

Supplementary figures and tables

Manuscript title: Phosphatidylcholine synthesis through cholinephosphate cytidyltransferase is dispensable in *Leishmania major*

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Supplementary figure legends

Fig. S1. Southern blot confirms the targeted deletion of *CPCT* (full-size, unedited images for Figure 2). (A) 8-hour exposure for the blot hybridized with the *CPCT* ORF probe. (B) Ethidium bromide staining of the DNA gel used in A. (C) 48-hour exposure for the blot hybridized with the *CPCT* FR probe. (D) Ethidium bromide staining of the DNA gel used in C.

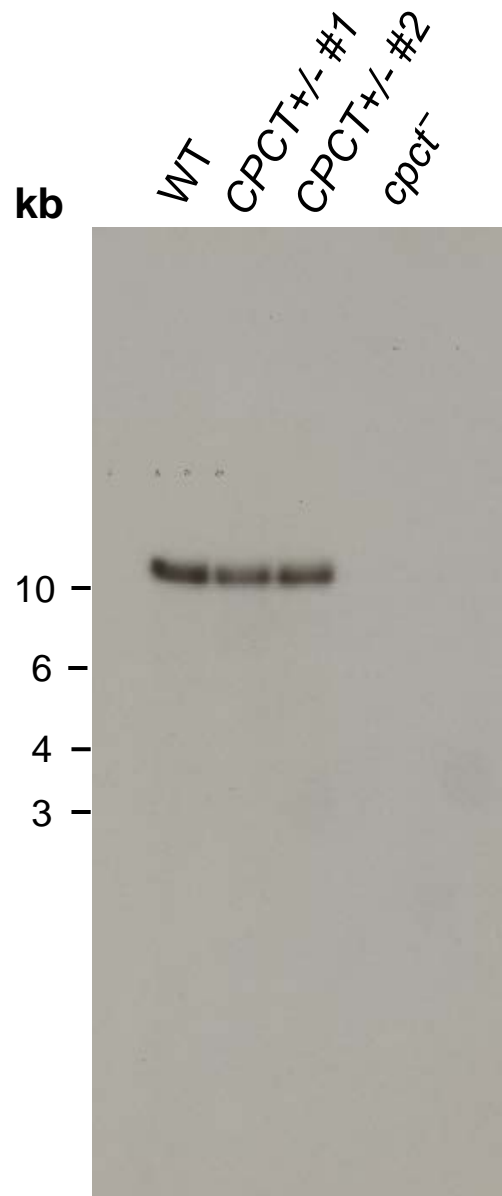
Fig. S2. Validation of GFP-tagged *CPCT* fusion proteins by Western blot. Cell lysates from log phase WT, WT/+ GFP (27 kDa), *c14dm*⁻/+*c14DM-GFP* (81 kDa), *cpct*⁻/+*GFP-CPCT* (92 kDa) and *cpct*⁻/+*CPCT-GFP* (92 kDa) parasites were analyzed by Western blot using antibodies against GFP (A, 5-second exposure) or α -tubulin (B, 5-second exposure).

