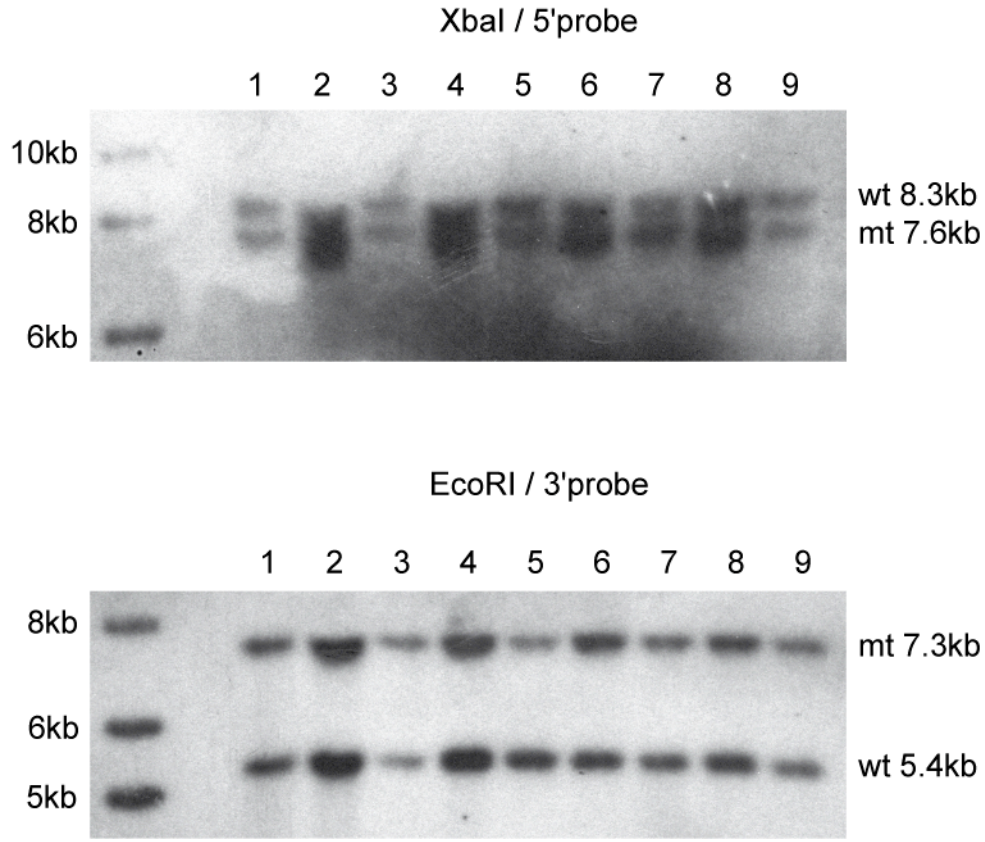
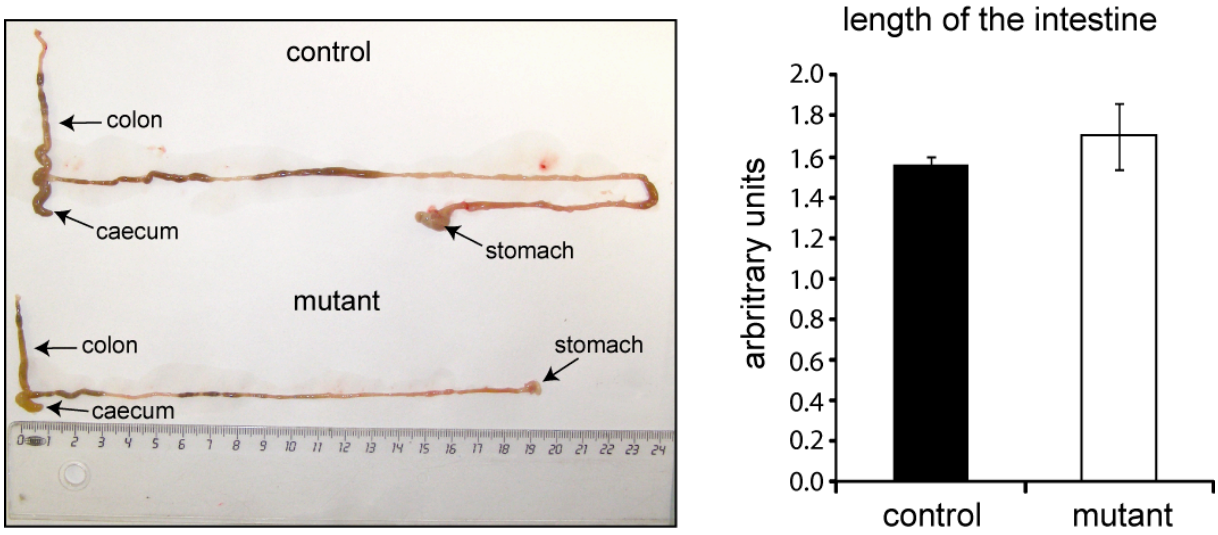


