

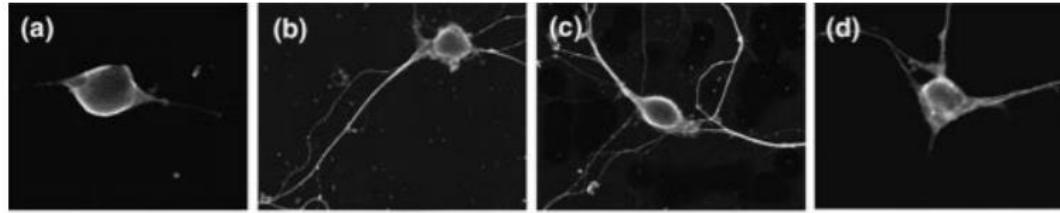
Integrative Nuclear FGFR1 Signaling (INFS) Overview

- FGF-2 and FGFR1 as nuclear proteins
- **Integrative Nuclear FGFR1 Signaling (INFS) in cell development**
- **Targeting nuclear FGFR1 signaling to control neuronogenesis.**
- Reconstitution of INFS in Cancer cells
- Nuclear FGFR1 as an essential gene activator
- INFS genome programming

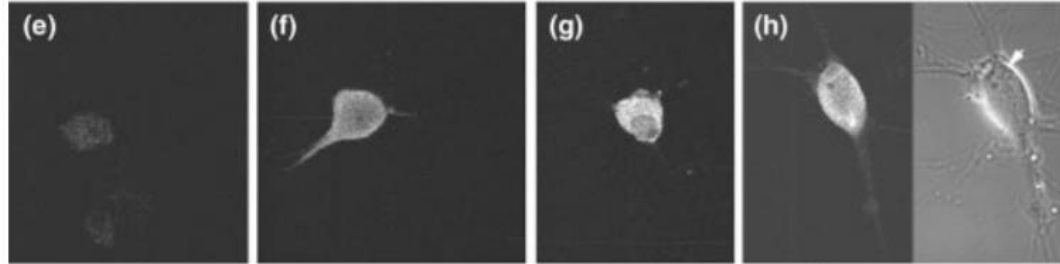
BMP7 directs postmitotic development of sympathetic neurons and activates INFS

In vitro
SCG

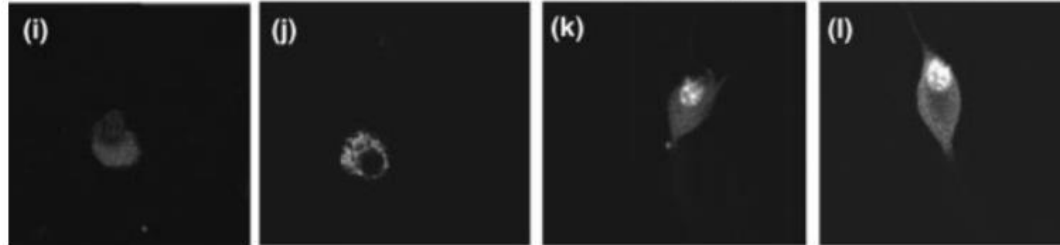
Neurite outgrowth



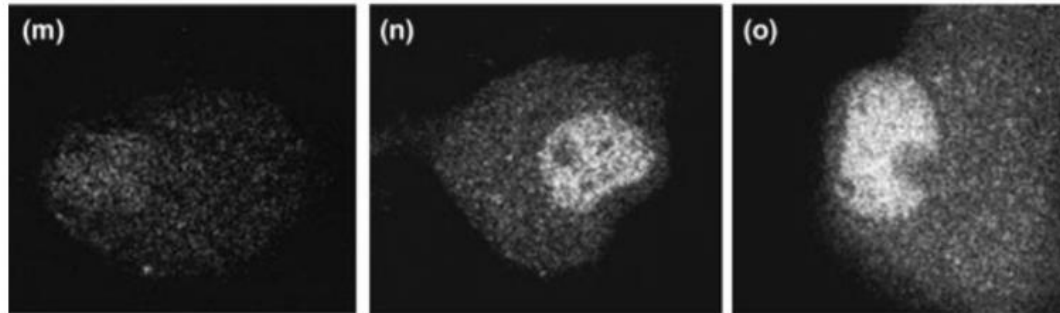
Upregulation of FGF-2



Nuclear accumulation of FGFR1



FGFR1



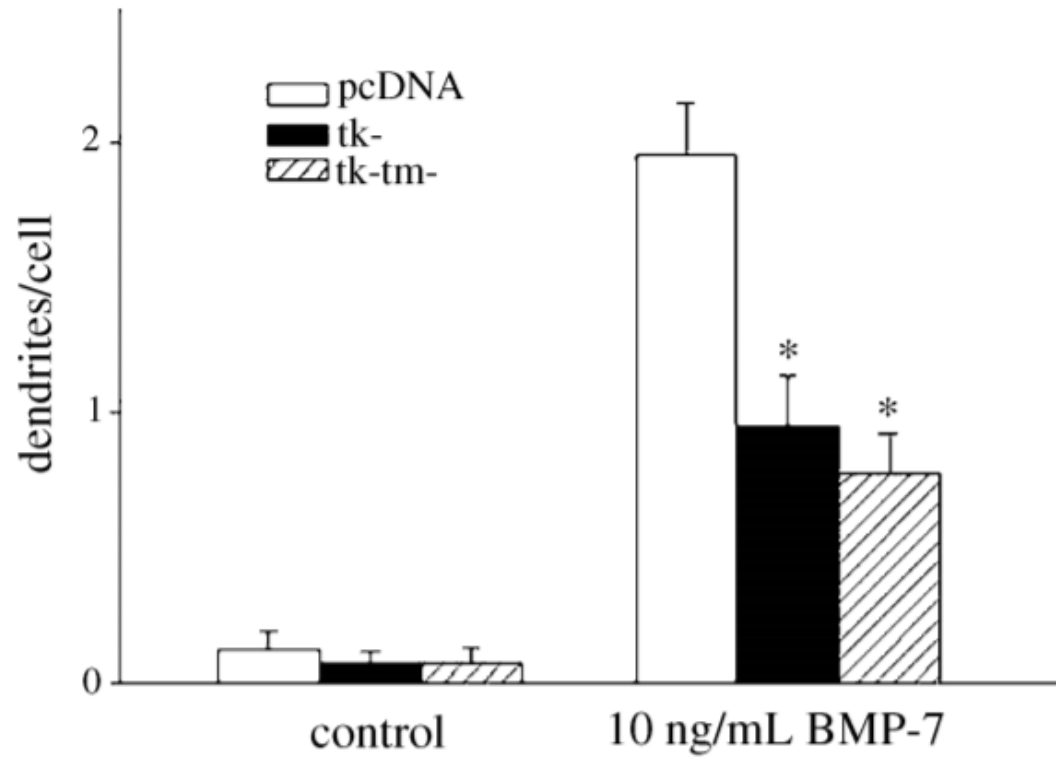
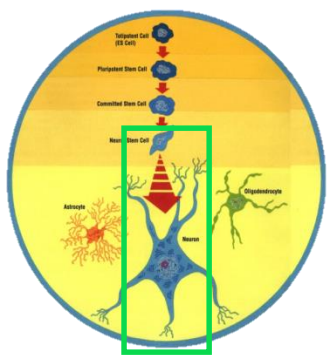
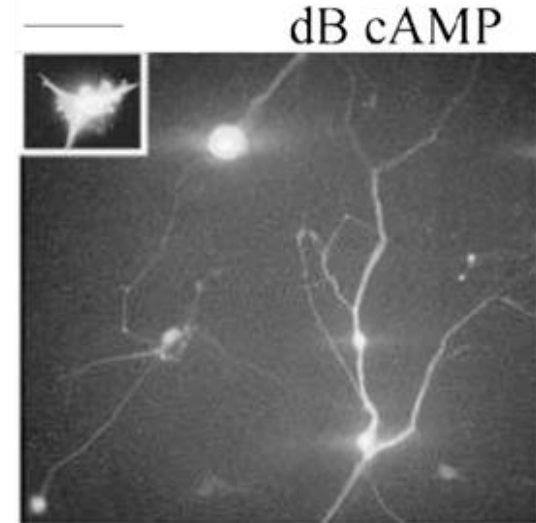
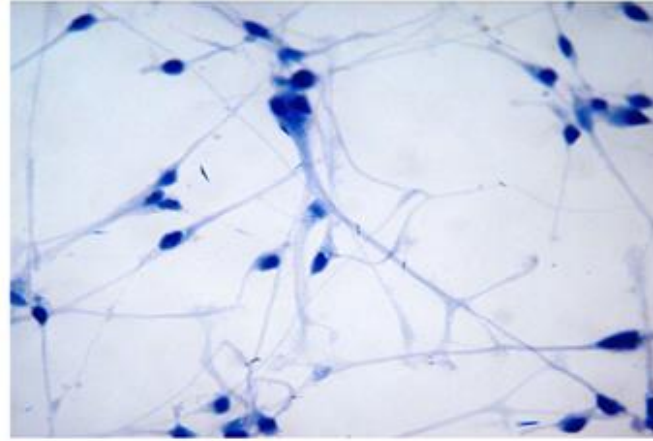


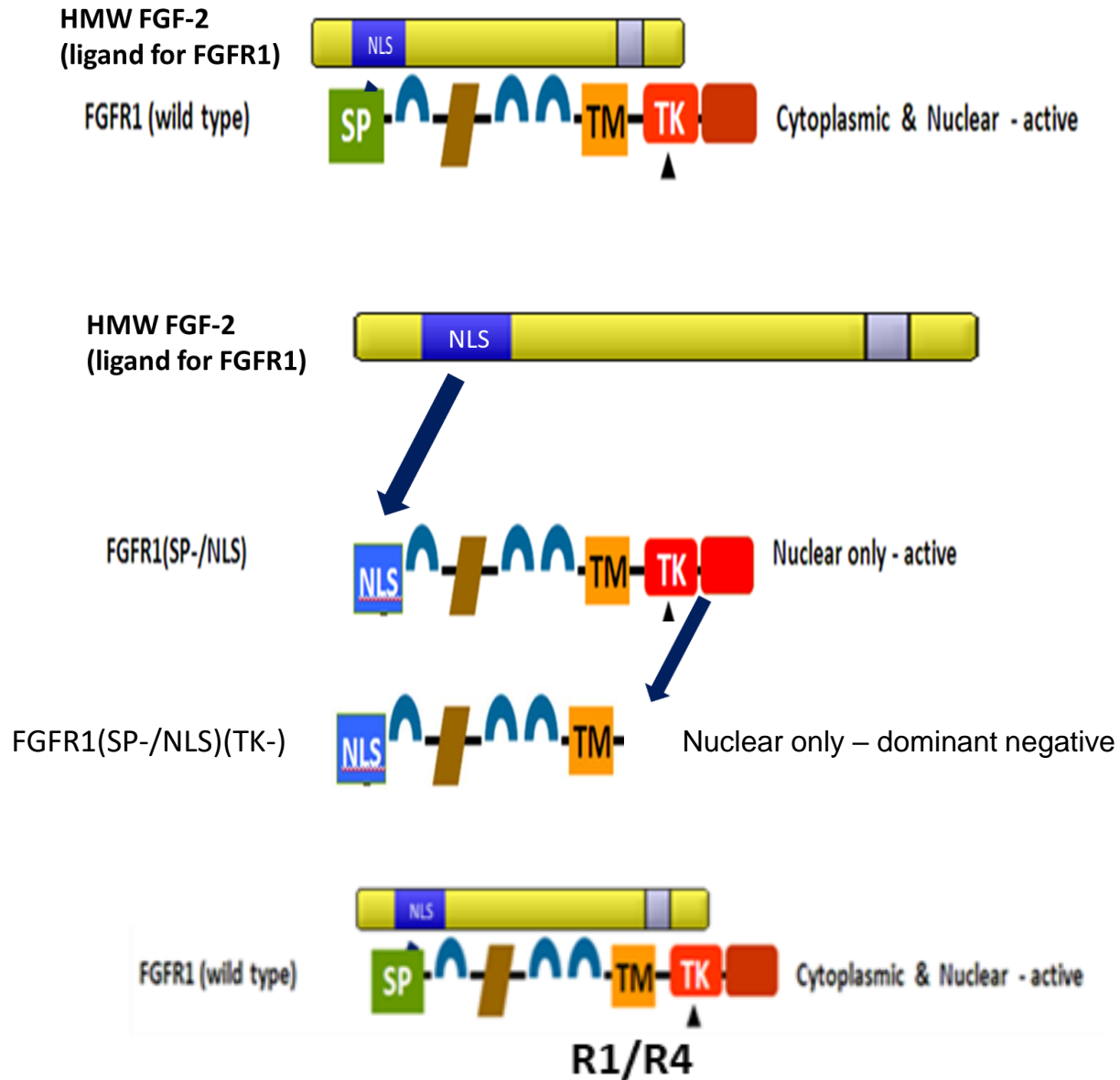
Fig. 4 FGFR1(TK-TM-) and FGFR1(TK-) inhibit BMP-7 signaling. After 5 days *in vitro*, sympathetic neurons were cotransfected with pEGFP/pcDNA3.1 (white bars), pEGFP/FGFR1(TK-) (black bars), or pEGFP/FGFR1(TK-/TM-) (hatched bars). BMP-7 treatment (10 ng/mL) began 3 days after transfection and continued for 5 days thereafter. For assessment of dendritic growth, neurons were then

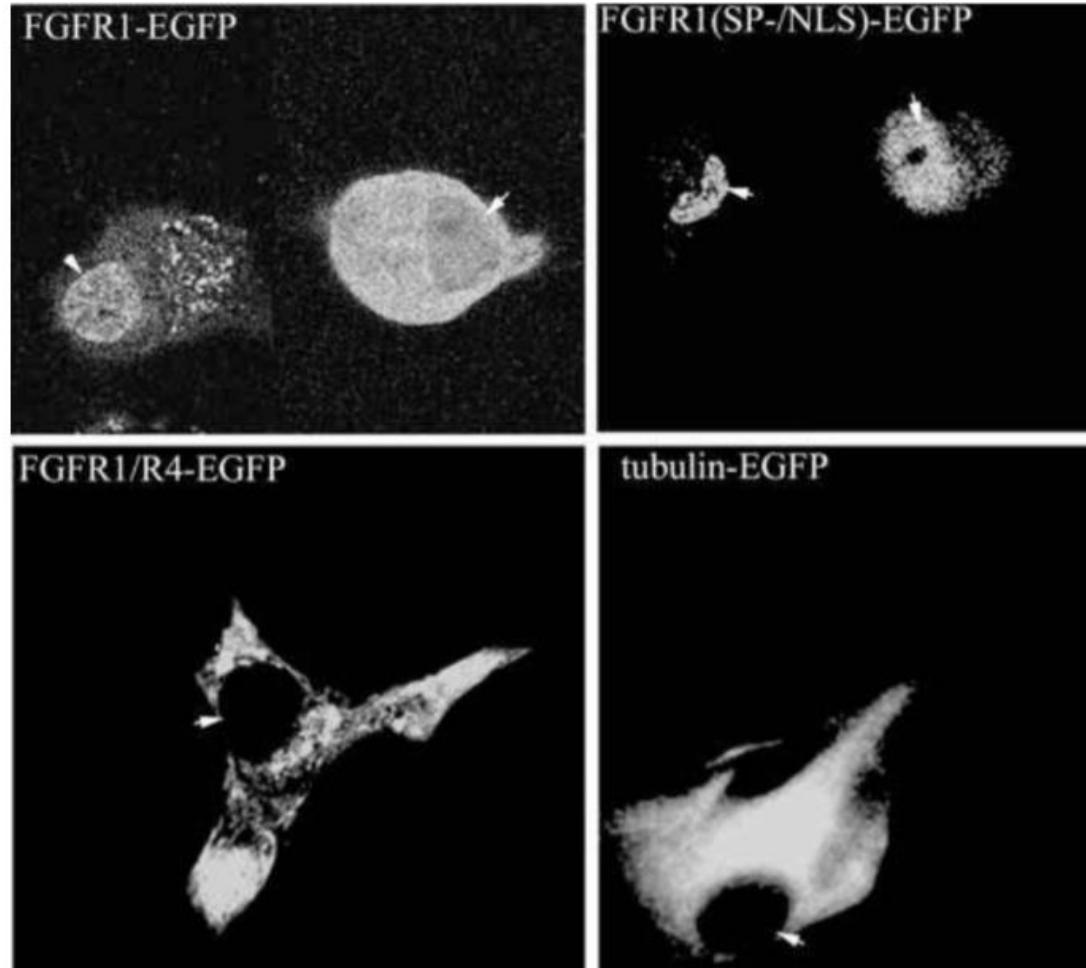
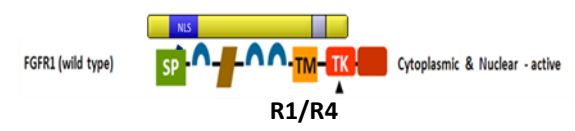
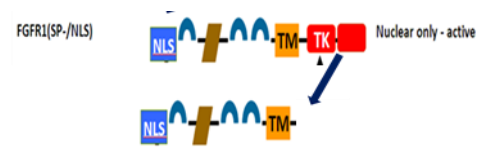


