

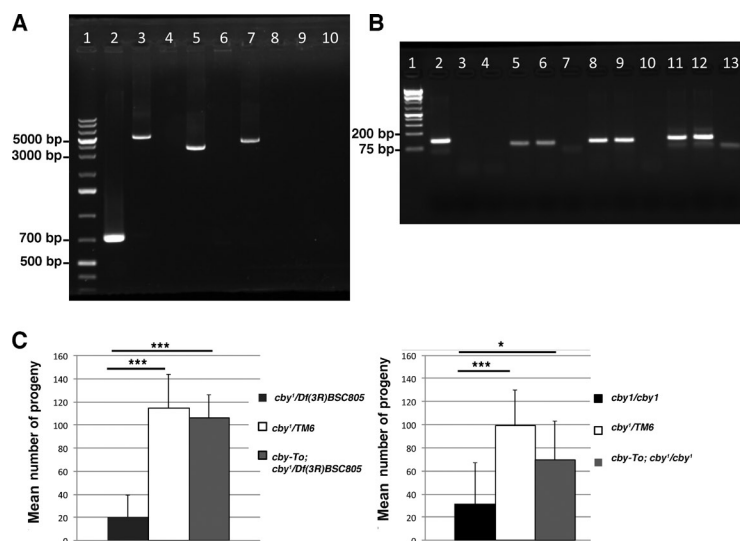
Enjolras et al., <http://www.jcb.org/cgi/content/full/jcb.201109148/DC1>

Figure S1. ***cby¹* is a null allele of *cby*, and *cby^{1/1}* flies are hypofertile.** (A) Genomic DNA from *w¹¹¹⁸* and *cby^{1/1}* flies was amplified by PCR using three sets of primers described in Fig. 4 A. Lane 1: 0.35 μ g of GeneRuler 1-kb Plus DNA Ladder (Thermo Fisher Scientific); lanes 2, 3, and 8: primers A/B; lanes 4, 5, and 9: primers C/D; lanes 6, 7, and 10: primers E/F; lanes 8–10: control with no DNA; lanes 2, 4, and 6: *w¹¹¹⁸* genomic DNA; lanes 3, 5, and 6: *cby^{1/1}* genomic DNA. The sizes of the PCR products are as follows: 720 bp in *w¹¹¹⁸* flies and 5,129 bp in *cby^{1/1}* flies for A/B primers; no product in *w¹¹¹⁸* and 3,603 bp in *cby^{1/1}* flies for C/D primers; no product in *w¹¹¹⁸* and 4,275 bp in *cby^{1/1}* flies for E/F primers. (B) PCR was performed on cDNA from *w¹¹¹⁸* (lanes 2, 5, 8, and 11) or *cby^{1/1}* (lanes 3, 6, 9, and 12) testes with the following primers: *cby* a/b (127 bp) in lanes 2–4; CG6569 a/b (94 bp) in lanes 5–7; CG31174 a/b (106 bp) in lanes 8–10; and CG9874 a/b (125 bp) in lanes 11–13. Lane 1: 0.5 μ g of GeneRuler 1-kb Plus DNA Ladder; lanes 4, 7, 10, and 13: no cDNA. No *cby* transcript was detected in *cby^{1/1}* flies. The expression of the two neighboring genes CG6569 and CG31174 was not affected as well as CG9874/*TBP*, a housekeeping gene control. (C) The fertility of flies was assessed by measuring the mean number of adult progeny issued from crosses ($n > 15$) of individual males with control *w¹¹¹⁸* females. *cby^{1/1}* and *cby¹/Df(3R)BSC805* males are hypofertile; some males are fertile but give less progeny than control males, and many males are sterile. One copy of the *cby-Tomato* transgene is sufficient to rescue fertility for both genotypes. (left) P-values for Student's *t* test are as follows: ***, *cby¹/Df(3R)BSC805* versus *cby¹/TM6* = $4.79\text{E}-08$ and *cby¹/Df(3R)BSC805* versus *cby-To*; *cby¹/Df(3R)BSC805* = $7.06\text{E}-09$. (right) P-values for Student's *t* test are as follows: ***, *cby^{1/1}* versus *cby¹/TM6* = $1.17\text{E}-06$; *, *cby^{1/1}* versus *cby-To*; *cby^{1/1}* = 0.0097. Error bars represent SD.

