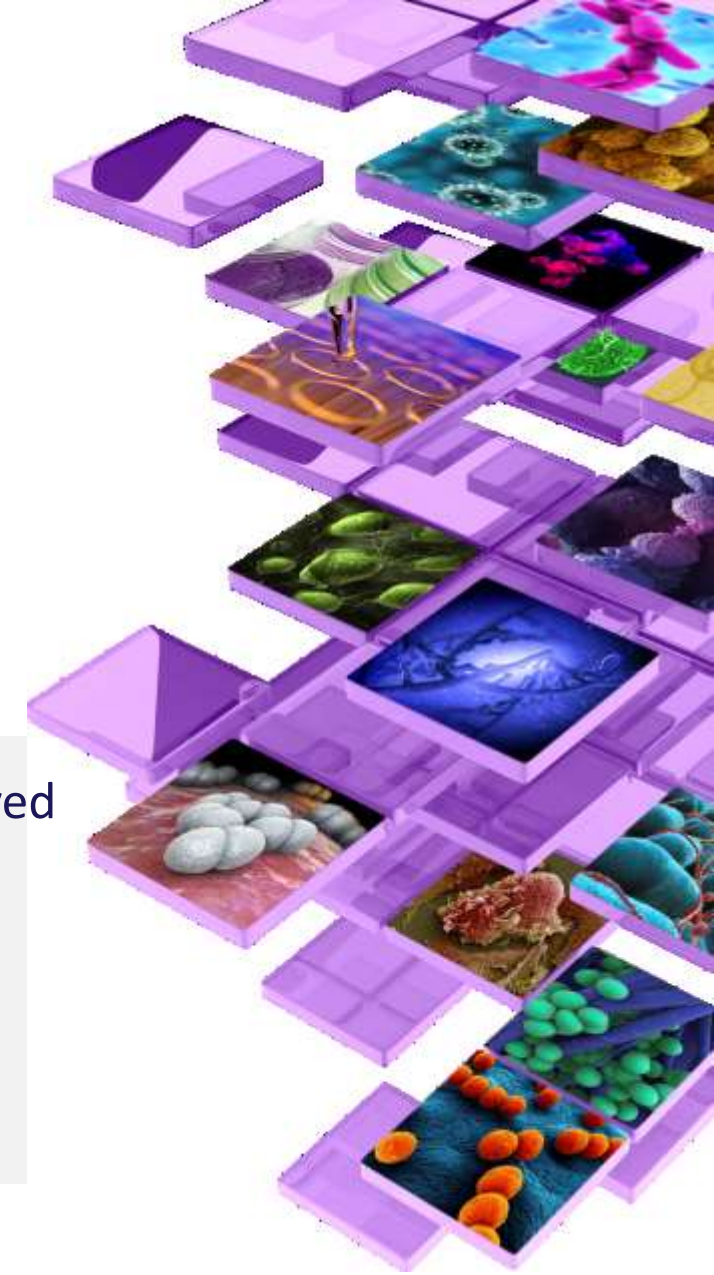
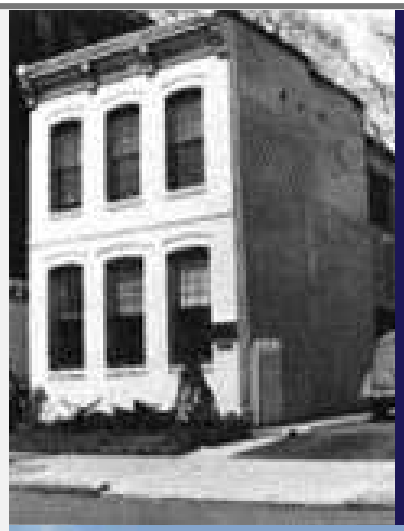


Functionally characterized human PBMCs: An improved *in vitro* model of human immune response

Alexei Miagkov, Ph.D.
Senior Scientist, Immunology, ATCC
June 30th, 2016

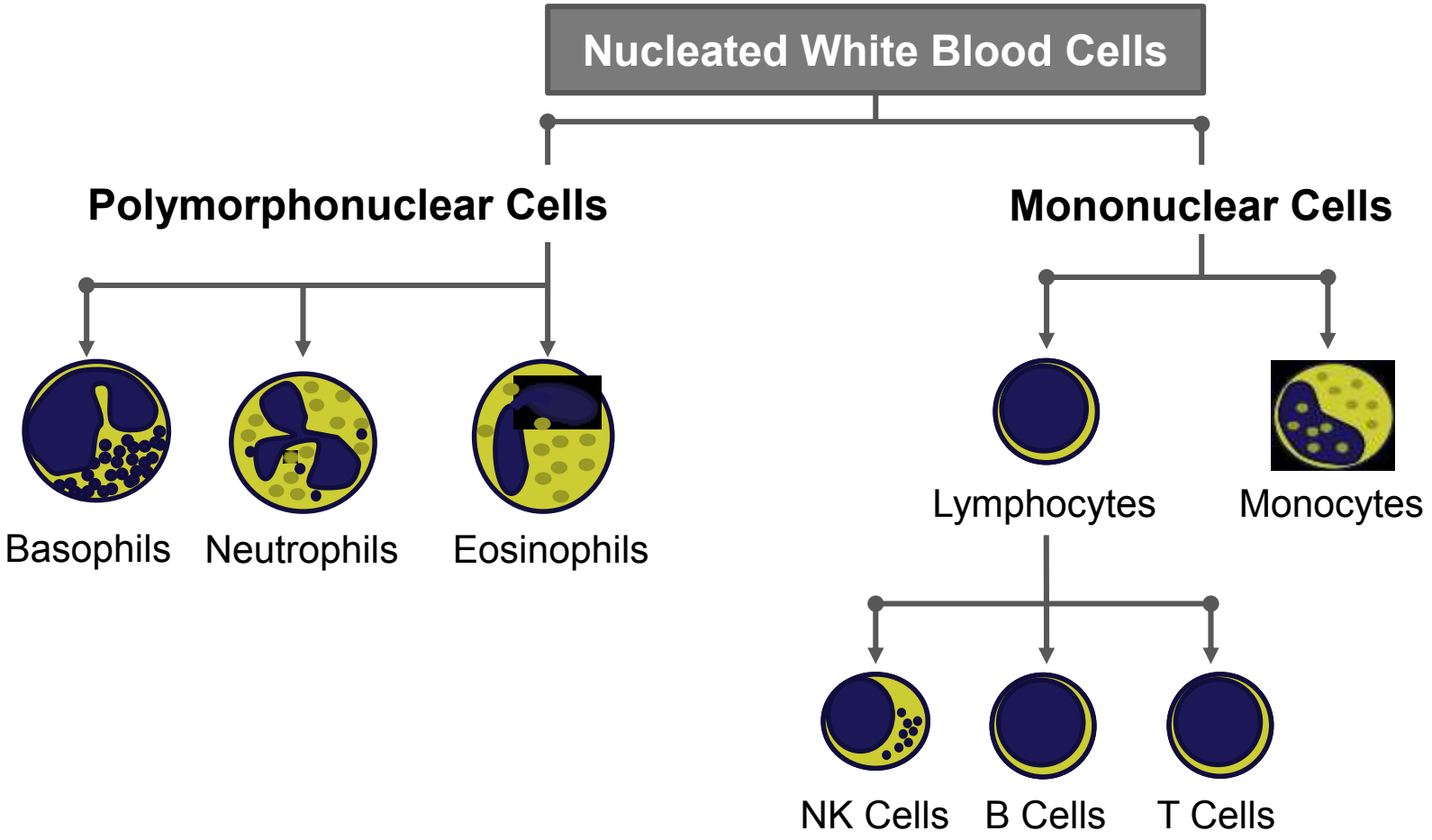
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one-third with advanced degrees



Established partner to global researchers and scientists

Peripheral blood mononuclear cells (PBMC): Cellular composition

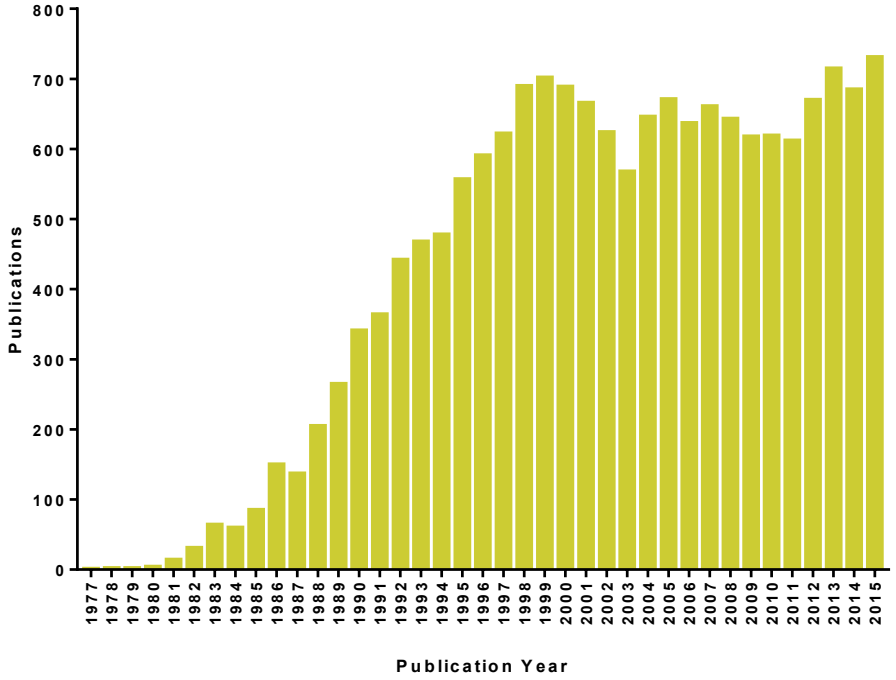


Peripheral blood mononuclear cells

The main source of the primary human cells in immunology research

Currently used in:

- Basic research
- High-throughput drug screening
- Development of vaccines, biologics, and cellular therapeutics
- Assessment of immunogenicity and immuno-toxicity of new drug candidates



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Functional activity of human PBMC is extremely variable

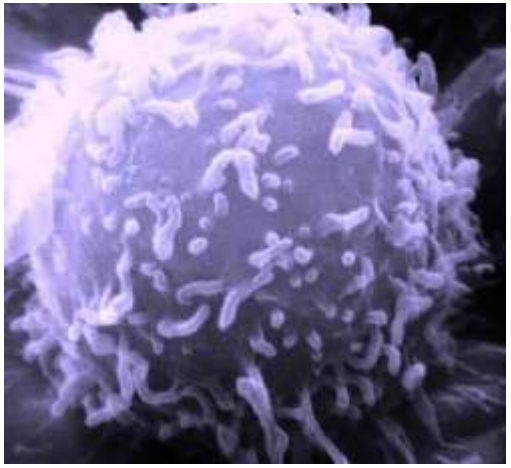
In this webinar, we will discuss the variability of PBMC functional activity and the main approaches to minimizing the impact of PBMC variability on your research

Salmonella invading an immune cell, photo credit: NIAID

Factors affecting PBMC assay variability

Donor Variability

- Genetic diversity
- Environmental factors
  - Immunizations
  - Nutrition
  - Latent infections



Cells/Assay Variability

- Cryopreservation technique
- Cell viability
- Cellular composition (immunophenotype)
- Medium composition
- Quality of cell activating reagents

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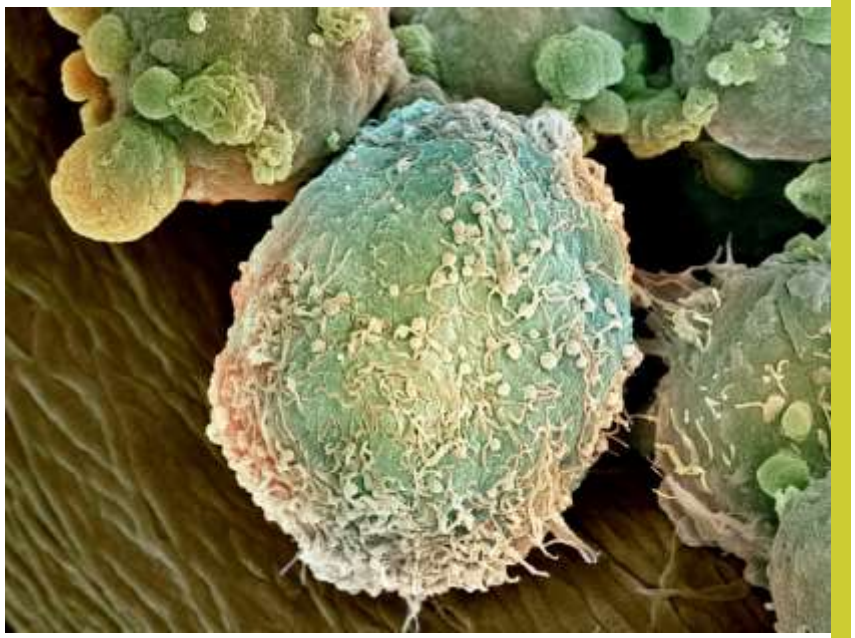
Minimizing the impact of donor variability

