E-MAP DIRECTED ANALYSIS OF EFFECTOR PROTEIN FUNCTION IN *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

E-MAP Directed Analysis of Effector Protein Function in *Salmonella enterica* Serovar Typhimurium

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Six effector proteins of *Salmonella enterica* serovar Typhimurium - SseC, SseG, SseI, SseK1, SteB, and SopD - were studied in order to determine their molecular contributions to virulence. Data collected from a high-throughput quantitative genetic interaction screen in budding yeast, called an E-MAP, was used to generate a list of GO terms for each effector protein. The E-MAP used in this analysis involved crossing yeast strains expressing the *Salmonella* effector proteins with single deletion mutant yeast from a deletion library of 4800 non-essential genes. To determine the validity of the proposed GO terms, unbiased experiments were conducted in mammalian cells. Immunoprecipitation was used to determine effector protein interacting partners, and the results were analyzed by Mass Spectrometry. Immunofluorescence Microscopy was used to observe localization patterns. It was found that SseC interacts with the retromer, a protein complex functioning in retrograde protein trafficking that assembles on endosomal membranes. The current hypothesis is that SseC is involved in promotion of complex disassembly from endosomes. SseG was shown to colocalize with the Golgi through Immunofluorescence data, supporting a hypothesis that SseG functions by interfering with host

trafficking processes. The results from these unbiased mammalian experiments align with the GO terms generated from the E-MAP, providing support for the E-MAP as an efficient means of uncovering effector protein function in pathogenic bacteria.

DEDICATION

I would like to dedicate this work to Dr. Robert Watson and Dr. Kristin Patrick. Their guidance and support brought this project to fruition, and I am incredibly grateful. They encourage me to be a better scientist in every way.

ACKNOWLEDGEMENTS

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NOMENCLATURE

E-MAP	Epistatic Mini Array Profile
GO	Gene Ontology
IF	Immunofluorescence Microscopy
IP	Immunoprecipitation
MS	Mass Spectrometry
OD	Optical Density
SCV	Salmonella Containing Vacuole
SLIC	Sequence and Ligation Independent Cloning
SPI	Salmonella Pathogenicity Island
T3SS	Type III Secretion System

BACKGROUND

Host-pathogen interactions are a vital component of understanding the manifestation of disease. The intracellular bacterial pathogen *Salmonella enterica* serovar Typhimurium exploits its eukaryotic host in order to evade destruction in lysosomes and establish its replicative niche [1]. *S. typhimurium* is frequently used as a model organism to study effector protein secretion. As it stands, *S. typhimurium* invades the vacuoles of eukaryotic hosts via a type III secretion system (T3SS) and modifies host vacuole membrane proteins to survive and replicate in a favorable environment [1]. T3SSs are needle-like organelles anchored in both membranes of some gram-negative bacteria [2]. The T3SS recognizes a signal sequence on the N terminus of effector proteins meant for secretion, and injects the proteins directly from the bacterial cytoplasm into the host through a highly specific mechanism [2]. In *Salmonella*, two types of T3SSs are encoded in the genome in areas called Salmonella pathogenicity islands, or SPIs [3]. SPI-1 contributes to invasion of host cells, while SPI-2 facilitates the maintenance of *Salmonella* containing vacuoles (SCVs) [3]. Modification of host cell pathways and creation of SCVs are the result of abundant *Salmonella* effector protein secretion [4]. *S. typhimurium* commonly targets the epithelial cells of the digestive tract, leading to gastro-intestinal problems in infected individuals [5].

Given the vastness and complexity of host-pathogen interactions, researchers have turned to genetic systems that can screen for effector protein function. In this study, E-MAPs (Epistatic Mini Array Profiles) were generated in yeast and employed to begin to identify the functions and mechanisms of previously uncharted *Salmonella* effector proteins. Yeast are great heterologous systems for determining effector protein function because they contain many highly conserved

eukaryotic pathways and do not encode immunity mechanisms of other eukaryotes, allowing for stronger phenotype observation [6]. They are also a commonly used way to look at the way genes interact in the cell.

E-MAPs are high throughput genomic screens that quantitatively measure genetic interactions in yeast [7]. First, pairwise interactions are measured by crossing a query strain - single deletion mutant - with the entire yeast deletion library. The yeast deletion library contains data from deletions of 4800 non-essential genes in *Saccharomyces cerevisiae* [7]. Numerical scores are digitally assigned to each colony by comparing the size of individual colony (as measured by the pixel count on a digital image) to the average growth of all colonies on the plate [7]. A positive, or alleviating, score suggests the two genes of interest are involved in the same genetic pathway based on the fact that yeast with two defects in the same pathway will not be as sick as the baseline that had defects in two different pathways. [7]. A positive score corresponds to a yellow bar on the E-MAP. A negative, or aggravating, score suggests the two genes of interest are involved in parallel pathways. So, a double mutant would show signs of a synthetic sick/lethal phenotype and appear blue on the E-MAP [7]. Double mutant yeast with neutral scores appeared black on the E-MAP, and they are slightly sicker than single mutants because they have defects in two pathways. However, the colonies are not as sick as colonies with a negative score. In addition to the pairwise genetic interactions, the interaction profiles of query strains were compared to those of the single deletion mutants. If the profile of the query strain showed similarities to the profile of a functionally defined single deletion mutant, it is suspected that the query strain single deletion mutant may be functioning in the same pathway as the functionally defined deleted gene [8]. The correlation of interaction profiles is highly significant because it shows how one gene interacts with lots of different pathways in the cell. The majority of the

interactions on both maps are neutral, suggesting these dramatic changes in growth phenotype are only observed with specific groups of genes.

Computational biologists have developed GO terms as a way of placing genes into functional categories. GO terms can be generated from the interactions shown in the E-MAP and sorted for enrichment with each query strain. These GO terms are highly specific and provide great insight into effector protein function.

The very first E-MAP was conducted by researchers at UCSF back in 2008 [7]. In the years since, the entire yeast deletion library has been crossed against itself and lots of E-MAPs have been created. Current work is being done to characterize effector proteins of *Brucella, Coxiella,* and *Salmonella* species using these vast amounts of genetic data.

CHAPTER I

INTRODUCTION

The intricate study of pathogenesis in mammalian cells is an essential component of developing novel treatments for infectious diseases. One bacterial pathogen, *Salmonella enterica* serovar Typhimurium, invades its host via a T3SS [3]. The underlying infection mechanisms of this bacterium can be analyzed through studying the functions of its many effector proteins. *S. typhimurium* manipulates host cellular trafficking events to establish infection in epithelial cells, causing gastro-intestinal inflammation.

