Colorectal cancer (CRC) is the third-leading cause of cancer mortality in the United States and other industrialized countries. A hypoxic microenvironment is a hallmark for solid tumors. The hypoxia-induced signal transduction is transcriptionally mediated by hypoxia-inducible factor (HIF). Three major HIF isoforms, HIF-1α, HIF-2α, and HIF-3α, are present in the intestine. Our previous work demonstrates that HIF-2α is essential for CRC growth and progression. However, the mechanisms mediating cell proliferation after hypoxia or HIF-2α activation in CRC are unclear. Data mining of RNA-Seq experiments with mouse models of intestinal HIF-2α or YAP1-overexpressed protein 1 (YAP1) overexpression indicates a significant overlap of genes in these conditions. YAP1 is a transcriptional co-activator in the Hippo signaling pathway, and YAP1-induced transcriptional responses are essential in cancer cell proliferation. Here, we report that HIF-2α robustly increases YAP1 expression and activity in CRC-derived cell lines and in mouse models. The potentiation of YAP1 activity by HIF-2α was not via canonical signaling mechanisms such as Src (non-receptor tyrosine kinase), PI3K, ERK, or MAPK pathways. Moreover, we detected no direct interaction of HIF-2α with YAP1. Of note, YAP1 activation was critical for cancer cell growth under hypoxia. Our findings indicate that HIF-2α increases cancer cell growth by up-regulating YAP1 activity, suggesting that this pathway might be targeted in potential anti-cancer approaches for treating CRC patients.

In response to hypoxia, tumor cells activate genes that are critical in angiogenesis, cell survival, cell proliferation, and glucose metabolism (3). Hypoxia-induced responses are transcriptionally mediated by hypoxia-inducible factor-1α (HIF-1α), HIF-2α, and HIF-3α, which are members of the Per-ARNT-Sim family of basic helix-loop-helix transcription factors that bind hypoxia response elements at target gene loci under hypoxic conditions (4, 5). Among the HIFs, HIF-2α is essential in CRC growth and progression. Our recent work has demonstrated an essential role of HIF-2α-induced epithelial-elicited inflammation and regulation of intratumoral iron homeostasis as important mechanisms leading to increase in colon cancer (6, 7). In cancer-derived cell lines and in renal carcinomas, HIF-2α induces proliferation via an increase in c-Myc activity (8, 9). However, in CRC, mechanisms of hypoxic and HIF-2α potentiation of cancer cell growth are unclear.

Hippo signaling is a central pathway that regulates intestinal growth. Yes-associated protein 1 (YAP1) is a downstream effector in the Hippo signaling pathway, which functions as an essential regulator of proliferation, organ size, and cell differentiation (10, 11). In response to a decrease in Hippo signaling, YAP1 translocates into the nucleus and acts as transcriptional co-activator to the transcriptional enhancer factors (TEA) domain (TEAD) family of transcription factors (TEAD1–4) (12, 13). YAP1 is dispensable for normal intestinal homeostasis but is absolutely required for intestinal regeneration after tissue injury (14). Recent work has demonstrated that the growth-promoting role of YAP1 is critical for the proliferative response observed during intestinal inflammation as well as in sporadic CRC (15, 16). Interestingly, intestine-specific YAP1-overexpressing mice restrict β-catenin activation, and under radiation-induced injury, deletion of intestinal YAP1 leads to hyper-proliferation (17). This discrepancy is perhaps due to the complex interaction of YAP1 with APC/β-catenin signaling in intestinal epithelial cells (18–22). However, several studies have demonstrated an important role of YAP1 as an essential transcription factor in mouse models of colon cancer (14, 15, 23). The role of HIF-2α in YAP1 signaling pathway has not been studied.

In the current study we found a novel role of hypoxia via HIF-2α in activating YAP1. Moreover, the increase in YAP1 by HIF-2α is essential for cell growth during hypoxic stress in colon-derived cell lines. The study provides a novel HIF-2α/
YAP1-signaling pathway that maybe targeted in cancer treatment.

