Supplementary Information

Supplementary figure 1

A

\[ \alpha\text{-GFP/}\alpha\text{-myc} \]

Brightfield

GFP-Kif5bA350

myc-SKIP

B

GFP

mCherry-Cathepsin B

Brightfield

GFP alone

GFP-SKIP

C

siRNA

NT

R27a

Kif5b-7

GFP-SKIP

Brightfield
Figure S1. Expression of motor-less Kif5b (GFP-Kif5bΔ350) and myc/GFP-tagged SKIP in melanocytes. melan-a cells were transiently transfected with plasmid vectors allowing expression of the indicated fusion proteins, fixed 48 hours later, processed for immunofluorescence and imaged using a fluorescence microscope (as described in materials and methods). Fluorescence and bright-field images show the distribution of heterologously expressed/endogenous protein and melanosomes in transfected cells. A) Neither motor-less Kif5b (GFP-Kif5bΔ350) nor myc-tagged SKIP co-localise with melanosomes. B) Expression of SKIP in melanocytes disperses lysosomes but not melanosomes. C) melan-a cells transfected with siRNA (as indicated) were infected with adenovirus vectors allowing expression of GFP-SKIP 72h after transfection and fixed and processed for immunofluorescence 24h later (as described in materials and methods). Scale bar (A) = 50μm, (B) = 20μm and (C) =100μm.
Supplementary Figure 2

A

<table>
<thead>
<tr>
<th></th>
<th>GFP</th>
<th>Bright-field</th>
<th>Inverted bright-field</th>
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<tbody>
<tr>
<td>Mini-K5b</td>
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<tr>
<td>K6b-MVo</td>
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<td>Myosin-Va</td>
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B

![Graph showing pigment coverage](image)

- **GFP/****ns****ns**
Figure S2. The function of the K5b-MVa, but not the mini-K5b, fusion protein is dependent upon melanophilin expression. Melanophilin null (melan-ln) cells were infected with adenovirus vectors allowing expression of the indicated GFP fusion proteins, fixed 24 hours later, processed for immunofluorescence and imaged using a confocal microscope (as described in material and methods). A) Fluorescence (green in merge), bright-field, inverted bright-field (magenta in merge) and merged images showing the distribution of the GFP fused motor proteins and melanosomes in representative fields of infected melan-ln cells. Scale bar = 20µm. B) A scatter plot showing the pigment distribution as reported by pigment coverage (% pigment filled area/total cell area) was calculated as described previously (Hume et al 2006). Horizontal bars show the median and 25th and 75th percentile of each population. The significance of differences in pigment coverage for each population compared with the GFP and mini-Va are displayed above and below each scatter (**** indicates p=<0.0001), respectively, as determined by one-way ANOVA. No other significant differences were observed. Data are from one of three independent experiments and are representative of the results of all experiments. Number of cells analysed; GFP = 11, myosin-Va = 9, mini-K5b = 10, mini-Va = 8 and K5b-MVa = 8.
Figure S3. Kif5b containing motors are dependent upon microtubule integrity for function. melan- 
d1 cells were infected with adenoviruses allowing expression of the indicated GFP fusion proteins then 24 hours later fixed, processed for immunofluorescence and imaged using a confocal microscope (as described in material and methods). For 1 hour prior to, and throughout adenovirus incubation, cells were maintained in medium supplemented with 10μM nocodazole to deplete microtubules. A) Fluorescence and bright field images showing the intracellular distribution of GFP fusions, tubulin and melanosomes. For mini-K5b and K5b-MVa cell outline traces have been overlaid onto bright-field images to highlight clustered melanosome distribution. Scale bar = 20μm. B) A scatter plot showing the pigment distribution in as reported by pigment coverage (% pigment filled area/total cell area) was calculated as described previously [Hume 2006]. Horizontal bars show the median and 25th and 75th percentile of the each population. The significance of differences in pigment coverage for each population compared with the GFP are displayed above each scatter (**** indicates p=<0.0001), respectively, as determined by one-way ANOVA. Data are from one of three independent experiments and are representative of the results of all experiments. Number of cells analysed; GFP = 8, myosin-Va = 8, mini-K5b = 9, mini-Va = 9 and K5b-MVa = 5.
Supplementary Figure 4

A) Mini-K5b

- GFP
- Phase contrast (melanosomes)
- Inverted phase contrast
- GFP/inverted phase contrast

B) Rab1aQ70L

- GFP
- Phase contrast (melanosomes)
- Inverted phase contrast
- GFP/inverted phase contrast
Figure S4. Expression of melanosome targeted mini-K5b (A) and constitutively active Rab1aQ70L (B) in melanocytes. A) mini-K5b drives hyper-dispersion of melanosomes to the tips of dendrites in melanocytes. melan-a cells were infected with adenovirus allowing expression of the mini-K5b or GFP-Rab1aQ70L melanosome targeted active Kif5b, fixed 24h later, prepared for immunofluorescence and imaged using a confocal microscope as described in material and methods. B) Constitutively active Rab1aQ70L is localised to melanosomes in melanocytes. melan-a cells were transfected with plasmid vectors allowing expression, fixed 48 hours later, prepared for immunofluorescence and the distribution of fluorescence recorded using a confocal microscope, as described in materials and methods. White boxes in left-hand images indicate the region shown in high magnification images on the right-hand images. Scale bar = 20µm.
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