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SUPPLEMENTARY INFORMATION

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Dietary salt promotes neurovascular and cognitive dysfunction through a gut-initiated TH17 response

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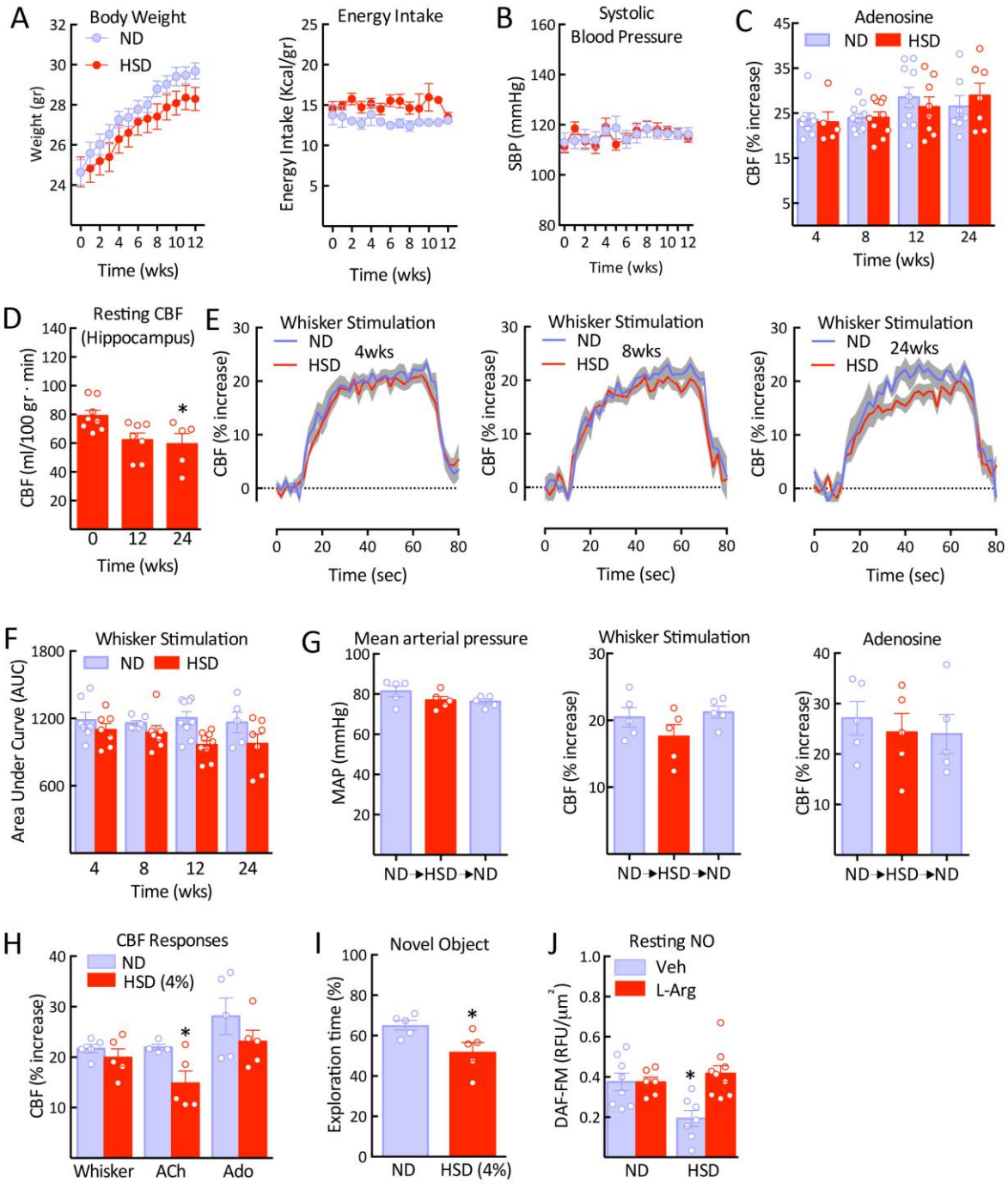
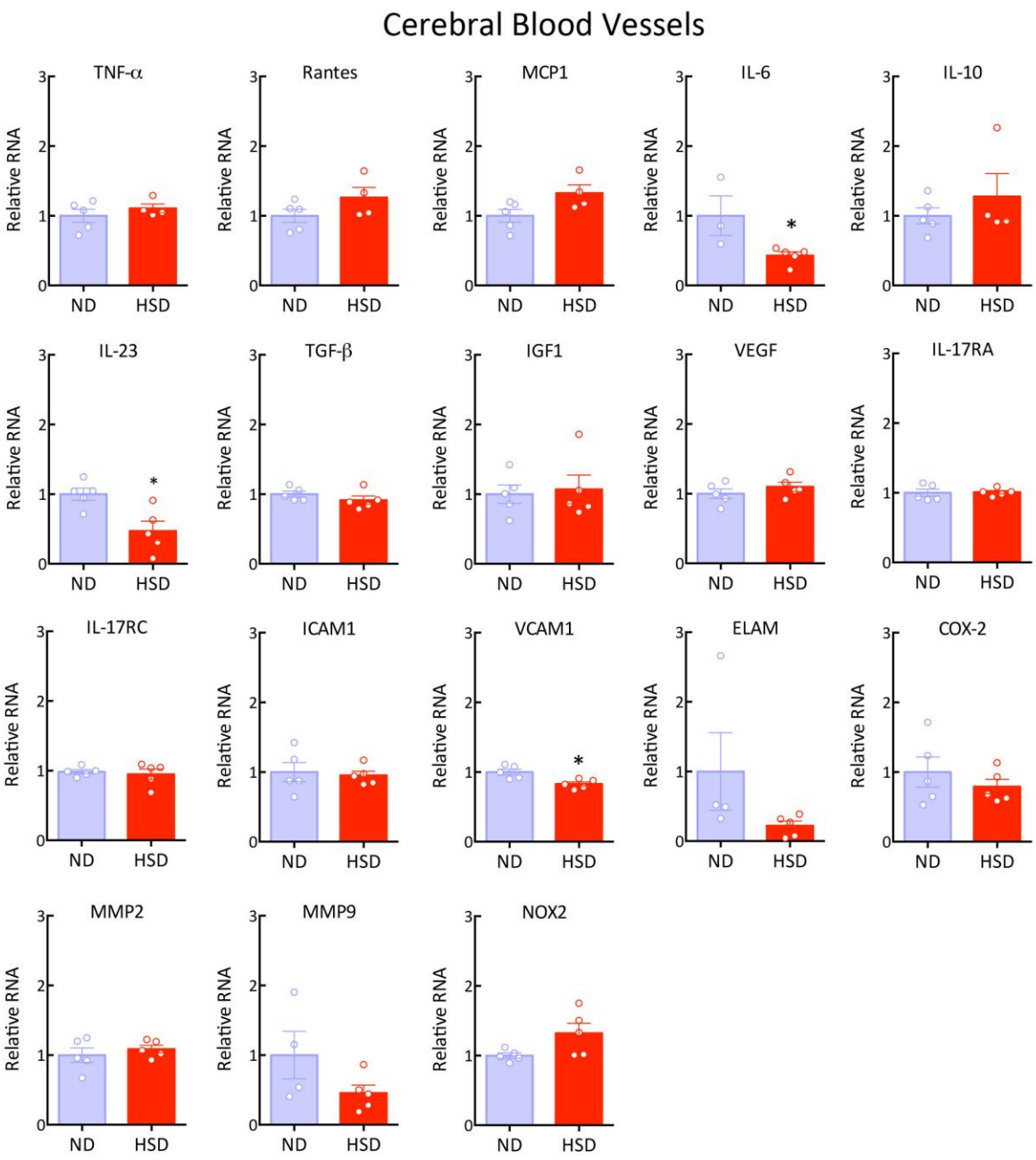

A
Body Weight
ND
HSD
Weight (gr)
Time (wks)
Energy Intake
Energy Intake (Kcal/gr)
Time (wks)
B
Systolic
Blood Pressure
SBP (mmHg)
Time (wks)
C
Adenosine
ND
HSD
CBF (% increase)
Time (wks)
D
Resting CBF
(Hippocampus)
CBF (ml/100 gr · min)
Time (wks)
E
Whisker Stimulation
ND
HSD
4wks
8wks
24wks
CBF (% increase)
Time (sec)
F
Whisker Stimulation
ND
HSD
Area Under Curve (AUC)
Time (wks)
G
Mean arterial pressure
MAP (mmHg)
ND→HSD→ND
Whisker Stimulation
CBF (% increase)
ND→HSD→ND
Adenosine
CBF (% increase)
ND→HSD→ND
H
CBF Responses
ND
HSD (4%)
CBF (% increase)
Whisker
ACh
Ado
I
Novel Object
Exploration time (%)
ND
HSD (4%)
J
Resting NO
Veh
L-Arg
DAF-FM (RFU/μm²)
ND
HSD

Supplementary Figure 1

Effect of HSD on body weight, energy intake, systolic blood pressure, hippocampal resting CBF and CBF response to adenosine and whisker stimulation.

