SUPPLEMENTARY FIGURES

Figure S1

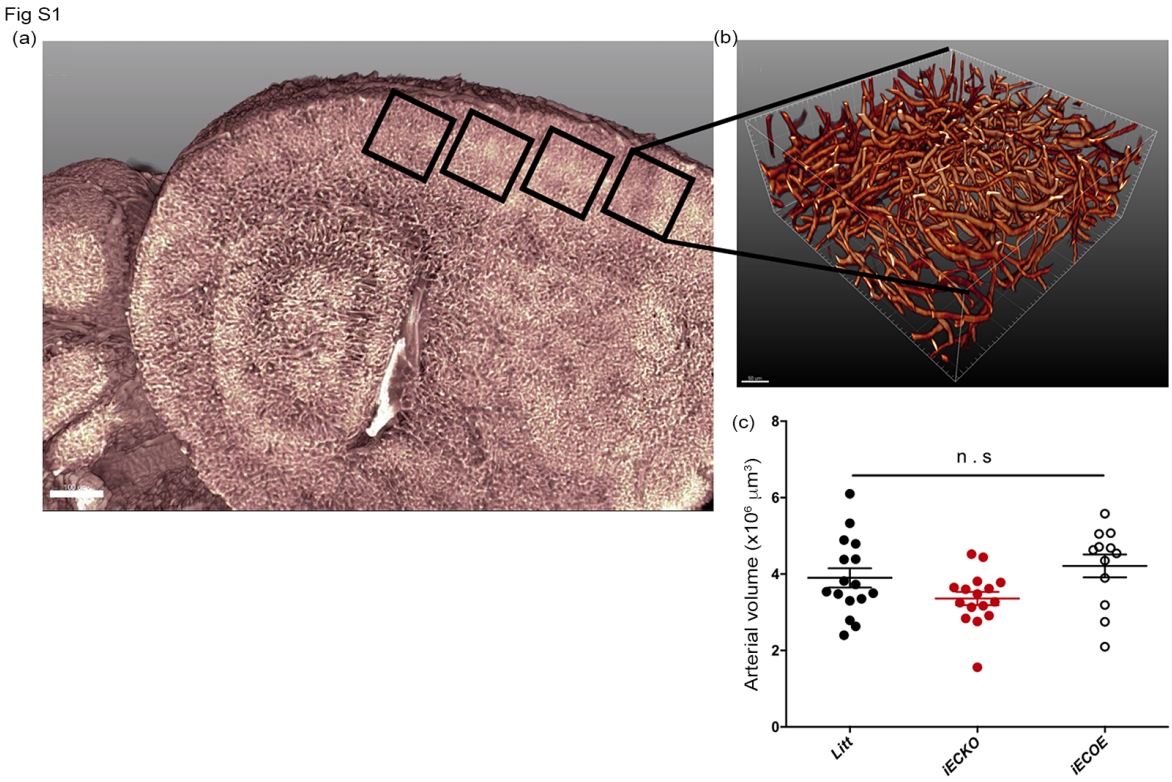


Fig S2

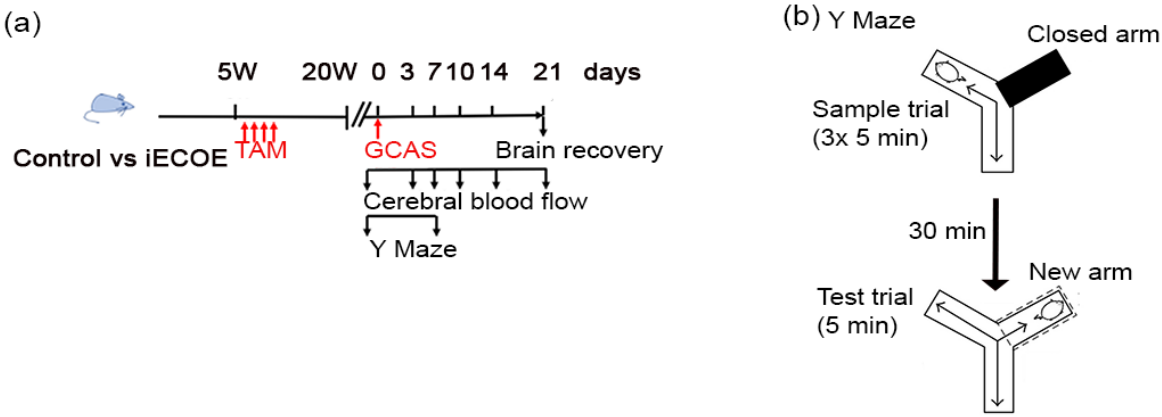

(a)
5W
20W
0
3
7
10
14
21
days
Control vs iECOE
TAM
GCAS
Brain recovery
Cerebral blood flow
Y Maze
(b) Y Maze
Closed arm
Sample trial
(3x 5 min)
30 min
New arm
Test trial
(5 min)

