# Hypoxia Impacts Histone Modifications in Immune Cells

Natalie A. Dennis, Department of Neuroscience
Sunny Virk, Master's Student, Division of Biomedical Sciences
Kathy Pham, Ph.D., Division of Biomedical Sciences
Erica C. Heinrich, Ph.D., Division of Biomedical Sciences

## ABSTRACT

Hypoxia, or reduced oxygen availability, influences immune cell function and contributes to pathologies such as cancer and lung disease. This study investigates how hypoxia impacts global histone modification patterns and the expression of histone-modifying enzymes in peripheral immune cells. Modifications on histone proteins influence gene expression, particularly for genes involved in mediating rapid cellular responses to stressors such as hypoxia. Therefore, we hypothesized that hypoxic stress induces (1) differential expression of histone-modifying enzyme genes, and (2) significant changes in global histone modification levels, particularly those linked to the hypoxic stress response.

We analyzed blood samples from 15 healthy individuals at sea level and after 3 days of high-altitude hypoxia (3800 m). We identified significant changes in histone-modifying enzyme gene expression, including decreased HDAC1 and HDAC3, and increased KDM3A and SIRT1. In addition, we cultured peripheral immune cells in hypoxia (1% $O_2$) and identified a significant increase in global histone modification levels across the 21 modifications studied. These findings suggest that histone modifications are significantly altered by hypoxic stress and may play a critical role in rapid cellular adaptation to oxygen limitation. Future work will map the genomic locations of these modifications to uncover mechanisms driving cellular and physiological responses to hypoxia.

KEYWORDS: hypoxia, histone modifications, epigenetics, RNA-seq, histone H3, energy conservation

**NATALIE DENNIS**

Natalie Dennis earned her B.S. in neuroscience in March 2025. Since 2023, she has researched hypoxia's effects on epigenetic regulation in Dr. Heinrich's lab. With four years as a medical assistant, she aims to blend clinical experience and research in her path toward becoming a physician-scientist.

**FACULTY MENTOR - Dr. Erica C. Heinrich, Division of Biomedical Sciences**

Dr. Erica Heinrich is an assistant professor in the Division of Biomedical Sciences at the UC Riverside School of Medicine at UC Riverside. She completed her Ph.D. at the University of California, Irvine with Dr. Timothy Bradley and her postdoctoral fellowship at the University of California, San Diego with Dr. Frank Powell and Dr. Tatum Simonson. Her research examines the integrative physiology of hypoxic stress in humans, with a specific focus on the impact of hypoxemia on immune function.

# Hypoxia Impacts Histone Modifications in Immune Cells

## INTRODUCTION

### Hypoxia and Its Biological Impact

Hypoxia, defined as a reduction in oxygen availability, is a critical stressor that influences cellular behavior across physiological and pathological contexts. Adaptation to hypoxia is vital for processes such as embryonic development, high-altitude acclimatization, and wound healing; it also plays a role in disease progression, including cancer, ischemia, and chronic lung diseases (Batie et al., 2019). Cells respond to hypoxia by activating transcriptional programs that include metabolic reprogramming, angiogenesis, and cell cycle regulation, primarily mediated by hypoxia-inducible factors (HIFs). These transcription factors are regulated by modifications that alter histone proteins and enable the regulation of genes involved in oxygen adaptation.

### Histones and Chromatin Dynamics

Histones are core components of nucleosomes, the fundamental units of chromatin. These proteins not only compact DNA but also dynamically regulate access to genetic information. This regulation is achieved through post-translational modifications (PTMs) that occur on histone tails, including methylation, acetylation, and phosphorylation, among others (Bannister & Kouzarides, 2011). These PTMs create a "histone code" that dictates chromatin states, enabling transcriptional activation or repression, and facilitating rapid cellular responses to different conditions.

PTMs of histones, such as methylation, acetylation, and phosphorylation, provide a versatile means of modulating gene activity. Histone methylation, particularly at lysine residues, has emerged as a key epigenetic mechanism regulating gene expression. Modifications like H3K9me2 and H3K9me3 are associated with transcriptional repression and heterochromatin formation (Greer & Shi, 2012). The balance of methylation is maintained by histone methyltransferases and demethylases, which can be sensitive to environmental cues, including oxygen levels (Batie et al., 2019). Acetylation neutralizes the positive charge of lysine residues on histones, weakening their interaction with negatively charged DNA. This process, often associated with transcriptional activation, allows chromatin to adopt a more open conformation (Ozawa, 2008). Phosphorylation of histone H3 can lead to either chromatin condensation during mitosis or chromatin relaxation associated with transcriptional activation and is overall highly context-dependent (Sawicka & Seiser, 2012).

