Conserved cross-species network modules elucidate Th17 T-cell differentiation in human and mouse

ECCB 14 BioNetVisA Workshop

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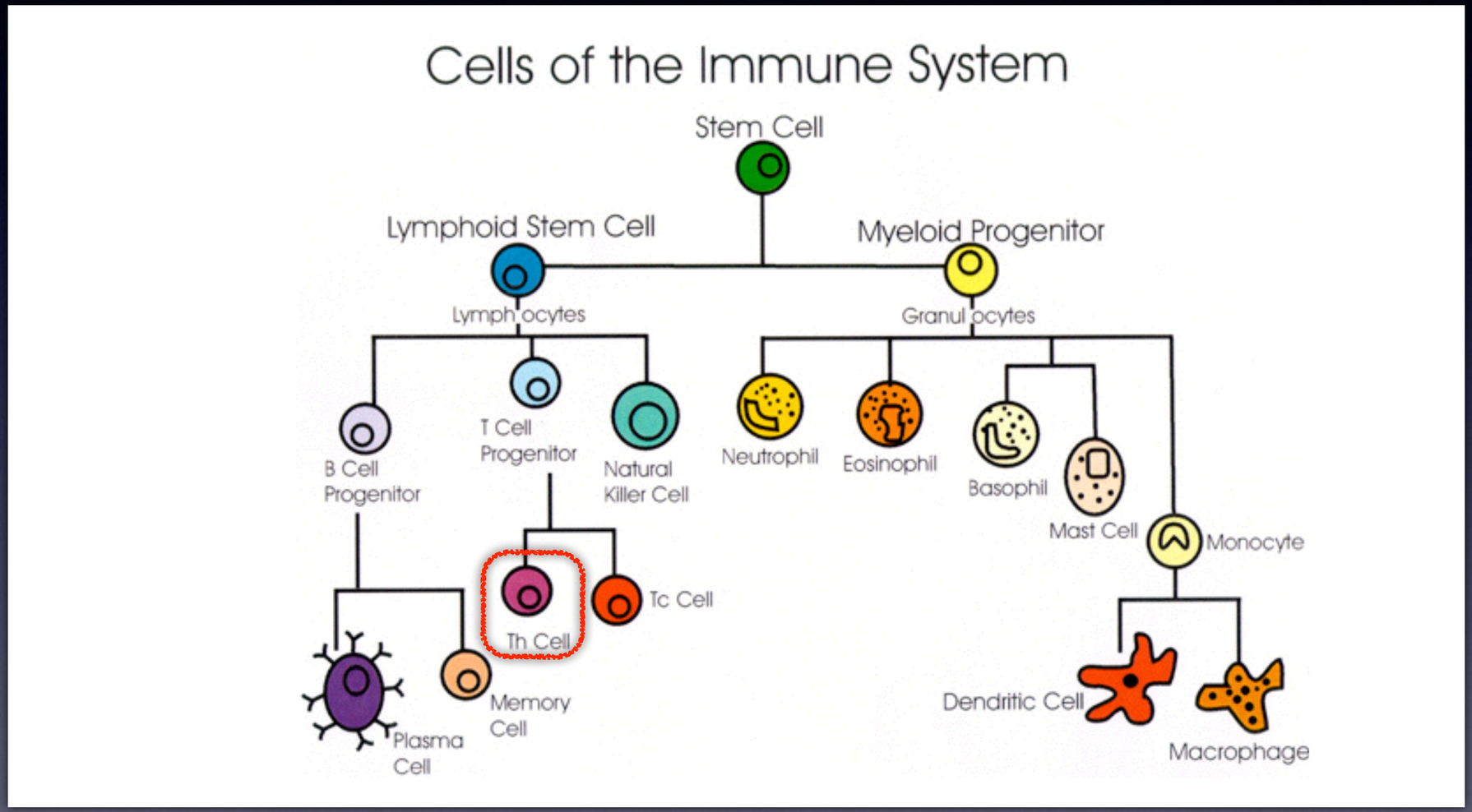
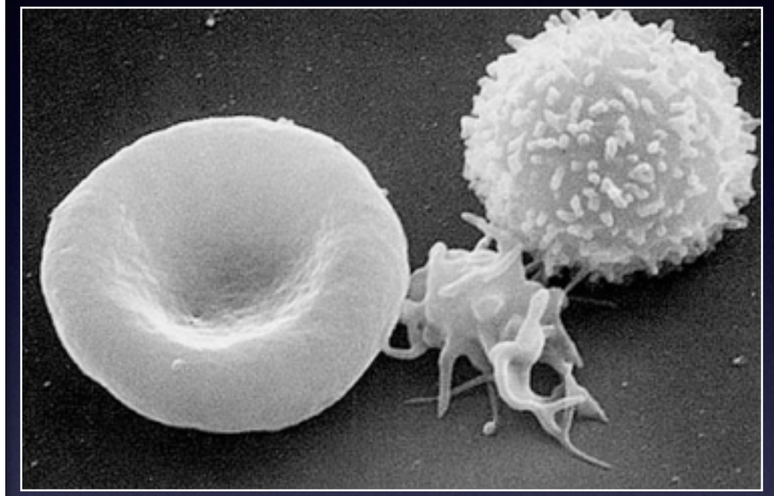
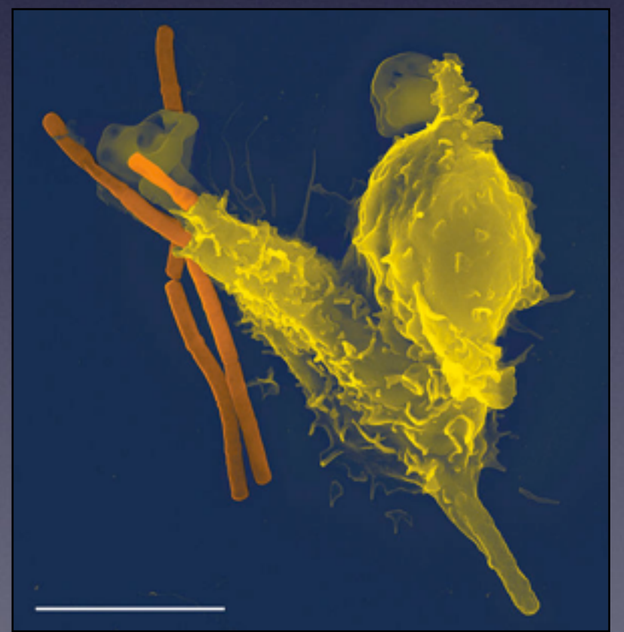
"Trouble with mice is you always kill 'em."
— John Steinbeck, Of Mice and Men

T Cells

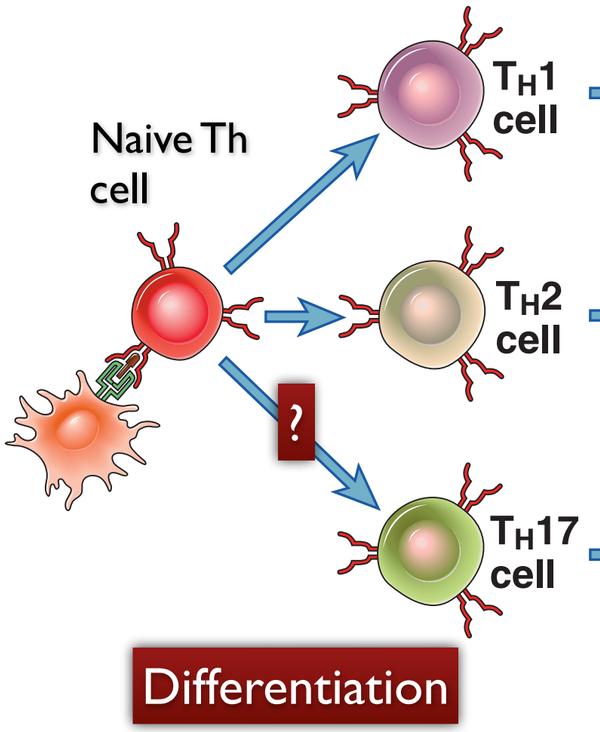






Wikimedia commons
Abbas, Cellular & Molecular Immunology, 7th ed

T Helper subsets



	Signature cytokines	Immune reactions	Host defense	Role in diseases
T_H1 cell	IFNγ	Macrophage activation; IgG production	Intracellular microbes	Autoimmune diseases; tissue damage associated with chronic infections
T_H2 cell	IL-4 IL-5 IL-13	Mast cell, eosinophil activation; IgE production; "alternative" macrophage activation	Helminthic parasites	Allergic diseases
T_H17 cell	IL-17A IL-17F IL-22	Neutrophilic, monocytic inflammation	Extracellular bacteria; fungi	Organ-specific autoimmunity

Abbas, Cellular & Molecular Immunology, 7th ed

Th17 cells: Clinical relevance

- Mucosal immunology : Th17 respond to bacterial and fungal antigens
- Th17 cells imbalance associated with several autoimmune diseases (Rheumatoid Arthritis, MS, psoriasis, lupus, CD)
- IL-17-deficient mice are more susceptible to the development of lung melanoma
- HIV infection specifically depletes Th17 population

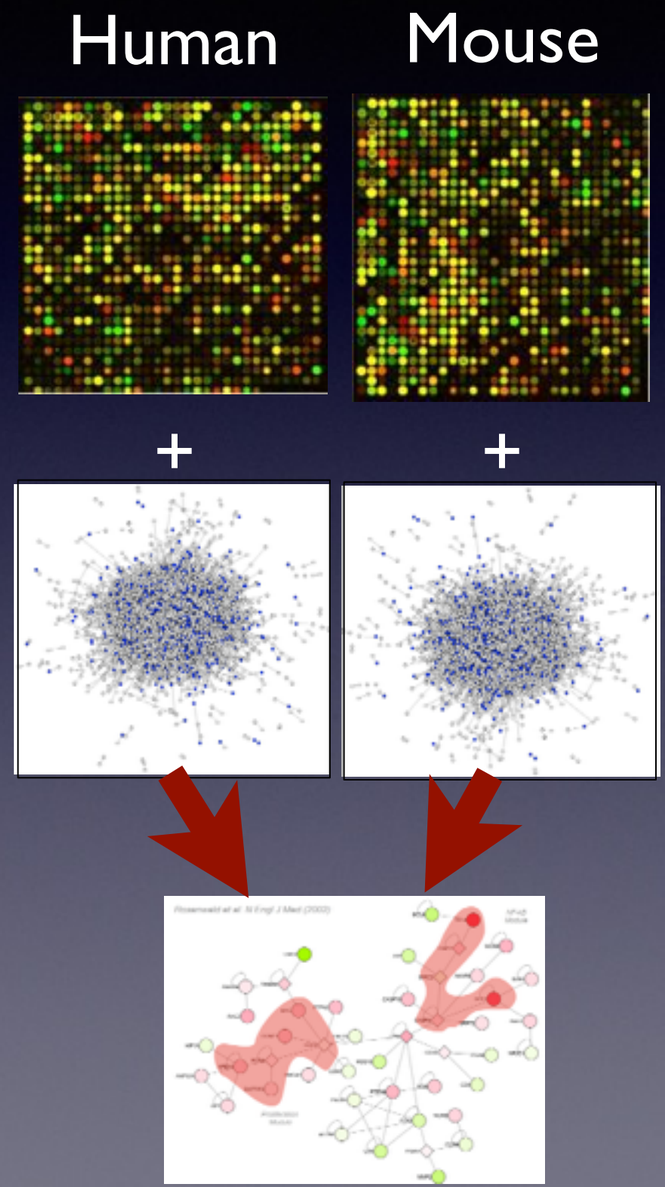
Main questions

- How to modulate Th17 response to self?
- What are the regulators of Th17 balance?
- What are the proteins and pathways responsible for proper differentiation of Th17 cells?

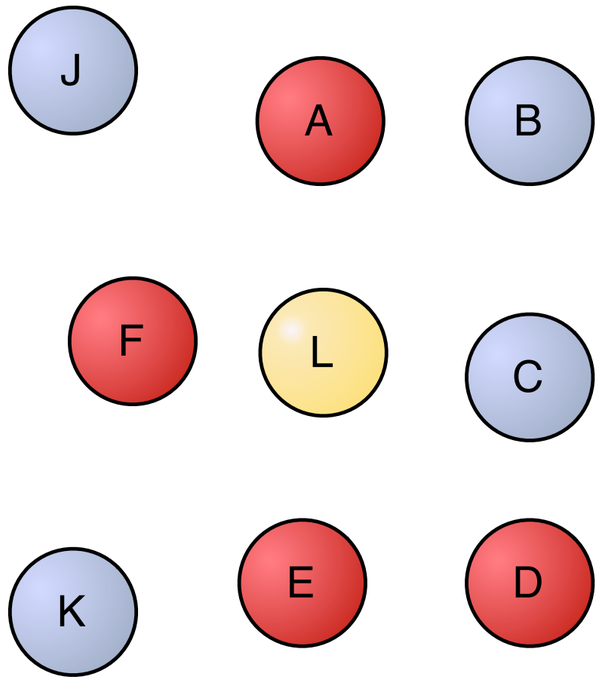
How well findings in mouse are transferrable to human immunology?

Our strategy

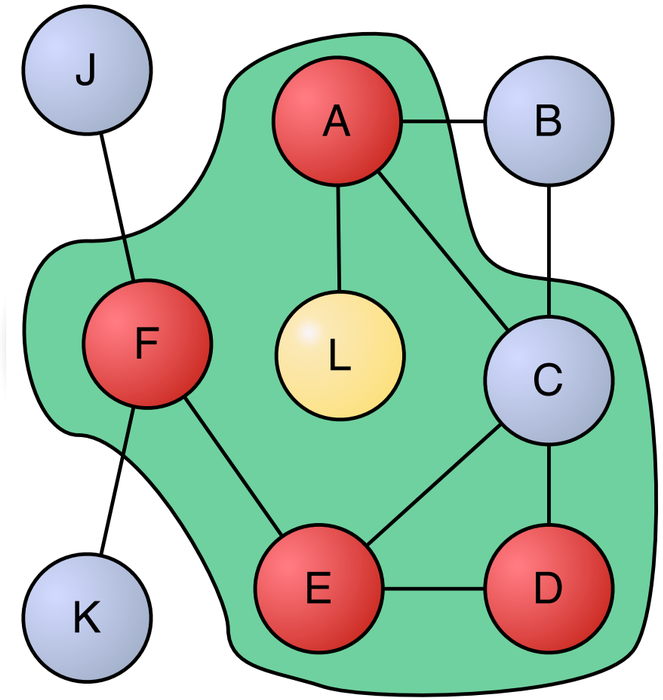
- Let's combine:
  - Human and Mouse Th17 differentiation transcriptomics data
  - Human and Mouse PPI networks
  - Orthology information between Human and Mouse
- Using an optimization framework
- To identify *conserved cross-species active modules*



