# Role of MED13/MED13L in the Induction of HIF-1α Target Genes

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Departmental Honors Thesis

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November 11, 2013

**ABSTRACT**

In cancer the over proliferation of cells leads to the growth of solid tumors in tissues. Hypoxia or a low oxygen environment is a characteristic found in all solid tumors. There is a family of transcription factors that respond to hypoxia commonly named Hypoxia Inducible Factors (HIFs). HIFs regulate many phenotypes that are hallmarks for cancer. CDK8, the enzymatic subunit of the Mediator complex was recently identified as a key co-activator of many HIF-1α target genes. Three of the four proteins in the CDK module have paralogous pairs; CDK8/CDK19, MED12/MED12L, and MED13/MED13L. MED13 and likely MED13L is the subunit that connects the CDK module with the core Mediator complex. This association is likely to be important for CDK8 to induce HIF-1α target genes through the Mediator complex. Therefore, I hypothesized that the MED13 and/or MED13L is required for the full induction of HIF-1α target genes under hypoxic conditions. Using a loss-of-function approach, I show that the MED13 and MED13L subunits of the Mediator complex are required in a gene-dependent manner in the induction of HIF-1α target genes. MED13 and MED13L have similar effects on HIF-1α target genes.

## INTRODUCTION

The regulation of transcription by Hypoxia-Inducible Factors (HIFs) is vital to the understanding of solid cancers because hypoxia has been identified as a hallmark of solid tumors as well as other diseases such as atherosclerosis, and pulmonary hypertension (Semenza, 2012). Cancer cells rely on this function used in normal physiology and development to facilitate their survival in a low oxygen environment (Licht et al., 2006). Further research into the transcriptional mechanism of the main transcription factor Hypoxia-Inducible Factor- 1α (HIF-1α) will aid in the development for further studies.

In cancer the response to hypoxia can induce several genes that allow the tumor cells to perform angiogenesis, proliferation, evasion of apoptosis, and many others (Dengler et al., 2013). Recent studies have identified new HIF-1α cofactors in addition to the widespread known cofactors CBP and p300 which have also been shown to only be important for select hypoxia genes (Kasper et al., 2005). CDK8, an enzymatic subunit of the Mediator complex, was recently discovered as a key co-activator of HIF-1α required for the induction of many HIF-1α target genes by alleviating RNAPII pausing (Galbraith et al., 2013). The Mediator complex is a multi-subunit complex that plays a critical role in gene regulation through its interaction with RNA Polymerase II (RNAPII) (Conaway & Conaway, 2012; Sato et al., 2004). The CDK-module of Mediator contains the only known enzymatic function. Three of the four proteins in the CDK module have paralogous pairs; CDK8/CDK19, MED12/MED12L, and MED13/MED13L (Sato et al., 2004). Not all of the paralogs are confirmed to be not functionally redundant; however, studies show that CDK8/CDK19 are non-redundant. For example, CDK8, but not its paralog CDK19 activates the transcription of HIF-1α target genes (Galbraith et al., 2013). MED13 is required for the association of the CDK module to the Mediator core, but this is unknown for

MED13L. This association is likely to be important for CDK8 to induce HIF-1α target genes through the Mediator complex. The preferential use of one paralog over the other very likely applies to the other module members. Therefore, I hypothesize that the MED13 and/or MED13L is required for the full induction of HIF-1α target genes under hypoxic conditions.

## BACKGROUND

### Hypoxia and its Role in Cancer

Cancer is a disease of accumulated mutations resulting in uncontrolled proliferation of a tissue. Cancer is the second leading cause of death in the United States of America with an expected 580,350 deaths in 2013 according to the American Cancer Society (Siegel et al., 2013). There are over 100 kinds of cancer; lung, breast/prostate, and colorectal being the highest cause of death. The over proliferation of cells leads to the growth of solid tumors in tissues. These tumors can cause other problems however, are not usually life threatening by themselves depending on their location. Tumors are considered cancerous and life threatening when they metastasize or invade other tissues. In order for these tumors to grow and proliferate they continue to acquire mutations that allow them to gain new functions and hijack normal functions that are used during development.

As tumors proliferate, they often outgrow their nutrient supply and oxygen-supplying blood vessels, causing these regions to become hypoxic. Hypoxia or a low oxygen environment is a characteristic found in all solid tumors (Semenza, 2012). In order for the tumor to survive this environment the hypoxic response is induced to activate genes required to aid in the hypoxic environment. The hypoxia-inducible genes regulate many phenotypes that are hallmarks for cancer; such as angiogenesis, proliferation, evading apoptosis, metastasis, and others (Dengler et al., 2013). For example, target genes up regulated during hypoxia such as Vascular Endothelia

Growth Factor (VEGF) act to induce angiogenesis and increase oxygen supply as well as promoting metastasis (Takeda et al., 2004). Many other genes are induced to activate metasis such as Stanniocalin-2 (STC2), Lysyl Oxidase (LOX), LOXL2, and LOXL4 (Tsai, et al. 2012). Many of these activated genes aid in the tumor's response to hypoxia and allow it to survive and even thrive in this environment (Figure 1).

