SUPPLEMENTAL METHODS

Immunohistochemistry: In general, immunohistochemistry images in embryonic pups and pre-weaned pups were obtained from the duodenum and proximal small intestine. In adults, 3-month old mice ileum is shown. Primary antibodies were visualized using a biotinylated secondary antibody, Vectastain Elite ABC kit and DAB chromagen (Vector Labs, Burlingame, CA) or by fluorescence with Cy3 and Cy2 (Jackson ImmunoResearch Laboratories, West Grove, PA) [1]. For histochemical staining, cryostat sections of intestine were incubated with 5-Bromo-4-chloro-3-indoxyl-α– D glycopyranoside (Biosynth, Itasca, IL) in pH 5.0 citrate-phosphate buffer for Sucrase-isomaltase, or 5-Bromo-4-chloro-3-indolyl- β D fucopyranoside (Sigma, St Louis, MO) in pH6.0 citrate-phosphate buffer for Lactase, with 50 mM Potassium Ferricyanide and 50mM Potassium Ferrocyanide. Oil Red O stain was applied to cryostat sections of the intestine and fecal pellets smeared on slides to detect fat. High iron diamine (HID) staining was as previously described[2]. The Phyloxine/Tartrazine staining was performed as described (http://stainsfile.info/stainsFile/stain/micro/phyloxtart/end.html). Primary antibodies utilized were Cdx1 CPSP [1], BrdU (Upstate Biotechnology, Lake Placid, NY), monoclonal Cdx2 (Biogenex, San Ramon, CA), Sucrase Isomaltase (gift of K. Yeh, LSU Shreveport. LA), Chromogranin A (Immunostar, Hudson, WI), Lysozyme (Dako, Carpinteria, CA), and Anti-Flag tag (Sigma, St. Louis, MO), Sox9 (Chemicon, Ramona, CA), β-catenin (Transduction laboratories, Lexington, KY) . Hes1 antibody was a gift of Ben Stanger, University of Pennsylvania. Klf4 antibody was a gift of Jonathon Katz, University of Pennsylvania. The Fatp4 antibody was kindly provided by Andreas Stahl, University of California Berkeley. Math1 antibody was a gift of Jane E. Johnson, University of Texas Southwestern. Additional

antibodies were obtained for Dgat2, Mttp, and Ihh (all from Abcam, Cambridge, MA), CD36 (Beckman Coulter, Brea, CA), Ki67 (Novocastra Laboratories, Bannockburn, IL), and cyclin D1 (Neomarkers, Fremont, CA). EphB2, EphB3 (R&D Systems, Minneapolis, MN) required amplification with TSA (Perkin Elmer Life Sciences, Shelton, CA) followed by streptavidin-Cy3. Alcian blue staining[1] and High Iron Diamine Staining[2] was as previously described.

Extent of transgene expression villi counts: The small intestine was divided into three equal lengths, fixed in 4% PFA overnight, and embedded as Swiss rolls. Sections were stained with Anti-Flag tag antibody. Duodenum counts began just distal to the Brunner's glands and proceeded distally. Ileum and jejunum counts began at the distal end of the each section and proceeded toward the proximal end. Because transgene expressing and non-expressing crypts adjacent to each other produce villi with one side positive and the other side negative, 200 half-villi in each section (as consecutive as possible) were scored as either positive or negative for Flag expression.

Cell count studies: Paneth cell numbers were counted in transgene expressing and non-expressing crypts with β-catenin co-stain to identify cell edges. Three transgenic and two non-transgenic Line 1 3-month old littermates were used. Nomarski optics visualized the Paneth cell granules. Goblet cells were counted in FLAG-Cdx2 and Alcian Blue stained tissue sections. Mice were injected with BrdU (Zymed, San Francisco, CA) 1 hour before sacrifice, and BrdU(+) cells were identified by immunofluorescence.

