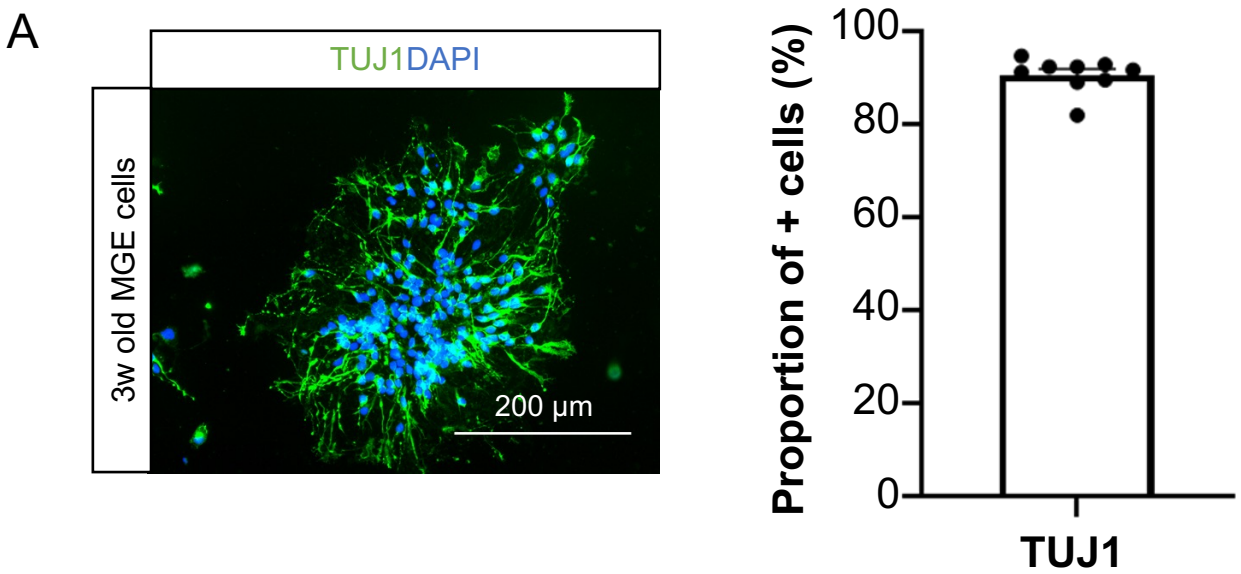
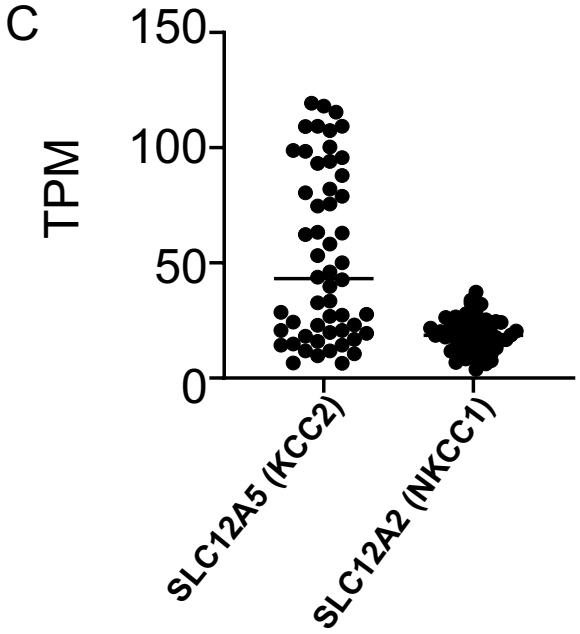
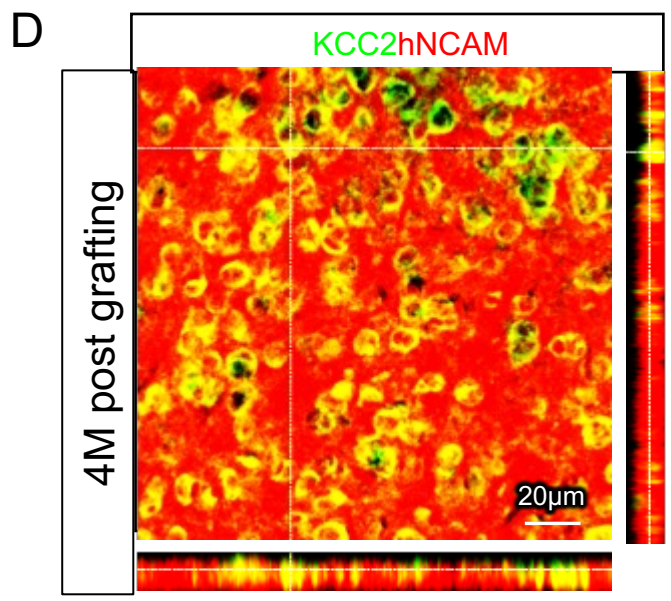
Fig. S1


A
TUJ1DAPI
3w old MGE cells
200 µm
Proportion of + cells (%)
100
80
60
40
20
0
TUJ1
B
iPSC
6 week cIN
OCT4 DAPI
13 µm
13 µm


C
TPM
150
100
50
0
SLC12A5 (KCC2)
SLC12A2 (NKCC1)


D
KCC2hNCAM
4M post grafting
20µm

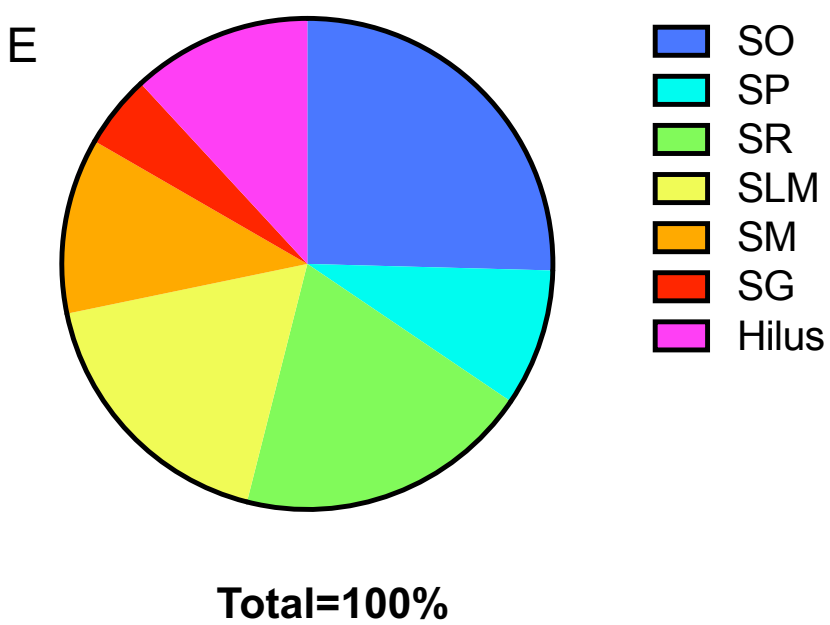
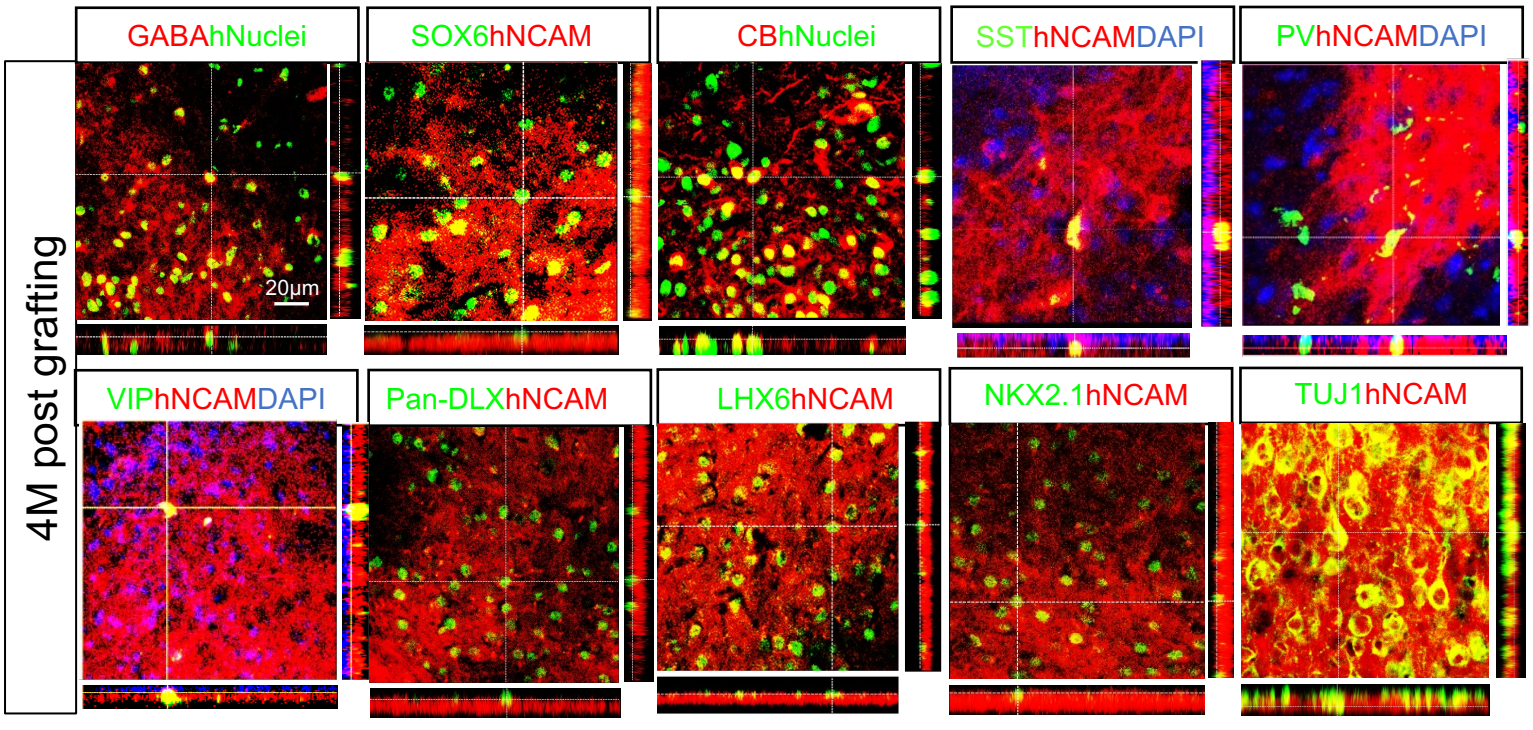
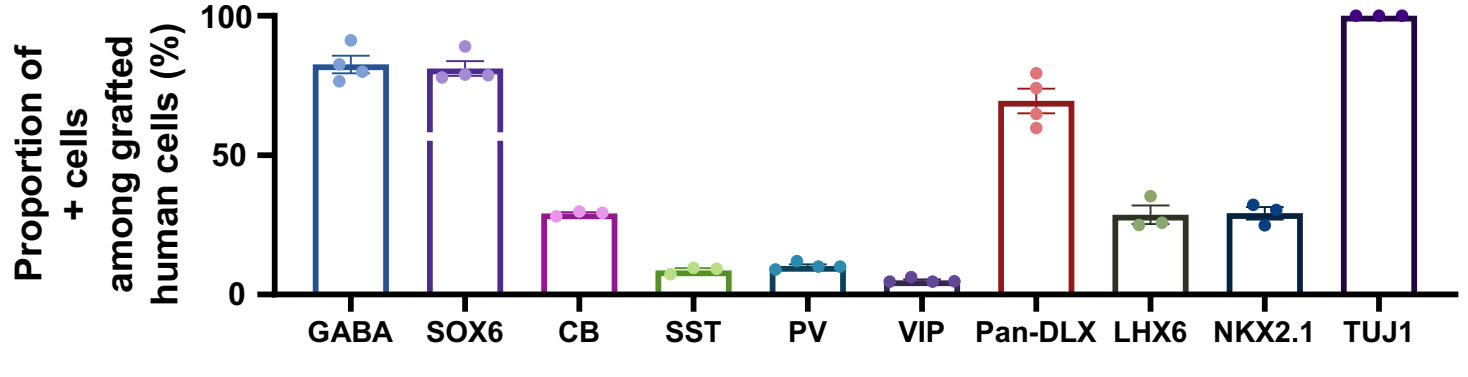

