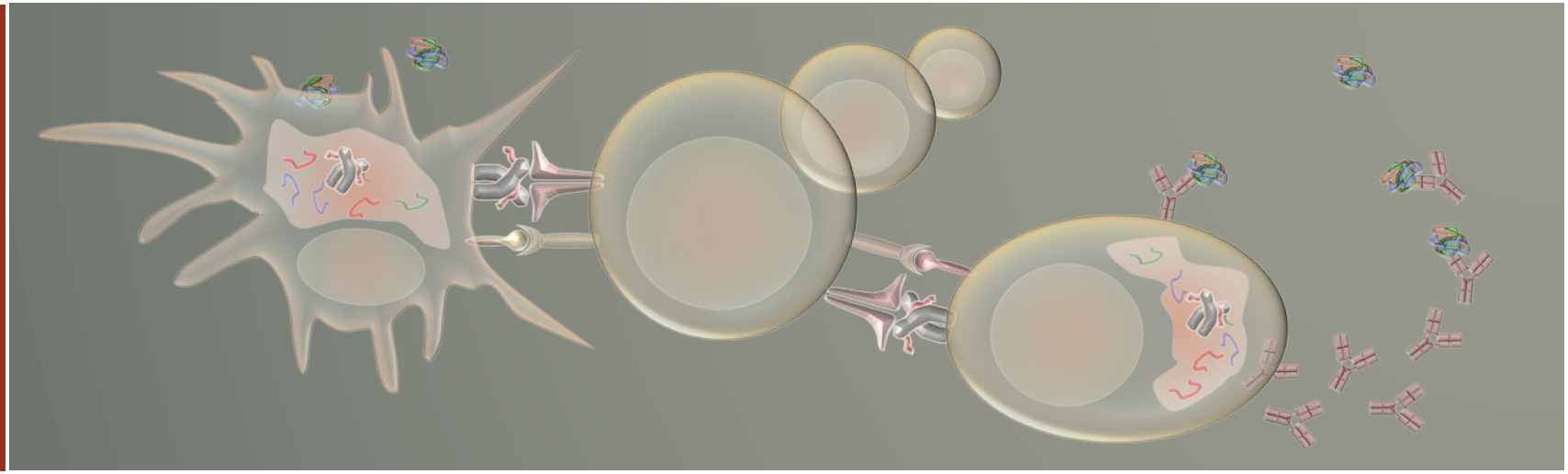

Aggregation of Human Recombinant Monoclonal Antibodies Enhances Their Presentation by Dendritic Cells In Vitro

Sebastian Spindeldreher

Novartis Institutes for Biomedical Research / DMPK-Biologics

7th Open Scientific EIP Symposium, Lisbon, February 24, 2015

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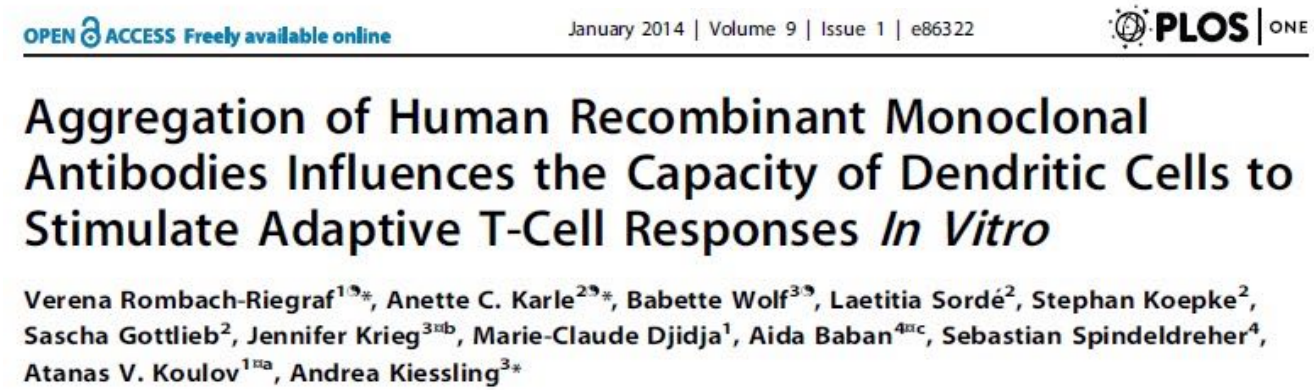
Aims of this study

- Do proteinaceous SVP have increased immunogenic potential?
- Why (mechanism)?
- Which factors influence immunogenicity:
  - Size?
  - Type of stress/structure?



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Aggregation of Human Recombinant Monoclonal Antibodies Influences the Capacity of Dendritic Cells to Stimulate Adaptive T-Cell Responses *In Vitro*

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MAPPs data

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DC data:

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Babette Wolf

Jennifer Krieg*

Stewart Jones*

T cell data

Aida Baban

Aggregates generation and phys/chem characterization:

Verena Rombach-Riegraf

Atanas Koulov*

Bahman Ossuli

Dolores Tinhan

Philippe Simeoni

Marie-Claude Djidja

Kamal Egodage

Markus Blümel

Margit Jeschke

Maria Brauchle

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* Left Novartis

Selected stress conditions

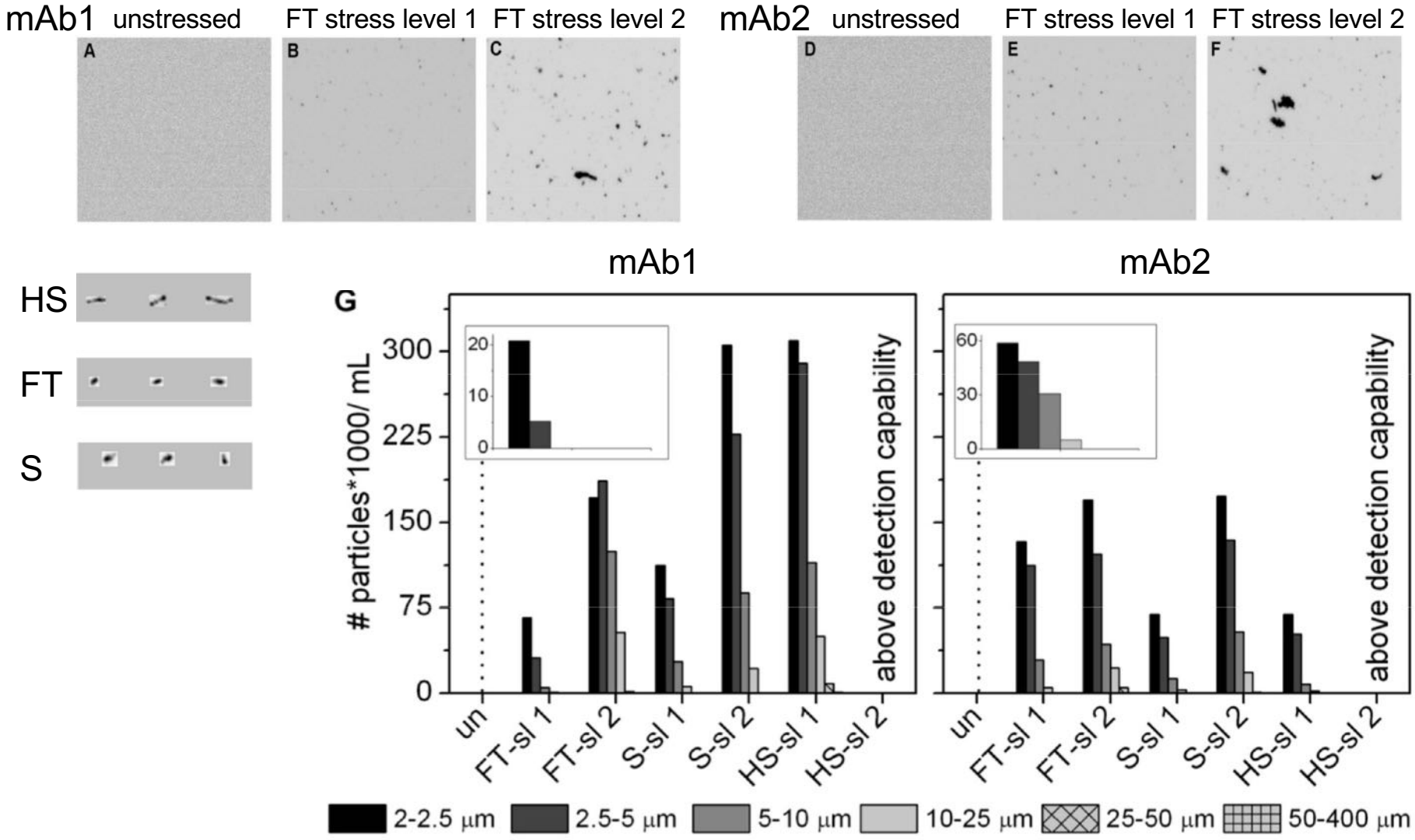
- 2 model antibodies: mAb1 and mAb2 (terminated projects)
- Both IgG1 subclass targeting soluble plasma proteins
- Very different biophysical properties from one another with regard to isoelectric point, melting temperature, surface hydrophobicity, colloidal stability
- Three stress conditions
  - Heat/shake (HS)
    - Unstressed
    - Stress level 1 (sl1) — 10 min at 65°C and 1400 rpm
    - Stress level 2 (sl2) — 6 min at 80°C and 1400 rpm
  - Shear stress (S)
    - Unstressed
    - Stress level 1 (sl1) — draw/empty syringe once
    - Stress level 2 (sl2) — draw/empty syringe four times
  - Freeze Thaw (FT)
    - Unstressed
    - Stress level 1 (sl1) — 3 FT cycles
    - Stress level 2 (sl2) — 10 FT cycles

