Evaluation of Postural Steadiness before and after Propofol Sedation

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Fitness for Ambulation

- Important criterion for discharge
- Impaired by sedation/anesthesia
- Recovery rates different
- No objective test

Postural Steadiness

- Complex interaction of multiple systems
- Complexity gives rise to chaotic behavior
- Chaos can be measured by nonlinear tools such as entropy

Previous Work

- AP sway acquired from Nintendo Wii® via Bluetooth
- Measurement of chaos via Fuzzy Sample Entropy (FSE)
- Can distinguish pre- and post- sedation states with Midazolam, Sleep Deprivation

Tietäväinen A, Gates FK, Meriläinen A, Mandel JE, Hæggström E. Nintendo® Wii Fit based sleepiness tester detects impairment of postural steadiness due to 24°of wakefulness. Medical Engineering & Physics. 2013: in press.

Hypothesis

FSE of postural sway can detect return towards baseline state during recovery from procedural sedation with propofol.

Methods

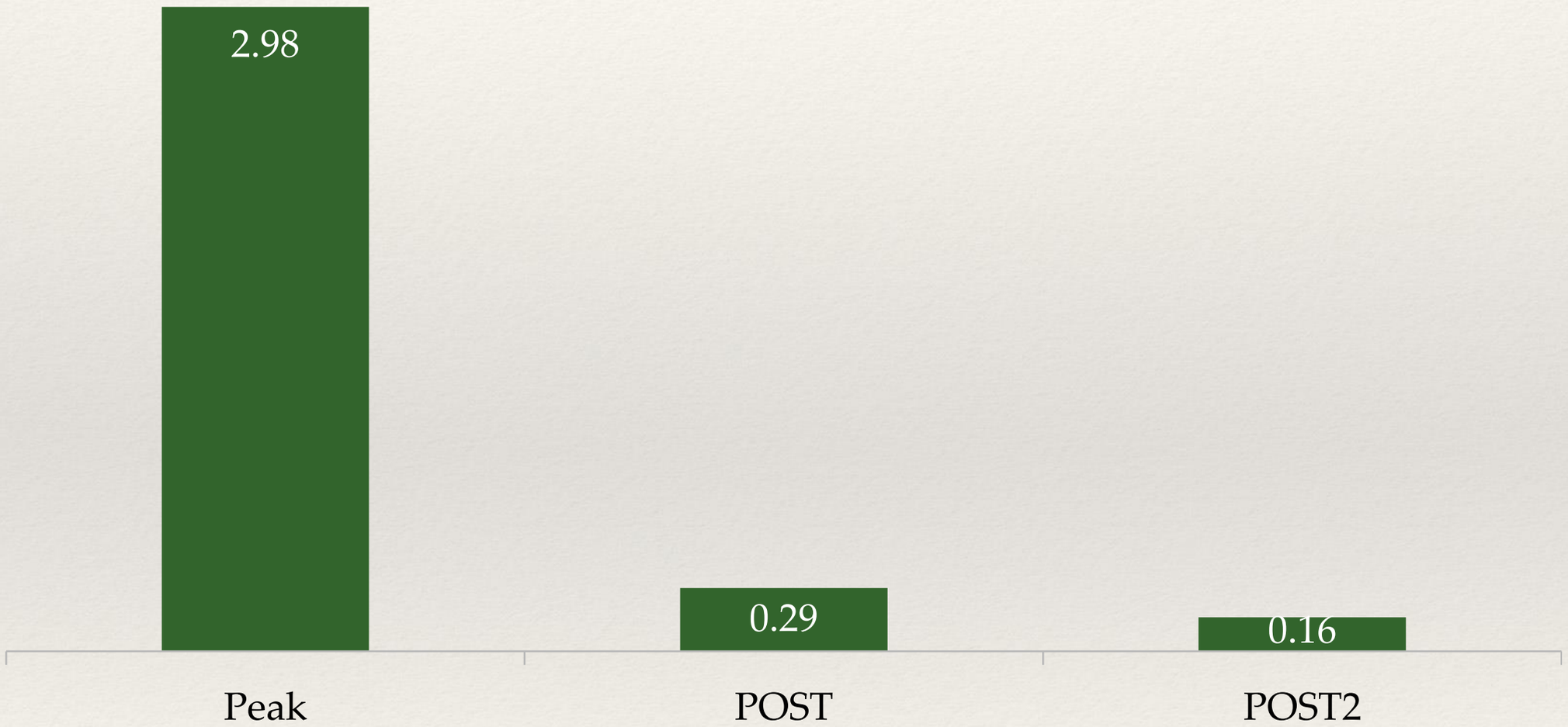
- IRB approval, informed consent
- 131 patients undergoing colonoscopy/EGD
- Assessed at 3 times:
  - Prior to procedure (PRE)
  - When first able to stand (POST1)
  - Appx. fifteen minutes later (POST2)
- Propofol administration obtained from EMR
- Propofol effect site estimates via Cortinez model
- Postural sway measured by [illegible]mpared by paired T test

Cortínez LI, Anderson BJ, Penna A, Olivares L, Muñoz HR, Holford NHG, Struys MMRF, Sepulveda P. Influence of obesity on propofol pharmacokinetics: derivation of a pharmacokinetic model. Br J Anaesth. 2010;105:448-56.

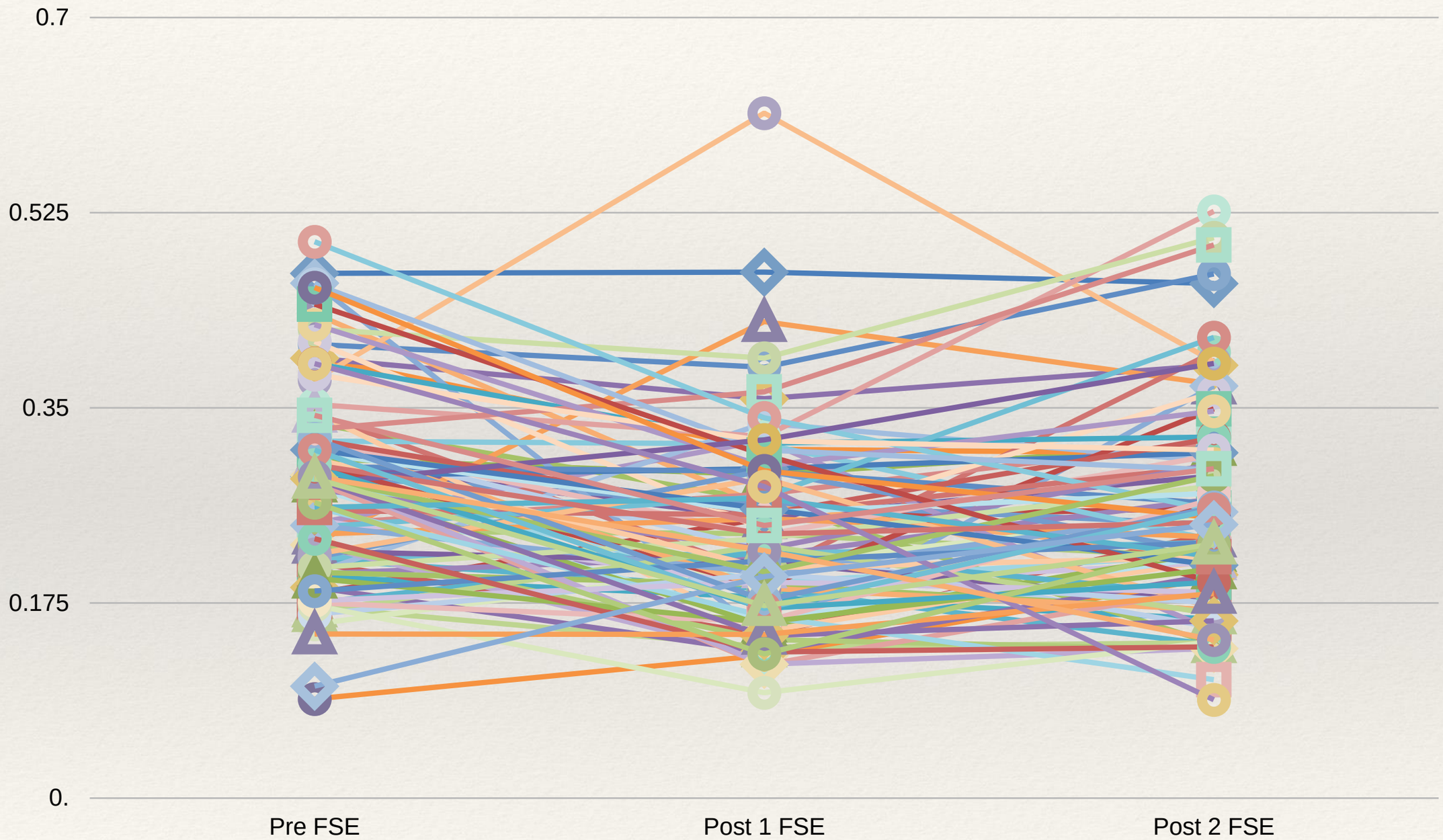
Patient Characteristics

Age (years)	54.3 ± 14.5
Height (cm)	170.39 ± 11.44
Weight (kg)	83.94 ± 23.27
Time to POST (min)	33.9 ± 12.1
Time to POST2 (min)	53.1 ± 14.2

Propofol Effect Site Estimates (ug/mL)

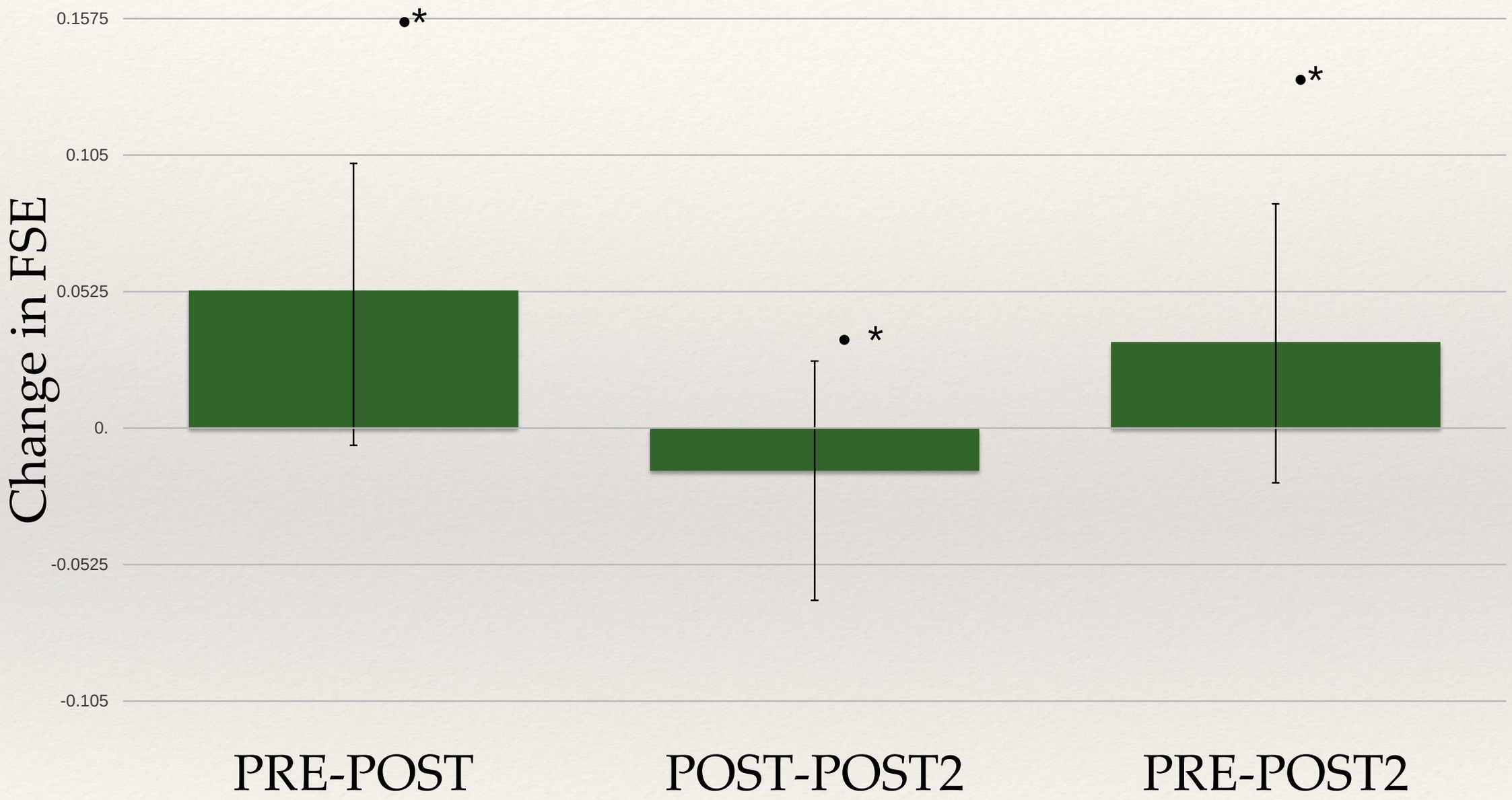


Fuzzy Sample Entropy (Individual)



*$P < 0.05$ vs PRE † $P < 0.05$ vs POST2

Fuzzy Sample Entropy (Change)



**P < 0.05, Paired T, N=93*

Results

- FSE was decreased from PRE at POST
- FSE was increased from POST to POST2
- FSE at POST2 is still below PRE
- NO Correlation between
  - Δ FSE and Age, Height, Weight
  - Δ FSE and Propofol peak, POST, POST2
  - Time to POST and Age, Height, Weight

Discussion

- Propofol is associated with a marked decrement in FSE of postural sway.
- This effect is still measurable at time of discharge.
- Implications for risk of falls unknown.
- There is significant variation between patients not attributable demographic differences.
- No subgroups stood out as being remarkably fast or slow at regaining stability.