E
SO
SP
SR
SLM
SM
SG
Hilus
Total=100%

Fig. S1. Immunocytochemistry and gene expression analysis of hESC-derived cINs *in vitro*, Related to Fig.1.
(A) H9-derived cINs were analyzed for the expression of TUJ1 after three weeks of differentiation (mean ± SEM), analyzed from nine pictures from three independent differentiation batches.
(B) H9-derived cINs were analyzed for the expression of OCT4 after six weeks of differentiation with CDP treatment. Human iPSCs were also stained for OCT4 expression as a positive control.
(C) Expression of SLC12A5 (KCC2) and SLC12A2 (NKCC1) in human iPSC-derived cINs after eight weeks of differentiation as in our previous study [S1] (n=56 batches of independent differentiations from 28 independent iPSC-derived human cINs with 2 independent differentiations per line). RNA-seq data were deposited in GEO (https://www.ncbi.nlm.nih.gov/geo/) with accession numbers GSE121376.
(D) Grafted human cINs were analyzed by immunohistochemistry for expression of KCC2. Right side and bottom panel of the micrograph shows z-stack side views.
(E) Proportion of grafted human cINs in each hippocampal layer (Total=100%). SO: stratum oriens, SP: stratum pyramidale, SR: stratum radiatum, SLM: stratum lacunosum moleculare, SM: stratum moleculare, SG: stratum granulosum.

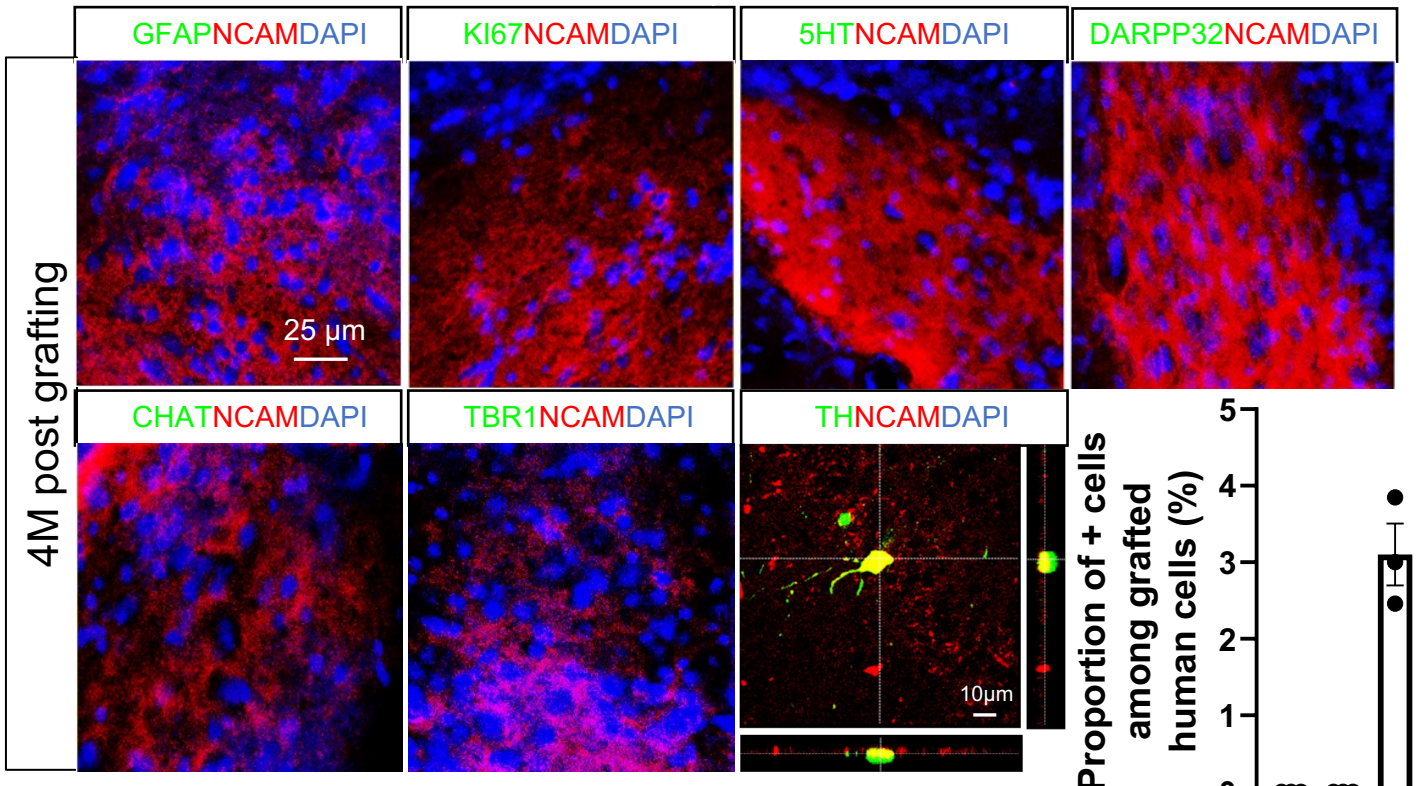
Fig. S2

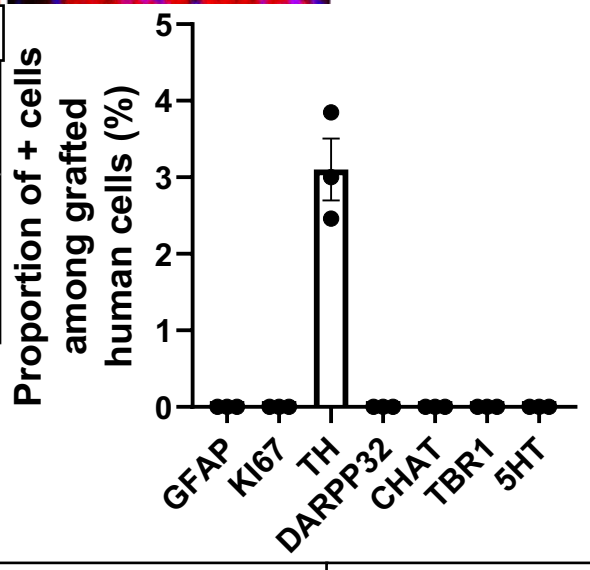
A


GABAhNuclei
SOX6hNCAM
CBhNuclei
SSThNCAMDAPI
PVhNCAMDAPI
4M post grafting
20µm
VIPhNCAMDAPI
Pan-DLXhNCAM
LHX6hNCAM
NKX2.1hNCAM
TUJ1hNCAM


Proportion of + cells among grafted human cells (%)
100
50
0
GABA
SOX6
CB
SST
PV
VIP
Pan-DLX
LHX6
NKX2.1
TUJ1

B


GFAPNCAMDAPI
KI67NCAMDAPI
5HTNCAMDAPI
DARPP32NCAMDAPI
4M post grafting
25 µm
CHATNCAMDAPI
TBR1NCAMDAPI
THNCAMDAPI
10µm


Proportion of + cells among grafted human cells (%)
5
4
3
2
1
0
GFAP
KI67
TH
DARPP32
CHAT
TBR1
5HT

C

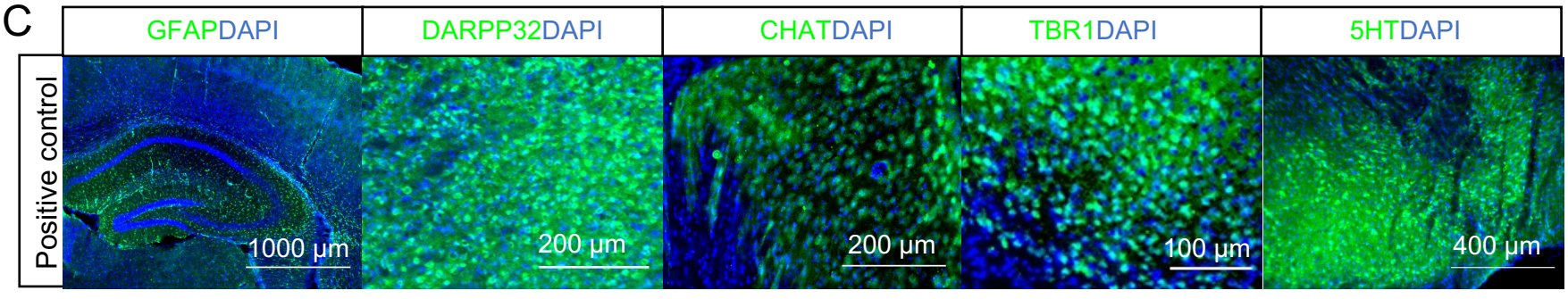

GFAPDAPI
DARPP32DAPI
CHATDAPI
TBR1DAPI
5HTDAPI
Positive control
1000 µm
200 µm
200 µm
100 µm
400 µm

Fig. S2. Immunohistochemistry and cell counting analysis of hESC-derived cINs four months after transplantation into PILO-TLE mouse hippocampus, Related to Fig .1.
(A-B) Grafted human cINs were analyzed by immunohistochemistry and cell counting (mean ± SEM), analyzed from every 12th 40µm coronal brain section from 3-4 mice per group. Right side and bottom panel of each micrograph shows z-stack side views.
(C) Representative immunostaining images of GFAP, DARPP32, CHAT, TBR1 and 5HT as positive controls.

