MYC is a proto-oncogene with a well-documented essential role in the pathogenesis and maintenance of several types of cancer. As a transcription factor, MYC binds to specific E-box sequences in the genome to regulate expression of adjacent genes. However, there is no universal set of MYC targets, as many of them are cell type- and developmental stage-specific. To date, a comprehensive analysis of direct MYC targets with essential roles in different types of cancer is missing.

In this study we generated a lentiviral single guide RNA (sgRNA) library to destroy MYC-bound E-box sequences. The catalogue of E-box sequences was based on the publicly available data from MYC-chromatin immunoprecipitation sequencing (MYC-ChIP-Seq) in four different types of MYC-addicted cell lines: MCF7 (breast cancer), K562 (chronic myelogenous leukemia), HepG2 (hepatocellular carcinoma) and Burkitt lymphoma (BL) cell lines. In parallel, we performed a screen with the Brunello CRISPR library to knock out protein-coding genes. Intersection of the results from the MYC-CRISPR and Brunello libraries indicated MYC binding sites and corresponding target genes essential for growth of cancer cells in general, as well as specific for the studied cancer subtypes.

Considering the combined effect of all sgRNAs targeting a given gene or E-box, 354-1.992 genes and 56-97 E-boxes were identified as essential for growth of selected cancer cells, while 3-9 E-boxes and 5-18 genes were significantly enriched. Moreover, analysis of essential E-boxes revealed that 20-32% were localized close to genes essential for cancer cells, and 42-49% of adjacent genes are well-known MYC-regulated targets. GO and GSEA analyses revealed that genes from Brunello library and genes localized near E-boxes were involved in processes such as metabolism, ribosome biogenesis, metabolism of nucleic acids, splicing, translation etc. Next, we validated our approach in K562 cell line. We confirmed that E-box disruption affected adjacent genes expression, cell growth and MYC binding. In addition, our results indicated potential critical hubs in MYC-regulated pathways and uncovered specific features of essential E-boxes. Altogether, we established a unique, well-validated tool to identify MYC-regulated target genes relevant for growth of malignant cells. Our findings provide novel insights into MYC-dependent vulnerabilities in cancer cells.