Conclusions

- Fuzzy Sample Entropy shows promise for tracking recovery of postural steadiness
- Recovery may not be as simple as measuring the propofol concentration
- The technology permits inexpensive and safe collection of large amounts of data

Thank You

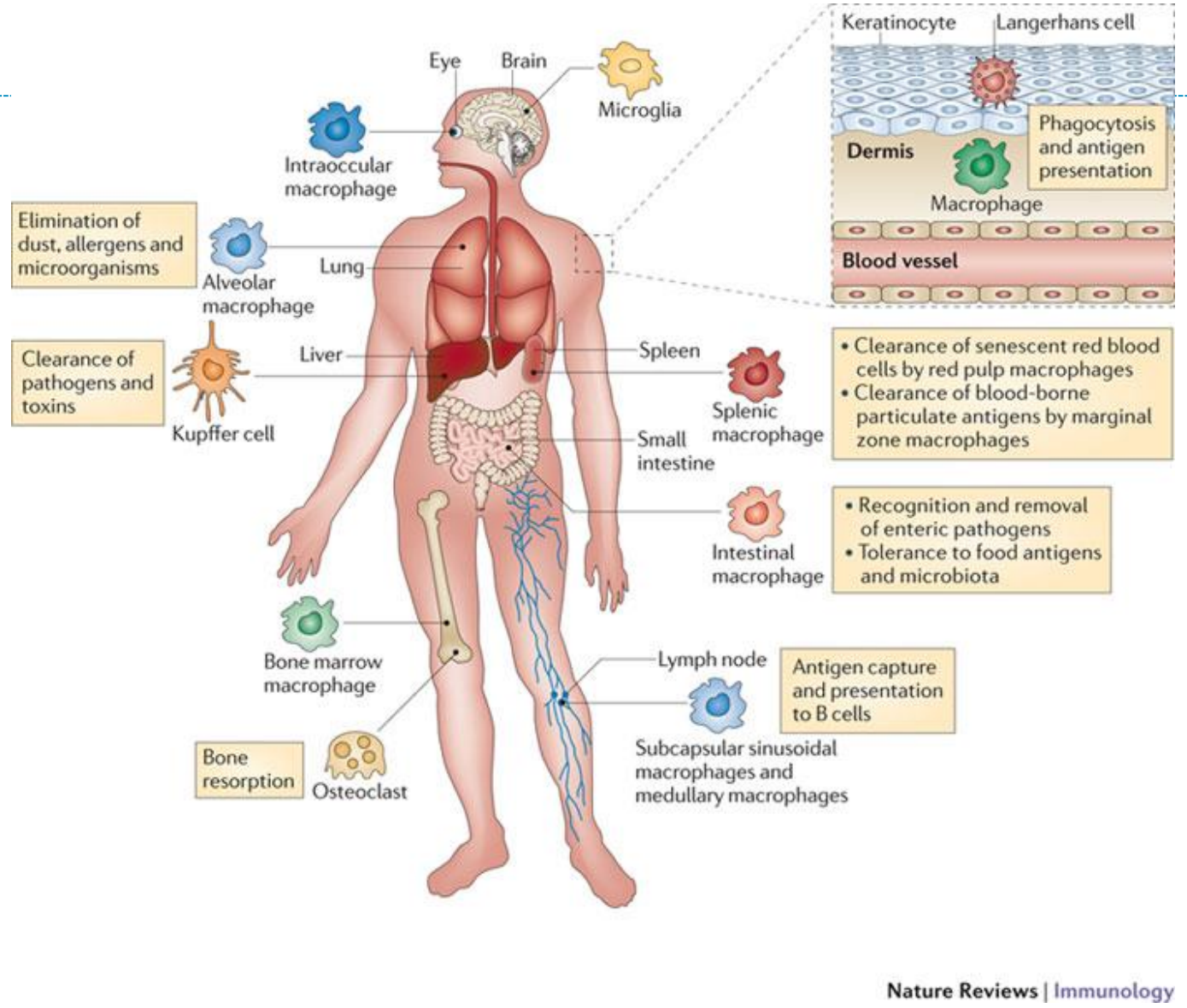
- FAER
- Department of Anesthesiology & Critical Care, Perelman School of Medicine at the University of Pennsylvania
- Jeff E Mandel MD MS

Macrophages and Long Noncoding RNA

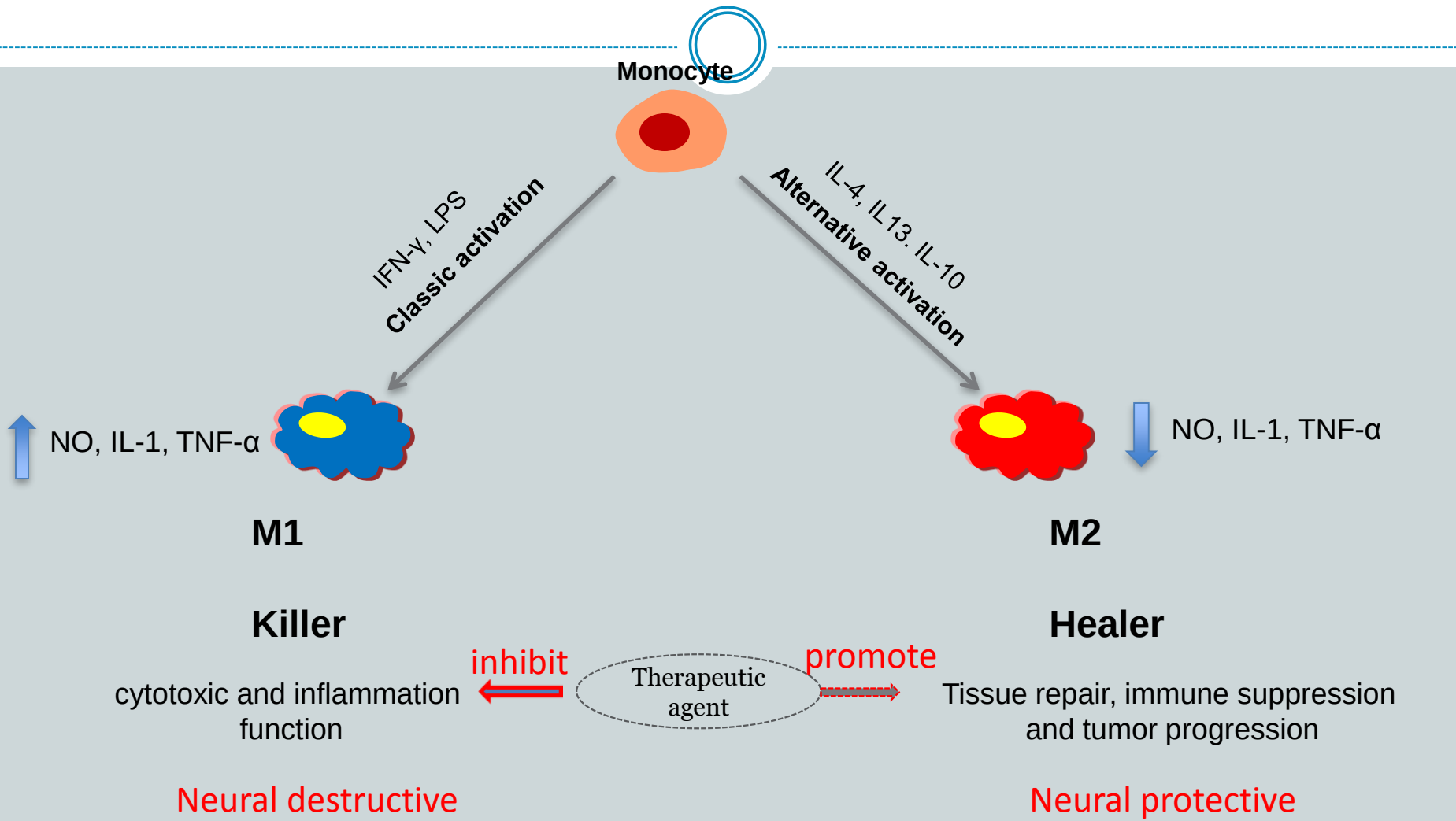
Objectives

- Investigate long noncoding RNA that are differently expressed in classically activated macrophages (M1), and myelin-laden macrophages.
- More specifically examine the expression of long noncoding RNA, TUG1, which is known to have a repressive effect on classical macrophage activation

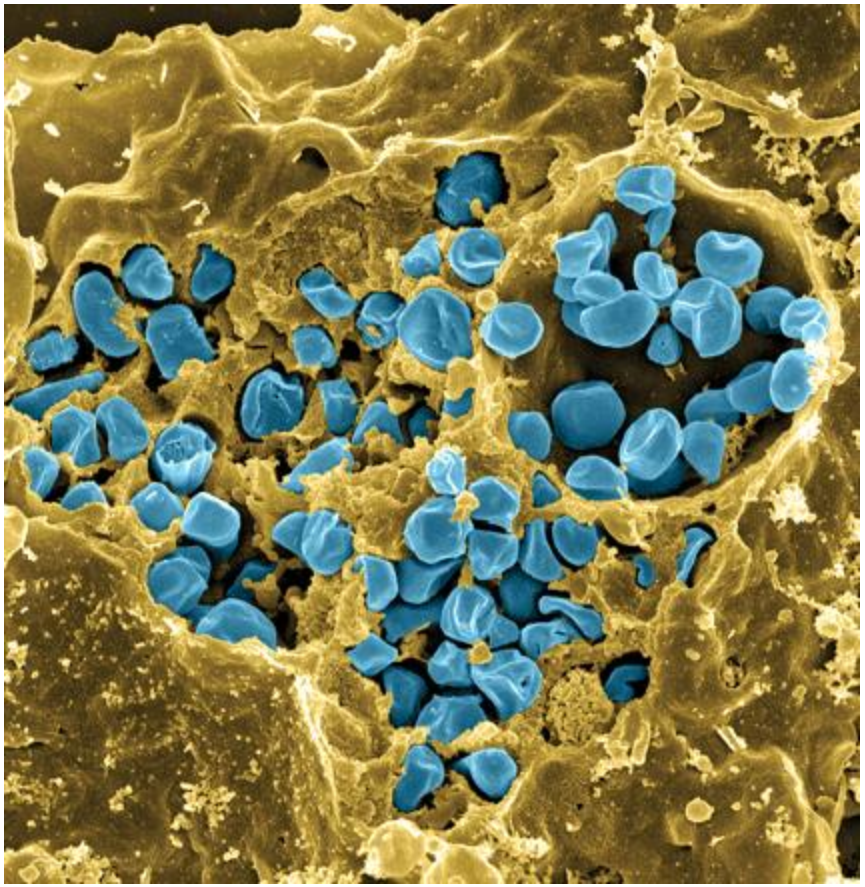
Macrophage


Keratinocyte
Langerhans cell
Dermis
Phagocytosis and antigen presentation
Macrophage
Blood vessel
Eye
Brain
Microglia
Intraoccular macrophage
Elimination of dust, allergens and microorganisms
Alveolar macrophage
Lung
Clearance of pathogens and toxins
Kupffer cell
Liver
Spleen
Splenic macrophage
• Clearance of senescent red blood cells by red pulp macrophages
• Clearance of blood-borne particulate antigens by marginal zone macrophages
Small intestine
Intestinal macrophage
• Recognition and removal of enteric pathogens
• Tolerance to food antigens and microbiota
Bone marrow macrophage
Lymph node
Antigen capture and presentation to B cells
Subcapsular sinusoidal macrophages and medullary macrophages
Bone resorption
Osteoclast
Nature Reviews | Immunology

Macrophage Activation

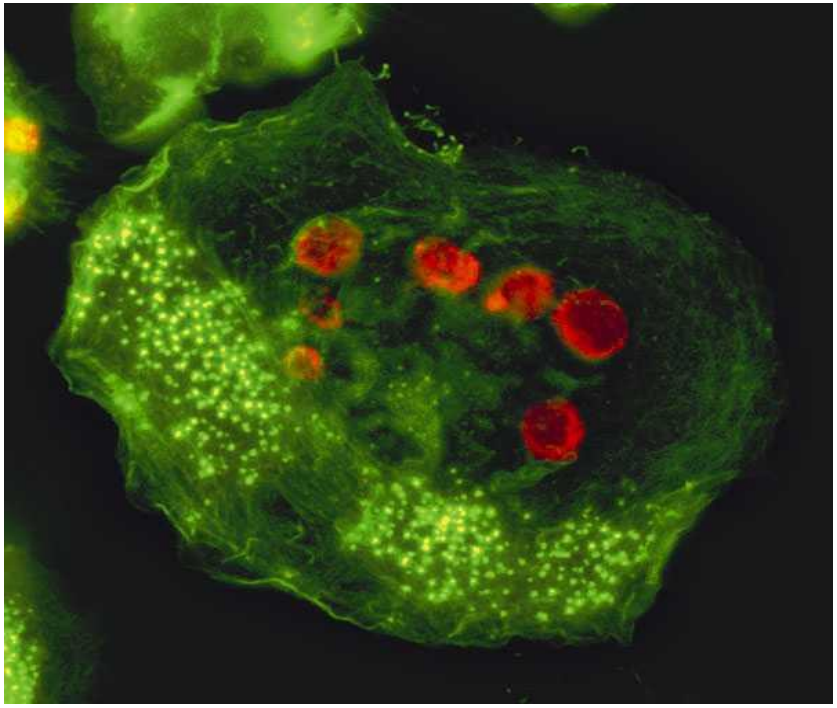


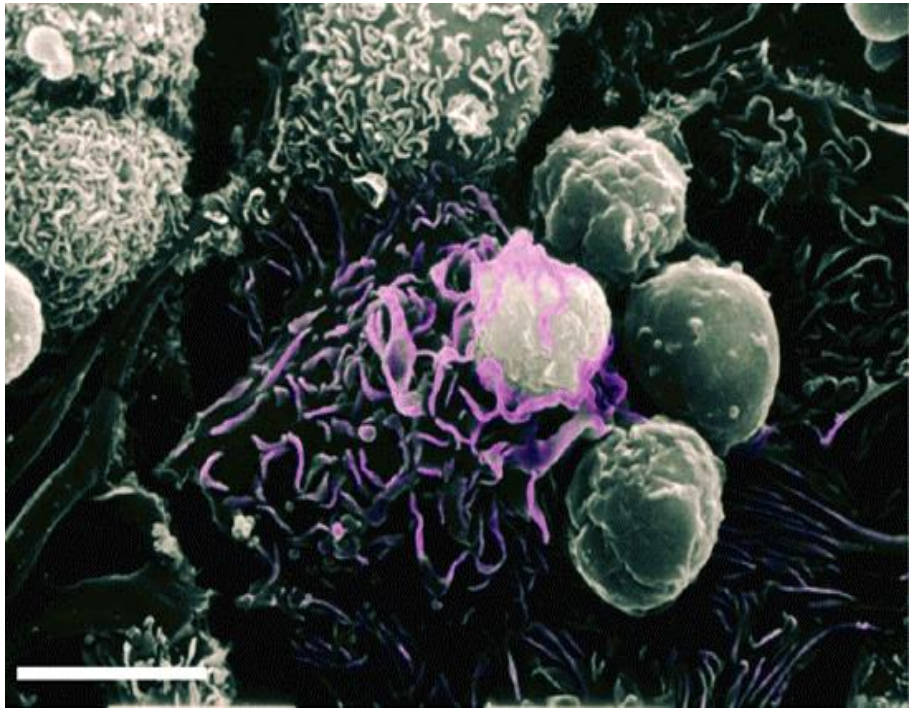
Scanning Electron Micrograph of a Macrophage Infected with *Francisella tularensis*



