Dennis et al., http://www.jcb.org/cgi/content/full/jcb.201605090/DC1

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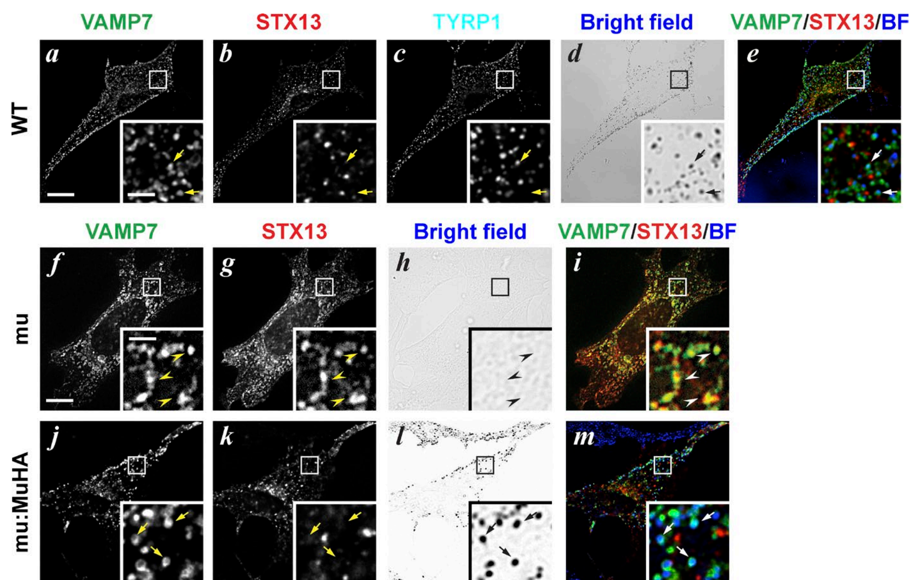


Figure S1. **VAMP7 is a BLOC-1 cargo.** WT melan-Ink4a (a–e), BLOC-1-deficient melan-mu (mu; f–i), or melan-mu melanocytes stably expressing Muted-HA (mu:muHA; j–m) were transiently transfected to express GFP-VAMP7 (green) and mCh-STX13 (red), fixed 24 h later, and analyzed by deconvolution immuno-FM. BF images in d, h, and l are pseudocolored blue in merged images e, i, and m. In a–e, cells were also immunolabeled for TYRP1 with Alexa Fluor 647 secondary antibody (cyan; not included in merge). Insets are boxed regions magnified five times. Arrows show melanosomes with GFP-VAMP7 (and TYRP1 in a–e) but lacking mCh-STX13. Arrowheads show GFP-VAMP7 retained in mCh-STX13–positive endosomes. Bars: (main) 10 µm; (insets) 2 µm.

