

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)

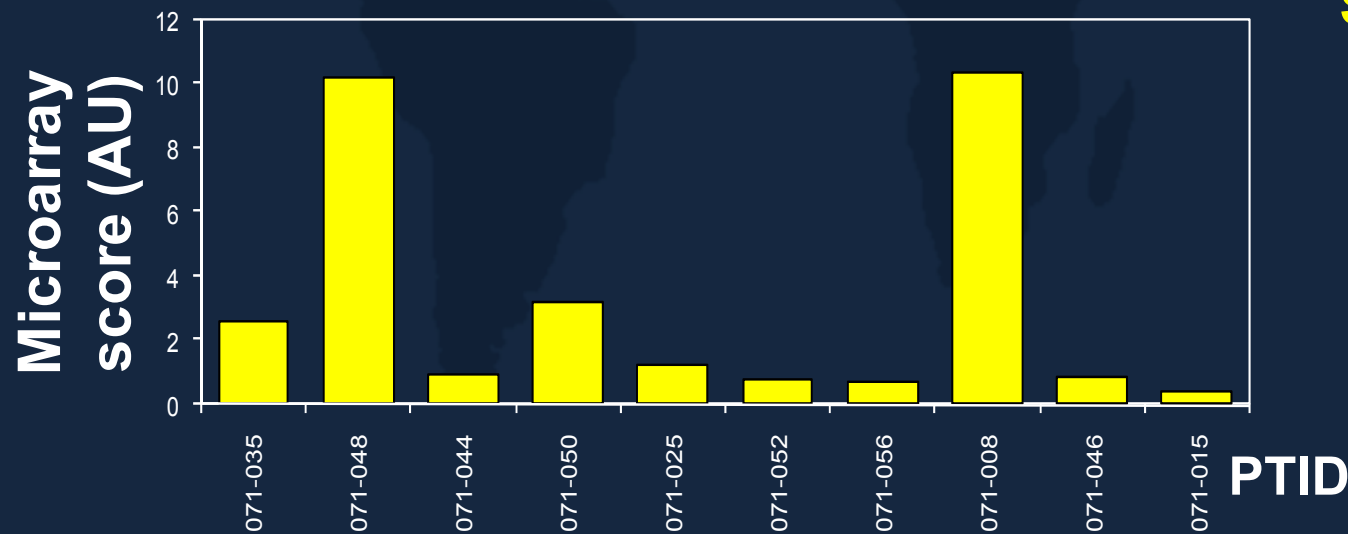
- 10 subjects vaccinated with trivalent MRKAd5 HIV-1 gag/pol/nef from the Step trial
(Erica Andersen-Nissen, Julie McElrath, FHCRC)
- Subjects had varying pre-vaccination immunity to vaccine vector (Ad5)
- Microarray profiling PBMC innate immune responses 6, 24, 72, AND 168hr after primary vaccination (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⁺ subjects be predicted directly from microarray analysis of vaccine response?
 - What is the innate immune gene expression signature for Ad5⁺ subjects?
- Expression signatures for vaccine-induced HIV-specific CD8⁺ T cell polyfunctionality

Identifying Ad5⁺ subjects by microarray analysis

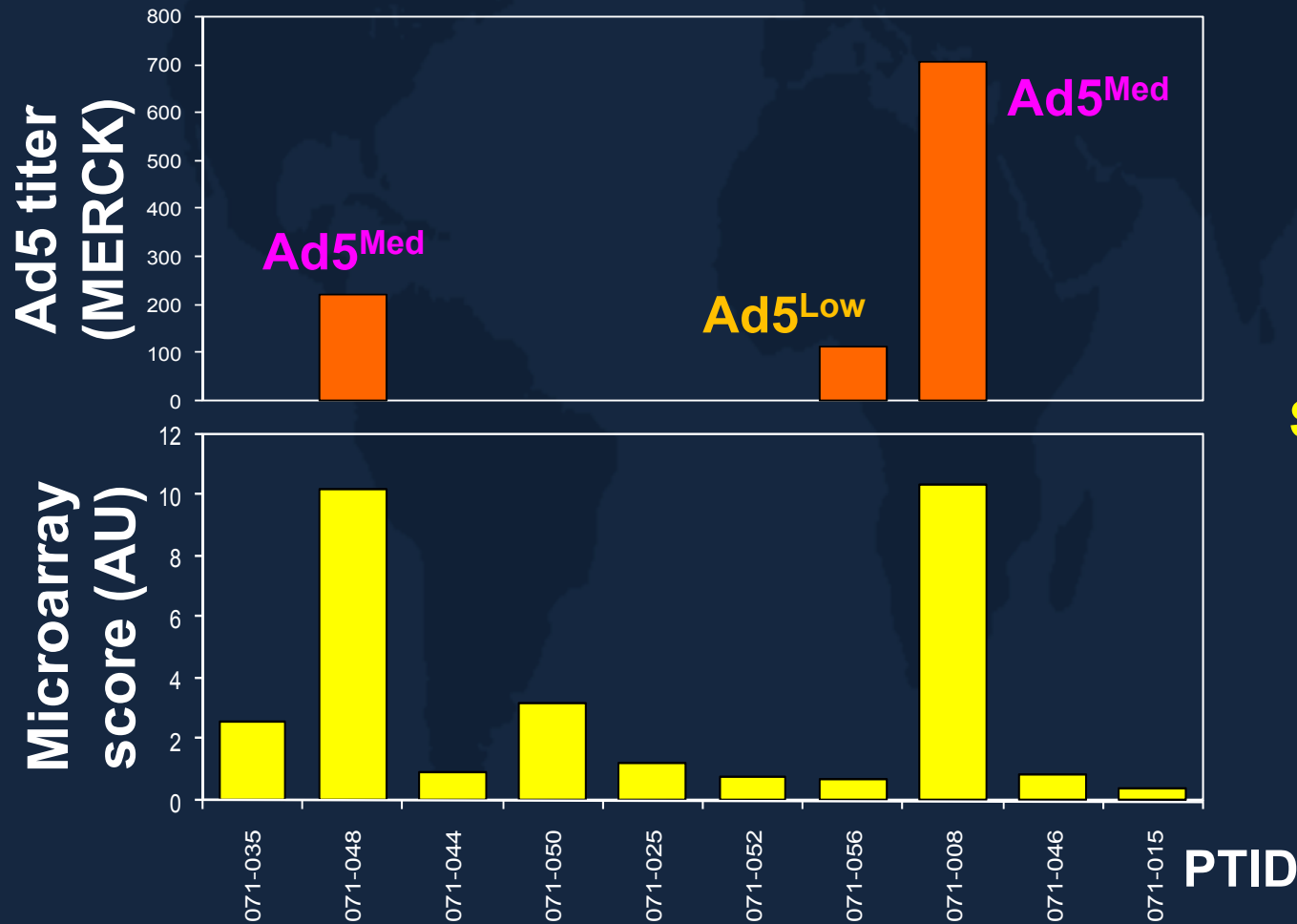
Assume Ad5 immune subjects will have attenuated responses to the vaccine, as measured by PBMC microarray response



