Figure S1

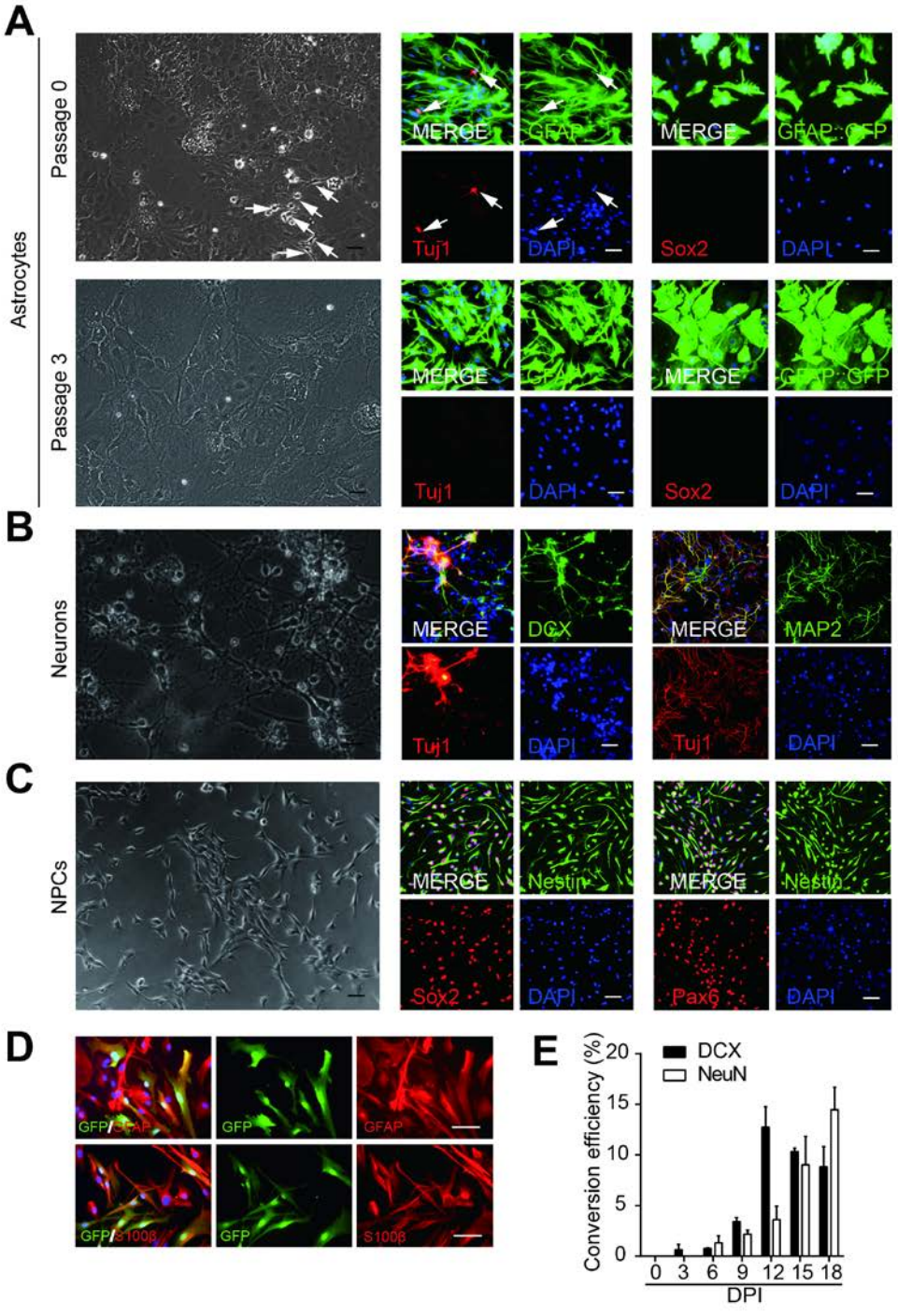
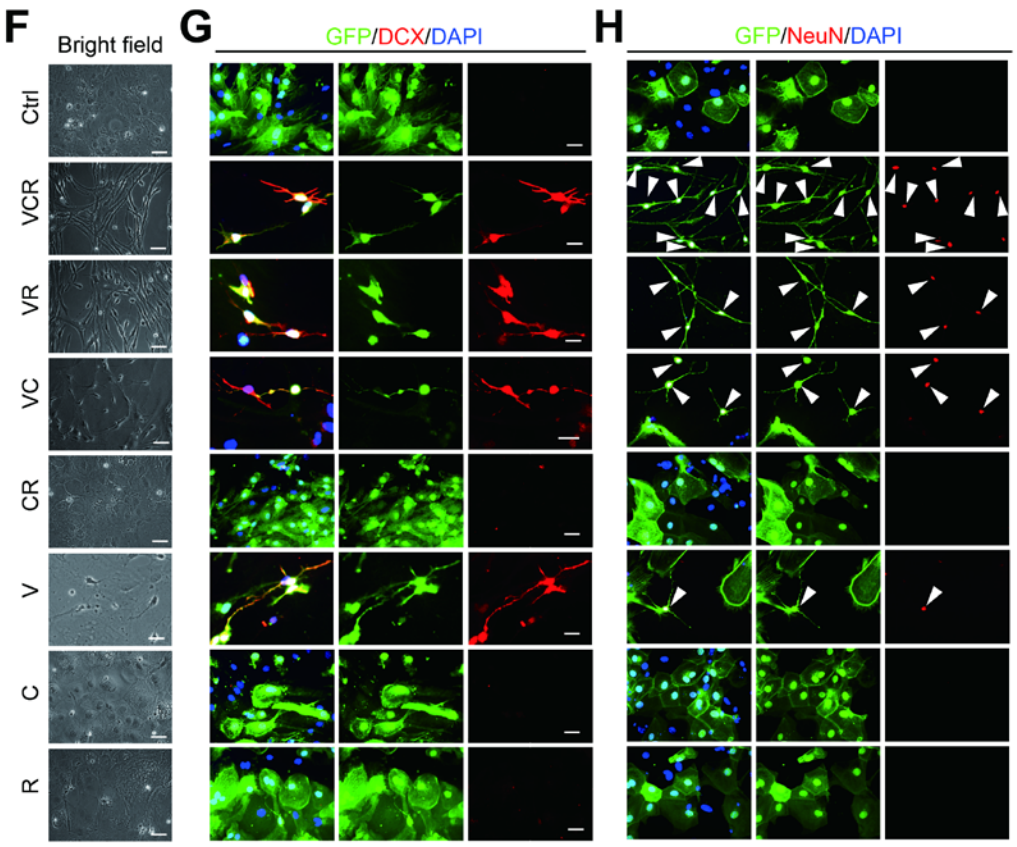

