

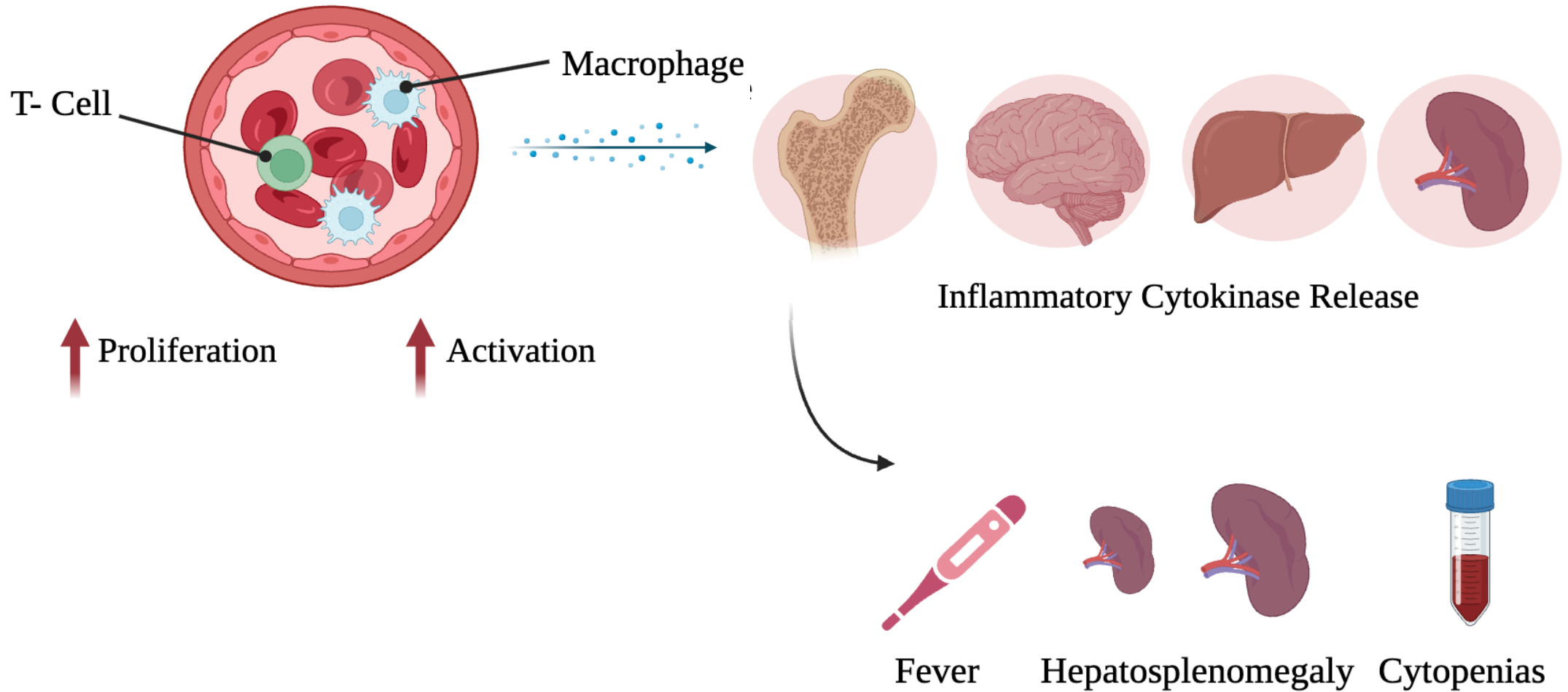
Gene- PRF1



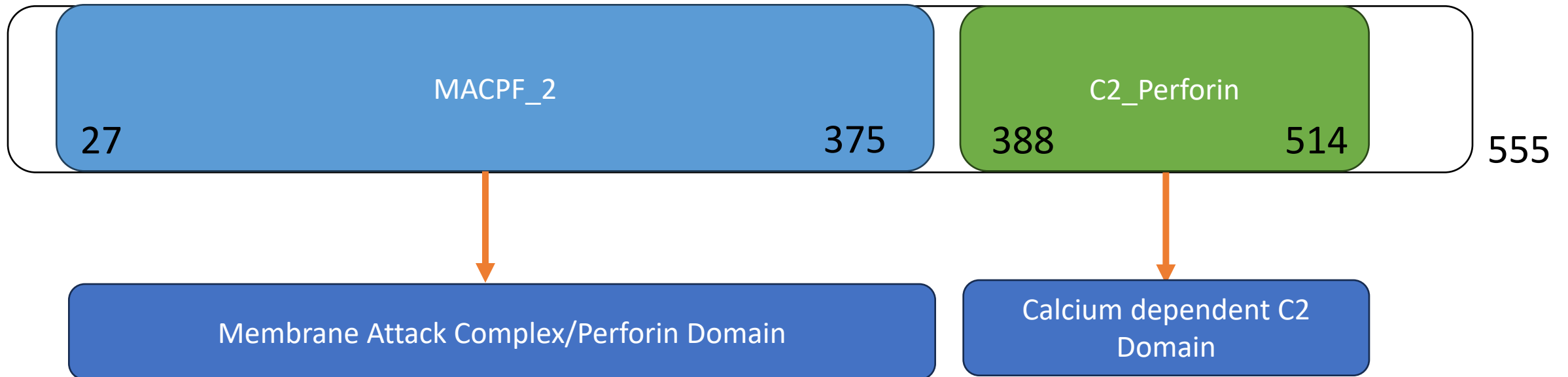
Madeline Blum

# Familial Hemophagocytic Lymphohistiocytosis

# Overactivation of T-cells and macrophages results in symptoms of HLH

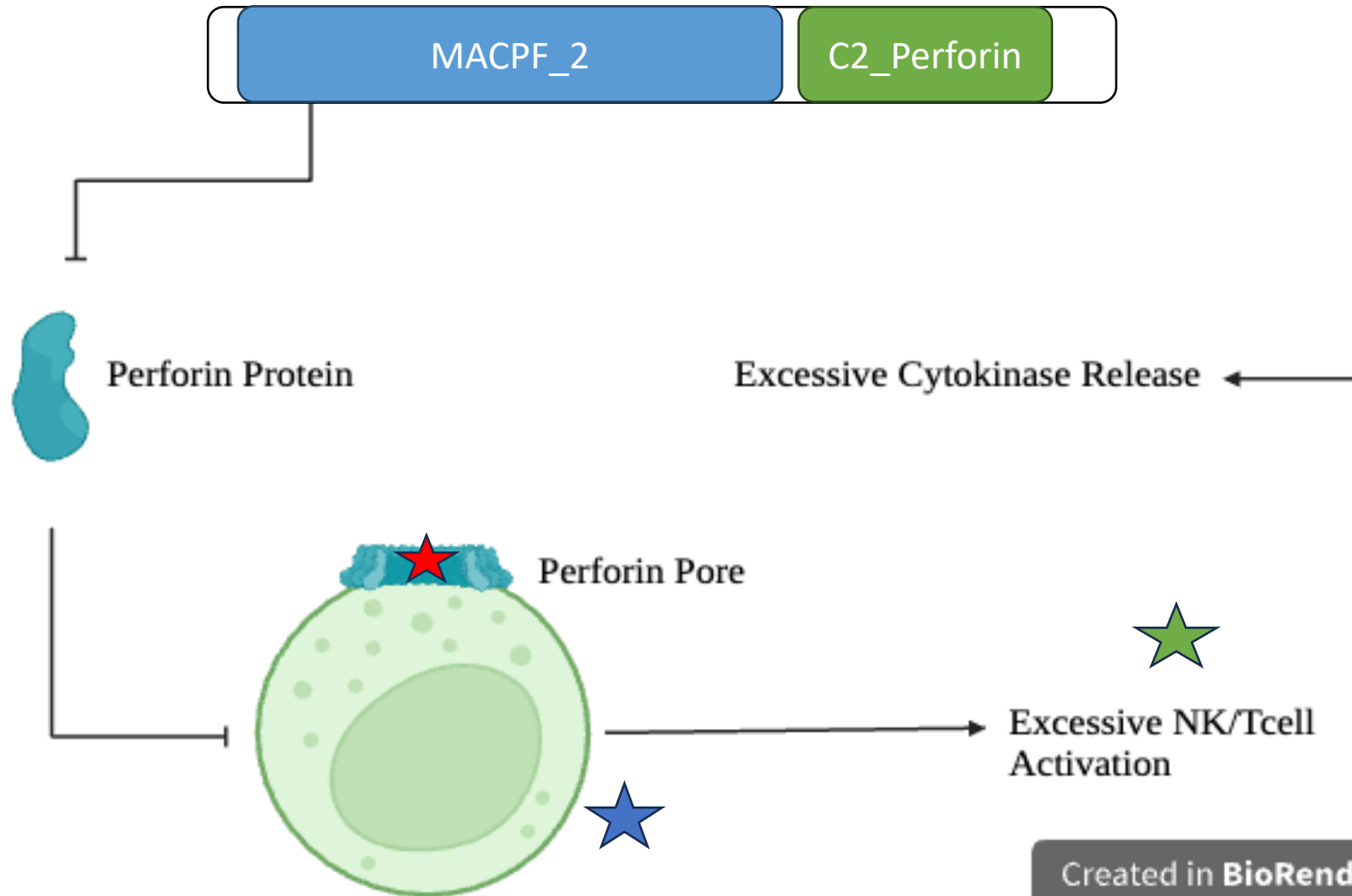


