***SUB1* targets are enriched with *MYC* target genes and pathways.**

Among genes whose 3’UTR regions have *SUB1* eCLIP sites, we observed significant enrichment of functional categories including *MYC* targets, oxidative phosphorylation, and spliceosome. *MYC* activation induces an increase in total precursor messenger RNA synthesis, which increases the burden on the core spliceosome to process pre-mRNA 1. Also, *MYC* activation can stimulate oxidative phosphorylation, which fulfills the bio-energetic demands of cancer cells 2. These results together indicate that *SUB1* may stabilize the *MYC* target genes and pathways to promote the malignant growth of cancer cells.

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**Supplementary Figure X. Functional enrichment analysis of SUB1 target genes.** **(a)** Function enrichment for genes with SUB1 binding sites on 3’UTR regions. For each category in MSigDB Hallmark set or KEGG set, a logistic regression analysis was applied to analyze its enrichment among SUB1 targets with the 3’UTR AU content, network degree, and gene annotation frequency as background. The z-scores (regression coefficient/stderr) for all categories were ranked and plotted with top enriched terms highlighted with black dots. **(b)** Gene set enrichment analysis for the differential gene expression profile after knocking down SUB1. All genes are ranked the log-fold change (logFC) ratio of gene expression value between shRNA knockdown (KD) condition and control condition. The gene members within each functional category are labeled with black lines. The enrichment score represents the degree to which the function category is over-represented at the top or bottom of a logFC ranked list.

**Mutation in enhancer region may affect TF activity.**

I analyzed the association between TF mutations in extended gene region and TF regulatory activity in three cancer types (breast, liver, and leukemia). Between each pairs of mutation type (e.g., ENH1, TF, eCLIP, UTR) and cancer type, I tested the association between mutation status and TF regulatory activity by two-sided rank-sum test and converted the *p*-values into FDRs by Benjamini-Hochberg procedure. Only the combination between liver cancer and ENH1 mutation has statistically significant results (FDR < 0.25, panel a). A mutation in the enhancer region of DPF2 or RELA indicates a lower TF regulatory activity (panel b). These results indicate that mutations in enhancers may cause TF loss-of-function in certain cancer types.



**Supplementary Figure X. Mutations in level one enhancers affects the activity of nearby TFs**. **(a)** The association between TF regulatory activity and mutation in enhancer regions. For each cancer type, the association between TF regulatory activity computed using ChIP-seq data and mutation status of nearby enhancer region was tested by two-sided rank-sum test. Only liver cancer has significant associations (FDR < 0.25) for TF DPF2 and RELA, and the results for liver cancer are shown with volcano plot. X-axis represents the z-score of rank-sum test and Y-axis represents the negative log p-values. **(b)** The regulatory activities of significant TFs in panel a in tumors with mutated or wild-type TF genes. The comparison between two groups was done by two-sided rank-sum test.

**Reference**

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