Supplementary Information
Investigating functional implication of reinforcing feedback loops in transcriptional regulatory network†

Yue Li,[*ab‡] **Cheng Liang,**[c‡] **Steve Easterbrook,**[a] **Jiawei Luo,**[c] **Zhaolei Zhang**[abd]

Supporting Information

Supporting Tables

Table S1 TF regulators per miRNA family. (TabS1_mirfamily_TF_regulation.xlsx)

Table S2 Full motif analysis results. Each subgraph is associated with a "Subgraph_label", where the three FBLs listed in Table 1 in the main text are highlighted; "occupancy" is the fraction of subgraph occurrence in the whole subgraph set. "mean_frequency" and "standard_deviation" are mean frequency and standard deviation of motif occurrence in 1000 randomized networks; "ZScore" was calculated in Eq 18; "pval_enrich" and "pval_deplet" indicate the enrichment p-value (Eq 19) and depletion p-value (Eq 20); "frequency_in_real_network" is observed motif frequency in the ENCODE+miRNA regulatory network. (TabS2_motif_analysis.xlsx)

Table S3 Functional enrichment of each node in the 9 rFBL subgraphs. Hypergeometric test was performed to examine the enrichment of each GO-BP term or pathway for the target set from each regulator. The columns are self-explanatory. (TabS3_function_enrich.xlsx)

Table S4 Full pairwise overlap between regulators within each rFBL. The columns are self-explanatory. (TabS4_sig_overlap_test.xlsx)

Supporting Figures

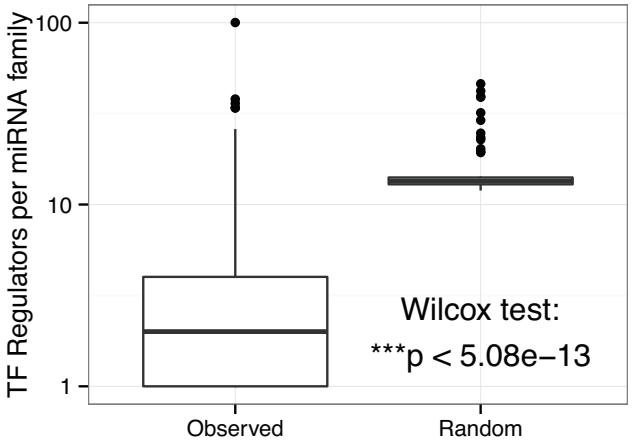

Fig. S1 TF regulators per miRNA family. The number of TF regulators per miRNA family were compared between observed TF→ miRNA and randomly shuffled TF→ miRNA. One-sided Wilcox rank-sum test was used to compute the p-value between the two distributions. As shown, the observed number of TF regulators per miRNA family is significantly lower than expected, indicating that miRNAs from the same family tend to be regulated by a common set of the TFs.

[a] *Department of Computer Science, University of Toronto, Toronto, Ontario M5S 3G4, Canada; E-mail: yueli@cs.toronto.edu; sme@cs.toronto.edu*
[b] *The Donnelly Centre, University of Toronto, Toronto, Ontario M5S 3E1, Canada; E-mail: zhaolei.zhang@utoronto.ca*
[c] *College of Information Science and Engineering, Hunan University, Changsha, Hunan, 410082, China; E-mail: cheng.liang@utoronto.ca; luojiawei@hnu.edu.cn*
[d] *Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5S 3E1, Canada; E-mail: zhaolei.zhang@utoronto.ca*
‡ These authors contributed equally to this work.

