Supplemental movies of CPE secretory vesicles in live cells in different conditions.

Supplemental movie #1 (Fig3video1): Live cell images of *in vivo* movement of CPE-GFP secretory vesicles in AtT20 cells expressing RFP alone. Each image was taken at a time interval of 1.97 sec. Movie speed is 8 frames/sec.
Supplemental movie #2 (Fig3video2): Live cell images of *in vivo* movement of CPE-GFP secretory vesicles in AtT20 cells expressing RFP-CPE_{C10}. Each image was taken at a time interval of 1.97 sec. Movie speed is 8 frames/sec.
Supplemental movie #3 (Fig4video1): Live cell images of *in vivo* movement of CPE-RFP secretory vesicles in primary anterior pituitary cells expressing GFP alone. Each image was taken at a time interval of 0.98 sec. Movie speed is 8 frames/sec.
Supplemental movie #4 (Fig4video2): Live cell images of *in vivo* movement of CPE-RFP secretory vesicles in primary anterior pituitary cells expressing GFP-CPE_{C25}. Each image was taken at a time interval of 0.98 sec. Movie speed is 8 frames/sec.

Fig3videos; AtT20 cells

Fig3video1

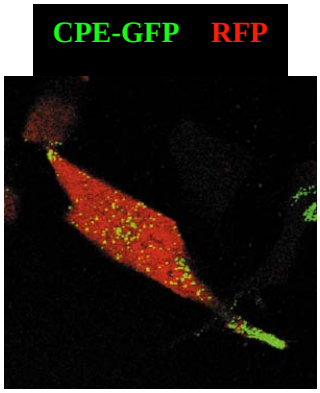


Fig3video2

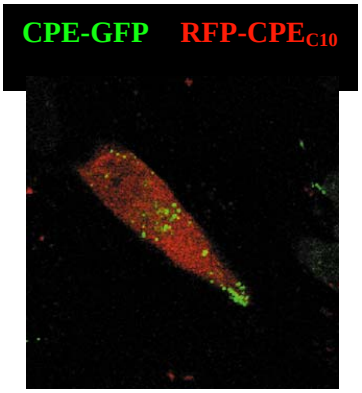


Fig4videos; Primary cells from anterior pituitary gland

Fig4video1

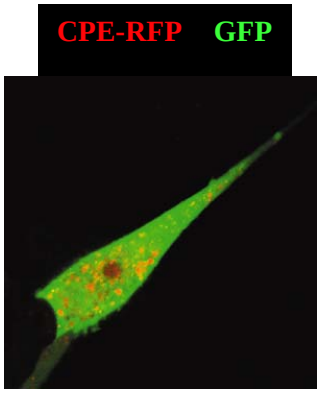
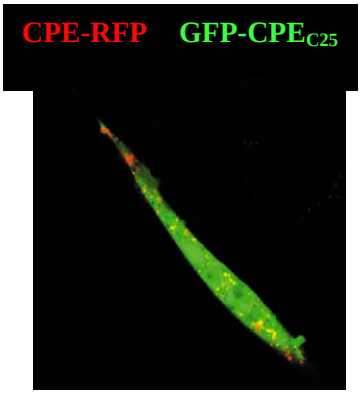
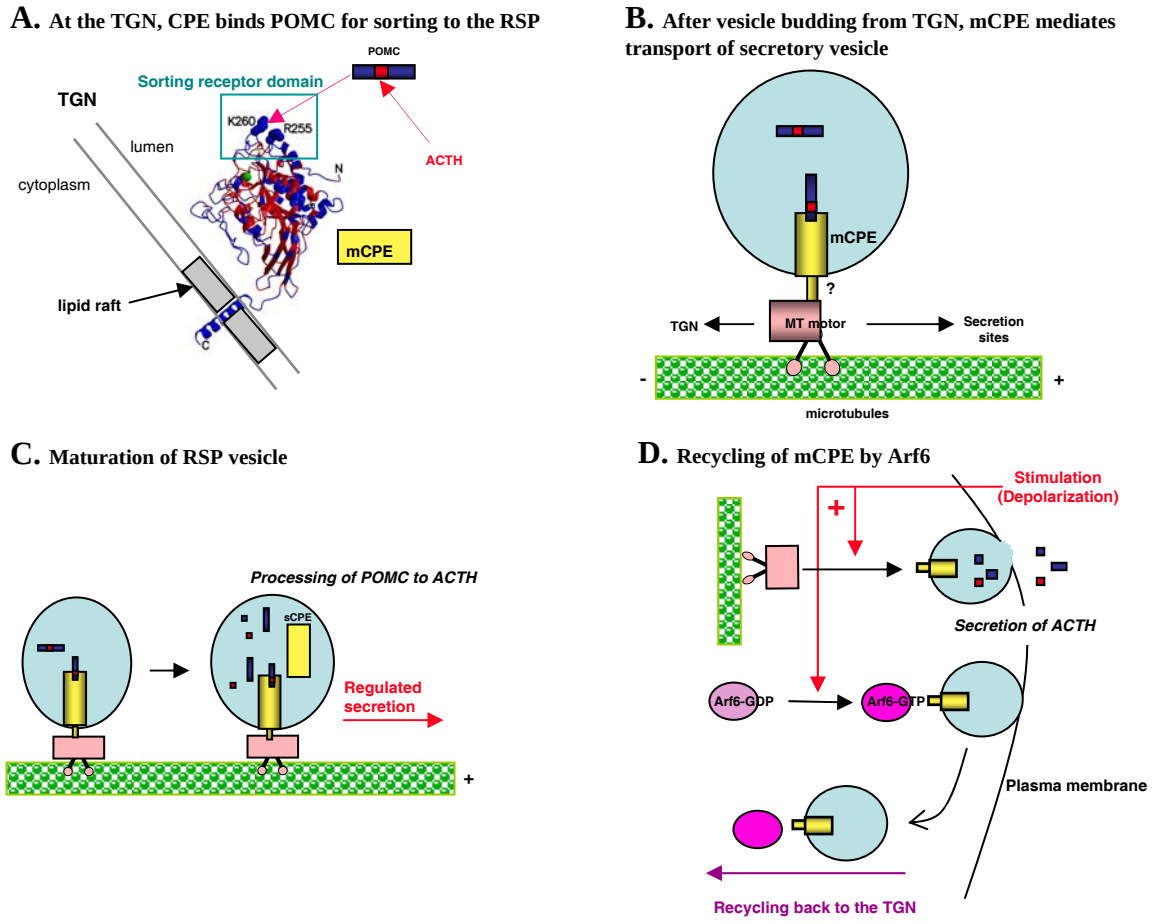


