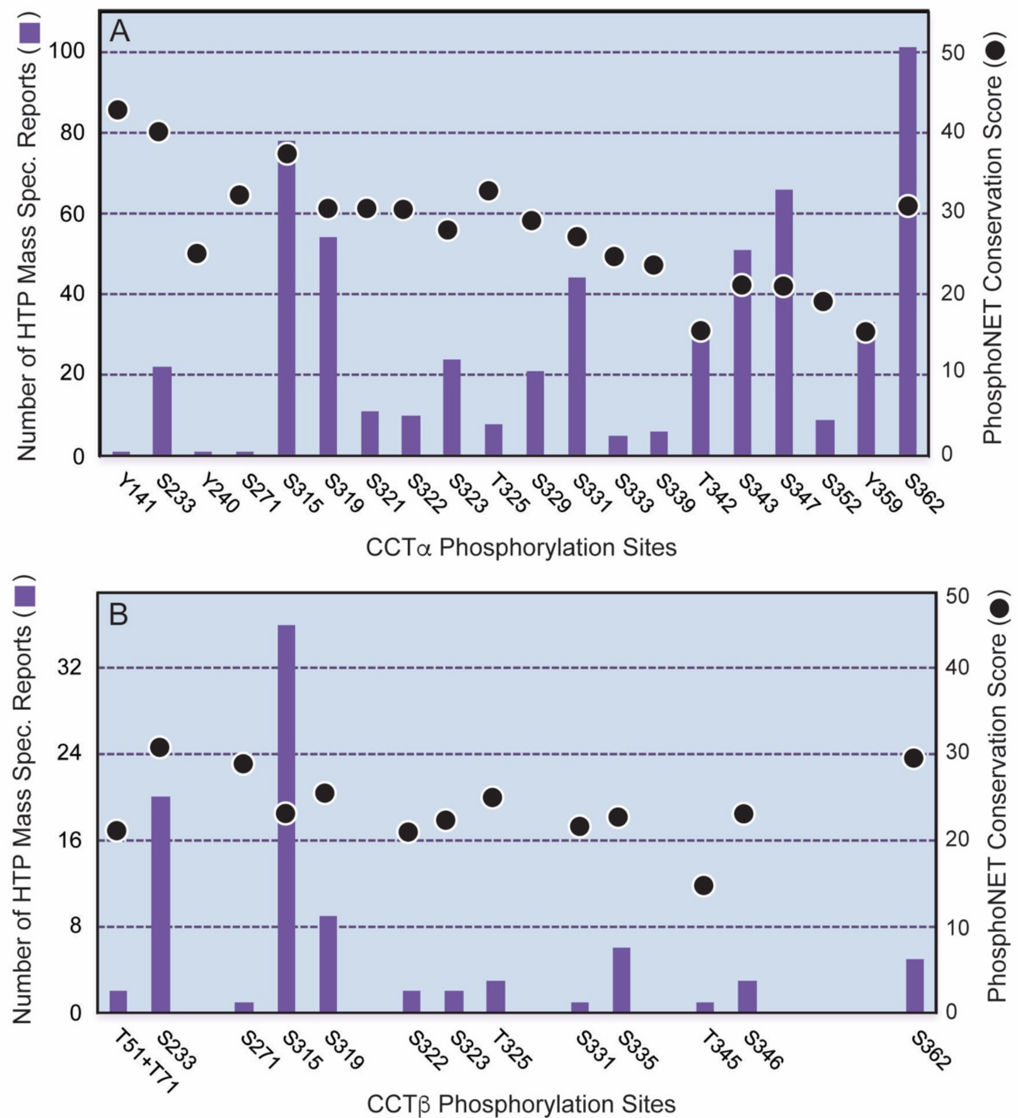
Supplemental Materials

