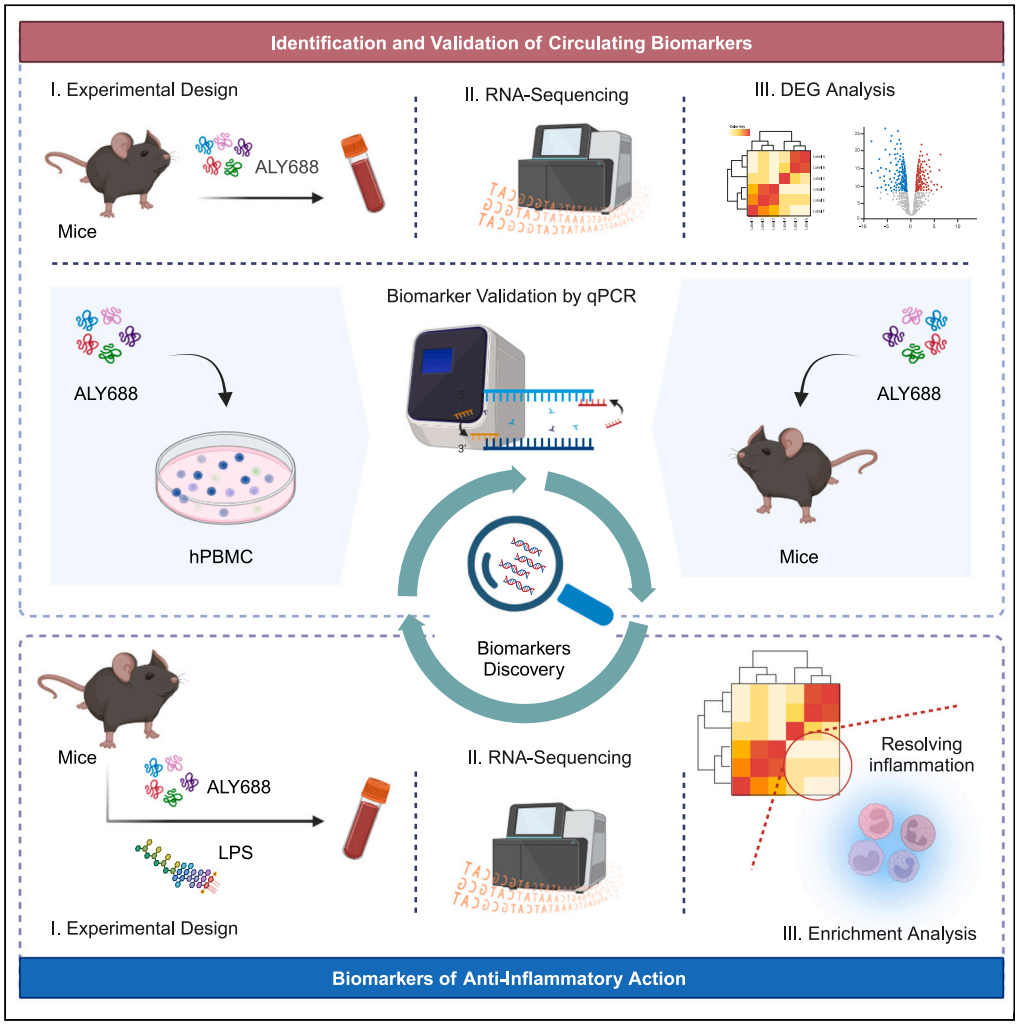
iScience

CellPress
OPEN ACCESS

Article

Development of a non-invasive bioassay for adiponectin target engagement in mice



Jialing Tang, Yubin Lei, Angelica Pignalosa, Henry H. Hsu, Ali A. Abdul-Sater, Gary Sweeney

aasater@yorku.ca (A.A.A.-S.)
gsweeney@yorku.ca (G.S.)

Highlights

Identified a cluster of genes as biomarkers to monitor ALY688 activity in blood

Validate the reliability of proposed biomarkers in human PBMC

Discovered ALY688 conferred anti-inflammatory effects in LPS-induced sepsis model

Tang et al., iScience 27, 110994
October 18, 2024
https://doi.org/10.1016/j.isci.2024.110994

iScience

CellPress
OPEN ACCESS

Article

Development of a non-invasive bioassay for adiponectin target engagement in mice

Jialing Tang,[1] Yubin Lei,[1] Angelica Pignalosa,[2] Henry H. Hsu,[2] Ali A. Abdul-Sater,[3,*] and Gary Sweeney[1,4,*]

SUMMARY

Adiponectin-based therapeutic strategies are promising for managing metabolic diseases and reducing inflammation, prompting the development of adiponectin receptor agonists. However, monitoring their pharmacodynamic actions in clinical applications is challenging. This study aimed to identify peripheral biomarkers to monitor adiponectin actions using ALY688, an adiponectin receptor agonist peptide. RNA sequencing analysis of whole blood identified a cluster of genes that were significantly increased in the ALY688-treated group compared to the control. This gene cluster was validated by qPCR and further confirmed in human peripheral blood mononuclear cells treated with ALY688 *ex vivo*. We also confirmed a functional outcome of ALY688 action in mice as our study also demonstrated the anti-inflammatory effect of ALY688 in a sublethal LPS mouse model. In summary, a newly identified gene cluster signature is suitable for assessing the pharmacodynamic action of adiponectin or its mimetics in blood samples.

INTRODUCTION

Adiponectin is a protein synthesized by adipocytes, exhibiting robust effects on lipid metabolism, insulin sensitivity, and cardiovascular regulation.[1–3] Subsequently, it was discovered that adiponectin is expressed not only by adipocytes but also by skeletal muscle cells, cardiac myocytes, and endothelial cells. Adiponectin exists in two forms: the full-length protein and a proteolytic cleavage fragment. This fragment comprises the globular C-terminal domain, commonly referred to as globular adiponectin. The cleavage process believed to generate the globular fragment of adiponectin is thought to be mediated by leukocyte elastase, which is secreted by activated monocytes and/or neutrophils. Adiponectin has gained a lot of interest due to its insulin sensitizing, anti-inflammatory, and antiapoptotic properties.[4,5] Adiponectin serves as a pivotal regulator of the innate immune system and plays a significant role in the development of inflammation and metabolic disorders.[6,7] Clinical studies have revealed an independent and inverse correlation between circulating adiponectin levels and various components of the metabolic syndrome. This includes insulin resistance, body weight, blood pressure, and serum lipids. Furthermore, adiponectin has been found to be negatively associated with cardiovascular diseases such as atherosclerosis and myocardial infarction.[8–11]

After more than a decade of research, the functions of adiponectin and its signaling pathways have been largely identified, primarily through two specific adiponectin receptors (AdipoR1 and AdipoR2).[12,13] The molecular actions of adiponectin imply that the molecule itself or agonists targeting its receptors hold potential for the treatment of obesity and its associated comorbidities.[12,13] Indeed, in rodent models, the administration of adiponectin has shown to enhance insulin sensitivity and glucose metabolism, elevate insulin secretion, and decrease body weight,[14,15] as well as demonstrated the role of preventing cardiac remodeling and improving cardiac function after myocardial infarction.[16,17] Targeting its receptors, a recent demonstration revealed that AdipoRon, an orally active synthetic small-molecule adiponectin receptor agonist, markedly enhanced insulin sensitivity and glucose tolerance in mice,[18] and demonstrated cardioprotective effect in the ischemic mouse model.[19] Conversely, the inhibition of AdipoR1 activation, coupled with pharmacological administration of adiponectin, demonstrated the reversal of post-myocardial infarction remodeling and mitigation of heart failure progression.[20] Consequently, there is substantial pharmaceutical interest in developing compounds that target the adiponectin receptor to mimic adiponectin actions. One such compound is ALY688, a 10-amino acid peptide spanning the active site of the adiponectin globular domain. Studies have demonstrated its ability to bind to AdipoR1 and AdipoR2 and induce adiponectin-like effects in several cellular and animal models.[21–24]

As therapeutic interventions mimicking adiponectin progress toward the initiation of clinical trials, the establishment of a dependable target engagement marker becomes imperative for monitoring pharmacodynamics in patients. Such an assay would also have significant value in research studies of animal models. The objective of this study was to develop a bioassay suitable for both clinical and experimental applications, based on a small blood sample. The implementation of such an assay stands to facilitate the non-intrusive, temporal monitoring of adiponectin-like effects. In this study, our goal was to develop such an assay via identifying and validating a cluster of genes that were

[1]Department of Biology, York University, 4700 Keele Street, Toronto, ON M3J 1P3, Canada
[2]Allysta Pharmaceuticals Inc., 500 108th Avenue NE, Suite 1100, Bellevue, WA 98004, USA
[3]School of Kinesiology and Health Science, York University, 4700 Keele Street, Toronto, ON M3J 1P3, Canada
[4]Lead contact
*Correspondence: aasater@yorku.ca (A.A.A.-S.), gsweeney@yorku.ca (G.S.)
https://doi.org/10.1016/j.isci.2024.110994

significantly altered by ALY688. This was achieved via whole-genome RNA sequencing, followed by validation in human peripheral blood mononuclear cells (PBMC). Our investigation also encompassed examining the impact of ALY688 on inflammatory responses in mice subjected to the potent pro-inflammatory endotoxin, lipopolysaccharide (LPS).

