

# Development of CAR-Macrophages (CAR-M) as a Potential Therapeutic to Facilitate Amyloid Clearance

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# Disclosure Information

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*Manasi Balachandran, University of Tennessee Graduate School of  
Medicine*

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*I have the following financial relationships to disclose:*

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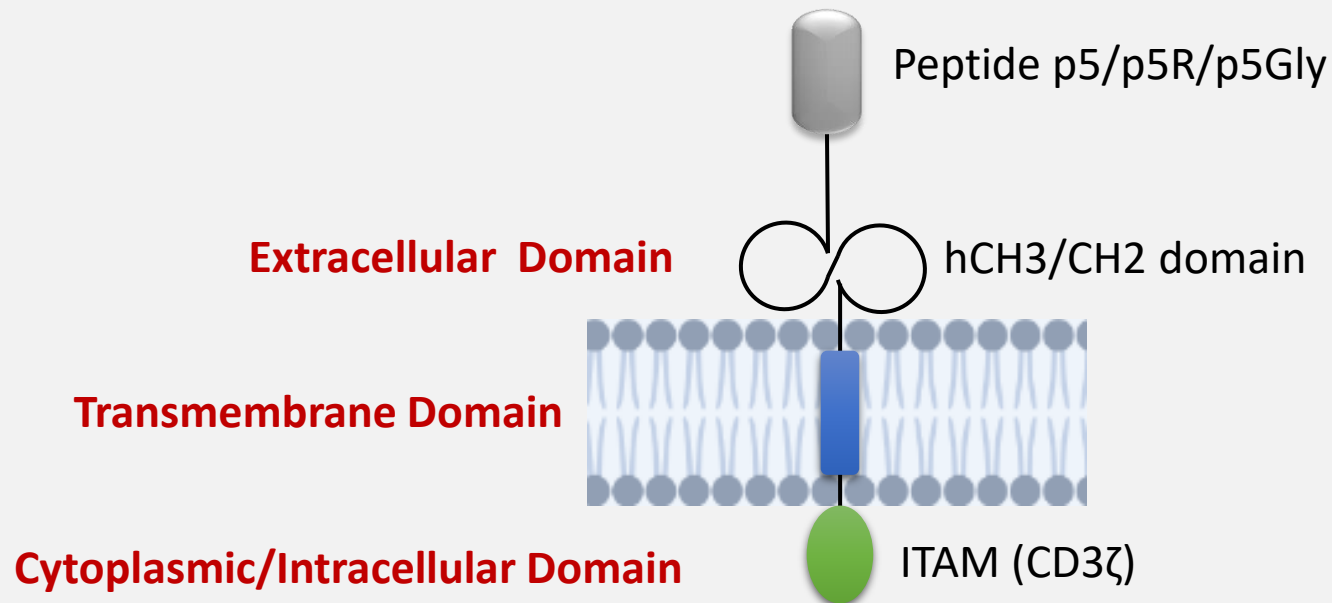
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I will not discuss off-label use in my presentation

# The Exemplary CAR Structure for Amyloid Binding

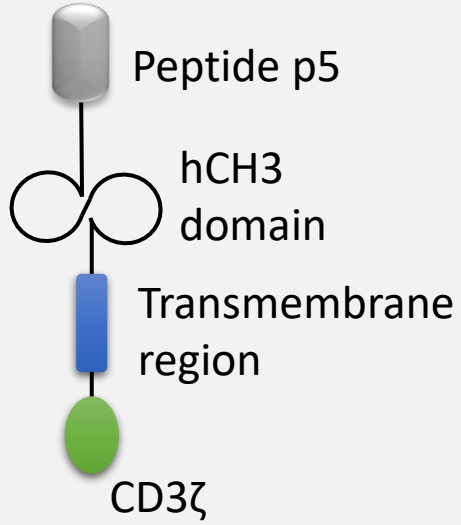
- CAR technology originally developed for cancer immunotherapy (CAR-T and CAR-M)
- The amyloid-reactive CAR incorporates the pan amyloid-reactive peptide p5 (lysine version) or p5R (arginine version). The uncharged p5Gly (glycine version) serves as a negative control

## Amyloid-binding CAR

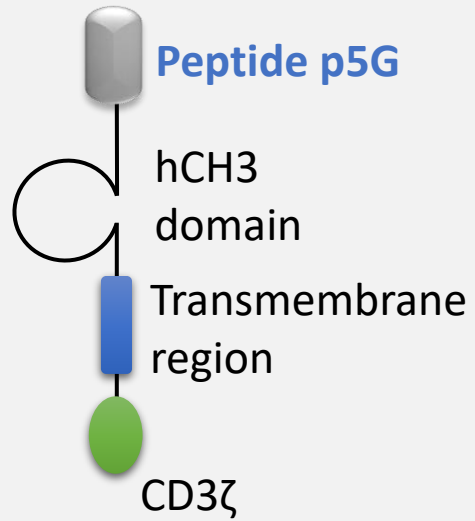


- The IgG1 heavy chain domains, hCH3 and hCH2, provide rigidity to the structure and may facilitate complement activation
- The intracellular signal activation domain CD3 $\zeta$  has three ITAMs and is a phagocytosis signal enhancer

