

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

BACH1 governs macrophage plasticity and niche adaptation during
skeletal muscle regeneration

by Noemí Caballero Sánchez

Supervisor: László Nagy, PhD, DSc, MHAS



UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF MOLECULAR CELLULAR AND IMMUNE BIOLOGY

DEBRECEN, 2026

BACH1 governs macrophage plasticity and niche adaptation during skeletal muscle regeneration

By Noemí Caballero Sánchez, MSc

Supervisor: László Nagy, PhD, DSc, MHAS

Doctoral School of Molecular Cellular and Immune Biology, University of Debrecen

Head of the **Defense Committee**:

Dr. Andrea Szegedi, PhD, DSc, MHAS

Reviewers:

Dr. Gábor Koncz, PhD, DSc

Dr. Bénédicte Chazaud, PhD

Members of the Defense Committee:

Dr. Beáta Lontay, PhD, DSc

Dr. Zoltán Jakus, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 1:00 p.m., on 4 September 2026

1. Introduction

Tissue repair is a complex biological process that depends on the precise coordination between inflammatory responses and regenerative mechanisms. While inflammation is essential for clearing damaged tissue, it must be carefully resolved to allow proper healing. Macrophages play a central role in this process due to their remarkable plasticity, enabling them to transition across a spectrum of functional states that support both early defense and later tissue restoration. Increasing evidence suggests that these transitions are not binary but are instead dynamically regulated over time and space, highlighting the importance of understanding the underlying control mechanisms.

Skeletal muscle provides a valuable model to study these processes because of its strong regenerative capacity and well-defined interaction between immune cells and tissue-resident progenitors. Within this context, transcriptional regulation has emerged as a key determinant of macrophage behavior. Factors such as BACH1, traditionally associated with oxidative stress responses, are now being explored for their broader roles in immune regulation. Understanding how such regulators influence macrophage function is important not only for clarifying fundamental aspects of tissue repair but also for informing therapeutic approaches aimed at improving regeneration and controlling inflammation in disease.

2. Literature overview

2.1. From host defense to tissue-driven inflammation

Inflammation has long been understood as a protective response aimed at eliminating pathogens and restoring tissue integrity. Classical immunological frameworks describe how innate immune cells recognize invading microorganisms through conserved molecular patterns and initiate rapid defensive responses. While this perspective has been fundamental for understanding antimicrobial immunity, it represents only one dimension of inflammatory biology. It is now clear that inflammation is not exclusively triggered by infection but also arises in response to endogenous signals released from damaged or stressed tissues.

These two contexts, infectious and sterile inflammation, originate from distinct sources but converge on common molecular mechanisms. Pattern recognition receptors detect both pathogen-associated molecular patterns and damage-associated molecular patterns, activating shared signaling pathways that lead to the production of cytokines, chemokines, and other inflammatory mediators. As a result, the early phases of inflammation, regardless of the trigger, are characterized by leukocyte recruitment, activation of innate immune cells, and amplification of inflammatory signaling. Importantly, inflammation is not a static or unidirectional process. Following the initial phase of pathogen clearance or debris removal, the immune response must transition toward a state that supports tissue recovery. This transition, often referred to as resolution, is an active and tightly regulated process rather than a passive decline in inflammation. It involves suppression of further immune cell recruitment, clearance of apoptotic cells, and the initiation of tissue repair mechanisms. When this transition fails or becomes dysregulated, inflammation can persist, leading to chronic disease, fibrosis, or impaired regeneration. Therefore, understanding how inflammatory responses are properly terminated and redirected toward repair is central to deciphering immune function in both health and disease.

