



**Supplemental Figure 1.** ProPEAMT1:GUS expression in roots and leaves of WT and *pah1 pah2* plants.

Histochemical staining for GUS activity was performed on four-week-old roots and leaves of WT and DM (*pah1 pah2*) plants containing a ProPEAMT1:GUS construct without or with a functional uORF in the 5' UTR. Single insertion site transgenic lines were first created by transforming WT and the T-DNAs were then introduced into the *pah1 pah2* background by crossing. Scale bars = 0.5 cm for leaves and 0.2 mm for roots.

**Supplemental Table 1.** Analysis of gene expression for genes associated with phospholipid synthesis in *pah1 pah2* roots.

| Enzyme  | Name          | AGI       | Fold change |
|---|---------------|-----------|-------------|
| Phosphoethanolamine <i>N</i> -methyltransferase | <i>PEAMT1</i> | At3g18000 | 1.2 ±0.3    |
|   | <i>PEAMT2</i> | At1g48600 | 1.4 ±0.4    |
|   | <i>PEAMT3</i> | At1g73600 | 0.9 ±0.2    |
| Choline kinase                                  | <i>CKI</i>    | At1g71697 | 1.2 ±0.3    |
|   | <i>CKI-L1</i> | At4g09760 | 0.7 ±0.3    |
|   | <i>CKI-L2</i> | At1g74320 | 0.9 ±0.5    |
| CTP-phosphocholine cytidyltransferase           | <i>CCT1</i>   | At2g32260 | 1.3 ±0.4    |
|   | <i>CCT2</i>   | At4g15130 | 1.0 ±0.2    |
| Aminoalcoholphosphotransferase                  | <i>AAPT1</i>  | At1g13560 | 0.9 ±0.2    |
|   | <i>AAPT2</i>  | At3g25585 | 1.4 ±0.3    |
| Phospholipid <i>N</i> -methyltransferase        | <i>PLMT</i>   | At1g80860 | 1.1 ±0.4    |
| Ethanolamine kinase                             | <i>EKI-L</i>  | At2g26830 | 1.5 ±0.3    |
| CTP-phosphoethanolamine cytidyltransferase      | <i>ECT</i>    | At2g38670 | 1.4 ±0.5    |

Real-time PCR analysis of transcript abundance for genes associated with phospholipid synthesis in *pah1 pah2* double mutant roots. Values are the mean ± SE ( $n = 4$ ) of measurements on roots of three-week-old plants grown on agar plates. Values are normalized using the level of *ACT2/ACT8* expression as a constitutive control.

**Supplemental Table 2.** Kinetic parameters for recombinant CCT1 activity in the presence and absence of lipids vesicles containing a 1:1 ratio of PC to either oleic acid or PA.

| Condition | $V_{\max}$ | $K_m$ P-Cho | $K_m$ CTP  |
|-----------|------------|-------------|------------|
| No lipid  | 3.9 ±0.3   | 0.61 ±0.07  | 0.19 ±0.03 |
| PC:OE     | 24.2 ±1.8* | 0.57 ±0.12  | 0.23 ±0.06 |
| PC:PA     | 18.1 ±1.0* | 0.59 ±0.11  | 0.22 ±0.04 |

Kinetic parameters were determined by regression analysis of the Michaelis-Menten hyperbola using Hyper32 freeware. Values are the mean ± SE of three separate experiments, each using five different concentration of substrate. Asterisk denotes a significant difference from the no lipid control ( $P < 0.05$ ).

**Supplemental Table 3.** Primers used for cloning.

| Primer  | Sequence   |
|---|--|
| AttB1-PAH1S                                       | GGGGACAAGTTTGTACAAAAAAGCAGGCTATGAGTTTGGTTG<br>GAAGAGTTGGGAG    |
| AttB2-PAH1A<br>(minus stop codon)                 | GGGGACCACTTTGTACAAGAAAGCTGGGTCTTCAACCTCTTC<br>TATTGGCAGTTTCC   |
| AttB2-PAH1A<br>(plus stop codon)                  | GGGGACCACTTTGTACAAGAAAGCTGGGTTCATTCAACCTCT<br>TCTATTGGCAGTTTCC |
| PAH1 <sup>ΔcatS</sup><br>(mutagenesis)            | CACCAAGATAGTGATTTTCAGAGGTTGAGGGAACTATAACTAA<br>ATCTG           |
| PAH1 <sup>ΔcatA</sup><br>(mutagenesis)            | CAGATTTAGTTATAGTTCCCTCAACCTCTGAAATCACTATCTT<br>GGTG            |
| AttB1-CCT1S                                       | GGGGACAAGTTTGTACAAAAAAGCAGGCTATGAGCAACGTTA<br>TCGGCGATCGC      |
| AttB2-CCT1A<br>(plus stop codon)                  | GGGGACCACTTTGTACAAGAAAGCTGGGTTTACTTGTTGTCT<br>TTAGCATCCGTC     |
| AttB2-CCT1 <sup>-211</sup> A<br>(plus stop codon) | GGGGACCACTTTGTACAAGAAAGCTGGGTTTACTGGAGTTTC<br>TTTAGCCTCATA     |