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| **AWARD NUMBER:** | W81XWH-22-1-0770 |
| **TITLE:** | Using Single-Cell CRISPR Screen to Decipher the Function of Core Transcription Factors in Driving NF1-Deficient MPNST |
| **PRINCIPAL INVESTIGATOR:** | Dr. Xiyuan Zhang |
| **CONTRACTING ORGANIZATION:** | The Geneva Foundation<br>950 Broadway, Suite 307<br>Tacoma, WA 98402-4437 |
| **REPORT DATE:** | AUGUST 2023 |
| **TYPE OF REPORT:** | Annual |
| **PREPARED FOR:** | U.S. Army Medical Research and Development Command<br>Fort Detrick, Maryland 21702-5012 |
| **DISTRIBUTION STATEMENT:** | Approved for Public Release;<br>Distribution Unlimited |

# REPORT DOCUMENTATION PAGE

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*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| August 2023 | Annual report | 1 August 2022 - 31 July 2023 |

**4. TITLE AND SUBTITLE**
Using Single-Cell CRISPR Screen to Decipher the Function of Core Transcription Factors in Driving NF1-Deficient MPNST

**5a. CONTRACT NUMBER** W81XWH-22-1-0770

**5b. GRANT NUMBER** W81XWH-22-1-0770

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Dr. Xiyuan Zhang
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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

The Geneva Foundation
950 Broadway, Suite 307
Tacoma, WA 98402-4437

**8. PERFORMING ORGANIZATION REPORT NUMBER**

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

U.S. Army Medical Research and Development 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**
In Aim 1, I will use the CRISPR-KO system that was previously established by transducing Cas9 vector into MPNST cell lines. Next, a pooled dual-gRNA library that targets the DNA binding domains of 85 candidate CRC TFs (plus 5 non-targeting controls) will be designed, synthesized and cloned into lentiviral vectors and used to transduce the Cas9- expressing MPNST cells. The transcriptional phenotypes of these cells will be sequenced on a single-cell level and compared to the known oncogenic signature of MPNST cells. In Aim 2, I will establish the CRISPRa system by transducing the dCas9- VPR vector into the NF1-/- Schwann cells, which will be transduced with a pooled dual-gRNA library targeting the promoters of the 85 MPNST-specific TFs. Next, these Schwann cells will be cultured in soft agar with or without the selecting pressure of single-agent topotecan, etoposide, or doxorubicin. Any colonies formed will be subjected to nuclei isolation and transcriptomic profiling.

**15. SUBJECT TERMS**
Oncology, rare cancer, neurofibroma

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRDC |
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**Standard Form 298 (Rev. 8-98)**
**Prescribed by ANSI Std. Z39.18**

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**Page**

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose, and scope of the research.*

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder that affects ~1/3500 newborns with a germline mutation in tumor suppressor gene *NF1*. A life-threatening complication of having NF1 is the development of an aggressive and highly metastatic malignant peripheral nerve sheath tumor (MPNST) at an earlier age compared to the general population. Currently, there are no effective treatments for MPNST other than complete surgical resection with wide margins. Patients with NF1 develop MPNSTs through the malignant transformation that is accompanied by a genetic model: 1) About 50% of NF1 patients exhibit plexiform neurofibromas, which are caused by the loss-of-function in *NF1* and the associated hyper activated Ras signaling pathway, 2) Atypical neurofibromas arise from PNs and they exhibit loss of *CDKN2A*, and 3) approximately 13% of NF1 patients develop MPNSTs, in which recurrent mutations in *SUZ12* and/or *EED*, two key components of the polycomb repressive complex 2 (PRC2), were identified, leading to loss of tri-methylation of histone H3 lysine 27 (H3K27me3) in these tumors. Like many other cancer subtypes, abnormal transcriptional addiction has been observed in MPNST. To fully characterize the transcriptional network that drives and sustains the MPNST, I profiled the epigenetic landscapes in MPNSTs and found that histone marker of super enhancers, acetylated-H3K27 (H3K27ac), was gained as H3K27me3 was lost, indicating the potential impact of super enhancer-defined core transcriptional regulatory circuitry (CRC) in driving MPNST tumorigenesis.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Neurofibromatosis, malignant transformation, malignant peripheral nerve sheath tumor, transcription factor, enhancer, CRISPR, single-cell sequencing

