# Restraint stress in hypertensive rats activates the intestinal macrophages and reduces of intestinal barrier Accompanied by intestinal flora dysbiosis

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**Supplementary**

Figure S1

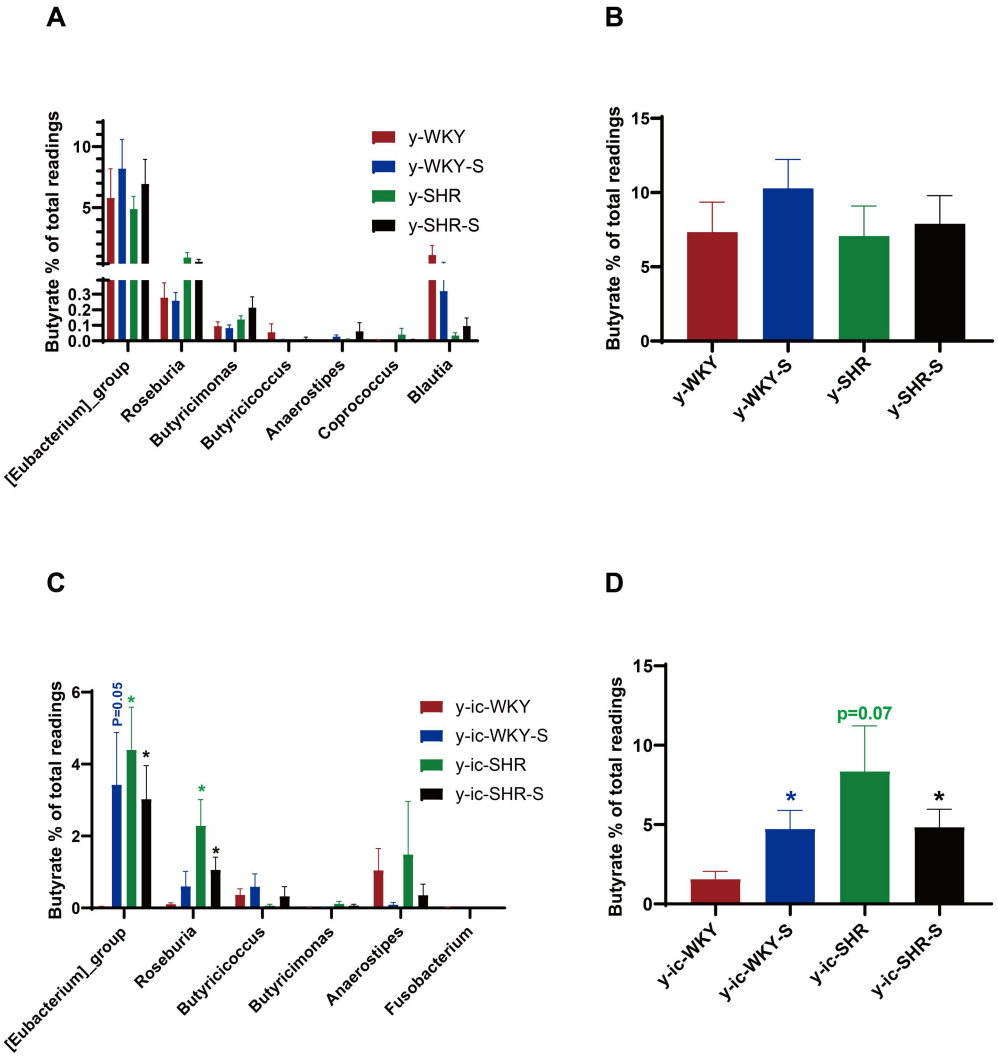


**Figure S1** The content of Butyric acid-related intestinal flora from the colon and ileocecum in different groups. **(A)** Intestinal flora associated with butyrate production in colons in different groups. **(B)** The bar graph illustrates the content of intestinal flora related to butyrate production in colons in different groups. **(C)** Intestinal flora associated with butyrate production in ileocecum in different groups. **(D)** The bar graph illustrates the total content of intestinal flora related to butyrate production in ileocecum in different groups. Data were expressed as mean ± SEM. (n = 4-5 per group). Results were compared by Unpaired t-test; *p<0.05 (black y-ic-SHR-S *vs* y-ic-WKY; blue y-ic-WKY-S *vs* y-ic-WKY; green y-ic-SHR *vs* y-ic-WKY).