- Stable, pre-qualified pool of adult healthy volunteer donors
- Complete donor information
  - Age, gender, ethnicity, blood type, and HLA class I and II
- At the time of collection, all donors were screened and certified to be free of:
  - Syphilis
  - Hepatitis B virus (HBV)
  - Human T-lymphotropic virus (HTLV)
  - Human immunodeficiency virus (HIV)
  - West Nile virus (WNV)
  - *Trypanosoma cruzi*



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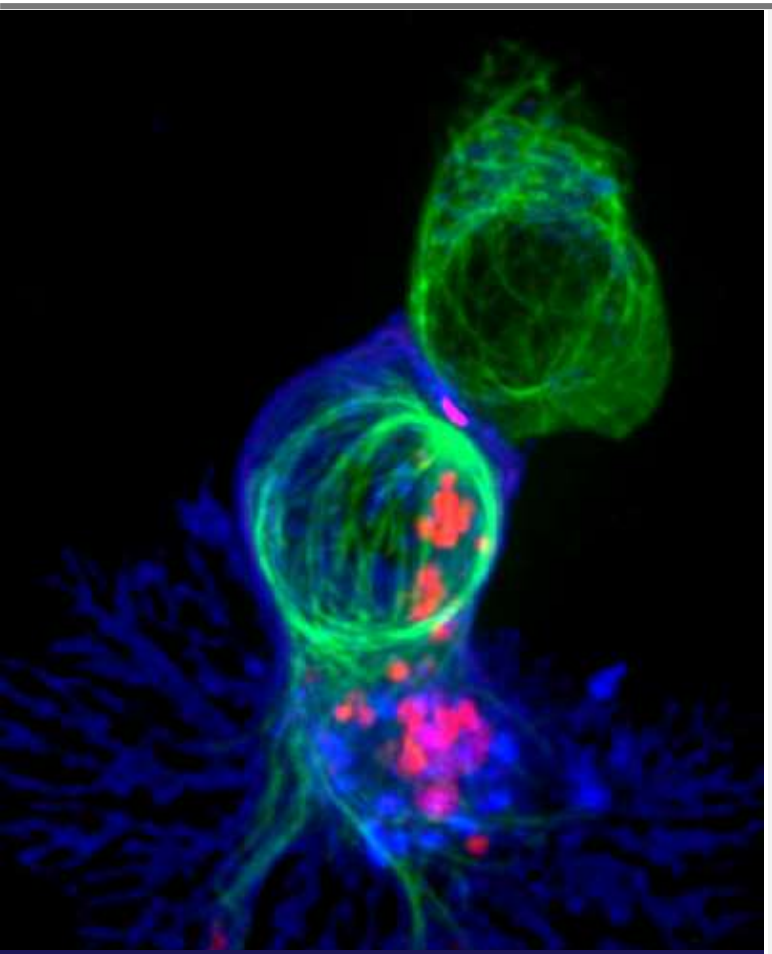
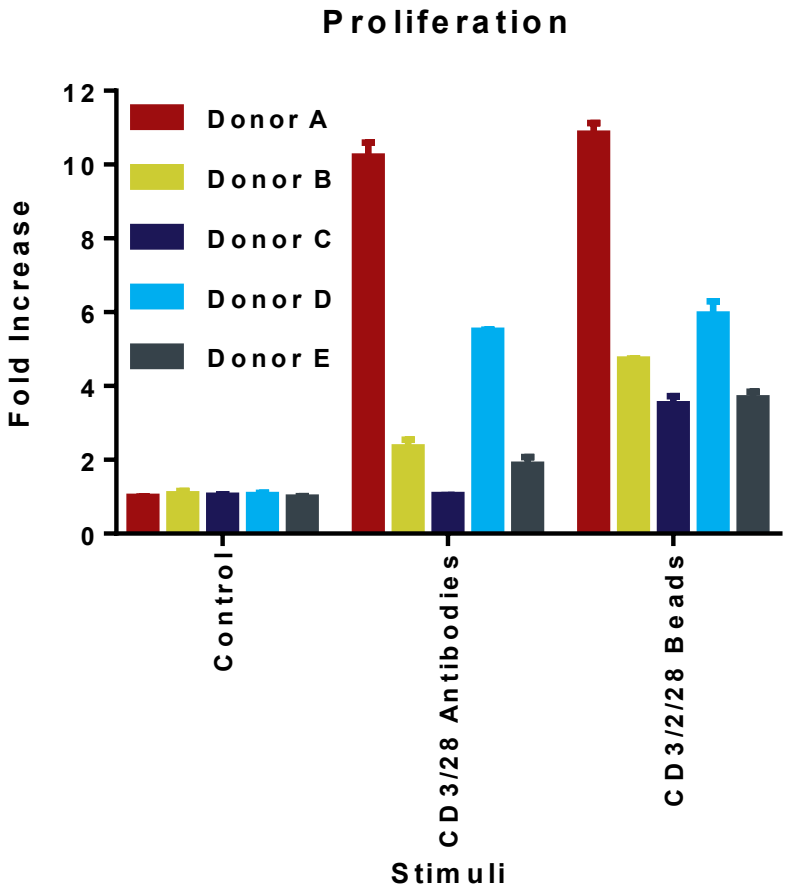
Ensuring quality of the collected cells



Killer T cells, photo credit: Alex Ritter

- Cells purified by gradient centrifugation
- Greater than 95% viability at the time of collection
- Fully validated cryopreservation protocol
  - Optimized protein-free cryopreservation solution
  - Fully controlled freezing and storage conditions
- Comprehensive immunophenotype characterization of the collected cells
- Greater than 90% post-thaw viability

Functional assay



- Select lots of PBMCs can be also characterized for their functional activity in commonly used immunological assays
- We will discuss results of such characterization and discuss how the generated data can help you to select the PBMCs that are most appropriate for your experiment

Functional assay: Donor profile

Group of genetically diverse donors

ID	Age	Gender	Ethnicity	Blood Type
Donor A	43	Male	Caucasian	A+
Donor B	51	Male	Caucasian	B-
Donor C	50	Female	Hispanic	O+
Donor D	30	Male	Hispanic	A-
Donor E	36	Male	Samoan	B+

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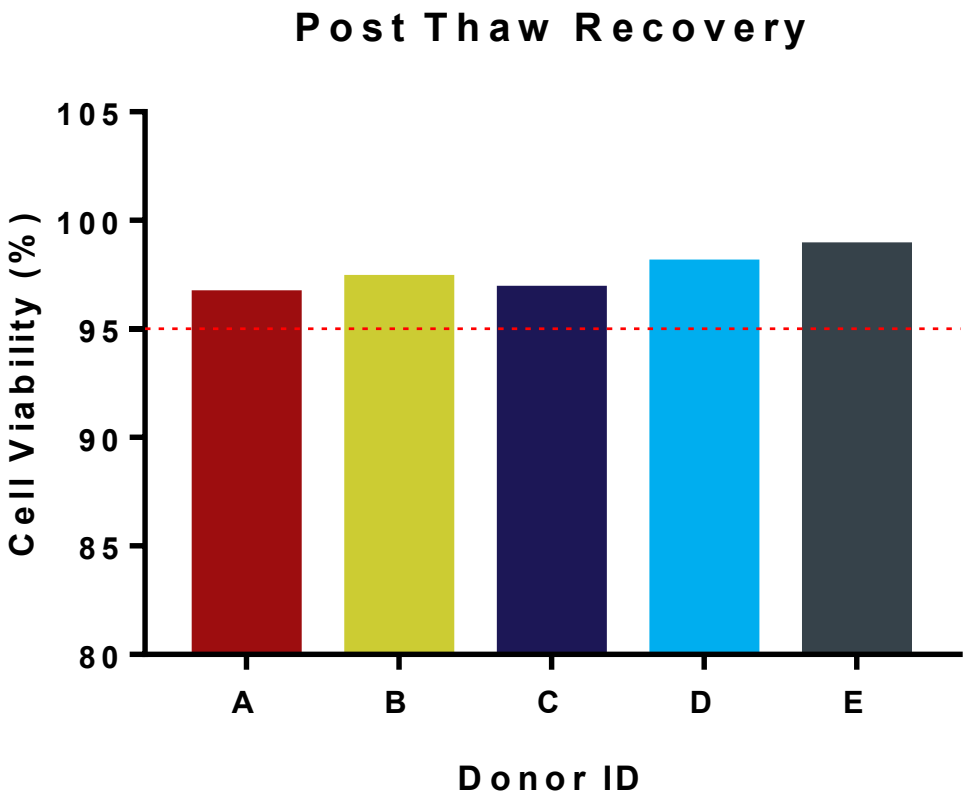
Functional assay: Cell viability



Post-thaw viability of the tested PBMCs was greater than 95%

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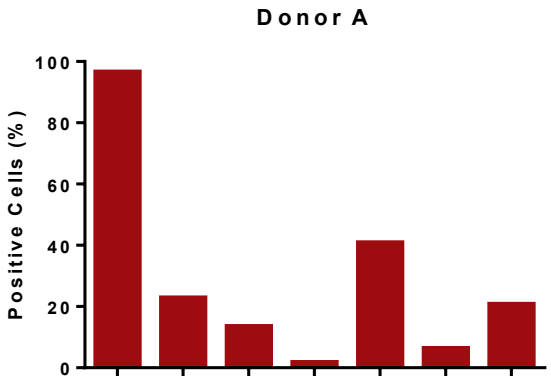
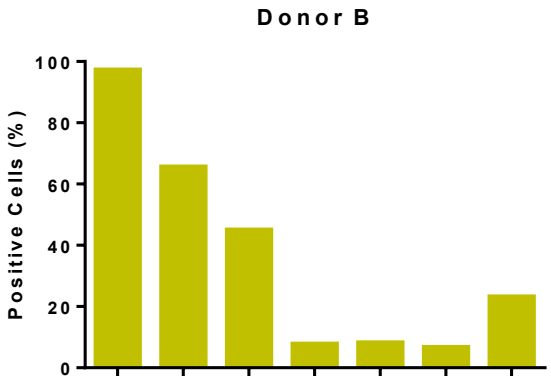
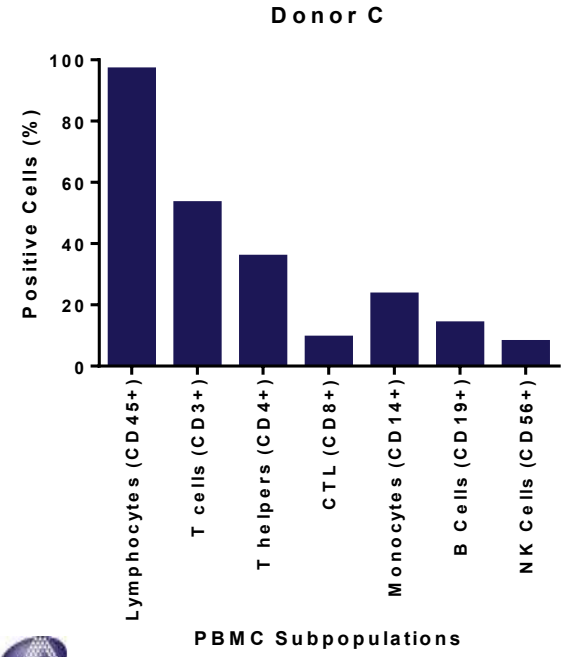
Functional assay: Immunophenotype

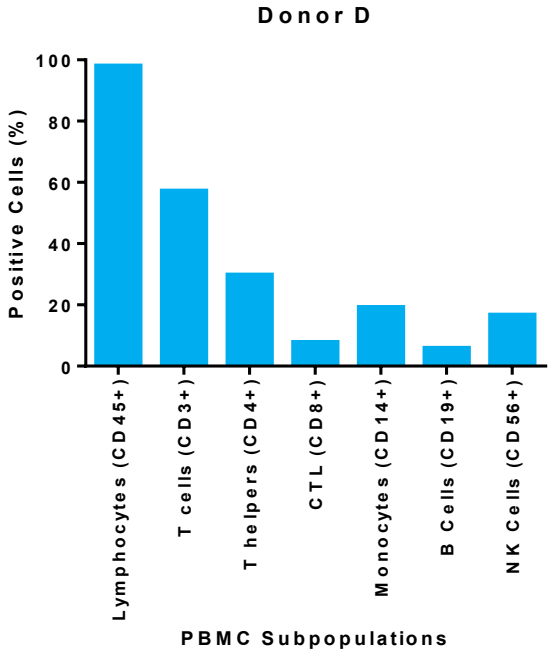
All cells are tested for:

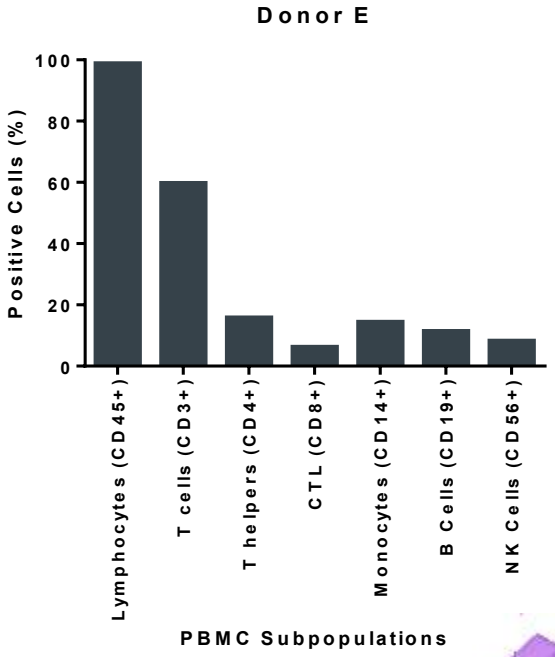
- CD45
- CD3
- CD4
- CD8
- CD14
- CD19
- CD56











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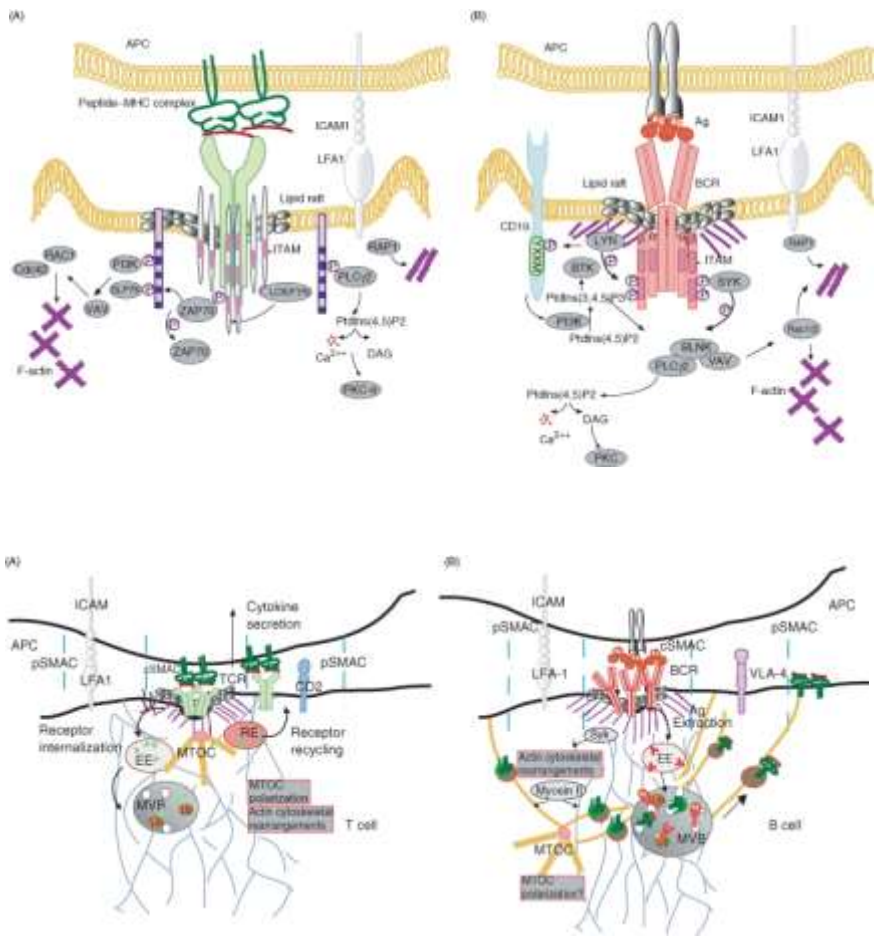
Functional assay: Cell treatment



Yuseff MI, *et al.* Traffic 10(6): 629-636, 2009.

Cells are stimulated with commonly used and commercially available activators of immune response including:

- Soluble and bead-bound T cell specific mitogenic antibodies:
  - Anti-CD3/CD28 soluble
  - Anti-CD2/CD3/CD28 bead-bound
- Plant-derived mitogens:
  - Phytohemagglutinin (PHA)
  - Pokeweed mitogen (PWM)
- Agonists of Toll-like receptors:
  - polyIC (TLR3 agonist)
  - LPS (TLR4 agonist)
  - R-848 (agonist of TLRs 7 and 8)

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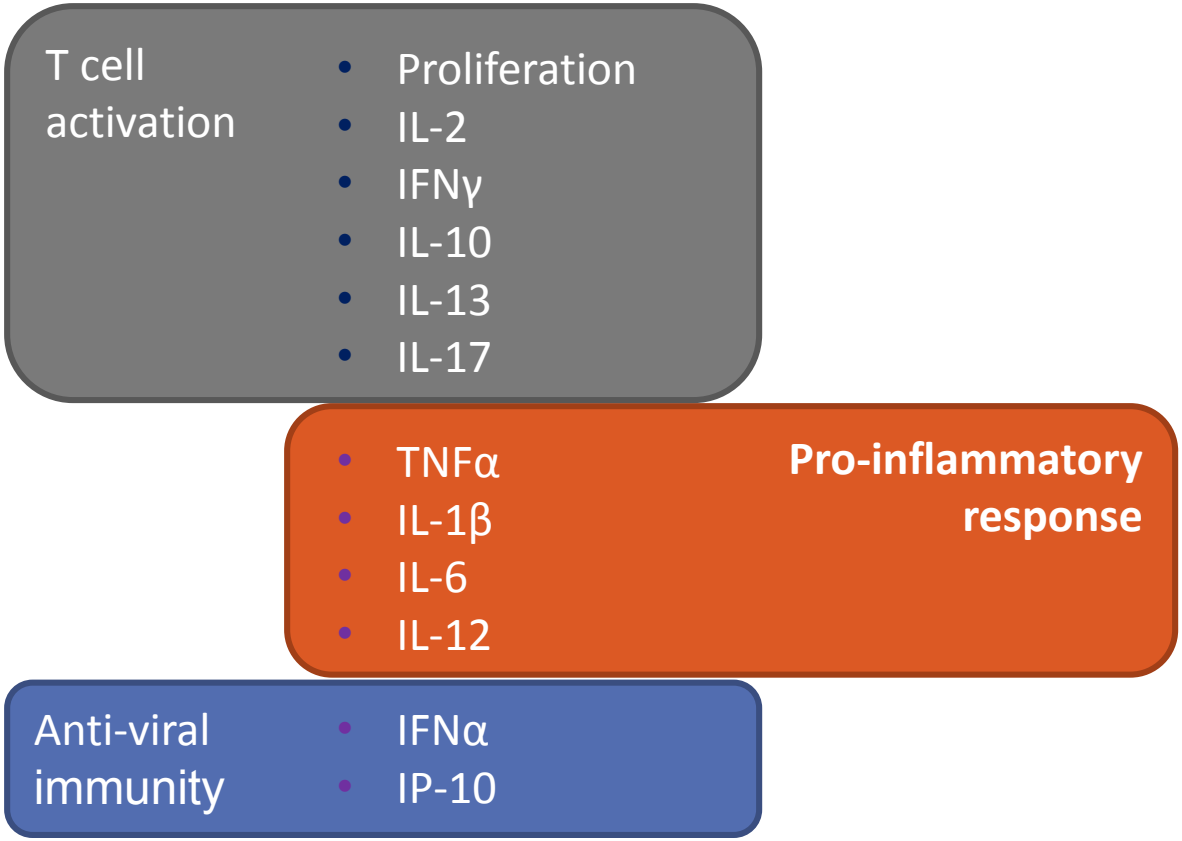
Functional assay: Assay readouts

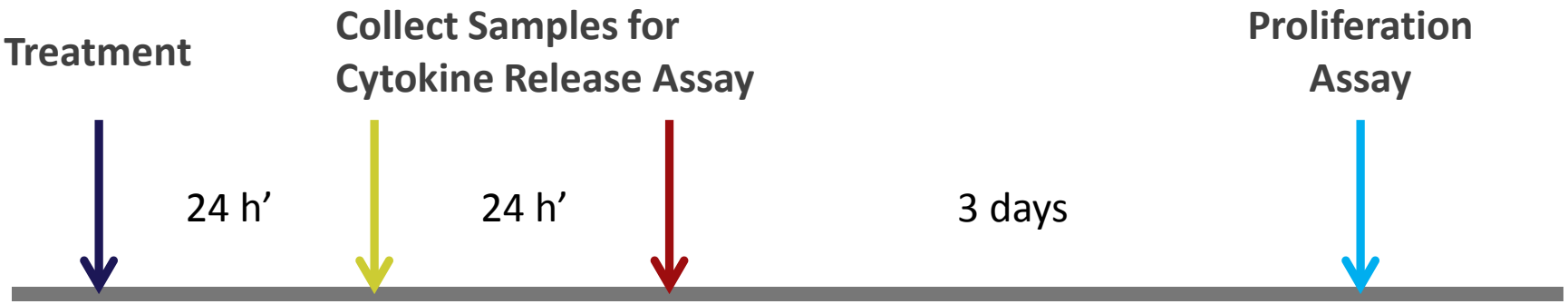
We employed cell proliferation and cytokine expression as the primary assay readouts



The stimuli and the assay readouts were selected to demonstrate an activation of innate and adaptive immune cells within PBMCs

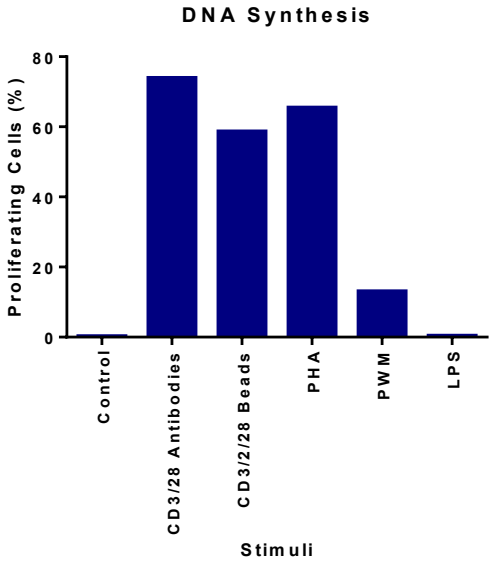
Functional assay: Experimental design

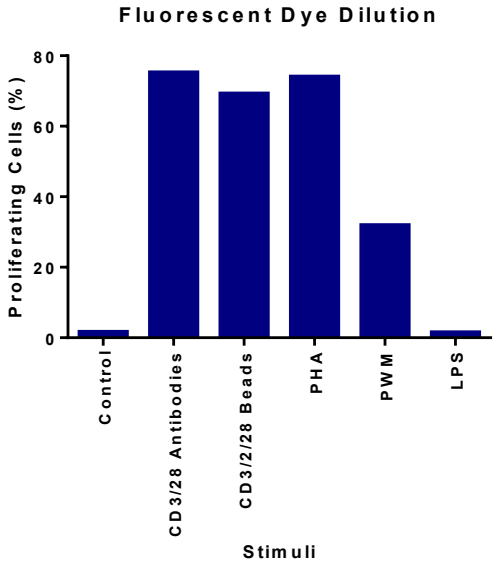
Cells were treated and cultured in chemically defined, serum-free medium

