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Supplemental Information

**Salt-Responsive Metabolite, β -Hydroxybutyrate,
Attenuates Hypertension**

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SUPPLEMENTAL FILES

Supplemental Figure 1

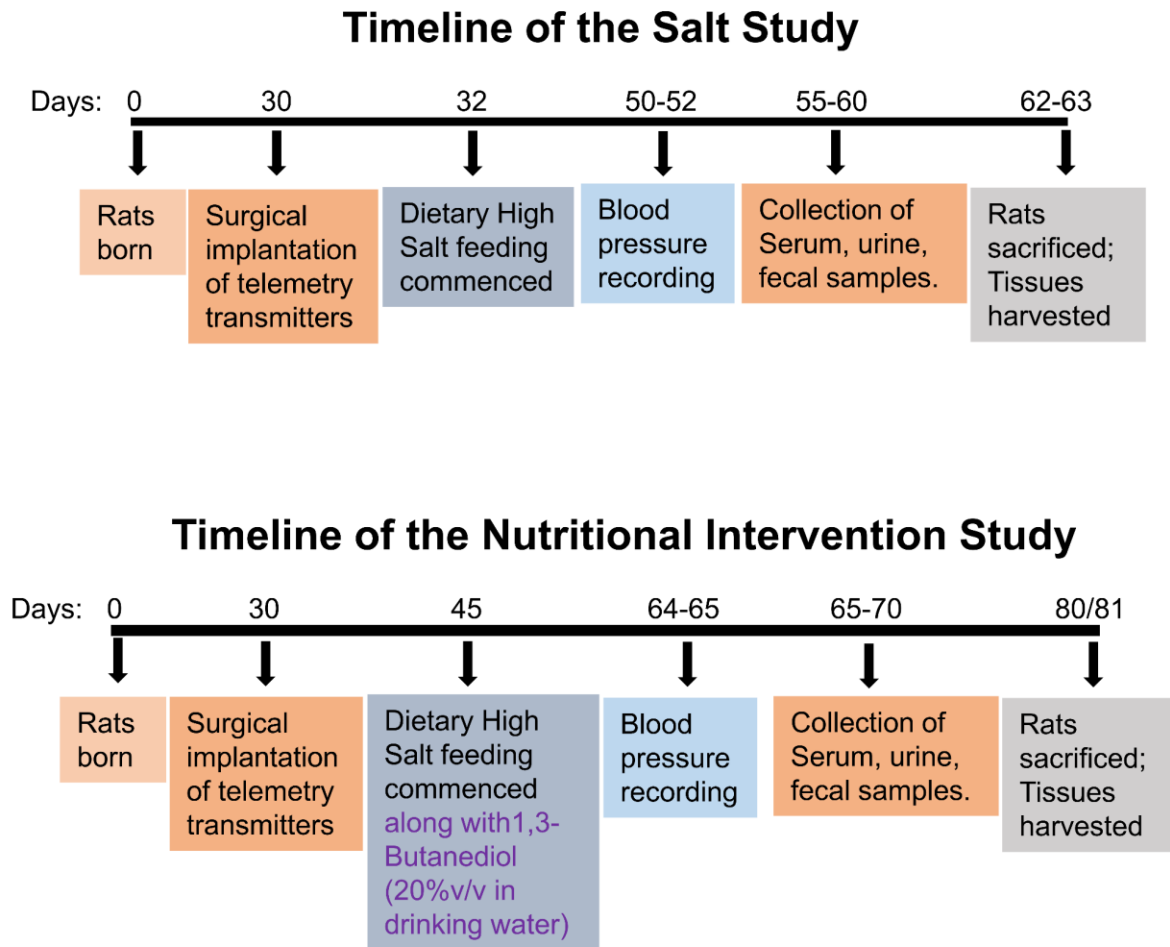


Figure S1. Timeline of the Salt and Nutritional Intervention study. Related to Figures 1 and 3.

