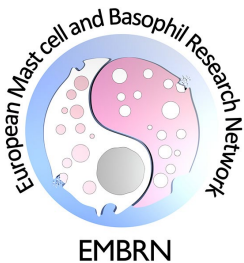


# Siglec-6 Interacts with KIT/CD117, Recruits Shp Phosphatases and Inhibits SCF-Mediated Inflammation

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Naomi Freitas, Ken Luehrsen and Bradford A. Youngblood

Allakos Inc., San Carlos CA

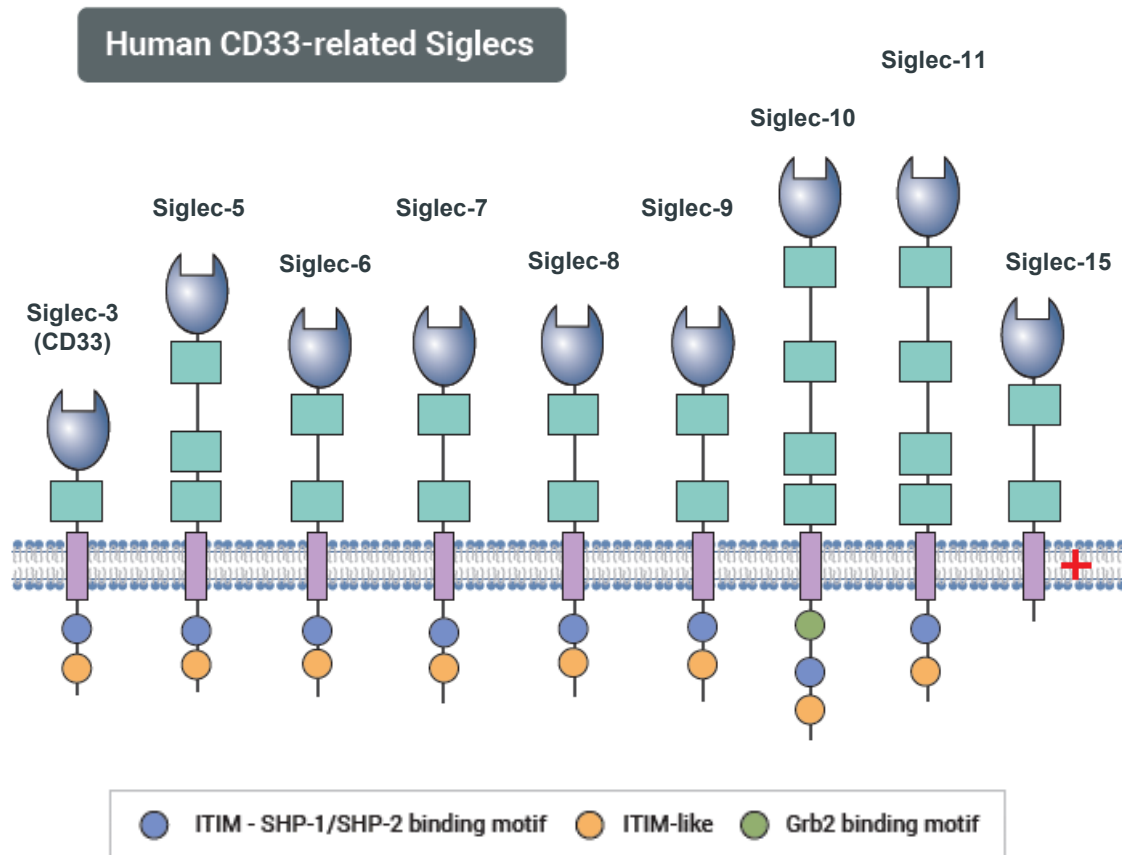
10<sup>th</sup> EMBRN International Mast Cell and Basophil Meeting  
July 11<sup>th</sup> – 13<sup>th</sup>, 2022



