



THE UNIVERSITY OF
TENNESSEE
HEALTH SCIENCE CENTER.

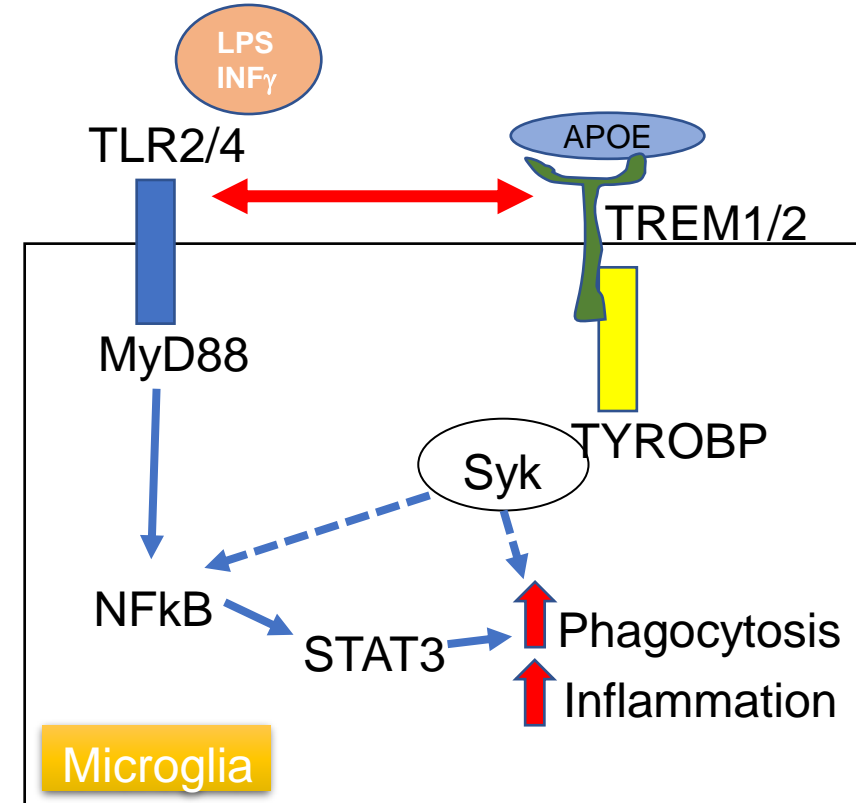
The Effects of Exosomal Derived TSG-6 on Microglia Activation

Presenter: Jonathan Martinez

Mentor: Rajashekhar Gangaraju, Ph.D.

