Pancreatic SEC23B deficiency is sufficient to explain the perinatal lethality of germline SEC23B deficiency in mice

Rami Khoriaty1, Lesley Everett2, Jennifer Chase3, Guojing Zhu3, Mark Hoenerhoff6, Brooke McKnight5,†, Matthew P. Vasievich2,‡, Bin Zhang6, Kärt Tomberg7, John Williams8, Ivan Maillard1,3 & David Ginsburg1,3,7,9,10

In humans, loss of function mutations in SEC23B result in Congenital Dyserythropoietic Anemia type II (CDAII), a disease limited to defective erythroid development. Patients with two nonsense SEC23B mutations have not been reported, suggesting that complete SEC23B deficiency might be lethal. We previously reported that SEC23B-deficient mice die perinatally, exhibiting massive pancreatic degeneration and that mice with hematopoietic SEC23B deficiency do not exhibit CDAII. We now show that SEC23B deficiency restricted to the pancreas is sufficient to explain the lethality observed in mice with global SEC23B-deficiency. Immunohistochemical stains demonstrate an acinar cell defect but normal islet cells. Mammalian genomes contain two Sec23 paralogs, Sec23A and Sec23B. The encoded proteins share ~85% amino acid sequence identity. We generate mice with pancreatic SEC23A deficiency and demonstrate that these mice survive normally, exhibiting normal pancreatic weights and histology. Taken together, these data demonstrate that SEC23B but not SEC23A is essential for murine pancreatic development. We also demonstrate that two BAC transgenes spanning Sec23b rescue the lethality of mice homozygous for a Sec23b gene trap allele, excluding a passenger gene mutation as the cause of the pancreatic lethality, and indicating that the regulatory elements critical for Sec23b pancreatic function reside within the BAC transgenes.

Approximately 8,000 mammalian proteins are transported from the endoplasmic reticulum (ER) to the Golgi apparatus via coat protein complex II (COPII)-coated vesicles12, before reaching their final destination in the plasma membrane, intracellular organelles, or extracellular space. The COPII coat is composed of 5 core components, SAR1, SEC23, SEC24, SEC13 and SEC312–4. COPII coat assembly begins on the ER cytosolic surface when the guanine nucleotide exchange factor SEC12 converts GDP-bound SAR1 to GTP-bound SAR15,6. SAR1-GTP recruits the SEC23-SEC24 heterodimer to the ER surface through direct binding to SEC237,8. SAR1-SEC23-SEC24 form the inner layer of the COPII coat. Following cargo recruitment9–12, SEC13-SEC31 heterotetramers form the outer layer of the COPII coat, facilitating budding of the COPII vesicle from the surface of the ER13–16. The COPII components are conserved throughout eukaryote evolution, but unlike yeast, mammals exhibit multiple paralogs for most subunits, including 2 SEC23 paralogs, SEC23A and SEC23B, encoding two highly similar proteins (~85%). In humans, SEC23A missense mutations result in cranio-lenticulo-sutural-dysplasia (CLSD), an autosomal recessive disease characterized by skeletal abnormalities, late closure of fontanelles, dysmorphic features,
and sutural cataracts, while multiple loss of function SEC23B mutations have been identified in patients with congenital dyserythropoietic anemia type II (CDAII). CDAII is an autosomal recessive disease characterized by moderate anemia, increased bi/multi-nucleated erythroblasts in the bone marrow (BM), a double red blood cell (RBC) membrane by electron microscopy, and a faster than normal migration of the RBC membrane protein band 3 on SDS-PAGE.8,15–22 No other non-hematologic abnormalities have been reported to result from SEC23B mutations, though a recent report suggested that germline heterozygous SEC23B variants are associated with Cowden syndrome and apparently sporadic thyroid cancer. Patients with two nonsense SEC23B mutations have never been reported, raising the possibility that complete SEC23B deficiency might be lethal.

We previously reported that mice homozygous for a Sec23b gene–trap allele (Sec23bgt/gt) die perinatally, with analysis at embryonic day 18.5 (E18.5) demonstrating degeneration of the pancreas and other professional secretory tissues. Lethally irradiated mice transplanted with hematopoietic stem cells (HSC) deficient for SEC23B did not exhibit anemia or other CDAII characteristics, and Sec23bgt/gt HSC exhibited no competitive disadvantage at reconstituting the BM erythroid lineage. We also recently reported that mice homozygous for a Sec23a gene trap allele die at mid–embryogenesis, exhibiting a neural tube defect and impaired collagen secretion, reminiscent of the human phenotype.

We now confirm and extend our previous findings, demonstrating that mice with erythroid specific (Epo–R Cre) and pan–hematopoietic (Vav1–Cre) Sec23b deletion support a normal erythroid and hematopoietic compartment. We also show that two BAC transgenes comprising Sec23b rescue the phenotype of Sec23bgt/gt mice, ruling out a passenger gene mutation and indicating that key Sec23b regulatory elements reside within these BAC transgenes. Finally, we demonstrate that loss of SEC23B expression exclusively in the pancreas is sufficient to explain the lethality of mice with germline deletion of Sec23b, and show that pancreatic SEC23A, unlike SEC23B, is dispensable for normal murine pancreatic development.

