Mechanistic understanding of the relationships between oxidative and electrophilic stress in allergic skin sensitisation

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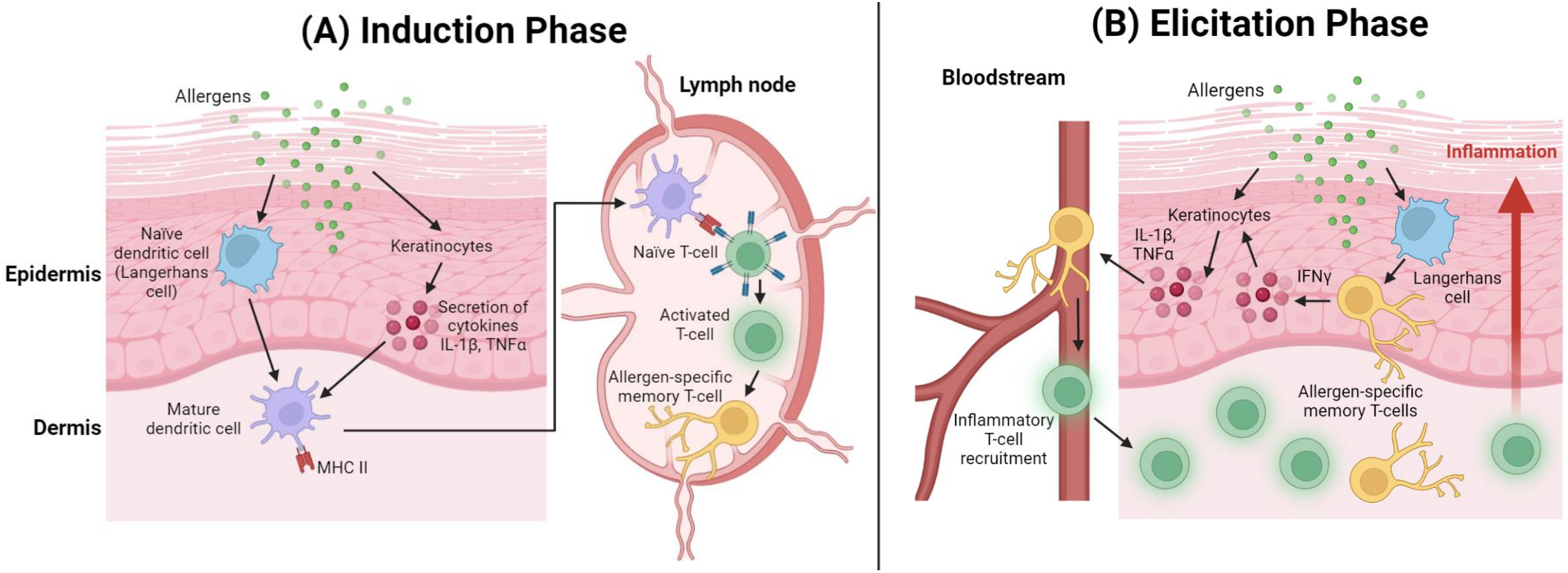
Background

- Allergic skin sensitisation manifests clinically as allergic contact dermatitis (ACD)
- ACD affects ~20% of European population[1]
- Individual variation in sensitisation not fully explained[2]
- We hypothesise that a state of oxidative stress could affect sensitiser potency
- Aim to investigate the effects of reactive oxygen species (ROS) induced oxidative stress on proteomic action of sensitiser 2,4-dinitrochlorobenzene (DNCB) *in vitro*

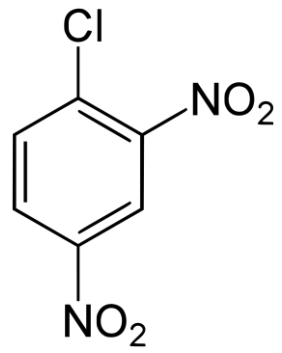
1. Peiser, M. *et al.* (2012) Allergic contact dermatitis: epidemiology, molecular mechanisms, in vitro methods and regulatory aspects. *Cellular and Molecular Life Sciences.* **69**(5): 763-781.
2. Gilmour, N. *et al.* (2019) Skin sensitization: Uncertainties, challenges, and opportunities for improved risk assessment. *Contact Dermatitis.* **80**(3): 195-200.

