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Supplemental Information

Efficient Generation of CA3 Neurons from Human Pluripotent Stem Cells Enables Modeling of Hippocampal Connectivity *In Vitro*

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Supplementary Figure legends

Figure S1. Characterization of hpNPCs and hCA3, Related to Figures 1-2

(A) Log2fold change in expression of cortical hem genes (Wnt2b, Wnt3a,Zic2, Emx2) in hpNPCs compared to panNPCs by RNAseq, FDR < 0.05 and log2fold >1

(B) Relative abundance of Grik4 mRNA in low WNT3A-treated hCA3, high WNT3A-treated hDG and non-treated neurons by real time qPCR. *** p< 0.001 n=3 neuronal cultures. Right, fold change mRNA of Pvrl3, Dkk3 and Tgfβ2 in hCA3 (6 WIV) population compared to hpNPC population (6 WIV) by qRT-PCR. ****P=0.0004. Results are presented as mean ± SEM.

(C) Relative expression of CA3-specific genes (Elavl2, Grik4, Dkk3, Scgn) in hCA3 compared to hpNPC, panNPC and pan neurons. ****P<0.001. N≥ 3 per group.

(D-E) Presence of CTIP2 (right, nuclear staining, green, arrows) in $MAP2^+$ hCA3s (D)and CALB1 (right, nuclear staining, green, arrows) and in $NESTIN^+$ hpNPCs (E).

Results are presented as mean ± SEM. Scale bar 10 um.

Figure S2. Single cell RNA-Seq of hCA3 population and SCGN expression, Related to Figures 3-4

(A-B) Normalized read counts for all (A) and high quality cells (B)

(C) tSNE plot showing the high quality cells.

(D-F) tSNE plot showing the expression of NPC merkers S100B (D) , Nestin (E)and . DCX expression. Graded red to black color indicate higher to lower expression. Graded green to black color indicate higher to lower expression.

(G) Top 100 Up and 100 down regulated genes in the green cluster (FDR < 0.05 and log2fold >1)

(H) SCGN (red, white arrowheads) and CALB1 (green) immunohistochemistry in postmortem human hippocampus. Green arrowhead shows the area where $CALB1^+$ mossy fiber expression tapers off, near area CA3 –CA2 boundary. Cellular SCGN expression in CA2 and CA3 (middle) and neuropil staining in CA1 (right) in human hippocampus. Scale bar 100 um.

(I) SCGN expression in mouse nucleus accumbens, hypothalamus and amygdala (B) and hippocampus (C). Scale bar 50 um.

(J) Relative intensity of SCGN signal in nucleus accumbens (NAc), ventral pallidum (VP), dorsal pallidum (DP), septum (Sep), hypothalamus (Hypoth), DG and CA2 in adult mouse.

(K-L) $SCGN^+$ neurons (6 WIV) have relatively smaller soma compared to $ELAVL2^+$ neurons in the hCA3 population as well as in postmortem human brain. N≥30, ****P<0.0001.

Data are presented as mean ± SEM. The schematic of human brain indicates postmortem human hippocampus; the tissue culture plate indicates hCA3s.

Figure S3. Functional maturation of hCA3, Related to Figure 5

(A) log_2fold change in gene expression in AMPA receptors upregulated in hCA3 at 2 (gray) and 4 WIV (black). >5 fold log_2fold change, $P<0.05$. Data are shown as mean ± SEM

(B) Schematic of an excitatory synapse showing the proteins coded by up-regulated genes ($P<0.05$, $log_2fold \geq 1$) in hCA3s at 4WIV.

(C-D) Enriched GO terms in 4-WIV hCA3 transcriptome compared to hpNPCs (C) and 2WIV hCA3s (D); $P<0.01$.

Figure S4. Functional maturation of hCA3, Related to Figure 5

(A) Schematics showing the design of the LV-GRIK4-eGFP and LV-ELAVL2-GFP.

(B) Grik4-eGFP+ and Elavl2-eGFP+ cells in hpNPCs and hCA3 (6WIV) respectively. MAP2+ neurons are shown in red.

