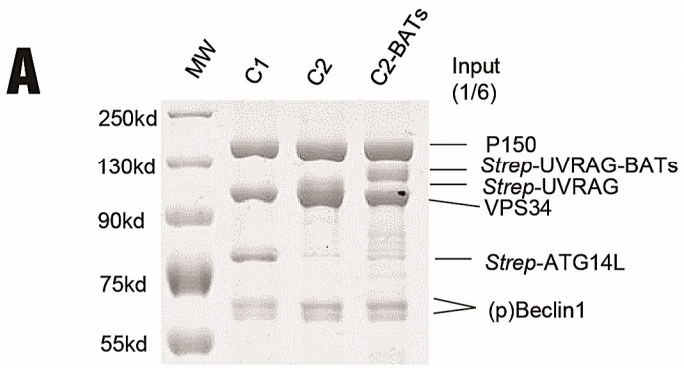
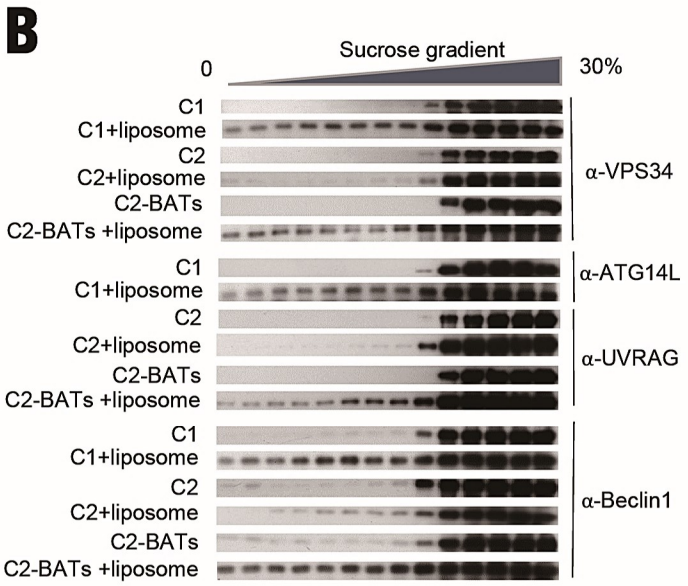
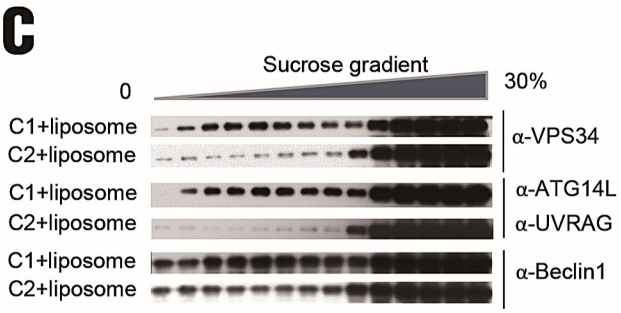
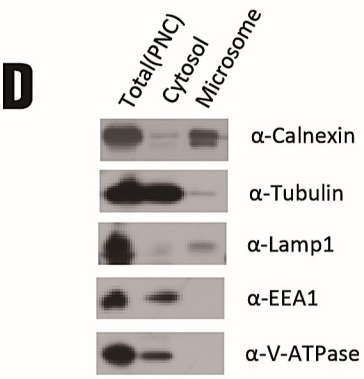
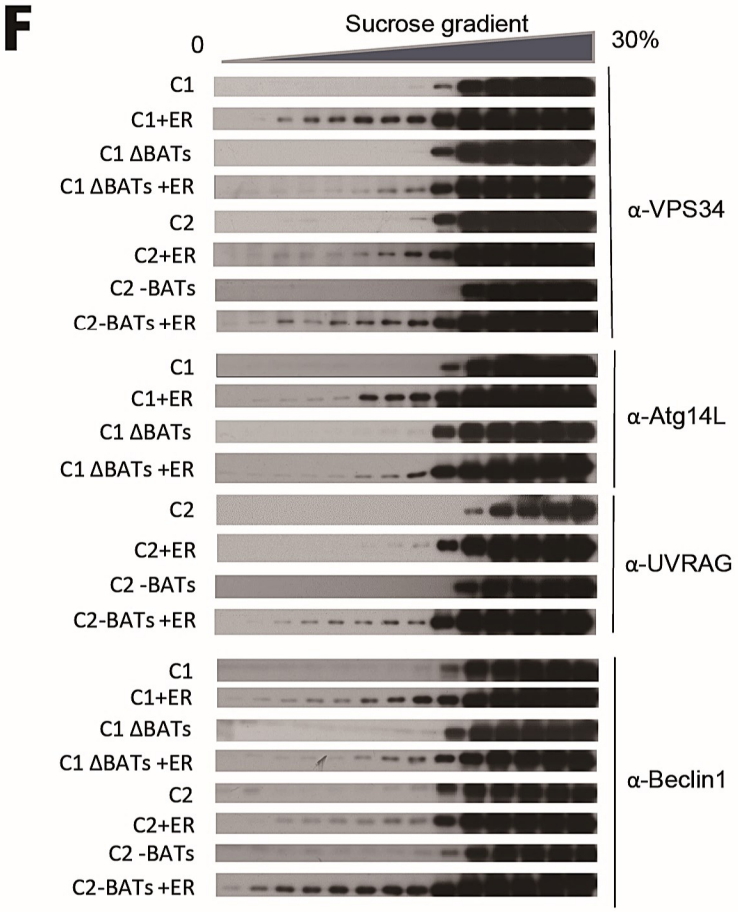

