

## **Supplementary Information**

### **Human Neural Organoid Microphysiological Systems Show the Building Blocks Necessary for Basic Learning and Memory**

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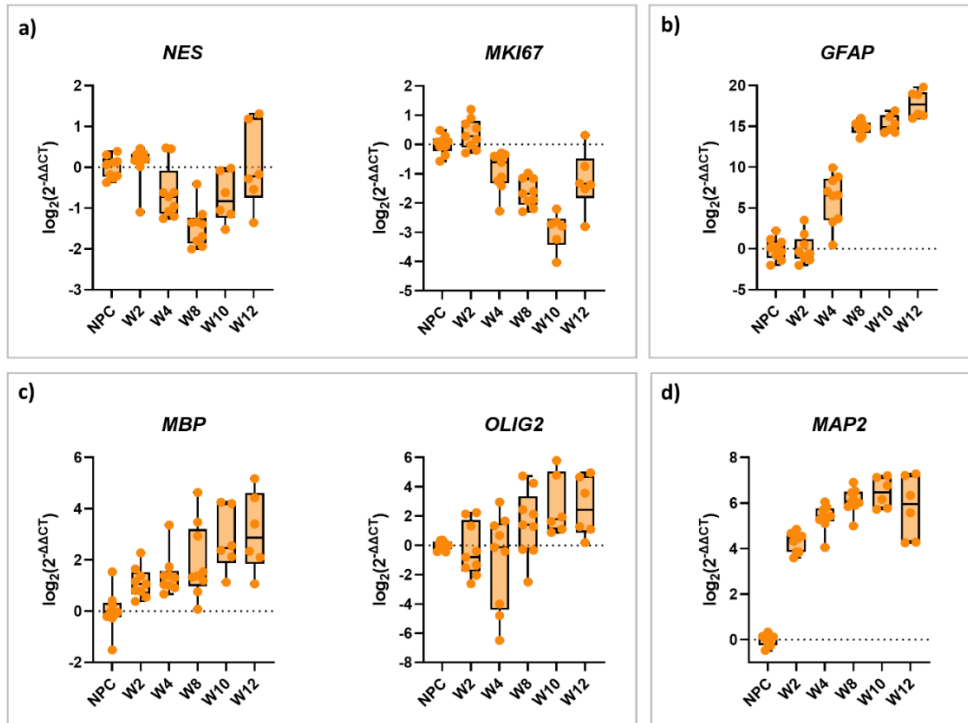
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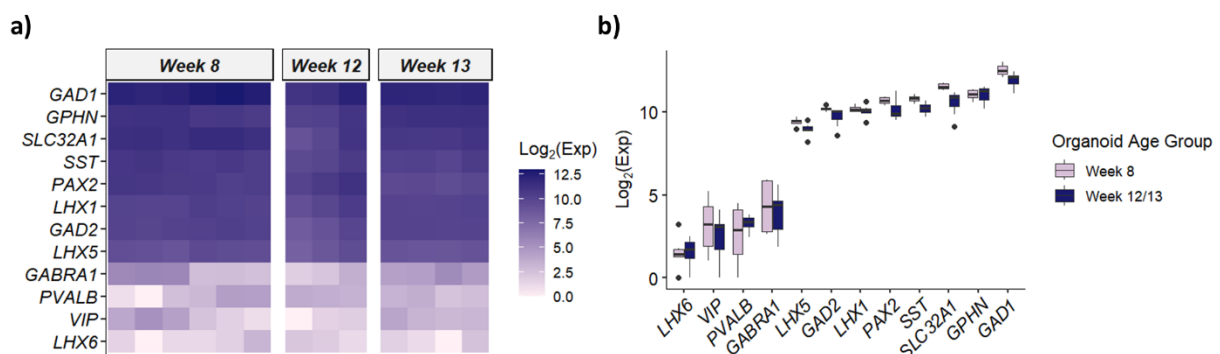
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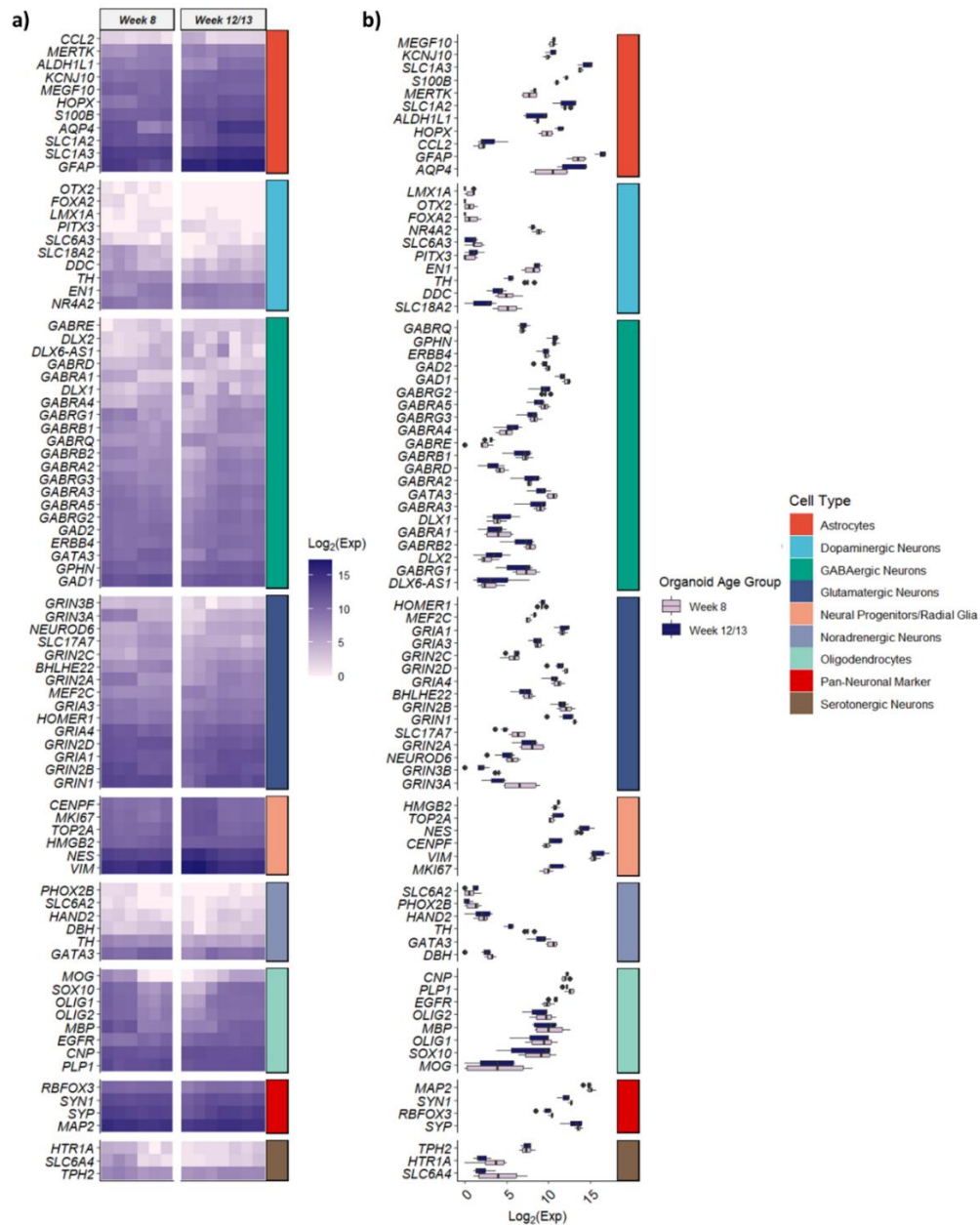
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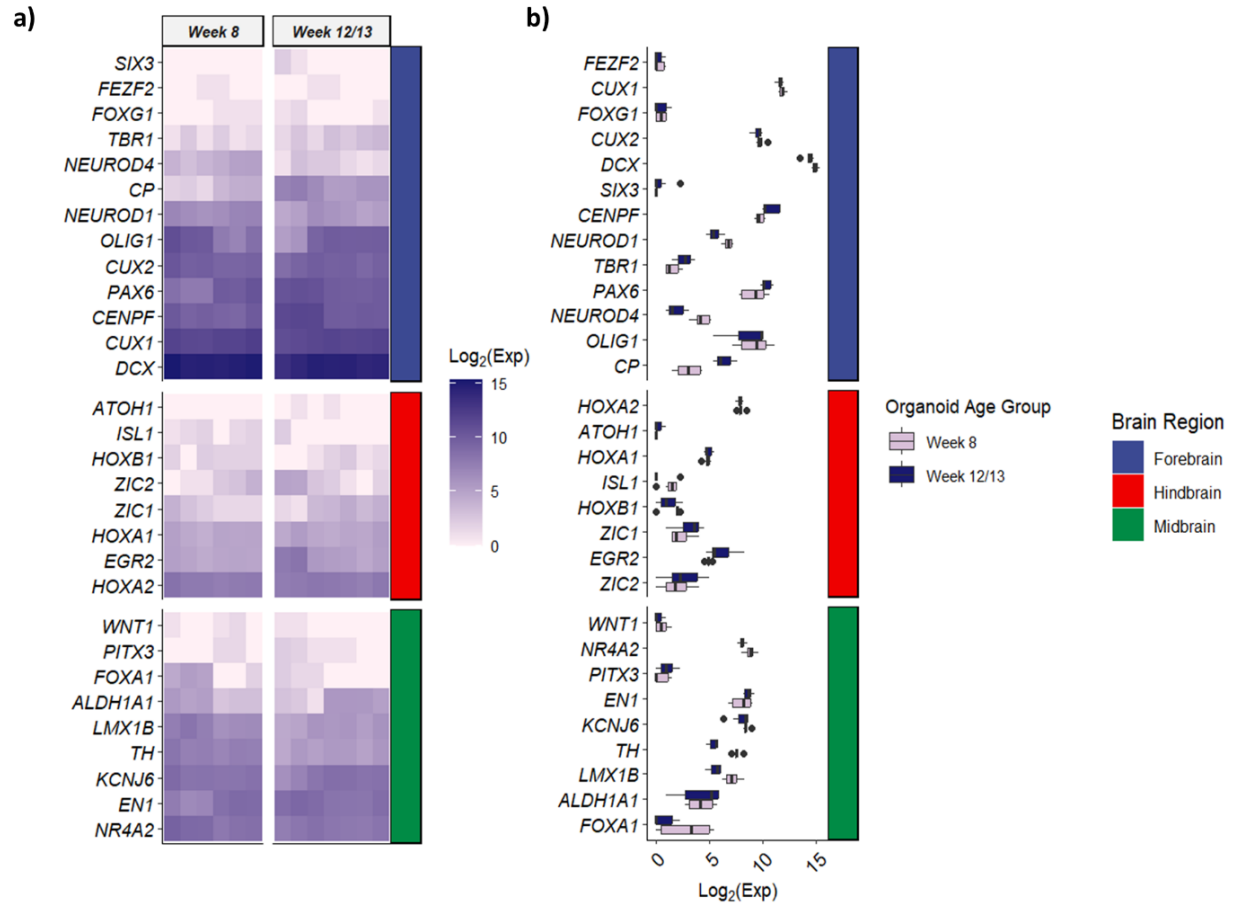
**Supplementary Figure 1. Model characterization via gene expression.** **a)** Expression of NPC (*NES*, Nestin) and proliferation (*MKI67*, *Kiel 67*) markers, **b)** astrocyte marker (*GFAP* - Glial Fibrillary Acidic Protein), **c)** oligodendrocyte markers (*MBP*- Myelin Based Protein, *OLIG2* - Oligodendrocyte Transcription Factor 2) **d)** neuronal dendritic marker (*MAP2*- Microtubule-associated protein 2) at weeks (W) 2, 4, 8, 10 and 12 of organoid differentiation. Data is shown as a box and whisker plot (with the box extending from the 25th to 75th percentiles) represented as  $\log_2$  of the difference in expression, normalised to NPCs. *ACTB* was used as a reference gene. Data represents 2-3 independent experiments with 3 technical replicates each.



