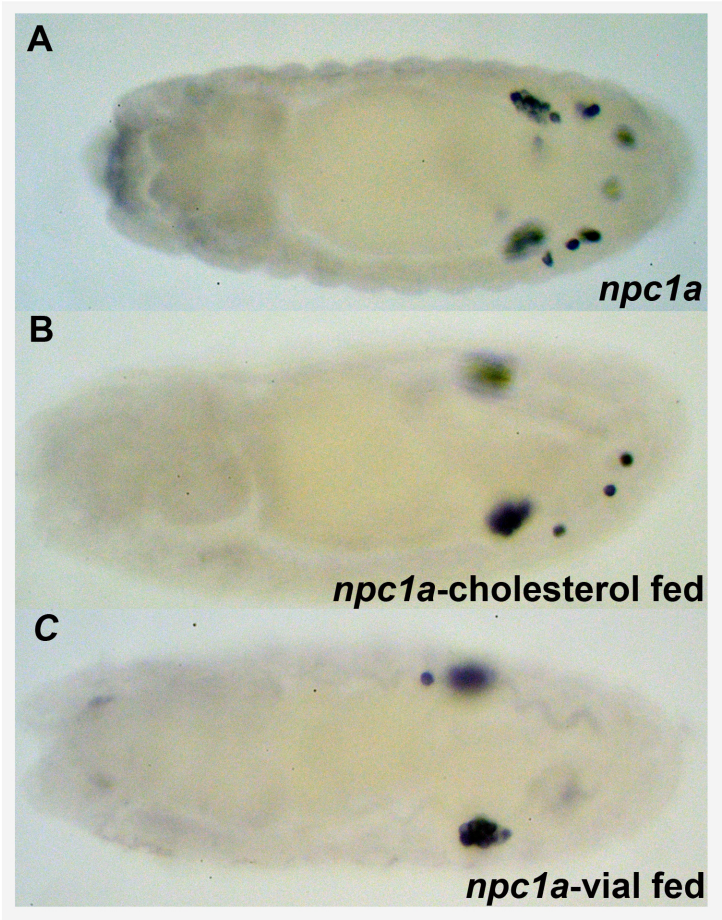

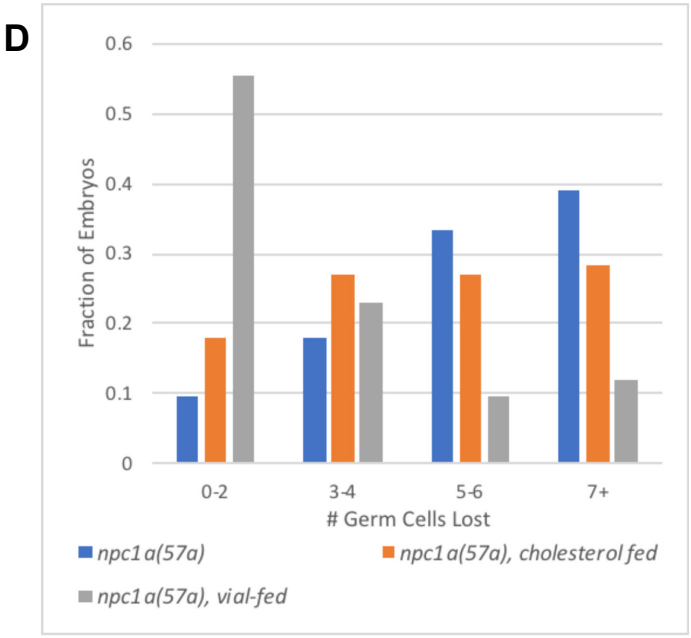

Figure S1. Cholesterol rich diet can rescue *NPC1a* mutant phenotype.

Embryos carrying mutant *NPC1a* allele raised on normal diet and cholesterol-rich diet in two different ways were screened for germ cell migration defects. The parents of "vial-fed" embryos were fed excess cholesterol for a longer period of time than those of "cholesterol-fed" embryos. Consistently vial-fed *NPC1a* embryos had the least defects. Embryos between stages 13-15 were analyzed.

