

Siglec-6 and Siglec-8 Show Distinct Differences in Regulating Mast Cell Function

Wouter Korver, Zachary Benet, Alan Wong, Gian Luca Negri, Katherine Chang, John Leung, Thuy Luu, Naomi Freitas, Robert Sanchez, Julia Schanin, Bradford A. Youngblood

Allakos Inc., San Carlos CA

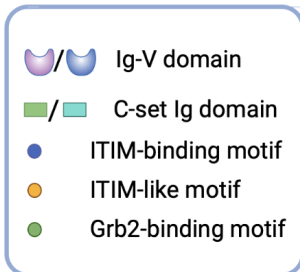
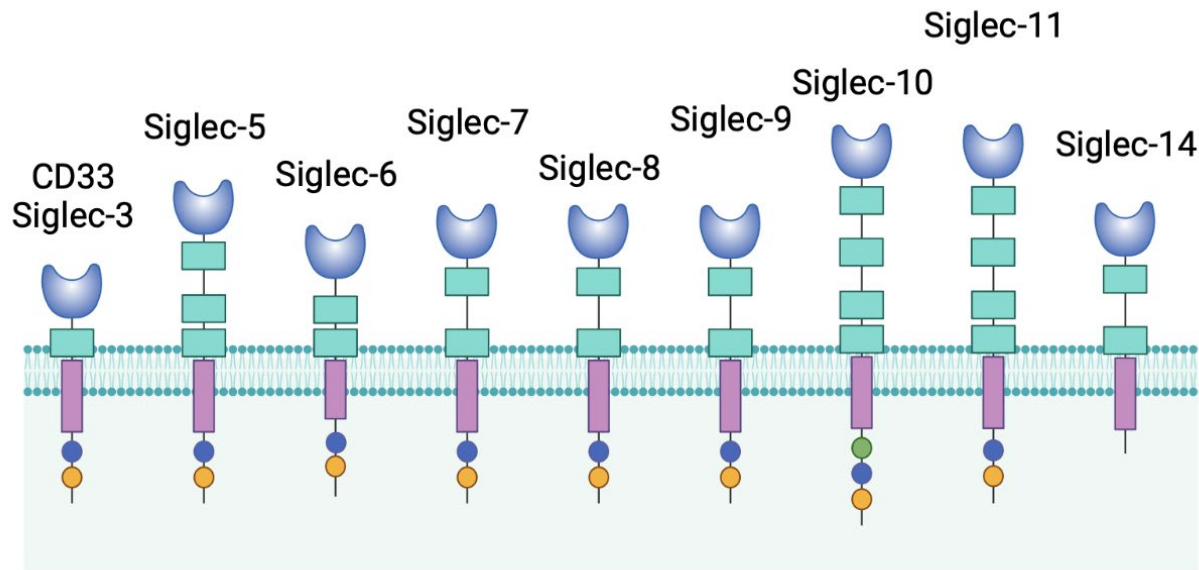
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Disclosure

- Employee of Allakos Inc.

Siglecs Represent Attractive Therapeutic Targets on Immune Cells

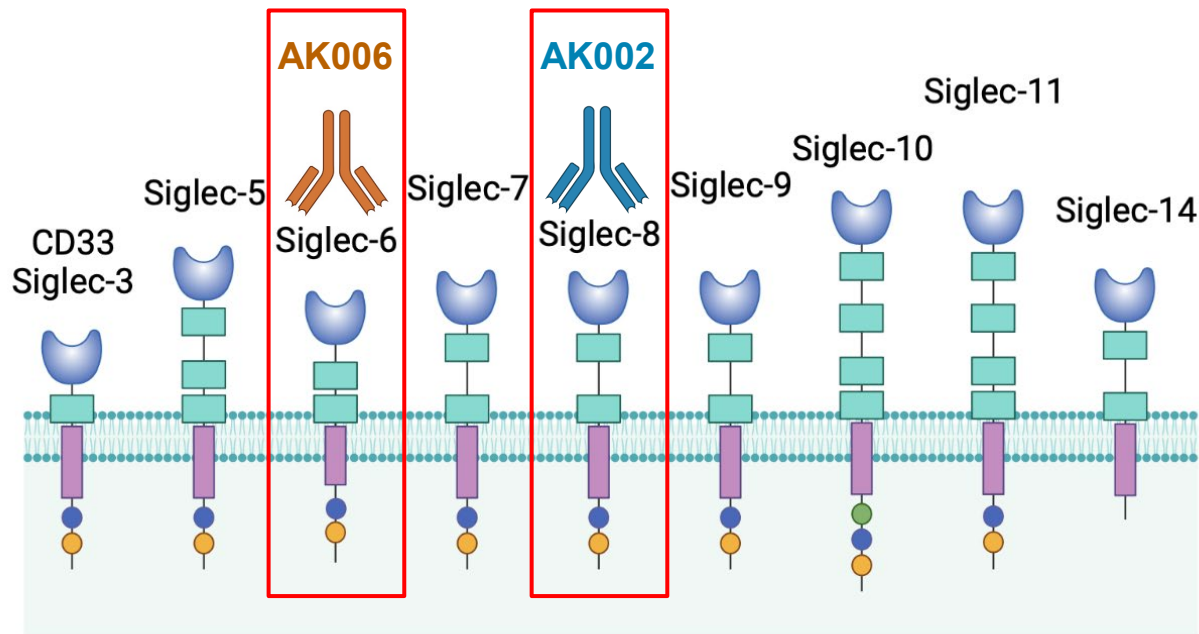
Human CD33-Related Siglecs



- Siglecs are inhibitory receptors selectively expressed on key immune cells
- Ability to selectively suppress immune cell activation via agonist antibodies to reduce chronic inflammation (i.e., AK002 and AK006)
- Targeting Siglecs provides an opportunity to directly modulate key pathogenic cells in different disease states