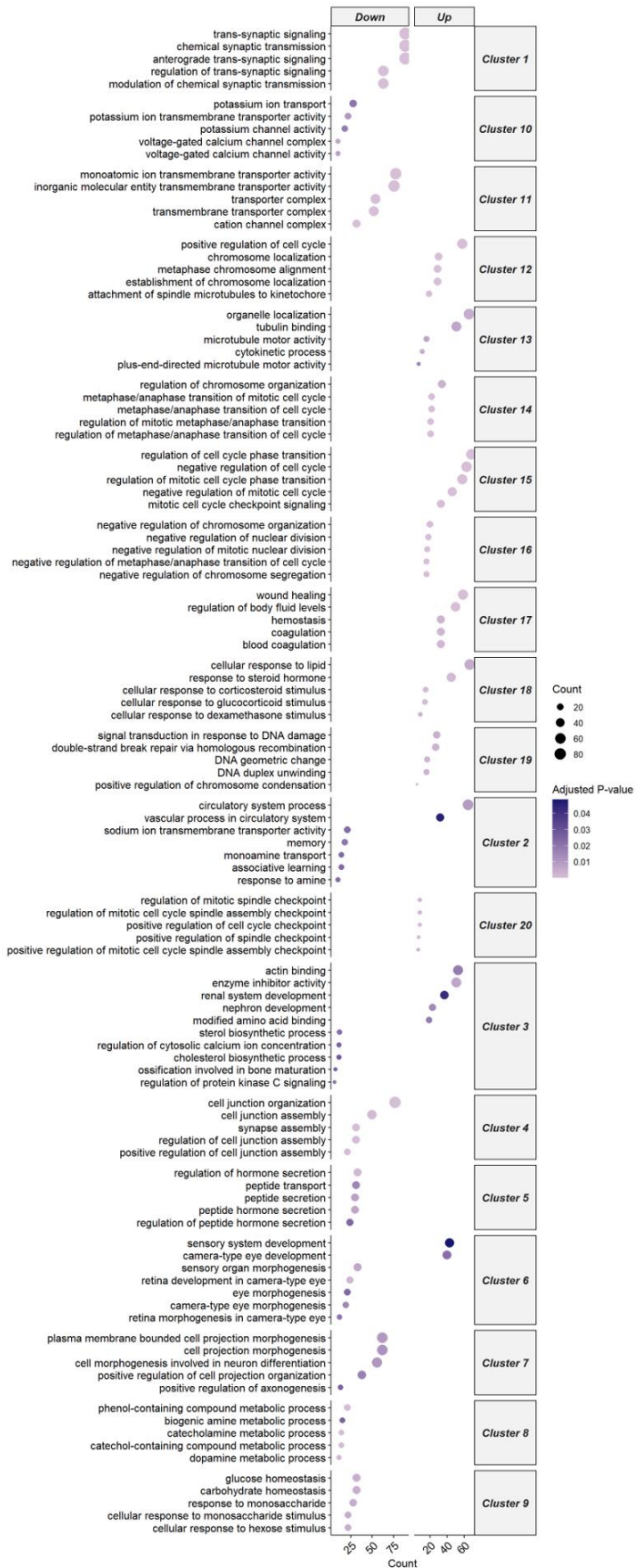
**Supplementary Figure 2. Gene expression of GABAergic markers in week 8, 12, and 13 organoids using RNA-seq data.** **a)** Heatmap and **b)** boxplot with interquartile range of distribution showing the  $\log_2$ -transformed gene expression of GABAergic neuronal markers across week 8, 12, and 13 neural organoids. Each time point includes at least 3 biological replicates: N = 6 for week 8, N = 3 for week 12, and N = 4 for week 13.



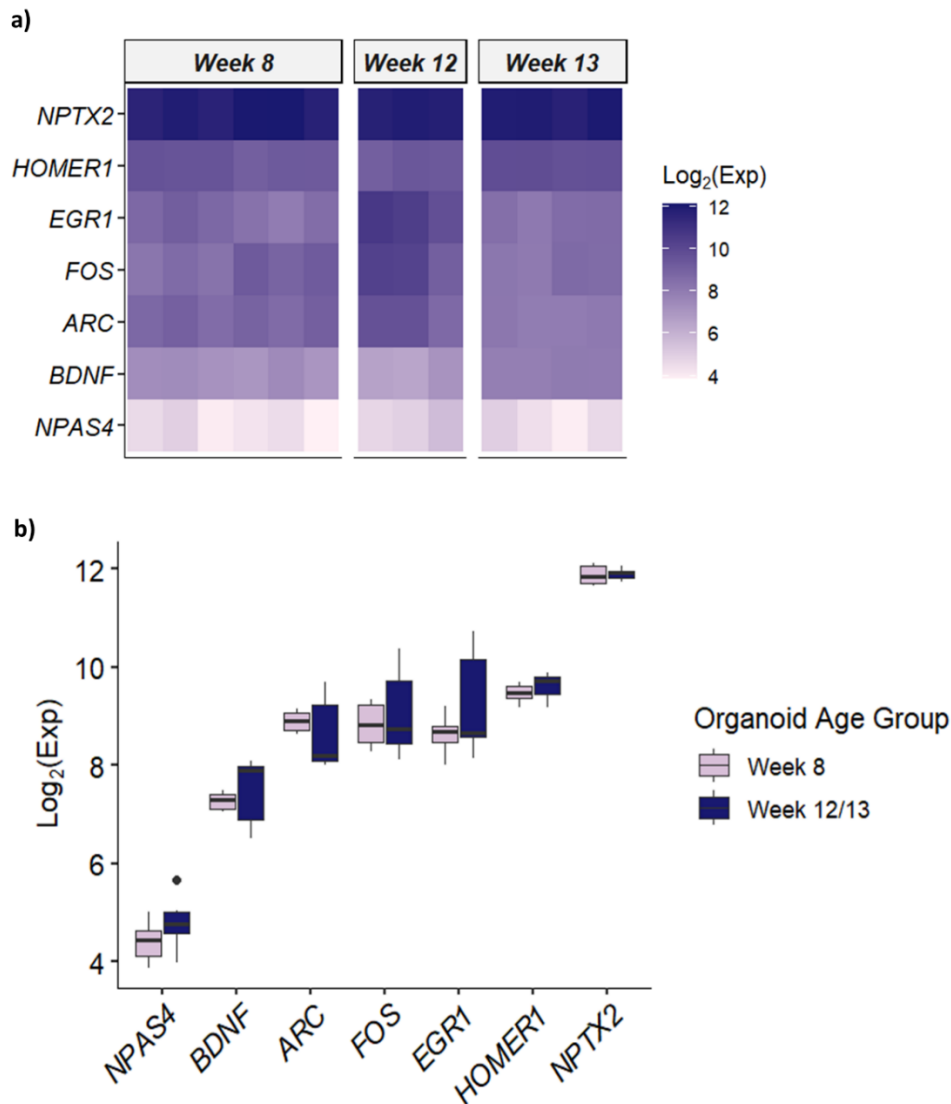
**Supplementary Figure 3. Heatmap and boxplots of the log<sub>2</sub>-transformed expression of cell type-specific markers comparing week 8 organoids to week 12/13 organoids using RNA-seq Data. a)** Heatmap showing the expression of cell type-specific markers for both week 8 and week 12/13 organoids. **b)** Data is shown as boxplot with interquartile range of distribution of gene expression of cell type-specific markers for both age groups, with expression values shown as Log<sub>2</sub>(Exp). Orange group represents astrocyte-specific genes, light blue- dopaminergic neurons; green – GABAergic neurons; dark purple – glutamatergic neurons; peach – neural progenitors/Radial glia; light purple – noradrenergic neurons; light green – oligodendrocytes; red – pan-neuronal markers; brown – serotonergic neurons. Each time point includes at least six biological replicates: N = 6 for week 8 and N = 7 for the combined week 12/13 group. The full names of genes are listed in Supplementary Data 4.



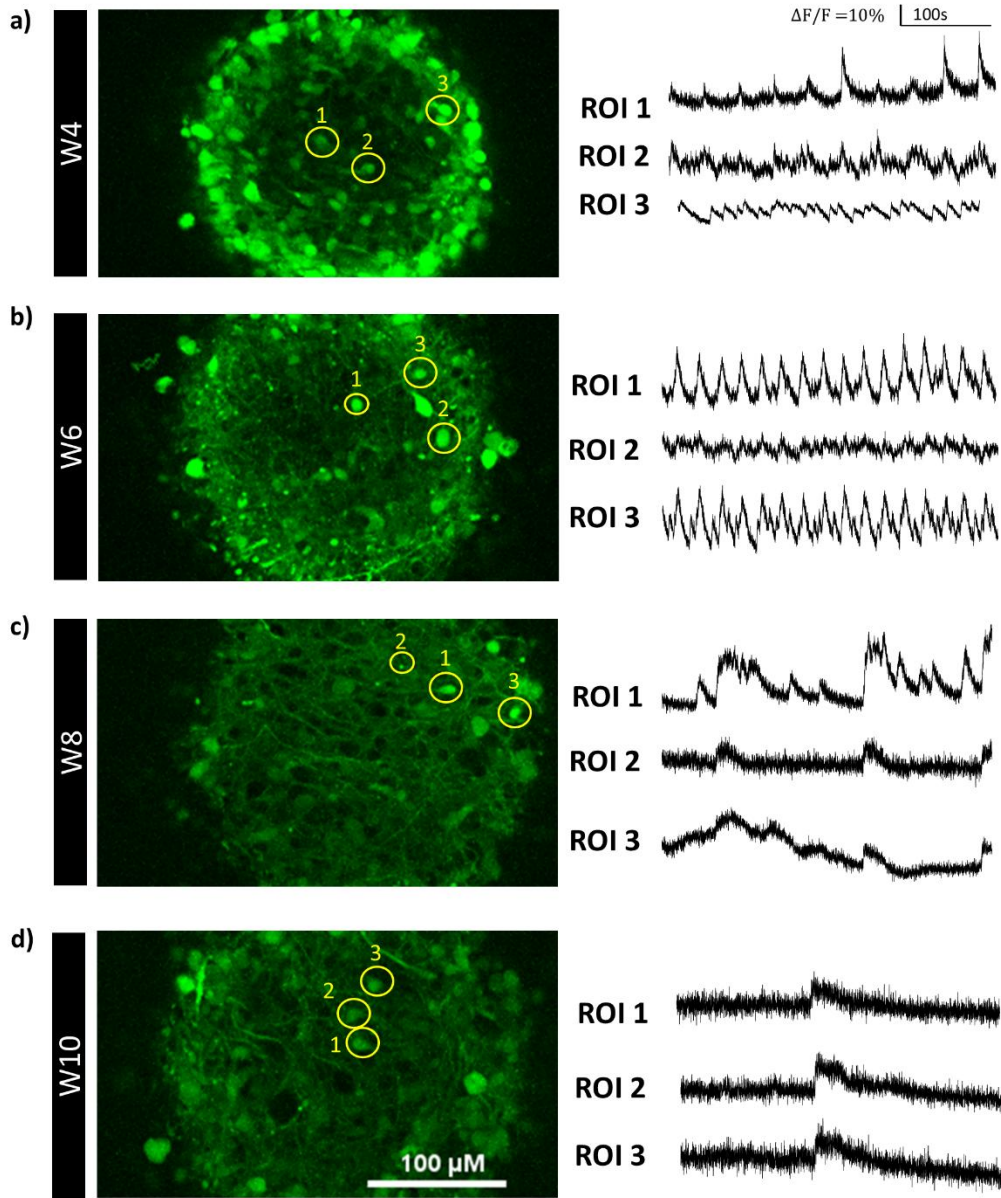
**Supplementary Figure 4. Heatmap and boxplots of the log<sub>2</sub>-transformed expression of brain region-specific markers, comparing week 8 organoids to week 12/13 organoids using RNA-seq Data. a) Heatmap showing the expression of brain region specific markers for both week 8 and week 12/13 organoids. b) Data is shown as a boxplot with interquartile range of distribution of gene expression of brain region specific markers for both age groups, with expression values shown as Log<sub>2</sub>(Exp). Dark purple group represent Forebrain genes, red group – hindbrain, green group - midbrain. Each time point includes at least six biological replicates: N = 6 for week 8 and N = 7 for the combined week 12/13 group. The full names of genes are listed in Supplementary Data 4.**



**Supplementary Figure 5. Dot Plot of enriched terms comparing week 12/13 organoids to week 8 organoids using RNA-seq data.** Downregulation and upregulation at week 12/13 are determined relative to the week 8 samples. Color shade represent the level of significance, while the size of the circles number of altered genes associated with the term Each time point includes at least six biological replicates: N = 6 for week 8 and N = 7 for the combined week 12/13 group.