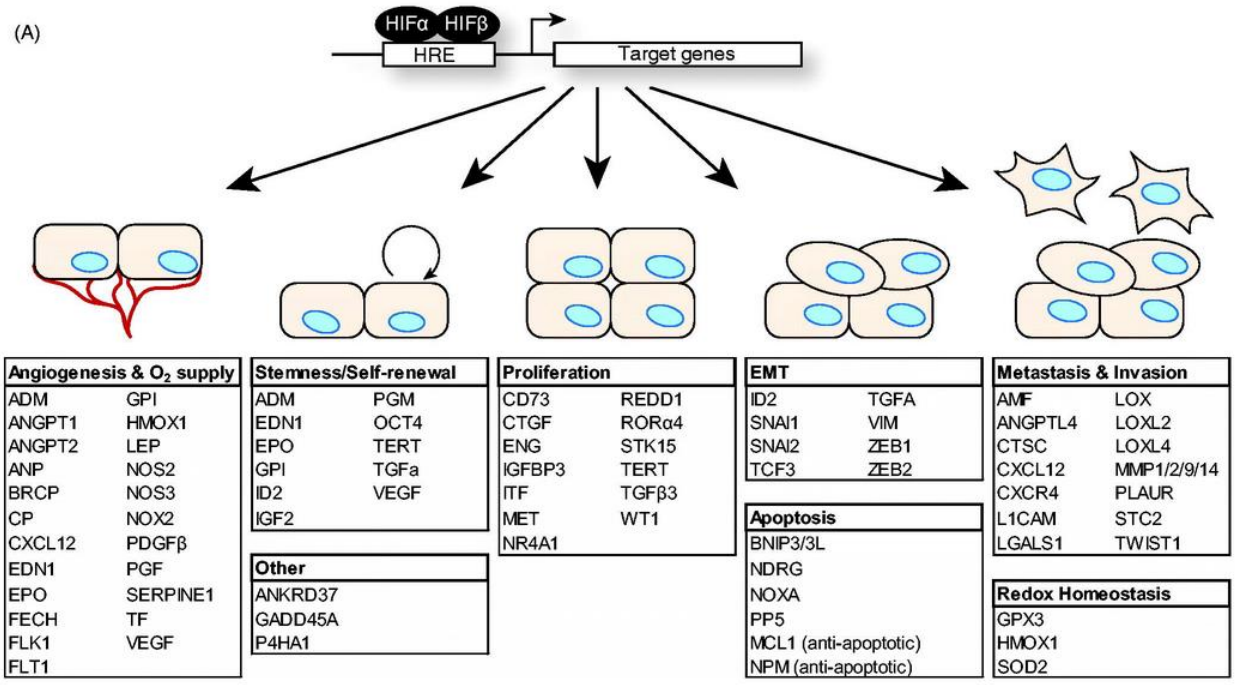


**Figure 1: There are many HIF target genes that promote the hallmarks of cancer.** HIF-1α and HIF-1β target genes for angiogenesis, self-renewal, proliferation, epithelial to mesenchymal transition (EMT), apoptosis, metastasis and invasion, and redox homeostasis (figure from Dengler et al., 2013).

There is a family of transcription factors that respond to hypoxia commonly named Hypoxia Inducible Factors (HIFs), which includes HIF-1α, 2α, 3α and β subunits. HIF-1β is a constitutively expressed nuclear protein (Greer et al., 2012). HIF-1α and HIF-2α stability is dependent on the concentration of oxygen (Ivan et al., 2001; Semenza 2007). Under normal oxygen HIF-1α is hydroxylated on proline residues in the oxygen dependent degradation domain

and the asparagine C-terminal domain by prolyl hydroxylase domain proteins (PHDs) and Factor Inhibiting HIF-1α (FIH). PHDs use oxygen, oxo-ketoglutarate, and iron as co-substrates. Hydroxylation prevents binding of HIF-1α co-activators and allows von Hippel Lindau (VHL) to bind, leading to polyubiquination of HIF-1α which targets the protein for proteasome degradation (Ivan et al., 2011). In levels of low oxygen the PHDs are inhibited allowing HIF-1α to translocate to the nucleus, form a heterodimer with HIF-1β, bind to co-activators bind to target DNA sequences, and recruit RNA Polymerase II (RNAPII) for the transcription of hypoxia inducible genes (Kenneth & Rocha, 2008) (Figure 2).

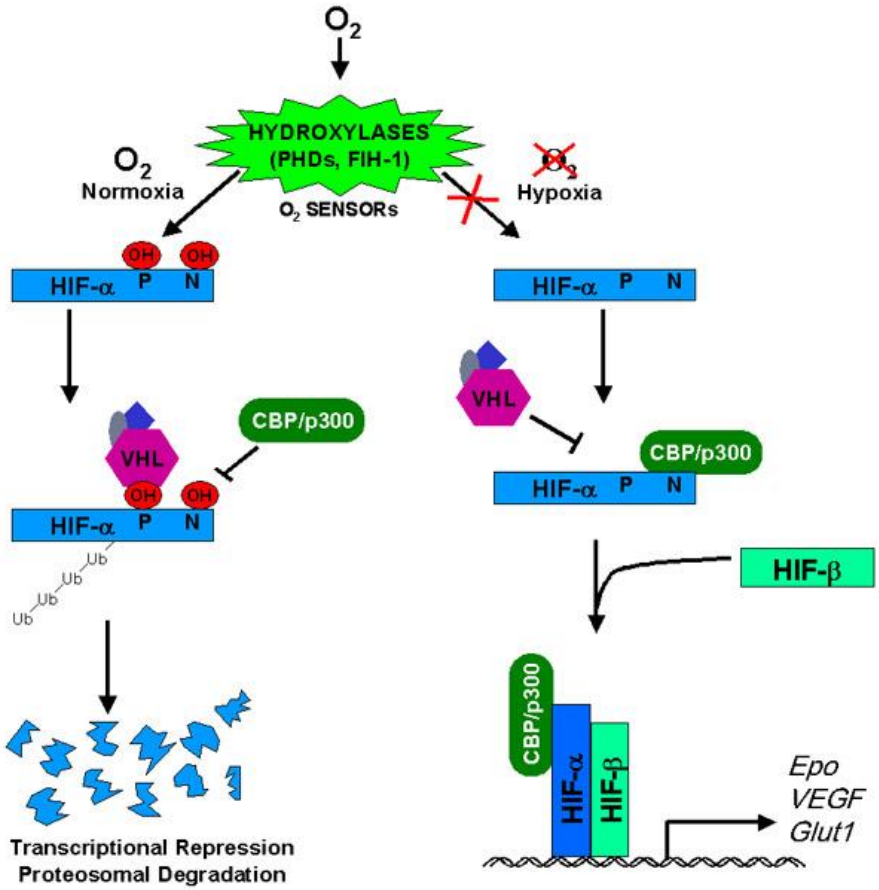


**Figure 2: Schematic diagram of the mechanism of HIF-1α in normoxic and hypoxic conditions.** In normoxic conditions HIF-1α is hydroxylated and then polyubiquinated by VHL for proteosomal degradation. In hypoxic conditions HIF-1α is not hydroxylated and VHL is unable to bind allowing HIF-1α to translocate to the nucleus form a heterodimer with HIF-1β and bind its co-activators (Antony et al. 2002).

**Co-activators**

In order for HIF-1α to activate transcription it translocates to the nucleus, forms a heterodimer with HIF-1β, and binds target genes and necessary co-factors. Recently the key co-factors were thought to be primarily p300 and CBP. HIF-1α binds p300 and CBP through the C-TAD domain to increase transcriptional activity of HIFs at the target genes (Arany, et al., 1996). However, recent studies have indicated that p300 and CBP co-activators enhance transcription rather than driving it, and are essential for only a few target genes (Kasper et al., 2005). A mutation in the binding region of HIF with p300 and CBP only impairs a limited amount of target genes suggesting the existence of other HIF co-activators. Other co-activators that have been linked to HIF-1α transcription include Nuclear Receptor Co-activator 1 (NCOA1), NCOA2, NCOA3 (Carrero et al., 200) and Pyruvate Kinase M2 (PKM2) (Luo et al., 2011).