A fundamental understanding of effector protein function is necessary in order to determine how *S. typhimurium* is capable of infection. To date, a handful of *Salmonella* effector proteins have been characterized but not all of their roles are fully understood [8]. While *S. typhimurium* is a well-studied organism, its effector protein library is vast and nearly untraversable without a source of direction. The complexity of the pathogenic proteome is infinitely compounded when examining interactions with host proteins, so it can prove difficult to begin directed experiments without the use of preliminary data. The use of E-MAPs is a way of efficiently generating large amounts of data that can be used to provide insight into discovering effector protein function.

The aim of this research was to identify the molecular mechanisms of *Salmonella* infection in mammalian cells through distinguishing the functions of various effector proteins. To achieve this, 18 *Salmonella* effector proteins and 2 viral proteins were used as query strains in an E-MAP. The effector proteins investigated in these experiments were SseC, SseG, SseI, SseK1, SteB, and SopD. Each query strain – yeast mutant expressing the bacterial effector - was crossed with the entire yeast deletion library, and the pairwise interactions were quantified (Appendix

A). Then, the interaction profiles of each effector were compared to the interaction profiles of functionally defined yeast mutants (Appendix B). Positive profile correlations between yeast expressing a particular effector and single deletion mutants suggest that the effector protein is targeting a conserved pathway involving the deleted gene.

GO terms were generated and sorted by significance of enrichment with each effector. A sample list of GO terms is shown for the effector SseC (Appendix C). The GO terms with highest enrichment for SseC involved protein trafficking pathways, including retrograde transport. To confirm the validity of these GO terms, unbiased experiments were conducted in mammalian cells. Immunoprecipitation experiments were used to reveal unique interacting partners of the *Salmonella* effectors, and localization patterns of the effectors were observed using Immunofluorsence Microscopy.

CHAPTER II

METHODS

Gateway™ Cloning and Expression of Effector Proteins

In order to express the *Salmonella* effector proteins in mammalian cells, the genes were inserted into a vector that is suitable for uptake by mammalian cells. Additionally, the effector proteins were tagged with a marker that facilitated visualization of the protein in the presence of antibody. This project used the 3X FLAG-tag system in the IPs and IF.

Protocol

sseC, sseG, sseI, sseK1, steB, and sopD were amplified via polymerase chain reaction. Highly specific forward and reverse primers were used. sopD amplified best at an annealing temperature of 60°C, while the rest of the effectors amplified best at an annealing temperature of 55°C. The gel-purified products were cloned into the Gateway™ entry vector pENTR1A no ccdB using SLIC protocol. The entry vector was altered to encode an N terminal 3x FLAG epitope tag. The vector was cut and purified, then treated with T4 ligase. Plasmid preps isolated from dh5α cells grown in the presence of kanamycin were digested with SalI and EcoRI, and then sent for sequence verification. The insert and tag were excised from the entry vector and inserted into the Gateway™ lentiviral destination vector pDEST CMV that contained a strong eukaryotic constitutive promoter using LR reactions. Plasmid preps isolated from stbl3 cells grown in the presence of ampicillin were purified using the OMEGA Bio-tek E.Z.N.A endonuclease free plasmid DNA mini kit II. To verify protein expression, 293T cells were lysed at 80% confluency using 2x loading sample buffer (Bio-rad) 48 hours post transfection with 2 μg of DNA. After boiling for 10 minutes and sonicating for 7 minutes, standard western blots were performed. The

primary antibody used was mouse monoclonal anti-flag M2 880 (Sigma: F1804) diluted 1:5000 and the secondary antibody used was goat monoclonal anti mouse (LI-COR: 926-32210) diluted 1:10000.

Immunoprecipitation

Immunoprecipitation was used to quantify protein-protein interactions. 3X FLAG tagged GFP was used as a control in all experiments. The results of the IP experiments were analyzed using Western Blots and silver stains to check for protein expression. The samples generated from the IP were sent to the Texas A&M Department of Chemistry's Protein Chemistry Laboratory for analysis. A list was returned that detailed all the human proteins each effector IPed with.

Protocol

293T cells were transfected with 10 µg of DNA at 80% confluency in 10 cm dishes using 3:1 PolyJet as the transfection reagent. Cells were grown in DMEM supplemented with 5% HEPES and 10% FBS. The IP was initiated at 48 hours post transfection. Cells were harvested in PBS+0.5M EDTA. Cells were lysed in lysis buffer with detergent containing 5% 1M Tris at pH 7.4, 3% NaCl, 0.2% 0.5M EDTA, and 0.26% 20% NP40. 50 µl of Flag resin was washed using lysis buffer without detergent containing 5% 1M Tris at pH 7.4, 3% NaCl, and 0.2% 0.5M EDTA. 1000 µl of the cleared lysate was added to the resin and inverted for 2 hours at 4°C. The flag resin was washed with 1000 µl of IP wash containing 5% 1M Tris at pH 7.4, 3% NaCl, 0.2% 0.5M EDTA, and 0.5% 20% NP40. Elutions were performed at room temperature for 15 minutes each using 3x FLAG peptide 25X diluted to 5X with lysis buffer without detergent. The performance of the IPs were verified using standard western blots and silver stains. The western blots were performed on the samples using mouse monoclonal anti-flag M2 880 as the primary antibody (Sigma: F1804) diluted 1:5000 and goat monoclonal anti mouse (LI-COR: 926-32210)

as the secondary antibody diluted 1:10000. Silver stains were performed using the Pierce Silver Stain Kit (ThermoScientific: 24612). Images were processed using LICOR imaging software.

Immunofluorescence Microscopy

Immunofluorescence Microscopy was used to visualize the expression patterns of the effector proteins. The N terminal 3x FLAG epitope tag added to the entry vector during cloning allowed for fluorescent antibodies to bind to the resultant protein. Each antibody fluoresced at a specific wavelength and this allowed for different colors to be observed on the images. Using one color antibody for the protein of interest and a different color for the organelle of interest allowed for distinguishing the localization patterns. From there, it could be determined if the protein of interest colocalized with specific organelles in the cell.

