

**Title: “Losartan-induced gut microbial changes has an antihypertensive effect”**

**Running title: Losartan restored gut microbiota in SHR**

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## **Supplemental Figure Legend**

**Figure S1. Losartan (Los) does not induce changes in the microecological parameters of gut microbiota of spontaneously hypertensive rats (SHR).** Changes in alpha-diversity parameters (i.e., Shannon diversity, Chao richness, Pielou evenness, and the observed species) (**A**), and the principal coordinate analysis of the Phyla (**B**) were examined in the gut microbiota of Wistar Kyoto rats (WKY), SHR and SHR treated with Los (SHR-Los). Data are expressed as mean  $\pm$  SEM. \* $P < 0.05$  significant differences compared with WKY.

**Figure S2. Distinct changes in the gut microbiota between Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).** Cladograms show the significantly enriched taxa, the taxa are identified on the key to the right of each panel. Larger circles represent greater differences in abundance between groups (**A**). Comparisons of microbiome changes in WKY *versus* SHR. Linear discriminant analysis effect size (LEfSe) identified significantly different bacterial taxa enriched in each cohort at LDA Score  $> 2$ ,  $p < 0.05$  (green bars represent WKY-enriched taxa, red bars represent SHR-enriched taxa) (**B**).  $n = 6$  rats per experimental group in each comparison.

**Figure S3. Losartan (Los) induced different behaviors of gut microbiota in spontaneously hypertensive rats (SHR).** Comparisons of microbiome changes in SHR *versus* SHR-Los. Cladograms show the significantly enriched taxa, the taxa are identified on the key to the right of each panel. Larger circles represent greater differences in abundance between groups (**A**). Linear discriminant analysis effect size (LEfSe) identified significantly different bacterial taxa enriched in each cohort at LDA

Score > 2,  $p < 0.05$  (red bars SHR enriched, green bars SHR-Los enriched) (B).  $n = 6$  rats per experimental group in each comparison.

**Figure S4. Losartan (Los) induced changes at the family level in the gut microbiota of spontaneously hypertensive rats (SHR).** Principal coordinate analysis of families (A) in the gut microbiota from all experimental groups. Samples clustered by experimental group demonstrated that the experimental conditions resulted in distinct populations of bacteria at the family level. Heat map showing differences in the relative abundance of the bacterial families between the three groups studied (B). The colours of the heat map represent the relative percentage of microbial family assigned within each sample. The most significantly modified bacterial families (C) in the gut microbiota in Wistar Kyoto rats (WKY), SHR and SHR treated with Los (SHR-Los). Values are expressed as mean  $\pm$  SEM ( $n = 6$ ). \* $P < 0.05$  significant differences compared with WKY. # $P < 0.05$  significant differences compared with untreated SHR.

**Figure S5. Losartan (Los) induced changes at the genus level in the gut microbiota of spontaneously hypertensive rats (SHR).** Principal coordinate analysis of genera (A) in the gut microbiota from all experimental groups. Samples clustered by experimental group demonstrated that the experimental conditions resulted in distinct populations of bacteria at the genus level. Heat map showing differences in the relative abundance of the bacterial genera between the three groups studied (B). The colours of the heat map represent the relative percentage of microbial genus assigned within each sample. The most significantly modified bacterial genera (C) in the gut microbiota in Wistar rats (WKY), SHR and SHR treated with Los (SHR-Los). Values are expressed as mean  $\pm$  SEM ( $n = 6$ ). \* $P < 0.05$  significant differences compared with WKY. # $P < 0.05$  significant differences compared with untreated SHR.

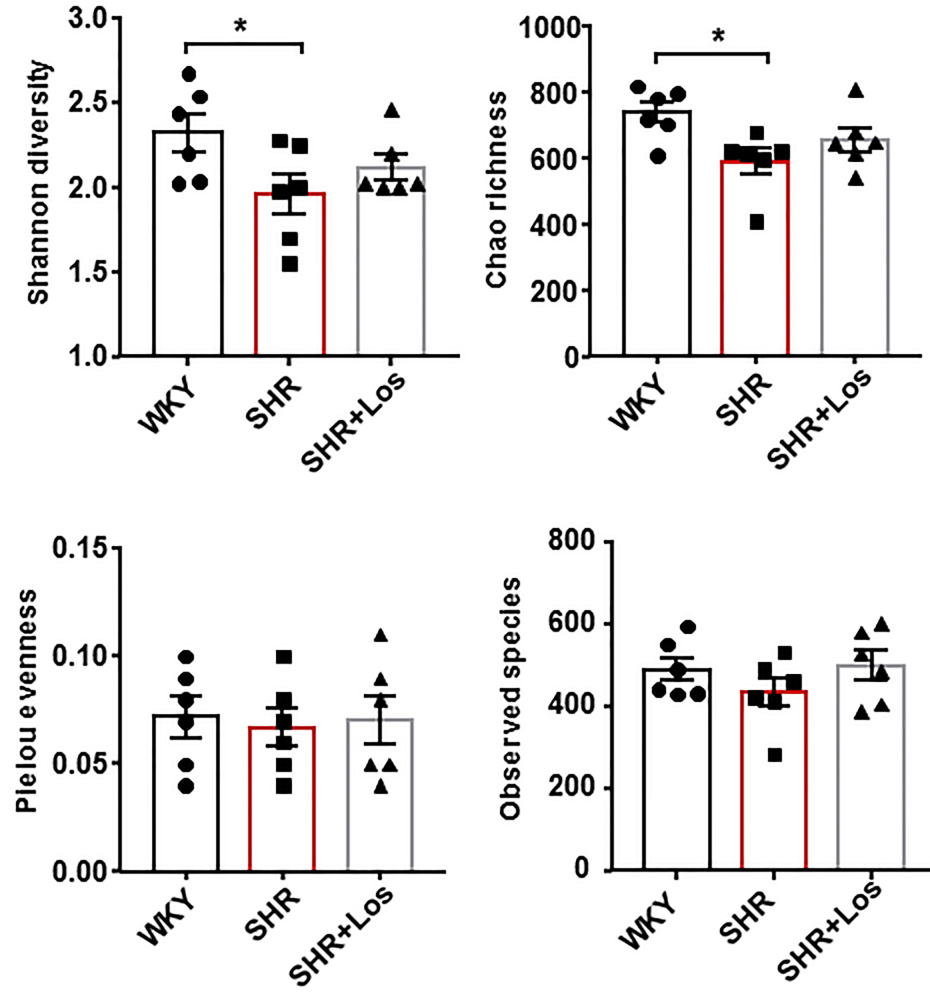
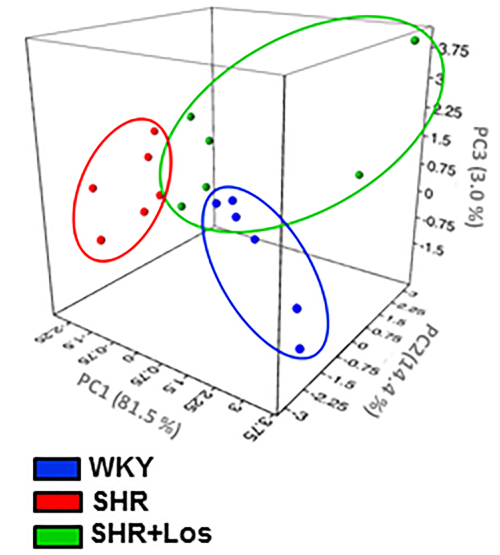
**Figure S6. Protective effects of Losartan (Los) in vascular function and oxidative stress in spontaneously hypertensive rats (SHR).** Systolic blood pressure (SBP, A) was measured by tail-cuff plethysmography in all experimental groups. Vascular relaxation responses induced by acetylcholine (ACh) in endothelium-intact aortas pre-contracted by phenylephrine in the absence (B) and in the presence of the NADPH oxidase inhibitor apocynin ( $10 \mu\text{M}$ ). Pictures on the left show aortic rings incubated in the presence of dihydroethidium (DHE), which produces a red fluorescence when oxidized to ethidium by reactive oxygen species. Pictures on the right show blue fluorescence of the nuclear stain 4,6-diamidino-2-phenylindole dichlorohydrate (DAPI;  $\times 400$  magnification). Red ethidium fluorescence values normalized to blue DAPI fluorescence, averaged and expressed as mean  $\pm$  SEM (C). Lucigenin-enhanced chemiluminescence measurement of NADPH oxidase activity (D) in Wistar Kyoto rats (WKY), SHR and SHR treated with Los (SHR-Los). Values are expressed as mean  $\pm$  SEM. \* $P < 0.05$  significant differences compared with WKY. # $P < 0.05$  significant differences compared with untreated SHR.