Supplemental Figure 2

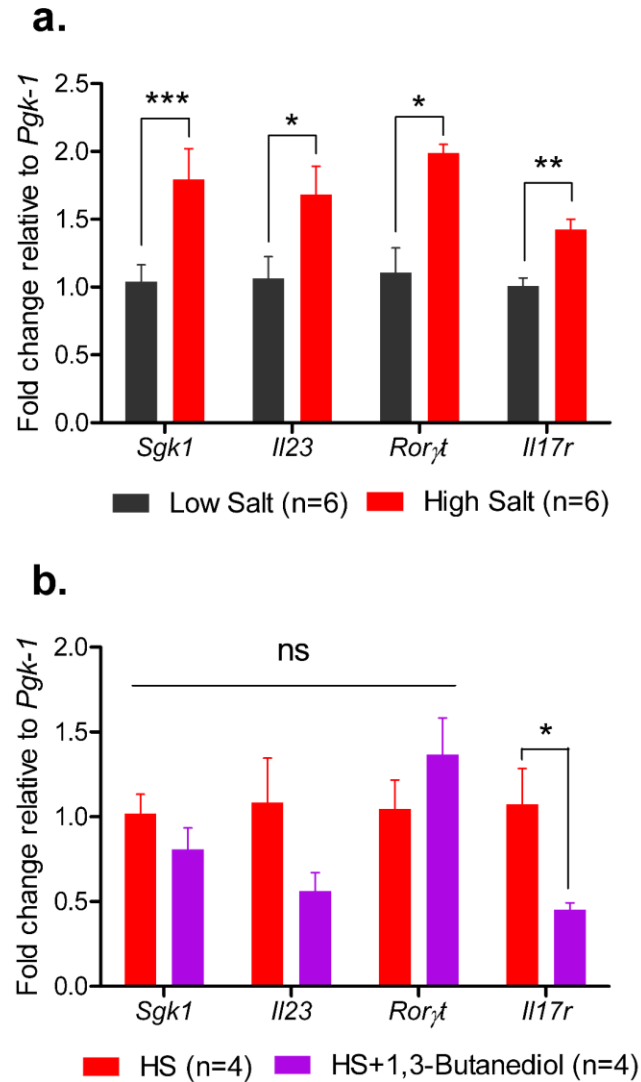


Figure S2. (a) Aberrant renal profile of markers associated with salt induced Th17 activation. * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$. (b) Effect of nutritional intervention with β OHB on renal profile of markers associated with salt induced Th17 activation. HS: High Salt; ns: not significant, * $p < 0.05$. Related to Figures 4 and 5.**

