Comparative Plasma Proteomics Reveals Divergent Responses to SIV in Natural and Nonnatural Hosts

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Background

- Sooty mangabeys (SMs), natural hosts of SIV, sustain high viral loads without developing AIDS.
- Rhesus macaques (RMs), non-natural hosts, develop AIDS-like disease similar to human HIV infection.
- Non-pathogenic SIV infection in SMs is characterized by limited immune activation[1], preserved CD4⁺ T cells[2], and reduced microbial translocation.
- The systemic molecular pathways underlying these divergent outcomes remain incompletely defined.

Central Question:
What plasma proteomic features distinguish pathogenic (RM) from non-pathogenic (SM) responses to SIV infection?

Methods

- Study Design: Plasma was collected from chronically SIV-infected (no ART) rhesus macaques and sooty mangabeys and from matched uninfected controls (n = 6 per group)
- Sample Processing: Plasma proteins processed using PreOmics® ENRICH-iST, including denaturation, reduction, alkylation, enzymatic digestion, and peptide cleanup.
- Data Acquisition: Peptides analyzed by DIA-PASEF LC–MS/MS on a Bruker timsTOF fleX coupled to a nanoElute 2 system.
- Data Analysis: DIA proteomic data processed using FragPipe / DIA-NN and interpreted using network, differential abundance, and functional enrichment analyses.

Experimental Design

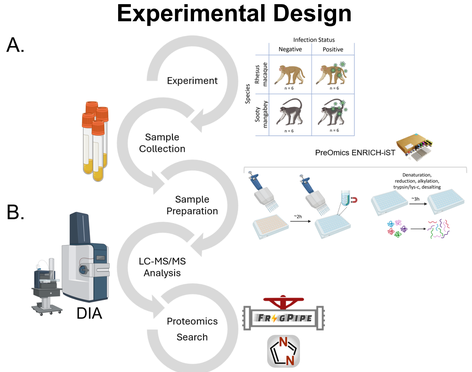


Figure 1. Experimental and bioinformatics workflow for DIA-based proteomics from Rhesus macaque or Sooty mangabey plasma. (A.) Plasma was collected from Rhesus macaques (*Macaca mulatta*) and Sooty mangabeys (*Cercocebus atys*) with defined infection status. Plasma samples were prepared using the PreOmics ENRICH-iST kit and analyzed by LC–MS/MS using a Bruker nanoElute 2 and timsTOF FLeX using a data-independent acquisition (DIA) acquisition method. **(B.)** Bruker .d files were processed through FragPipe with DIA-NN to generate protein abundance matrices. Data were log2 transformed and median-centered using TAMPOR using the human canonical FASTA, followed by bootstrap regression against covariates (age, sex, and acquisition batches), and weighted gene co-expression network analysis. Downstream analyses included differential expression, gene ontology, and pathway visualization.

Results

Distinct plasma protein networks are differentially regulated by SIV infection.

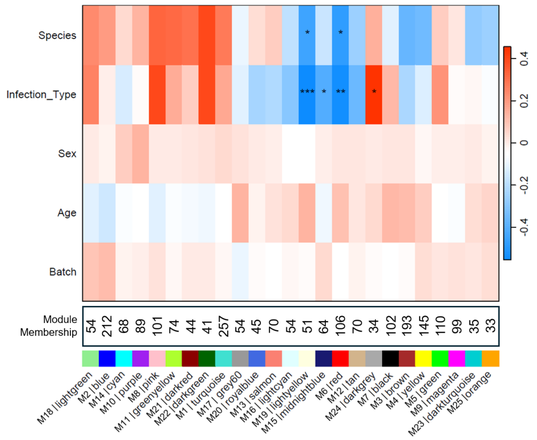


Figure 2. Network analysis reveals species specific response. Module-trait correlation heatmap showing biweight midcorrelation coefficients (bicor *r*) with experimental variables after weighted co-expression network analysis (WGCNA). 2,205 of 2,341 proteins were utilized with WGCNA. Box color indicates correlation directionality and significant associations are indicated as P *<0.05, **<0.01, ***<0.005. Module membership refers to number of proteins within module after co-expression clustering. Infection_Type = Noninfected vs SIV-infected.

Lipid metabolism proteins are decreased in SIV-infected SM but not in SIV-infected RM.