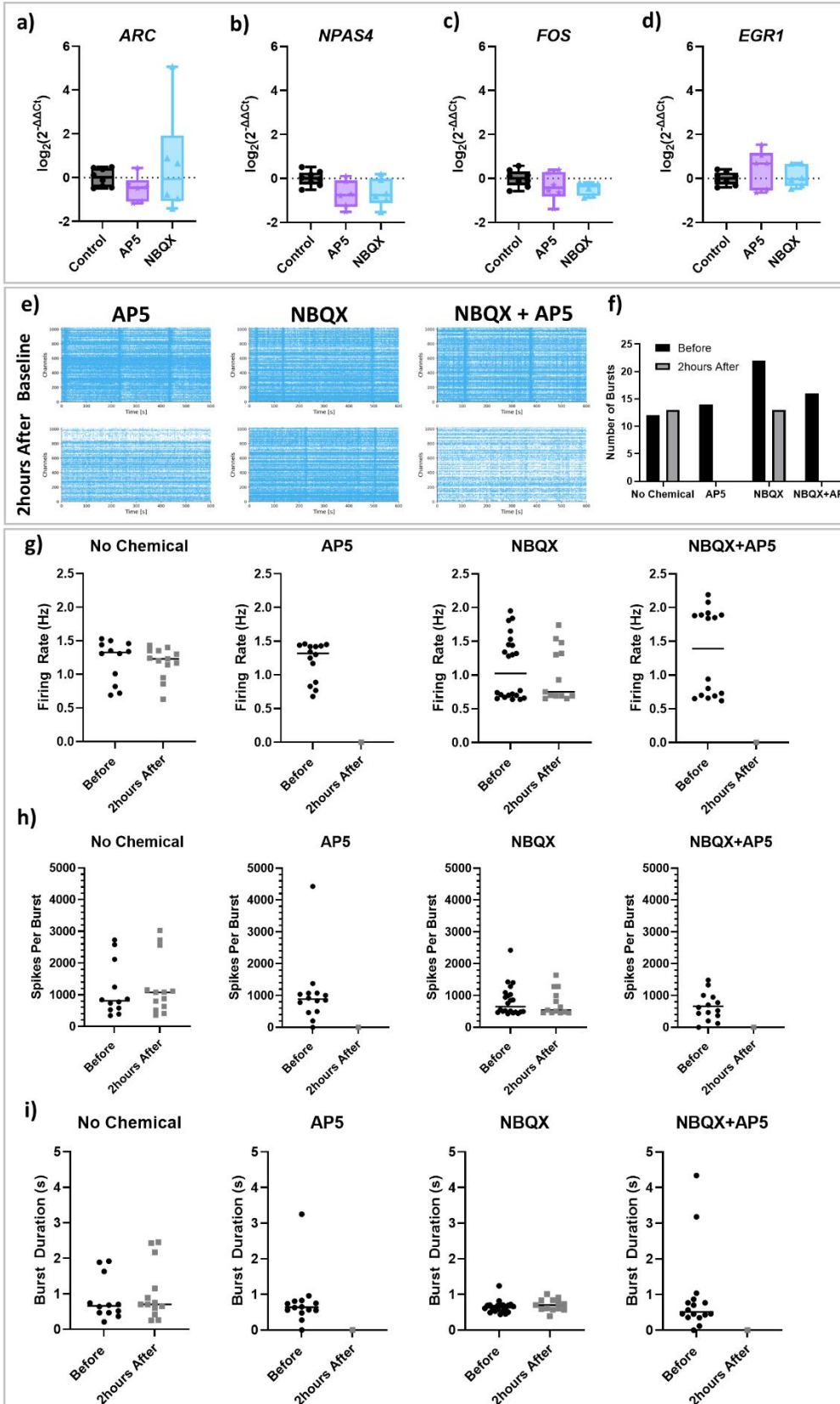


**Supplementary Figure 6.** Expression of Immediate Early Gene/Synaptic Plasticity-related markers at week 8 and 12 of differentiation. **a)** Heatmap and **b)** boxplots with interquartile range of distribution of the  $\text{log}_2$ -transformed gene expression comparing week 8 organoids to week 12/13 organoids using RNA-seq data. Each time point includes at least 3 biological replicates: N = 6 for week 8, N = 3 for week 12, and N = 4 for week 13. The full names of genes are listed in Supplementary Data 4.

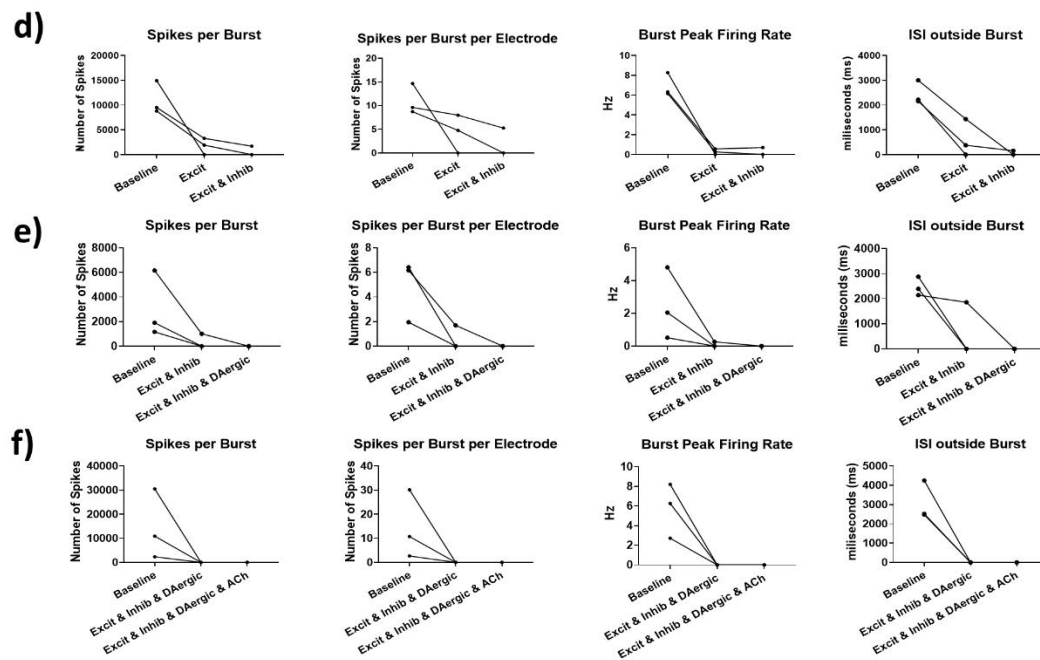
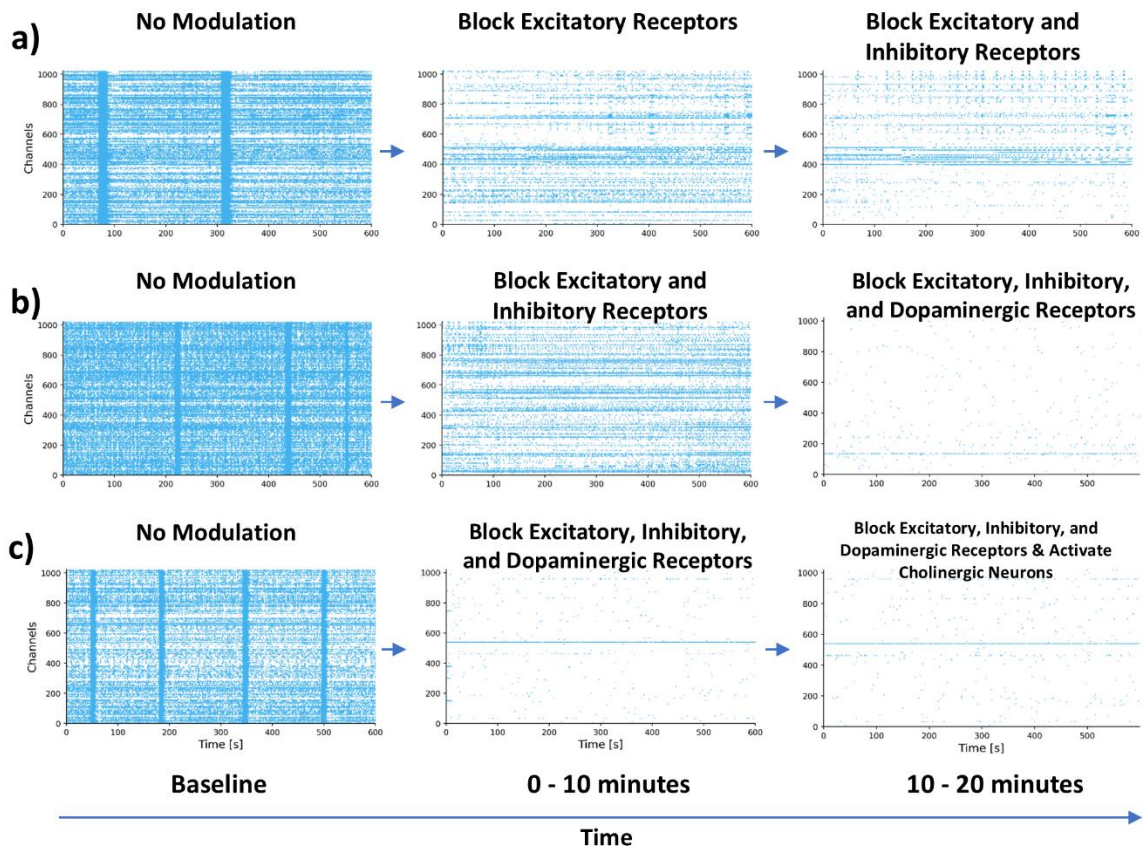


**Supplementary Figure 7. Single neuron calcium imaging analysis during development.**

Maximum intensity z-projection from time course calcium imaging videos are shown with the neurons that were analysed. Three neurons from representative organoids were quantified for change in fluorescence over baseline fluorescence and their fluorescent trace over time is shown on the right. Cultures at **a)** week 4 (W4), **b)** week 6 (W6), **c)** week 8 (W8), and **d)** week 10 (W10) are shown. ROI – region of interest.

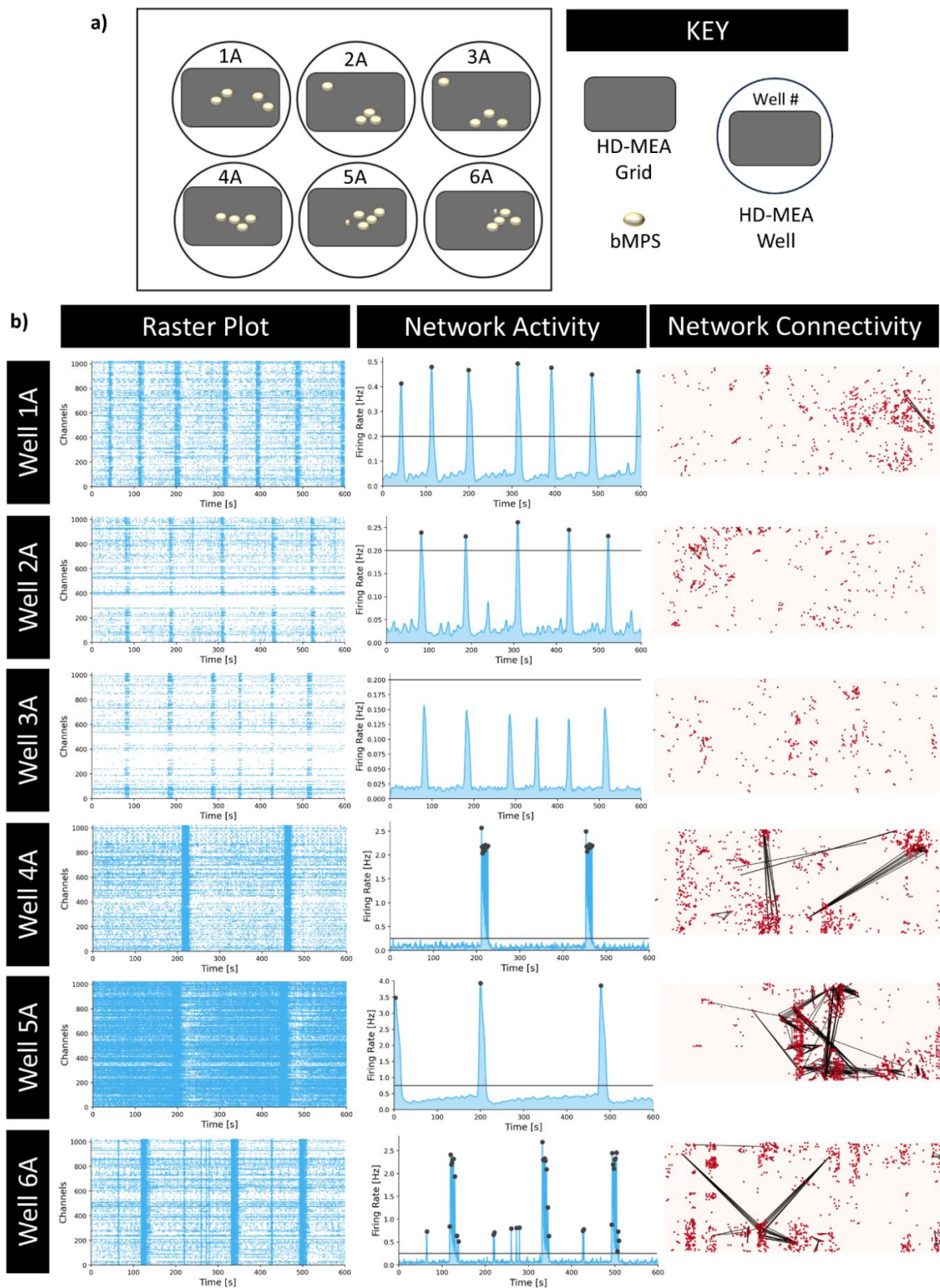


**Supplementary Figure 8. Pharmacological characterization of IEG and synaptic transmission changes after AP5 and NBQX exposure.** **a) *ARC* b) *NPAS4* c) *FOS* d) *EGR1*** expression in 13-week old organoids after a 2 hour exposure to 20  $\mu$ M AP5 (purple box plot), and 20  $\mu$ M NBQX (blue box plot) represented as  $\log_2$  of the difference in expression, normalized to negative control (no chemical treatment – 2h Control). Data is shown in a box and whisker plot (with the *box* extending from the 25th to 75th percentiles) and represents 3 independent experiments with 2 replicates each. Statistical significance was calculated from the average value from each independent experiment with One-way ANOVA with post-hoc Dunnett's tests \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . **e)** Representative raster plots, **f)** Number of bursts, **g)** Firing rate, **h)** Spikes per Burst, and **i)** Burst Duration are shown before and after exposure for all chemical combinations. For MEA data, data was collected across a 10-minute recording for 1 well per condition. *ARC* - Activity-Regulated Cytoskeleton-Associated Protein; *NPAS4* – Neuronal PAS Domain Protein 4; *FOS* - Fos proto-oncogene AP-1 transcription factor subunit; *EGR1* - Early Growth Response Protein 1; AP5 - 2-Amino-5-phosphonopentanoic acid (an NMDA receptor antagonist); NBQX – 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (an AMPA receptor antagonist).

