

# Application of systems biology to identify predictors of HIV vaccine immunogenicity

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# Overview

- **Objective**: identify innate immune response genes that are associated with enhanced HIV vaccine immunogenicity
- **Approach**: Seek correlations between expression profiles (microarray data) and functional T cell assays
  - *Human studies*: HVTN 071 subjects (trivalent MRKAd5 gag/pol/nef)
  - *Non-human primate (NHP) studies*: SIV gag protein + TLR ligand adjuvants
- *See Alan Aderem's talk for an overview of his lab's Systems Immunology efforts (Today, 5:40pm, Room 2.40)*

## Human studies: HVTN 071 Trial

*See Erica Andersen-Nissen's talk for more detail (#3– this session)*

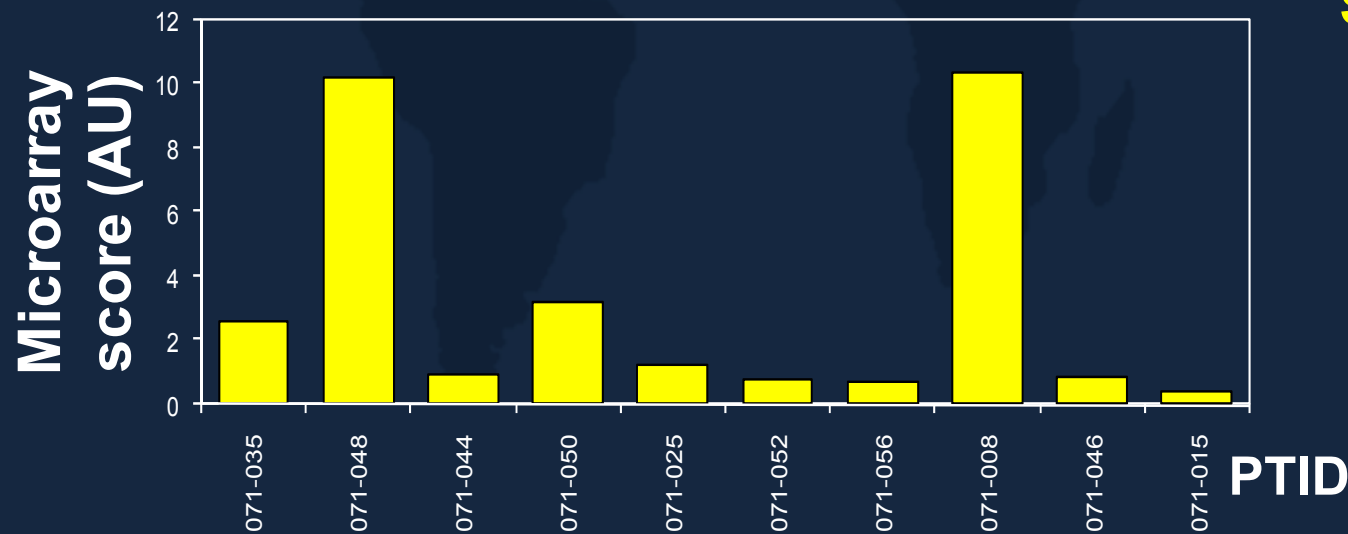
- 10 subjects vaccinated with trivalent MRKAd5 HIV-1 gag/pol/nef from the Step trial  
(Erica Andersen-Nissen, Julie McElrath, FHCRC)
- Subjects had varying pre-vaccination immunity to vaccine vector (Ad5)
- Microarray profiling PBMC innate immune responses 6, 24, 72, AND 168hr after primary vaccination (ISB)
  - Affymetrix GeneChip® Human Exon 1.0 ST Array
- Intracellular Cytokine Staining (ICS) profiling HIV-specific T cell responses (Erica Andersen-Nissen, Julie McElrath, FHCRC)

# Objectives

- Expression signatures for pre-existing immunity to vector (Ad5):
  - Can Ad5<sup>+</sup> subjects be predicted directly from microarray analysis of vaccine response?
  - What is the innate immune gene expression signature for Ad5<sup>+</sup> subjects?
- Expression signatures for vaccine-induced HIV-specific CD8<sup>+</sup> T cell polyfunctionality

# Identifying Ad5<sup>+</sup> subjects by microarray analysis

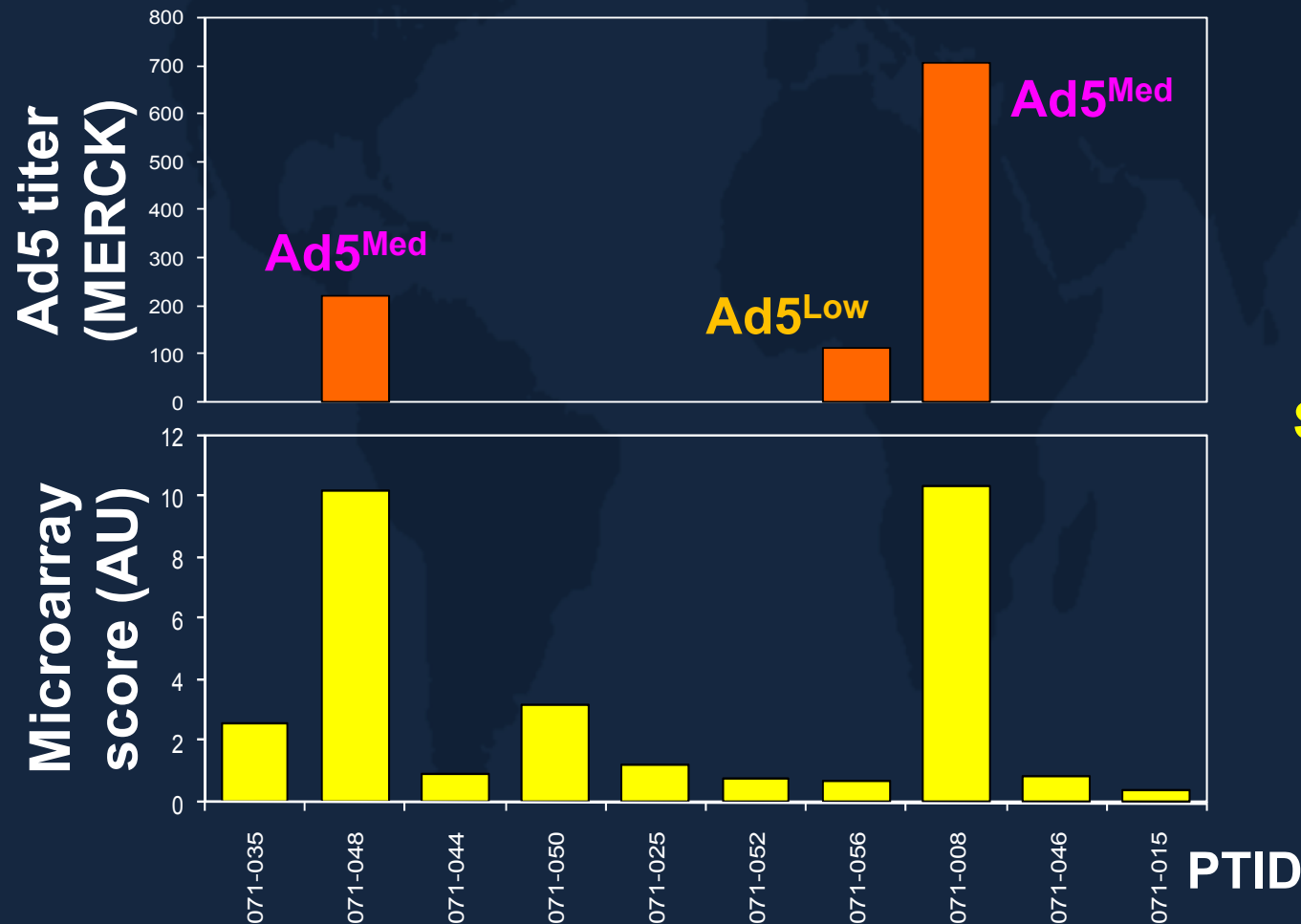
Assume Ad5 immune subjects will have attenuated responses to the vaccine, as measured by PBMC microarray response



