**Supplemental information**

**Table S1.** Composition of the diets

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| --- | --- | --- |
|  | AIN-93G | WD |
| Casein (g/kg) | 200 | 232 |
| l-Cysteine (g/kg) | 3 | 3 |
| Corn starch (g/kg) | 397.48 | 305.63 |
| Maltodextrin (g/kg) | 132 | 95 |
| Sucrose (g/kg) | 100 | 116 |
| Soybean oil (g/kg) | 70 | – |
| Anhydrous milk fat (g/kg) | – | **132.8** |
| Canola oil (g/kg) | – | 55.4 |
| Sunflower oil (g/kg) | – | 11.8 |
| Cellulose (g/kg) | 50 | 20 |
| Calcium (g/kg) | 5 | 0.5 |
| Folic acid (mg/kg) | 2 | 0.2 |
| Vitamin D (IU/kg) | 1000 | 100 |
| kcal from proteins (%) | 18.8 | 18.5 |
| kcal from carbohydrates (%) | 63.9 | 42.3 |
| kcal from fat (%) | 17.2 | 39.2 |



**Figure S1.** Related to *Western diet increases weight and induces neoplastic changes in colon* paragraph in the main text. **A** Mouse weight in different experimental groups. Mice were separated according to sex, N = 3-7 mouse per each group, \* p < 0.05. **B** Expected tumors with 95% confidence intervals. WD increases progressively number of colon tumors (neoplasms and hyperplastic growths) in mice with aging.



**Figure S2.** Venn diagrams of proteins detected and identified with two different proteomics platforms: 2D-DIGE and LS-MS.



**Figure S3.** Related to Figure 2. Extended histological analysis of colon crypts. **A** Number of cells in colon crypts in different experimental groups. **B** Size of the colon cell in different experimental groups. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, \*\*\*\* p < 0.0001, a denotes p < 0.001 significant difference between diet groups at 12 months and diet groups at 18 months.



**Figure S4.** Related to Figure 3. Changes in bile acid transport pathway. **A** Validation of FABP6 expression with three independent quantitative Western blots and an accompanying representative image. **B** LS-MS quantitation of FABP6. **C** Expression of the *Fxr* gene, bile acid sensing nuclear receptor. **D** Expression of FXR target *Shp* on mRNA level indicate that activity of FXR is significantly reduced in colon tissue of mice fed with WD. Western blots were performed in three independent assays. Error bars are SEM, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, \*\*\*\* p < 0.0001



**Figure S5.** Related to Figure 4.Volcano plot of metabolomics analysis of bile acids. Comparison of bile acid concentrations and similar compounds in colon **A** between diet groups at 18 months and **B** between 12m AIN and 18m AIN. Comparison of bile acid concentrations and similar compounds in content of colon lumen **C** between diet groups at 18 months and **D** between 12m AIN and 18m AIN. Gray letters denote fatty acids. Blue dots show significantly reduced, and red dots significantly increased concentrations of bile acids and fatty acids, respectively. Significance threshold p < 0.05 is indicated with red horizontal line. Concentration is described on x-axis as log2 value. Groups N = 8-10 animals, p < 0.05.

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**Figure S6.** Heatmap of comparison between fold changes in protein abundance detected in our study with 2D DIGE and RNAseq data (GSE67342) from (1). Gene expression and protein abundance in these two studies show striking similarity. Heatmap was generated by calculating correlation between fold changes and complete distance.

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**Figure S7.** Network analysis of LC-MS proteomics data at 12 months, combined with metabolomics data showing expression of proteins and concentration of intracellular bile acids in WD mice, supports inactivation of SREBF2 and HNF4A, both involved in regulation of *Asbt* transcription.



**Figure S8.** Abundance of MUC2 measured with LC-MS revealed increase of MUC2 in 12m WD group and reduction in 18m WD when compared to control groups. Western blots were performed in three independent assays. Error bars are SEM, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, \*\*\*\* p < 0.0001

1. Beyaz S, Mana MD, Roper J, Kedrin D, Saadatpour A, Hong S-J, et al. High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. Nature. 2016;531:53–8.