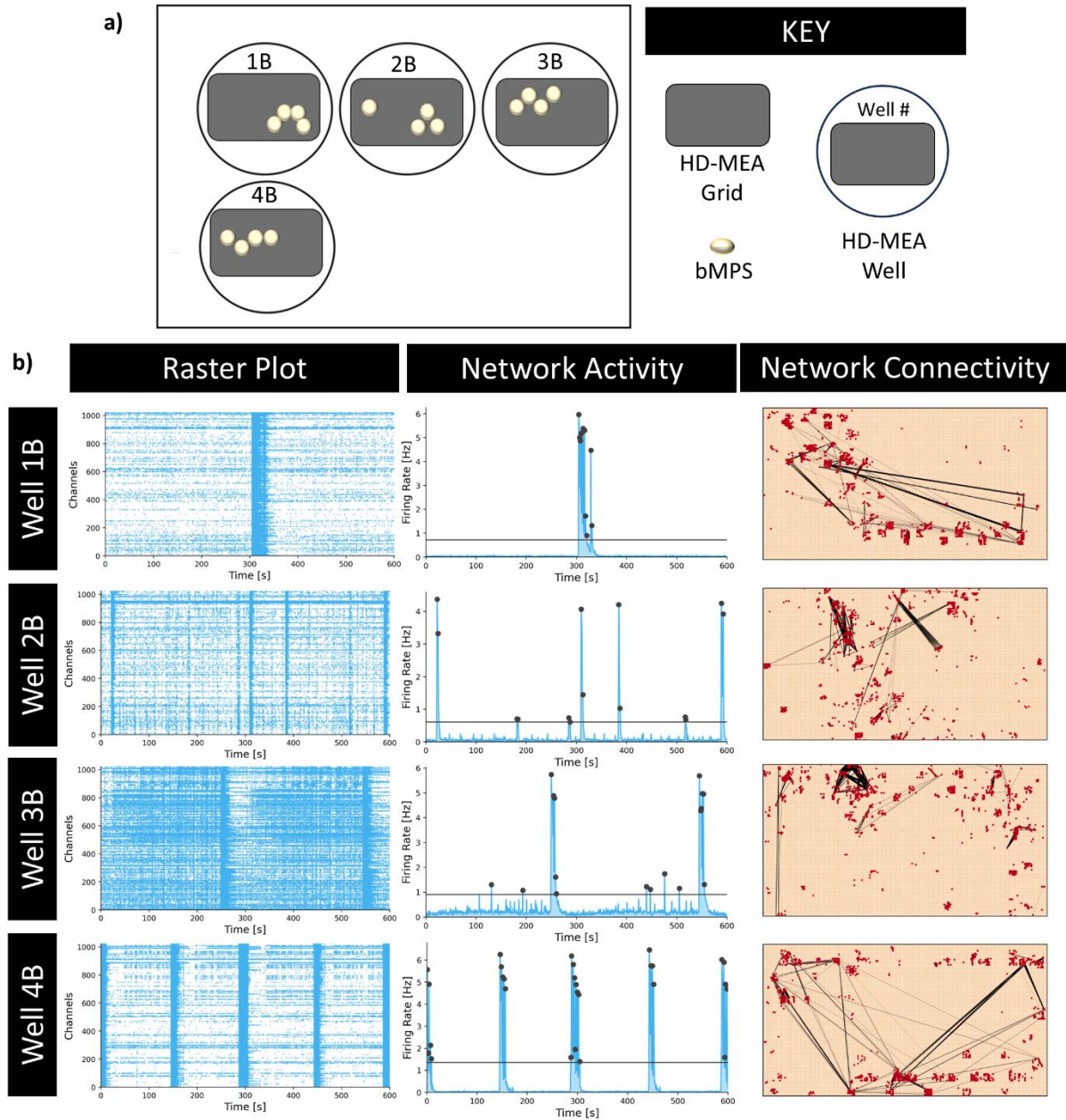


**Supplementary Figure 9. Pharmacological characterization of synaptic transmission changes after modulating excitatory, inhibitory, dopaminergic, cholinergic, and serotonergic receptors.**

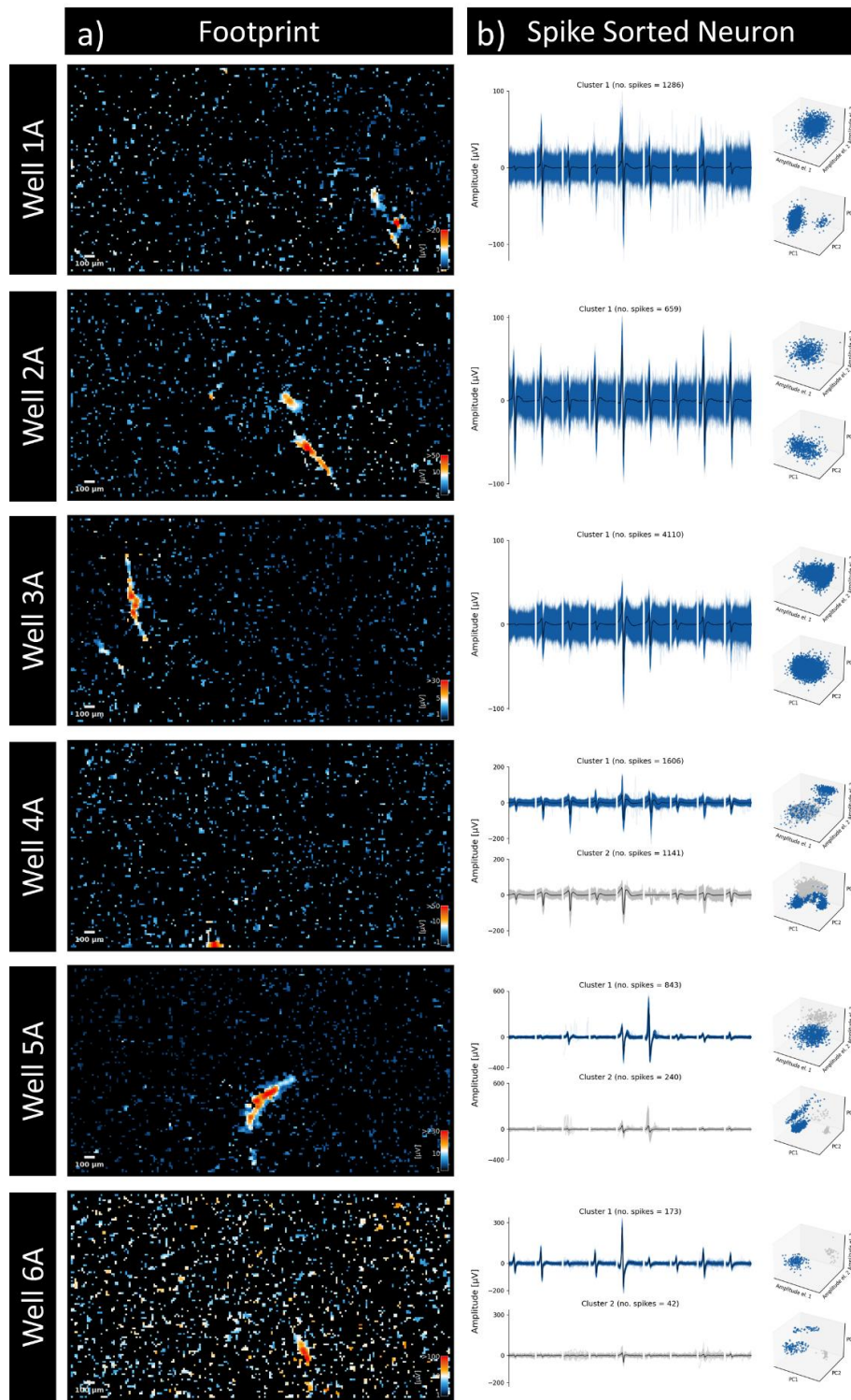
**a)** Example raster plot showing baseline activity (no modulation) followed by the immediate effect of 40  $\mu$ M NBQX and AP5, recorded for 10 minutes. Blocking AMPA and NMDA receptors abolishes bursting. Subsequent application of 10  $\mu$ M bicuculline and CPG 55845, which block GABA(A) and GABA(B) receptors, further reduces spiking activity. **b)** Example raster plot of baseline activity and the immediate effect of 40  $\mu$ M NBQX and AP5, 10  $\mu$ M bicuculline, and CPG 55845, showing a complete absence of bursting and a reduction in spiking. The next raster plot to the right illustrates the additional effect of 5  $\mu$ M 6-OHDA and 10  $\mu$ M haloperidol, which block dopamine D2 receptors and induce lesions in dopaminergic (and noradrenergic) neurons. This results in an even more pronounced reduction in spiking activity. **c)** Example raster plot showing baseline activity and the immediate effect of 40  $\mu$ M NBQX and AP5, 10  $\mu$ M bicuculline, CPG 55845, 5  $\mu$ M 6-OHDA, and 10  $\mu$ M haloperidol. Blocking AMPA, NMDA, GABA(A), GABA(B), and dopamine D2 receptors, along with dopaminergic (and noradrenergic) neuron lesions induced by haloperidol, leads to a drastic reduction in spiking activity. Following the addition of 1 nM CPF-Oxone, no further change in spiking activity is observed. **d, e, and f)** quantifies the corresponding changes in bursting and spiking metrics for **a, b** and **c**, respectively.



**Supplementary Figure 10. Overview of chip location and baseline activity for experiment A. a)** Schematic of the location and number of organoids per well used for TBS experiment. **b)** Baseline raster plot, network activity, and connectivity for each well used for TBS experiment.

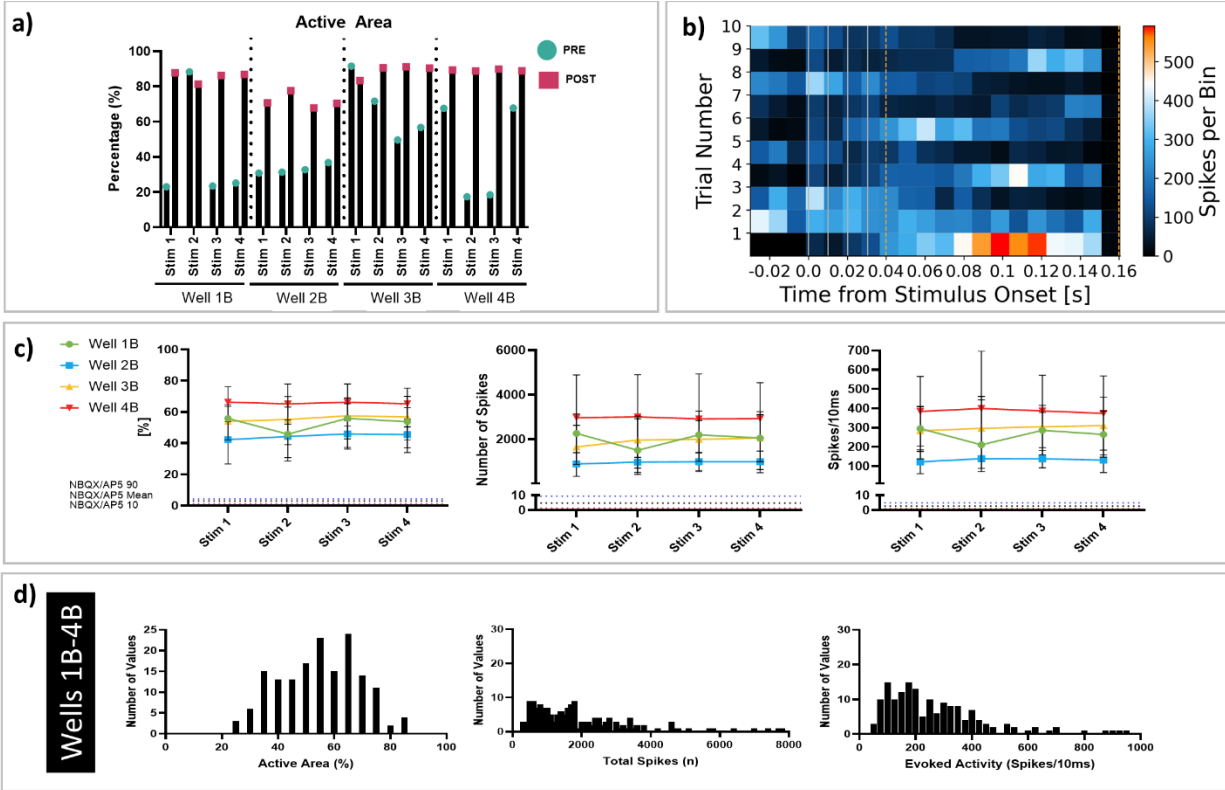


**Supplementary Figure 11. Overview of chip location and baseline activity for experiment B.** **a)** Schematic of the location and number of organoids per well used for TBS experiment. **b)** Baseline raster plot, network activity, and connectivity for each well used for TBS experiment.

