University of Southampton

Investigation of cellular responses to specific protein haptenation by low molecular weight chemical sensitisers

Dr Alexander Lester

Introduction – Adverse Outcome Pathway

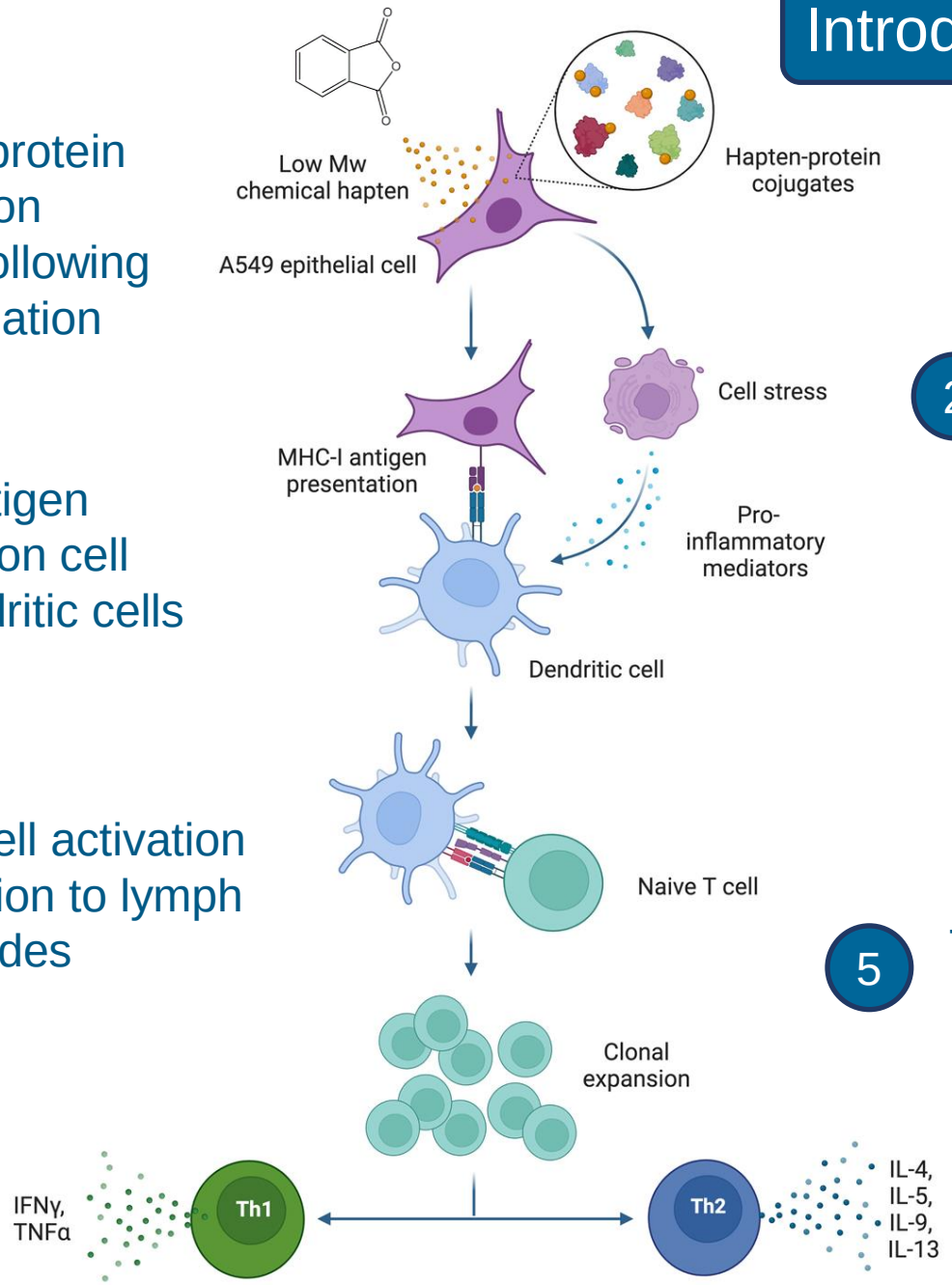
1 Endogenous protein modification (haptenation) following cell internalisation

2 Cellular response following exposure

3 Peptide antigen presentation on cell surface for dendritic cells

4 Dendritic cell activation and migration to lymph nodes

5 T cell activation, proliferation and differentiation

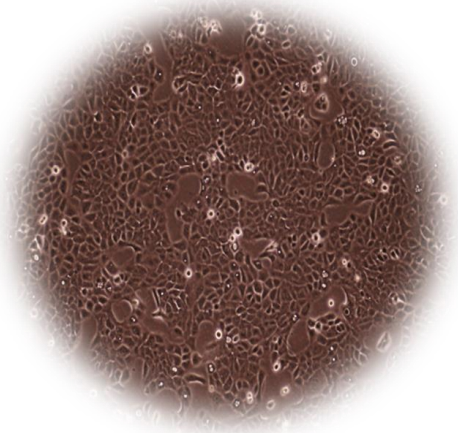


Type 1 immune response – skin sensitisers

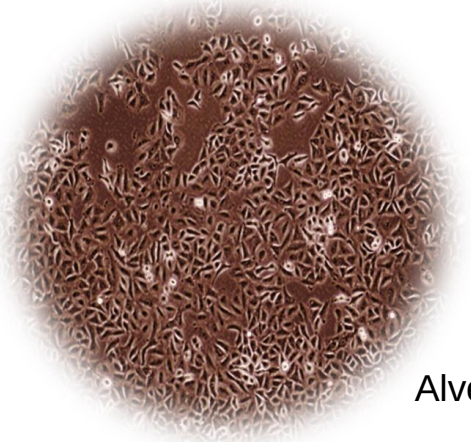
Type 2 immune response – respiratory sensitisers

Adapted from: *An Adverse Outcome Pathway for sensitization of the respiratory tract by low-molecular-weight chemicals*. Sullivan *et al* (2017).

