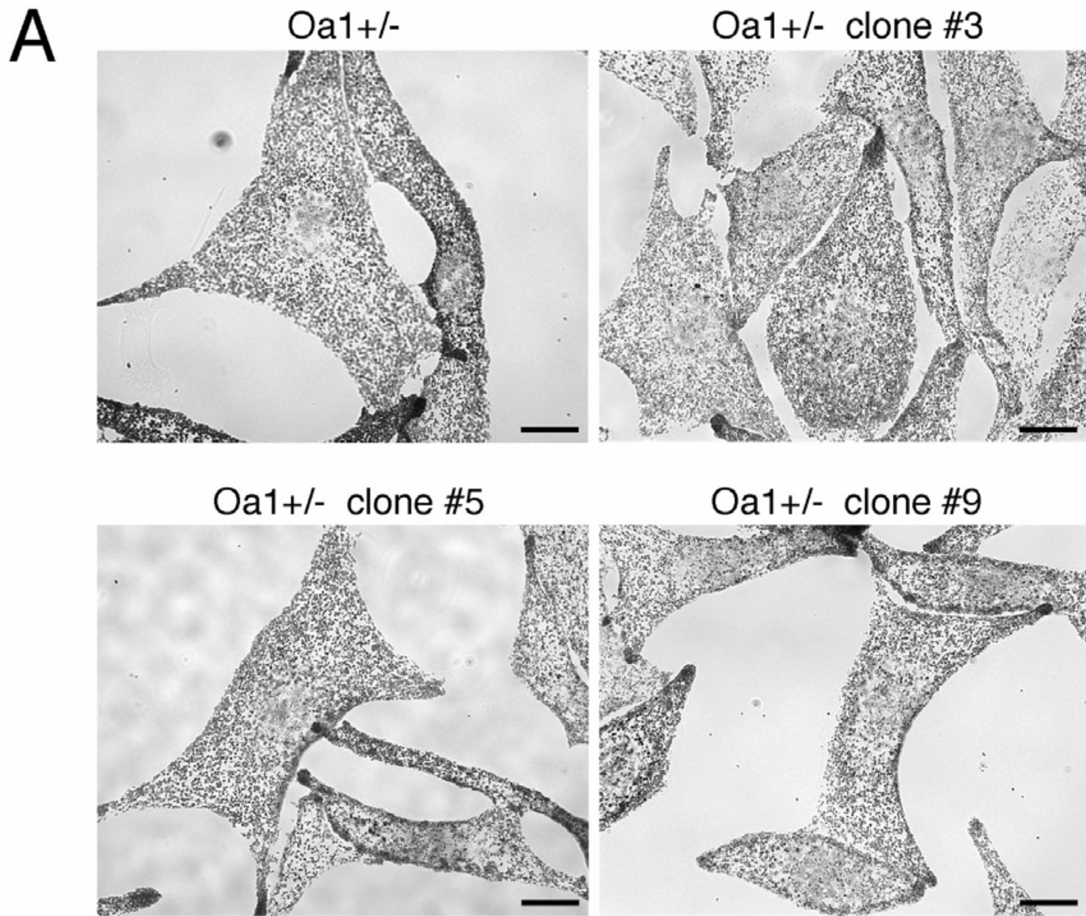
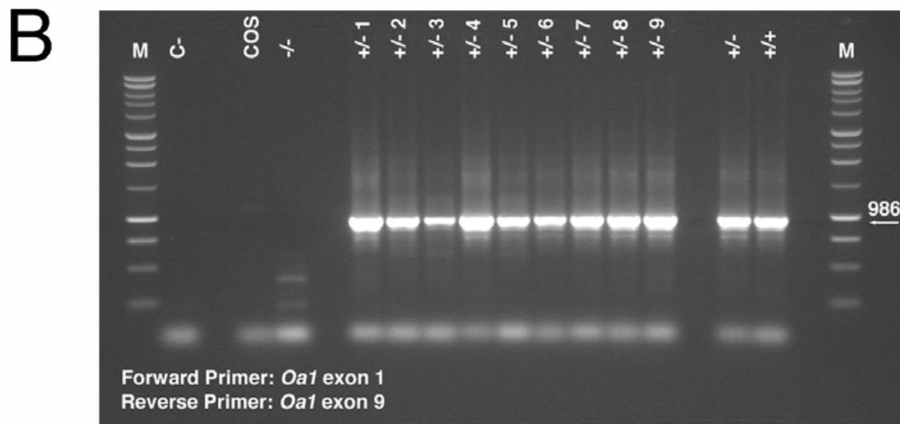
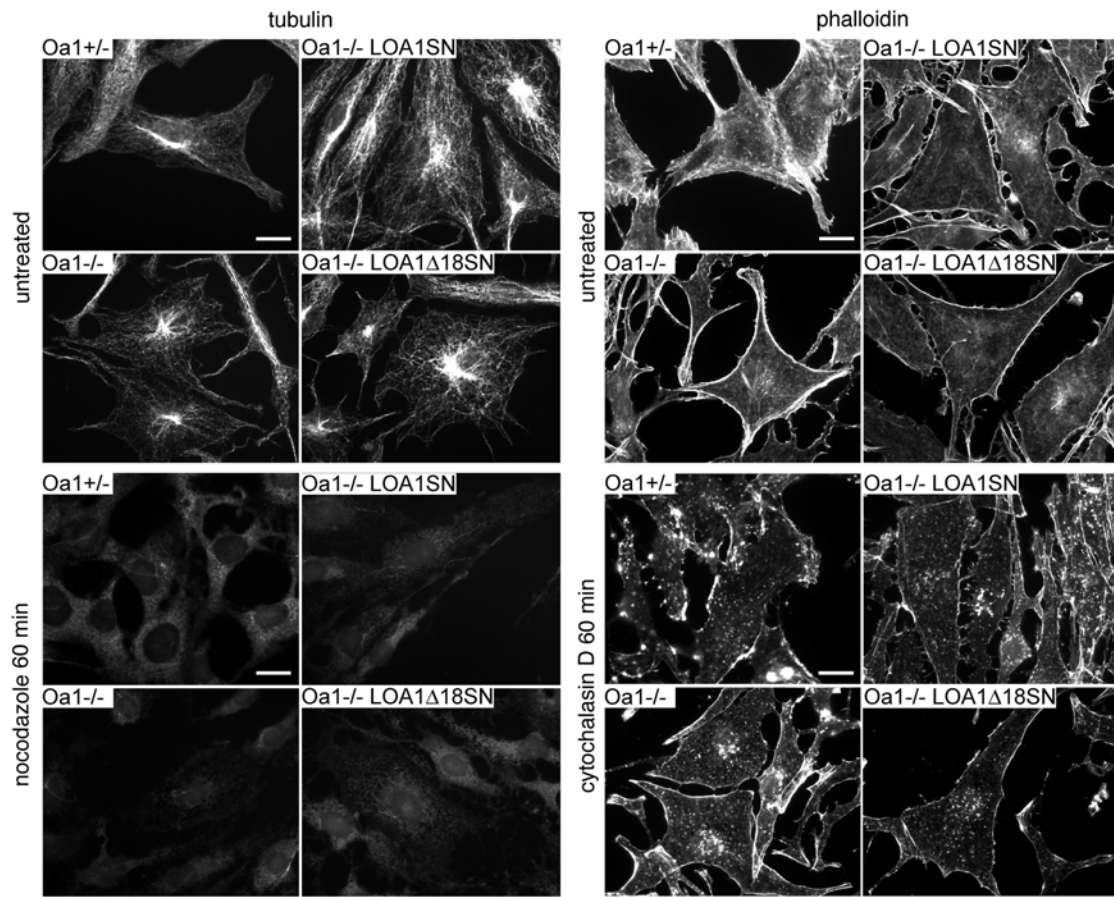
LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental Figure 1. Characterization of *Oa1*+/- melanocytes by subcloning and subsequent molecular and morphological analyses. (**A**) Bright field images of parental *Oa1*+/- melanocytes and of subclone number 3, 5 and 9, showing comparable size and distribution of melanosomes. Bars, 15 µm. (**B**) RT-PCR products obtained by amplifying the *Oa1* transcript from COS7 cells, *Oa1*-/-, *Oa1*+/- subclones #1 to 9, *Oa1*+/- and *Oa1*+/+ melanocytes. cDNAs were amplified with primers located on the first and last exon of *Oa1* and separated by about 1 kb (986 bp) on the spliced transcript (20-25 kbp in genomic DNA). M, molecular weight marker; C-, amplification performed in the absence of template. All clones are able to express full-length *Oa1*, as *Oa1*+/- and *Oa1*+/+ melanocytes and differently from *Oa1*-/- cells.

