

NTZ ALLEVIATES STRESS-INDUCED HEPATOCYTE CELL DEATH THROUGH MODULATION OF OXIDATIVE STRESS AND DNA DAMAGE SIGNALING PATHWAYS IN ACLF MODELS

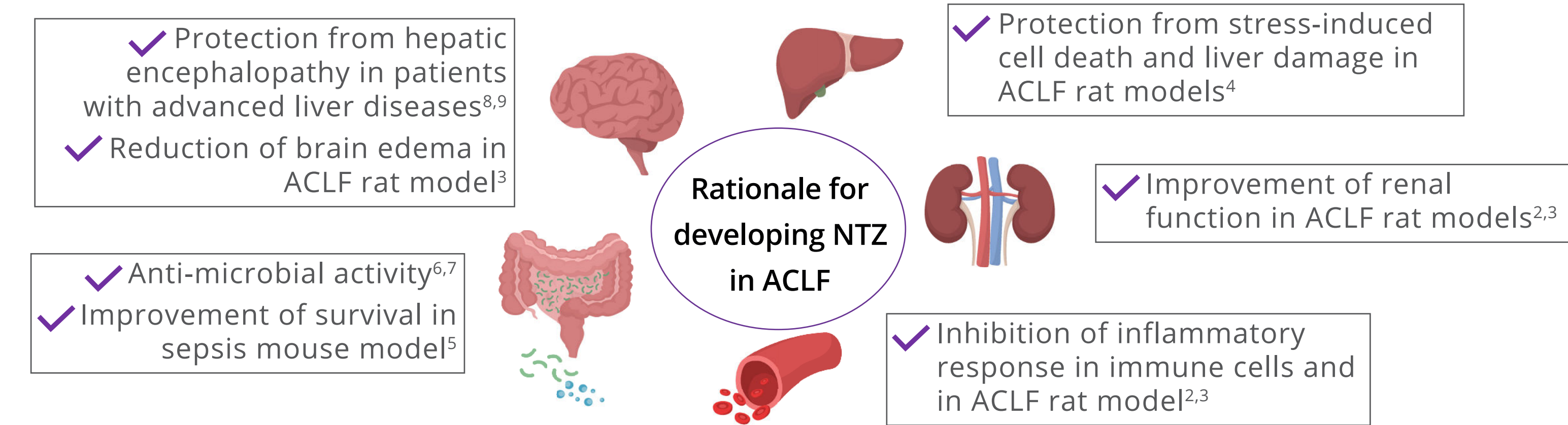
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BACKGROUND & AIM

- Nitazoxanide (NTZ) is an FDA approved anti-parasitic drug that is currently being investigated for the treatment of Acute-on-Chronic Liver Failure (ACLF), a severe syndrome affecting patients with acutely decompensated cirrhosis¹
- We previously demonstrated that NTZ alleviates systemic inflammation and organ damage in disease models of ACLF²⁻⁵



- In human hepatocytes, tizoxanide (TZ, the active metabolite of NTZ), blunted stress-induced apoptosis in a dose-dependent manner. Moreover, NTZ modulated cell-death associated gene signature in the liver of a BDL+LPS rat model⁴

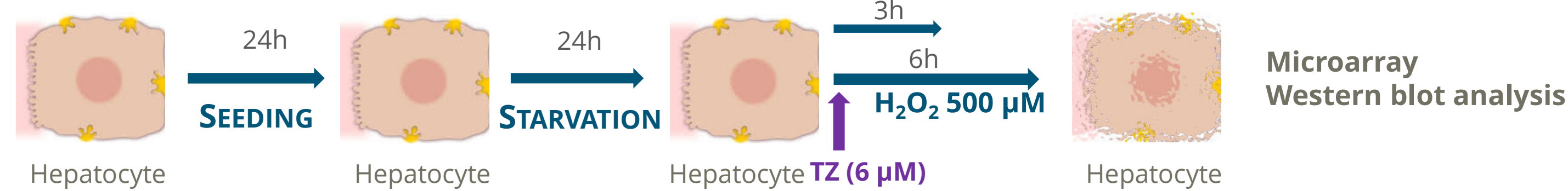
To decipher the molecular mechanisms by which NTZ and TZ protect hepatocytes from cell death and liver damage, we performed transcriptomics analyses in two preclinical models:

- In vitro:** human hepatocytes stressed with H₂O₂
- In vivo:** liver from fibrotic rats who underwent LPS challenge (ACLF model)

