AWARD NUMBER: W81XWH-21-1-0785

TITLE: The STING Paradox in Serous Ovarian Cancers

PRINCIPAL INVESTIGATOR: Dr. Dawn Cochrane, PhD

CONTRACTING ORGANIZATION: BC Cancer, Provincial Health Services Authority

REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2023	Annual	15SEPT2022 - 14SEPT2023

4. TITLE AND SUBTITLE

The STING Paradox in Serous Ovarian Cancers

5a. CONTRACT NUMBER
W81XWH-21-1-0785

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Dawn Cochrane, PhD
E-Mail: dcochrane@bccrc.ca

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

BC Cancer
675 West 10th Ave,
Vancouver BC, V5Z 1L3

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Marvland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Low grade serous ovarian cancer (LGSC), a rare subtype of ovarian cancer is characterized by a slow growth rate, resistance to treatment regimens, multiple recurrences and poor survival. The stimulator of interferon genes (STING), is a receptor which initiates a signaling cascade that results in an inflammatory response to cytoplasmic DNA. LGSC express high levels of STING compared to other ovarian cancers, but low levels of downstream proteins, suggesting an inactive STING pathway. We set out to characterize the defective STING signaling and whether the lack of an active STING pathway renders these tumors particularly sensitive to oncolytic viral therapy. Using proteomic profiling, we found STIM1 is more highly expressed in LGSC compared to other types of ovarian cancer. High STIM1 can anchor STING in the endoplasmic reticulum, thus preventing signaling. We have tested several oncolytic viruses and determined that the SKV vaccinia virus has superior killing compared to other vaccinia strains. Additionally, we mined our proteomic data to understand the biological differences between LGSC and its precursor lesion, serous borderline tumor (SBT). Fibroblast activation protein (FAP), a protein expressed in cancer associated fibroblasts, is abundantly expressed in LGSC. Tregs and M2 macrophages are more abundant in the stroma of LGSC compared to SBT. Together these data suggest that the tumor microenvironment provides a supportive environment for LGSC tumorigenesis and progression and that targeting the tumor microenvironment of LGSC may be a viable therapeutic strategy.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	18	

TABLE OF CONTENTS

1. INTRODUCTION

Low grade serous ovarian cancer (LGSC) is a rare subtype of ovarian cancer, characterized by a slow growth rate, resistance to current treatment regimens, multiple recurrences and poor survival. The stimulator of interferon genes (STING), is a receptor which initiates a signaling cascade that results in the transcription of type I interferon (IFN) genes and inflammatory cytokines in response to cytoplasmic DNA. LGSC consistently express high levels of STING compared to other ovarian cancers, however there are low levels of downstream proteins, suggesting that the STING pathway is inactive in LGSCs. We set out to determine in where the defect lays in the STING pathway and whether we could restore STING signaling to LGSC cell lines. Furthermore, we hypothesized that defective STING signaling renders LGSC particularly sensitive to oncolytic viral therapy. We tested several types of oncolytic viruses on LGSC to determine viral efficacy. These are the first pre-clinical studies using oncolytic viruses to treat this type of tumor and could pave the way to clinical trials.

2. KEYWORDS

Low grade serous ovarian cancer
STING
Interferon
Oncolytic virus

3. ACCOMPLISHMENTS

Major goals of the project (as per SOW):

Major Task 1 Characterization of cGAS/STING pathway proteins and phosphorylation events in LGSC
Major Task 2 Restoration of STING signaling to LGSC cell lines
Major Task 3 Determine whether LGSC cell lines are sensitive to oncolytic viruses
Major Task 4 Determine whether oncolytic virus treatment of LGSC can activate immune cells.

Accomplishments under these goals

Major Task 1 Characterization of cGAS/STING pathway proteins and phosphorylation events in LGSC
Subtask 1 – Optimization of isodoping peptides for proteomics (Months 1-2).
STATUS: Complete (detailed on previous technical report)

Subtask 2 - Proteomic profiling of 21 each LGSC, high grade serous (HGSC) and serous borderline ovarian tumor (SBT) tumors. (Months 3-12)
We performed proteomic profiling on HGSC, LGSC and SBT samples, separating the SBT into classical SBT, and micropapillary SBT (mSBT). The significance of the mSBT is that it is in indication of a more invasive for of SBT. Global proteomic analysis of our 56 samples identified 7,507 unique proteins, and 5,826 (77.6%) proteins detected across all samples. We assessed the spread and clustering of the samples based on their log2 protein abundance using principal component analysis (PCA) and *k*-means clustering (Fig. 1a). The unsupervised centroid-based clustering suggests that the variance captured by PCA largely separates samples by histotype. The first cluster consists solely of HGSC (n = 14/19) tumours. LGSC (n = 9/11) is the main histotype in the second cluster, accompanied by HGSC (n = 3/19) and SBT (n = 1/19). Cluster 3 is primarily defined by putative precursors of LGSC, both SBT (n = 18/19) and mSBT (n = 7/7).

We further characterized the global proteomic landscape of our samples by performing unsupervised hierarchical clustering on the top 25% variable proteins (n = 1,090), determined by median absolute deviation (Fig. 1b). The generated dendrogram collapsed into 3 clusters, determined by visual inspection of the consensus matrix, item consensus, and relative change in area under the cumulative distribution function curve (Supplementary Fig. 2). The first cluster captured most HGSC (n = 16/19) samples, accompanied by a single mSBT (n = 1/7). The second cluster is marked by the putative LGSC precursors, SBT (n = 18/19) and mSBT (n = 5/7). The third cluster contains all LGSC samples (n = 11/11) and the remaining SBT (n = 1/19) and mSBT (n = 2/7) samples.

Using the semi-targeted approach for proteomics, where we spiked in peptides related to the STING pathway, we found a protein called STIM1 to be higher in LGSC compared to HGSC (Fig 2a). STIM1 is a protein that resides in the endoplasmic reticulum and when levels are high enough, it can anchor STING and prevent signaling. This was validated using immunohistochemical (IHC) staining of a tissue microarray (TMA) containing different ovarian cancer histotypes (Fig 2b). Western blotting of LGSC cell lines further confirms that LGSC express high levels of STIM1 in comparison to their STING levels (Fig 2c). Conversely the HGSC cell line has higher levels of STING compared to STIM1. We propose that the high STIM1 levels in LGSC is preventing STING from translocating into the golgi and effectively signaling.

We further mined our proteomic data to determine if there were any proteins that could further our understanding of LGSC biology. We found that the biggest difference between LGSC and HGSC or LGSC and its precursor, SBT, lies in proteins involved in the tumor microenvironment (TME), including fibroblast activation protein (FAP) (Fig 3a) and proteins in inflammation pathways (Fig 3b). Since the TME is important for response to oncolytic viral therapy, we investigated this further.

