

1 Supplement to:

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3 *In Vitro* and *In Vivo* Activities of Zinc Linolenate, a Selective Antibacterial Agent against

4 *Helicobacter pylori*

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21 **Table S1.** Broth microdilution MIC of metal complex of unsaturated fatty acid for *H. pylori*
 22 strains. (MIC: µg/ml)

Strain	Drug sensitivity	BiLla	FeLla	ZnOa	ZnSa	ZnPa	ZnMa
HP2665	S	128	>128	>128	>128	>128	>128
NSH57	S	64	>128	>128	>128	>128	>128
G27	S	64	>128	>128	>128	>128	>128
HP159	L, C, M (R)	128	>128	>128	>128	>128	>128
HP163	L, M (R)	64	>128	>128	>128	>128	>128
HP160	M (R)	64	128	>128	>128	>128	>128
HP161	C (R)	128	>128	>128	>128	>128	>128
HP162	L (R)	64	128	>128	>128	>128	>128

23 Abbreviations: L, levofloxacin; C, clarithromycin; M, metronidazole; S, Drug sensitive; R,
 24 Drug resistance; BiLla, bismush linolenate; FeLla, iron linolenate; ZnOa, zinc oleate; ZnSa,
 25 zinc stearate; ZnPa, zinc palmitate; ZnMa, zinc myristate.

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Table S2. The toxicity of ZnLla after oral gavage.

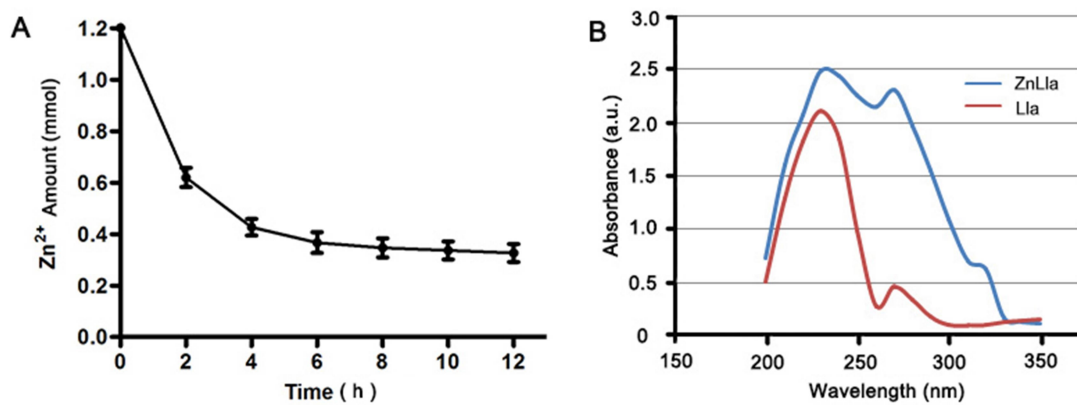
Groups	ALT (U/L)	AST (U/L)	BUN (mmol/L)	CREA (μ mol/L)
Infected (PBS)	22.00 \pm 4.36	110.00 \pm 14.73	13.90 \pm 3.72	16.67 \pm 1.15
Infected (OPZ+AC)	34.33 \pm 3.43	220.00 \pm 18.40	8.77 \pm 1.23	17.00 \pm 1.00
Infected (OPZ+ZnLla)	19.67 \pm 1.53	117.00 \pm 12.61	10.27 \pm 0.32	16.33 \pm 0.58
Infected (ZnLla)	20.00 \pm 4.12	182.33 \pm 14.74	14.97 \pm 3.28	18.33 \pm 1.06
Uninfected (10-fold dosage of ZnLla)	23.33 \pm 0.58	138.33 \pm 13.18	11.90 \pm 0.79	15.00 \pm 2.00
Uninfected (PBS)	29.00 \pm 6.93	231.00 \pm 17.25	13.13 \pm 1.42	18.33 \pm 1.69

27 Abbreviations: ALA, Alanine aminotransferase; AST, Aspartate aminotransferase; BUN,
28 urea nitrogen; CREA, creatinine.

