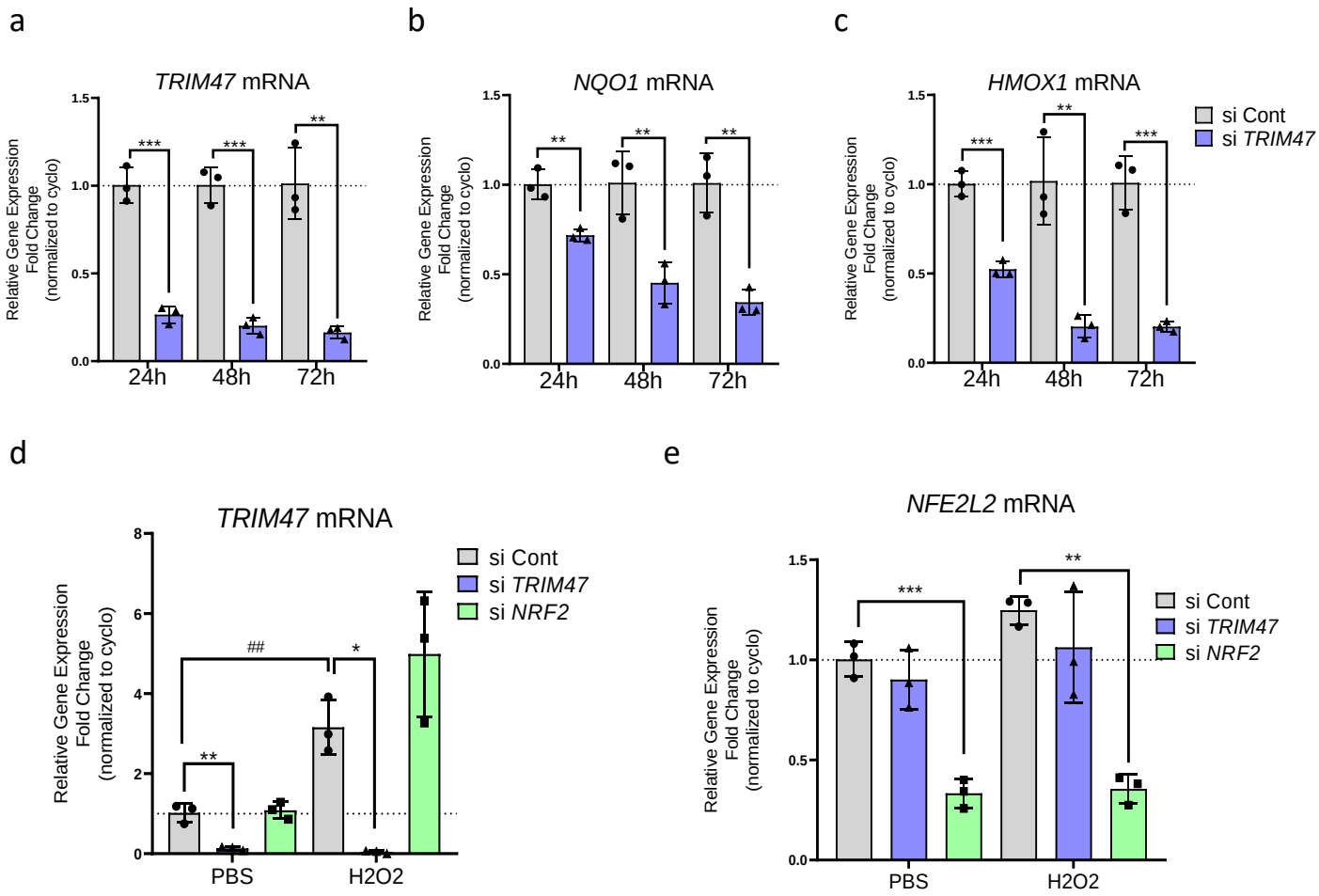
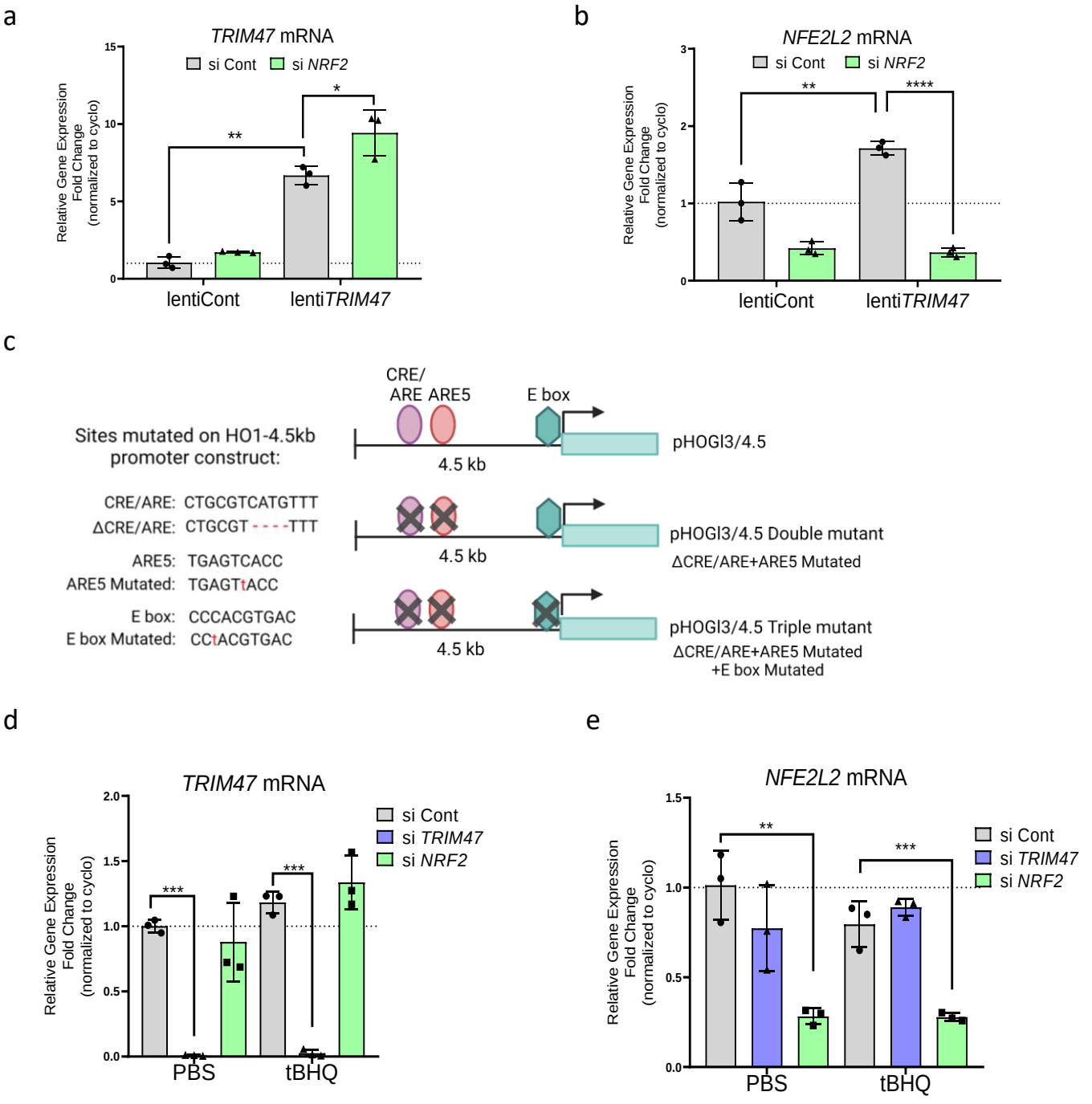
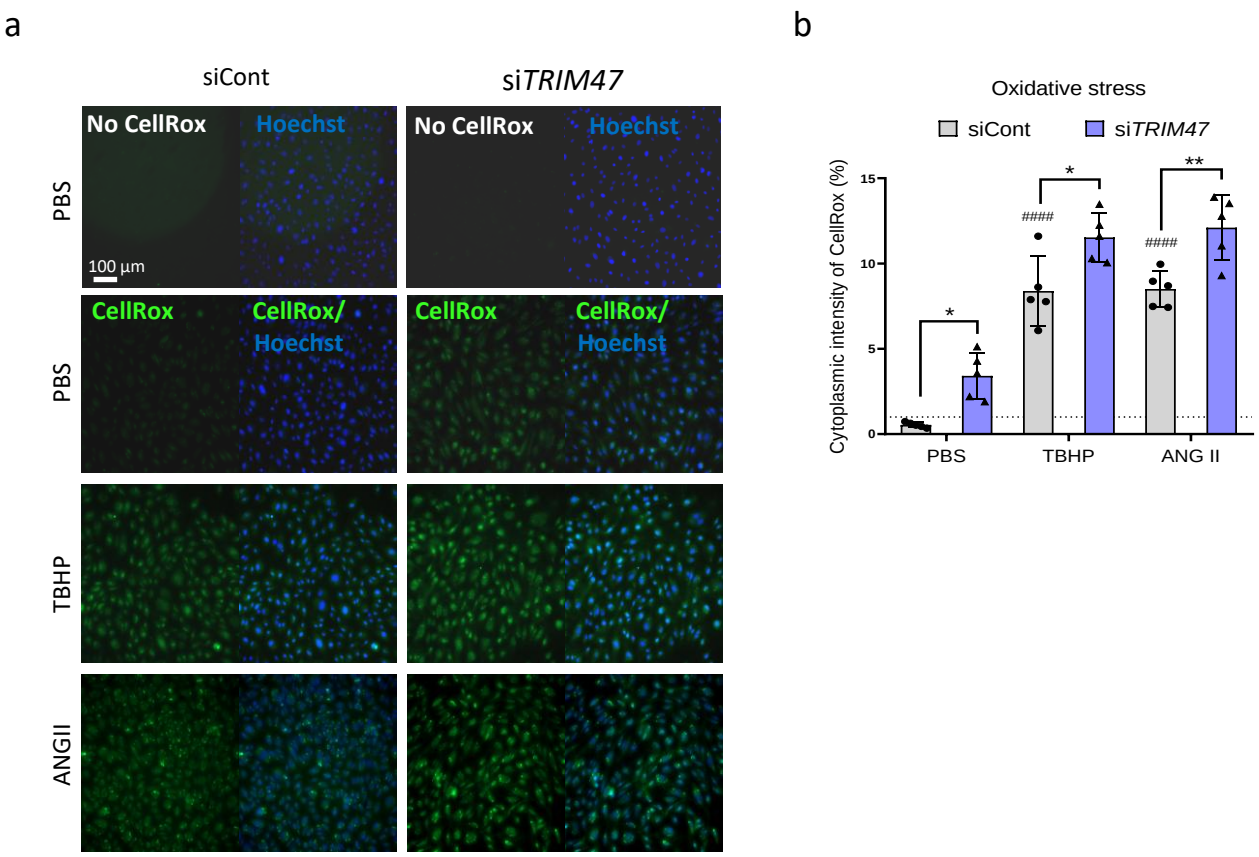


