

p63 Drives Head and Neck Squamous Cell Carcinoma

Head and Neck Squamous Cell Carcinoma (HNSCC) is a heterogeneous group of epithelial malignancies with significant mortality and limited treatment options. The lack of improvement in patient survival and targeted therapies is in part due to paucity of key drivers and biomarkers that underpin the molecular landscape of HNSCC. To address this knowledge void and to get a better understanding of the molecular etiology of HNSCC, we have focused our studies on p63, a master transcription factor (TF) that is overexpressed and intimately linked with the pathology and progression of HNSCC.

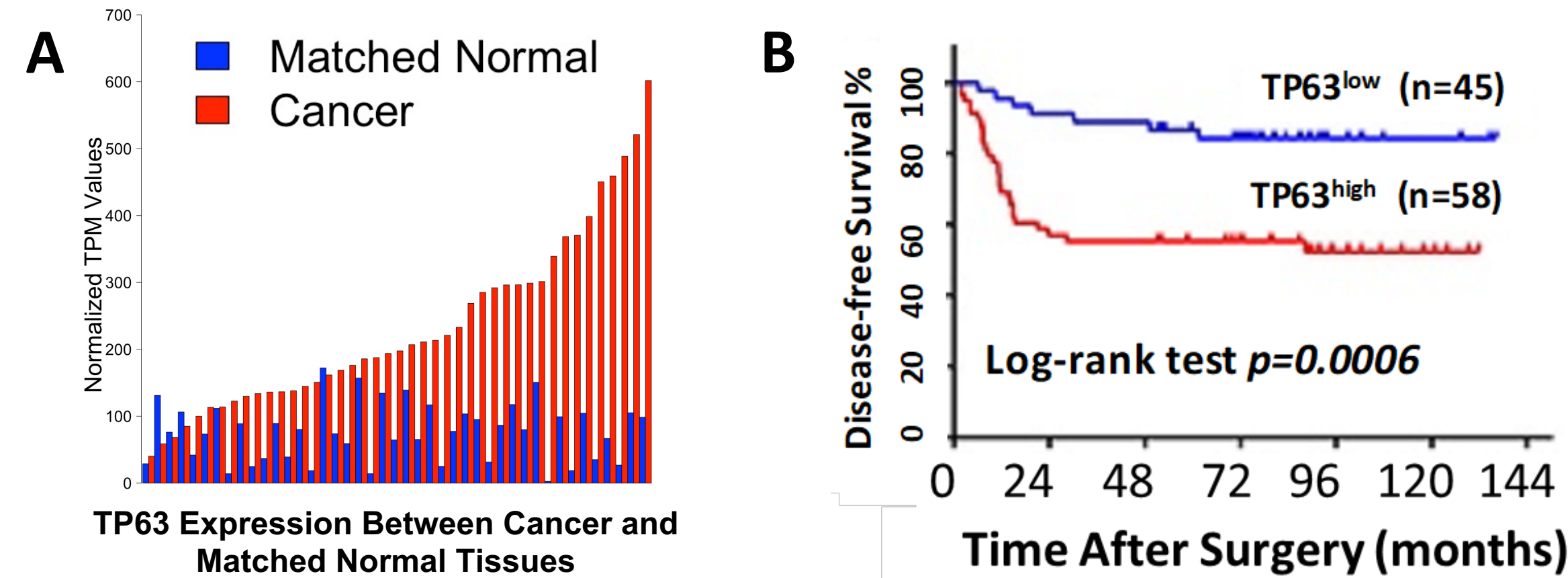


Figure 1: (A) TP63 expression between cancer and matched normal tissues. (B) Kaplan-Meier Analysis of p63 expression shows that higher levels of p63 is associated with lower disease free survival as compared to tumors with low p63 expression.¹

