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Supplemental information

Astrocyte calcium dysfunction causes early network hyperactivity in Alzheimer's disease

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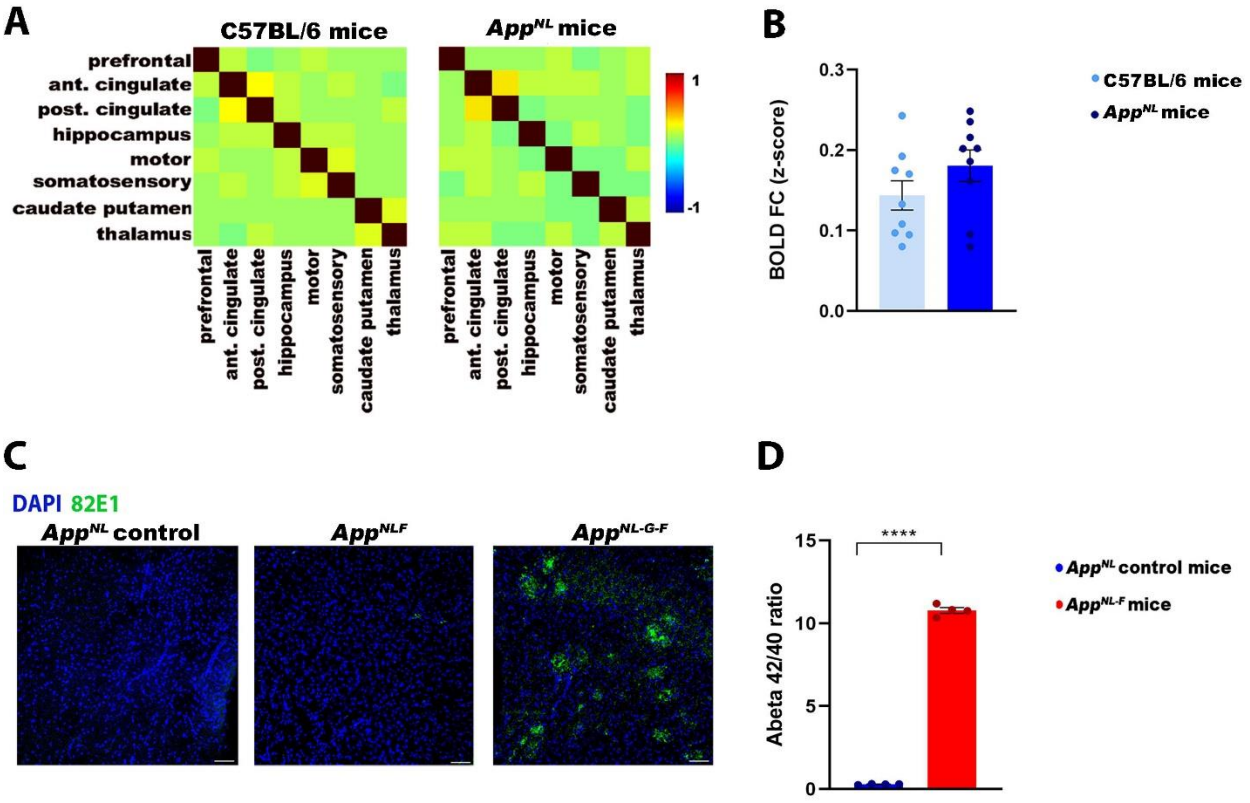


<u>Figure S1, related to Figure 1:</u> RsfMRI controls and amyloid pathology in *App* mice. A) Mean BOLD FC matrices show correlation between BOLD signals in C57BL/6 mice and *App*^{NL} mice (N=9/group). There were no differences between groups (two-sample T-test, FDR correction). Color scale shows z-scores. **B)** Graph shows BOLD FC between anterior-posterior cingulate cortex (z-scores±SEM) for each group. **C)** DAPI and 82E1 staining confirm lack of amyloid plaques in the cingulate cortex of 3 months old *App*^{NL} and *App*$^{NL-F}$ mice. We used 3 months old *App*$^{NL-G-F}$ mice as a positive control for the plaque staining. Scale bar=50 µm. **D)** Graph shows amyloid-beta 42/40 ratio's±SEM in the cingulate cortex of 3 months old *App*^{NL} and *App*$^{NL-F}$ mice (N=4/group). ****p<0.0001, two-sample T-test.