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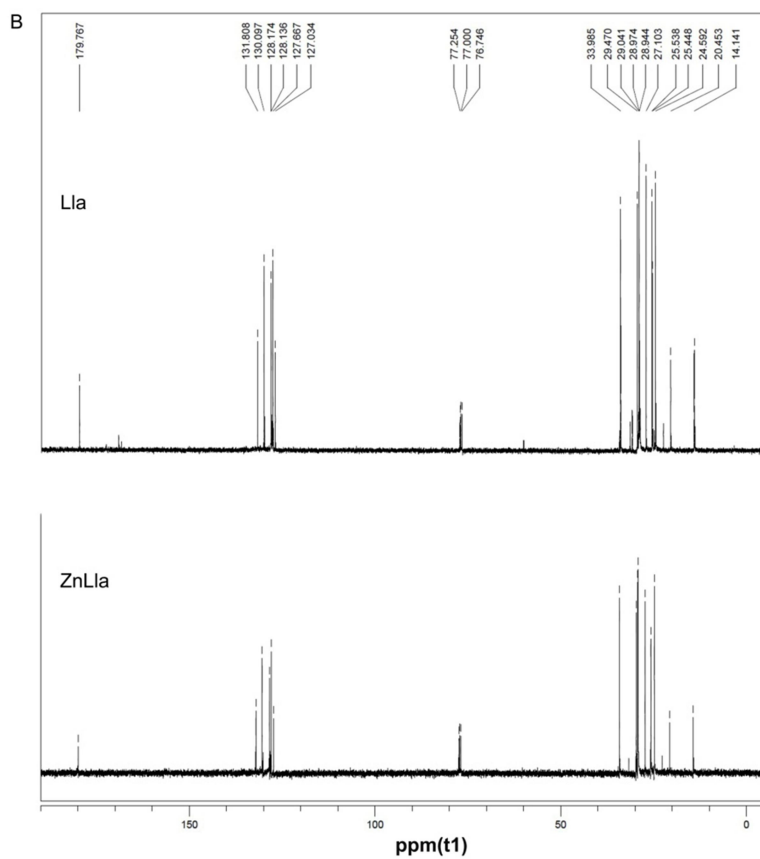
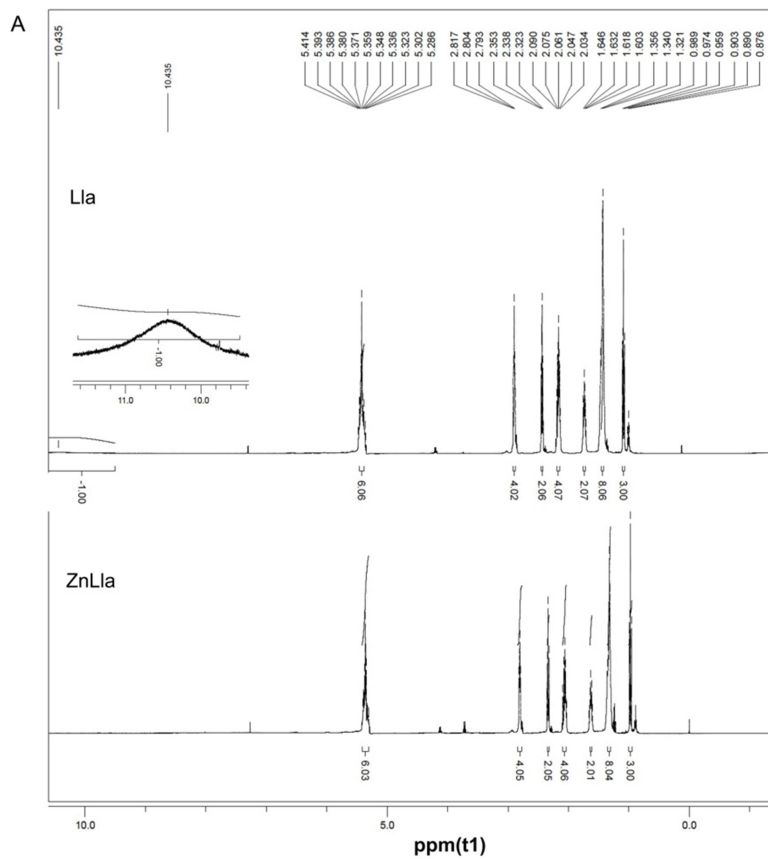