Protocol

HeLa cells were transfected with 500 ng of plasmid DNA at 80% confluency. Cells were grown in DMEM supplemented with 5% HEPES and 10% FBS. Fixing was initiated 24 hours post transfection. Cells were fixed for 10 minutes at room temperature with 4% PFA solution, then washed with PBS. To stain, the cells were blocked for 5 minutes in 50 µl of a milk and saponin solution. The milk and saponin solution was prepared by adding 9.5 ml of 5% powdered milk in PBS and 0.5 ml of 1% saponin in PBS. After blocking, the cells were incubated for 2 hours at room temperature with 50 µl of mouse monoclonal anti-flag M2 880 (Sigma: F1804) as the primary antibody diluted 1:500 in the milk and saponin solution. Cells were washed thrice with PBS before addition of secondary antibody. Cells were incubated in the following secondary antibodies for one hour in the dark, diluted 1:500 in the milk saponin solution. Alexas 488 goat anti-mouse IgG (lifetechnologies: A1101), Alexas 594 goat anti-mouse IgG (lifetechnologies: A11005), and Alexas 647 goat anti-mouse IgG (lifetechnologies: A21235). PDI was used as an

endoplasmic reticulum marker, SFA was used as a Golgi marker, and EEA1 was used as an early endosome marker.

CHAPTER III

RESULTS

GO Terms of SseC

Table 1. E-MAP generated list of GO terms sorted for enrichment with SseC.

GO TERM	Z SCORE SIGNIFICANCE
Golgi apparatus	3.28E-15
Protein transport	5.40E-12
Vesicle-mediated transport	3.34E-08
Endosome	2.22E-06
Cytoplasmic vesicle	2.66E-06
Transport	4.43E-06
Golgi to vacuole transport	9.34E-06
Protein targeting to vacuole	1.55E-05
Endosome membrane	2.60E-05
Retrograde transport, endosome to Golgi	3.16E-05
Intracellular protein transport	3.39E-05
Golgi membrane	4.13E-05

Note: List of GO terms sorted by enrichment with SseC. A more complete list can be found in Appendix C. Smaller Z scores correspond to more significant enrichment, and thus higher probability of SseC function.

These GO terms were generated from the E-MAP. From this list, SseC is predicted to be involved in something related to protein trafficking and transport. With these GO terms in mind, unbiased experiments (IP/MS, IF) were performed in mammalian cells.

Immunoprecipitation of SseC

The MS works by cleaving the bait protein (the effector) and its interacting partners into smaller peptides. These peptides are sent through a machine that records their molecular weights and sequences. The peptide sequences are cross-referenced against a peptide database of whatever organism you specify, and matches appear as an ordered list. The western and silver stain of the samples sent for analysis can be found in Appendix D.

The MS results indicate that SseC interacts with hundreds of eukaryotic proteins, however four specific targets warrant further investigation as shown in Figures 1 and 2.

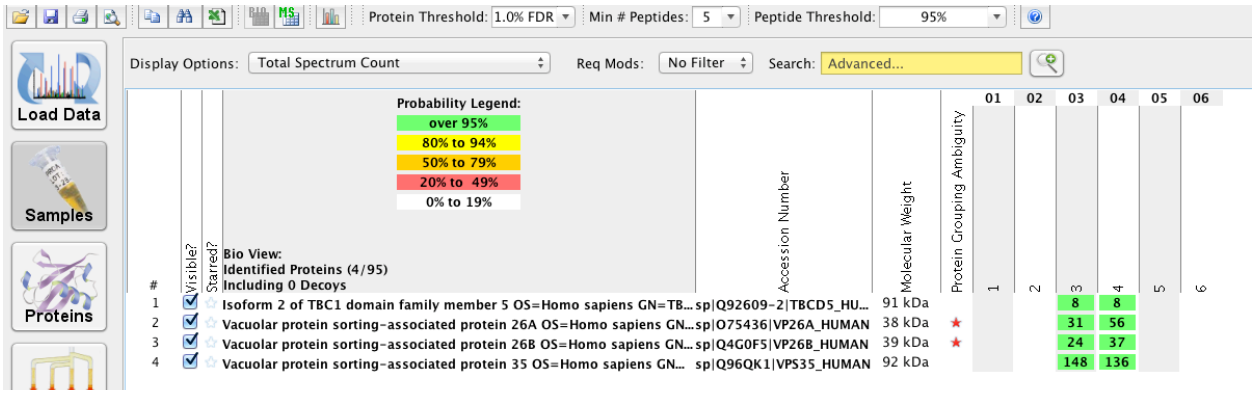


Figure 1: Abbreviated list of SseC IP/MS analysis highlighting the four unique interacting partners. A more detailed account of how to interpret these results can be found in Appendix E.

The IP results of SseC show that SseC interacts with the following four unique partners: Vacuolar protein sorting-associated protein 35 (Vps35), Vacuolar protein sorting-associated protein 26A (Vps26A), Vacuolar protein sorting-associated protein 26B (Vps26B), and Isoform 2 of TBC1 domain family member 5 (Tbc1d5). These proteins were only detected in samples containing SseC.

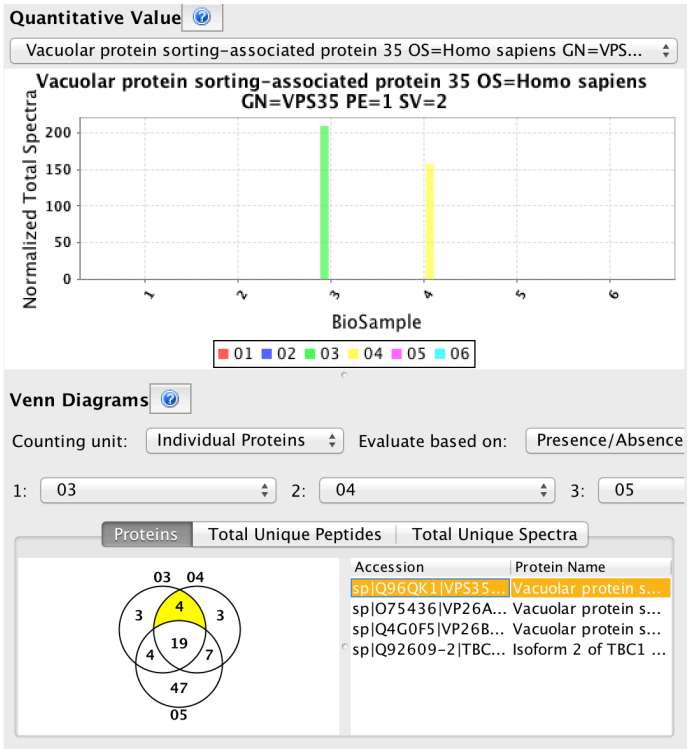
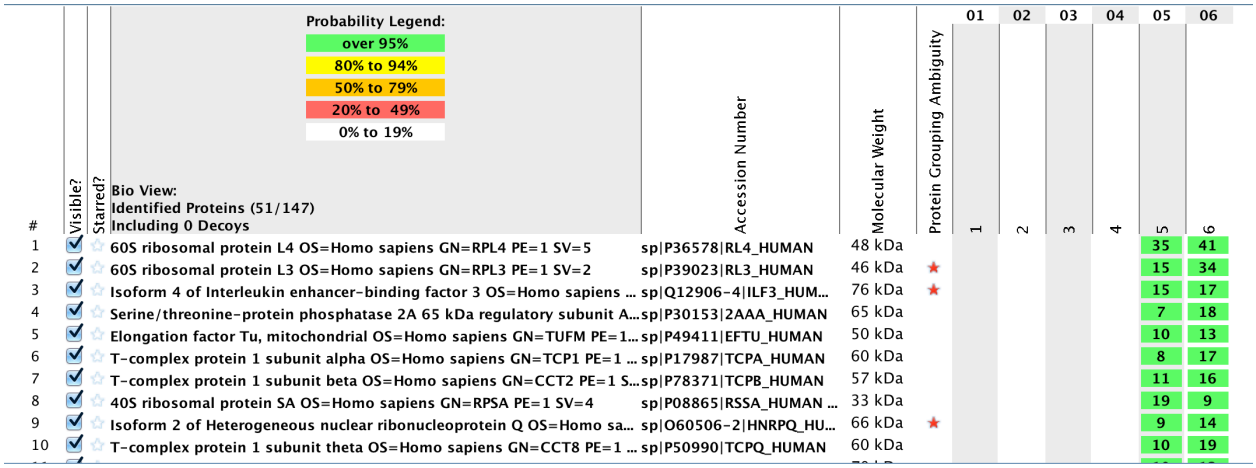