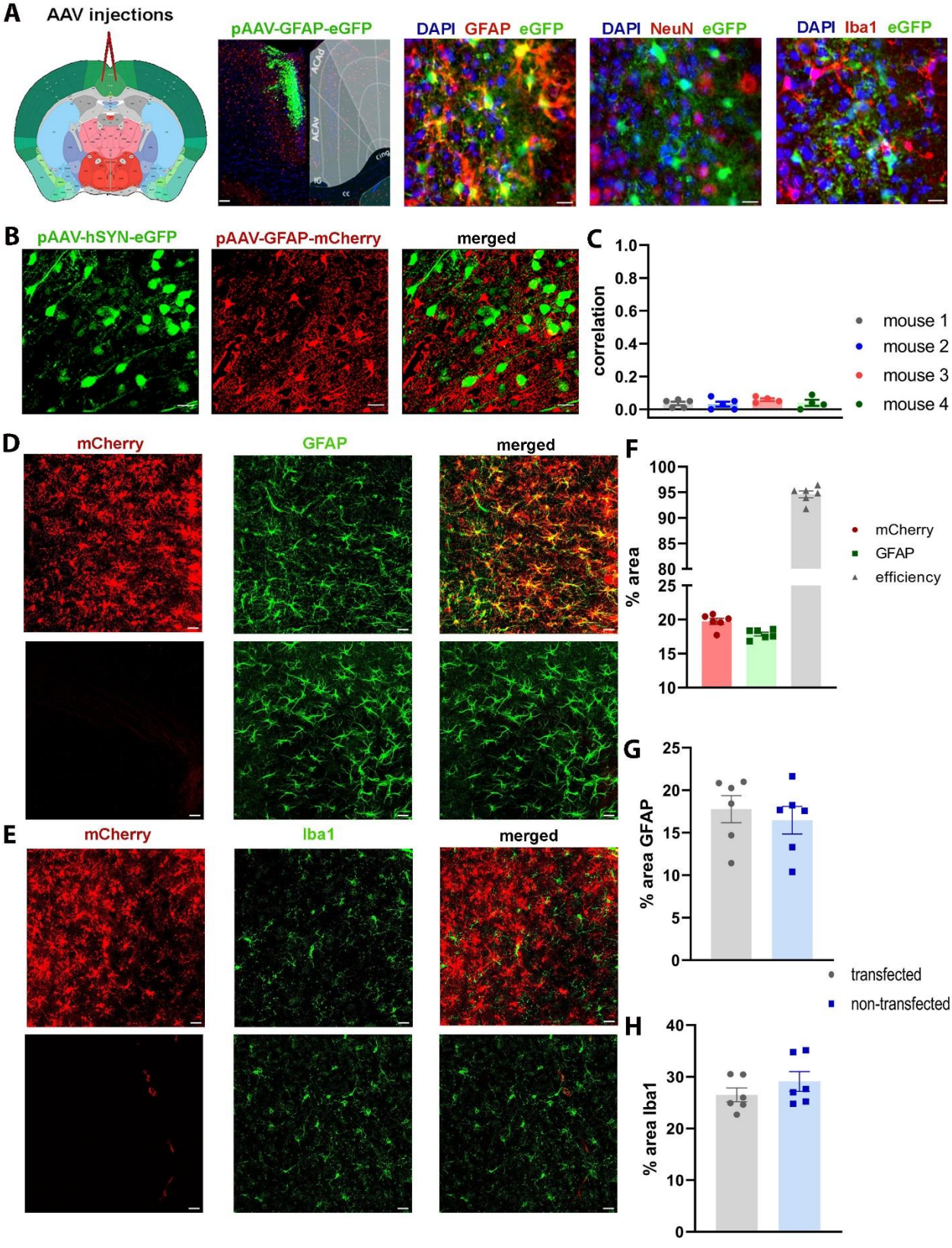

A
AAV injections
pAAV-GFAP-eGFP
DAPI GFAP eGFP
DAPI NeuN eGFP
DAPI Iba1 eGFP
B
pAAV-hSYN-eGFP
pAAV-GFAP-mCherry
merged
C
correlation
1.0
0.8
0.6
0.4
0.2
0.0
mouse 1
mouse 2
mouse 3
mouse 4
D
mCherry
GFAP
merged
E
mCherry
Iba1
merged
F
% area
100
95
90
85
80
25
20
15
10
mCherry
GFAP
efficiency
G
% area GFAP
25
20
15
10
5
0
transfected
non-transfected
H
% area Iba1
40
30
20
10
0

