

Figure S1. Characterization of mouse ES neural differentiation. (A) Representative immunofluorescence images at NS and ND cell stages for neural progenitor (nestin), neuronal (TUJ1 and NeuN) and astroglial (GFAP) markers. (B) Gene expression analysis by qPCR of projection neuronal markers (Tbr1, Bcl11b, Er81, Cux2, Uchl1 and Satb2 was performed from NS stage (12 DIV) to ND cells (18 DIV and 21 DIV). (C) Representative images for vGLUT1 and vGAT1 immunofluorescence and quantification of glutamatergic and GABAergic cells referred to total DAPI counterstained cells. (D) Representative image of TUJ1 and vGLUT1 immunofluorescence. (E) BCL11B ${ }^{+}$and SATB2 ${ }^{+}$ neurons were quantified after immunofluorescence in ND cells and referred to the total number of cells counterstained with DAPI. (F) Electrophysiological characterization of ES-derived neurons was performed by patch-clamp recordings in current- and voltage-clamp mode. (G) Representative biocytinlabelled cell during patch-clamp analysis, after immunofluorescence with an anti-ER81 antibody. The analyses were done in mRNA extracts and ES-derived cultures from $n=4$ independent experiments. Scale bar, $50 \mu \mathrm{~m}(\mathrm{~A}, \mathrm{C}, \mathrm{E})$ and 10 $\mu \mathrm{m}$ (D, G). Statistics: *** $p<0.001$.


Figure S2. Characterization of $\mathrm{CB}_{1}$ receptor expression in knockdown and control ES cells. (A) Western blot analysis of $\mathrm{CB}_{1}$ receptor transcripts and levels after ES nucleofection with shCB1 receptor and shControl and analysed at ES and ND stages. (B) qPCR quantification of $C B_{1}$ mRNA levels in shCB $1_{1}-R 1$ and shCtrl-R1 cells transfected with pcDNA-Control or pcDNA-CB ${ }_{1}$ expression vectors. (C) Western blot analyses of $\mathrm{CB}_{1}$ protein levels in cell extracts as
above. (D) $C B B_{1}$ receptor immunoreactivity determined in ND cells after immunofluorescence from shCtrl- and shCB $\mathrm{CB}_{1}$-R1 cells and transfection with pcDNA-Control or pcDNA-CB1 expression vectors. Representative images are shown. (E) Western blot analyses of phosphor-ERK and ERK in the shCtrl-R1 and shCB1-R1 cells, at 21 DIV, as above, after 15 min stimulation with THC. The analyses were done in cells from $n=3$ and mRNA extracts from $n=4$ independent ES-differentiations. Statistics: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (vs shCtrl-R1 at ES stage or shCtrl-R1+pcDNA or shCtrl-R1+pcDNA - Veh); \#\# $\mathrm{p}<0.01$, \#\#\# $p<0.001$ (vs shCtrl-R1 at ND stage or shCB $1+\mathrm{pcDNA}$ or shCtrl-R1+pcDNA-CB1 - Veh); $\$ \$ p<0.01$ (vs shCB $1-R 1+$ pcDNA $^{2} C_{1}$ - Veh). Scale bar, $15 \mu \mathrm{~m}(\mathrm{~A}), 25 \mu \mathrm{~m}(\mathrm{C})$.


Figure S3. Genetic ablation of the $\mathrm{CB}_{1}$ receptor at NS stage interferes with deep cortical neuronal generation. $(\mathrm{A}, \mathrm{B})$ Representative immunofluorescence images of $\mathrm{BCL11B}^{+}$and SATB2 $^{+}$cells at ND stage in shCB 1 and shControl cells. (C, D) Quantification of BCL11B ${ }^{+}$and SATB2 ${ }^{+}$cells in GFP ${ }^{+}$nucleofected cells and quantification of neuronal marker BCL11B and SATB2 immunoreactivity in the same cells. (E) qPCR quantification of $C B_{1}, T u j 1, B c / 11 b$ and Satb2 mRNA levels in ND differentiated cells. The analyses were done from $n=3-6$ independent ES-differentiations. Statistics: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (vs shControl).