Supplemental Figure 1

Selected ES cell clones show a correctly recombined conditional *Ngn3* allele

Genotype analysis by Southern blots of nine independent recombinated ES cell clones by hybridization with a 5'- and 3'-external genomic probe. wt, wild type; mt, mutant; the molecular weight of the wild type and mutant bands are indicated.

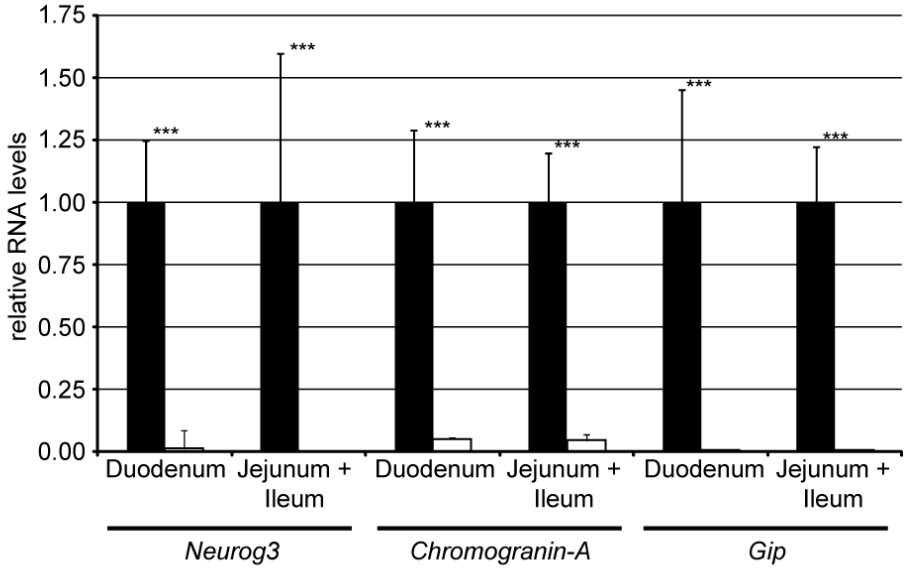


Supplemental Figure 2

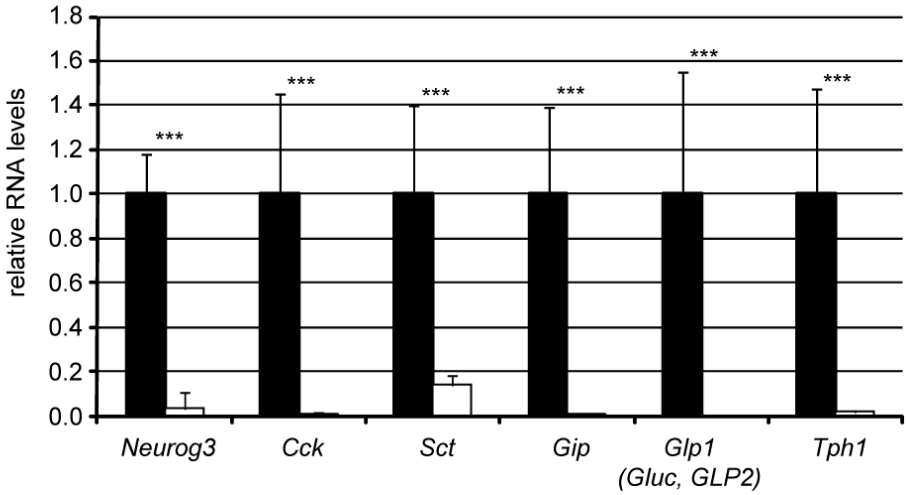
The length of the intestine is not altered in *Ngn3*$^{\Delta int}$ mice.

Representative photograph showing the intestinal tract of control and *Ngn3*$^{\Delta int}$ mutant mice. Mutant and control mice show no statistical significant difference in the length of their intestinal tract (graph). n=4, the measured length of the intestine was normalized to the weight of the mouse analyzed. Age of the mice = 24-26 month.

