Tong et al., http://www.jcb.org/cgi/content/full/jcb.200811071/DC1

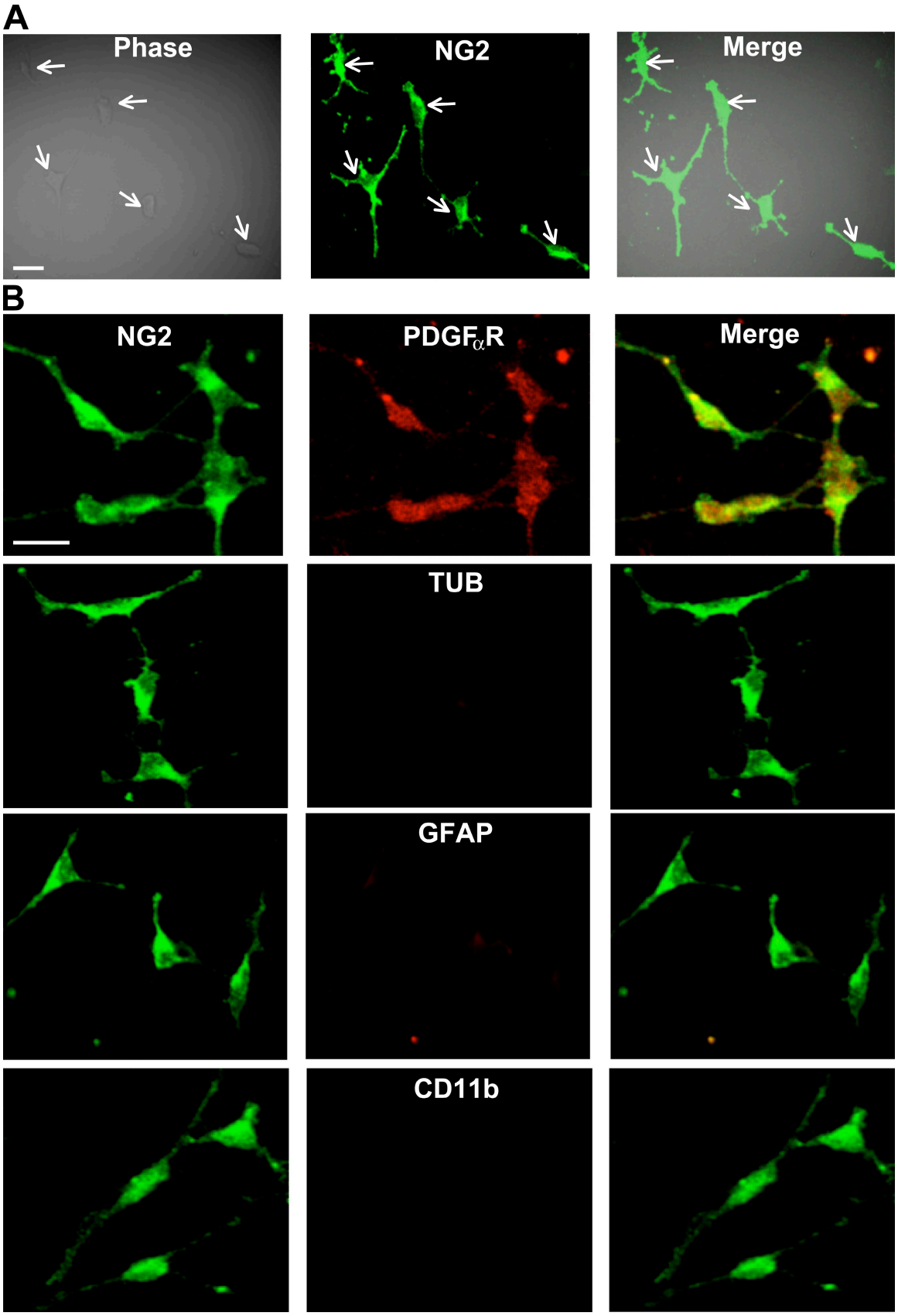


Figure S1. **Purity of cultured NG2 cells identified by immunostaining.** (A) Phase (left) and fluorescence (middle) images showing that all the cells (arrows) in the imaging field are NG2 positive (green). (B) Double immunostaining of cultured NG2 cells showing that NG2-positive cells (left, green) are also immunopositive for anti-PDGFα receptors (another characteristic marker of NG2 cells), but are negative for anti-βIII tubulin (TUB; neuronal marker), anti-GFAP (astrocyte marker), or anti-CD11b (microglia marker). Bars, 20 μm.

