SUPPLEMENTARY INFORMATION.

Supplementary Methods

Electron microscopy. Melan-Ink4a cells that were stably transduced with retrovirus to express OCA2-AA23 (following selection with 400 μg/ml hygromycin) were fixed with 2% paraformaldehyde/ 0.2% glutaraldehyde in PHEM buffer (60 mM PIPES, 25 mM HEPES, 2 mM $MgCl_2$, 10 mM EGTA pH 6.9) and the processed for cryosectioning as described (Raposo *et al*., 2001). Ultrathin cryosections were prepared with an Ultracut FCS ultracryomicrotome (Leica, Vienna, Austria), and single or double immunogold labeled using a rat anti-HA11 antibody (Roche Diagnostics) with or without rabbit anti-γ-adaptin (anti-AP-1; Seaman *et al*., 1996) and protein A conjugated to 10 – or 15-nm gold (PAG10 or PAG15). Sections were analyzed with a Philips CM120 electron microscope (FEI, Eindoven, The Netherlands), and digital acquisitions were made with a Keen View numeric camera (Soft Imaging System, Münster, Germany).

Supplementary Reference

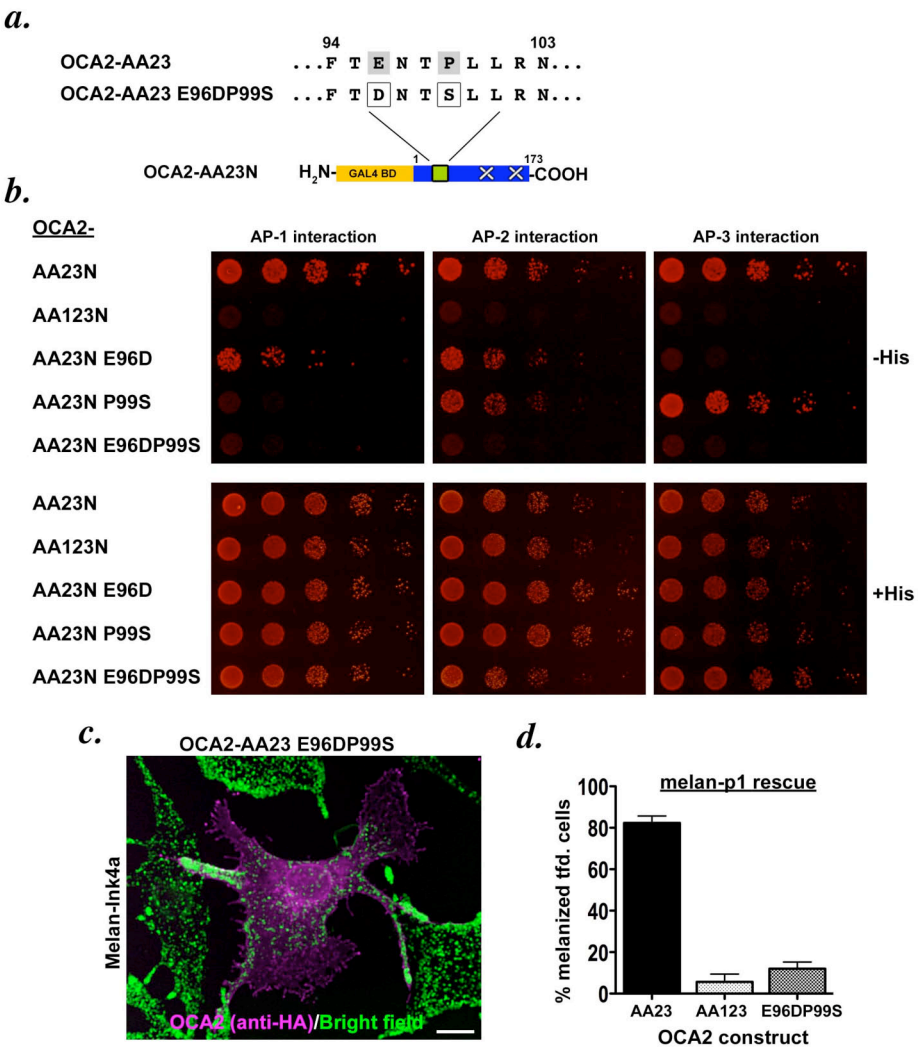
Seaman, M. N., Sowerby, P. J., and Robinson, M. S. (1996). Cytosolic and membrane-associated proteins involved in the recruitment of AP-1 adaptors onto the trans-Golgi network. J. Biol. Chem. *271*, 25446–25451

Supplementary Table 1. Conservation of LL1 motifs among OCA2 homologs in vertebrates