Figure S4. JZL-184 induced eCB levels and promoted ES-derived deep layer neuronal differentiation. (A) Levels of the eCBs 2AG and AEA were determined in JZL-184- and vehicle-treated ND cells. (B) Representative images of $\mathrm{BCL11B}^{+}$, ER81 $^{+}$and SATB2 ${ }^{+}$cells derived from JZL-184- and vehicle-treated shCB1-R1 or shCtrl-R1 cells. (C) Quantification of BCL11B ${ }^{+}$, ER81+ and SATB2 ${ }^{+}$cells in GFP ${ }^{+}$neurons derived from shControl or shCB $_{1}$ transfected R1-derived NS cells. The analyses were done in mRNA extracts and immunofluorescence experiments from $n=3$ independent ES-


Figure S5. HU-210-induced $\mathrm{CB}_{1}$ receptor activation promotes ES neuronal differentiation. (A-D) mES cells were differentiated in the presence of HU-210 (100 nM) in the presence or absence of AM-251 (1 $\mu \mathrm{M}$ ) or vehicle, and neuronal differentiation was characterized by the analysis of nestin, TUJ1, TBR1 and NeuN immunofluorescence. Quantification is shown in Table S1. (E)

Cannabinoid induced changes in NeuN, Bcl11b, Er81, Satb2 and Cux2 transcript levels determined by real-time PCR as compared to vehicle treated ES cells. ES cells were treated with HU-210, THC combined or not with AM-251 as above. The analyses were done in mRNA extracts and ES-derived cultures from $n=3-5$ independent experiments. Statistics: * $p<0.05,{ }^{* *} p<0.01$, *** $p<$ 0.001 (vs vehicle). Scale bar, $50 \mu \mathrm{~m}$.


Figure S6. Characterization of $\mathrm{CB}_{1}$ receptor-mediated regulation of Bcl 11 b transcriptional activity. (A, B) R1 mES cells were treated during neuronal differentiation with the CB1 receptor agonist THC $(2 \mu \mathrm{M})$ or JZL-184 ( $1 \mu \mathrm{M}$ ) in the presence and absence of AM-251 (1 $\mu \mathrm{M}$ ), and luciferase activity of MAR-A4 Bcl11b and MAR1-Dcc constructs were determined. (C) HiB5 cells were transfected with 500 ng of pCAG-Satb2, $2 \mu \mathrm{~g}$ of pCMV-Ski and pSatb2, $1 \mu \mathrm{~g}$ MAR-A4-pfosluc of Bc/11b and 40 ng Renilla vectors and luciferase activity determined 24h after vehicle and HU-210 (100 nM) treatment. (D) Luciferase activity of the MAR-A4 Bcl11b reporter in vehicle and HU-210 treated cells in the presence of different pharmacological modulators UO126 (1 $\mu \mathrm{M}$ ), AktiX (2.5 $\mu \mathrm{M})$, rapamycin (100 nM ), SP600125 (12.5 $\mu \mathrm{M}$ ) and dibutiryl cyclic AMP (2.5 $\mu \mathrm{M})$, MG-132 $(5 \mu \mathrm{M})$. The analyses were done in ES-derived cultures from $n=$ 3-6 independent experiments. Statistics: *p<0.05, ** $p<0.01$, *** $p<0.001$ (vs vehicle), $\# p<0.05$, $\# \# p<0.01$, $\# \# \# p<0.001$ (vs HU-210).
A

C


B


Figure S7. Lack of toxicity in mES-derived cells and hiPS-derived organoids at the usage concentrations. $(A, B)$ Representative images and quantification of Cleaved caspase-3 on ND cells derived from mES in the presence of THC $(2 \mu \mathrm{M})$, JZL-184 ( $1 \mu \mathrm{M}$ ) and AM-251 (1 $\mu \mathrm{M})$. (C) Representative images and quantification of Cleaved caspase-3 on human iPS cell-derived organoids in the presence of THC $(2 \mu \mathrm{M})$, JZL-184 (1 $\mu \mathrm{M}$ ) and AM-251 (1 $\mu \mathrm{M})$. (D) Representative images and quantification of organoid area in the presence of HU-210 (100 nM) and THC $(2 \mu \mathrm{M})$. Scale bar: $25 \mu \mathrm{~m}(\mathrm{~A}, \mathrm{C}) ; 0.3 \mathrm{~mm}(\mathrm{D})$.