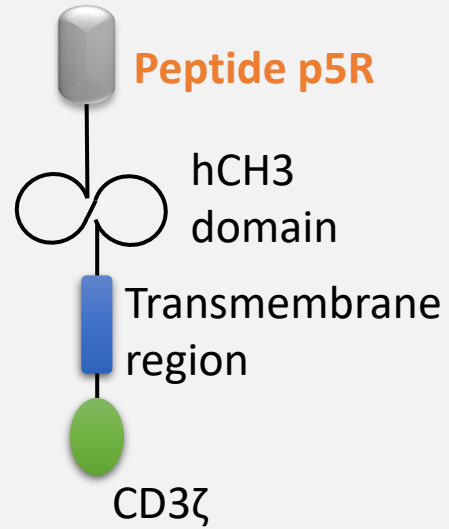
CARM-2



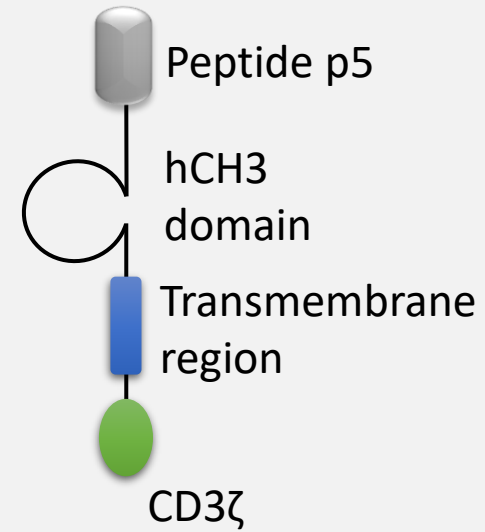
CARM-Gly



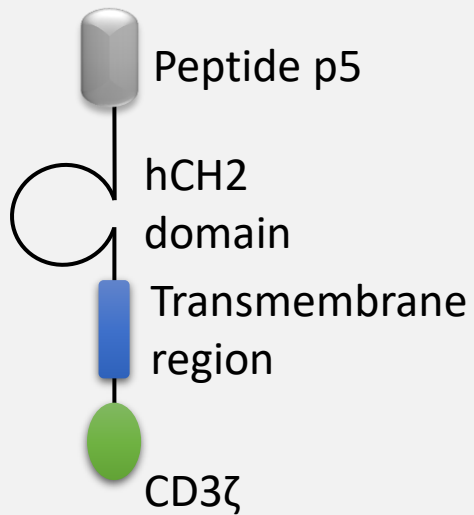
CARM-3



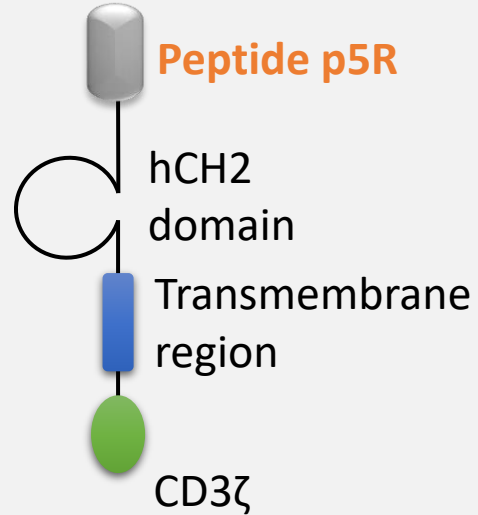
CARM-4



