## SUPPLEMENTARY DATA



Figure S1 The effect of deleting the *ACB1* gene on the species profiles of phospholipids in yeast. Wild type (WT) and *acb1* cells were cultured in SL medium, harvested at late log-phase, and total lipid extracts were prepared. Phospholipid classes were separated by HPLC, and subjected to MS(/MS) to determine the species compositions of PC, PE, PI, and PS, as indicated in the respective panels. The major  $[M+H]^+$  species of PC, PE and PS, as well as the major  $[M-H]^-$  species of PI are indicated by their *m/z* values. The species labels indicate total acyl carbons:total acyl double bonds. In each panel, the intensity of the highest peak was set at 100 %. See Table S2 for the molecular species assignment per cluster.

<u>Table S1</u> Relative abundance of fatty acids (mol%,  $\pm$  SD, n = 4) in total lipid extracts of WT, *acb1*, *crd1* and *crd1acb1* strains grown in SL medium. Lipids were transesterified and the fatty acid methyl esters were quantified by gas chromatography.

Strain	Fatty acids								
	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1		
WT	$0.6\pm0.2$	$1.2\pm0.1$	$0.4 \pm 0.0$	$17.1 \pm 1.3$	$52.0 \pm 1.4$	$5.0\pm0.9$	$23.7\pm1.6$		
acb1	$2.0\pm0.2$	$2.3\pm0.9$	$2.5\pm0.6$	$14.0 \pm 1.2$	$58.5\pm0.2$	$3.3\pm0.3$	$17.6\pm0.8$		
crd1	$0.5\pm0.2$	$1.1 \pm 0.1$	$0.3\pm0.0$	$17.0\pm0.3$	$50.2 \pm 2.6$	$5.3\pm1.6$	$25.7\pm1.2$		
crd1acb1	$1.8 \pm 0.4$	$2.5 \pm 0.1$	$2.7 \pm 0.1$	$12.7\pm0.5$	$60.2 \pm 1.4$	$2.6 \pm 0.2$	$17.4 \pm 0.7$		

Cluster	# double	Possible acyl chain compositions <sup>a</sup>		m/z for indicated phospholipid class					
	bonds		PC	PE	PI	PS	$PG^{b}$		
C26	0	(16:0)(C10:0), (C14:0)(C12:0)	650	608	725	652	637		
	1	(16:1)(C10:0), (C14:1)(C12:0)	648	606	723	650	635		
C28	0	(C18:0)(C10:0), (C16:0)(C12:0), (C14:0) <sub>2</sub>	678	636	753	680	665		
	1	(C18:1)(C10:0), (C16:1)(C12:0),							
		(C14:1)(C14:0)	676	634	751	678	663		
	2	$(C14:1)_2$	674	632	749	676	661		
C30	0	(C18:0)(C12:0), (C16:0)(C14:0)	706	664	781	708	693		
	1	(C18:1)(C12:0), (C16:1)(C14:0),							
		(C16:0)(C14:1)	704	662	779	706	691		
	2	(C16:1)(C14:1)	702	660	777	704	689		
C32	0	(C18:0)(C14:0), (C16:0) <sub>2</sub>	734	692	809	736	721		
	1	(C18:1)(C14:0), (C16:1)(C16:0),							
		(C18:0)(C14:1)	732	690	807	734	719		
	2	$(C18:1)(C14:1), (C16:1)_2$	730	688	805	732	717		
C34	0	(C18:0)(C16:0)	762	720	837	764	749		
	1	(C18:1)(C16:0), (C18:0)(C16:1)	760	718	835	762	747		
	2	(C18:1)(C16:1)	758	716	833	760	745		
C36	0	$(C18:0)_2$	790	748	865	792	777		
	1	(C18:1)(C18:0)	788	746	863	790	775		
	2	$(C18:1)_2$	786	744	861	788	773		

<u>Table S2</u> Molecular species assignment for PC, PE, PI, PS and PG per cluster with the theoretical m/z values indicated

<sup>a</sup> Only combinations of C10-C18 acyl chains are listed. Note: not all combinations are present in equal amounts, since some acyl chains are more abundant than others (see also Table S1). <sup>b</sup> For peak assignments in Figure 8A, the listed m/z values should be increased by 5 to account for the presence of d5-glycerol in labeled PG.