METHODS & STATISTICS

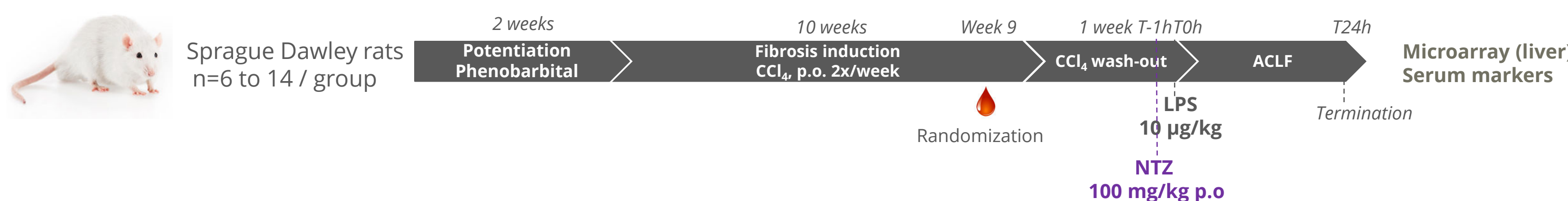
Evaluation of TZ in human hepatocytes stressed with H₂O₂

Immortalized human hepatocytes were stressed with 0.5 mM H₂O₂, a method commonly used to trigger cellular oxidative damage and cell death. Tizoxanide (TZ, 6 μM) was added concomitantly with H₂O₂ for 3 or 6h. Caspase 3 cleavage was assessed by Western blot analysis



Evaluation of NTZ in ACLF rats

Male Sprague Dawley rats received phenobarbital for 2 weeks then CCl₄ p.o. (uptitration to 0.85 mL/kg) twice a week for 10 weeks. LPS (10 μg/kg, i.p.) was injected one week after the last CCl₄ dose. NTZ (100 mg/kg) or vehicle was administered by oral gavage 1h before LPS injection. Surviving rats (17/27 in vehicle and 10/21 in NTZ group, respectively) were euthanized 24h after LPS injection. Serum markers of liver injury and function were measured using the Daytona plus automate



Transcriptomics analyses

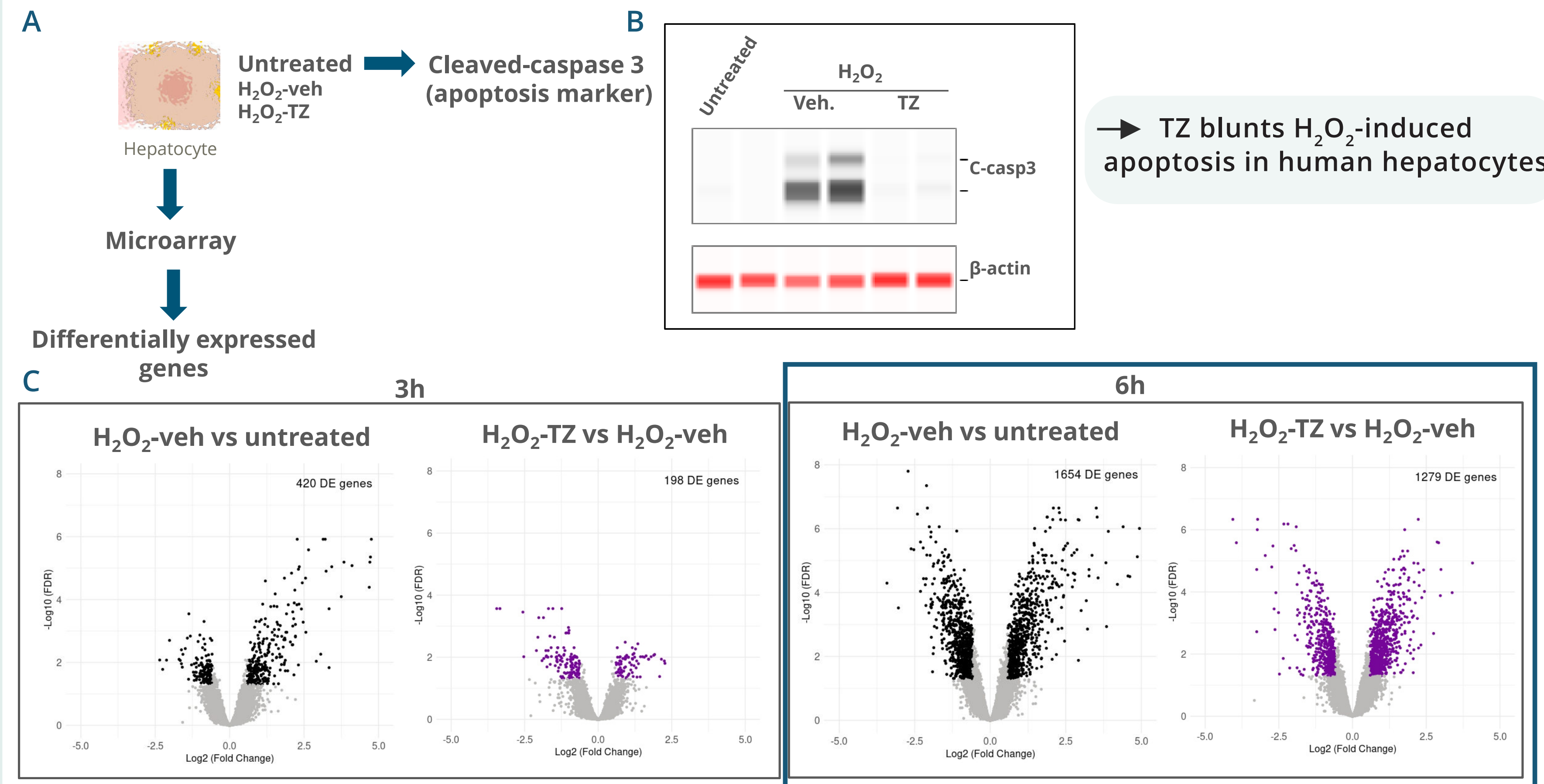
Hepatocytes and whole liver mRNA were extracted for RT-qPCR analyses (CFX96 Touch™) or for oligonucleotide microarray (GeneChip™ System 3000). Differentially expressed (DE) genes were identified using TAC software (Qiagen) followed by Limma analysis. Pathway analysis was performed with IPA (Qiagen)