Figure S2, related to Figure 2: AAV controls. A) Injection site of local AAV injections in the cingulate cortex shown on the Allen Brain Atlas. pAAV-GFAP-eGFP expression was observed in the target region, i.e. anterior cingulate cortex and there was co-staining with astrocyte marker GFAP, but not with neurons (NeuN) or microglia (Iba1). Scale bar=10µm. **B)** Stereotactical co-injection of pAAV-hSYN-eGFP and pAAV-GFAP-mCherry shows no co-staining, confirming specificity of each AAV. Scale bar=20µm. **C)** Quantification of co-expression (correlation±SEM) between pAAV-hSYN-eGFP and pAAV-GFAP-mCherry (N=4 mice, 5 slices per mouse). **D-E)** Co-staining of GFAP (**D**) and Iba1 (**E**) with pAAV-GFAP-mCherry in transfected (upper panel) and non-transfected areas (lower panel). Scale bar=20µm. **F)** Efficiency of AAV-GFAP-mCherry transfection is shown (% area±SEM), by quantifying mCherry+GFAP/total GFAP staining (N=6 mice, 5 slices per mouse). **G-H**) Quantification of GFAP and Iba1 staining (% area±SEM) in transfected and non-transfected regions (N=6 mice, 5 slices per mouse).

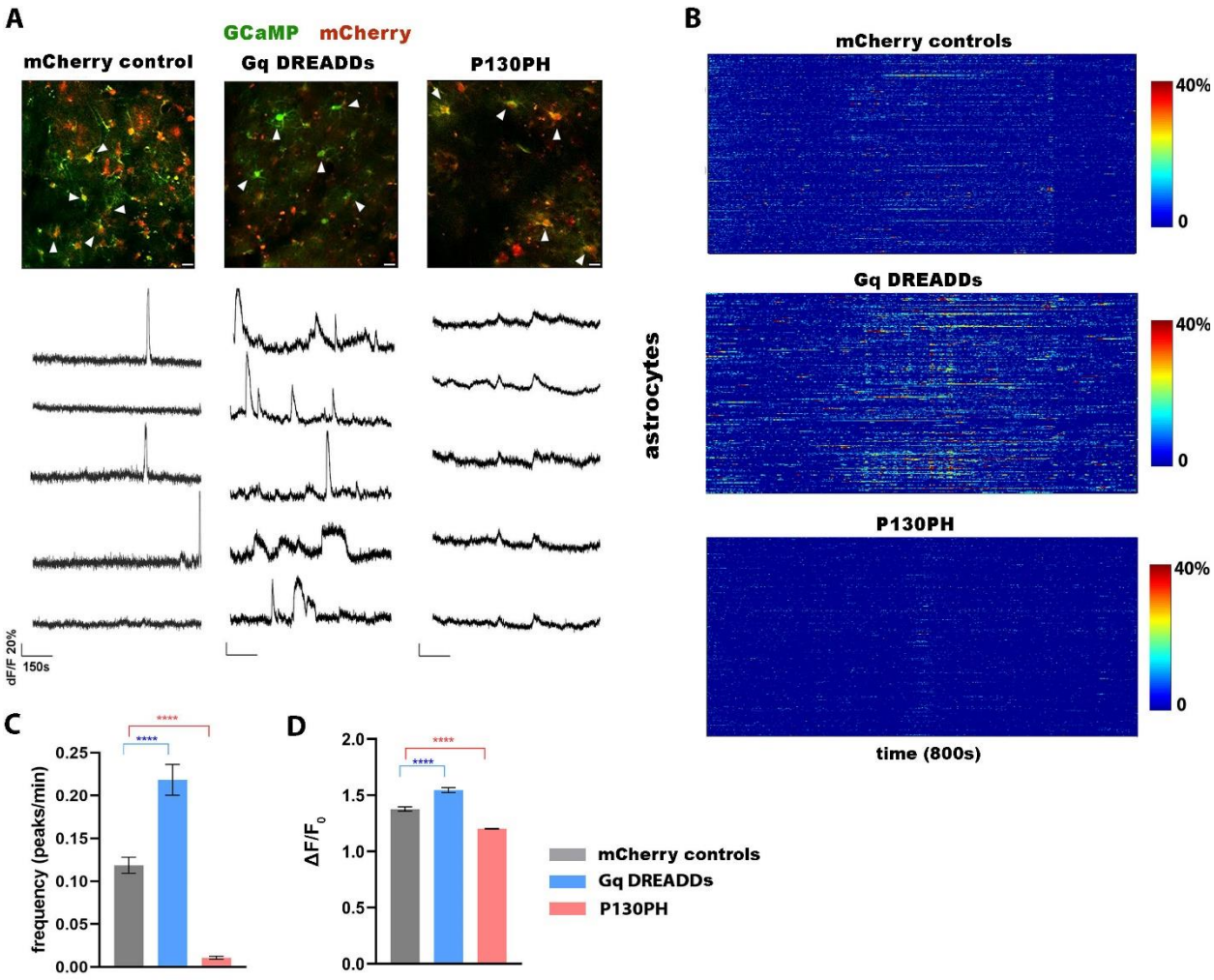


Figure S3, related to Figure 2: Modulation of astrocyte calcium signaling in-vivo. A) Representative $\Delta F/F_0$ calcium traces of astrocytes expressing calcium indicator GCaMP6f, in addition to either DREADDs or P130PH (GFAP promotor). DREADDs elicit increased calcium signaling in astrocytes upon CNO injection (3mg/kg) and P130PH expression causes decreased calcium signaling (4 weeks expression) compared to C57BL/6 mice expressing mCherry. Scale bar=20µm. **B)** Heat maps showing astrocyte calcium signals ($\Delta F/F_0$ exceeding 2*SD baseline, Y-axis) over time (800s, X-axis) in C57BL/6 mice expressing mCherry (N=5 mice, 329 cells), DREADDs (N=5 mice, 263 cells), and P130PH (N=5 mice, 353 cells). **C-D)** Graphs show quantification of frequency (number of peaks per minute±SEM) and signal amplitude ($\Delta F/F_0$±SEM), ****p<0.0001, one-way ANOVA with Sidak correction for multiple comparisons.