Figure 2: Alternate view of unique interacting partners of sseC. Two proteins on this list were also found in samples containing SteB, so they were excluded from the targeted list.

Vps35 has the highest amount of peptide overlap, meaning SseC and its interacting partners have the most peptides in common with Vps35 according to the MS analysis. Vps26A and Vps26B both have moderate amounts of overlap, and Tbc1d5 has a small amount of unique peptide overlap.

Immunoprecipitation of SteB

The MS results show that there are about 30 unique interacting partners whose locations range from the cytosol to inside the mitochondria to the nucleus as shown in Figures 3 and 4.



Probability Legend:
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

#	Visible?	Starred?	Bio View: Identified Proteins (51/147) Including 0 Decoys	Accession Number	Molecular Weight	Protein Grouping Ambiguity	01	02	03	04	05	06
1	☑	☆	60S ribosomal protein L4 OS=Homo sapiens GN=RPL4 PE=1 SV=5	sp|P36578|RL4_HUMAN	48 kDa						35	41
2	☑	☆	60S ribosomal protein L3 OS=Homo sapiens GN=RPL3 PE=1 SV=2	sp|P39023|RL3_HUMAN	46 kDa	★					15	34
3	☑	☆	Isoform 4 of Interleukin enhancer-binding factor 3 OS=Homo sapiens ...	sp|Q12906-4|ILF3_HUM...	76 kDa	★					15	17
4	☑	☆	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A...	sp|P30153|2AAA_HUMAN	65 kDa						7	18
5	☑	☆	Elongation factor Tu, mitochondrial OS=Homo sapiens GN=TUFM PE=1...	sp|P49411|EFTU_HUMAN	50 kDa						10	13
6	☑	☆	T-complex protein 1 subunit alpha OS=Homo sapiens GN=TCP1 PE=1 ...	sp|P17987|TCPA_HUMAN	60 kDa						8	17
7	☑	☆	T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 S...	sp|P78371|TCPB_HUMAN	57 kDa						11	16
8	☑	☆	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4	sp|P08865|RSSA_HUMAN ...	33 kDa						19	9
9	☑	☆	Isoform 2 of Heterogeneous nuclear ribonucleoprotein Q OS=Homo sa...	sp|O60506-2|HNRPQ_HU...	66 kDa	★					9	14
10	☑	☆	T-complex protein 1 subunit theta OS=Homo sapiens GN=CCT8 PE=1 ...	sp|P50990|TCPQ_HUMAN	60 kDa						10	19

Figure 3: Abbreviated list of SteB interacting partners with the top unique interactions boxed in red. A more detailed account of how to read these results can be found in Appendix E.

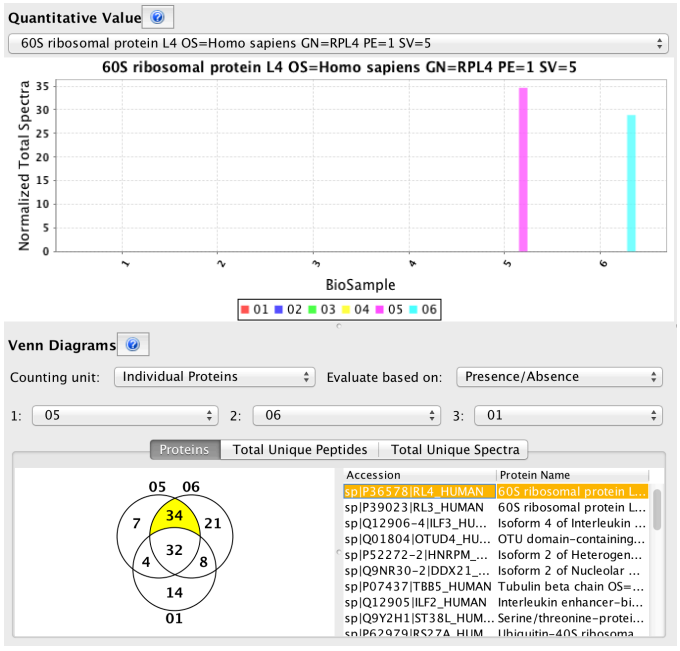


Figure 4: Alternate view of unique interacting partners of SteB. There are significantly more interacting partners than observed in SseC, and the peptide overlap sequences are shorter.

The MS results show that SteB interacts with around 30 unique eukaryotic proteins. However, the peptide sequences that arose from the IP/MS analysis don't correlate highly with specific peptides from the database. The most significant interactions are between SteB and 60S

ribosomal proteins, and heat shock proteins with about 33% of the amino acids matching sequences found in the database.

Immunofluorescence Microscopy

The following images in Figures 5 and 6 were obtained from the Immunofluorescence experiments. IF was performed to observe localization patterns of the *Salmonella* effectors. We predicted that SseG may localize to the Golgi and SseC may localize to early endosomes. To test this, IF costaining was performed by labeling the effectors and specific organelles in HeLa cells.

