SUPPLEMENTAL DATA

Fig. S1. Clc-5 is highly expressed in the macrophages and weakly expressed in the colonic mucosa of WT mice, but not in peripheral blood monocytes (PBMCs). Peritoneal macrophages (MΦ) and peripheral blood monocytes (PBMCs), were isolated from both WT and *Clcn5* KO mice (6-8 weeks old). Equal amount of proteins (60 μg per lane) from purified macrophages and PBMCs were separated by SDS-PAGE and blotted for Clc-5 using anti-Clc-5 polyclonal antibody (1:1000 dilution) (**A**). Lane 1, PBMCs isolated from WT mice; land 2, empty lane (no sample was loaded); lane 3, MΦ isolated from WT mice; lanes 4, 5, 10, and 11, colonic mucosa isolated from WT mice; lanes 6 &7, colonic mucosa isolated from *Clcn5* KO mice (a negative control, in which no Clc-5 is expressed); lanes 8 &9, total extracts from kidney cortex of WT mice (as positive controls for Clc-5). Clc-5 was highly expressed in the macrophages (compare lane 3 with lanes 8 &9; ~1/3 of that in kidney), but not in PBMCs (lane 1). Colonic mucosa of WT mice express a low level of Clc-5 expression, which is more visible after prolonged exposure [compare lanes 10 &11 (long exposure) to lanes 4 &5 (short exposure) of the same blot]. The same blot was also probed for actin as a sample loading control (**B**). Representative of at least three independent experiments was shown.

Fig. S2. Elevated levels of IL-6 protein expression were observed in the kidney, but not the colon, of *Clcn5* KO mice. Protein expression of IL-6 in the kidney cortex of *Clcn5* KO and WT mice was analyzed by SDS-PAGE/Western blot and immunoflourescence, as

described in **Fig. S2.** (A) Significant increase in IL-6 protein expression in the kidney cortex of *Clcn5* KO relative to WT mice, suggesting the novel finding that IL-6 may contribute to an immunopathogenic role. Statistical analysis of the expression of IL-6 by normalization to Actin is also shown. (B) Immunofluorescence analysis of expression and localization of renal IL-6 expression using frozen sections of kidney cortex of WT and *Clcn5* KO mice. IL-6 was significantly elevated in the kidney cortex of *Clcn5* KO mice compared to WT. In WT kidney, low level of IL-6 expression was localized to the tubular basement membrane. However, in the *Clcn5* KO kidney, there was increased expression of IL-6 in the basement membrane, upregulation in the interstitium, surrounding renal tubules, as well as in epithelial cells of the proximal tubules, which was not observed in WT kidney sections. Representatives of at least 3 independent experiments are shown in A and B. Statistical significance is denoted by ** for p values less than 0.001.

Fig S3. Major differences in Z-diet when compared to the H-diet. A group of mice were subjected to a separate NIH-31 Modified Open Formula Diet, denoted Z-diet from birth to evaluate the influence of diet on the development of experiment colitis. As shown, this diet contains greater amounts of Vit-D, Vit B12, selenium, and choline and lower amounts of Iodine, Vit-K3, Inositol, Pantothenic Acid, Vit B2, Vit B6, Vit B7 and Vit E (among others) when compared to the H-Diet (Harlan Teklad Diet), which is the regular diet used at our mouse facility.