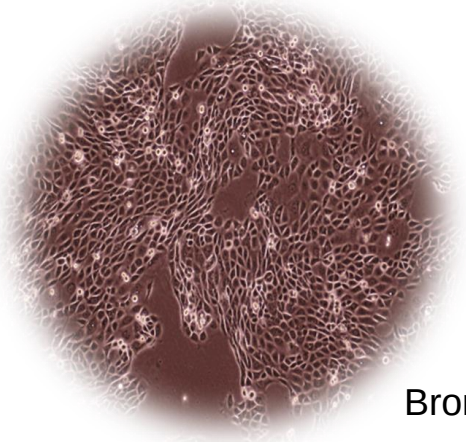
Cell Lines & Chemical Sensitisers



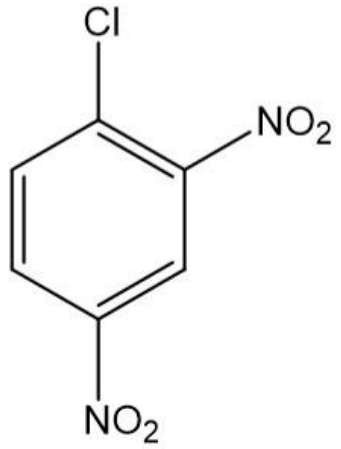
HaCaT
Keratinocytes



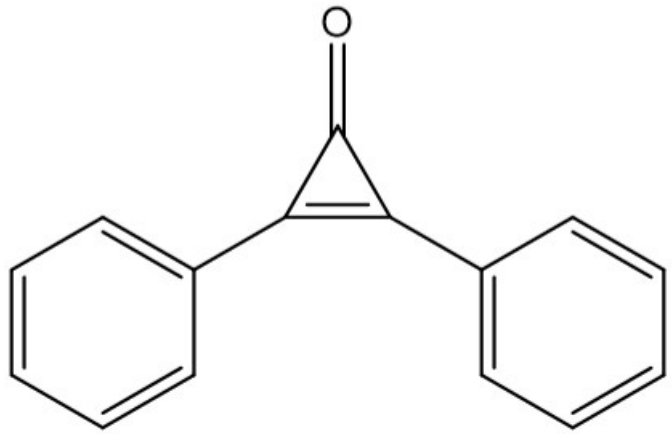
A549
Alveolar Epithelial Cells



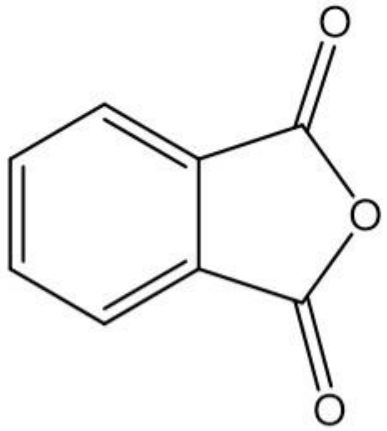
16HBE14o^{-}
Bronchial Epithelial Cells



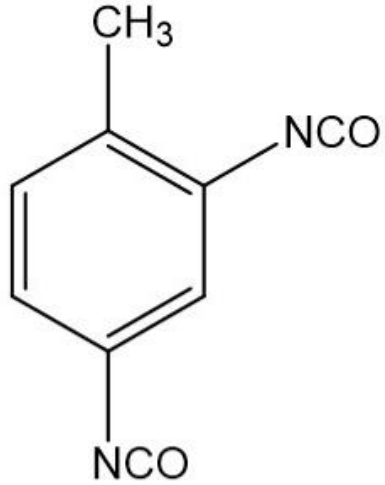
2-4-Dinitrochlorobenzene | **DNCB**
(Skin Sensitiser)



Diphenylcyclopropenone | **DPCP**
(Skin Sensitiser)



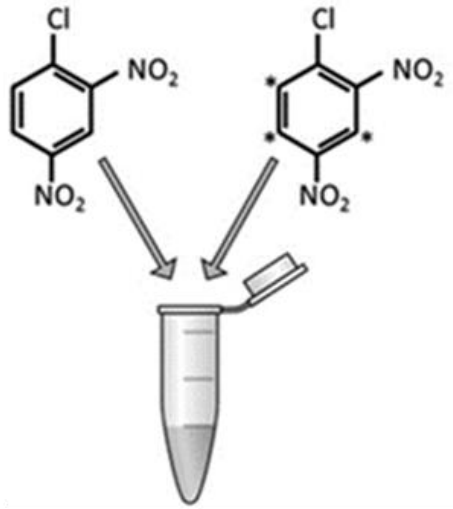
Phthalic Anhydride | **PA**
(Respiratory Sensitiser)



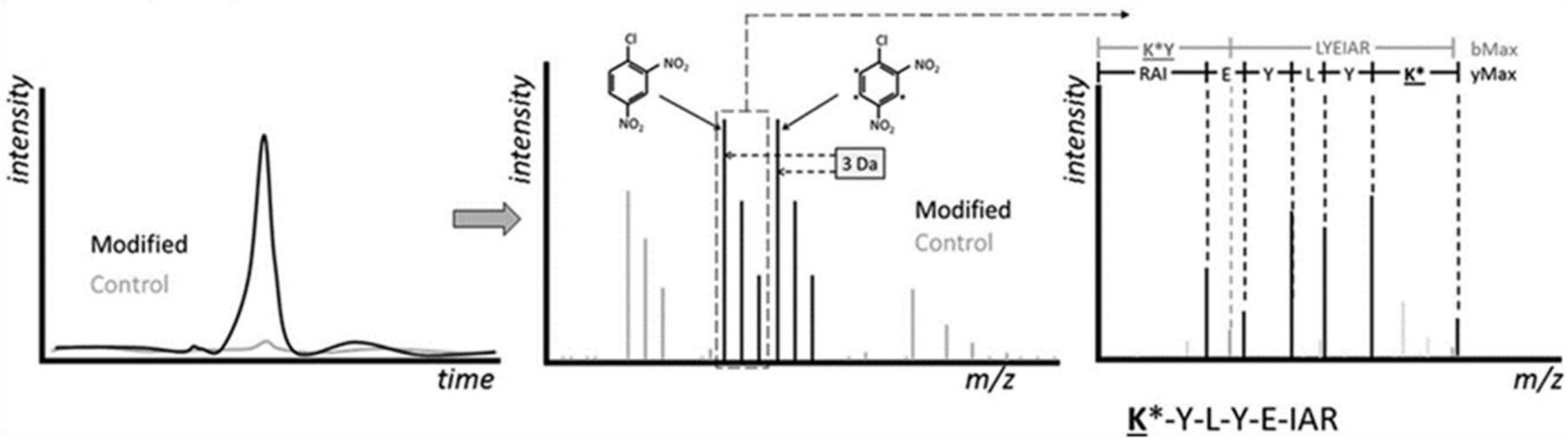
Toluene 2,4-Diisocyanate | **TDI**
(Respiratory Sensitiser)

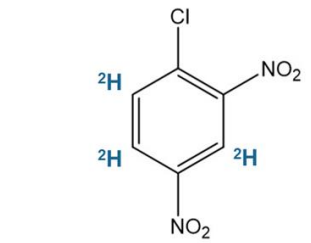
Cell Viability Profiles After Sensitisation

Dual Isotope Labelling LC-MS/MS Technique

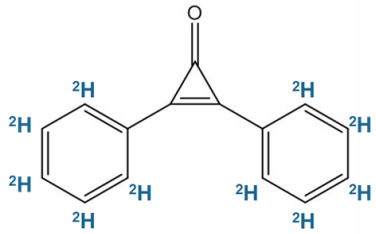


Dithiothreitol reduction
Iodoacetamide alkylation
Tryptic digest
Peptide purification

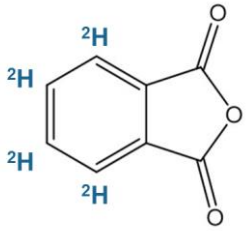




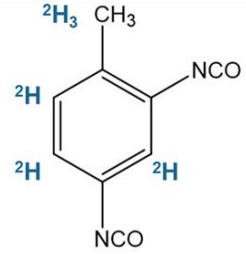
2,4-Dinitrochlorobenzene (DNCB)



Diphenylcyclopropenone (DPCP)



