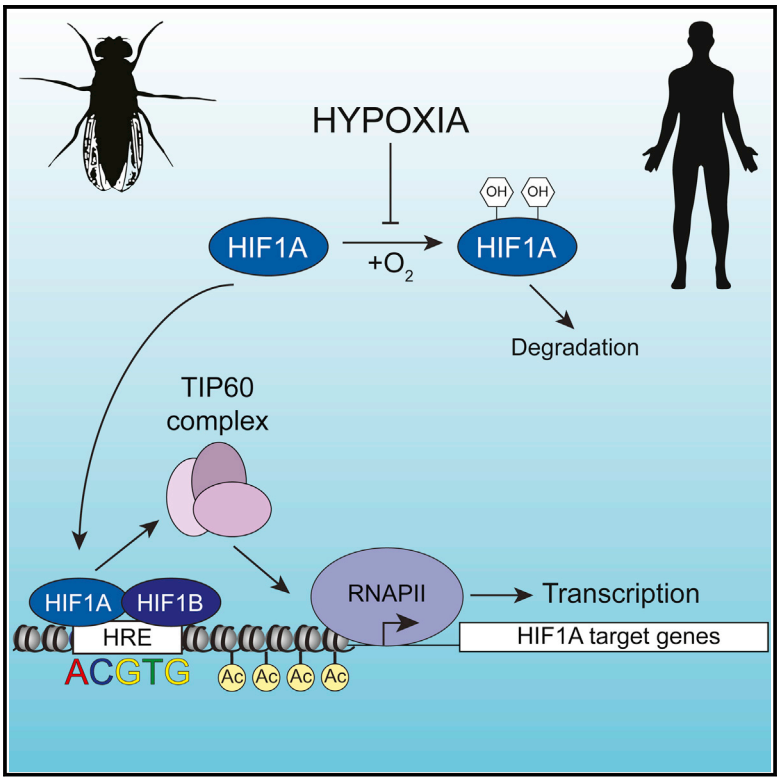
Report

Cell Reports

The TIP60 Complex Is a Conserved Coactivator of HIF1A

Graphical Abstract



Highlights

- TIP60 is required for expression of HIF1-dependent genes in *Drosophila*
- The role of TIP60 at HIF1-dependent genes is conserved in human colorectal cancer cells
- HIF1A interacts with and recruits TIP60 to chromatin
- Many HIF1A targets require TIP60, CDK8, or both for full expression in hypoxia

Authors

Joel I. Perez-Perri, Veronica L. Dengler, K. Audrey Audetat, ..., Pablo Wappner, Matthew D. Galbraith, Joaquín M. Espinosa

Correspondence

matthew.galbraith@ucdenver.edu (M.D.G.), joaquin.espinosa@ucdenver.edu (J.M.E.)

In Brief

Hypoxia-inducible factors (HIFs) are critical regulators of the cellular response to hypoxia. In this study, Perez-Perri et al. uncover a conserved role for the TIP60 complex in HIF-dependent gene expression in flies and human cancer cells. Further work demonstrates that HIF1A interacts with and recruits TIP60 to chromatin. Global transcriptome analysis reveals that most HIF1A targets require either TIP60, the CDK8-Mediator complex, or both as coactivators for full expression in hypoxia

Accession Numbers

GSE68297

Perez-Perri et al., 2016, Cell Reports *16*, 37–47
June 28, 2016
http://dx.doi.org/10.1016/j.celrep.2016.05.082

CellPress

Cell Reports
Report

The TIP60 Complex Is a Conserved Coactivator of HIF1A

Joel I. Perez-Perri,[4,6,7] Veronica L. Dengler,[3,6] K. Audrey Audetat,[3] Ahwan Pandey,[2,3] Elizabeth A. Bonner,[2] Marjeta Urh,[5] Jacqui Mendez,[5] Danette L. Daniels,[5] Pablo Wappner,[4] Matthew D. Galbraith,[2,3,*] and Joaquín M. Espinosa[1,2,3,*]
[1]Linda Crnic Institute for Down Syndrome, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA
[2]Department of Pharmacology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA
[3]Howard Hughes Medical Institute and Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO 80309, USA
[4]Instituto Leloir, 1405 Buenos Aires, Argentina
[5]Promega Corporation, Madison, WI 53711, USA
[6]Co-first author
[7]Present address: European Molecular Biology Laboratory, Meyerhofstrasse 1, Heidelberg 69117, Germany
*Correspondence: matthew.galbraith@ucdenver.edu (M.D.G.), joaquin.espinosa@ucdenver.edu (J.M.E.)
http://dx.doi.org/10.1016/j.celrep.2016.05.082

SUMMARY

Hypoxia-inducible factors (HIFs) are critical regulators of the cellular response to hypoxia. Despite their established roles in normal physiology and numerous pathologies, the molecular mechanisms by which they control gene expression remain poorly understood. We report here a conserved role for the TIP60 complex as a HIF1 transcriptional cofactor in *Drosophila* and human cells. TIP60 (KAT5) is required for HIF1-dependent gene expression in fly cells and embryos and colorectal cancer cells. HIF1A interacts with and recruits TIP60 to chromatin. TIP60 is dispensable for HIF1A association with its target genes but is required for HIF1A-dependent chromatin modification and RNA polymerase II activation in hypoxia. In human cells, global analysis of HIF1A-dependent gene activity reveals that most HIF1A targets require either TIP60, the CDK8-Mediator complex, or both as coactivators for full expression in hypoxia. Thus, HIF1A employs functionally diverse cofactors to regulate different subsets of genes within its transcriptional program.

INTRODUCTION

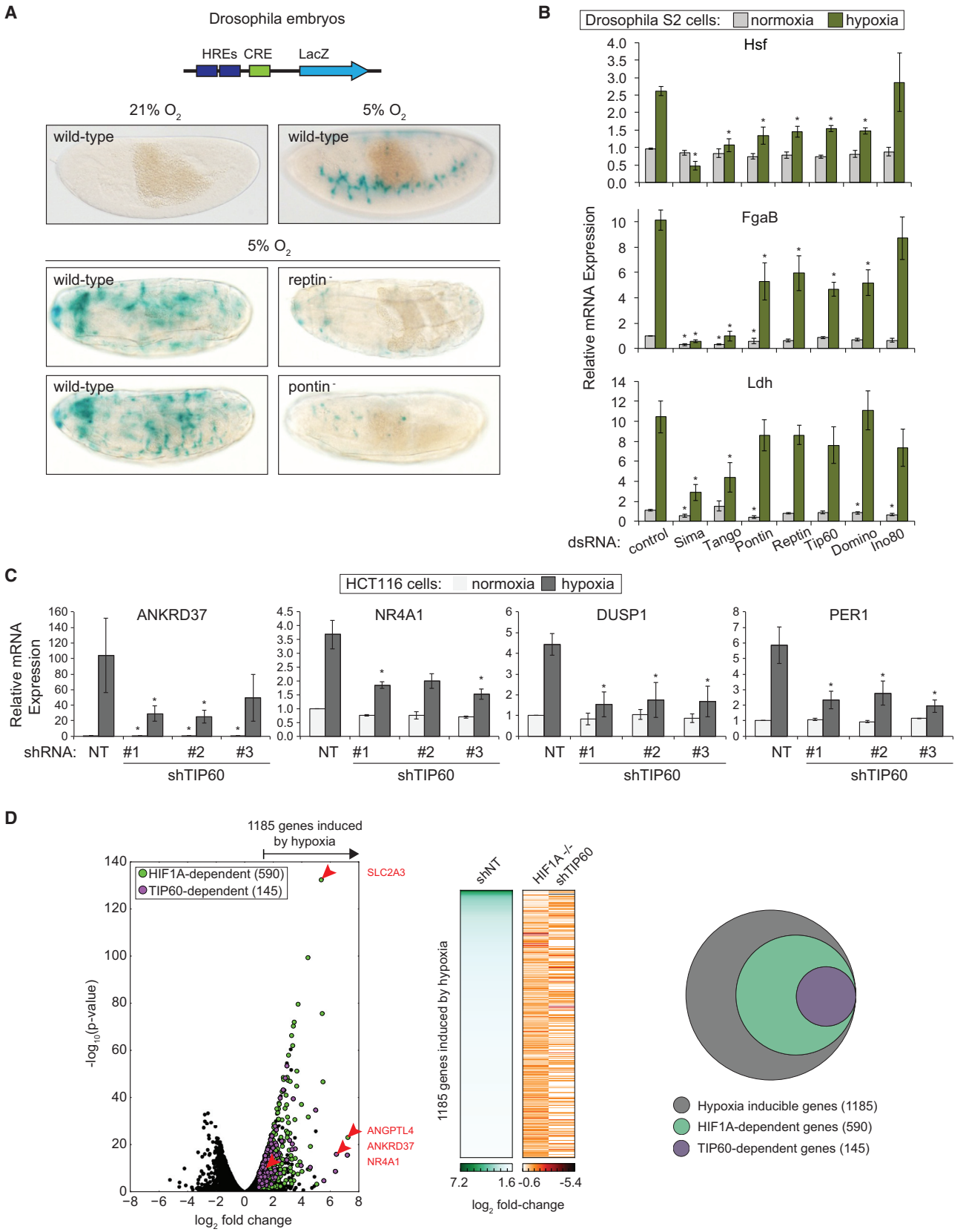
The cellular response to hypoxia is essential for normal physiological processes, such as embryonic development and stem cell maintenance (Dunwoodie, 2009; Mazumdar et al., 2009), but is also involved in diverse human pathologies, including cancer, stroke, and heart failure (Majmundar et al., 2010; Semenza, 2012a). At the transcriptional level, the response to hypoxia is largely governed by hypoxia-inducible factors (HIFs) (Dengler et al., 2014; Semenza, 2009). In human cells, numerous studies have delineated how the oxygen-sensitive subunits HIF1A and HIF2A are stabilized and activated in hypoxia and have identified hundreds of their target genes, but less is known about the mechanisms employed by HIFs to stimulate RNAPII activity.