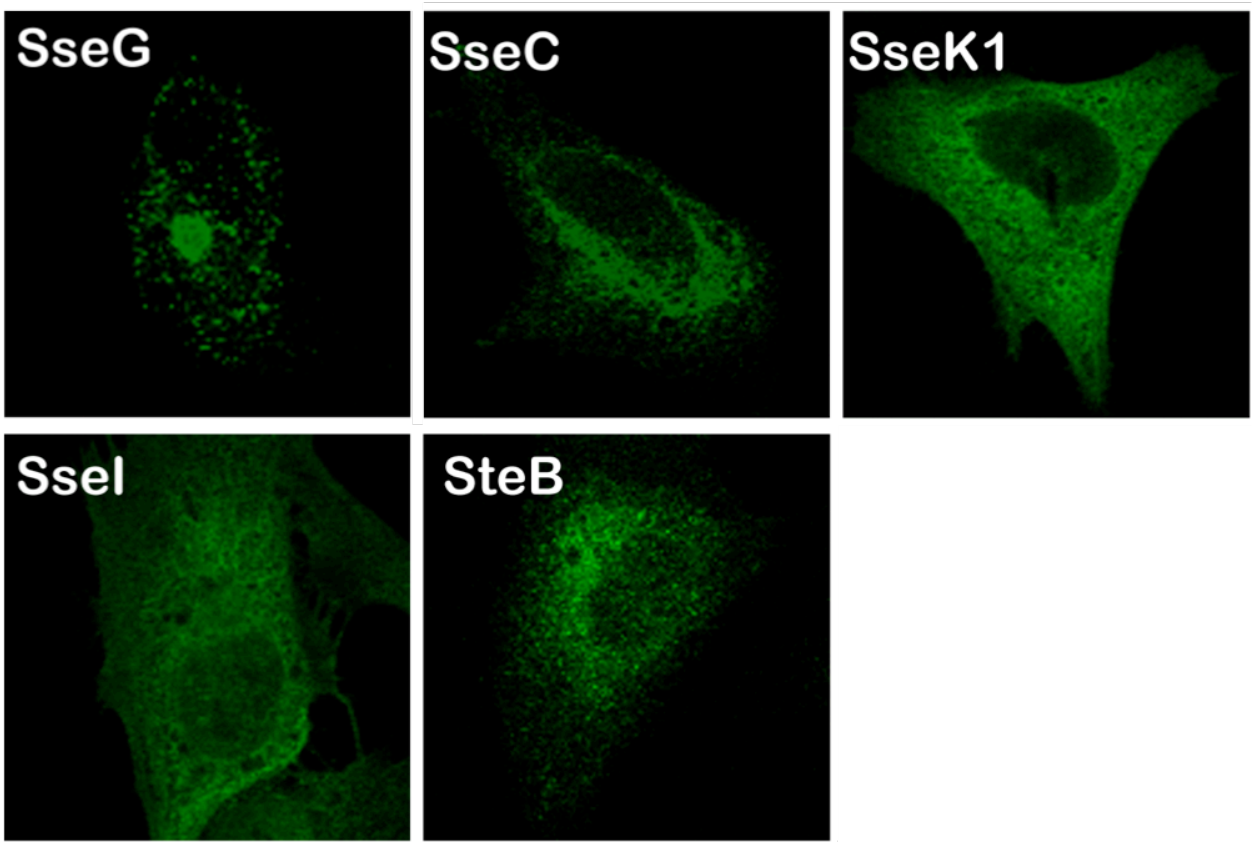


Figure 5: Immunofluorescence of *Salmonella* effectors in HeLa cells. Proteins were stained with fluorescent antibodies against 3x FLAG N-terminus epitope tags 48 hours post transfection.

Overlap was observed with SseG and the Golgi, however overlap was not observed with SseC and the early endosome marker EEA1. Colocalization occurs between SseG and the golgi, but does not occur between SseC and early endosomes.

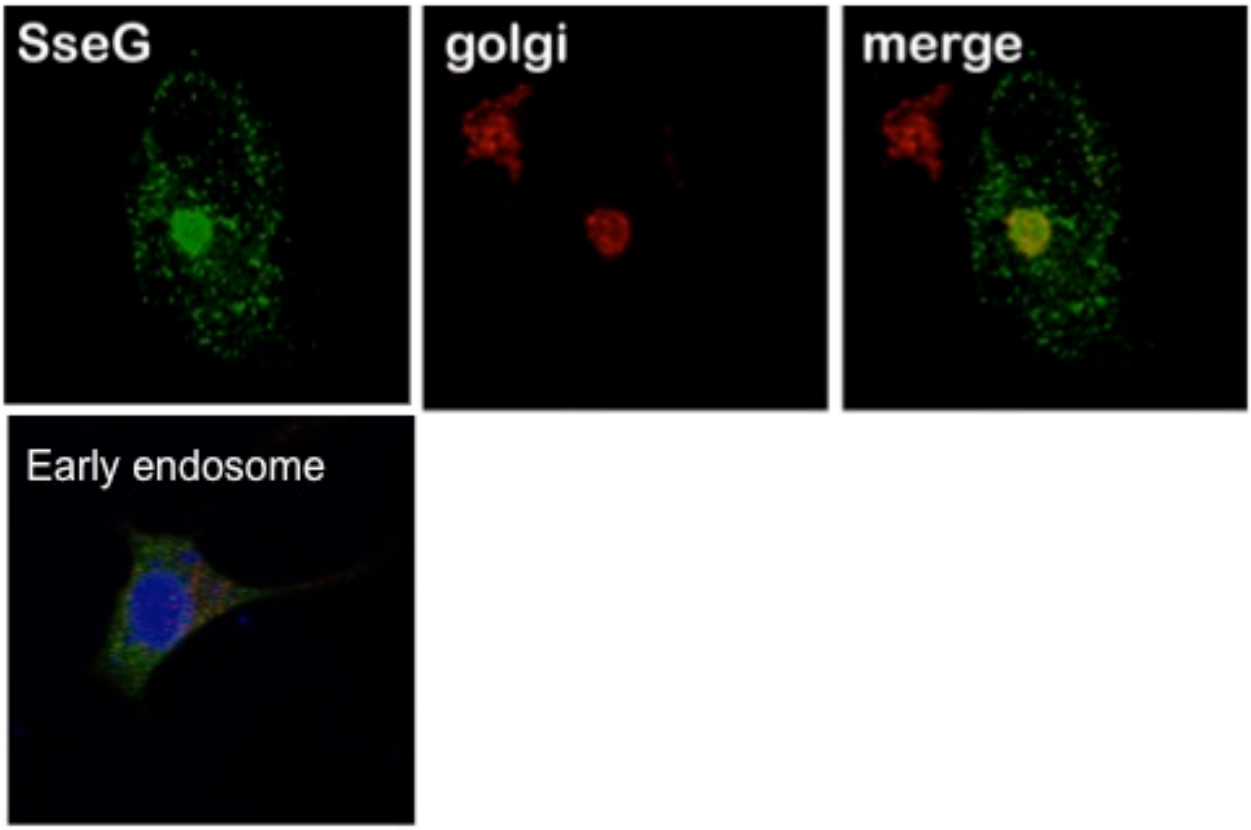


Figure 6: Colocalization of SseG with the Golgi and SseC with early endosomes in HeLa cells. SseG and sseC were stained with green fluorescent antibody against a 3x FLAG GFP N-terminus epitope. Nuclei were stained with DAPI. The Golgi was stained with SAF, and early endosomes were stained with EEA1 (Thermofischer Scientific). Colocalization is shown in yellow.