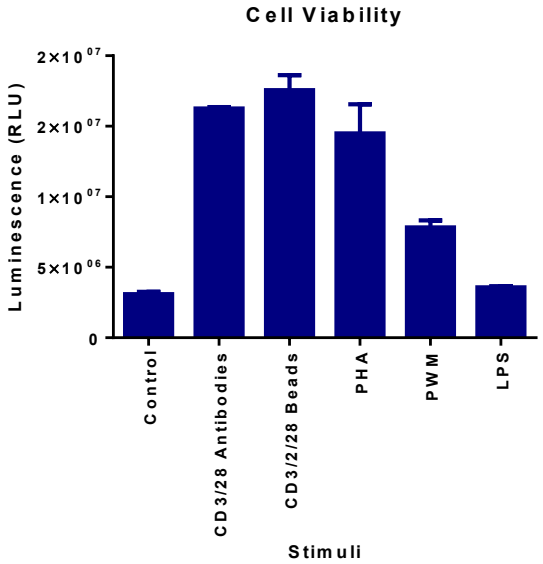


Functional assay: Cell proliferation

Readout selection: DNA synthesis, fluorescent dye dilution, cell viability







All methods provide very robust results; however, the majority of cell viability assays are faster and easier to perform

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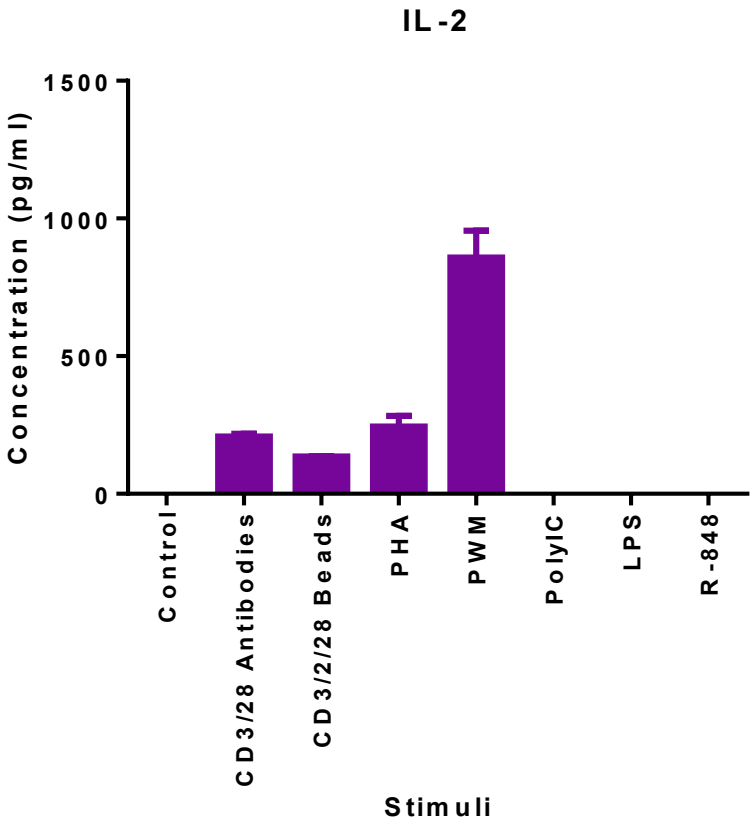
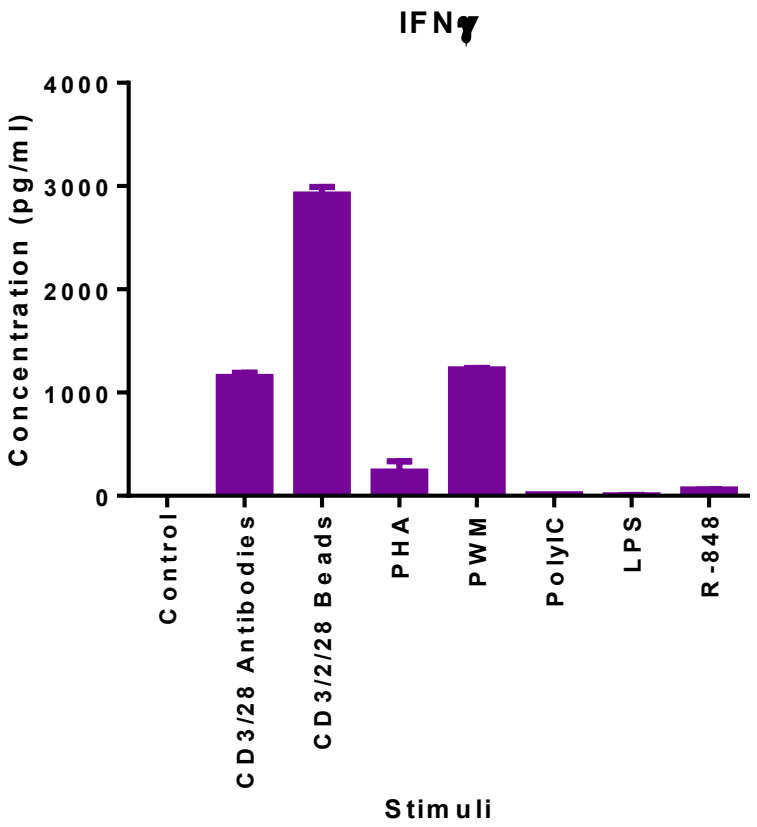
Functional assay: Reagent validation