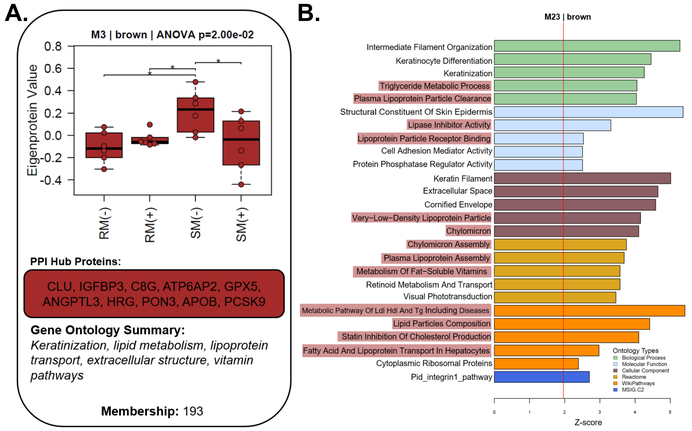


Figure 3. SIV tolerance is associated with lipid metabolic remodeling. (A.) Module M3 (brown) eigengene values and analyses. Boxplots show median ± IQR; significance by one-way ANOVA. Hub proteins include APOB, APOA2, PCSK9, CLU, GPX5, and ANGTPL3. **(B.)** Enriched Gene Ontology (GO) and Reactome terms indicate roles in keratinization, lipid metabolism, lipoprotein transport, and extracellular organization. Dashed lines denote significance thresholds; bars in enrichment plots are colored by ontology type (Biological Process, Molecular Function, Cellular Component, Reactome, or WikiPathways). Highlighted GO terms indicate association with lipids and lipid metabolism.

Ubiquitin-based proteostasis and autophagy proteins are increased in SIV-infected SM but not in SIV-infected RM.

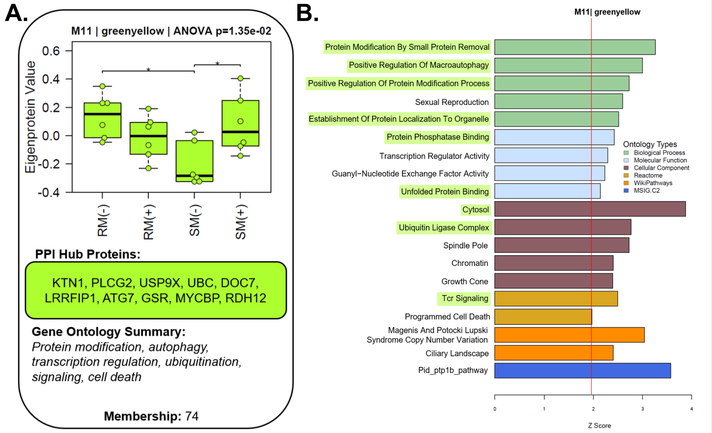
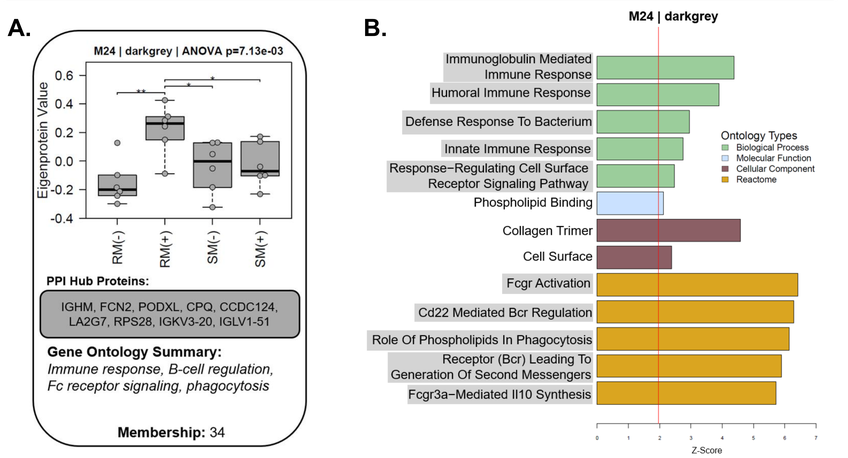


Figure 4. SIV tolerance is associated with adaptations to ubiquitin-mediated proteostasis. (A.) Module M11 (greenyellow) eigengene values showing increased expression in SM(+) relative to SM(–), whereas RM shows no infection-associated change. Boxplots display median ± IQR; significance determined by one-way ANOVA. Hub proteins include KTN1, PLCG2, USP9X, UBC, DOC7, ATG7, and GSR. **(B.)** Enriched Gene Ontology and Reactome terms for M11 highlight pathways involved in protein modification, autophagy, ubiquitination, transcriptional regulation, and cell-death signaling. Bars are colored by ontology type (Biological Process, Molecular Function, Cellular Component, Reactome, WikiPathways, or MSigDB C2). Dashed lines indicate statistical thresholds; enrichment magnitude is represented by Z-score. Highlighted GO terms indicate association with proteostasis and autophagy.