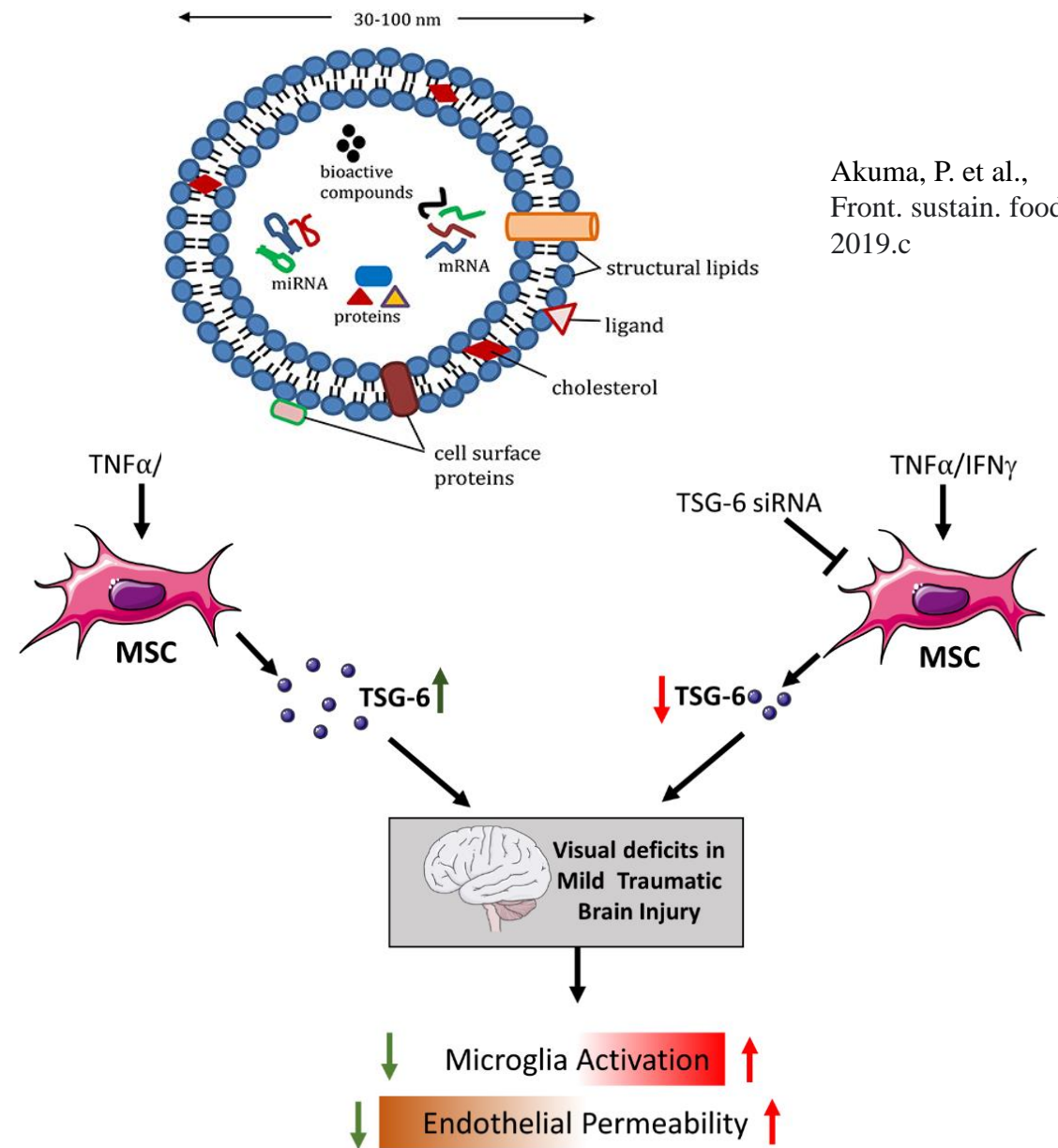
Background

- Microglia are macrophages located in CNS and retina
- Regulate neuronal function and clearance of cell debris for neuronal regeneration and growth
- “Disease associated microglia” (DAM) are overactive microglia in neurodegenerative states and brain injuries that release pro-inflammatory cytokines and phagocytose viable neurons
- Distinguished by increased expression DAM genes and phagocytic activity



Hypothetical model of TLR and TyroBP signaling in microglia to regulate inflammation, phagocytosis.

- Traumatic brain injury (TBI) causes visual deficits and is observed to increase levels of overactive DAM
- Stem cell therapies are being developed as therapeutics for TBI visual deficits
- Human mesenchymal stem cells (MSC) can be stimulated with inflammatory cytokines to secrete secretomes (conditioned medium)
- MSC conditioned media shown to decrease phagocytosis of mouse microglia in-vitro, decrease visual deficits in TBI model mice
- Prior studies suggest within the secretome, exosomes (transport vesicle) containing anti-inflammatory protein TSG-6 are therapeutic



Akuma, P. et al.,
Front. sustain. food syst.,
2019.c

Jha & Gangaraju et al., SCRT, 2019.

Hypothesis

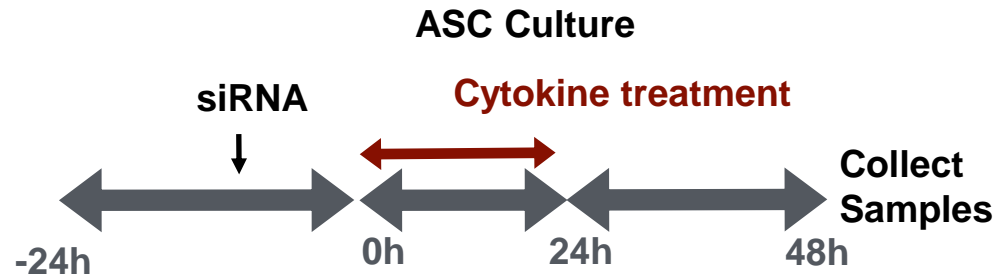
- Exosomal derived TSG-6 will decrease activation of stimulated microglia