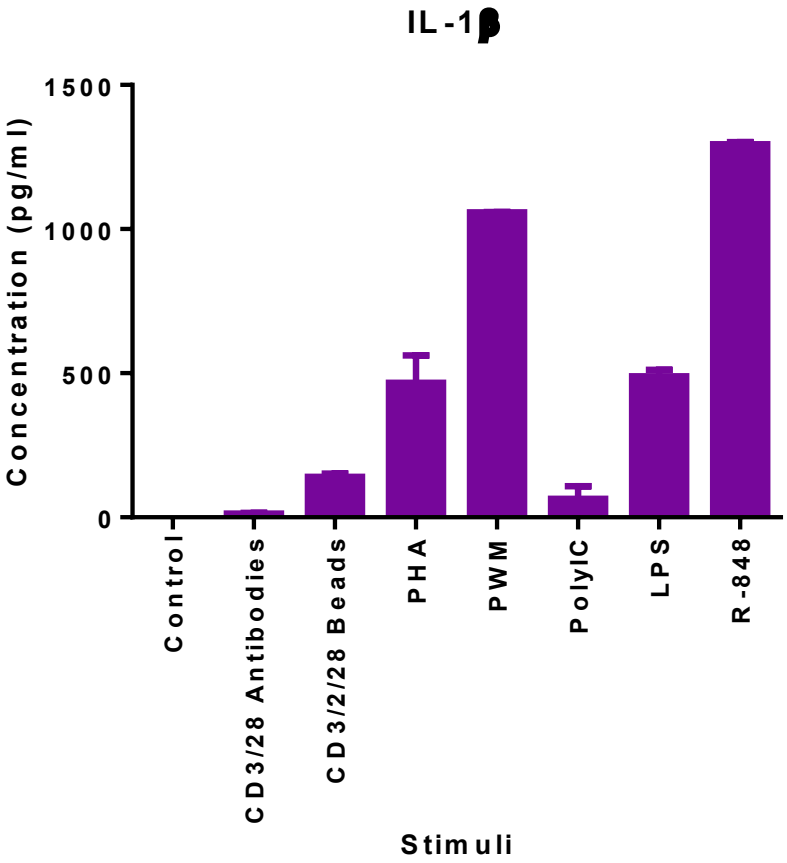


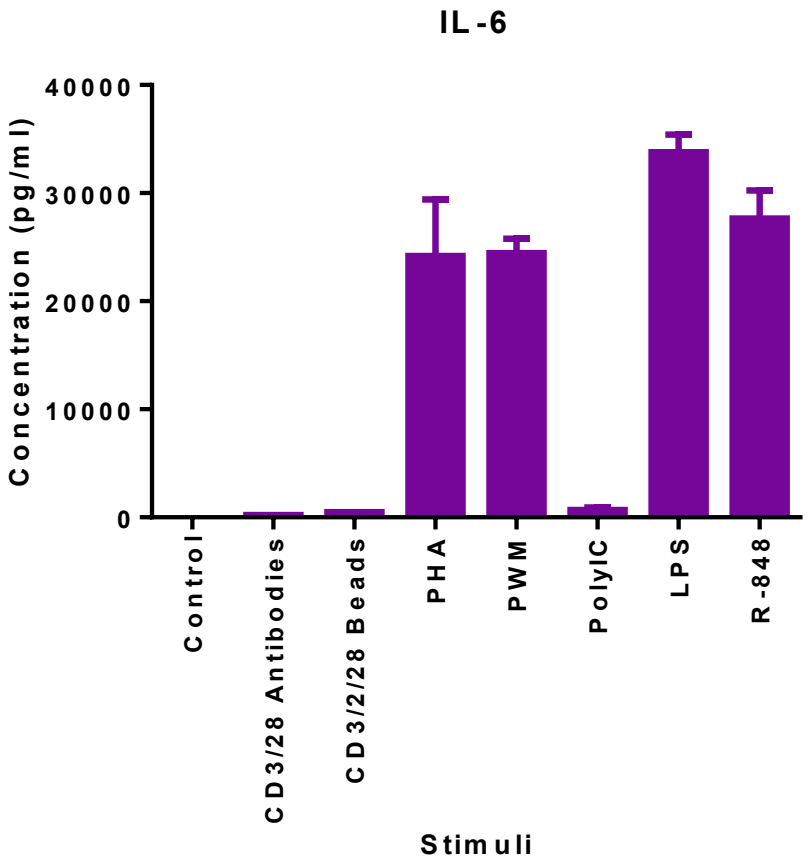


T cell dependent cytokines are induced exclusively by known T cell activators

ATCC

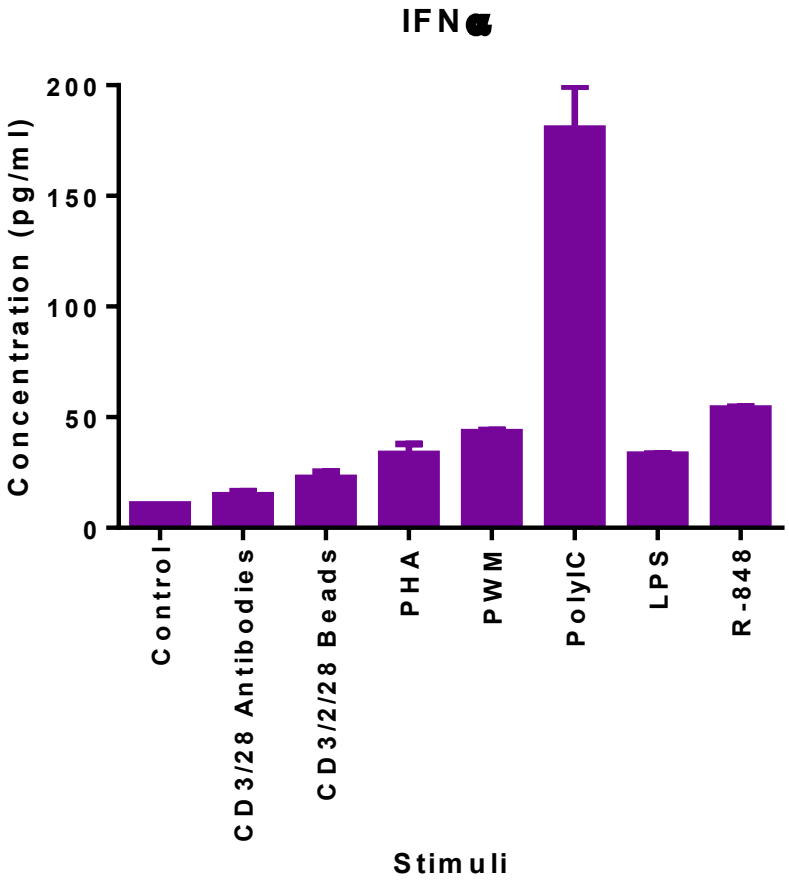
Functional assay: Reagent validation

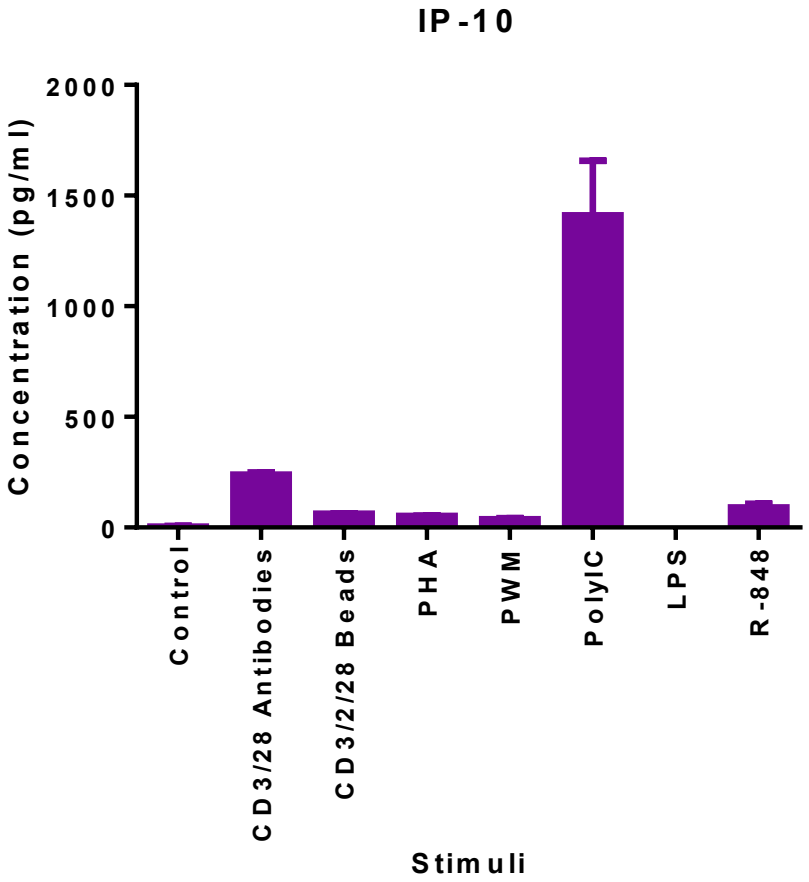




Pro-inflammatory cytokines are induced by plant-derived lectins and TLR ligands

Functional assay: Antiviral cytokines





IFNα and IP-10 expression is induced primarily by TLR3 activation

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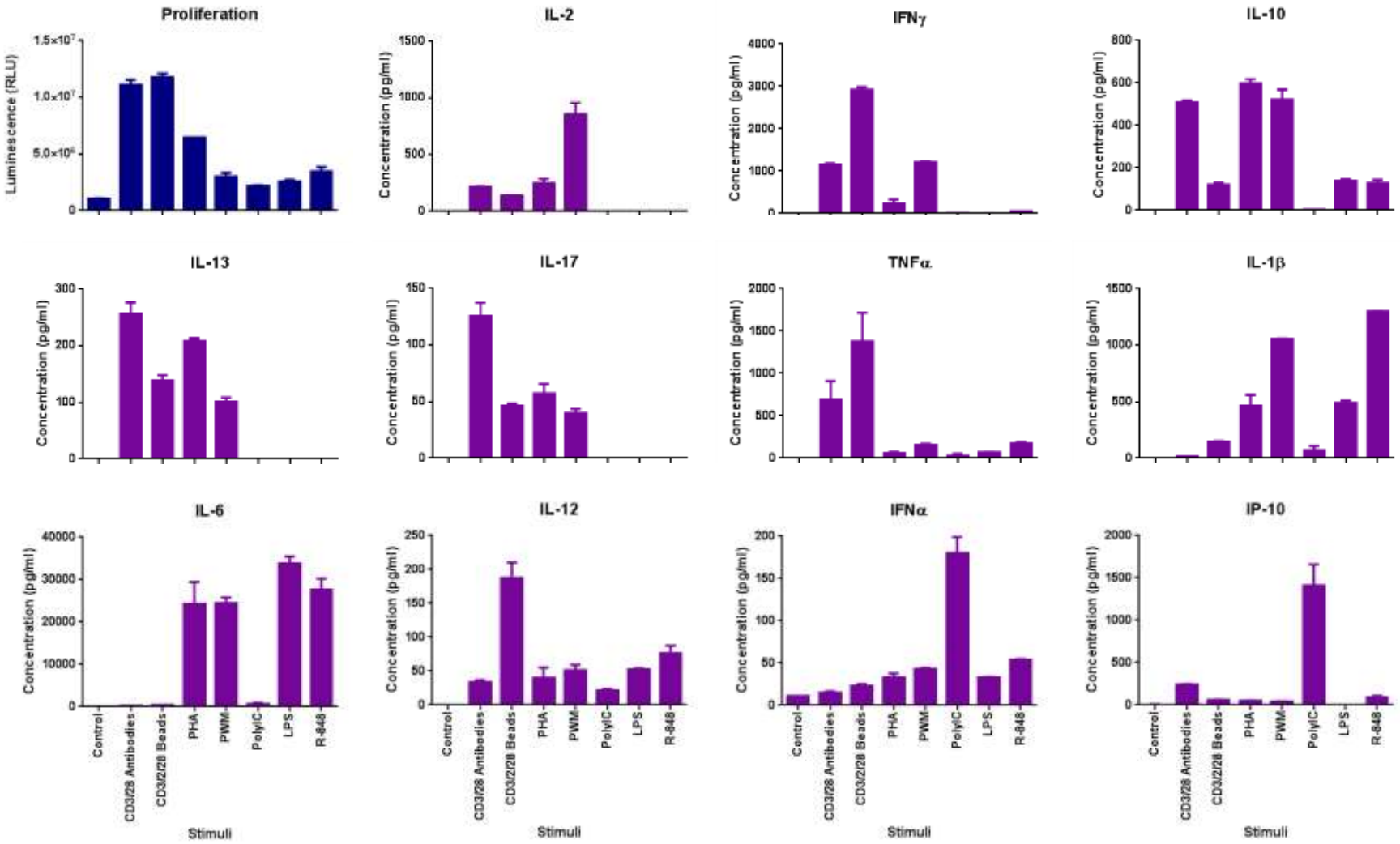
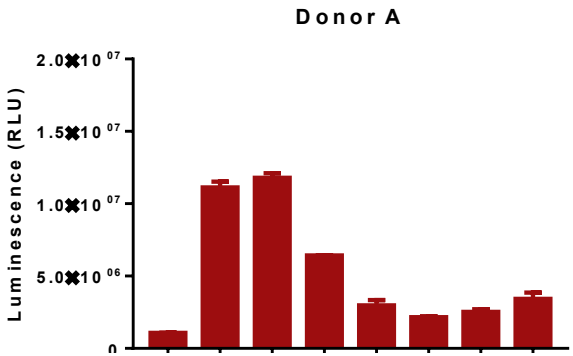
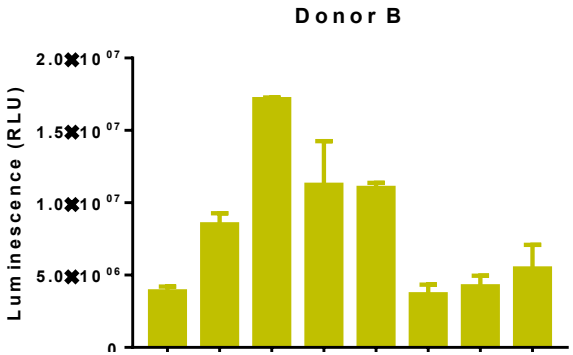
Functional assay: Complete data set for a single donor

