

Supplementary data

Activation of the hypoxia response protects mice from amyloid- β accumulation

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Short title: Hypoxia and amyloid- β

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Supplemental Table 1. Pearson correlation coefficients of anthropometric and metabolic parameters and mRNA levels of all mice. WAT, white adipose tissue weight; liver, liver weight; b, blood; C, cortical; A β , amyloid- β ; trigly, triglycerides; glyc, glycogen; chol, cholesterol; WAT macroph, WAT macrophage aggregates; H, hippocampal. Significant associations ($P < 0.05$) are indicated by bold.

4 mo correlation		WAT	Liver	b-Glucose	b-Lactate	s-Insulin	b-Hemoglobin	Body weight	HOMA-IR	C A β	H A β	Liver Trigly	Liver Glyc	s-Trigly	s-Chol	s-HDL	C Xbp1	C Hspa5	C Atf6	C Eif2ak3	H Xbp1	H Hspa5	H Atf6	H Eif2ak3	C Trem2	H Trem2	C cFOS	H cFOS	C BTG2	H BTG2	C Arc	H Arc	C Dusp1	H Dusp1					
WAT																																							
Liver		0.56																																					
b-Glucose		0.41	0.30																																				
b-Lactate		-0.09	-0.39	-0.07																																			
s-Insulin		0.53	0.43		0.10																																		
b-Hemoglobin		-0.18	-0.22	-0.15	0.21	-0.04																																	
Body weight		0.37	0.56	0.05	-0.44	0.22	-0.27																																
HOMA-IR		0.62	0.50	0.46	0.08	0.90	-0.10	0.20																															
C A β		-0.02	0.04	-0.46	-0.35	0.40	0.04	0.42	0.12																														
H A β		0.07	0.06	-0.40	-0.35	0.45	0.19	0.32	0.17	0.63																													
Liver Trigly		0.52	0.20	0.36	-0.14	0.20	-0.16	0.00	0.34	-0.25	-0.07																												
Liver Glyc		0.42	0.46	0.35	-0.07	0.33	-0.27	0.26	0.45	0.13	0.17	0.10																											
s-Trigly		0.08	0.07	-0.07	-0.05	0.33	-0.03	0.12	0.23	0.48	0.46	-0.05	0.23																										
s-Chol		0.67	0.58	0.62	-0.18	0.23	-0.12	0.26	0.46	-0.48	-0.36	0.41	0.51	0.04																									
s-HDL		0.53	0.42	0.53	0.00	0.21	-0.19	0.21	0.40	-0.24	-0.25	0.12	0.54	0.19	0.77																								
C Xbp1		0.32	0.52	0.10	0.00	0.20	-0.24	0.15	0.32	-0.05	0.11	-0.11	0.71	0.07	0.20	0.05																							
C Hspa5		0.31	0.56	0.05	-0.07	0.14	-0.63	0.41	0.18	0.13	0.04	-0.10	0.55	0.08	0.21	0.09	0.81																						
C Atf6		0.11	0.41	0.23	0.08	0.11	-0.21	-0.04	0.31	0.03	-0.06	-0.01	0.58	0.16	0.26	0.05	0.75	0.73																					
C Eif2ak3		0.01	0.31	0.08	-0.10	-0.03	-0.16	-0.04	0.10	0.08	-0.07	-0.15	0.57	0.06	0.03	0.09	0.71	0.68	0.77																				
H Xbp1		0.14	0.36	0.08	-0.06	0.10	-0.28	0.13	0.20	0.04	-0.10	-0.37	0.57	0.17	-0.01	-0.12	0.79	0.73	0.67	0.59																			
H Hspa5		0.49	0.54	0.13	0.13	0.32	-0.50	0.33	0.48	0.04	-0.02	-0.17	0.58	-0.01	0.30	0.13	0.76	0.81	0.59	0.48	0.65																		
