UNTARGETED AND TARGETED PROTEOMICS REVEAL THE ANTI-INFLAMMATORY POTENTIAL OF A NOVEL NRF2-ACTIVATOR IN AN ULCERATIVE COLITIS MODEL

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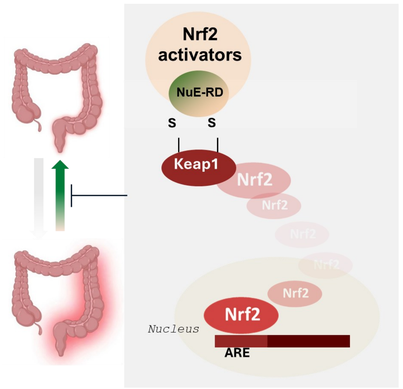
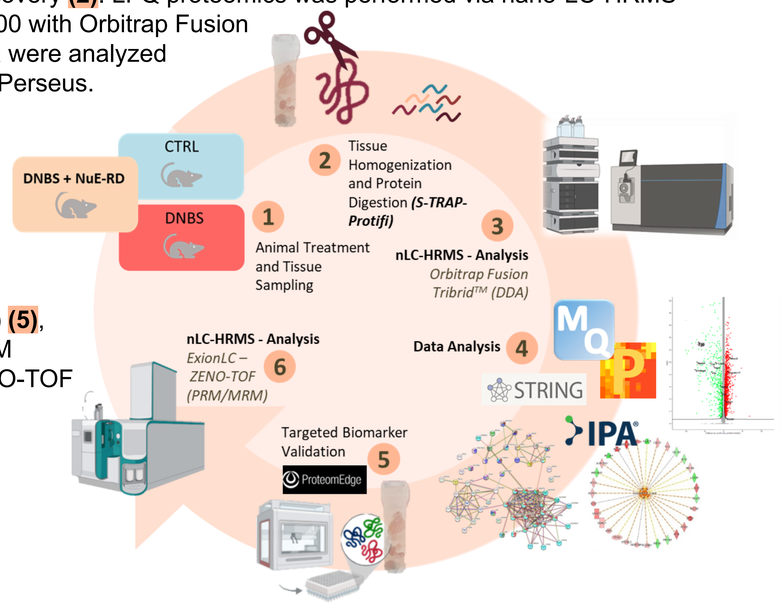
Introduction

Ulcerative colitis (UC) is a multifactorial IBD sustained by oxidative stress and inflammation, which activate **Nrf2** and **NF-κB** in a self-perpetuating loop. Current therapies offer limited relief and have drawbacks, underscoring the need for safer, more effective options. **Nrf2 activators** can break this loop; among them, moderately electrophilic derivatives interact with Keap1 thiols, triggering Nrf2 activation. A nucleophile-based derivative facilitates controlled electrophile release (**NuE-RD, *Nucleophile-Electrophile Releasing Derivative***) was designed to enhance antioxidant defenses and modulate NF-κB. This proteomics-guided study elucidates NuE-RD's mechanism, supporting its potential as a novel therapeutic strategy for UC.



Methods

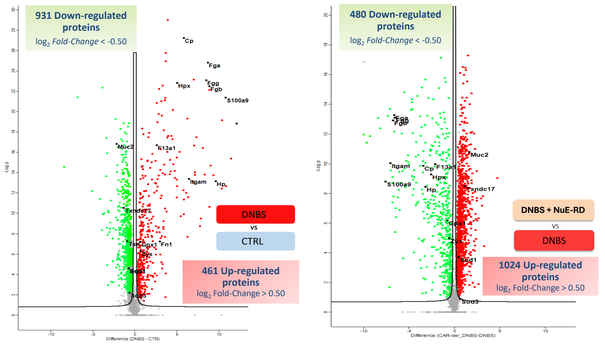
NuE-RD was evaluated in a DNBS-induced colitis murine model including three groups: control (saline), DNBS (UC phenotype), and DNBS/NuE-RD (oral treatment during DNBS exposure) **(1)**. Colon tissues were processed using an optimized S-TRAP-Protifi protocol to enhance peptide recovery **(2)**. LFQ proteomics was performed via nano-LC-HRMS (Dionex Ultimate 3000 with Orbitrap Fusion Tribrid) **(3)**, and data were analyzed with MaxQuant and Perseus. STRING and IPA provided pathway Insights **(4)**. Targeted validation was carried out with the ProteomEdge kit (DiscoveryEdge175) **(5)**, using PRM and MRM on an ExionLC-ZENO-TOF platform to ensure precision and reproducibility **(6)**.