Figure S2

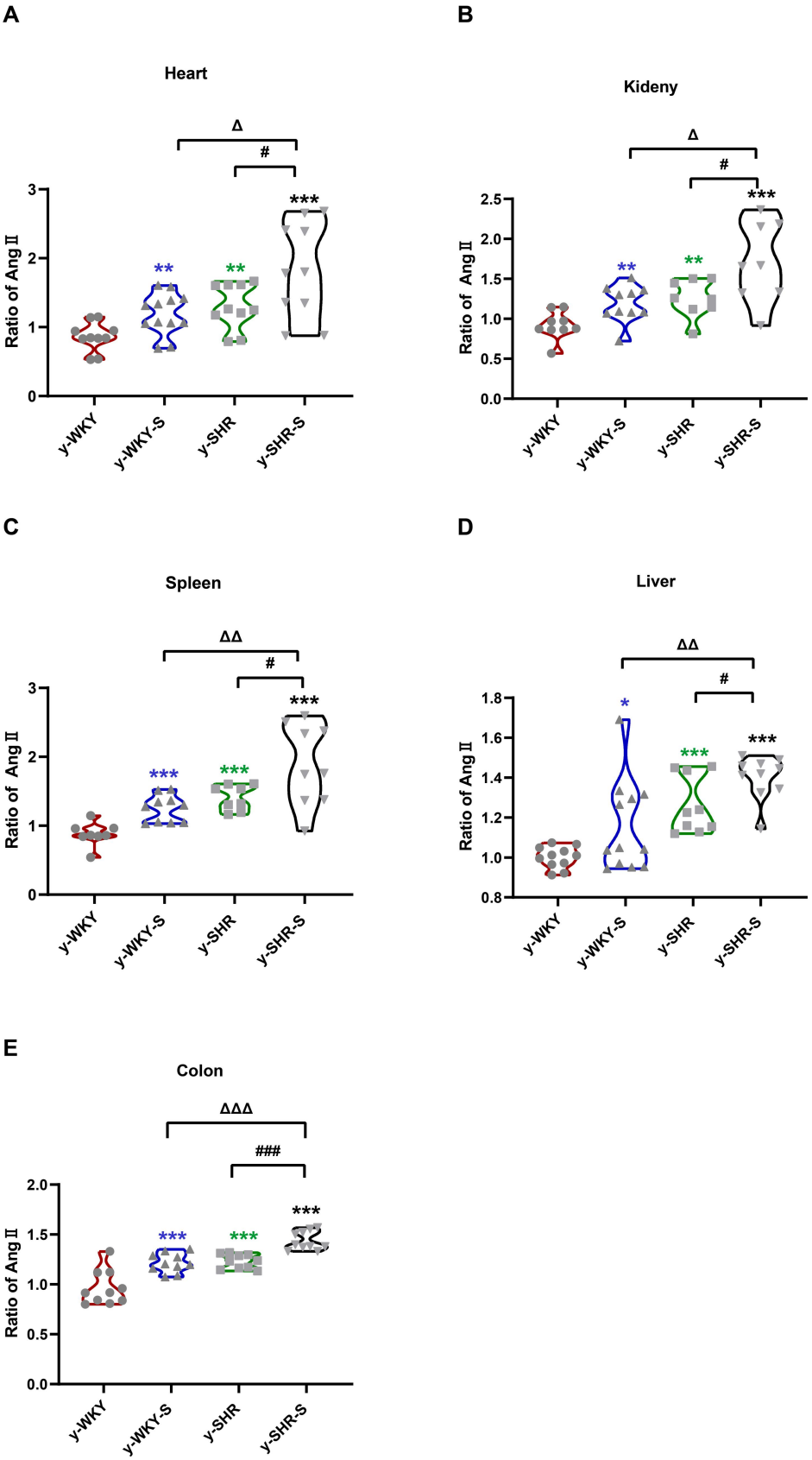
A
Heart
Ratio of Ang II
y-WKY
y-WKY-S
y-SHR
y-SHR-S
B
Kideny
Ratio of Ang II
C
Spleen
Ratio of Ang II
D
Liver
Ratio of Ang II
E
Colon
Ratio of Ang II

