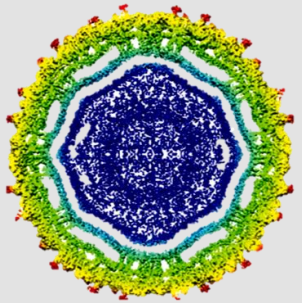
A high content screening platform to evaluate viral infection in human brain cells

Karina Karmirian; Pitia Ledur; Carolina Pedrosa; Leticia Souza; Luiza Higa; Amilcar Tanuri; Stevens Rehen

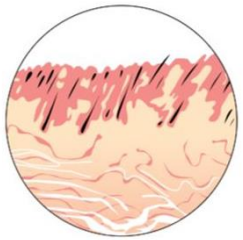
D'Or Institute for Research and Education
Federal University of Rio de Janeiro



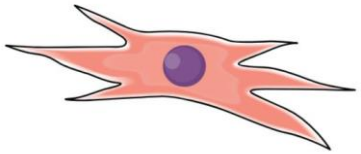
UFRJ

INSTITUTO D'OR
PESQUISA E ENSINO

Automated platform to evaluate cellular responses to viral infection in human neural cells derived from induced pluripotent stem (iPS) cells

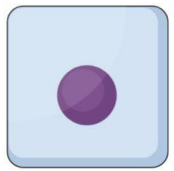


Skin Biopsy



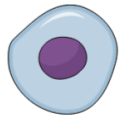
Human Fibroblast

Urine Samples

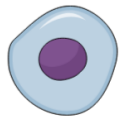


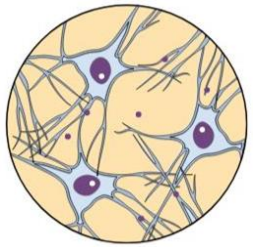
Human
Urine Epithelial Cell

Reprogramming

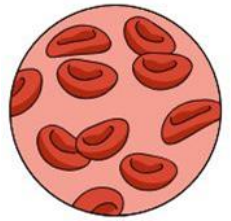


Human iPS cells

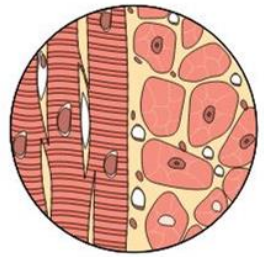




Neural Cells



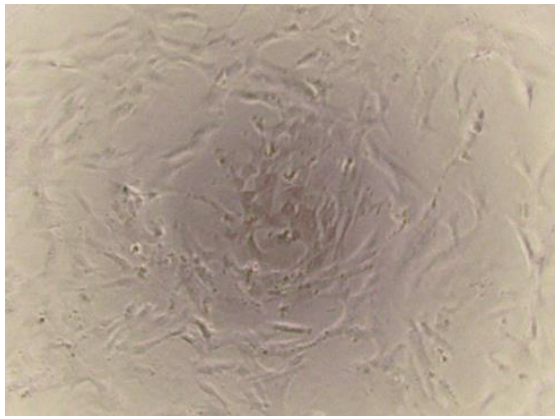
Blood Cells



Cardiac Cells

Automated analysis system

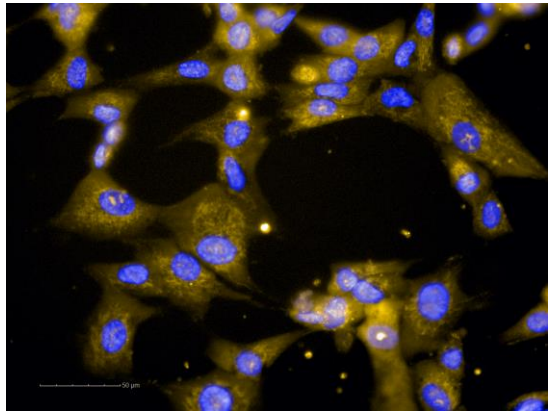
Brain cells derived from human iPS cells



