



Klotho in Aging and Disease

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INTRODUCTION

- Klotho is a regulatory anti-aging protein that is significantly expressed in the choroid plexus, plays an important role in regulating the immune response in the CNS, and diminishes with age
- Klotho deficiency is characterized by significant neuroinflammation, oxidative damage in the neural parenchyma, and decline in neurocognitive functions
- The choroid plexus is a reservoir for HIV, and as HIV infection can cause HIV-associated neurocognitive disorders (HAND)—an inflammaging phenotype, we hypothesized that klotho may play a role in the development of this neurocognitive deficit
- Approximately 30-60% of adults with HIV develop HAND (Rosca 2021)

OBJECTIVES

- In this project, we sought to characterize patterns of age-related klotho decline in primates as well as to determine if there was a correlation between klotho expression in the choroid plexus and HIV infection.

METHODS

Immunofluorescence:

- Choroid plexus samples of *Rhesus macaques* of various ages and SIV infection status were studied
- The tissues were stained using immunohistochemistry for polyclonal klotho and ZO-1
- Tissues were first blocked with 5% normal goat serum
- 200uL of primary antibody solution were applied to each slide for 1 hour in a dark setting
- The previous step was then repeated with the respective secondary antibody solution
 - Polyclonal Klotho primary antibody (Invitrogen), 1:50 dilution of 1mg/mL stock
 - Klotho secondary goat ant– rabbit 488 (Invitrogen), 1:1000 dilution of 2 mg/mL stock
 - ZO-1 primary antibody (Invitrogen), 1:100 dilution of .5 mg/mL stock
 - ZO-1 secondary goat anti- mouse 568 (Invitrogen), 1:1000 dilution of 2mg/mL stock

Quantification of Mean Fluorescence Intensity:

- To measure MFI, 10 images were taken of the choroid plexus at 40x on each slide
- SIV analysis images were captured on a Nikon Ti2 microscope, while the aging analysis images were obtained using a Keyence BZ-X810
- For each image, 10 choroid plexus epithelial cells were selected and analyzed in Fiji
- The background fluorescence was also measured and subtracted from the epithelial fluorescence
- All 10 calculations of corrected total cell fluorescence (CTCF) were then averaged for the final MFI value

Statistics:

- All statistical analyses of counts were performed with GraphPad Prism 7. α -klotho mean fluorescence intensity was analyzed using a Mann-Whitney one- and two-tailed t-test. Significance was defined a $p < 0.05$

RESULTS

- Although klotho expression was varied in the uninfected *Rhesus macaques*, there was an overall decreasing trend with the advancement of age past adulthood
- In SIV-infected *Rhesus macaques*, the choroid plexus had significantly less klotho expression than the uninfected control group regardless of age ($p = 0.01$)