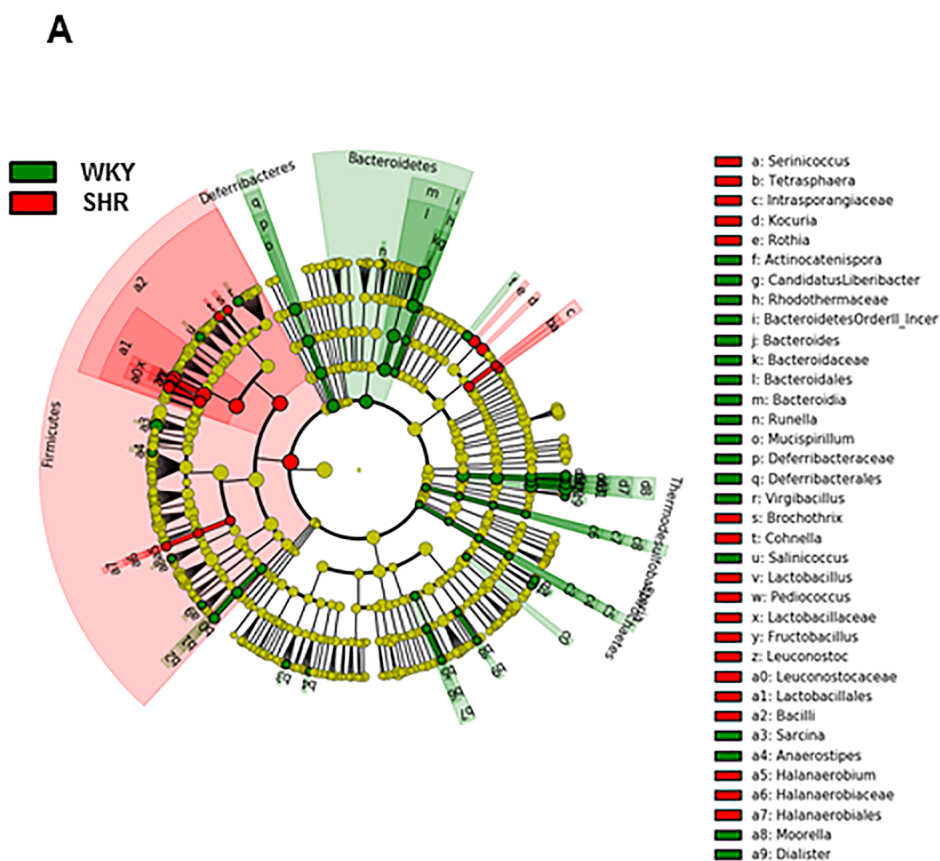
**Figure S7. Effects of faecal microbiota transplantation from spontaneously hypertensive rat (SHR)-losartan to SHR on different behaviours of the gut microbiota of SHR.** Faecal samples were collected at the end of FMT experiment (SHR with stool transplant from SHR (S-S) or from SHR treated with losartan (S-SLOS)) and bacterial 16S ribosomal DNA was amplified and sequenced to analyse the composition of microbial communities. Phylum breakdown of the 5 most abundant bacterial communities in the faecal samples obtained from all experimental groups (A). The *Firmicutes/Bacteroidetes* ratio (F/B ratio) was calculated as a biomarker of gut dysbiosis (B). Relative proportions of acetate-, butyrate-, lactate-, and propionate-producing bacteria (C). Anaerobic and aerobic bacteria expressed as relative proportions (D) in the gut microbiota. Values are expressed as mean  $\pm$  SEM. # $P < 0.05$  significant differences compared with S-S group.

**Figure S8. Comparisons of gut microbiome changes in SHR with stool transplant from SHR (S-S) versus from SHR treated with losartan (S-SLOS).** Cladograms show the significantly enriched taxa, the taxa are identified on the key to the right of each panel. Larger circles represent greater differences in abundance between groups (A). Linear discriminant analysis effect size (LEfSe) identified significantly different bacterial taxa enriched in each cohort at LDA Score > 2, p<0.05 (red bars S-S enriched, green bars S-SLOS enriched) (B). n = 7-6 rats per experimental group in each comparison.

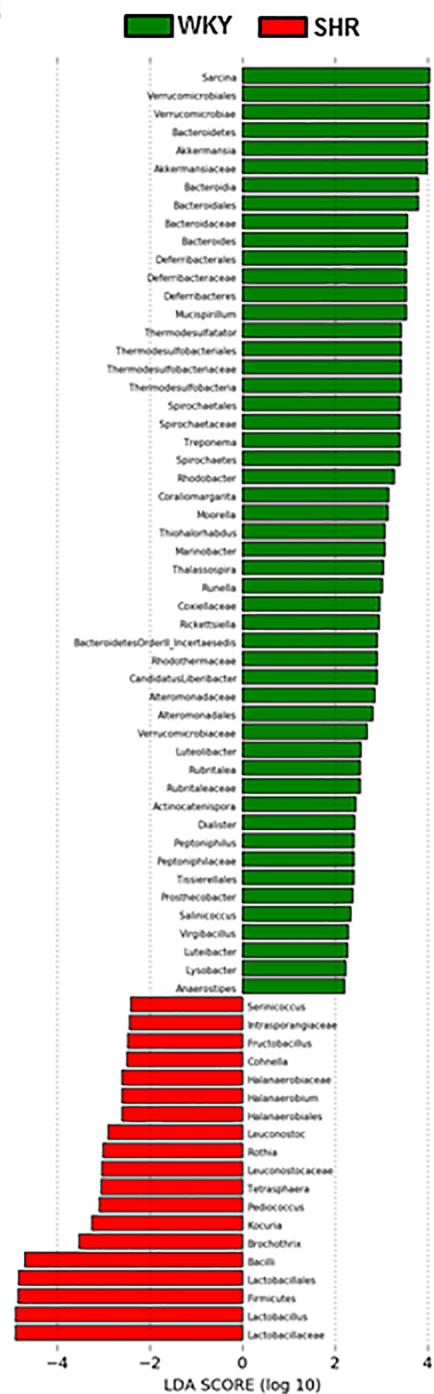
**Figure S9. Effects of faecal microbiota transplantation from spontaneously hypertensive rat (SHR)-losartan at the genus level in the gut microbiota of SHR.** Faecal samples were collected at the end of FMT experiment (SHR with stool transplant from SHR (S-S) or from SHR treated with losartan (S-SLOS)). Principal coordinate analysis of genera (A) in the gut microbiota from all experimental groups. Samples clustered by experimental group demonstrated that the experimental conditions resulted in distinct populations of bacteria at the genus level. (B) Heat map showing differences in the relative abundance of the bacterial genera between the three groups studied. The colours of the heat map represent the relative percentage of microbial genus assigned within each sample. The most significantly modified bacterial genera (C) and species (D) in the gut microbiota. Values are expressed as mean ± SEM. #P< 0.05 significant differences compared with S-S group.

