

NODALITY

INTEGRATING BIOLOGY AND THERAPEUTICS FOR PATIENT BENEFIT

Single Cell Network Profiling Technology and  
Applications in Immunological Monitoring

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# Presenter Disclosure Information



Diane Longo

- The following relationships exist related to this presentation:
  - Nodality Inc., salary/shareholder, full time employee

# Presentation Topics

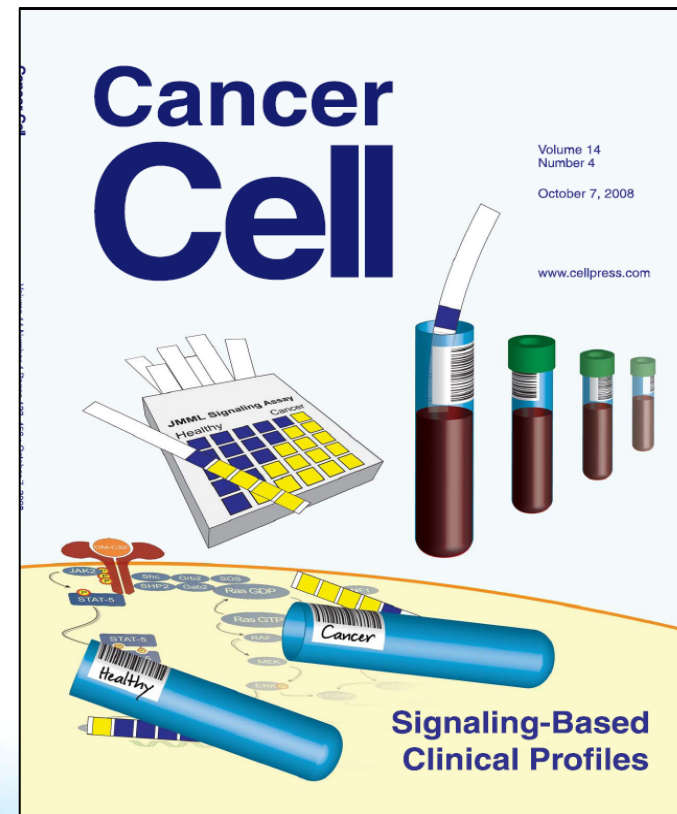
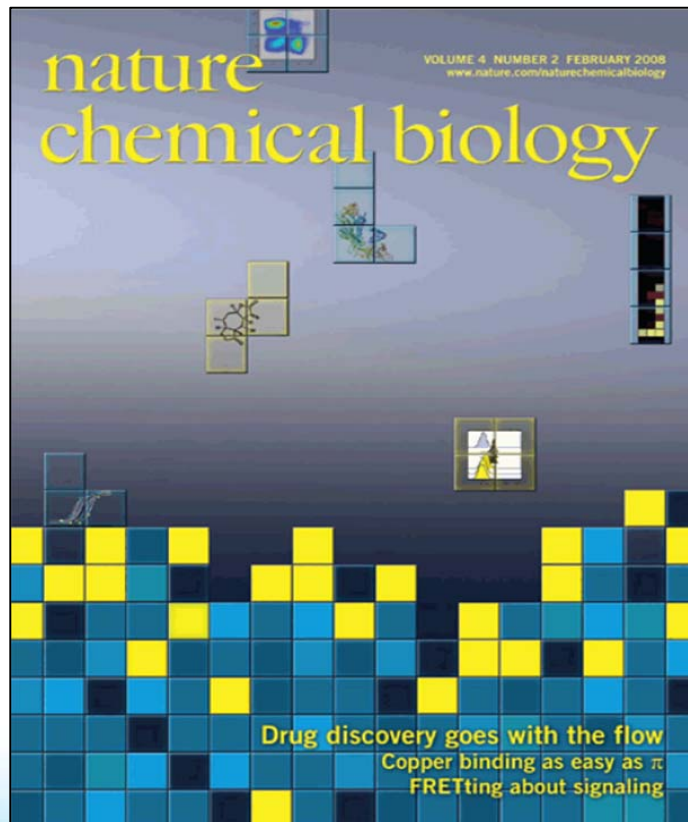


- Introduction to Single Cell Network Profiling (SCNP) Technology
- SCNP Applications
- Immunological Monitoring with SCNP

# SCNP Foundation Technology



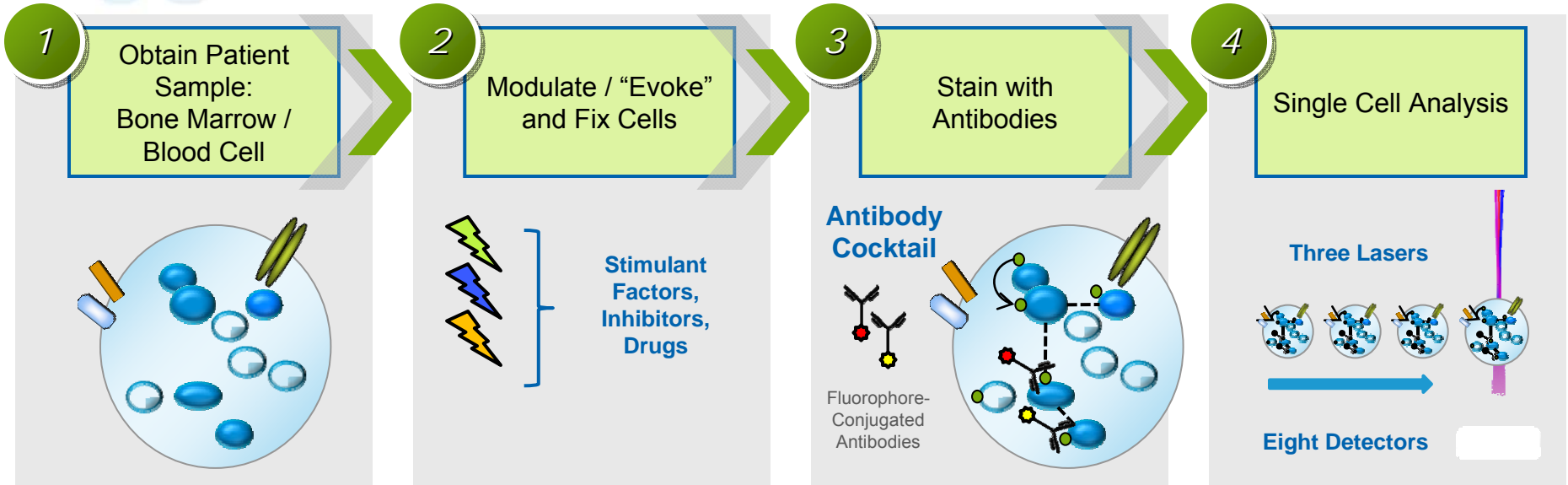
- Proprietary phosphoflow signaling technology developed in Dr. Garry Nolan's lab at Stanford University