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

**What were the major goals of the project?**

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

| **Specific Aim 1. Characterize the interconnected transcriptional regulatory network formed by CRC TFs using single-cell CRISPR screen in malignant peripheral nerve sheath tumor (MPNST) cells.** | **Timeline** | **Site** |
|---|---|---|
| **Major Task 1: Single cell CRISPR-seq using the CRISPR-KO system** | **Months** | |
| Subtask 1: Seek HRPO review and approval for the human cell lines that we obtained from collaborators and repository prior to their use in this study. | 1 | X |

| | | |
|---|---|---|
| Subtask 2: Generation of a customized dual-gRNA library that will be used to effectively knockout candidate core transcription factors (TFs) and produce lenti-virus for transduction.<br>The sequences of pairs of gRNAs (dual-gRNAs) that target the DNA binding domain of 85 candidate core TFs have been obtained from the "Vakoc Human DNA Binding Domain-Focused CRISPR Knockout Library" (Addgene: pooled library #123334) by choosing the pairs of gRNAs that span ~80-1000 base pair of the genome, so the functional domain of a given TF will be knocked out when these dual-gRNAs are used. These dual-gRNAs will be synthesized and cloned as described in a recent publication (PMID: 32231336) and they are compatible for direct-capture of the dual-gRNAs on a 10X single-cell RNA sequencing platform. Next, the dual-gRNA library will be packaged into lenti-virus and used for transduction.<br>#dual-gRNA library will be purchased from Sigma<br>#Cell lines used: Lenti-X 293T cells (from Takara Bio) | 2-4 | X |
| Subtask 3: Carry out the CRISPR screen in MPNST cells that express nuclease Cas9, followed by direct-capture and cell barcoding for single-cell RNAseq using the 10X platform.<br>Cas9-expressing T265 cells will be transduced with lenti-virus of the dual-gRNA library and treated with puromycin before FACS sorting for blue fluorescent protein-expressing cells. A week post selection, perturbed cells will be harvested for direct-capture of the dual-gRNAs and transcriptomic profiling on a single-cell level.<br>#Cell lines used: CRISPR-KO system in which T265 cells (from Dr. Karlyne Reilly) were previously transfected with the Cas9 vector. Single clones of these Cas9-expressing cells were selected and validated for gene editing when used with gRNAs. | 4-6 | X |
| Subtask 4: Library preparation and sequencing at the core facility. | 6-7 | X |
| *Milestone(s) Achieved: all CRISPR-KO screened samples submitted for single-cell sequencing.* | 7 | |

Achieved (yes/no, date): Yes. Completed on Jan. 31, 2023. IRB approval was obtained. Dual-sgRNA library was received and amplified. Lentivirus was made and titered in each cell lines. A pilot experiment in T265 cells was completed and the cells were submitted and sequenced at the Single-cell analysis facility.

| **Major Task 2: Data analysis, integration and validation** | | |
|---|---|---|
| Subtask 1: Use standard CellRanger (10X Genomics) pipeline to analyze the raw single-cell RNAseq data obtained from Major Task 1. | 7 | X |
| Subtask 2: Apply in-house developed, customized R packages and other bioinformatic software to analyze the single-cell CRISPR-seq result. | 7-10 | X |

| | | |
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| #I predict to observe a group of super-enhancer-driven TFs regulate each other's transcription as well as the oncogenic transcriptional program of MPNST cells. | | |