# Disclosures

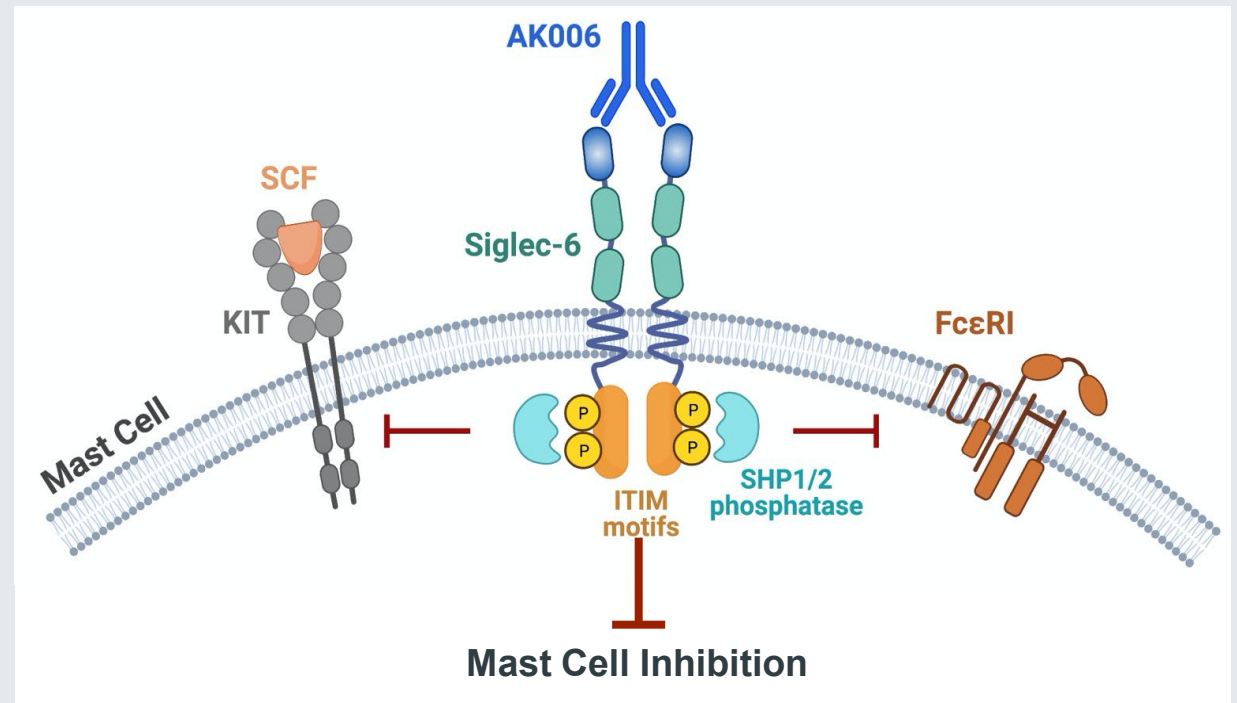
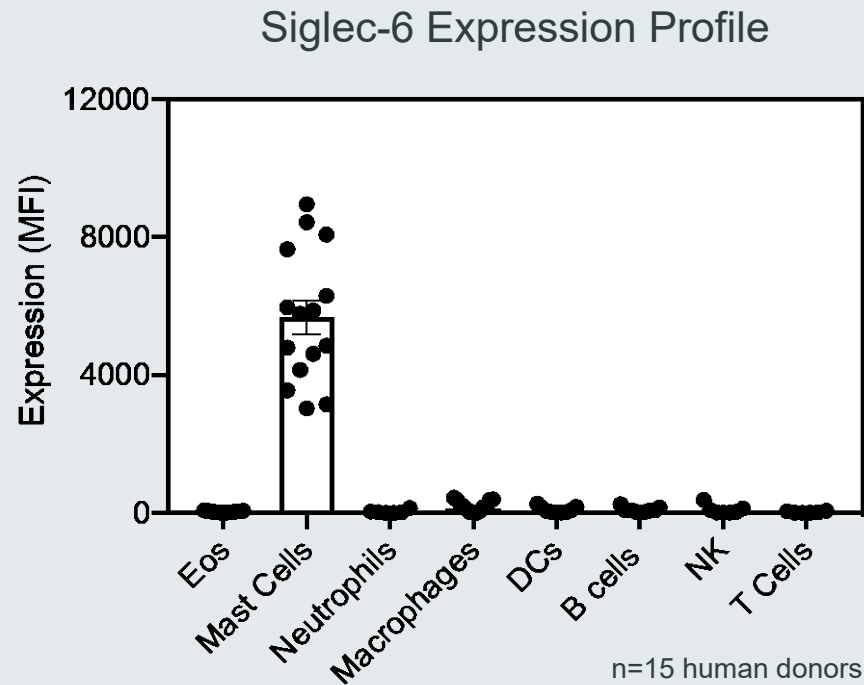
- Employee of Allakos Inc.

# Siglecs Represent Attractive Therapeutic Targets on Immune Cells



- Siglecs are inhibitory receptors selectively expressed on key immune cells
- Ability to selectively suppress immune cell activation via agonistic antibodies to reduce chronic inflammation (i.e., anti-Siglec-8 - AK002/lirentelimab)
- Opportunity to selectively activate immune cells through neutralizing antibodies to increase anti-tumor immunity
- Targeting Siglecs provides an opportunity to directly modulate key pathogenic cells in different disease states

