Supporting Information

Wang et al. 10.1073/pnas.1017668108

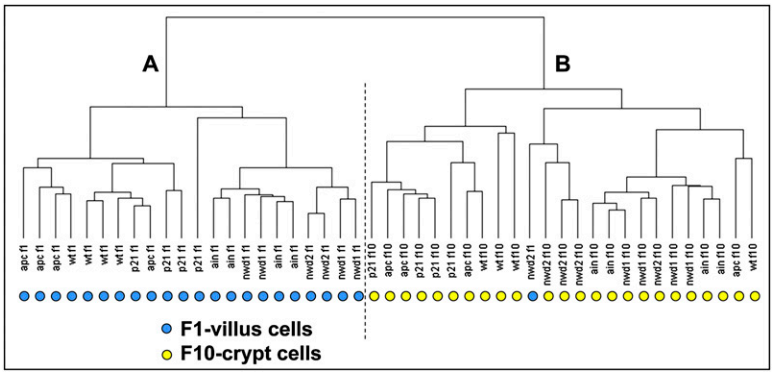

Fig. S1. Unsupervised clustering of all Affymetrix gene expression data from crypt and villus cells of all mice (47 arrays) of the dietary and genetic risk groups clusters the data into two main brances, *A* and *B*. The array intensity values were filtered by spot filter and gene filter, globally normalized, and center-clustered using centered correlation. Villus and crypt cell data cluster separately, regardless of genotype or diet. Total RNA from each epithelial cell fraction was isolated using the Qiagen RNeasy Mini kit, and RNA integrity was verified by denaturing agarose gel electrophoresis. Methods. Five micrograms total RNA from each of fraction 1 (F1; upper villus) and fraction 10 (F10; lower crypt) were reverse-transcribed into cDNA (one-cycle cDNA synthesis kit; Affymetrix). The Affymetrix GeneChip IVT labeling kit was used to generate biotin-labeled cRNA. Fragmented cRNA was then hybridized to an Affymetrix 430 2.0 mouse expression array, and chips were subsequently scanned by the Genomics Core Facility of the Albert Einstein College of Medicine. Data analysis. CHP files generated by GeneChip Operating Software (GCOS; Affymetrix) were imported into BRB-ArrayTools (http://linus.nci.nih.gov/BRB-ArrayTools.html). Probes for which there were absent detection calls and probe sets for which all arrays were flagged as absent were removed by spot filter and gene filter, respectively. Signal intensities were log-transformed (base 2), and global median normalization was performed. Expression datasets were processed by Cluster 3.0 and visualized using Treeview (Eisen Laboratory, Stanford University, Stanford, CA). Two classes of alterations were identified: significantly changed sequences between different groups defined by the class comparison function integrated in BRB-ArrayTools using combined criteria of both $P < 0.05$ and greater than twofold change and risk-regulated sequences defined as those for which expression was absent/present in one fraction (F1 or F10) of all control mice (e.g., WT or AIN76A) but present/absent in the same fraction of all $Apc^{1638N/+}$-, $p21^{-/-}$-, or NWD1-fed mice.

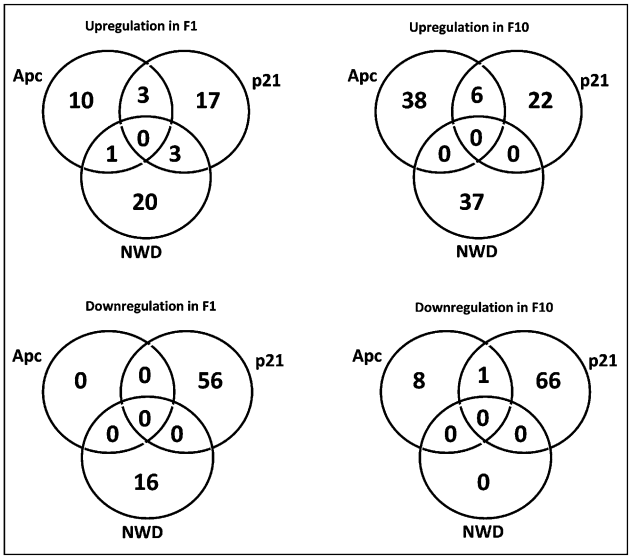

Fig. S2. Limited overlap of functional groups enriched by each risk factor in both villus and crypt cells. Biological categorization of sequences was by gene ontology (GO) enrichment analysis using GO-Elite (Gladstone Institutes, University of California at San Francisco, San Francisco, CA) with overrepresentation and permutation algorithms to identify nonredundant GO terms and pathways associated with a gene set.