Lipid	Cluster	Possible acyl chain compositions <sup>a</sup>		m/z for indicated # of double bonds				
			0	1	2	3	4	
MLCL	C38	$(C18)(C10)_2, (C16)(C12)(C10), (C14)_2(C10),$						
		$(C14)(C12)_2$	486	485	484	-	-	
	C40	(C18)(C12)(C10), (C16)(C14)(C10),						
		$(C16)(C12)_2, (C14)_2(C12)$	500	499	498	-	-	
	C42	(C18)(C14)(C10), (C18)(C12) <sub>2</sub> , (C16) <sub>2</sub> (C10),						
		(C16)(C14)(C12), (C14) <sub>3</sub>	514	513	512	511	-	
	C44	(C18)(C16)(C10), (C18)(C14)(C12),						
		$(C16)_2(C12), (C16)(C14)_2$	528	527	526	525	-	
	C46	$(C18)_2(C10), (C18)(C16)(C12), (C18)(C14)_2,$						
		$(C16)_2(C14)$	542	541	540	539	-	
	C48	(C18) <sub>2</sub> (C12), (C18)(C16)(C14), (C16) <sub>3</sub>	556	555	554	553	-	
	C50	$(C18)_2(C14), (C18)(C16)_2$	570	569	568	567	-	
	C52	$(C18)_2(C16)$	584	583	582	581	-	
	C54	(C18) <sub>3</sub>	598	597	596	595	-	
CL	C52	$(C18)(C14)(C10)_2, (C18)(C12)_2(C10),$						
		$(C16)_2(C10)_2, (C16)(C14)(C12)(C10),$						
		$(C16)(C12)_3, (C14)_3(C10), (C14)_2(C12)_2$	591	590	589	588	-	
	C54	(C18)(C16)(C10) <sub>2</sub> , (C18)(C14)(C12)(C10),						
		(C18)(C12) <sub>3</sub> , (C16) <sub>2</sub> (C12)(C10),						
		$(C16)(C14)_2(C10), (C16)(C14)(C12)_2,$						
		$(14)_{3}(C12)$	605	604	603	602	-	
	C56	(C18) <sub>2</sub> (C10) <sub>2</sub> , (C18)(C16)(C12)(C10),						
		$(C18)(C14)_2(C10), (C18)(C14)(C12)_2,$						
		$(C16)_2(C14)(C10), (C16)_2(C12)_2,$						
		$(C16)(C14)_2(C12), (C14)_4$	619	618	617	616	615	
	C58	(C18) <sub>2</sub> (C12)(C10), (C18)(C16)(C14)(C10),						
		$(C18)(C16)(C12)_2, (C18)(C14)_2(C12),$						
		$(C16)_3(C10), (C16)_2(C14)(C12), (C16)(C14)_3$	633	632	631	630	629	
	C60	$(C18)_2(C14)(C10), (C18)_2(C12)_2,$						
		$(C18)(C16)_2(C10), (C18)(C16)(C14)(C12),$						
		$(C18)(C14)_3, (C16)_3(C12), (C16)_2(C14)_2$	647	646	645	644	643	
	C62	$(C18)_2(C16)(C10), (C18)_2(C14)(C12),$						
		$(C18)(C16)_2(C12), (C18)(C16)(C14)_2,$						
		$(C16)_3(C14)$	661	660	659	658	657	
	C64	$(C18)_3(C10), (C18)_2(C16)(C12), (C18)_2(C14)_2,$						
		$(C18)(C16)_2(C14), (C16)_4$	675	674	673	672	671	
	C66	(C18) <sub>3</sub> (C12), (C18) <sub>2</sub> (C16)(C14), (C18)(C16) <sub>3</sub>	689	688	687	686	685	
	C68	$(C18)_3(C14), (C18)_2(C16)_2$	703	702	701	700	699	
	C70	$(C18)_3(C16)$	717	716	715	714	713	
	C72	$(C18)_4$	731	730	729	728	727	

<u>Table S3</u> MLCL and CL molecular species assignment per cluster with the theoretical m/z values indicated

<sup>a</sup> Only compositions based on C10-C18 acyl chains are listed. Note: not all combinations are present in equal amounts, since some acyl chains are more abundant than others (see also Table S1).