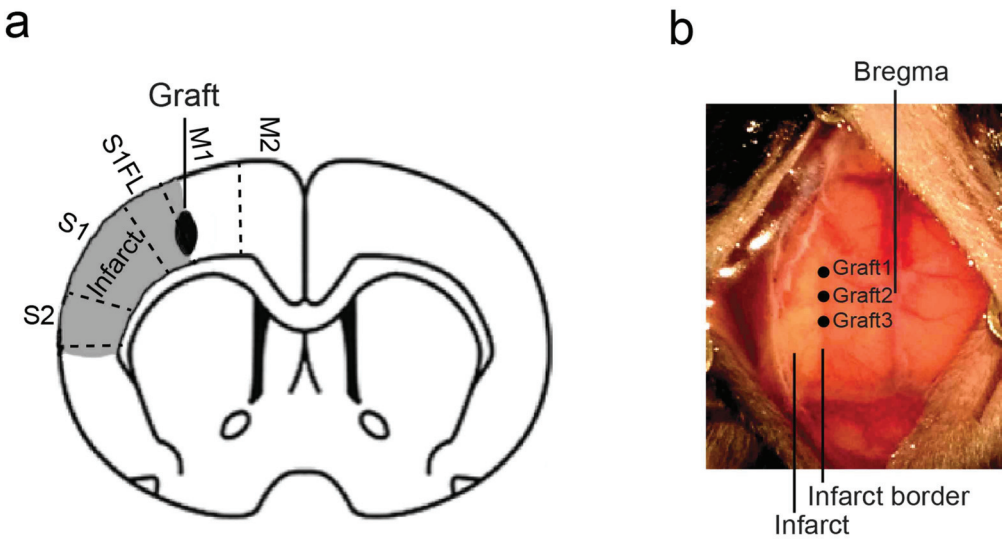
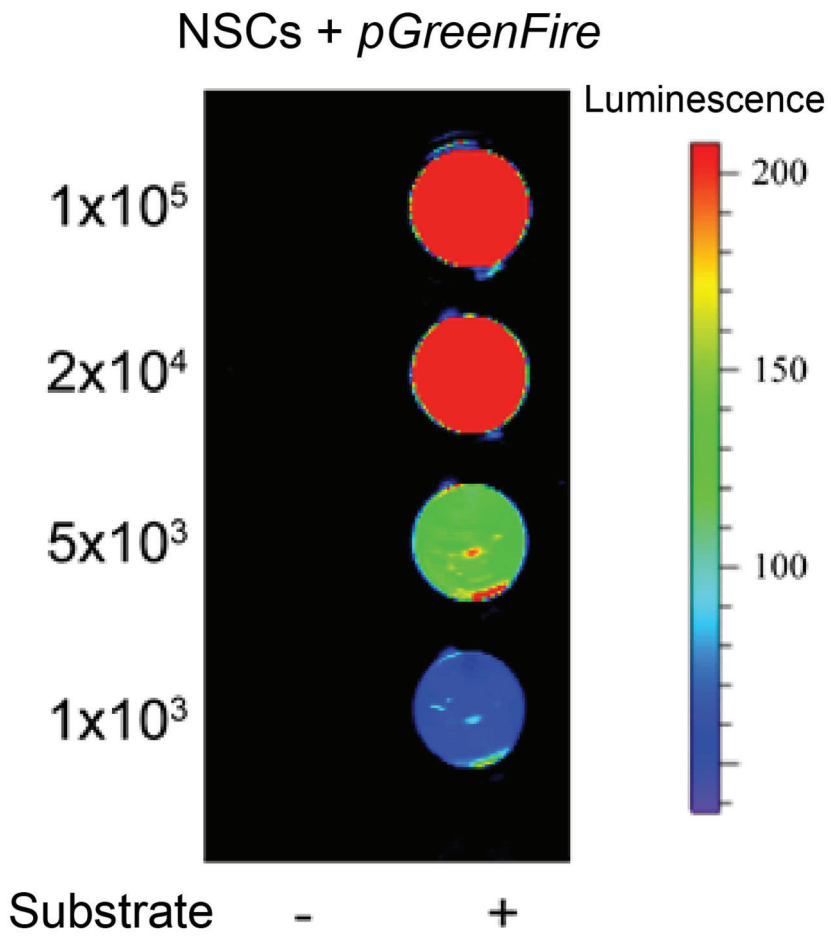
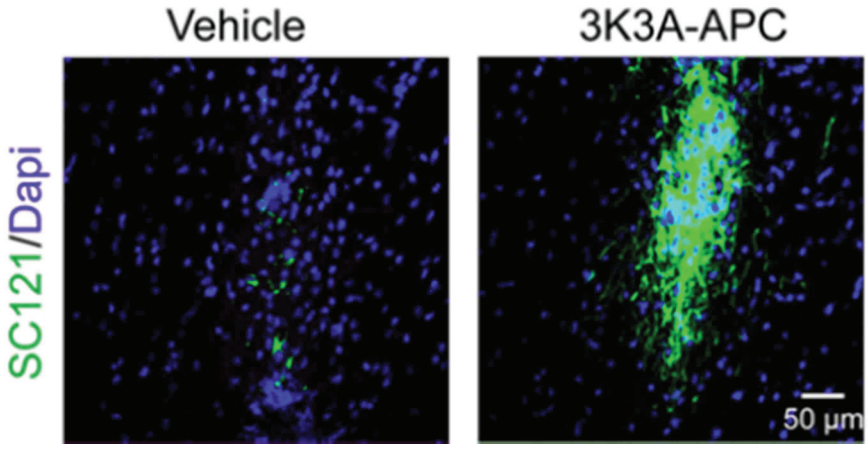
SUPPLEMENTARY INFORMATION



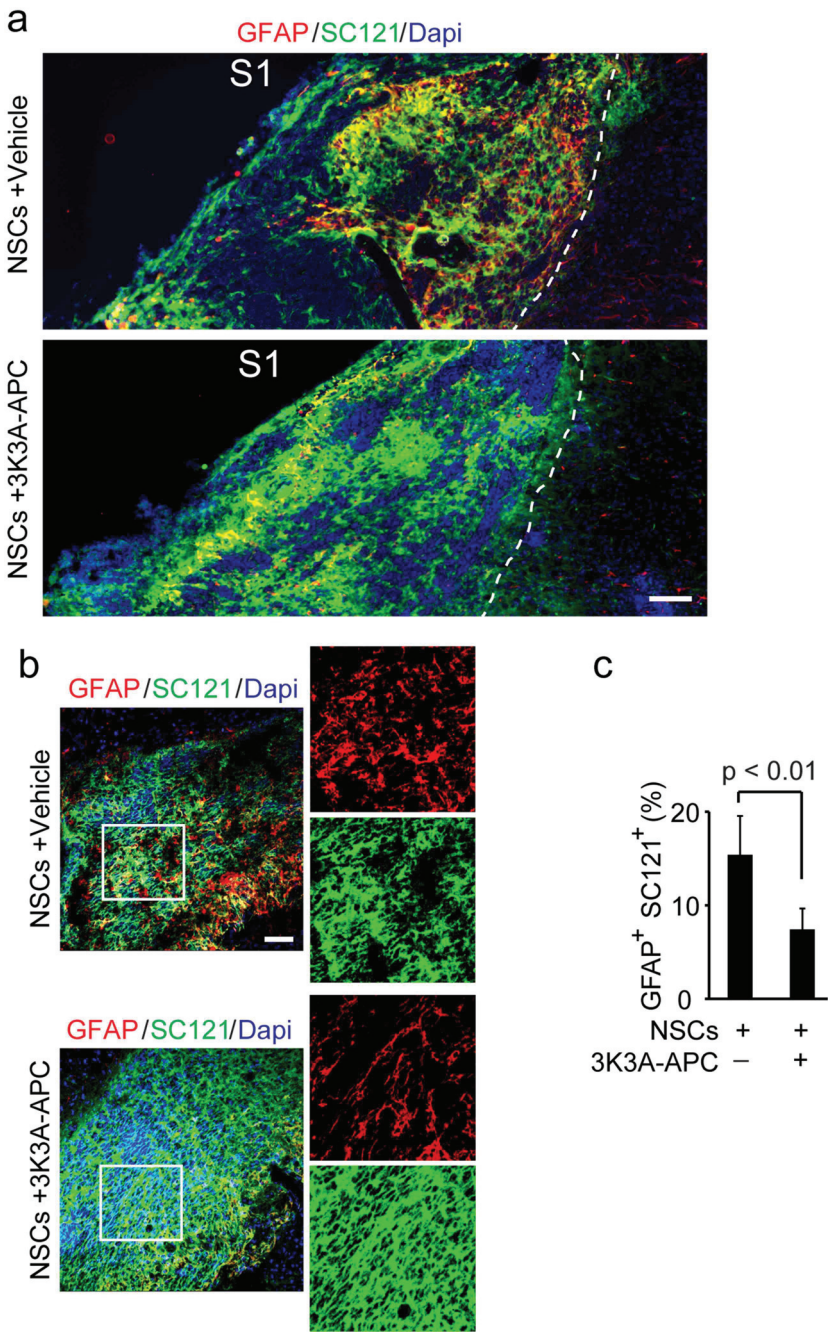
Supplementary Figure 1: The location of the transplanted NSCs. (**a**) Schematic location of the graft at a coronal section at the level of optic chiasm showing the distribution of ischemic infarct 7 d after dMCAo. M1, primary motor cortex; M2, secondary motor cortex; S1, primary somatosensory cortex; S1FL, primary somatosensory cortex forelimb region; S2, secondary somatosensory cortex. (**b**) Grafting points over the skull.