CHAPTER IV

DISCUSSION

Based on the GO term analysis, the E-MAP predicted that SseC contributes to *Salmonella* infection by interfering with host trafficking processes. To follow up on this hypothesis, an unbiased investigation of protein-protein interactions was initiated in mammalian cells. The GO terms sorted for enrichment with SseC show that retrograde transport and endosome membranes are two of the most significant hits. The retromer is a complex directly involved in retrograde transport. The alignment of the GO terms generated from the E-MAP data with the unbiased approach in mammalian cells indicates that the E-MAP is an efficient and powerful way of providing direction to effector protein investigations.

Immunoprecipitation

The MS data returned a list of SseC's interacting partners. The four unique interacting partners, Vps35, Vps26A, Vps26B, and Tbc1d5 are all involved in a larger protein complex called the retromer [9]. The retromer is a complex that assembles on the endosomal membrane and is involved in retrograde cellular trafficking [9]. Cells can send cargo forwards or backwards through protein trafficking pathways. Normally, cells can recycle proteins and other cargo by sending them from endosomes back to the Golgi. According to the data, SseC immunoprecipitated with 3 subunits of the complex involved in cargo selection (Vps35, Vps26A, and Vps26B) and a GAP responsible for recruitment of the complex to the endosome (TBC1D5). It is currently known that activation of TBC1D5, and by extension the GTPase Rab7, promotes disassembly of the retromer with the endosomal membrane [9]. Further experiments will be needed to determine specific interactions between SseC and the retromer complex.

SteB was shown to interact with proteins in all different components of the cell. Heat shock proteins and ribosomal subunits were found in high amounts. This may be a result of overexpressing SteB for too long. A definitive function of SteB could not be predicted from the MS data.

Immunofluorescence Microscopy

SseG is shown to colocalize with the Golgi, supporting the experiments of Salcedo and Holden [10]. While IP data was not analyzed for SseG, the colocalization provides strong support that SseG is directly involved with trafficking in the Golgi. SseK1, SseI, and SopD do not show distinct localization patterns. SseC doesn't appear to colocalize with early endosomes, so a possible hypothesis might be that SseC is promoting disassembly of the complex with the endosomal membrane.

CHAPTER V

CONCLUSION

Highly specific GO terms generated from a high throughput genetic screen in *Saccharomyces cerevisiae* resulted in terms that were sorted for enrichment with each *S. typhimurium* effector protein. To determine the validity of these proposed GO terms, unbiased experiments were conducted in 293T cells. Through IP/MS, it was determined that one effector, SseC, interacts with the retromer complex. The retromer is directly involved in host retrograde transport. This interaction provides support for the GO term analysis, as retrograde transport is listed as a GO term with high enrichment for SseC. Moving forward, this finding gives us confidence that the E-MAP is a powerful way to begin investigations into bacterial effector protein function. E-MAPs cannot only be used to explain host/pathogen interactions, but they may also uncover new host cell biology along the way. The results of these experiments are not limited to one organism- these methods can translate to many bacterial pathogens.

FUTURE DIRECTIONS

We hope to gain a better understanding of exactly how SseC is interacting with the retromer during infection. We will begin infection studies of wild type and sseC mutant *Salmonella* in mammalian cells to determine differences in *Salmonella* infection capabilities. We are also creating knockdown of retromer components, and will begin directed immunoprecipitation experiments to further clarify which retromer components SseC is interacting with. Furthermore, we have plans to observe SseC and retromer localization patterns at different time points during *Salmonella* infection.

As well as these more directed experiments involving SseC, we will be continuing investigation into 5 other effectors – SseG, SseI, SseK1, SteB, and SopD. In addition, further E-MAPs will be used to investigate the effectors of other pathogenic bacteria such as *Coxiella* and *Brucella*.

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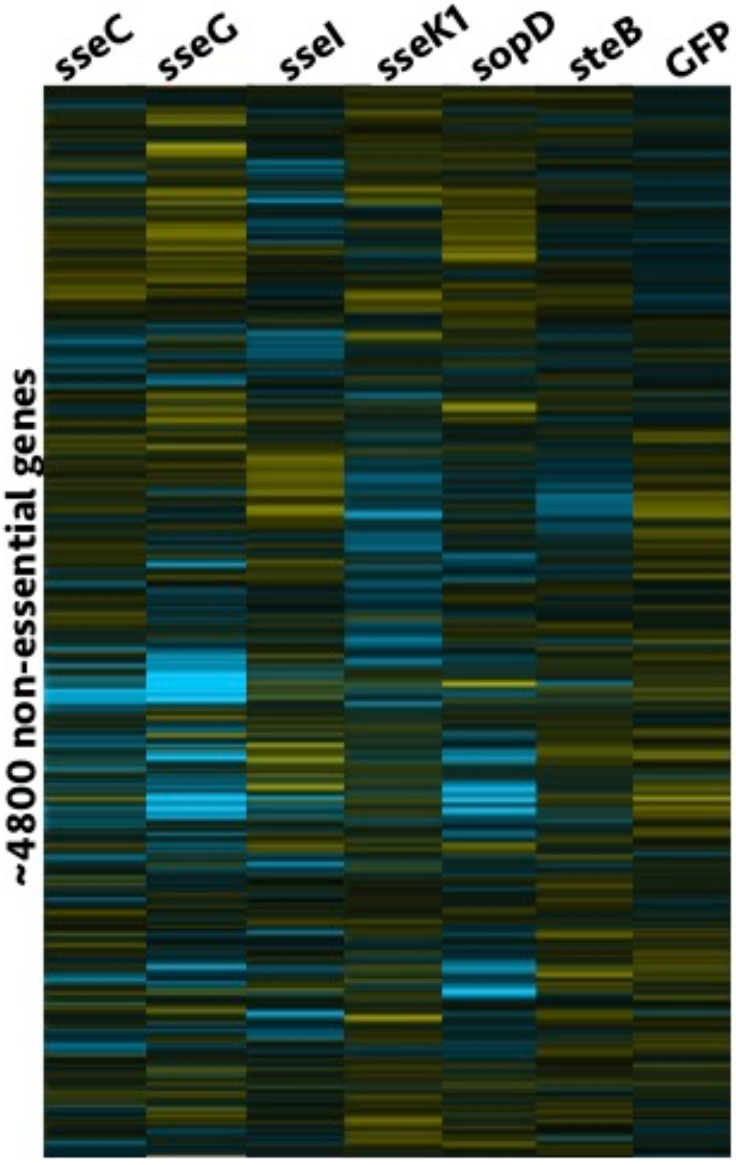
APPENDIX A