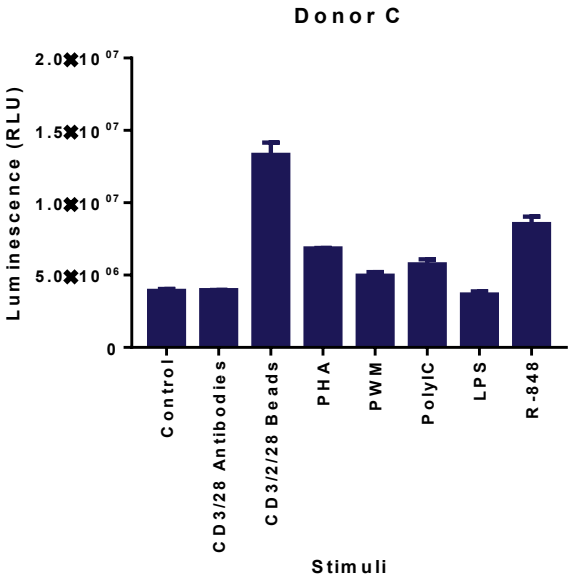


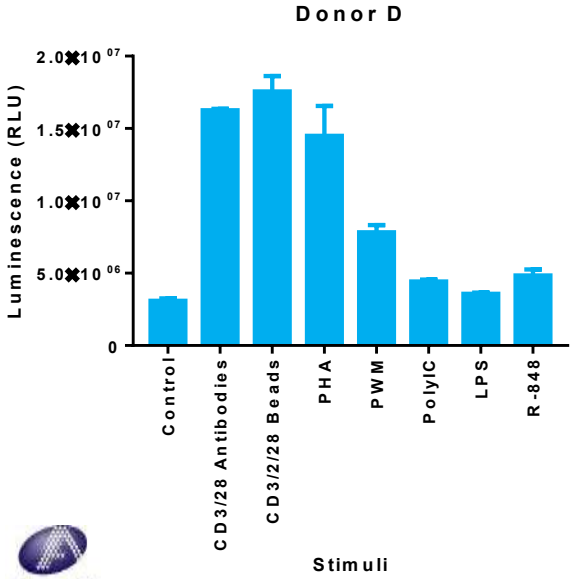
ATCC

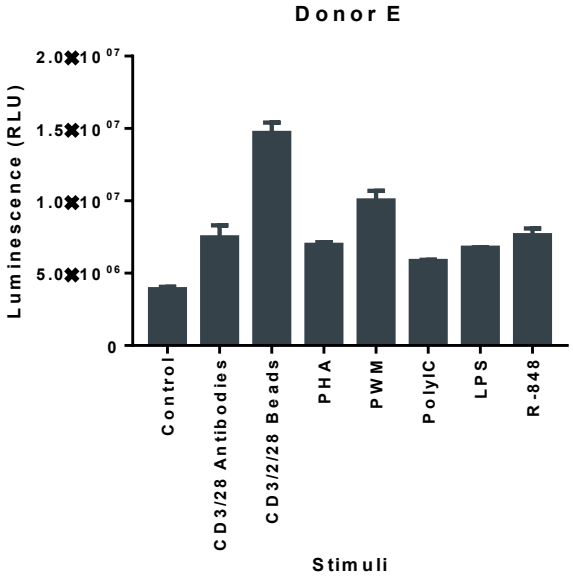
Functional assay: Cell proliferation











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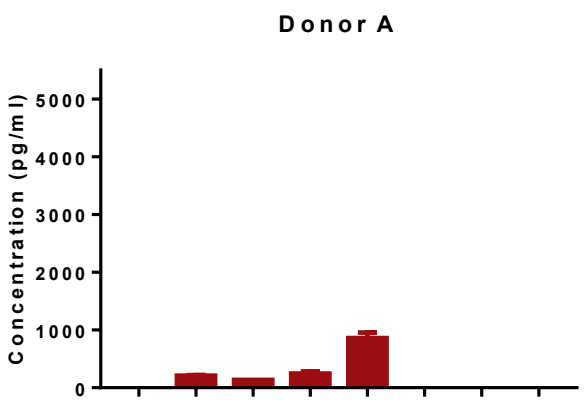
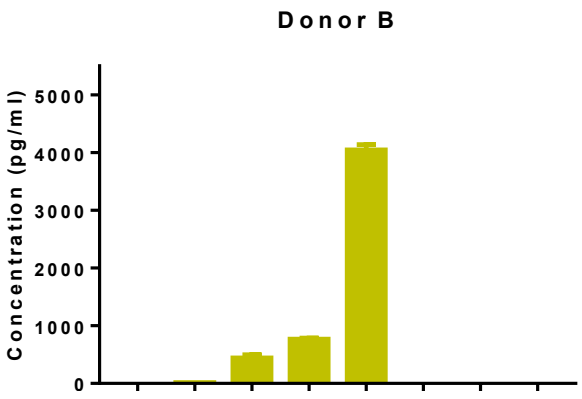
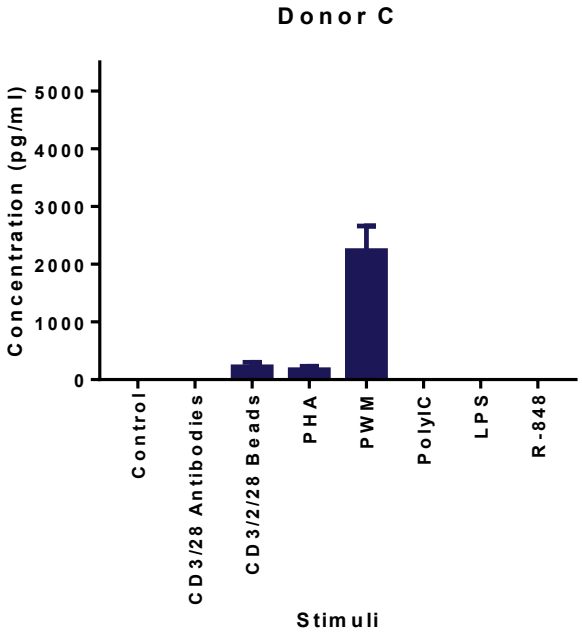
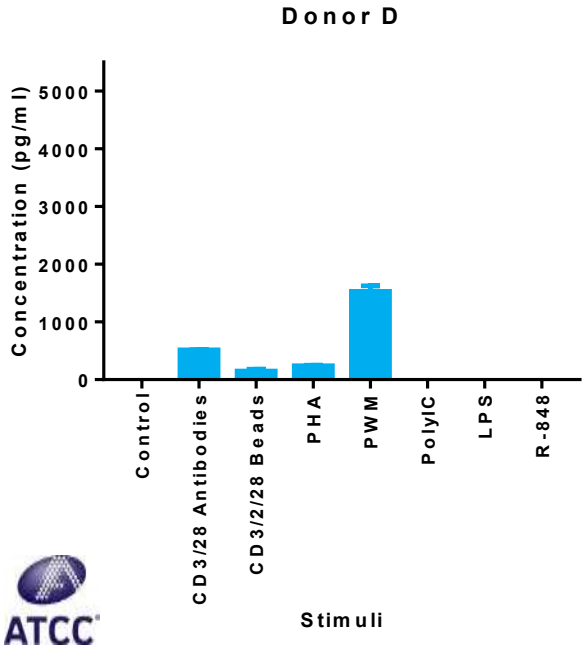
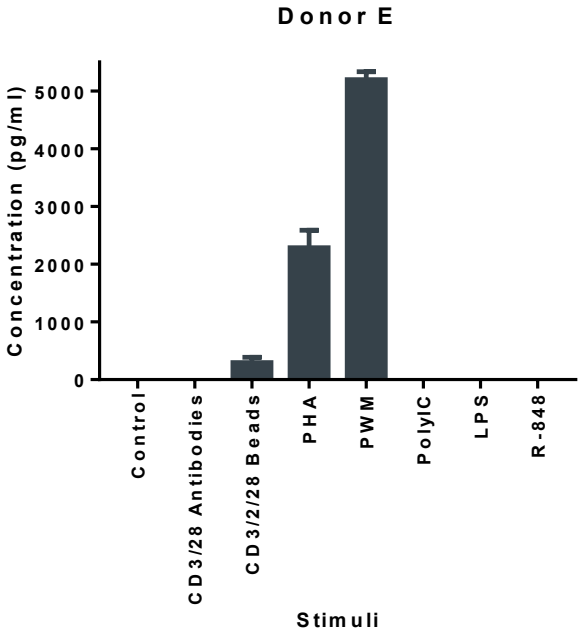
Functional assay: IL-2 expression