**Score subjects by enhancement in signal/noise observed when they are excluded from the analysis**

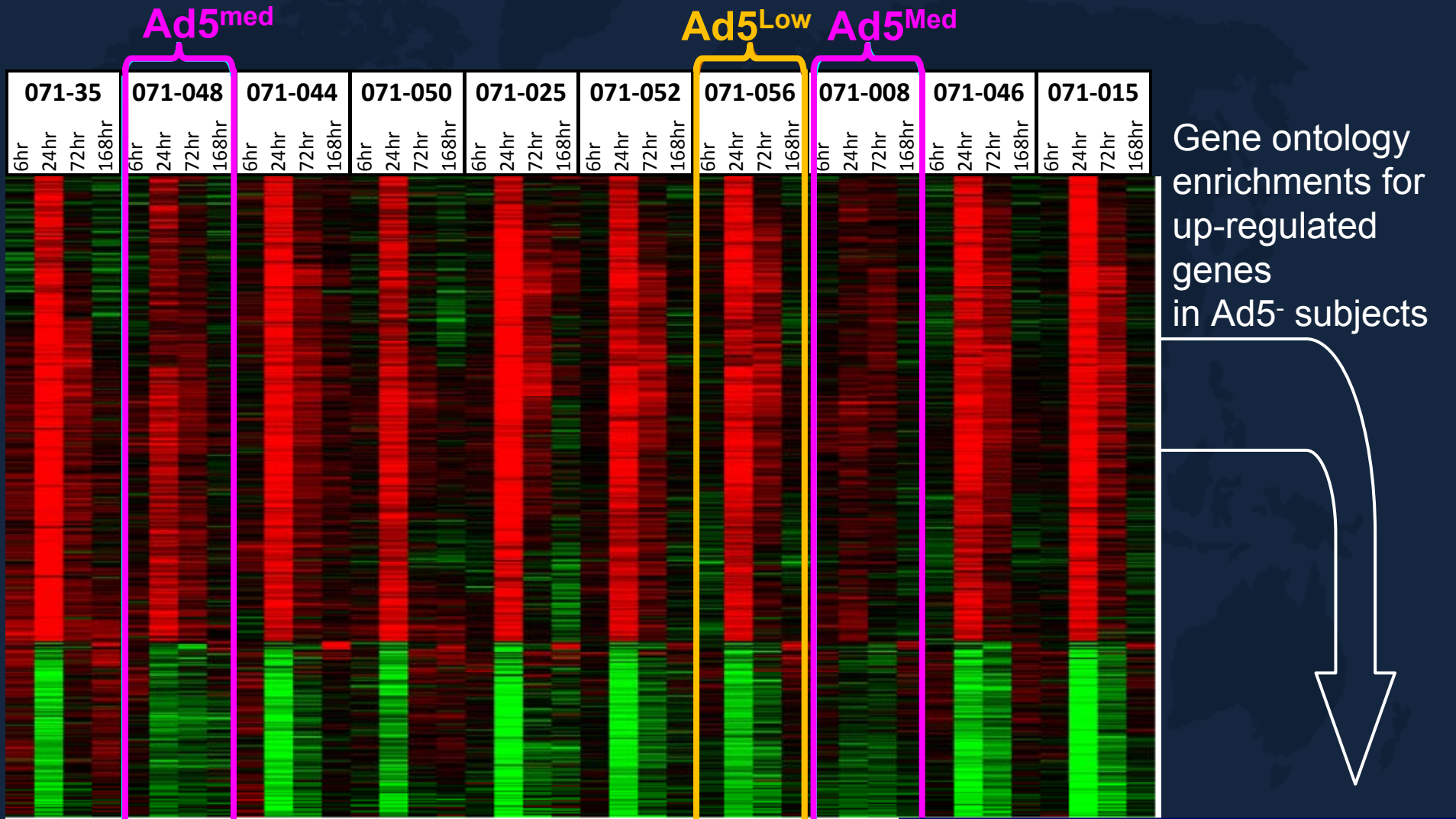
# Identifying Ad5<sup>+</sup> subjects by microarray analysis

Assume Ad5 immune subjects will have attenuated responses to the vaccine, as measured by PBMC microarray response



Score subjects by enhancement in signal/noise observed when they are excluded from the analysis

# Ad5<sup>-</sup> profiles enriched for innate antiviral response



- Up-regulation in response to vaccine
- Down-regulation in response to vaccine
- 490 genes at  $p < 1 \times 10^{-6}$  (one-way ANOVA)

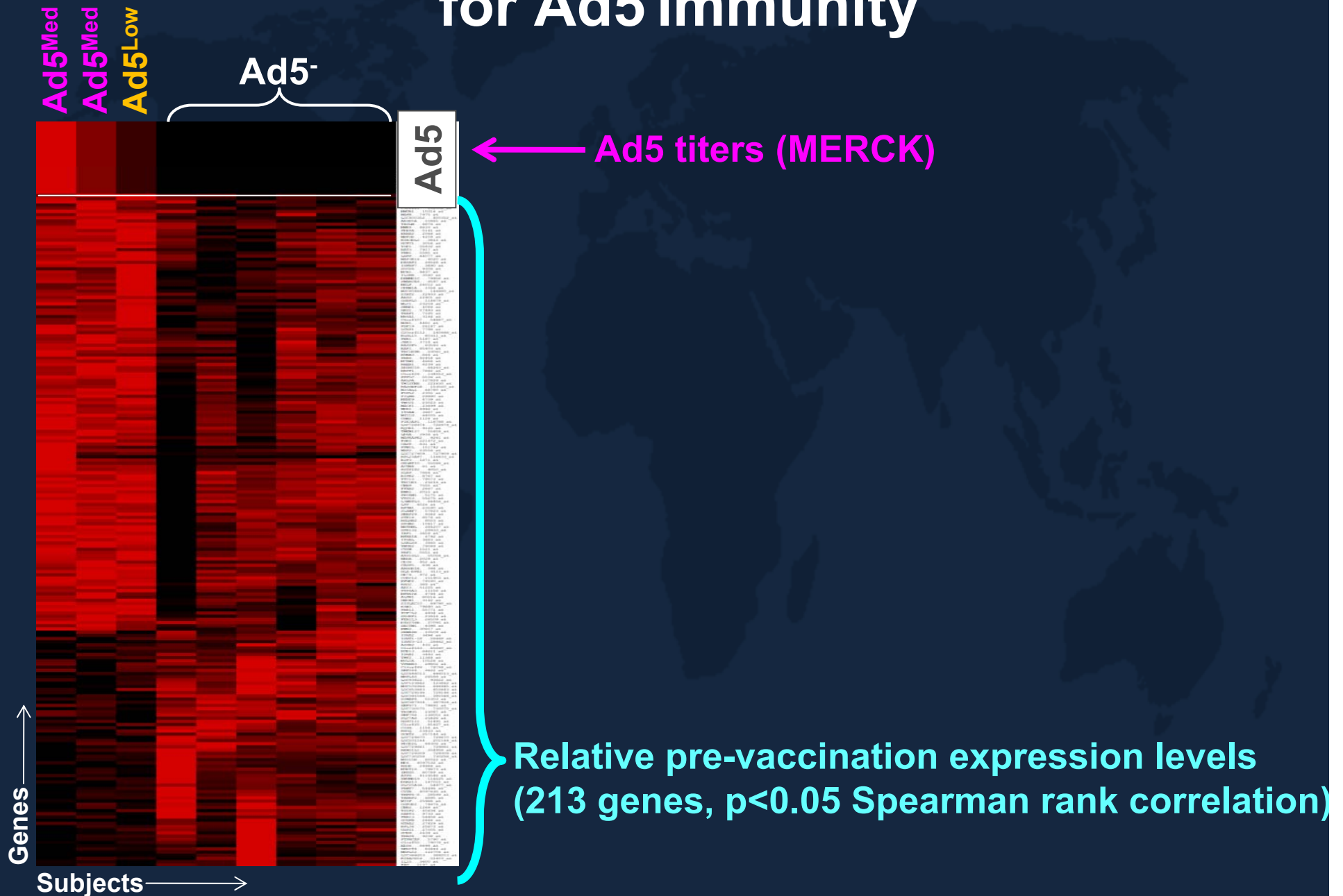
p-value	Description
6.85E-26	immune response
3.70E-19	response to virus

