INVESTICE
DO ROZVOJE
VZDĚLÁVÁNÍ

Podpora vytváření excelentních výzkumných týmů a intersektorální mobility na Univerzitě Palackého v Olomouci II

CZ.1.07/2.3.00/30.0041

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Comparative analysis of AhR expression and its target CYP genes in Neuroblastoma, hESC and hiPSC based neurons and related cell lineages

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Faculty of Science,
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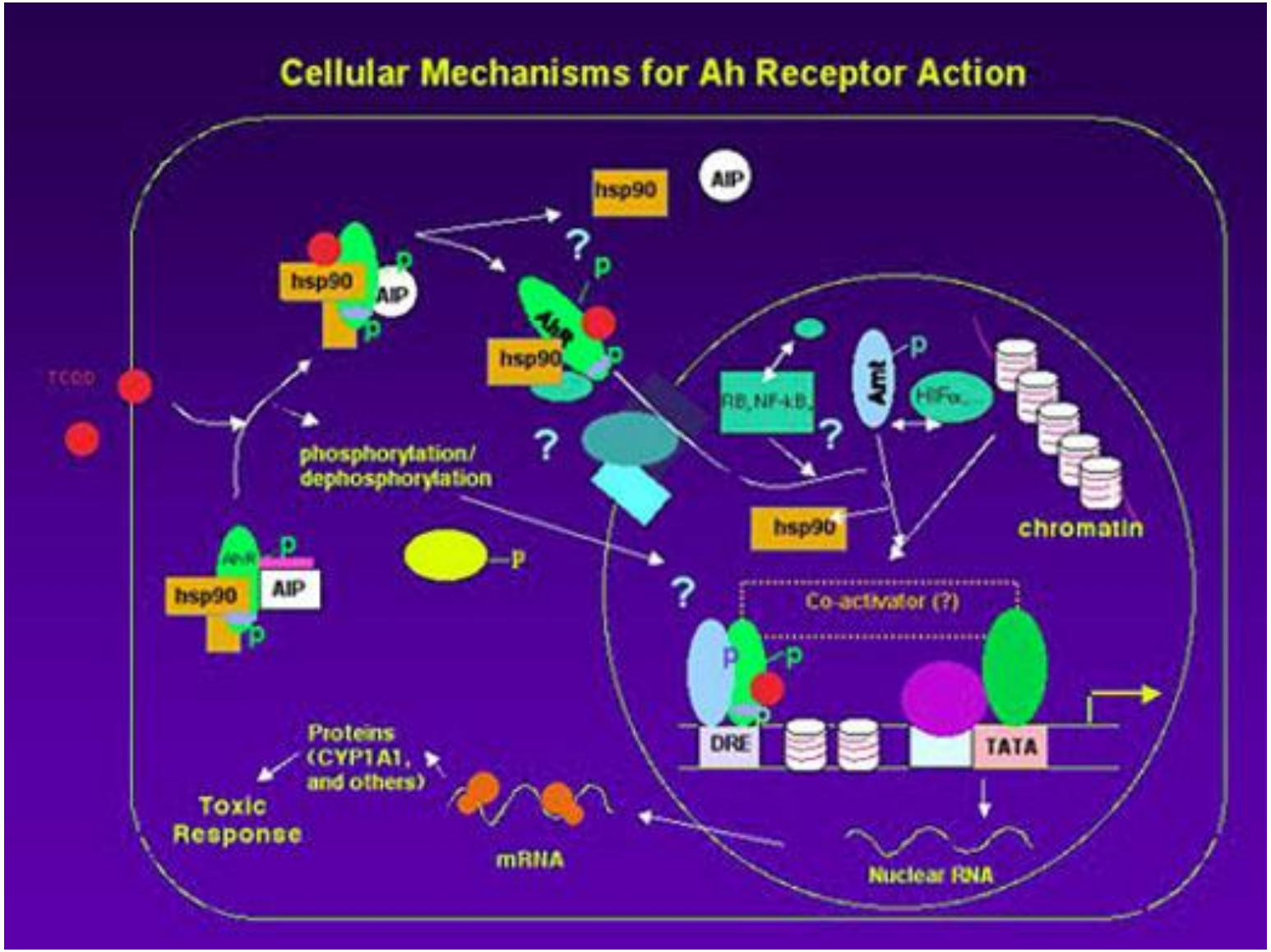
Why AhR?

- **Epidemiological studies link impaired mental status of children with heavily polluted reagions !!!**
- Hydrocarbon binds Ah receptor in cytosol
- Complex translocates → nucleus
- → Specific recognition sites on DNA for complex
- Increase expression of several genes, e.g. for biotransformation
  - Phase I - **CYP1A1** (Aryl hydrocarbon hydroxylase), **CYP1B1**, **AhRR**
  - Phase II enzyme


Cellular Mechanisms for Ah Receptor Action
hsp90
AIP
hsp90
AIP
AhR
hsp90
TCDD
phosphorylation/
dephosphorylation
RB, NF-kB,
Arnt
HIFα
chromatin
hsp90
AhR
hsp90
AIP
Co-activator (?)
DRE
TATA
Proteins
(CYP1A1,
and others)
Toxic
Response
mRNA
Nuclear RNA

AhR mediated toxicity can cause..

- Epithelial cell changes
  - Hyperplasia – Increased cell number with increased cell division
  - Hypoplasia – Decreased cell division → decreased number of proliferating cells
  - Important to changes in several organs
    - Hepatomegaly
    - Gastric mucosal changes
      - → Ulceration, hemorrhage
      - Species specific

Cytochromes P450 (CYPs):

- Were found in brain (abundant expression in liver, intestine, etc.).
- Catalyze metabolic activation and detoxification of a large number of Xenobiotics.
- metabolise several endogenous chemical, e.g. fatty acids, hormones, neurotransmitters, steroids, cholesterol and vitamins
- play important role in controlling brain activities, behavior, susceptibility and are involved in various brain disorders

Differentiation of selected cellular models and monitoring of AhR-mediated gene expression upon dioxin treatment

1. **Neuroblastoma cell line (SH-SY5Y)**
2. **Induced pluripotent stem cells (iPSCs)**
3. **Embryonic stem cells (ESCs)**

SHSY5Y Differentiation model to study NR expression:

Seeding
Density $2X10^4$ cells/cm^2

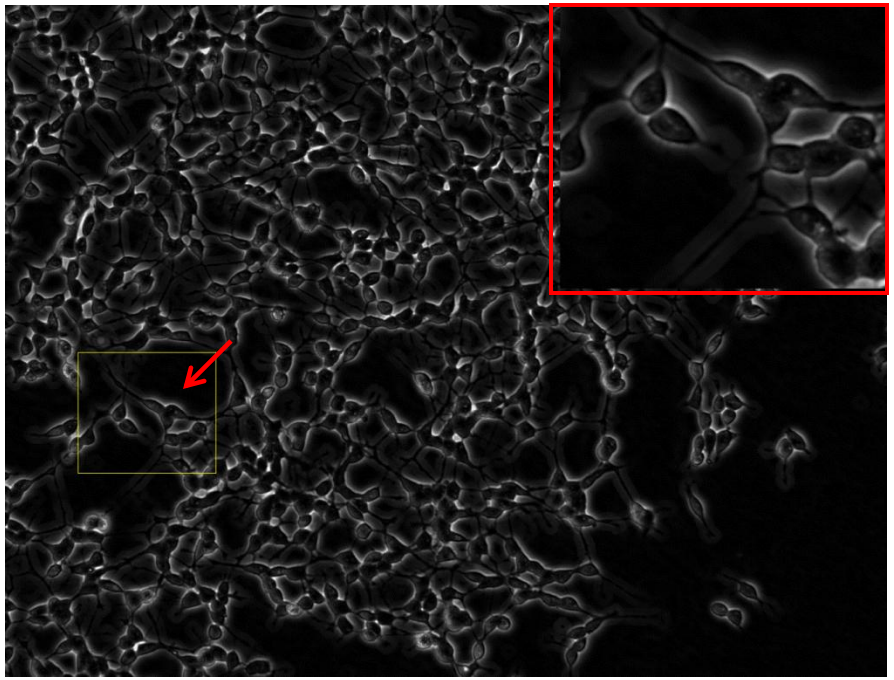
Retinoic Acid (10 µM)

Medium: DMEM/F12 with Glutamax, 10% FBS, 1% Pen strep, Laminin

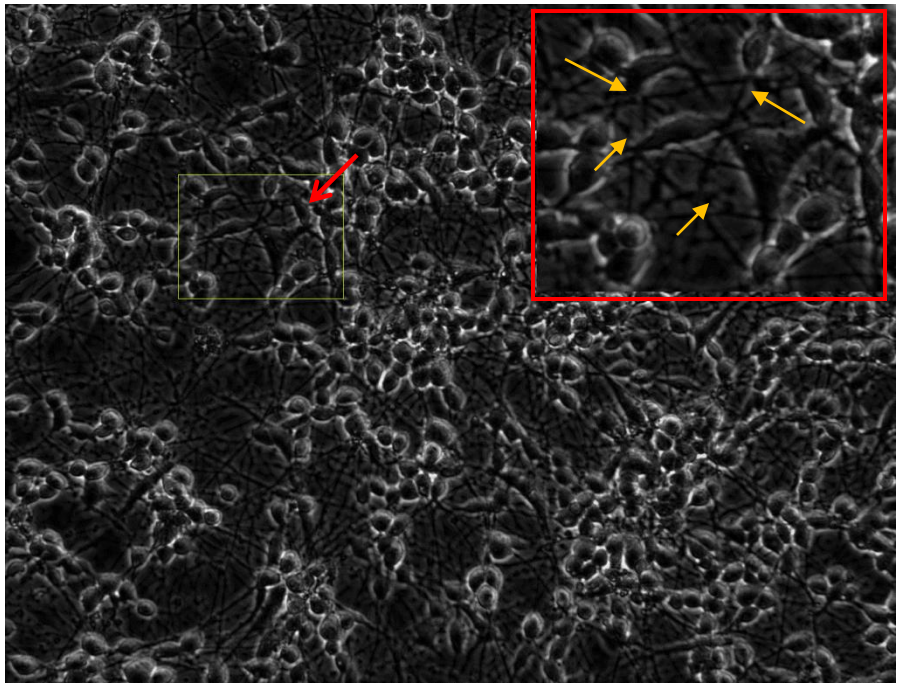
BDNF (50 ng/ mL)