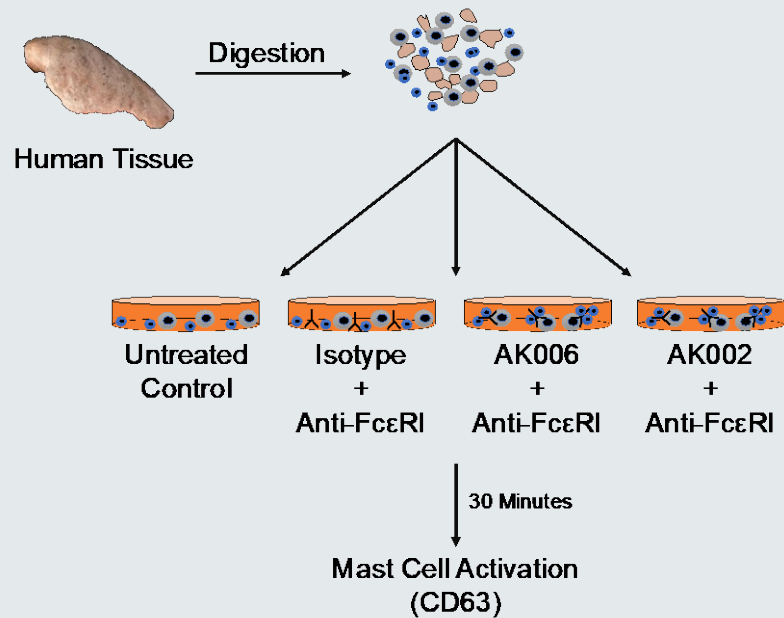
# Siglec-6 is Selectively Expressed on Human Tissue Mast Cells



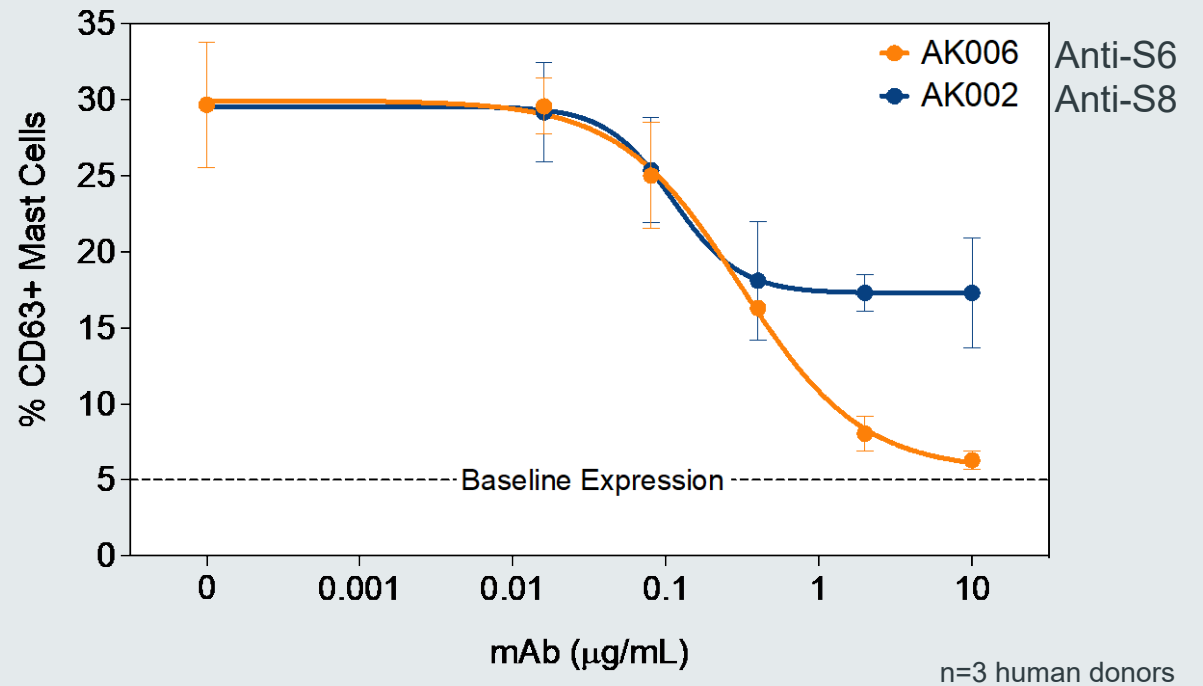
- These findings are consistent with previously published studies using single cell sequencing<sup>1</sup> and proteomic<sup>2</sup> approaches
- Agonistic anti-Siglec-6 antibody AK006 broadly inhibits mast cells