Supplementary Figure 1: Regulation of NRF2 target genes by TRIM47 in human brain microvascular endothelial cells (HBMEC). **a-c**. qPCR analysis of (**a**) *TRIM47*, (**b**) *NQO1* and (**c**) *HMOX1* expression in control (siCont) and *TRIM47*-deficient (si*TRIM47*) HBMEC after 24, 48 and 72 hours siRNA treatment. Data were normalized to cyclophilin (n=3 experiments). **: p<0.01; ***: p<0.001, Student's *t*-test. **d-e**. qPCR analysis of (**d**) *TRIM47* and (**f**) *NFE2L2* (NRF2) gene expression in control, *TRIM47* or *NRF2* siRNA-treated HBMEC for 72h and treated with either PBS or H2O2 (200 uM for 3h). Data were normalized to *cyclophilin* (n=3 experiments). * P<0.05; ** P<0.01; *** P<0.001, One-way ANOVA, ## P<0.01, Student's *t*-test. All Graphical data are mean ± s.d.

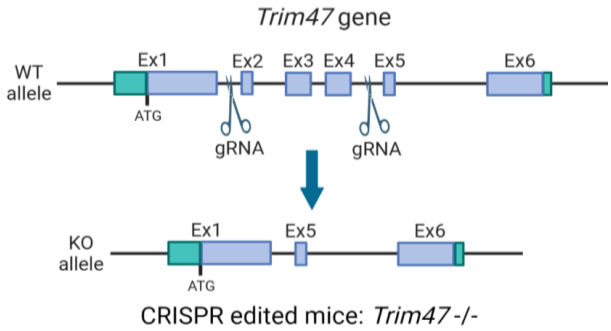


Supplementary Figure 2: Controls and tools for the experiments showing TRIM47 cooperation with the transcription factor NRF2 in HBMEC. **a-b**. qPCR analysis of (**a**) *TRIM47* and (**b**) *NFE2L2* expression in siCont or si*NRF2* treated HBMEC and co-transduced with a control or TRIM47 lentivirus for 24h. Data were normalized to *cyclophilin* (n=3 experiments). ** P<0.01; *** P<0.001; One-way ANOVA. **c.** Cartoon depicted the HO1 tools used for promoter luciferase reporter assay in HeLa. Wild type HO1 promoter-luciferase construct (HO1-4.5bp), construct mutated for CRE/ARE and ARE5 sites (HO1-4.5bp double mutant) and construct mutated for CRE/ARE, ARE5 sites and E box (HO1-4.5bp triple mutant, used as a control for the experiment). **d-e**. qPCR analysis of (**d**) *TRIM47* and (**e**) *NFE2L2* expression in HBMEC treated with control, *TRIM47* or *NRF2* siRNA for 72h and with tBHQ for 24h (n=3 experiments). ** P<0.01; *** P<0.001; One-way ANOVA. All Graphical data are mean ± s.d.