Identifying MED15 as an Important Target of p63

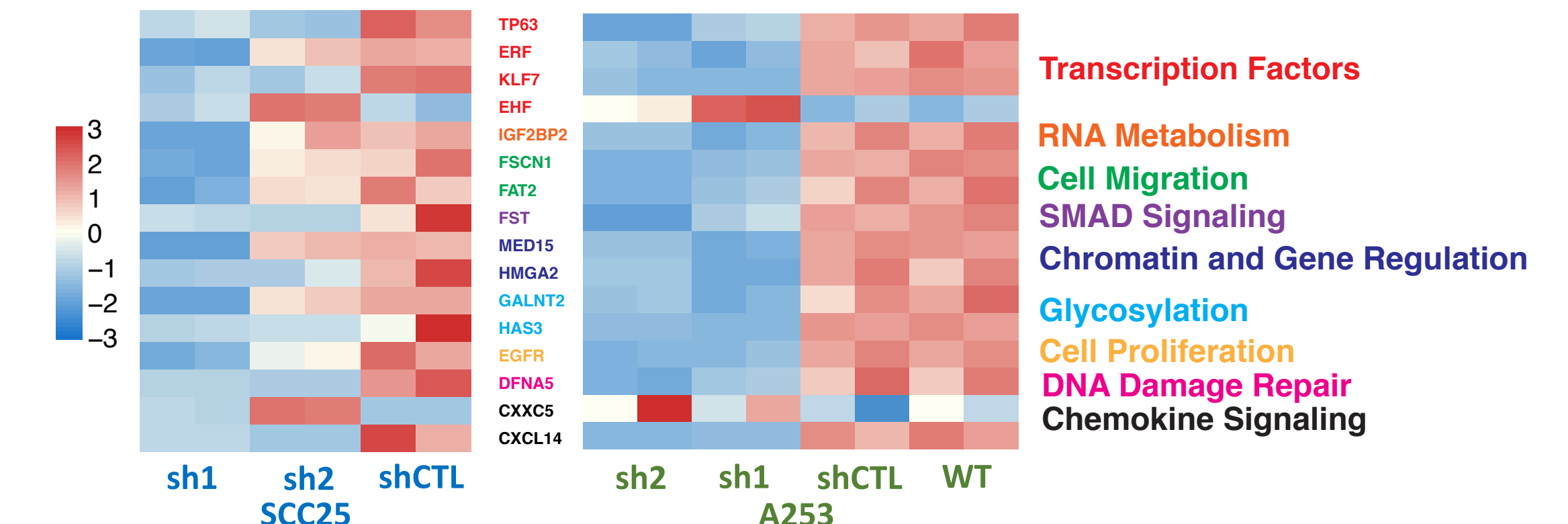


Figure 2: Multiple HNSCC cell lines in which p63 expression was ablated using two shRNAs were generated. RNA-sequencing and differential expression analysis identified differentially expressed genes (DEGs) whose expression was affected by p63 knockdown. The heatmap shown above is a selection of these genes and the pathways associated with them.