Fig. S3

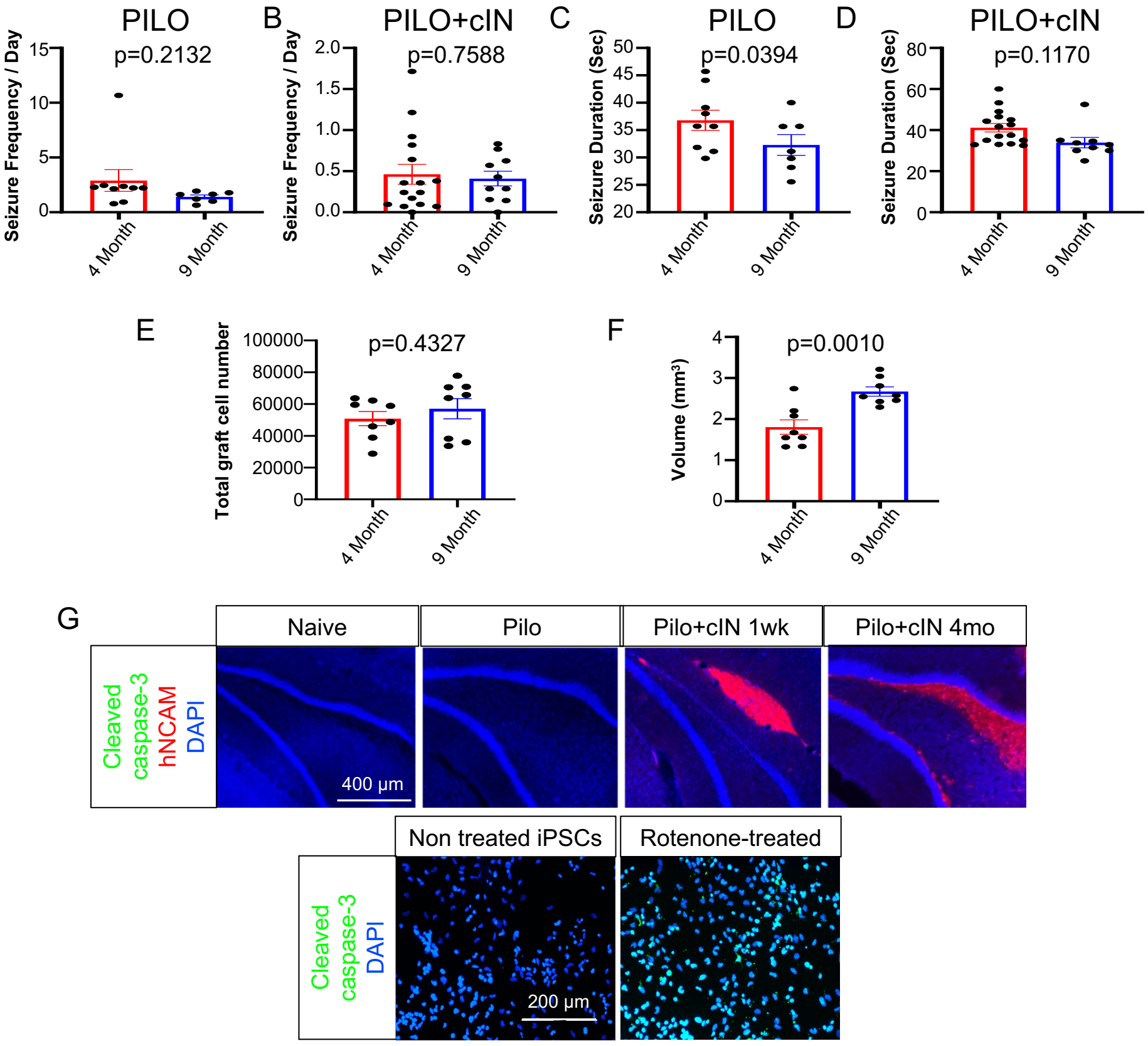
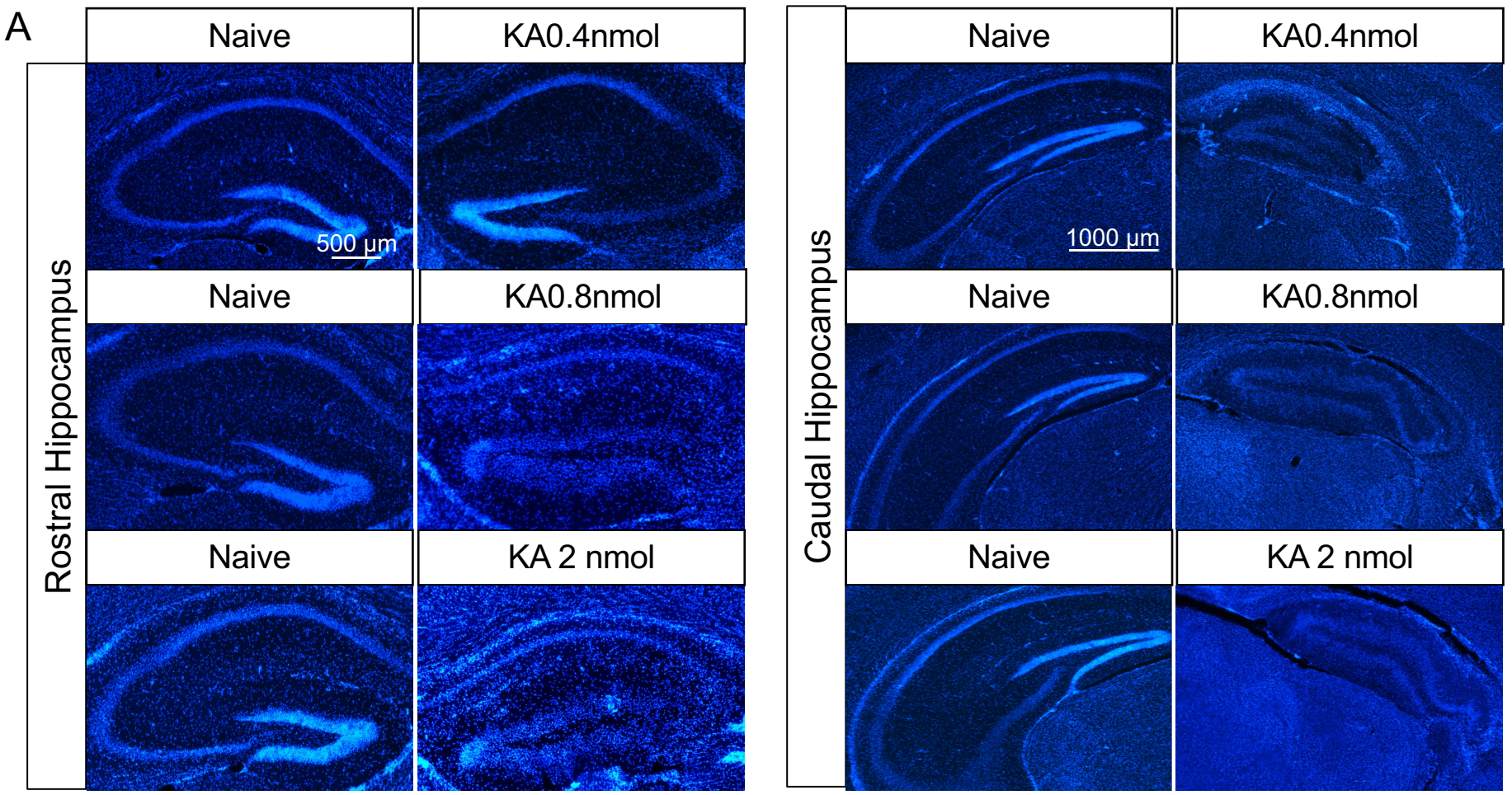

A
PILO
p=0.2132
Seizure Frequency / Day
15
10
5
0
4 Month
9 Month
B
PILO+cIN
p=0.7588
Seizure Frequency / Day
2.0
1.5
1.0
0.5
0.0
4 Month
9 Month
C
PILO
p=0.0394
Seizure Duration (Sec)
50
45
40
35
30
25
20
4 Month
9 Month
D
PILO+cIN
p=0.1170
Seizure Duration (Sec)
80
60
40
20
0
4 Month
9 Month
E
p=0.4327
Total graft cell number
100000
80000
60000
40000
20000
0
4 Month
9 Month
F
p=0.0010
Volume (mm³)
4
3
2
1
0
4 Month
9 Month
G
Naive
Pilo
Pilo+cIN 1wk
Pilo+cIN 4mo
Cleaved caspase-3
hNCAM
DAPI
400 µm
Non treated iPSCs
Rotenone-treated
Cleaved caspase-3
DAPI
200 µm

Fig. S3. Analysis of PILO-TLE models with human cIN grafts, Related to Fig.2.
(A) Seizure frequency of control PILO-TLE mice at four months and nine months post-transplant were analyzed by non-parametric Mann-Whitney test (mean ± SEM; n=9 mice at four months and n=7 at nine months).
(B) Seizure frequency of cIN-grafted PILO-TLE mice at four months and nine months post-transplant were analyzed by non-parametric Mann-Whitney test (mean ± SEM; n=16 mice at four months and n=9 at nine months).
(C) Seizure duration of control PILO-TLE mice at four months and nine months post-transplant were analyzed by non-parametric Mann-Whitney test (mean ± SEM; n=9 mice at four months and n=7 at nine months).
(D) Seizure duration of cIN-grafted PILO-TLE mice at four months and nine months post-transplant were analyzed by parametric two-tailed unpaired t-test (mean ± SEM; n=16 mice at four months and n=9 at nine months).
(E) Total grafted cell numbers at four months and nine months post-transplant were analyzed by parametric two-tailed unpaired t-test (mean ± SEM; n=8 bilateral grafts from four mice).
(F) Total graft volume at four months and nine months post-transplant were analyzed by parametric two-tailed unpaired t-test (mean ± SEM; n=8 bilateral grafts from 4 mice).
(G) Cell death analysis of naïve, KA-TLE, KA-TLE + cIN transplantation mice brains using cleaved caspase-3 antibody (Scale bar: 400 μm). Rotenone-treated iPSCs were used as a positive control (Scale bar: 200 μm).