Supplementary Figure 3: TRIM47 displays antioxidant properties in HBMEC.
a-b. Representative immunofluorescence image (**a**) and quantification (**b**) of CellRox dye (green) in HBMEC transfected with siCont or si*TRIM47* for 24 h and treated with PBS or tert-butyl hydroperoxide (TBHP, 200 µM for 1 h) or Angiotensin II (ANGII, 500 nM for 2h); nuclei are identified by Hoechst (blue). Scale bar 100 µm. Quantification represents the mean percentage of cytoplasmic area of Cellrox dye per field (n=5 wells from 3 independent experiments). * P<0.05, ** P<0.01, One-way ANOVA; #### P<0.001: compared to siCont + THBP without drug treatment. Graphical data are mean ± s.d.

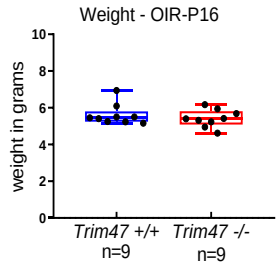
a



b

genotype	+/+	-/-	Total
% observed	23	22	100
% -mendelian ratio	25	25	100

c



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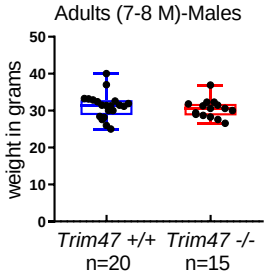

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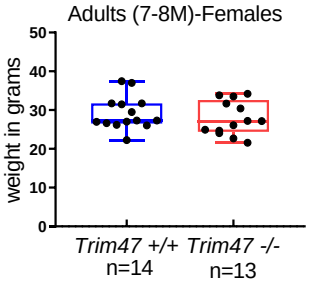

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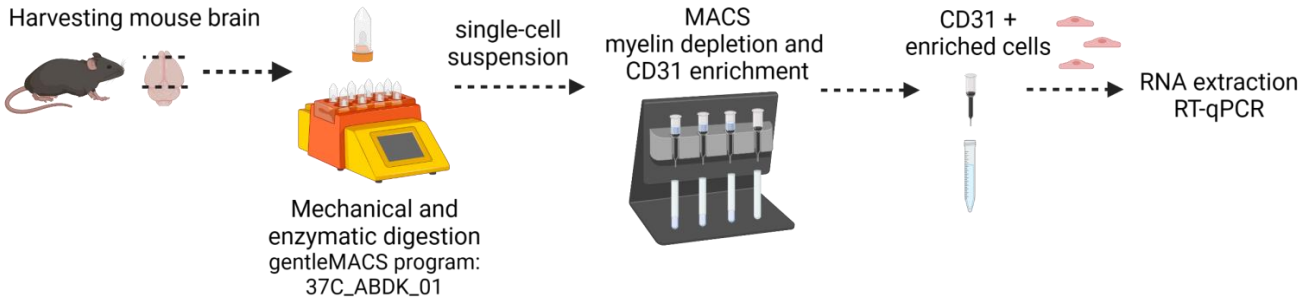
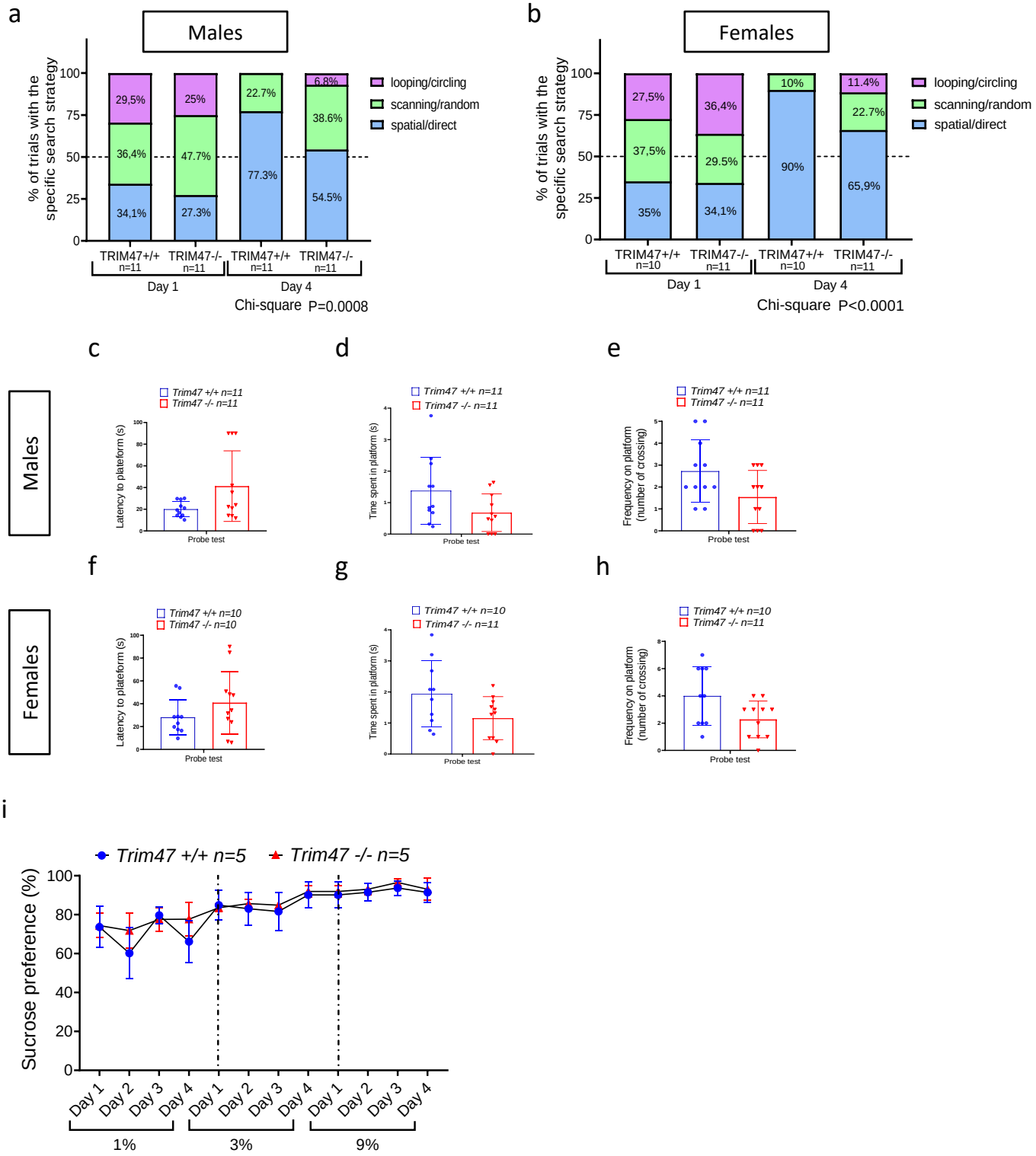
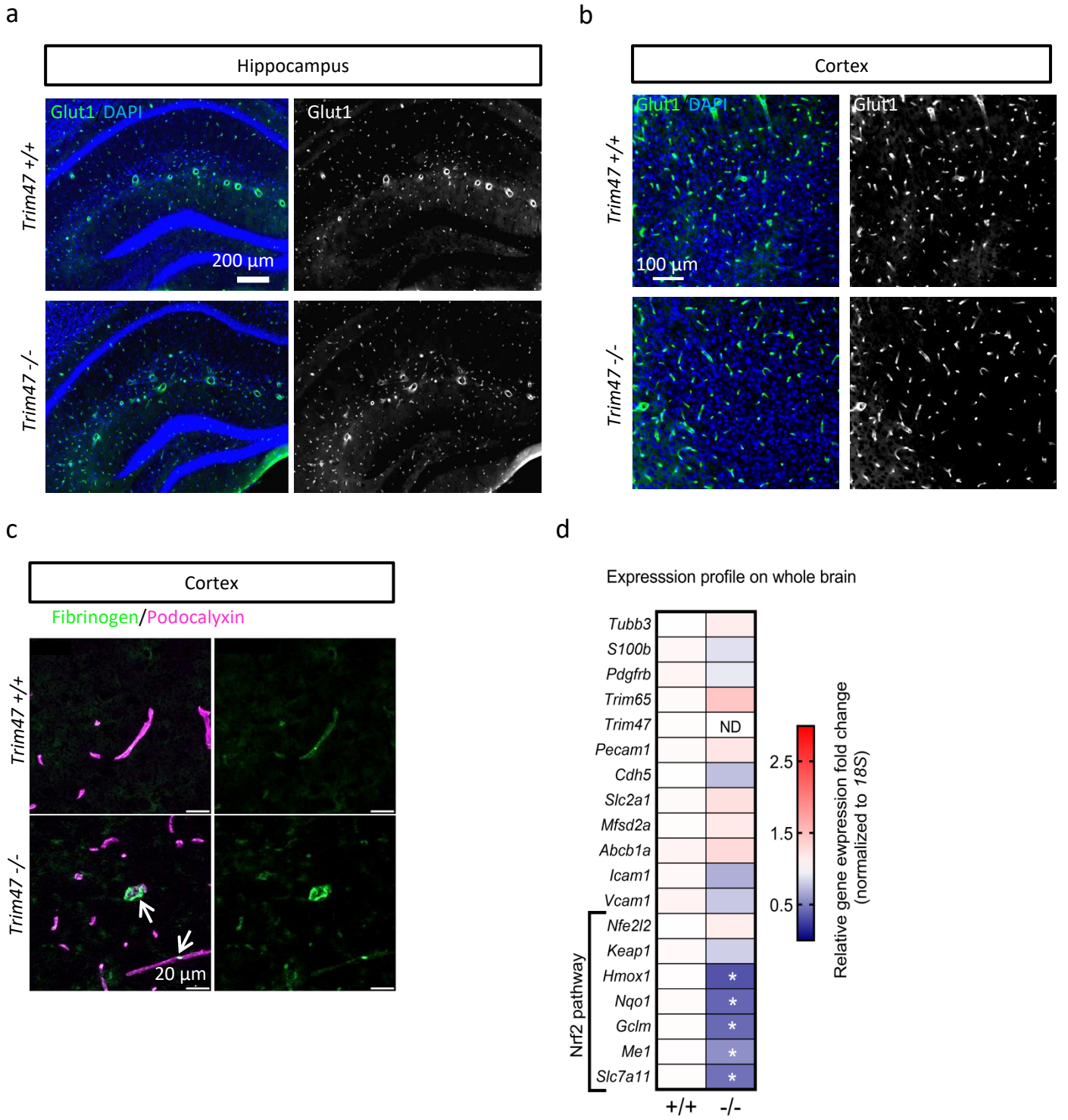