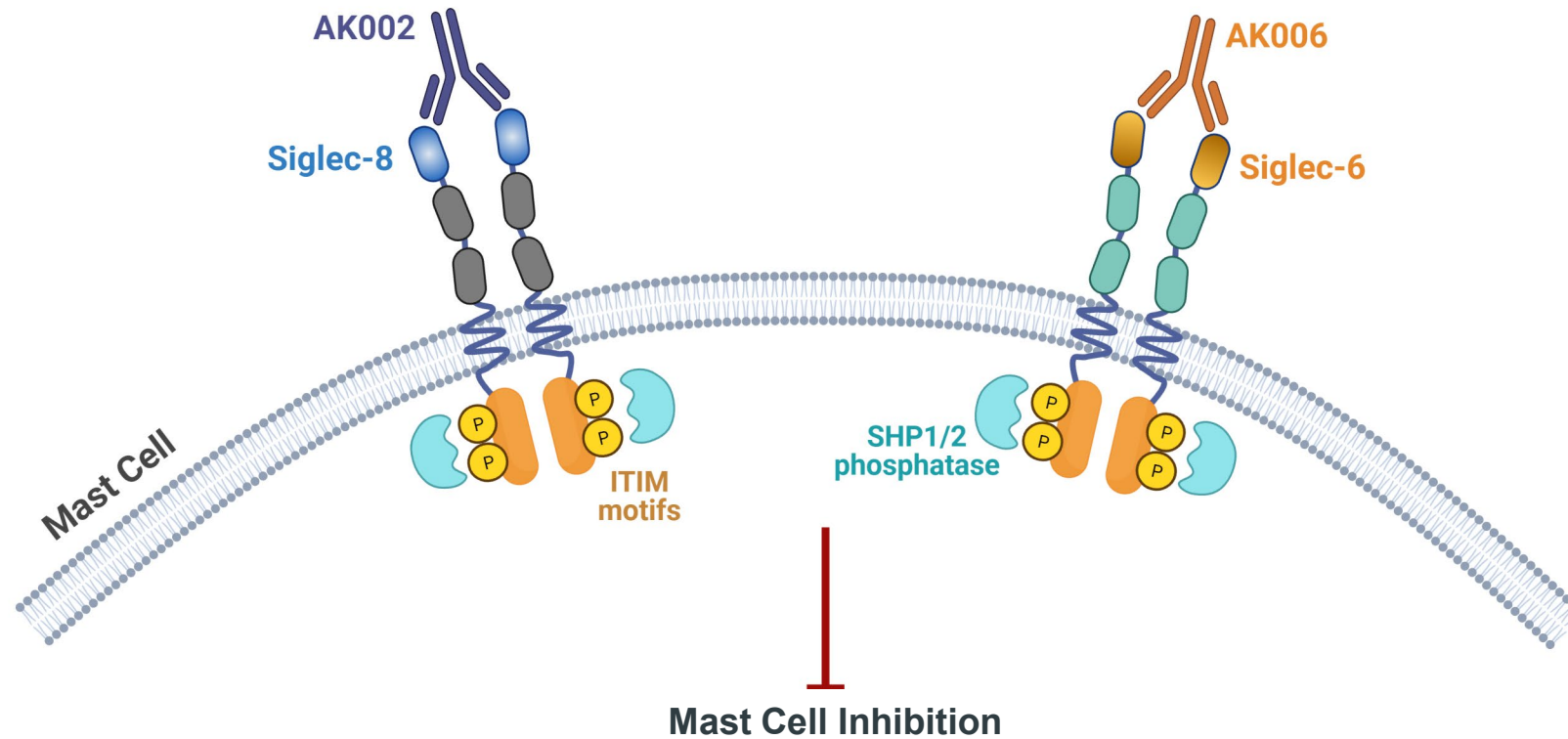
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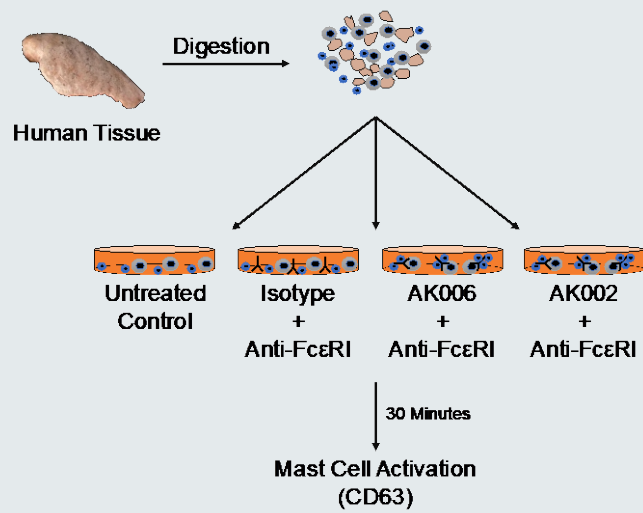
AK002 and AK006 Induce Mast Cell Inhibition via Siglec-8/6



- How do Siglec-8 and Siglec-6 induce broad mast cell inhibition?
- Are there differences between Siglec-8 and Siglec-6 inhibitory function via AK002 or AK006?