Applied technologies

- Phys/Chem analytics
  - Microflow Imaging
  - Size Exclusion Chromatography
  - Dynamic Light Scattering
  - Capillary Electrophoresis Sodium Dodecyl Sulfate
  - LC/MS peptide mapping
- Biological assays:
  - DC activation assay
  - MAPPs assay
  - T cell assay

Particle size distribution

Determined via Microflow imaging



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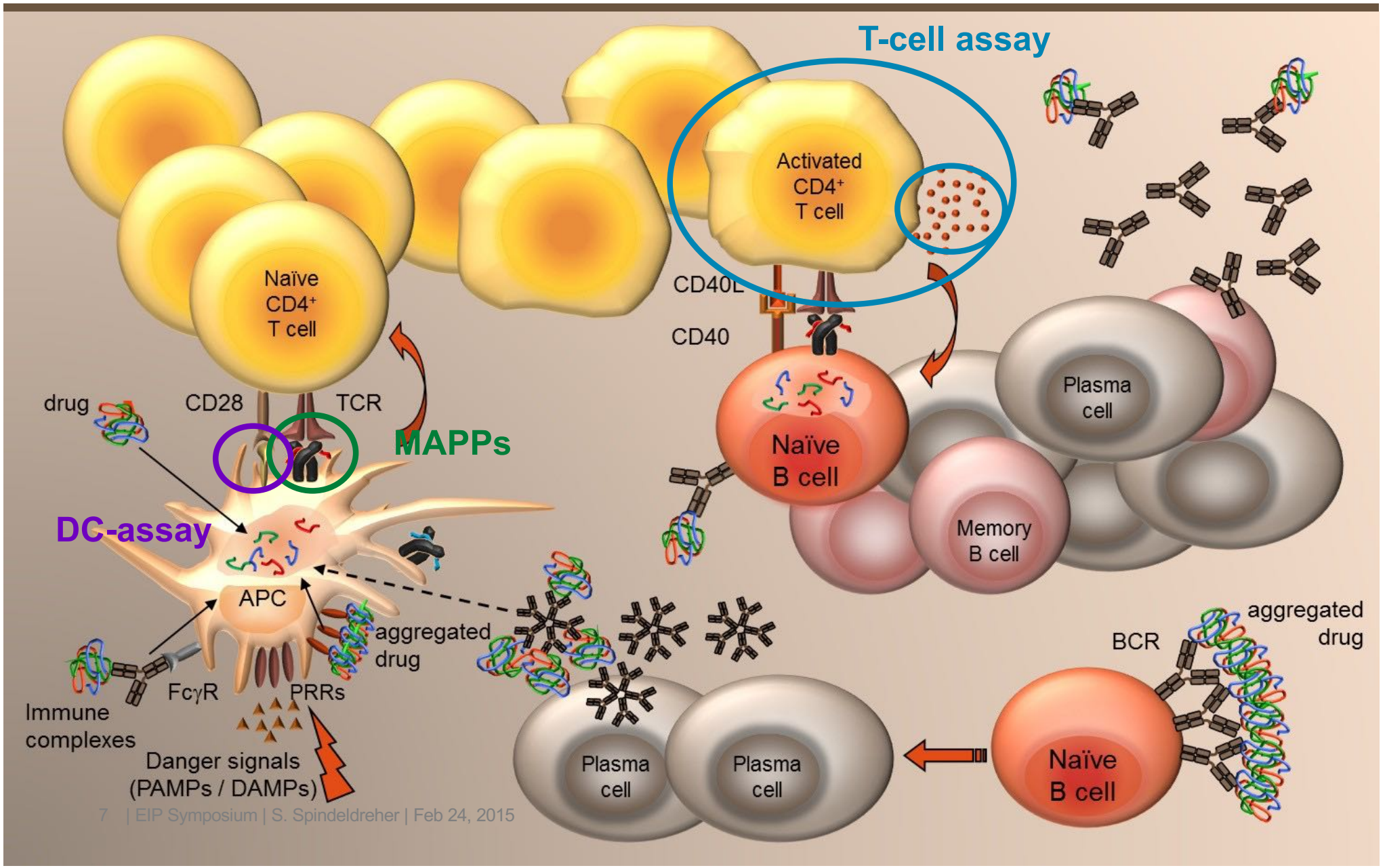
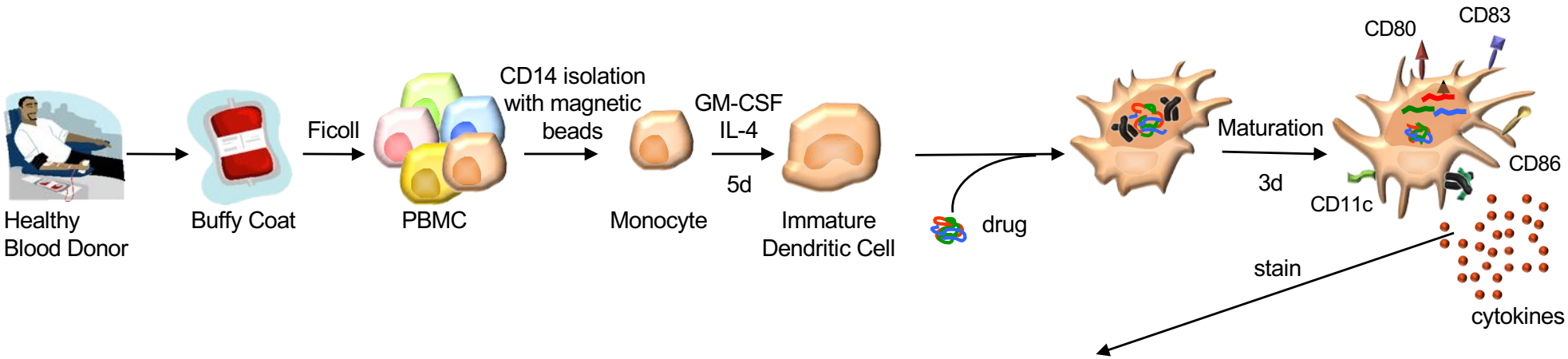
Immune cell interplay