Medium: DMEM/F12 with Glutamax, 1% Penstrep, Laminin

D1 D6 D10

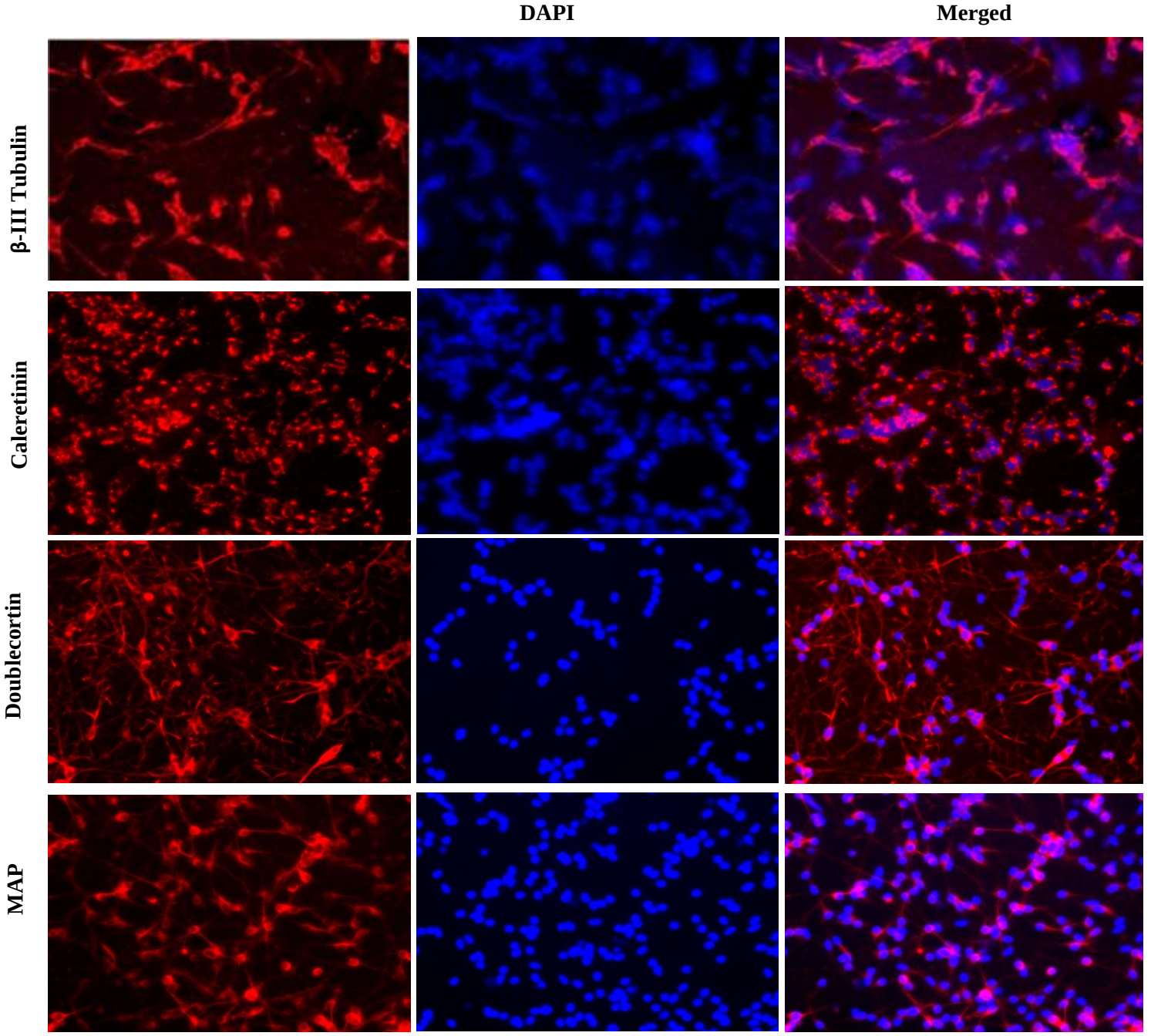


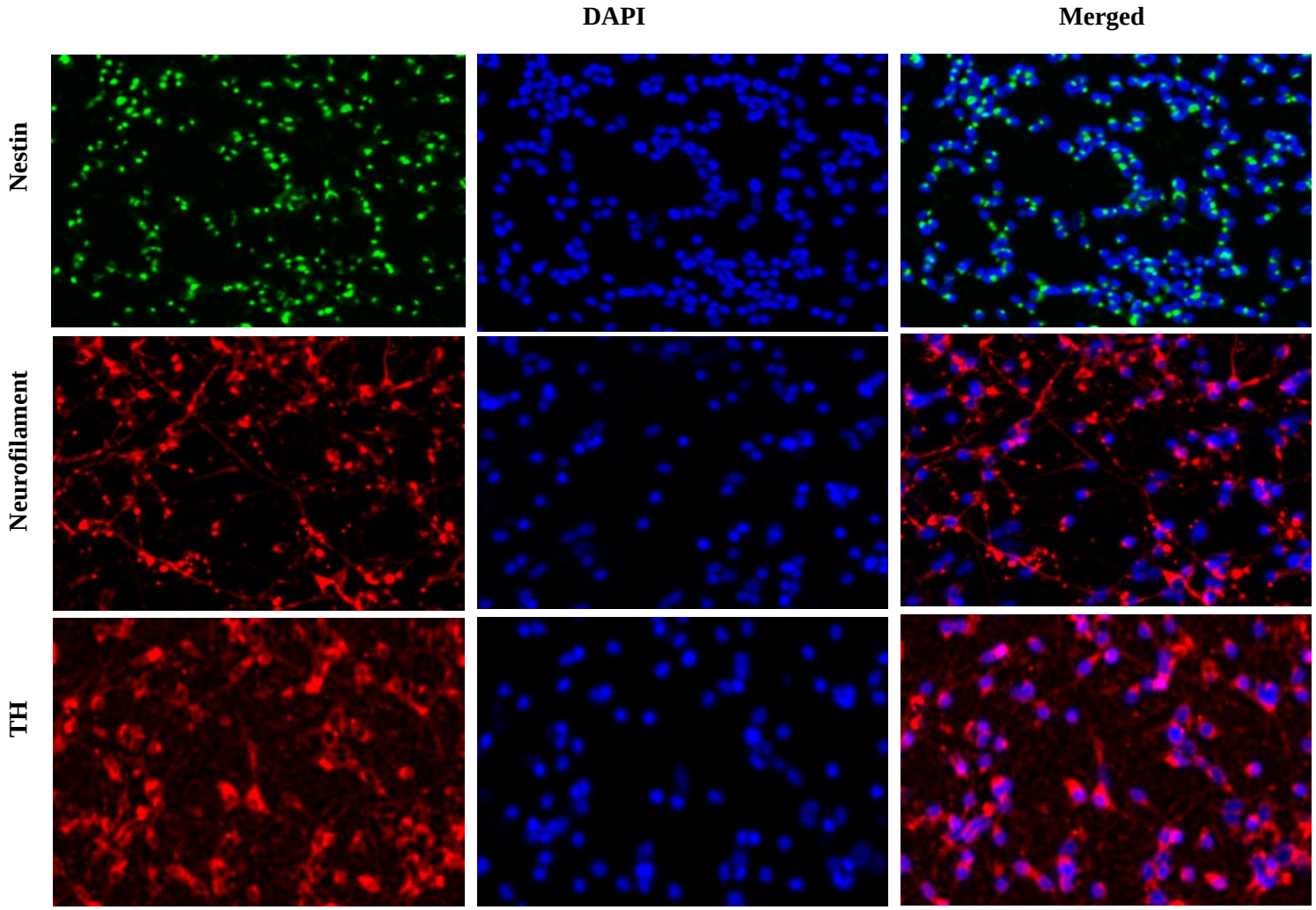
Undifferentiated SHSY5Y cells (D1)

