Polysialylation Of GPR15 Is Required For T Cell Migration Towards GPR15L: Implications For Autoimmune Diseases Psoriasis and Scleroderma

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T cells are critical components of the immune response

- There are many different kinds of T cells:
  - $CD4^+$ cells are akin to orchestra conductors – they tell other cells what to do through cytokine secretion and direct interactions
    - Subtypes include T_H1, T_H2, T_H9, T_H17, T_H22, T_{FH}, T_{REG}
  - $CD8^+$ cells are primarily cytotoxic but also secrete cytokines
- Upon exposure to antigen, T cells differentiate from a naïve phenotype into memory and effector T cells
  - Activated T cells can have pro- or anti-inflammatory properties, depending on the kind of T cell and mechanism of stimulation
- Dysregulation of T cells leads to numerous chronic diseases, including cancers and autoimmune diseases.

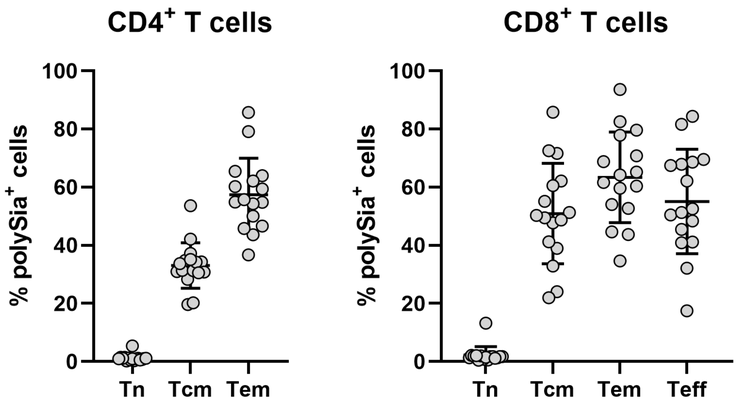
The role of polysialic acid in T cell biology is understudied

- Polysialic acid (polySia) is a linear polymer of up to 400 sialic acid residues
  - Fewer than a dozen proteins are known
- In healthy humans, it is expressed in cells of the nervous, immune, and reproductive systems
  - It is dysregulated in cancer and mental health disorders
- PolySia influences immune responses through several mechanisms, including by directing cell migration

Hypothesis

- Characterizing the expression of polySia in T cells will shed light on how it influences the adaptive immune response in health and disease

PolySia is only found on antigen-exposed T cells in human peripheral blood



Flow cytometry analysis of T cells from human PBMCs. GFP-$EndoN_{DM}$ was used to identify $polySia^+$ cells. Naive (Tn), central memory (Tcm), effector memory (Tem), and effector (Teff) T cells were analyzed. PolySia is not found on naïve T cells. 30 – 60% of antigen-exposed T cells in both $CD4^+$ and $CD8^+$ compartments are polysialylated.

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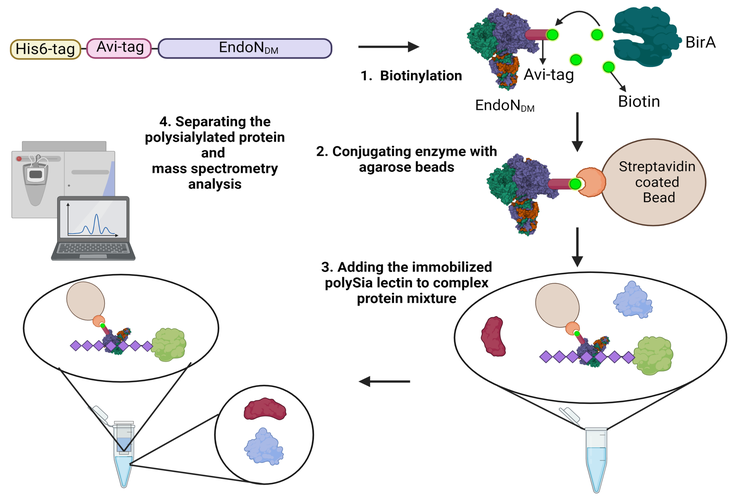
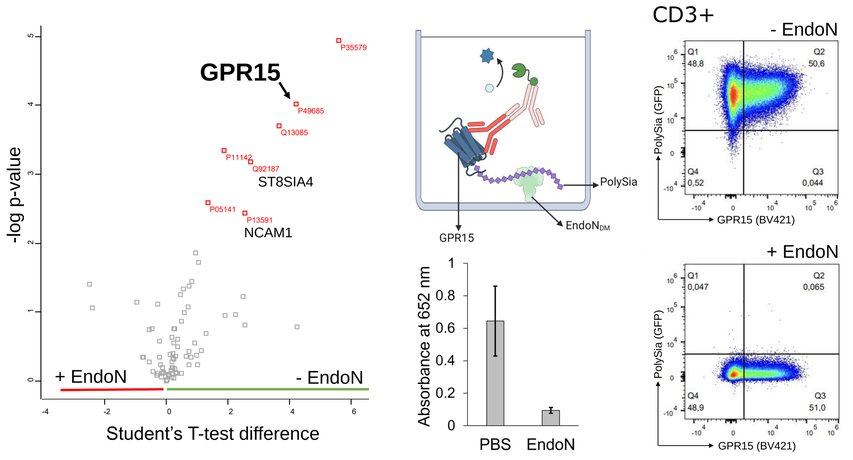
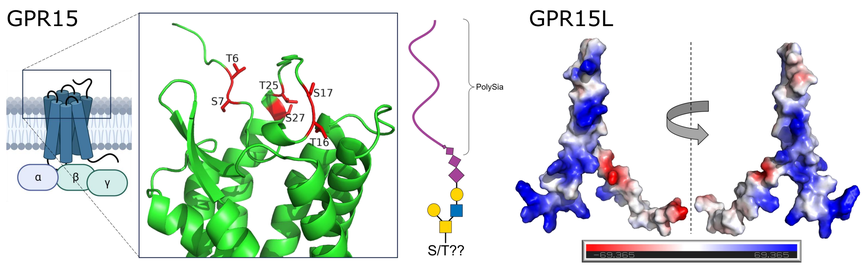
GPR15 is polysialylated in activated T cells