**Figure S2** The ratio of Ang II in different tissues. **(A)** Ratio of cardiac-derived Ang II. **(B)** Ratio of kidney-derived Ang II. **(C)** Ratio of spleen-derived Ang II. **(D)** Ratio of liver-derived Ang II. **(E)** Ratio of colon-derived Ang II. The results were compared by Unpaired t-test, (n = 5-6 per group); *p<0.05, **p<0.01, ***p<0.001 (black y-SHR-S *vs* y-WKY; blue y-WKY-S *vs* y-WKY; green y-SHR *vs* y-WKY); $^{\#}$p<0.05, $^{\#\#\#}$p<0.001 (y-SHR-S *vs* y-SHR); $^{\triangle}$p<0.05, $^{\triangle\triangle}$p<0.01, $^{\triangle\triangle\triangle}$p<0.001 (y-SHR-S *vs* y-WKY-S).

**Table S1**

**Guaranteed value of product composition analysis (calculated per kilogram of feed)**

| Component | Content % | Component | Content % | Component | Content% | Component | Content % |
|---|---|---|---|---|---|---|---|
| Protein | ≥180g | Histidine | ≥4g | Vitamin K | ≥3.0mg | Vitamin C | - |
| Fat | ≥40g | Trp | ≥1.9g | Vitamin $B_1$ | ≥8.0mg | Magnesium(Mg) | ≥2.0g |
| Fiber | ≤50g | Phe+Tyr | ≥11g | Vitamin $B_2$ | ≥10mg | Potassium (K) | ≥5.0g |
| Ash | ≤80g | Thr | ≥6.5g | Vitamin $B_6$ | ≥6.0mg | Sodium(Na) | ≥2.0g |
| Moisture | ≤100g | Leu | ≥14.4g | Niacin | ≥45mg | Iron(Fe) | ≥100mg |
| Calcium(Ca) | 10-18g | Ile | ≥7.0g | Vitamin $B_5$ | ≥17mg | Manganese(Mn) | ≥75mg |
| Phosphorus(P) | 6-12g | Valine | ≥8.4g | Folic acid | ≥4.0mg | Copper(Cu) | ≥10mg |
| Lysine | ≥8.2g | Vitamin A | ≥7000IU | Biotin | ≥0.1mg | Zinc(Zn) | ≥30mg |
| Met + Cys | ≥5.3g | Vitamin D | ≥800IU | Vitamin $B_{12}$ | ≥0.02mg | Iodine(I) | ≥0.5mg |
| Arginine | ≥9.9g | Vitamin E | ≥60IU | Choline | ≥1250mg | Selenium(Se) | 0.1-0.2mg |
| The upper limit of vitamins and minerals is 2 times the lower limit | | | | | | | |

| | | | |
|---|---|---|---|
| Phenylalanine | Phe | Tyrosine | Tyr |
| Tryptophan | Trp | Methionine | Met |
| Cystine | Cys | Leucine | Leu |
| Threonine | Thr | Isoleucine | Ile |

**Specimen processing**

(1) The rats were weighed and anaesthetized with 10% chloral hydrate; (2) The abdominal cavity was opened to take blood from the abdominal aorta. After standing for 30 min with or without anticoagulant, the blood was taken (with or without anticoagulant), centrifuged at 4°C and 6000 RPM for 40 min. The supernatant, namely serum (plasma), was taken and stored at -80°C; (3) Take tissue from anus to ileocecus, put it on a white paper and measure the length of colon with a ruler; (4) Feces from colon and ileocecal parts were placed in EP tubes and quickly frozen in liquid nitrogen respectively, and then stored at -80°C for 16S rDNA sequencing; (5) The colon was taken and rinsed with K-HS solution, the excess water was absorbed with filter paper, placed in an EP tube, quickly placed in liquid nitrogen and quickly frozen, which was kept in an ultra-low temperature refrigerator at -80°C for protein extraction; (6) At the same time take part of the colon tissue fixed in 4% paraformaldehyde fixed liquid 48 hours, and then fixed with 95% ethanol 3.5 hours, 2 hours soaked in anhydrous ethanol, a total of 2 times, with 30 minutes of xylene, a total of 3 seconds, finally in 65 °C for 30 minutes in liquid paraffin, a total of three times, finally and embedded in paraffin slice as 5 μm is used.