RESULTS

Identification of potential biomarkers of ALY688 administration in mouse peripheral blood

In order to determine whether expression of certain genes can be used as a blood-based biomarker for ALY688 administration *in vivo*, whole blood was collected from mice 24 h post ALY688 (15 mg/kg) or vehicle (PBS) followed by RNA sequencing analysis (Figure 1A). Differentially expressed genes (DEGs) analysis was performed by screening read counts mean value more than 10, adjusted $p \leq 0.05$, fold change ≥ 2, including a total of 170 DEGs, 13 were downregulated and 157 were upregulated in ALY688 treated mice compared to vehicle (PBS) group (Figures 1B and 1C). Hierarchical clustering showed that the gene expression profile of ALY688 was distinct from PBS (Figure 1D). We then ranked genes by the expression to identify the top 20 ALY688-induced genes, with an adjusted $p \leq 0.05$ (Table 1; Figure 1E), and top 10 ALY688-induced genes with a higher significance (adjusted $p \leq 0.01$) (Table 2). Remarkably, Gene Ontology (GO) term analysis illustrated that these ALY688-induced genes are associated with processes involved in the regulation of T cell differentiation, regulation of inflammatory responses, regulation of polyunsaturated fatty acid metabolic process, DNA damage, collagen formation, copper-dependent protein binding, and ubiquitin-proteasome dependent proteolysis (Figures 1F and 1G). Among the top upregulated genes, *Rorc*, *Myb* enriched adaptive immune response and T cell differentiation, lymphocyte differentiation pathways, *C1qc* enriched immunoglobulin-mediated immune response pathway, and *Jag2* was found in the T cell differentiation pathway (Table S3). In essence, these genes can serve as biomarkers for ALY688 activity.

Validation of the potential biomarker in human PBMCs and mouse peripheral blood

To determine whether the biomarker candidates identified in mice are clinically relevant, healthy human PBMCs were treated with ALY688 *ex vivo* (Figure 2A) to examine gene expression of the top DEGs identified in our RNA sequencing analysis in mice. Remarkably, expression of 16 out of 20 genes that were examined were significantly increased in the ALY688 treated PBMCs compared to those treated with PBS (Figures 2B–2Q). *Aclam*, *Igha*, *Msmo1*, and *Zbtb20* were unchanged following ALY688 treatment (Figures S1A–S1D). Then PLS-DA (Partial Least Squares Discriminant Analysis) was performed, showing the degree of separation between clusters of different treatment, PBS and ALY688 (Figure 2R). Based on the proximity within the genes altered by ALY688, which suggesting the high intra-group similarity, we then proposed the group of genes, *Tgfb2*, *Rorc*, *Myb*, *Prg4*, and Il17rb, as a candidate panel of biomarkers to assess the action of ALY688 in circulation (Table 3). To further verify the reliability of these 6 genes expression upon ALY688 stimulation, blood from mice subjected to PBS or ALY688 injection was collected. Expression of the proposed biomarkers were then measured and suggested that *Tgfb2*, *Rorc*, *Prg4*, and *Il17rb* demonstrated significant increase upon ALY688, and there is a trend of increase in *Myb* ($p = 0.0576$), together intensifying the feasibility of this assay (Figure 3).

ALY688 administration inhibits LPS-induced inflammatory gene expression in mice whole blood

To assess potential anti-inflammatory effects of ALY688, we injected mice with a sublethal dose of LPS[25] (Figure 4A). RNA sequencing analysis from whole blood samples demonstrated that 2994 genes were differentially expressed in LPS treated mice compared to control (PBS) (Figure 4B). DEGs highlighted a strong distinction between the two groups, as evidenced by the heatmap (Figure 4C). Besides, among the DEGs, it's suggested that 1536 genes were upregulated by the stimulation of LPS, while 1,458 genes showed downregulation (Figures 4B and 4D). Among DEGs, significantly increased the output of pro-inflammatory cytokines (*Il2ra*, *Il-1β*, *Il15ra*), *Tnfα*, chemokines interferon was observed, and together contributes to the pathophysiology of septic shock. The analysis of GO terms through binary cut clustering provided additional confirmation of the validity of our LPS-induced sepsis mouse model. This analysis revealed a significant enrichment in pathways associated with lymphocyte cell adaptive responses and immune activation (Figure S2A), further validating the reliability of our RNA sequencing methodology.

Next, to determine the biological impact of administering adiponectin on inflammation, mice were injected with 15 mg/kg ALY688 or vehicle (PBS) 15 min prior to LPS injection (Figure 4A). Whole blood was collected after 24 h and RNA sequencing analysis was conducted to assess alterations in whole blood. Using similar analytical approaches to those in Figure 1, we identified a total of 76 genes that were differentially expressed between LPS injected mice with or without ALY688 treatment (Figures 4E and 4F). Among the 76 DEGs, 56 were upregulated and 20 were downregulated in the ALY+LPS group compared to the PBS+ LPS group (Figures 4E and 4G). Specifically, the increase of pro-inflammatory cytokines *Il27*, *Traip* (TRAF-interacting protein), *Tnfrsf9* (tumor necrosis factor receptor superfamily, member 9) and *Irf2bp1* (Interferon regulatory factor 2 binding protein 1) induced by LPS was reversed by ALY688 treatment (Figures 3A–3D). Taken together, our data suggested that ALY688 administration reversed the LPS-induced genes profile in whole blood cells.

DISCUSSION

Adiponectin, a multifunctional protein with a multitude of beneficial metabolic and anti-inflammatory properties, has garnered attention for its therapeutic potential in addressing cardiometabolic disorders.[5,26,27] In this study, we used the adiponectin receptor agonist ALY688 which will soon enter phase I clinical trials. We first demonstrated the adiponectin-mimetic action of ALY688 via an anti-inflammatory effect in a

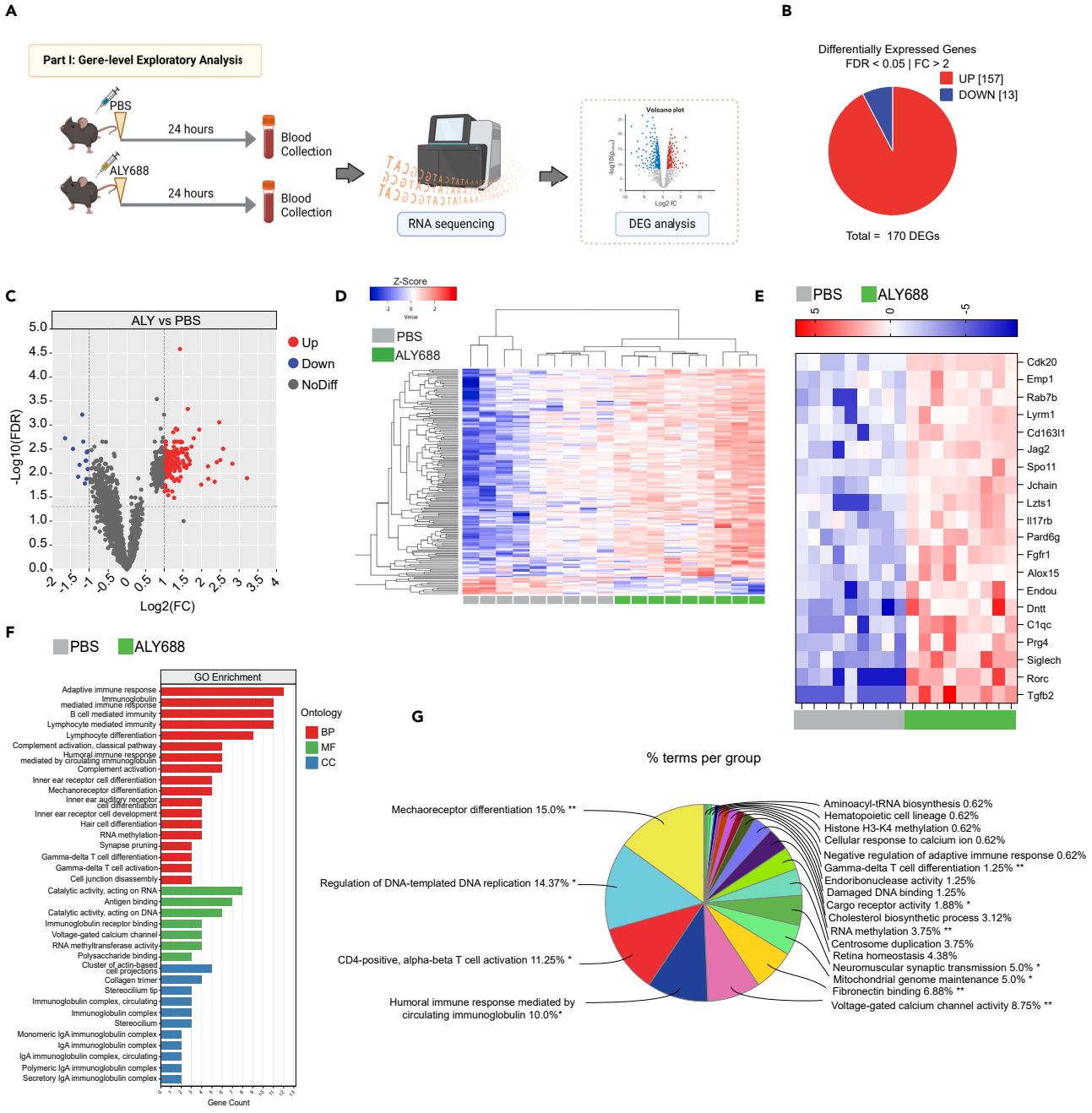


Figure 1. Identification of differentially expressed genes upon ALY688 treatment enriched in inflammatory processes

(A) Study design: mice were subjected to 15 mg/kg ALY688 or PBS via *sq.* injection. After 24 h, cardiac puncture was performed for blood collection for RNA sequencing analysis.

(B) Pie chart summarizes the number of genes upregulated and downregulated when comparing ALY688 with PBS.

(C) Volcano plot of gene expression changes in gene expression in the ALY688 versus PBS. The volcano plot represents the estimated log2 fold change as a function of the mean of normalized counts. Significant genes were selected when adjusted *p* value lower than 0.05 and fold-change ≥2.