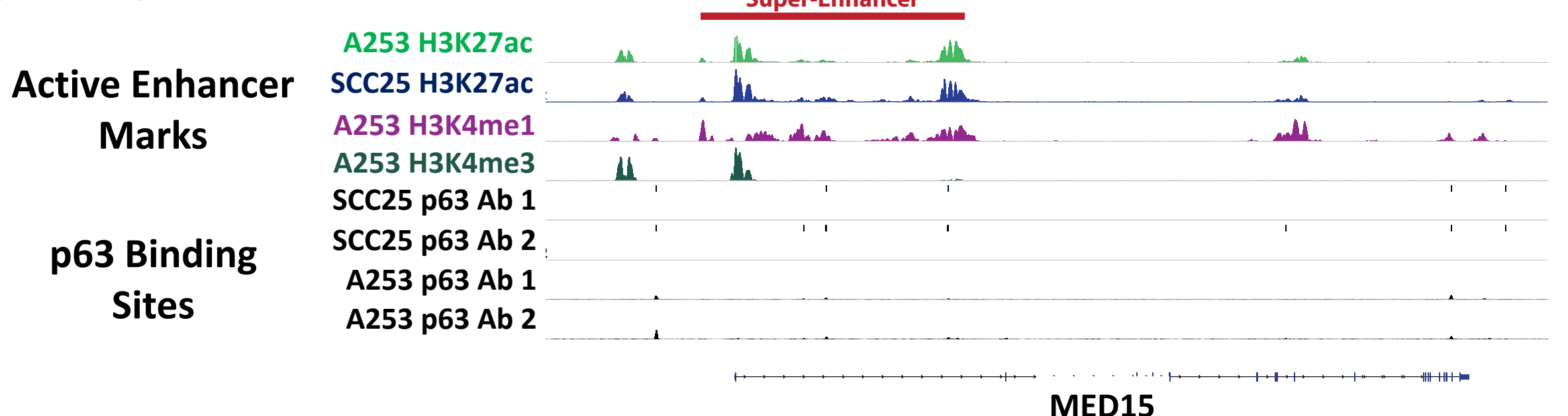


Figure 3: Histone ChIP-seq data and p63 ChIP-seq data from HNSCC cell lines A253 and SCC25 were utilized to identify a core p63 network. ChIP-seq of H3K27ac was utilized to identify the super-enhancer network of our cell lines. The figures above showcases multiple p63 binding sites in and around the MED15 locus which is also associated with a super-enhancer, suggesting its importance to HNSCC.

MED15 is a Mediator of Chromatin and Gene Regulation

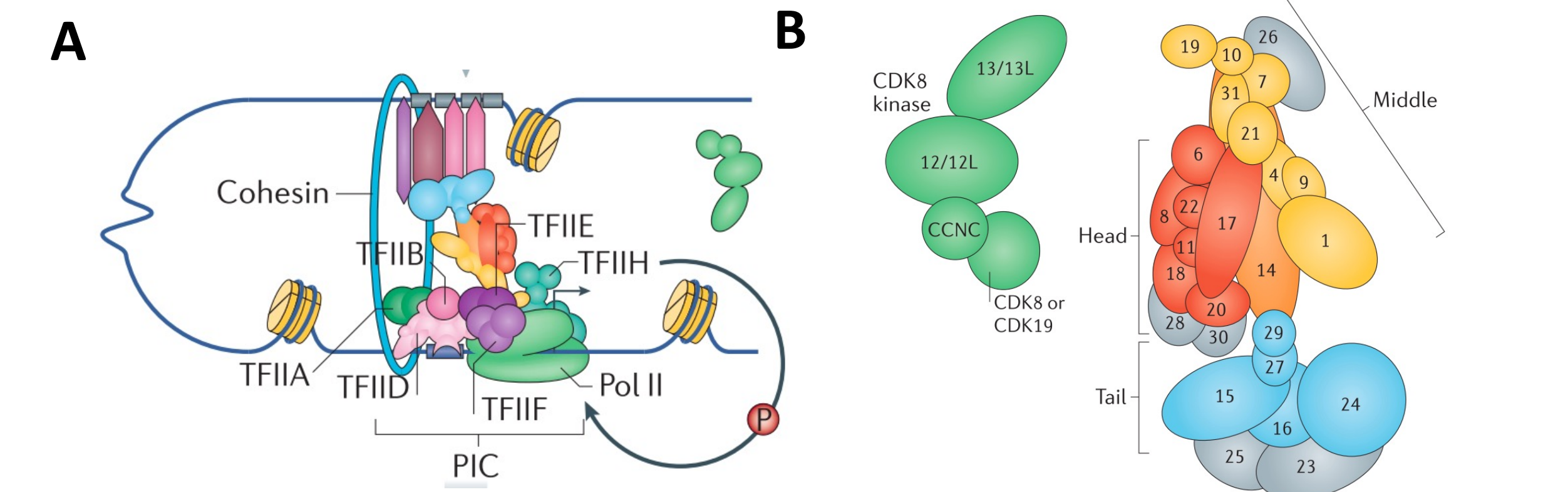


Figure 4: (A) Mediator of RNA Polymerase II transcription (Mediator) is independently recruited to enhancer regions through direct interactions with TFs bound to enhancers. Following chromatin looping, Mediator interacts with pre-initiation complex (PIC) components promoting PIC assembly.² (B) Mediator is subdivided into four modules which are involved in different functions of the complex. MED15 is a member of the tail module, involved in regulating Mediator activity through its interactions with TFs at enhancer sites.²

