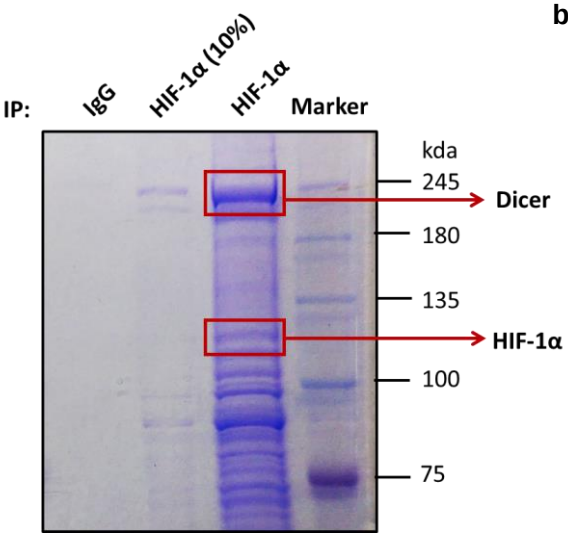
A



B

Peptides covering the entire sequence of Dicer identified by LC-MASS/MASS analysis of HIF-1α immunoprecipitates

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VGEYSNLEVN ASWTKERWNQ EFTKHQVLIM TCYVALNVLK NGYLSLSDIN LLVFDECHLA
ILDHPYREIM KLCENCPSCP RILGLTASIL NGKCDPEELE EKIQKLEKIL KSNAETATDL
VVLDRYTSQP CEIVVDCGPF TDRSGLYERL LMELEEALNF INDCNISVHS KERDSTLISK
QILSDCRAVL VVLGPWCADK VAGMMVRELQ KYIKHEQEEL HRKFLLFTDT FLRKIHALCE
EHFSPASLDL KFVTPKVIKL LEILRKYKPY ERQQFESVEW YNNRNQDNYV SWSDSEDDDE
DEEIEEKEKP ETNFPSPFTN ILCGIIFVER RYTAVVLNRL IKEAGKQDPE LAYISSNFIT
GHGIGKNQPR NKQMEAEFRK QEEVLRKFRA HETNLLIATS IVEEGVDIPK CNLVVRFDLP
TEYRSYVQSK GRARAPISNY IMLADTDKIK SFEEDLKTYK AIEKILRNKC SKSVDTGETD
IDPVMDDDDV FPPYVLRPDD GGPRVTINTA IGHINRYCAR LPSDPFTHLA PKCRTRELPD
GTFYSTLYLP INSPLRASIV GPPMSCVRLA ERVVALICCE KLHKIGELDD HLMPVGKETV
KYEEELDLHD EEETSVPGRP GSTKRRQCYP KAIPECLRDS YPRPDQPCYL YVIGMVLTTP
LPDELNFRRR KLYPPEDTTR CFGILTAKPI PQIPHFPVYT RSGEVTISIE LKKSGFMLSL