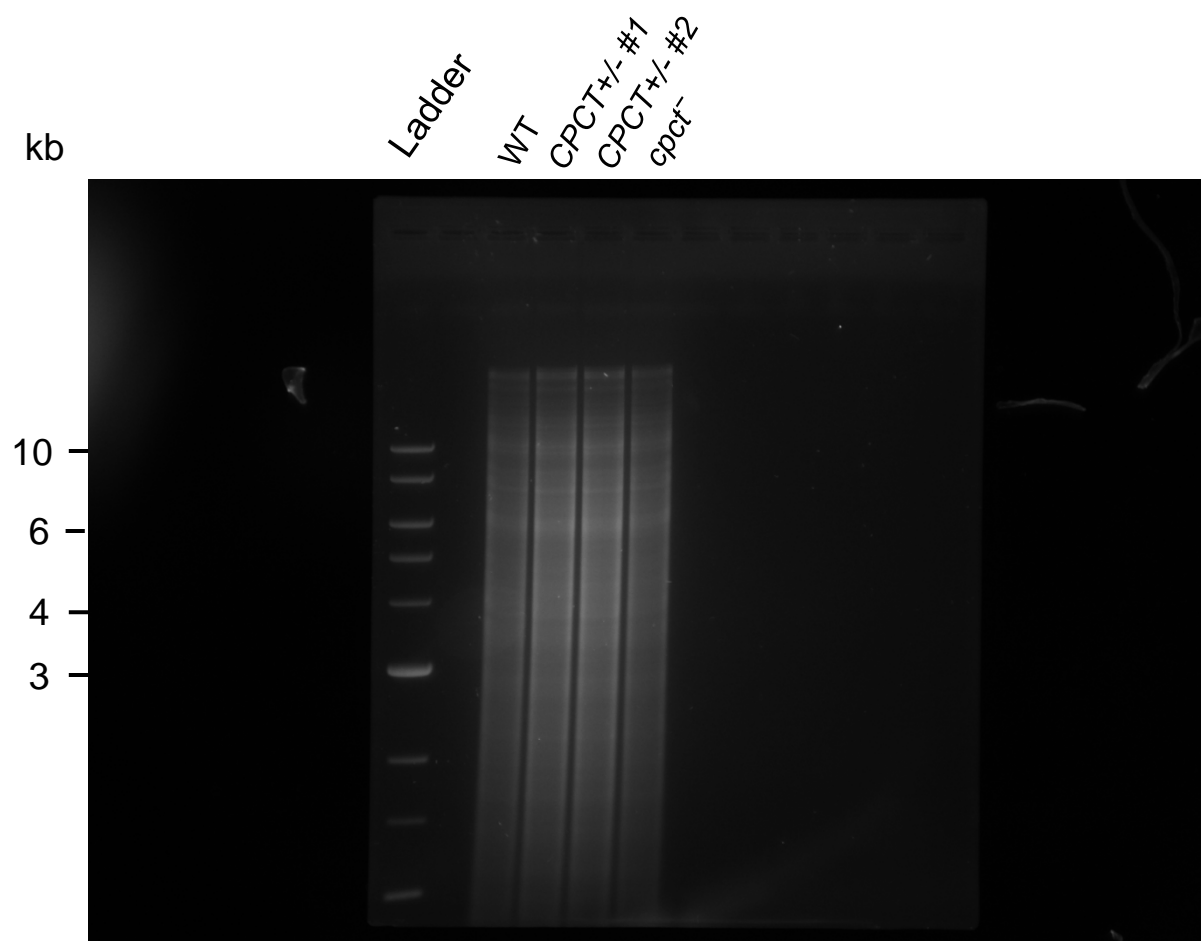
Fig. S3. *CPCT* is required for incorporating choline to PC (full-size, unedited images for Figure 4). (A) 10-hour exposure (Bio-Rad Personal Molecular Imager) of the *CPCT* assay using *E. coli* lysates (boiled and not boiled). (B-C) 24-hour exposure (autoradiography) of TLC analyses of metabolic labeling with [¹⁴C]-labeled choline (B) or [³H]-labeled EtN (C).

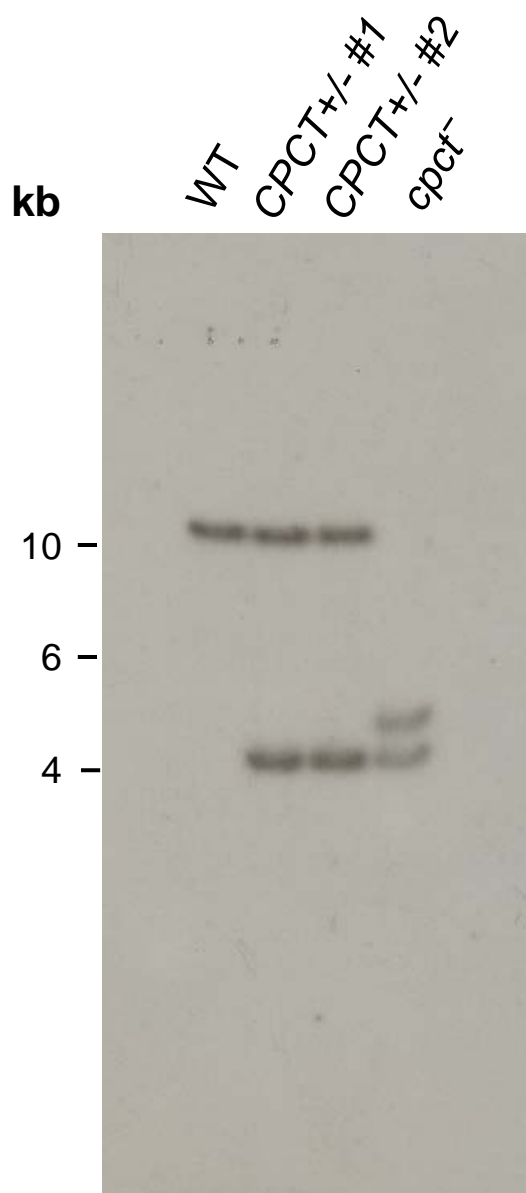
Fig. S4. Western blot analysis of LPG in *cpct*⁻ mutants. Log phase and stationary phase (day 1 and day 3) promastigotes were washed once in PBS and resuspended at 5.0×10^7 cells/ml in SDS sample buffer. After SDS-PAGE and transfer, blots were probed with antibodies against LPG (A) or α -tubulin (B), followed by HRP-conjugated secondary antibodies. Six independent experiments were performed and one representative image is shown here.

