

Fig. S1. Molecular character change of hiPSC-derived neurons depend on culture day. (A) Bright field microscopy images by day; bar= $50 \mu \mathrm{~m}$. (B) Immunocytochemistry images of hiPSC-derived neurons. $\mathrm{bar}=50 \mu \mathrm{~m}$. (C) Quantification of glutamatergic neurons and gabaergic neurons. Number of vGlut + cells or GABA + cells were depicted as ratio to number of DAPI + cells. (D) Time-course qPCR analysis of neuronal markers in hiPSC-neurons (mean $\pm$ SEM, $n=3$ ). All the values were normalized to the value of hiPSCs.


Fig. S2. Electrophysiological character of hiPSC derived neuron depend on culture day. (A) Time-course MEA Index in hiPSC-neuron (Mean $\pm$ SEM, $n=15$ ). (B) Changes of number of active electrode during 4 weeks (Mean $\pm$ SEM, $n=15$ ). (C) Representative images of raster plot for 100 secs of firing rate. Blue boxes were indicated single electrode burst while Pink ractangles were indicated network burst. (D) Representative images of synchronization scale in 3th week and 4th week. (E) Temporal response (the first 10 mins of recording after remove of first 2 mins ) measured by MEA according to the different neurotransmitters. ( $\mathrm{N}=10 \sim 13, \mathrm{M}=5$, *for L -glutamic acid and acetylcholine, ${ }^{\text {"for }}$ dopamine and gaba, ${ }^{\text {s }}$ for epinephrine and serotonin).

Table S1. wMFR values normalized by control(baseline) on figure S2E

| Concentration <br> $(\mu \mathrm{M})$ | Neurotranmitter (wMFR normalized by control, \%) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | L-glutamic acid | Dopamine | Epinephrine | Acetylcholine | GABA | Serotonin |
| 0.03 | - | $102.6 \pm 14.9$ | - | - | - | - |
| 0.1 | - | $101.6 \pm 8.8$ | $102.6 \pm 2.1$ | $100.8 \pm 16.8$ | - | - |
| 0.3 | $107.6 \pm 5.9$ | $109.5 \pm 18.6$ | $122.5 \pm 3.6$ | $96.3 \pm 5.5$ | $98.9 \pm 11.6$ | $102.6 \pm 20.7$ |
| 1 | $115 \pm 8.2$ | $135.8 \pm 10.1$ | $59.7 \pm 12.9$ | $88.1 \pm 10.3$ | $93.3 \pm 8.9$ | $105.8 \pm 9$ |
| 3 | $136.1 \pm 21.1$ | $140.2 \pm 9.9$ | $40.5 \pm 11.3$ | $77.3 \pm 8.4$ | $86.7 \pm 5.9$ | $71.3 \pm 9.2$ |
| 10 | $160.8 \pm 9.9$ | $183.4 \pm 28.7$ | $30.1 \pm 5.4$ | $66.7 \pm 14.3$ | $44.3 \pm 19.9$ | $58.7 \pm 11.3$ |
| 30 | $239.4 \pm 53.8$ | $76.2 \pm 5.5$ | $16.9 \pm 10$ | $43.1 \pm 12$ | $18.2 \pm 22.2$ | $34.3 \pm 8.7$ |
| 100 | $298.5 \pm 63.9$ | $62.7 \pm 4.6$ | $8.4 \pm 2.4$ | $19.2 \pm 16.3$ | $16.9 \pm 13$ | $7.6 \pm 4.6$ |
| 300 | $67.3 \pm 12.8$ | - | $4.9 \pm 4.2$ | $17.2 \pm 8.2$ | $12.9 \pm 9.6$ | $4.9 \pm 4$ |
| 1000 | $59.6 \pm 16.9$ | - | - | - | $7.3 \pm 2.5$ | $1.1 \pm 0.8$ |