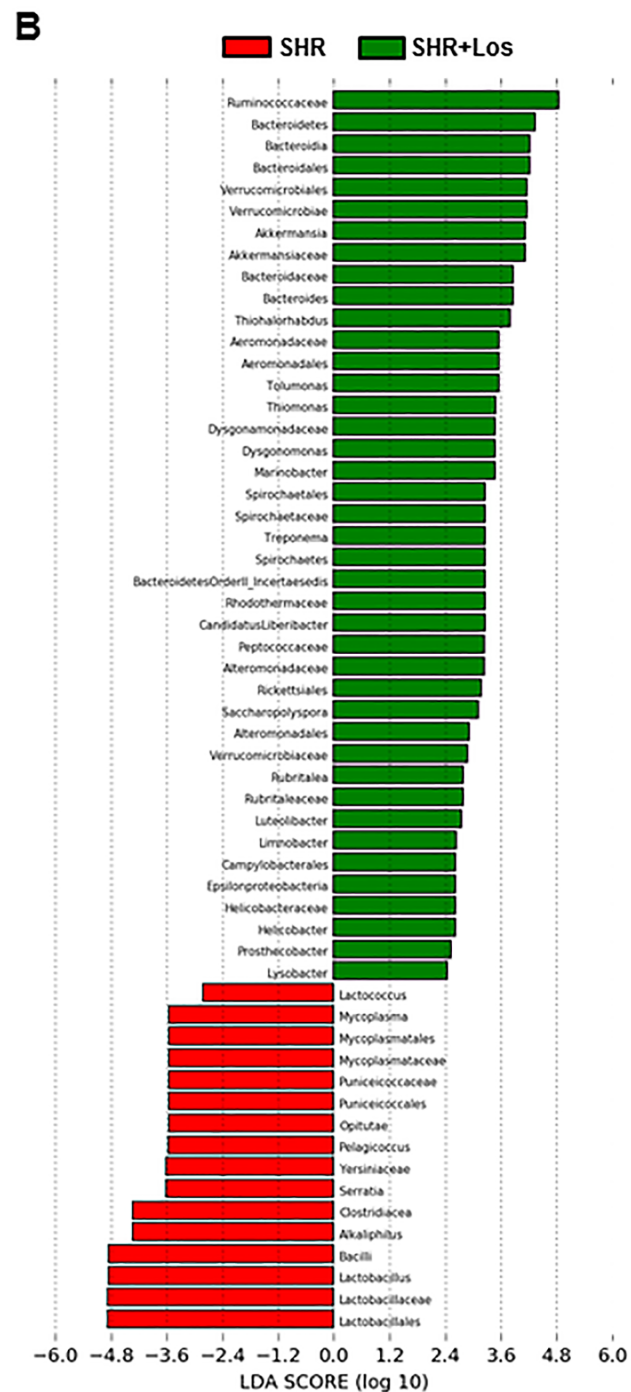
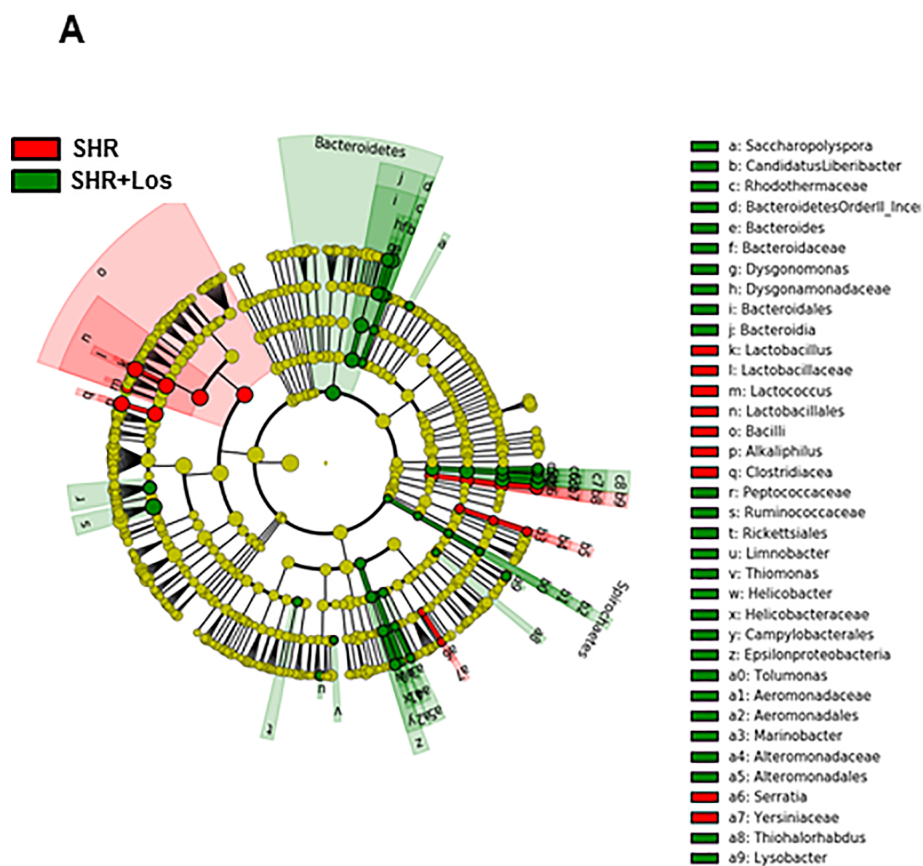
**Figure S10. Summary of mechanistic changes involving gut microbiota in the antihypertensive effects of Losartan in spontaneously hypertensive rat.**