A



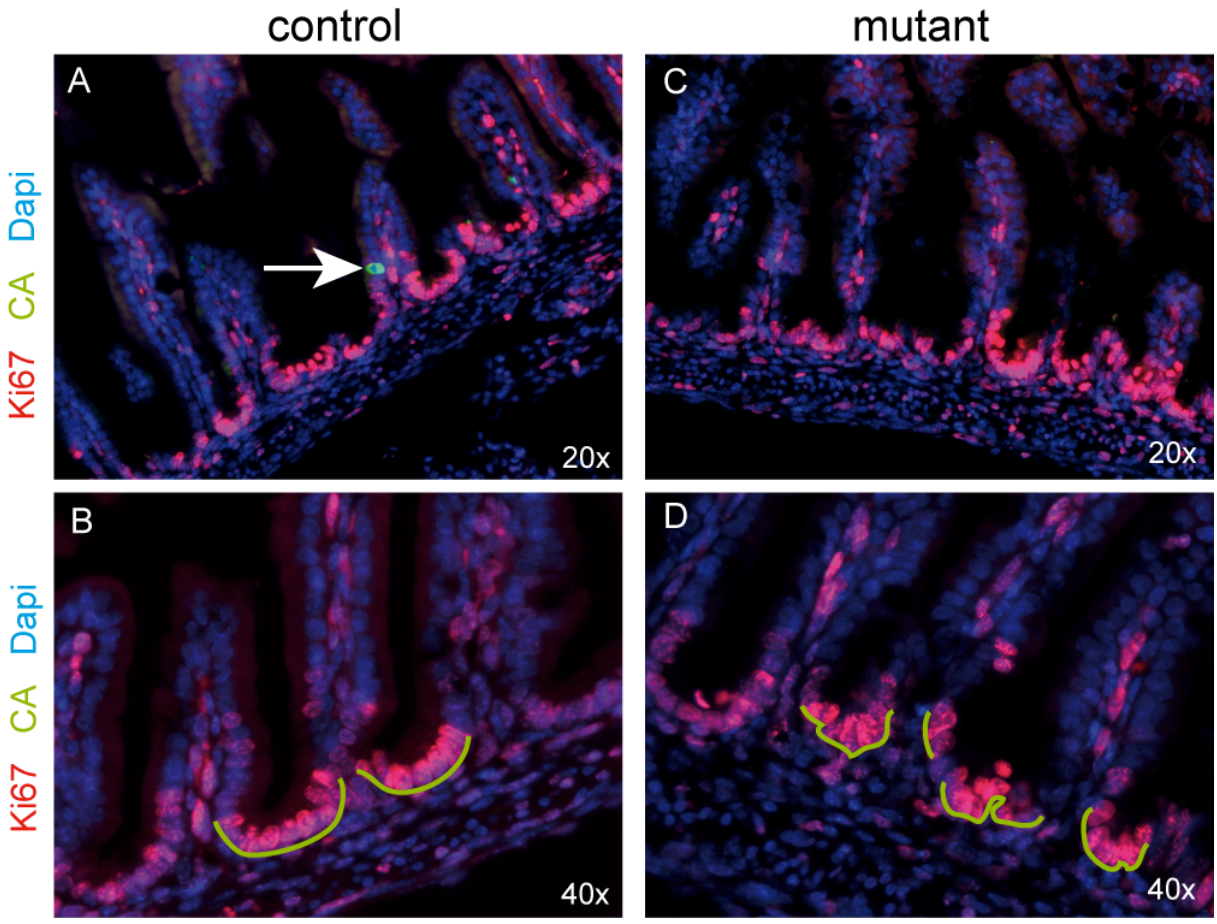
B



Supplemental Figure 3

Depletion of enteroendocrine cells in the intestine of *Ngn3$^{\Delta int}$* mice.

Total RNA was prepared from the duodenum and jejunum+ileum of E19.5 (A) or the epithelium of the small intestine of 8-10 week old (B) *Ngn3$^{\Delta int}$* and control tissue, respectively. The relative mRNA levels of Neurog3 (A, B), Chromogranin-A (A), Cck (B), Secretin (B, Sct), Gip (A, B), Glp1 (B, Gluc, Glp-1 and Glp-2 are encoded by the