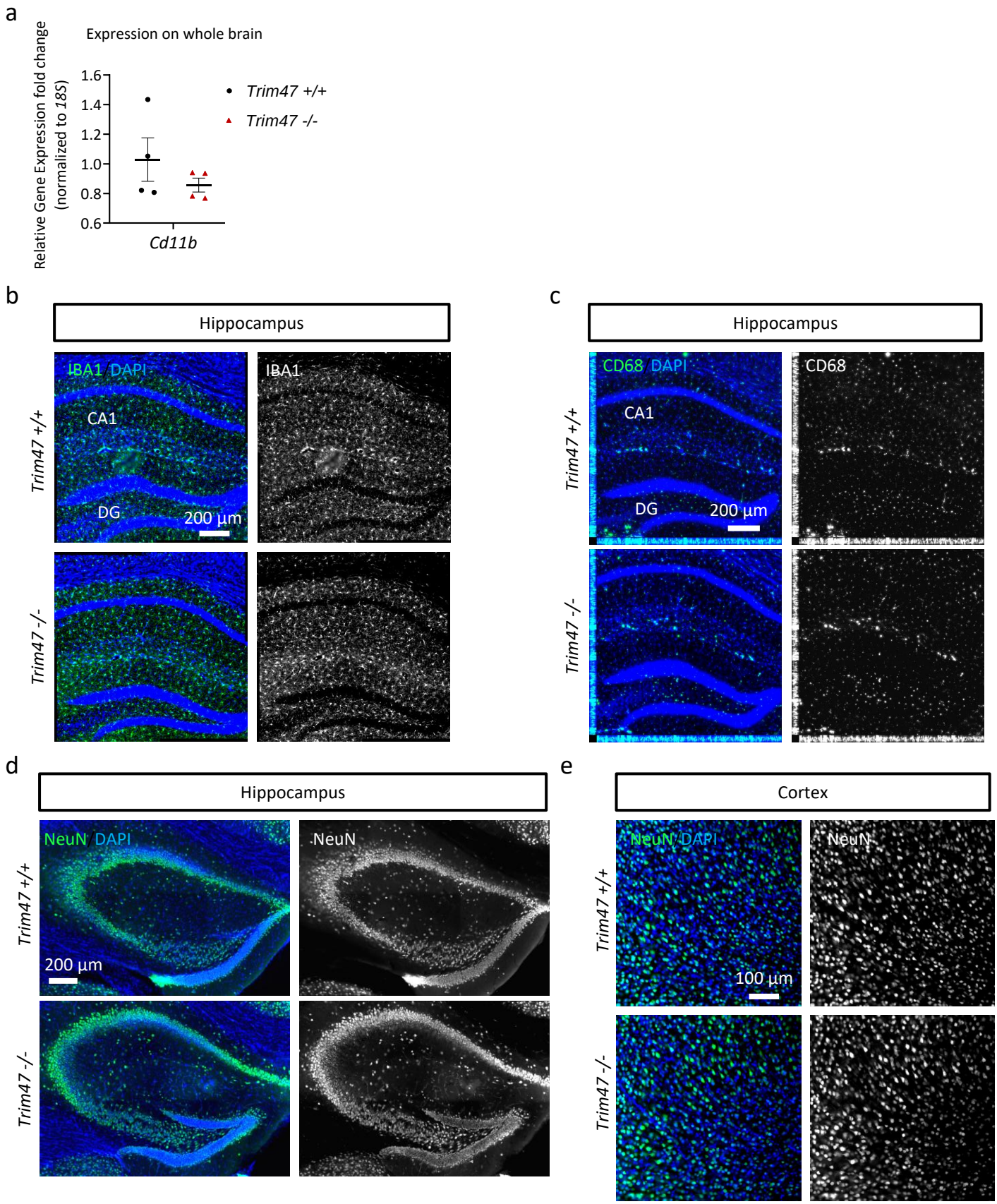
Supplementary Figure 4: Generation and characterization of mice deleted for *Trim47* in all tissues. **a.** Cartoon depicting the strategy for generating mice globally deleted for *Trim47* using CRISPR deletion of exons 2 to 4. **b**. Expected Mendelian ratio and percentage of mice observed with +/+ or -/- genotype is reported in the table. **c-e.** Body weight (in grams) of (**c**) postnatal day 16 mice after OIR model, adult (**d**) males and (**e**) females +/+ and -/- mice. **f.** Workflow of mouse brain endothelial cells isolation protocol using gentle MACS for mechanical and enzymatic digestion, then myelin removal step and enrichment with CD31 magnetic beads. Graphical data are mean ± s.d.