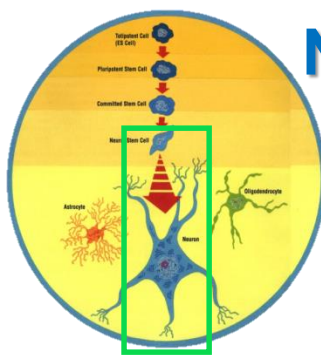
Nuclear FGFR1 mediates neuronal differentiation of Multipotent Human Brain Neural Progenitor Cells



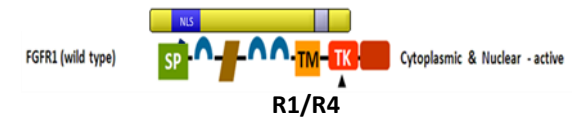
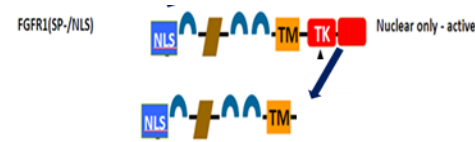
Engineering constitutive active and dominant negative nFGFR1



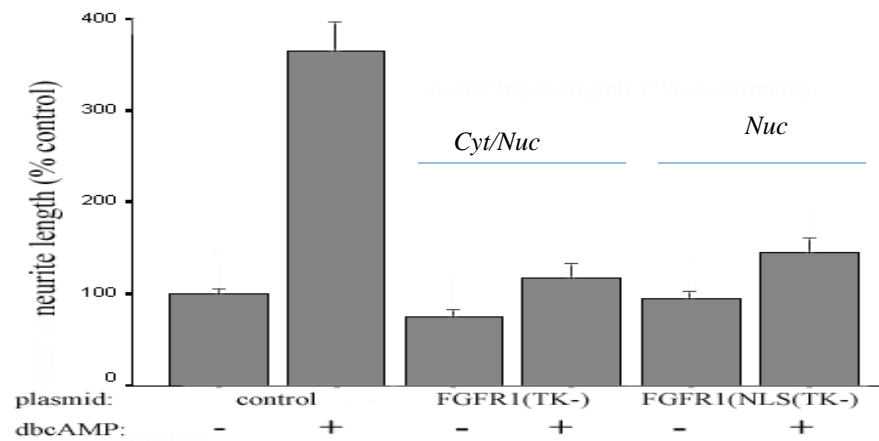




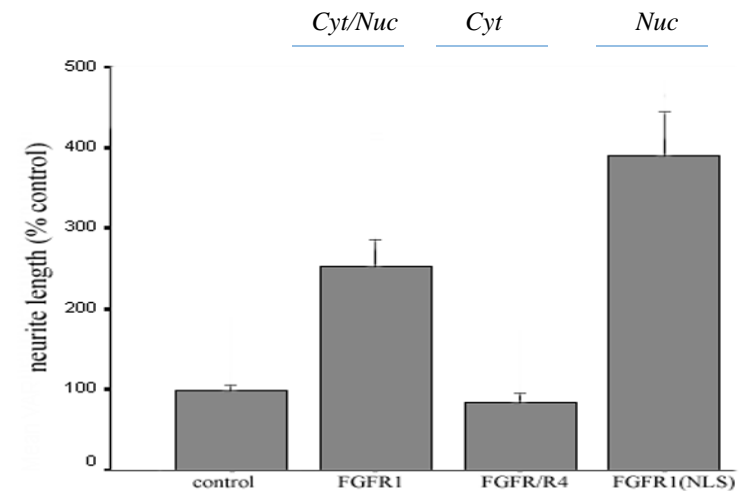
Nuclear FGFR1 mediates neuronal differentiation of Multipotent Human Brain Neural Progenitor Cells



cAMP-induced neuronal differentiation is blocked by dominant negative nuclear FGFR1

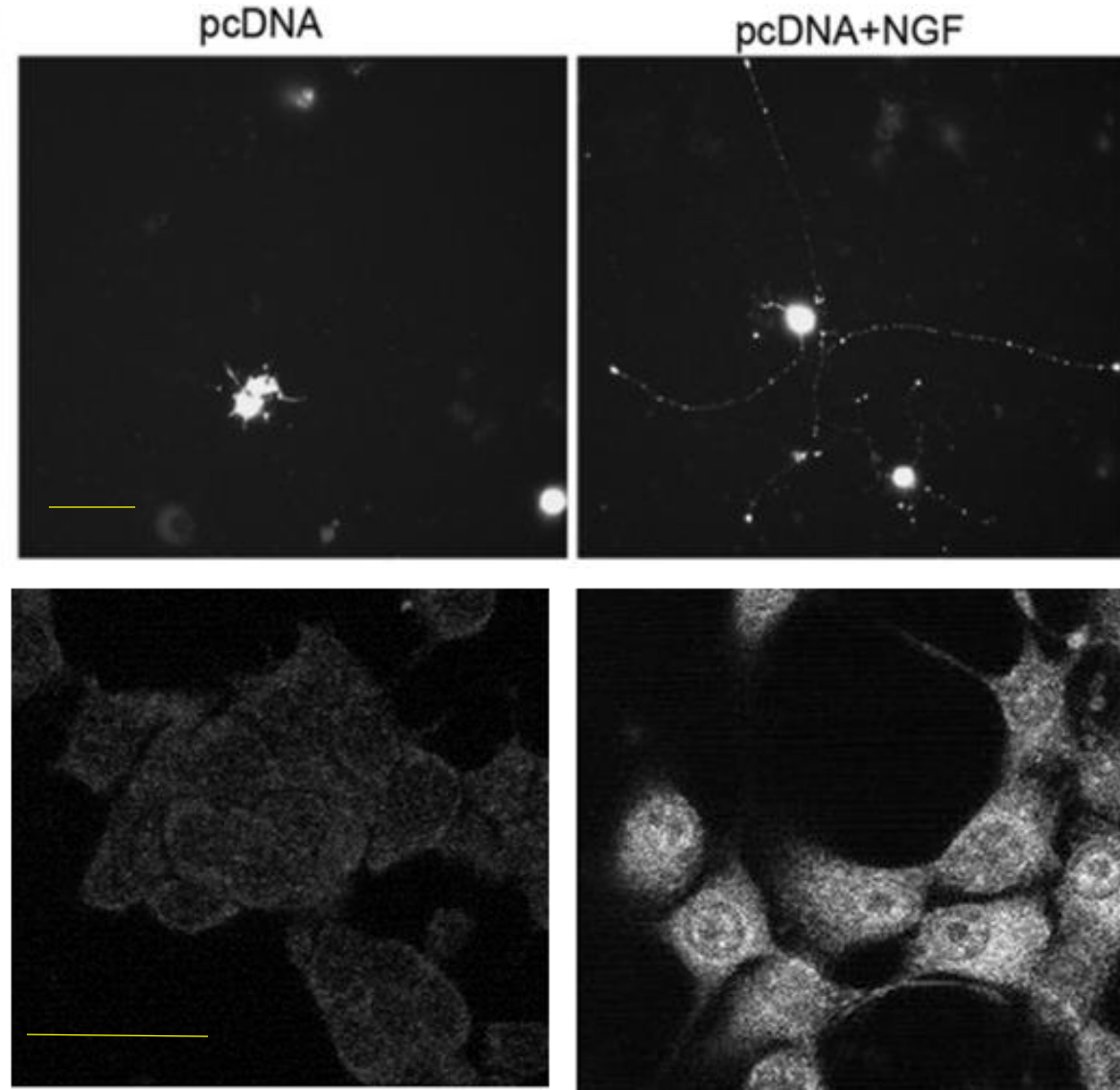


Nuclear accumulation of FGFR1 is sufficient to induce neuronal differentiation

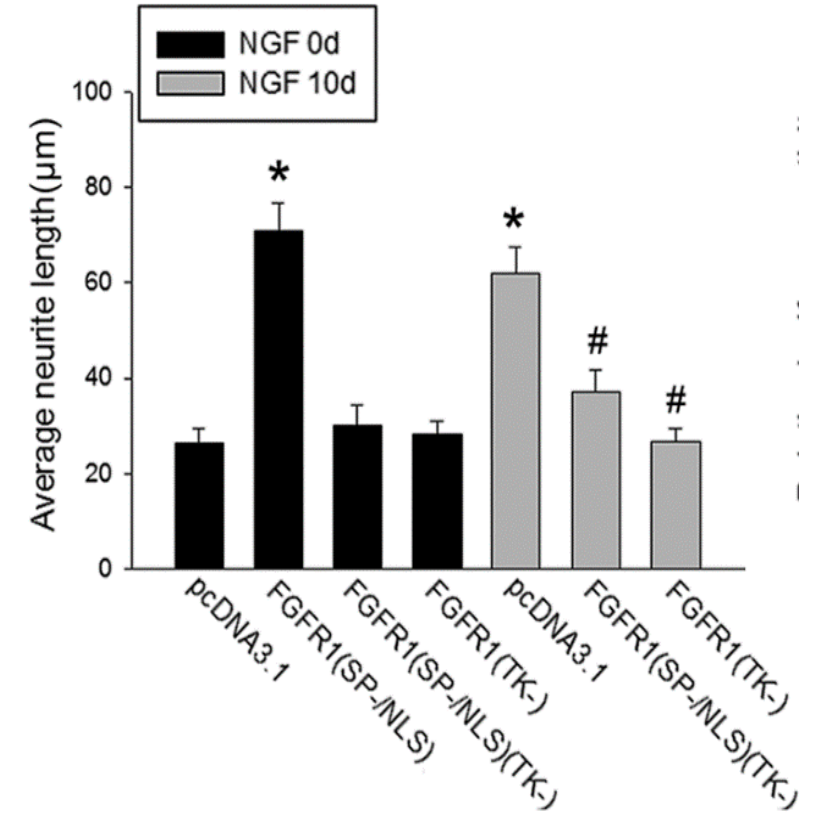
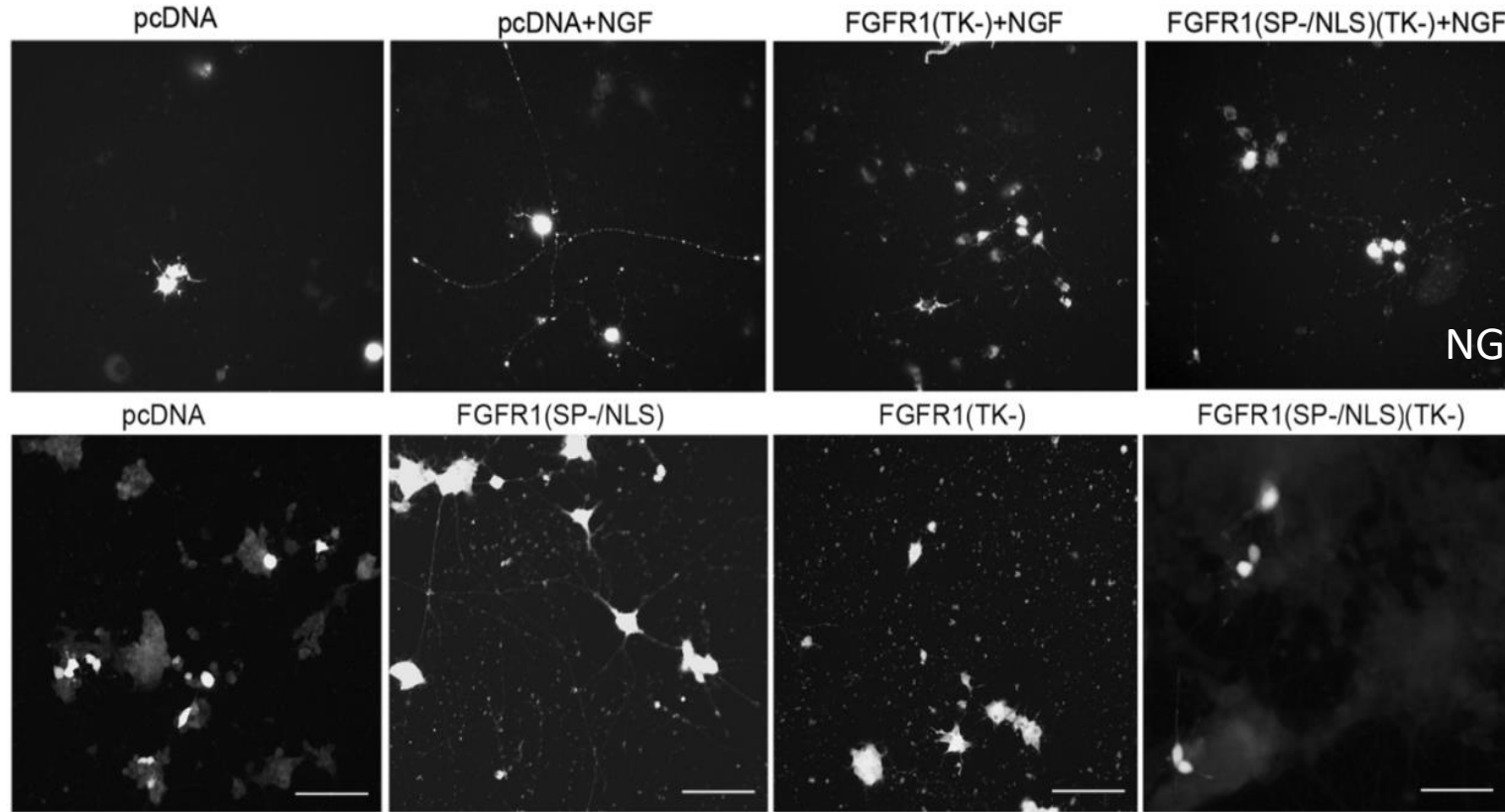


Nuclear FGFR1 mediates neuronal differentiation of Tumor cells

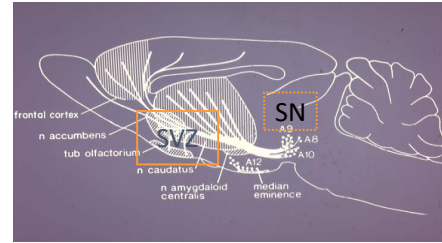
Nerve Growth Factor (NGF) in rat PC12 cells



Nuclear FGFR1 mediates neuronal differentiation of Tumor cells

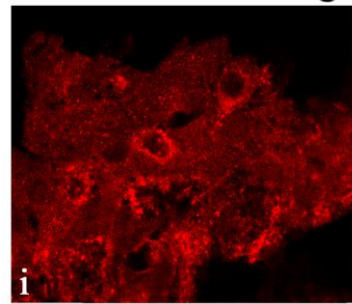


Role of INFS in early postmitotic neuronal development *in vivo* – transgenic mice



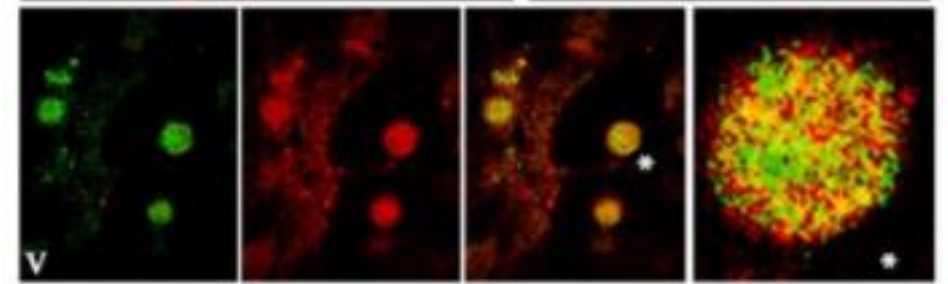
Proliferating Neural Stem Cells (SVZ):

Differentiating DA neurons (SN):



TH-
FGFR1_{cyt}

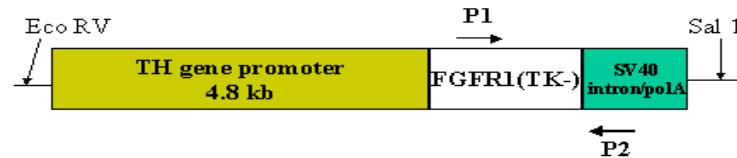
TH+
FGFR1_{nuc}



CBP

FGFR1

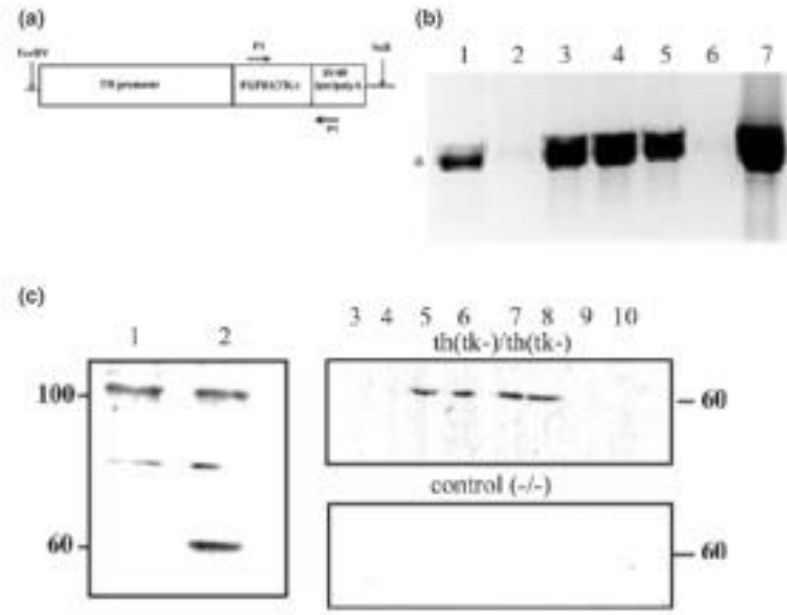
CBP+FGFR1



Strategy:

Blocking *FGFR1* signaling with dominant negative *FGFR1(TK-)* during neuronal differentiation.