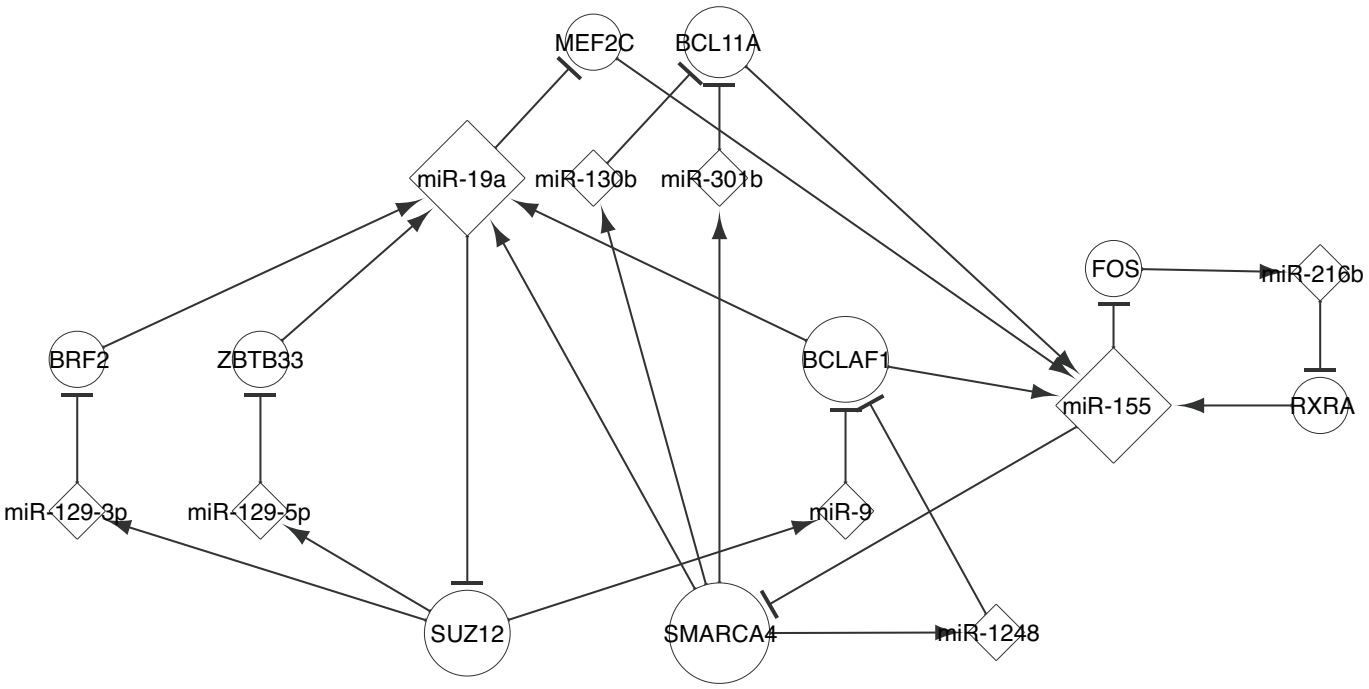

Fig. S2 Connectivities between nodes across the 9 reinforcing feedback loop subgraphs. The rFBL network derived from Figure 3 is visualized in Cytoscape.[1] miRNAs are diamonds and TFs are circles. The nodes are arranged as a hierarchy based on their in/out degree. The size of the nodes is also proportion to their in/out degrees.