Immune response proteins, especially those associated with the complement cascade, are increased in SIV-infected RM but not in SIV-infected SM.



C.

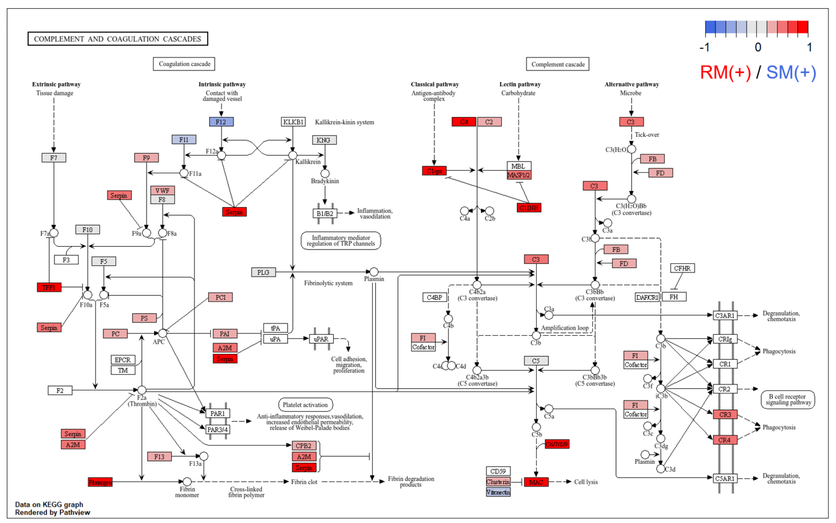


Figure 5. Pathogenic infection is characterized by humoral immune activation. (A.) Module eigengene M24 (dark grey) values across groups showing increased expression in RM(+) relative to RM(–) and Sooty mangabey groups. Boxplots show median ± IQR; significance by one-way ANOVA. Key hub proteins within the module include IGHM, FCN2, PODXL, CPQ, CCDC124, and IGKV3-20. **(B.)** Functional enrichment analysis of M24 members indicating overrepresentation of immune-related terms including immunoglobulin-mediated response, Fc-receptor signaling, and phagocytosis. Bars are colored by ontology type (Biological Process, Molecular Function, Cellular Component, Reactome, WikiPathways, or MSigDB C2). Highlighted GO terms indicate association with immune response. **(C.)** Pathway visualization of complement activation between RM(+) and SM(+). Color scale represents $\log_2$ fold-change (RM(+)/SM(+)); red indicates increased abundance in RM(+), blue indicates increased abundance in SM(+). Pathway visualizations were generated using Pathview (v1.48.0).

Conclusions

- Plasma proteomics with network analysis reveal distinct host strategies underlying pathogenic versus non-pathogenic outcomes during SIV infection (Fig. 2).
- Infected sooty mangabeys (SM+) limit humoral activation while remodeling lipid metabolism (Fig. 3) and ubiquitin-based proteostasis (Fig. 4), which may be associated with their well-described tolerance to high viral loads without progression to AIDS-like disease.
- Infected rhesus macaques (RM+) exhibit a dominant immune-centric humoral response consistent with chronic immune activation and progression to an AIDS-like disease (Fig. 5).

Summary

Central Finding

- Non-pathogenic SIV infection is defined by metabolic and proteostatic adaptation, whereas pathogenic infection is dominated by heightened humoral immune activation.

Plain Language

- Some primates infected with HIV-like viruses remain healthy despite high levels of virus, while others develop AIDS-like disease, similar to HIV infection in humans.
- By comparing blood proteins, this study shows that resistant primates rely on alternative biological strategies, such as lipid metabolism and protein quality control systems, which may help prevent disease progression and inform future therapies.

Additional Key Information

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