### Hypoxia and Epigenetic Regulation

Emerging evidence suggests that hypoxia significantly alters chromatin through PTMs of histones. Histone-modifying enzymes, such as Jumonji C (JmjC)-domain histone demethylases, require oxygen as a cofactor, making them highly sensitive to oxygen availability (Batie et al., 2019). Under hypoxic conditions, inhibition of these enzymes leads to an accumulation of methylation marks, such as H3K36me3, which are linked to adaptive transcriptional responses. Similarly, hypoxia has been shown to reduce histone acetylation, potentially contributing to a transcriptionally repressive chromatin environment to conserve energy (Bannister & Kouzarides, 2011).

Previous research has shown that hypoxia leads to increased global histone methylation, particularly at repressive marks like H3K9me3 and H3K27me3, suggesting a shift toward transcriptional repression (Kim et al., 2022).

### Clinical Impact of Hypoxia-Driven Histone Modifications

Hypoxia, a hallmark of diseases such as cancer, ischemia, and chronic pulmonary diseases, significantly alters histone modification patterns, impacting gene expression involved in cell survival, inflammation, and metabolism. These hypoxia-driven epigenetic changes can promote disease progression, influence patient prognosis, and contribute to therapy resistance. Clinically, understanding these modifications offers potential diagnostic and prognostic biomarkers. Importantly, therapeutic targeting of hypoxia-induced histone alterations, such as through histone deacetylase inhibitors, presents promising avenues for novel treatment strategies. Thus, clarifying the epigenetic mechanisms linking hypoxia and histone modifications is crucial for improving patient outcomes.

While it is known that hypoxia alters histone modifications, gaps remain in our understanding of the role histone modifications contribute to adaptation under physiological hypoxic stress and how the level of these modifications change in disease progression. The purpose of our

# Hypoxia Impacts Histone Modifications in Immune Cells

study is to address this gap by examining changes in histone-modifying enzyme expression in humans using a high-altitude hypoxia model and verifying if these enzymatic changes result in measurable alterations in histone modifications via *in vitro* hypoxia cell cultures. We hypothesize that if histone modifications play a crucial role in hypoxia adaptation, then we expect to observe differential expression of histone-modifying enzymes and measurable changes in histone modification patterns.

## METHODS

### Study Design and Sample Collection

This study consists of two separate experiments including an in vivo cohort study in which participants were exposed to environmental sustained hypoxia via high-altitude travel, as well as an in vitro cell culture study to examine direct effects of hypoxia on immune cells. RNA sequencing data from the high-altitude cohort was previously published by Pham et al. (2022) and is further explored in this study.

### Experiment 1: In Vivo High-Altitude Exposure

The study included 15 healthy participants (5 women and 10 men) aged 19–32 years, recruited from the University of California, Riverside campus through word of mouth and flyers. Participants had no known history of cardiopulmonary disease, sleep disturbances (including obstructive sleep apnea), or abnormal findings on ECG or pulmonary function testing. They had not traveled to elevations above 2500 m within one month of the study and were nonsmokers and nonpregnant. The mean age of participants was 25 ± 4 years for men and 26 ± 5 years for women, with BMI averages of 26.7 ± 5.4 kg/m² for men and 28.4 ± 6.9 kg/m² for women.

*Study design:* The study design involved pre-ascent screenings at UC Riverside (400 m elevation) to collect demographic data, medical history, and baseline physiological measurements, including blood pressure, peripheral oxygen saturation ($SpO_2$), and Acute Mountain Sickness (AMS) scores. Participants traveled by car to Barcroft Station, located at 3800 m on White Mountain, over a 6.5-hour period. Systolic and diastolic blood pressure, heart rate, oxygen saturation, AMS scores, and fasting blood samples were taken at sea level and each morning for three consecutive days at high altitude (within one hour of waking and before 09:00). An overview of the experiment is shown in **Figure 1**. End-tidal CO concentrations were also measured nightly.

