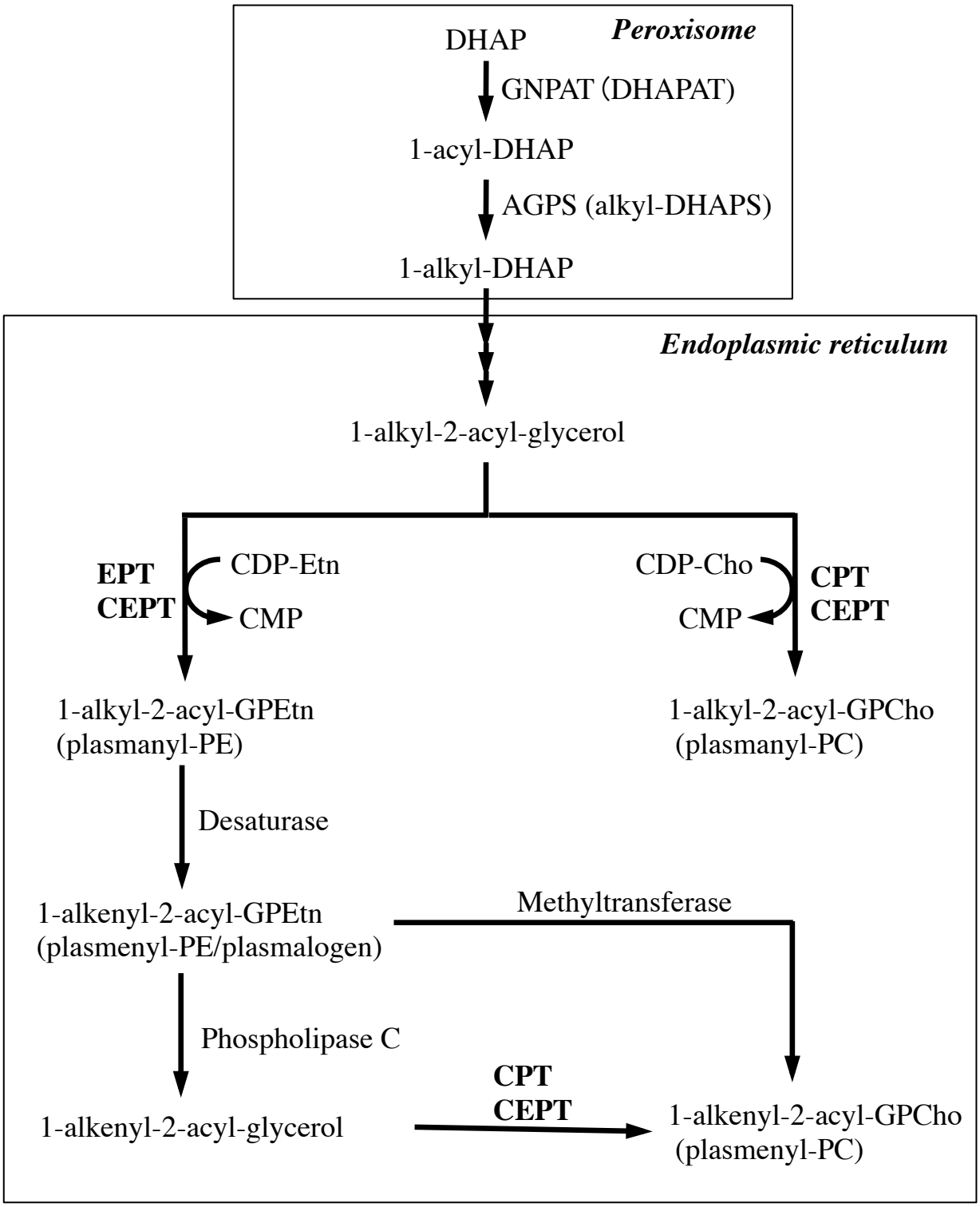
Supplemental Figure S1

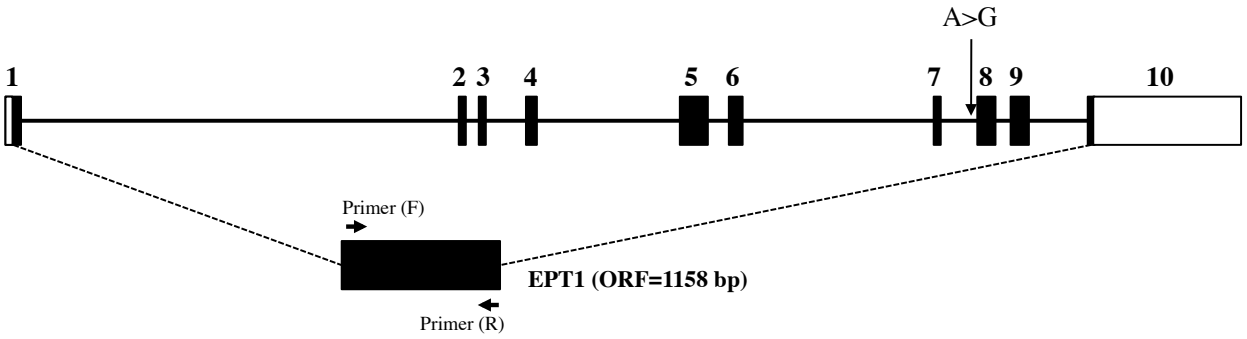


Supplemental Fig. S1. Biosynthesis pathway of ether-linked phospholipids.

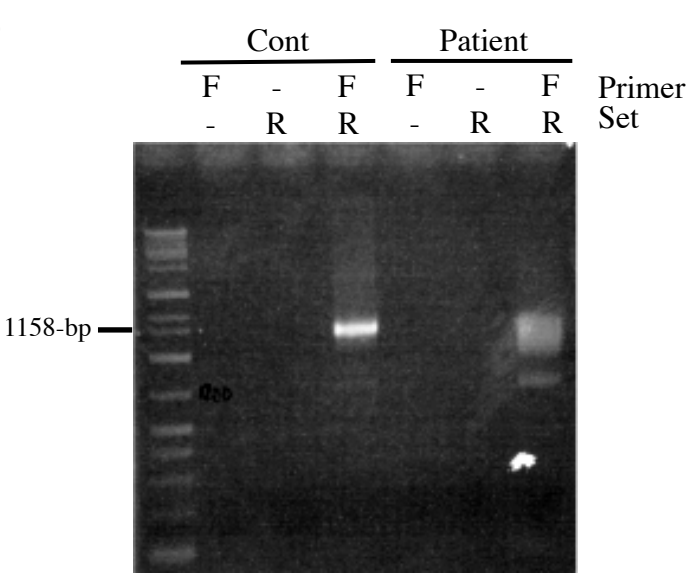
Alkyl-dihydroxyacetonephosphate (DHAP) is synthesized from DHAP by glyceronephosphate O-acyltransferase (GNPAT) and alkylglycerone phosphate synthase (AGPS) in the peroxisome. These enzymes are also named DHAP acyltransferase (DHAPAT) and alkyl-DHAP synthase (alkyl-DHAPS), respectively. Alkyl-DHAP is then converted to 1-alkyl-2-acyl-glycerol in the ER by three enzymatic reactions: the reduction, acylation, and removal of the phosphate group. EPT and CEPT synthesize 1-alkyl-2-acyl-glycerophosphoethanolamine (GPEtn) (plasmanyl-PE) by transferring phosphoethanolamine from CDP-ethanolamine to 1-alkyl-2-acyl-glycerol with the release of CMP. The 1-alkyl group is then desaturated to form the 1-alkenyl group by plasmanyl-PE desaturase to produce 1-alkenyl-2-acyl-GPEtn (plasmenyl-PE/ plasmalogen). Plasmenyl-PE is hydrolyzed by phospholipase C to form free 1-alkenyl-2-acyl-glycerol, which is then modified by CPT and CEPT to produce plasmenyl-PC. This lipid can be synthesized by methylation of the ethanolamine group in plasmenyl-PE. 1-Alkyl-2-acyl-GPCho (plasmanyl-PC) is synthesized from CDP-choline and 1-alkyl-2-acyl-glycerol by CPT and CEPT.