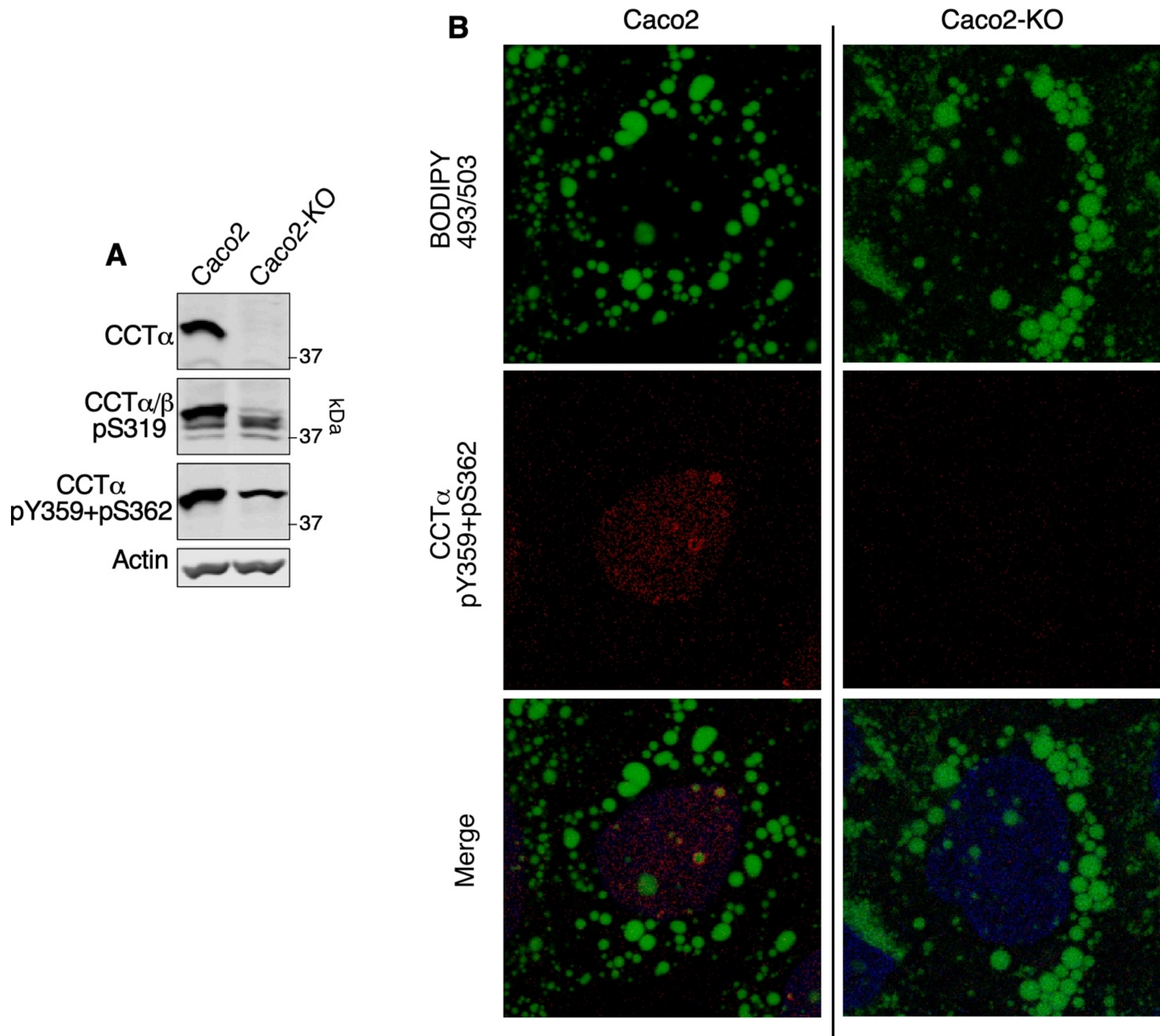
Molecular Biology of the Cell

Yue et al.