H Atf6		0.01	0.14	-0.12	-0.59	0.04	-0.02	0.13	0.45	0.00	-0.10	-0.11	0.33	0.18	-0.27	-0.22	0.38	0.46	0.37	0.63	0.55	0.22				0.70													
H Eif2ak3		0.05	0.35	0.01	-0.64	-0.16	-0.24	0.24	-0.21	0.16	0.38	-0.10	0.44	0.27	0.02	-0.03	0.44	0.46	0.32	0.48	0.48	0.12	0.70																
C Trem2		0.14	0.13	-0.10	-0.42	0.06	-0.12	0.38	-0.01	0.46	0.00	-0.10	0.00	-0.16	-0.16	-0.05	0.09	0.34	0.06	0.47	0.14	0.12	0.68	0.27															
H Trem2		0.24	0.15	-0.31	-0.49	0.38	0.02	0.57	0.12	0.76	0.54	-0.07	0.14	0.39	-0.39	-0.19	-0.04	0.12	-0.14	0.00	0.15	-0.02	0.62	0.36	0.53														
C cFOS		-0.16	-0.27	0.00	-0.15	-0.16	0.06	-0.38	-0.14	0.08	-0.34	0.43	-0.32	-0.13	0.00	-0.06	-0.42	-0.17	0.02	0.17	-0.39	-0.27	0.19	-0.17	0.39	-0.09													
H cFOS		-0.20	-0.14	0.07	-0.29	-0.30	-0.24	-0.22	-0.36	-0.17	-0.15	0.52	-0.27	-0.23	-0.12	-0.13	-0.05	0.13	-0.03	0.26	-0.11	-0.10	0.30	0.22	0.34	-0.14	0.61												
C BTG2		-0.26	-0.46	-0.22	0.09	0.05	0.22	-0.40	-0.07	0.15	-0.22	0.38	-0.57	-0.09	-0.19	-0.27	-0.64	-0.37	-0.18	-0.15	-0.50	-0.42	-0.01	-0.33	0.16	-0.05	0.88	0.41											
H BTG2		-0.14	-0.23	0.00	-0.37	-0.10	0.06	-0.20	0.12	0.01	0.51	-0.37	-0.11	-0.34	-0.20	-0.26	-0.11	-0.21	0.18	-0.26	-0.36	0.40	0.19	0.55	0.19	0.58	0.84	0.47											
C Arc		0.05	0.37	0.40	-0.15	-0.08	-0.45	-0.07	0.10	-0.19	-0.19	0.37	0.32	0.20	0.33	0.21	0.10	0.23	0.26	0.11	0.05	0.34	0.00	-0.04	-0.19	-0.26	0.54	0.50	0.22	0.11									
H Arc		-0.28	-0.30	-0.09	-0.31	-0.18	-0.01	-0.25	-0.32	-0.02	-0.21	0.37	-0.34	-0.30	-0.26	-0.27	-0.24	-0.06	-0.24	0.15	-0.14	-0.18	0.41	0.16	0.46	0.03	0.68	0.85	0.58	0.79	0.35								
C Dusp1		-0.16	-0.33	0.03	-0.10	-0.21	0.09	-0.50	-0.19	-0.22	-0.41	0.54	-0.34	-0.30	-0.04	-0.13	-0.26	-0.19	-0.10	0.10	-0.22	-0.30	0.18	-0.05	0.17	-0.21	0.80	0.78	0.67	0.69	0.63	0.84							
H Dusp1		-0.23	0.15	-0.03	-0.07	-0.19	-0.34	-0.08	-0.13	-0.20	-0.73	-0.08	-0.17	-0.40	-0.04	-0.11	-0.11	0.05	0.55	0.48	0.08	0.23	-0.26	-0.61	-0.18	-0.51	0.65	0.11	0.56	-0.16	0.51	0.22	0.40						
10 mo correlation		WAT	Liver	b-Glucose	b-Lactate	s-Insulin	b-Hemoglobin	Body weight	HOMA-IR	C A β	H A β	C Bace1	Liver Trigly	Liver Glyc	s-Trigly	s-Chol	s-HDL	s-LDL	WAT macroph	C Xbp1	C Hspa5	C Atf6	C Eif2ak3	H Xbp1	H Hspa5	H Atf6	H Eif2ak3	C Trem2	H Trem2	C cFOS	H cFOS	C BTG2	H BTG2	C Arc	H Arc	C Dusp1	H Dusp1	C GLUT1	
WAT																																							
Liver		0.68																																					
b-Glucose		0.53	0.49																																				
b-Lactate		0.18	0.07	0.33																																			
s-Insulin		0.61	0.49	0.51	-0.01																																		
b-Hemoglobin		-0.08	0.05	-0.10	-0.18	0.08																																	
Body weight		0.93	0.69	0.50	0.14	0.70	0.01																																
HOMA-IR		0.63	0.55	0.72	0.12	0.95	0.06	0.67																															
C A β		0.12	0.00	-0.07	-0.06	0.0																																	