Supplemental Figure S2

A



B



C

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WT              MAGYEYVSPEQLAGFDKYKYSAVDTNPLSLYVMHPFWNTIVKVFPTWLAPNLITFSGFLLVVFNFLLMAYFDPDFYASAPGHKHVPDWVW    90
Variant 1       MAGYEYVSPEQLAGFDKYKYSAVDTNPLSLYVMHPFWNTIVKVFPTWLAPNLITFSGFLLVVFNFLLMAYFDPDFYASAPGHKHVPDWVW    90
Variant 2       MAGYEYVSPEQLAGFDKYKYSAVDTNPLSLYVMHPFWNTIVKVFPTWLAPNLITFSGFLLVVFNFLLMAYFDPDFYASAPGHKHVPDWVW    90
Variant 3       MAGYEYVSPEQLAGFDKYKYSAVDTNPLSLYVMHPFWNTIVKVFPTWLAPNLITFSGFLLVVFNFLLMAYFDPDFYASAPGHKHVPDWVW    90
                ****************************************************************************************

WT              IVVGILNFVAYTLDGVDGKQARRTNSSTPLGELFDHGLDSWSCVYFVVTVYSIFGRGSTGVSVFVLYLLLWVVLFSFILSHWEKYNTGIL   180
Variant 1       IVVGILNFVAYTLDGVDGKQARRTNSSTPLGELFDHGLDSWSCVYFVVTVYSIFGRGSTGVSVFVLYLLLWVVLFSFILSHWEKYNTGIL   180
Variant 2       IVVGILNFVAYTLDGVDGKQARRTNSSTPLGELFDHGLDSWSCVYFVVTVYSIFGRGSTGVSVFVLYLLLWVVLFSFILSHWEKYNTGIL   180
Variant 3       IVVGILNFVAYTLDGVDGKQARRTNSSTPLGELFDHGLDSWSCVYFVVTVYSIFGRGSTGVSVFVLYLLLWVVLFSFILSHWEKYNTGIL   180
                ****************************************************************************************

WT              FLPWGYDISQVTISFVYIVTAVVGVEAWYEPFLFNFLYRDLFTAMIIGCALCVTLPMSLLNFFRSYKNNTLKLNSVYEAMVPLFSPCLLF   270
Variant 1       FLPWGYDISQVVVHYV----------------------------------------------------------------------------   196
Variant 2       FLPWGYDISQVTISFVYIVTAVVGVEAWLCIMCDSSNEFIKLFQRGSFGHLQIF------------------------------------   234
Variant 3       FLPWGYDISQVVVHYV----------------------------------------------------------------------------   196
                ***********.: :*

WT              ILSTAWILWSPSDILELHPRVFYFMVGTAFANSTCQLIVCQMSSTRCPTLNWLLVPLFLVVLVVNLGVASYVESILLYTLTTAFTLAHIH   360
Variant 1       ----------------------------------------------------------------------------------------
Variant 2       ----------------------------------------------------------------------------------------
Variant 3       ----------------------------------------------------------------------------------------