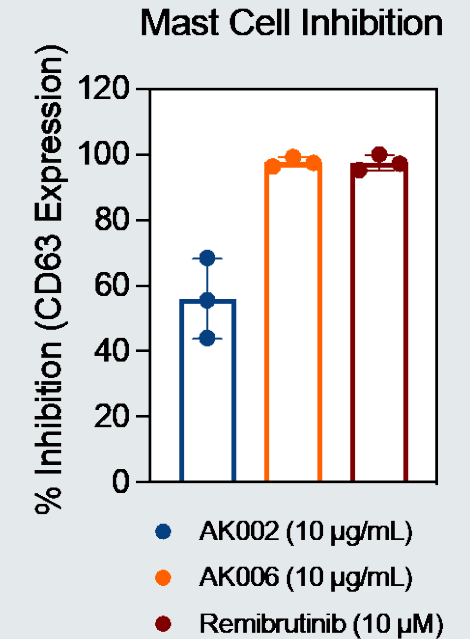
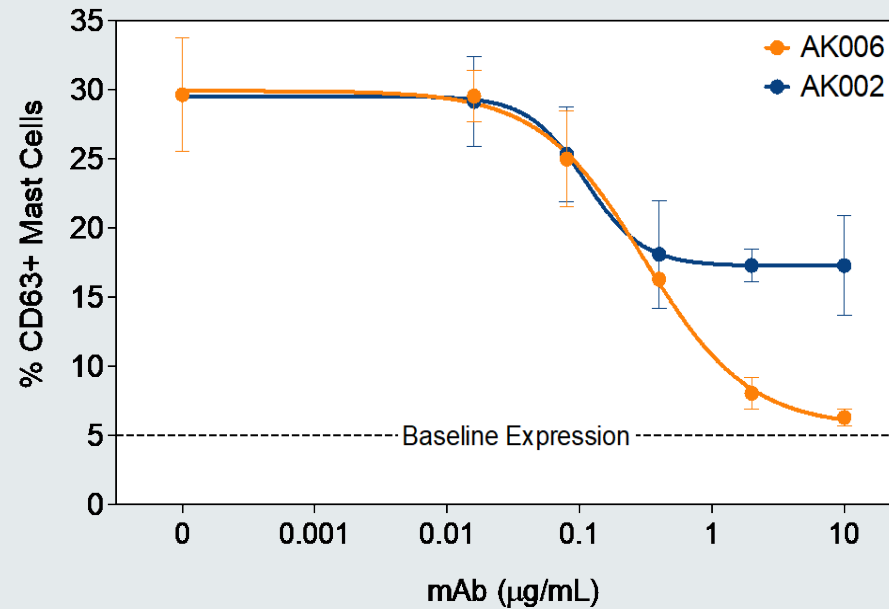
AK002 and AK006 Inhibit Mast Cell Activation in Human Tissues