## Results

### The key regulatory sequences required for Sec23b pancreatic function reside within a 127 kb spanning the Sec23b gene.

Mice homozygous for a Sec23b gene–trap allele (Fig. 1A) were previously reported to exhibit massive pancreatic degeneration with uniform perinatal lethality. In contrast, pancreatic abnormalities have not been observed in SEC23B deficient humans. Two independent mouse BAC transgenes (Fig. 1B) spanning the Sec23b gene were crossed onto the Sec23b+ line. Both Sec23b BAC transgenes demonstrated full rescue of the Sec23bgt/gt lethality, with the expected numbers of Sec23bgt/gt Tg− mice observed at weaning (Table 1). Sec23bgt/gt Tg+ mice exhibited normal survival (observed for 300+ days), and fertility, with no apparent pancreatic or other abnormalities on routine necropsy (Supplementary Fig. 1). BAC transgene expression in the relevant tissues is inferred from the dramatic phenotype rescue. These data demonstrate that the key regulatory elements necessary for Sec23b pancreatic function are located within the minimum ~127 kb region shared by the two BAC transgenes (Fig. 1B).

An intercross between mice heterozygous for the Sec23b− allele (Fig. 1C, Table 1) generated no Sec23b−/− mice at weaning (p < 0.0001), though Sec23b−/− mice were present at the expected frequency at E17.5 (p > 0.9) and E18.5 (p > 0.7), consistent with the previously reported perinatal lethality. Sec23b−/− mice were present at the expected numbers at weaning (Table 1), and exhibited no gross or microscopic abnormalities. E18.5 Sec23b−/− embryos were significantly smaller than their littermates (Supplementary Fig. 2A), with massive pancreatic hypoplasia uniformly observed in the former (Supplementary Fig. 2B), as well as variable vacuolation of salivary glands, gastric pit epithelial cells, and nasal glands (Supplementary Fig. 2C).

### Loss of pancreatic SEC23B expression is sufficient to account for the perinatal mortality observed in Sec23b−/− mice.

Pancreatic–specific Sec23b deficient mice were generated, using the Pdx–Cre transgene. Out of 74 progeny generated from a Sec23b+/− Pdx–Cre+ × Sec23b−/− cross, only 2 Sec23b−/− Pdx–Cre+ mice were observed at weaning (compared to 9/74 mice expected, p < 0.016, Table 2). Analysis of pancreatic tissues from these 2 mice detected a high level of residual functional non-excised Sec23b+ alleles (Supplementary Fig. 3), likely explaining their extended survival, either due to incomplete excision of Sec23b and/or selection of non-excised cells during pancreatic development.

Analysis of a similar cross for a second pancreatic–specific Cre transgene, p48–Cre, resulted in 0/148 Sec23b−/− p48–Cre+ mice observed at weaning (p < 0.001, Table 2), though Sec23b−/− p48–Cre+ mice were present at the expected numbers at E18.5 (p > 0.5, Table 2). Four mice from this cross died within 1 day of birth, all of which were genotyped as Sec23b−/− p48–Cre+ mice (Table 2).

Taken together, these data demonstrate that loss of SEC23B exclusively in the pancreas is sufficient to explain the perinatal mortality observed in mice with germline deletion of Sec23b.

### Pancreatic SEC23B deficiency results in loss of pancreatic acini.

Histologic evaluation of pancreatic tissues harvested from E18.5 Sec23b−/− p48–Cre+ embryos demonstrated hypoplastic pancreatic remnants with degeneration of pancreatic acini (Fig. 2A, supplementary Table 2, p < 0.0001). Acinar cells were smaller than normal, with scant to minimal eosinophilic cytoplasm that was often vacuolated, and separated by clear space and prominent interlobular stroma. Though pancreatic islets could not be identified by H&E staining due to parenchymal collapse, immunostaining for insulin or glucagon demonstrated normal islet cell morphology, while immunohistochemistry for amylase confirmed loss of acinar cells (Fig. 2B).

### Mice with pancreas–specific SEC23A deficiency survive to adulthood and lack a pancreatic phenotype.

Mice homozygous for a Sec23a gene trap allele die at mid–embryogenesis exhibiting neural tube opening and abnormal development of extra-embryonic membranes. This early lethality precluded the evaluation of the effect of SEC23A deficiency on pancreatic development. To assess the impact of pancreatic SEC23A deficiency...
on pancreatic development, we generated mice heterozygous for a conditional Sec23a floxed allele (Sec23a\(^{+/-}\)) and for a Sec23a null allele (Sec23a\(^{-/-}\)) (Fig. 1D–G). Mice with pancreatic deficiency of SEC23A were generated by crossing either Sec23a\(^{+/-}\) p48-Cre\(^{+}\) or Sec23a\(^{-/-}\) p48-Cre\(^{+}\) mice to Sec23a\(^{+/+}\) mice. These crosses yielded the expected number of Sec23a\(^{+/+}\) and Sec23a\(^{-/-}\) p48-Cre\(^{+}\) offspring (Table 3).