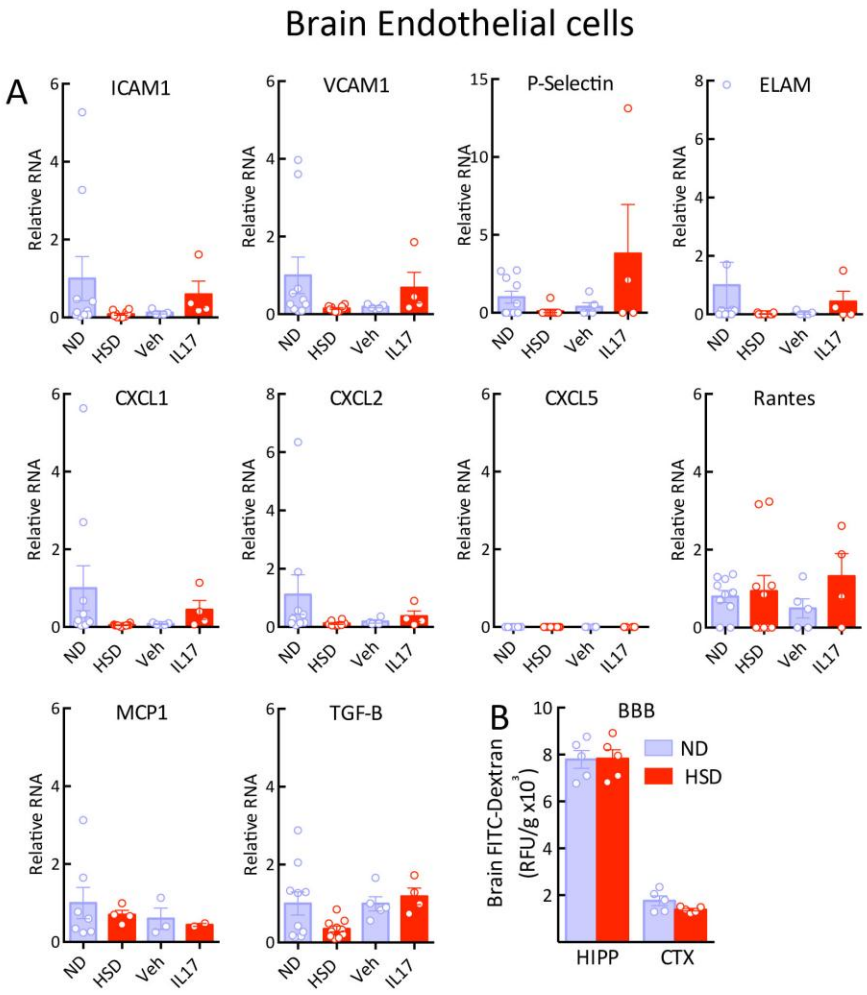
A: HSD tends to reduce body weight and increase caloric intake, but the effect is not statistically significant; Diet: p=0.2668, Time: p<0.0001; ND/HSD n=15/20 mice/group (Two-way ANOVA and Tukey's test). **B**: HSD does not alter systolic blood pressure measured in awake animals by tail-cuff plethysmography. Diet: p=0.9417, Time: p=0.1382; ND/HSD n=15 mice/group (Repeated two-way ANOVA and Bonferroni's test). **C:** HSD does not alter the CBF response to the smooth muscle relaxant adenosine; Diet: p=0.9659, Time: p=0.0549; 4 weeks: ND/HSD n=8/5; 8 weeks: ND/HSD n=10; 12 weeks: ND/HSD n=10/8; 24 weeks: ND/HSD n=7 mice/group (Two-way ANOVA and Tukey's test). **D**: HSD reduces resting CBF in the hippocampus. * p=0.0245; 0 weeks n=8, 12 weeks n=7, 24 weeks n=5 mice/group (One way ANOVA and Tukey's test). **E:** Temporal profile of CBF increase induced by whisker stimulation after 4, 8 and 24 weeks of ND or HSD; 4 weeks: ND/HSD n=7/7; 8 weeks: ND/HSD n=6/7; 12 weeks: ND/HSD n=9; 24 weeks: ND/HSD n=5/7 mice/group. The dotted line indicates the baseline CBF whereas the shaded area represents the standard error. **F**: CBF increase to whisker stimulation assessed as area under the curve shows a diet effect (Diet: p=0.0013, Time: p=0.6687), which did not reach statistical significance at the Tukey's test; 4 weeks: ND/HSD n=7/7; 8 weeks: ND/HSD n=6/7; 12 weeks: ND/HSD n=9; 24 weeks: ND/HSD n=5/7 mice/group (Two-way ANOVA and Tukey's test). **G**: Return to normal diet does not alter MAP and CBF responses to whisker stimulation and adenosine. MAP: p=0.2406; Whisker: p=0.2340; Adenosine: p=0.8010, n=5 mice/group (One-way ANOVA and Tukey's test). **H:** Endothelial dysfunction is also observed in mice fed a 4% HSD for 12 weeks. ACh: * p<0.0376 vs ND; ND/HSD n=4/5 mice/group (unpaired t-test, two-tailed). **I:** A 4% HSD impairs performance at the novel object test (p=0.0350 vs ND, unpaired t-test, two tailed). **J:** Resting NO production, assessed by DAF-FM, is reduced in pial microvascular preparation from mice fed a HSD. The effect is reversed by administration of the NO precursor L-arginine. * p=0.0024; microvessels isolated from 8 (ND Veh), 6 (ND L-Arg), 7 (HSD Veh) and 9 (HSD L-Arg) mice/group (One-way ANOVA and Tukey's test). Data are expressed as mean±SEM.


Cerebral Blood Vessels
TNF-α
Rantes
MCP1
IL-6
IL-10
IL-23
TGF-β
IGF1
VEGF
IL-17RA
IL-17RC
ICAM1
VCAM1
ELAM
COX-2
MMP2
MMP9
NOX2
Relative RNA
ND
HSD
*

Supplementary Figure 2

Effect of HSD on mRNA levels of proinflammatory mediators in cerebral microvessels

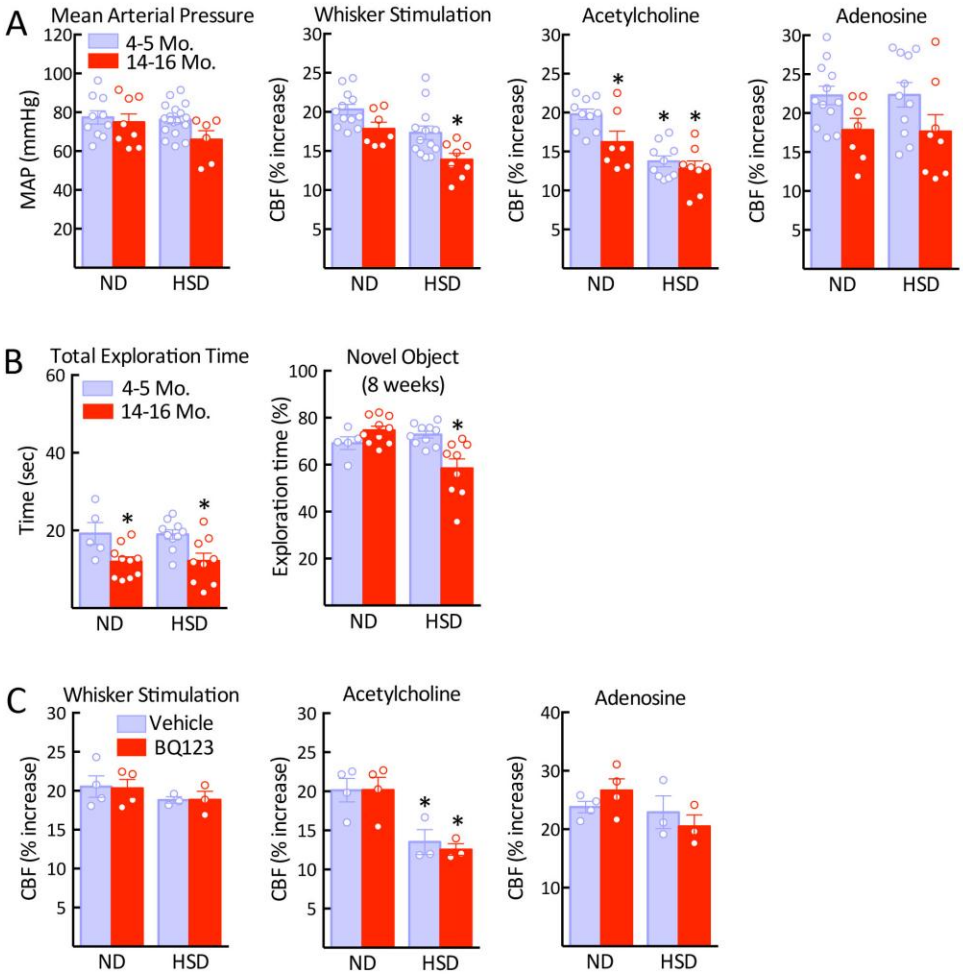
HSD does not induce upregulation of cytokines, adhesion molecules and other proinflammatory molecules in isolated pial microvascular preparations of mice fed a HSD for 12 weeks. IL-6: * $p<0.0411$ vs ND, ND/HSD n=3/5; VCAM1: * $p=0.0102$ vs ND, ND/HSD n=5 mice/group (unpaired t-test, two-tailed). Data are expressed as mean±SEM.


