Tumor Heterogeneity, Signaling Cascades, and Drug Targets

Ananth Grama

Department of Computer Science, Purdue University.

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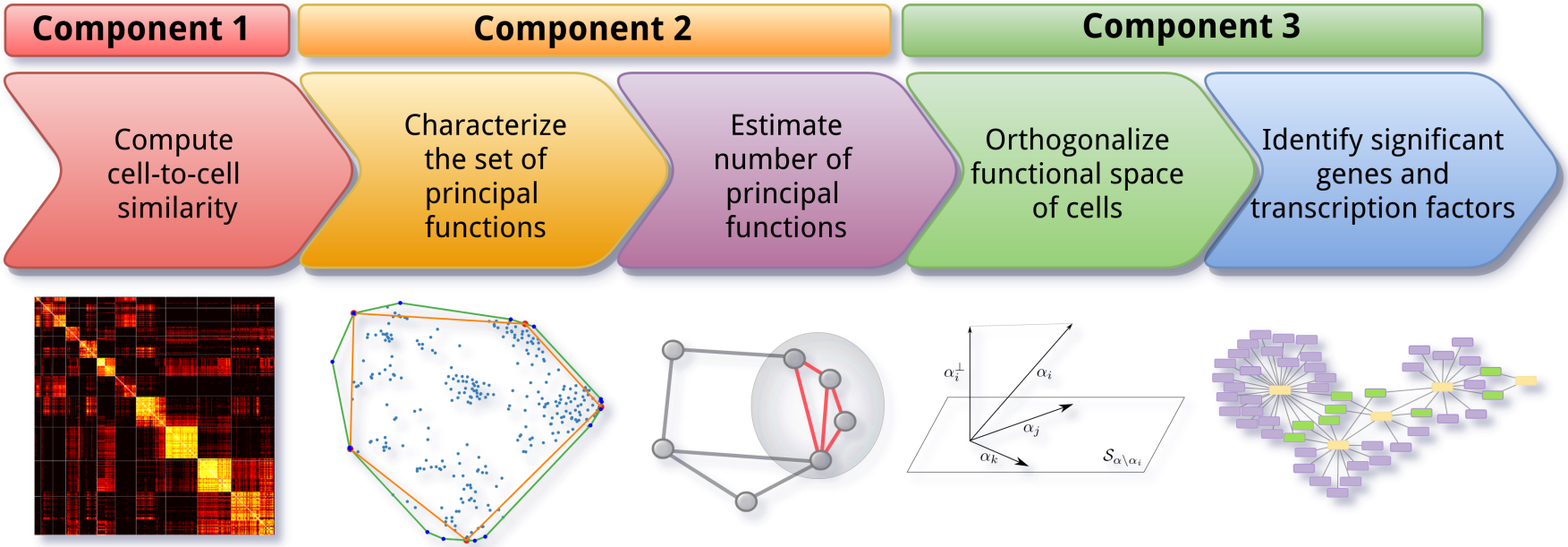
Overall Workflow



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Underlying hypothesis

Transcriptional profile of cells is dominated by housekeeping genes, whereas their functional identity is determined by a combination of weak but preferentially expressed genes.

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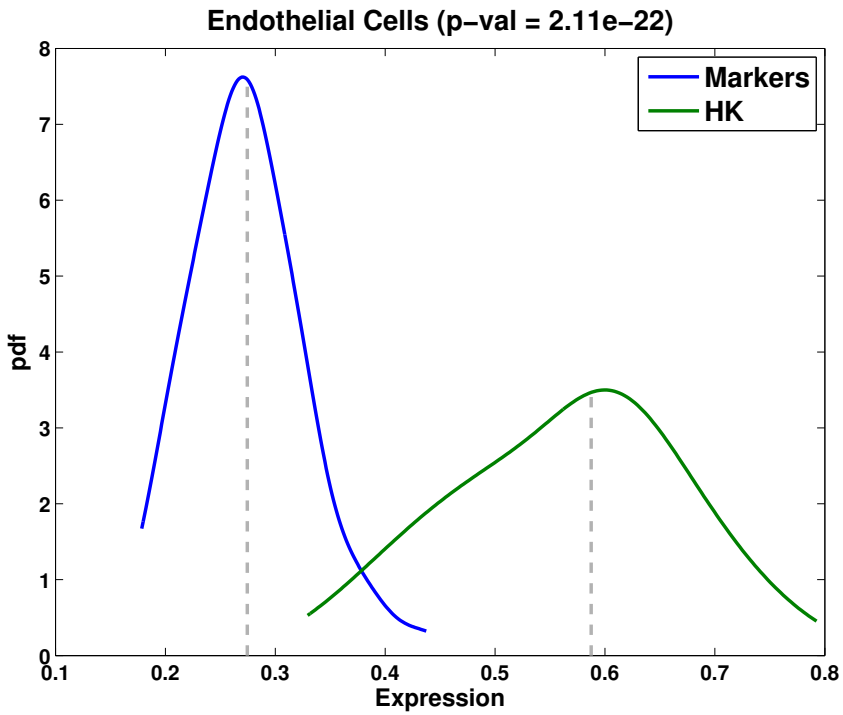