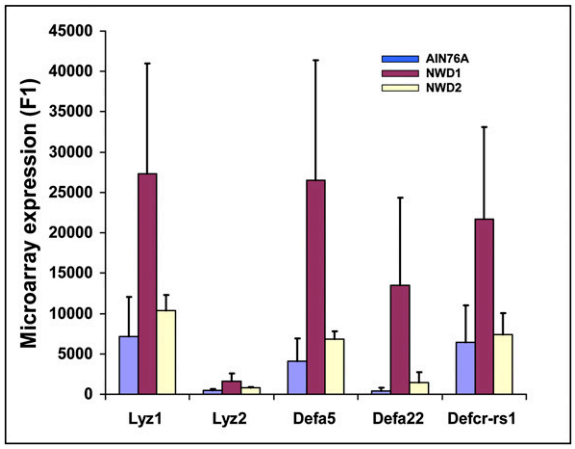

Fig. S3. Dietary effects on expression of Paneth cell markers calculated from the gene expression data generated using Affymetrix 430 2.0 mouse microarrays.

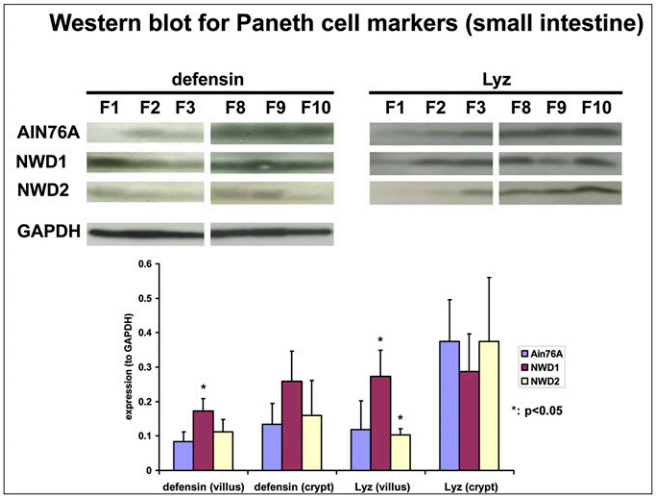

Fig. S4. Western blot analysis of defensin and lysozyme in cells isolated from the villus (F1–F3) and crypt (F8–F10) of C57BL/6 mice fed AIN76A, NWD1, or NWD2 from weaning for 1 y. The method of analysis was as described in ref. 1.

1. Wang D, et al. (2010) Altered dynamics of intestinal cell maturation in Apc1638N/+ mice. *Cancer Res* 70:5348–5357.

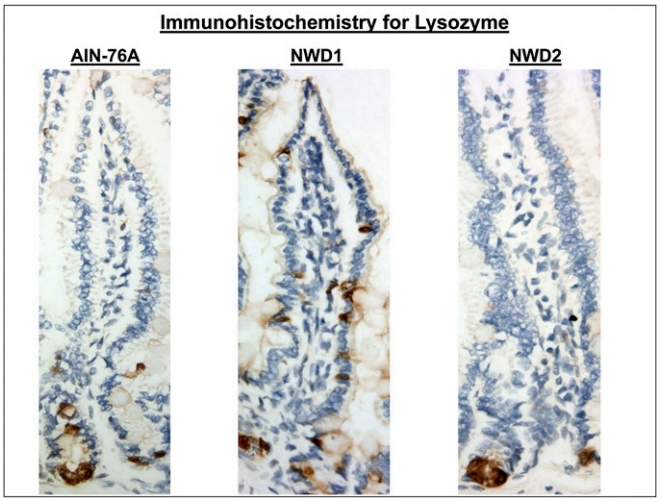

Fig. S5. Immunohistochemical detection of the Paneth cell marker lysozyme in the intestinal mucosa of mice maintained on AIN76A, NWD1, or NWD2 from weaning for 1 y. Positive staining appears brown and is localized to Paneth cells in the crypt for mice fed AIN76A or NWD2, but it also characterizes some cells in the villus of mice fed NWD1, with faint staining also seen lining the vacuoles of goblet cells.