Checroun *et al.* ***PNAS*** 2006 103 (39) 14578

A Human Monocyte-derived Macrophage Ingesting Multiple Apoptotic Bodies

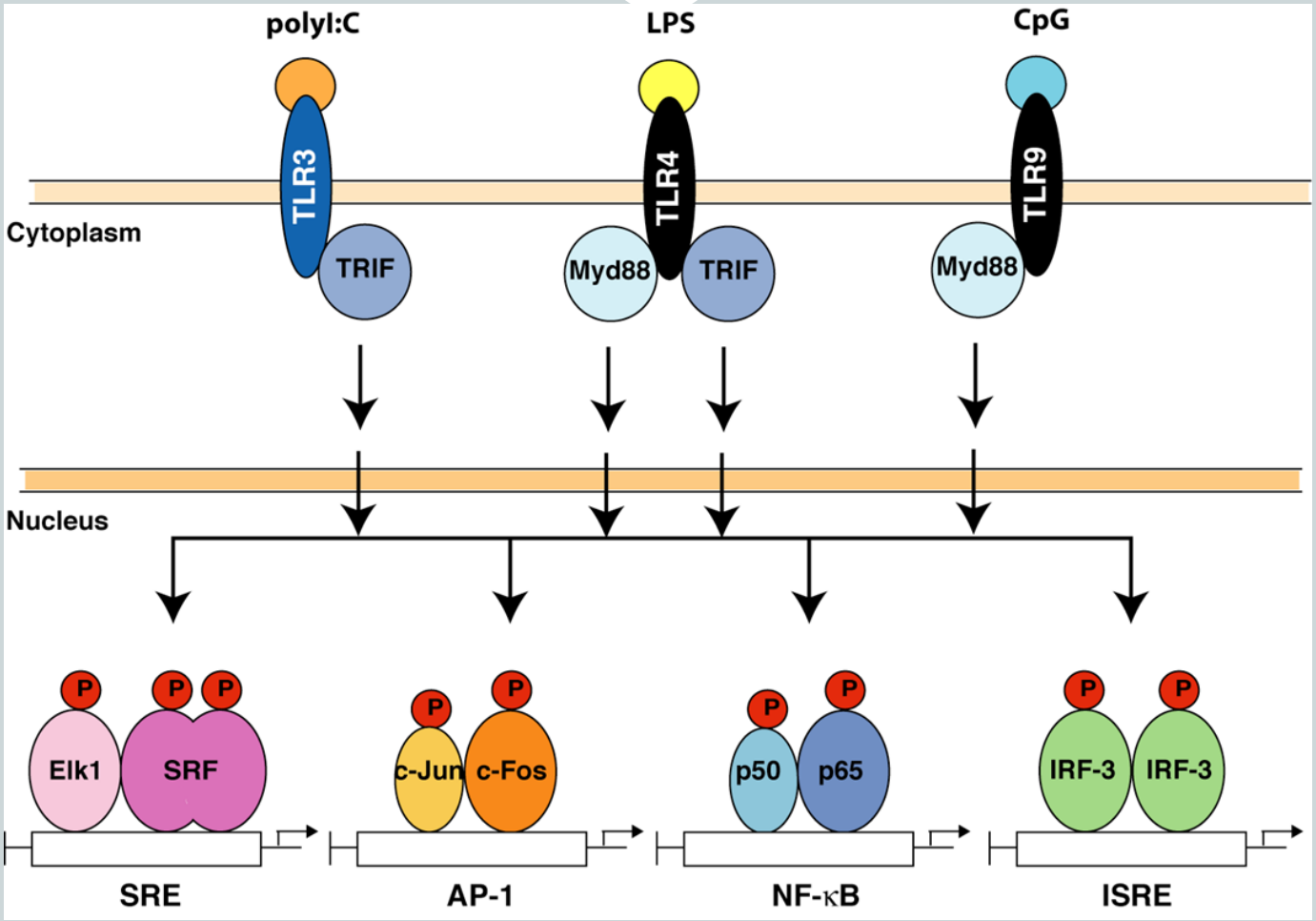




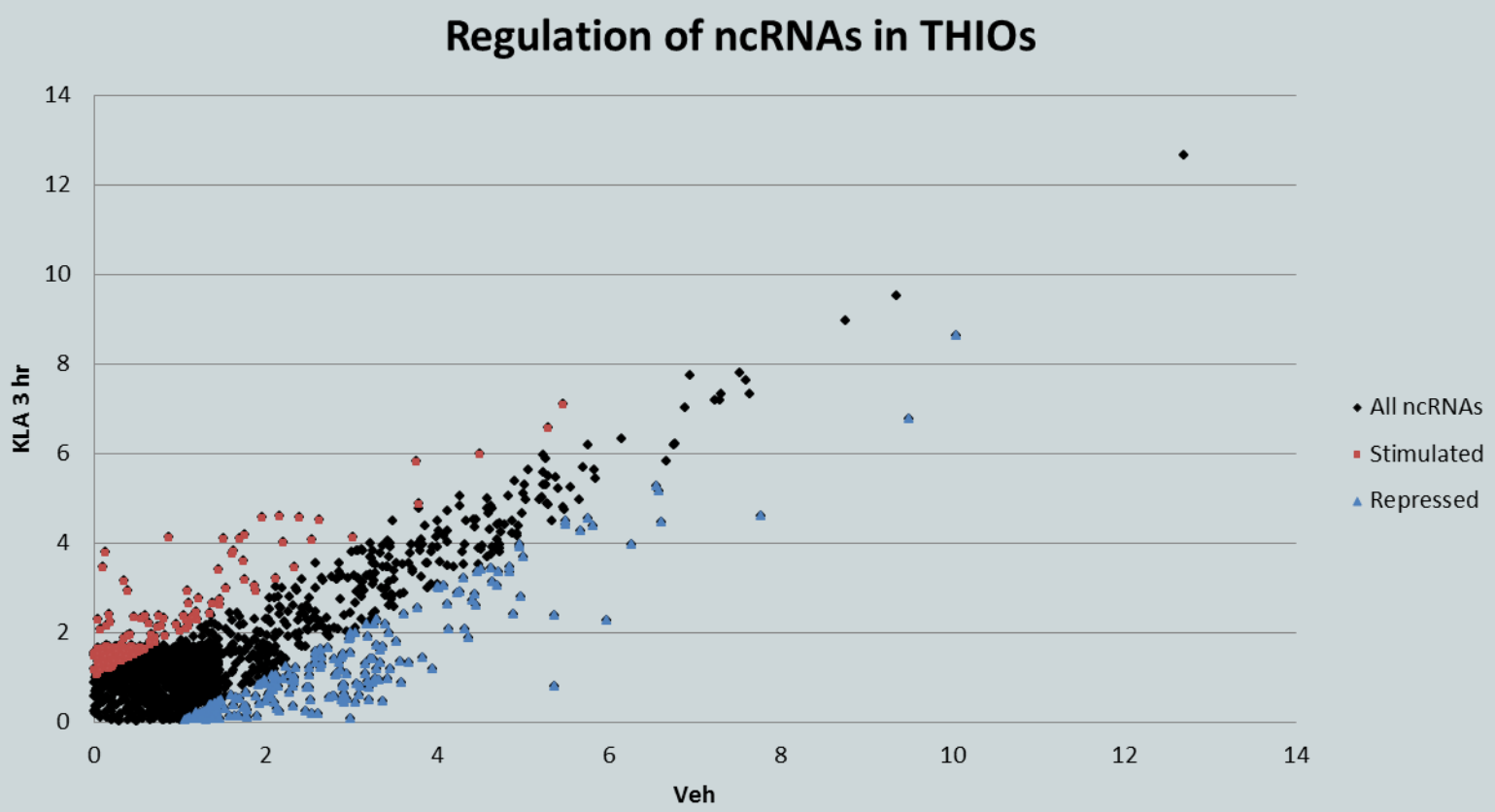
Dead men may tell no tales,
but dead cells certainly do,
the macrophage having the last word. ------Sir John Savill

Savill & Fadok. ***Nature.*** 2000. 407

TLR Activation Promotes Pro-inflammatory Gene Activation



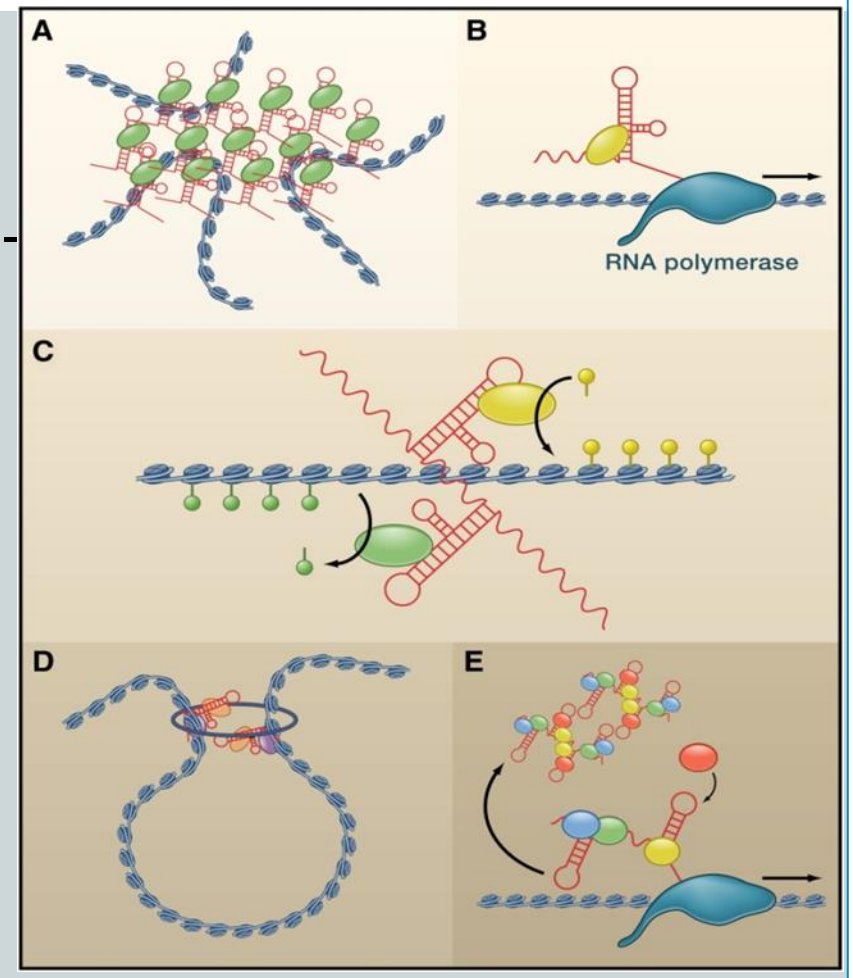
Response to Toll Like Receptor Stimulation



Dr. Joshua Stender UCSD

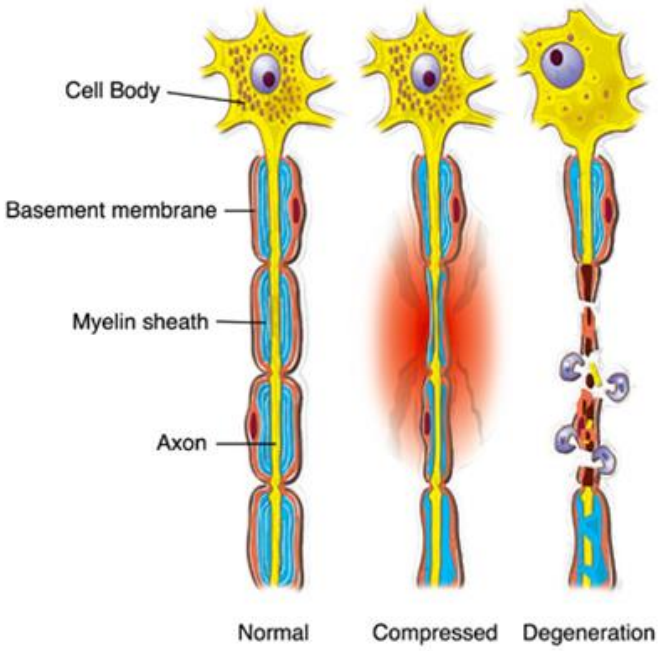
Known Role of Non Coding RNA

- Cis-Acting non coding RNA(ncRNA)-
- local silencer
- Trans-long non coding RNA (lncRNA)-
- Transcriptional regulator
- ncRNA as Histone Modifier Scaffolds
- Enhancer related RNAs



