Figure S2

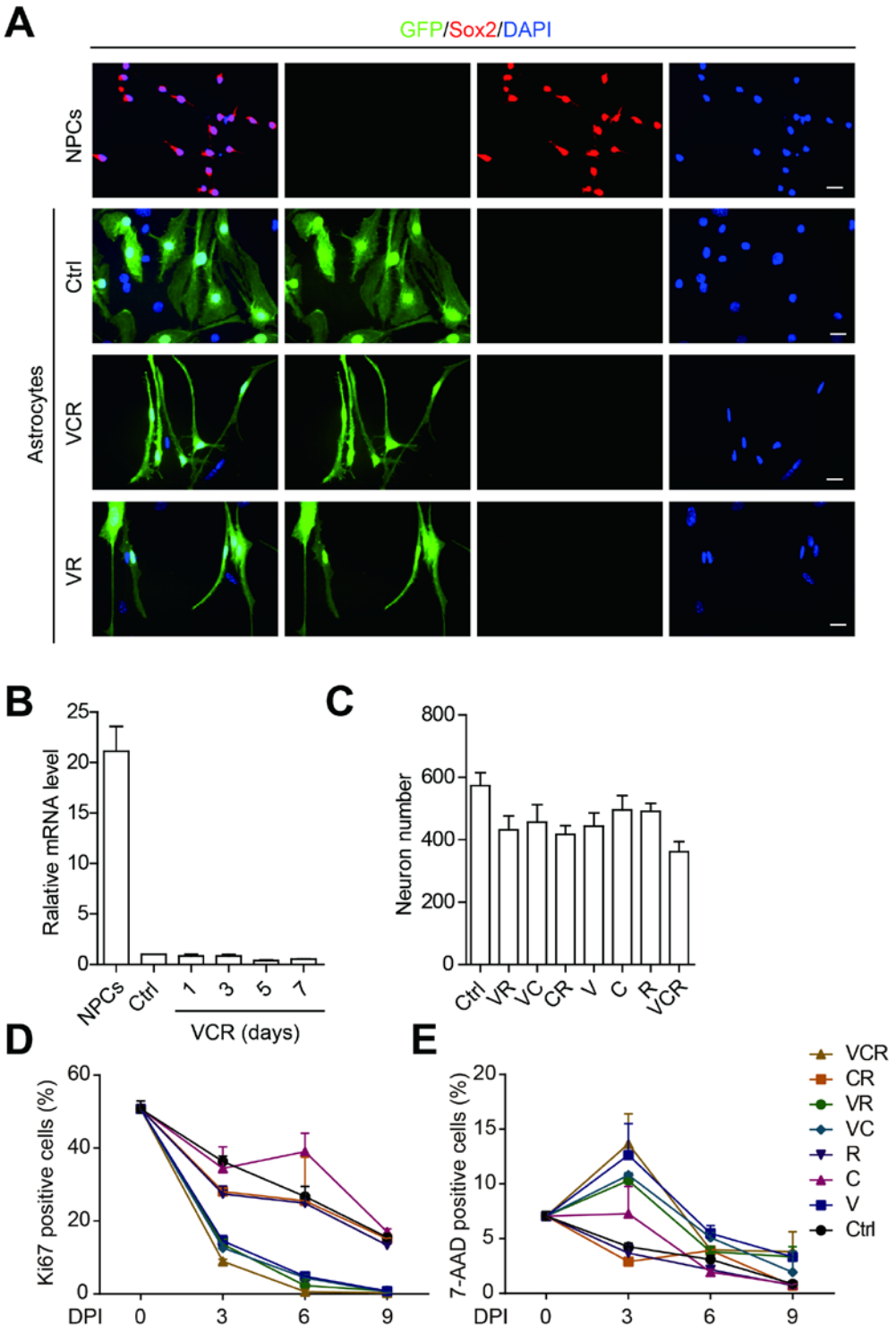
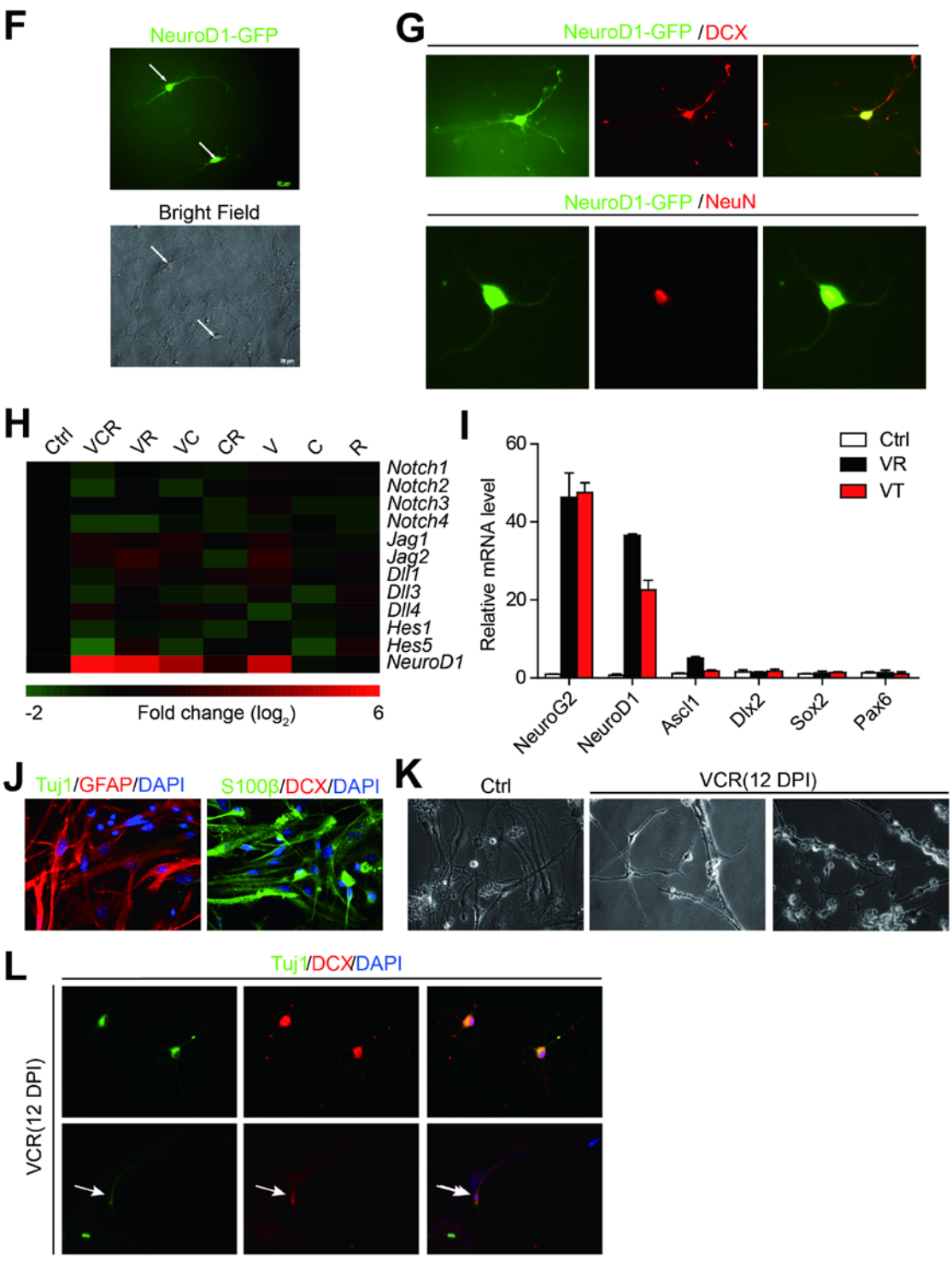