Methods

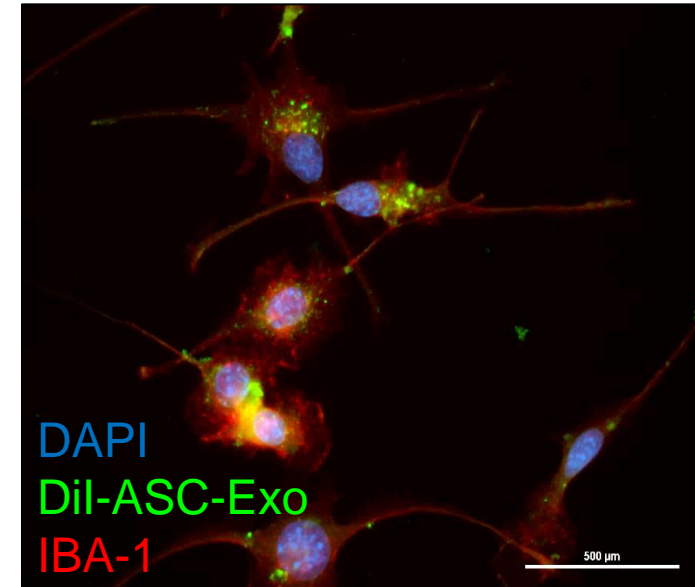
- Cell line: HMC3 human fetal microglia commercial line
- Exosomes: containing TSG-6, collected from adipose stem cell media
- Conditions:
 - APOE transfected HMC3
 - LPS IFN- γ stimulated HMC3
- Experiment:
 - Phagocytosis Assay
 - PCR Gene Expression