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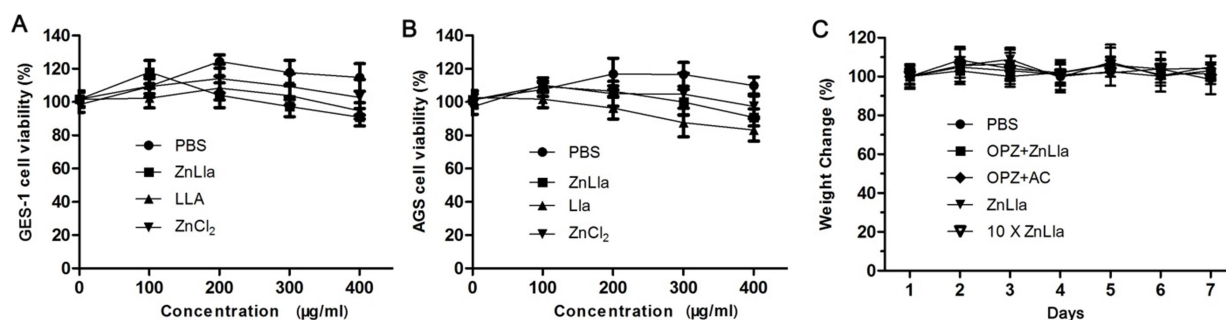
33 **Figure S1.** A. The amount change of Zn²⁺ during the reaction process of ZnLla. B. UV-Vis
34 spectrum of ZnLla (blue line) and Lla (red line).

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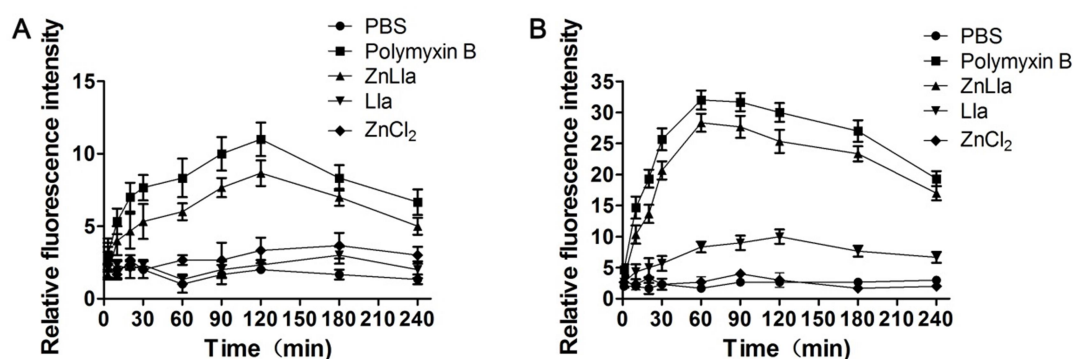
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39 **Figure S2.** A. ^1H NMR spectrum of Lla and ZnLla in CDCl_3 (500 MHz, CDCl_3), δ 5.40 ~
 40 5.30 (m, 6H), 2.81 (t, $J = 6.5$ Hz, 4H), 2.34 (t, $J = 7.5$ Hz, 2H), 2.09 ~ 2.03 (m, 4H), 1.66 ~
 41 1.60 (m, 2H), 1.36 ~ 1.32 (m, 8H), 0.97 (t, $J = 7.5$ Hz, 3H); B. ^{13}C NMR spectrum of Lla and
 42 ZnLla in CDCl_3 (125 MHz, CDCl_3), δ 179.9, 132.0, 130.3, 128.4, 127.9, 127.2, 34.2, 29.7,
 43 29.2, 29.1, 27.3, 25.7, 24.8, 20.6, 14.3.