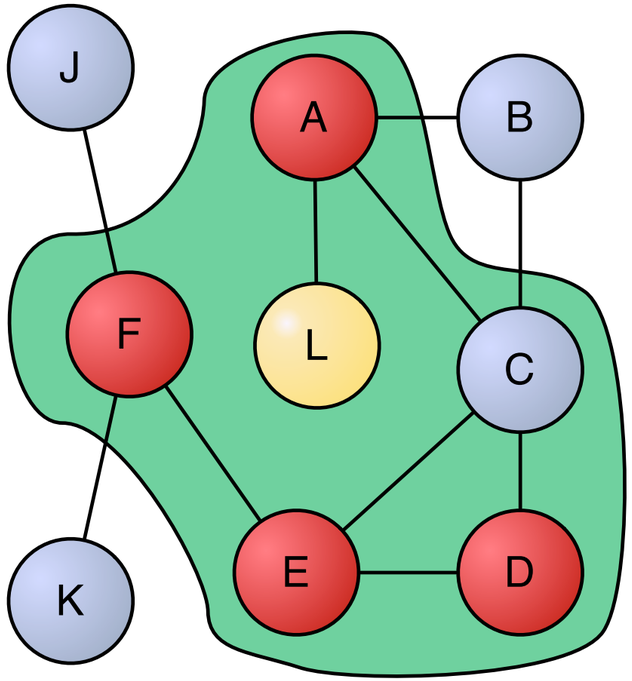
CCSAM:(1) Activity

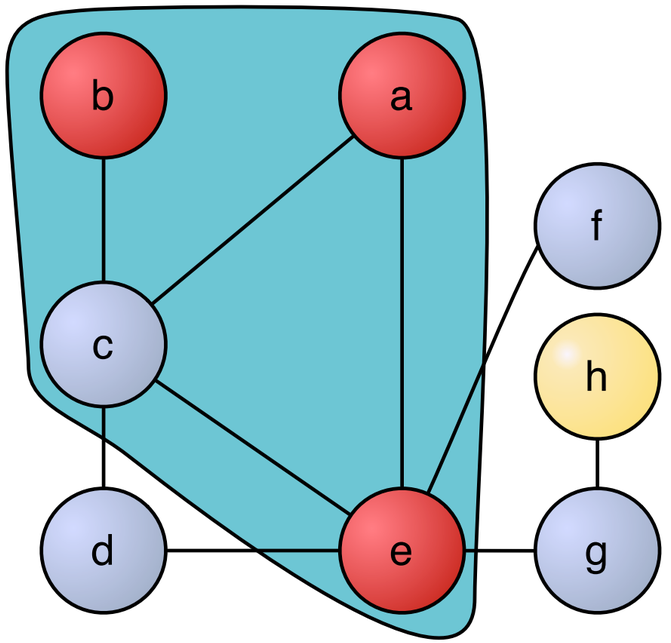


CCSAM:(11) Modularity

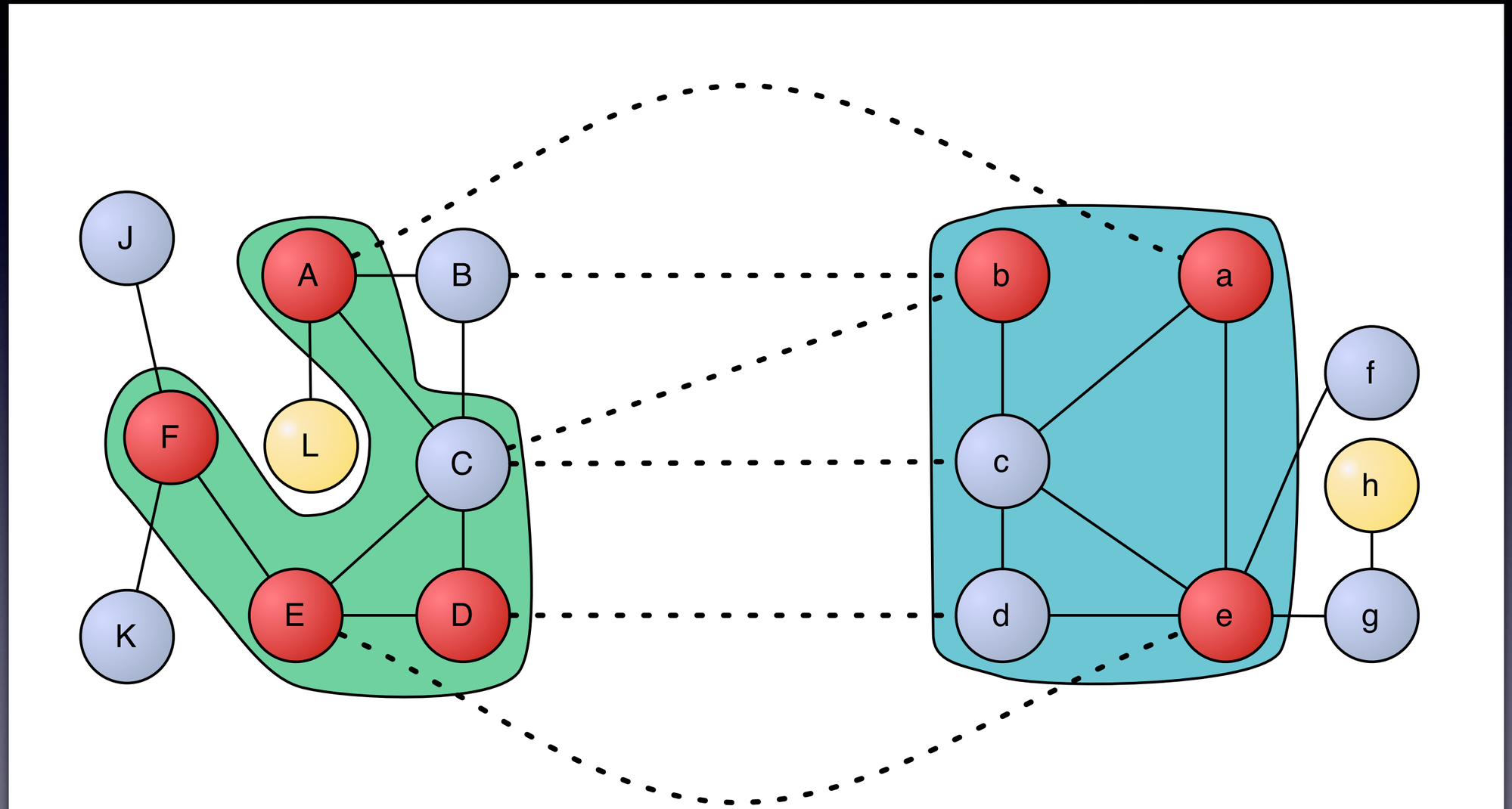


CCSAM:(11) Modularity^2





CCSAM:(III) Conservation



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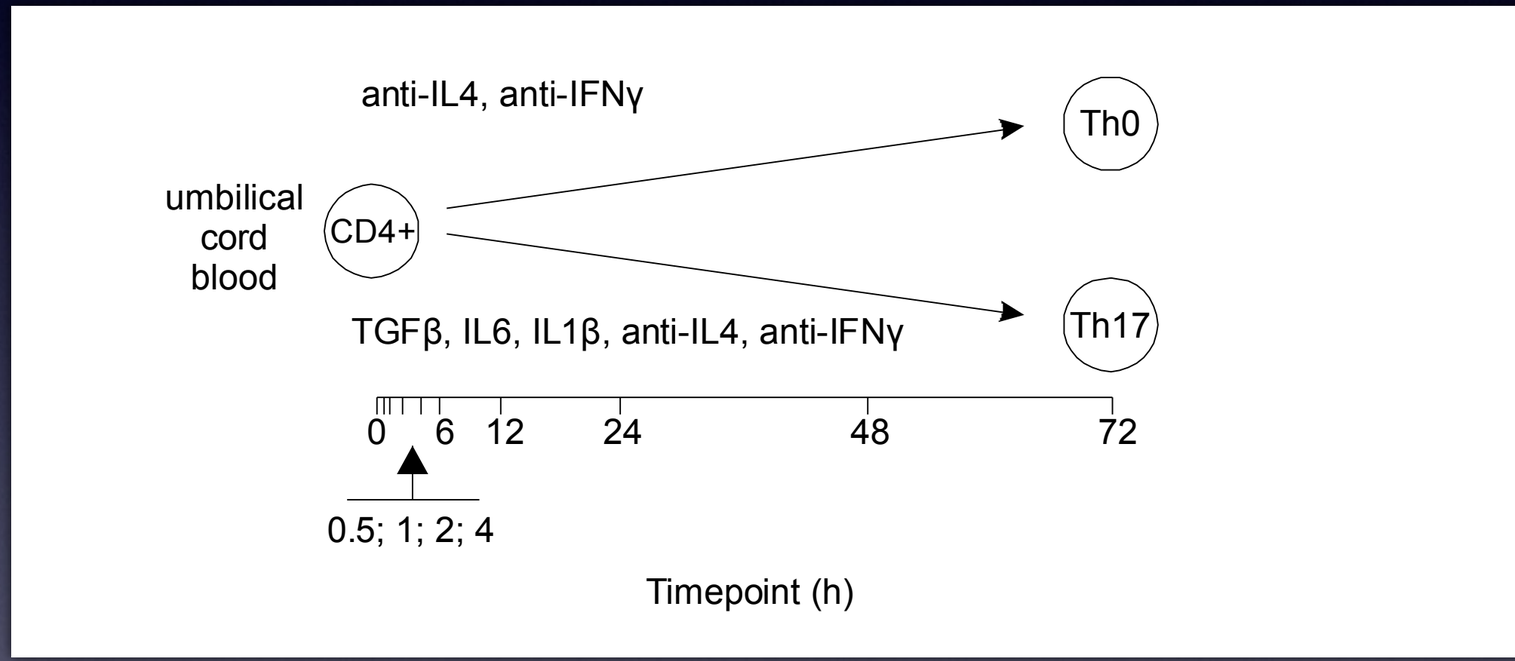
M&M recipe:

- For each species individually:
  - Process RNA-Seq => Count matrix
  - Fit a GLM => estimated coefs
  - For each time point,
    - For each gene:
      - call for DE => p.value
      - Fit a BUM => activity score
  - Get PPI network & orthology relations

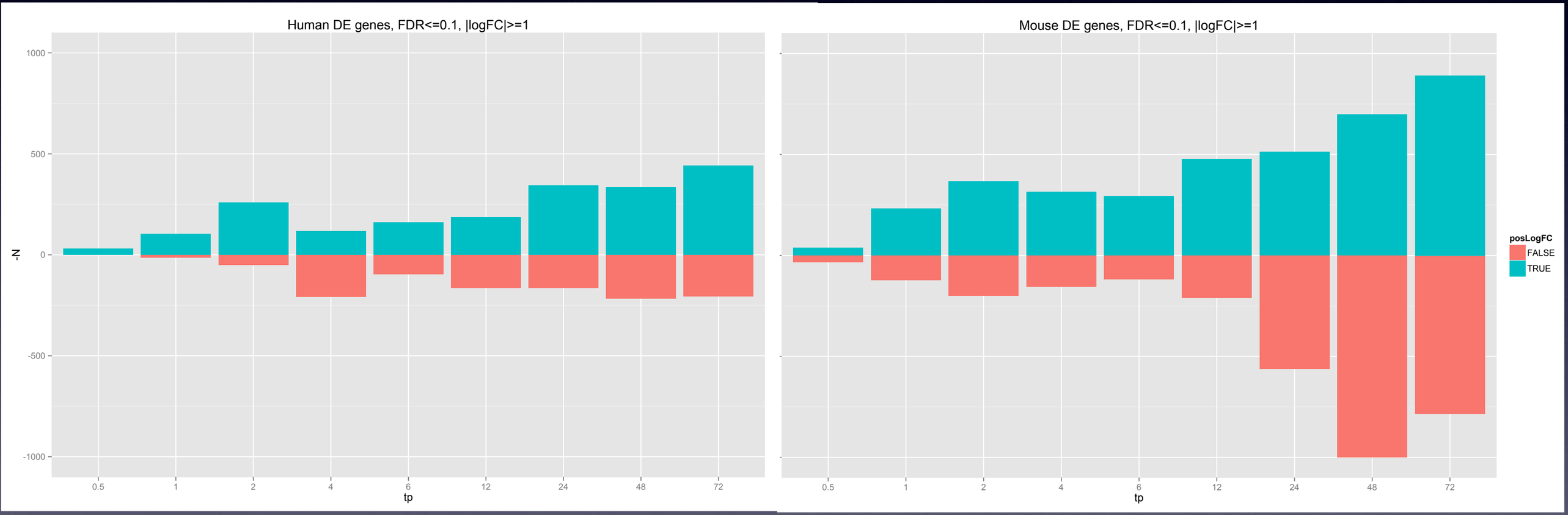
Transcriptional profiling of Human & Mouse Th17 cells

- Control Th0 vs Th17
- 9 time points, RNA-Seq
- Human: 14,338 mRNAs quantified in the first 72h
- Mouse: 11,751 mRNAs quantified in the first 72h
- Matched time points
- DE called with an edgeR GLM



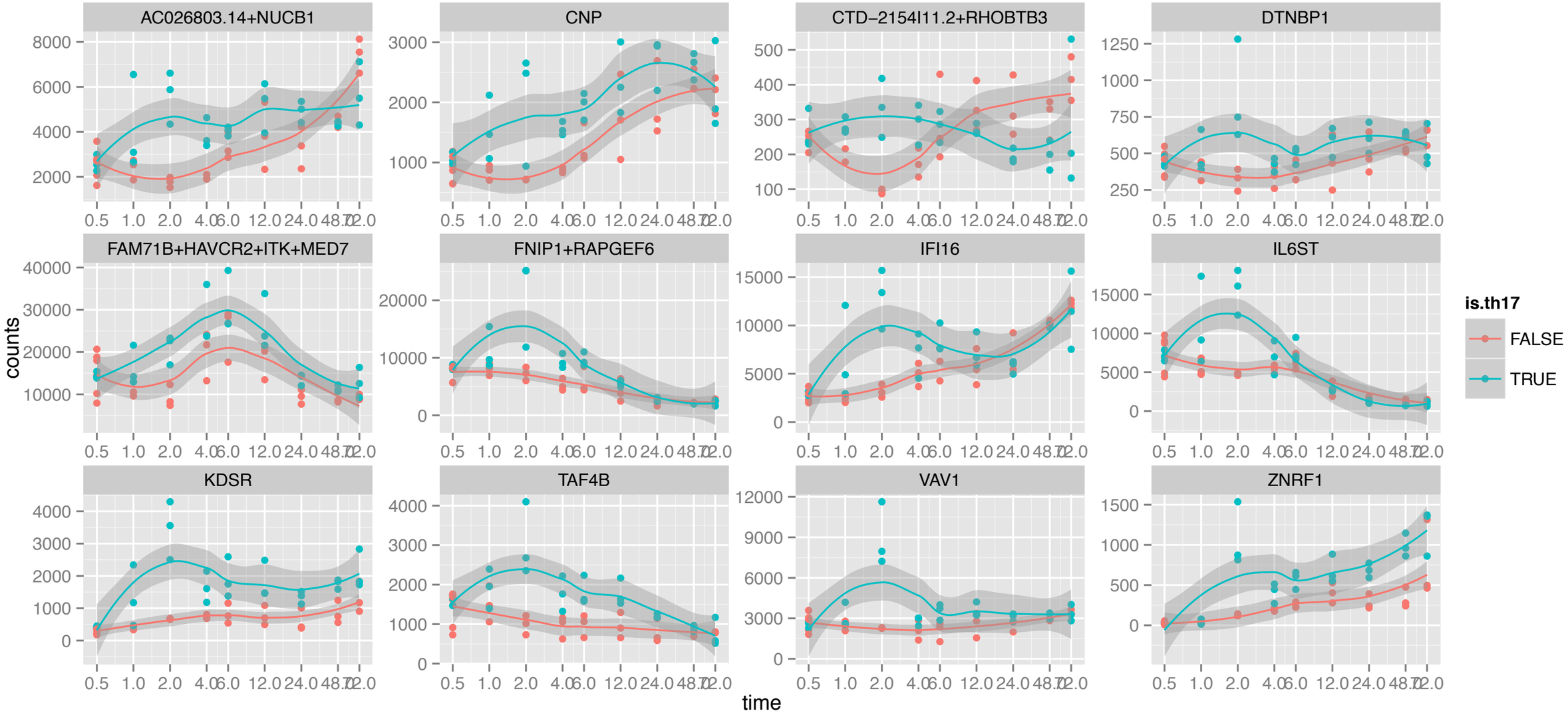
Tuomela et al., 2014

Transcription dynamics



- Biphasic in both species
- Seems “stronger” in the mouse samples
- earliest changes (1/2h!) visible

Contrasting: Genes DE at time 2

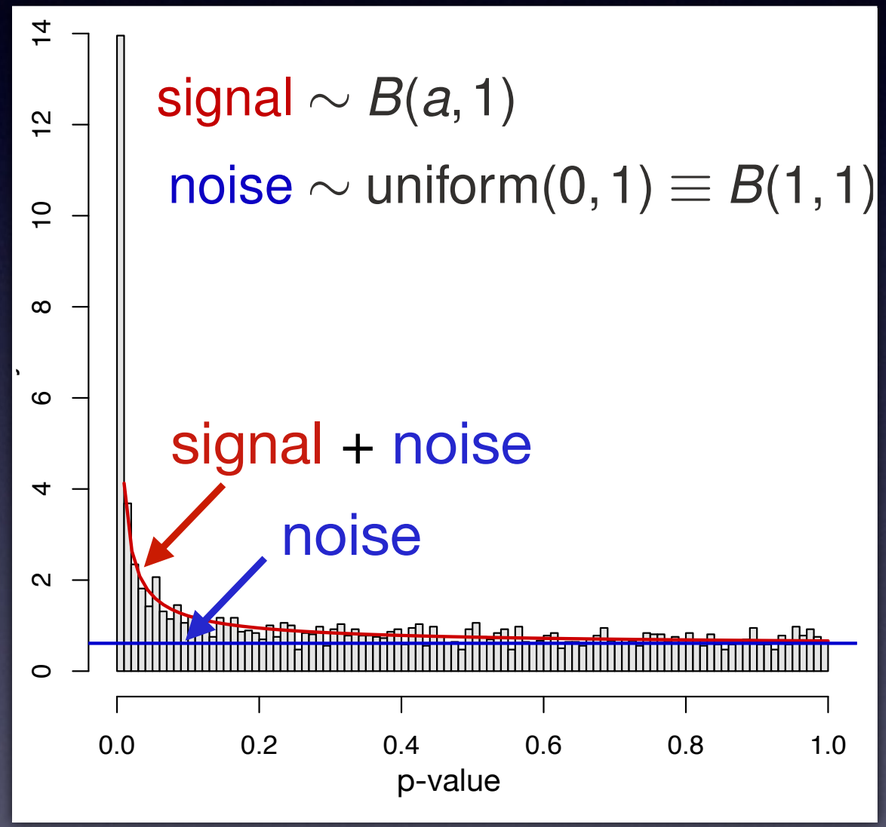


Scores: BUM Model

- Activity (positive and negative) scores derived from
  - p-values distribution
  - described with a beta-uniform mixture model;
  - controlling the FDR
  - using an adj. LL ratio:

$$s(x, \mathrm{FDR}) = \log \frac{\hat{a} x^{\hat{a}-1}}{\hat{a}\tau(\mathrm{FDR})^{\hat{a}-1}}$$

$$= (\hat{a} - 1)\left(\log(x) - \log(\tau(\mathrm{FDR}))\right)$$



Estimating the occurrence of false positives and false negatives in microarray studies by approximating and partitioning the empirical distribution of p-values. Pounds 2003

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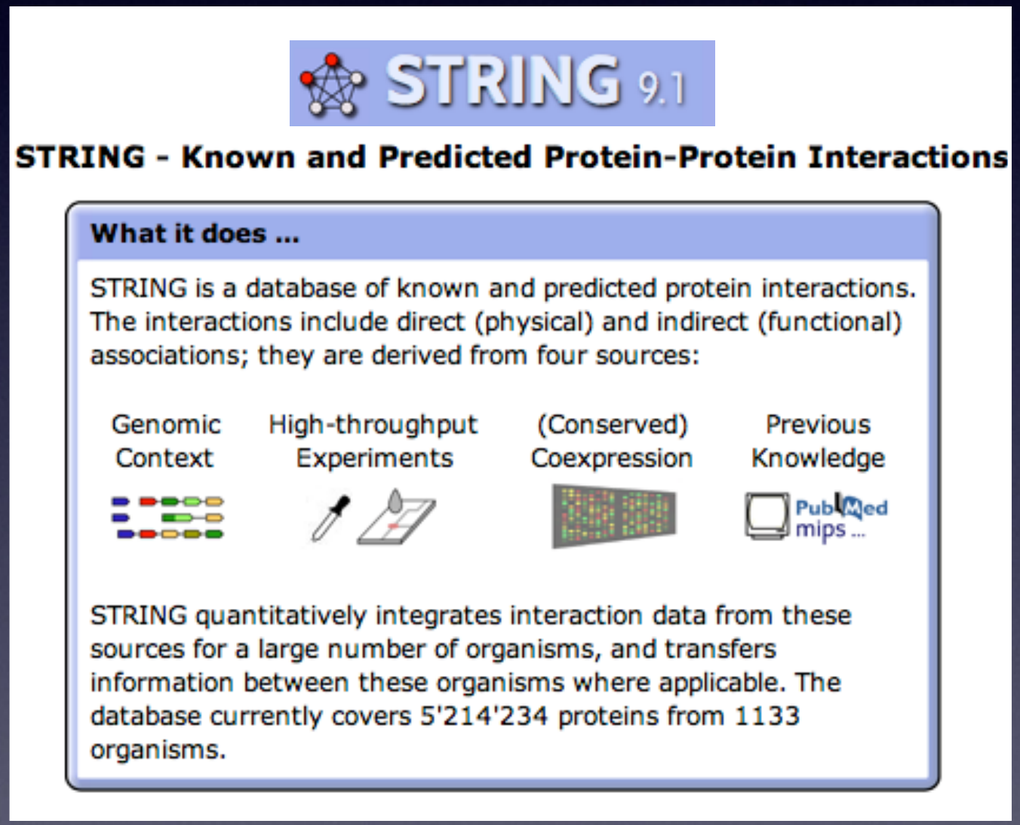
PPI Networks

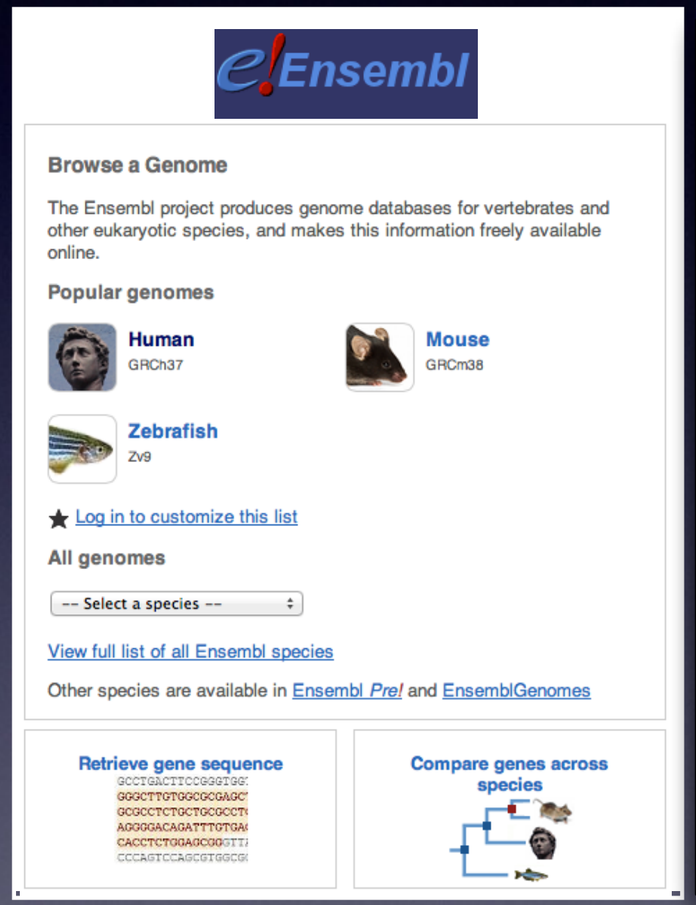
- Obtained from the STRING db
- Only kept *physical interactions*
- Mouse network: 12,121 nodes and 176,462 edges
- Human network : 14,280 nodes and 197,649 edges