Nagano and Fisher Cell Volume 145 Issue 2

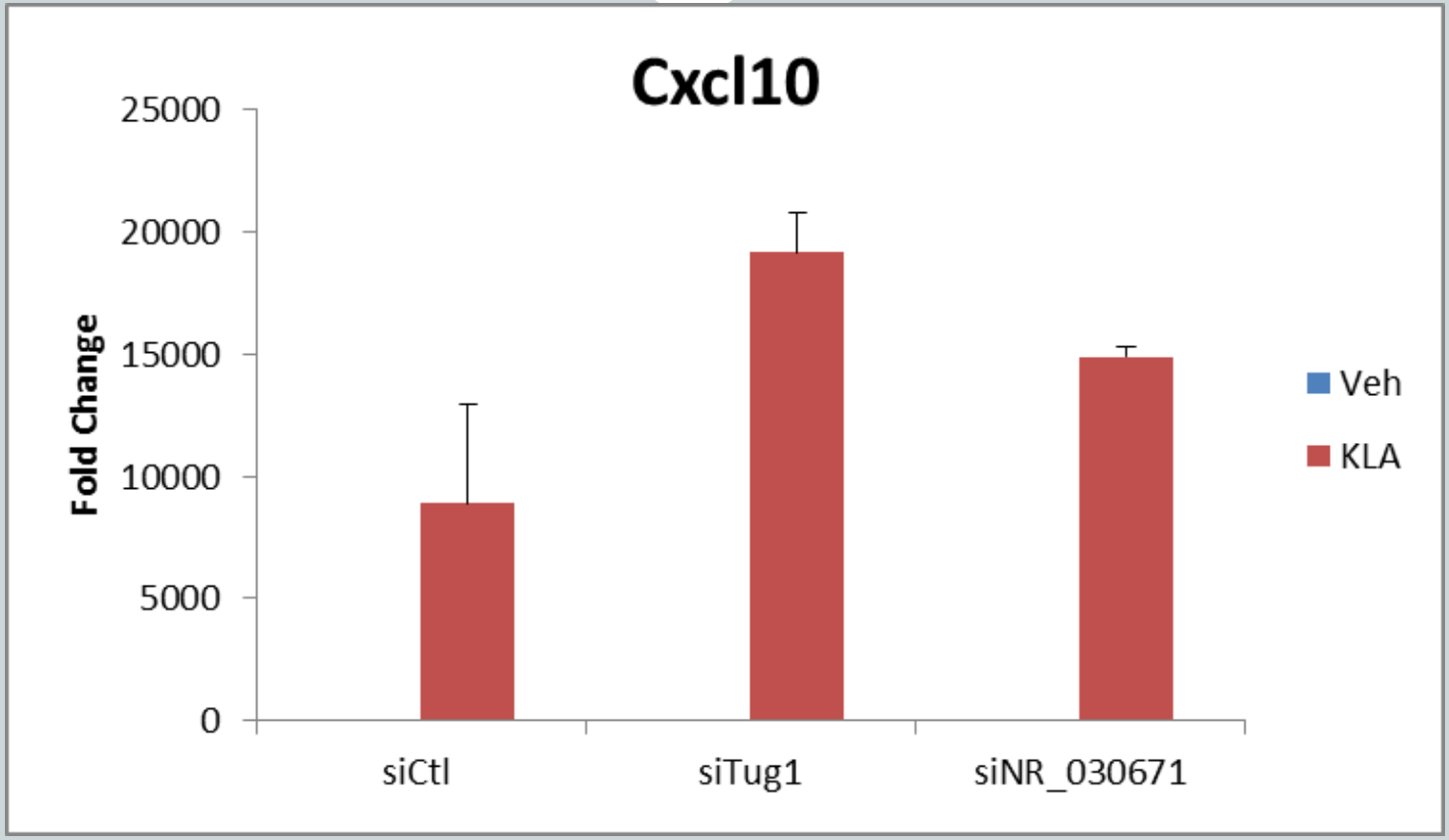
Myelin

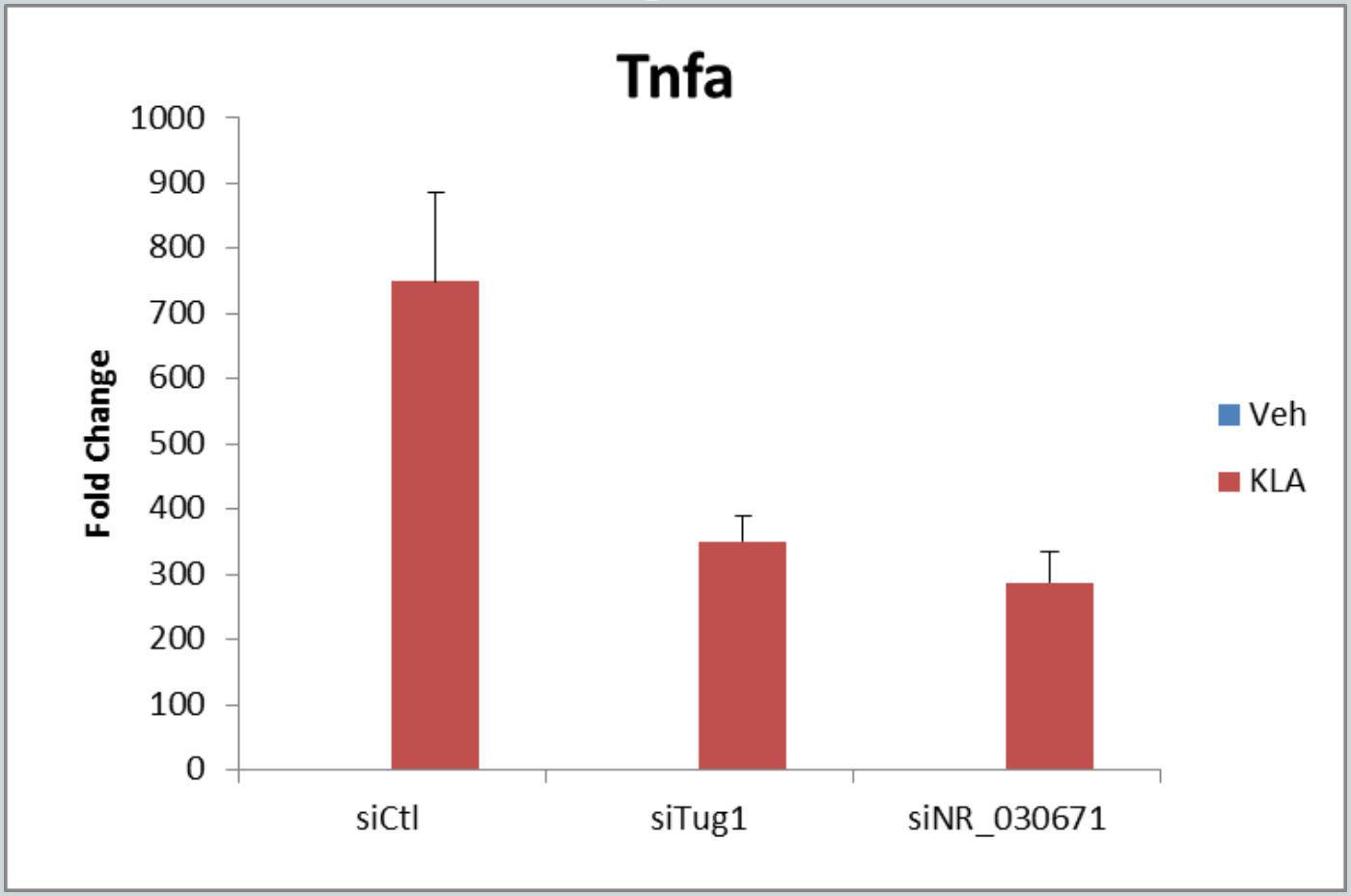


- **Composed of *lipids* and *proteins* (myelin basic protein, MBP; proteolipid protein, PLP; myelin-associated glycoprotein, MAG; myelin-oligodendrocyte glycoprotein, MOG)**
- **Myelin debris is an inhibitory signal for regeneration**
- **No direct evidence that myelin -debris can stimulate inflammation**

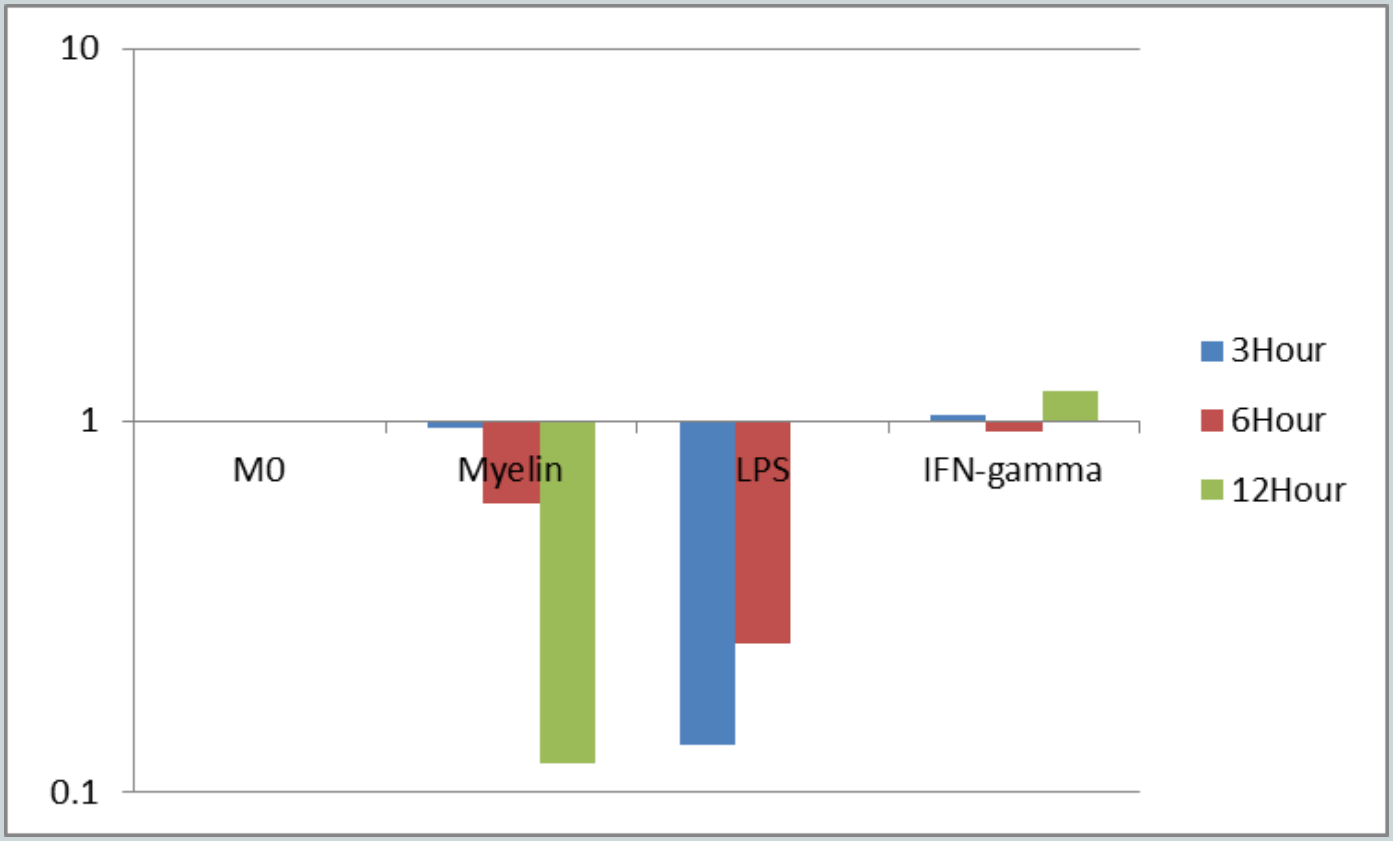
Methodology

1. Extract hematopoietic stem cells from mice bone marrow.
2. Culture the hematopoietic stem cells in a medium that promotes macrophage differentiation.
3. Culture a sufficient number of macrophages for multiple trials of experiment.
4. Culture macrophages with IFN-gamma, LPS and myelin debris. The myelin debris is to represent myelin after traumatic spinal cord injury.
5. Isolate RNA from the cytoplasm of each group at 3, 6, and 12hours.
6. Analyze the RNA by use of quantitative real time PCR.


Cxcl10
Fold Change
25000
20000
15000
10000
5000
0
siCtl
siTug1
siNR_030671
Veh
KLA


Tnfa
Fold Change
1000
900
800
700
600
500
400
300
200
100
0
siCtl
siTug1
siNR_030671
Veh
KLA

TUG1 Expression



Discussion

- TUG1 expression has anti-inflammatory effect
- TUG1 silencing increases the expression of several pro-inflammatory proteins
- In LPS exposed and myelin laden macrophages, TUG1 expression is repressed. This suggests that myelin-laden macrophages have pro-inflammatory characteristics.
- This also suggests that TUG1 is an important mediator for the pro-inflammatory state of macrophages at sites of spinal cord injury

Future Directions

- The next step in the project would be to observe the phenotypical changes involved with TUG1 silencing and overexpression.

Acknowledgements

- FSU College of Medicine and the Division of Research, Graduate and Undergraduate Programs
- Dr. Ren and the Ren Lab

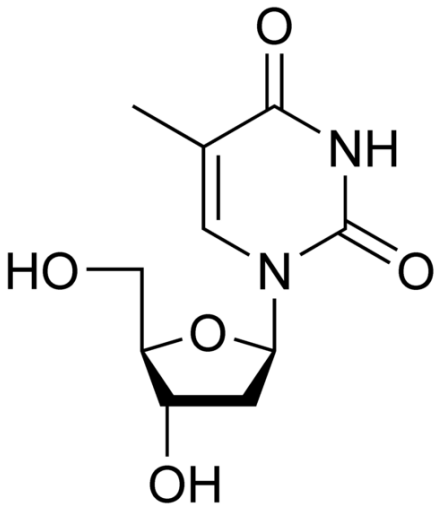
Differential Incorporation Rates of the S-Phase Markers Bromodeoxyuridine and Ethynyldeoxyuridine

Grand Rounds 2014

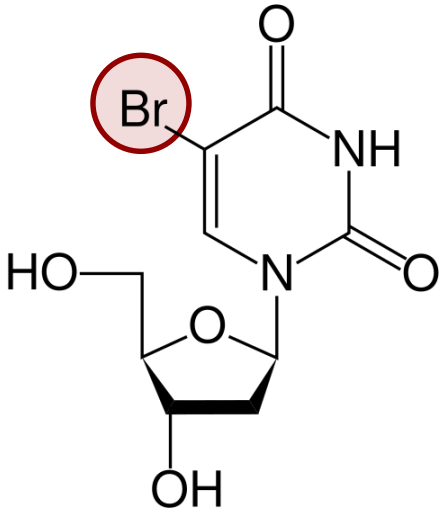
Princess Urbina
Eric Laywell, Ph.D.