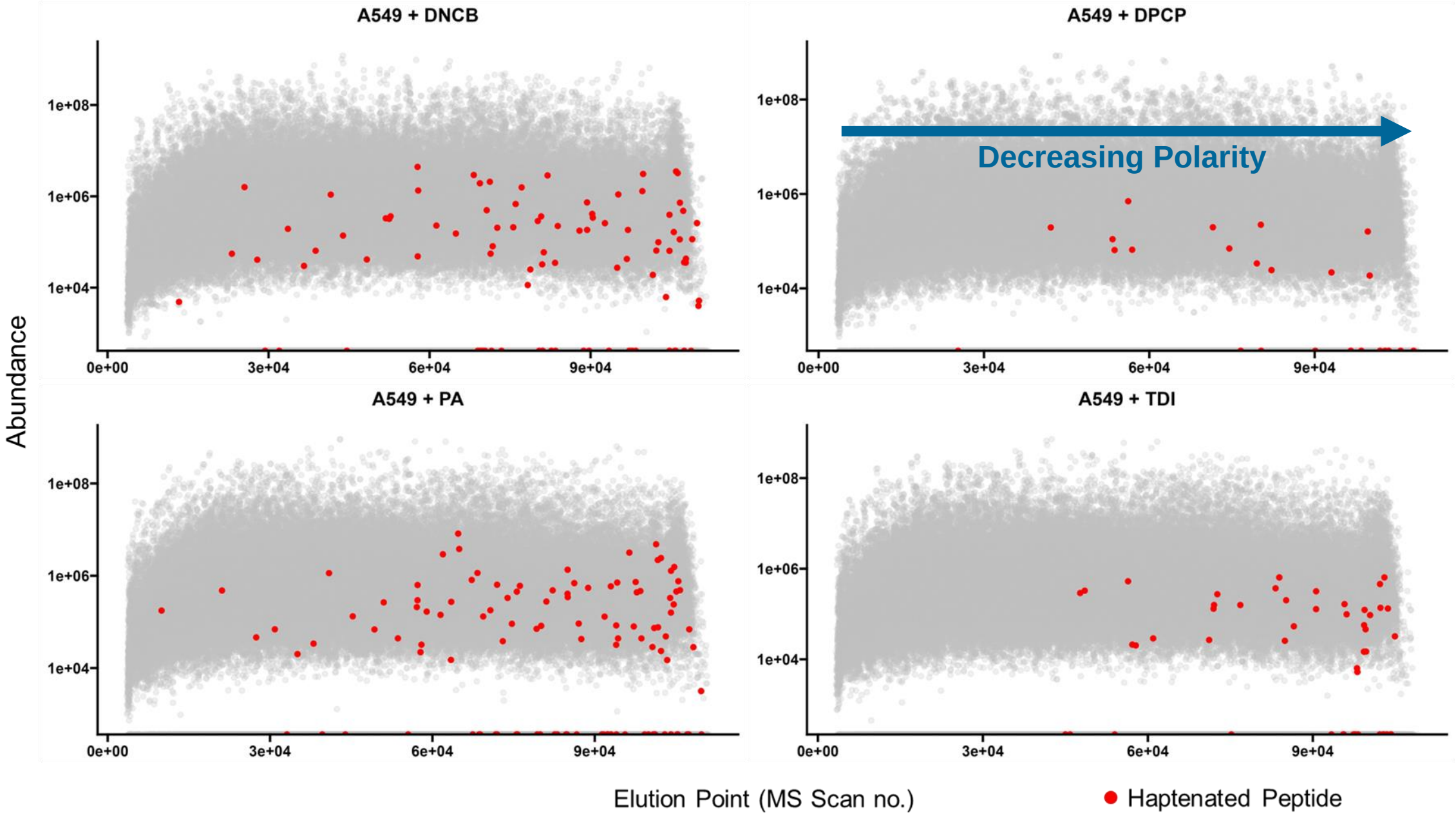
Phthalic Anhydride (PA)



Toluene 2,4-Diisocyanate (TDI)

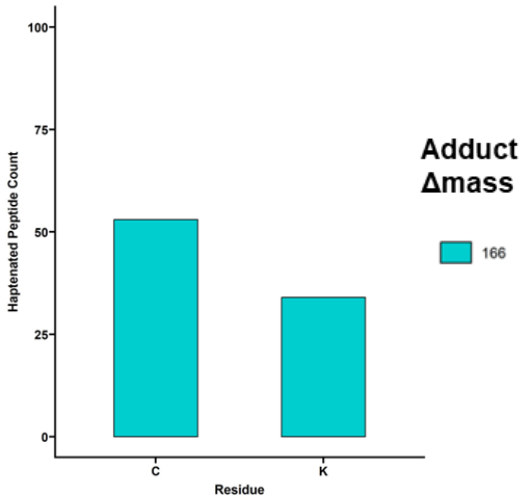
Adapted from: Parkinson *et al*. Stable Isotope Labeling Method for the Investigation of Protein Haptenation by Electrophilic Skin Sensitisers. *Toxicological Sciences.* 2014.