# Familial HLH is caused by loss-of-function mutation in PRF1



# Loss of PRF1 decreases perforin pore formations and associated cytotoxic responses

Two Pathogenic Alleles Required

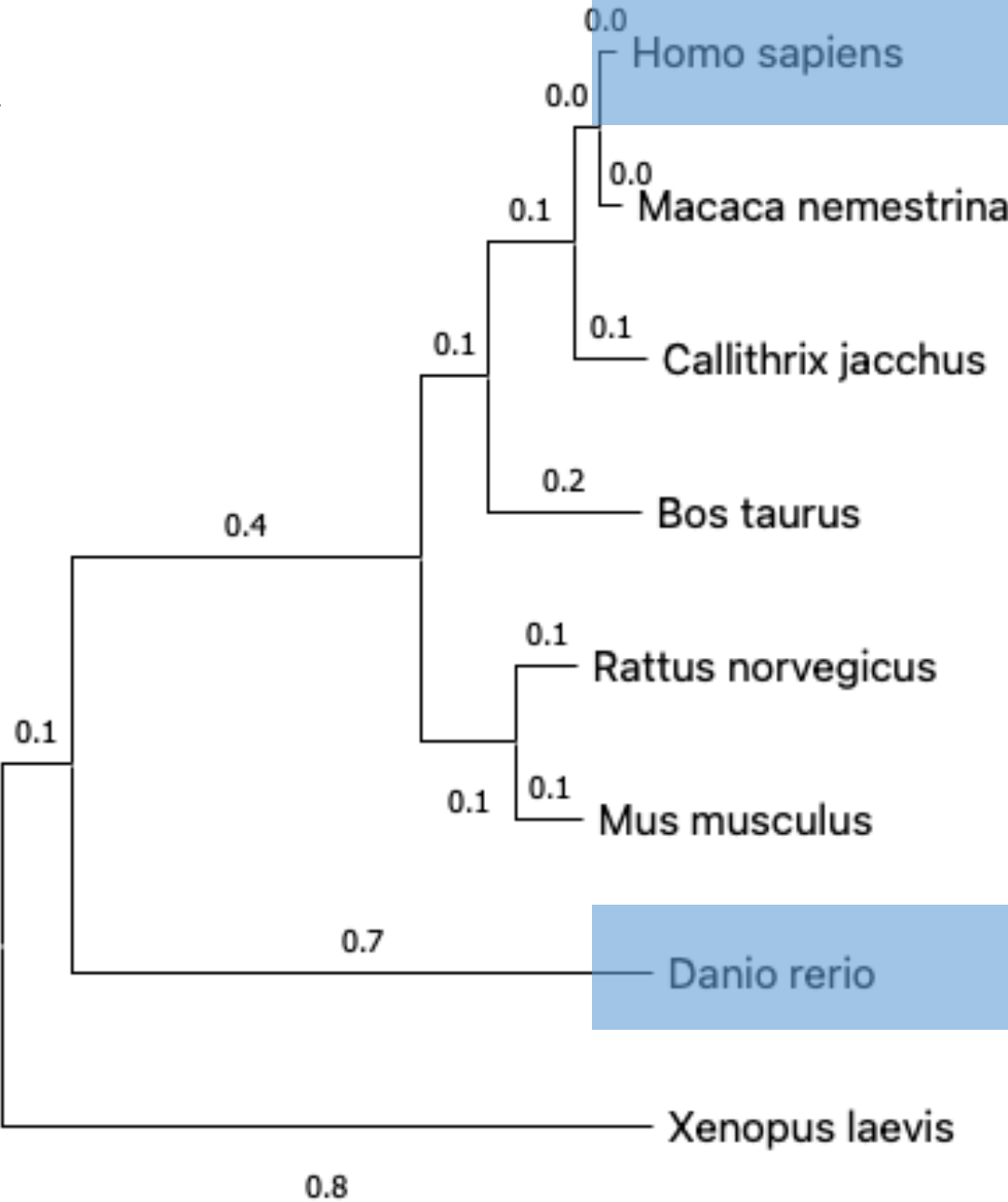


