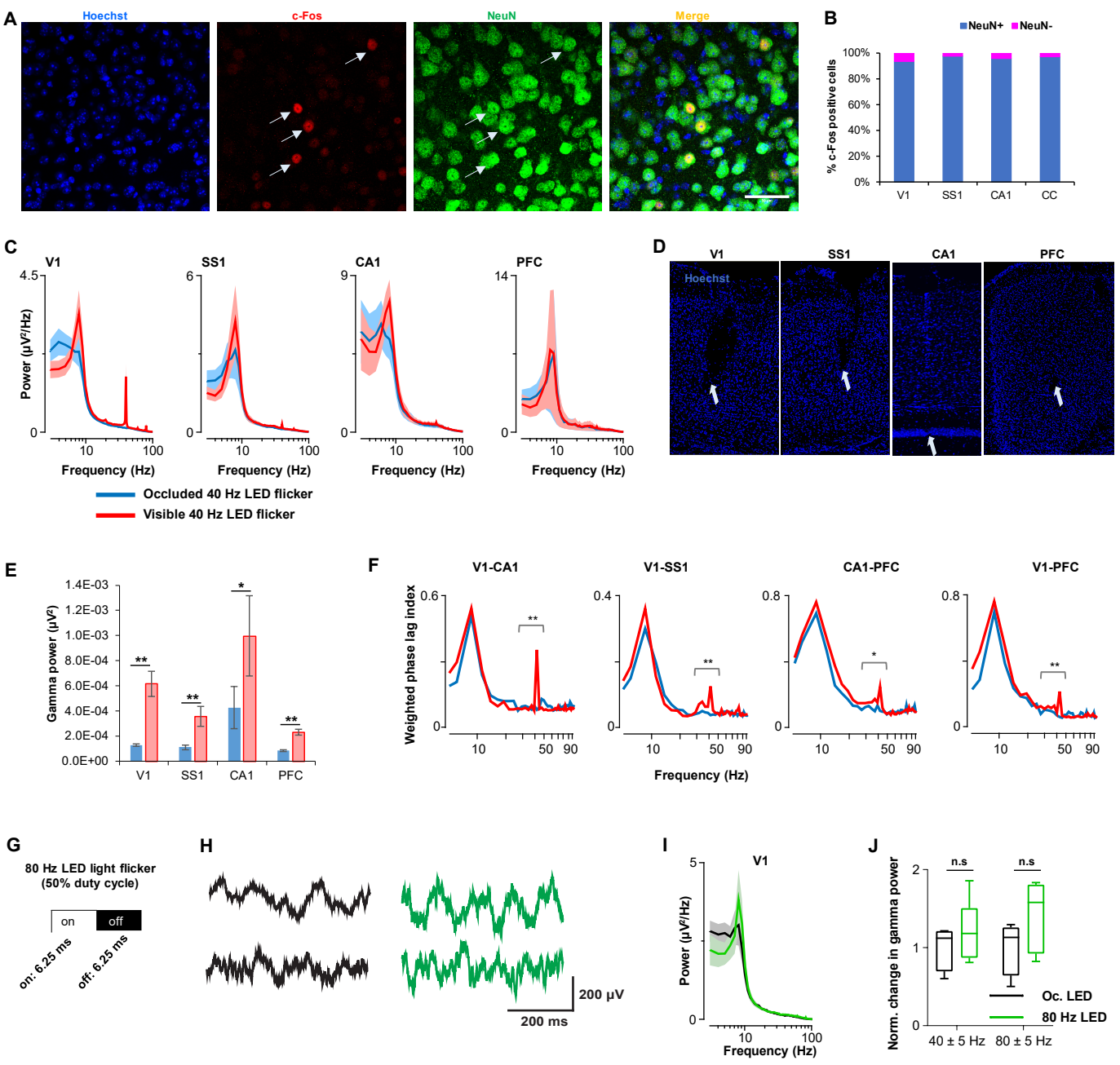

A
Hoechst
c-Fos
NeuN
Merge
B
NeuN+
NeuN-
% c-Fos positive cells
100%
80%
60%
40%
20%
0%
V1
SS1
CA1
CC
C
V1
SS1
CA1
PFC
Power (μV²/Hz)
Frequency (Hz)
Occluded 40 Hz LED flicker
Visible 40 Hz LED flicker
D
V1
SS1
CA1
PFC
Hoechst
E
Gamma power (μV²)
V1
SS1
CA1
PFC
F
V1-CA1
V1-SS1
CA1-PFC
V1-PFC
Weighted phase lag index
Frequency (Hz)
G
80 Hz LED light flicker
(50% duty cycle)
on
off
on: 6.25 ms
off: 6.25 ms
H
200 μV
200 ms
I
V1
Power (μV²/Hz)
Frequency (Hz)
J
n.s
n.s
Norm. change in gamma power
Oc. LED
80 Hz LED
40 ± 5 Hz
80 ± 5 Hz

Figure S1. Acute and chronic 40 Hz light flicker stimulation entrains gamma oscillations beyond visual cortex, Related to Figure 1.

(A) Representative images show co-localization of c-Fos (red) and NeuN (green) from V1, with Hoechst labeling of cell nuclei (blue) (Scale bar 50 μm).

(B) Bar chart shows the percentage overlap of c-Fos and NeuN. We observed that 93.3, 97.7, 95.9, 97.3 percentage of c-Fos positive cells are NeuN positive from V1, SS1, CA1 and CC, respectively.

(C) Power spectra from C57BL/6J mice LFPs recorded from V1, SS1, CA1 and PFC. LFP power was compared between visible (red line) and occluded 40 Hz light flicker (blue line; LED array was covered to occlude light; see STAR Methods; N = 7 mice). Data are mean ± sem. Related to **Figure 1C-1E**.

(D) Representative images show the site of recordings in V1, SS1, CA1 and PFC. Related to **Figure 1C- 1E**

(E) LFP recorded from No Stim or 40 Hz visual stimulation C57BL/6J mice on day 43, following 1 h/day for 6 weeks of No Stim or 40 Hz visual stimulation. Significant increases in 40 Hz power were observed in V1 (Wilcoxon-Ranksum test; $p < 0.01$), SS1 ($p < 0.001$), CA1 ($p = 0.04$) and PFC ($p < 0.001$) during visible stimulation compared to occluded 40 Hz light flicker.

(F) WPLI of 30-50 Hz low gamma coherence in C57BL/6J mice on day 43, between V1-CA1 (Wilcoxon-Ranksum test; $p = 0.002$), V1-SS1 ($p = 0.008$), CA1-PFC ($p = 0.04$), V1-PFC ($p = 0.002$) during visible 40 Hz stimulation.

(G) Schematic of 80 Hz LED light delivery with 50% duty cycle (6.25 ms light on and 6.25 ms light off).