MED15 Expression is Increased in HNSCC and Higher Expression is Associated with Poor Survival

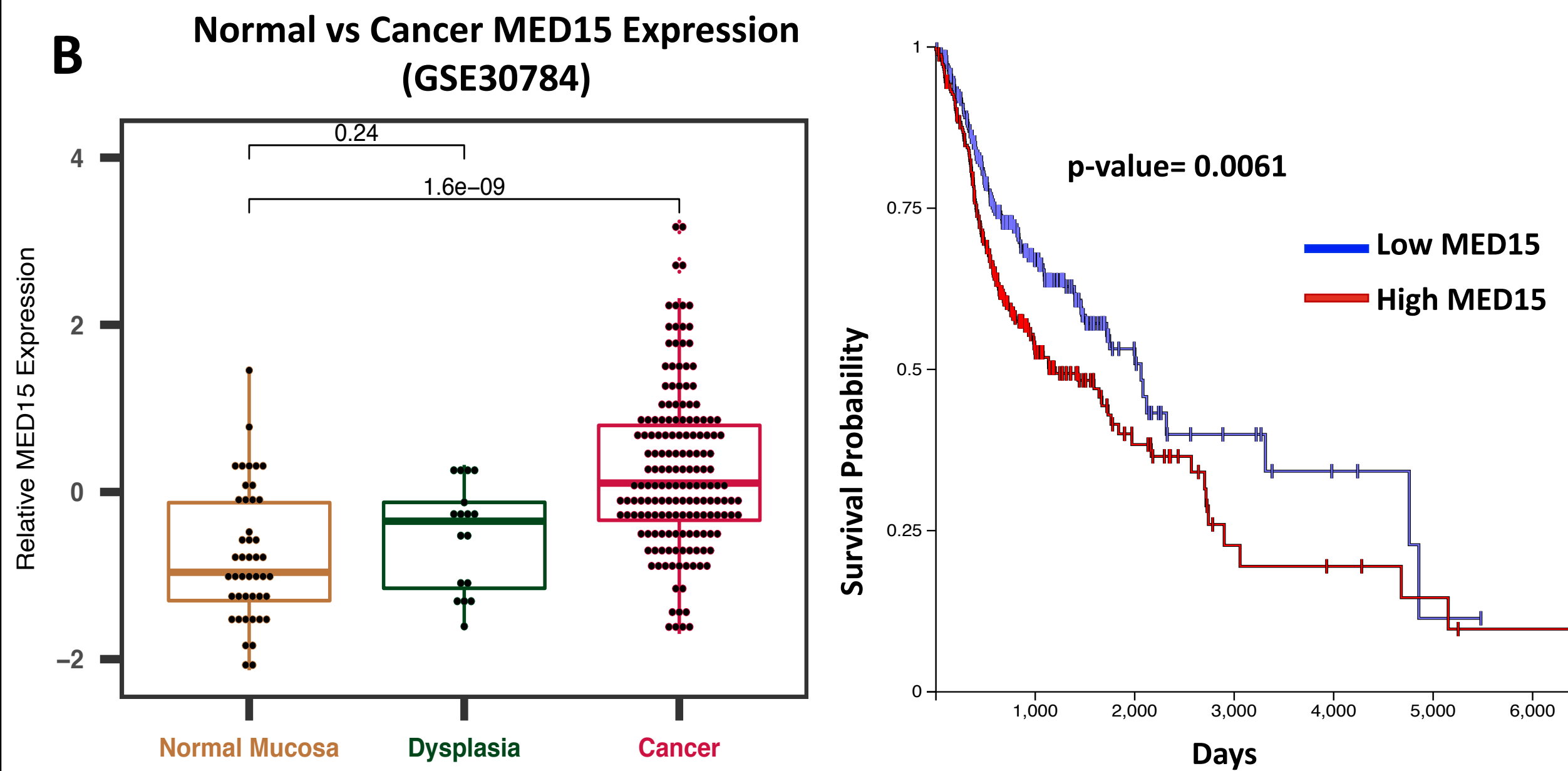


Figure 5: (A) Analysis of GSE30784 normal, dysplasia, and carcinoma microarray datasets reveals that MED15 expression increases as the tissue progresses through tumorigenesis.³ (C) Kaplan Meier Analysis of the TCGA HNSCC dataset showed that high levels of MED15 are correlated with worse overall survival.⁴