Fig S3

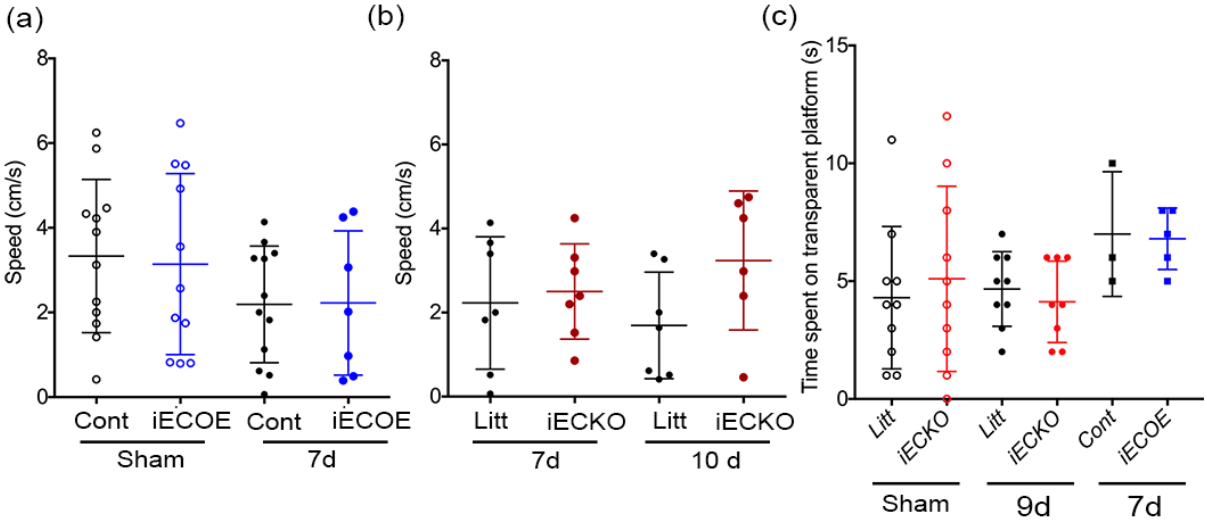
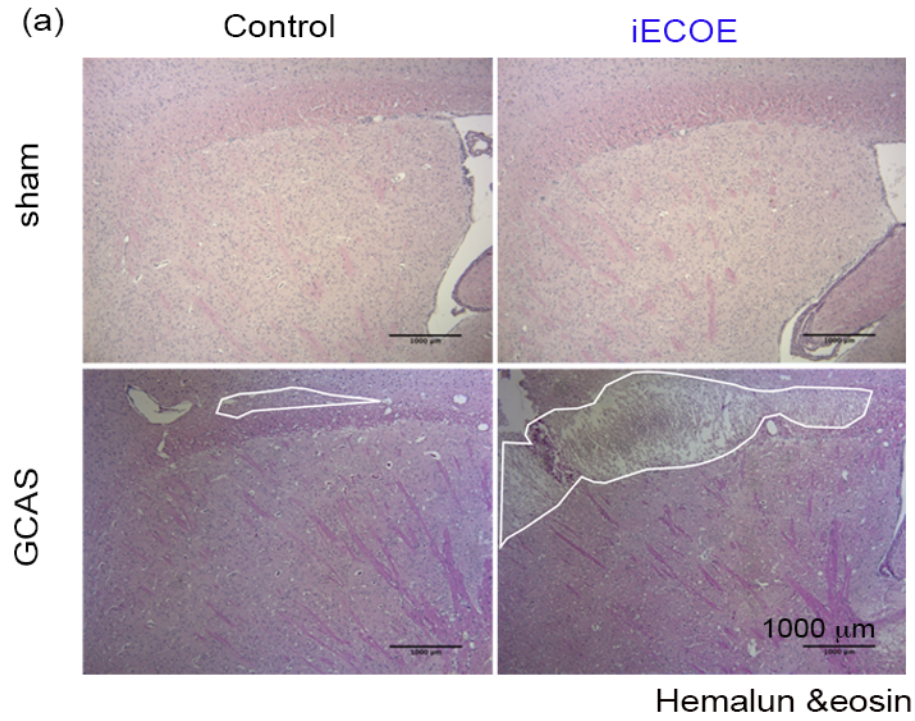