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

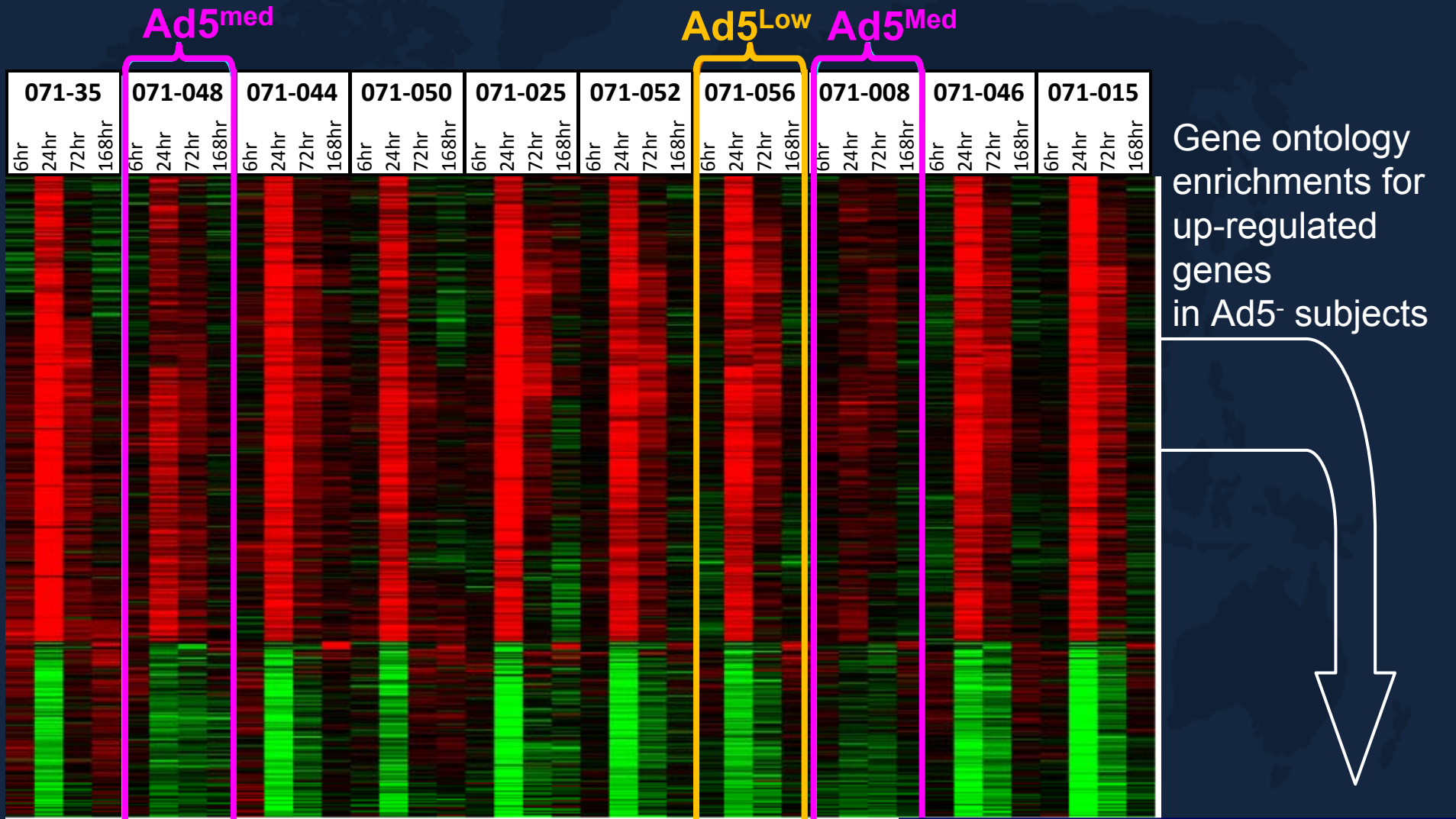
Identifying Ad5⁺ subjects by microarray analysis

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⁻ profiles enriched for innate antiviral response



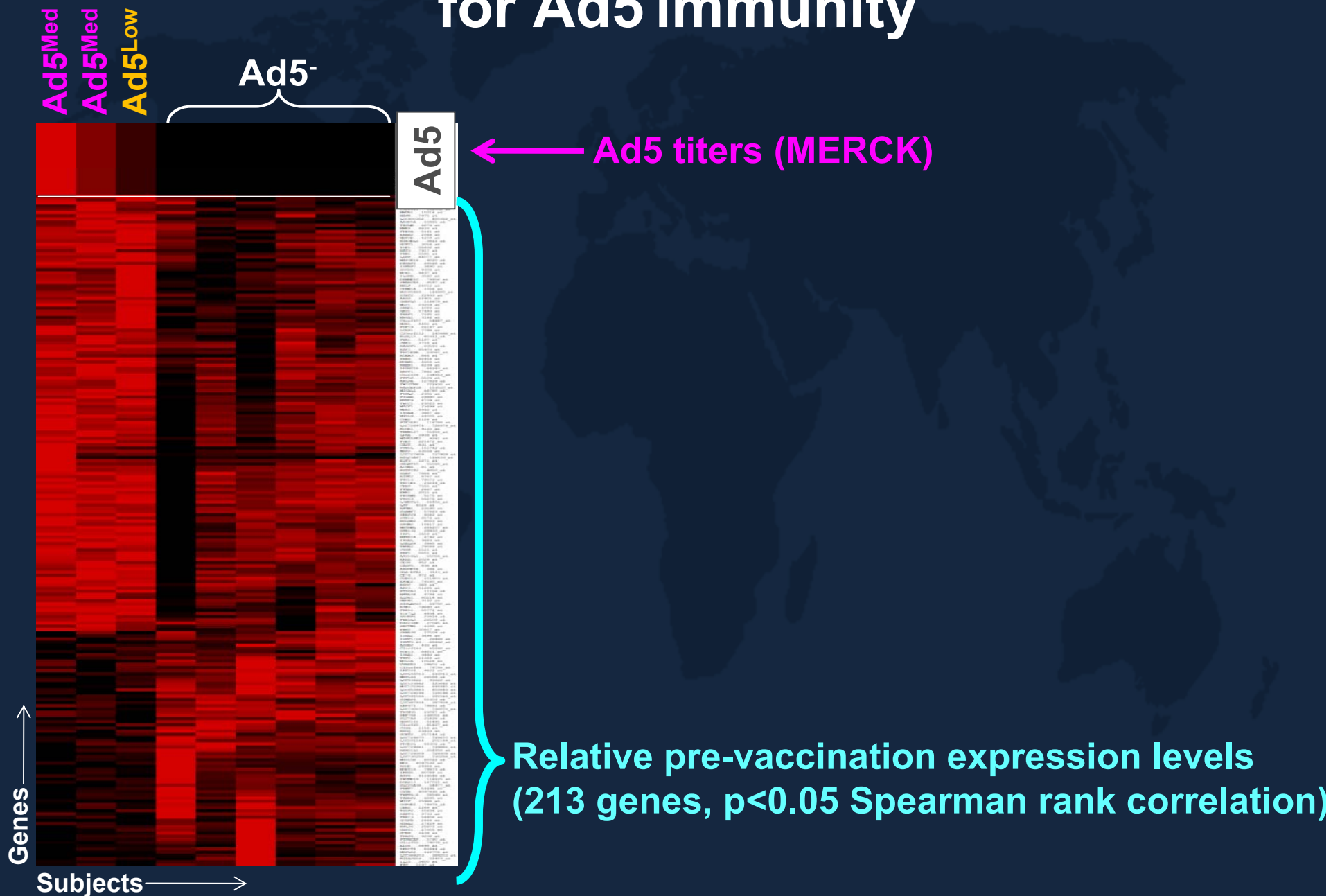
- Up-regulation in response to vaccine
- Down-regulation in response to vaccine
- 490 genes at $p < 1 \times 10^{-6}$ (one-way ANOVA)

