AWARD NUMBER: W81XWH-17-1-0239

TITLE: DNA Polymerase Zeta Inactivation in Prostate Cancer

PRINCIPAL INVESTIGATOR: Richard Wood, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center

REPORT DATE: August 2020

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Aug 2020	Annual	15 Jul 2019 – 14 Jul 2020

4. TITLE AND SUBTITLE

DNA Polymerase Zeta Inactivation in Prostate Cancer

5a. CONTRACT NUMBER
W81XWH-17-1-0239

5b. GRANT NUMBER
GRANT12258091

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)

Richard Wood, Ph.D.

E-Mail: rwood@mdanderson.org

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

The University of Texas MD
Anderson Cancer Center
1515 Holcombe Blvd., Unit 116
Houston, TX 77030-4009

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 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

Not all prostate cancer patients respond in the same way to therapies. For example, some cancers respond well to hormone therapies, and others to radiation therapy. A major reason for these differences is that different genetic changes underlie individual cancers. In order to personalize therapy and make it much more effective, it is important to take advantage of genetic analyses and determine, as early as possible during treatment, the therapeutic strategies that will be most effective to cure or control the cancer. Although it is the most common cancer in American men, more than a quarter of primary prostate cancers of both good and poor clinical prognosis are driven by unknown molecular changes in the genome. Recently, in the course of our studies of DNA repair, we analyzed prostate cancer genome data and discovered that the gene for an important DNA repair enzyme called DNA polymerase zeta (abbreviated "pol zeta") is deleted in 13% of primary prostate cancers. This is very significant because identification of cancers with deletion of the pol zeta gene is highly likely to be useful in diagnosis and therapy. This is because suppressing pol zeta sensitizes cells to DNA damage. The absence of pol zeta is likely very important for improving therapy in these cancers, but it has never been investigated.

15. SUBJECT TERMS

pol ζ, pol δ, pol ϵ, pol η, TAG, MMC, ICL, HR, EJ WT, Het, KO, Puro, µg, µl, IHC, STR, ATCC, NOD-SCID

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	20	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

The major objective of the research is to develop the idea that prostate cancers with pol zeta deletions are specifically sensitive to therapeutic DNA damaging agents, including radiation. This could lead to individualized treatment of an important group of prostate cancers. It will also indicate the usefulness of DNA damaging chemotherapy for a previously unrecognized major group of prostate cancers. Because we know that normal cells do not grow well in the absence of pol zeta, we also intend in this research to identify genetic alterations that allow cells to proliferate in the absence of pol zeta. This will be a practically important advance because it will help identify the pol zeta-deleted class of cancers.

2. KEYWORDS:

DNA polymerase, DNA repair, mitomycin C, cisplatin, radiation, cell lines, gene deletion

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Determine the DNA damage sensitivity conferred by disruption of pol ζ in prostate cancer cells		
Major Task 1: *REV3L* will be inactivated by targeted genetic deletion in prostate cancer cell lines and specific mutations will be tested	Months	% comple-tion
Inactivate REV3L	1-6	100
Toxicity measurements	7-12	100
Make specific mutations in cDNA	7-12	100
Complementation assays using mouse MEFs	13-20	100

Specific Aim 2: Identify suppressor mutations that allow cells to proliferate in the absence of pol ζ		
Major Task 1: Candidate suppressor genes suggested by preliminary studies will be tested in REV3L-deficient human prostate cancer cells.	Months	MDA
Make targeted deletions in cell lines	1-12	70
Toxicity measurements	6-18	30

Major Task 2: Identify genes that, when downregulated, alleviate the growth defects in REV3L-defective human cancer cells.		
Subtask 1: Make targeted deletions in cell lines and measure growth rates	1-12	60

Subtask 2: A genome-wide shRNA screen for growth of REV3L-defective cells	12-24	20

Specific Aim 3: Determine radiation and chemosensitivity of a pol ζ-defective prostate cancer model in mice		
Major Task 1: Establish xenograft model and determine the response to ionizing radiation and cisplatin-based treatment will be quantified.	Months	MDA
Subtask 1: Obtain mice and establish xenografts	6-18	0
Subtask 2: Test drug and radiation resistance of xenografts	9-20	0

What was accomplished under these goals?

Major Activities and Specific Objectives:

A major objective was to test the functionality and consequence of cancer-associated mutations in REV3L, the catalytic subunit of DNA polymerase ζ. We focused on measurement of sensitivity to the chemotherapeutic agent cisplatin, which produces lesions including DNA interstrand crosslinks. Cell lines were generated in REV3L KO cell lines REV3L 4(-/Cre)TAg MEF and REV3L 3(+/Cre)TAg MEF. Cancer associated mutations in REV3L included the planned P2744S and R2523C and the more recently reported R187W mutation found in a cohort study of 40 Spanish families with colorectal cancer. The parental cell lines are REV3L 4(-/Cre)TAg MEF and REV3L 3(+/Cre)TAg MEFs. Successfully transfected clones are isolated by fluorescence activated flow-sorting. They are then confirmed by checking plasmid integration using PCR and immunoblotting.

We constructed the following complemented MEF cell lines, several independent clones of each:

pCDH-FH-TR4-2 ASM REV3L KO
pCDH-FH-TR4-2 R2523C REV3L KO
pCDH-FH-TR4-2 P2744S REV3L KO
pCDH-FH-TR4-2 R187W REV3L KO
pCDH-FH-TR4-2 4A REV3L KO

Methodology:

To test sensitivity to chemical DNA damaging agents, the immortalized MEFs were plated into white 96-well plates (immortalized MEFs– 5,000 cells/well). The following day, various concentrations of cisplatin (Sigma) were added to the wells, and the cells were incubated for 48 hr. Then the cells were lysed, a reagent was added that emits light in the presence of ATP (ATPLite One Step, Perkin Elmer), and luminescence was measured using a plate reader (Biotek Synergy II). The luminescence measurement was normalized to undamaged control. The ATP content measured by luminescence provides a measure of survival.