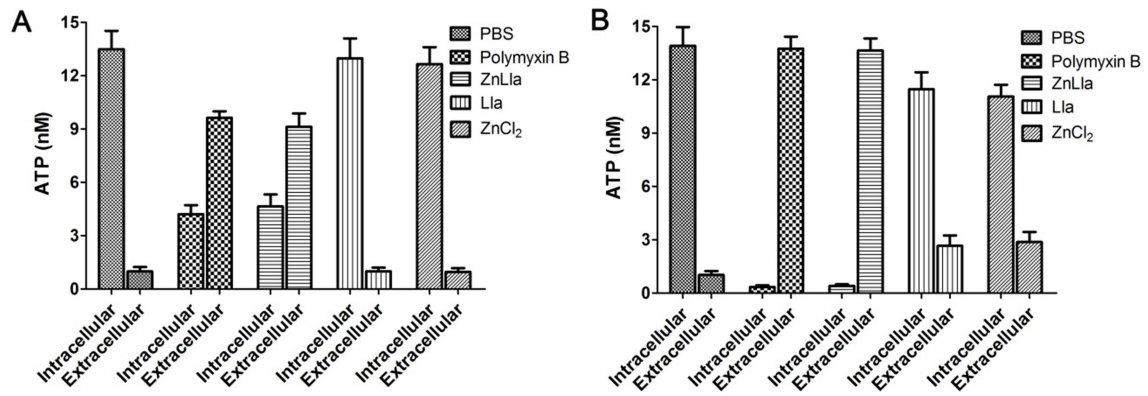


47 **Figure S3.** Cytotoxicity assays of ZnLla and effects on mouse weight. A. Cell viability test
 48 on GES-1 cells. B. Cell viability test on AGS cells. C. The effects on mice weight change.



56 **Figure S4.** Uptake of N-phenyl naphthylamine (NPN) by *H. pylori* G27 cells after treatment of
 57 PBS (negative control), 16 µg/ml (A) and 400 µg/ml (B) of polymyxin B (positive control),
 58 ZnLla, Lla or ZnCl₂.

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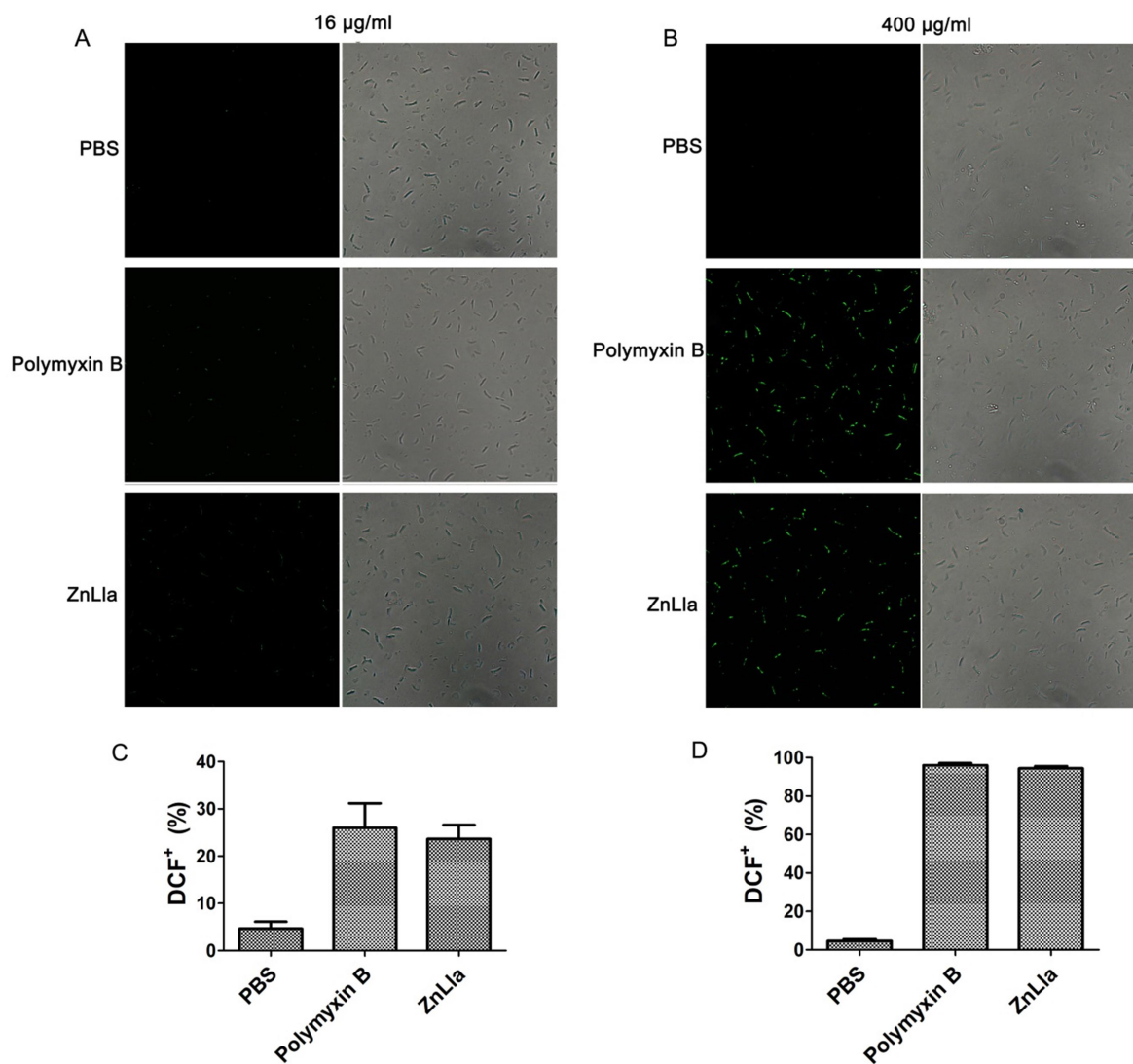