2.2. Dynamic adaptation of macrophages to environmental requirements

Among immune cells, macrophages are uniquely positioned to coordinate the progression of inflammation. These cells act as central integrators of environmental signals and exhibit remarkable functional flexibility, allowing them to adapt to changing tissue conditions. During the early stages of inflammation, macrophages contribute to host defense by producing pro-

inflammatory cytokines, reactive oxygen species, and antimicrobial mediators. As the tissue environment evolves, these same cells undergo a functional reprogramming that enables them to support resolution and repair. Historically, macrophage activation has been described using the M1/M2 polarization framework, which classifies macrophages into pro-inflammatory or anti-inflammatory states. While this model has provided a useful conceptual starting point, it does not fully capture the complexity of macrophage behavior in vivo. Advances in single-cell transcriptomics have demonstrated that macrophages exist along a continuum of transcriptional states rather than as discrete subsets. These states are shaped by a combination of temporal, spatial, and environmental factors, reflecting the dynamic nature of immune responses within tissues. Rather than switching between fixed phenotypes, macrophages continuously adjust their functional programs in response to local milieu. This adaptability allows them to coordinate different phases of inflammation, from initial defense to eventual resolution. Consequently, macrophage biology is better understood as a process of regulated state transitions rather than binary polarization. Defining the mechanisms that control these transitions represents a critical challenge for understanding how immune responses are orchestrated in complex tissue environments.

2.3. Immune coordination of tissue repair in regenerative inflammation

The role of macrophages in coordinating inflammation becomes particularly evident in tissues with high regenerative capacity. Skeletal muscle provides a well-established model to study these processes, as it undergoes a highly organized sequence of events following acute injury. This sequence includes immune cell infiltration, clearance of necrotic tissue, activation of muscle stem cells, and reconstruction of functional tissue architecture. Macrophages are central regulators of this process. In the early phase of injury, they adopt a pro-inflammatory profile that promotes efficient debris clearance and amplifies immune signaling. These early macrophages also influence the activation and proliferation of muscle stem cells, linking immune responses to regenerative processes. As regeneration progresses, macrophages transition toward pro-resolving phenotypes that suppress inflammatory signaling and support tissue repair through the production of growth factors and anti-inflammatory mediators. This temporal coordination is essential for successful regeneration. If macrophages fail to transition appropriately between these functional states, tissue repair can be compromised, resulting in fibrosis or chronic inflammation. Such dysregulation is observed in a variety of pathological conditions, including muscular dystrophies and chronic inflammatory diseases, where persistent inflammatory signaling disrupts normal regenerative processes. These observations highlight that the outcome of tissue injury is not determined solely by the presence of immune cells, but by how their functional states are regulated over time.

2.4. Transcriptional control of macrophage states and emerging role of BACH1

The ability of macrophages to adapt to changing environments is governed by tightly regulated transcriptional and epigenetic mechanisms. Lineage-determining transcription factors establish the foundational chromatin landscape of macrophages, defining accessible regulatory regions that enable cell-type-specific gene expression. In response to external stimuli, additional transcription factors dynamically regulate gene expression programs that drive functional changes. These processes are further modulated by chromatin accessibility, histone modifications, and higher-order genome organization, which together shape the responsiveness of macrophages to environmental changes. While significant progress has been made in understanding transcriptional activation in macrophages, the mechanisms that regulate repression and coordinate transitions between functional states remain less well defined. In particular, how macrophages suppress inflammatory programs while activating reparative ones, and how these processes are temporally controlled, are key unresolved questions.

In this context, BACH1 has emerged as a potential regulator of macrophage plasticity. Traditionally described as a heme-sensitive transcriptional repressor involved in oxidative stress responses, BACH1 is best known for its role in regulating genes such as HMOX1. However, recent evidence suggests that its function extends beyond this canonical pathway. BACH1 has been implicated in controlling chromatin accessibility, enhancer activity, and transcriptional networks, positioning it as a candidate regulator of broader gene expression programs. These properties raise the possibility that BACH1 may play a central role in coordinating macrophage state transitions, particularly in environments where metabolic and inflammatory signals intersect. Understanding whether and how BACH1 regulates these processes is essential for defining the molecular mechanisms that govern inflammatory resolution and tissue repair.

To address this, the present work investigates the role of BACH1 in macrophage plasticity during skeletal muscle regeneration. By integrating *in vivo* models, single-cell transcriptomics, and functional analyses, this study aims to define how transcriptional regulation shapes macrophage behavior and determines the outcome of tissue injury.