Florida State University
College of Medicine

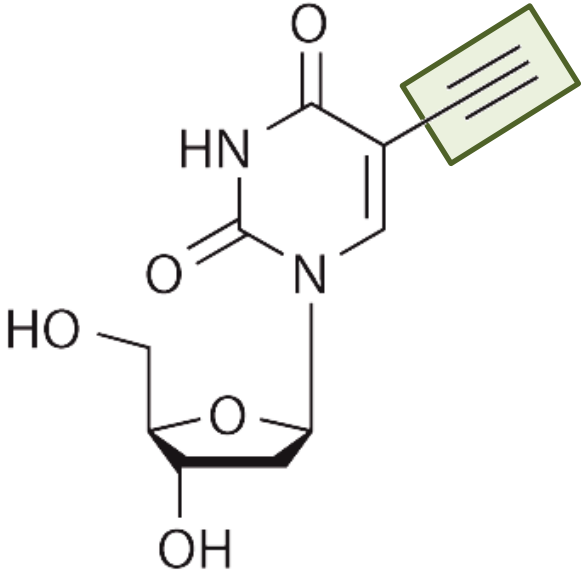
Thymidine



BrdU

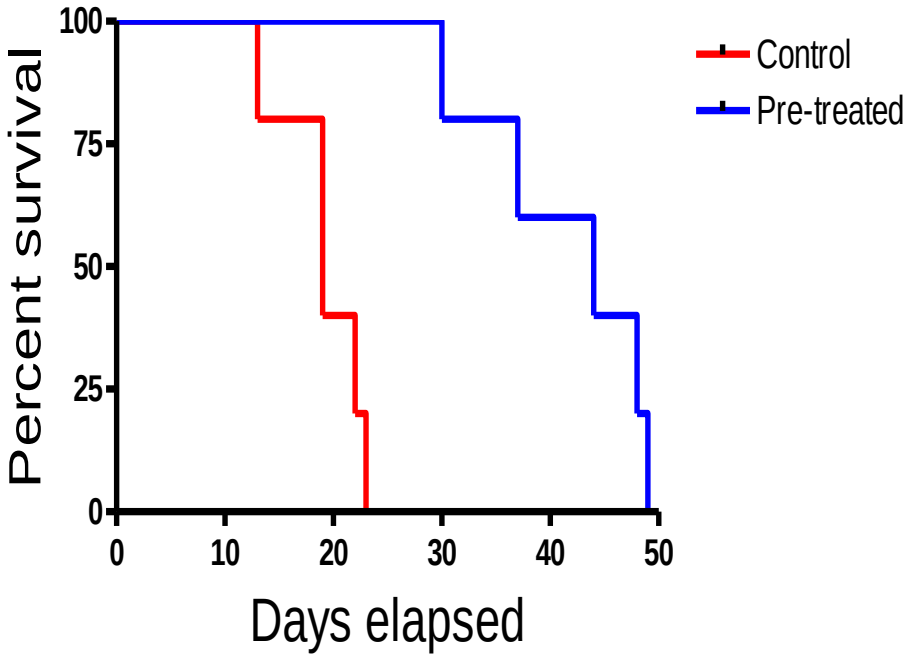


EdU



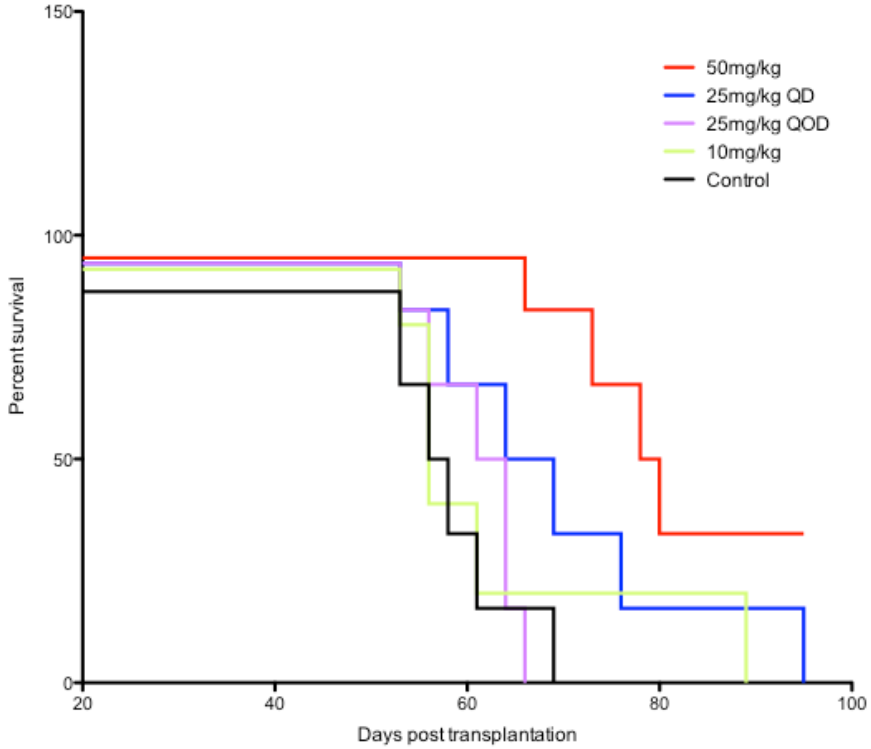
BrdU and EdU delay tumor progression in rodent models of glioma

BrdU



syngeneic rat glioma

EdU



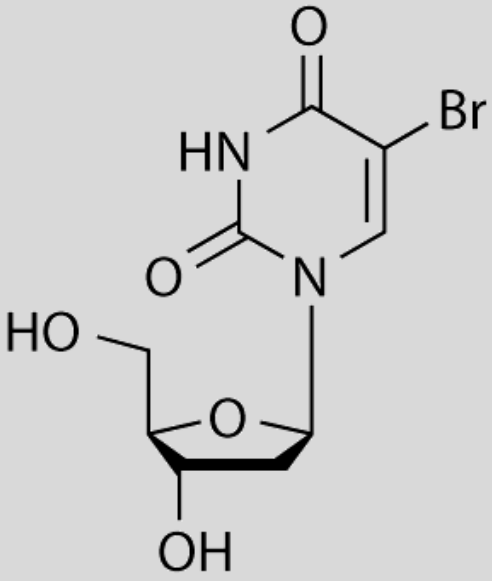
human GBM in mouse xenograft

- **BrdU has a long history of use as an s-phase marker in cell birthdating studies and proliferation assays.**

- **EdU is beginning to replace BrdU as the preferred s-phase marker, since it is faster and easier to detect.**

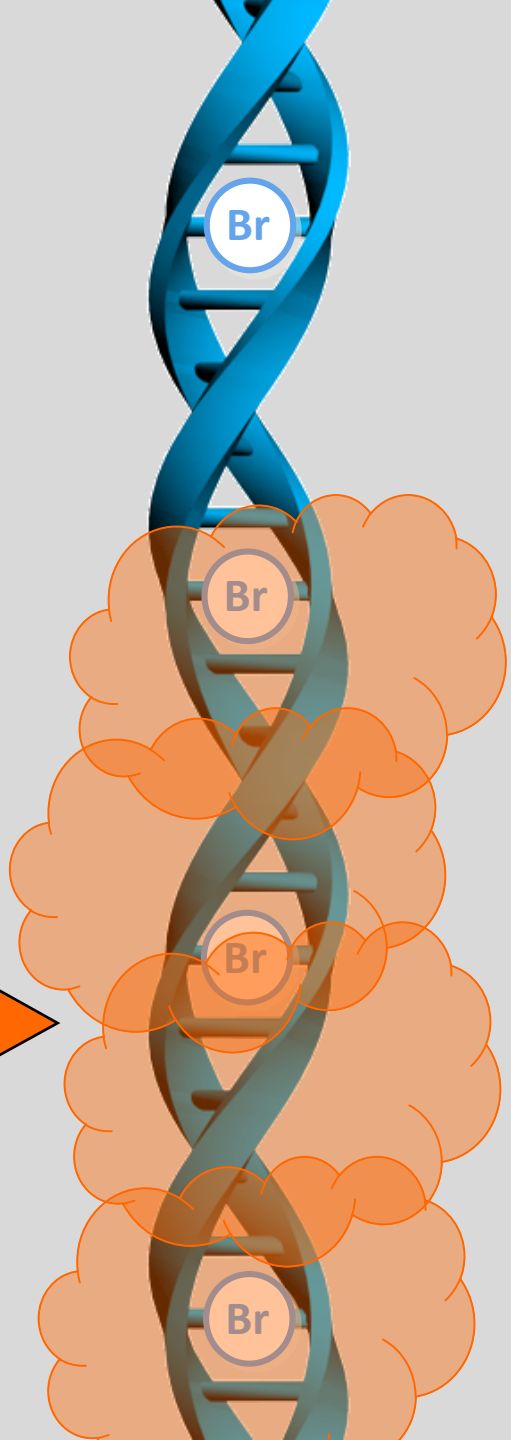
- **Both BrdU and EdU have shown potential as tumor inhibitors.**

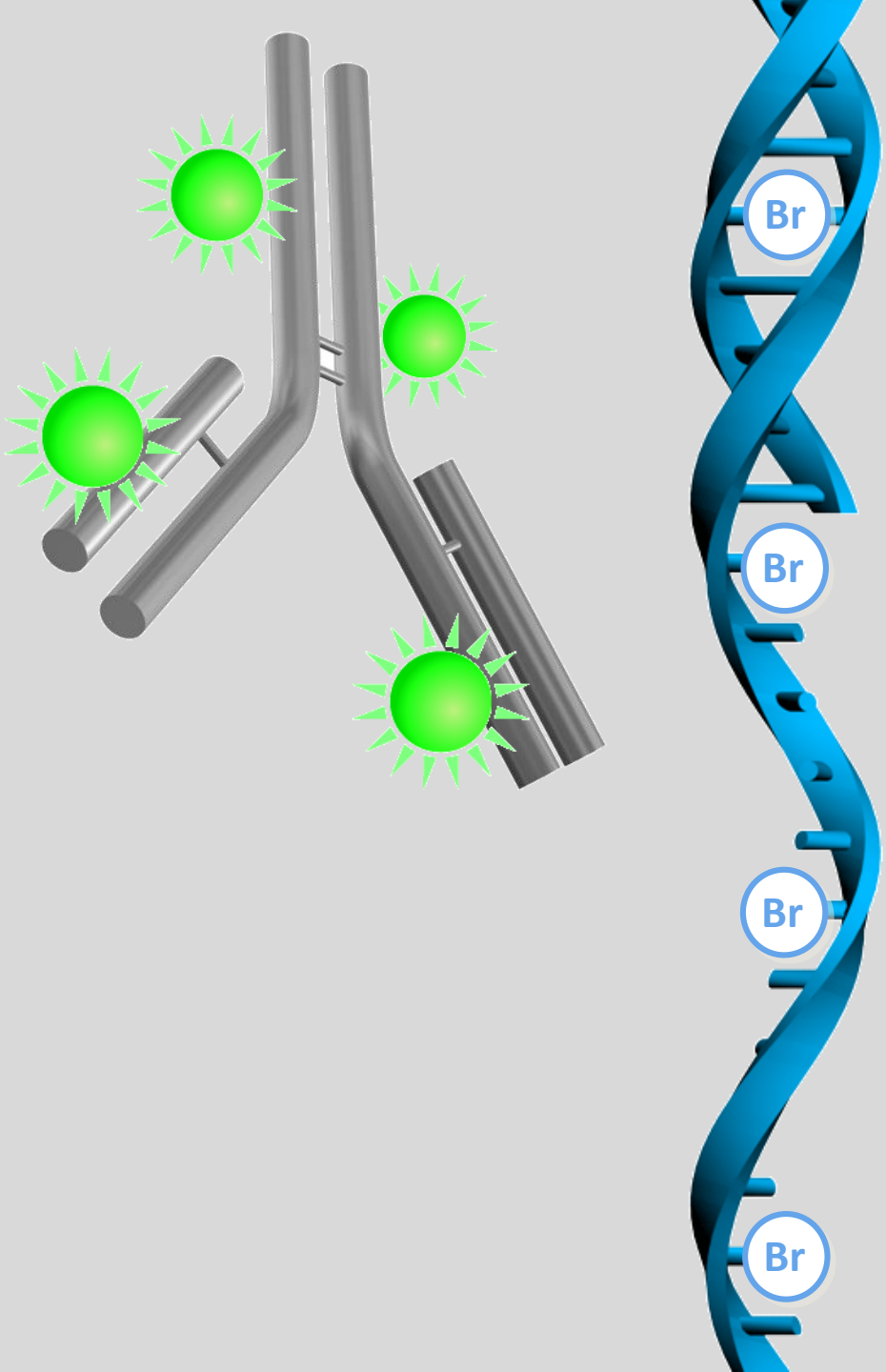
- **Therefore, studies of their uptake kinetics will provide needed information that may influence their use as both experimental s-phase markers, and as possible adjunctive cancer therapeutics.**