same mRNA) and Tph1 (B) were measured by RT-qPCR using mouse specific TaqMan gene expression assays. *Ngn3$^{\Delta int}$* mice show a more than 90% decrease of *Ngn3*, *Chromogranin-A*, *CcK*, *Gip*, *Glp1* and *TpH1* expression. *Sct* expression is reduced more than 80%. For mutant (white columns) and control (black columns) n=5-6; ***:p<0.001.

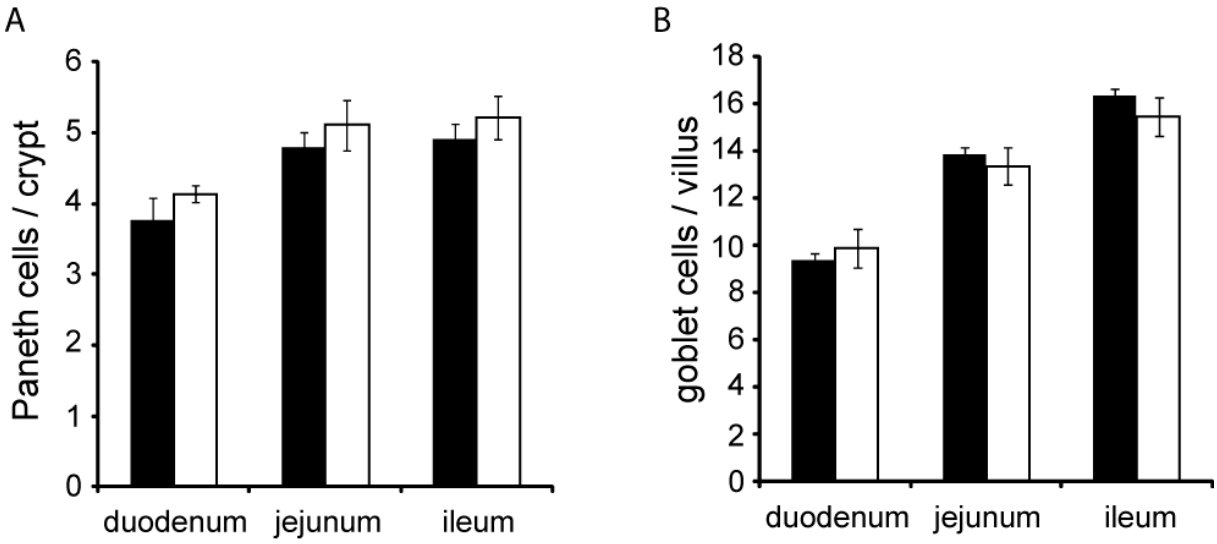


Supplemental Figure 4

Intestinal epithelial cell proliferation is not altered in *Ngn3*$^{\Delta int}$ embryos.