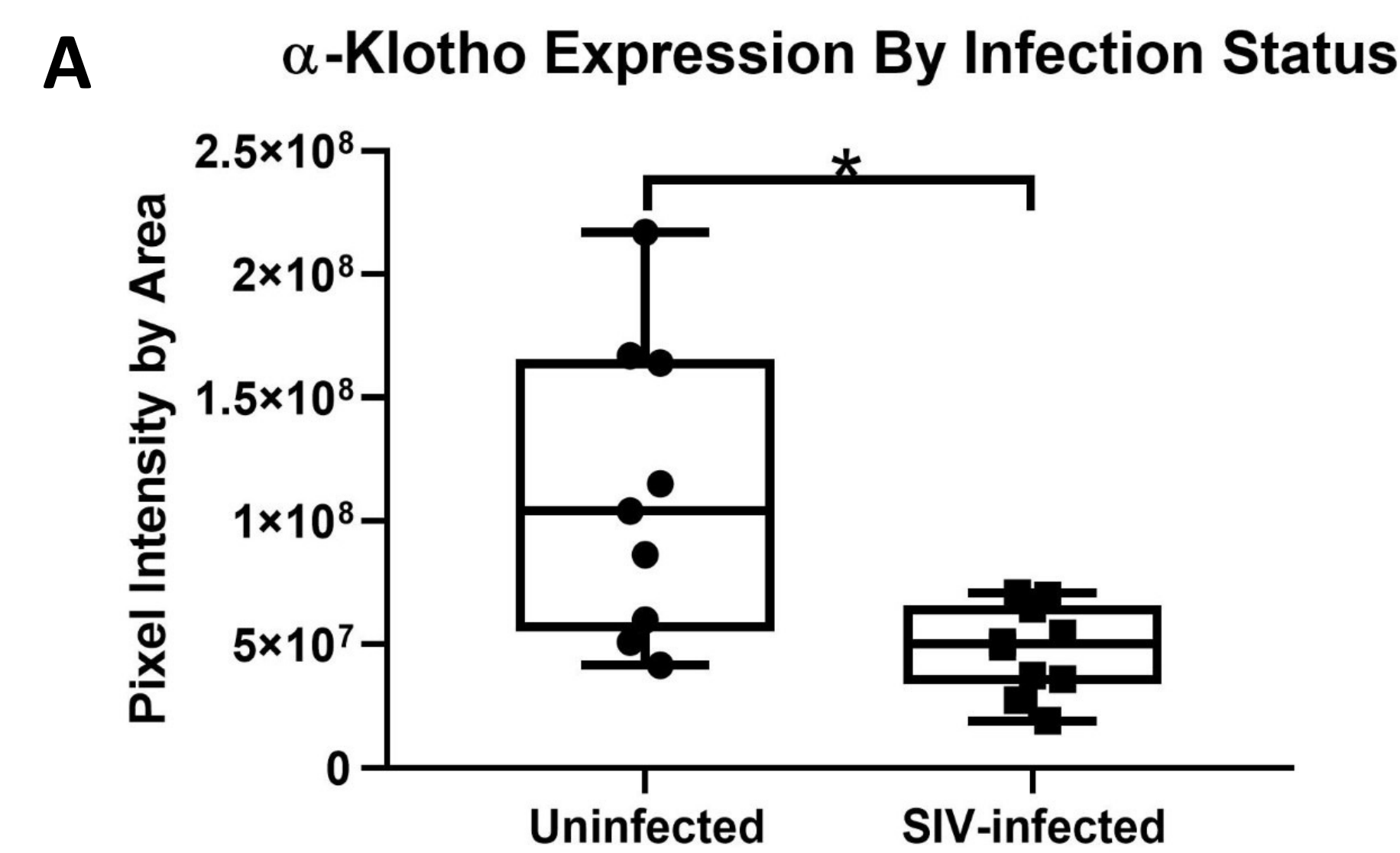


Figure A shows the dramatic decrease in klotho expression in SIV infected individuals compared to the uninfected control group. The SIV infected group had much more narrow range of klotho expression compared to the uninfected. This could be due to natural genetic variance in the uninfected.