Global Haptenome - Repertoire

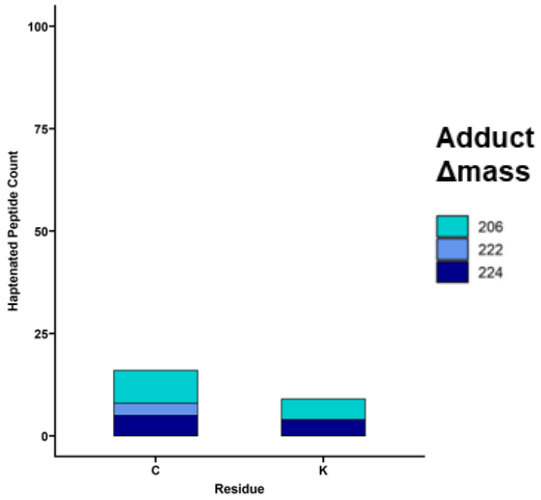


Global Haptenome - Residue Proportions

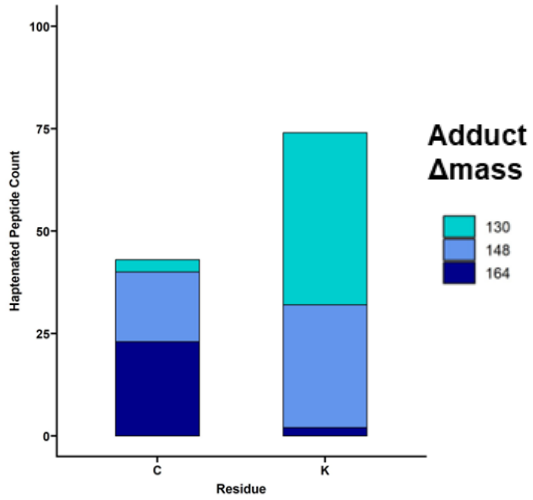
DNCB



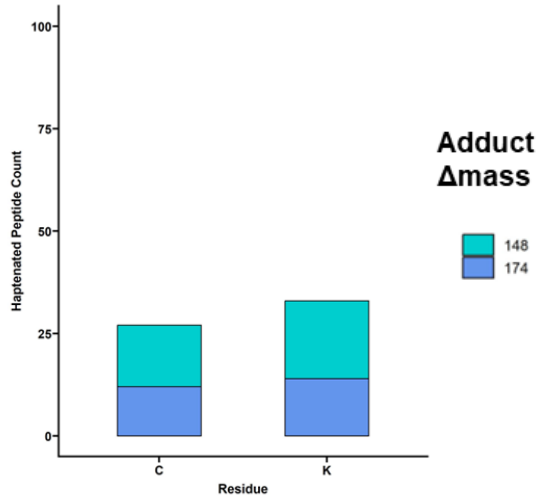
DPCP



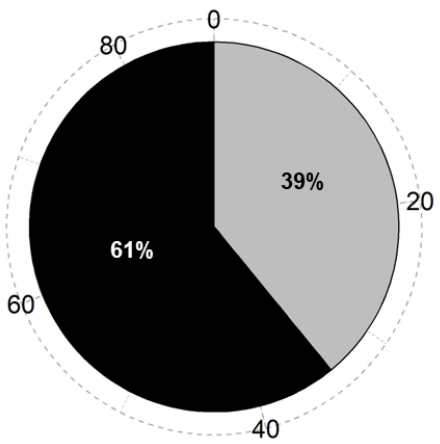
PA



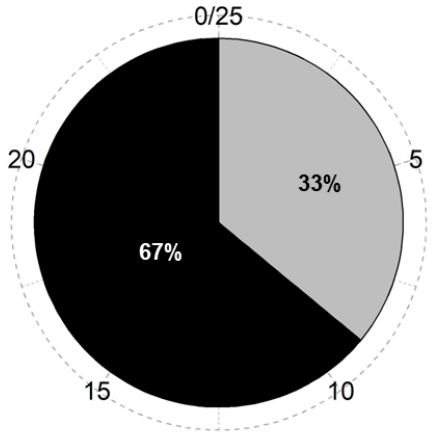
TDI



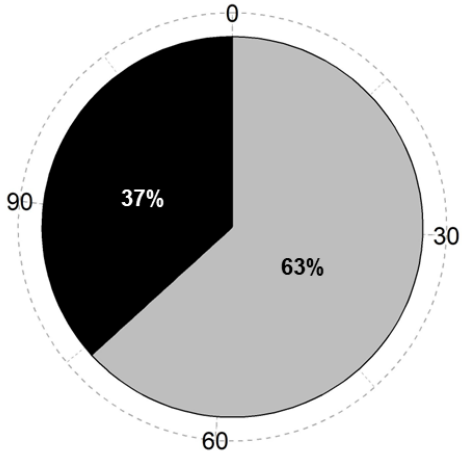
DNCB
n = 87



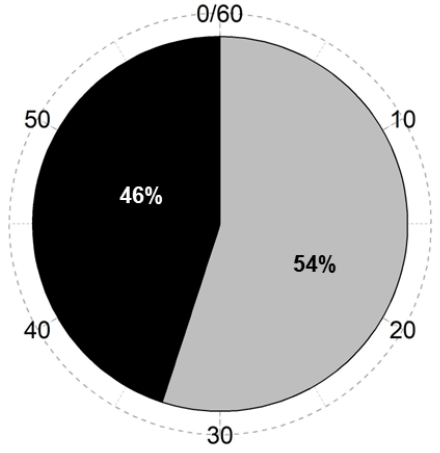
DPCP
n = 24



PA
n = 106

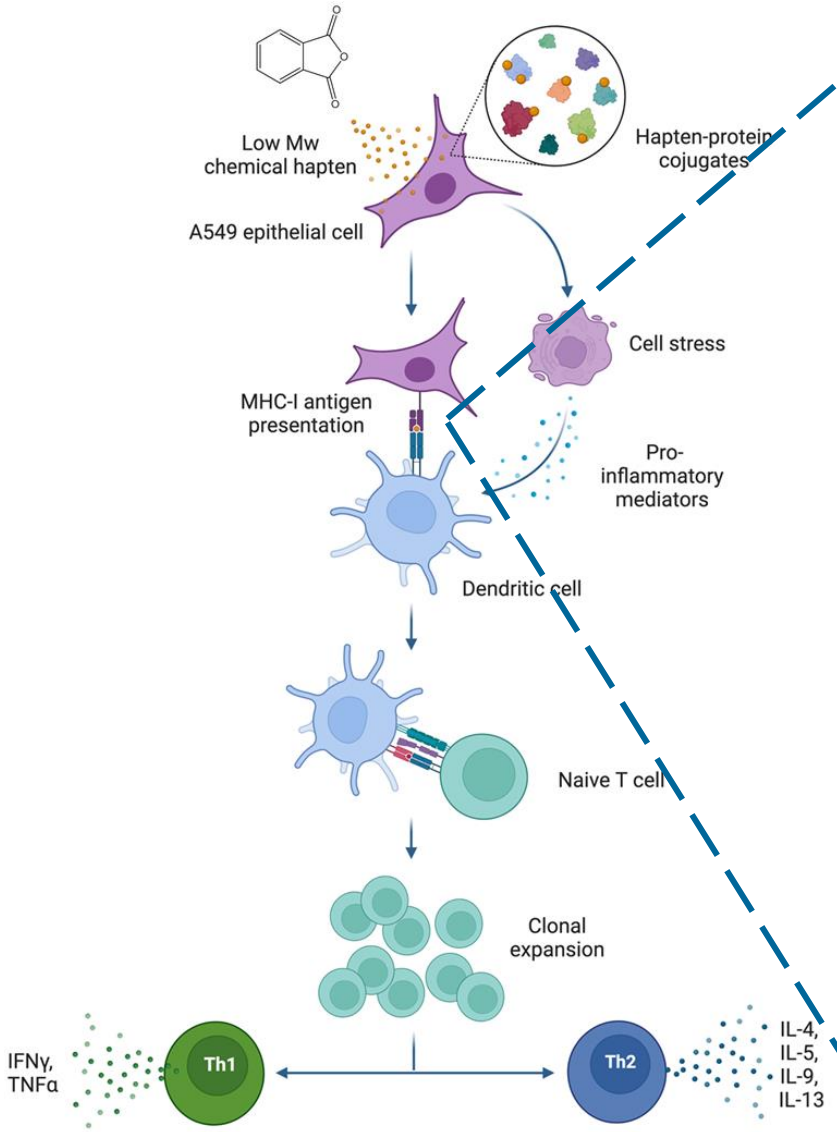


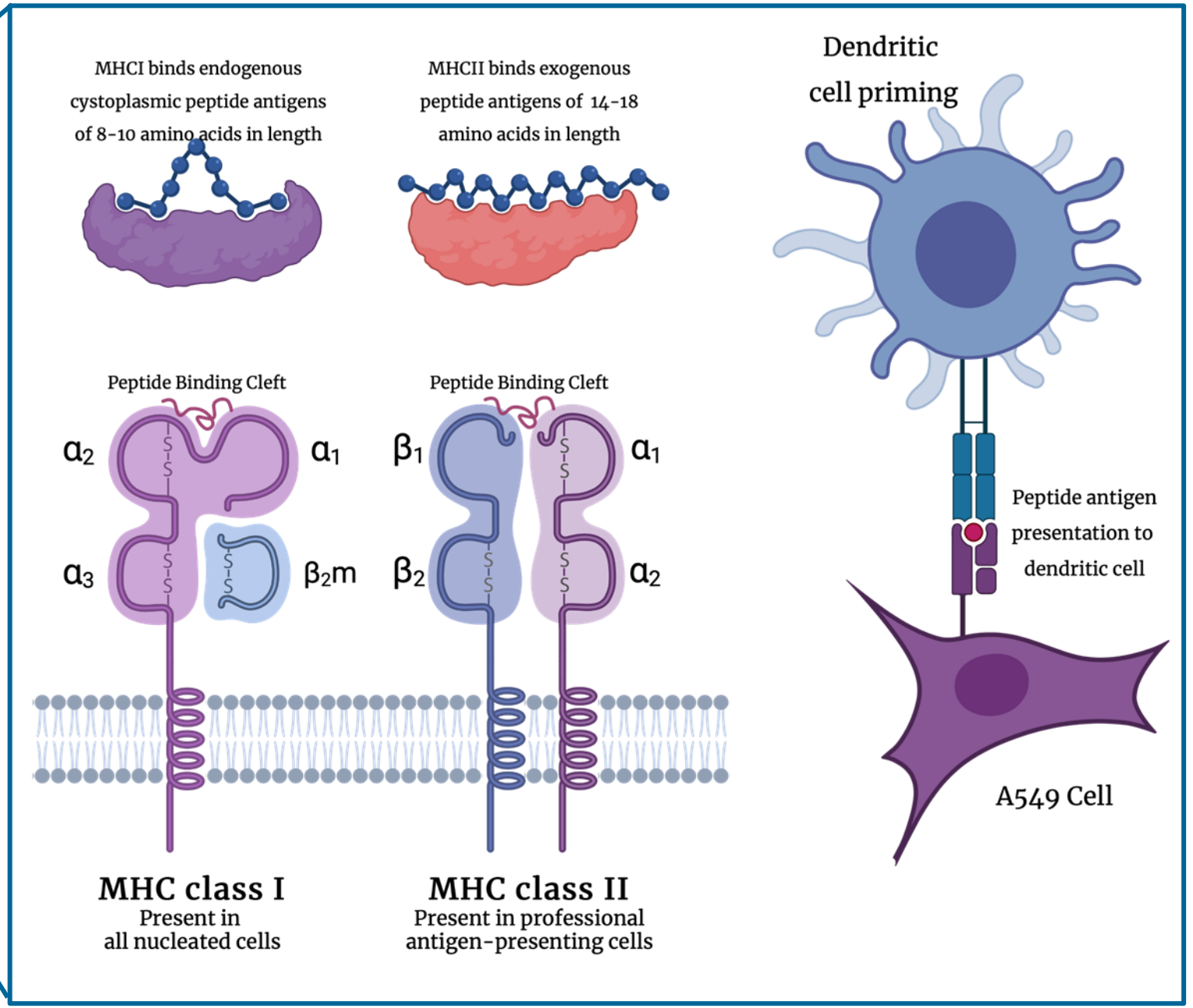
TDI
n = 57