Figure: Endothelial Cells

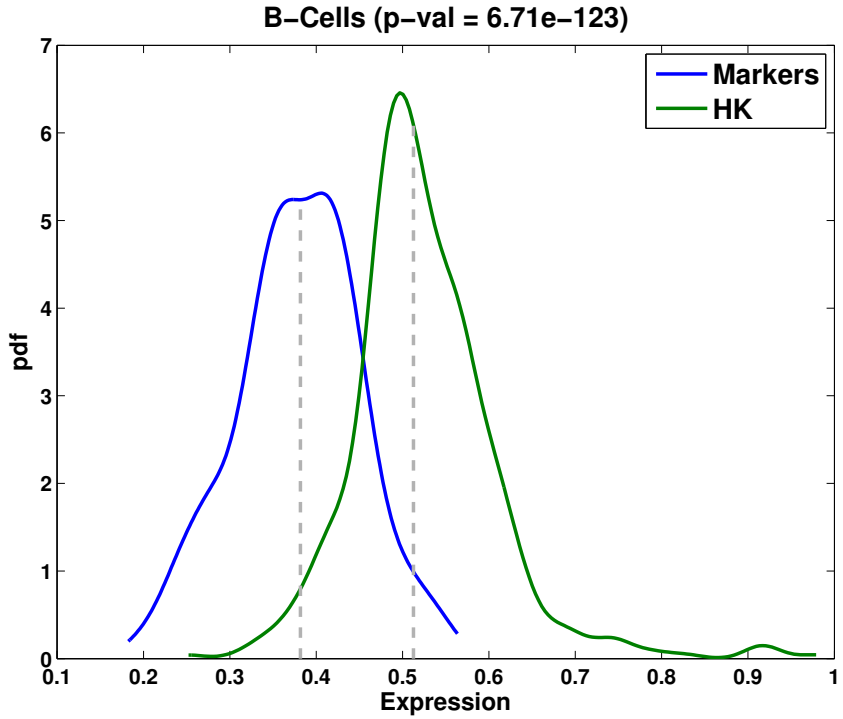

Figure: B-Cells

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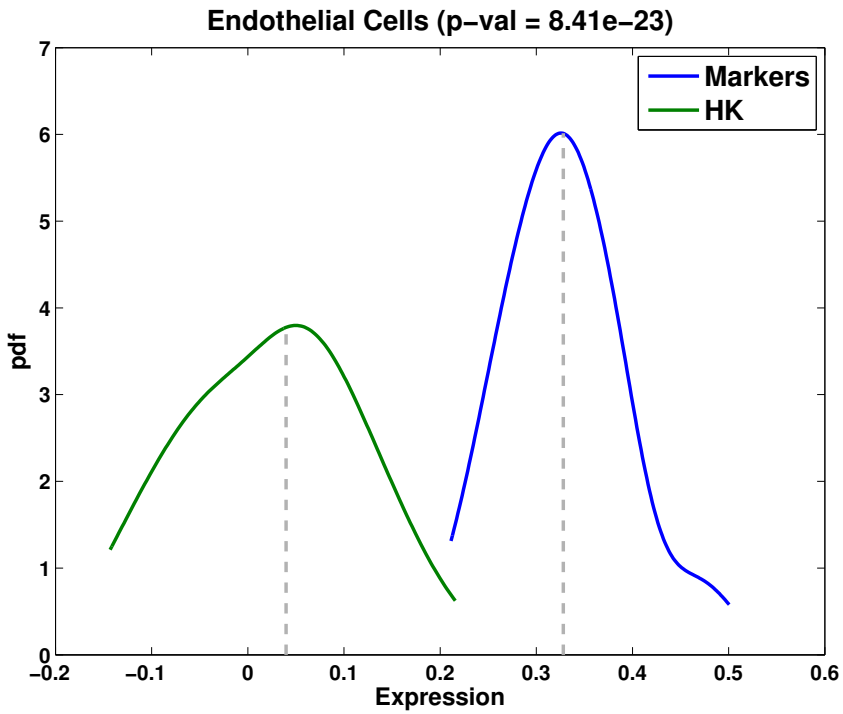

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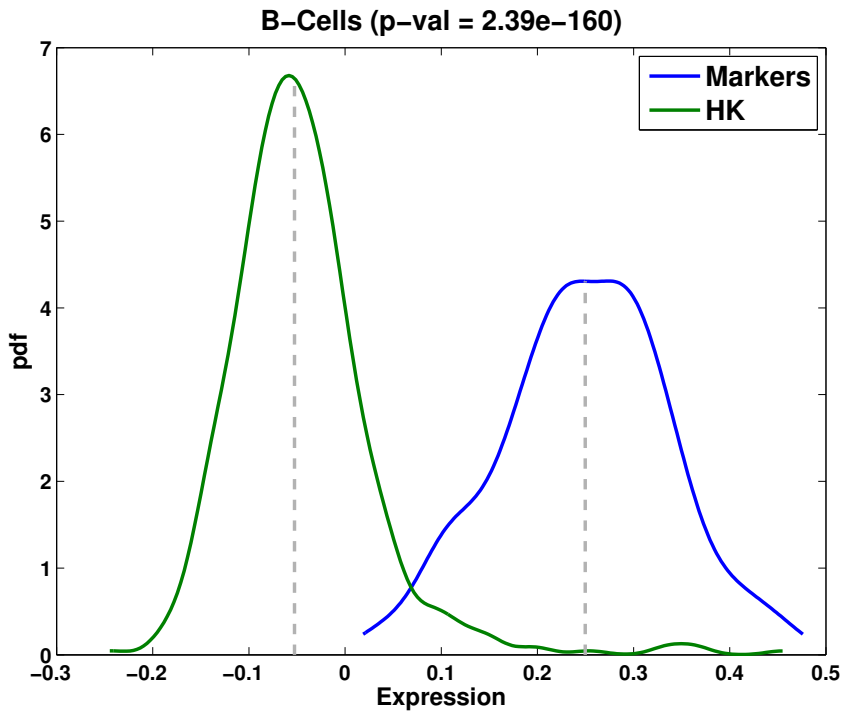