3. Hypothesis

Macrophages coordinate the transition from inflammation to tissue repair through tightly regulated transcriptional programs that enable dynamic adaptation to changing microenvironments. While activation pathways driving inflammatory responses have been extensively characterized, the mechanisms that govern the timely suppression of these programs and the transition toward reparative states remain incompletely understood. Based on the emerging role of BACH1 as a chromatin-associated transcriptional regulator, we hypothesize that BACH1 functions as a key regulator of macrophage plasticity by controlling transcriptional networks that govern state transitions during regenerative inflammation. Specifically, BACH1 is proposed to modulate the balance between inflammatory and reparative programs, thereby ensuring proper temporal coordination of macrophage responses following tissue injury. We further hypothesize that loss of BACH1 disrupts these regulatory programs, leading to altered macrophage activation dynamics, impaired resolution of inflammation, and defective tissue regeneration.

4. Aims

To test this hypothesis, the following objectives were pursued:

1. Define macrophage heterogeneity and activation programs during regenerative inflammation
 - Characterize immune cell populations in skeletal muscle following acute injury.
 - Identify macrophage subsets and their transcriptional states using single-cell transcriptomics.
 - Determine how macrophage states are organized along inflammatory-to-reparative trajectories.
2. Determine the impact of BACH1 deficiency on macrophage activation and state transitions
 - Assess how loss of BACH1 affects macrophage composition and dynamics during tissue repair.
 - Analyze trajectory progression and differentiation pathways in control and BACH1-deficient conditions.
 - Evaluate whether BACH1 regulates the timing and directionality of macrophage state transitions.
3. Identify BACH1-dependent transcriptional programs in macrophages
 - Define gene expression changes associated with BACH1 deficiency at both basal and stimulated conditions.

- Determine how BACH1 regulates inflammatory and reparative gene networks.
 - Investigate the role of BACH1 in shaping transcription factor activity and regulatory pathways.
4. Assess the functional consequences of BACH1-mediated regulation on tissue regeneration
- Evaluate how BACH1-dependent macrophage programs influence muscle stem cell function and differentiation.
 - Determine the impact of altered macrophage signaling on myogenesis using conditioned media approaches.
 - Assess tissue-level outcomes, including regeneration efficiency and structural recovery, *in vivo*.

5. Materials and Methods

Experimental model of skeletal muscle injury

To investigate macrophage dynamics during regenerative inflammation, acute skeletal muscle injury was induced in mice using a cardiotoxin-based model. Intramuscular injection of cardiotoxin into the tibialis anterior muscle results in rapid and synchronized myofiber necrosis while preserving the basal lamina and resident stem cell populations, allowing robust and reproducible regeneration. This model enables precise temporal analysis of immune cell infiltration, macrophage activation, and tissue repair processes. Muscle tissues were collected at defined time points following injury, corresponding to early inflammatory and later regenerative phases. These time points were selected to capture the transition of macrophages from pro-inflammatory to pro-resolving states.

Isolation and characterization of immune cells

Single-cell suspensions were prepared from injured skeletal muscle through enzymatic digestion and mechanical dissociation. CD45⁺ immune cells were isolated to focus the analysis on hematopoietic populations involved in the inflammatory response. Flow cytometry was used to characterize immune cell composition and to quantify macrophage populations during regeneration. Cells were stained with fluorophore-conjugated antibodies targeting lineage and activation markers, allowing identification of monocytes, macrophages, and other immune subsets. Viability dyes were included to exclude dead cells, and absolute cell numbers were determined using counting beads where appropriate.

Single-cell RNA sequencing and computational analysis

To resolve macrophage heterogeneity and transcriptional dynamics, single-cell RNA sequencing (scRNA-seq) was performed on CD45⁺ immune cells isolated from injured muscle at selected time points. Libraries were generated using a droplet-based 3' end sequencing platform, enabling transcriptome profiling at single-cell resolution. Data processing included quality control, normalization, and dimensionality reduction. Cell clustering was performed to identify distinct immune populations, and macrophage subsets were further analyzed to define transcriptional states. Trajectory inference and RNA velocity analyses were applied to reconstruct dynamic transitions between macrophage states over time. Graph-based approaches were used to assess connectivity and directionality of differentiation pathways.

