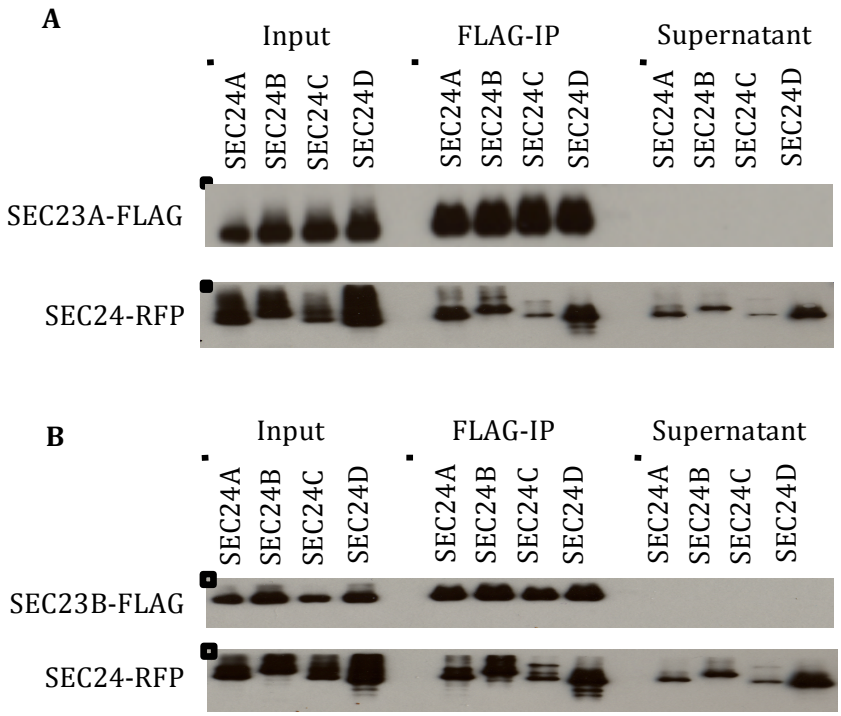

Figure S1. SEC23A and SEC23B interaction with the four SEC24 paralogs. (A, B) FLAG-tagged SEC23A or SEC23B together with RFP-tagged either SEC24A, SEC24B, SEC24C, or SEC24D were co-expressed in HEK293T cells. FLAG immunoprecipitation and immunoblotting with anti-RFP antibody demonstrated equivalent interactions between (A) SEC23A or (B) SEC23B and all four SEC24 paralogs.