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Figure S5. The release of ATP from *H. pylori* cells after treatment of PBS (negative control), 16 µg/ml (A) and 400 µg/ml (B) of polymyxin B (positive control), ZnLla, Lla or ZnCl₂.



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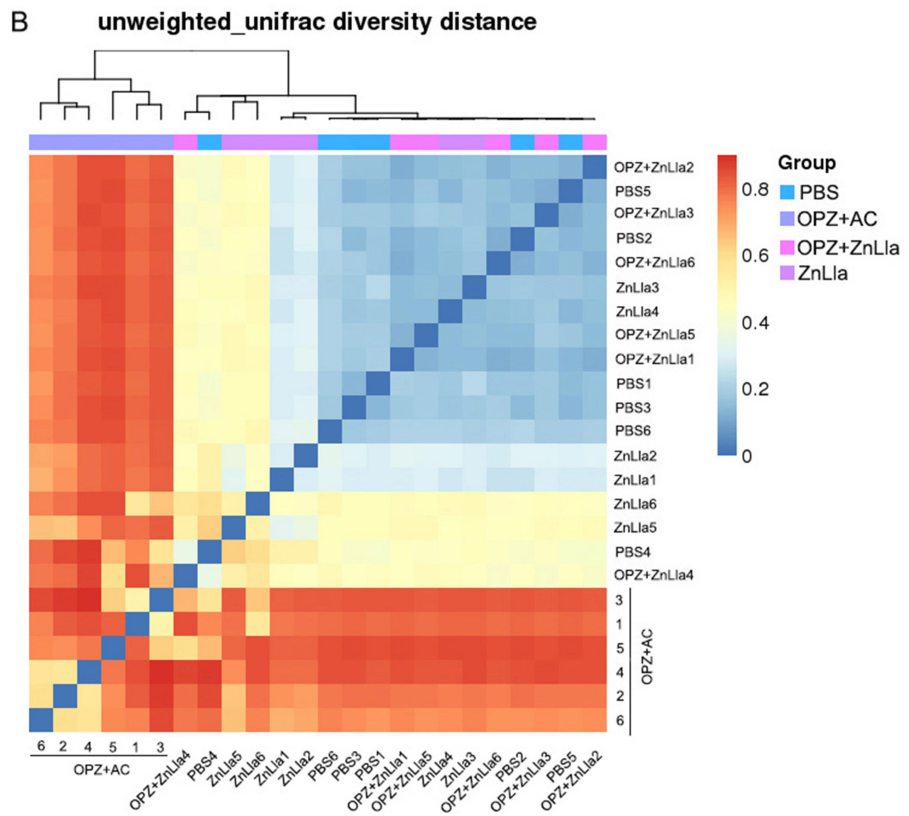
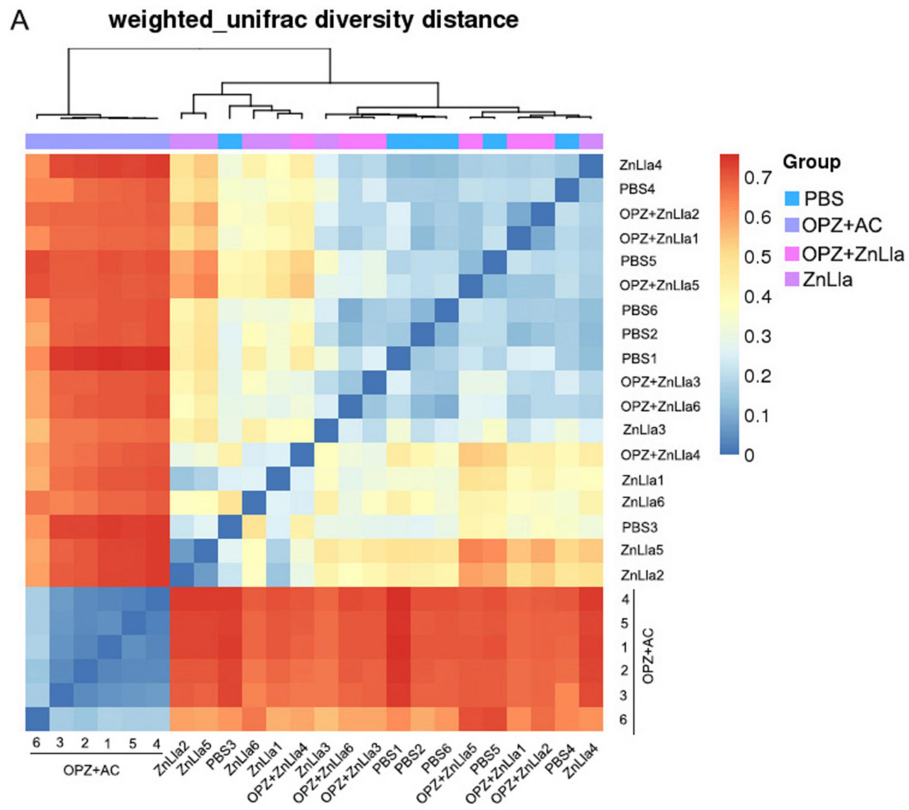
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Figure S6. Fluorescence microscopic images (left) and bright field images (right) of DCF⁺ labeled *H. pylori* stained with DCFDA after the incubation with PBS, 16 µg/ml (A) and 400 µg/ml (B) of polymyxin B or ZnLla. C and D. The ratios of DCF⁺ cells incubated with each