Establishing MED15 Knockdown Cell Lines

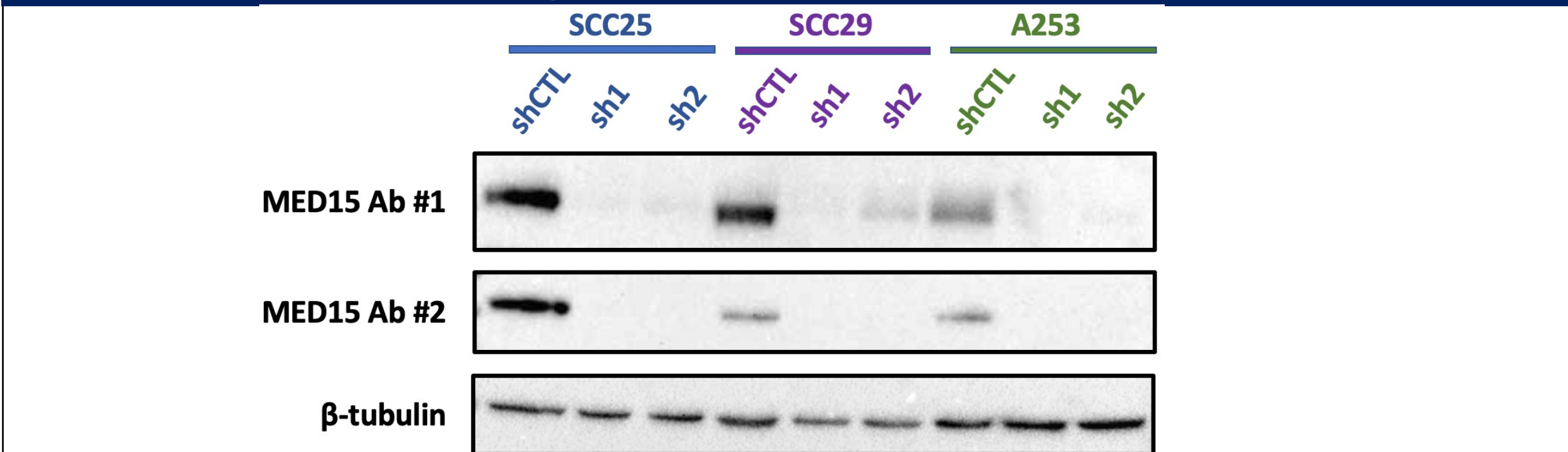


Figure 6: (A) Western blot of MED15 expression in 3 representative HNSCC cell lines with MED15 knockdown. MED15 expression was ablated using two independent, stable, shRNAs and compared against cells infected with non-silencing control shRNA.

MED15/p63 Controlled Gene Network Affects Pathways Including EMT, Stemness, and Immune Responses

