

# Detecting FOXM1 gene targets in K562 cells through the integration of ATAC-seq, ChIP-seq, and RNA-seq data

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Forkhead box M1 (FOXM1) transcription factor is implicated in many types of cancer (6)

Overexpression generally leads to poor prognosis in cancer patients

Which genes does FOXM1 target?

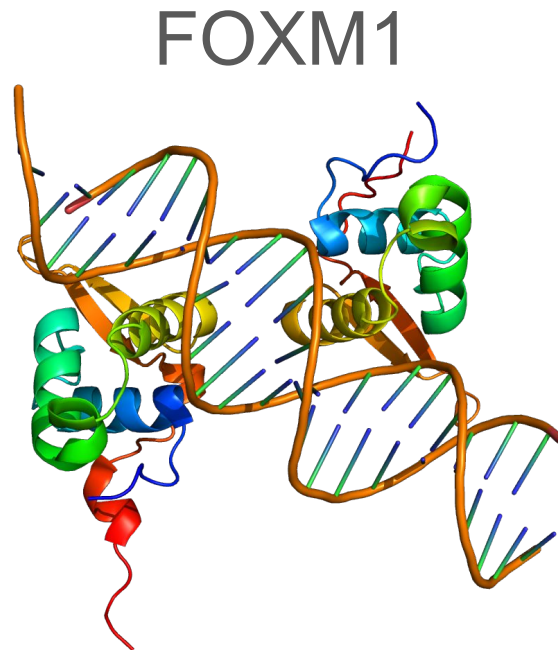
RNA-seq, ATAC-seq, and ChIP-seq data from K562 cells: an immortalized cell line from a female CML patient

RNA-seq: treated with CRISPRi targeting FOXM1 (2 replicates); untreated (2 replicates)

ATAC-seq: untreated (3 replicates)

ChIP-seq: untreated (2 replicates)

All datasets publicly available from ENCODE database



# METHODOLOGY

All datasets were trimmed for adapter content and base quality using Trim Galore

Conversion from SAM to sorted BAM

**RNA-seq:** Alignment with HG38 using HISAT2. Count matrix created using *featureCounts* in R's *Rsubread* package. Differential expression analysis conducted using *DESeq2*: 0.05 p-value threshold / log<sub>2</sub> FC threshold of 1

**ATAC-seq:** Alignment with HG38 using BOWTIE2, peak calling using MACS2 (both broad and narrow peaks), replicate peak files sorted/merged. Peak annotation using HOMER.

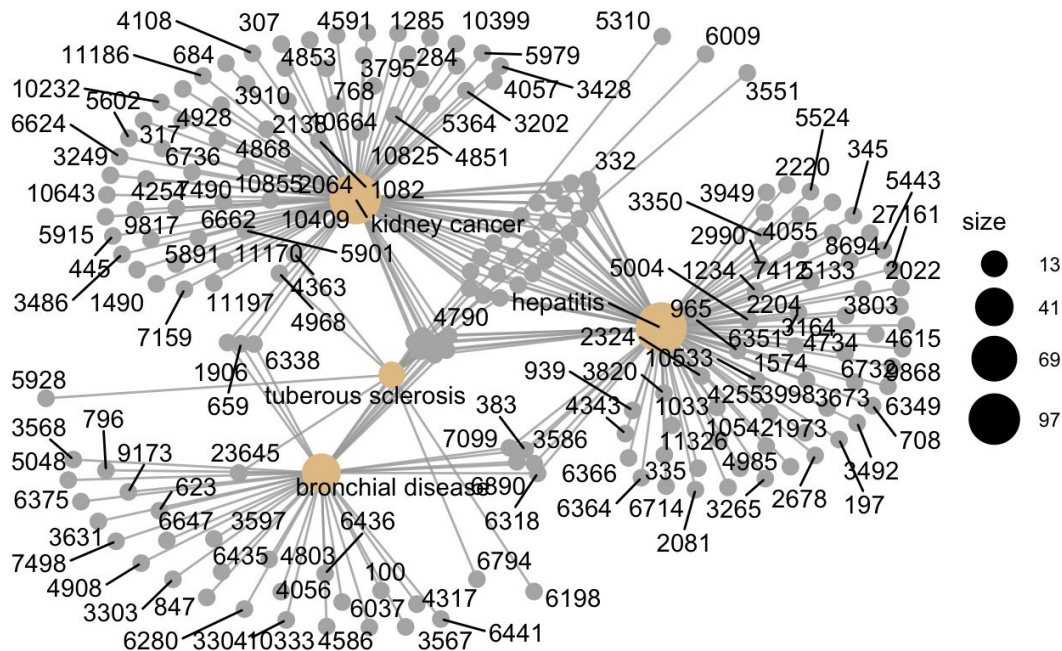
**ChIP-seq:** Alignment with HG38 using BOWTIE2, peak calling using MACS2 (only narrow peaks), replicate peak files sorted/merged. Peak annotation using HOMER.