|  | Positive cells/DAPI ( $\pm$ SEM) |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | Vehicle | HU-210 | AM-251 | AM-251+HU-210 |
| Nestin | $38.24 \pm 1.57$ | $30.47 \pm 2.59$ | $39.42 \pm 2.28$ | $37.83 \pm 2.90$ |
| TUJ1 | $42.82 \pm 3.20$ | $55.20 \pm 4.90$ | $41.80 \pm 2.37$ | $39.87 \pm 4.48$ |
| NeuN | $36.33 \pm 1.90$ | $47.95 \pm 1.27$ | $37.45 \pm 2.25$ | $25.50 \pm 5.37$ |
| TBR1 | $8.31 \pm 0.34$ | $18.47 \pm 2.30$ | ND | ND |

Table S1. Quantification of neuronal cell population changes determined after immunofluorescence for the indicated antibodies in ES-differentiated neurons in the presence of HU-210 (100 nM) in the presence or absence of AM-251 ( $1 \mu \mathrm{M}$ ) and vehicle treated cells. Representative images are shown in Supplementary Fig. 2. (ND) not determined. The analyses were done in cells from $n=4$ independent ES-differentiations.

| Antigen | Species reactivity | Clonality | Dilution (IF/WB) | Reference |
| :---: | :---: | :---: | :---: | :---: |
| $\alpha$-TUBULIN | Mouse | Monoclonal | $\begin{gathered} \text { Sigma } \\ \text { (n.e./1:5000) } \end{gathered}$ | T9026 |
| $\beta$-ACTIN | Mouse | Monoclonal | $\begin{gathered} \text { Sigma } \\ \text { (n.e. } / 1: 5000 \text { ) } \end{gathered}$ | A5441 |
| $C B_{1}$ | Guinea pig | Polyclonal | Frontier Institute (1:500/n.e) | CB1-GP-Af530-1 |
| $\mathrm{CB}_{1}$ | Rabbit | Polyclonal | Frontier Institute (n.e./1:500) | CB1-Rb-Af380-1 |
| CTIP2 | Rat | Monoclonal | Abcam (1:500/n.e.) | ab18465 |
| DAGL | Guinea pig | Polyclonal | Frontier Institute (1:200/n.e.) | DGL $\alpha$-GP-Af380 |
| ER81 | Rabbit | Polyclonal | Abcam (1:500/n.e.) | ab184120 |
| ERK 1/2 | Mouse | Monoclonal | Cell Signalling (n.e./1:1000) | 4696 |
| FAAH | Rabbit | Polyclonal | Chemicon (1:50/n.e.) | AB5644P |
| GFAP | Mouse | Polyclonal | Invitrogen (1:400/n.e.) | PA5-16291 |
| GFP | Rabbit | Polyclonal | Abcam (1:1000/n.e.) | ab290 |
| MAP2 | Mouse | Monoclonal | Sigma (1:500/n.e.) | M9942 |
| MGL | Rabbit | Polyclonal | Cayman Chem (1:100/n.e.) | 100035 |
| NAPE-PLD | Guinea pig | Polyclonal | Frontier Institute (1:200/n.e.) | NAPE-PLD-GP-Af720 |
| Nestin | Mouse | Monoclonal | Chemicon (1:500/n.e.) | MAB353 |
| Nestin | Rabbit | Polyclonal | Covance (1:200/n.e.) | 839801 |
| NeuN | Mouse | Monoclonal | Chemicon (1:500/n.e.) | MAB377 |
| OCT4 | Rabbit | Polyclonal | Abcam (1:500/n.e.) | ab19857 |
| p-ERK 1/2 | Rabbit | Polyclonal | Cell Signalling (n.e./1:1000) | 9101 |
| SATB2 | Mouse | Monoclonal | Abcam (1:50/n.e.) | ab51502 |
| TBR1 | Rabbit | Polyclonal | Abcam (1:500/n.e.) | ab23345 |
| TUJ1 | Mouse | Monoclonal | Chemicon (1:500/n.e.) | MAB1637 |
| TUJ1 | Rabbit | Polyclonal | BioLegend (1:500/n.e.) | 802001 |
| vGLUT1 | Rabbit | Polyclonal | Synaptic Systems (1:250/n.e.) | 135303 |
| vGAT1 | Rabbit | Polyclonal | Synaptic Systems (1:250/n.e.) | 131003 |

Table S2. Primary antibodies used in this study. Dilution employed for immunofluorescence (IF) and western blot (WB) are indicated, (n.e.) application not employed.