Supplemental Figure 3

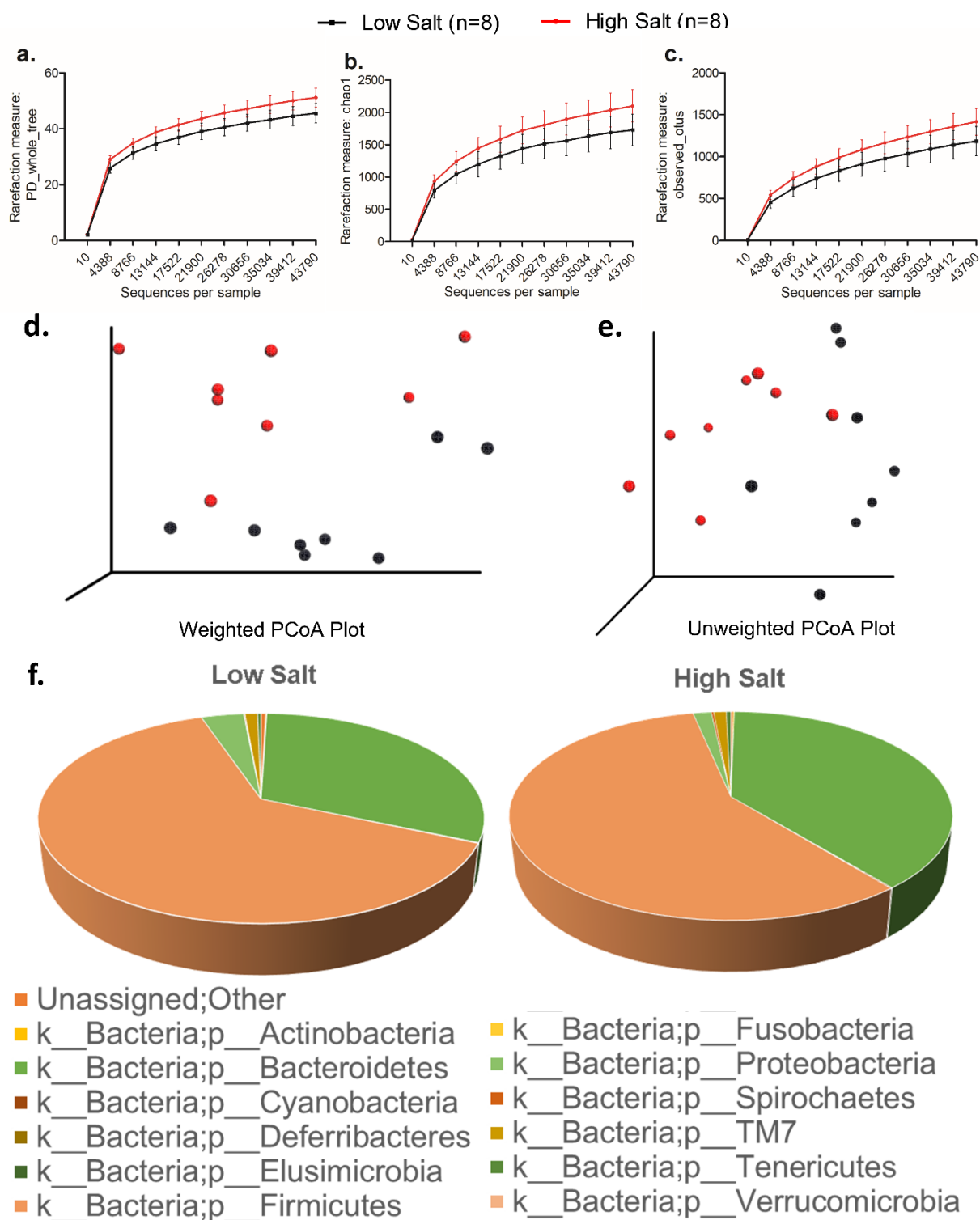


Figure S3. Salt loading results in alterations in gut microbiota. Related to Figure 4

Supplemental Figure 4

— High Salt (n=12) — HS+1,3-Butanediol (n=12)