| | | |
|---|---|---|
| Subtask 3: Validate the observed inter-connected regulatory network formed by a group of TFs in additional MPNST cell lines by repeating the single-cell CRISPR screen using the same dual-gRNA library.<br>#Cell lines used: Cas9-transduced S462 (PRC2-deficient, from Dr. Karlyne Reilly), PT2002 (PRC2-deficient, from the Johns Hopkins NF1 biospecimen repository), ST88-14 (PRC2-deficient, from Dr. Nancy Ratner), STS-26T (PRC2-intact, from Dr. Steven Porcelli), and MPNST724 (PRC2-intact, from Dr. Nancy Ratner) cells. | | |
| Subtask 4: Validate the observed inter-connected regulatory network formed by a group of TFs by knocking out or transcriptionally silencing them individually using additional gRNAs. I will plan to measure the transcriptomic outcome in future experiments. | 10-12 | X |
| *Milestone(s) Achieved: A group of candidate core TFs will be validated to form a feed forward core transcriptional regulatory circuitry.* | 12 | |

Achieved (yes/no, date): Yes. Completed on July 31, 2023. Data analysis in T265 cells' pilot study was completed. Repeat of the CRISPR-single-cell-seq in multiple MPNST cell lines was done using the high-throughput GEX kit and two capture lanes were done for each cell line, aiming to capture ~24000 cells per cell line.

| | | |
|---|---|---|
| **Specific Aim 2. Identify TFs and TF combinations that cause the malignant transformation of neoplastic Schwann cells and contribute to the chemotherapy resistance of MPNST.** | | |
| **Major Task 3: Generation of CRISPRa system using the *NF1$^{-/-}$*;*CDKN2A$^{-/-}$* neoplastic Schwann cells** | | |
| Subtask 1: Establish human Schwann cell lines that express dCas9-VPR.<br>#Cell lines used: *NF1$^{-/-}$* immortalized Schwann cells that were previously isolated from human patient plexiform neurofibroma. They were purchased from ATCC. These cells will be transfected with the dCas9-VPR (Addgene, Plasmid #63798) vector and selected with antibiotic drug (G418).<br>#Cell lines used: CRL-3387, CRL-3388, CRL-3389, CRL-3390, CRL-3391, CRL-3392 (all purchased from ATCC). | 1 | X |
| Subtask 2: Validate the transcriptional activating function of the CRISPRa system by using individual gRNAs targeting the promoters of a few genes.<br>#I have previously used gRNAs targeting the promoters of several oncogenic TFs in MPNST cells. I will use these gRNAs in this Schwann cell-CRISPRa system and validate their activation by qRT-PCR. | 2-3 | X |
| *Milestone(s) Achieved: CRISPRa-engineered Schwann cells are validated and ready to be used.* | 3 | |

Achieved (yes/no, date): No. Subtask 1 (transducing the immortalized Schwann cells) was completed. They will next be used to test the effect of CRISRP activation with the guidance of sgRNA targeting the promoter of gene-of-interest followed by qRT-PCR.

| | | |
|---|---|---|
| **Major Task 4: *In vitro* single nuclei CRISPRa screen in soft agar** | | |

| | | |
|---|---|---|
| Subtask 1: Generation of the customized dual-gRNA library that will be used to induce the endogenous expression of candidate core TFs and produce lenti-virus.<br>#dual-gRNAs targeting the promoters of the 85 candidate oncogenic TFs will be designed, synthesized, and cloned into the vector that allows direct-capture of their sequences in the following single-nuclei RNA sequencing experiment. The use of two gRNAs targeting the same gene will significantly increase the efficacy of transcriptional activation. This dual-gRNA library will be packaged into lenti-virus.<br>#dual-gRNA library will be purchased from Sigma<br>#Cell lines used: Lenti-X 293T cells (from Takara Bio) | 1-2 | X |