Figure 1. Sec23 mutant alleles and locations of the bacterial artificial chromosome (BAC) transgenes and microsatellite markers on mouse chromosome 2. (A) Sec23b\(^{+}\): Sec23b wild type allele; Sec23b\(^{-}\): the original Sec23 gene trap allele containing a gene trap insertion in intron 19. (B) BAC transgenes RP23 and RP24 contain the entire Sec23 gene as well as upstream and downstream sequences as indicated. The relative locations of the microsatellites markers used for microsatellite genotyping are depicted by stars. (C) A second, independent, Sec23b conditional gene trap allele (Sec23b\(^{gt}\)) contains a gene trap insertion in intron 4. FLP mediated excision yields the conditional Sec23b floxed allele (Sec23b\(^{fl}\)), and subsequent Cre mediated excision results in the Sec23b null allele (Sec23b\(^{-}\)). (D) The Sec23a wild type allele is referred to as Sec23a\(^{+}\). The Sec23a conditional gene trap (Sec23a\(^{gt}\)) allele was crossed to a FLP mouse, resulting in the Sec23a\(^{fl}\) allele (Sec23a\(^{fl}\)). Subsequent Cre mediated excision gives rise to the Sec23a\(^{-}\) allele (Fig. 1D). The locations of genotyping primers are depicted by arrows. (E) Competitive PCR assay with 1 forward primer (primer A) and 2 reverse primers (primers B and B4) yields a 179 base pair (bp) product from the wild type allele (primers A and B4), and a 260-bp product from the Sec23a\(^{gt}\) allele (primers A and B). (F) PCR assay using primers A and E2 results in a 231 bp product from the wild type allele and a 335 bp product from the Sec23b\(^{fl}\) allele. (G) PCR assay using primers A and D results in a 482 bp product from the Sec23b\(^{-}\) allele. No PCR product is generated with these primers from a WT allele due to the large distance between the primers.

No RBC abnormalities observed in mice with erythroid-specific and hematopoietic specific SEC23B deficiency. Mice with erythroid-specific or pan-hematopoietic SEC23B-deficiency were generated by crossing the Sec23b\(^{-}\) allele to mice expressing Cre-recombinase driven by the EpoR promoter or the Vav1 promoter, respectively. Sec23b\(^{-}\) EpoR-Cre\(^{+}\) and Sec23b\(^{-}\) Vav1-Cre\(^{+}\) mice were observed in the expected Mendelian ratios at weaning (Table 2), appeared grossly normal, and exhibited normal survival and fertility, as well as normal RBCs, with none of the features of human CDAII. Specifically, these mice exhibited no anemia...
disorders17,18,23. approaches to modifying the expression of the paralogs in CDAII and other SEC23 regulatory elements critical for physiologic pancreatic expression of Sec23b, more exocrine pancreatic enzymes, which when retained in the ER results in pancreatic degeneration.

result of an acinar cell defect, potentially due to delayed ER exit of a specific secretory cargo(s), possibly one or abnormal acinar cells but normal islet cells. These data suggest that the observed pancreatic degeneration is a cell-autonomous defect in the pancreatic cell itself. Immunohistochemical stains demonstrate morphologically

with germline deficiency for SEC23B. These data exclude a previously unidentified pathology, ectopic to the pancreas (Fig. 4A,B), no increased bi/multi-nucleated erythroid precursors in the bone marrow (Fig. 4C), and no increased mobility of band 3 on SDS PAGE (Fig. 4D). Vav1-Cre mediated excision of the Sec23b floxed allele appeared complete in bone marrow cells (Fig. 4E).

Murine hematopoietic SEC23B deficiency does not result in a block of B-lymphocyte development. In a previous report44, a subtle B-cell defect was suggested in cells derived from SEC23B-deficient hematopoietic precursors. To further characterize the B-lineage development in SEC23B null mice, C57BL/6J mice ubiquitously expressing GFP (UBC-GFP Tg/) were lethally irradiated and transplanted with fetal liver cells (FLC) isolated from E16.5 Sec23b−/− or WT embryos. Eight weeks post-transplantation, reconstituted BM hematopoietic cells were GFP(−), confirming donor HSC engraftment. The percentages of GFP(−) BM pro-B cells, pre-B cells, recirculating B cells, and immature B cells were equivalent in mice transplanted with Sec23b−/− FLC and mice transplanted with WT FLC (Fig. 4F,G), thereby arguing against a major defect in B-lymphocyte development resulting from SEC23B deficiency.