(D) *Z* score heatmap of RNA-seq transcriptome analysis composed of the 170 differentially expressed genes identified.

(E) Expression of top 20 upregulated DEGs sorted by highest fold change with adjusted *p* value ≤ 0.05.

(F) Functional enrichment analysis of robust DEGs. Gene ontology (GO) enrichment analysis of robust DEGs is visualized in a bar plot ($p < 0.05$). BP: Biological Process; CC: Cellular Component; MF: Molecular Function.

(G) An overview chart with functional ontologies and pathways enriched from the differentially expressed genes in ALY688 treated animal. $n = 9$ per group.

Table 1. Top 20 upregulated DEGs sorted by highest fold changes (adjusted *p* value ≤ 0.05, mean value of raw count ≥10)

Gene	Official name	Mean of count (PBS)	Mean of count (ALY)	Fold-changes
Tgfb2	transforming growth factor β 2	0.33	14.67	18.05
Rorc	RAR related orphan receptor C	3.89	20.44	10.05
Siglech	CD33	9.00	49.56	7.47
Prg4	proteoglycan 4	8.89	76.89	6.13
C1qc	complement C1q C chain	4.11	15.44	5.64
Dntt	DNA nucleotidylexotransferase	4.22	34.67	5.50
Endou	endonuclease, poly(U) specific	7.22	24.22	4.87
Alox15	arachidonate 15-lipoxygenase	35.56	97.78	3.92
Fgfr1	Fibroblast Growth Factor Receptor 1	7.89	35.67	3.69
Pard6g	par-6 family cell polarity regulator gamma	4.67	11.44	3.54
Il17rb	interleukin 17 receptor B	5.44	11.56	3.37
Lzts1	leucine zipper tumor suppressor 1	3.44	8.33	3.34
Jchain	Joining chain of multimeric IgA and IgM	14.44	57.89	3.31
Spo11	DNA topoisomerase	8.78	17.89	3.16
Jag2	jagged canonical Notch ligand 2	6.00	31.33	3.03
Cd163L1	CD163 molecule like 1	5.44	11.22	2.96
Lyrm1	LYR Motif Containing 1	4.33	18.89	2.96
Rab7b	Ras-Related Protein Rab-7b	5.00	19.00	2.96
Emp1	Epithelial Membrane Protein 1	8.11	16.89	2.87

mouse model treated with LPS. Our RNA sequencing analysis of whole blood in a sublethal LPS mouse model pretreated with or without ALY688 demonstrated that the adiponectin receptor agonist elicited a substantial impact on pro-inflammatory gene expression. In the LPS injected group, whole blood cell RNA sequencing revealed the significant increase of *Tnf*, *Il-1β*, and interferon, which confirmed the phenotype of septic model.[28] The decrease in pro-inflammatory genes such as *Il27*, and *Traip* observed with ALY688 in this LPS model suggests that ALY688 may mitigate the deleterious impacts of inflammation during acute inflammatory conditions, which is in line with previously reported adiponectin anti-inflammatory effect in various diseases model[29–32] The inflammatory cytokines, including interferon-γ, and tumor necrosis factor found suppressed by adiponectin signaling in the disease model like, obese, atherosclerosis, and NASH were also observed in the sequencing analysis of ALY+LPS versus LPS.[33–37] In conclusion, using this well-established model of acute inflammation we demonstrated that pretreatment of mice with ALY688 successfully attenuated induction of various pro-inflammatory factors. We also performed discovery-based research using RNA sequencing (RNAseq) of whole blood to identify DEGs in wild-type mice treated with ALY688 versus vehicle. The goal of this approach was to develop an assay biomarking adiponectin receptor activation that could be used not only in animal research but would be amenable to implementation in human clinical study sites. Thus, our investigation focused on the identification of potential blood-based biomarkers altered in response to ALY688 treatment. Based on DEG analysis derived from whole blood sample RNA sequencing datasets, we were able to identify 157 genes significantly upregulated by ALY688 treatment.

Table 2. Top 10 upregulated DEGs sorted by highest fold changes (adjusted *p* value ≤ 0.01, mean value of raw count ≥10)

Gene	Official name	Mean of count (PBS)	Mean of count (ALY)	Fold-changes
Siglech	CD33	8.67	93.56	7.49
Prg4	Proteoglycan 4	8.89	76.89	6.13
Alox15	Arachidonate 15-lipoxygenase	29.00	139.67	3.92
Fgfr1	Fibroblast Growth Factor Receptor 1	7.89	35.67	3.69
Jchain	Joining chain of multimeric IgA and IgM	14.44	57.89	3.31
Igha	Immunoglobulin heavy constant alpha	103.78	403.56	3.08
Jag2	Jagged canonical Notch ligand 2	6.00	31.33	3.03
Zc3h6	Zinc finger CCCH-type containing 6	20.67	73.89	3.46
Igkj5	Immunoglobulin Kappa Joining 5	25.22	82.33	2.65
Myb	MYB proto-oncogene	37.44	117.56	2.54

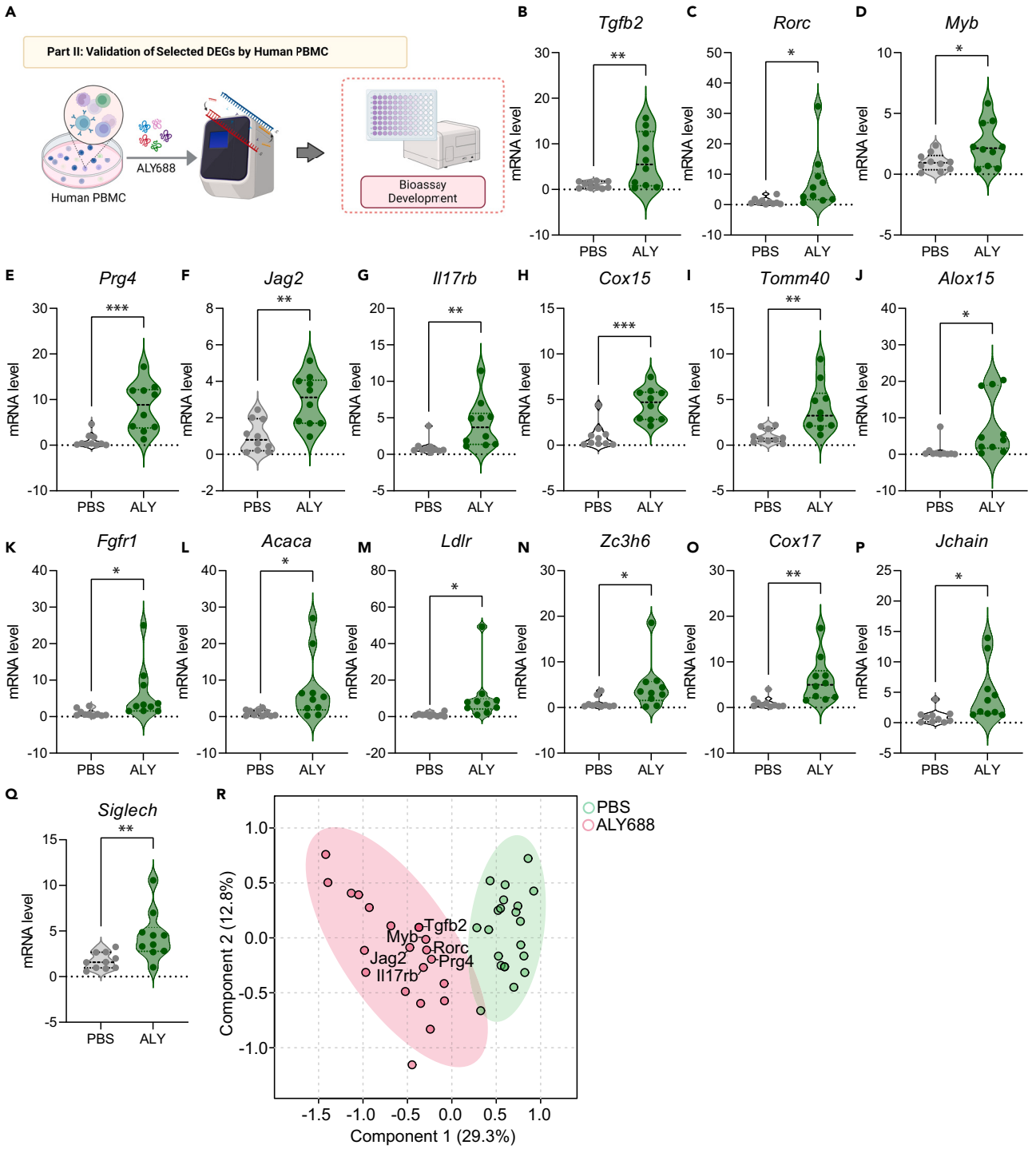


Figure 2. Verification of RNA sequencing findings in human PBMC

(A) Study design: DEGs selected by RNA sequencing analysis were further validated in human PMBC treated with or without ALY688 by qPCR. Gene expressions of (B) *Tgfb2*, (C) *Rorc*, (D) *Myb*, (E) *Prg4*, (F) *Jag2*, (G) *Il17rb* (H) *Cox15*, (I) *Tomm40*, (J) *Alox15*, (K) *Fgfr1*, (L) *Acaca*, (M) *Ldlr*, (N) *Zc3h6*, (O) *Cox17*, (P) *Jchain*, and (Q) *Siglech* in PBMC treated with or without 300 nM ALY688 for 24 h.

(R) PLSDA analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compare with PBS, $n = 10$ per group. Data are presented as fold change to PBS. Ordinary One-way ANOVA with Tukey's multiple comparison was run for statistical analysis.