(H) Raw LFPs from V1 of baseline activity with light occluded (black) and during visible 80 Hz visual stimulation (green).

(I) Power spectra of V1 LFP in C57BL/6J mice subjected to light occluded (black) or visible 80 Hz visual stimulation (green).

(J) The area power centered on 40 Hz (±5 Hz) or 80 Hz (±5 Hz) was not significantly different with 80 Hz visual stimulation (N = 5 mice, Wilcoxon-Ranksum test, 40 Hz, $Z = 0.668$, $p = 0.38$; 80 Hz, $Z = 1.2$, $p = 0.22$; n.s. = not significant).

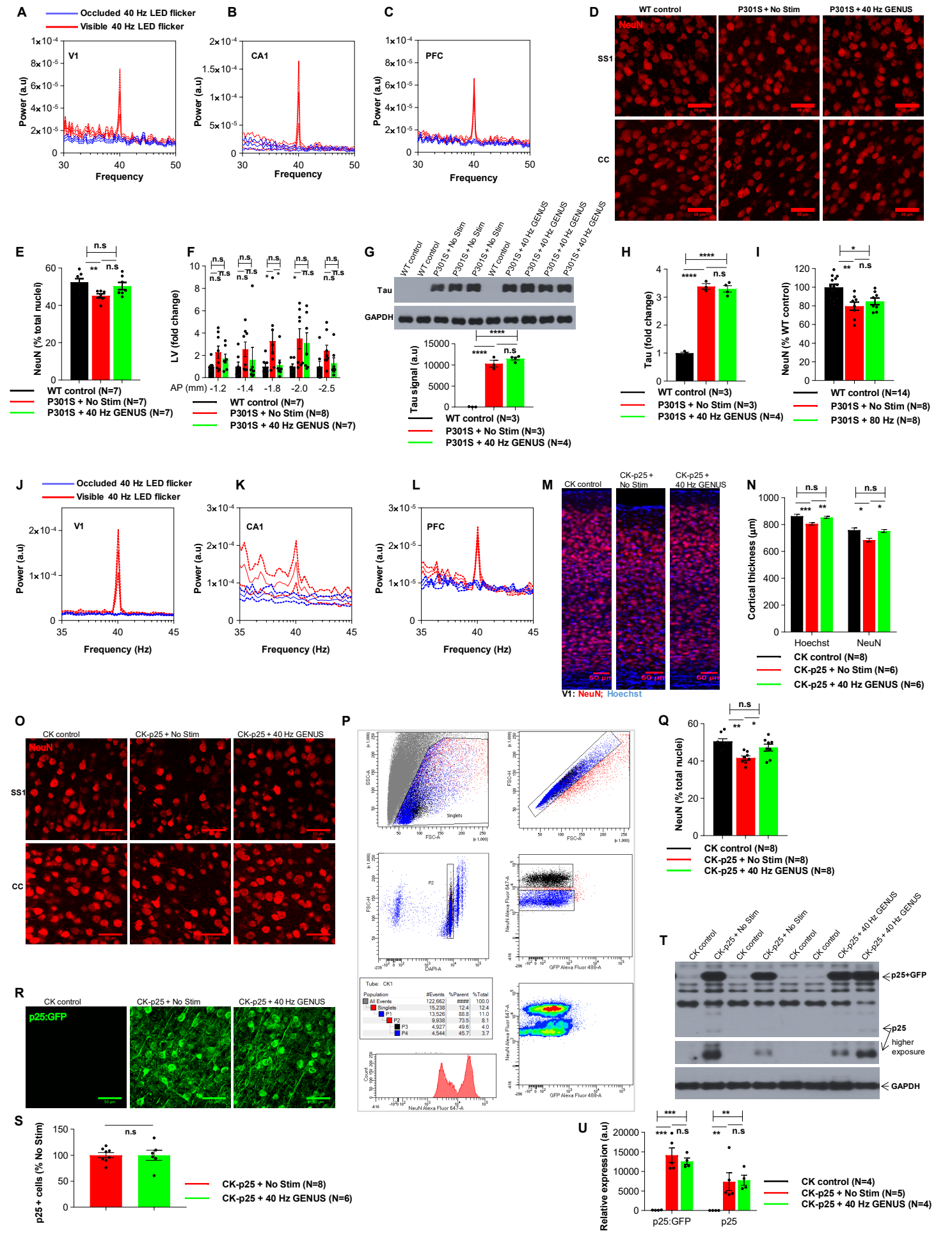

A
Occluded 40 Hz LED flicker
Visible 40 Hz LED flicker
V1
Power (a.u)
Frequency
B
CA1
C
PFC
D
WT control
P301S + No Stim
P301S + 40 Hz GENUS
NeuN
SS1
CC
E
NeuN (% total nuclei)
WT control (N=7)
P301S + No Stim (N=7)
P301S + 40 Hz GENUS (N=7)
F
LV (fold change)
AP (mm) -1.2 -1.4 -1.8 -2.0 -2.5
WT control (N=7)
P301S + No Stim (N=8)
P301S + 40 Hz GENUS (N=7)
G
Tau
GAPDH
Tau signal (a.u)
WT control (N=3)
P301S + No Stim (N=3)
P301S + 40 Hz GENUS (N=4)
H
Tau (fold change)
WT control (N=3)
P301S + No Stim (N=3)
P301S + 40 Hz GENUS (N=4)
I
NeuN (% WT control)
WT control (N=14)
P301S + No Stim (N=8)
P301S + 80 Hz (N=8)
J
Occluded 40 Hz LED flicker
Visible 40 Hz LED flicker
V1
Power (a.u)
Frequency (Hz)
K
CA1
L
PFC
M
CK control
CK-p25 + No Stim
CK-p25 + 40 Hz GENUS
V1: NeuN; Hoechst
N
Cortical thickness (µm)
Hoechst
NeuN
CK control (N=8)
CK-p25 + No Stim (N=6)
CK-p25 + 40 Hz GENUS (N=6)
O
CK control
CK-p25 + No Stim
CK-p25 + 40 Hz GENUS
NeuN
SS1
CC
P
Singlets
Tube: CK1
Population #Events %Parent %Total
All Events 122,662 ##### 100.0
Singlets 15,238 12.4 12.4
P1 13,526 88.8 11.0
P2 9,938 73.5 8.1
P3 4,927 49.6 4.0
P4 4,544 45.7 3.7
Q
NeuN (% total nuclei)
CK control (N=8)
CK-p25 + No Stim (N=8)
CK-p25 + 40 Hz GENUS (N=8)
R
CK control
CK-p25 + No Stim
CK-p25 + 40 Hz GENUS
p25:GFP
S
p25+ cells (% No Stim)
CK-p25 + No Stim (N=8)
CK-p25 + 40 Hz GENUS (N=6)
T
CK control
CK-p25+ No Stim
CK control
CK-p25+ No Stim
CK control
CK control
CK-p25+ 40 Hz GENUS
CK-p25+ 40 Hz GENUS
p25+GFP
p25
higher exposure
GAPDH
U
Relative expression (a.u)
p25:GFP
p25
CK control (N=4)
CK-p25 + No Stim (N=5)
CK-p25 + 40 Hz GENUS (N=4)

Figure S2. 40 Hz light flicker entrains gamma oscillations in mouse models of neurodegeneration and chronic GENUS reduces neurodegeneration, Related to Figure 2.

