Supplementary Information

Supplementary Text

CAGE-derived genomic regions selection

The user can select an expression level threshold by specifying either the relative expression (corresponding to expression specificity) or a combination of the raw tag count and the normalized number of tags per million. CAGE peaks that meet or exceed the provided expression characteristics in any of the selected set of FANTOM5 samples are retrieved from the underlying database to be used for defining the foreground set of genomic regions. This selected set of CAGE peaks can be further filtered based on proximity to specific genes (using a 500 bp maximal distance between CAGE peaks and Ensembl transcription start sites (Cunningham et al., 2015) for user-supplied peaks or using the peak-to-gene associations for FANTOM5 peaks from (The FANTOM Consortium, 2014)), overlap with user-supplied regions of interest (such as ChIP-seq peak regions in a BED-formatted file), or prediction as true TSSs by the TSS classifier in (The FANTOM Consortium, 2014) for FANTOM5 CAGE-peaks.

TF binding profile clusters

Clusters of JASPAR (version 2016 (Mathelier et al., 2015)) TF binding profiles were computed using the *matrix-clustering* tool from RSAT (version 2015 (Medina-Rivera et al., 2015; Castro-Mondragon et al., in preparation)). We used the *average* linkage method with the *Ncor* similarity metric to compute the clusters with the following thresholds: *Ncor=0.55* and *cor=0.75*. The tool computed 136 clusters of TF binding profiles. CAGEd-oPOSSUM uses these clusters to combine TFBSs predicted from TF binding profiles in the same cluster and to compute the enrichment scores associated to the clusters as implemented in the TFBS Cluster Analysis of oPOSSUM3 (Kwon, Arenillas, Hunt, & Wasserman, 2012).

Precomputation of TFBSs for time efficiency

The flanking regions of 2,000 bp were applied to each FANTOM5 CAGE peak and overlapping regions were merged to create a set of maximal spanning, non-overlapping CAGE-derived genomic regions. The genomic sequences were extracted from Ensembl (Cunningham et al., 2015) and scanned with the TF binding profiles from JASPAR (Mathelier et al., 2016). TFBSs were predicted where the corresponding position weight matrix relative score was above 80% (as in oPOSSUM3 (Kwon et al., 2012)).

Examples of application

The default parameters used for the three case examples were as follows. A CAGE peak relative expression level of at least 1 was used to select transcription start sites specific to the samples.

Flanking regions of 500 bp upstream and downstream were extracted. Background sequences matching the %GC composition and length of selected regulatory regions were generated with HOMER (Heinz et al., 2010). All JASPAR 2016 CORE vertebrate profiles (Mathelier et al., 2016) with a minimum information content of 8 bits were used to predict TFBSs with a relative score of at least 85% for the oPOSSUM3 analysis.

The most enriched profile predicted by both oPOSSUM3 (using the Fisher scores accounting for the number of genomic regions containing at least one predicted TFBS) and HOMER is associated with the HNF4A TF for the liver sample (Supplementary Figure 1 and Supplementary Data). The HNF4A TF is a well-characterized TF involved in the regulation of several biological functions in liver (Babeu & Boudreau, 2014). Using the three samples corresponding to CD19-positive B-cells, the most enriched profiles (from both oPOSSUM3 and HOMER) are associated with ETS-related factors (Supplementary Figure 2 and Supplementary Data). Several of these ETS-related factors profiles are associated with TFs already known to be critical for B-cell development such as GABPA (Xue et al., 2007), ETS1 (Eyquem et al., 2004), PU.1/SPI1 (Sokalski et al., 2011), and SPIB (Sokalski et al., 2011). Of the top scoring TFs, RELA is the only non ETS-related factor predicted; it is known to regulate the development of B-cells and has a critical role in the regulation of B-cell survival (Prendes, Zheng, & Beg, 2003). Finally, from the testis samples, CAGEd-oPOSSUM identified RFX-related factors as the most enriched profiles with both oPOSSUM3 and HOMER (Supplementary Figure 3 and Supplementary Data). RFX TFs have already been described to be important in testis during spermatogenesis (Morotomi-Yano et al., 2002; Wolfe, van Wert, & Grimes, 2006; Wolfe, Vanwert, & Grimes, 2008).

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Authors' contributions

AM and WWW were responsible for project conception and oversight. DJA implemented the CAGEd-oPOSSUM web tool. TL was responsible for tag mapping. HK managed the data handling. ARRF was responsible for FANTOM5 management and its concept. DJA, WWW, and AM wrote the manuscript.