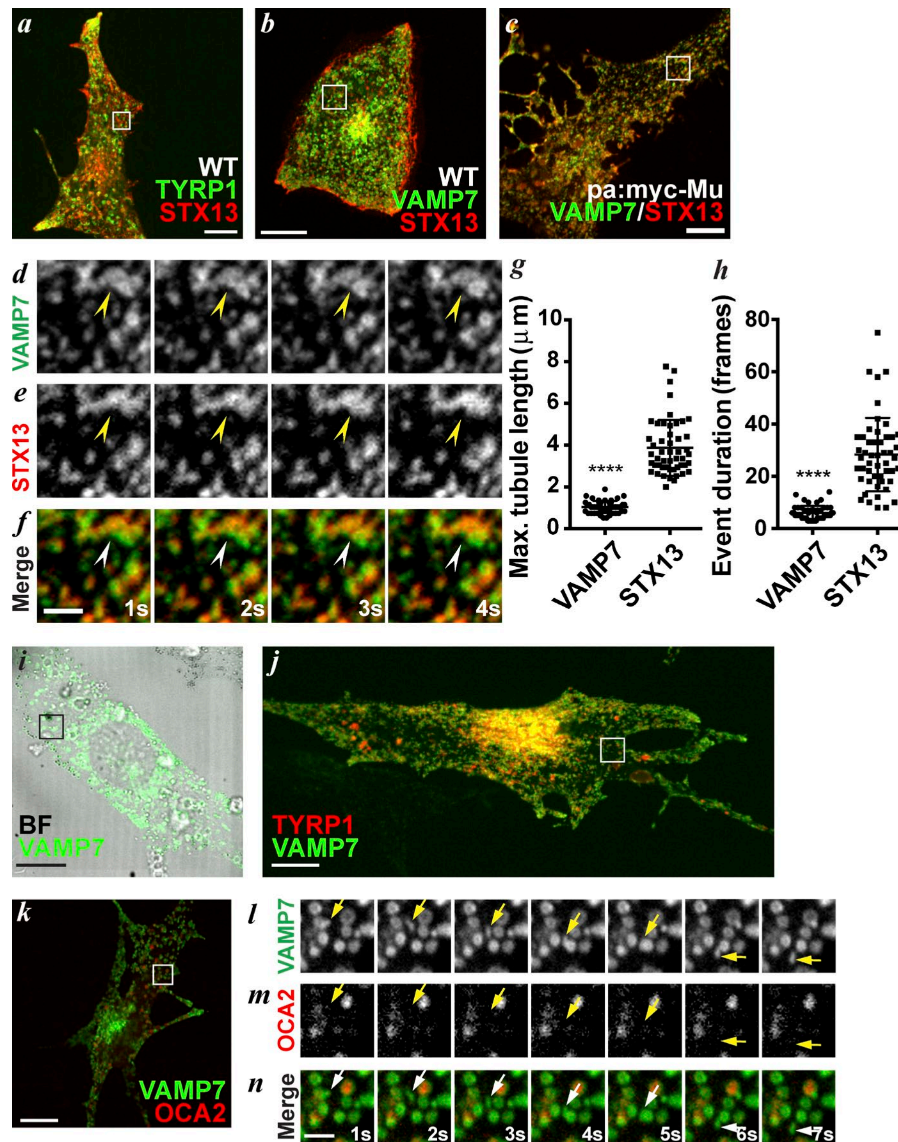


Figure S2. **VAMP7-containing tubules are distinct from anterograde STX13-containing tubules and lack melanosomal cargoes TYRP1 and OCA2, and VAMP7 requires BLOC-1 to exit from endosomes.** (a and b) WT melan-Ink4a cells transiently cotransfected with mCh-STX13 (red) and either TYRP1-GFP (a) or GFP-VAMP7 (b; green) were analyzed 24 h later by spinning-disk confocal microscopy. Shown are single frames of a whole cell with region of interest corresponding to images in Fig. 4 (a–c in panel a; and d–f in panel b). Bars, 10 µm. (c–f) BLOC-1–deficient melan-pa melanocytes transiently transfected with myc-Muted (pa:myc-Mu; "mock rescue"), mCh-STX13 (STX13, red), and GFP-VAMP7 (VAMP7, green) were analyzed 24 h later by spinning-disk confocal microscopy at ~1 fps. (c) Single frame of a representative cell showing overlap of mCh-STX13 with GFP-VAMP7. Bar, 10 µm. (d–f) Image sequences from the boxed region in c are magnified 3.5 times. Arrowheads indicate mCh-STX13–labeled endosomes containing GFP-VAMP7. Elapsed time is indicated (in seconds) at the lower right. Bar, 2 µm. (g and h) Quantification of length (g) and stability (h) of VAMP7- and STX13- labeled tubules in WT melanocytes. WT melan-Ink4a cells transiently cotransfected with mCh-STX13 and GFP-VAMP7 were analyzed 24 h later by spinning-disk microscopy. For at least 50 tubules labeled by each protein, the maximum tubule length and number of frames (captured at ~1 fps) in which a given tubule was visible were manually quantified using ImageJ. Individual data points are plotted on a dot plot with mean ± SD indicated. ****, $P < 0.0001$. (i–n) WT melan-Ink4a melanocytes transiently transfected with GFP-VAMP7 (green) alone (i) or with either TYRP1-mRFP (j) or mRFP-OCA2 (k–n; red) were analyzed 24 h (i–j) or 48 h (k–n) later by spinning-disk confocal microscopy at ~1 fps. (i and j) Single frame from whole cells with ROI corresponding to images in Fig. 5 (a–c in panel i; and h–j in panel j). Bars, 10 µm. (k) Single frame of representative cell showing overlap of GFP-VAMP7 with mRFP-OCA2. Bar, 10 µm. (l–n) Image sequences from the boxed region in k are magnified 3.5 times. Arrows, a GFP-VAMP7–labeled tubule exits from an mRFP-OCA2– and GFP-VAMP7–labeled melanosome. Elapsed time is indicated (in seconds) at the lower right. Bar, 2 µm.