A
Astrocytes
Passage 0
Passage 3
MERGE
GFAP
GFAP::GFP
Tuj1
DAPI
Sox2
B
Neurons
DCX
MAP2
C
NPCs
Nestin
Pax6
D
GFP/GFAP
GFP
GFAP
GFP/S100β
S100β
E
Conversion efficiency (%)
DCX
NeuN
0
5
10
15
20
0 3 6 9 12 15 18
DPI

Figure S1


F
Bright field
G
GFP/DCX/DAPI
H
GFP/NeuN/DAPI
Ctrl
VCR
VR
VC
CR
V
C
R

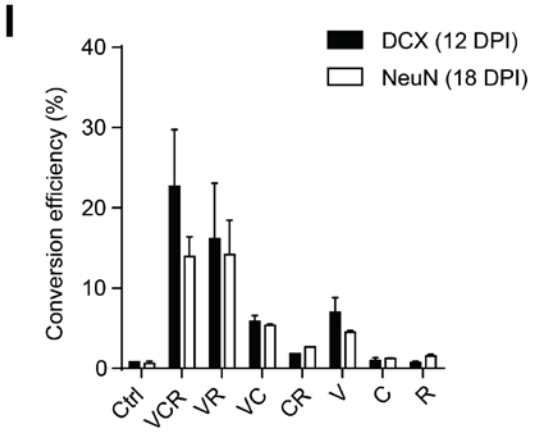
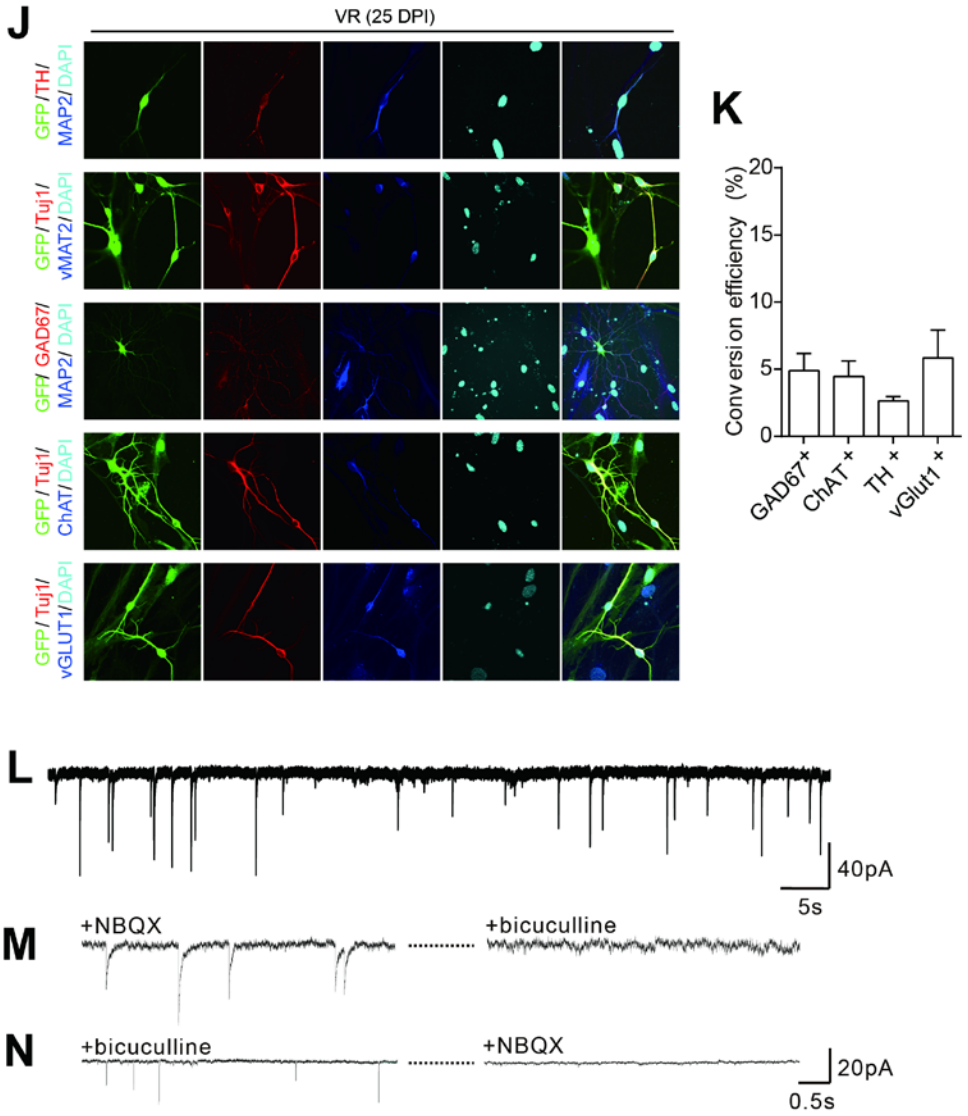