(A): $NPC1a^{57A}$ (n=72).

(B): *NPC1a* embryo derived from 'cholesterol-fed' parents (n=364; $p<0.05$).

(C): $NPC1a^{57A}$ embryo derived from 'vial-fed' parents at stage 15 with 1 mis-migrated cells (n=83; $p<0.005$).

(D): Distribution of germ cell migration defects in $NPC1a^{57A}$, cholesterol fed $NPC1a^{57A}$ embryos, and vial-fed $NPC1a^{57A}$ embryos.

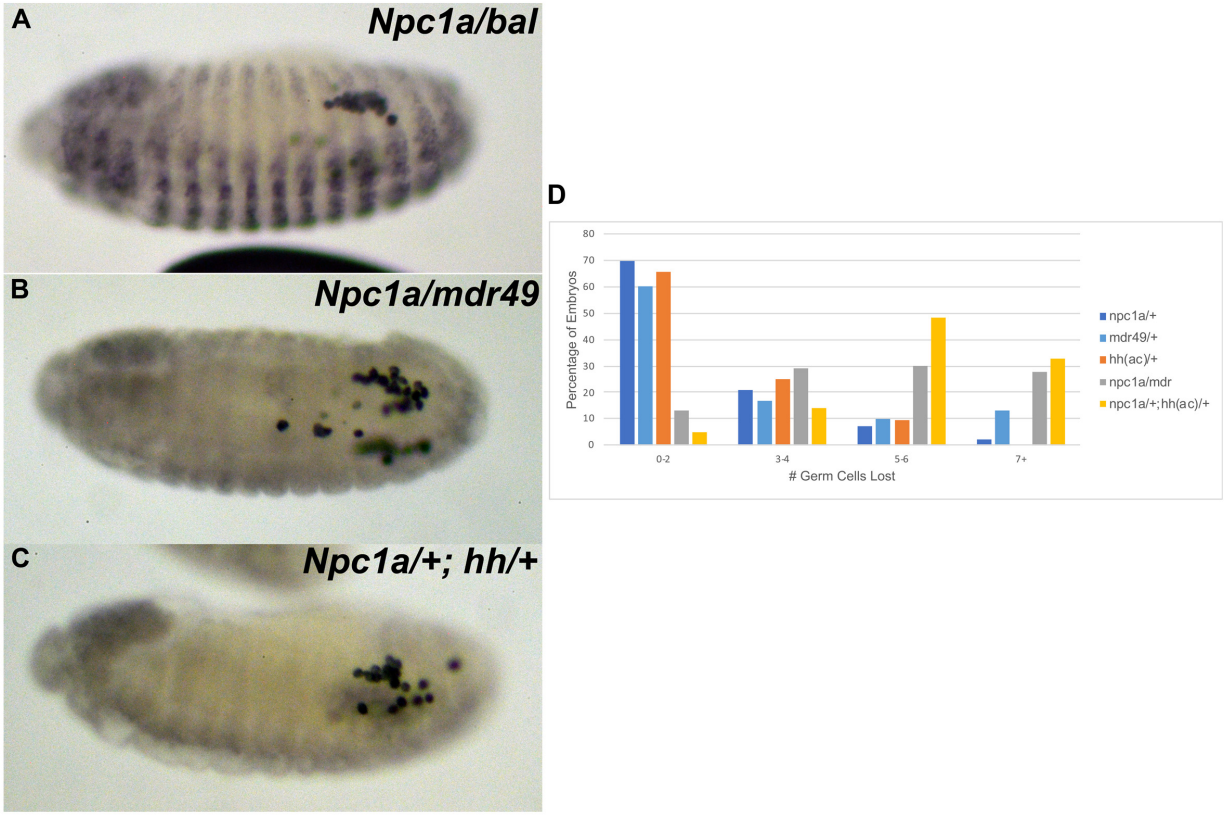

Figure S2. Genetic interaction between *NPC1a* and *mdr49 or hh* during germ cell migration.

