(Ap-GAL4) promoter. Icmt deficiency in the developing wing phenocopies the terminal vein bifurcation (arrows) and thickened crossvein (arrowhead) observed in the wings of Delta (Dl) flies that are deficient for the Notch ligand. (C) GAL4 expression driven instead with an eye-specific (GMR-GAL4) promoter. The rough eye phenotype is also consistent with that seen with Notch loss-of-function alleles, including Dl. An adult eye expressing GMR-GAL4 alone is shown in the lower left as a control because this GAL4 driver has been reported to induce a mild rough eye phenotype. (D) Wing imaginal discs from 3rd instar larvae expressing shSte14 in heat-shock induced clones marked with GFP and stained for Cut, a Notch-dependent gene product. Where the GFP positive clone intersects the line of Cut staining there is a decrease in the number of Cut positive cells (enlargement). Bar depicts 50 µm. See also Fig. S7.

Supplemental Figure 1. Icmt deficiency affects neither the endocrine pancreas nor pancreatic development. (A) Glucose tolerance tests (left panel) were carried out on female (F) and male (M) mice of the indicated genotypes that also carried a PDX-1-Cre allele. No significant difference in the area under the curve (AUC) was detected (right panel). (B) Representative H&E stained sections of pancreata from 25 month old mice of the indicated genotypes. Bars indicate 100 µm. (C) Icmtflx/+;PDX-1-Cre and Icmtflx/flx;PDX-1-Cre mice were crossed with animals harboring an LSL-lacZ transgene expressed from the ROSA26 locus to yield the indicated genotypes. Shown are frozen sections of pancreata from 10 week old mice treated with X-gal to stain cells that express β-galactosidase (blue) and counterstained with eosin (red). Whereas almost all visible pancreatic cells stained blue in sections from Icmtflx/+;PDX-1-Cre;ROSA26-LSL-LacZ pancreata, some β-galactosidase negative cells can be seen in pancreata from Icmtflx/flx;PDX-1-Cre;ROSA26-LSL-LacZ mice (arrow). Blue cells indicate that Icmt deficient cells contribute to the developing pancreatic tissue. Non-pancreatic tissues, such as blood vessels (arrowhead) and lymph nodes, are β-galactosidase negative. Bars indicate 500 µm. (D) Icmt enzymatic activity in membrane fractions of pancreatic homogenates from 10 week old mice of the indicated genotypes. Data shown are mean ± SEM, n=3, p<0.05.
Supplemental Figure 2. Icmt deficiency does not cause pancreatic neoplasia in the absence of oncogenic K-Ras even when p53 is inactivated. Representative H&E stained paraffin sections are shown of pancreata of 12 month old mice with the indicated genotypes. No PanINs or other pathology were found in the pancreata of either genotype. Bars represent 100 µm.

Supplemental Figure 3. Icmt deficiency results in more extensive PanIN lesions and fibrosis in PDX-1-Cre;LSL-KRAS<sup>G12D</sup> mice. (A) Sections of Pancreas from mice of the indicated genotypes were stained with Alcian Blue to visualize the mucin content characteristic of PanIN lesions. Right panel shows plots of the mean percent area of Alcian Blue staining in 5 fields of view. Bars represent 100 µm. Data shown are mean ± SEM of sections taken from 6 pairs of littermate mice \( p < 0.05 \). (B) Sections of pancreas from 3 month old mice of the indicated genotypes were stained by IHC for α-smooth muscle actin.

Supplemental Figure 4. Icmt is not required for Ras activity and signaling. (A) GST-RBD pulldown of GTP loaded Ras in lysates of HeLa cells ±ICMT knockdown stimulated with 10 ng/ml of EGF for 3 min. Cells were transfected with siRNA targeting ICMT or a non-targeting siRNA 72 hrs before lysis. Data shown are mean ± SEM (n=3). (B) Immunoblots for total Erk (tErk), phospho-Erk (pErk), and Icmt in lysates of HeLa cells transfected with either siRNA targeting ICMT or non-targeting (NT) siRNA for 72 hrs, or dominant negative GFP-H-Ras17N for 24 hrs then stimulated with 5 ng/ml of EGF for the indicated times before lysis. The amount of pERK/tErk for each condition was normalized to the maximum stimulation for each experiment. Data shown are mean ± SEM (n=4). (C) in vitro Icmt activity towards N-acetyl-S-farnesyl-L-cysteine of 10 µg of a total membrane fraction isolated from HeLa cells that had been transfected with either siRNA targeting ICMT or non-targeting siRNA for 72 hrs.

