Optimized Conditions to Identify SGSM2-Interacting Proteins

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Introduction

- Type 2 diabetes affects millions throughout the U.S.
- Diabetic individuals experience dysregulation of insulin secretion
- Genetic variants in and near *SGSM2* are associated with plasma proinsulin, suggesting an effect on insulin processing or secretion
- Identifying proteins that interact with SGSM2 can reveal mechanisms by which it affects insulin processing or secretion
- **Aim:** Optimize pull-down conditions to effectively isolate SGSM2-6xHis and its potential interactors for identification by affinity-purification mass spectrometry

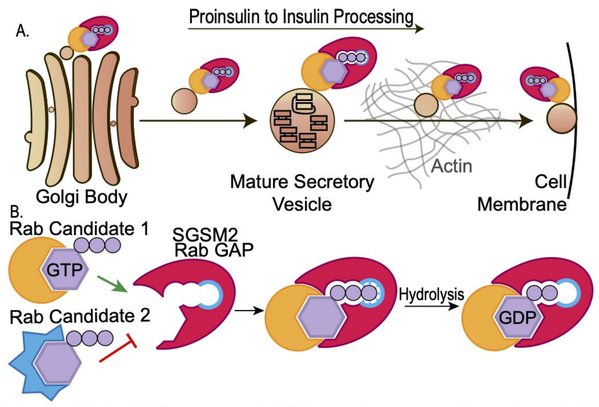


Figure 1. Roles of Rab GTPases and GTPase Activating Proteins (GAPs) in insulin processing and secretion. (A) Rab GAPs are found in the Golgi Body, secretory vesicles, and plasma membrane during insulin processing. (B) Rab GAPs (red) promote the deactivation of the partner Rabs (yellow, blue) by encouraging hydrolysis of GTP to GDP (purple).

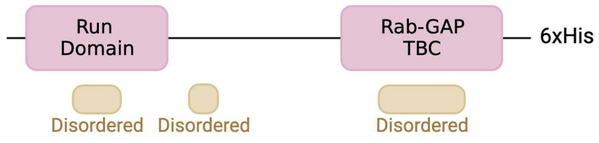


Figure 2. Domains of the cloned SGSM2 construct. We overexpressed human SGSM2 protein with a C-terminally linked 6xHis affinity tag.

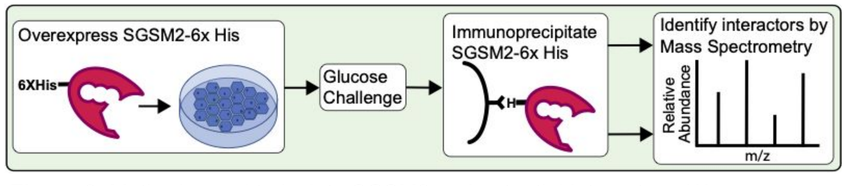


Figure 3. Methods used to isolate SGSM2 and potential interactors. We over-expressed SGSM2-6xHis in MIN6 murine pancreatic beta cells, used glucose to stimulate insulin secretion, collected lysate, and used nickel-cobalt beads to isolate SGSM2 and interacting proteins.

Results

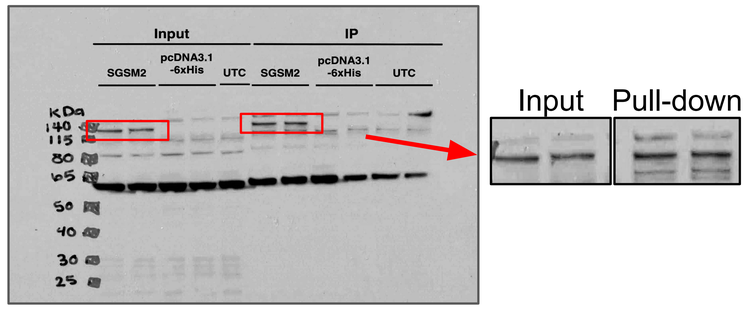


Figure 4. SGSM2 was successfully isolated from the protein lysate in a pull-down when the lysate was incubated with beads for 30 minutes