Statistical analysis

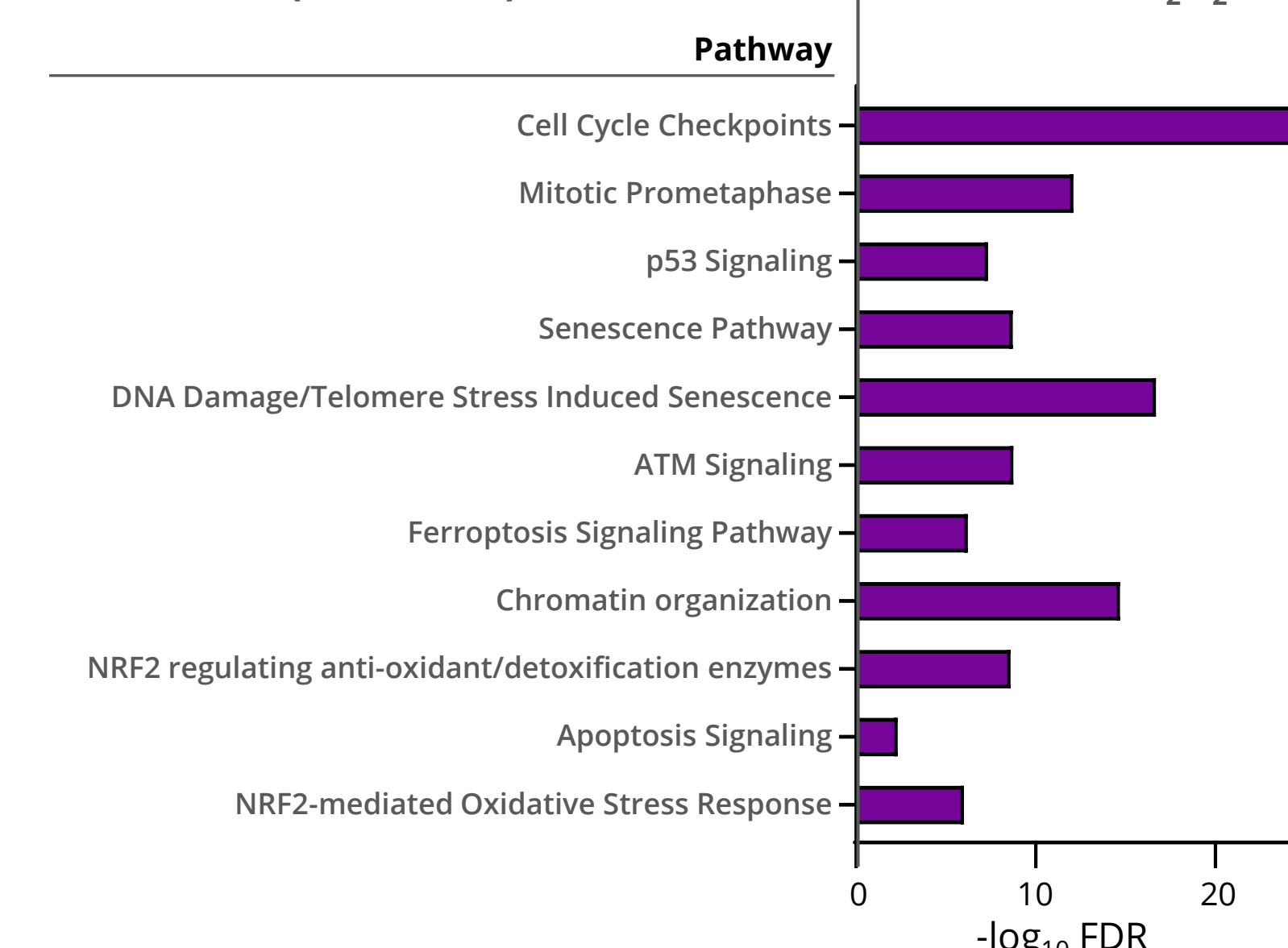
For microarray, the p-values were adjusted using Benjamini-Hochberg correction. Box plots show the median as a line, 25th to the 75th percentile as a box and min and max as whiskers. #: p<0.05; ##: p<0.01; ###: p<0.001; two-tailed Student T test for data following a normal distribution. *: p<0.05; **: p<0.01; ***: p<0.001; two-tailed non-parametric Mann-Whitney for non-normally distributed variables