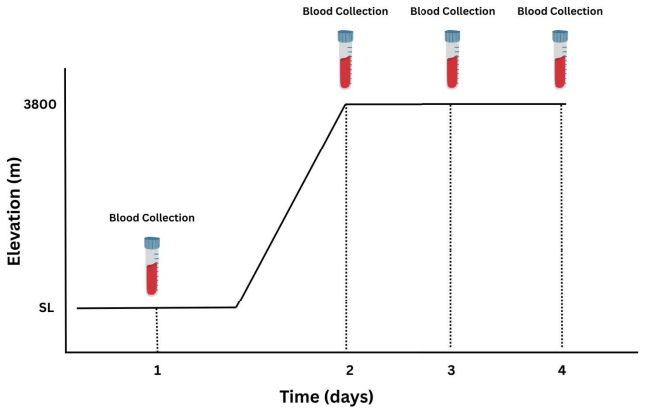


**Figure 1.** Fasting blood samples were collected once at sea level (SL) and then collected at high altitude (3800 meters) consecutively for 3 days.

Participants' blood samples were then analyzed via RNA sequencing and NanoString analyses to examine changes in the expression of histone-modifying enzymes involved in cellular response to hypoxia.

### RNA Sequencing

RNA sequencing (RNA-seq) was employed to analyze gene expression changes in response to acute hypoxia exposure. Peripheral blood samples were collected and processed using the Quiagen PaxGene Blood RNA Kit to ensure high-quality RNA extraction. Libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit, with modifications for optimal purification and size selection. Sequencing was performed on an Illumina NovaSeq 6000 platform, generating 50 bp paired-end reads. Data processing involved alignment to the GRCh38/hg38 reference genome using Rsubread, and gene expression quantification was conducted with featureCounts. Differential gene expression analysis was carried out using DESeq2, applying the Benjamini–Hochberg correction to control for false discovery rates.

# Hypoxia Impacts Histone Modifications in Immune Cells

### Experiment 2: In Vitro Peripheral Blood Mononuclear Cell Culture

For *in vitro* studies, primary peripheral immune cells taken from 3 healthy donors were collected during fasting. Blood was drawn into EDTA tubes and stored at room temperature until processing within 4 hours. Donors were instructed to abstain from caffeine intake 12 hours prior to blood collection. This study was performed in compliance with the Declaration of Helsinki and in accordance with approved UCR IRB protocols (22088).

### PBMC Culture and Hypoxia Treatment

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral venous blood using a density gradient separation protocol with Histopaque 1077. Following centrifugation at 400 g for 30 minutes at room temperature, the PBMC layer was carefully extracted, washed with PBS, and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and streptomycin and penicillin. Cells were seeded at a density of 700,000 cells per flask and incubated under normoxic (18% $O_2$, 5% $CO_2$) or hypoxic conditions (1% $O_2$, 5% $CO_2$) for 24 hours at 37°C. Hypoxic conditions were achieved using a sealed StemCell™ Hypoxia Chamber flushed with calibration gas containing 1% $O_2$. **Figure 2** demonstrates an overview of Experiment 2.

### Histone Extraction and Quantification

Histone proteins were extracted from cultured PBMCs using the EpiQuik™ Total Histone Extraction Kit. Following cell lysis in pre-lysis buffer and centrifugation at 4°C, histones were extracted in lysis buffer, quantified using a Bradford assay and NanoDrop Spectrophotometer, and stored at -80°C until further analysis. Histone H3 modifications were quantified using the EpiGenTek™ Histone H3 Modification Multiplex Assay Kit. Samples (50–100 ng per well) were added to strip wells pre-coated with antibodies specific to 21 histone H3 modifications. The signal was developed using a colorimetric substrate and read at 450 nm and 650 nm with a microplate reader.

### Statistical Analysis

Histone modification data were normalized to total histone H3 levels using internal controls provided in the multiplex assay kit. Fold changes in modification levels between hypoxic and normoxic conditions were calculated to assess epigenetic responses to hypoxia.

## RESULTS

### Experiment 1: In Vivo High-Altitude Exposure

To confirm participants were hypoxic at high altitude, physiological measurements were taken. Table 1 shows participants' mean systolic (Psys) and diastolic (Pdia) blood pressure, heart rate (HR), oxygen saturation (SpO2), Acute Mountain Sickness (AMS), and end tidal CO measurements (CO), with a significant decrease in peripheral oxygen saturation and increase in Acute Mountain Sickness scores ($p < 0.001$) compared to sea level.