Supplementary Figure 5: Assessment of cognitive functions and anhedonia of mice deleted for *Trim47*.**a-b.**Search strategy use in water maze was affected in *Trim47-/-* mice. Males (**a**) and females (**b**) were analyzed separately and classified in 3 categories: spatial/direct, scanning/random and looping/circling. Data from all trials were combined for this analysis and expressed as percentage of trials using each strategy for each genotype at days 1 and 4 of training. *Trim47+/+* n=44 trials (11 males)*; Trim47-/-* n=44 trials (n=11 males) (4 trials/mouse). *Trim47+/+* n=40 trials (10 females)*; Trim47-/-* n=44 trials (n=14 females). Males: p=0.008 and females: p<0.0001. Chi-square test. **c-h.** Probes trials were performed 3 days after the last training on males (**c-e**) and females (**f-h**). The 3 additional following parameters were measured: (**c-f**) latency to reach platform area (seconds), (**d-g**) time spent in platform area (seconds) and (**e-h**) frequency on platform (number of platform crossings). In line with an increase in proximity measure, a trend towards increased latency to reach platform area, decreased time spent in platform area and number of crossings were observed in *Trim47-/-* mice. *Trim47+/+* males: n=11, *Trim47-/-* males: n=11.males, *Trim47+/+* females: n=10, *Trim47-/-* females: n=11. * P<0.05, Mann-Whitney test. **i.** Sucrose preference test was performed to asses anhedonia and anxiety in adults +/+ and -/- mice (males, 8 months, n=5 mice per genotype) and is expressed as the percentage of sucrose intake normalized to the total amount of liquid (water and sucrose) intake. Graphical data are mean ± s.d.

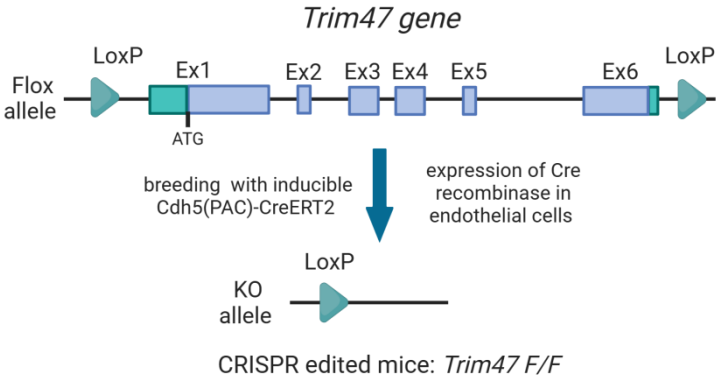


Supplementary Figure 6: Histological and transcriptomic analysis of *Trim47+/+* and *Trim47-/-* adults mice.
a-b. Representative images of Glut1 (green or grayscale) in brain sections (coronal, 50 um) from *Trim47*+/+ and *Trim47*-/- mice showing blood vessels in (**a**) hippocampus and (**b**) cortical regions. Nuclei are stained with DAPI (blue). Scale bars 200 or 100 µm, as noted. **c**. Representative confocal images of fibrinogen leakage (green) in brain cryosections from *Trim47*+/+ and *Trim47*-/- mice. Tissues are co-stained for podocalyxin (blood vessels, pink). Scale bars 20 µm. White arrows highlight fibrinogen extravasation in *Trim47-/-* mice. **d.** qPCR screening of whole brain from *Trim47*+/+ and *Trim47*-/- mice (7-10 months). Data normalized to *18S* (n=4 *Trim47*+/+, n=4 *Trim47* /-). ND: not detectable; * P<0.05, Mann-Whitney.



Supplementary Figure 7: Effects of *Trim47* deletion on non-endothelial cells in adult mouse brain.
a. mRNA expression profile of the microglia marker *Cd11b* in whole brain lysates from *Trim47+/+* and *Trim47-/-* mice (7-10 months). Data normalized to *18S* (n=4 *Trim47+/+,* n=4 *Trim47-/-*). **b-c**. Representative images of (**b**) Iba1 (microglia) (green/grayscale) and (**c**) Cd68 (macrophages) (green/grayscale) immunostaining in brain sections (coronal, 50 um, hippocampus) from *Trim47+/+* and *Trim47-/-* mice. Scale bars 200 µm. CA1: cornu ammonis region 1, DG: dentate gyrus **d-e**. Representative images of Neun staining (neurons) (green/grayscale) in brain cryosections from *Trim47+/+* and *Trim47-/-* mice in (**d**) hippocampus and in (**e**) cortex region; nuclei identified by DAPI (blue). Scale bars 200 or 100 µm. All Graphical data are mean ± s.d.