Results

**HIF-2α increases YAP1 transcriptional activity**

To investigate the mechanisms of HIF-2α-induced cell proliferation, RNA-sequencing data were analyzed in a mouse model of HIF-2α overexpression specifically in intestinal epithelial cells (HIF2αLSL/LSL;VillinCre) (24). Interestingly, the data showed an increased expression of several well-characterized YAP1 target genes: Cyr61, Ctgf, Tgfbr2, and Tead1. To assess the global regulation of YAP1 target genes by HIF-2α, data were compared from the HIF2αLSL/LSL;VillinCre mice and global gene expression profiles from mice, which have a constitutive activated YAP1 in intestinal epithelial cells (17). 484 genes (279 down-regulated and 205 up-regulated genes) were commonly regulated in YAP1- and HIF-2α-overexpressing mice (Fig. 1, A and B). This suggests a potential cross-talk between HIF-2α and YAP1 signaling. These commonly regulated genes by HIF-2α and YAP1 were involved in not only Hippo signaling pathway but also several other pathways including MAPK, PI3K-AKT, and TGF-β signaling pathways (Fig. 1, A and B). To confirm these changes Western blot analyses and quantitative PCR (qPCR) were performed in HIF2αLSL/LSL;VillinCre mice for YAP1 signaling and target genes. In the HIF2αLSL/LSL;VillinCre mice a significant increase in total-YAP1 protein levels and YAP1 target genes, Cyr61, Ctgf, and Tgfbr2 were observed, whereas HIF-1α overexpression decreased YAP1 target genes Tgfbr2 and Tead1 (Fig. 2A). This indicates that HIF-2α, but not HIF-1α, increases YAP1 activity. To demonstrate this was a cell autonomous function of HIF-2α, an oxygen-stable HIF-2α was stably overexpressed in HCT116 colon cancer cell line (Fig. 2B). Consistent with in vivo data, the overexpression of HIF-2α in HCT116 significantly increased YAP1 target genes Cyr61 and Ctgf expression (Fig. 2C). Western blotting and immunofluorescent staining showed that HIF-2α overexpression significantly increased both total YAP1 (T-YAP1) and phospho-YAP1 (p-YAP1) protein levels and nuclear YAP1 localization without altering the p-YAP/T-YAP1 ratio (Fig. 1, E and F). These data confirm that HIF-2α increases the expression of YAP1 target genes.

**HIF-2α potentiates YAP1 activity**

The Cyr61 promoter luciferase (25) and a synthetic promoter consisting of 8 × tandem TEAD-binding sites (GTIIC) upstream of a luciferase reporter (26) were assessed for YAP1 activity. Consistent with a previous study, YAP1 significantly increased Cyr61 and GTIIC promoter activities in all of the cell lines assessed (Fig. 3, A and B). Interestingly, HIF-2α increased basal Cyr61 activity in SW480 and HEK293 cell lines (Fig. 3A) and potentiated the YAP1 transcriptional activity on the Cyr61
and GTIIC promoter in the cell lines assessed (Fig. 3, A and B). These data indicated that HIF-2α potentiates YAP1 activity.

Improved YAP1 activity in sporadic tumors is HIF-2α independent

YAP1 is important for cell proliferation, adenoma formation, and cancer development (27–29). In addition, previous reports suggested that YAP is part of β-catenin signal cascade and is directly activated after the loss of APC (18, 20–22). To further understand if HIF-2α is essential for YAP1 activation after APC disruption, a novel mouse model was generated using a tamoxifen-inducible colon-specific Cre (CDX2ERT2cre) (30). The CDX2ERT2cre mice were crossed to the Apc floxed mice (CDXERT2Cre/ApcF/F) or crossed to the Apc and Hif2α/β floxed mice (CDXERT2Cre/ApcF/F/Hif2α/β). 7-Days after tamoxifen treatment, Hif2α and HIF-2α target gene Scl11a2 were significantly decreased in Hif2α floxed mice (CDXERT2Cre/ApcF/F/Hif2α/β) compared with CDXERT2Cre/ApcF/F cell lines (Fig. 4A). Consistent with previous work (31), Cyr61 and CTGF genes were induced in both CDXERT2Cre/ApcF/F and CDXERT2Cre/ApcF/F/Hif2α/β mice compared with littermate controls ApcF/F mice, but there was no significant change in YAP1 target genes in the CDXERT2Cre/ApcF/F/Hif2α/β mice compared with CDXERT2Cre/ApcF/F cell lines (Fig. 4A). Moreover, the increase of p-YAP1 and T-YAP1 protein after Apc disruption was not altered in mice with a HIF-2α disruption (Fig. 4B). Immunofluorescence staining with Ki67 and H&E staining showed increased cell proliferation in CDXERT2Cre/ApcF/F mice, whereas HIF-2α knock-out did not alter the tissue proliferation (Fig. 4, C and D). These results indicate that HIF-2α is not essential for the increase of YAP1 and its target gene expression in sporadic CRC models.

