

Supplemental Materials

Molecular Biology of the Cell

Abraham et al.

SUPPLEMENTAL FIGURES

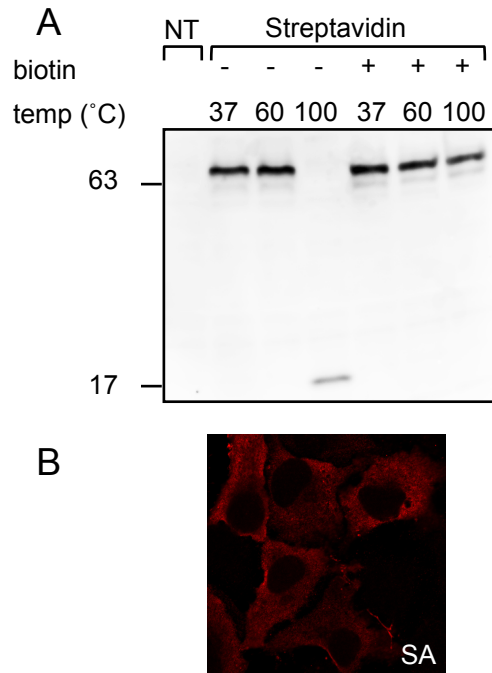


Figure S1. Expression of streptavidin in HeLa cells. (A) Western blot analysis using antibody that recognized both native and denatured SA (Abcam polyclonal, 1:5,000). Cell extracts were treated with SDS sample buffer with or without 1 mM biotin for 5 min at the indicated temperature. The observed 68 KD form represents the tetrameric form, which resists denaturation with the exception of heating at 100°C in the absence of biotin (Humbert *et al.*, 2005). (B) Indirect immunofluorescence microscopy of cells 24h after transfection with SA.

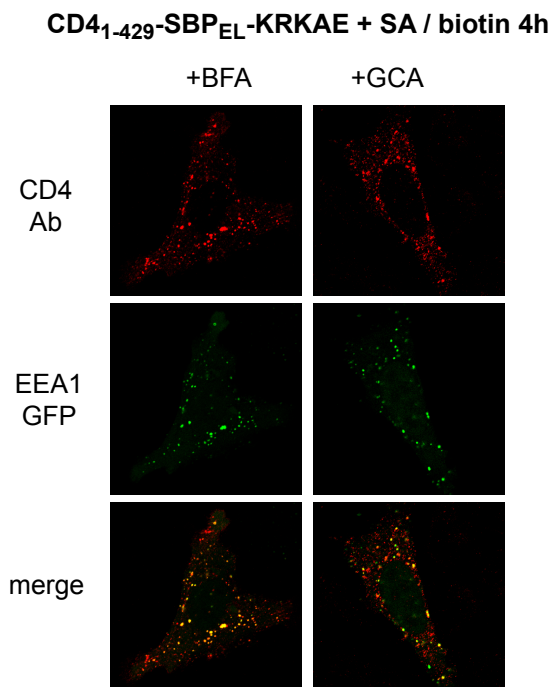


Figure S2. In the presence of BFA/GLA, biotin treatment induces redistribution of CD4₁₋₄₂₉-SBP_{EL}-KRKAE to a punctate pattern that resembles that of cotransfected EEA1-GFP. Conditions as in Figure 9C.

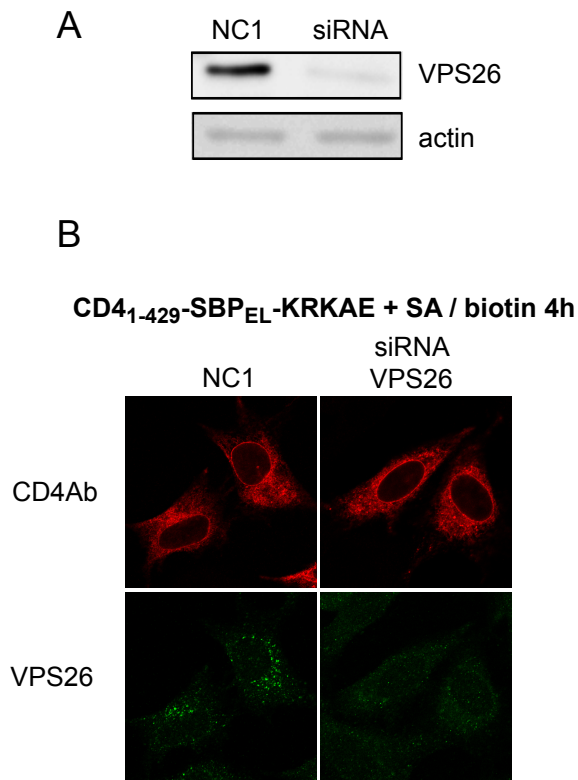


Figure S3. Knockdown of VPS26 has no effect on PM to ER transport of CD4₁₋₄₂₉-SBP_{EL}-KRKAE. The VPS26 siRNA was prepared according to Ref. (Du *et al.*, 2012). (A) Western blot analysis of the VPS26 siRNA effect 3 days after transfection. Scrambled siRNA NC1 was used as a control. Quantification of the bands indicated knockdown efficiency of 95%. (B) Cells were pretreated with or without siRNA for 2 days and were subsequently co-transfected with CD4₁₋₄₂₉-SBP_{EL}-KRKAE and SA; 24h later they were incubated for 4h with mouse anti-CD4 antibodies and biotin, then stained with anti-VPS26 antibodies followed by secondary antibodies.

REFERENCES

- Du X, Kazim AS, Brown AJ, Yang H (2012). An essential role of Hrs/Vps27 in endosomal cholesterol trafficking. *Cell Rep* 1, 29-35.
- Humbert N, Zocchi A, Ward TR (2005). Electrophoretic behavior of streptavidin complexed to a biotinylated probe: a functional screening assay for biotin-binding proteins. *Electrophoresis* 26, 47-52.