Expression of FGFR1(TK-) in Substantia Nigra of transgenic mice



th-fgfr1(tk-) mice have hypoplastic DA neurons but increased DA release I striatum

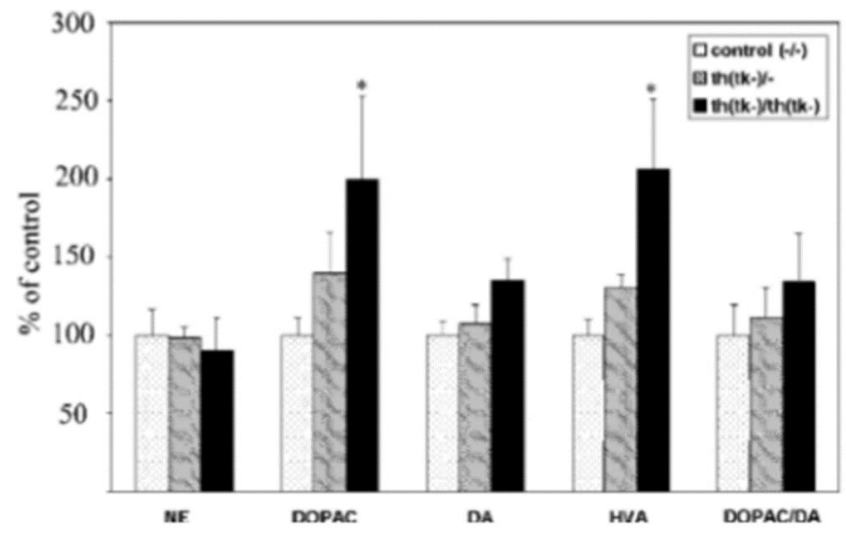
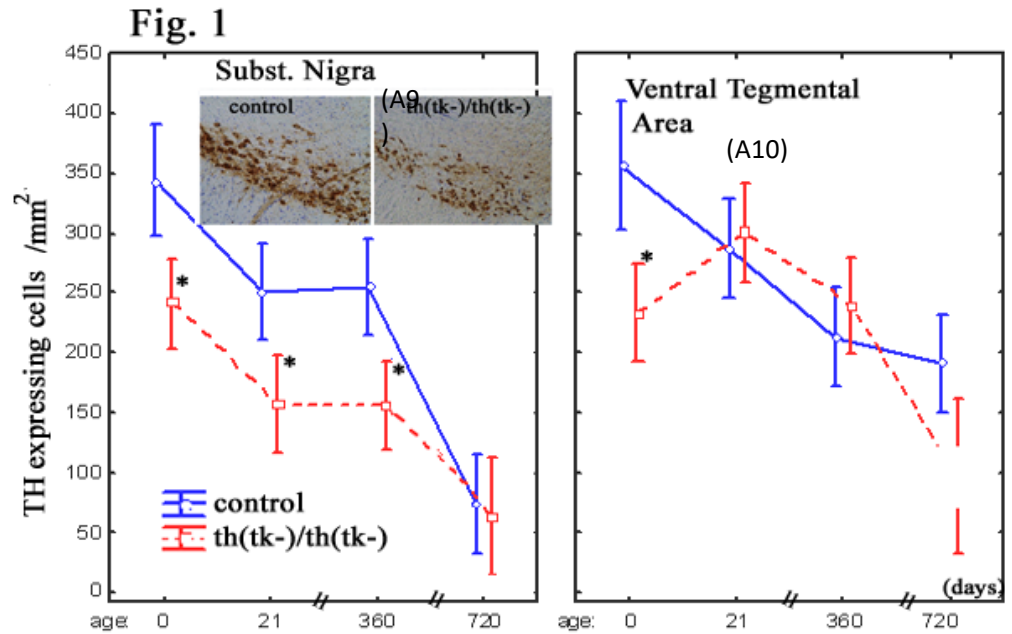
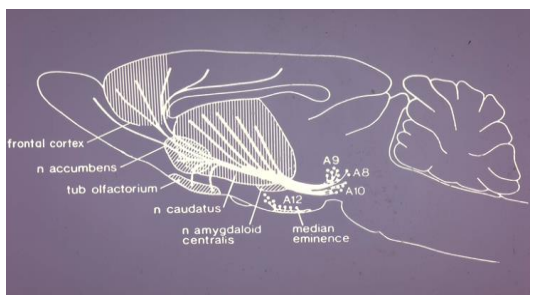
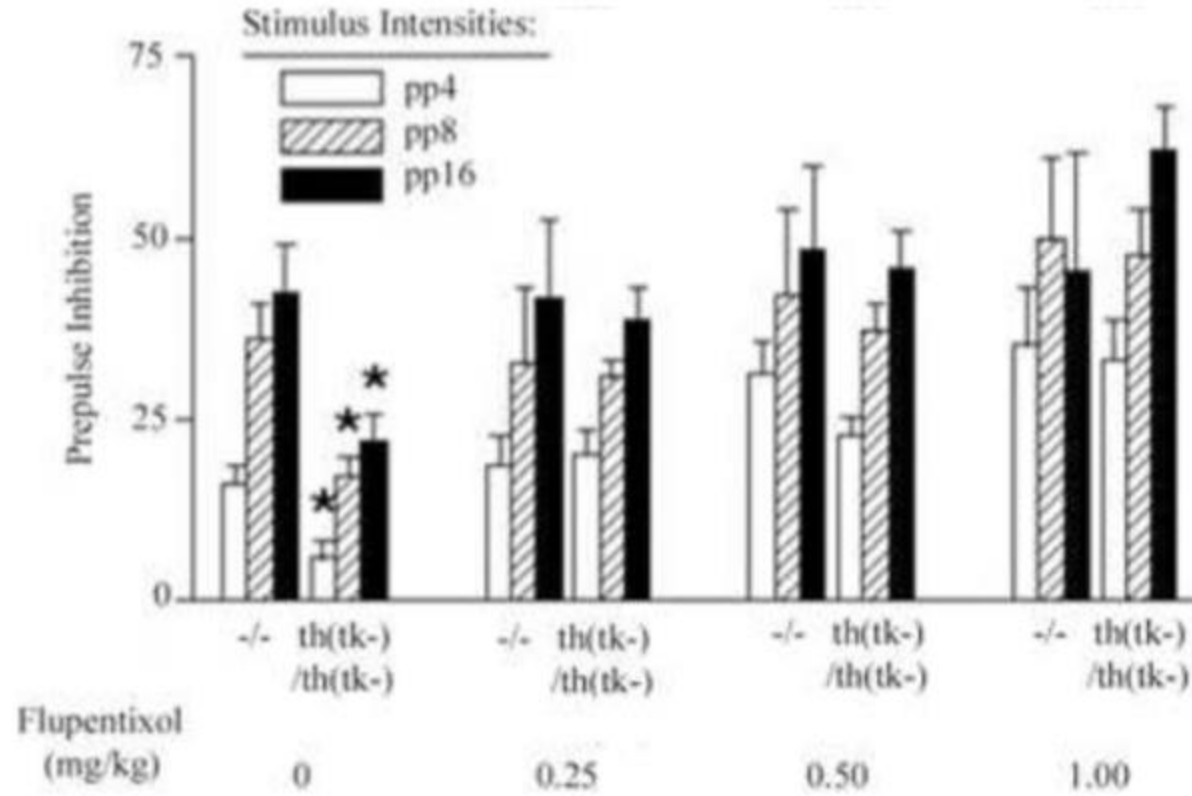


Fig. 4 Continued.

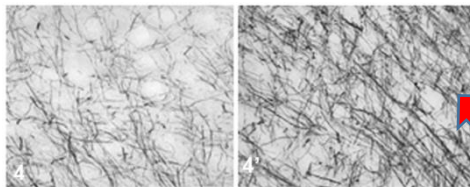
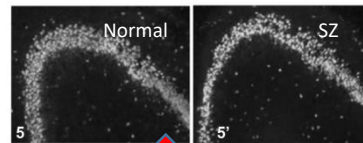
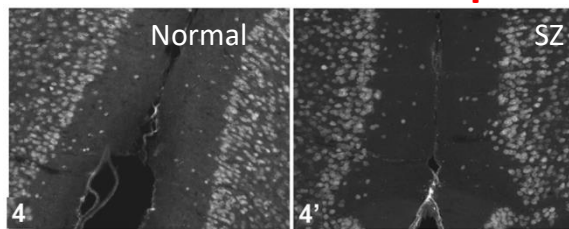
th-fgfr1(tk-) mice have reduced Pre-Pulse Inhibition (PPI)



Dominant Negative FGFR1(TK-) impairs brain development and models schizophrenia

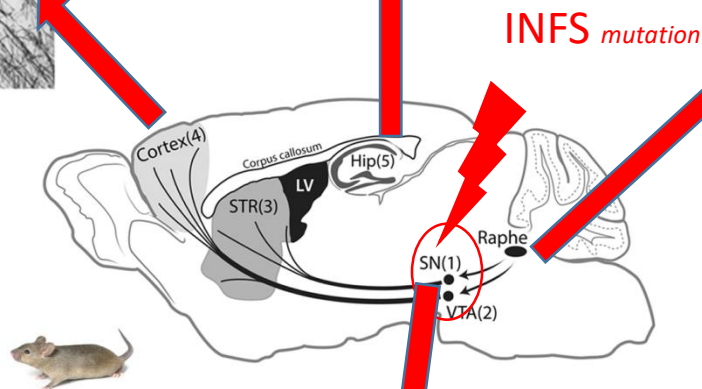
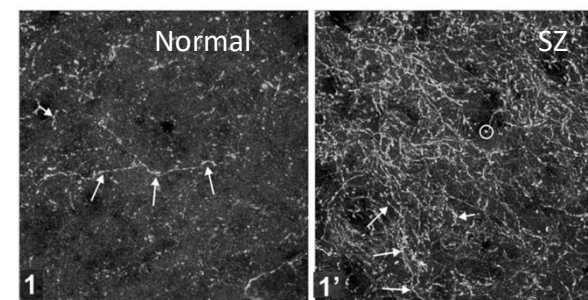
Glutamate & GABA neurons

Impaired Learning

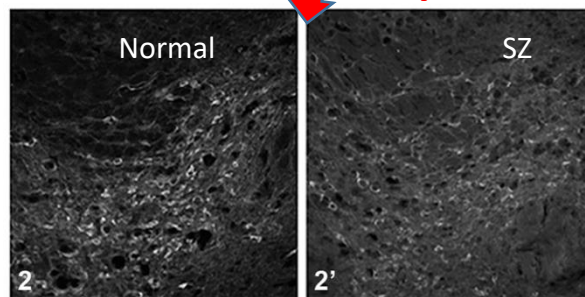


Serotonin neurons

Impaired Social Interactions

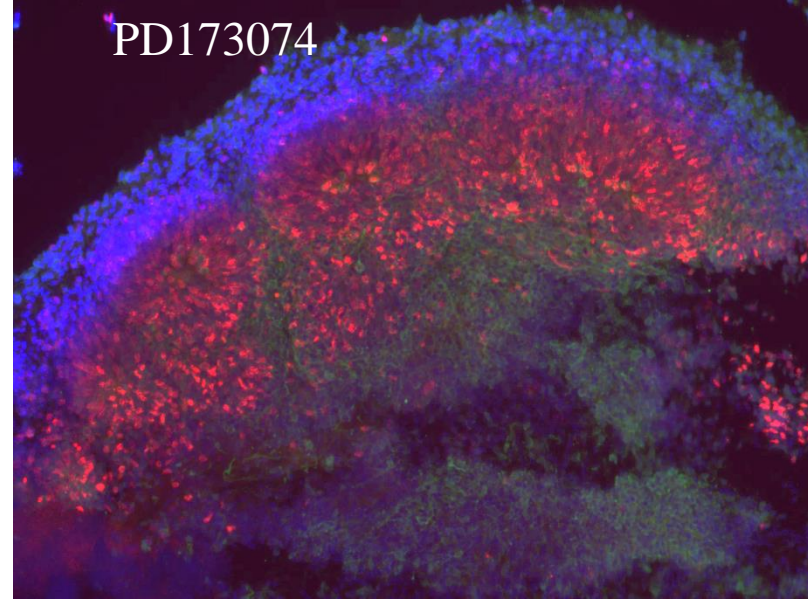
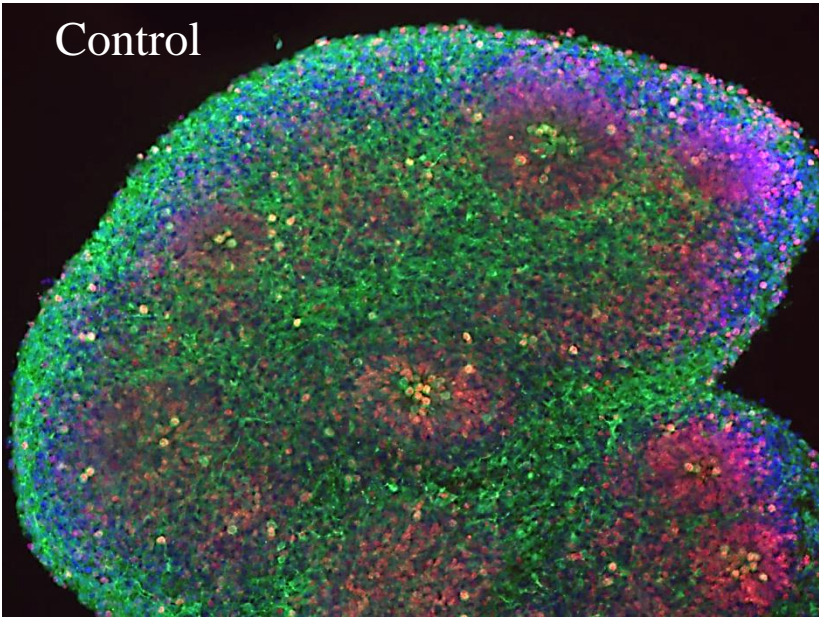


