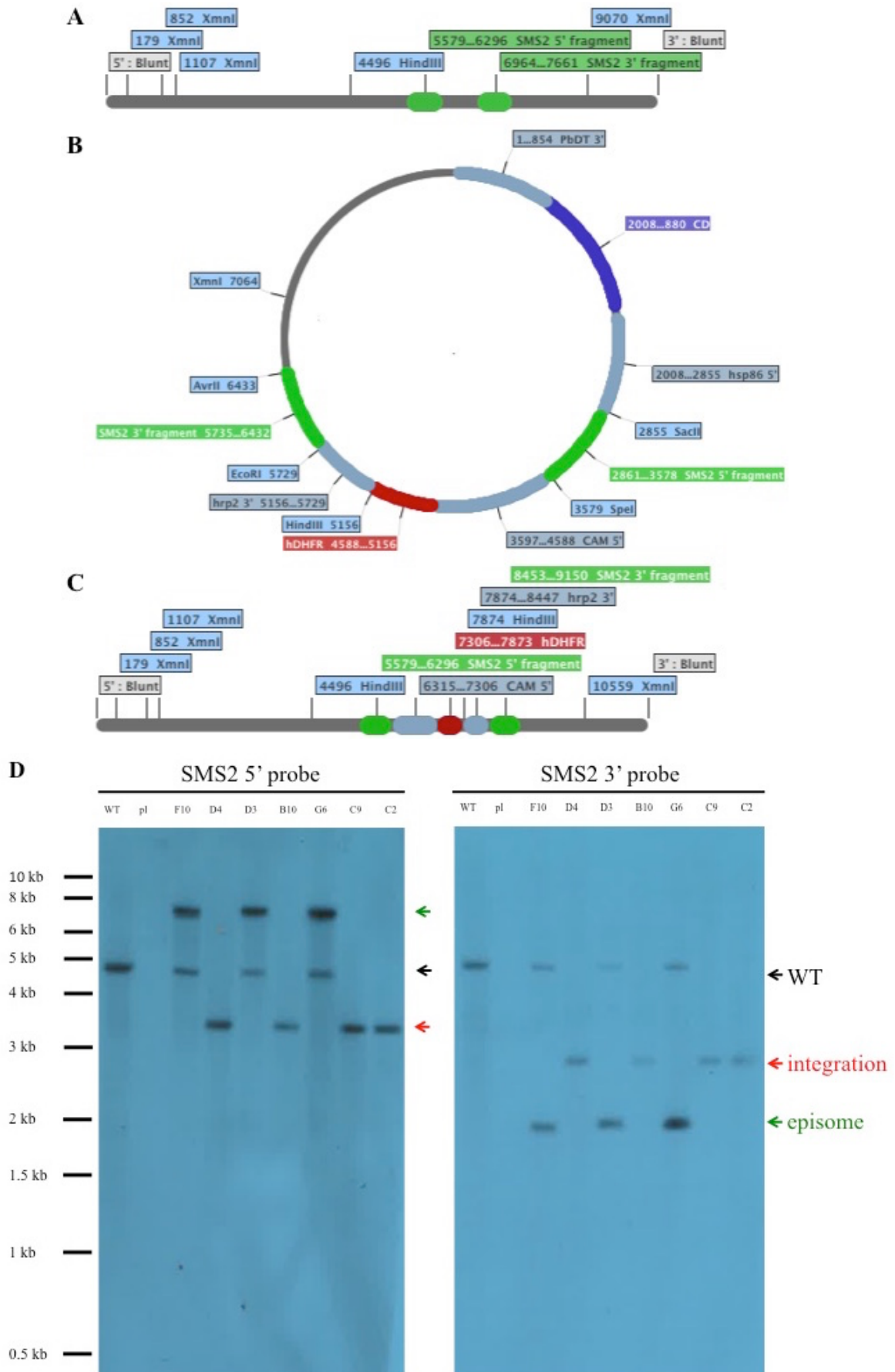
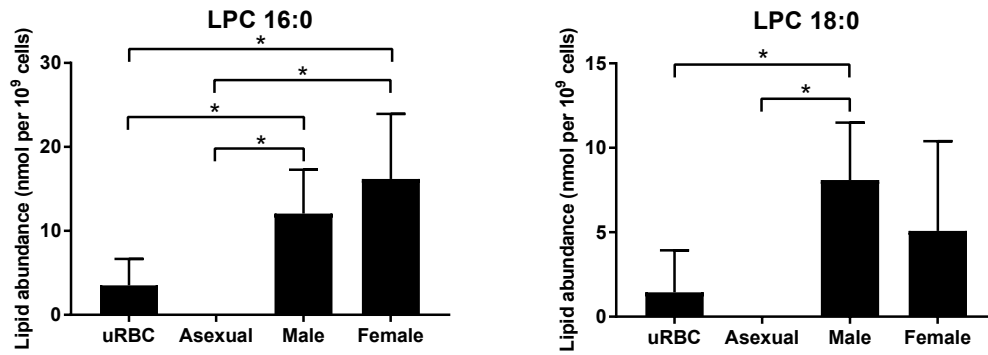