(C) Elavl2-eGFP+ and Grik4-eGFP+ cells express ELAVL2+ELAVL4 and only a small fraction express CALB1. Arrowheads show double positive cells.

(D) Trace showing excitatory postsynaptic currents (with a test pulse) in hCA3s at -60mV. The EPSC in hCA3 can be blocked by 20 μM APV and 40 uM CNQX.

(E-T) Electrophysiological properties of a total of 10 eGFP+ hCA3 neurons at 6 WIV measured with patch-clamping. AP= Action Potentials, Vm rest = resting membrane potential measured at 0pA in current clamp mode; AP freq = max frequency of APs evoked in current clamp with a 500ms depolarizing pulse from -70mV and rising above -10mV; Rm = Membrane resistance; Cap = capacitance; AP Thresh = AP Threshold; AP AHP = AP After Hyperpolarization Potential.

(U) GFP^+ hCA3 are positioned on hippocampal pyramidal layer.

(U) $ELAVL2^+$ hCA3 graft (green) in mouse brain. Scale bar 100 um

(V) Immunohistochemistry of CALB1 (left, red) and CTIP2 (right, magenta) on mouse hippocampal tissue containing hCA3 graft (green). Scale bar 100 um.

(W) $SCGN^+$ neurons (yellow arrows) in transplanted LV-GRIK4-GFP carrying hCA3 in mouse. White arrows show SCGN-negative hCA3s. Scale bar 50 um.

(X) hCA3s show characteristic pyramidal neuron morphology with primary dendrites. Yellow box is shown in higher magnification on the right. Yellow stars show puncta on $SCGN^+$ hCA3s. Scale bars 10 um.

(Y) Bright-field images of hCA3 and hDG neurons on a MEA plate showed no apparent difference in cell numbers.

(Z) Principal component analysis on spontaneous MEA data from differentiating hDGs and hCA3s. PC1 = 98%.

Figure S5. Electron microscopy of hDG and hCA3 co-culture, Related to Figure 6

(A) Design of the two compartment microfluidic devices.

(B-C) Examples of transmission electron microscopy images of hDG-hCA3 co-culture (8 WIV) showing dendritic structures (blue arrows), numerous synaptic vesicles (red arrowheads) and electron dense postsynaptic densities (PSDs, red arrows). Scale bar 1um.

Figure S6. Differentiation of hCA3s from iPSCs, Related to Figure 7

(A-D) Comparison of hCA3s differentiated from two progenitor clones from iPSCs derived from individuals with healthy (C,D) and SZ (A,B) genetic background at 4WIV. Scale bar 50um

(E-F) HuES6 and iPSC derived hCA3 show similar number of ELAVL2+ELAVL4 and CALB1 positive neurons.

(G-H) iPSC clones showing equivalent differentiation of ELAVL2+ELAVL4 and CALB1.

Figure S7. Comparisons of healthy and SZ-iPSC derived hCA3s, Related to Figure 7

(A) Equivalent numbers of SCGN in healthy and SZ groups. $p > 0.05$

(B) Equivalent number of cells on MEA plates used for recording in healthy and SZ groups. $p > 0.05$

(C) Representative images of MAP2, ELAVL2 and CALB1 positive neurons in hDG-hCA3 co-culture derived from healthy and SZ iPSCs.

(D) Average spikes per burst and spikes per network burst over 10 minutes of MEA recordings in hCA3-hDG co-culture (6 WIV) derived from healthy iPSCs are not significantly different than SZ iPSCs.

(E-F) Equivalent numbers of GABA+ interneurons between healthy and SZ group: ns $p > 0.05$;

(E) Examples of GABA neurons (arrows) in healthy and SZ lines. Scale bar 10 um.

(H) Comparison of average spikes per burst (ns $P > 0.05$) and spikes per network burst (*$P=0.02$) over 10 minutes of MEA recordings in hCA3 culture (6 WIV) derived from healthy and SZ iPSCs. Spikes per network bursts in SZ-hCA3s are significantly reduced compared to healthy-hCA3s.