Sections of E19.5 control (A, B) and mutant (C, D) intestine were examined for the status of the proliferative inter-villus compartment by immunofluorescence staining for the proliferative cell marker Ki67. *Ngn3*$^{\Delta int}$ mutant embryos show only a mild disordered (green line underlining the intervillus region in control (B) and mutant (D) intestine) but no enlarged proliferative inter-villus compartment. Immunofluorescence staining for Chromogranin-A was used as control for the presence (flashes in A) or absence of enteroendocrine cells.

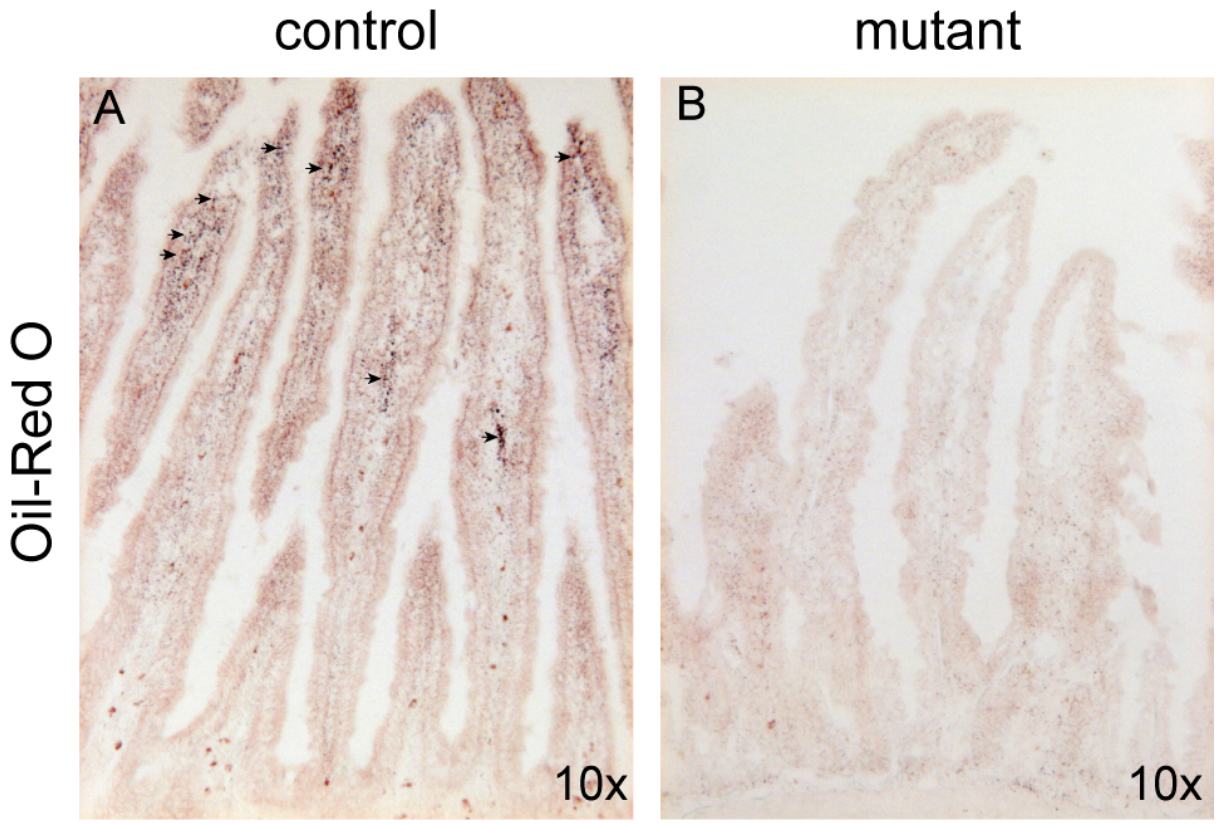
Supplemental Figure 5



Supplemental Figure 5

Paneth and Goblet cell numbers are not altered in *Ngn3*$^{\Delta int}$ mice.