Figure: B-Cells

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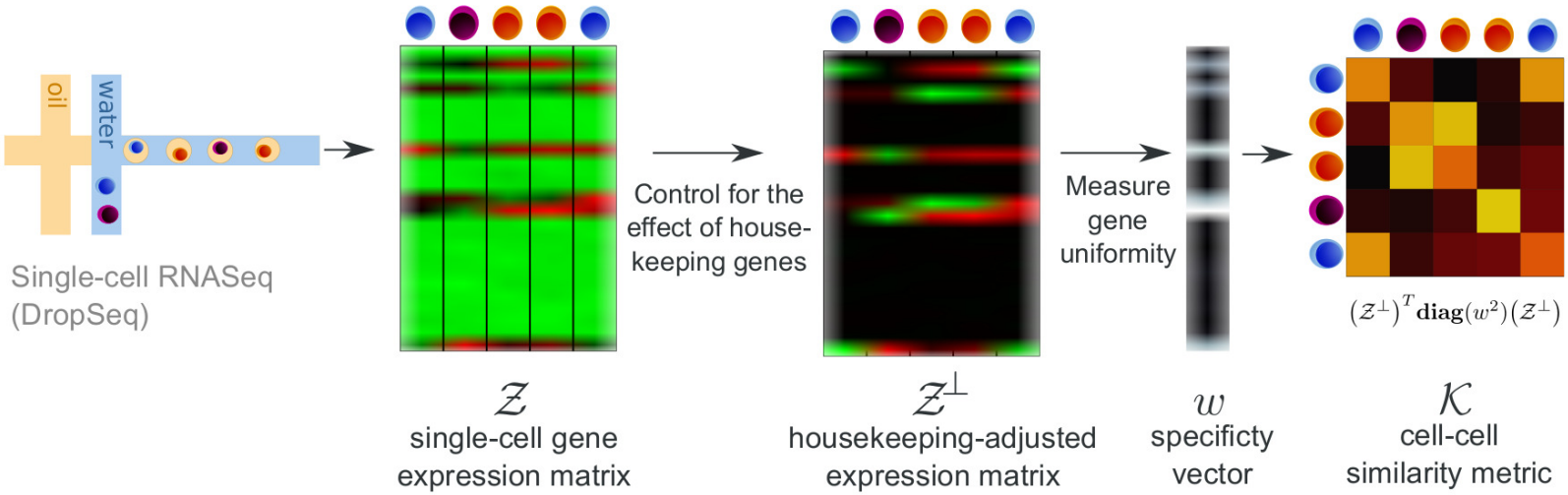

- The main steps involved in identifying similarity between cells

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Goal: Identify the shared subspace of genes

Low-rank decomposition

$$A = U_r \Sigma_r V_r = \sum_{i=1}^{r} \sigma_i u_i v_i^T,$$

Example decomposition choices:

- Mean vector
  - *Optimal in a least-square sense when the chance of observing a gene is uniform across all cells.*
- Singular Value Decomposition (SVD)
- Nonnegative Matrix Underapproximation (NMU)
- Sparse NMU

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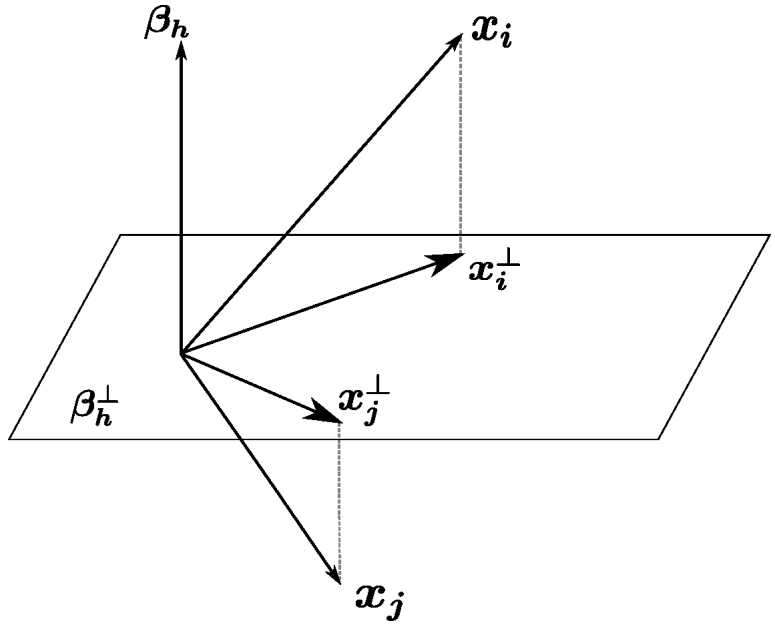
Goal: Remove the effect of common subspace

- $\boldsymbol{x}_i$ and $\boldsymbol{x}_j$: tissues/cell types i and j
- z-score normalize $\boldsymbol{x}_i$ to compute $\boldsymbol{z}_i$
- $\boldsymbol{\beta}_h$: the common signature
- z-score normalize $\boldsymbol{\beta}_h$ to compute $\boldsymbol{z}_h$
- Project to the orthogonal subspace:

$$\boldsymbol{z}_i^{\perp} = \left(\mathbf{I} - \frac{\boldsymbol{z}_h \boldsymbol{z}_h^T}{\|\boldsymbol{z}_h\|_2^2}\right)\boldsymbol{z}_i.$$



Similar in nature to the partial Pearson's correlation

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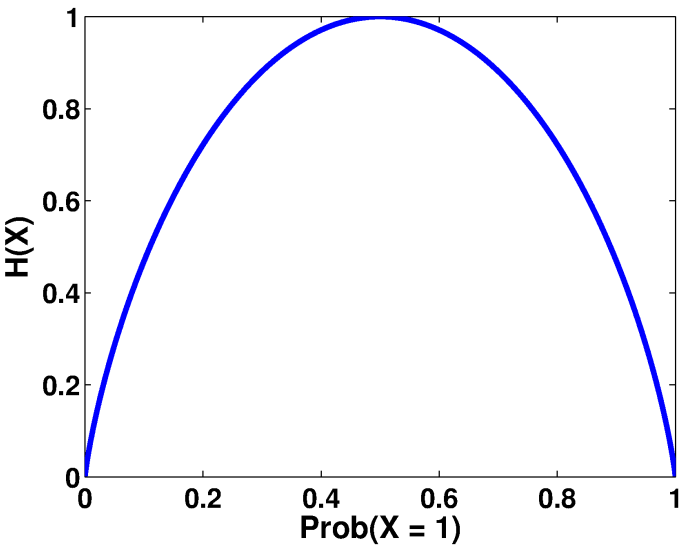
Goal: Estimate expression-specificity of genes across different cells



- Entropy as a measure of expression uniformity: $H(i) = -\sum_j p_{ij} log(p_{ij})$
- How informative observing a gene is with respect to the cell type that it came from
- Maximum entropy when probability of a gene coming from all cell types is equal
- For each gene i, compute a specificity factor w_i.