**CDK8-Mediator: a major regulator and co-activator of HIF-1α target genes.**

CDK8 is a component of the Mediator complex. It comprises the CDK submodule of Mediator along with MED12/MED12L, MED13/ MED13L, and Cyclin C. The kinase member of the CDK module is the only predicted enzymatic subunit of Mediator (Sato et al., 2004). CDK8 was previously thought to be a transcriptional repressor but was found to function as both a repressor by inhibiting phosphorylating RNAPII in the C-terminal domain repeats, and a positive regulator of transcription (Donner et al., 2010) by regulating the assembly of the RNAPII elongation complex. CDK8 has oncogenic characteristics and has been identified as a potent oncogene in colorectal carcinomas (Firestein et al., 2008). CDK8 was recently identified as a key co-activator of many HIF-1α target genes (Galbraith et al., 2013). CDK8 is recruited to the promoter-proximal regions of target genes in response to hypoxia. CDK8 is required for the recruitment of the Super Elongation Complex in response to hypoxia in order to release RNAPII

pausing, a majority of hypoxia-inducible genes have RNAPII at the promoters and are paused prior to activation (Galbraith et al., 2013). Therefore, in hypoxia HIF-1α binds HIF-1β and recruits histone acetyl-transferases and CDK8-Mediator along with the Super Elongation complex (Figure 3). The CDK8-Mediator co-activation step leads to the release of the paused RNAPII (Galbraith et al., 2013).

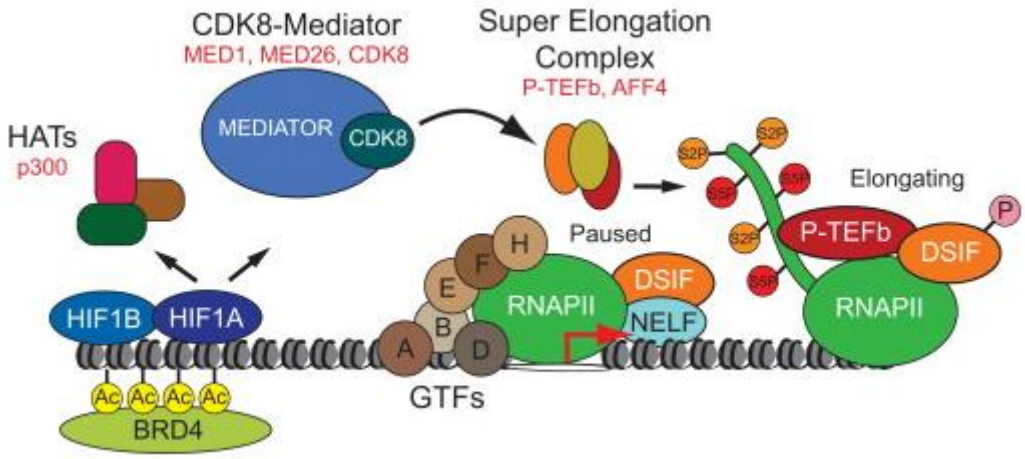


**Figure 3: A Revised Model of HIF-1α Transactivation.** During normoxia RNAPII is paused at many hypoxia genes. During hypoxia HIF-1α recruits histone acetyl transferases, CDK8- Mediator and Super Elongation Complex. In absence of CDK8, HIF-1α cannot stimulate transcriptional elongation (figure from Galbratih et al., 2013).

## The Mediator Complex

First identified in yeast the Mediator is a physical bridge between activators that can directly stimulate transcription and elongation through recruitment and activation of RNAP II and other general transcription factors (Wang et al., 2005). Conserved from yeast to humans, the mammalian Mediator complex is composed of 25-30 subunits. The multi-subunit complex promotes the activation of transcription and the RNAPII initiation complex through direct interactions with DNA binding transcription factors (Conaway & Conaway 2012). The Mediator complex is divided into four modules; the head, the middle, the tail, and the CDK module. The

CDK Module contains CDK8/19, MED12/MED12L, MED13/MED13L and Cyclin C. In vertebrates the CDK module is more complex, three of the four proteins have paralogs due to gene duplications.

**Paralog Pairs**

CDK8 and its paralog CDK19 are not functionally redundant and may have different effects on the Mediator complex. For example, CDK8 and not CDK19 was identified as an oncogene in colon cancer (Firestein et al. 2008). It is also unknown if there are differential effects of MED12/MED12L and MED13/MED13L on Mediator function. The interactions of these paralogs have been shown to be mutually exclusive with each other but not with other CDK-module subunits (Figure 4) (Daniels et al., 2013).

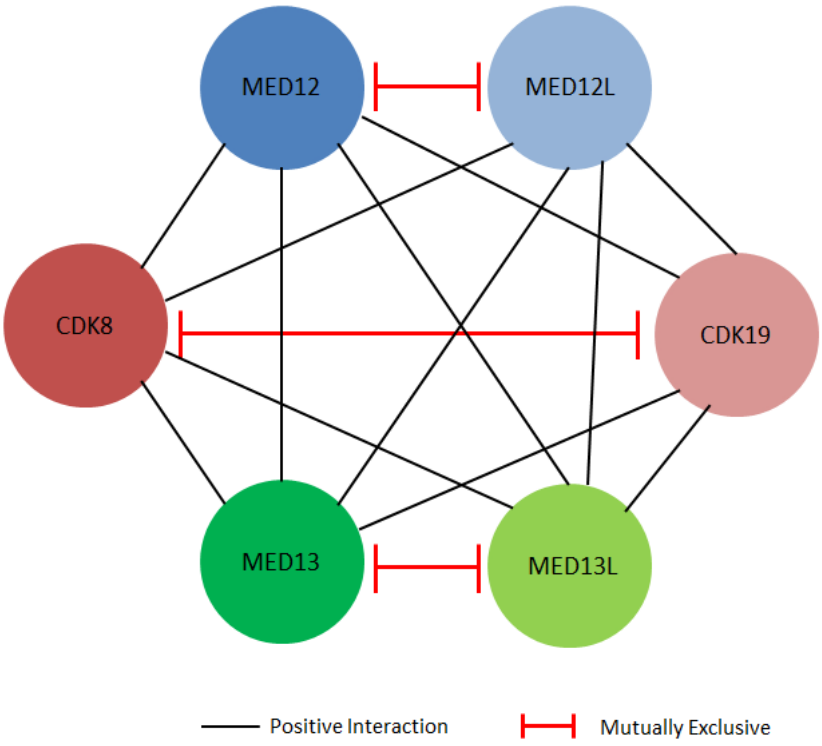


**Figure 4: Module of interactions within the CDK-Mediator.** Schematic drawing showing that paralogs are mutually exclusive with each other, but not with other subunits in the Mediator complex (Daniels et al., 2013).