Discussion
In humans, homozygous or compound heterozygous loss of function mutations in SEC23B result in a phenotype limited to the erythroid compartment, with no other non-hematologic abnormalities reported. In contrast, mice homozygous for a Sec23b genetrap allele die perinatally exhibiting extensive pancreatic degeneration, and mice with hematopoietic deficiency for SEC23B exhibit no RBC phenotype.

The rescue of the SEC23B deficiency phenotype by either of two BAC transgenes demonstrates that the rescue effect on a nearby gene (passenger gene mutation) segregating with the Sec23b allele. We also show that mice with either erythroid specific or pan-hematopoietic Sec23b deletion do not exhibit a phenotype. These data are consistent with previously reported HSC transplantation results44, and exclude an artifact from HSC transplantation in our prior report as the cause of the lack of RBC phenotype in mice transplanted with SEC23B deficient HSCs44. Furthermore, we demonstrate that the stages of B-lymphocyte development in the BMs of mice transplanted with SEC23B deficient HSCs are indistinguishable from those in BMs of control mice transplanted with wild type HSCs, thereby ruling out a defect in B-lymphocyte development resulting from SEC23B deficiency.
Table 2. Results of matings to generate mice with pancreas specific Sec23b deletion using Pdx-Cre (A) and p48-Cre (B), and to generate mice with erythroid specific (C) or pan-hematopoietic (D) Sec23b deletion using Epor-Cre or Vav1-Cre, respectively. *P-value calculated for Sec23b<sup>−/−</sup> Pdx-Cre(+) versus all other genotypes. ^P-value calculated for Sec23b<sup>−/−</sup> p48-Cre(+) versus all other genotypes. A P-value calculated for Sec23b<sup>−/−</sup> Epor-Cre(+) versus all other genotypes. !P-value calculated for Sec23b<sup>−/−</sup> Vav1-Cre(+) versus all other genotypes.

Materials and Methods

Generation of Sec23b mutant mouse lines. Two independent Sec23b mutant mouse lines, one with a gene trap insertion in intron 19 of the gene (Sec23b<sup>gt</sup>), and another line with a conditional gene trap (flanked by FRT sites) insertion in intron 4 of Sec23b (Sec23b<sup>fl/fl</sup>) were previously described<sup>22,23</sup> (Fig. 1A,C). Mice expressing FLP recombinase driven by the human β-actin promoter (β-actin FLP) (Jackson laboratory stock # 005703) were crossed to the Sec23b<sup>gt</sup> allele to excise the gene trap cassette, resulting in the Sec23b floxed allele (Sec23b<sup>−/−</sup>), with exons 5 and 6 flanked by loxP sites. The Sec23b<sup>gt</sup> allele was crossed to mice ubiquitously expressing Cre recombinase under the control of an Elia promoter (Elia-Cre) (Jackson laboratory stock # 003724) to excise exons 5 and 6 and generate a null Sec23b allele (Sec23b<sup>−/−</sup>), resulting in a frameshift and early stop codon in exon 7 (Fig. 1C). Sec23b<sup>−/−</sup> mice were back-crossed with C57BL/6J mice to remove the Elia-Cre transgene. Mice with a pancreas-specific SEC23B knock-out were generated by crossing Sec23b<sup>−/−</sup> or Sec23b<sup>−/−</sup> mice to mice expressing Cre recombinase driven by either the p48 promoter (generous gift from Dr. Christopher Wright) or the Pdx1 promoter<sup>27</sup>. Mice with erythroid-specific and pan-hematopoietic SEC23B-deficiency were generated by crossing the Sec23b<sup>−/−</sup> allele to Epor-Cre mice<sup>28</sup> (generous gift from Dr. Ursula Klingmüller) and Vav1-Cre mice (Jackson laboratory stock # 008610), respectively. Sec23b<sup>−/−</sup> mice used in this study were backcrossed to C57BL/6J mice for >10 generations. The Sec23b<sup>−/−</sup> allele was derived from C57BL/6J ES cell, and the Sec23b<sup>−/+</sup> and Sec23b<sup>−/−</sup> alleles were maintained on a pure C57BL/6J background. All Cre mice were back-crossed to C57BL/6J mice for >6 generations. Mice were housed at the Life Sciences Institute of the University of Michigan, and all experiments were approved by and performed in accordance with the regulations of the University Committee on Use and Care of Animals.