Allergic skin sensitisation

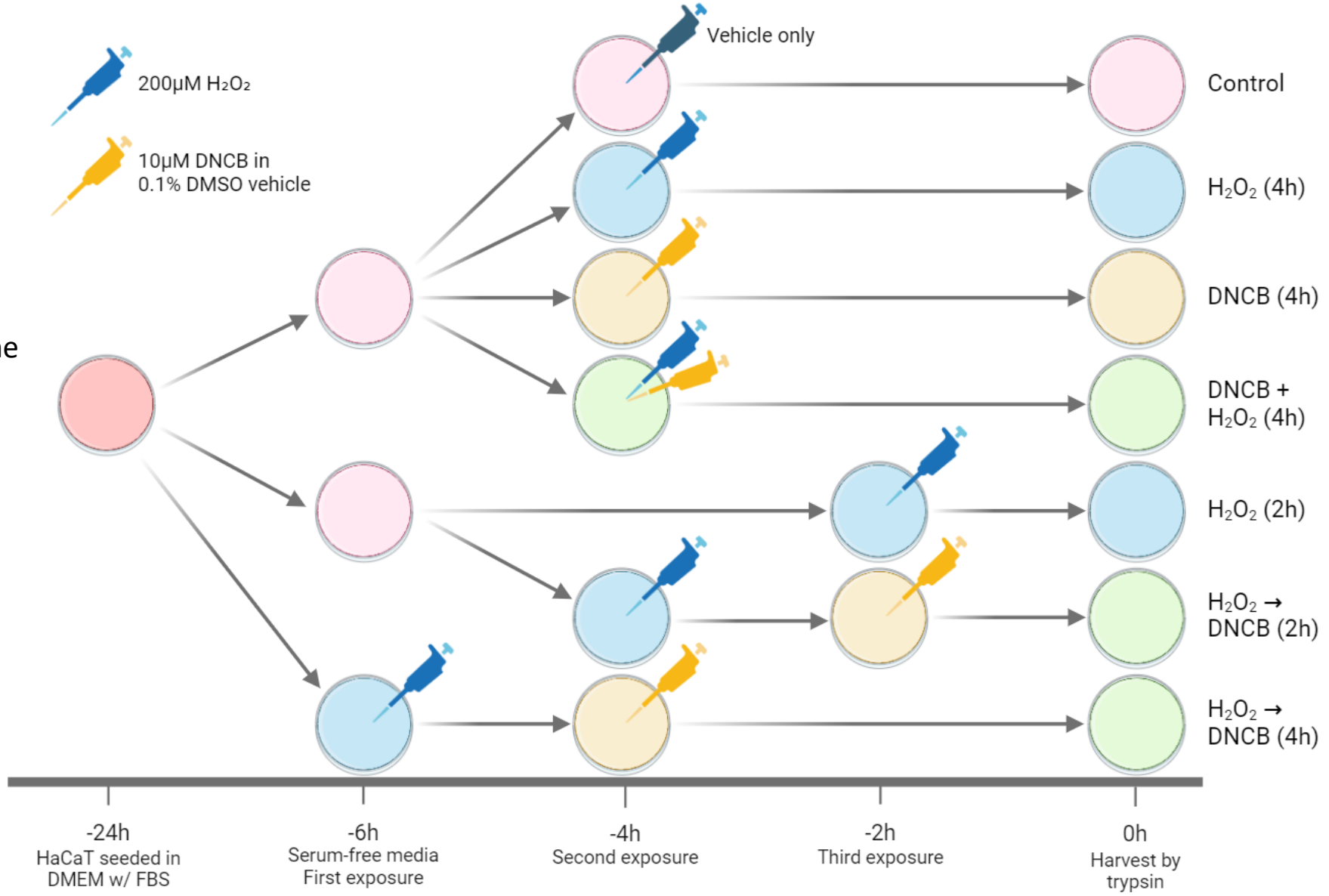


Experimental design

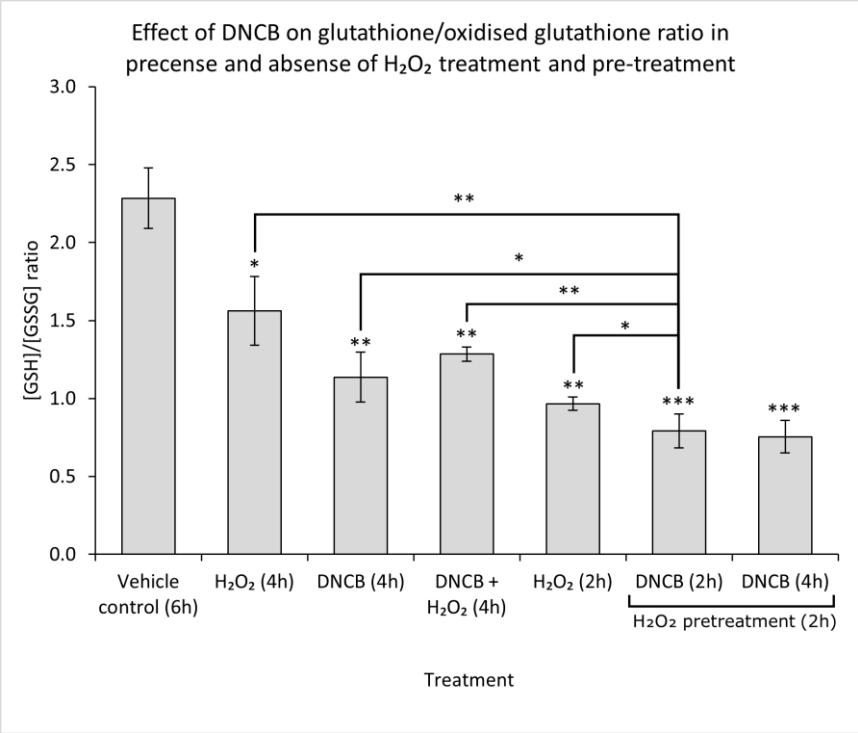
- HaCaT keratinocytes cultured in DMEM media
- Model ROS H_2O_2
- Model sensitiser DNCB
- Comparing antioxidant enzyme activity, protein expression between samples