(A) LFP in V1 from multi-site LFP probe implanted in 8-months old No Stim P301S mice shows significant increase in gamma power (40 Hz) during visible (red) versus occluded (blue) 40 Hz light stimulation ($p < 0.001$) (N = 4 P301S mice).

(B) LFP power spectra from CA1. Significant increase in gamma power (40 Hz) was observed during visible (red) versus occluded (blue) 40 Hz light stimulation in CA1 ($p < 0.001$).

(C) LFP power spectra from PFC. Significant increase in gamma power (40 Hz) was observed during visible (red) versus occluded (blue) 40 Hz light stimulation in PFC ($p < 0.001$).

(D) Representative images showing neuronal marker NeuN in SS1 and CC from WT control, No Stim P301S, and GENUS P301S mice. Related to **Figure 2B, 2C**.

(E) Bar chart of quantification of NeuN+ cells using FACS (ANOVA $F(2,18) = 4.97$, $p = 0.0191$. Bonferroni's post hoc test; WT control Vs No Stim P301S, $p = 0.0203$; WT control Vs GENUS P301S mice, $p > 0.99$; No Stim Vs GENUS P301S, $p = 0.125$). See also **Figure S2P** for FACS gating strategy**.**

(F) Bar chart of size of lateral ventricles (N = 7, WT control; 8, No Stim P301S; and 7, GENUS P301S. Two-way repeated measures ANOVA effect between groups $F(2, 19) = 14.22$, $p = 0.0002$. Post-hoc test, WT control Vs No Stim P301S mice: AP from bregma (mm): -1.2, $p = 0.339$; -1.4, $p = 0.208$; -1.8, $p = 0.0350$; -2.0, $p = 0.0186$; -2.5, $p = 0.2725$; WT control Vs GENUS P301S: -1.2, $p = 0.722$; -1.4, $p = 0.795$; -1.8, $p = 0.989$; -2.0, $p = 0.0689$; -2.5, $p = 0.943$; No Stim Vs GENUS P301S: -1.2, $p = 0.811$; -1.4, $p = 0.554$; -1.8, $p = 0.0495$; -2.0, $p = 0.895$; -2.5, $p = 0.450$; n.s. = not significant).

(G) Top: Full length western blots of tau and GAPDH. Bottom: Bar chart shows the signal intensity of tau immunoblot (One way ANOVA, $F(2,7) = 173.3$, $p < 0.0001$; Bonferroni's post hoc test, WT control Vs No Stim P301S, $p < 0.0001$; WT control Vs GENUS P301S, $p < 0.0001$; No Stim Vs GENUS P301S, $p = 0.2281$).

(H) Bar chart shows the relative levels of tau protein detected in LC-MS/MS (One way ANOVA, $F(2,7) = 186.1$ $p < 0.0001$; Bonferroni's post hoc test, WT control Vs No Stim P301S, $p < 0.0001$; WT control Vs GENUS P301S mice, $p < 0.0001$. No Stim Vs GENUS P301S, $p = 0.789$).

(I) 7-months old P301S mice was subjected to no stimulation or 80 Hz flicker stimulation (1h/d for 22 days). Mice were then sacrificed and neuronal density were quantified using 40 µm coronal brain slices immunohistochemically (ANOVA $F(2, 27) = 9.403$, $p = 0.0008$.

Bonferroni's post hoc test, WT control Vs No Stim P301S, p = 0.0013; WT control Vs 80 Hz P301S mice, p = 0.018. No Stim Vs 80 Hz P301S, p = 0.99).

(J) CK-p25 mice exposed to No Stim or GENUS during 6-weeks of p25 induction, followed by multi-electrode probe implantation and LFP recording. 40 Hz visual stimulation increased 40 Hz power in V1 in 6-weeks induced CK-p25 mice (Wilcoxon-Rank sum; p = 0.0022).

(K) 40 Hz visual stimulation increased 40 Hz power in CA1 in 6-weeks induced CK-p25 mice (Wilcoxon-Rank sum; p = 0.001).

(L) 40 Hz visual stimulation increased 40 Hz power in PFC in 6 weeks induced CK-p25 mice (Wilcoxon-Rank sum; p = 0.002).

(M) Representative images showing the thickness of visual cortex (nuclear stain Hoechst is shown in blue and neuronal marker NeuN in red).

(N) Bar chart of visual cortex thickness (Two-way ANOVA between group effect F (2, 36) = 12.93, p < 0.0001. Bonferroni's multiple comparisons test, ***p < 0.001, **p < 0.01, *p < 0.05).

(O) Representative images showing neuronal marker NeuN in SS1 and CC. Related to **Figure 2H, 2I**.

(P) Neuronal nuclei separation strategy in FACS from representative CK control mice. FACS plots show the gating strategy for isolating NeuN-positive cells. Top row: The entire gating tree for one representative sample. Nuclei were separated from most of the debris based on forward scatter area (FSC-A) and side scatter (SSC-A). Events that could represent more than one nuclei were excluded based on the forward scatter height (FSC-H) and DAPI positive nuclei (DAPI-A). Middle and bottom right: NeuN- Alexa Fluor 647 positive nuclei (P3) were selected and sorted. We also obtained a similar separation profile from P301S mice **(Figure S2E**).

(Q) Bar graph of NeuN+ nuclei (ANOVA F (2, 21) = 9.284, p = 0.0013. Bonferroni's post hoc test, CK control Vs No Stim CK-p25, p = 0.0010; CK control Vs GENUS CK-p25, p = 0.388; No Stim Vs GENUS CK-p25, p = 0.0417).

(R) Representative images show p25:GFP (p25 transgene with GFP reporter; fusion protein) positive cells. Note that there was no p25:GFP positive cells detected in CK control mice.

(S) Bar graph of number of p25:GFP+ cells in V1 (t-test; t = 0.0202, p = 0.984).

(T) Westernblots of p25 and GAPDH from visual cortex.