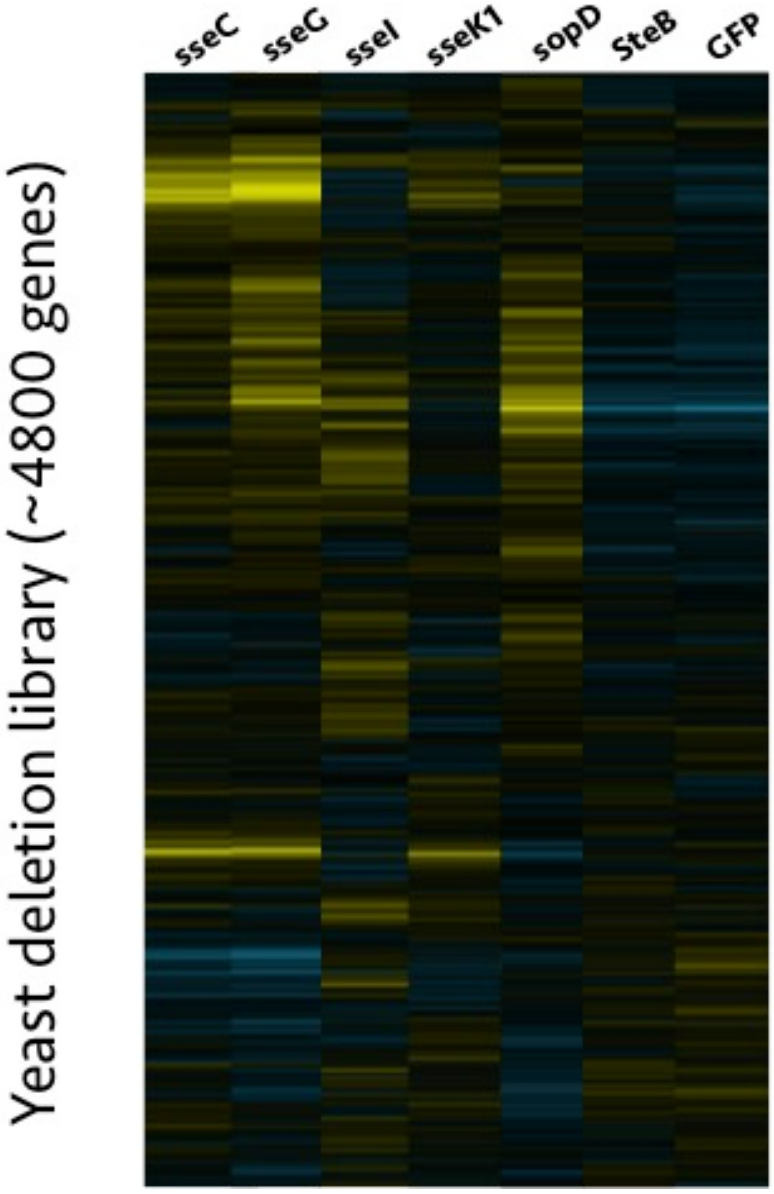
PAIRWISE INTERACTIONS E-MAP



Appendix A: Pairwise interactions of yeast double mutants. Double mutants were created by crossing yeast expressing each *Salmonella* effector with single deletion mutants of 4800 non-essential genes. Yellow represents positive genetic interactions, blue represents negative genetic interactions, and black represents neutral interactions. GFP was used as a negative control. Courtesy of Jason Wojcechowskyj, Krogan Lab, UCSF.

APPENDIX B

INTERACTION PROFILES OF EFFECTORS



Appendix B: Interaction profiles of effectors. Interaction profiles were created by comparing the profiles of each effector to profiles of single deletion mutants of 4800 non essential genes in *Saccharomyces cerevisiae*. Positively correlated profiles are yellow and negatively correlated profiles are blue. Very weakly correlated or uncorrelated profiles are black. GFP was used as a negative control. Courtesy of Jason Wojcechowskyj, Krogan Lab, UCSF.

APPENDIX C

GO TERMS OF SSEC

A	O
term	sseC
Golgi apparatus	3.28E-15
protein transport	5.40E-12
vesicle-mediated transport	3.34E-08
endosome	2.22E-06
cytoplasmic vesicle	2.66E-06
transport	4.43E-06
Golgi to vacuole transport	9.34E-06
protein targeting to vacuole	1.55E-05
endosome membrane	2.60E-05
retrograde transport, endosome to Golgi	3.16E-05
intracellular protein transport	3.39E-05
Golgi membrane	4.13E-05
posttranslational protein targeting to memb	0.0001165
vesicle fusion with Golgi apparatus	0.0001165
AP-3 adaptor complex	0.00019869
intra-Golgi vesicle-mediated transport	0.00021312
vesicle fusion	0.00021312
endoplasmic reticulum	0.00032782
ER to Golgi transport vesicle membrane	0.0003295
sterol biosynthetic process	0.00039338
SNAP receptor activity	0.00066991
ER to Golgi vesicle-mediated transport	0.00067071
CVT pathway	0.00083576
SNARE complex	0.000853
TRC complex	0.00093971