Brain Endothelial cells
A
ICAM1
VCAM1
P-Selectin
ELAM
CXCL1
CXCL2
CXCL5
Rantes
MCP1
TGF-B
Relative RNA
ND
HSD
Veh
IL17
B
BBB
Brain FITC-Dextran (RFU/g x10³)
ND
HSD
HIPP
CTX

Supplementary Figure 3

Effect of HSD or systemic administration of IL-17 on mRNA levels of proinflammatory mediators in brain endothelial cells and on the BBB

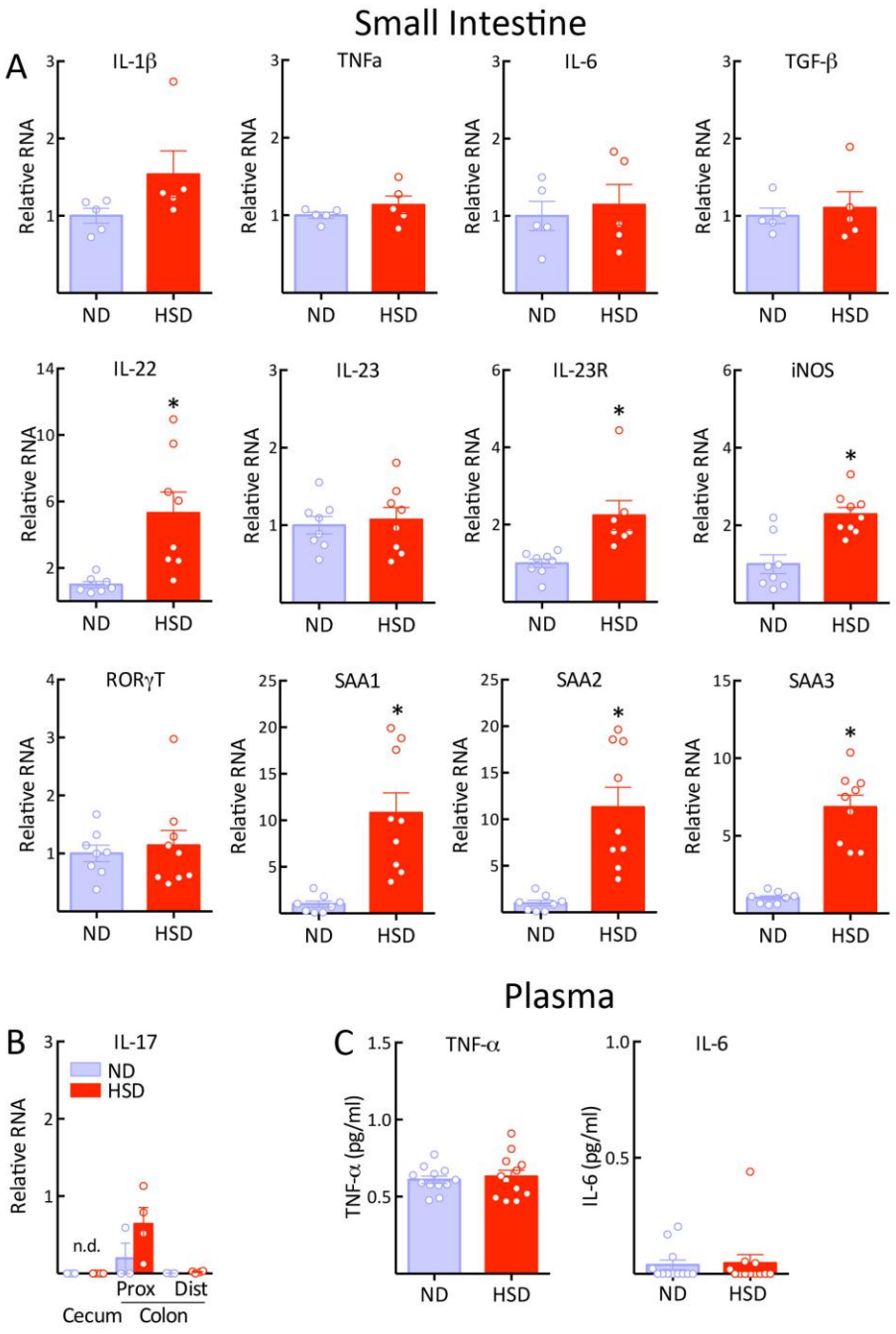
A: HSD or systemic administration of IL-17 do not upregulate cytokines, adhesion molecules and other proinflammatory molecules in endothelial cells FACS-sorted from the brain. ND/HSD n=10; Veh/IL-17 n=5/4 mice/group (One-way ANOVA and Tukey's test). **B:** HSD does not increase BBB permeability to FITC-dextran (3kDa) both in cortex and hippocampus. p=0.1143 and p=0.9624 vs ND; n=5 mice/group (unpaired t-test, two-tailed). Data are expressed as mean±SEM.


A
Mean Arterial Pressure
4-5 Mo.
14-16 Mo.
MAP (mmHg)
ND
HSD
Whisker Stimulation
CBF (% increase)
Acetylcholine
Adenosine
B
Total Exploration Time
Time (sec)
Novel Object
(8 weeks)
Exploration time (%)
C
Whisker Stimulation
Vehicle
BQ123
Acetylcholine
Adenosine

Supplementary Figure 4

Effect of aging or ET_A receptor antagonism on cerebrovascular and cognitive dysfunction induced by HSD

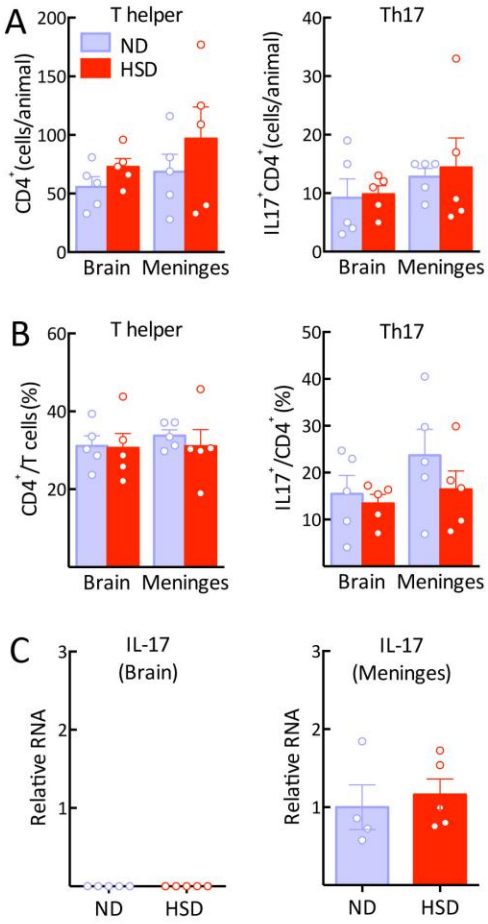
A: HSD (8 weeks) does not affect MAP in aged mice but, at variance with young mice, significantly attenuates the CBF response induced by whisker stimulation; Diet: * p=0.0010, Age: * p=0.0003; ND young/old n=13-8, HSD young/old n=12/8 mice/group (Two-way ANOVA and Tukey's test). HSD does not further decrease the CBF response to ACh in aged mice; Diet: * p<0.0001, Age: * p=0.0195 ND young/old n=10-7, HSD young/old n=10/8 mice/group (Two-way ANOVA and Tukey's test). **B**: Worsening of the performance at the novel object recognition task occurs earlier (8 weeks of HSD), in aged mice than in young mice (12 weeks for HSD)(see Fig. 2A). Total Exploration Time, Diet: * p<0.0001, Age: p=0.1935; NOR, Diet: * p=0.048, Age: * p=0.0096; ND young/old n=8-10, HSD young/old n=10/9 mice/group (Two-way ANOVA and Tukey's test). **C**: Neocortical superfusion of the ET_A receptor antagonist BQ123 fails to reverse the cerebrovascular effects of HSD. ACh: Diet * p<0.0008, Treatment p=0.7523; ND Veh/BQ123 n=4/4, HSD Veh/BQ123 n=3/3 mice/group (Two-way ANOVA and Tukey's test). Data are expressed as mean±SEM.


Small Intestine
A
IL-1β
TNFa
IL-6
TGF-β
IL-22
IL-23
IL-23R
iNOS
RORγT
SAA1
SAA2
SAA3
Relative RNA
ND
HSD
*
B
IL-17
n.d.
Prox
Dist
Cecum
Colon
Plasma
C
TNF-α
IL-6
TNF-α (pg/ml)
IL-6 (pg/ml)

Supplementary Figure 5

HSD increases mRNA levels of factors required for Th17 polarization

A: HSD induces upregulation of IL-22, IL-23R, iNOS, SAA1-3 in distal small intestine of mice fed a HSD. IL-22: * p=0.0073 vs ND; ND/HSD n=7/8; IL-23R: * p=0.0057 vs ND; ND/HSD n=8/7; iNOS: * p=0.0005 vs ND; ND/HSD n=8/9; SAA1: * p=0.0007 vs ND; ND/HSD n=8/9; SAA2: * p=0.0005 vs ND; ND/HSD n=8/9; SAA3: * p<0.0001 vs ND; ND/HSD n=8/9 mice/group (unpaired t-test, two-tailed). **B:** HSD does not increase IL-17A mRNA levels in the colon. mRNA levels are normalized to IL-17A mRNA levels in the distal small intestine of mice fed a ND. Diet: p=0.1461, ND/HSD n=3/4 mice/group (Two-way ANOVA and Tukey's test). **C**: Plasma levels of TNF-α and IL-6 are not increased after HSD. TNF-α: p=0.6581 vs ND; IL-6: p=0.8592 vs ND; n=12 mice/group (unpaired t-test, two-tailed). n.d: not detectable. Data are expressed as mean±SEM.