HIF-2α potentiation of YAP1 activity is not via kinase signaling or protein-protein interaction

Src family kinases can promote proliferation of intestinal epithelial cells through activation of YAP1, which contributes to the proper regulation of intestinal epithelial cell turnover and intestinal homeostasis (32). To assess if HIF-2α potentiates YAP1 through Src, YAP1 reporter assays were performed after treatment with the Src inhibitor, SU6656. Cyr61 promoter luciferase was significantly increased when the cells were transfected with both HIF-2α and YAP1, but this potentiation was not inhibited when treated with SU6656 (Fig. 5A, left panel). The PI3K-AKT

**Figure 2. HIF-2α increased YAP1 target genes.** A, YAP1 protein levels (left panel) and YAP1 target gene expression (right panel) in the colons of HIF2α/βLSL/LSL;VillinCre (HIF2α/βLSL/LSL group: n = 4; HIF2αLSL/LSL;VillinCre group: n = 5) or HIF1α/βLSL/LSL;VillinCre (n = 3 in each group) mice. B, HIF-2α protein levels in control and HCT116 cells that overexpress HIF-2α (HIF-2αOE). GAPDH was used as a loading control. C, Cyr61 and Ctgf gene expression in HCT116 control cell line (n = 3) and HIF-2αOE cell line (n = 3). The gene expression was normalized to β-actin. D, total (T)-YAP1 and phospho (P)-YAP1 protein levels in control or HIF-2αOE HCT116 cell lines. E, immunofluorescent staining for YAP1 in control or HIF-2αOE HCT116 cell lines. *, p < 0.05; **, p < 0.01 compared with HIF2αLSL/LSL, HIF1αLSL/LSL or control. The cell line work was performed in triplicate and repeated at least three times.

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Figure 3. HIF-2α potentiated Cyr61 and GTIIC luciferase activity. Shown are Cyr61 promoter luciferase (A) or GTIIC promoter luciferase (B) activity in HCT116, SW480, and HEK293A. The cells were transfected with EV, YAP1, oxygen stable HIF-2α (HIF-2α-TM), or co-transfected with both YAP1 and HIF-2α-TM for 48 h. *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared with EV; #, p < 0.05; ##, p < 0.01 compared with YAP1. The experiments were performed in triplicate and repeated at least three times.

pathway is essential for cell survival (33). AKT also alters YAP1 activity (34); however, AKT (MK2206) inhibitor did not affect the potentiation of YAP1 activity by HIF-2α (Fig. 5B, left panel). The phosphorylated AKT (p-AKT) inhibition by MK2206 was confirmed using Western blot analysis (Fig. 5B, right panel). Recently we showed a novel cross-talk of HIF-2α with MEK1-ERK1/2 signaling (35). MAPK pathways increase YAP1 activity (36). Inhibition of MEK1 using two different inhibitors (GSK1120212 or PD325901) significantly decreased ERK activity, as the phosphorylated ERK (p-ERK) was inhibited (Fig. 5C, right panel). The inhibition of MEK1 using GSK1120212 significantly decreased the CYR61 promoter activity induced by YAP1, but neither inhibitor decreased the HIF-2α-induced potentiation to levels observed with YAP1 transfection (Fig. 5C, left panel). To test if HIF-2α and YAP1 could directly interact, FLAG-tagged YAP1 (YAP1-FLAG) and HIF-2α plasmids were co-transfected into HCT116 cells. A pulldown of FLAG and Western blot analysis for HIF-2α did not demonstrate any interaction (Fig. 5D). Similarly, cells were transfected with NTAP (N-terminal affinity purification)-tagged HIF-2α (HIF-2α-NTAP) and YAP1-FLAG plasmids. A pulldown of NTAP and blotting for FLAG also indicated no interaction between HIF-2α and YAP1 (Fig. 5D). These data indicated that there is no direct protein binding between HIF-2α and YAP1.