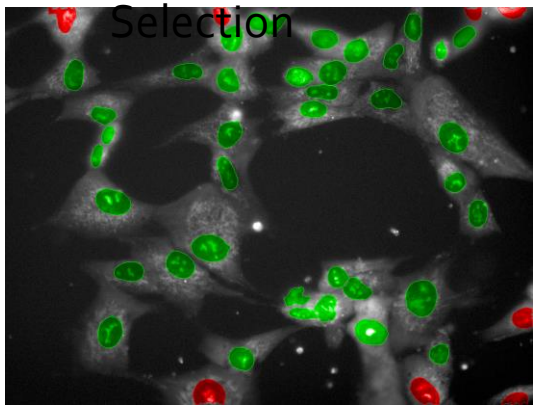
Dye incubation

Image Acquisition

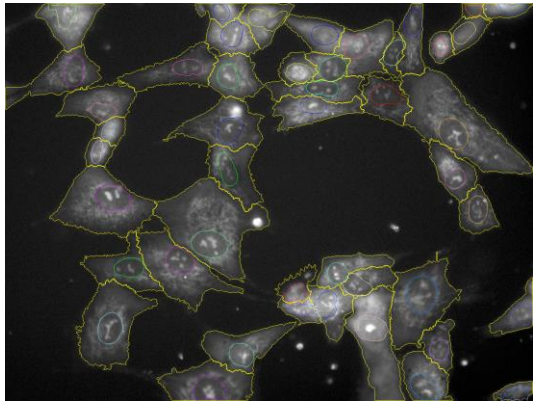
High-content screening microscope



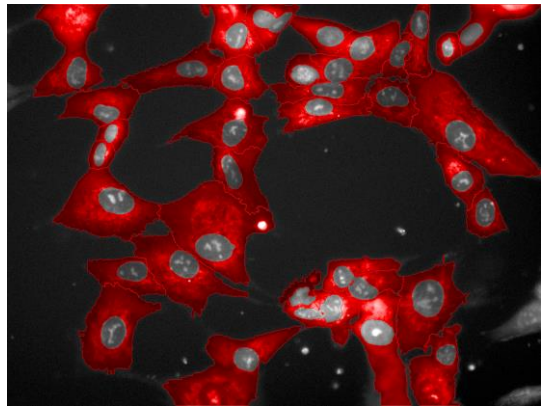
Nuclei Selection

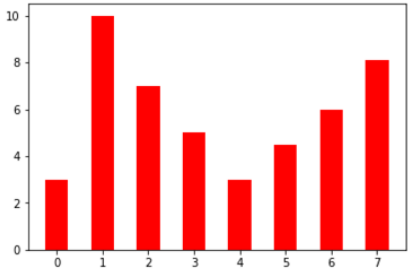


Cytoplasm Selection

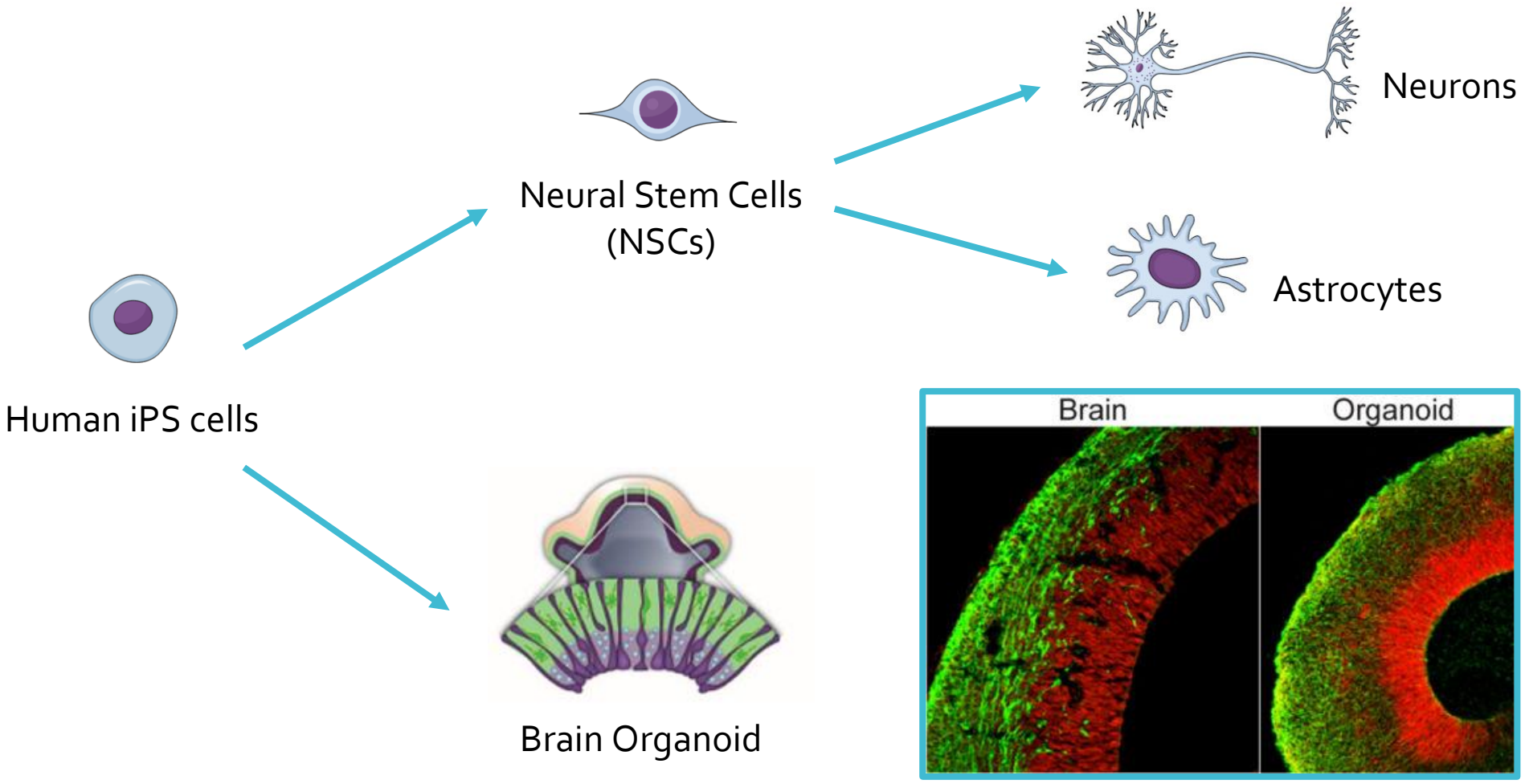


Calculate Intensity Properties



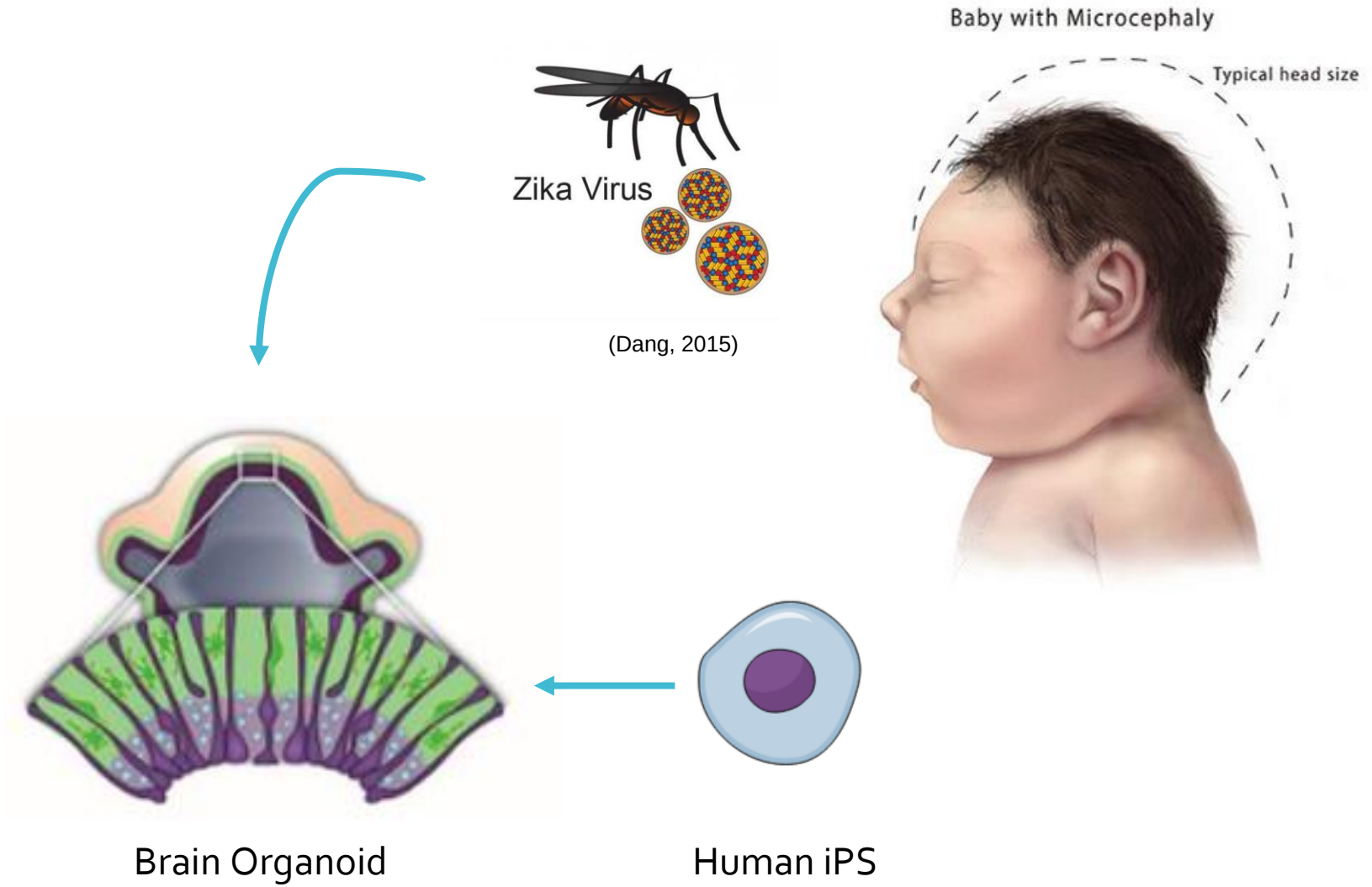