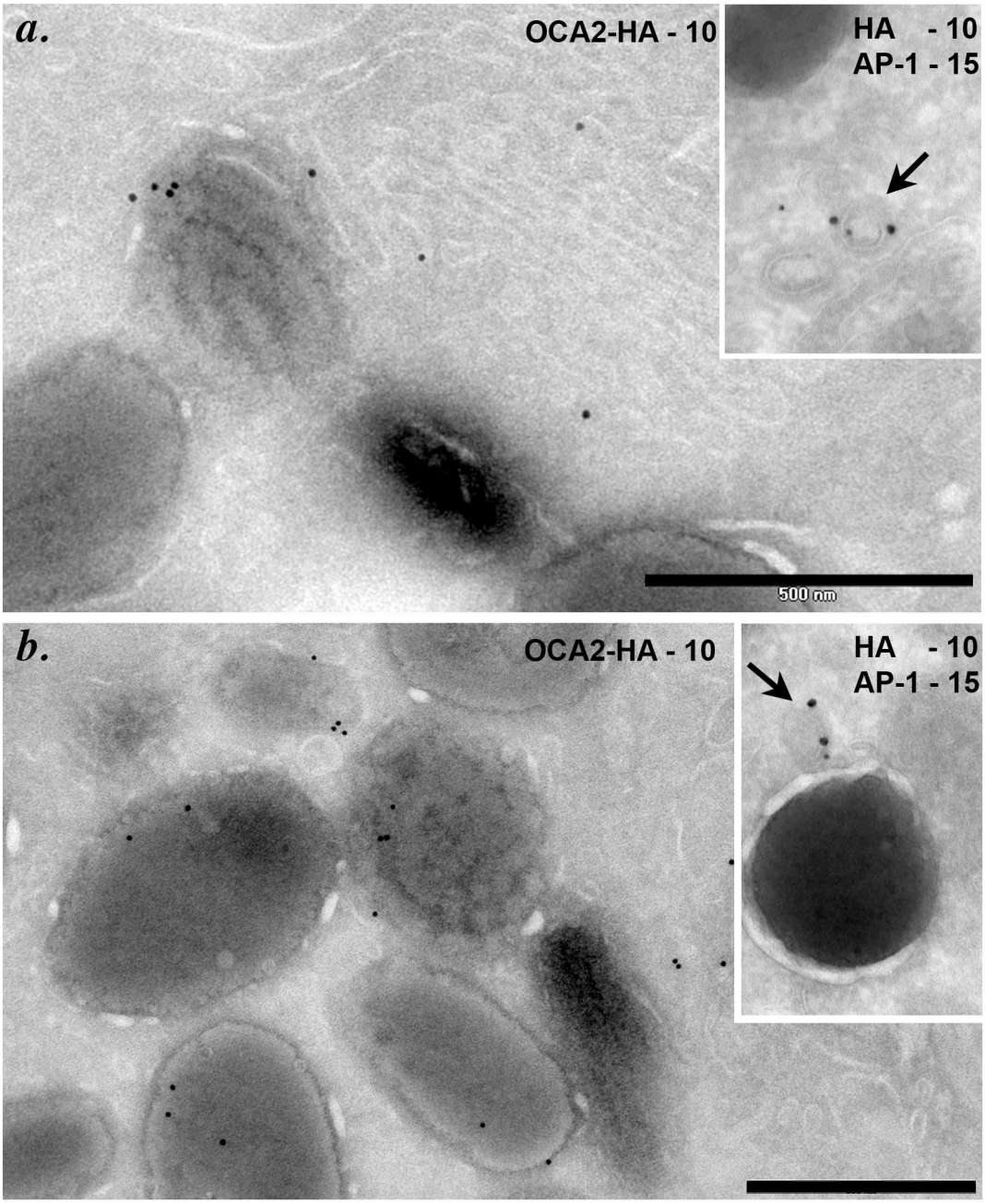
Organism	Protein Accession	First amino acid	Sequence
Human (*Homo sapiens*)	NP_000266	96	**E**NT**PLL**
Chimpanzee (*Pan troglodytes*)	XP_001162129	96	**E**NT**PLL**
Gibbon (*Nomascus leucogenys*)	XP_003272870	96	**E**NT**PLL**
Marmoset (*Callithrix jacchus*)	XP_002806937	152	**E**NT**PLL**
Rhesus macaque (*Macaca mulatta*)	XP_002804735		-[a]
Eur. rabbit (*Oryctolagus cuniculus*)	XP_002718259	103	**E**NT**PLL**
Mouse (*Mus musculus*)	NP_068679	92	**E**DT**PLL**
Rat (*Rattus norvegicus*)	XP_002725672	101	**E**DT**PLL**
Pig (*Sus scrofa*)	NP_999259	103	**E**NT**PLL**
Cattle (*Bos taurus*)	XP_002685246	103	**E**NT**PLL**
Horse (*Equus caballus*)	XP_001494337	53	**E**NT**PLL**
Dog (*Canis lupus familiaris*)	XP_545800	20	**E**NT**PLL**
Panda (*Ailuropoda melanoleuca*)	XP_002930355	49	**E**NT**PLL**
Opossum (*Monodelphis domestica*)	XP_001366525	188	DFG**PLL**[a]
Chicken (*Gallus gallus*)	XP_425579		-[a]
Turkey (*Meleagris gallopavo*)	XP_003203180	109	**E**RT**PLL**
Zebra finch (*Taenopygia guttata*)	XP_002194840	179	**E**KT**PLL**
Clawed frog (*Xenopus (Silurana) tropicalis*)	XP_002937026	106	**E**KT**PLL**
Green anole (*Anolis carolinensis*)	XP_003218820	34	**E**RS**PLL**
Mexican cavefish (*Astyanax mexicanus*)	ABB29299	97	**E**RS**PLL**
Japanese medaka (*Oryzias latipes*)	NP_001098262	101	**E**RS**PLL**
Zebrafish (*Danio rerio*)	XP_695807	97	**E**RT**PLL**