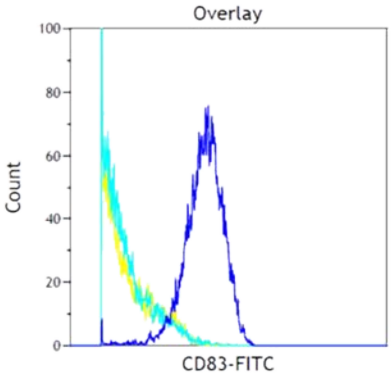


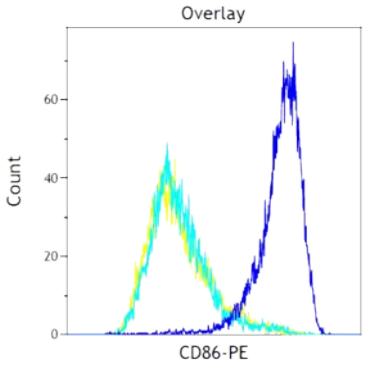
DC maturation assay

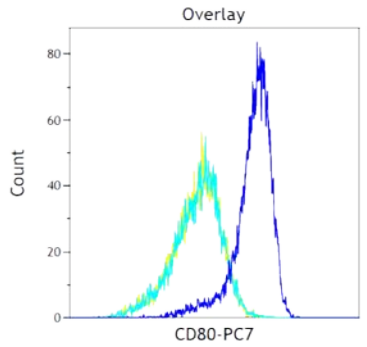
Assay procedure



- 72h maturation cocktail
- 72h medium control
- 72h DPBS







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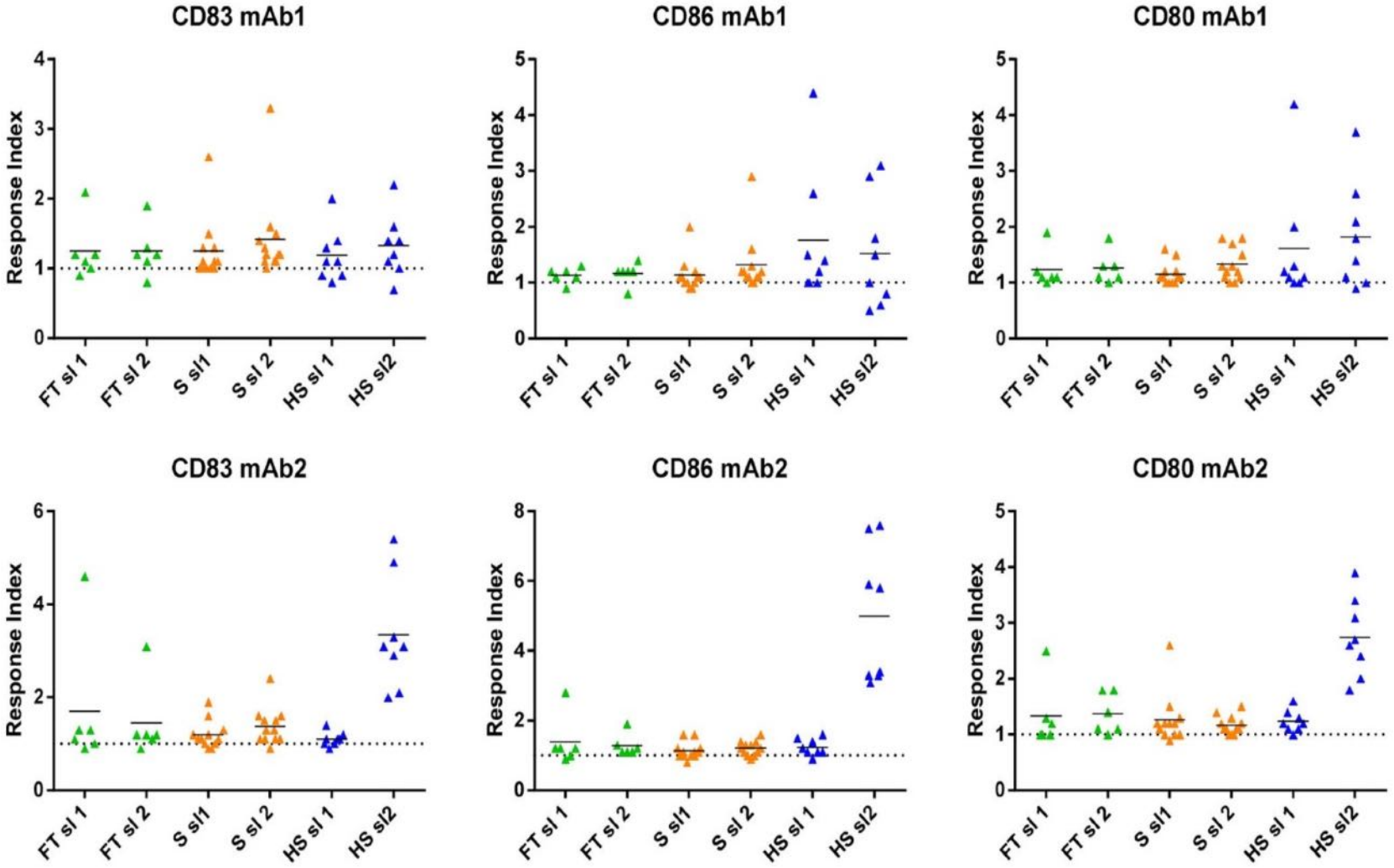
Induction of DC maturation by stressed mAb materials



Response index: response relative to unstressed condition

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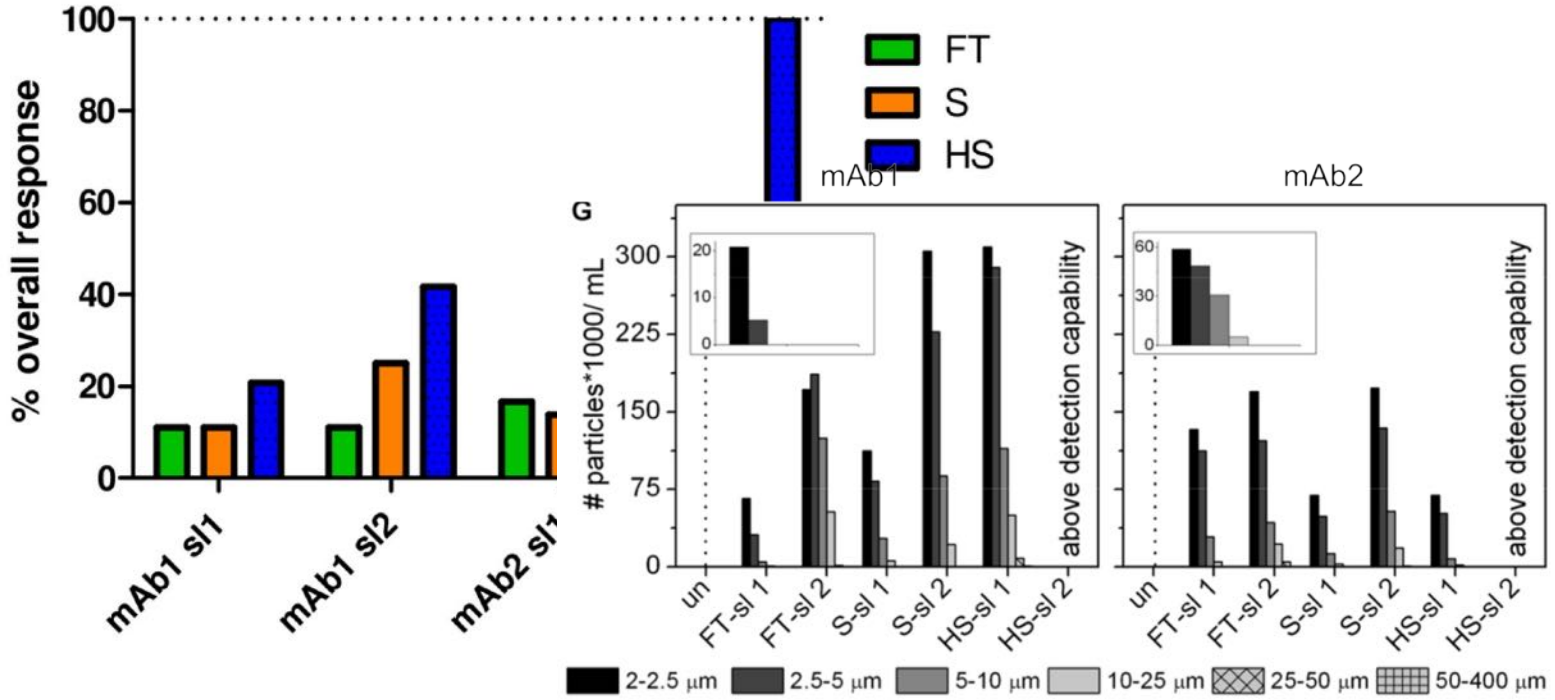
Relative number of positive responder with response index at least 1.5