(U) Bar chart of the expression levels of p25 (one way ANOVA, F (2,12) = 13.065, p = 0.002), and p25:GFP levels (one way ANOVA, F (2, 12) = 3.581, p = 0.025. Bonferroni's multiple comparisons test, ***p < 0.001, **p < 0.01, *p < 0.05, n.s = not significant).

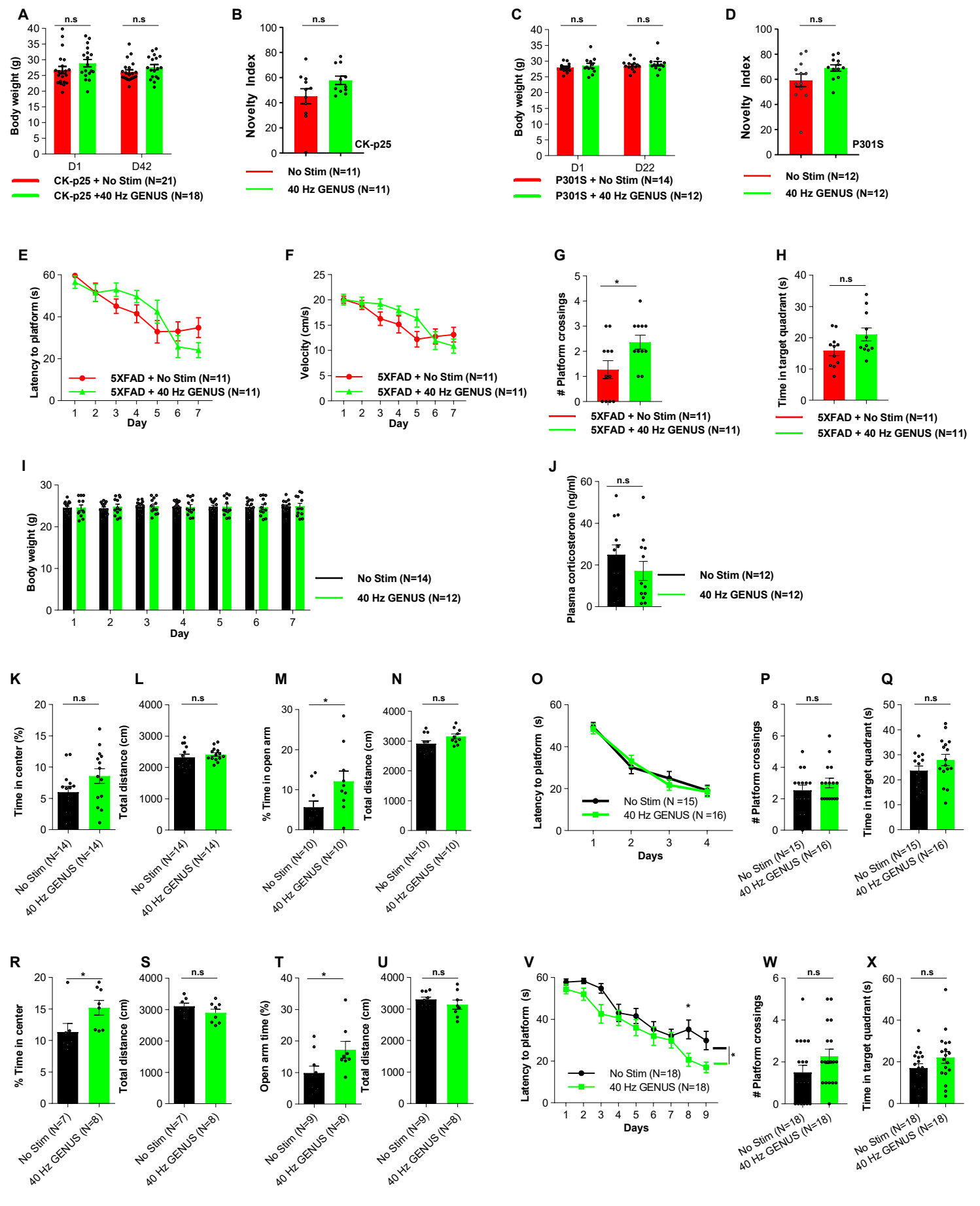

A
n.s
n.s
Body weight (g)
D1
D42
CK-p25 + No Stim (N=21)
CK-p25 +40 Hz GENUS (N=18)
B
n.s
Novelty Index
CK-p25
No Stim (N=11)
40 Hz GENUS (N=11)
C
n.s
n.s
Body weight (g)
D1
D22
P301S + No Stim (N=14)
P301S + 40 Hz GENUS (N=12)
D
n.s
Novelty Index
P301S
No Stim (N=12)
40 Hz GENUS (N=12)
E
Latency to platform (s)
5XFAD + No Stim (N=11)
5XFAD + 40 Hz GENUS (N=11)
Day
F
Velocity (cm/s)
5XFAD + No Stim (N=11)
5XFAD + 40 Hz GENUS (N=11)
Day
G
*
Platform crossings
5XFAD + No Stim (N=11)
5XFAD + 40 Hz GENUS (N=11)
H
n.s
Time in target quadrant (s)
5XFAD + No Stim (N=11)
5XFAD + 40 Hz GENUS (N=11)
I
Body weight (g)
No Stim (N=14)
40 Hz GENUS (N=12)
Day
J
n.s
Plasma corticosterone (ng/ml)
No Stim (N=12)
40 Hz GENUS (N=12)
K
n.s
Time in center (%)
No Stim (N=14)
40 Hz GENUS (N=14)
L
n.s
Total distance (cm)
No Stim (N=14)
40 Hz GENUS (N=14)
M
*
% Time in open arm
No Stim (N=10)
40 Hz GENUS (N=10)
N
n.s
Total distance (cm)
No Stim (N=10)
40 Hz GENUS (N=10)
O
Latency to platform (s)
No Stim (N =15)
40 Hz GENUS (N =16)
Days
P
n.s
Platform crossings
No Stim (N=15)
40 Hz GENUS (N=16)
Q
n.s
Time in target quadrant (s)
No Stim (N=15)
40 Hz GENUS (N=16)
R
*
% Time in center
No Stim (N=7)
40 Hz GENUS (N=8)
S
n.s
Total distance (cm)
No Stim (N=7)
40 Hz GENUS (N=8)
T
*
Open arm time (%)
No Stim (N=9)
40 Hz GENUS (N=8)
U
n.s
Total distance (cm)
No Stim (N=9)
40 Hz GENUS (N=8)
V
*
*
Latency to platform (s)
No Stim (N=18)
40 Hz GENUS (N=18)
Days
W
n.s
Platform crossings
No Stim (N=18)
40 Hz GENUS (N=18)
X
n.s
Time in target quadrant (s)
No Stim (N=18)
40 Hz GENUS (N=18)

Figure S3. Chronic GENUS modifies the behavior but does not affect body weight or plasma corticosterone levels, Related to Figure 3.

