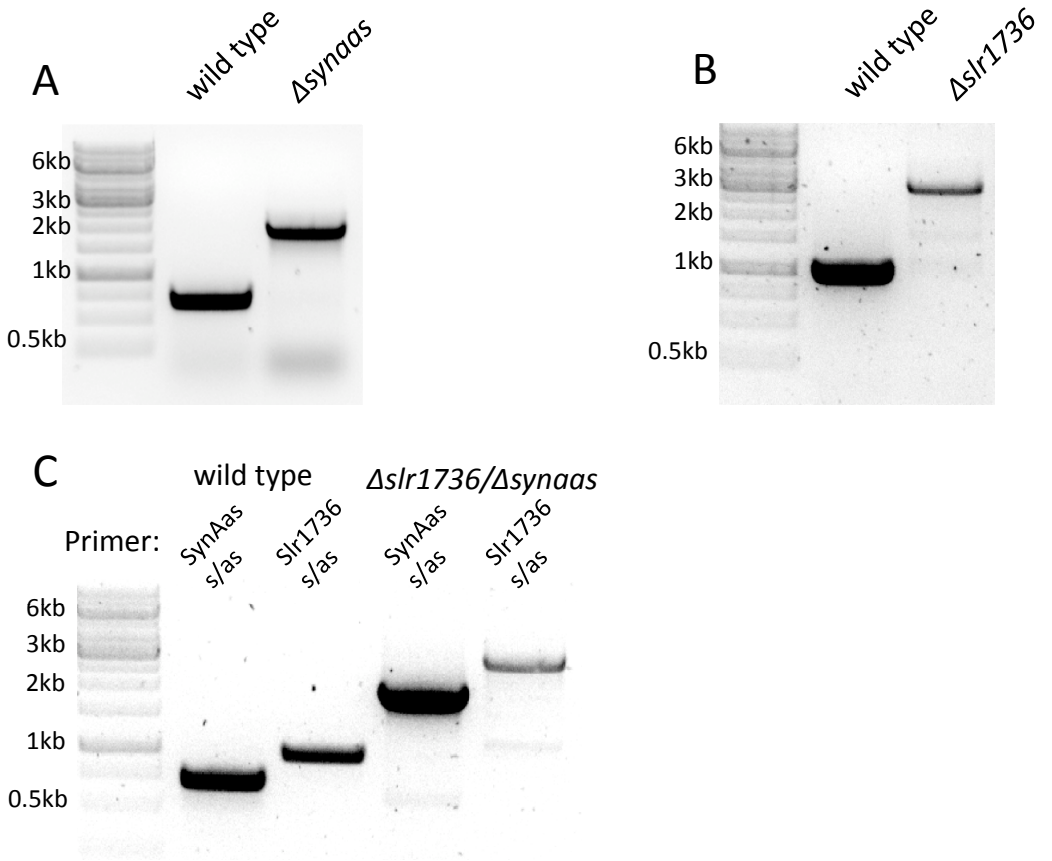
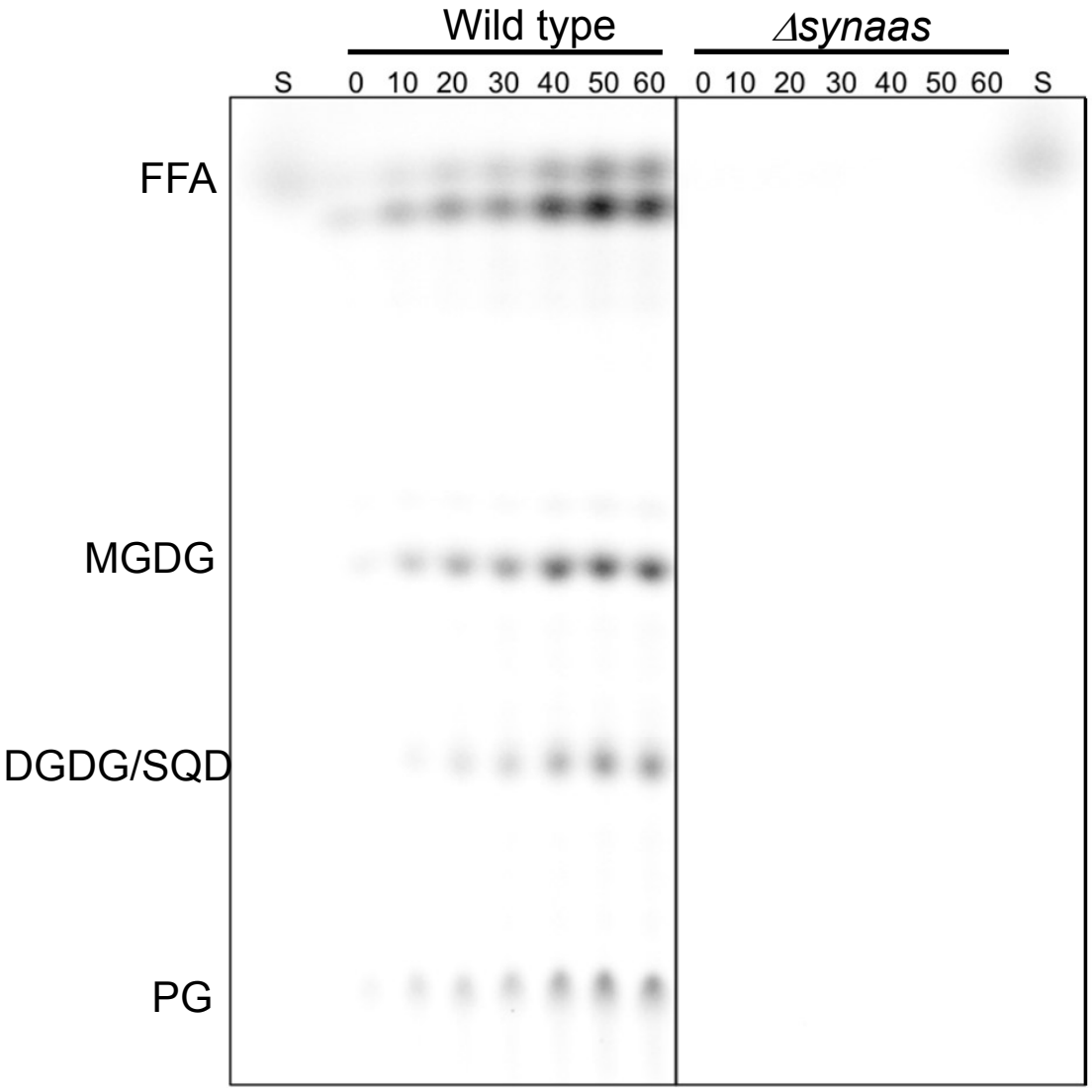
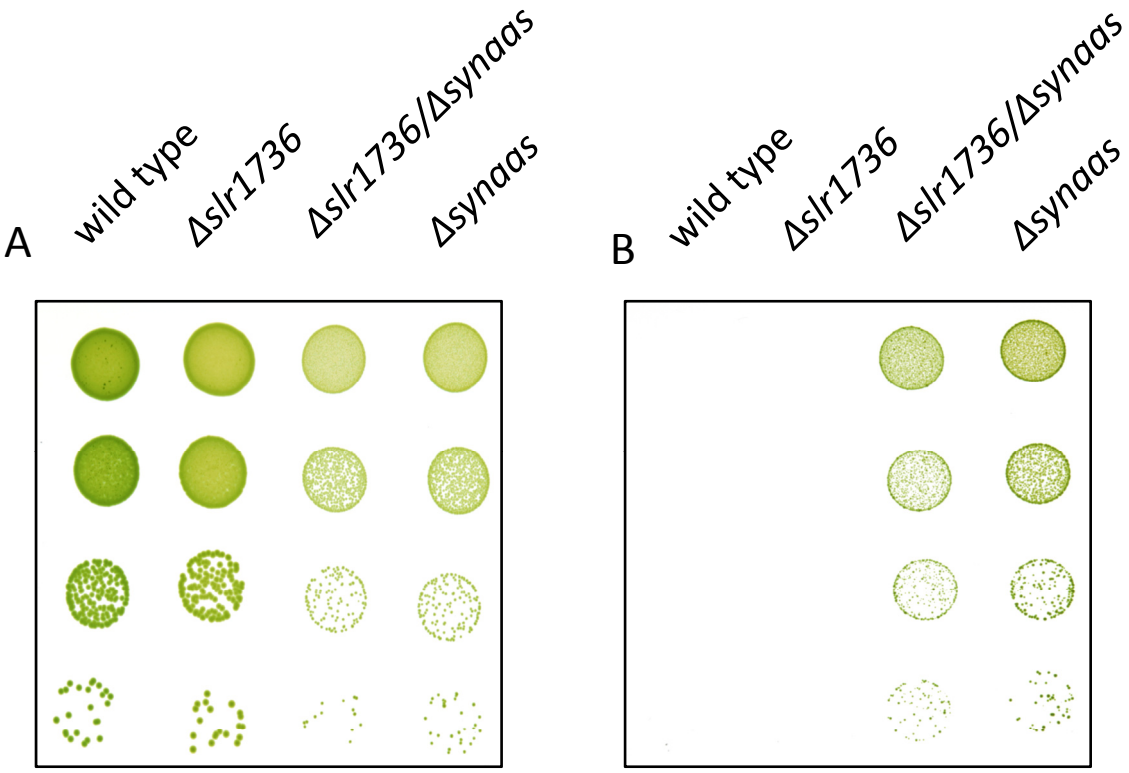