Generation of a Sec23a conditional mutant mouse line. Mice heterozygous for a Sec23a conditional gene trap (flanked by FRT sites) insertion into intron 2 of Sec23a (Sec23a<sup>−/+</sup>) were derived from an embryonic stem (ES) cell clone (EPD0072_1_B09) and live mice were obtained from the Knock-Out Mouse Project (KOMP) Repository. The Sec23a<sup>−/−</sup> allele was derived from C57BL/6J ES cells and subsequently maintained on a pure C57BL/6J background. Sec23a<sup>−/−</sup> mice were crossed to β-actin FLP mice to excise the gene-trap cassette, resulting in the Sec23a<sup>−/−</sup> allele, with exon 3 flanked by LoxP sites (Fig. 1D). Sec23a<sup>−/−</sup> mice were back-crossed with C57BL/6J mice to remove the FLPs transgene. Sec23a<sup>−/−</sup> mice were crossed to Elia-Cre transgenic mice to excise exon 3, resulting in a null Sec23a allele (Sec23a<sup>−/−</sup>) with a frameshift and an early stop codon. Sec23a<sup>−/−</sup> mice were

| A. Genotypes: | Sec23b<sup>−/−</sup> Pdx-Cre(+) | Sec23b<sup>−/−</sup> Pdx-Cre(−) | Sec23b<sup>−/−</sup> Pdx-Cre(+) | Sec23b<sup>−/−</sup> Pdx-Cre(−) | Sec23b<sup>−/−</sup> Pdx-Cre(+) | Sec23b<sup>−/−</sup> Pdx-Cre(−) | p-value<sup>3</sup> |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------|
| Sec23b<sup>−/−</sup> x Sec23b<sup>−/−</sup> Expected ratios | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% |
| Observed at weaning (n=74) | 16% (12) | 11% (8) | 16% (12) | 11% (8) | 16% (12) | 12% (9) | 3% (2) | 15% (11) | <0.016 |

| B. Genotypes: | Sec23b<sup>−/−</sup> p48-Cre(+) | Sec23b<sup>−/−</sup> p48-Cre(−) | Sec23b<sup>−/−</sup> p48-Cre(+) | Sec23b<sup>−/−</sup> p48-Cre(−) | Sec23b<sup>−/−</sup> p48-Cre(+) | Sec23b<sup>−/−</sup> p48-Cre(−) | p-value<sup>3</sup> |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------|
| Sec23b<sup>−/−</sup> x Sec23b<sup>−/−</sup> Expected ratios | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% |
| Observed at weaning (n=148) | 17% (25) | 11% (17) | 12% (18) | 17% (25) | 14% (21) | 14% (20) | 0% (0) | 15% (22) | <0.0001 |
| Observed at E.18.5% (n=52) | 10% (5) | 8% (4) | 13% (7) | 12% (6) | 17% (9) | 13% (7) | 15% (8) | 12% (6) | >0.5 |
| Postnatal death within 1 day of birth (n=4) | 0% (0) | 0% (0) | 0% (0) | 0% (0) | 0% (0) | 100% (4) | 0% (0) | <0.0001 |

| C. Genotypes: | Sec23b<sup>−/−</sup> Epor-Cre(+) | Sec23b<sup>−/−</sup> Epor-Cre(−) | Sec23b<sup>−/−</sup> Epor-Cre(+) | Sec23b<sup>−/−</sup> Epor-Cre(−) | Sec23b<sup>−/−</sup> Epor-Cre(+) | Sec23b<sup>−/−</sup> Epor-Cre(−) | p-value<sup>3</sup> |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------|
| Sec23b<sup>−/−</sup> x Sec23b<sup>−/−</sup> Epor-Cre(+) Expected ratios | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% | 12.5% |
| Observed at weaning (n=80) | 12% (10) | 10% (8) | 10% (8) | 16% (13) | 10% (8) | 13% (10) | 15% (12) | 14% (11) | >0.7 |

| D. Genotypes: | Sec23b<sup>−/−</sup> Vav1-Cre(+) | Sec23b<sup>−/−</sup> Vav1-Cre(−) | Sec23b<sup>−/−</sup> Vav1-Cre(+) | Sec23b<sup>−/−</sup> Vav1-Cre(−) | Sec23b<sup>−/−</sup> Vav1-Cre(+) | Sec23b<sup>−/−</sup> Vav1-Cre(−) | p-value<sup>3</sup> |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------|
| Sec23b<sup>−/−</sup> x Sec23b<sup>−/−</sup> Vav1-Cre(+) Expected ratios | 25% | 25% | 25% | 25% | 25% | 25% | >0.4 |
| Observed at weaning (n=43) | 21% (9) | 19% (8) | 28% (12) | 32% (14) | 28% (12) | 32% (14) | 28% (12) | 32% (14) | >0.4 |
back-crossed with C57BL/6 J mice to remove the EIIA-Cre transgene. Mice with pancreas-specific SEC23A deficiency were generated by crossing the Sec23a<sup>fl</sup> allele to p48-Cre<sup>+</sup>, as above.