(A) Bar graph of body weight (Two-way ANOVA between groups effects, $F(1, 74) = 3.318$, $p = 0.0726$).

(B) Bar graph of novel object preference (t-test, $t = 1.826$, $p = 0.082$).

(C) Bar graph of body weight (Two-way ANOVA between groups effects, $F(1, 48) = 1.071$, $p = 0.306$).

(D) Bar graph of novel object preference (t-test, $t = 1.746$, $p = 0.094$).

(E) 9-months old 5XFAD mice with or without GENUS (1h per day for 22 days) was tested for MWM performance. MWM was conducted during the third week of stimulation. Latency to find the platform (Repeated measures ANOVA between groups effect, $F(1, 140) = 0.05011$, $p = 0.8274$).

(F) Line plot of swimming velocity (Repeated measures ANOVA between groups effect, $F(1,140) = 2.203$, $p = 0.140$).

(G) Bar graph of platform crossing (Mann-Whitney $U = 30$ $p = 0.0422$).

(H) Bar graph of time spent in the target quadrant in the probe test (independent samples two-sided t-test, $t=1.974$, $p = 0.0623$).

(I) C57BL/6J mice (3.5-months old) subjected to No Stim or 40 Hz visual stimulation. No difference in exploratory behavior during the first 10 min pre-stimulation baseline period between No Stim and GENUS was observed (N = 6 mice per group. T-test, $T = 0.3173$, $p = 0.7576$). Similarly, no systematic changes in velocity throughout the 40 Hz stimulation period (every minute for the 30 minutes) were detectable when compared to the no-stimulation group (Two-way repeated measures ANOVA interaction between No Stim and GENUS WT mice, $F(29, 290) = 0.9354$, $p = 0.5652$ (data not shown)). These mice underwent no stimulation or GENUS 1h/d for 7 days. The body weight of mice was measured 1h before the stimulation paradigm every day for 7 days and 1-day post stimulation regimen. Bar chart show the body weight across 7 days (N = 14 No Stim and 12 GENUS stimulated mice; there was an overall increase in body weight across days in both groups ($F(6,144) = 2.889$, $P = 0.011$), however; there was no difference in body weight between No Stim and GENUS ($F(6, 144) = 1.327$, $P = 0.249$)).

(J) C57BL/6J mice (3.5-months old) subjected to No Stim or GENUS 1h/d for 7 days. Bar graph of plasma corticosterone levels (independent samples two-sided t-test, $t = 1.17$, $p = 0.2546$).

(K) C57BL/6J mice (3.5-months old) subjected to No Stim or GENUS 1h/d for 7 days followed by open field test. Bar graph of percentage of time spent in the center of an arena (independent samples two-sided t-test, t = 1.731, p = 0.0953).

(L) Bar graph of total distance travelled in the open field test (t = 0.7733, p = 0.446).

(M) C57BL/6J mice (3 months) subjected to No Stim or GENUS 1h/d for 7 days followed by EPM. Bar graph of percentage of time spent in the open arm (independent samples two-sided t-test, t = 2.163, p = 0.0433).

(N) Bar graph of distance travelled in EMP (independent samples two-sided t-test, t = 1.852, p = 0.0806).

(O) MWM performance of young C57Bl6 (4-months old) mice with or without 40 Hz GENUS for 1h/d for 7 days. Line plot of latency to find platform (repeated measures ANOVA between groups effect F (1, 29) = 0.030, p = 0.864).

(P) Bar graph of number of platform crossings in the probe test (independent samples t-test, t = 1.034, p = 0.309).

(Q) Bar graph of time spent in the target quadrant (independent samples two-sided t-test, t = 1.469, p = 0.1527).

(R) 17-months old C57BL/6J mice subjected to No Stim or GENUS 1h/d for 5 weeks and then to open field test. Bar graph of percentage of time spent in the center of an arena (independent samples two-sided t-test, T = 2.178, p = 0.0484).

(S) Bar graph of distance travelled in the open field test (t = 1.301, p = 0.2160).

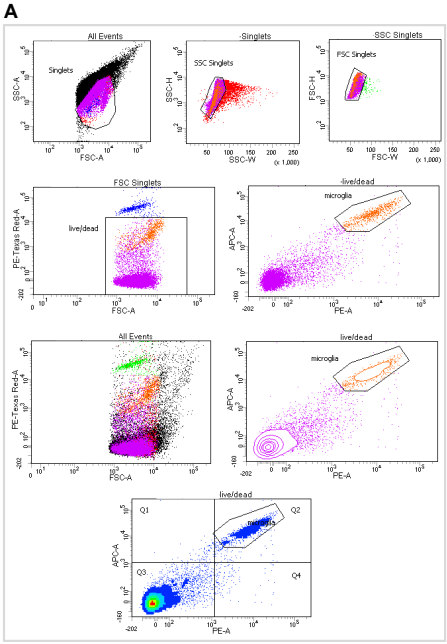
(T) C57BL/6J mice (17-months old) subjected to No Stim or GENUS 1h/d for 5 weeks followed by elevated plus maze test. Bar graph of percentage of time spent in the open arm (independent samples two-sided t-test, t = 2.173, p = 0.0462).

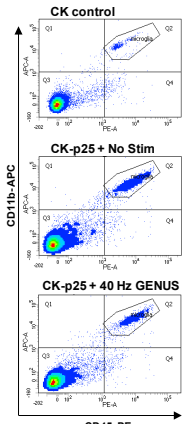
(U) Bar graph of distance travelled (independent samples two-sided t-test, t = 1.039, p = 0.3153).

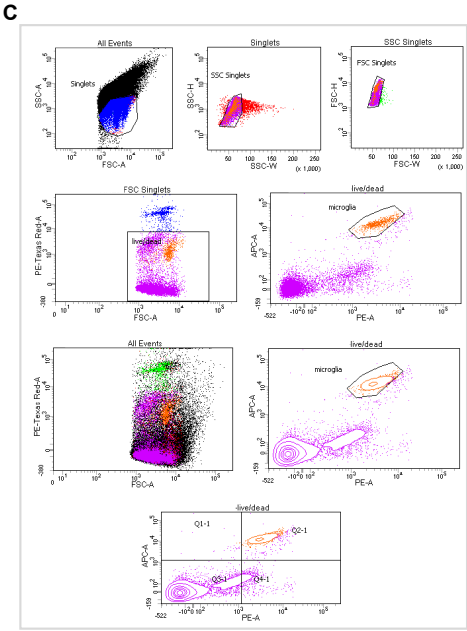
(V) MWM performance of aged C57Bl6 (17-months old) mice with or without 40 Hz GENUS for 1h/d for 4 weeks. MWM was conducted in the last week of stimulation. Line plot of latency to find platform (N = 18 mice/group, Repeated measures ANOVA between groups effect F (1, 34) = 6.954, p = 0.013).

