Figure S6. Purification of the ATP8A1-CDC50A complex from HEK293T cells.
Wild-type (WT) ATP8A1 and the E191Q mutant each containing a 9 amino acid 1D4 tag were co-expressed with CDC50A in HEK293T cells. The CHAPS-solubilized cell lysate was applied to a Rho1D4-Sepharose matrix. After extensively washing the matrix to remove unbound protein, the ATP8A1-CDC50A complex was eluted with the 1D4 peptide for the experiments in Fig 2D, 4A and 4B. The cell lysate (input) and peptide eluted fraction (elution) were analyzed on SDS gels stained with Coomassie blue or by Western blots with anti-Rho1D4 antibody (ATP8A1) and anti-CDC50A antibody (CDC50A). Both the WT and E191Q mutant were expressed and co-purified with CDC50A at similar levels. Please be noted that CDC50A is not readily detected by Coomassie Blue stain because of its highly glycosylated extracellular domain (Coleman & Molday, 2011).