Fig. S4



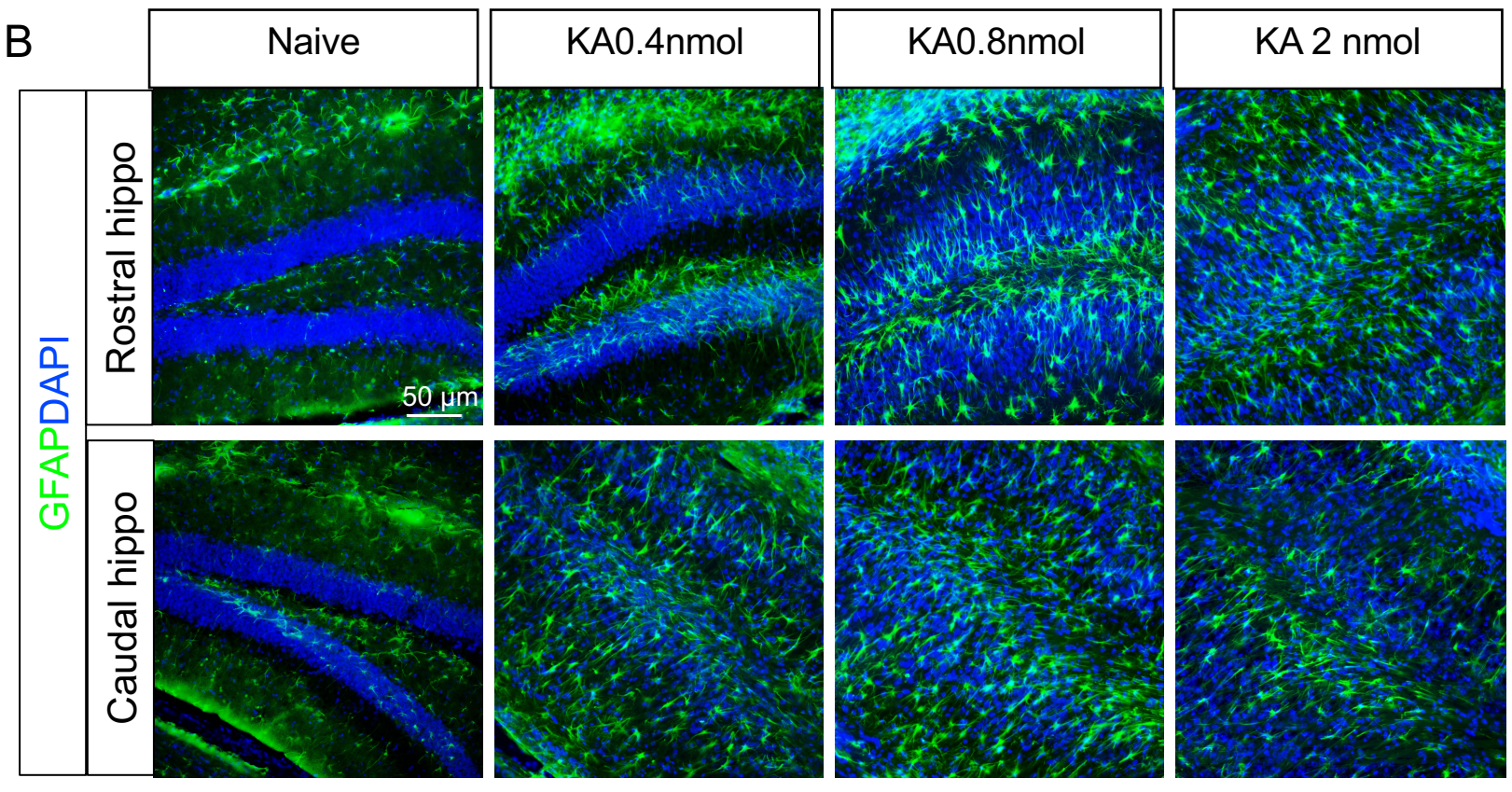


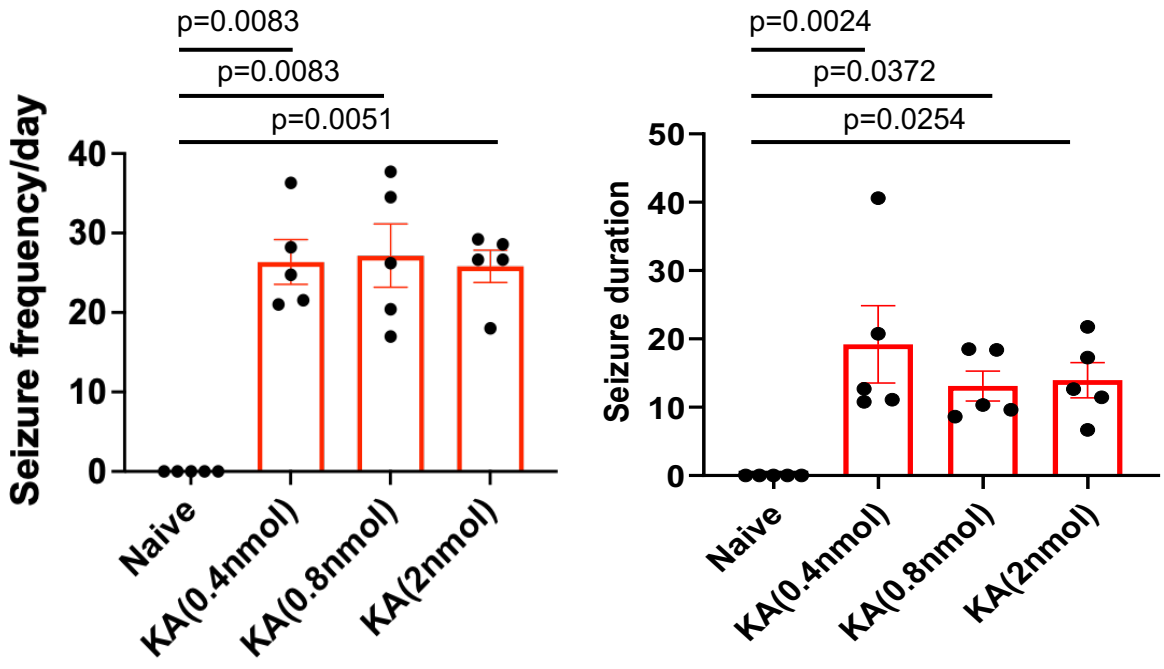
Fig. S4. Dose dependent effect of KA injection on granule cell dispersion and astrogliosis in the hippocampus, Related to Fig.4.

(A) Representative images of DAPI staining of rostral hippocampus (**Left**) and caudal hippocampus (**Right**), showing granular dispersion with 0.4 nmol, 0.8 nmol and 2 nmol of KA injection into hippocampus. Scale bar: 500 μm and 1000 μm.

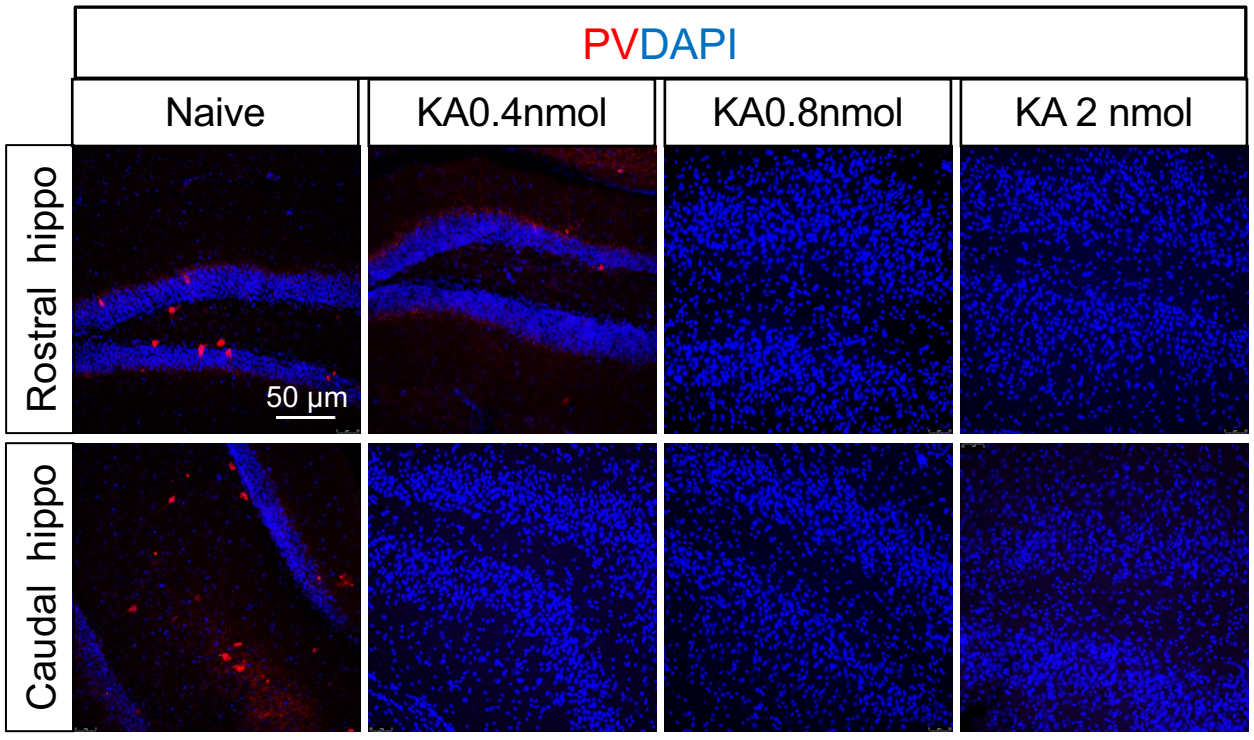
(B) Representative images showing immunostaining of GFAP in the rostral and caudal hippocampus with different doses of KA injection. Scale bar: 50 μm.

Fig. S5

A


p=0.0083
p=0.0083
p=0.0051
Seizure frequency/day
40
30
20
10
0
Naive
KA(0.4nmol)
KA(0.8nmol)
KA(2nmol)
p=0.0024
p=0.0372
p=0.0254
Seizure duration
50
40
30
20
10
0
Naive
KA(0.4nmol)
KA(0.8nmol)
KA(2nmol)

B


PVDAPI
Naive
KA0.4nmol
KA0.8nmol
KA 2 nmol
Rostral hippo
Caudal hippo
50 μm

C

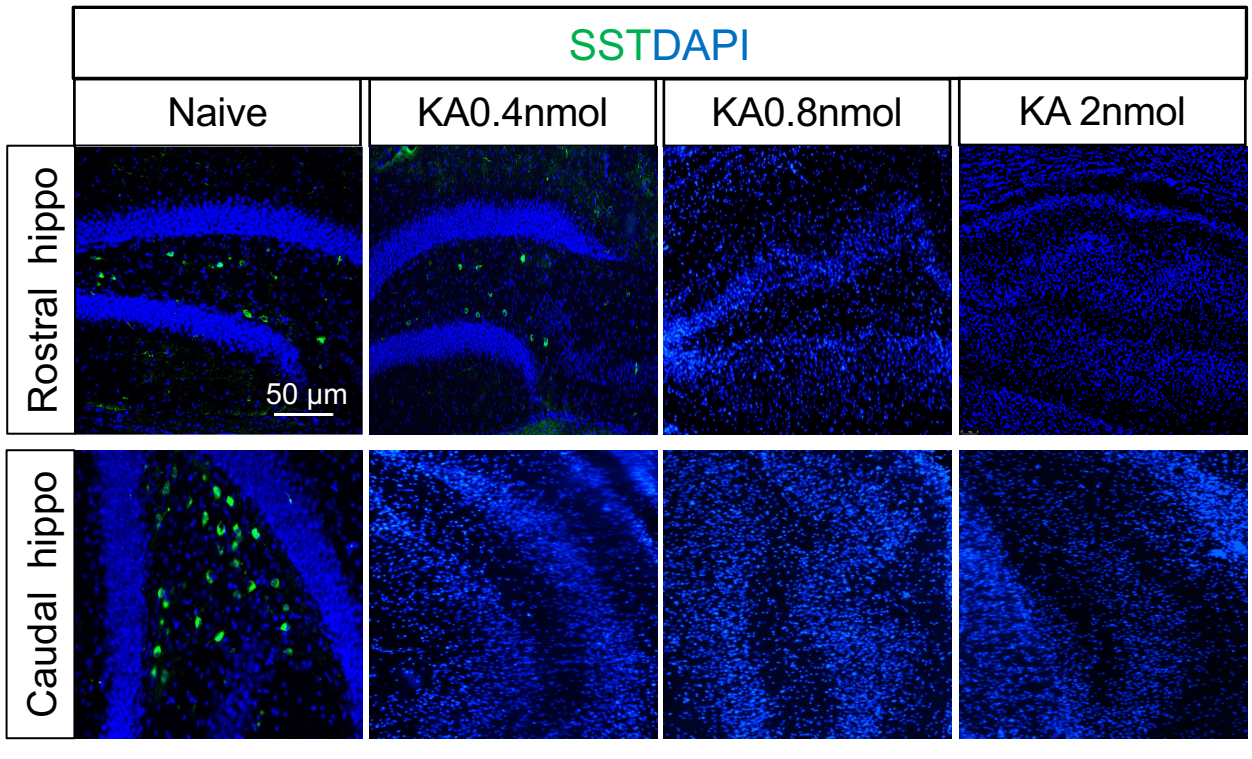

SSTDAPI
Naive
KA0.4nmol
KA0.8nmol
KA 2nmol
Rostral hippo
Caudal hippo
50 μm

Fig. S5. Dose dependent effect of KA injection on seizure activity and interneuron loss, Related to Fig.4.

