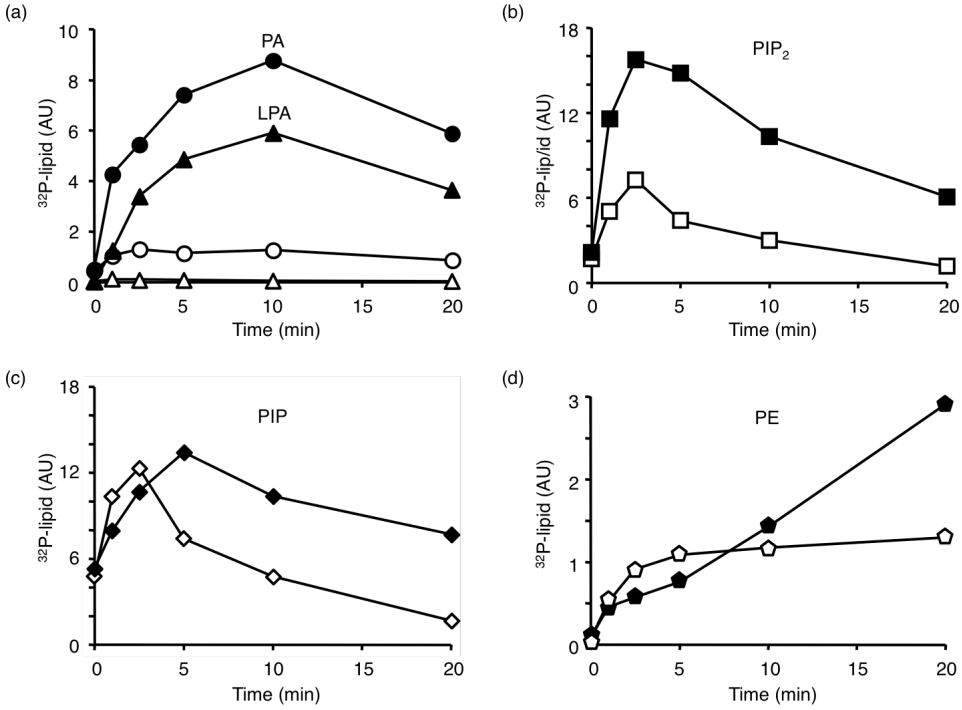
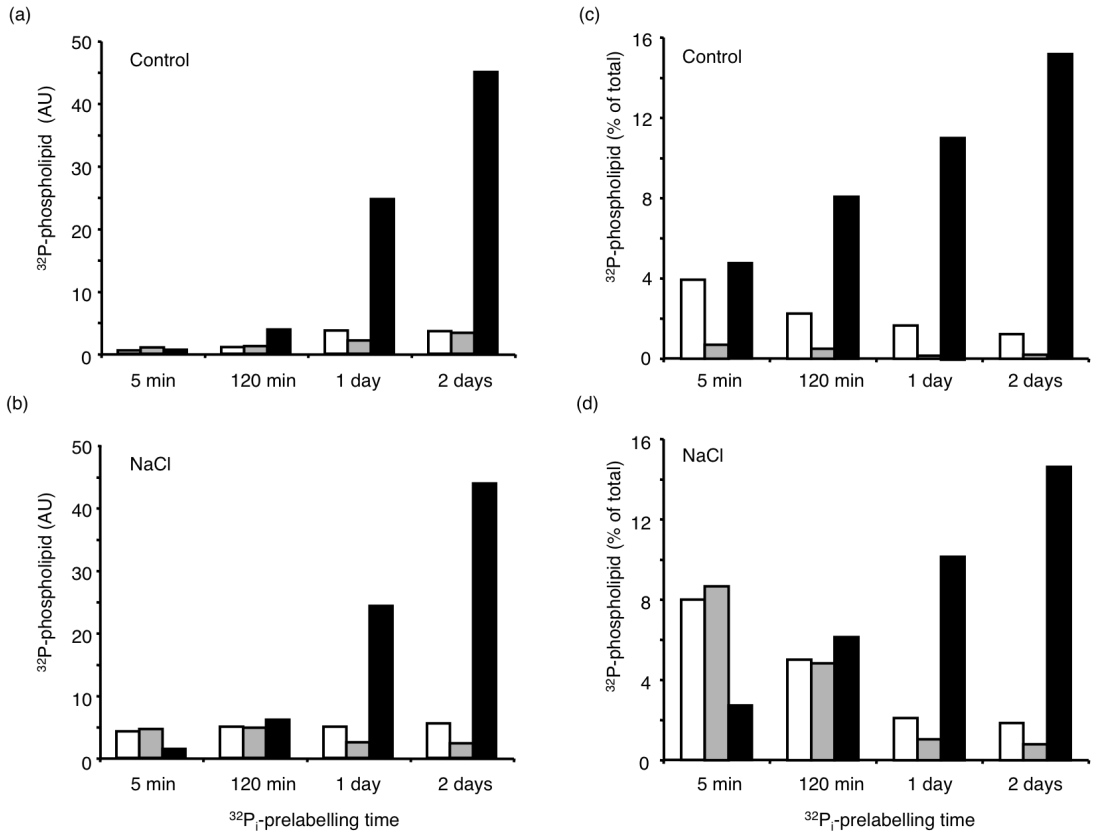
Supplement