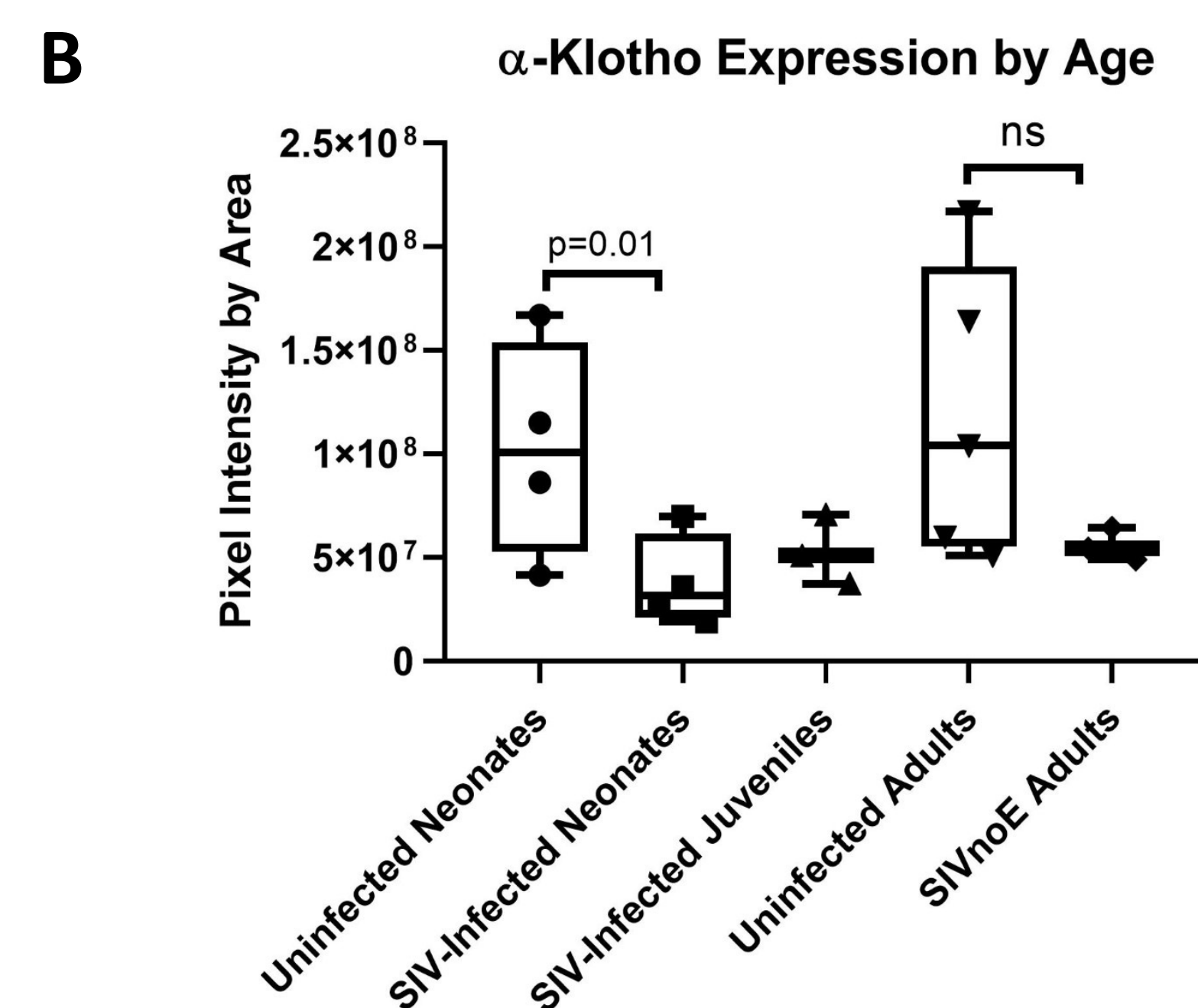


Figure B compares klotho expression groups separated by both SIV status and age. Both uninfected neonates and uninfected adults had significantly higher klotho expression compared to their infected counterparts.