A
MW
C1
C2
C2-BATs
Input
(1/6)
250kd
130kd
90kd
75kd
55kd
P150
Strep-UVRAG-BATs
Strep-UVRAG
VPS34
Strep-ATG14L
(p)Beclin1


B
Sucrose gradient
0
30%
C1
C1+liposome
C2
C2+liposome
C2-BATs
C2-BATs +liposome
α-VPS34
α-ATG14L
α-UVRAG
α-Beclin1


C
Sucrose gradient
0
30%
C1+liposome
C2+liposome
α-VPS34
α-ATG14L
α-UVRAG
α-Beclin1


D
Total(PNC)
Cytosol
Microsome
α-Calnexin
α-Tubulin
α-Lamp1
α-EEA1
α-V-ATPase


F
Sucrose gradient
0
30%
C1
C1+ER
C1 ΔBATs
C1 ΔBATs +ER
C2
C2+ER
C2 -BATs
C2-BATs +ER
α-VPS34
α-Atg14L
α-UVRAG
α-Beclin1

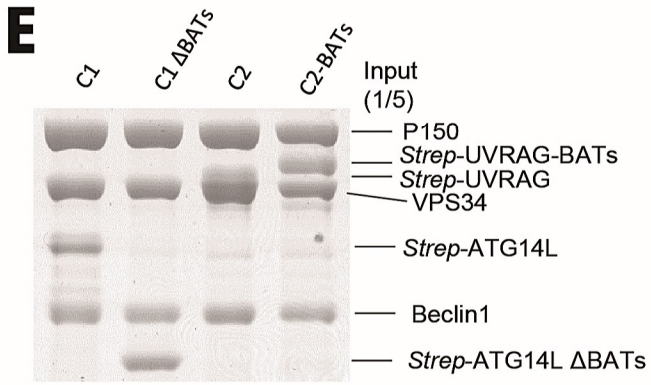

E
C1
C1 ΔBATs
C2
C2-BATs
Input
(1/5)
P150
Strep-UVRAG-BATs
Strep-UVRAG
VPS34
Strep-ATG14L
Beclin1
Strep-ATG14L ΔBATs

Figure S5 The BATs domain determines the ER-binding capacity of PI3KC3 complexes *in vitro*. (A) 1/6 of the protein input for the flotation assays shown in **B** and **C**. **(B)** Flotation assay to examine the interaction of C1, C2 and C2-BATs with 6% PI-containing liposomes in a sucrose gradient (from top to bottom: 0, 20%, 25%, 30%). All 14 fractions from top to bottom were analyzed by immunoblotting using antibodies against VPS34, ATG14L, UVRAG and Beclin1. **(C)** Flotation assay to examine the interaction of C1 and C2 with 6% PI3P-containing liposomes in a sucrose gradient (from top to bottom: 0, 20%, 25%, 30%). All 14 fractions from top to bottom were analyzed using antibodies against VPS34, ATG14L, UVRAG and Beclin1. **(D)** Subcellular fractionation was performed on 293F cells to obtain the post-nuclear supernatant fraction (Total(PNC)), the cytosolic fraction (Cytosol) and the ER fraction (Microsome). Equivalent amounts of each fraction were subjected to immunoblotting with antibodies against specific organelle markers. **(E)** 1/5 of the input protein for the flotation assay in **F**. **(F)** Flotation assay to examine the interaction of C1, C1 ΔBATs, C2 and C2-BATs with the ER fraction in a sucrose gradient (from top to bottom: 0, 20%, 25%, 30%). All 14 fractions from top to bottom were analyzed using antibodies against VPS34, ATG14L, UVRAG and Beclin1.