Human neural cells derived from induced pluripotent stem (iPS) cells

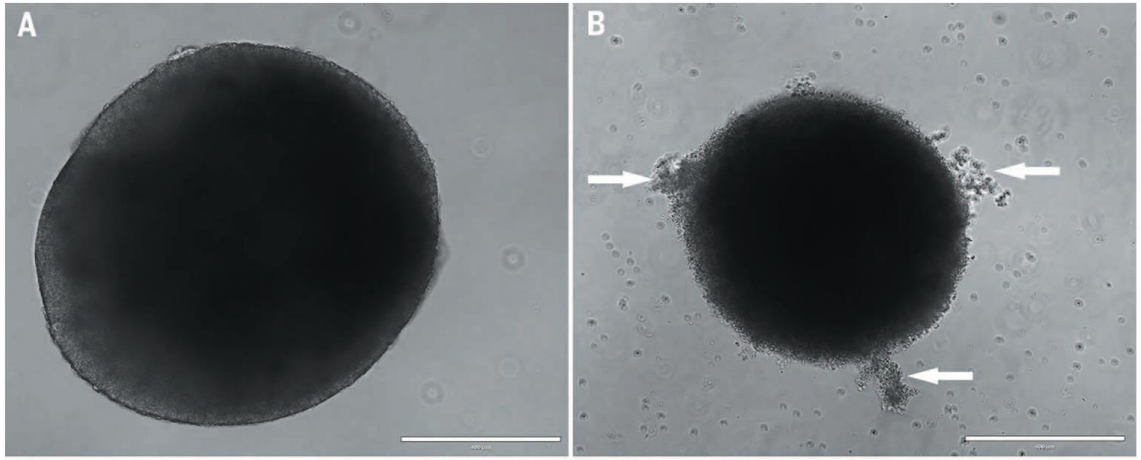


Lancaster,2015

Zika virus (ZIKV) impairs brain organoid growth



Zika virus (ZIKV) impairs brain organoid growth



Science

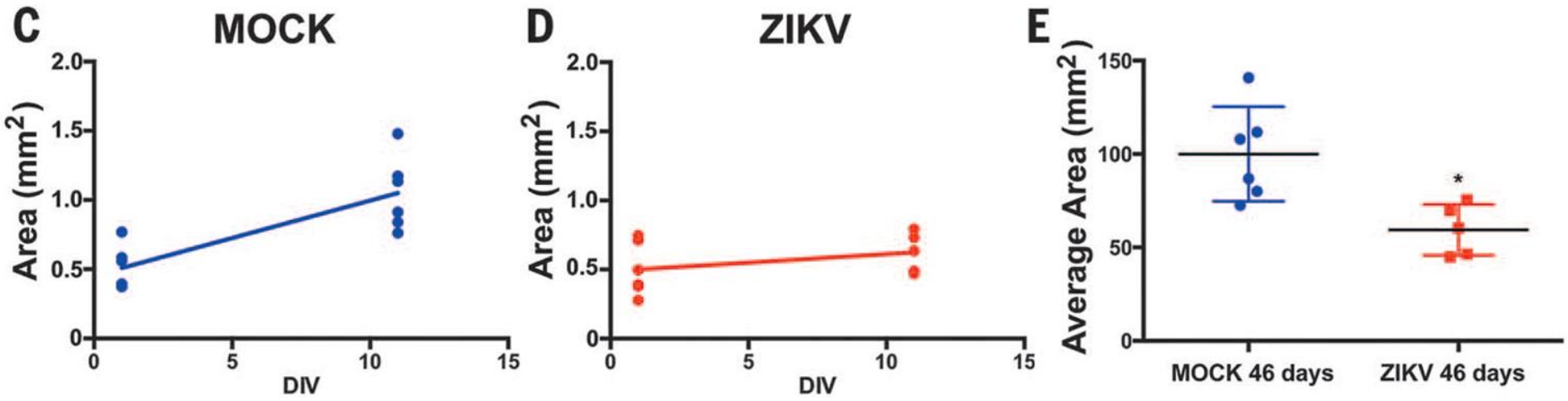
REPORTS

Cite as: Garcez *et al.*, *Science* 10.1126/science.aaf6116 (2016).