**MED13/MED13L**

MED13 is the subunit that connects the CDK module with the core Mediator complex. MED13L probably has a similar role however, this has not been confirmed (Malik et al., 2005). Therefore, MED13/MED13L is critical in the structure and interaction of CDK-Mediator (Knuesel et al., 2009; Tsai et al., 2013). Depending on the mobility of MED13 the CDK module can adopt several conformations resulting in structural flexibility however, its paralog MED13L was not included in the study (Wang et al., 2013). It is likely that the paralogs MED13 and MED13L have different downstream transcription effects because MED26, critical in the interactions with transcriptional machinery complexes, co-purifies with MED13L but not MED13 suggesting they are not functionally redundant (Daniels et al., 2013). During hypoxia, along with CDK8, MED26 is recruited to HIF-1α target genes (Galbraith et al., 2013). This suggests that they both allow CDK module to bind core Mediator. More evidence that suggests MED13/MED13L are non-redundant is both MED13 and MED13L mutations contribute to different severe defects in humans. MED13L expression is higher in the aorta, and MED13L mutations contribute to congenital heart defects (Asadollahi et al., 2013; Muncke et al., 2003). This supports the conclusion that MED13L has a role in cardiovascular development (Grueter, et al., 2013). A mutation in MED13 has an effect on a number of organs including the heart and the brain. MED13 mutations contribute to the cyanotic congenital heart disease (Boutry-Kryza et al., 2012). Haploinsufficiency in MED13 results in developmental aberrations in the brain such as cognitive defects (Boutry-Kryza et al., 2012). This suggests that MED13 and MED13L are functionally different, and have different expression in selective tissues.

## PRELIMINARY STUDIES

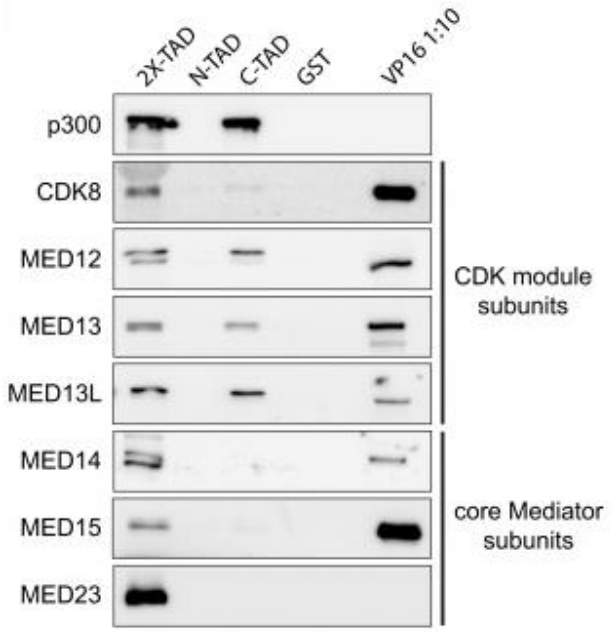


**Figure 5: Western Blot analysis of proteins purified by their affinity for GST HIF-1α transactivation domains.** The transactivation domain of HIF-1α physically associates with CDK8- Mediator (figure from Galbraith et al., 2013).

### HIF-1α exhibits strong interaction with CDK8-Mediator

A HIF-1α TAD interaction was performed finding the HIF-1α 2X-TAD region interacts with CDK-Mediator, and the widespread co-activators p300. The other Mediator subunits that the 2X-TAD bait efficiently pulled down included MED12, MED13, and MED13L. This interaction is preserved in the C-TAD and not the N-TAD domain of HIF-1α. This illustrates that the transactivation domain of HIF-1α associates with CDK-Mediator (Figure 5) (Galbraith et al., 2013).

## RESEARCH AIMS

The preliminary studies have provided a physical basis for recruitment of CDK-Mediator to the hypoxia inducible target gene loci. For the CDK module to fully assemble and associate with core Mediator, MED13 or likely MED13L is required. My hypothesis is MED13 and/or MED13L is required for the induction of HIF-1α target genes under hypoxic conditions.

**Aim1: Investigate the requirement for MED13 for the induction of HIF-1α target genes by hypoxia.**

I will generate two cell lines containing different stably expressing shRNAs against MED13. The level of knockdown will be validated by western blot or alternatively by QRT-PCR. I will use QRT-PCR to determine expression levels of selected HIF-1α target genes in the knockdown cell lines for normoxic and hypoxic conditions. The genes investigated are known HIF-1α genes that have a high induction upon hypoxia exposure (Galbraith et al., 2013).

**Aim2: Investigate the requirement for MED13L for the induction of HIF-1α target genes by hypoxia.**

I will generate two cell lines containing stably expressing different shRNAs against MED13L. The level of knockdown will be validated by western blot or alternatively by QRT-PCR. I will use QRT-PCR to determine expression levels of selected HIF-1α target genes in the knockdown cell lines for normoxic and hypoxic conditions. The target genes that will be investigated are known key target genes.

## METHODS

### Cell Culture

Cells were grown in McCoy's 5A Medium (Sigma-Aldrich) supplemented with 10% FBS (Sigma-Aldrich) and 1% Antibiotic Antimycotic solution (Sigma-Aldrich) at 37˚C/ 5% CO2.

### shRNA mediated knockdown

Commercially available shRNAs precloned into the pLKO.1-Puro vector were purchased from the Functional Genomics Facility. A non-targeting shRNA cell line was generated in the HCT116 cell line as a control (shc). Lentiviral particles were produced in HEK293FT packaging cells. HCT116 cells were transduced with .45µm-filtered viral supernatants. Selection of stably transduced cells was carried out for 2-7 days with 10.0 µg/mL Puromycin.

| Gene | TRC Number | Sequence |
|---|---|---|
| MED13 | 0000234901 | CCGGACAAGATCAGTGCACTAATTTCTCGAGAAATTAGTGCACTGATCTTGTTTTTTG |
| MED13 | 0000234903 | CCGGTCATGAGGAAGTACCTAATATCTCGAGATATTAGGTACTTCCTCATGATTTTTG |
| MED13L | 0000233505 | CCGGCATCACCTAGCACCTTATTTACTCGAGTAAATAAGGTGCTAGGTGATGTTTTTG |
| MED13L | 0000233506 | CCGGATATAGTCACAACGGAAATATCTCGAGATATTTCCGTTGTGACTATATTTTTTG |

### Hypoxia Treatment

All cells were plated at a density of $2.5x10^5$ cells/mL on $10cm^2$ plates 24 hours prior to exposure to normoxic/hypoxic conditions. For normoxic conditions cells were kept in a humidified incubator with 5% $CO_2$ at 37˚C. For hypoxic conditions cells were transferred to a hypoxia chamber and exposed to two flushes of 200mL of a mixture of 1% $O_2$, 5% $CO_2$, 94% $N_2$ separated by a one hour incubation period.