69 treatment calculated by manually counting under a microscope. Data represent medians \pm SD
 70 of three independent experiments.

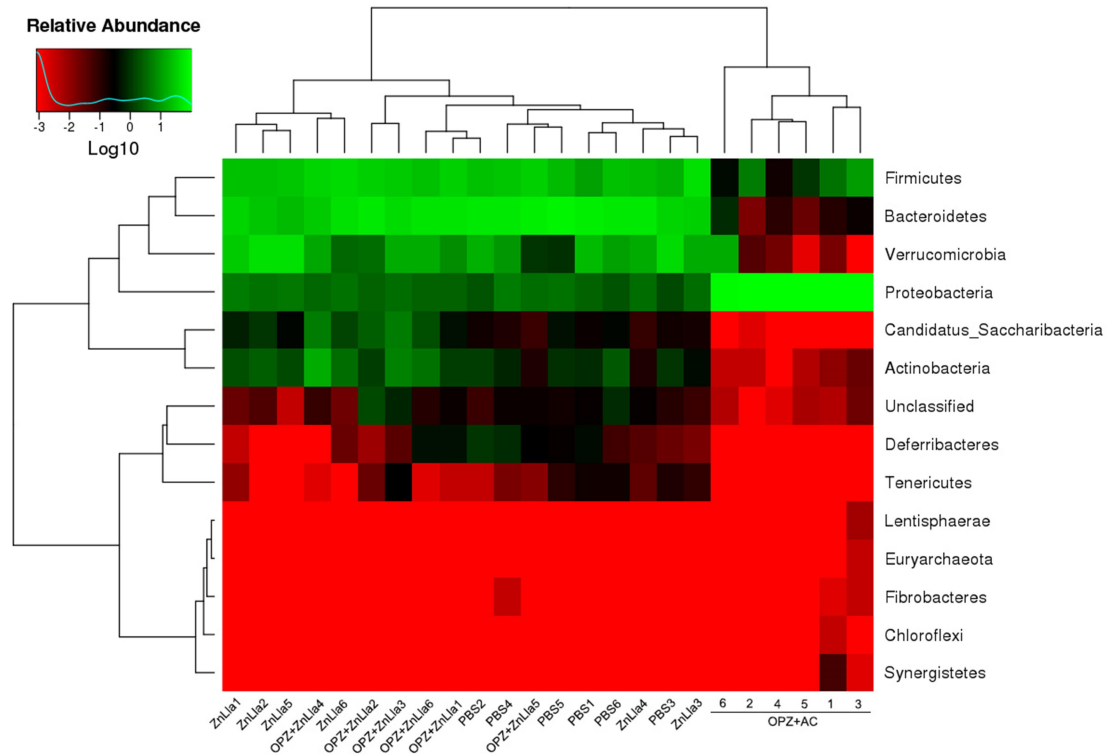
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73 **Figure S7.** Beta diversity measurements of each sample receiving different treatments based
 74 on weighted (A) and unweighted (B) Unifrac analysis.