Significant Results:

Aim 1: Representative survival results were summarized in earlier reports. The cancer-associated R2523C, P2744S, and R187W mutations did not affect the functionality of the TR4-2 REV3L.

However, it is significant that inactivation of the four REV7 binding sites in REV3L (in the 4A mutant) was sufficient to inactivate REV3L function (**Fig 1A**). To obtain a readout of genetic instability in REV3L-defective cells for future use in assessing REV3L defects, we assayed micronuclei, which are diagnostic of unresolved double strand breaks in chromosomes (**Fig 1D**).

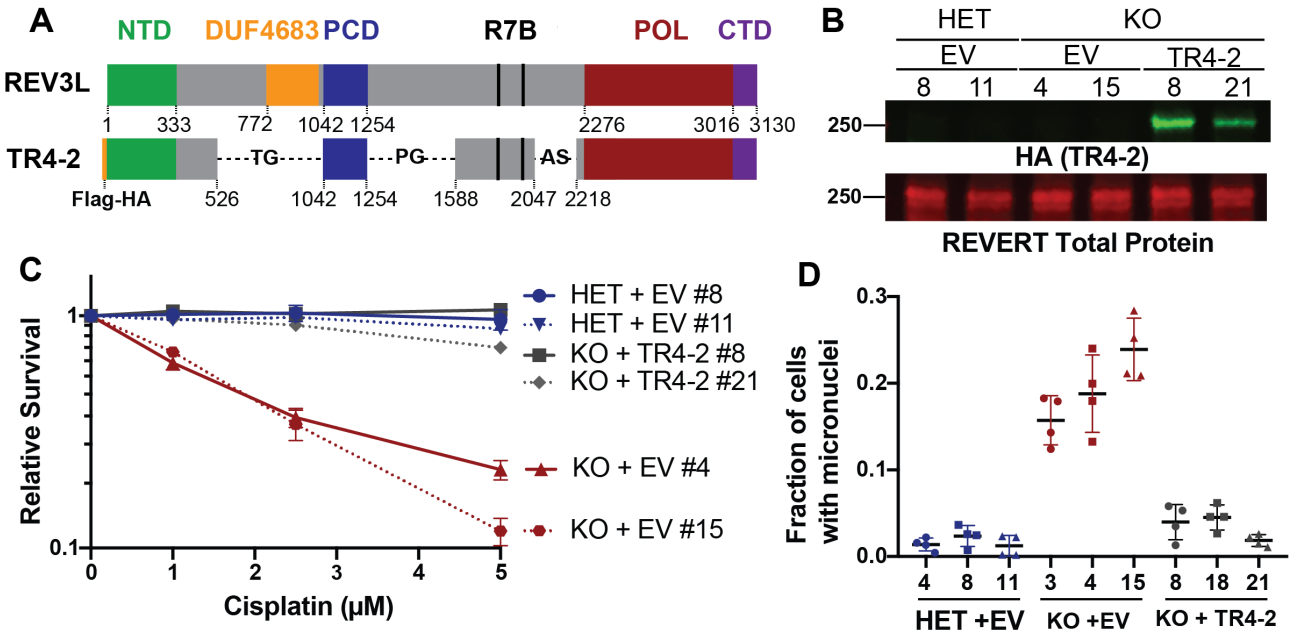


Figure 1: Shortened REV3L construct rescues phenotypes of pol ζ disruption
A) Schematic of full-length human REV3L and human REV3L construct, TR4-2. TR4-2 retains most conserved domains and binding sites of REV3L including the regions that coordinate interactions with the accessory subunits of pol ζ, the C-terminal domain (CTD, purple) the two REV7 binding sites (R7B, black), and a positively charged domain (PCD) of uncertain function. The B-family catalytic core is formed by folding of the N-terminal domain (NTD, green) and the polymerase domain (POL, red). B) Immunoblot with HA antibody showing stable expression of TR4-2 with an N-terminal Flag-HA tag in *Rev3l* KO MEF clones. C) Stable expression of TR4-2 in *Rev3l* KO MEF clones reverses hypersensitivity to cisplatin. MEFs were exposed to the indicated cisplatin concentrations for 48 h and relative survival was quantified with the ATPlite assay. D) Stable expression of TR4-2 decreases micronuclei formation in unchallenged *Rev3l* KO MEF clones.

Aim 2: A major objective of Specific Aim 2 is to construct REV3L knockout cell line(s) in prostate cancer cells. As described in last year's report, we did extensive screening of Crispr-Cas9 targeted clones in DU145 and PC3 prostate cancer cell cells. None of the many candidate clones had full REV3L deletion. The inviability of these cells following REV3L deletion was, in some ways expected according to our current hypothesis, because neither cell line harbors the large deletion on chromosome 6q that encompasses REV3L and surrounding genes. We also cultured REV3L knockout cells from the human Jurkat cell line for a total 12 months to attempt to find clones that regained cell growth speed sufficient for CRISPR-Cas9 screening, but we did not find such cell lines.

These results show that there is very strong selection to retain REV3L function in primary cells and in most cancer cells. We have been careful in our genetic analysis, in contrast to some other reports in the field. For example, a recently published manuscript describes REV3L knockout cells from human HEK293T cells (Su et al 2019, J. Biol Chem. PMID: 30842261 10.1074/jbc.RA119.007925). We examined the deleted DNA sequences given in this paper. They do not prove that both alleles of REV3L are inactivated. One of the targeted mutations eliminates a splice site and part of an intron, leaving open the strong possibility that alternative splicing may permit *REV3L* function in that cell line.