### RNA isolation and QRT-PCR Analysis

Cells were washed with PBS and harvested by scraping and centrifugation. Total RNA was extracted using Trizol Reagent (Sigma Aldrich) following the manufacturer's instructions. Reverse Transcription reactions were performed with a qScript cDNA synthesis kit (Quanta

Biosciences). Primers were designed against sequences of the indicated mRNAs using Primer BLAST (NCBI, http://www.ncbi.nlm.nih.gov/tools/primer-blast). PCR was carried out containing 1× SYBR Select Green Mix (Applied Biosystems), 0.1 ng of cDNA, and 500 nM primers. Standard curves from 0.001-10 ng of cDNA pooled were used. Values were normalized to those of 18s rRNA. Equal amounts of total cDNAs were used in PCR reactions alongside standard curves of total cDNA to determine fold enrichment. All QRT-PCR analysis was performed using the Applied Biosystems ViiA7 QRT-PCR machine (Life Technologies). Primers used for QRT-PCR were as follows (5'to 3'):

18S-fwd, GCCGCTAGAGGTGAAATTCTTG; 18S-rev, CTTTCGCTCTGGTCCGTCTT; ALDOC-fwd, TCATCAAGCGGGCTGAGGTGAA; ALDOC-rev, TGTGCTGCTGCTCCACCATCTT; ANKRD37-fwd, TGTGTTGCCGTGCTCAGACAGA; ANKRD37-rev, ACCCACGTGACATCAGCACTTC; P4HA1-fwd, AGCAAAACCAAGGCTGAGCCGA; P4HA1-rev, TTCATAGCCAGAGAGCCAGGCA; STC2-fwd, ACTTGCTGCTGCACGAACCCTA; STC2-rev TCACACTGAACCTGCACGCTGT.

**Protein Isolation and Immunoblot Analysis**

Cells were washed with PBS and harvested by scraping and centrifugation. Protein was extracted with RIPA lysis buffer solution. Samples were sonicated for 15s and spun down to remove any insoluble materials. Protein in the supernatant was quantified by a BCA assay (Pierce), and 30ug protein was loaded on an 8% SDS-PAGE gel. The blots were transferred to a .45µm PVDF membrane. Primary antibodies: MED13 diluted at 1:500 (Bethyl A301-278A), MED13L diluted at 1:1000 (Bethyl A302-421A), Tubulin diluted at 1:20000(Santa Cruz sc-23950), CDK8 diluted at 1:10000 (Santa Cruz sc −1521), and HIF-1α diluted at 1:1000 (BD

Transduction 610959); and developed with peroxidase-conjugated secondary antibodies (Santa Cruz and ECL detection agents using an ImageQuant LAS4000 digital camera system.

**Statistical Methods**

All statistical analysis for quantitative data was done in Excel. Standard error of the mean was used to calculate the error between biological replicates for each experiment. The formula used was the standard deviation of all included normalized samples divided by the square root of the number of the included samples with n=3. The Students T-test was used for statistical testing and all significant values with a $p < .05$ are marked with an asterisk (*).

## RESULTS

### Aim1: Investigate the requirement for MED13 for the induction of HIF-1α target genes by hypoxia.

In order to identify the requirement for MED13, I established stable knockdown cell lines for MED13 in HCT116 colorectal cancer cells. Two cell lines expressing different shRNAs against MED13 were used to rule out any off target effects. To validate the shRNA only effected the expression of the targeted MED13 and not its paralog MED13L a Western Blot analysis was performed (Figure 6).

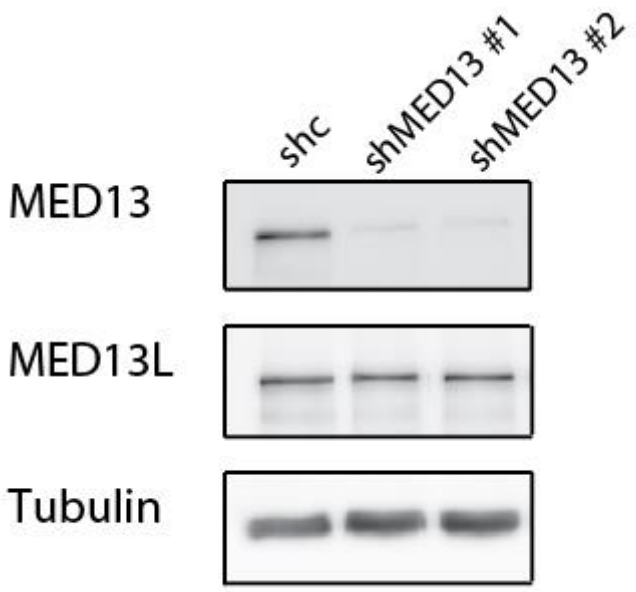


**Figure 6:** Western Blot validation of MED13 knockdown in HCT116. The knockdown of MED13 does not impair MED13L protein levels (n=3). Tubulin was used as a loading control.

Upon exposure to hypoxia HIF-1α had a very strong induction at the protein level. Interestingly the levels of CDK8 varied between the knockdown cell lines in multiple western blots (n=3). CDK8 is at a lower level in the cells of the second shRNA which also has a better knockdown of MED13 (Figure 7). This interference with CDK8 is not entirely unexpected because CDK8 and MED13 are very closely associated and may be required for the stability of CDK8.

