# Generation and rapid maturation of cortical layer V glutamatergic neurons from human iPSCs

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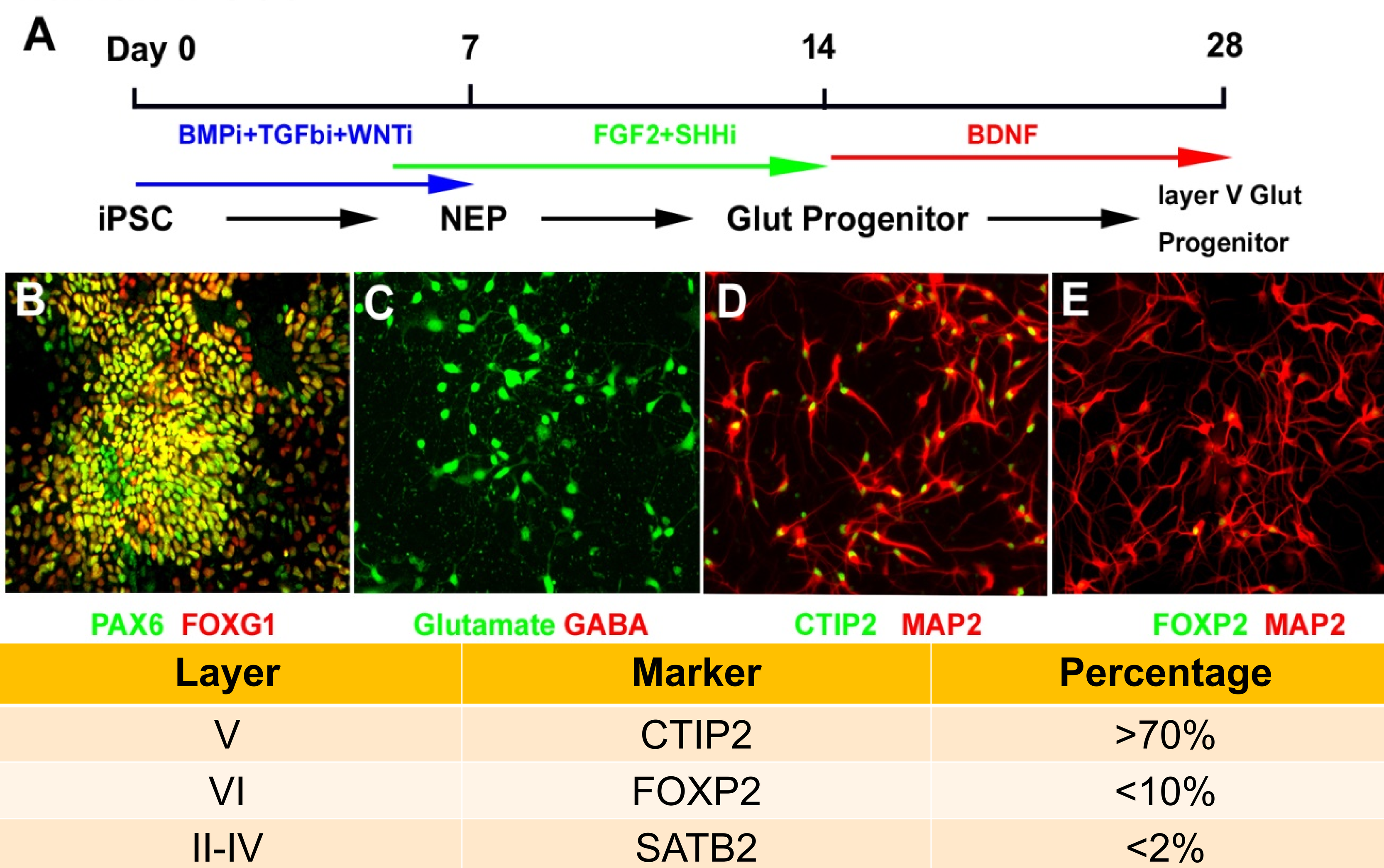
## Introduction

Numerous neurological and psychiatric disorders involve glutamatergic neurons specific to one or more of the six cortical layers. Death or malfunction of these specific neurons underlies disease pathophysiology and disrupts higher cognitive function. Study of these neurons may reveal the molecular mechanisms behind their vulnerability and enable development of more relevant disease models. Toward this goal, we developed a protocol to efficiently produce enriched cortical layer V glutamatergic neurons. Both normal and patient iPSCs were first induced to forebrain neuroepithelial progenitors, then patterned to cortical neuronal progenitors (>90% FOXG1+/PAX6+), and finally to layer V progenitors. Using novel combinations of small molecules, layer V neural progenitors can be expanded up to 500-fold, which allows for production of large and consistent batches of neurons. The progenitors were plated in a medium containing a specialized maturation supplement that rapidly promotes morphological and functional maturation. After treating with this supplement, neurons displayed extensive neurite outgrowth within 3 days. Seven days post-plating, the cultures were >90% neurons (MAP2+) with the following breakdown by layer identity: >70% layer V (CTIP2+/FOXP2-/SATB2-), <10% layer VI (FOXP2+), and <2% layer II-IV (SATB2+). These layer V glutamatergic neurons expressed mature synaptic markers within 7 days and exhibited electrophysiological activity within 2 weeks. Thus, our novel differentiation protocol generates a pure neuronal culture that is highly enriched for cortical glutamatergic neurons with a layer V identity. Coupled with the ability to generate very large batches (>1 billion neurons) and bring about rapid maturation, layer V cortical neurons present a highly relevant model system for drug discovery and development.

## BrainXell Cortical Layer V Glutamatergic Neurons

### 1. Directed differentiation of layer V neural progenitors from human iPSCs

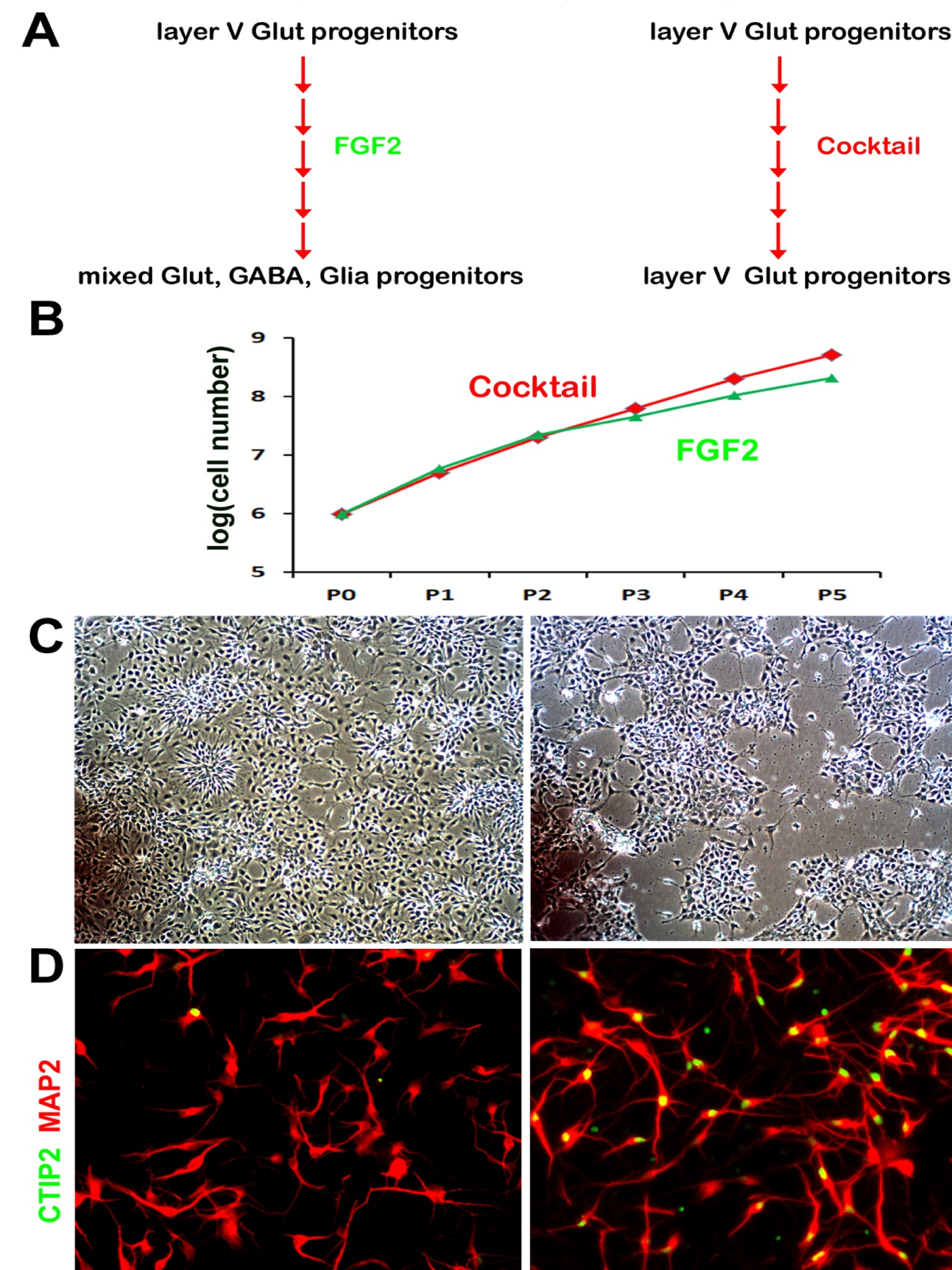



| Layer | Marker | Percentage |
|---|---|---|
| V | CTIP2 | >70% |
| VI | FOXP2 | <10% |
| II-IV | SATB2 | <2% |

A) Diagram showing the protocol for generating layer V glutamatergic progenitors from human iPSCs in 28 days. (B) The cortical progenitors at two weeks showed expression of progenitor markers PAX6 (green) and FOXG1 (red). (C) The layer V glutamatergic progenitors were differentiated into neurons for another week and showed expression of glutamate (green, C) and MAP2 (red, D, E). These neurons mostly expressed the layer V marker CTIP2 (green, D) with limited expression of the layer VI marker FOXP2 (green, E).

## BrainXell Cortical Layer V Glutamatergic Neurons

### 2. Expansion of layer V neural progenitors by novel cocktail




(A) Diagrams showing the expansion of layer V progenitors for 5 passages with FGF2 and a novel cocktail. (B) The cell number expands from 1 million to 0.5 billion (about 500 fold) during this phase. (C) FGF2 expansion produces a mixed population, but the novel cocktail yields a homogeneous population. (D) FGF2-expanded progenitors generated few CTIP2+ neurons (green), but >70% CTIP2+ neurons were produced by the novel cocktail-expanded progenitors.

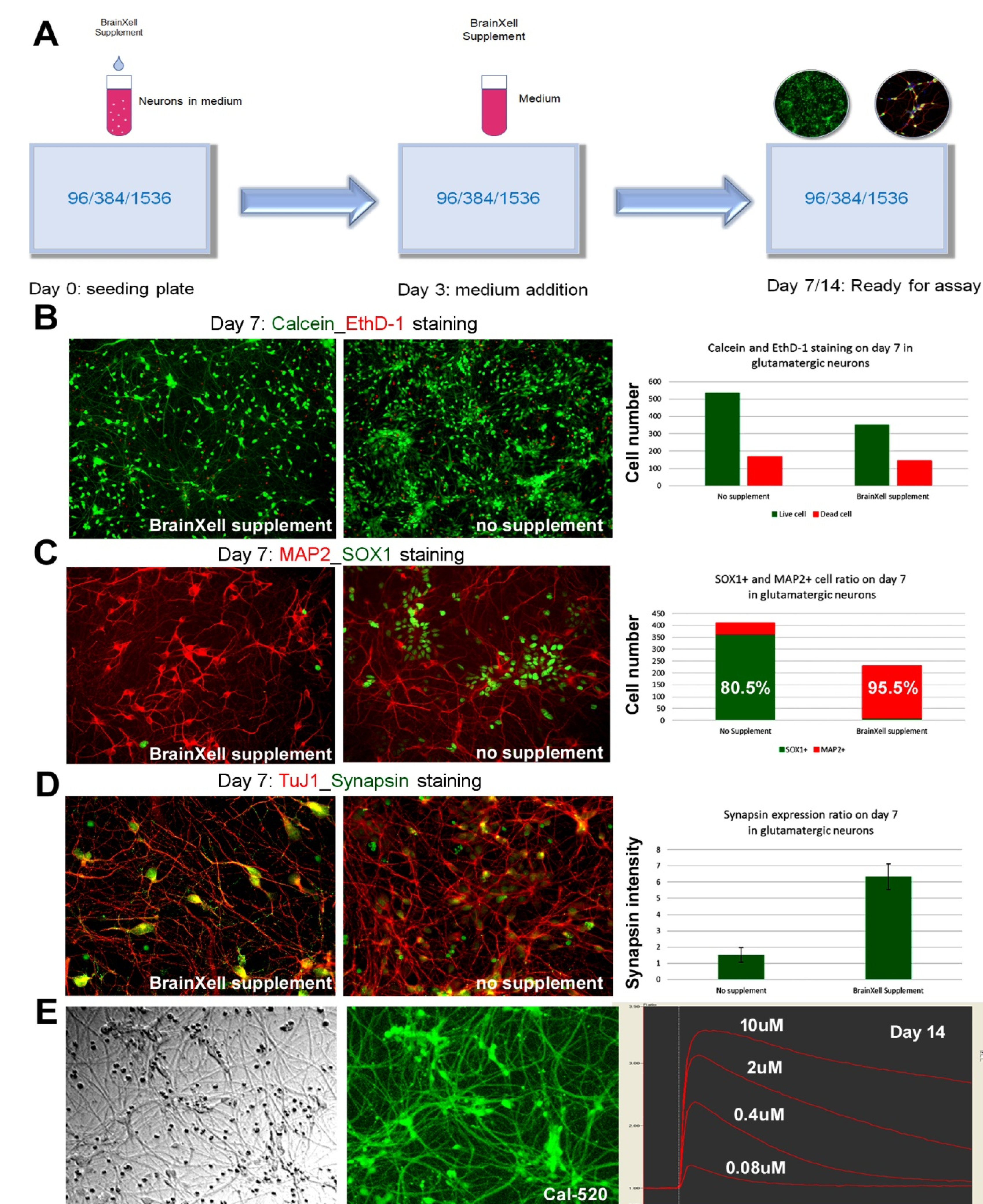
### Summary

1. We can generate highly enriched cortical layer V glutamatergic neurons (>70% pure) at a large-scale (> 1 billion neurons).

2. Our maturation supplement rapidly promotes morphological and functional maturation in 1-2 weeks.

3. We are applying the same strategy for developing cortical superficial layer II-III neurons.

### 3. BrainXell supplement accelerates cortical layer V glutamatergic neuron maturation




(A) Diagrams show the process of BrainXell maturation supplement treatment. (B) Layer V glutamatergic neurons were stained with the live/dead dyes calcein AM and EthD-1 on day 7, showing similar number of dead cells (<30%) in the no-supplement condition comparing to the BrainXell supplement condition. (C) 80.5% of cells in the no-supplement condition were still SOX1+ progenitors, but 95.5% of cells were MAP2+ mature neurons in BrainXell supplement condition. (D) The synaptic marker synapsin had expression on the neurites that was 4.2-fold higher in the BrainXell supplement condition than in no-supplement condition. (E) The BrainXell supplement promoted the functional maturation of layer V glutamatergic neurons in 14 days, which is showed by the calcium response from 0.08 μM to 10μM glutamate.

### Acknowledgement

This study was supported by NIH-NIMH SBIR grant (R44MH110301).