Supporting information

Bioinspired Nanocomplex for Spatiotemporal Imaging of Sequential mRNA Expression In Differentiating Neural Stem Cells

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Supplementary experimental methods:

Texture feature analysis of gene expression heterogeneity obtained from dynamic imaging.

The spatial heterogeneity of the images was analyzed using co-occurrence matrix based on conditional probability density function P(i, j; a, d). The value of ith, jth element in the co-occurrence matrix is the probability that intensity levels i and j occur in two voxels by distance (d) and direction (a) in the images. Here we defined d as one pixel size and used 26-connection in 3D space. The intensity level of i and j is 16 and the pixels of images are 512×512. The P(i, j; a, d) was calculated using reference [*].Co-occurrence feature of local homogeneity (LH) was deduced by [**].

*R. Haralick et al. Texture features for image classification, IEEE Trans. Syst. Man Cybern. S MC 3 (1973) 610 – 621

**I. El Naqa et al. / Pattern Recognition 4 2 (2009) 1162 – 1171

Program Code:

```
%-- read the 3D image to 3D matrix
Aimage=zeros(512,512,71);
for i=1:71
   if(i<10) %i=1,2,3...,9
      image_name=['day 8-1 z stack_Z00' num2str(i) '.jpg'];
   else
      image_name=['day 8-1 z stack_Z0' num2str(i) '.jpg'];
   end
   image_tmp=imread(image_name);
   image_gray=rgb2gray(image_tmp);
   Aimage(:,:,i)=im2double(image_gray(1:2:1024, 1:2:1024));
end


%-- load the 3D image to 3D matrix
```

```
Y=Aimage;
clear Aimage
Ymin=min(min(min(Y)));
Ymax=max(max(max(Y)));
X1_16=(floor((Y-Ymin)*15./(Ymax-Ymin)+1.5));

%-- read the 3D image to 3D matrix
Pall=zeros(16,16);
for i=2:511
   for j=2:511
      for k=2:70
         %--- top layer 9 pixels
         Pall(X1_16(i,j,k),X1_16(i-1,j-1,k+1))=Pall(X1_16(i,j,k),X1_16(i-1,j-1,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i-1,j,k+1))=Pall(X1_16(i,j,k),X1_16(i-1,j,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i-1,j+1,k+1))=Pall(X1_16(i,j,k),X1_16(i-1,j+1,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i,j-1,k+1))=Pall(X1_16(i,j,k),X1_16(i,j-1,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i,j,k+1))=Pall(X1_16(i,j,k),X1_16(i,j,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i,j+1,k+1))=Pall(X1_16(i,j,k),X1_16(i,j+1,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i+1,j-1,k+1))=Pall(X1_16(i,j,k),X1_16(i+1,j-1,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i+1,j,k+1))=Pall(X1_16(i,j,k),X1_16(i+1,j,k+1))+1;
         Pall(X1_16(i,j,k),X1_16(i+1,j+1,k+1))=Pall(X1_16(i,j,k),X1_16(i+1,j+1,k+1))+1;
         %---

         %--- middle 8 pixels
```

```
        Pall(X1_16(i,j,k),X1_16(i-1,j-1,k))=Pall(X1_16(i,j,k),X1_16(i-1,j-1,k))+1;
        Pall(X1_16(i,j,k),X1_16(i-1,j,k))=Pall(X1_16(i,j,k),X1_16(i-1,j,k))+1;
        Pall(X1_16(i,j,k),X1_16(i-1,j+1,k))=Pall(X1_16(i,j,k),X1_16(i-1,j+1,k))+1;
        Pall(X1_16(i,j,k),X1_16(i,j-1,k))=Pall(X1_16(i,j,k),X1_16(i,j-1,k))+1;
        Pall(X1_16(i,j,k),X1_16(i,j+1,k))=Pall(X1_16(i,j,k),X1_16(i,j+1,k))+1;
        Pall(X1_16(i,j,k),X1_16(i+1,j-1,k))=Pall(X1_16(i,j,k),X1_16(i+1,j-1,k))+1;
        Pall(X1_16(i,j,k),X1_16(i+1,j,k))=Pall(X1_16(i,j,k),X1_16(i+1,j,k))+1;
        Pall(X1_16(i,j,k),X1_16(i+1,j+1,k))=Pall(X1_16(i,j,k),X1_16(i+1,j+1,k))+1;
        %---

        %--- bottom layer 9 pixels
        Pall(X1_16(i,j,k),X1_16(i-1,j-1,k-1))=Pall(X1_16(i,j,k),X1_16(i-1,j-1,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i-1,j,k-1))=Pall(X1_16(i,j,k),X1_16(i-1,j,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i-1,j+1,k-1))=Pall(X1_16(i,j,k),X1_16(i-1,j+1,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i,j-1,k-1))=Pall(X1_16(i,j,k),X1_16(i,j-1,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i,j,k-1))=Pall(X1_16(i,j,k),X1_16(i,j,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i,j+1,k-1))=Pall(X1_16(i,j,k),X1_16(i,j+1,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i+1,j-1,k-1))=Pall(X1_16(i,j,k),X1_16(i+1,j-1,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i+1,j,k-1))=Pall(X1_16(i,j,k),X1_16(i+1,j,k-1))+1;
        Pall(X1_16(i,j,k),X1_16(i+1,j+1,k-1))=Pall(X1_16(i,j,k),X1_16(i+1,j+1,k-1))+1;
      end
    end
end
```

```
Pall=Pall./sum(sum(Pall));
figure;
 surf(Pall);
 axis([0,16, 0,16, 0,0.25]);
 colorbar;
 xlabel('i-level'); ylabel('j-level'); zlabel('Co-occurrence probility');

%--local homogeneity
PLH=0;
for i=1:16
    for j=1:16
        PLH=PLH+Pall(i,j)./(1+(i-j).^2);
    end
end
PLH
```