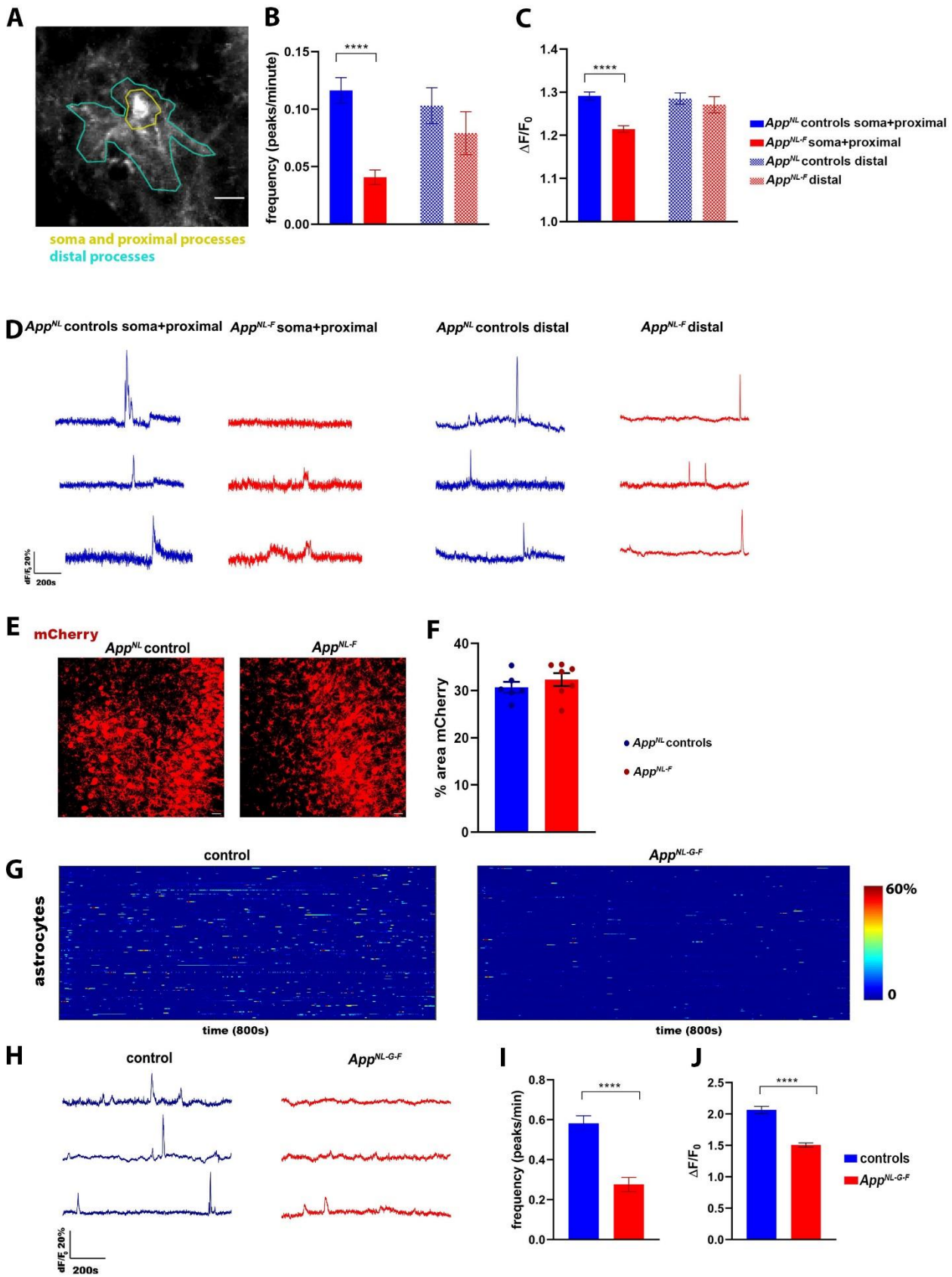

A
soma and proximal processes
distal processes
B
frequency (peaks/minute)
0.15
0.10
0.05
0.00
****
C
ΔF/F0
1.4
1.3
1.2
1.1
1.0
****
AppNL controls soma+proximal
AppNL-F soma+proximal
AppNL controls distal
AppNL-F distal
D
AppNL controls soma+proximal
AppNL-F soma+proximal
AppNL controls distal
AppNL-F distal
dF/F0 20%
200s
E
mCherry
AppNL control
AppNL-F
F
% area mCherry
40
30
20
10
0
AppNL controls
AppNL-F
G
control
AppNL-G-F
astrocytes
time (800s)
time (800s)
60%
0
H
control
AppNL-G-F
dF/F0 20%
200s
I
frequency (peaks/min)
0.8
0.6
0.4
0.2
0.0
****
J
ΔF/F0
2.5
2.0
1.5
1.0
0.5
0.0
****
controls
AppNL-G-F

Figure S4, related to Figure 3: Astrocyte calcium signaling in *App* mice. A) Representative image showing delineation of soma and proximal processes (yellow) and distal processes (blue). Scale bar=10µm. **B-C)** Graphs show frequency (peaks per minute±SEM) and amplitude (dF/F_0±SEM) for each analyzed domain of astrocytes in 3 months old control and APP^{NL-F} mice. *p<0.05, ****p<0.0001, Kruskal-Wallis test with Dunn's correction. **D)** representative time traces of calcium signals in the different astrocyte domains for controls and APP^{NL-F} mice. **E)** Representative immunofluorescence images showing expression of pAAV-GFAP-mCherry in 3 months old App^{NL} control and App^{NL-F} mice. Scale bar=30µm. **F)** Graph shows pAAV-GFAP-mCherry expression (% area±SEM) for App^{NL} control (N=6, 5 slices per mouse) and App^{NL-F} mice (N=7, 5 slices per mouse). **G)** Heat maps show astrocyte calcium activity (>2*SD of baseline, Y-axis) over time (800s, X-axis) for 6 weeks old control mice (N=4 mice, 238 cells) and App^{NL-G-F} mice (N=4 mice, 277 cells) expressing GCaMP6f in astrocytes (GFAP promoter). **H)** Representative $\Delta F/F_0$ calcium traces of astrocytes in the cingulate cortex of control and App^{NL-G-F} mice. **I-J)** Graphs show frequency (number of peaks per minute±SEM) and signal amplitude ($\Delta F/F_0$±SEM). ****p<0.0001, Kruskal-Wallis test with Dunn's correction.

