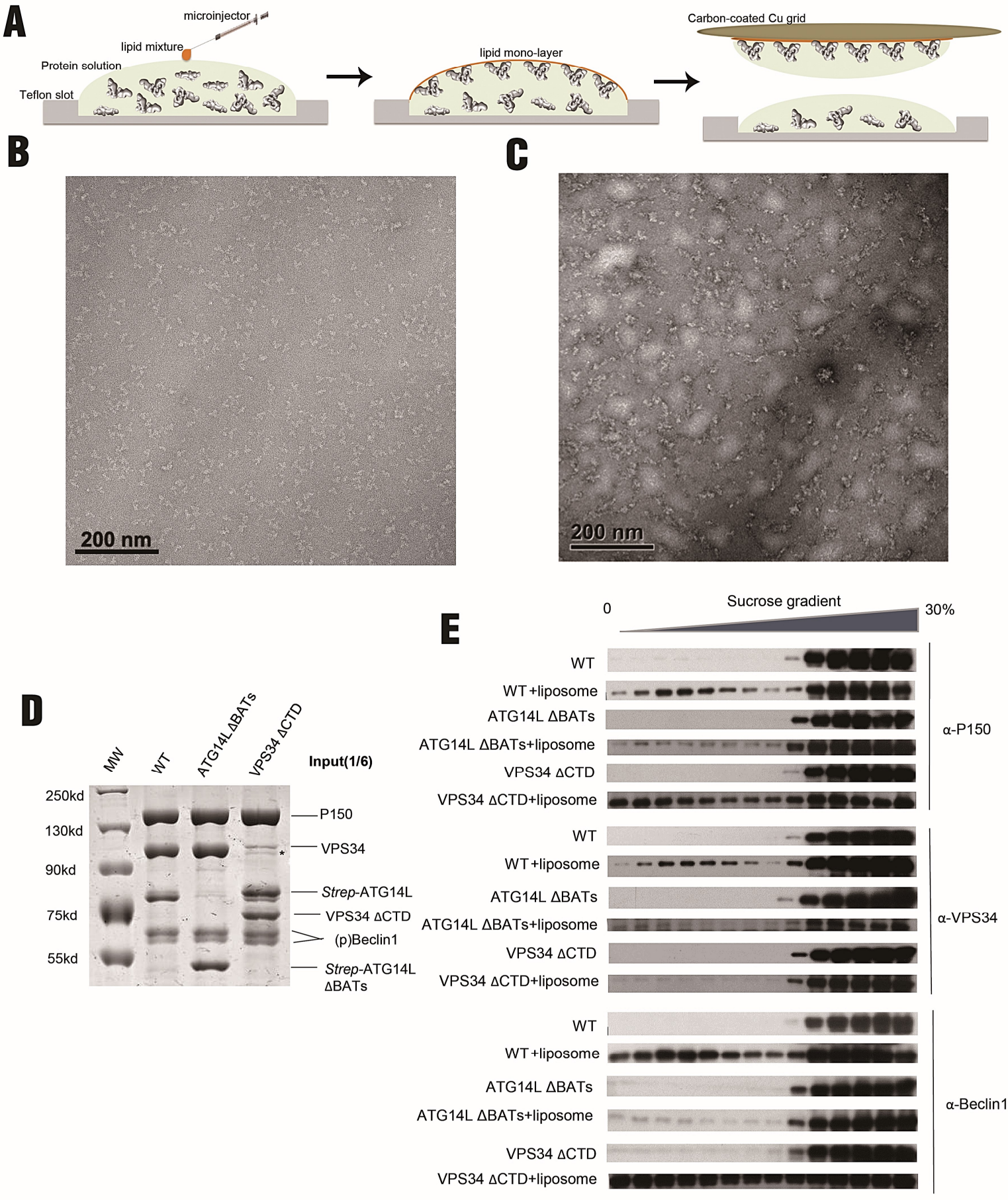
A
microinjector
lipid mixture
Protein solution
Teflon slot
lipid mono-layer
Carbon-coated Cu grid
B
200 nm
C
200 nm
D
MW
WT
ATG14L ΔBATs
VPS34 ΔCTD
Input(1/6)
250kd
130kd
90kd
75kd
55kd
P150
VPS34
Strep-ATG14L
VPS34 ΔCTD
(p)Beclin1
Strep-ATG14L ΔBATs
E
0
Sucrose gradient
30%
WT
WT+liposome
ATG14L ΔBATs
ATG14L ΔBATs+liposome
VPS34 ΔCTD
VPS34 ΔCTD+liposome
α-P150
α-VPS34
α-Beclin1

Figure S4 Membrane-binding orientation of C1. (A) Work-flow for EM sample preparation of C1 on membranes. The necessary materials are labeled in the left panel. Cartoons of the Teflon slot, protein solution and lipid solution are colored in grey, light green and orange, respectively. The protein complexes are shown as EM models embedded in the solution. The micro-injector is also presented at the top. The blue ellipse in the right panel represents the Cu grid with a supported carbon layer on the surface, which interacts with the lipid monolayer. **(B)** The classical image of C1 on the carbon surface prepared by negative staining. **(C)** The classical image of C1 bound to lipid monolayers prepared by the procedure shown in **A**. **(D)** 1/6 of the protein input for the flotation assay in **E**. **(E)** Flotation assay to examine the interaction of C1 WT, C1 VPS34 ΔCTD and C1 ATG14L ΔCTD with liposomes containing 6% PI in a sucrose gradient (from top to bottom: 0, 20%, 25%, 30%). All 14 fractions from top to bottom were immunoblotted using antibodies against P150, VPS34 and Beclin1.