**Generation of BAC transgenic mice.** Two bacterial artificial chromosome (BAC) clones spanning the Sec23b gene, RP23-70J9 (RP23) and RP24-371A4 (RP24), were purchased from the BACPAC Resources Center at Children's Hospital Oakland Research Institute. BAC DNA constructs were expanded in One Shot TOP10 Escherichia coli and purified using the NucleoBond BAC100 kit (Machery-Nagel). BAC DNA was injected into zygotes generated from a cross between C57BL/6J x SJL F1 females and Sec23b<sup>+/+</sup> male mice. RP23 and RP24 transgenic founders (Sec23b<sup>+/+Tg<sup>+</sup></sup>) were crossed to Sec23b<sup>−/−</sup> mice, and the Sec23b<sup>−/−Tg<sup>+</sup></sup> progeny were crossed to Sec23b<sup>−/−</sup> C57BL/6 J mice to generate potential Sec23b<sup>−/−Tg<sup>+</sup></sup> "rescue mice". Mice were genotyped for the

---

**Figure 2. Pancreas tissues obtained from Sec23b<sup>−/−</sup>p48-Cre<sup>+</sup>E18.5 embryos.** (A) Hematoxilin and eosin staining demonstrates that the pancreata of Sec23b<sup>−/−</sup>p48-Cre<sup>+</sup> embryos are hypoplastic, with shrunken acini containing small exocrine epithelial cells with scant, often vacuolated, cytoplasms. The ducts and supporting stroma are more prominent in the Sec23b<sup>−/−</sup>p48-Cre<sup>+</sup> compared to wild type pancreas tissues. In contrast, islets cells appear histologically normal in the Sec23b<sup>−/−</sup>p48-Cre<sup>+</sup> pancreata. An image is selected from one of six mice evaluated per genotype. (B) Immunohistochemistry for amylase, glucagon, and insulin demonstrate that the pancreatic defect in Sec23b<sup>−/−</sup>p48-Cre<sup>+</sup> mice appears to be confined to the acinar cells. An image is shown from one of six mice evaluated per genotype. Scale bar indicated 25 μm.
Sec23b allele and for the presence of the BAC transgene. Standard genotyping methods are unable to differentiate between the endogenous Sec23b allele and the Sec23b gene present on the BAC transgene. Therefore, microsatellite genotyping was used (see below) to distinguish Sec23b+/gt Tg+ mice from Sec23bg/gt Tg+ mice.

**PCR genotyping.** DNA was isolated from mouse tail biopsies and genotyping was performed using the Go-Taq Green Master Mix (Promega). Genotyping for the Sec23b,gfl, Sec23b, and Sec23b alleles and for the various Cre transgenes was performed as previously described24,27,28. Location of the Sec23a genotyping primers is shown in Fig. 2A, and their sequences are shown in Supplementary Table 1. The Sec23a gt allele was genotyped in a three-primer PCR assay using a forward primer (primer A) located in Sec23a intron 2, upstream of the gene trap insertion site and two reverse primers, one (primer B) located in the gene trap insertion cassette between the two FRT sites and the second (primer B4) located in intron 2 downstream of primer A (the genomic sequence to which primer B4 anneals is absent from Sec23a,gfl). This PCR product was resolved by 2% (weight/volume) agarose gel electrophoresis (Fig. 1E). Genotyping for the Sec23a allele was performed with a PCR assay consisting of the forward primer A and a reverse primer located in intron 2 between the two LoxP sites (primer E2) (Fig. 1F). This reaction does not yield a PCR product for the Sec23a− allele. Confirmation of the excision of

Table 3. Results of matings to generate mice with pancreas specific Sec23a deletion, using p48-Cre.

| Genotypes: | Sec23a+/p48-Cre(+) | Sec23a+/p48-Cre(−) | Sec23a−/p48-Cre(+) | Sec23a−/p48-Cre(−) | p-value* |
|------------|---------------------|---------------------|---------------------|---------------------|----------|
| Sec23a+/p48-Cre(+) x Sec23a−/p48-Cre(+) Expected ratios | 25% | 25% | 25% | 25% | >0.6 |
| Observed at weaning % (n=33) | 36.4% (12) | 27.3% (9) | 15.1% (5) | 21.2% (7) | >0.6 |

| Genotypes: | Sec23a+/p48-Cre(+) | Sec23a−/p48-Cre(−) | Sec23a−/p48-Cre(+) | Sec23a−/p48-Cre(−) | p-value* |
|------------|---------------------|---------------------|---------------------|---------------------|----------|
| Sec23a+/p48-Cre(+) x Sec23a−/p48-Cre(+) Expected ratios | 25% | 25% | 25% | 25% | >0.7 |
|Observed at weaning % (n=29) | 34.4% (10) | 27.6% (8) | 20.7% (6) | 17.3% (5) | >0.7 |

**Figure 3. Sec23a+/− p48-Cre+ mice do not exhibit a pancreatic phenotype.** Pancreas tissues harvested from Sec23a+/− p48-Cre+ mice (A) do not have detectable SEC23A protein by western blot, (B) have normal weights, (C) and appear histologically normal by Hematoxilin and eosin stain (6 mice per genotype were evaluated). Scale bar indicated 50 μm in the top panels and 25 μm in the lower panels. (D) SEC23B protein expression is increased by ~33% in SEC23A-deficient pancreata, as measured by quantitative western blots (infrared fluorescent detection). (E) SEC23B mRNA expression is indistinguishable in SEC23A-deficient compared to WT pancreata.
Fetal Liver cells transplants. Timed matings were performed by intercrossing Sec23b+/− mice as previously described24. Pregnant females were euthanized at E16.5 postcoitus, and fetal liver cells were isolated and transplanted into lethally irradiated recipient mice as previously described24.