Table 3. Proposed panel of genes as biomarkers of ALY688 action

Gene	Official name
Tgfb2	Transforming growth factor β 2
Myb	MYB proto-oncogene
Rorc	RAR related orphan receptor C
Jag2	Jagged canonical Notch ligand 2
Prg4	Proteoglycan 4
Il17rb	Interleukin 17 receptor B

The identified genes, including *Tgfb2*, *Rorc*, *Myb*, *Prg4*, *Jag2*, and *Il17rb*, selected by magnitude of fold change and clustering, were thus selected as promising candidates for clinical target engagement monitoring. Among this selected cluster genes, *Tgfb2* belongs to the family of the transforming growth factor β (TGF-β), and is a versatile cytokine that induces responses which have been correlated with an increased risk of developing various hallmarks of metabolic syndrome.[38,39] TGF-β can originate from multiple sources, primarily

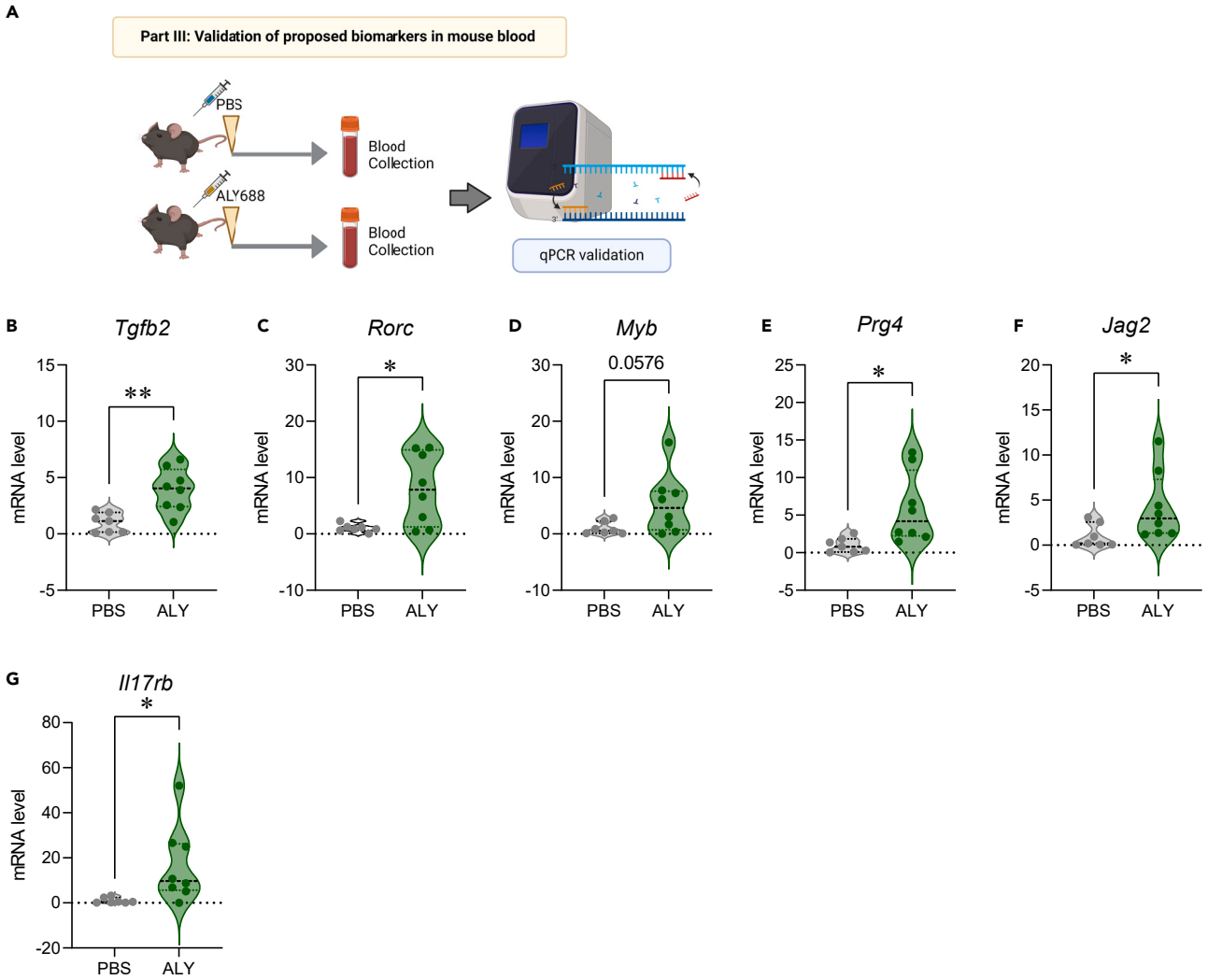


Figure 3. Biomarkers validation in mouse blood

(A) Study design: Proposed biomarkers were validated in whole blood cells isolated from mice subjected to PBS or ALY688 injection. Gene expressions of (B) *Tgfb2*, (C) *Rorc*, (D) *Myb*, (E) *Prg4*, (F) *Jag2*, (G) *Il17rb*. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compare with PBS, n = 7–8 per group. Data are presented as fold change to PBS. Ordinary One-way ANOVA with Tukey's multiple comparison was run for statistical analysis.

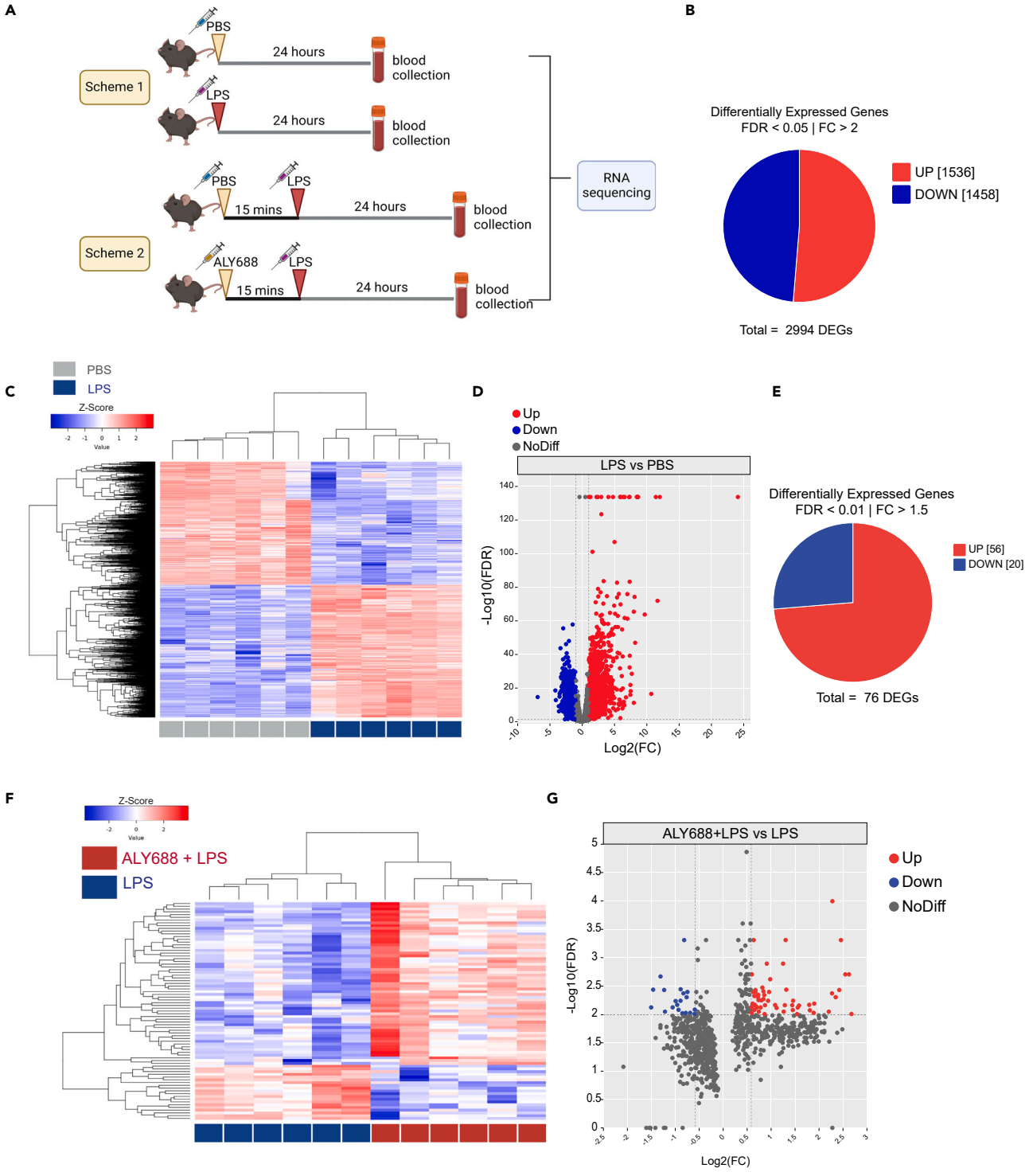


Figure 4. Altered gene expressions uncovered from ALY688 treatment upon LPS stimulation versus LPS

(A) Schematic diagram. Scheme 1: Mice were subjected to 15 mg/kg ALY688 vis sq. 15 min prior to 10 mg/kg LPS or PBS i.p. injection. Scheme 2: Mice were subjected to 10 mg/kg LPS or PBS i.p. injection. After 24 h, cardiac puncture was performed for blood collection for RNA sequencing analysis.

(B) Pie chart summarizes the number of genes upregulated and downregulated when comparing LPS with PBS.

(C) *Z* score heatmap of RNA-seq transcriptome analysis composed of the 2994 differentially expressed genes identified.