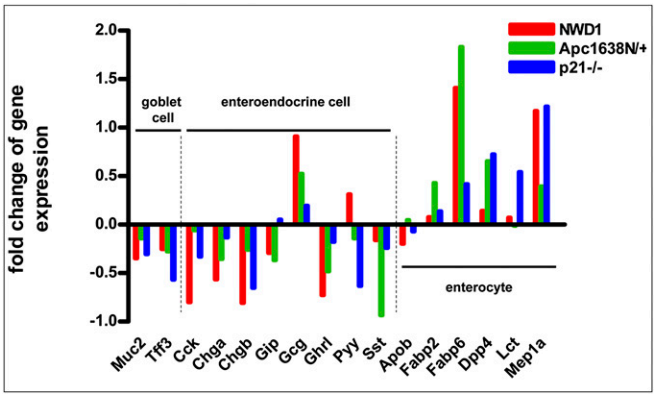

Fig. S6. Altered expression of markers of goblet cells, enteroendocrine cells, and enterocytes in villus cells for each of the dietary and genetic risk groups. Expression levels relative to GAPDH were determined by quantitative RT-PCR. The data for the $Apc^{1638N/+}$ mice were previously published (1), and they are presented for comparison with the other groups.

1. Wang D, et al. (2010) Altered dynamics of intestinal cell maturation in Apc1638N/+ mice. *Cancer Res* 70:5348–5357.

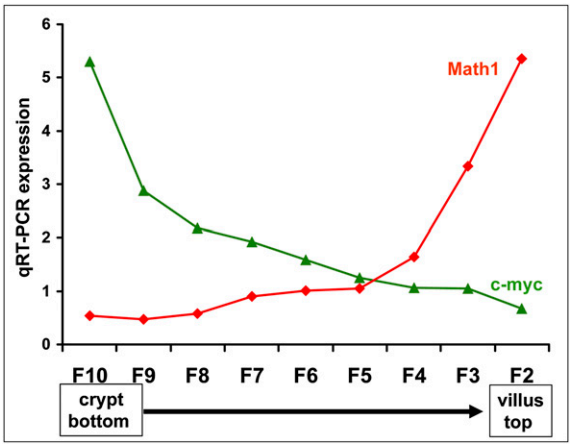

Fig. S7. Expression of c-myc and Math 1 in cells isolated according to position along the crypt (fraction 10) to villus (fraction 2) axis. Fraction 1, the first eluting cells from the tip of the villus, was not assayed because of limited numbers of cells collected and used for the array experiments herein. The method of cell isolation was a function of position along the crypt–villus axis. Intestines are dissected, flushed with wash solution, everted, tied off at one end, distended by filling with PBS, and then tied off at the other end. The everted organ is then incubated for 5 min at 37 °C in buffer A (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 1 mM DTT, pH 7.1–7.4). Sequential elution of cells from the top to the bottom of the villus–crypt axis is then accomplished by the following 10 sequential incubations at 37 °C in buffer B (1× PBS, 1.5 mM EDTA, 0.5 mM DTT, 0.1% BSA, pH 7.1–7.4): 10, 10, 6, 5, 5, 9, 10, 15, 25, and 30 min/elution. After each period of incubation on a rocking platform, cells are harvested by centrifugation, washed in cold PBS, snap-frozen in liquid N_2, and stored at −80 °C until use.

Table S1. Differentially expressed sequences for all pair-wise comparisons, including statistically significantly changed sequences (criteria: greater than twofold change and $P < 0.05$) and risk-specific regulated sequences (absent vs. present in all mice in given groups)

Table S1

Table S2. Functional groups altered for each of the dietary and genetic risk factors based on the overall expression data

Table S2 (DOCX)

Table S3. Primers for quantitative PCR analysis

Table S3 (DOC)