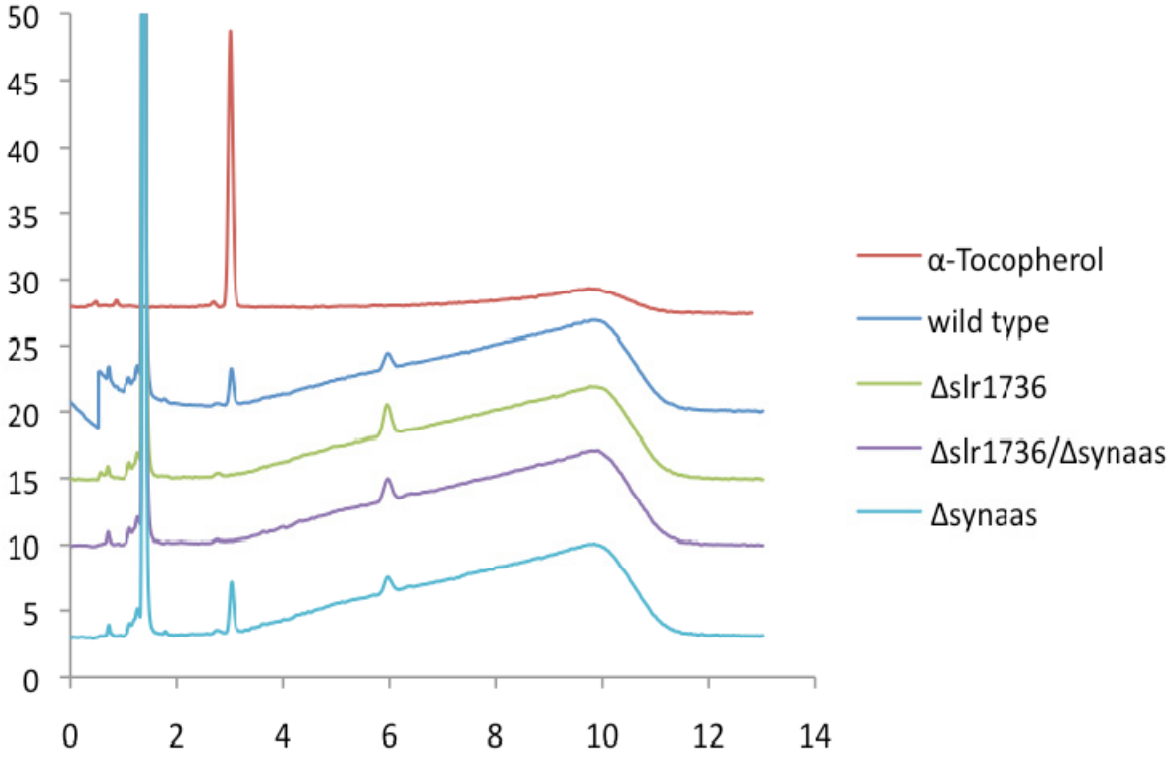
Supplemental Figure 1
Confirmation of the inserted kanamycin and spectinomycin cassette and full segregation of the *Δslr1736*, *Δslr1609* and *Δslr1736/Δslr1609* mutant.