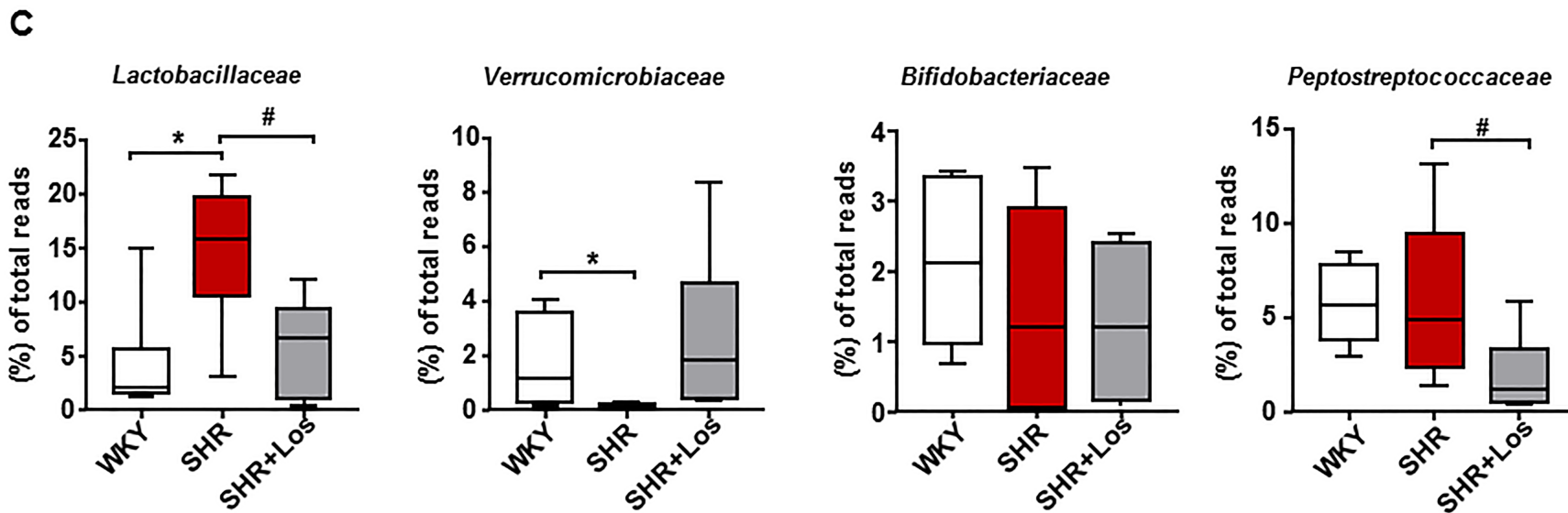
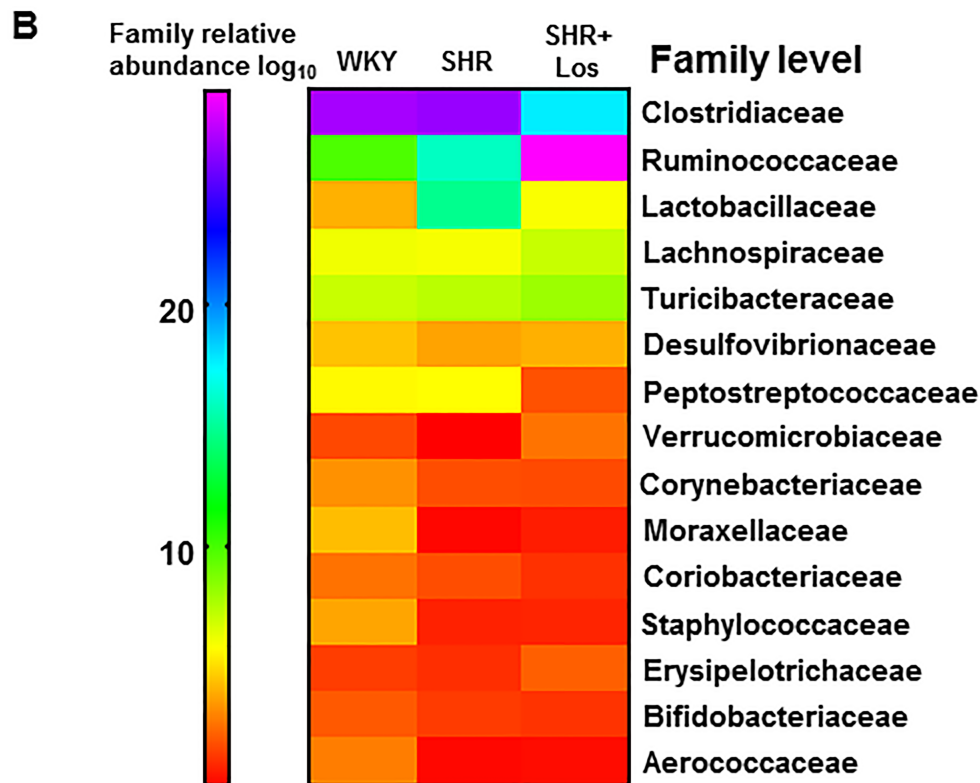
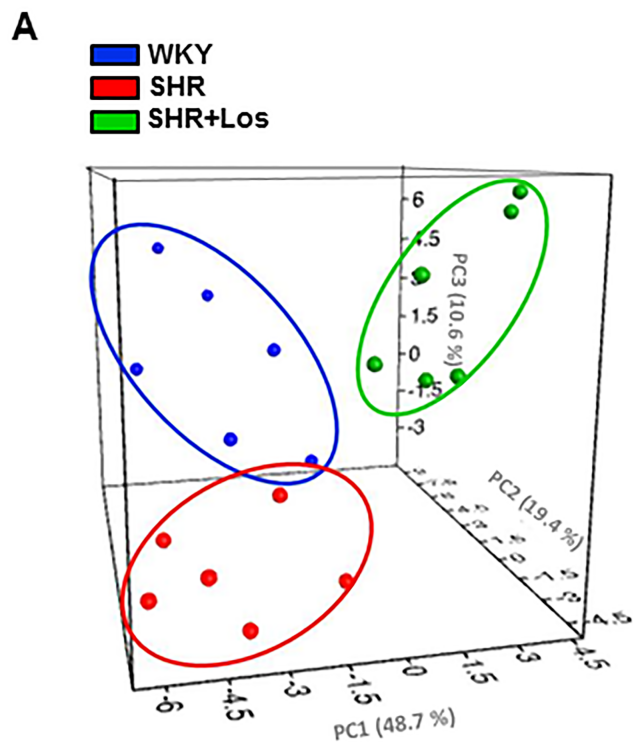
**A****B**

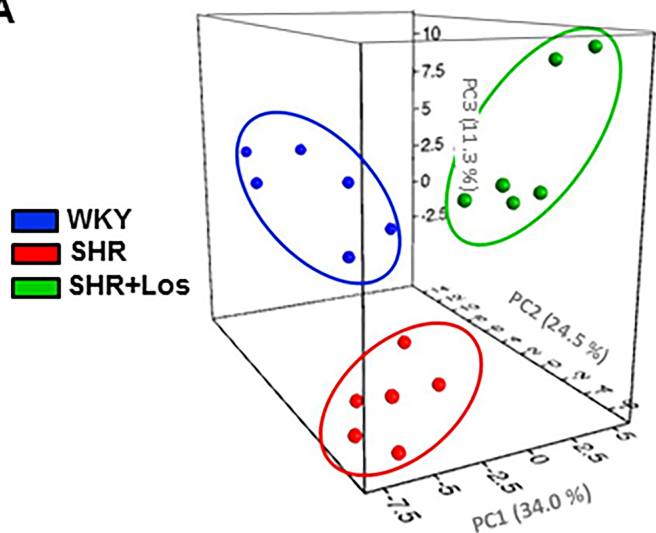
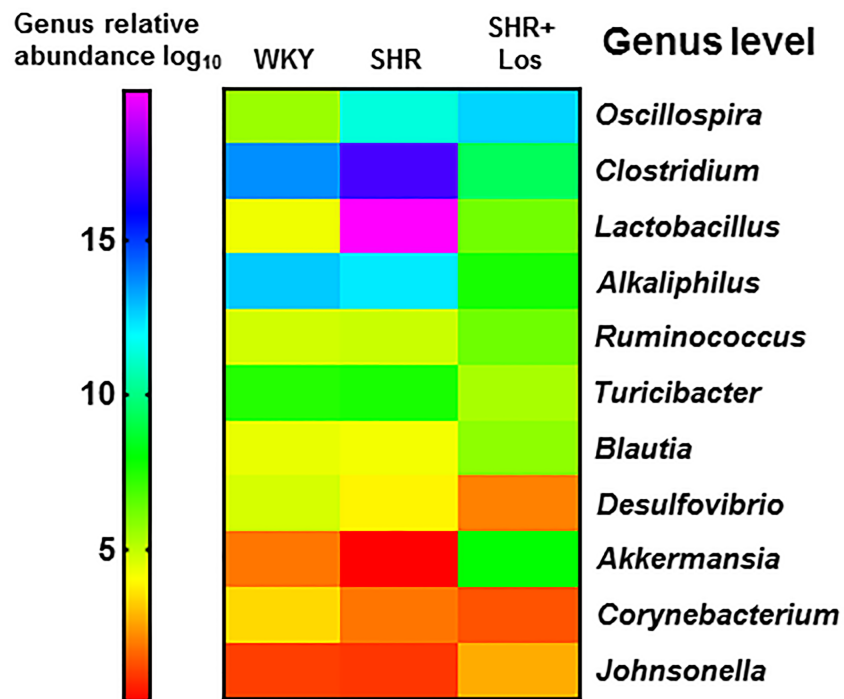
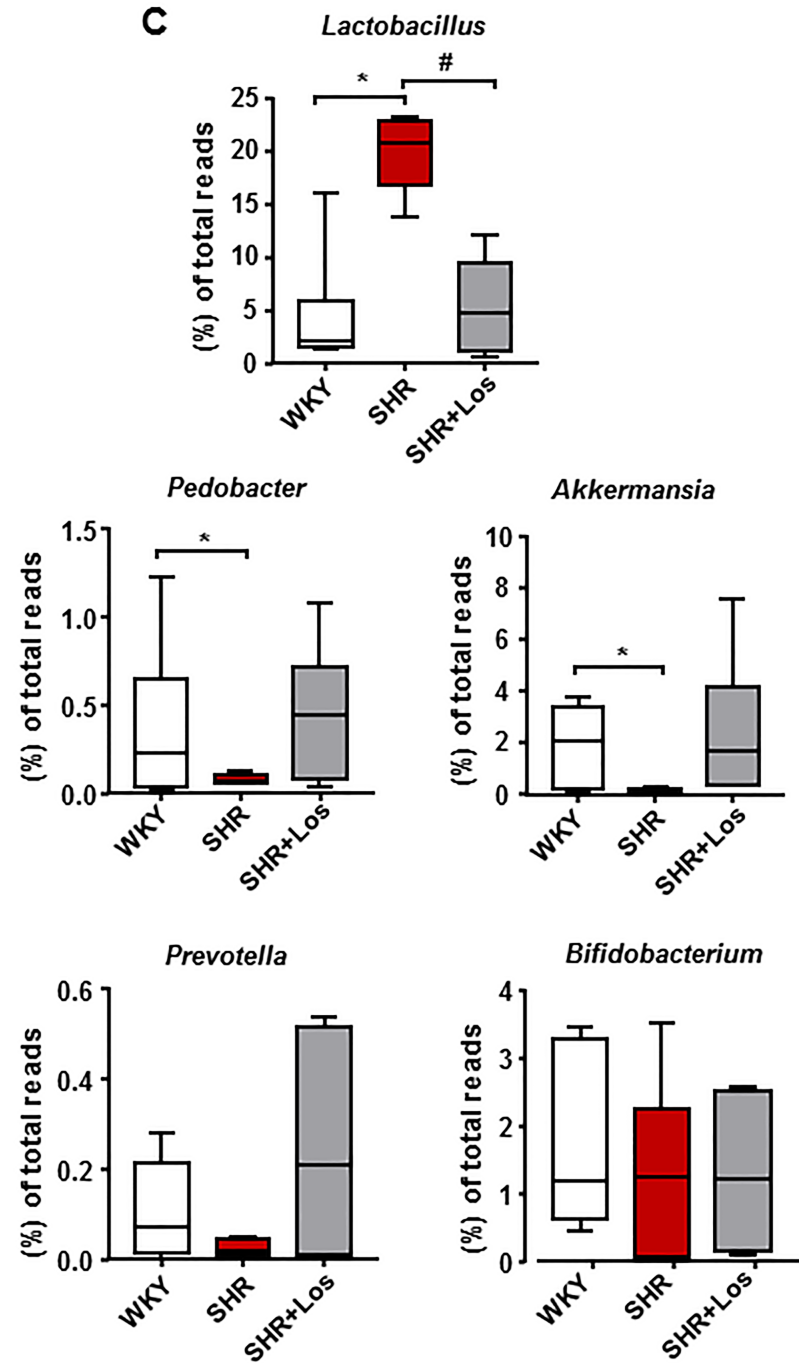