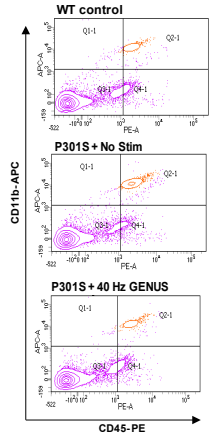
(W) Bar graph of platform crossings in the probe test (Mann-Whitney U = 113, p = 0.114).

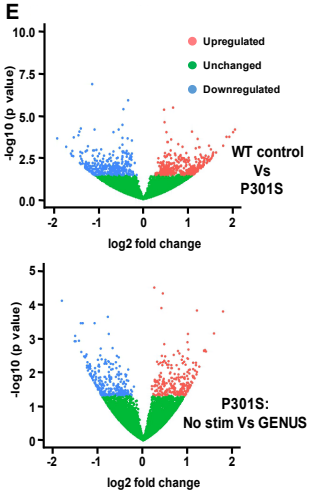
(X) Bar graph of time spent in the target quadrant in the probe test (t-test, t = 1.441, p = 0.158). N in all graph represents number of animals.

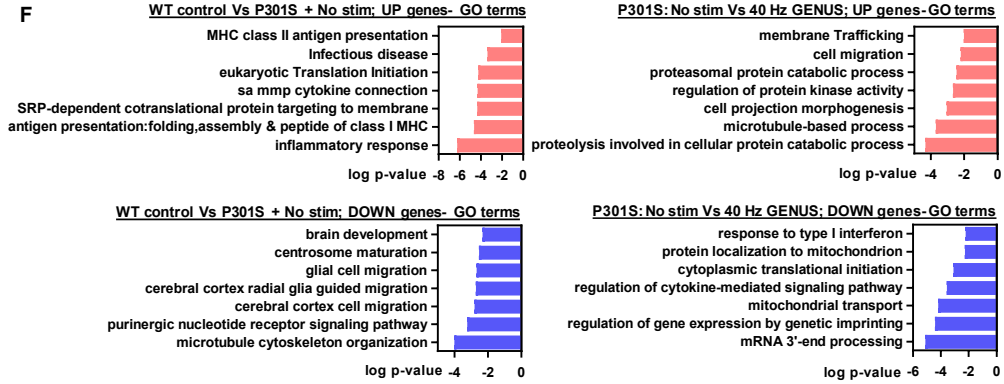


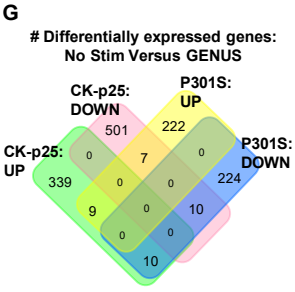












H

CK-p25:UP Vs P301S:UP

Chtf8	Zfand3	Atp2b2	Dedd2	2310033P09Rik
Gpx1	Eif5b	Rpl9	Akt2	

CK-p25:UP Vs P301S:DOWN

Eno1b	Ccnd1	Jrk	Mrps25	Arl10
Ncam1	Rrad	Pold2	Cpsf4	Psmd7

CK-p25:DOWN Vs P301S:UP

Ssfa2	Atg2a	Impact	Dpp8	Cdyl
Gbp9	Gm14420			

CK-p25:DOWN Vs P301S:DOWN

C330027C09Rik	Ndfip2	Flna	Ctbs	Sars2
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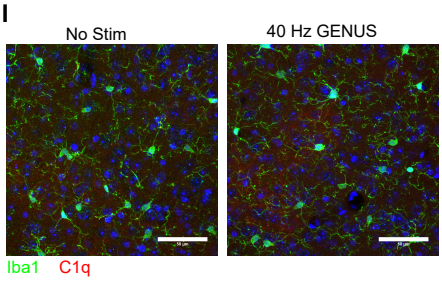
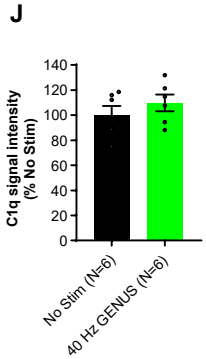
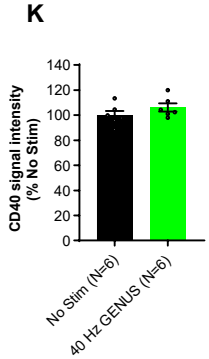
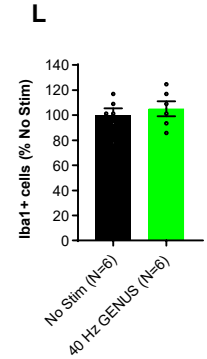








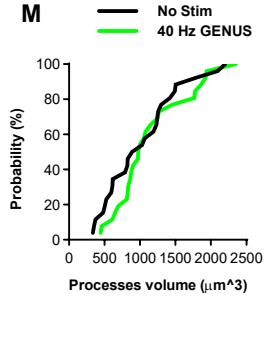


Figure S4. Chronic GENUS reduces microgliosis and modifies microglia mediated inflammatory response, Related to Figure 4.

A. Microglia separation strategy in FACS from a representative mouse. Related to **Figure 4A, 4B**. FACS plots show the gating strategy for isolating CD11b and CD45 double positive cells. The entire gating tree for one representative sample. Intact cells were separated from most of the debris based on forward scatter area (FSC-A) and side scatter (SSC-A). Events that could represent more than one cell were excluded based on the side scatter width (SSC-W) and the forward scatter width (FSC-W). Propidium iodide negative cells (viable cells) were selected. CD11b-APC and CD45-PE-positive cells were selected and sorted.

B. Microglia separation profile in FACS from representative CK control, No Stim and GENUS CK-p25. There was a significant increase in the CD11b and CD45 double positive cells in the No Stim CK-p25 compared to CK controls ($p = 0.019$), whereas GENUS significantly reduced the CD11b and CD45 double positive cells in CK-p25 mice compared to No Stim CK-p25 ($p = 0.0226$).

C. Microglia separation strategy in FACS from a representative mouse.

D. Microglia separation profile in FACS from representative WT control, No Stim and GENUS P301S mice. (ANOVA $F(2, 13) = 4.765$, $p = 0.0280$. WT control Vs No Stim P301S, $p = 0.603$; WT control Vs GENUS P301S, $p = 0.2558$; non-stimulation Vs GENUS P301S, $p = 0.0267$).

E. Volcano plots of differentially expressed genes (DEGs). Group comparisons are shown to the right (N = 5 mice/group).

F. Top 7 processed gene ontology (GO) terms for biological processes associated with the identified DEGs. Group comparisons are shown to the top.