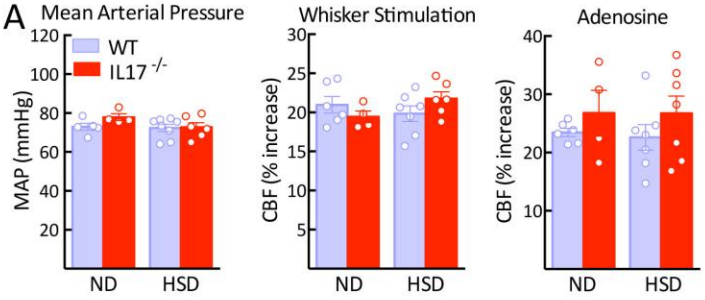

A
T helper
ND
HSD
CD4+(cells/animal)
200
150
100
50
0
Brain
Meninges
Th17
IL17+CD4+(cells/animal)
40
30
20
10
0
Brain
Meninges
B
T helper
CD4+/T cells(%)
60
40
20
Brain
Meninges
Th17
IL17+/CD4+ (%)
50
40
30
20
10
Brain
Meninges
C
IL-17
(Brain)
Relative RNA
3
2
1
ND
HSD
IL-17
(Meninges)
Relative RNA
3
2
1
ND
HSD

Supplementary Figure 6

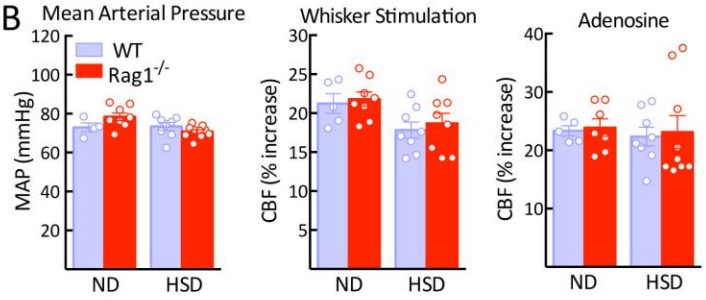
HSD does not increase *Il17a* mRNA levels or TH17 cells in both brain and meninges

A: HSD does not alter number and **B:** frequency of T helper and TH17 cells in brain and meninges. Brain T helper: p=0.1671 vs ND and p=0.9157 vs ND; Meningeal T helper: p=0.3882 vs ND and p=0.5669 vs ND; Brain TH17 cells: p=0.8709 vs ND and p=0.6491 vs ND; Meningeal TH17 cells: p=0.7677 vs ND and p=0.3192 vs ND; n=5/group, 2 mice/samples (unpaired t-test, two-tailed). **C**: IL-17 mRNA levels are not increased in the brain or meninges of mice fed a HSD. Meninges: p=0.6465 vs ND; ND/HSD n=4/5 mice group (unpaired t-test, two-tailed). Data are expressed as mean±SEM.

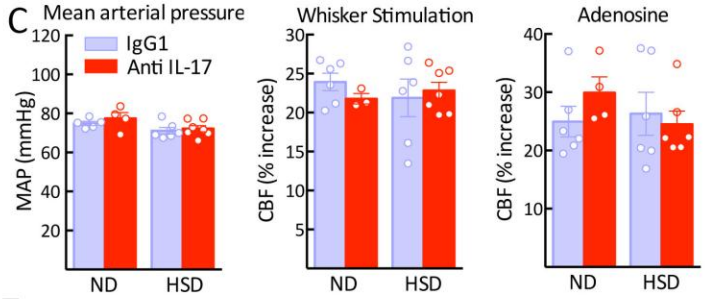
IL17$^{-/-}$



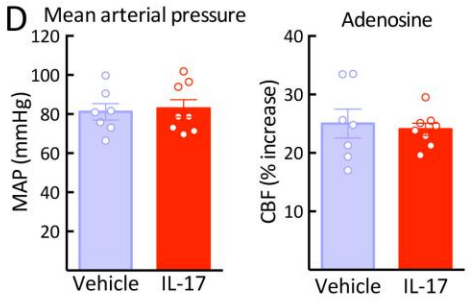
Rag1$^{-/-}$



Anti-IL-17 Antibody



IL-17

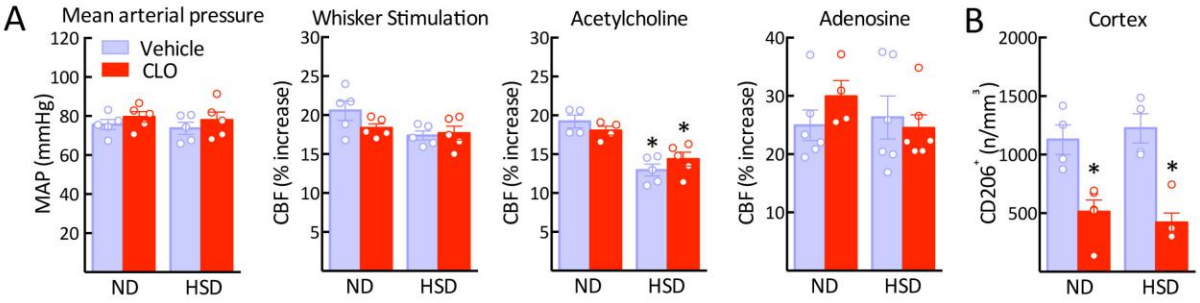


Supplementary Figure 7

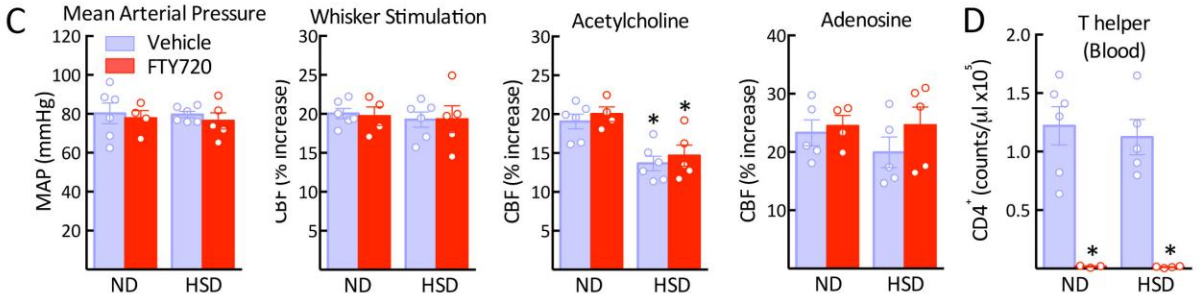
MAP and CBF responses in *Il17a*$^{-/-}$ or *Rag1*$^{-/-}$ mice, in WT mice treated with IL-17-neutralizing antibodies and in WT mice treated with IL-17

A-C: HSD does not alter MAP or CBF responses to whisker stimulation and adenosine in IL17$^{-/-}$ and Rag1$^{-/-}$ mice, or in WT mice receiving IL17 neutralizing antibodies. IL17$^{-/-}$, MAP, Diet: p=0.1864, Genotype: p=0.2153; ND WT/IL17-/- n=5/4, HSD WT/IL17$^{-/-}$ n=8/6; Whisker, Diet: p=0.5651, Genotype: p=0.8474, WT/IL17$^{-/-}$ n=6/4, HSD WT/IL17$^{-/-}$ n=7/6; Adenosine, Diet: p=0.8710, Genotype: p=0.1652; WT/IL17$^{-/-}$ n=6/4, HSD WT/IL17$^{-/-}$ n=7/7 mice/group. Rag1$^{-/-}$, MAP, Diet: p=0.0619, Genotype: p=0.4521; ND WT/Rag1$^{-/-}$ n=4/8, HSD WT/Rag1$^{-/-}$ n=8/10; Whisker, Diet: * p=0.0094, Genotype: p=0.5461, WT/Rag1$^{-/-}$ n=5/8, HSD WT/Rag1$^{-/-}$ n=8/8; Adenosine, Diet: p=0.6543, Genotype: p=0.7647; WT/Rag1$^{-/-}$ n=5/7, HSD WT/Rag1$^{-/-}$ n=8/9 mice/group (Two-way ANOVA and Tukey's test). **D**: Administration of IL-17 in WT mice does not affect MAP or the increase in CBF produced by adenosine; MAP: p=0.7772 vs Veh; Veh/IL-17 n=7-8/mice; Adenosine: p=0.7102 vs Veh; Veh/IL-17 n=7-8/mice group (unpaired t-test, two-tailed). Data are expressed as mean±SEM.