Supplemental Table 2 RNA sequencing (RNASeq) analysis of the cerebral cortex of the 4-month-old hypoxia and normoxia treated APP/PSEN1 mice (n = 2 + 2). Genes with lowest adjusted *P* values for a difference in expression are indicated. The Log2FoldChange displays the difference in expression level between hypoxia mice and normoxia. The adjusted *P* value displays the significance of the difference in expression levels.

Gene	Log2FoldChange	<i>P</i> value adjusted
<i>Mfrp</i>	8.4104	0.0054
<i>Dusp1</i>	1.0289	0.0110
<i>Shox2</i>	4.8264	0.0112
<i>Btg2</i>	1.1773	0.1052
<i>c-Fos</i>	1.0255	0.1119
<i>Eif2s3y</i>	-1.0067	0.2517
<i>Ciart</i>	1.2509	0.2517
<i>Arc</i>	0.6472	0.2517

Supplemental Table 3 Primers used in the quantitative PCR analyses.

Gene	Forward primer (5' -> 3')	Reverse primer (5' -> 3')
<i>Arc</i>	AAGTGCCGAGCTGAGATGC	CGACCTGTGCAACCCTTTC
<i>Atf6</i>	TCGCCTTTTAGTCCGGTTCTT	GGCTCCATAGGTCTGACTCC
<i>Bace1</i>	GGAACCCATCTCGGCATCC	TCCGATTCCTCGTCGGTCTC
<i>Btg2</i>	ATGAGCCACGGGAAGAGAAC	GCCCTACTGAAAACCTTGAGTC
<i>Dusp1</i>	GTTGTTGGATTGTCGCTCCTT	TTGGGCACGATATGCTCCAG
<i>Eif2ak3</i>	AGTCCCTGCTCGAATCTTCCT	TCCAAGGCAGAACAGATATACC
<i>c-Fos</i>	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
<i>Hif-p4h-2</i>	GCGTCCAGTCTTTATTTAGATA	CTGGGCAACTACAGGATAAAC
<i>Hspa5</i>	ACTTGGGGACCACCTATTCT	ATCGCCAATCAGACGCTCC
<i>Trem2</i>	TGGAACCGTCACCATCACTC	TGGTCATCTAGAGGGTCCTCC
<i>Xbp1</i>	AGCTTTTACGGGAGAAACTCAC	CCTCTGGAACCTCGTCAGGA