Embryos between stages 13–15 of the indicated genotype were stained with anti-Vasa and β-galactosidase antibody. Total number of germ cells that failed to coalesce and remained scattered were counted per embryo. Nearly 70% of *NPC1a/+* embryos show 0–2 lost germ cells (blue and red bars respectively). But when embryos are simultaneously compromised for both *NPC1a* and *mdr49 or hh*, ~ 60% of the embryos have 5 or more lost germ cells.

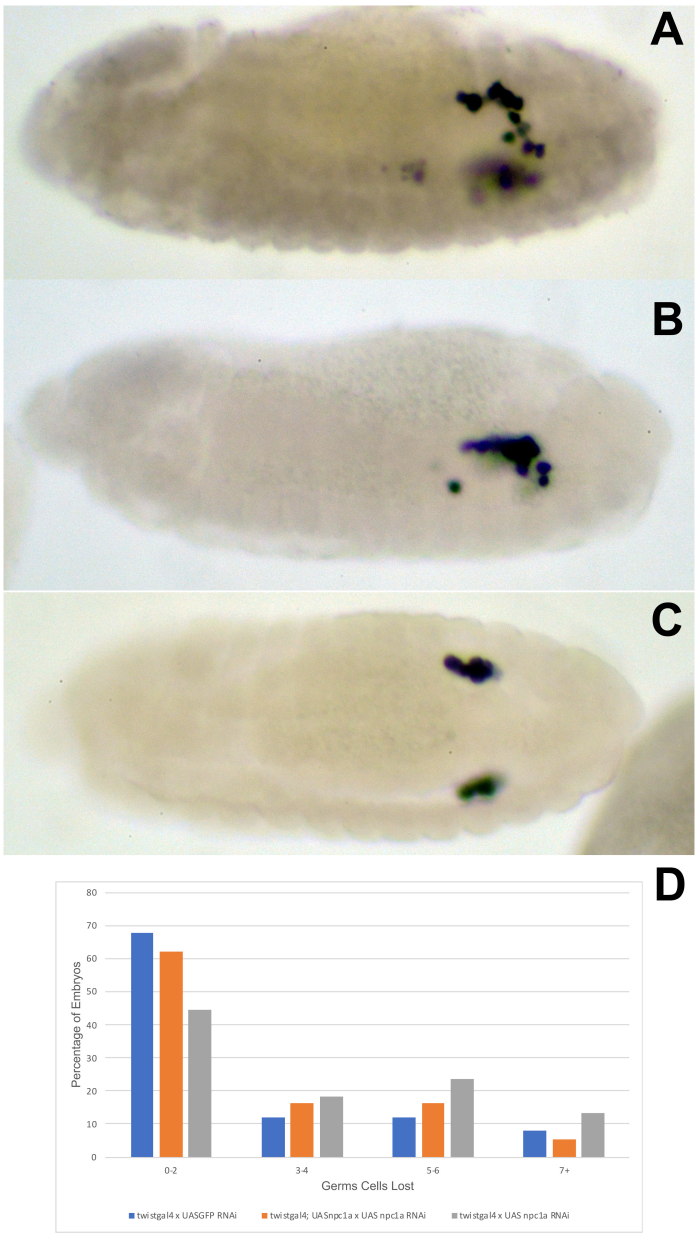

Figure S3. Overexpression of *NPC1a* can rescue aberrant germ cell migration induced by mesodermal inactivation of *NPC1a*.

Simultaneous expression of *UAS-NPC1a* and *UAS-NPC1a RNAi* in the embryonic mesoderm mitigates the germ cell migration defects induced by *NPC1aRNAi*.

(A): *twist-GAL4: UAS-NPC1a-RNAi* (n=38; p<0.05).

(B) and (C): *twist-GAL4*; *UAS-NPC1a/UAS-NPC1a-RNAi* (n=37; ***The difference between control and the 'rescued' sample is statistically insignificant (p>0.1) indicating the specific and efficient nature of the rescue).***

(D): Comparison of germ Cell migration defects observed in the two genotypes.