# Protein Domains Across Species

Homo  
Sapiens



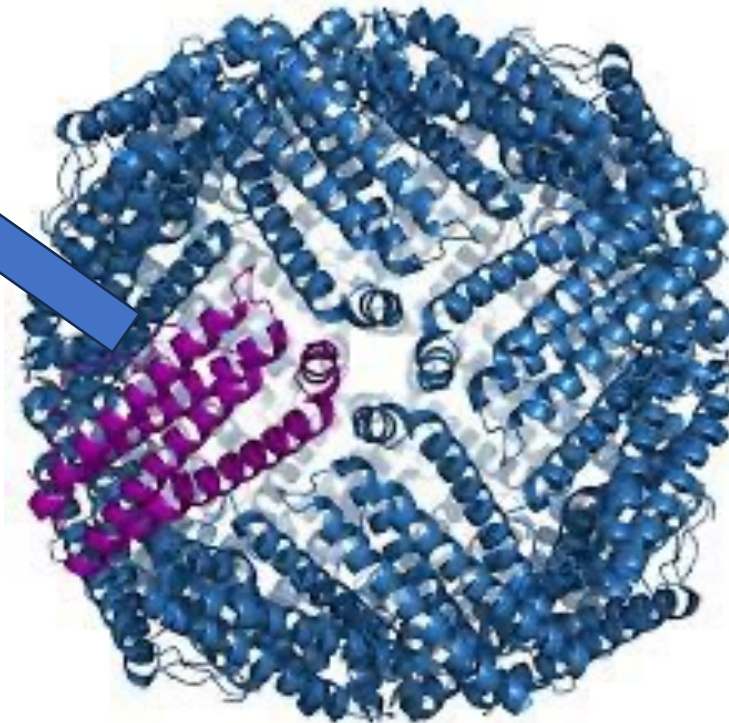
# PRF1 Phylogeny





# The relationship between hyperferritinemia and PRF1 mutation is unclear

Iron Stores



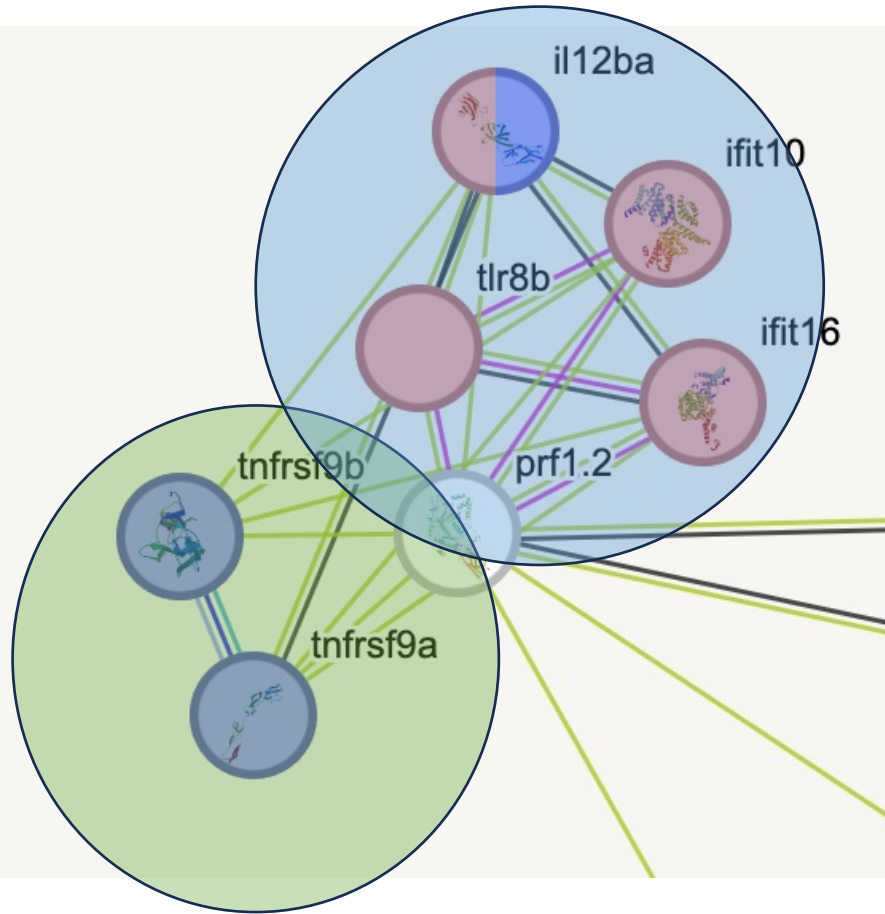
