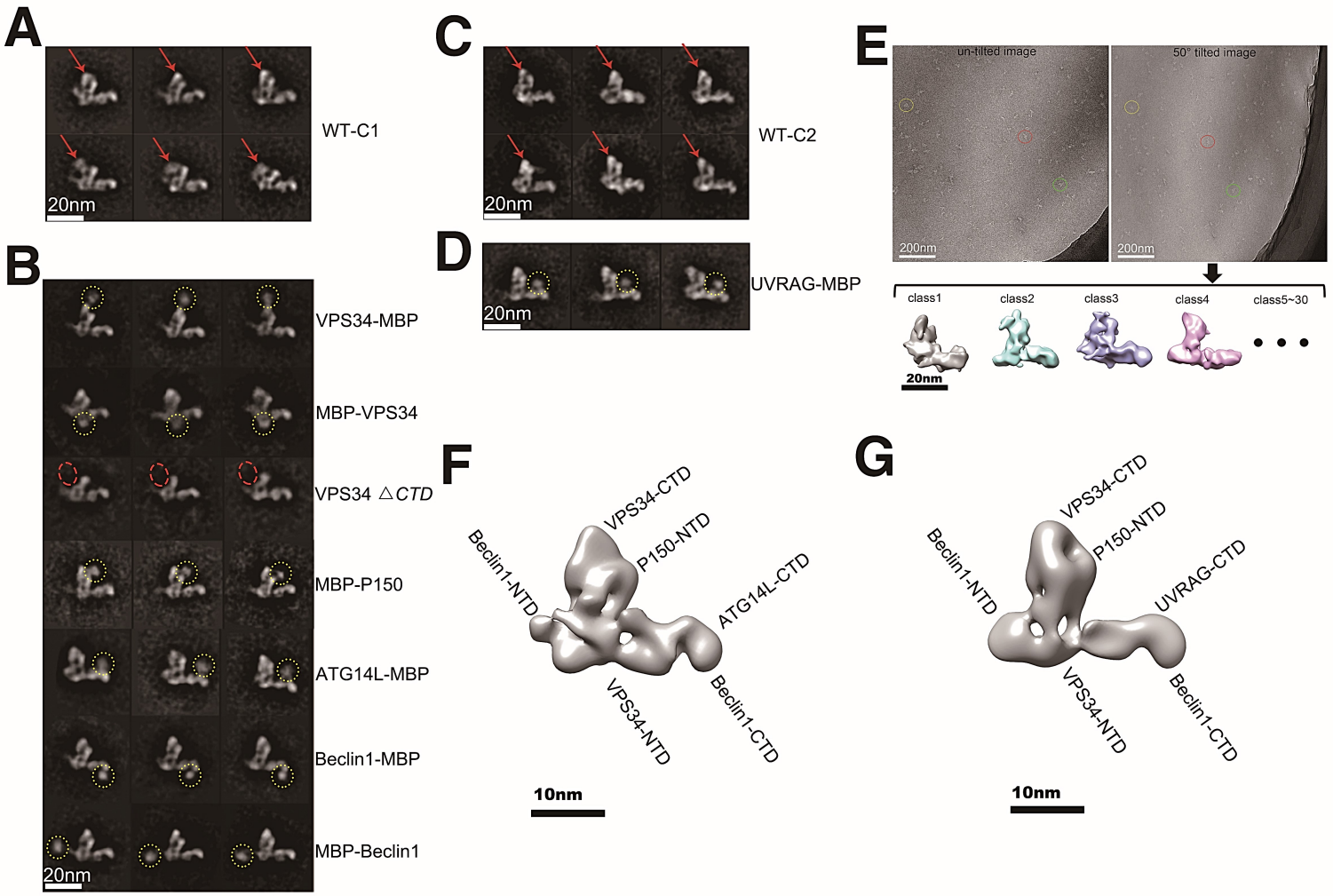
A
WT-C1
20nm
B
VPS34-MBP
MBP-VPS34
VPS34 △CTD
MBP-P150
ATG14L-MBP
Beclin1-MBP
MBP-Beclin1
20nm
C
WT-C2
20nm
D
UVRAG-MBP
20nm
E
un-tilted image
50° tilted image
200nm
200nm
class1
class2
class3
class4
class5~30
20nm
F
VPS34-CTD
P150-NTD
Beclin1-NTD
ATG14L-CTD
VPS34-NTD
Beclin1-CTD
10nm
G
VPS34-CTD
P150-NTD
Beclin1-NTD
UVRAG-CTD
VPS34-NTD
Beclin1-CTD
10nm