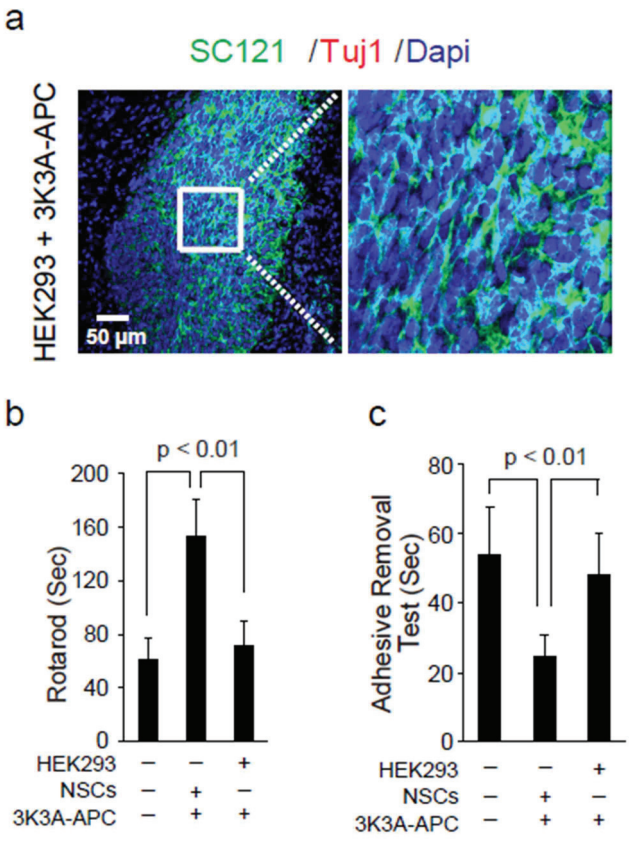
Supplementary Figure 2: Lentiviral transduction of NSCs. NSCs were transduced with *pGreenFire* lentivirus to express GFP and Luciferase. The cells were seeded in the 96 well plates at different density (number per well as indicated), and imaged with or without substrate. The luminescence intensity correlates with the number of NSCs seeded.



