Supplementary Materials

Figure S1: Depletion of VAPs has no effect on endosome and lysosome morphology and microtubule organization. The effect of VAP-knockdown on endosomes (A) lysosomes (B,C), and microtubule organization (D) was determined by confocal microscopy analysis of control and VAP-depleted HeLa grown in the absence or presence of 25OH, as indicated. As shown, VAP-knockdown had no effect on the number, distribution, or morphology of the endosomes, as determined by immunostaining with anti-EEA1 antibody (A), of the lysosomes, as determined by Lysotracker staining (B) or Lamp-1 localization (C), and on microtubule organization, as determined by immunostaining with anti-β-tubulin antibody (D). Bar, 10 μm.

Figure S2: Redistribution of VAP proteins to the perinuclear region in response to 25OH treatment. HeLa cells were treated with 25OH for 12 h as indicated, fixed, and double-immunostained with either anti-VAP (red) and anti-p115 (green) antibodies (A), anti-PDI (red) and anti-p115 (green) antibodies (B), or with anti-Calnexin (red) and anti-GRASP65 (green) antibodies (C). Shown are representative confocal images demonstrating the partial co-localization of VAPs with p115 in 25OH-treated cells. Bar, 10 μm.

Figure S3: Depletion of VAPs reduces the DAG level in Golgi as indicated by GFP-PKD targeting. Control and VAP RNAi cells were transiently transfected with a mammalian expression vector encoding PKD fused to GFP and 8 h later were treated with 25OH for 12 h as indicated. The cells were fixed, immunostained with anti-p115 antibody (red) and analyzed by confocal microscopy. Bar, 10 μm.

Figure S4: The level of PI4P in the Golgi of Nir2-depleted cells is markedly reduced in response to 25OH treatment. Control and Nir2 RNAi cells were transfected with GFP-PH-OSBP. The cells were treated with 25OH as indicated, fixed and analyzed by confocal microscopy. The distribution of GFP-PH-OSBP in control and Nir2-knockdown cells is shown. Bar, 10 μm.

Figure S5: The PI-transfer domain of Nir2 failed to restore the targeting of CERT and OSBP to the Golgi of 25OH-treated VAP-depleted cells. HeLa cells were transiently transfected with either scrambled (control) or VAPs siRNA duplexes. Three days later, the cells were transfected with mammalian expression vectors encoding either the wild-type Nir2-HA, or a Myc-tagged Nir2 PI-transfer domain (PI-TD) (aa 1-277). The cells were treated with 25OH for

12 h, fixed, and double-immunostained with either anti-HA or anti-Myc antibodies (green) and antibodies against CERT (red) or OSBP (red). Shown are representative confocal images of VAPs RNAi and control cells. Bar, 10 μm.

Figure S6: ET-18-OCH_3 affects the DAG level in the Golgi of Nir2-depleted cells.

Control or Nir2-RNAi treated HeLa cells were transiently transfected with the GFP-PKCη-C1b reporter, and eight hours later were treated with 25OH for 12 hr as indicated, fixed and co-immunostained with anti-Nir2 and anti-p115 antibodies. Where indicated, the cells were treated with ET-18-OCH_3 (10 μM) for two hours before fixation. Shown are representative confocal images demonstrating the distribution of GFP-PKCη-C1b and p115 (red) under the described growth conditions. Bar, 10 μm.

Movie M1: Export of YFP-VSV-G from the TGN of control HeLa cells. HeLa cells were transfected with YFP-VSV-G (tsO45) and incubated overnight at 40°C. The cells were shifted to 20°C for 2.5 h to accumulate VSV-G in the TGN. Export of YFP-VSV-G from the TGN was monitored in living cells following shifting the temperature to 32°C by confocal microscope equipped with a heat stage. 25OH was present in the last 12 h of the experiment. The movie was taken at 2 sec/frame and shown at 25 frames/sec.

Movie M2: Export of YFP-VSV-G from the TGN of VAP-depleted HeLa cells. VAP RNAi HeLa cells were transfected with YFP-VSV-G (tsO45) and incubated at 40°C overnight. The cells were shifted to 20°C for 2.5 h to accumulate VSV-G in the TGN, and VSV-G export was monitored as described above for the control movie. The movie was taken at 2 sec/frame and shown at 25 frames/sec. Note the tubular YFP-VSV-G-labeled structures that emanate from the TGN of these cells.