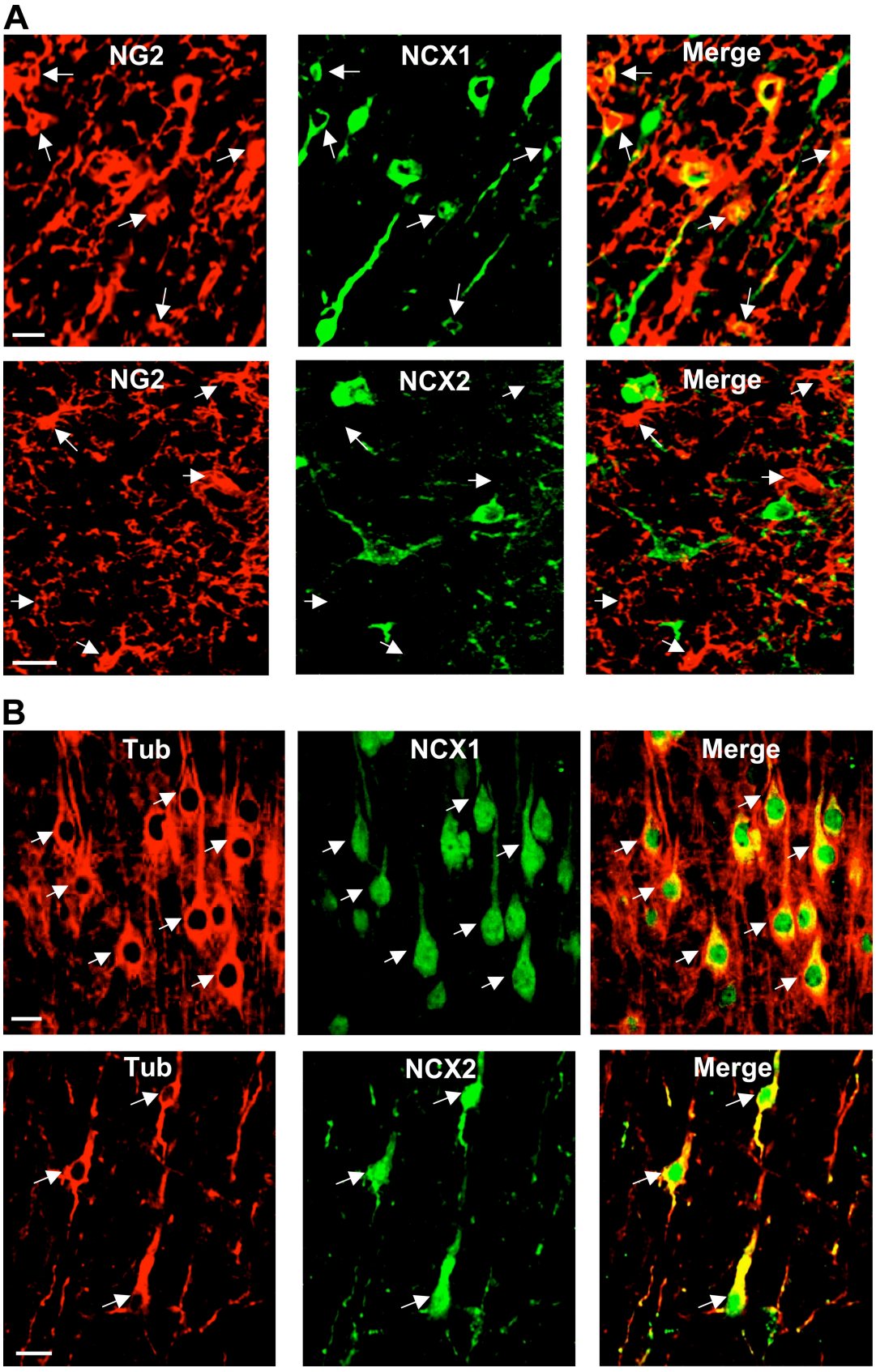


Figure S2. **Expression of NCX1 and NCX2 in neurons and NG2 cells in rat cortical slices.** (A) Coimmunostaining of anti-NG2 with anti-NCX1 (top) or anti-NCX2 (bottom). Note the apparent expression of NCX1 but not that of NCX2 in NG2-positive cells (arrows). Bars, 20 μm. (B) Coimmunostaining of anti-βIII tubulin (TUB; neuronal marker) with anti-NCX1 (top) or anti-NCX2 (bottom) showing apparent expression of both NCX1 and NCX2 in neurons (arrows). Bars, 20 μm.

