# Suppl. Fig 1 $-1^{0}$ Ab $1^{0}$ 1

# **Rabbit IgG**





*p*p65<sup>276</sup>





**(A)** 



**(B)** 







<u>is anna c</u>

Suppl. Figure 7





B)



**A**)

### Suppl. Figure 8







#### **1 SUPPLEMENTARY FIGURE LEGENDS**

Supplementary Figure 1. Immunohistochemistry either in the absence of primary
antibody (upper panel) or with rabbit-IgG (lower panel) did not reveal any specific
staining (Bar = 100µm; n = 3 independent experiment).

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6 Supplementary Figure 2. Kinetics of p65 subunit phosphorylated at Ser-276 in the 7 crypt denuded *lamina propria*. Paraffin embedded sections prepared from the crypt-8 denuded *lamina propria* of uninfected normal (N) and days 1-7 post-infected mice were 9 stained with antibody for p65 phosphorylated at Ser-276 ( $pp65^{276}$ ) and analyzed with 10 light microscopy. Magnification = 400x; n = 3 independent experiments).

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Supplementary Figure 3. Lymphocytes, monocytes and mononuclear cells stain 12 positive for p65 subunit phosphorylated at Ser-276 (pp65<sup>276</sup>). Crypt-denuded lamina 13 propria of uninfected normal (N) and days 1-7 post-infected C3H mice were collected 14 and processed for paraffin embedding and sections were stained with antibody for p65 15 phosphorylated at Ser-276 and were analyzed with light microscopy. Please note 16 significant staining for pp65<sup>276</sup> in lymphocytes and monocytes/macrophages (Eos, 17 eosinophils; Gran, granulocytes; Lymph, lymphocytes; Meta, metamyelocytes; Mono, 18 19 monocytes; Magnification = 400x; n = 3 independent experiments).

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Supplementary Figure 4. Confirmation of increases in CD3+ve T-cells and F4/80+ve
 macrophages in the crypt-denuded lamina propria during TMCH. Crypt-denuded
 *lamina propria* of uninfected normal (N) and days 1-7 post-infected C3H mice were

collected and processed for paraffin embedding and sections were stained with antibodies for CD3 (**A**) and F4/80 (**B**) to label T-cells and macrophages, respectively. Sections were analyzed with light microscopy. Insets in **A** and **B** demonstrate presence of these cells in areas denuded for crypts. Magnification = 100x, insets, 400x; n = 3 independent experiments).

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Supplementary Figure 5. Effect of p38 and p44/42 inhibitors on recruitment of Tcells and macrophages. Representative photomicrographs of paraffin embedded sections stained with antibodies to CD3 and F4/80 to detect T-cells and macrophages, respectively. N, uninfected normal; CR, CR-infected; CR+PD or CR+SB, CR-infected and treated with specific ERK1/2 and p38 inhibitors. (Scale bar =  $50\mu$ m; n = 3 independent experiments).

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Supplementary Figure 6. Anti-inflammatory properties of dietary pectin and 37 curcumin. A and B. Paraffin embedded sections prepared from the distal colons of 38 uninfected normal (N), CR infected (day 9, D9) and CR infected (D9) + treated with 6% 39 pectin or 4% curcumin diets were stained with antibodies for CD3 (A) and F4/80 (B) to 40 label T-cells and macrophages, respectively. Staining was analyzed with light 41 microscopy. Scale bar =  $75\mu$  (A) and  $50\mu$  (B); n = 3). C. Measurement of histology 42 score. H&E stained sections from the above group of animals were subjected to 43 measurement of inflammation and/or colitis score by an observer blinded to the treatment 44 groups according to the morphological criteria described in Materials and Methods. 45  $^{+*}P$  < 0.05 vs. control (†); \*♥P < 0.05 vs. D9 (†\*); n = 3 independent experiments. 46

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Supplementary Figure 7. Both pectin and curcumin diets exhibit significant anti-48 inflammatory properties in vivo. A. Expression of pro-inflammatory cytokines and 49 chemokines in the distal colonic homogenates of uninfected normal (N) and CR infected 50 and CR infected + treated with 6% pectin or 4% curcumin mice were measured using 51 Bio-Plex Cytokine assay Kit as described by the manufacturer. Samples were analyzed 52 on a Bio-Rad 96 well plate reader using the Bio-Plex Array system and Bio-Plex 53 Manager software. Each bar represents mean±SD. [†\*, p<0.05 vs. control (†); \*•, p<0.05 54 vs. CR ( $\dagger^*$ ); n = 3 independent experiments]. **B. Effect of dietary intervention on** 55 myeloperoxidase (MPO) activity. MPO activity was measured in the colonic 56 homogenates of uninfected normal (N) and CR infected and CR infected + treated with 57 6% pectin or 4% curcumin mice using Fluoro MPO detection kit (Cell Tech. Inc. CA, 58 USA) as was instructed in the manufacturer's protocol. Each bar represents mean±SD. 59  $[\dagger^*, p < 0.05 \text{ vs. control}(\dagger); \bullet, p < 0.05 \text{ vs. CR}(\dagger^*); n = 3 \text{ independent experiments}].$ 60

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Supplementary Figure 8. LPS staining to detect CR. A. Immunofluorescence 62 detection of LPS as a surrogate for *Citrobacter* presence in the distal colon isolated from 63 64 uninfected normal (N) or CR-infected (D9) or pectin (D9+P) and curcumin (D9+Cur)treated animals. Paraffin-embedded sections were deparaffinized, subjected to antigen 65 66 retrieval and incubated overnight at 4°C with anti-LPS antibody. Following incubation with secondary antibody conjugated with fluorescein isothiocyanate (FITC), slides were 67 68 analyzed by fluorescent microscopy using Axiophot 2 microscope [Carl Zeiss, Germany; Bar =  $125\mu m$ ; n = 5 independent experiments. Green staining represents bacterial 69

- 70 presence. B. Bacterial counts. Neither pectin nor curcumin diet had any effect on
- 71 bacterial counts (n = 5 independent experiments).

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