Similar formulation have been previously used for marker detection.

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ACTION-adjusted cell signatures

$$\mathbf{Y} = \boldsymbol{diag}(\boldsymbol{w})\mathcal{Z}^{\perp}$$

ACTION metric (kernel)

$$\begin{aligned} \mathbf{K}_{ACTION} &= \mathbf{Y}^T\mathbf{Y} \\ &= \left(\mathcal{Z}^{\perp}\right)^T \boldsymbol{diag}(\boldsymbol{w}^2)\left(\mathcal{Z}^{\perp}\right) \end{aligned}$$

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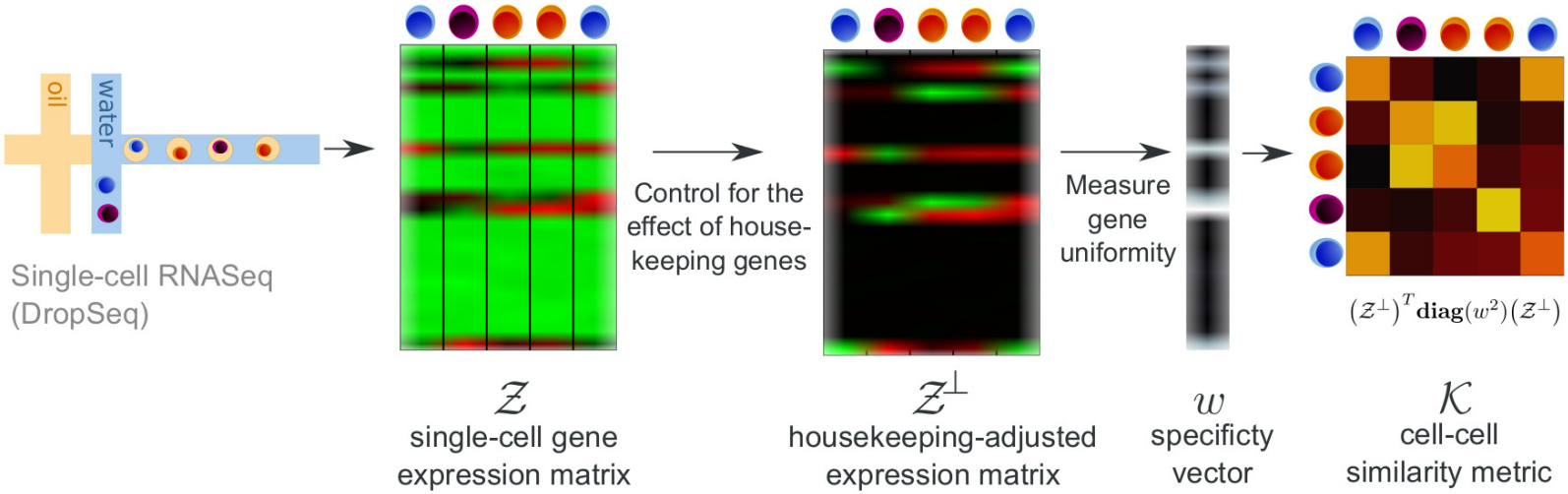

- Now we have computed the ACTION kernel

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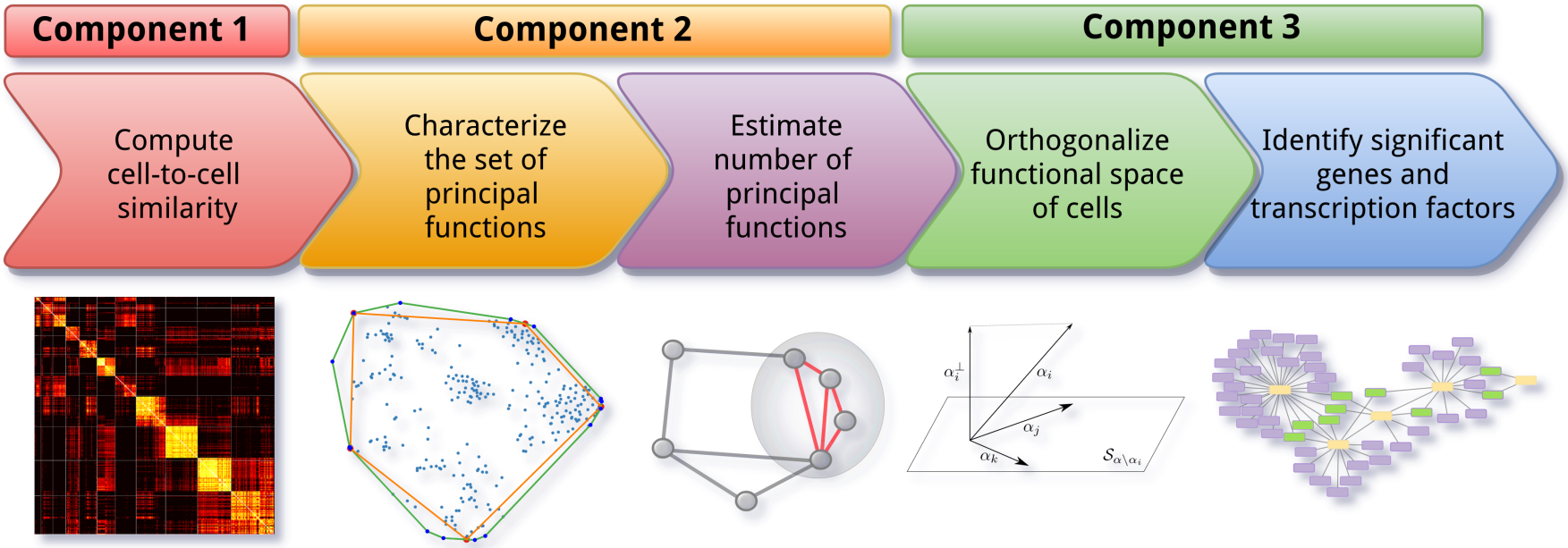
Overall Workflow