Haptenated Residue
C
K

Immunopeptidomics – Background





Immunopeptidomics – Workflow

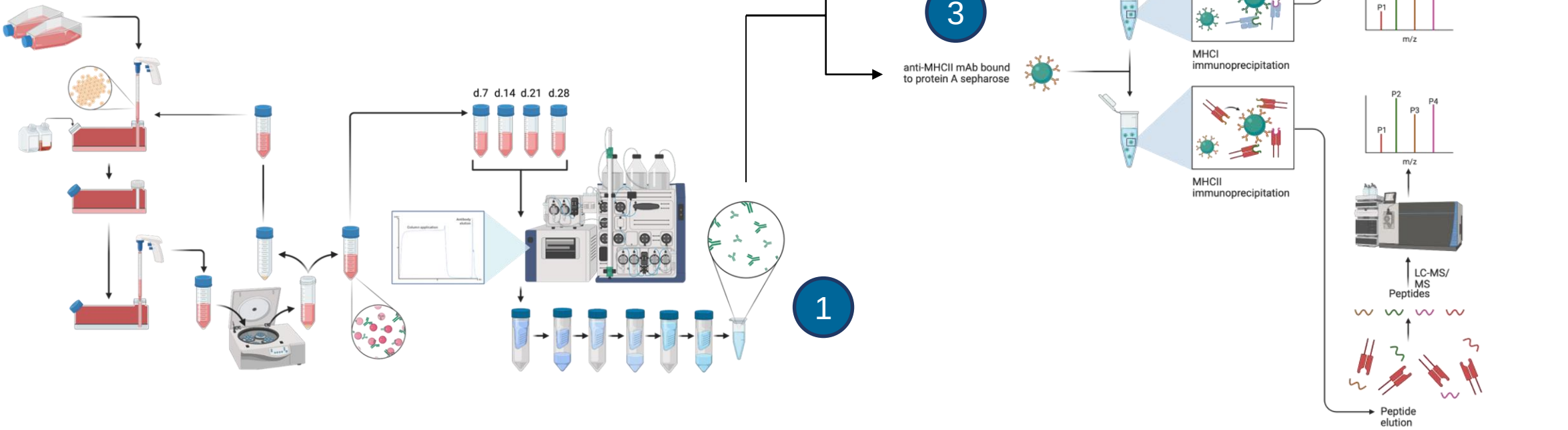
1. Anti-MHC monoclonal antibody extraction weekly from bioreactors, with automated purification and quantitfication of pooled mAb.

2. Dual isotope labelling of living alveolar epithelial cell cultures

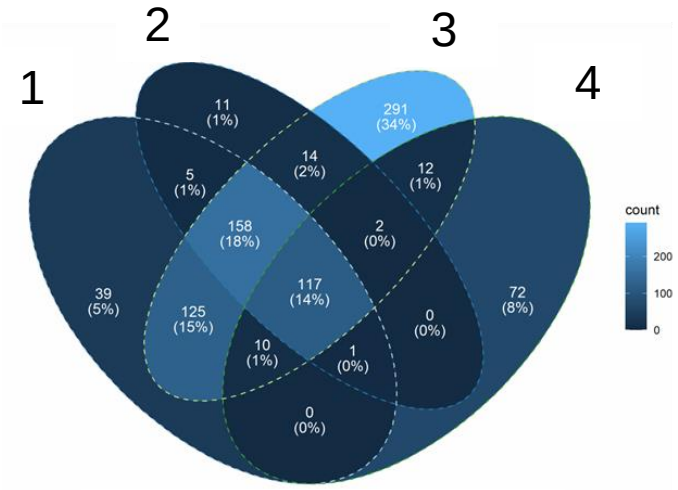
3. Immunoprecipitation

4. Immunopeptide elution and purification.

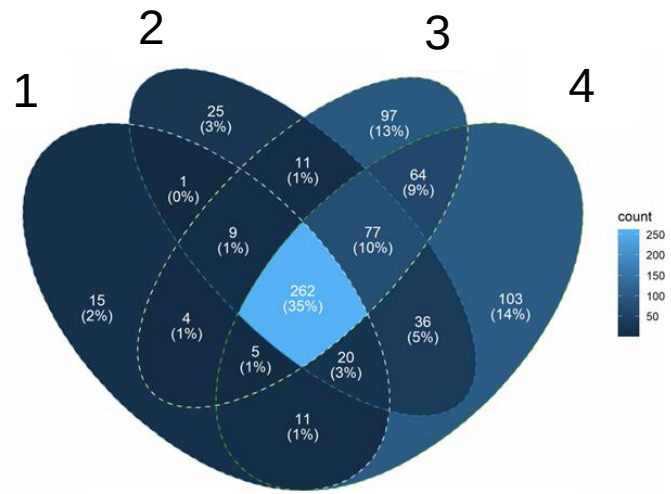
5. Tandem mass spectrometry analysis.



