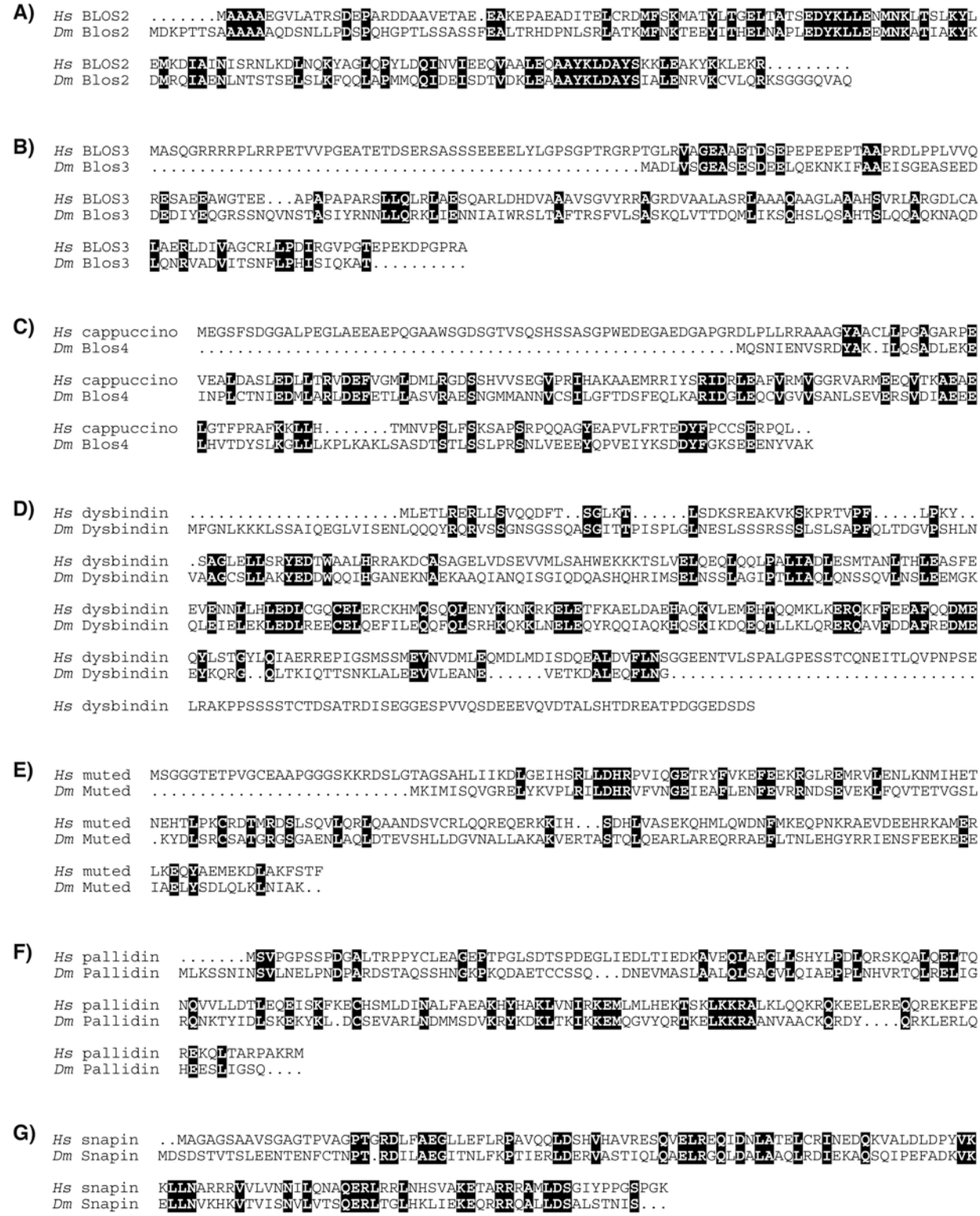


**Figure S1.** Sequence alignments of the BLOC-1 subunits BLOS2 (**A**), BLOS3 (**B**), cappuccino (**C**), dysbindin (**D**), muted (**E**), pallidin (**F**) and snapin (**G**) from *Homo sapiens* (*Hs*), with homologues from *Drosophila melanogaster* (*Dm*). Identical amino acid residues are highlighted.