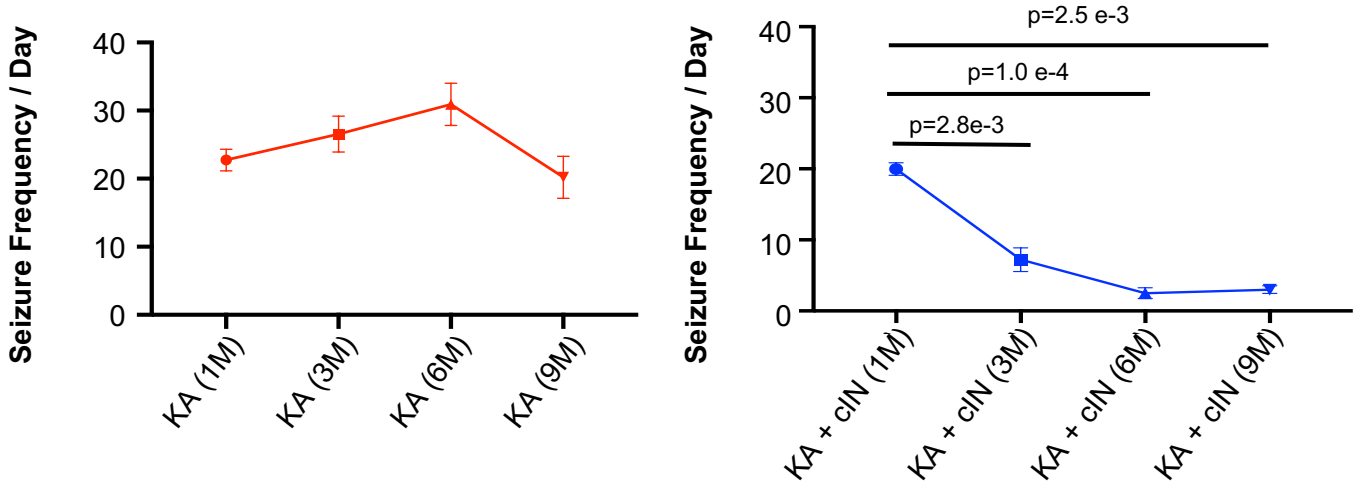
(A) Seizure frequency and duration analysis in KA-TLE that received different doses of (n=5 mice per group). Differences among groups were analyzed by non-parametric Kruskal-Wallis test (p=0.0122 for seizure frequency and p=0.0088 for seizure duration), followed by post hoc analysis using Dunnett's multiple comparisons test for seizure frequency and seizure duration (mean ± SEM). This analysis achieved 100% (seizure frequency) and 99% (seizure duration) power to reject the null hypothesis of equal means with a significance level of 0.05 and the observed effects.

(B) Representative images showing PV^+ interneurons in the rostral and caudal hippocampus at different doses of KA. Scale bar: 50 μm.

(C) Representative images showing SST^+ interneurons in the rostral and caudal hippocampus at different doses of KA. Scale bar: 50 μm.

Fig.S6

A



B

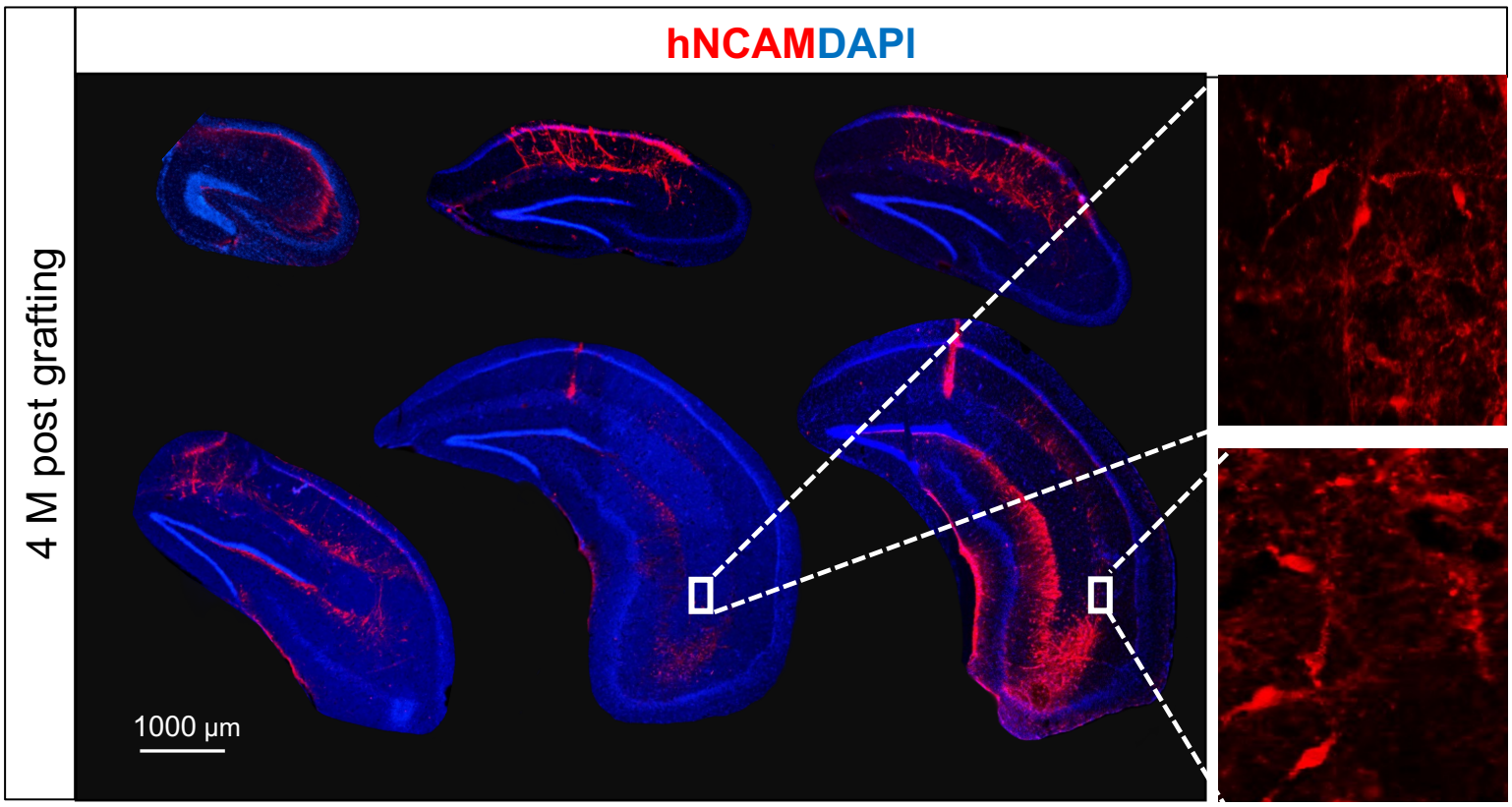
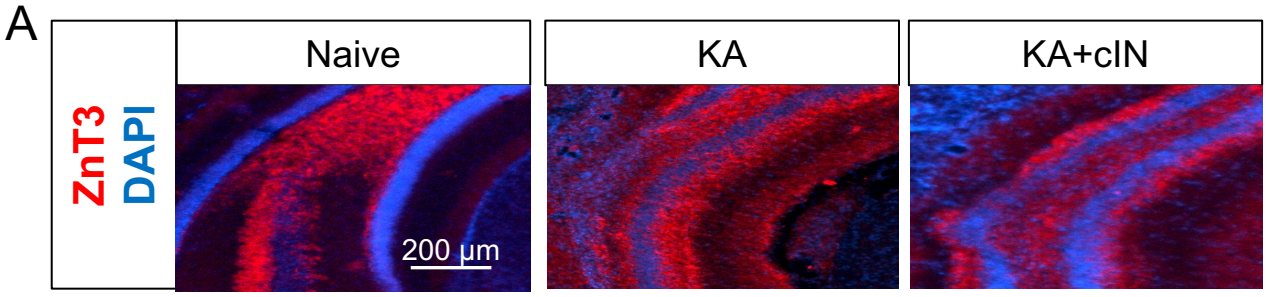


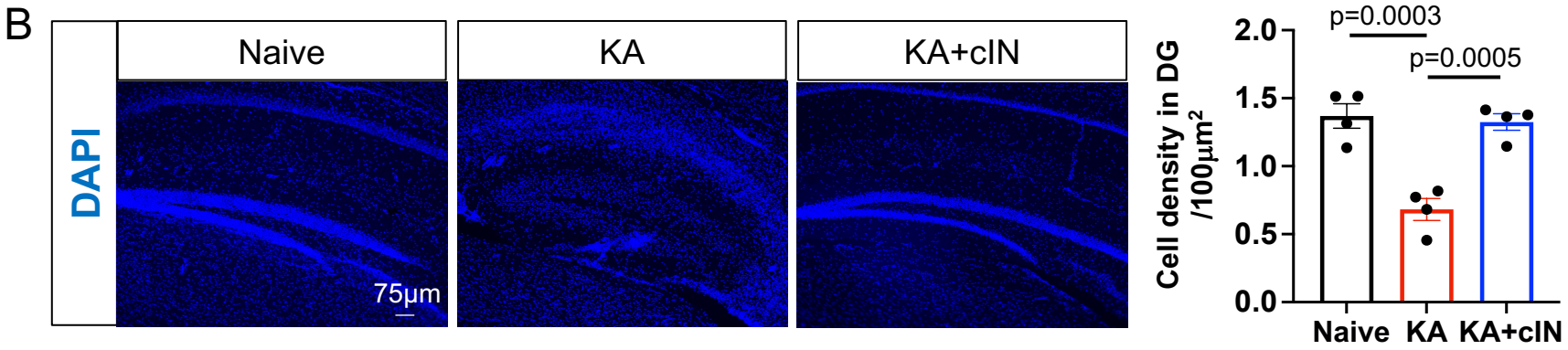
Fig. S6. Seizure analysis and histological analysis in KA-TLE models with human cIN grafts, Related to Fig.4.