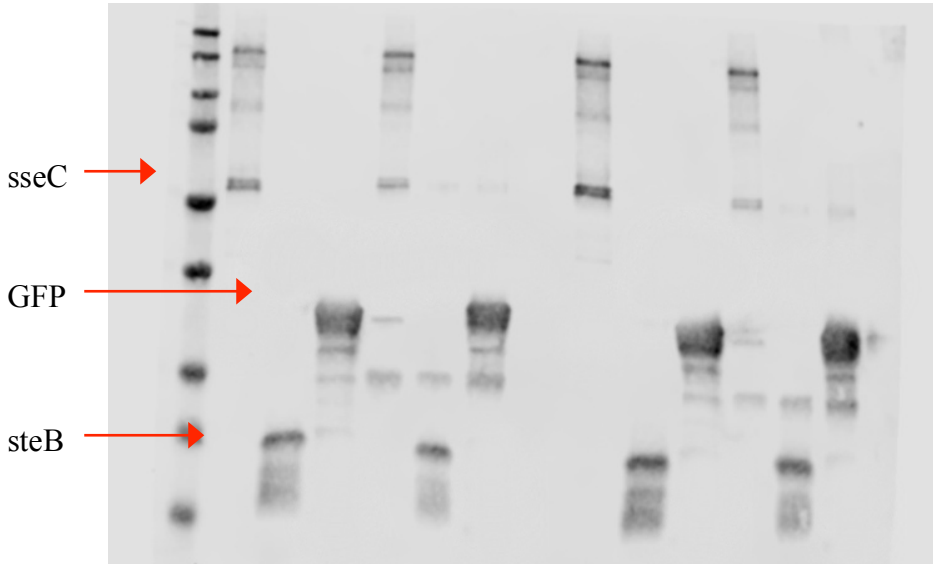
Z-scores

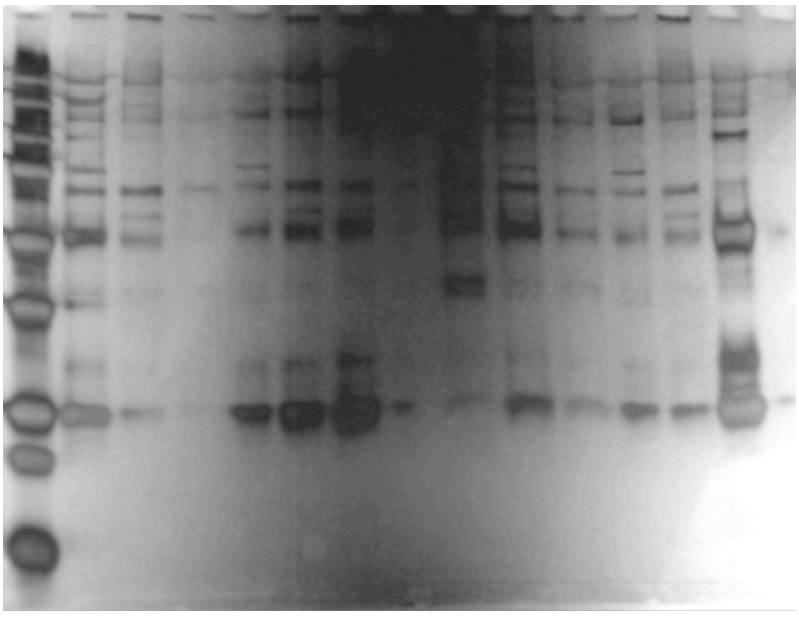
Appendix C: Gene Ontology terms sorted by significance of enrichment with the *Salmonella* effector protein SseC.

The more significant GO terms are shown in red, and the less significant GO terms are shown in green.

APPENDIX D

WESTERN BLOT AND SILVER STAIN OF MS-IP





Appendix D: Western blot and silver stain of samples sent for analysis. With the epitope tag used for cloning, SseC is 55 kDa, GFP is 35 kDa, and SteB is 20 kDa. Lane 1, protein ladder; Lane 2, SseC elution 1; Lane 3, SteB elution 1; Lane 4, GFP elution 1; Lane 5, SseC beads 1; Lane 6, SteB beads 1; Lane 7, GFP beads 1; Lanes 9-14, biological replicate.

APPENDIX E

INTERPRETATION OF MS RESULTS

Probability Legend:
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

Bio View:
Identified Proteins (129/147)
Including 0 Decoys

#	Visible?	Starred?		Accession Number	Molecular Weight	Protein Grouping Ambiguity	01	02	03	04	05	06
							1	2	3	4	5	6
1	☑	☆	Vacuolar protein sorting-associated protein 35 OS=Homo sapiens GN...	sp|Q96QK1|VPS35_HUMAN	92 kDa				148	136		
2	☑	☆	Heat shock 70 kDa protein 1A OS=Homo sapiens GN=HSPA1A PE=1 SV...	sp|P0DMV8|HS71A_HUM...	70 kDa	★	63	38	95	91	105	128
3	☑	☆	Kinesin-like protein KIF11 OS=Homo sapiens GN=KIF11 PE=1 SV=2	sp|P52732|KIF11_HUMAN	119 kDa		35	24	46	86	99	88
4	☑	☆	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 ...	sp|P11021|GRP78_HUMAN	72 kDa	★	8		33	38	30	29
5	☑	☆	SWISS-PROT:P02768-1 Tax_Id=9606 Gene_Symbol=ALB Isoform 1 of S...	P02768-1 (+1)	69 kDa	★	39	31	32	19	21	32
6	☑	☆	Vacuolar protein sorting-associated protein 26A OS=Homo sapiens GN...	sp|O75436|VP26A_HUMAN	38 kDa	★			31	56		
7	☑	☆	Protein phosphatase 1B OS=Homo sapiens GN=PPM1B PE=1 SV=1	sp|O75688|PPM1B_HUMAN	53 kDa	★	41	19	28	41	63	78
8	☑	☆	Methylosome protein 50 OS=Homo sapiens GN=WDR77 PE=1 SV=1	sp|Q9BQA1|MEP50_HUM...	37 kDa	★	44	25	26	37	45	50
9	☑	☆	Vacuolar protein sorting-associated protein 26B OS=Homo sapiens GN...	sp|Q4G0F5|VP26B_HUMAN	39 kDa	★			24	37		
10	☑	☆	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1	sp|P63261|ACTG_HUMAN	42 kDa	★	34	6	15	6	23	24
11	☑	☆	Isoform Short of Heterogeneous nuclear ribonucleoprotein U OS=Hom...	sp|Q00839-2|HNRPU_HU...	89 kDa		33	23	9	16	55	64
12	☑	☆	Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A ...	sp|P62979|RS27A_HUMAN	18 kDa				8	8	4	5
13	☑	☆	Isoform 2 of TBC1 domain family member 5 OS=Homo sapiens GN=TB...	sp|Q92609-2|TBCD5_HU...	91 kDa				8	8		
14	☑	☆	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subu...	sp|P04844|RPN2_HUMAN	69 kDa				8			

Appendix E: Abbreviated MS list of proteins that interact with each sample. The top 14 results are displayed, but the complete list includes nearly 150 proteins. Samples 01 and 02 were processed from cells overexpressing GFP. Samples 03 and 04 were processed from cells overexpressing SseC. Samples 05 and 06 were processed from cells overexpressing SteB. The green boxes indicate that the probability of a peptide correctly matching with the database is extremely accurate, and the numbers in the boxes correspond to the number of amino acids that aligned with the given database peptide. Larger numbers suggest more significant interactions between the bait and its interacting partners. Keratin and trypsin were removed from the list for clarity.