Blue residues differ from the human sequence, ENTPLL.

[a]The conservation between these proteins and human OCA2 is very poor throughout the cytoplasmic N-terminal domain or the NCBI database in this region is incomplete.



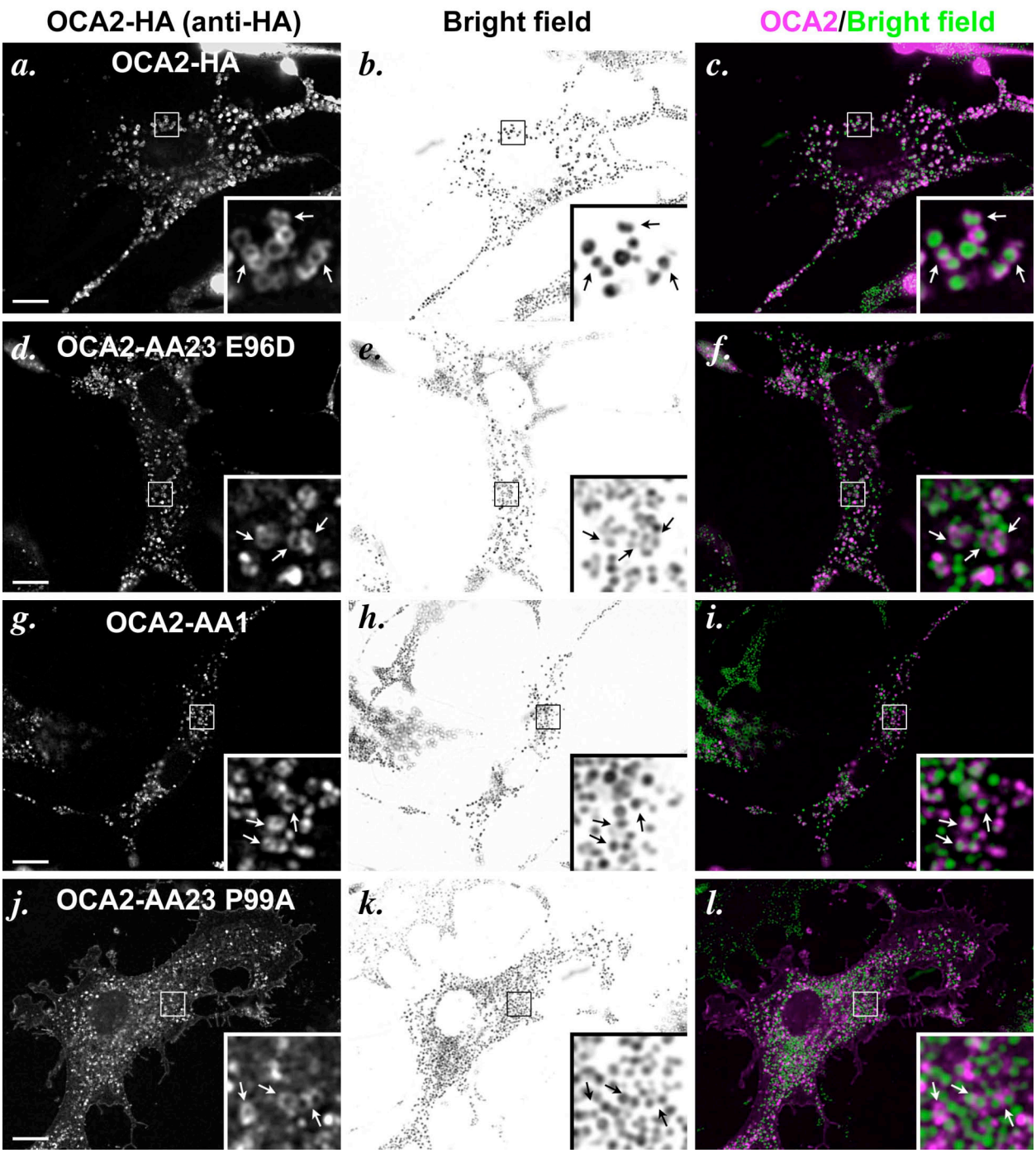
<u>Supplementary Figure S1.</u> The E96DP99S combined dileucine mutant is nonfunctional.
(a) The OCA2 LL1 motif was subjected to two amino acid substitutions in the OCA2-AA23N yeast three-hybrid construct to create the OCA2-AA23N E96DP99S mutant. (b) Yeast three-hybrid results show that the E96DP99S construct does not interact with the three AP complexes. (c) Full-length OCA2-AA23 E96DP99S was transiently transfected into melan-Ink4a melanocytes. The transgene is visualized by anti-HA antibodies (magenta) and pigmented melanosomes are visualized by Bright field microscopy (inverted and colored green). Bar = 10 μm. (d) OCA2-AA23 E96DP99S was transiently transfected into melan-p1 melanocytes and assayed for rescue of melanin synthesis as in Figure 2j. The graph shows the results from three independent transfections.