RNA detection by quantitative RT-PCR: In general, qPCR was performed on RNA obtained from the duodenum and proximal small intestine in embryonic pups and pre-weaned pups. In

adult 3-month old mice the distal jejunum was sampled. Sections of the intestine were stored in RNALater (Ambion, Austin, TX). Total RNA was isolated from the tissue with a RNAeasy Midi kit (Qiagen, Valencia, CA). cDNA was prepared from one microgram of total RNA using Superscript II (Invitrogen, Calsbad, CA) and random hexamers. Quantitative RT-PCR was performed on an ABI 7000 (Applied Biosystems, Foster City, CA), with Syber Green used as the fluorescent dye. Primers were designed using Primer Express software (Applied Biosystems). Primers used are listed in Supplemental Table S1. mHes1, mHes5, and mHey1 primers were a gift of Ben Stanger, University of Pennsylvania. Klf4 primers were a gift of Jonathon Katz, University of Pennsylvania. Standard PCR conditions with dissociation curves were utilized, and the PCR results analyzed using the Ct analysis as described[3]. 36B4 was used as the housekeeping control gene.

Proliferation and cell count studies: Mice were injected with BrdU (Zymed, San Francisco, CA) 1 hour before sacrifice. After BrdU and Lysozyme immunofluorescence stain, BrdU labeled nuclei were counted in 10 full-length ileal crypts that reached the muscularis from both transgene expressing crypts (Paneth cell negative) and non-expressing (Paneth cell positive) crypts, and expressed as the percentage of BrdU(+) cells per total cells in the crypt unit. Neuroendocrine cells were identified with Chromogranin A staining. Nomarski optics were used to visualize the Paneth cell granules. For migration studies, Line1 littermates were injected with BrdU and sacrificed at 24, 48, 72, and 96 hours after BrdU injection.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. FLAG-Cdx2 transgene expression in Line 1 and 5 Villin-Cdx2 transgenic mice. A. The FLAG-Cdx2 transgene is first detected at E15 in Line 5 mice. **B.** α-FLAG staining in Wt and transgenic colon. **C. and D.** Mosaic FLAG-Cdx2 expression in the ileum of 3-month old female **C.** Line 1 and **D.** Line 5 transgenic mice. **E.** Percent of Villi expressing Flag-Cdx2 transgene in Line 1 (**L1**), 4 (**L4**), and 5 (**L5**) mice. Duodenum: **blue bars;** Jejunum: **Red Bars;** Ileum: **yellow bars. F.** Real-time RT-PCR quantification of total Cdx2 mRNA levels in small intestine of non-transgenic (**White bar**) and transgenic (**Blue bar**) littermates. Quantification of endogenous Cdx2 and Cdx1 mRNA levels in the jejunum of non-transgenic (**White**) and transgenic Line 1 (**Blue**) male mice at post-partum day 3 (**P3**) and female Line 1 mice at 3 months of age. Also shown is Cdx1 staining in the ileum by immunohistochemistry in transgene expressing (crypts with goblet cells, **G**) and non-expressing crypts (with Paneth cells, **P**) at 3 months.

Supplemental Figure S2. FLAG-Cdx2 transgene expression in Line 5 Villin-Cdx2 transgenic mice. A. The non-transgenic wild-type littermate intestine stained with anti-FLAG antibody. **B.** FLAG-Cdx2 transgene is expressed extensively in male Line 5 mice at two days post-partum (**P2**). **C.** FLAG-Cdx2 transgene is expressed extensively in female Line 5 mice at P2. **D.** Mosaic pattern of FLAG-Cdx2 expression in duodenum of 3-month old Line 5 female mice. **E.** Wild-type and transgenic littermates at P2 and P10. **F.** Kaplan-Meier survival curve for Line 5 mice representing 167 mice from 24 litters; Wild-type male and female (**WtM** and **WtF**), transgenic male and female (**TgM, TgF**), and mice of unknown (**Unk**) genotype that

disappeared before they could be genotyped. Survival curves are significantly different by Logrank testing $p<0.0001$. **G.** Growth curve for Line 5 female littermate mice n=2 mice each.