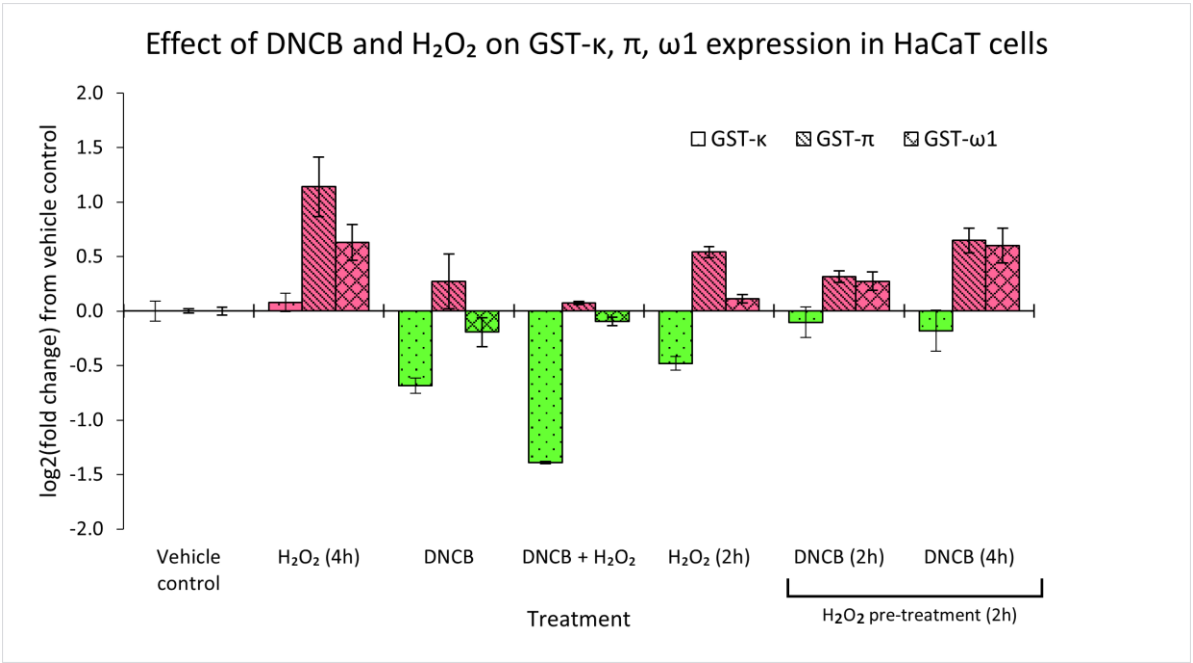


2,4-dinitrochlorobenzene (DNCB)



H_2O_2 pre-exposure decreases Glutathione (GSH) availability, increases GSH-s-transferase omega (GST-ω) expression following DNCB exposure

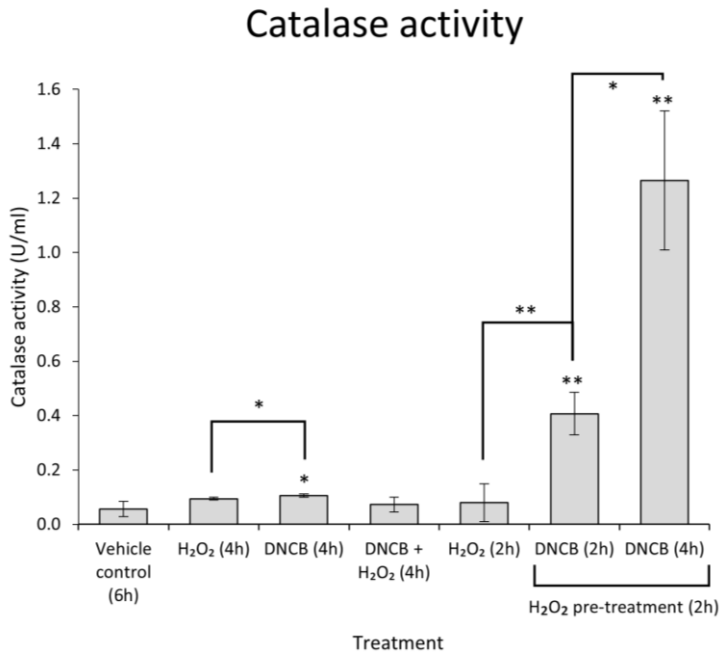




- Ratio of [GSH]/[GSSG] found by luminescence assay
- Glutathione (GSH) oxidises into GSSG