We performed IHC for FAP on TMAs for the proteomic, as well as independent validation cohorts. FAP is solely observed in the stromal compartment of LGSC cores (Fig. 4a). Borderline tumors commonly lack FAP staining in their fibrovascular core (Fig. 4a). In our validation cohort (“Stanford cohort”), all LGSC cores were positive in the stromal compartment, consistent with the approximately 90% observed in the proteomic cohort (Fig. 4b).

Since the proteomics suggested an inflammatory response in LGSC, we performed multiplex IHC for immune cell markers, including CD8+ T cells (CD3+/CD8+), CD8- T cells (CD3+/CD8-), macrophages (CD68+), B cells (CD20+/CD79A+), and plasma cells (CD20-/CD79a+). The stromal compartment of LGSC exhibited a significantly higher density of macrophages and CD8- T cells compared to SBT (Fig. 5a, b). The stromal area of LGSC demonstrated higher levels of these cells compared to the corresponding tumor area (Fig. 5c, d).

We further explored the distribution of immunosuppressive macrophage and CD8- T cell subtypes. Strikingly, the stroma of LGSC exhibited a higher proportion of these immunosuppressive subtypes, specifically M2-polarized macrophages (CD163+ macrophages) and regulatory T cells (Tregs) (Fig. 6a-f).

STATUS: Complete

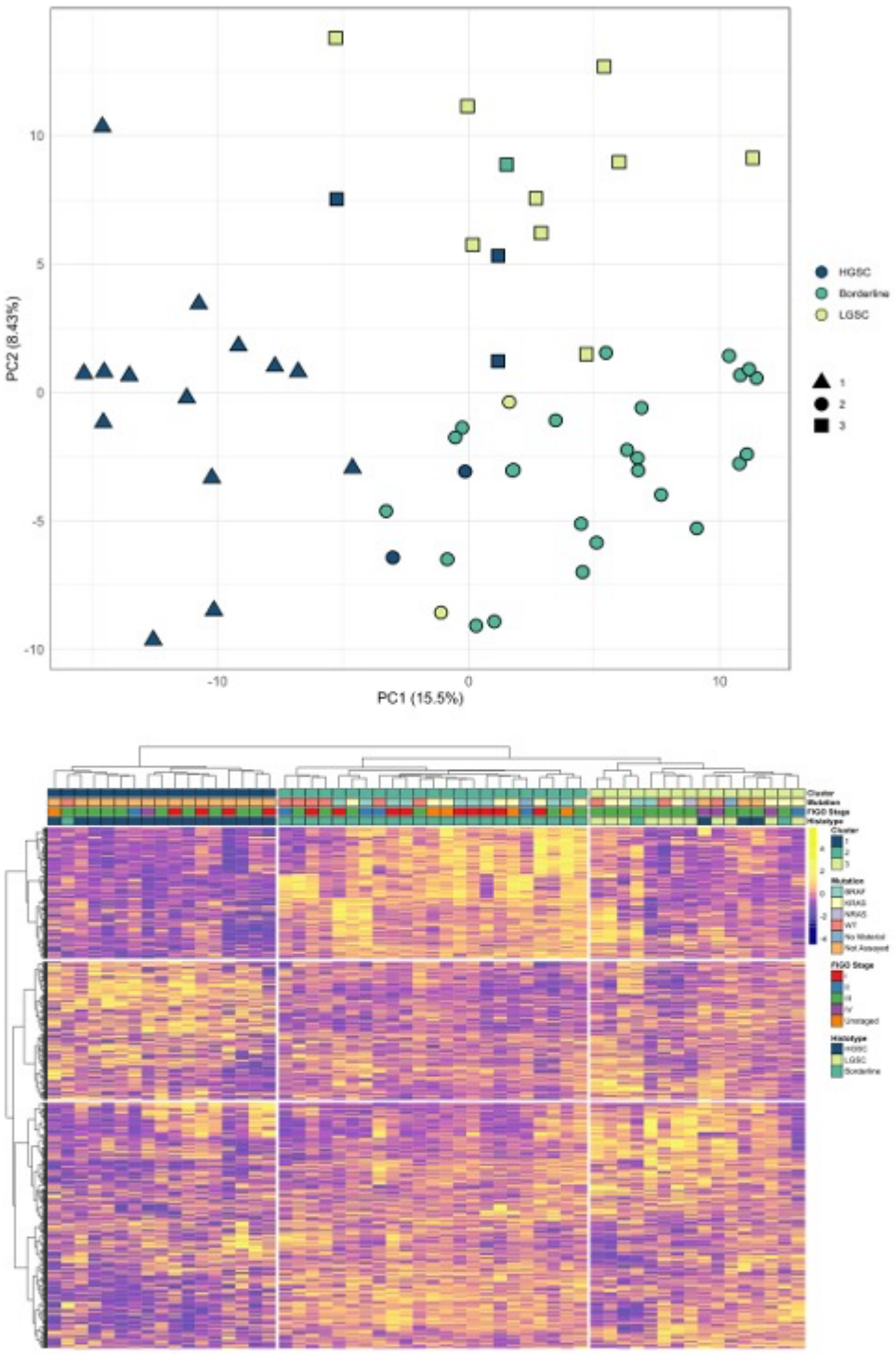

Figure 1. Clustering of proteomics samples. Our proteomic cohort from 56 FFPE archival tissue blocks, constituting HGSC (n = 19), LGSC (n = 11), micropapillary SBT (mSBT; n = 7), and SBT (n = 19). **a)** Principal component analysis (PCA) and *k*-means clustering of the samples based on their log2 protein abundance. The unsupervised centroid-based clustering suggests that the variance captured by PCA can separate the samples by histotype, or closest etiological histotype. **b)** unsupervised hierarchical clustering on the top 25% variable proteins (n = 1,090), determined by median absolute deviation. The first cluster captured most HGSC (n = 16/19) samples, accompanied by a single mSBT (n = 1/7). The second cluster is marked by the putative LGSC precursors, SBT (n = 18/19) and mSBT (n = 5/7). The third cluster contains all LGSC samples (n = 11/11) and the remaining SBT (n = 1/19) and mSBT (n = 2/7) samples.