Bulk RNA sequencing and gene expression analysis

To complement single-cell analyses and characterize transcriptional programs at the population level, bulk RNA sequencing was performed on bone marrow-derived macrophages and tissue-derived samples under different conditions. Total RNA was extracted, and sequencing libraries were prepared using standard protocols followed by high-throughput sequencing. Differential gene expression analysis was conducted to identify genes and pathways regulated by BACH1. Functional annotation and pathway enrichment analyses were

performed to interpret transcriptional changes and to define biological processes associated with inflammatory and reparative macrophage states.

In vitro macrophage culture and stimulation

Bone marrow-derived macrophages were generated by differentiating bone marrow cells in the presence of macrophage colony-stimulating factor. Fully differentiated macrophages were subjected to different stimulation conditions to model inflammatory and activation responses. Cells were treated with lipopolysaccharide to induce pro-inflammatory activation, and additional stimuli were applied where indicated to assess functional responses. In selected experiments, inflammasome activation was triggered using a two-step stimulation protocol involving priming and activation signals, enabling assessment of cytokine processing and secretion.

Functional assays and cytokine measurements

To evaluate macrophage function, cytokine production was measured in cell culture supernatants using enzyme-linked immunosorbent assays. Particular attention was given to inflammatory mediators associated with macrophage activation and tissue repair. To assess the impact of macrophage-derived signals on muscle regeneration, conditioned media experiments were performed. Supernatants from macrophage cultures were applied to myogenic cells to evaluate their effects on differentiation and cellular behavior. These assays provided functional insights into how macrophage states influence tissue regeneration.

Histological and tissue-level analysis

Regeneration outcomes were assessed through histological analysis of skeletal muscle tissue. Muscles were harvested, processed, and sectioned for staining to evaluate tissue architecture, myofiber morphology, and regeneration status. Quantitative measurements, including myofiber size and indicators of regeneration, were performed to compare tissue repair between experimental conditions. These analyses enabled correlation of molecular and cellular findings with functional outcomes at the tissue level.

Integration of multi-level datasets

To obtain a comprehensive view of macrophage regulation, data from single-cell transcriptomics, bulk RNA sequencing, flow cytometry, and functional assays were integrated. This multi-layered approach allowed the identification of consistent patterns across different experimental systems and provided a robust framework for linking transcriptional regulation to cellular behavior and tissue-level outcomes.

6. Results

6.1. Transcriptional landscape reveals macrophage heterogeneity during regenerative inflammation

To characterize immune cell dynamics during tissue repair, single-cell RNA sequencing was performed on CD45⁺ immune cells isolated from skeletal muscle following acute injury. This approach enabled high-resolution profiling of the cellular composition and transcriptional states associated with regenerative inflammation. Dimensionality reduction and clustering analyses identified multiple immune cell populations, with macrophages representing a dominant and highly heterogeneous compartment. Rather than forming discrete subsets, macrophages displayed a continuum of transcriptional states, reflecting progressive adaptation to the evolving tissue environment. These states were characterized by distinct gene expression programs associated with inflammatory activation, phagocytosis, and tissue repair.

Trajectory inference analysis revealed that macrophages are organized along a dynamic axis spanning early inflammatory to later reparative states. Early-stage macrophages exhibited transcriptional signatures consistent with pro-inflammatory activation, including expression of cytokines and chemokines involved in immune recruitment and amplification of inflammation. In contrast, macrophages located at later stages of the trajectory displayed gene expression patterns associated with resolution and tissue remodeling, including factors linked to angiogenesis, extracellular matrix organization, and cellular differentiation. Notably, gene expression patterns suggested the presence of regulatory axes associated with alternative activation and tissue repair. These included transcriptional programs linked to IL-4-responsive pathways and angiogenic signaling, indicating that macrophage states are shaped by both inflammatory and regenerative signals. Importantly, these findings support the concept that macrophage activation *in vivo* is not adequately described by binary polarization models but instead reflects a continuous and context-dependent spectrum of functional states.