Zika virus impairs growth in human neurospheres and brain organoids

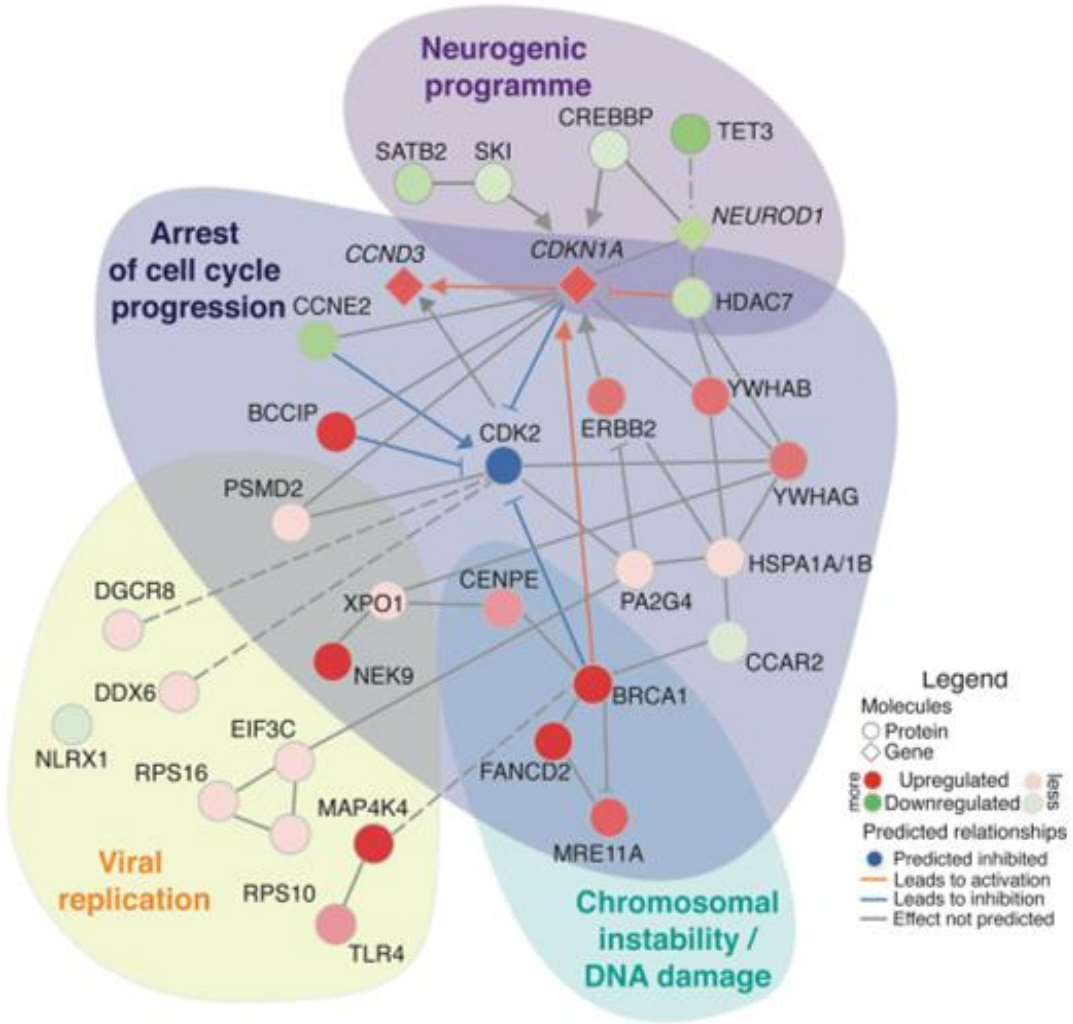
Patricia P. Garcez,[1,2]* Erick Correia Loiola,[2]† Rodrigo Madeiro da Costa,[2]† Luiza M. Higa,[3]† Pablo Trindade,[2]† Rodrigo Delvecchio,[3] Juliana Minardi Nascimento,[2,4] Rodrigo Brindeiro,[3] Amilcar Tanuri,[3] Stevens K. Rehen[2,1]*

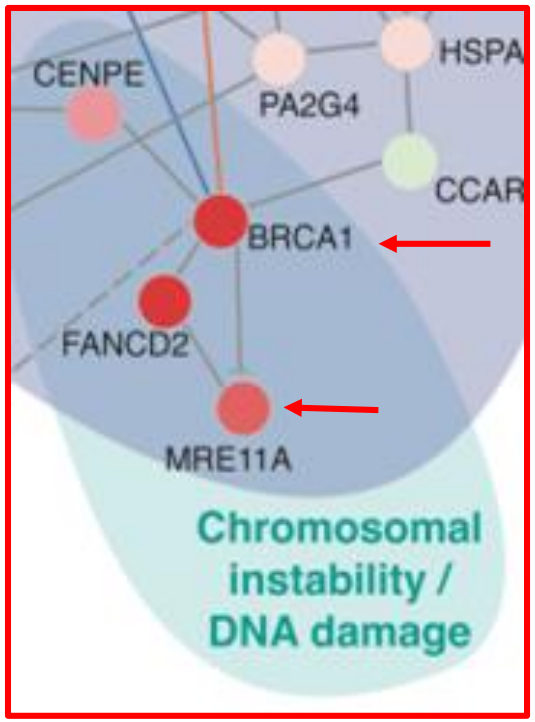
[1]Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. [2]D'Or Institute for Research and Education (IDOR), Rio de Janeiro, Brazil. [3]Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. [4]Institute of Biology, State University of Campinas, Campinas, Brazil.



Garcez, 2016

Proteomic analysis of ZIKV-infected neurospheres shows upregulation of DNA damage signaling

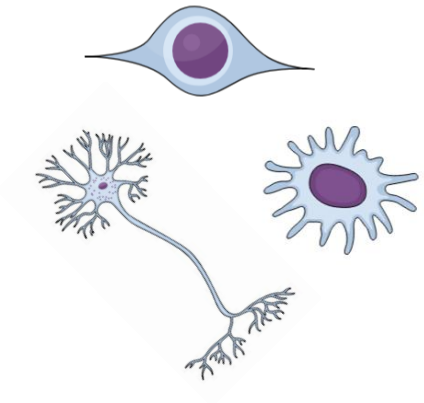




Reactive Oxygen Species (ROS)?