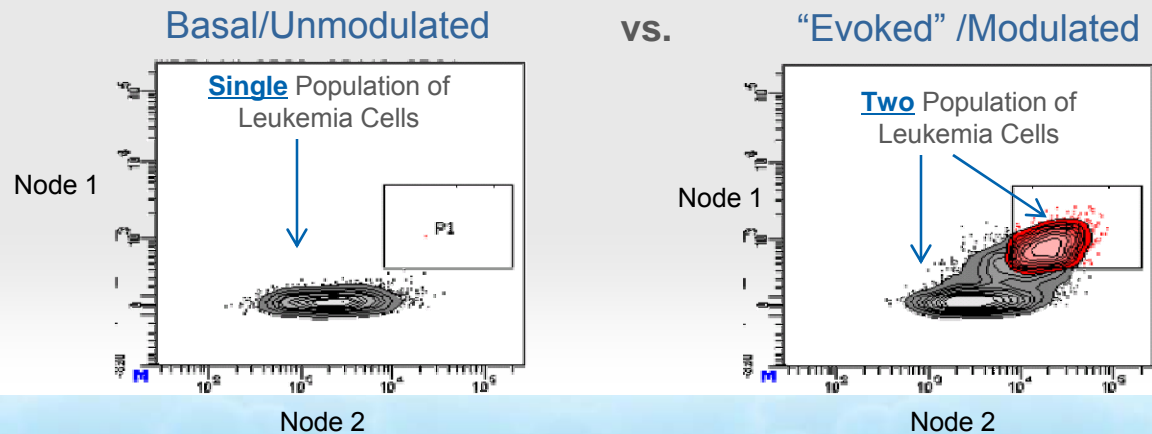
# Key Features of SCNPN

- Measures signaling in single cells
- Measures protein pathways and networks
  - Signaling molecules (nodes) measured simultaneously
    - In the same pathway
    - In different pathways
- Measures baseline and evoked signaling
- Resistance versus sensitivity
- Rare cells
  - Signaling heterogeneity

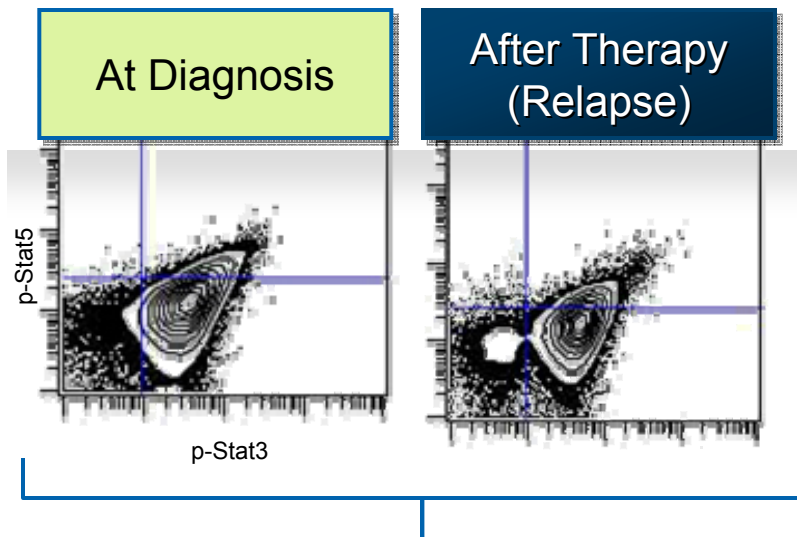
# SCNP: Unmasking Disease Biology to Reveal New, Relevant Information



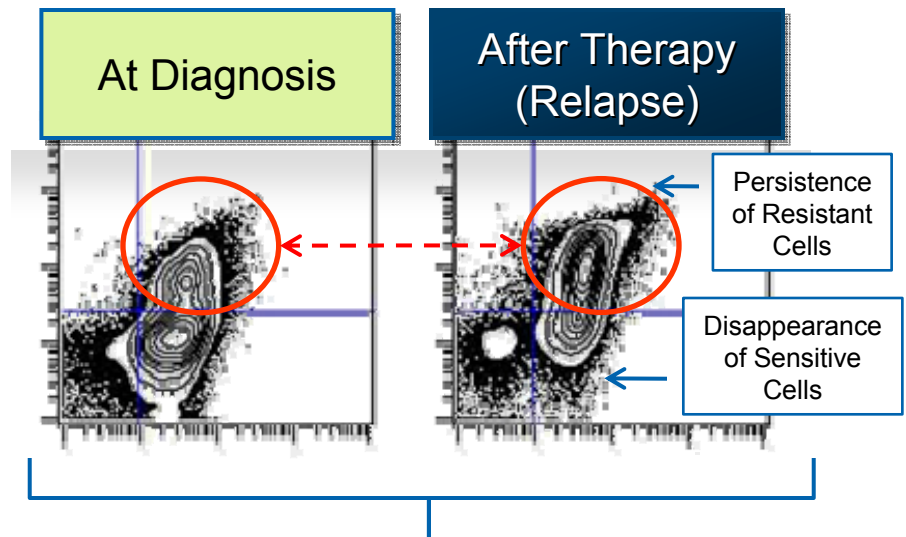
## 5 THE RESULT



# Value of Functional Characterization in Drug Resistance



No Key Differences Seen in Tumor Samples . . .