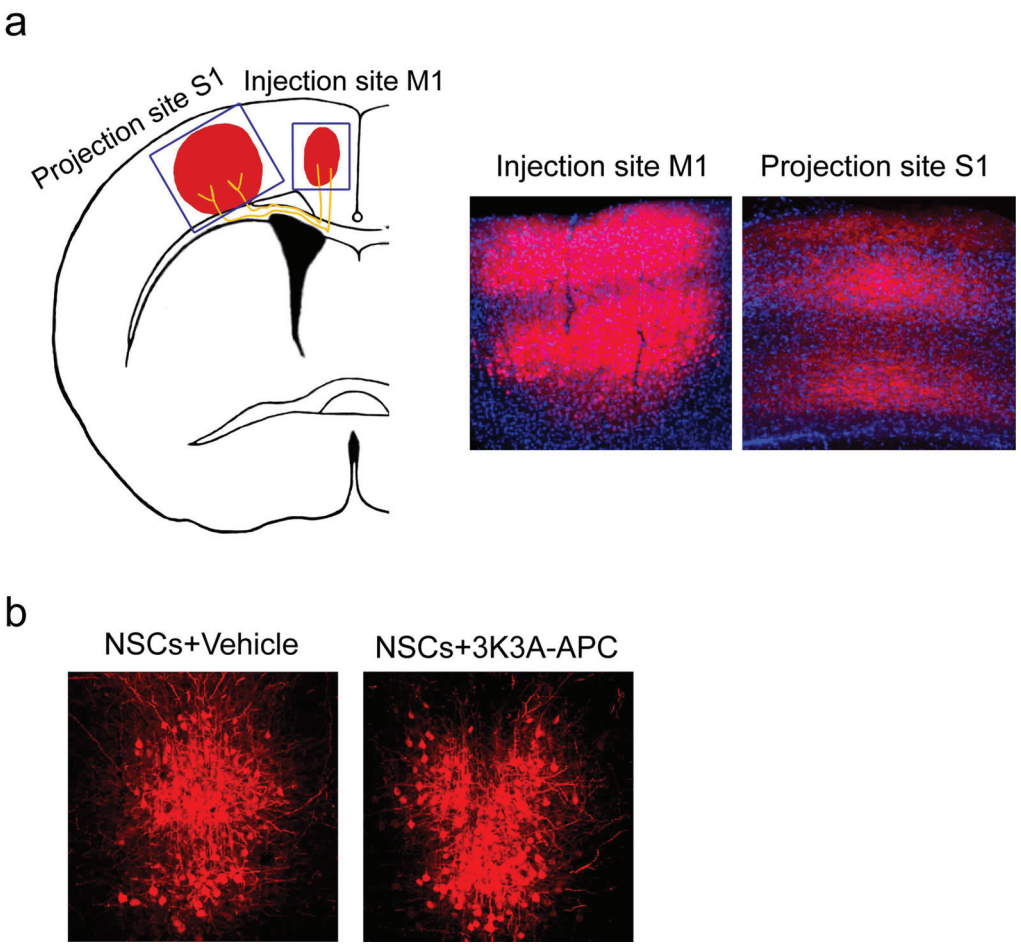
Supplementary Figure 3: Intracerebral transplantation of NSCs in control mice. Confocal images of human NSCs transplanted into the mouse cortex and detected with human-specific SC121 antibody (green) 1 week after transplantation.



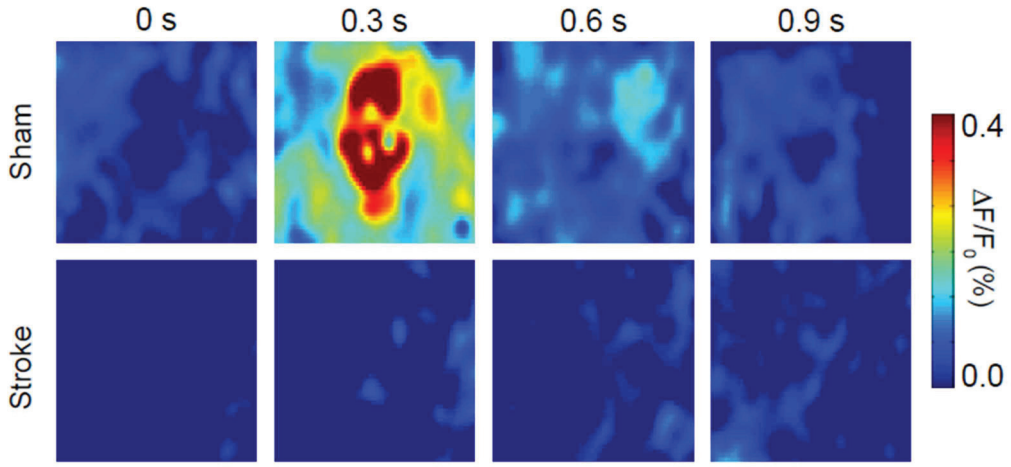
Supplementary Figure 4: Inhibition of astroglial differentiation from NSCs by 3K3A-APC 35 d after dMCAo. (**a-c**) 3K3A-APC suppresses astroglial differentiation of transplanted NSCs. Representative images of dual immunostaining for SC121 (green) and GFAP (red) at low magnification (**a**) and high magnification in stroke damaged S1 region (**b**), and quantification of $GFAP^+$ $SC121^+$ double positive cells expressed as the percentage of all positive $SC121^+$ cells (**c**). Mean ± s.d., n = 5 mice per group; statistical significance by Student's-t test.