Table S1. Gene sequences used in this study

Names of DNA	Sequences
Tubb3 recognizing sequence	5'-NH_2-mUmCmC mAAG TCC ACC AGA ATmG mGmCmC AAA AAA-dithiol-3'
Tubb3 Reporter	5'-Alexa488-TGG CCA TTC TGG-3'
Tubb3 DNA target	5'-GGC CAT TCT GGT GGA CTT GGA-3'
Tubb3 DNA target with one mismatch	5'-GGC CAT TCT G*C*T GGA CTT GGA-3'
Fox3 recognizing sequence	5'-NH2-mCmAmU mUTT AAC AAG CGT TTmG mCmUmC AAA AAA-dithiol-3'
Fox3 reporter	5'-Cy3-TGA GCA AAC GCT-3'
Fox3 DNA target	5'-GAG CAA ACG CTT GTT AAA ATG-3'
Fox3 DNA target with one mismatch	5'-GAG CAA ACG C*A*T GTT AAA ATG-3'
Survivin	5'-CAAGGAGCTGGAAGGCTG-3'
β-actin	5'-GCTACAGCTTCACCACCACAG-3'
Nestin	5'-GTCTCAGGACAGTGCTGAGCCTTC-3'
GAPDH	5'-GGTCTCCTCTGACTTCAACA-3'
CXCR4	5'-CGGCAGCAGGTAGCAAAGTGAC-3'

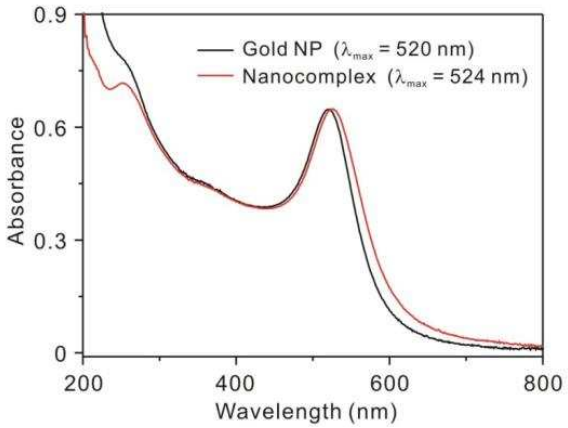

Figure S1. UV-Vis spectra of pure gold nanoparticles (black) and multifunctional nanocomplex (red).