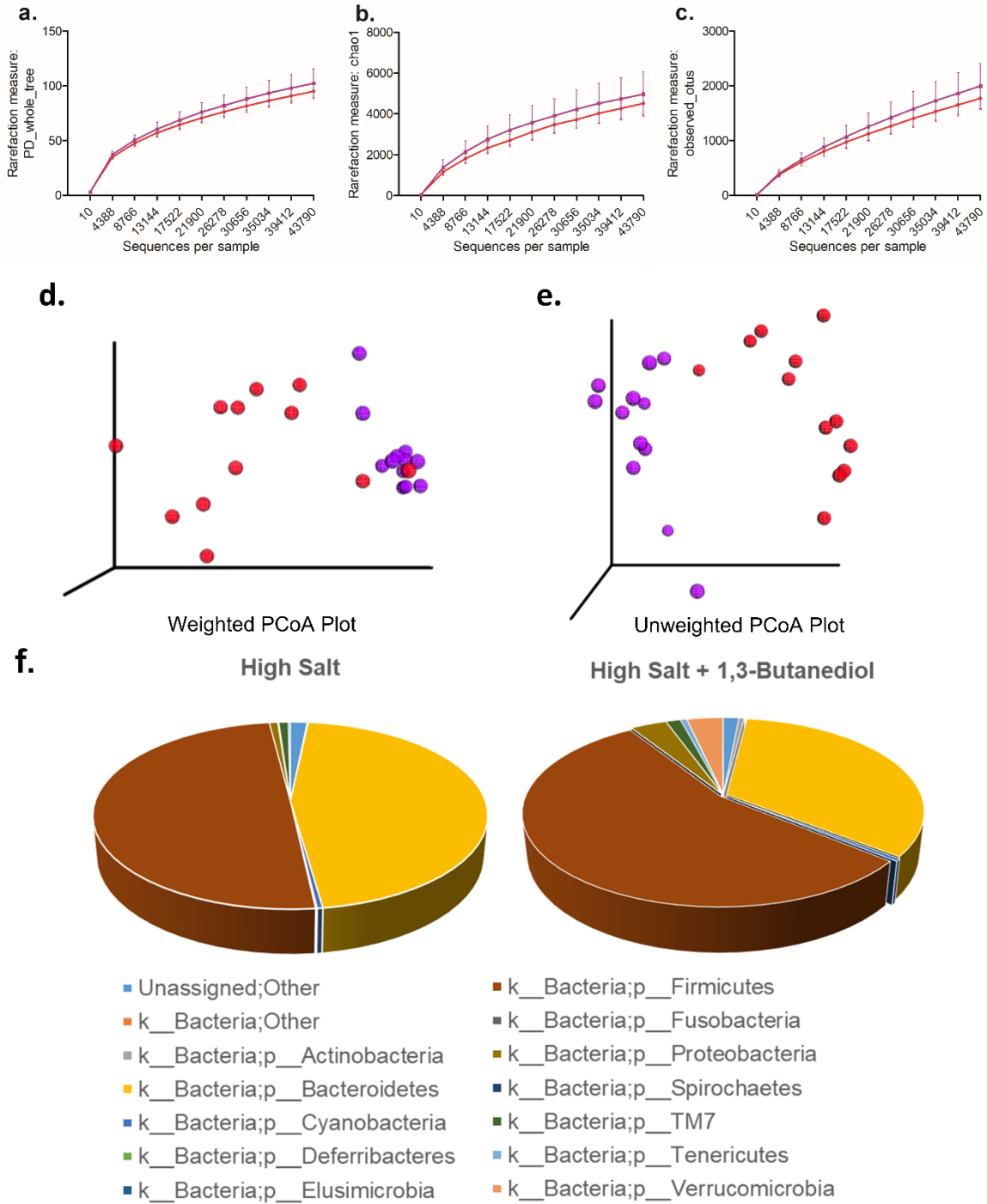


Figure S4. Nutritional intervention with β OHB leads to alterations in gut microbiota.

Related to Figure 5.