I
DCX (12 DPI)
NeuN (18 DPI)
Conversion efficiency (%)
40
30
20
10
0
Ctrl
VCR
VR
VC
CR
V
C
R

Figure S1



Supplementary information, Figure S1 Small molecules induce astrocytic-to-neuronal cell conversion *in vitro*. (A-D) Characterization of primary astrocytes isolated from mouse cortex. (A) Cortical astrocytes were isolated from mouse pups one day after birth. In order to exclude the contamination of neuronal cells, mixed cells were passaged for three generations. No exist of $Tuj1^+$ neurons or $Sox2^+$ neural progenitor cells in cultured astrocytes at passage 3. Pure astrocytes

showed typical astrocytic shape demonstrated by cell morphology and immunofluorescent staining of well-established astrocyte marker GFAP, which were highly different from primary neurons (B) and primary neural progenitor cells (NPCs) (C). Arrows highlight individual neurons in mixed primary cells. Scale bars: 20 μm. (D) Cultured astrocytes expressing GFAP or S100β were labelled by retrovirus expressing GFAP::GFP. pGFAP-GFP-IRES-GFP retroviral vector was generated by replacing *CAG* promoter of pCAG-GFP-IRES-GFP (gift of Prof. Fred Gage) with the human *GFAP* promoter cloned from genome. Retrovirus was produced by transfection of plat-E cells with retroviral vectors for further infection. (E) Efficiency of neuronal cell conversion from astrocytes by VCR was monitored and calculated on different days post induction. (F-I) Screening of essential small molecule(s) in VCR inducing astrocytes into neuronal cells. (F) Changes in cell morphology of cultured astrocytes treated with diverse small molecules, removing one or two component(s) from VCR, under conditional medium 12 days post induction. Treatment of VCR, VC, VR, or V induced the obvious changing from astrocytic shape to neuronal cell shape. (G) These chemical-treated astrocytes were further fixed by 4% PFA for immunofluorescent staining of neuroblast marker DCX. (H) Cultured astrocytes treated with diverse small molecules under conditional medium 18 days post induction were fixed for immunofluorescent staining of mature neuron marker NeuN. Treatment of VCR, VR, VC or V alone resulted in generation of $NeuN^+$ neurons from astrocytes *in vitro*, but not the treatment of CR, C or R. Arrows highlight individual cells co-expressing GFP and NeuN. V, VPA; C, CHIR99021; and R, Repsox. Scale bars: 20 μm. (I) Efficiency

of neuron conversion from astrocytes by removing chemical(s) from VCR. (J-N) VR converts astrocytes into specific types of neurons. (J) Cultured astrocytes treated with VR and maintained under defined conditions for 25 days were converted into mature neurons expressing markers of specific-type neurons. Tyrosine hydroxylase (TH) and vesicular monamine transporter (vMAT2), makers of dopaminergic neuronal cell. GAD67, a marker for GABAergic neuron. ChAT, a marker of motor neuron. Vesicular glutamate transporter 1 (vGLUT1), glutamatergic neuron marker. (K) Quantification of neuron converting efficiency in (J). (L) Spontaneous PSCs was recorded from neurons derived from astrocytes. (M) Slow PSCs were recorded after applying NBQX. The slow PSCs were completely blocked by bicuculline. (N) Fast PSCs were recorded after applying bicuculline. The fast PSCs were completely blocked by NBQX.