# AK006 Inhibits Mast Cell Activation in Human Tissues

## Human Tissue Mast Cell Activation Assay

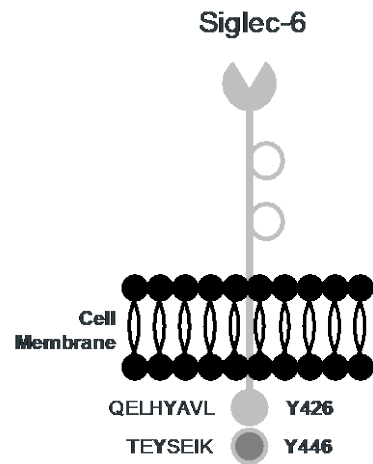


## IgE-Activated Human Tissue Mast Cells

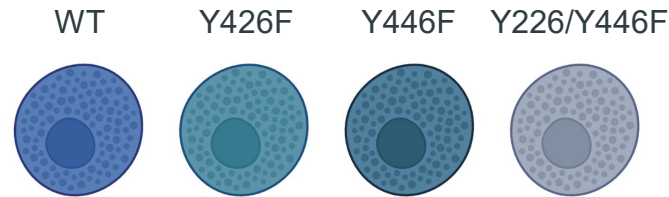


AK006 potently inhibits IgE-mediated mast cell activation

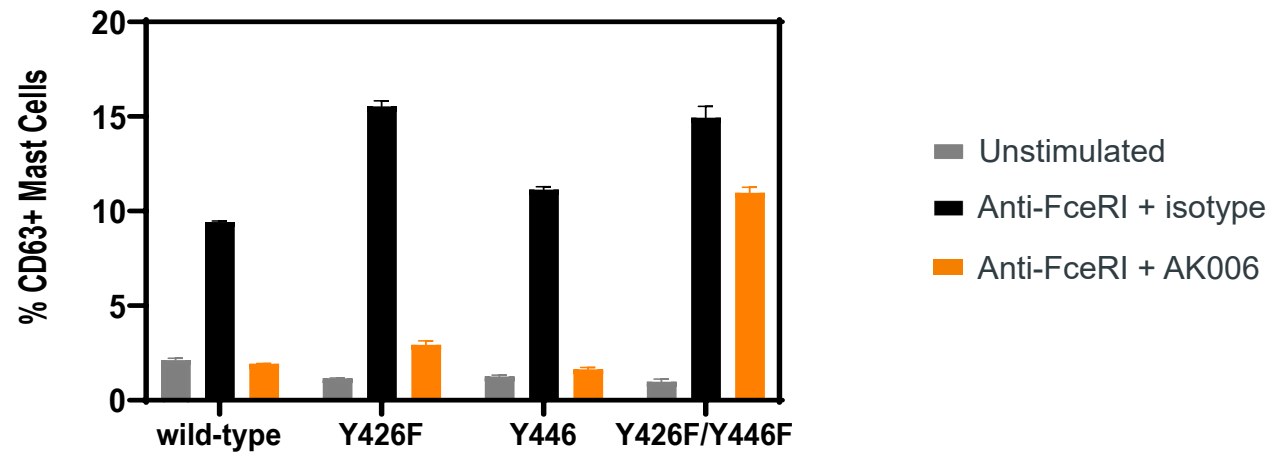
# Siglec-6-Mediated Inhibition Requires Both ITIMs