Isolation of polysialylated proteins using immobilized EndoN. Polysialylated proteins were isolated from purified human $CD3^+$ cells that were activated for 7 days *in vitro* using α-CD2/3/28 in the presence of IL-2. As a control, we pretreated cells with EndoN to hydrolyze polySia.



This is the first time polySia has been found on GPR15, making it a novel polysialylated protein.

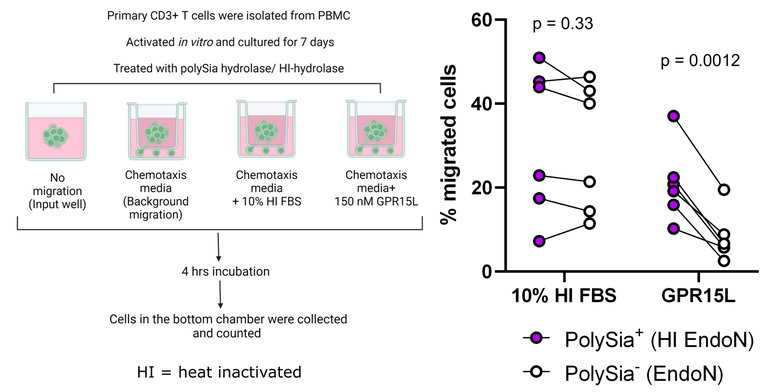


Alphafold models of GPR15 and its chemokine ligand GPR15L.

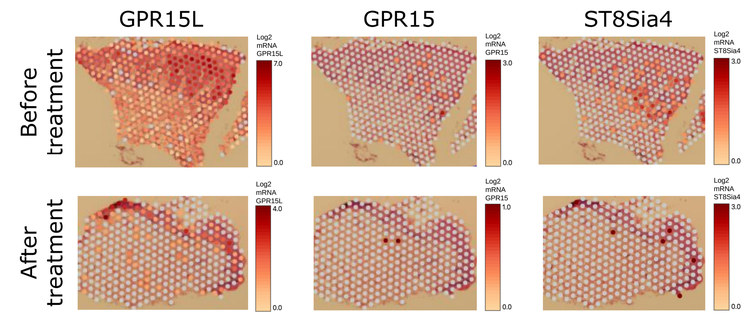
GPR15:
- G protein-coupled receptor
- Expressed primarily on T_H17 and T_{REG}
  - $GPR15^+$ cells are substantially increased in patients with autoimmune diseases
- Mediates migration of T cells towards GPR15L
- Has 6 predicted O-linked glycosylation sites in its N-terminal end

GPR15L:
- Expressed primarily in gastrointestinal tract, skin, and lymph nodes
- Basic residues (blue) account for 28% of the protein sequence while acidic residues (red) are only 3.5%
  - Highly basic nature may facilitate binding to anionic polySia?

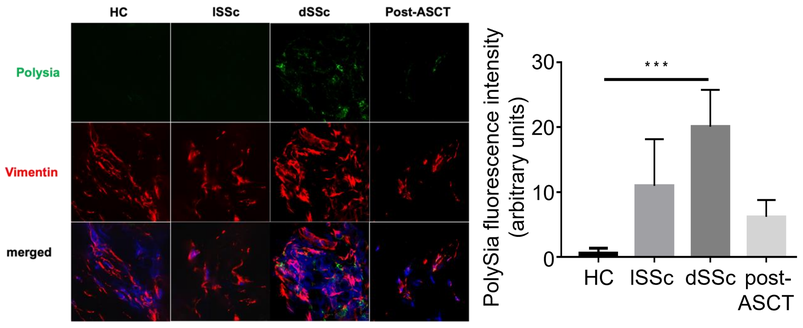
PolySia is required for GPR15-mediated T cell migration



PolySia occurs in skin of patients with T_H17-mediated autoimmune skin diseases



Spatial RNAseq of skin biopsies from patients with psoriasis before and after treatment with systemic biologic drugs. Each circle represents 55 µm area. Sections were stained with H&E.



Microscopy of skin biopsies from patients with scleroderma (SSc). Limited SSc (lSSc) is a milder form of the disease whereas diffuse SSc (dSSc) is more severe. Autologous stem cell transplantation (ASCT) is the only curative therapy but few patients qualify for treatment and success rates are low.

Current model