Exosomal collection

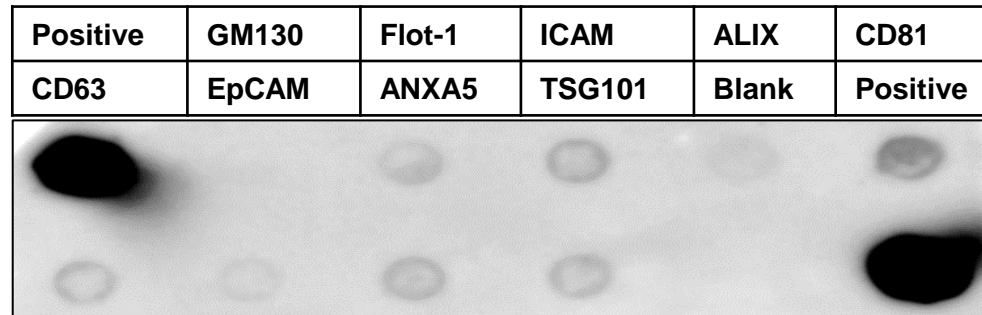
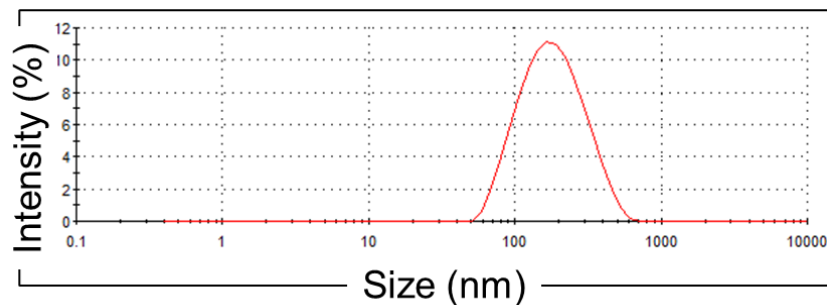
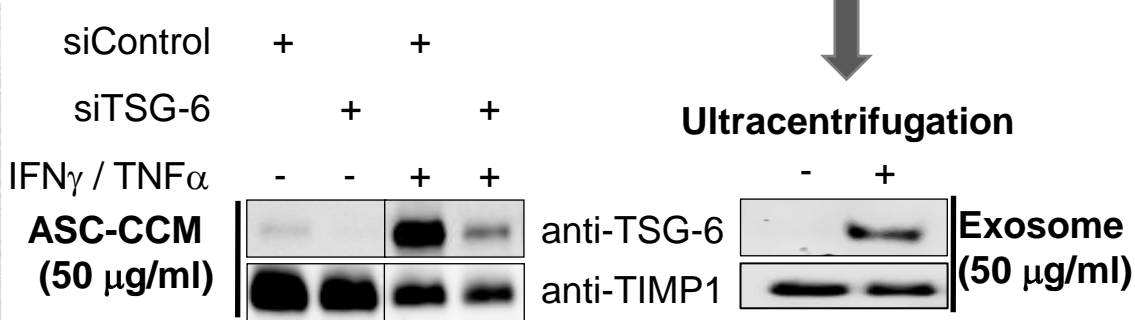
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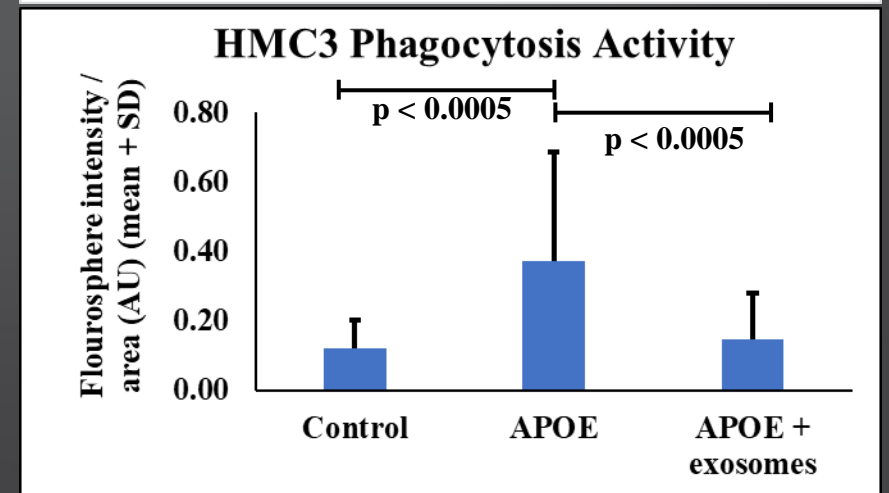
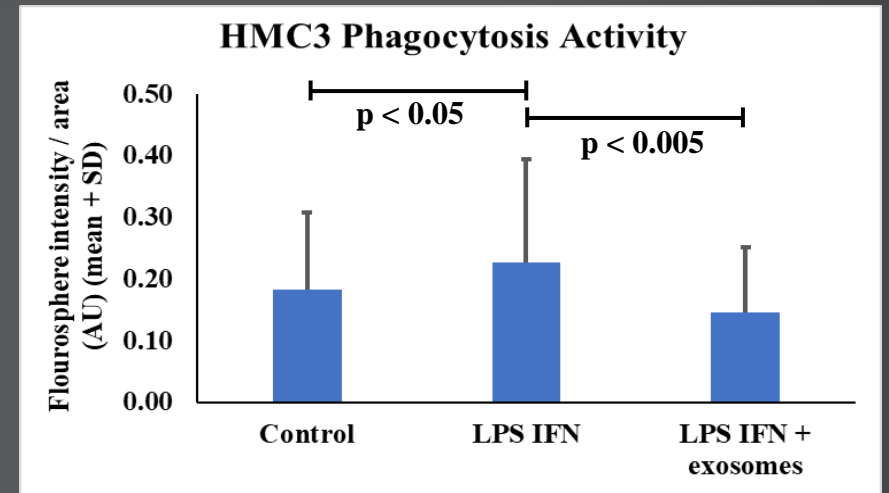
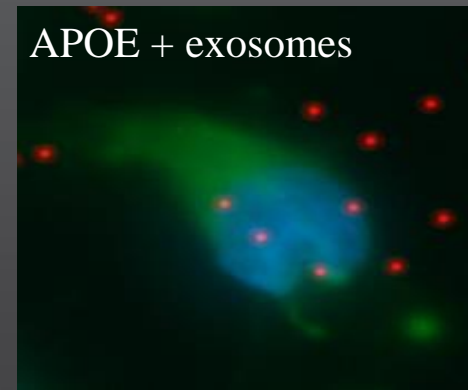