Transfect BMDCs with  
WT and ITIM mutant Siglec-6



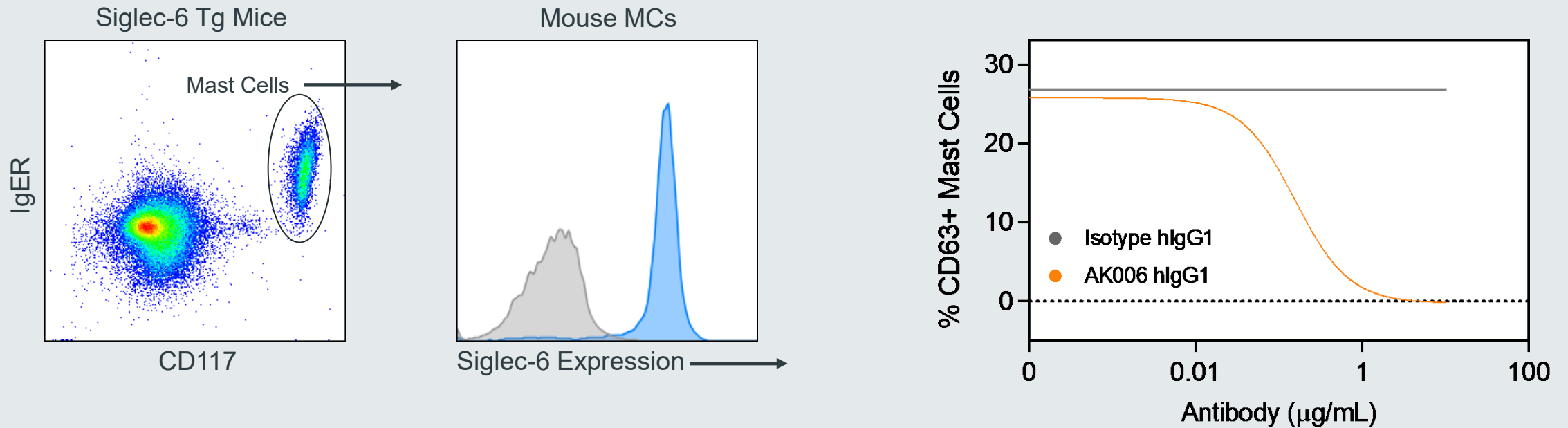
Siglec-6-mediated MC Inhibition



# Siglec-6 Transgenic Mice Express Functional Receptor on Mouse MCs

## Siglec-6 Tg Mice

## Siglec-6-mediated MC Inhibition on PL MCs

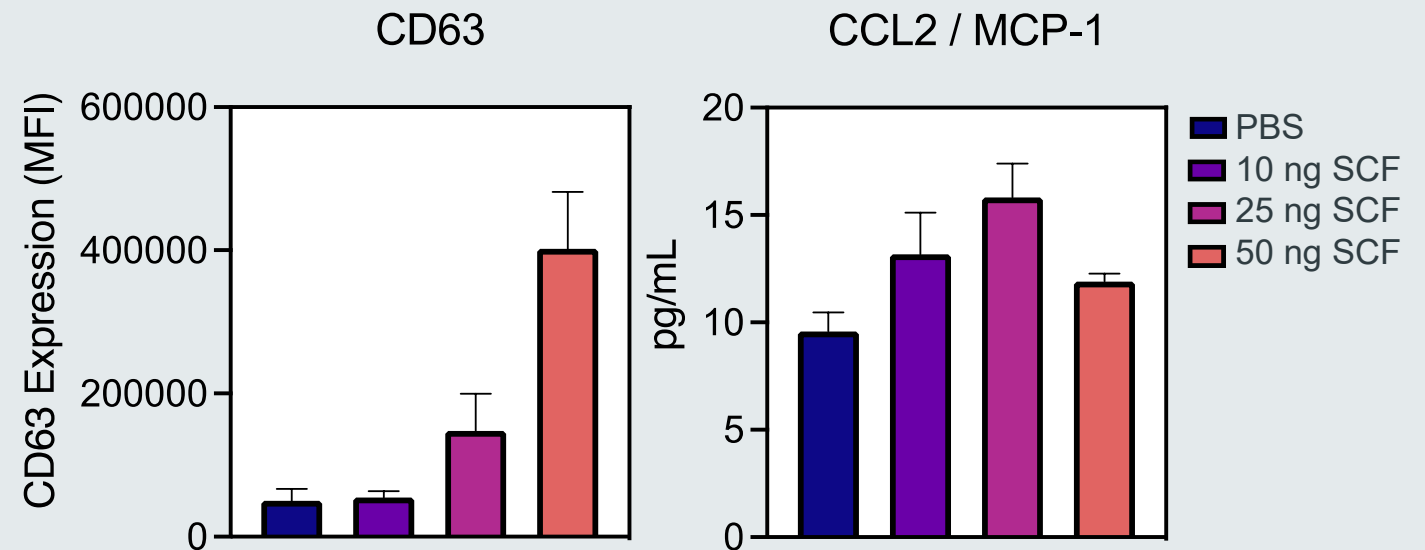
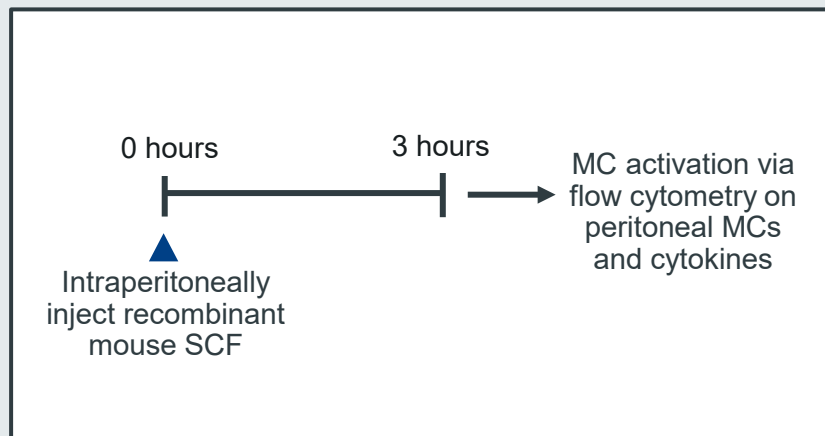


- Siglec-6 tg mice were created on C57BL/6 background
- Mouse mast cells in peritoneal cavity (and other tissues) express Siglec-6
- Engaging Siglec-6 on peritoneal MCs with AK006 induces inhibition of IgE-mediated activation