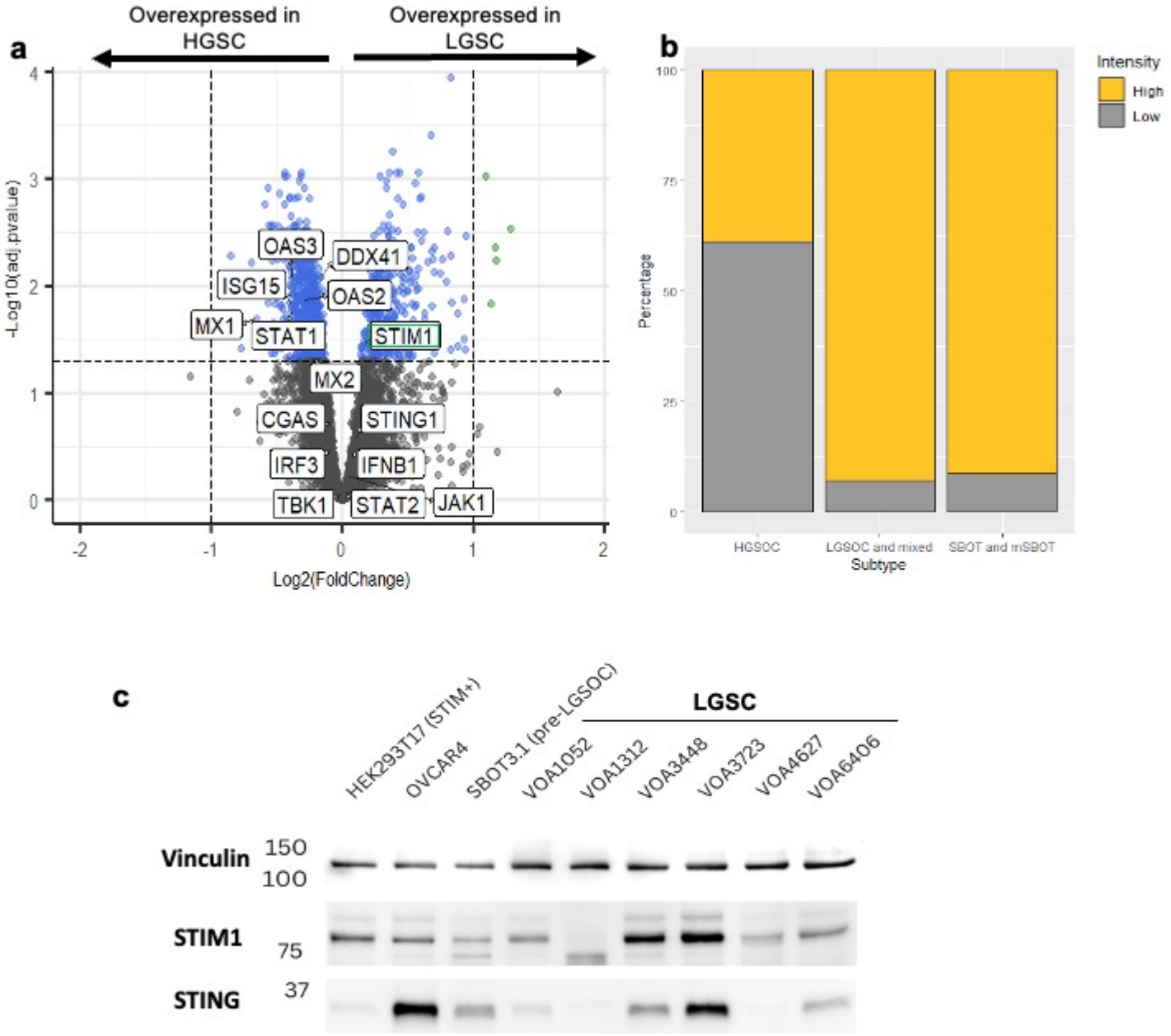

Figure 2. STIM1 is highly expressed in LGSC. a) Volcano plot of differential protein abundance in LGSC and HGSC from semi-quantitative proteomic analysis. Labeled proteins were targeted for mass spectrometry analysis using spiked in peptides. STIM1 is highlighted in green. **b)** Immunohistochemistry (IHC) for STIM1 was performed on a tissue microarray (TMA) containing different ovarian cancer histotypes. The intensity was scored as low (negative or weak staining) or high (moderate or intense staining). LGSC and SBT tumors express high levels of STIM1. c) Western blot for STING and STIM1 in HGSC line (OVCAR4), SBOT and LGSC lines. The ratio of STIM1 to STING is higher in LGSC lines than in HGSC.