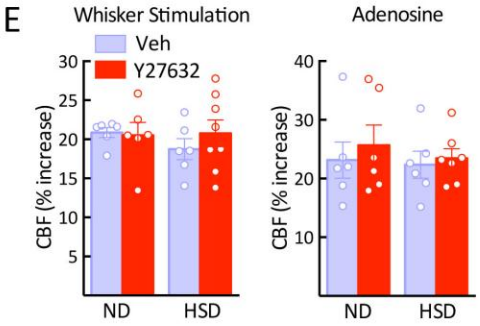
Clodronate



FTY720



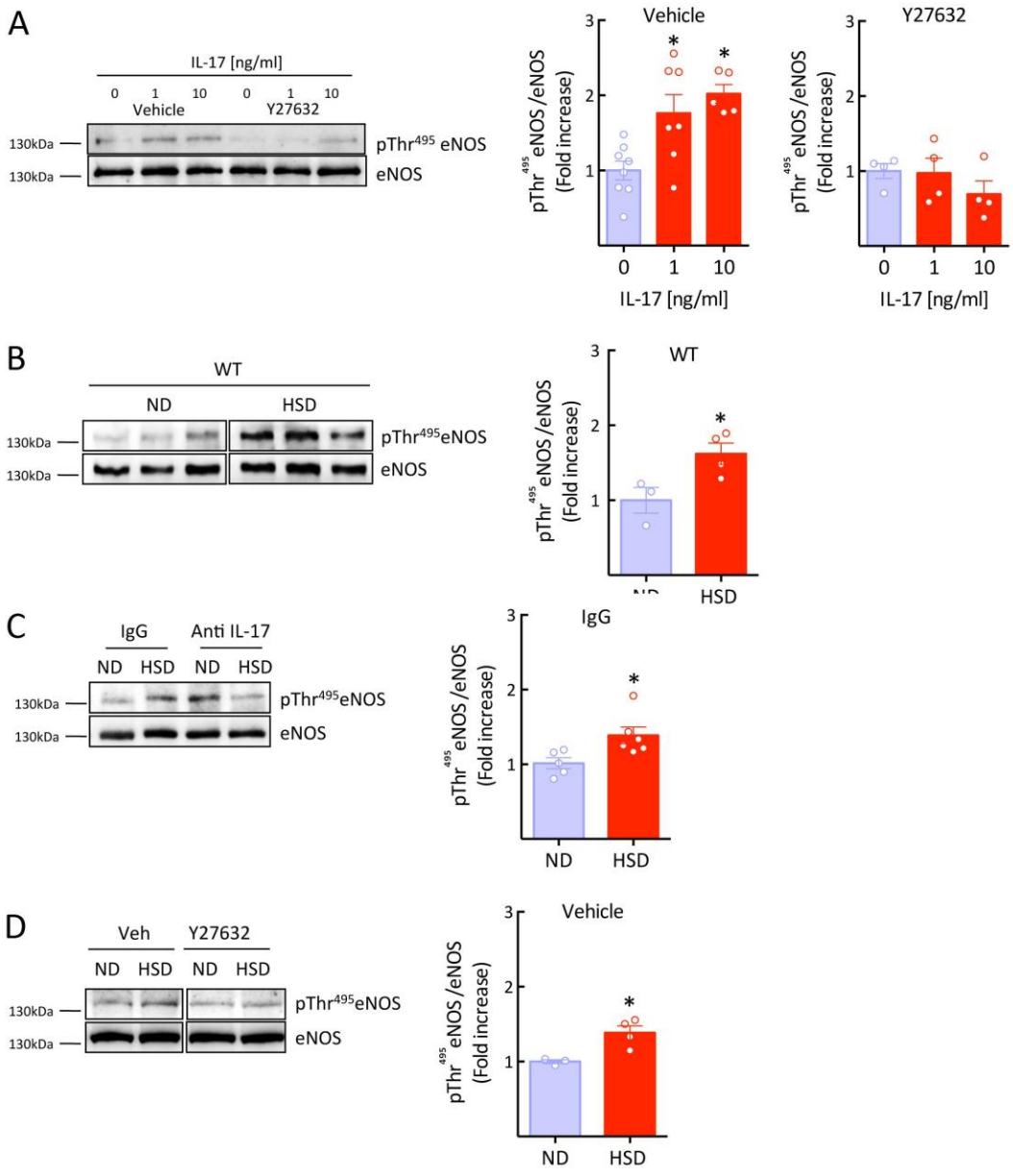
Y27632



Supplementary Figure 8

Effect of depletion of brain perivascular macrophages and of FTY720 or Y27632 on CBF responses in mice fed HSD

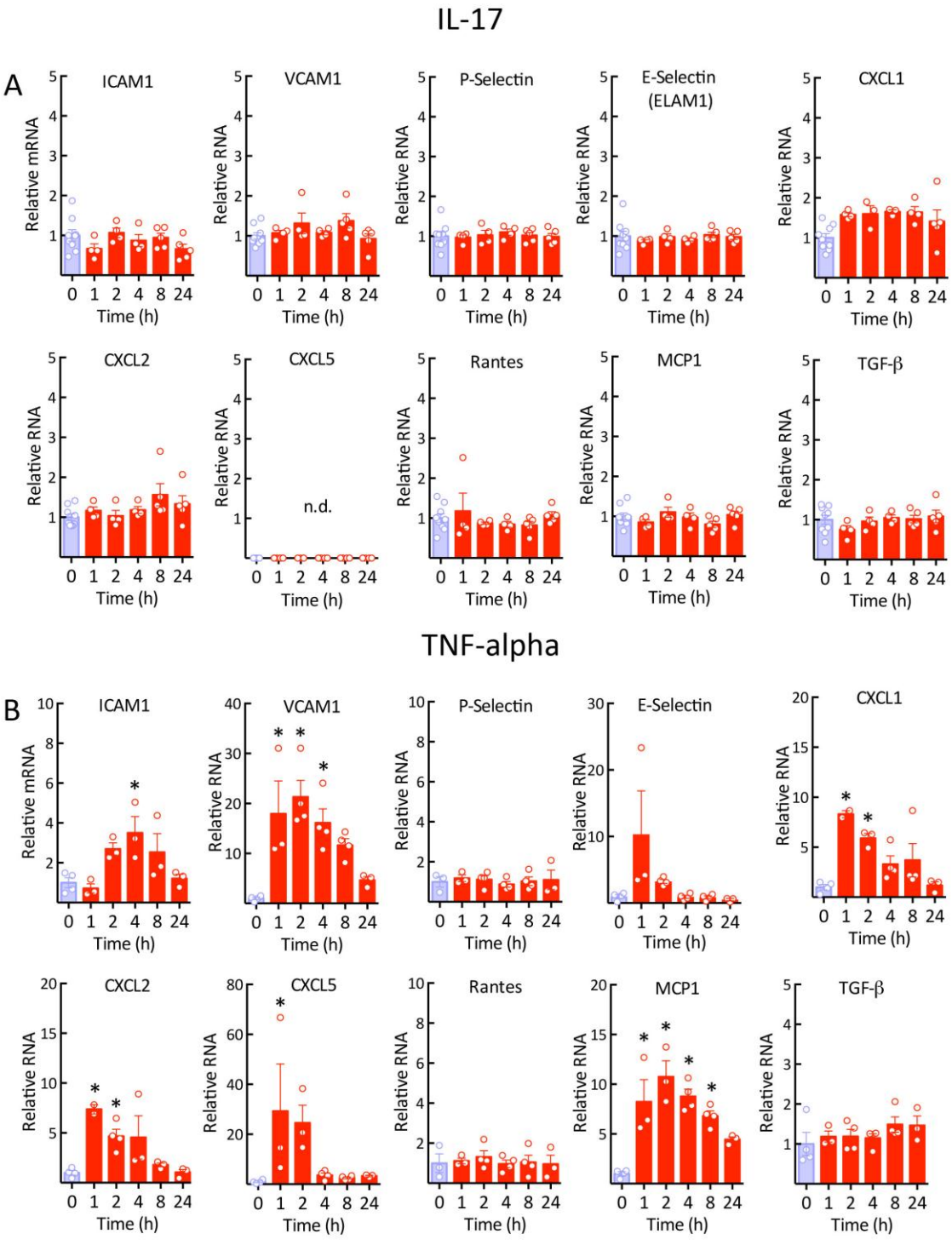
A: PVM depletion by clodronate does not ameliorate the endothelial dysfunction in mice fed a HSD diet; Diet: * $p<0.0001$, Treatment: $p=0.8889$; ND Veh/ND CLO n=4, HSD Veh/HSD CLO n=5 mice/group (Two-way ANOVA and Tukey's test). **B**: Clodronate (i.c.v.) depletes brain PVM in the somatosensory cortex of both ND and HSD-fed mice; Diet: $p=0.9780$, Treatment: * $p<0.0001$; ND Veh/ND CLO n=4, HSD Veh/HSD CLO n=5 mice/group (Two-way ANOVA and Tukey's test). **C**: FTY720 has no effect on the endothelial dysfunction induced by HSD; Diet: * $p=0.0001$, Treatment: $p=0.3789$; ND Veh/ND FTY720 n=6/4, HSD Veh/HSD FTY720 n=6/5 mice/group (Two-way ANOVA and Tukey's test). **D**: FTY720 administration reduces blood T-helper lymphocytes in both ND and HSD-fed mice. Diet: $p=0.7357$, Treatment: $p<0.0001$; ND Veh/ND FTY720 n=6/3, HSD Veh/HSD FTY720 n=5/4 mice/group (Two-way ANOVA and Tukey's test). **E**: The CBF responses to whisker stimulation or adenosine are not altered by Y27632; $p>0.05$ vs ND; Whisker, Diet: $p=0.5397$, Treatment: $p=0.5804$; ND Veh/Y27632 n=6/6, HSD Veh/Y27632 n=6/8; Adenosine, Diet: $p=0.5712$, Treatment: $p=0.4964$; ND Veh/Y27632 n=6/6, HSD Veh/Y27632 n=6/7 mice/group (Two-way ANOVA and Tukey's test). Data are expressed as mean±SEM.