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

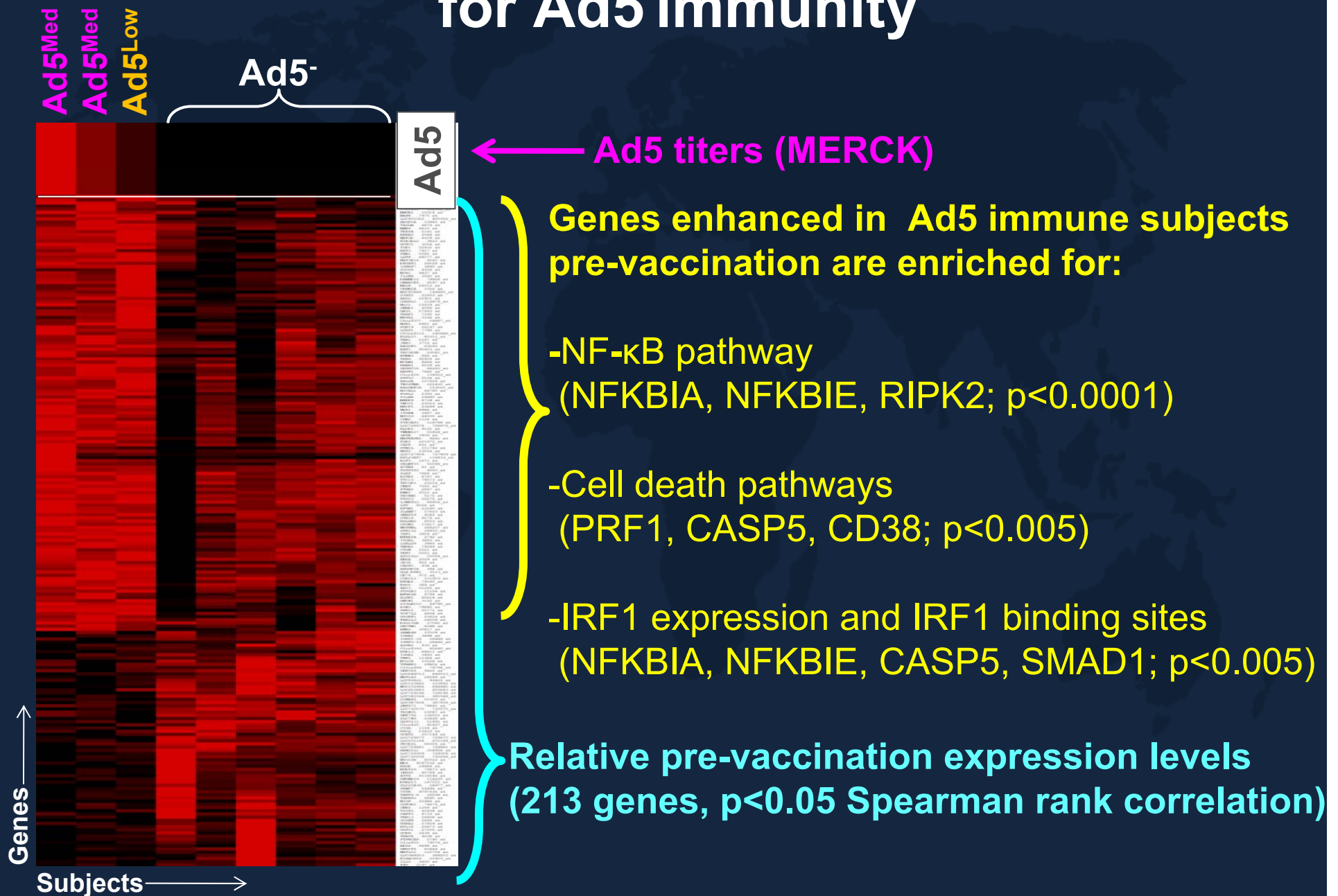
Pre-vaccination expression signatures for Ad5 immunity



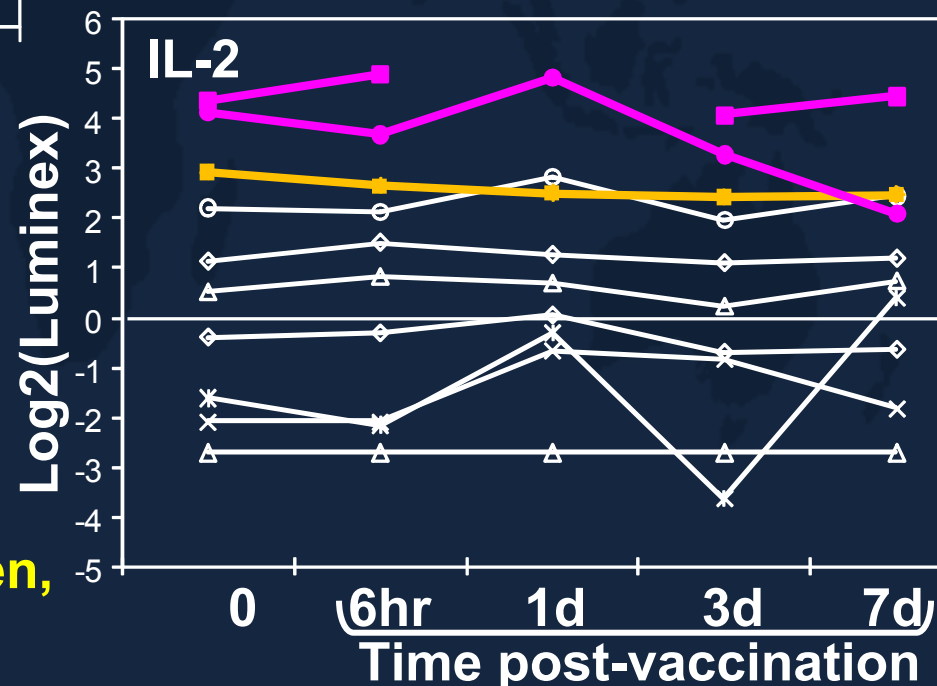
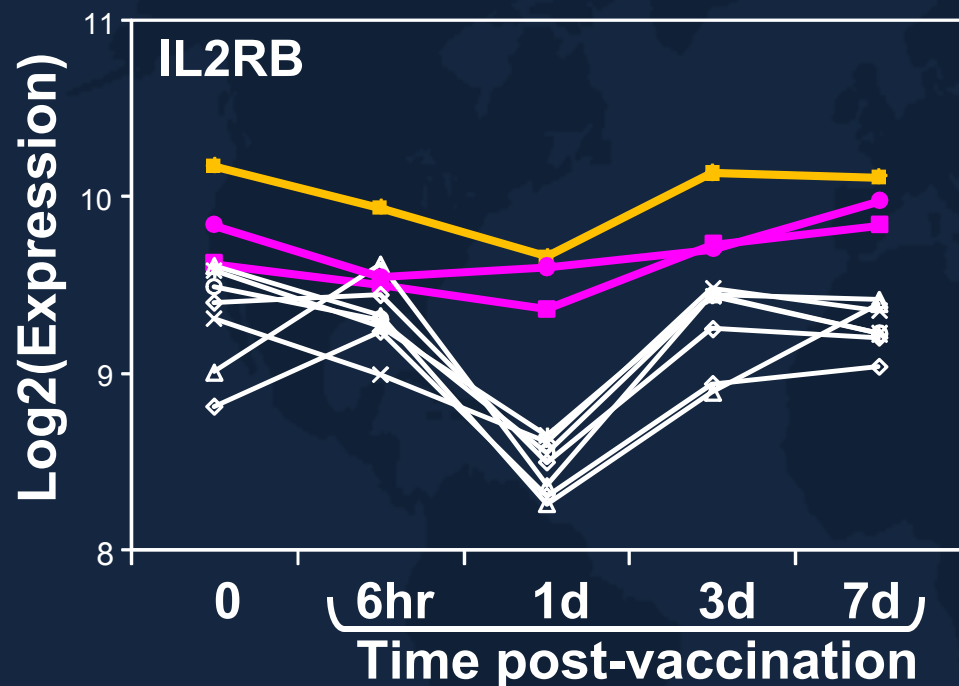
Pre-vaccination expression signatures for Ad5 immunity



Pre-vaccination expression signatures for Ad5 immunity



Enhanced pre-vaccination IL-2RB expression and enhanced IL-2 in serum of Ad5⁺ subjects