Human Mast Cell Activation Assay



n=3 human donors

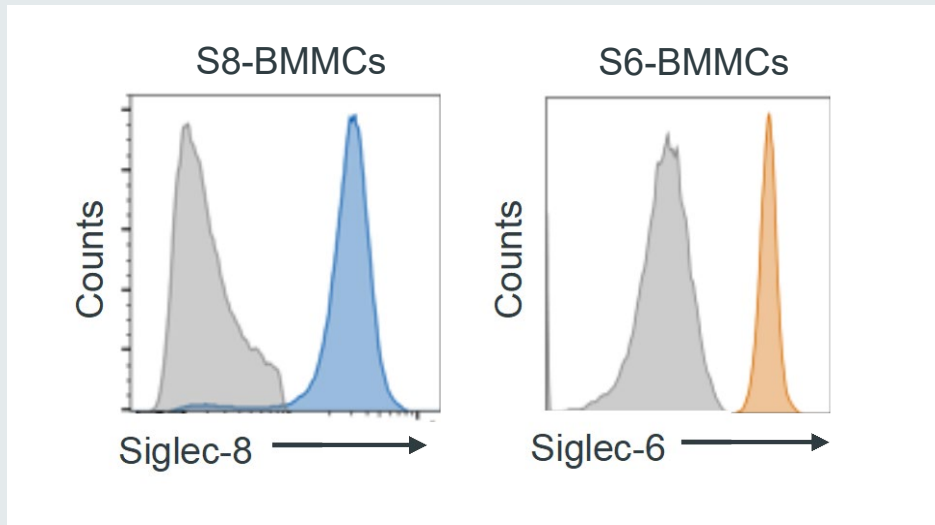
IgE-Activated Human Tissue Mast Cells



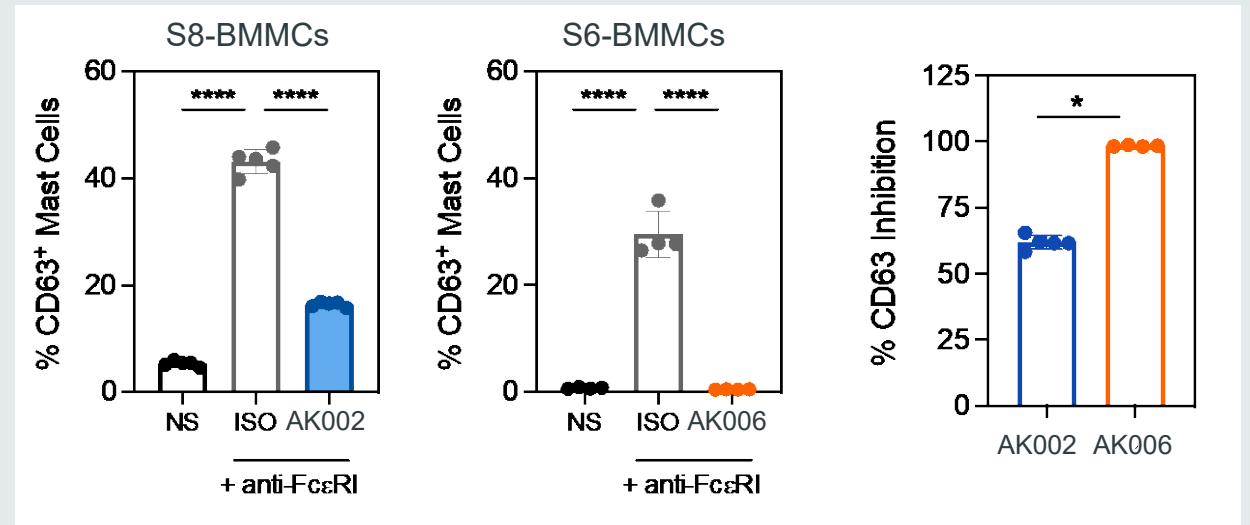
AK006 displays deeper inhibition of IgE-mediated mast cell activation than AK002 and has similar activity as remibrutinib

BMMCs From Transgenic Mice Express Functional Siglec-8 and Siglec-6