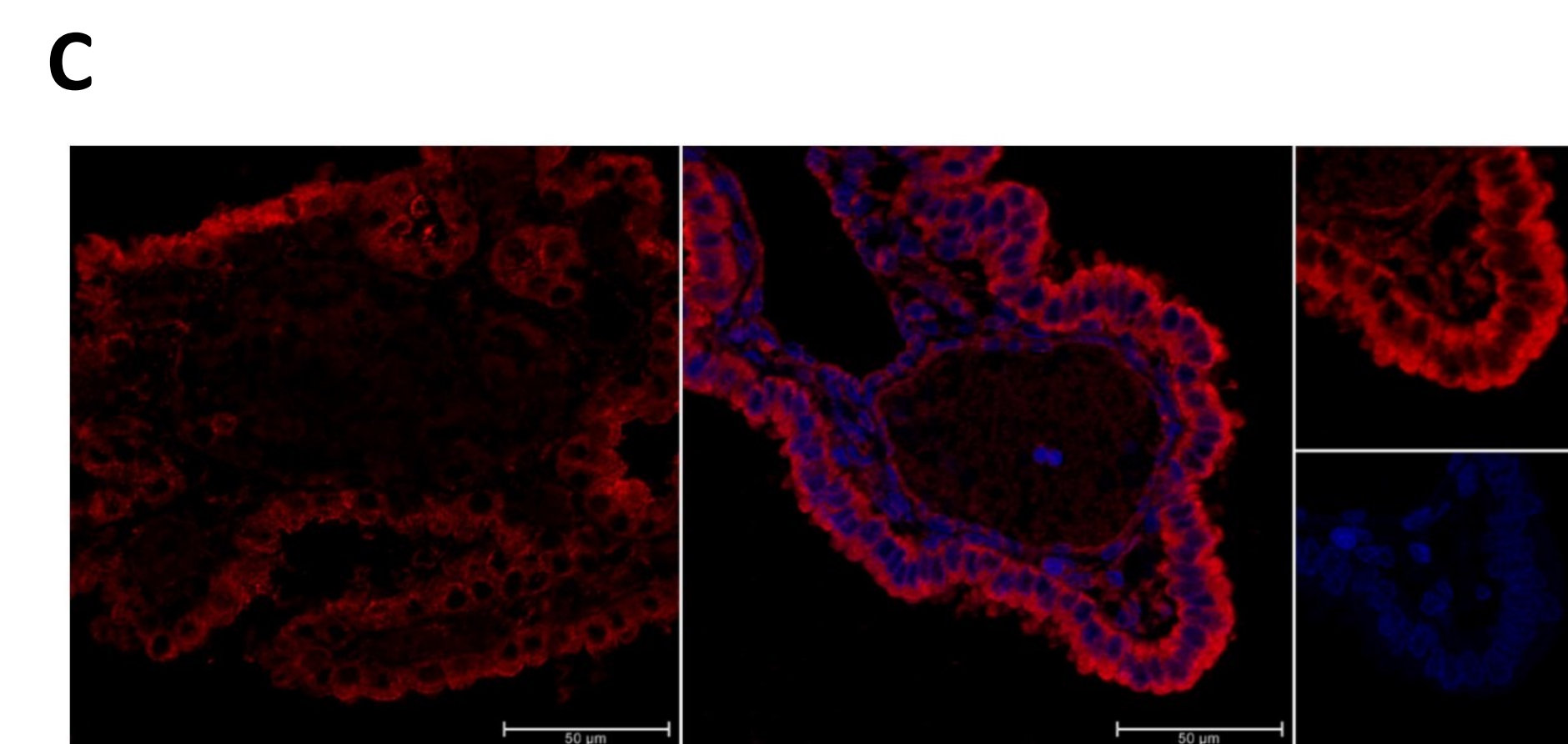


Figure C is a comparison of klotho expression between SIV positive (left) and negative (middle) subjects. The SIV positive image has significantly diminished fluorescence compared to the SIV negative.