RESULTS

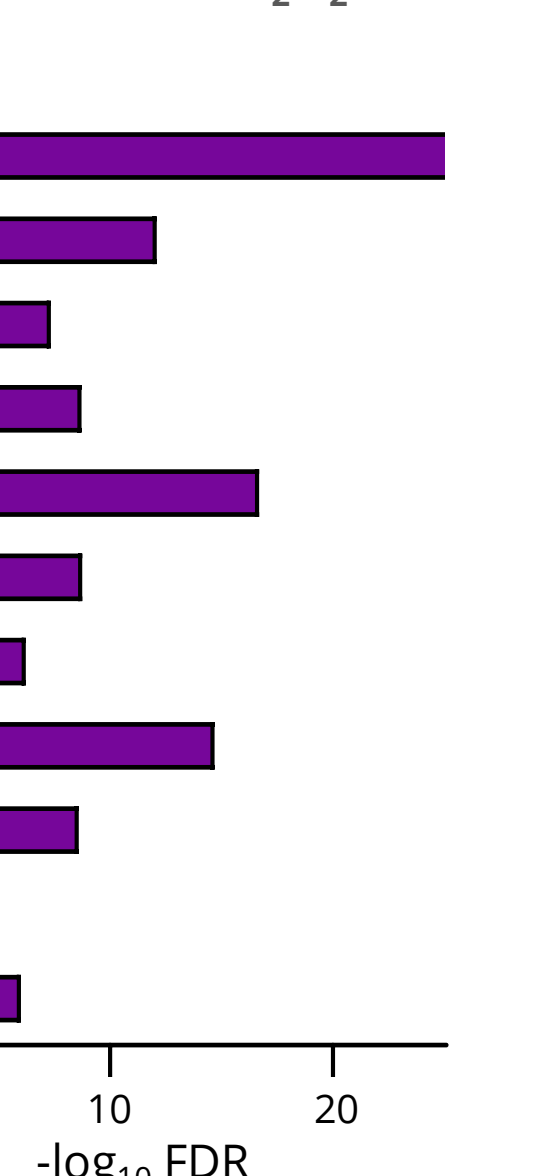
TZ ALLEVIATES H₂O₂-INDUCED APOPTOSIS IN HUMAN HEPATOCYTES THROUGH MODULATION OF OXIDATIVE STRESS, DNA DAMAGE AND CELL CYCLE PATHWAYS