Expression of Siglecs on BMMCs



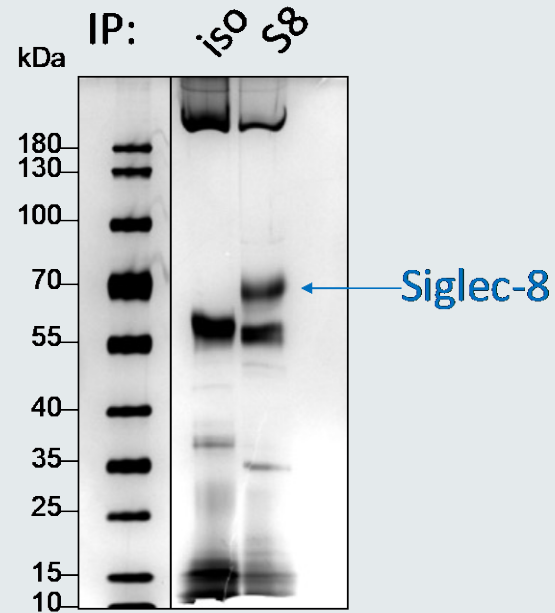
IgE-Mediated Activation of BMMCs



BMMCs: bone marrow derived mast cells generated from transgenic mice

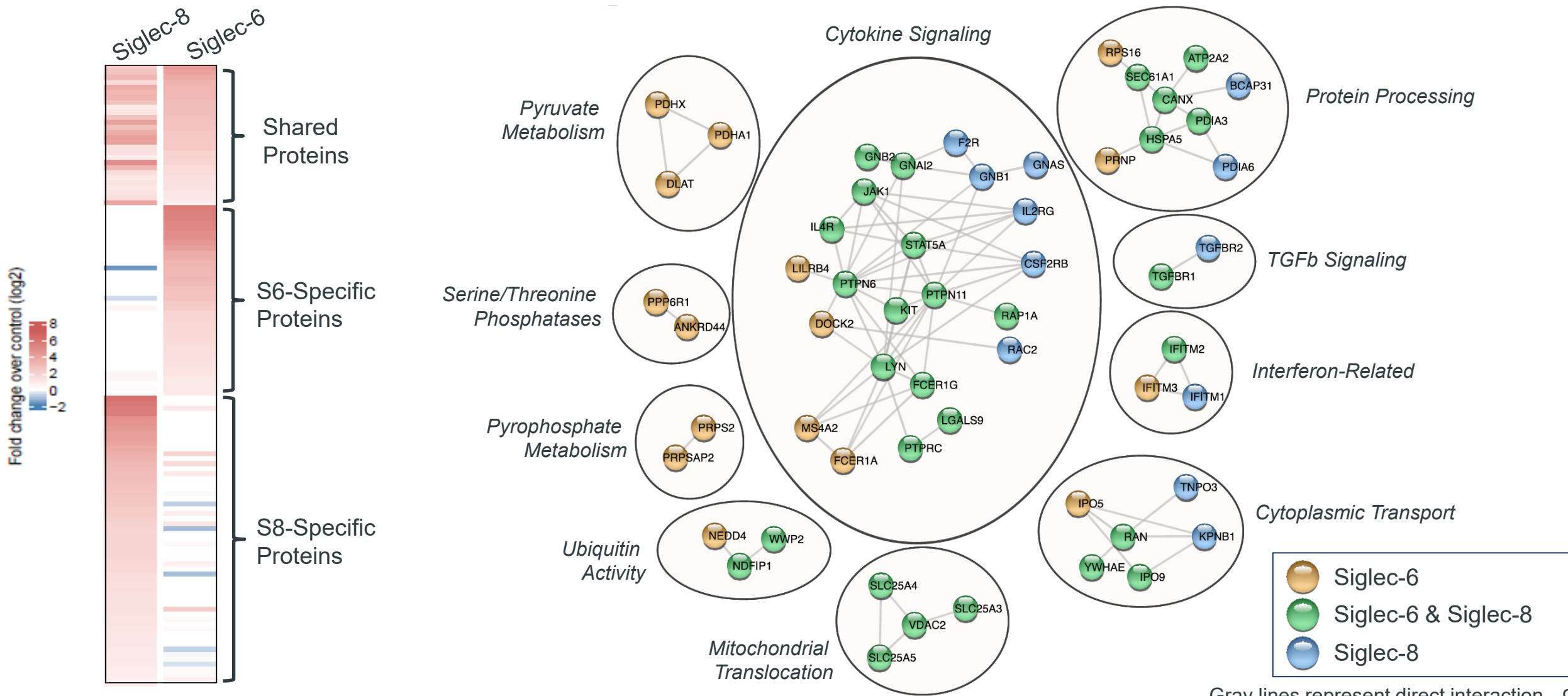
Expression and inhibitory activity in BMMCs from transgenic mice mirror Siglec-8/6 in primary human mast cells