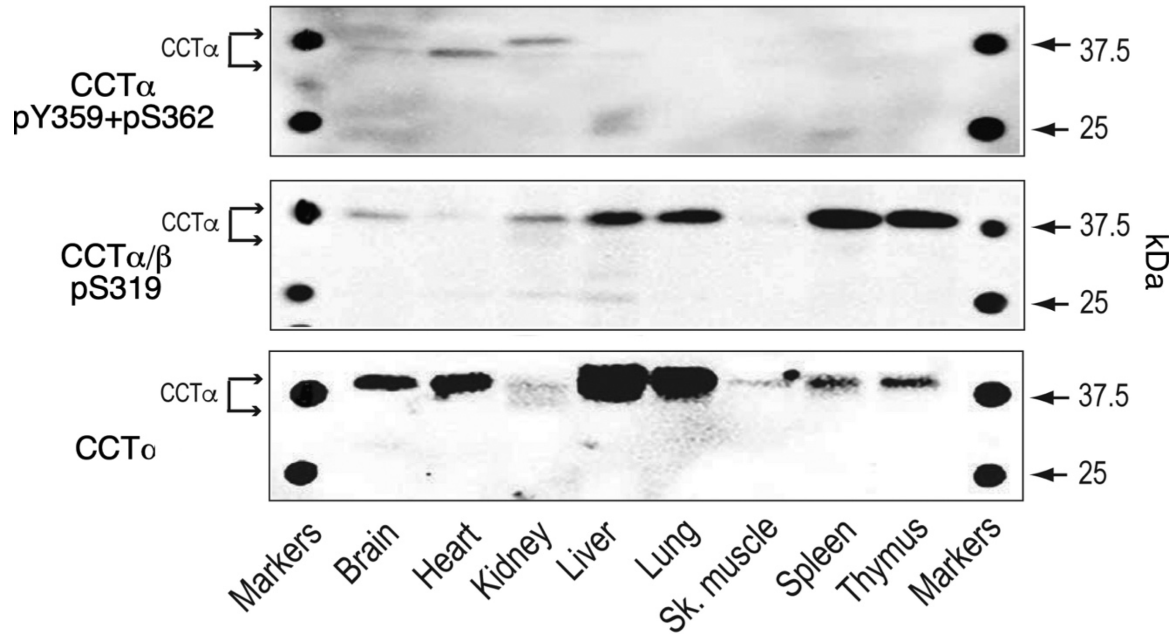
Yue *et al.* Supplemental Figure S1



SUPPLEMENTAL FIGURE S1. Mass spectrometry frequency and conservation of human CCTα and CCTβ phosphorylation sites. The distribution and number of separate high throughput (HTP) mass spectrometry reports of phosphorylation sites listed for human CCTα (**A**) and CCTβ (**B**) on the PhosphositePlus (www.phosphosite.org) website (Hornbecker *et al.,* 2015) was determined (August 31, 2019). The conservation score (% retention in the analogous CCT phosphosites of 24 diverse species from human to red bread mold) was determined from the PhosphoNET (www.phosphonet.ca) website Evolutionary module.