6.2. Loss of BACH1 alters basal macrophage states and primes activation responses

To investigate the role of BACH1 in macrophage regulation, transcriptional profiles of BACH1-deficient macrophages were analyzed and compared to control conditions. At baseline, loss of BACH1 resulted in widespread alterations in gene expression, indicating that BACH1 contributes to maintaining macrophage homeostasis even in the absence of external stimulation. BACH1-deficient macrophages exhibited a primed transcriptional state characterized by the upregulation of genes associated with immune activation, cytokine signaling, and cellular metabolism. This suggests that BACH1 normally acts to restrain activation programs, preventing premature or excessive inflammatory responses. Upon stimulation, BACH1-deficient macrophages displayed altered responsiveness compared to controls. While the magnitude of gene expression changes was comparable, the identity of regulated genes differed substantially, indicating that BACH1 influences not only the baseline state but also the trajectory of activation. In particular, genes associated with inflammatory and metabolic pathways were differentially regulated, highlighting the role of BACH1 in shaping stimulus-dependent transcriptional outputs. These findings demonstrate that BACH1 functions as a key regulator of macrophage activation dynamics, controlling both basal transcriptional states and responses to environmental signals.

6.3. BACH1 controls macrophage state transitions during regenerative inflammation

To determine how BACH1 influences macrophage behavior *in vivo*, single-cell datasets from control and BACH1-deficient conditions were integrated and analyzed jointly. This approach allowed direct comparison of macrophage states and their progression along differentiation trajectories. Trajectory and RNA velocity analyses revealed that macrophage transitions from inflammatory to reparative states follow a defined directional progression in control conditions. Early monocyte-derived cells progressively acquire macrophage identity and transition toward resolution-associated states during the course of regeneration. In contrast, BACH1 deficiency altered this trajectory. Macrophages lacking BACH1 displayed shifts in their distribution along the inferred trajectory, indicating disruptions in the timing and progression of state transitions. In particular, the balance between early inflammatory and later reparative states was perturbed, suggesting that BACH1 is required for proper coordination of macrophage differentiation during tissue repair.

Graph-based analyses further supported these observations by demonstrating changes in connectivity between macrophage states in BACH1-deficient conditions. These alterations indicate that BACH1 contributes to maintaining the structure and directionality of macrophage state transitions. Importantly, these effects were not solely attributable to changes in cell composition but reflected intrinsic differences in transcriptional programs governing macrophage behavior. Together, these findings establish BACH1 as a regulator of

macrophage trajectory progression and highlight its role in coordinating the transition from inflammation to resolution *in vivo*.

6.4. BACH1 regulates transcriptional networks underlying macrophage function

To define the molecular basis of BACH1-dependent regulation, transcriptional programs were analyzed at both single-cell and bulk levels. Differential gene expression analysis identified extensive changes in genes associated with immune signaling, metabolism, and tissue remodeling. BACH1-dependent gene regulation extended beyond canonical targets such as oxidative stress-responsive genes, indicating a broader role in controlling macrophage transcriptional networks. Genes involved in cytokine production, chemokine signaling, and extracellular matrix remodeling were differentially regulated, suggesting that BACH1 integrates multiple aspects of macrophage function.

Further analysis revealed that BACH1 influences transcription factor networks, modulating the activity of pathways associated with inflammatory and reparative responses. These findings support a model in which BACH1 acts as a higher-order regulator of transcriptional programs rather than a gene-specific repressor. In addition, integration with chromatin-related datasets indicated that BACH1 may contribute to shaping the regulatory landscape of macrophages, influencing enhancer activity and transcriptional accessibility. This is consistent with a role for BACH1 in coordinating gene expression programs at the level of chromatin organization. Overall, these results demonstrate that BACH1 regulates complex transcriptional networks that govern macrophage identity and function across different activation states.

6.5. BACH1-dependent macrophage programs influence tissue repair outcomes

To assess the functional consequences of BACH1-mediated regulation, the impact of macrophage-derived signals on tissue repair processes was evaluated. Cytokine measurements revealed that BACH1 deficiency affects the production of key inflammatory mediators, including factors involved in the regulation of immune responses and tissue regeneration. In particular, alterations in the expression and secretion of cytokines associated with inflammatory activation suggested that BACH1-deficient macrophages exhibit dysregulated signaling profiles. These changes have the potential to influence downstream cellular interactions within the regenerative niche. To directly test this, conditioned media experiments were performed to evaluate the effect of macrophage-derived factors on myogenic differentiation. Supernatants from BACH1-deficient macrophages impaired the differentiation of muscle precursor cells compared to controls, indicating that macrophage signaling plays a critical role in regulating myogenesis. Consistent with these findings, *in vivo* analysis revealed that loss of BACH1 leads to defective muscle regeneration. Histological assessment demonstrated alterations in tissue architecture and regeneration efficiency, including changes in myofiber size and indicators of delayed repair. Together, these results establish a functional link between BACH1-dependent macrophage regulation and tissue-level outcomes. By controlling transcriptional programs that govern macrophage behavior, BACH1 ensures proper coordination of inflammatory resolution and tissue regeneration.