Figure S1

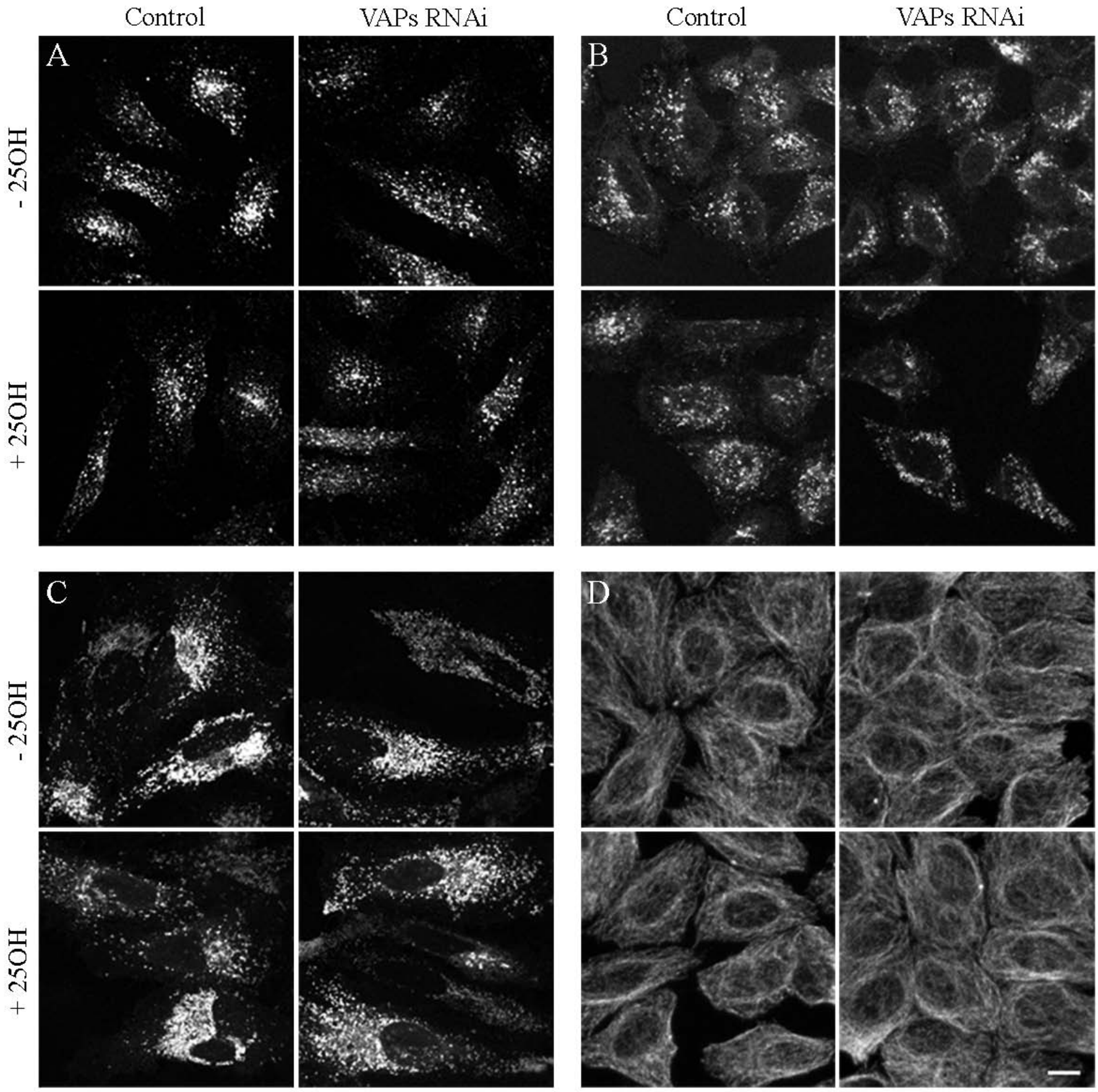

Control
VAPs RNAi
Control
VAPs RNAi
A
B
C
D
- 25OH
+ 25OH
- 25OH
+ 25OH

Figure S2