# SCF Induces Dose-dependent MC Activation *In Vivo*

## Mast Cell Activation

### Model of SCF-mediated MC Activation



SCF, stem cell factor; n=5 mice/group

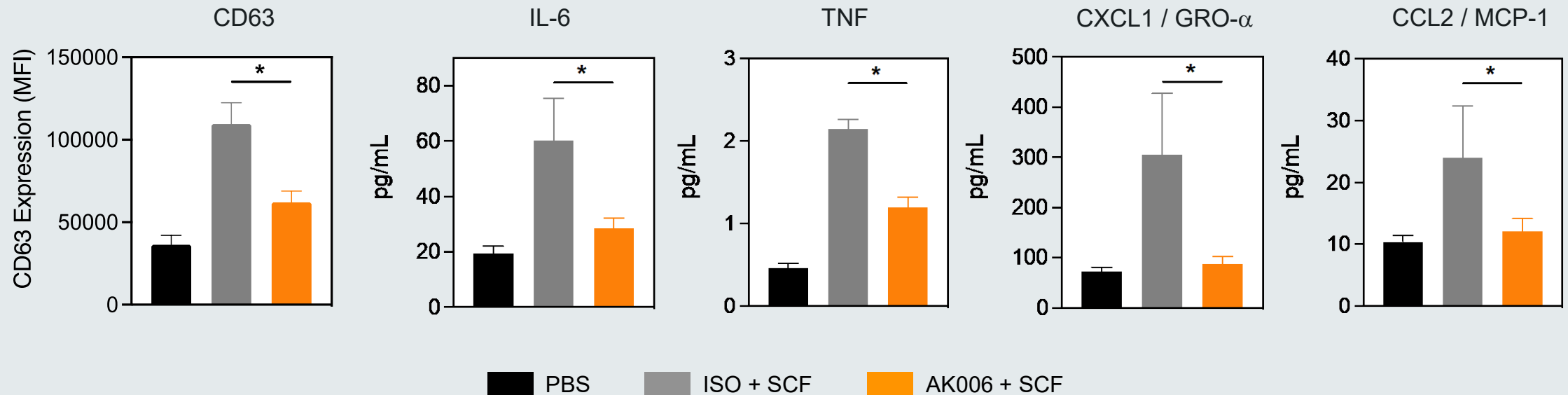
- Intraperitoneal administration of recombinant SCF induced dose-dependent MC activation on PL MCs as shown by elevated CD63 expression
- SCF administration also induced elevated levels of cytokines and chemokines, such as CCL2



# AK006 Inhibits KIT-mediated Mast Cell Activation in Siglec-6 Transgenic Mice

## Mast Cell Activation

## Cytokines and Chemokines

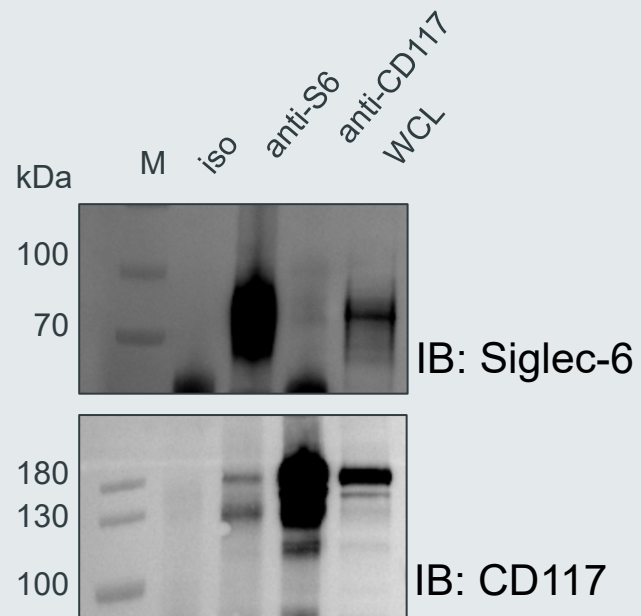


SCF, stem cell factor; \*  $p < 0.01$ ; n=7-8 mice/group

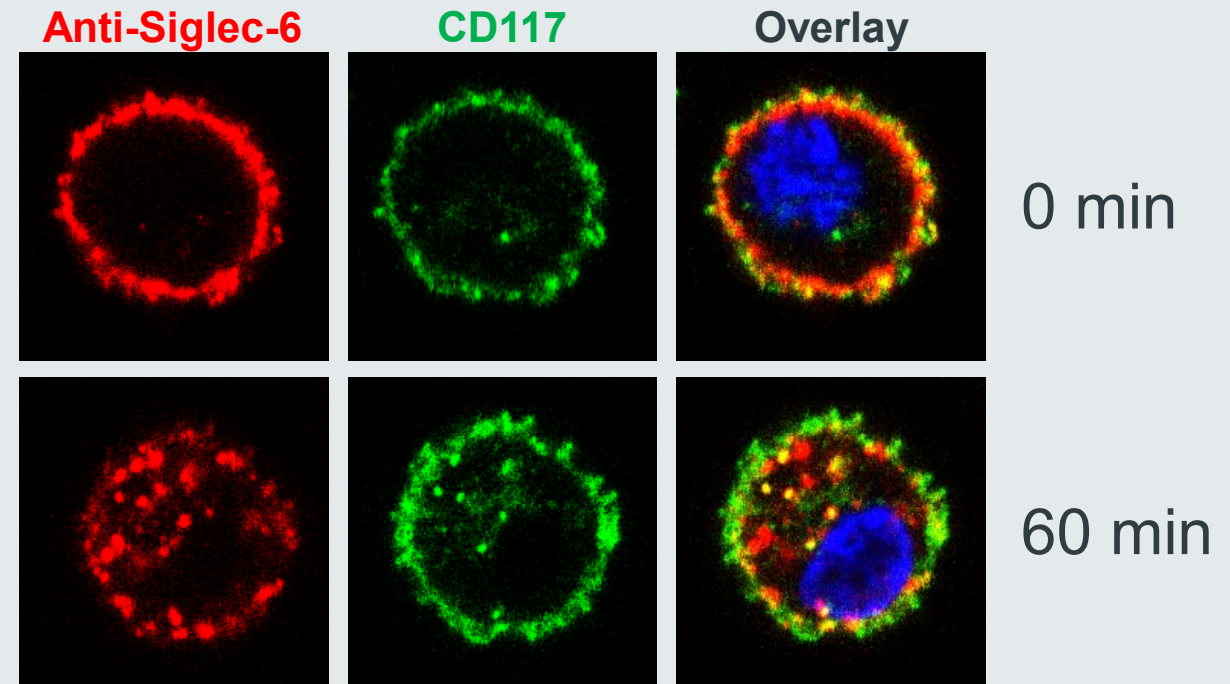
Treatment with AK006 reduced SCF-induced MC activation and mediator production

