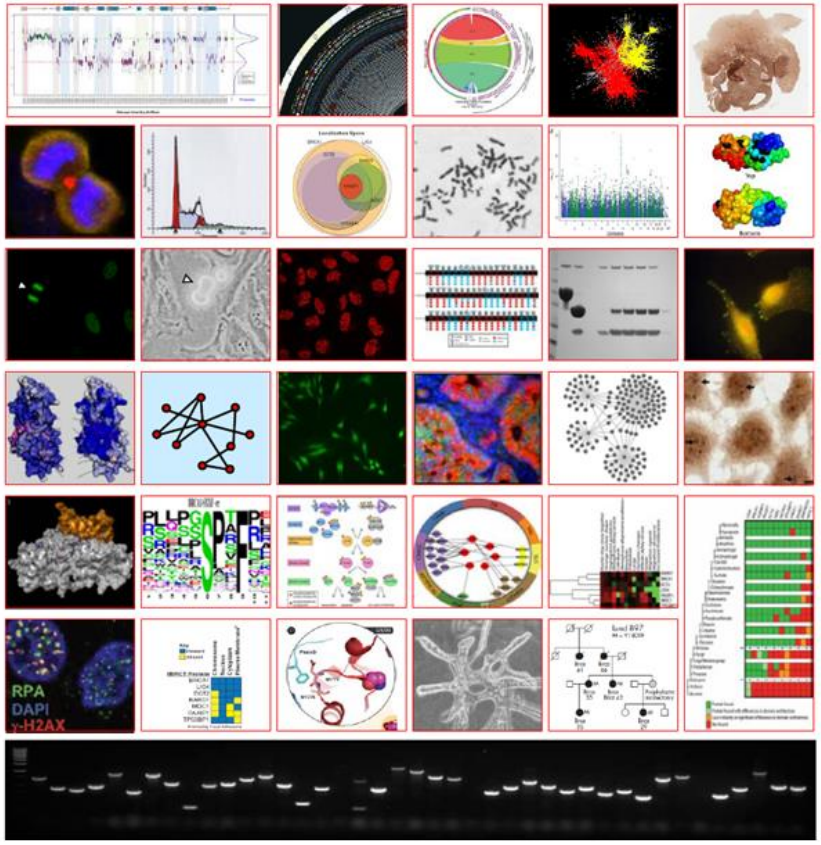
Cellular targets of Zika-encoded proteins and microcephaly (7ZK29)

Volha Golubeva[1], Thales C. Nepomuceno[2], Rafael Mesquita[3], Xueli Li[1], Victoria Izumi[4,5], John Koomen[4,5], Marcelo A. Carvalho[2,6], Alvaro N.A. Monteiro[1]

[1]Cancer Epidemiology Program, H. Lee Moffitt Cancer Center, Tampa, FL, USA; [2]Programa de Pesquisa Clínica, Instituto Nacional de Câncer, Rio de Janeiro, Brazil; [3]Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro; [4]Molecular Oncology Department, H. Lee Moffitt Cancer Center, Tampa, FL, USA; [5]Proteomics Shared Facility; H. Lee Moffitt Cancer Center, Tampa, FL, USA [6]Instituto Federal do Rio de Janeiro, Rio de Janeiro, Brazil

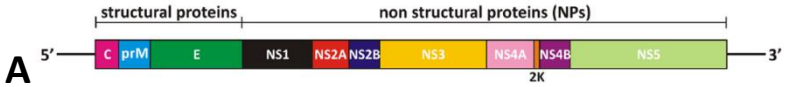


Hypothesis: *ZIKV-encoded proteins specifically target host proteins in neural progenitor cells leading to microcephaly; and that missense ZIKV variants isolated in Brazil may do so with a higher affinity.*

Proteins encoded by viruses can bind and inactivate host cell proteins leading to attenuation of cell growth, gene expression dysregulation, induction of apoptosis, and abrogation of DNA damage responses

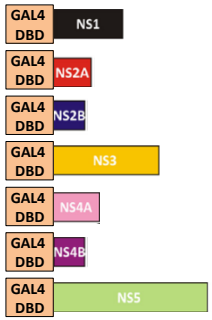
Unpublished protein-protein interaction data from our lab indicates that the BRCT domains of the microcephaly protein MCPH1 interact with the catalytic subunit of the Anaphase Promoting Complex (APC/C)

Overal strategy for the rapid pilot



B

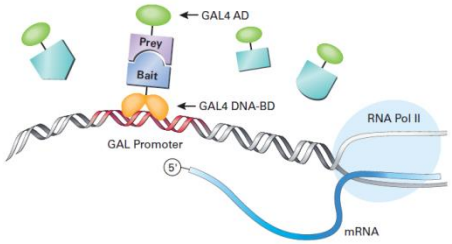
GAL4 DNA-DB : Bait (ZIKV proteins)