a



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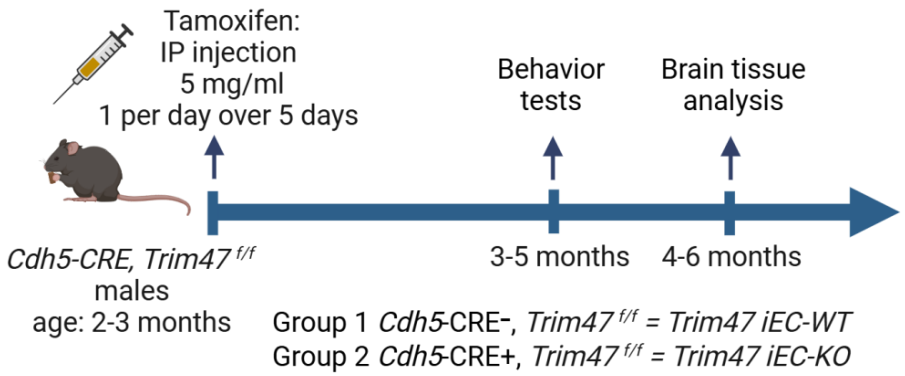

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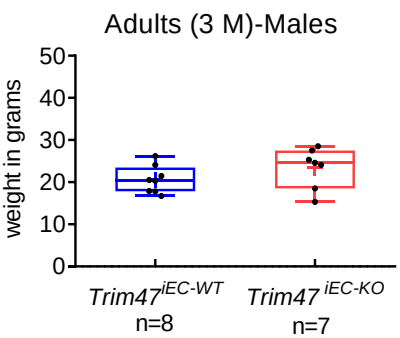

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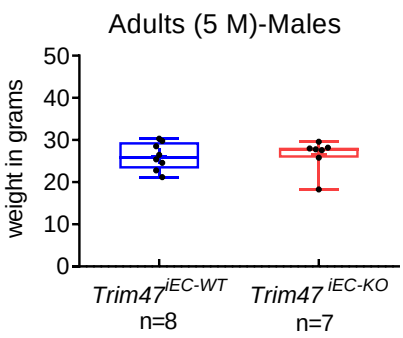
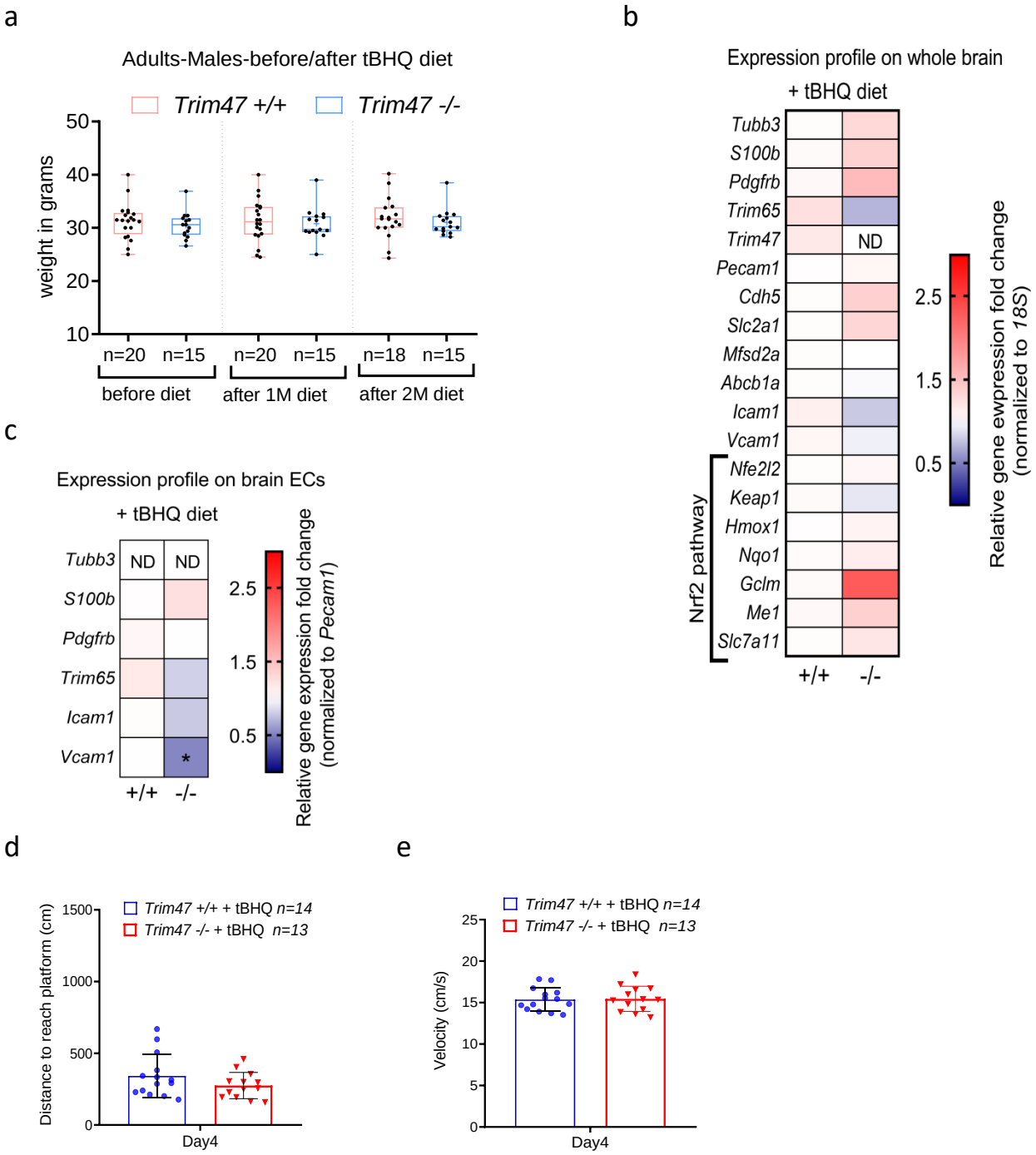


Supplementary Figure 8: Generation and characterization of mice deleted for *Trim47* specifically in endothelial cells. **a.** Cartoon depicting the strategy for generating mice deleted for *Trim47* in endothelial cells using *Trim47 F/F* mice (Flox sequences flanking exons 1 to 6) bred with *Cdh5*-Cre mice. **b**. Timeline for induction of *Trim47* deletion in EC following tamoxifen injection in adult mice (2 months). Behavioral tests, brain function assessment and qPCR screening were performed on adults *Trim47* $^{iEC\text{-}WT}$ and *Trim47* $^{iEC\text{-}KO}$ males. **c.** Body weight expressed in grams of 3 months males (n=8 *Trim47* $^{iEC\text{-}WT}$ and n=7 *Trim47* $^{iEC\text{-}KO}$). **c.** Body weight expressed in grams of 5 months males (n=8 *Trim47* $^{iEC\text{-}WT}$ and n=7 *Trim47* $^{iEC\text{-}KO}$).