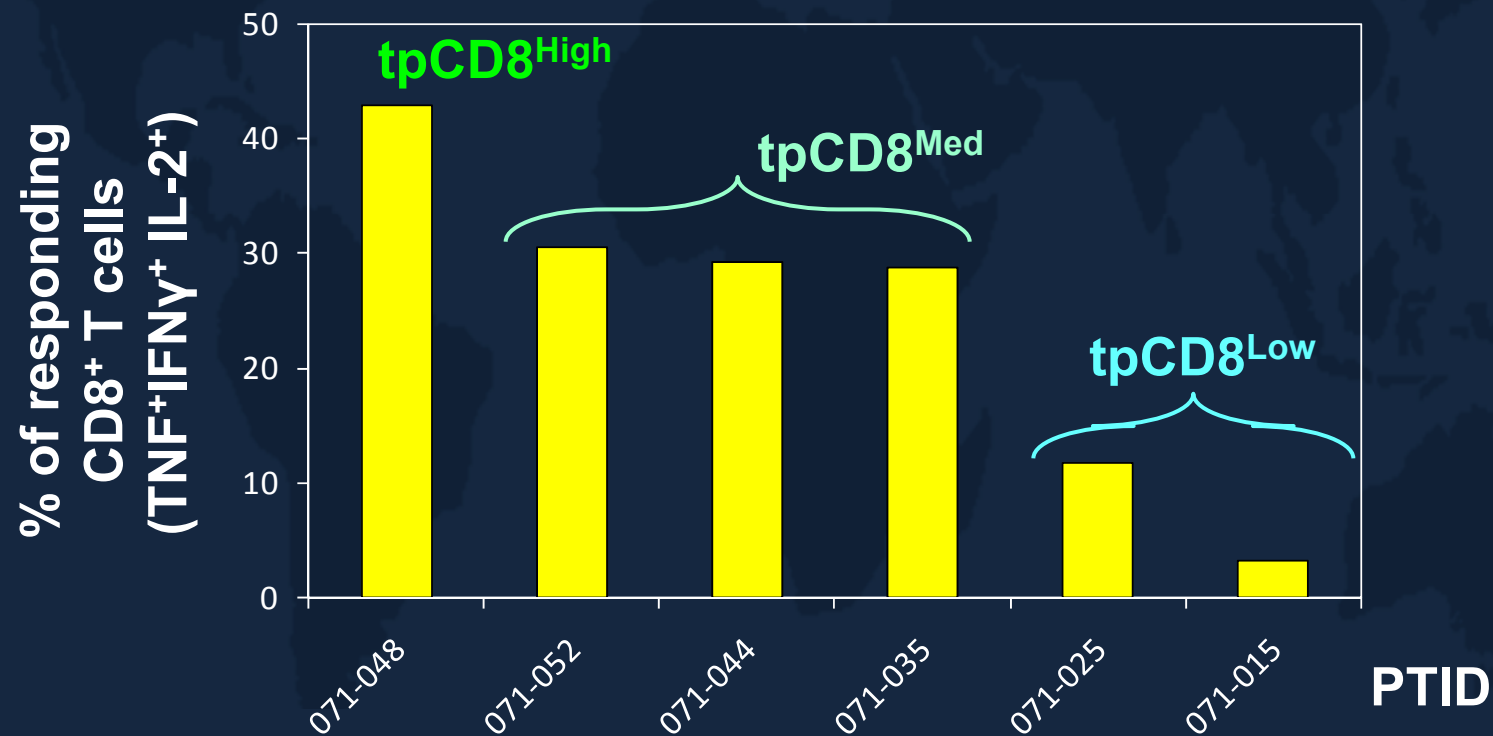
Cytokine data: Erica Andersen-Nissen, Julie McElrath, FHCRC

CD8⁺ T cell response polyfunctionality:

% triple-positive (TNF⁺IFN γ ⁺IL-2⁺, “tpCD8”)