It is generally accepted that the lysine (K) acetyl-transferases (KATs) p300/CBP are key HIF transcriptional coactivators (Arany et al., 1996; Ebert and Bunn, 1998; Ruas et al., 2002, 2005). However, abrogation of the interaction between HIF1A and p300/CBP affects the expression of only a few HIF target genes (Kasper et al., 2005). Here, we report the identification of a conserved role for the TIP60 chromatin-modifying complex as a HIF1A transcriptional cofactor. We show that HIF1A utilizes TIP60 (KAT5) for full induction of specific target genes and for histone acetylation and RNAPII activation upon hypoxia at these loci. We find that HIF1A physically associates with components of the TIP60 complex and is required for TIP60 recruitment to chromatin. Global analyses of gene expression in human cells depleted of HIF1A, TIP60, or CDK8 revealed that, across much of its transcriptional program, HIF1A employs TIP60, CDK8-Mediator, or both as gene-specific coactivators. Altogether, our results illuminate the orchestrated action of functionally diverse cofactors during the transcriptional response to hypoxia.

RESULTS

Components of the TIP60 Complex Modulate HIF Target Gene Activation in *Drosophila*

We previously carried out a genome-wide screen in *Drosophila* S2 cells and identified Pontin and Reptin as two of the strongest regulators of HIF-dependent transcription using a HIF reporter system (Dekanty et al., 2010). Pontin (RUVBL1 and TIP49) and Reptin (RUVBL2 and TIP48) are AAA+ ATPases with diverse cellular functions, including transcriptional regulation (Gallant, 2007). Here, we analyzed their requirement in vivo using *Drosophila* transgenic lines bearing a HIF-dependent LacZ reporter (Lavista-Llanos et al., 2002) and null mutations at the *pontin* (*pont*) or *reptin* (*rept*) loci. Whereas the reporter is highly induced in wild-type *Drosophila* embryos subjected to hypoxia (5% O_2 4 hr), its activity is severely compromised in *pontin* and *reptin* mutants (Figure 1A).



(legend on next page)

Pontin and Reptin are components of multiple complexes with roles in transcription, including the TIP60 and INO80 complexes (Jha et al., 2013; Jónsson et al., 2004; Sapountzi et al., 2006). To determine whether these complexes are involved in HIF-dependent transcription, we tested the effect of depleting shared and specific subunits on expression of known HIF targets in S2 cells under normoxia and hypoxia (*Hsf*, *FgaB* (*Hph-RA*), and *Ldh* (*ImpL3*); Figures 1B and S1A). Depletion of Pontin, Reptin, or two different subunits specific to the TIP60 complex, Tip60 (KAT5) and Domino (p400), impaired the induction of two of three genes tested (Figure 1B). In contrast, depletion of Ino80 had a lesser effect. Depletion of the *Drosophila* homologs of HIF1A (Sima) and HIF1B/ARNT (Tango) confirmed HIF1-dependent induction of these genes. Together, these results suggest a role for the Pontin- and Reptin-containing *Drosophila* Tip60 complex as a gene-specific HIF transcriptional coactivator.

TIP60 Depletion Impairs Expression of Specific HIF1A Target Genes in Human Cells

We next asked whether this role of the TIP60 complex is conserved in human cells. We first depleted the catalytic subunit KAT5 using three independent short hairpin RNAs (shRNAs) in HCT116 colorectal carcinoma cells (shTIP60) and confirmed that TIP60 knockdown did not affect HIF1A stabilization in hypoxia (1% O_2 24 hr; Figures S1B and S1C). Examination of the effect on expression of a panel of HIF1A-dependent genes revealed gene-specific TIP60 requirements (Figures 1C and S1D–S1F). For example, whereas induced expression of *ANKRD37* and *NR4A1* is reduced by >50% (Figure 1C), expression of *ALDOA* and *JMJD1A* remains unaffected (Figure S1F). A similar gene-specific requirement for TIP60 was also observed in a different colon-cancer-derived cell type, SW480 (Figure S1G).

To define the contribution of TIP60 to the global HIF1A-driven transcriptional response, we used RNA-seq to measure mRNA levels in *HIF1A*$^{-/-}$ and shTIP60 HCT116 cells under normoxic and hypoxic conditions. From ~14,000 expressed genes, our analysis identified 1,185 genes significantly induced by hypoxia, of which half (590; 49.7%) showed significantly reduced expression during hypoxia in *HIF1A*$^{-/-}$ cells (Figure 1D; Table S1). In turn, ~25% (145) of these HIF1A-dependent genes relied on TIP60 for full expression during hypoxia, demonstrating that TIP60 makes a substantial contribution to the HIF1A-driven expression program.

TIP60 Complex Subunits Are Recruited to HIF1A Target Genes

To explore the mechanistic basis for TIP60 requirement in HIF1A-driven gene expression, we performed quantitative chromatin immunoprecipitation (ChIP) analysis of the *ANKRD37* locus, a hypoxia-responsive gene strongly affected by TIP60 depletion. Analysis of HIF1A binding to the *ANKRD37* locus demonstrated that TIP60 knockdown does not alter HIF1A association with chromatin upon hypoxia (Figure 2A).

We next asked whether the TIP60 complex localizes to this gene by assessing occupancy of TIP60, Reptin, and TRRAP (Figures 2B–2D). All three subunits were found to associate with the *ANKRD37* promoter, with their occupancy peaking in hypoxia. Interestingly, Reptin and TRRAP exhibit relatively strong enrichment even in normoxic conditions. This could be explained by their reported roles within other chromatin-associated complexes distinct from the TIP60 complex or by basal but undetectable recruitment of TIP60 itself in normoxia (see next section). Note that TIP60 depletion modestly affects occupancy of both Reptin and TRRAP (Figures 2C and 2D), and TIP60 enrichment is expectedly low in TIP60-depleted cells (Figure 2B).

TIP60 Depletion Compromises HIF1A-Dependent RNAPII Activation and Histone Acetylation

Many HIF1A target genes, including *ANKRD37*, exhibit promoter-proximal paused RNAPII during normoxia, and their activation under hypoxia requires conversion of paused RNAPII into an elongation-competent form (Galbraith et al., 2013). This process typically involves phosphorylation of the C-terminal domain (CTD) of RNAPII. Therefore, we tested the influence of TIP60 on total and phosphorylated RNAPII. Expectedly, hypoxia provokes an increase in total RNAPII levels throughout the body of the *ANKRD37* gene with a peak near the transcription start site (Figure 2E) and stimulates RNAPII activation, as indicated by CTD serine 5 phosphorylation (S5P) (Figure 2F). Whereas TIP60 depletion has a small effect on normoxic levels, it causes a substantial reduction in induced enrichment of both total and S5P-RNAPII across the *ANKRD37* locus. This suggests that

Figure 1. Subunits of the TIP60 Complex Modulate HIF Target Gene Expression in *Drosophila* and Human Cells

(A) Schematic diagram of the murine *Ldha* enhancer-derived hypoxia-response element (HRE)-LacZ reporter construct inserted into the fly genome. CRE, cAMP response element. Wild-type and *pontin*$^{-}$ or *reptin*$^{-}$ mutant embryos subjected to normoxia or hypoxia (5% O_2 4 hr) were stained with X-gal (blue) to visualize reporter activity.