Prostaglandin E2 (PGE2) is not essential for HIF-2α potentiation of YAP1 activity in CRC

A recent study demonstrates that PGE2 and the synthase needed for PGE2 production, cyclooxygenase-2 (COX2), are essential in YAP1 expression and transcriptional activity in CRC (37). The COX2-PGE2 pathway is critical in CRC progression and non-steroidal inflammatory drugs, which are potent and selective inhibitors of COX2, are effective chemopreventative agents in CRC (38, 39). PGE2 production is highly elevated in CRC via a HIF-2α-dependent activation of COX2 (7). To assess if HIF-2α leads to potentiation of YAP1 via COX2-PGE2 signaling loop, a colon tumor model dependent on HIF-2α activation was assessed (6, 7). Mice with an intestine-specific disruption of Von Hippel Lindau (VHL) tumor suppressor protein led to constitutive HIF activation and an increase in HIF1α and HIF2α target genes Sla1a2, DcxIB, Vegf, PDK1, and Ankrd37 (Fig. 6A) (40). These mice crossed to a sporadic intestinal tumor model Apcmin/+ leads to a robust increase in colon tumors that is dependent on HIF-2α (but not HIF-1α) (VhlIEmin/Apcmin/+). Moreover, our previous study demonstrates that intestinal epithelial cell activation of HIF-1α does not increase carcinogenesis or progression of colon cancer (41). VhlIEIE;Apcmin/+ and littersmate control mice (VhlIEIE;Apcmin+) were treated with the COX2 inhibitor nimesulide. Tumor tissue from both VhlIEIE;Apcmin/+ and VhlIEIE;Apcmin+ mice had significant increases of Cyr61 and Ctgf gene expression compared with normal tissue (Fig. 6B). Nimesulide treatment significantly suppressed YAP1 target gene expression in VhlIEIE;Apcmin+ tumor tissue (Fig. 6B). HIF-2α stabilization led to a further increase in Cyr61 and Ctgf, consistent with our data showing that HIF-2α can potentiate YAP1 signaling. Nimesulide did not decrease the HIF-2α potentiation of YAP1 target genes in tumor tissues (Fig. 6B). This suggests that the cross-talk between HIF-2α and YAP1 was PGE2-independent.

YAP1 is important for HIF-2α-mediated cancer cell growth

Currently the mechanism by which HIF-2α leads to YAP1 potentiation is not clear. However, to understand if YAP1 activation is essential in hypoxia or HIF-2α-induced colon cancer...
cell growth, proliferation assays were assessed. RKO, SW480, and KM12 cell lines were incubated in either normoxia (21% O₂) or hypoxia (1% O₂) conditions. All three cell lines demonstrated a significant increase in HIF-1α and HIF-2α protein expression in hypoxia (Fig. 7A). YAP1 was shown to be essential in hypoxic growth, as using our previously validated YAP1 siRNAs (siYAP1) (31) (Fig. 7B). To address the specific role of HIF-2α and YAP1 in hypoxic growth, HCT116 cells were assessed that expressed both HIF-1α and HIF-2α (Fig. 7A). HCT116-overexpressing HIF-2α or siYAP1 knockdown did not lead to significant changes in growth in normoxia. Under hypoxic conditions (1% O₂), cell growth was significantly inhibited, whereas the growth was increased in cells that overexpressed HIF-2α. Cells that overexpressed HIF-2α but had a knockdown of YAP1 demonstrated a decrease in cell growth under hypoxia (Fig. 7C). Interestingly, knockdown of YAP1 not only reversed the growth-protective effect of HIF-2α under hypoxia but also led to a decrease in growth under hypoxia. This result demonstrates that YAP1 is essential for HIF-2α and hypoxia-mediated growth.

**Discussion**

Hypoxia is a hallmark of solid tumors. Hypoxia can activate the expression of numerous genes involved in cell metabolism, cell survival, cell proliferation, and cell apoptosis. In the intestine, activation of HIF-2α but not HIF-1α promotes the development of CRC (6). However, how HIF-2α contributes to proliferation is still not clear. Here, we demonstrate that HIF-2α promotes CRC cell growth via regulating YAP1 activity. The hippo pathway can be activated by cell density or mechanical cues (42, 43), which leads to phosphorylation and degradation of the YAP/TAZ transcription factors (11). A decrease in Hippo signaling leads to YAP and TAZ nuclear localization and activation of gene expression that promotes cell proliferation and survival. The persistent activation of YAP1 leads to tissue overgrowth and tumor formation in a broad range of tissues (27).