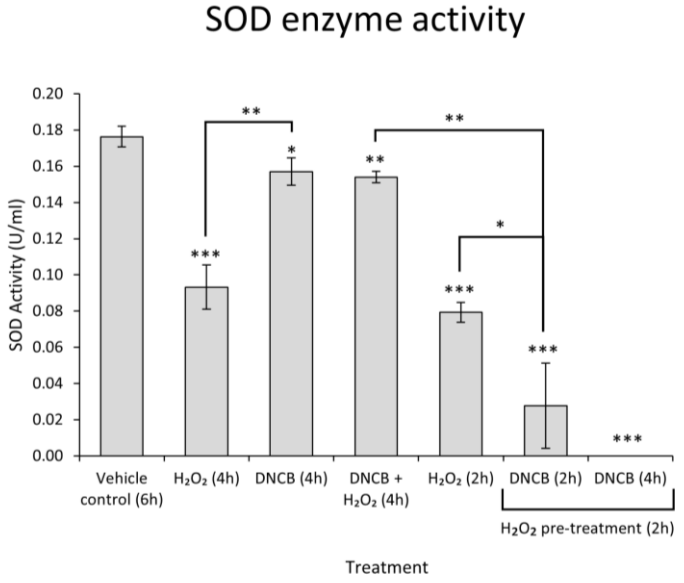
- Protein analysis by LC-MS/MS
- Comparative protein expression of glutathione-S-transferases (GST)

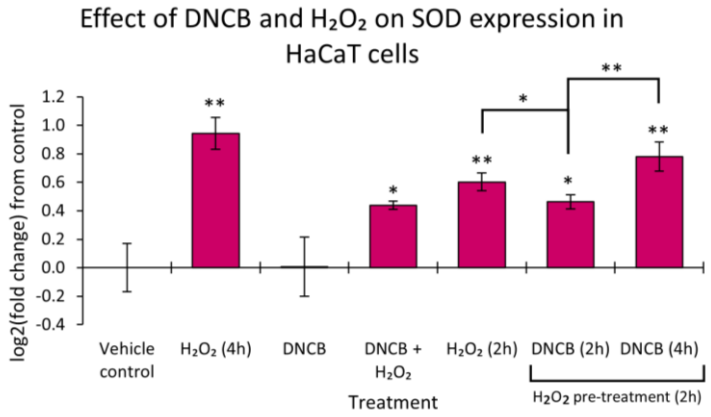
H_2O_2 pre-exposure increases catalase activity



- ELISAs measuring catalase, superoxide dismutase (SOD) enzyme activity in HaCaTs
- SOD protein expression changes matches from LC-MS/MS data

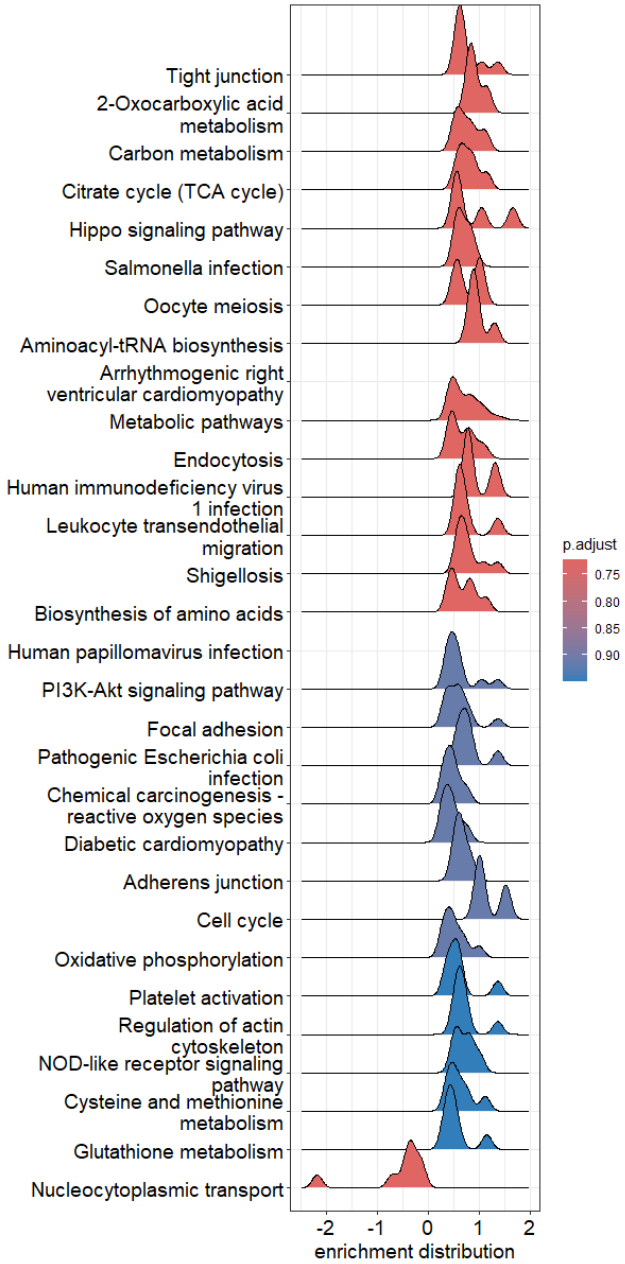
H_2O_2 pre-exposure decreases SOD activity, increases SOD expression