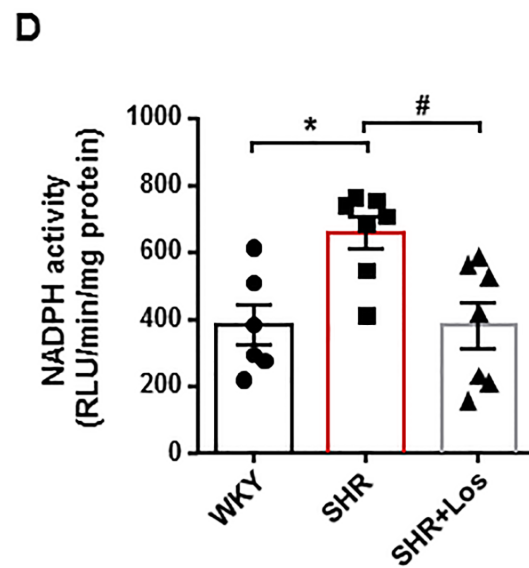
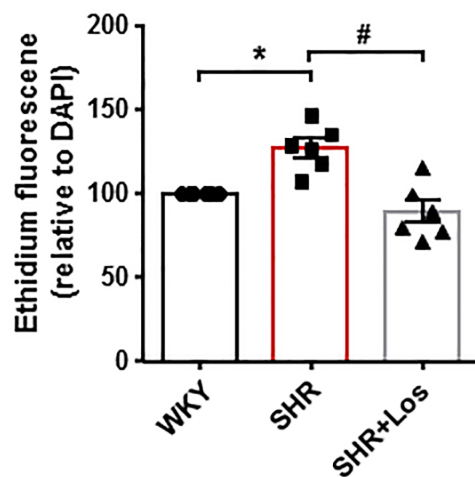
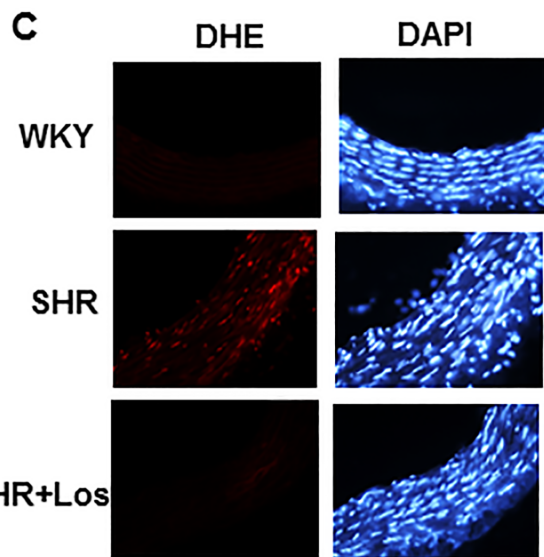
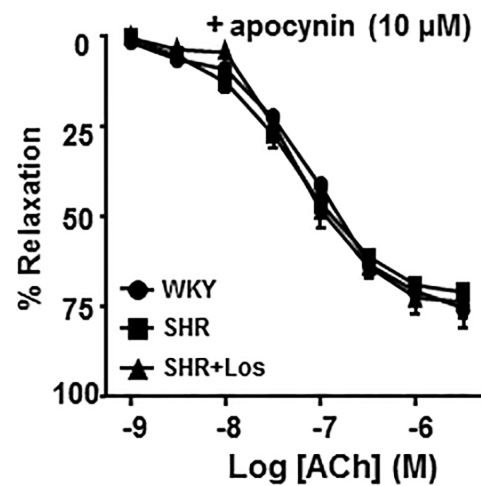
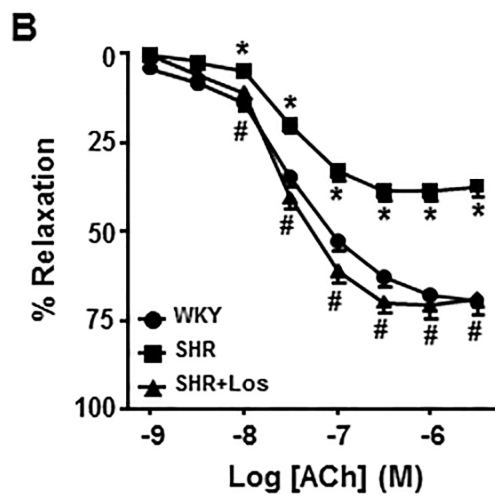
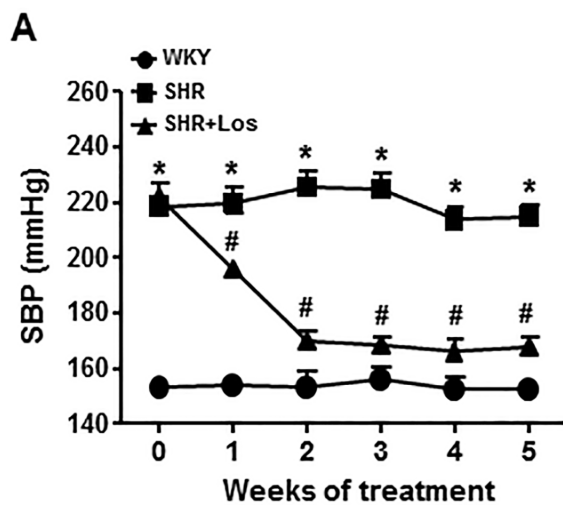
**B**