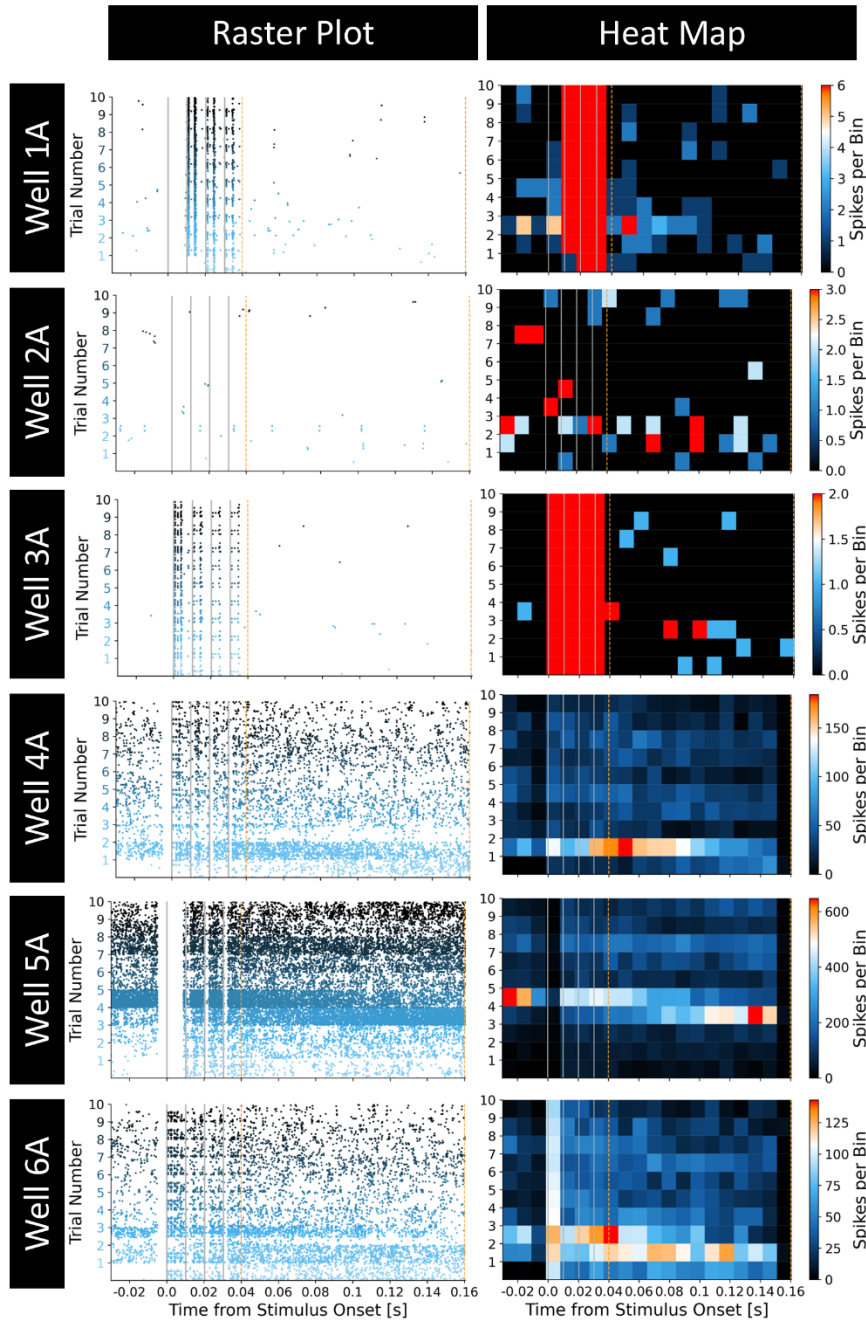


**Supplementary Figure 12. Tracking of stimulated neurons in experiment A.** **a)** The footprint of the neuron stimulated by Theta-Burst Stimulation and **b)** the spike sorted neurons are shown for wells 1A-6A using the axon tracking assay in MaxLab-Live.

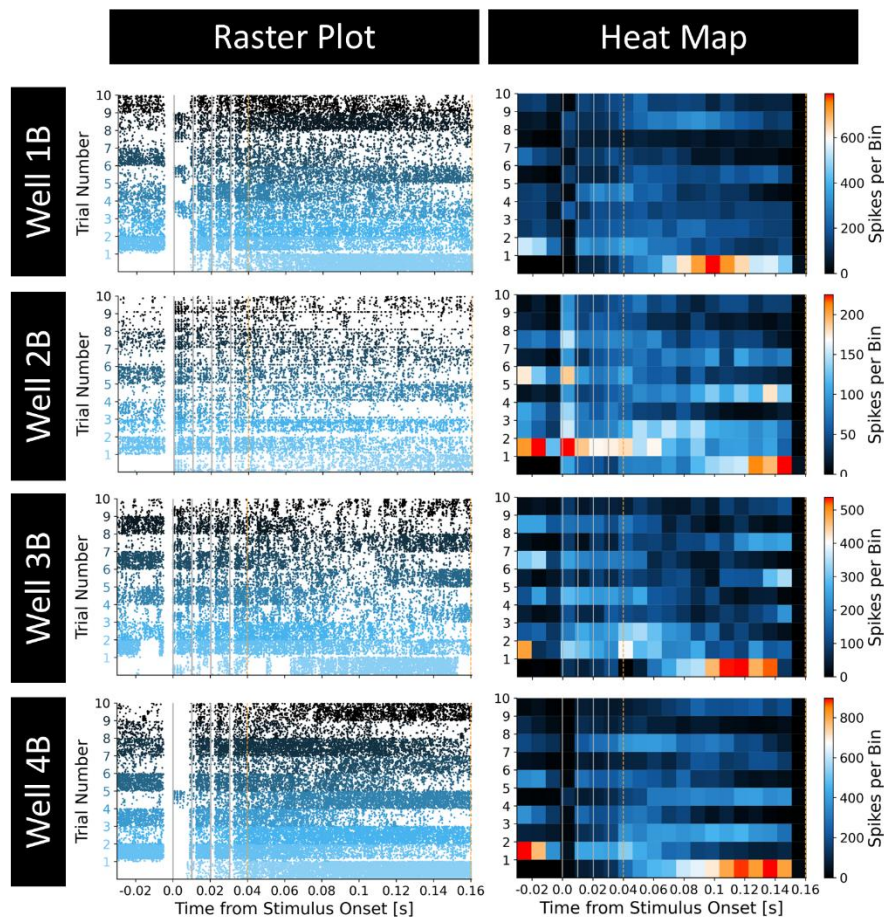




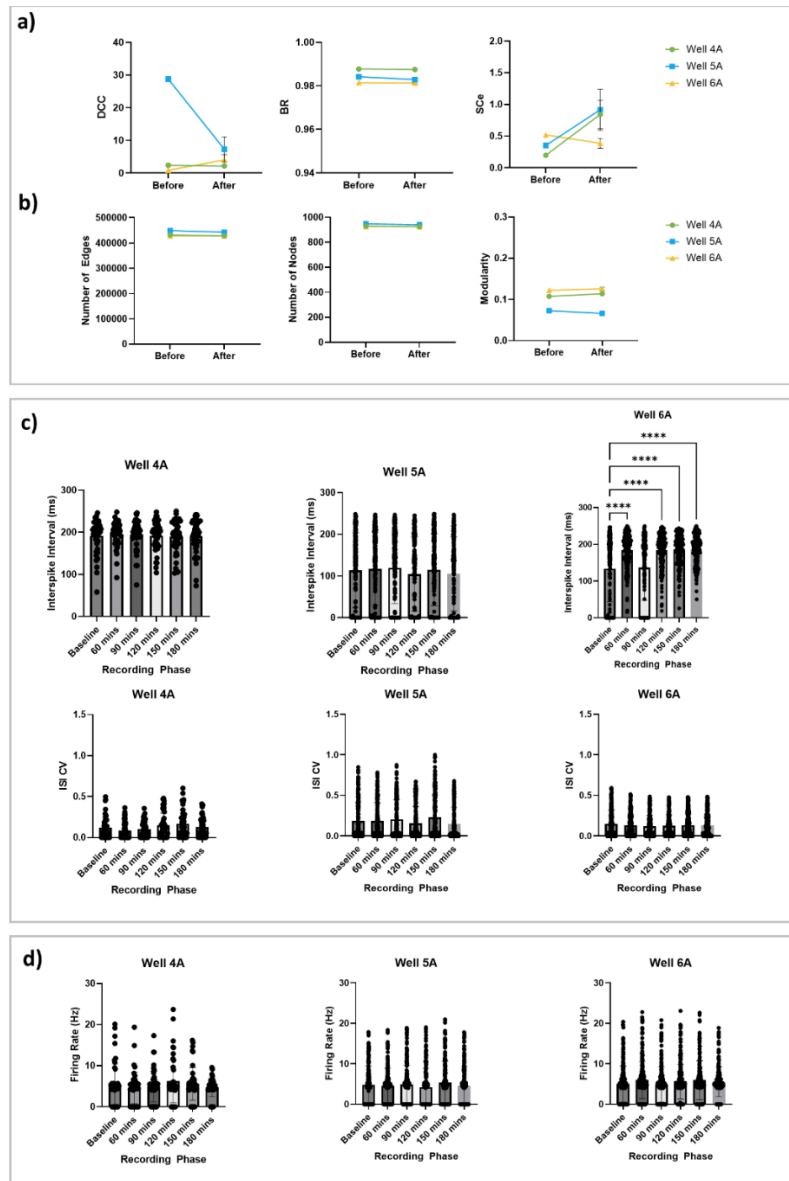
**Supplementary Figure 14: Theta-Burst stimulation modulated short-term plasticity for experiment B.** **a)** Percent active area before and after stimulation across all 6 wells. Wells 1B-4B show consistent increase or decrease in active area in response to stimulation. **b)** Representative heat map of evoked activity response. Bin size is equal to 10 ms. The stimulation pulses are the light grey vertical lines, and the dashed orange lines indicate the start/stop time of the analysis window for calculating evoked activity. **c)** Percent of active electrodes, total spikes, and evoked activity for wells 1B (green), 2B (blue), 3B (yellow) and 4B (red). Data represents the mean with p10 to p90 for each well. The 90<sup>th</sup> percentile response of a well, treated with NBQX/AP5 before and during stimulation is shown in the dark blue dash line overlayed on all graphs. Data represents the mean with p10 to p90 for each well. The 90<sup>th</sup> percentile response of a well, treated with NBQX/AP5 before and during stimulation is shown in the dark blue dash line overlayed on all graphs. The mean response of a well, treated with NBQX/AP5 before and during stimulation is shown in black dashed line overlayed on all graphs. The 10<sup>th</sup> percentile response of a well, treated with NBQX/AP5 before and during stimulation is shown in red dashed line overlayed on all graphs. Responses above this NBQX/AP5 region indicate responses generated by glutamatergic receptors. **d)** Histograms of total evoked activity per bin (bin size of 10 ms), total spikes, and total active area. The graphs show data aggregated across all electrodes for all 4 TBS for wells 1B-4B.



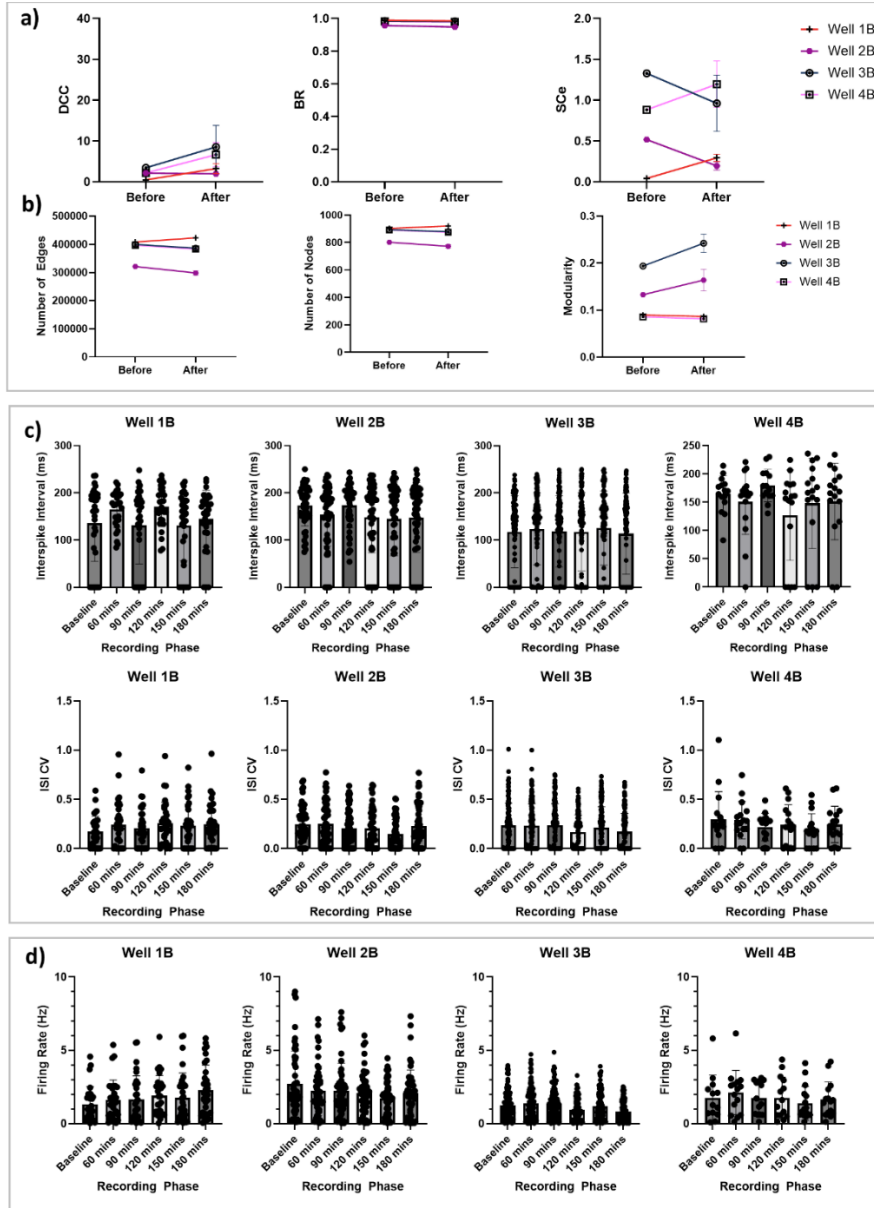
**Supplementary Figure 15. Representative raster plots and heat map of evoked activity response for wells 1A-6A.** Bin size is equal to 10 ms. The stimulation pulses are the light grey vertical lines, and the dashed orange lines indicate the start/stop time of the analysis window for calculating evoked activity.