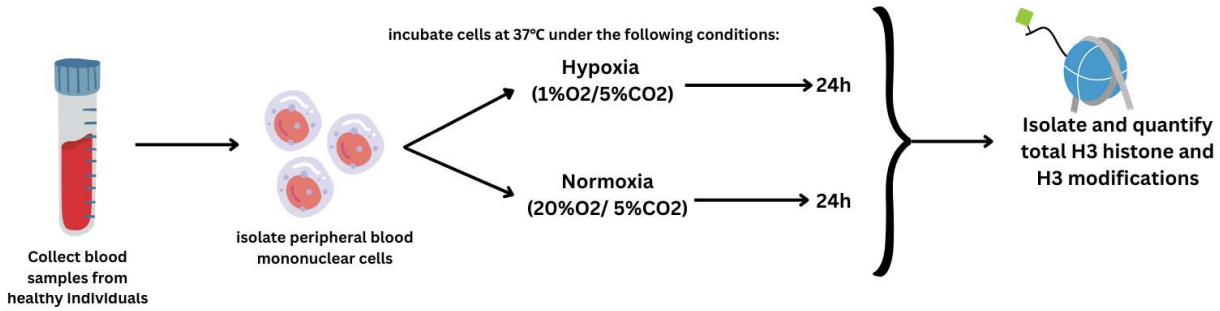


**Figure 2.** Blood samples were collected from two healthy individuals at sea level. Peripheral blood mononuclear cells (PBMCs) were isolated, cultured in either a hypoxic or normoxic environment and incubated for 24 hours each. H3 Histones were isolated and assayed to quantify both total H3 and H3 modifications.

# Hypoxia Impacts Histone Modifications in Immune Cells

### Histone-Modifying Enzyme Expression in Hypoxia

RNA sequencing analysis of blood samples from individuals exposed to high-altitude hypoxia revealed significant changes in histone-modifying enzyme expression. Particularly, histone deacetylases HDAC1 and HDAC3 were downregulated at both Day 1 (HA1) and Day 3 (HA3) of high-altitude exposure. Conversely, expression levels of the histone demethylase KDM3A were upregulated by Day 1, and sirtuin deacetylase SIRT 1 levels were upregulated by Day 1 and Day 3 of high-altitude exposure. These results are shown in Figure 3, with gene expression count on the y-axis and timepoint on the x-axis.

### Experiment 2: In Vitro Peripheral Blood Mononuclear Cell Culture

*In vitro* experiments using cultured peripheral blood mononuclear cells (PBMCs) further confirmed that hypoxia alters histone modification patterns. Figure 4 shows ELISA analyses of 4 different histone modification types under both hypoxic and control conditions, with 3 ELISA plates per modification type. Cells exposed to 1% $O_2$ for 24 hours most notably showed a global increase in levels of histone modifications H3K9me3, H3K36me3, H3K79me3, and H3K4me3, as shown in **Figure 4**.

## DISCUSSION

The goal of this study was to explore how histone modifications contribute to adaptation under chronic hypoxia by quantifying global changes in these marks through both in vivo and in vitro models.

Of the many enzymes we analyzed via RNA sequencing, we chose to focus on genes HDAC1, HDAC3, SIRT1, and KDM3A. HDAC1 and HDAC3 are class I histone deacetylases that remove acetyl groups from histones, leading to chromatin condensation and gene repression. Under hypoxia, their activity is often suppressed, contributing to widespread transcriptional reprogramming necessary for cell survival and energy conservation. SIRT1, a class III $NAD^{+}$-dependent deacetylase, is upregulated in many hypoxic contexts and plays a protective role by regulating genes involved in metabolism, inflammation, and stress resistance — functions that are critical in both adaptation and disease progression. KDM3A is a histone demethylase that specifically removes repressive H3K9 methylation marks and is known to be hypoxia-inducible through HIF-1α signaling. It facilitates the activation of genes required for glycolysis, angiogenesis, and survival under low oxygen conditions. We found that high-altitude hypoxia induces differential

| Variable | SL | HA 1 | HA 2 | HA3 | ANOVA P |
|---|---|---|---|---|---|
| $P_{sys}$ | 128 ± 7 | 125 ± 12 | 126 ± 45 | 126 ± 13 | 0.537 |
| $P_{dia}$ | 79 ± 10 | 83 ± 9 | 83 ± 7 | 85 ± 7 | 0.054 |
| HR | 78.0 ± 8.1 | 88.3 ± 13.2 | 89.7 ± 12.1* | 95.6 ± 12.8*** | **<0.001** |
| $SpO_2$ | 94.8 ± 1.6 | 85.0 ± 4.4*** | 83.7 ± 2.5*** | 86.1 ± 2.5*** | **<0.001** |
| AMS | 0.2 ± 1.4 | 3.1 ± 1.8*** | 2.3 ± 2.0** | 0.7 ± 1.2 | **<0.001** |
| CO | 3.9 ± 1.4 | 5.2 ± 1.5 | 5.0 ± 1.9 | | 0.080 |

