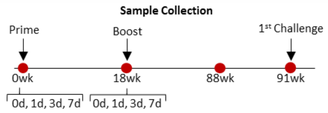
Transcriptomic Host Responses to RhCMV/SIV Vaccination in Rhesus Macaques

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BACKGROUND

The rhesus cytomegalovirus (RhCMV) strain 68-1 vaccine against simian immunodeficiency virus (SIV) induces a T cell response that protects over 50% of vaccinated rhesus macaques (RM) to clear infection against multiple SIV challenge including distinct challenge routes. To define the molecular features of the host response to vaccination and the underlying gene signature of vaccine protection we assessed the transcriptional responses of protected and non-protected RMs following vaccination prior to challenge.

A



B

Protection Outcome		
Animal Group	Delivery	Protection
Group 1	Oral	9/15 (60%)
Group 2	SubQ (3 sites)	8/15 (53%)

C

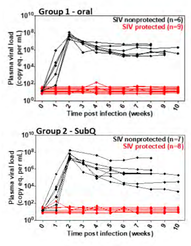
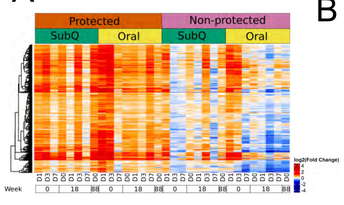


Fig 1) *Study design. (A). Sample collection. (B). Proportions of protection outcome for Subcutaneous and Oral administration groups. (C). Plasma viral loads following infection.*

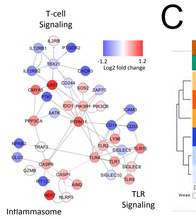
STUDY DESIGN

Two groups of 15 RMs were administered 68-1 RhCMV/SIV vaccine via oral or subcutaneous delivery (Fig. 1A). Following vaccination, RMs were subjected to repeated limiting dose intrarectal SIVmac239 challenge until infected as detected as plasma virus or de novo development of SIV Vif-specific T cell response. During vaccination phase, blood samples were collected at time points near prime, boost, and before challenge. mRNA-seq was performed followed by bioinformatics analysis.

A



B



C

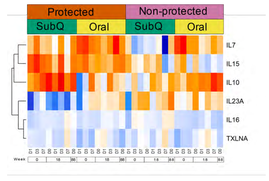
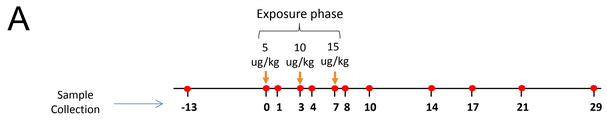


Fig 2) *Gene correlates of protection. All colors indicate log2(fold change) over corresponding baseline samples (A). Protective correlates heatmap (FDR < 0.05, absolute fold-change > 1.5. (B). Interactions between DE genes within pathways significantly upregulated in protective correlates (p-value < 0.05, Ingenuity Pathway Analysis). (C). Interleukin genes that passed background count threshold.*

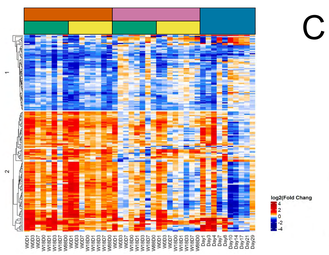
LINEAR MODELING RESULTS

Using linear modeling, we identified a subset of genes that correlate with protection and are sustained throughout the time series (Fig. 2A). Enriched pathways included Toll-like Receptor, Inflammasome, T-Cell Receptor Signaling (Fig. 2B), and Interleukin Signaling where only *Il15* differentiated by protective outcome (Fig. 2C). We then assessed the correlates of protection in a naïve cohort of RM treated with IL-15 at increasing doses (Fig. 3A). We identified 195 genes that correspond with both protection and IL-15 treatment response (Fig. 3B), wherein co-expression analysis revealed enrichment in innate immune and cytokine signaling responses (Fig. 3C,D).