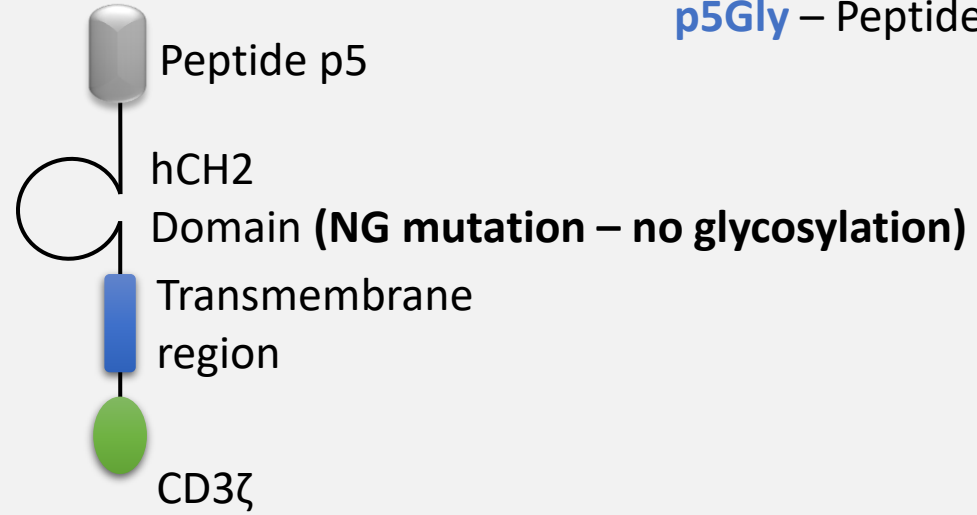
CARM-5



CARM-6

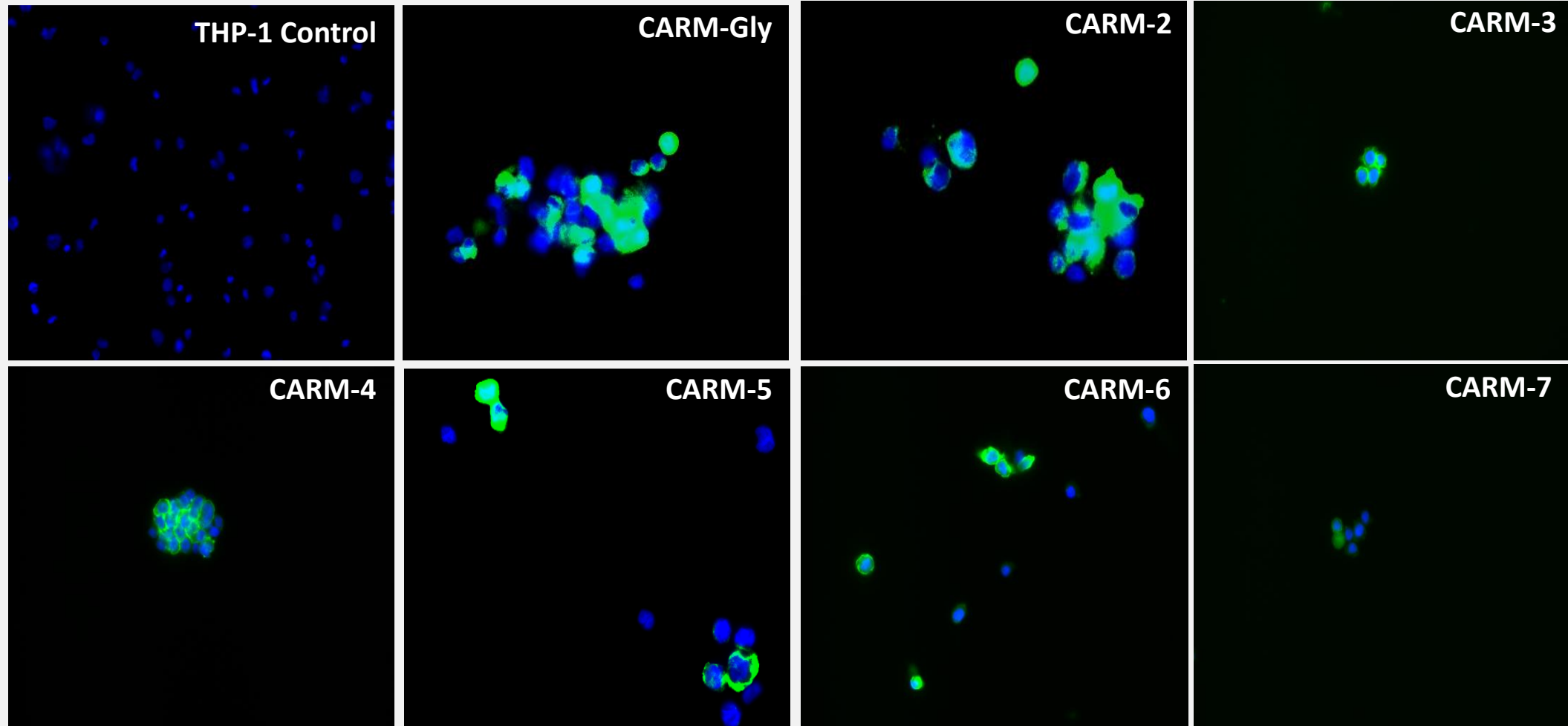


CARM-7



p5 – Peptide p5 Lysine version  
 p5R – Peptide p5 Arginine version  
 p5Gly – Peptide p5 Glycine version