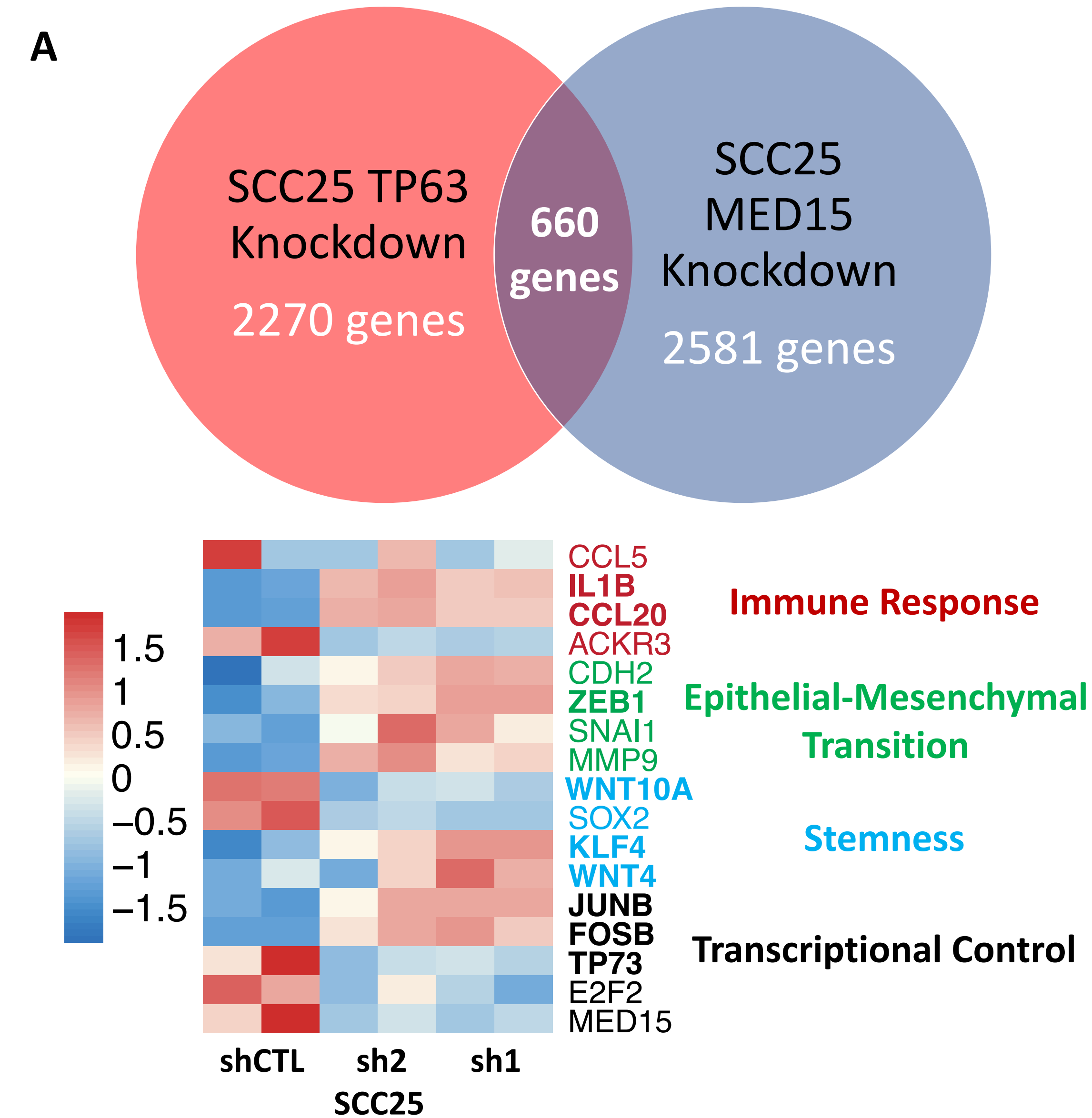


Figure 7: (A) Analysis of our MED15 knockdown RNA-seq data revealed a large pool of shared DEGs with our SCC25 p63 knockdown suggesting a potential MED15/p63 interconnected network. (B) Within our MED15 DEGs we observed an enrichment of Epithelial-Mesenchymal Transition (EMT)-associated pathways, immune response pathways, and stemness pathways. Shown above is a heatmap of some of the associated MED15 KD DEGs associated with these pathways as well as a few genes (**bolded**) which are shared targets of MED15 and p63. (C) Western blots show that some proteins traditionally associated with EMT are changing in our MED15 knockdowns, but some like Vimentin are not, suggesting MED15 knockdown may not lead to a full epithelial to mesenchymal transition. Proteins in red are associated with a mesenchymal phenotype and proteins in green are associated with an epithelial phenotype.