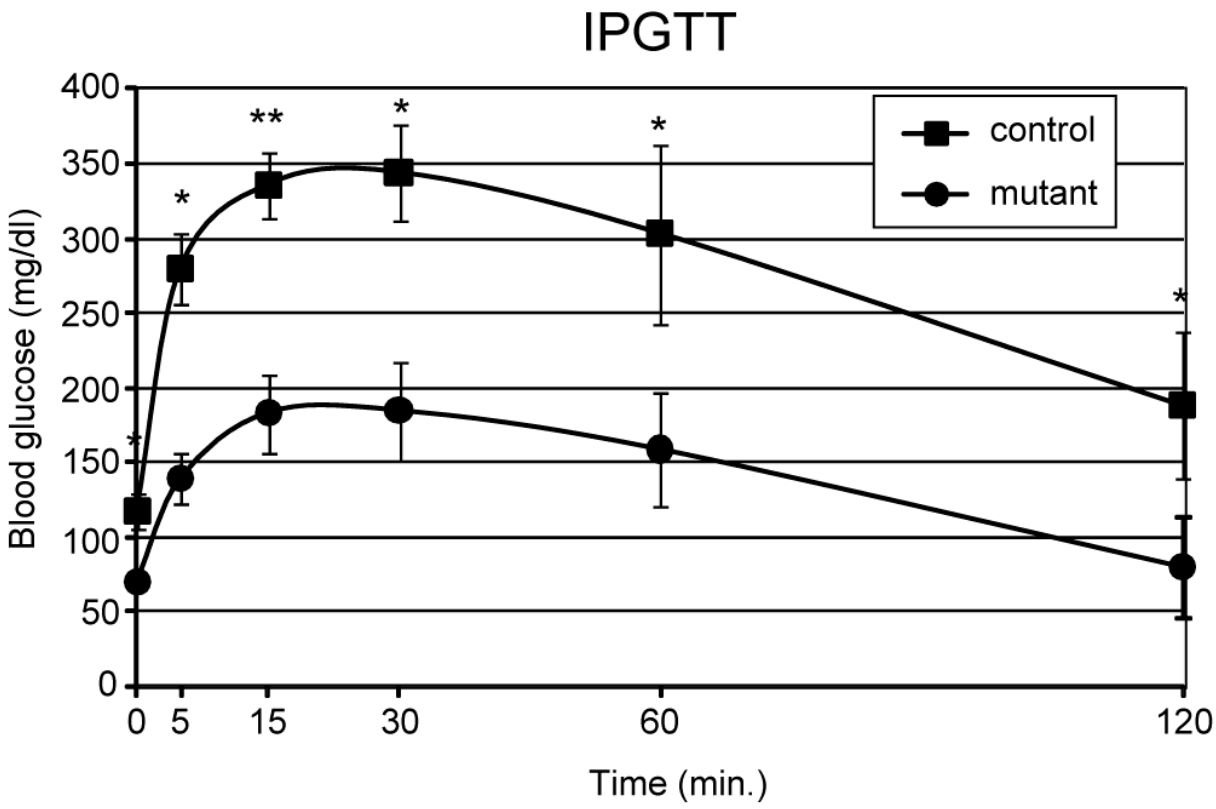
Sections of adult wild type and mutant duodenum, jejunum and ileum were examined for the number of Paneth (A) and Goblet cells (B) per villus or crypt, respectively. Mutant mice show no difference in the number of Paneth (A) or Goblet cells (B) compared to wild type mice. A, B) For mutant (white columns) and control (black columns) n=4, with an average of 100-150 villi and 100-140 crypts analyzed per animal, respectively. A) Cell counts were normalized to the length of the villus analyzed. A, B) Age of the mice analyzed = 10-12 weeks.