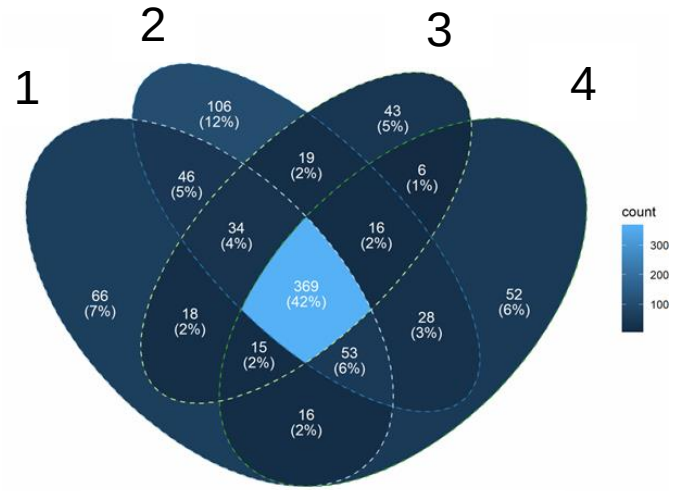
Immunopeptidomics Results: Overview



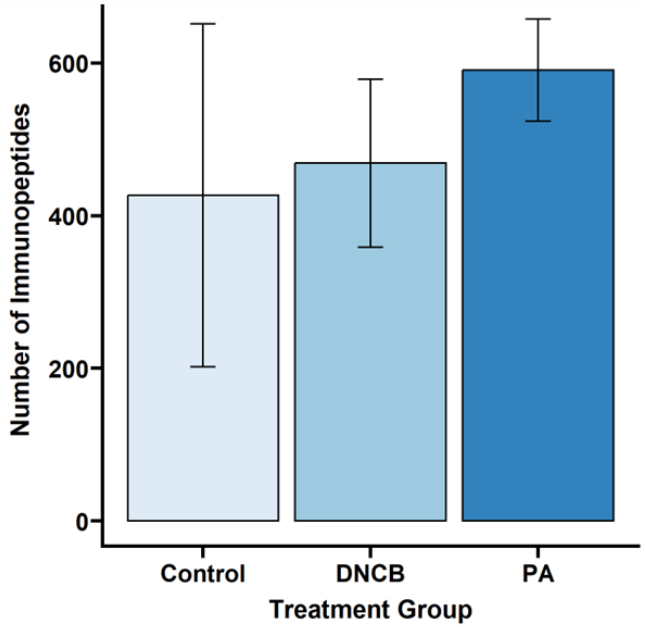
Control Replicates



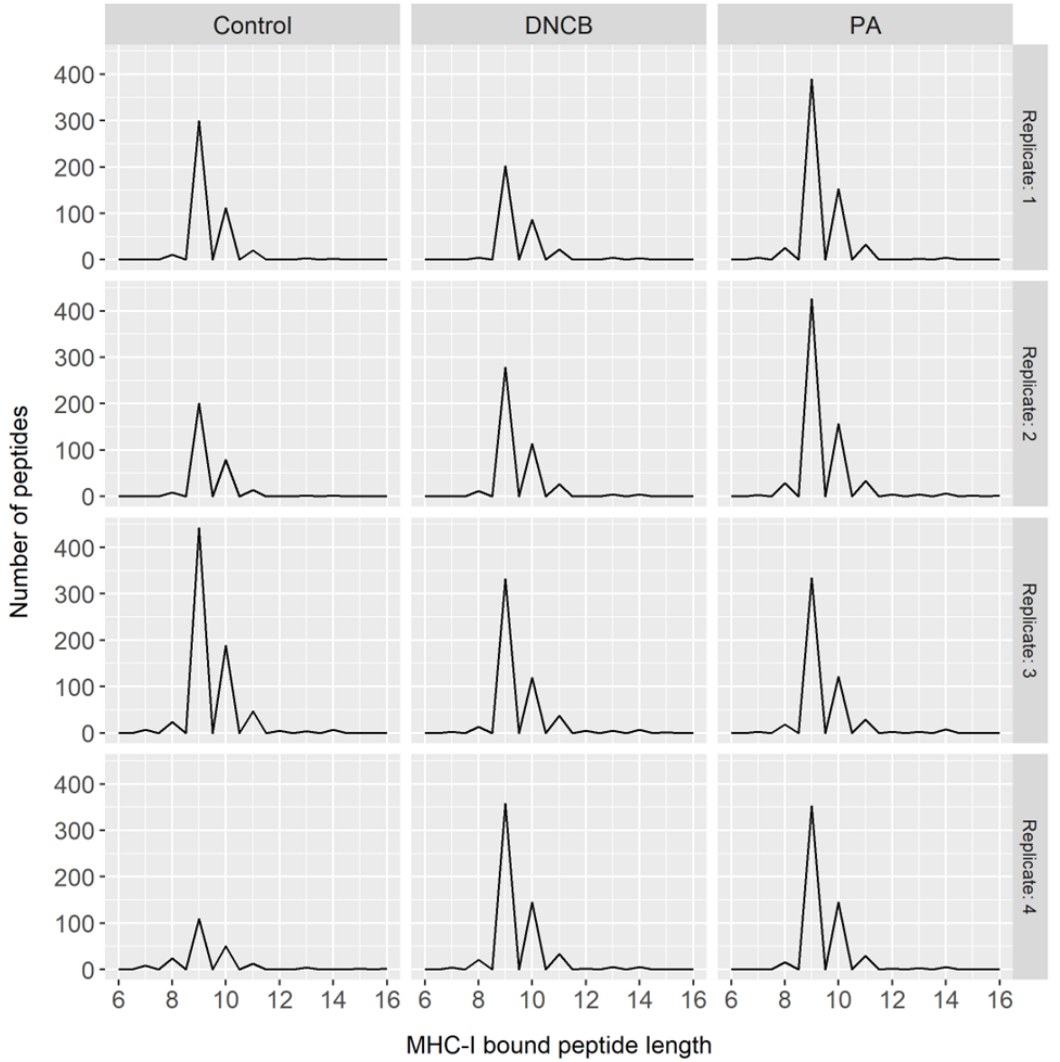
DNCB Replicates



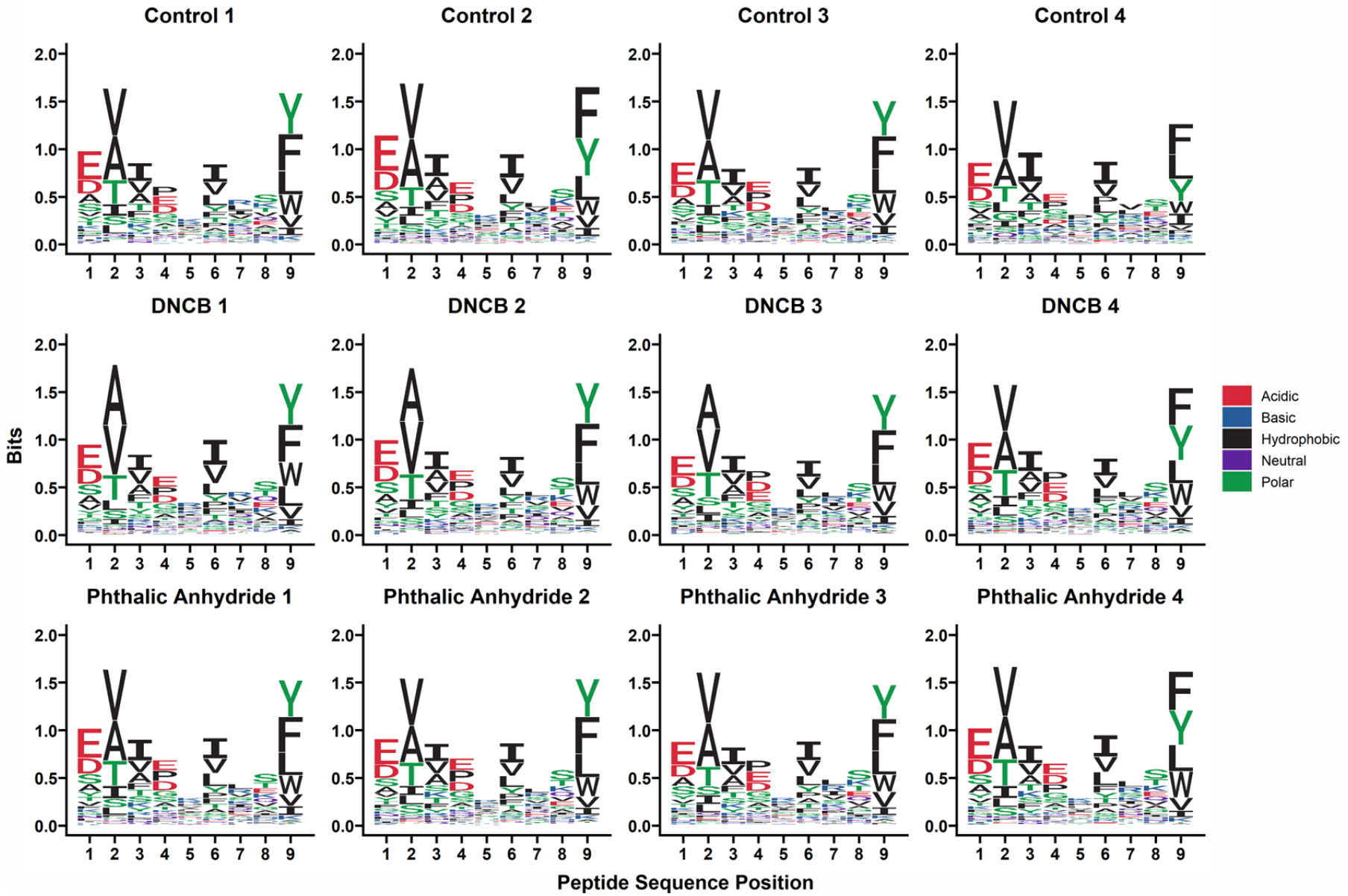