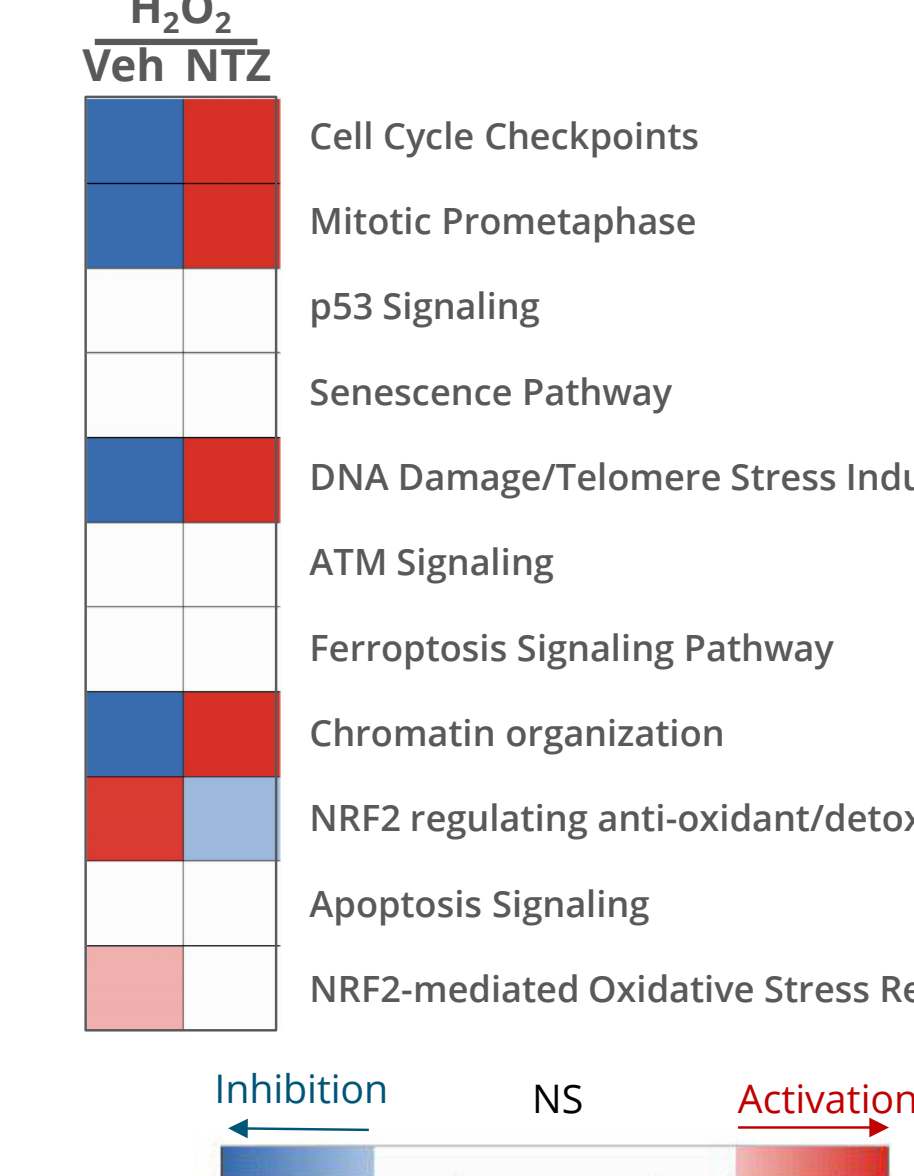
Top pathways enriched in H₂O₂ vs untreated at 6h (FDR<5.10⁻⁷)