Supplemental Figure 6

Impaired lipid absorption in *Ngn3*$^{\Delta int}$ mutant mice.

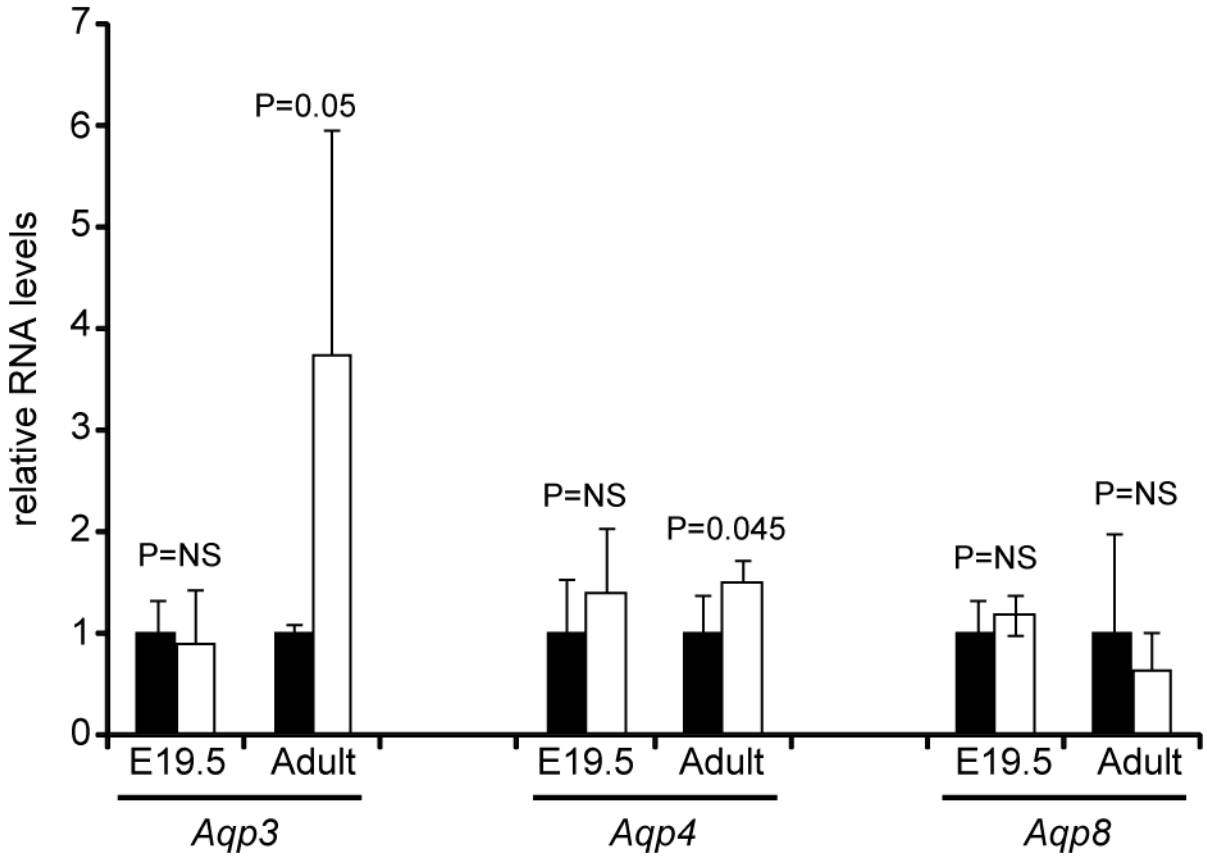
Oil red O, which stains neutral fats, was used to visualize lipid droplets in control (A) and mutant (B) tissue. Mutant small intestine clearly shows a strong reduction in the amount of lipid droplets (dark dots in A and indicated by some flashes) compared to control tissue.