7. Discussion

The ability of the immune system to coordinate the transition from inflammation to tissue repair is fundamental for maintaining tissue integrity. Macrophages are central to this process due to their capacity to dynamically adapt their functional programs in response depending on the tissue environment. While significant progress has been made in characterizing macrophage activation, the mechanisms that regulate transitions between inflammatory and reparative states remain incompletely understood. The findings presented in this thesis identify BACH1 as a key regulator of these transitions and provide new insights into the transcriptional control of macrophage plasticity.

A central observation of this work is that macrophage states during regenerative inflammation are not discrete but organized along a continuum. Single-cell transcriptomic analysis revealed progressive transitions from early inflammatory to later reparative states, supporting a model in which macrophage function is defined by dynamic trajectories rather than fixed phenotypes. This conceptual framework challenges the traditional M1/M2 paradigm and emphasizes the importance of temporal and environmental context in shaping macrophage behavior. Within this dynamic system, BACH1 emerges as a critical regulator of macrophage identity. Loss of BACH1 alters basal transcriptional programs, leading to a primed state characterized by increased expression of activation-associated genes. This suggests that BACH1 plays a role in maintaining macrophage homeostasis by restraining premature or excessive activation. Importantly, these baseline alterations influence subsequent responses to environmental stimuli, indicating that BACH1 regulates not only gene expression but also the trajectory of macrophage activation.

At the tissue level, BACH1 was found to control macrophage transitions during regenerative inflammation. Trajectory and RNA velocity analyses demonstrated that BACH1 deficiency disrupts the progression from inflammatory to reparative states, resulting in altered distribution of macrophage populations along the differentiation continuum. These findings highlight that BACH1 is not simply involved in defining individual macrophage states, but rather in coordinating the timing and directionality of state transitions. This distinction is critical, as proper sequencing of macrophage functions is essential for successful tissue repair. Mechanistically, BACH1 regulates transcriptional networks that extend beyond its classical role in oxidative stress responses. The data presented here show that BACH1 influences gene expression programs associated with inflammation, metabolism, and tissue remodeling, suggesting a broader role as a regulator of macrophage transcriptional identity. Furthermore, evidence supporting its involvement in chromatin regulation and enhancer activity positions BACH1 as a higher-order regulator capable of shaping the transcriptional landscape of macrophages.

The functional consequences of BACH1-dependent regulation are evident at the level of tissue repair. BACH1-deficient macrophages exhibit altered cytokine production and impaired ability to support myogenic differentiation. These changes translate into defective muscle regeneration *in vivo*, demonstrating that transcriptional dysregulation at the cellular level has direct consequences for tissue-level outcomes. These findings reinforce the concept that macrophage plasticity is not only a feature of immune regulation but also a determinant of regenerative capacity. An important implication of this work relates to the therapeutic targeting of BACH1. Given its association with pathological conditions, BACH1 has often been considered a candidate for inhibition. However, the results presented here indicate that complete loss of BACH1 function disrupts essential regulatory processes required for proper tissue repair. This suggests that BACH1 should not be viewed solely as a detrimental factor but rather as a context-dependent regulator whose activity must be finely controlled. Future therapeutic strategies may therefore benefit from approaches that modulate, rather than abolish, BACH1 activity, allowing selective tuning of macrophage responses depending on the inflammatory context.