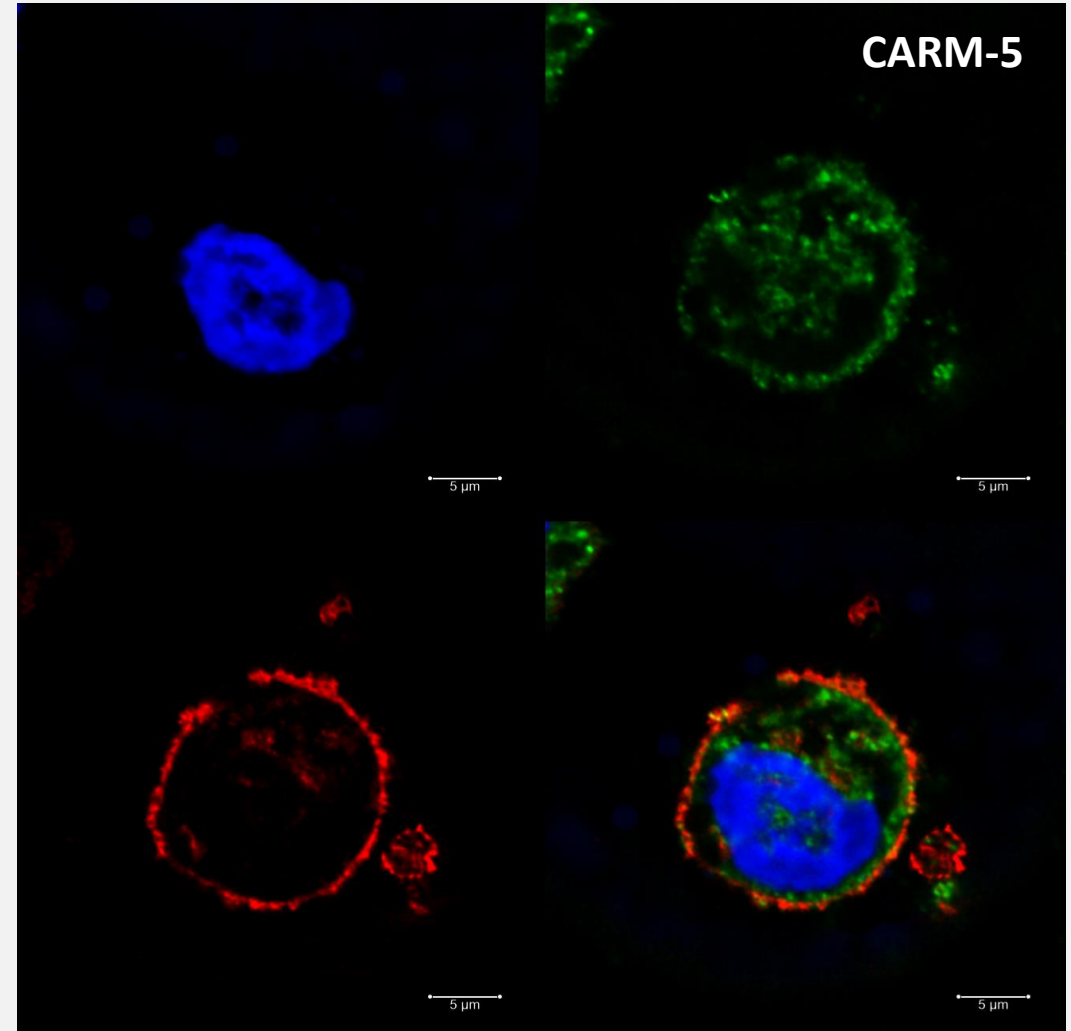
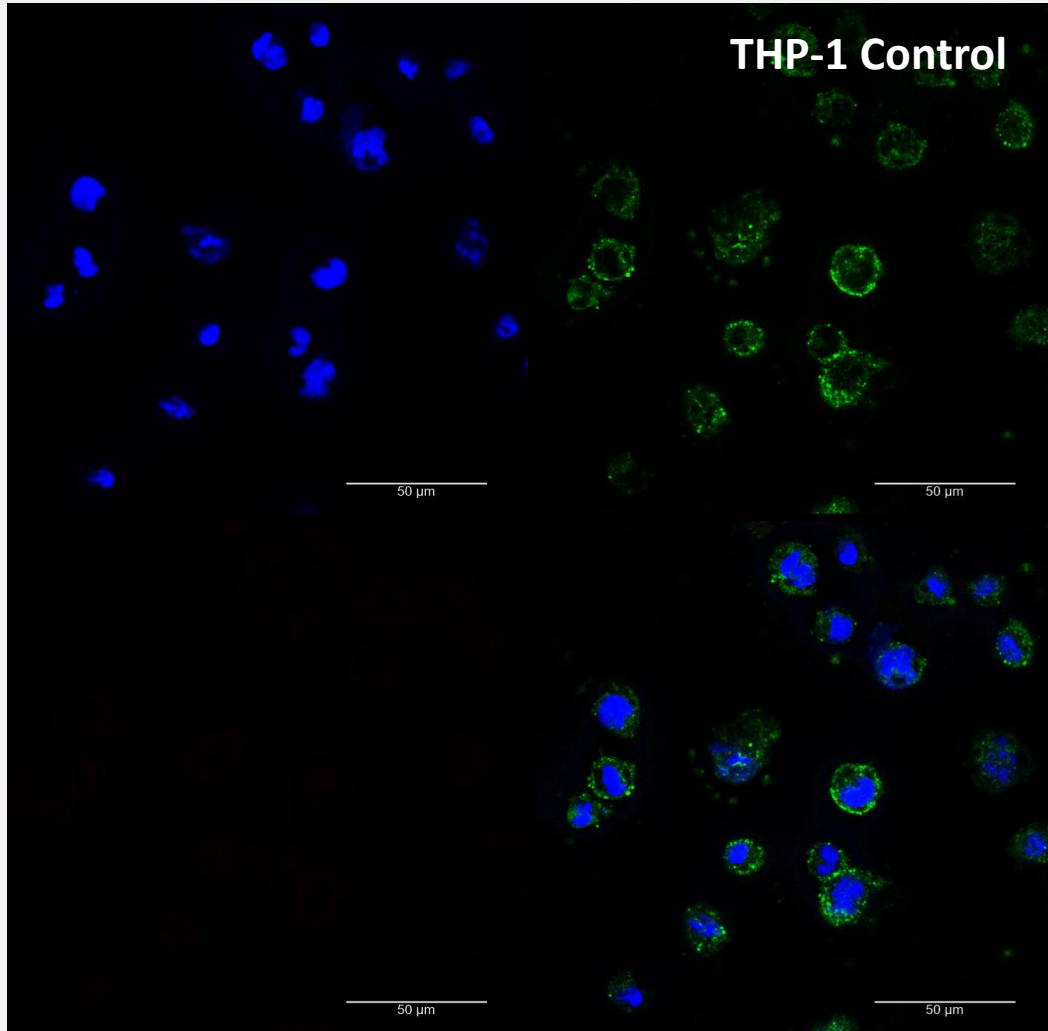
# Staining of CAR-M Pools with Anti-Human IgG AlexaFluor488



Blue – Hoechst Stain (nuclei)

Green – Anti-Human IgG (CAR)