Garcez, 2017

ZIKV preferentially infects human astrocytes *in vitro*

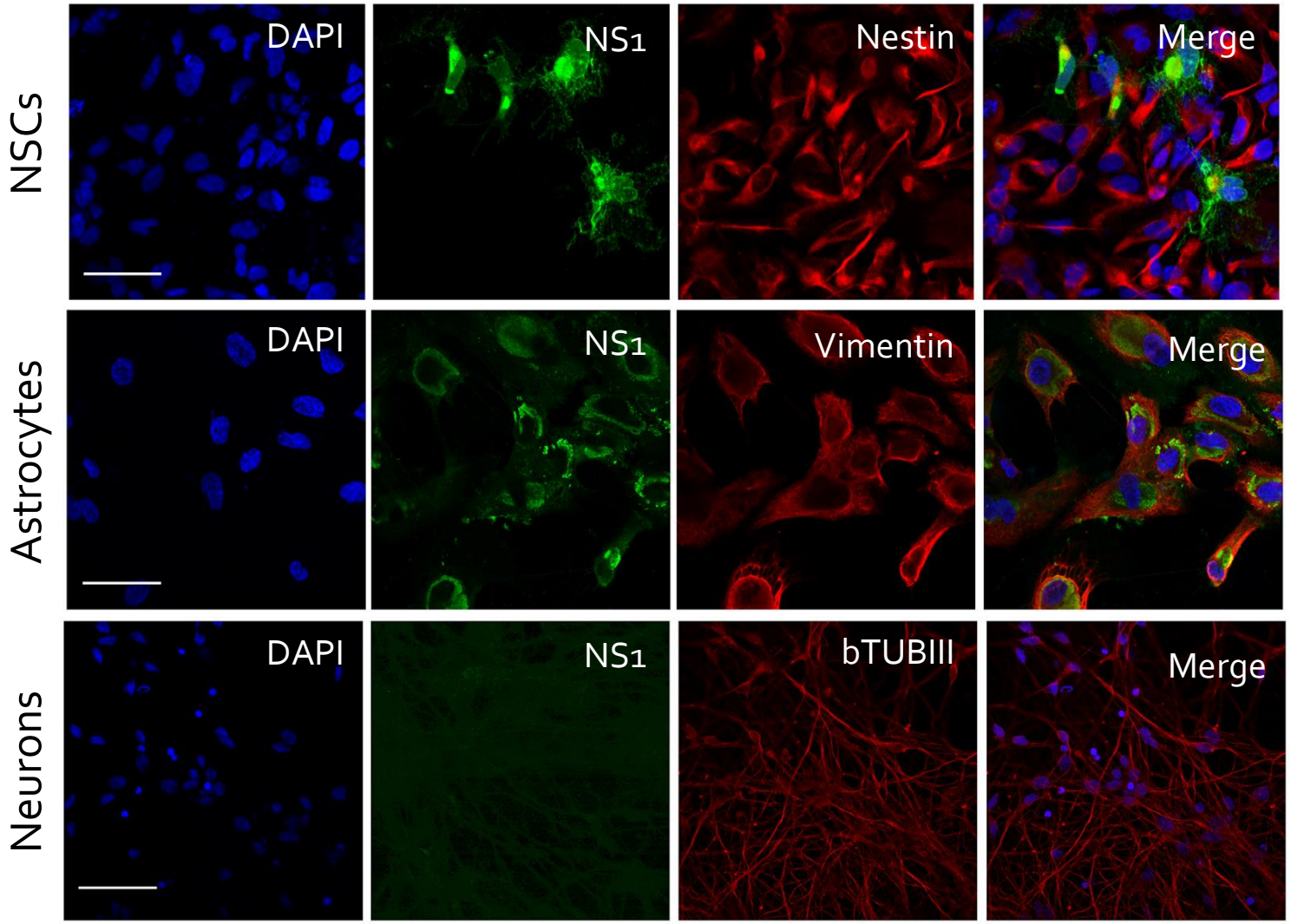


ZIKV infection

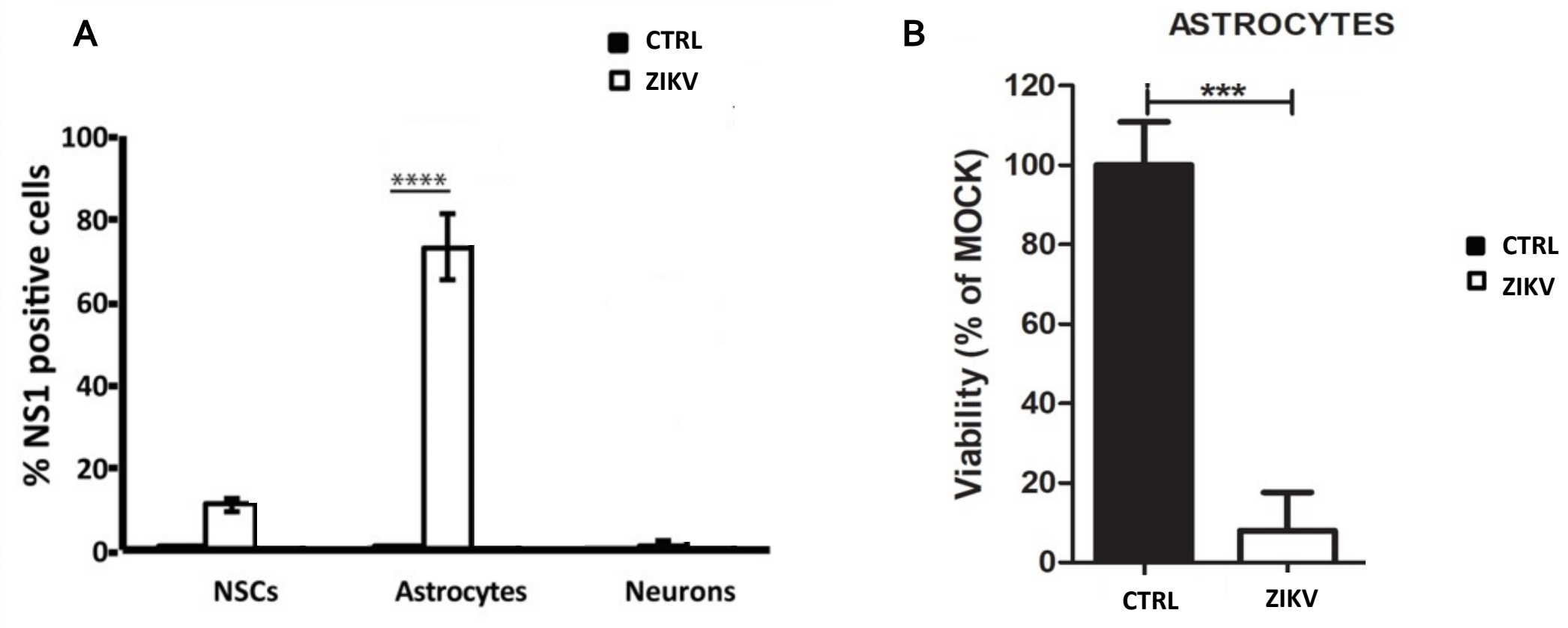
2h

72h

Infection rate and cell viability analysis

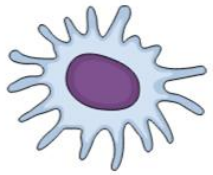


ZIKV preferentially infects human astrocytes *in vitro*



ZIKV preferentially infects human astrocytes *in vitro*

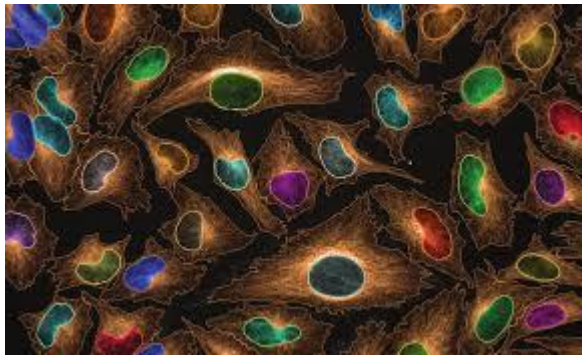
ZIKV infection leads to reactive oxygen species (ROS)

