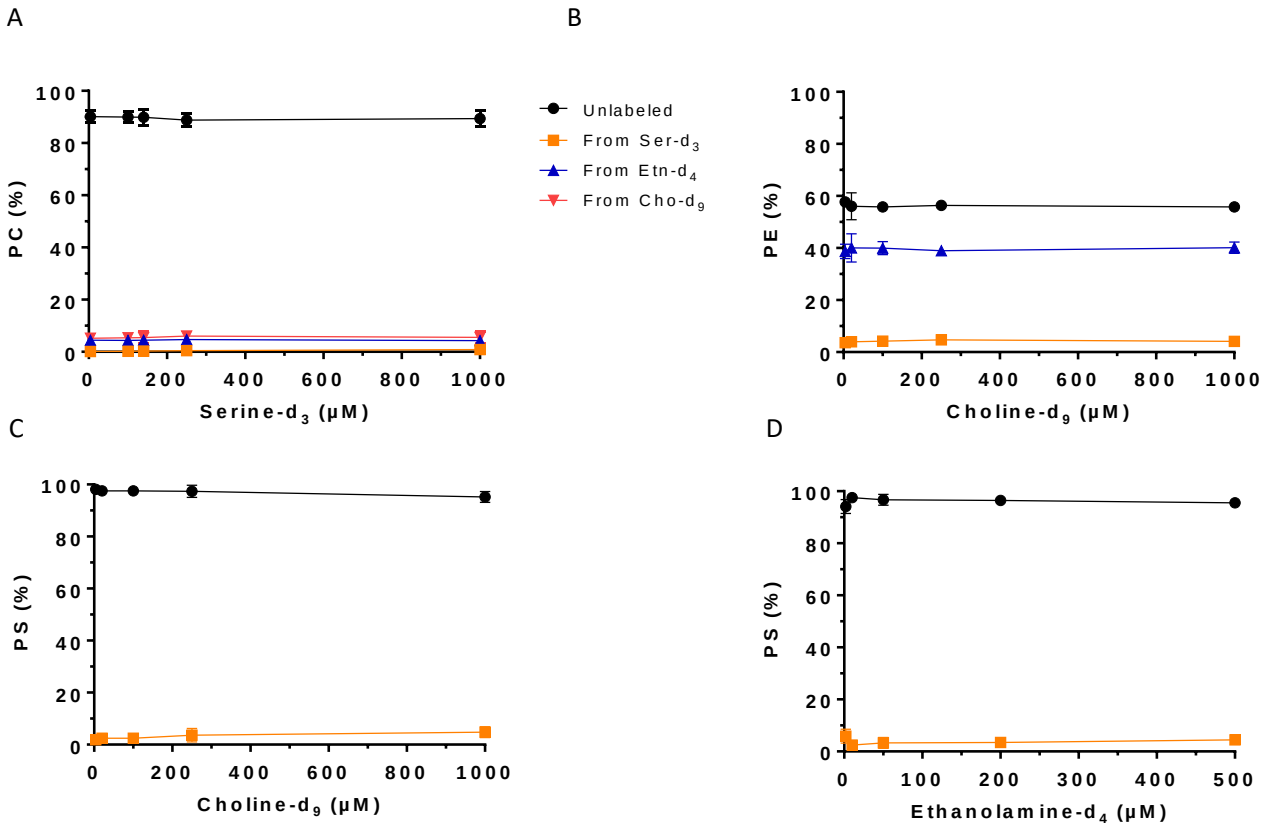
	PS-d_0		**PS-d_3**		**Significant difference**
	mean (%)	**s.e.m (%)**	**mean (%)**	**s.e.m (%)**	
PS 16:0-18:0	**4.91**	0.65	**0.00**	0.00	**
PS 16:0-18:1	**12.04**	1.52	**18.14**	0.58	*
PS 16:0-18:2	**5.57**	0.77	**7.82**	1.89	ns
PS 18:0-18:1	**15.88**	1.49	**19.49**	1.53	ns
PS 18:0-18:2	**11.25**	0.09	**11.62**	0.90	ns
PS 18:0-20:3	**5.55**	0.15	**3.98**	1.01	ns
PS 18:0-20:4	**30.26**	1.65	**21.09**	1.06	**
PS 18:0-22:4	**3.65**	0.41	**0.00**	0.00	***
PS 18:1/18:1	**3.44**	0.21	**6.94**	0.08	****
PS 18:1-18:2	**3.29**	0.11	**4.64**	0.26	**
PS 18:1-20:4	**4.16**	0.40	**6.28**	0.59	*
Total	100%		100%		



Supplemental Figure S1: Principle of deuterium labeling of *P. falciparum* PL metabolism.
P. falciparum-iRBCs were incubated 48 h or 96 h in several culture media in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, PL and reaction intermediates were extracted and quantified by LC-MS/MS. **(A)** Molecules labeled with "d_9" were synthesized from Cho-d_9. **(B)** Molecules labeled with "d_4" were synthesized from Etn-d_4. **(C)** Molecules labeled with "d_3" were synthesized from Ser-d_3. The contributions of Cho-d_9, Etn-d_4 and Ser-d_3 to biosynthesis of PLs can thus be determined.



Supplemental Figure S2: Quantification and origin of PC, PE and PS in *P. falciparum*-iRBC and isolated parasites. *P. falciparum*-iRBC were incubated 48h in culture medium supplemented with 0.5% albumax I in the presence of 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, a fraction was treated with saponin to obtain isolated parasites. After extraction, phospholipids and reaction intermediates were quantified by LC-MS/MS. **(A)** Quantification of total PC, PE and PS in iRBC. **(B to D)** Quantification of the precursors Cho (B), Etn (C) and serine (D) in culture medium (CM) and RBC in the presence of 10% human serum (HS) or 0.5% albumax (Albx) **(E)** Quantification of total PC, PE and PS in isolated parasites. The same color code is used for all the panels.



Supplemental Figure S3: Effect of varying precursor concentrations on the PC, PE and PS biosynthesis pathways in *P. falciparum*-iRBC. *P. falciparum*-iRBCs were incubated 96 h in culture medium supplemented with 10% human serum in the presence of increasing concentrations of Cho-d_9, Etn-d_4 or Ser-d_3 while the two others labeled precursors remained at fixed concentrations. Fixed concentrations were 20 µM Cho-d_9, 10 µM Etn-d_4 and 140 µM Ser-d_3. After incubation, PLs were quantified by LC-MS/MS. **(A)** Impact of Ser-d_3 concentration on PC biosynthesis. **(B)** Impact of Cho-d_9 concentration on PE biosynthesis. **(C)** Impact of Cho-d_9 and Etn-d_4 **(D)** concentrations on PS biosynthesis. Results are shown as means ± s.d. of three independant experiments. The same color code was used for all the panels.