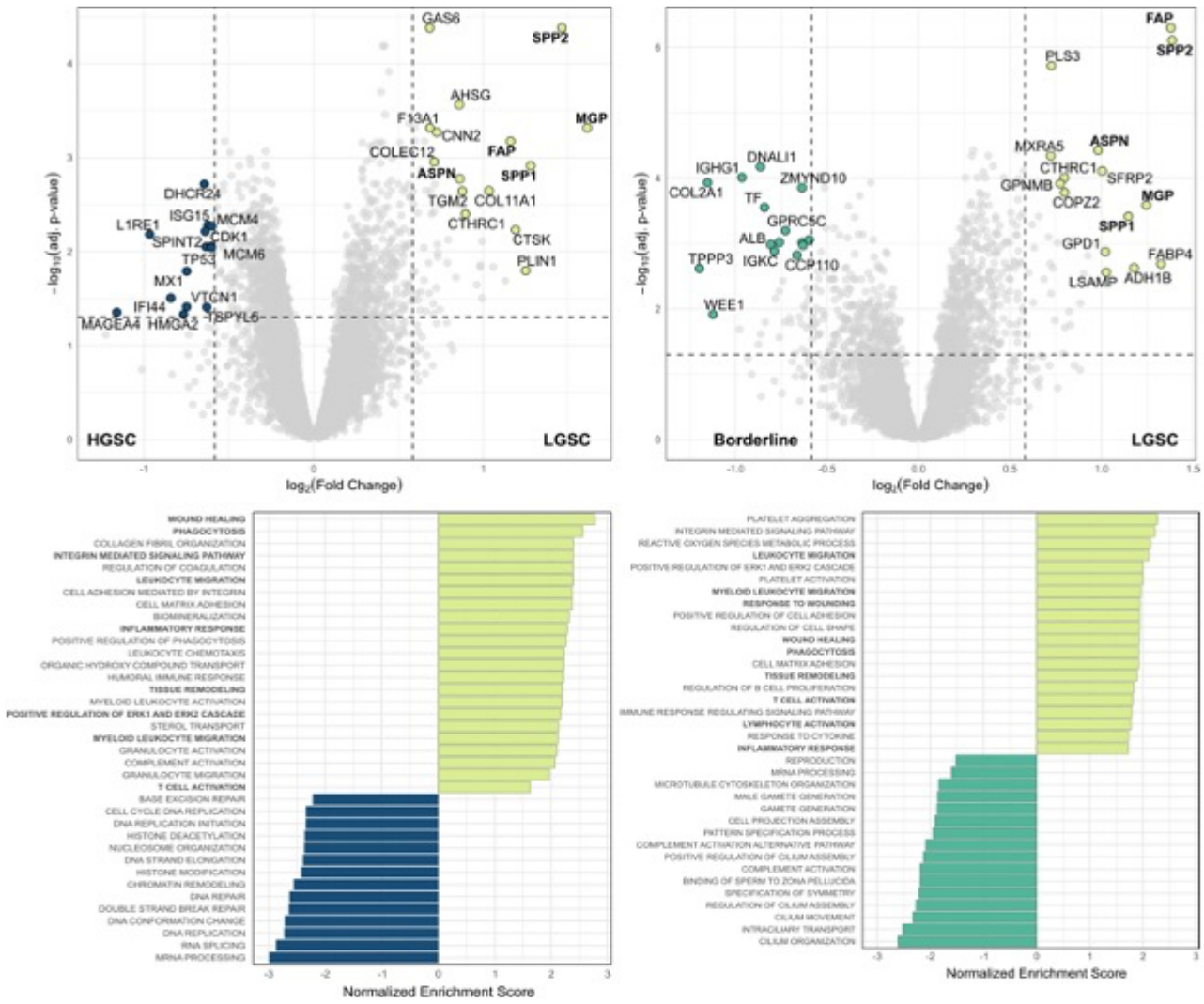

Figure 3. Differential protein expression and pathway analysis of proteomics data. Volcano plots demonstrating relative protein abundance of LGSC compared to HGSC (left) and SBT (right). GSEA pathway analysis was performed. Pathways involved in inflammation are highlighted.

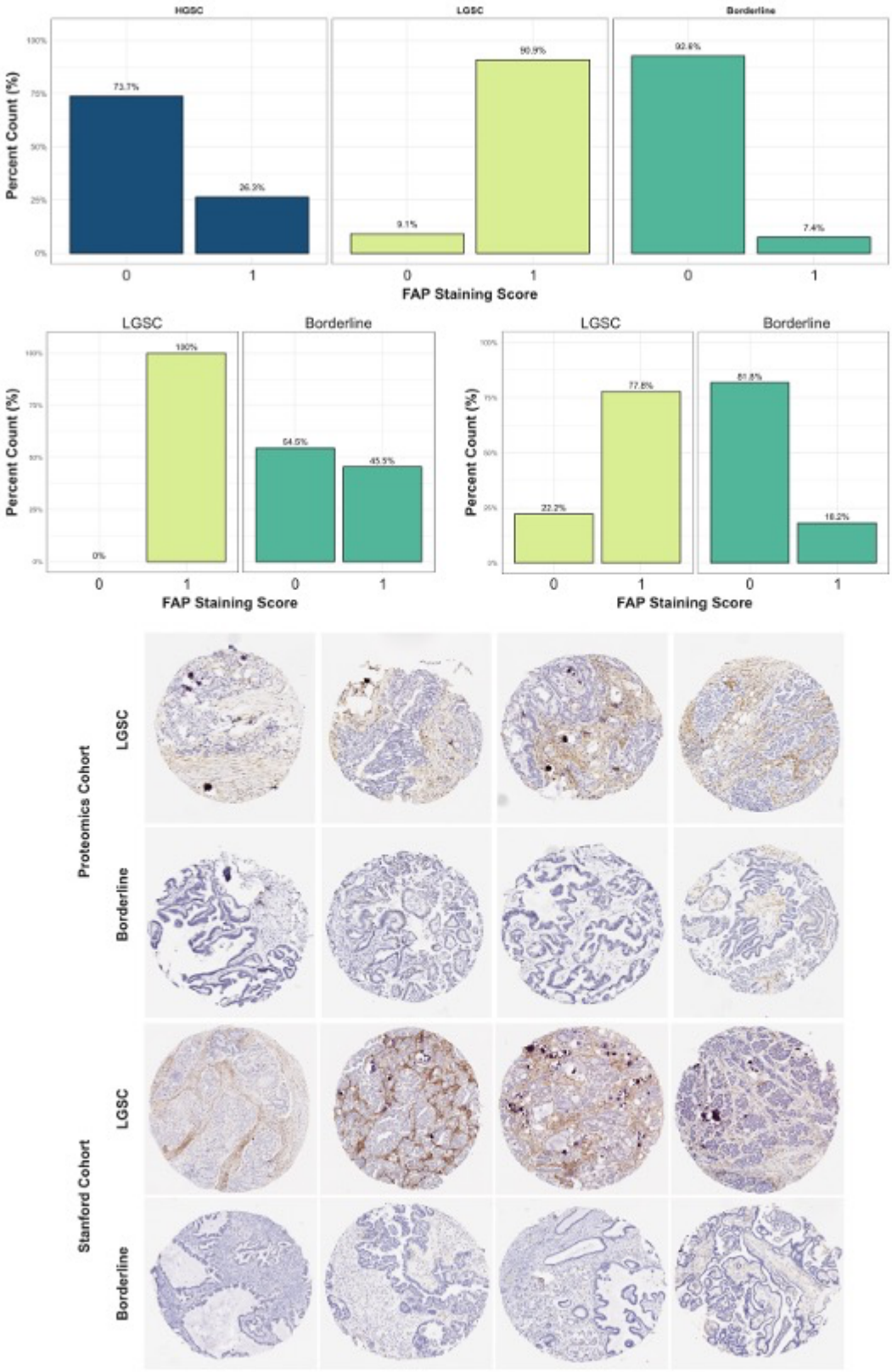

Figure 4. FAP is highly expressed in the stroma of LGSC tumors. TMAs for the proteomic cohort (top) and validation cohorts (below) were stained for fibroblast activation protein (FAP). LGSC expresses FAP in the stroma, while SBT and HGSC less frequently express FAP. Stromal expression of FAP was scored as 0 for negative or 1 for positive. Below are representative images of staining.