# Siglec-6 Interacts with CD117/KIT

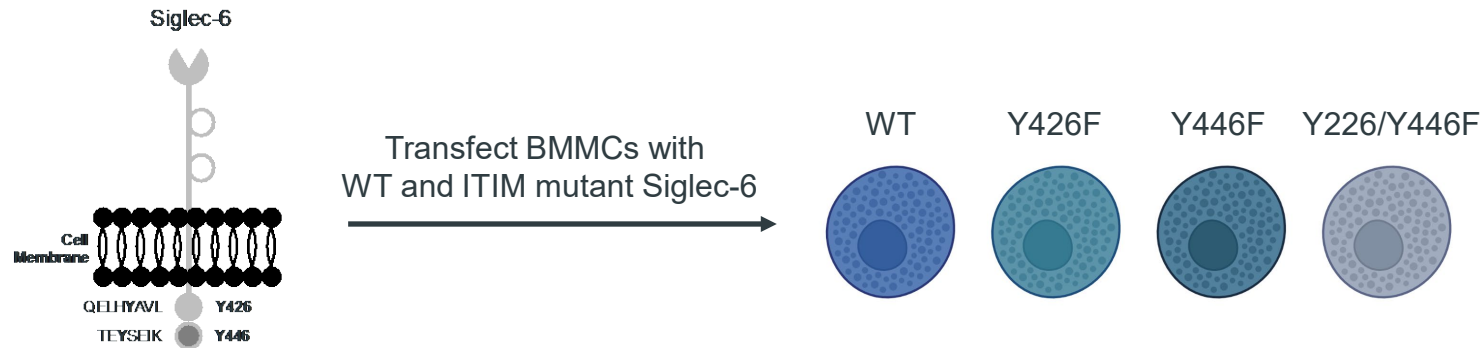
## IP-Western Blot



## Treatment with anti-Siglec-6 mAb

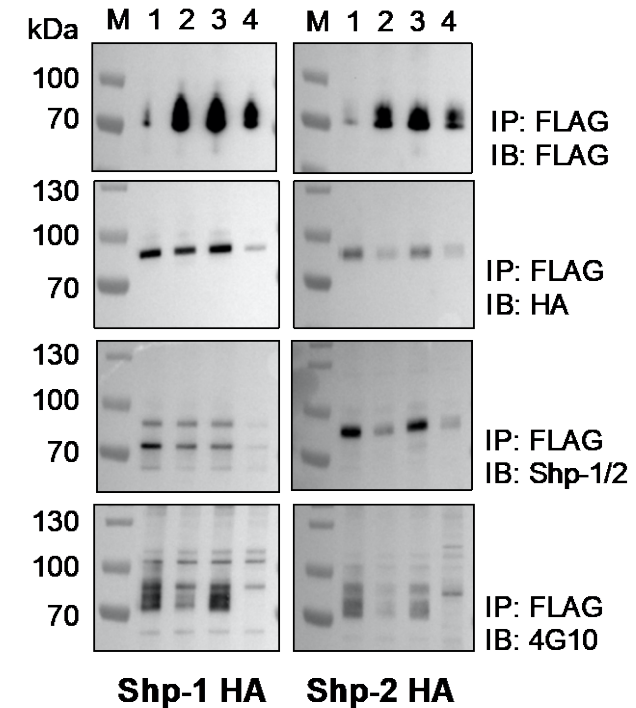
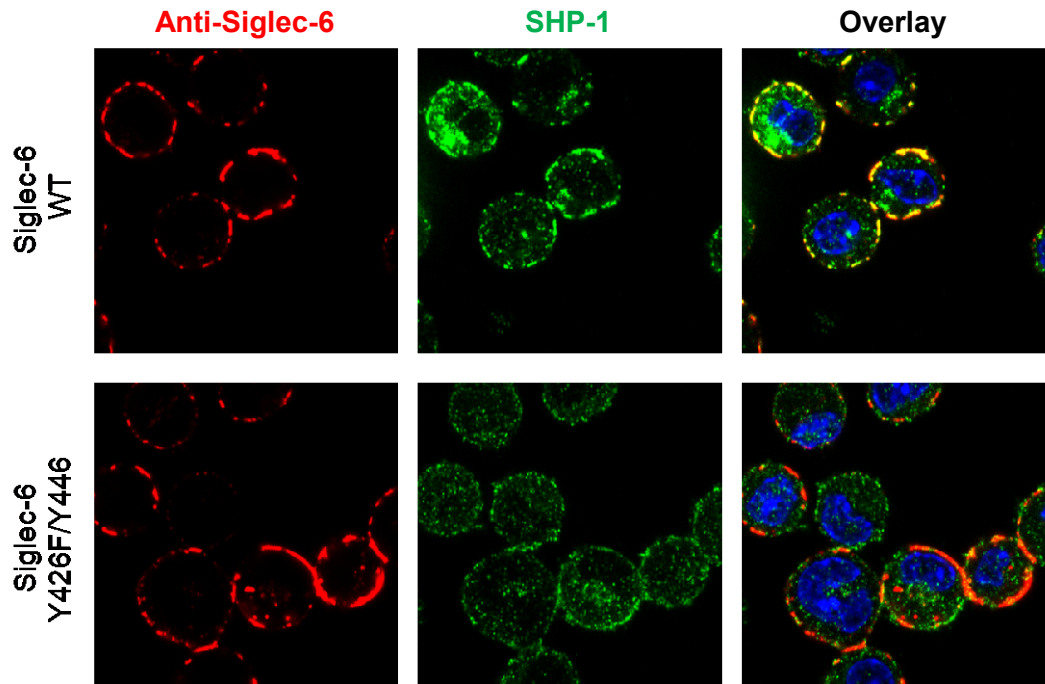