(B) Relative mRNA levels for HIF target genes, as assessed by qRT-PCR, in *Drosophila* S2 cells treated with the indicated dsRNAs and maintained under normoxia or hypoxia (1% O_2 20 hr). Expression values were normalized to Rpl29 RNA and are expressed relative to the control normoxia value. Data are represented as mean ± SEM from at least three independent replicates. Asterisks indicate p values $\leq$ 0.05 by one-way ANOVA.

(C) Relative mRNA levels for HIF1 target genes, as assessed by qRT-PCR, for HCT116 cells stably expressing shRNAs targeting TIP60 and subjected to normoxia or hypoxia (1% O_2 24 hr). Expression values were normalized to 18S rRNA and are expressed relative to the control normoxia value. Data are represented as mean ± SEM from at least three independent replicates. Asterisks indicate p values $\leq$ 0.05 by one-way ANOVA.

(D) RNA-seq analysis of global mRNA levels in shNT control, *HIF1A*$^{-/-}$, and shTIP60 HCT116 cells subjected to hypoxia. Left: volcano plot of $\log_2$ fold change against $-\log_{10}$ p value for all genes in normoxia versus hypoxia is shown. Green circles, HIF1A-dependent hypoxia-inducible genes; purple circles, HIF1A-dependent genes that are also TIP60 dependent. Selected genes of interest are indicated by red arrows, with labels on right. Middle: heatmap of mRNA $\log_2$ fold change values is shown. Right: Venn diagram representing the genes induced by hypoxia (gray) and subsets that require HIF1A (green) or both HIF1A and TIP60 (purple) for hypoxic induction is shown. In all cases, hypoxic induction is defined as ratio (shNT normoxia/shNT hypoxia) $\geq$ 2, false discovery rate (FDR)-adjusted p value < 10% and requirement for HIF1A or TIP60 is defined as ratio (*HIF1A*$^{-/-}$ or shTIP60 hypoxia/shNT hypoxia) $\leq$ 0.9, FDR-adjusted p value < 10%.
See also Figure S1.

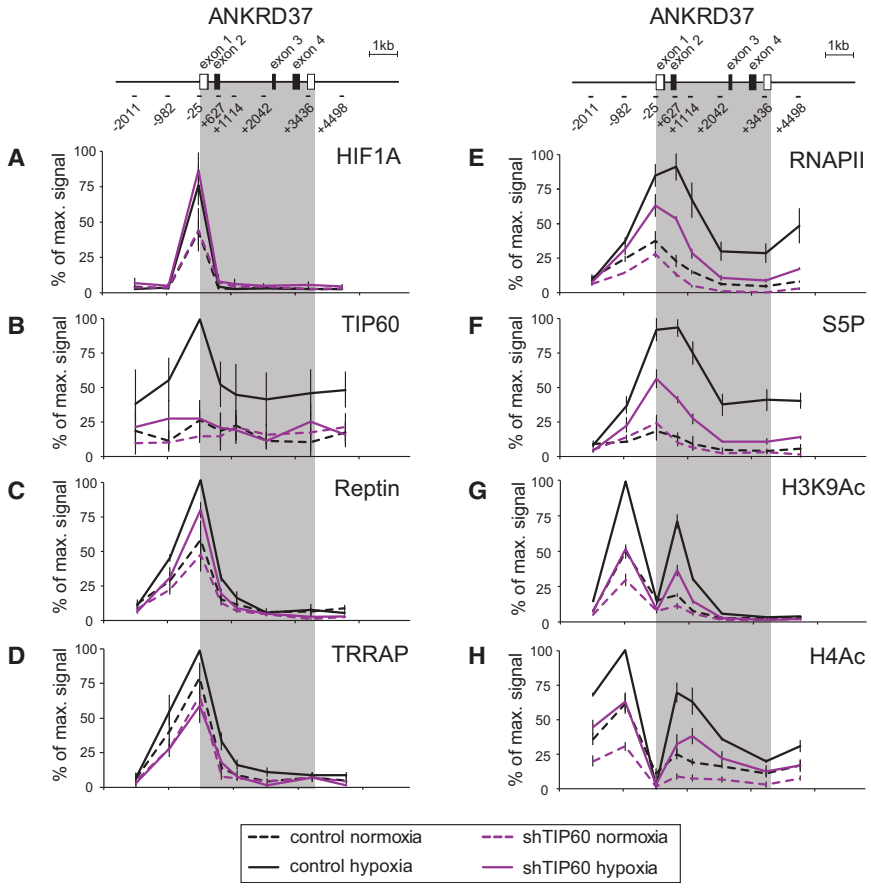


Figure 2. TIP60 Is Required for RNAPII Activation and Histone Acetylation at the *ANKRD37* Locus

Quantitative ChIP analysis of (A) HIF1A, (B) TIP60, (C) Reptin, (D) TRRAP, (E) total RNAPII, (F) serine-5-phosphorylated RNAPII CTD (S5P), and (G and H) histone acetylation (H3K9ac and pan-H4ac) at the *ANKRD37* locus in control and shTIP60 HCT116 cells in normoxia or hypoxia (1% O_2 24 hr). To represent profiles across the locus, values are plotted as percentage of maximum signal for each epitope. Data are represented as mean ± SEM from three independent replicates. Gray area indicates the transcribed region.

TIP60 is a direct positive regulator of RNAPII activity at HIF1A-responsive genes.

Because histone acetylation is associated with HIF1A-dependent gene activation (Johnson et al., 2008) and the TIP60 complex has well-established KAT activity, we next asked whether this complex promotes HIF1A-dependent histone acetylation. We found that TIP60 depletion diminishes acetylation of histone H3 lysine 9 (H3K9) and histone H4 (Figures 2G and 2H), two acetylation events previously shown to depend on HIF1A (Galbraith et al., 2013). Although TIP60 recruitment is maximal during hypoxia, its depletion also impairs histone acetylation during normoxia. This might reflect a low basal recruitment of the TIP60 complex, which is consistent with levels of HIF1A, Reptin, and TRRAP observed during normoxia at the *ANKRD37* locus (Figures 2A, 2C, and 2D). Notably, *ANKRD37* is one of a minority of HIF1A-dependent genes whose normoxic expression is affected by depletion of TIP60. Overall, these results demonstrate that TIP60 is required for maximal HIF1A-dependent RNAPII activation and histone hyper-acetylation at the *ANKRD37* locus.

HIF1A Directs the TIP60 Complex to Chromatin

We next asked whether HIF1A is required for recruitment of the TIP60 complex to chromatin. Using ChIP in isogenic wild-type and *HIF1A*$^{-/-}$ HCT116 cells, we found that maximal occupancy of the *ANKRD37* locus by the TIP60, Reptin, and TRRAP subunits of the TIP60 complex under hypoxia requires HIF1A (Figure 3A).

To test whether HIF1A associates physically with the TIP60 complex, we performed HaloTag (HT) pull-down and mass spectrometry analysis of HIF1A-interacting proteins from HEK293T cells expressing Halo-tagged-HIF1A or control HT alone and treated with desferrioxamine (DFX) to induce HIF1A stabilization. Using this approach, in addition to known HIF1A interactors, such as HIF1B (ARNT) and VHL, we detected Reptin and Pontin (Figures 3B and S2A; Table S2). Detection of an interaction with VHL, a subunit of the E3 ligase complex that targets HIF1A for degradation when hydroxylated at key residues during normoxia, suggests that, in this context, some HT-HIF1A remains hydroxylated, despite treatment with DFX. Also present, albeit at lower abundance, were three other potential TIP60 complex subunits, TRRAP, BAF53A (ACTL6a), and p400 (EP400), as well as p300 (EP300).