(D) Volcano plot of gene expression changes in gene expression in the LPS versus PBS. The volcano plot represents the estimated log2 fold change as a function of the mean of normalized counts. Significant genes were selected when adjusted *p* value lower than 0.05 and fold-change ≥2.

Figure 4. *Continued*

(E) Pie chart summarizes the number of genes upregulated and downregulated when comparing ALY688+LPS versus LPS.

(F) *Z* score heatmap of RNA-seq transcriptome analysis composed of the 76 differentially expressed genes identified.

(G) Volcano plot of gene expression changes in gene expression in the ALY688+LPS versus LPS. The volcano plot represents the estimated log2 fold change as a function of the mean of normalized counts. Significant genes were selected when adjusted *p* value lower than 0.01 and fold-change $\geq$1.5. $n = 6$ per group.

fibroblasts and, to a lesser extent, resident macrophages, contributing to the promotion of fibrosis.[40] TGF-β plays a key role in inhibiting immune responses, either directly by impeding the functions of Th1 and Th2 $CD4^+$ effector cells and NK cells or indirectly through the promotion and activation of Tregs.[41,42] Notably, recent studies have demonstrated that ALY688 increased TGF-β protein following muscle damage in a Duchenne muscular dystrophy model.[43] Our findings suggest TGF-β level is increased by ALY688 and that this could convey an anti-inflammatory outcome, which may explain the reduction of pro-inflammatory cytokines evoked by LPS stimulation, and in line with adiponectin's anti-inflammatory effect in various models.[29,44–47] RNA sequencing data also identified the receptor of IL-17, *Il17rb*, as one of the most predominant upregulated genes upon ALY688 administration. IL-17 family cytokines have been shown to play a pivotal role in host defense against extracellular pathogens and inflammatory responses.[48] Among this cytokine family, IL-25 acts as an alarm signal, generated in response to cell injury or tissue damage. IL-25 activates immune cells through its interaction with the IL-17RA and IL-17RB receptors, thereby initiating immune responses.[49] Splenocytes from IL-17RB-KO ($Il17rb^{-/-}$) mice ceased to produce numerous inflammatory factors (e.g., IL-5 or IL-13) upon *in vitro* stimulation with IL-25.[50] Thus, IL-17 stands out as a pivotal cytokine produced by TH17 cells, playing a central role in regulation of gut immunity.[51,52] Its significance extends beyond infection control, as TH17 cells actively contribute to the safeguarding of epithelial and mucosal tissues.[53–55] *Jag2*, a member of the Notch signaling pathway, has been implicated in adipogenesis and adipose tissue inflammation and, interestingly, adiponectin was shown to activate Notch signaling in the hippocampus through upregulating ADAM10 and Notch1.[56] While the direct relationship between *Jag2* and adiponectin remains unclear, our data suggest further investigation of this understudied association is warranted. Furthermore, Treg cell expansion required cell-to-cell contact and Notch3 signaling, which was mediated selectively through the Notch ligand *Jag2* expression.[57] For example, in experimental autoimmune encephalomyelitis Treg expansion is regulated by signaling through Jag2.[58] *Rorc* is the lineage-specific transcription factor for Th17 cells whose upregulation in developing Th17 cells is critically regulated by IL-6 and TGF-β.[57,59] Mice lacking the transcription factor *Myb* in Tregs succumbed to a multi-organ inflammatory disease.[60,61] Overall, the cluster gene of 6 presented robust and consistent upregulation in response to ALY688 treatment in both murine and human samples, as well as strong correlation of adiponectin mimetic effect in immune response, position them as panel of potential non-invasive biomarkers for assessing the pharmacokinetics of ALY688 action.

Translating our findings to human relevance, we validated the identified biomarkers based on the highest fold changes in RNA sequencing analysis, in human PBMC. The significant alignment between mouse and human studies, particularly the consistent upregulation of key genes, suggests the potential applicability of these markers across species.

Analysis using functional annotation tools revealed enrichment in pathways related to immune response and inflammation, aligning with the anticipated effects of adiponectin-based therapy.[44,62–64] To refine our biomarker selection, we scrutinized the most significantly upregulated genes, pinpointing those involved in T cell differentiation, inflammation, fatty acid metabolism, DNA damage, collagen formation, and ubiquitin-proteasome-dependent proteolysis.[65–67] The subsequent GO term analysis emphasized the immune response-related pathways enriched among the top upregulated genes, such as *Myb*, *Rorc*, and *Il17rb* enriched in the pathway regulating CD4, alpha-beta T cell activation, and Th17 cell differential and immune response. Interestingly, it's well known that Th17 cells secrete IL-17A, IL-17F, and express master transcription factor RORγt, encoded by *Rorc*, in response to diseases.[68–70] Multiple pre-clinical studies have shown the protective effect of ALY688, featuring its anti-inflammatory property. In a mouse model of metabolic dysfunction-associated steatohepatitis (MASH), ALY688 administration elicited the suppression effect of pro-inflammatory genes including *Tnfα*, *Mcp1*, *IL-1β*.[71] ALY688 also exerted a cardioprotective effect in part by lowering *IL-6*, *TLR4*, and *IL-1β* gene expression in heart from a preclinical mouse model of heart failure with reduced ejection fraction induced by pressure overload.[72] Circulating IL-6 level, as well as the skeletal muscle expression of TNFα and IL-1β which are enhanced by D2.*mdx* mouse model of Duchenne muscular dystrophy was decreased by ALY688 treatment.[73,74] Together, our findings reinforce the regulatory potential of ALY688 upon inflammation.

In conclusion, our study demonstrated anti-inflammatory effects of ALY688, and identified a gene signature indicating adiponectin action using whole blood samples, which were validated in human PBMC. The identified biomarker candidates offer a foundation for future clinical applications, enabling the non-intrusive monitoring of adiponectin-like effects. New knowledge from our study and the future use of the bioassay emerging from this work will be valuable in the clinical development of adiponectin-based therapeutic strategies.

Limitations of the study

We acknowledge a potential limitation of this study in using only lipopolysaccharide (LPS) to induce acute inflammation. Alternative approaches to consider include peritoneal contamination and infection (PCI) or and cecal ligation and puncture (CLP). LPS and PCI models demonstrate similar degrees of immune response, indicated by serum pro-inflammatory cytokines and oxidative stress and apoptosis in spleen, whereas a delayed and a lower degree of response are typically found in CLP. Considering the purpose of this study, the temporal nature and size of response made LPS the most suitable model to mimic the characteristic features of human systemic inflammation.

RESOURCE AVAILABILITY

Lead contact

Further information and reasonable requests for resources and reagents should be directed to the lead contact, Dr. Gary Sweeney (gsweeney@yorku.ca).

Material availability

This study did not generate any unique materials. The full RNAseq dataset has been deposited at GEO (see information above).

Data and code availability

RNA-seq data have been deposited to GEO with the identifier GSE275088. This study did not generate any new code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

These studies were supported in part via a MITACS Accelerate industry partnership grant to G.S. Related work in G.S. and A.A.S. laboratories is supported by Canadian Institutes of Health Research and Canada Foundation for Innovation and in G.S. lab by Heart & Stroke Foundation of Canada.

AUTHOR CONTRIBUTIONS

G.S. and A.A.S. conceptualized the research and designed the research framework; J.T., developed the methodology and acquired data; and J.T. and Y.L. performed the data analysis; J.T. drafted the manuscript, with contributions from G.S., A.A.S., and Y.L.

DECLARATION OF INTERESTS

These studies were supported in part via a research contract with Allysta Pharmaceuticals Inc. H.H.H. and A.P. are employees of Allysta and G.S. and A.A.S. consult for Allysta.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Animal model
  - Cell culture
- METHOD DETAILS
  - RNA isolation and RT-PCR
  - RNA sequencing
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110994.

Received: June 11, 2024
Revised: July 23, 2024
Accepted: September 16, 2024
Published: September 19, 2024

REFERENCES

1. Langer, H.T., Rohm, M., Goncalves, M.D., and Sylow, L. (2024). AMPK as a mediator of tissue preservation: time for a shift in dogma? Nat. Rev. Endocrinol. *20*, 526–540. https://doi.org/10.1038/s41574-024-00992-y.
2. Valenzuela, P.L., Carrera-Bastos, P., Castillo-García, A., Lieberman, D.E., Santos-Lozano, A., and Lucia, A. (2023). Obesity and the risk of cardiometabolic diseases. Nat. Rev. Cardiol. *20*, 475–494. https://doi.org/10.1038/s41569-023-00847-5.
3. Straub, L.G., and Scherer, P.E. (2019). Metabolic Messengers: Adiponectin. Nat. Metab. *1*, 334–339. https://doi.org/10.1038/s42255-019-0041-z.
4. Fasshauer, M., Blüher, M., and Stumvoll, M. (2014). Adipokines in gestational diabetes. Lancet Diabetes Endocrinol. *2*, 488–499. https://doi.org/10.1016/S2213-8587(13)70176-1.
5. Turer, A.T., and Scherer, P.E. (2012). Adiponectin: mechanistic insights and clinical implications. Diabetologia *55*, 2319–2326. https://doi.org/10.1007/s00125-012-2598-x.
6. Zelechowska, P., Brzezinska-Blaszczyk, E., Kusowska, A., and Kozlowska, E. (2020). The role of adipokines in the modulation of lymphoid lineage cell development and activity: An overview. Obes. Rev. *21*, e13055. https://doi.org/10.1111/obr.13055.
7. Luo, Y., and Liu, M. (2016). Adiponectin: a versatile player of innate immunity. J. Mol. Cell Biol. *8*, 120–128. https://doi.org/10.1093/jmcb/mjw012.
8. Wu, Z.J., Cheng, Y.J., Gu, W.J., and Aung, L.H.H. (2014). Adiponectin is associated with increased mortality in patients with already established cardiovascular disease: a systematic review and meta-analysis. Metabolism *63*, 1157–1166. https://doi.org/10.1016/j.metabol.2014.05.001.
9. Cote, M., Cartier, A., Reuwer, A.Q., Arsenault, B.J., Lemieux, I., Despres, J.P., Wareham, N.J., Kastelein, J.J., Boekholdt, S.M., and Khaw, K.T. (2011). Adiponectin and risk of coronary heart disease in apparently healthy men and women (from the EPIC-Norfolk Prospective Population Study). Am. J. Cardiol. *108*, 367–373. https://doi.org/10.1016/j.amjcard.2011.03.053.
10. Kadowaki, T., Yamauchi, T., Okada-Iwabu, M., and Iwabu, M. (2014). Adiponectin and its receptors: implications for obesity-associated diseases and longevity. Lancet Diabetes Endocrinol. *2*, 8–9. https://doi.org/10.1016/S2213-8587(13)70120-7.