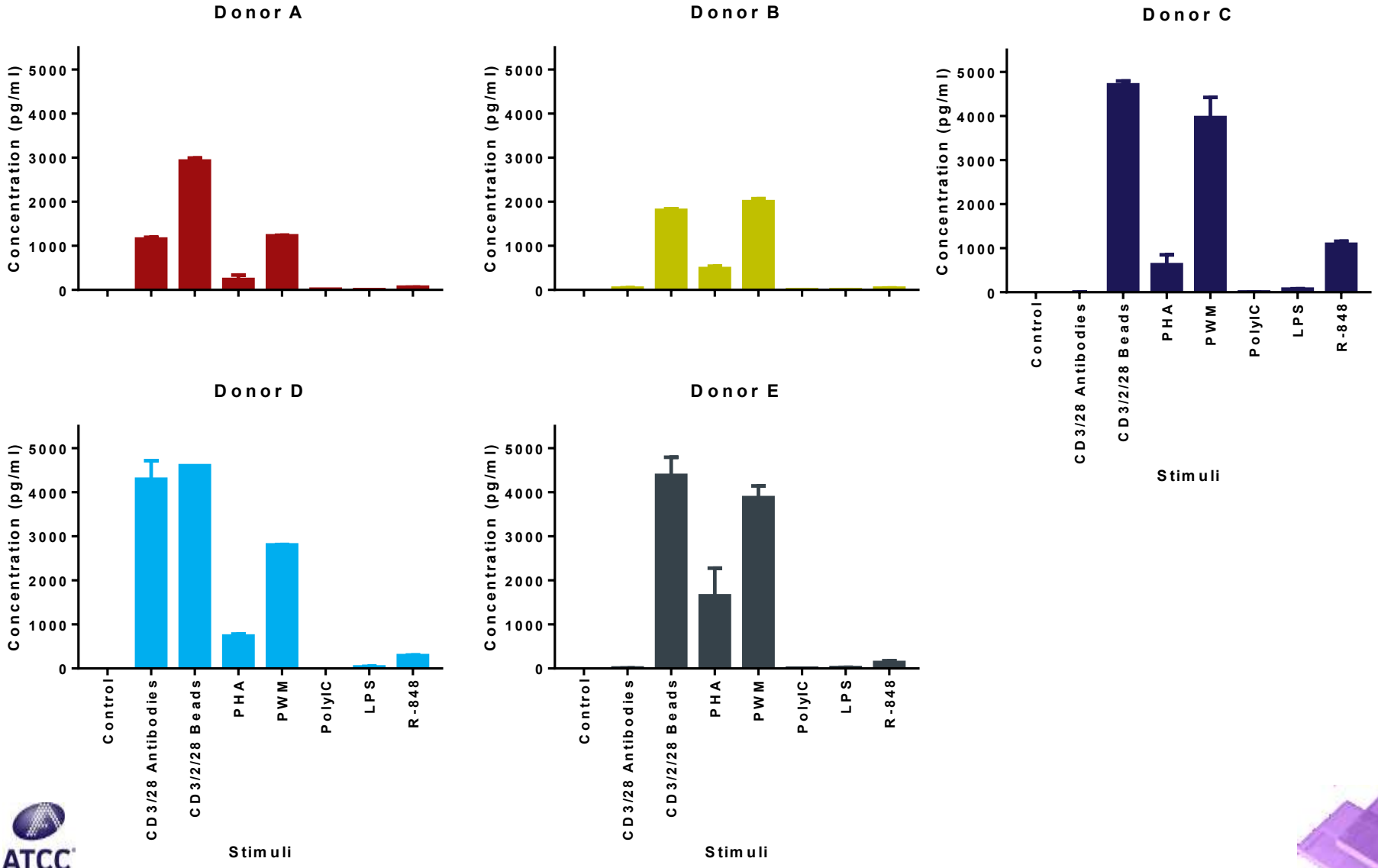




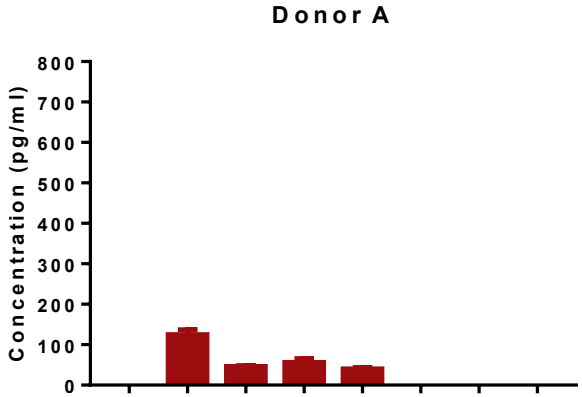


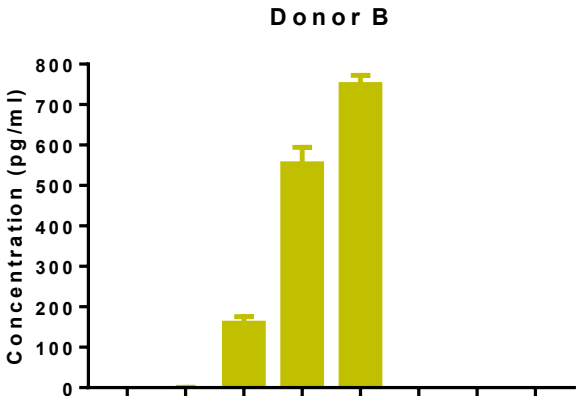


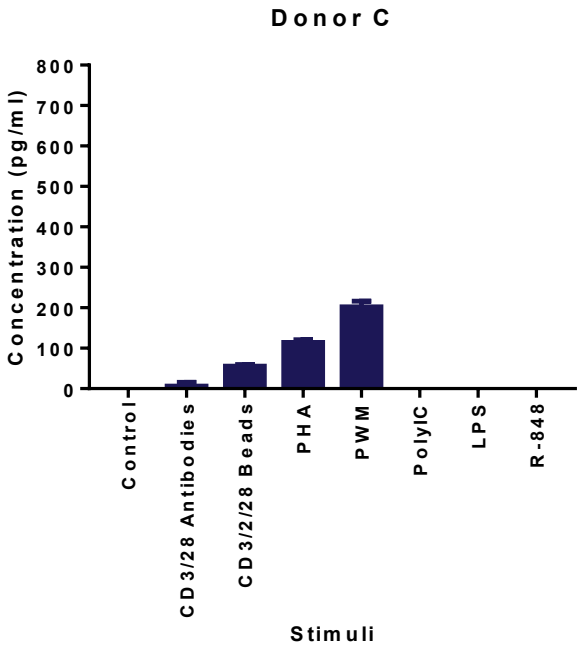
Functional assay: IFNγ expression

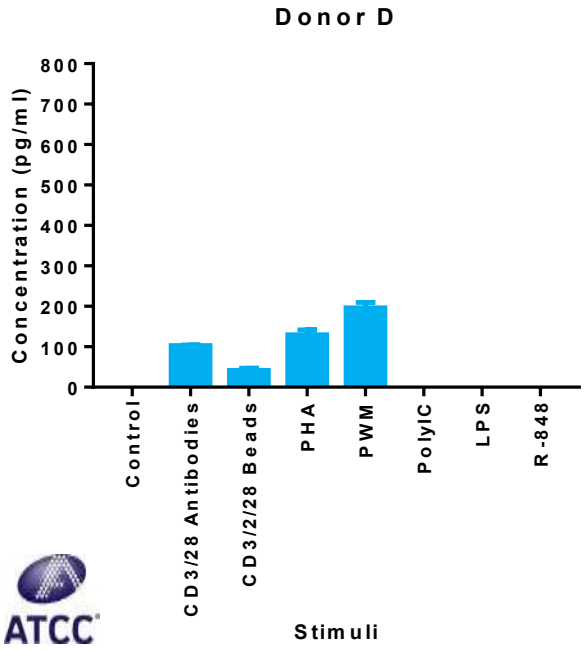


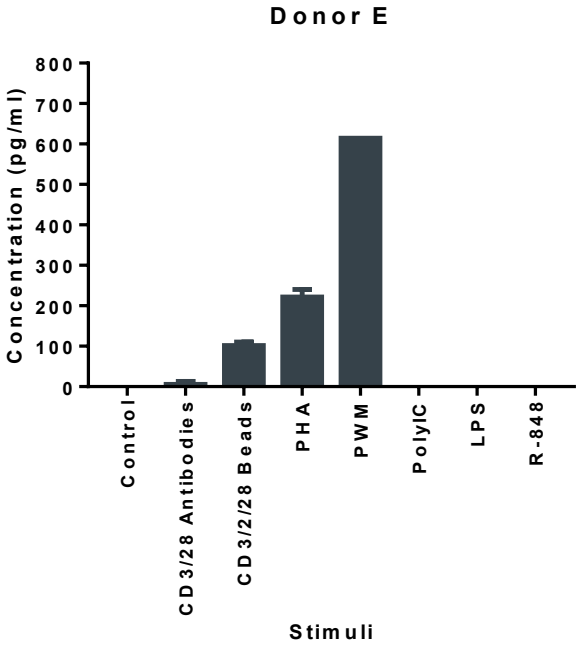
Functional assay: IL-17 expression











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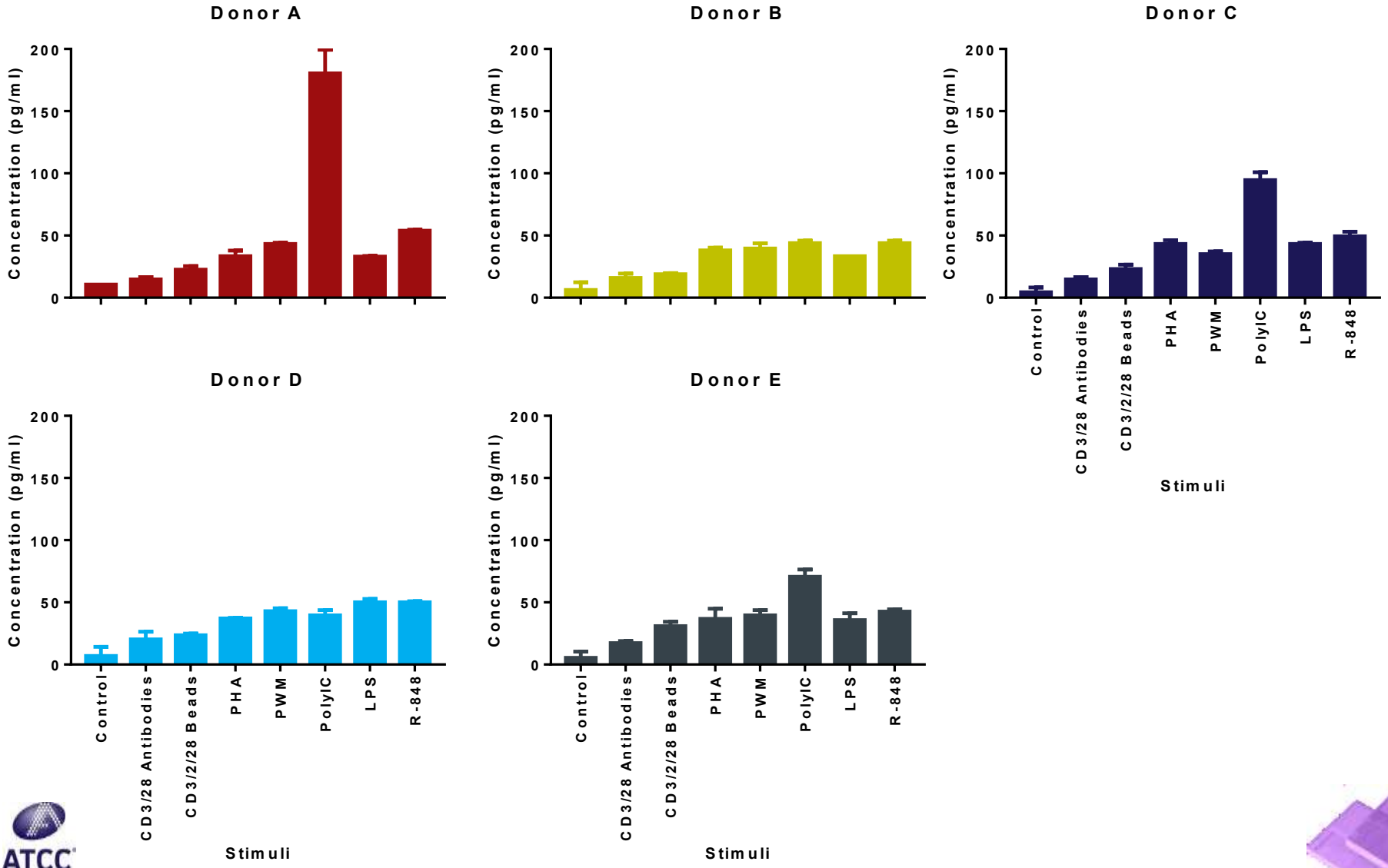
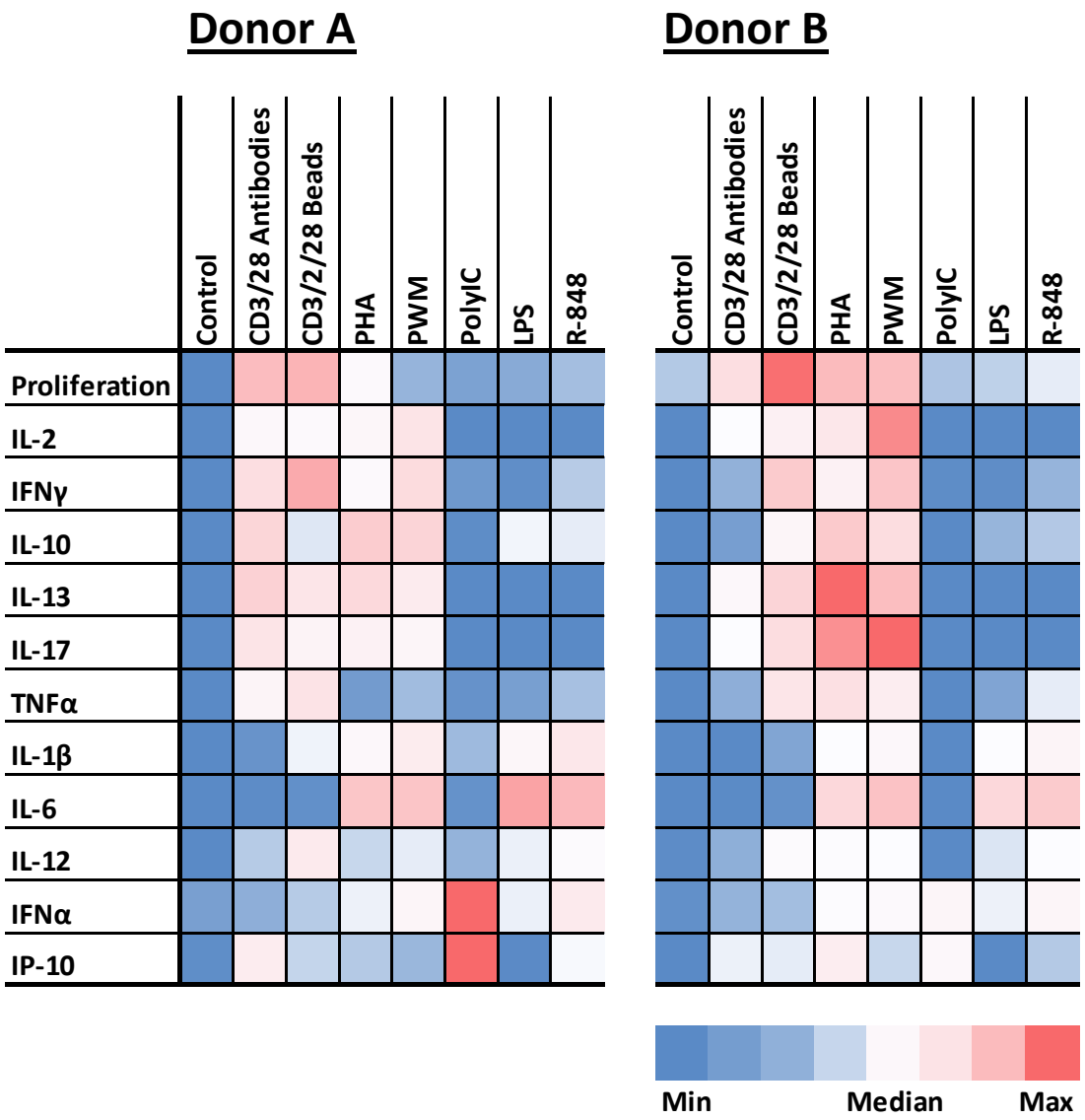
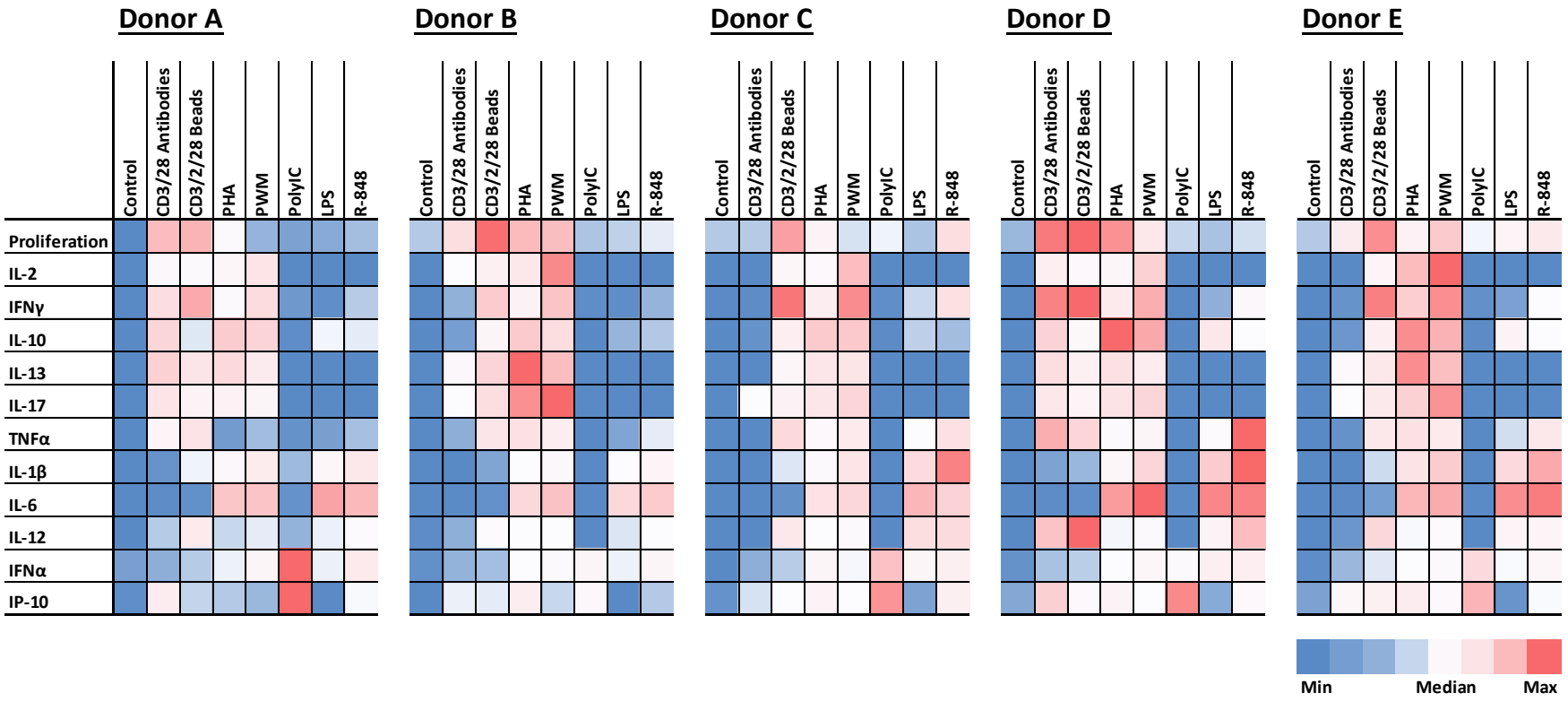
Functional assay: IFNα expression