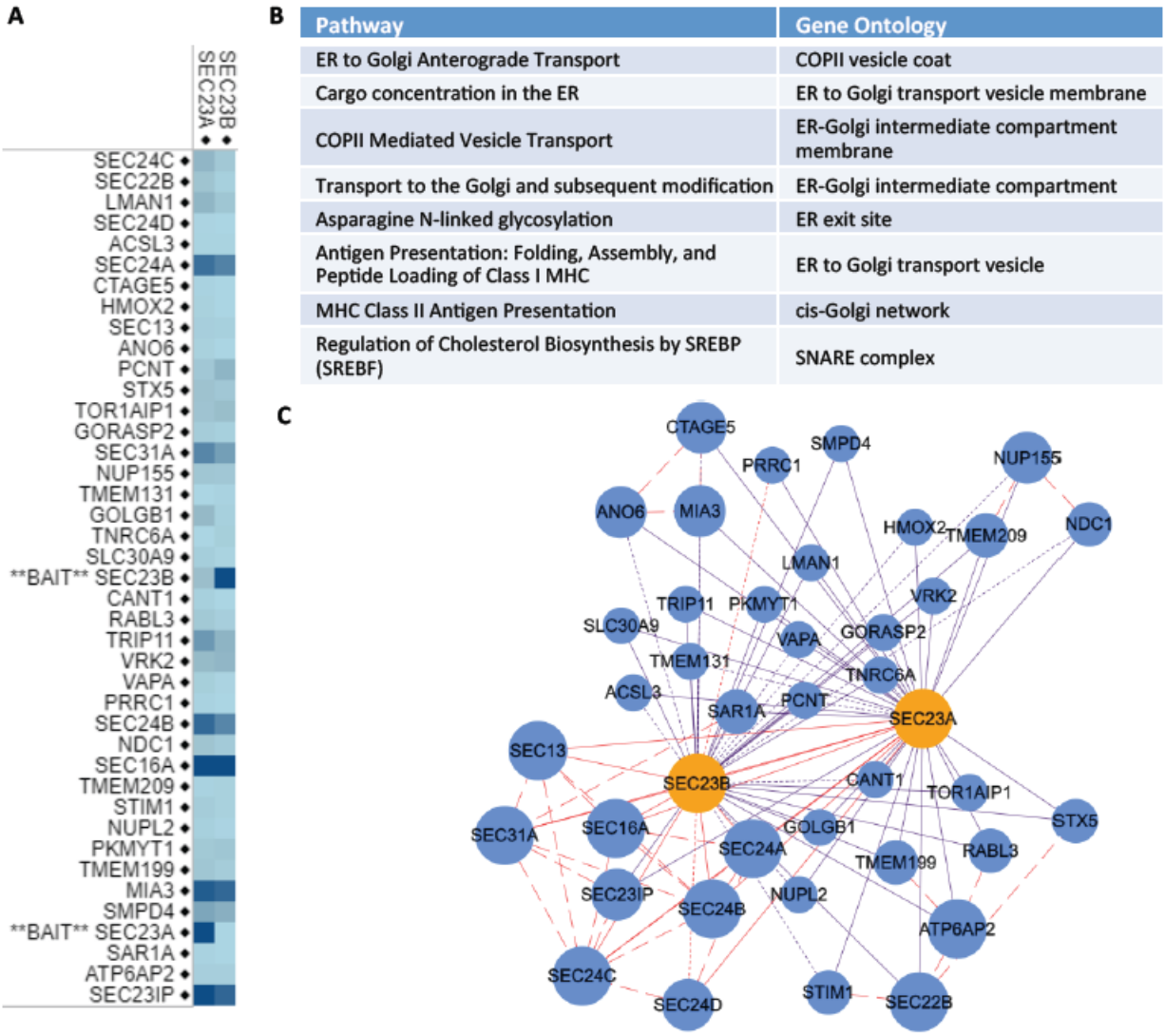

Figure S2. SEC23A and SEC23B have overlapping interactomes in HEK293 cells.

(A**)** Heatmap of the top interacting proteins with SEC23A or SEC23B in HEK293 cells. Though similar patterns are observed for each paralog, SEC23A and SEC23B baits are more highly biotinylated by their own BirA* tag (auto-biotinylation). (B) “Pathway” and “Gene Ontology” analyses demonstrate enriched pathways based on the functional annotation of the SEC23 interactomes. (C) SEC23A and SEC23B yield identical interacting protein networks. Blue lines indicate literature evidence for the interactions, while red lines indicate that the interactions have not been previously described.

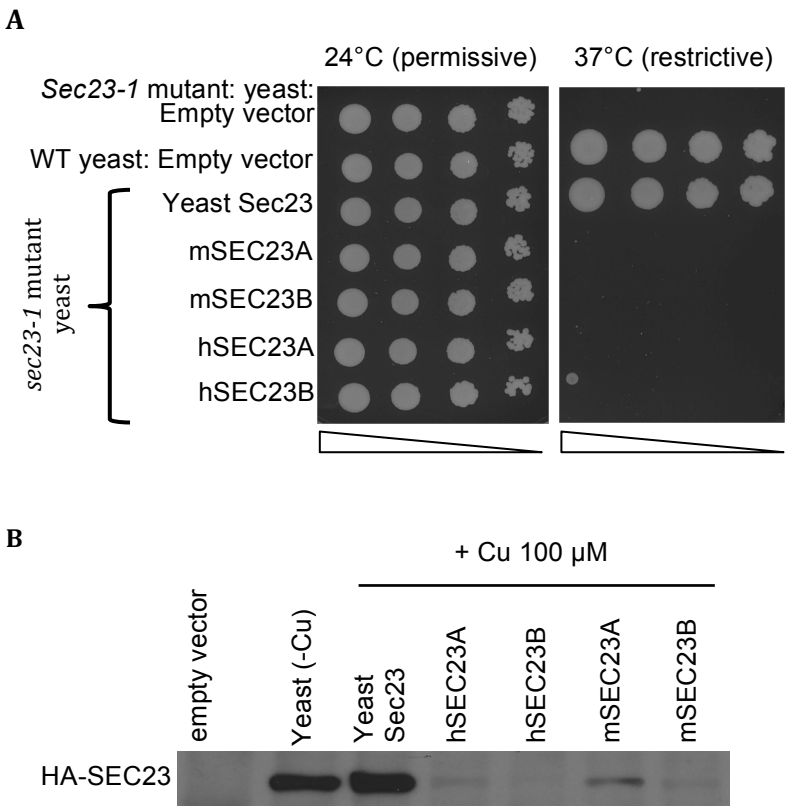

Figure S3. Expression of the mammalian SEC23 paralogs from a copper-inducible promoter.