Supplemental Figure 5

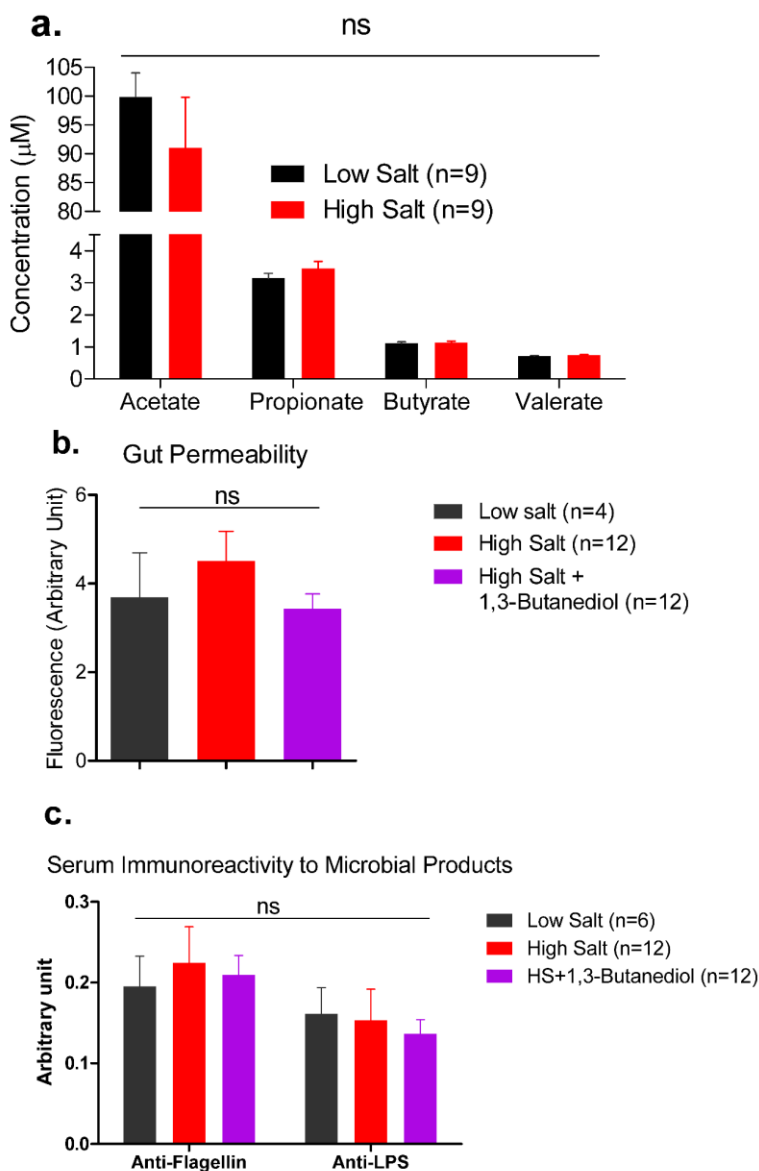


Figure S5. (a) Salt-loading did not alter plasma microbial derived short-chain fatty acids. ns: not significant. (b) Gut permeability was unchanged by salt loading or intervention of the high salt group with 1,3-butanediol. ns: not significant. (c) Serum immunoreactivity to bacterial products were unchanged between the 3 groups. HS: High Salt; ns: not significant. Related to Figures 4 and 5.