Ferritin

Hypothesis: iron homeostasis in fHLH will result from varying expression of ferritin encoding genes [FTL, FTH], and subsequent protein concentrations

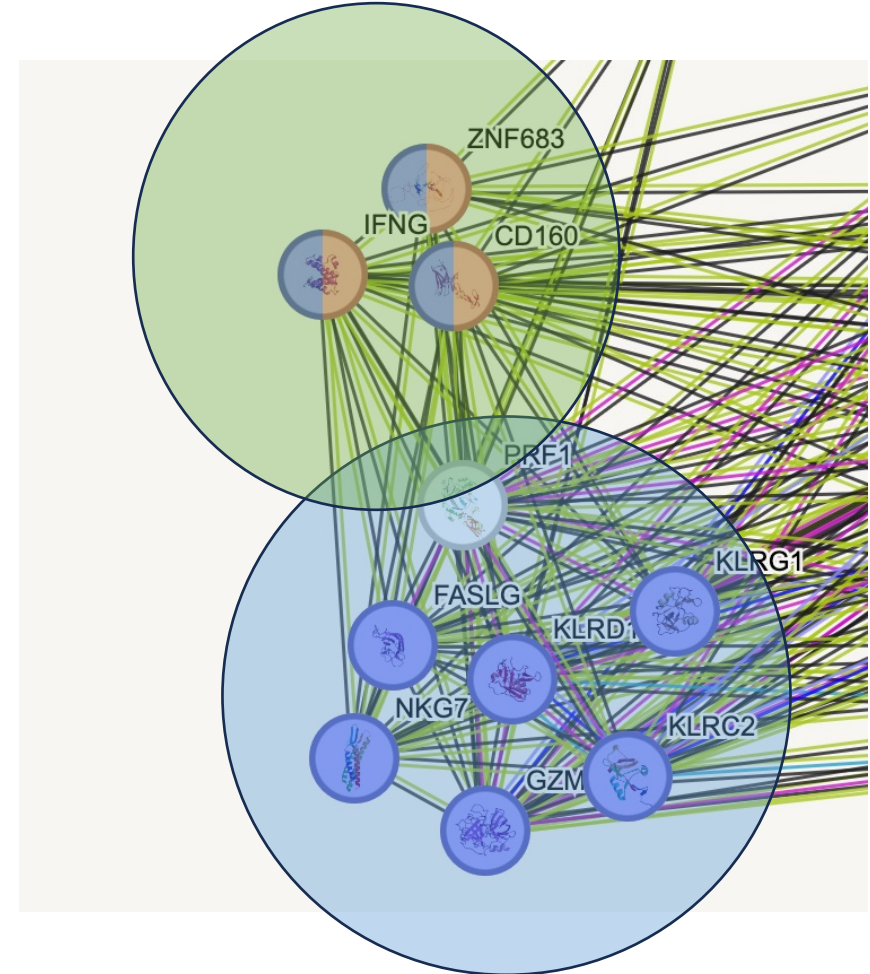
# **Danio Rerio will be used as model organism**