(I) Equivalent number of average spike over 10 minutes MEA recording at 3-5 weeks in hCA3 culture derived from healthy and SZ iPSCs. $p > 0.05$

(J) Na+ and K+ current of iPSC derived SZ and healthy hCA3s.

(K) ES and iPS derived hCA3 show similar total and maximum evoked APs. Two-way T-test was performed using weighted mean and weighted standard deviation. $P= 0.18$ and 0.19 respectively. Each dot is eGFP+ hCA3 neuron.

For all graphs, each dot shows an individual. Data are presented as mean ± SEM. N≥3 iPSCs in healthy and SZ group. At least ≥3 neuronal cultures per individual were used for (A, D-G), 6 neuronal cultures per individual were used for (C, H-I). ns $p > 0.05$.

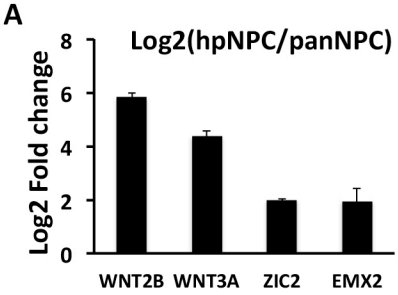
Supplementary Tables

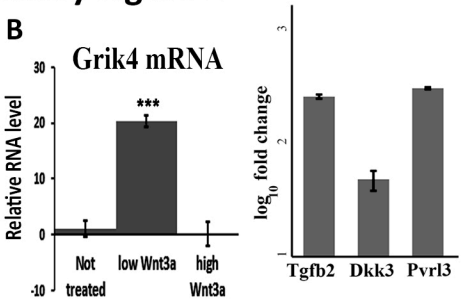
Table S1
Differential gene expression list of panNPC and hpNPC.
Related to Figure 1.

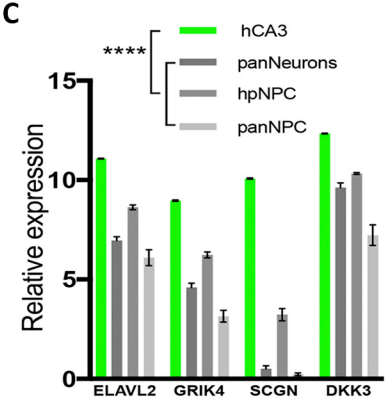
Table S2
Normalized RNAseq count of hpNPC, hCA3s at 2- 6 week.
Related to Figure 2.

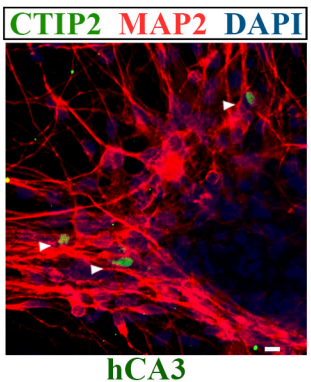
Table S3
Up and down regulated gene list of all Green, Red and Blue clusters.
Related to Figures 3-4.