We next tested for physical proximity between HIF1A and TIP60 complex subunits in live cells using a nano-luciferase bioluminescent resonance energy transfer (NanoBRET) assay (Machleidt et al., 2015) in HCT116 and HEK293 cells (Figures 3C and S2B). In agreement with our mass spectrometry analysis, we detected energy transfer from HIF1A-NanoLuc (HIF1A-NL) to HT-Pontin and HT-Reptin. These interaction signals were not significantly enhanced by DFX treatment, which could be explained by the fact that ectopic expression of HIF1A-NL bypasses the need for hypoxia-induced stabilization. Notably, we detected a stronger signal for HT-TIP60 than for HT-Pontin and HT-Reptin. This suggests that, despite not being detected by mass spectrometry, TIP60 can also interact with HIF1A in cells. This could be explained by poor performance or low relative abundance of TIP60-derived peptides in the mass spectrometry assay. Of note, nanoBRET ratios are influenced by many factors, including close proximity, relative affinity, occupancy, and expression level, and therefore, higher values do not necessarily indicate a higher affinity interaction. Importantly, all signals were greater than for the HT control (Figure S2C). To confirm the HIF1A-TIP60 interaction, we performed HT pull-down from

HCT116 cells co-expressing HT-TIP60, HT-Pontin, HT-Reptin, or HT control with HIF1A-NL (Figures 3D and S2D). After isolation, complexes were assayed for luciferase activity as a measure of interaction with HIF1A-NL. We observed an enrichment in luciferase signal, over the HT control, for all three pull-downs, with HT-TIP60 again displaying the strongest signal (Figure 3D). Treatment with DFX induced a small, albeit not statistically significant, increase in signal. The interaction between HT-TIP60 and HIF1A-NL was also observed in HEK293 cells (Figure S3D). Taken together, our data suggest that HIF1A can physically interact with components of the TIP60 complex and direct their recruitment to chromatin during hypoxia.

HIF1A Employs TIP60 and CDK8-Mediator as Coactivators across Much of Its Transcriptional Program

We recently discovered that HIF1A employs the CDK8-Mediator complex as a coactivator at many of its target genes (Galbraith et al., 2013). To define the relative contributions of both TIP60 and CDK8 to the transcriptional response during hypoxia, we compared RNA-seq data for HCT116 cells depleted of CDK8 (shCDK8) to our previous data for *HIF1A*$^{-/-}$ and shTIP60 HCT116 cells (Figure 4A; Table S1). This analysis revealed that TIP60 and CDK8 affect distinct but overlapping sets of genes and, collectively, contribute to the induced expression of >60% (363 out of 590) of genes across the HIF1A network. We defined four classes of HIF1A-dependent genes. Class I genes require HIF1A, but not TIP60 or CDK8, for full expression. Class II genes require HIF1A and CDK8, but not TIP60. Class III genes require HIF1A, CDK8, and TIP60. Class IV genes require HIF1A and TIP60, but not CDK8. Each class contains known HIF1A target genes displaying differential requirement for CDK8 and TIP60 (Figure 4B). The differential requirement for TIP60 across select genes in classes I, III, and IV (*PDK1*, *FAT4*, and *EMP1*) was confirmed by qRT-PCR (Figure S3A). Next, we examined markers of transcriptional activity at these loci upon TIP60 knockdown (Figure S3B). Depletion of TIP60 largely abrogated enrichment of total and phosphorylated RNAPII at *FAT4* (class III) and *EMP1* (class IV), but not *PDK1* (class I). Interestingly, TIP60 depletion also affected histone H4 acetylation at *PDK1*, a TIP60-independent gene, suggesting that TIP60 may be recruited and active even at loci where it is not required for RNAPII activity or full mRNA induction. We also confirmed that these genes are likely direct HIF1A target genes, as HIF1A depletion led to reduced levels of total and phosphorylated RNAPII, TIP60 recruitment, and histone acetylation (Figure S3C). We also analyzed a hypoxia-inducible gene that does not require HIF1A, TIP60, or CDK8 (*CYR61*; Figures S3A–S3C). TIP60 and HIF1A depletion did not affect total RNAPII at this locus (Figures S3B and S3C). However, their depletion did impact Ser5 phosphorylation, and as observed for the class I gene *PDK1*, TIP60 knockdown reduced histone acetylation at *CYR61* despite not being necessary for *CYR61* mRNA expression. HIF1A depletion had minor effects on TIP60 and histone acetylation at this locus (Figure S3C). Altogether, these results indicate that TIP60 is recruited to many classes of hypoxia-inducible genes, where it contributes to histone acetylation, yet it is only required for RNAPII activity and mRNA production at classes III and IV. Note that the requirement for TIP60 is not simply defined by its chromatin occupancy and that its ultimate effects on RNAPII at a given locus are likely defined by other, unknown variables (see below). Furthermore, because our ChIP analyses may not have captured the site of maximum TIP60 occupancy at each locus, a quantitative relationship between TIP60 association and its impact on RNAPII activity cannot be established. Because histone H4 acetylation is affected by TIP60 knockdown at all genes analyzed, TIP60 effects could be driven by other acetylation targets in the transcriptional apparatus. In fact, several non-histone TIP60 targets, including transcription factors, have been identified (Patel et al., 2004; Sun et al., 2005; Tang et al., 2006, 2008; Xiao et al., 2014).

Of note, the differential requirements for TIP60 and CDK8 across the HIF1A-driven gene expression program are not simply a consequence of a general effect on transcription, as their overall contributions to global mRNA levels in normoxic cells are much smaller, with TIP60 affecting many more genes than CDK8 (Figure S4A). These results suggest that alternative coactivator usage by HIF1A could confer regulatory flexibility to the transcriptional response to hypoxia. Moreover, the differential coactivator requirements of subsets of genes might depend in part on the ability of HIF1A to cooperate with other transcription factors (Dang et al., 2008; Gray et al., 2005; Xia and Kung, 2009). To search for factors with potential to differentially regulate subsets of HIF1A target genes, we identified known upstream transcriptional regulators of each gene class in our RNA-seq dataset using Ingenuity Pathway Analysis (IPA) (Figure S4B). Expectedly, HIF1A was the top prediction for all HIF1-dependent hypoxia-inducible genes, as well as for the four classes within this group, but not for HIF1A-independent genes. HIF2A is the second inferred regulator for HIF1A-dependent genes as well as a top prediction for gene classes I and II, which likely reflects its many shared target genes with HIF1A. When comparing the top ten enriched regulators across each of the four gene classes, we identified transcription factors that are predicted to regulate genes within specific classes (Figure S4C). For example, whereas SP3 may regulate only genes in class I, STAT6 could regulate genes in classes II and III, both of which require CDK8 for full expression. Thus, we propose that the combinatorial action of HIF1A with other transcription factors may enable the fine-tuning of the transcriptional response to hypoxia to favor diverse biological responses in a context-dependent fashion. Indeed, analysis of the different gene classes identified here also revealed distinct functional pathways controlled by this putative mechanism (Figure S4D).

Finally, to examine the roles of TIP60 and CDK8 in oncogenic properties of colorectal cancer cells, we measured the ability of non-targeting shRNA (shNT) control, shTIP60, shCDK8, and *HIF1A*$^{-/-}$ HCT116 cells to initiate and sustain growth of three-dimensional colonies in soft agar, which quickly reach a size large enough to create hypoxic conditions at their core (Indovina et al., 2007; Lin et al., 2012; Meng et al., 2012; Sutherland et al., 1986). These assays are considered a good surrogate of the "tumor-initiating ability" of cancer cells (i.e., "stemness") and their ability to adapt to hypoxic conditions. Importantly, this assay is exquisitely sensitive to HIF1A activity in multiple cell types