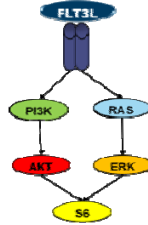
...But Stimulation Reveals Distinct Cell Subsets – One Sensitive and One Resistant to Chemotherapy



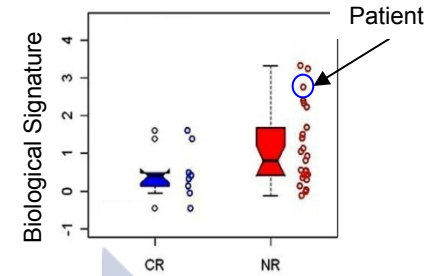
# Industrialization of SCNP Technology



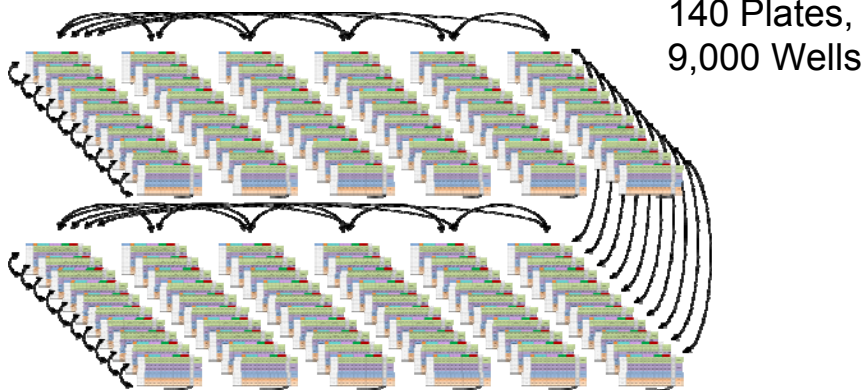
Provide accurate biological characterization of a patient that can be translated to actionable clinical information



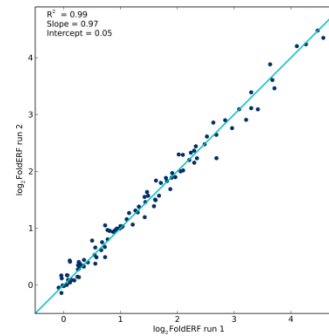
Single Cell Network Profiling (SCNP)