Supplementary Figure 9: Generation and characterization of mice deleted for *Trim47* specifically in endothelial cells.
a. Monitoring of mouse body weight (in grams) before, after 1 month or 2 months of tBHQ diet showing no weight loss over time. **b-c**. qPCR screening on (**b**) whole brain and (**c**) brain EC isolated from *Trim47+/+ and Trim47-/-* males (8-10 months) with tBHQ diet (n=5 replicates/genotype). *: p<0.5; Mann-Whitney. ND: not detectable. **d-e.** Water maze test was performed on *Trim47+/+ and Trim47-/-* males with tBHQ diet (n=14 *Trim47+/+* + tBHQ and n=13 *Trim47+/+* + tBHQ). (**d**) Graph shows the distance to reach platform (expressed in cm) for the day 4. (**e**) Data represent the velocity expressed as cm per second (swimming speed) of each mouse at day 4 during water maze. All Graphical data are mean ± s.d.

	WMH					BG-PVS					WM-PVS				
	beta	**se**	**pval**	**pFDR**	**Qpval**	**beta**	**se**	**pval**	**pFDR**	**Qpval**	**beta**	**se**	**pval**	**pFDR**	**Qpval**
HMOX1	0,001	0,023	9,79E-01	1,00E+00	2,64E-01	0,044	0,019	1,84E-02	2,02E-01	1,58E-01	0,039	0,019	3,75E-02	3,72E-01	7,34E-01
GCLM	0,015	0,031	6,36E-01	1,00E+00	3,54E-01	0,048	0,028	8,33E-02	5,83E-01	4,99E-01	0,042	0,028	1,26E-01	6,30E-01	5,16E-01
TXNRD1	0,053	0,025	3,72E-02	3,72E-01	2,92E-01	0,053	0,02	6,41E-03	7,69E-02	4,91E-01	0,07	0,02	3,29E-04	4,94E-03	**3,06E-02**
GSR	0,042	0,061	4,88E-01	1,00E+00	3,62E-01	0,156	0,054	3,65E-03	**4,75E-02**	8,54E-01	0,063	0,053	2,34E-01	9,36E-01	9,36E-01
PRDX6	-0,036	0,018	4,76E-02	3,81E-01	4,64E-01	0,029	0,01	3,08E-03	**4,13E-02**	5,65E-01	0,017	0,01	8,94E-02	5,83E-01	5,75E-02

Supplementary Table 1: Association analysis of protein levels in plasma with MRI markers of cSVD, meta-analysis of 3C-Dijon and the UK Biobank (N=5,523). WMH: white matter hyperintensities, PVS: perivascular spaces; BG: basal ganglia, WM: white matter.

Target	Company	Sequence
siControl	Eurogentec	SR-CL000-005
siTRIM47 #1	Eurogentec	CCAGGGACUAUUUCCUCAA55
siTRIM47 #2	Eurogentec	GCAGCUGUUUGGAACCAAA55
siNRF2	Eurogentec	AUUGAUGUUUCUGAUCUAUCACU55

Supplementary Table 2: Sequences of siRNA used for HBMEC and HeLa transfection

Antibody (host)	Company	Catalogue number	Application and dilution
CD68 (rat)	Biolegend	137001	IF (mouse brain, 1/200)
Claudin5-488 (mouse)	ThermoFisher	352588	IF (mouse brain, 1/200)
Fibrinogen (rabbit)	Dako	A0080	IF (mouse brain, 1/200)
GFAP (rabbit)	ThermoFisher	OPA1-06100	IF (mouse brain, 1/400)
Glut1 (rabbit)	ThermoFisher	PA1-1063	IF (mouse brain, 1/200)
HO1 (mouse)	Enzo life Science	ADI-OSA-110	WB (HBMEC, 1/1000)
IBA1 (rabbit)	Fujifilm Wako	019-19741	IF (mouse brain, 1/200)
Isolectin B4-FITC	Sigma	L 2895	IF (mouse retina, 1/200)
KEAP1 (rabbit)	Abcam	ab227828	WB (HBMEC, 1/1000), Co-IP (Hek293, 2 µg for 500 µg of protein lysates)
Myelin Basic Protein (rat)	Abcam	ab7349	IF (mouse brain, 1/200)
Myc (mouse)	Upstate	05-419	WB (Hek293, 1/1000)
Myc (mouse)	Millipore	05-724	Co-IP (Hek293, 2 µg for 500 µg of protein lysates)
NeuN (rabbit)	Millipore	ABN78	IF (mouse brain, 1/200)
NRF2 (rabbit)	Abcam	ab62352	WB (HBMEC, 1/1000)
Podocalyxin (goat)	R&D Systems	AF1556	IF (mouse brain, 1/400)
TRIM47 (rabbit)	Invitrogen	PA5-110521	WB (HBMEC , 1/1000)
TRIM47(rabbit)	Proteintech	26885-1-AP	WB (mouse brain, 1/1000)
α-tubulin (mouse)	Sigma	T5168	WB (HBMEC, 1/30000)

Supplementary Table 3: List of antibodies used for this study on HBMEC and mouse tissue. IF: Immunofluorescence; WB: Western-Blot, Co-IP: co-immunoprecipitation. Dilution of the antibodies used for each specific application is specified in brackets.

Target	Forward	Reverse
PPIA/cyclophilin	AGCTAGACTTGAAGGGGAATG	ATTTCTTTTGACTTGCGGGC
TRIM47	TGAGCAGTCCAAAGTCCTGA	CTACGGCTGCACTCTTGATG
NFE2L2/NRF2	CACATCCAGTCAGAAACCAGTGG	GGAATGTCTGCGCCAAAGCTG
KEAP1	CCAACTTCGCTGAGCAGATT	GCTGATGAGGGTCACCAGTT
HMOX1/HO1	CCAGGCAGAGAATGCTGAGTTC	AAGACTGGGCTCTCCTTGTTGC
NQO1	GAAGAGCACTGATCGTACTGGC	GGATACTGAAAGTTCGCAGGG

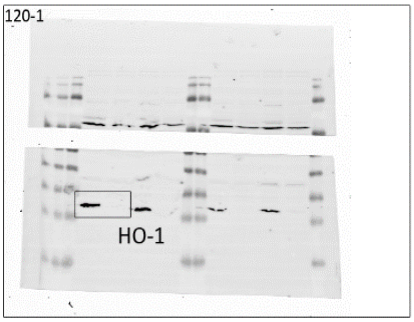
Supplementary Table 4: List of human oligonucleotides used for qPCR