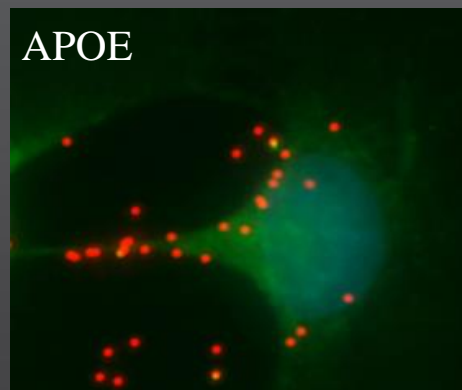
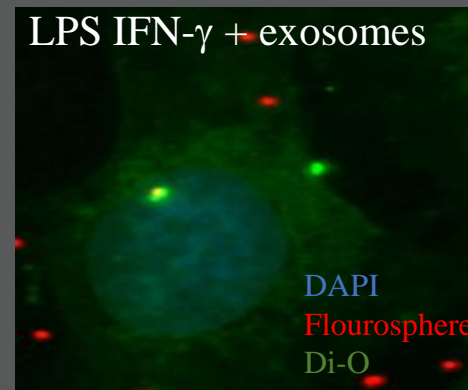
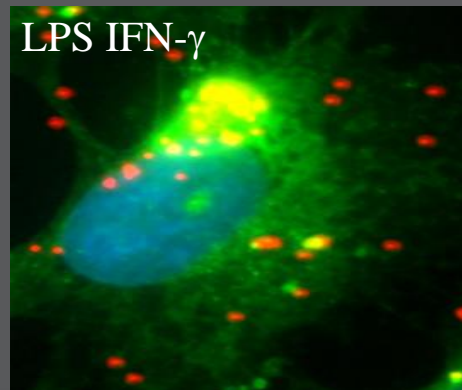
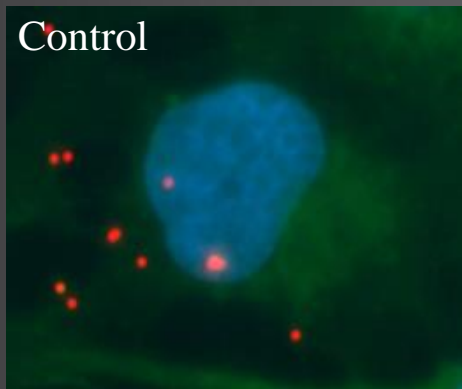
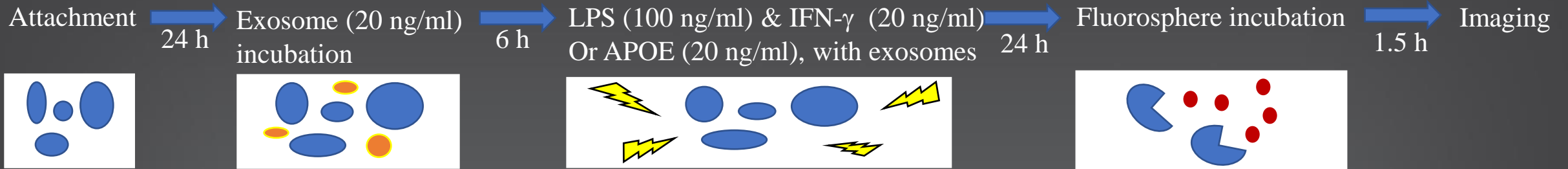
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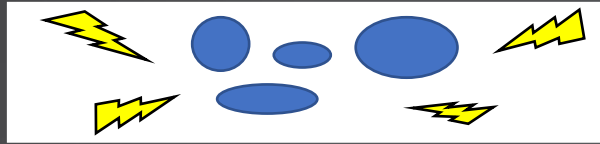