Component 2



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General framework

$$\begin{aligned} \underset{\mathbf{C},\mathbf{H}}{\text{argmin}} \quad & \| \mathbf{Y} - \underbrace{\mathbf{YC}}_{\mathbf{W}} \mathbf{H} \| \\ \text{subject to:} \quad & \| \mathbf{C}(:,i) \|_1 = 1. \\ & \| \mathbf{H}(:,i) \|_1 = 1. \\ & 0 \leq \mathbf{C}, 0 \leq \mathbf{H} \end{aligned}$$

Various algorithms can be cast using this formulation

- K-means: $\mathbf{C} \in \mathbb{R}^+, \mathbf{H} \in \{0,1\}$
- K-medoids: $\mathbf{C} \in \{0,1\}, \mathbf{H} \in \{0,1\}$

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Convex NMF

$$\begin{aligned} \underset{\mathcal{K},\mathbf{H}}{\text{argmin}} \quad & \| \mathbf{Y} - \mathbf{Y}(:,\mathcal{S})\mathbf{H} \| \\ \text{subject to:} \quad & \| \mathbf{H}(:,i) \|_1 = 1, \mathbf{H} \in \mathbb{R}^+. \end{aligned}$$

- It uses the same formulation as k-medoid, but relaxes the hard assignment of cells: $\mathbf{C} \in \{0,1\}, \mathbf{H} \in \mathbb{R}^n$
- Unlike k-medoid and k-means, it has an optimal global solution.
  - Under near-separability assumption: there exists for each cell type an ideal example in the population.
- A modification of the *Gram Schmidt* process.

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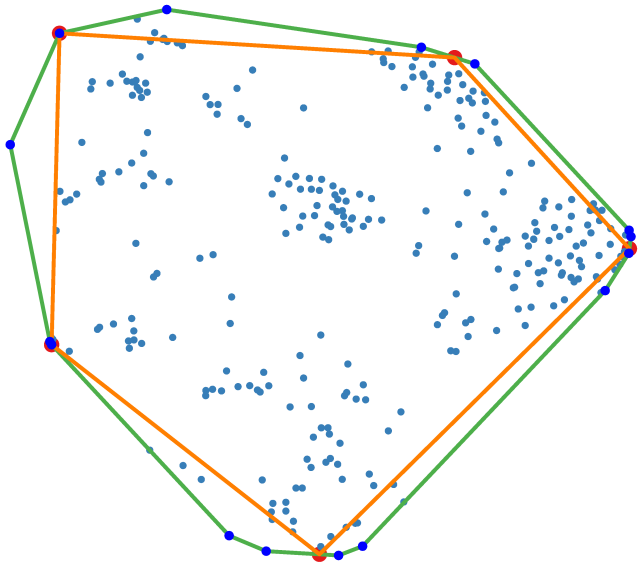

Geometry of functional space: each point is a cell and red points are the "pure cells"

- Picking k corner points/archetypes from the convex hull of the cells, such that they optimally "contain" the rest of cells.
- Each archetype is an ideal example of a cell type with a distinct set of principal functions.

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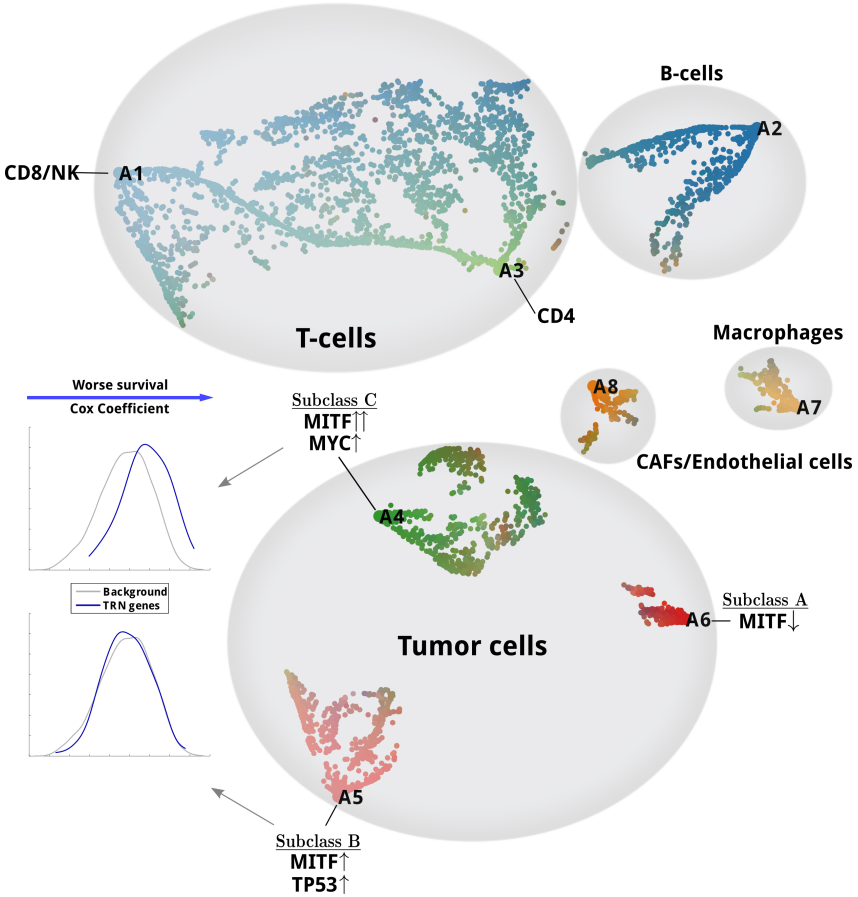
Continuous view

Case study in the Melanoma dataset



- T-cells reside in a continuum of states (Thogerson *et al.*).
- Tumor cells form compact groups.
- Two subclasses of MITF-associated tumors significantly differ in terms of their survival.