(A, B) Growth of temperature-sensitive *sec23-1* mutant yeast expressing HA-tagged human (h) or mouse (m) SEC23 paralogs (or yeast Sec23) from a copper-inducible promoter at the permissive and restrictive temperatures. (B) Immunoblotting for HA demonstrates that the expression of mammalian SEC23s is considerably lower than the yeast protein, explaining the lack of rescue by the mammalian SEC23 paralogs.

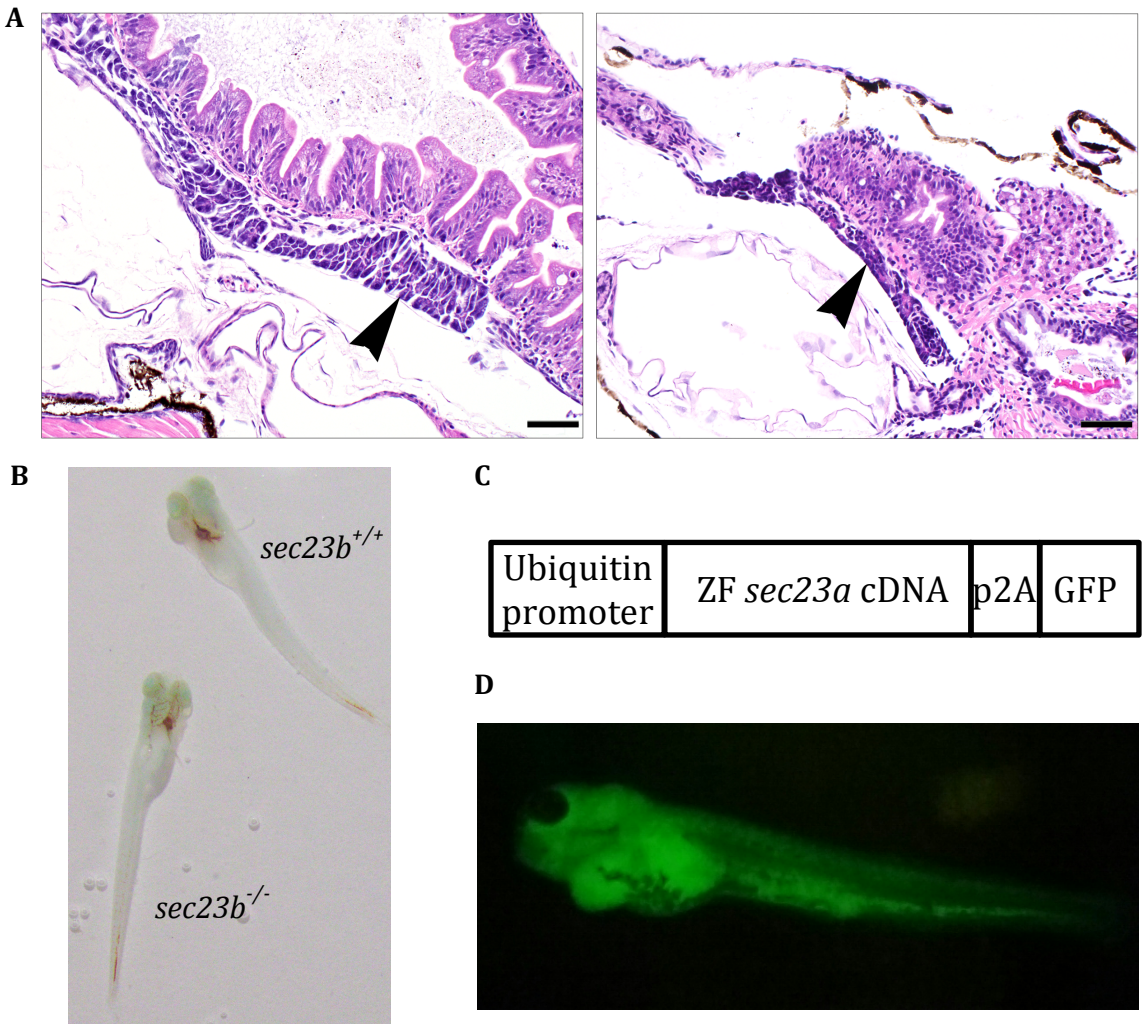

Figure S4. *sec23b*$^{-/-}$ ZF histology and GFP expression of ZF injected with a vector (designed to integrate into the ZF genome) expressing Sec23a and GFP from a ubiquitin promoter.