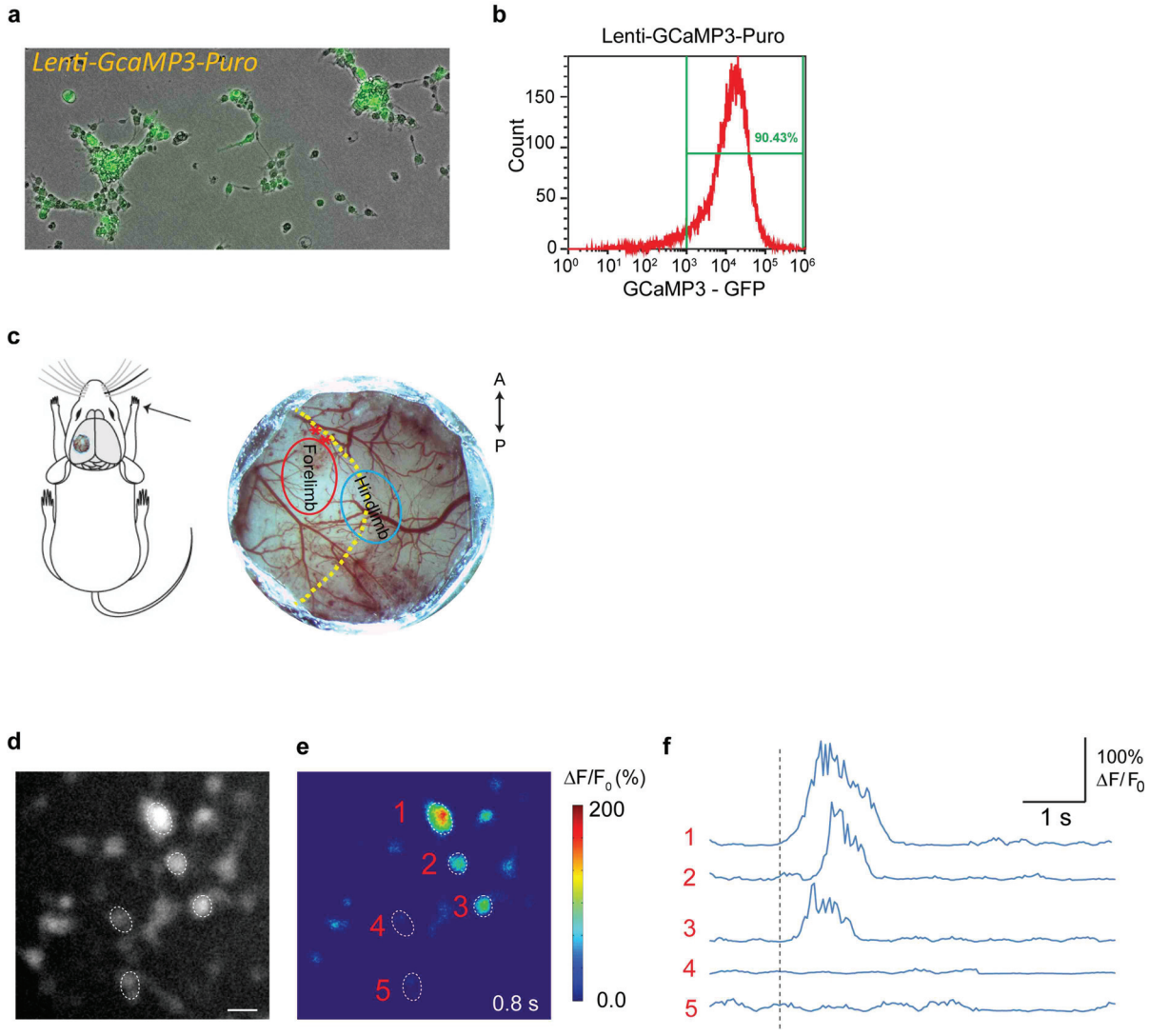
Supplementary Figure 5: Combined treatment of HEK293 transplantation and 3K3A-APC after stroke in mice. HEK293 cells were transplanted 7 d after dMCAo as in **Supplementary Fig. 1** and **Fig. 1a-b**. 3K3A-APC (0.2 mg/kg i.v.) or vehicle was administered 7, 9, 11 and 13 d after stroke. (**a**) Representative fluorescent confocal images of brain sections of the ischemic region 5 weeks after stroke showing immunostaining for SC121 (green), negative immunostaining for Tuj1, a neuronal marker (red), and Dapi (blue). (**b-c**) Behavioral analysis with the rotarod test (**b**) and adhesive removal test (**c**) were performed 5 weeks after stroke and compared with functional outcome of NSCs plus 3K3A-APC treatment. Mean ± s.d., n = 5 mice per group. Statistical significance by one-way ANOVA followed by Bonferroni's post hoc test.