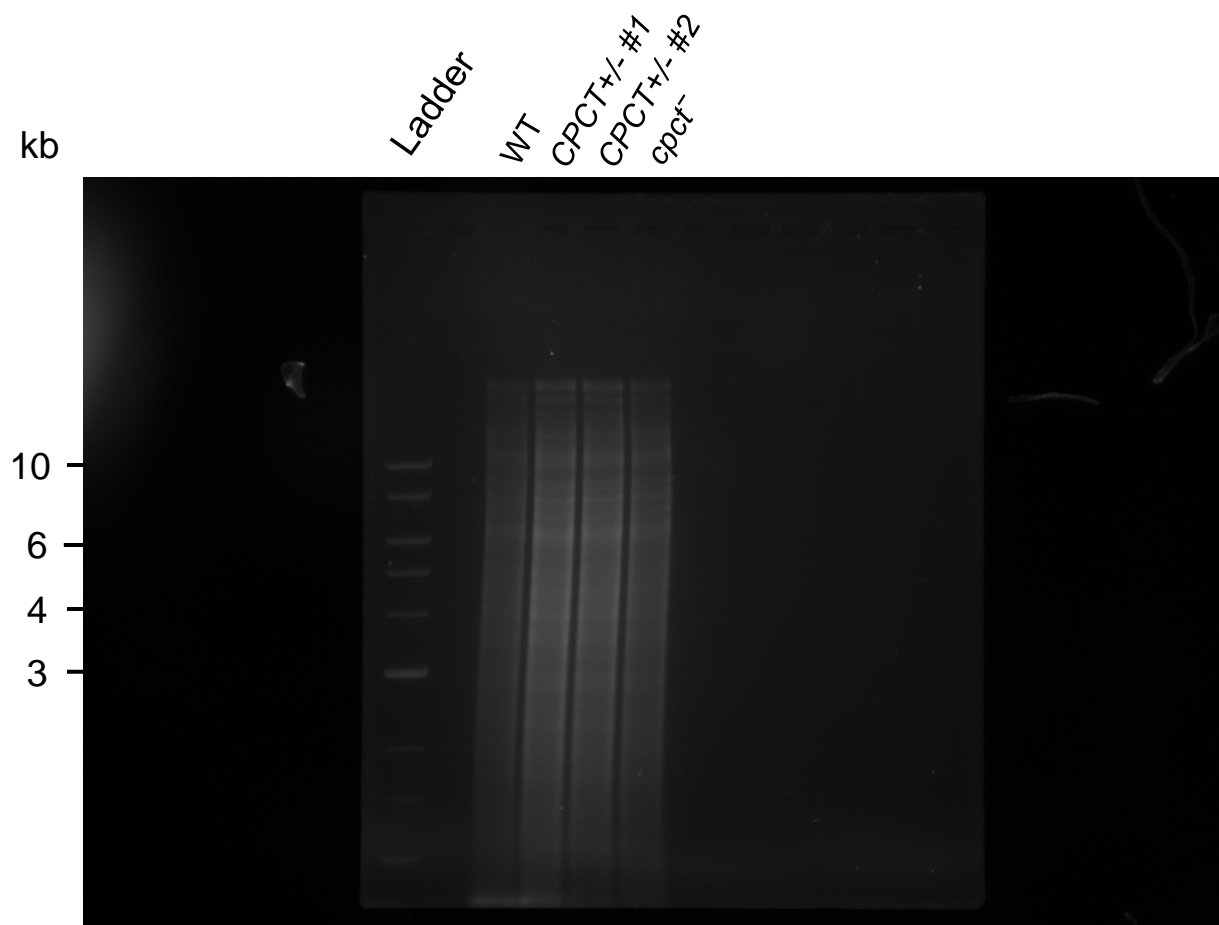
Table S1. Summary results of randomly selected *cpct*⁻/+*GFP-CPCT* cells labeled with anti-*T. brucei* BiP antibody (ER marker). Images were analyzed by the JaCOP Image J software and the Pearson correlation coefficient (PCC) between the localizations of BiP and GFP was determined. A complete overlap is 1.00 and no overlap is 0. Average of 8 images (30 cells) = 0.85. Standard deviation = 0.056.