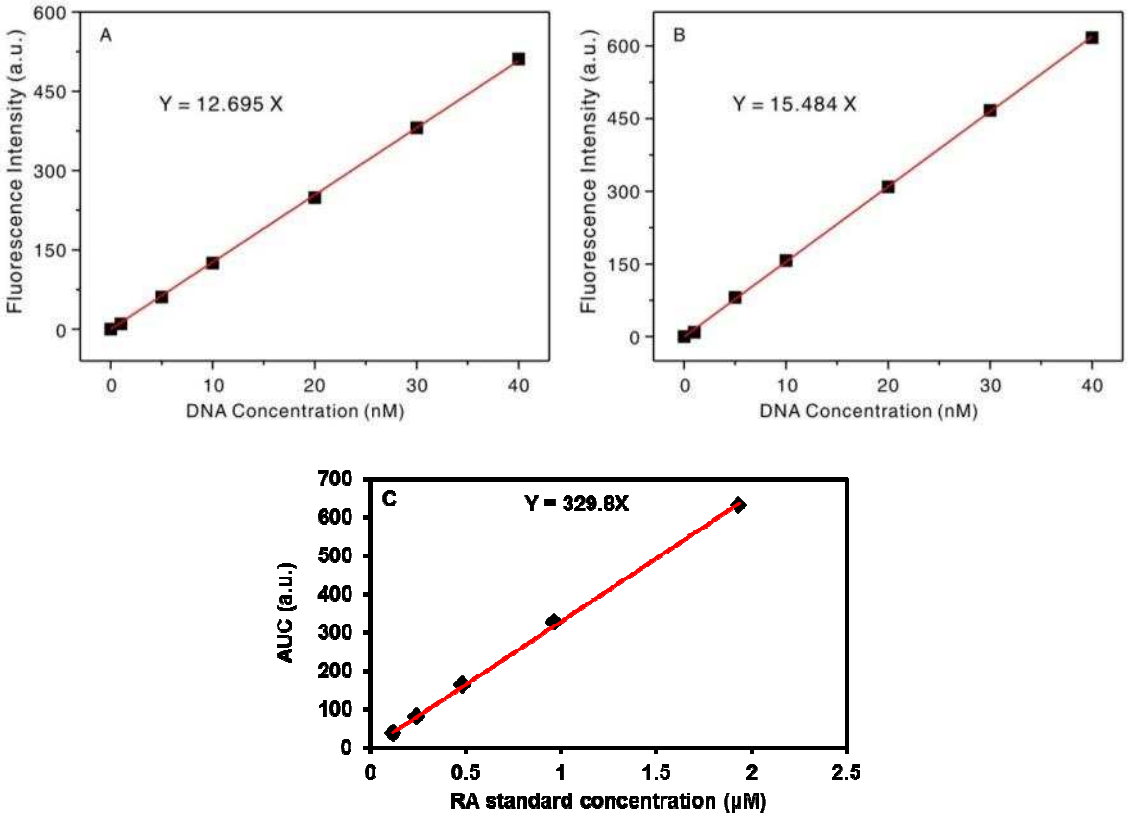

Figure S2. Calibration curves (A) Plot of fluorescence intensity as the function of DNA concentration of Alexa 488-modified *Tubb3* oligo. (B) Plot of fluorescence intensity as the function of DNA concentration of Alexa 488-modified *Tubb3* oligo of Cy3-modified *Fox3* oligo. (C) Calibration curve of area under curve values from HPLC analysis to retinoic acid standard concentration.