Loss of polymerase ζ alters the transcriptome

In order to uncover the type of stress occurring in cells lacking pol ζ, we performed genome-wide mRNA sequencing on a controlled set of immortalized clones: *Rev3l* HET + empty vector, *Rev3l* KO + empty vector and *Rev3l* KO + TR4-2. To focus on major changes, we set a strict threshold (> |2| $\log_2$ fold change and FDR < 0.05). Expression analysis of 17,346 mapped transcripts revealed that 1117 transcripts were either upregulated or downregulated in the *Rev3l* KO + empty vector relative to the *Rev3l* HET + empty vector MEFs (Fig 2A). The majority (~68%) were upregulated (Fig 2A). These upregulated or downregulated genes displayed no statistically significant enrichment or depletion for DNA replication or canonical DNA damage sensing pathways. This is not completely unexpected given that p53 promotes much of the transcriptional response to DNA damage, while our MEFs have inactivated p53 due to large T-antigen immortalization. In these immortalized high passage cells, we are likely to observe stable transcriptional alterations, rather than an acute response.

Instead, the upregulated genes displayed an enrichment in immune system-related pathways, as revealed by gene ontology analysis (Fig 2B). Upstream regulator analysis was used to analyze all differentially expressed genes. This revealed that the alterations in the transcriptome are consistent with activation of positive regulators of the interferon response, including key transcription factors in this pathway, IRF3 and IRF7 (Fig 2C). Importantly the predicted activation of IRF3 and IRF7 was reversed by expression of the *Rev3l* TR4-2 cDNA, showing that these results stem from a function of *Rev3l.* The same trends were also observed by applying a substantially lower threshold ($\log_2$ FC > |0.5|) for differentially expressed genes. This increased the dataset to 2071 differentially expressed transcripts. Pathway analysis showed a negative correlation with predicted TRIM24 activation. TRIM24 suppresses interferon-stimulated gene expression [16], confirming that our data is consistent with expression of interferon stimulated genes.

To explore whether our data set is in fact consistent with an interferon response, we analyzed a curated set of 25 known interferon stimulated genes and observed increased expression in the *Rev3l* KO relative to the *Rev3l* HET MEFs, which was partially reduced by TR4-2 stable expression (Fig 2D). Together our results reveal that disruption of pol ζ promotes induction of interferon-stimulated genes.

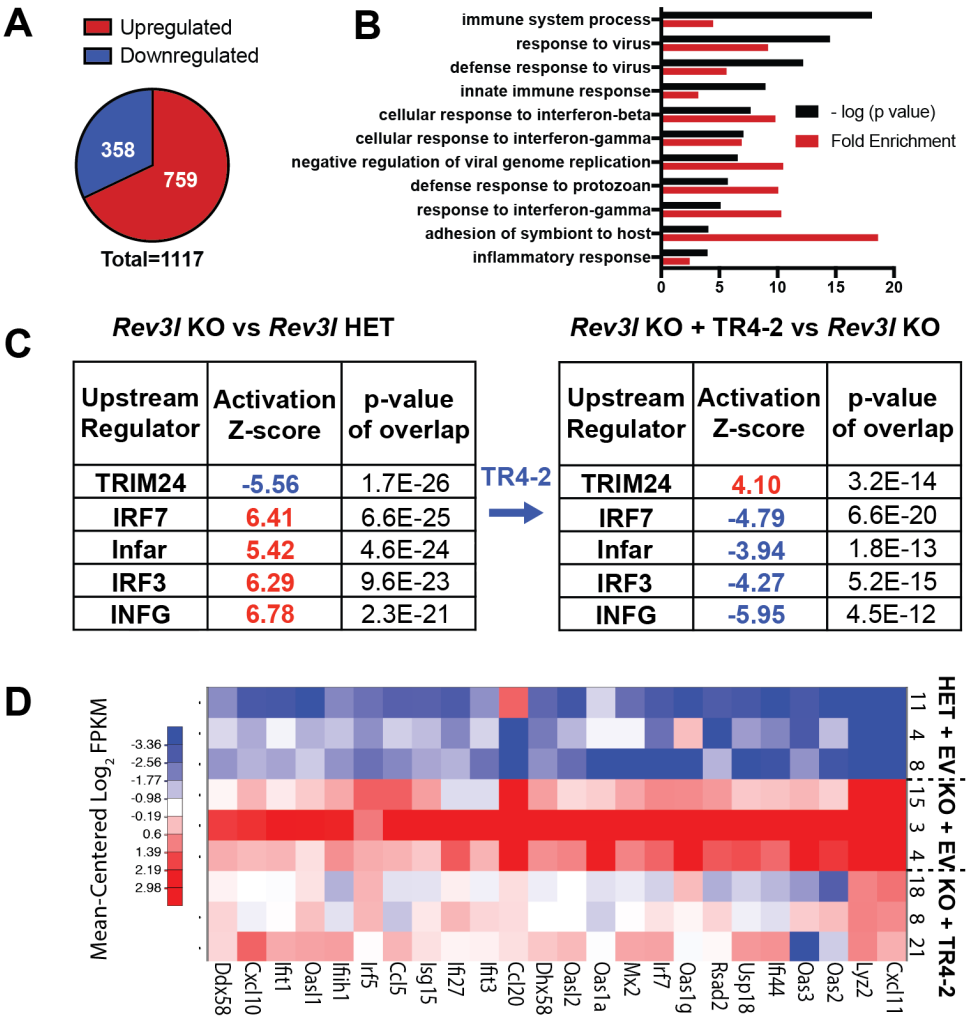


Figure 2: Cells deficient in DNA polymerase ζ have an altered transcriptome.

A) Differentially expressed genes in *Rev3l* KO + empty vector relative to *Rev3l* HET + empty vector using the threshold of a $\log_2$ fold change of > |2| and a false discovery rate of < 0.05. B) Top 10 GO (Gene Ontology) terms reveal enrichment of immune system related genes in upregulated genes in *Rev3l* KO MEFs. C) Upstream regulator analysis reveals the data set is consistent with predicted activation of positive regulators of an interferon response in *Rev3l* KO MEFs. D) Heatmap of a set of 25 known interferon-stimulated genes.

Disruption of *Rev3l* induces interferon-stimulated gene expression driven by the cGAS-STING axis

Given that our complemented cell lines were generated using lentivirus constructs and grown continually under selection, we moved to validate the results in the parental *Rev3l* KO and *Rev3l* HET MEF cell lines and one additional set of cell lines to limit extraneous variables. We confirmed an increase in mRNA expression of specific interferon stimulated genes in the *Rev3l* KO MEFs relative to the control cell lines, including key chemokines (Fig 3A)..