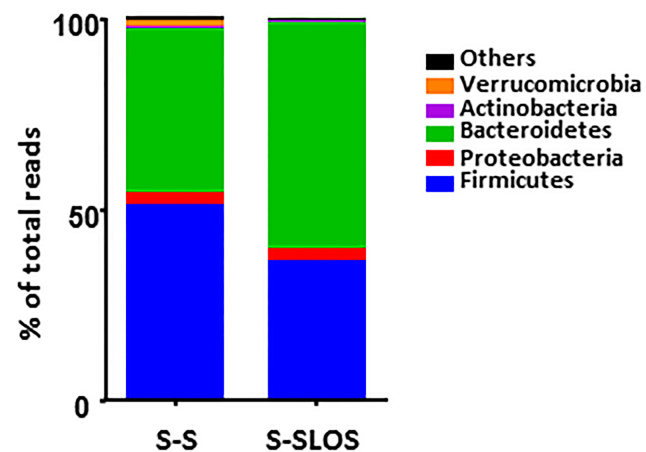


**A****B****C**

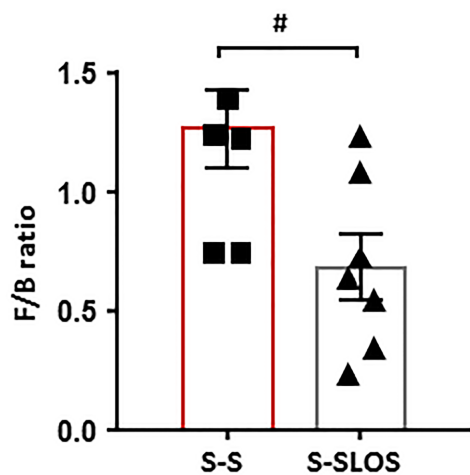


A

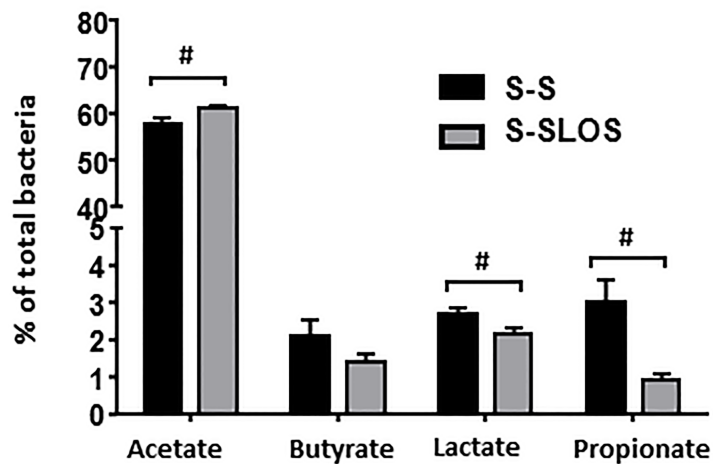
Phylum	S-S (n=7)	S-SLOS (n=6)
Firmicutes	51.20 ± 3.3	36.9 ± 5.2#
Bacteroidetes	42.8 ± 3.5	59.2 ± 5.2#
Proteobacteria	3.1 ± 0.7	2.8 ± 0.5
Verrucomicrobia	1.6 ± 0.7	0.4 ± 0.0#
Actinobacteria	0.6 ± 0.2	0.6 ± 0.3



