CRISPR-based transcriptional repression to perform immunomodulation *in vivo*

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Overview

Background:

- Myeloid differentiation primary response 88 (MyD88) is a key node in innate and adaptive immune responses, acting as an essential adaptor molecule for several signaling pathways.
- Recent repurposing of the Clustered Regularly Interspace Short Palindromic Repeat (CRISPR) system for transcriptional modulation has opened new avenues for developing novel therapeutic opportunities.

Hypothesis:

- Transcriptional control over endogenous *Myd88*, can be an effective and readily available strategy as a de-immunization modality for viral gene therapy.

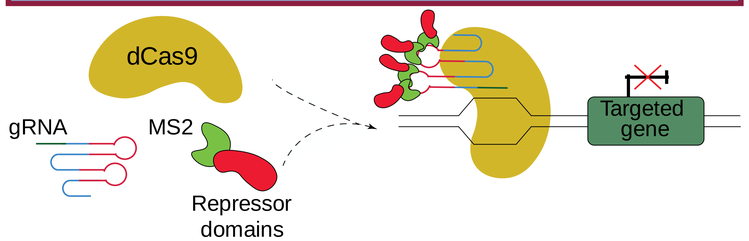
Method:

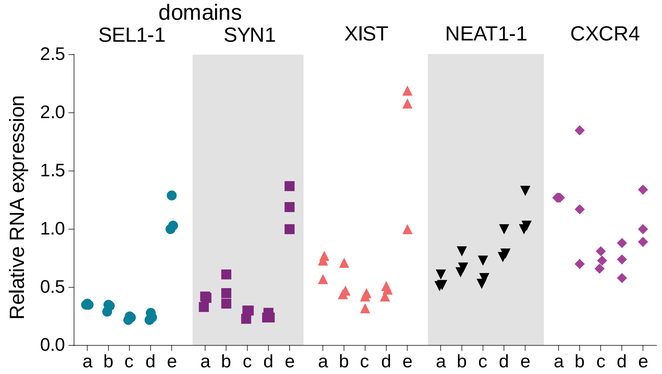
- We developed a potent CRISPR-based transcription repressor.
- Our engineered repressor relies on simultaneous employment of two repressor-domains, fused to MS2 coat protein and truncated guide RNA (gRNA) from 5' end, which enables Cas9 protein to perform transcriptional modulation of the targeted gene.
- We used this system to achieve synthetic immunomodulation through regulation of endogenous *Myd88* levels *in vivo*.

Results:

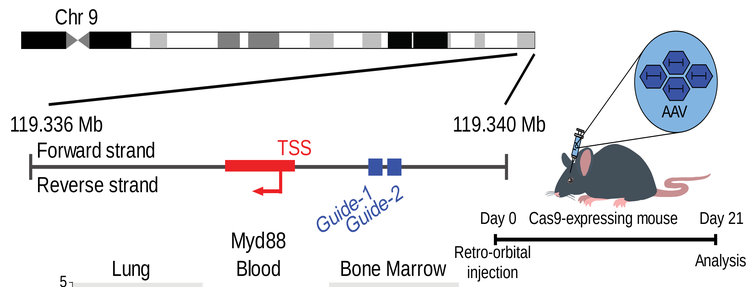
- We show using this system we can efficiently achieve a functionally relevant phenotype through transcriptional repression of targeted gene *in vivo* (above 60% in different organs).
- Our data demonstrate modulation of general immunoglobulin expression patterns followed by receiving the CRISPR based synthetic repressor *in vivo*.
- Our data presents an exciting opportunity to modulate humoral immunity against AAV possibly through prophylactic repression of *Myd88*.

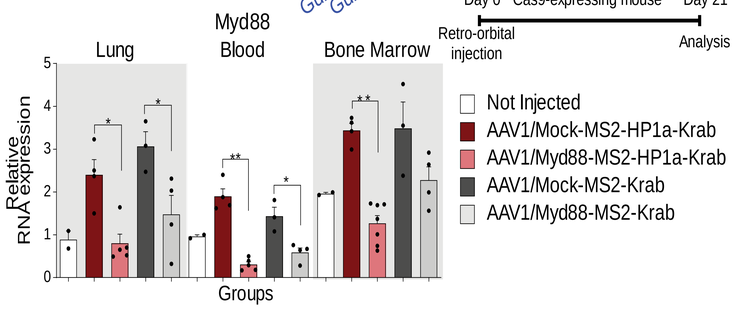
Aptamer-mediated CRISPR repression *in vitro* targeting a set of genes in HEK293FT cells.