ATAC-seq (broad and narrow) and ChIP-seq peaks were intersected before annotation; and annotation was performed separately on each, then the gene lists were intersected. All three were integrated with differentially expressed genes from RNA-seq.

# RESULTS

RNA-seq network plot: 1) Kidney cancer  
2) Hepatitis  
3) Bronchial disease

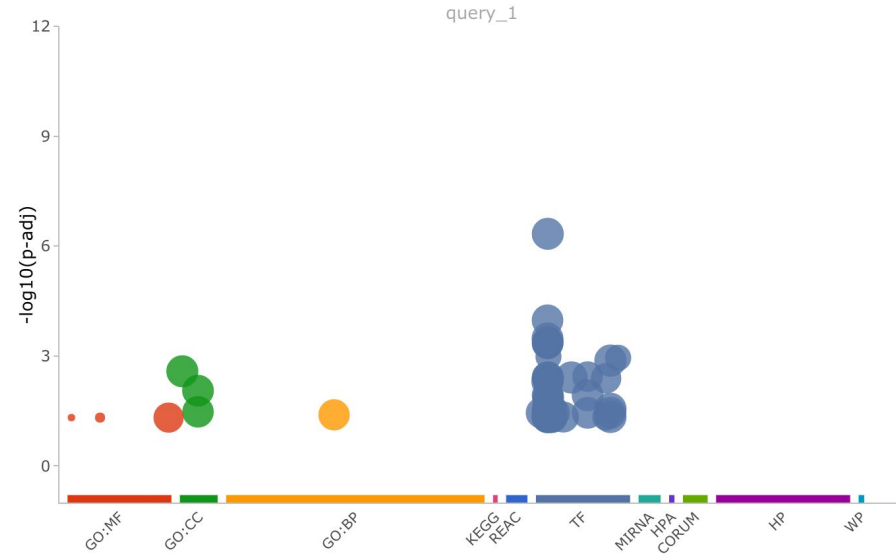
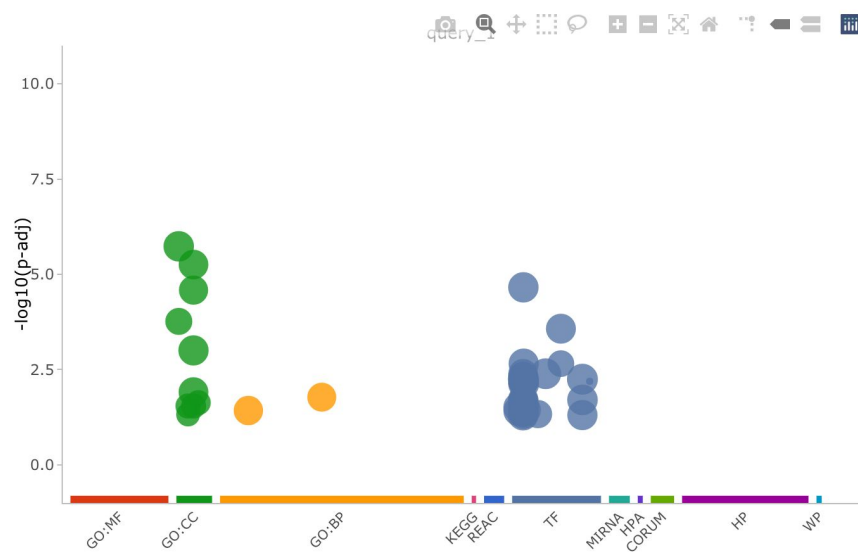
6177 differentially expressed genes.



# Associating ATAC-seq/ChIP-seq with RNA-seq

Integration results: 567 (broad); 412 (narrow).