**Fig. S1.** Disruption of SMS1 by homologous recombination. A-C) Schematic of WT SMS1 locus with surrounding fragment of genomic DNA (A), pCC-1/SMS1 plasmid (B) and disrupted SMS1 locus with surrounding fragment of genomic DNA (C). SMS1 gene is disrupted by double homologous recombination at the 5' and 3' extremities of the gene in green (SMS1 5' fragment between SacII and SpeI restriction sites and SMS1 3' fragment between EcoRI and AvrII restriction sites), which inserts positive selection marker human dihydrofolate reductase

(hDHFR, red) under the control of the calmodulin promoter (CAM 5') and histidine rich protein 2 3' untranslated region (hrp2 3'). The plasmid also contains negative selection marker cytosine deaminase (CD, purple) under the control of heat shock protein 86 promoter (hsp86 5') and *P. berghei* dihydrofolate reductase 3' untranslated region (PbDT 3') that are lost by double recombination. AflIII and PacI restriction digest sites used for Southern blot are also indicated. D) Southern blot showing disruption of SMS1 locus. DNA from 3D7 wild type (WT), SMS1 KO plasmid (pl), polyclonal 3D7 transfected with SMS1 KO plasmid (pol) or clonal 3D7 transfected with SMS1 KO plasmid (G7, E10, D4, E6, G2) digested with AflIII and PacI restriction enzymes and probed with 5' homologous flank probe (SMS1 5' probe, left) or 3' homologous flank probe (SMS1 3' probe, right). Expected SMS1 5' probe band lengths: WT locus: 4,515 bp (black arrow), episomal locus: 2,606 bp (green arrow), integrated locus: 2,726 bp (red arrow). Expected SMS1 3' probe band lengths: WT 2 locus: 4,515 bp (black arrow), episomal locus: 6,243 bp (green arrow), integrated locus: 3,068 bp (red arrow).



**Fig. S2.** Disruption of SMS2 by homologous recombination. A-C) Schematic of WT SMS2 locus with surrounding fragment of genomic DNA (A), pCC-1/SMS2 plasmid (B) and disrupted SMS2 locus with surrounding fragment of genomic DNA (C). SMS2 gene is disrupted by double homologous recombination at the 5' and 3' extremities of the gene in green (SMS2 5' fragment between SacII and SpeI restriction sites and SMS2 3' fragment between EcoRI and AvrII restriction sites), which inserts positive selection marker human dihydrofolate reductase (hDHFR, red) under the control of the calmodulin promoter (CAM 5') and histidine rich protein 2 3' untranslated region (hrp2 3'). The plasmid also contains negative selection maker cytosine deaminase (CD, purple) under the control of heat shock protein 86 promoter (hsp86 5') and *P. berghei* dihydrofolate reductase 3' untranslated region (PbDT 3') that are lost by double recombination. XmnI and HindIII restriction digest sites used for Southern blot are also indicated. D) Southern blot showing disruption of SMS2 locus. DNA from 3D7 wild type (WT), SMS2 KO plasmid (pl), or clonal 3D7 transfected with SMS2 KO plasmid (G7, E10, D4, E6 and G2) digested with XmnI and Hind III restriction enzymes probed with 5' homologous flank probe (SMS2 5' probe, left) or 3' homologous flank probe (SMS2 3' probe, right). Expected SMS2 5' probe band lengths: WT locus: 4,574 bp (black arrow), episomal locus: 7,036 bp (green arrow), integrated locus: 3,378 bp (red arrow). Expected SMS2 3' probe band lengths: WT locus: 4,574 bp (black arrow), episomal locus: 1,908 bp (green arrow), integrated locus: 2,685 bp (red arrow).



**Fig. S3.** Abundance of lysophosphatidylcholine (LPC) 16:0 (left) and LPC 18:0 (right) in uninfected red blood cells (uRBC) and red blood cells infected with asexual stage parasites (Asexual), male gametocytes (Male) or female gametocytes (Female) with results from student t tests (\*:  $p < 0.1$ ; nothing indicated where  $p > 0.1$ ).

**Table S1.** Primers for construct preparation. Lowercase letters in the primer sequences correspond to restriction enzyme sites while uppercase letters refer to the gene sequence.