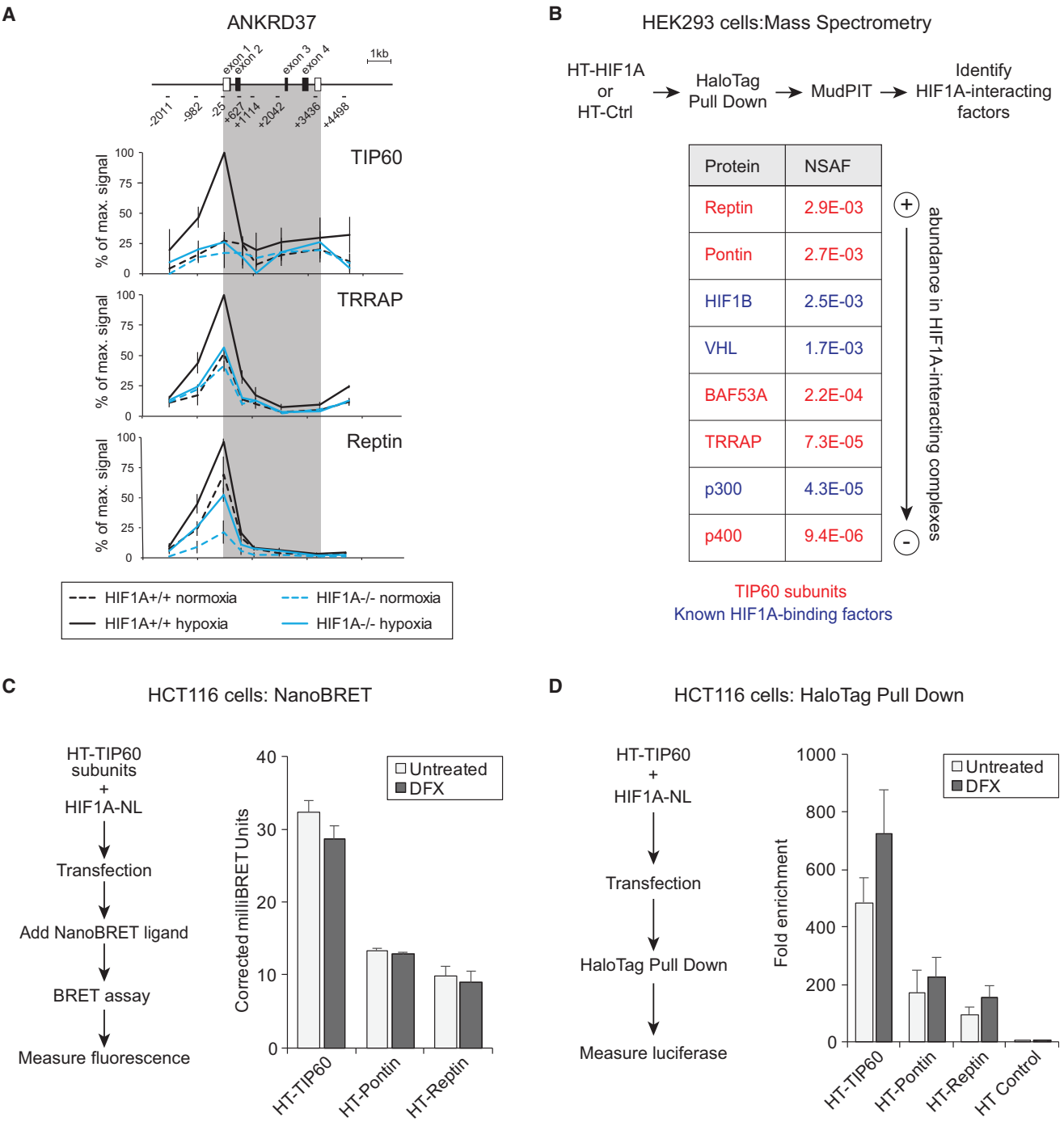


Figure 3. HIF1A Directs the TIP60 Complex to Chromatin

(A) Quantitative ChIP analysis of TIP60, TRRAP, and Reptin at the *ANKRD37* locus in wild-type and *HIF1A*$^{-/-}$ HCT116 cells in normoxia or hypoxia (1% O_2 24 hr). To represent profiles across the locus, values are plotted as percentage of maximum signal for each epitope. Data are represented as mean ± SEM from three independent replicates. Gray area indicates the transcribed region.

(B) Identification of HaloTag (HT)-HIF1A interactors by pull-down and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Average normalized spectral abundance factors (NSAFs) from two biological replicates are shown for selected proteins isolated in complex with HT-HIF1A from desferrioxamine (DFX)-treated HEK293T cells. Known HIF1A interactors are shown in blue, and subunits of the TIP60 complex are shown in red.

(C) NanoBRET assays measuring proximity of HIF1A to TIP60, Pontin, and Reptin. HCT116 cells were co-transfected with HIF1A-NL (donor) and either HT-TIP60, HT-Pontin, or HT-Reptin (acceptors). Graph shows corrected milliBRET units for each acceptor fusion protein when combined with the HIF1A-NL donor. Higher

(legend continued on next page)

(Dang et al., 2006; Leek et al., 2005; Onnis et al., 2013; Rohwer et al., 2008), including when incubated at 21% oxygen (Dang et al., 2006). Whereas HCT116 *HIF1A*$^{-/-}$ cells do not show significant proliferation defects in monolayers, they completely fail to form colonies in soft agar (Figures 4C and 4D). Therefore, this assay provides a great opportunity to gauge the contribution of TIP60 and CDK8 to a cellular process that is fully dependent on HIF1A. Hypoxia is known to stimulate "stemness" and clonogenicity, along with induction of HIF1A target genes (Lee and Simon, 2012; Yeung et al., 2011). We therefore performed the assay under conditions of normoxia and hypoxia. In wild-type HCT116 cells, introducing hypoxia from the time of seeding stimulates colony formation efficiency, as seen by increased colony numbers, although without reaching statistical significance (Figures 4C and 4D). The stimulatory effect of hypoxia is completely lost in *HIF1A*$^{-/-}$ cells, consistent with the ability of HIF1A to promote stemness in colon cancer cells (Yeung et al., 2011). However, the stimulatory effect of hypoxia can be observed, to varying degrees, in TIP60- and CDK8-depleted cells, which can be explained by the fact that these cells retain the ability to induce a fraction of the HIF1A-dependent transcriptional program (Figure 4A). In fact, the amount of colonies formed under hypoxia inversely correlates with the contributions of HIF1A, TIP60, and CDK8 to the hypoxia-inducible program. Altogether, these data suggest that HIF1A, TIP60, and CDK8 may promote tumorigenesis through their contributions to a common transcriptional program.

DISCUSSION

The cellular response to hypoxia is important in normal physiology and disease (Semenza, 2012a). In particular, hypoxia favors tumor progression by promoting angiogenesis, epithelial-to-mesenchymal transition, and metabolic reprogramming (Mucaj et al., 2012; Semenza, 2012b). It is therefore imperative to understand the molecular basis by which cells respond to this stress. The transcription factors HIF1A and HIF2A are the key factors governing the transcriptional response to hypoxia, yet the mechanisms by which they regulate RNAPII have yet to be fully elucidated (Dengler et al., 2014). Here, we identified a conserved role for the TIP60 complex in HIF1A-dependent transactivation and investigated differential requirements for TIP60 and CDK8 as coactivators in the regulation of HIF1A target genes in human cancer cells.

Repeatedly, the related KATs p300 and CBP have been reported as key HIF1A coactivators (Arany et al., 1996; Cho et al., 2007; Ebert and Bunn, 1998; Ema et al., 1999; Kallio et al., 1998; Ruas et al., 2010). However, mutation of their CH1 domains to disrupt HIF interaction was shown to affect expression of only a small number of HIF target genes (Kasper et al., 2005). Our data show that a different KAT, TIP60, contributes to the induction of specific HIF1A targets and suggest that it may be a common driver of HIF1A-driven histone acetylation. We found that TIP60 is required for HIF1-dependent gene expression in *Drosophila* and two different human cancer cell lines, HIF1A interacts with and recruits TIP60 to chromatin, and TIP60 is dispensable for HIF1A association with its target genes but is required for HIF1A-dependent chromatin modification and RNAPII activation in hypoxia.

It has been suggested that Pontin and Reptin play opposite roles in HIF1-dependent transcription (Lee et al., 2010, 2011). However, we found here that both Pontin and Reptin are required for HIF1 transcriptional activity in *Drosophila* cultured cells and embryos (Figures 1A and 1B). This contrasting requirement could be explained by locus-specific functions and the critical role of Pontin and Reptin in assembly of the TIP60 complex and its acetyl-transferase activity (Jha et al., 2013). In agreement with this notion, we found that depletion of the Tip60 or Domino (p400) subunits reproduced the gene-specific effects of Pontin and Reptin depletion in S2 cells (Figure 1B). Thus, we focused our investigation on the defining subunit of the complex, TIP60 (KAT5).

Recently, we demonstrated that a specific variant of the Mediator complex plays a widespread role in HIF1A coactivation (Galbraith et al., 2013). Our transcriptome analysis shows that HIF1A employs TIP60, CDK8-Mediator, or both as coactivators across much of its transcriptional program. Together, these two coactivators contribute to induced expression of >60% of HIF1A-driven genes. It would be interesting to determine in our system what fraction of HIF1A targets requires other known coactivators, such as PKM2 (Luo et al., 2011).