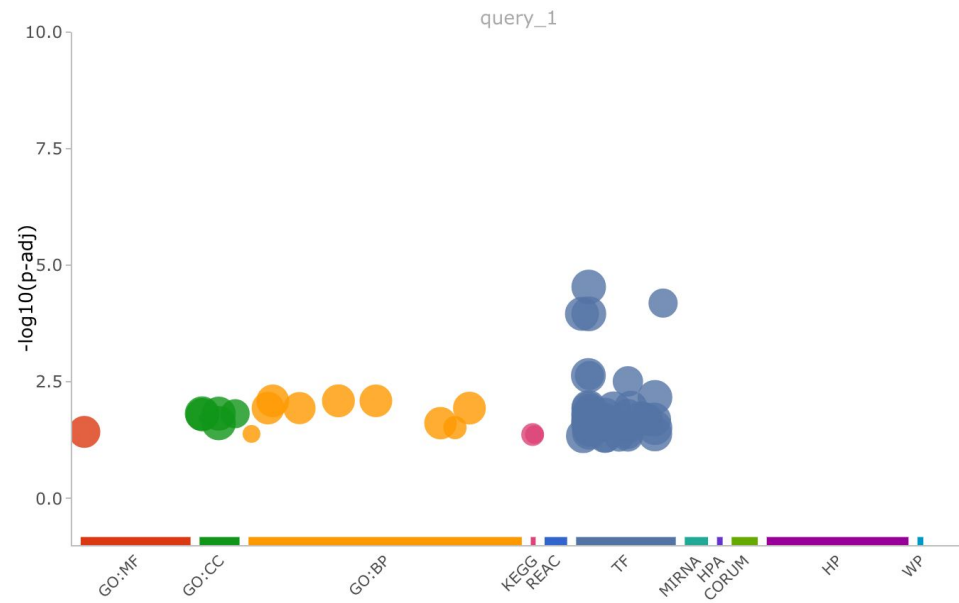
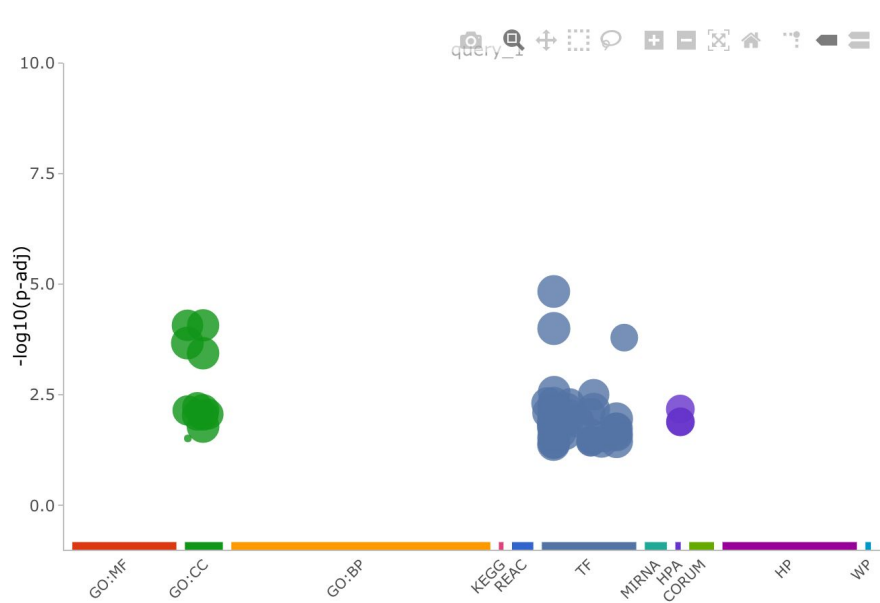
Functional enrichment using FDR threshold 0.05: GO:0005622, intracellular anatomical structure; GO:0043229, intracellular organelle; and GO:0043231, intracellular membrane-bounded organelle; E2F-2 TF



# Likely FOXM1 targets from integrating ATAC-seq and ChIP-seq peaks with differentially expressed genes

Integration results: 512 (broad); 381 (narrow).

Functional enrichment using FDR threshold 0.05: GO:0005634, nucleus; and GO:0043231, intracellular membrane-bounded organelle; E2F-2 and E2F-3 transcription factors



# INTERPRETATION

Three most significant DE categories (kidney cancer, hepatitis, and bronchial disease) make sense (5,7).

GO term results from both conditions (broad and narrow ATAC-seq peaks) of both integration experiments also not surprising given FOXM1's role in cell cycle progression and as a proto-oncogene.

E2F-2 TF was significantly represented in every case: "plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming proteins of small DNA tumor viruses" (1)

Other significant TFs:

-E2F-3: regulates cell cycle via retinoblastoma protein, implicated in "a number of human cancers" (2)

-CDX2: regulates intestine-specific genes involved in cell growth, associated with inflammation and tumorigenesis (3)

-ZNF418: zinc finger protein associated with ovarian squamous cell carcinoma, related to gene expression and HSV1 infection

- (1) <https://www.genecards.org/cgi-bin/carddisp.pl?gene=E2F2>
- (2) <https://www.genecards.org/cgi-bin/carddisp.pl?gene=E2F3>
- (3) <https://www.genecards.org/cgi-bin/carddisp.pl?gene=CDX2>
- (4) <https://www.genecards.org/cgi-bin/carddisp.pl?gene=ZNF418>
- (5) Chou, L.-F.; Chen, C.-Y.; Yang, W.-H. et al. (2020) Suppression of hepatocellular carcinoma progression through FOXM1 and EMT inhibition via hydroxygenkwanin-induced miR-320a expression. *Biomolecules*. volume 10, issue 1, p. 1-15. <https://doi.org/10.3390/biom10010020>
- (6) Myatt, SS; Lam, E W-F (2007). The emerging roles of forkhead box (Fox) proteins in cancer. *Nature Reviews Cancer*. Volume 7, p. 847--859. <https://doi.org/10.1038/nrc2223>
- (7) Zhang, J.; Zhang, J.; Cui, X. et al. (2015) FoxM1: a novel tumor biomarker of lung cancer. *International Journal of Clinical and Experimental Medicine*. Volume 8, issue 4, p. 3136--3140.