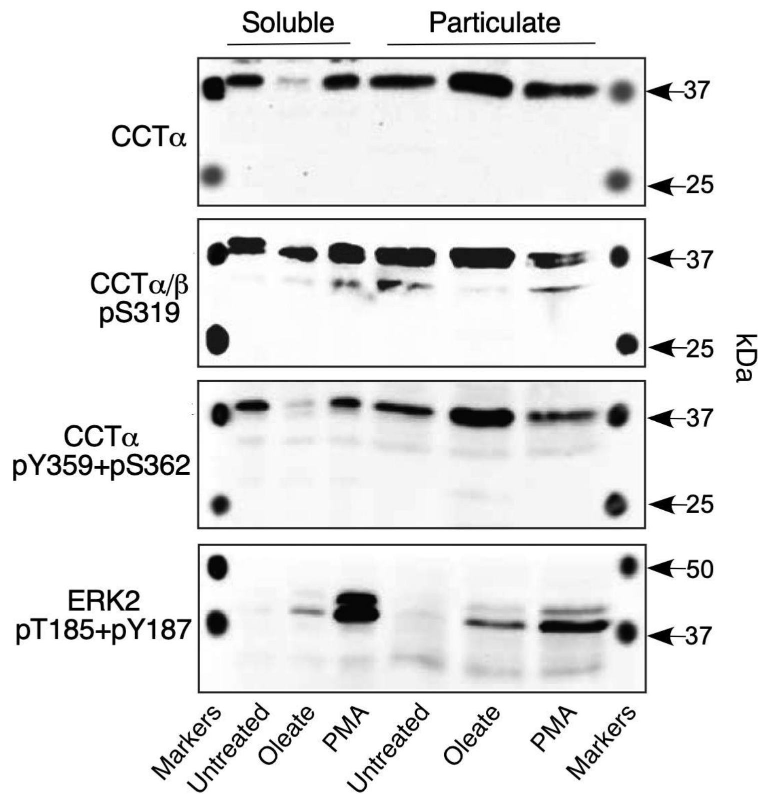


SUPPLEMENTAL FIGURE S2. Specificity of phosphosite-specific antibodies for human CCTα. (A) Lysates from Caco2 and Caco2 CCTα knockout cells (Caco2-KO) were immunoblotted with anti-CCTα/β-pS319, anti-CCTα-pY359+pS362 and anti-CCTα. (B) CaCo2 and CaCo2-KO cells were immunostained with anti-CCTα-pY359+pS362, and LDs and nuclei were visualized with BODIPY 493/503 and DAPI, respectively. Images are confocal sections (1 μm).



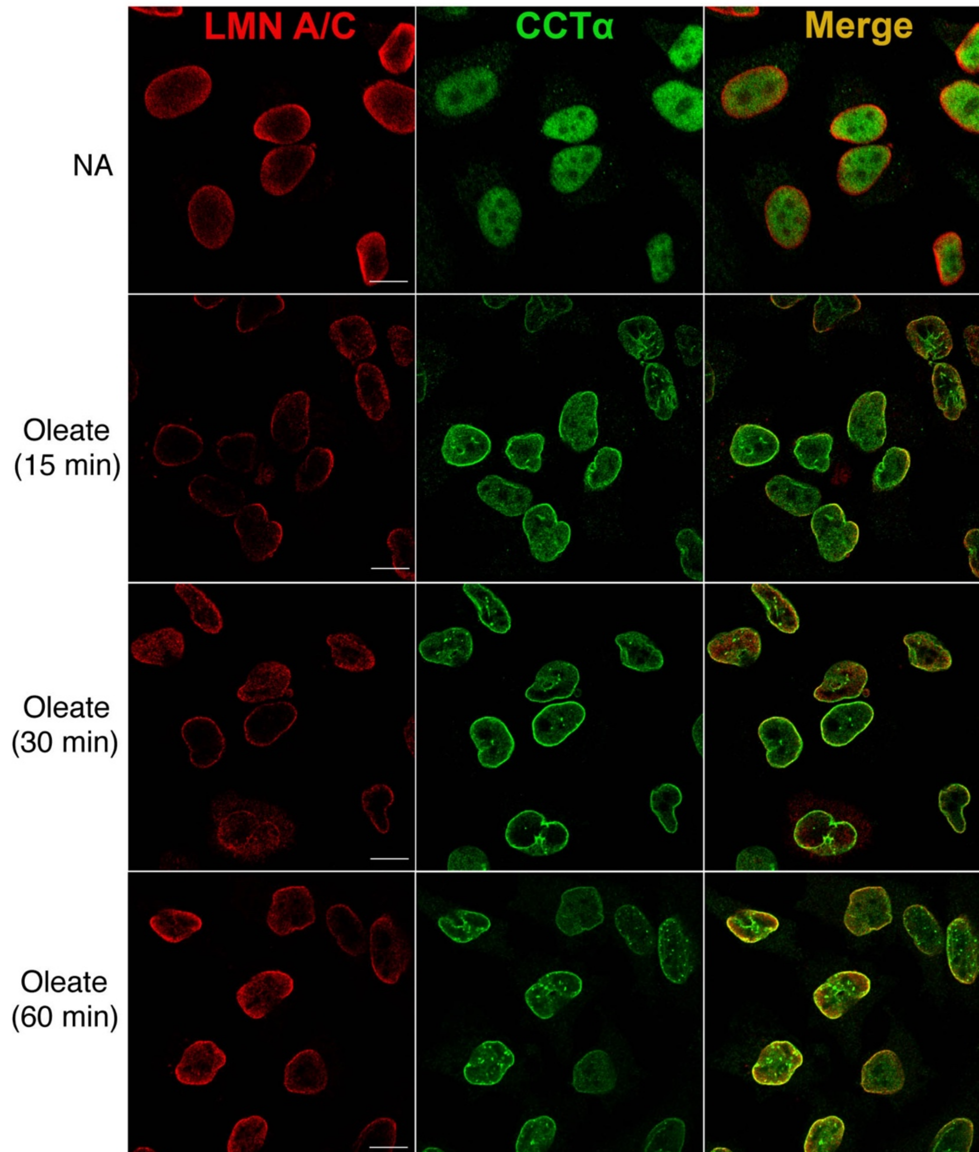
SUPPLEMENTAL FIGURE S3. Analysis of CCTα expression and phosphorylation in mouse tissues. Mouse tissue lysates (~40 μg per lane) were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with anti-CCTα/β-pS319, anti-CCTα-pY359+pS362 and anti-CCTα.