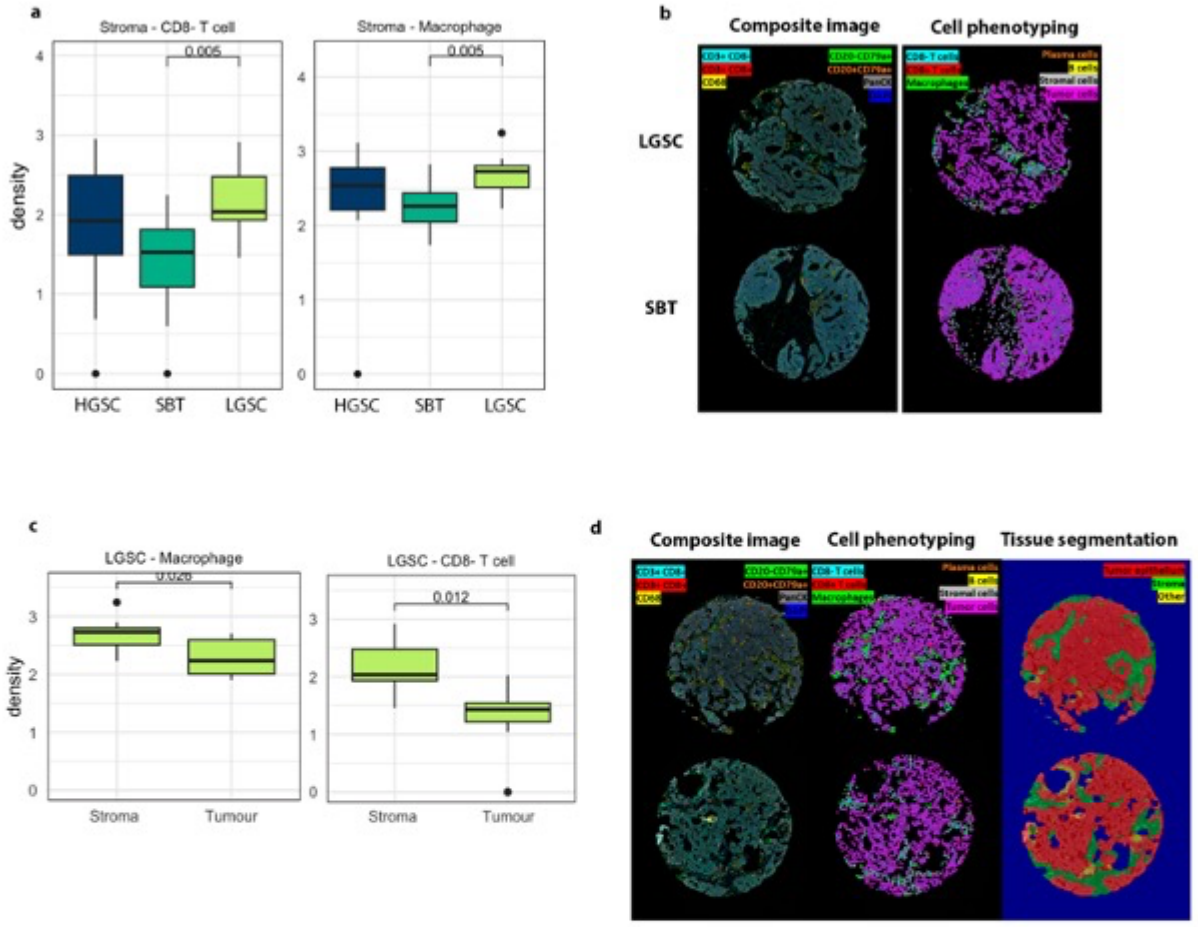

Figure 5. High levels of T cells and macrophages in the LGSC stroma. Multiplex IHC was performed on a TMA of the samples in the proteomic cohort. **a)** Higher levels of CD8- T cells and macrophages are found in the stroma of LGSC compared to SBT. Representative image of multiplex IHC is shown. **b)** The relative levels of macrophages and CD8- T cells are higher in the stroma of LGSC compared to the tumor. Representative image of the multiplex IHC, with the tumor and stroma masking, are shown.

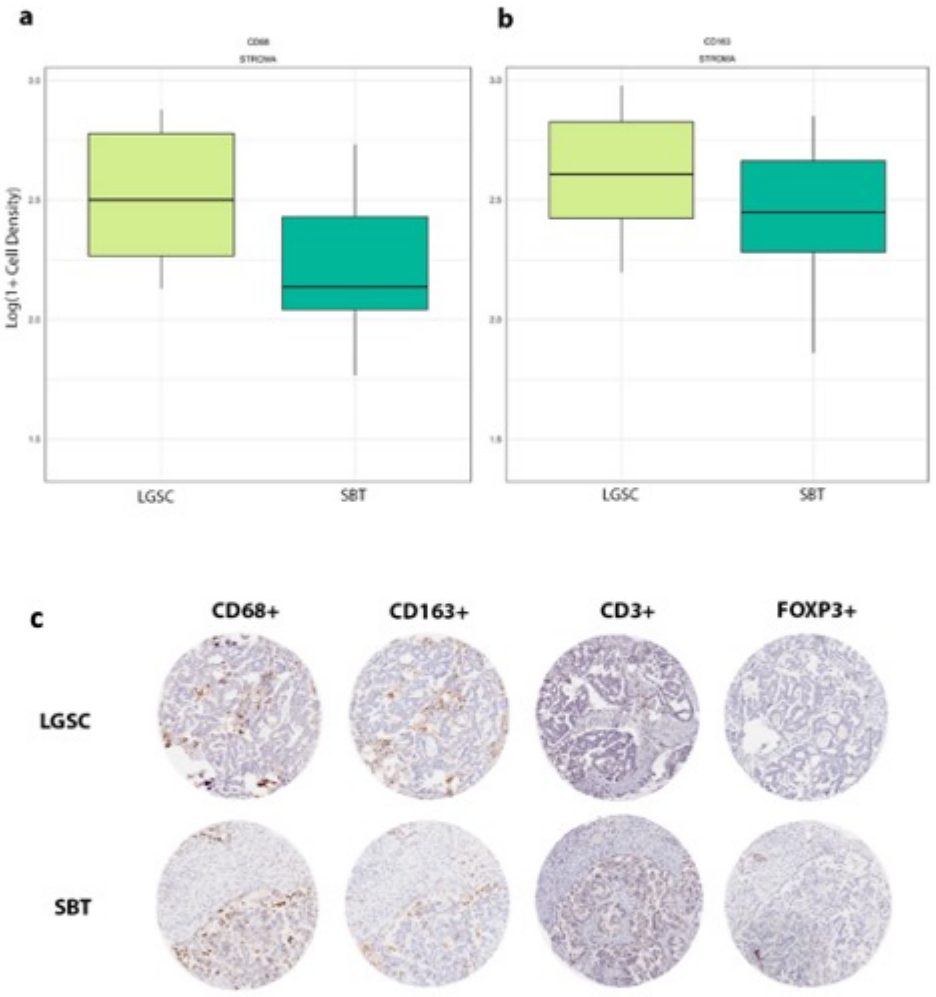

Figure 6. Tregs and M2 macrophages are higher in LGSC than in SBT. Quantification of **a)** CD68 and **b)** CD163 positive cells in LGSC and SBT shows higher levels of M2 macrophages (CD68+, CD163+) in LGSC. Similar results were observed for Tregs (CD3+, FOXP3+, quantification not shown) **c)** Representative images are shown below.