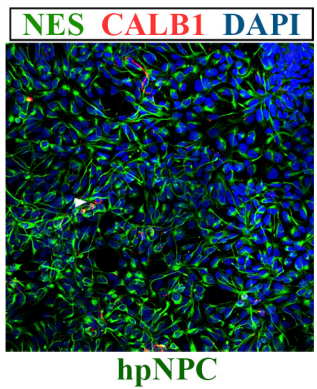
Supplementary Figure 1



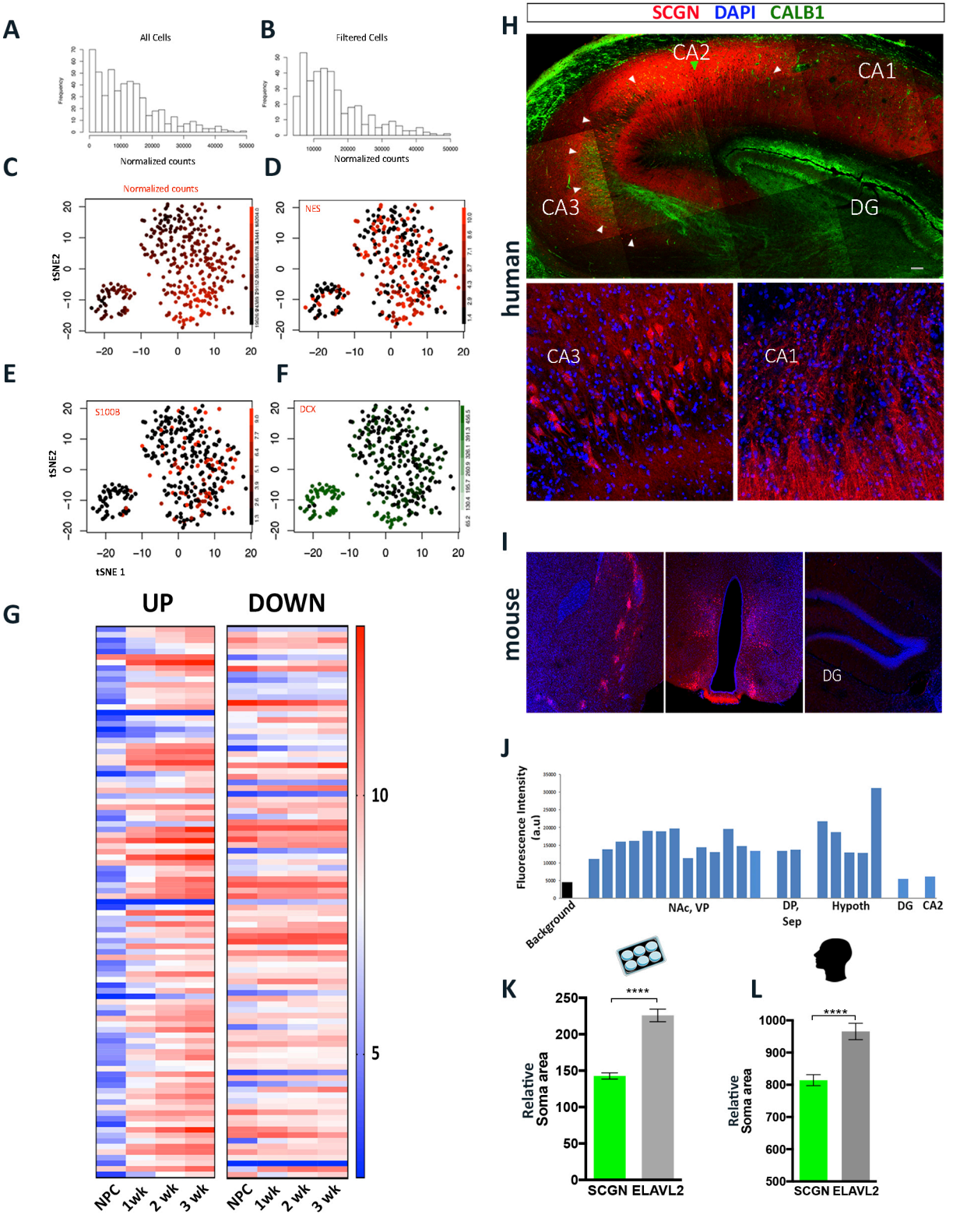