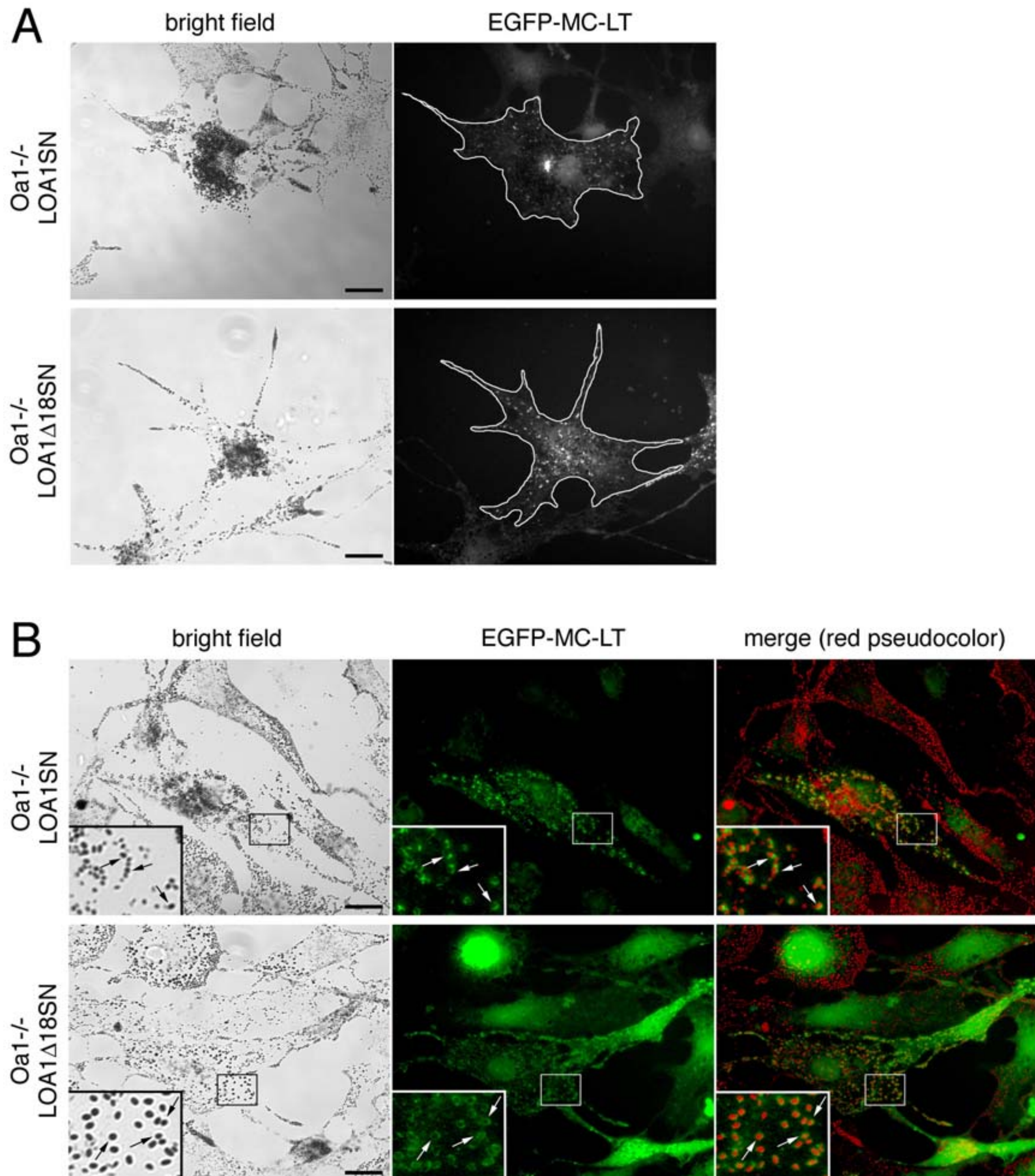

A
Oa1+/-
Oa1+/- clone #3
Oa1+/- clone #5
Oa1+/- clone #9


B
M
C-
COS
-/-
+/- 1
+/- 2
+/- 3
+/- 4
+/- 5
+/- 6
+/- 7
+/- 8
+/- 9
+/-
+/+
M
986
Forward Primer: Oa1 exon 1
Reverse Primer: Oa1 exon 9

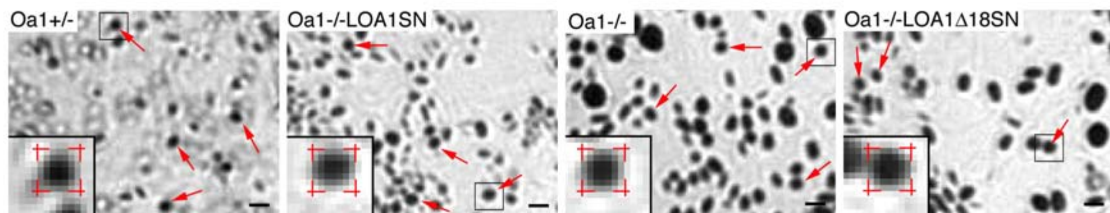
Supplemental Figure 2. Disruption of the MT and AF cytoskeleton in parental and transduced melanocyte lines. Representative optical pictures of the indicated cell lines prior to treatment (untreated), or treated with 30 µM nocodazole or 1 µM cytochalasin D for 60 min at 37°C. To visualize MTs and AFs, cells were fixed and stained with anti-tubulin antibodies and secondary fluorescent antibodies, or with rhodaminated phalloidin, respectively. Cytochalasin D induces reorganization of the actin cytoskeleton in short actin patches and nocodazole leads to complete depolymerization of MTs. Similar results were obtained 30 min after drug addition (not shown). In untreated conditions, note the very similar cell morphology and phalloidin staining of transduced *Oa1*-/- LOA1SN and LOA1Δ18SN melanocytes and parental *Oa1*-/- cells. Bars, 15 µm.