Effect of TZ on top pathways induced by H₂O₂



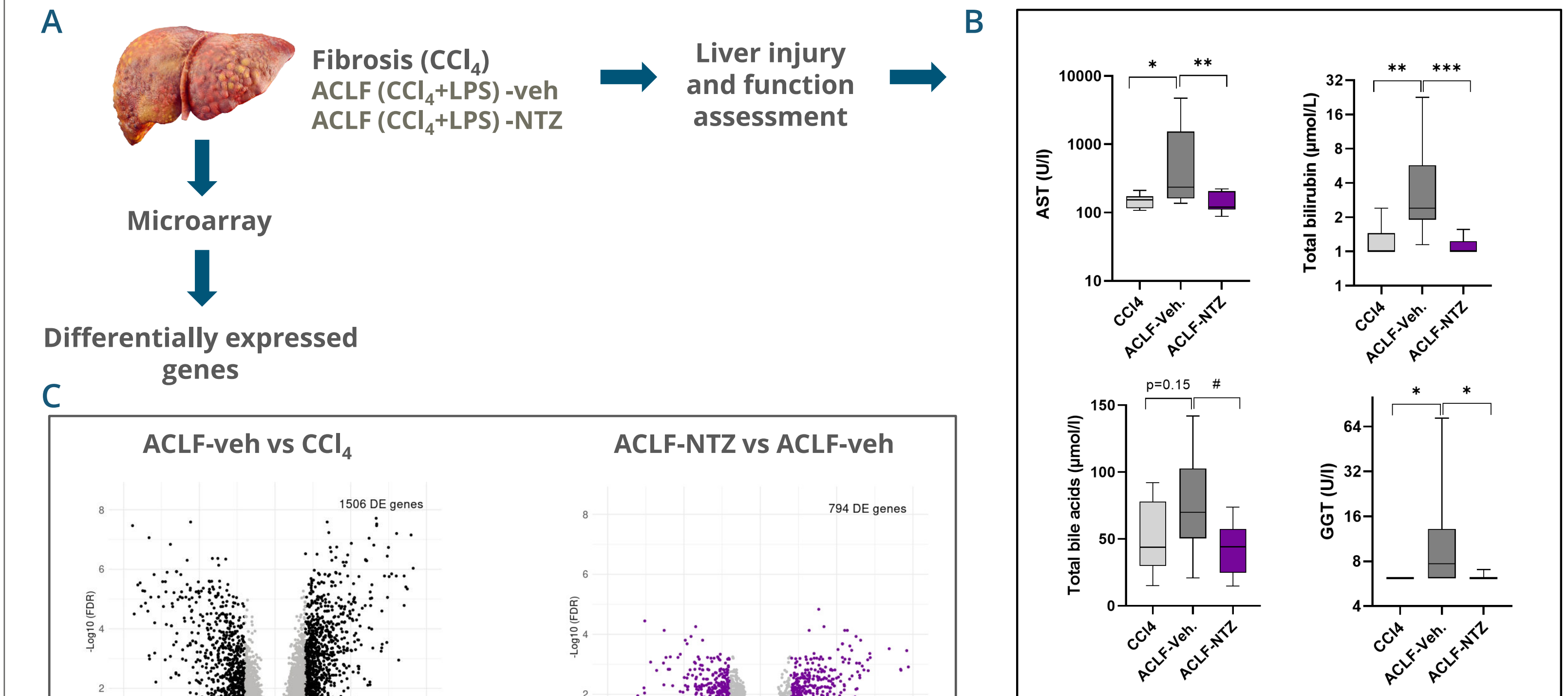
Predictive pathway activation score



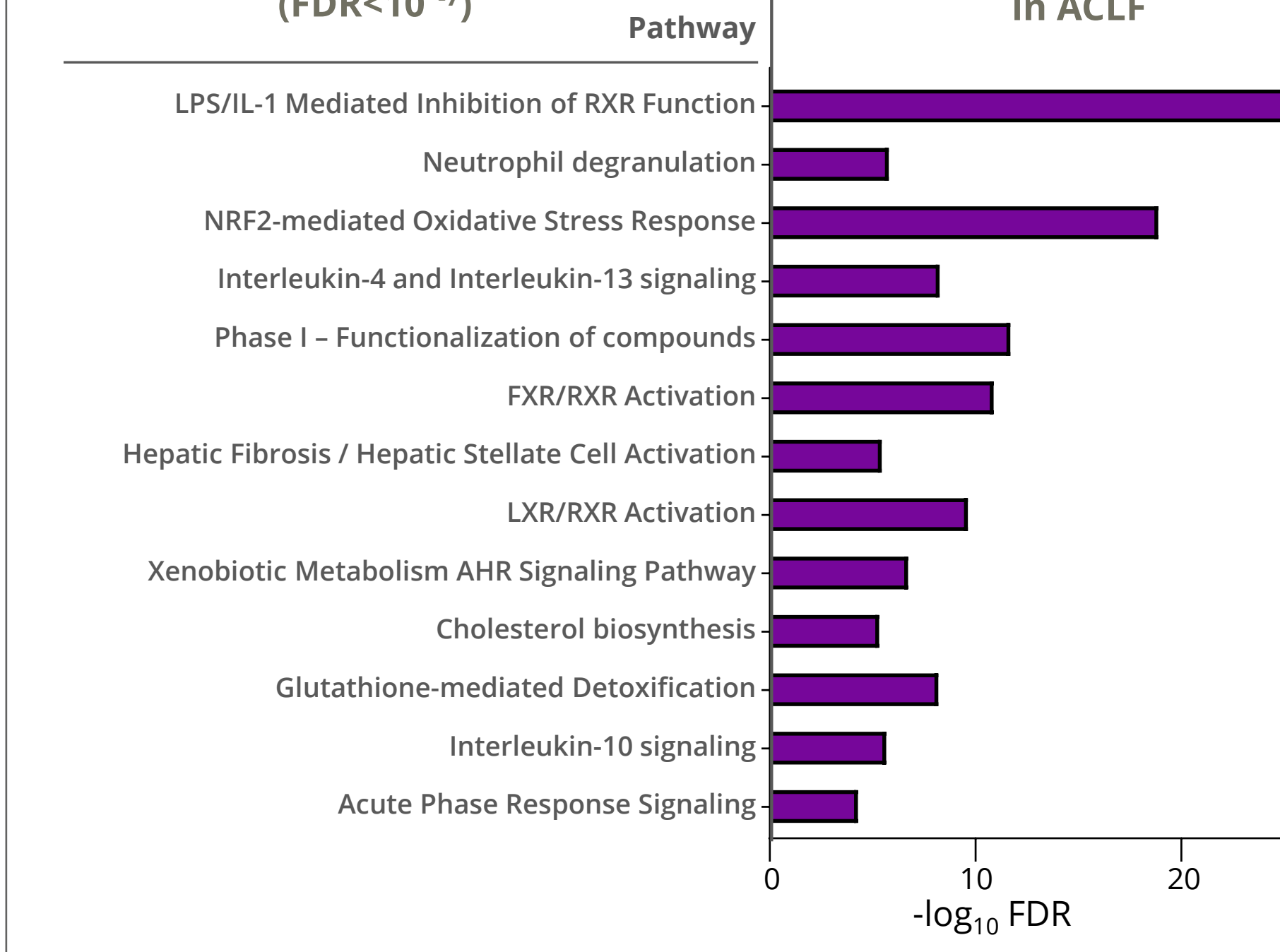
- H₂O₂-induces a transcriptomic remodeling, which is more pronounced after 6h H₂O₂ stimulation. H₂O₂-modulated genes are mostly related to cell cycle, DNA damage, cell death and oxidative stress
- TZ strongly modulates the H₂O₂-induced gene signature, by reversing the effect of H₂O₂