# Confocal Microscopy to Map CAR



50  $\mu$ m

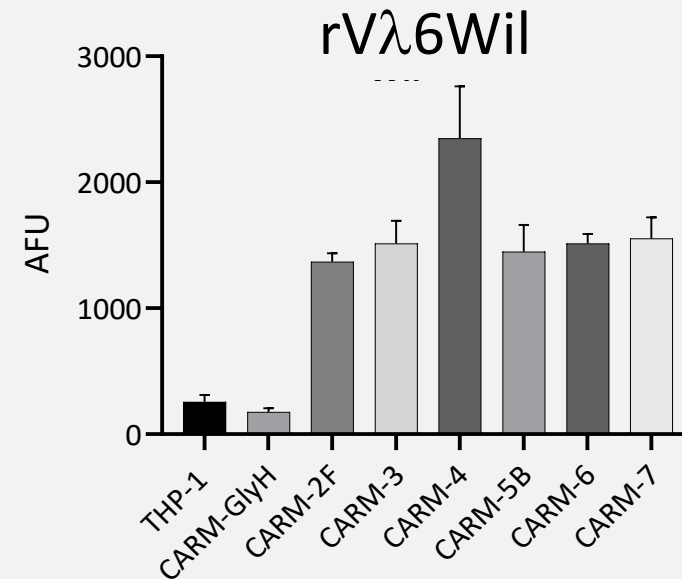
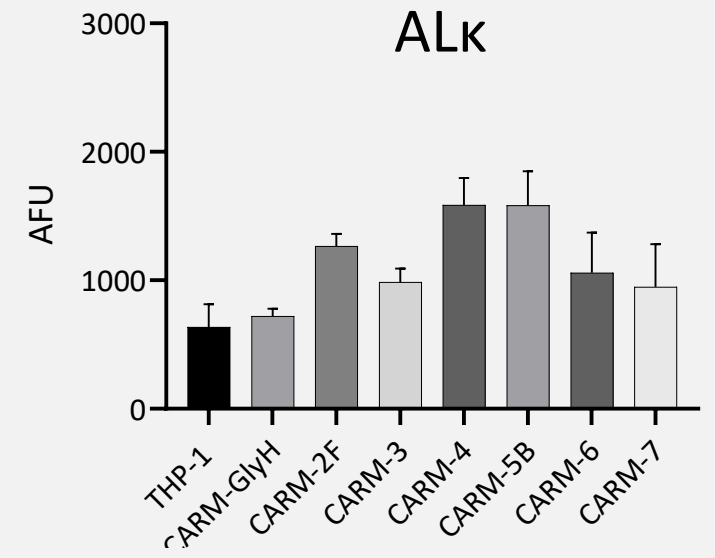
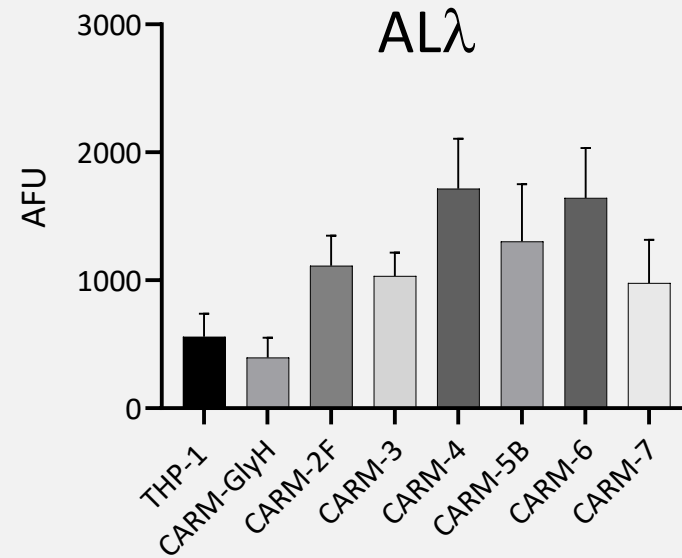
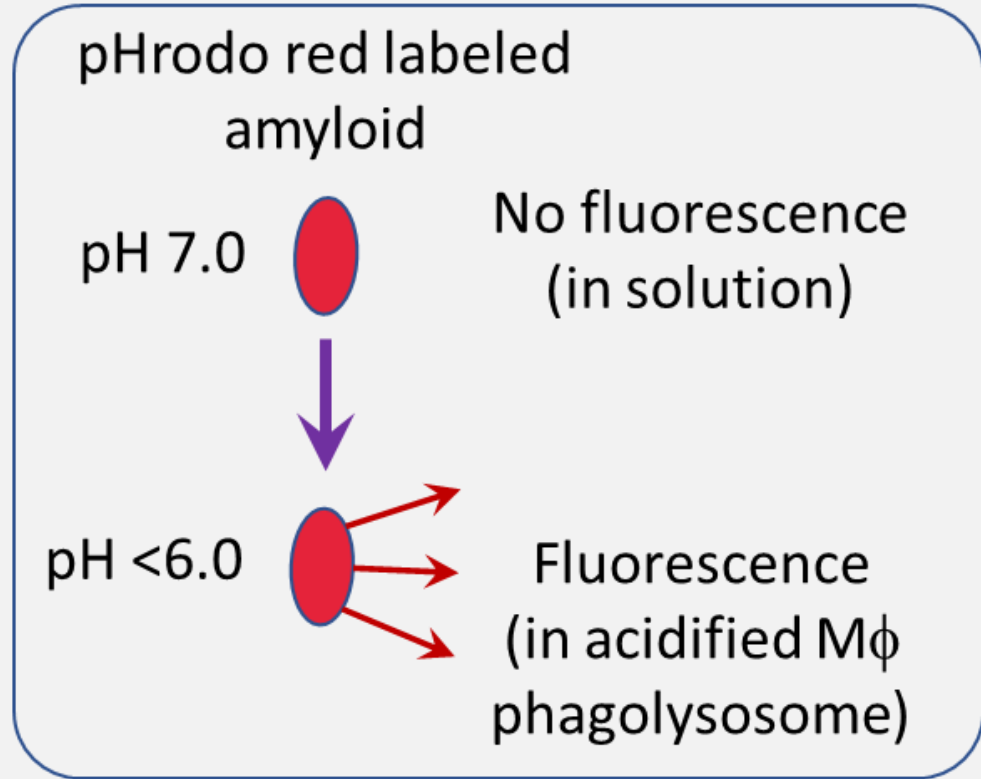
5  $\mu$ m

Blue – Hoechst Stain (nuclei)