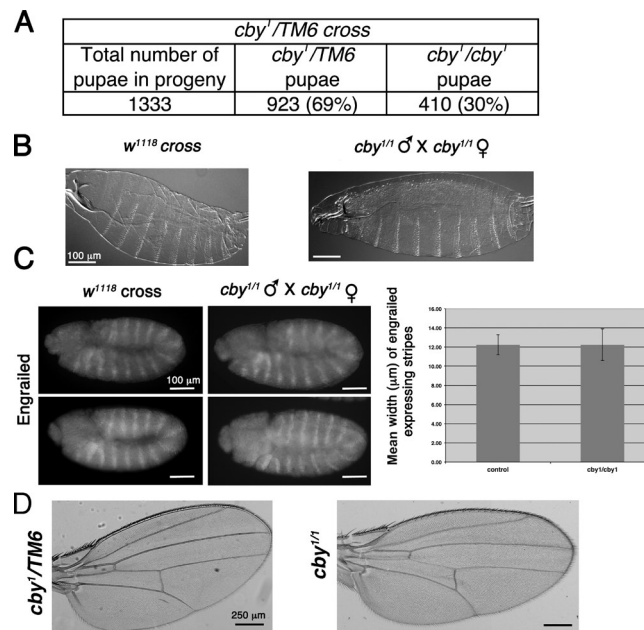


Figure S2. **No Wg-associated phenotypes are observed in *cby*^{1/1} flies.** (A) Survival rates of the progeny issued from *cby*¹/*TM6* heterozygous flies. *cby*^{1/1} flies are present in Mendelian ratios. (B) Embryos derived from homozygous *cby*^{1/1} females and *cby*^{1/1} males have normally patterned cuticles compared with control (*w*¹¹¹⁸), showing that even in absence of maternally provided CBY, embryos develop normally. (C) Engrailed expression in embryos issued from *w*¹¹¹⁸ males and females or from *cby*^{1/1} males and females. No differences of the engrailed expression domains can be observed. The width (in micrometers) of the engrailed expression domain was measured and shows no differences. Error bars represent SD. (D) No Wg gain- or loss-of-function-associated phenotypes are observed in wings of *cby*^{1/1} flies. The wings of *cby*^{1/1} and control *cby*¹/*TM6* have identical bristle, vein, and hair patterns.

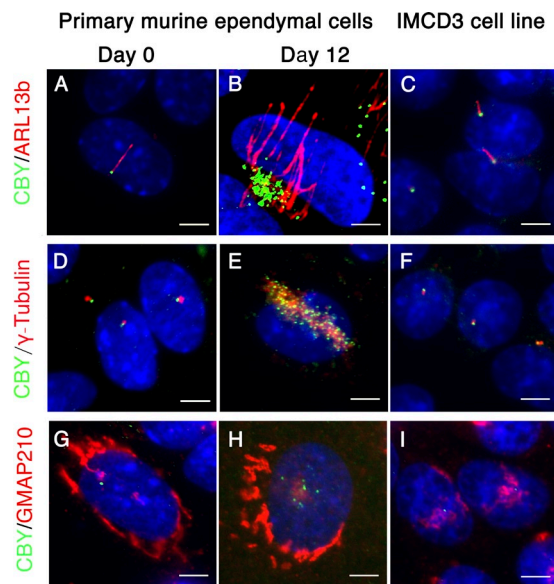


Figure S3. **CBY is associated with basal bodies in mouse ciliated cells.** CBY localization was followed by immunolabeling of different types of mouse ciliated cells. (A, D, and G) Ependymal cells derived from newborn mouse brains. Cells were fixed just before serum deprivation. (B, E, and H) Ependymal cells derived from newborn mouse brains. Cells were fixed after 12 d of serum deprivation. (C, F, and I) Mouse IMCD3 cell lines fixed after 48 h of serum deprivation. (A–C) CBY localizes at the base of the cilium labeled with an anti-ARL13b antibody. (D–F) CBY protein is apposed to the centriole, as observed with γ -tubulin staining. (G–I) CBY is not associated with the cis-Golgi compartment. However, some GMAP210 and CBY staining can be observed at the base of the cilia in multiciliated ependymal cells. Bars, 5 μ m.

Table S1. Primers used for transgenic constructs and molecular characterization of the *cby* locus

Primer	Sequence
F-3'cby/SphI	5'-TAATCC GCATGC TTTGATATACTGAATATTTTATTGTCA-3'
R-5'cby/Ascl	5'-TAATCC GGCGCGCC AAACGGAAAGTTGACCACATT-3'
3'cby/NotI	5'-TAATCC GGGGCCGC GAGTTCCGGACGACAAAGAC-3'
F-5'cby/BsiWI	5'-TAATCC CGTACG GCCCCATTAAACATTAATTGAGC-3'
CG 11356-PRO3	5'-GGA AGATCT ACAAGAAGTGGACTCACCTTTTCG-3'
CG 11356-PRO5	5'-CGG GGTACCC AATAGGCCAGTAGGTCAGTGGC-3'
F-14870/BamHI	5'-TAATC GGATCC GGTTCAACACACGACGCTCG-3'
R-14870/NotI	5'-TTATC GGGGCCGC AGGGAGTGCCCAATGGTAGCCCCA-3'
Cby-PRO3/Agel	5'-TAATC ACCGGT CTTTTCCTTGGCTTCAGCTCA-3'
Cby-pro5	5'-GACCTAAAATT GAATTC CGAAAACG-3'
Cby-pro3/BamHI	5'-CG GGATCC TTAGTCCGTCGCGCTCGGCCAGCAG-3'
A	5'-CGTGCAGCAAGGAGTTCTCT-3'
B	5'-AGATTTTAGCATTTTATTAGTGAAATC-3'
C	5'-TGGATTGTTCAATTGAACAATGG-3'
D	5'-TGCAGGTCGACTCTAGAGGA-3'
E	5'-GTTTGAATTGAATTGACGCTCC-3'
F	5'-AATACCGCTCCGTCACATTC-3'
cbya	5'-CACCGAGGATCTGGATGACT-3'
cbyb	5'-GTTCAGCCGAAGCATATCAT-3'
CG6569a	5'-TTACCACCATAAAGGAGAAGCTG-3'
CG6569b	5'-TTTTGCTCCAGGCGATTG-3'
CG31174a	5'-TGGAGGAACAACAAGCTGAA-3'
CG31174b	5'-TCATCCATGTCTCTTGAAGG-3'
CG9874a	5'-ACTCCAGACTGGCAGCGAGAAAGTA-3'
CG9874b	5'-CAAGCGTATGGGGAACCTTGACATC-3'

Bold letters represent cloning restriction sites.