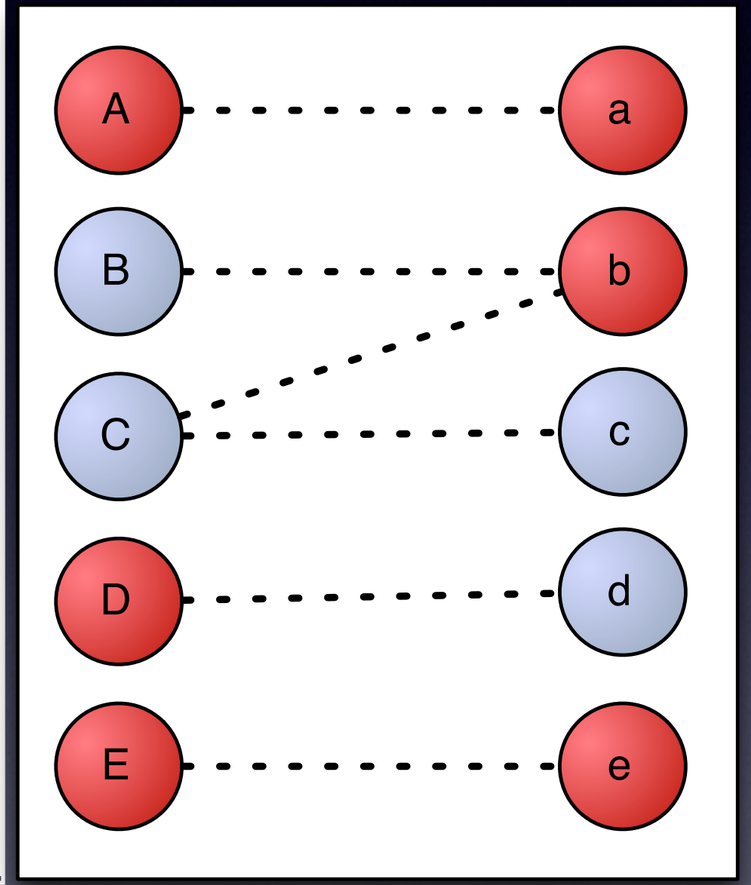


Orthology relations

- Obtained from ENSEMBL orthology
- Represented as a bi-partite graph M
- 85,640 human proteins
- 49,717 mouse proteins
- linked by 125,685 edges
- grouped in 16,736 bicliques

  (avg size of 8.08, median of 6, SD of 5.97)





M&M recipe (recap)

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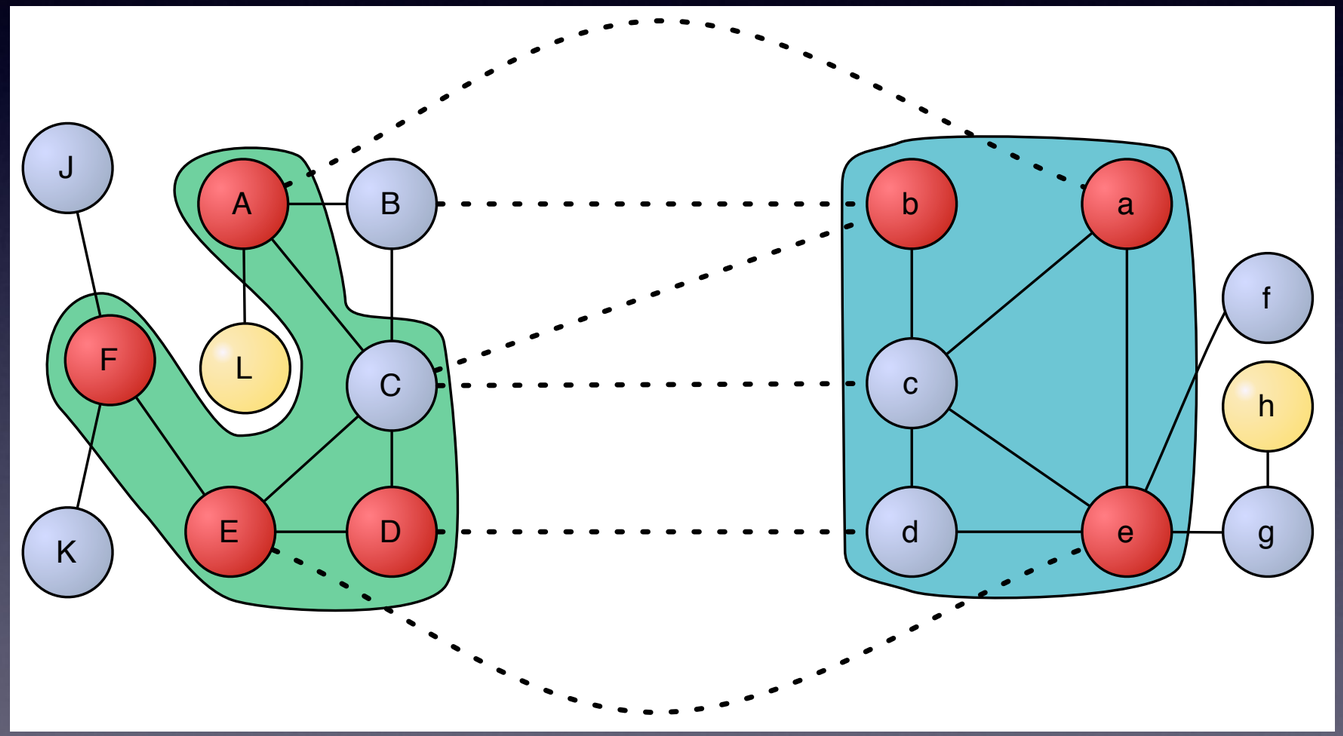
Conserved active modules

- Formalized using a constraint modeling approach over boolean variables
- Constraints are linearized => MILP
- The MILP is then solved using CPLEX with a *branch-and-cut* algorithm



MILP:?

- A formulation, with:
  - an *objective function*
  - subject to *linear constraints*
  - where variables can be constrained to discrete domains ($\{0,1\}, \mathbb{N}$)
  - much harder than on $\mathbb{R}$
  - for which exact solutions can be found efficiently in practice

$$\max_{x_1, x_2} S_1 \cdot x_1 + S_2 \cdot x_2$$

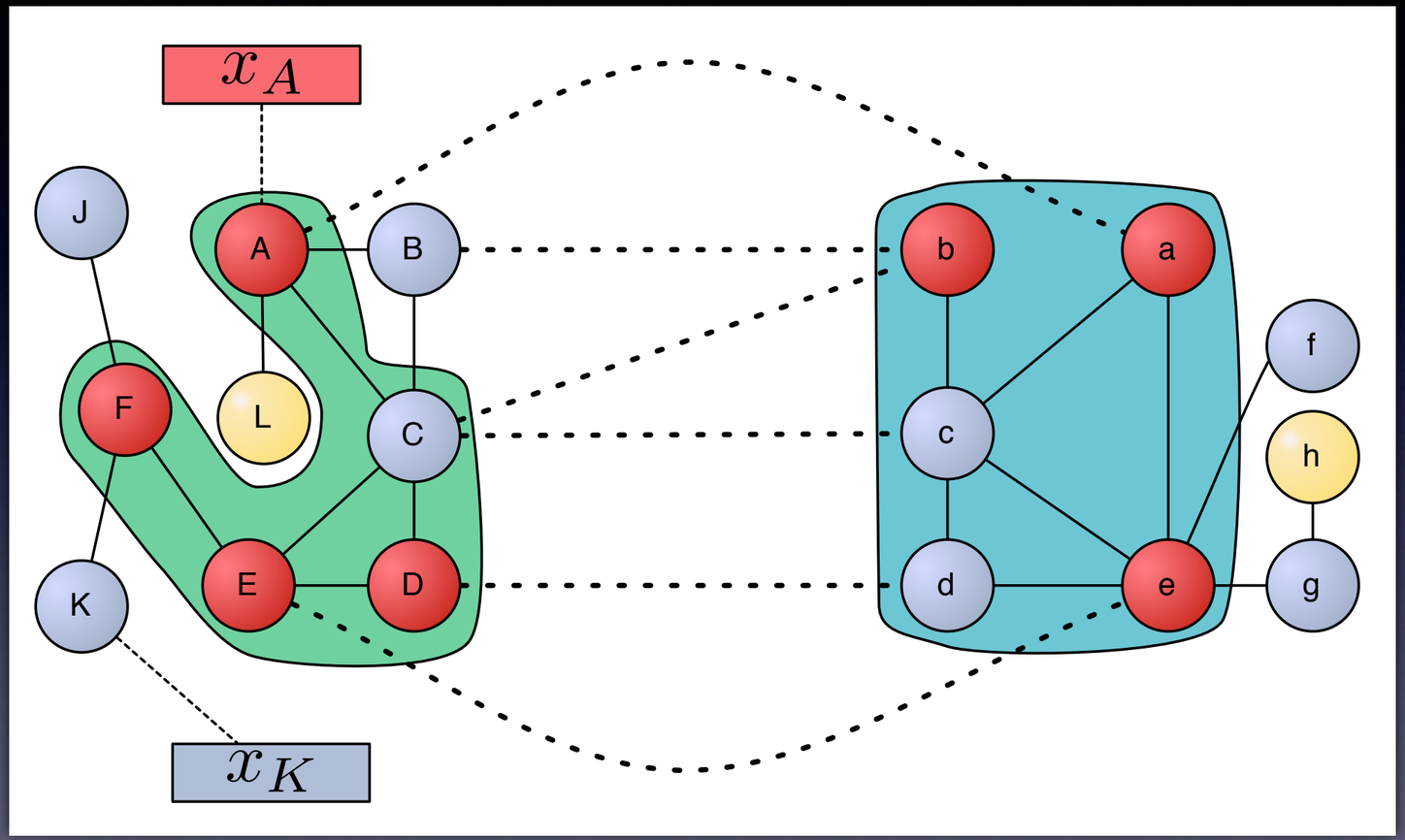
$$\text{Subject to: } x_1 + x_2 \leq L$$

$$F_1 \cdot x_1 + F_2 \cdot x_2 \leq F$$

$$P_1 \cdot x_1 + P_2 \cdot x_2 \leq P$$

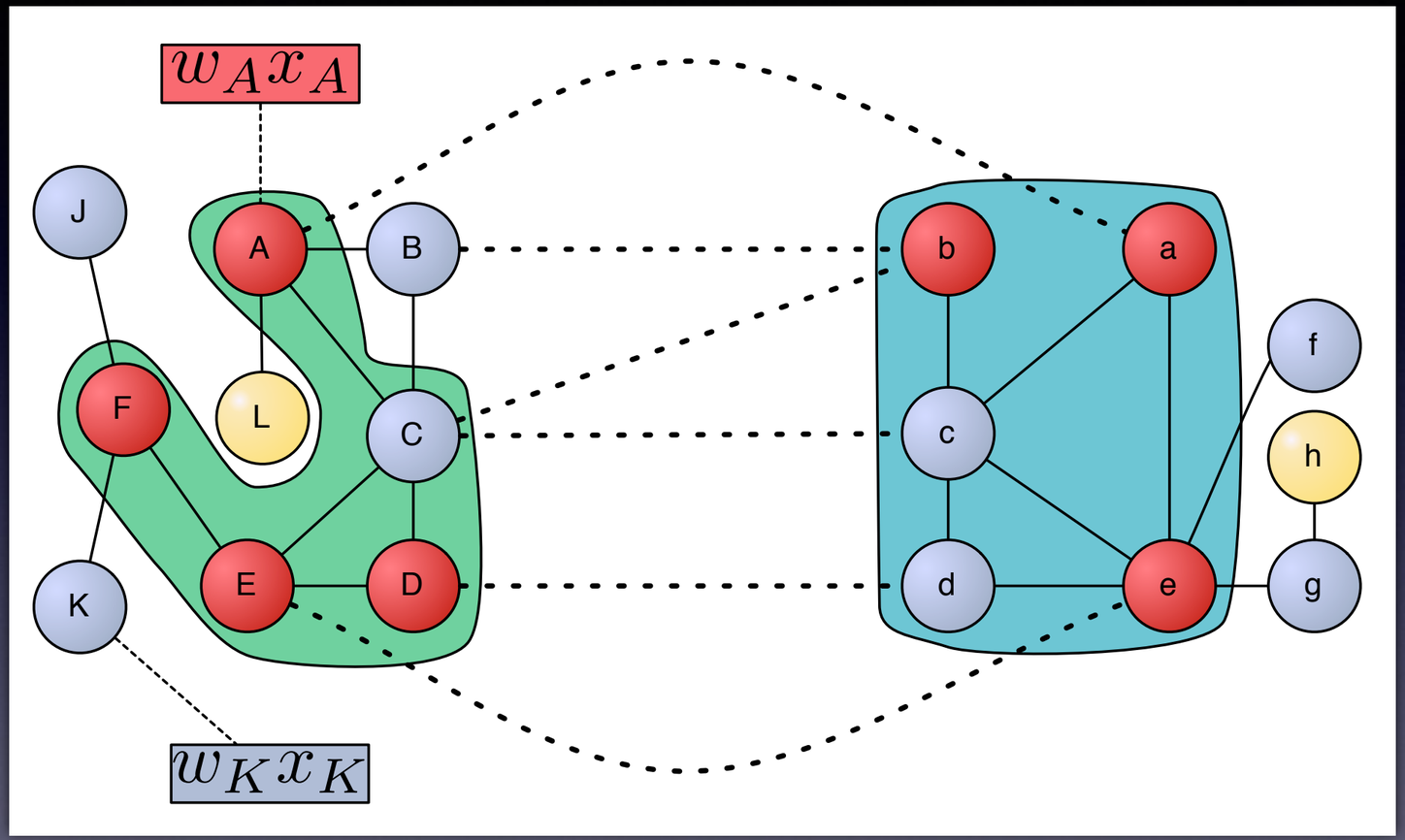
$$x_1 \geq 0, x_2 \geq 0$$

MILP: Variables



Boolean variables for nodes in solution

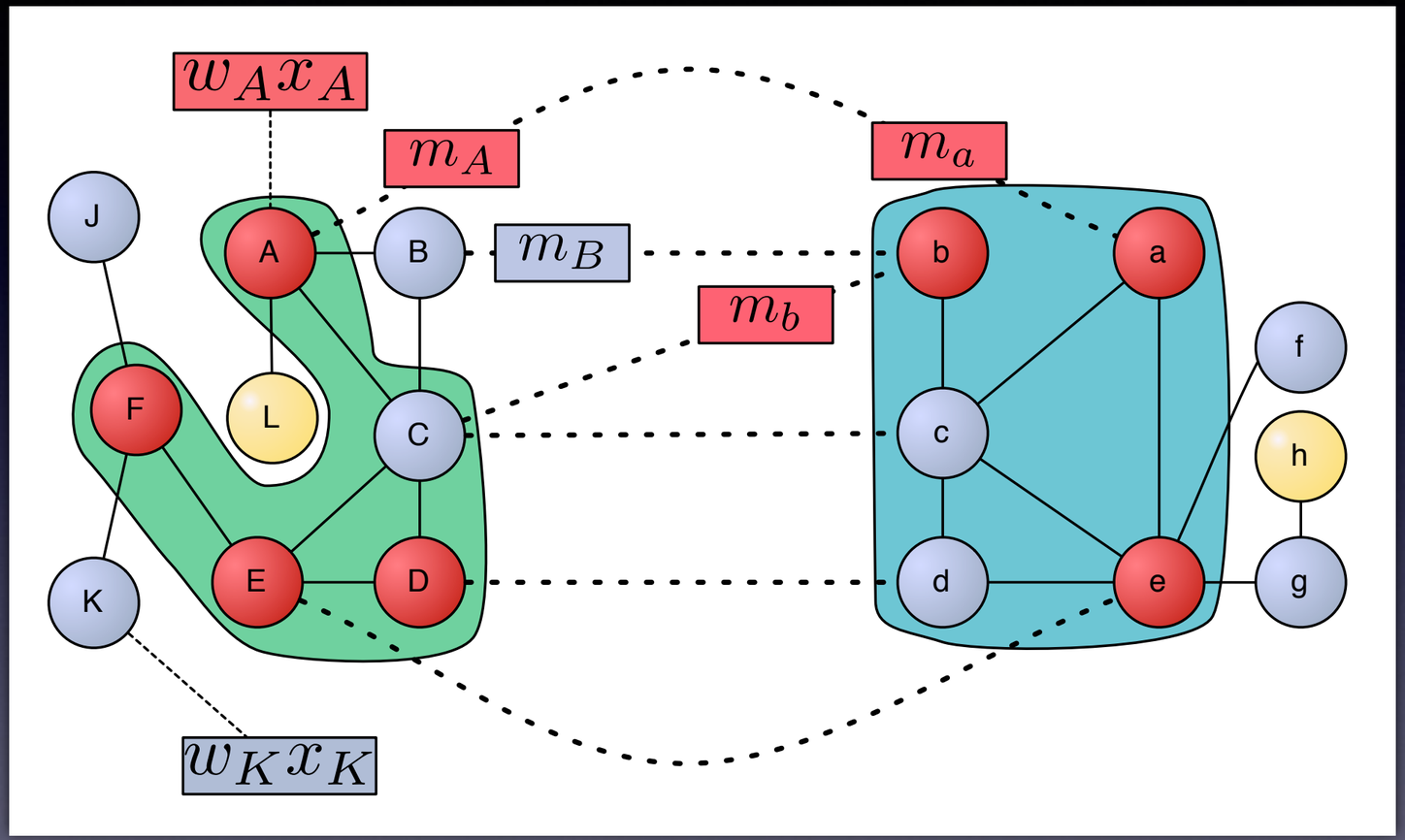
MILP: Variables



Weighted boolean variables for nodes in solution, objective function:

$$\max \sum_{v \in V_1 \cup V_2} w_v x_v$$

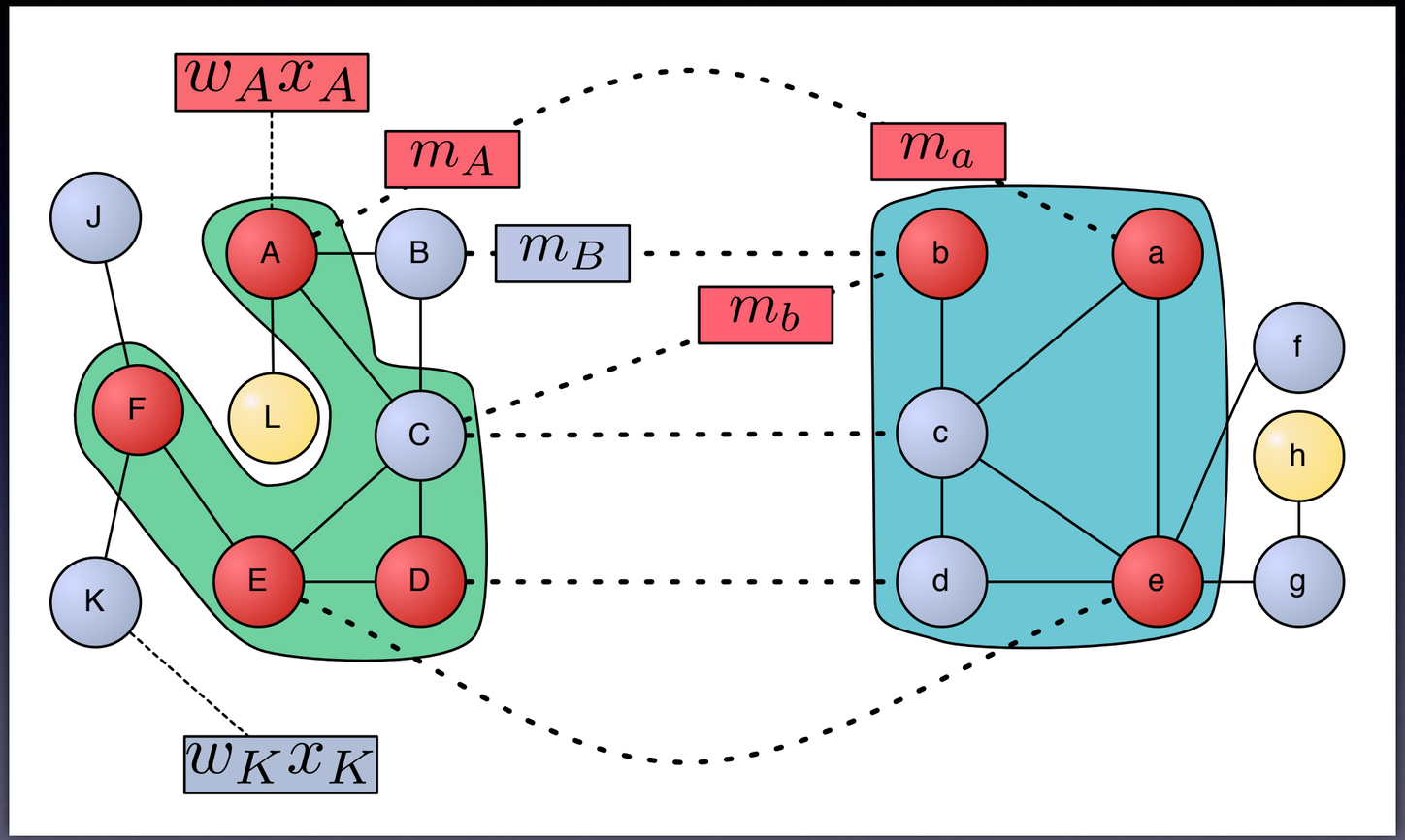
MILP:Variables



Boolean variables for conserved nodes

$$m_u = \max_{uv \in M} \{x_u x_v\}$$

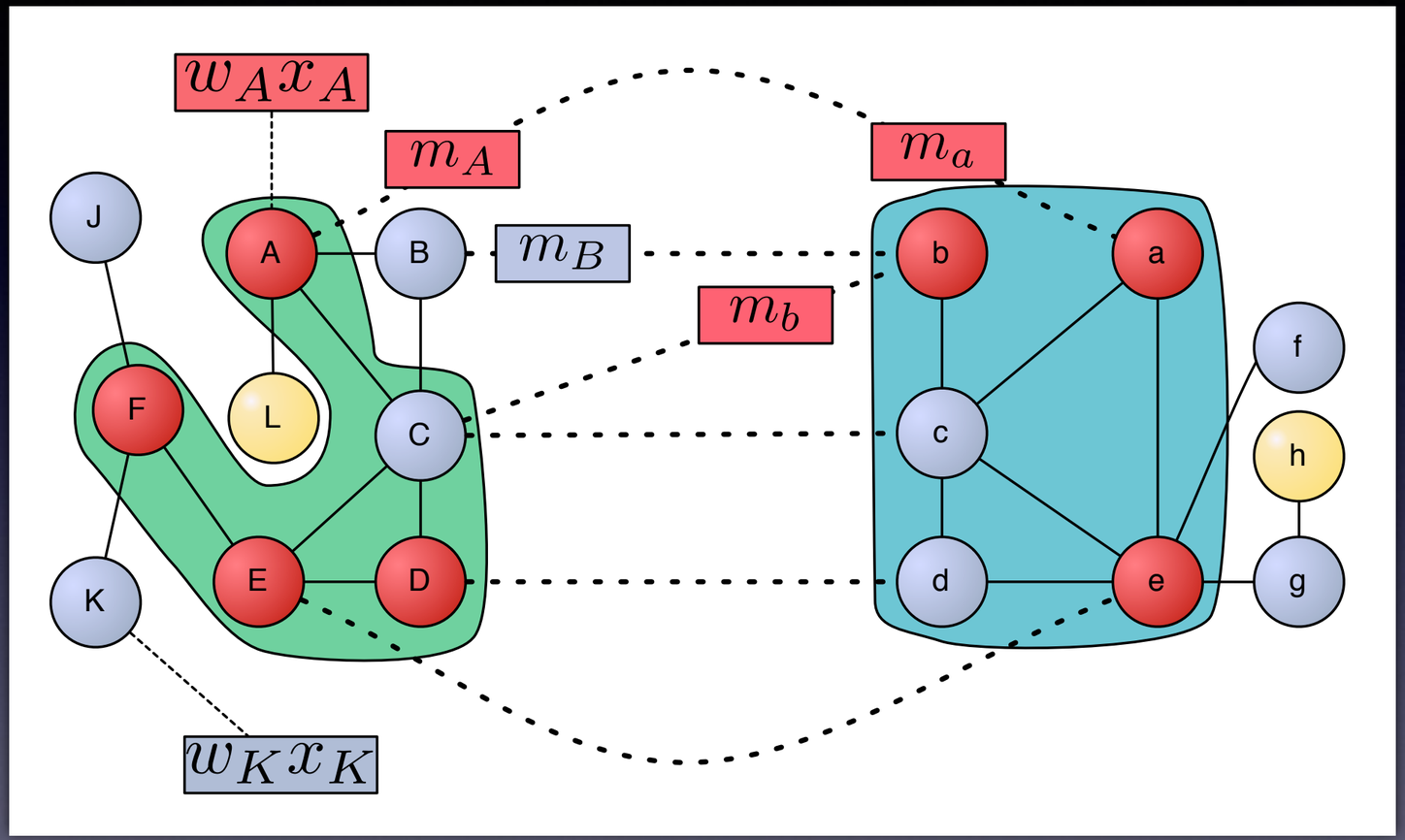
MILP: degree of conservation



constrained by having e.g more than $\alpha = 50\%$ of nodes being conserved

$$\sim \frac{\sum m_v}{\sum x_v} \geq 50\%$$

MILP: connectivity



- And satisfying the connectivity constraint:
  - Possibly an exponential number of constraints
  - Constraints added as needed during optimization

MILP: Formulation

$$\max \sum_{v \in V_1 \cup V_2} w_v x_v \tag{1}$$

$$\text{s.t. } m_u = \max_{uv \in M} \{x_u x_v\} \qquad u \in V_1 \tag{2}$$

$$m_v = \max_{uv \in M} \{x_u x_v\} \qquad v \in V_2 \tag{3}$$

$$\sum_{v \in V_1 \cup V_2} m_v \geq \alpha \sum_{v \in V_1 \cup V_2} x_v \tag{4}$$

$$G_1[\mathbf{x}] \text{ and } G_2[\mathbf{x}] \text{ are connected} \tag{5}$$

$$x_v, m_v \in \{0, 1\} \qquad v \in V_1 \cup V_2 \tag{6}$$

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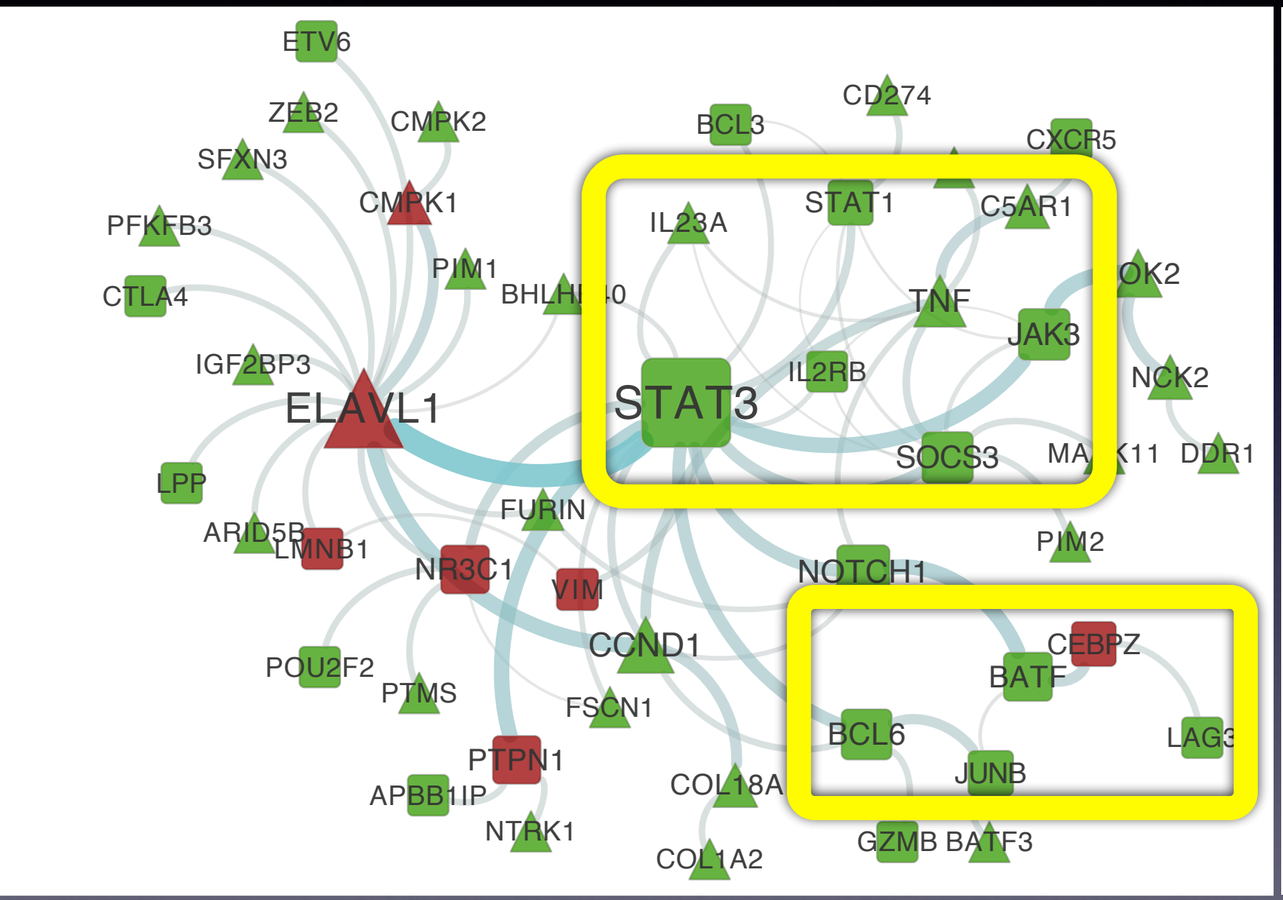
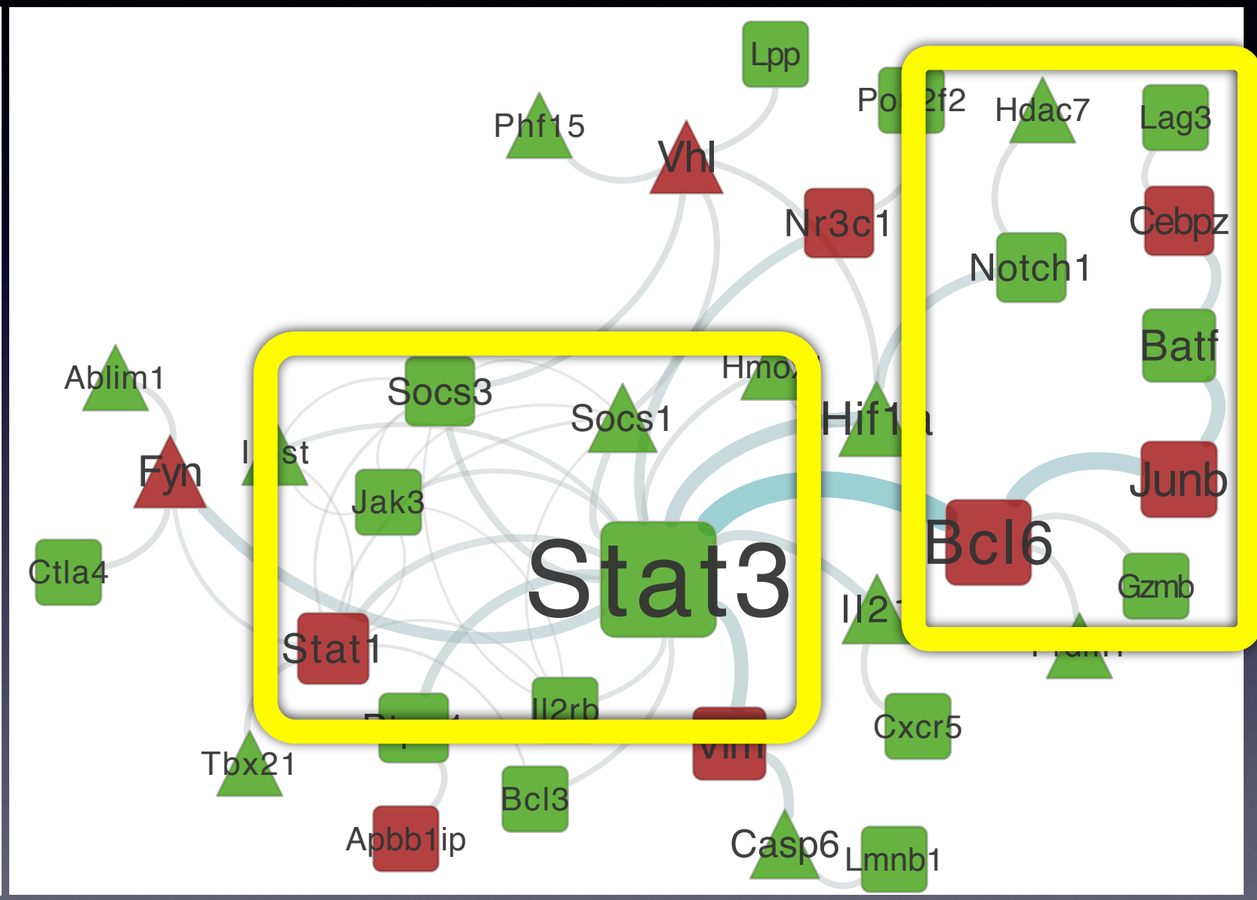
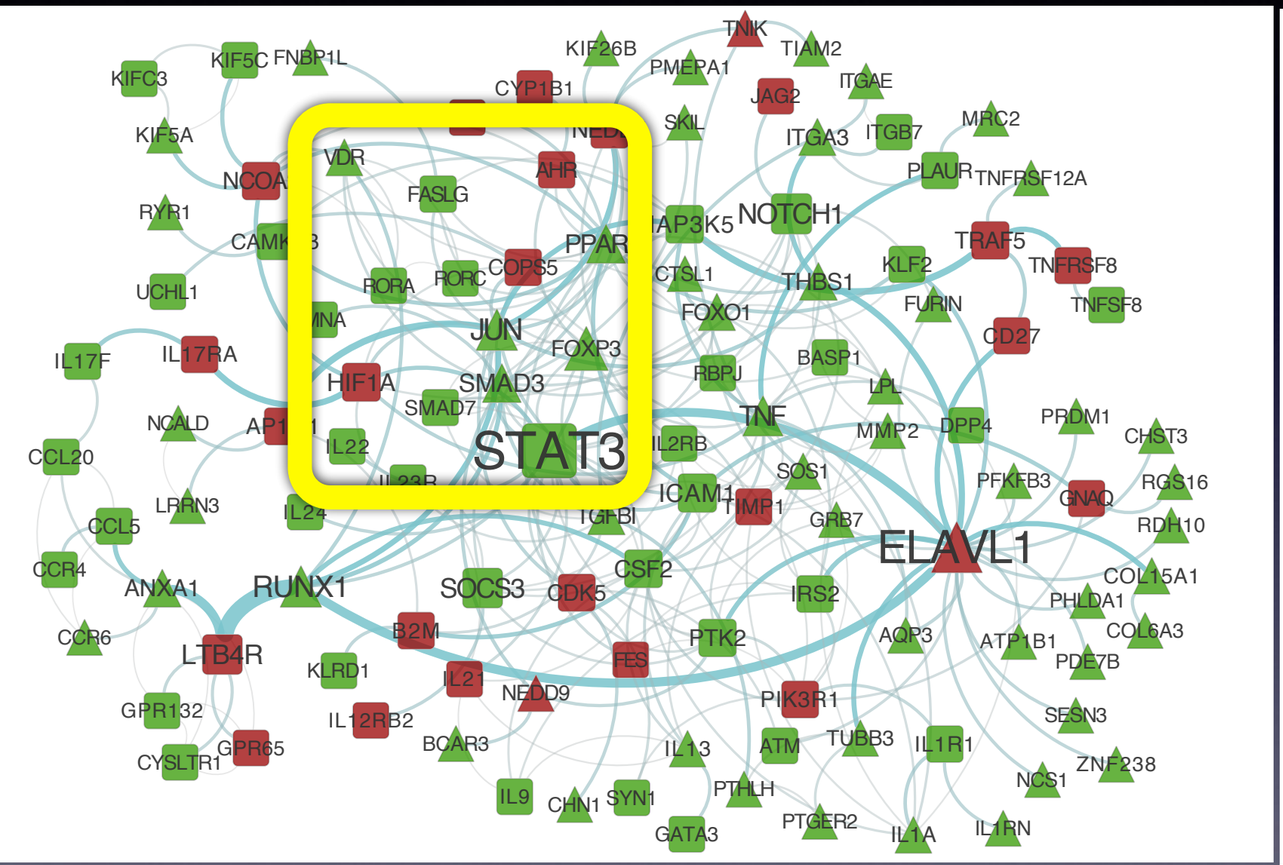
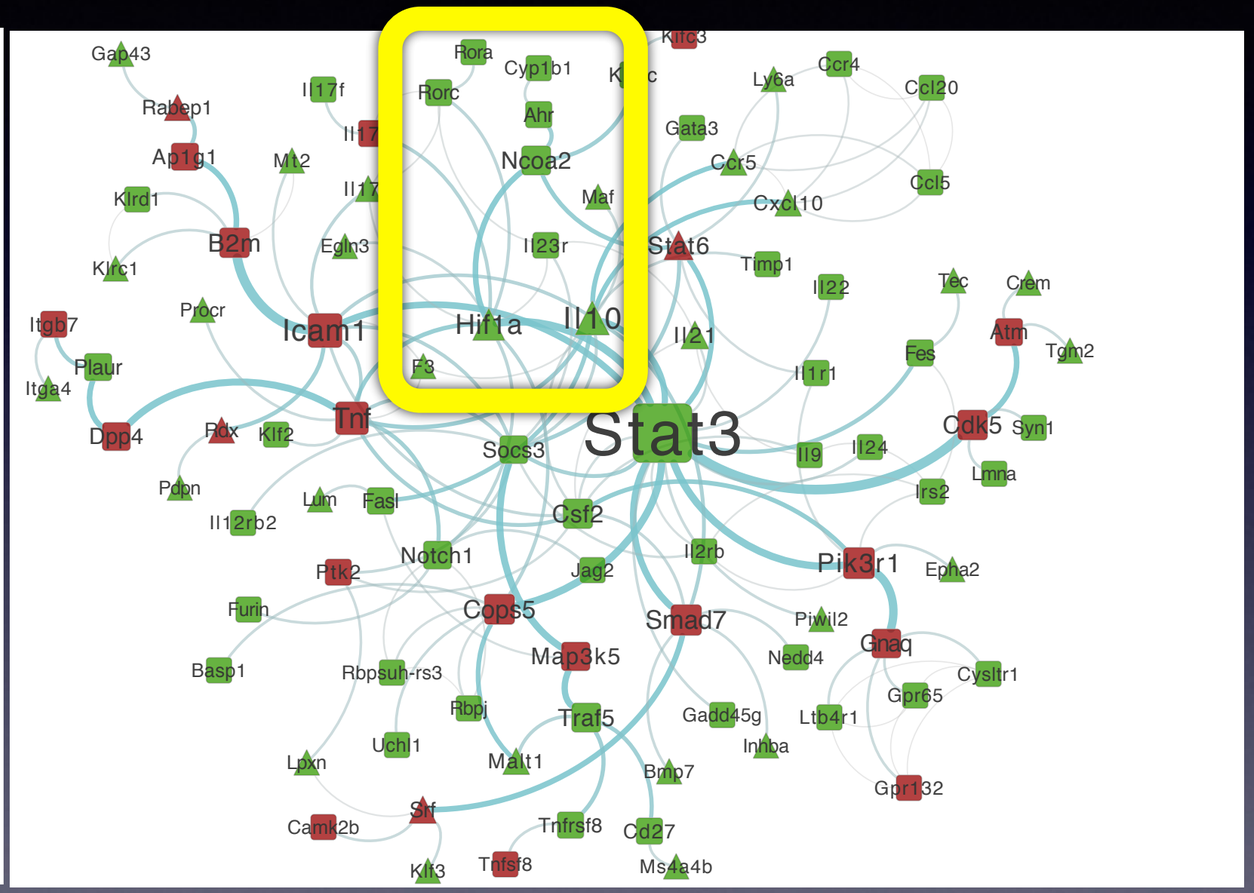
2h conserved module