A
IL-17 [ng/ml]
0 1 10 0 1 10
Vehicle
Y27632
130kDa
130kDa
pThr495 eNOS
eNOS
Vehicle
Y27632
pThr495 eNOS /eNOS (Fold increase)
IL-17 [ng/ml]
B
WT
ND
HSD
pThr495eNOS
eNOS
C
IgG
Anti IL-17
D
Veh
Y27632

Supplementary Figure 9

Effect of IL-17 administration on eNOS phosphorylation in human cerebral endothelial cells (HBEC.5i)

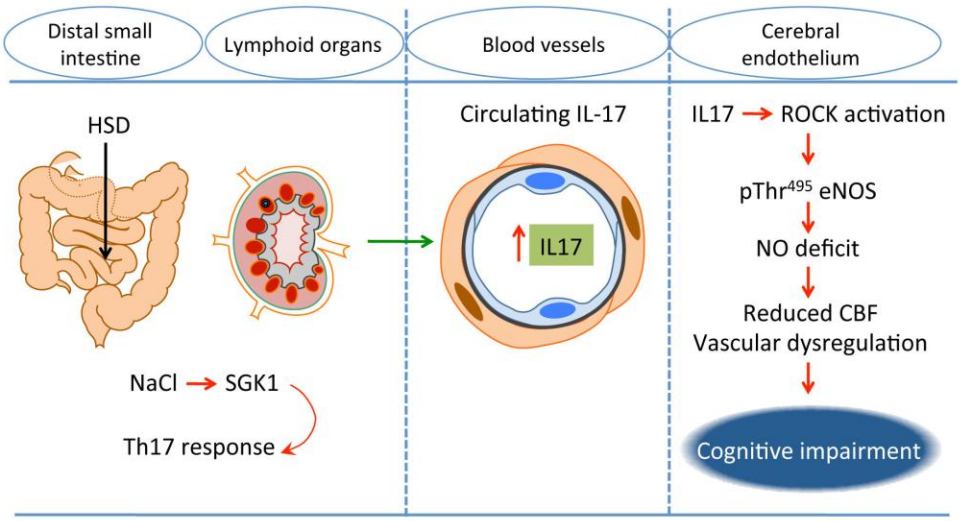
A: IL-17 (1-10ng/mL) increases phosphorylation of eNOS on Thr495, an effect abrogated by the administration of the ROCK inhibitor Y27632 (5µM). * p=0.0026; Vehicle: IL-17 0ng/ml n=8; IL-17 1ng/ml n=7, IL-17 10ng/ml n=5; Y27632: IL-17 0-10ng/ml n=4 independent experiments/group (One-way ANOVA and Tukey's test). **B-D**: HSD increases the inhibitory phosphorylation of eNOS at Thr495 in isolated pial microvascular preparations isolated from WT mice and mice injected with an IgG control antibody or vehicle. WT: * p=0.0380 vs ND; ND/HSD n=3/6; IgG: * p=0.0272 vs ND; ND/HSD n=5/6; Veh: * p=0.0177 vs ND; ND/HSD n=3/4 mice/group (unpaired t-test, two-tailed). Data are expressed as mean±SEM. Immunoblots in A, B, C and D are cropped. Full gel pictures for immunoblots are shown in Supplementary Fig 14.


IL-17
A
ICAM1
VCAM1
P-Selectin
E-Selectin (ELAM1)
CXCL1
CXCL2
CXCL5
n.d.
Rantes
MCP1
TGF-β
Relative mRNA
Relative RNA
Time (h)
0 1 2 4 8 24
TNF-alpha
B
ICAM1
VCAM1
P-Selectin
E-Selectin
CXCL1
CXCL2
CXCL5
Rantes
MCP1
TGF-β
Relative mRNA
Relative RNA
Time (h)
0 1 2 4 8 24

Supplementary Figure 10

Effect of IL-17 and TNF-α administration on mRNA levels of proinflammatory mediators in brain endothelial cells (bEnd.3)

A: IL-17 (10ng/mL) does not induce upregulation of cytokines, adhesion molecules and other pro-inflammatory molecules in cultures of bEnd.3 cells. $p>0.05$ vs 0; n=4 independent experiments/group (One-way ANOVA and Tukey's test). **B**: TNF-α (10ng/mL) induces upregulation of cytokines and adhesion molecules in cultures of bEnd.3 cells. ICAM1: * $p=0.0124$ vs 0; VCAM1: * $p<0.0001$ vs 0; CXCL1: * $p=0.0024$; CXCL2: * $p=0.0035$ vs 0; CXCL5: * $p=0.0372$ vs 0; MCP1: * $p<0.0001$ vs 0; n=3 independent experiments/group (One-way ANOVA and Tukey's test). Data are expressed as mean±SEM.

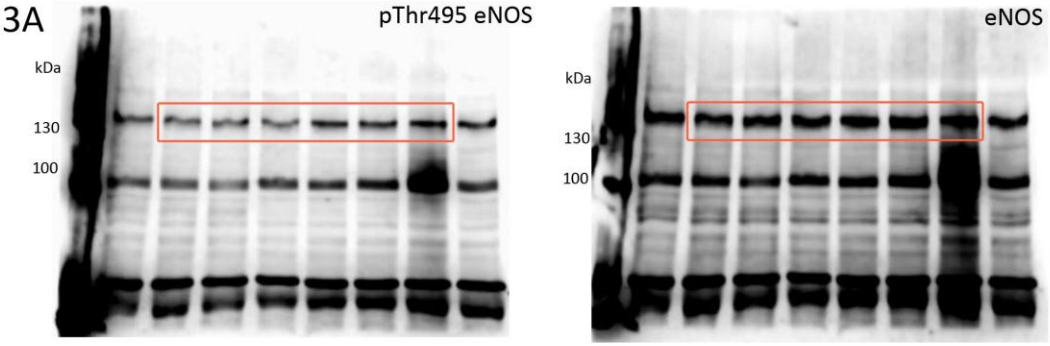

Distal small intestine
Lymphoid organs
Blood vessels
Cerebral endothelium
HSD
NaCl → SGK1
Th17 response
Circulating IL-17
IL17
IL17 → ROCK activation
pThr495 eNOS
NO deficit
Reduced CBF
Vascular dysregulation
Cognitive impairment

Supplementary Figure 11

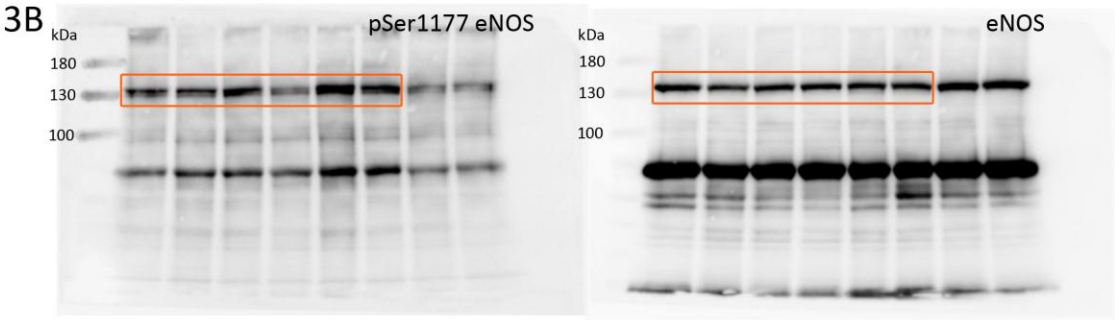
Cartoon illustrating the mechanisms of neurovascular and cognitive dysfunction induced by HSD

HSD induces a TH17 response in the distal small intestine by presumably activating serum glucocorticoid-regulated kinase 1 (SGK1). TH17 cells lead to an increase in circulating IL17, which, in turn, acts on cerebral endothelial cells to induce ROCK activation, inhibitory eNOS phosphorylation, and reduction in eNOS catalytic activity. The resulting reduction in endothelial NO leads to cerebral hypoperfusion, vascular dysregulation and cognitive impairment.