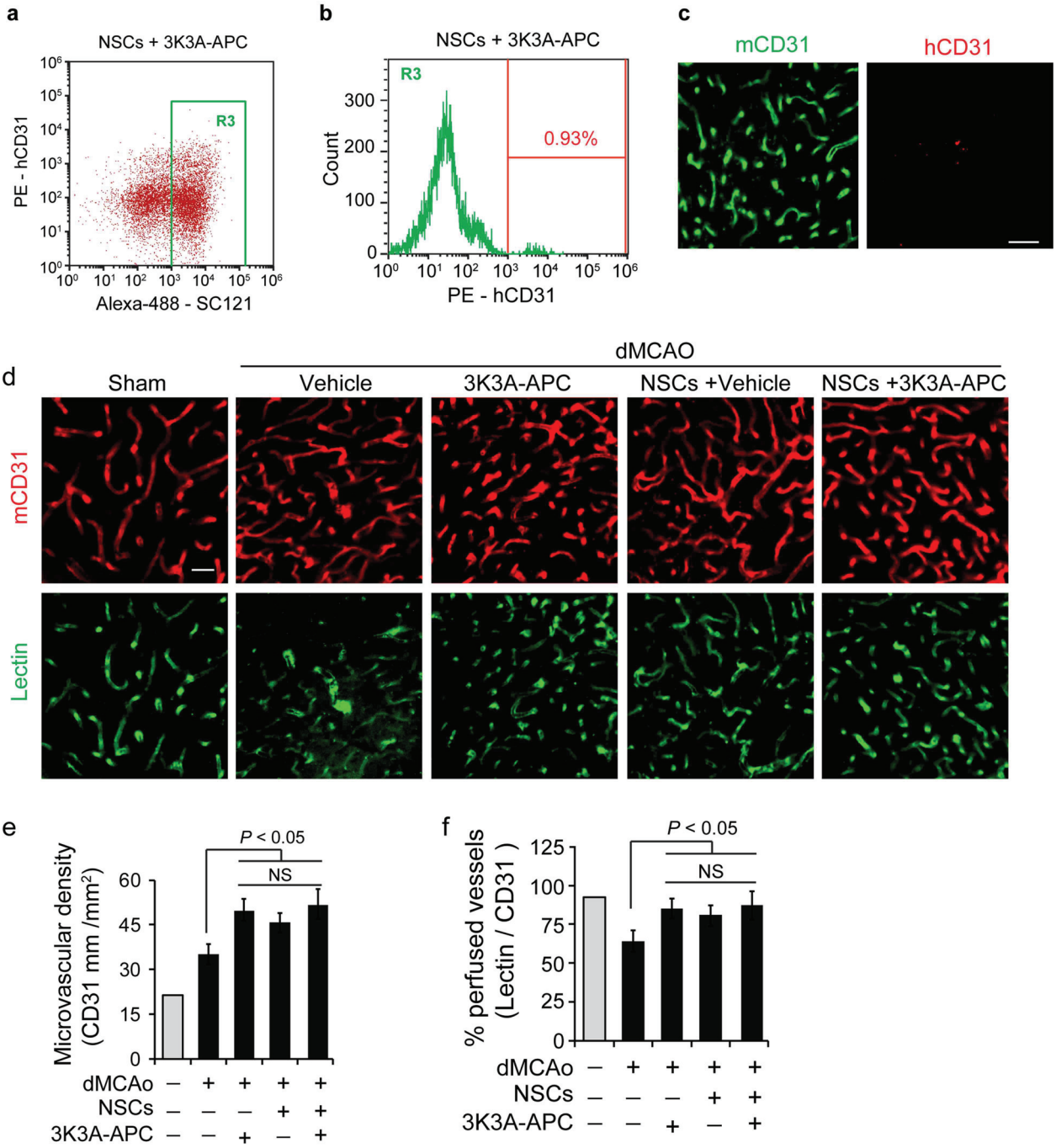
Supplementary Figure 6: Adeno-associated viral transduction of cortical neurons to label neural circuits. (**a**) A diagram showing the projections from the primary motor cortex (M1) to the adjacent somatosensory area (S1), and viral injection site in the motor cortex on coronal sections of the mouse brain (*left*). Representative images of the viral labeling in the injection site (M1) and projection site (S1) (*right*). (**b**) Representative images showing a comparable viral labeling in the injection sites with NSCs plus vehicle and NSCs plus 3K3A-APC in the experiment shown in **Figure 3 a-c**.