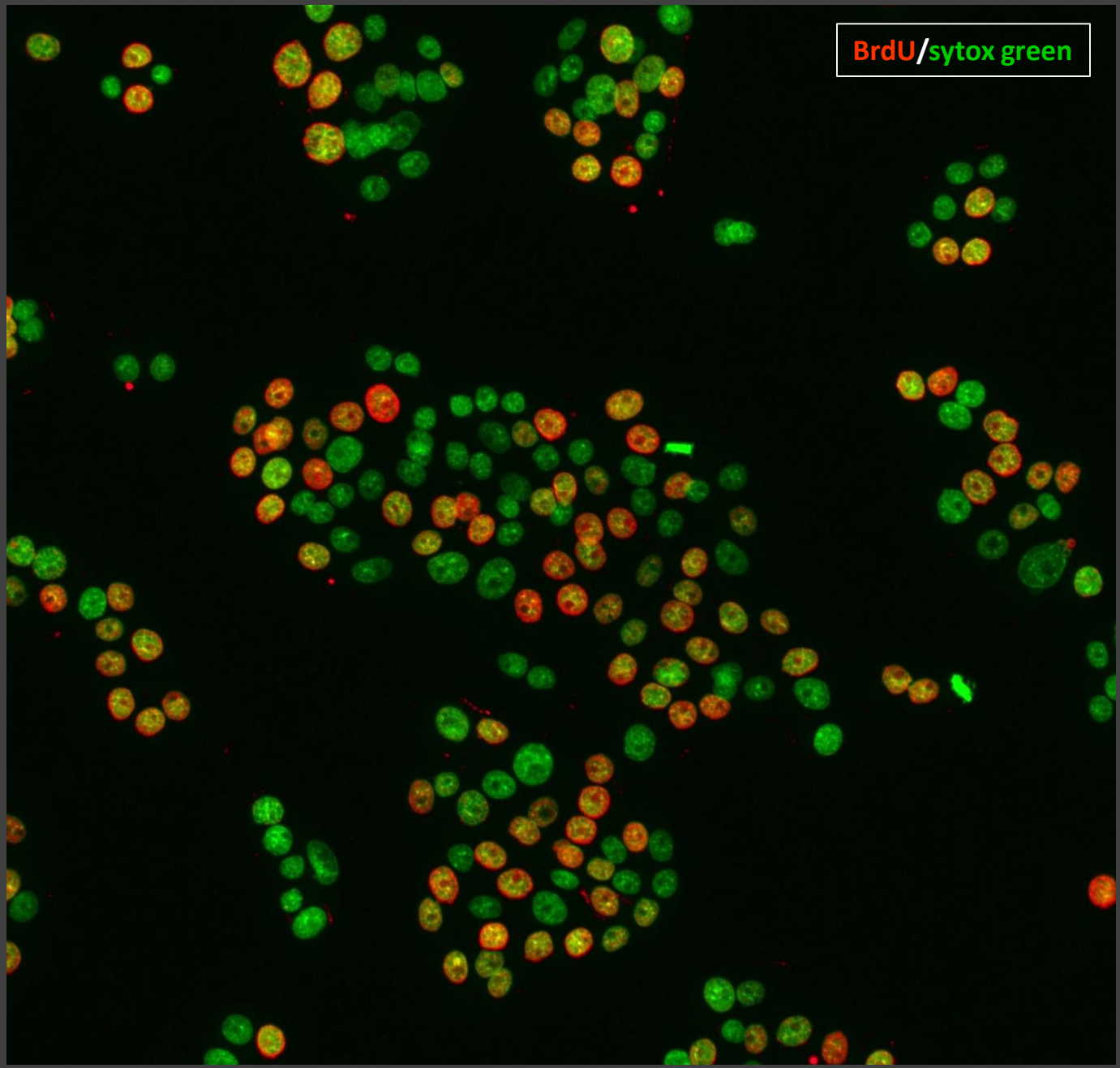

O
Br
HN
O
N
HO
O
OH


Br
Br
Br
Br

ACID OR DNAse

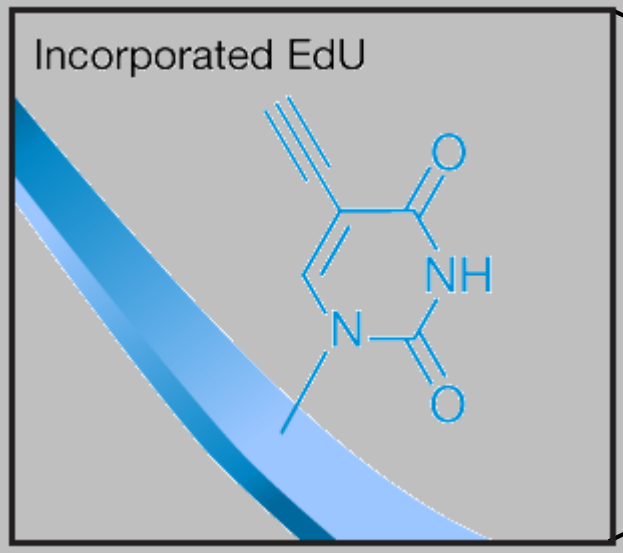


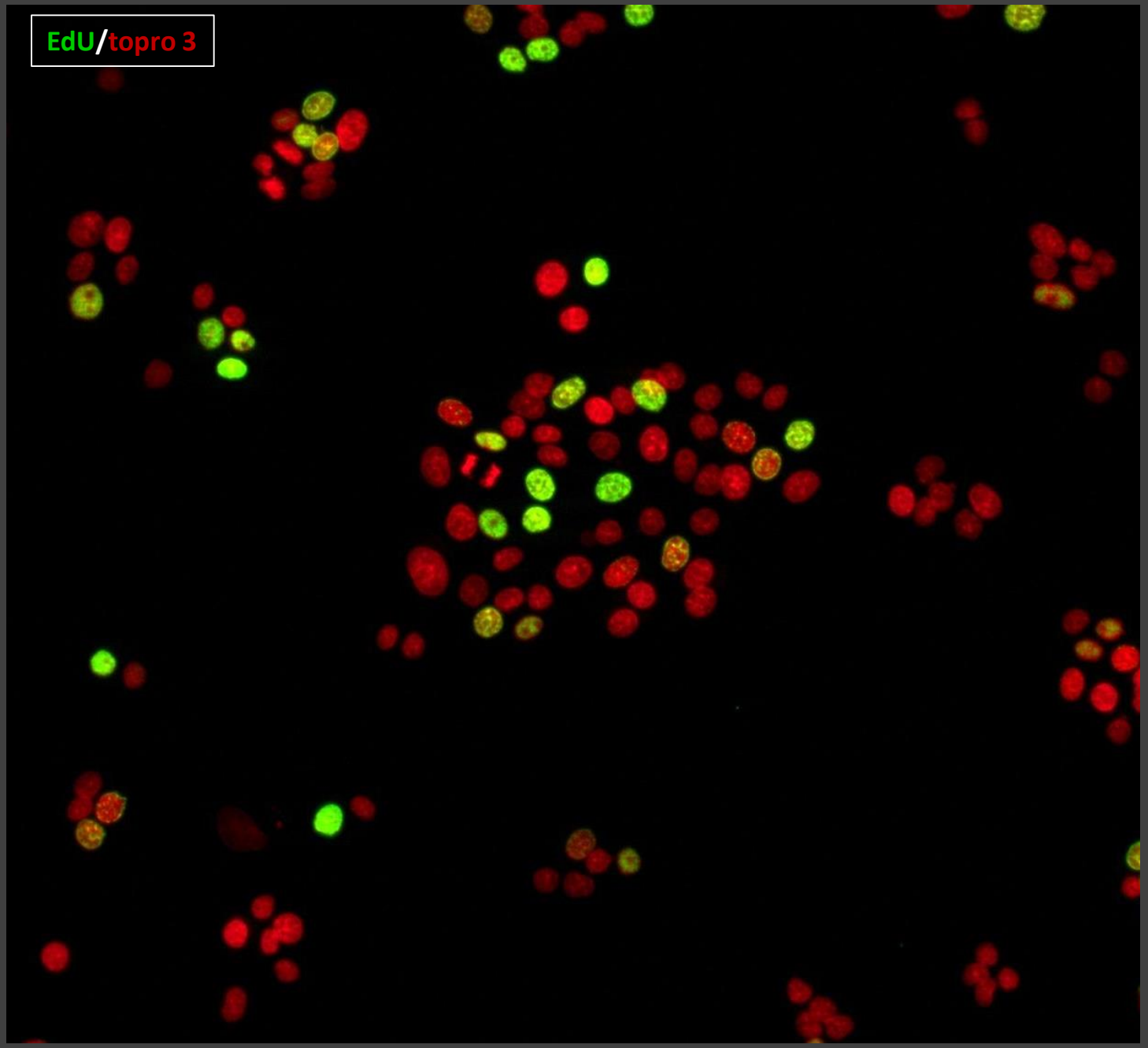

Br
Br
Br
Br


BrdU/sytox green

Click-iT™ EdU

EdU contains an alkyne which reacts with an azide (Alexa fluor 488), forming a very stable covalent bond.



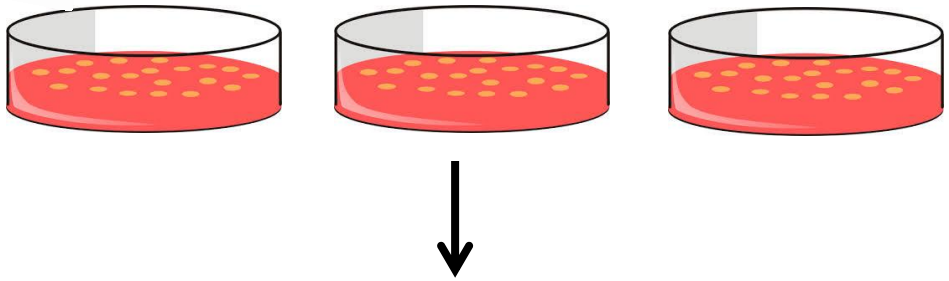

EdU/topro 3

Goal

Compare the rates of BrdU and EdU incorporation in cells in vitro and in two areas of persistent neurogenesis in vivo.

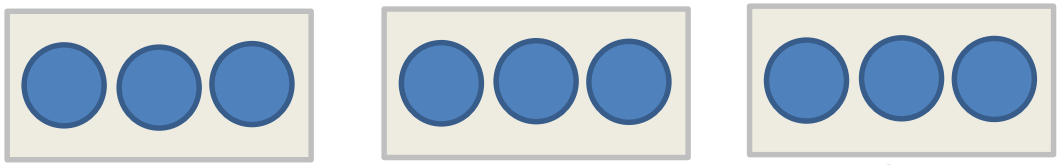
In Vitro

1) Treat:
10uM BrdU or EdU



Human
SaOS cells

2) Fix



5 min. 2 hrs. 6 hrs.