| | | |
|---|---|---|
| Subtask 2: Transduce the neoplastic Schwann cells that express dCas9-VPR with the customized dual-gRNA library, followed by antibiotic selection (puromycin), FACS sorting (blue fluorescent protein will express), and culturing in soft agar.<br>#Low melting agar will be used to ensure the isolation of intact nuclei at the end of this experiment.<br>#I will transduce the CRISPRa-engineered Schwann cells with the dual-gRNA library at a multiplicity of infection (MOI) of 0.3 (each cell will contain one vector), 1, 2 and 4 (each cell will be randomly transduced with more than one vectors that target different combinations of TFs).<br>#Schwann cells are sensitive to chemotherapy and MPNST cells are resistant. Cell in the soft agar will be treated with DMSO, topotecan (topoisomerase I inhibitor), etoposide (topoisomerase II inhibitor) or doxorubicin (alkylating agent). Since I hypothesize that the activation of oncogenic TF or TF combination will cause malignant transformation of the neoplastic Schwann cells and maintain a chemo resistant phenotype, I expect to observe the growth of colonies as a result of activating oncogenic TF(s) in soft agar that is treated with or without chemotherapy drugs. | 2-3 | X |
| Subtask 3: Change culturing media twice a week and monitor the soft agar for colony formation. Harvest any formed colonies for nuclei extraction.<br>#Isolated nuclei can be used for direct-capture and downstream single-nuclei transcriptomic profiling (10X Genomics). | 4-6 | X |
| *Milestone(s) Achieved: colony formation upon activation of oncogenic core TF and TF combination and nuclei ready to be submitted for sequencing.* | 6 | |

Achieved (yes/no, date): No.

| **Major Task 5: Data analysis, oncogenic core TF identification and validation** | | |
|---|---|---|
| Subtask 1: Submit nuclei for direct-capture and sequencing of the gRNA library and gene expression library on a single-cell level. | 6-7 | X |
| Subtask 2: Obtain sequencing result and analyze with in-house developed bioinformatic tools (as described in Major Task 2). Activation of a MPNST-specific oncogenic program will be confirmed using the gene signature that is unique to human MPNST cells. | 7-9 | X |
| Subtask 3: Perform validating transformation experiments with the identified TF and TF combinations. | 9-12 | X |

| | | |
|---|---|---|
| *Milestone(s) Achieved: identification of TFs or TF combinations that have the capacity to transform neoplastic Schwann cells to form MPNST-like colonies; get ready for publication of 1 peer reviewed paper.* | 12 | |

Achieved (yes/no, date): No.

**What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

The dual-sgRNA library was synthesized and amplified by the Genome Modification Core facility of the NCI Frederick National Laboratory for Cancer Research. In this library, each sgRNA construct has two validated sgRNAs that target the same gene. Since these target genes are human transcription factors (TF), to ensure the efficacy of disrupting their functions, sgRNA pairs that target the DNA binding domains of each TF were used. As shown in Figure 1, sgRNA pairs were cloned in pJR85 (addgene plasmid #140095) plasmid, which is resistant to puromycine (puro) in mammalian cell culture. The puro-resistant cells are also positive of blue fluorescent protein (BFP) that could be detected in flow cytometer.