Phagocytosis Assay



PCR Gene Expression

LPS (100 ng/ml) & IFN- γ (20 ng/ml)
Or APOE (20 ng/ml), with exosomes



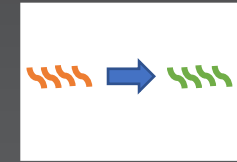
Cell lysate collection
24 h



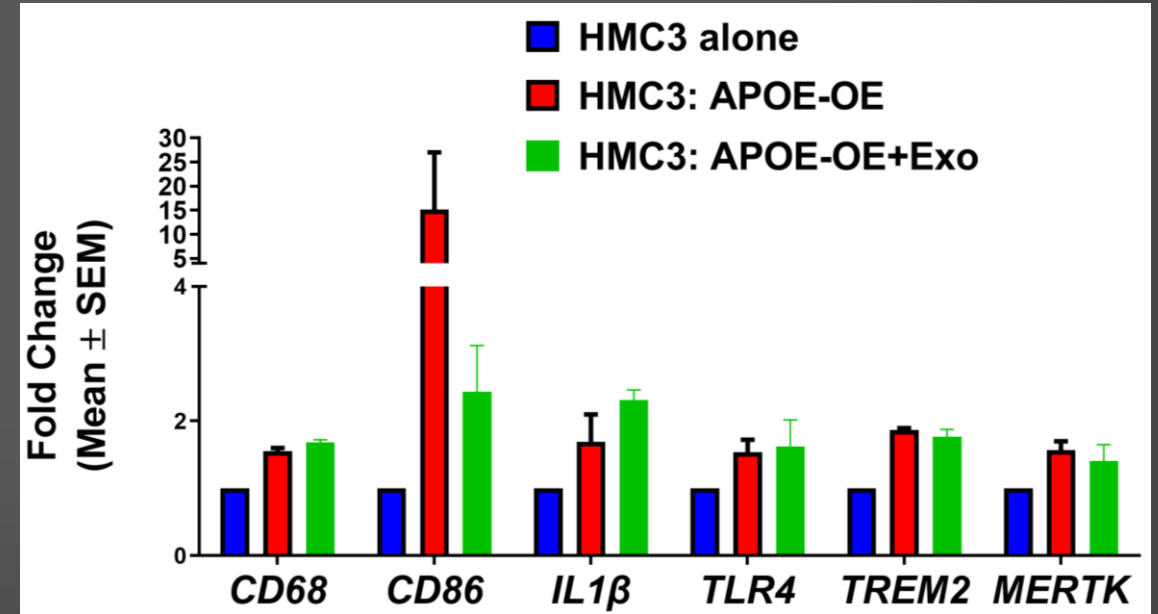
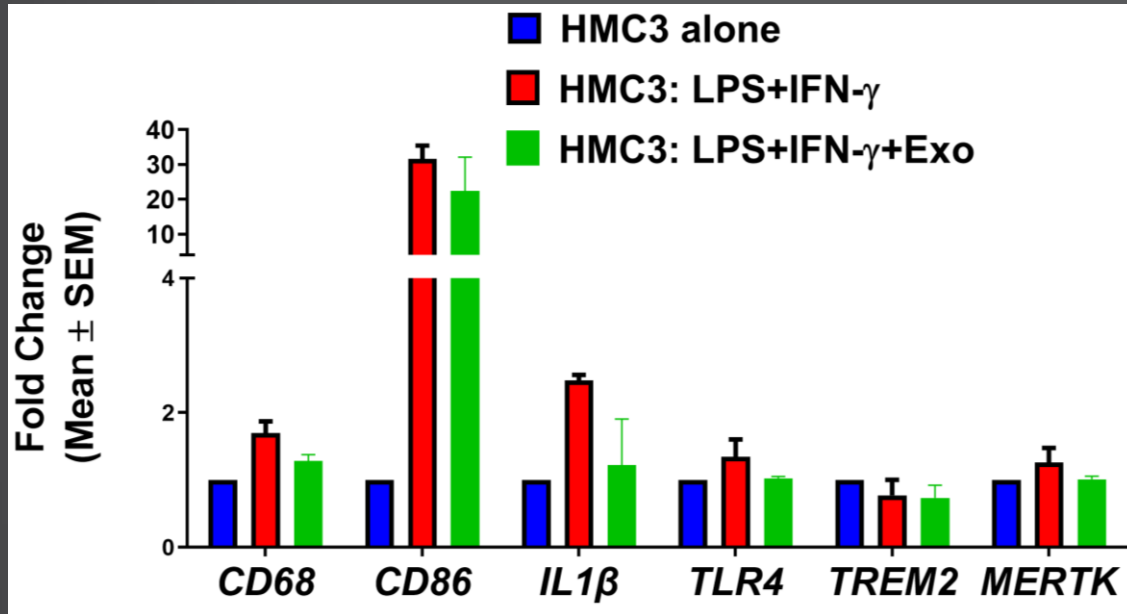
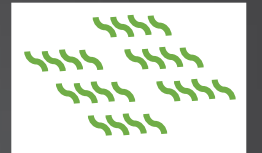
RNA
isolation



cDNA
conversion



PCR



Conclusions:

- LPS IFN- γ and APOE stimulation significantly increased phagocytic activity, exosomal derived TSG-6 significantly decreased phagocytic activity
- LPS IFN- γ and APOE stimulation increased DAM gene expression, exosomal derived TSG-6 showed decreased DAM gene expression

Future Directions:

- Adjust stimulation period and exosome concentration for gene expression
- Utilize engineered cells containing isolated TSG-6 exosomes

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Diadem Biotherapeutics Inc.

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