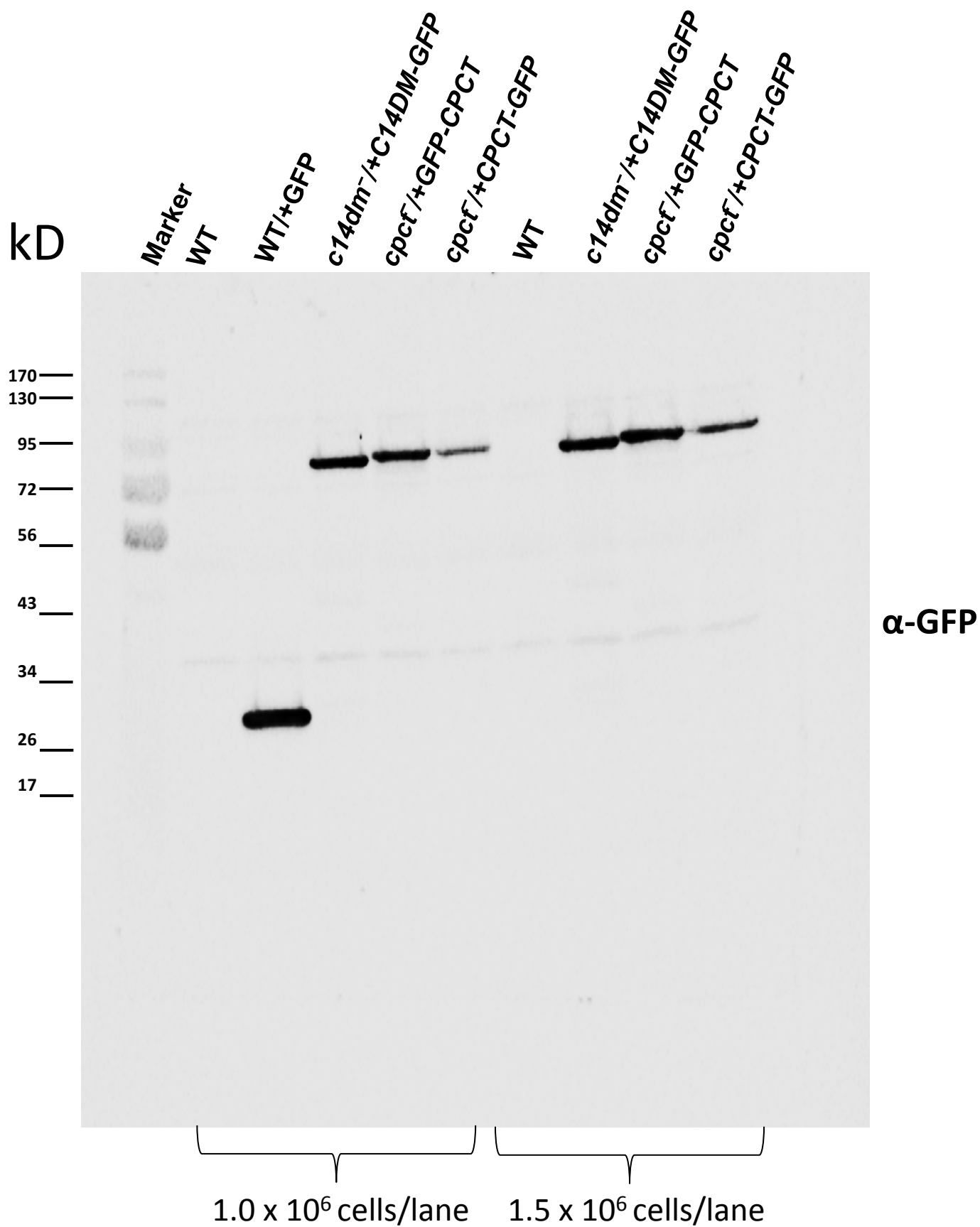
Figure S1A











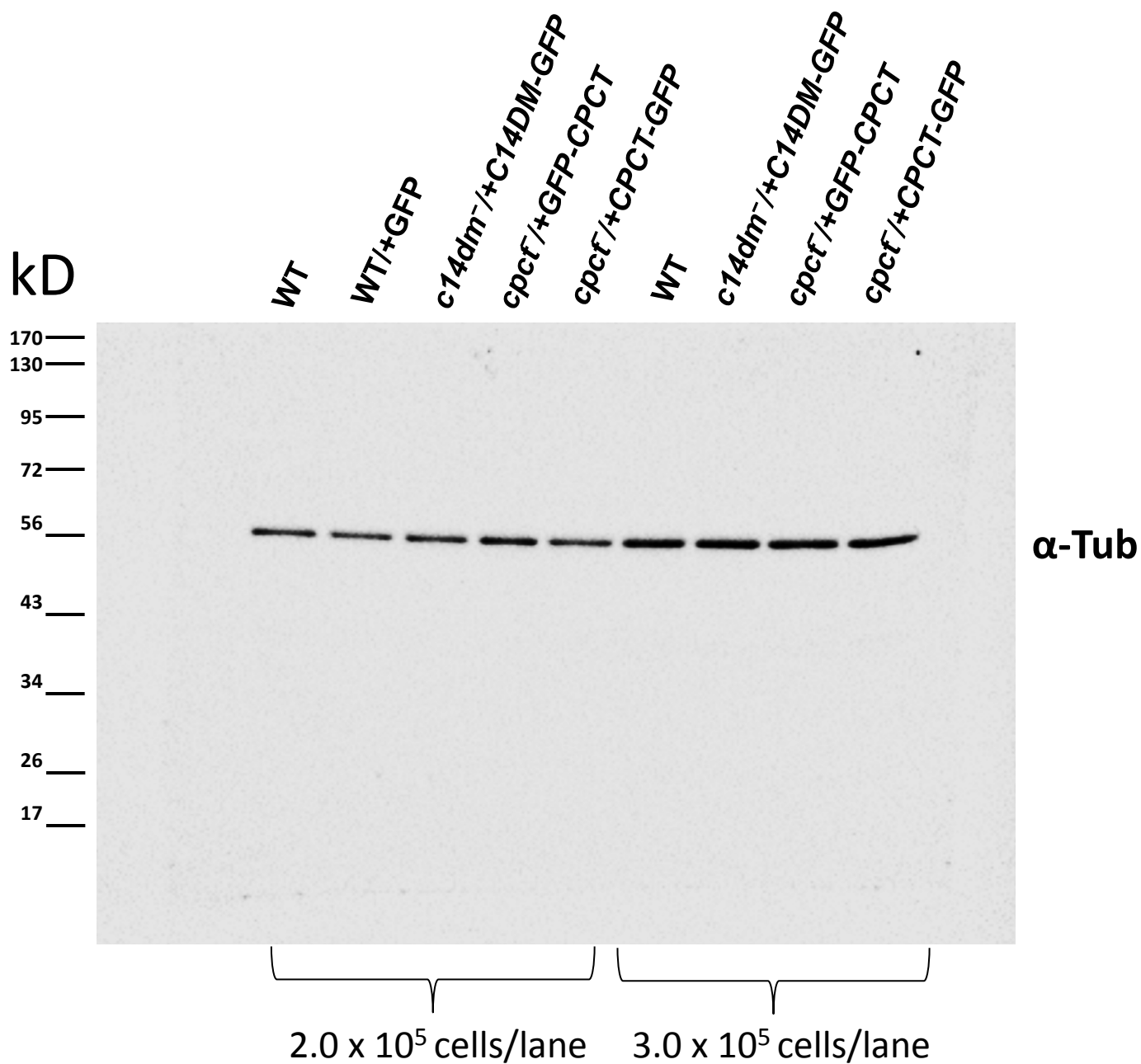


Figure S3A

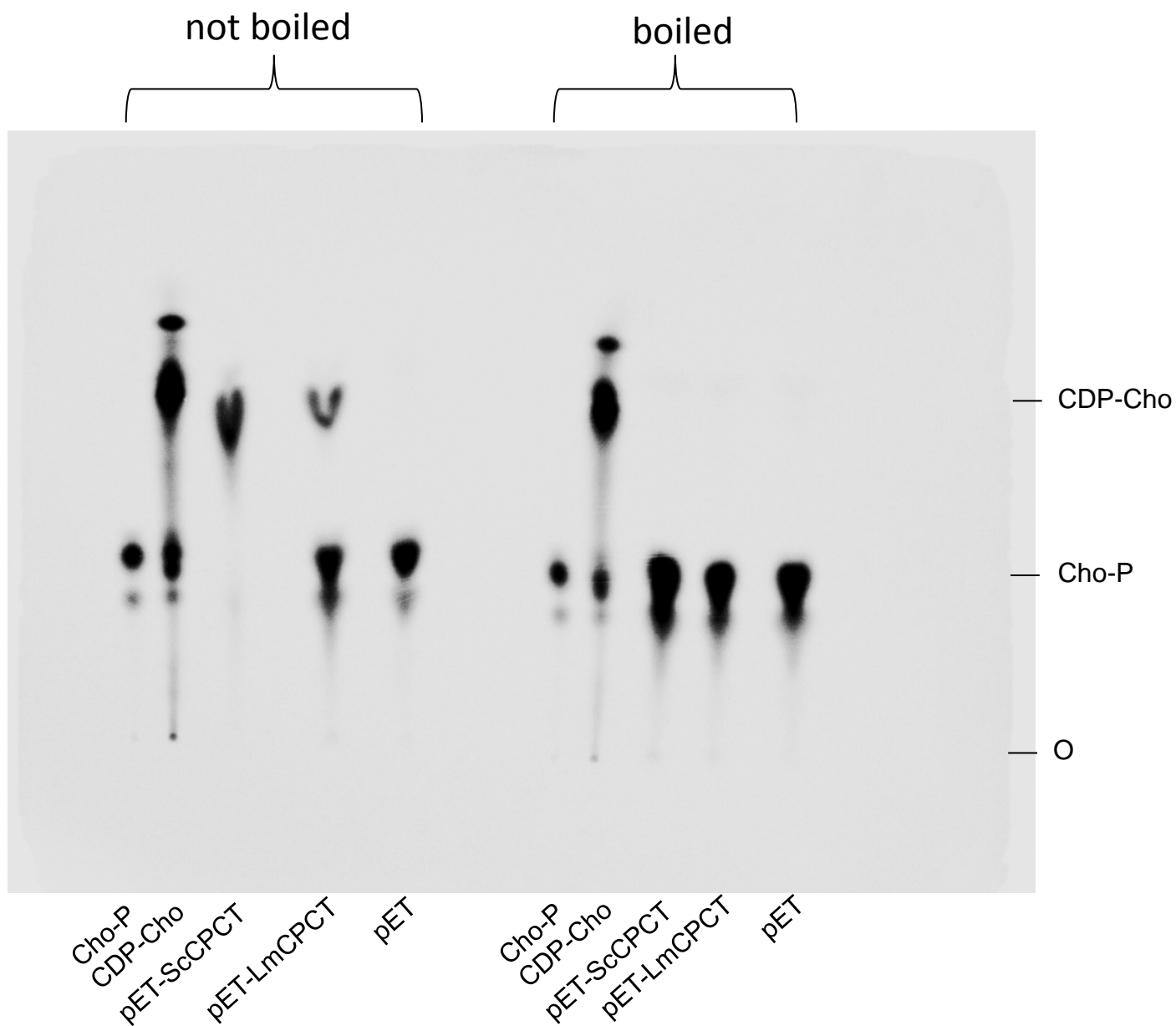


Figure S3B

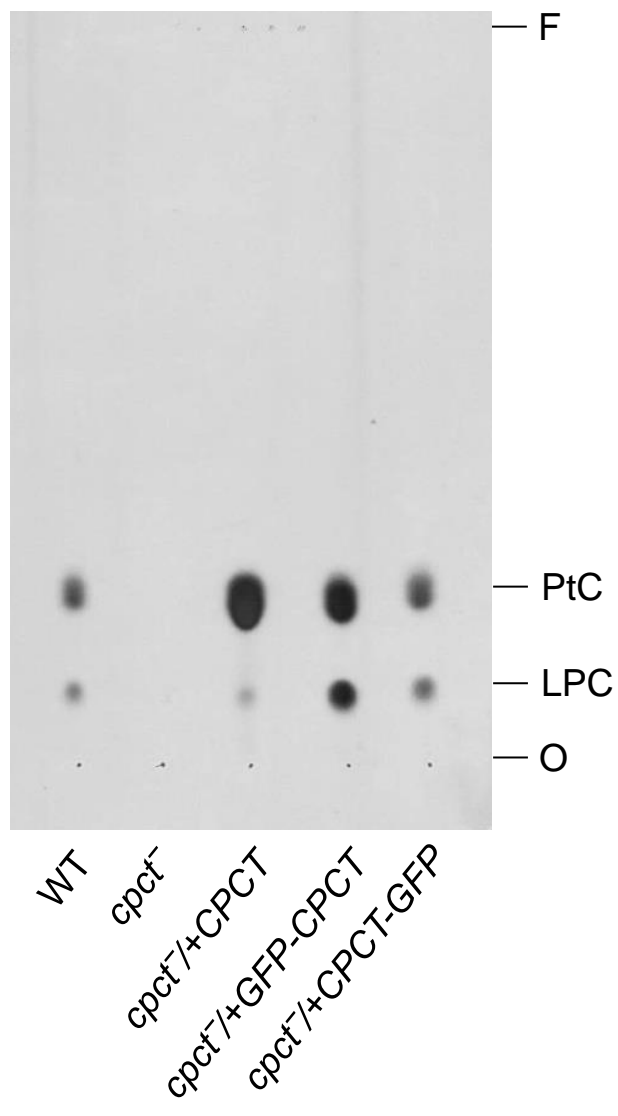