Fig S1

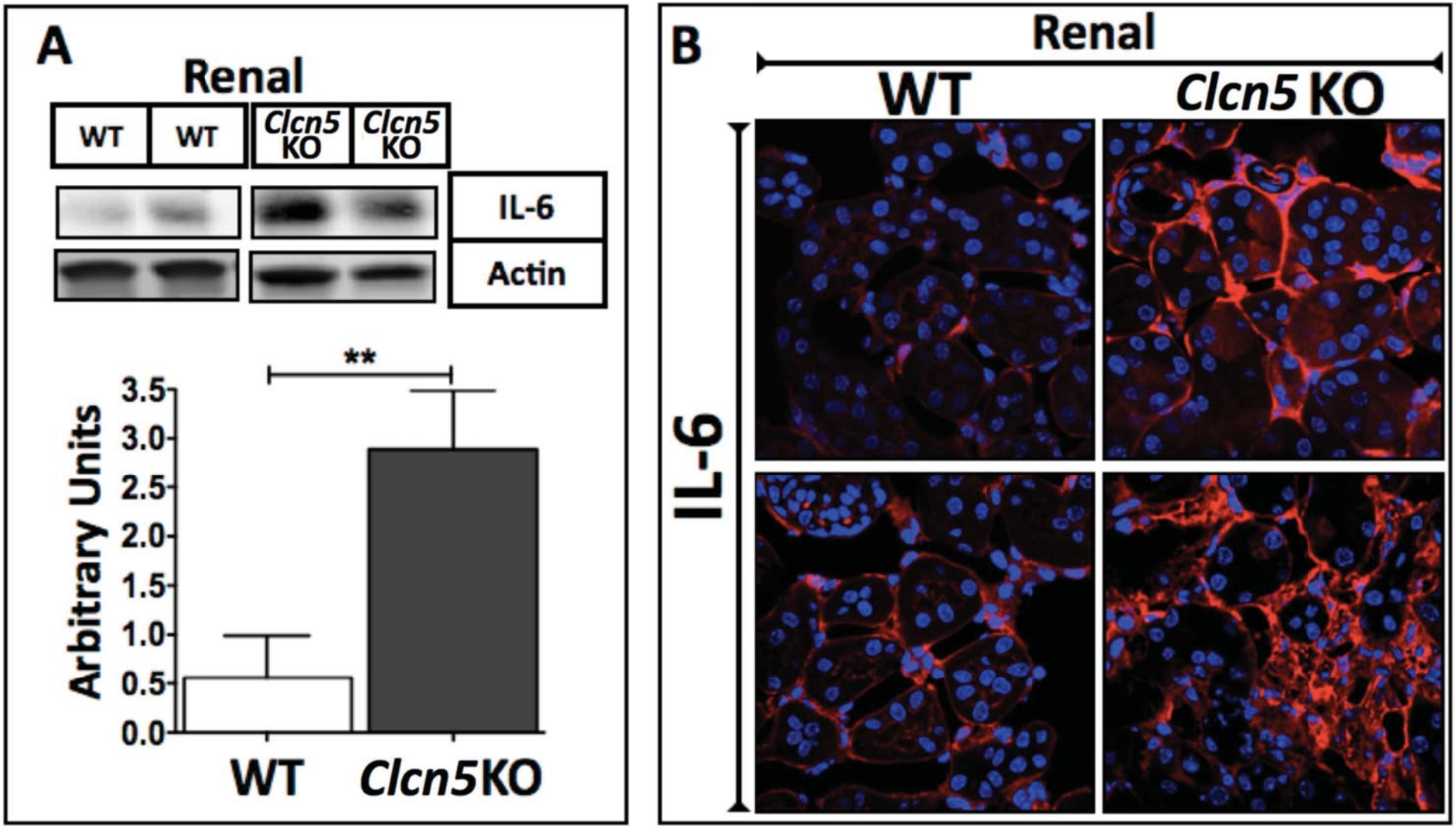

A
Renal
WT
WT
Clcn5 KO
Clcn5 KO
IL-6
Actin
**
Arbitrary Units
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
WT
Clcn5KO
B
Renal
WT
Clcn5 KO
IL-6