<u>Supplementary Figure S2.</u> OCA2 localizes to melanosomes, the TGN and AP-1-coated vesicles in melanocytic cells.

HA-tagged OCA2-AA23 was stably expressed in wild-type melan-ink4a melanocytes after retroviral transduction and selection with hygromycin. Ultrathin cryosections of fixed cells were immunogold labeled with anti-HA and 10 nm protein A gold (OCA2-HA 10) alone (a, b) or with anti-γ-adaptin and 15 nm protein A gold (AP-1 15; insets). a, note labeling over the *trans* Golgi network and nearby pigmented melanosomes. b, note the predominant labeling over the lumen

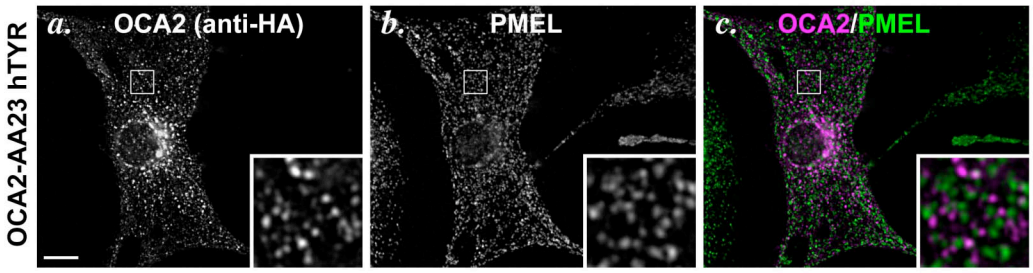
and limiting membrane of melanosomes. Insets, note labeling on coated vesicle structures that are in close proximity with and/ or fusing with melanosomes.



<u>Supplementary Figure S3.</u> OCA2 variants are trapped on enlarged melanosomes in some AP-3-deficient melanocytes.

HA-tagged human OCA2 (a-c), OCA2-AA23 E96D (d-f), OCA2-AA1 (g-i), or OCA2-AA23 P99A (j-l) was transiently transfected into AP-3-deficient melan-pe melanocytes. The transgene was visualized with anti-HA antibodies (a, d, g, j) and melanosomes were visualized by Bright

field microscopy (b, e, h, k). OCA2 (magenta) and Bright field (green) images were merged in (c, f, i, l). All insets show 5X magnified images of the boxed region. Arrows point to regions of overlap between OCA2 and pigmented melanosomes. Bar = 10 μm.



<u>Supplementary Figure S4.</u> The dileucine motif does not determine BLOC-1 dependence. The OCA2-AA23 hTYR construct was transiently transfected into BLOC-1-deficient melanpallid melanocytes. The transgene was visualized with anti-HA antibodies (a) and hypopigmented melanosomes were visualized by antibodies to PMEL (b). OCA2 (magenta) and PMEL (green) images were merged in (c). All insets show 5X magnified images of the boxed region. Bar = 10 μm.