**Supplementary Figure 16. Representative raster plots and heat map of evoked activity response for wells 1B-4B.** Bin size is equal to 10 ms. The stimulation pulses are the light grey vertical lines, and the dashed orange lines indicate the start/stop time of the analysis window for calculating evoked activity.



**Supplementary Figure 17. Modulation of long-term network dynamics in neural organoids by Theta-Burst Stimulation for wells 4A-6A.** **a)** Deviation from criticality coefficient, branching ratio, and shape collapse error before, and after all 4 TBS (data from 60-180 minutes is grouped for the after time point). **b)** Number of edges, number of nodes, and modularity is shown before and after all 4 TBS (data from 60-180 minutes is grouped for the after time point). Green line with circle - well 4A, blue line with square – well 5A, yellow line with triangle -well 6A. Exact p-values can be seen in supplemental tables 28-33. Mean and SEM is plotted for panels **a** and **b**. Two-tailed paired t-test was performed for panels **a** and **b**. **c)** Spike sorted units were used to plot interburst interval, interburst interval coefficient of variation, and **d)** firing rate for all units across wells 4A-6A before stimulation, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes after stimulation. Mean with SD is plotted for Panels **c** and **d**. Kruskal-Wallis test with Dunns multiple comparisons test was performed for panels **c** and **d**. Exact p-values for well 6A ISI (as it was the only well to show significance) can be seen in supplemental table 34 and significance can be seen in the figures. \*\*\*\*p < 0.0001



**Supplementary Figure 18. Modulation of long-term network dynamics in neural organoids by Theta-Burst Stimulation for wells 1B-4B** **a)** Deviation from criticality coefficient, branching ratio, and shape collapse error before, and after all 4 TBS (data from 60-180 minutes is grouped for the after time point). **b)** Number of edges, number of nodes, and modularity is shown before and after all 4 TBS (data from 60-180 minutes is grouped for the after time point). Red line with cross represents - well 1B, dark pink line with circle – well 2B, dark blue with open circle -well 3B, light pink with open square – well 4B. Exact p-values can be seen in supplemental tables 28-33. Mean and SEM is plotted for panels **a** and **b**. Two-tailed paired t-test was performed for panels **a** and **b**. **c)** Spike sorted units were used to plot Interburst interval, interburst interval coefficient of variation, and **d)** Firing Rate for all units across wells 1B-4B before stimulation, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes after stimulation. Mean with SD is plotted for Panels **c** and **d**. Kruskal-Wallis test with Dunns multiple comparisons test was performed for panels **c-d** and nothing was significant.

Supplementary Table 1. Primary antibodies used for Immunostaining.

| <b>Antibody</b>                                   | <b>Host</b> | <b>Type</b> | <b>Source</b>      | <b>Catalogue number</b> | <b>Dilution</b> |
|---------------------------------------------------|-------------|-------------|--------------------|-------------------------|-----------------|
| ARC                                               | Rabbit      | Polyclonal  | Gift of Worley Lab | N/A                     | 1:100           |
| BDNF                                              | Rabbit      | Polyclonal  | Invitrogen         | PA5-15198               | 1:10            |
| B-III-tubulin                                     | Mouse       | Monoclonal  | Sigma              | T5076                   | 1:1500          |
| CREB1                                             | Rabbit      | Monoclonal  | Cell Signaling     | 9197S                   | 1:600           |
| Gephyrin                                          | Mouse       | Monoclonal  | Synaptic Systems   | 147 011                 | 1:1000          |
| GFAP                                              | Chicken     | Polyclonal  | Aves               | GFAP                    | 1:500           |
| Homer1                                            | Mouse       | Monoclonal  | Synaptic Systems   | CI 268                  | 1:100           |
| MAP2                                              | Chicken     | Polyclonal  | Invitrogen         | PA1-10005               | 1:5000          |
| Nestin                                            | Rabbit      | Polyclonal  | Sigma              | N5413-100UG             | 1:200           |
| NeuN                                              | Mouse       | Monoclonal  | Phospho Solutions  | 583-FOX3                | 1:500           |
| NPTX2                                             | Rabbit      | Polyclonal  | Gift of Worley Lab | N/A                     | 1:200           |
| PSD95                                             | Mouse       | Monoclonal  | Invitrogen         | MA1-046                 | 1:500           |
| PSD95                                             | Rabbit      | Monoclonal  | Invitrogen         | 700902                  | 1:1000          |
| SOX2                                              | Mouse       | Monoclonal  | Invitrogen         | MA1-014                 | 1:200           |
| Synapsin 1                                        | Rabbit      | Polyclonal  | EMD Millipore      | AB1543P                 | 1:400           |
| Synaptophysin                                     | Chicken     | Polyclonal  | Synaptic Systems   | 101006                  | 1:500           |
| Alexa Fluor™ 488 goat anti-mouse IgG (H+L)        | Goat        | Polyclonal  | Invitrogen         | A11001                  | 1:500           |
| Alexa Fluor™ 568 goat anti-mouse IgG (H+L)        | Goat        | Polyclonal  | Invitrogen         | A11001                  | 1:500           |
| Alexa Fluor™ Plus 647 goat anti-chicken IgY (H+L) | Goat        | Polyclonal  | Invitrogen         | A32933                  | 1:500           |

Supplementary Table 2. Overview of SYBR® Green primers designed for this study.

| Name of gene | Forward and Reverse primer sequence | Slope  | Efficiency | Product length |
|--------------|-------------------------------------|--------|------------|----------------|
| ARC          | F: 5'-GCACCATCAGAGCCAGGAGA-3'       | -3.144 | 108.016    | 68             |
|              | R: 5'-CCAGCACAGCAGCAAAGACT-3'       |        |            | 68             |
| EGR1         | F: 5'-AGCCCTACGAGCACCTGAC-3'        | -3.346 | 98.993     | 92             |
|              | R: 5'-GGTTTGGCTGGGGTAACTGG-3'       |        |            | 92             |
| FOS          | F: GCGGAGACAGACCAACTAGAAGATG        | -3.519 | 92.374     | 105            |
|              | R: TCGGTGAGCTGCCAGGATGAA            |        |            | 105            |
| BDNF-all     | F: GCGGCGAGACAAAAAGACTG             | -2.753 | 130.816    | 197            |
|              | R: CGTACGACTGGGTAGTTCGG             |        |            | 187            |
| HOMER1       | F: 5'-GGTGGGGGTGGAAAAGCAACT-3'      | -3.278 | 101.871    | 139            |
|              | R: 5'-AGCTCGAGTGCTGAAGATAGGT-3'     |        |            | 139            |
| NPAS4        | F: 5'-ACACTCGCAAGGGCGTCTTC-3'       | -3.288 | 101.431    | 102            |
|              | R: 5'-CGGGTAGTGCCGCTACGAT-3'        |        |            | 102            |
| NPTX2        | F: 5'-TCACCGGCAGAAGACCGAGA-3'       | -3.097 | 110.321    | 108            |
|              | R: 5'-AGGGACACCTTGAACGCATCT-3'      |        |            | 108            |
| CREB1        | F: 5'-GCGGCCCAGCCATCAGTTAT-3'       | -3.466 | 94.309     | 95             |
|              | R: 5'-GAAATCTGTGTTCCGGAGAAAAGTC-3'  |        |            | 95             |
| SYNGAP1      | F: TCCCCGCGATGTCCTATGC              | -3.568 | 90.671     | 131            |
|              | R: CAGCTGGTTCCCCGAGATGA             |        |            | 131            |
| ACTB         | F: 5'-CCTCGCCTTTGCCGATCC-3'         | -3.048 | 112.86     | 70             |
|              | R: 5'-CGCGGCGATATCATCATCCAT-3'      |        |            | 70             |
| MAP2         | F: GCGGAAAACCACAGCAGCA              | -3.742 | 85.025     | 145            |
|              | R: CCGAGGAGGGAGAATGGAGG             |        |            | 145            |
| MKI67        | F: ACTTGACGAGCGGTGGTTCG             | -3.384 | 97.489     | 109            |
|              | R: GGGGAAGGCCAGGTATAATCCG           |        |            | 109            |
| CAMK2A       | F: GCGAAGGTGTGTGAAGGTGC             | -3.372 | 97.932     | 144            |
|              | R: AGTCGGACGATGTTGGGGTG             |        |            | 144            |
| GRIA1        | F: AACGAGCAGGGGCTTTTGA              | -3.666 | 87.394     | 106            |
|              | R: GGCTCAGAGCGCTTGTCTTG             |        |            | 106            |
| RBFOX3       | F: TGAGAACTCCCGGCTGCAAA             | -3.624 | 88.776     | 99             |
|              | R: CCCTCTGGGGTCTAGGGAA              |        |            | 99             |

Supplementary Table 3. Overview of TaqMan and miRCURY LNA primers.

| <b>Assay ID</b> | <b>Assay Type</b>             | <b>Catalogue Number</b> | <b>Assay Name</b>     |
|-----------------|-------------------------------|-------------------------|-----------------------|
| Hs00609557      | TaqMan™ Gene Expression Assay | 4331182                 | <i>GRIN1</i>          |
| Hs00168219      | TaqMan™ Gene Expression Assay | 4331182                 | <i>GRIN2A</i>         |
| Hs01002012      | TaqMan™ Gene Expression Assay | 4331182                 | <i>GRIN2B</i>         |
| Hs00300164      | TaqMan™ Gene Expression Assay | 4331182                 | <i>OLIG2</i>          |
| Hs04187831      | TaqMan™ Gene Expression Assay | 4331182                 | <i>NES</i>            |
| Hs00909233      | TaqMan™ Gene Expression Assay | 4331182                 | <i>GFAP</i>           |
| Hs00971228      | TaqMan™ Gene Expression Assay | 4331182                 | <i>GABRA1</i>         |
| Hs99999901      | TaqMan™ Gene Expression Assay | 4331182                 | <i>18S</i>            |
| Hs00921945      | TaqMan™ Gene Expression Assay | 4331182                 | <i>MBP</i>            |
| YP00206026      | miRCURY LNA™ miRNA PCR Assay  | 339306                  | <i>hsa-miR-124-3p</i> |
| YP00205989      | miRCURY LNA™ miRNA PCR Assay  | 339306                  | <i>hsa-miR-132-3p</i> |
| YP00206035      | miRCURY LNA™ miRNA PCR Assay  | 339306                  | <i>hsa-miR-134-5p</i> |
| YP00203902      | miRCURY LNA™ miRNA PCR Assay  | 339306                  | <i>SNORD44 (hsa)</i>  |

Supplementary Table 4. Statistics for Average Amplitude calculated from calcium imaging data. At least 8 individual organoids across at least 3 independent experiments were imaged and quantified for each time point. Statistics was performed using One-way ANOVA and a Tukey post-hoc test. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff.   | Below threshold? | Summary | Adjusted P Value |
|-----------------------------------|------------|----------------------|------------------|---------|------------------|
| W4 vs. W6                         | -0.01871   | -0.1367 to 0.09925   | No               | ns      | 0.9969           |
| W4 vs. W8                         | -0.1048    | -0.2100 to 0.0004939 | No               | ns      | 0.0517           |
| W4 vs. W10                        | 0.04432    | -0.06479 to 0.1534   | No               | ns      | 0.8309           |
| W4 vs. W12                        | 0.1182     | -0.02769 to 0.2641   | No               | ns      | 0.1746           |
| W4 vs. W14                        | 0.1063     | -0.01606 to 0.2286   | No               | ns      | 0.1222           |
| W6 vs. W8                         | -0.08606   | -0.1951 to 0.02302   | No               | ns      | 0.1973           |
| W6 vs. W10                        | 0.06303    | -0.04977 to 0.1758   | No               | ns      | 0.5635           |
| W6 vs. W12                        | 0.1369     | -0.01176 to 0.2856   | No               | ns      | 0.087            |
| W6 vs. W14                        | 0.125      | -0.0006514 to 0.2506 | No               | ns      | 0.0519           |
| W8 vs. W10                        | 0.1491     | 0.04964 to 0.2485    | Yes              | ***     | 0.0007           |
| W8 vs. W12                        | 0.223      | 0.08416 to 0.3618    | Yes              | ***     | 0.0003           |
| W8 vs. W14                        | 0.211      | 0.09724 to 0.3249    | Yes              | ****    | <0.0001          |
| W10 vs. W12                       | 0.07387    | -0.06787 to 0.2156   | No               | ns      | 0.6348           |
| W10 vs. W14                       | 0.06196    | -0.05541 to 0.1793   | No               | ns      | 0.6222           |
| W12 vs. W14                       | -0.01191   | -0.1641 to 0.1402    | No               | ns      | 0.9999           |