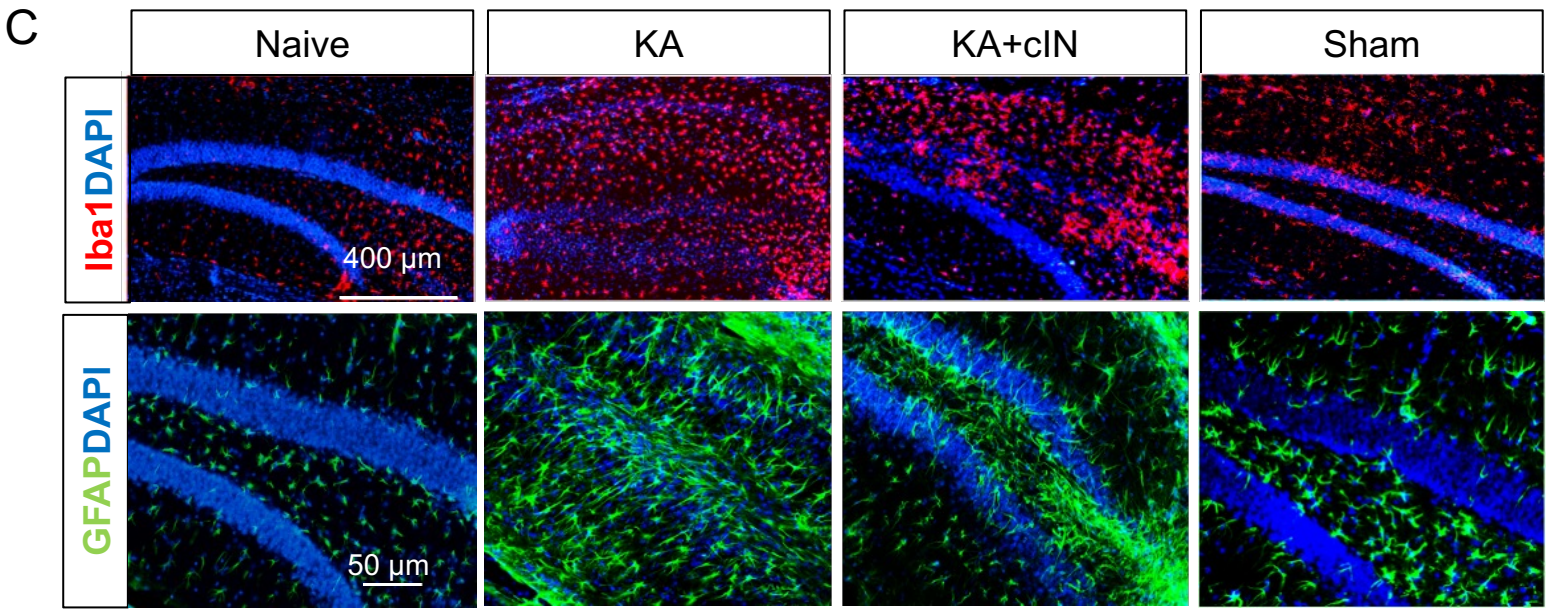
(A) Seizure frequency analysis with EEG at one month, three months, six months and nine months post-transplant in KA-TLE (Left Panel; n=11 mice for one month, n=8 mice for three and six months and n=5 mice for nine months) and KA-TLE + cIN transplantation (Right Panel; n=13 mice for one month, n=8 mice for three and six months and n=5 mice for nine months) groups. The differences among the group means were analyzed with One-way ANOVA for repeated measures (p=0.1480, F(1.610, 15.03)= 2.232 for KA group and p=6.1e-6, F(2.370, 14.22) = 41.72 for KA + cIN transplantation group), followed by post hoc analysis using Bonferroni's multiple comparisons test (mean ± SEM).

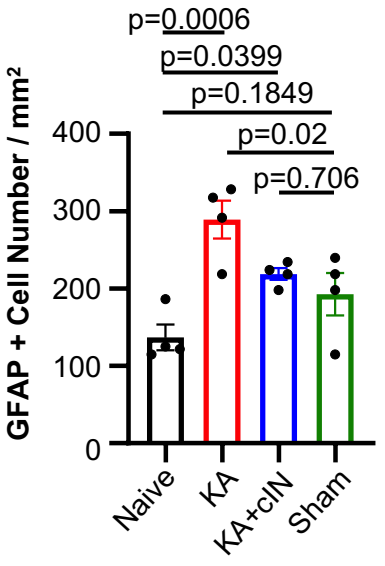
(B) Migration and integration of iPSC-derived human cINs into the KA-TLE mouse hippocampus, analyzed by human-specific NCAM antibody four months after transplantation. Scale bar: 1000 µm.

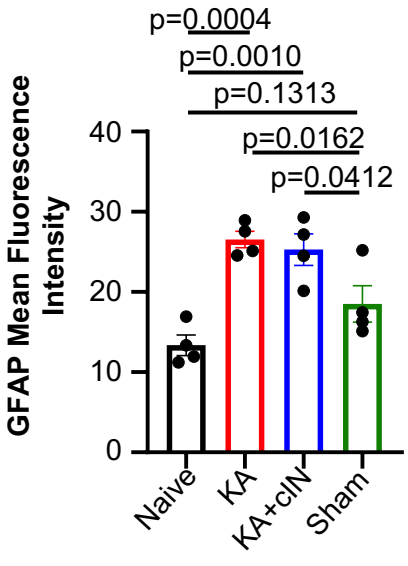
Fig. S7

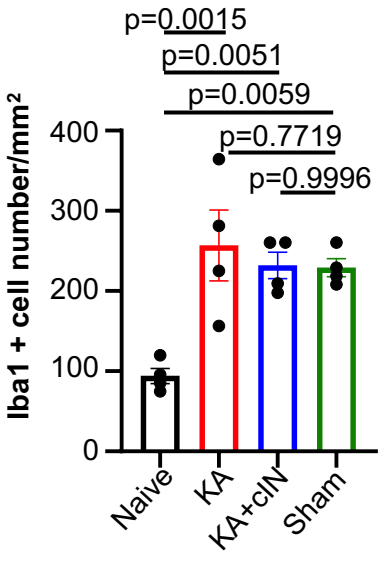

A
Naive
KA
KA+cIN
ZnT3
DAPI
200 µm

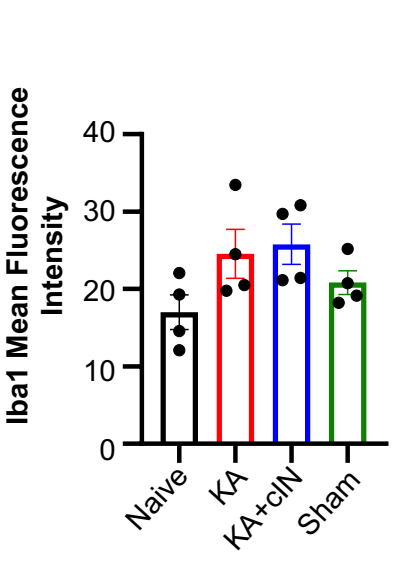

B
Naive
KA
KA+cIN
DAPI
75µm
p=0.0003
p=0.0005
Cell density in DG /100µm²
2.0
1.5
1.0
0.5
0.0
Naive
KA
KA+cIN


C
Naive
KA
KA+cIN
Sham
Iba1 DAPI
400 µm
GFAP DAPI
50 µm


p=0.0006
p=0.0399
p=0.1849
p=0.02
p=0.706
GFAP + Cell Number / mm²
400
300
200
100
0
Naive
KA
KA+cIN
Sham


p=0.0004
p=0.0010
p=0.1313
p=0.0162
p=0.0412
GFAP Mean Fluorescence Intensity
40
30
20
10
0
Naive
KA
KA+cIN
Sham


p=0.0015
p=0.0051
p=0.0059
p=0.7719
p=0.9996
Iba1 + cell number/mm²
400
300
200
100
0
Naive
KA
KA+cIN
Sham


Iba1 Mean Fluorescence Intensity
40
30
20
10
0
Naive
KA
KA+cIN
Sham

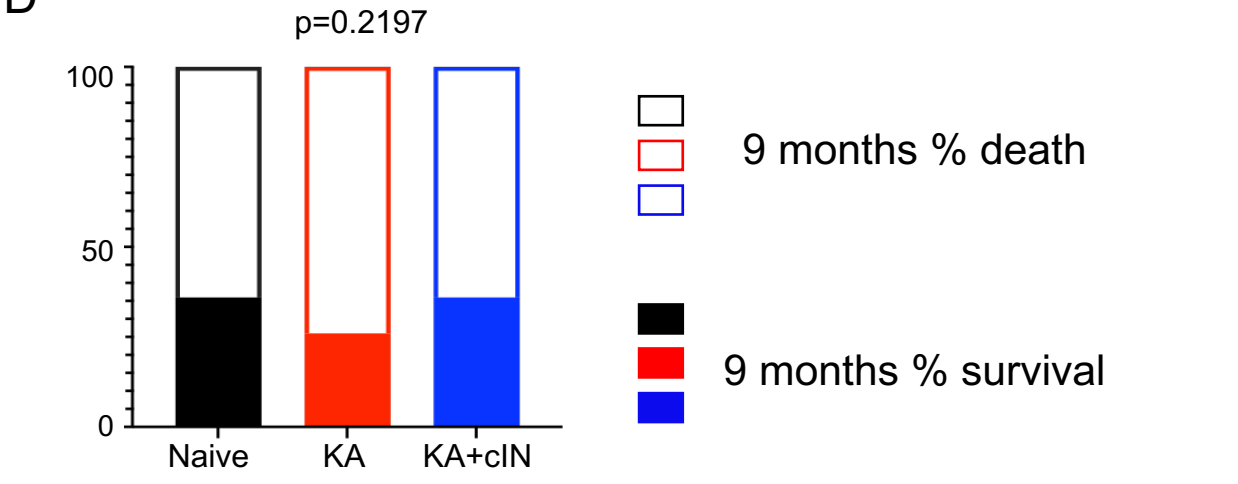

D
p=0.2197
100
50
0
Naive
KA
KA+cIN
9 months % death
9 months % survival

Fig. S7. Analysis of KA-TLE models with human cIN grafts, Related to Fig.4.
(A) Mossy fiber sprouting shown by ZnT3 staining in naïve, KA-TLE vs KA-TLE + cIN transplantation mice. Scale bar: 200 μm.
(B) Granule cell dispersion shown by DAPI staining in naïve, KA-TLE vs KA-TLE + cIN transplantation mice. Scale bar: 400 μm. **Right Panel:** Cell densities in the dentate gyrus (/100um^2) in naïve, KA-TLE and KA-TLE + cIN transplantation groups. Differences in group means were analyzed by One-way ANOVA ($p=0.0002$, $F(2, 9)=24.05$), followed by post hoc analysis using Dunnett's multiple comparisons test (mean ± SEM, n=4 mice). This analysis achieved 100% power to reject the null hypothesis of equal means with a significance level of 0.05 and the observed effects.
(C) Microglial activation shown by Iba1 staining (Scale bar: 400μm) and astrocyte activation shown by GFAP staining (Scale bar: 50 μm) in in naïve, KA-TLE, KA-TLE + cIN, and sham transplantation mice (mean ± SEM; n=4 mice). GFAP$^+$ cell numbers were analyzed using One-way ANOVA ($p=0.0018$, $F(3, 12)=9.410$), followed by posthoc analysis using Dunnett's multiple comparisons test. GFAP intensity was analyzed using One-way ANOVA ($p=0.0005$, $F= (3, 12) = 12.88$), followed by posthoc analysis using Dunnett's multiple comparisons test. Iba1$^+$ cell numbers were analyzed using One-way ANOVA ($p=0.0022$, $F(3, 12)= 8.916$), followed by posthoc analysis using Dunnett's multiple comparisons test. Iba1 intensity are analyzed One-way ANOVA ($p=0.1002$, $F(3, 12)=2.603$. These analyses achieve 100% power for GFAP cell number, 100% power for GFAP intensity, 100% power for IBA1 cell number and 89% power for IBA1 intensity to reject the null hypothesis of equal means with a significance level of 0.05 and the observed effects.
(D) Mortality analysis of naïve, KA-TLE vs KA-TLE + cIN transplantation mice at nine months post-transplant, as analyzed using Chi-square test ($p=0.2197$).