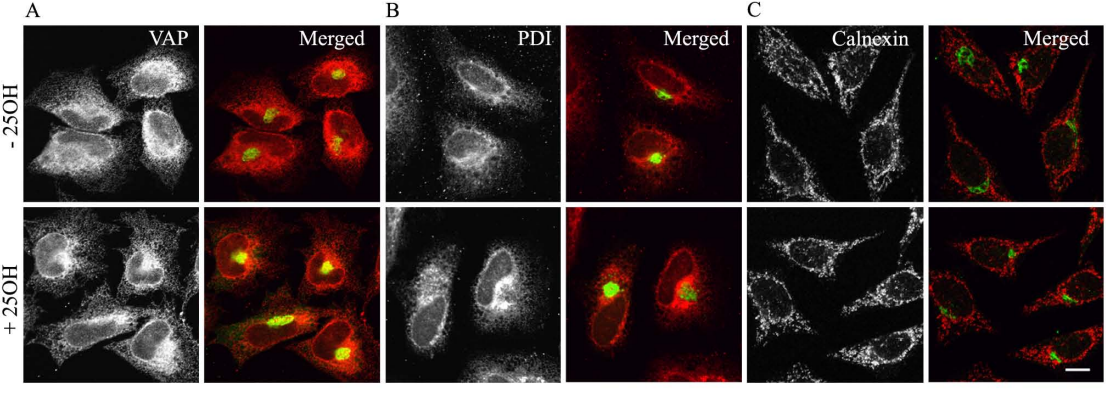


Figure S3

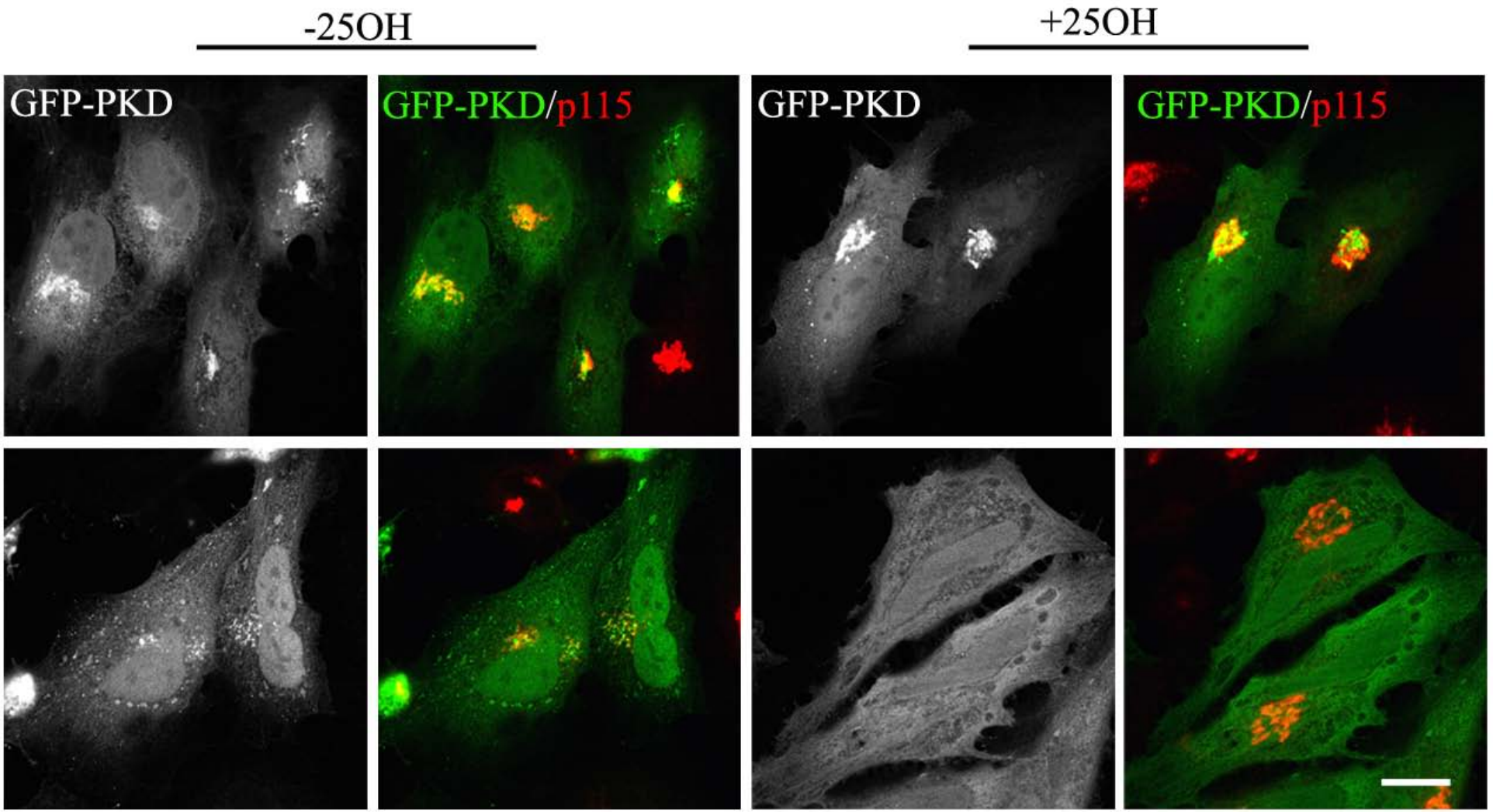

-25OH
+25OH
GFP-PKD
GFP-PKD/p115
GFP-PKD
GFP-PKD/p115