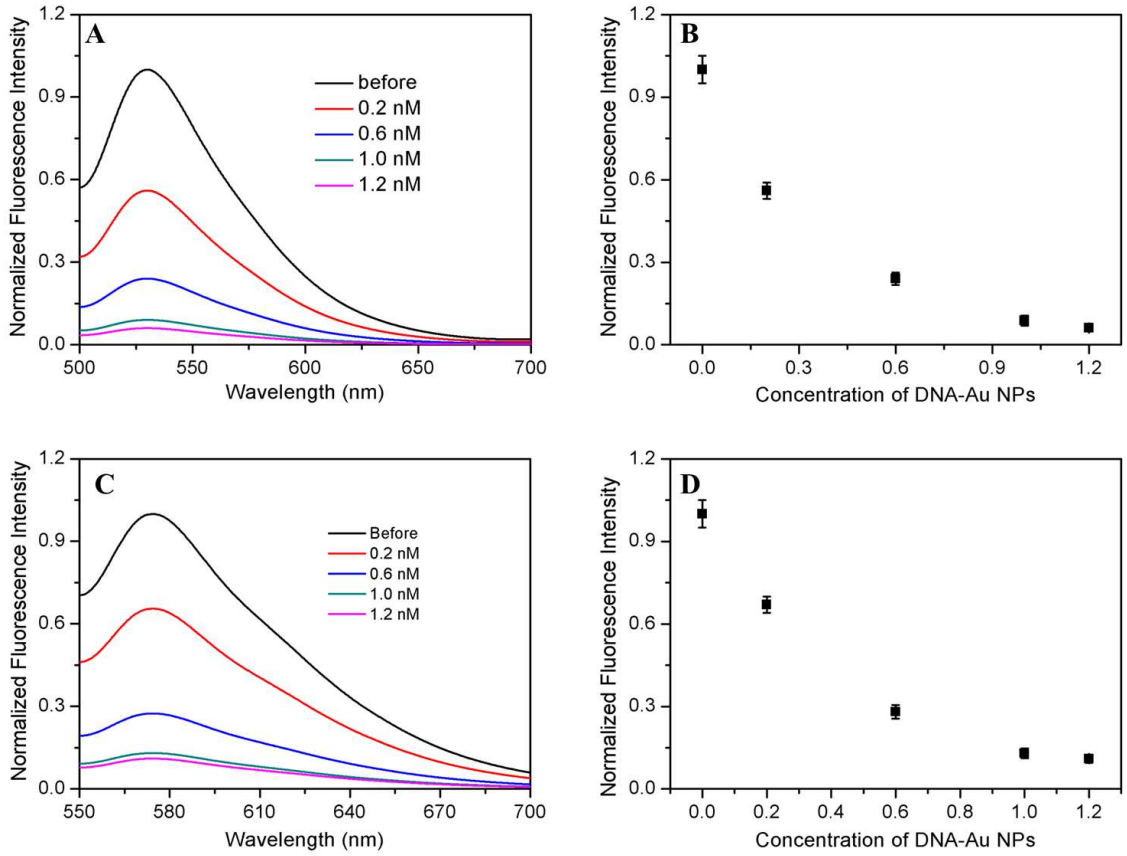

Figure S3. Quenching effects of AuNP to fluorophores. (A) Fluorescence spectrum of Alexa 488 linked oligo reporter (*Tubb3*) in hybridization with corresponding recognizing sequence modified AuNP. (B) The peak fluorescence (wavelength=520nm) decay of Alexa 488 caused by AuNP quenching effect. (C) Fluorescence spectrum of Cy3 linked oligo reporter (*Fox3*) in hybridization with corresponding recognizing sequence modified AuNP. (D) The peak fluorescence (wavelength=570nm) decay of Cy3 caused by AuNP quenching effect. (n=3)

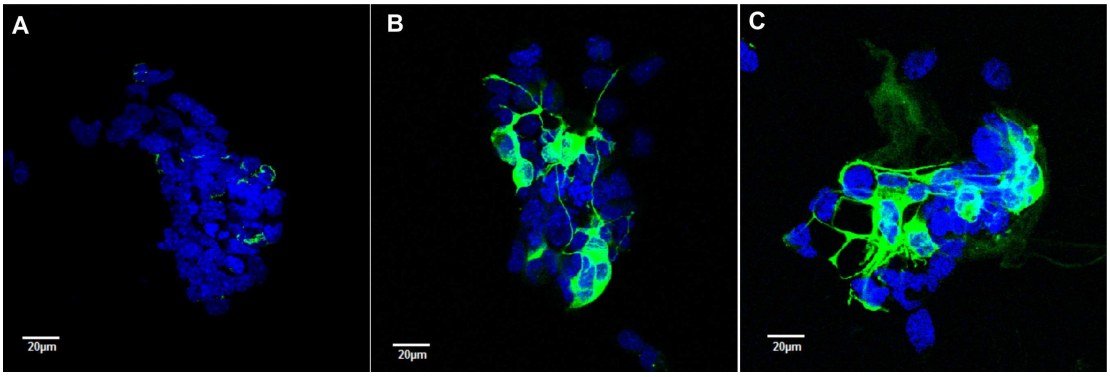

Figure S4. Immunofluorescence staining of βIII tubulin expression in differentiating neural stem cells at different treatment conditions over 8 days: (A) 15 nM RA dissolved in medium; (B) 1 μM RA dissolved in medium; (C) 15 nM RA attached on fNC. Scale = 20 μm. Blue: DAPI.