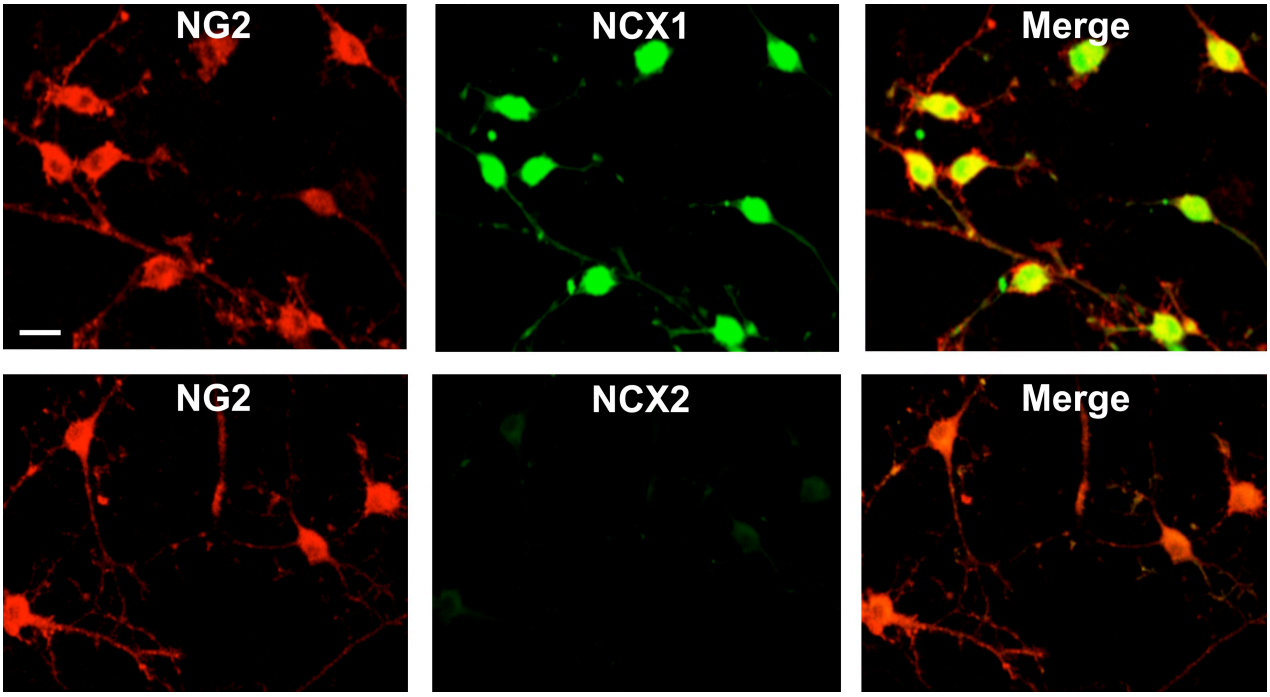


Figure S3. **Apparent expression of NCX1 but not NCX2 in cultured NG2 cells.** Purified NG2 cell cultures immunostained with anti-NG2 (red), anti-NCX1(green; top), and anti-NCX2 (green; bottom). Bar, 20 μm.