Control
VAPs RNAi

Figure S4

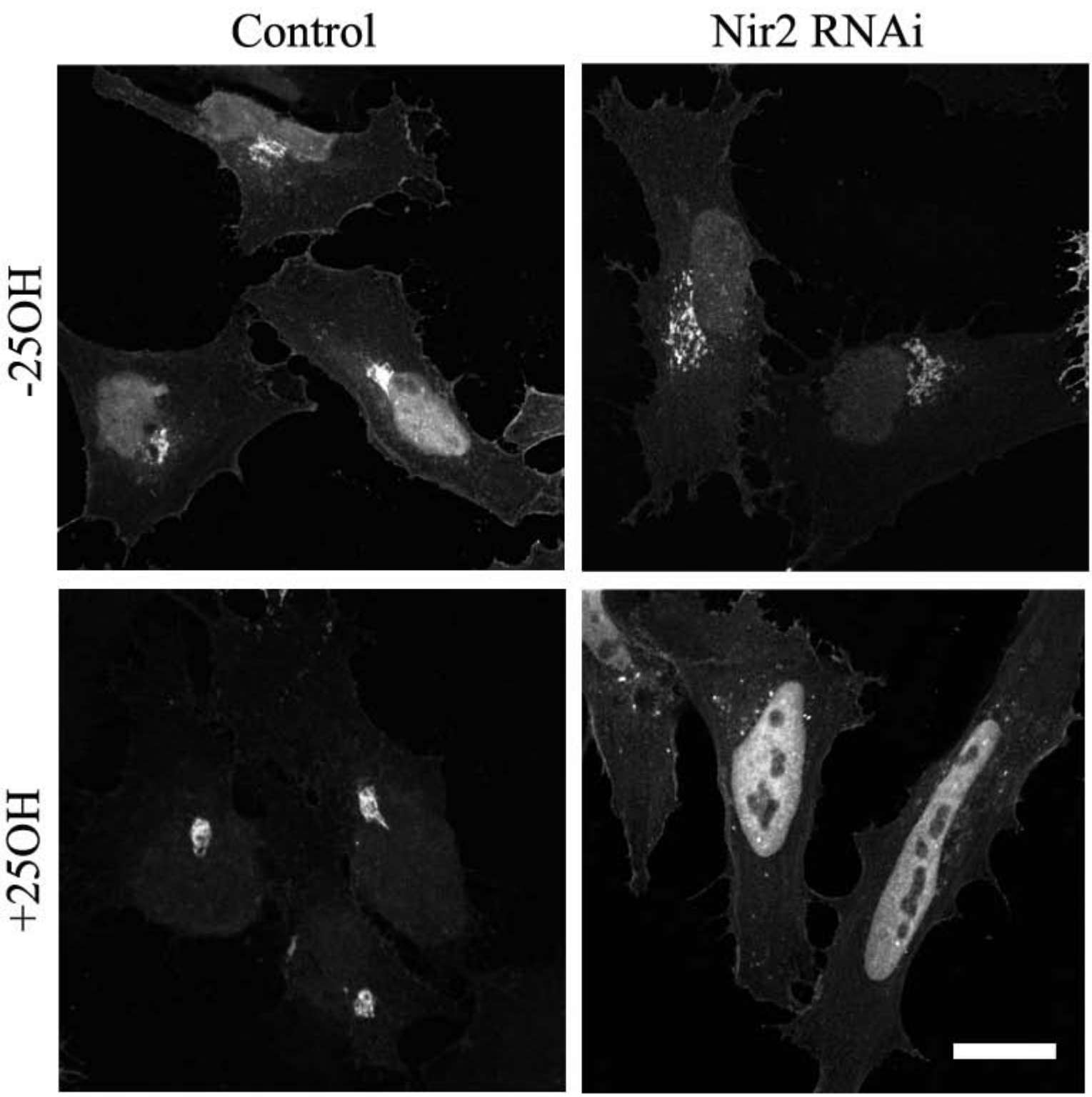


Figure S5

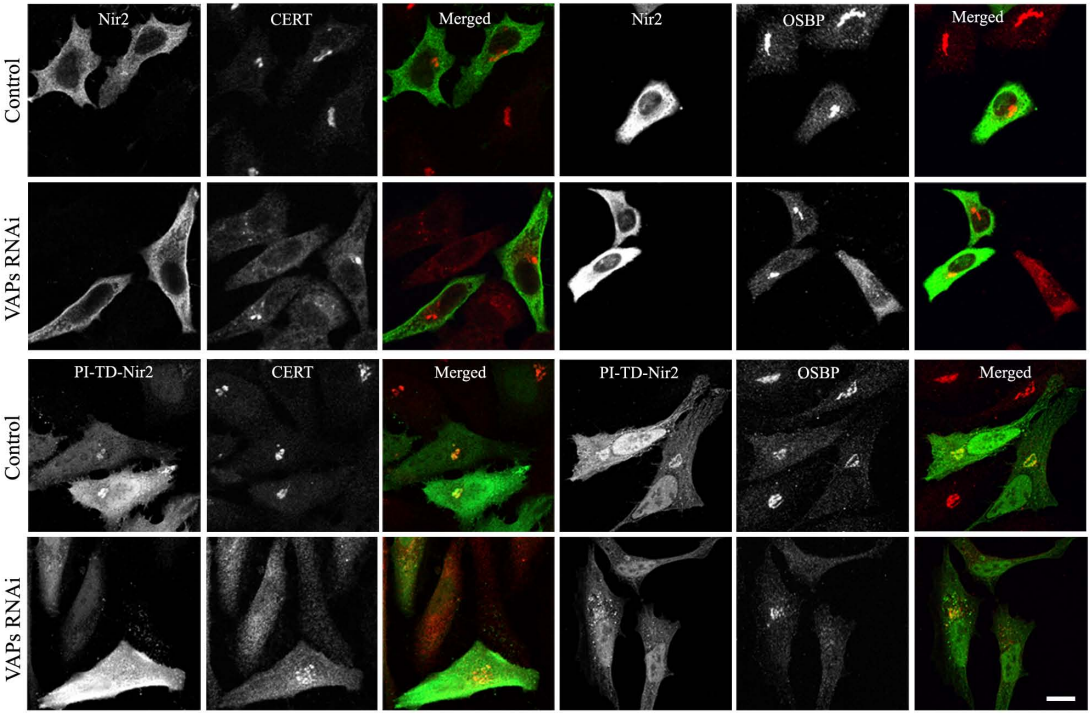