(a)
Speed (cm/s)
0
2
4
6
8
Cont
iECOE
Cont
iECOE
Sham
7d
(b)
Speed (cm/s)
0
2
4
6
8
Litt
iECKO
Litt
iECKO
7d
10 d
(c)
Time spent on transparent platform (s)
0
5
10
15
Litt
iECKO
Litt
iECKO
Cont
iECOE
Sham
9d
7d

Fig S4


(a)
Control
iECOE
sham
GCAS
1000 μm
Hemalun &eosin

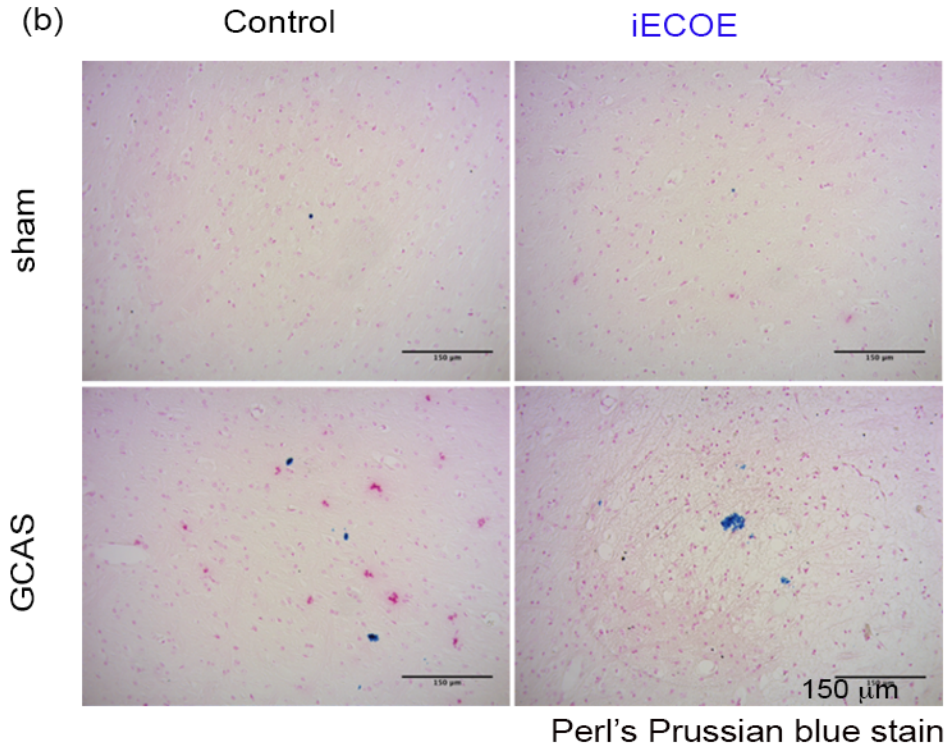
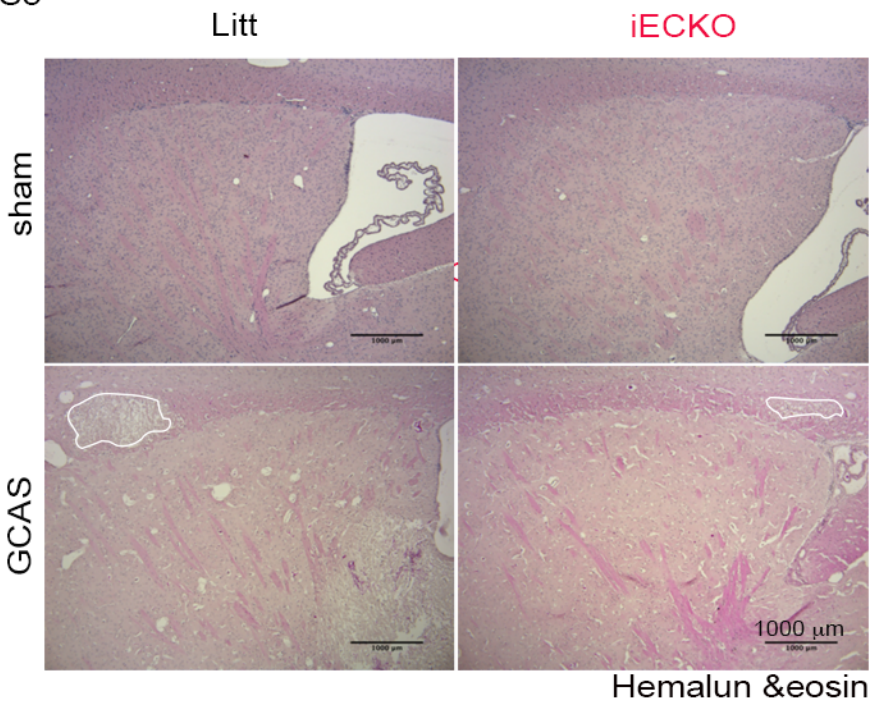