Subtask 3 – Proteomic profiling of 3 LGSC cell lines treated with and without STING agonist (dsDNA90).

As we believe that it would be more informative to do profiling on LGSC cell lines +/- STIM1 knock down in the presence or absence of dsDNA90, we have modified our original SOW to reflect this.

STATUS: In progress. We are currently developing the STIM1 knock out lines. We anticipate that this will be complete in 6 months.

Subtask 4 – Western blots on 3 LGSC cell lines treated with STING agonist (dsDNA90).

In this subtask we sought to figure out where the defect in STING signaling lies. We first treated LGSC cell lines with dsDNA90 a STING agonist and performed qPCR for IFN response genes. As a positive control, we included HGSC cell lines (OVCAR4 and OVCAR5) which have intact STING signaling. As a negative control, we included HEK293, which are negative for STING signaling. The cells were treated with polyIC, which is a double stranded RNA that activates the RIG pathway, which also produces an interferon response, without signaling through STING. All cell lines produced IFN gene response when treated with polyIC, indicating that there is no defect in the transcription of IFN response genes in LGSC. However, when treated with dsDNA90, the STING agonist, the HGSC cell lines produced a response, while the LGSC did not (Fig 7a). To test the phosphorylation of proteins in the STING pathway, we performed western blotting on LGSC treated with dsDNA90 and probed for total and phospho STING, TBK1 and IRF3. In the LGSC cell lines (VOA6406, VOA3723 and VOA3448), all proteins were phosphorylated in response to dsDNA90 (Fig 7b). To determine whether there was a defect in translocation of STING between the endoplasmic reticulum and the golgi, we performed immunofluorescent staining for STING in cell lines treated with dsDNA90. We found that while STING translocation is apparent in the HGSC cell line, it is muted or absent in the LGSC cell lines (Fig 7c).

STATUS: Complete

Major Task 2 Restoration of STING signaling to LGSC cell lines

Subtask 1 - Creation of vectors to reintroduce members of the STING pathway back into LGSC cell lines.

We have designed gRNAs targeting STIM1 and are currently cloning them into a lentiviral CRISPR-cas9 vector.

STATUS: In progress. We anticipate this being completed within 1 month.

Subtask 2 – Transfections in 3 LGSC cell lines and selection of cells (using fluorescent marker and cell sorting or antibiotic selection).

STATUS: Not started yet. Once the CRISPR lentiviral vectors are complete, the infection and sorting should be complete in 2 months.

Subtask 3 – Treatment of cells with STING agonist (dsDNA90), western blotting, real time PCR.

STATUS: Not started yet. Should be complete 2-3 months after completion of subtask 2.

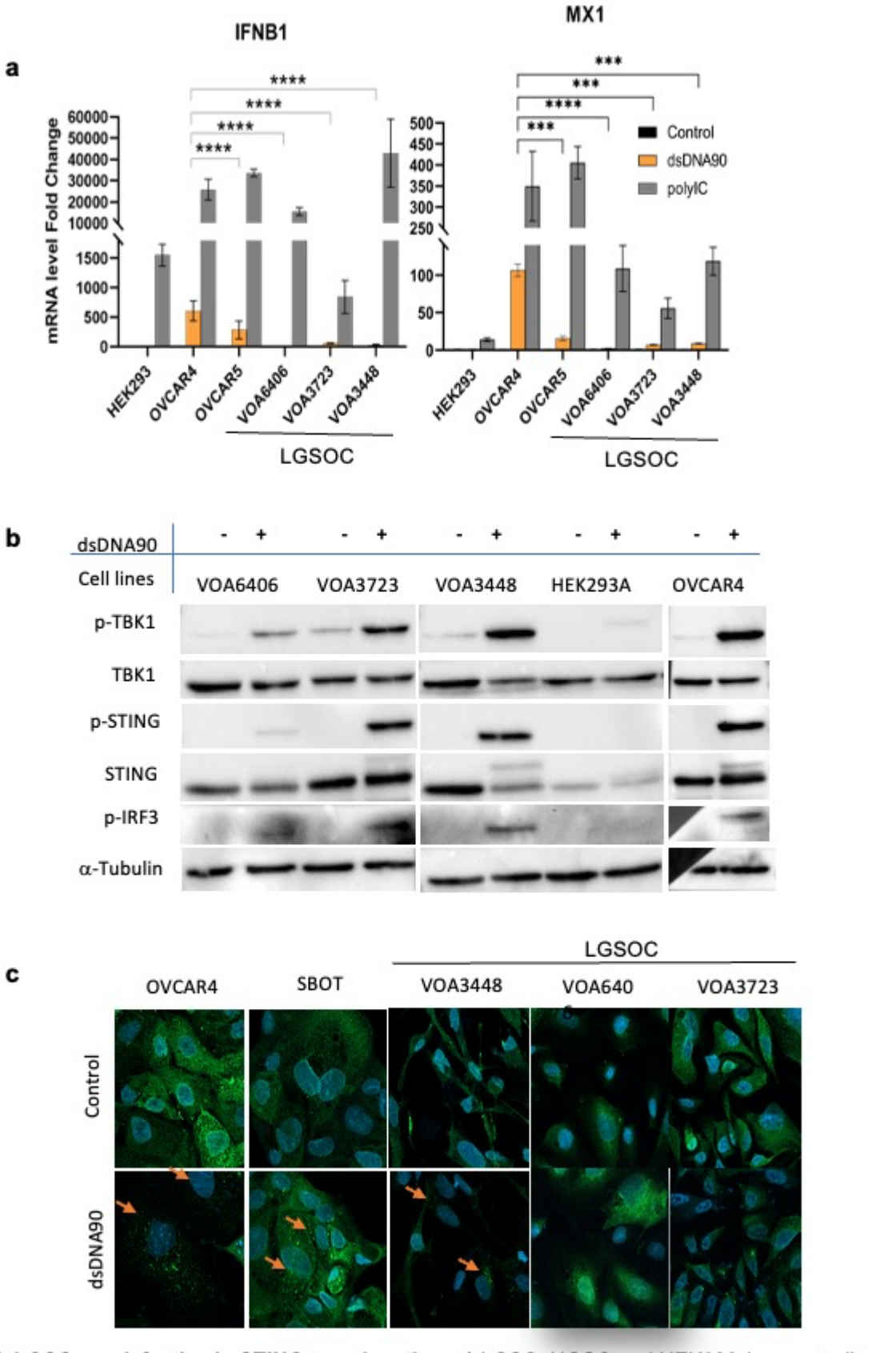

Figure 7. LGSC are defective in STING translocation. a) LGSC, HGSC and HEK293 (-ve control) cells were treated with dsDNA90 (STING agonist), polyIC (RIG pathway agonist) or vehicle control. qPCR for IFN response genes, IFNB1 and MX1, was performed. **b)** Western blot for IRF3, STING and TBK1 and their phospho-proteins was performed on cell lines treated with or without dsDNA90. **c)** Immunofluorescent staining for STING was performed on cell lines treated with dsDNA90.Translocation from the ER into the Golgi appears as punctate, peri-nuclear staining (shown by arrows).