| Gene | Forward primer ( $5^{\prime}$-3') | Reverse primer ( $3^{\prime}-5^{\prime}$ ) |
| :---: | :---: | :---: |
| Abhd4 | GGGCTTGTTTACTATGGCTGA | CAAGTGGGGAGCCAGCTA |
| Abhd6 | CTCCTATGTCCGCTtCAAGG | GAATGCGAACATCGACAAGA |
| Abhd12 | GGATGATGTGACTATTGGAGTCTG | CACATCTGGTCCTTCCCTTG |
| CB1 | GGGCAAATTTCCTTGTAGCA | GGCTCAACGTGACTGAGAAA |
| Ctip2 | ACCCACGAAAGGCATCTGT | GCTGGAAGGCTCATCTTTACC |
| Cux2 | TCAGTCAACAGCTCCATTCG | GCCCTGAACACAGAGCAAAG |
| Dagla | CTTTTCCTCTTGGGCATCAT | GCATCGTGCATTCCTTATCA |
| Dcc | tgtcgaggagagccacaig | CGCTCAAGTCATCCTGTTCA |
| Er81 | ATGGAGAAAAGTGCCTGTACAAT | GGTGTAGTGGGGACACTGGA |
| Faah | GCAGGTGGGCTGTTCAGT | AAGCAGGGATCCACAAAGTC |
| MgI | tGATGTCTGCAGCCTGTCTC | GCCGTtGTACAAAAGGATTGT |
| Rbfox 3 (NeuN) | AAGAAGCCTGGGAACCCATA | GGCCCATAGACTGTTCCTACC |
| Satb2 | TTTAGCCAGCTGGTGGAGAC | CACCTCCCTAGCTTGATTATTCC |
| Tbr1 | CAAGGGAGCATCAAACAACA | GTCCTCTGTGCCATCCTCAT |
| Tuj1 | GCGCATCAGCGTATACTACAA | CATGGTTCCAGGTTCCAAGT |
| Uchl1 | GCCCTTTCCAGTGAACCAT | tGAATTCTCTGCAGACCTTGG |
| Unc5C | TCCAAGAACTGCACTGATGG | CCACGTAGAGAGCCACATCAT |

Table S3. Primers used for qPCR in this study.