72h conserved module





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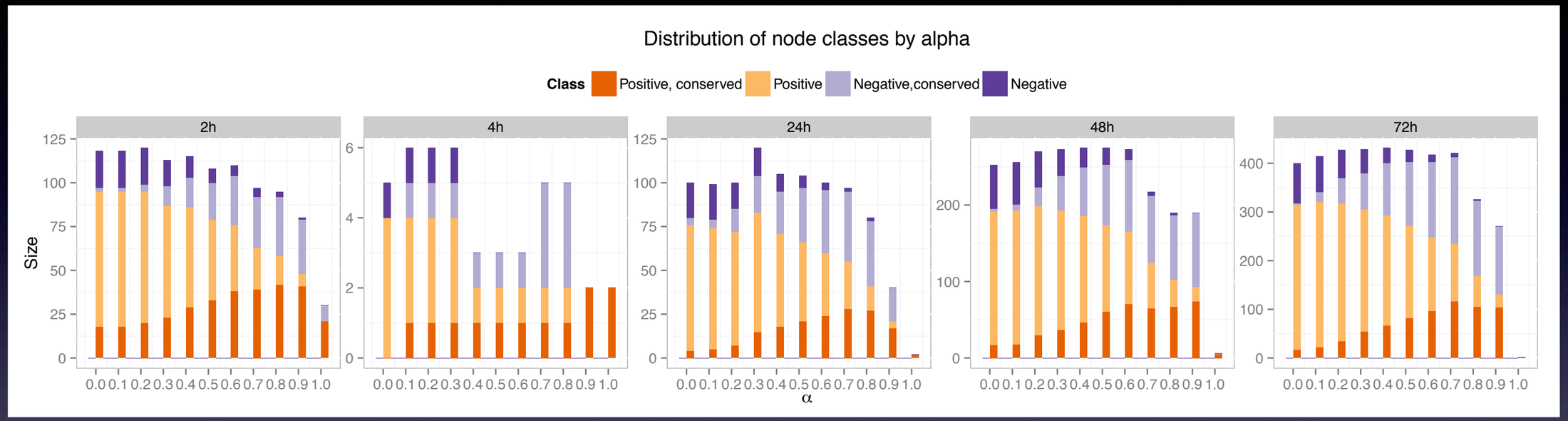
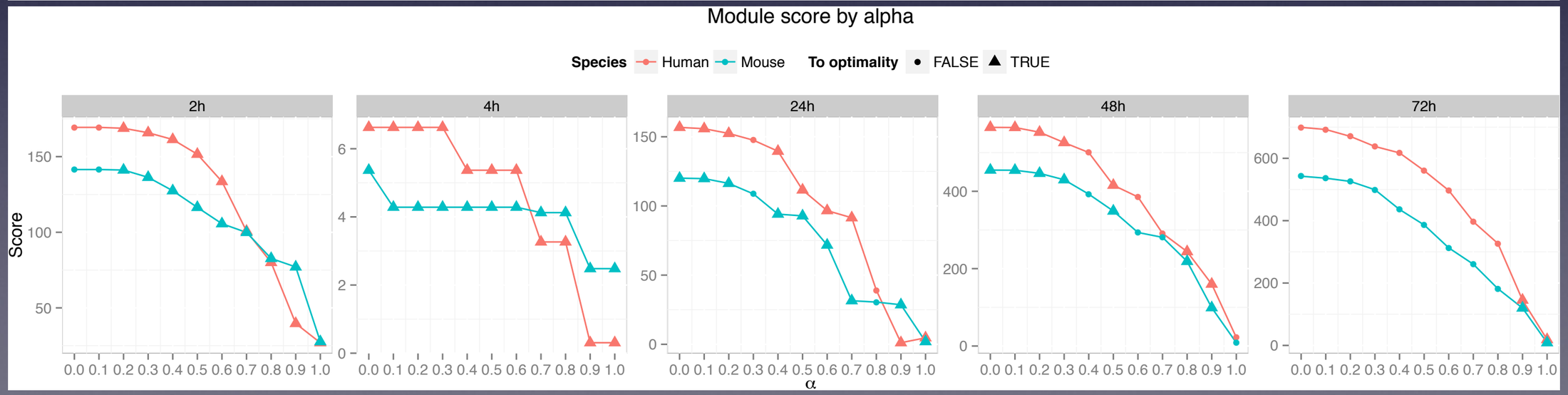
Overall dynamics and solutions





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Conclusions

- Mouse and Human Th17 differentiation processes are well conserved during the first 72h
- Differentiation happens in two phases, very early (0h--4h) and late (12h--72h)
- We provide the first formulation of the *conserved active module problem* as well as an efficient MILP solver
- Code and recipes available there: http://software.cwi.nl/xheinz
- PS: We got the same results on an independent data set!

Future work

- Theoretical results on the computational complexity for specific network topologies
- Novel formulation for bi-conservation: Conservation between species and across time
- Non-supervised formulation: Clustering of samples based on conserved active modules

Thanks!

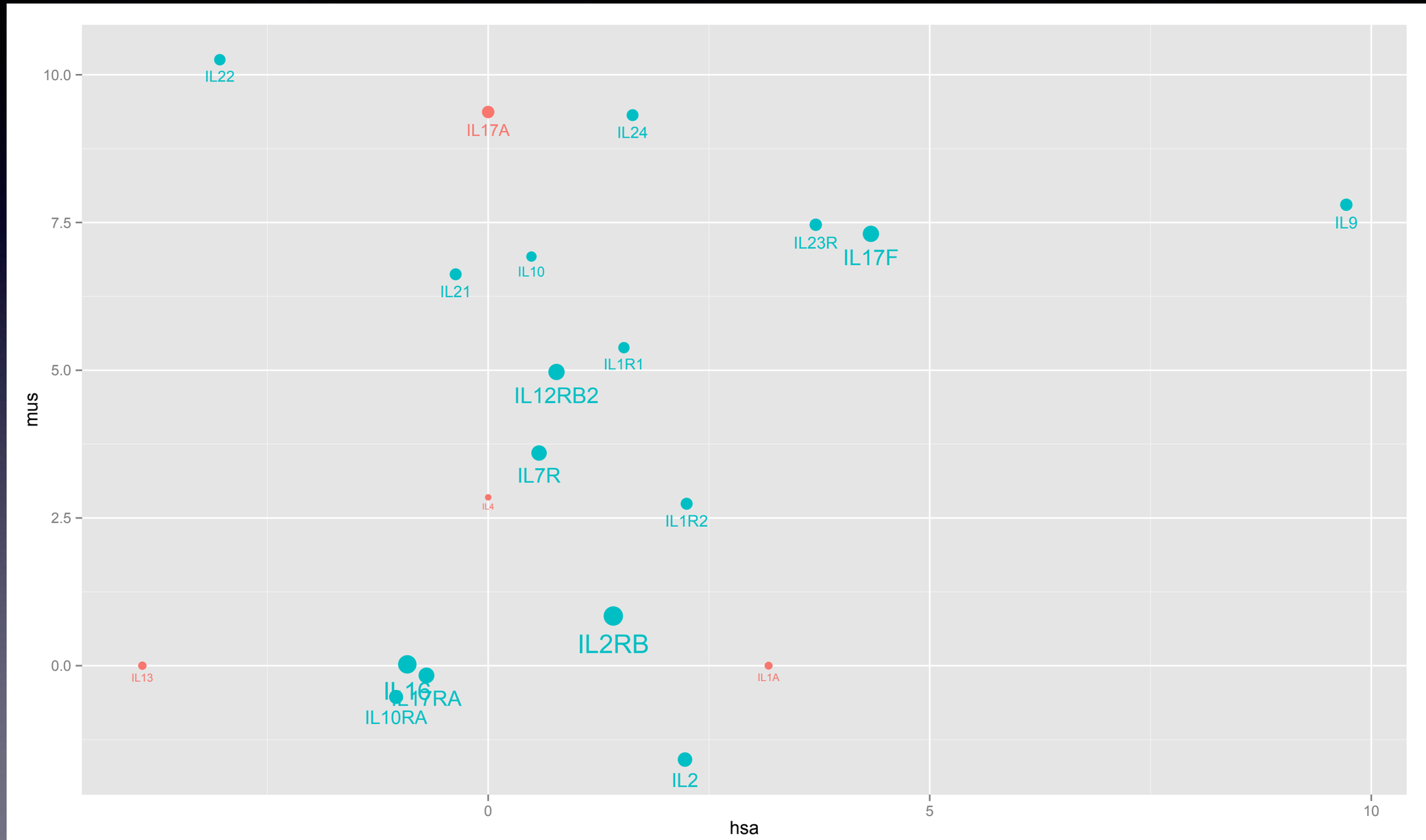
and see you @ poster 7!

CWI

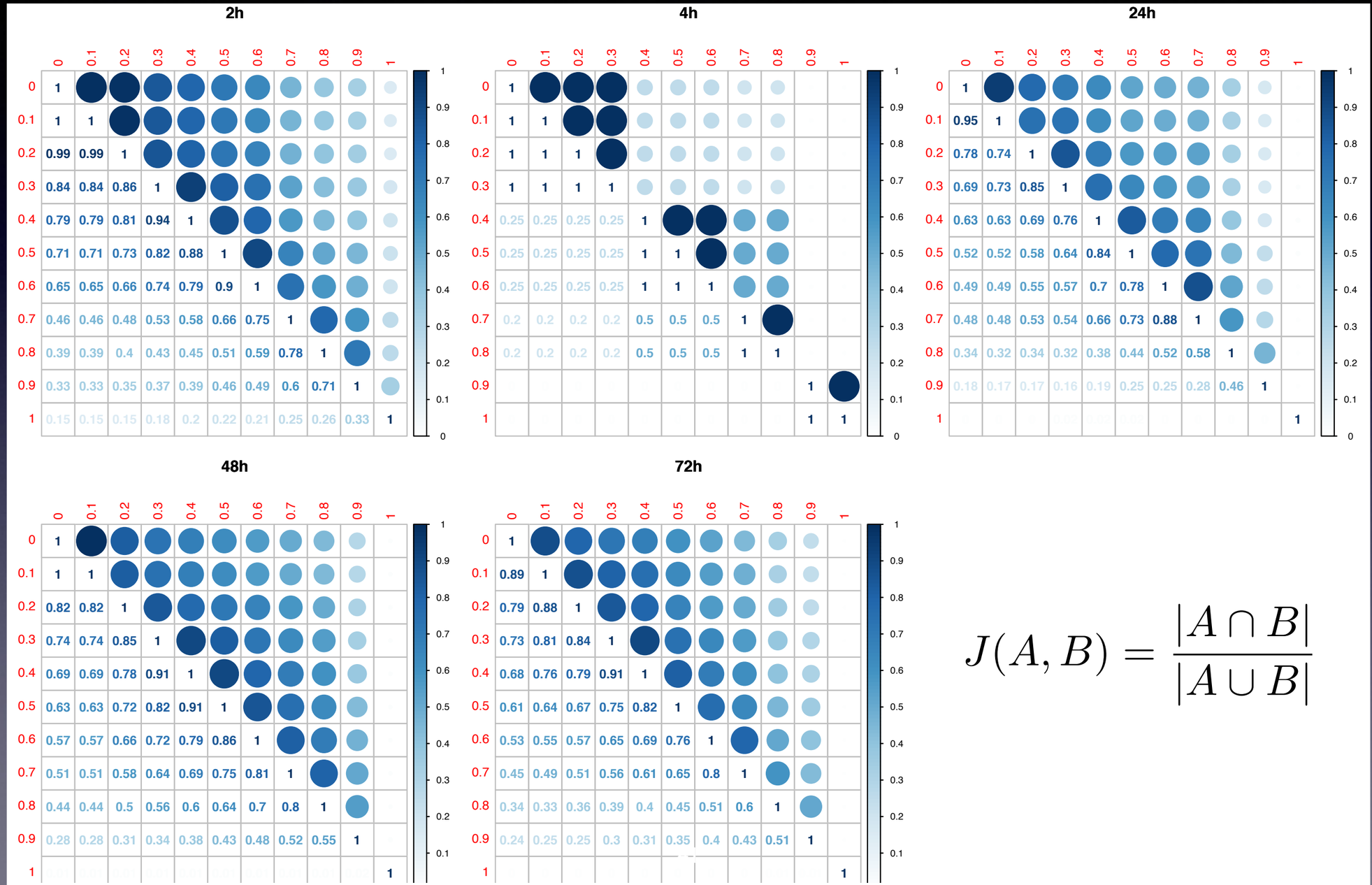
- Wessels group / NKI
- CWI for the expertise
- CBIB/CGFB for the environment (and computing power!)
- VU Centre for Integrative Bioinformatics
- ERASysBio for the funding
- Riitta Laheesma's group for the data
- And you for your attention... and for trying the tool: http://software.cwi.nl/xheinz

ERASysBio Plus

72h: What are the DE IL?

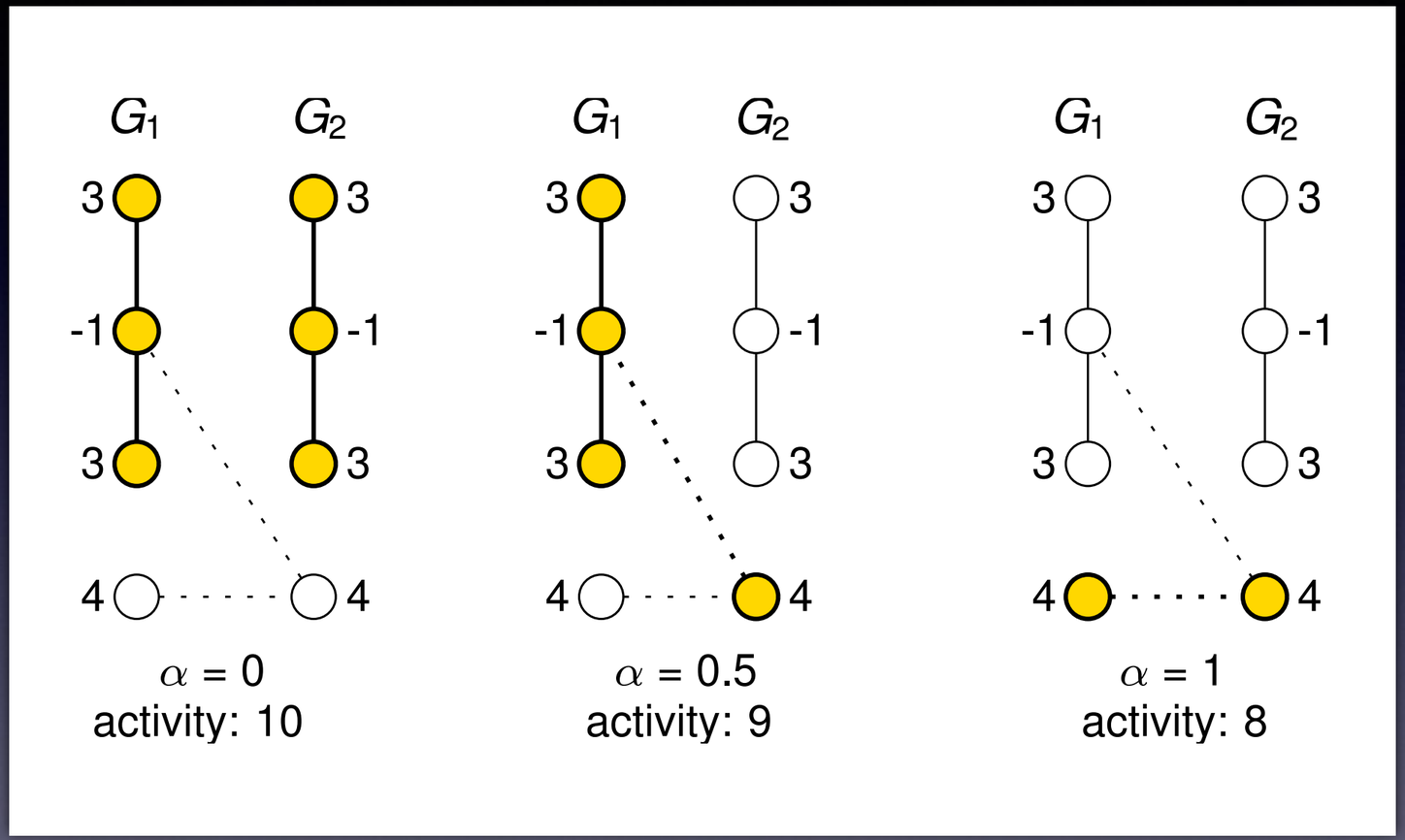


Influence of conservation on content



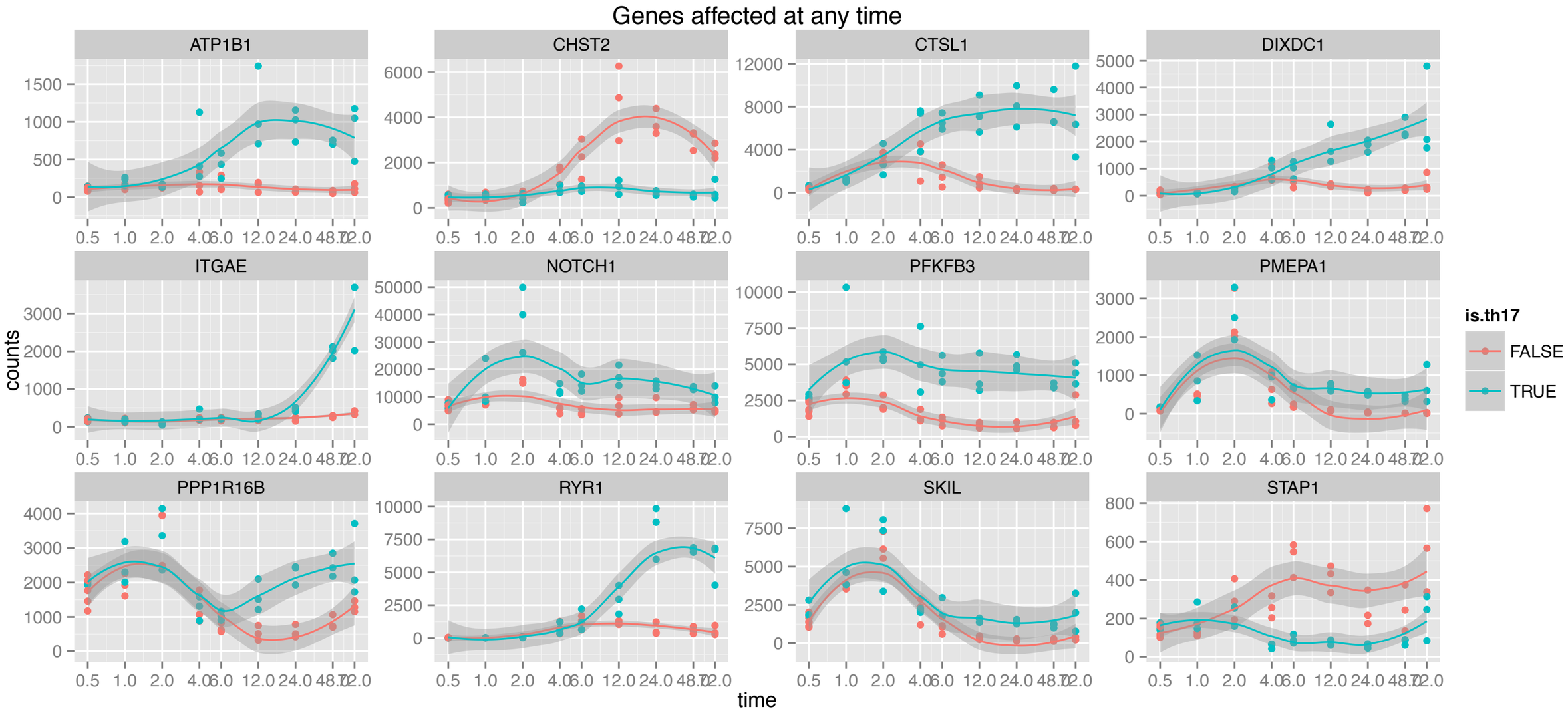
$$J(A,B) = \frac{|A \cap B|}{|A \cup B|}$$