Several logistic and technical challenges need to be met to achieve this goal



High throughput and high content with many internal relationships



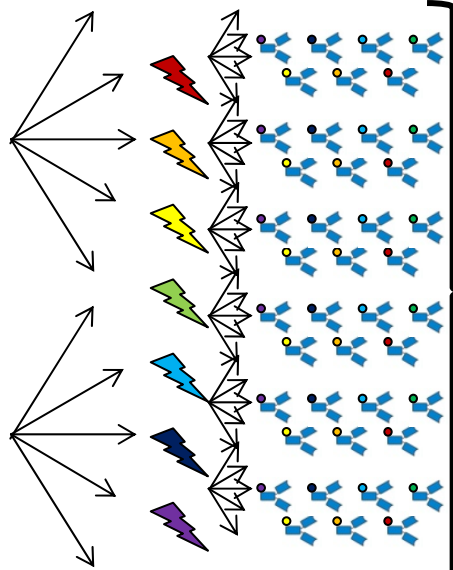
Robust and accurate assay



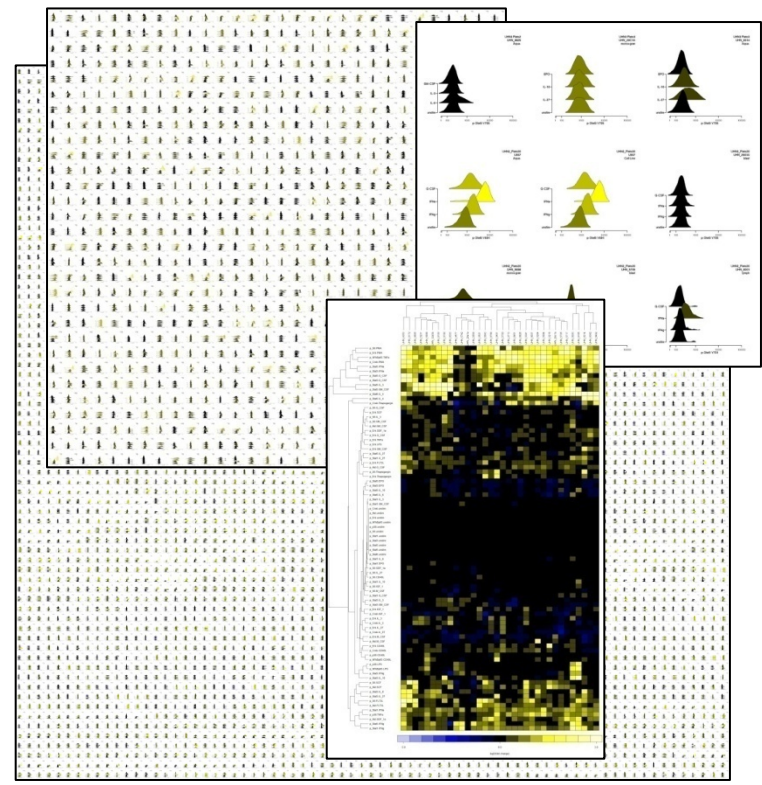
Temporal stability



# Large amounts of data & multiple types of relationships



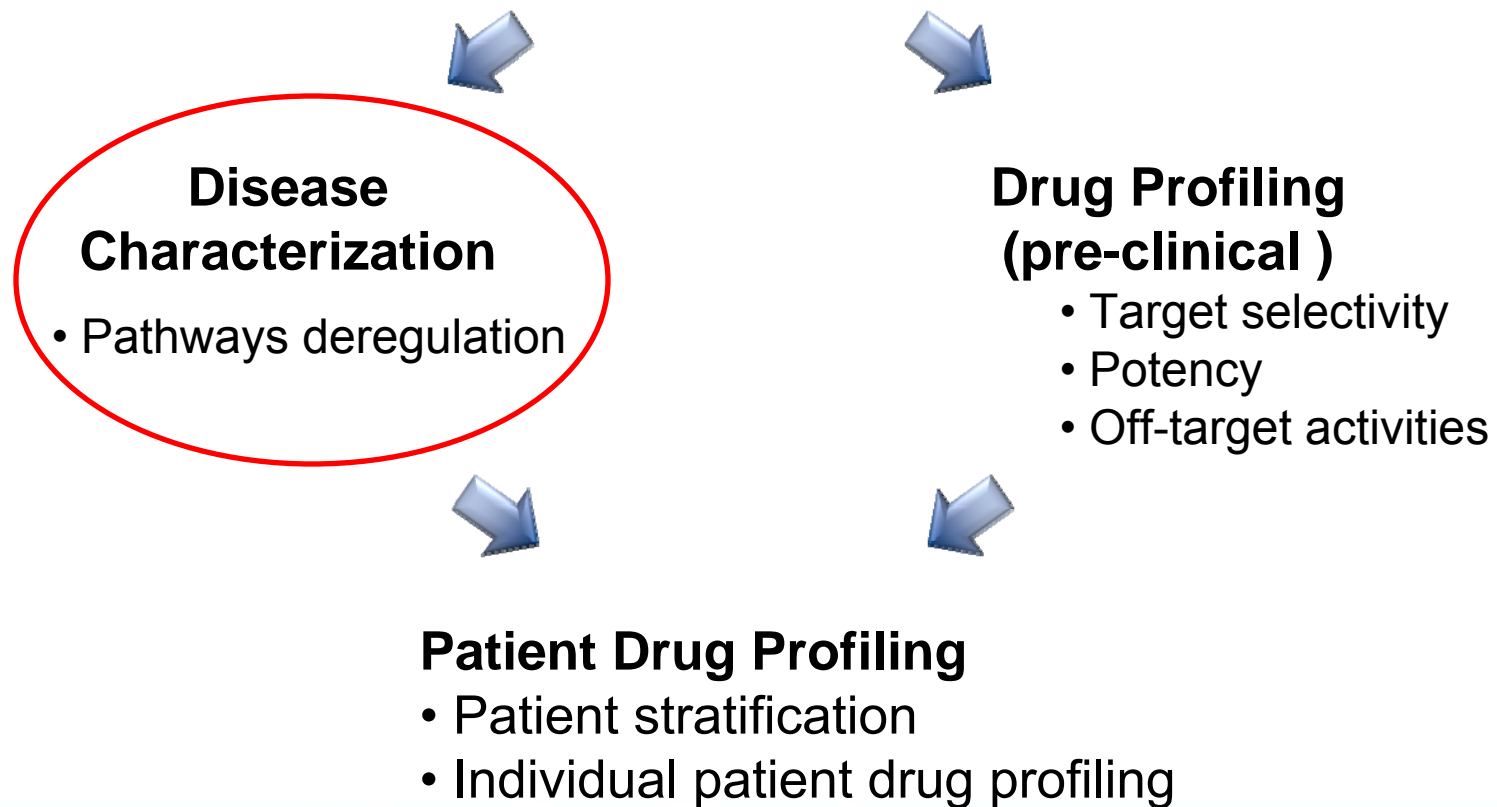
| Cell ID | CD20 | CD3  | CD5  | p-2-Ak | p-Syk | p-Slnk |
|---------|------|------|------|--------|-------|--------|
| 1       | 525  | 2.29 | 5.75 | 0.45   | 1.77  | 0.77   |
| 2       | 228  | 1.41 | 2.75 | 1.20   | 1.15  | 2.62   |
| 3       | 264  | 2.27 | 2.09 | 2.48   | 5.32  | 5.20   |
| 4       | 400  | 1.10 | 2.14 | 5.48   | 1.54  | 3.01   |
| 5       | 217  | 5.63 | 5.23 | 2.49   | 2.69  | 2.22   |
| ...     | ...  | ...  | ...  | ...    | ...   | ...    |
| NA-2    | 2.00 | 2.09 | 2.21 | 5.47   | 1.22  | 0.74   |
| NA-2    | 2.62 | 2.82 | 2.42 | 1.72   | 2.48  | 2.24   |
| NA-2    | 4.18 | 4.57 | 2.43 | 1.34   | 0.82  | 1.47   |
| N       | 2.92 | 5.29 | 1.80 | 5.21   | 1.42  | 1.01   |



100 samples per expt  $\times$   $10^6$  cells per patient  $\times$  20 modulators per expt  $\times$  20 antibody/fluorophores per expt  $>10^9$  data points per experiment

- Each experiment is related to all other experiments
- Keep track of every single cell and all readouts
- Do all this efficiently

## SCNP



- Disease Characterization
  - Hematological malignancies
    - AML
    - MDS
    - CLL
    - NHL
  - Immunological based diseases and conditions
    - RA
    - Lupus
    - Vaccine development
    - Immunotherapy