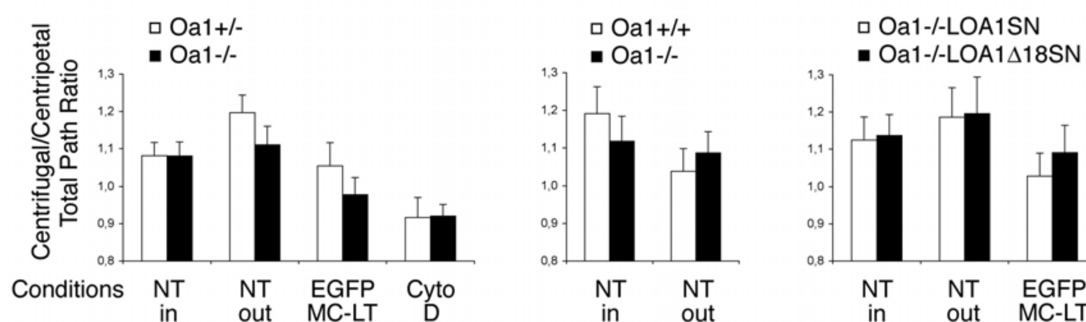
Supplemental Figure 3. Melanosome redistribution upon disruption of myosin Va function and colocalization with its tail domain in transduced melanocyte lines. (**A**) Representative optical pictures showing the redistribution of melanosomes upon expression of a dominant-negative construct for myosin Va. Transduced *Oa1*-/-LOA1SN and *Oa1*-/-LOA1Δ18SN cells were transfected with pEGFP-MC-LT, driving the expression of the EGFP-tagged melanocyte-specific tail domain of myosin Va. After 24 h, the distribution of melanosomes in bright field was assessed in EGFP-positive cells. The edges of transfected cells are marked with a white line. The *dilute*-like phenotype was observed in a similarly high percent of *Oa1*-/-LOA1SN and *Oa1*-/-LOA1Δ18SN cells. Bars, 15 μm. (**B**) Representative optical pictures showing the colocalization of melanosomes with the melanocyte-specific tail domain of myosin Va. *Oa1*-/-LOA1SN and *Oa1*-/-LOA1Δ18SN cells were transfected with pEGFP-MC-LT and analyzed by optical microscopy before the appearance of a complete *dilute*-like phenotype. Melanosomes appear typically surrounded by a ring of fluorescence corresponding to the EGFP-MC-LT construct in both *Oa1*-/-LOA1SN and *Oa1*-/-LOA1Δ18SN cells. In the insets (3x magnification), arrows point to examples of colocalization. Bars, 15 μm.


A
bright field
EGFP-MC-LT
Oa1-/-
LOA1SN
Oa1-/-
LOA1Δ18SN
B
bright field
EGFP-MC-LT
merge (red pseudocolor)
Oa1-/-
LOA1SN
Oa1-/-
LOA1Δ18SN

Supplemental Figure 4. Bright field pictures of parental and transduced cell lines showing examples of melanosomes selected for manual tracking (red arrows). To ensure that organelles of equivalent size were compared, only melanosomes with major and minor diameters fitting within the range of 7-9 ± 1 pixels (corresponding to about 0.7-0.9 ± 0.1 μm) were chosen and their sizes determined. In the insets (3x magnification), examples of melanosomes measuring 7x7 pixels are shown. Bars, 1 μm.



Supplemental Figure 5. Average centrifugal/centripetal path ratio of melanosomes from the indicated cell lines in various conditions. Results represent the mean ± SEM of the data from 40-100 melanosomes (considering one melanosome path ratio as one data point). Although path ratios display some variation, in particular reflecting a slight prevalence of centripetal movements in the absence of AF-based transport, no significant differences were found between each wild-type line and its *Oa1*-deficient counterpart in the same conditions. Similar results were obtained by considering movements ≥0.4 μm/s only (data not shown).



Supplemental Movie 1. Motility of melanosomes, visualized by bright field optical microscopy, in an *Oa1*+/+ melanocyte analyzed in untreated conditions. Movements are more evident nearby the nucleus, although peripheral organelles move considerably as well. Frame rate: 20 fps (180 frames captured in 90 s).

Supplemental Movie 2. Motility of melanosomes, visualized by bright field optical microscopy, in an *Oa1*+/- melanocyte analyzed in untreated conditions. Movements are more evident nearby the nucleus, although peripheral organelles move considerably as well. Frame rate: 20 fps (180 frames captured in 90 s).

Supplemental Movie 3. Motility of melanosomes, visualized by bright field optical microscopy, in an *Oa1*-/- melanocyte analyzed in untreated conditions. Movements are more evident nearby the nucleus, despite the presence of fewer melanosomes in this area and despite an overall reduced motility compared to wild-type, while organelles at the periphery appear stationary. Note the heterogeneity of melanosome size and the lower motility of larger melanosomes. Frame rate: 20 fps (180 frames captured in 90 s).

Supplemental Movie 4. Motility of melanosomes, visualized by bright field optical microscopy, in an *Oa1*-/- melanocyte analyzed in untreated conditions. Movements are more evident nearby the nucleus, despite the presence of fewer melanosomes in this area and an overall reduced motility compared to wild-type, while organelles at the periphery appear stationary. In this particular cell, melanosome size is quite homogeneous and clearly giant melanosomes are not observed. Frame rate: 20 fps (180 frames captured in 90 s).