(b)
Control
iECOE
sham
GCAS
150 μm
Perl's Prussian blue stain

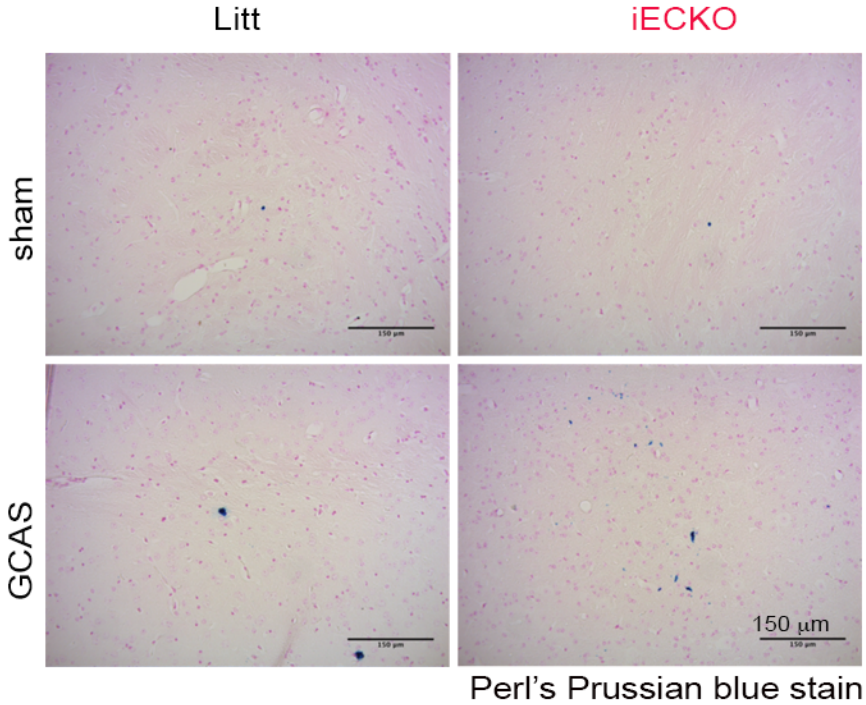
Fig S5

(a)



Hemalun &eosin

(b)



Perl's Prussian blue stain

Fig S6

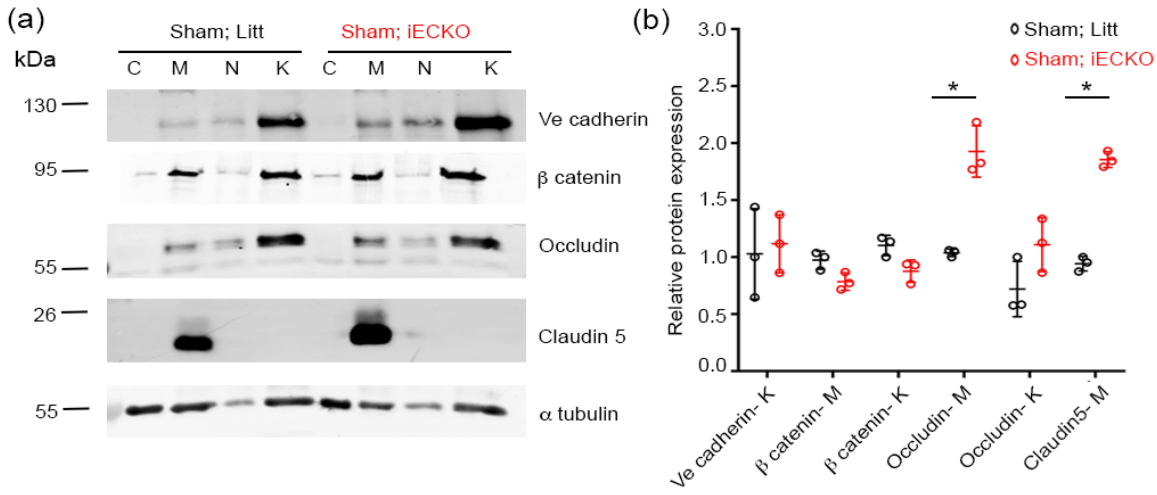

(a)
Sham; Litt
Sham; iECKO
kDa
C M N K C M N K
130
95
55
26
55
Ve cadherin
β catenin
Occludin
Claudin 5
α tubulin
(b)
Sham; Litt
Sham; iECKO
Relative protein expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Ve cadherin- K
β catenin- M
β catenin- K
Occludin- M
Occludin- K
Claudin5- M