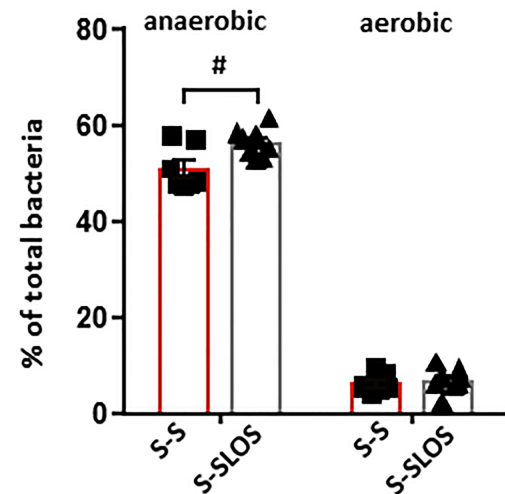
B



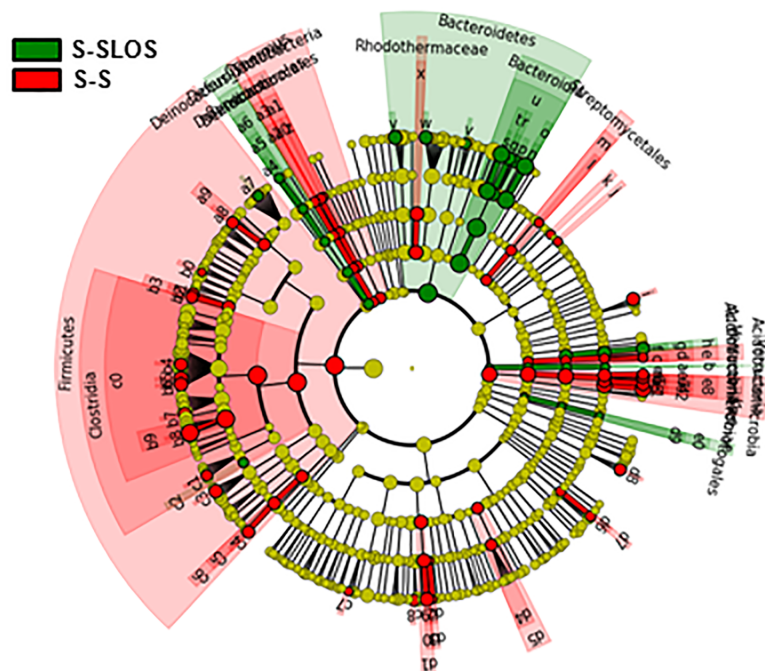
C



D



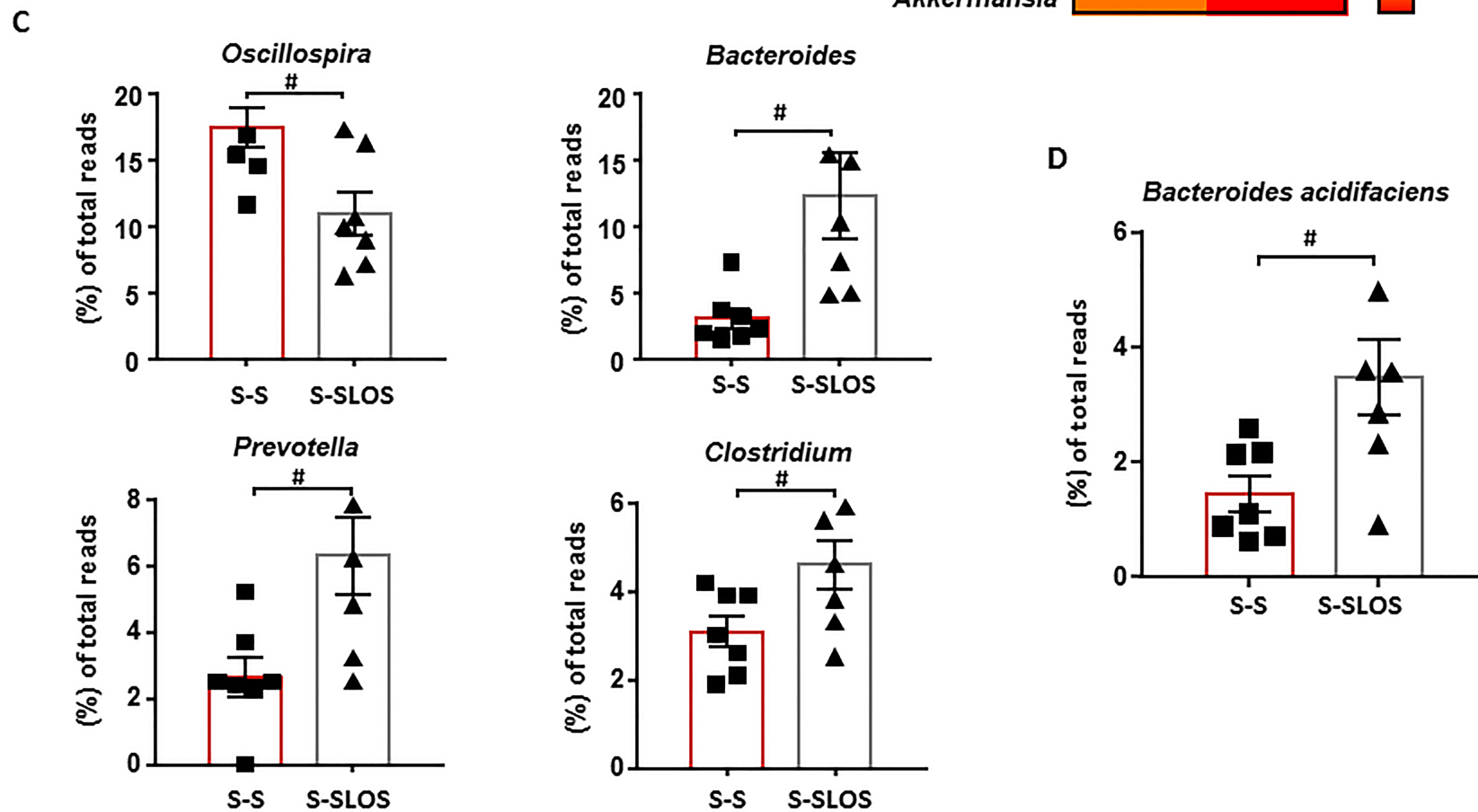
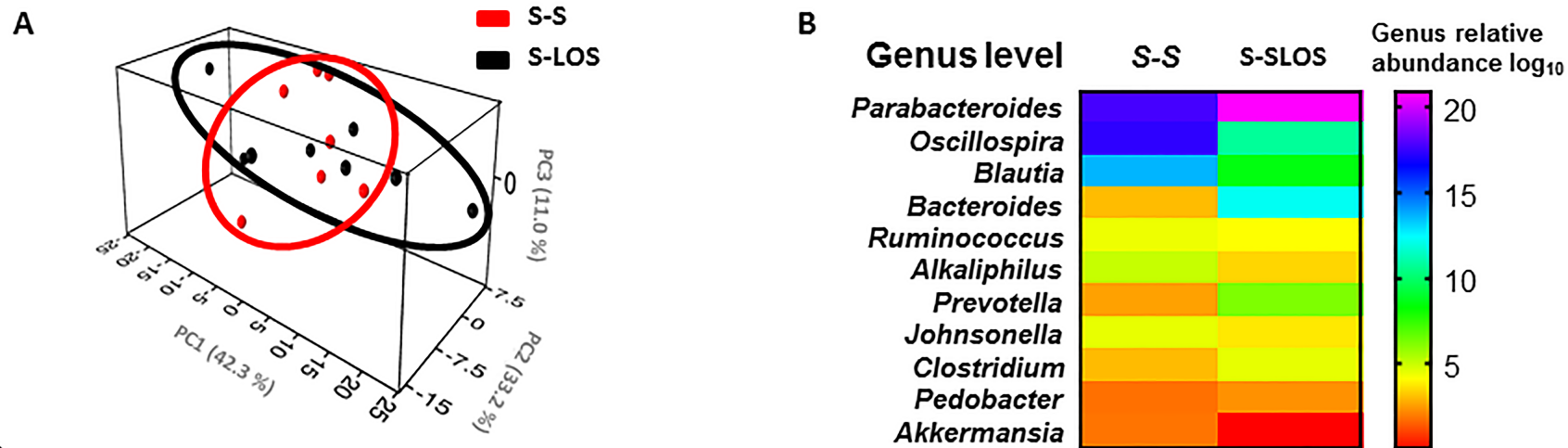
A

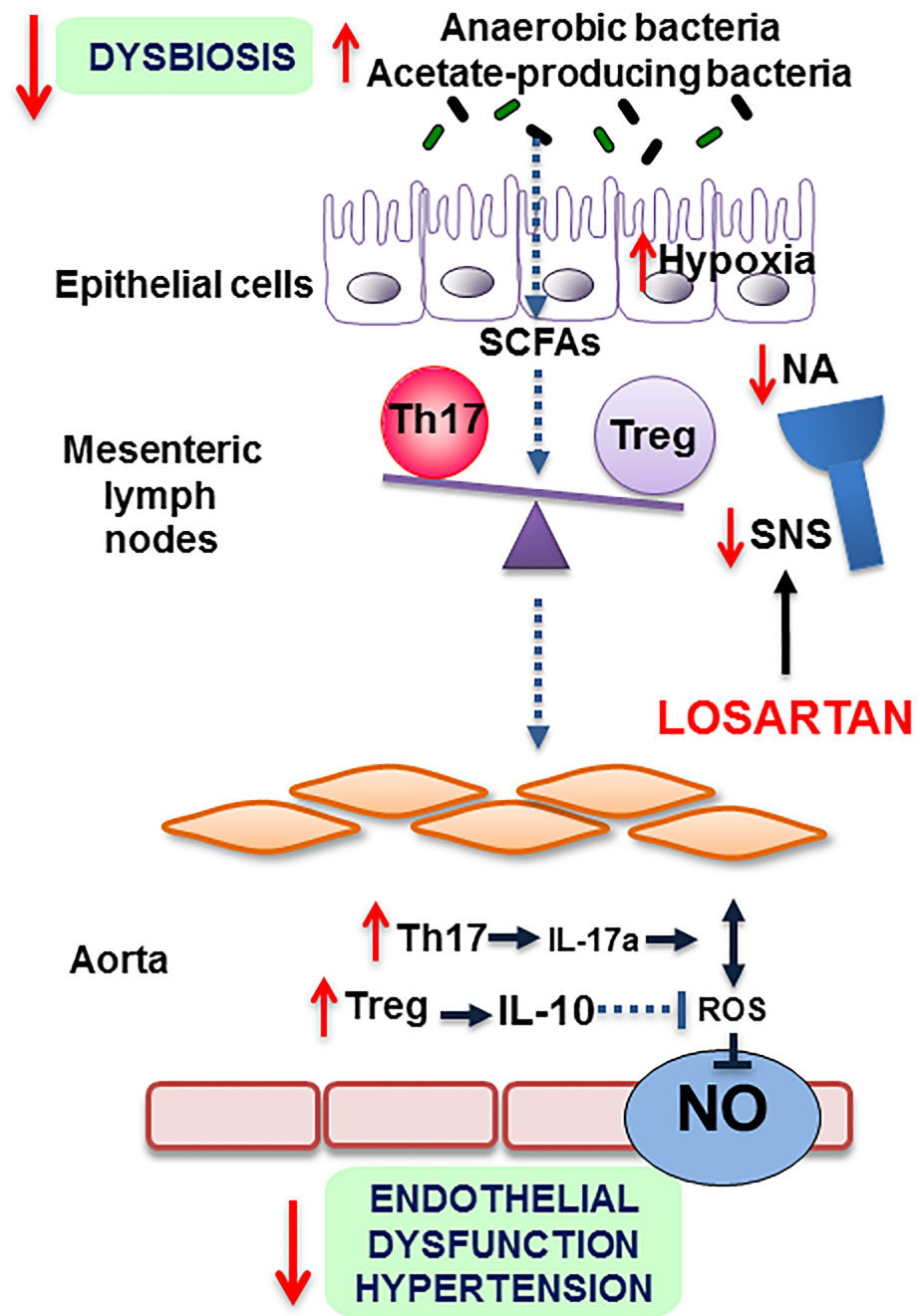
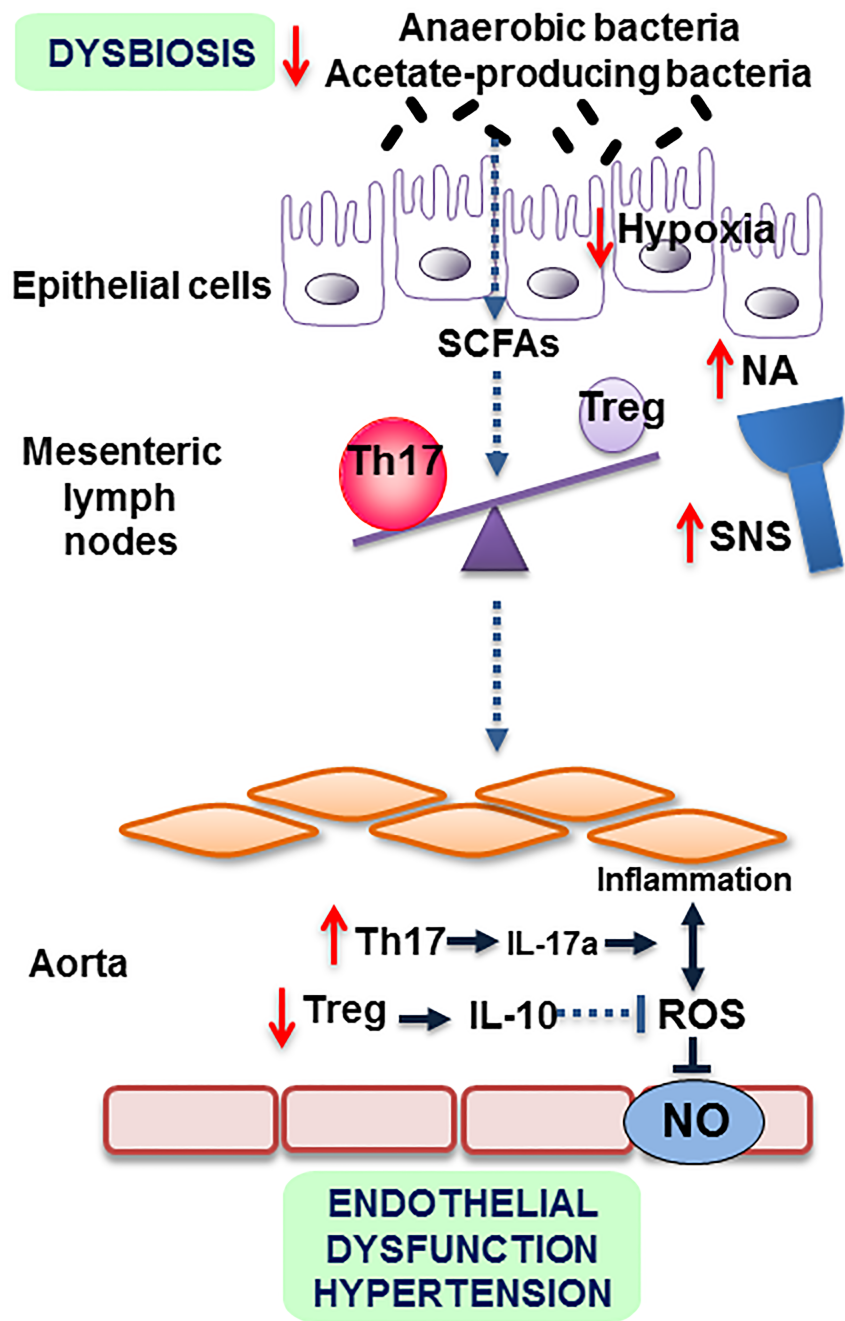