Supplemental Figure S3. Phenotypes associated with Villin-Cdx2 transgene are dosage-dependent. Line 6 mice, heterozygous for the transgene, were inbred and it was observed that approximately one-fourth of the mice had poor weight gain prior to weaning, and this effect was associated with early demise (not shown). **A.** Picture of litter from Line 6 inbreeding, day 8 post partum, with the poor growing pup indicated by **blue arrow**. **B.** Quantification of total Cdx2 mRNA levels in the jejunum of non-transgenic (**Blue**) and transgenic (**orange**) mice at post-partum day 3 from normal-growing and slow-growing mouse pups. **C.** Cdx2 transgene expression by FLAG immunohistochemistry in P3 mice from normal growth and slow-growth littermates.

Supplemental Figure S4. Sucrase isomaltase activity and serum triglyceride levels are reduced in Villin-Cdx2 transgenic mice. A. Sucrase isomaltase enzymatic activity detected histochemically in fresh frozen intestinal tissue from 1 day-old mice and E20.5 embryos. **B.** levels of SI mRNA by qPCR in P1 and E20.5 mice. **Black bar**: wild-type mice; **grey bar**: Villin-Cdx2 mice. n=3 mice; p value determined by Student's T test. **C.** Serum triglyceride and cholesterol levels in pooled specimens from day 0 to 3 day old line 1 male mice. **Blue line**: wild-type littermate; **Pink line**: Villin-Cdx2 mice. 3 to 6 littermates were exsanguinated and their blood pooled for each serum sample.

Supplemental Figure S5. Cdx2 expression alters the levels of critical factors involved in intestinal lipid absorption. A. CD36 mRNA and protein levels are reduced in P2 male Line 1 mice. **Black bar**: wild-type mice; **White bar**: Villin-Cdx2 mice. n=3 mice; p value determined by Student's T test. **B.** Similar study except Fatp4 levels were determined. **C.** Similar study except Mttp levels were determined by qPCR and immunohistochemistry. **D.** MTTP levels remained diminished in adult Line 1 female mouse at 3 months by immunofluorescence staining. Mice were fed normal chow during this period. **E.** Indian hedgehog (**Ihh**) levels by qPCR in wild-type (**Black bar**) and Villin-Cdx2 (**White bar**) male mice at P2. n=3 mice; p value determined by Student's T test.

Supplemental Figure S6. Line 6 mice when inbred yield mice with loss of Paneth cell markers as observed in Lines 1, 4, and 5. Line 6 mice were inbred and it was observed that approximately one-fourth of the mice had poor weight gain prior to weaning, and this effect was associated with early demise (not shown). **A.** These mice were shown to have increased Cdx2 levels compared to non-inbred Line 6 mice. Immunohistochemistry for FLAG-Cdx2 in 3-month old adult mice demonstrates increased expression of the transgene protein in the inbred, poor growth pup intestine. **B.** Lysozyme staining of the same tissue demonstrates significant loss of mature Paneth cells in the inbred, poor growth Line 6 mice at 3 months.

Supplemental Figure S7. Enteroendocrine (EE) cell numbers, Sox 9 levels, and BrdU labeling are unchanged with expression of the Villin-Cdx2 transgene.
Immunohistochemistry with Anti-ChromograninA antibody in **A.** transgene non-expressing (**TgNE**) and **B.** Transgene expressing (**TgE**) crypts. **C.** Quantification of total EE cell numbers in

20 TgNE and TgE crypts from Line 1 and Line 5 female mice (3 each) at three months, separating crypt EE cells from villi cells. Controls included EE cell numbers from Wt littermates. **D.** Sox9 mRNA levels by qPCR in 3 month old Line 1 female mice, n=3. P value was determined by Student's T test. **E.** Immunofluorescence co-staining for lysozyme and BrdU. **F.** Quantification of BrdU incorporation in TgNE, TgE, and Wt crypts at 3 months.