11. Borges, M.C., Lawlor, D.A., de Oliveira, C., White, J., Horta, B.L., and Barros, A.J.D. (2016). Role of Adiponectin in Coronary Heart Disease Risk: A Mendelian Randomization Study. Circ. Res. *119*, 491–499. https://doi.org/10.1161/CIRCRESAHA.116.308716.
12. Wang, Z.V., and Scherer, P.E. (2016). Adiponectin, the past two decades. J. Mol. Cell Biol. *8*, 93–100. https://doi.org/10.1093/jmcb/mjw011.
13. Achari, A.E., and Jain, S.K. (2017). Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int. J. Mol. Sci. *18*, 1321. https://doi.org/10.3390/ijms18061321.
14. Okamoto, M., Ohara-Imaizumi, M., Kubota, N., Hashimoto, S., Eto, K., Kanno, T., Kubota, T., Wakui, M., Nagai, R., Noda, M., et al. (2008). Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. Diabetologia *51*, 827–835. https://doi.org/10.1007/s00125-008-0944-9.
15. Li, X., Zhang, D., Vatner, D.F., Goedeke, L., Hirabara, S.M., Zhang, Y., Perry, R.J., and Shulman, G.I. (2020). Mechanisms by which adiponectin reverses high fat diet-induced insulin resistance in mice. Proc. Natl. Acad. Sci. USA *117*, 32584–32593. https://doi.org/10.1073/pnas.1922169117.
16. Meng, Z., Zhang, Z., Zhao, J., Liu, C., Yao, P., Zhang, L., Xie, D., Lau, W.B., Tsukuda, J., Christopher, T.A., et al. (2023). Nitrative Modification of Caveolin-3: A Novel Mechanism of Cardiac Insulin Resistance and a Potential Therapeutic Target Against Ischemic Heart Failure in Prediabetic Animals. Circulation *147*, 1162–1179. https://doi.org/10.1161/CIRCULATIONAHA.122.063073.
17. Zhou, Z., Liu, C., Xu, S., Wang, J., Guo, F., Duan, S., Deng, Q., Sun, J., Yu, F., Zhou, Y., et al. (2022). Metabolism regulator adiponectin prevents cardiac remodeling and ventricular arrhythmias via sympathetic modulation in a myocardial infarction model. Basic Res. Cardiol. *117*, 34. https://doi.org/10.1007/s00395-022-00939-2.
18. Okada-Iwabu, M., Yamauchi, T., Iwabu, M., Honma, T., Hamagami, K.i., Matsuda, K., Yamaguchi, M., Tanabe, H., Kimura-Someya, T., Shirouzu, M., et al. (2013). A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature *503*, 493–499. https://doi.org/10.1038/nature12656.
19. Zhang, Y., Zhao, J., Li, R., Lau, W.B., Yuan, Y.X., Liang, B., Li, R., Gao, E.H., Koch, W.J., Ma, X.L., and Wang, Y.J. (2015). AdipoRon, the first orally active adiponectin receptor activator, attenuates postischemic myocardial apoptosis through both AMPK-mediated and AMPK-independent signalings. Am. J. Physiol. Endocrinol. Metab. *309*, E275–E282. https://doi.org/10.1152/ajpendo.00577.2014.
20. Zhu, D., Zhang, Z., Zhao, J., Liu, D., Gan, L., Lau, W.B., Xie, D., Meng, Z., Yao, P., Tsukuda, J., et al. (2022). Targeting Adiponectin Receptor 1 Phosphorylation Against Ischemic Heart Failure. Circ. Res. *131*, e34–e50. https://doi.org/10.1161/CIRCRESAHA.121.319976.
21. Otvos, L., Jr., Haspinger, E., La Russa, F., Maspero, F., Graziano, P., Kovalszky, I., Lovas, S., Nama, K., Hoffmann, R., Knappe, D., et al. (2011). Design and development of a peptide-based adiponectin receptor agonist for cancer treatment. BMC Biotechnol. *11*, 90. https://doi.org/10.1186/1472-6750-11-90.
22. Otvos, L., Jr. (2019). Potential Adiponectin Receptor Response Modifier Therapeutics. Front. Endocrinol. *10*, 539. https://doi.org/10.3389/fendo.2019.00539.
23. Sung, H.K., Mitchell, P.L., Gross, S., Marette, A., and Sweeney, G. (2022). ALY688 elicits adiponectin-mimetic signaling and improves insulin action in skeletal muscle cells. Am. J. Physiol. Cell Physiol. *322*, C151–C163. https://doi.org/10.1152/ajpcell.00603.2020.
24. Da Eira, D., Jani, S., Sung, H., Sweeney, G., and Ceddia, R.B. (2020). Effects of the adiponectin mimetic compound ALY688 on glucose and fat metabolism in visceral and subcutaneous rat adipocytes. Adipocyte *9*, 550–562. https://doi.org/10.1080/21623945.2020.1817230.
25. Abdul-Sater, A.A., Edilova, M.I., Clouthier, D.L., Mbanwi, A., Kremmer, E., and Watts, T.H. (2017). The signaling adaptor TRAF1 negatively regulates Toll-like receptor signaling and this underlies its role in rheumatic disease. Nat. Immunol. *18*, 26–35. https://doi.org/10.1038/ni.3618.
26. Kita, S., Maeda, N., and Shimomura, I. (2019). Interorgan communication by exosomes, adipose tissue, and adiponectin in metabolic syndrome. J. Clin. Invest. *129*, 4041–4049. https://doi.org/10.1172/JCI129193.
27. Ghadge, A.A., Khaire, A.A., and Kuvalekar, A.A. (2018). Adiponectin: A potential therapeutic target for metabolic syndrome. Cytokine Growth Factor Rev. *39*, 151–158. https://doi.org/10.1016/j.cytogfr.2018.01.004.
28. Li, W., Yang, S., Kim, S.O., Reid, G., Challis, J.R.G., and Bocking, A.D. (2014). Lipopolysaccharide-Induced Profiles of Cytokine, Chemokine, and Growth Factors Produced by Human Decidual Cells Are Altered by Lactobacillus rhamnosus GR-1 Supernatant. Reprod. Sci. *21*, 939–947. https://doi.org/10.1177/1933719113519171.
29. Meacham, C.E., Jeffery, E.C., Burgess, R.J., Sivakumar, C.D., Arora, M.A., Stanley, A.M., Colby, E.M., Crane, G.M., Zhao, Z., and Morrison, S.J. (2022). Adiponectin receptors sustain haematopoietic stem cells throughout adulthood by protecting them from inflammation. Nat. Cell Biol. *24*, 697–707. https://doi.org/10.1038/s41556-022-00909-9.
30. Masamoto, Y., Arai, S., Sato, T., Yoshimi, A., Kubota, N., Takamoto, I., Iwakura, Y., Yoshimura, A., Kadowaki, T., and Kurokawa, M. (2016). Adiponectin Enhances Antibacterial Activity of Hematopoietic Cells by Suppressing Bone Marrow Inflammation. Immunity *44*, 1422–1433. https://doi.org/10.1016/j.immuni.2016.05.010.
31. Qi, G.M., Jia, L.X., Li, Y.L., Li, H.H., and Du, J. (2014). Adiponectin suppresses angiotensin II-induced inflammation and cardiac fibrosis through activation of macrophage autophagy. Endocrinology *155*, 2254–2265. https://doi.org/10.1210/en.2013-2011.
32. Takemura, Y., Ouchi, N., Shibata, R., Aprahamian, T., Kirber, M.T., Summer, R.S., Kihara, S., and Walsh, K. (2007). Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. J. Clin. Invest. *117*, 375–386. https://doi.org/10.1172/JCI29709.
33. Lee, H., Tu, T.H., Park, B.S., Yang, S., and Kim, J.G. (2019). Adiponectin Reverses the Hypothalamic Microglial Inflammation during Short-Term Exposure to Fat-Rich Diet. Int. J. Mol. Sci. *20*, 5738. https://doi.org/10.3390/ijms20225738.
34. Kantartzis, K., Rittig, K., Balletshofer, B., Machann, J., Schick, F., Porubska, K., Fritsche, A., Häring, H.U., and Stefan, N. (2006). The relationships of plasma adiponectin with a favorable lipid profile, decreased inflammation, and less ectopic fat accumulation depend on adiposity. Clin. Chem. *52*, 1934–1942. https://doi.org/10.1373/clinchem.2006.067397.
35. van Stijn, C.M.W., Kim, J., Barish, G.D., Tietge, U.J.F., and Tangirala, R.K. (2014). Adiponectin expression protects against angiotensin II-mediated inflammation and accelerated atherosclerosis. PLoS One *9*, e86404. https://doi.org/10.1371/journal.pone.0086404.
36. Bao, L., Yin, J., Gao, W., Wang, Q., Yao, W., and Gao, X. (2018). A long-acting FGF21 alleviates hepatic steatosis and inflammation in a mouse model of non-alcoholic steatohepatitis partly through an FGF21-adiponectin-IL17A pathway. Br. J. Pharmacol. *175*, 3379–3393. https://doi.org/10.1111/bph.14383.
37. Handa, P., Maliken, B.D., Nelson, J.E., Morgan-Stevenson, V., Messner, D.J., Dhillon, B.K., Klintworth, H.M., Beauchamp, M., Yeh, M.M., Elfers, C.T., et al. (2014). Reduced adiponectin signaling due to weight gain results in nonalcoholic steatohepatitis through impaired mitochondrial biogenesis. Hepatology *60*, 133–145. https://doi.org/10.1002/hep.26946.
38. Travis, M.A., and Sheppard, D. (2014). TGF-beta activation and function in immunity. Annu. Rev. Immunol. *32*, 51–82. https://doi.org/10.1146/annurev-immunol-032713-120257.
39. Batlle, E., and Massagué, J. (2019). Transforming Growth Factor-beta Signaling in Immunity and Cancer. Immunity *50*, 924–940. https://doi.org/10.1016/j.immuni.2019.03.024.
40. Ahuja, S., and Zaheer, S. (2024). Multifaceted TGF-beta signaling, a master regulator: From bench-to-bedside, intricacies, and complexities. Cell Biol. Int. *48*, 87–127. https://doi.org/10.1002/cbin.12097.
41. Wan, Y.Y., and Flavell, R.A. (2007). Yin-Yang' functions of transforming growth factor-beta and T regulatory cells in immune regulation. Immunol. Rev. *220*, 199–213. https://doi.org/10.1111/j.1600-065X.2007.00565.x.
42. Bellissimo, C.A., Gandhi, S., Castellani, L.N., Murugathasan, M., Delfinis, L.J., Thuhan, A., Garibotti, M.C., Seo, Y., Rebalka, I.A., Hsu, H.H., et al. (2024). The slow-release adiponectin analog ALY688-SR modifies early-stage disease development in the D2.mdx mouse model of Duchenne muscular dystrophy. Am. J. Physiol. Cell Physiol. *326*, C1011–C1026. https://doi.org/10.1152/ajpcell.00638.2023.
43. Bellissimo, C.A., Gandhi, S., Castellani, L.N., Murugathasan, M., Delfinis, L.J., Thuhan, A., Garibotti, M.C., Seo, Y., Rebalka, I.A., Sweeney, G., et al. (2023). The adiponectin analogue ALY688-SR attenuates diaphragm fibrosis, atrophy and mitochondrial stress in a mouse model of Duchenne muscular dystrophy. bioRxiv *2023*, 541826. https://doi.org/10.1101/2023.05.22.541826.
44. Qiu, W., Wu, H., Hu, Z., Wu, X., Tu, M., Fang, F., Zhu, X., Liu, Y., Lian, J., Valverde, P., et al. (2021). Identification and characterization of a novel adiponectin receptor agonist adipo anti-inflammation agonist and its anti-inflammatory effects in vitro and in vivo. Br. J. Pharmacol. *178*, 280–297. https://doi.org/10.1111/bph.15277.
45. Qiu, W., Wang, Z., Chen, Z., Sun, Q., Wu, H., Chen, Z., Luan, K., Liu, Z., Ding, D., Tu, Q.,