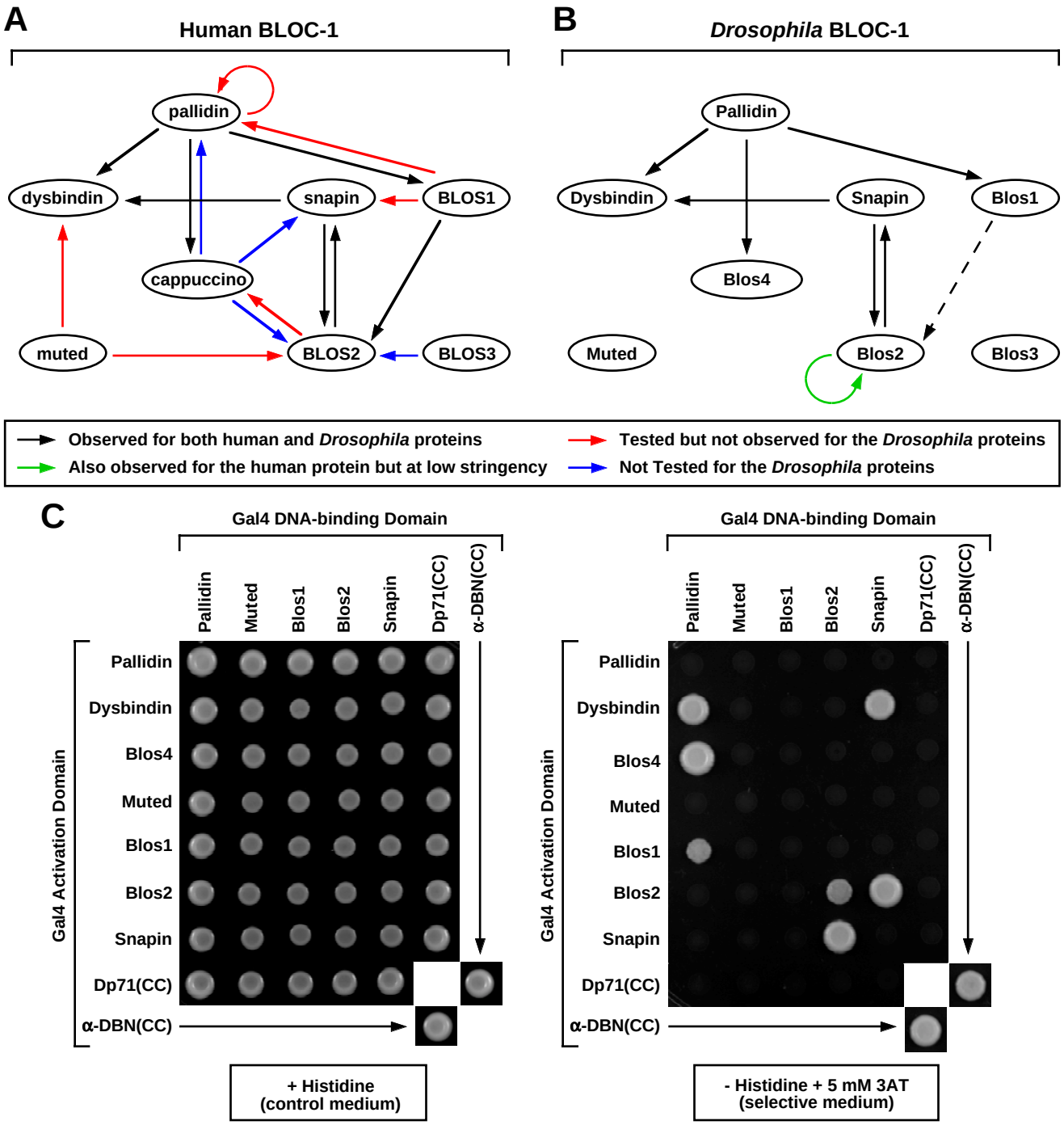


**Figure S2.** Interaction maps inferred from yeast-two-hybrid (Y2H) analyses of subunits of BLOC-1 from humans (**A**) and their homologues from *Drosophila melanogaster* (**B**). Arrows denote the combination of constructs (DNA-binding domain → activation domain) that yielded positive results. The map shown in (A) was inferred from data reported elsewhere [Starcevic, M. and Dell'Angelica, E.C. (2004) Identification of snapin and three novel proteins (BLOS1, BLOS2, and BLOS3/reduced pigmentation) as subunits of biogenesis of lysosome-related organelles complex-1 (BLOC-1). *J. Biol. Chem.*, **279**, 28393-28401], while the map shown in (B) was inferred from data reported herein except for the dashed arrow, which represents a result from a recent study [Schwartz, A.S., Yu, J., Gardenour, K.R., Finley, R.L., Jr. and Ideker, T. (2009) Cost-effective strategies for completing the interactome. *Nat. Methods*, **6**, 55-61]. (**C**) Y2H of BLOC-1 subunit homologues