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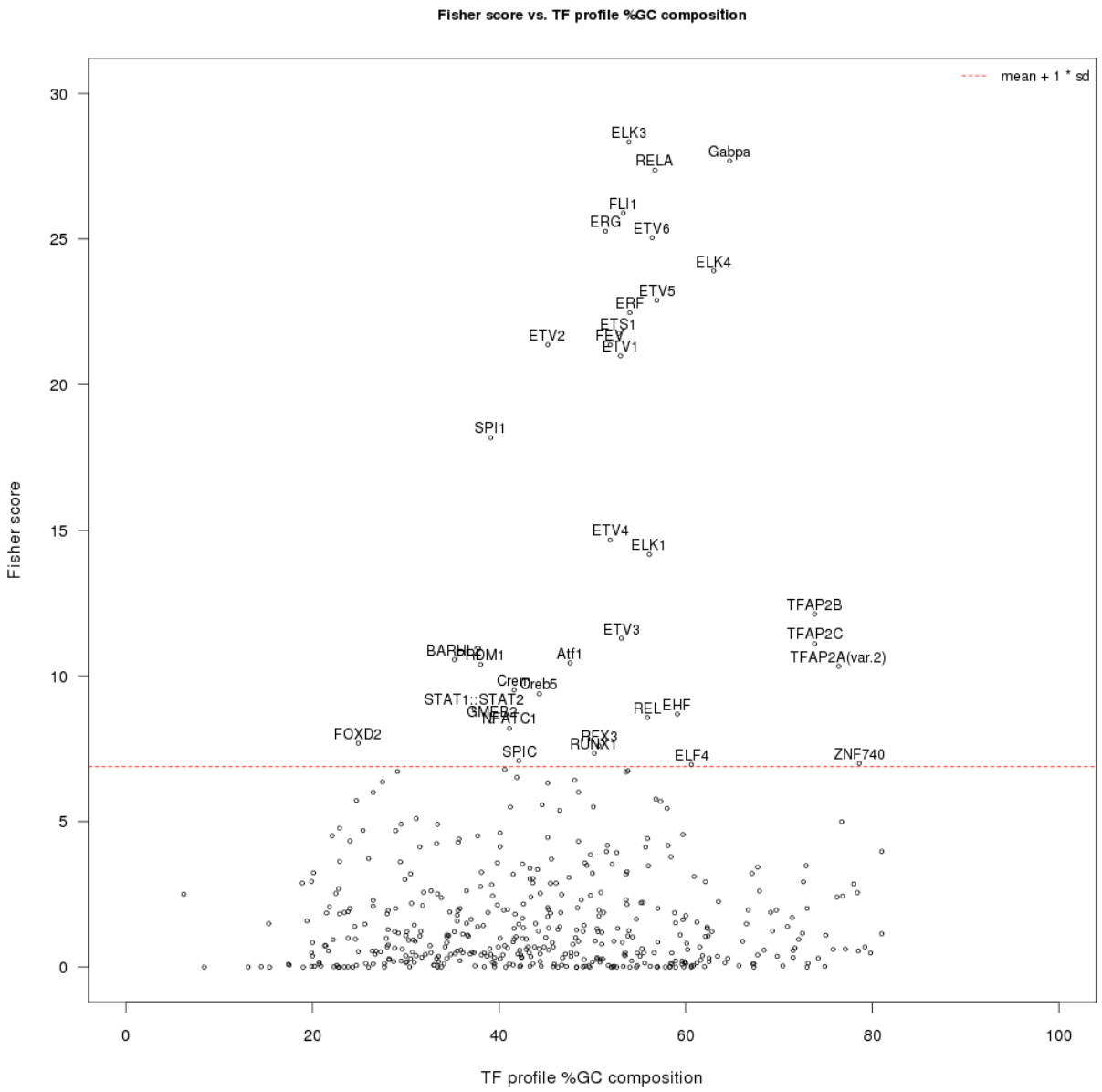
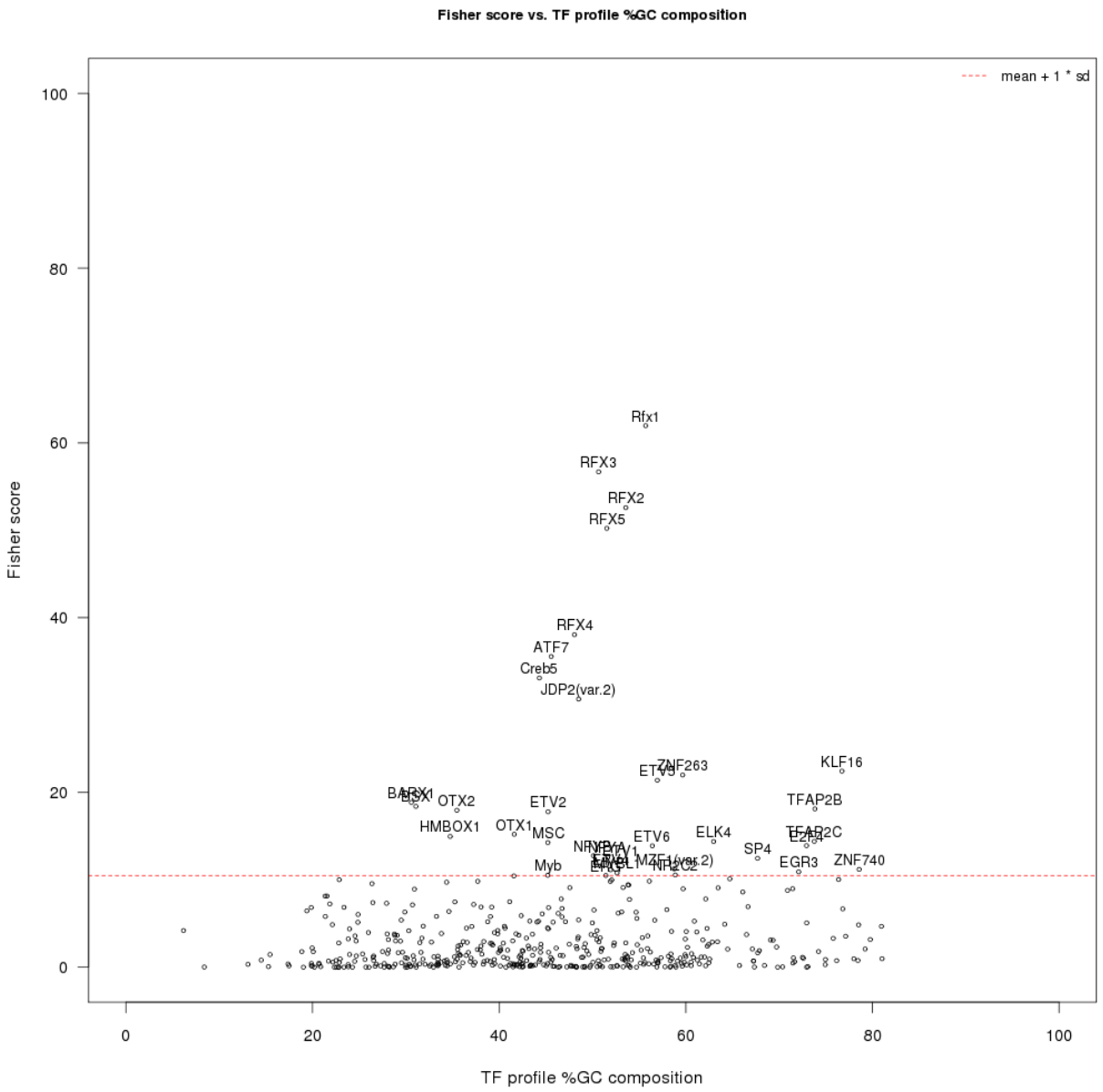
Supplementary Figures