Fig. S8

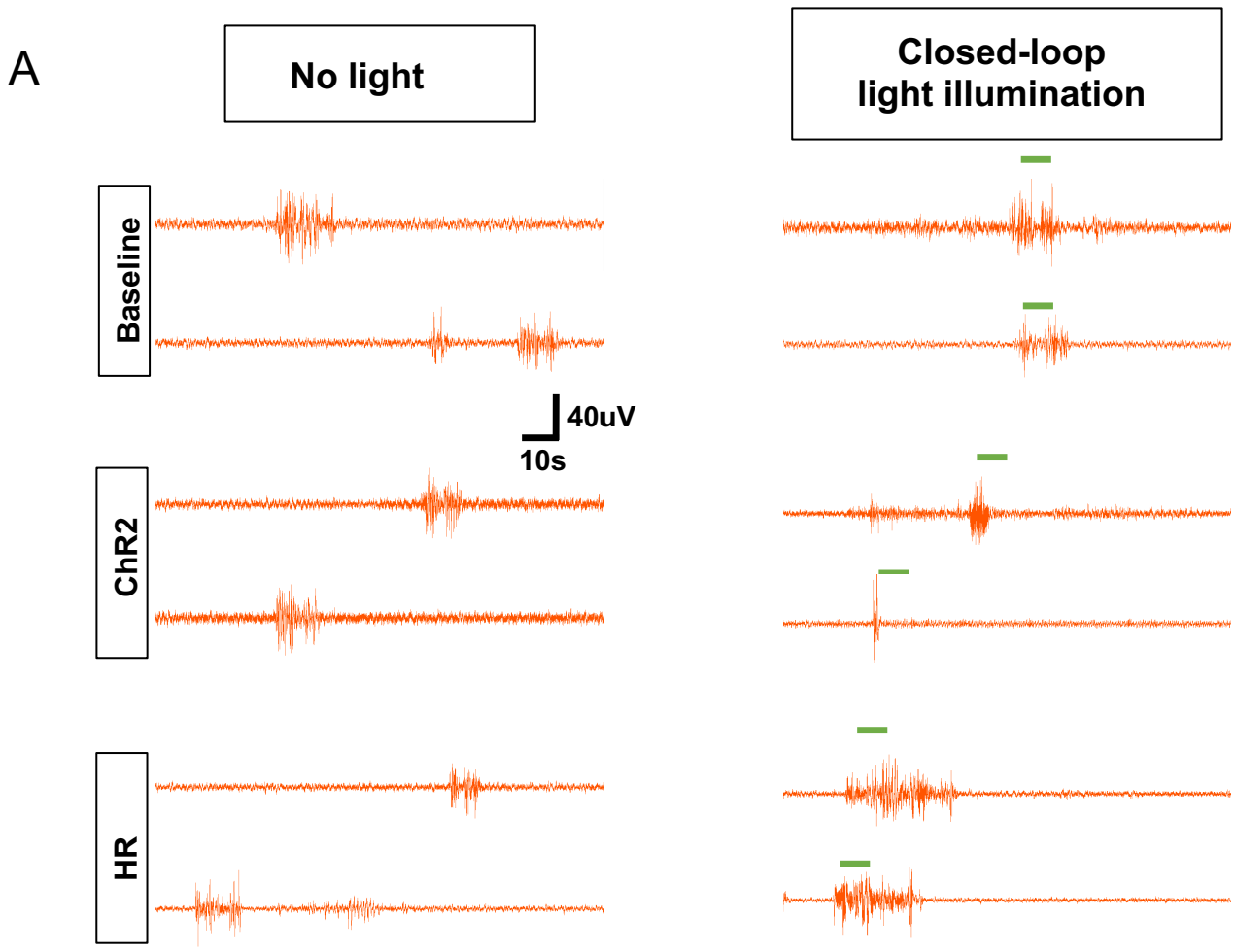

A
No light
Closed-loop
light illumination
Baseline
ChR2
HR
40uV
10s
B
Baseline
ChR2
HR
Seizure Duration (Sec)
p=0.2557
p=2.8e-8
p=3.7e-9
No Light
Light
C
Pre-light
Seizure duration (Sec)
Baseline
ChR2
HR
D
Post-light
p=5.3e-5
p=1.7e-5
p=1.0e-14

Fig. S8. Closed loop optogenetic modulation of grafted human cINs in KA-TLE model in NSG mice, Related to Fig.4.
(A) Representative EEG tracing at baseline (one week post-transplant) or one month after grafting with Chr2-expressiong human cINs or HR-expressing human cINs, either without light illumination or with closed-loop light illumination.
(B) Total seizure duration comparisons between no light control group vs closed loop optogenetic modulation group (mean ± SEM, n=64 seizure events), analyzed by Mann-Whitney test.
(C) Pre-light seizure duration comparisons among baseline, Chr2-cIN vs HR-cIN groups (mean ± SEM, n=64 seizure events), analyzed by Kruskal-Wallis test (p=0.0789).
(D) Post-light seizure duration comparisons among baseline, Chr2-cIN vs HR-cIN groups (mean ± SEM, n=64 seizure events), analyzed by non-parametric Kruskal-Wallis test (p=1.0e-9), followed by post hoc analysis using Dunnett's multiple comparisons test.

Fig. S9

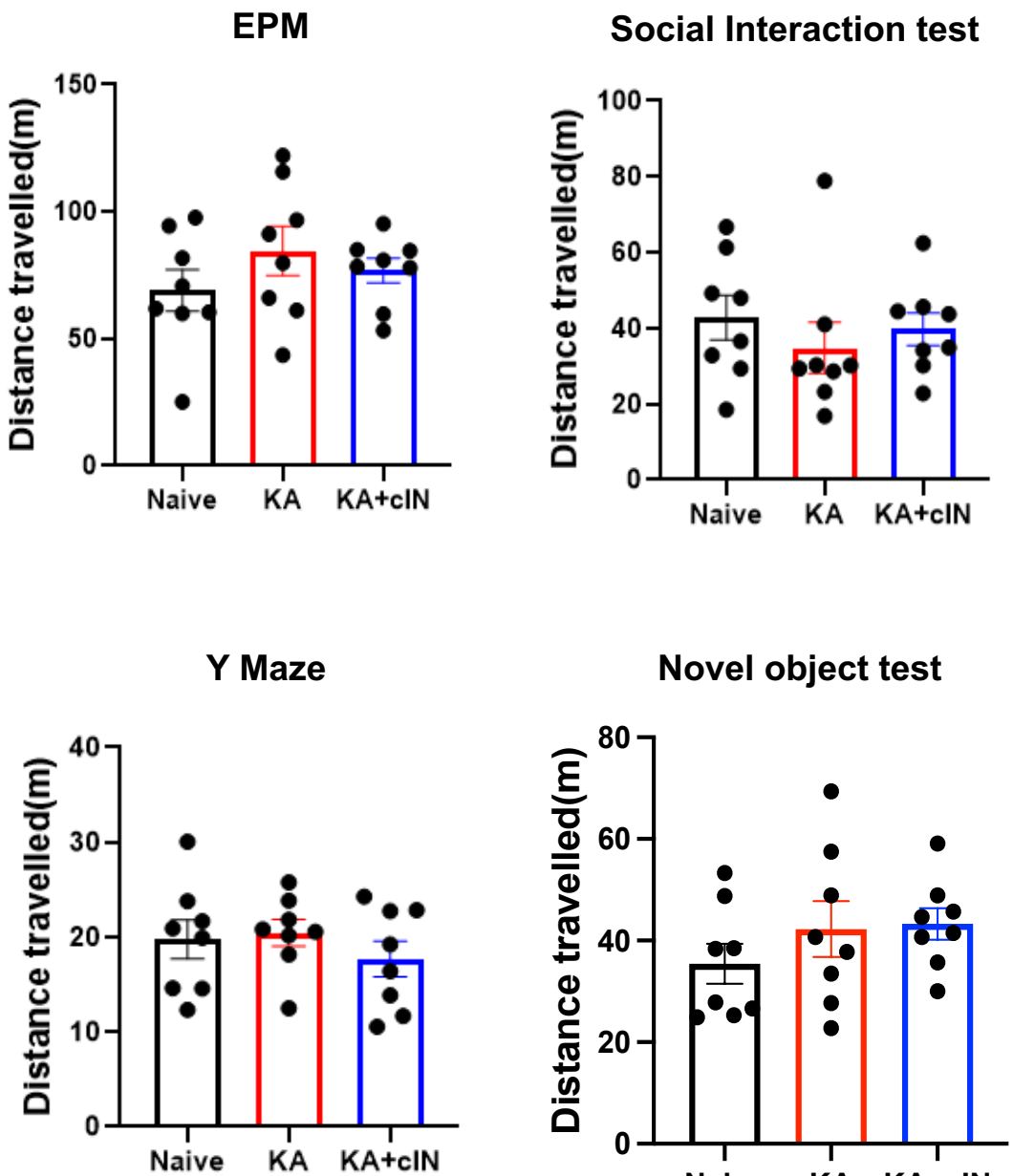
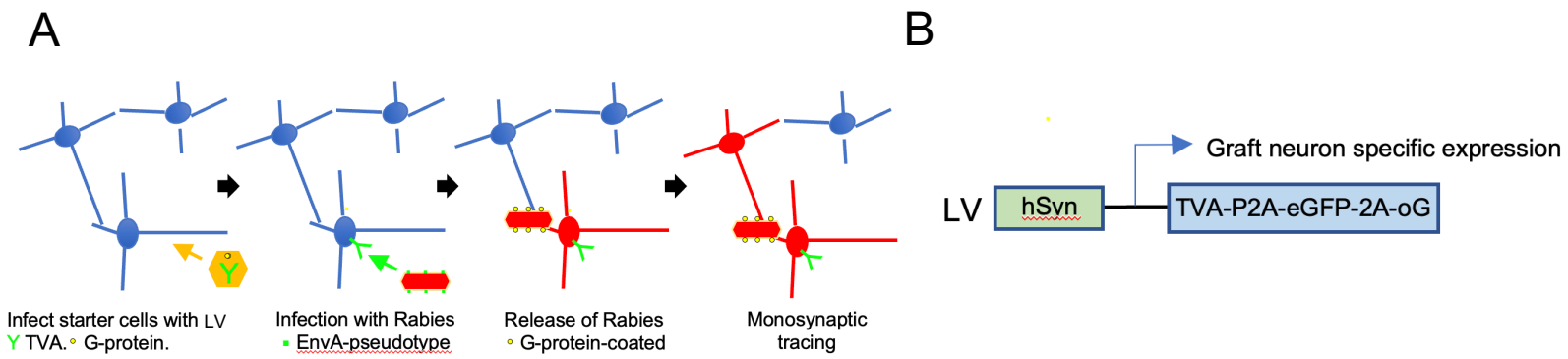
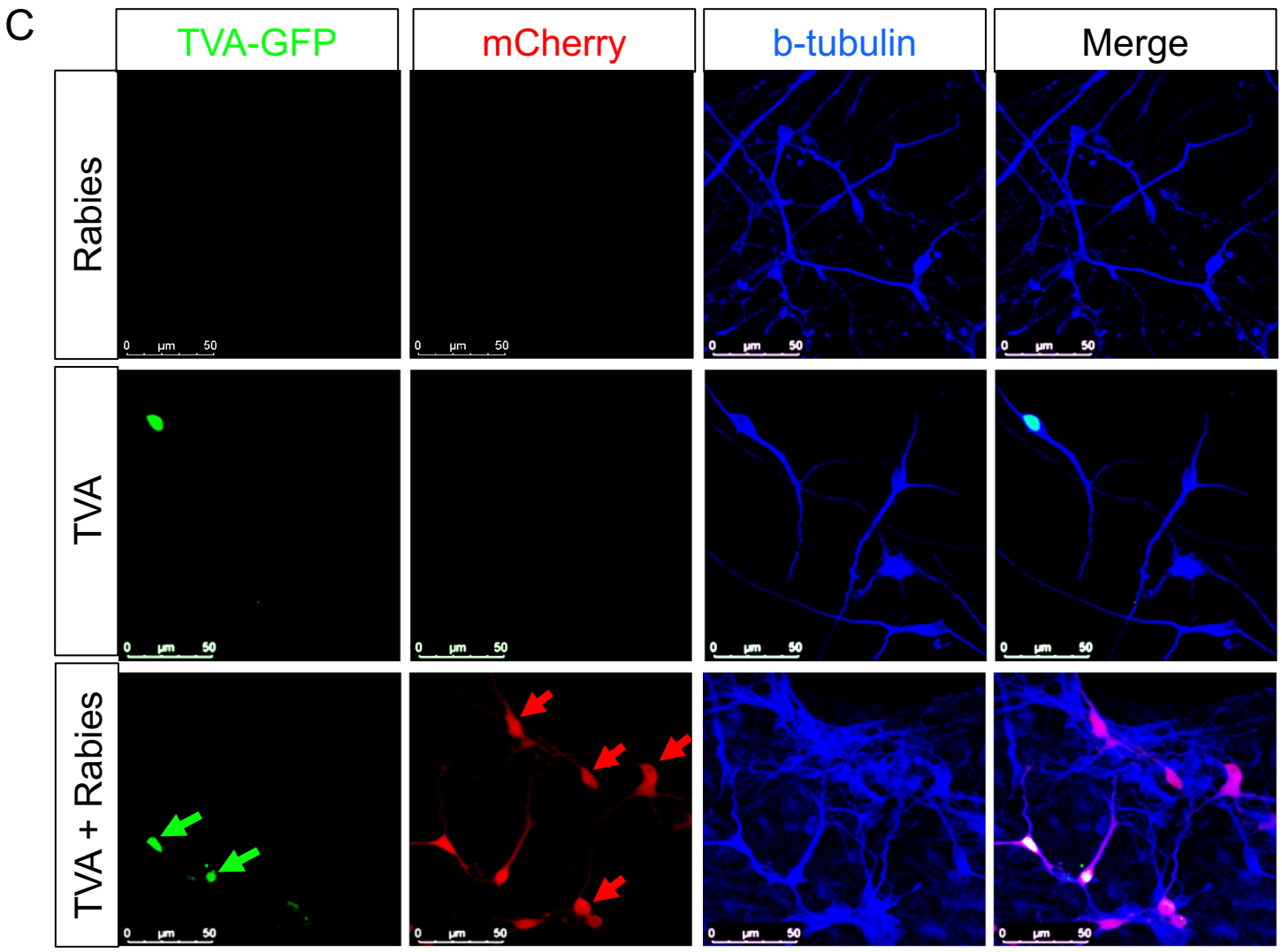