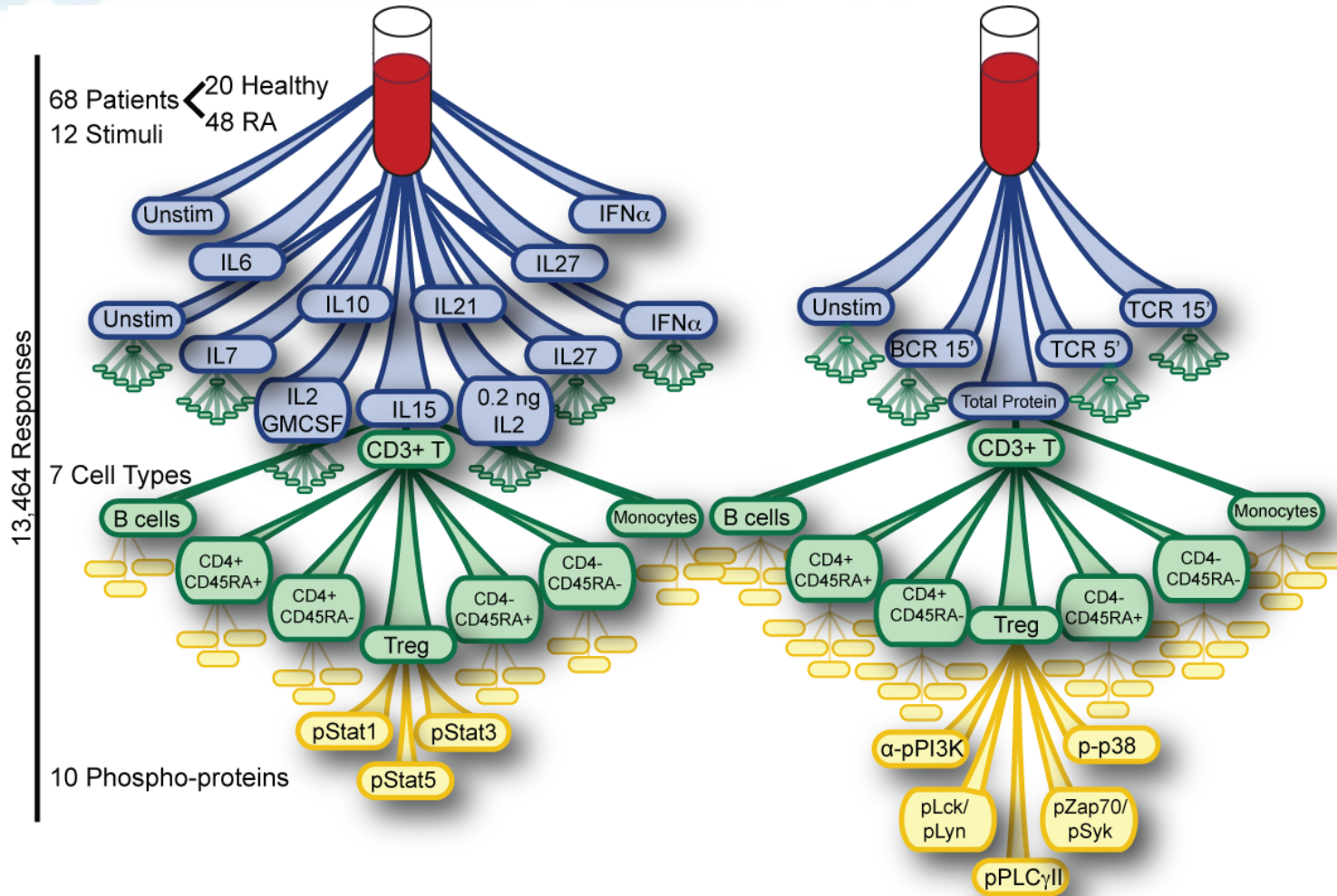
# SCNP Applications in Immunobiology: Rheumatoid Arthritis



- RA is a chronic, systemic autoimmune disease of unknown etiology
  - Cytokines drive and maintain inflammation
  - **T cells** are essential mediators of RA
  - **B cells** produce autoantibodies that target self-antigens
  - **Monocytes/macrophages** can become osteoclasts and destroy bone and cartilage
- Disease characterization with SCNP
  - Identify signaling pathway alterations in RA
  - Classify disease activity or treatment response based on signaling profiles