Several studies have shown that HIF-2α induces proliferation via an increase in c-Myc activity (8), inflammation (6, 44), or disrupted iron homeostasis (45). However, it has been suggested that hypoxia hinders cell growth (46–49) and increases...
apoptosis (49, 50), which is consistent with the decreased cell proliferation in our RKO, SW480, KM12, and HCT116 cells under hypoxia conditions. Here, we showed that HIF-2α/H9251 is an activator for YAP1, which increases colon cancer cell growth. Our data showed that stabilized HIF-2α/H9251 significantly increased YAP1 protein levels and downstream target gene Cyr61, Ctgf, and Tgfrb2 expression in Hif2αLSL/LSL;VillinCre mice as well as in the HIF-2α-overexpressing HCT116 human colon cancer cell line. Indeed, HIF-2α-induced cancer cell growth was inhibited when YAP1 was knocked down in various colon cancer cell lines, indicating that HIF-2α-YAP1 cross-talk is important for tumorigenesis. Future work will focus on how the different

Figure 5. YAP1 and HIF-2α cross-talk was not via canonical kinase signaling or direct protein interaction. CYR61 promoter luciferase in HCT116 cells transfected with EV, YAP1, and/or oxygen stable HIF-2α (HIF-2α-TM) and treated with or without 10 μM Src inhibitor, SU6656 (A), 100 nM Akt inhibitor (MK2206) (B), or 100 nm MEK1 inhibitors, GSK (GSK1120212 or trametinib) or PD (PD325901) (C) for 24 h after transfection. RLU, relative light units. Right panels for A–C, Western blot analysis in HCT116 cells for total (T)-Src, phospho (P)-Src T-AKT, p-AKT, T-ERK, and p-ERK after inhibitor treatment. D, co-immunoprecipitation for YAP1 and HIF-2α. HEK293A cells were co-transfected with FLAG tagged YAP1 (YAP1-FLAG) and/or NTAP-tagged HIF-2α (NATP-HIF-2α) plasmids. A pulldown of FLAG or NTAP and Western blot analysis for HIF-2α or YAP1 is shown. *, p < 0.05; **, p < 0.01 compared with the indicated bars. The experiment was performed in triplicate and repeated at least three times. n.s., not significant.
HIF-2α-induced growth proliferative mechanisms integrate and are regulated to increase CRC progression.

The mechanism by which HIF-2α induced YAP1 expression and activity was not through direct protein binding or promoter activation of YAP1 target genes but via indirect mechanism. The possible mechanisms that led to potentiation are not clear but are a major future focus. In our sporadic colon cancer mouse model, we demonstrated that the activation of YAP1 is HIF-2α-independent. This is possibly due to other parallel pathways such as increased β-catenin resulting from tumor suppressor APC mutation. The dysregulation of YAP phosphorylation and nuclear localization has been extensively studied (51–53). However, recent data have revealed that cancer samples have increased total YAP abundance (23, 54). Our data support this recently proposed idea and provide a clinically relevant mechanism by which YAP activation by HIF-2α is increased in human colon cancers.

Previous studies have demonstrated that the activation of YAP1 leads to hyper-proliferation and spontaneous tumor formation in certain tissues, including the intestine (23, 55). Recently, YAP-TEAD complex suppressor Verteporfin has shown potential as an anticancer treatment (31, 56). However, YAP1 is a critical component of the β-catenin degradation complex, and disruption of YAP1 leads to an enhanced β-catenin activation and increased proliferation in the intestine (17). Therefore, better therapeutic methods are necessary. Hypoxia can regulate YAP1 activity in hepatocellular carcinoma-derived cell lines independent of HIF-1α; however, HIF-2α was not assessed (57). Moreover, a complimentary pathway was recently shown in which HIF-1α induces the Hippo coactivator TAZ and can serve as transcriptional co-activator (58). Here, we demonstrate a novel mechanism of YAP1 regulation by HIF-2α, which can serve as a potential therapeutic target for YAP1-associated tumorigenesis.