Dopamine neurons
Impaired Sensory Processing

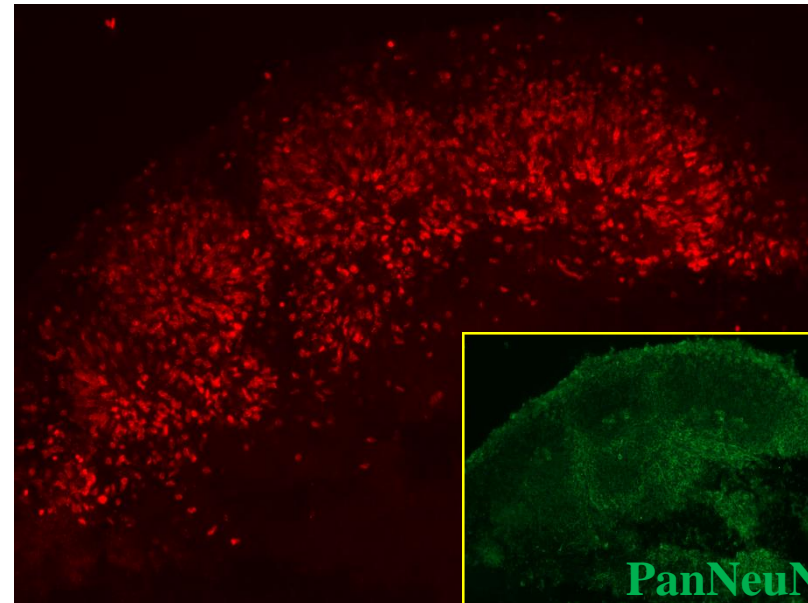
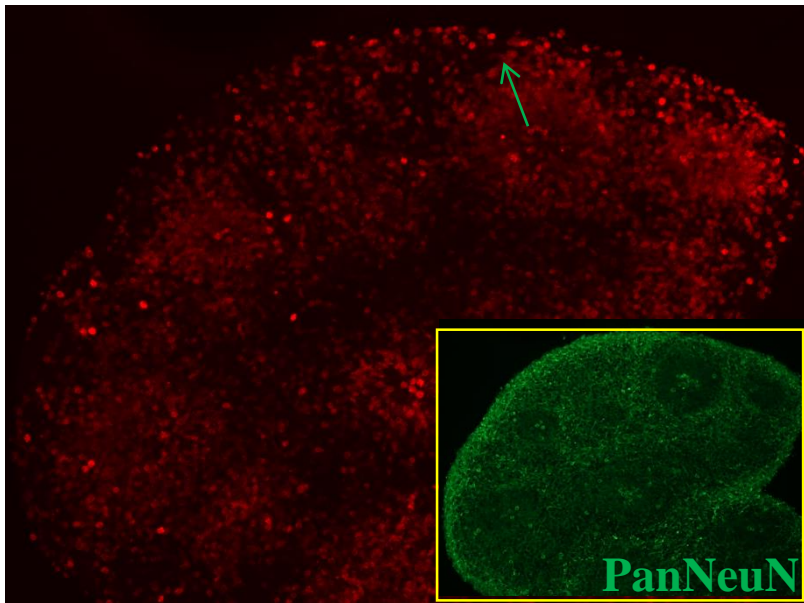


PD173074 –IMPAIRS MIGRATION & DIFFERENTIATION OF NEW CORTICAL NEURONS
(mimics changes in schizophrenia)

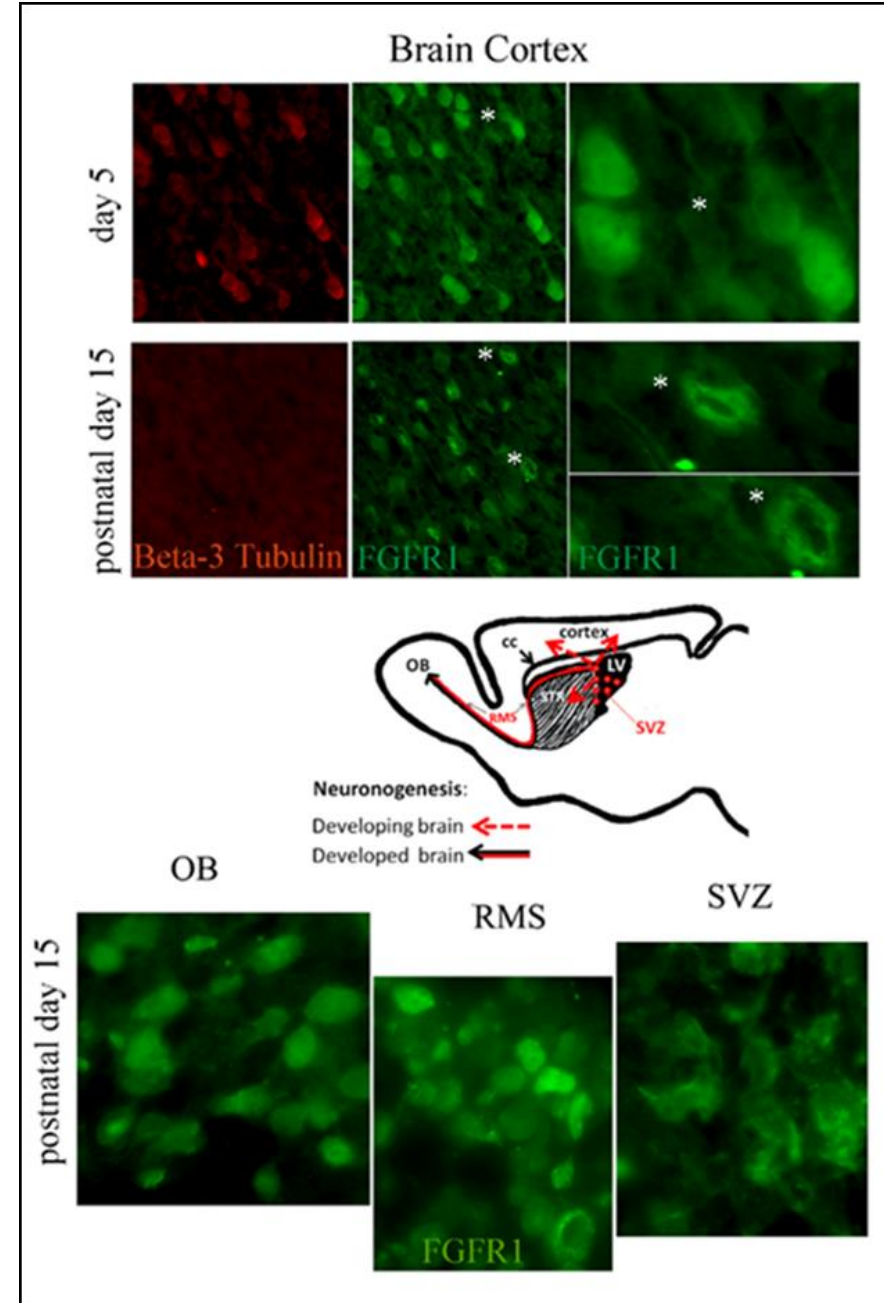
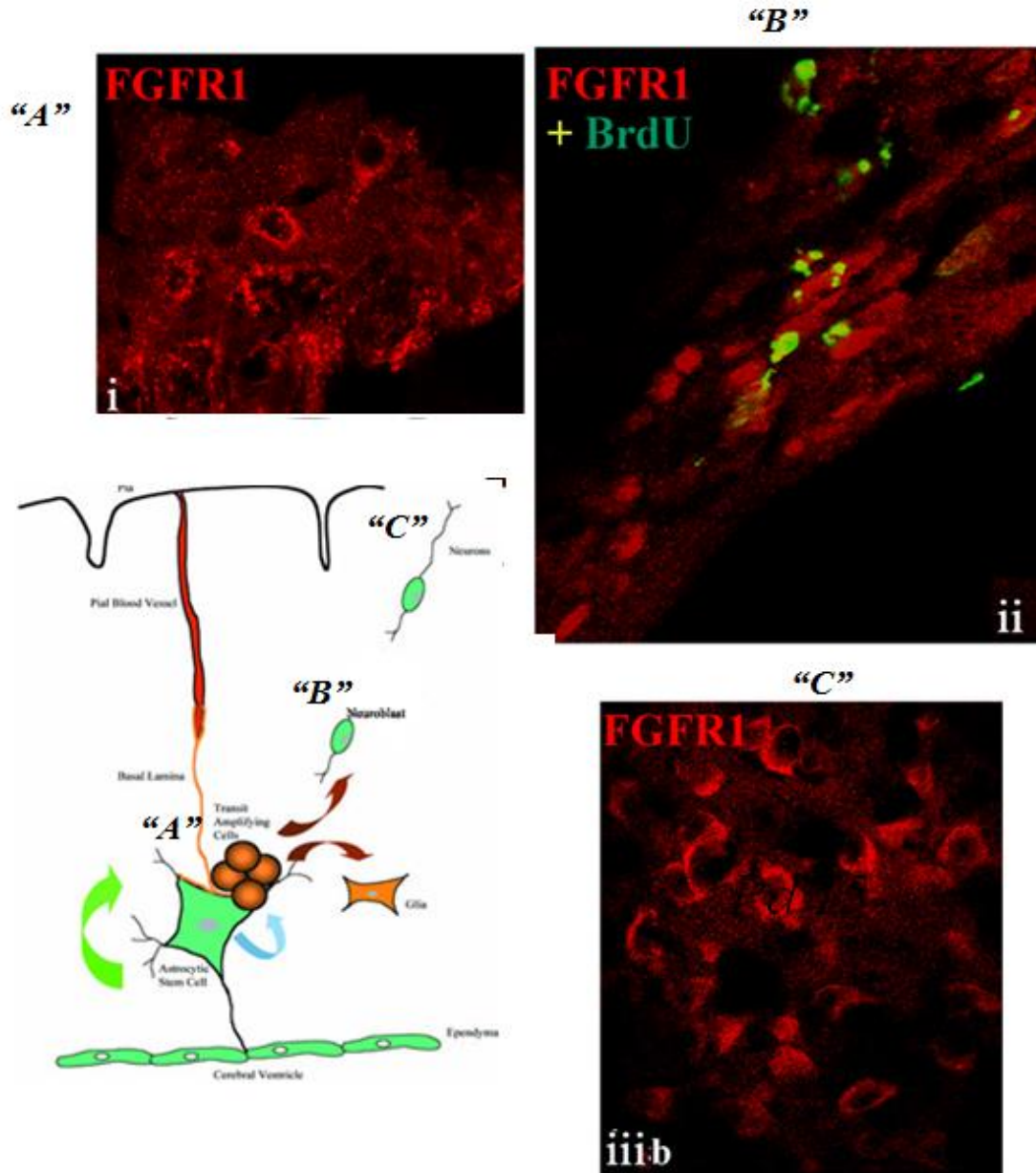
BrDu/PanNeuN



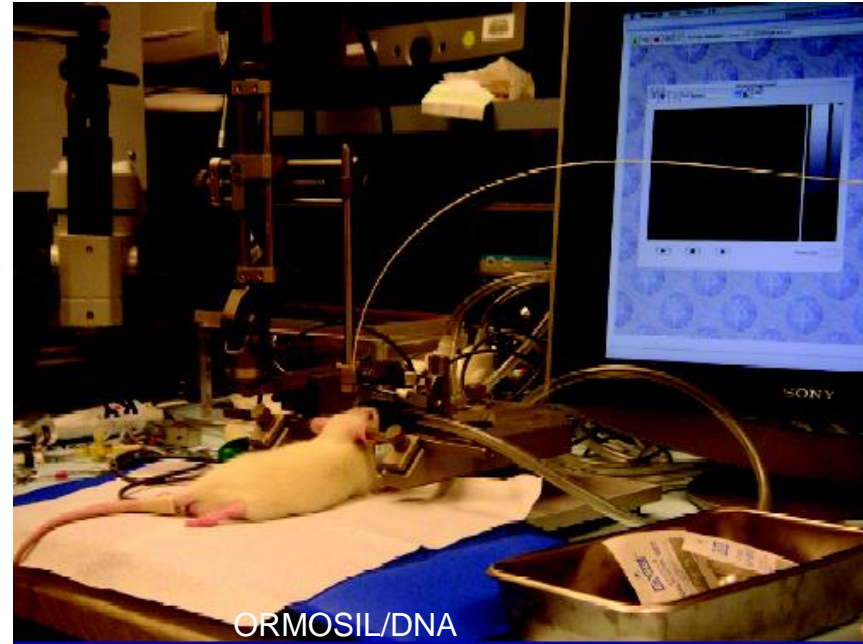
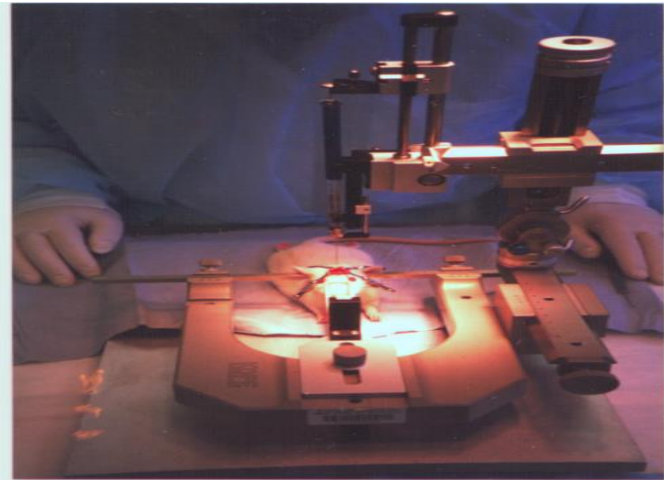
BrDu



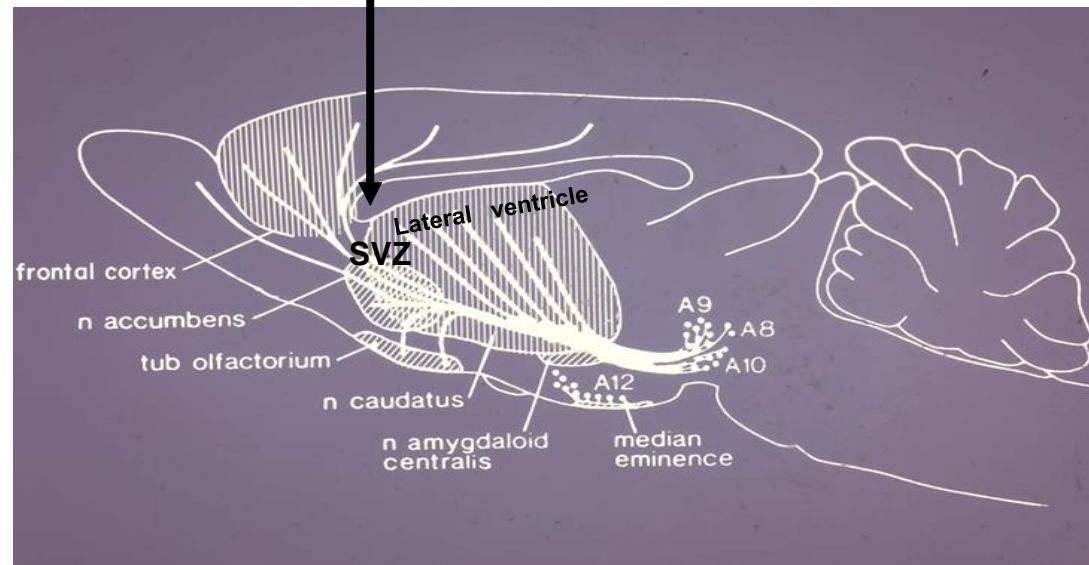
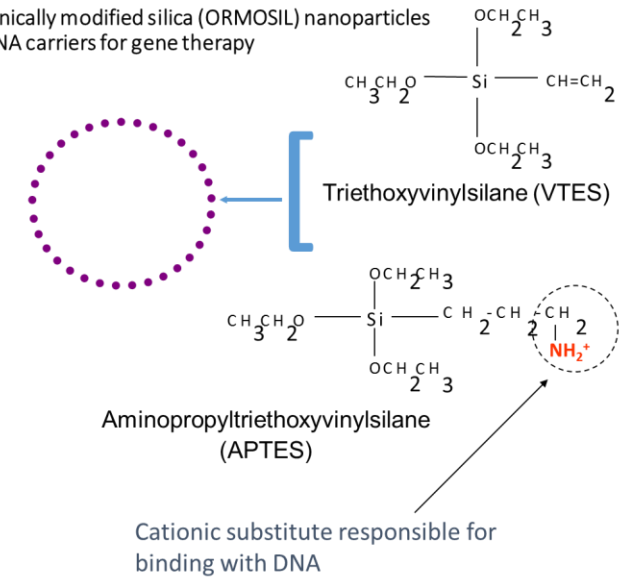
Transient Activation of INFS in Developing Brain



Role of FGFR1 in neuronal development in vivo via direct gene transfer into the brain.