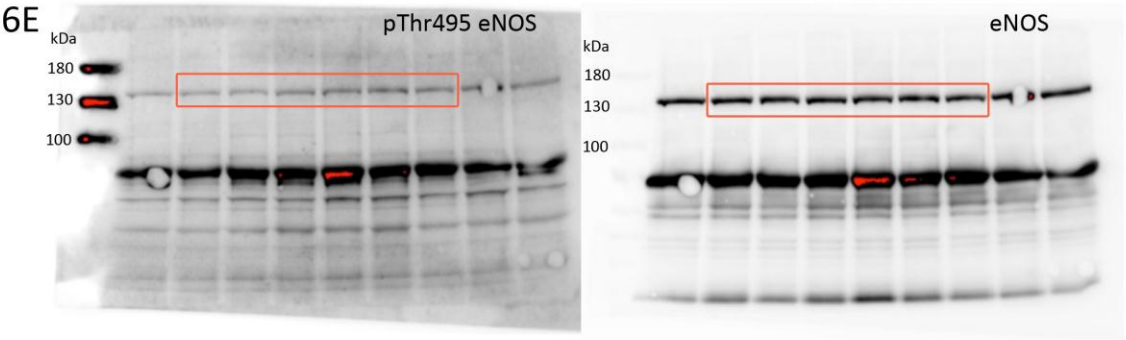
3A


pThr495 eNOS
kDa
130
100
eNOS
kDa
130
100

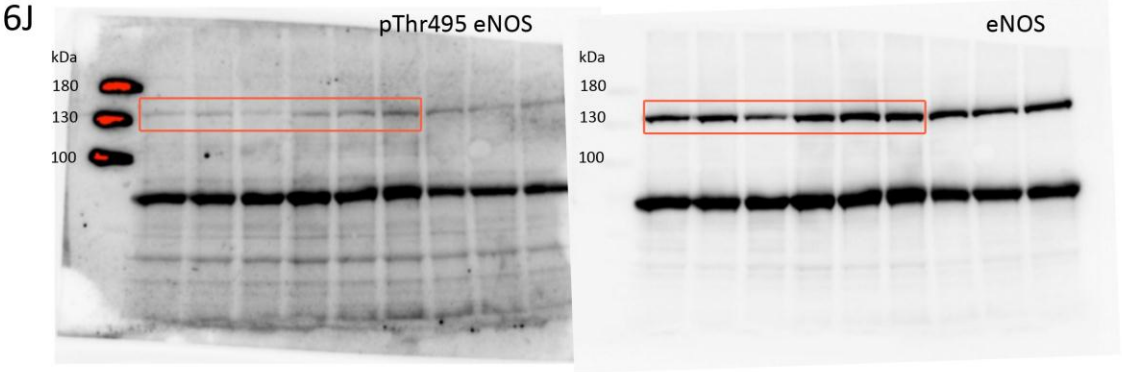
3B


pSer1177 eNOS
kDa
180
130
100
eNOS
kDa
180
130
100

6E


pThr495 eNOS
kDa
180
130
100
eNOS
kDa
180
130
100

6J

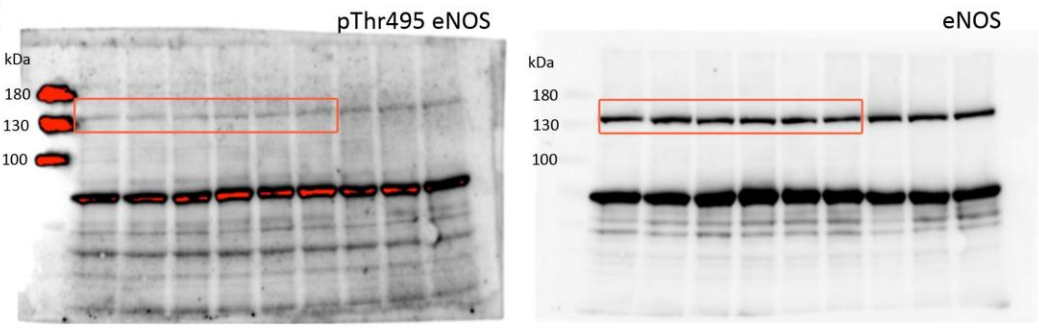

pThr495 eNOS
kDa
180
130
100
eNOS
kDa
180
130
100

Supplementary Figure 12

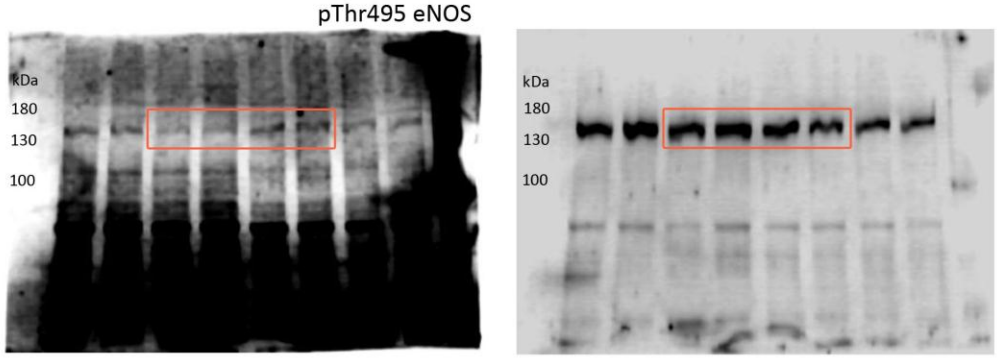
Full immunoblots image of panels in Figs. 3 and 6

Membranes were incubated with anti-pThr495 eNOS antibody (1:500) or anti-pSer1177 eNOS antibody (1:1000) overnight. After incubation with secondary antibody, protein bands were visualized with Super Signal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) on a Bio Rad ChemiDoc MP Imaging System. After washes with TBS/0.1% Tween-20 (TBST), membranes were incubated with anti-eNOS antibody (1:1000) overnight. eNOS bands were visualized with Clarity Western ECL Substrate (BioRad). Samples from microvessels preparations (≈30µg of protein/lane).

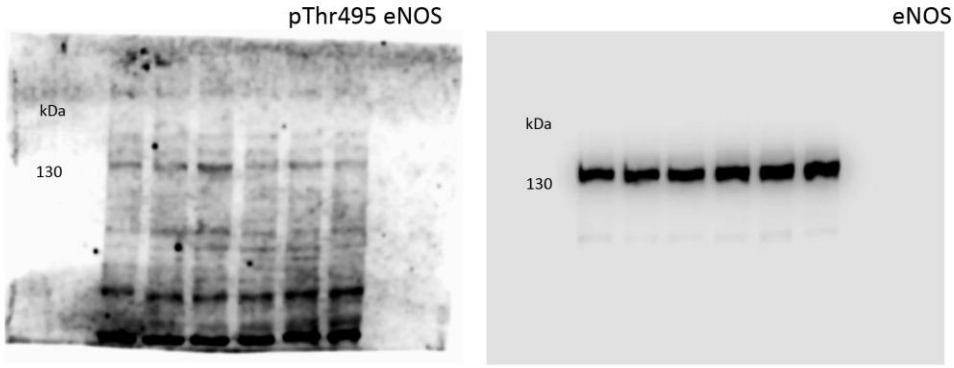
7C


pThr495 eNOS
eNOS
kDa
180
130
100

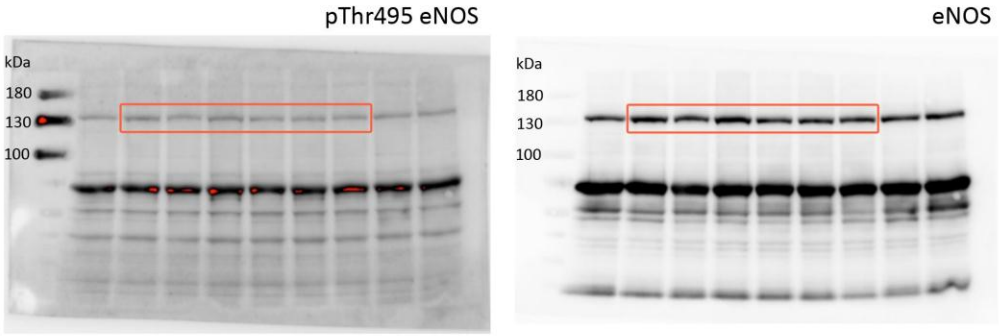
7G


pThr495 eNOS
kDa
180
130
100

8A


pThr495 eNOS
eNOS
kDa
130

8H

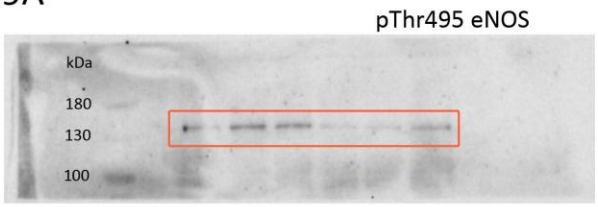

pThr495 eNOS
eNOS
kDa
180
130
100

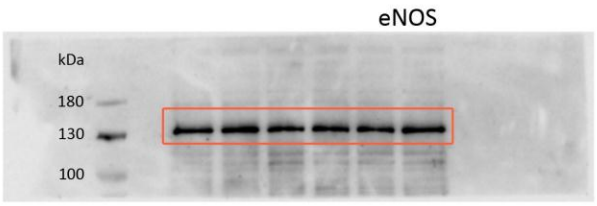
Supplementary Figure 13

Full immunoblots image of panels in Figs. 7 and 8

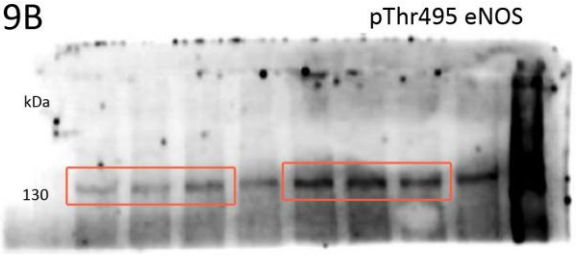
Membranes were incubated with anti-pThr495 eNOS antibody (1:500) overnight. After incubation with secondary antibody, protein bands were visualized with Super Signal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) on a Bio Rad ChemiDoc MP Imaging System. After washes with TBS/0.1% Tween-20 (TBST), membranes were incubated with anti-eNOS antibody (1:1000) overnight. eNOS bands were visualized with Clarity Western ECL Substrate (BioRad). Samples from microvessels preparations and cultures of mouse brain endothelial cells (≈30µg of protein/lane).