MILP: Conservation tradeoff

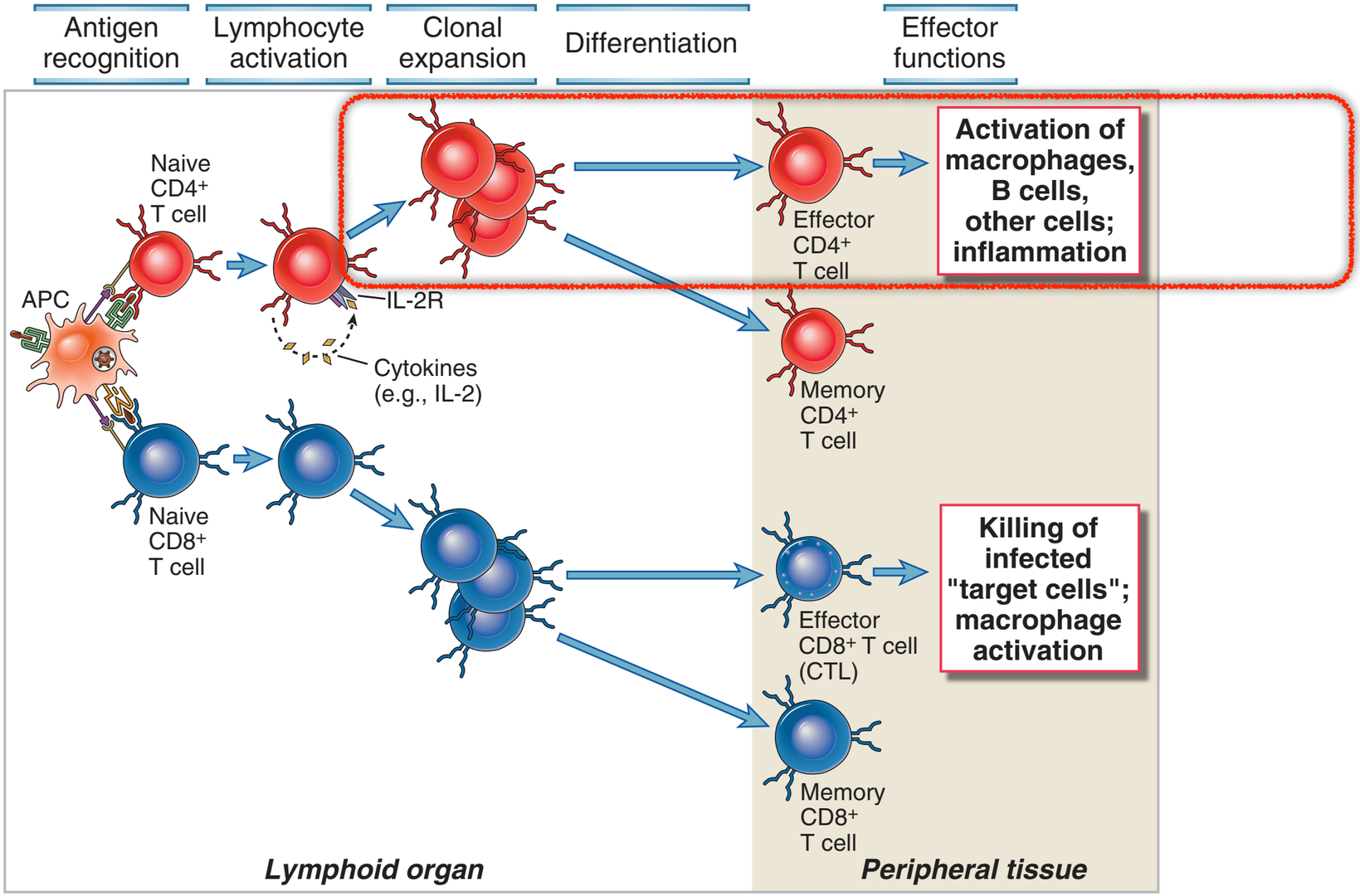


Controls tradeoff between overall activity and conservation

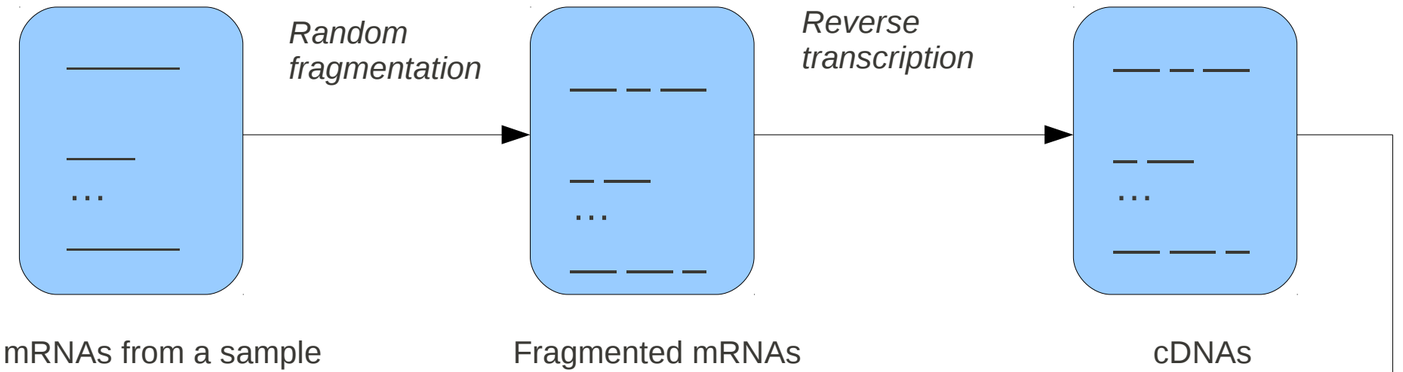
Genes affected at any time point



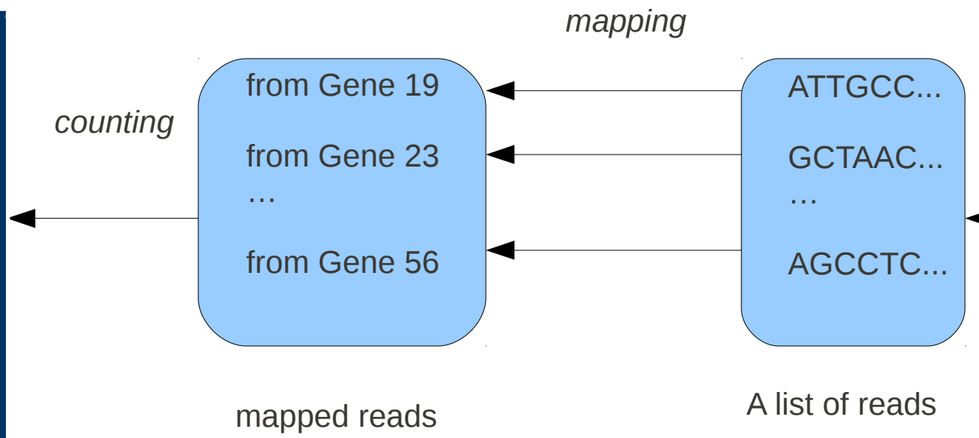
T cells response



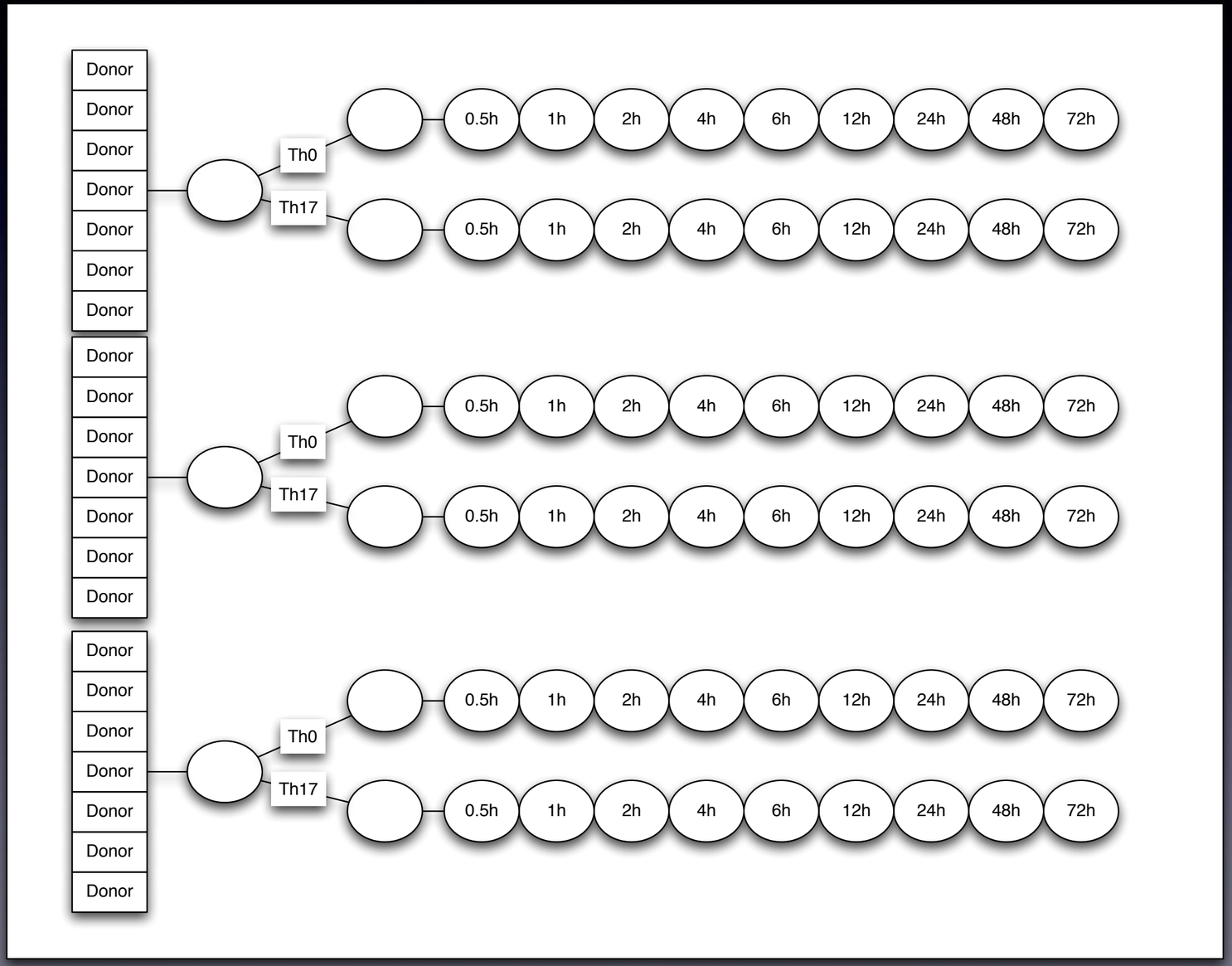
RNA-Seq



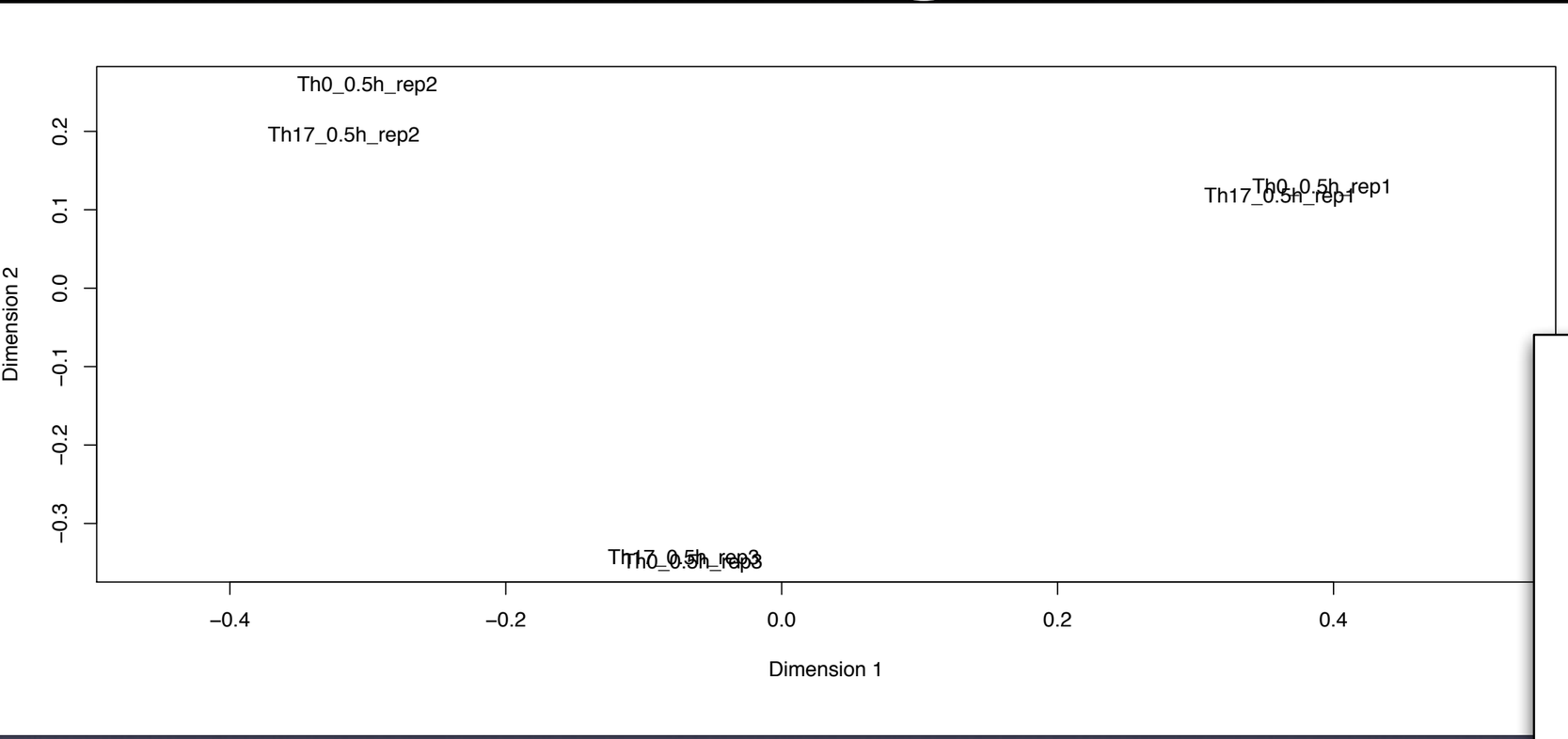
gene	Thp_rep1	Th0_0.5h_rep1	Th0_1h_rep1	Th0_2h_rep1
TAS2R42	0	0	0	0
RP11-140L24.3	6	7	1	6
MIR125B1	0	0	0	0
TRIM27	0	0	0	0
FBLN1	0	0	1	0
AC007919.19	0	0	0	0
EPS15L1	521	593	545	712
CTD-2334D19.1	0	0	0	0
CTA-714B7.5+CTA-714B7.6	12	11	12	10
ZBTB45	154	221	165	254
BAZ1A	2393	2987	3221	4764
FAM83C	0	0	0	0

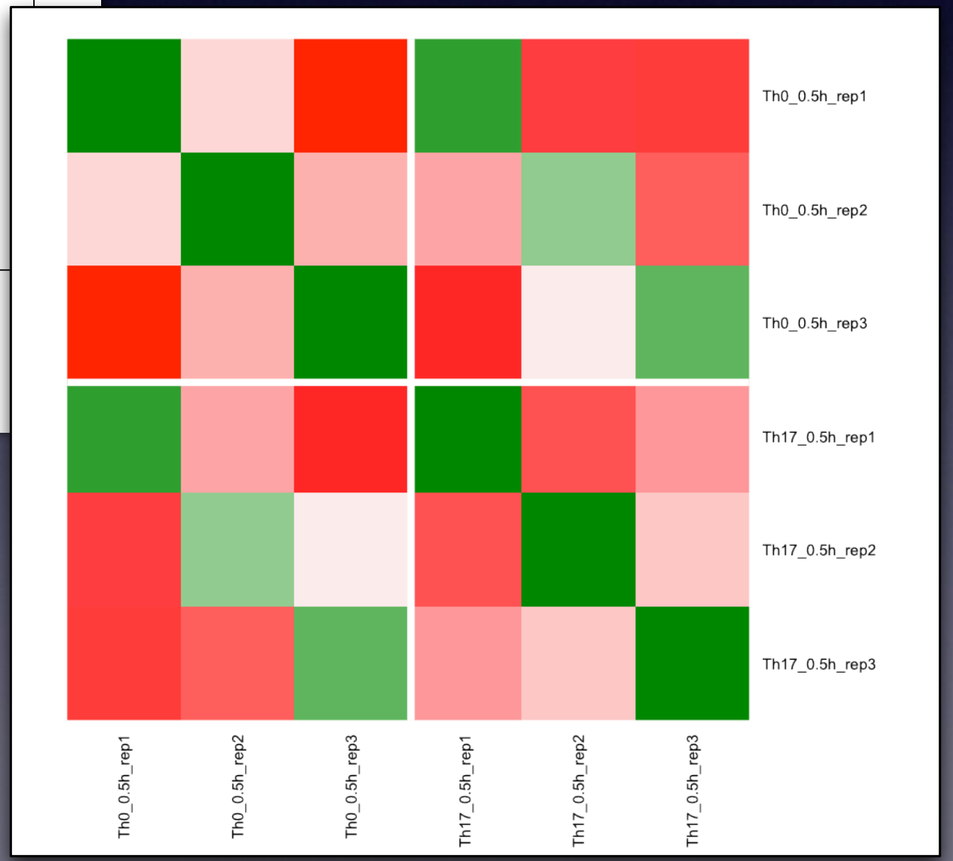


Human exp. design:

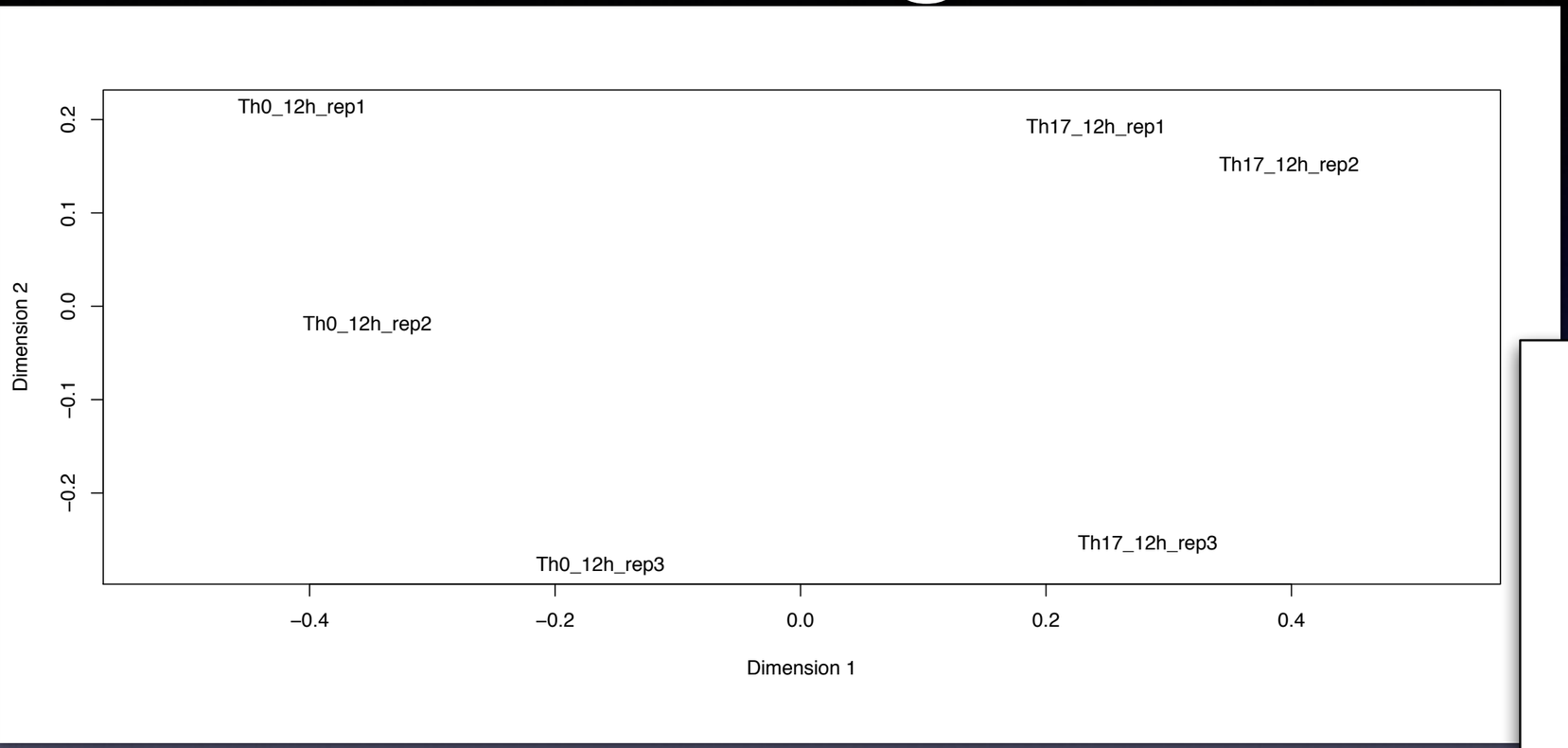


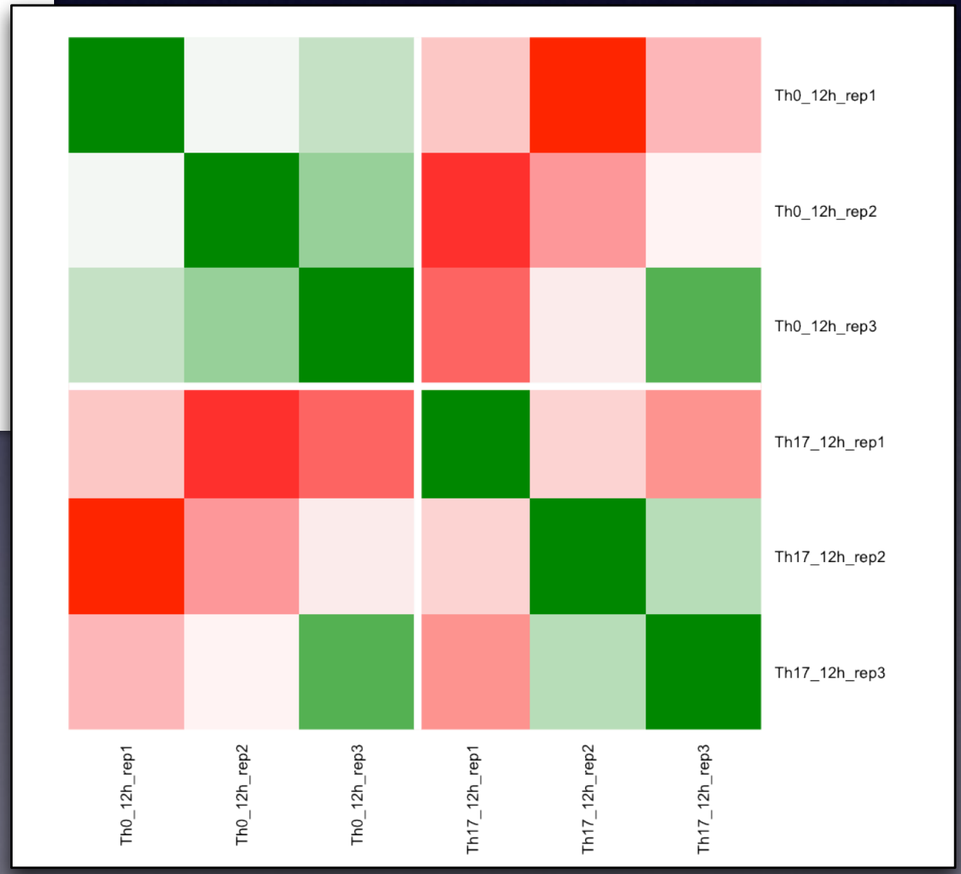
Strongest effects: 0.5h



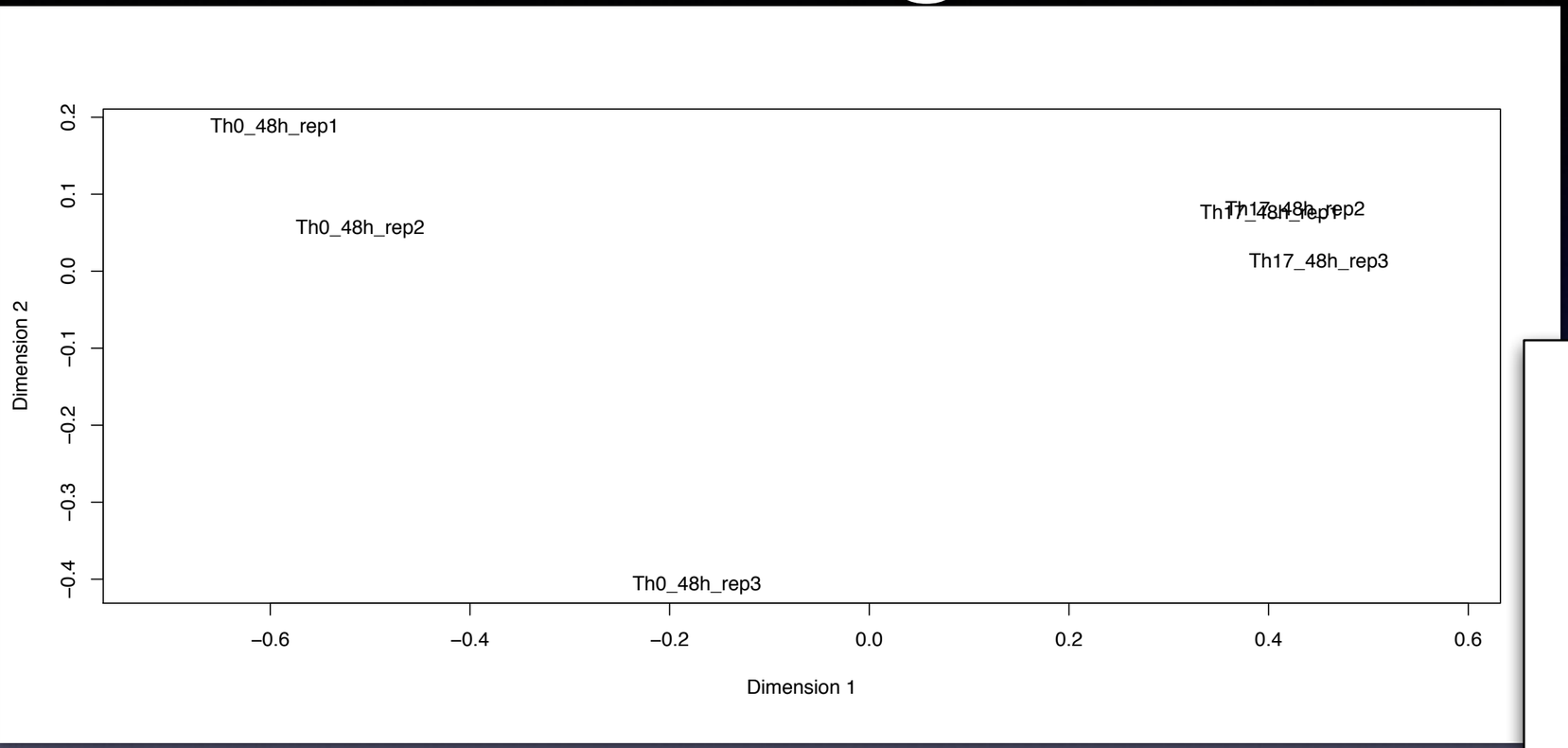


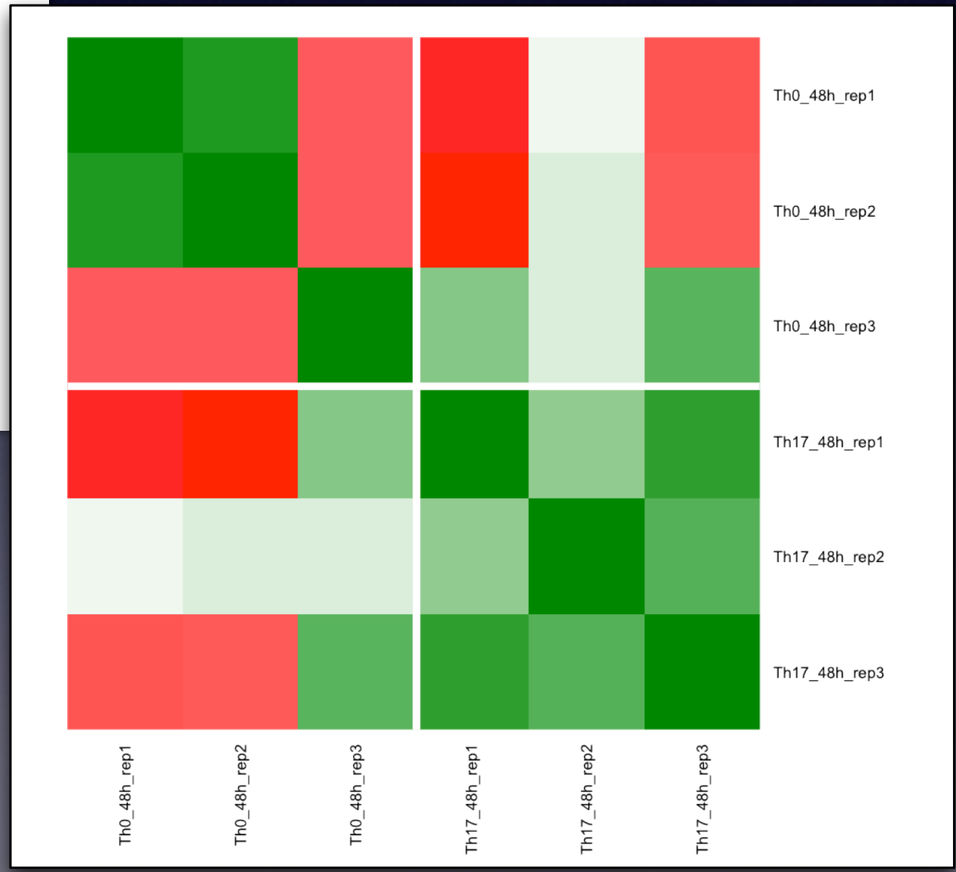
Strongest effects: 12h



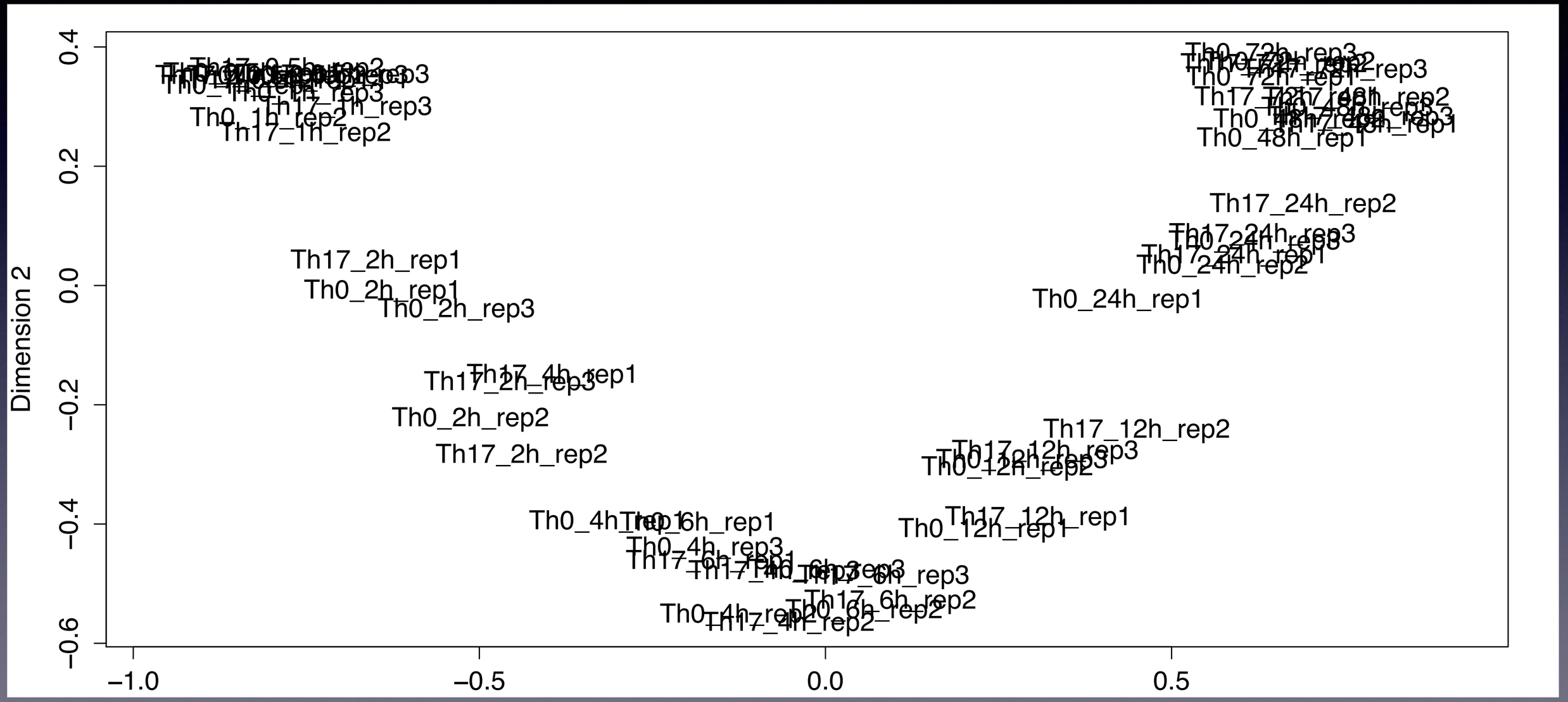


Strongest effects: 48h

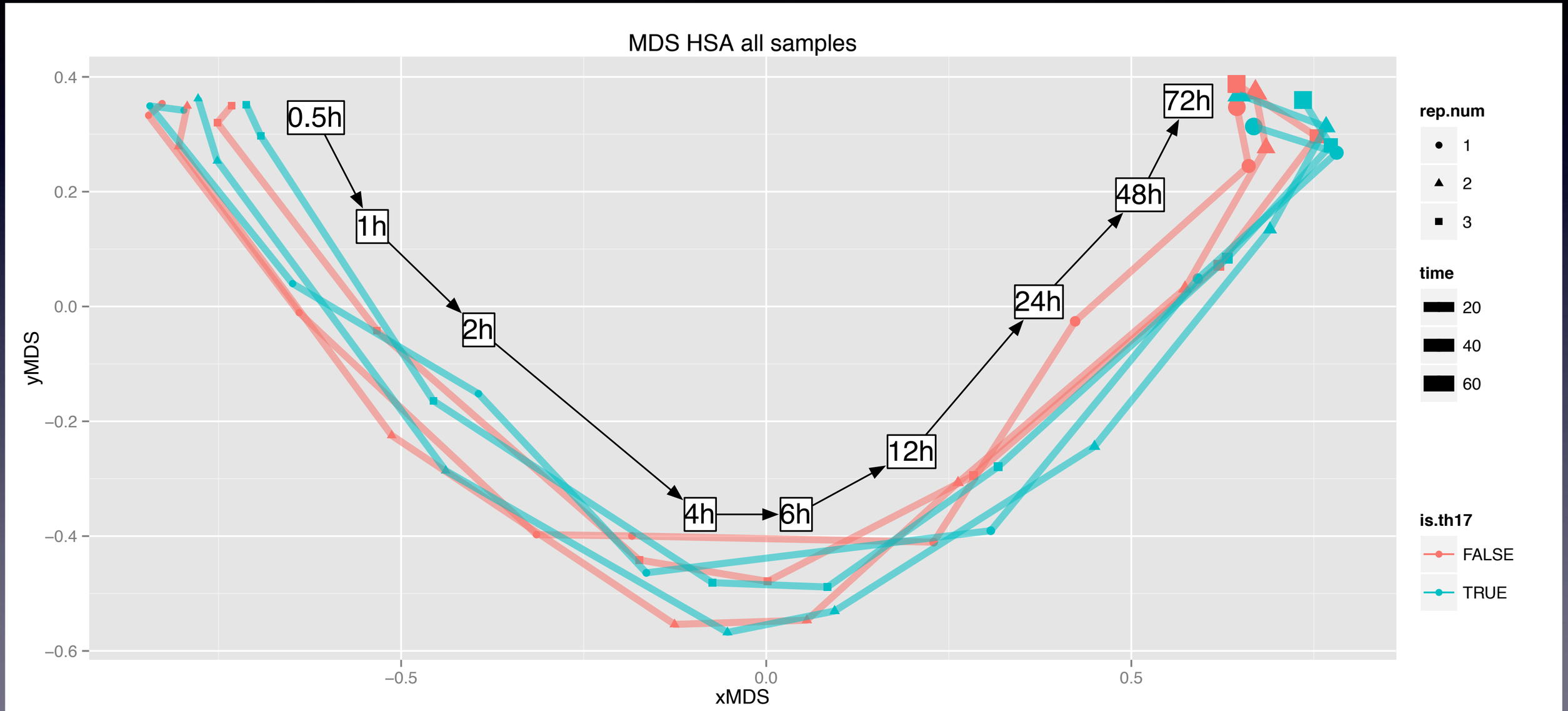




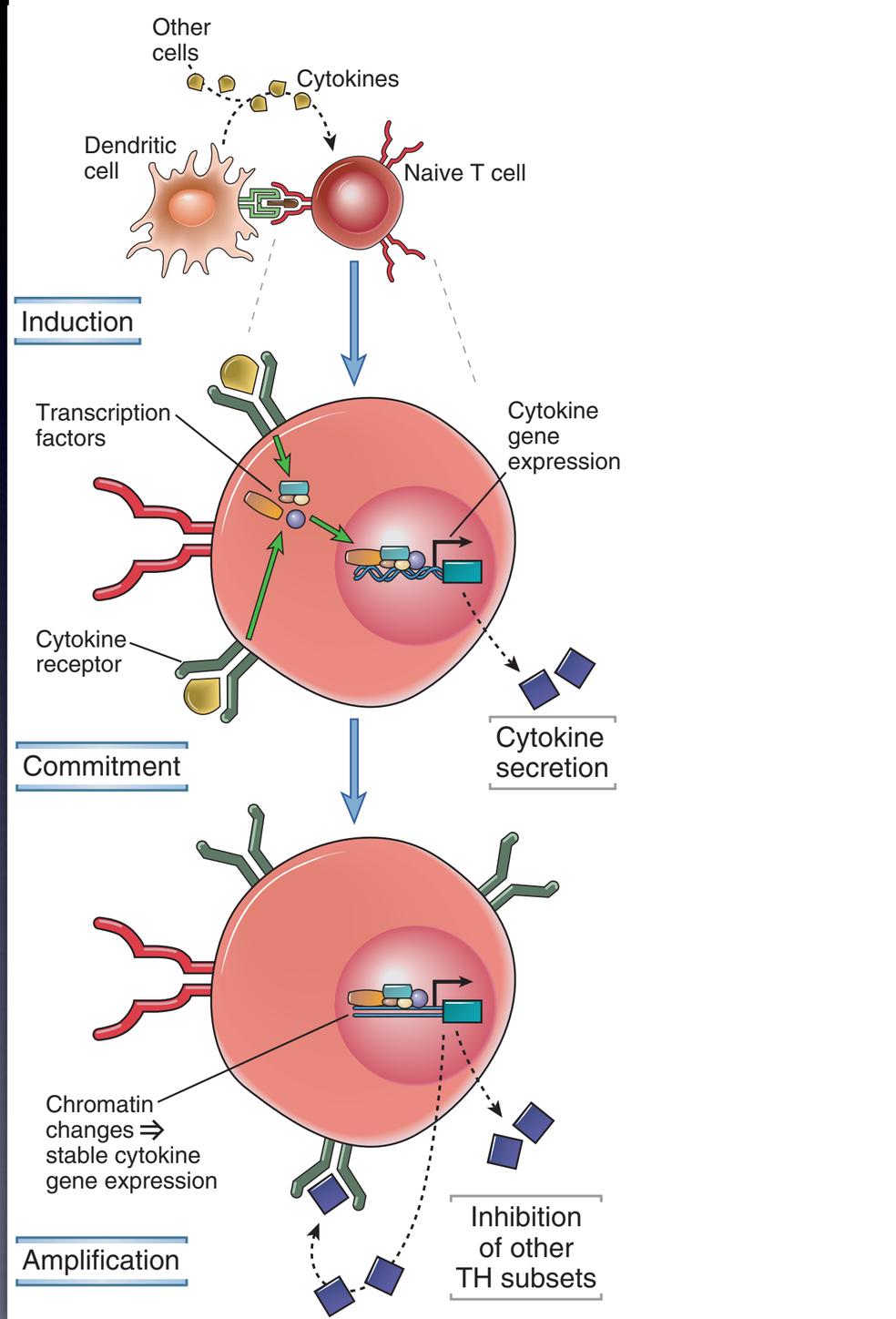
Between all samples



Between all samples

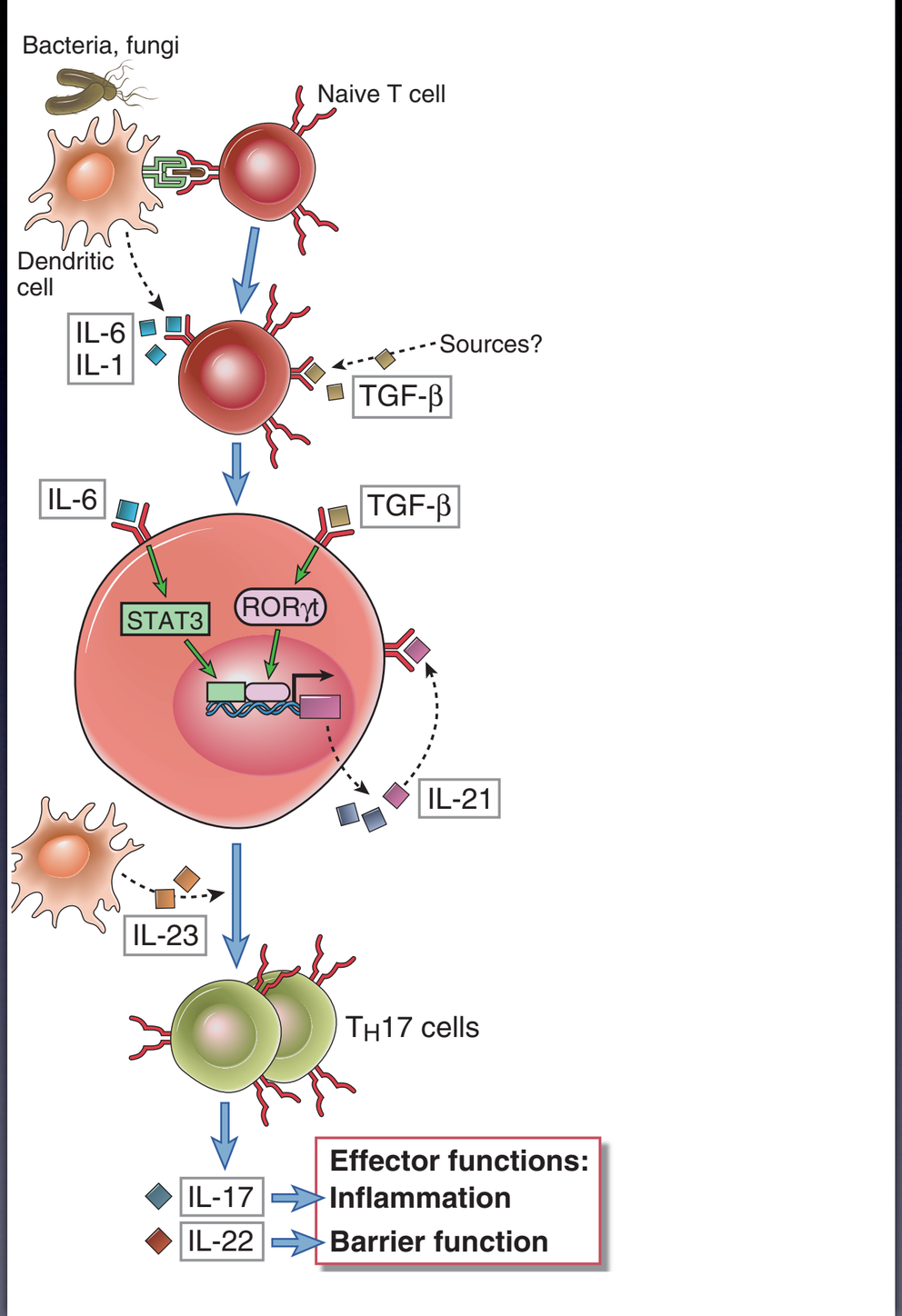


Th cells development & differentiation



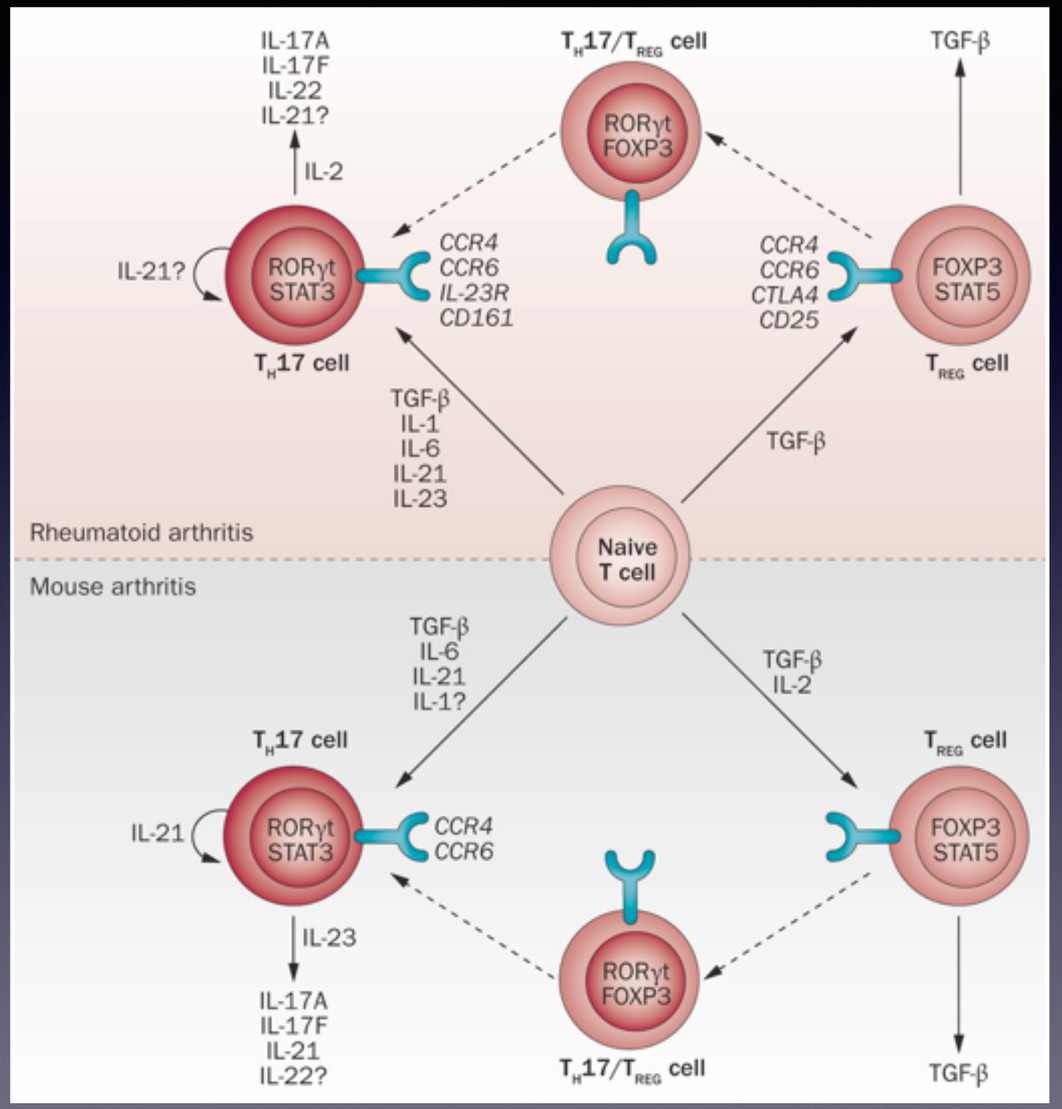
Abbas, Cellular & Molecular Immunology, 7th ed

Th17 cells development & differentiation



Mice vs Men

- Cells origin?
- Role of TGFB?
- Secreted cytokines?



Kobezda et al. (2014). Of mice and men: how animal models advance our understanding of T-cell function in RA. Nat. Reviews. Rheumatology

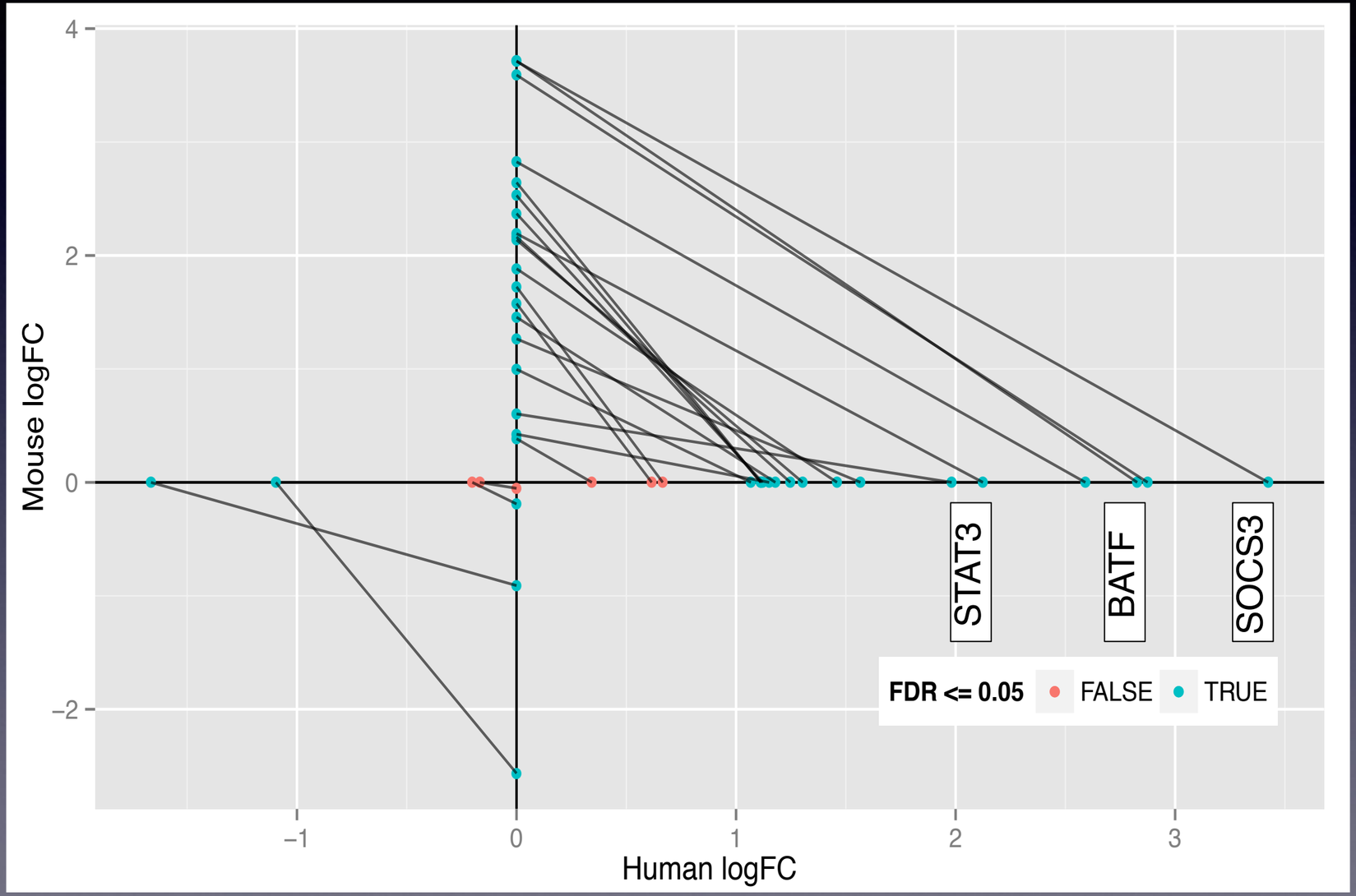
edgeR GLM