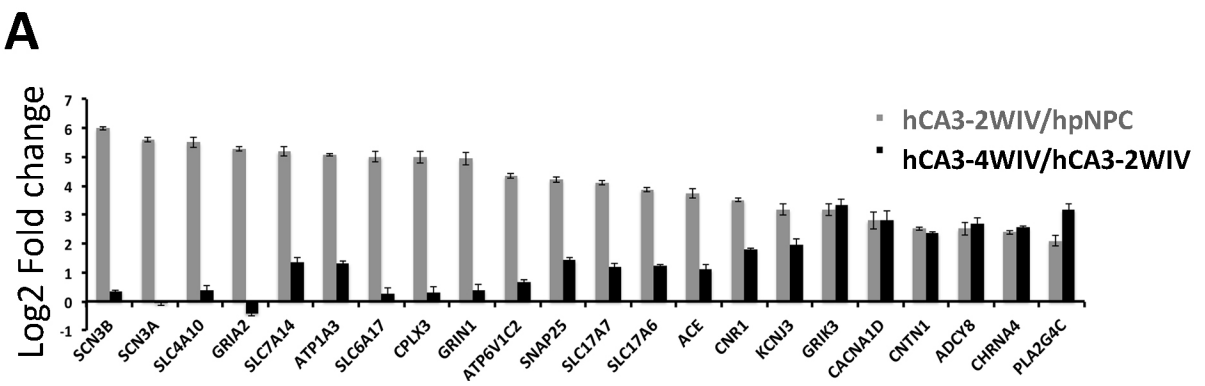


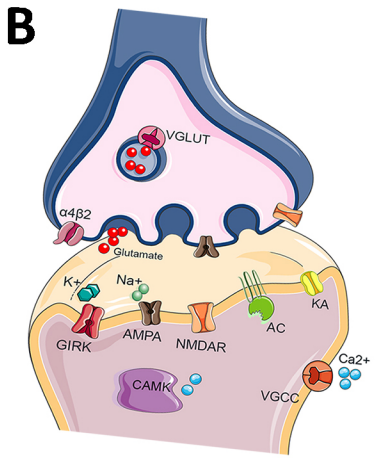


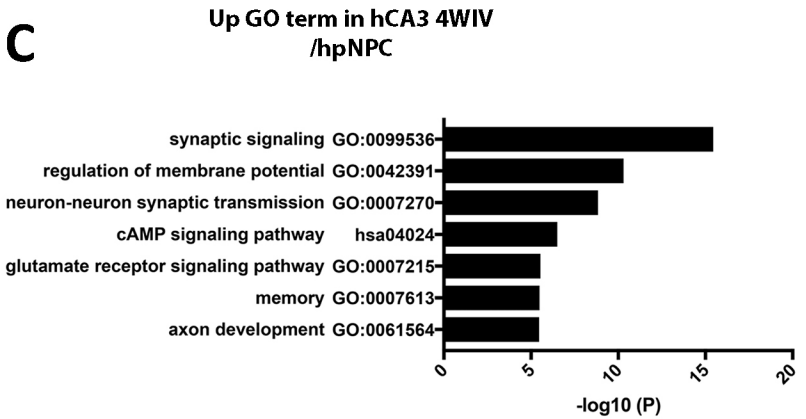
Supplementary Figure 2