**Table 1.** Participants' mean systolic (Psys) and diastolic (Pdia) blood pressure, heart rate (HR), oxygen saturation (SpO2), Acute Mountain Sickness (AMS), and end tidal CO measurements (CO) were taken at sea level (SL) and high altitude (HA) for three consecutive days. Overall p-values for repeated measures ANOVA are provided. Asterisks indicate significant differences from SL at p<0.05 (*), and p<0.001 (***) levels via post-hoc pairwise comparisons with Bonferroni adjusted p-values.

# Hypoxia Impacts Histone Modifications in Immune Cells

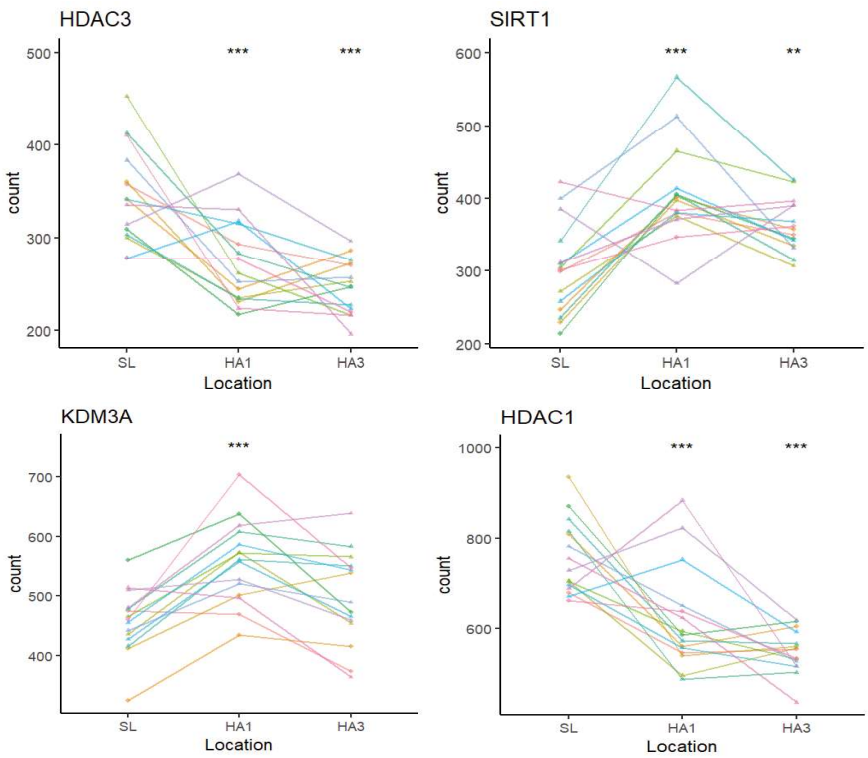


**Figure 3.** Expression levels for genes encoding key histone-modifying enzymes HDAC1 (Histone Deacetylase 1), HDAC3 (Histone Deacetylase 3), KDM3A (Lysine-specific Demethylase 3A), and SIRT1 (Sirtuin 1) were taken at Sea Level (SL), High Altitude on Day 1 (HA1) and High Altitude on Day 3 (HA3). Significance is indicated by **, with p<0.01 and ***, with p <0.001 denoting a significant difference in expression levels compared to sea level.