(A) *sec23b*$^{-/-}$ ZF exhibit normal pancreas histology at day 16 and (B) normal staining with o-dianisidine at day 7 compared to WT clutchmate controls. (C) The *sec23a* cDNA is expressed from a ubiquitin promoter with a P2A sequence separating it from a sequence encoding GFP. (D) Ubiquitous Sec23A expression (approximated by GFP), as expected from vector integration into one-cell stage embryos.

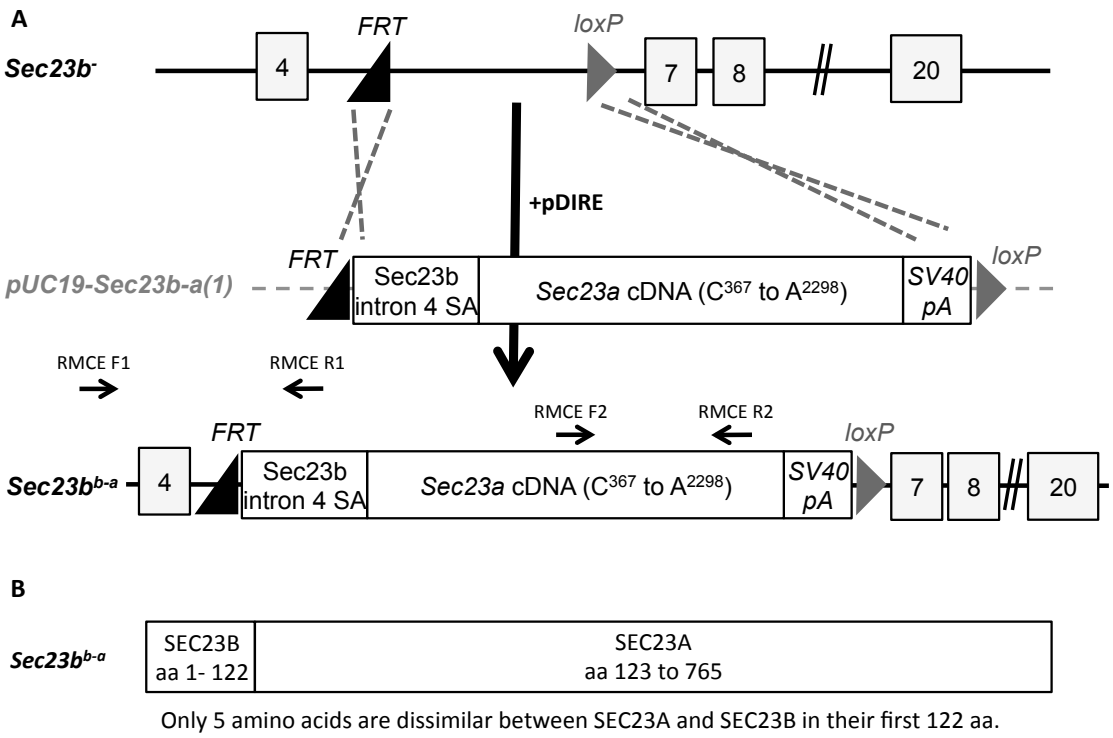

Figure S5. Direct zygote injection of the replacement vector and structure of the SEC23B-A fusion protein resulting from the *Sec23*$^{b-a}$ allele.

(A) The replacement vector pUC19-Sec23b-a(1) containing the *Sec23b* intron 4 splice acceptor (SA), the *Sec23a* coding sequence beginning with C^{367} and ending in A^{2298}, and the SV40 polyA termination signal (SV40 pA) was injected with pDIRE into 93 zygotes from a cross between *Sec23b*$^{+/-}$ and WT mice. The location of the genotyping primers are indicated in the figure. (B) The SEC23B-A fusion protein resulting from the *Sec23*$^{b-a}$ allele is composed of the first 122 SEC23B amino acids followed by amino acids 123-765 of SEC23A.