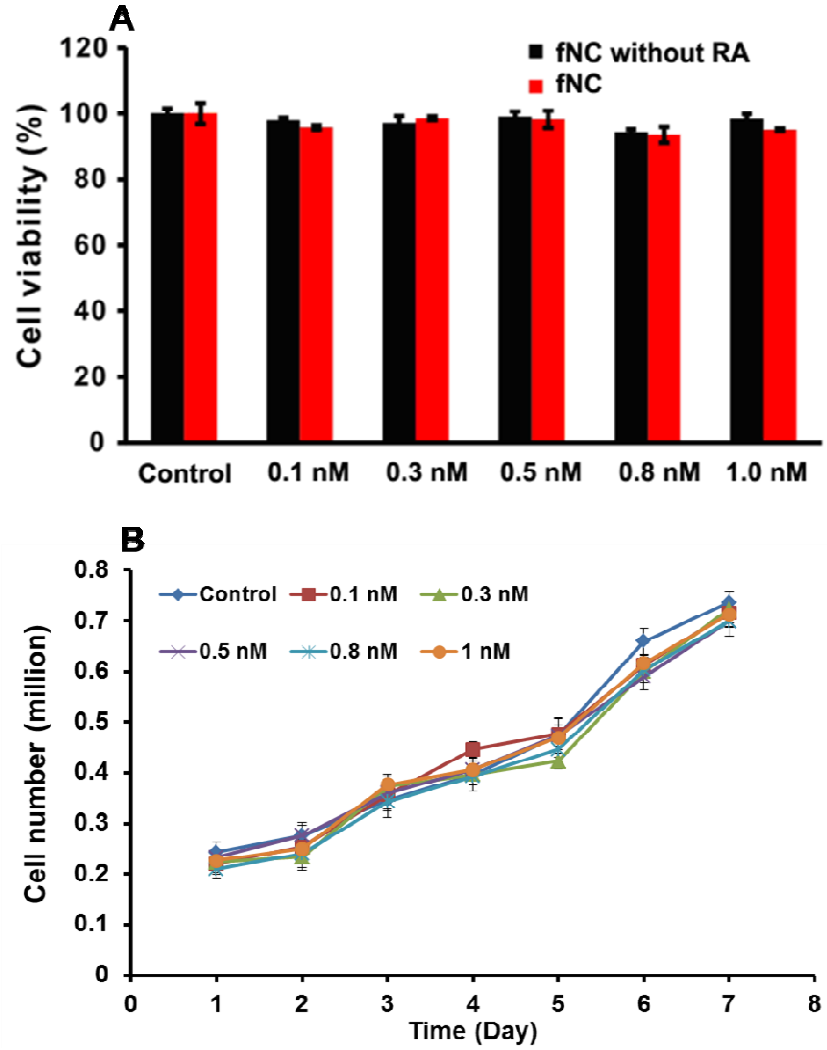

Figure S5. Cytotoxicity assays of fNC to neural stem cells. (A) MTT assay of fNC with and without RA from 0.1-1 nM after 48h incubation. (n=6) (B) Neural stem cell self-renewing rate in different concentrations of fNC without RA over 7 days. (n=3)