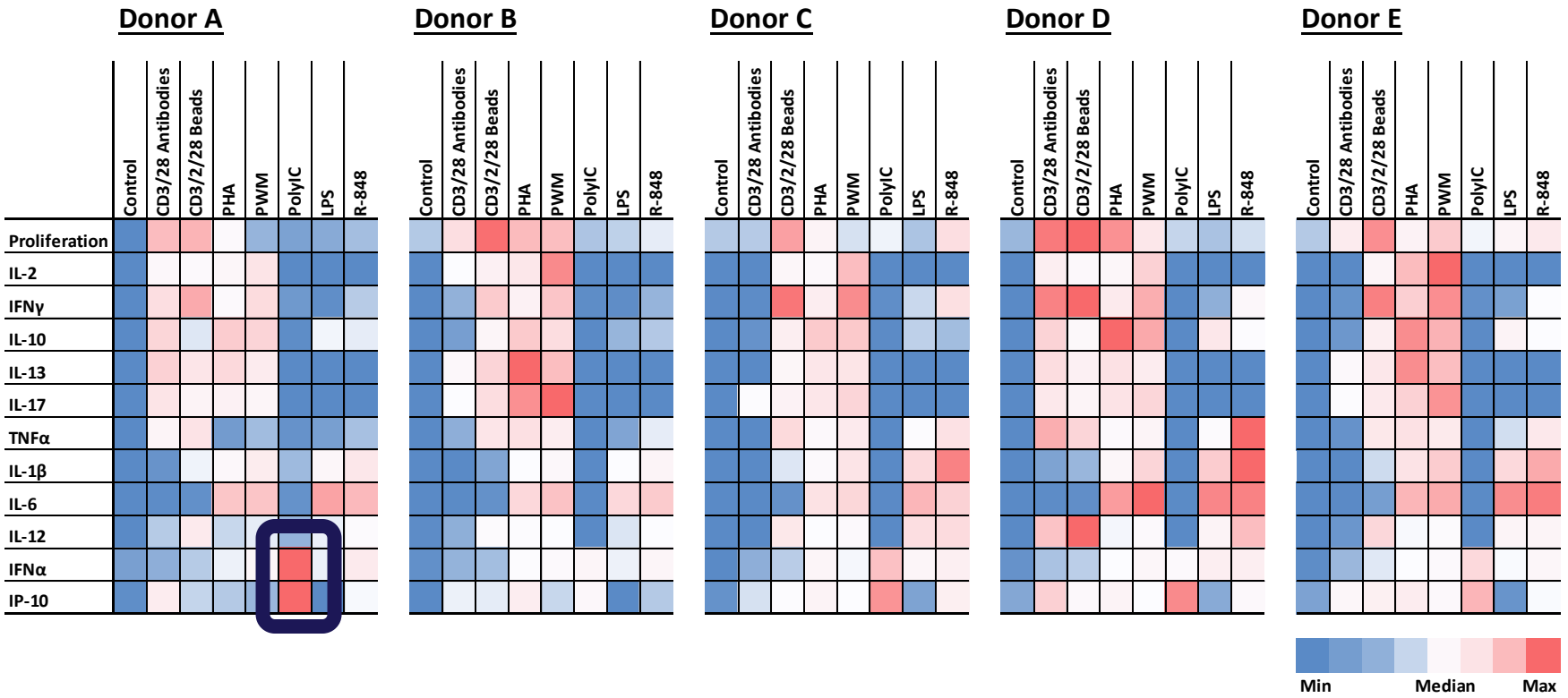
Functional assay: Complete data set



Functional assay: 5 donors complete data set

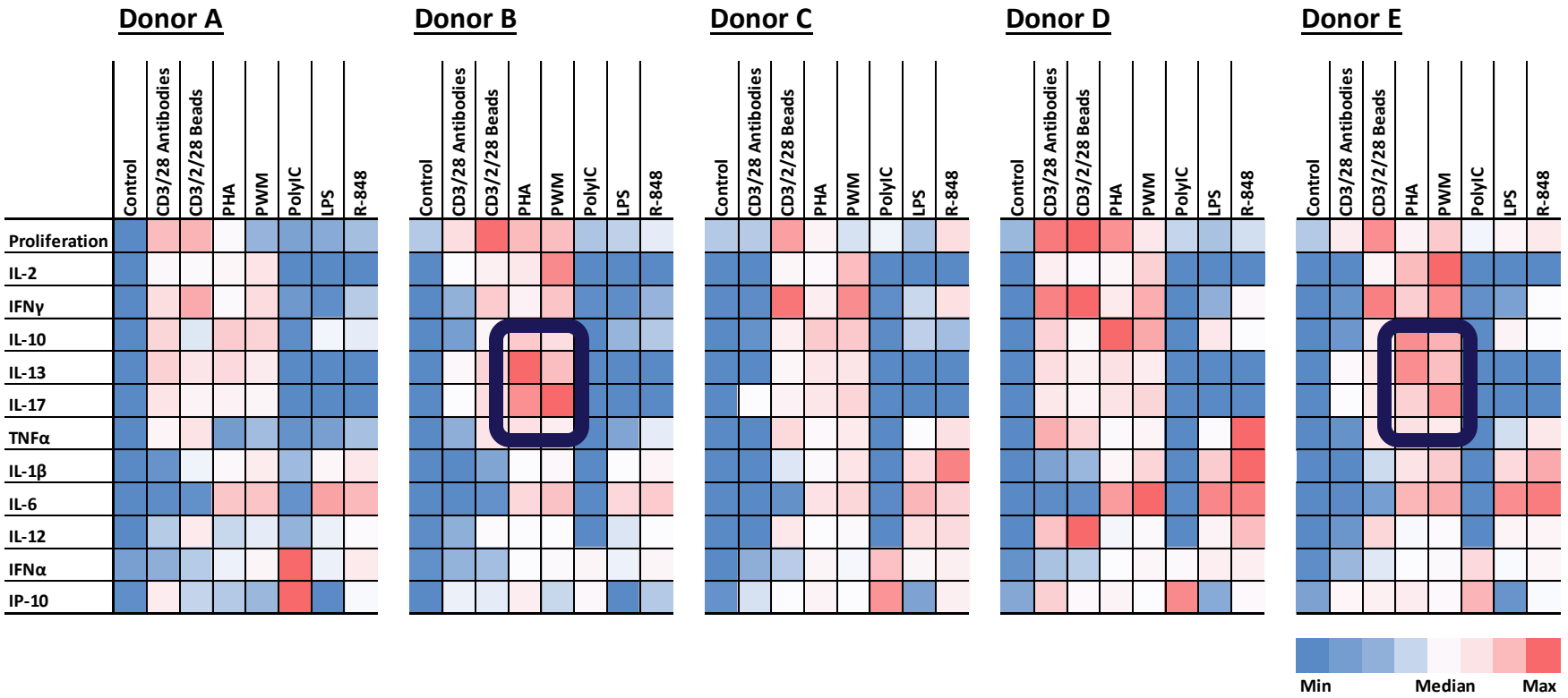


Data driven donor selection



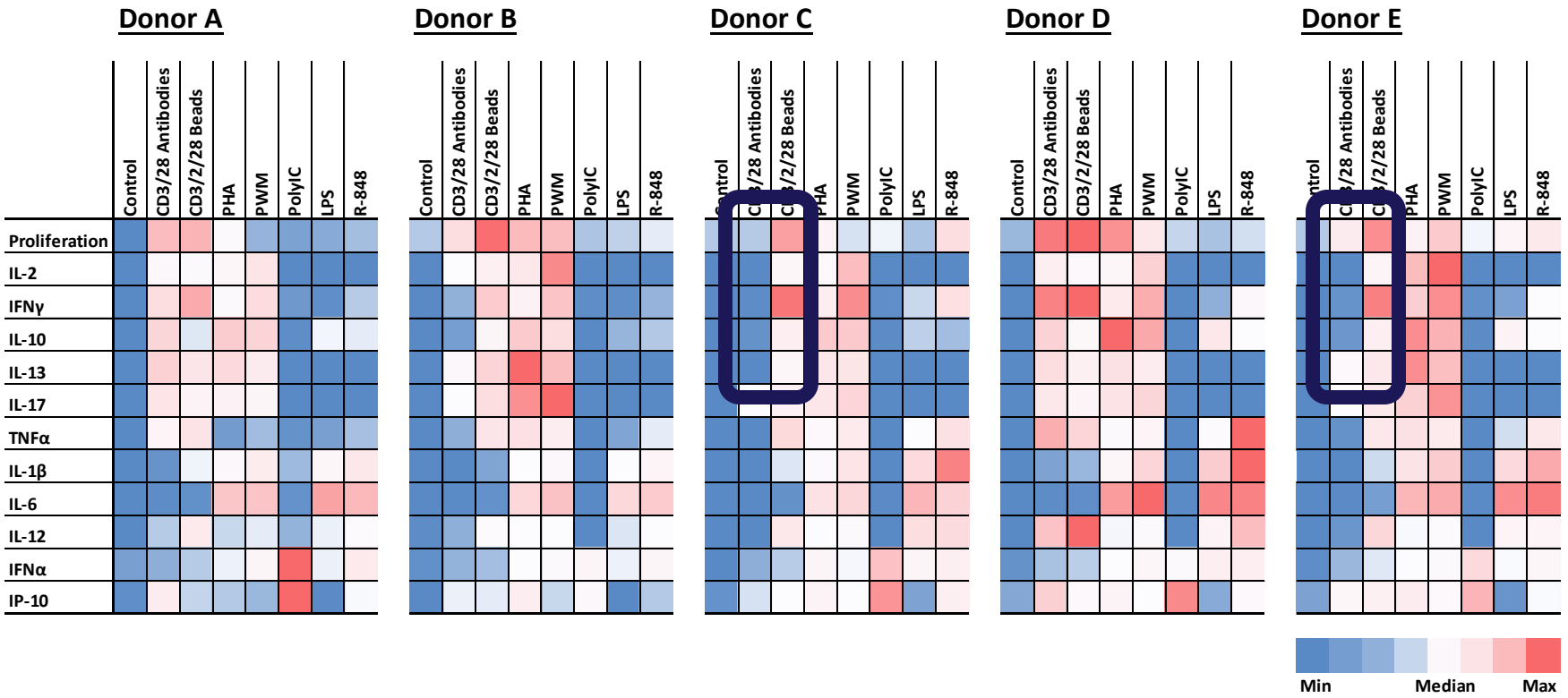
Donor A is the most appropriate for investigation of IFNα expression

Data driven donor selection



Donors B and E are more suitable for investigation of IL-13 and IL-17 expression

Data driven donor selection



There is significant variability in cell responsiveness to commonly used stimuli

Conclusion

1. Functional activity of human PBMC *in vitro* is extremely variable

2. Factors contributing to this variability can be divided into two groups:

I. Can be controlled

- Cryopreservation technique
- Cell viability
- Medium composition
- Quality of cell activating reagents

II. Have to be accepted (Uncontrollable)

- Genetic diversity
- Environmental factors
  - Immunizations
  - Nutrition
  - Latent infections

3. To minimize assay variability we recommend to:

- Use serum- or protein-free cryopreservation solution
- Ensure high viability of cells at the time of experiment
- Use prequalified FBS, autologous plasma/serum, or serum-free medium
- Validate cell activating (control) reagents

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Conclusion (cont'd)

4. **Use of functionally characterized cells can significantly minimize assay variability and improve reproducibility of experimental results**

5. **When selecting a specific lot of characterized cells one should consider:**
   - Assay readout
   - Cell reactivity (ability to respond to a specific stimuli)
   - Magnitude of the response

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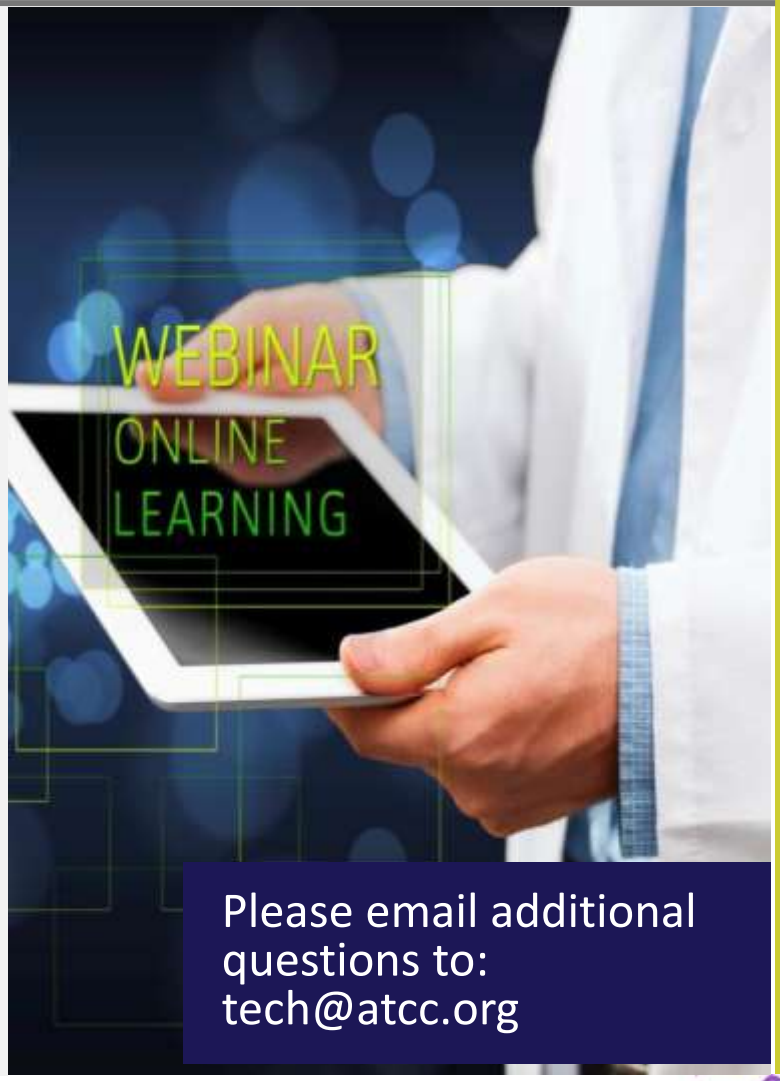
- **July 14, 2016**
  12:00 PM EST
  James Clinton, Ph.D., *Scientist,* ATCC
  Discovering ATCC Hematopoietic Progenitor Cells – Model Systems to Study the Immune and Cardiovascular Systems



Please email additional questions to: tech@atcc.org