Primer code	Primer description		Primer sequence
al398	SMS1 5'	forward	atcccgcggCACACATTTGTACCTCTC
al399		reverse	gatactagtATCTGAGAAATTGGAACGC
al400	SMS1 3'	forward	atcgaattcGCTGCAAGAAGATATGC
al401		reverse	gatcctaggAAAAAGAGTTTGTAGGTG
al406	SMS2 5'	forward	atcccgcggGTTTAATACACGTGAG
al407		reverse	gatactagtCGATCACTTAATGGTTGCG
al408	SMS2 3'	forward	atcgaattcTATACCTTAGATTATGCC
al409		reverse	gatcctaggAAAAACGACATTTAGGG

**Table S2.** Fitness competition primers to quantify wild type (SMS1 or SMS2 “cut out”) and knock out (hDHFR) parasite genotype abundance relative to total parasite abundance (reference).

Target	Gene ID	Primers (forward/reverse)
SMS1 “cut out”	PF3D7_0625000	ACTGTTGATGTGTTAATGGGATATG ATCTTCTTGCAGCTACATCTACTA
SMS2 “cut out”	PF3D7_0625100	TTCATGCAAAGCCATTCTTTCT TGGCATAATCTAAGGTATAATTCAATCC
hDHFR		GAACTCAAGGAACCTCCACAA ACAGAACTGCCACCAACTATC
Reference	PF3D7_0317300	AATAGTCGAAGCGGGAAGTG CGAATTGGATTCTCCCAAATAACC

**Table S3.** Primers for Southern blot probe synthesis

Primer code	Primer description		Primer sequence
al 514	SMS1 5'	forward	CACACATTTGTACCTCTCTTA
al515		reverse	CCTTATGGTTGTAATGTTTGTC
al516	SMS1 3'	forward	TCACAGACAGATTCCAAGTGTT
al517		reverse	GGTACCATTTCCAGCGTATGA
al518	SMS2 5'	forward	GTAAATTAAGACATGCACGTGAGA
al519		reverse	CGCTATCTGTTACTGACTCTTCAT
al520	SMS2 3'	forward	ACCCATAACAGAGGATAA
al521		reverse	ACGACATTTAGGGATTAAA

**Table S4.** List of detected lipid species other than free cholesterol in *P. falciparum*. CE: cholesteryl ester; DAG: diacylglycerol; TAG: triacylglycerol; LPC: lyso-phosphatidylcholine; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PS: phosphatidylserine; Cer: ceramide; DHSM: dihydrosphingomyelin; SM: sphingomyelin.

Neutral Lipids		
CE 16:0	DAG (34:0) 16:0 18:0	TAG 52:1
CE 16:1	DAG (34:1) 16:0 18:1	TAG 52:2
CE 18:1	DAG (36:1) 18:0 18:1	TAG 52:3
CE 18:2	DAG (36:2) 18:1 18:1	TAG 54:2
CE 18:3	TAG 48:0	TAG 54:3
CE 20:4	TAG 50:0	TAG 54:6
CE 20:5	TAG 50:1	TAG 56:6
DAG (32:0) 16:0 16:0	TAG 50:2	

<b>Phospholipids</b>		
LPC 16:0	PC 38:7	PE 38:4
LPC 18:0	PC 40:4	PE 38:5
PC 32:0	PC 40:5	PE 38:6
PC 32:1	PC 40:6	PE 40:5
PC 34:0	PC 40:7	PE 40:6
PC 34:1	PC O-32:0	PG (34:1) 16:0 18:1
PC 34:2	PC O-34:1	PG (34:2) 16:0 18:2
PC 34:3	PC O-36:2	PG (36:1) 18:0 18:1
PC 36:0	PC O-38:4	PG (36:2) 18:0 18:2
PC 36:1	PC O-38:5	PG (36:2) 18:1 18:1
PC 36:2	PE 34:1	PG (36:3) 18:1 18:2
PC 36:3	PE 34:2	PS 34:1
PC 36:4	PE 36:1	PS 36:1
PC 36:5	PE 36:2	PS 36:2
PC 38:3	PE 36:3	PS 38:4
PC 38:4	PE 36:4	PS 38:5
PC 38:5	PE 36:5	PS 40:5
PC 38:6	PE 38:3	PS 40:6

<b>Sphingolipids</b>		
Cer 16:0	DHSM 25:0	SM 22:2
Cer 18:0	SM 14:0	SM 23:0
Cer 19:0	SM 15:0	SM 23:1
Cer 22:0	SM 16:0	SM 24:0
Cer 24:0	SM 16:1	SM 24:1
Cer 24:1	SM 17:0	SM 24:2
Cer 24:2	SM 18:0	SM 24:3
DHSM 16:0	SM 18:1	SM 25:0
DHSM 17:0	SM 19:0	SM 25:1
DHSM 18:0	SM 20:0	SM 26:0
DHSM 19:0	SM 20:1	SM 26:1
DHSM 20:0	SM 21:0	SM 26:2
DHSM 22:0	SM 22:0	
DHSM 24:0	SM 22:1	