Siglec Interactomes Elucidated by Mass Spectrometry

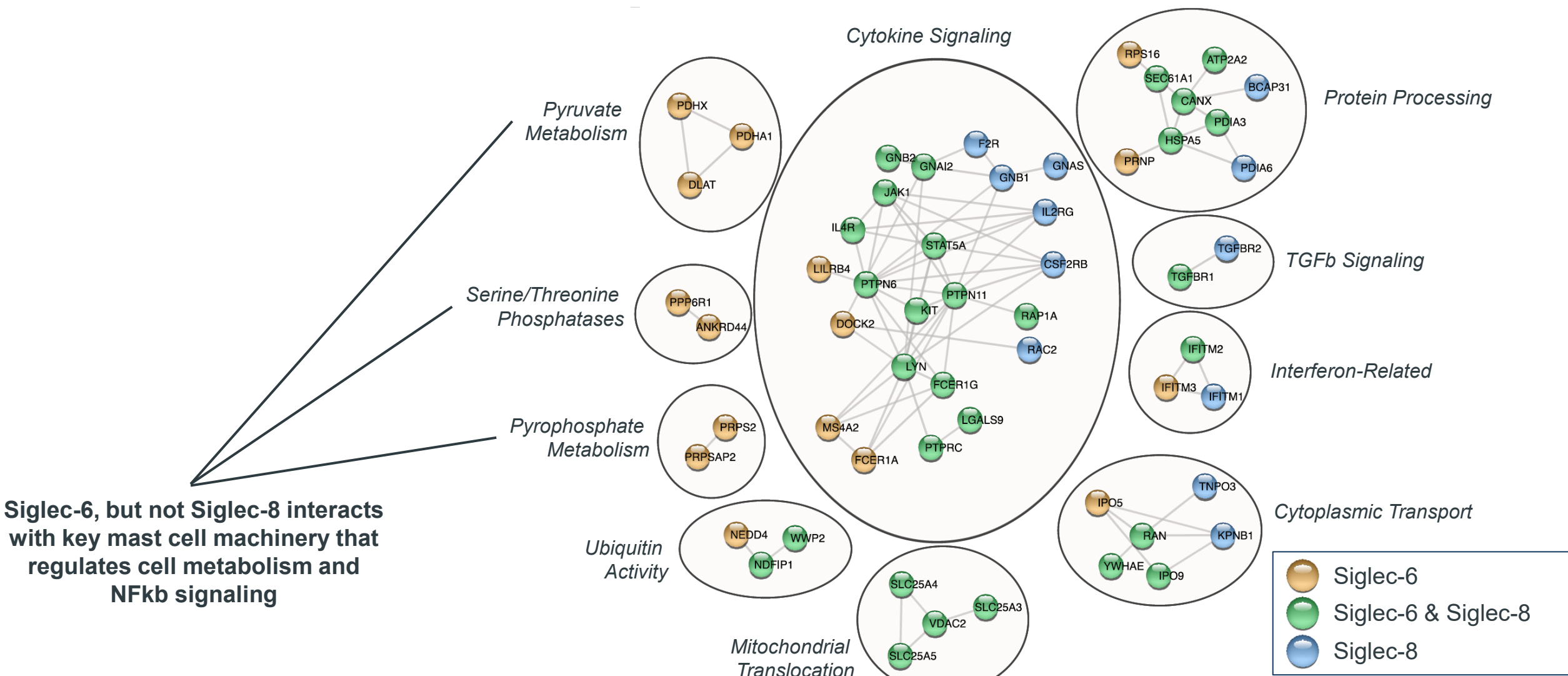


Quantification of proteins in Siglec immuno-precipitations by LC-MS

Siglec-8 and Siglec-6: Similarities and Differences in Protein Interactions






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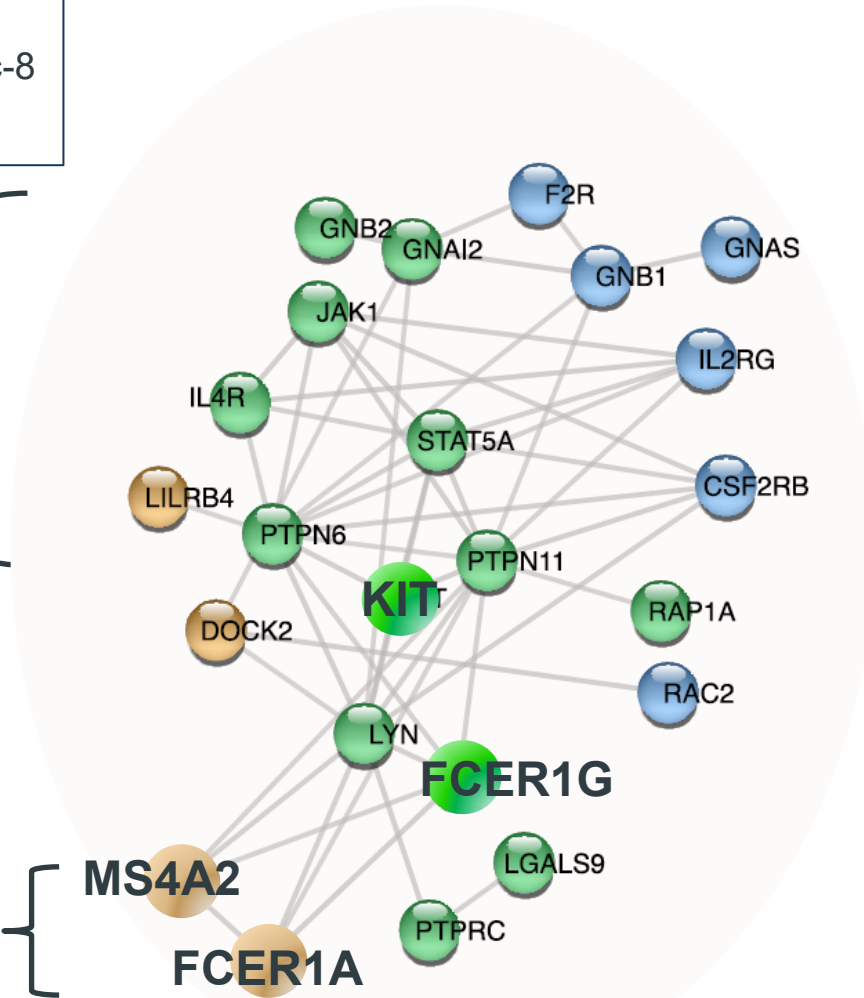
Siglec-6, but not Siglec-8 interacts with key mast cell machinery that regulates cell metabolism and NFkb signaling