Figure S3C

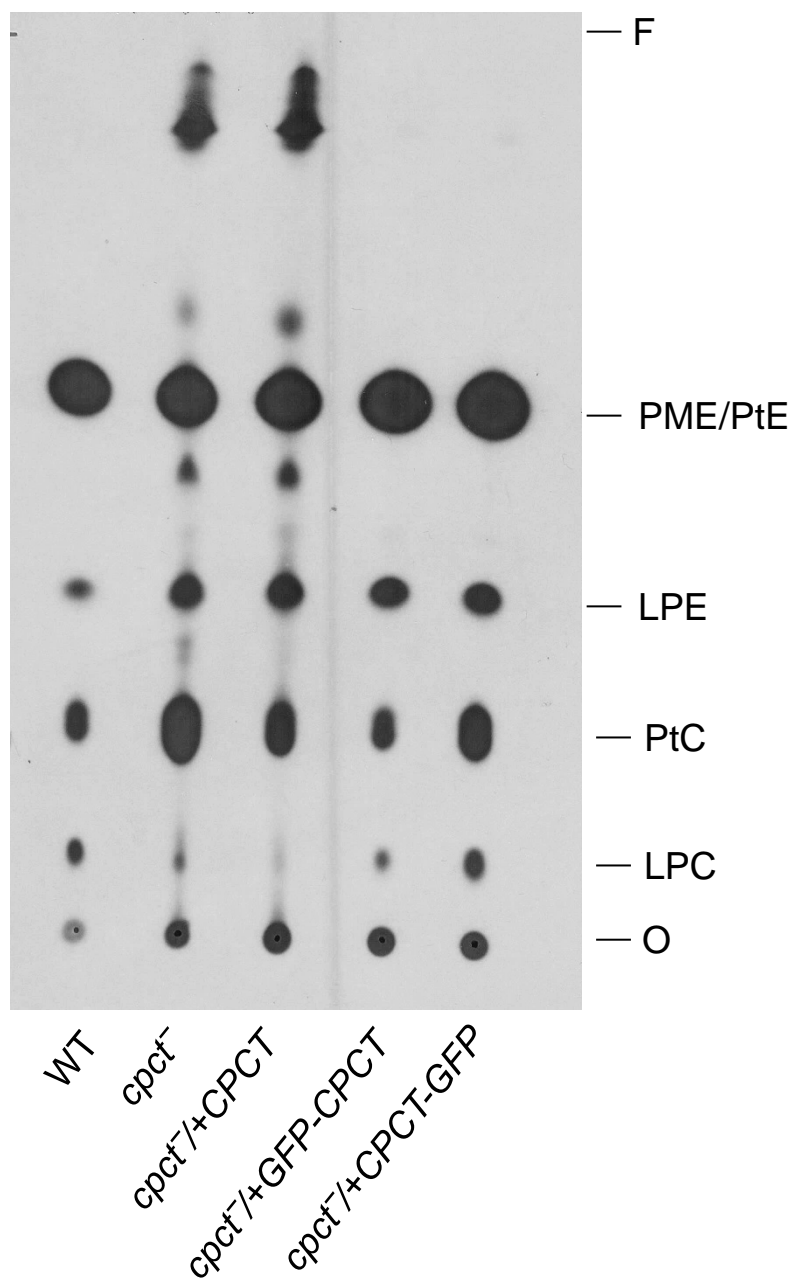


Figure S4A

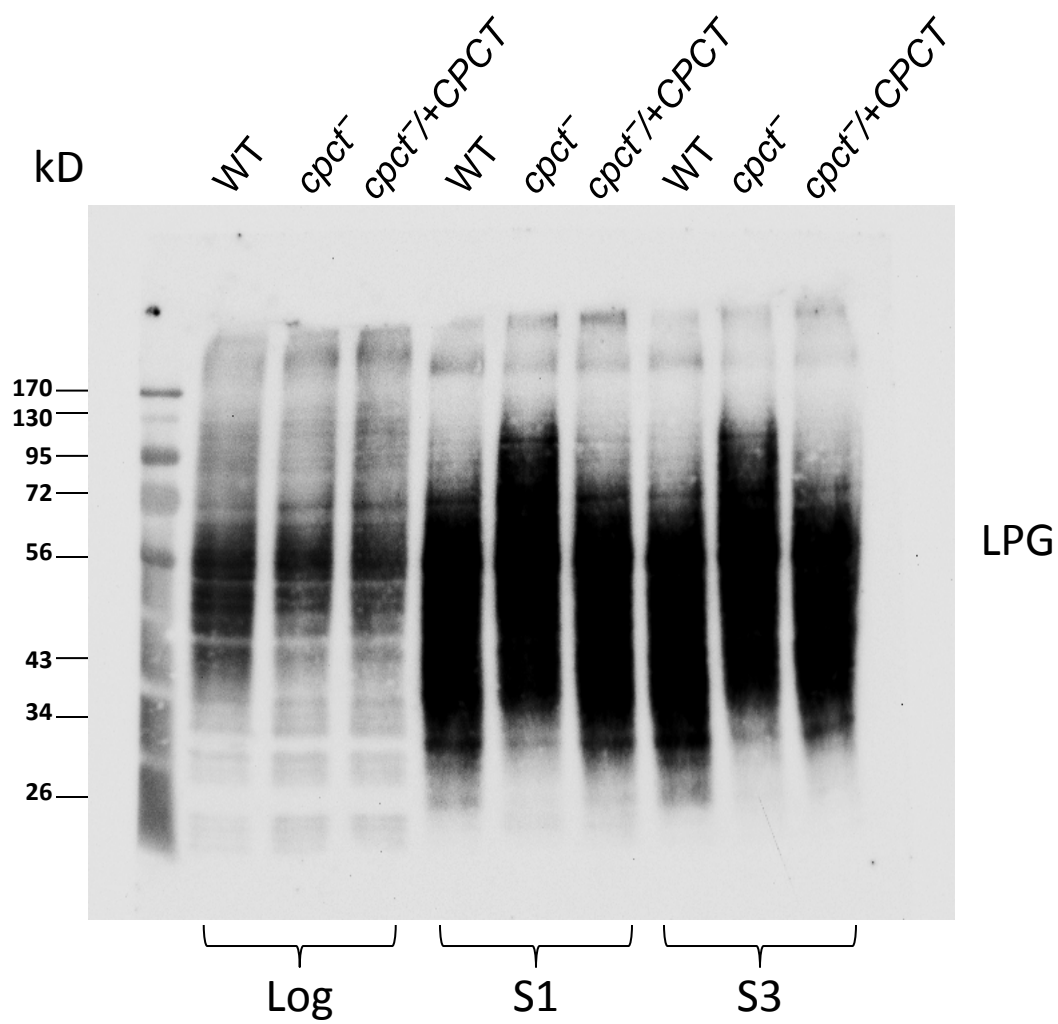


Figure S4B

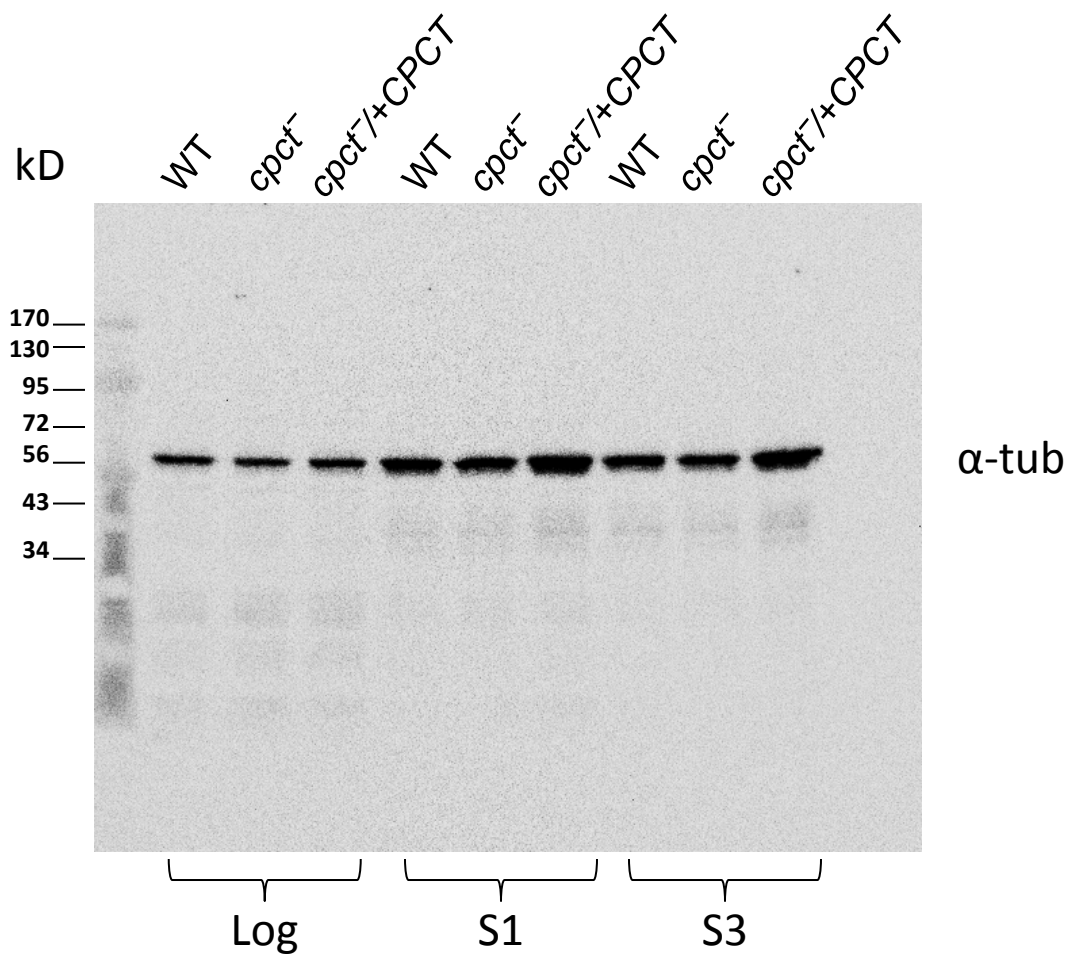


Table S1. Summary results of randomly selected *cpct*^{-/+}GFP-CPCT cells labeled with anti-*T. brucei* BiP antibody (ER marker). Images were analyzed by the JaCOP Image J software and the Pearson correlation coefficient (PCC) between the localizations of BiP and GFP was determined. A complete overlap is 1.00 and no overlap is 0. Average of 8 images (30 cells) = 0.85. Standard deviation = 0.056.

Image #	PCC
1 (average of 3 cells)	0.80
2 (average of 5 cells)	0.76
3 (average of 2 cells)	0.89
4 (average of 6 cells)	0.84
5 (average of 2 cells)	0.86
6 (average of 6 cells)	0.87
7 (average of 2 cells)	0.94
8 (average of 4 cells)	0.83