et al. (2023). The adiponectin receptor agonist AdipoAI attenuates periodontitis in diabetic rats by inhibiting gingival fibroblast-induced macrophage migration. Br. J. Pharmacol. *180*, 2436–2451. https://doi.org/10.1111/bph.16103.

46. Bobbert, P., Scheibenbogen, C., Jenke, A., Kania, G., Wilk, S., Krohn, S., Stehr, J., Kuehl, U., Rauch, U., Eriksson, U., et al. (2011). Adiponectin expression in patients with inflammatory cardiomyopathy indicates favourable outcome and inflammation control. Eur. Heart J. *32*, 1134–1147. https://doi.org/10.1093/eurheartj/ehq498.
47. Wang, X., Chen, Q., Pu, H., Wei, Q., Duan, M., Zhang, C., Jiang, T., Shou, X., Zhang, J., and Yang, Y. (2016). Adiponectin improves NF-kappaB-mediated inflammation and abates atherosclerosis progression in apolipoprotein E-deficient mice. Lipids Health Dis. *15*, 33. https://doi.org/10.1186/s12944-016-0202-y.
48. Wilson, S.C., Caveney, N.A., Yen, M., Pollmann, C., Xiang, X., Jude, K.M., Hafer, M., Tsutsumi, N., Piehler, J., and Garcia, K.C. (2022). Organizing structural principles of the IL-17 ligand-receptor axis. Nature *609*, 622–629. https://doi.org/10.1038/s41586-022-05116-y.
49. Yuan, Q., Peng, N., Xiao, F., Shi, X., Zhu, B., Rui, K., Tian, J., and Lu, L. (2023). New insights into the function of Interleukin-25 in disease pathogenesis. Biomark. Res. *11*, 36. https://doi.org/10.1186/s40364-023-00474-9.
50. Rickel, E.A., Siegel, L.A., Yoon, B.R.P., Rottman, J.B., Kugler, D.G., Swart, D.A., Anders, P.M., Tocker, J.E., Comeau, M.R., and Budelsky, A.L. (2008). Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. J. Immunol. *181*, 4299–4310. https://doi.org/10.4049/jimmunol.181.6.4299.
51. Eddens, T., Elsegeiny, W., Garcia-Hernadez, M.d.l.L., Castillo, P., Trevejo-Nunez, G., Serody, K., Campfield, B.T., Khader, S.A., Chen, K., Rangel-Moreno, J., and Kolls, J.K. (2017). Pneumocystis-Driven Inducible Bronchus-Associated Lymphoid Tissue Formation Requires Th2 and Th17 Immunity. Cell Rep. *18*, 3078–3090. https://doi.org/10.1016/j.celrep.2017.03.016.
52. Gheitasi, R., Keramat, F., Khosravi, S., Hajilooi, M., Pletz, M.W., and Makarewicz, O. (2021). Evaluation of Th2 and Th17 Immunity-Related Factors as Indicators of Brucellosis. Front. Cell. Infect. Microbiol. *11*, 786994. https://doi.org/10.3389/fcimb.2021.786994.
53. Duan, J., Matute, J.D., Unger, L.W., Hanley, T., Schnell, A., Lin, X., Krupka, N., Griebel, P., Lambden, C., Sit, B., et al. (2023). Endoplasmic reticulum stress in the intestinal epithelium initiates purine metabolite synthesis and promotes Th17 cell differentiation in the gut. Immunity *56*, 1115–1131.e9. https://doi.org/10.1016/j.immuni.2023.02.018.
54. Zheng, D., Liwinski, T., and Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. Cell Res. *30*, 492–506. https://doi.org/10.1038/s41422-020-0332-7.
55. Spivak, I., Fluhr, L., and Elinav, E. (2022). Local and systemic effects of microbiome-derived metabolites. EMBO Rep. *23*, e55664. https://doi.org/10.15252/embr.202255664.
56. You, J., Sun, L., Wang, J., Sun, F., Wang, W., Wang, D., Fan, X., Liu, D., Xu, Z., Qiu, C., et al. (2021). Role of Adiponectin-Notch pathway in cognitive dysfunction associated with depression and in the therapeutic effect of physical exercise. Aging Cell *20*, e13387. https://doi.org/10.1111/acel.13387.
57. Yan, Y., Ramanan, D., Rozenberg, M., McGovern, K., Rastelli, D., Vijaykumar, B., Yaghi, O., Voisin, T., Mosaheb, M., Chiu, I., et al. (2021). Interleukin-6 produced by enteric neurons regulates the number and phenotype of microbe-responsive regulatory T cells in the gut. Immunity *54*, 499–513.e5. https://doi.org/10.1016/j.immuni.2021.02.002.
58. Elyaman, W., Bassil, R., Bradshaw, E.M., Orent, W., Lahoud, Y., Zhu, B., Radtke, F., Yagita, H., and Khoury, S.J. (2012). Notch receptors and Smad3 signaling cooperate in the induction of interleukin-9-producing T cells. Immunity *36*, 623–634. https://doi.org/10.1016/j.immuni.2012.01.020.
59. Chang, D., Xing, Q., Su, Y., Zhao, X., Xu, W., Wang, X., and Dong, C. (2020). The Conserved Non-coding Sequences CNS6 and CNS9 Control Cytokine-Induced Rorc Transcription during T Helper 17 Cell Differentiation. Immunity *53*, 614–626.e4. https://doi.org/10.1016/j.immuni.2020.07.012.
60. Wang, F., Qi, Z., Yao, Y., Yu, G., Feng, T., Zhao, T., Xue, H.H., Zhao, Y., Jiang, P., Bao, L., and Yu, S. (2021). Exploring the stage-specific roles of Tcf-1 in T cell development and malignancy at single-cell resolution. Cell. Mol. Immunol. *18*, 644–659. https://doi.org/10.1038/s41423-020-00527-1.
61. Dias, S., D'Amico, A., Cretney, E., Liao, Y., Tellier, J., Bruggeman, C., Almeida, F.F., Leahy, J., Belz, G.T., Smyth, G.K., et al. (2017). Effector Regulatory T Cell Differentiation and Immune Homeostasis Depend on the Transcription Factor Myb. Immunity *46*, 78–91. https://doi.org/10.1016/j.immuni.2016.12.017.
62. Choi, S.R., Lim, J.H., Kim, M.Y., Kim, E.N., Kim, Y., Choi, B.S., Kim, Y.S., Kim, H.W., Lim, K.M., Kim, M.J., and Park, C.W. (2018). Adiponectin receptor agonist AdipoRon decreased ceramide, and lipotoxicity, and ameliorated diabetic nephropathy. Metabolism *85*, 348–360. https://doi.org/10.1016/j.metabol.2018.02.004.
63. Jenke, A., Yazdanyar, M., Miyahara, S., Chekhoeva, A., Immohr, M.B., Kistner, J., Boeken, U., Lichtenberg, A., and Akhyari, P. (2021). AdipoRon Attenuates Inflammation and Impairment of Cardiac Function Associated With Cardiopulmonary Bypass-Induced Systemic Inflammatory Response Syndrome. J. Am. Heart Assoc. *10*, e018097. https://doi.org/10.1161/JAHA.120.018097.
64. Lindfors, S., Polianskyte-Prause, Z., Bouslama, R., Lehtonen, E., Mannerla, M., Nisen, H., Tienari, J., Salmenkari, H., Forsgård, R., Mirtti, T., et al. (2021). Adiponectin receptor agonist AdipoRon ameliorates renal inflammation in diet-induced obese mice and endotoxin-treated human glomeruli ex vivo. Diabetologia *64*, 1866–1879. https://doi.org/10.1007/s00125-021-05473-9.
65. Li, J., Zhao, J., Chen, L., Gao, H., Zhang, J., Wang, D., Zou, Y., Qin, Q., Qu, Y., Li, J., et al. (2023). alpha-Synuclein induces Th17 differentiation and impairs the function and stability of Tregs by promoting RORC transcription in Parkinson's disease. Brain Behav. Immun. *108*, 32–44. https://doi.org/10.1016/j.bbi.2022.10.023.
66. O'Connor, K.S., Read, S.A., Wang, M., Schibeci, S., Eslam, M., Ong, A., Weltman, M.D., Douglas, M.W., Mazzola, A., Craxì, A., et al. (2016). IFNL3/4 genotype is associated with altered immune cell populations in peripheral blood in chronic hepatitis C infection. Genes Immun. *17*, 328–334. https://doi.org/10.1038/gene.2016.27.
67. Long, J., Wang, X., Du, X., Pan, H., Wang, J., Li, Z., Liu, H., Li, X., and Zheng, Z. (2019). JAG2/Notch2 inhibits intervertebral disc degeneration by modulating cell proliferation, apoptosis, and extracellular matrix. Arthritis Res. Ther. *21*, 213. https://doi.org/10.1186/s13075-019-1990-z.
68. Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell *126*, 1121–1133. https://doi.org/10.1016/j.cell.2006.07.035.
69. Chang, S.H., and Dong, C. (2007). A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. Cell Res. *17*, 435–440. https://doi.org/10.1038/cr.2007.35.
70. Knochelmann, H.M., Dwyer, C.J., Bailey, S.R., Amaya, S.M., Elston, D.M., Mazza-McCrann, J.M., and Paulos, C.M. (2018). When worlds collide: Th17 and Treg cells in cancer and autoimmunity. Cell. Mol. Immunol. *15*, 458–469. https://doi.org/10.1038/s41423-018-0004-4.
71. Huang, Z., Sung, H.K., Yan, X., He, S., Jin, L., Wang, Q., Wu, X., Hsu, H.H., Pignalosa, A., Crawford, K., et al. (2024). The adiponectin-derived peptide ALY688 protects against the development of metabolic dysfunction-associated steatohepatitis. Clin. Transl. Sci. *17*, e13760. https://doi.org/10.1111/cts.13760.
72. Cho, S., Dadson, K., Sung, H.K., Ayansola, O., Mirzaesmaeili, A., Noskovicova, N., Zhao, Y., Cheung, K., Radisic, M., Hinz, B., et al. (2024). Cardioprotection by the adiponectin receptor agonist ALY688 in a preclinical mouse model of heart failure with reduced ejection fraction (HFrEF). Biomed. Pharmacother. *171*, 116119. https://doi.org/10.1016/j.biopha.2023.116119.
73. Bellissimo, C.A., Castellani, L.N., Finch, M.S., Murugathasan, M., Gandhi, S., Sweeney, G., Abdul-Sater, A.A., MacPherson, R.E.K., and Perry, C.G.R. (2023). Memory impairment in the D2.mdx mouse model of Duchenne muscular dystrophy is prevented by the adiponectin receptor agonist ALY688. Exp. Physiol. *108*, 1108–1117. https://doi.org/10.1113/EP091274.
74. Dubuisson, N., Versele, R., Davis-Lopez de Carrizosa, M.A., Selvais, C.M., Noel, L., Planchon, C., Van den Bergh, P.Y.K., Brichard, S.M., and Abou-Samra, M. (2023). The Adiponectin Receptor Agonist, ALY688: A Promising Therapeutic for Fibrosis in the Dystrophic Muscle. Cells *12*, 1. https://doi.org/10.3390/cells12162101.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
ALY688	Allysta Pharmaceuticals	Cat. #ALL.1188.BR.01
Lipopolysaccharides from Escherichia coli O55:B5	Millipore Sigma	Cat. #L2880
AIM V culture medium	ThermoFisher	Cat. #12055091
Fetal bovine serum	Wisent	Cat. #080-150
Penicillin and Streptomycin	ThermoFisher	Cat. #15070063
Critical commercial assays		
TriZol Reagent	ThermoFisher	Cat. #15596026
PureLink™ RNA Mini Kit	ThermoFisher	Cat. #12183018
RevertAid RT Reverse Transcription Kit	ThermoFisher	Cat. #K1691
SsoAdvanced Universal SYBR Green Supermix	BioRad	Cat. #1725274
Deposited data		
Mouse whole blood cell RNA sequencing	This paper	GSE275088
Experimental models: cell lines		
Human peripheral blood mononuclear cells	StemCell Technology	Cat. #70025.3
Experimental models: organisms/strains		
C57BL/6	Charles River	Strain #027
Oligonucleotides		
Please see Tables S1 and S2		
Software and algorithms		
GraphPad Prism version 9	GraphPad Software, Inc.	V9.4
R studio	R	V4.3.0