Fig4video2



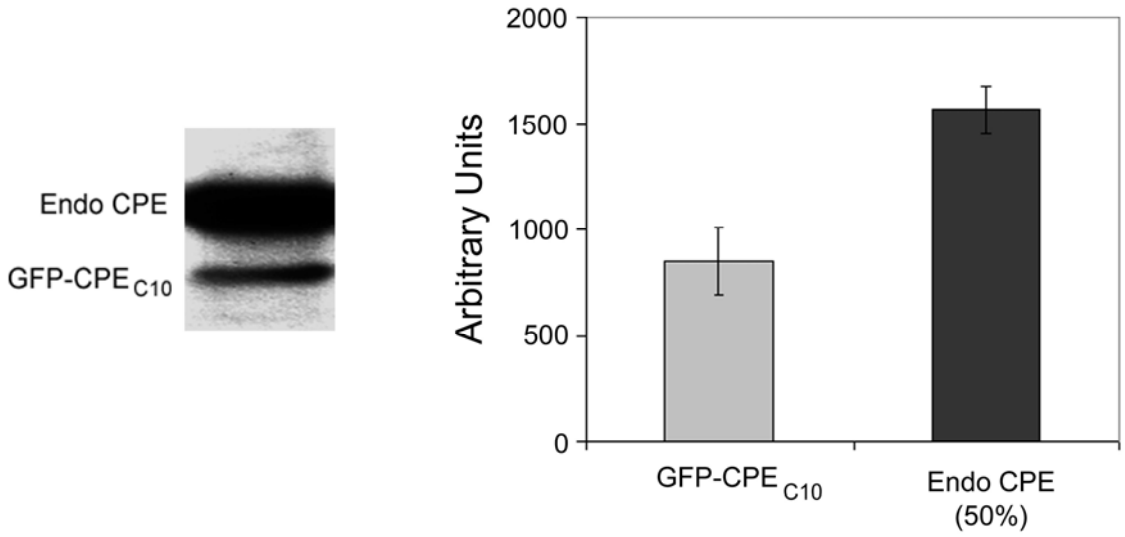
SUPPLEMENTAL DATA

Figure S1. Sequential function of membrane CPE (mCPE) in the regulated secretory pathway (RSP).



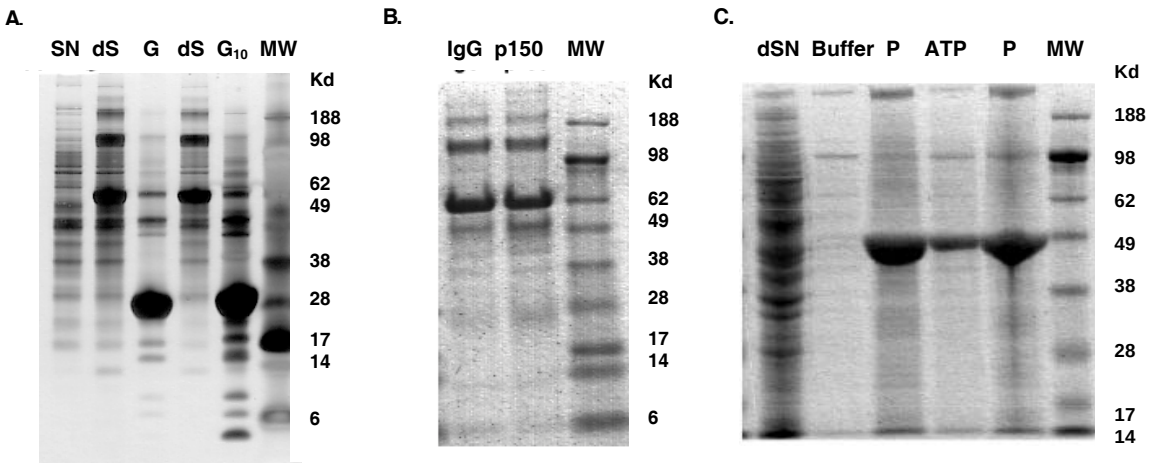
A. POMC binds to the basic residues on the luminal (receptor) domain of lipid-raft-associated membrane carboxypeptidase E (mCPE) which sorts it to the regulated secretory pathway (RSP) by vesicle budding at the Trans Golgi Network (TGN). POMC that does not bind mCPE enters by default into vesicles that bud off the TGN and are secreted constitutively. B. Subsequently, we propose that the cytoplasmic tail of mCPE recruits microtubule-based motors that, in turn, transport the RSP vesicles to the sites for activity-dependent secretion. C. Inside of maturing RSP vesicles, soluble CPE (sCPE), in which the membrane-associated C-terminus is removed by processing enzymes, mediates processing of POMC to ACTH and other derivatives. D. RSP vesicles at the secretion sites release its content, including ACTH, upon stimulation. Arf6 (GTP-bound form), then, binds to the cytoplasmic tail of mCPE and initiates recycling of mCPE from the plasma membrane to the TGN.

Figure S2. Comparison of levels of endogenous CPE and expressed exogenous CPE_{C10}.



Left panel: Immunoblot of GFP-CPE_{C10} and endogenous CPE (endo CPE) in the cell extracts from AtT20 cells transfected with GFP-CPE_{C10}. Anti-CPE tail antibody was used to detect GFP-CPE_{C10} and endogenous CPE. Right panel: Bar graphs show mean band intensities of exogenous CPE_{C10} and 50% of the endogenous CPE (since transfection efficiency for GFP-CPE_{C10} was approximately 50%) (n=3, mean±SEM).