WT              YGVRVVKQLSSHFQIYPFSLRKPNSD   386
Variant 1       -------------------------
Variant 2       -------------------------
Variant 3       -------------------------
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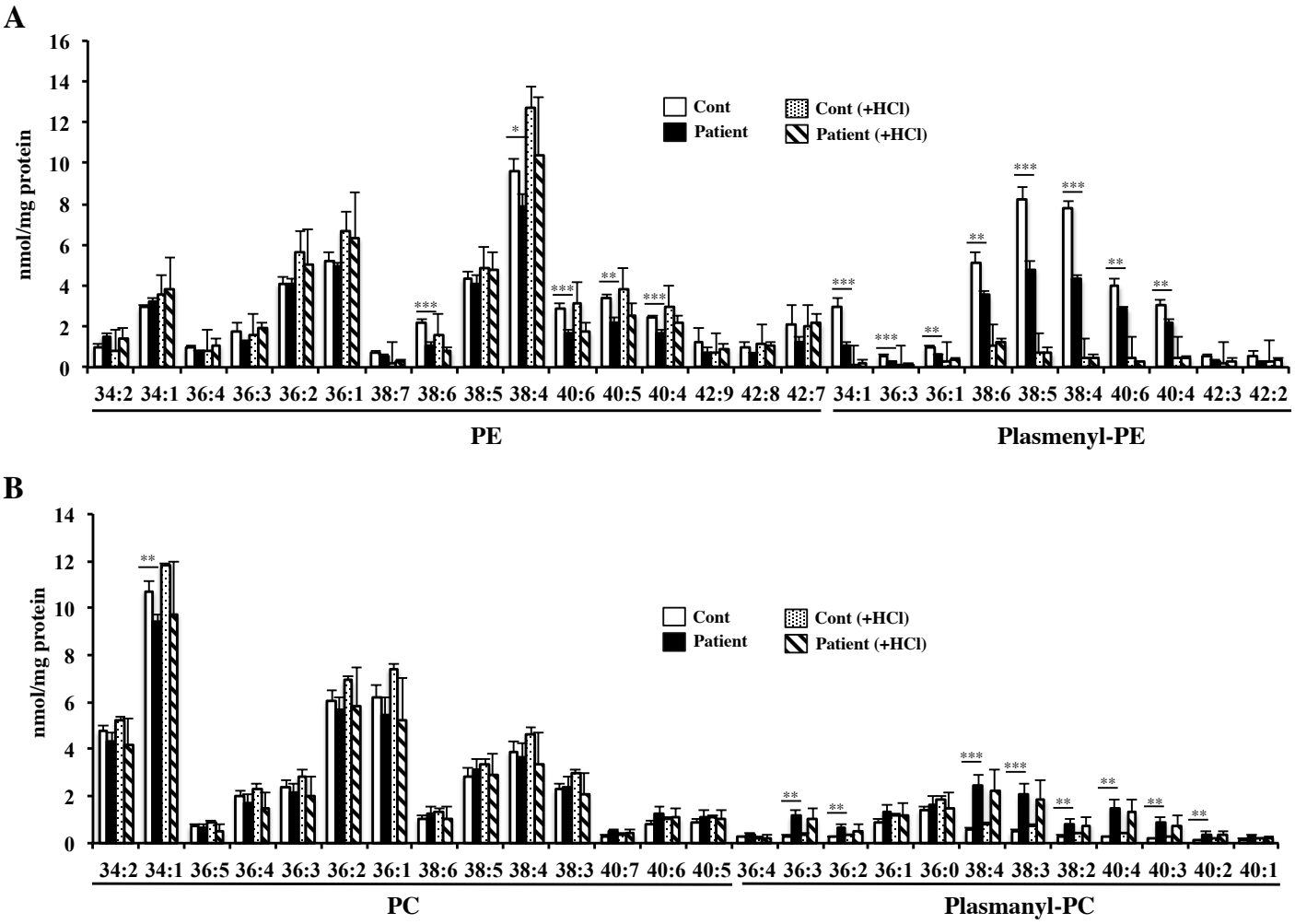
Supplemental Fig. S2. RT-PCR of EPT1 with the patient's fibroblast

(A) Exon-intron structure of human EPT1. Boxes are exons. Non-coding exons are shown in white. The ORF of EPT1 is 1158-bp. The F and R primers encode the N and C-terminus of EPT1, respectively. (B) Agarose gel electrophoresis of RT-PCR product in the healthy control and the patient. Note smeared bands in the patient. (C) Amino acid alignment of WT and splicing variants found in the patient. Identical amino acids are indicated by asterisks, and gaps inserted into the sequences are indicated by dashed lines. Amino acid residues conserved in the CDP-alcohol phosphatidyltransferase motif are shown on a black background.