# Experimental Design

## Profile Variables



### Biochemistry

163 signaling responses  
11,084 responses collected



### Clinic

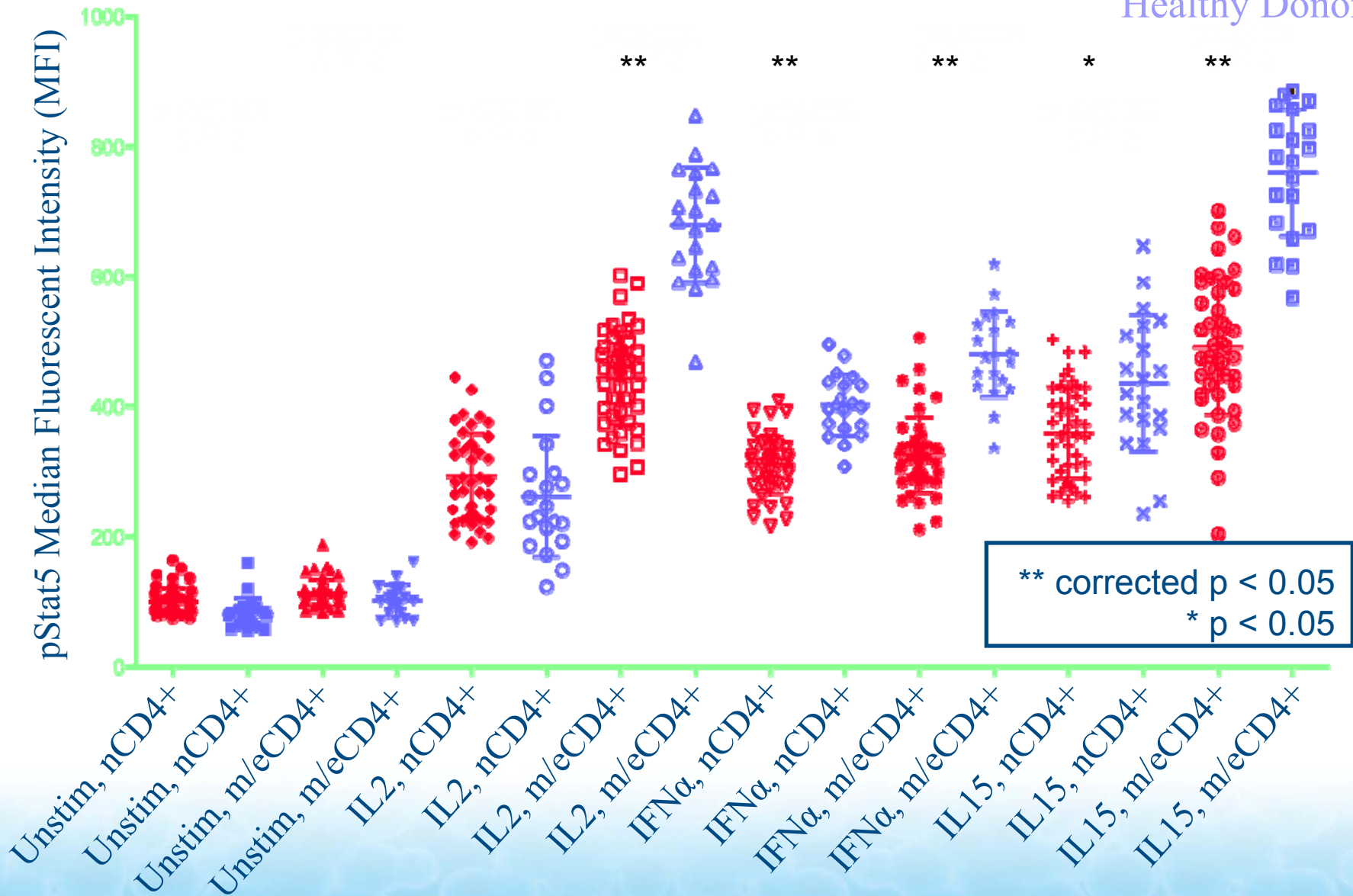
68 clinical measurements  
3,264 clinical data points