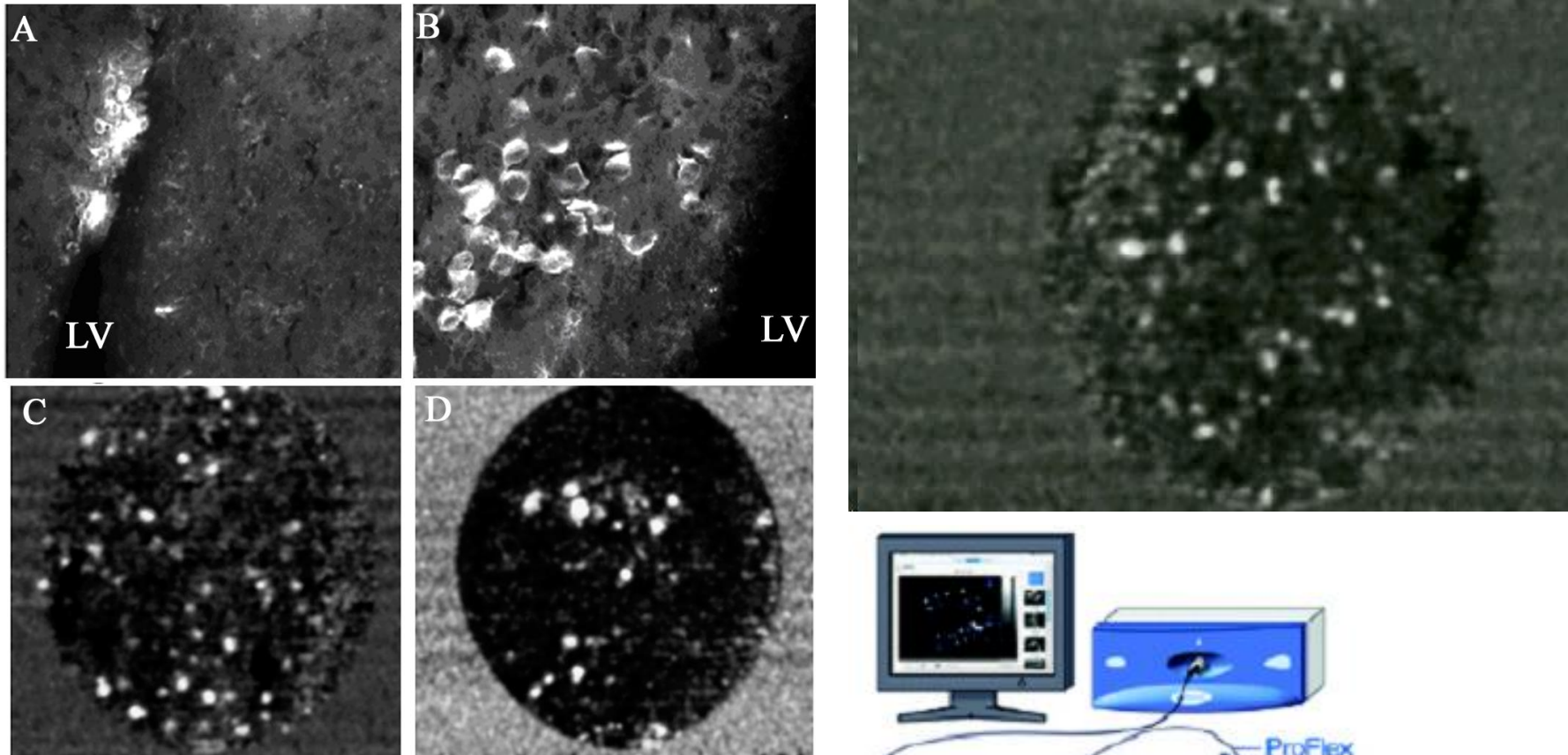
Organically modified silica (ORMOSIL) nanoparticles as DNA carriers for gene therapy



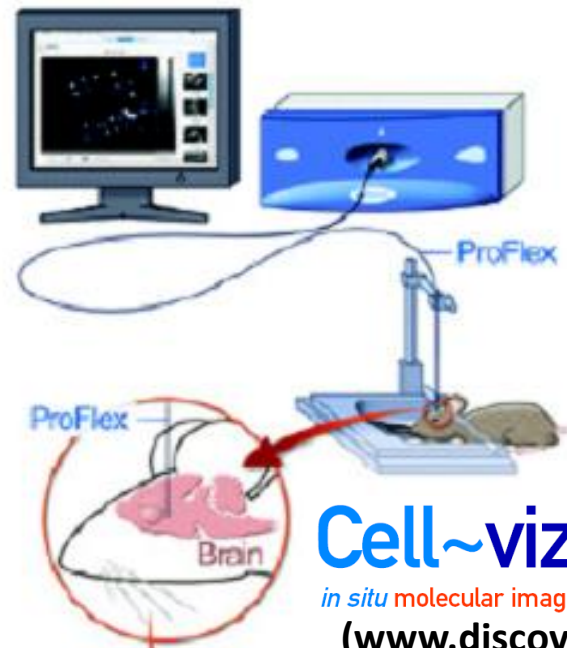
E.K. Stachowiak, I. Roy, Yu-Wei Lee, M. Capacchietti, J.M. Aletta, P.N. Prasad, and M.K. Stachowiak.: **Integrative BiolD.**

Bharali, I. Klejbor, E.K. Stachowiak, I. Roy, P.N. Prasad, and M.K. Stachowiak.: **PNAS 2005.**
gy, 2009, 1, 394 – 403.

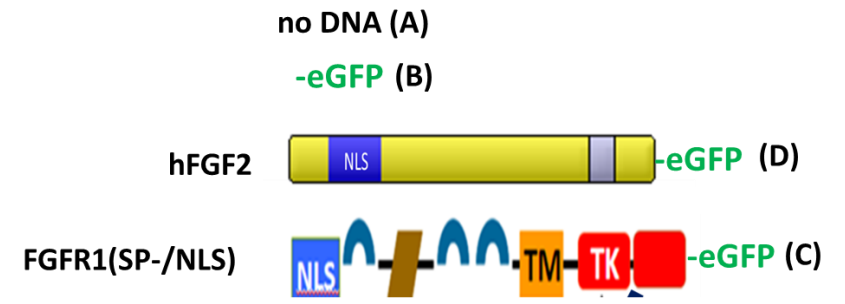
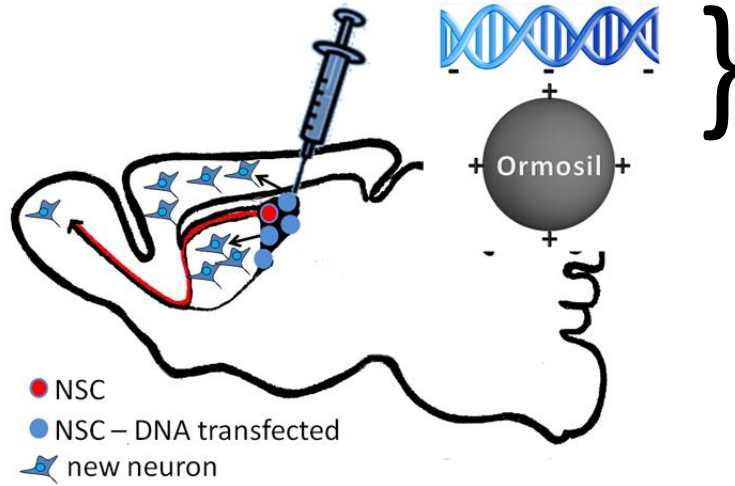
In vivo Imaging of EGFP Expression



(A & B) Immunostaining of lateral ventricle
(C & D) In vivo imaging of EGFP fluorescence in cells in the lateral ventricle.

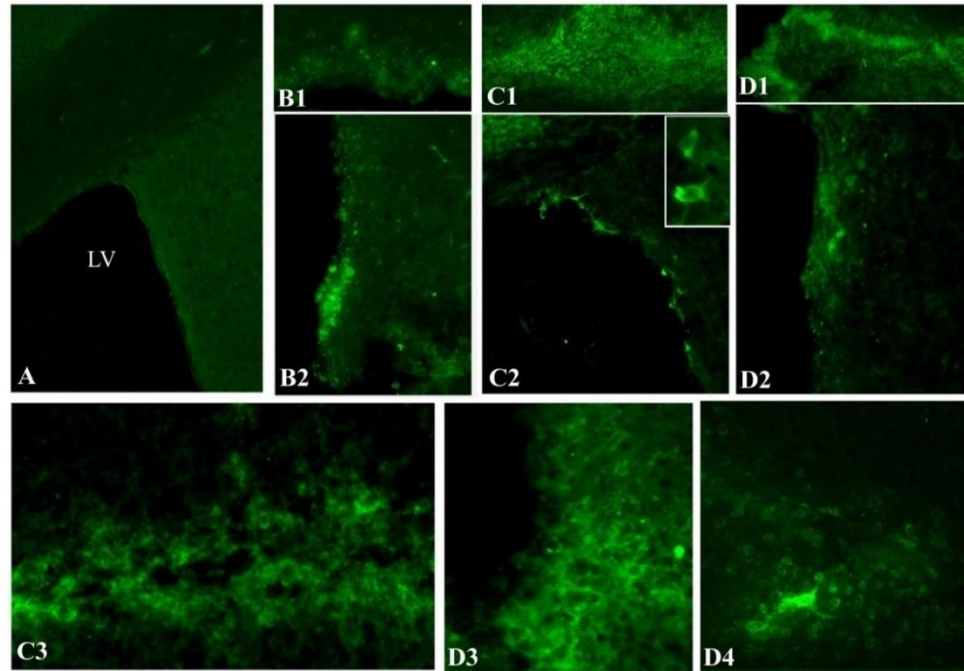
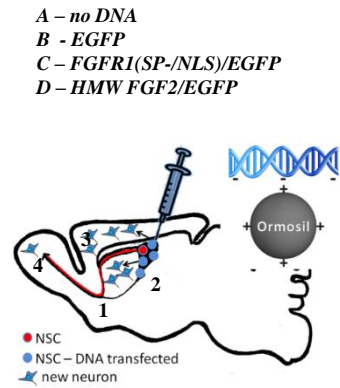


Targeting INFS mechanism in brain stem cells by nanoplex-mediated gene transfers



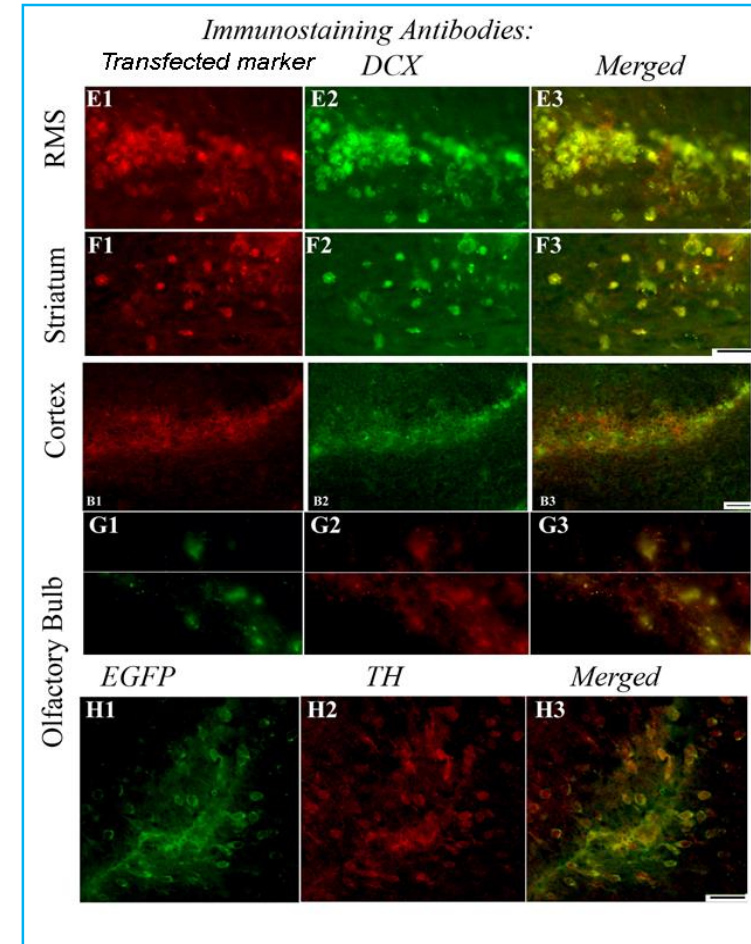
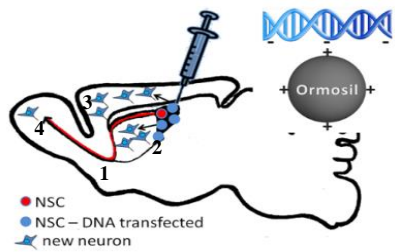
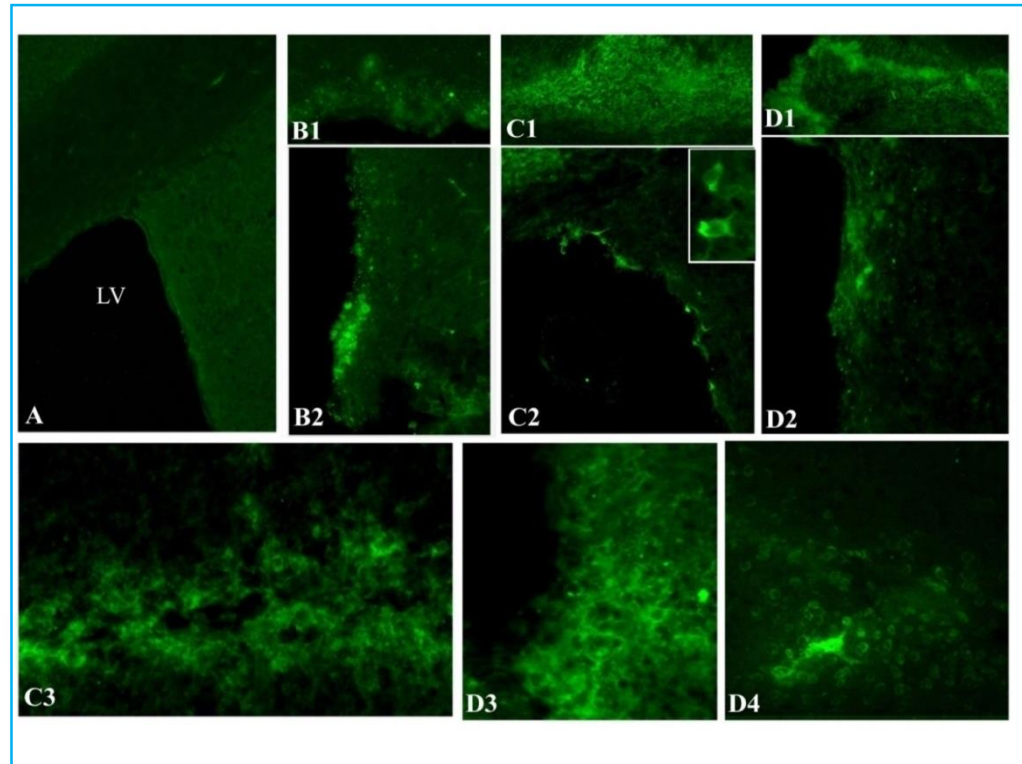
Targeting INFS in brain stem cells by nanoplex-DNA - migration of transfected cells from SVZ into the brain

Fig. 1

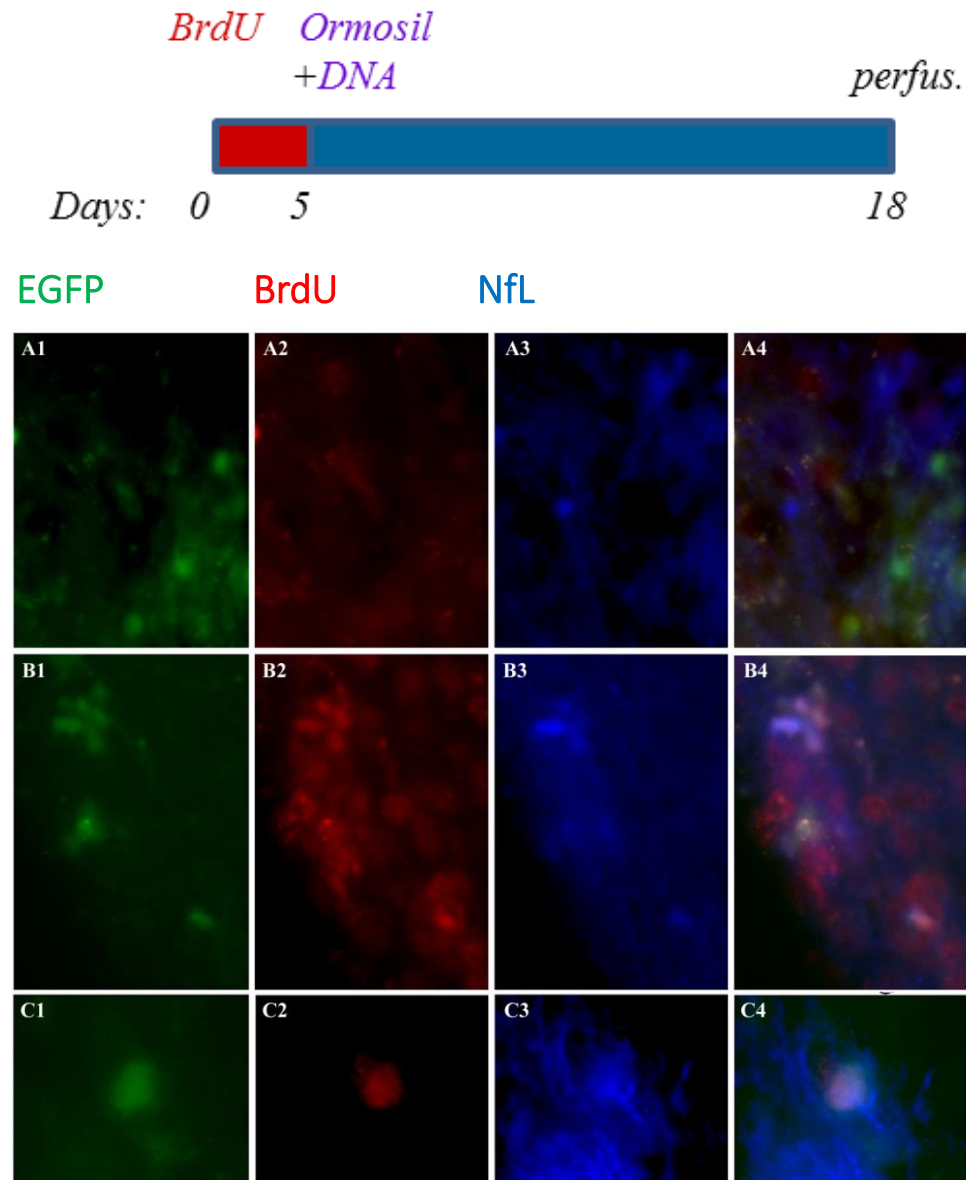


D. Bharali, I. Klejbor, E.K. Stachowiak, I. Roy, P.N. Prasad, and M.K. Stachowiak.: *PNAS* 2005.;
 E.K. Stachowiak, I. Roy, Yu-Wei Lee, M. Capacchietti, J.M. Aletta, P.N. Prasad, and M.K. Stachowiak.: *Integrative Biology*, 2009, 1, 394 - 403.

Nuclear FGFR1 transfection: Migration of cells from SVZ



Differentiation of newly generated SVZ cells into neurofilament L (NfL) expressing neurons.

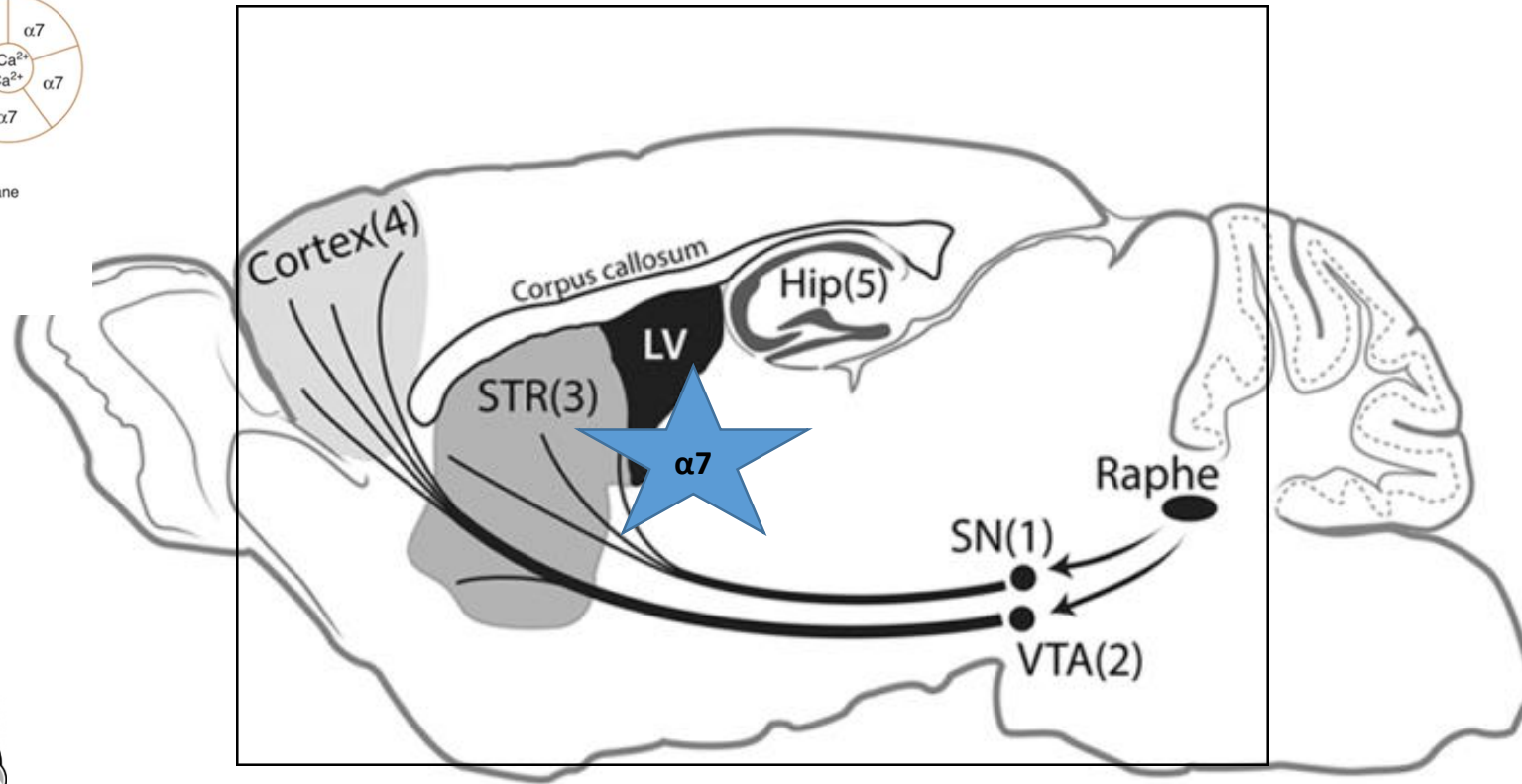
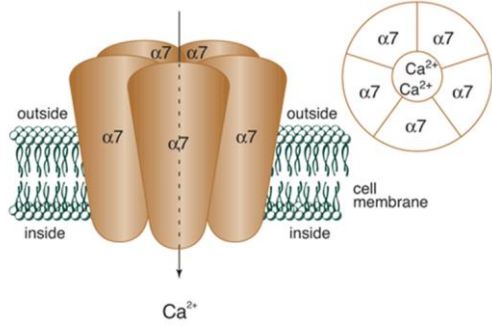


**Can neurogenesis be reinstated
in the adult brain by
pharmacological reactivation of
INFS?**

Small Molecules for INFS activation

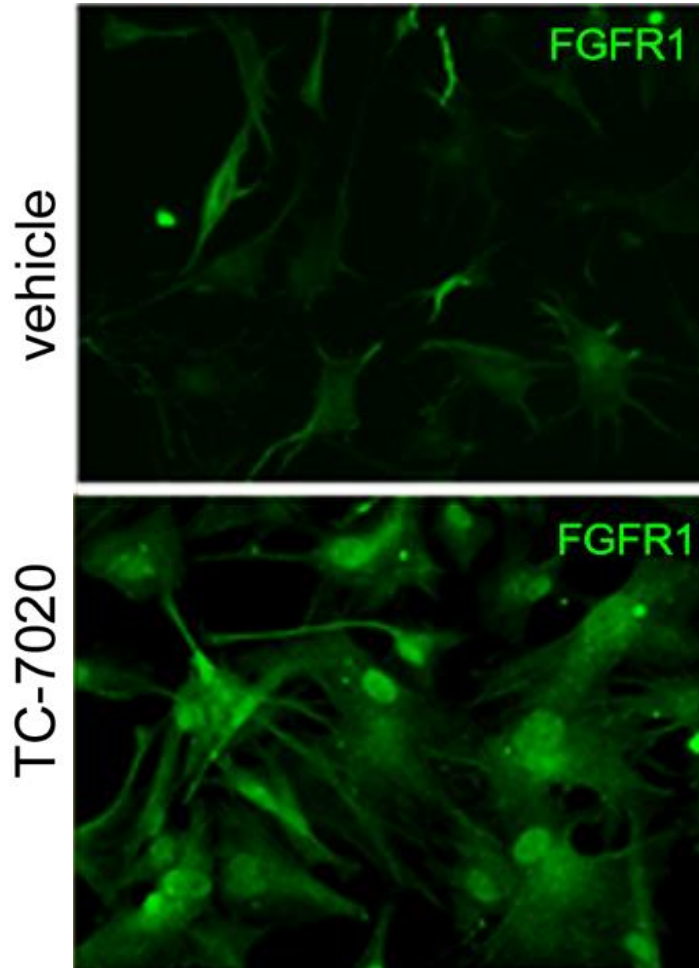
Developmental $\alpha 7$ Nicotinic Receptors are preserved in Adult Brain

Five $\alpha 7$ subunits form an $\alpha 7$ homo-oligomeric nAChR

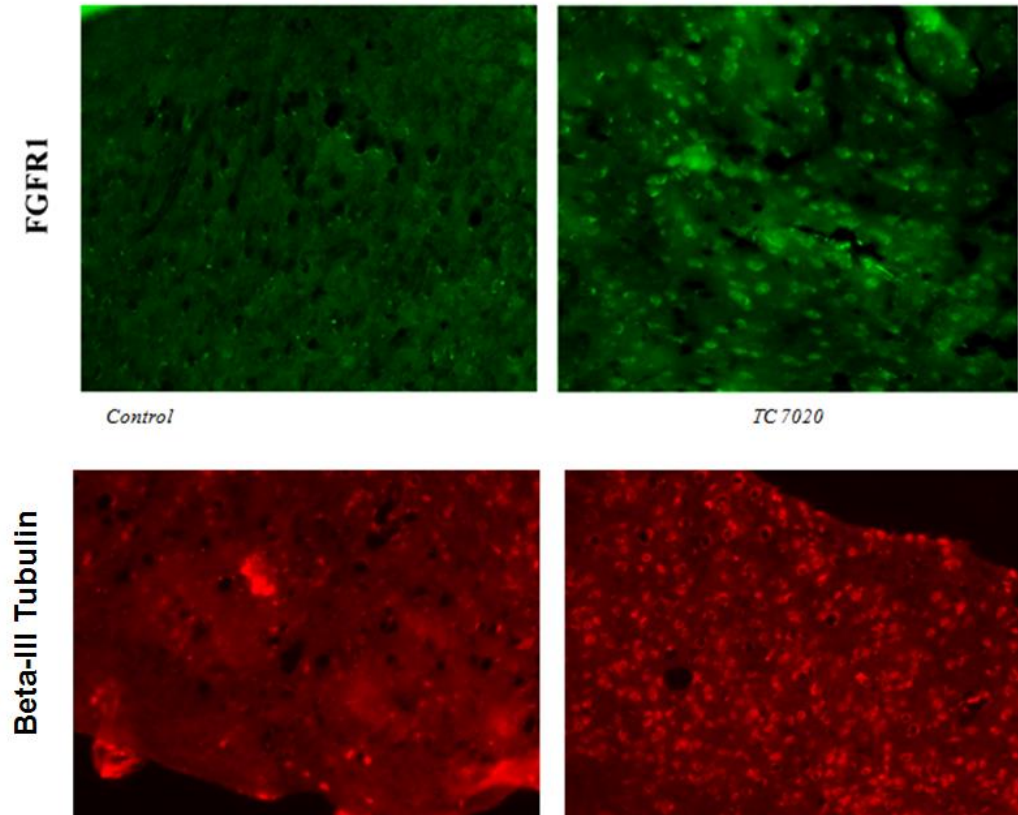


$\alpha 7$ Nicotinic Agonist (*TC7020: Targacept Inc*) activates INFS and neurogenesis

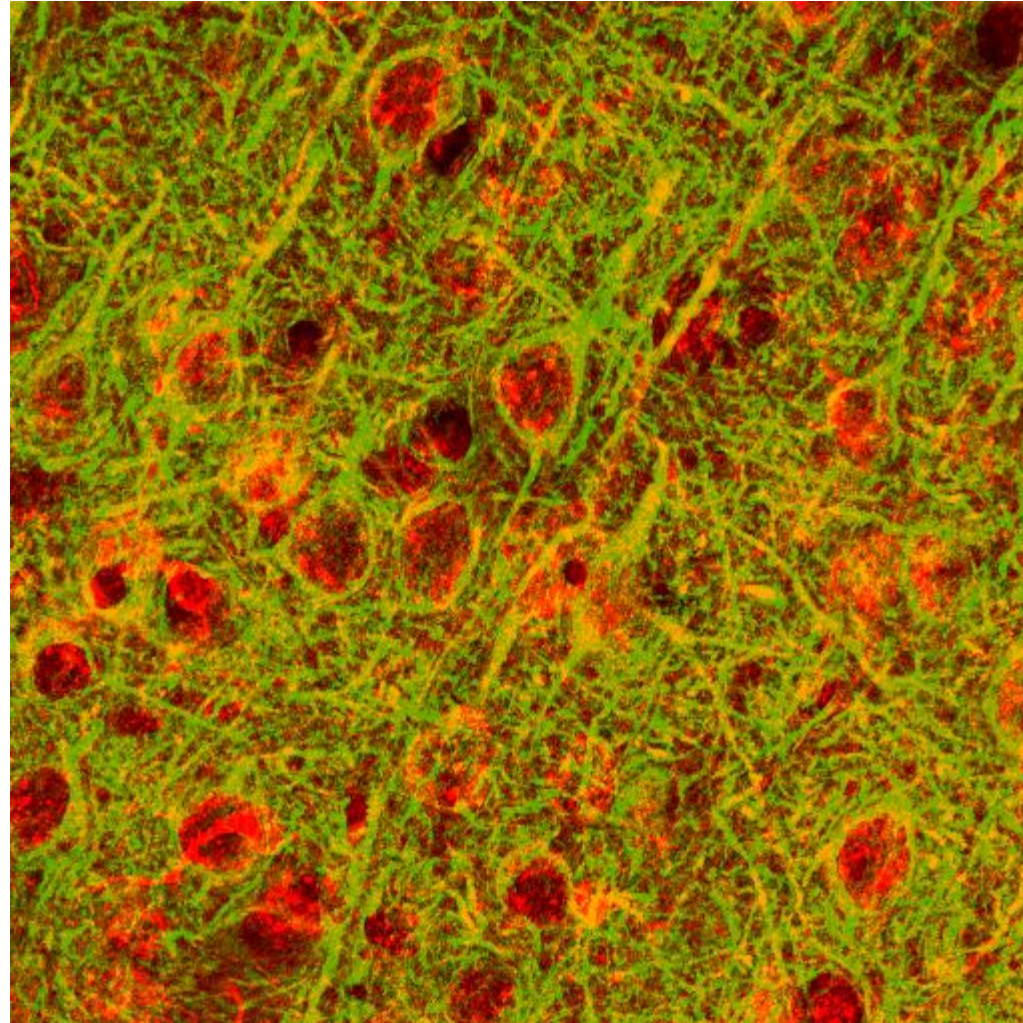
Human Neural Progenitors
- Cell culture



Cortical Neurogenesis in adult brain



nuclear **FGFR1** accumulation in **β III-Tubulin+** young neurons of developing brain



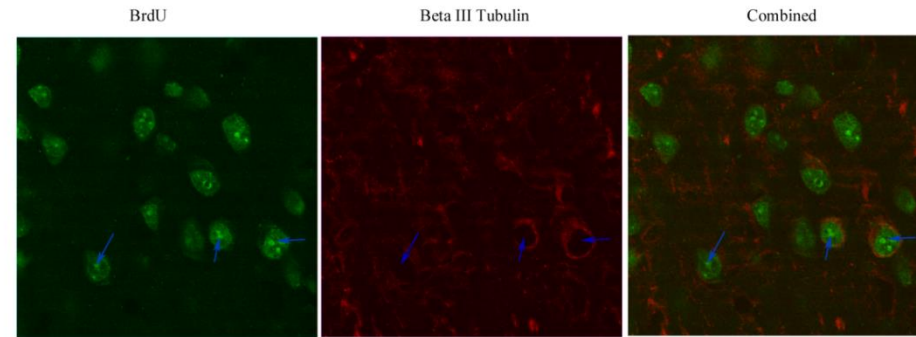
$\alpha 7$ Nicotinic Agonist reactivation of Neuronogenesis in adult mouse brain



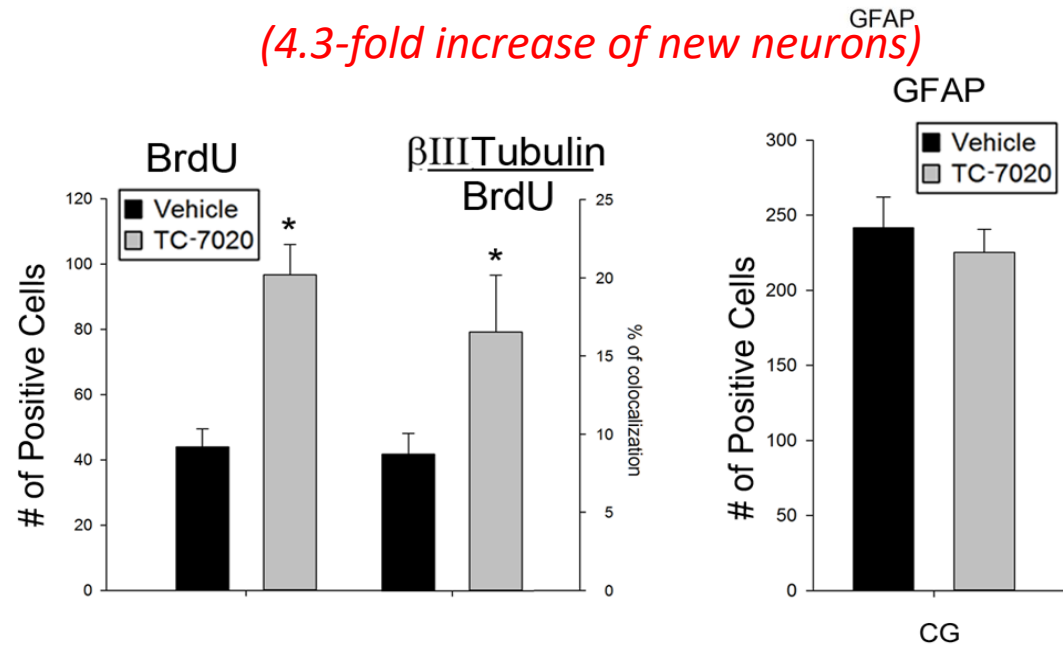
4 Month old Male Mice

- BrdU positive cells in non-neurogenic regions of the brain
- Colocalization with neuronal markers

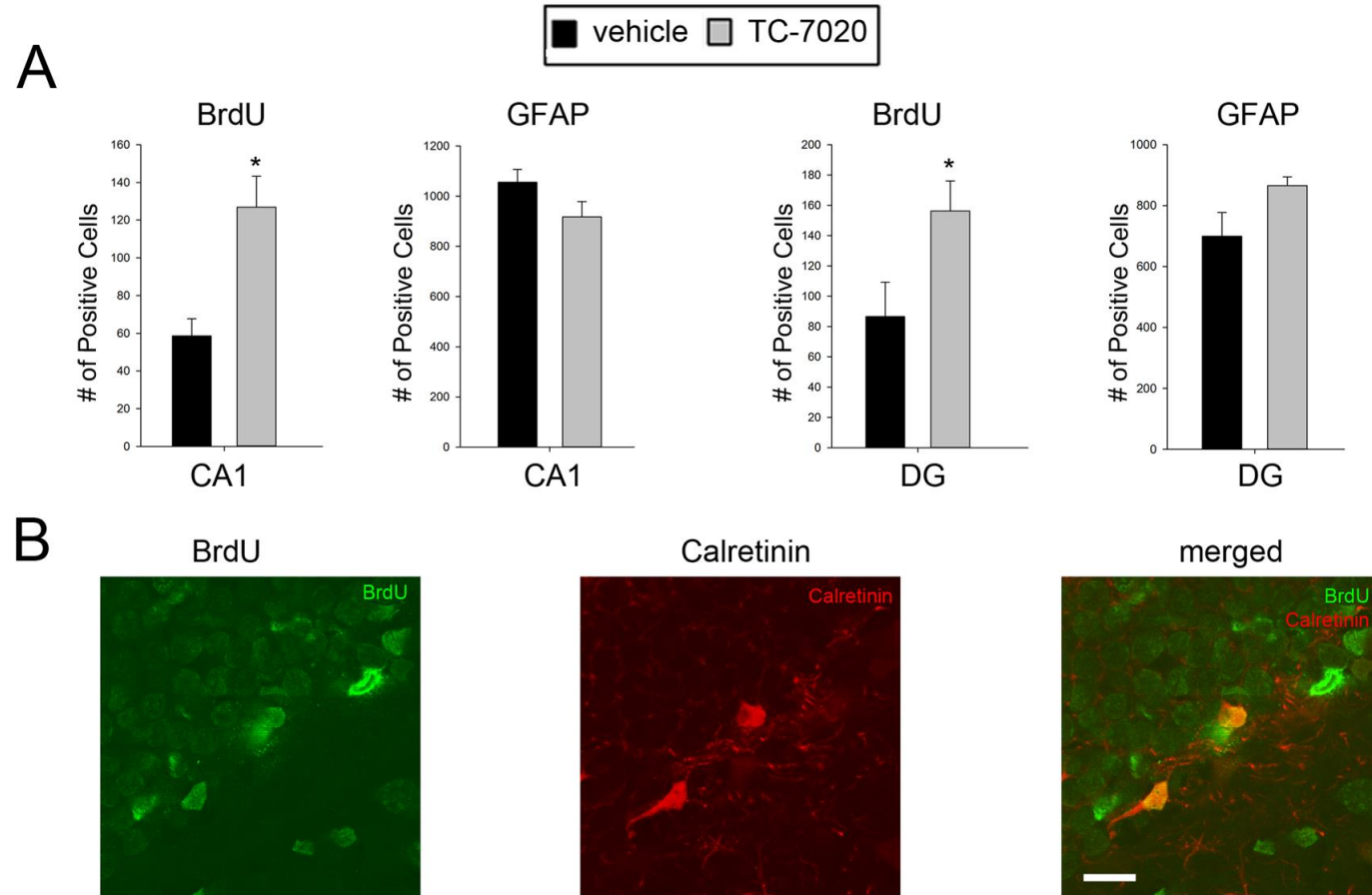
TC7020 Promotes Neuronal Differentiation of BrdU pre-labeled cells in brain cortex



(4.3-fold increase of new neurons)

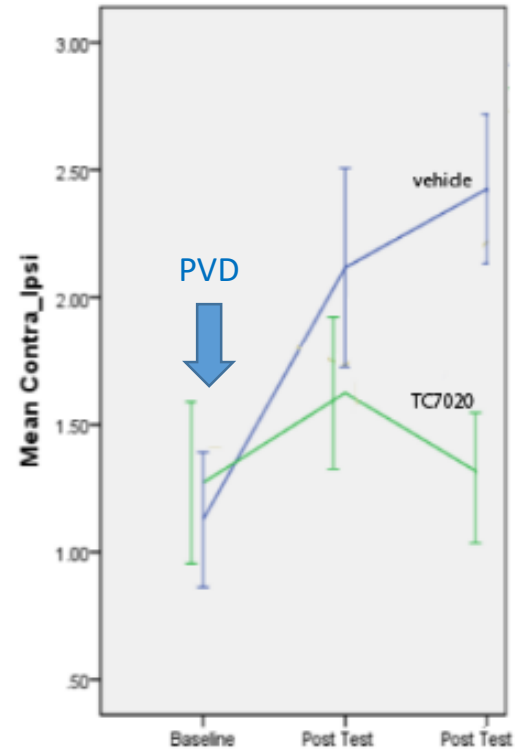
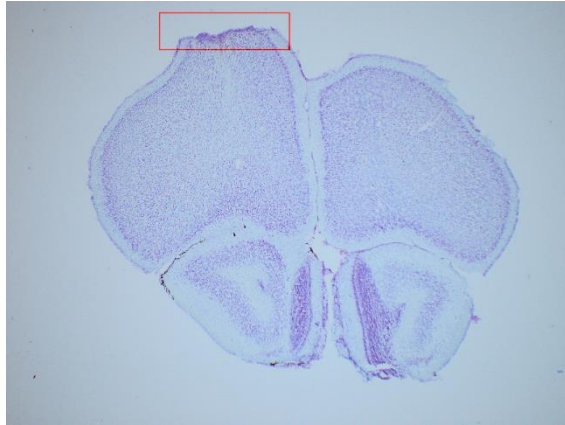


$\alpha 7$ Nicotinic Agonist (*TC7020*) activation of *Hippocampal* neurogenesis



$\alpha 7$ Nicotinic Agonist (TC7020) preserves motor coordination after Ischemic injury

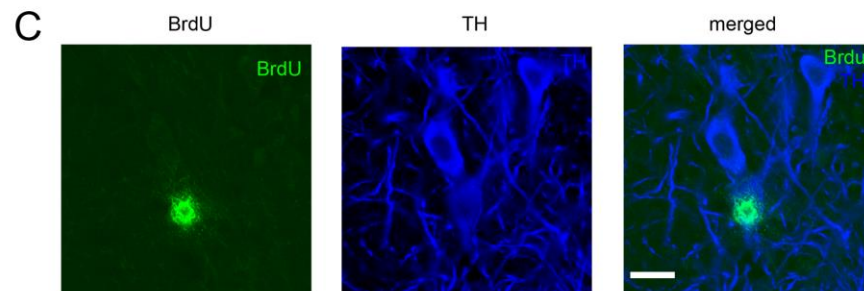
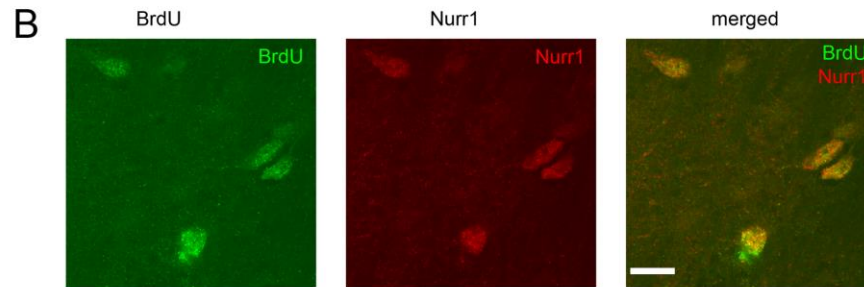
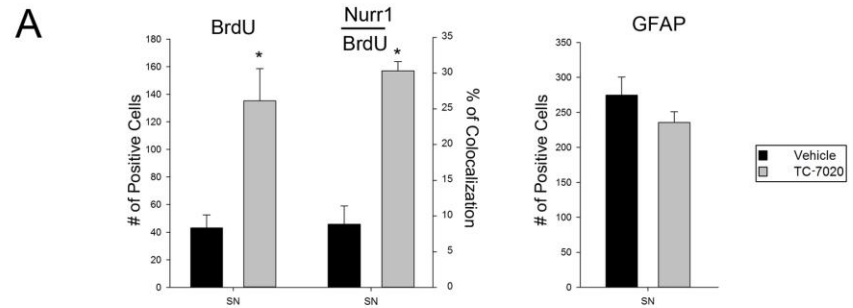
Pia Vessel Destruction
(PVD)



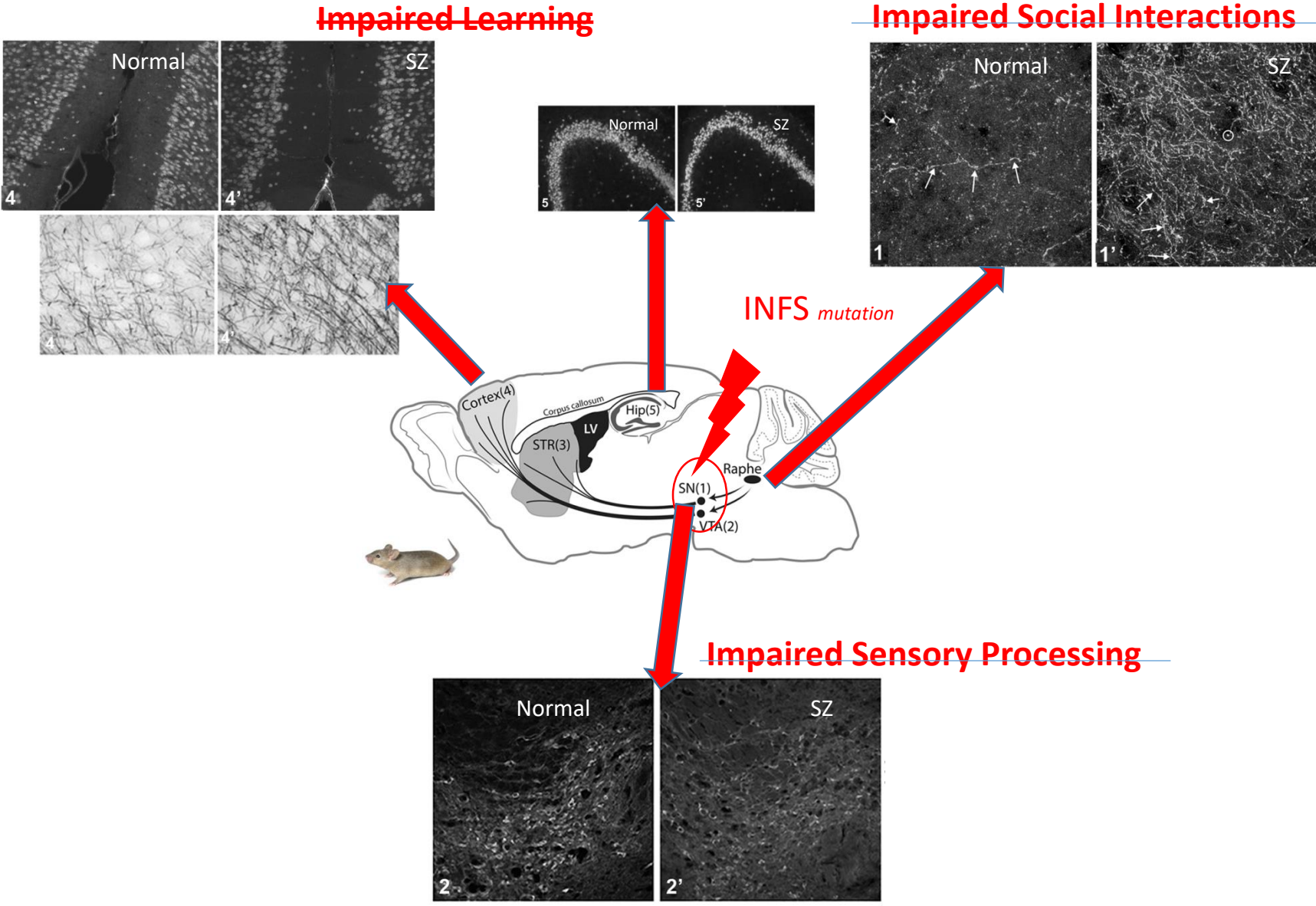
$\alpha 7$ Nicotinic Agonist (*TC7020*) activation of neuronogenesis in *S. Nigra*



(12-fold increase of new Nurr1 neurons)



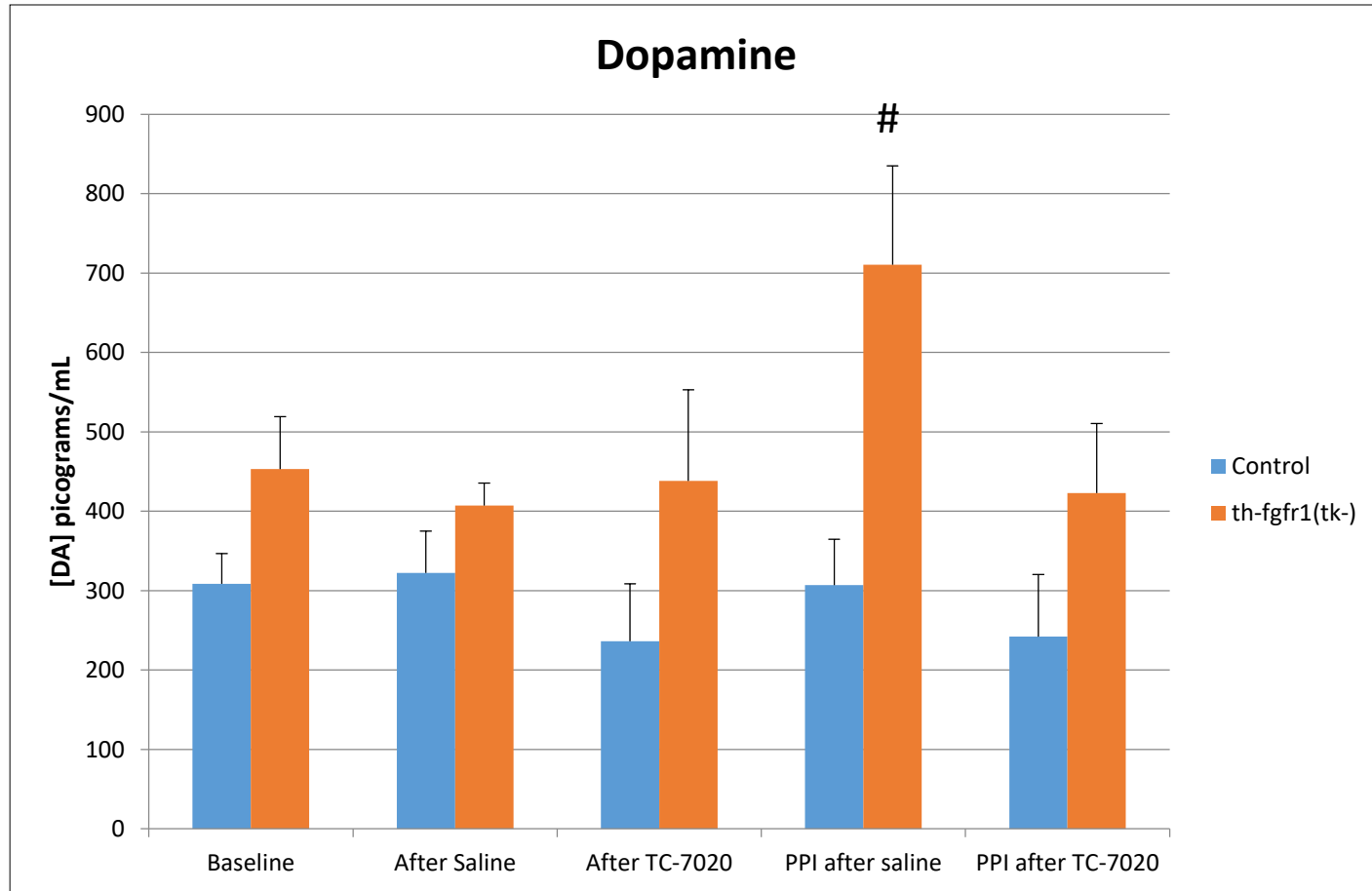
Schizophrenia-like symptoms reversed by TC7020 in transgenic mouse



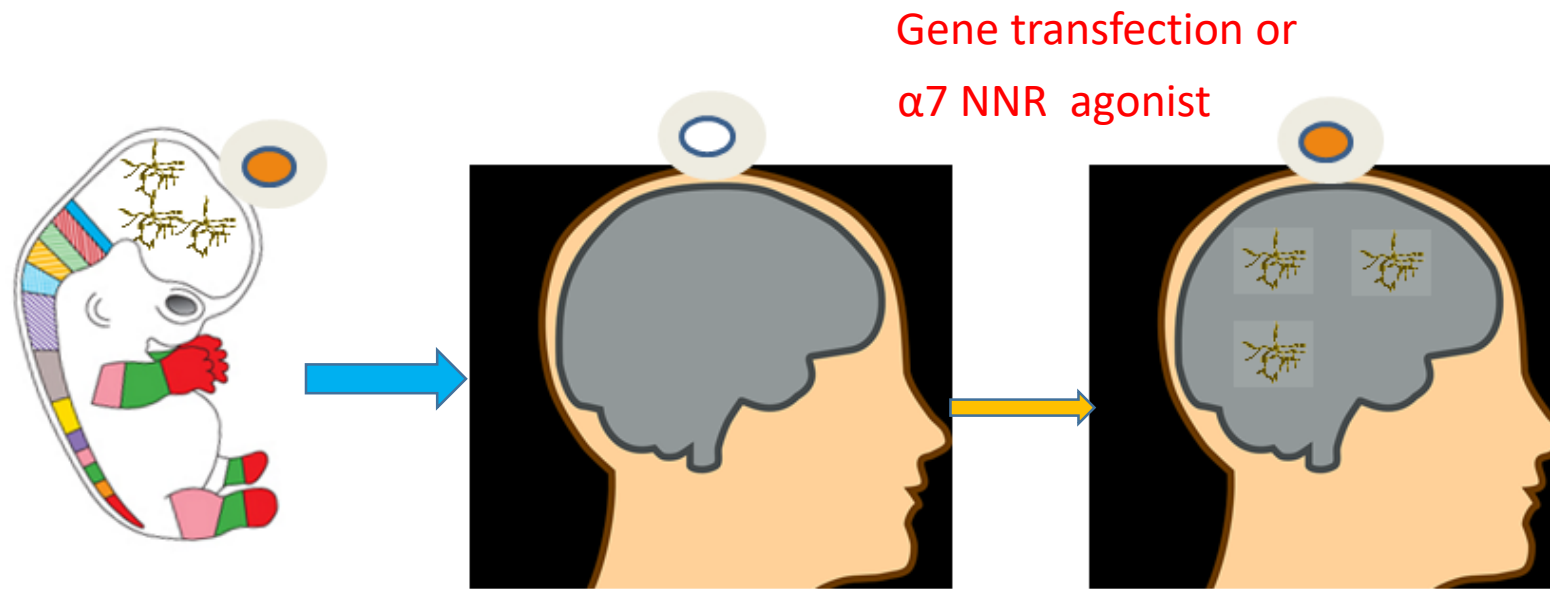
In vivo DA release in striatum microdialysis experiment.



TC-7020 normalizes Dopamine release in the striatum during acoustic PPI test th-fgfr1(tk-) mice



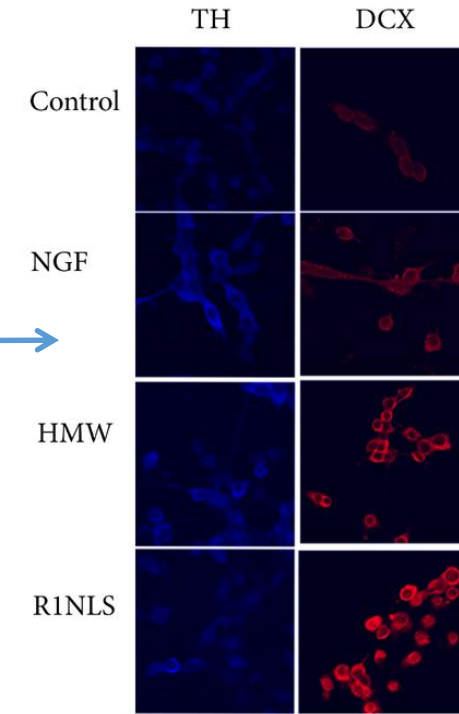
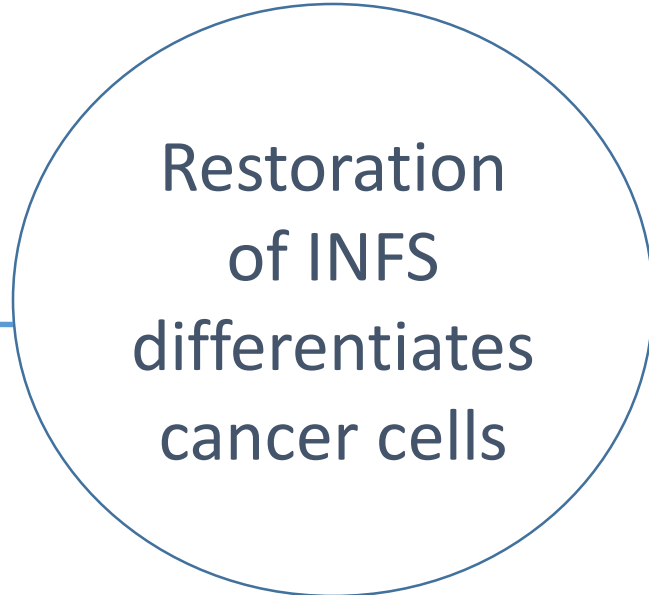
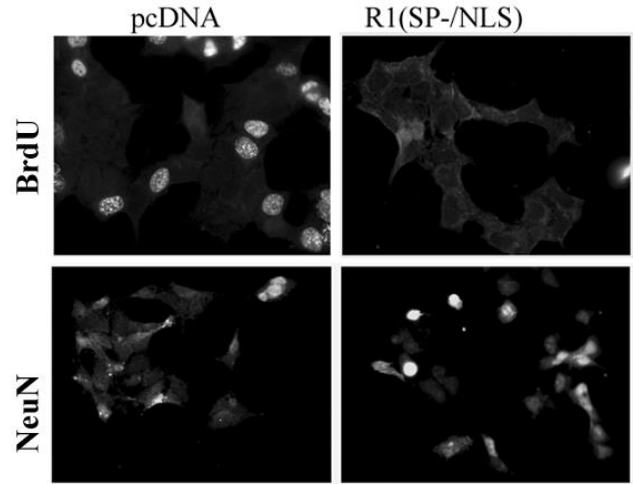
Overview



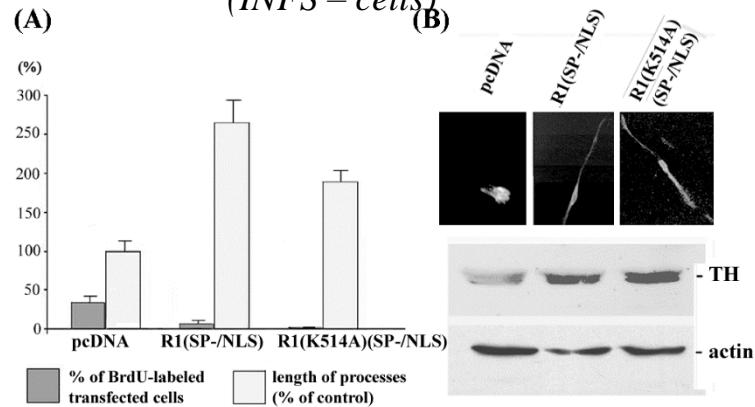
- nFGFR1 activation and neurogenesis can be reinstated in the adult brain by $\alpha 7$ nAChR agonist, TC-7020
- Results suggest a potential pharmacological therapy for neurodegenerative diseases and brain injury

Rat pheochromocytoma (PC12)
(INFS+ cells)

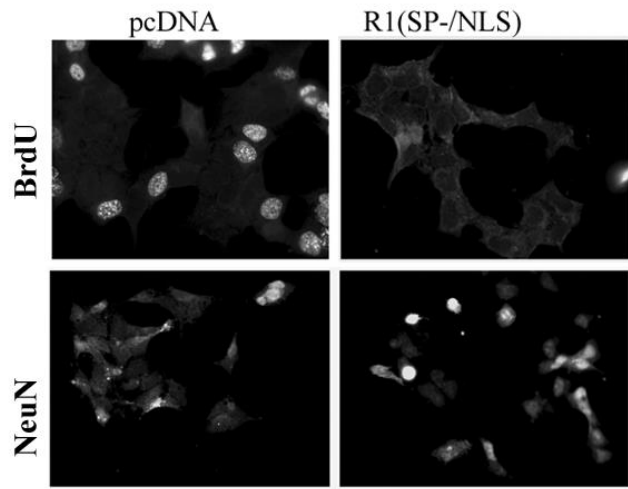
Human Neuroblastoma
(INFS+ cells)



Human Medulloblastoma (TE671)
(INFS - cells)

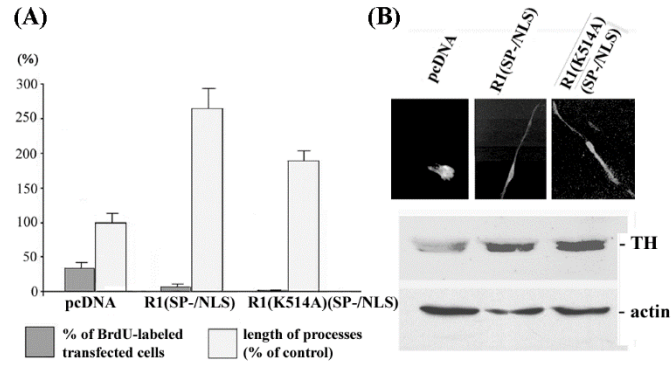


Human Neuroblastoma



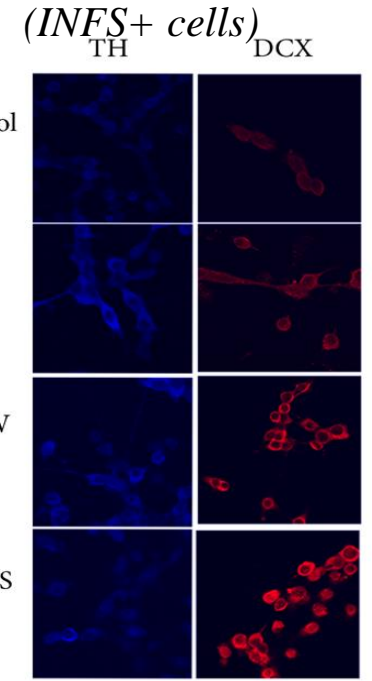
Mol. Biol. Cell, 2009, 20, 2401 – 2412.

Human Medulloblastoma (TE671)

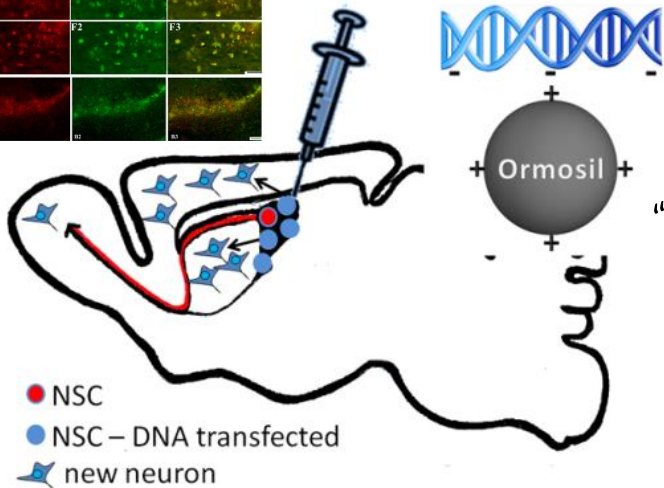
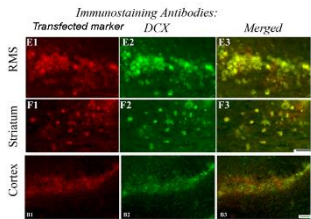
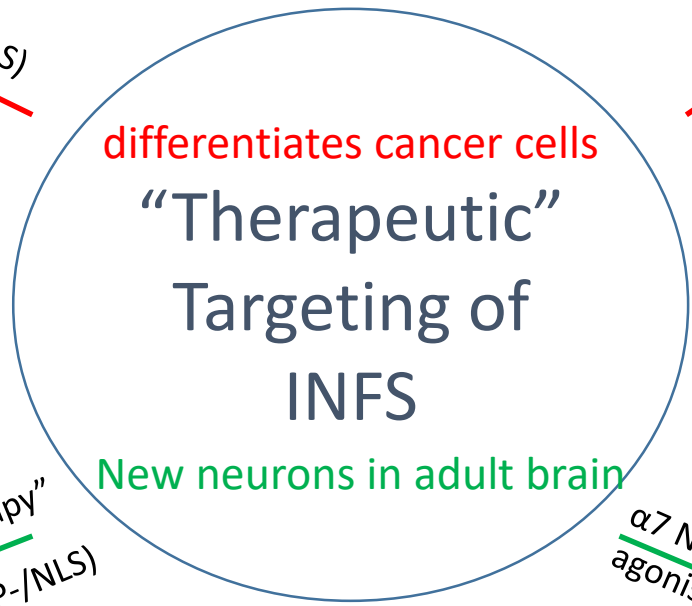


J. Biol. Chem. – 280(31):28451-62.

Rat pheochromocytoma (PC12)



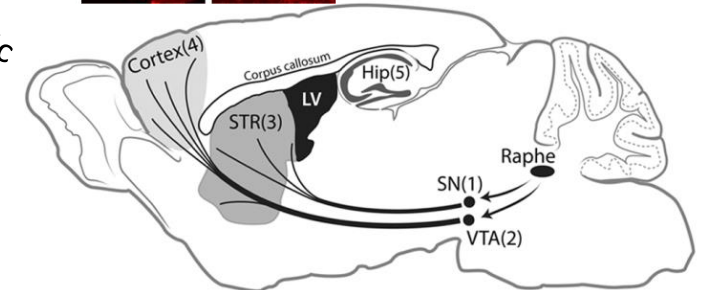
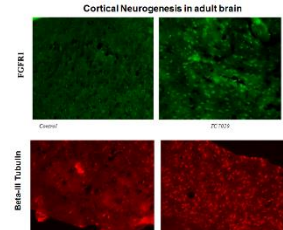
PloS one. 2013, 8:e68931



“Gene therapy”
FGFR1(SP-/NLS)

FGFR1(SP-/NLS)

α7 Nicotinic agonist



Stem cells translational medicine 2013, 2:776-788.

The End