$$\log \mu_{gi} = \mathbf{x}_i^T \beta_g + \log N_i$$

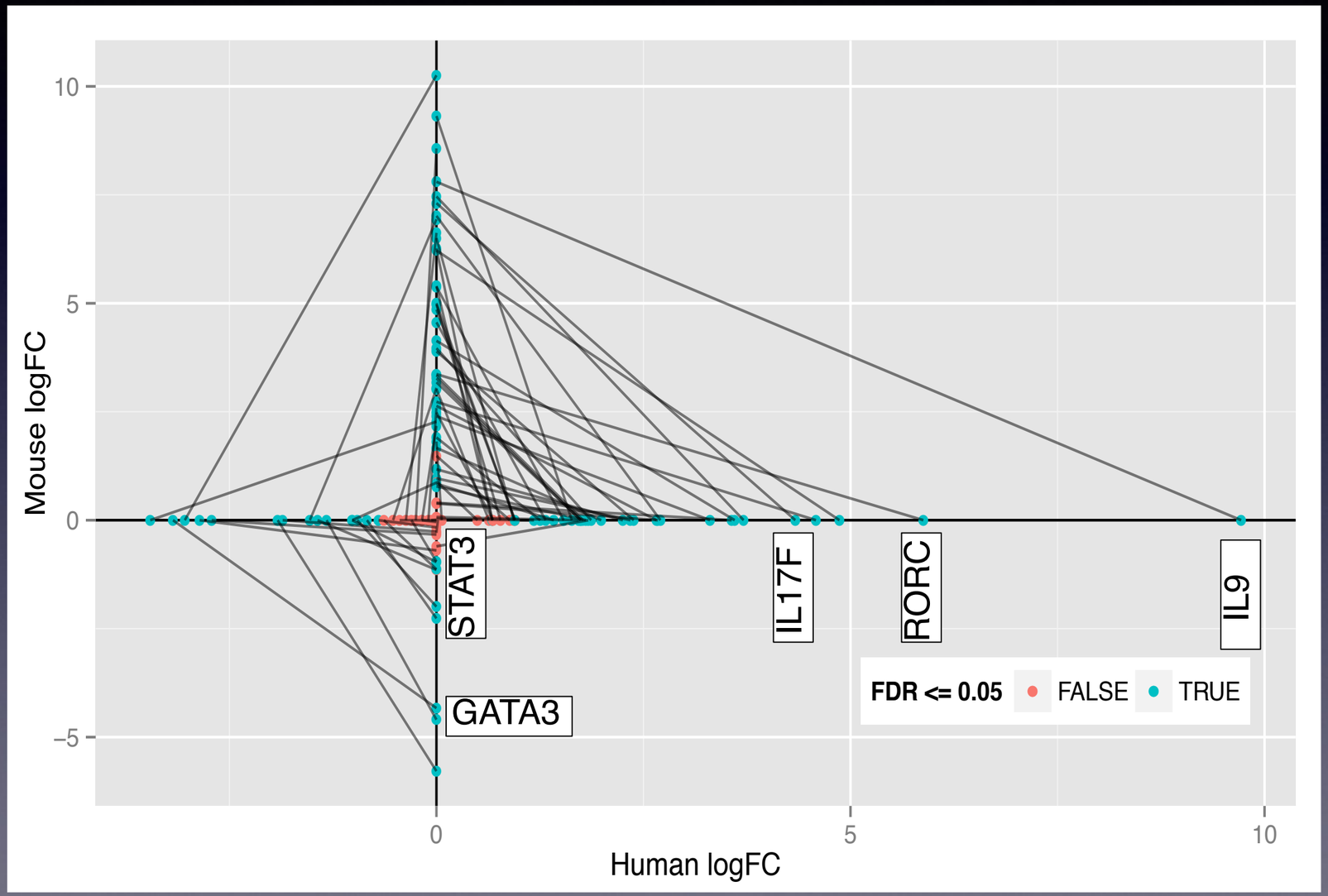
- We fit a model of the like :
- Here: mean ~ donor + time + treat:time
- Test for DE by contrasting <=> H0: treat:time ==0
  - LRT, compare models

counts ~ donor + time VS counts ~ donor + time + time:treat

2h dynamics of conserved module

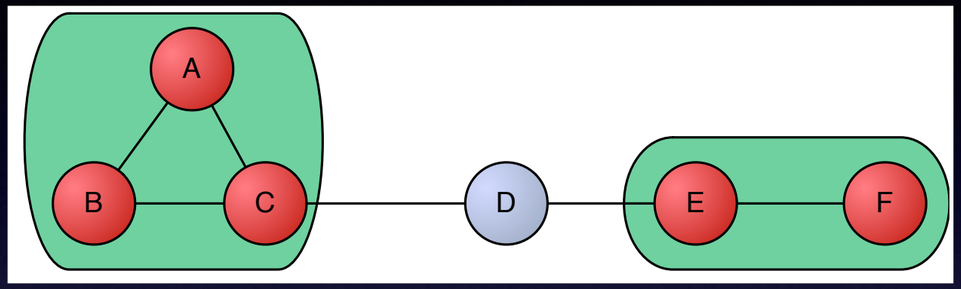


72h dynamics of conserved module



MILP: connectivity constraints

- For each connected component of the current solution S
- Determine its neighborhood not in S
- Formulate the two alternatives:
  - It's expanded towards other CCs
  - Or it'll be the final module (new y_v variables)



$$x_A \leq x_D + y_A + y_B + y_C$$
$$x_B \leq x_D + y_A + y_B + y_C$$
$$x_C \leq x_D + y_A + y_B + y_C$$

$$x_E \leq x_D + y_E + y_F$$
$$x_F \leq x_D + y_E + y_F$$

$$\text{with} \quad y_v \leq x_v \text{ and } \sum_{v \in V} y_v \leq 1;$$