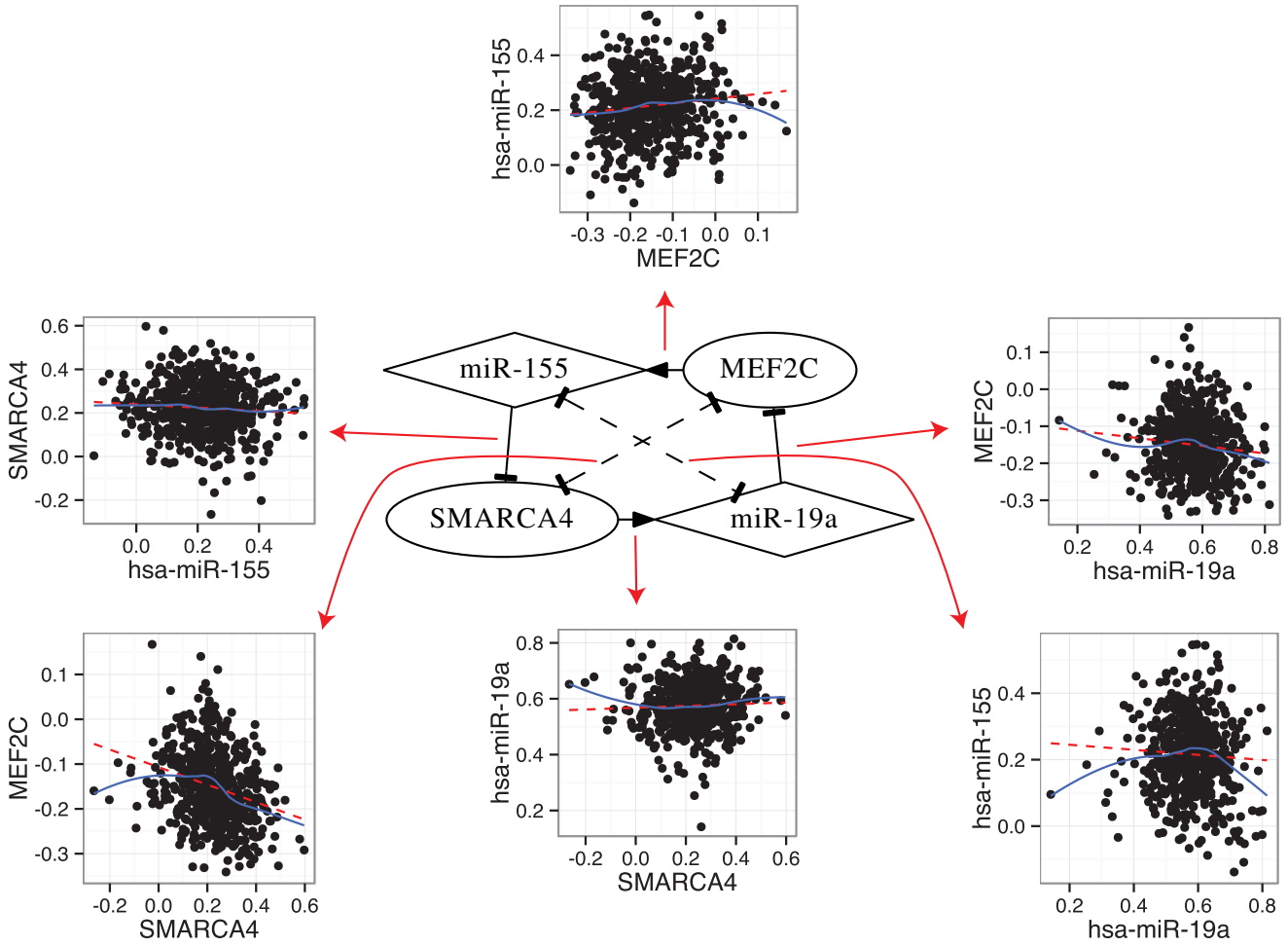

Fig. S3 Expression correlation of a select rFBL subgraph in ovarian cancer. We associated each edge with a scatter plot of the corresponding expression from over 500 ovarian cancer samples from TCGA. The inhibition edges (direct solid or indirect dashed) are coherently associated with negative expression correlation trends, whereas the activation edges are coherently associated with positive expression trends, thus supporting the predicted rFBL.