Yue et al. Supplemental Figure S4

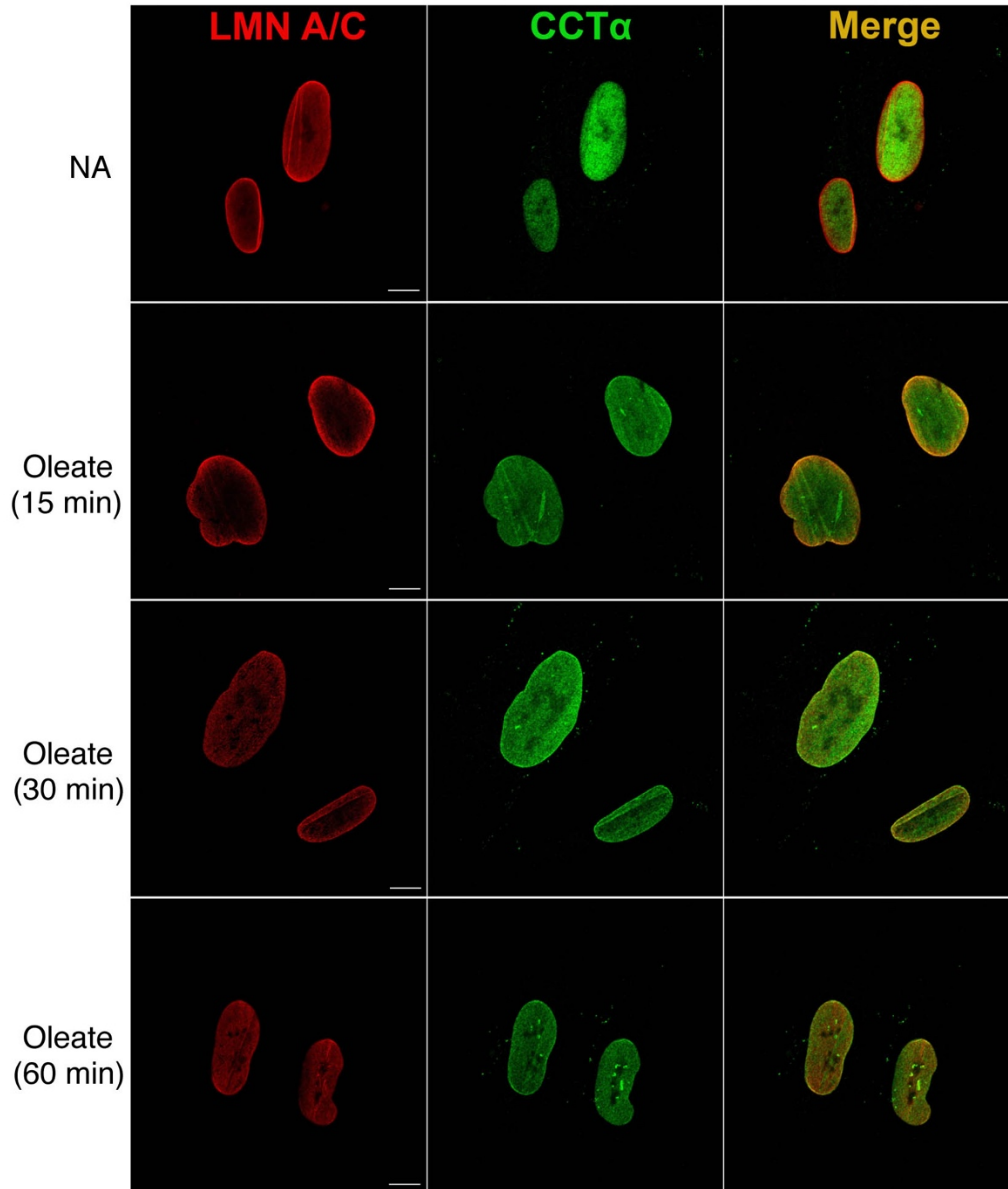


SUPPLEMENTAL FIGURE S4. Phosphorylation status and subcellular localization of CCTα in oleate- and phorbol ester-treated HeLa cells. Serum-maintained HeLa cells were untreated or treated with 1 mM sodium oleate for 20 minutes or 20 ng/ml PMA for 15 min. Cells were homogenized and ultracentrifuged at 90,000 x g for 30 min in detergent-free buffer. The supernatant (soluble fraction) was removed and the pellet was re-homogenized in buffer containing 1% Triton-X100 and subjected to ultracentrifugation to recover the detergent solubilized supernatant (particulate fraction). Fractions (25 µg protein per lane) were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with the following primary anti-CCTα/β-pS319, anti-CCTα-p-Y359+pS362; anti-CCTα P-domain pan-specific; and anti-ERK2-pT185 +pY187.