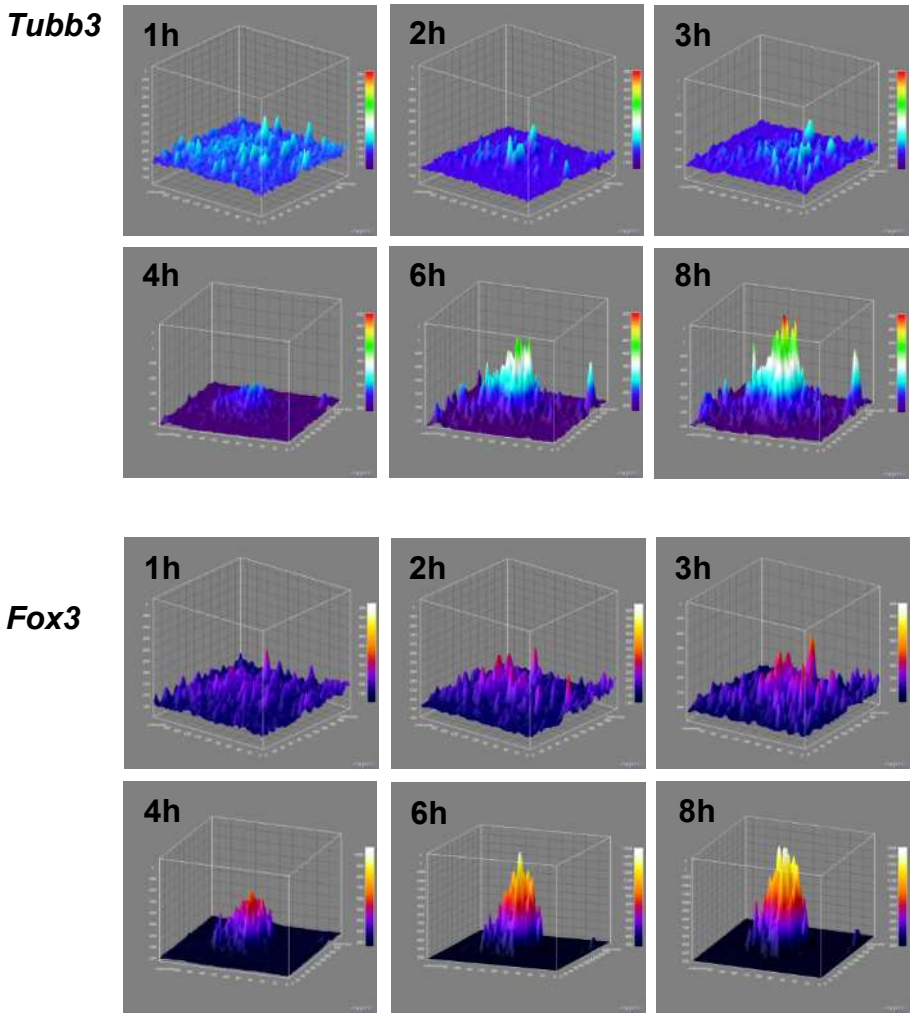

Figure S6. Hot map analysis of mRNA imaging results of differentiated NSCs at different time points on day 8. Top panel: *Tubb3*; bottom panel: *Fox3*.