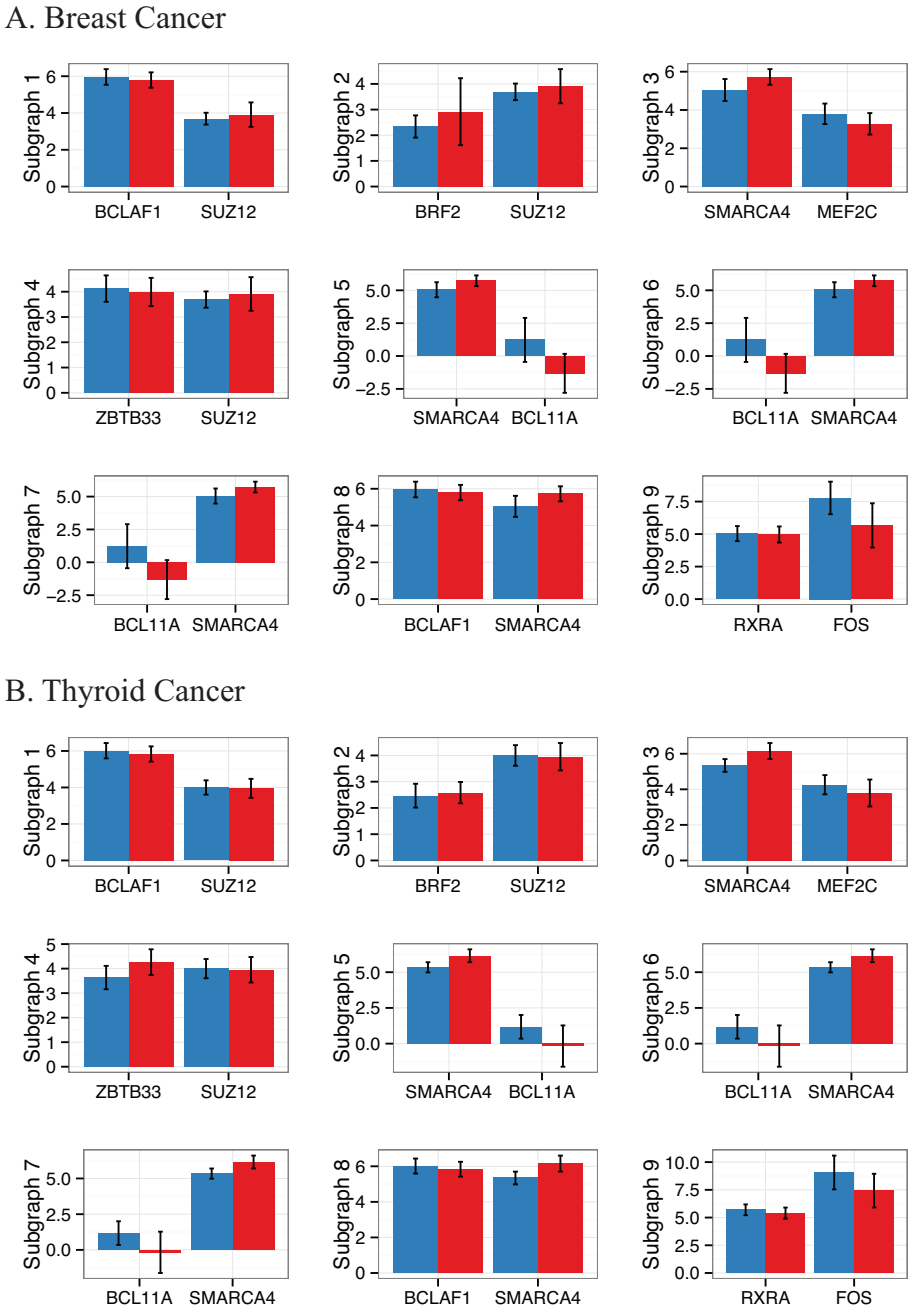

Fig. S4 Matched TCGA tumor/normal expression comparison in the 9 rFBL subgraphs. **A**. We extracted the expression profiles from the 14 matched tumor/normal samples of breast cancer patients from TCGA. The averaged expression of TFs within the same subgraph in normal (blue) and tumor (blue) were plotted with error bars indicating the standard deviations. **B**. Same as **A** but using the 58 matched tumor/normal samples from thyroid cancer patients from TCGA.