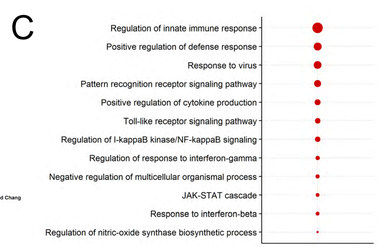
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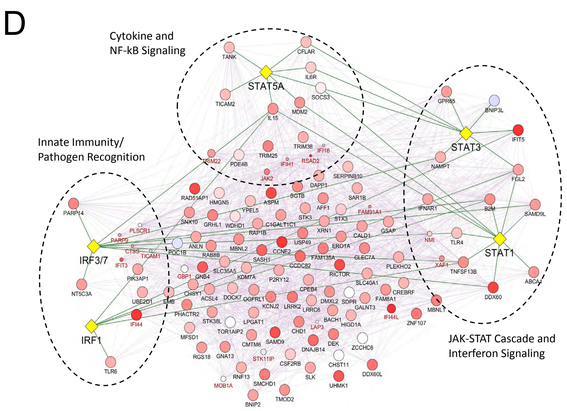
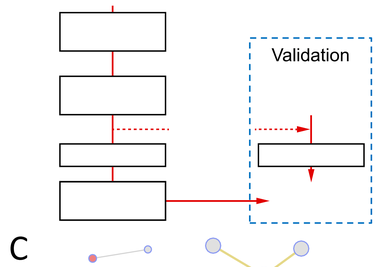


Fig 3) *IL-15 and protection correlates. (A). Study design. Five naïve RM were treated with increasing doses of IL-15 administered subcutaneously. (B) Heatmap of IL-15 protective signature. (C) Enriched pathways in IL-15 protective signature. (D) IL-15 protective signature co-expression network. Predicted transcription factors are yellow diamonds with canonically regulated genes connected by green edges*

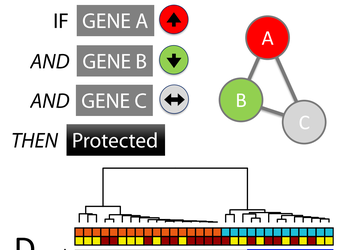
RULE BASED MODELING

To determine if transcriptomic profiles could predict vaccine protection, we used a machine learning approach. First, we trained a rule-based model (RBM) based on the 30 RhCMV/SIV vaccinated RMs. The resulting RBM showed over 90% accuracy using cross-validation. We also validated the RBM using a training set of 6 RhCMV/SIV vaccinated RMs.

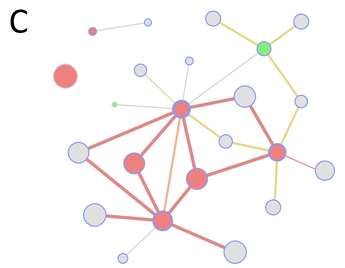
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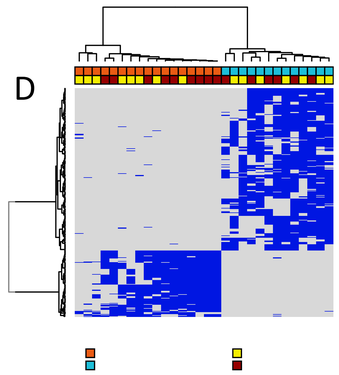


Fig 3) *Rule-based modeling. (A) Machine learning pipeline. (B) Example rule with "co-classifying" genes. (C) Rule network of top classifiers. (D) Rule heatmap, showing clustering between RMs based on RBM.*

CONCLUSIONS

Our results indicate that vaccine-elicited protection is associated with transcriptional responses of innate (TLR, inflammasome) and adaptive immune pathways (T-cells) in conjunction with IL-15 cytokine signaling. We have also created a machine learning model that predicts protection outcome with high accuracy.

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