D α -Klotho Expression by Age (Years) in Uninfected *Rhesus macaques*

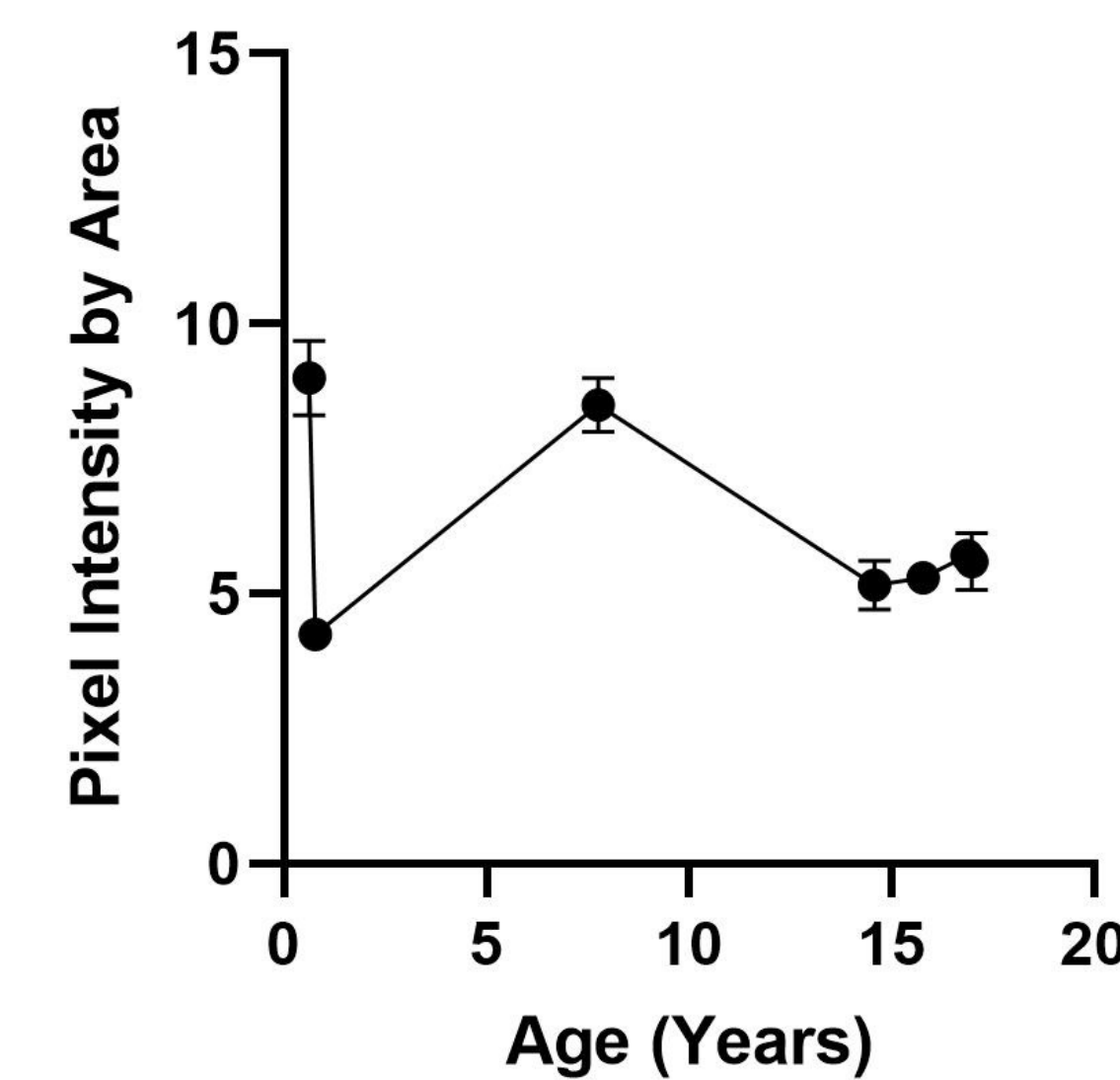


Figure D shows klotho expression in the uninfected based on age. There seems to be a general decrease in klotho expression past adulthood. However, the neonatal data is unclear as there is significant difference between the two neonates.