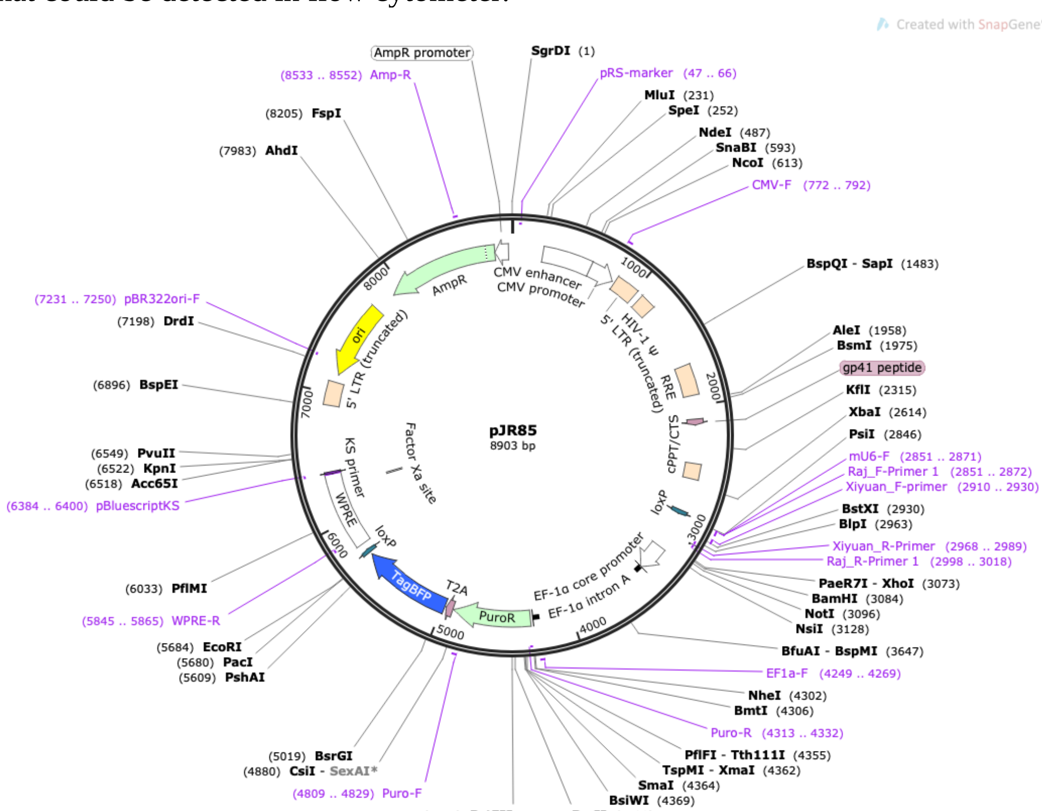


Figure 1. Plasmid map showing that sgRNA constructs are resistant to puromycine and BFP positive.

Lentivirus of the sgRNA library was made using the Lenti-X HEK cells. MPNST cell line T265 that was previously transduced with the doxycycline (Dox) – inducible Cas9 virus was transduced with the lentivirus and selected with puro for a week (1 μg/mL) and then sorted with a Sony cell sorter for BFP+ cells. The puro-resistant and BFP+ Cas9-expression T265 cells were next treated with Dox (0.5 μg/mL) for one week before captured for single-cell RNA sequencing. A total of 7598 cells were captured in one lane using the 10x Genomic platform. After quality control to get rid of low-quality cells (nFeature_RNA > 500 & nFeature_RNA < 8000 & percent.mt <15), a total of 7179 cells were retained in downstream analysis. After removing cells that either did not contain a detectable sgRNA or contained more than one detected sgRNA, a total of 3250 cells were retained for the analysis of perturbation effect. Next, R package Seurat and the MixScape pipeline were used to analyze the data. Because of the limited cell number in this pilot study, about 5-100 cells per target gene were available for analysis. This problem would be solved in the actual experiment when more cells were to be captured and sequenced. Shown in Figure 2, the top50 differentially expressed genes in cells with MYC perturbation shows the perturbing effect of MYC knockout and included other enhancer-driven TF.

