



Figure S7 Characterization of mitochondria binding by Beclin 1 ECD. **(A)** The WT Beclin 1 ECD, but not the finger mutant (F359D/F360D/W361D), was localized to mitochondria. NRK cells were transfected with YFP tagged Beclin 1 ECD and ECD-FFW/DDD. 24 hours after transfection, cells were stained with Mito-tracker red and observed by confocal microscopy. Scale bar, 5 μ m. **(B)** The WT Beclin 1 ECD, but not the mutant, predominantly bound to mitochondria. NRK cells were

fractionated into mitochondrial and clear cytosolic fractions. These fractions, together with total cell lysate, were subjected to Western blot analysis. T: total cells; M: mitochondria; C: cytosol. (C) Beclin 1 ECD is associated with the outer membrane of mitochondria. Isolated mitochondria were incubated with proteinase K in the absence or presence of the solubilizing detergent Triton X-100. The YFP-tagged Beclin 1 ECD was completely degraded in both cases, whereas TIM23 (located on the inner membrane) and AIF (located in the inter-membrane space of mitochondria) were only degraded by proteinase K in the presence of Triton X-100.