# PRF1 Interactions



T cell Proliferation



Innate Immune Response

# Aim 1 Identifying Protein Domain Essential for Pore Formation in Danio Rerio

Identify Perforin/Perforin-like domain through GO



Membrane Attack Complex/Perforin Domain



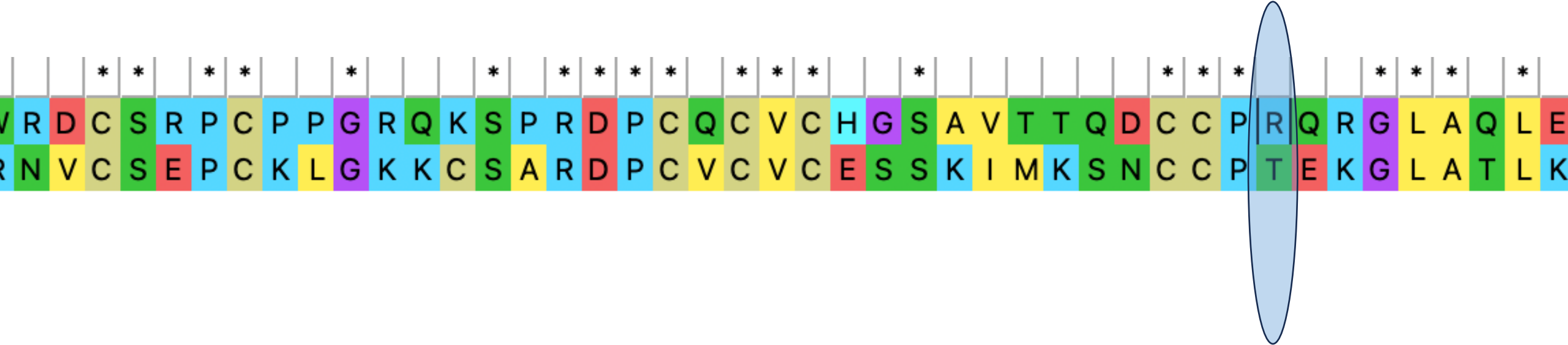
InterPro

NCBI Protein Blast

GO Analysis

Confirmation By Screening

# Aim 1 Identifying Protein Domain Essential for Pore Formation in Danio Rerio



Multiple Sequence Alignment

GO Analysis

Confirmation By Screening

(Via Mega11)

# Aim 1 Identifying Protein Domain Essential for Pore Formation in Danio Rerio



NCBI Protein Blast

GO Analysis

Confirmation By Screening

# Aim 2

## Identifying Gene Expression in PRF1 and Iron Homeostasis Genes

RNA Sequencing

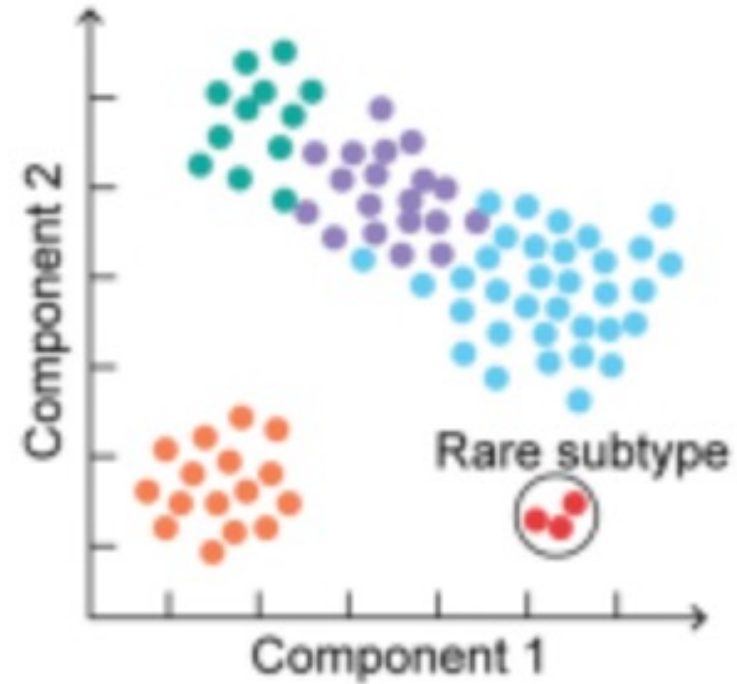
Cluster Analysis

Confirmation By Screening

# Aim 2

## Identifying Gene Expression in PRF1 in Iron Homeostasis Genes

Identify Gene Clusters relating to Cytotoxicity and Iron Homeostasis



(Hwang et al., 2018)