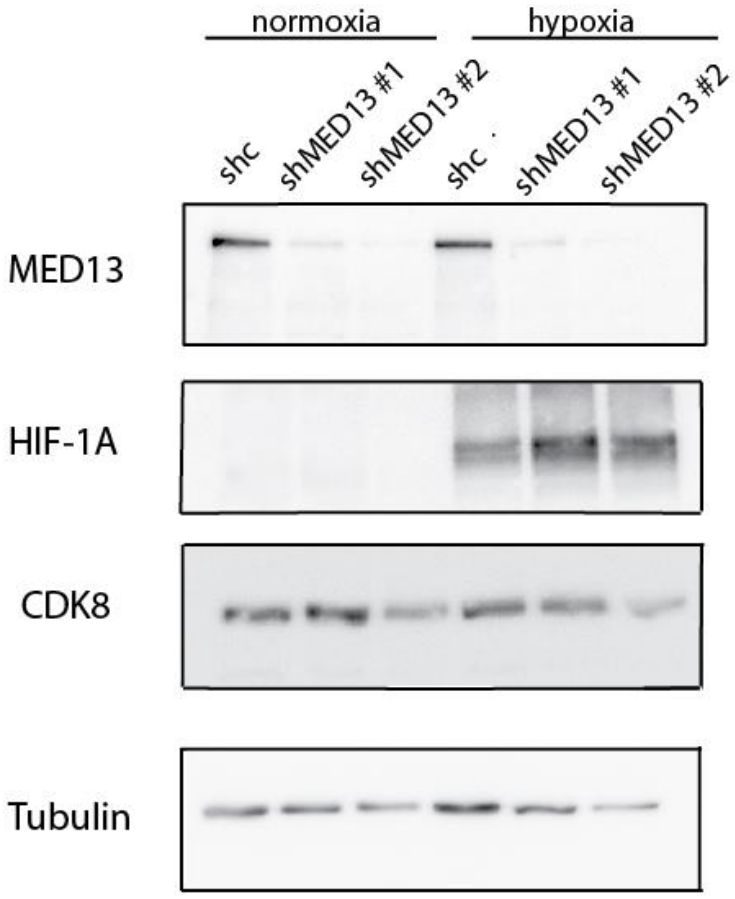


**Figure 7:** Western blot analysis indicating the activation of HIF-1α after hypoxia treatment and MED13 knockdown (n=3).

Following the generation of the cell lines with the stable shRNA against MED13, I examined the effects of MED13 knockdown on the induction select genes; ANKRD37, ALDOC, STC2, and P4HA1. These genes are known HIF-1α genes that have a strong induction upon hypoxia exposure (Galbraith et al., 2013). Based on the preliminary findings, and MED13/MED13L association with CDK8, it was hypothesized that MED13 knockdown might inhibit the induction of these key genes. Interestingly, some genes exhibited a modest effect and others had a much stronger effect (Figure 8). ANKRD37 mRNA expression was down-regulated in both shMED13 cell lines (Figure 8-A). The second shRNA targeting hairpin had a stronger

effect, which may be due to the quality of the knockdown. This suggests that MED13 has a regulatory effect on ANKRD37 expression. The MED13 knockdown cell lines had a modest decrease in ALDOC mRNA levels indicating that MED13 has some effect, but lack of MED13 does not fully impair the expression of ALDOC (Figure 8-B). STC2 mRNA levels had about a 4-fold decrease upon MED13 knockdown (Figure 8-C). P4HA1 had a slight decrease in mRNA expression indicating a weak requirement of MED13 (Figure 8-D). There was no variation in the mRNA levels of the expression of the target genes in the normoxic conditions. In summary, MED13 knockdown caused a decreased in mRNA expression for some genes gives suggesting a gene specific dependence for MED13.

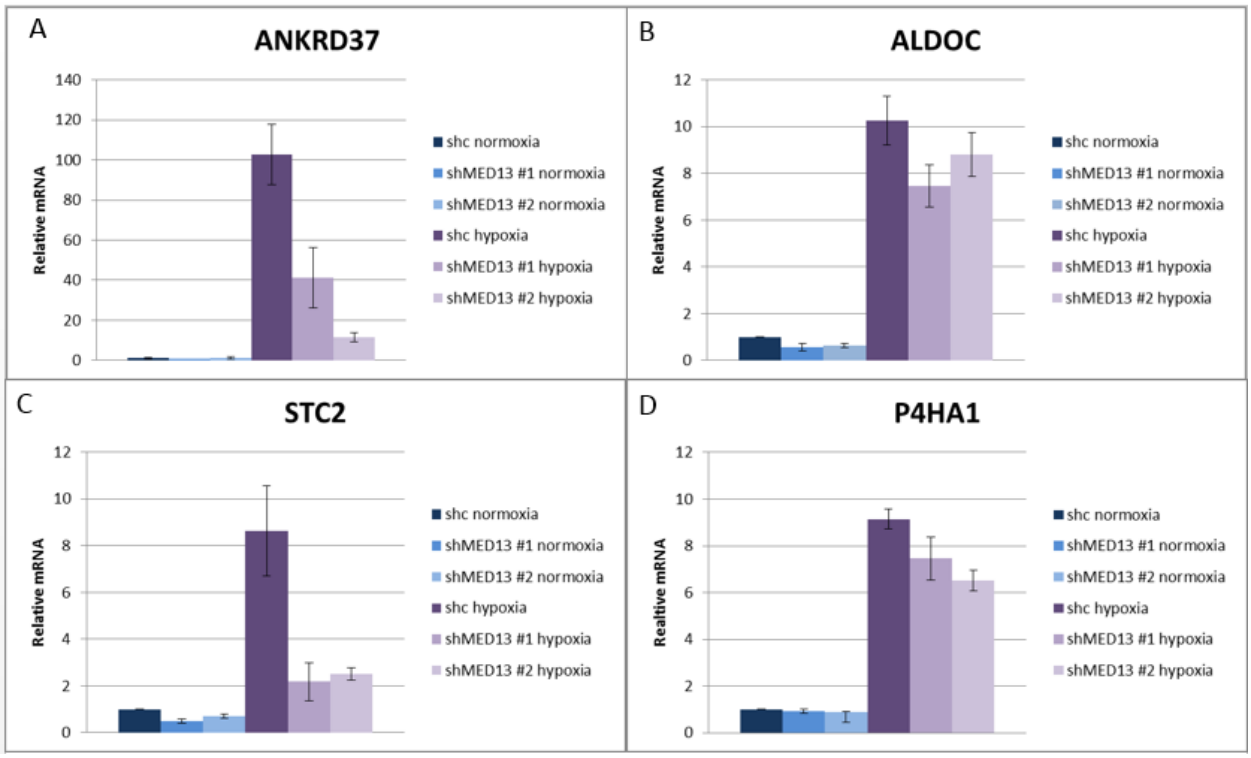


**Figure 8:** QRT-PCR analysis shows that the knockdown MED13 affects expression of (A) ANKRD37* and (C) STC2. The knockdown of MED13 has a modest effect on (B) ALDOC and (D) P4HA1 (n=3)

**Aim2: Investigate the requirement for MED13L for the induction of HIF-1α target genes by hypoxia.**

To test the requirement for MED13L cell lines expressing stable shRNA for MED13L were generated. Once again two cell lines with two different MED13L hairpins were generated (Figure 9). The levels of MED13 were unchanged in the MED13L knockdown cells as determined by Western Blot analysis (Figure 9).