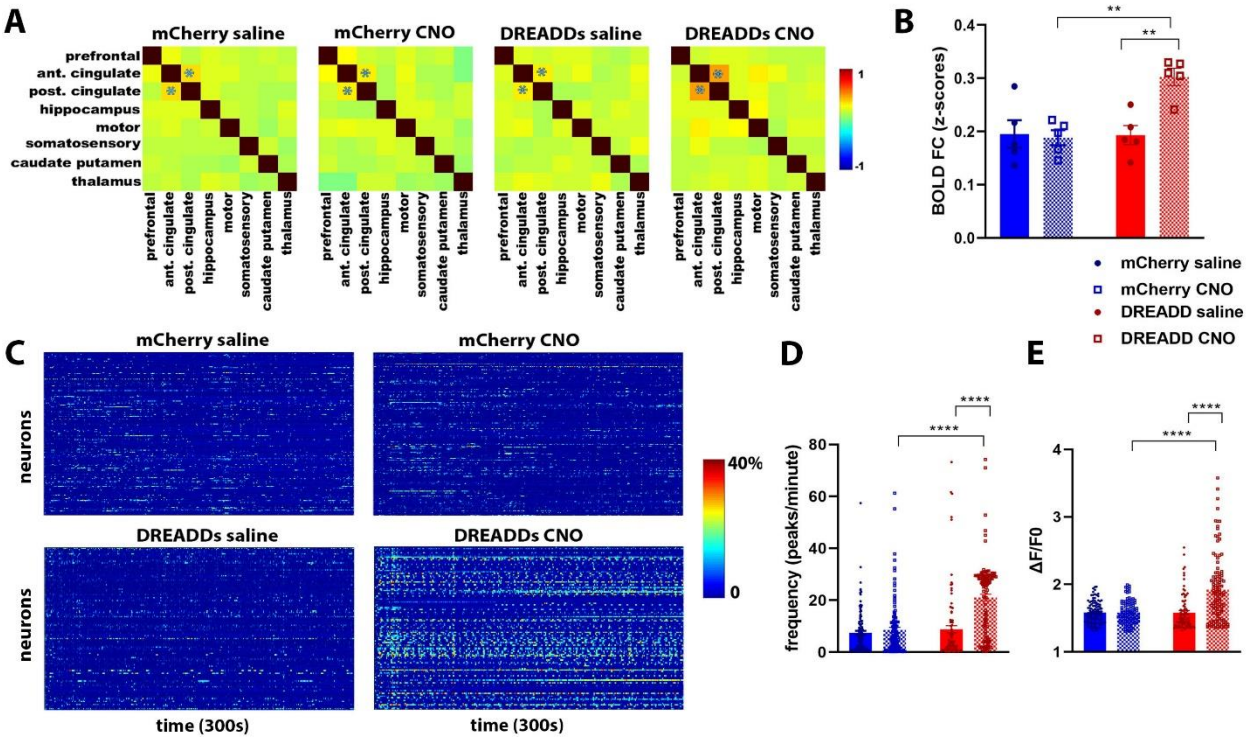


Figure S5, related to Figure 4: Saline and CNO controls for in-vivo studies. A) Mean BOLD FC matrices show correlation between BOLD signals in C57BL/6 mice expressing mCherry or DREADDs after injection of saline or CNO (3mg/kg) (N=5/group). Color scale shows z-scores. BOLD FC between the anterior and posterior cingulate cortex is indicated by *. **B)** Graph shows BOLD FC between anterior-posterior cingulate cortex (z-scores±SEM) for each condition. **p<0.01, one-way ANOVA with Sidak correction. **C)** Heat maps show neuronal calcium activity ($\Delta F/F_0$ exceeding 2*SD baseline, Y-axis) over time (300s, X-axis) in C57BL/6 mice expressing mCherry injected with saline (N=3 mice, 104 neurons), or CNO (3mg/kg, N=3 mice, 106 neurons), and C57BL/6 mice expressing DREADDs injected with saline (N=3 mice, 86 neurons) or CNO (3mg/kg, N=3 mice, 112 neurons). **D-E)** Graphs show frequency (number of peaks per minute±SEM) and signal amplitude ($\Delta F/F_0$±SEM). ****p<0.0001, Kruskal-Wallis test with Dunn's correction.