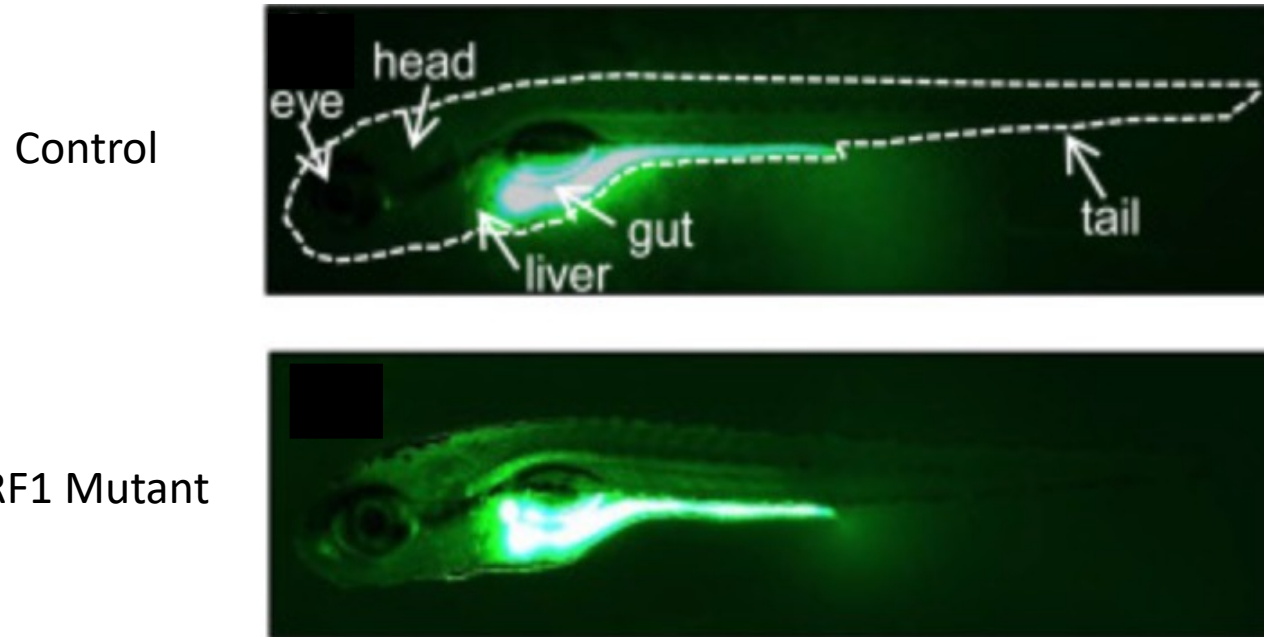
RNA Sequencing

Cluster Analysis

Confirmation By Screening

# Aim 2

## Identifying Gene Interactions in PRF1 and Iron Homeostasis Genes



Screen for Ferritin in vivo with fluorescence assay

(Ayaat et al., 2020)

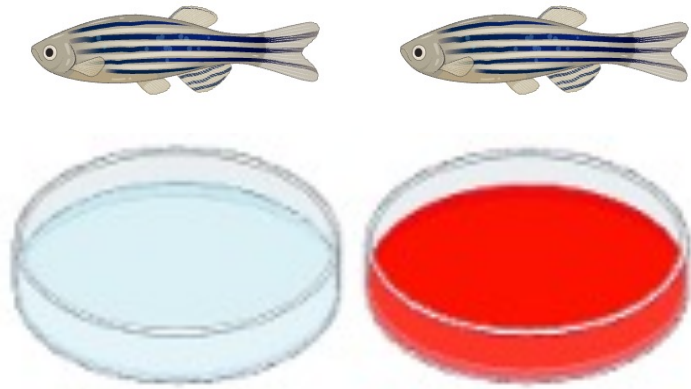
RNA Sequencing

Cluster Analysis

Confirmation By Screening



# Aim 3 Analysis of proteome via metabolic labeling of control/KO tissues



(Hwang et al., 2018)