- ACTION sheds light on the underlying topology of cell types

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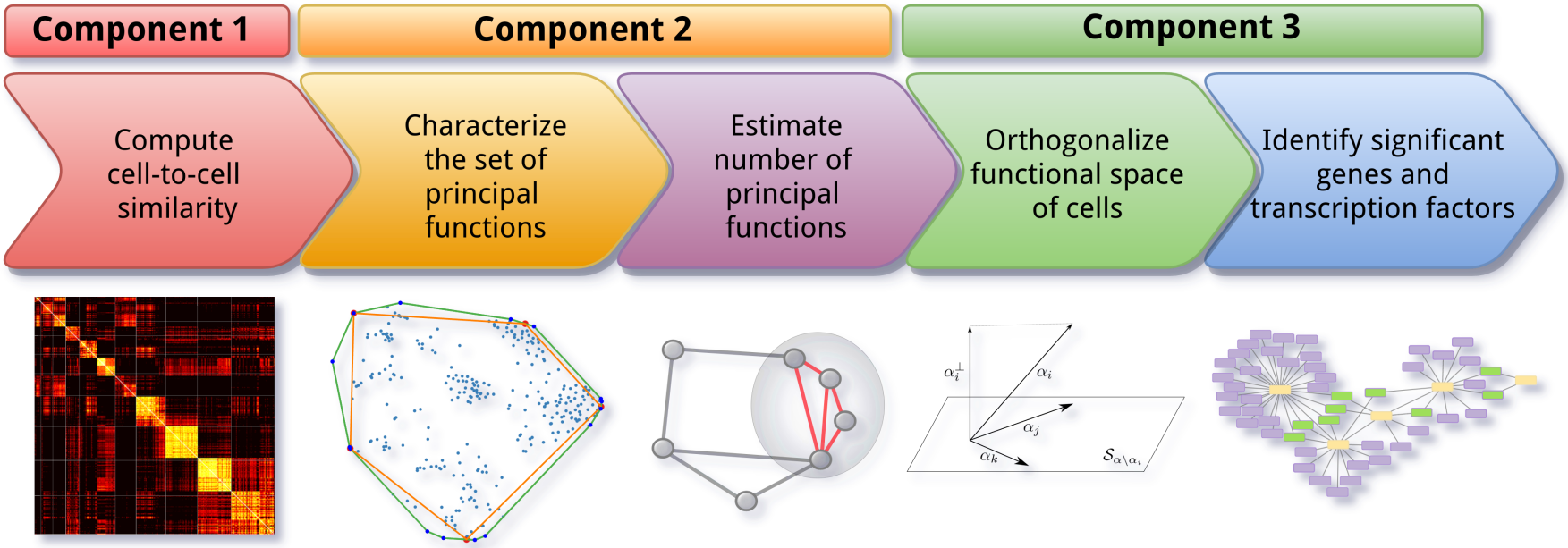
Overall Workflow

Component 3



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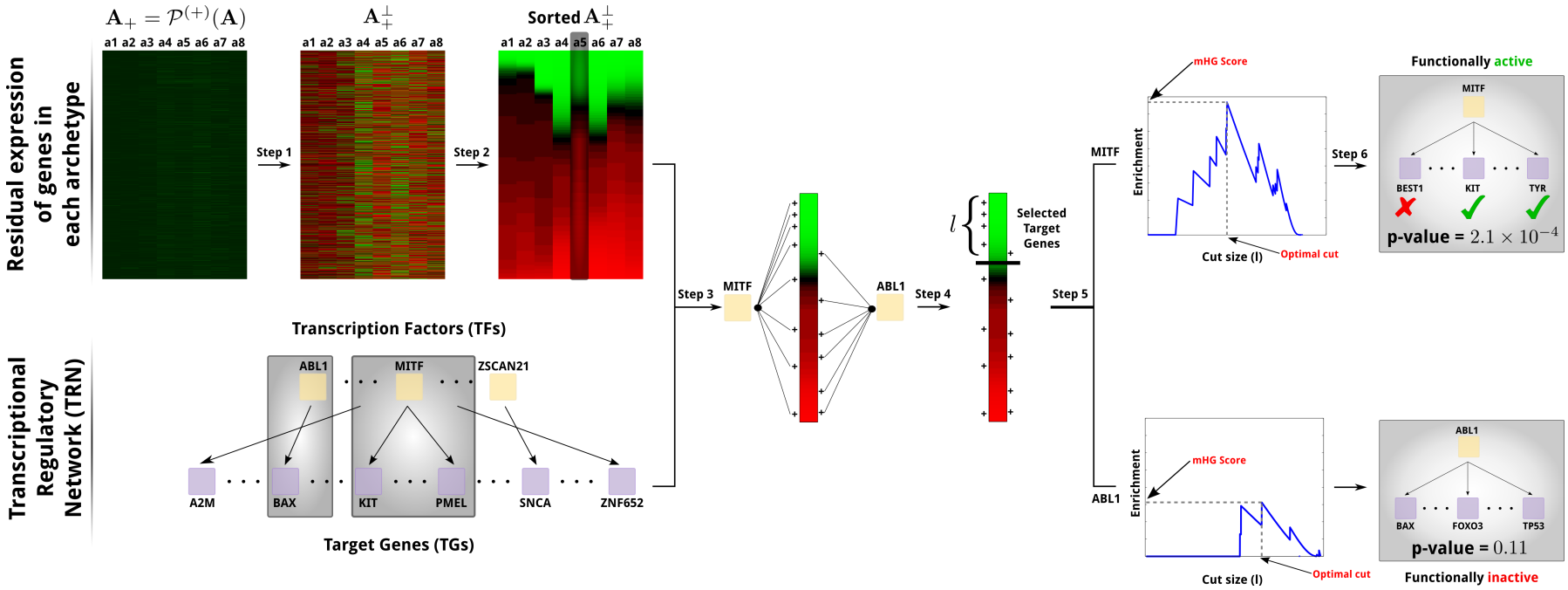
Component 3
Constructing TRN