Fig S2

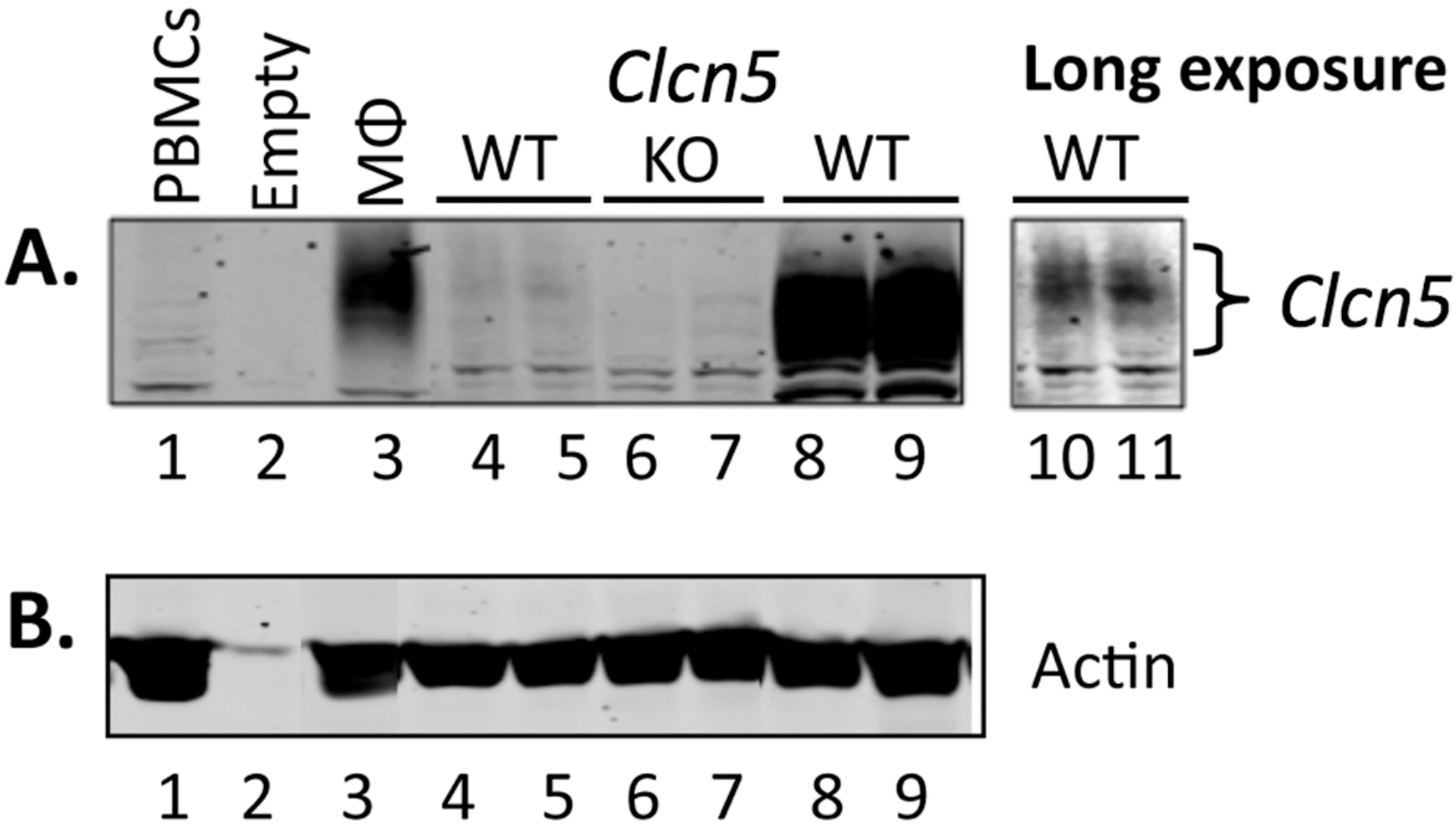
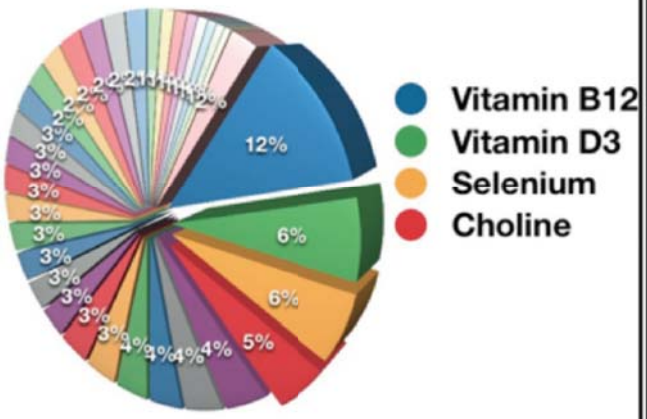

PBMCs
Empty
MΦ
Clcn5
WT
KO
WT
Long exposure
WT
A.
Clcn5
1 2 3 4 5 6 7 8 9 10 11
B.
Actin
1 2 3 4 5 6 7 8 9

Fig S3

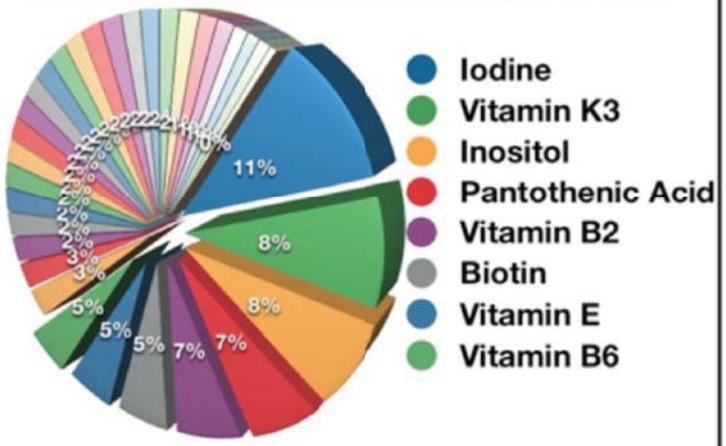
Differences in Z-Diet when compared to H-Diet

Increases in Z-Diet relative to H-Diet



Constituents	Fold Increase
Vitamin B12	3.72
Vitamin D3	2.03902439
Selenium	2
Choline	1.70625

Decreases in Z-Diet relative to H-Diet



Constituents	Fold Decrease
Iodine	5.86
Vitamin K3 (menadione)	4.58
Inositol	4.40
Pantothenic Acid	3.69
Vitamin B2 (riboflavin)	3.61
Avail. Biotin	2.96
Vitamin E (a-tocopherol)	2.69
Vitamin B6 (pyridoxine)	2.51