# Reduced IFN $\alpha$ , IL2, IL7, IL15 Stat5 Activation in RA

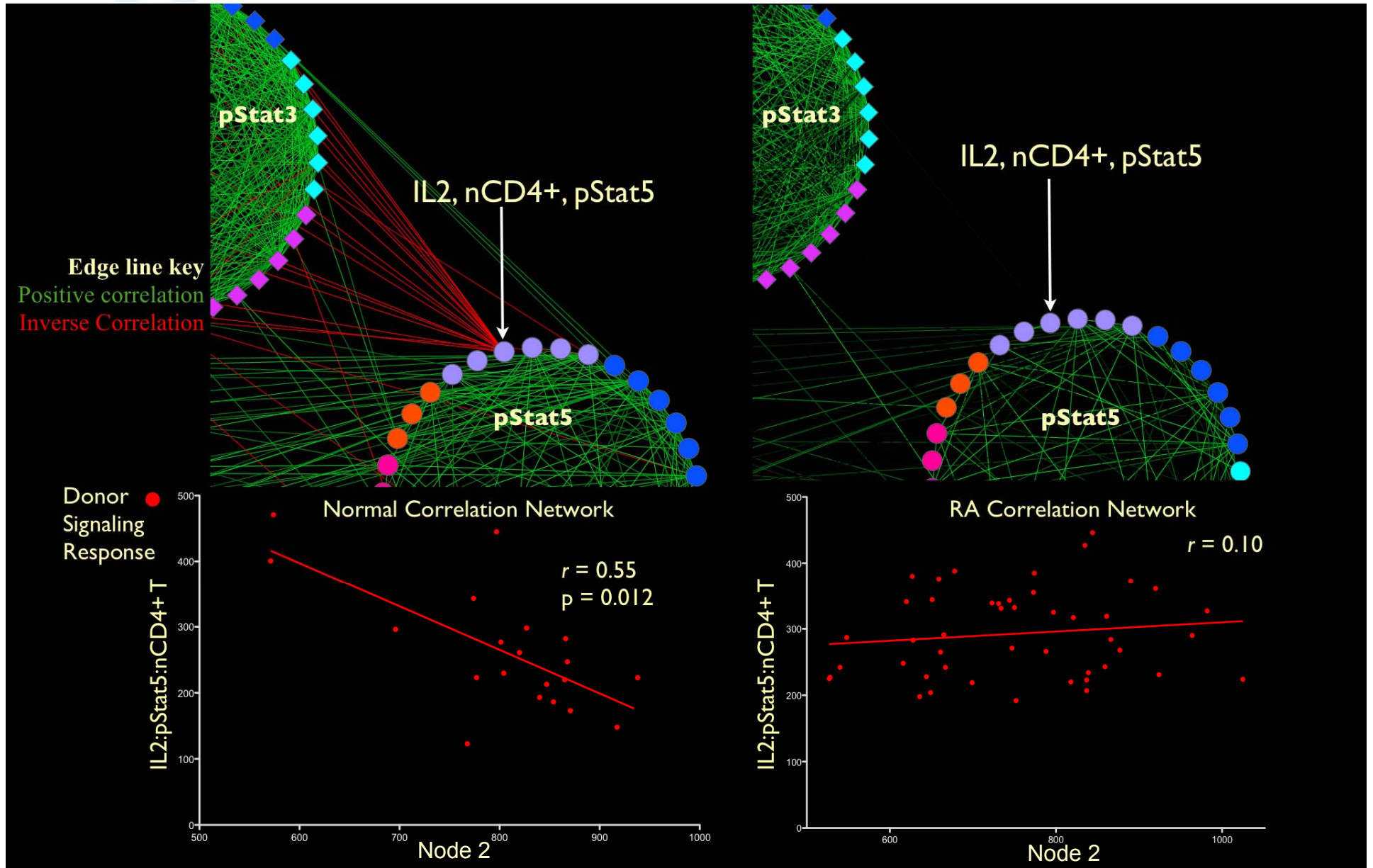
RA Donor

Healthy Donor





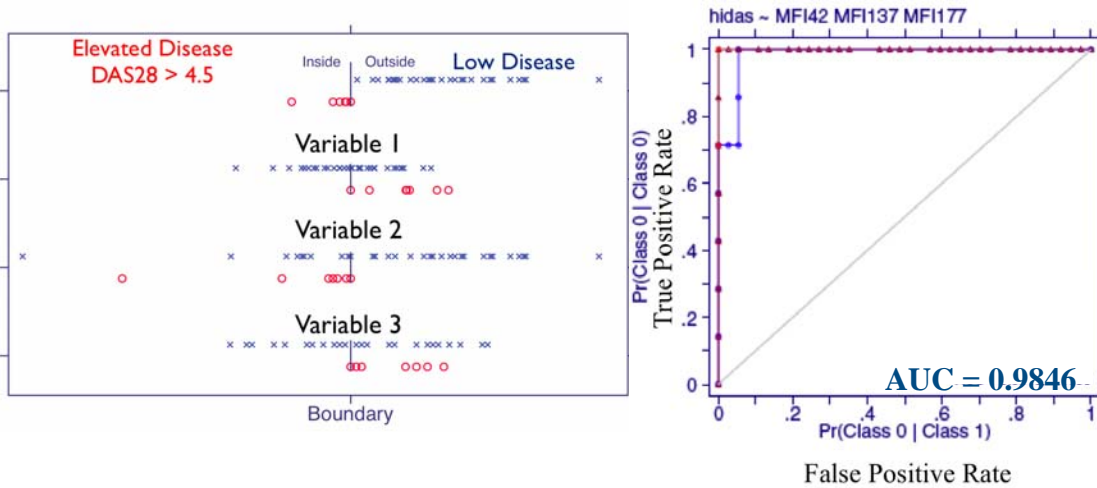
# Inferring loss of regulation by mapping disease reorganization of signaling



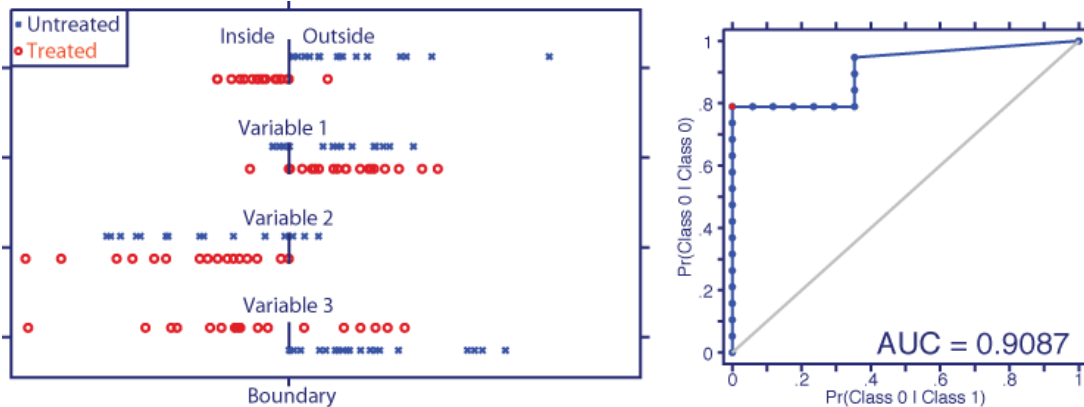
# Signaling potential is predictive of disease activity and therapeutic intervention



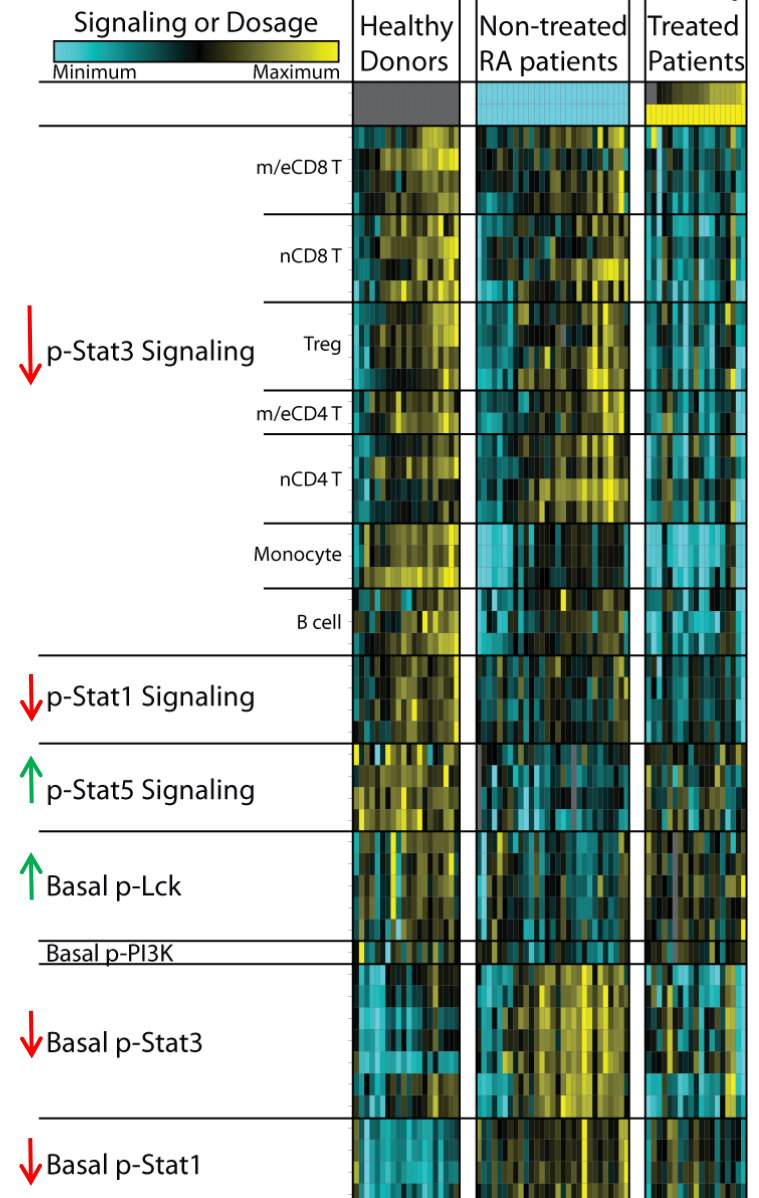
Signaling correlates to the Disease Activity Score (DAS28)