PA Replicates



Immunopeptide Length Distributions

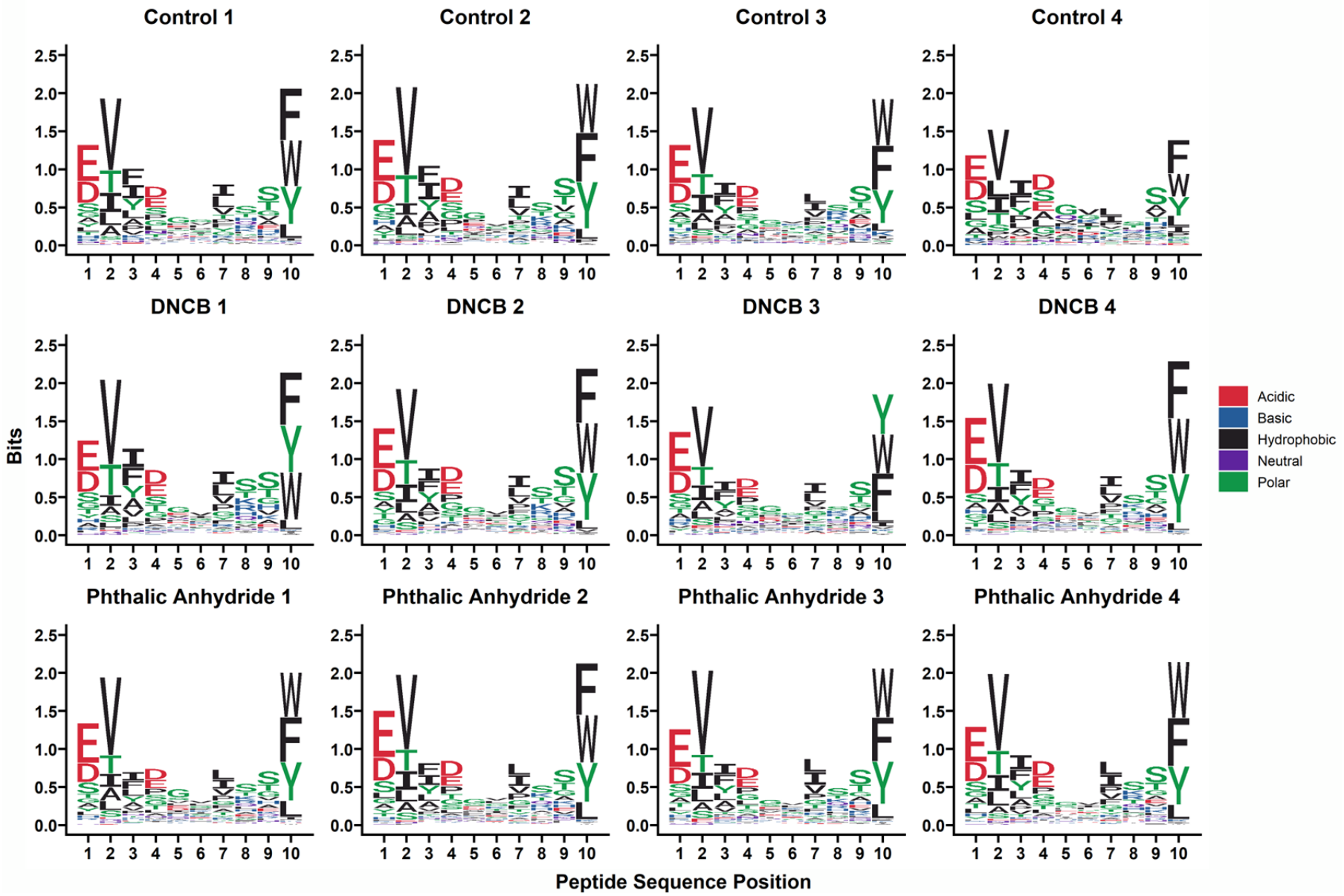


Immunopeptidomics Results: MHCI **9mers**



HLA class I immunopeptidome: HLA-A*25:01, A*30:01, B*18:01, B*44:03, C*12:03, C*16:01