Supplemental Figure 5. Icmt deficiency does not affect cerulein-induced acute or chronic pancreatitis. (A) H&E stained paraffin sections of pancreata of 3 month old mice of the indicated genotypes in which acute pancreatitis was induced by cerulein as described in
Experimental Procedures. Sections from pancreata on days 0, 3 and 7 relative to the cessation of cerulein are shown. Differences among genotypes were observed neither in the extent of inflammation (scored as interstitial infiltrates within acinar tissue) nor in the rate or extent of recovery. (B) H&E stains of pancreata from the indicated genotypes after induction of chronic pancreatitis as described in Experimental Procedures showing no apparent differences. Bars indicate 100 µm.

Supplemental Figure 6. Ste14 deficiency inhibits Notch1 signaling in D. melanogaster wing development. Adult wings of D. melanogaster transgenic for UAS-shSte14, a GAL4 responsive hairpin that silences Icmt. GAL4 expression was driven with a wing-specific promoter (MS1069-GAL4). Icmt deficiency in the developing wing phenocopies the terminal vein bifurcation (arrows) observed in the wings of Delta (Dl) flies that are deficient for the Notch ligand.

Supplemental Figure 7. Ste14 deficiency inhibits Notch1 signaling in the developing D. melanogaster wing. Wing imaginal discs from 3rd instar larvae expressing shSte14 in heat-shock induced clones marked with GFP and stained for Cut, a Notch-dependent gene product. (A) As described (41), cells stain for Cut in a band of 3-4 cells along the dorsal-ventral boundary of the disc. (B) Where the GFP positive clone intersects the line of Cut staining there is a decrease in the number of Cut positive cells (arrow). (C) A disc in which heat shock activated UAS-shSte14 and GFP throughout the tissue shows no staining of Cut. Bar depicts 50 µm. See also Fig. S8D.

Supplemental Figure 8. Pharmacologic inhibition of Notch signaling blocks expression of p16INK4A in cultured pancreatic ductal epithelial cells. PDECs harvested from wild-type mice were grown in 3D matrigel culture before shifting to 2D culture on plastic. Cells were treated with or without 1 µM of the γ secretase inhibitor compound E (CE) and analyzed on day 8 by immunoblot for Notch1, Delta4, β-tubulin and p16INK4A as indicated. Results from the
quantification by Li-Cor Odyssey scanner of immunoblots from three independent experiments are plotted in the right panel (mean±SEM, normalized to control).

**Supplemental Figure 9. Icmt deficiency enhances Wnt signaling.** Immunohistochemical staining for β-catenin in paraffin sections of pancreata from 2 month old mice of the indicated genotypes imaged with 10x and 40x objectives. In the Icmt deficient pancreas there is an accumulation of cytoplasmic and nuclear β-catenin in PanIN lesions indicating enhanced Wnt signaling. Bars indicate 100 µm.
Figure S1

A. Blood glucose levels over time in Icmt flx/+ and Icmt flx/flx mice with PDX-1-Cre. The area under the curve for each group is also shown.

B. Histological images of liver tissue from Icmt flx/+ and Icmt flx/flx mice with PDX-1-Cre.

C. Immunohistochemical staining of liver tissue from Icmt flx/+ and Icmt flx/flx mice with PDX-1-Cre; ROSA26-LSL-LacZ.

D. Histogram showing [3H] CPM for Icmt flx/+ and Icmt flx/flx mice with PDX-1-Cre; ROSA26-LSL-LacZ. The difference is significant (p<0.05).
Figure S2
A. ICMT flx/+; PDX-1-Cre; LSL-KRAS G12D

ICMT flx/flx; PDX-1-Cre; LSL-KRAS G12D

B. ICMT flx/+; PDX-1-Cre; LSL-KRAS G12D

ICMT flx/flx; PDX-1-Cre; LSL-KRAS G12D

Figure S3
Figure S4
Figure S5
Wild-Type
UAS-shSte14

Icmt Deficient
MS1096-GAL4>UAS-shSte14

Icmt Deficient
MS1096-GAL4>UAS-shSte14

Figure S6
Figure S7
Figure S8
Figure S9

Icm^{flx/flx}; PDX-1-Cre; LSL-KRAS^{G12D}

Icm^{flx/flx}; PDX-1-Cre; LSL-KRAS^{G12D}