A
GFP/Sox2/DAPI
NPCs
Astrocytes
Ctrl
VCR
VR
B
Ralative mRNA level
25
20
15
10
5
0
NPCs
Ctrl
1
3
5
7
VCR (days)
C
Neuron number
800
600
400
200
0
Ctrl
VR
VC
CR
V
C
R
VCR
D
Ki67 positive cells (%)
60
40
20
0
DPI
0
3
6
9
E
7-AAD positive cells (%)
20
15
10
5
0
DPI
0
3
6
9
VCR
CR
VR
VC
R
C
V
Ctrl

Figure S2



Supplementary information, Figure S2. Potential mechanism and application of the

small molecule-induced neuronal conversion from cultured astrocytes. (A and B) Astrocytic-to-neuronal transition *in vitro* is unrelated with neural progenitor cell state. (A) No exist of neural progenitor cell *in vitro* post chemical treatment in astrocytes. Immunofluorescent staining of Sox2 in astrocytes treated with VCR or VR for four days *in vitro*. Scale bars: 20 μm. (B) No significant increase of *Sox2* expression level during the entire cell transition from astrocytes to neuronal cells at day 1, 3, 5 and 7 post VCR treatment. All sample data are normalized to that of control (Ctrl), which is considered as 1. Data are presented as mean ± s.e.m.. (C-E) Testing the effect of small molecules and their combination on neurons and astrocytes. (C) Cell number of primary neurons was calculated post chemical treatment for three days. (D) Analysis of cell proliferation marker Ki67 for astrocytes at different days post chemical treatment. (E) Cell death of astrocytes under chemical treatments was also monitored by flow cytometry of 7-Aminoactinomycin D (7-AAD). (F and G) Conversion of cultured astrocytes into neuronal cells by NeuroD1. Retrovirus expressing NeuroD1 and GFP were generated by transfecting plat-E with retroviral vector pCAG-NeuroD1-IRES-GFP then for further infection. (F) Morphology changing of astrocytes 4 days post NeuroD1 overexpression (highlight by arrows). (G) Immunostaining of DCX 4 days post NeuroD1 overexpression. NeuN positive cells were detected 6 days after overexpressing NeuroD1 in astrocytes. (H) Heat map depicting the relative fold change of expression of genes involved in Notch signaling pathways, including Notch receptors *1*, *2*, *3* and *4*, and Notch ligands *Jagged 1* and *2*, *Dll 1* and *2*, and *Hes 1* and 5, in astrocytes under chemical treatment with one week *in vitro*. The value in the color denotes $\log_2$-transformed fold changes (relative to *HPRT* and normalized to ctrl). (I) Application of Q-PCR for detecting expression level of transcription factors

reported to convert astrocytes into neuronal cells. Cultured astrocytes were treated with indicated chemical cocktails VR or VT for 3 days. All sample data are normalized to that of ctrl, which is considered as 1. (J-L) Conversion of astrocytes derived from adult mouse into neuronal cells by VCR. (J) Characterization of astrocytes from adult mouse by immunofluorescent staining analysis. (K) Adult mouse astrocytes showed morphological changing from astrocytic shape into neuronal cell-like type under VCR treatment. (L) Verification of induced neuronal cells from adult mouse astrocytes by immunostaining of neuronal cell markers DCX and Tuj1.