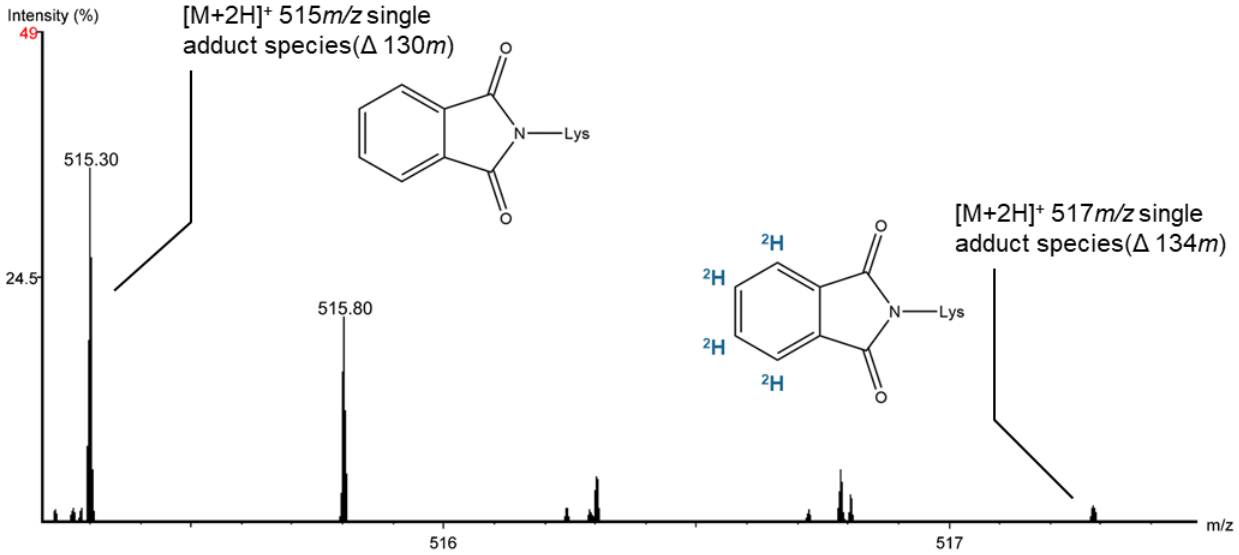
Immunopeptidomics Results: MHCI **10mers**



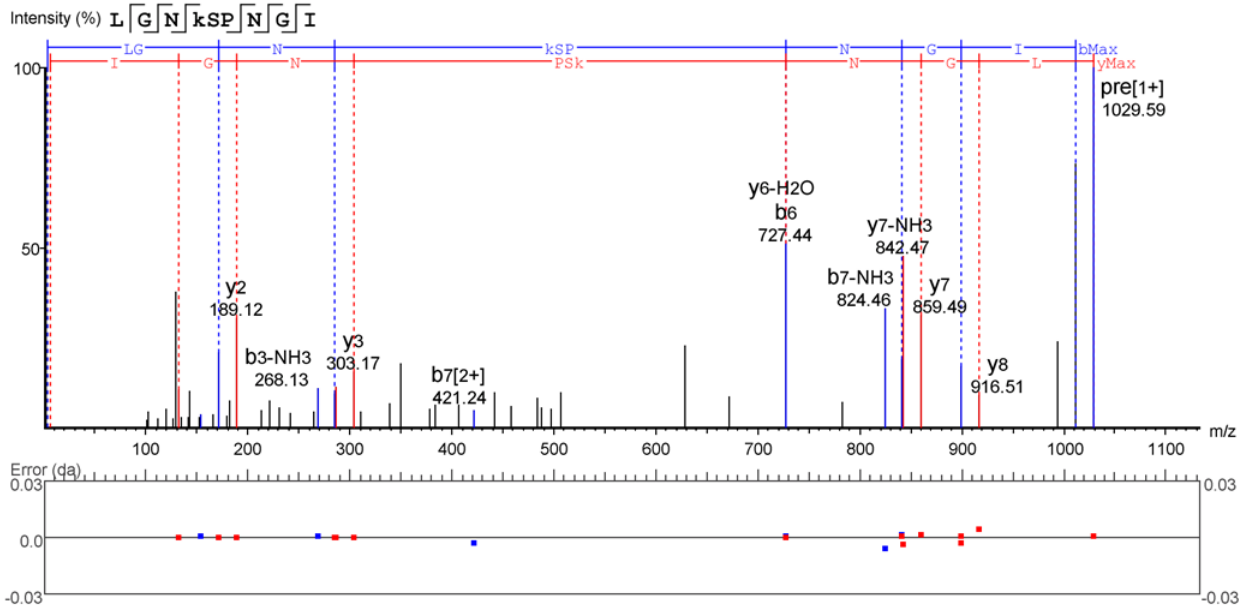
HLA class I immunopeptidome: HLA-A*25:01, A*30:01, B*18:01, B*44:03, C*12:03, C*16:01

Immunopep. Results: Validated Haptenation

MS^1 Precursor peptide ion scan



MS^2 Fragmentated peptide ion scan



PA Δ130 cyclised adduct haptenation of **sentrin-specific protease 2 (SENP2)** lysine residue.

Protease catalysing essential functions of the SUMO pathway, with nuclear localisation and export signal motifs.

SUMOylation has recently emerged as an important regulator of oxidative stress and hypoxic response pathways (Filippopoulou *et al*, 2020).

Interplay of SUMOylation and immune signalling/development pathways with the potential to shape specificity and recognition of immune responses has recently been outlined (Sajeev *et al*, 2021).

Involvement here highlights pathways potentially driving the divergence in adverse outcome pathway models.

- Notable difference in dose response identified in cells exposed to skin & respiratory sensitiser chemicals *in vitro*.

- Novel protein haptenation targets identified with respiratory sensitiser chemicals. Provides haptenomes for comparison to those previously generated from similar studies with skin sensitisers.

- *In vitro* haptenation adheres to the Cys v Lys chemical reactivity duality typically observed *in chemico*.

- Characterisation of the A549 alveolar epithelial cell immunopeptidome identified a reproducible, novel haptenated immunopeptide (SENP2) after exposure to the respiratory sensitiser phthalic anhydride.

Acknowledgements

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