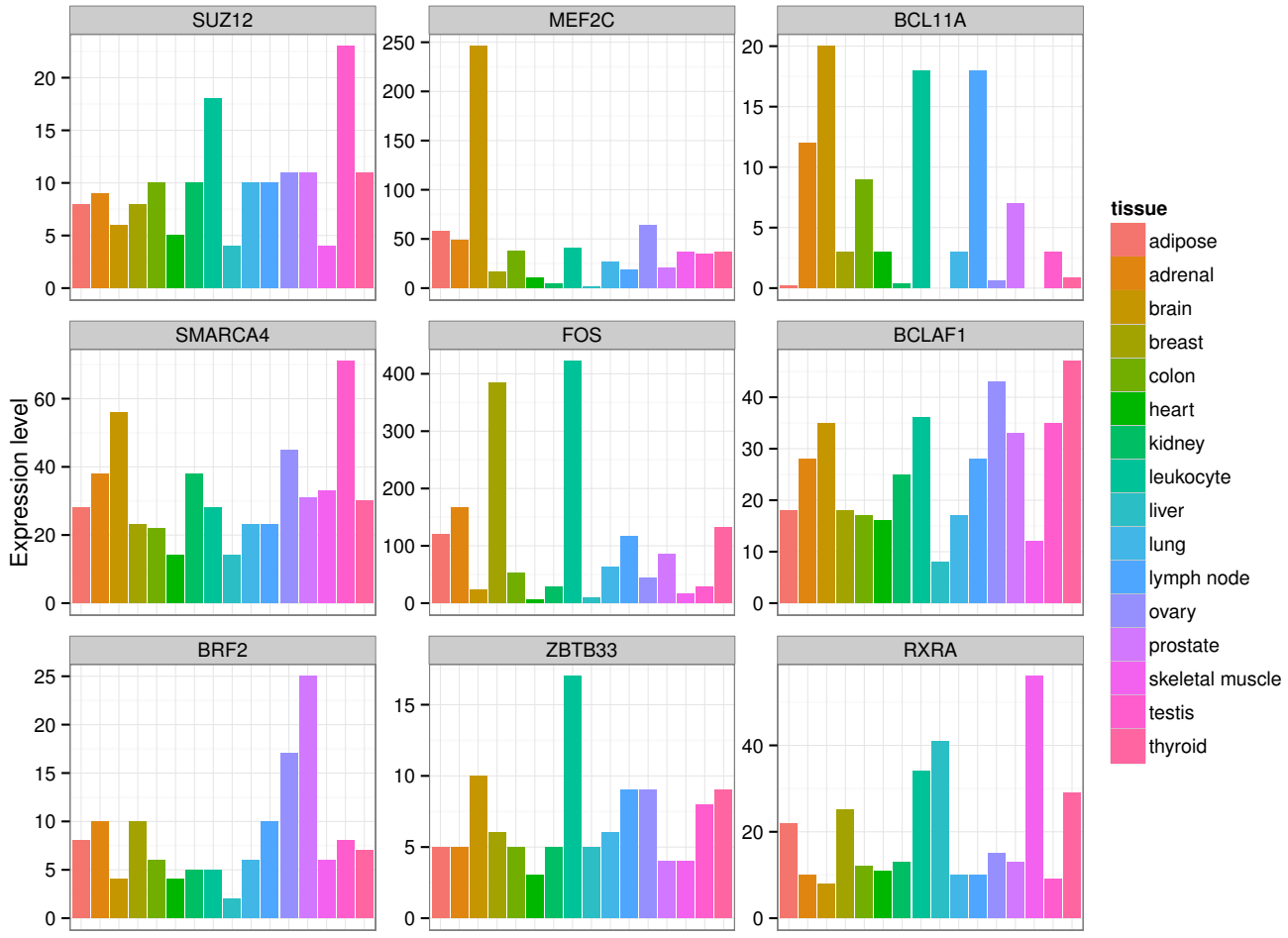

Fig. S5 Tissue-specific expression of TF regulators in the identified rFBLs. Data were obtained from EMBL-EBI Expression Atlas (http://www.ebi.ac.uk/gxa/home).[2]

References

1 P. Shannon, A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski and T. Ideker, *Genome research*, 2003, **13**, 2498–2504.

2 R. Petryszak, T. Burdett, B. Fiorelli, N. A. Fonseca, M. Gonzalez-Porta, E. Hastings, W. Huber, S. Jupp, M. Keays, N. Kryvych, J. McMurry, J. C. Marioni, J. Malone, K. Megy, G. Rustici, A. Y. Tang, J. Taubert, E. Williams, O. Mannion, H. E. Parkinson and A. Brazma, *Nucleic Acids Research*, 2014, **42**, D926–D932.