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

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



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

Signatures for CD8⁺ T cell polyfunctionality

tpCD8^{High}

tpCD8^{Med}

tpCD8^{Med}

tpCD8^{Med}

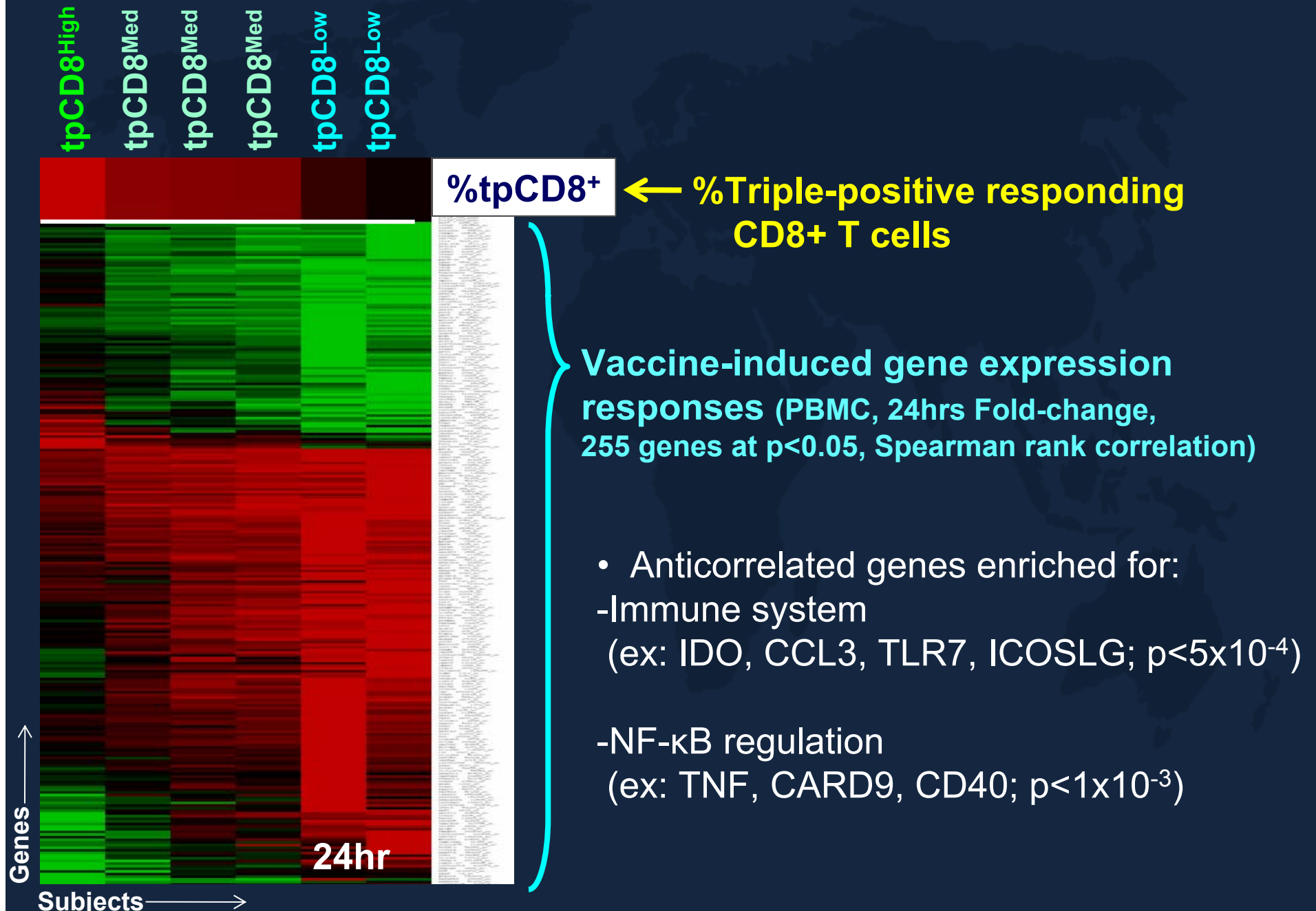
tpCD8^{Low}

tpCD8^{Low}

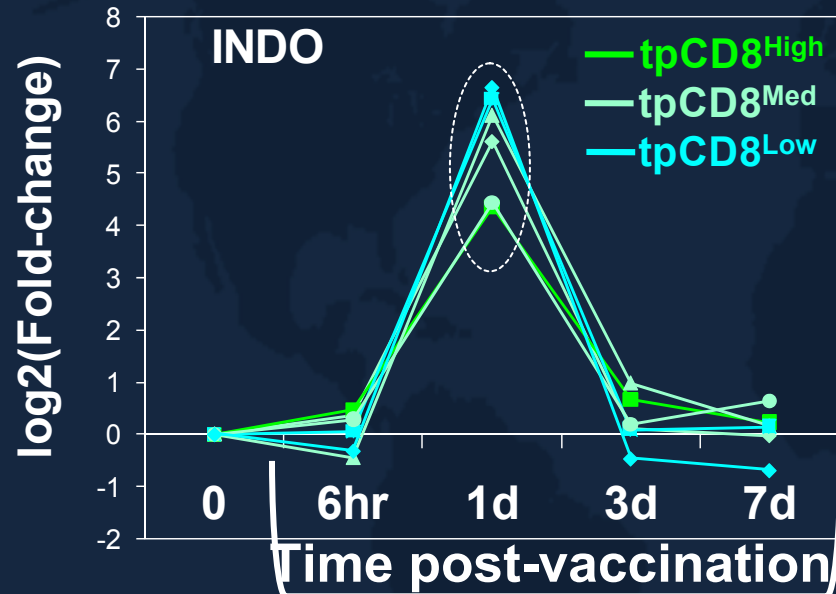


← %Triple-positive responding CD8⁺ T cells

Signatures for CD8⁺ T cell polyfunctionality

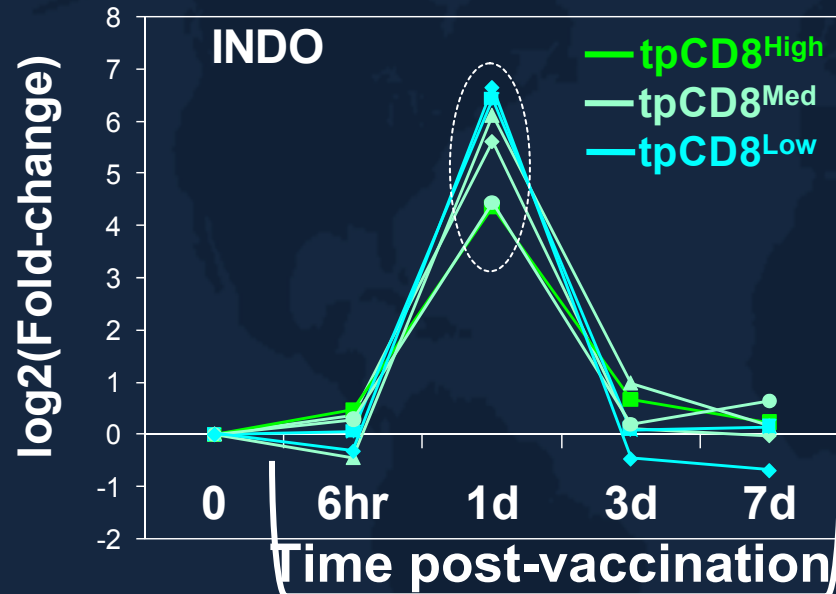


CD8⁺ T cell polyfunctionality signature: IDO/INDO T cell inhibitor

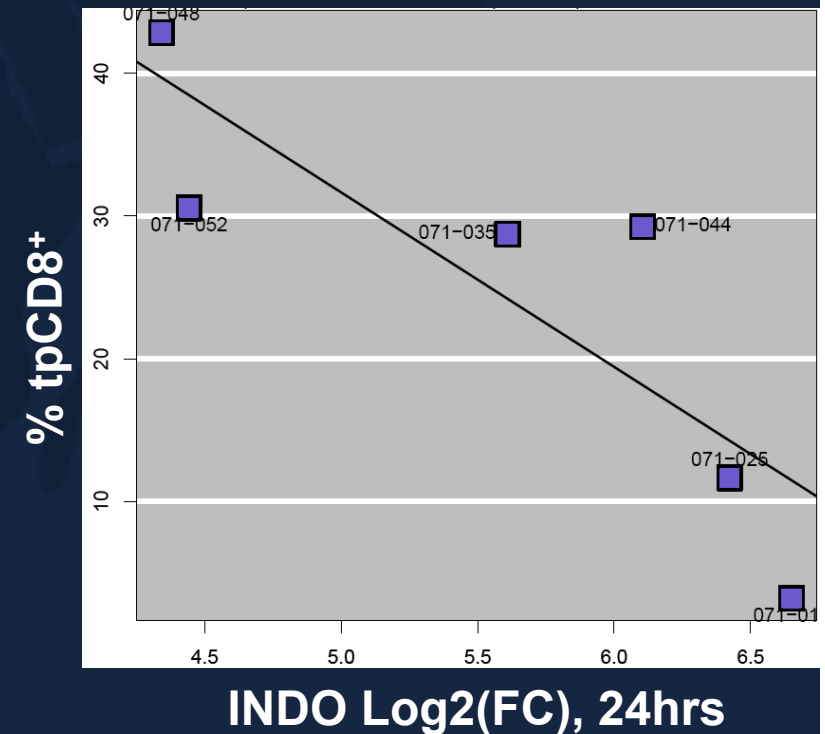


IDO/INDO activity inhibits T cells, particularly CD8⁺ T cell proliferation
(Forouzandeh et al., 2008)

CD8⁺ T cell polyfunctionality signature: IDO/INDO T cell inhibitor



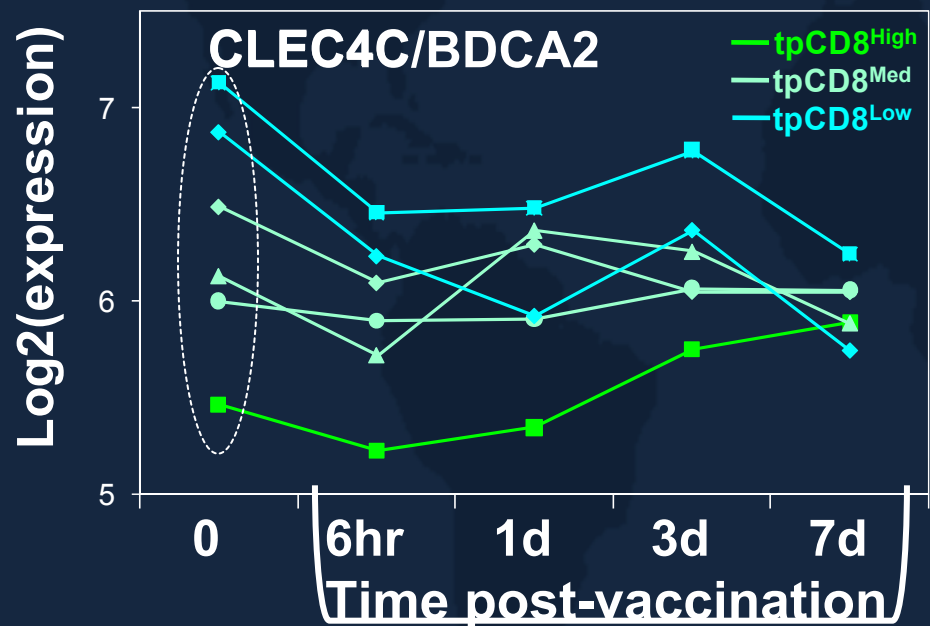
%tpCD8⁺ is negatively correlated with INDO induction, 24 hrs post-vac.