Yue *et al.* Supplemental Figure S5

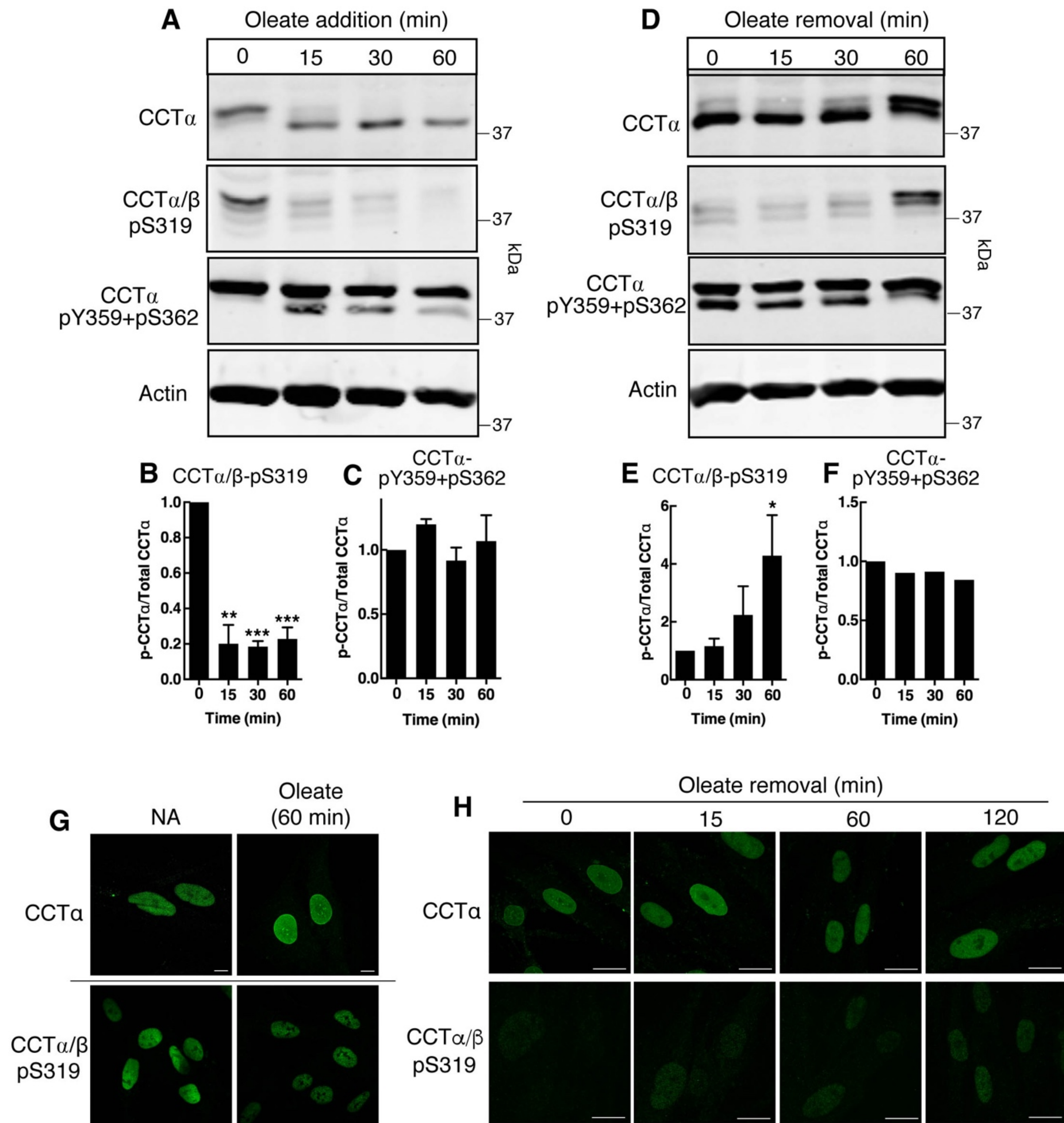


SUPPLEMENTAL FIGURE S5. Oleate treatment induces CCTα translocation from the nucleoplasm to the nuclear envelope in HeLa cells. Cells were cultured in serum-free DMEM and 500 μM oleate/BSA for 0 to 60 min prior to fixation, permeabilization and probing with primary antibodies for CCTα and LMNA/C, followed by AlexaFluor-488 and AlexaFluor-594 secondary antibodies. All images are 0.8 μm confocal sections. Bar, 10 μm.