Fig S7

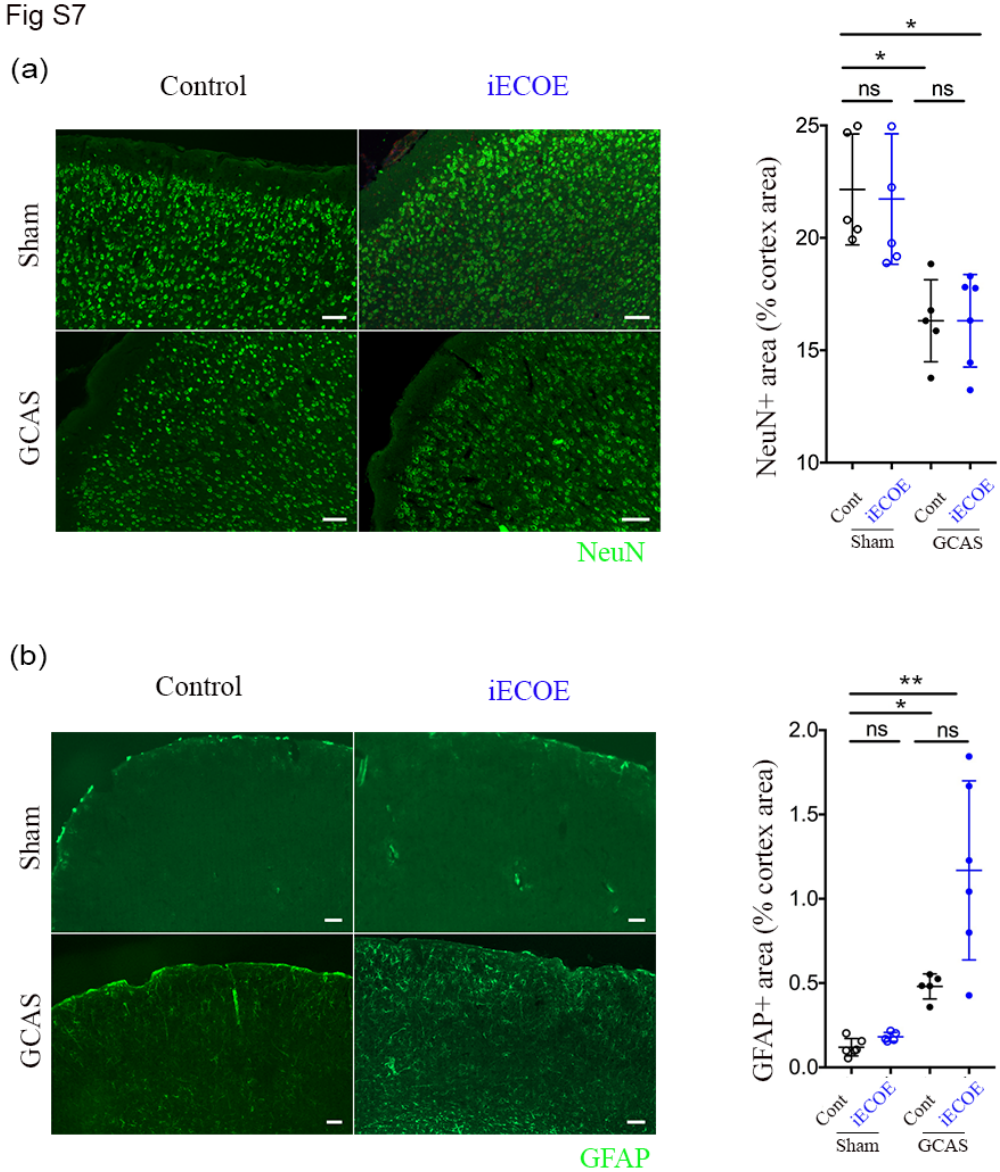

(a)
Control
iECOE
Sham
GCAS
NeuN
NeuN+ area (% cortex area)
25
20
15
10
*
*
ns
ns
Cont
iECOE
Cont
iECOE
Sham
GCAS
(b)
Control
iECOE
Sham
GCAS
GFAP
GFAP+ area (% cortex area)
2.0
1.5
1.0
0.5
0.0
**
*
ns
ns
Cont
iECOE
Cont
iECOE
Sham
GCAS