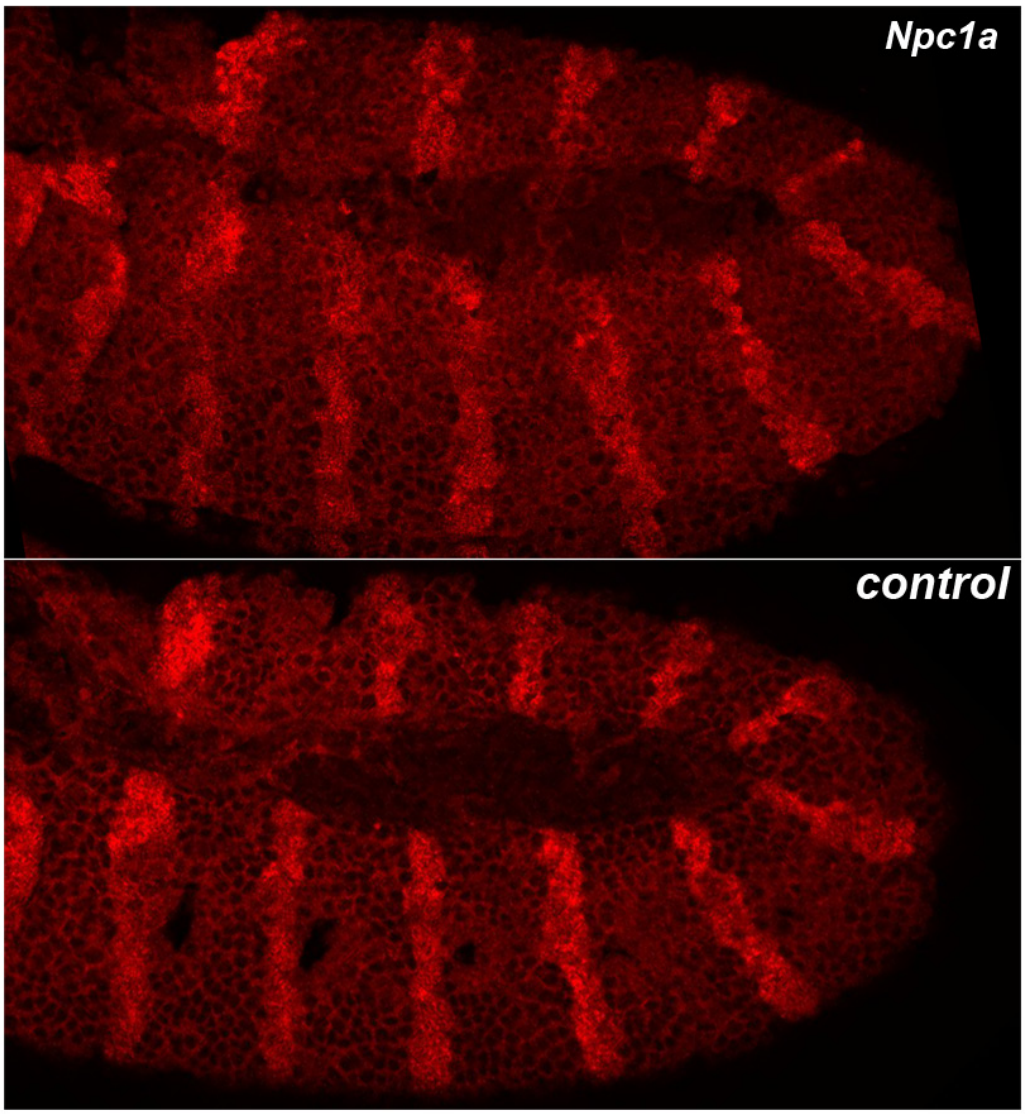

Figure S4. Engrailed expression is properly maintained in NPC1a embryos. Embryos from the *NPC1a*^{57A}*/ Cy0, en:LacZ* stock were collected and fixed using standard procedure. Embryos were genotyped by simultaneously staining them with β-galactosidase (imaged in green: not shown) and En (imaged in red) antibodies. Both control (*Balancer*) and mutant embryos show strong En specific expression in 14 stripes. Pixel intensities were compared between several pairs of embryos at identical stage and no significant difference in En (or Wg; not shown) protein levels was observed.