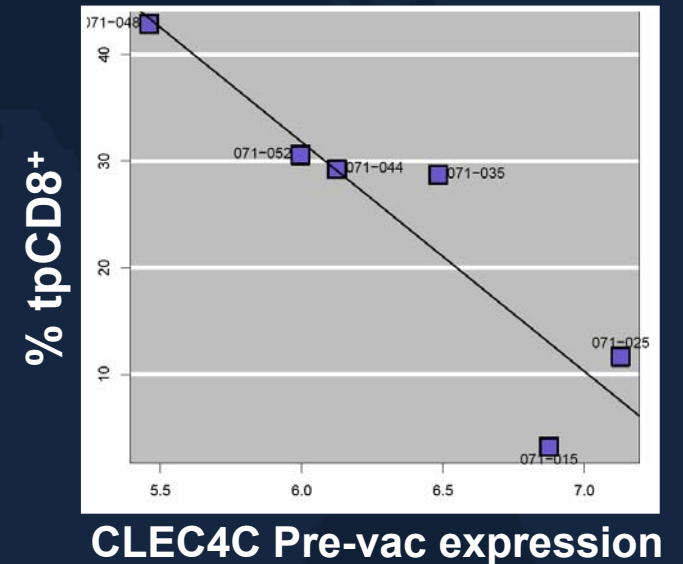
IDO/INDO activity inhibits T cells, particularly CD8⁺ T cell proliferation
(Forouzandeh et al., 2008)

pDC CD8⁺ T cell polyfunctionality signature:

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



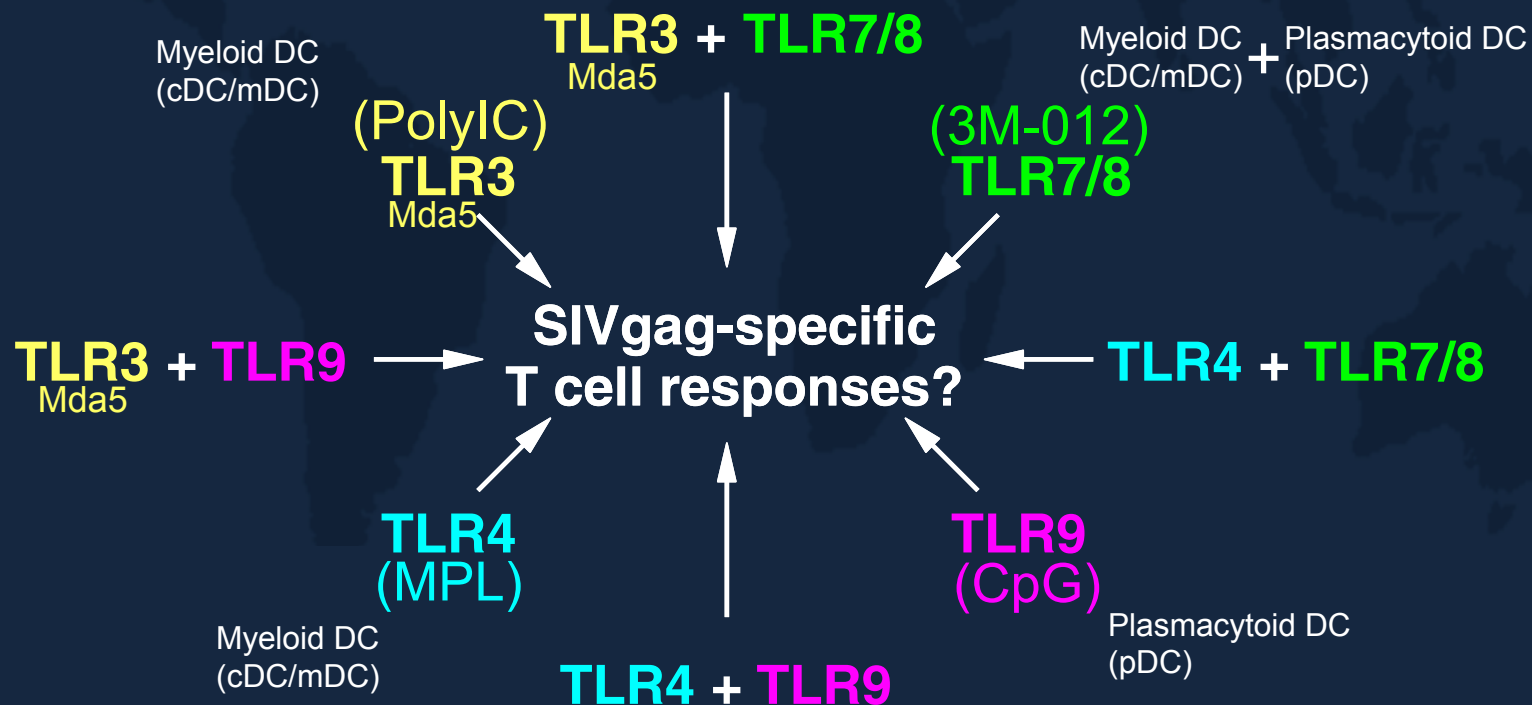
— Corr.
to Pre-vac.



NHP studies: TLR ligands as adjuvants

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

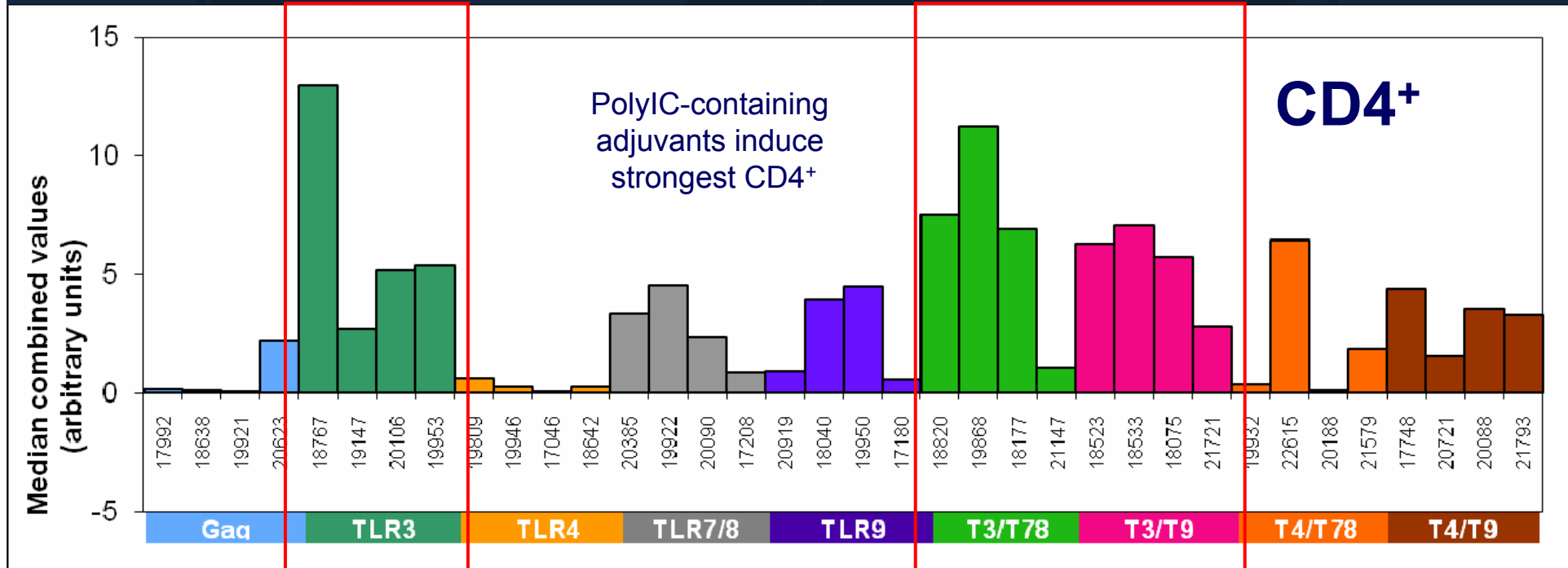
- NHP immunized with SIV Gag protein + Montanide + TLR ligands (Haesun Park & Louis Picker-OHSU, Bob Seder-NIH/VRC)
- Profile SIVgag-specific CD4⁺ & CD8⁺ T cell responses (Haesun Park & Louis Picker-OHSU, Bob Seder-NIH/VRC)
- Profile gene expression responses in PBMC and LN (ISB)