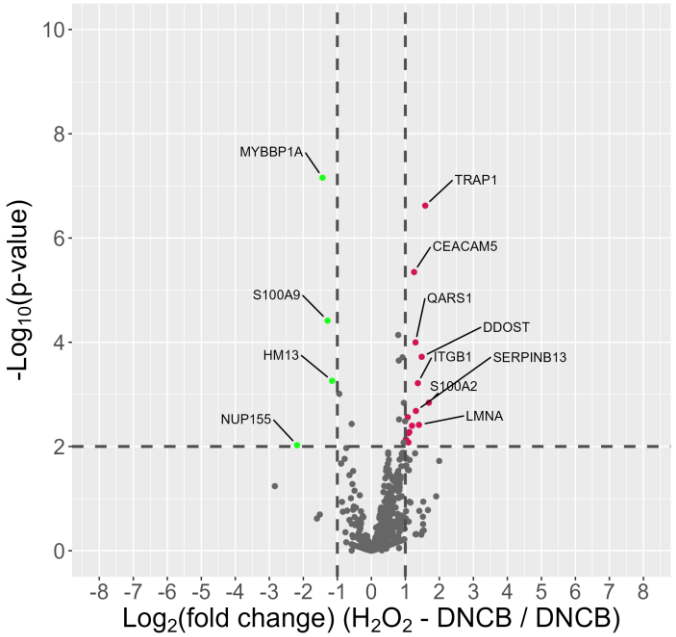




H_2O_2 pre-exposure, DNCB exposure upregulate proteins in key oxidative stress pathways compared to DNCB-only exposure

- Whole proteome analysis of HaCaT samples
- MS data processed in R by gene set enrichment analysis, Kegg pathway analysis
- Figures compare protein expression in Kegg pathways between samples exposed DNCB with and without H_2O_2 pre-exposure