Why do different HIF1A target genes display differential requirement for TIP60, CDK8, or other coactivators? Using publicly available ENCODE data for the HCT116 cell line, we looked for differences in regulatory chromatin modifications but did not find any striking differences between our gene classes that might explain differential cofactor requirement. Another possibility is that coactivator requirement is defined by the combinatorial action of HIF1A and its partner transcription factors (Dang et al., 2008; Gray et al., 2005; Xia and Kung, 2009). Our bioinformatics analysis supports this notion by predicting multiple transcription factors in addition to HIF1A as upstream regulators of our various gene classes (Figures S4B and S4C). Mechanistically, the coordinated binding of HIF1A and a specific partner transcription factor may allow recruitment of different coactivators that are required at specific genes. In turn, this layered action of partner transcription factors and coactivators may provide flexibility to the HIF1A-driven transcriptional program, veering it toward different cellular outcomes in a context-dependent manner. Furthermore, we observed enrichment of different cellular pathways between our gene classes (Figure S4D), and it would therefore be interesting to examine the role of HIF1A coactivators and

values indicate closer proximity. Data are represented as mean + SEM from three independent replicates. None of the comparisons between untreated and DFX values gave p values < 0.05 by t test.

(D) HT pull-down from HCT116 cells co-expressing HT-TIP60, HT-Pontin, HT-Reptin, or HT control with HIF1A-NL. Pull-down of HIF1A-NL by physical association with HT-TIP60, or HT control, was measured by detection of luciferase activity. Data are represented as mean + SEM from at least three independent replicates. None of the comparisons between untreated and DFX values gave p values < 0.05 by t test.

See also Figure S2.

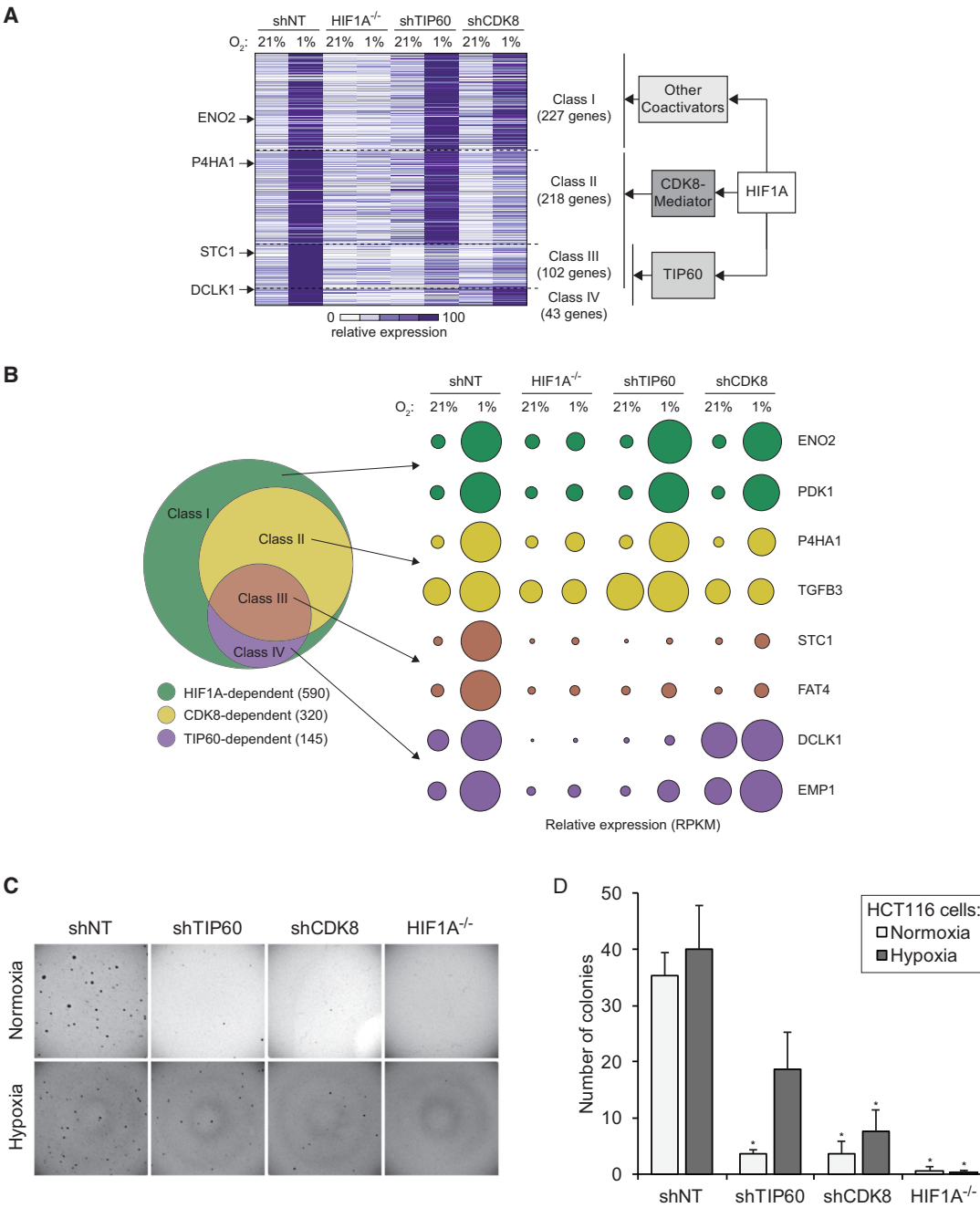


Figure 4. TIP60 and CDK8 Are Required for Expression of Specific Subsets of HIF1A Target Genes

Differential coactivator requirement as determined by RNA-seq analysis (includes datasets used in Figure 1).

(A) Comparison of mRNA levels for hypoxia-inducible genes in shNT control, *HIF1A*$^{-/-}$, shTIP60, and shCDK8 HCT116 cells in normoxia and hypoxia (1% O_2 24 hr). HIF1A-dependent hypoxia-inducible genes were divided into four non-overlapping classes based on their coactivator dependency: CDK8- and TIP60 independent (class I); CDK8 dependent (class II); TIP60 dependent (class IV); and dependent on both TIP60 and CDK8 (class III). Hypoxia-inducible genes were defined as ratio (shNT normoxia/shNT hypoxia) ≥ 2, FDR-adjusted p value < 10%; cofactor requirement was defined as ratio ≤ 0.9, FDR-adjusted p value < 10% for *HIF1A*$^{-/-}$, shTIP60, or shCDK8 over shNT cells in hypoxia. Heatmap shows mRNA levels as RPKM (reads per kilobase per million) for all HIF1A-dependent hypoxia-inducible genes, normalized to the maximum signal for that gene.

(B) Left: Venn diagram showing the relative size of each class. Right: bubble plots showing relative mRNA levels for example known direct HIF1A target genes within each class are shown. Surface area corresponds to RPKM values relative to shNT control cells in hypoxia.

(C and D) Anchorage-independent growth assay showing the ability of shNT control versus shTIP60, shCDK8, or *HIF1A*$^{-/-}$ HCT116 cells to form colonies in soft agar. Data are represented as mean + SEM. Asterisks indicate p values ≤ 0.05 by t test against shNT in normoxia or hypoxia.

See also Figures S3 and S4.

putative partner transcription factors (TFs) in regulation of these pathways and phenotypic outcomes during hypoxia.

Many cancer cells experience hypoxia due to uncontrolled proliferation and aberrant blood supply within tumors. In hypoxic cancer cells, HIFs regulate genes involved in metabolic reprograming, angiogenesis, stemness, epithelial-to-mesenchymal transition, invasion, metastasis, apoptosis, and resistance to radiation and chemotherapy (Dengler et al., 2014; Semenza, 2012b). Accordingly, hypoxic tumors expressing high levels of HIFs are known to be more aggressive and resistant to various therapies. Our data here suggest that TIP60 and CDK8, by regulating a fraction of the HIF1A transcription program, may contribute to survival and proliferation of cancer cells during hypoxia. Because both TIP60 and CDK8 are potentially amenable to pharmacological inhibition (Cee et al., 2009; Ghizzoni et al., 2012), it is possible to envision strategies for therapeutic remodeling of the hypoxic response by selectively blocking the activity of these HIF1A coactivators.