E α -Klotho Expression in Uninfected *Rhesus macaques*

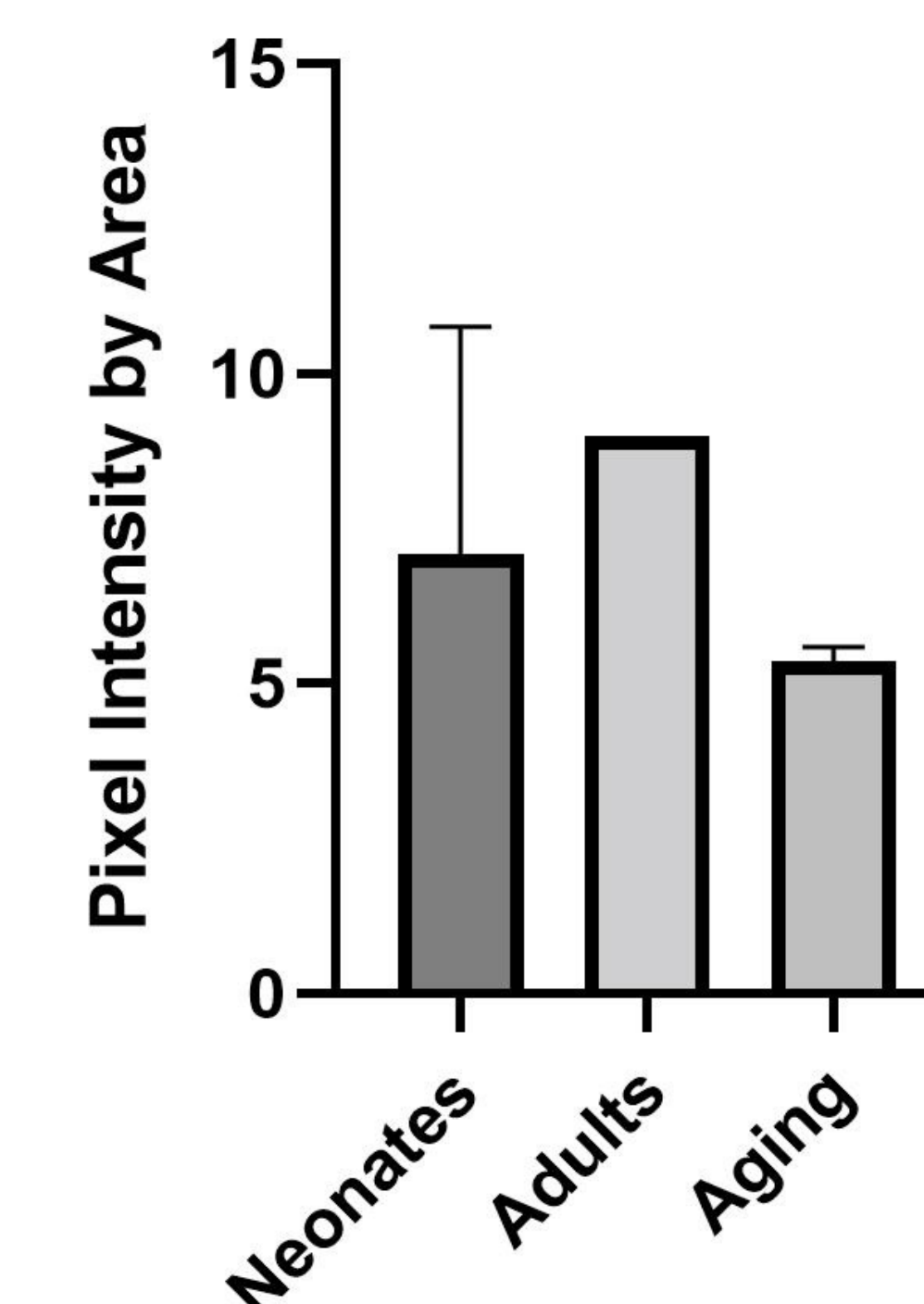


Figure E shows klotho expression in the uninfected based on different age groups. There was an increase in klotho expression from neonates to adults and a subsequent decrease between adults and aging groups.