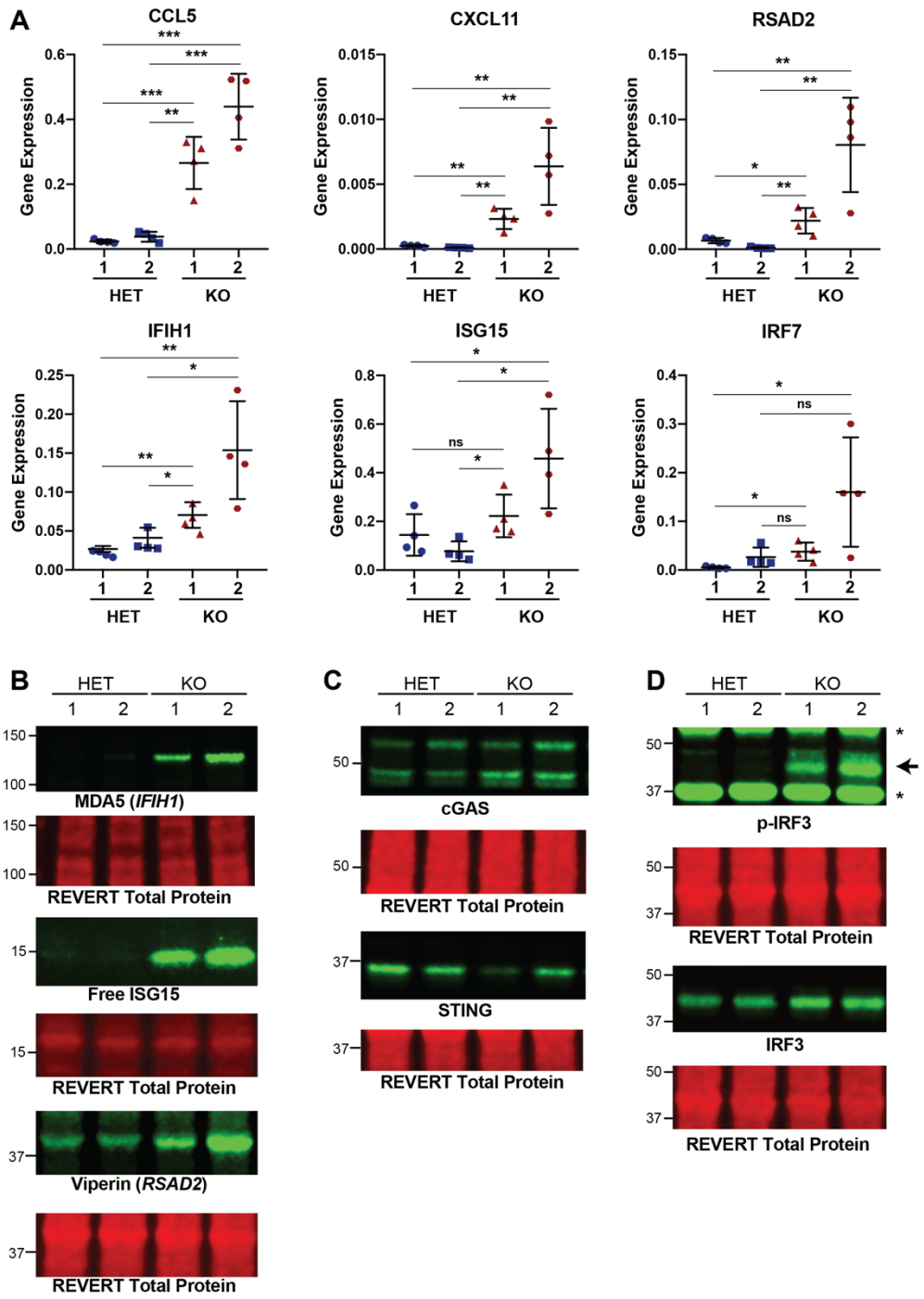


Figure 3: Disruption of *Rev3l* results in increased expression of interferon stimulated genes and proteins. A) Gene expression ($2^{-\Delta Ct}$) of selected interferon stimulated genes normalized to HPRT detected by qRT-PCR. Error bars represent standard deviation. Unpaired student t-test, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$. B) Immunoblots showing increased protein levels of interferon stimulated genes, MDA5 and ISG15. C) Immunoblot showing presence of components of the innate immune system, cGAS and STING, in MEFs, with reduced STING in pol ζ knockout cells. D) Enhanced phosphorylation of S888 of IRF3 (corresponding to S396 in humans) in *Rev3l* KO MEFs.

In order to extend these findings to the protein level, we examined interferon stimulated gene products by immunoblotting. Corresponding to an increase in mRNA levels, we also observed an increase in protein levels of known interferon stimulated genes, MDA5 (encoded by the *IFIH1* gene), ISG15, and viperin (encoded by the *RSAD2* gene) (Fig 3B). Together these data indicate that an interferon branch of the innate immune system may be activated due to disruption of pol ζ function. Since it seems unlikely that pol ζ plays a direct role in transcriptional regulation, the next obvious question is how and why loss of pol ζ induces the expression of interferon stimulated genes

The major consequence of pol ζ disruption in unchallenged mammalian cells is increased genomic instability as evidenced by multiple markers including γ-H2AX foci, chromosome fragmentation and aberrations, and micronuclei (Fig 1D). Therefore, it seems likely that this transcriptional response ultimately stems from the vast genomic damage induced by loss of pol ζ function. Consistent with this hypothesis, the innate immune system not only can recognize and mount an interferon response to foreign DNA, but also can respond to endogenous DNA that has escaped from the nucleus. In some instances, this response can halt cell growth providing organisms to shut down propagations of virally infected cells and cells with dangerously fragmented genomes.

