Supplementary Information

Fig S1. Further analysis of *D. discoideum* inositol lipids by mass spectrometry

A. Accurate mass determination of *D. discoideum* PIP2

A Thermo LTQ-Orbitrap Velos Pro FT full scan of methylated PIP2. Methylated lipid extracts (40 µl per injection) were separated by HPLC on a ACE Excel 2 C18 amide 150mm x 2.1 mm column. The mobile phase A consisted of water plus 0.1% formic acid, mobile phase B consisted of 60% methanol/acetonitrile plus 0.1 % formic acid. The following gradient was used at a flow rate of 0.4ml/min: 0 min (70% B), 5 min (70% B), 15 min (80% B), 30 min (100% B), 40 min (100% B). Fractions were collected (100 ul each) between 23 min and 25 min. These were then re-injected into the QTRAP mass spectrometer using the standard method to identify the fractions which contained the desired material. The fractions identified as of interest were then introduced into a Thermo LTQ-Orbitrap Velos Pro mass spectrometer using static nanospray emitters (Thermo Scientific) via a nanoelectrospray ion source (Proxeon). Full-scan spectra were acquired at the highest resolution setting (nominally 100,000 at *m/z* 400). The instrument was externally calibrated to a mass accuracy <10ppm and an internal lock mass (polysiloxane *m/z* 445.120025) was automatically applied to all spectra to give a final mass accuracy of <1 ppm.

B. Fragmentation of *D. discoideum* PIP2

A QTRAP4000 EPI scan of methylated PIP2 (*m/z* 1053.55). Mass spectrometer parameters were as follows: Q1 unit resolution, Q2 low resolution, CE 35, CAD medium, IS 4500, TEM 300, CUR 20, GS1 18, GS2 20, ihe ON, DP 100.

C. NLS of lipid extracts prepared from *D. discoideum* grown on bacteria

Lipid extracts were prepared from *D. discoideum* grown on bacteria, then methylated with TMS-diazomethane and analysed by HPLC-ESI mass spectrometry. Neutral loss scans are shown which describe the major species of PI, PIP and PIP2 present

D. NLS of lipid extracts prepared from *D. discoideum* grown on SIH defined medium

Lipid extracts were prepared from *D. discoideum* grown on SIH defined medium, then methylated with TMS-diazomethane and analysed by HPLC-ESI mass spectrometry. Neutral loss scans are shown which describe the major species of PI, PIP and PIP2 present

Fig S2. Time course of D2-palmitol incorporation into *D. discoideum* PIP2.

Methylated lipid extracts were prepared from *D. discoideum* grown in axenic medium in the presence or absence of D2-hexadecan-1-ol for the indicated periods of time and then analysed by HPLC-ESI mass spectrometry. The centroid mass traces and the relative abundances of the 1053.6 (endogenous) and 1055.6 (D2-labelled) species (inset) demonstrate both the time course and extent of incorporation of both deuterium nuclei into the major species of PIP2.

Fig S3. Measurement of the relative abundance of the major molecular species of PS, PC and PE in *D. discoideum*

Methylated lipid extracts were prepared from *D. discoideum* grown under axenic conditions and analysed by HPLC-ESI mass spectrometry. Neutral loss or parent ion scans were integrated (a 5% cutoff applied) to provide relative abundances of the major species of PS, PC and PE present.

Fig S4. Measurement of the relative intensity of selected DG species

Methylated lipid extracts were prepared from *D. discoideum* grown under axenic conditions and analysed by HPLC-ESI mass spectrometry. MRM traces were integrated to provide relative abundances of the selected species of DG. The 18:1a-16:0e species is equivalent to the C34:1e backbone described in other Figs and the text. DAGs were measured using the following MRM transitions: 16:0e/18:1a (581>299.3, loss of 18:1a); 16:0/18:2 (593.5>313.3, loss of 18:2); 18:0e/18:2a (607.6>327.3, loss of 18:2a); 18:1/18:2 (619.5>337.3, loss of 18:1); 18:1/18:2 (619.5>339.3, loss of 18:2); 18:1/18:1 (621.5>339.3, loss of 18:1); 18:0/18:2 (621.5>341.3, loss of 18:2). None of these transitions can be used to determine which position (sn1 or sn2) the fatty acids can be found on. Where two species of the same parent mass are found, the different transitions recorded were found at different HPLC retention times.

Fig S5. Examples of MRM traces for inositol phospholipids.

The parental *Ax2* strain of *D. discoideum* was starved and rendered competent to respond to cAMP. Cells were then stimulated with 10 μM cAMP or vehicle for 5 sec. Methylated lipid extracts were prepared and analysed by HPLC-ESI mass spectrometry. Typical MRM traces are shown from which integrated values for the relative intensities of PI, PIP, PIP2 and PIP3 were derived (see Figs 6-8).