# Siglec-6 Recruits Inhibitory Phosphatases via ITIMs

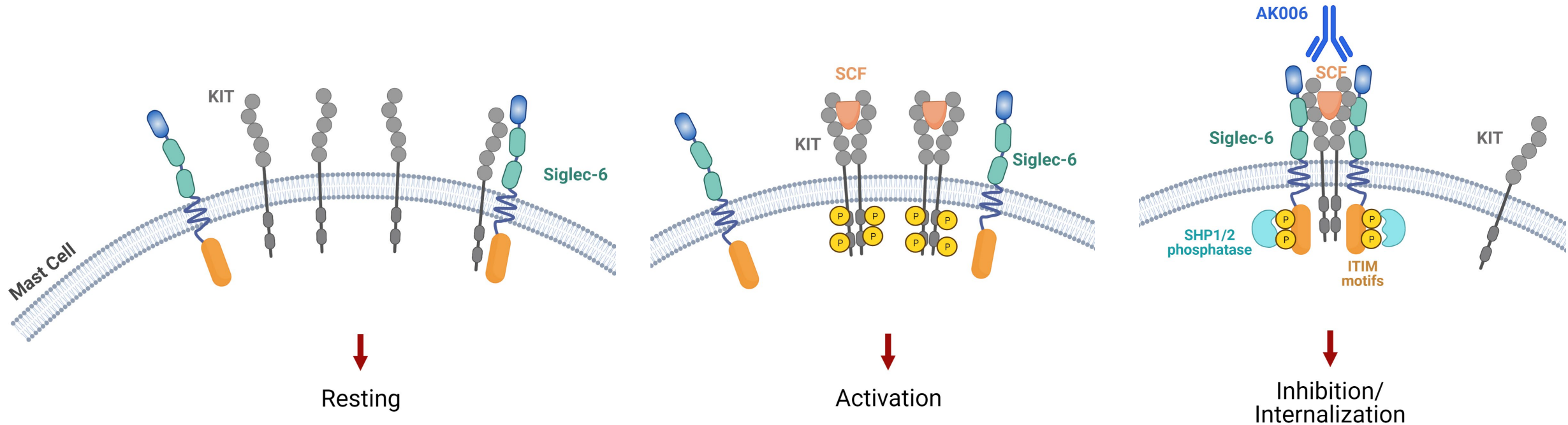


- 1 = S6 wt
- 2 = S6 Y426F
- 3 = S6 Y446F
- 4 = S6 Y426F Y446F

S6 = FLAG-tagged  
Shp-1/2 = HA-tagged



# Siglec-6-Mediated Inhibition of KIT Activation in Mast Cells



- Siglec-6 interacts with KIT, recruits Shp phosphatases and inhibits SCF-mediated mast cell activation
- AK006 is a humanized IgG1 agonistic Siglec-6 mAb that selectively targets and broadly inhibits mast cells

# Acknowledgements

## Allakos Research Team



## Scientific Advisory Board

- Bruce Bochner
- Bob Schleimer

## University of Mass Medical School

- Mike Brehm