# Pre-vaccination expression signatures for Ad5 immunity

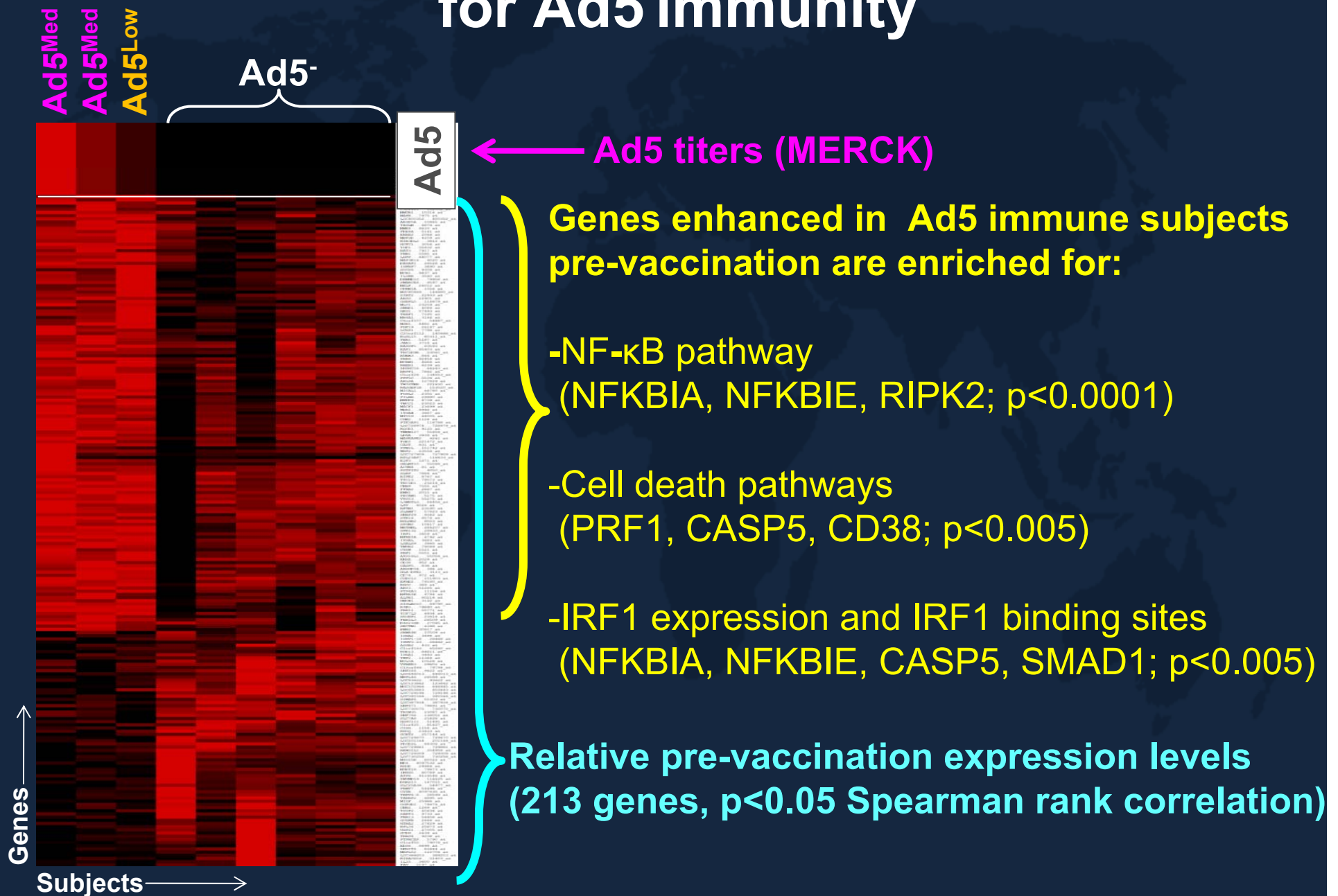




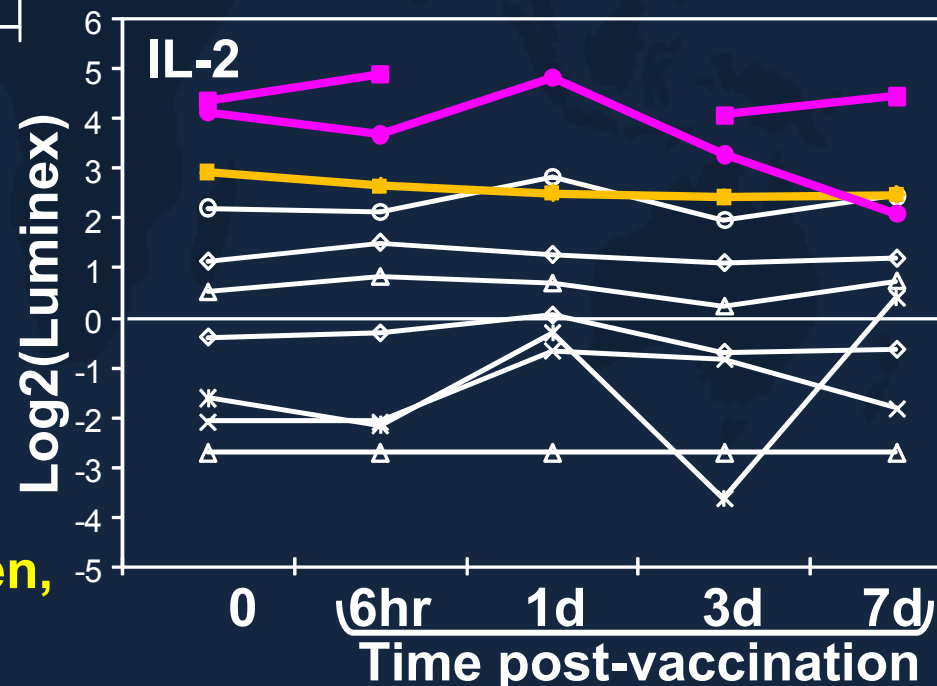
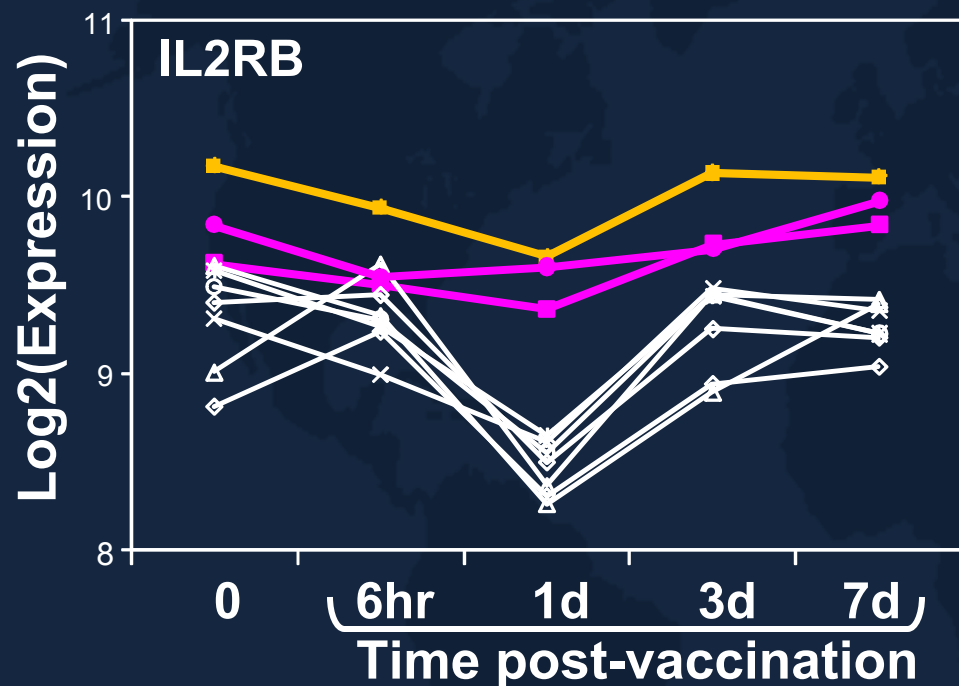
# Pre-vaccination expression signatures for Ad5 immunity



# Pre-vaccination expression signatures for Ad5 immunity



# Enhanced pre-vaccination IL-2RB expression and enhanced IL-2 in serum of Ad5<sup>+</sup> subjects



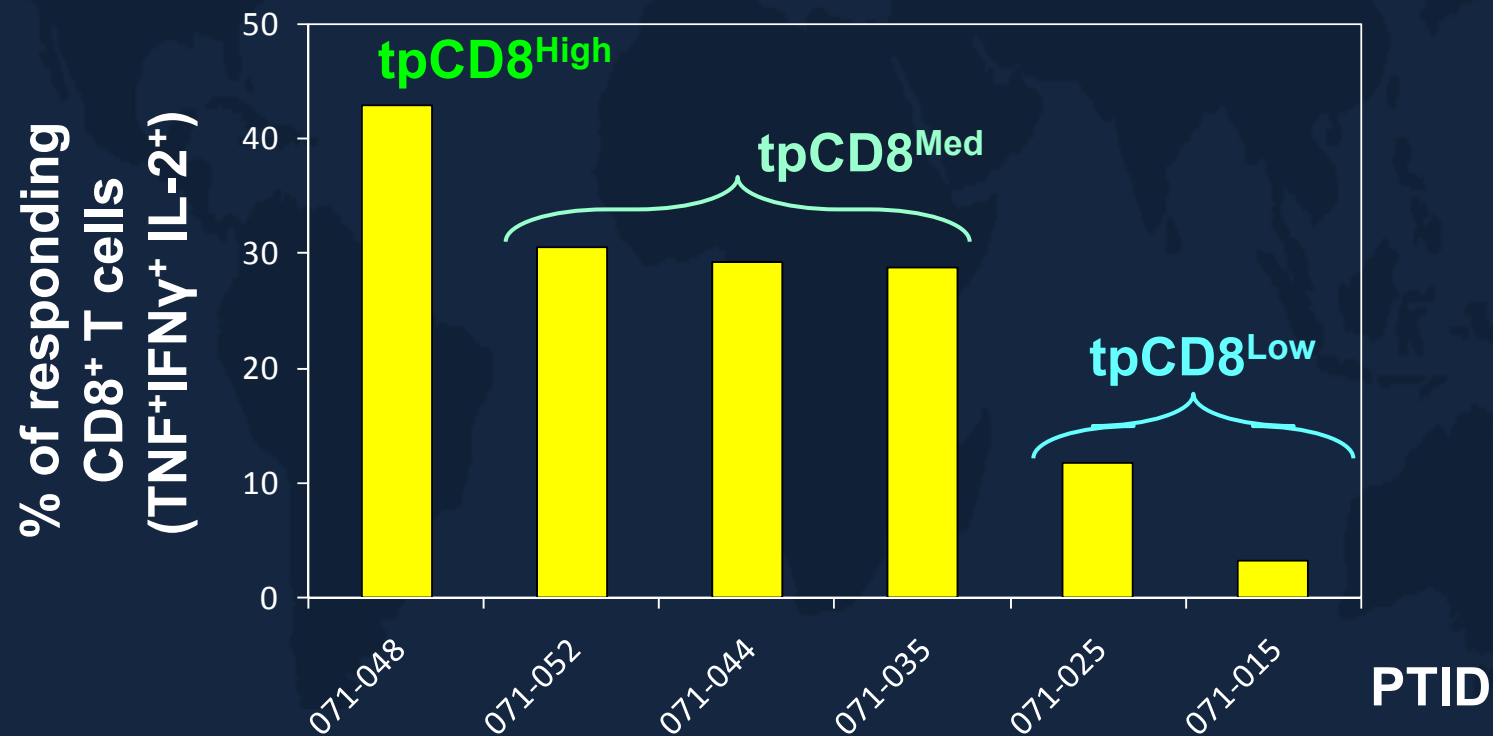
Cytokine data: Erica Andersen-Nissen, Julie McElrath, FHCRC

# CD8<sup>+</sup> T cell response polyfunctionality:

% triple-positive (TNF<sup>+</sup>IFN $\gamma$ <sup>+</sup>IL-2<sup>+</sup>, “tpCD8”)

(data from Erica Andersen-Nissen, Julie McElrath, FHCRC)

Non-progressors consistently maintain higher levels of polyfunctional HIV-specific CD8<sup>+</sup> T cells (Betts et al., 2006)



ICS of %HIV-gag responding CD8<sup>+</sup> T cells (TNF<sup>+</sup>, IFN $\gamma$ <sup>+</sup>, or IL-2<sup>+</sup>) that are triple-positive (“tpCD8”) in PBMCs, 28 days post-primary vaccination

# Signatures for CD8<sup>+</sup> T cell polyfunctionality

tpCD8<sup>High</sup>

tpCD8<sup>Med</sup>

tpCD8<sup>Med</sup>

tpCD8<sup>Med</sup>

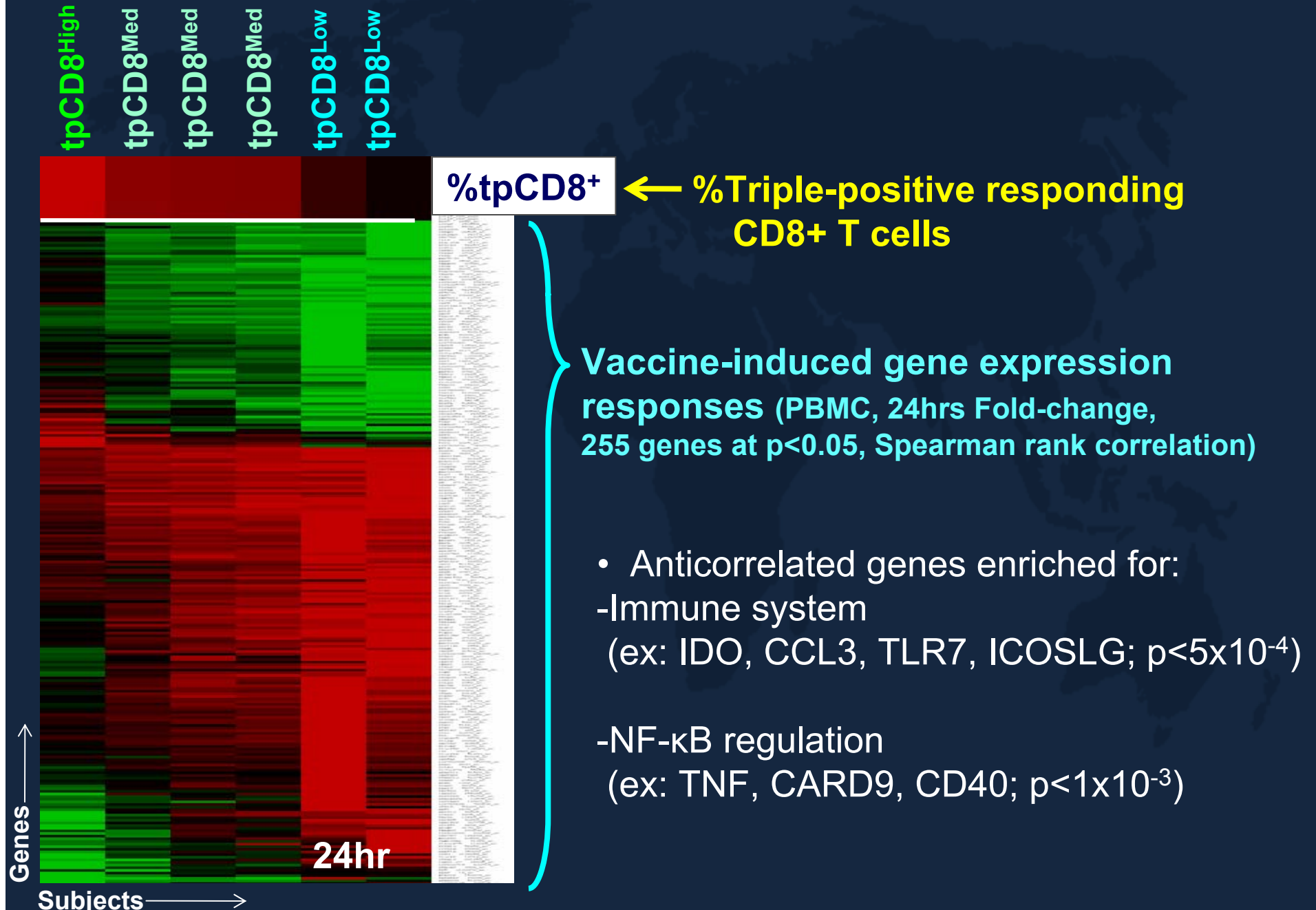
tpCD8<sup>Low</sup>

tpCD8<sup>Low</sup>

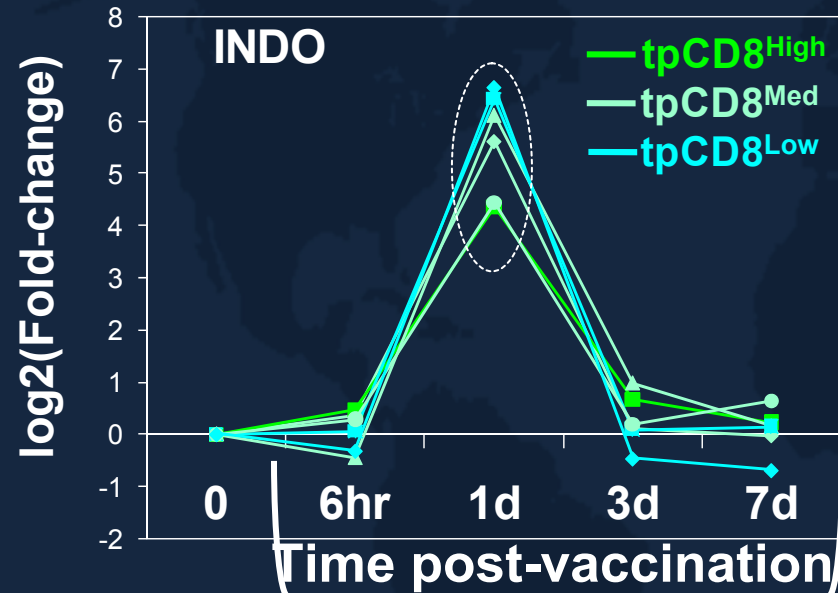


← %Triple-positive responding CD8<sup>+</sup> T cells

# Signatures for CD8<sup>+</sup> T cell polyfunctionality

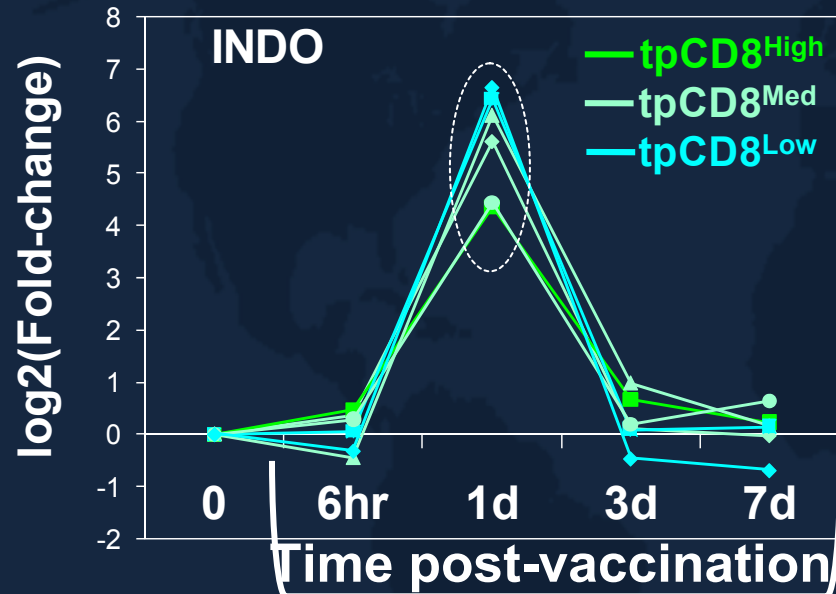


# CD8<sup>+</sup> T cell polyfunctionality signature: IDO/INDO T cell inhibitor

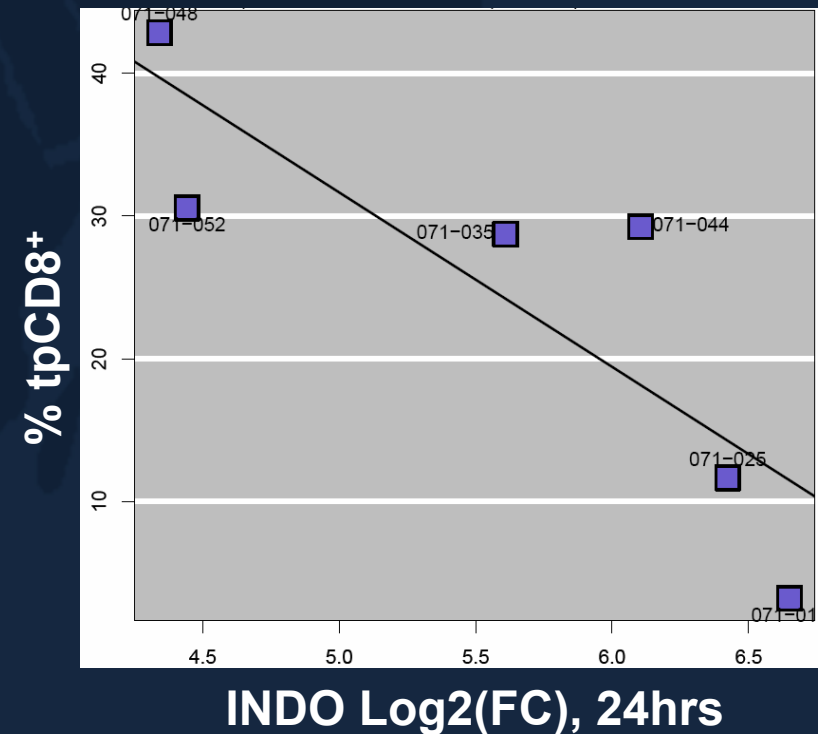


**IDO/INDO activity inhibits T cells, particularly CD8<sup>+</sup> T cell proliferation**  
(Forouzandeh et al., 2008)

# CD8<sup>+</sup> T cell polyfunctionality signature: IDO/INDO T cell inhibitor



%tpCD8<sup>+</sup> is negatively correlated with INDO induction, 24 hrs post-vac.

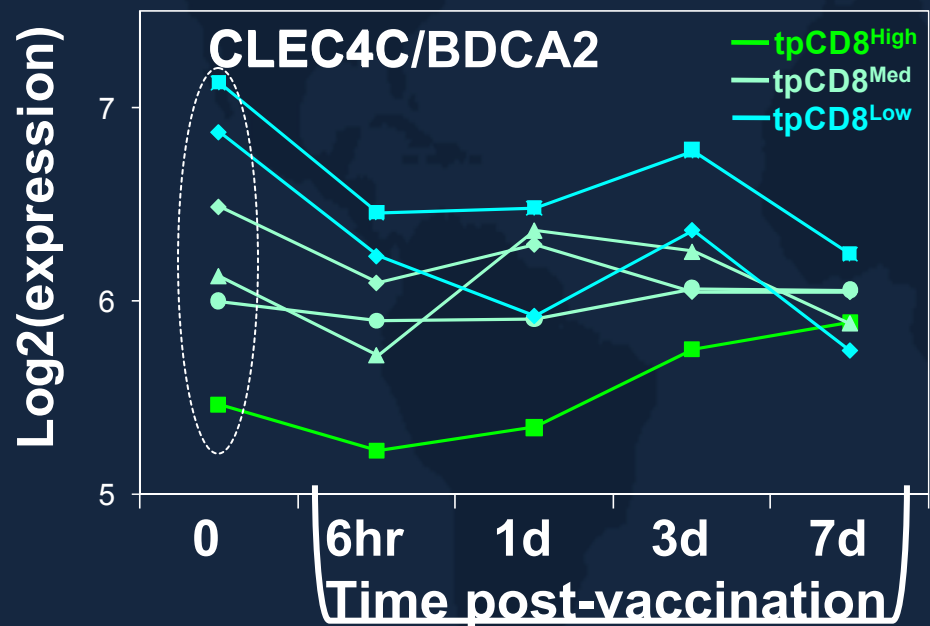


**IDO/INDO activity inhibits T cells, particularly CD8<sup>+</sup> T cell proliferation**  
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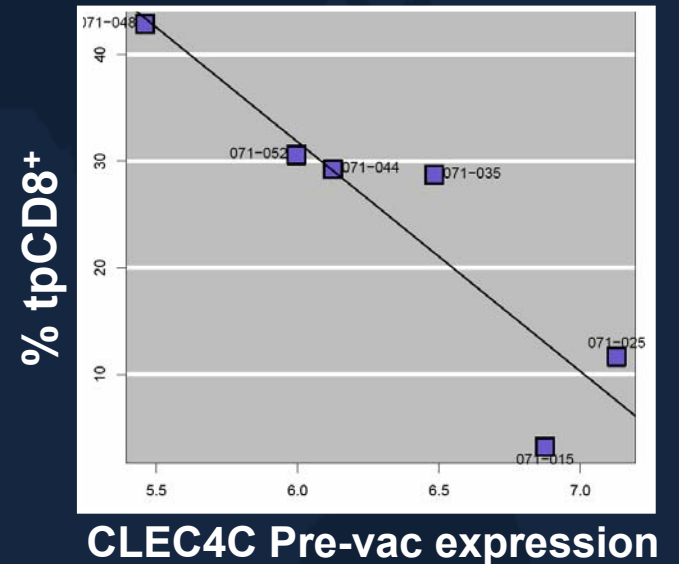


# pDC CD8<sup>+</sup> T cell polyfunctionality signature:

BDCA2 (plasmacytoid DC marker, Ag uptake/ inhibitory receptor)



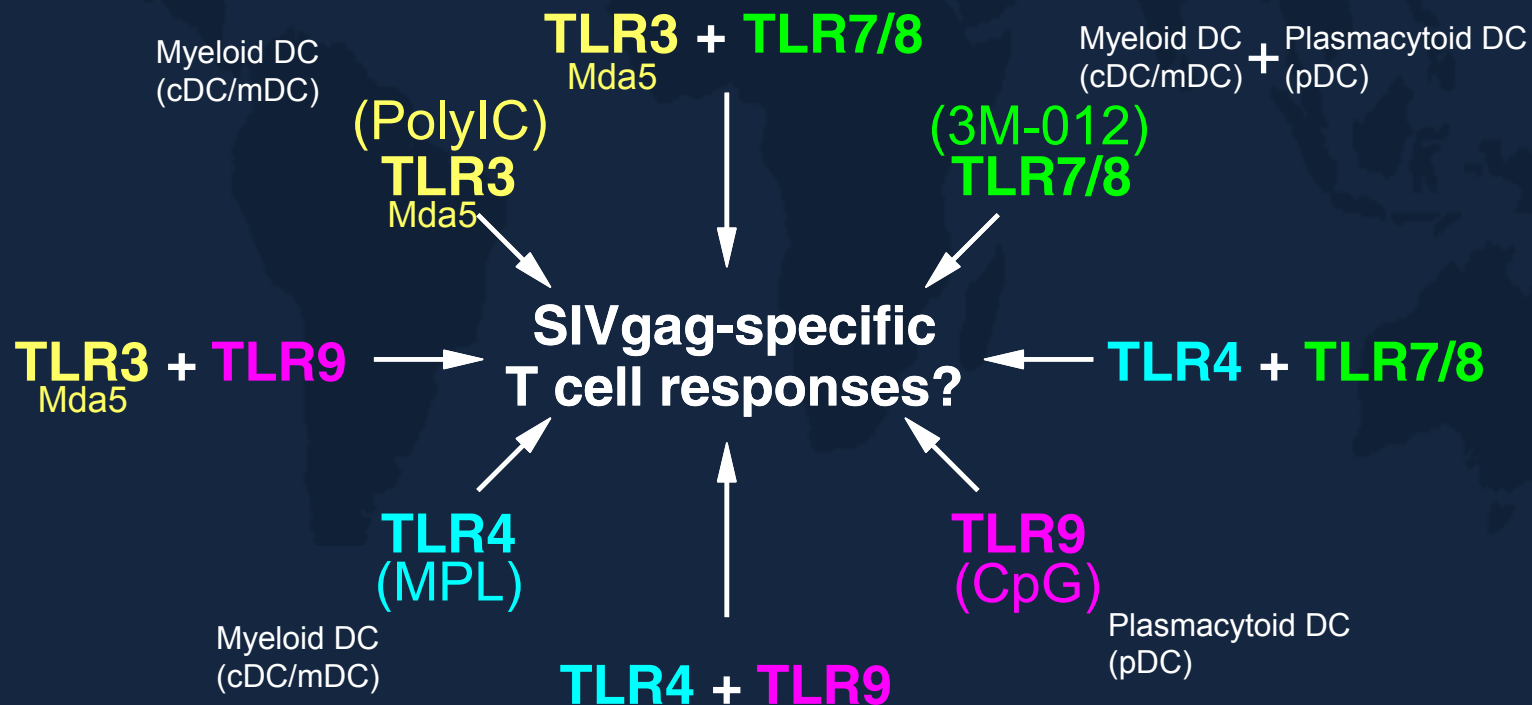
— Corr.  
to Pre-vac.



# NHP studies: TLR ligands as adjuvants

See Bob Seder's talk for more details (Today, 5:20pm, Room 1.40)

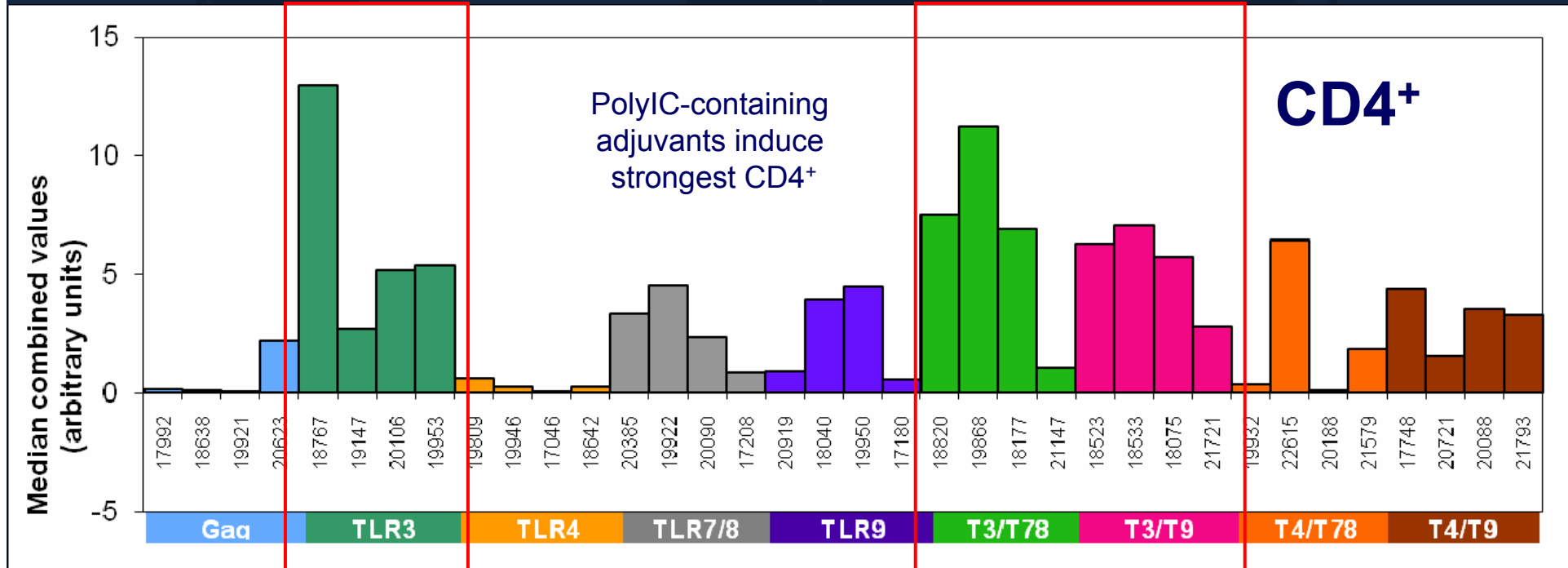
- NHP immunized with SIV Gag protein + Montanide + TLR ligands (Haesun Park & Louis Picker-OHSU, Bob Seder-NIH/VRC)
- Profile SIVgag-specific CD4<sup>+</sup> & CD8<sup>+</sup> T cell responses (Haesun Park & Louis Picker-OHSU, Bob Seder-NIH/VRC)
- Profile gene expression responses in PBMC and LN (ISB)



# PolyIC-containing adjuvants (TLR3) induce strongest CD4<sup>+</sup> responses

(data from Haesun Park & Louis Picker-OHSU, Bob Seder-NIH/VRC)

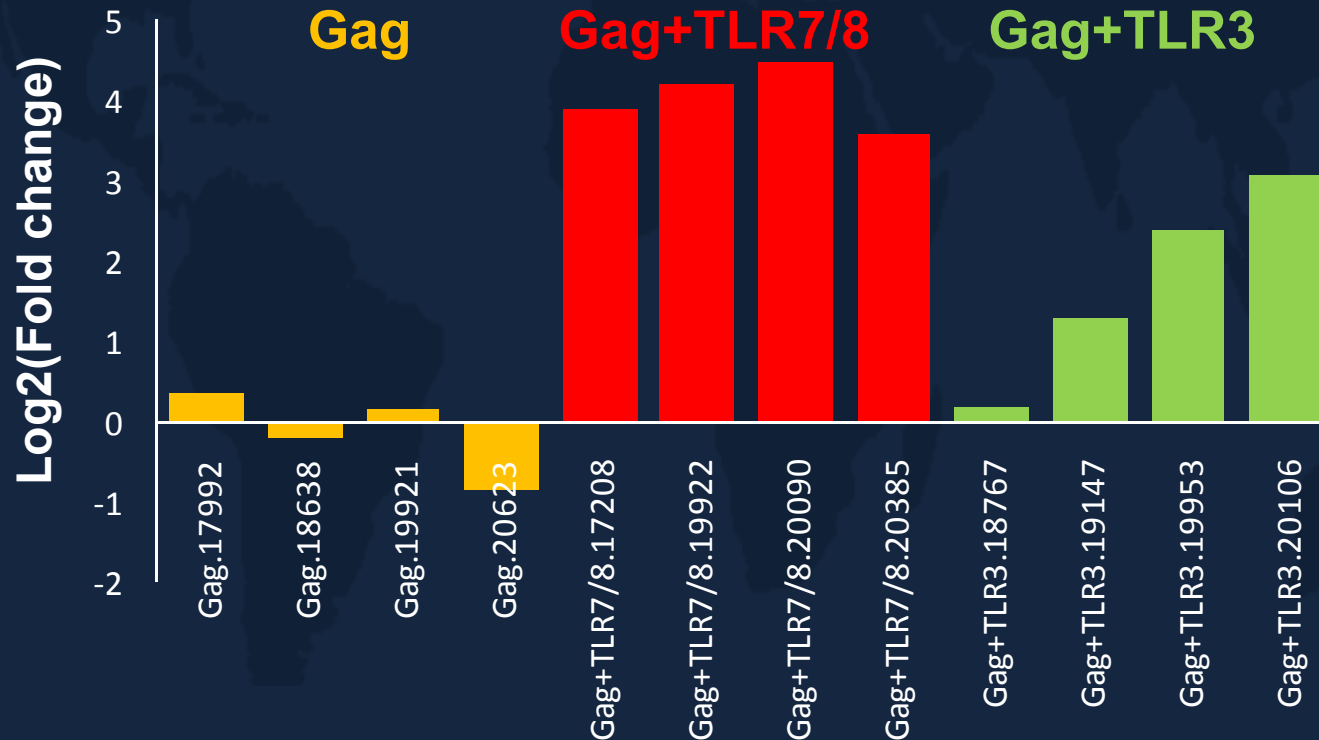
%CD4<sup>+</sup> responding to SIV gag with TNF or IFN $\gamma$



Median-scaled values over time for %CD4<sup>+</sup> T cells responding to SIV gag with TNF or IFN $\gamma$  in BAL in response to primary vaccination. Up to 60% responding.

# TLR7/8 more potently induces Interferon Stimulated Genes than TLR3/PolyIC (Day 2 post-vaccination PBMC, qRT-PCR)

## DDX58/RIG-I



Equivalent results obtained for *IFIH1/MDA5* and *RSAD2/Viperin*

# Identify novel predictors of immunity:

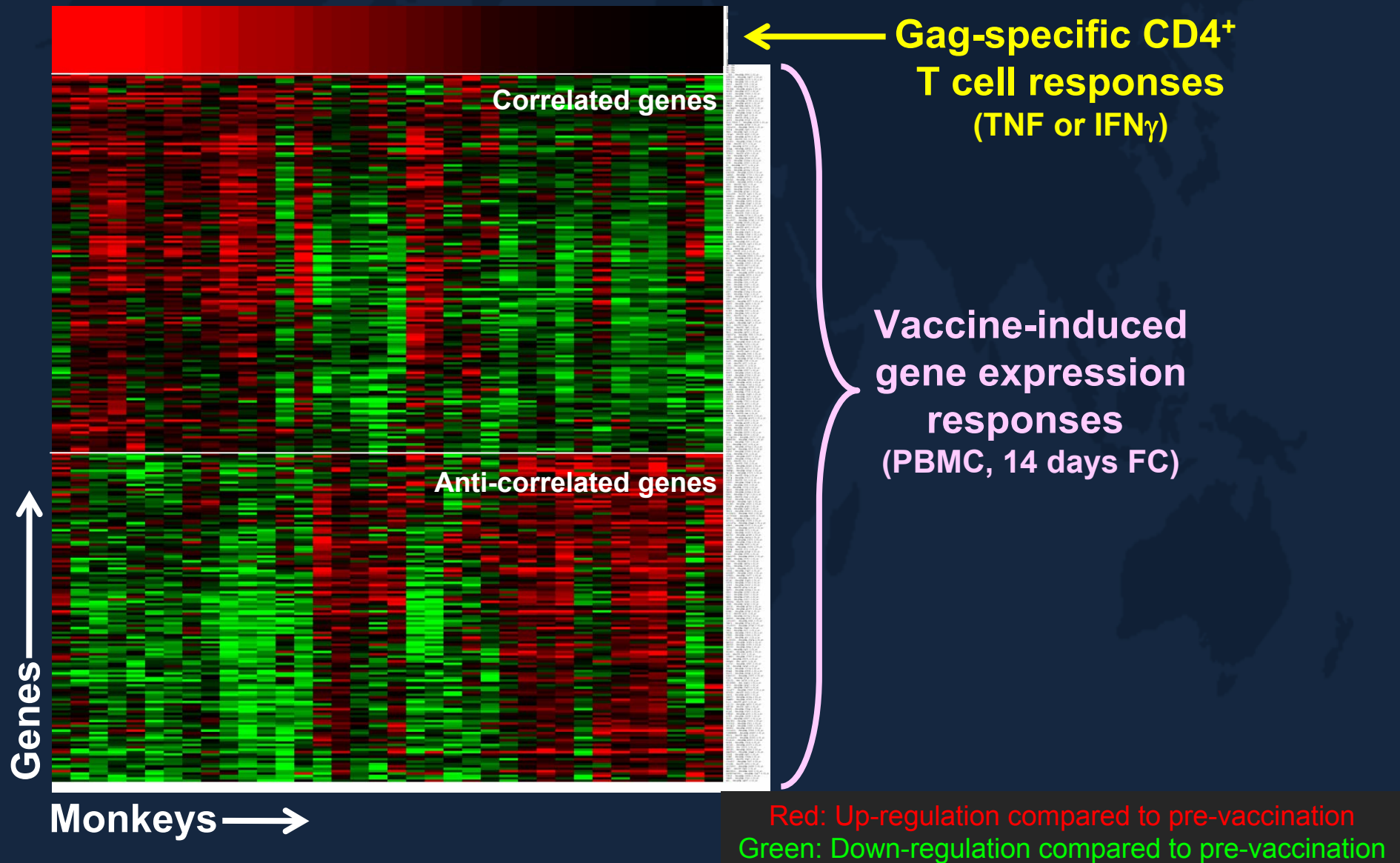
Correlate T cell response magnitudes  
with gene expression responses



← **Gag-specific CD4<sup>+</sup>  
T cell responses  
(TNF or IFN $\gamma$ )**

# Identify novel predictors of immunity:

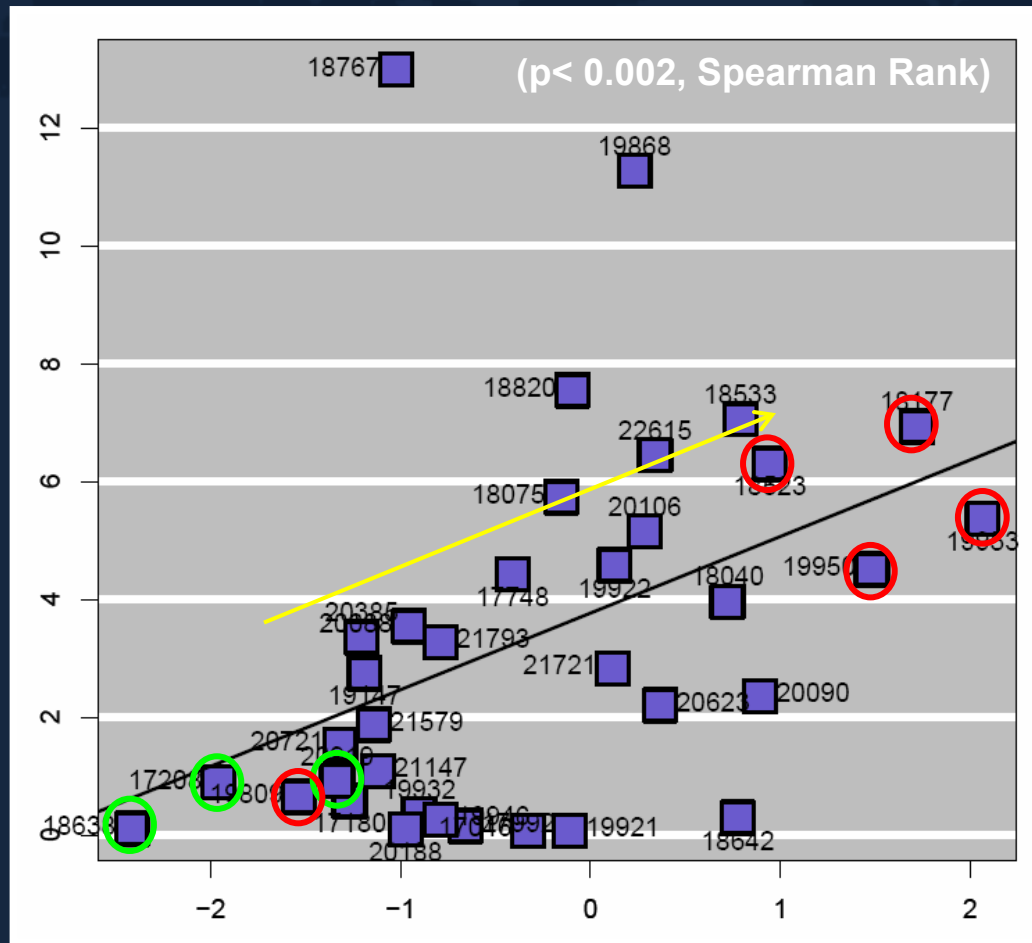
Correlate T cell response magnitudes  
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# Signature for CD4<sup>+</sup> T cell response

Vaccine-induced CCR2B expression (PBMC, 14 days)

Gag-specific CD4<sup>+</sup> T cell response  
(%responding TNF or IFN $\gamma$ )



CCR2B expression  
log<sub>2</sub>(Fold-change) compared to pre-vaccination

## CCR2:

-MCP-1 receptor, minor HIV-1 co-receptor

-CCR2-64I SNP associated with protection from early AIDS progression (Ioannidis et al., 2001; Mulherin et al., 2003)

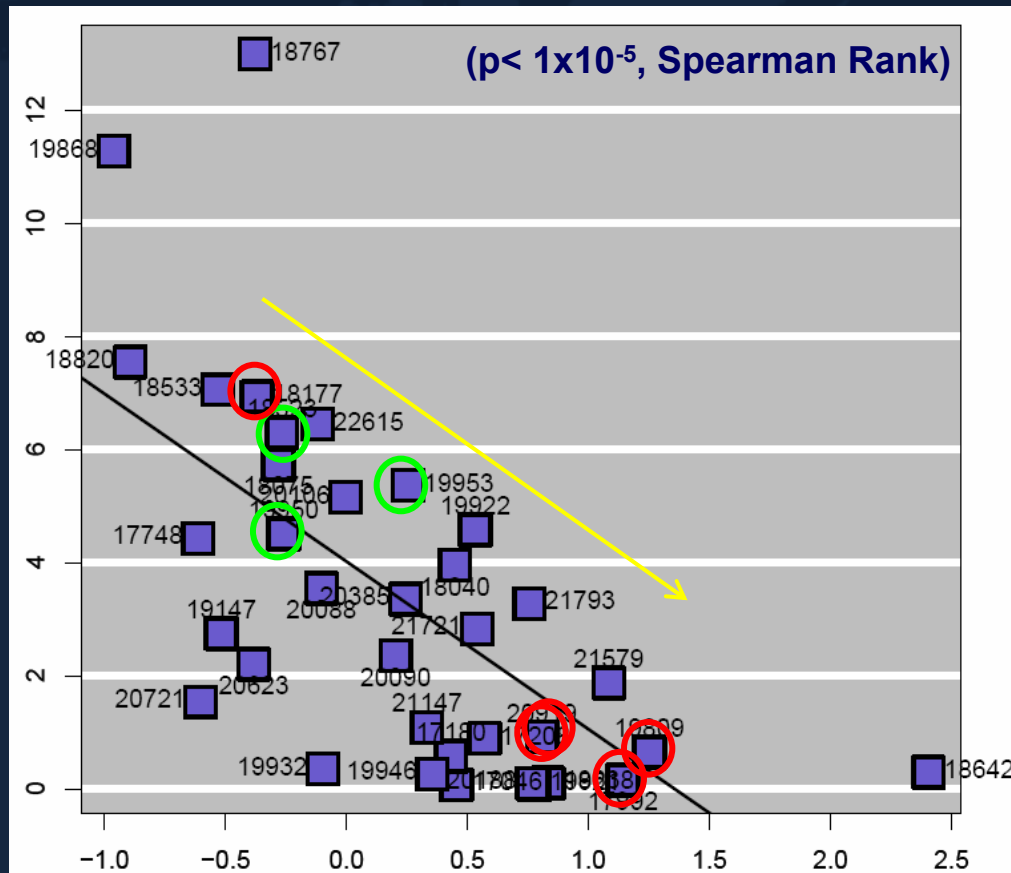
-Important for CD4<sup>+</sup> T cell recruitment to lung during influenza infection (Wareing et al., 2007)

**Stronger induction of CCR2B is associated with stronger CD4<sup>+</sup> T cell responses**

# Signature for CD4<sup>+</sup> T cell response

## Vaccine-induced KLF5 expression (PBMC, 14 days)

Gag-specific CD4<sup>+</sup> T cell response  
(%responding TNF or IFN $\gamma$ )



KLF5 expression  
log<sub>2</sub>(Fold-change) compared to pre-vaccination

**KLF5:**  
Transcription factor that regulates TNF and LPS-induced pro-inflammatory cytokines in various cell lines (Changevalap et al., 2006; Kumekawa et al., 2008)

**Stronger repression of KLF5 is associated with stronger CD4<sup>+</sup> T cell responses**



# Summary

- We have integrated expression profiling and functional measurements to identify novel signatures of immunogenicity
  - Attenuated response to MRKAd5 gag/pol/nef in Ad5<sup>+</sup> subjects
  - CD8<sup>+</sup> T cell polyfunctionality signature: ex, IDO, pDCs
  - Signatures for CD4<sup>+</sup> T cell response: ex, CCR2B and KLF5
- We made some surprising observations
  - Baseline Ad5<sup>+</sup> expression signature: pre-existing altered expression of immune relevant genes, potential roles for IL-2, IRF1
  - CD8<sup>+</sup> polyfunctionality: negative association with inflammatory gene expression responses
  - NHP: most immunogenic adjuvant (PolyIC) does not induce the strongest expression responses

# Next steps

- Incorporate additional functional assays
  - CD8<sup>+</sup> magnitude, CD4<sup>+</sup> polyfunctionality, viral load after challenge (NHP), etc.
- Validate baseline Ad5<sup>+</sup> signatures by profiling additional Ad5<sup>+</sup> and Ad5<sup>-</sup> individuals
- Identify signatures that are consistent across vaccine platforms (and species)
  - Profiling expression and T cell responses to successful vaccines in humans (ex: YFV, HBV) and alternative HIV vaccine candidates
  - Seek global correlations across multiple platforms
- Identify cell-type specific signatures by integrating with expression profiles of sorted cellular subsets
  - CD8<sup>+</sup> signatures, CD4<sup>+</sup> signatures, DC subsets, NK-cells, etc.



# Thank you!

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