A. Experimental workflow (n=3 / group)
B. Cleaved caspase 3 expression in hepatocytes stressed with H₂O₂ for 6h
C. Volcano plots displaying significant DE genes (FC>1.5 and FDR<0.05) between H₂O₂ vs untreated groups (black dots) and between H₂O₂-TZ vs H₂O₂-veh groups (purple dots) after 3h or 6h H₂O₂
D. Top enriched pathways using the list of DE genes between H₂O₂-veh and untreated group at 6h (1654 genes)
E. Enrichment analysis using the list of DE genes obtained between H₂O₂-TZ and H₂O₂-veh groups at 6h (1279 genes)
F. Activation score predicted by IPA using pathways analysis described in D. and E. Non significant (NS) Z-score (-2 to 2) are displayed in white

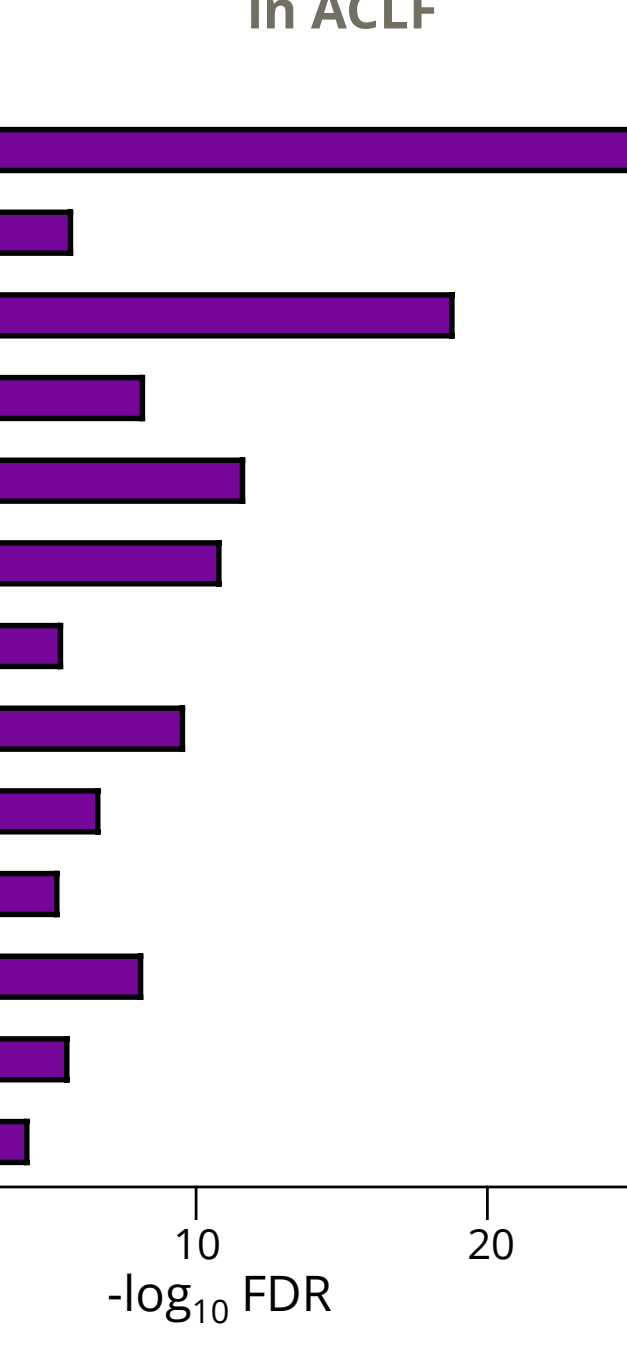
NTZ MODULATES LPS-INDUCED GENE SIGNATURE IN ACLF RAT LIVER, THEREBY PROTECTING FROM HEPATIC DAMAGE