Supplemental Figure 6

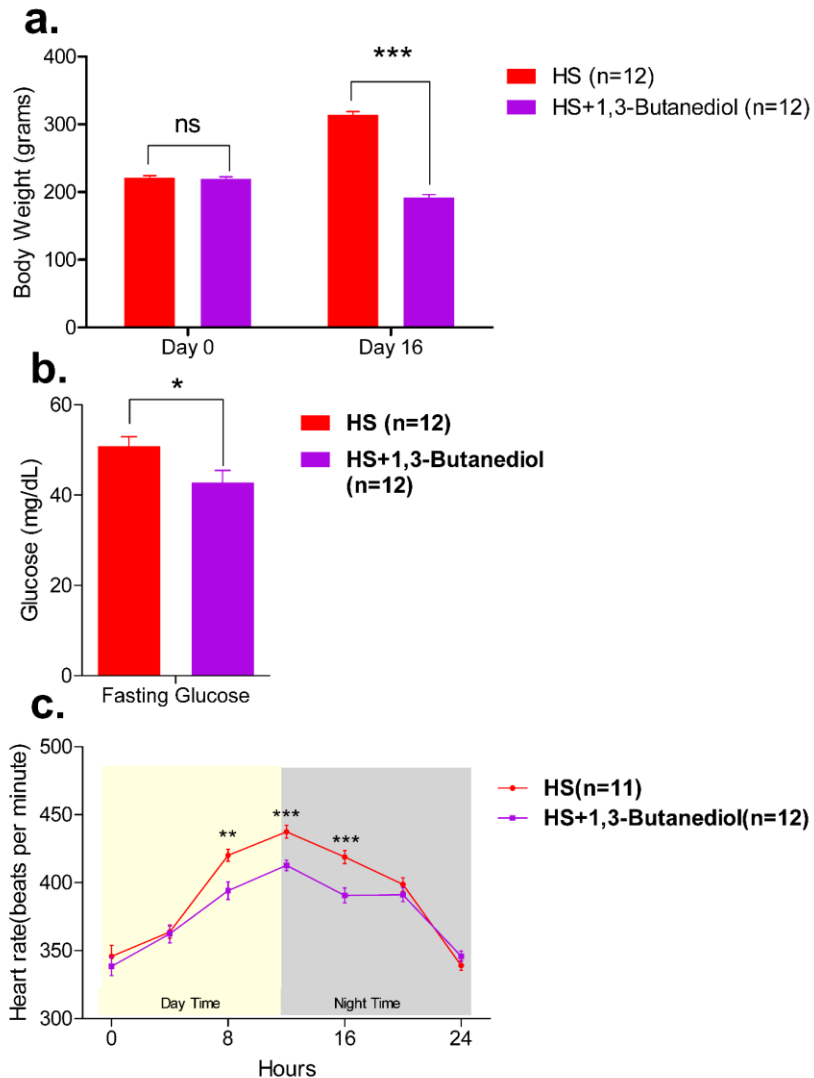


Figure S6. Nutritional intervention with 1,3-Butanediol lowered body weight and fasting glucose. (a) Body weight of rats at day 0 (starting point of high salt and nutritional intervention) and day 16 (post 16 days of administration of β OHB with 1,3-Butanediol). (b) Fasting glucose level of HS and HS+1,3-Butanediol post 3 weeks of 1,3-Butanediol administration. (c) Heart rate of HS and HS+1,3-Butanediol post 3 weeks of 1,3-Butanediol administration. HS: High salt; ns: not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **Related to Figures 3 and 6.**

Supplemental Figure 7

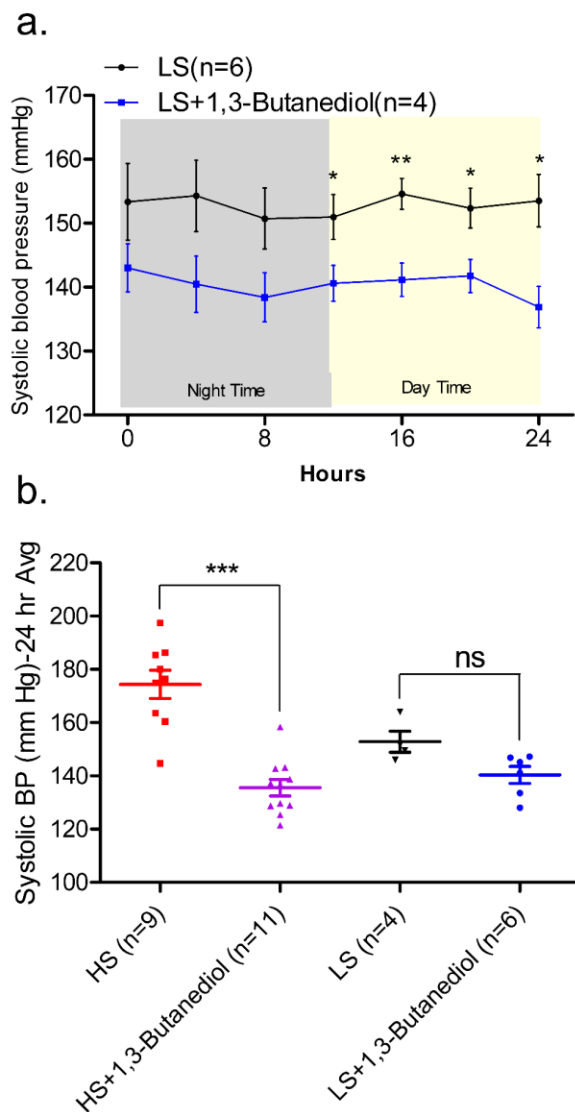


Figure S7. Systolic blood pressure comparison in Salt and 1,3-Butanediol intervention. (a) Systolic blood pressure of LS and LS+1,3-Butanediol post 3 weeks of intervention. (b) 24 hour average systolic blood pressure of HS, HS+1,3-Butanediol, LS, LS+1,3-Butanediol post 3 weeks of 1,3-Butanediol administration. LS: Low salt, HS: High salt; ns: not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. **Related to Figures 1 and 3**

Supplemental Table 1: List of mean of all the significantly different metabolites' mean height peaks generated through GC-TOF-MS based untargeted metabolomics. **Related to Figure 2.**