More broadly, these findings contribute to a growing understanding of how transcriptional and epigenetic mechanisms regulate immune cell plasticity. By identifying BACH1 as a regulator of macrophage state transitions, this work provides a framework for investigating how similar mechanisms may operate in other tissues and disease settings. Dysregulated macrophage responses are a common feature of chronic inflammatory diseases, fibrosis, and cancer, suggesting that the principles uncovered here may have broad relevance. In summary, this thesis demonstrates that BACH1 governs macrophage plasticity by controlling transcriptional programs that regulate state transitions during regenerative inflammation. By linking transcriptional regulation to functional outcomes in tissue repair, this work advances our

understanding of immune-mediated regeneration and highlights new avenues for therapeutic intervention.

8. Summary

Our research demonstrates that BACH1 is a crucial hierarchical epigenomic and transcriptional modulator of macrophage identity and functional plasticity in steady-state and regenerative inflammatory conditions. Through distinct mechanisms such as pioneer repression and the shaping of chromatin accessibility at MARE/TRE motifs, BACH1 controls cell-intrinsic gene expression programs and niche adaptation. Given its specific role in balancing inflammatory and reparative trajectories, BACH1 presents a potential target for therapeutic intervention using fine-tuned modulation rather than complete inhibition to preserve tissue homeostasis and enhance skeletal muscle repair.

As summary, in our findings we demonstrated that (1) BACH1 acts as a hierarchical regulator of macrophage priming, where its deficiency induces a basal pro-inflammatory state and exaggerated responsiveness even in the absence of stimuli and (2) as a repressor during early regeneration (dpi2), it binds to MARE/TRE motifs to remodel the chromatin landscape, establishing the regulatory framework for subsequent functional states. (3) It serves as a critical determinant of competitive fitness and niche adaptation, enabling myeloid cells to adapt to within specialized environments such as the lung and peritoneal cavity and (4) coordinates the temporal sequence of the monocyte-to-macrophage transition, preventing an accelerated but incomplete differentiation trajectory that would otherwise lead to premature accumulation of non-functional resolving populations. (5) Regenerative inflammatory events are modulated by myeloid BACH1 to maintain a transcriptional balance between pro-inflammatory programs and interferon-responsive signatures, leading to a sustained inflammatory environment characterized by increased production of TNF, IL-6, and IL-1 β when BACH1 is lost. (6) Myeloid-specific loss of BACH1 manifests in impaired skeletal muscle regeneration, where elevated macrophage-derived IL-1 β directly inhibits myoblast differentiation, resulting in reduced myofiber size, disrupted tissue architecture, and persistent necrosis.

9. Acknowledgments

I would like to sincerely thank my colleagues and collaborators at the University of Debrecen for creating such a stimulating and supportive scientific environment. In particular, I am grateful to Krisztian, Petros, Gergely and Andreas for the many insightful discussions and ideas that greatly contributed to the development of this thesis. I would also like to thank Timea for her invaluable help during experiments and for making long experimental days in the lab far more enjoyable. My thanks also go to Dóra, my office colleague, whose help and encouragement were especially appreciated during the many frustrating moments of troubleshooting coding errors. I am deeply grateful to Zsolt for his constant availability, enthusiasm for science, and valuable suggestions, which were key throughout this journey. Lastly, I would like to acknowledge the vast resources available at the University of Debrecen, particularly the Sequencing Facility, led by Dr. Szilárd Poliska, and the Animal Facility, headed by Dr. Dávid Deák, together with its excellent technicians, Vicky and Péter, whose work was indispensable for this work.

My deepest gratitude goes to my supervisor, László, for introducing me to the fascinating world of macrophage biology and for guiding me through the complexity of their diverse roles in physiology and disease. It has been truly inspiring to work with a supervisor whose medical background constantly bridges basic science with its potential applications in medicine. His vision of connecting fundamental research with in vivo biology and translational relevance has been a major source of motivation throughout this work.

I would also like to thank our collaborators from the Renoir Marie Curie network, especially Matthew Borok and Frédéric Relaix, who taught me how to isolate and culture MuSCs and whose expertise greatly inspired the final section of this thesis on cell-cell interactions.

Finally, I would like to express my deepest gratitude to my family. I am especially thankful to my uncle Manolo for the many thoughtful conversations shared over a glass of wine. To my brother, Eleazar C. Sánchez, scientist and coach, thank you for your constant support during the most challenging moments and for always encouraging me to never give up on sport and discipline, an inspiration that even found its way into the first sentences of Section 1.2.2 of the Introduction. And of course, I cannot forget my mother, who has always been a role model of hard work and perseverance, qualities that are essential in science and that have found the basis of who I am today.