Mammalian cells have a host of cytosolic nucleic acid sensors that patrol the cytosol for DNA. One of these, cGAS, is increasingly recognized to be of paramount importance in the induction of an interferon response to both exogenous and endogenous cytosolic DNA. When cGAS binds to double stranded DNA, it activates its enzymatic activity and results in the production of cGAMP, a cyclic dinucleotide. cGAMP binds to the STING receptor on the membrane of endoplasmic reticulum, resulting in activation of kinases including TBK1 which can in turn phosphorylate and activate IRF3, a central transcription factor in the interferon response.

Given that cGAS-STING axis has been implicated specifically in responding to endogenous DNA damage and has been correlated with micronuclei formation, we asked whether cGAS-STING promotes the induction of expression of interferon stimulated genes due to loss of function of pol ζ. Consistent with most MEFs having a functional innate immune system, both *Rev3l* KO and HET MEF cell lines expressed both cGAS and STING (Fig 3C). We noted a decrease in STING expression in *Rev3l* KO MEFs, which is consistent with a constitutive activation of the cGAS-STING pathway, as cGAS activation leads to a negative feedback loop resulting in STING degradation [19,20]. Importantly, we detected an increase of IRF3 phosphorylated at S888 (corresponding to S396 in humans) indicative of IRF3 activation in *Rev3l* KO MEFs (Fig 3D).

This led us to investigate if cGAS-STING drives expression of interferon-stimulated genes upon loss of pol ζ. Knockdown of either cGAS or STING significantly reduced the mRNA expression of selected interferon stimulated genes as well as the protein levels (Fig 4A-D). In addition, depletion of cGAS or STING in *Rev3l* KO MEFs markedly reduced S888 phosphorylation of IRF3 (Fig 4E). Together this indicates that disruption of pol ζ function promotes activation of an innate immune response driven by the cGAS-STING axis.

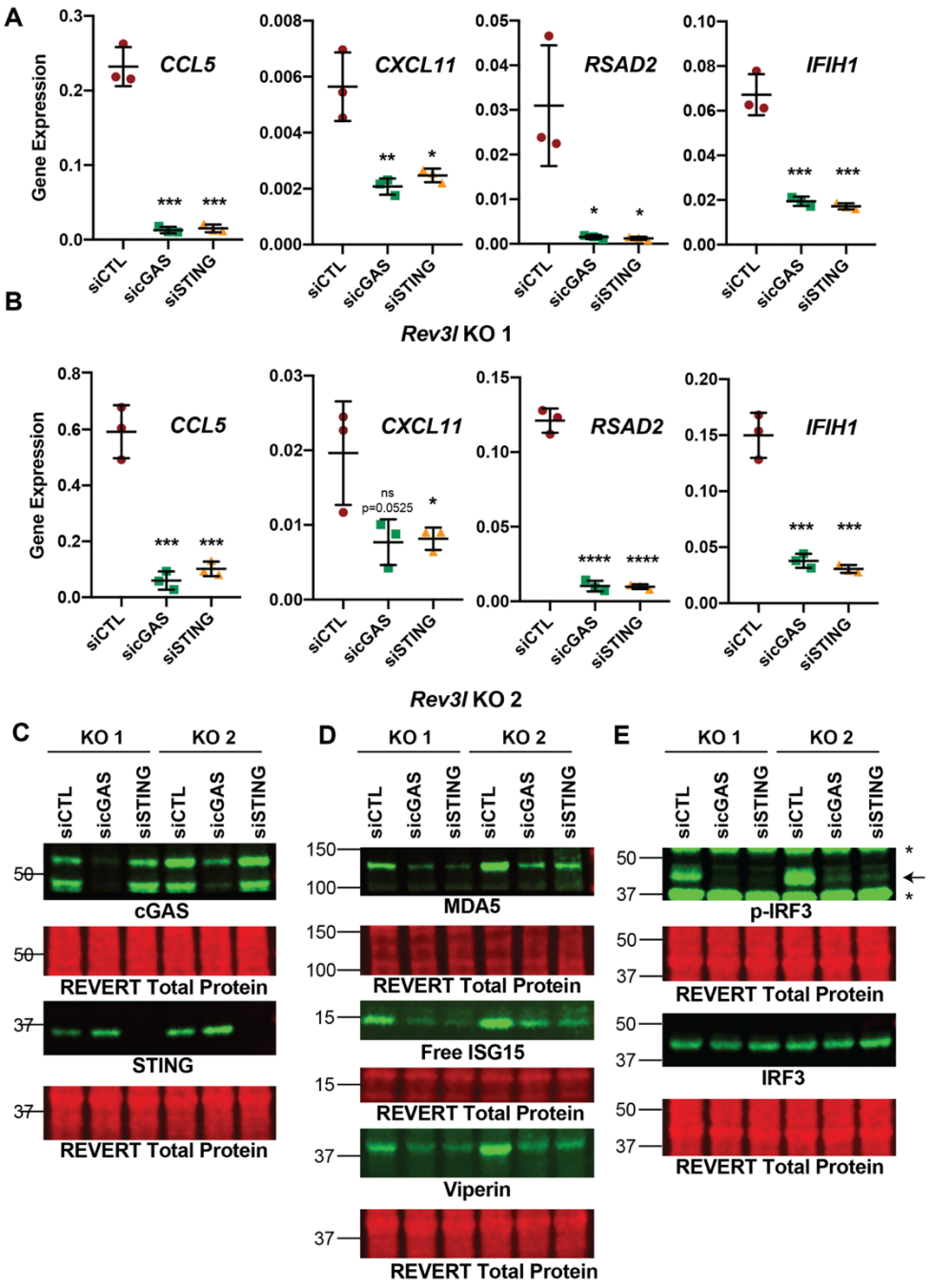


Figure 4: The cGAS-STING axis promotes expression of interferon stimulated genes due to loss of pol ζ function. A) Knockdown of cGAS or STING reduces mRNA expression of *CCL5, CXCL11, RSAD2* (which encodes Viperin protein), *IFIH1* (which encodes MDA5) as detected by qRT-PCR in *Rev3l* KO 1. Gene expression ($2^{-\Delta Ct}$) of selected interferon stimulated genes normalized to HPRT detected by qRT-PCR. Error bars represent standard deviation. Unpaired student t-test, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, and **** = $p < 0.0001$. B) Same as in A except with *Rev3l* KO 2. C) Efficient knockdown of cGAS or STING protein levels. D) MDA5, ISG15, and Viperin protein levels decrease with cGAS and STING knockdown. E) Phosphorylation of S888 in mouse (analogous to the human S396) of IRF3 in *Rev3l* KO MEFs decrease with knockdown of cGAS and STING.