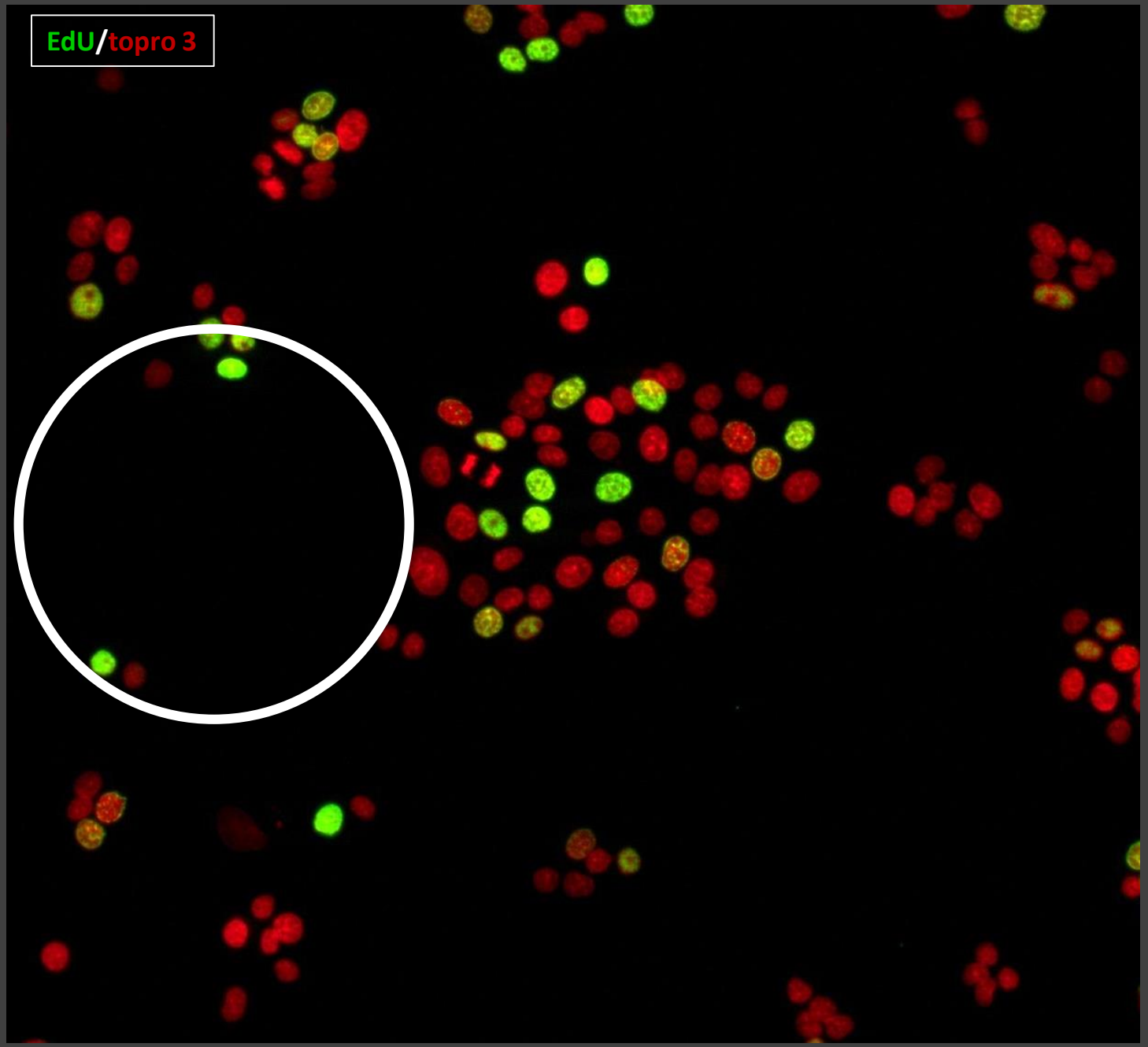
3) Stain

BrdU
Immunostaining

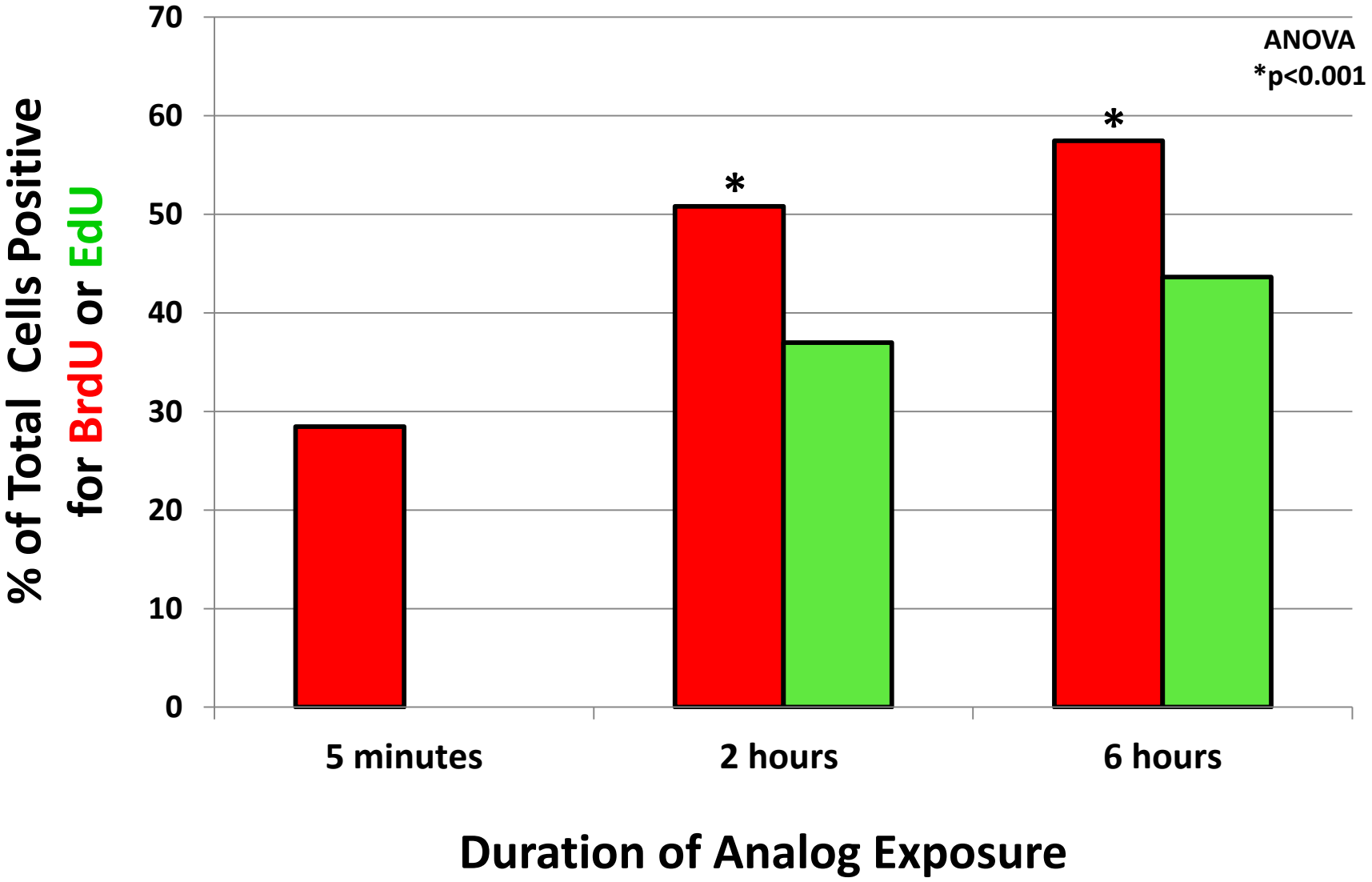
or

EdU
Click-It Kit

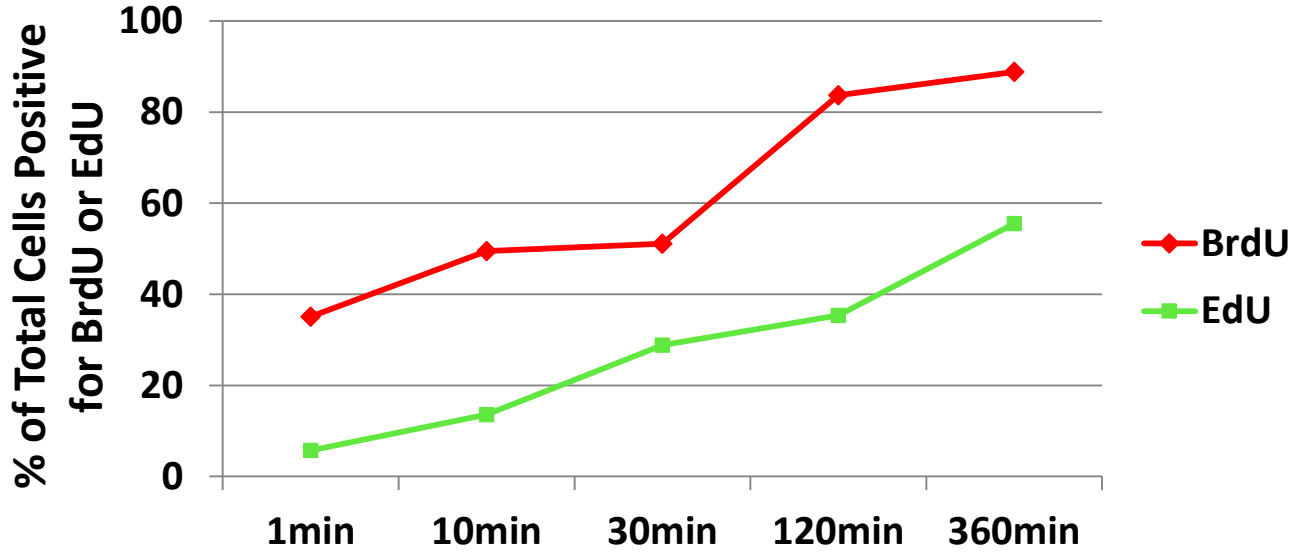
4) Quantify


EdU/topro 3

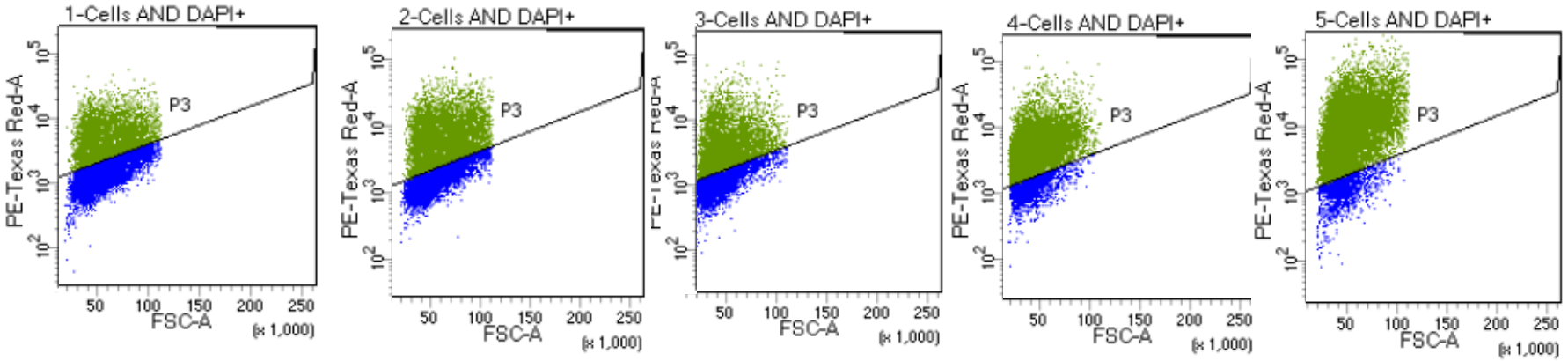
EdU incorporates more slowly than BrdU in vitro



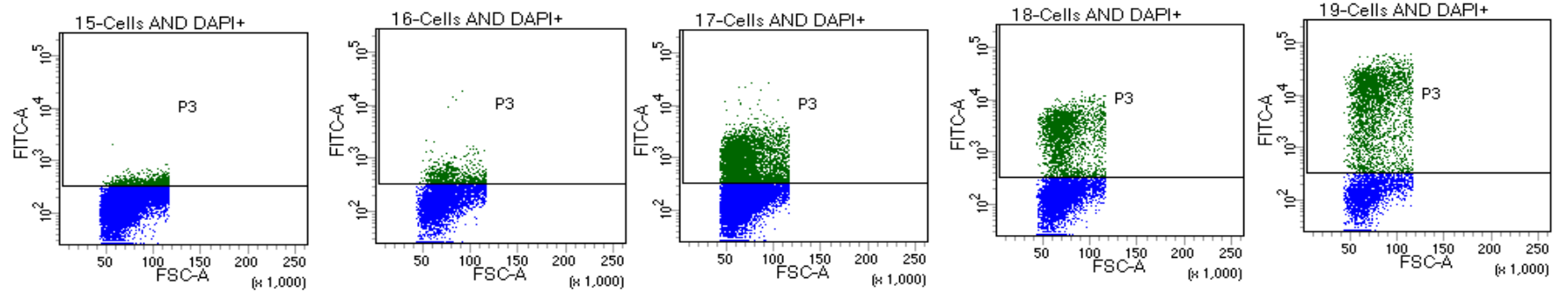
Flow Cytometry



BrdU

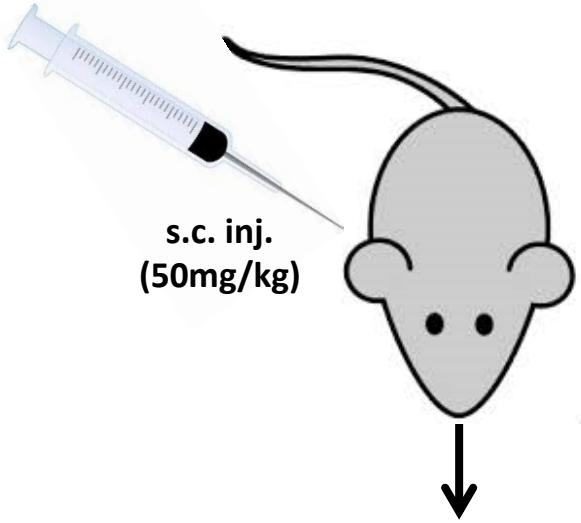


EdU



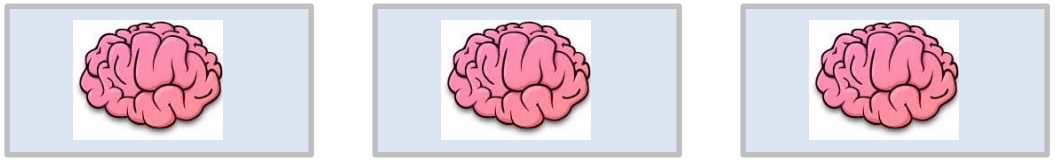
In Vivo

1) Treat:
BrdU or EdU



Adult Male
C57BL/6 Mice

2) Sacrifice, collect, section



5 min. 20 min. 3 hrs.