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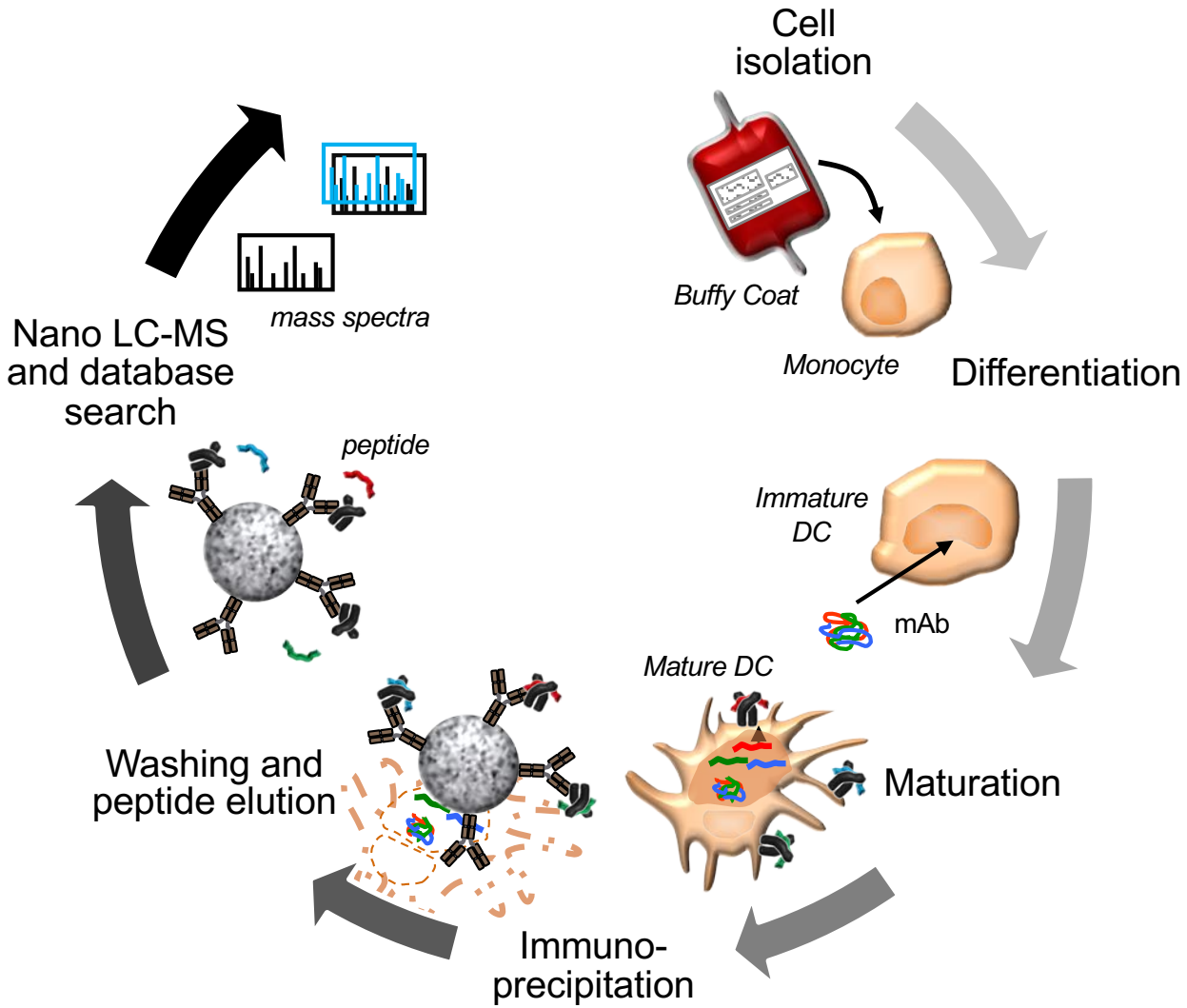
Identification of naturally processed HLA peptides

MHC-associated Peptide Proteomics (MAPPs)



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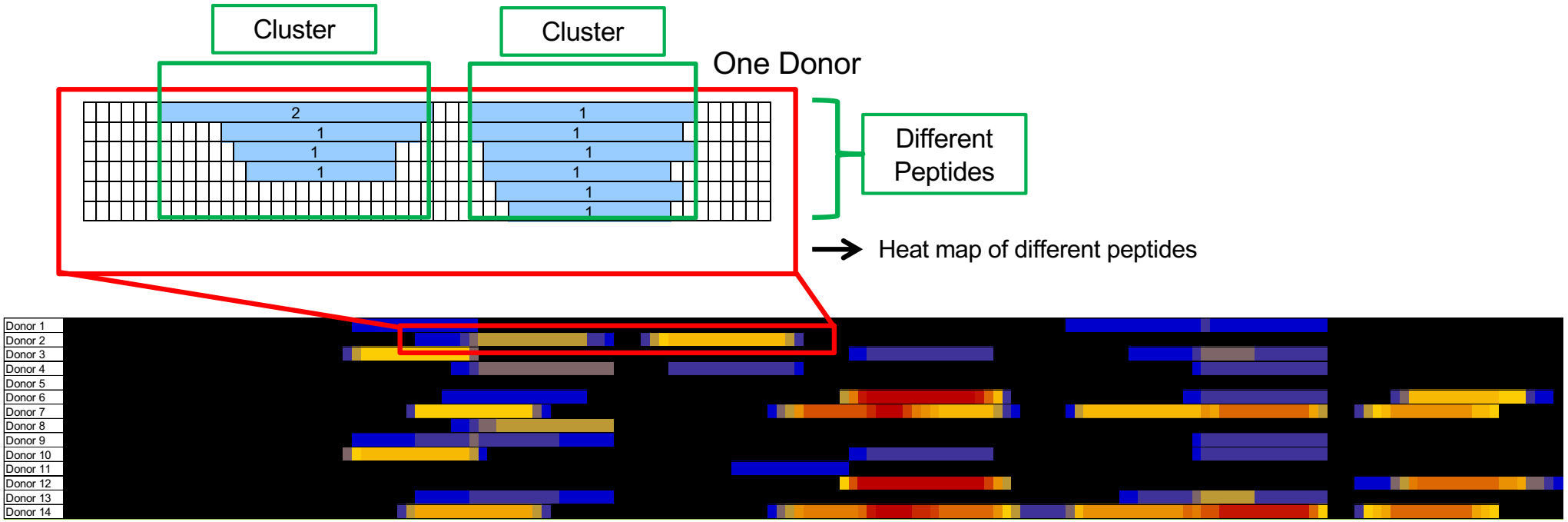
MAPPs

example: clusters in human interferon beta



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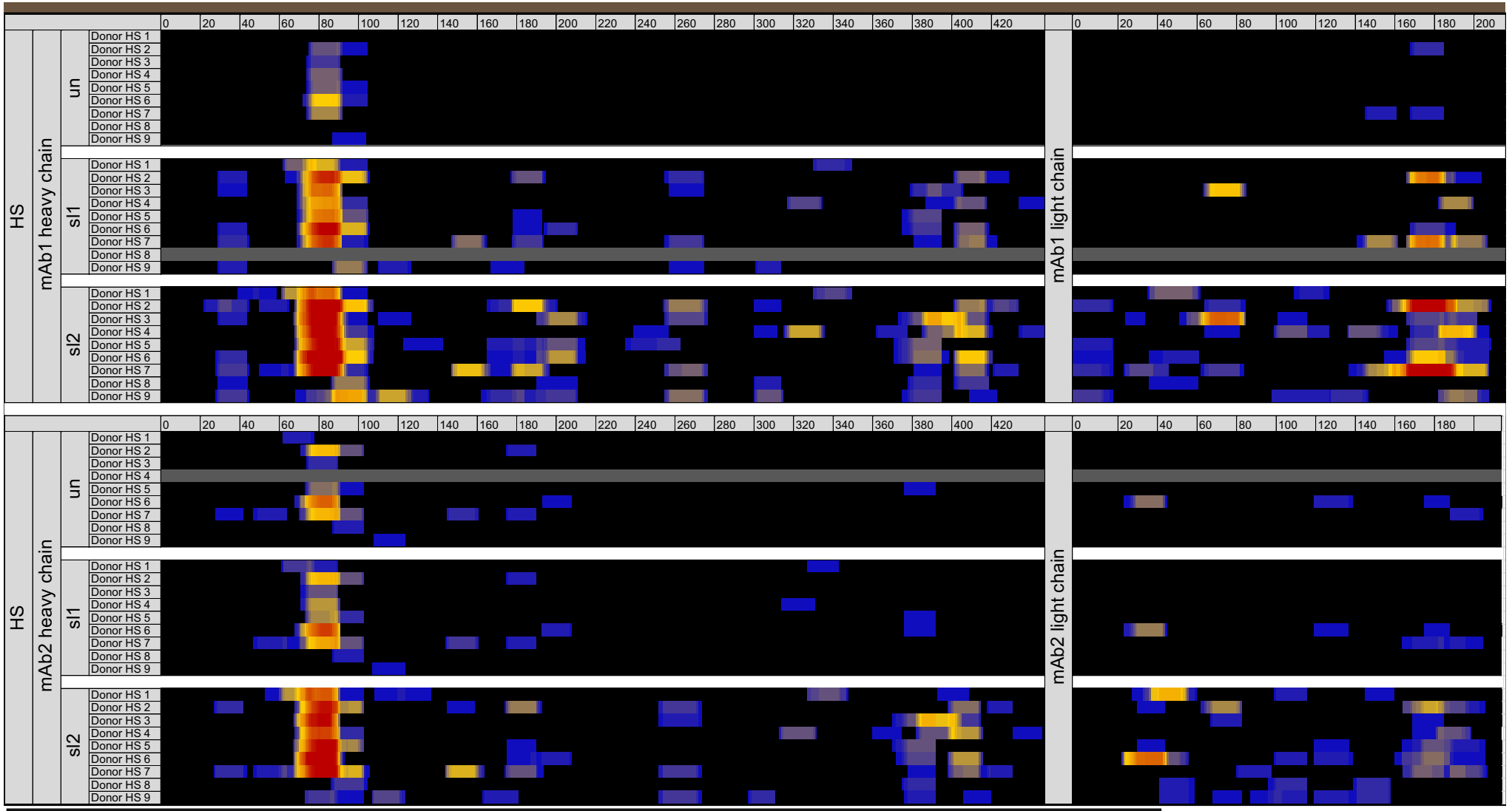
Peptide clusters presented on DCs from heat stressed mAb1 and mAb2