Primer SynAAS_s and SynAAS_as were used for the confirmation of the Δ*synaas* mutant (A), for the confirmation of the *Δslr1736* mutant SLR1736_s and SLR1736_as were used (B). Both primer pairs were used to confirm the *Δslr1736*/*Δslr1609* double mutant (C). Size of wild-type PCR products are 710 bp for SynAas and 974 bp for Slr1736 and 1936 bp and 3019 bp for mutant PCR products respectively.



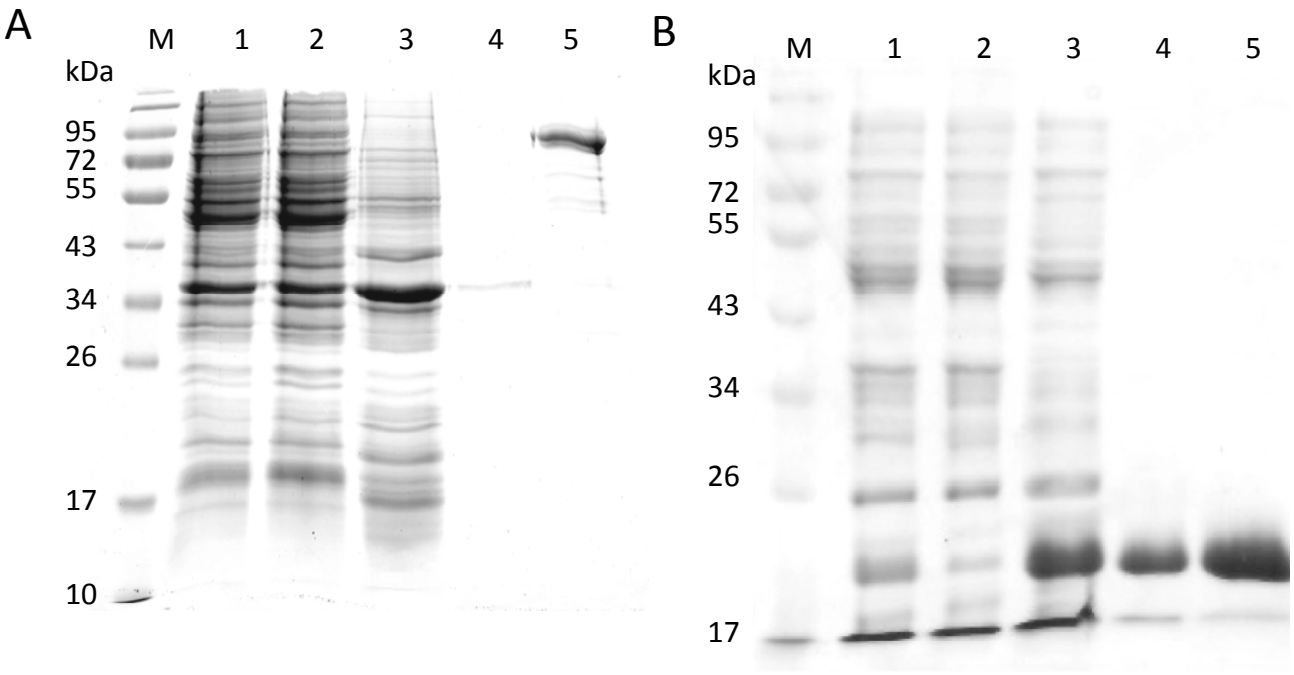
Supplemental Figure 2
Autoradiography of total lipid extracts from α-[^{14}C]linolenic acid-treated wild-type and *Δsynaas* cells separated by thin layer chromatography
300 µl of *Synechocystis* suspension culture (OD_{750}= 3.0) were incubated for up to 60 minutes with 33 µM final concentration α-linolenic acid at a 1:10 dilution with α-[^{14}C]-linolenic acid (Hartmann Analytic) at 30°C in the light. For each time point 30 µl cells were harvested and lipids were extracted with 30 µl MeOH/Chloroform (1:1/ v:v) and 15 µl 1 M KCl, 0.2 M H_3PO_4. Soluble phase and lipid phase were separated by centrifugation and 5 µl of the lipid phase were spotted on a TLC plate. Lipids were separated on silica plates using acetone:toluene:H_2O (91:30:8 v/v/v) as solvent. α-[^{14}C]-linolenic acid (Hartmann Analytic) was used as a standard (S). Spots were visualized by an Image Analyser (FLA-3000, Fujifilm).