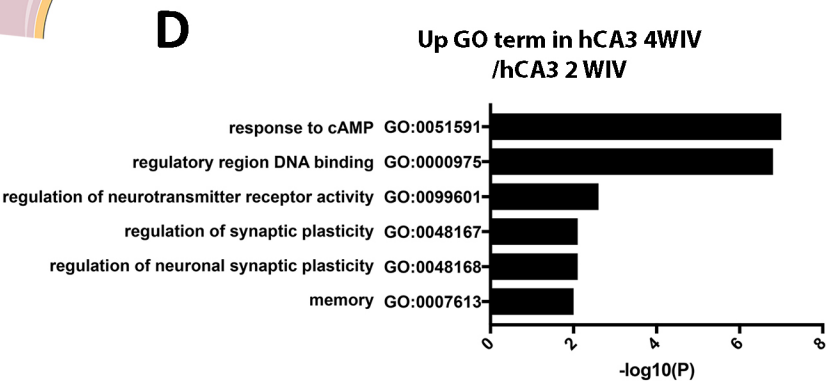


Supplementary Figure 3

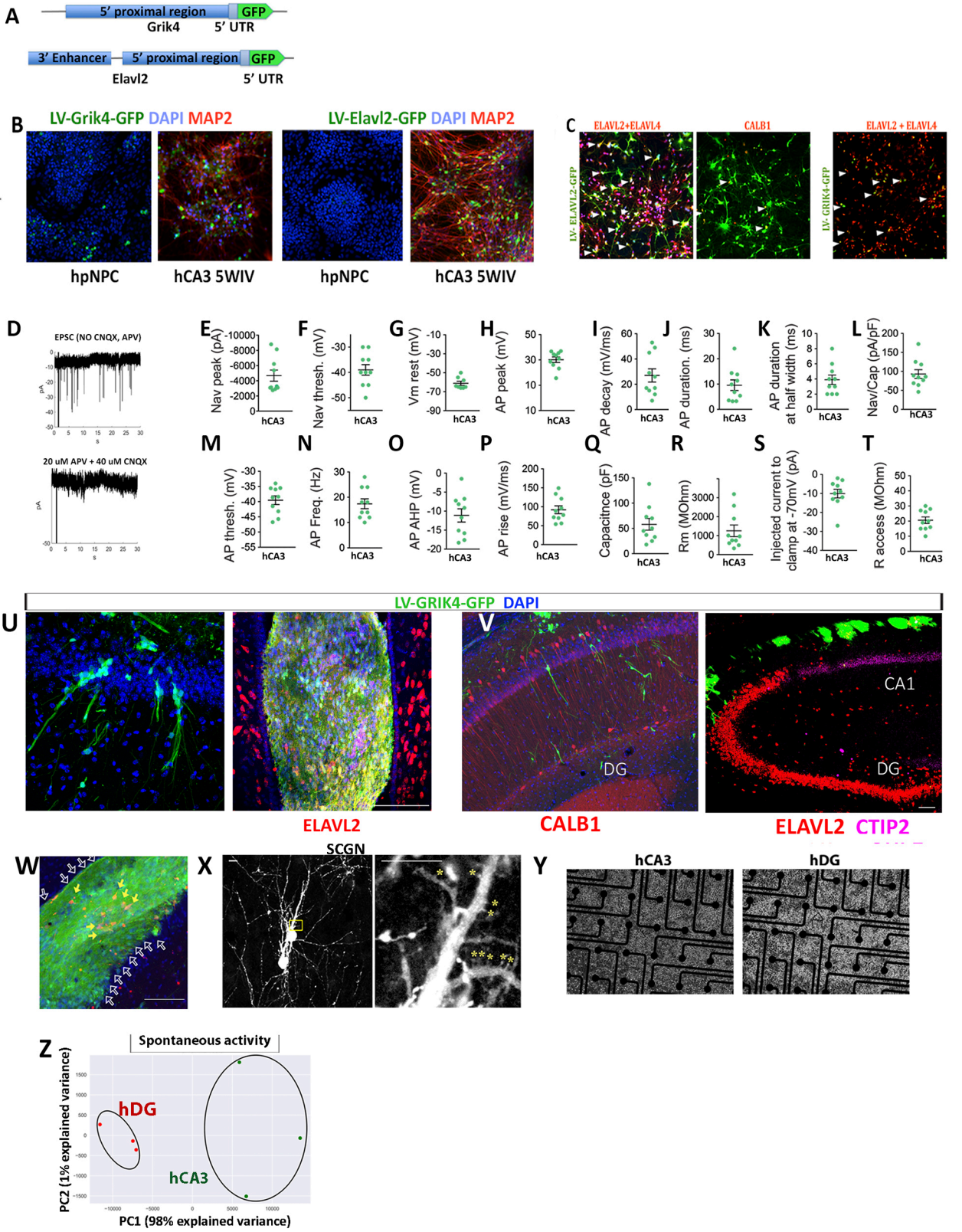