Supplemental Figure 7

Altered glucose homeostasis in *Ngn3*$^{\Delta int}$ mice.

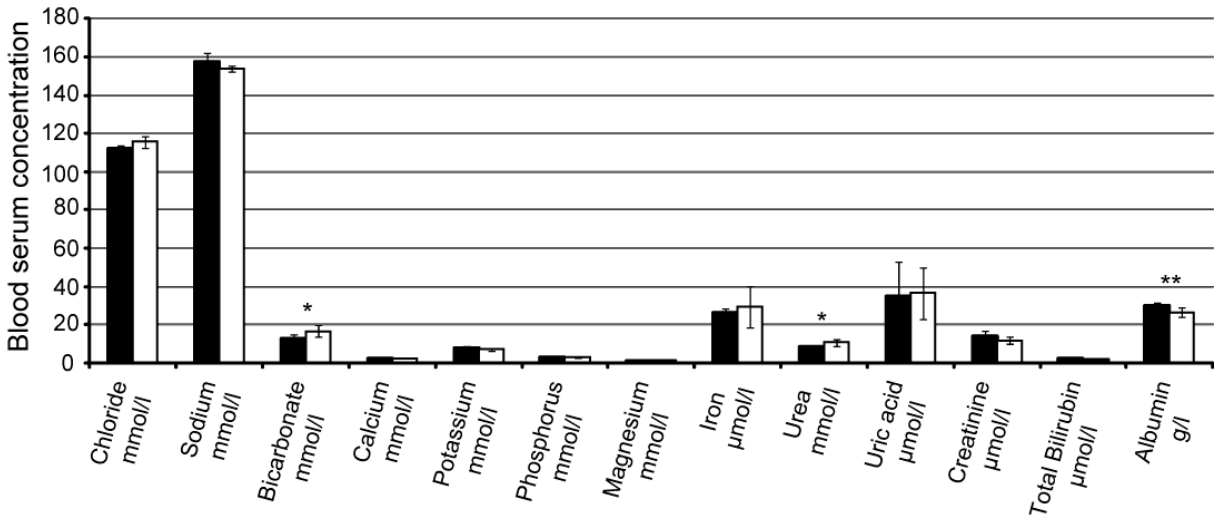
Control (filled squares) and mutant mice (filled circles) were subjected to an intraperitoneal (IPGTT) glucose challenge. Blood glucose levels were than measured at the indicated time points. At all time points measured blood glucose levels of mutant mice do not rise to the same levels as in control mice. For control and mutant mice n=4-6. 0 = blood glucose level before the glucose challenge. *:$p<0.05$, **:$p<0.01$. Age of the mice analyzed = 14 weeks.



Supplemental Figure 8

The expression levels of Aqp3, Aqp4 and Aqp8 are not reduced in *Ngn3$^{\Delta int}$* mice.

Total RNA was prepared from the colon of E19.5 or 8-10 week old *Ngn3$^{\Delta int}$* and control tissue, respectively. The relative mRNA levels of Aqp3, Aqp4 and Aqp8 were measured by RT-qPCR using mouse specific TaqMan gene expression assay. For mutant (white columns) and control (black columns), n=6; NS:non significant.



Supplemental Figure 9

Ion blood levels are not changed in *Ngn3$^{\Delta int}$* mice.

Chloride, Sodium, Calcium, Potassium, Phosphorus, Iron and Magnesium levels are unaltered in *Ngn3$^{\Delta int}$* mice whereas they show slightly increased Bicarbonate and Urea and decreased Albumin levels. For mutant (white columns) and control (black columns) n=7; *:p<0.05, **:p<0.01. Age of the mice analyzed 10-12 weeks.