An innate immune response cause by loss of pol ζ function

Pol ζ stands apart from the other translesion polymerases in that it is required for mammalian development and proliferation of primary cells. Now we can add that in addition to activating p53-dependent responses, disruption of pol ζ function invokes a prominent innate immune response promoted by the cGAS-STING pathway.

It is remarkable that disruption of an enzyme commonly thought of as a specialized transleion synthesis polymerase can lead to a constitutive innate immune response. Recently, cells with loss of function of key DNA repair enzymes, RNaseH2, BRCA2, and BLM have been shown to have an elevated cGAS-STING response that correlates with an increase in micronuclei that colocalize with cGAS. There are several sources of DNA damage that may give rise to a sustained response including cytosolic mitochondrial DNA and cytosolic DNA arisen from stalled and processed replication forks. DNA stress may continually arise from likely collapse of DNA replication forks in the absence of pol ζ, which could promote formation of micronuclei and also release small fragments of DNA. Further, some nuclear genes including pol ζ

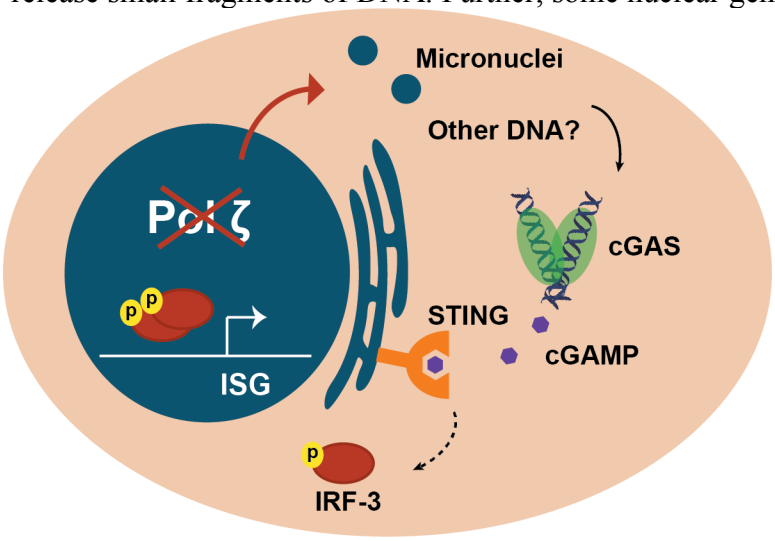


Figure 5: Model for disruption of pol ζ triggering an innate immune response. Loss of pol ζ induces genomic damage that results in accumulation of forms of cytosolic DNA, including micronuclei at a minimum. This results in DNA binding of cGAS and activation of STING which indirectly promotes phosphorylation and activation of IRF-3. This results in expression of interferon stimulated genes (ISG).

control mitochondrial DNA integrity, and there is evidence that mitochondrial function is compromised without pol ζ [29]. It remains to be seen whether micronuclei are the primary source of interferon signalling in cells lacking pol ζ, or whether they are more of an indicator of DNA degradation.

An interferon response can result in shutting down cell growth. Specifically, cGAS has been tied to promoting senescence in primary cells [30,31]. Our experiments were performed in T-antigen immortalized MEFs. In addition to blunting p53 activity, large T-antigen has been implicated in impairing an interferon response to nucleic acids [32]. Loss of pol ζ would likely induce an innate immune response of even greater magnitude in primary MEFs. Primary MEFs lacking pol ζ only make it approximately two cell divisions before cell growth completely halts, which is accompanied by an increase in senescent cells [11]. An essential function of pol ζ is also evident from the failure of *Rev3l*-defective embryos to develop, and the from the inability of *Rev3l*-defective primary keratinocytes to proliferate in a mouse model [2,10,11]. It is possible that cGAS-STING drives this severe growth arrest in primary cells due to loss of pol ζ function.

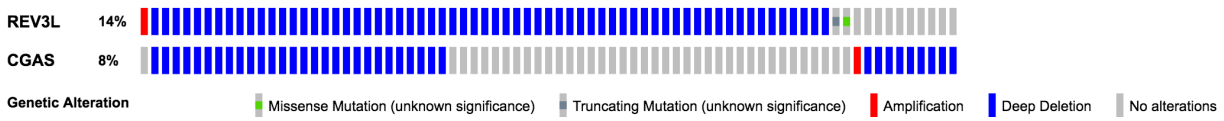
In addition to widening our understanding of the lengths cell go to protect themselves from the genomic damage induced by impairment of pol ζ, these results could have impact on translational approaches. A potential approach, suggested by experiments in laboratory settings, has been to disrupt pol ζ function to enhance chemotherapeutic effectiveness. For example, an inhibitor that impairs the interaction of pol ζ with the master regulator REV1 has been developed that sensitizes cancer cells and xenograft tumors to cisplatin treatment. Our work suggests that such inhibitors might also induce an interferon response. This approach would have multiple advantages for therapy by enhancing DNA damage sensitivity, limiting induced mutations, and potentially enhancing a cytotoxic immune response on targeted cells.

This is exciting and highly relevant to prostate cancer because our genetic analysis shows that *CGAS* is one of the co-deleted genes with *REV3L* in human primary prostate cancer, as it is located at human chromosome 6q13 in the deleted region (**Fig 7**). It is possible that *CGAS* deletion may allow *REV3L* defective cells to survive, and we are currently testing this candidate gene.