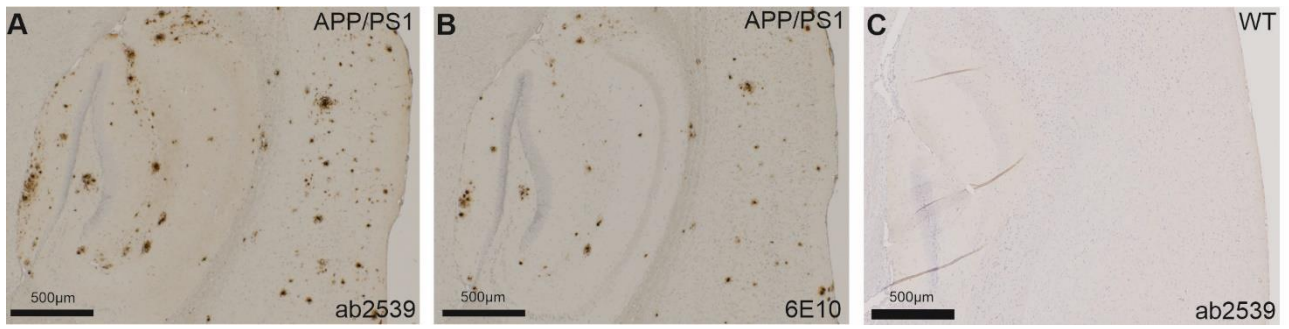


Fig. S1. Validation of the A β antibody. (A) Mouse/human A β binding antibody ab2539 detects amyloid plaques consisting of mixed mouse and human A β correspondingly to (B) human A β specific 6E10 in the cortex and hippocampus of the APP/PSEN1 mice but not in wild type (WT) (C).

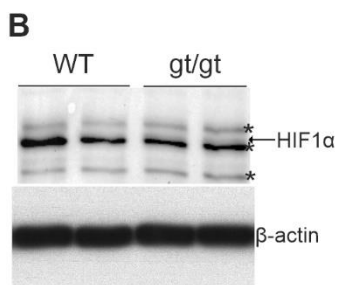
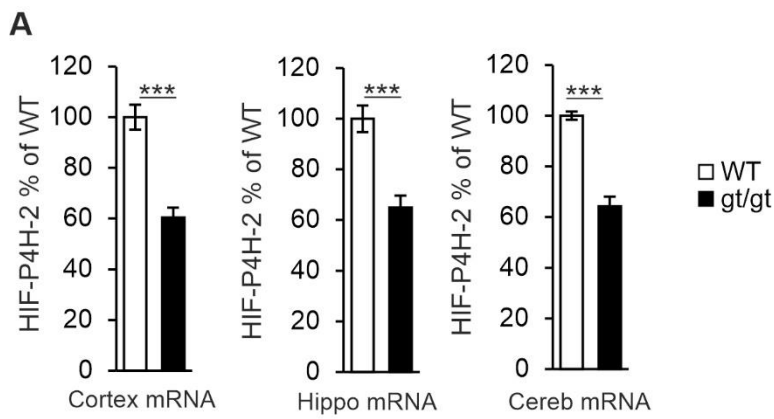


Fig. S2. HIF1 α is stabilized in *Hif-p4h-2^{gt/gt}* brain. HIF stabilisation in wild-type (WT) and *Hif-p4h-2^{gt/gt}* (gt/gt) brain. (A) qPCR analysis of *Hif-p4h-2* mRNA in cortex, hippocampus and cerebellum of WT and gt/gt mice. Data are means \pm SEM. *** $P < 0.005$ in T-test. $n = 10$ WT, 10 gt/gt. (B) Western blot analysis of HIF1 α in hippocampus of WT and gt/gt mice. *indicates non-specific bands. β -actin was used as a loading control.