Fig S8

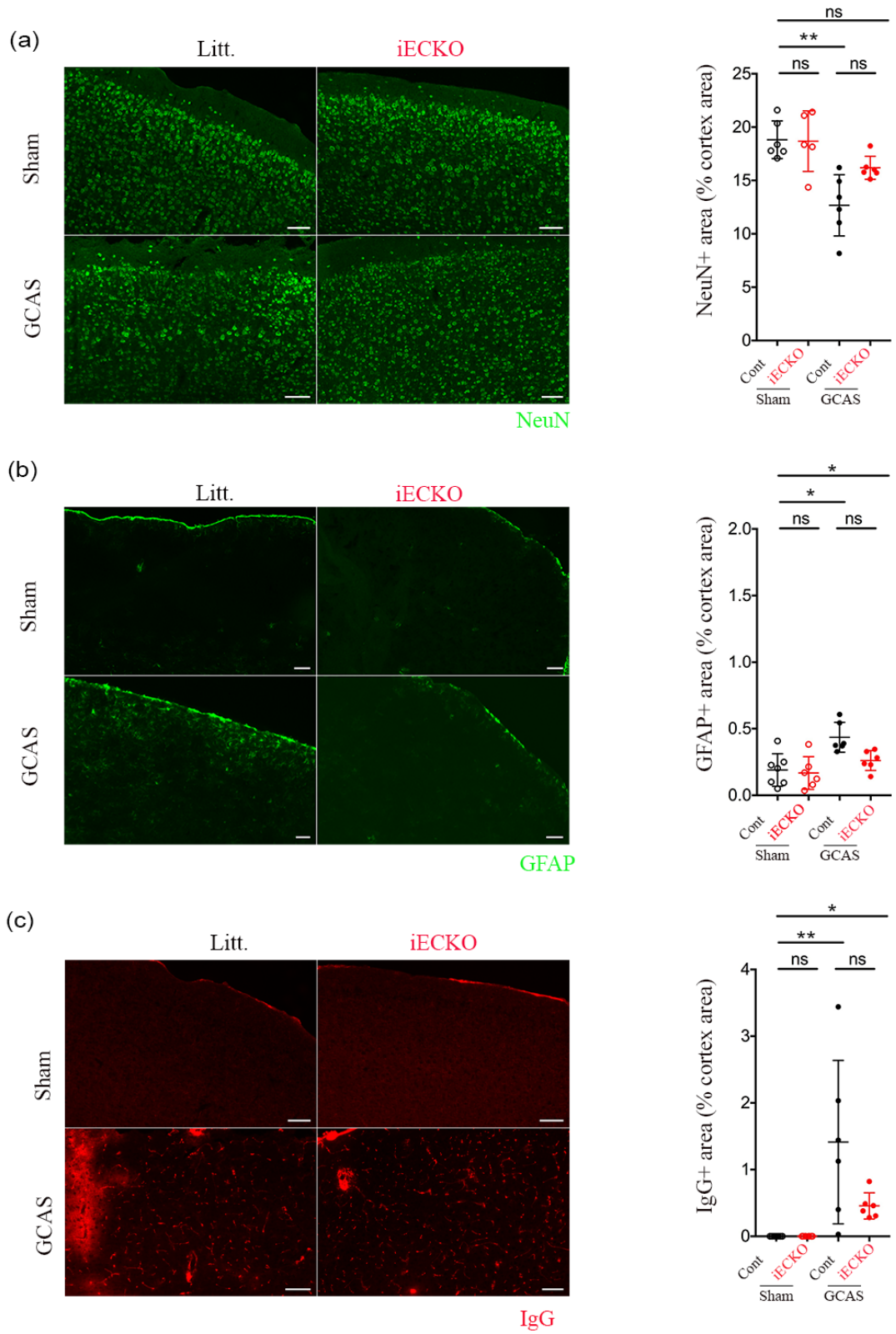
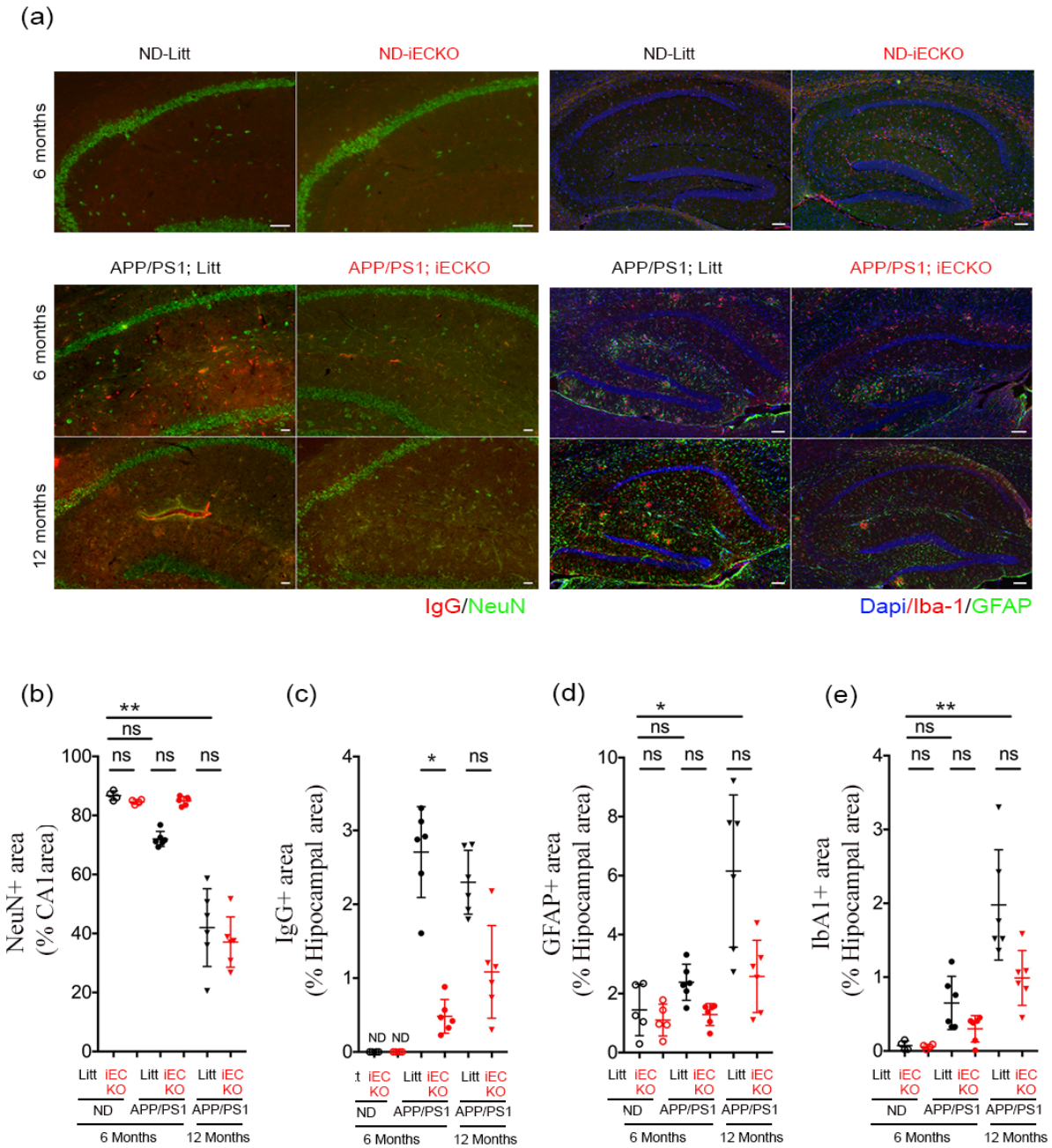