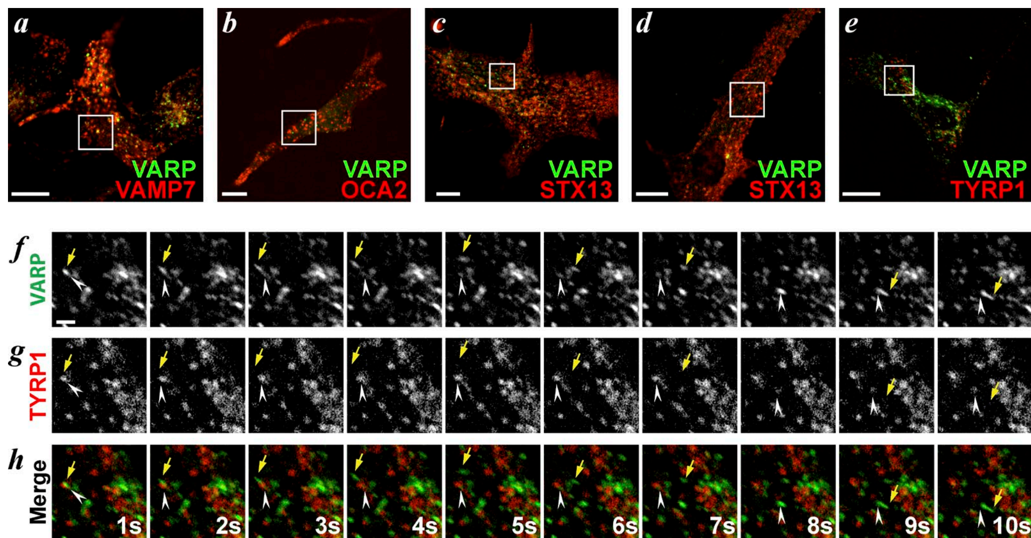


Figure S3. **VARP departs TYRP1-labeled melanosomes in VAMP7-labeled tubules.** WT melan-Ink4a cells transiently transfected with VARP-GFP (green) and either mCh-VAMP7 (a), mRFP-OCA2 (b), mCh-STX13 (c and d), or TYRP1-mRFP (e–h; red) were analyzed 48 h later by spinning-disk confocal microscopy at ~1 fps. Bars, 10 µm. (a–e) Single frame from whole cells with regions of interest corresponding to images in Fig. 6 (j-l in panel a; m–o in panel b; p–r in panel c; or s–u in panel d). (e–h) Single frame (e) and image sequence from boxed region in the periphery (f–h; magnified 3.5 times) of a representative cell showing localization of VARP-GFP (green) relative to TYRP1-mRFP (red). Arrows show a VARP-GFP–labeled tubule departs from a TYRP1-mRFP–labeled melanosome (at arrowhead). Elapsed time is indicated (in seconds) at the lower right. Bars: (a–e) 10 µm; (f–h) 2 µm.