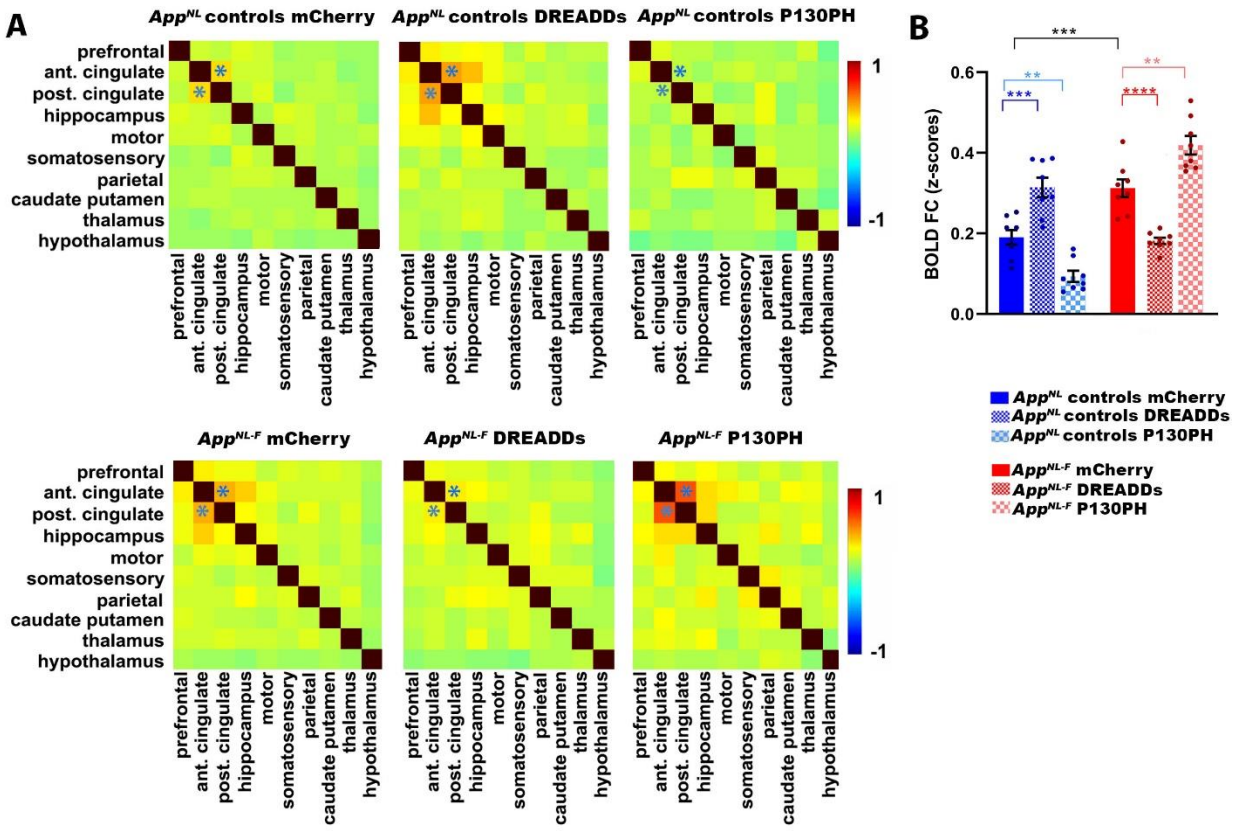