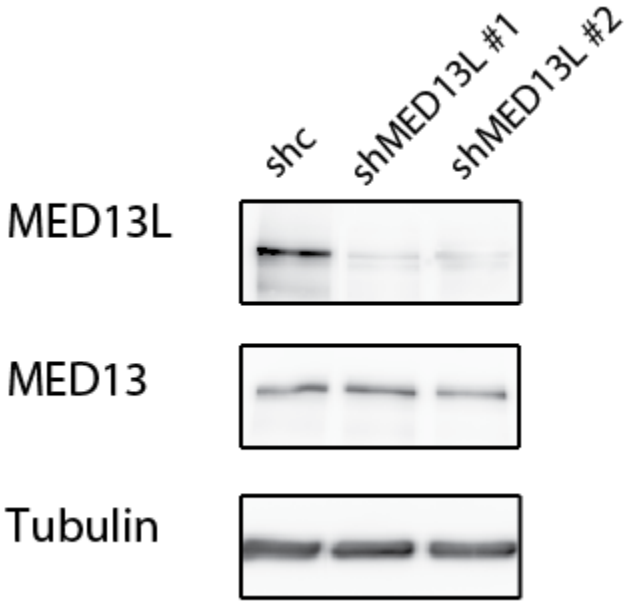


**Figure 9:** Western Blot analysis of HCT116 verifying the expression of shRNA against MED13L. The knockdown of MED13L does not impair MED13 protein levels. Tubulin was used as a loading control (n=3).

Upon hypoxia exposure HIF-1α was induced to the same extent in the shMED13L as in the non-targeting control. The levels of CDK8 did not vary between the knockdown cell lines unlike the cell lines containing the shRNA for MED13 (Figure 10).

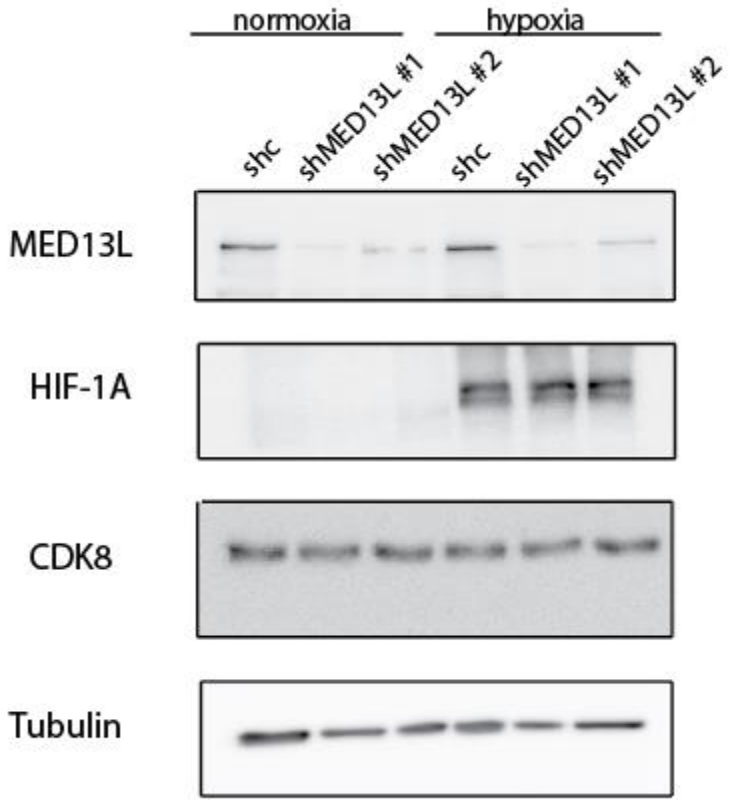


**Figure 10:** Western blot analysis indicating the activation of HIF-1α after hypoxia treatment and MED13L knockdown. Tubulin was used as a loading control (n=3).

Following the generation of the cell lines with the stable shRNA against MED13L, the effects of MED13L knockdown was examined on the same select genes tested for shMED13; ANKRD37, ALDOC, STC2, and P4HA1. Similar to MED13, some genes showed a modest reduction in expression while others were much more affected. ANKRD37 mRNA expression was down-regulated by an approximate two-fold in both shMED13L cell lines (Figure 11-A). shMED13L had a modest decrease in ALDOC mRNA levels indicating that MED13L has some effect but the MED13L knockdown does not fully impair the expression (Figure 11-B). The first hairpin has a stronger effect on the induction of ALDOC which correlates to the better knockdown. STC2 mRNA levels had strong decrease upon MED13L knockdown (Figure 11-C).

P4HA1 had very minor decrease in mRNA expression (Figure 11-D). There was scarcely any variation in the mRNA levels of the expression of the target genes in the normoxic conditions. In summary, MED13L knockdown appears to be gene dependent similar to the effect of shMED13.