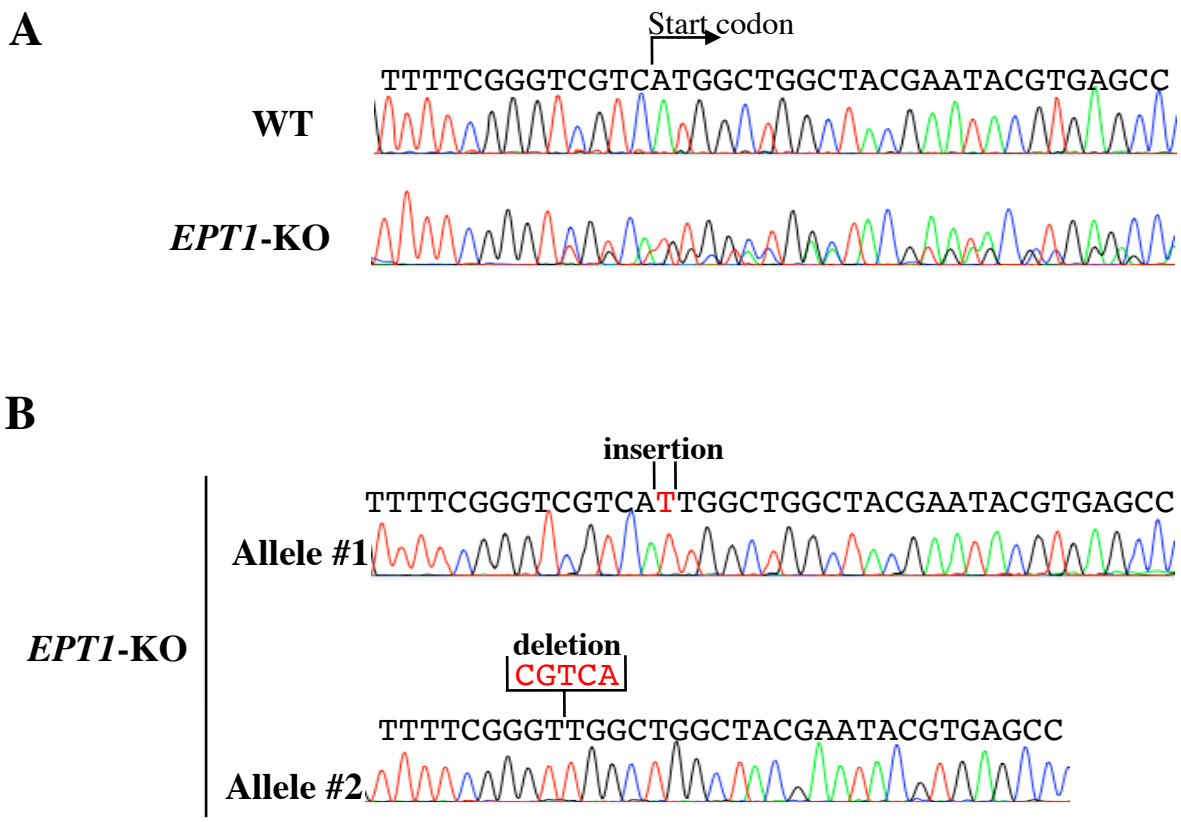
Supplemental Figure S3



Supplemental Fig. S3. Quantification of ethanolamine and choline glycerophospholipids in the skin fibroblasts after treatment with HCl.

After extraction from the skin fibroblasts using the Bligh-Dyer method, the total lipids were treated with HCl (0.15 N) for 2 h at room temperature to degrade plasmenyl-PE and plasmenyl-PC. The amounts of PE and plasmenyl-PE (A), PC and plasmanyl-PC (B) were determined using LC-MS/MS. Values were normalized against the amount of protein in the cells. Each experiment was repeated at least three times with similar results. Data are means ± S.D. from quadruplet analyses in one experiment. Each experiment was repeated at least three times with similar results. *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$ as compared with control cells, respectively.