Loss of MED15 Expression Increases Invasiveness and Stemness in HNSCC

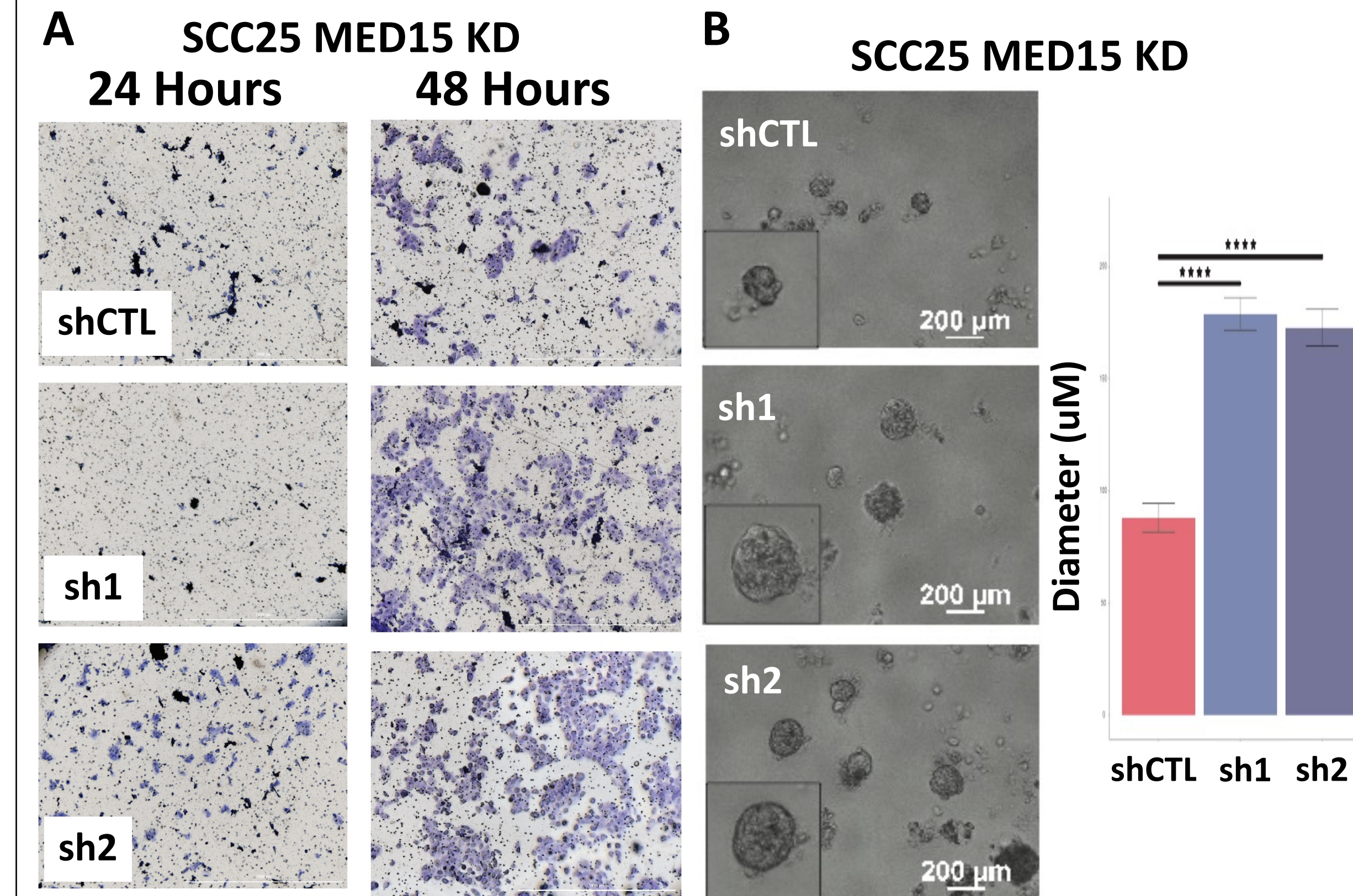


Figure 8: (A) Transwell invasion assays show increased invasion of SCC25 cells with MED15 knockdown in agreement with the EMT phenotype. (B) Spheroid formation assay performed with SCC25 MED15 knockdown cells shows that loss of MED15 expression leads to formation of larger spheroids in agreement with a stem cell-like phenotype.