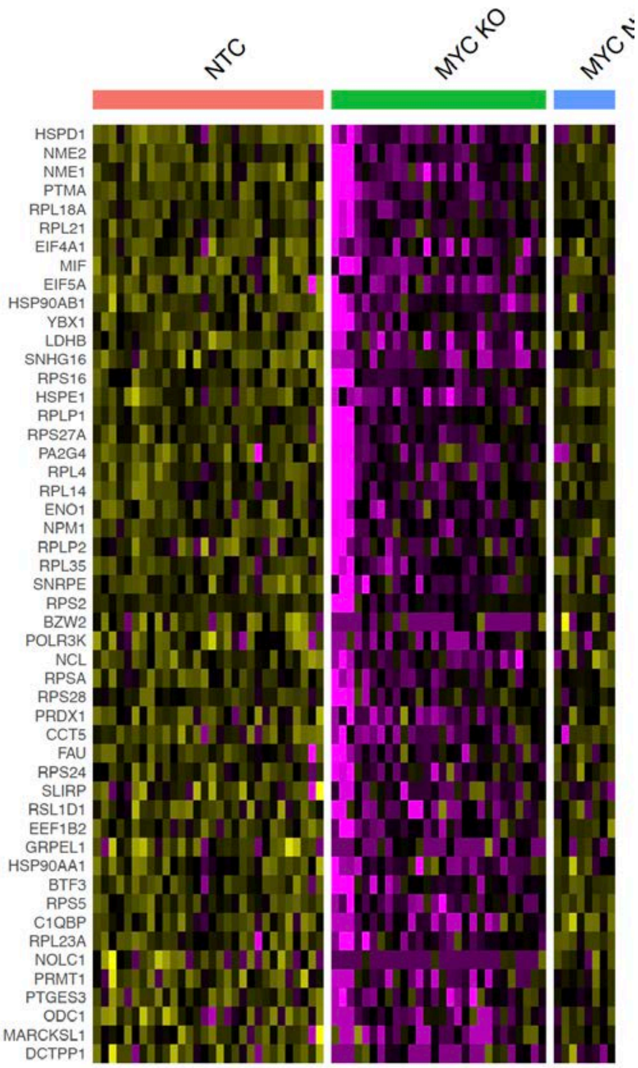


Figure 2. Heatmap showing the top 50 differentially expressed genes in control cells, and cells harboring MYC perturbation.

After seeing the success of this pilot experiment, I went ahead with the repeat of the perturb-seq in multiple MPNST cells using a high-throughput gene expression system (10x Genomics). Similarly, cells that previously transduced with a Dox-inducible Cas9 virus were transduced with the dual-sgRNA library, selected with puro, sorted for the BFP positivity, recovered, and treated with Dox for one week before captured for transcriptomic profiling on a single-cell level.

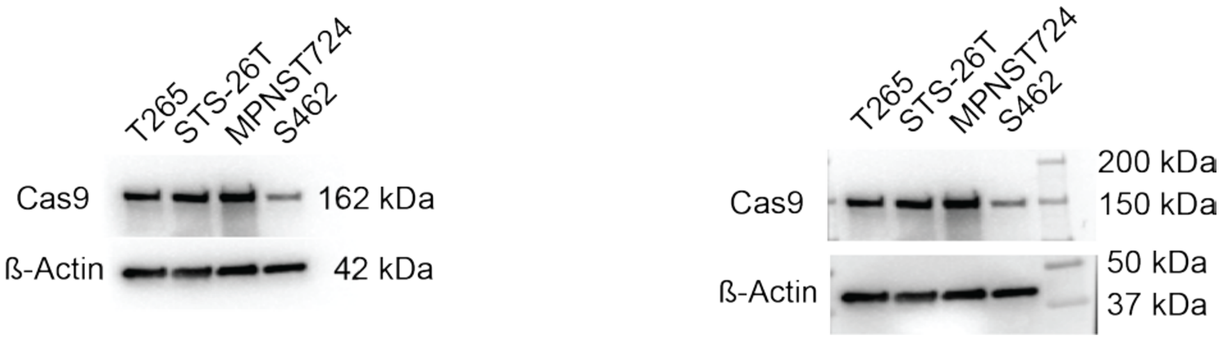


Figure 3 Western blotting result of Cas9 in MPNST cell lines showing that they expressed Cas9 at the time of capture.