G. Venn diagram of overlap of number of microglia specific DEGs. No statistically significant overlap was observed with Fisher's exact test.

H. Table shows the name of overlap genes, and the comparisons are indicated on the top (related to Figure S4G).

I. Representative Iba1 (green) and C1q (red) images in V1 from No Stim and GENUS aged WT mice.

J. Bar graph of C1q signal intensity (N = 6 mice/group. $t = 0.984$, $p = 0.348$).

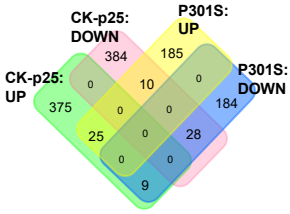
K. Bar graph of CD40 signal intensity (N = 6 mice/group. $t = 1.287$, $p = 0.227$).

L. Bar graph of number of Iba1+ cells (N = 6 mice/group. $t = 0.642$, $p = 0.535$).

M. Distribution plot of the volume of the processes of microglia (N = 6 mice/group. Kolmogorov-Smirnov $D = 0.23$, $p = 0.493$).

A

Differentially expressed genes: No Stim Versus GENUS



B

CK-p25, UP:P301S, UP

Chmp2a	Coro6	Dgkg	Efr3a	Got2
Hint1	Hsp90ab1	Hspa8	Igfbp5	Iqgap2
Jun	Maml3	Map4k2	Ndrg2	Rgl1
Rmrp	Smarca2	Snord22	Sparcl1	Specc1
Stac2	Trpc4	Tshz2	Unc13c	Ywhag

CK-p25, DOWN:P301S, UP

Arc	Dusp1	Midn	Nr4a1	Prkar2b
Rasl11b	Tm9sf3	Zfp658	Zfp72	Zfp758

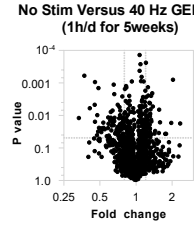
CK-p25, UP:P301S, DOWN

4833420G17Rik	8430427H17Rik	Fgd4	Mblac2	Nfkb1
Pex14	Ptprm	Uvrag	Zkscan5	

CK-p25, DOWN:P301S, DOWN

2810049E08Rik	5730522E02Rik	Arhgap29	Arhgap42	Cntln
Col6a1	Ddx17	Drp2	Entpd4	Grm3
Herc6	Igfn1	Ipo9	Kcnk9	Lama4
Myo1b	Myo5c	Nyap2	Pak7	Pamr1
Pias4	Pla2g4e	Pou6f2	Rsrp1	Sorbs2
Tmem132d	Vldlr	Zfp763		

C



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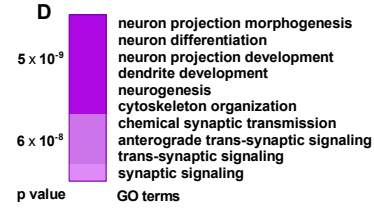

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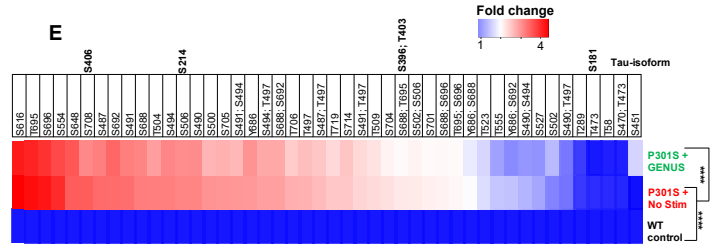
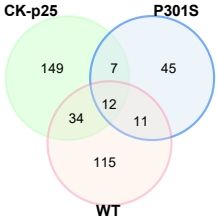


F

Differentially phosphorylated proteins: No Stim Versus GENUS



G

WT:CK-p25:P301S

Aak1	Ank2	Caskin1	Dlgap3	Dlgap4	Dnm1
Gap43	Map1b	Map2	Shank2	Srcin1	Srrm2

WT:CK-p25

Abi1	Agap2	Ankrd34b	Arhgap39	Cacna1a	Camkk1
Camkv	Clasp1	Cnksr2	Ctnnd2	Dclk1	Dlgap1
Dlgap2	Eef2	Eif4b	Gprin1	Macf1	Map1a
Map7d1	Marcks	Mbp	Mink1	Myh10	Pclo
Plxna1	Ppm1e	Sh2d3c	Shank1	Snph	Syn1
Syn2	Syn3	Tnik	Wipf2		

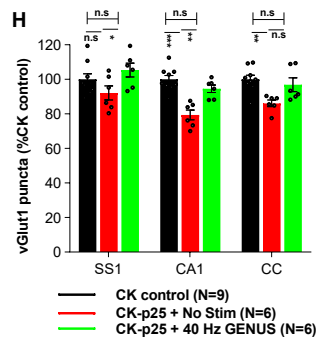
WT:P301S

Gsk3b	Mff	Myo18a	Palm	Phf24	Pitpnm1
Plppr4	Slc39a10	Slc4a10	Syngap1	Q8BH50	

CK-p25:P301S

Cep170	Kcnb1	Mapt	Pdha1	Sipa1l1	Sphkap
Tnks1bp1					

H



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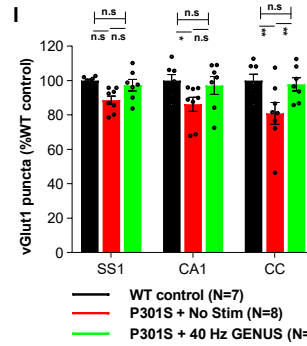

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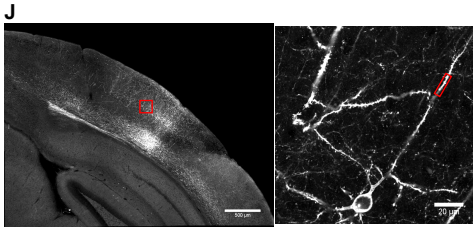

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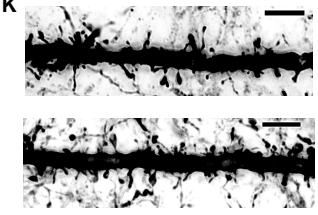

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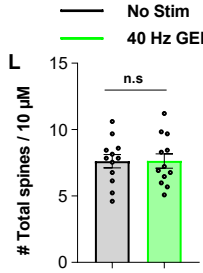

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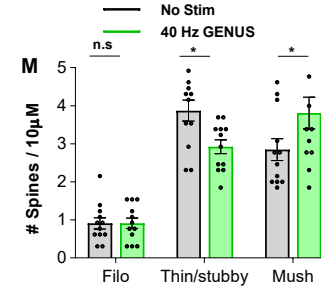

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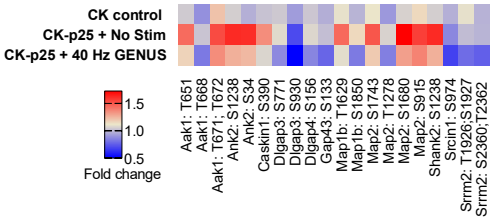

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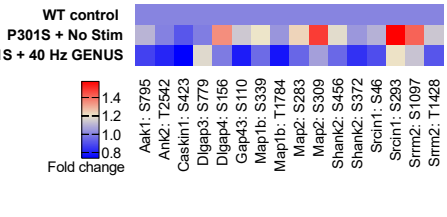

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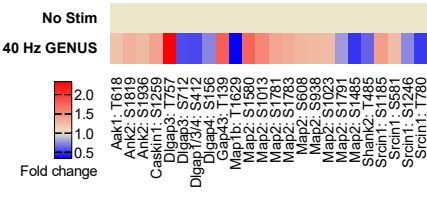

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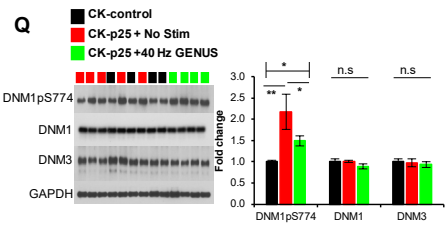

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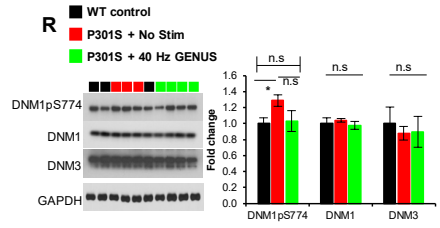

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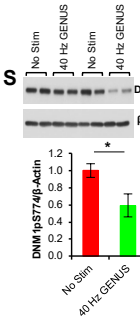

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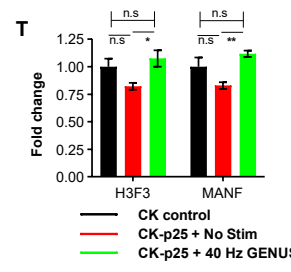

Figure S5. Chronic GENUS preserves synaptic density and improves the expression of synaptic genes, Related to Figure 5.

(A) Venn diagram of overlap of number of neuron specific DEGs between P301S and CK-p25 mice after chronic GENUS (statistically significant effect was observed in P301S and CK-p25 mice in commonly upregulated and downregulated genes with p = 1.27E-10 and 1.40E-12, respectively (Fisher's exact test). No other comparisons showed any significant overlap).

(B) Table shows the name of overlap genes, and the comparisons are shown on the top.

(C) Volcano plot of differentially S/T phosphorylated proteins in GENUS aged wildtype compared to No Stim aged wildtype mice.

(D) GO term of the biological processes associated with the differentially S/T phosphorylated proteins in GENUS aged WT mice compared to No Stim aged WT mice.

(E) Heat map of phosphorylation levels of different residues of tau protein (Two way ANOVA interaction $F(90, 322) = 22.57$, $p < 0.0001$. General post-hoc multiple comparison effect between groups, WT control Vs No Stim P301S, $p < 0.0001$; WT control Vs GENUS P301S, $p < 0.0001$; Overall, there was a significant effect of GENUS on tau phosphorylation status, No Stim P301S Vs GENUS P301S, $p < 0.0001$).

(F) Venn diagram of overlap of number of differentially S/T phosphorylated proteins across P301S, CK-p25 and aged wild type mice after chronic GENUS (statistically significant effect was observed in commonly S/T phosphorylated proteins with p = 1.06E-17, 3.42E-43 and 5.5E-25 (Fisher's exact test), for between CK-p25 and P301S, CK-p25 and aged WT, and P301S and aged WT mice, respectively).

(G) Table shows the name of overlap proteins, and the comparisons are shown on the top. Performing enrichment analysis using these proteins again revealed that they regulate synaptic function and vesicular trafficking.

(H) Group data comparing the expression of vGlut1 (N = 9, CK control; 6, No Stim CK-p25; 6, GENUS CK-p25 mice. Two-way ANOVA between groups effect $F(2, 54) = 18.83$, $p < 0.0001$).

(I) Group data comparing the expression of synaptic puncta vGlut1 (N = 7, WT control; 8, No Stim P301S; 7, GENUS P301S mice. Two-way ANOVA between groups effect $F(2, 57) = 11.77$, $p < 0.001$).

(J) Representative images. GENUS stimulation activated neurons in visual cortex were tagged (pseudo-colored EGFP) using cFoc-CreER^{T2} mice x AAV5-DIO-EYFP.

(K) Higher magnification images as in **Figure S5J**. *Top*: No Stim. *Bottom*: 40 Hz GENUS.

(L) Bar chart of total number of spines (t-test, $t = 0.1737$, $p = 0.986$).

(M) Bar graph shows different types of spines (filopodia, thin/stubby and mushroom; Two way repeated measures ANOVA, effect between groups and types of spines, $F(2, 22) = 8.544$, $p = 0.0018$. Post-hoc multiple comparison, no stim Vs GENUS: filopodia, $p > 0.999$; thin/stubby spines, $p = 0.0245$; mushroom spines, $p = 0.022$).

(N,O, and P) Heat maps of S/T residues of proteins commonly affected by GENUS in CK-p25 (N), P301S (O) and aged wild type (P) mice (related to **Figure S5F, S5G**). Dynamin 3 is not included in the graph as is shown in the subsequent graphs (S5Q, S5R, S5S).

(Q) Western blots of DNM1pS774, total DNM1, DNM3, and GAPDH *Right:* Bar graph quantifying western blots for DNM1pS774/DNM1 ($F(2, 10) = 5.836$, $p = 0.02$) total DNM1/GAPDH, DNM3/GAPD.

(R) Western blots of DNM1pS774, total DNM1, DNM3 and GAPDH from WT control, No Stim and GENUS P301S mice. *Right:* Bar graph quantifying western blots for DNM1pS774/DNM1, total DNM1/GAPDH, DNM3/GAPD.

(S) Western blots of DNM1pS774, and β-actin from visual cortex of wild type mice exposed to No Stim or GENUS ($t = 2.626$, $p = 0.039$).

(T) Expression of neuroprotective proteins histone3.3 (H3F3) and MANF was significantly higher in V1 in GENUS CK-p25 mice compared to No Stim CK-p25 mice (Two-way ANOVA $F(2,14) = 10.7$, $p = 0.0015$. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; n.s = not significant).