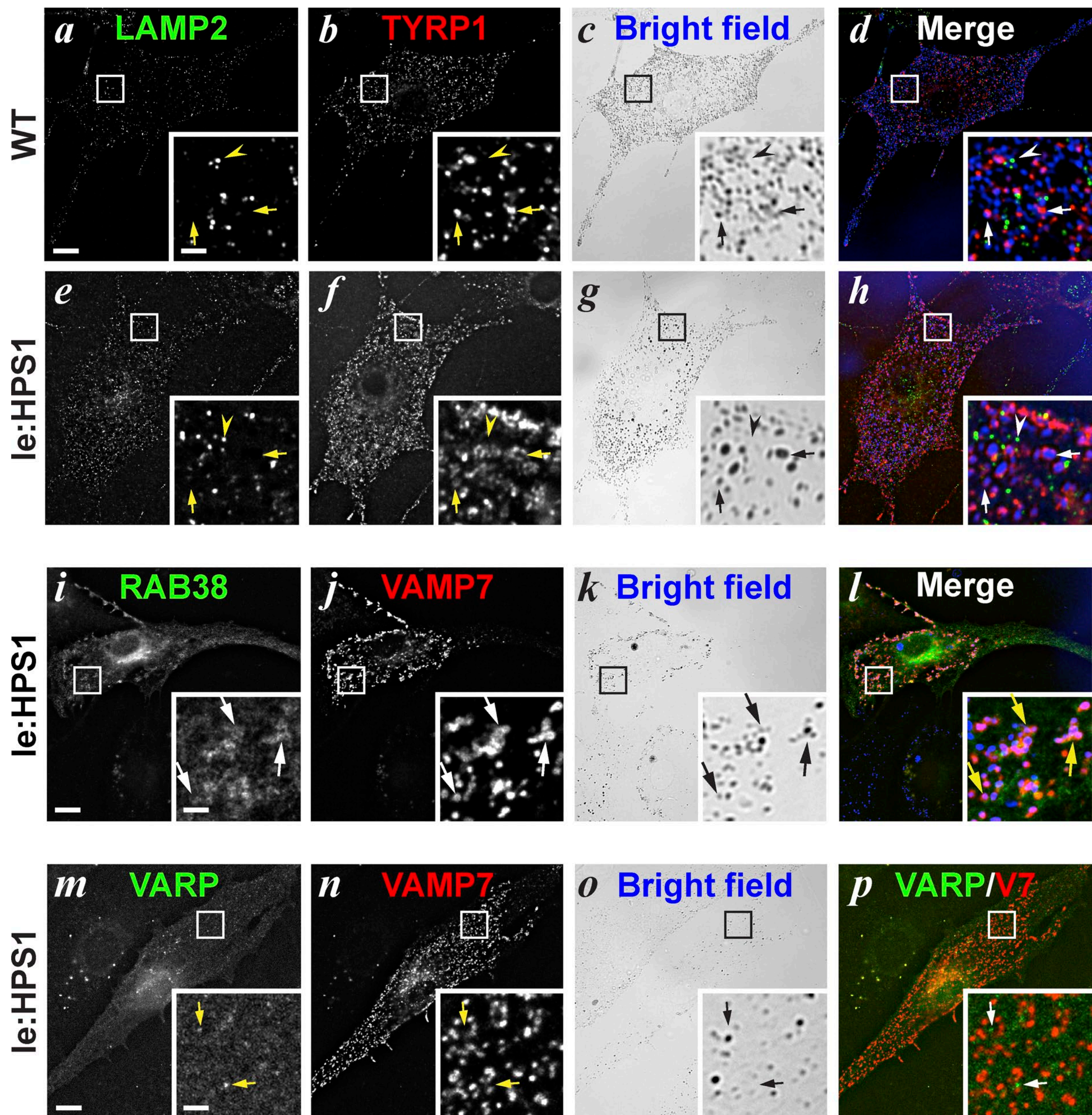


Figure S4. **VAMP7 and TYRP1, but not RAB38 and VARP, localize normally to melanosomes in mock-rescued BLOC-3-deficient *light ear* melanocytes.** WT melan-Ink4a melanocytes (a–d), and melan-le cells stably expressing HA-HPS1 (le:HPS1 or "mock rescued", e–h) were fixed and immunolabeled for LAMP2 (green) and TYRP1 (red) and analyzed by deconvolution immuno-FM. BF images in c and g are pseudocolored blue in the merged images (d and h). Insets are boxed regions magnified five times. Bars: (main) 10 µm; (insets) 2 µm. (i–p) Mock-rescued melan-le cells stably expressing HA-HPS1 (le:HPS1) transiently transfected with mCh-VAMP7 (red) and either GFP-RAB38 (i–l) or VARP-GFP (m–p; green) were fixed 24 h (i–l) or 48 h (m–p) after transfection and analyzed by deconvolution immuno-FM. BF images in k and o are pseudocolored blue in the merged images (l and p). Insets are boxed regions magnified five times. Bars: (main) 10 µm; (insets) 2 µm. (i–l) Arrows show mCh-VAMP7–labeled melanosomes lacking GFP-RAB38. (m–p) Arrows show VARP-GFP puncta not associated with melanosomes.

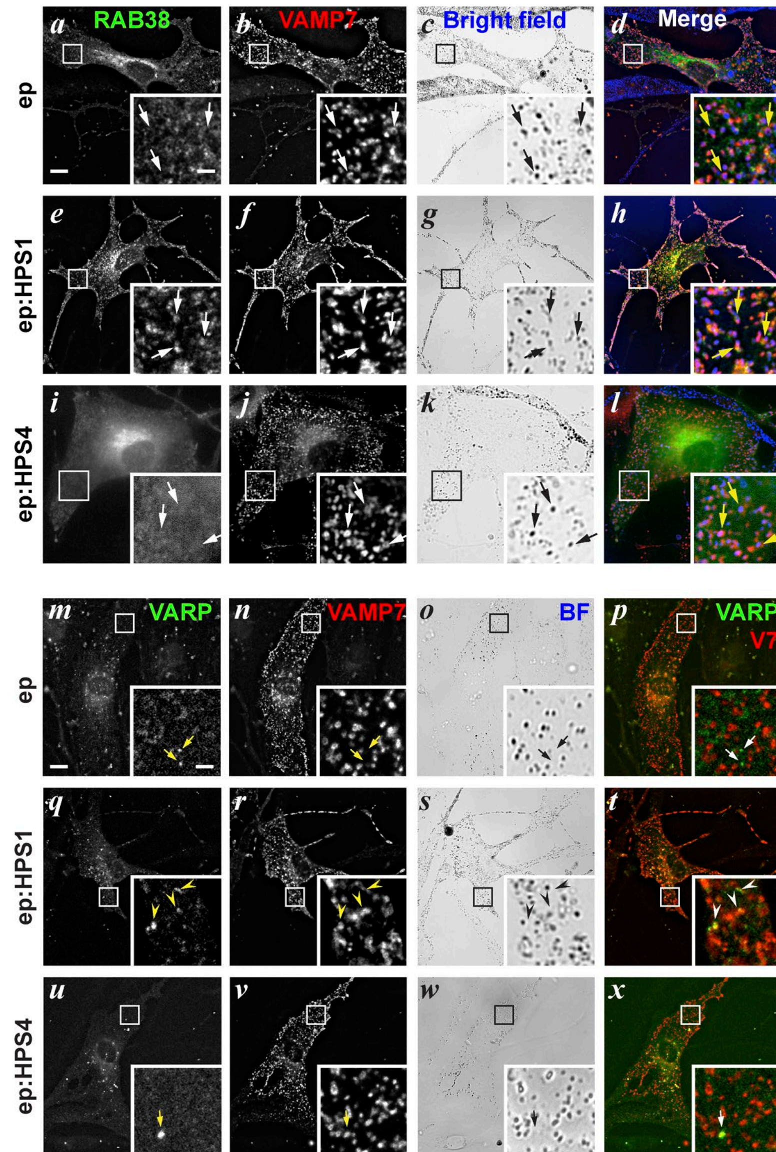
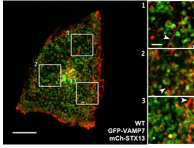
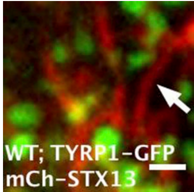