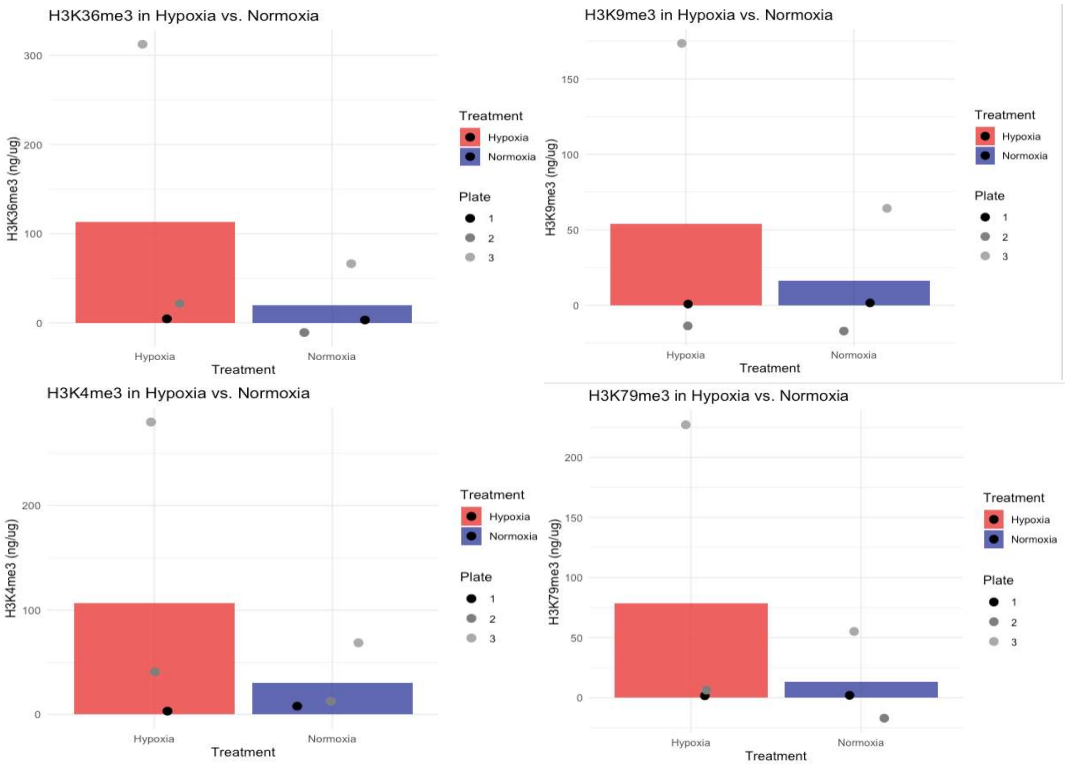


**Figure 4.** H3 histone modification levels of H3K36me3, H3K9me3, H3K4me3, and H3K79me3 collected across 3 ELISA assays post-24-hour incubation in either hypoxic (1% $O_2$) or normoxic (18% $O_2$) conditions.

expression of these enzymes, including downregulation of HDAC1 and HDAC3, and upregulation of SIRT1 and KDM3A, suggesting a selective epigenetic response to sustained low-oxygen conditions.

In parallel, *in vitro* hypoxia exposure resulted in increased levels of histone methylation marks, most notably H3K36me3, H3K9me3, H3K4me3, and H3K79me3. We chose to examine the level of these histone modifications to help explain how epigenetic remodeling supports adaptation and contributes to disease. H3K36me3 and H3K79me3 are marks of active transcription and are often enriched under hypoxia due to reduced demethylase activity, supporting stress-adaptive gene expression. H3K9me3 is a repressive mark linked to heterochromatin formation and global transcriptional silencing, consistent with hypoxia-induced energy conservation. H3K4me3, typically associated with active promoters, is often reduced in hypoxia, reflecting the downregulation of non-essential gene programs. These findings support the idea that hypoxia drives coordinated changes in both enzyme expression and chromatin structure as part of an adaptive transcriptional program.

Overall, our findings support the hypothesis that hypoxia induces widespread epigenetic remodeling, evidenced by a global increase in histone modification levels alongside changes in histone-modifying enzyme expression. This broad upregulation may reflect a cellular strategy to rapidly reprogram gene expression in response to oxygen limitation. Importantly, such epigenetic shifts may not be transient; in the context of chronic or repeated hypoxia exposure, they could contribute to the onset or progression of diseases such as cancer, pulmonary hypertension, or chronic inflammatory conditions. By identifying specific enzymes and modifications altered under hypoxia, our study points to potential biomarkers of hypoxic stress and highlights histone-modifying enzymes as possible therapeutic targets. These results underscore the critical role of epigenetic plasticity in hypoxia adaptation and its relevance to long-term health outcomes.