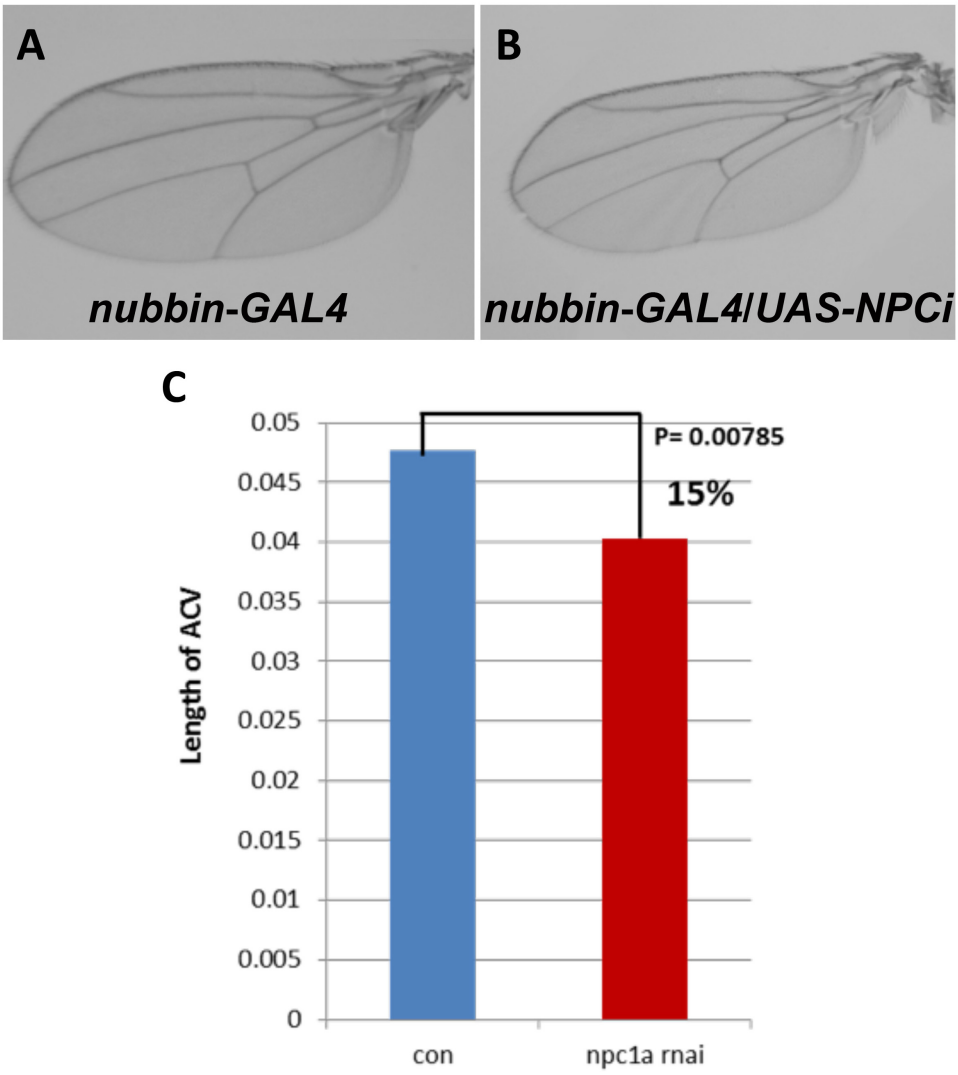

Figure S5. Compromised levels of NPC1a result in adult wing abnormalities characteristic of reduced Hh signaling.

UAS-dicer; nub-Gal4 flies were mated with *UAS-NPC1a-RNAi* flies. Panels A and B show adult wings of the specified genotypes.

(A): *UAS-dicer*; *nubbin-GAL4; UAS-GFP*.

(B): *UAS-dicer; nubbin-GAL4*/*UAS-NPC1a-RNAi; UAS-NPC1a-RNAi*.

(C): Relative estimation of the average length of the anterior cross vein (ACV) from the control and NPC1a knockdown adult wings. (n=60, three independent trials were conducted).