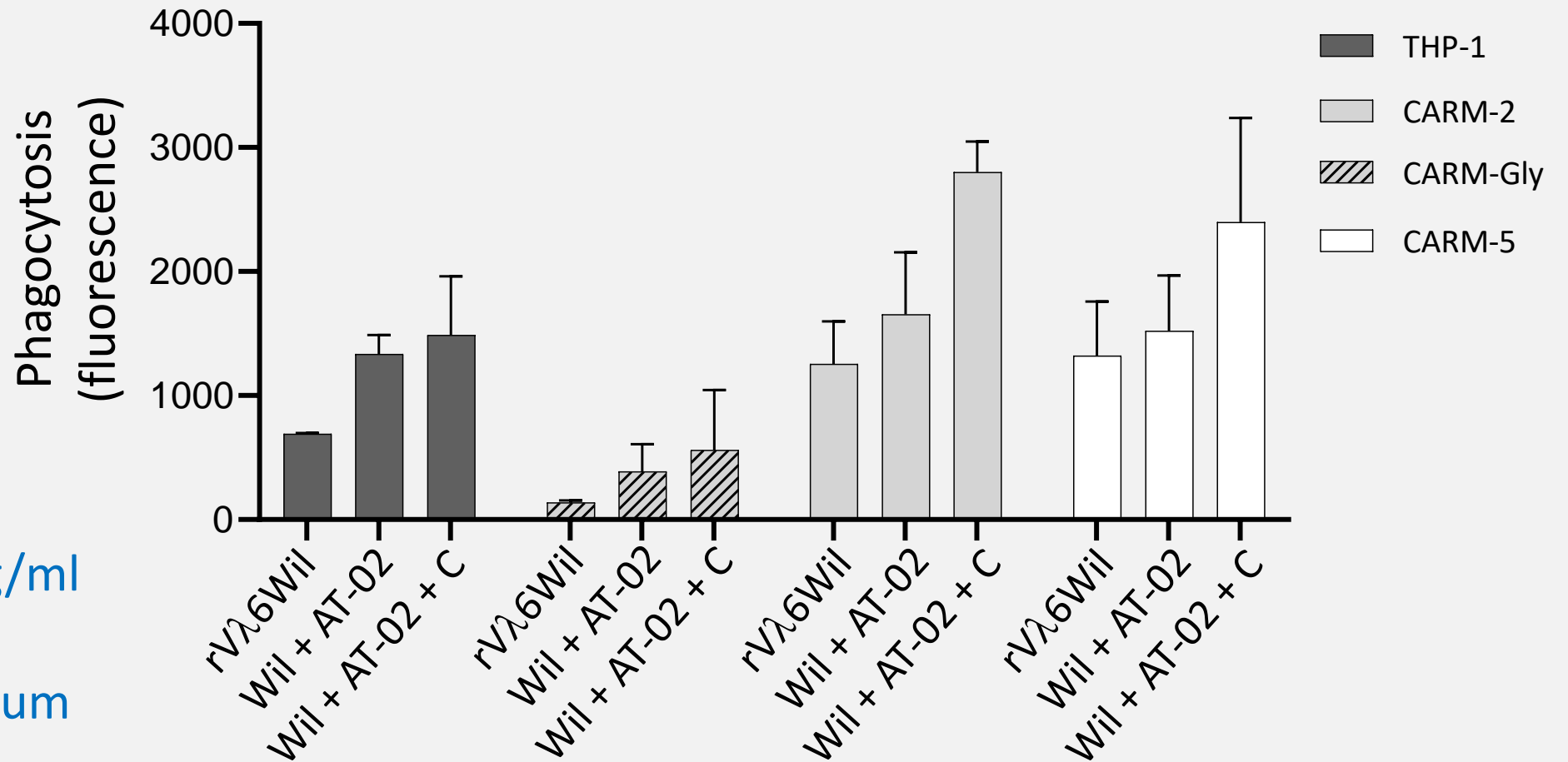
Green – Phalloidin

Red – Anti-Human IgG (CAR)

# Phagocytosis of pHrodo red labeled rV $\lambda$ 6W $\mu$ l fibrils and AL $\lambda$ and AL $\kappa$ amyloid extracts



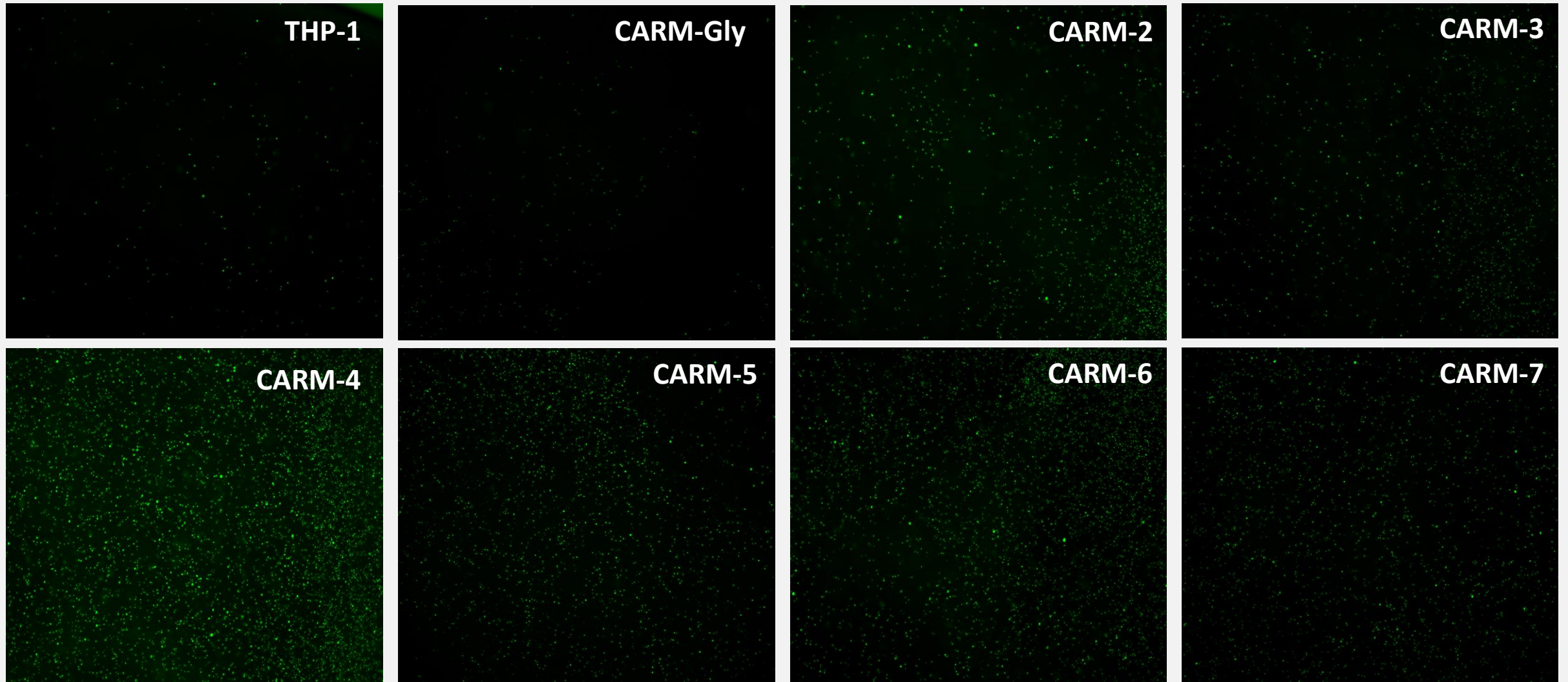
# Effect of Opsonization with (AT-02) and Complement (C) on Phagocytosis of pHrodo Wil