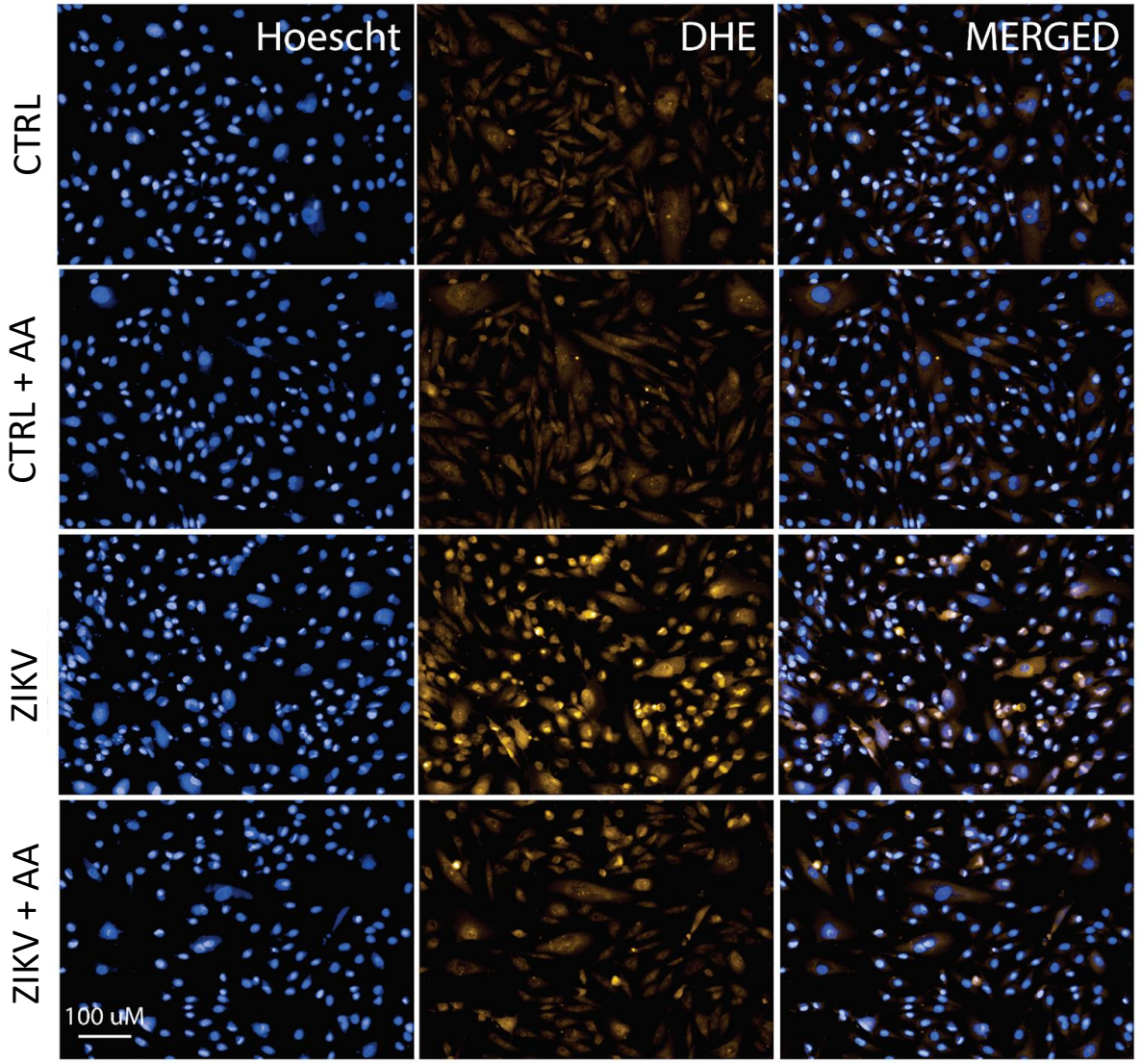


48 hours post infection

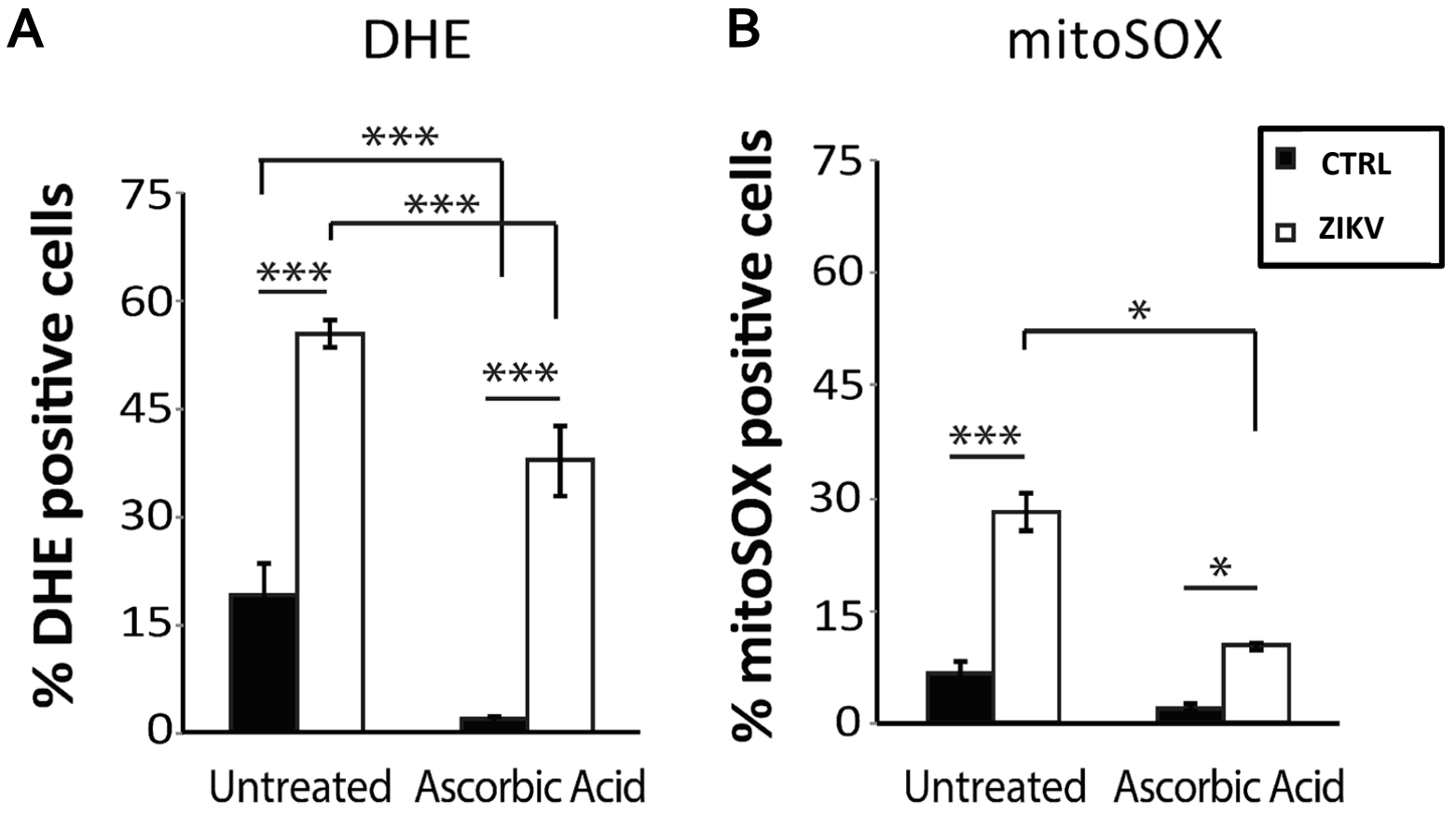
Superoxide indicator dyes: DHE and mitoSOX™

Live cell imaging – High content microscope





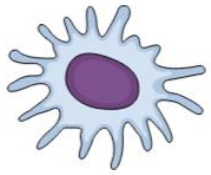
ZIKV leads to ROS production in human astrocytes