Proposed Model of MED15 Action

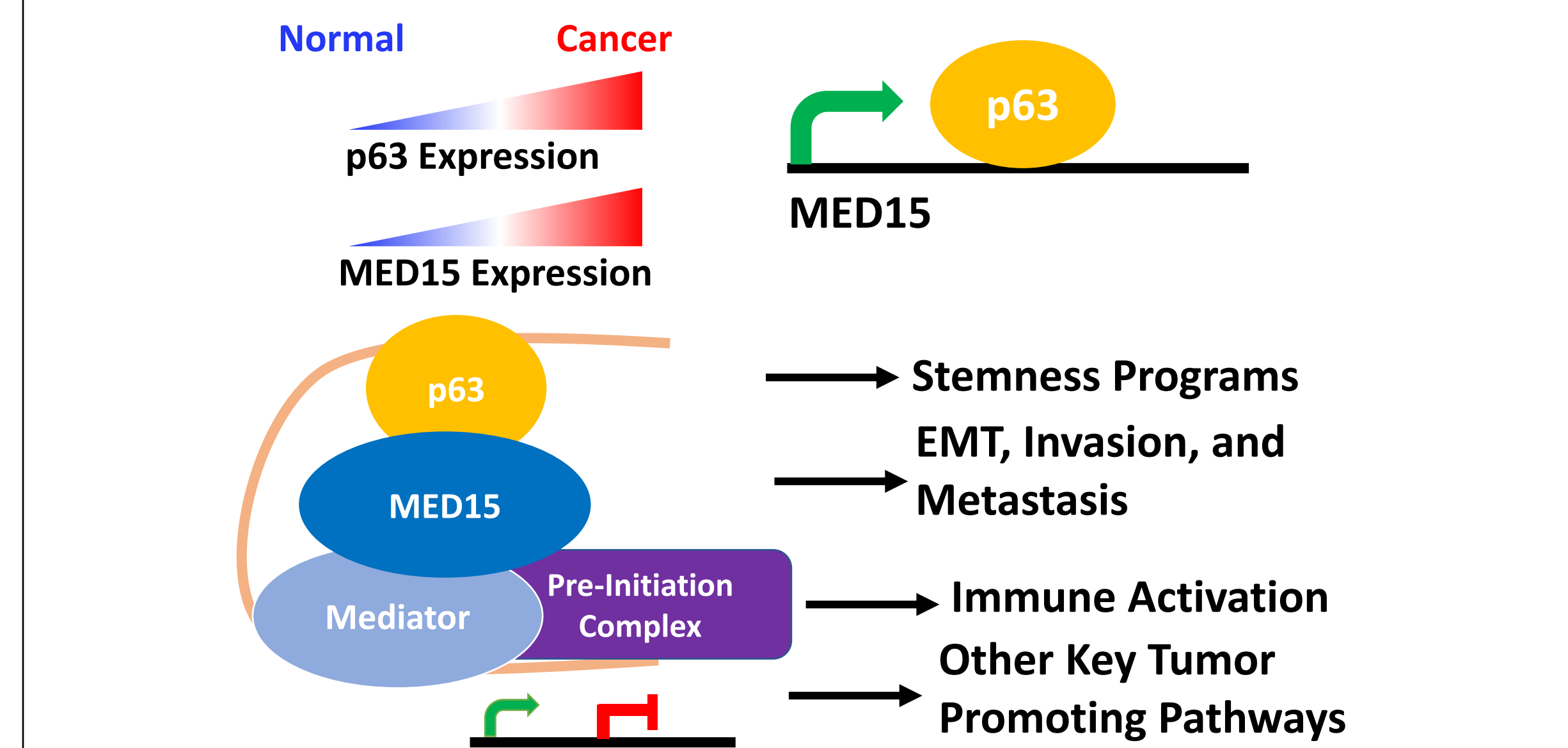


Figure 9: Our proposed model of MED15 action. Increased expression of p63 in HNSCC drives MED15 expression. Increased levels of MED15 allow for more interactions with p63 at p63 controlled enhancers, which downstream affects p63 target gene expression. Our findings so far suggest that a coordinated and interdependent p63/MED15 axis drives broad transcriptional addiction mechanisms that regulate such processes as immune activation, stemness, and metastasis. Our work offers novel insights into both p63 and Mediator biology, and identifies potentially vulnerable target genes and pathways for the development of new therapeutics.