We are also testing *ATG5* as a candidate gene, deleted in a similar fraction of primary prostate adenocarcinoma. This is a collaboration with Dr. Bratton in our department.

If we obtain suitable human knockout cell lines for screening, we will use Crispr-Cas9 libraries as proposed.

Figure 7. Oncoprint generated from cBioportal for TCGA data of prostate adenocarcinoma (492 patients). About 13% of all patients have a deep deletion of *REV3L*, and about half of these also have a deep deletion of *CGAS*.



cBioPortal uses the GISTIC algorithm on copy number segments to determine if a gene has a deep deletion. We are finding that this algorithm overcalls deletions by looking at the underlying copy number segment data.

Another approach is to use copy number segment data and RNA-seq (**Figure 8**). This figure shows that copy number calls as deep (homozygous deletion) in TCGA are questionable for these data. Even the lowest copy number calls are not correlated with exceptionally low expression. Another view is the color-coded plot in **Figure 9**, covering the area of chromosome 6 harboring REV3L. The gradated spectrum of blue color shows that making a distinct call for homozygous deletion is not possible from these data. If one chooses only those patients (120) where coverage of the REV3L gene is < 0.2 (log2), it is interesting that those with some REV3L deletion show lower survival (**Figure 10**). We are investing this data by further bioinformatic analysis.

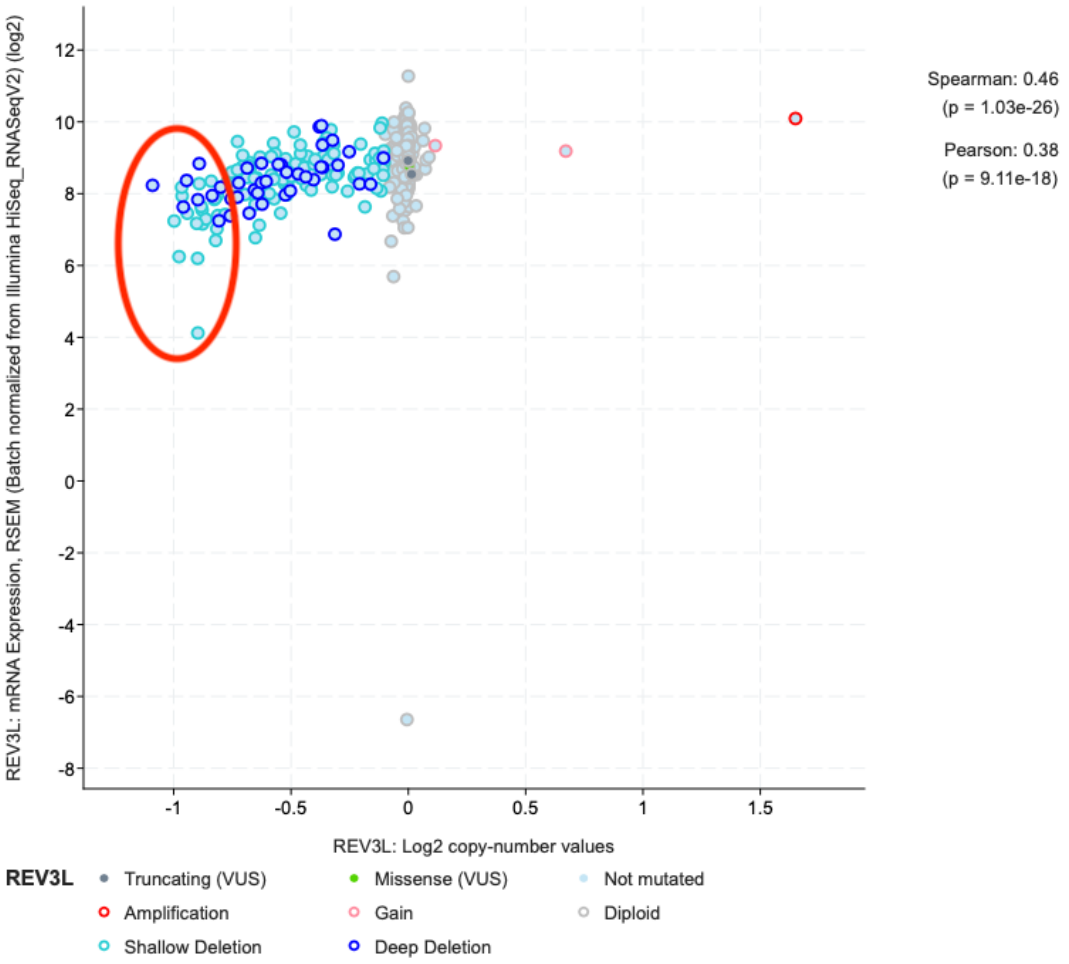


Figure 8. TCGA data for 499 Prostate Adenocarcinoma samples, plotting TCGA copy number values vs mRNA expression for *REV3L*. The points/samples within and around the red-circled area are those with multiple lines of evidence that there is a potential deep deletion. Ideally, one would like to identify samples in the extreme bottom left corner.

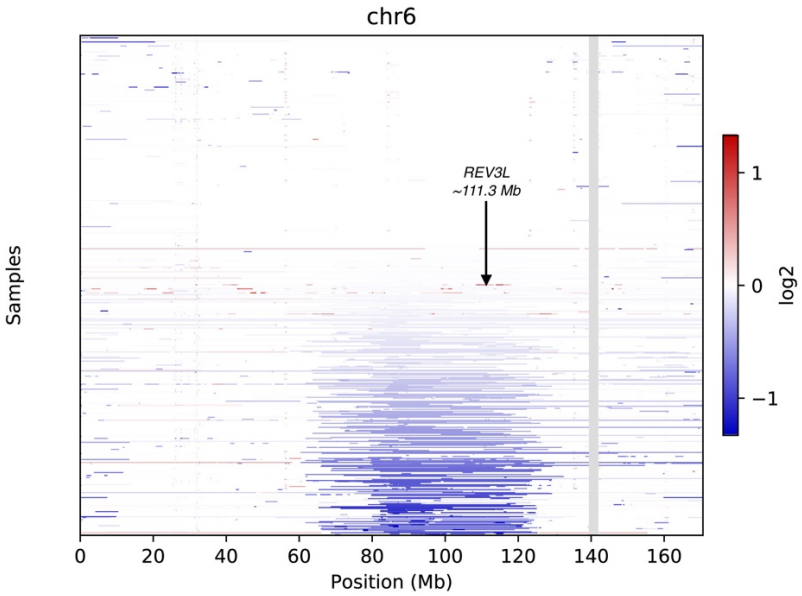


Figure 9. TCGA data for 499 Prostate Adenocarcinoma samples, plotting TCGA copy number values vs chromosome 6 location. *REV3L* is located at 111.3 to 111.4 Mb as indicated by the black arrow. The spectrum of blue color shows the difficulty in definitively assigning homozygous deletion calls from this type of data.