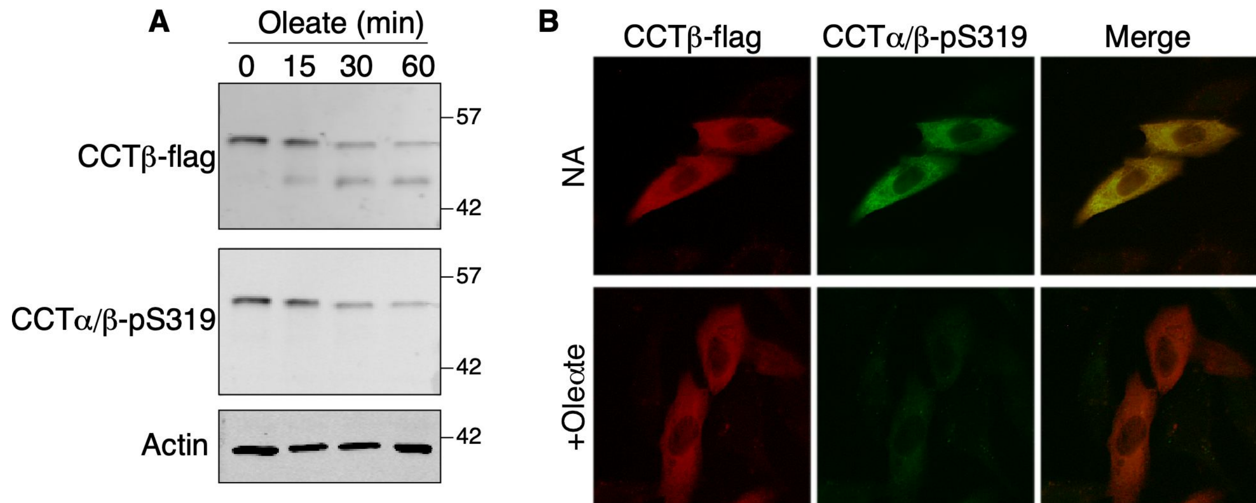
SUPPLEMENTAL FIGURE S6. Oleate treatment induces CCTα translocation from the nucleoplasm to the nuclear envelope in F8 human fibroblasts. Cells were cultured in serum-free DMEM and 300 μM oleate/BSA for 0 to 60 min prior to fixation, permeabilization and probing with primary antibodies for CCTα and LMNA/C and AlexaFluor-488 and AlexaFluor-594 secondary antibodies, respectively. All images are 0.8 μm confocal sections. Bar, 10 μm.