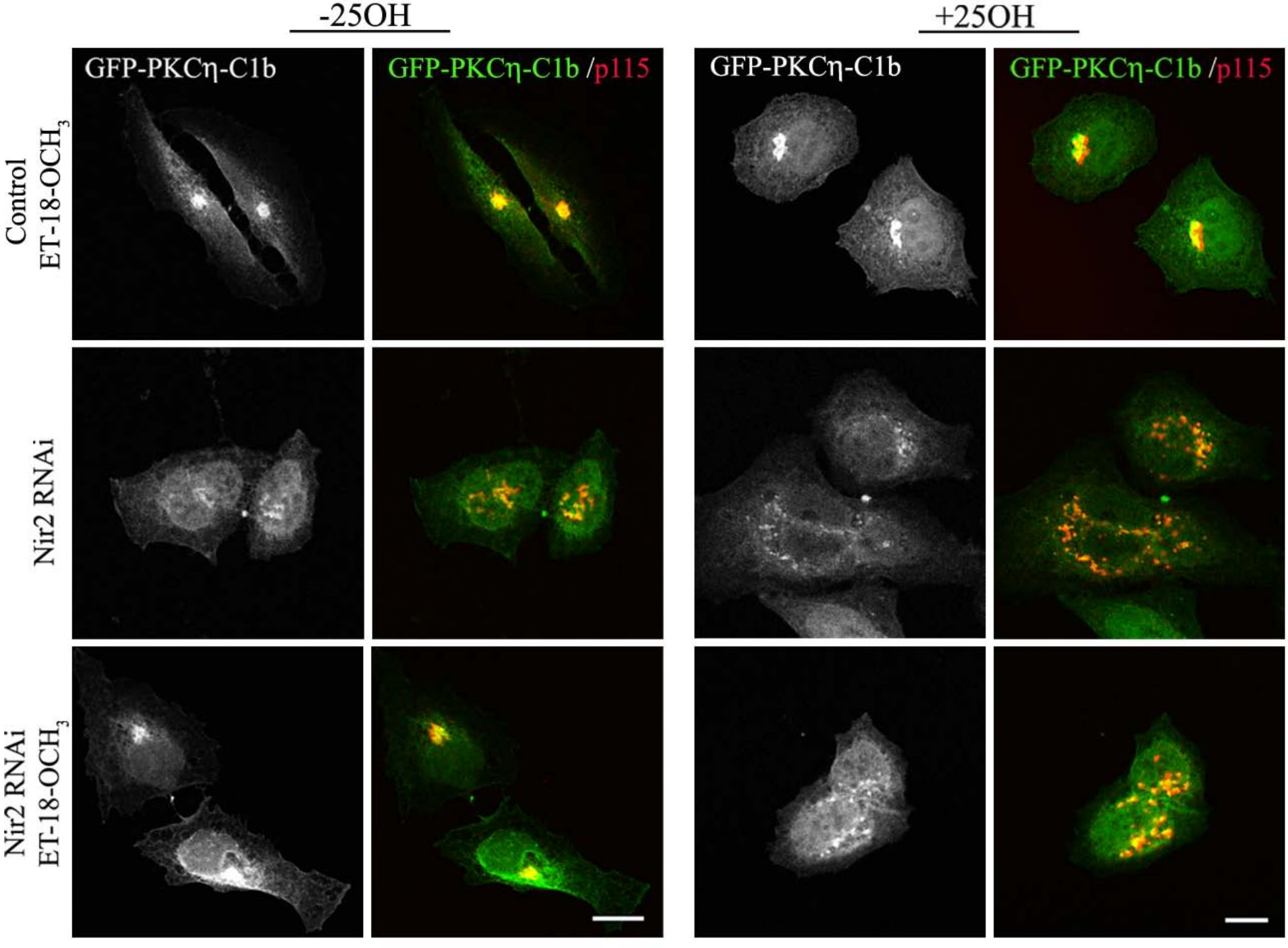

Control
VAPs RNAi
Control
VAPs RNAi
Nir2
CERT
Merged
Nir2
OSBP
Merged
PI-TD-Nir2
CERT
Merged
PI-TD-Nir2
OSBP
Merged

Figure S6


-25OH
+25OH
GFP-PKCη-C1b
GFP-PKCη-C1b /p115
GFP-PKCη-C1b
GFP-PKCη-C1b /p115
Control
ET-18-OCH3
Nir2 RNAi
Nir2 RNAi
ET-18-OCH3