Fig. S9. Total distances traveled in various behaivoral assays, Related to Fig.5. Total distances traveled among naïve mice group, KA-TLE group vs KA-TLE + cIN transplantation group in various behavioral assays at three months post-transplant, presented as the mean ± the SEM (n=8 mice). The mean difference among groups was analyzed by One-way ANOVA tests for EPM ($p=0.3886$, $F(2, 21)=0.9891$), Y maze ($p=0.5437$, $F(2, 21)=0.6274$) and Novel object recognition ($p=0.3904$, $F(2, 21)=0.9841$) and Kruskal-Wallis test for social interaction test ($p=0.2851$).

Fig. S10


A
Infect starter cells with LV
Y TVA. ◦ G-protein.
Infection with Rabies
▪ EnvA-pseudotype
Release of Rabies
◦ G-protein-coated
Monosynaptic
tracing
B
Graft neuron specific expression
LV
hSyn
TVA-P2A-eGFP-2A-oG


C
TVA-GFP
mCherry
b-tubulin
Merge
Rabies
TVA
TVA + Rabies
0 µm 50

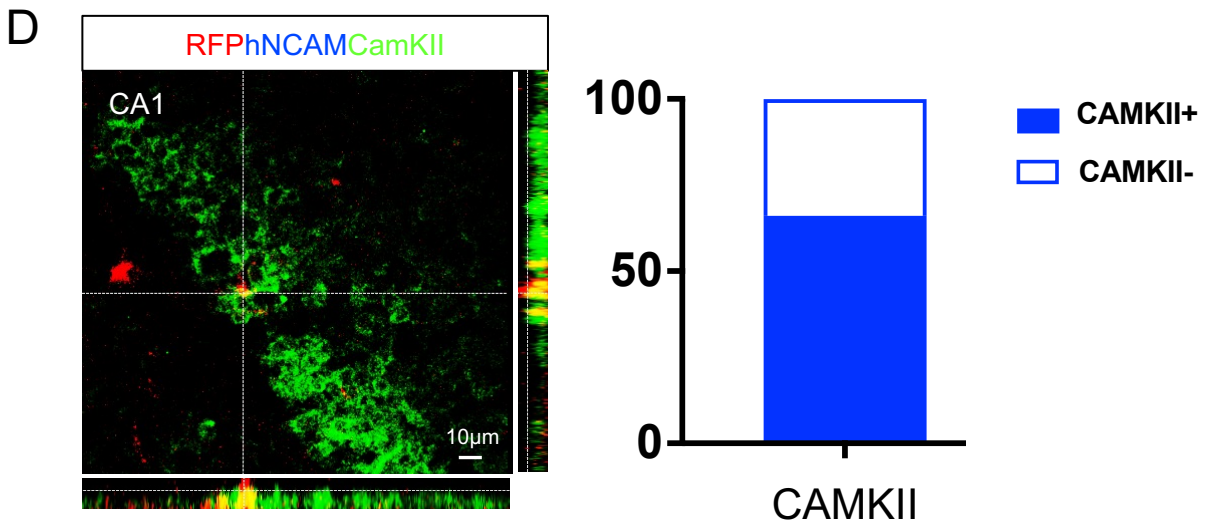

D
RFPhNCAMCamKII
CA1
10µm
100
50
0
CAMKII
CAMKII+
CAMKII-

Fig. S10. Rabies monosynaptic tracing, Related to Fig.6.
(A) Schematic diagram of rabies monosynaptic tracing.
(B) Lentiviral (LV) construct used to infect human interneurons for grafting.
(C) Human cINs traced using Rabies monosynaptic tracing system in vitro. Green arrows indicate starter neurons and red arrows indicates neurons which were monosynaptically traced which innervate starter cINs.
(D) Host CamKII^{+} glutamatergic neurons innervate onto grafted human cINs. Bar graph showing the proportion of CamKII^{+} vs CamKII^{-} cells among traced host neurons (total 103 traced host neurons).

Table. S1 Statistical analysis used for each experiment, Related to the STAR Methods.

Figure number	Analysis ID	Normal Distribution (Shapiro Wilks test)	F test	Statistical test used	Sample Size	Post hoc Power (significance level of 0.05)
Fig. 1E	Total graft cell numbers	*		Non-parametric Mann-Whitney test	n=10 bilateral grafts from five mice	
Fig.2B	4 months Seizure frequency	*		Non-parametric Mann-Whitney test	n=9 mice for the PILO-TLE group, n=16 mice for the PILO-TLE + cIN transplantation group	100%
	4-months Seizure duration			Unpaired parametric t-test, Two-tailed	n=9 mice for the PILO-TLE group, n=16 mice for the PILO-TLE + cIN transplantation group	
Fig.2C	Open Zone time			Unpaired parametric t-test, Two-tailed	n=7 mice for the PILO-TLE group, n=13 mice for the PILO-TLE + cIN transplantation group	
	Total distance travelled			Unpaired parametric t-test, Two-tailed	n=7 mice for the PILO-TLE group, n=13 mice for the PILO-TLE + cIN transplantation group	
Fig.2D	9-months Seizure frequency			Unpaired parametric t-test, Two-tailed	n=7 for the PILO-TLE group, n=9 mice for the PILO-TLE + cIN transplantation group	99%
	9-months Seizure duration	*		Non-parametric Mann-Whitney test	n=7 for the PILO-TLE group, n=9 mice for the PILO-TLE + cIN transplantation group	
Fig.2F	Inhibitory synapse density			One-way ANOVA ($p=0.0015$, $F(2,21)=9.015$), followed by posthoc analysis using Dunnett's multiple comparisons test	n=8 pictures collected from four mice per group	
	Excitatory synapse density			One-way ANOVA ($p=0.0015$, $F(2, 21)=5.718$), followed by posthoc analysis using Dunnett's multiple comparisons test	n=8 pictures collected from four mice per group	

Fig.3H	sIPSC frequency		*	Unpaired t test with Welch's correction	n=11 cells from control group, n=14 cells from transplanted group	98%
	sIPSC amplitude			Unpaired parametric t-test, Two-tailed	n=11 cells from control group, n=14 cells from transplanted group	
Fig. 3I	sIPSC frequency			Unpaired parametric t-test, Two-tailed	n=7 cells	
Fig.4B	1 month Seizure frequency	*		Non-parametric Mann-Whitney test	n=11 KA-TLE mice, n=13 KA-TLE + cIN mice	89%
	3-months Seizure frequency			Unpaired parametric t-test, Two-tailed	n=8 mice per group	100%
	6-months Seizure frequency	*		Non-parametric Mann-Whitney test	n=8 mice per group	100%
	9-months seizure frequency			Unpaired parametric t-test, Two-tailed	n=5 mice per group	100%
Fig.5A	3 Months Open Zone Time	*		One-way ANOVA using Kruskal-Wallis test (p=6.0e-4), followed by posthoc analysis using Dunnett's multiple comparisons test	n=8 mice per group	100%
	9 Months Open Zone Time	*		One-way ANOVA using Kruskal-Wallis test (p=6.0e-4), followed by posthoc analysis using Dunnett's multiple comparisons test	n=6 mice per group	100%
Fig.5B	3 Months Sucrose preference test			Ordinary One-way ANOVA (p=5.3e-5, F(2, 21)= 22.85), followed by posthoc analysis using Dunnett's multiple comparisons test	n=8 mice per group	100%

	9 Months Sucrose preference test			Ordinary One-way ANOVA (p=1.9e-4, F(2, 15) = 24.26), followed by posthoc analysis using Dunnett's multiple comparisons test	n=6 mice per group	100%
Fig.5C	3 Months Social interaction Index	*		One-way ANOVA using Kruskal-Wallis test (p=1.6e-5), followed by posthoc analysis using Dunnett's multiple comparisons test	n=8 mice per group	100%
	9 Months Social interaction Index			Ordinary One-way ANOVA (p=5.1e-5, F(2, 15) =41.73), followed by posthoc analysis using Dunnett's multiple comparisons test	n=6 mice per group	100%
Fig.5D	3 Months Alternation (%)			Ordinary One-way ANOVA (p=9.6e4, F(2, 21) = 14.82), followed by posthoc analysis using Dunnett's multiple comparisons test	n=8 mice per group	100%
	9 Months Alternation (%)			Ordinary One-way ANOVA (p=0.0002, F(2, 15) = 15.42), followed by posthoc analysis using Dunnett's multiple comparisons test	n=6 mice per group	99%
Fig.5E	3 Months Discrimination index	*		One-way ANOVA using Kruskal-Wallis test (p=7.8e-6), followed by posthoc analysis using Dunnett's multiple comparisons test	n=8 mice per group	100%
	9 Months Discrimination index			Ordinary One-way ANOVA (P=7.2e-8, F(2, 15)= 83.78), followed by posthoc analysis using Dunnett's multiple comparisons test	n=6 mice per group	100%

Supplemental References

S1. Shao, Z., Noh, H., Bin Kim, W., Ni, P., Nguyen, C., Cote, S.E., Noyes, E., Zhao, J., Parsons, T., Park, J.M., et al. (2019). Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. Nat Neurosci *22*, 229-242. 10.1038/s41593-018-0313-z.