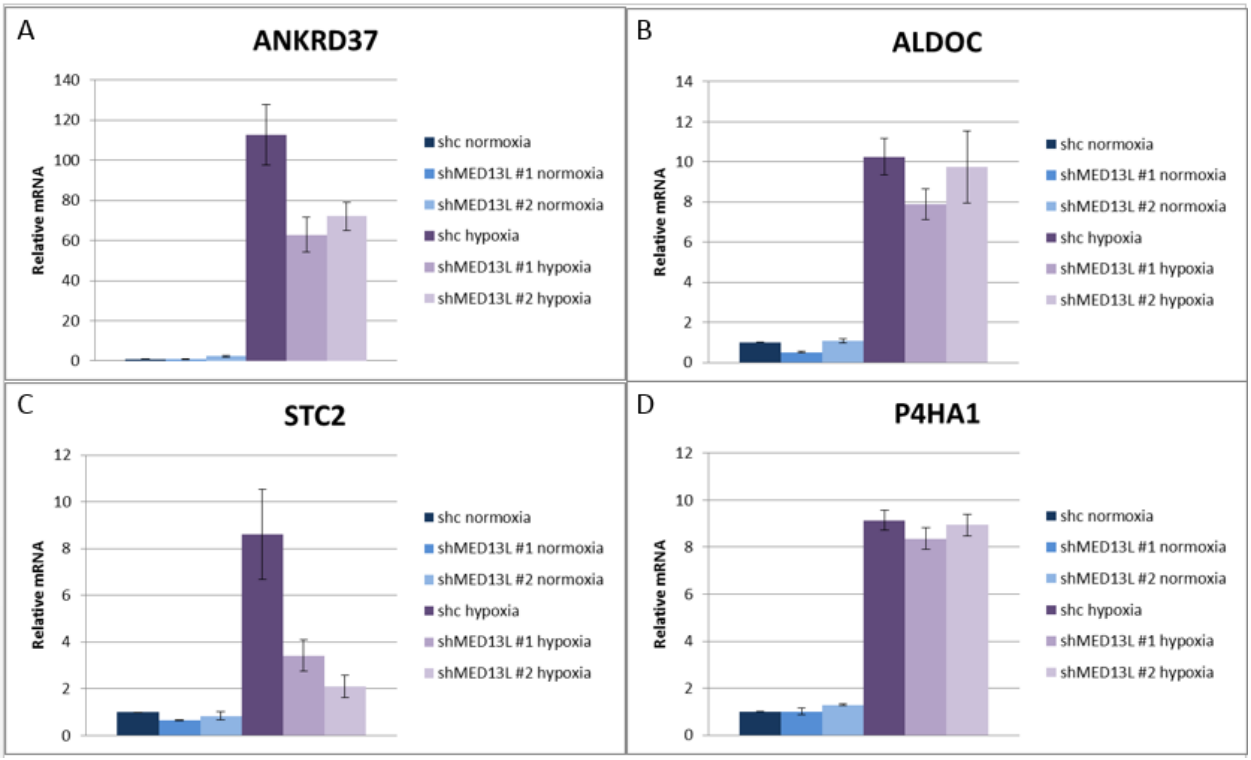


**Figure 11:** QRT-PCR analysis shows that the knockdown MED13L affects expression of (A) ANKRD37 and (C) STC2. The knockdown of MED13L has little/no affect on (B) ALDOC and (D) P4HA1 (n=3)**.**

## DISCUSSION

The response to hypoxia has some beneficial purposes such as wound healing, and development; however, cancer cells exploit this mechanism to survive and thrive in a low oxygen environment (Harris, 2002; Licht, et al., 2006; Zhong et al., 1999). This hypoxic stress induced the activation of hypoxia-inducible factors. In cancer the response to hypoxia can induce several genes that allow the tumor cells to perform angiogenesis, proliferation, evasion of apoptosis, and many others (Dengler et al., 2013). HIF-1α and HIF-1β are the main transcription

factors in the hypoxia response. CDK8, the enzymatic subunit of the Mediator complex, is a key co-activator of HIF-1α required for the induction of many HIF-1α target genes (Galbraith et al., 2013). The Mediator complex is a multi-subunit complex that plays a critical role in gene regulation (Conaway et al., 2012; Sato et al., 2004). Three of the four proteins in the CDK module have undergone gene duplication and have paralog pairs; CDK8/CDK19, MED12/MED12L, and MED13/MED13L (Sato et al., 2004). CDK8, but not its paralog CDK19 activates the transcription of HIF-1α target genes (Galbraith et al., 2013). MED13 and likely MED13L is required for the association of the CDK module to the Mediator core. This association is likely to be important for CDK8 to induce HIF-1α target genes through the Mediator complex.

Using a loss-of-function approach, I show that the MED13 and MED13L subunits of the Mediator complex are required in a gene-dependent manner in the induction of HIF-1α target genes. Both knockdowns of MED13/MED13L impaired the induction of ANKRD37. There seems to be a preference towards MED13, but this preference could be due to the level of knockdown. However, unlike CDK8 MED13/MED13L the impairment in the gene induction is not as strong as the knockdown for CDK8 (Galbraith et al., 2013). ANKRD37 still has a 10 fold increase at the lowest level of mRNA induction. Also in STC2 induction there was a decrease in mRNA expression in the knockdown cell lines for both MED13 and MED13L. In both MED13 and MED13L knockdowns there is a modest decrease in the induction of ALDOC. Perhaps the induction of ALDOC has no requirement to either MED13 or MED13L. Similar to P4HA1 there was a modest decrease in the levels of expression, this decrease was more pronounced in the MED13 knockdown cell line however, this could be due to the knockdown level. It is not entirely unexpected that MED13 and MED13L have similar effects on HIF-1α target genes

because in the preliminary data both of the subunits were pulled down with the transactivation domain of HIF-1α (Figure 5). These results suggest that the requirement for MED13/MED13L is gene dependent in the induction of HIF-1α target genes.

Still little is known about the Mediator paralogs and their requirement for the transcription of genes. To further investigate the gene dependence of MED13/MED13L, other HIF-1α target genes will be tested by QRT-PCR. Also an RNA-sequencing analysis can be performed to determine the widespread gene dependency of MED13/MED13L on HIF-1α target genes. Further examination into the recruitment of MED13/MED13L is necessary by quantitative ChIP analysis. It is expected that MED13/MED13L effects the CDK8 recruitment, but I hypothesize not for HIF-1α recruitment. The other paralog MED12/MED12L can undergo similar experiments to determine its role in the induction of HIF-1α target genes. Similar to this experiment, knockdowns of MED12/MED12L could be generated to examine the effect on HIF-1α target genes. The interaction of CDK-Mediator and HIF-1α is key to the regulation of hypoxia inducible genes and is vital to the understanding of solid tumors as well as other diseases. Further studies about the transcription of the HIF-1α will aid in the basis for developing therapeutic techniques for these diseases.

**ACKNOWLEDGEMENTS**

Thank you to Dr. Joaquín Espinosa for the wonderful opportunity to work in his laboratory and his support throughout my research. His passion and devotion to search for the cure for cancer is very noble and has inspired me to be a better scientist. As I worked directly with Dr. Matthew Galbraith this project would not have been possible without him. I appreciate his invaluable project guidance, assistance with experimental design. Thank you to Audrey Audetat for her enthusiastic support, always making me laugh, and making time to help me even though she had her own project. Her knowledge and guidance helped me achieve my goal. Thank you to Dr. Hestia Mellert for moral support and project suggestions; and Veronica Dengler for helping me with my scientific techniques.

Thank you to the entire Espinosa lab for their endless support throughout my research and sharing their knowledge. They are a lab of intelligent, devoted, and supportive scientists that have shown me how to learn and grow as a research scientist.

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