Major Task 3 Determine whether LGSC cell lines are sensitive to oncolytic viruses

Subtask 1 – Treat three LGSC, three HGSC and an immortalized ovarian surface epithelial cell line with oncolytic viruses (1. Oncolytic virus that exploits lack of STING and 2) oncolytic viruses that express cyclic dinucleotides)

We have grown and tittered 6 vaccina-based oncolytic viruses, 3 parental viruses (Cop-TK, SKV-TK and Tian Tian-Tk), and each of these three viruses that also express the cyclic dinucleotide, DISA (Cop-DISA, SKV-DISA and Tian Tian DISA). The purpose of expressing DISA is that it will be released into the microenvironment, activating an innate immune response, increasing clearance of the LGSC tumor cells. We have tittered the viruses to determine the optimal time and multiplicity of infection (MOI) to be used in the experiments (example shown in Fig 8a). We have begun testing the parental viruses on LGSC cell lines, with the SKV-TK showing the highest efficacy (Fig 8b).

STATUS: In progress. There is one more virus to be made and tittered. Once all viruses are made, the treatment of the LGSC cell lines should take 2-3 months.

Subtask 2 – Characterization of transcriptional response of LGSC to oncolytic virus treatment

STATUS: Not yet started. Once subtask 1 is complete, this subtask is anticipated to take 3-4 months to complete.

Major Task 4 Determine whether oncolytic virus treatment of LGSC can activate immune cells.

Subtask 1 – Supernatants of virus treated LGSC cells will be used in a luciferase IFN reporter assay

STATUS: Not yet started. This subtask can be started relatively quickly once the viruses have all been tittered (Major task 3, subtask 1). We anticipate this will take 2 months to complete once started.

Subtask 2 – Co-culture of LGSC treated with oncolytic viruses and peripheral blood mononuclear cells from the same patient.

We have so far accrued 12 LGSC tissue samples with matched peripheral blood mononuclear cells (PBMCs). We will continue to accrue and use fresh tissue/PBMCs whenever possible.

STATUS: In progress. We anticipate this to take 4-6 months to complete once started.

Opportunities for training and professional development

Nothing to report

Dissemination of results to communities of interest

Some of this data was presented recently at the AACR Ovarian Cancer Conference. This conference included participation of ovarian cancer patient advocates.

Plans to complete goals through next reporting period

These were outlined under accomplishments for each of the major tasks and subtasks (see above).

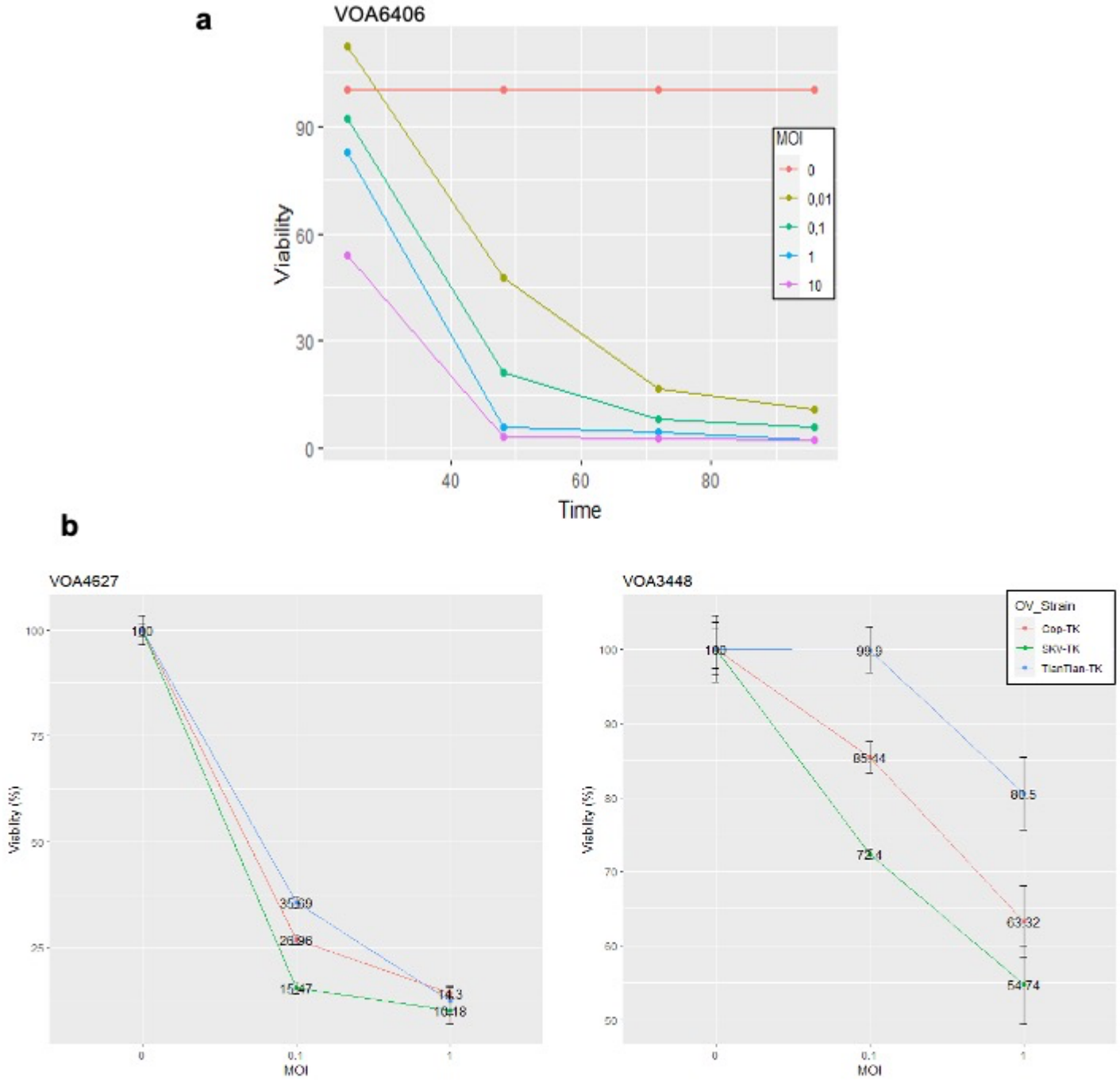

Figure 8. Optimization of Vaccinia oncolytic viruses. a) The vaccinia virus Cop-Tk was tittered on VOA6406 cells. Cells were treated with different MOIs of virus over 96 hours. Crystal violet cell viability assays were performed at regular intervals. **b)** Two LGSC cell lines were treated with three different vaccinia oncolytic viruses at different MOIs for 48 h before crystal violet cell viability assays were performed.