- pHrodo Wil – 20  $\mu\text{g/ml}$
- AT-02 – 60nM
- 20% v/v human serum (complement)

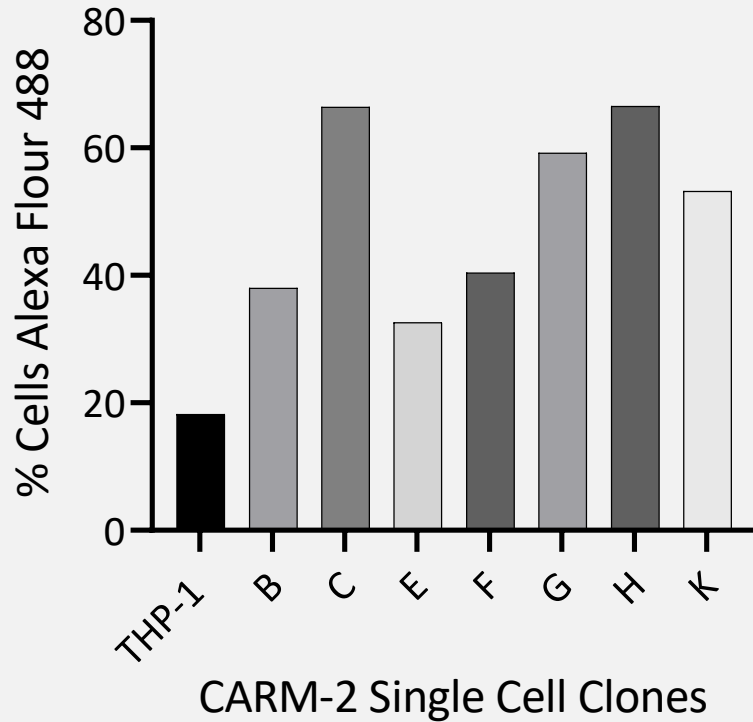


# Heparin Binding by CAR Macrophages

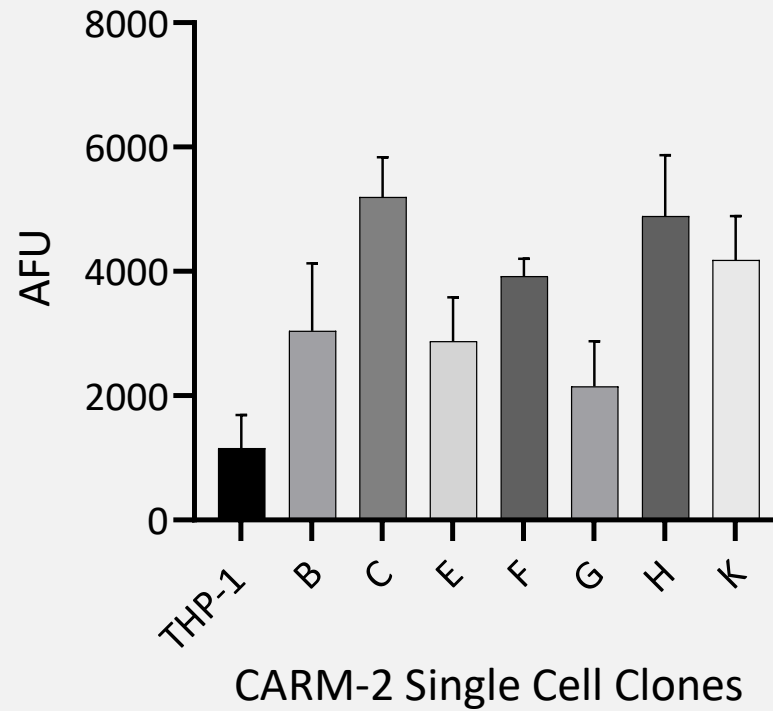


- Peptide p5 binds heparin
- CMFDA-labeled THP-1 and CAR-M added to heparin-coated plates