Complete blood counts (CBC) and bone marrow (BM) analysis. Blood (20 or 70 microliters) was collected from the retro-orbital venous sinuses of anesthetized mice via anticoagulant-coated capillary tubes.
Blood was diluted in 5% bovine serum albumin in phosphate buffered saline (pH 7.4) to a total volume of 200 microliters. CBCs were determined as previously described23. BM cells were collected from hind limbs of euthanized mice, cytopsins prepared and stained as previously described24, and the latter examined under light microscopy by an investigator blinded to the BM genotype.

Flow cytometry and RBC ghost preparation. BM cells were stained with the following antibodies obtained from BioLegend: anti-B220, anti-CD19, anti-CD43, anti-CD93 (AA4.1), and anti-sIgM. Analysis was performed using a FACSAria III (Becton Dickinson Biosciences). Non-viable cells were excluded with 4′ 6-diamidino-2-phenylindole (Sigma). Files were analyzed with FlowJo (Tree Star).

RBC ghosts were prepared from peripheral blood RBC and stored at −80°C in lysis buffer, as previously described24.

Western blot and qRT-PCR. Total cell lysates were prepared as previously described24. Western blots (film visualization with chemiluminescent detection) and quantitative western blot (Infrared fluorescent detection) were performed as previously described22. For quantitative western blots, band intensities were quantified using the Image Studio software (LI-COR Biosciences) and normalized to GAPDH, and the secondary antibodies utilized were IRDye 680RD or IRDye 800CW. qRT PCR was performed as previously described22.

Antibodies. Rabbit Anti-mouse SEC23B and anti-mouse SEC23A antibodies were generated as previously described22,23. Mouse anti-GAPDH and anti-Band3 antibodies were purchased from Millipore. Anti-Actin antibody was purchased from Santa Cruz.

Hematoxylin and eosin staining and Immunohistochemistry. At necropsy, tissues were collected and fixed in aqueous buffered zinc formalin (Z-fix, Anatech) for histologic and immunohistochemical analysis. Tissues were routinely processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). For immunohistochemistry, rabbit polyclonal antibodies to pancreatic amylase (ab21156, 1:1000; Abcam, Cambridge MA), insulin (C27C9, 1:800; Cell Signaling Technology, Danvers MA), and glucagon (D16G10, 1:100; Cell Signaling Technology, Danvers MA) were used. Following antigen retrieval, quenching of endogenous peroxidases and rodent block, primary antibodies were applied. After primary antibody incubation and washing, rabbit polymer HRP secondary antibody (Biocare, Concord CA), was applied. Negative controls were performed as previously described24. Tissues were routinely processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin, dehydrated through graded alcohols, immersed in xylene, and mounted with coverslips. Histologic evaluation was performed by an investigator blinded to the genotype of the evaluated mice.

Statistical analysis. The Chi-square test was used to calculate the statistical significance of the deviation of the genotypes of a given cross from the expected Mendelian ratios. The statistical significant differences between blood count parameters in test cohorts and controls were calculated using the student’s T-test.