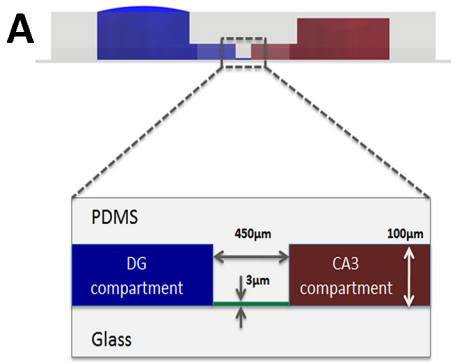


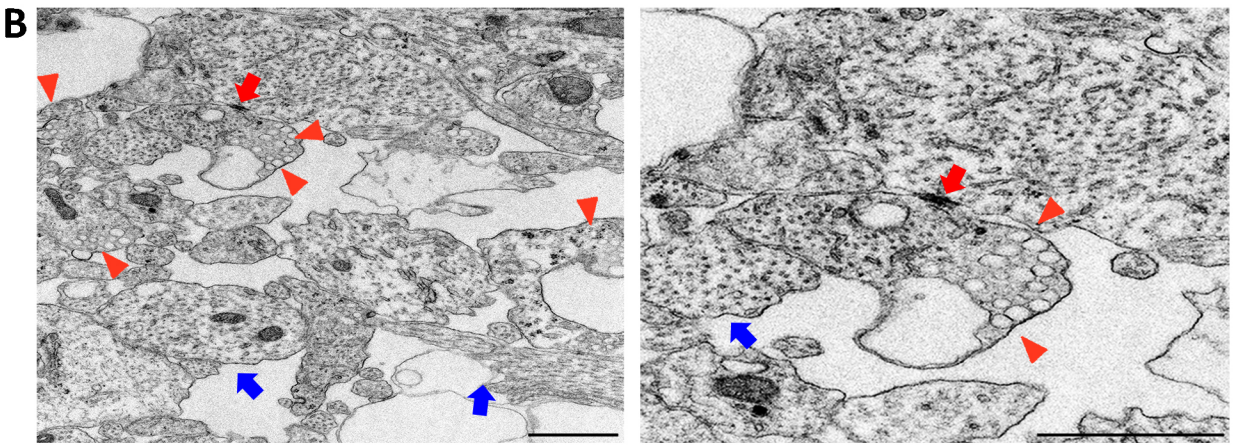


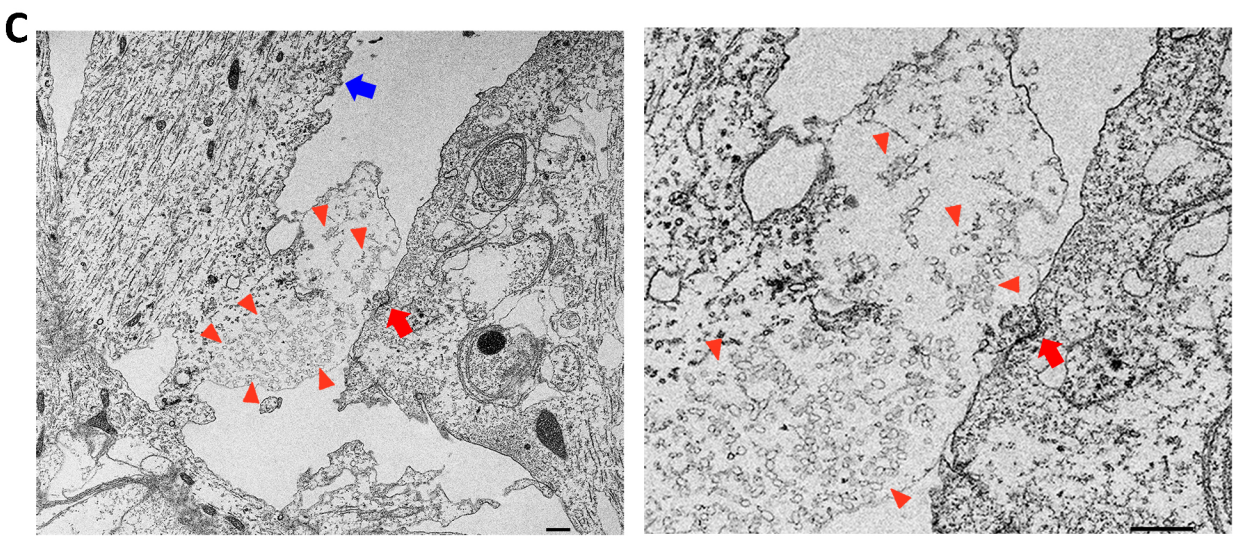
Supplementary Figure 4