Yue *et al.* Supplemental Figure S7



SUPPLEMENTAL FIGURE S7. Oleate treatment of human skin fibroblasts induces reversible dephosphorylation of CCTα S319 but not Y359 or S362. (A-C) Total cell lysates prepared from F8 human fibroblasts cultured in serum-free DMEM containing 500 μM oleate/BSA were immunoblotted with CCTα/β-pS319, CCTα-pY359+pS362, CCTα or actin antibodies. Phosphorylation of S315 (B) and Y359+S362 (C) was quantified relative

to total CCTα protein. (D-F) Fibroblasts were cultured in serum-free DMEM with 300 μM oleate/BSA for 30 min before replacing with serum-free DMEM for the indicated times. Total cell lysates were immunoblotted as described in panel A, and phosphorylation of S315 (E) and Y359+S362 (F) was quantified relative to total CCTα protein. Results are the mean and SEM of 3 experiments. Statistical comparisons were made with 0 h controls. (G) Fibroblasts cultured in serum-free DMEM with 300 μM oleate/BSA for 30 min were immunostained with CCTα and CCTα/β-pS319 antibodies. (G) Fibroblasts were cultured in serum-free DMEM with 300 μM oleate/BSA for 30 min Oleate-containing media was then replaced with serum-free DMEM and cells were incubated for the indicated times before immunostaining with CCTα and CCTα/β-pS319 antibodies. Confocal images are shown (0.8 μm sections). Bar, 10 μm.



SUPPLEMENTAL FIGURE S8. Oleate-induced dephosphorylation of S319 in human CCTβ. (A) CHO-MT58 cells transiently expressing human CCTβ-Flag were treated with 300 μM oleate for up to 60 min. Total cell lysates were immunoblotted with Flag monoclonal, CCTa/b-pS319 and actin. (B) Cells expressing CCTβ-Flag and treated with oleate (300 μM) for 60 min were immunostained with anti-Flag and anti-CCTα/β-pS319, followed by AlexaFluor-594 and -488-conjugated secondary antibodies, respectively.

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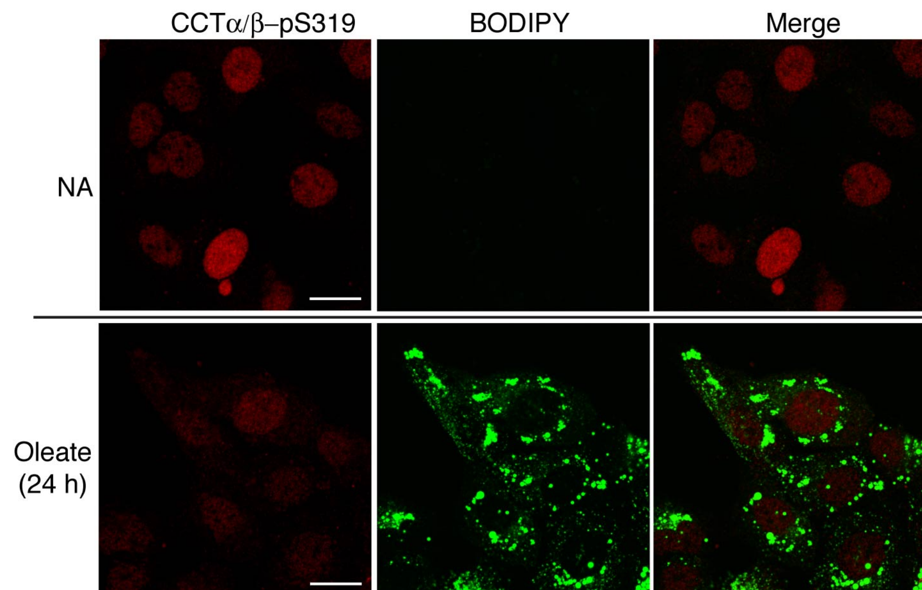

SUPPLEMENTAL FIGURE S9. U2OS cells treated with oleate have reduced nuclear CCTα S319 phosphorylation. U2OS cells were cultured in medium with no additions (NA) or in the presence of 300 μM oleate/BSA for 24 h, fixed and immunostained with primary CCTα/β-S319 and secondary Alexafluor-594 antibodies. Nuclear lipid droplets were visualised with BODIPY493/503. Confocal images are shown (0.8 μm sections). Bar,20 μm.