Supplemental Movie 5. Motility of melanosomes, visualized by bright field optical microscopy, in an *Oa1*+/- melanocyte analyzed upon pEGFP-MC-LT transfection. Movements are more evident at the periphery due to the huge aggregate in the perinuclear area. Frame rate: 20 fps (180 frames captured in 90 s).

Supplemental Movie 6. Motility of melanosomes, visualized by bright field optical microscopy, in an *Oa1*-/- melanocyte analyzed upon pEGFP-MC-LT transfection. Movements are more evident at the periphery due to the huge aggregate in the perinuclear area. Note that all size melanosomes aggregate toward the centrosome. Frame rate: 20 fps (180 frames captured in 90 s).

Supplemental Table I. Size of tracked melanosomes in wild-type and *Oa1*-deficient melanocyte lines in various conditions.

Condition	Oa1+/-			Oa1-/-			t-test
NT in	average		n	average		n	
Major diameter (µm)	0.84	±0.11	103	0.85	±0.10	103	0.884
Minor diameter (µm)	0.74	±0.09	103	0.73	±0.08	103	0.419
NT out	average		n	average		n	
Major diameter (µm)	0.83	±0.10	100	0.85	±0.09	100	0.148
Minor diameter (µm)	0.75	±0.08	100	0.76	±0.08	100	0.356
Nocodazole	average		n	average		n	
Major diameter (µm)	0.82	±0.12	90	0.83	±0.10	90	0.504
Minor diameter (µm)	0.74	±0.08	90	0.75	±0.08	90	0.560
EGFP-MC-LT	average		n	average		n	
Major diameter (µm)	0.84	±0.09	50	0.87	±0.11	50	0.168
Minor diameter (µm)	0.72	±0.07	50	0.76	±0.11	50	0.055
Cytochalasin D	average		n	average		n	
Major diameter (µm)	0.97	±0.11	50	0.96	±0.11	50	0.725
Minor diameter (µm)	0.82	±0.10	50	0.82	±0.08	50	0.933

Condition	Oa1+/+			Oa1-/-			t-test
NT in	average		n	average		n	
Major diameter (µm)	0.88	±0.10	44	0.89	±0.09	44	0.527
Minor diameter (µm)	0.77	±0.08	44	0.79	±0.08	44	0.262
NT out	average		n	average		n	
Major diameter (µm)	0.83	±0.10	50	0.86	±0.09	50	0.091
Minor diameter (µm)	0.75	±0.08	50	0.75	±0.07	50	1.000

Condition	Oa1-/-LOA1SN			Oa1-/-LOA1Δ18SN			t-test
NT in	average		n	average		n	
Major diameter (µm)	0.93	±0.08	50	0.93	±0.10	50	0.904
Minor diameter (µm)	0.82	±0.08	50	0.81	±0.09	50	0.698
NT out	average		n	average		n	
Major diameter (µm)	0.98	±0.09	44	1.01	±0.09	41	0.088
Minor diameter (µm)	0.82	±0.10	44	0.85	±0.10	41	0.193
Nocodazole	average		n	average		n	
Major diameter (µm)	0.85	±0.08	50	0.88	±0.07	50	0.114
Minor diameter (µm)	0.78	±0.08	50	0.80	±0.07	50	0.189
EGFP-MC-LT	average		n	average		n	
Major diameter (µm)	0.86	±0.09	50	0.84	±0.09	50	0.410
Minor diameter (µm)	0.76	±0.08	50	0.76	±0.08	50	1.000

NT in and out, melanosomes selected inside and outside of the perinuclear area, respectively, in untreated melanocytes; nocodazole e cytochalasin D, cells treated with the indicated drugs; EGFP-MC-LT, cells expressing the dominant-negative mutant of myosin Va. n, number of melanosomes analyzed. t-test, *P* values resulting from unpaired Student's t-test assuming equal variances.