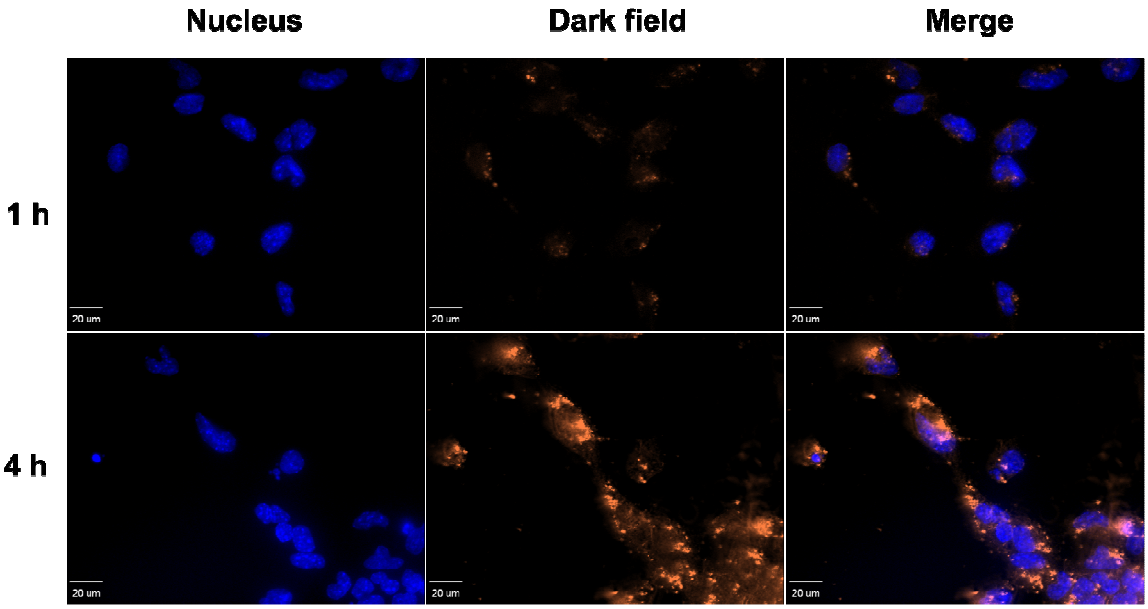

Figure S7. Dark field imaging of the cellular uptake of the nanocomplex after 1h and 4h incubation. Blue: Hoechst 33342. Scale bar = 20 μm

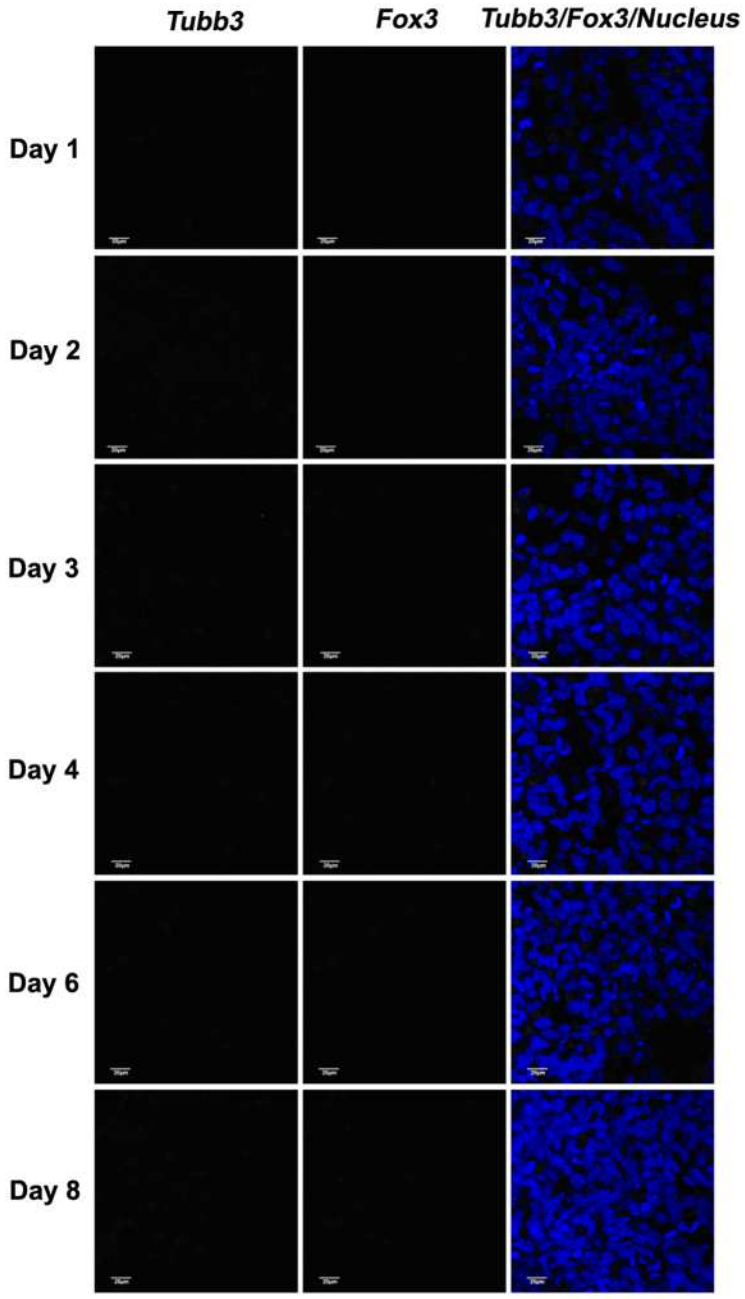

Figure S8. Negative control of fNC without RA motif for detection of *Tubb3* and *Fox3* mRNA expressions over 8 days. Scale = 20 μm. Blue: Hoechst 33342. The morphology of original epithelial shape of NSCs was not changed and minimal background noise, if any, could be detected for both mRNAs after 4 day incubation onwards.