10. List of publications related to dissertation



UNIVERSITY of
DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN

H-4002 Egyetem tér 1, Debrecen

Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/269/2026.PL
Subject: PhD Publication List

Candidate: Noemí Caballero-Sánchez

Doctoral School: Doctoral School of Molecular Cellular and Immune Biology

List of publications related to the dissertation

1. Tzerpos, P., Bojcsuk, D., Bene, K., **Caballero-Sánchez, N.**, Parteka-Tojek, Z., Banecki, K., Korsak, S., Pritchett, S. M., Patsalos, A., Oleksak, D., Berger, W. K., Sarris, M. E., Giannakis, N., Chantalat, S., Silye-Cseh, T., Papachristou, E. K., Erdélyi, F., Máté, Z., Szabó, G., Nagy, G., Carroll, J. S., Spilianakis, C. G., Schwabe, J. W. R., Plewczynski, D., Nagy, L.: BACH1 acts as a pioneer repressor to enable macrophage plasticity and adaptation across tissues and inflammatory contexts.
Immunity. "Accepted by Publisher", 2026.
IF: 26.3 (2024)
2. **Caballero-Sánchez, N.**, Tzerpos, P., Bene, K., Halász, L., Bojcsuk, D., Nagy, G., Ali, M. A., Silye-Cseh, T., Pólska, S., Borok, M. J., Scherer, J., Relaix, F., Saez, E., Czimmerer, Z., Patsalos, A., Nagy, L.: BACH1 orchestrates macrophage state transitions to coordinate regenerative inflammation.
J. Immunol. "Accepted by Publisher", 2026.
DOI: <http://dx.doi.org/10.1093/jimmun/vkag101>
IF: 3.4 (2024)
3. Domokos, A., Varga, Z., Jambrovics, K., **Caballero-Sánchez, N.**, Szabó, E., Nagy, G., Scholtz, B., Halász, L., Váradi, E., Bene, K., Türk-Mázló, A., Bácsi, A., Jeney, V., Szebeni, G. J., Nagy, L., Czimmerer, Z.: The transcriptional control of the VEGFA-VEGFR1 (FLT1) axis in alternatively polarized murine and human macrophages.
Front. Immunol. 14, 1-20, 2023.
DOI: <http://dx.doi.org/10.3389/fimmu.2023.1168635>
IF: 5.7





List of other publications

4. Baráth, B. R., Bojcsuk, D., Bene, K., **Caballero-Sánchez, N.**, Silye-Cseh, T., de Freitas, J. C., Tzerpos, P., Tóth, M., Tang, Z., Guller, S., Krasznai, Z. T., Neuperger, P., Szebeni, G. J., Nagy, G., Deli, T., Nagy, L.: Distinct transcriptional and epigenomic programs define Hofbauer cells in term placenta.
JCI Insight. 11 (3), 1-21, 2025.
DOI: <http://dx.doi.org/10.1172/jci.insight.195801>
IF: 6.1 (2024)
5. **Caballero-Sánchez, N.**, Alonso-Alonso, S., Nagy, L.: Regenerative inflammation: When immune cells help to re-build tissues.
FEBS J. 291 (8), 1593-1614, 2024.
DOI: <http://dx.doi.org/10.1111/febs.16693>
IF: 4.2
6. Alonso-Alonso, S., Vázquez, N., Chacón, M., **Caballero-Sánchez, N.**, Del Olmo-Aguado, S., Suárez, C., Alfonso-Bartolozzi, B., Fernández-Vega-Cueto, L., Nagy, L., Merayo-Llodes, J., Meana, A.: An effective method for culturing functional human corneal endothelial cells using a xenogeneic free culture medium.
Sci. Rep. 13 (1), 1-11, 2023.
DOI: <http://dx.doi.org/10.1038/s41598-023-46590-2>
IF: 3.8

Total IF of journals (all publications): 49,5

Total IF of journals (publications related to the dissertation): 35,4

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

12 May, 2026