Supplementary Figure 7: Voltage-sensitive dye imaging (VSD). Representative VSD pseudo-colored image sequence of neuronal activity in the somatosensory region in response to stimulus 35 d after stroke (dMCAo) and sham-operated group mice.



Supplementary Figure 8: Stable transduction of NSCs with *Lenti-GCaMP3-Puro* viruses and *in vivo* two-photon imaging of GCaMP3-based responses. NSCs were transduced with *Lenti-GCaMP3-Puro* lentiviruses and selected with puromycin (see supplementary methods) for stable expression of GCaMP3 Calcium indicator. (**a**) Representative image showing the majority NSCs express GCaMP3 (green). (**b**) Flow cytometry analysis of transduced NSCs 3 d after puromycin selection showing that > 90% of cells are positive for GCaMP3. (**c**) Diagram showing the position of cranial window and forelimb stimulation (arrow), and a top view of the cranial window showing the ischemic border, injection sites for transplantation, and sensory cortical regions responding to forelimb or hindlimb stimulation. A-P: anterior – posterior. (**d**) Representative *in vivo* two-photon imaging of individual responses of GCaMP3-NSCs in the cortical layers II-III to forelimb stimulus 35 d after dMCAo in mice treated with NSCs plus 3K3A-APC. Bar = 20 µm. (**e**) Pseudo-colored frames from images shown in **d** demonstrating evoked calcium responses based on GCaMP3 fluorescence change (ΔF / F) in individual GCaMP3-NSCs after forelimb stimulation. (**f**) Representative averaged traces of GCaMP3 responses in the selected 5 NSCs shown in **d-e**. Dash line indicates the onset of forelimb stimulation (see supplementary methods).



Supplementary Figure 9: Neovascularization after stroke. (**a**) Multicolor flow cytometry analysis of cell populations in the grafted cortical region of mice treated with NSCs plus 3K3A-APC showing SC121 human antigen-positive NSCs 5 weeks after transplantation (ROI: R3), but minimal expression of human endothelial cell marker CD31 (hCD31), using a specific human CD31 monoclonal antibody (Clone: WM59). (**b**) Multicolor flow cytometry analysis of cell populations in the grafted cortical region of mice treated with NSCs plus 3K3A-APC showing that < 1% of GCaMP3-expressing NSCs express human endothelial marker CD31 in (R3), as

shown in **a**. (**c**) Representative confocal images showing minimal hCD31 immunoreactivity in murine CD31-positive microvessels (mCD31) in the ischemic brain region with NSCs transplantation. Immunostaining was performed using murine-specific CD31 antibody (mCD31 that does not cross-react with human CD31) and human-specific CD31 antibody (hCD31 with minimal cross-reactivity with murine CD31) (see supplementary methods for details). Bar= 20 μm. (**d-f**) Representative confocal images of murine CD31-positive microvessels (upper) and *in vivo* systemic FITC-lectin angiography (bottom) in the S1 cortex 35 d after dMCAo. (**e-f**) Quantification of microvascular density based on mCD31 immunostaining (**e**) and the percentage of perfused brain microvessels based on lectin signal over the mCD31 vascular profiles after *in vivo* lectin angiography (**f**) in sham-operated mice and dMCAo stroked mice treated with vehicle, 3K3A-APC alone, NSCs plus vehicle or NSCs plus 3K3A-APC. Mean ± s.e.m., n = 5 mice/group. $P < 0.05$, statistical significance by one-way ANOVA followed by Bonferroni's post hoc test. Bar = 20 μm.