Altogether, the results presented here demonstrate a conserved role for the TIP60 complex in the transcriptional response to hypoxia, alone or in combination with CDK8-Mediator, at HIF1A target genes. This paves the road for future studies aimed at defining the mechanistic basis and biological implications of this functional relationship between three well-recognized players in gene expression control.

EXPERIMENTAL PROCEDURES

Fly Stocks and β-Galactosidase Assay

Flies were yw; yw, LDH-LacZ (Lavista-Llanos et al., 2002). Pont5.1/TM3 (*pontin*-) and rept35/TM3 (*reptin*-) flies were generously provided by Dr. Peter Gallant (Julius Maximilians University of Würzburg). To measure β-galactosidase activity of the hypoxia-responsive reporter, embryos were exposed to 21% or 5% O_2 for 4 hr. Embryos were then bleach-dechorionated, fixed with 0.5% glutaraldehyde, washed with PBS + 1% Triton, and incubated with a solution of X-gal.

Drosophila S2 Cell Culture, dsRNA Synthesis, and RNAi

Drosophila S2 cells were maintained at 25°C in Schneider medium (Sigma), supplemented with 10% fetal bovine serum (GIBCO), 50 μg/ml streptomycin, and 50 units/ml penicillin. Double-stranded RNAs (dsRNAs) were synthesized from cDNA with the T7 Megascript kit (Ambion). See Table S3 for dsRNA sequences. Transfection of S2 cells with dsRNAs was performed in 24-well plates (Greiner) using the "bathing" method as previously described (Clemens et al., 2000). After 4 days, cells were exposed to 1% O_2 or kept in normoxia for 20 hr. RNA was extracted using Trizol (Life Technologies), cDNA synthesized using the SuperScript III First-Strand Synthesis System (Life Technologies), and gene expression analyzed by real-time PCR in a Stratagene Mx3005P system (Agilent Technologies). Gene expression was normalized to RPL29 levels.

Mammalian Cell Culture and Stable shRNA Knockdown Cell Lines

Cells were cultured in McCoy's 5A (GIBCO/Life Technologies) or DMEM medium (Sigma) supplemented with 10% fetal bovine serum and antibiotic-antimycotic mixture (GIBCO/Life Technologies) under 5% CO_2 at 37°C. HCT116 *HIF1A*$^{-/-}$ cells were created by disrupting exons 3 and 4 of the *HIF1A* locus using adeno-associated virus-mediated homologous recombination, resulting in a 226-bp deletion with translation stop codons in all three reading frames (Dang et al., 2006). Cells were plated 24 hr prior to experimental treatments and harvested in radioimmunoprecipitation assay (RIPA) lysis buffer for protein or Trizol for total RNA. Hypoxia treatments were carried out in incubation chambers (Billups-Rothenberg) by flushing twice with 120 l of a mixture of 1% O_2/5% CO_2/94% N_2 (Airgas) and incubated for 24 hr at 37°C. Individual knockdown cell lines were generated using Sigma Mission shRNA lentiviral plasmids (pLKO.1-puro), as described previously (Galbraith et al., 2013). See Table S3 for shRNA sequences.

RNA-Seq Analysis, ChIP, HT Pull-Down, and NanoBRET Assay

See Supplemental Experimental Procedures.

ACCESSION NUMBERS

The accession number for the RNA-seq data reported in this paper is GEO: GSE68297.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.05.082.

AUTHOR CONTRIBUTIONS

J.I.P.-P. and V.L.D. conceived and designed experiments; acquired, analyzed, and interpreted data; and drafted the article. K.A.A., M.U., J.M., and D.L.D. acquired and analyzed data. A.P. analyzed data. P.W. conceived the study and revised the article. M.D.G. conceived and designed experiments; acquired, analyzed, and interpreted data; and drafted and revised the article. J.M.E. conceived and designed experiments, interpreted data, and drafted and revised the article.

ACKNOWLEDGMENTS

We are grateful to all members of the J.M.E. lab for invaluable advice and reagents and Bruce Dickinson for helpful discussions. We thank Dr. Dylan Taatjes for the TRRAP antibody and Dr. Bruno Amati for TIP60 antibodies. We thank Long Dang for the HCT116 *HIF1A*$^{-/-}$ cells. This work was supported primarily by a grant from the National Science Foundation (NSF MCB-1243522) and by NIH grant 5R01CA117907, NIH training grant T32 GM08759 to V.L.D., and NIH grant P30-CA046934-27. M.U., J.M., and D.L.D. are employees of Promega Corporation. Promega Corporation is the commercial owner by assignment of patents of the HaloTag technology and its applications.

Received: October 15, 2015
Revised: March 9, 2016
Accepted: May 19, 2016
Published: June 16, 2016

REFERENCES

Arany, Z., Huang, L.E., Eckner, R., Bhattacharya, S., Jiang, C., Goldberg, M.A., Bunn, H.F., and Livingston, D.M. (1996). An essential role for p300/CBP in the cellular response to hypoxia. Proc. Natl. Acad. Sci. USA *93*, 12969–12973.

Cee, V.J., Chen, D.Y., Lee, M.R., and Nicolaou, K.C. (2009). Cortistatin A is a high-affinity ligand of protein kinases ROCK, CDK8, and CDK11. Angew. Chem. Int. Ed. Engl. *48*, 8952–8957.

Cho, H., Ahn, D.R., Park, H., and Yang, E.G. (2007). Modulation of p300 binding by posttranslational modifications of the C-terminal activation domain of hypoxia-inducible factor-1alpha. FEBS Lett. *581*, 1542–1548.

Clemens, J.C., Worby, C.A., Simonson-Leff, N., Muda, M., Maehama, T., Hemmings, B.A., and Dixon, J.E. (2000). Use of double-stranded RNA interference in Drosophila cell lines to dissect signal transduction pathways. Proc. Natl. Acad. Sci. USA *97*, 6499–6503.

Dang, D.T., Chen, F., Gardner, L.B., Cummins, J.M., Rago, C., Bunz, F., Kantsevoy, S.V., and Dang, L.H. (2006). Hypoxia-inducible factor-1alpha promotes nonhypoxia-mediated proliferation in colon cancer cells and xenografts. Cancer Res. *66*, 1684–1936.

Dang, C.V., Kim, J.W., Gao, P., and Yustein, J. (2008). The interplay between MYC and HIF in cancer. Nat. Rev. Cancer *8*, 51–56.

Dekanty, A., Romero, N.M., Bertolin, A.P., Thomas, M.G., Leishman, C.C., Perez-Perri, J.I., Boccaccio, G.L., and Wappner, P. (2010). Drosophila genome-wide RNAi screen identifies multiple regulators of HIF-dependent transcription in hypoxia. PLoS Genet. *6*, e1000994.

Dengler, V.L., Galbraith, M.D., and Espinosa, J.M. (2014). Transcriptional regulation by hypoxia inducible factors. Crit. Rev. Biochem. Mol. Biol. *49*, 1–15.

Dunwoodie, S.L. (2009). The role of hypoxia in development of the mammalian embryo. Dev. Cell *17*, 755–773.

Ebert, B.L., and Bunn, H.F. (1998). Regulation of transcription by hypoxia requires a multiprotein complex that includes hypoxia-inducible factor 1, an adjacent transcription factor, and p300/CREB binding protein. Mol. Cell. Biol. *18*, 4089–4096.

Ema, M., Hirota, K., Mimura, J., Abe, H., Yodoi, J., Sogawa, K., Poellinger, L., and Fujii-Kuriyama, Y. (1999). Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. EMBO J. *18*, 1905–1914.

Galbraith, M.D., Allen, M.A., Bensard, C.L., Wang, X., Schwinn, M.K., Qin, B., Long, H.W., Daniels, D.L., Hahn, W.C., Dowell, R.D., and Espinosa, J.M. (2013). HIF1A employs CDK8-mediator to stimulate RNAPII elongation in response to hypoxia. Cell *153*, 1327–1339.

Gallant, P. (2007). Control of transcription by Pontin and Reptin. Trends Cell Biol. *17*, 187–192.

Ghizzoni, M., Wu, J., Gao, T., Haisma, H.J., Dekker, F.J., and George Zheng, Y. (2012). 6-alkylsalicylates are selective Tip60 inhibitors and target the acetyl-CoA binding site. Eur. J. Med. Chem. *47*, 337–344.

Gray, M.J., Zhang, J., Ellis, L.M., Semenza, G.L., Evans, D.B., Watowich, S.S., and Gallick, G.E. (2005). HIF-1alpha, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. Oncogene *24*, 3110–3120.