Figure S3. Coomassie staining of proteins in co-immunoprecipitation and MT copelleting assays.

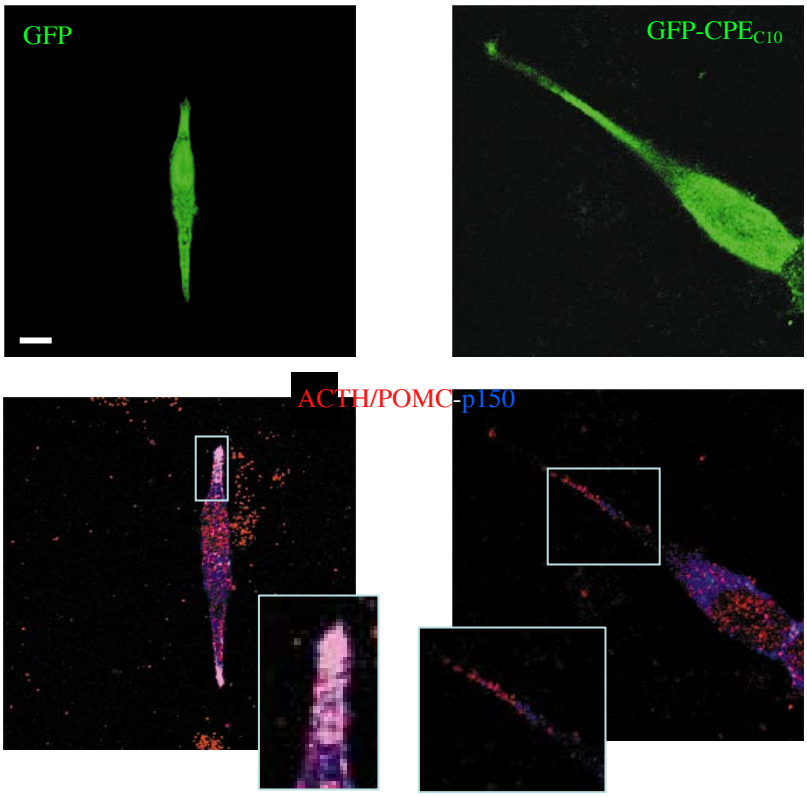


A. Silver staining of SDS-PAGE gel containing proteins co-precipitated by GST tag alone (G), GST tagged CPE_{C10} (G_{10}). Bacterially expressed GST-tagged proteins were added to cytosol from AtT20 cells. (SN: starting supernatant, dS: supernatant depleted by GST of GST-CPE_{C10}).

B. Coomassie staining of SDS-PAGE gel containing proteins co-immunoprecipitated with dynactin (p150). Anti-p150 antibody or IgG was added to cytosol from AtT20 cells for co-immunoprecipitation. (IP: immunoprecipitation, IgG: rabbit IgG, p150: anti-p150 antibody). The 3 bands at ~62, 120 and >188kD are BSA monomer, dimer and trimer, and the 50 and 25 kD bands are the IgGs.

C. Coomassie staining of SDS-PAGE gel containing proteins bound to microtubules and released by buffer alone or by ATP-containing buffer. Microtubule co-pelleting assay was performed to examine the microtubule-binding nature of CPE, motor proteins and their associated proteins in AtT20 cell cytosol. Proteins in each fraction were detected by immunoblotting. CPE was released from the microtubule pellet with buffer wash or ATP wash. (Buffer: wash with buffer alone, P: washed microtubule pellet, ATP: wash with buffer containing ATP). The tubulin band at 49 kD indicative of microtubule depolymerization with the ATP wash is relatively low compared to that in the pellet.

Figure S4. Colocalization between dynactin (p150) and ACTH in AtT20 cells expressing GFP or GFP-CPE$_{C10}$.



AtT20 cells transfected with GFP or GFP-CPE$_{C10}$ were stained with anti-p150 (dynactin) antibody (Cy3:red) and anti-ACTH antibody (Cy5: blue). Colocalization (pink) correlation R for dynactin and ACTH vesicles was measured in cells expressing GFP or GFP-CPE$_{C10}$. (scale bar = 5μm)