| Figure |  | Statistical analysis | Comparison | p -value ( n experiments) |
| :---: | :---: | :---: | :---: | :---: |
| Figure 1 | B | One-way ANOVA <br> (Dagla, p=0.085; <br> Mgl, p<0.0001; <br> Abhd6, p = 0.0029; <br> Abhd12, $p=0.019$ ) | ES vs ND | Post-hoc: $\mathrm{p}=0.039$ (Dagla, $\mathrm{n}=5), \mathrm{p}<0.001(\mathrm{Mgl}, \mathrm{n}=6), \mathrm{p}=0.0012$ (Abhd6, $\mathrm{n}=4)$ and $\mathrm{p}=0.0092($ Abhd12, $\mathrm{n}=4)$ |
|  | C | One-way ANOVA (Nape-pld, p=0.37; Faah, p < 0.0001; Abhd4, $\mathrm{p}=0.002$ | ES vs ND | Post-hoc: $\mathrm{p}=0.77$ (Nape-pld, $\mathrm{n}=0.77), \mathrm{p}<0.0001$ (Faah, $\mathrm{n}=6$ ) and $\mathrm{p}<0.0001$ (Abhd4, $\mathrm{n}=4$ ) |
|  | D | $\begin{aligned} & \text { One-way ANOVA } \\ & (\mathrm{p}=0.021) \\ & \hline \end{aligned}$ | ES vs NS | Post-hoc: $\mathrm{p}=0.008$ ( $\mathrm{CB}_{1}, \mathrm{n}=5$ ) |
|  |  |  | ES vs ND | Post-hoc: $\mathrm{p}<0.0001$ ( $\mathrm{CB}_{1}, \mathrm{n}=5$ ) |
|  | E | One-way ANOVA (2AG, p = 0.0064; <br> AEA, $p=0.0015$; <br> OEA, $\mathrm{p}=0.0074$; <br> PEA, $p=0.0014$ ) | ES vs NS | Post-hoc: $\mathrm{p}=0.039(2 \mathrm{AG}, \mathrm{n}=3), \mathrm{p}=0.0005($ AEA, $\mathrm{n}=3), \mathrm{p}=0.0025(0 E A, n=3)$ and $\mathrm{p}=0.0007($ PEA, $n=3)$ |
|  |  |  | ES vs ND | Post-hoc: $\mathrm{p}=0.0021$ (2AG, $\mathrm{n}=3$ ), $\mathrm{p}=0.027$ (AEA, $\mathrm{n}=3$ ), $\mathrm{p}=0.076$ (OEA, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0014$ (PEA, $\mathrm{n}=3$ ) |
| Figure 3 | B | One-way ANOVA (BCL11B, p = 0.0003; ER81, p < 0.0001; SATB2 p < 0.0001) | shCtrl+pcDNA vs shCtrl+pcDNA-CB ${ }_{1}$ | Post-hoc: $\mathrm{p}=0.0018$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}<0.0001$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0004$ (SATB2, $\mathrm{n}=3$ ) |
|  |  |  | shCrrilpcDNA vs shCB ${ }_{1}+$ pcDNA | Post-hoc: $\mathrm{p}=0.031$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}=0.0014$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0025$ (SATB2, $\mathrm{n}=3$ ) |
|  |  |  | shCtrl+pCDNA vs shCB1+pCDNA-CB2 | Post-hoc: $\mathrm{p}=0.0036$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}=0.0021$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0003$ (SATB2, $\mathrm{n}=3$ ) |
|  |  |  | shCB ${ }_{1}+$ pcDNA vs shCB1+pcDNA-CB ${ }^{\text {a }}$ | Post-hoc: $\mathrm{p}=0.0002$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}<0.0001$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}<0.0001$ (SATB2, $\mathrm{n}=3$ ) |
|  | C | One-way ANOVA (BC111b, p = 0.0002; Er81, $\mathrm{p}=0.0002$; Satb2, $\mathrm{p}<0.0001$ Cux2, p = 0.0002) | shCtrl+pcDNA vs shCtrl+pcDNA-CB ${ }^{\text {d }}$ | Post-hoc: $\mathrm{p}=0.0019$ (Bcl11b, $\mathrm{n}=4$ ), $\mathrm{p}=0.0011$ (Er81, $\mathrm{n}=4), \mathrm{p}=0.0014$ (Satb2, $\mathrm{n}=4$ ) and $\mathrm{p}=0.030$ (Cux2, $\mathrm{n}=4$ ) |