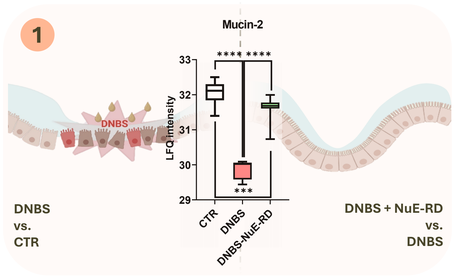


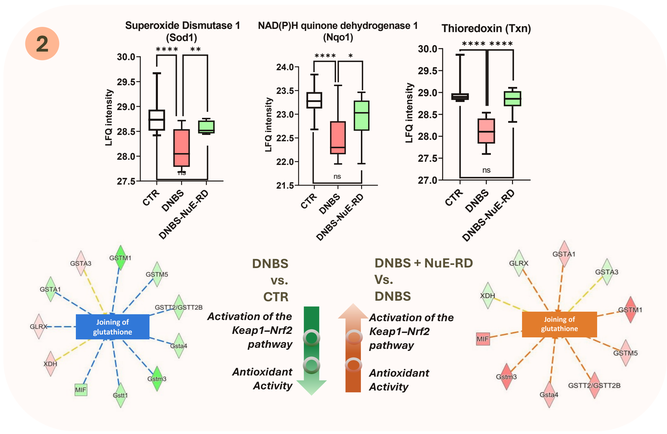
Results

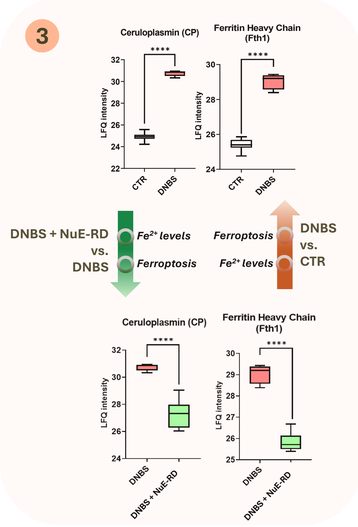
Summary: This proteomic study not only validated the murine model of DNBS-induced colitis but also revealed the potent anti-inflammatory effects of NuE-RD.

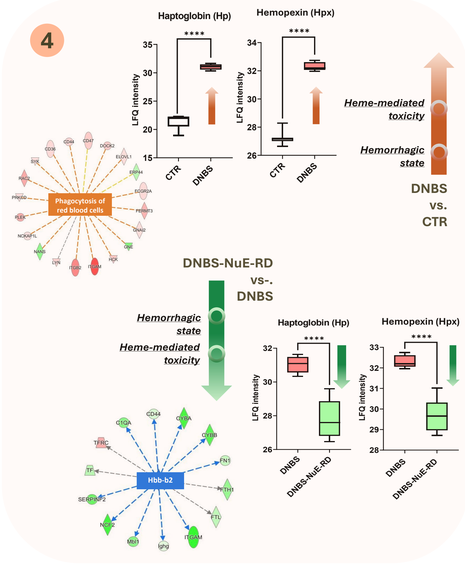
By modulating key molecular pathways involved in **intestinal homeostasis (1-4)**, **oxidative stress (2-3)**, and **immune response (5)**, NuE-RD emerges as a promising therapeutic candidate for ulcerative colitis (UC).

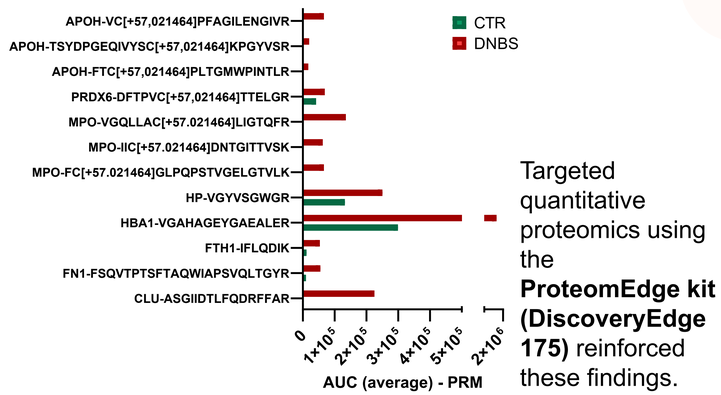




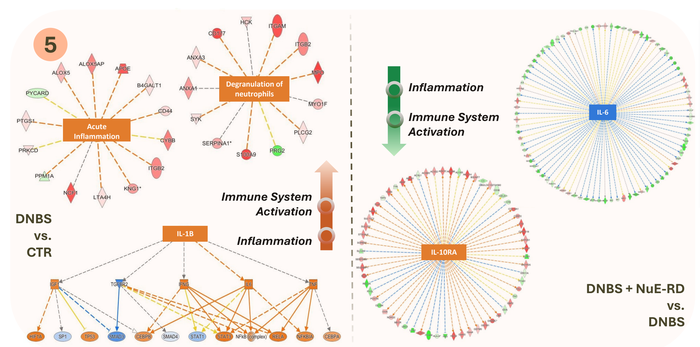








Targeted quantitative proteomics using the **ProteomEdge kit (DiscoveryEdge 175)** reinforced these findings.



Conclusion

This multi-layered analytical strategy enhanced proteomic resolution, enabling the confident identification of novel biomarkers and therapeutic targets. The integrated workflow proved robust and reproducible, highlighting the translational relevance of NuE-RD as a potential UC therapy. NuE-RD selectively activates Nrf2 and restores intestinal homeostasis, as revealed by advanced quantitative proteomics.