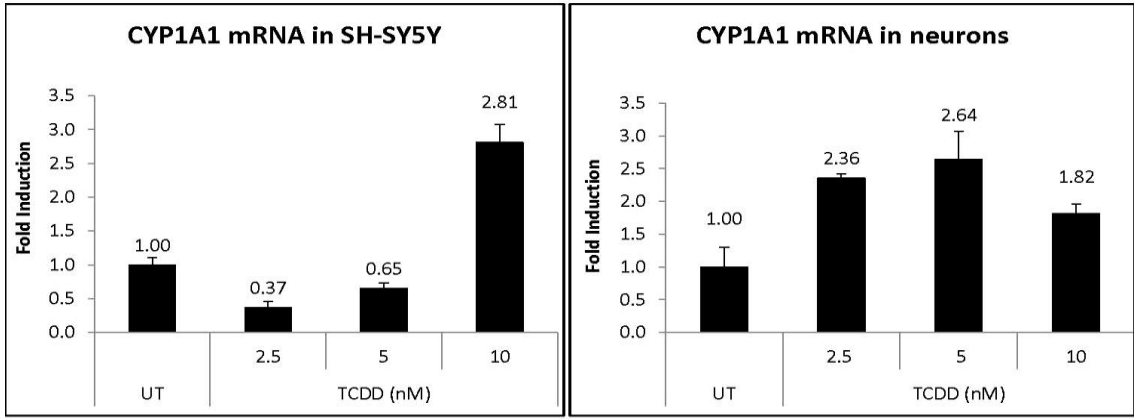


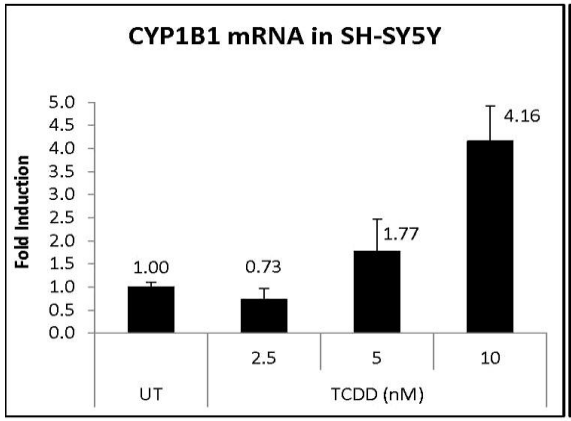
Differentiated SHSY5Y cells (D10)

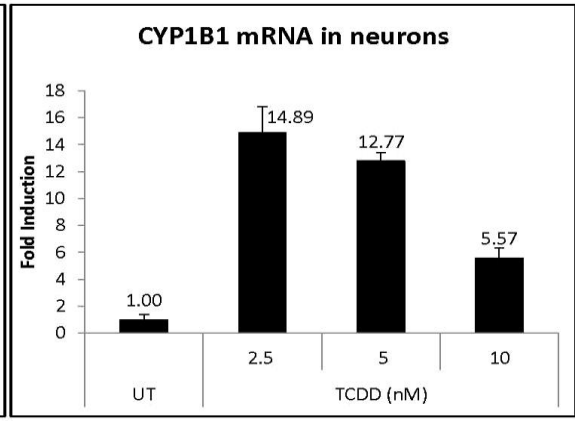
Expression of neuron-specific proteins in differentiated cells

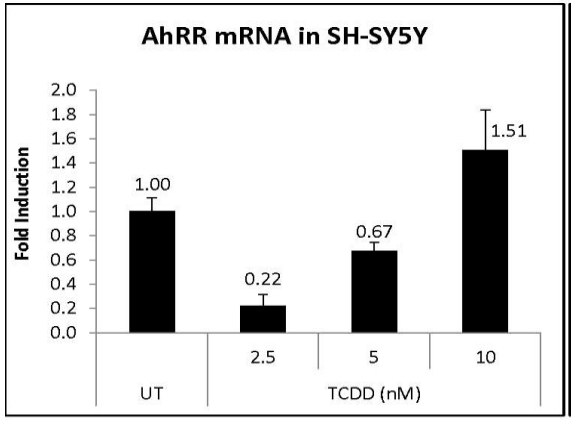


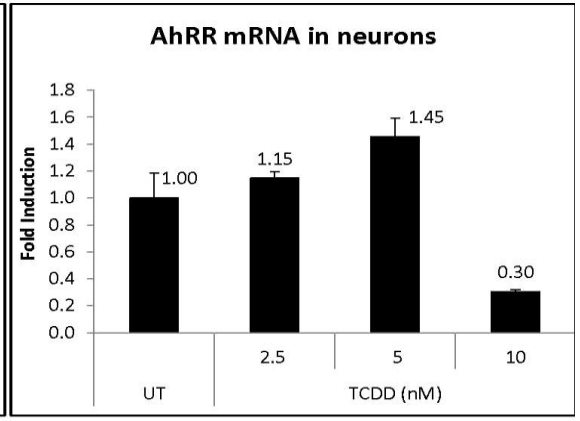

DAPI
Merged
Nestin
Neurofilament
TH











Submitted Paper

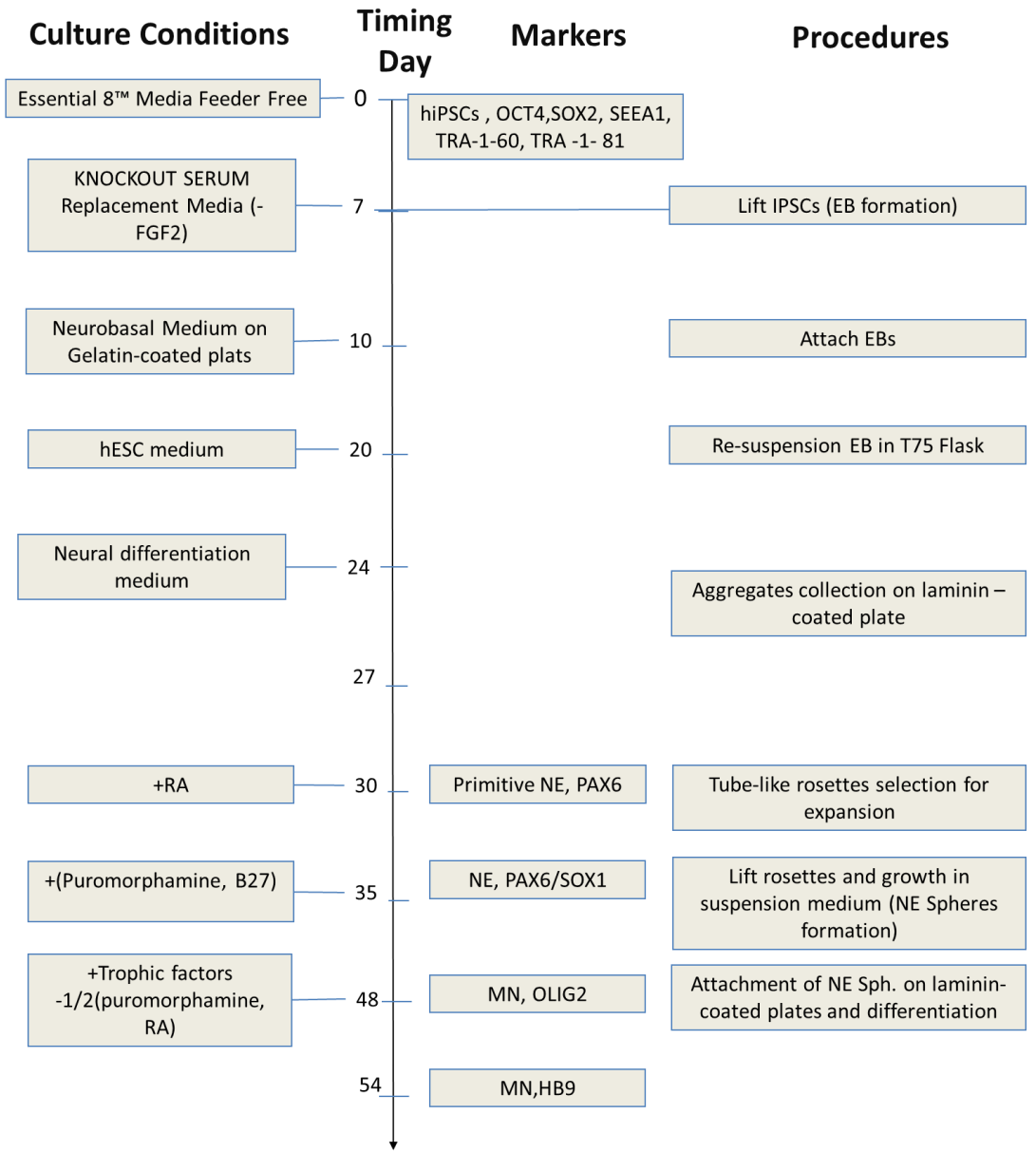
Saima Imran, Patrizia Ferretti, Radim Vrzal, Different regulation of aryl hydrocarbon receptor-regulated genes in response to dioxin in undifferentiated and neuronally differentiated human neuroblastoma SH-SY5Y cells. Neurotoxicology (Under review)

Differentiation of selected cellular models and monitoring of AhR-mediated gene expression upon dioxin treatment

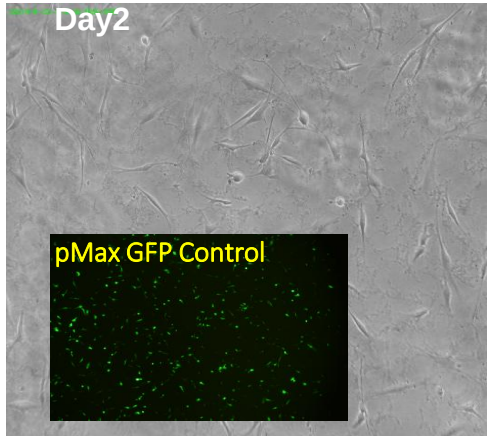
1. Neuroblastoma cell line (SH-SY5Y)
2. Induced pluripotent stem cells (iPSCs)
3. Embryonic stem cells (ESCs)

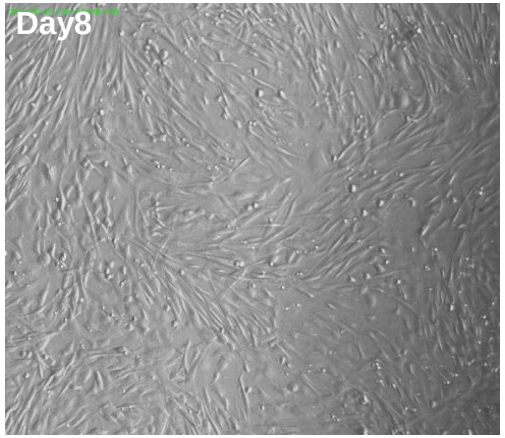
Differentiation of hiPSCs into motor neurons- model to study AhR expression

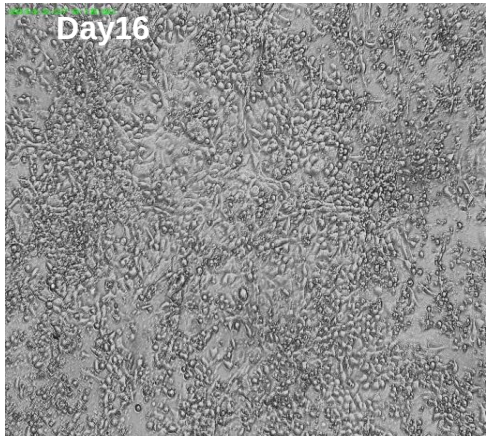
Differentiation Protocol Scheme

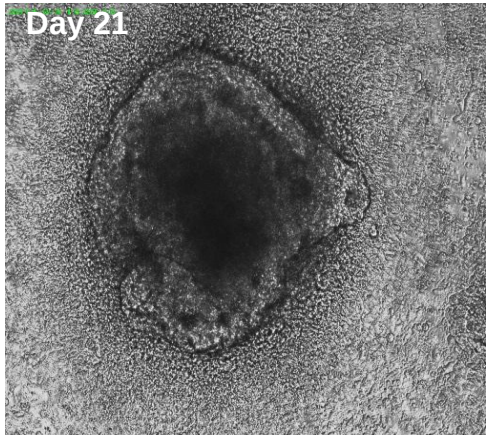


hiPSCs Colonies Development from human neonatal skin fibroblast

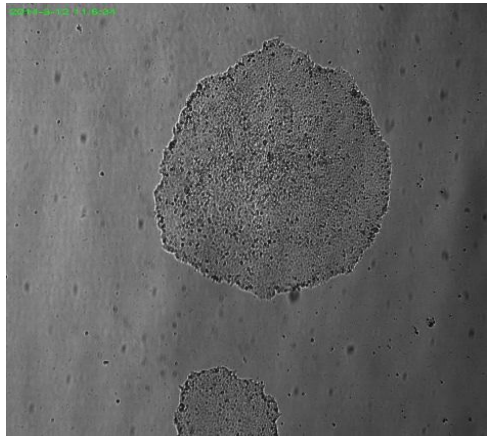


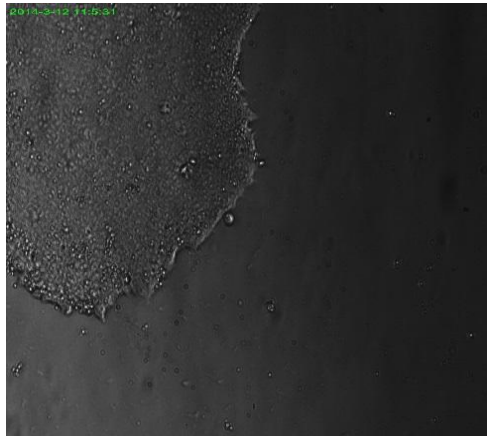


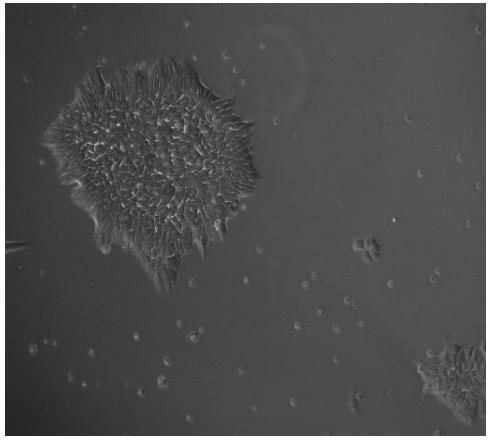


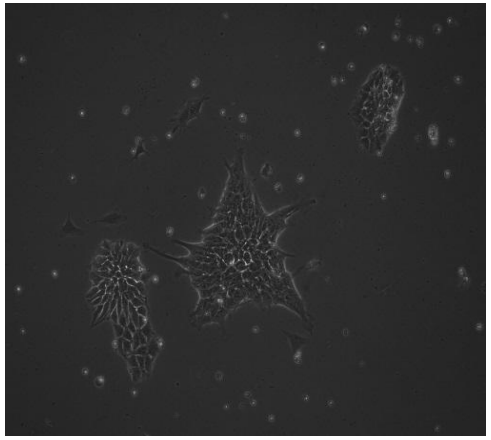


hiPSCs Colonies on feeder-free medium condition (Passage 1-9)

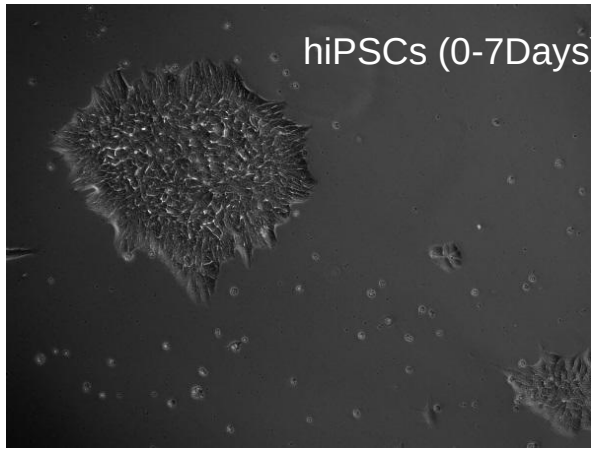


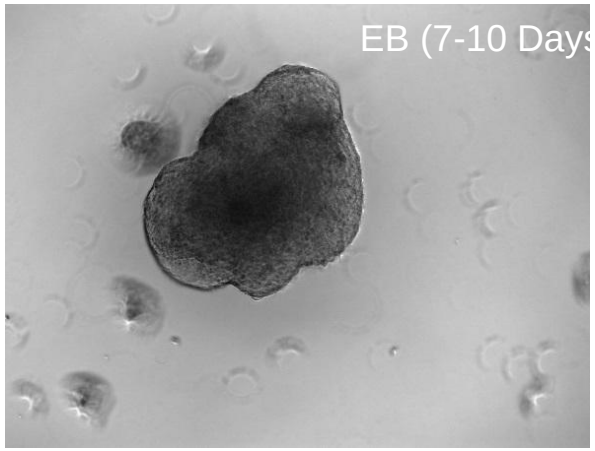


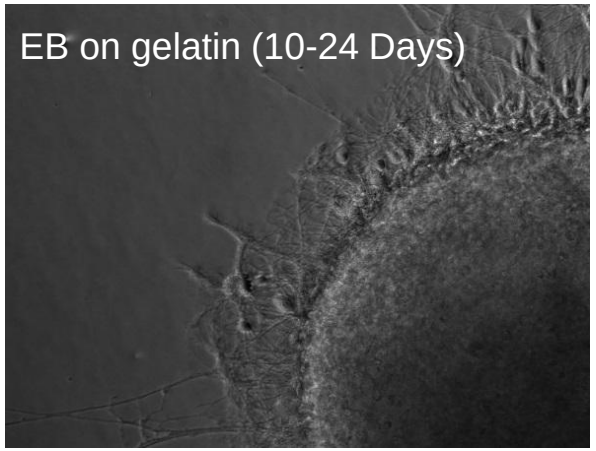


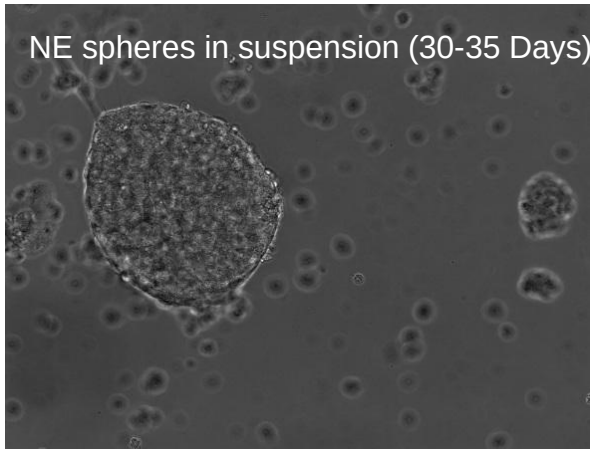


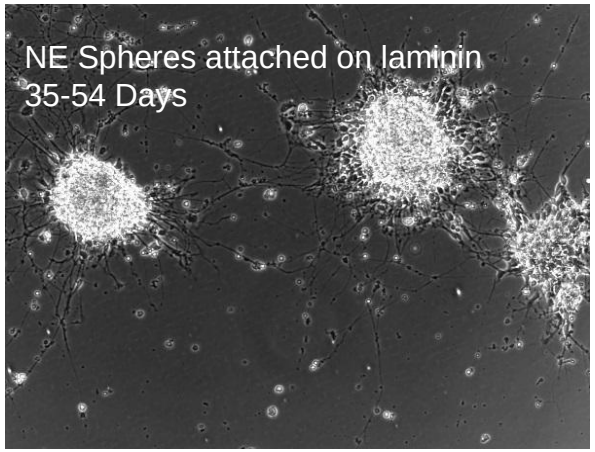
Differentiation of hiPSCs into matured neurons

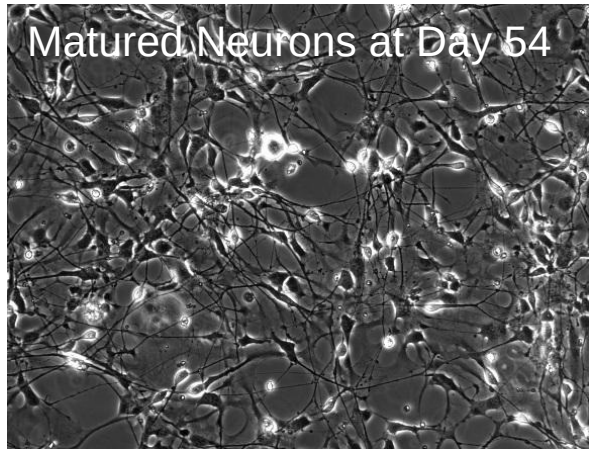












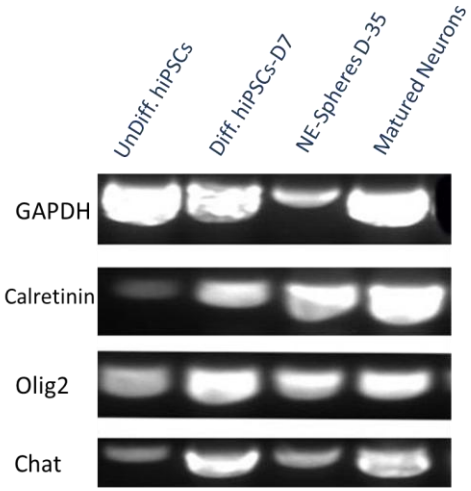
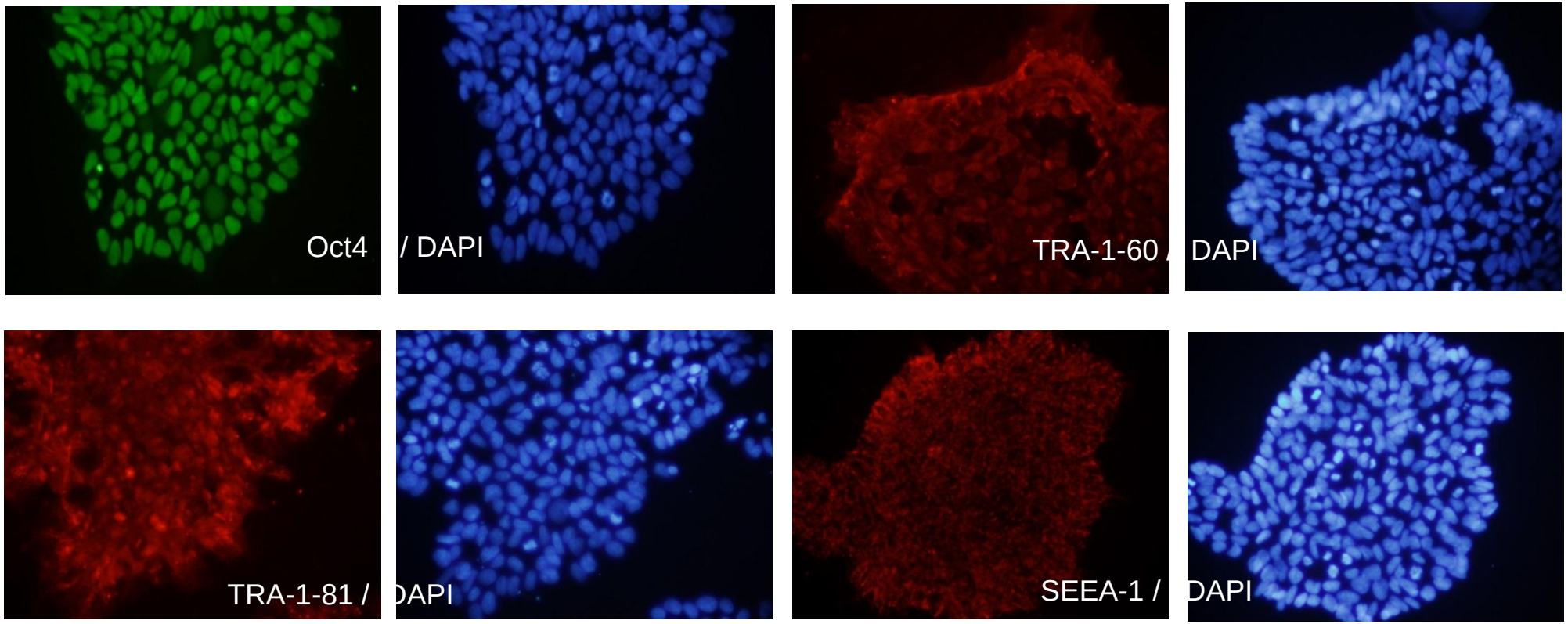
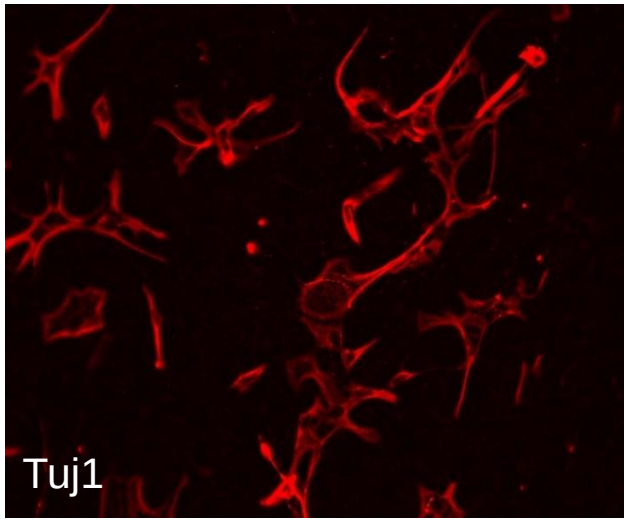


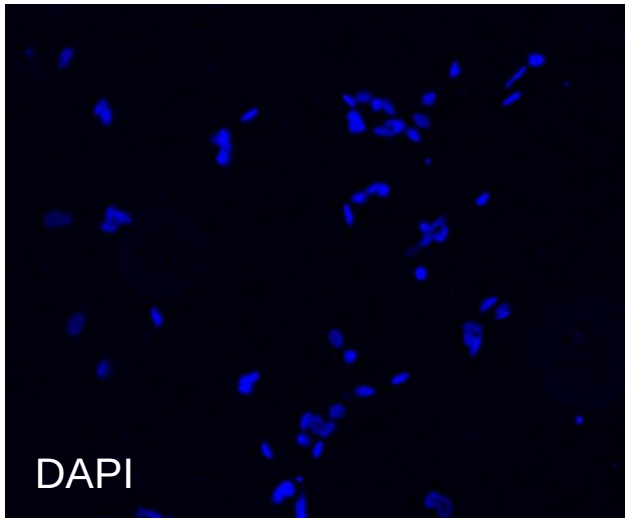
Fig. 8: qRT-PCR gel electrophoresis results showing motor neuron specific markers expression on mRNA level. qRT-PCR was carried out on Light Cycler 480 II apparatus (Roche Diagnostic Corporation, Prague, Czech Republic). The levels of all mRNAs were determined using primers with SYBR Green (Roche Diagnostic Corporation, Prague, Czech Republic) using protocol described elsewhere (7).

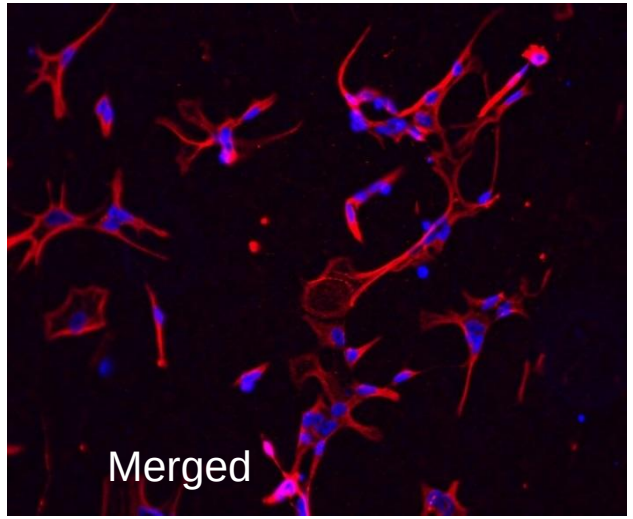
Immunofluorescence results of pluripotency markers in hiPSCs generated from Human Neonatal Fibroblasts.

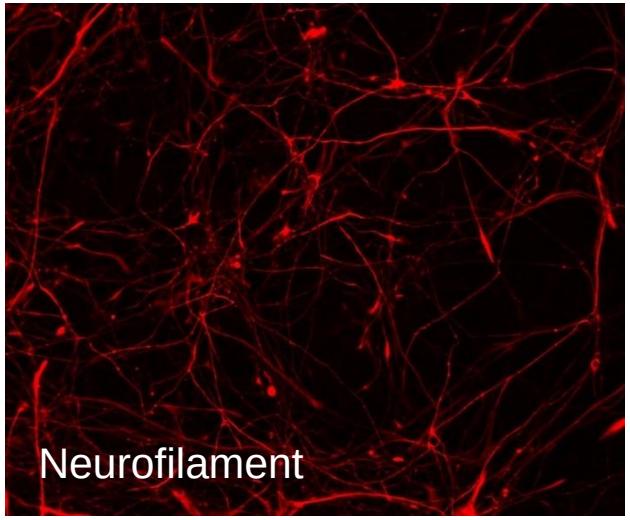


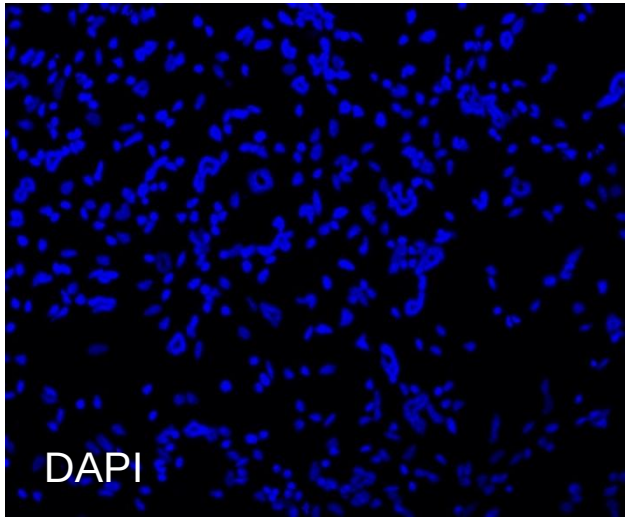
Immunofluorescence results of Neuronal markers in 6 weeks matured neurons

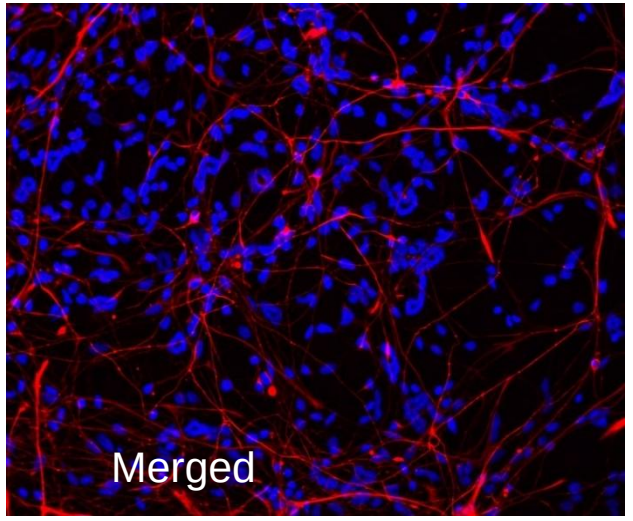


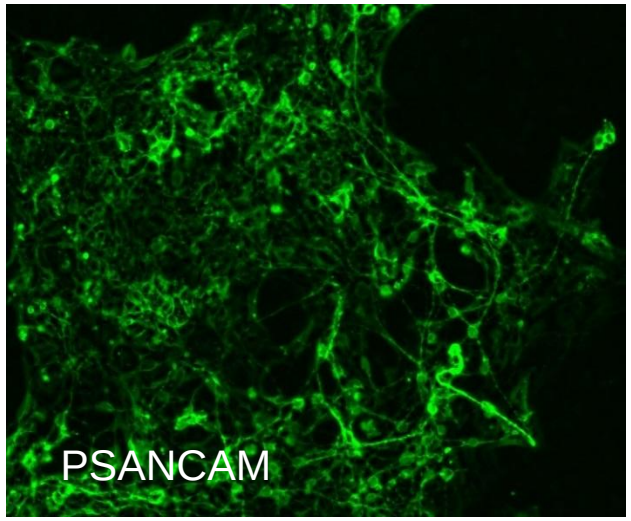


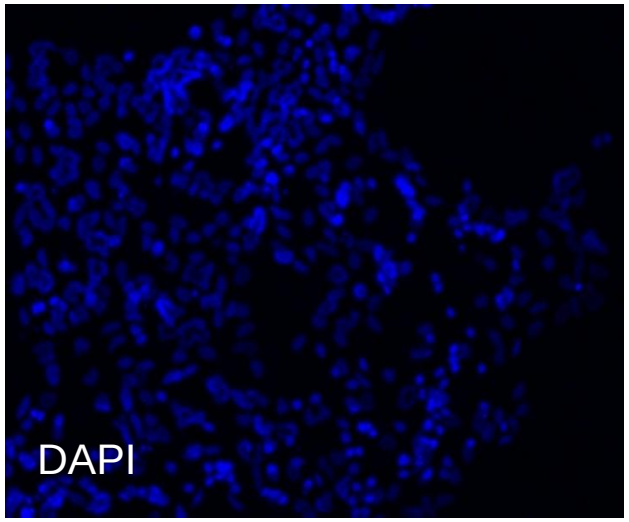


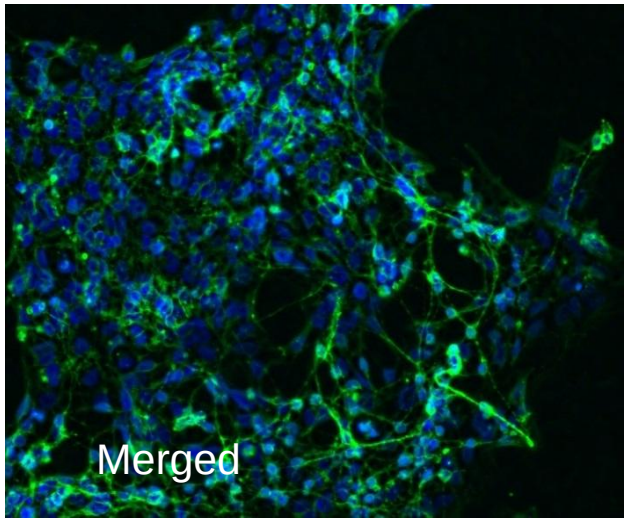


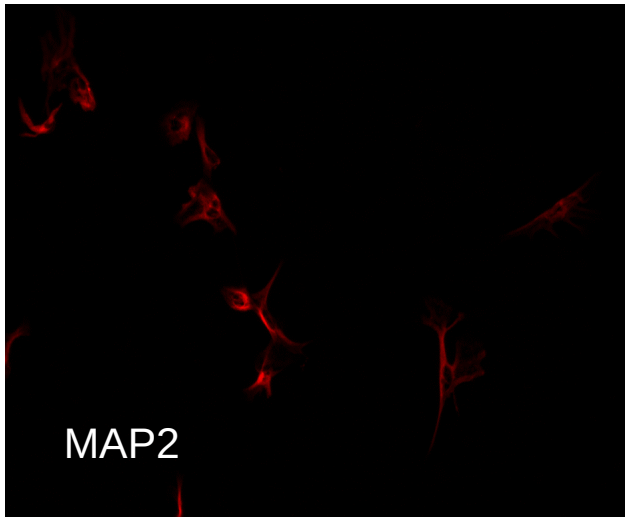


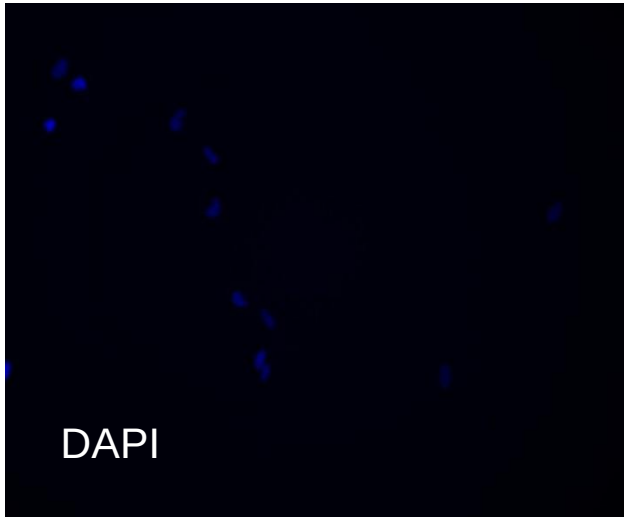


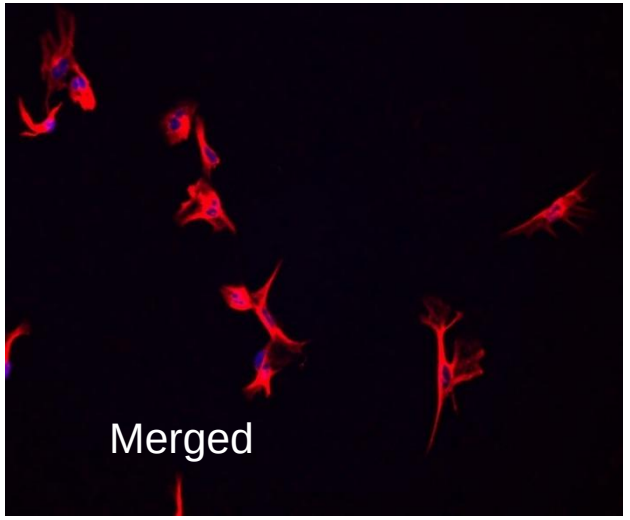

PSANCAM


DAPI


Merged


MAP2


DAPI

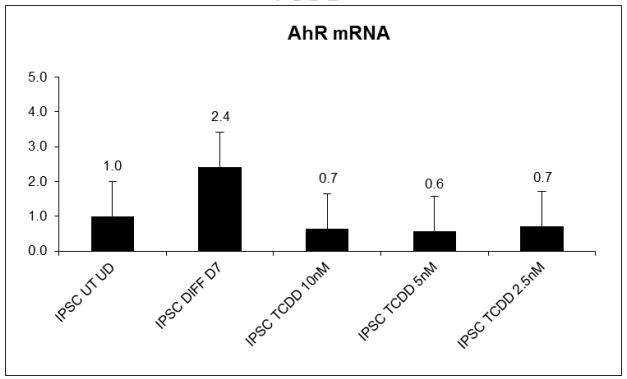

Merged

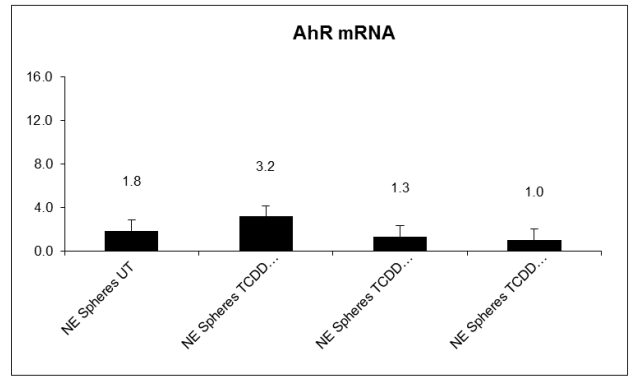
qPCR results for the expression of mRNA level of AhR Receptor and its target genes CYP1A1 and CYP1B1

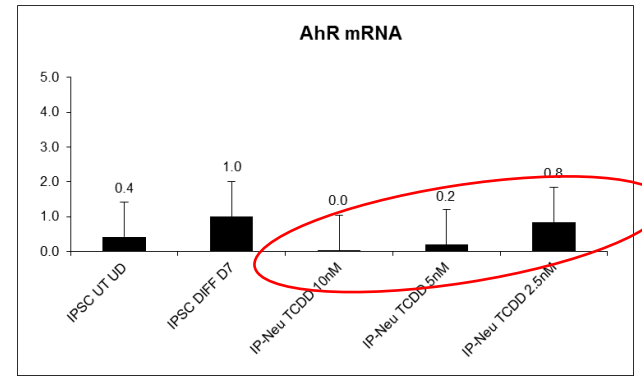
(a) Undifferentiated hiPSCs, differentiated hiPSCs at Day7 and hiPSCs treated with 10, 5 and 2.5nM TCDD

(b) Untreated and treated NE Spheres with 10, 5 and 2.5nM TCDD

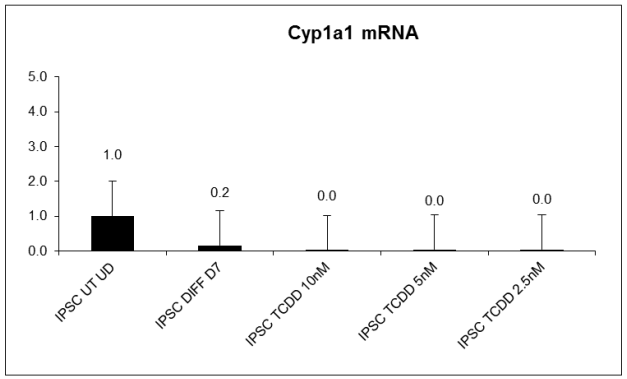
(c) Untreated 56 days matured neurons and Treated Neurons with 10, 5 and 2.5nM TCDD

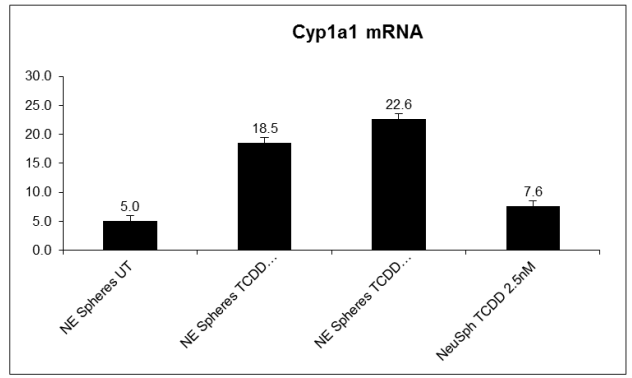


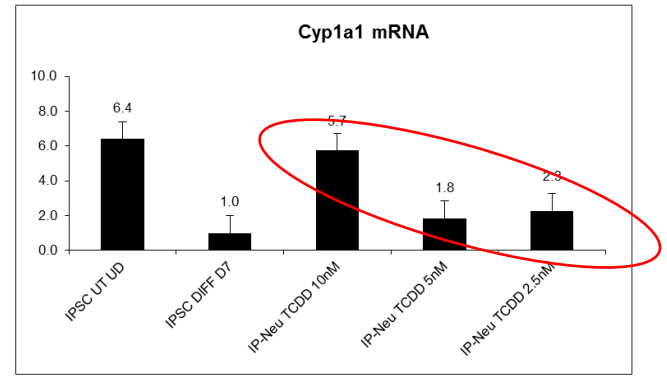




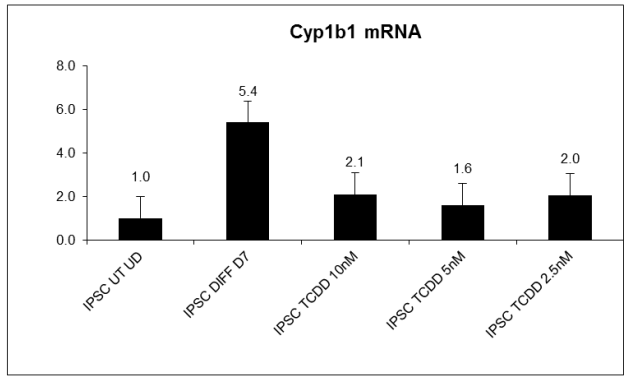
Downregulation in dose-dependent manner !!!!

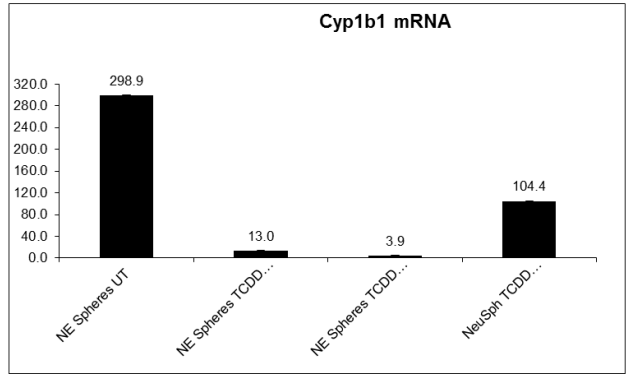


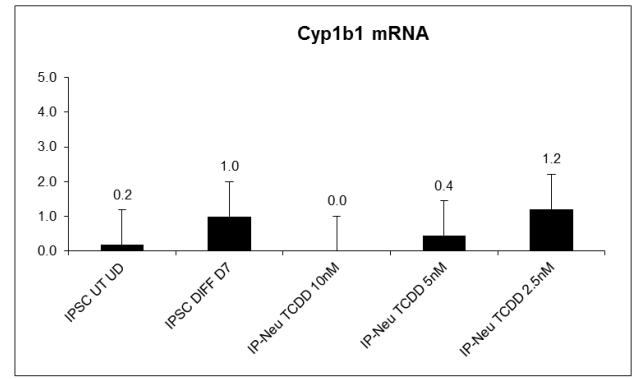




Induction in dose-dependent manner !!!!





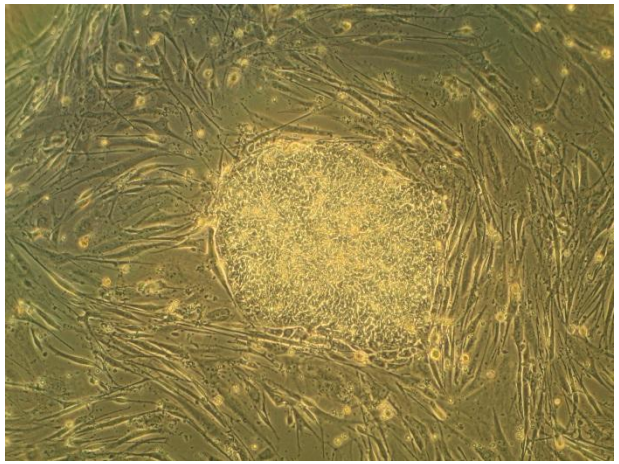


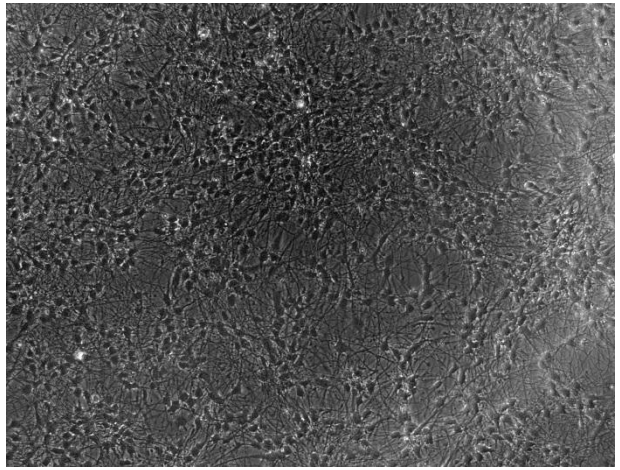
Differentiation of selected cellular models and monitoring of AhR-mediated gene expression upon dioxin treatment

1. Neuroblastoma cell line (SH-SY5Y)
2. Induced pluripotent stem cells (iPSCs)
3. Embryonic stem cells (ESCs)

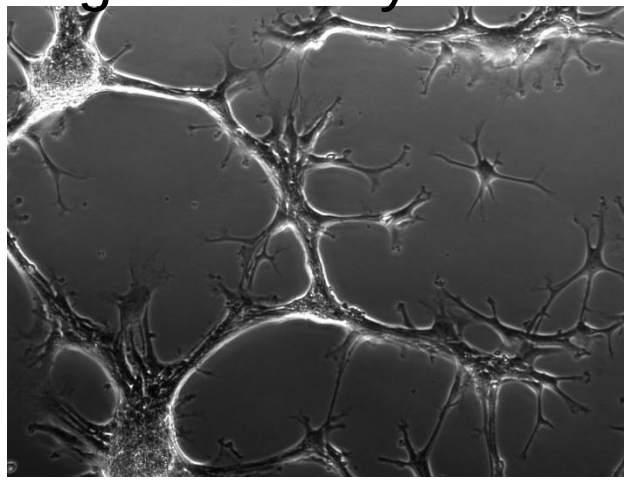
Matured Neurons

Human Embryonic Stem Cell (ESCs)



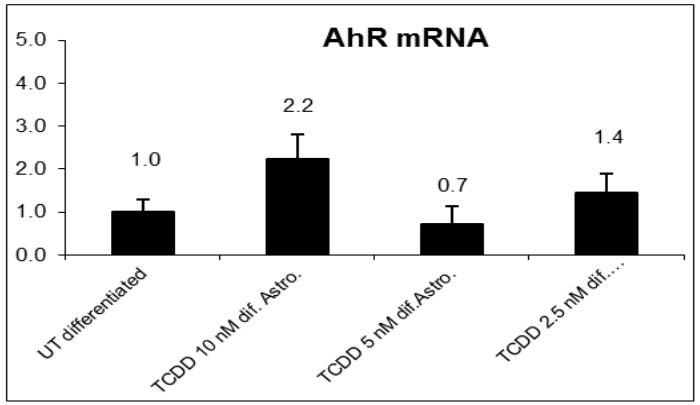


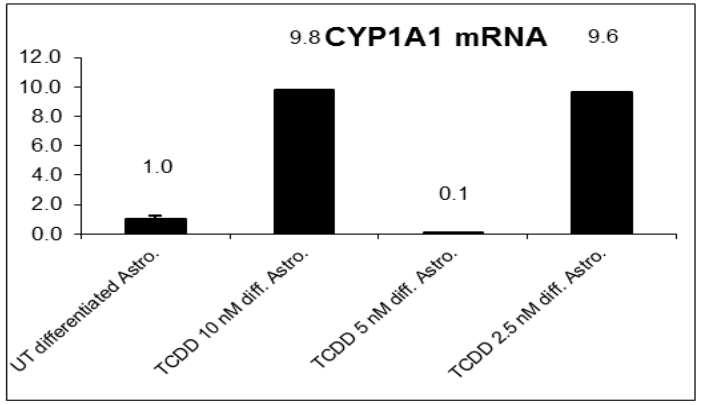
Oligodendrocytes

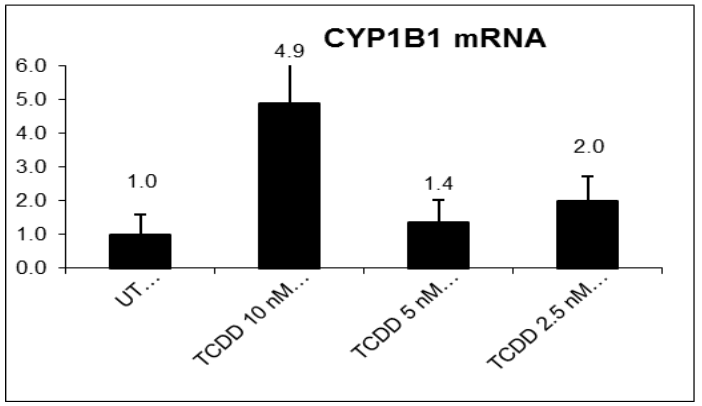


qPCR results for the expression of mRNA level of AhR Receptor and its target genes CYP1A1 and CYP1B1

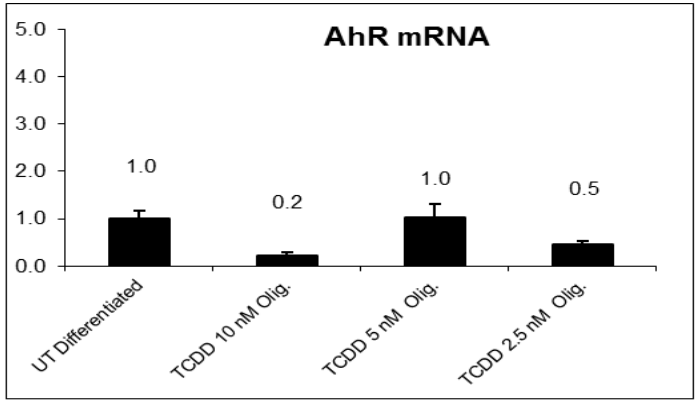
Astrocytes

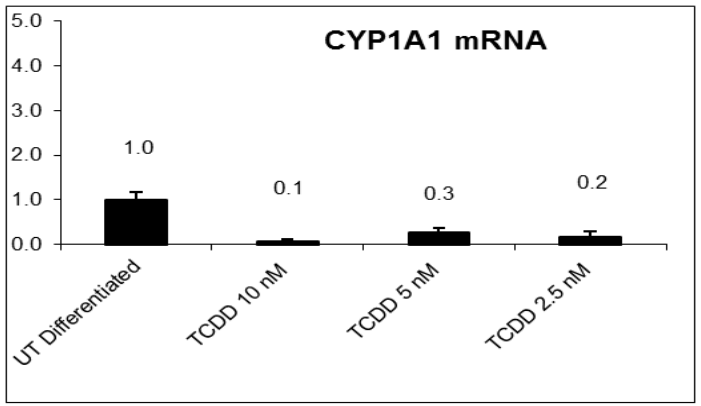


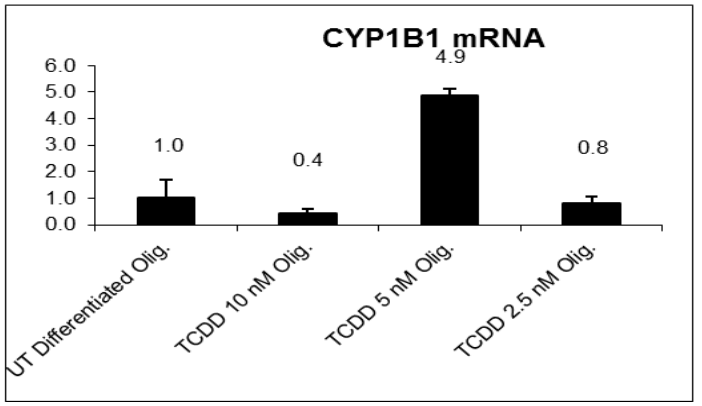




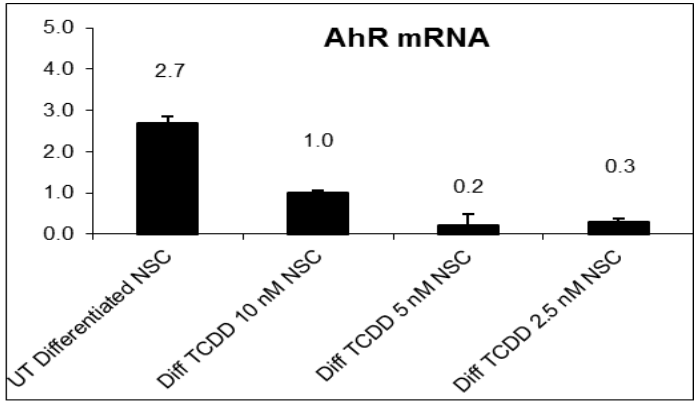
Oligodendrocytes

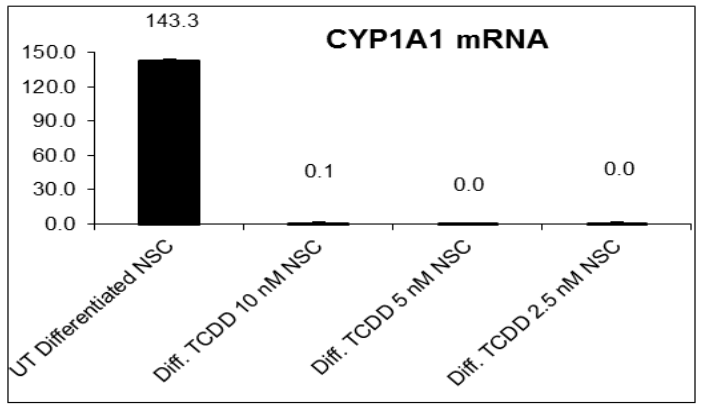


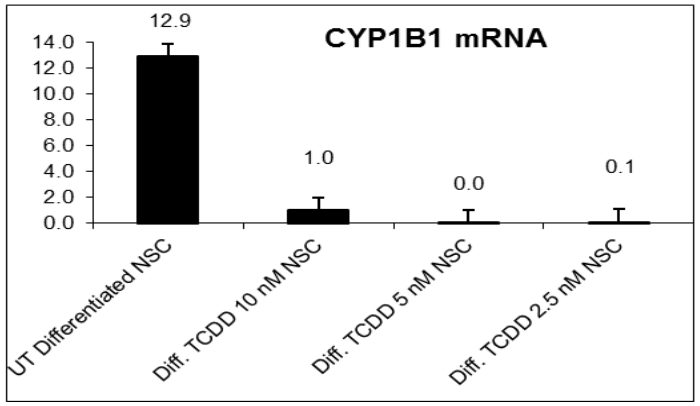




Neurons







Publications under process:

SAIMA J. IMRAN, PATRIZIA FERRETTI, **RADIM VRZAL**. Induced pluripotent stem cells (iPSCs)-differentiated neurons can be a suitable tool for neurotoxicological monitoring of aryl hydrocarbon receptor (AhR)-mediated signalling (Manuscript In preparation)

SAIMA J. IMRAN, PATRIZIA FERRETTI, BARBORA VAGASKA, **RADIM VRZAL**. Cell-specific aryl hydrocarbon receptor(AhR)-mediated signalling in matured neurons, oligodendrocytes and astrocytes differentiated from embryonic neural stem cells (Manuscript In preparation)

Conclusion

- ✓ Three different models suggested to study AhR expression and its targeted selected genes.
- ✓ SHSY5Y cell lines- differently behaving cell lines, did not followed the classical pathway for Ah Receptor expression
- ✓ hiPSCs model- not studied previously for this mechanism.
- ✓ hESCs model- not useful to study AhR-mediated signalling.