|  |  |  | shCrrl+pCDNA vs shCB ${ }_{1}+$ pcDNA | Post-hoc: $\mathrm{p}=0.019$ (BCl11b, $n=4), \mathrm{p}=0.040$ (Er81, $\mathrm{n}=4), \mathrm{p}<0.0001$ (Satb2, $\mathrm{n}=4$ ) and $\mathrm{p}=0.0014$ (Cux2, $\mathrm{n}=4)$ |
|  |  |  | shCB ${ }_{1}+$ pcDNA vs shCB1+pcDNA-CB ${ }_{2}$ | Post-hoc: $\mathrm{p}=0.012$ (BCl11b, $\mathrm{n}=4)$, $\mathrm{p}=0.011($ Er81, $\mathrm{n}=4), \mathrm{p}<0.0001($ Satb2, $\mathrm{n}=4)$ and $\mathrm{p}=0.0011($ Cux2, $\mathrm{n}=4)$ |
| Figure 4 | B | One-way ANOVA <br> (BCL11B, p = 0.0064 <br> ER81, $\mathrm{p}=0.029$; <br> SATB2, $\mathrm{p}=0.010$ | Veh vs JIL-184 | Post-hoc: $\mathrm{p}=0.0023$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}=0.0075$ (ER81, $\mathrm{n}=3-4$ ) and $\mathrm{p}=0.0057$ (SATB2, $\mathrm{n}=3-4$ ) |
|  | C | One-way ANOVA (BCL11B, p < 0.0001; ER81, $\mathrm{p}<0.0001$; SATB2, p < 0.0001) | Veh-shCtrl Vs IZL-shCtrl | Post-hoc: $\mathrm{p}<0.0001$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}<0.0001$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}<0.0000$ (SATB2, $\mathrm{n}=3$ ) |
|  |  |  | Veh-shCtrlvs Veh-shCB ${ }_{1}$ | Post-hoc: $\mathrm{p}=0.0095$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}=0.0003$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0002$ (SATB2, $\mathrm{n}=3$ ) |
|  |  |  | Veh-shCtrivs JZL-shCB ${ }_{1}$ | Post-hoc: $\mathrm{p}=0.012$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}=0.0003($ ER81, $\mathrm{n}=3)$ and $\mathrm{p}=0.0012$ (SATB2, $\mathrm{n}=3$ ) |
|  | D | One-way ANOVA (Bcl11b, p < 0.0001; <br> Er81, p < 0.0001; <br> Satb2, $\mathrm{p}<0.0001$ <br> Cux2, $\mathrm{p}=0.0002$ ) | Veh-shCtrl Vs JZ-shCtrl | Post-hoc: p < 0.0001 (BCl111b, $\mathrm{n}=3$ 3), $\mathrm{p}=0.0004$ (Er81, $\mathrm{n}=3$ ), $\mathrm{p}=0.0004$ (Satb2, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0077$ (Cux2, $\mathrm{n}=3$ ) |
|  |  |  | Veh-shCtrrlvs Veh-shCB ${ }_{1}$ | Post-hoc: $\mathrm{p}=0.0031$ (BCl11b, $\mathrm{n}=3$ ), $\mathrm{p}=0.0046$ (Er81, $\mathrm{n}=3), \mathrm{p}<0.0001$ (Satb2, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0058$ (Cux2, $\mathrm{n}=3$ ) |
|  |  |  | Veh-shCtrl vs JIZ-shCB ${ }_{1}$ | Post-hoc: p = 0.0096 (Bcl11b, $\mathrm{n}=3$ ), p $=0.015$ (Er81, $\mathrm{n}=3$ ), p < 0.0001 (Satb2, $\mathrm{n}=3$ ) and p $=0.0063$ (Cux2, $\mathrm{n}=3$ ) |
| Figure 5 | B | One-way ANOVA (BCL11B, p = 0.0092; ER81, p = 0.0003; SATB2 $p=0.15$ ) | Veh vs THC | Post-hoc: $\mathrm{p}=0.0057($ (BCL11B, $\mathrm{n}=5), \mathrm{p}=0.0004($ (ER81, $\mathrm{n}=4)$ and $\mathrm{p}=0.039($ SATB2, $\mathrm{n}=4)$ |
|  | C | One-way ANOVA <br> (Dcc, p=0.071; <br> Unc5C, $p=0.10$ ) | Veh vs THC | Post-hoc: $\mathrm{p}=0.016$ ( $\mathrm{Dcc}, \mathrm{n}=5$ ) and $\mathrm{p}=0.038(\mathrm{Unc5C}, \mathrm{n}=4)$ |
| Figure 6 | C | Student's t test | Veh vs THC | $\mathrm{p}=0.0004$ [AP threshold, $\mathrm{n}=42$ (Veh) and $\mathrm{n}=44$ (THC) cells] and $\mathrm{p}<0.0001$ [ [nward current, $\mathrm{n}=42$ (Veh) and $\mathrm{n}=44$ (THC) cells] |