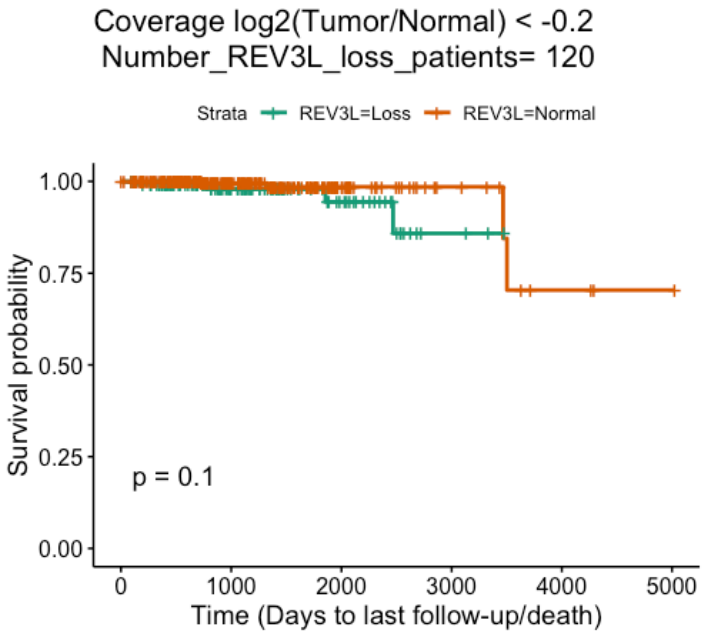


Figure 10. TCGA data for 120 Prostate Adenocarcinoma samples, where the coverage log 2 (Tumor/Normal) is less that -0.2. with p = 0.1, those samples with *REV3L* loss have a lower probability of survival than those with normal copy numbers of *REV3L*.

Aim 3: For Specific Aim 3, we plan to begin the mouse xenograft tumor work after we obtain *REV3L* knockout human prostate cancer cell lines and when the new postdoctoral fellow is in place.

What opportunities for training and professional development has the project provided?

The overall purpose of the project is not specifically to foster training and professional development. However, two undergraduate students have been able to work with the key personnel on this project, and learn about mechanism of pol zeta action. They presented their work at the annual undergraduate symposium in the department.

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Carry out experiments as planned in the SOW, specifically the remainder of Specific Aim 2, and Specific Aim 3. We intend to determine suppressor genes that allow growth of prostate cancers in the absence of DNA pol ζ.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Scientific feedback from our conference presentations on the ongoing work (see Section 6 below) show that the prospects for significant impact are high, if we can identify the most relevant suppressor genes, and REV3L-deficient prostate cancers.

What was the impact on other disciplines?

Nothing to Report (research in progress, publications not yet prepared)

What was the impact on technology transfer?

Nothing to Report (research in progress, publications not yet prepared)

What was the impact on society beyond science and technology?

Nothing to Report (research in progress, publications not yet prepared)

5. CHANGES/PROBLEMS:

Changes in approach and reason for change:

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Several factors combined to create a delay in scientific completion of the project, most of them related to the ongoing COVID-19 pandemic (i) Because of the pandemic, our laboratories shut down on March 20 and reopened with shift work a few months later. All mouse work had to be stopped and could not be restarted in the interim (ii) We could not hire new positions, even grant funded, during this interim due to an institutional rule. US Government rules and uncertainties for obtaining a J1 visa for candidates are introducing further delays. We therefore requested a no costs extension to conclude all studies, and this extension was approved.

Changes that had a significant impact on expenditures

No significant changes.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Significant changes in use or care of human subjects

No significant changes.

Significant changes in use or care of vertebrate animals

No significant changes.

Significant changes in use of biohazards and/or select agents

No significant changes.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report. Initial manuscript submitted to Cell Reports, currently under revision for resubmission.

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.

Invited Speaker, DNA Polymerases and Cancer, 4th DNA Repair/Replication Structures and Cancer Conference, Nassau, Bahamas, 2/16/2020

Invited Speaker, A DNA polymerase for stress relief and cancer suppression, Gordon Research Conference on DNA Damage, Mutation and Cancer, Ventura, CA, 3/5/2020

Invited Speaker, University of Pittsburgh, Biological Sciences, Pittsburgh, PA, 11/18/2019

- **Website(s) or other Internet site(s)**

Nothing to report (research in progress).

- **Technologies or techniques**

Nothing to report (research in progress).

- **Inventions, patent applications, and/or licenses**

Nothing to Report (research in progress).

- **Other Products**

Nothing to Report (research in progress).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Richard D. Wood (No Change)

Name: Sarita Bhetawal
Project Role: Senior Research Assistant
Nearest person month worked: 5 CM
Contribution to Project: Technical assistance with all experiments

Name: Sara Martin
Project Role: Postdoctoral Fellow
Nearest person month worked: 6 CM, Paid from CPRIT Fellowship
Contribution to Project: Technical assistance with all experiments

Name: Yuzhen Li
Project Role: Postdoctoral Fellow
Nearest person month worked: 8 CM
Contribution to Project: Technical assistance with all experiments

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ***N/A***
QUAD CHARTS: N/A

9. APPENDICES: