Figure 16

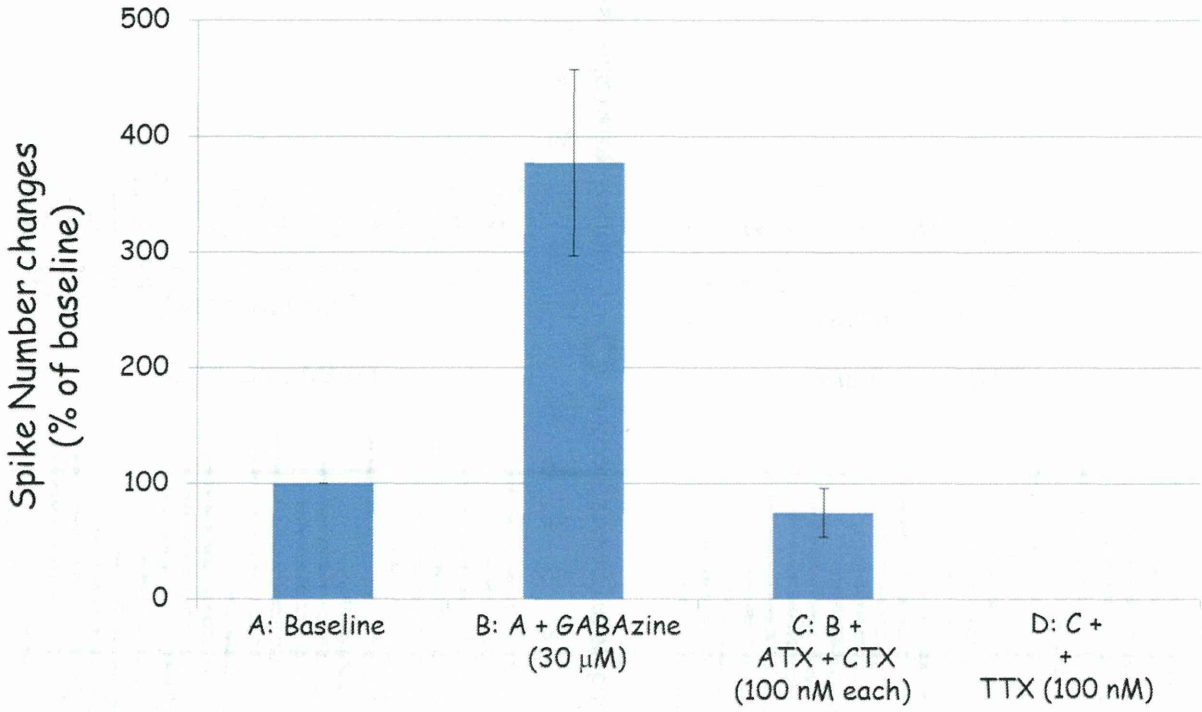


Fig. 16 Synchronized burst spikes are eliminated by neurotransmitter release blockers
The spontaneous spikes were monitored by MEA from iCell Neurons cultured in iCell neuron maintenance medium with mouse astrocyte-conditioned medium for 2 months. Baseline spike numbers were determined by using 100 nM TTX treated background value (A). Altered baseline spike numbers are shown after 3 min treatment with 30 μM GABAzine (B), and simultaneous addition of 100 nM ω-agatoxin (ATX) and 100 nM ω-conotoxin (CTX), then followed by the addition of 100 nM of TTX (D).

Figure 17

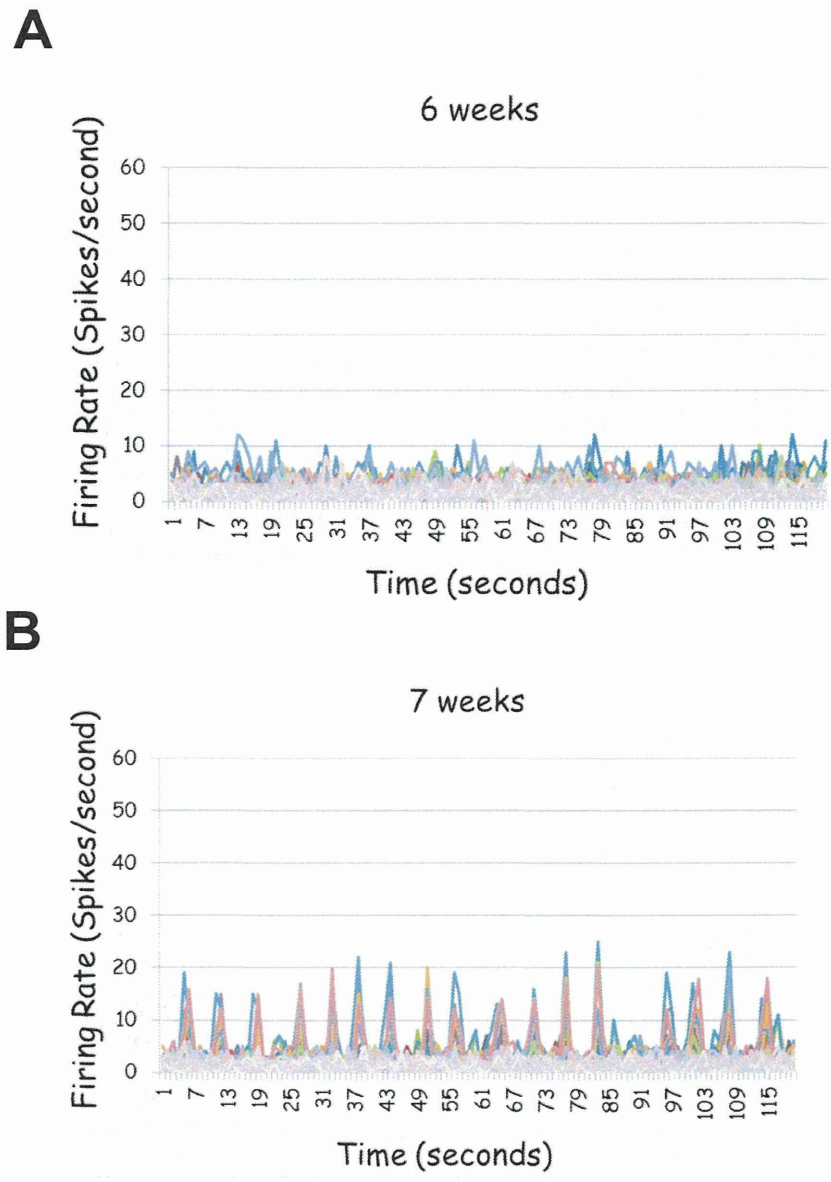
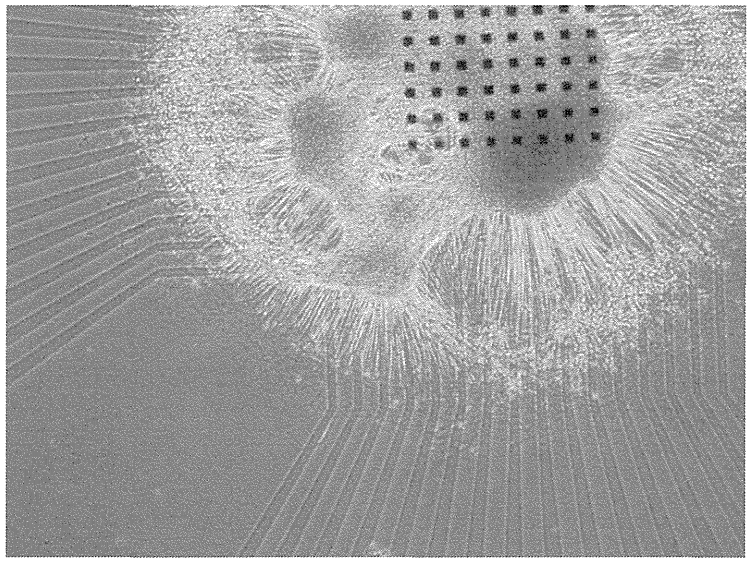


Fig. 17 The periodic spontaneous synchronized burst spike generation is required for more than 6 weeks with astrocyte's stimuli

The spontaneous spikes were monitored by MEA every week from iCell neurons cultured in iCell neuron maintenance medium with mouse astrocyte-conditioned medium. Baseline spike numbers were determined by the value of RMS400 with untreated signals. Periodic synchronized burst spikes among multiple probes weren't observed in 6 weeks (A), but was observed from 7 weeks cultured iCell Neurons (B).

Figure 18

A



B

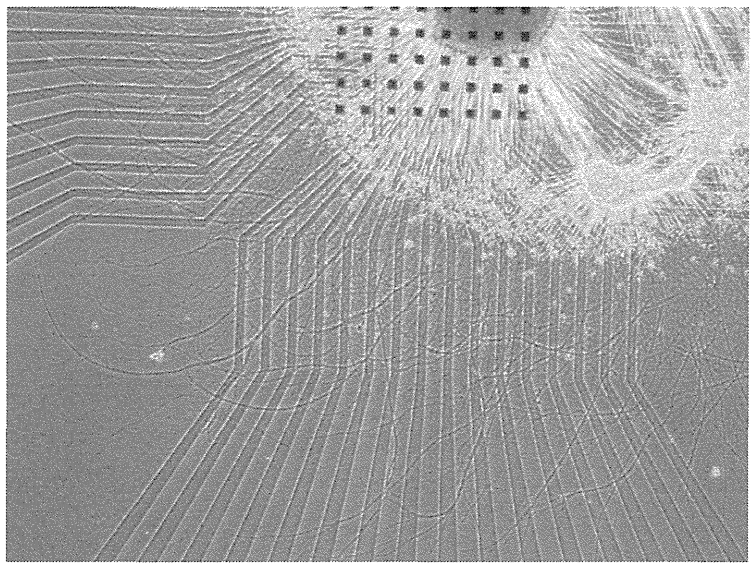


Fig. 18 Neurite outgrowth of iCell Neurons are potently enhanced by human iPS Ccll-derived astrocyte-conditioned medium

iCell Neurons were cultured in iCell neuron maintenance medium with mouse astrocyte-conditioned medium (A) or human iPS Ccll-derived astrocyte-conditioned medium (B) for a week. Neurite outgrowth was observed by microscopy.

Figure 19

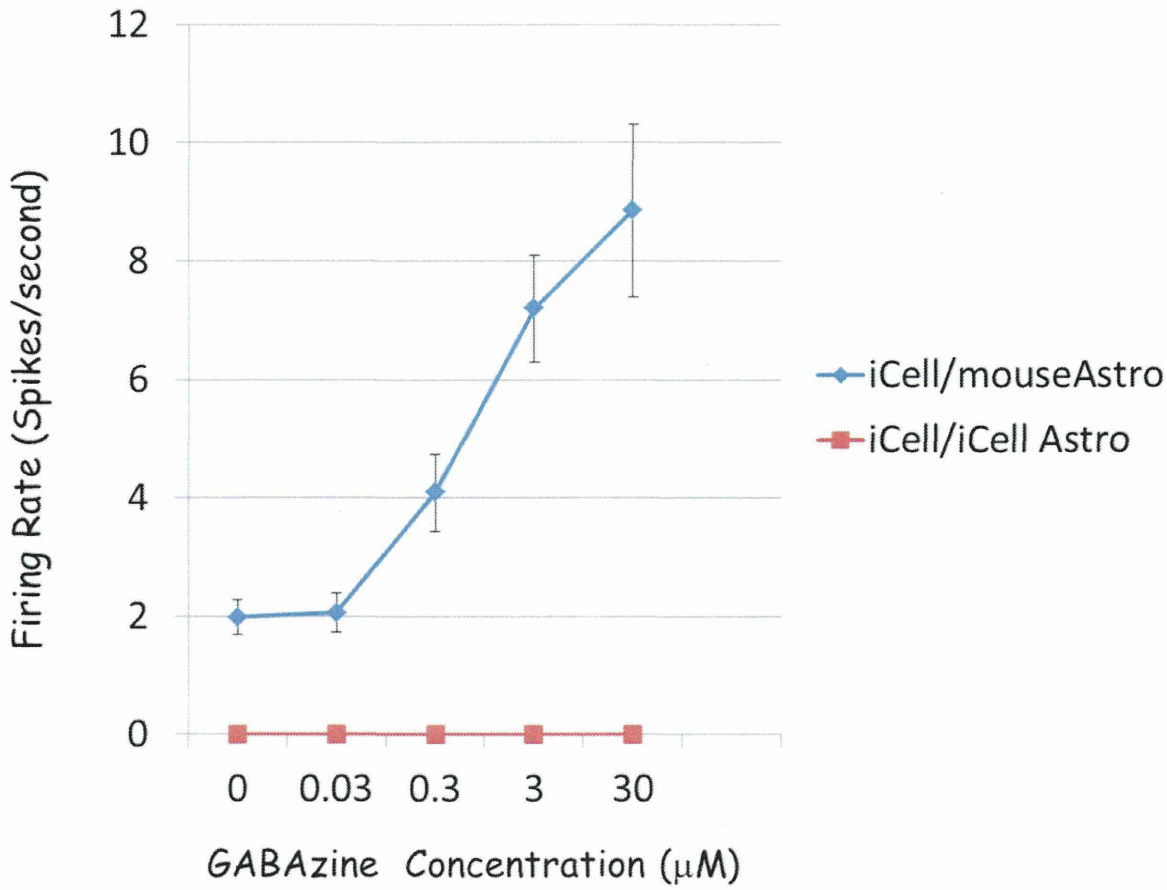


Fig. 19 Human iPS Ccll-derived astrocyte-conditioned medium could not generate spontaneous spikes in iCell Neurons

iCell Neurons were cultured in iCell neuron maintenance medium with mouse astrocyte-conditioned medium or human iPS Ccll-derived astrocyte-conditioned medium for 2 months. The baseline activity was determined by subtraction of background value which was determined after 100 nM TTX treatment. Probes over 1 Hz of spike numbers were selected to data analysis.

Figure 20

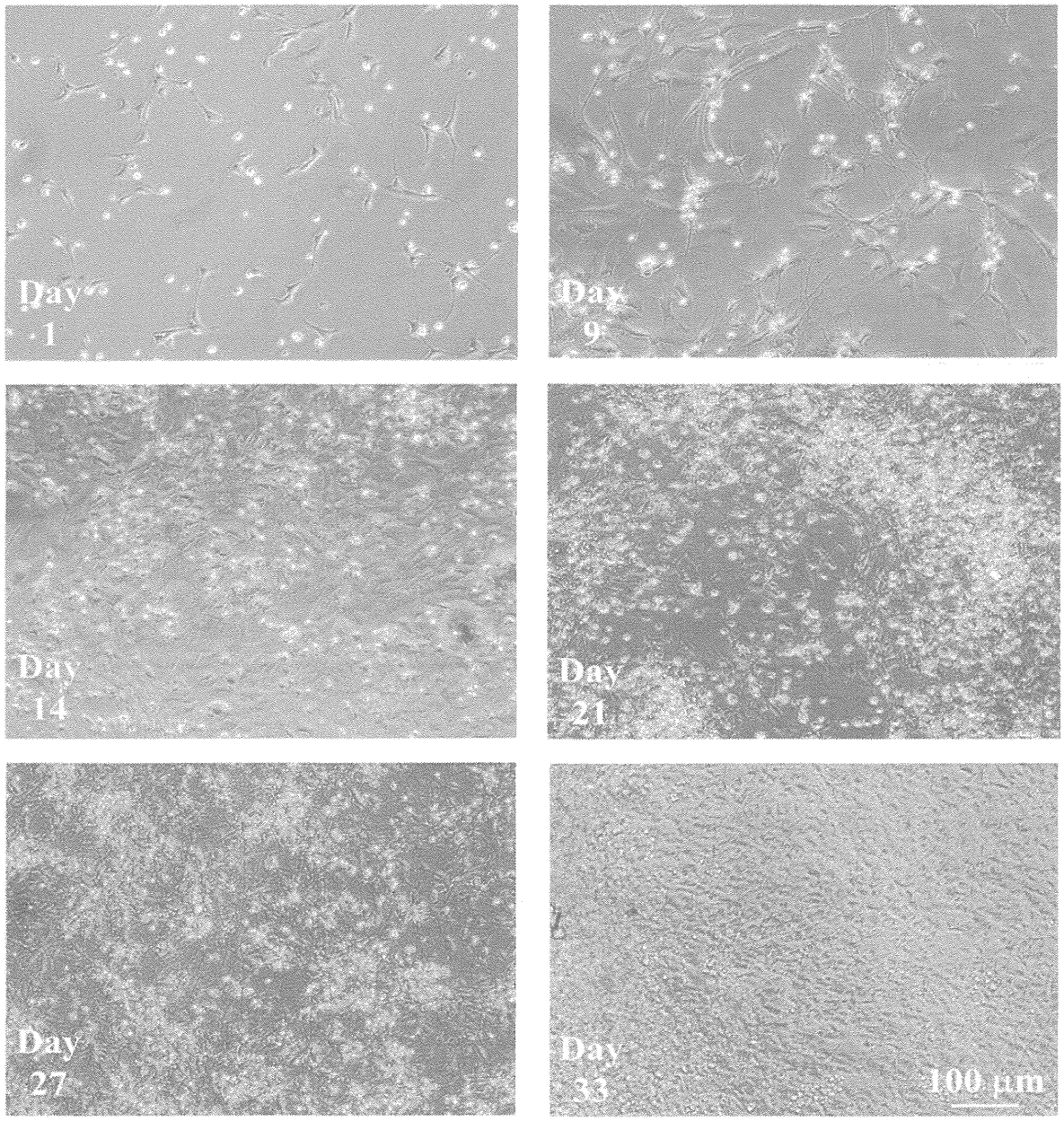
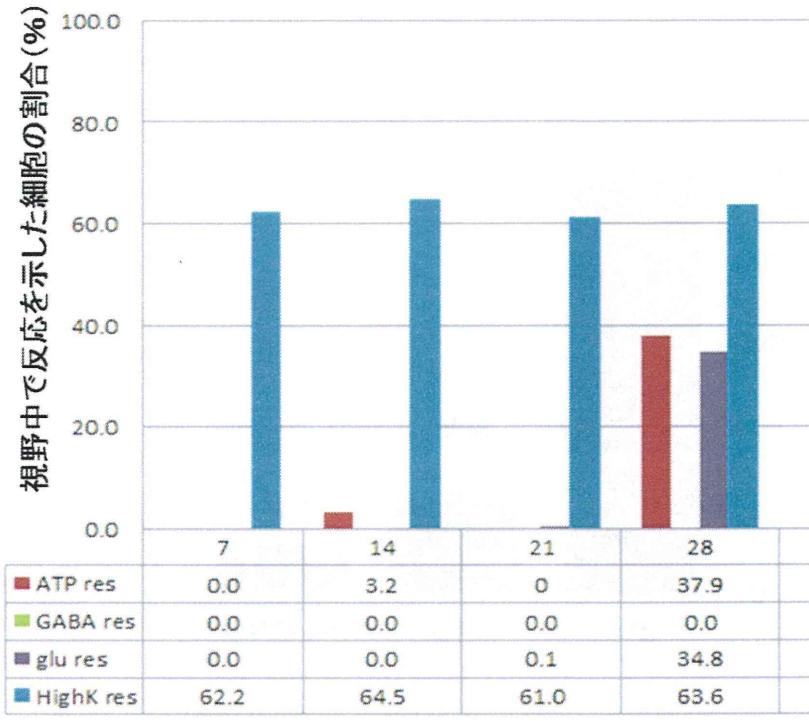


Fig. 20 Phase-contrast images of Repro-Glu until DIV33
The proliferation of the cells became remarkable at DIV14. After DIV27, it became hard to discriminate the morphology of each cell.

Figure 21

A



	7	14	21	28
ATP res	0.0	3.2	0	37.9
GABA res	0.0	0.0	0.0	0.0
glu res	0.0	0.0	0.1	34.8
HighK res	62.2	64.5	61.0	63.6

B

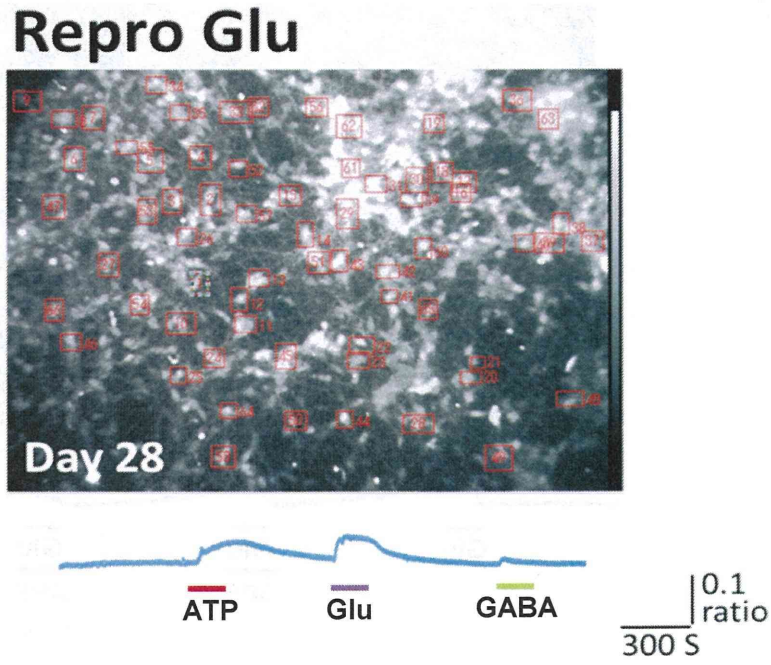


Fig. 21 Ca^{2+} responses of Repro-Glu to ATP, L-Glu, and GABA.

A. Time course of the responses. The cells did not respond to the ligands until day 21. On day 28, there appeared the cells which responded to ATP (100 μM) and L-Glu (100 μM). The responses to GABA (100 μM) was not observed.

B. The typical Ca^{2+} influx traces of Repro-Glu on day 28.

Figure 22

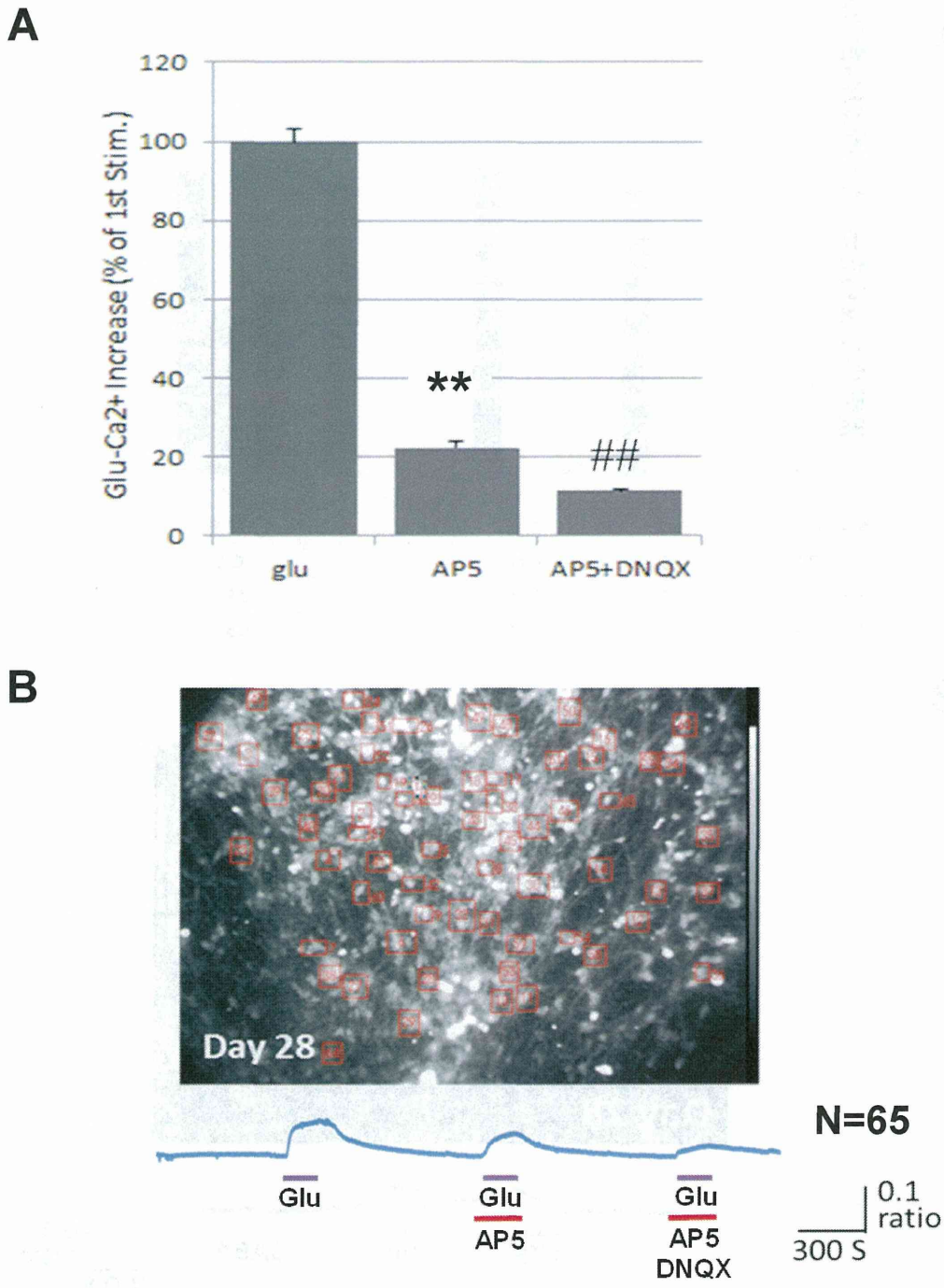


Fig. 22 Effects of AP5 and DNQX on the L-Glu-induced Ca^{2+} responses.

A. In the cells which responded to L-Glu, AP-5 strongly suppressed the Ca^{2+} responses to L-Glu. DNQX+AP5 further suppressed the L-Glu-induced Ca^{2+} responses.

B. Typical trace of the Ca^{2+} responses of this pharmacological **experiment.**

: **p<0.01 vs L-Glu-treated group, ##: p<0.01 vs L-Glu+AP5-treated group, Tukey's test following ANOVA. Error bars represent s.e.m.