|  |  | Welch's test |  | $\mathrm{p}<0.0001$ [AP amplitude, $\mathrm{n}=42$ (Veh) and $\mathrm{n}=44$ (THC) cells] |
|  | F | Student'st test | Veh vs JZL-184 | p<0.0001 [AP threshold, $n=9$ (Veh) and $\mathrm{n}=12$ (IZL) cells] and $p=0.0047$ [Inward current, $\mathrm{n}=29$ (Veh) and $\mathrm{n}=41$ (IZL) cells] |
|  |  | Welch's test |  | $\mathrm{p}<0.0001$ [AP amplitude, $\mathrm{n}=29$ (Veh) and $\mathrm{n}=41$ (JZL) cells] |
| Figure 7 | D | One-way ANOVA (BCL11B, p < 0.0001; SATB2, p < 0.0001) | Veh vs HU-210 | Post-hoc: $\mathrm{p}<0.0001$ [BCL11B, $\mathrm{n}=13$ (Veh) and $\mathrm{n}=24$ (HU-210) ventricles] and $\mathrm{p}=0.0003$ [SATB2, $\mathrm{n}=12$ (Veh) and $\mathrm{n}=10$ (HU-210) ventricles] |
|  |  |  | Veh vs THC | Post-hoc: $\mathrm{p}=0.0003$ [BCL11B, $\mathrm{n}=13$ (Veh) and $\mathrm{n}=24$ (THC) ventricles] and $\mathrm{p}<0.0001$ [SATB2, $\mathrm{n}=12$ (Veh) and $\mathrm{n}=10$ (THC) ventricles] |
| Figure S1 | C | Student'st test | Glu vs GABA | $\mathrm{p}<0.0001$ ( $\mathrm{n}=4$ ) |
| Figure S2 | A | $\begin{aligned} & \text { One-way ANOVA } \\ & (\mathrm{p}<0.0001) \end{aligned}$ | - | Post-hoc: $\mathrm{p}=0.0016$ (ES-shCtrrl vs ES-shCB, $\mathrm{n}=3$ ), $\mathrm{p}=0.0016$ (ES-shCtrlvs ND-shCtrl, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0001$ ( ND -shCtrlvs ND -shCB $1, \mathrm{n}=3$ ) |
|  | B | $\begin{aligned} & \text { One-way ANOVA } \\ & (\mathrm{p}<0.0001) \end{aligned}$ | - | Post-hoc: $p=0.0007$ (shCtrl+pcDNA vs shCtrl+pcDNA-CB $1, n=4$ ), $p=0.006$ (shCtrl+pcDNA vs shCB ${ }_{1}+$ pcDNA, $n=4$ ) and $\mathrm{p}=0.0003\left(\mathrm{shCB}_{1}+\mathrm{pcDNA}\right.$ vs shCB $1+\mathrm{pcDNA}^{2}-\mathrm{CB}_{1}, \mathrm{n}=4$ ) |
|  | C | $\begin{aligned} & \text { One-way ANOVA } \\ & (p=0.0005) \end{aligned}$ | - | Post-hoc: $\mathrm{p}=0.0015$ (shCtrl+pcDNA vs shCtrl+pcDNA-CB ${ }_{1}, \mathrm{n}=3$ ), $\mathrm{p}=0.021$ (shCtrl+pcDNA vs shCB ${ }_{1}+\mathrm{pcDNA}, \mathrm{n}=3$ ) and $\mathrm{p}=0.010\left(\right.$ shCB $_{1}+\mathrm{pcDNA}$ vs shCB1 1 pcDNA $-\mathrm{CB}_{1}, \mathrm{n}=3$ ) |
|  | D | $\begin{aligned} & \text { One-way ANOVA } \\ & (\mathrm{p}<0.0001) \end{aligned}$ | - | Post-hoc: $\mathrm{p}=0.0002$ (shCtrl+pcDNA vs shCtrl+pcDNA-CB $1, \mathrm{n}=3$ ), $\mathrm{p}=0.0014$ (shCtrl +pcDNA vs shCB ${ }_{1}+\mathrm{pcDNA}, \mathrm{n}=3$ ) and $\mathrm{p}=0.0001\left(\mathrm{shCB}_{1}+\mathrm{pcDNA}\right.$ vs shCB $1+\mathrm{pcDNA}^{2} \mathrm{CB}_{1}, \mathrm{n}=3$ ) |
|  | E | $\begin{aligned} & \text { One-way ANOVA } \\ & (\mathrm{p}<0.0001) \end{aligned}$ | - | Post-hoc: p 0.0001 (Veh-shCtrl+pcDNA vs THC-shCtrl+pcDNA, $n=3$ ), p $<0.0001$ (Veh-shCtrl+pcDNA-CB ${ }_{1}$ vs THC-shCtrI + pcDNA-CB ${ }_{1}, \mathrm{n}=3$ ) and $\mathrm{p}=0.0087$ (Veh-shCB1 pcDNA -CB 1 vs THC-shCB1+pcDNA-CB $1, \mathrm{n}=3$ ) |