BinBase name	Fasting Plasma Metabolites		t-test	Mean(HS)-Mean(LS)	Ranked based on value of Mean(HS)-Mean (LS)
	Mean Low Salt	Mean High Salt			
3-hydroxybutyric acid	740065.6	518790.7	0.033568	-221274.9	1
stearic acid	503116.1	418287.3	0.042409	-84828.78	3
7458	8427.556	4298.778	0.00296	-4128.778	9
xylose	9477.667	5606	0.029512	-3871.667	10
133026	7591.333	4967.667	0.043349	-2623.667	14
4721	4850.556	2708.333	0.03389	-2142.222	17
4723	4771	2821.222	0.008215	-1949.778	18
14736	3946.444	2343.222	0.047469	-1603.222	21
heptadecanoic acid	5542.889	4260	0.038143	-1282.889	23
41862	623.8889	908.5556	0.045398	284.66667	29
130466	655.7778	955.3333	0.030354	299.55556	28
5935	418.4444	740.3333	0.017416	321.88889	27
240510	1644	2049.556	0.006502	405.55556	26
209686	884.2222	1303.778	0.035521	419.55556	25
4747	356.5556	827.3333	0.002345	470.77778	24
phosphoenolpyruvate	828.3333	2368.889	0.02539	1540.5556	22
240048	4214.667	5924.667	0.001249	1710	20
247704	2590.778	4400.222	0.040984	1809.4444	19
allantoic acid	2368.444	4774	0.002219	2405.5556	16
beta-sitosterol	3858	6452	0.008491	2594	15
93947	3033.667	5711.111	0.002887	2677.4444	13
arachidonic acid	7939.444	10789.67	0.033295	2850.2222	12
conduritol-beta-epoxide	5873.222	8997.667	0.00191	3124.4444	11
4956	12942.22	18188.33	0.027428	5246.1111	8
aspartic acid	35553.78	46841.67	0.048754	11287.889	7
ethanolamine	18684.11	32835.89	0.039953	14151.778	6
219478	57249.89	75430.11	0.011592	18180.222	5
threonine	184105.3	216072	0.039557	31966.667	4
creatinine	203515.7	309223.6	0.036295	105707.89	2

Supplemental Table 2: List of all primers with forward and reverse sequence. **Related to STAR methods, Figures 6, and 7.**

Gene		Sequence (5'-3')
<i>Casp1</i>	Forward	CCGGGCAAGCCAGATGTTTA
	Reverse	CCAGGAAATGCGCCACCTTC
<i>Nlrp3</i>	Forward	AATGGGGAGGAGAAGGCGTG
	Reverse	AAGGCTGTCTTCCTGGCACA
<i>IL1β</i>	Forward	GCACTGCAGGCTTCGAGATG
	Reverse	CCCAAGGCCACAGGGATTTTG
<i>IL18</i>	Forward	AGAGGACTGGCTGTGACCCT
	Reverse	GTTGTGTCCTGGCACACGTT
<i>Lcn2</i>	Forward	GAACCAAGGGGCTGTCCGAT
	Reverse	ACGCTCACCGTCTGTTCAGT
<i>Hif1α</i>	Forward	GGCGGCGAGAACGAGAAGAA
	Reverse	AGCTCACGTTGTGGGGAAGT
<i>Tgfb1</i>	Forward	GGCAGTGGCTGAACCAAGGA
	Reverse	GGAGCAGGAAGGGTTCGGTTC
<i>Serpine1</i>	Forward	TTCAAGCTCTTCCGGACCAC
	Reverse	AATAGAGGGCGTTCACCAGC
<i>Col1a2</i>	Forward	CTGCTGGCCCAATGGATTT
	Reverse	CCATCACACGACTTCCAGC
<i>RoryT</i>	Forward	GCTGGCTGCAAAGAAGACCC
	Reverse	CTGACGGGTGCAGGAGTAGG
<i>IL23</i>	Forward	TGCACACACACCAGTGGGAC
	Reverse	AAACACCAGACCTTGGCGGAT
<i>Sgk1</i>	Forward	GAGCGAGTCCGTCTGCTAA
	Reverse	TGGCGTTCATAAGTTCGGGC
<i>IL17r</i>	Forward	CTGGCCACGGGTCGTC
	Reverse	GAAGACGGCGTCAGGTTTCG
16s rRNA (V3-V4 region)	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT