**Virologica Sinica**

**Supplementary Data**

**Antibiotic-induced gut bacteria depletion has no effect on HBV replication in HBV immune tolerance mouse model**

Yanan Bua, Kaitao Zhaoa, Zaichao Xua, Yingcheng Zhenga, Rong Huaa, Chuanjian Wua,Chengliang Zhub, Yuchen Xiaa,\*, Xiaoming Chengc,\*

*a State Key Laboratory of Virology and Hubei Province Key Laboratory of Allergy and Immunology, Hubei Jiangxia Laboratory, Institute of Medical Virology, TaiKang Center for Life and Medical Sciences, TaiKang Medical School, Wuhan University, Wuhan, 430071, China*

*b Department of Clinical Laboratory, Renmin Hospital of Wuhan University, Wuhan, 430060，China*

*c Department of Pathology, Center for Pathology and Molecular Diagnostics, Hubei Clinical Center and Key Laboratory of Intestinal and Colorectal Diseases, Zhongnan Hospital of Wuhan University, TaiKang Medical School, Wuhan University, Wuhan, 430071, China*

\* Corresponding authors.

*E-mail addresses*: yuchenxia@whu.edu.cn (Y. Xia), xiaoming.cheng@whu.edu.cn (X. Cheng)

**Supplementary Table S1** Primer sequences for qPCR assay.

|  |  |
| --- | --- |
| Primer name | Sequence(5′–3′) |
| HBV pgRNA fwd | CTGGGTGGGTGTTAATTTGG |
| HBV pgRNA rev | TAAGCTGGAGGAGTGCGAAT |
| HBV total RNA fwd | CCGTCTGTGCCTTCTCATCTGC |
| HBV total RNA rev | ACCAATTTATGCCTACAGCCTCC |
| Total bacteria fwd | TCCTACGGGAGGCAGCAGT |
| Total bacteria rev | GACTACCAGGGTATCTAATCCTGTT |
| *Escherichia* *coli* fwd | CATGCCGCGTGTATGAAGAA |
| *Escherichia coli* rev | CGGGTAACGTCAATGAGCAAA |
| [*klebsiella pneumoniae*](javascript:;) fwd | ATTTGAAGAGGTTGCAAACGAT |
| [*klebsiella* *pneumoniae*](javascript:;) rev | TTCACTCTGAAGTTTTCTTGTGTTC |
| *Enterococcus* fwd | CCCTTATTGTTAGTTGCCATCATT |
| *Enterococcus* rev | ACTCGTTGTACTTCCCATTGT |
| *Lactobacillus* fwd | AGCAGTAGGGAATCTTCCA |
| *Lactobacillus* rev | CACCGCTACACATGGAG |
| Mouse IFN-γ fwd | GCCACGGCACAGTCATTGA |
| Mouse IFN-γ rev | TGCTGATGGCCTGATTGTCTT |
| Mouse Actb fwd | GGCTGTATTCCCCTCCATCG |
| Mouse Actb rev | CCAGTTGGTAACAATGCCATGT |

**Supplementary Table S2** Antibodies.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Antibody | Company | Catalog no. | Host | Dilution |
| β-actin | Cell Signaling Technology | 4970 | Rabbit | 1:10,000 |
| HBc | Gene Technology | GB058629 | Rabbit | 1:1000 |
| APC/Cyanine 7 anti-CD3 | Biolegend | 100222 | Mouse | 1:800 |
| FITC anti-CD8a | Biolegend | 100706 | Mouse | 1:100 |
| PE anti-IFN-γ | Biolegend | 505808 | Mouse | 1:500 |
| PE IgG1, κ Isotype Control Antibody | Biolegend | 400408 | Rat | 1:500 |

## 

## Supplementary Fig. S1 The effect of antibiotic-induced gut bacteria depletion on HBV immune response in male HDI mouse model. A The repeated experiment was conducted, during which mice were euthanatized at indicated time points for HBV immune response evaluation (water: n = 16, ABX: n = 16 at the starting point). B Serum HBsAg level was measured with 2000-fold dilution in PBS via ELISA (*P* = 0.057, cut off value = 0.1). C Spleen IFN-γ mRNA level was analyzed by qPCR assay with mouse *Actb* as the reference gene (*P* = 0.078). The relative level was calculated by the calibrator-normalized Relative Quantification of LightCycler 480 instrument operator’s manual. Data are presented as mean ± standard error of mean. Statistical analysis was performed by Student’s unpaired two-tailed *t*-test.

****

**Supplementary Fig. S2** The effect of antibiotics on gut microbiota in established female AAV-HBV1.3 mouse model. **A** More gut bacteria with lower abundance at the genus level were displayed *via* the heat map. Red and blue block indicated high and low abundance, respectively. **B** The bar plot depicted enriched gut microbiota by LEfSe analysis at the genus level in two groups (LDA threshold value ≥ 4). **C, D** The cecum size and weight were shown at 14 and 38 weeks post treatment, respectively. Data are presented as mean ± standard error of mean. Statistical analysis was performed by Student’s unpaired two-tailed *t*-test, *\*\*P* < 0.01.