References
1. Khoriaty, R., Vasievich, M. P. & Ginsburg, D. The COPII pathway and hematologic disease. Blood 120, 31–38 (2012).
2. Zanetti, G., Pahuja, K. B., Studer, S., Shim, S. & Schekman, R. COPII and the regulation of protein sorting in mammals. Nat Cell Biol 14, 20–28 (2012).
3. Brandizzi, F. & Barlowe, C. Organization of the ER-Golgi interface for membrane traffic control. Nature reviews. Molecular cell biology 14, 382–392 (2013).
4. D’Arcangelo, J. G., Stahmer, K. R. & Miller, E. A. Vesicle-mediated export from the ER: COPII coat function and regulation. Biochim Biophys Acta 1833, 2464–2472 (2013).
5. Barlowe, C. & Schekman, R. SEC12 encodes a guanine-nucleotide-exchange factor essential for transport vesicle budding from the ER. Nature 365, 347–349 (1993).
6. Nakano, A. & Muramatsu, M. A novel GTP-binding protein, Sar1p, is involved in transport from the endoplasmic reticulum to the Golgi apparatus. J Cell Biol 109, 2677–2691 (1989).
7. Bl, X., Mancias, J. D. & Goldberg, J. Insights into COPII coat nucleation from the structure of Sec23. Sar1 complexed with the active fragment of Sec31. Dev Cell 13, 635–645 (2007).
8. Matsuo, K., et al. COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. Cell 93, 263–275 (1998).
9. Aridor, M., Weissman, J., Bannikh, S., Nuofer, C. & Balch, W. E. Cargo selection by the COPII budding machinery during export from the ER. J Cell Biol 141, 61–70 (1998).
10. Kuehn, M. J., Herrmann, J. M. & Schekman, R. COPII-cargo interactions direct protein sorting into ER-derived transport vesicles. Nature 391, 187–190 (1998).
11. Miller, E. A. et al. Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. Cell 114, 497–509 (2003).
12. Mosessova, E., Bickford, L. C. & Goldberg, J. SNARE selectivity of the COPII coat. Cell 114, 483–495 (2003).
13. Fath, S., Mancias, J. D., Bi, X. & Goldberg, J. Structure and organization of coat proteins in the COPII cage. Cell 129, 1325–1336 (2007).
14. Lederkremer, G. Z. et al. Structure of the Sec23p/24p and Sec13p/31p complexes of COPII. Proc Natl Acad Sci USA 98, 10704–10709 (2001).
15. Salama, N. R., Yeung, T. & Schekman, R. W. The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. EMBO J 12, 4073–4082 (1993).
16. Stagg, S. M. et al. Structure of the Sec13p/31p COPII coat cage. Nature 439, 234–238 (2006).
17. Brothers, E. S. A. et al. Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. Nat Genet 38, 1192–1197 (2006).
18. Schwarz, K. et al. Mutations affecting the secretory COPII coat component SEC23B cause congenital dyserythropoietic anemia type II. Nat Genet 41, 936–940 (2009).
19. Alloisio, N. et al. The cisternae decorating the red blood cell membrane in congenital dyserythropoietic anemia (type II) originate from the endoplasmic reticulum. *Blood* 87, 4433–4439 (1996).

20. Heimpel, H. et al. Congenital dyserythropoietic anemia type II: epidemiology, clinical appearance, and prognosis based on long-term observation. *Blood* 102, 4576–4581 (2003).

21. Iolascon, A., Heimpel, H., Wahlin, A. & Tamary, H. Congenital dyserythropoietic anemias: molecular insights and diagnostic approach. *Blood* 122, 2162–2166 (2013).

22. Satchwell, T. J. et al. Characteristic phenotypes associated with congenital dyserythropoietic anemia type II (CDAII) manifest at different stages of erythropoiesis. *Haematologica* 98, 1788–1796 (2013).

23. Yehia, L. et al. Germline Heterozygous Variants in SEC23B Are Associated with Cowden Syndrome and Enriched in Apparently Sporadic Thyroid Cancer. *Am J Hum Genet* 97, 661–676 (2015).

24. Khoriaty, R. et al. Absence of a red blood cell phenotype in mice with hematopoietic deficiency of SEC23B. *Molecular and cellular biology* (2014).

25. Tao, J. et al. SEC23B is required for the maintenance of murine professional secretory tissues. *Proc Natl Acad Sci USA* 109, E2001–E2009 (2012).

26. Zhu, M. et al. Neural tube opening and abnormal extraembryonic membrane development in SEC23A deficient mice. *Scientific reports* 5, 13471 (2015).

27. Kawaguchi, Y. et al. The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* 32, 128–134 (2002).

28. Hingorani, S. R. et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer cell* 4, 437–450 (2003).

29. Heinrich, A. C., Pelanda, R. & Klingmuller, U. A mouse model for visualization and conditional mutations in the erythroid lineage. *Blood* 104, 659–666 (2004).

30. Baines, A. C., Adams, E. J., Zhang, B. & Ginsburg, D. Disruption of the Sec24d gene results in early embryonic lethality in the mouse. *PLoS One* 8, e61114 (2013).

31. Gelfand, Y., Rodriguez, A. & Benson, G. TRDB–the Tandem Repeats Database. *Nucleic acids research* 35, D80–D87 (2007).

Acknowledgements
This work was supported by National Institute of Health Grants R01 HL039693 and P01-HL057346 (DG), K08 HL128794 (RK), R01 AI091627 (IM), and R01 HL094505 (BZ). Rami Khoriaty is a recipient of the American Society of Hematology Scholar Award. David Ginsburg is a Howard Hughes Medical Institute investigator. The authors would like to acknowledge Elizabeth Hughes, Keith Childs, and Thomas Saunders for preparation of gene targeted mice and the Transgenic Animal Model Core of the University of Michigan’s Biomedical Research Core Facilities. Core support was provided by the University of Michigan Cancer Center (P30 CA046592).

Author Contributions
R.K. and D.G. conceived the study and designed the experiments. R.K. performed most of the experiments. L.E., J.C., G.Z., M.H., B.M., M.P.V. and K.T. contributed to the execution of the experiments. R.K. and D.G. wrote the paper. All authors contributed to the integration and discussion of the results.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Khoriaty, R. et al. Pancreatic SEC23B deficiency is sufficient to explain the perinatal lethality of germline SEC23B deficiency in mice. *Sci. Rep.* 6, 27802; doi: 10.1038/srep27802 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/