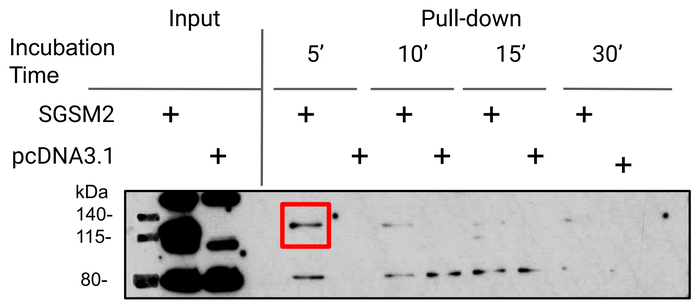
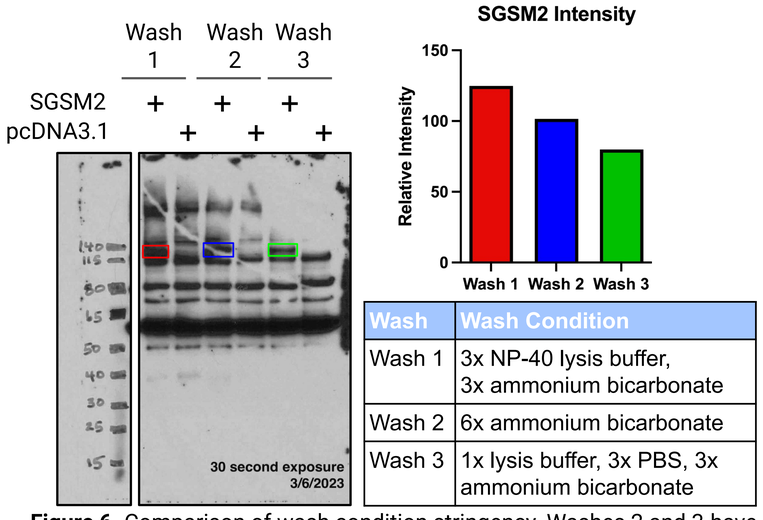


Figure 5. Protein retention following incubation at several time points. SGSM2 was best isolated following a five minute incubation period as shown using a 5 minute film exposure.



Wash	Wash Condition
Wash 1	3x NP-40 lysis buffer, 3x ammonium bicarbonate
Wash 2	6x ammonium bicarbonate
Wash 3	1x lysis buffer, 3x PBS, 3x ammonium bicarbonate

Figure 6. Comparison of wash condition stringency. Washes 2 and 3 have significantly lower background binding than wash 1. It is unclear whether or not the decreased binding affinity of higher weight proteins for wash 3 is a true reduction.

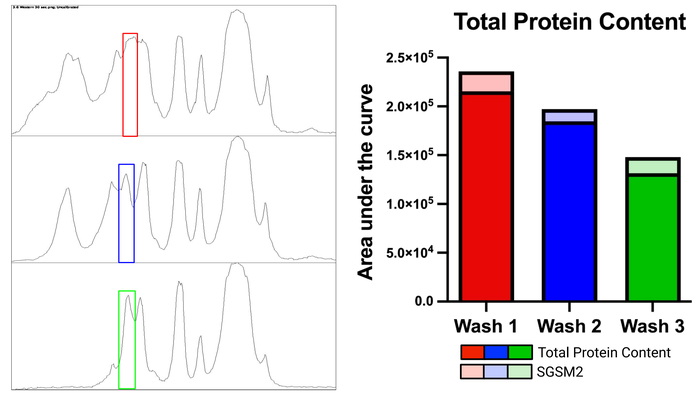


Figure 7. Intensity of the SGSM2 band across the different wash conditions.

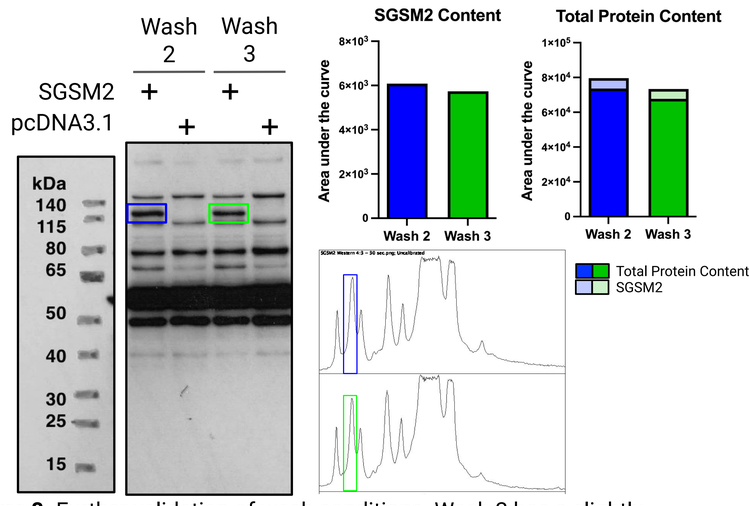


Figure 8. Further validation of wash conditions. Wash 2 has a slightly cleaner banding for SGSM2 and decreased binding of high molecular weight proteins (>140 kDa).

Conclusions

- We successfully generated and pulled down SGSM2-6xHis
- We successfully optimized conditions for pull-down with SGSM2
  - 5 minute incubation period allows for the strongest SGSM2 band intensity
  - 1X lysis buffer/3X PBS/3X ammonium bicarbonate wash decreased non-specific banding and increased SGSM2 band intensity
- Future mass spectrometry data will identify potential interactors of SGSM2 and provide insight into SGSM2’s role in insulin processing and secretion