Supplemental Figure 3
Synechocystis wild type and mutant growth assay on plates at higher α-linolenic acid concentrations.
Liquid culture of wild type, *Δslr1736, Δsynaas, Δslr1736/Δsynaas* cultivated in low light (15 µE m^{-2} s^{-1}) conditions were adjusted to OD_{750} 0.05 and dropped on plates containing 0.1% EtOH (A) or 20 µM α-linolenic acid (B). Plates were subsequently grown for 7 day under high light (300 µE m^{-2} s^{-1}) conditions. The *Δsynaas* and *Δslr1736/Δsynaas* mutants are viable under these conditions while wild type and *Δslr1736* cells are unable to grow.



Supplemental Figure 4
α-Tocopherol measurement.
α-Tocopherol was extracted from wild type, *Δslr1736*, *Δslr1736*/*Δsynaas* and *Δsynaas* cells and subjected to HPLC. Purified α-tocopherol (Sigma Aldrich, St. Louis, USA) was used as standard. The specific α-tocopherol peak is missing in the *Δslr1736* and *Δslr1736*/*Δsynaas* mutant.



Supplemental Figure 5
Purification of 6x His-tagged SynAas from *Synechocystis sp. 6803* and Acyl Carrier Protein from *Escherichia coli* using Ni-NTA affinity chromatography.
SynAas protein (A) and Acyl Carrier Protein (B) were overexpressed in yeast (InvSC1) and *E. coli* (BLR) cells, respectively. (M) molecular mass marker, (1) crude extract, (2) flow through, (3) first wash step, (4) second wash step, (5) Elution of the protein. SynAas and ACP have an expected molecular mass of 82.5 and 9 kDa including a 6xHis-tag respectively. Please note that ACP is known to run at an apparent molecular weight of approx. 20 kDa in SDS-PAGE due to a high density of anionic amino acid residues (Chan DI, Vogel HJ (2010) Current understanding of fatty acid biosynthesis and the acyl carrier protein. Biochem J **430**:1-19).

Supplemental Methods

Tocopherol measurement

10 ml of cells OD_{750} = 1.0 were collected by centrifugation at 8000 g for 10 min. Tocopherols were extracted in 500 µl MeOH and 1 mg ml^{-1} butylhydroxytoluene (BHT) at 4 °C. Cell debris was removed by centrifugation and 5 µl of the supernatant was subjected to HPLC analysis (Ultimate 3000, Dionex with fluorescence detector RF 200, Dionex) using a Phenomenex column (Hyperclone ODS 3 µm, C18, length 150 mm, width 4.6 mm). A Methanol (A)-Isopropanol (B) gradient was used (0-8 min 98% A, 2% B; 8-13 min 100%A). Tocopherols were detected by fluorescence using 290 nm excitation and 325 nm emission. Purified α-tocopherol purchased from Sigma-Aldrich was used as a standard.