Supplementary Figure 1. TF binding profiles over-representation results on the liver FANTOM5 sample (FF:10018-101C9). For each TF binding profile analyzed, we plot the Fisher-score on the y-axis along with the %GC composition of the TF binding profile on the x-axis. The name of the TFs associated with the profiles are provided when the Fisher-score is above a defined threshold (plot as a dashed red line). The threshold corresponds to *mean* + 1 * *sd* where *mean* and *sd* correspond to the average mean and standard deviation, respectively, of the distribution of all Fisher-scores. We note that the most over-represented profile is associated with the HNF4A TF, a known regulator in liver.



Supplementary Figure 2. TF binding profiles over-representation results on CD19-positive B cells, donor 1, 2 & 3 (FF:11544-120B5, FF:11624-122B4, and FF:11705-123B4) FANTOM5 samples. For each TF binding profile analyzed, we plot the Fisher-score on the y-axis along with the %GC composition of the TF binding profile on the x-axis. The name of the TFs associated with the profiles are provided when the Fisher-score is above a defined threshold (plot as a dashed red line). The threshold corresponds to *mean* + 1 * *sd* where *mean* and *sd* correspond to the average mean and standard deviation, respectively, of the distribution of all Fisher-scores. We note that ETS-related factors are the most enriched profiles.



Supplementary Figure 3. TF binding profiles over-representation results on testis FANTOM5 samples (FF:10026-101D8 and FF:10096-102C6). For each TF binding profile analyzed, we plot the Fisher-score on the y-axis along with the %GC composition of the TF binding profile on the x-axis. The name of the TFs associated with the profiles are provided when the Fisher-score is above a defined threshold (plot as a dashed red line). The threshold corresponds to *mean* + 1 * *sd* where *mean* and *sd* correspond to the average mean and standard deviation, respectively, of the distribution of all the Fisher-scores. We note that profiles associated with RFX TFs are the most enriched profiles.