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Development of an ELISpot Assay for the Assessment of AAV Peptides to Examine Immune Safety

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Life forward

Acknowledgements

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Development of an Enzyme-Linked Immunosorbent Spot Assay for the Assessment of Adeno-Associated Virus Peptides to Examine Immune Safety

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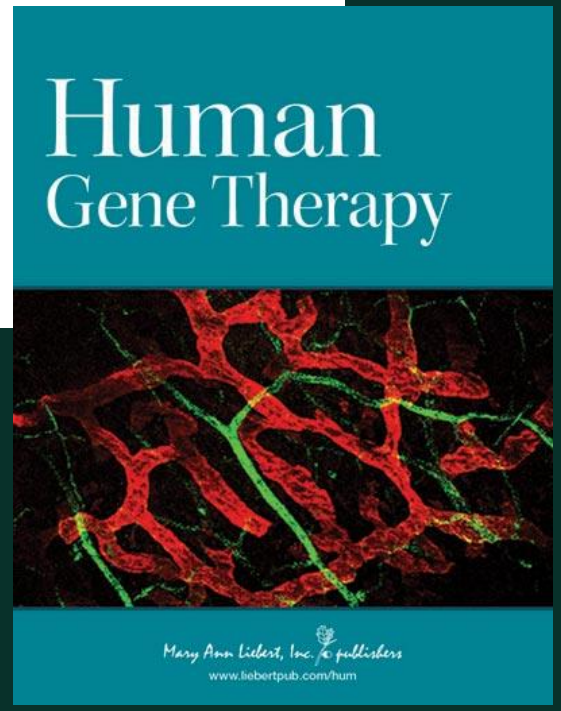

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Outline

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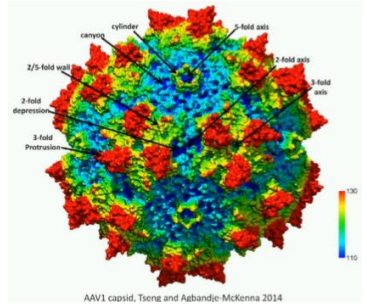
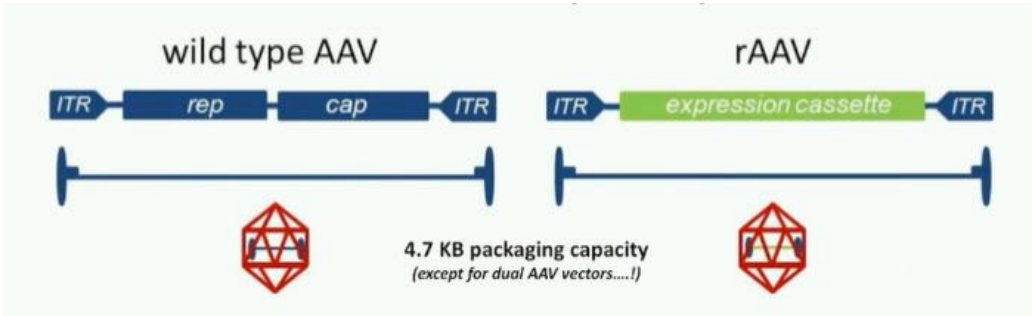
Recombinant AAV (rAAV) Vectors

- rAAV vectors, like wild-type AAVs, are non-replicative
- rAAV capsids are composed of the same proteins (VP1, VP2, VP3) as wild-type AAV
- Selection of capsid serotype can direct transgene expression to targeted tissues
- rAAV have demonstrated sustained transgene expression in specific tissues (lung, liver, muscle, CNS, etc.)
- Pre-existing immune responses to AAV can interfere with rAAV transduction, safety, and efficacy





Sanford Boye, Director of Vecotr Core, Powell Gene Therapy Center University of Florida, Chief Technology Officer Atsena Therapeutics. **Workshop B: Translating Next Generation Vectors for the Eye. 2021 Gene Therapy for Ophthalmic Disorders**

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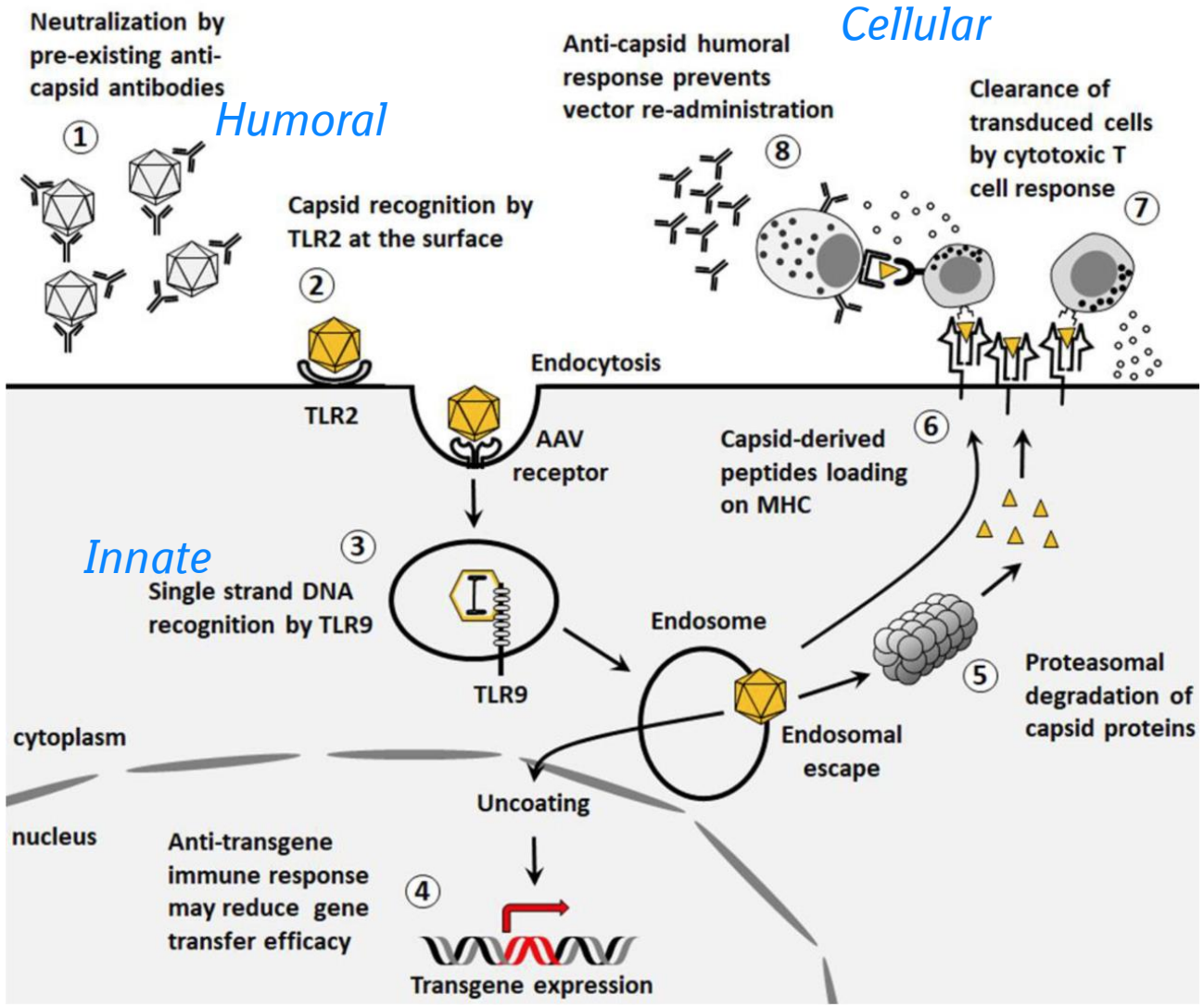
Immunogenicity/ Immune safety overview



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Adapted from Ronzitti, G., *et al* (2020) *Front Immunol*

Impact of Immunogenicity on AAV Gene Therapies

- **Humoral responses:**
  - anti-AAV anitbodies can reduce efficacy
    - Pre-existing anti-AAV antibodies might prevent the use of AAV gene therapy[1]
    - Neutralizing antibodies may prevent repeat vector administration[2]
    - Depletion of anti-AAV antibodies can limit transduction inhibitors[3]

- **Cellular responses:**
  - T cell response to capsid or transgene could result in significant decline in pharmacology
    - High unmethylated CpG content in vector = high CTL response and low persistence[4]
    - Anti-capsid CD8 T cell response led to clearance of transduced target cells[5]

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[1]Mendell, J.R., *et al* (2022) *Mol Ther Methods Clin Dev*
[2]Nathwani, A.C., *et al* (2014) *N Engl J Med*
[3]Orlowski, A., *et al* (2020) *Mol Ther Methods Clin Dev*
[4]Wright, J.F. (2020) *Mol Ther*
[5]Mingozzi, F., *et al* (2007) *Nat Med*

Assessment of Immune Responses to AAV Gene Therapies

- AAV therapies can affect innate, humoral, and cellular immune responses
- More traditional assays, like qPCR, anti-drug antibody assays, and neutralizing antibody assays can be employed to assess some bioanalysis and immunogenicity topics
- Unique assays to AAV therapies include T cell assessments, typically done by ELISpot
- The AAV field continues to evolve, and the safety and immunogenicity strategies will be continually re-evaluated to meet new understanding

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Assessment of T cell Responses to AAV Gene Therapies by ELISpot

- **AAV-specific T cell responses:**
  - Clearance of transduced cells
  - Loss of transgene expression
  - Induction of neutralizing antibodies to prevent subsequent vector administration
- **ELISpot:**
  - Sensitive and reproducible assay
  - Assess T cell activation in response to AAV gene therapies
    - AAV8 and AAV9 capsid-derived peptides
    - IL-2 and IFNγ
- **ELISpot will refine our understanding of the T cell response to AAV, which could aid in improvements to vector design, patient safety, and treatment efficacy.**

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T cell ELISpot Assay

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Advantages of ELISpot

ELISpot	Flow Cytometry
Commonly measures only one cytokine – can multiplex with Fluorospot and multi-color ELISpot	Can measure multiple cytokines
Information on specific cell type (T-cell subsets, NK cells), only when pre-separated	Information on specific cell type producing cytokines intracellularly, at the single cell level
Relatively easy to qualify/validate	Challenging to validate – more commonly used for exploratory
Wide quantitative range and unique sensitivity at the single-cell level to detect rare events	Semi-quantitative; large number of events or in vitro cellular expansion needed to detect rare populations
Detects cells that actively secrete cytokine in response to antigen	Detects cells that produce cytokine intracellularly
Detects responses under relatively more physiologically relevant conditions/stimuli	Requires addition of Brefeldin A and/or Monensin to block cytokine secretion; toxic to cells

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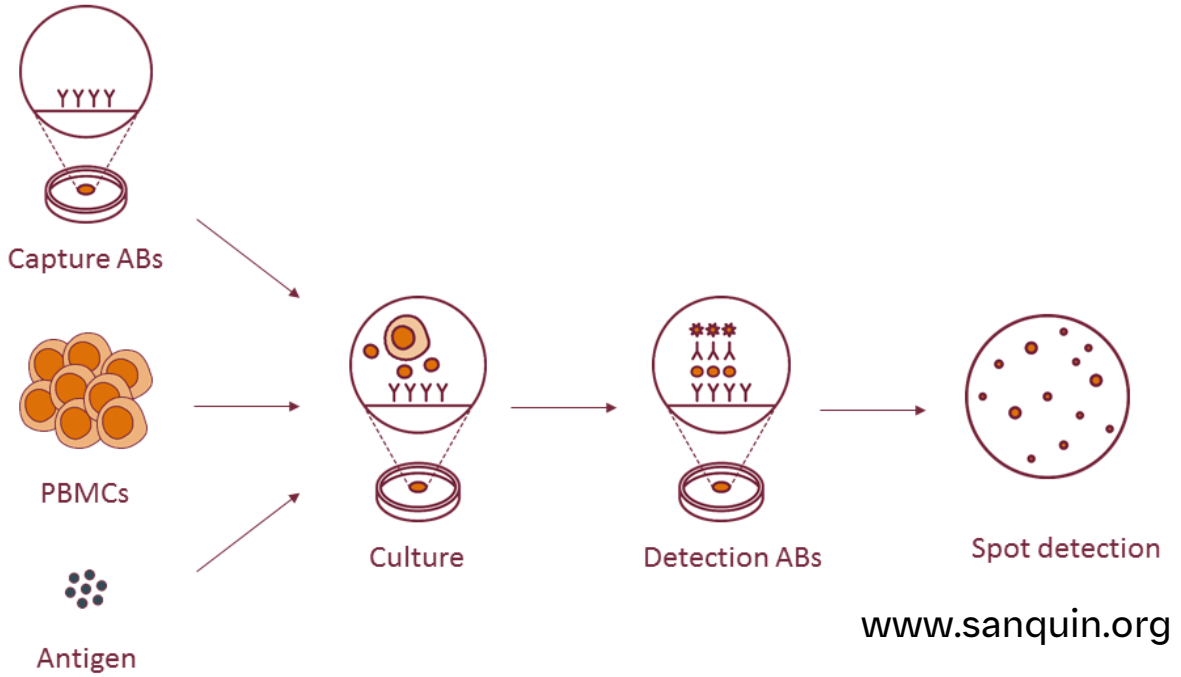
Adapted from IQ Consortium

ELISpot Assay for T cell Responses to AAV

- Assess anti-capsid T cell responses
- IFNγ and IL-2, to give a broader view of the anti-AAV T cell response
- PBMCs
- Overlapping peptide pools to AAV capsid
  - Individual peptides could also be screened for further elucidation of immunogenic epitopes



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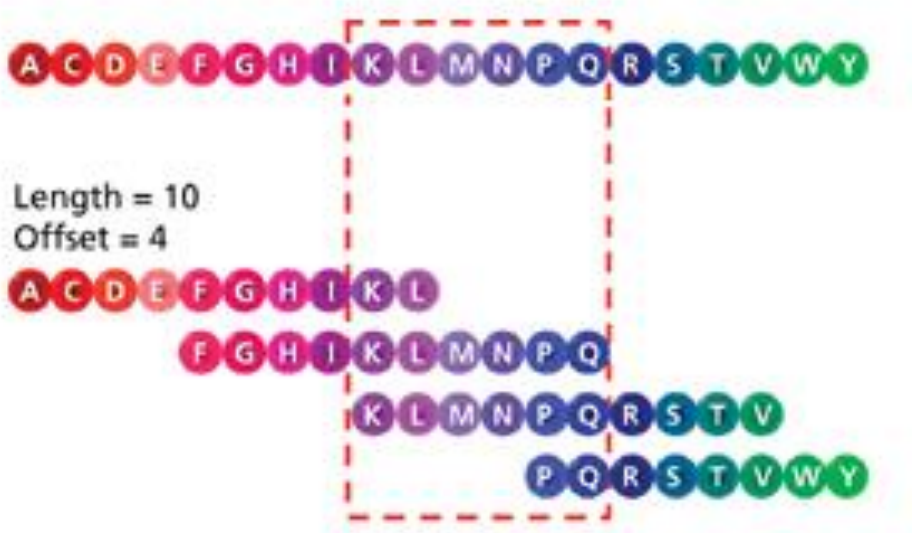
ELISpot Considerations

Peptide pools

- Synthetic peptides derived from AAV capsid
- Individual peptides are of 15 amino acids (aa) in length, with overlapping sequencing
- Overlap is 10aa = offset is 5aa
- Arranged in 16 pools



Overlapping Peptide Library example
adapted from www.ProImmune.com

Positive Controls

- PBMCs from untreated donors, stimulated with anti-CD3 and anti-CD28 / common antigens CEF/CEFT (CMV, EBV, Influenza, Tetanus)

Other Considerations

- Viability and recovery of frozen PBMCs
- Positive response defined as above the LOD; high response defined as 2x LOD (per individual donor)

- Pre-existing T cell responses to AAV capsids are not unexpected

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T cell Response to AAV8 and AAV9

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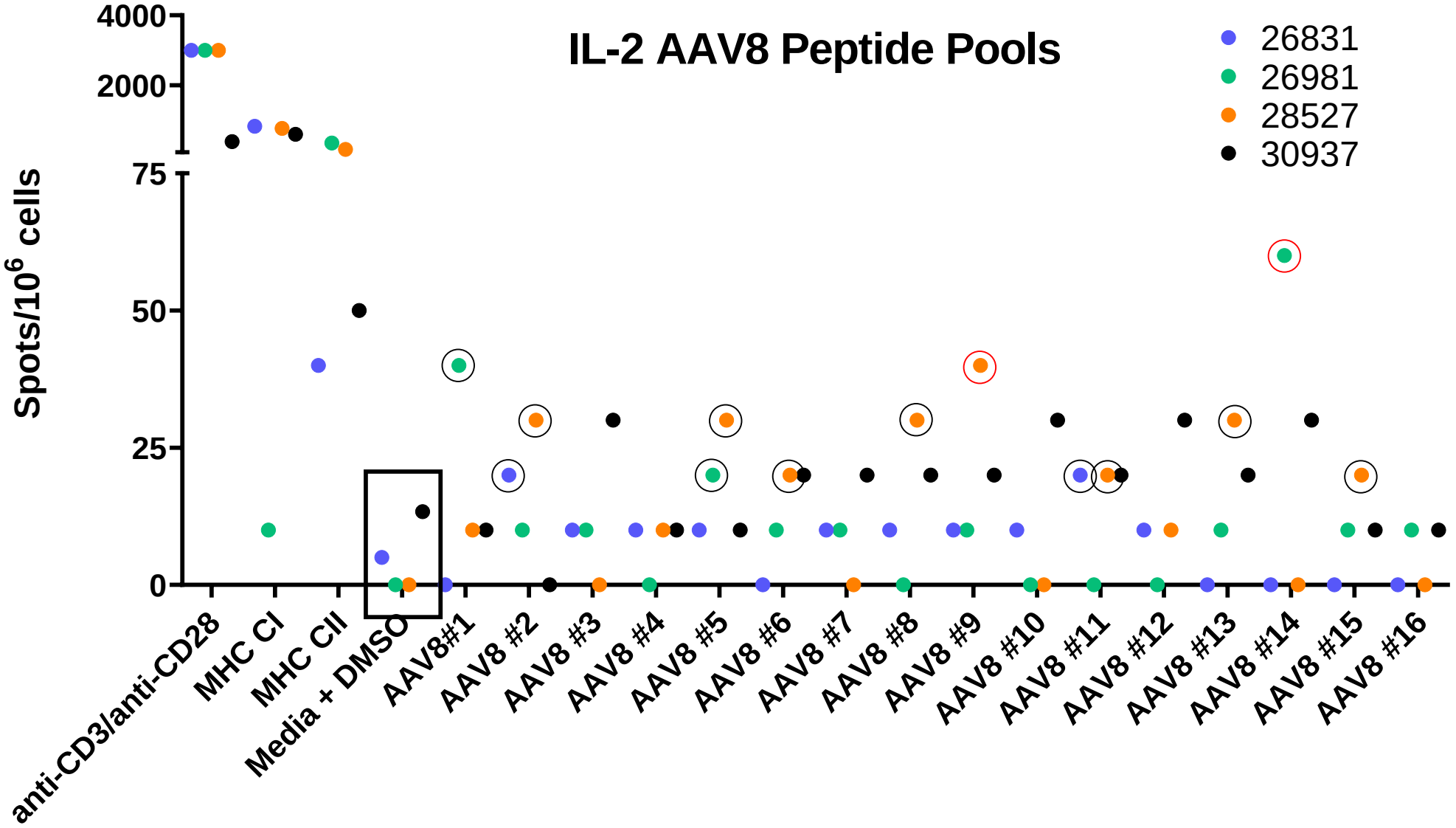

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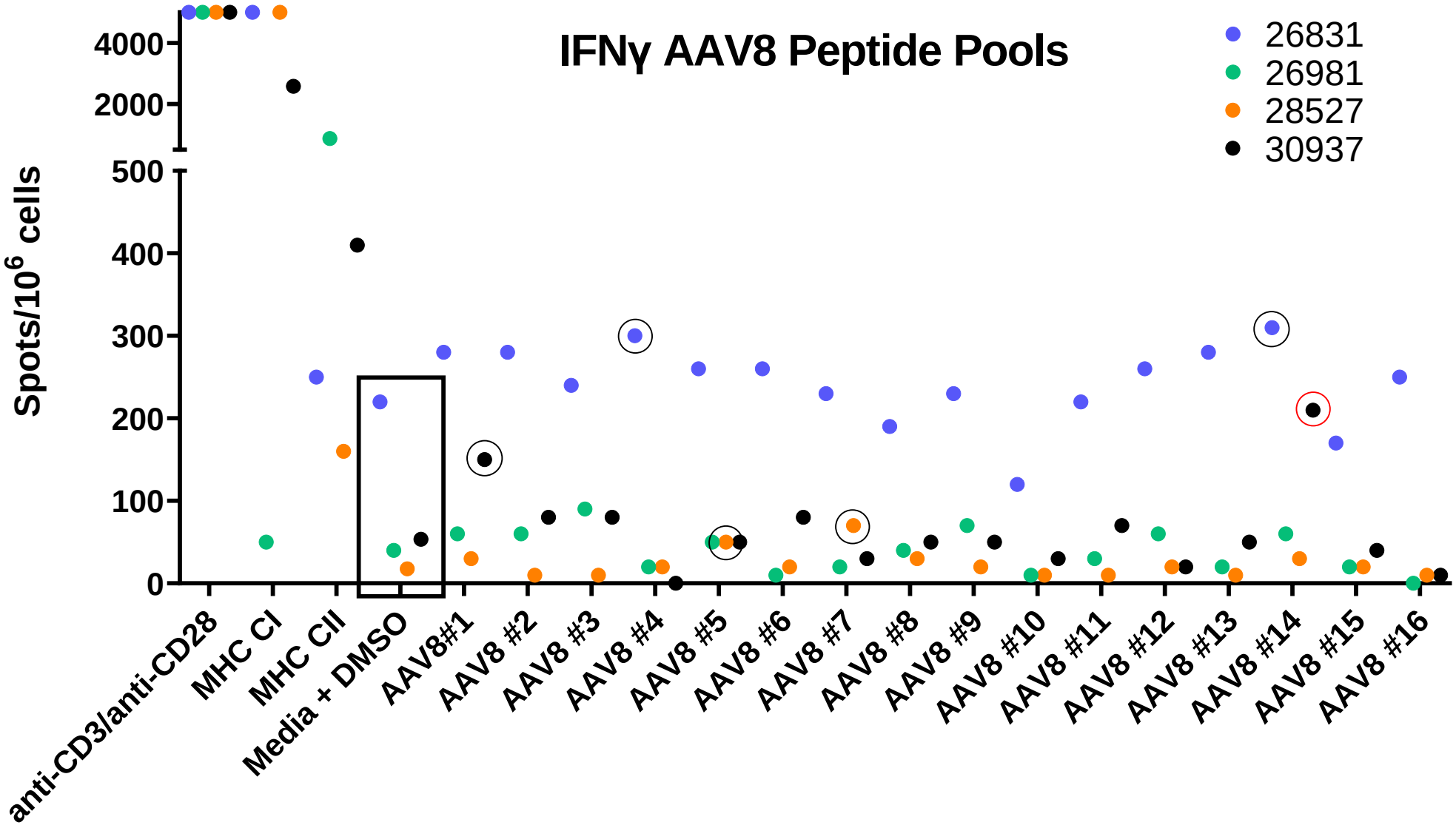

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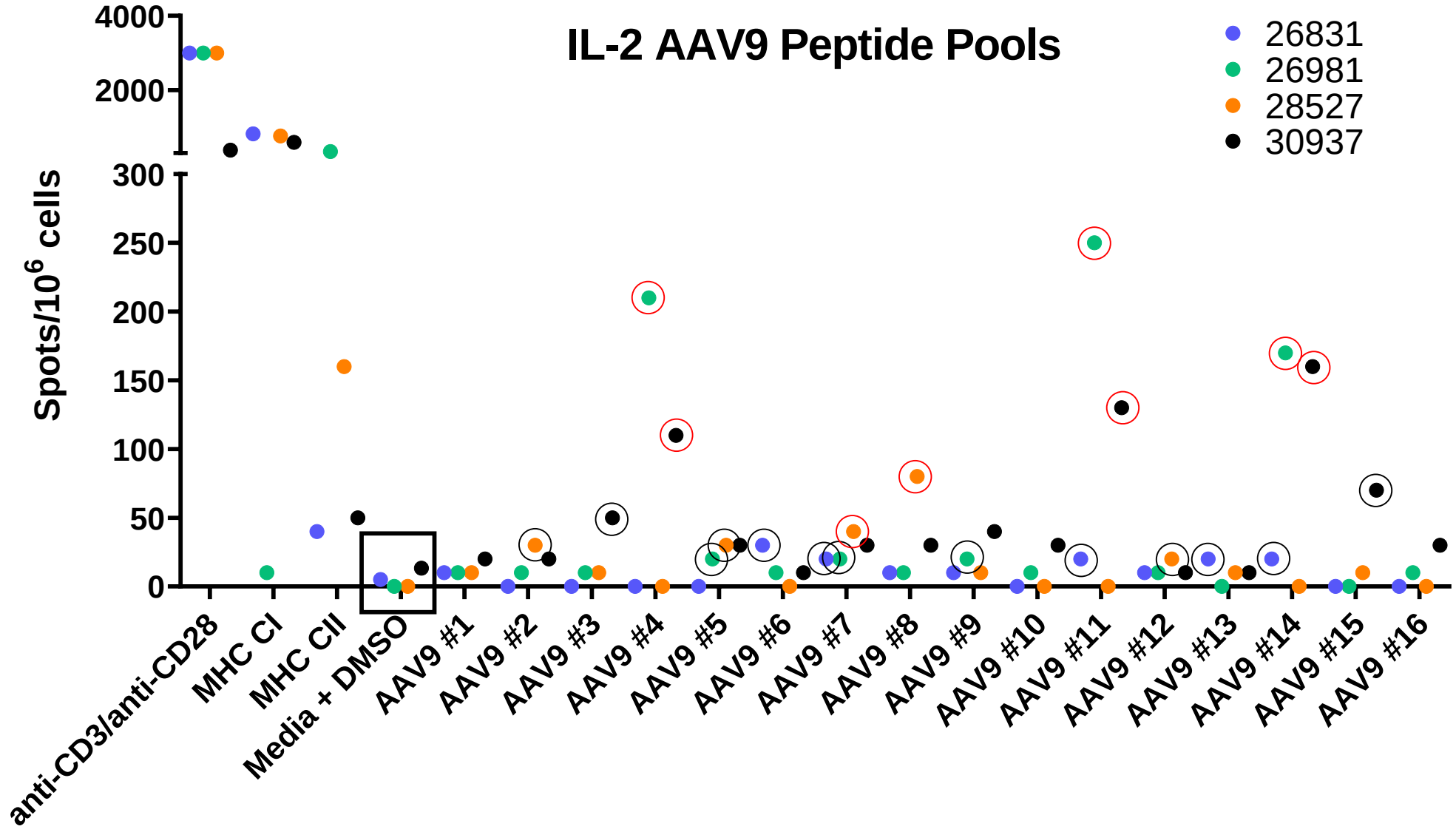

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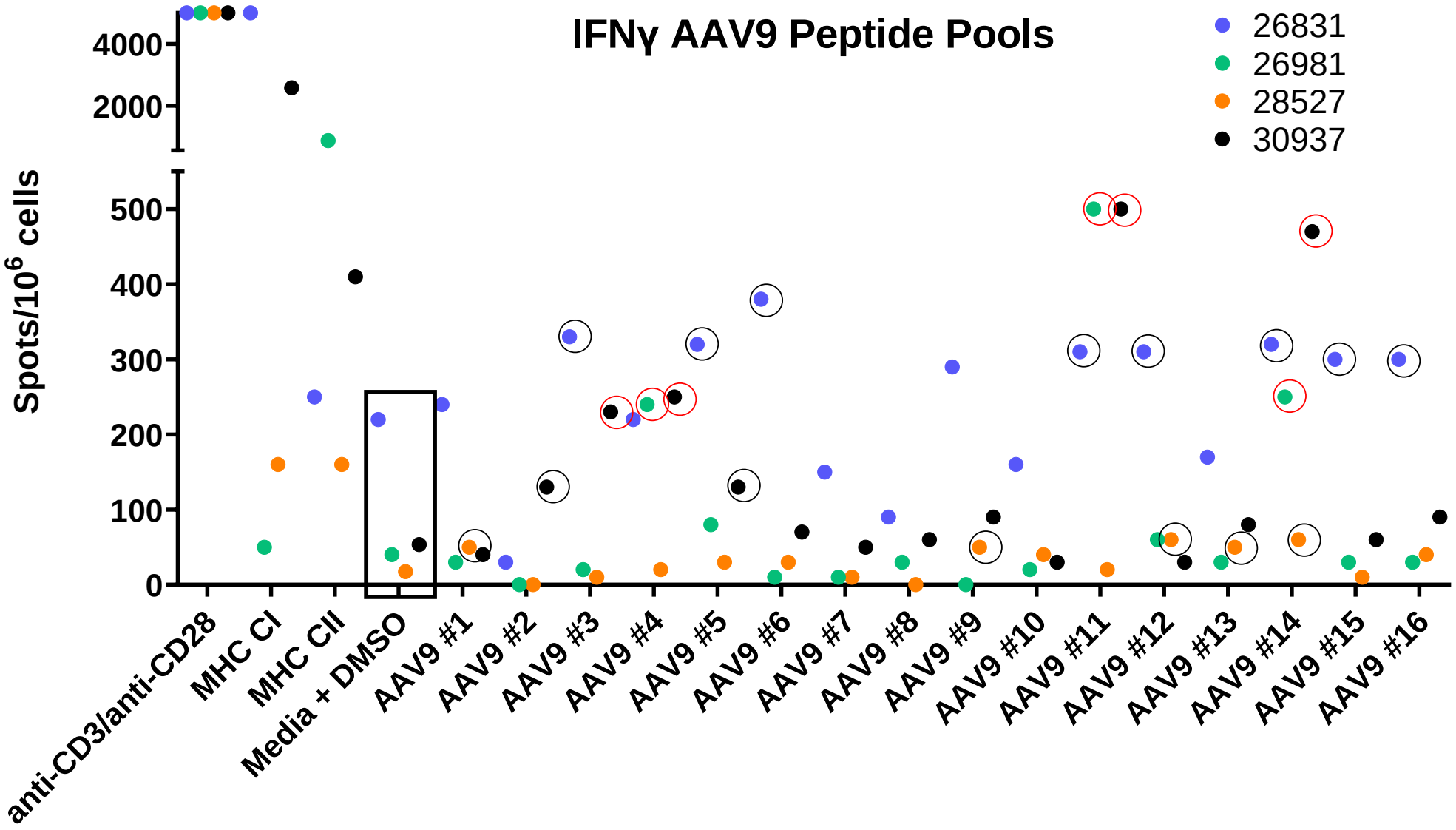

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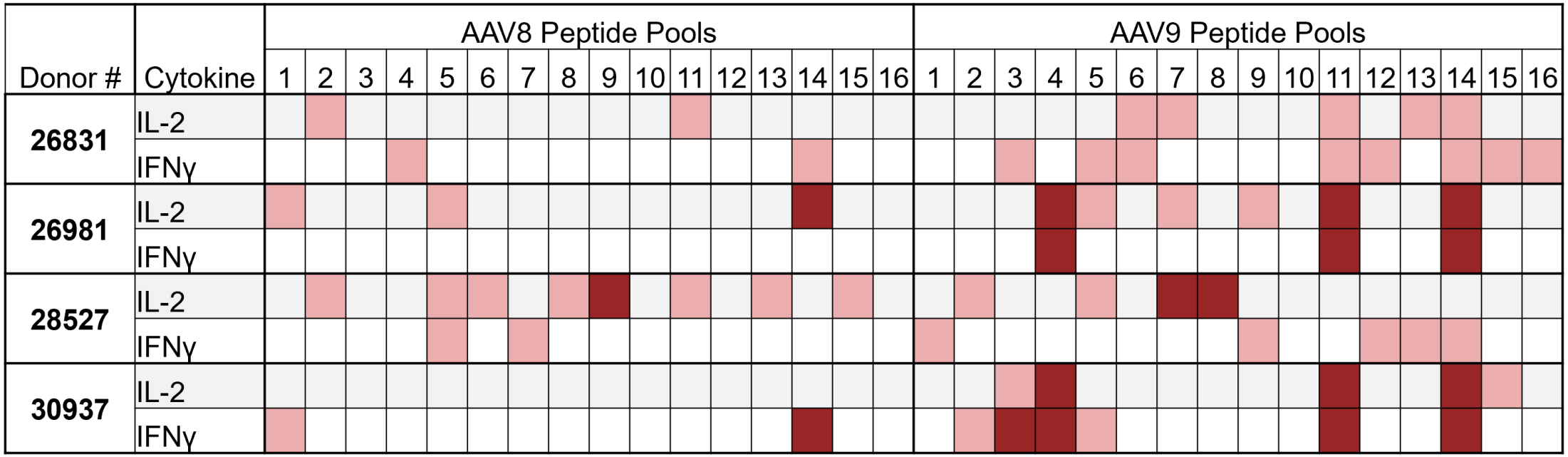
Summary of T cell responses (IL-2 and IFNγ) to AAV8 and AAV9 Peptide Pools



	IL-2	IFNγ	Total
AAV8	13 (2)	6 (1)	19 (3)
AAV9	21 (8)	22 (7)	43 (15)
Total	34 (10)	28 (8)	62 (18)

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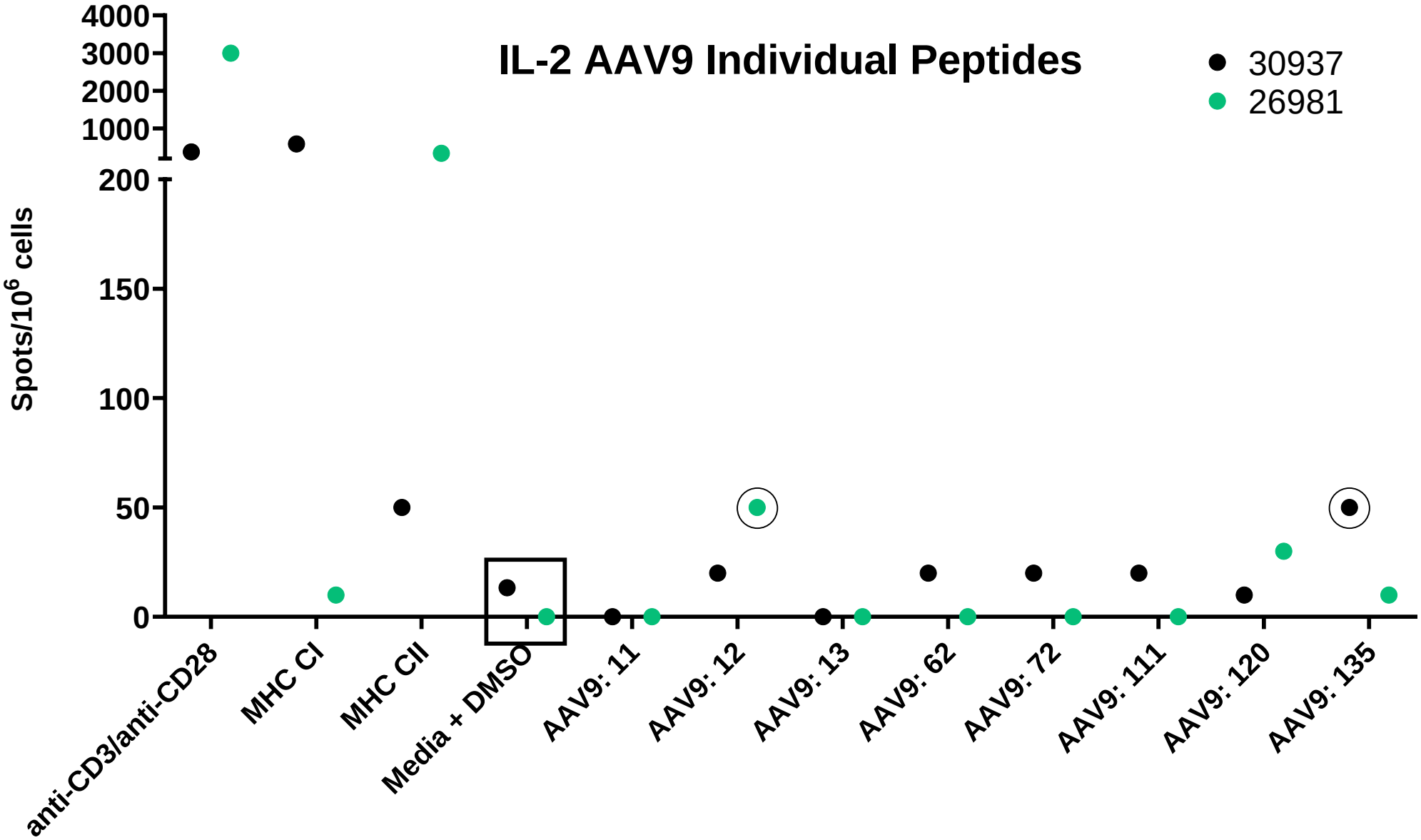

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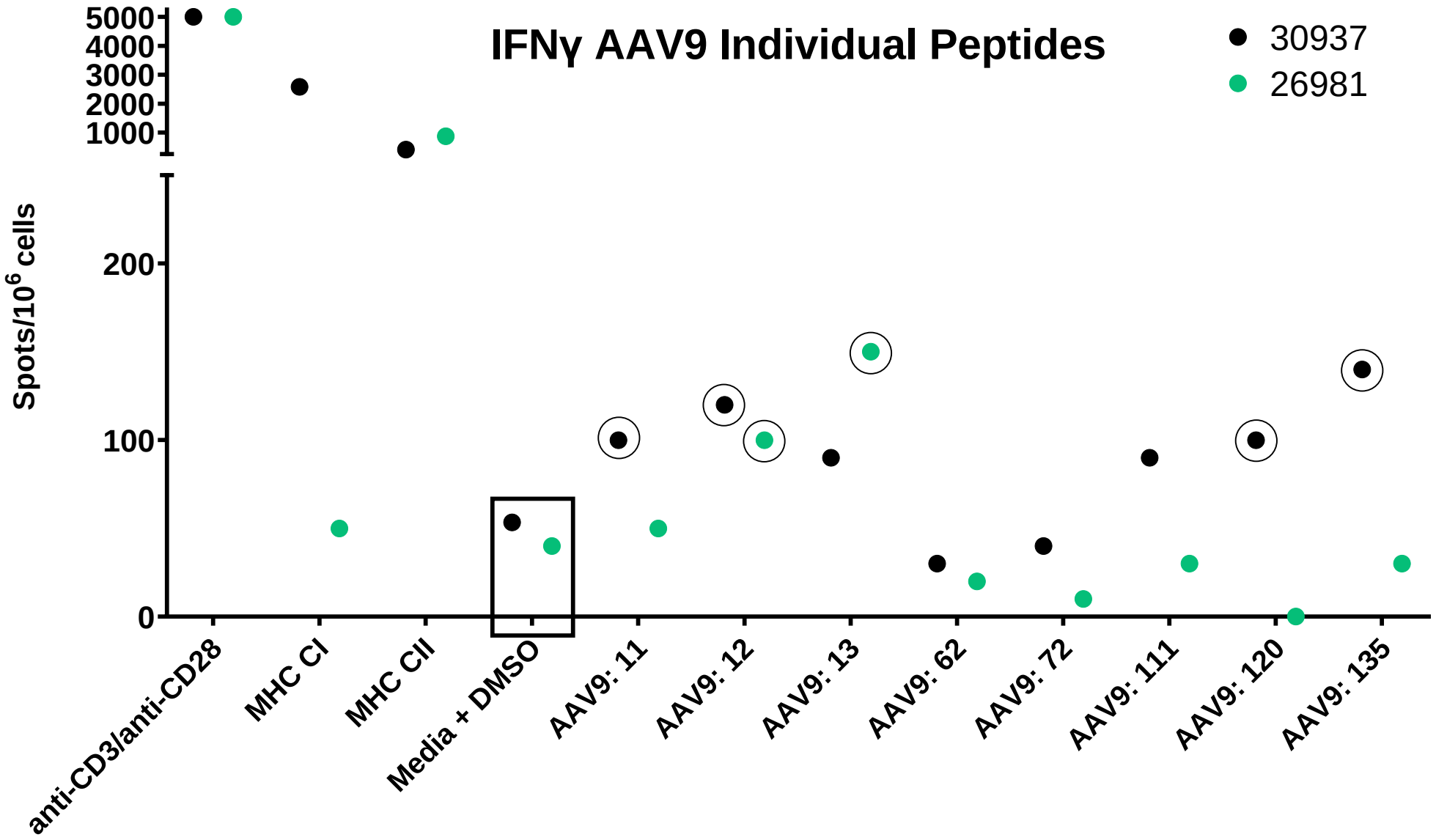

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Summary of T cell Responses to AAV9 Peptides

T cell ELISpot

- Both donors responded to AAV9 peptide 12, and either peptide 11 or peptide 13 with an IFNγ response
- One donor also responded to AAV9 peptide 12 with and IL-2 response

- Peptides 11, 12, and 13 overlap across a 25 a.a. sequence
- Confirmed two known epitopes:
  - MHC I 9-mer (KYLGPGNGL)*
  - MHC II 15-mer (LPGYKYLGPGNGLDK)**
- Predicted one novel epitope:
  - 15-mer (GNGLDKGEPVNAADA)

AAV9 Peptides

11: VLPGYKYLGPGNGLD

12: KYLGPGNGLDKGEPV

13: GNGLDKGEPVNAADA

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*Bing, SJ, *et al* (2022) *Mol Ther Methods Clin Dev*

** Brito-Sierra CA, *et al* (2022) *Front Immunol*

Summary

- Challenge: De-risking immune responses to AAV
- Value Added: Better understanding of AAV-specific T Cell responses

- Main Findings:
  - Both AAV peptide pools and individual peptides elicited a T cell response
    - Variable responses (specificity and strength) across donors and peptides
    - More IL-2 and IFNγ responses to the AAV9 peptide pools
  - Repeated IL-2/IFNγ responses to AAV9 individual peptides highlighted a 25 a.a. region in the capsid
    - A known MHC Class I epitope, a known MHC Class II epitope
    - A novel MHC Class II 15-mer

ELISpot description of the T cell response to AAV could aid improvements to patient safety and treatment efficacy

- Pre-screening and monitoring of developing T cell response
- Improved vector design

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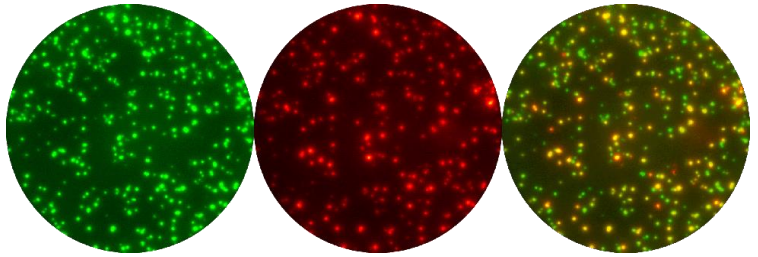
Future Directions and Challenges

- Responses across HLAs could vary
- Capsids could potentially be engineered to exclude known epitopes
- ELISpot can be used for other viral programs, enzyme therapies, protein cargo
- Translate to NHP and other animal models
- Monitoring T Cell Response to treatment
  - Humans and NHPs
- Can be multiplexed to examine a broader T cell response while limiting sample demand
  - Fluorospot: Multiplex allows detection of multiple cytokines e.g. IFNγ, TNF-α, Granzyme B



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