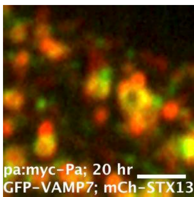
Figure S5. **HPS1-deficient melan-ep melanocytes are phenotypically similar to HPS4-deficient melan-le cells.** BLOC-3–deficient melan-ep (ep, HPS1-deficient, a–d and m–p) and melan-ep cells stably expressing either HA-HPS1 (ep:HPS1 or "rescued", e–h and q–t) or HA-HPS4 (ep:HPS4 or "mock rescued", i–l and u–x) and transiently expressing mCh-VAMP7 (red) and either GFP-RAB38 (a–l) or VARP-GFP (m–x; green) were fixed and analyzed by deconvolution immuno-FM. BF images in c, g, and k are pseudocolored blue in the merged images in d, h, and l. Insets are boxed regions magnified five times (all panels except i–l) or three times (i–l). Bars: (main) 10 µm; (insets) 2 µm. (a–l) Arrows point to mCh-VAMP7–labeled melanosomes. Note minimal localization of GFP-RAB38 to melanosomes except in "rescued" ep:HPS1 cells. (m–x) Arrows point to VARP-GFP puncta not associated with melanosomes, and arrowheads show VARP-GFP puncta associated with mCh-VAMP7–labeled melanosomes.



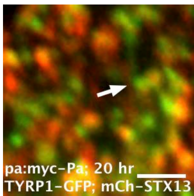
Video 1. **GFP-VAMP7 and mCh-STX13 label separate tubule populations in WT melanocytes.** A WT melan-Ink4a melanocyte transiently expressing GFP-VAMP7 (green) and mCh-STX13 (red) was imaged by spinning-disk confocal microscopy 24 h after transfection. Images were acquired at ~1 fps. (left) 60 frames of whole cell with boxes corresponding to regions of interest in Fig. 5 (d–g). (right) 30 frames of magnified insets of boxes. White arrowheads show mCh-STX13-labeled tubules, and yellow arrows show GFP-VAMP7-labeled tubules. Video plays at 5× real time. Bars: (main) 10 µm; (insets) 2 µm.



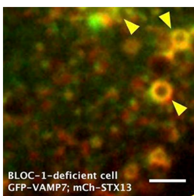
Video 2. **The melanosomal cargo TYRP1-GFP is not enriched in mCh-STX13 tubules in WT melanocytes.** A WT melan-Ink4a melanocyte transiently expressing TYRP1-GFP (green) and mCh-STX13 (red) was imaged by spinning-disk confocal microscopy 24 h after transfection. Video is taken from the same cell shown in Fig. 4 (a–c). Images were acquired at ~1 fps. Arrows show mCh-STX13 tubules lacking TYRP1-GFP. Video plays at 5× real time. Bar, 1 µm.



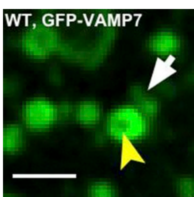
Video 3. **GFP-VAMP7 traffics to melanosomes via BLOC-1–dependent STX13 tubules.** A BLOC-1–deficient melan-pa melanocyte transiently transfected with myc-Pallidin (to rescue BLOC-1 activity), GFP-VAMP7 (green), and mCh-STX13 (red) was imaged by spinning-disk confocal microscopy 20 h after transfection. Images were acquired at ~1 fps. Arrow shows a tubule containing both GFP-VAMP7 and mCh-STX13. Frames from a video of a different cell are shown in Fig. 4 (g–j). Note the presence of mCh-STX13 tubules dependent on BLOC-1 function (compare to Video 5) and visualization of GFP-VAMP7 trafficking through mCh-STX13 tubules, which is undetectable in WT melanocytes (compare to Video 1). Video plays at 5× real time. Bar, 2 µm.