| Figure S3 | C | Student'st test | shControl vs shCB ${ }_{1}$ | $p=0.0004$ (BCL11B, $n=6$ ) and $p=0.0007$ (SATB2, $n=6$ ) |
|  | D | Student's test | shControl vs shCB ${ }_{1}$ | $p=0.0015$ (BCL11B, $n=3$ ) and $p=0.010$ (SATB2, $n=3$ ) |
|  | E | Student's t test | shControl vs shCB ${ }_{1}$ |  |
| Figure S4 | A | Student's t test | Veh vs JIL-184 | $p=0.023$ (2AG, $n=3$ ) and $p=0.007$ (AEA, $n=3$ ) |
|  | C | One-way ANOVA (BCL11B, p < 0.0001; ER81, p $=0.0001$ SATB2, $\mathrm{p}<0.0001$ ) | Veh-shControl vs SZL-shCtrI | Post-hoc: $\mathrm{p}<0.0001$ (BCL11b, $\mathrm{n}=3$ ), $\mathrm{p}=0.004$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}=0.0001$ (SATB2, $\mathrm{n}=3$ ) |
|  |  |  | Veh-shControl vs Veh-shCB ${ }_{1}$ | Post-hoc: $\mathrm{p}<0.0001$ (BCL118, $\mathrm{n}=3), \mathrm{p}=0.0068$ (ER81, $\mathrm{n}=3$ ), and $\mathrm{p}<0.0001$ (SATB2, $\mathrm{n}=3$ ) |
|  |  |  | Veh-shControl vs JZL-shCB ${ }_{1}$ | Post-hoc: $\mathrm{p}<0.0001$ (BCL11B, $\mathrm{n}=3$ ), $\mathrm{p}=0.0026$ (ER81, $\mathrm{n}=3$ ) and $\mathrm{p}<0.0001$ (SATB2, $\mathrm{n}=3$ ) |
|  |  | Student's t test | Veh vs HU-210 | $\begin{aligned} & \mathrm{p}<0.0001[\mathrm{NeuN}, \mathrm{n}=5(\text { Veh }) \text { and } \mathrm{n}=4(\mathrm{HU}-210)], \mathrm{p}=0.0068[\mathrm{Bcl11b,n}=5(\text { Veh }) \text { and } \mathrm{n}=5(\mathrm{HU}-210)], \mathrm{p}=0.010[\mathrm{Er} 81, \mathrm{n}=4(\text { Veh }) \text { and } \mathrm{n}=4(\mathrm{HU}-210)], \\ & \mathrm{p}<0.0001[\text { Satb2, } \mathrm{n}=5(\text { Veh }) \text { and } \mathrm{n}=5(\mathrm{HU}-210)], \text { and } p=0.010[\mathrm{Cux} 2, \mathrm{n}=5(\text { Veh }) \text { and } \mathrm{n}=4(\mathrm{HU}-210)] \end{aligned}$ |
| Figure 55 | E | One-way ANOVA <br> (NeuN, p = 0.0029; <br> Bcl11b, p=0.10; <br> Er81, p = 0.059; <br> Satb2, $\mathrm{p}=0.032$; <br> Cux2, $\mathrm{p}=0.15$ ) | Veh vs THC |  |
| Figure S6 | A | One-way ANOVA (Bcl11b, p = 0.0008; Dcc, $p=0.0048$ ) | Veh vs THC | Post-hoc: $\mathrm{p}=0.0001(\mathrm{Bcl11b}, \mathrm{n}=6)$ and $\mathrm{p}=0.0098(\mathrm{Dcc}, \mathrm{n}=4)$ |
|  | B | One-way ANOVA (Bcl11b, p < 0.0001; Dcc, $p=0.0034$ ) | Veh vs JJL-184 | Post-hoc: $\mathrm{p}<0.0001$ (Bc111b, $\mathrm{n}=6$ ) and $\mathrm{p}=0.0024$ ( $\mathrm{Dcc}, \mathrm{n}=4$ ) |
|  | C | $\begin{aligned} & \text { One-way ANOVA } \\ & (\mathrm{p}<0.0001) \end{aligned}$ | - | Post-hoc: p $=0.0009$ (pcDNA-Veh vs pcDNA-HU, $n=3$ ), $\mathrm{p}=0.018$ (pcDNA-Veh vs Satb2-HU, $\mathrm{n}=3$ ), $p=0.0062(p c D N A-V e h ~ v s ~ S k i-V e h, ~ n=3)$ and $p=0.0004(p c D N A-V e h ~ v s ~ S a t b 2+S k i-V e h, ~ n=3) ~$ |
|  | D | $\begin{aligned} & \text { One-way ANOVA } \\ & \text { (p }<0.0001) \end{aligned}$ | - |  |

Table S4. Detailed statistical analyses per figure, containing relevant test performed, p-values and