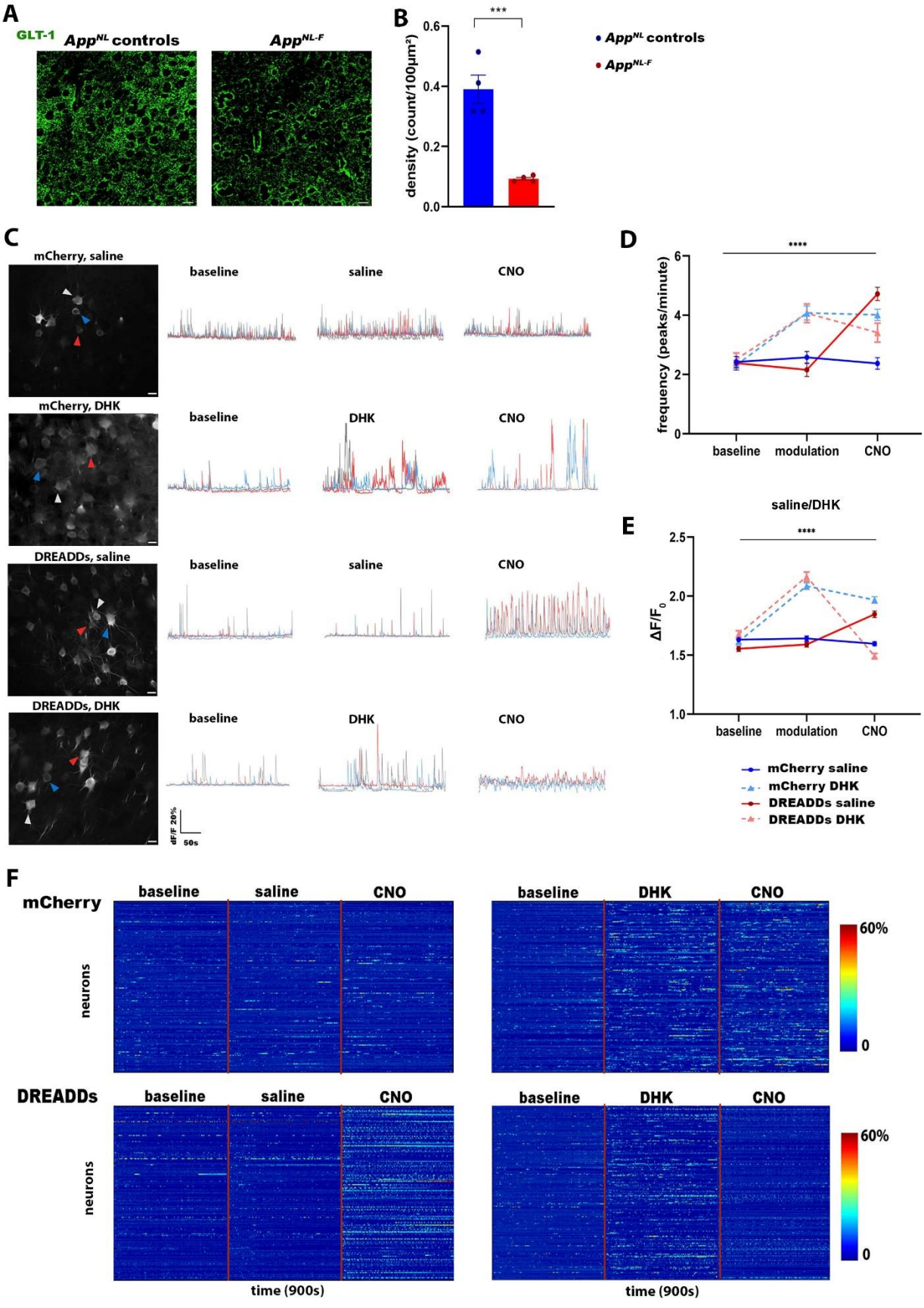