B

- a: Edaphobacter
- b: Acidobacteriaceae
- c: Acidimicrobium
- d: Acidimicrobiaceae
- e: Acidimicrobiales
- f: Bifidobacterium
- g: Bifidobacteriaceae
- h: Bifidobacteriales
- i: Slackia
- j: Kribbella
- k: Amycolatopsis
- l: Kitasatospora
- m: Streptomycetaceae
- n: Bacteroides
- o: Bacteroidaceae
- p: Butyrivomona
- q: Porphyromonas
- r: Porphyromonadaceae
- s: Prevotella
- t: Prevotellaceae
- u: Bacteroidales
- v: Runella
- w: Tenacibaculum
- x: Rhodothermus
- y: Sphingobacterium
- z: Arthronema
- a0: Leptolyngbya
- a1: Leptolyngbyaceae
- a2: Deferribacter
- a3: Deferribacteraceae
- a4: Thermus
- a5: Thermaceae
- a6: Thermales
- a7: Geobacillus
- a8: Paenibacillus
- a9: Paenibacillaceae

■ S-SLOS ■ S-S



**Table S1.** Primers for real-time RT-PCR.

<b>mRNA targets</b>	<b>Descriptions (Gene ID)</b>	<b>Forward</b>	<b>Reverse</b>
<i>TNF-<math>\alpha</math></i>	tumor necrosis factor-alpha (24835)	ACGATGCTCAGAAACACACG	CAGTCTGGGAAGCTCTGAGG
<i>IL-6</i>	interleukin-6 (24498)	GATGGATGCTTCCAAACTGG	AGGAGAGCATTGGAAGTTGG
<i>IL-10</i>	interleukin-10 (25325)	GAATCCCTGGGAGAGAAGC	GCTCCACTGCCTTGCTTTTA
<i>IL-17a</i>	interleukin-17a (301289)	CTTCACCTGGACTCTGAGC	TGGCGGACAATAGAGGAAAC
<i>IFN<math>\gamma</math></i>	interferon gamma (29197)	GCCCTCTGGCTGTTACTG	CCAAGAGGAGGCTCTTTCT
<i>T-bet</i>	T-bet (303496)	CCACCAGCACCAGACAGAGA	AACATCCTGTAATGGCTCGTG
<i>GATA-3</i>	GATA-3 (85471)	GCCTGCGGACTCTACCATAA	GTCTGACAGTTTCGCACAGGA
<i>FOXP3</i>	forkhead box P3 (317382)	AGGCACTTCTCCAGGACAGA	CTGGACACCCATTCCAGACT
<i>ROR<math>\gamma</math></i>	ROR-gamma 1(9885)	GCCTACAATGCCAACAACCACACA	TGATGAGAACCAAGGCCGTGTAGA
<i>Occludin</i>	Occludin (83497)	AGCCTGGGCAGTCGGGTTGA	ACACAGACCCAGAGCGGCA
<i>Muc2</i>	mucin-2 (24572)	CGATCACCACCATTGCCACTG	ACCACCATTACCACCCTCAG
<i>MUC3</i>	mucin-3 (687030)	CACAAAGGCAAGAGTCCAGA	AGTGTCTTGGTGCTGCTGAATG
<i>ZO-1</i>	zonula occludens-1 (292994)	GCCAGCCAGTTCGCCTCTG	AGGGTCCC GGTTGGTG
<i>Th</i>	tyrosine hydroxylase (25085)	GATTGCTACCTGGAAGGAGGT	AGTCCAATGTCTGGGAGAAC
<i>RNP1-2</i>	alpha defensin RNP1-2 (613220)	GGACGCTCACTCTGCTTACC	TGGATTCTTCTGGTCGGAG
<i>RNP3</i>	alpha defensin RNP3 (498659)	AAGAGCGCTGTGTCTCTTGC	CAACAGAGTCGGTAGATGCG
<i>RNP4</i>	alpha defensin RNP4 (286958)	TCTGCTCATCACCCTTCTCC	AACAGAGACGGTAGATGCGG
<i>RNP5</i>	alpha defensin RNP5 (28699)	ACCAGGCTTCAGTCATGAGG	CATCCATTGGTTCTTGGTC
<i>GAPDH</i>	glyceraldehyde-3-phosphate dehydrogenase (28383)	GTCGGTGTGAACGGATTT	ATGGGTTTCCGTTGATG