Siglec-8 and Siglec-6: Similarities and Differences in Protein Interactions

-  Siglec-6
-  Siglec-6 & Siglec-8
-  Siglec-8

Cytokine receptor signaling

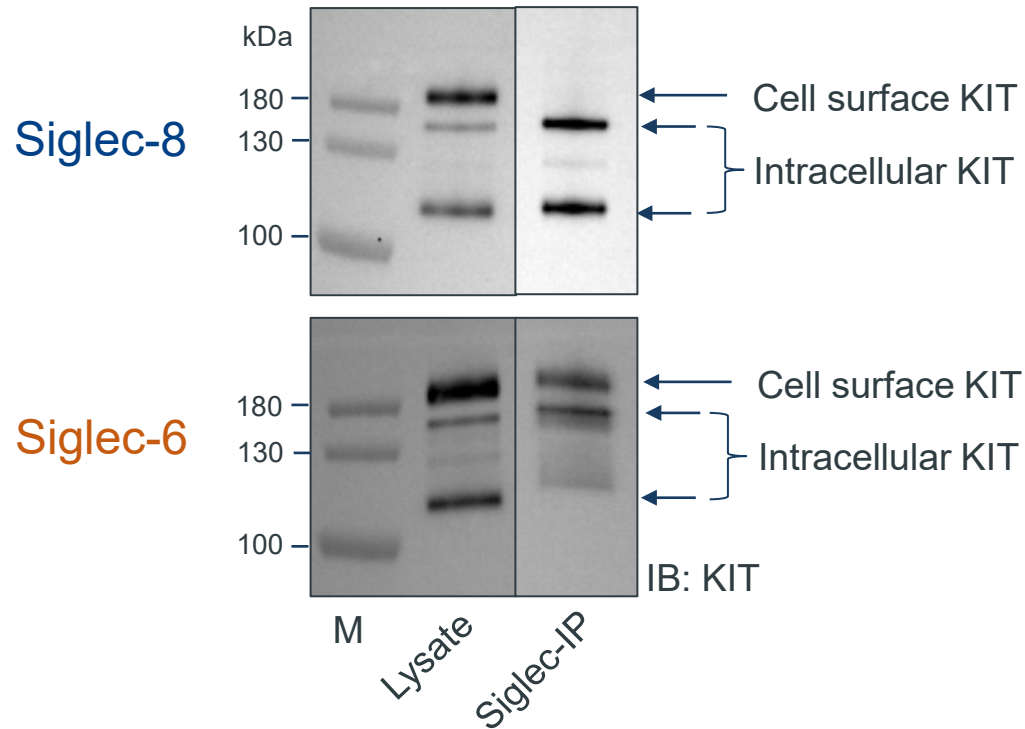
FcεRI receptor subunits



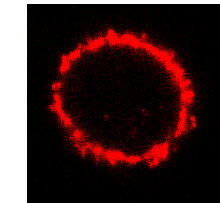
- Siglec-6 and Siglec-8 interact with multiple key activating receptors and signaling molecules in mast cells, including IL-4R, FcεRI, LYN, STAT5A, and JAK1
- Additionally, Siglec-6 and Siglec-8 both interact with the inhibitory phosphatases, SHP-1 and SHP-2
- Siglec-8 specifically interacts with IL-2R and the common beta chain (CSF2RB)
- Specific Siglec-6 interactions are observed with additional subunits of the FcεRI complex and inhibitory signaling molecules

Siglec-6 but not Siglec-8 Interacts with Cell Surface KIT

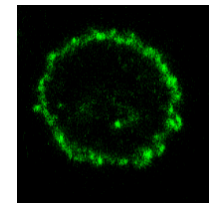
KIT Pulldown through Siglec-6 or Siglec-8



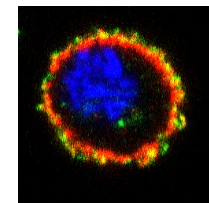
KIT-S6 Interaction on MC Membrane



Anti-Siglec-6

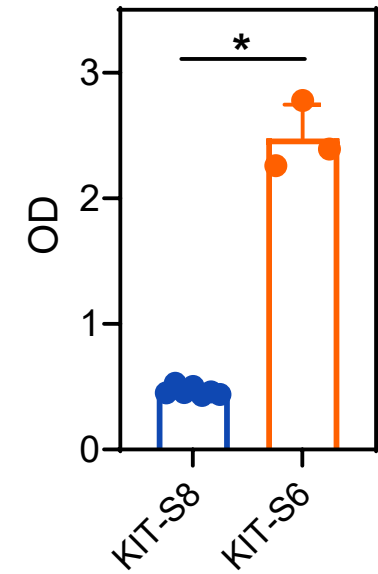


KIT



Overlay

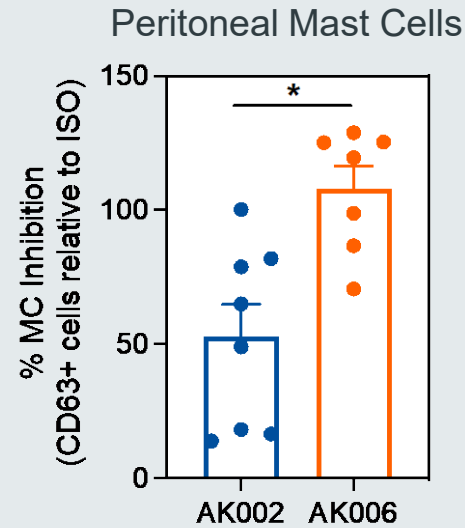
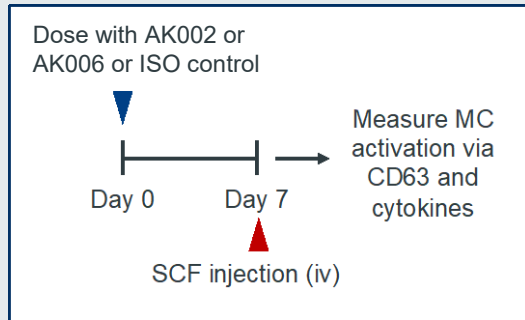
KIT Binding to Recombinant Siglecs



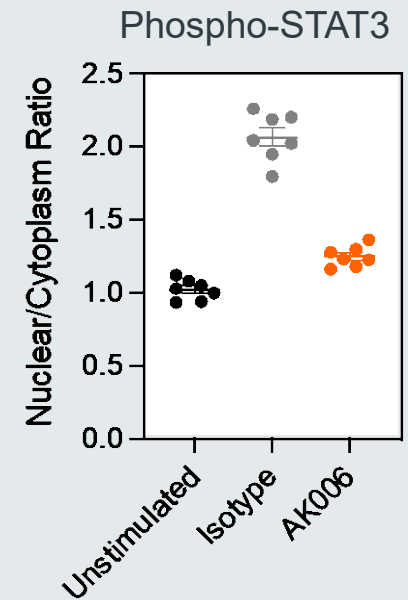
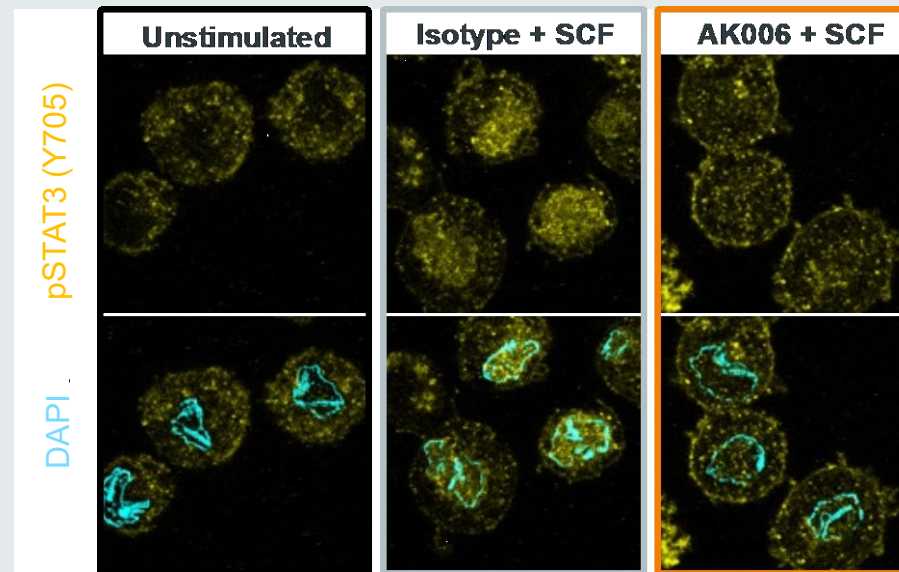
Siglec-6 interacts with mature KIT at the cell surface