(a)
Litt.
iECKO
Sham
GCAS
NeuN
ns
**
ns
ns
NeuN+ area (% cortex area)
25
20
15
10
5
0
Cont
iECKO
Cont
iECKO
Sham
GCAS
(b)
Litt.
iECKO
Sham
GCAS
GFAP
*
*
ns
ns
GFAP+ area (% cortex area)
2.0
1.5
1.0
0.5
0.0
Cont
iECKO
Cont
iECKO
Sham
GCAS
(c)
Litt.
iECKO
Sham
GCAS
IgG
*
**
ns
ns
IgG+ area (% cortex area)
4
3
2
1
0
Cont
iECKO
Cont
iECKO
Sham
GCAS

Fig S9


(a)
ND-Litt
ND-iECKO
ND-Litt
ND-iECKO
6 months
APP/PS1; Litt
APP/PS1; iECKO
APP/PS1; Litt
APP/PS1; iECKO
6 months
12 months
IgG/NeuN
Dapi/Iba-1/GFAP
(b)
NeuN+ area (% CA1area)
(c)
IgG+ area (% Hipocampal area)
(d)
GFAP+ area (% Hipocampal area)
(e)
IbA1+ area (% Hipocampal area)
Litt
iEC KO
ND
APP/PS1
6 Months
12 Months
ns
*
**

Supplementary figure legends

Fig. S1. 3D view of brain vascular pattern in mutant mice

(a) large view on brain capillaries in hemi brain. (b) Raw maximum intensity projection and reconstructed tomato lectin-positive blood vessels (c) Vascular volumes quantification with the Imaris Filament Tracer tool in a region of interest chosen of the cortex from littermates (n=4), Pdzrn3 iECKO (n=4)and iECOE (n=3) mice. For each mouse, 4 cortical square (500 x 500 µm) were quantified. Bars denote mean ± SD; One-way ANOVA was performed followed by Bonferroni post hoc test.