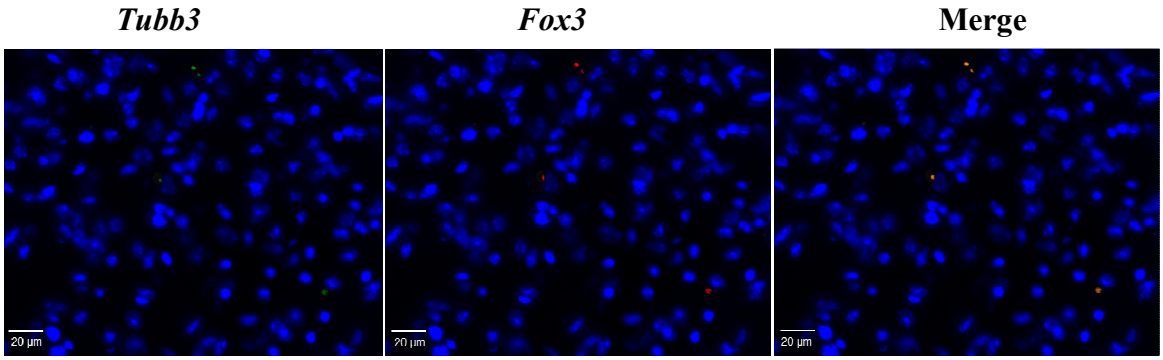

Figure S9. Fluorescence of brain tissues adjacent to lateral ventricle on day 8. Scale bar = 20 µm. Green: *Tubb3* mRNA imaging; Red: *Fox3* mRNA imaging; Yellow/orange: Merge.

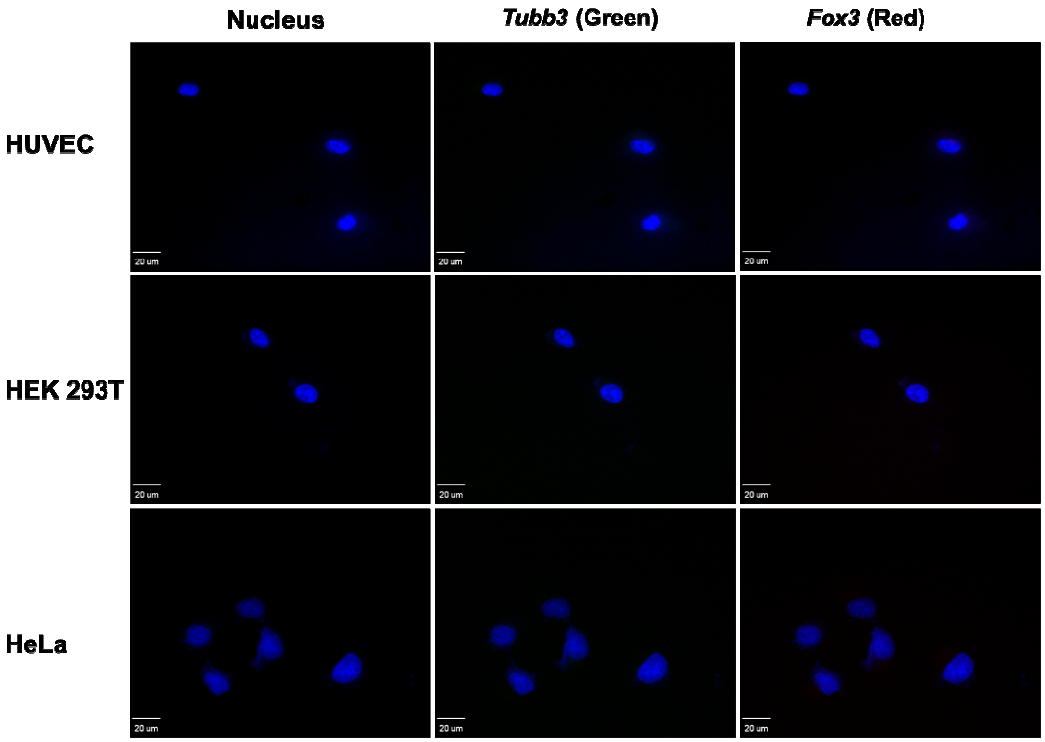

Figure S10. *In vitro* assay of off-target fluorescence of fNC in different cell types. Green: Alexa 488; Red: Cy3.