In summary, we discovered that there is novel cross-talk between HIF-2α and YAP1, which is one of the mechanisms that HIF-2α can increase tumor genesis. Future studies focusing on YAP1 deletion in HIF-2α-induced cancer models are needed to further understand this cross-talk.

**Experimental procedures**

**Animals and treatments**

Intestine-specific HIF-2α-overexpressing mice (Hif2αLSL/LSLVillinCre) and sporadic colon tumor mouse models including CDXERT2Cre/ApcF/F, CDXERT2CreApcF/F/HIF2αF/F, VhlIE/Apcmin/+ and VhlF/F/Apcmin/+ were maintained in standard cages in a light- and temperature-controlled room and...
were fed with standard chow and water *ad libitum*. For the PGE2/COX2 inhibition study, normal tissue and tumor tissue samples were obtained from VhlF/F/Apcmin/H11001 and VhlF/F/Apcmin/H11001 mice fed with powdered laboratory rodent diet 5001 (PMI Nutrition International LLC, Brentwood, MO) with or without 400 mg/kg nimesulide (Sigma) for 8 weeks. All the ani-

Figure 7. YAP1 was essential for cell growth under hypoxia. A, HIF-1α and HIF-2α protein levels after incubation in hypoxia in SW480, RKO, KM12, and HCT116 cells. Shown is an MTT cell viability assay at 0, 24, 48, and 72 h under normoxia (21% O2) or hypoxia (1% O2) in scrambled (siScr) or siRNA specific for YAP1 (siYAP1) in SW480, RKO, and KM12 cells (B) or in HCT116 EV or HIF-2αOE cells (C). *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared with the groups indicated. The experiment was performed in triplicate and repeated at least three times.
The cross-talk between HIF-2α and YAP1

| Table 1
| QPCR primers sequence list |
| Forward (5' → 3') | Reverse (5' → 3') |

**Mouse qPCR primers**

- **β-Actin**
  - Forward: TGAAACGAGCATCAGGAAGG
  - Reverse: CGAACTCTGAAACCTGGG

- **Ctgf**
  - Forward: GCTTGCAGGTTACCTGCTG
  - Reverse: CAGTGTCCAGCTGCTGCTG

- **Cyr61**
  - Forward: CACCTGACTGGAGGCTGCTG
  - Reverse: ACCTAGAGAGAGAGAGAGAGAG

- **Tgfβr2**
  - Forward: TGGCGCAGAGGCTGCTGCTG
  - Reverse: CAGTGTCCAGCTGCTGCTG

- **Tead1**
  - Forward: AACTCTGAGCTGGAGGCTGCTG
  - Reverse: ACCTAGAGAGAGAGAGAGAGAG

- **Axin2**
  - Forward: TGGCGCAGAGGCTGCTGCTG
  - Reverse: CAGTGTCCAGCTGCTGCTG

- **Hif2a**
  - Forward: CACCTGACTGGAGGCTGCTG
  - Reverse: ACCTAGAGAGAGAGAGAGAGAG

- **Sle11a2**
  - Forward: TGGCGCAGAGGCTGCTGCTG
  - Reverse: CAGTGTCCAGCTGCTGCTG

- **Dcty1**
  - Forward: AACTCTGAGCTGGAGGCTGCTG
  - Reverse: ACCTAGAGAGAGAGAGAGAGAG

- **Vegf**
  - Forward: TGGCGCAGAGGCTGCTGCTG
  - Reverse: CAGTGTCCAGCTGCTGCTG

- **Pdk1**
  - Forward: TGGCGCAGAGGCTGCTGCTG
  - Reverse: CAGTGTCCAGCTGCTGCTG

- **Ankrd37**
  - Forward: CACCTGACTGGAGGCTGCTG
  - Reverse: ACCTAGAGAGAGAGAGAGAGAG

**Human qPCR primers**

- **β-Actin**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Ctgf**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Cyr61**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Tgfβr2**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Tead1**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Axin2**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Hif2a**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Sle11a2**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Dcty1**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Vegf**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Pdk1**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

- **Ankrd37**
  - Forward: GCTTGCAGGCGTAGGGCG
  - Reverse: GCACAGACCTGGCTCTT

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