PolyIC-containing adjuvants (TLR3) induce strongest CD4⁺ responses

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

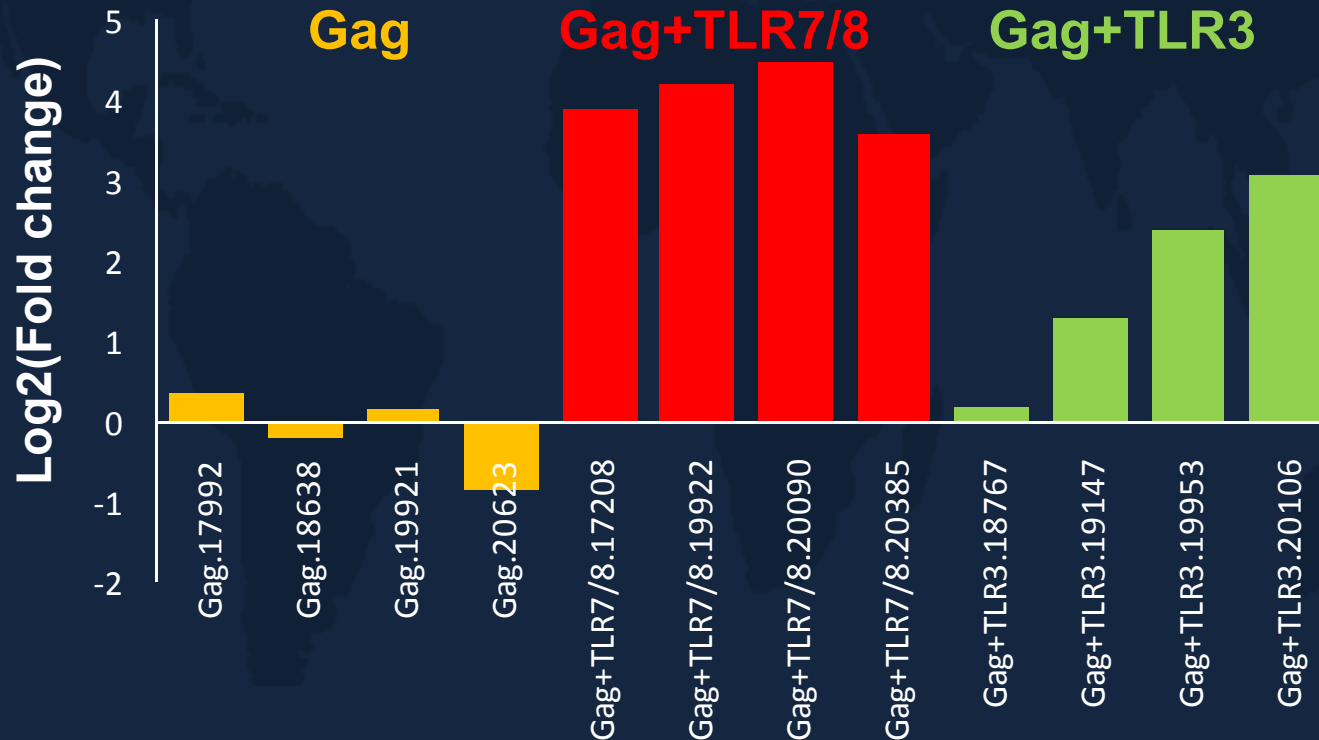
%CD4⁺ responding to SIV gag with TNF or IFN γ



Median-scaled values over time for %CD4⁺ T cells responding to SIV gag with TNF or IFN γ 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:

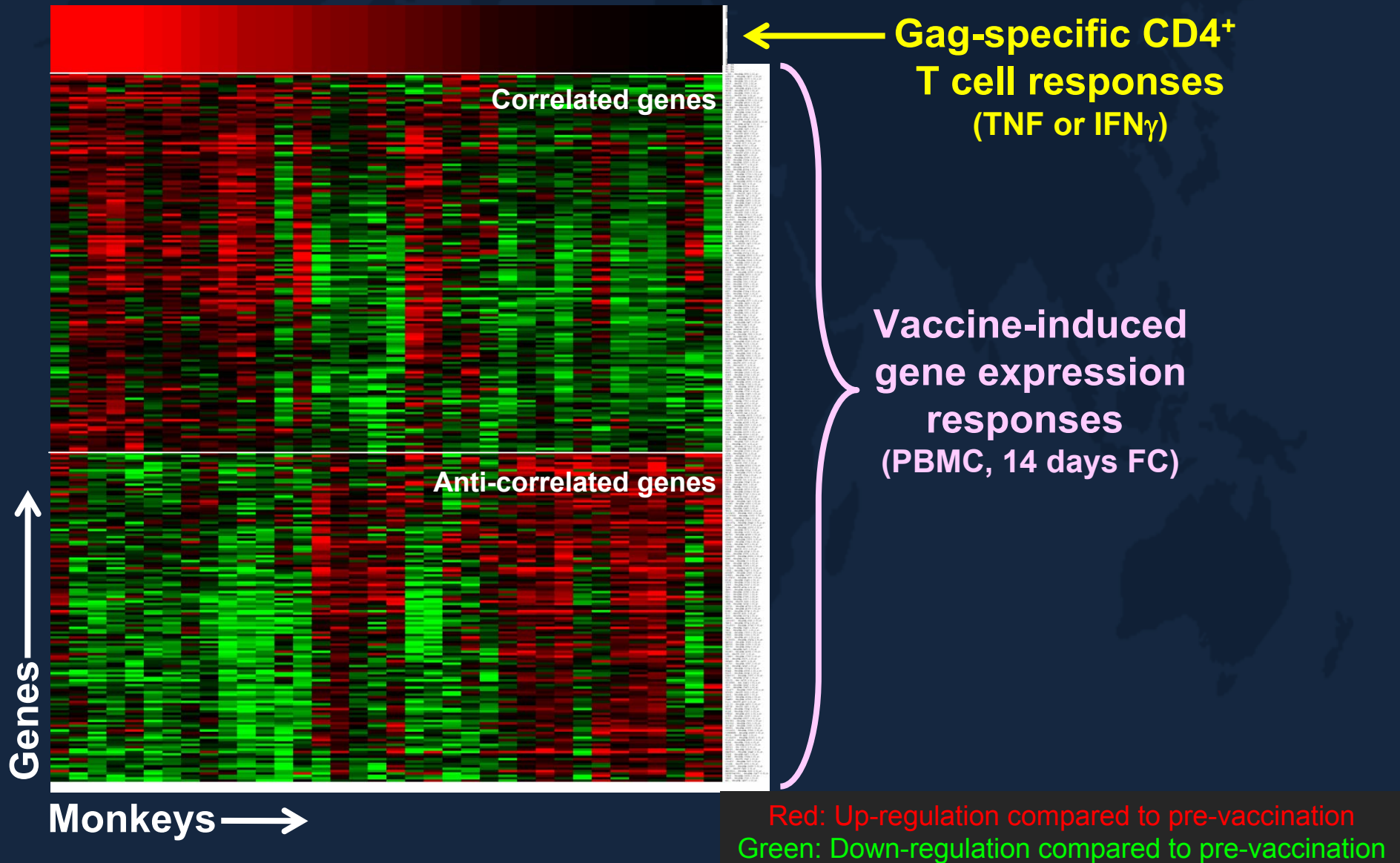
Correlate T cell response magnitudes
with gene expression responses