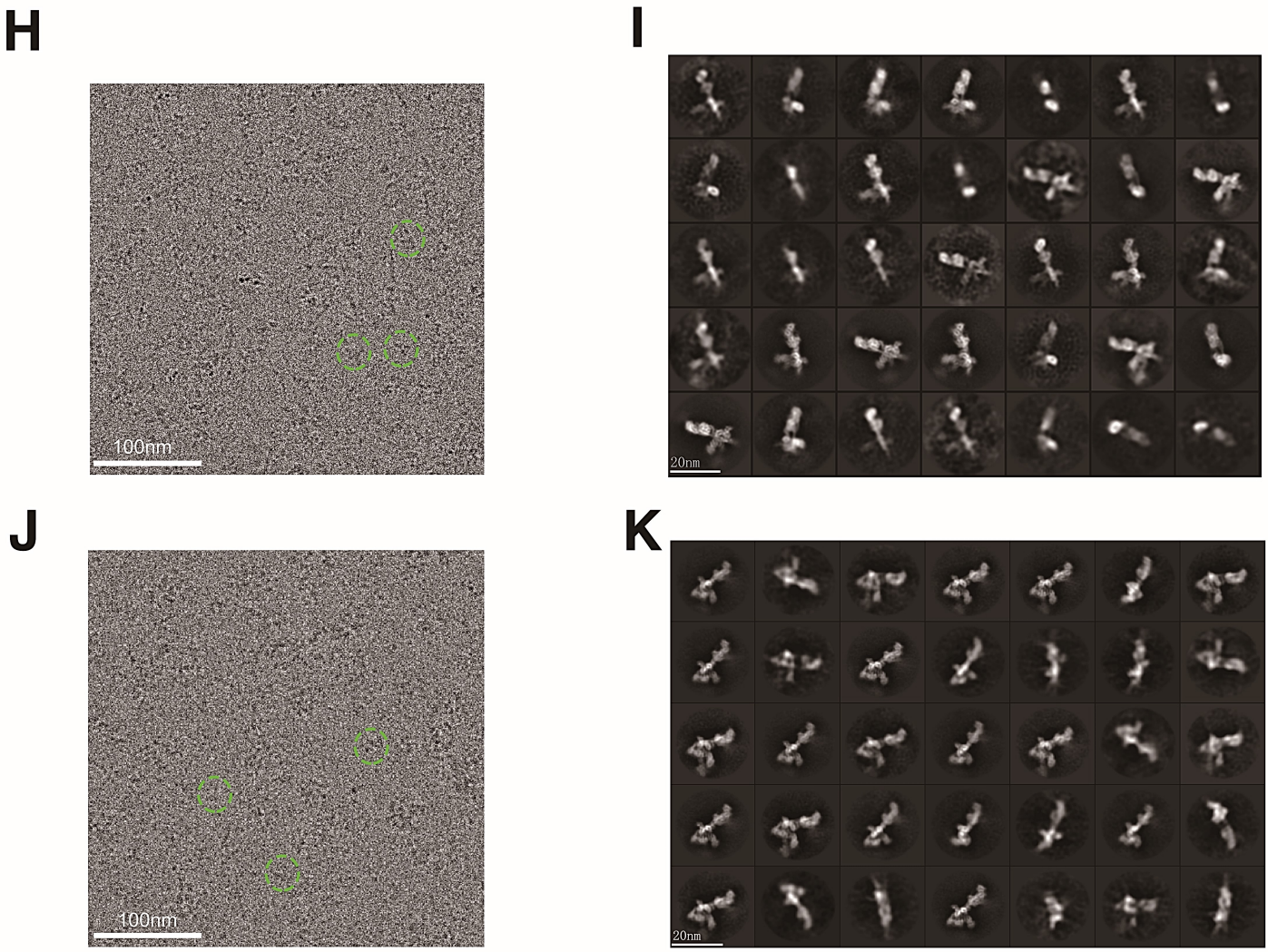
H
100nm
I
20nm
J
100nm
K
20nm

Figure S1 Architectural definition of C1 and C2. (A) 2D class averages of negative-stain EM images of wild-type C1. Six classical views are presented with red arrows indicating the C-terminal domain of VPS34. **(B)** 2D class averages of C1 with different MBP-tailed or deleted subunits. The modified subunits are labeled at the right. For each modification, three 2D side views are presented. In VPS34-MBP, ATG14L-MBP and Beclin1-MBP, the MBP peptide was fused to the C-terminus of the subunit. In MBP-VPS34, MBP-P150 and MBP-Beclin1, the MBP peptide was added to the N-terminus of the subunit. In the VPS34 ΔCTD sample, the C-terminal domain of VPS34 was deleted. The yellow dashed rings mark the MBP densities, and the red ones indicate the location of the deleted VPS34 C-terminal domain. **(C)** 2D class averages of wild-type C2 by negative staining. Six classical views are presented with red arrows indicating the C-terminal domain of VPS34. **(D)** 2D-class averages of C1 with MBP-tailing on the C-terminus of the UVRAG protein. The yellow dashed rings mark the MBP densities. The scale bar in the 2D images is 20 nm. **(E)** Initial models generated by the random conical tilt method. 60 tilted/un-tilted pairs were collected with 50-degree tilt, and about 30 similar models were reconstructed from the tilted particles. Four representative models are shown. **(F)** The architecture of C1. The reconstructed map was segmented and colored in pink, cyan and green. The locations of the N-terminal and C-terminal domains (NTD and CTD) of each subunit, which were defined by our results, are marked on the volume. **(G)** 3D refinement models of C2 by negative staining. Different domains are marked based on MBP-tailing analysis and the structural similarity with C1. **(H)** Cryo-EM image of C1 collected by a Titan Krios cryo-electron microscope fitted with a K2 camera. The green dashed rings mark the C1 particles. **(I)** 2D analysis of C1 by cryo-EM. The class averages were

calculated based on cryo-EM images of particles collected as in **H**. **(J)** Cryo-EM image of C2 collected by a Titan Krios microscope with a K2 camera. The green dashed rings mark the C2 particles. The scale bar in the original image is 100 nm. **(K)** 2D analysis of C2 by cryo-EM. The class averages were calculated based on cryo-EM images of particles collected as in **J**.