Fig. S2. Experimental scheme

(a) Experimental scheme for tamoxifen administration to postnatal Pdzrn3 IECOE mice; schedule to evaluate CBF and Y-maze test before and after implantation of ameroid constrictors (GCAS). (b) Y-maze exploration test.

Fig. S3. Speed evaluation during Y-maze exploration test of Pdzrn3 iECKO mice 7 and 10 days after GCAS of Pdzrn3 iECOE mice

(a) and Pdzrn3 iECKO (b) before and at time indicated days after GCAS. Each point represents a different mouse. Data are represented as individual values plus means ± SD. (c) Evaluation of time spent on transparent platform of Pdzrn3 iECKO mice before and 9 days after GCAS, and of Pdzrn3 iECOE mice 7 days after GCAS.

Fig S4: coloration of representative brain section images

(a) for Hemalum and eosin (H&E) staining and (b) for Perl's Prussian blue stain from iECOE vs their age-matched control littermates after GCAS. Scale bar represents 1000 µm for H&E and 150 µm for Perl's coloration.

Fig S5: coloration of representative brain section images

(a) for Hemalum and eosin staining and (b) for Perl's Prussian blue stain from iECKO vs their age-matched littermates (Litt) mice after GCAS. Scale bar represents 1000 µm for H&E and 150 µm for Perl's coloration.

Fig S6: analysis of junction protein levels in brain vessels from sham;iECKO vs sham;littermate mice

(a) Representative immunoblot images and (b) quantification of TJ proteins Occludin, Claudin5, Ve cadherin and of b catenin in subcellular brain microvessel fractions from sham;Litt vs sham;iECKO mice (n=3). Cytosol (C), Membrane (M), nucleus (N), cytoskeleton (K) (n=4). Data are represented as mean ± SD. Mann-Whitney test was performed. * $p<0.05$.

Fig S7: Representative cortical immunofluorescent staining for NeuN and GFAP before (Sham) and after GCAS at 14 days in control and iECOE mice.

(a) NeuN+ and (b) GFAP + areas were quantified as a percentage of the area occupied in the cortical area non-operated (sham) and after GCAS from iECOE vs their respective control littermate mice. Data shown in the graphs are obtained in sham condition, from 6 mice per group and in GCAS condition from n=6 iECOE vs n=5 control mice; data for four brain slices per mouse were recorded and averaged to produce a single value for each mouse. One-way ANOVA was performed. Data are represented as individual values plus means ± SD. ns, non significant, * $p<0.05$, ** $p<0.001$. Scale bar represents 100 μm.

Fig S8: Representative cortical brain section images stained for (a) NeuN, (b) GFAP and (c) IgG from non-operated littermate (Sham) and after GCAS at 14 days in littermate and iECKO mice

(**a**) NeuN, (**b**) GFAP and (c) IgG + areas were quantified as a percentage of the area occupied in cortical region. Each point represents a different mouse. Data are represented as individual values plus means ± SD. n=5-6 mice/ group. One-way ANOVA was performed. ns, non significant, * $p<0.05$, ** $p<0.001$. Scale bar represents 100 μm.

Fig S9: representative hippocampal immunohistochemical staining for (a) IgG (red) and NeuN (green) in left panel and for Iba1 (red) and GFAP (green) in right panel in APP/PS1; iECKO vs their age-matched littermates APP/PS1; Litt at 6 and 12 months vs non demented (ND) iECKO vs their age-matched littermate groups at 6 months.

(**b**) NeuN+ cells were quantified as a percentage of the area occupied in the CA1 area (**c**) IgG (**d**) GFAP and (e) Iba1 + areas were quantified as a percentage of the area occupied in hippocampal region. Each point represents a different mouse. Data are represented as individual values plus means ± SD. n=6 mice/APP/PS1 group at 6 months; n=5-6 mice/ group. One-way ANOVA was performed. ns, non significant, * $p<0.05$, ** $p<0.001$. Scale bar represents 100 μm.

(**g**) and (**h**) quantification for GFAP before (sham) and at 21 days after GCAS in littermate and iECKO mice. Data shown in the graphs are obtained in sham condition, from 3 mice per group. Data shown in the graphs are obtained in sham condition, from 3 mice per group; in GCAS condition, from n=10 IECKO vs n=11 littermate mice. Data for four brain slices per mouse were recorded and averaged to produce a single value for each mouse.