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animal model

All study protocols were approved by the Animal Care Committee of York University under permit 2022-03. C57BL/6C male mice (Charles River Laboratories, Canada) were maintained with *ad libitum* access to water and regular chow diet until 8 weeks of age when they were randomly separated into each group (n = 6–10 per group). All animals were housed in temperature and humidity-controlled rooms (21 ± 2°C, 35–40%) with a daily 12 h light/dark cycle in the animal care facility of York University in accordance with the guidelines of the Canadian Council on Animal Care. LPS (Sigma) and ALY688, a slow-release formulation of the drug ALY688 adapted for daily subcutaneous injection (Allysta Pharmaceuticals) were dissolved in PBS accordingly. ALY688 or PBS was subcutaneously injected to mice at a dose of 15 mg/kg. After 24 h of injection, blood was collected by cardiac puncture with EDTA coated tube. LPS induced sepsis model was then induced by LPS i.p. injection at a dose of 10 mg/kg or same volume of PBS. And ALY688 anti-inflammatory effect was evaluated in the mouse model of ALY688 subcutaneous injection at a dose of 15 mg/kg, 15 min prior to LPS i.p. injection at a dose of 10 mg/kg or same volume of PBS. For genes validation experiment with mice blood, mice subjected to 3 days ALY688 at a dose of 15 mg/kg. All the control group mice underwent subcutaneous injection of PBS in a volume equivalent to that of ALY688, followed by intraperitoneal administration of PBS in an equal quantity of LPS. Blood was collected in the same way as mentioned above.

Cell culture

Human peripheral blood mononuclear cells (PBMC) were purchased from Stem Cell Technologies after authenticated by the company. PBMC were thawed and resuspended in AIM V culture medium (ThermoFisher) culture medium, supplement with 10% FBS (Wisent) and 1% Penicillin and Streptomycin (ThermoFisher) and recovered for 2 h. After recovery, culture medium was replaced by 0.5% FBS supplemented AIM V culture medium and treated with or without 300 nM ALY688 (Allysta Pharmaceutica) for 24 h (*n* = 10 donors per group).

METHOD DETAILS

RNA isolation and RT-PCR

Total RNA from blood sample and PBMC was extracted with Trizol reagent (ThermoFisher) and purified with PureLink RNA Mini Kit (ThermoFisher) as the manufacture's instruction. expressed. For RT-PCR, cDNA Synthesis Kit (ThermoFisher) was used to synthesize first strand cDNA. qPCR was performed in SYBR Green Supermix (Bio-Rad, 1725274) method. The primers used were listed in Tables S1 and S2. The data were normalized to *Prlp0* in whole blood samples and *Gapdh* in PBMC samples, and the fold change was calculated via the $2^{-\Delta\Delta Ct}$ method Based on the untreated group, the relative concentration of mRNA was expressed in arbitrary units, and its assigned value was 1.

RNA sequencing

RNA sequencing was performed by NovoGene Co. Ltd. Ater cDNA synthesis, adaptor ligated sequencing library preparation then perform sequencing on Illumina, a high-throughput platform. After alignment and assemble data, raw data were further selected by featured counts. Principal components analysis and normalizing counts by median ratio normalization were carried out for raw data trimming. An R language-based algorithm, DeSeq2 were used for differential gene expression analysis, together with Gene Ontology (GO) and KEGG pathway enrichment analysis. To understand the biological function and association of the DEGs, GO and KEGG enrichment analysis were performed and visualized using ClueGO, and plug-in of Cytoscape. The p value <0.05 was identified as the significant ontology/pathway.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 9. For comparison between two experimental groups, unpaired two-tailed Student's t test or nonparametric test was performed, and details can be found in each figure legend. $p < 0.05$ was considered statistically significant.