← **Gag-specific CD4⁺
T cell responses
(TNF or IFN γ)**

Identify novel predictors of immunity:

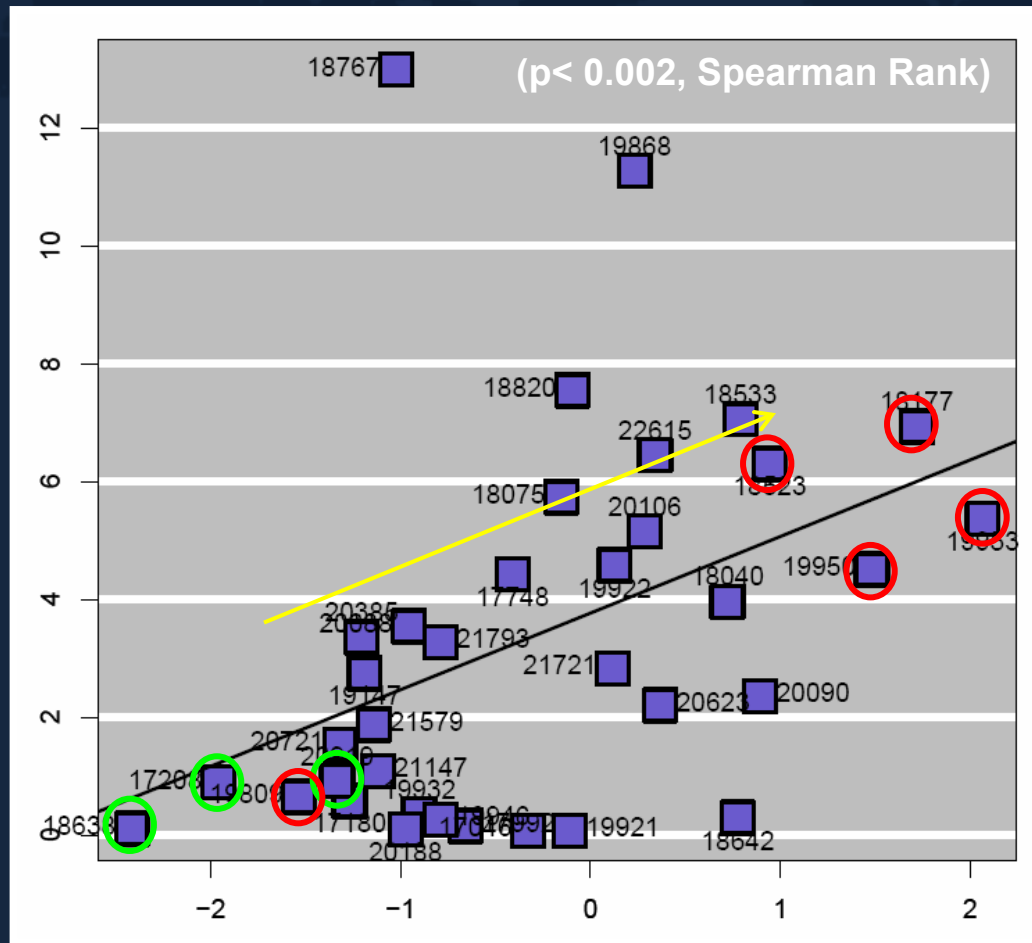
Correlate T cell response magnitudes
with gene expression responses



Signature for CD4⁺ T cell response

Vaccine-induced CCR2B expression (PBMC, 14 days)

Gag-specific CD4⁺ T cell response
(%responding TNF or IFN γ)



CCR2B expression
log₂(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)

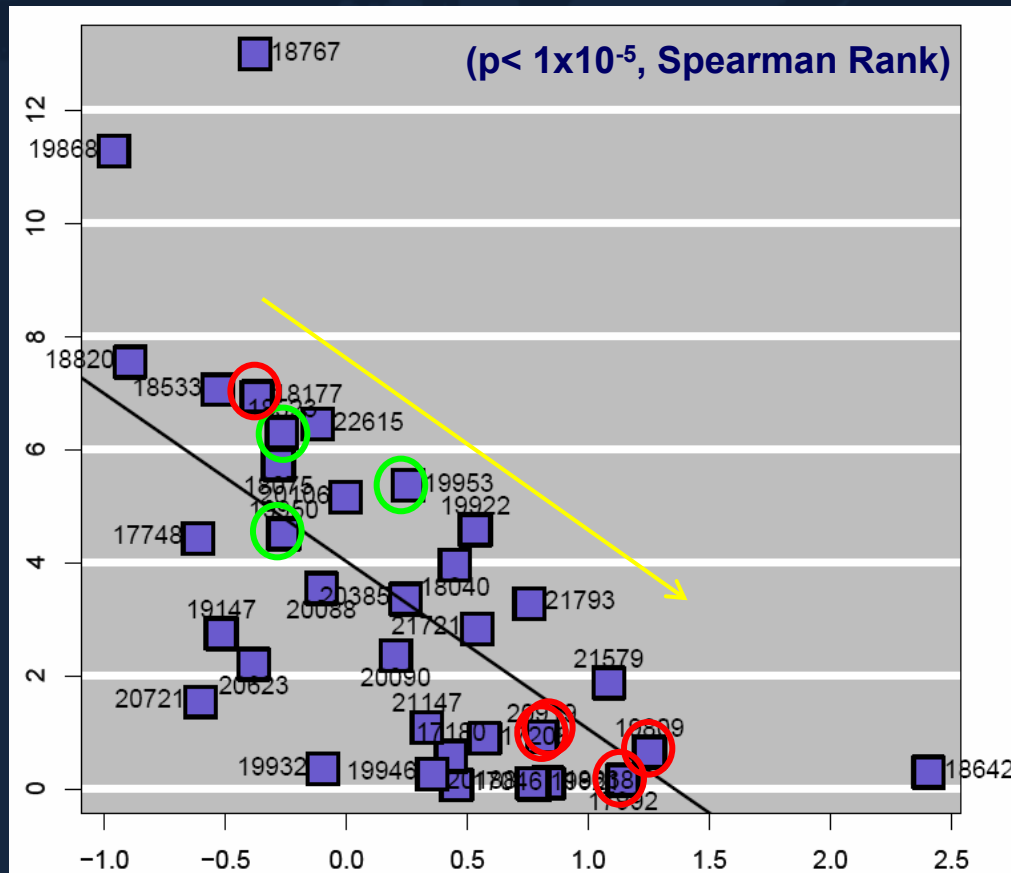
-Important for CD4⁺ T cell recruitment to lung during influenza infection (Wareing et al., 2007)

Stronger induction of CCR2B is associated with stronger CD4⁺ T cell responses

Signature for CD4⁺ T cell response

Vaccine-induced KLF5 expression (PBMC, 14 days)

Gag-specific CD4⁺ T cell response
(%responding TNF or IFN γ)



KLF5 expression
log₂(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⁺ 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⁺ subjects
 - CD8⁺ T cell polyfunctionality signature: ex, IDO, pDCs
 - Signatures for CD4⁺ T cell response: ex, CCR2B and KLF5
- We made some surprising observations
 - Baseline Ad5⁺ expression signature: pre-existing altered expression of immune relevant genes, potential roles for IL-2, IRF1
 - CD8⁺ 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⁺ magnitude, CD4⁺ polyfunctionality, viral load after challenge (NHP), etc.
- Validate baseline Ad5⁺ signatures by profiling additional Ad5⁺ and Ad5⁻ 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⁺ signatures, CD4⁺ signatures, DC subsets, NK-cells, etc.



Thank you!

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