Target	Forward	Reverse
Tubb3/Tuj1	TCAGCGATGAGCACGGCATA	CACTCTTTCCGCACGACATC
S100b	CTGGAGAAGGCCATGGTTGC	CTCCAGGAAGTGAGAGAGCT
Gfap	AACCGCATCACCATTCCTGT	TGGCAGGGCTCCATTTTCAA
Cd11b/Itgam	TACTTCGGGCAGTCTCTGAGTG	ATGGTTGCCTCCAGTCTCAGCA
Pdgfrb	GCTAGCTGGTTGGCTAGCTG	CTTCCGGTGTCTAAATGTGGGT
Trim65	AGGAGCAACGCAGTCGGATTGA	GCCTGCTTCTTGGCTACCTCTA
Trim47	CTACAGAAACTCGGCTCAGAAGAT	GACTCCGGGTAGTTGATGGG
Pecam1	TCATTGGAGTGGTCATCGCC	TGTTGGAGTTCAGAAGTGGAGCAG
Cdh5	CCTGTAGGGAAAGAGTCCATTGTG	ACTTGACCGTGATGTTGGCG
Slc2a1/Glut1	TCTCTGTCGGCCTCTTTGTT	GCAGAAGGGCAACAGGATAC
Abcb1a	TCCTCACCAAGCGACTCCGATA	ACTTGAGCAGCATCGTTGGCGA
Mfsd2a	GCTCTGTCACCTCCTCACTG	ACGTTTCTACATTAGTGTTCCGAG
Nfe2l2/Nrf2	TAGATGACCATGAGTCGCTTGC	GCCAAACTTGCTCCATGTCC
Keap1	ATCCAGAGAGGAATGAGTGGCG	TCAACTGGTCCTGCCCATCGTA
Hmox1/Ho1	CACTCTGGAGATGACACCTGAG	GTGTTCCTCTGTCAGCATCACC
Nqo1	AGGATGGGAGGTACTCGAATC	AGGCGTCCTTCCTTATATGCTA
Gclm	AGGAGCTTCGGGACTGTATCC	GGGACATGGTGCATTCCAAAA
Me1	GTCGTGCATCTCTCACAGAAG	TGAGGGCAGTTGGTTTTATCTTT
Slc7a11	CTTTGTTGCCCTCTCCTGCTTC	CAGAGGAGTGTGCTTGTGGACA
Icam1	TGGCCTGGGGGATGCACACT	CCACCGGGCTGTAGGTGGGT
Vcam1	CGTACACCATCCGCCAGGCA	TAGAGTGCAAGGAGTTCGGGCG
Il1ß	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
Il6	CACTTCACAAGTCGGAGGCT	CTGCAAGTGCATCATCGTTGT
Cldn5	ACGGGAGGAGCGCTTTAC	GTTGGCGAACCAGCAGAG
Cldn11	GCCTGGAGTGGCCAAGTA	AGATGGTGGCGACAATGG
Ocln	GTCCGTGAGGCCTTTTGA	GGTGCATAATGATTGGGTTTG
Tjp1	AAGTTGGCAAGAGAGGAGCC	CAACCGCATTTGGCGTTACA
Plvap	GTTGACTACGCGACGTGAGATG	AGCTGTTCCTGGCACTGCTTCT
18S	CGCGGTTCTATTTTGTTGGT	AGTCGGCATCGTTTATGGTC

Supplementary Table 5: List of mouse oligonucleotides used for qPCR

Supplementary data 6: Uncropped immunoblots underlying Figures

Uncropped immunoblots underlying Figure 1d



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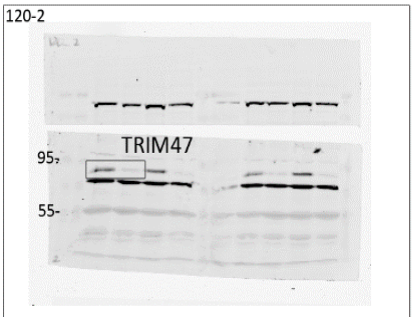

6% Acrylamide Gel

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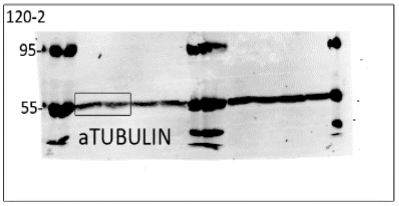

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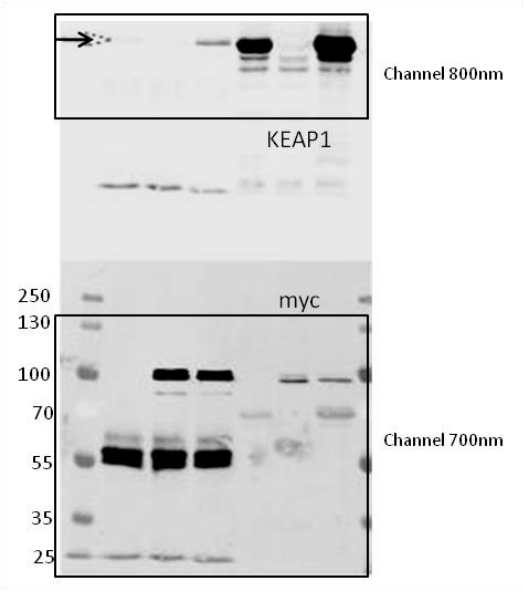
Uncropped immunoblots underlying Figure 2d



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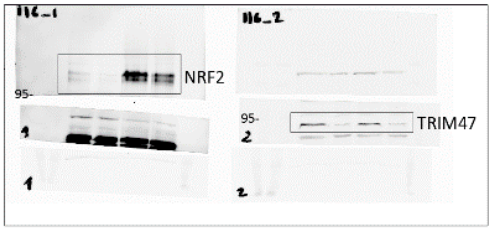
Channel 700nm

Uncropped immunoblots underlying Figure 2e



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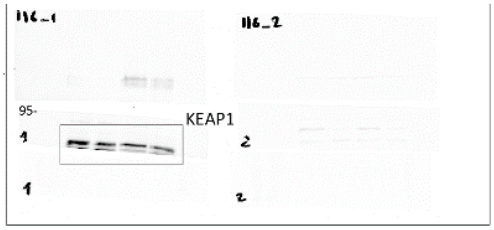

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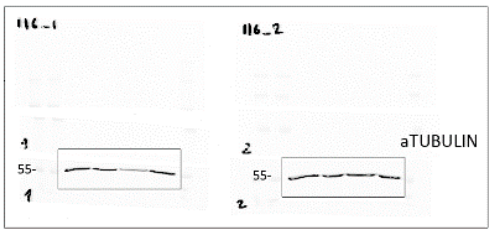

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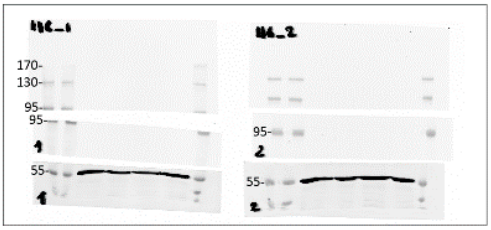

6% Acrylamide

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