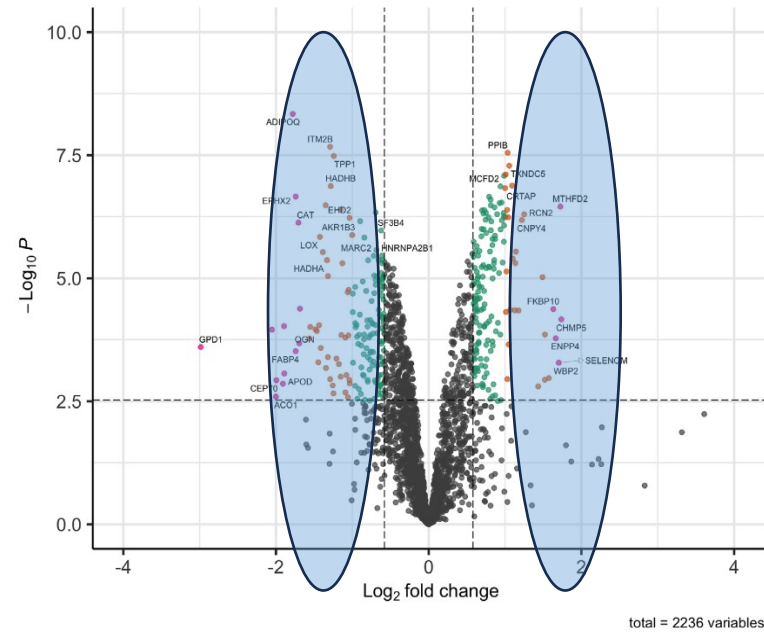
Isotopic Labeling

Analysis

Confirmation By Screening

# Aim 3 Analysis of proteome via metabolic labeling of KO tissues

Identify Ferritin in increased expression regions



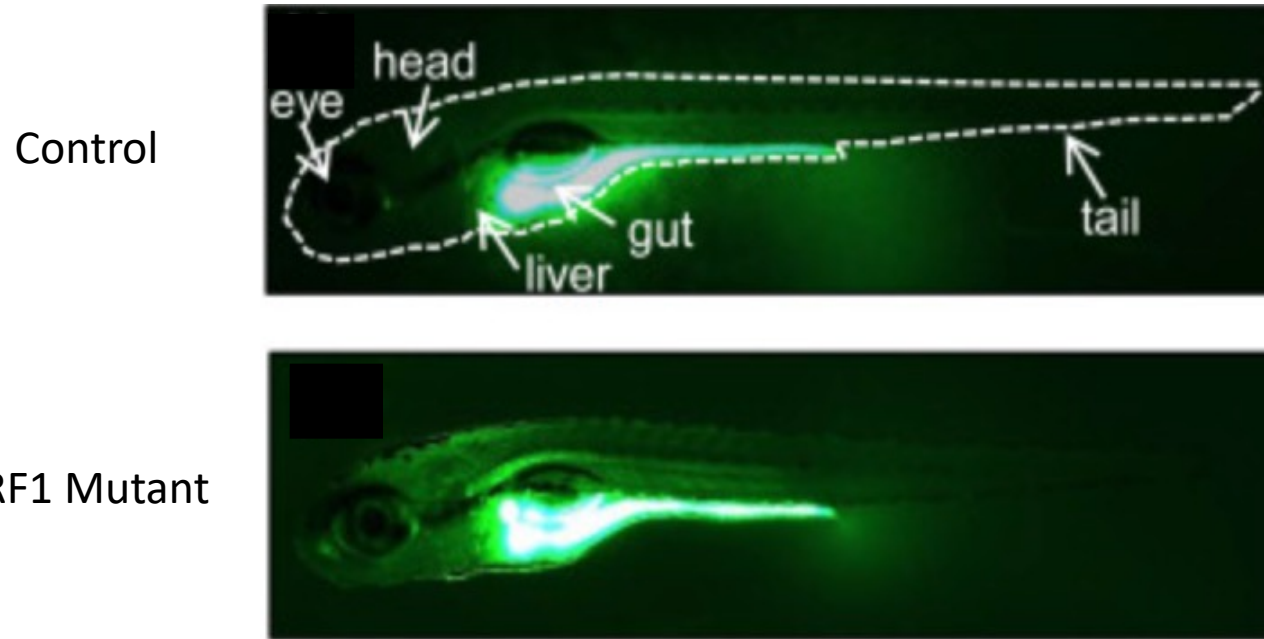
(Hwang et al., 2018)

Isotopic Labeling

Analysis

Confirmation By Screening

# Aim 3 Analysis of proteome via metabolic labeling of KO tissues



Screen for Ferritin in vivo with fluorescence assay

(Ayaat et al., 2020)