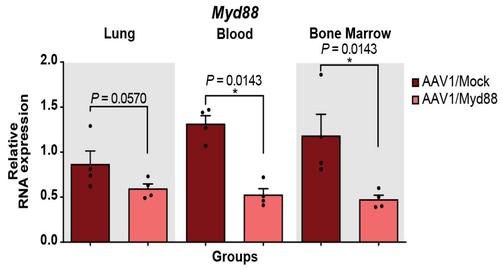


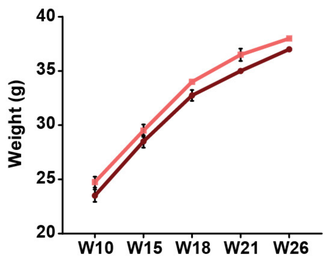
CRISPR-based targeted *Myd88* repression *in vivo* using MS2 repressors.

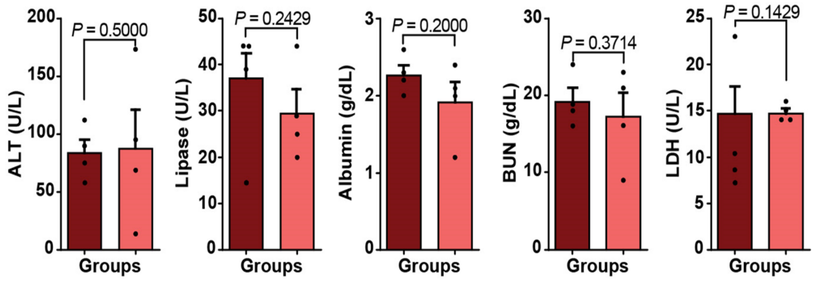




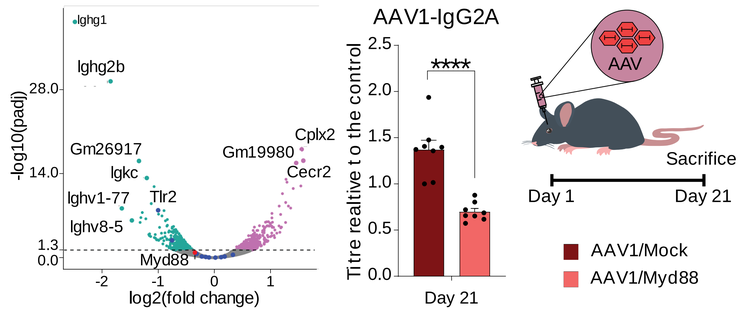
CRISPR-based repression of Myd88 in vivo was safe and lacked long term adverse events



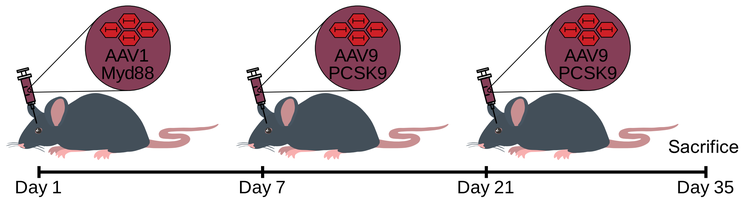




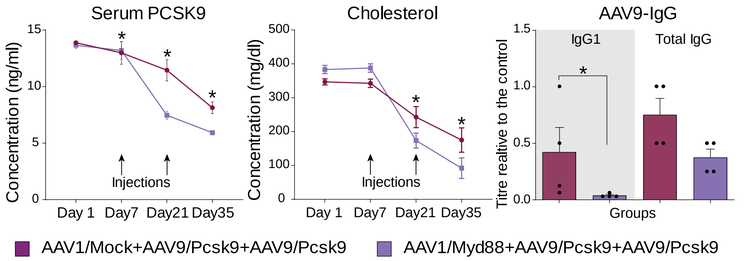
Administration of AAV1/Myd88 *in vivo* leads to modulation of humoral immunity against AAV.



Modulating humoral immunity against AAV using CRISPR-based *Myd88* repression *in vivo* leads to enhanced AAV-based gene therapies.



Analysis of plasma



Summary

- We developed and tested a novel strategy for application of CRISPR-based transcriptional regulators to control immune response *in vivo*.
- We report that CRISPR-mediated endogenous repression of *Myd88* is a promising strategy to reprogram inflammatory responses implicated in innate immunity and was shown to be safe.
- We demonstrate that transient transcriptional repression of Myd88 via CRISPR was effective as de-immunization strategy, along with increased transgene expression and has implication for reduced dosing concentration with optimized schedules.

Acknowledgements

This work was primarily supported by startup fund by the School of Biological and Health Systems Engineering of Ira. A Fulton Schools of Engineering at Arizona State University as well as an RO1 grant from National Institute of Biomedical Imaging and Bioengineering (R01EB024562) to S.K. and M.R.E. and DARPA Young Faculty Award (D16AP00047) to S.K. A.C. is supported by a Career Awards for Medical Scientists from the Burroughs Welcome Fund.