4. IMPACT

Impact on the principal discipline

The major areas that have been impacted by the studies are as follows:

1. Confirming that STING signaling is aberrant in LGSC. Since resistance and clearance of oncolytic viruses can be due to STING, we anticipate that this provides further evidence that oncolytic viral therapy will be successful in these tumors.
2. Identification of STIM1 as a possible reason for defective STING signaling. This provides further insight into the biology of this disease.
3. Identification of FAP as being expressed in fibroblasts of LGSC. This provides insight into the possible causes of transformation between the precursor lesions into LGSC. Furthermore, FAP could be a new therapeutic target in future studies.
4. Characterization of an immunosuppressive TME in LGSC. The immune microenvironment in LGSC has been poorly studied, and these are the first studies to identify high levels of Tregs and M2 macrophages in LGSC TME. This information is important for future design of targeted therapeutics, including new strategies for oncolytic virus therapy
5. Optimization of oncolytic viruses. These are the first studies that show that oncolytic viruses can be used to kill LGSC. Our further optimizations will refine our targeting strategies.

Impact on other disciplines

The data generated from these studies will be applicable to any other cancers that have defective STING signaling. Such tumors may also be sensitive to oncolytic viral therapy.

Impact on technology transfer

Nothing to report

Impact on society beyond science and technology

Nothing to report

5. CHANGES/PROBLEMS

Changes in Approach

The one major change in our approach has been to change proteomic profiling of LGSC cells with dsDNA90, to include LGSC cells that have STIM1 knocked down. In our studies we have identified STIM1 as being the possible reason for aberrant STING signaling. By including the knockout cells, we will obtain more information that without them.

Problems or delays

The generation of the oncolytic viruses has taken longer than expected. Because of this, the subsequent experiments were delayed. We have asked for and received a one year no-cost extension to be able to complete the experiments. This should be enough time to complete the experiments outlined.

6. PRODUCTS

Journal publications

Changes in the tumor microenvironment mark transition from serous borderline tumor to low grade serous carcinoma. Rodrigo Vallejos, Almira Zhantuyakova, Gian Luca Negri, Spencer Martin, Sandra E Spencer, Shelby Thornton, Samuel Leung, Branden Lynch, Yimei Qin, Christine Chow, Brooke Liang, Sabrina Zdravko, Maxwell Douglas, Katy Milne, Bridget Mateyko, Brad Nelson, Brooke Howitt, Lars-Christian Horn, Naveena Singh, Gregg Morin, David Huntsman and Dawn Cochrane. Manuscript in preparation, to be submitted to Nature Communications October 2023.

Books or other non-periodical, one-time publications

Nothing to report

Other publications, conference papers, and presentations

Almira Zhantuyakova "Deciphering Aberrant STING pathway and Exploring Oncolytic Viruses Therapy in Low Grade Serous Ovarian Carcinoma" - in-person poster presentation AACR annual meeting 2023

Almira Zhantuyakova “Deciphering aberrant DNA-sensing STING pathway in Low-Grade Serous Ovarian Carcinoma”- a **virtual poster** presentation at the 14th Biennial Virtual Ovarian Cancer Research Seminar Series presented by the Rivkin Center and the American Association for Cancer Research. 2022

Almira Zhantuyakova "Deciphering aberrancy in STING pathway in Low Grade Serous Ovarian Carcinoma"-in-person poster at BC Cancer Research Day 2022

Almira Zhantuyakova "The STING paradox: Deciphering aberrant DNA-sensing pathway in Low Grade Serous Ovarian Carcinoma" - in-person **poster** presentation Canadian Conference on Ovarian Cancer Research 2022

Almira Zhantuyakova " Deciphering aberrant DNA-sensing: Proteome of STING pathway in Low-Grade Serous Ovarian Carcinoma" - talk at GSC Proteomics Platform Workshop 2022

Almira Zhantuyakova "The STING paradox: Deciphering aberrant STING signaling in Low Grade Serous Ovarian Carcinoma"- virtual **poster** Keystone symposium "DAMPs Across the Tree of Life Inducing Innate Immunity" 2022

Almira Zhantuyakova "The STING paradox: Deciphering aberrancy in DNA-sensing pathway in Low Grade Serous Ovarian Carcinoma " - 5 minute **talk** at Gynecologic Cancer Initiative Trainee Research Day 2022

Almira Zhantuyakova "The STING paradox: Deciphering aberrancy in DNA-sensing pathway in Low Grade Serous Ovarian Carcinoma"- **poster** at OBGYN Academic Day 2022

Rodrigo Vallejos. "FAP Marks Invasion During the Development of Low Grade Serous Ovarian Carcinoma" - **poster** at UBC Pathology Day, 2022.

Rodrigo Vallejos. *“*Changes in the tumor microenvironment marks transition from serous borderline tumor to low grade serous carcinoma” – **talk** and **poster** at Northwest Gynecologic Cancer Symposium 2023

Dawn Cochrane “Defective STING signaling in low-grade serous ovarian cancer: An opportunity for oncolytic viruses therapy” **talk** and **poster** at Northwest Gynecologic Cancer Symposium 2023

Dawn Cochrane “Defective STING signaling in low-grade serous ovarian cancer: an opportunity for oncolytic viruses therapy” – **lightning talk** and **poster** at AACR Ovarian Cancer Conference 2023

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name: Dawn Cochrane
Project Role: PD/PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6
Contribution to Project: As project lead, Dr. Cochrane has provided supervision for Almira and Rodrigo, and overseen all experiments.

Name: Almira Zhantuyakova
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project: Almira has prepared samples for proteomic profiling and analyzed the results. She has done the follow up on STIM1 (IHC and western blotting). She has also helped with the follow up with multiplex IHC, staining and analysis of the M2 macrophages. She has performed western blotting, immunofluorescence and qPCR on LGSC cell lines treated with STING agonists. She has grown and titred oncolytic viruses. She is working with Rodrigo on preparation of the manuscript.

Name: Rodrigo Vallejos
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 4
Contribution to Project: Rodrigo has helped analyze the proteomics data (clustering, pathway analysis) and has done follow up on FAP (IHC staining and analysis). He has also done follow up on the immune microenvironment, specifically the Tregs. He is working with Almira to prepare the manuscript.

Change in active other support

Nothing to report

Other organizations

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES

None