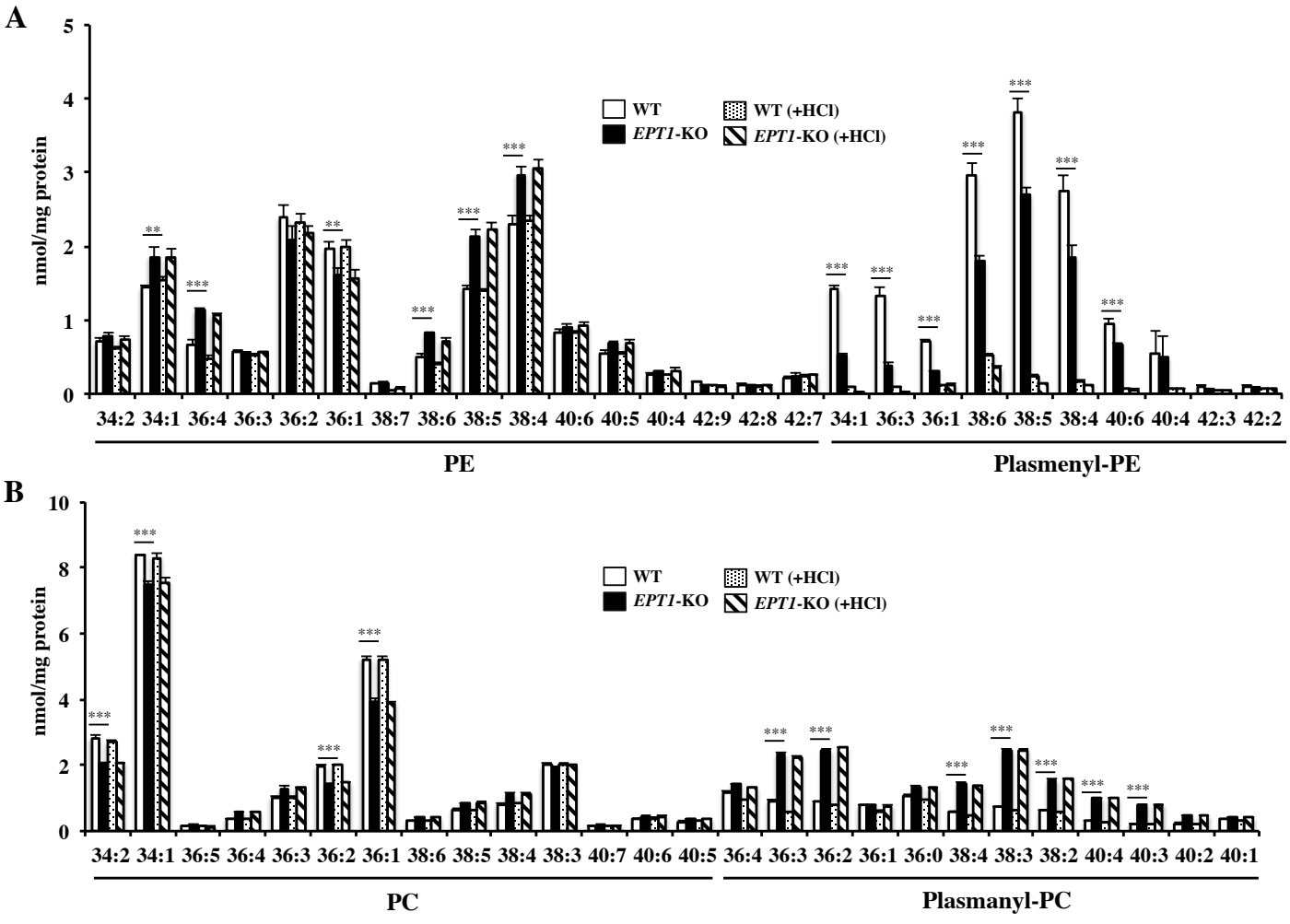
Supplemental Figure S4



Supplemental Fig. S4. Generation of *EPT1*-KO HeLa cells

The DNA fragments around the target regions in exon1 were amplified by PCR and directly sequenced (A). After the fragments were sub-cloned, the insert containing each allele was determined (B).

Supplemental Figure S5



Supplemental Fig. S5. Quantification of ethanolamine and choline glycerophospholipids in *EPT1*-KO HeLa cells after treatment with HCl.

After extraction from HeLa cells using the Bligh-Dyer method, the total lipids were treated with HCl (0.15 N) for 2 h at room temperature to degrade plasmenyl-PE and -PC. The amounts of PE and plasmenyl-PE (A), PC and plasmanyl-PC (B) were each determined using LC-MS/MS. Values were normalized against the amount of protein in the cells. Each experiment was repeated at least three times with similar results. Data are means ± S.D. from quadruplet analyses in one experiment. Each experiment was repeated at least three times with similar results. ** and *** indicate $P < 0.01$ and $P < 0.001$ as compared with WT cells, respectively.