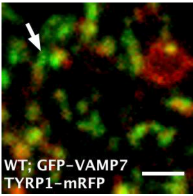
Video 4. **TYRP1-GFP traffics to melanosomes via BLOC-1–dependent STX13 tubules.** A BLOC-1–deficient melan-pa melanocyte transiently transfected with myc-Pallidin (to rescue BLOC-1 activity), TYRP1-GFP (green), and mCh-STX13 (red) was imaged by spinning-disk confocal microscopy 20 h after transfection. Images were acquired at ~1 fps. Arrows show tubules containing both TYRP1-GFP and mCh-STX13. The video corresponds to the same region shown in Fig. 4 (k–n). Note the presence of mCh-STX13 tubules that are dependent on BLOC-1 function (compare to Video 5) and the presence of TYRP1-GFP in mCh-STX13–labeled tubules, which is undetectable in WT melanocytes (compare to Video 2). Video plays at 5× real time. Bar, 2 µm.



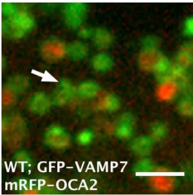
Video 5. **BLOC-1-deficient melanocytes lack STX13-labeled endosomal tubules and retain GFP-VAMP7 and mCh-STX13 in endosomes.** A BLOC-1–deficient melan-pa melanocyte transiently expressing GFP-VAMP7 (green) and mCh-STX13 (red) was imaged by spinning-disk confocal microscopy 24 h after transfection. Images were acquired at ~1 fps. Yellow arrowheads show enlarged endosomes containing both GFP-VAMP7 and mCh-STX13. Note the reduced mCh-STX13 tubulation from endosomes compared with WT melan-Ink4a melanocytes (Videos 1 and 2). Frames from a different video are shown in Fig. S2 (c–f). Video plays at 5× real time. Bar, 2 µm.



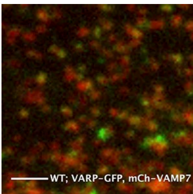
Video 6. **GFP-VAMP7–labeled tubules exit pigmented melanosomes.** A WT melan-Ink4a melanocyte transiently expressing GFP-VAMP7 (green) was imaged by spinning-disk confocal microscopy 24 h after transfection. Images were acquired at ~1 fps. The region shown corresponds to Fig. 5 (a–c) and Fig. S2 i. Yellow arrowhead indicates the position of a pigmented melanosome as viewed by BF microscopy (see Fig. 5 b); white arrow, GFP-VAMP7 tubule extending from and leaving the melanosome. Video plays at 5× real time. Bar, 2 µm.



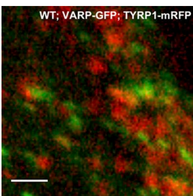
Video 7. **TYRP1-mRFP is undetectable in GFP-VAMP7 tubules leaving melanosomes.** A WT melan-Ink4a melanocyte transiently expressing GFP-VAMP7 (green) and TYRP1-mRFP (red) was imaged by spinning-disk confocal microscopy 24 h after transfection. Images were acquired at ~1 fps. The region shown corresponds to Fig. 5 (h–j) in the cell shown in Fig. S2 j. The arrow shows a GFP-VAMP7 tubule leaving a TYRP1-mRFP– and GFP-VAMP7–positive melanosome. Video plays at 5× real time. Bar, 2 µm.



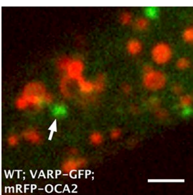
Video 8. **mRFP-OCA2 is undetectable in GFP-VAMP7 tubules leaving melanosomes.** A WT melan-Ink4a melanocyte transiently expressing GFP-VAMP7 (green) and mRFP-OCA2 (red) was imaged by spinning-disk confocal microscopy 48 h after transfection. Images were acquired at ~1 fps. The region shown corresponds to Fig. S2 (k–n). Arrow, GFP-VAMP7 tubule leaving an mRFP-OCA2– and GFP-VAMP7-positive melanosome. Video plays at 5× real time. Bar, 2 µm.



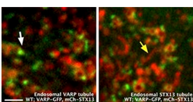
Video 9. **mCh-VAMP7 tubules are also labeled by VARP-GFP.** A WT melan-Ink4a melanocyte transiently expressing VARP-GFP (green) and mCh-VAMP7 (red) was imaged at ~1.4 fps by spinning-disk confocal microscopy 48 h after transfection. A Hamamatsu ORCA-Flash4.0 sCMOS camera on the PerkinElmer spinning-disk confocal was used to acquire this image sequence. Arrow shows a tubule labeled by both VARP-GFP and mCh-VAMP7 that extends and departs from an mCh-VAMP7–labeled melanosome. Frames from a different video are shown in Fig. 6 (j–l) in the cell shown in Fig. S3 a. Video plays at 5× real time. Bar, 2 µm.