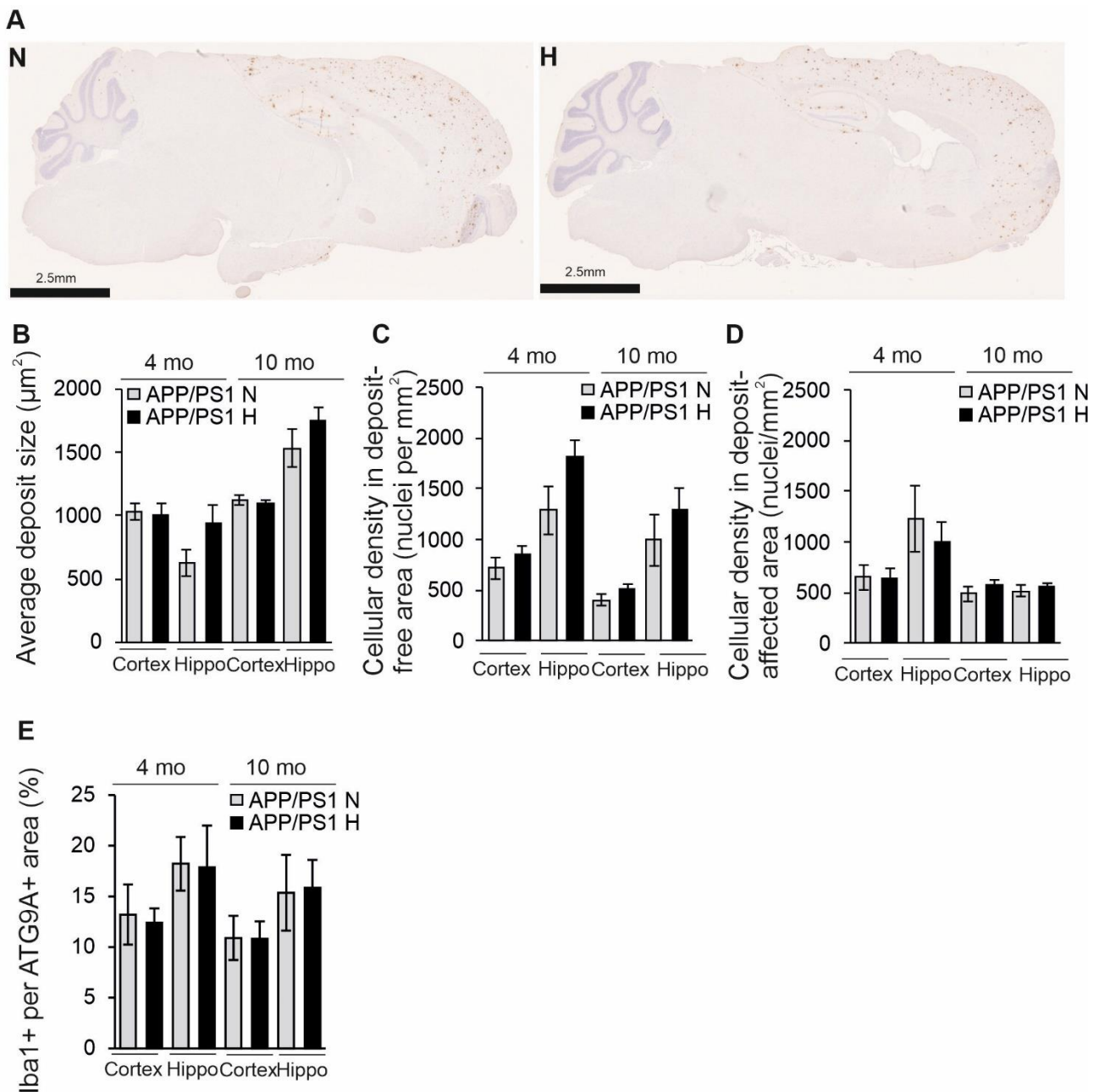


Fig. S3. Immunohistochemical analysis of A β plaques, nuclear density, dystrophic neurites and microglia in normoxia and hypoxia treated APP/PSEN1 cortex and hippocampus. (A) Examples of immunohistochemical stainings of sagittal brain sections of 10-month-old APP/PS1 mice with the human specific 6E10 antibody. (B) Quantification of average size of A β positive plaques. Total cellular density in deposit-free area (C) and deposit-affected area (D). IBA1-positive area in ATG9A-positive area (E). n = 8 APP/PSEN1 N 4 mo, n = 9-10 APP/PSEN1 H 4 mo, n = 8-9 APP/PSEN1 N

10 mo, n = 9-11 APP/PSEN1 H 10 mo. Abbreviations: Hippo, hippocampus; H, hypoxia; mo, months old; N, normoxia.