Indovina, P., Collini, M., Chirico, G., and Santini, M.T. (2007). Three-dimensional cell organization leads to almost immediate HRE activity as demonstrated by molecular imaging of MG-63 spheroids using two-photon excitation microscopy. FEBS Lett. *581*, 719–726.

Jha, S., Gupta, A., Dar, A., and Dutta, A. (2013). RVBs are required for assembling a functional TIP60 complex. Mol. Cell. Biol. *33*, 1164–1174.

Johnson, A.B., Denko, N., and Barton, M.C. (2008). Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. Mutat. Res. *640*, 174–179.

Jónsson, Z.O., Jha, S., Wohlschlegel, J.A., and Dutta, A. (2004). Rvb1p/Rvb2p recruit Arp5p and assemble a functional Ino80 chromatin remodeling complex. Mol. Cell *16*, 465–477.

Kallio, P.J., Okamoto, K., O'Brien, S., Carrero, P., Makino, Y., Tanaka, H., and Poellinger, L. (1998). Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha. EMBO J. *17*, 6573–6586.

Kasper, L.H., Boussouar, F., Boyd, K., Xu, W., Biesen, M., Rehg, J., Baudino, T.A., Cleveland, J.L., and Brindle, P.K. (2005). Two transactivation mechanisms cooperate for the bulk of HIF-1-responsive gene expression. EMBO J. *24*, 3846–3858.

Lavista-Llanos, S., Centanin, L., Irisarri, M., Russo, D.M., Gleadle, J.M., Bocca, S.N., Muzzopappa, M., Ratcliffe, P.J., and Wappner, P. (2002). Control of the hypoxic response in Drosophila melanogaster by the basic helix-loop-helix PAS protein similar. Mol. Cell. Biol. *22*, 6842–6853.

Lee, K.E., and Simon, M.C. (2012). From stem cells to cancer stem cells: HIF takes the stage. Curr. Opin. Cell Biol. *24*, 232–235.

Lee, J.S., Kim, Y., Kim, I.S., Kim, B., Choi, H.J., Lee, J.M., Shin, H.J., Kim, J.H., Kim, J.Y., Seo, S.B., et al. (2010). Negative regulation of hypoxic responses via induced Reptin methylation. Mol. Cell *39*, 71–85.

Lee, J.S., Kim, Y., Bhin, J., Shin, H.J., Nam, H.J., Lee, S.H., Yoon, J.B., Binda, O., Gozani, O., Hwang, D., and Baek, S.H. (2011). Hypoxia-induced methylation of a pontin chromatin remodeling factor. Proc. Natl. Acad. Sci. USA *108*, 13510–13515.

Leek, R.D., Stratford, I., and Harris, A.L. (2005). The role of hypoxia-inducible factor-1 in three-dimensional tumor growth, apoptosis, and regulation by the insulin-signaling pathway. Cancer Res. *65*, 4147–4152.

Lin, H.H., Li, X., Chen, J.L., Sun, X., Cooper, F.N., Chen, Y.R., Zhang, W., Chung, Y., Li, A., Cheng, C.T., et al. (2012). Identification of an AAA ATPase VPS4B-dependent pathway that modulates epidermal growth factor receptor abundance and signaling during hypoxia. Mol. Cell. Biol. *32*, 1124–1138.

Luo, W., Hu, H., Chang, R., Zhong, J., Knabel, M., O'Meally, R., Cole, R.N., Pandey, A., and Semenza, G.L. (2011). Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell *145*, 732–744.

Machleidt, T., Woodroofe, C.C., Schwinn, M.K., Méndez, J., Robers, M.B., Zimmerman, K., Otto, P., Daniels, D.L., Kirkland, T.A., and Wood, K.V. (2015). NanoBRET–a novel BRET platform for the analysis of protein-protein interactions. ACS Chem. Biol. *10*, 1797–1804.

Majmundar, A.J., Wong, W.J., and Simon, M.C. (2010). Hypoxia-inducible factors and the response to hypoxic stress. Mol. Cell *40*, 294–309.

Mazumdar, J., Dondeti, V., and Simon, M.C. (2009). Hypoxia-inducible factors in stem cells and cancer. J. Cell. Mol. Med. *13*, 4319–4328.

Meng, F., Evans, J.W., Bhupathi, D., Banica, M., Lan, L., Lorente, G., Duan, J.X., Cai, X., Mowday, A.M., Guise, C.P., et al. (2012). Molecular and cellular pharmacology of the hypoxia-activated prodrug TH-302. Mol. Cancer Ther. *11*, 740–751.

Mucaj, V., Shay, J.E., and Simon, M.C. (2012). Effects of hypoxia and HIFs on cancer metabolism. Int. J. Hematol. *95*, 464–470.

Onnis, B., Fer, N., Rapisarda, A., Perez, V.S., and Melillo, G. (2013). Autocrine production of IL-11 mediates tumorigenicity in hypoxic cancer cells. J. Clin. Invest. *123*, 1615–1629.

Patel, J.H., Du, Y., Ard, P.G., Phillips, C., Carella, B., Chen, C.J., Rakowski, C., Chatterjee, C., Lieberman, P.M., Lane, W.S., et al. (2004). The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. Mol. Cell. Biol. *24*, 10826–10834.

Rohwer, N., Welzel, M., Daskalow, K., Pfander, D., Wiedenmann, B., Detjen, K., and Cramer, T. (2008). Hypoxia-inducible factor 1alpha mediates anoikis resistance via suppression of alpha5 integrin. Cancer Res. *68*, 10113–10120.

Ruas, J.L., Poellinger, L., and Pereira, T. (2002). Functional analysis of hypoxia-inducible factor-1 alpha-mediated transactivation. Identification of amino acid residues critical for transcriptional activation and/or interaction with CREB-binding protein. J. Biol. Chem. *277*, 38723–38730.

Ruas, J.L., Poellinger, L., and Pereira, T. (2005). Role of CBP in regulating HIF-1-mediated activation of transcription. J. Cell Sci. *118*, 301–311.

Ruas, J.L., Berchner-Pfannschmidt, U., Malik, S., Gradin, K., Fandrey, J., Roeder, R.G., Pereira, T., and Poellinger, L. (2010). Complex regulation of the transactivation function of hypoxia-inducible factor-1 alpha by direct interaction with two distinct domains of the CREB-binding protein/p300. J. Biol. Chem. *285*, 2601–2609.

Sapountzi, V., Logan, I.R., and Robson, C.N. (2006). Cellular functions of TIP60. Int. J. Biochem. Cell Biol. *38*, 1496–1509.

Semenza, G.L. (2009). Regulation of oxygen homeostasis by hypoxia-inducible factor 1. Physiology (Bethesda) *24*, 97–106.

Semenza, G.L. (2012a). Hypoxia-inducible factors in physiology and medicine. Cell *148*, 399–408.

Semenza, G.L. (2012b). Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol. Sci. *33*, 207–214.

Sun, Y., Jiang, X., Chen, S., Fernandes, N., and Price, B.D. (2005). A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. Proc. Natl. Acad. Sci. USA *102*, 13182–13187.

Sutherland, R.M., Sordat, B., Bamat, J., Gabbert, H., Bourrat, B., and Mueller-Klieser, W. (1986). Oxygenation and differentiation in multicellular spheroids of human colon carcinoma. Cancer Res. *46*, 5320–5329.

Tang, Y., Luo, J., Zhang, W., and Gu, W. (2006). Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol. Cell *24*, 827–839.

Tang, Y., Zhao, W., Chen, Y., Zhao, Y., and Gu, W. (2008). Acetylation is indispensable for p53 activation. Cell *133*, 612–626.

Xia, X., and Kung, A.L. (2009). Preferential binding of HIF-1 to transcriptionally active loci determines cell-type specific response to hypoxia. Genome Biol. *10*, R113.

Xiao, Y., Nagai, Y., Deng, G., Ohtani, T., Zhu, Z., Zhou, Z., Zhang, H., Ji, M.Q., Lough, J.W., Samanta, A., et al. (2014). Dynamic interactions between TIP60 and p300 regulate FOXP3 function through a structural switch defined by a single lysine on TIP60. Cell Rep. *7*, 1471–1480.

Yeung, T.M., Gandhi, S.C., and Bodmer, W.F. (2011). Hypoxia and lineage specification of cell line-derived colorectal cancer stem cells. Proc. Natl. Acad. Sci. USA *108*, 4382–4387.