Supplemental Figure S8. Cdx2 overexpression does not result in significant changes to previously identified regulators of Paneth and goblet cell differentiation. A. Immunohistochemistry with for Hes1 demonstrated migration of Hes1+ cells to the crypt base when Paneth cells are absent. (**G**) goblet cell crypt; (**P**) Paneth cell + crypt. qPCR for Hes1, Hes5, and Hey1 mRNA levels in 3 month old Line 1 female mice. n=3; p values determined by Student's T test. **B.** Klf4 mRNA and protein levels by qPCR and immunohistochemistry in 3 month old Line 1 female mice. Immunohistochemistry for Klf4 was costained with phyloxine/tartrazine to identify Paneth cell granules and counterstained with hematoxylin. For qPCR, n=3; p values determined by Student's T test. **C.** Math1 mRNA and protein levels by qPCR and immunohistochemistry. (**G**) goblet cell crypt; (**P**) Paneth cell + crypt. D. Spdef and Lkb1 mRNA levels by qPCR in 3 month old female Line 1 mice; n=3; p values determined by Student's T test.

Supplemental Table S1 PCR primers used in this study.

Genotyping primers:

Villin 4937	5’ CTGTGTGTGGGGACAGAGAAC 3’
TreCdx2B2	5’ GAGGACTGACAAAGTTCTGCG 3’

Sexing primers

SMC4-1	5’ TGAAGCTTTTGGCTTTGAG 3’
SMCX1	5’ CCGCTGCCAAATTCTTTGG 3’

Transgene specific primers:

FVMCex1	5’ TGGCTGCCTCTTCCAGACA 3’
RCdx2 323	5’ GCACGGAGCTAGGATACATGCT 3’

Total Cdx2 primers

qFCdx2 840	5’ CGATACATCACCATCAGGAGG 3’
qRCdx2 977	5’ TGGCTCTGCGGTTCTGAAA 3’

Endogenous Cdx2 primers:

FCdx2UTR	5’ CCTACCCACGAACAGCATCTACT 3’
RCdx2UTR	3’ CCTGAGGTCCATAATTCCACTCA 3’

Cdx1 primers

qFCdx1UTR	5' CTAGGACAAGTAGCTTGCCCT 3'
qRCdx1UTR	5' TCCAACAGGCTCACCACACA 3'
FmSI	5' CGTCCTCCTCACGACTCAGAGT 3'
RmSI	5' CTTGTTATTTGGATCTGTGAGCTTGA 3'
FmLPH	5' CAGAAGCTGAGTGAATTGCAGAA 3'
RmLPH	5' GCAGTCCTGAGGTTTAGGGTGTA 3'
FmMttp	5' CAAGCTCACGTACTCCACTGAAG 3'
RmMttp	5' CAGTAACACAACGTCCACATCAGA 3'
FmSpdef	5' CACGTTGGATGAGCACTCGCTA 3'
RmSpdef	5' AGCCACTTCTGCACGTTACCAG 3'
FmSox9	5' GACAAGCGGAGGCCGA 3'
RmSox9	5' CCAGCTTGCACGTCGGTT 3'
FmLkb1	5' CTACTCCGAGGGATGTTGGA 3'
RmLkb1	5' GATAGGTACGAGCGCCTCAG 3'
FmFatp4	5' CCAGTAGTGTGGCCAACTTCCT 3'

RmFatp4	5' CCACAGACCCACAAACTCATTG 3'
FmIhh	5' GACTCATTGCCTCCCAGAACTG 3'
RmIhh	5' CCAGGTAGTAGGGTCACATTGC 3'
FmCD36	5' TGTGTTTGGAGGCATTCTCA 3'
RmCD36	5' TTTTGCACGTCAAAGATCCA 3'
FmDgat2	5' AGTGGCAATGCTATCATCATCGT 3'
RmDgat2	5' AAGGAATAAGTGGGAACCAGATCA 3'
m36B4F	5’ TTTATAACCCTGAAGTGCTCGACAT 3’
m36B4R	5’ TGGCACCGAGGCAACAG 3’

1. Crissey MA, Guo RJ, Fogt F, et al. The homeodomain transcription factor Cdx1 does not behave as an oncogene in normal mouse intestine. Neoplasia 2008;10:8-19.
2. Silberg DG, Sullivan J, Kang E, et al. Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice. Gastroenterology 2002;122:689-96.
3. Kong J, Nakagawa H, Isariyawongse B, et al. Induction of Intestinalization in Human Esophageal Keratinocytes is a Multi-step Process. Carcinogenesis 2009;30:122-130.