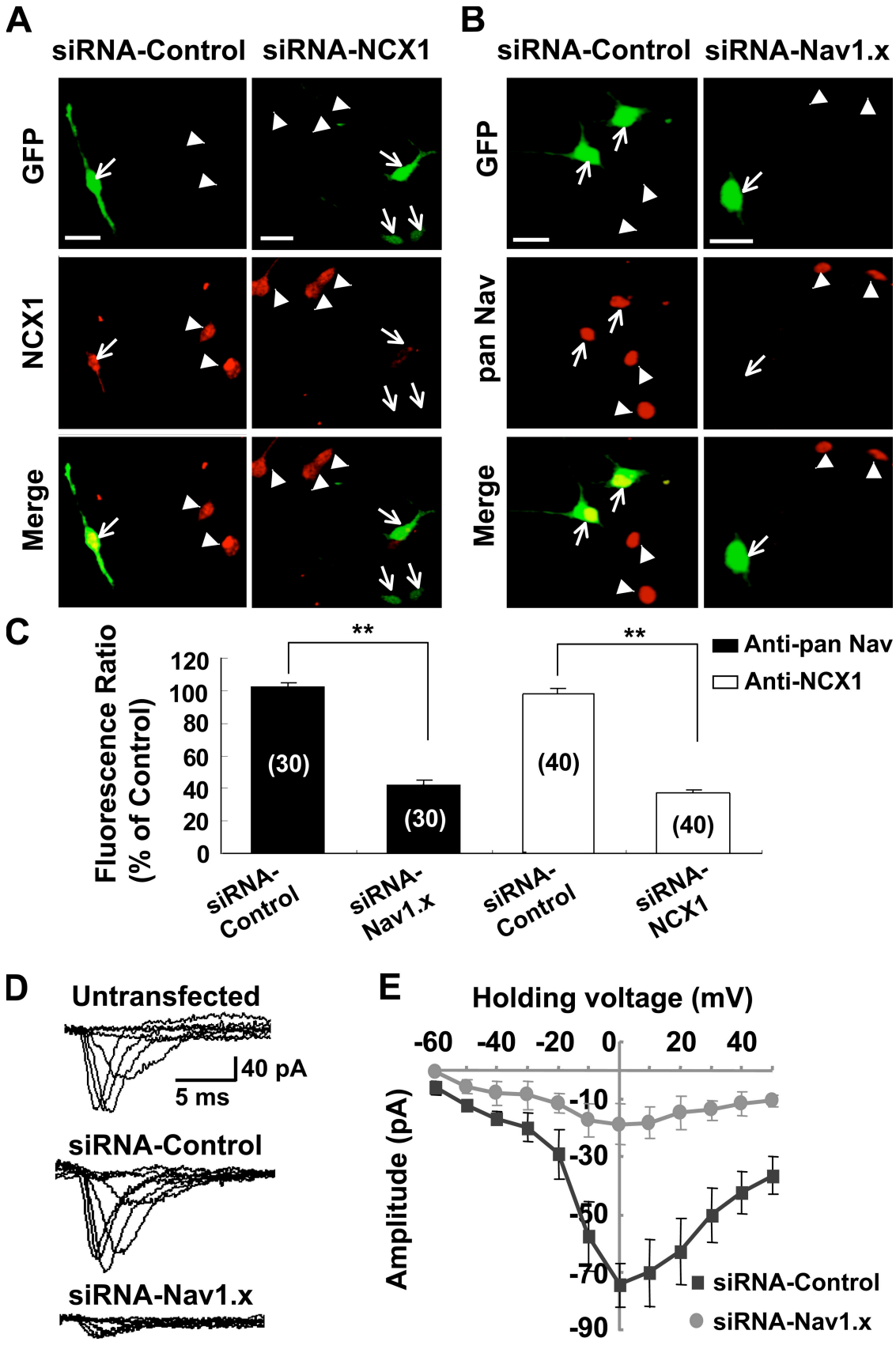

A
siRNA-Control
siRNA-NCX1
GFP
NCX1
Merge
B
siRNA-Control
siRNA-Nav1.x
GFP
pan Nav
Merge
C
Fluorescence Ratio (% of Control)
120
100
80
60
40
20
0
**
**
(30)
(30)
(40)
(40)
Anti-pan Nav
Anti-NCX1
siRNA-Control
siRNA-Nav1.x
siRNA-Control
siRNA-NCX1
D
Untransfected
40 pA
5 ms
siRNA-Control
siRNA-Nav1.x
E
Holding voltage (mV)
-60
-40
-20
0
20
40
Amplitude (pA)
-10
-30
-50
-70
-90
siRNA-Control
siRNA-Nav1.x

Figure S4. **siRNA knockdown of NCX1 and Na^+ channels in cultured NG2 cells.** (A) Confocal images showing cotransfection of GFP (green) + scrambled siRNA (left) or GFP + siRNA-NCX1 (right) in cultured NG2 cells. Immunostaining with anti-NCX1 (red) showed a significant decrease of NCX1 expression in cells transfected with siRNA-NCX1 (right, arrows), but not in cells transfected with scrambled siRNA (left, arrows) as compared with nontransfected cells (arrowheads). Bars, 20 µm. (B) Confocal images showing cotransfection of GFP (green) + scrambled siRNA (siRNA-Control; left) or GFP + siRNA- Nav1.x (right) in cultured NG2 cells. Immunostaining with pan Nav antibody (red) showed significant decrease of Nav1.x expression in cells transfected with siRNA- Nav1.x (right, arrows), but not in cells transfected with scrambled siRNA (left, arrows) as compared with nontransfected cells (arrowheads). Bars, 20 µm. (C) Percentage of fluorescence intensity of pan Nav or NCX1 immunostaining signaling in GFP-positive cells, as compared with the corresponding immunostaining signaling in nontransfected cells. The number associated with each column refers to the number of cells examined in each group. **, $P < 0.01$ compared with each scrambled siRNA group. (D) Example traces of transient Na^+ currents recorded from cultured NG2 cells untransfected (top) or transfected with GFP + scrambled siRNA (middle) and GFP + siRNA-Nav1.x (bottom) in response to voltage steps (100 ms, 10 mV increment) from – 60 to +50 mV, with a 300-ms prepulse of −110 mV. (E) Current–voltage (I-V) plots of Na^+ currents in cultured NG2 cells transfected with scrambled siRNA or with siRNA-Nav1.x, evoked by depolarizing voltage steps as shown in D. $n = 7$ for each group. Error bars represent mean ± SEM.