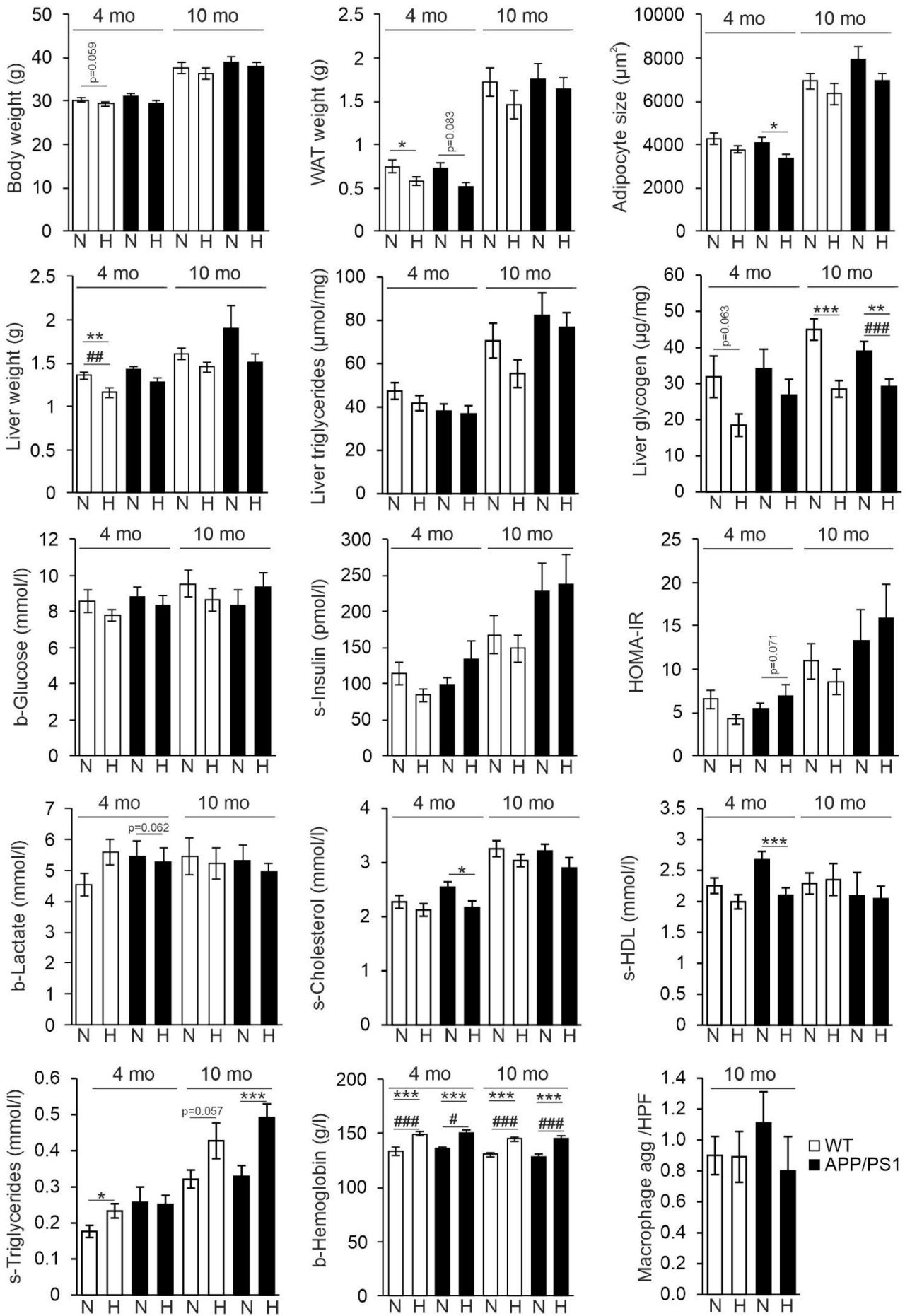


Fig. S4. Hypoxia treatment improves metabolic parameters in APP/PSEN1 mice. Metabolic parameters of wild-type (WT) and APP/PSEN1 mice after 6 weeks of hypoxia treatment. Data are means \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ in T-test, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.005$ in 2-way ANOVA. n = 14 WT N 4 mo, 12 WT H 4 mo, 8 APP/PSEN1 N 4 mo, 9-10 APP/PSEN1 H 4 mo, 13-14 WT N 10 mo, 10-11 WT H 10 mo, 9 APP/PSEN1 N 10 mo, 10-11 APP/PSEN1 H 10 mo. Abbreviations: agg, aggregates; b, blood; HPF, high power field; H, hypoxia; mo, months old; N, normoxia; s, serum; WAT, white adipose tissue.