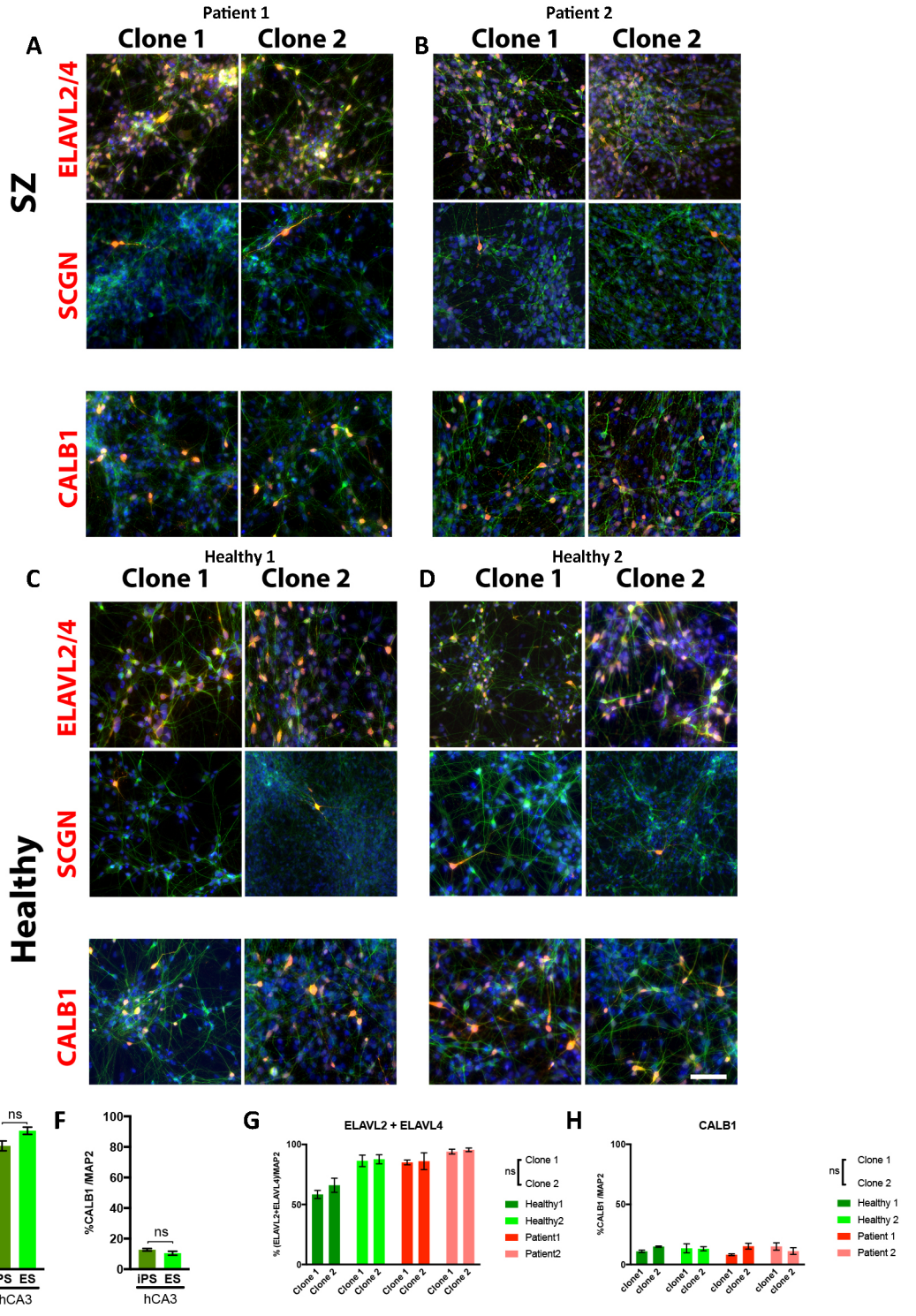
Supplementary Figure 5







Supplementary Figure 6



Supplementary Figure 7