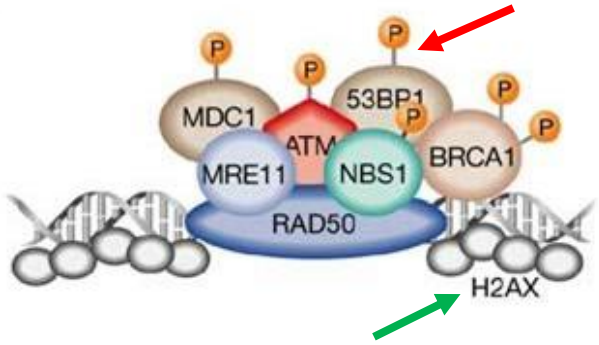
ZIKV preferentially infects human astrocytes *in vitro*

ZIKV leads to ROS production in human astrocytes

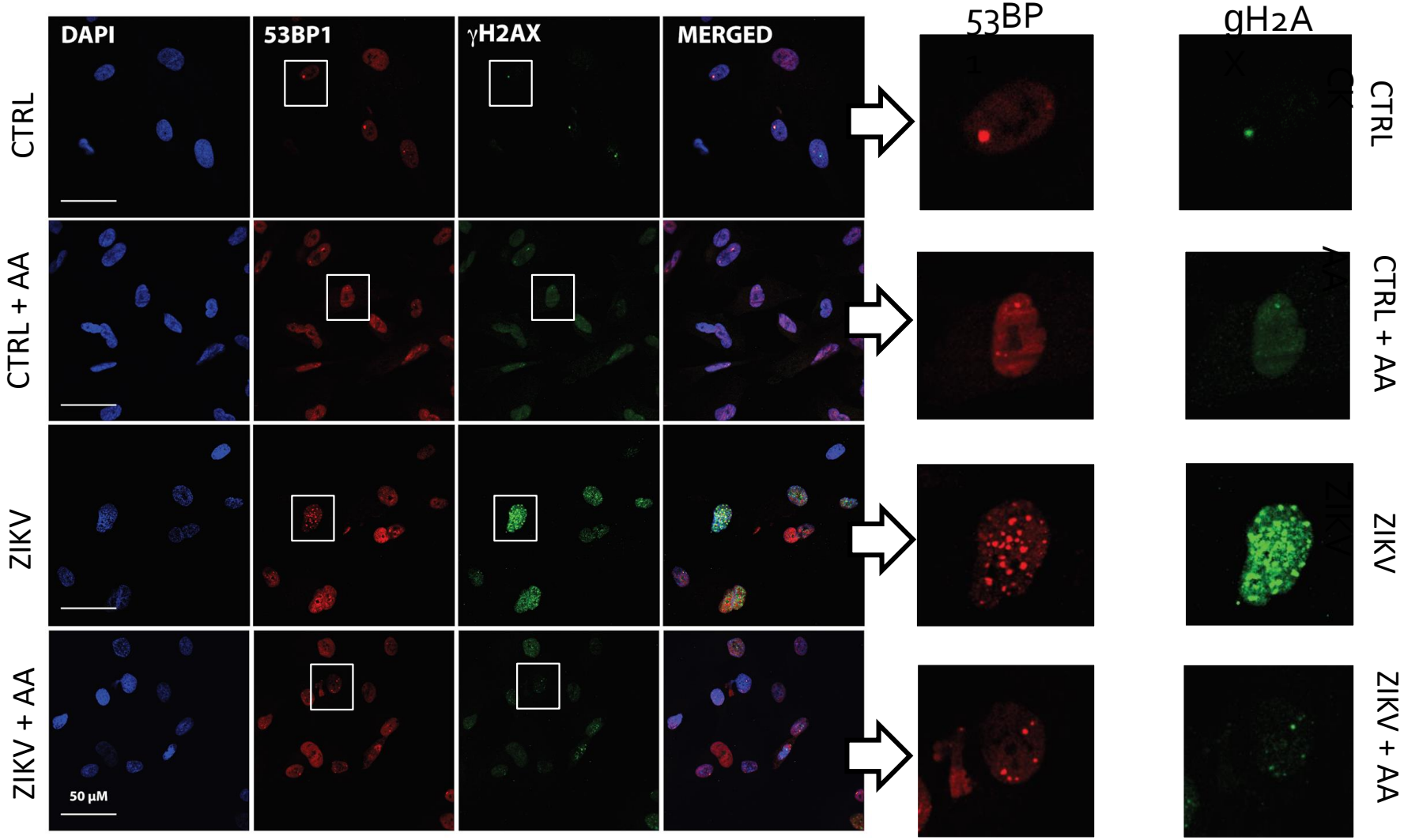
ZIKV causes DNA breaks and activates DDR signaling in astrocytes