Manuscript JLR/2011/016873, Arisz and Munnik (submitted).



Suppl. Fig. 1. $^{32}P_i$-pulse labeling kinetics of LPA and PA (a), PIP_2 (b), PIP (c) and PE (d) in response to salt stress in *Chlamydomonas*. Cells, prelabeled with carrier-free $^{32}P_i$ for 1 min, were treated for different periods of time with buffer (mock treatment, open symbols) or buffer supplemented with 300 mM NaCl (closed symbols), and subsequently lipids were extracted and their radioactivity determined. The rapid and transient labeling of LPA and PA is indicative of their origin in the PPIs via PLC and DGK activities rather than being derived from PLD-mediated hydrolysis of PE. Data represent a representative experiment of three, showing similar trends. Quantitation of this kind of labelling is particularly sensitive to variation e.g. due to differences in ^{32}P-label date, culture-age and the phosphoimaging technique, making it difficult to directly compare ^{32}P-quantitations from different experiments. Lipids were extracted and analyzed as described in Material and Methods.



Suppl. Fig. 2. Long-term $^{32}P_i$-labeling kinetics of ^{32}P-PA (white bars), ^{32}P-LPA (grey bars) and ^{32}P-PE (black bars). *Chlamydomonas* was prelabeled with $^{32}P_i$ plus carrier-P_i (1 mM K-P_i buffer at pH 7.4) for different periods of time, and subsequently mock treated for 5 min (a and c) or with buffer supplemented with 300 mM NaCl (b and d). Lipids were extracted and analyzed as described in Material and Methods and their radioactivity was either expressed in arbitrary units (AU; a and b), or as fraction of the total labeled phospholipids (c and d). Under these labelling conditions, ^{32}P-lipid levels reflect, over time, actual quantities rather than turnover rates (ref. 1). Consequently, radioactivity in PE (and other structural phospholipids), keeps on increasing for days. Under control conditions, also ^{32}P-PA and ^{32}P-LPA increase (a), reflecting extensive equilibration of the precursor pools in *de novo* synthesis. In contrast to these trends, NaCl-stimulated ^{32}P-LPA declined (b), arguing against it being derived from PE through PLD (followed by PLA_2), or from G3P through GPAT. The opposite ^{32}P-labelling trends of stimulated LPA and PE are even clearer when the data are compared as percentage of the total ^{32}P-lipid, as is usually done (c and d). The data are representative of three experiments with similar results.

Reference:
1. Arisz, S. A., J. A. van Himbergen, A. Musgrave, H. van den Ende, and T. Munnik. 2000. Polar glycerolipids of *Chlamydomonas moewusii*. *Phytochemistry* **53**: 265-270.