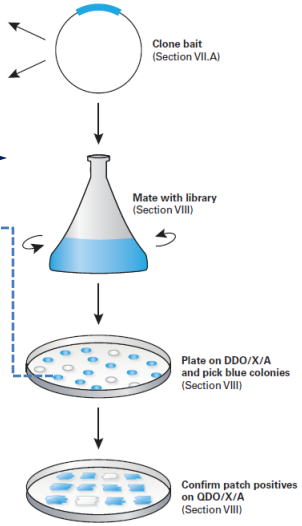
X

GAL4 AD : Prey (library)

cDNA library
Human brain



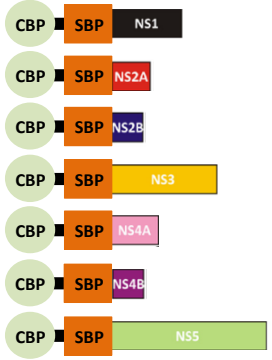
Screenings



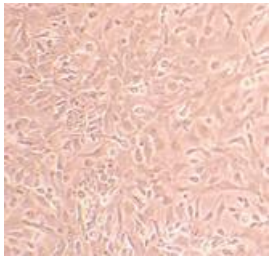
Validation

C

TAP (CBP and SBP): Bait (ZIKV proteins)