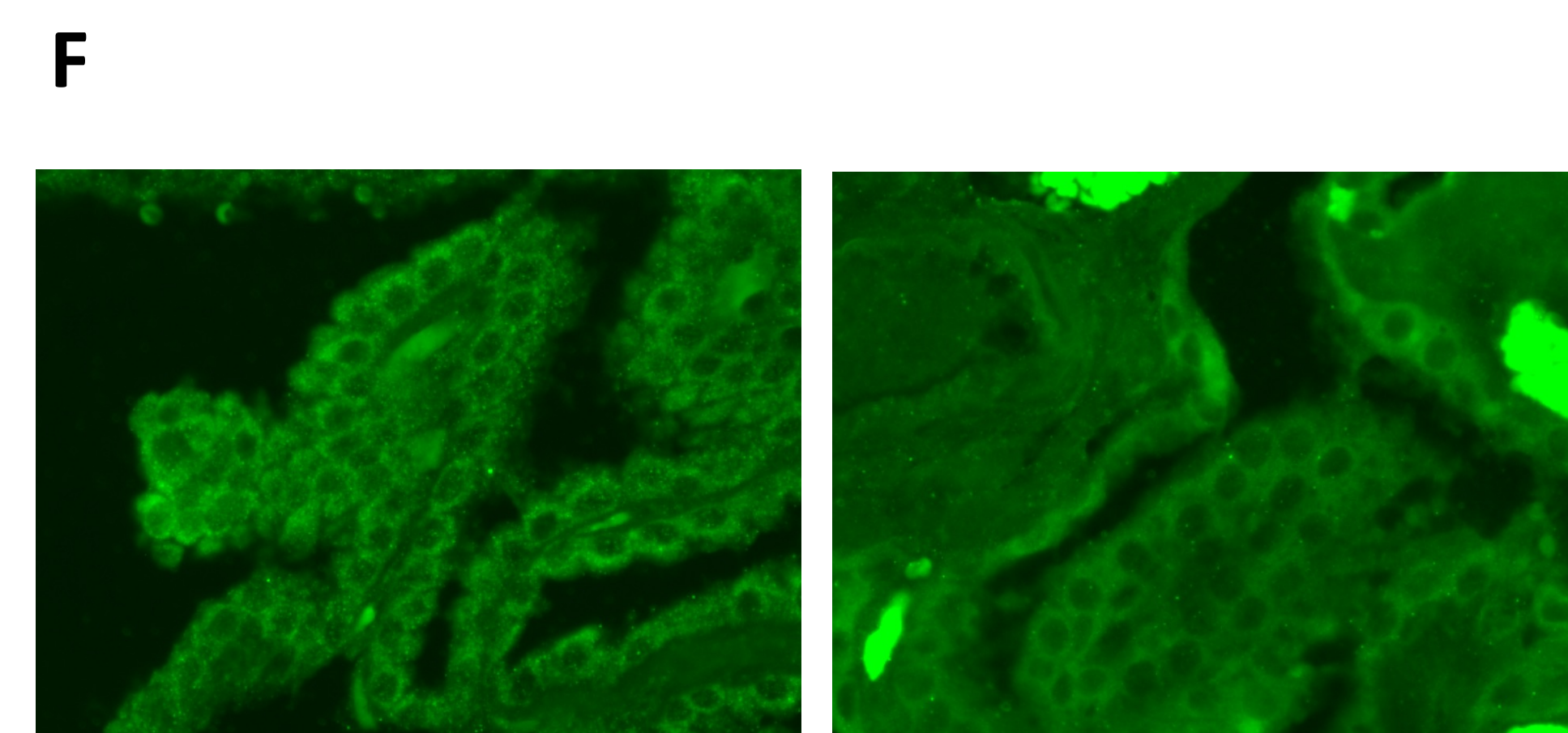


Figure F compares images of klotho staining between an uninfected adult (left) and aging (right) subject. The intensity of fluorescence is greater in the adult subject.

G

Animal ID	Status	Sex	SIV Strain	SIV (Age at Infection)	Length of Infection (Days)
16A076	Unin. Neonate	M	Uninfected	N/A	N/A
15A164	Unin. Neonate	M	Uninfected	N/A	N/A
15A202	Unin. Neonate	M	Uninfected	N/A	N/A
15A233	Unin. Neonate	M	Uninfected	N/A	N/A
15A302	Infected Neonate	M	SiVmac251	0	380
11A489	Infected Neonate	F	SiVmac251	0	76
11A490	Infected Neonate	M	SiVmac251	0	37
12A611	Infected Neonate	F	SiVmac251	0.01	62
10A718	SIV Juveniles	F	SiVmac251	0.36	79
11A067	SIV Juveniles	F	SiVmac252	0.21	154
11A204	SIV Juveniles	F	SiVmac251	0.34	209
14A100	Unin. Adults	M	Uninfected	N/A	N/A
14A318	Unin. Adults	M	Uninfected	N/A	N/A
14A319	Unin. Adults	M	Uninfected	N/A	N/A
14A644	Unin. Adults	M	Uninfected	N/A	N/A
14A745	Unin. Adults	M	Uninfected	N/A	N/A
11A439	SiVnoE Adults	M	SiVmac251	9.03	49
11A837	SiVnoE Adults	M	SiVmac251	7.68	223
13A307	SiVnoE Adults	F	SiVmac239	21.47	146
39413	Unin. Neonate	F	Uninfected	N/A	N/A
37747	Unin. Neonate	F	Uninfected	N/A	N/A
32752	Unin. Adults	M	Uninfected	N/A	N/A
28282	Unin. Adults	F	Uninfected	N/A	N/A
26813	Unin. Adults	F	Uninfected	N/A	N/A
19845	Unin. Adults	F	Uninfected	N/A	N/A
19691	Unin. Adults	F	Uninfected	N/A	N/A

Figure G presents additional information on the animals used in this study such as their age, gender, and SIV status.

CONCLUSIONS

- This significant decrease of klotho in SIV infected individuals show that SIV has a significant affect on klotho expression
- Compared to the uninfected, which had natural variances of klotho levels, the SIV infected all had similar levels of klotho despite being diverse in age
- The lower klotho level in uninfected neonates can be due to klotho expression not reaching its peak until adulthood. A study in mice found klotho levels increased after birth and into adulthood and decreased with aging (Vo 2018)
- One limitation to the study was that most of slides analyzed were that of female macaques. As macaques are considered an acutely scarce resource, we could not have a balance between male and female subjects
- Relationship between klotho and HIV could help to explain the development of HIV-associated neurocognitive disorders (HAND)
- However, the exact mechanism in which SIV diminishes klotho expression is unknown and additional studies are warranted to examine this phenomenon

REFERENCES

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