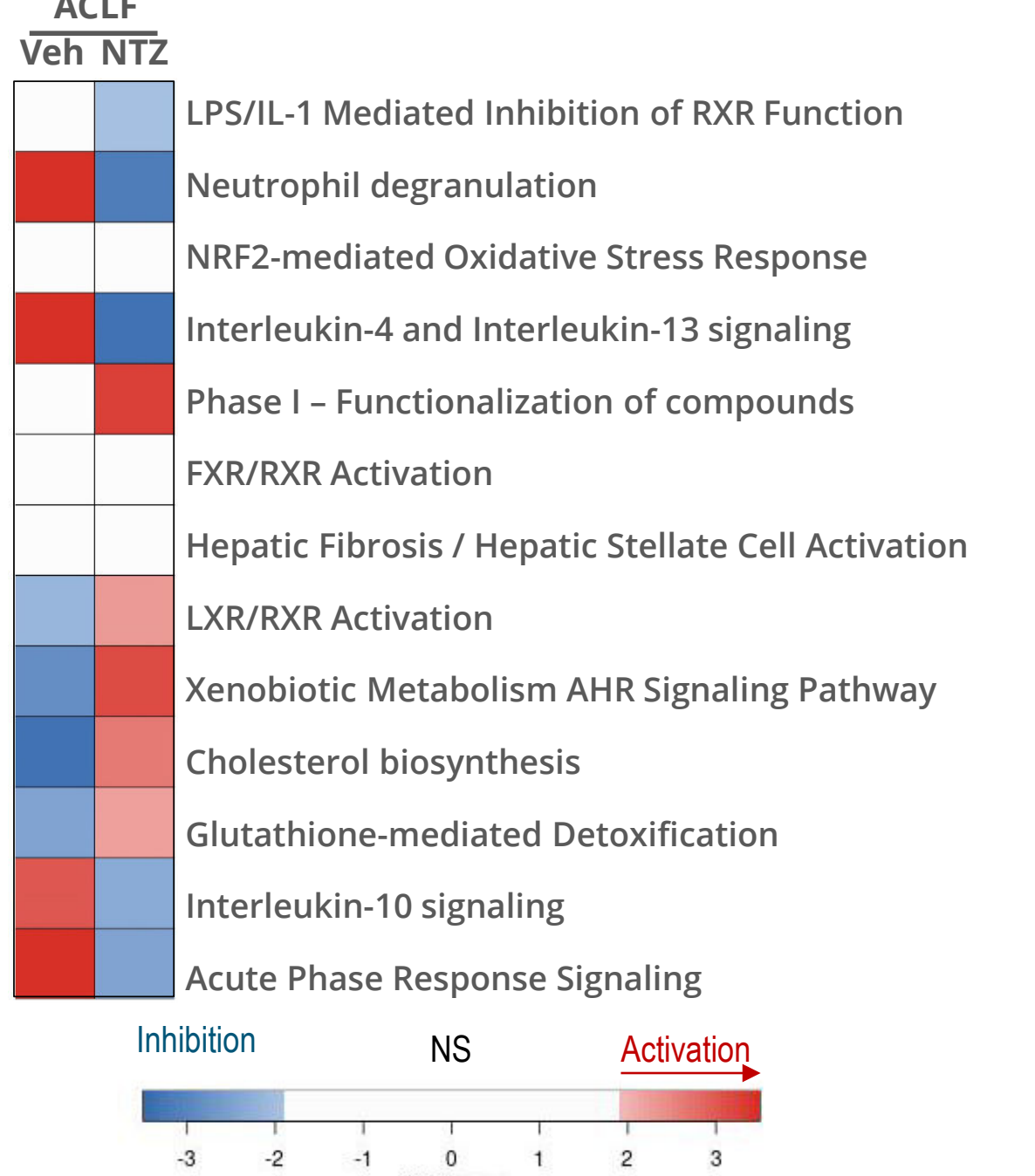
Top pathways enriched in ACLF vs CCl₄ (FDR<10⁻⁷)



Effect of NTZ on top pathways induced in ACLF



Predictive pathway activation score



- LPS induces a strong remodeling of fibrotic rat liver transcriptome, ACLF-modulated genes being mostly related to immune response, oxidative stress and metabolism
- NTZ further modulates the ACLF gene signature, by reversing the effect of LPS

A. Experimental workflow (n=5 to 6/group)
B. Serum levels of hepatic injury / function markers 24h after LPS in fibrotic rats
C. Volcano plots displaying DE genes (FC>2 and FDR<0.05) between ACLF-veh vs CCl4-veh groups (black dots) and between ACLF-NTZ vs ACLF-veh groups (purple dots)
D. Top enriched pathways using the list of DE genes between ACLF-veh and CCl4 groups (1506 genes)
E. Enrichment analysis using the list of DE genes obtained between ACLF-NTZ and ACLF-veh groups (794 genes)
F. Activation score predicted by IPA using pathways analyses described in D. and E. Non significant (NS) Z-score (-2 to 2) are displayed in white

CONCLUSION

- In two ACLF-related preclinical models, transcriptomic analyses show that NTZ counteracts the deregulation of pathways involved in immune response, metabolism and oxidative stress response, which could explain the protective role of NTZ against hepatocyte cell death and liver damage
- Combined with its anti-bacterial and anti-inflammatory activities, these findings further support the development of NTZ as a new therapeutic approach for ACLF

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