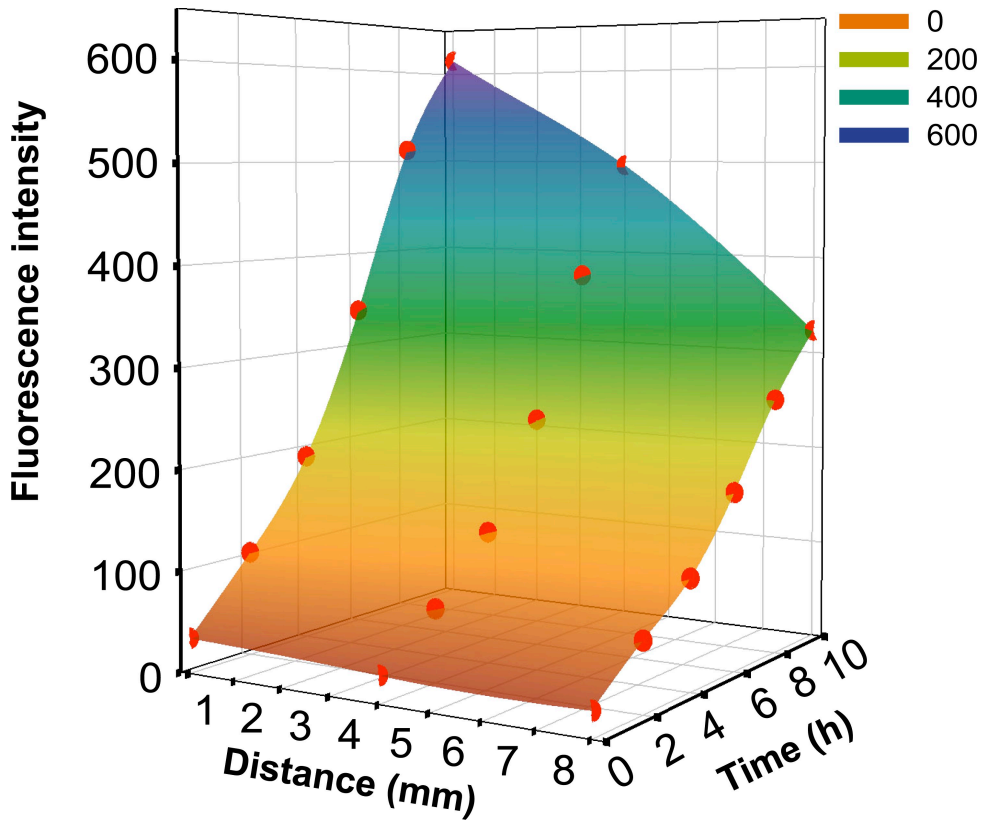
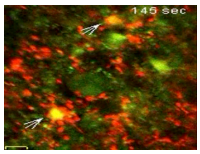
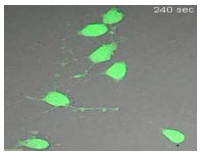


Figure S5. **Diffusion gradient in the medium surrounding the agarose block.** Diffusion gradient in the medium along the distance from the edge of agarose block, as assayed by fluorescence imaging of the Alexa Fluor 488 signal during different times after adding Alexa Fluor 488 (50 µl, 5 µM) to the small well of the agarose block (see also Fig. 9 A). The fluorescence intensity (y axis) decreased with distance (x axis) from the edge of the agar block, and increased with time (z axis) after application of the dye. Each data point (red circles) was averaged from three measurements.



Video 1. **GABA-induced $[Ca^{2+}]_i$ elevation in NG2 cells in hippocampal slices.** The slice was live stained with anti-NG2 antibody (red) and loaded with Ca^{2+} dye Fluo-4 AM (green). 1 mM GABA induced apparent $[Ca^{2+}]_i$ elevation in typical NG2 cells (indicated by arrows). Images were taken from the same slice shown at Fig. 3 B. 500 µM kynurenic acid was added to block potential secondary activation of glutamate receptors. Bar, 10 µm.



Video 2. **GABA-induced $[Ca^{2+}]_i$ elevation in cultured NG2 cells.** Fluorescent imaging of purified NG2 cells that were loaded with the Ca^{2+} dye Fluo-4 AM and perfused with 1 mM GABA. The first and the last image in the video were superimposed with the phase contrast picture. Bar, 10 µm.