from flies. Cells co-transformed with plasmids encoding the indicated proteins were cultured on control medium or medium lacking histidine and containing 3-amino-1,2,4-triazole (3AT), the latter to select for strong expression of the *HIS3* reporter gene.

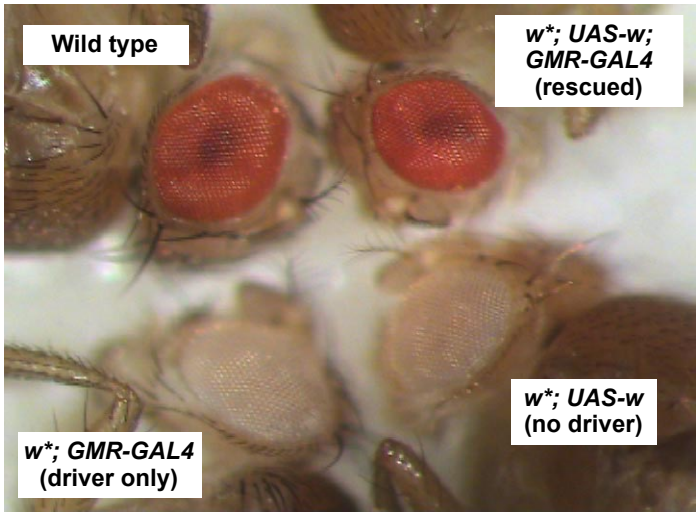


**Figure S3.** Genetic test to verify the activity of a modified *GMR-GAL4* driver transgene that lacks the *mini-white* marker gene. The photograph shows the eye pigmentation phenotype of Canton-S flies (wild type) and those of flies homozygous for an unspecified null allele of the *white* gene (*w**) and carrying the *UAS-w* transgene (for expression of the wild-type White protein under the control of the yeast *GAL4* transcription factor), the modified *GMR-GAL4* driver (for expression of *GAL4* in the developing eye), or both. Notice the lack of eye pigmentation in *w** flies carrying only the *GMR-GAL4* driver, and the full rescue of eye color defects in those also carrying the *UAS-w* transgene.