### Limitations & Future Directions

Because our analysis focuses on peripheral immune cells, the observed epigenetic changes may not reflect responses in other tissues. In addition, a limitation of the *in vitro* component of our study is the small sample size (n = 3), which may limit the generalizability of our findings. While these preliminary results offer important insight into hypoxia-induced histone modifications, further studies with larger and more diverse donor cohorts are needed to validate and expand upon these observations. To determine whether the observed changes in histone modification levels under hypoxia correspond to specific genomic loci, we will perform CUT&Tag targeting H3K9me3, H3K4me3, and other relevant marks to identify genome-wide localization patterns under normoxic and hypoxic conditions.

## CONCLUSION

Together, our findings demonstrate that sustained hypoxia, modeled through high-altitude exposure *in vivo* and low-oxygen culture *in vitro*, induces coordinated shifts in both histone-modifying enzyme expression and global histone modification patterns. The downregulation of HDAC1 and HDAC3, paired with the upregulation of SIRT1 and KDM3A, highlights an epigenetic reprogramming strategy that may enable cellular adaptation to oxygen deprivation. These enzymatic changes correspond with histone modifications such as increased H3K36me3, H3K9me3, H3K4me3, and H3K79me3. The coordinated expression of these enzymes suggests that cells actively remodel chromatin in response to oxygen levels, potentially regulating genes involved in stress, metabolism, and inflammation. Future CUT&Tag studies will help identify where these changes occur in the genome and clarify their functional relevance. Overall, our findings underscore the importance of histone modifications in hypoxia adaptation and highlight their potential as biomarkers and therapeutic targets in hypoxia-related diseases.

## ACKNOWLEDGMENTS

I am deeply grateful to Dr. Heinrich for her unwavering support, thoughtful guidance, and belief in me throughout the course of this project. I would also like to extend my sincerest thanks to Sunny Virk, whose generosity, patience, and insight made this experience truly collaborative.

# Hypoxia Impacts Histone Modifications in Immune Cells

## REFERENCES


**1.** Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, 21(3), 381–395. https://doi.org/10.1038/cr.2011.22

**2.** Batie, M., Frost, J., Frost, M., Wilson, J. W., Schofield, P., & Rocha, S. (2019). Hypoxia induces rapid changes to histone methylation and reprograms chromatin. *Science, 363*(6425), 1222–1226. https://doi.org/10.1126/science.aau5870

**3.** Kim, J., Lee, H., Yi, SJ. et al. Gene regulation by histone-modifying enzymes under hypoxic conditions: a focus on histone methylation and acetylation. *Exp Mol Med* 54, 878–889 (2022). https://doi.org/10.1038/s12276-022-00812-1

**4.** Greer, E. L., & Shi, Y. (2012). Histone methylation: A dynamic mark in health, disease, and inheritance. *Nature Reviews Genetics*, 13(5), 343–357. https://doi.org/10.1038/nrg3173

**5.** Ozawa, K. (2008). Reduction of phosphorylated histone H3 serine 10 and serine 28 cell cycle markers under hypoxic conditions. *Cytometry Part A*, 73(8), 681–688. https://doi.org/10.1002/cyto.a.20591

**6.** Wade, P. A., Jones, P. L., Vermaak, D., & Wolffe, A. P. (1997). A multiple subunit Mi-2 histone deacetylase from Xenopus laevis coexists with nucleosome remodeling activity and is required for transcriptional repression by the thyroid hormone receptor. *Genes & Development*, 11(23), 3065–3077. https://doi.org/10.1101/gad.11.23.3065

**7.** Pham, K., Frost, S., Parikh, K., Puvvula, N., Oeung, B., & Heinrich, E. C. (2022). Inflammatory gene expression during acute high-altitude exposure. *The Journal of Physiology*, 600(18), 4169–4186. https://doi.org/10.1113/JP282772

**8.** Sawicka, A., Seiser, C. (2012). Histone H3 phosphorylation – A versatile chromatin modification for different occasions, *Biochimie*, Volume 94, Issue 11, https://doi.org/10.1016/j.biochi.2012.04.018.

**9.** Cavalieri, V. (2021). The expanding constellation of histone post-translational modifications in the epigenetic landscape. *Genes*, 12(10), 1596. https://doi.org/10.3390/genes12101596

**10.** Monte-Serrano, E., Morejón-García, P., Campillo-Marcos, I., Campos-Díaz, A., Navarro-Carrasco, E., & Lazo, P. A. (2023). The pattern of histone H3 epigenetic posttranslational modifications is regulated by the VRK1 chromatin kinase. *Epigenetics & Chromatin*, 16(1), 18. https://doi.org/10.1186/s13072-023-00494-7