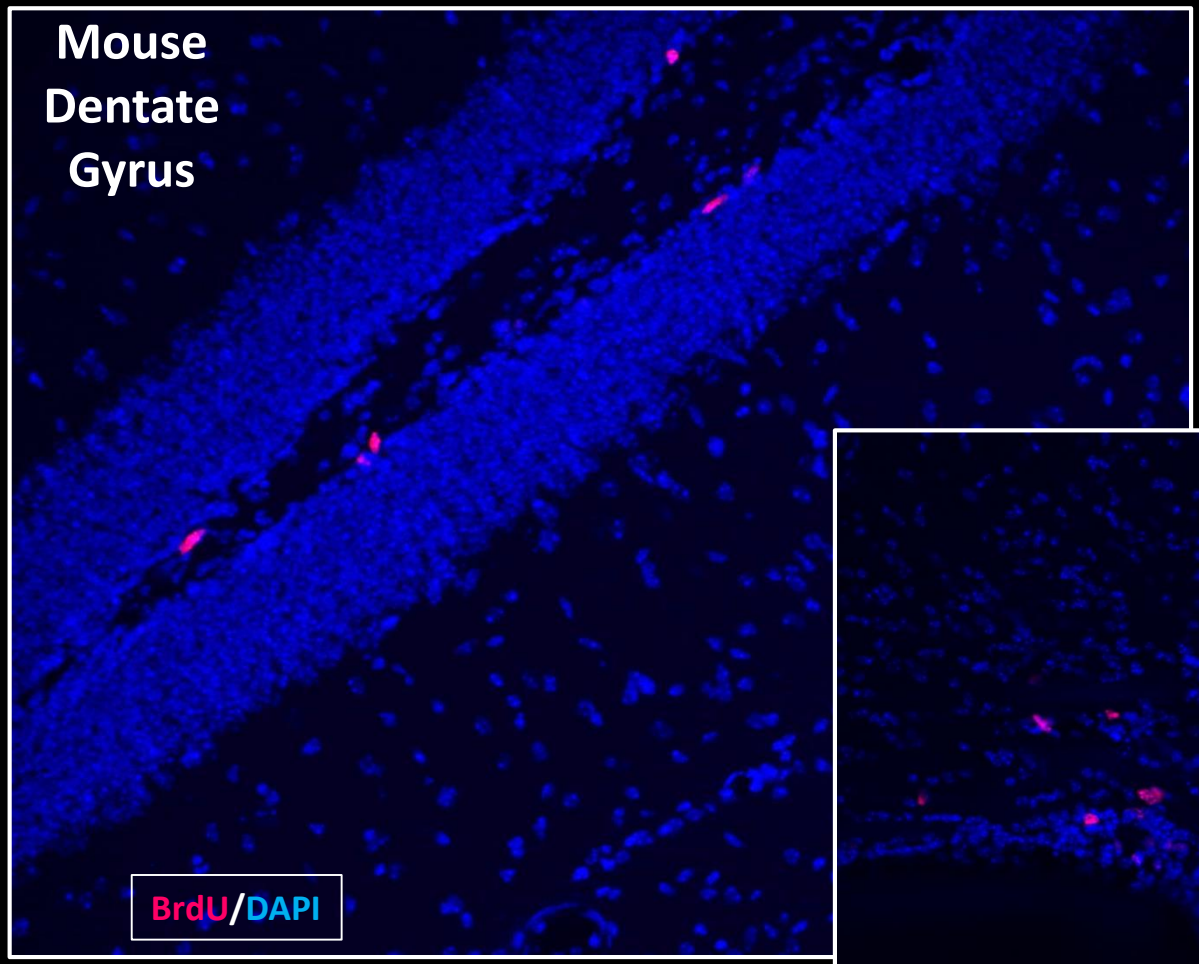
3) Stain

BrdU Immunostaining

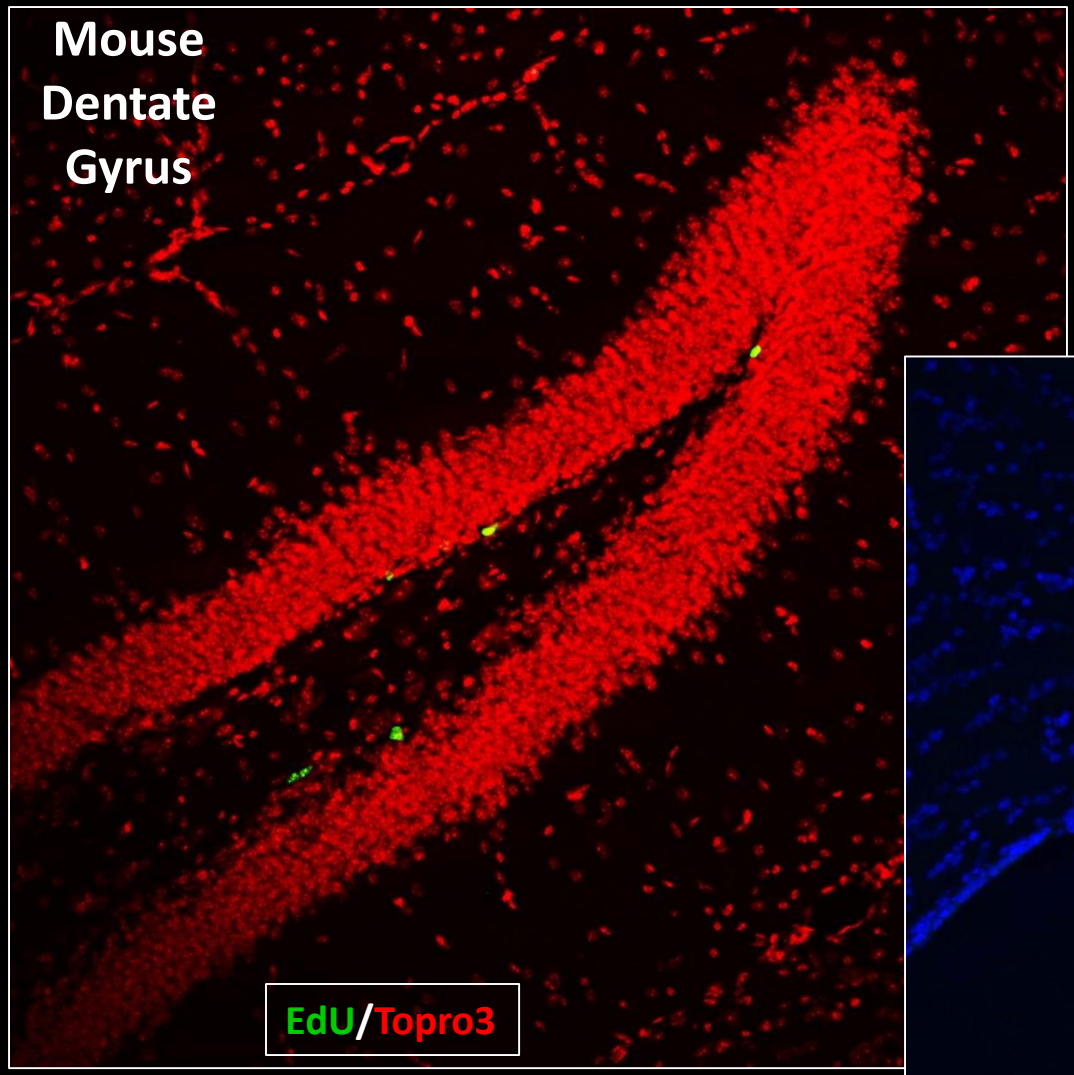
or

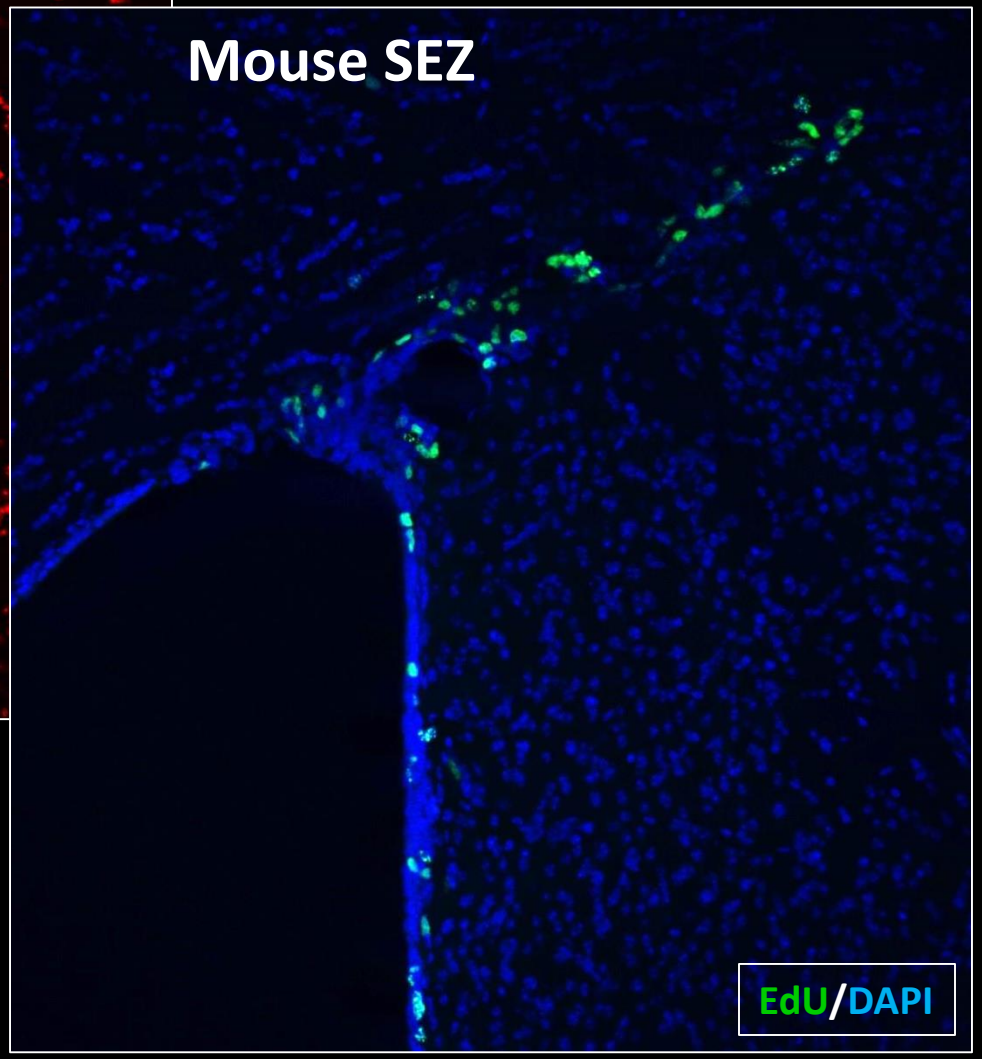
EdU Commercial Labeling Kit

4) Quantify

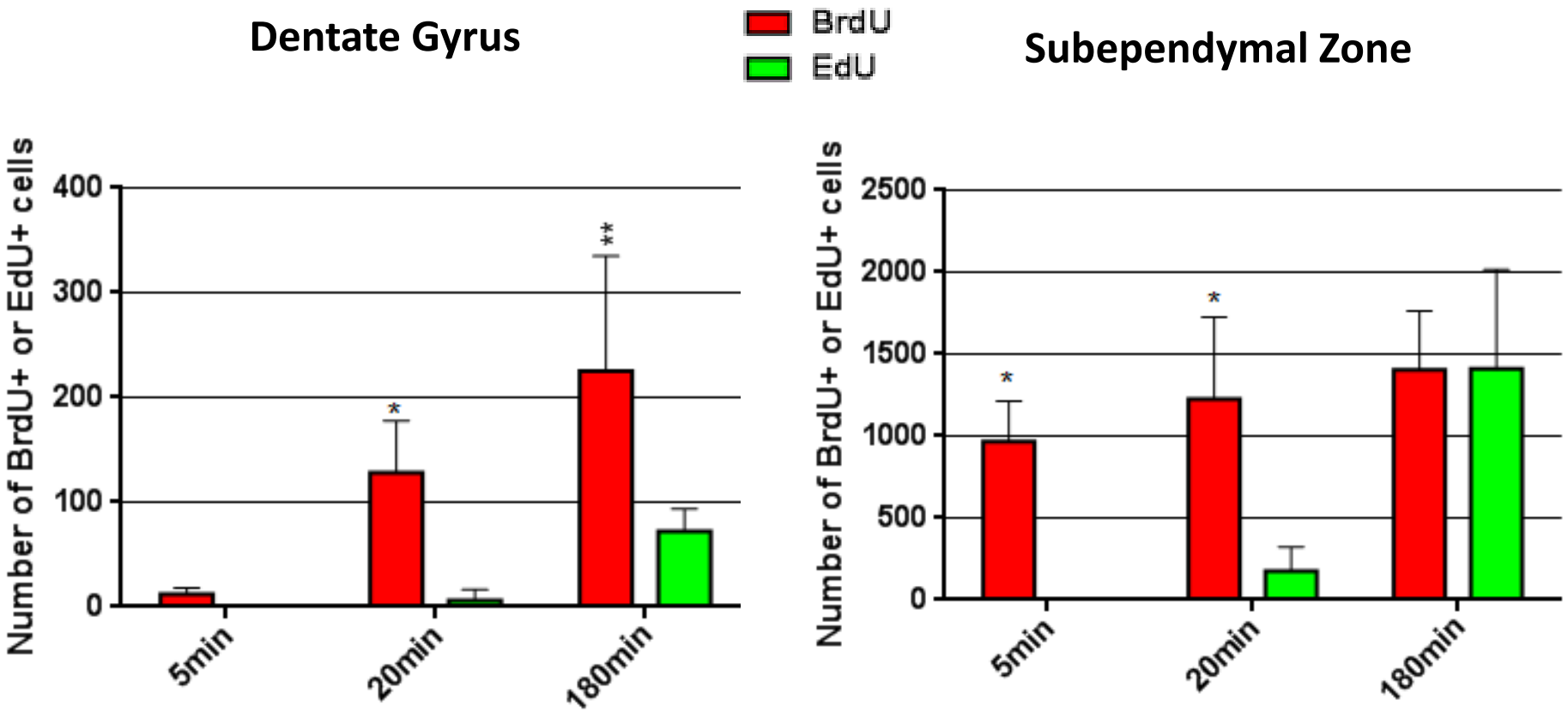

Mouse
Dentate
Gyrus
BrdU/DAPI


Mouse SEZ
BrdU/DAPI


Mouse
Dentate
Gyrus
EdU/Topro3


Mouse SEZ
EdU/DAPI

EdU incorporation lags behind BrdU in vivo



Duration of "Chase" Interval

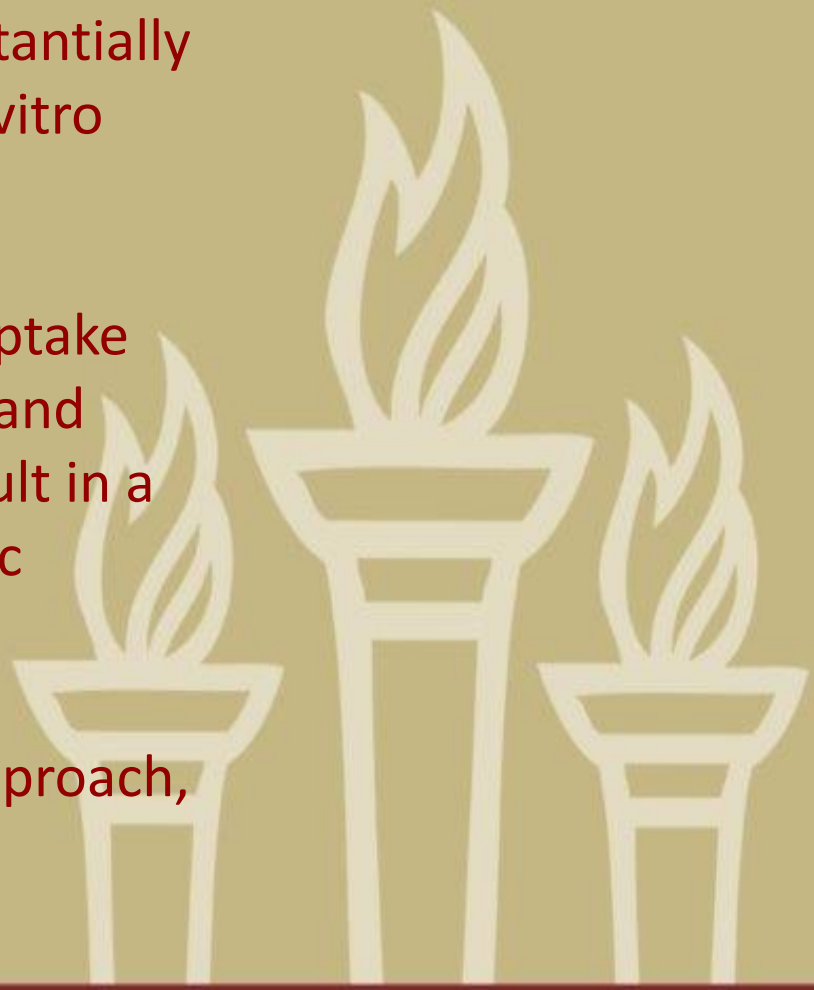
(survival time after injection)

Conclusions

- EdU incorporation consistently and substantially lags behind that of BrdU in SaoS cells in vitro and in newly-generated cells in vivo.

- Failure to appreciate these differential uptake kinetics when designing cell birthdating and proliferation index experiments may result in a drastic **under**estimation of DNA synthetic events.

- Conversely, from a chemotherapeutic approach, one risks **over**estimating EdU uptake.



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Future Directions

- Manuscript in progress
- Exact mechanism requires further investigation
  - Entry of analogs into cell?
  - Phosphorylation states?
  - Polymerase efficiency?

Florida State University
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Principal Investigator

Christina Chavarriaga
Lab Technician

Melody Wu
Lab Technician

Javier Ayo
FSU M1

Florida State University
College of Medicine

Thank you!

Questions?

Florida State University
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