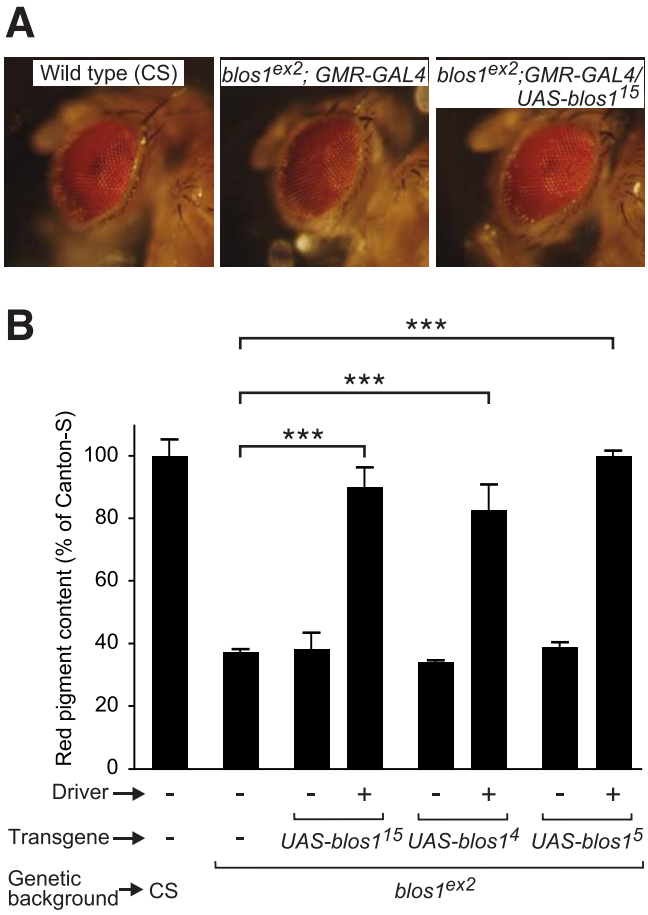


**Figure S4.** Transgenic rescue of the eye pigmentation phenotype of *blos1* mutant flies. (**A**) Eye pigmentation phenotypes of adult flies of the Canton-S (CS) line or homozygous for the $blos1^{ex2}$ allele and carrying an eye-specific driver (*GMR-GAL4*) with or without a transgene encoding wild-type Blos1 under the control of *GAL4*-dependent, upstream activating sequences (*UAS-blos1*). The eyes of both wild-type and rescued flies display a bright-red color with a characteristic dark spot known as the pseudopupil. (**B**) Quantification of red pigments extracted from the heads of young adult male flies (2-3 days after eclosion) of the indicated genotypes. The $UAS\text{-}blos1^{4}$, $UAS\text{-}blos1^{5}$ and $UAS\text{-}blos1^{15}$ alleles arose from independent insertions on chromosome 3 of the same *UAS-blos1* transgene. Data are expressed as percentages of the red pigment content of Canton-S flies and represent means ± SD. One-way ANOVA followed by Bonferroni comparison of selected group pairs: ***$p < 0.001$.

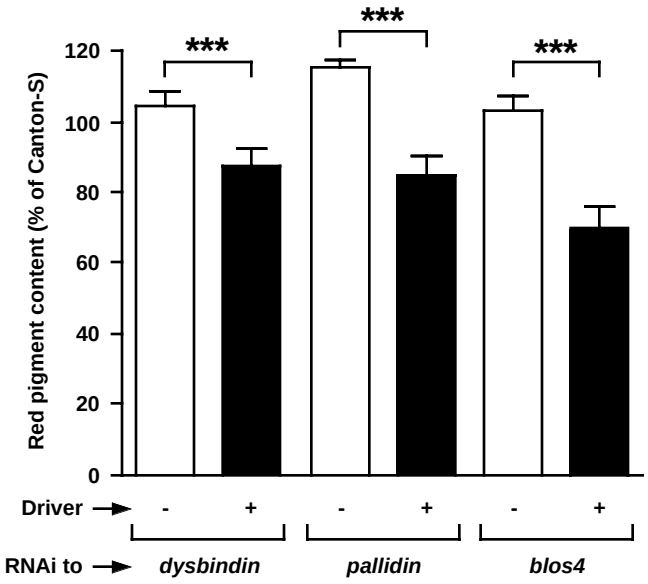


**Figure S5.** Effects of eye-specific RNA interference of selected *Drosophila* BLOC-1 subunits on red pigment content. Transgenic flies carrying RNAi constructs, which were designed to silence the products of the indicated fly genes in tissues expressing the transcription factor encoded by the yeast *GAL4* gene, were crossed with appropriate stocks to obtain adult male flies carrying a normal allele of the *white* gene plus one copy of the indicated RNAi construct, and without (empty bars) or with (solid bars) the *GMR-GAL4* driver for expression of *GAL4* in the developing eye. Red pigments were extracted from pools of 2-3-day-old male fly heads, and quantified by spectrophotometry. Data represent percentages of the values obtained in parallel for wild-type Canton-S flies, and are expressed as means ± SD. One-way ANOVA followed by Bonferroni tests: *** $p < 0.001$.