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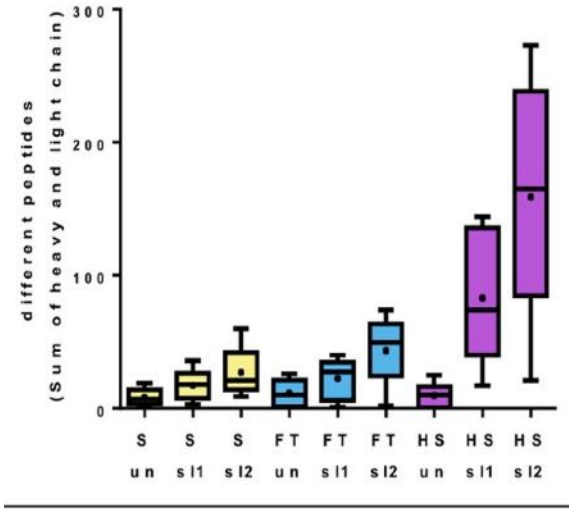
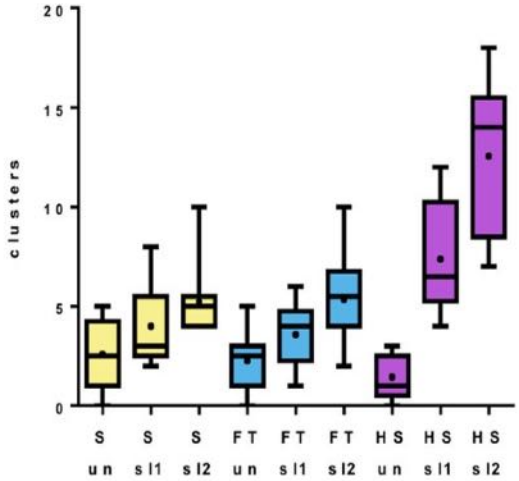
Naturally processed peptides: MAPPs assay

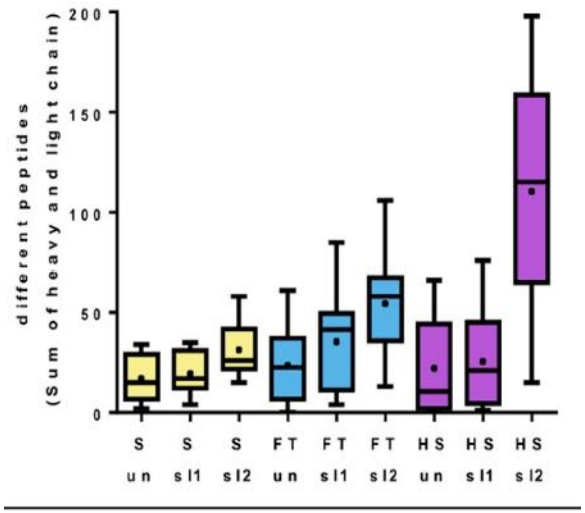
Number of different peptides and clusters

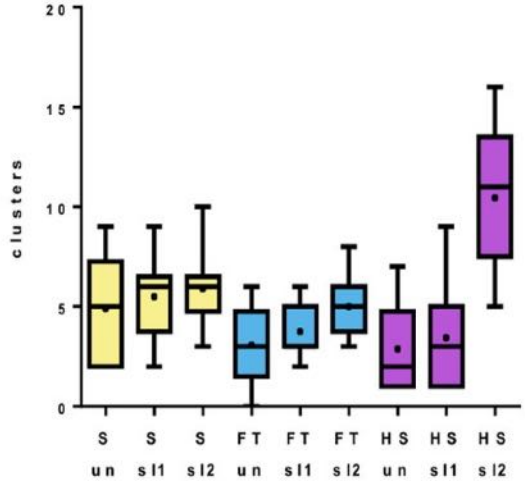
mAb1





mAb2





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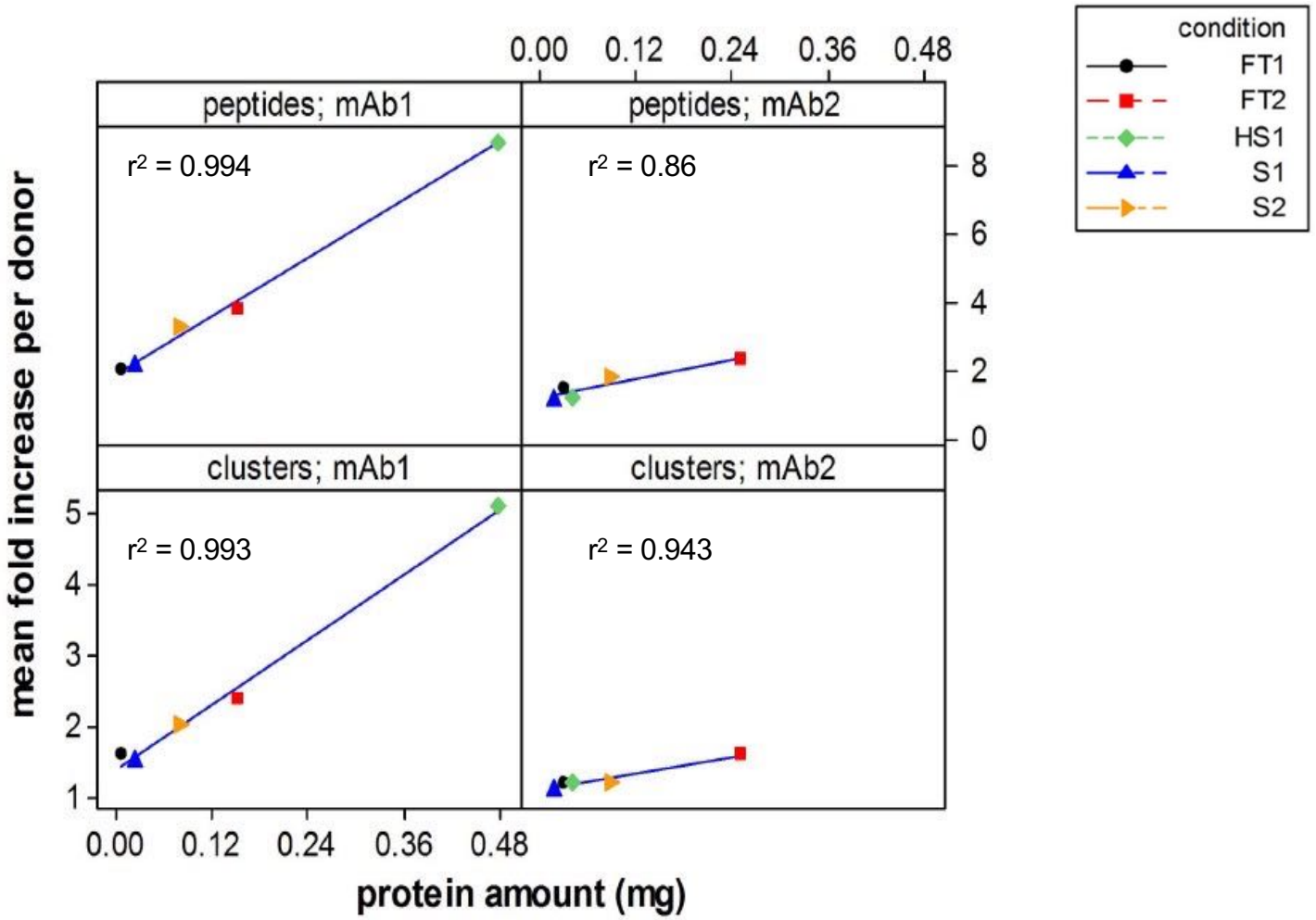
Correlation of peptide presentation and protein amount in particles



Linear regression analyses of increase of HLA-DR associated peptides/clusters as functions of the calculated amount of protein present in subvisible particles.

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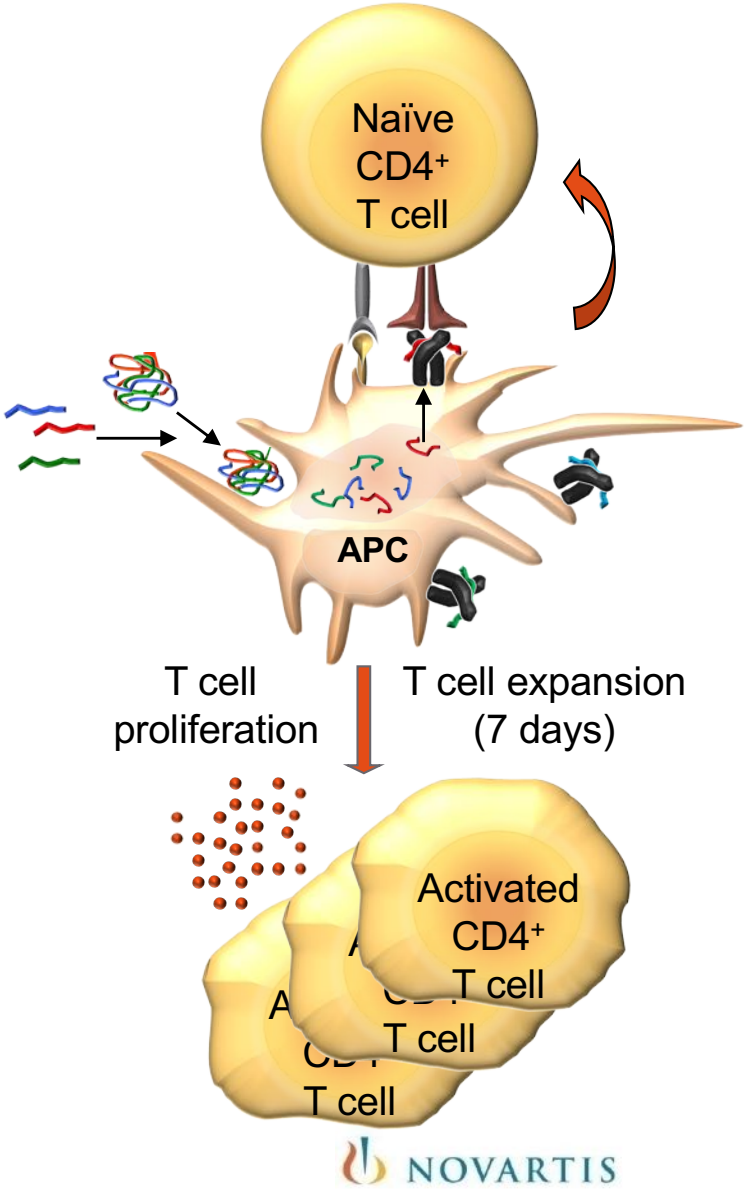
T cell assay

- **Aim:** Link to other studies that show enhanced T cell activation
- PBMCs prepared from buffy coats of healthy human donors by density gradient centrifugation.
- On day 0, challenge with different preparations of biotherapeutics at 200 μg/mL.
- IFN-γ ELISpot after 7 days in culture
- Limited no. of donors tested (8 blood donors tested for FT and 13 different for HS)
- MAb2 not tested due to direct interference in the assay
- Shear stress not tested



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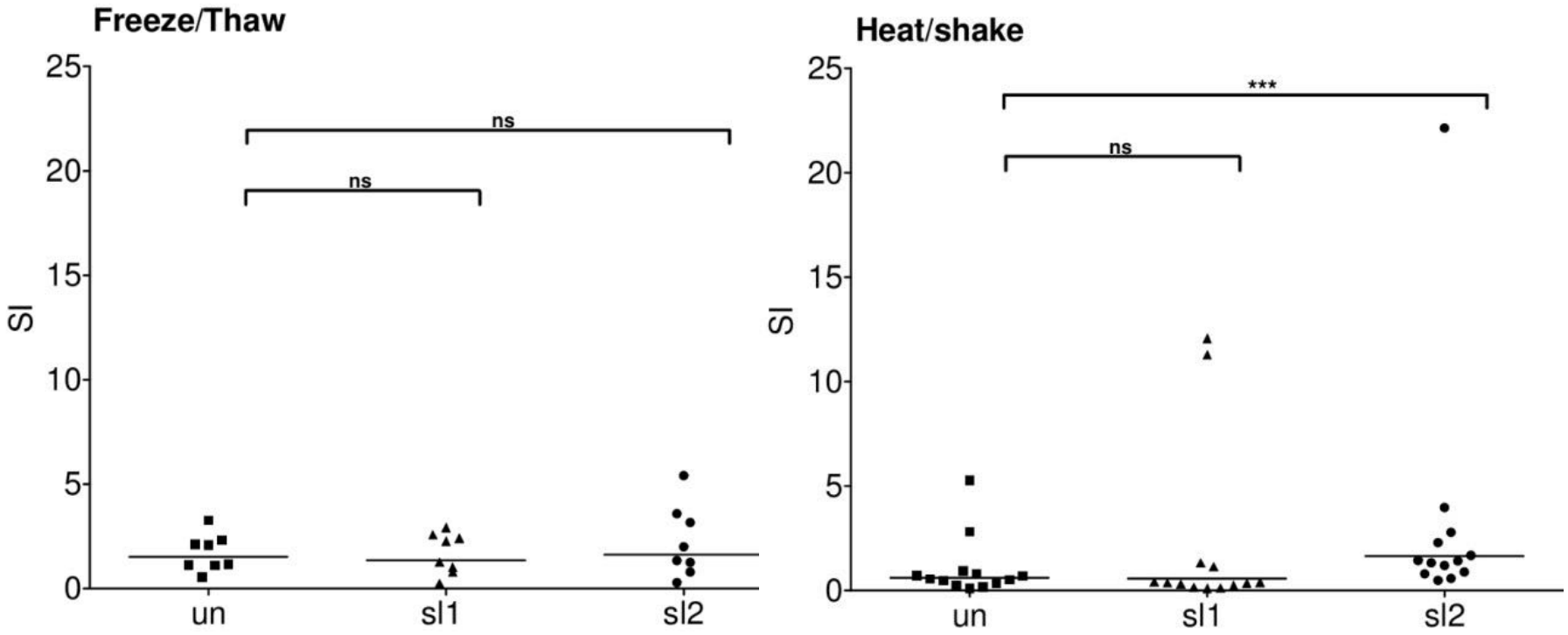
T cell assay results

mAb1 only due to direct interference of mAb2 in T cell assay



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Summary

- Aggregates can induce DC maturation. More particles induce stronger DC maturation in more donors
- Aggregates lead to increased HLA-restricted antigen presentation
- Extent of presentation correlates exceptionally well with calculated amount of protein contained within the subvisible particles
- Aggregates can lead to increased T cell responses
- Thus, highly aggregated proteins are able to induce adaptive immune responses

Summary cont'd

- Aggregates and subvisible particles are present, to a limited extent, in every biopharmaceutical product sold on the market today.
- The aggregation levels typically observed for marketed therapeutic antibodies are much lower than the levels generated for this study.
- To shed further light on the mechanistic involvement of aggregates during induction of immunogenicity additional investigations have been initiated as part of ABIRISK:
  - Additional aggregated monoclonal antibodies (marketed formulations) which are also part of clinical investigations in ABIRISK
  - Advanced assays performed by multiple labs

Anti-Biopharmaceutical Immunization: Prediction and analysis of clinical relevance to minimize the RISK

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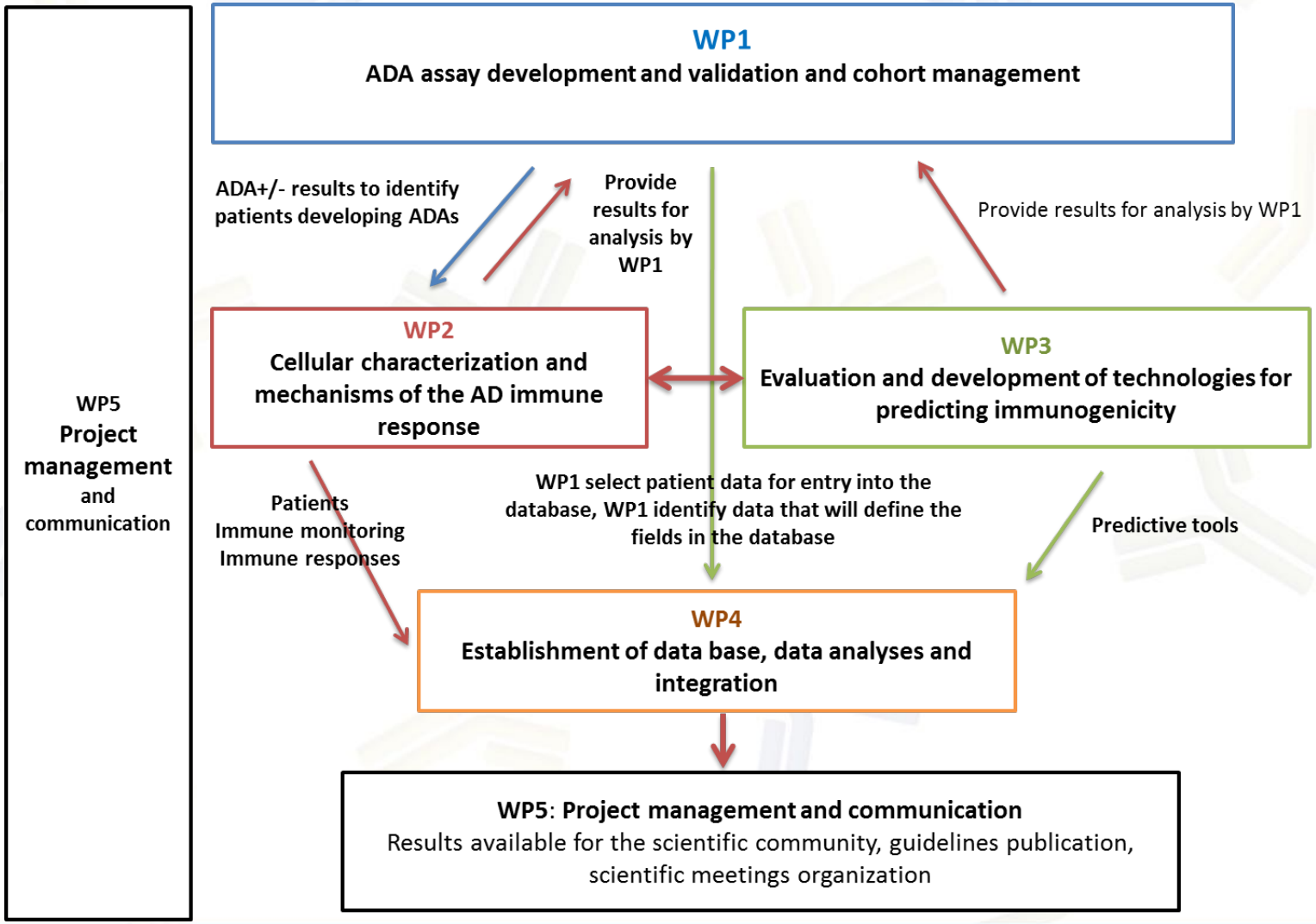

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Evaluation and development of technologies for predicting immunogenicity

Aim 1: Evaluate clinical relevance and gain a greater understanding of technologies of prediction of immunogenicity

Aim 2: Develop and assess novel prediction methods

Aim 3: Assess **effects of aggregation** on immunogenicity

Co-leaders

Bernard Maillere, CEA
Christian Pedersen Ross, Novo Nordisk
Sebastian Spindeldreher, Novartis Pharma

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Aim 3: Effect of aggregates

A sneak preview

- Aggregate generation and characterization
  - Focus on antibodies (rituximab, infliximab, natalizumab, adalimumab)
  - Syringe stress:
    - SS-L1: 3x "up and down“
    - SS-L2: 10x "up and down“
  - Heat stress:
    - HS-L1: 24 h @55 °C
    - HS-L2: 72 h @55°C
  - Physicochemical analytics : SEC, DLS, MFI and Turbidity
- Applied methods
  - MAPPs
  - DC activation assays
  - T cell assay

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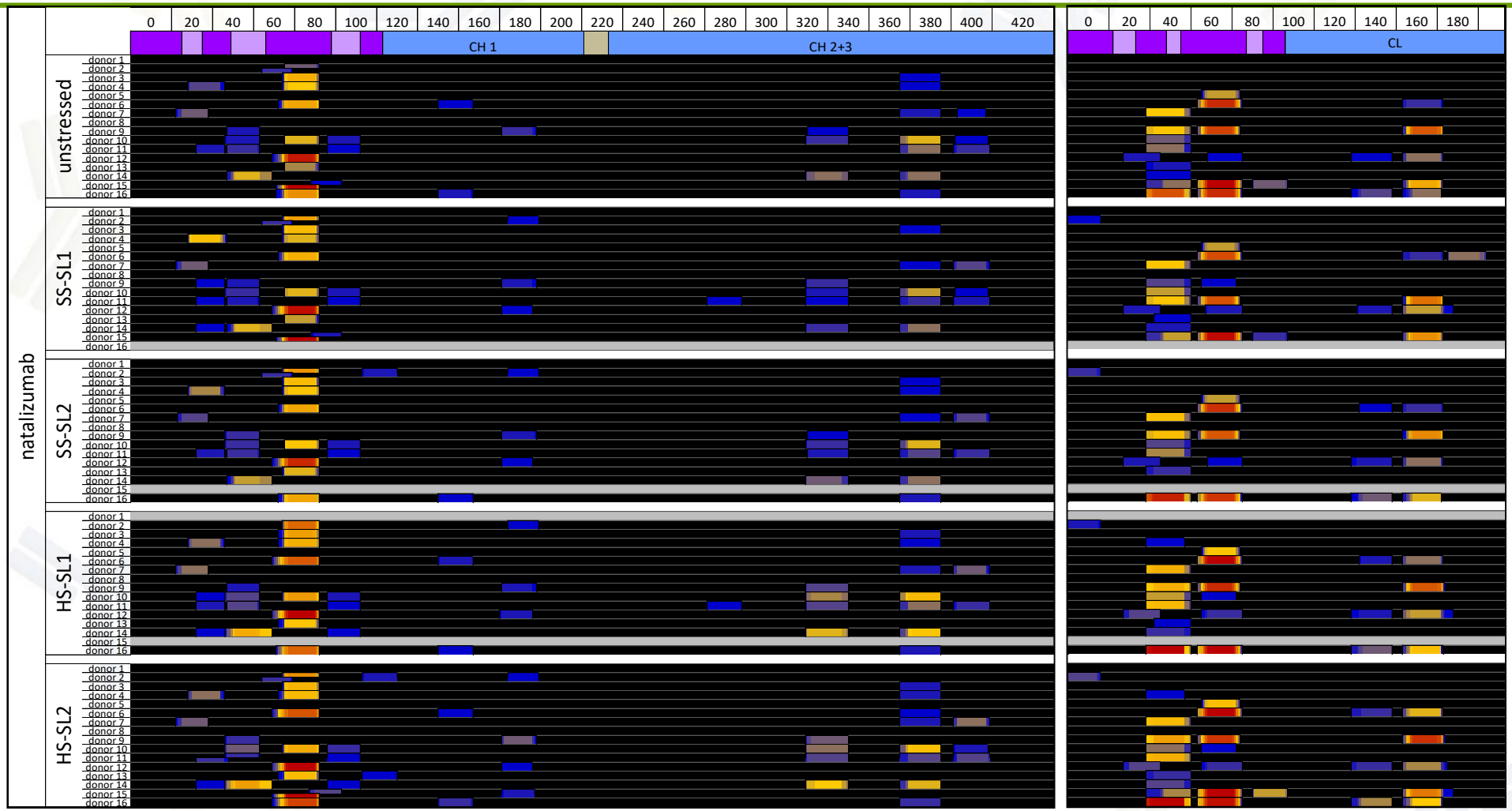
Natalizumab: Changes in peptide presentation



heatmap coloring based on peptide numbers

1	5	10	15	20	25	30	35	40	45	50	59

Legend for sequence coloring:

Constant framework	Hinge
Variable framework	CDR

heatmap coloring based on peptide numbers

1	5	10	15	20	25	30	35	40	45	50	58

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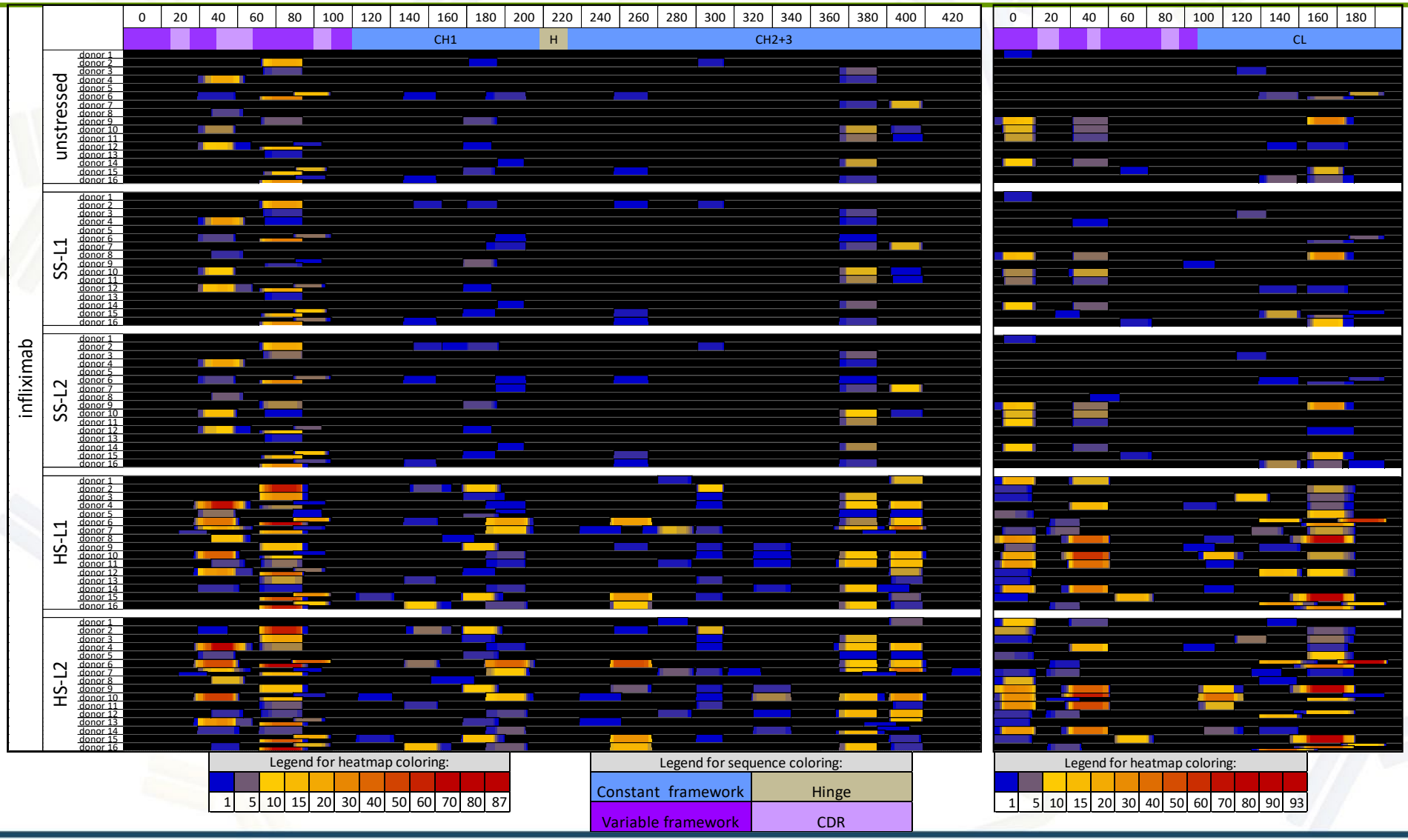
Infliximab: Changes in peptide presentation



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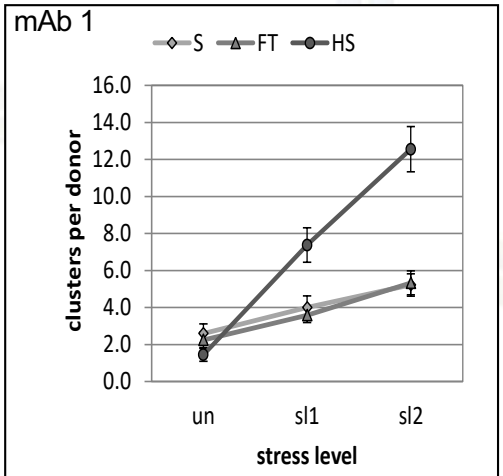
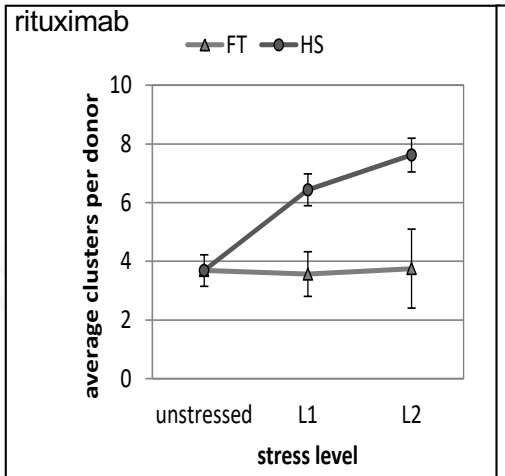
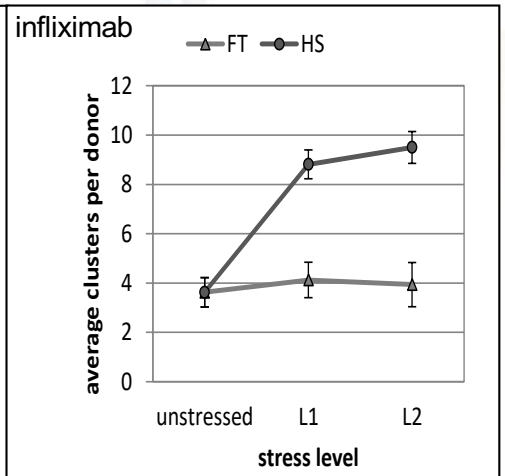
Increased stress leads to increased peptide presentation

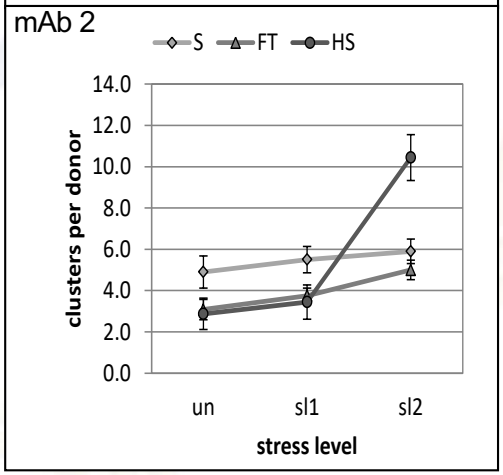
Novartis internal study

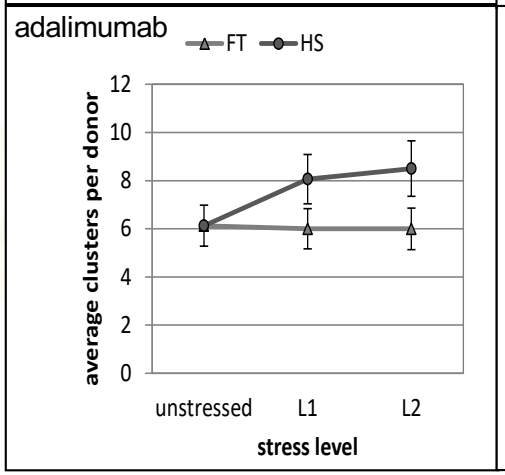
New IMI study

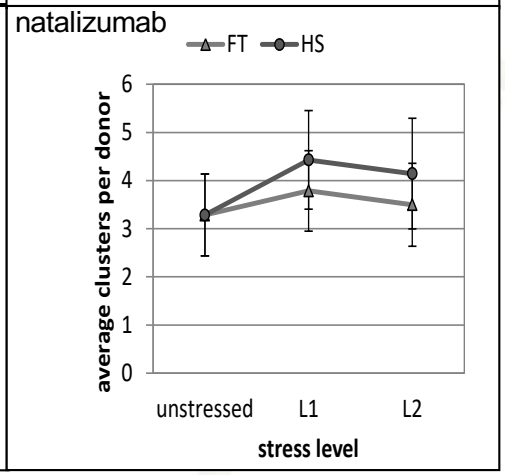












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Conclusion

- MAPPs on 4 marketed antibodies confirmed the findings of the Novartis study
  - The stronger the stress, the more peptides are being presented on HLA class II
  - The baseline presentation as well as the degree of change in peptide presentation depends on the molecule.
- Ongoing:
  - DC activation assays with multiple endpoints:
    - Cell surface activation markers
    - Cytokine secretion
    - Chemokine and cytokine transcription
    - Cell signaling (phosphorylation)
  - Selection of T cell assay setup for aggregate study

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