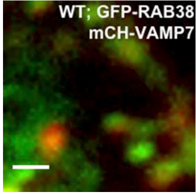
Video 10. **TYRP1-mRFP is undetectable in VARP-GFP–labeled tubules.** A WT melan-Ink4a melanocyte transiently expressing VARP-GFP (green) and TYRP1-mRFP (red) was imaged by spinning-disk confocal microscopy 48 h after transfection. Images were acquired at ~1 fps. The region shown corresponds to Fig. S3 (e–h). Arrows show tubules labeled by VARP-GFP but lacking TYRP1-mRFP that extend and depart from VARP-GFP– and TYRP1-mRFP–labeled melanosomes. Video plays at 5× real time. Bar, 2 µm.



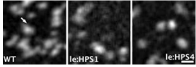
Video 11. **mRFP-OCA2 is undetectable in VARP-GFP–labeled tubules.** A WT melan-Ink4a melanocyte transiently expressing VARP-GFP (green) and mRFP-OCA2 (red) was imaged by spinning-disk confocal microscopy 48 h after transfection. Images were acquired at ~1 fps. The region shown corresponds to Fig. 6 (m–o) in the cell shown in Fig. S3 b. The arrow shows a tubule labeled by VARP-GFP but lacking mRFP-OCA2 extends and departs from a VARP-GFP- and mRFP-OCA2-labeled melanosome. Video plays at 5× real time. Bar, 2 µm.



Video 12. **VARP-GFP labels tubules that originate from STX13-labeled endosomes but lack STX13.** A WT melan-Ink4a melanocyte transiently expressing VARP-GFP (green) and mCh-STX13 (red) was imaged by spinning-disk confocal microscopy 48 h after transfection. Images were acquired at ~1 fps. (left) a VARP-GFP–labeled tubule (white arrow) lacking mCh-STX13 extends and departs from a mCh-STX13–positive endosome. Note the longer and more stable tubule dynamics relative to VARP-GFP–labeled tubules that emerge from melanosomes (in Videos 9, 10, and 11). (right) An mCh-STX13–labeled tubule lacking VARP-GFP (yellow arrow) extends from a mCh-STX13– and GFP-VARP–labeled endosome. The video corresponds to Fig. 6 (p–u); both panels are taken from the cell shown in Fig. 6 (p–r) and Fig. S3 c, and the video plays at 5× real time. Bar, 2 µm.



Video 13. **GFP-RAB38 is present on mCh-VAMP7–containing recycling tubules.** A WT melan-Ink4a melanocyte transiently expressing GFP-RAB38 (green) and mCh-VAMP7 (red) was imaged by spinning-disk confocal microscopy 24 h after transfection. Images were acquired at ~1 fps. White arrows, tubules labeled by both GFP-RAB38 and mCh-VAMP7 extend and depart from a GFP-RAB38– and mCh-VAMP7–labeled melanosome. The video corresponds to Fig. 7 (i–l) and plays at 5× real time. Bar, 1 µm.



Video 14. **Example of regions of interest used in quantification of GFP-VAMP7 tubules departing melanosomes in WT, BLOC-3–deficient, and BLOC-3 "rescue" cells.** A WT melan-Ink4a (WT; left), a BLOC-3–deficient "mock rescue" (le:HPS1; middle), and a "rescued" melan-le (le:HPS4; right) melanocyte each transiently expressing GFP-VAMP7 was imaged by spinning-disk confocal microscopy 24 h after transfection. Images were acquired at ~1 fps. White arrows, GFP-VAMP7–labeled tubules depart from melanosomes. The video corresponds to the regions shown in panels a–c of Fig. 10 and quantified in Fig. 10 d, and it plays at 5× real time. Bar, 1 µm.