Two capture lanes per cell line were used to aim for 24000 cells. The table below shows the number of cells captured per cell line, as well as the sgRNA detection rate in each capture lane.

| Sample Name | Estimated Number of Cells | Mean Reads per Cell | Median Genes per Cell | CRISPR: Guide Reads in Cells |
|---|---|---|---|---|
| **SCAF3226_T265_1** | 25,555 | 58,455 | 6,709 | 66.50% |
| **SCAF3227_STS_26T_1** | 14,345 | 66,080 | 7,092 | 85.40% |
| **SCAF3228_S462_1** | 11,940 | 81,165 | 8,122 | 82.70% |
| **SCAF3229_MPNST724_1** | 12,380 | 57,118 | 6,480 | 79.30% |
| **SCAF3230_T265_2** | 21,344 | 59,769 | 6,743 | 70.50% |
| **SCAF3231_STS_26T_2** | 12,459 | 78,730 | 7,773 | 91.70% |
| **SCAF3232_S462_2** | 13,659 | 91,908 | 8,323 | 86.80% |
| **SCAF3233_MPNST724_2** | 11,264 | 59,672 | 6,643 | 88.40% |

From the perturbation effect of these candidate core TFs, an interconnected transcriptomic loop was detected.

**What opportunities for training and professional development has the project provided?**
*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

| |
|---|
| *Nothing to Report.* |

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

| |
|---|
| *Nothing to Report.* |

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

*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

I will focus my investigation on the Specific Aim 2 to begin with the validation of CRISPRa-engineered human immortalized Schwann cell lines.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**What was the impact on the development of the principal discipline(s) of the project?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

*Nothing to Report.*

**What was the impact on other disciplines?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

*Nothing to Report.*

**What was the impact on technology transfer?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

| |
|---|
| *Nothing to Report.* |

**What was the impact on society beyond science and technology?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

| |
|---|
| *Nothing to Report.* |

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

*Nothing to Report.*

**Actual or anticipated problems or delays and actions or plans to resolve them**
*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

There was a delay in completing the aims outlined in the proposal that prompted a request for a no cost extension which was awarded on 23 June 2023. With the extension, the timeline is now back on track for timely completion.

**Changes that had a significant impact on expenditures**
*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

*Nothing to Report.*

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

*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

*Nothing to Report.*

**Significant changes in use or care of vertebrate animals**

*Nothing to Report.*

**Significant changes in use of biohazards and/or select agents**

*Nothing to Report.*

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

*Report only the major publication(s) resulting from the work under this award.*

**Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

| |
|---|
| *Nothing to Report.* |

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

| |
|---|
| *Nothing to Report.* |

**Other publications, conference papers and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

| |
|---|
| *Nothing to Report* |

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

  *List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

| |
|---|
| *Nothing to Report.* |

- **Technologies or techniques**
  *Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

| |
|---|
| *Nothing to Report.* |

- **Inventions, patent applications, and/or licenses**
  *Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

| |
|---|
| *Nothing to Report.* |

- **Other Products**
  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding,*

*prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

| |
|---|
| *Nothing to Report.* |

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".*

*<u>Example:</u>*

| | |
|---|---|
| *Name:* | *Mary Smith* |
| *Project Role:* | *Graduate Student* |
| *Researcher Identifier (e.g. ORCID ID):* | *1234567* |
| *Nearest person month worked:* | *5* |
| *Contribution to Project:* | *Ms. Smith has performed work in the area of combined error-control and constrained coding.* |
| *Funding Support:* | *The Ford Foundation (Complete only if the funding support is provided from other than this award.)* |

| | |
|---|---|
| Name: | Xiyuan Zhang |
| Project Role: | PI |
| Researcher Identifier: | 0000-0001-9949-5258 |
| Nearest person month worked: | 4 calendar months |
| Contribution to Project: | Dr. Zhang has supervised and performed all of the proposed work in this study. |
| Funding Support: | NIH intramural research program and the Early Investigator Research Award (DoD Neurofibromatosis Research Program). |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

| |
|---|
| *Nothing to Report.* |

**What other organizations were involved as partners?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

*Nothing to Report.*

8. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*