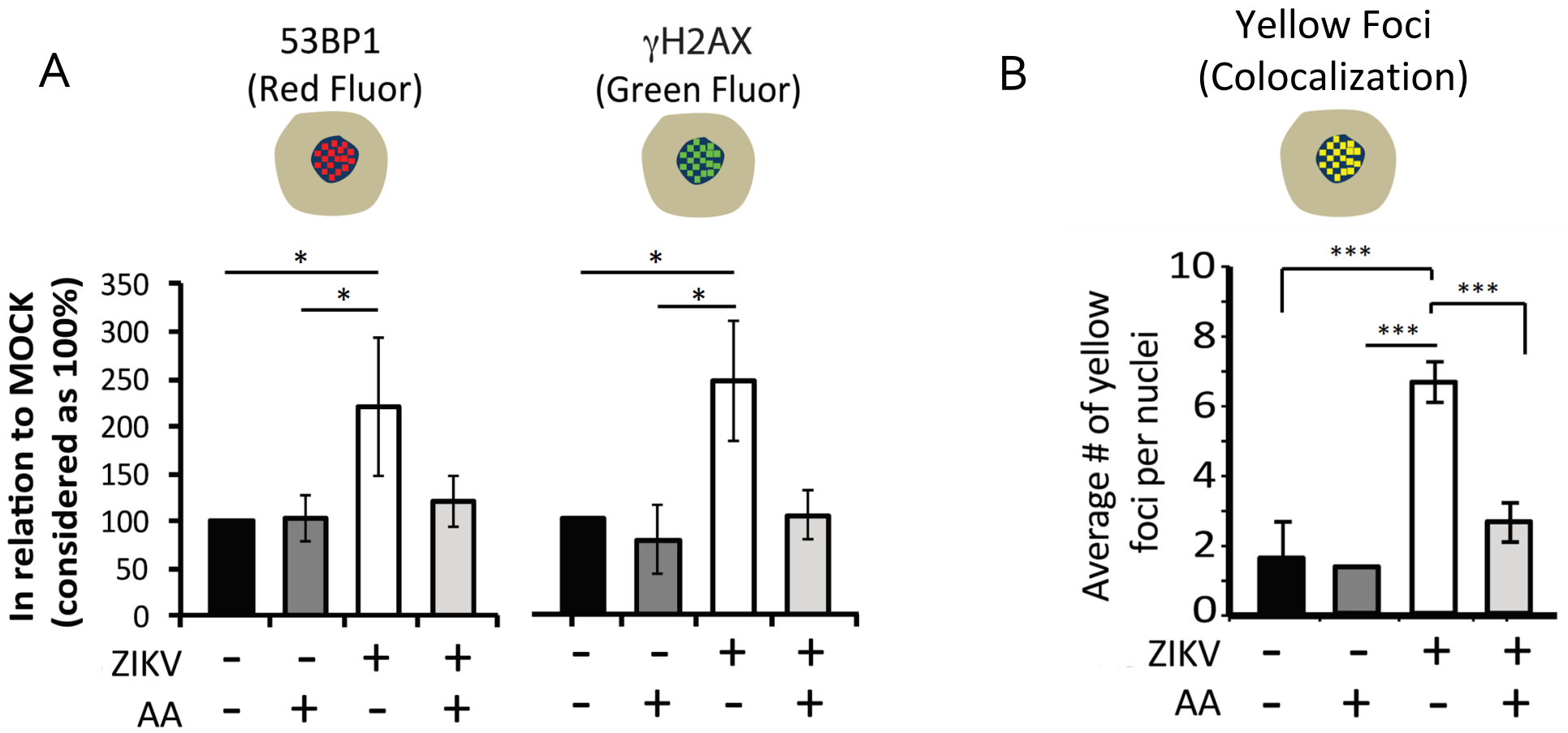
48 hours post infection



DNA Damage Response (DDR)



ZIKV-induced DNA damage can be rescued by ascorbic acid



ZIKV preferentially infects human astrocytes *in vitro*

ZIKV leads to ROS production in human astrocytes

ZIKV causes DNA breaks and activates DDR signaling in astrocytes

ZIKV-induced DNA damage can be rescued by ascorbic acid

SUMMARY

- Our **automated analysis system** combined to iPS can be applied to investigate molecular and cellular responses to pathogens and environmental factors anticipating future consequences;
- ZIKV preferentially infects **human astrocytes;**
- ZIKV infection leads to **ROS production;**
- ROS leads to **DNA damage** (53BP1 and γH2AX), which is rescued by ascorbic acid;
- We propose that oxidative stress and DNA damage response could be linked to consequences besides **microcephaly** and also to future neural disorders.

SPONSORS


Finep
INOVAÇÃO E PESQUISA


FAPERJ
Fundação Carlos Chagas Filho de Amparo
à Pesquisa do Estado do Rio de Janeiro


CNPq


CAPES


INSTITUTO D'OR
PESQUISA E ENSINO


BNDES

REHEN LAB