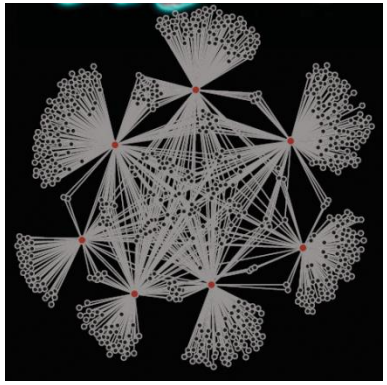
Transfection



Affinity purification and mass spectrometry

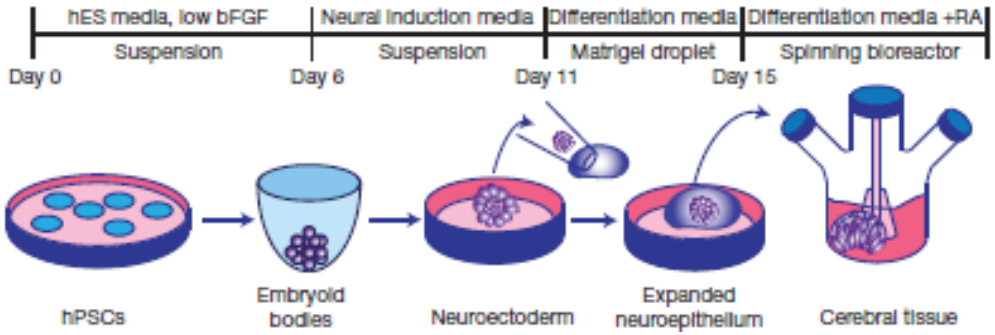
Validation

D



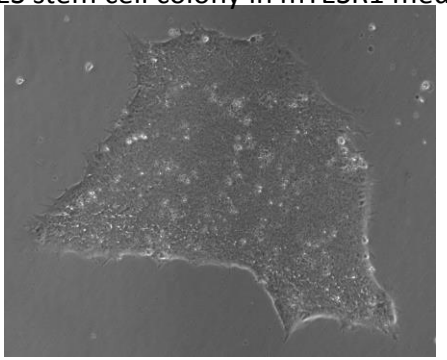
ZIKV-Human interaction network

Validation using 3D human brain organoid model

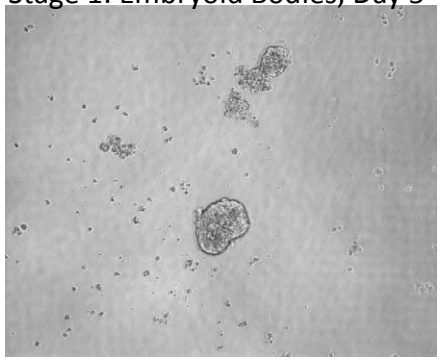


From Lancaster et al. Nature 2013; *501*, 373-379

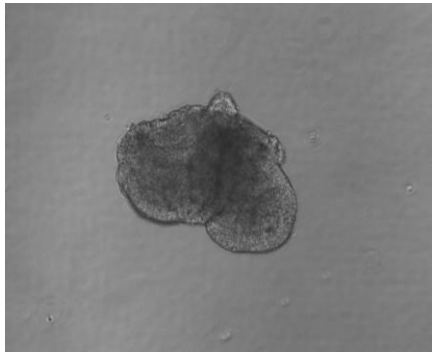
H9ES stem cell colony in mTESR1 media



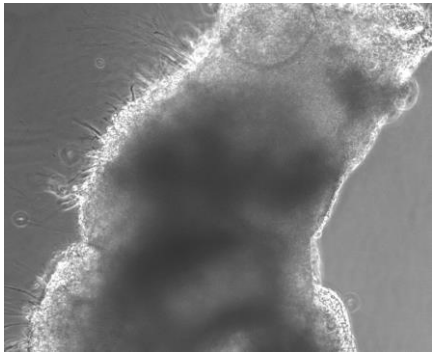
Stage 1: Embryoid Bodies, Day 5



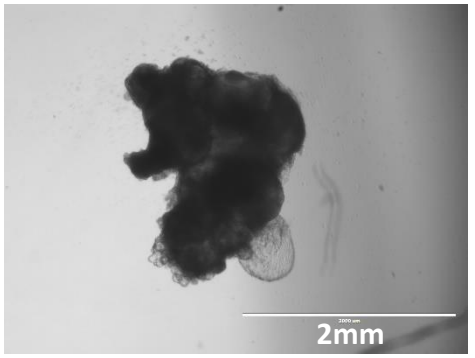
Stage 2, Neural Induction: Day 8



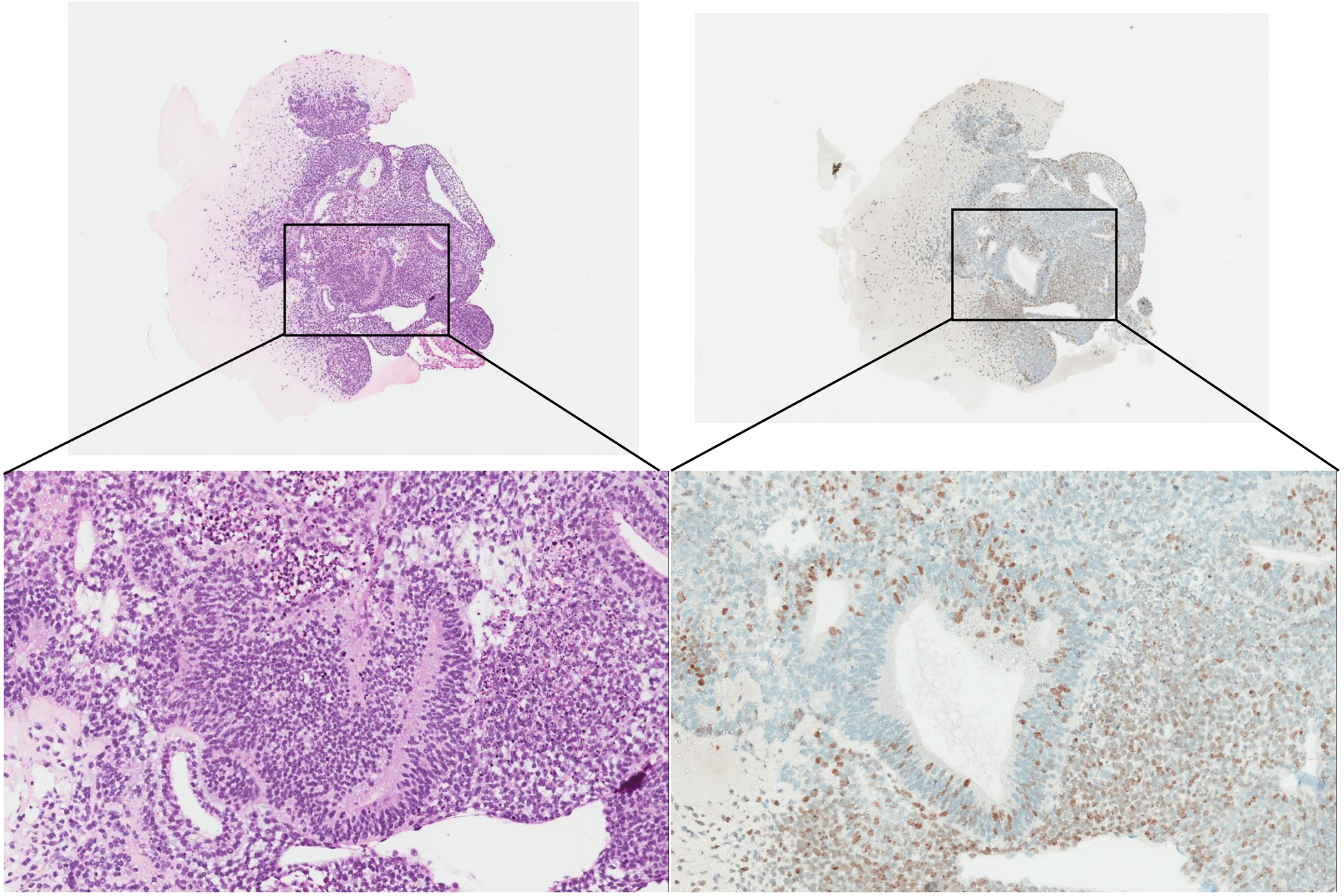
Stage 3: Matrigel Droplet, Day 15



Stage 4: Final Differentiation, Day 23 Stationary (2X mag)