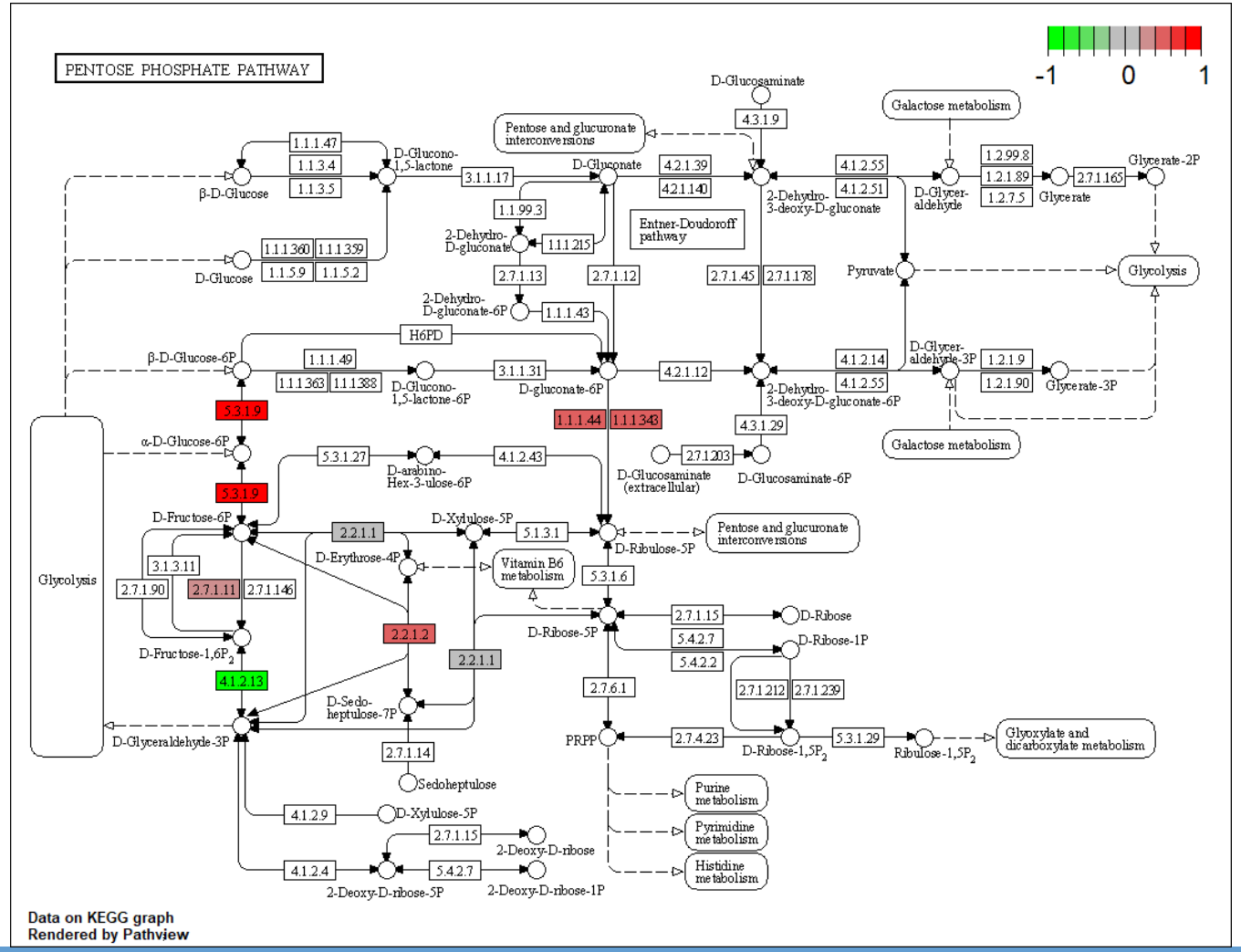




H_2O_2 pre-exposure, DNCB exposure upregulate proteins in key oxidative stress pathways compared to DNCB-only exposure

- Kegg pathway analysis in R
- Protein expression in HaCaTs pre-exposed to H_2O_2, exposed to DNCB versus HaCaTs exposed to DNCB only
- Red proteins upregulated
- Green proteins downregulated

- Key upregulations in TCA cycle, glycolysis, pentose phosphate pathways



Summary

- Pre-exposure to hydrogen peroxide alters the effect of DNCB on the HaCaT proteome
  - GSH/GSSG ratio is lower, indicating loss of available GSH
  - Superoxide dismutase activity is lower despite increased protein expression, suggesting inhibitory effect
  - Protein expression in key oxidative stress pathways are increased
- These data indicate that a pre-existing state of ROS induced oxidative stress could potentially increase risk of oxidative, electrophilic damage during sensitiser exposure

Ongoing work

- Determining impact of H_2O_2 pre-exposure on DNCB haptenation of HaCaT peptides
  - Data analysis ongoing
- Development of a novel multiplexed proteomic approach to measure protein carbonylation in HaCaTs following H_2O_2, DNCB exposure