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Continued Goal: Identifying key regulatory elements that drive each cell type

1. Archetype Orthogonalization ($\rightarrow$ Only over positive projection)

$$\boldsymbol{a}_i^{\perp} = \left(\mathbf{I} - \mathbf{A}_{-i}(\mathbf{A}_{-i}^T\mathbf{A}_{-i})^{-1}\mathbf{A}_{-i}^T\right)\boldsymbol{a}_i$$

2. Assessing significance of TFs/TGs

$$\begin{aligned} p\text{-value}(Z = b_l(\lambda)) &= \text{Prob}(b_l(\lambda) \leq Z) \\ &= \sum_{x=b_l(\lambda)}^{min(T,l)} \frac{\binom{T}{x}\binom{m-T}{l-x}}{\binom{m}{l}} \end{aligned}$$

Use Dynamic Programming to compute exact p-value.

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Functional activity of transcription factors (TFs)

Key point!

We identify "functional activity" of transcription factors (TFs) by aggregating transcriptional activity of their downstream targets, not the transcriptional level of TFs themselves. TFs can, and typically do, get regulated through post-translational mechanisms.

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- Both *Subtype A* and *Subtype C* exhibit high activity of *MITF* and *Sox10* transcription factors, which are canonical markers for melanoma cells in the "proliferative" (as opposed to "invasive") state (Verfaiilie *et al.*).
- These two subtypes are significantly enriched for marker genes in the proliferative state:
  - *Subtype A:* 9.3×10^{-14}
  - *Subtype B:* 7.9×10^{-11}
- Subtype A has higher MITF activity (according to its activated targets):
  - *GPNMB*, *M1ANA*, *PMEL*, and *TYR* are shared between two subtypes.
  - *ACP5*, *CDK2*, *CTSK*, *DCT*, *KIT*, and TRPM1/P1 are uniquely upregulated in subtype A.

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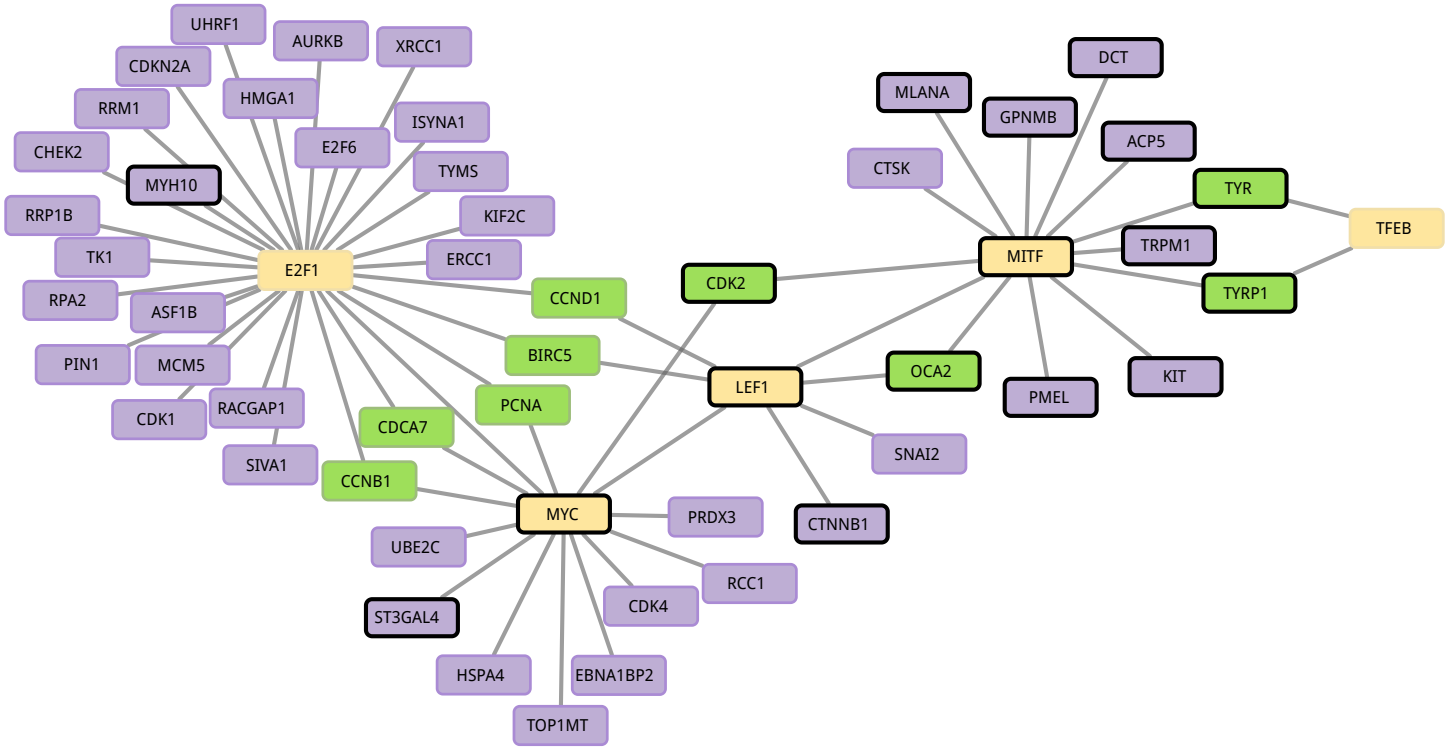
Dissecting transcriptional controls of Melanoma subclasses