Figure 23

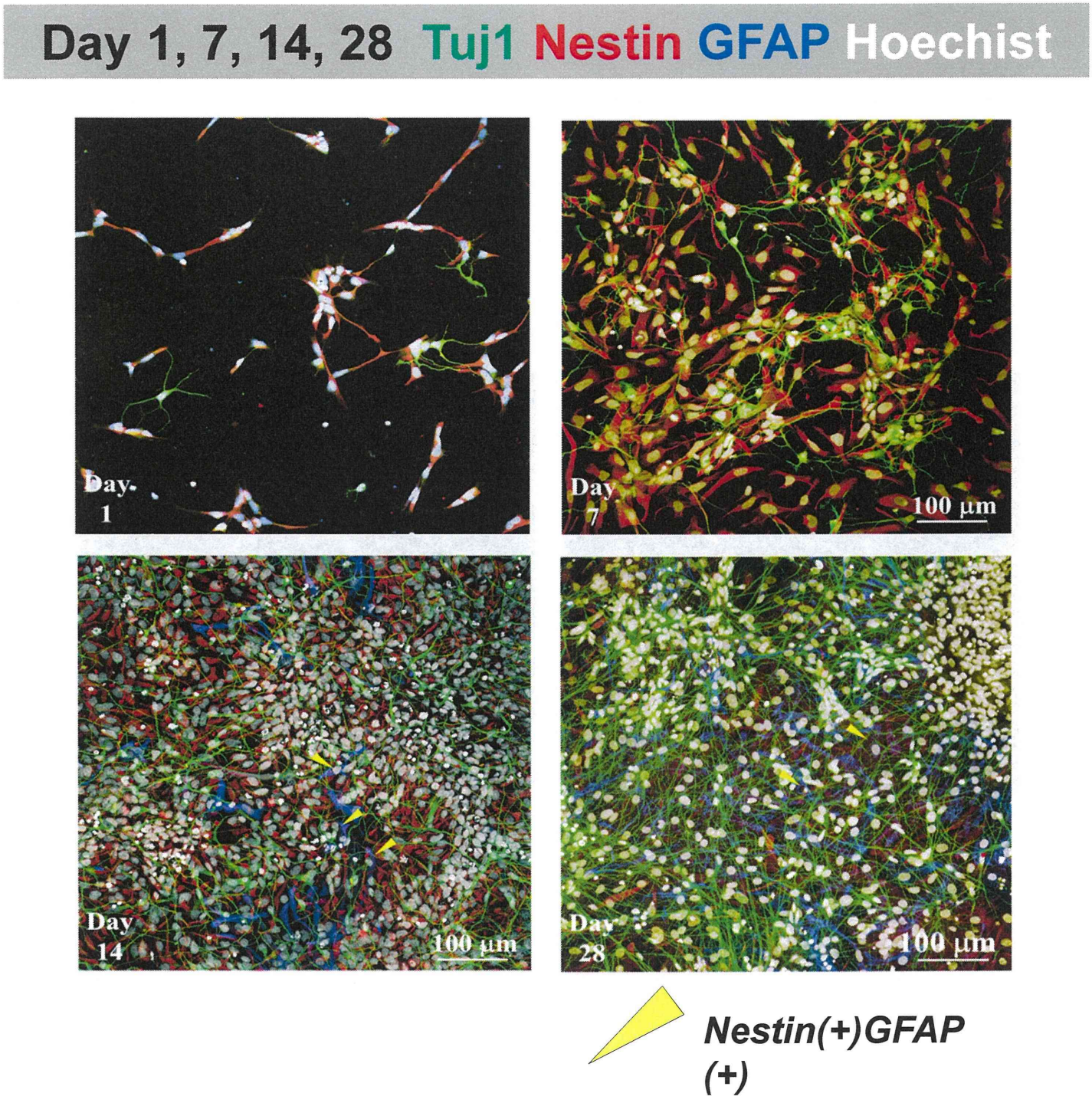


Fig. 23 Expression of Tuj1, Nestin, and GFAP in Repro-Glu (- day 28).
The existence of Tuj1(+) cells, Nestin(+) cells, GFAP(+) cells were confirmed by day 28. Nestin(+)GFAP(+) cells were still observed on day 28, suggesting the existence of radial glia.

Figure 24

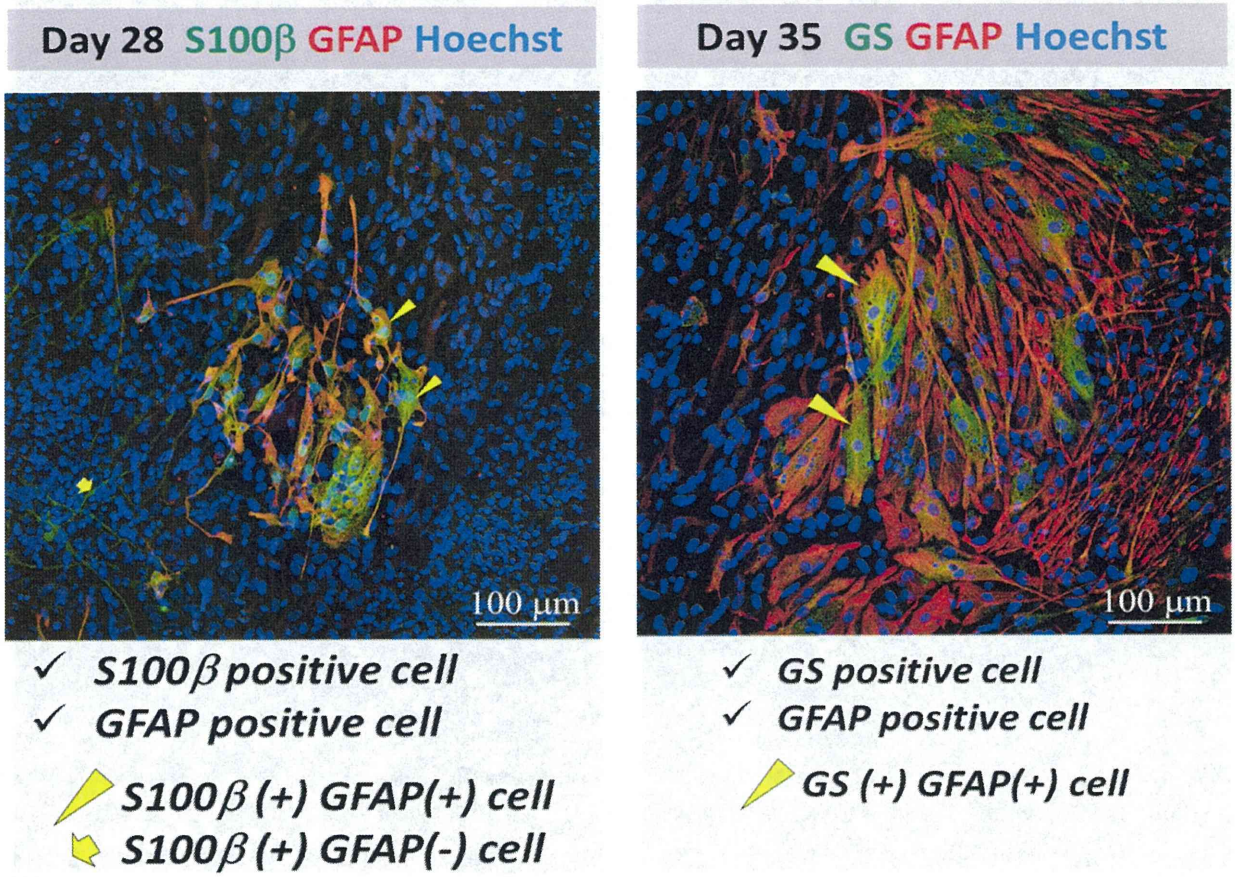


Fig. 24 Astrocytes were included in Repro-Glu
In GFAP(+) cells, S100b(+)GFAP(+) cells and GS(+)GFAP(+) cells are included, strongly suggesting that astrocytes are included in Repro-Glu.

Figure 25

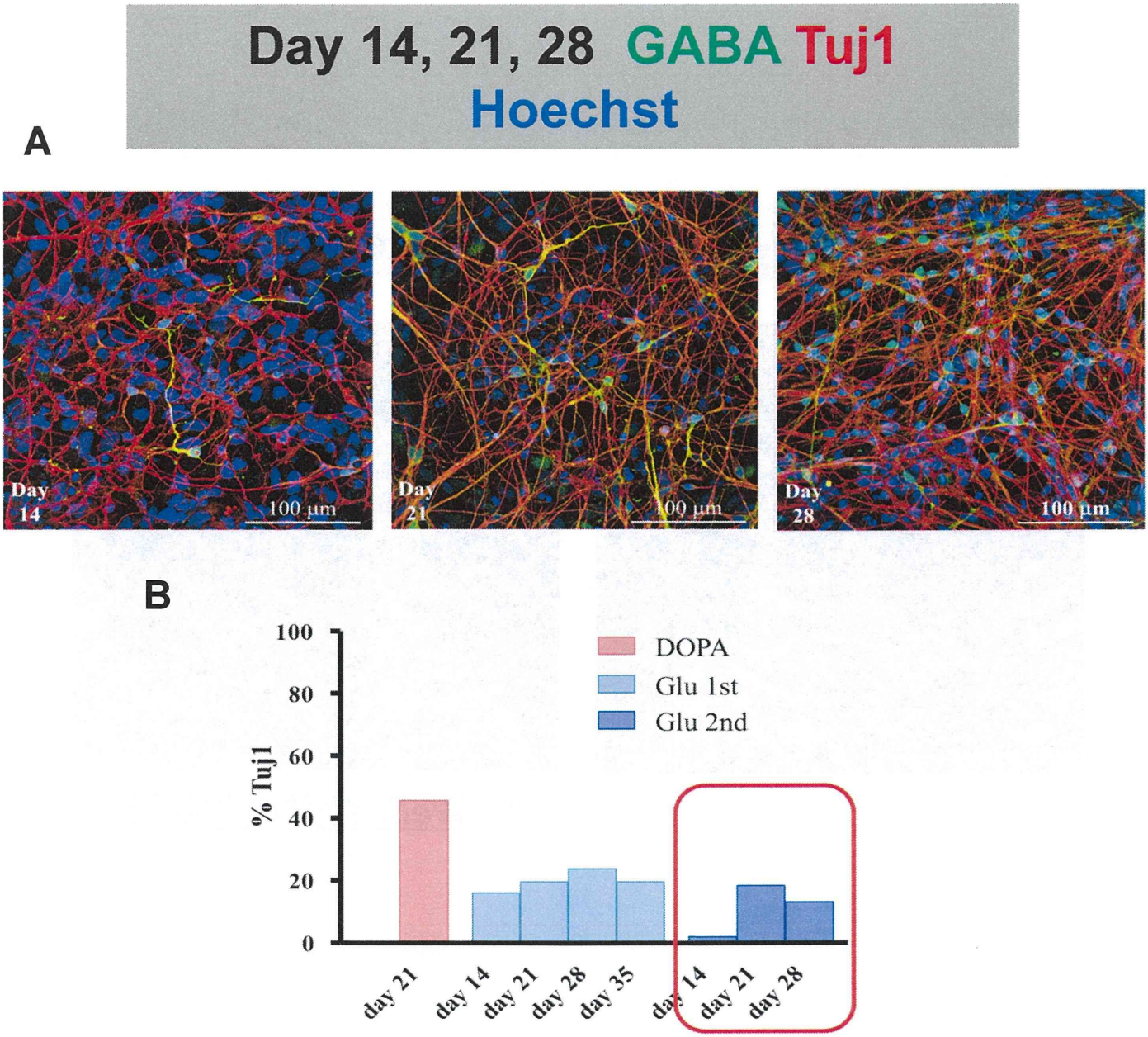


Fig. 25 The percentage of GABA neurons in Ripro-Glu.
The percentage of GABA neurons in the Ripro-Glu neurons was 10-20% (Glu), while that in the Ripro-DA neurons was 40-50% (DOPA). Duplicated data. The percentage did not change by day 28.