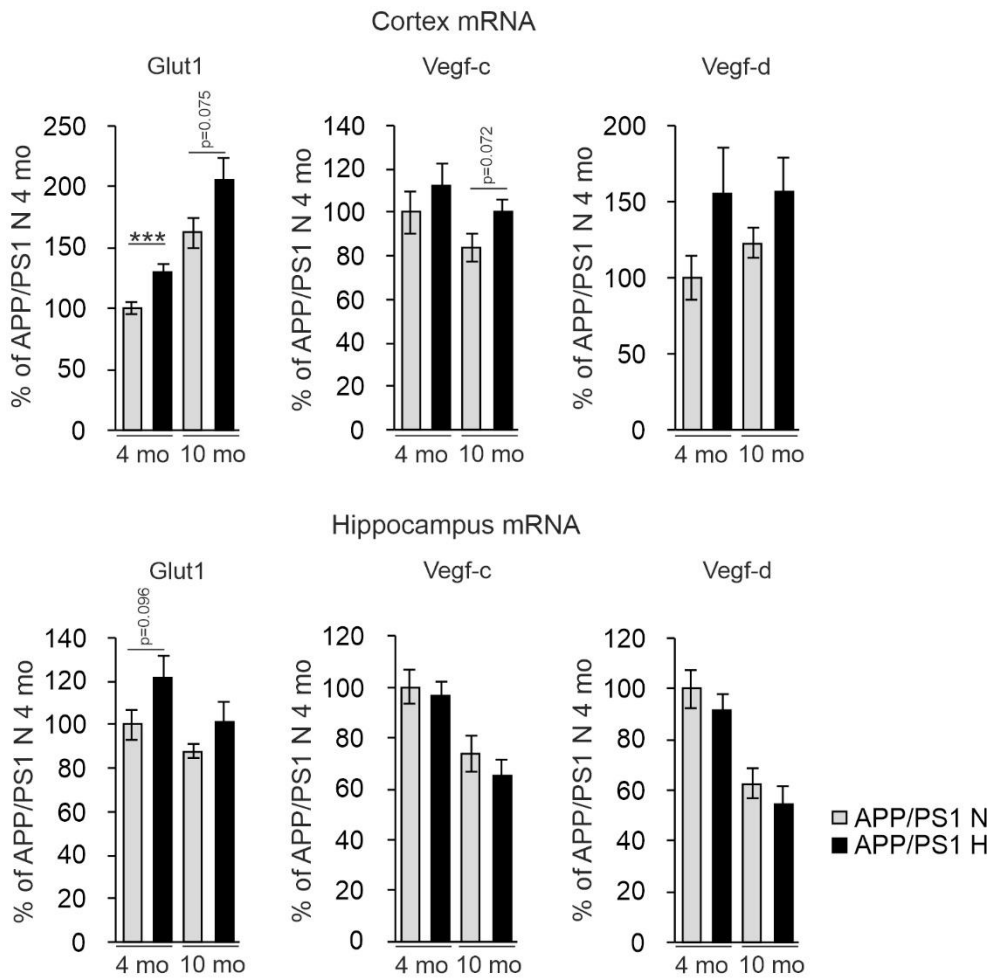


Fig. S5. Expression of angiogenic mRNAs in the APP/PSEN1 brain following hypoxic exposure. mRNA expression after 6 weeks of normoxia or hypoxia treatment in cortex and hippocampus of APP/PSEN1 mice. Data are means \pm SEM. *** P <0.005 in T-test. $n = 8$ N 4 mo, 9-10 H 4 mo, 8-9 N 10 mo, 10-11 H 10 mo. Abbreviations: H, hypoxia; mo, months old; N, normoxia.