Siglec-6 mAb Inhibits KIT-Mediated Mast Cell Activation

KIT-Driven Mast Cell Activation Mouse Model

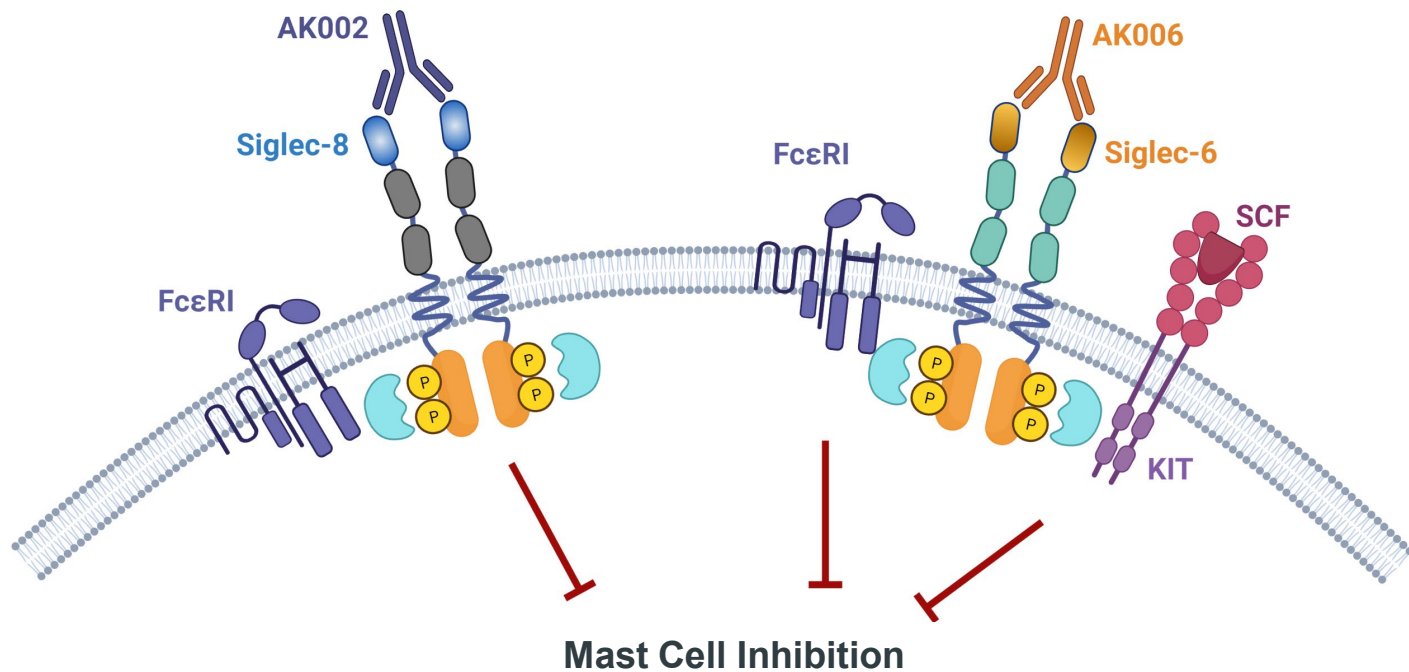


p-STAT3 Imaging in KIT-Activated Human Mast Cells



Siglec-6 reduces KIT-mediated MC activation via inhibition of STAT phosphorylation and translocation to the nucleus

Broad and Selective Mast Cell Inhibition Through Siglec-6



- Siglec-6 and Siglec-8 interact with multiple activating receptors and signaling molecules, highlighting a potential mechanism for broad mast cell inhibition
- Global interactome analyses suggest Siglec-6 and Siglec-8 may differentially regulate mast cell function
- Siglec-6, but not Siglec-8, interacts with cell surface KIT and demonstrates deeper KIT-mediated mast cell inhibition
- Targeting Siglec-6 with AK006 represents a novel approach to broadly inhibit mast cells in inflammatory diseases

Acknowledgements

Allakos Research Team



Scientific Advisory Board

- Bruce Bochner MD
- Bob Schleimer PhD