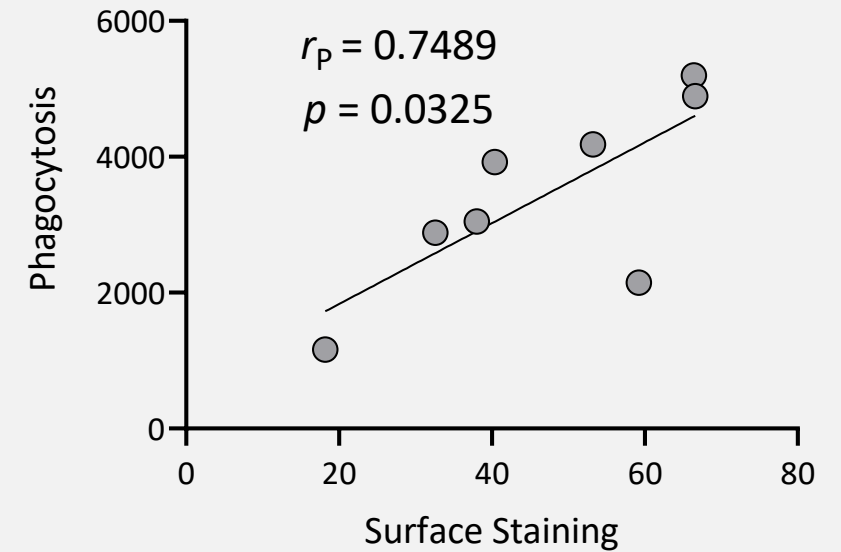
# CARM-2 Single Cell Clones – Comparison of Surface Expression of CAR with Phagocytosis



Surface staining to demonstrate CAR expression



Phagocytosis of pHrodo-rVλ6Wil to demonstrate CAR function

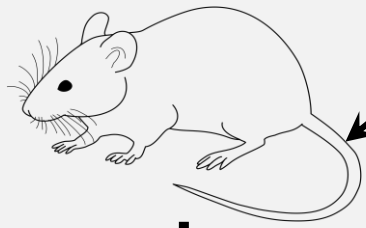


Correlation analysis indicates that higher the amount of CAR expression on the cell surface, better is the phagocytic ability

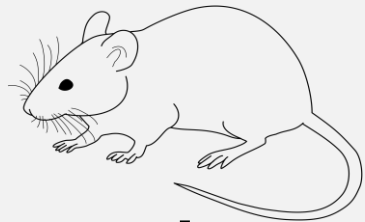
# Monocytes Are Actively Recruited to AA Amyloid-Laden Kidney

hIL-6 transgenic  
Develops AA amyloidosis in  
liver, spleen, kidney

CMFDA labeled  
THP-1 or CAR-M  
injected IV



48 h

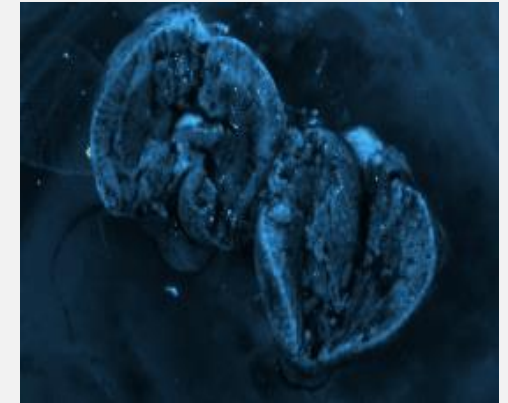
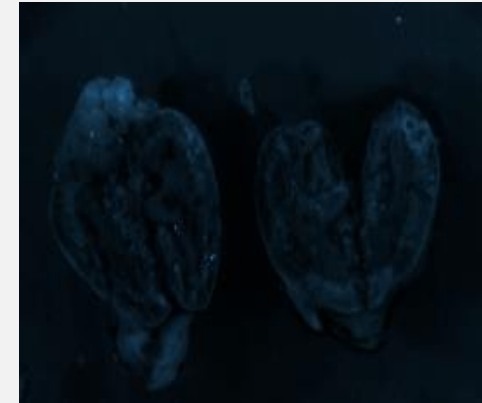


Euthanize mice. Harvest liver,  
spleen, and kidney and measure  
fluorescence by optical imaging

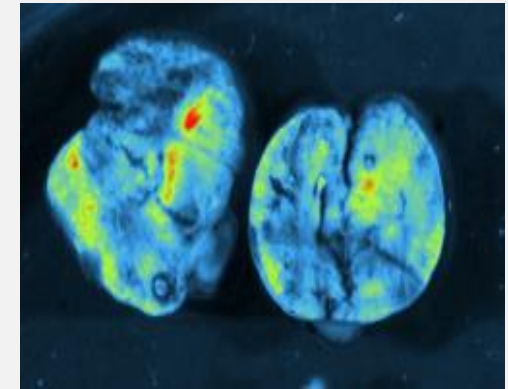
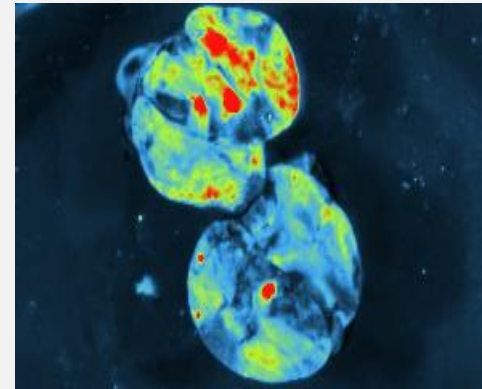
CARM-2 Pool

THP-1

Wild type  
mice  
(kidney)



AA amyloid  
mice  
(kidney)



No recruitment of the cells was seen in the  
liver or spleen in AA or WT mice

# Summary

- CAR with pan-amyloid-reactive peptides p5 and p5R as the amyloid-reactive moiety successfully expressed in human THP-1 cells.
- Correct orientation of the CAR in the plasma membrane has been confirmed by immunofluorescence imaging.
- Expression of CAR in human THP-1 cells enhances heparin binding indicating the p5 and p5R peptides are bioactive.
- Expression of CAR in human THP-1 cells enhances phagocytosis of human amyloid extracts which is enhanced in the presence of human complement (20% serum) and of an amyloid binding opsonin.
- In the AA mouse model, amyloid-laden kidneys sequester macrophages providing a model system to evaluate efficacy and support a rationale for use in patients.
- Amyloid-reactive CAR may provide a complimentary approach to the goal of tissue amyloid removal.

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