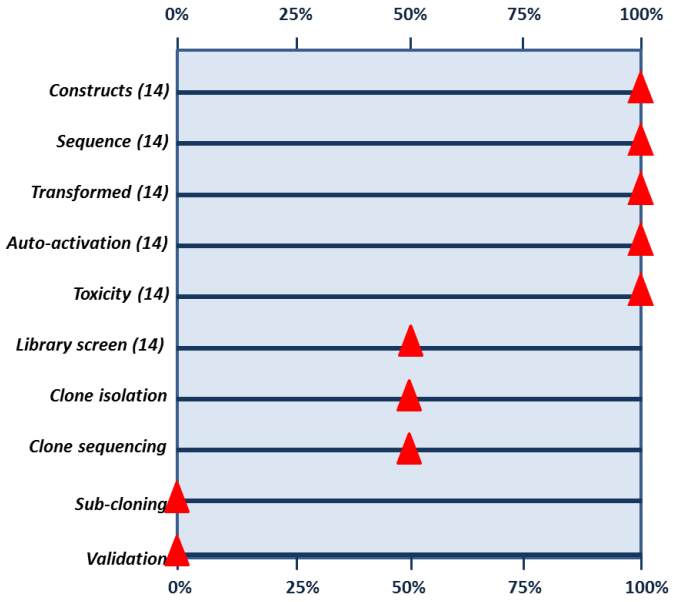
Validation using 3D human brain organoid model

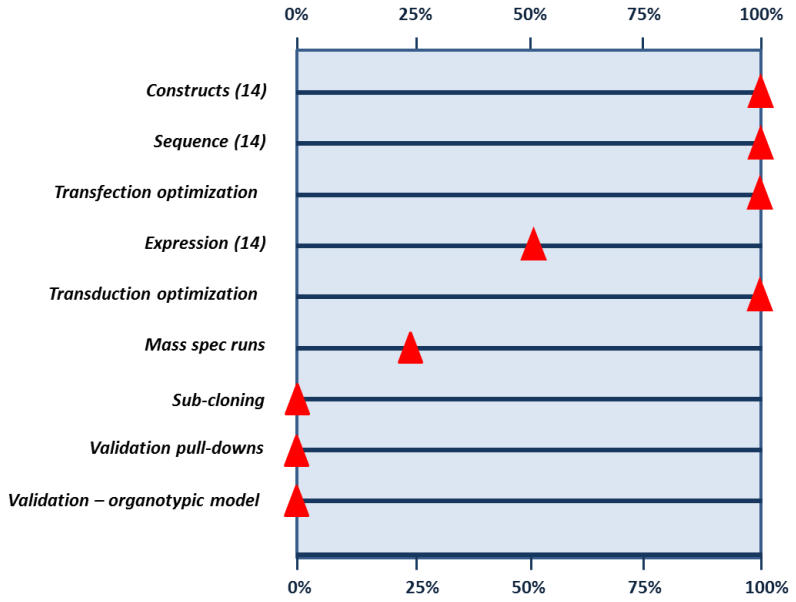


H&E

BRCA1

Progress to date (Mar – Sep 2017)

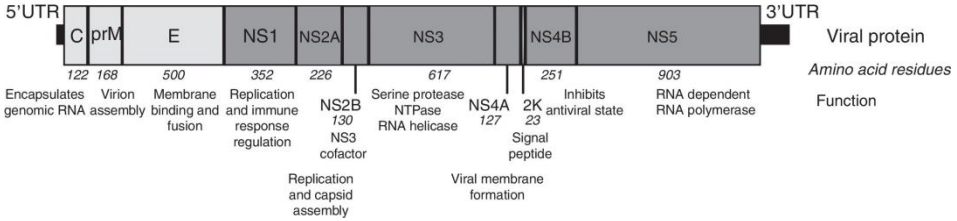




Preliminary results

Bait	Isolated Clones (Y2H)	Unique Interactions (Y2H)	High Confidence Interactions (TAP-MS)
NS1-Br	TBD	TBD	
NS2A-Br	13	1	
NS2B-Br	28	12	
NS3-Br	TBD	TBD	
NS4A-Br	49	6	43
NS4B-Br	46	3	44
NS5-Br	37	7	
NS5-As	80	10	

Preliminary results



K.A. Galán-Huerta et al. 2016