Isotopic Labeling

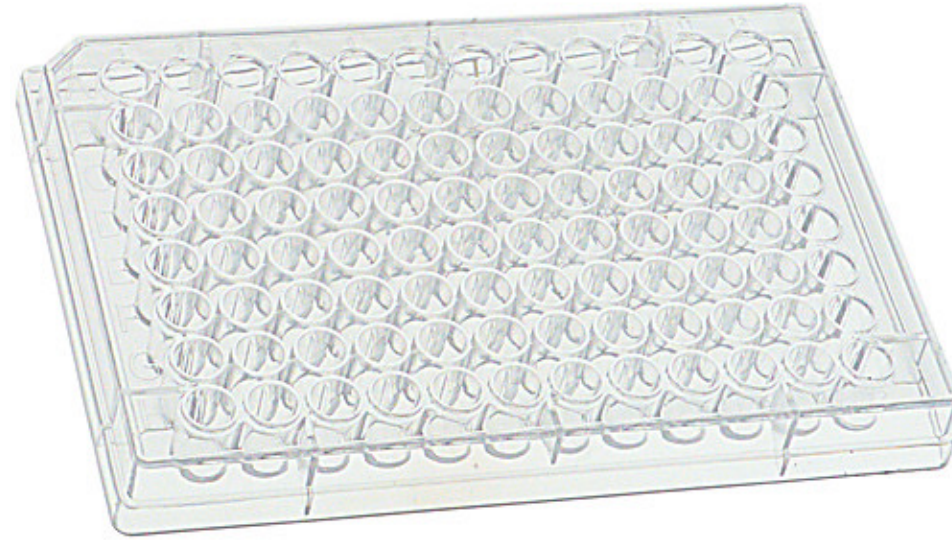
Cluster Analysis

Confirmation By Screening

# Summary

- Today, we identified what fHLH is and how PRF1 mutation causes damage to the immune response. The relationship between this condition and high iron homeostasis is unknown.
- We explored *Danio Rerio*, a model organism for PRF1 mutation, and how it can be used to elucidate this relationship
- We proposed multiple genomics/proteomics approaches to exploring the relationship between these pathways.

# Future Directions



# References

Al-Amrani, Safa, et al. "Proteomics: Concepts and Applications in Human Medicine." *World Journal of Biological Chemistry*, U.S. National Library of Medicine, 27 Sept. 2021, [www.ncbi.nlm.nih.gov/pmc/articles/PMC8473418/#:~:text=Proteomics%20is%20the%20study%20of,of%20the%20organism%20than%20genomics](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC8473418/#:~:text=Proteomics%20is%20the%20study%20of,of%20the%20organism%20than%20genomics).

Ayaat T. Hassan, et al. "The Neurophysiological Effects of Iron in Early Life Stages of Zebrafish." *Environmental Pollution*, Elsevier, 18 Sept. 2020, [www.sciencedirect.com/science/article/pii/S0269749120363132#abs0015](http://www.sciencedirect.com/science/article/pii/S0269749120363132#abs0015).

Bohauud, Candice, et al. "The Role of Macrophages during Zebrafish Injury and Tissue Regeneration under Infectious and Non-Infectious Conditions." *Frontiers*, Frontiers, 2 July 2021, [www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.707824/full](http://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2021.707824/full).

Glen Stecher, Koichiro Tamura, and Sudhir Kumar (2020) Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution* 37:1237-1239 (Publication PDF available at <https://www.megasoftware.net/citations>)

Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp Mol Med*. 2018 Aug 7;50(8):1-14. doi: 10.1038/s12276-018-0071-8. Erratum in: *Exp Mol Med*. 2021 May;53(5):1005. PMID: 30089861; PMCID: PMC6082860.

Janka, Gritta, and Udo zur Stadt. "Familial and Acquired Hemophagocytic Lymphohistiocytosis." *American Society of Hematology*, American Society of Hematology, 1 Jan. 2005, [ashpublications.org/hematology/article/2005/1/82/19290/Familial-and-Acquired-Hemophagocytic](http://ashpublications.org/hematology/article/2005/1/82/19290/Familial-and-Acquired-Hemophagocytic).

Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. *Int Immunol*. 2017 Nov 1;29(9):401-409. doi: 10.1093/intimm/dxx031. PMID: 28541437; PMCID: PMC5890889.

Murtha, Jill, et al. "Hematologic and Serum Biochemical Values for Zebrafish (Danio Rerio)." *Ingenta Connect*, 2003, [www.ingentaconnect.com/content/aalas/cm/2003/00000053/00000001/art00005?crawler=true&mimetype=application/pdf](http://www.ingentaconnect.com/content/aalas/cm/2003/00000053/00000001/art00005?crawler=true&mimetype=application/pdf).

Osińska, Iwona, et al. "Perforin: An Important Player in Immune Response." *Central-European Journal of Immunology*, U.S. National Library of Medicine, 2014, [www.ncbi.nlm.nih.gov/pmc/articles/PMC4439970/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4439970/).

Paysan-Lafosse T, Blum M, Chuguransky S, Grego T, Pinto BL, Salazar GA, Bileschi ML, Bork P, Bridge A, Colwell L, Gough J, Haft DH, Letunić I, Marchler-Bauer A, Mi H, Natale DA, Orengo CA, Pandurangan AP, Rivoire C, Sigrist CJA, Sillitoe I, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Bateman A. [InterPro in 2022](https://doi.org/10.1093/nar/gkac993). *Nucleic Acids Research*, Nov 2022, (doi: 10.1093/nar/gkac993)

Zhang, Kejian. "Familial Hemophagocytic Lymphohistiocytosis." *GeneReviews® [Internet]*, U.S. National Library of Medicine, 30 Sept. 2021, [www.ncbi.nlm.nih.gov/books/NBK1444/](http://www.ncbi.nlm.nih.gov/books/NBK1444/).