Supplementary Table 5. Statistics for Average Burst Duration calculated from calcium imaging data. At least 8 individual organoids across at least 3 independent experiments were imaged and quantified for each time point. Statistics was performed using One-way ANOVA and a Tukey post-hoc test. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-----------------------------------|------------|--------------------|------------------|---------|------------------|
| W4 vs. W6                         | 16.56      | -6.255 to 39.38    | No               | ns      | 0.2769           |
| W4 vs. W8                         | -17.51     | -37.88 to 2.848    | No               | ns      | 0.129            |
| W4 vs. W10                        | -10.28     | -31.39 to 10.82    | No               | ns      | 0.6978           |
| W4 vs. W12                        | -5.354     | -33.57 to 22.86    | No               | ns      | 0.9929           |
| W4 vs. W14                        | -2.805     | -26.47 to 20.86    | No               | ns      | 0.9992           |
| W6 vs. W8                         | -34.08     | -55.18 to -12.98   | Yes              | ***     | 0.0002           |
| W6 vs. W10                        | -26.84     | -48.66 to -5.025   | Yes              | **      | 0.0081           |
| W6 vs. W12                        | -21.92     | -50.67 to 6.839    | No               | ns      | 0.2288           |
| W6 vs. W14                        | -19.37     | -43.67 to 4.936    | No               | ns      | 0.1885           |
| W8 vs. W10                        | 7.233      | -12.00 to 26.47    | No               | ns      | 0.8715           |
| W8 vs. W12                        | 12.16      | -14.69 to 39.01    | No               | ns      | 0.7577           |
| W8 vs. W14                        | 14.71      | -7.304 to 36.72    | No               | ns      | 0.3655           |
| W10 vs. W12                       | 4.928      | -22.49 to 32.35    | No               | ns      | 0.9944           |
| W10 vs. W14                       | 7.477      | -15.23 to 30.18    | No               | ns      | 0.9222           |
| W12 vs. W14                       | 2.55       | -26.88 to 31.98    | No               | ns      | 0.9998           |

Supplementary Table 6. Statistics for Average Decay Time calculated from calcium imaging data. At least 8 individual organoids across at least 3 independent experiments were imaged and quantified for each time point. Statistics was performed using One-way ANOVA and a Tukey post-hoc test. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-----------------------------------|------------|--------------------|------------------|---------|------------------|
| W4 vs. W6                         | 8.432      | -12.65 to 29.51    | No               | ns      | 0.8396           |
| W4 vs. W8                         | -19.03     | -37.84 to -0.2162  | Yes              | *       | 0.046            |
| W4 vs. W10                        | -17.76     | -37.26 to 1.740    | No               | ns      | 0.0931           |
| W4 vs. W12                        | -6.351     | -32.42 to 19.72    | No               | ns      | 0.9779           |
| W4 vs. W14                        | -3.337     | -25.20 to 18.52    | No               | ns      | 0.9974           |
| W6 vs. W8                         | -27.46     | -46.95 to -7.966   | Yes              | **      | 0.0017           |
| W6 vs. W10                        | -26.19     | -46.35 to -6.033   | Yes              | **      | 0.0044           |
| W6 vs. W12                        | -14.78     | -41.35 to 11.78    | No               | ns      | 0.5678           |
| W6 vs. W14                        | -11.77     | -34.22 to 10.68    | No               | ns      | 0.6291           |
| W8 vs. W10                        | 1.269      | -16.50 to 19.04    | No               | ns      | >0.9999          |
| W8 vs. W12                        | 12.68      | -12.13 to 37.48    | No               | ns      | 0.6538           |
| W8 vs. W14                        | 15.69      | -4.647 to 36.03    | No               | ns      | 0.2174           |
| W10 vs. W12                       | 11.41      | -13.92 to 36.74    | No               | ns      | 0.762            |
| W10 vs. W14                       | 14.42      | -6.553 to 35.40    | No               | ns      | 0.3342           |
| W12 vs. W14                       | 3.014      | -24.18 to 30.20    | No               | ns      | 0.9994           |

Supplementary Table 7. Statistics for Average Firing Rate calculated from calcium imaging data. At least 8 individual organoids across at least 3 independent experiments were imaged and quantified for each time point. Statistics was performed using One-way ANOVA and a Tukey post-hoc test. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff.    | Below threshold? | Summary | Adjusted P Value |
|-----------------------------------|------------|-----------------------|------------------|---------|------------------|
| W4 vs. W6                         | -0.02399   | -0.03168 to -0.01631  | Yes              | ****    | <0.0001          |
| W4 vs. W8                         | 0.006389   | -0.0002246 to 0.01300 | No               | ns      | 0.0644           |
| W4 vs. W10                        | 0.00743    | 0.0009003 to 0.01396  | Yes              | *       | 0.0164           |
| W4 vs. W12                        | 0.0113     | 0.004683 to 0.01791   | Yes              | ****    | <0.0001          |
| W4 vs. W14                        | 0.01049    | 0.004212 to 0.01676   | Yes              | ****    | <0.0001          |
| W6 vs. W8                         | 0.03038    | 0.02329 to 0.03747    | Yes              | ****    | <0.0001          |
| W6 vs. W10                        | 0.03142    | 0.02441 to 0.03844    | Yes              | ****    | <0.0001          |
| W6 vs. W12                        | 0.03529    | 0.02820 to 0.04238    | Yes              | ****    | <0.0001          |
| W6 vs. W14                        | 0.03448    | 0.02770 to 0.04126    | Yes              | ****    | <0.0001          |
| W8 vs. W10                        | 0.001042   | -0.004780 to 0.006864 | No               | ns      | 0.9951           |
| W8 vs. W12                        | 0.004907   | -0.001008 to 0.01082  | No               | ns      | 0.1608           |
| W8 vs. W14                        | 0.004097   | -0.001436 to 0.009630 | No               | ns      | 0.2667           |
| W10 vs. W12                       | 0.003866   | -0.001956 to 0.009688 | No               | ns      | 0.3862           |
| W10 vs. W14                       | 0.003055   | -0.002378 to 0.008489 | No               | ns      | 0.573            |
| W12 vs. W14                       | -0.00081   | -0.006343 to 0.004723 | No               | ns      | 0.9981           |

Supplementary Table 8. Statistics for Average Number of Peaks calculated from calcium imaging data. At least 8 individual organoids across at least 3 independent experiments were imaged and quantified for each time point. Statistics was performed using One-way ANOVA and a Tukey post-hoc test. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001.

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-----------------------------------|------------|--------------------|------------------|---------|------------------|
| W4 vs. W6                         | -8.225     | -10.54 to -5.914   | Yes              | ****    | <0.0001          |
| W4 vs. W8                         | 3.267      | 1.277 to 5.256     | Yes              | ****    | <0.0001          |
| W4 vs. W10                        | 3.713      | 1.748 to 5.677     | Yes              | ****    | <0.0001          |
| W4 vs. W12                        | 5.2        | 3.313 to 7.087     | Yes              | ****    | <0.0001          |
| W4 vs. W14                        | 4.835      | 2.989 to 6.680     | Yes              | ****    | <0.0001          |
| W6 vs. W8                         | 11.49      | 9.358 to 13.62     | Yes              | ****    | <0.0001          |
| W6 vs. W10                        | 11.94      | 9.828 to 14.05     | Yes              | ****    | <0.0001          |
| W6 vs. W12                        | 13.43      | 11.39 to 15.46     | Yes              | ****    | <0.0001          |
| W6 vs. W14                        | 13.06      | 11.06 to 15.06     | Yes              | ****    | <0.0001          |
| W8 vs. W10                        | 0.4458     | -1.305 to 2.197    | No               | ns      | 0.976            |
| W8 vs. W12                        | 1.933      | 0.2690 to 3.598    | Yes              | *       | 0.0132           |
| W8 vs. W14                        | 1.568      | -0.04900 to 3.185  | No               | ns      | 0.0627           |
| W10 vs. W12                       | 1.488      | -0.1468 to 3.122   | No               | ns      | 0.0958           |
| W10 vs. W14                       | 1.122      | -0.4640 to 2.709   | No               | ns      | 0.3168           |
| W12 vs. W14                       | -0.3652    | -1.855 to 1.125    | No               | ns      | 0.9797           |

Supplementary Table 9. Statistics for Average Rise Time calculated from calcium imaging data. At least 8 individual organoids across at least 3 independent experiments were imaged and quantified for each time point. Statistics was performed using One-way ANOVA and a Tukey post-hoc test. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, and \*\*\*\*p < 0.0001

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-----------------------------------|------------|--------------------|------------------|---------|------------------|
| W4 vs. W6                         | 8.131      | -1.662 to 17.92    | No               | ns      | 0.155            |
| W4 vs. W8                         | 1.513      | -7.227 to 10.25    | No               | ns      | 0.9953           |
| W4 vs. W10                        | 7.476      | -1.582 to 16.53    | No               | ns      | 0.1596           |
| W4 vs. W12                        | 0.9969     | -11.11 to 13.11    | No               | ns      | 0.9999           |
| W4 vs. W14                        | 0.5322     | -9.624 to 10.69    | No               | ns      | >0.9999          |
| W6 vs. W8                         | -6.618     | -15.67 to 2.438    | No               | ns      | 0.2701           |
| W6 vs. W10                        | -0.6542    | -10.02 to 8.710    | No               | ns      | >0.9999          |
| W6 vs. W12                        | -7.134     | -19.47 to 5.208    | No               | ns      | 0.527            |
| W6 vs. W14                        | -7.598     | -18.03 to 2.832    | No               | ns      | 0.2733           |
| W8 vs. W10                        | 5.964      | -2.292 to 14.22    | No               | ns      | 0.2819           |
| W8 vs. W12                        | -0.5157    | -12.04 to 11.01    | No               | ns      | >0.9999          |
| W8 vs. W14                        | -0.9803    | -10.43 to 8.468    | No               | ns      | 0.9996           |
| W10 vs. W12                       | -6.479     | -18.25 to 5.287    | No               | ns      | 0.579            |
| W10 vs. W14                       | -6.944     | -16.69 to 2.800    | No               | ns      | 0.2959           |
| W12 vs. W14                       | -0.4647    | -13.10 to 12.17    | No               | ns      | >0.9999          |

Supplementary Table 10. Statistics for the percent of spikes within bursts for NBQX treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | 28.9       | 20.04 to 37.75     | Yes              | ***     | 0.0003           |
| Baseline vs. 2hour                  | 28.9       | 20.04 to 37.75     | Yes              | ***     | 0.0003           |
| Baseline vs. 4hour                  | 28.9       | 20.04 to 37.75     | Yes              | ***     | 0.0003           |

Supplementary Table 11. Statistics for the percent of spikes within bursts for Bicuculline treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | -17.71     | -33.09 to -2.329   | Yes              | *       | 0.0298           |
| Baseline vs. 2hour                  | -8.06      | -21.17 to 5.052    | No               | ns      | 0.2128           |
| Baseline vs. 4hour                  | -12.26     | -28.87 to 4.352    | No               | ns      | 0.1319           |

Supplementary Table 12. Statistics for the percent of spikes within bursts for 4-AP treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | -17.68     | -45.21 to 9.858    | No               | ns      | 0.1914           |
| Baseline vs. 2hour                  | -8.792     | -24.72 to 7.135    | No               | ns      | 0.2714           |
| Baseline vs. 4hour                  | -8.563     | -24.99 to 7.868    | No               | ns      | 0.3055           |

Supplementary Table 13. Statistics for the burst duration for NBQX treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | 2.228      | 1.940 to 2.516     | Yes              | ****    | <0.0001          |
| Baseline vs. 2hour                  | 2.228      | 1.940 to 2.516     | Yes              | ****    | <0.0001          |
| Baseline vs. 4hour                  | 2.228      | 1.940 to 2.516     | Yes              | ****    | <0.0001          |

Supplementary Table 14. Statistics for the burst duration for Bicuculine treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | 0.000      | -1.213 to 1.213    | No               | ns      | >0.9999          |
| Baseline vs. 2hour                  | -0.5450    | -2.100 to 1.010    | No               | ns      | 0.5647           |
| Baseline vs. 4hour                  | -0.3467    | -1.873 to 1.180    | No               | ns      | 0.8010           |

Supplementary Table 15. Statistics for the burst duration for 4-AP treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | 1.250      | -0.6116 to 3.112   | No               | ns      | 0.1707           |
| Baseline vs. 2hour                  | 1.747      | -0.3575 to 3.851   | No               | ns      | 0.0929           |
| Baseline vs. 4hour                  | 0.9917     | -1.172 to 3.156    | No               | ns      | 0.3870           |