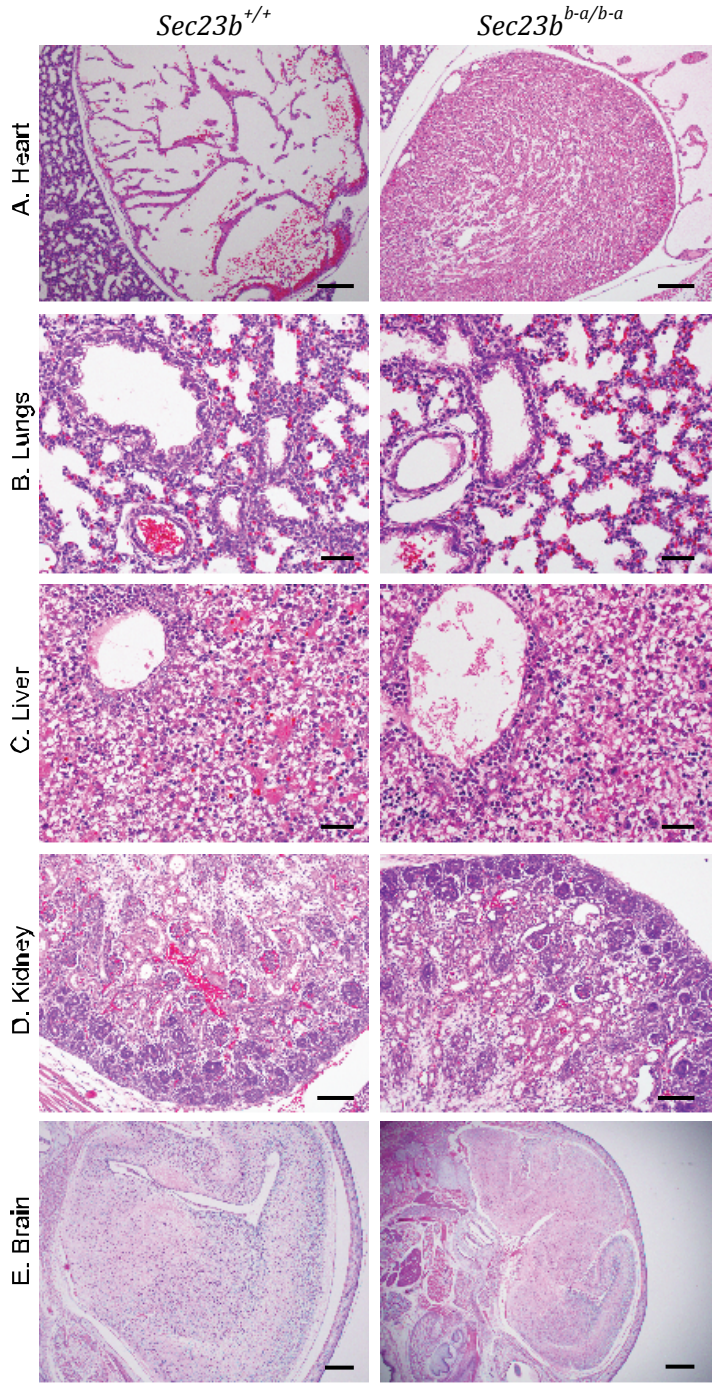

Figure S6. Histological evaluation of multiple tissues from mice expressing *Sec23a* from the *Sec23b* locus.

(A) *Sec23b*$^{b\text{-}a/b\text{-}a}$ mice exhibit normal heart (horizontal bar = 100 μm), (B) lung (horizontal bar = 20 μm), (C) liver (horizontal bar = 20 μm), (D) kidney (horizontal bar = 50 μm), and (E) brain (horizontal bar = 200 μm) histology compared to wild-type littermate controls.

Supplementary Information

Primers	**Sequences (5' to 3')**
RMCE F1	CCCAGCCATGATCTCTGTTTA
RMCE R1	CCAATGATGAGGACCCAGAG
RMCE F2	TGGTAATGGGTGACTCTTTCAA
RMCE R2	TCCAACCATCTTAGCACGTC
RMCE F3	GCCTGAAGAACGAGATCAGC
RMCE R3	CAGCAACCAGAAGCTTTCAA
RMCE A	CTTTAAAAGAACGCCCAGACTTAC
RMCE B	CAGAGCCTGCAAATCCTCAT
RMCE C	ACGTGTCAACCACATAGAGAAAGA
ZF *sec23b* CRISPR F	GCGTGGACCTCCAACCCC
ZF *sec23b* CRISPR R	GGCATTGGGCGGAAGGAGAC

Table S1. Primer sequences