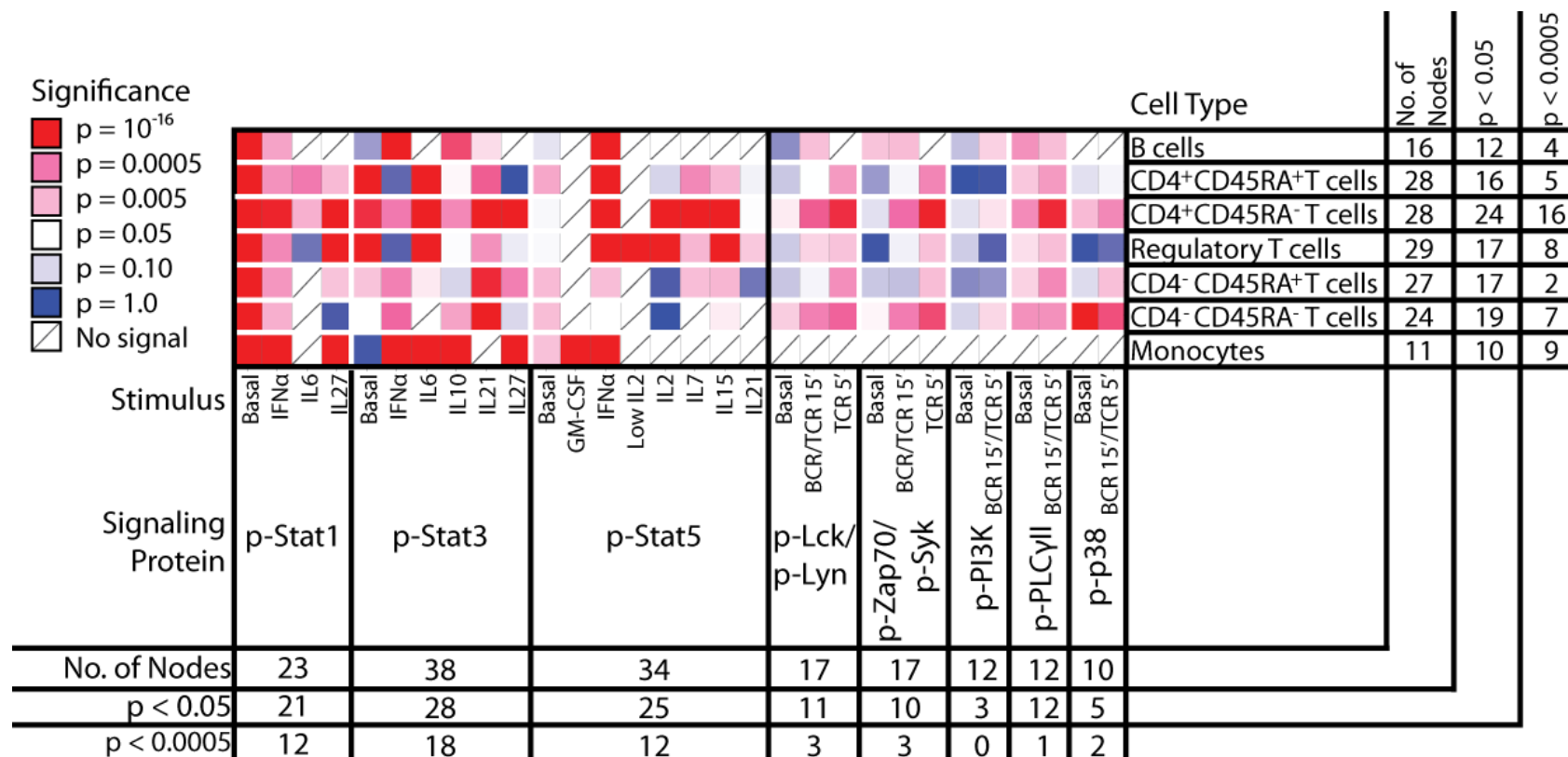
Signaling correlates to prednisone usage



Prednisone Heatmap



# Global analysis of signaling nodes altered by RA



- 163 potentiated and basal signaling nodes measured
- 115 nodes significantly different (p < 0.05)
- 51 nodes very significantly altered (p < 0.0005)

- Patients with active disease show a specific profile of potentiated signaling
- Interrogation of cell signaling allows a direct means to classify disease activity and response to treatment
- The relationships of signaling events to each other can be used to infer a structure to the immune system
- Changes in these relationships in an RA population suggest disease mechanisms and may provide novel targets in the treatment of RA
- Immunological monitoring with SCNP can be applied to other diseases and conditions:
  - Cancer vaccine development
  - Immunotherapy
  - Lupus