**Supplementary Table S1.** Effects of Auxilin missexpression on the eye pigmentation of wild-type and mutant flies

| Genetic background | Without *UAS-aux* | | Carrying the *UAS-aux* transgene | | |
|---|---|---|---|---|---|
| | Without driver | *GMR-GAL4* (+ driver) | Without driver | *GMR-GAL4* (+ driver) | +/- Driver ratio |
| Canton-S | 100.0 ± 5.8 | 112.9 ± 6.7 | 101 ± 10 | 72.2 ± 4.4 | 0.71 |
| $blos1^{ex2}$ | 43.0 ± 1.6 | 42.1 ± 4.5 | 42.8 ± 4.0 | 8.1 ± 1.8 | 0.19 |
| $or^{49h}$ | 11.3 ± 1.2 | 10.4 ± 0.6 | 11.3 ± 2.5 | 2.9 ± 0.4 | 0.26 |
| $g^{2}$ | 27.4 ± 1.1 | 25.1 ± 2.7 | 22.2 ± 1.1 | 2.7 ± 0.5 | 0.12 |
| $g^{53d}$ | 4.1 ± 0.3 | 3.4 ± 0.6 | 3.6 ± 1.2 | 0.7 ± 0.7 | 0.19 |
| $rb^{1}$ | 16.2 ± 0.9 | 18.9 ± 1.4 | 21.0 ± 1.0 | 2.1 ± 0.2 | 0.10 |
| $ltd^{1}$ | 38.3 ± 5.3 | 44.6 ± 2.8 | 36.9 ± 2.3 | 17.0 ± 2.8 | 0.46 |

Red pigments were extracted from the heads of male flies of the wild-type line (Canton-S), or homozygous for the indicated mutant alleles, and lacking or carrying the *UAS-aux* and *GMR-GAL4* transgenes for *GAL4*-dependent misexpression of Auxilin in the developing eye. The extracted pigments were quantified by spectrophotometry and expressed as percentages of the pigment content of Canton-S flies lacking any transgenic construct (mean ± SD). The extent of *GAL4*-dependent effect was estimated by calculating a ratio between the values obtained for flies carrying *UAS-aux* with and without the *GMR-GAL4* driver.

**Supplementary Table S2.** Summary of *post-hoc* Bonferroni's tests performed to compare the effects on eye pigmentation between selected pairs of misexpressed Auxilin constructs

| *Post-hoc* test | | | Genetic background | | |
|---|---|---|---|---|---|
| Transgenic construct | *vs* | Transgenic construct | *blos1*$^{ex2}$ (Fig. 8C) | *or*$^{49h}$ (Fig. 8D) | *g*$^{2}$ (Fig. 8F) |
| (none) | *vs* | FL | * | *** | *** |
| | | ΔC | NS | NS | *** |
| | | CJ | *** | *** | *** |
| | | ΔJ | —[a] | —[a] | *** |
| ΔC | *vs* | FL | *** | *** | *** |
| | | CJ$^{DLL/DPF}$ | NS | NS | ** |
| | | CJ$^{DLL}$ | —[a] | —[a] | *** |
| | | CJ$^{DPF}$ | NS | ** | *** |
| CJ | *vs* | CJ$^{DLL/DPF}$ | *** | *** | *** |
| | | CJ$^{DLL}$ | —[a] | —[a] | *** |
| | | CJ$^{DPF}$ | *** | *** | *** |

Bonferroni's multiple comparison tests were performed on the data represented in Fig. 8C, D and F, for which an initial ANOVA test had reached statistical significance: *p < 0.05; **p < 0.01; ***p < 0.001; NS, not significant.

[a] Not determined because all available ΔJ and CJ$^{DLL}$ construct transgenes were inserted on chromosome 2, which also harbors the *blos1* and *or* genes.