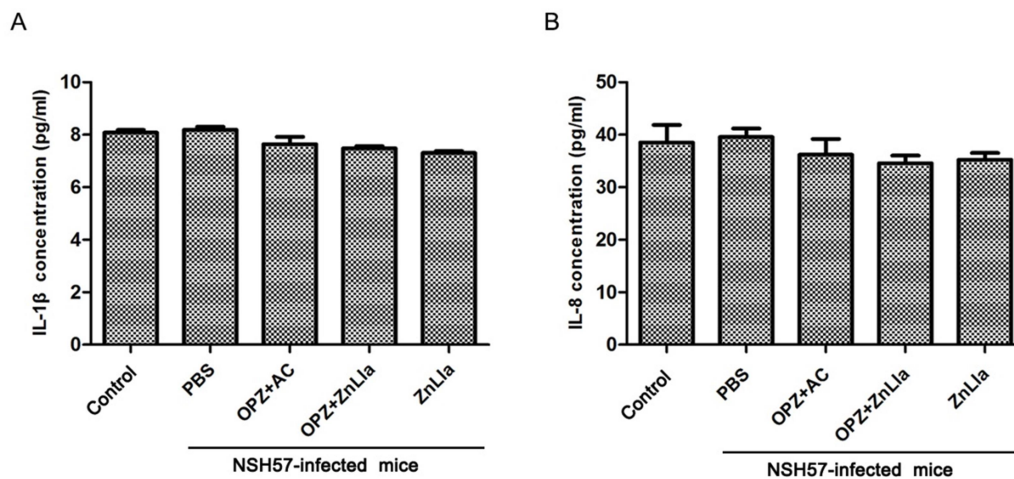
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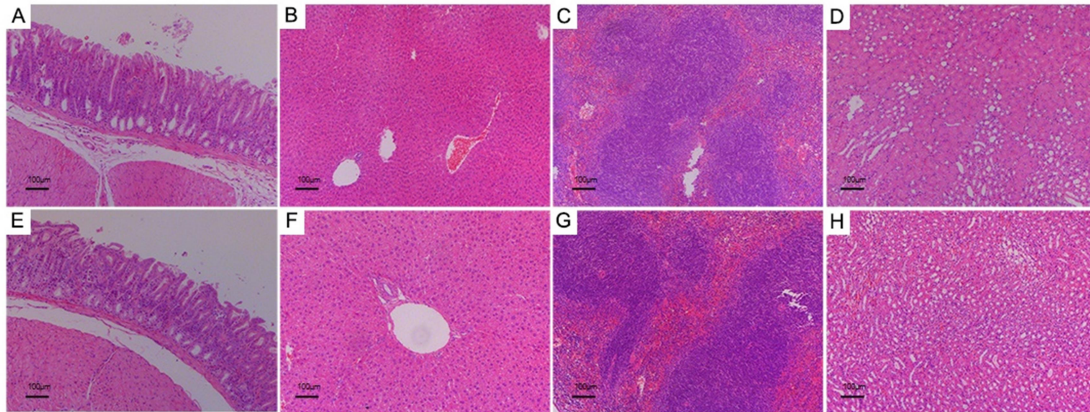
77 **Figure S8.** Log-scaled percentage heat map of Phylum-level in each sample receiving
 78 different treatments identified from the sequenced data.

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81 **Figure S9.** Proinflammatory cytokine production. Concentrations of serum IL-1 β (A) and
 82 IL-8 (B) levels were determined by ELISA kits from NSH57-infected mice (Normal) and
 83 mice receiving different treatments.



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85 **Figure S10.** The H&E-stained stomach (A and E), liver (B and F), spleen (C and G) and
86 kidney (D and H) from BHKS159-infected mice receiving 10-fold dosage of ZnL1a (scale
87 bar = 100 μm). A-D, ×100; E-H, ×200.

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