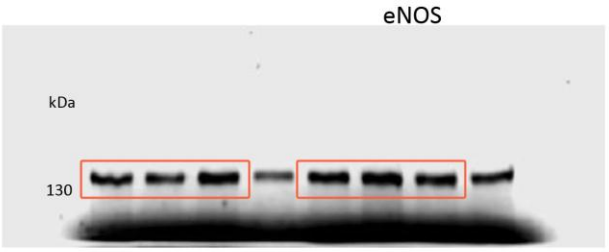
9A


pThr495 eNOS
kDa
180
130
100

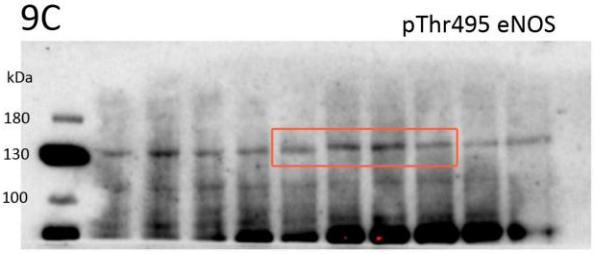

eNOS
kDa
180
130
100

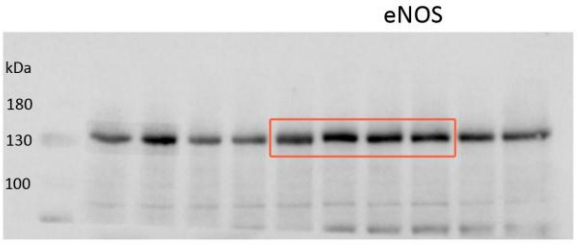
9B


pThr495 eNOS
kDa
130

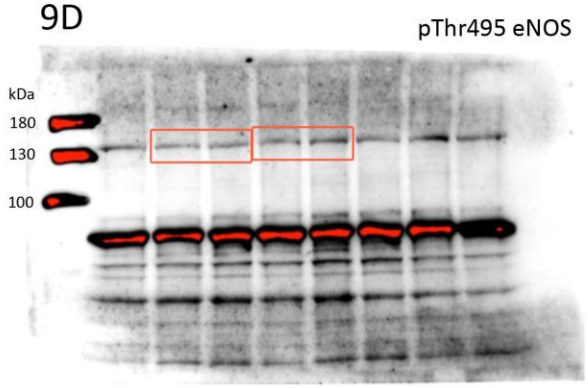

eNOS
kDa
130

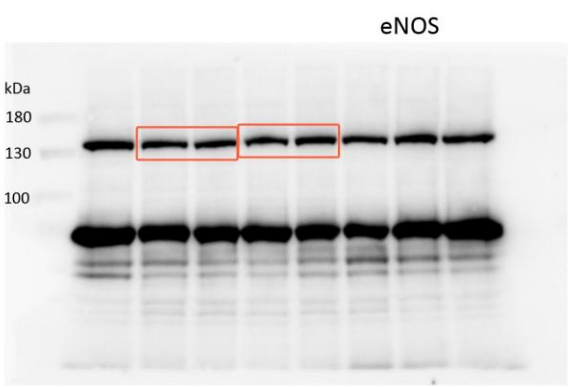
9C


pThr495 eNOS
kDa
180
130
100


eNOS
kDa
180
130
100

9D

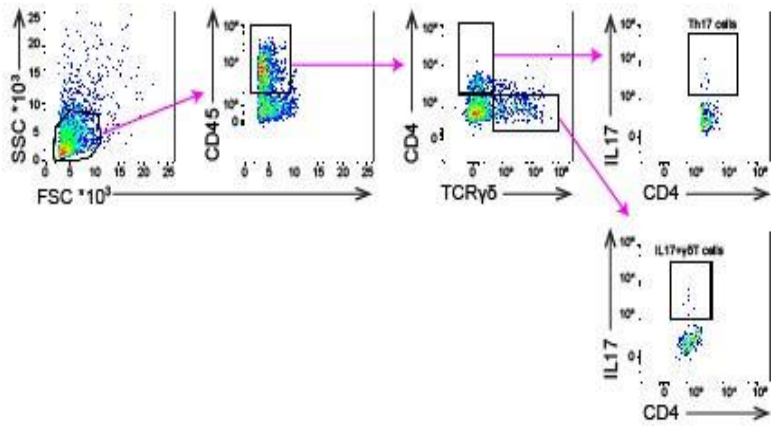

pThr495 eNOS
kDa
180
130
100


eNOS
kDa
180
130
100

Supplementary Figure 14

Full immunoblots image of panels in Supplementary Figure 9

Membranes were incubated with anti-pThr495 eNOS antibody (1:500) overnight. After incubation with secondary antibody, protein bands were visualized with Super Signal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) on a Bio Rad ChemiDoc MP Imaging System. After washes with TBS/0.1% Tween-20 (TBST), membranes were incubated with anti-eNOS antibody (1:1000) overnight. eNOS bands were visualized with Clarity Western ECL Substrate (BioRad). Samples from microvessels preparations and cultures of human brain endothelial cells (≈30µg of protein/lane).



Supplementary Figure 15

Flow cytometry gating strategy for IL-17^{+} cells.

Cells were discriminated by FSC-SSC gating and then gated for CD45+CD4+TCRγδ-IL17+ (Th17 cells) or CD45+CD4-TCRγδ+IL17+ (IL17+γδ T cells).

Plasma	ND	HSD 8wks	HSD 24wks
Na^+ (mEq/L)	150.2±0.6	149.6±1.0	150.4±1.0
K^+ (mEq/L)	7.7±0.2	7.1±0.4	7.7±0.4
Cl^- (mEq/L)	113.7±0.4	115.6±1.7	112.0±0.9
BUN (mg/dl)	28.5±1.2	25.8±0.6	26.8±0.7
Creatinine (md/dl)	0.14±0.01	0.13±0.01	0.12±0.005
Osmolality (mOsm/Kg)	338.0±0.9	335.8±2.1	336.0±1.9
Anion Gap (mEq/L)	33.3±0.4	30.6±0.9	35.6 ±1.4

Urine	ND	HSD 8wks
Creatinine (mg/dl)	60.9±6.5	21.7±3.6*
Total Proteins (mg/dl)	753±211	225±59
Na^+ (mEq/L)	63.5±11.6	205.3±14.6*
K^+ (mEq/L)	244.1±81.2	74.4±14.1
Cl^- (mEq/L)	121.0±22.9	230.5±20.3*

Supplementary Table 1. Plasma and urine chemistry in mice on a HSD. ND: normal diet; HSD high salt diet; BUN: Blood urea nitrogen. Creatinine: * p=0.0009 vs ND; Na^+: * p=0.0003 vs ND; Cl^-: * p=0.0117; n=4/group, unpaired t-test, two-tailed.

Gene	*Forward (5’-3’)*	*Reverse (5’-3’)*
IL-17A	CAGACTACCTCAACCGTTCCA	AGAATTCATGTGGTGGTCCAG
IL-17RA	CATGAGTGGATCTGTTGCCCT	GCCTCTTCCTGCTTCTCAAGT
IL-17RC	CAACCTCTGTGTCCAGGTGAG	GGCCGGTTTTCATCTCCACTA
IL-21	GGCAATGAAAGCCTGTGGAA	GGCAATGAAAGCCTGTGGAA
IL-22	GCTCAGCTCCTGTCACATCA	TTCCCCAATCGCCTTGATCTC
IL-23	CTCAAGGACAACAGCCAGTTC	CTCCCCTTTGAAGATGTCAGA
IL-23R	CTTCCCAGACAGTTTCCCAGG	CCAAGAAGACCATTCCCGACA
ROR-γT	GGAGGACAGGGAGCCAAGTT	CCGTAGTGGATCCCAGATGACT
SAA1	CATTTGTTCACGAGGCTTTCC	GTTTTTCCAGTTAGCTTCCTTCATGT
SAA3	TGTGTATCCCACAAGGTTTCAGA	TTATTACCCTCTCCTCCTCAAGCA
SAA2	CGCAGCACGAGCAGGAT	CCAGGATCAAGATGCAAAGAATG
TNF-α	TTGGAGTCATTGCTCTGTGAA	GGGTCAGAGTAAAGGGGTCAG
IL-1β	CGGGAGGAGACGACTCTAAAT	AGGTCGGTCTCACTACCTGTG
IL-6	ATGGATGCTACCAAACTGGAT	TGAAGGACTCTGGCTTTGTCT
GP91	AATCTCAGGCCAATCACTTTG	AACGCCTATTGTGGTGTTAGG
MCP-1	AGGTGTCCCAAAGAAGCTGTA	ATGTCTGGACCCATTCCTTCT
RANTES	ATATGGCTCGGACACCACTC	GTGACAAACACGACTGCAAGA
TGF-β1	GAGAGCCCTGGATACCAACTA	GACAGAAGTTGGCATGGTAGC
iNOS	TCACCACAAGGCCACATCGGATT	AGCTCCTCCAGAGGGGTAGGCT
COX2	TGGTGCCTGGTCTGATGATG	GTGGTAACCGCTCAGGTGTTG
IL-10	TCCCCTGTGAAAATAAGAGCA	TCATGGCCTTGTAGACACCTT
MMP-2	GAATGCCATCCCTGATAACCT	CAGCCAGTCTGATTTGATGCT
MMP-9	ATTCGCGTGGATAAGGAGTTC	CCTTGTTCACCTCATTTTGGA
ICAM1	GCCTTGGTAGAGGTGACTGAG	GACCGGAGCTGAAAAGTTGTA
VCAM	TGCCGAGCTAAATTACACATTG	CCTTGTGGAGGGATGTACAGA
ELAM	CTCACTCCTGACATCGTCCTC	ACGTTGTAAGAAGGCACATGG
P-Selectin	GACACTGACAATCCAGGAAGC	GGCATTTTCCATCATCTTTCTT
VEGF	CCAGGAGGACCTTGTGTGAT	GAGGAAGGGTAAGCCACTCAC
IGF-1	CCAGTCTCCTCAGATCACAGC	CTGGTGGATGCTCTTCAGTTC
CXCL1	ACCGAAGTCATAGCCACACTC	TTCTCCGTTACTTGGGGACAC
CXCL2	TGAACAAAGGCAAGGCTAACTG	GAGGCACATCAGGTACGATCC
CXCL5	CCCTACGGTGGAAGTCATAGC	AGCTTTCTTTTTGTCACTGCCC
HPRT	TGACACTGGTAAAACAATGCAA	CTGGCCTGTATCCAACACTTC

Supplementary Table 2. List of primers used for qRT-PCR