Figure S6, related to Figure 5: Exacerbation of astrocyte disruptions in *App*$^{NL-F}$ mice worsens hypersynchrony of BOLD signals in the cingulate cortex. A) BOLD FC matrices upon modulation of astrocyte calcium activity in *APP*^{NL} control and *App*$^{NL-F}$ mice expressing mCherry, DREADDs or p130PH (N=8/group). The connection between the anterior and posterior cingulate cortex is indicated by * on the matrices. **B)** Graphs show BOLD FC between the anterior and posterior cingulate cortex (z-scores±SEM) for each group. **p<0.01, ***p<0.001, ****p<0.0001, one-way ANOVA with Sidak correction for multiple comparisons.


A
GLT-1
App^NL controls
App^NL-F
B
***
density (count/100μm²)
0.6
0.4
0.2
0.0
App^NL controls
App^NL-F
C
mCherry, saline
baseline
saline
CNO
mCherry, DHK
baseline
DHK
CNO
DREADDs, saline
baseline
saline
CNO
DREADDs, DHK
baseline
DHK
CNO
dF/F 20%
50s
D
****
frequency (peaks/minute)
6
4
2
0
baseline
modulation
CNO
E
saline/DHK
****
ΔF/F0
2.5
2.0
1.5
1.0
baseline
modulation
CNO
mCherry saline
mCherry DHK
DREADDs saline
DREADDs DHK
F
mCherry
baseline
saline
CNO
baseline
DHK
CNO
neurons
60%
0
DREADDs
baseline
saline
CNO
baseline
DHK
CNO
neurons
60%
0
time (900s)
time (900s)

<u>Figure S7, related to Figure 4:</u> Intact calcium signaling in astrocytes allows regulation of neuronal activity in the healthy brain. A) Representative immunofluorescence images of GLT-1 expression in 3 months old App^{NL} control and App^{NL-F} mice. Scale bar=20µm. **B)** Graph shows GLT-1 expression (density/100µm²±SEM) for App^{NL} control and App^{NL-F} mice (N=4, 5 slices per mouse). **C)** Representative images and $\Delta F/F_0$ calcium time traces of neurons from C57BL/6 mice that were injected with dihydrokainic acid (DHK, 10mg/kg) or saline prior to injection of CNO (3mg/kg) to activate DREADDs in astrocytes (N=4/group, mCherry saline N=302 neurons, mCherry DHK N=290 neurons, DREADDs saline N=384 neurons, DREADDs DHK N=200 neurons). Scale bar=20µm. **D-E)** Quantification of frequency (number of peaks/min±SEM) and amplitude ($\Delta F/F_0$±SEM) for each condition. ****p<0.0001 group effect, p<0.0001 time effect, two-way ANOVA with Sidak correction for multiple comparisons. No significant differences were found for $\Delta F/F_0$ amplitude or frequency in mCherry mice injected with saline. Significant differences were observed in mCherry mice injected with DHK ($\Delta F/F_0$ and frequency: baseline versus DHK p<0.0001), while subsequent injection of CNO did not cause changes. Significant differences were observed for mice expressing DREADDs injected with CNO ($\Delta F/F_0$ and frequency, baseline or saline versus CNO, p<0.0001). Finally, significant differences were found for mice expressing DREADDs and injected with DHK ($\Delta F/F_0$ and frequency for baseline versus DHK p<0.0001; DHK versus CNO $\Delta F/F_0$ p<0.0001 and frequency p<0.05). **F)** Heatmaps show $\Delta F/F_0$ calcium time courses for all neurons ($\Delta F/F_0$ exceeding 2*SD baseline, Y-axis) over time (900s, X-axis). Red line indicates each 300 seconds scan session, i.e. baseline, after injection of saline or DHK, and after subsequent injection of CNO.

Table S1, related to STAR methods: Characteristics of F-PACK participants included for analysis. For each characteristics the mean±SEM are shown per group.

	controls	Amyloid accumulators
N	24	10
baseline CL	7.1±1.1	7.7±2.1
T1 CL	6.1±1.6	42±5.
Age (years)	78±1.0	77±2.0
gender	N=12 female, N=12 male	N=5 female, N=5 male
baseline education (years)	13±0.56	13±1.04
baseline MMSE	29±0.16	29±0.27
T1 MMSE	29±0.17	29±0.3

Table S2, related to STAR methods: Seizure scoring

score	Behaviour description
1	Movement arrest
2	Neck jerks
3	Clonic seizures, sitting position
4	Myotonic seizures, flat on belly
5	Clonic or clonic-tonic seizures, sitting position
6	Clonic or clonic-tonic seizures with loss of balance, lying on side