Case study in MITF⇑⇑/MYC↑ subtype



- 19 "functionally" active transcription factors in subtype A (p-value ≤ 0.05)
- We focus on the five most significant TFs and their targets (p-value $\leq 10^{-3}$)

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- MITF is one of the most well-known markers for classifying melanoma patients (Hartman *et al.*: MITF in melanoma: mechanisms behind its expression and activity).
- Overexpression of the E2F1 is common in high-grade tumors that are associated with poor survival in melanoma patients (Alla *et al.*: E2F1 in melanoma progression and metastasis).
- Melanoma cell phenotype switching, between proliferative an invasive states, is regulated by differential expression of LEF1/TCF4 (Eichhoff *et al.*:Differential LEF1 and TCF4 expression is involved in melanoma cell phenotype switching).
- Amplification and overexpression of the c-myc have been associated with poor outcome (Kraehn *et al.*: Extra c-myc oncogene copies in high risk cutaneous malignant melanoma and melanoma metastases).

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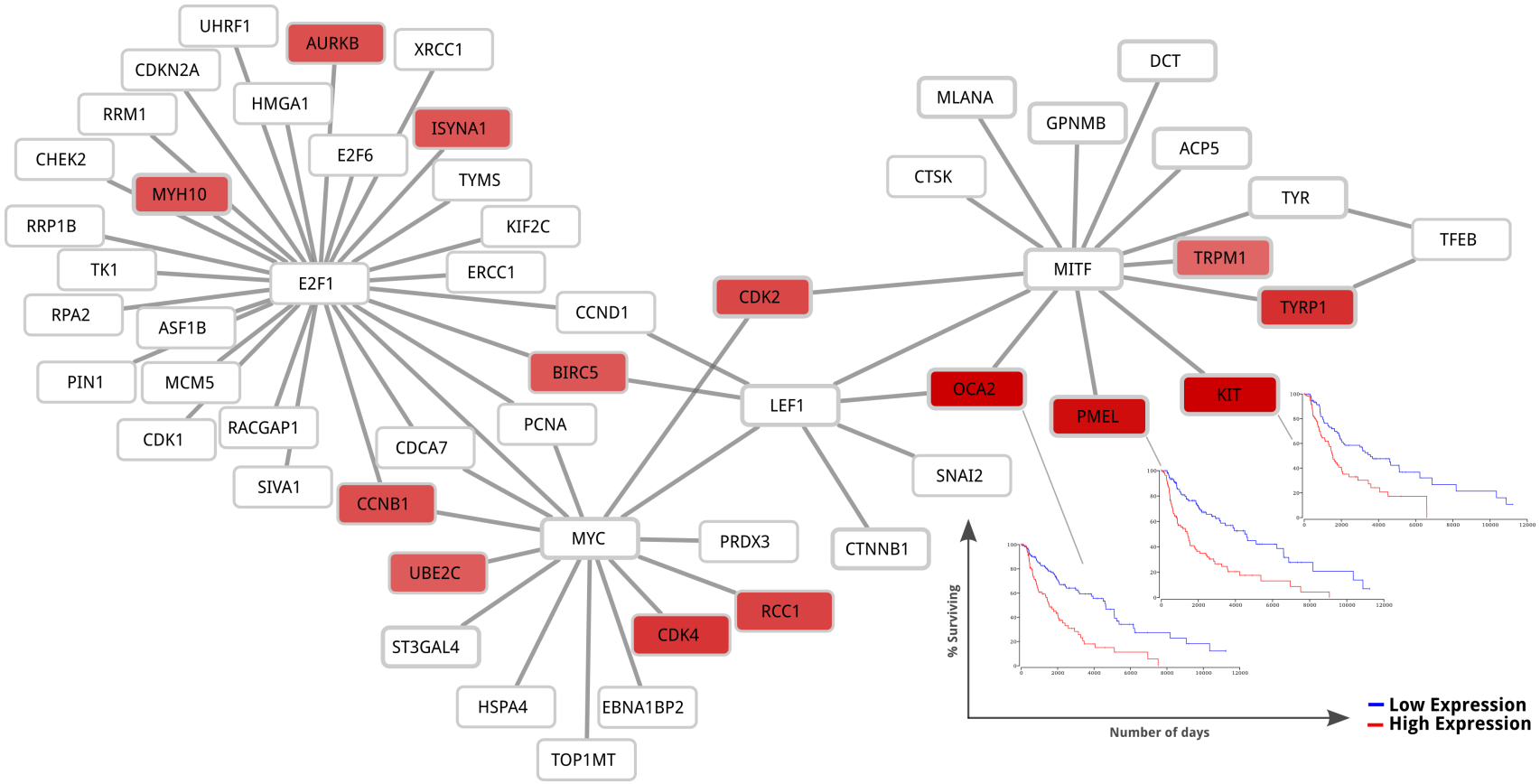
Case study in MITF⇑/MYC↑ subtype

Survival analysis revisited – Kaplan-Meier plots



1. Developed a novel cell similarity metric that is robust to biological noise, while at the same time is sensitive enough to identify weak cell type-specific signals
2. Characterized the functional identity of cells
   - Under the pure cell assumption, this metric induces a convex topology that embeds functional identity of cells
3. Utilized functional identity of cells to identify both discrete cell types and continuous cell states
4. Identified driving transcriptional controls that mediate the functional identity of cells

Clinical significance: Characterization of two MITF-associated subclasses of Melanoma patients, one of which has substantially worse outcomes, along with their underlying regulatory elements.

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Questions?

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