Supplementary Table 16. Statistics for the interburst interval coefficient of variation for NBQX treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | 1.107      | 0.6607 to 1.553    | Yes              | **      | 0.0011           |
| Baseline vs. 2hour                  | 1.107      | 0.6607 to 1.553    | Yes              | **      | 0.0011           |
| Baseline vs. 4hour                  | 1.107      | 0.6607 to 1.553    | Yes              | **      | 0.0011           |

Supplementary Table 17. Statistics for the interburst interval coefficient of variation for Bicuculline treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | -0.1083    | -0.7049 to 0.4882  | No               | ns      | 0.8790           |
| Baseline vs. 2hour                  | -0.04833   | -0.7707 to 0.6741  | No               | ns      | 0.9917           |
| Baseline vs. 4hour                  | -0.1550    | -0.8674 to 0.5574  | No               | ns      | 0.8183           |

Supplementary Table 18. Statistics for the interburst interval coefficient of variation for 4-AP treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | -0.09500   | -0.7534 to 0.5634  | No               | ns      | 0.9312           |
| Baseline vs. 2hour                  | -0.09500   | -0.8029 to 0.6129  | No               | ns      | 0.9429           |
| Baseline vs. 4hour                  | -0.1167    | -0.8836 to 0.6503  | No               | ns      | 0.9214           |

Supplementary Table 19. Statistics for the burst frequency for NBQX treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|--------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | 0.03       | 0.01527 to 0.04473 | Yes              | **      | 0.0027           |
| Baseline vs. 2hour                  | 0.03       | 0.01527 to 0.04473 | Yes              | **      | 0.0027           |
| Baseline vs. 4hour                  | 0.03       | 0.01527 to 0.04473 | Yes              | **      | 0.0027           |

Supplementary Table 20. Statistics for the burst frequency for Bicuculline treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff.  | Below threshold ? | Summary | Adjusted P Value |
|-------------------------------------|------------|---------------------|-------------------|---------|------------------|
| Baseline vs. 0min                   | 0.003333   | -0.1159 to 0.1226   | No                | ns      | 0.9994           |
| Baseline vs. 2hour                  | 0.01833    | -0.06897 to 0.1056  | No                | ns      | 0.8317           |
| Baseline vs. 4hour                  | 0.005      | -0.05849 to 0.06849 | No                | ns      | 0.9867           |

Supplementary Table 21. Statistics for the burst frequency for 4-AP treated wells calculated from HD-MEA data. Data represents 3 independent experiments with 2 HD-MEA wells per experiment per chemical (n=6). Statistical significance was calculated with repeated measures ANOVA with post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff.    | Below threshold? | Summary | Adjusted P Value |
|-------------------------------------|------------|-----------------------|------------------|---------|------------------|
| Baseline vs. 0min                   | -0.035     | -0.06288 to -0.007121 | Yes              | *       | 0.0213           |
| Baseline vs. 2hour                  | -0.01333   | -0.02722 to 0.0005523 | No               | ns      | 0.0576           |
| Baseline vs. 4hour                  | -0.00833   | -0.02812 to 0.01146   | No               | ns      | 0.443            |

Supplementary Table 22. Statistics for STP analysis of number of nodes for TBS HD-MEA data. Statistical significance was calculated with a Wilcoxon matched-pairs signed rank test.  $p < 0.05$  was considered significant.

|                                         |                                         |               |
|-----------------------------------------|-----------------------------------------|---------------|
| Wilcoxon matched-pairs signed rank test |                                         |               |
| Post vs Pre                             | P value                                 | <0.0001       |
|                                         | Exact or approximate P value?           | Exact         |
|                                         | P value summary                         | ****          |
|                                         | Significantly different ( $P < 0.05$ )? | Yes           |
|                                         | One- or two-tailed P value?             | Two-tailed    |
|                                         | Sum of positive, negative ranks         | 406.0 , 0.000 |
|                                         | Sum of signed ranks (W)                 | 406.0         |
|                                         | Number of pairs                         | 28            |
|                                         | Number of ties (ignored)                | 0             |

Supplementary Table 23. Statistics for STP analysis of number of edges for TBS HD-MEA data. Statistical significance was calculated with a Wilcoxon matched-pairs signed rank test.  $p < 0.05$  was considered significant.

|                                         |                                         |               |
|-----------------------------------------|-----------------------------------------|---------------|
| Wilcoxon matched-pairs signed rank test |                                         |               |
| Post vs Pre                             | P value                                 | <0.0001       |
|                                         | Exact or approximate P value?           | Exact         |
|                                         | P value summary                         | ****          |
|                                         | Significantly different ( $P < 0.05$ )? | Yes           |
|                                         | One- or two-tailed P value?             | Two-tailed    |
|                                         | Sum of positive, negative ranks         | 406.0 , 0.000 |
|                                         | Sum of signed ranks (W)                 | 406.0         |
|                                         | Number of pairs                         | 28            |
|                                         | Number of ties (ignored)                | 0             |

Supplementary Table 24. Statistics for STP analysis of modularity for TBS HD-MEA data. Statistical significance was calculated with a Wilcoxon matched-pairs signed rank test.  $p < 0.05$  was considered significant.

|                                         |                                         |                |
|-----------------------------------------|-----------------------------------------|----------------|
| Wilcoxon matched-pairs signed rank test |                                         |                |
| Post vs Pre                             | P value                                 | <0.0001        |
|                                         | Exact or approximate P value?           | Exact          |
|                                         | P value summary                         | ****           |
|                                         | Significantly different ( $P < 0.05$ )? | Yes            |
|                                         | One- or two-tailed P value?             | Two-tailed     |
|                                         | Sum of positive, negative ranks         | 0.000 , -406.0 |
|                                         | Sum of signed ranks (W)                 | -406.0         |
|                                         | Number of pairs                         | 28             |
|                                         | Number of ties (ignored)                | 0              |

Supplementary Table 25. Statistics for STP analysis of DCC for TBS HD-MEA data. Statistical significance was calculated with a Wilcoxon matched-pairs signed rank test.  $p < 0.05$  was considered significant.

|                                         |                                         |                |
|-----------------------------------------|-----------------------------------------|----------------|
| Wilcoxon matched-pairs signed rank test |                                         |                |
| Post vs Pre                             | P value                                 | 0.3502         |
|                                         | Exact or approximate P value?           | Exact          |
|                                         | P value summary                         | ns             |
|                                         | Significantly different ( $P < 0.05$ )? | No             |
|                                         | One- or two-tailed P value?             | Two-tailed     |
|                                         | Sum of positive, negative ranks         | 161.0 , -245.0 |
|                                         | Sum of signed ranks (W)                 | -84.00         |
|                                         | Number of pairs                         | 28             |
|                                         | Number of ties (ignored)                | 0              |

Supplementary Table 26. Statistics for STP analysis of BR for TBS HD-MEA data. Statistical significance was calculated with a Wilcoxon matched-pairs signed rank test.  $p < 0.05$  was considered significant.

|                                         |                                         |                |
|-----------------------------------------|-----------------------------------------|----------------|
| Wilcoxon matched-pairs signed rank test |                                         |                |
| Post vs Pre                             | P value                                 | 0.0017         |
|                                         | Exact or approximate P value?           | Exact          |
|                                         | P value summary                         | **             |
|                                         | Significantly different ( $P < 0.05$ )? | Yes            |
|                                         | One- or two-tailed P value?             | Two-tailed     |
|                                         | Sum of positive, negative ranks         | 315.0 , -63.00 |
|                                         | Sum of signed ranks (W)                 | 252.0          |
|                                         | Number of pairs                         | 27             |
|                                         | Number of ties (ignored)                | 0              |

Supplementary Table 27. Statistics for STP analysis of S<sub>Ce</sub> for TBS HD-MEA data. Statistical significance was calculated with a Wilcoxon matched-pairs signed rank test.  $p < 0.05$  was considered significant.

|                                         |                                         |                |
|-----------------------------------------|-----------------------------------------|----------------|
| Wilcoxon matched-pairs signed rank test |                                         |                |
| Post vs Pre                             | P value                                 | 0.4108         |
|                                         | Exact or approximate P value?           | Exact          |
|                                         | P value summary                         | ns             |
|                                         | Significantly different ( $P < 0.05$ )? | No             |
|                                         | One- or two-tailed P value?             | Two-tailed     |
|                                         | Sum of positive, negative ranks         | 194.0 , -131.0 |
|                                         | Sum of signed ranks (W)                 | 63.00          |
|                                         | Number of pairs                         | 25             |
|                                         | Number of ties (ignored)                | 0              |

Supplementary Table 28. Statistics for the number of edges before and after (60 – 180 minutes grouped) TBS HD-MEA data. Statistical significance was calculated with a two tailed paired t-test.  $p < 0.05$  was considered significant.

|                 |                                         |               |
|-----------------|-----------------------------------------|---------------|
| Paired t test   |                                         |               |
| Before vs After | P value                                 | 0.1992        |
|                 | P value summary                         | ns            |
|                 | Significantly different ( $P < 0.05$ )? | No            |
|                 | One- or two-tailed P value?             | Two-tailed    |
|                 | t, df                                   | t=1.443, df=6 |
|                 | Number of pairs                         | 7             |

Supplementary Table 29. Statistics for the number of nodes before and after (60 – 180 minutes grouped) TBS HD-MEA data. Statistical significance was calculated with a two tailed paired t-test.  $p < 0.05$  was considered significant.

|                 |                                         |               |
|-----------------|-----------------------------------------|---------------|
| Paired t test   |                                         |               |
| Before vs After | P value                                 | 0.1944        |
|                 | P value summary                         | ns            |
|                 | Significantly different ( $P < 0.05$ )? | No            |
|                 | One- or two-tailed P value?             | Two-tailed    |
|                 | t, df                                   | t=1.461, df=6 |
|                 | Number of pairs                         | 7             |

Supplementary Table 30. Statistics for modularity before and after (60 – 180 minutes grouped) TBS HD-MEA data. Statistical significance was calculated with a two tailed paired t-test.  $p < 0.05$  was considered significant.

|                 |                                         |               |
|-----------------|-----------------------------------------|---------------|
| Paired t test   |                                         |               |
| Before vs After | P value                                 | 0.2244        |
|                 | P value summary                         | ns            |
|                 | Significantly different ( $P < 0.05$ )? | No            |
|                 | One- or two-tailed P value?             | Two-tailed    |
|                 | t, df                                   | t=1.355, df=6 |
|                 | Number of pairs                         | 7             |

Supplementary Table 31. Statistics for branching ratio before and after (60 – 180 minutes grouped) TBS HD-MEA data. Statistical significance was calculated with a two tailed paired t-test.  $p < 0.05$  was considered significant.

|                 |                                         |               |
|-----------------|-----------------------------------------|---------------|
| Paired t test   |                                         |               |
| Before vs After | P value                                 | 0.0527        |
|                 | P value summary                         | ns            |
|                 | Significantly different ( $P < 0.05$ )? | No            |
|                 | One- or two-tailed P value?             | Two-tailed    |
|                 | t, df                                   | t=2.409, df=6 |
|                 | Number of pairs                         | 7             |

Supplementary Table 32. Statistics for deviation from criticality coefficient before and after (60 – 180 minutes grouped) TBS HD-MEA data. Statistical significance was calculated with a two tailed paired t-test.  $p < 0.05$  was considered significant.

|                 |                                         |                |
|-----------------|-----------------------------------------|----------------|
| Paired t test   |                                         |                |
| Before vs After | P value                                 | 0.8038         |
|                 | P value summary                         | ns             |
|                 | Significantly different ( $P < 0.05$ )? | No             |
|                 | One- or two-tailed P value?             | Two-tailed     |
|                 | t, df                                   | t=0.2596, df=6 |
|                 | Number of pairs                         | 7              |

Supplementary Table 33. Statistics for shape collapse error before and after (60 – 180 minutes grouped) TBS HD-MEA data. Statistical significance was calculated with a two tailed paired t-test.  $p < 0.05$  was considered significant.

|                 |                                         |                |
|-----------------|-----------------------------------------|----------------|
| Paired t test   |                                         |                |
| Before vs After | P value                                 | 0.4155         |
|                 | P value summary                         | ns             |
|                 | Significantly different ( $P < 0.05$ )? | No             |
|                 | One- or two-tailed P value?             | Two-tailed     |
|                 | t, df                                   | t=0.8745, df=6 |
|                 | Number of pairs                         | 7              |

Supplementary Table 34. Statistics for the interspike interval for well 6A from TBS HD-MEA data. Statistical significance was calculated with one way ANOVA and post-hoc Dunnett tests.  $p < 0.05$  was considered significant.

| Dunn's multiple comparisons test | Mean rank diff. | Significant? | Summary | Adjusted P Value |
|----------------------------------|-----------------|--------------|---------|------------------|
| Baseline vs. 60 mins             | -343.4          | Yes          | ****    | <0.0001          |
| Baseline vs. 90 mins             | -17.49          | No           | ns      | >0.9999          |
| Baseline vs. 120 mins            | -369.3          | Yes          | ****    | <0.0001          |
| Baseline vs. 150 mins            | -353.6          | Yes          | ****    | <0.0001          |
| Baseline vs. 180 mins            | -355.3          | Yes          | ****    | <0.0001          |