QMLELITRLH QYIFSHILRL EKPALEFKPT DADSAYCVLP LNVVNDSSTL DIDFKFMEDI
EKSEARIGIP STKYTKETPF VFKLEDYQDA VIIPRYRNFD QPHRFYVADV YTDLTPLSKF
PSPEYETFAE YYKTKYNLDL TNLNQPLLDV DHTSSRLNLL TPRHLNQKGK ALPLSSAEKR
KAKWESLQNK QILVPELCAI HPIPASLWRK AVCLPSILYR LHCLLTAEEL RAQTASDAGV
GVRSLPADFR YPNLDFGWKK SIDSKSFISI SNSSSAENDN YCKHSTIVPE NAAHQGANRT
SSLENHDQMS VNCRTLLSES PGKLHVEVSA DLTAINGLSY NQNLANGSYD LANRDFCQGN
QLNYYKQEIP VQPTTSYSIQ NLYSYENQPQ PSDECTLLSN KYLDGNANKS TSDGSPVMAV
MPGTTDTIQV LKGRMDSEQS PSIGYSSRTL GPNPGLILQA LTLSNASDGF NLERLEMLGD
SFLKHAITTY LFCTYPDAHE GRLSYMRSKK VSNCNLYRLG KKKGLPSRMV VSIFDPPVNW
LPPGYVVNQD KSNTDKWEKD EMTKDCMLAN GKLDEDYEEE DEEEESLMWR APKEEADYED
DFLEYDQEHI RFIDNMLMGS GAFVKKISLS PFSTTDSAYE WKMPKKSSLG SMPFSSDFED
FDYSSWDAMC YLDPSKAVEE DDFVVGFWNP SEENCGVDTG KQSISYDLHT EQCIADKSIA
DCVEALLGCY LTSCGERAAQ LFLCSLGLKV LPVIKRTDRE KALCPTRENF NSQQKNLSVS
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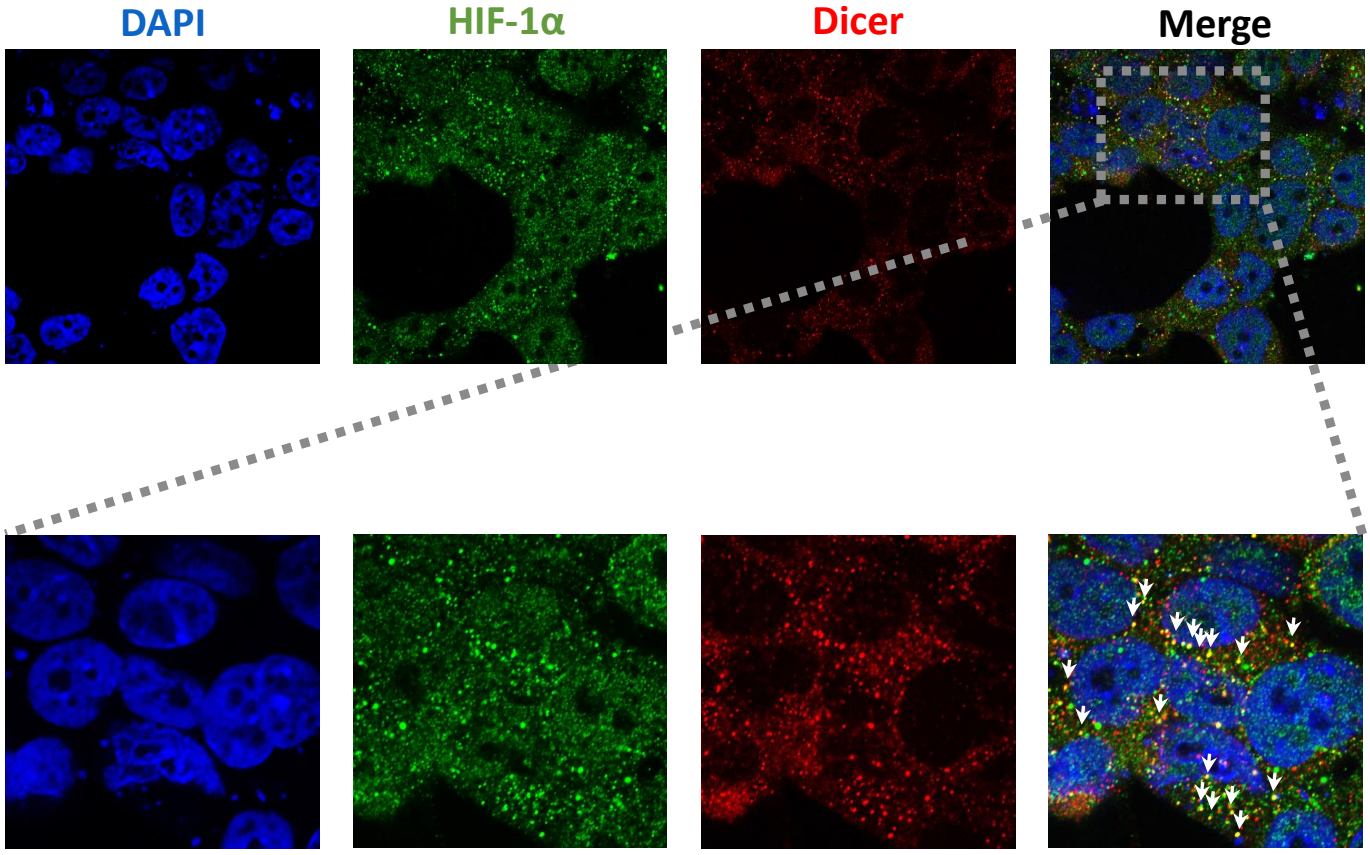
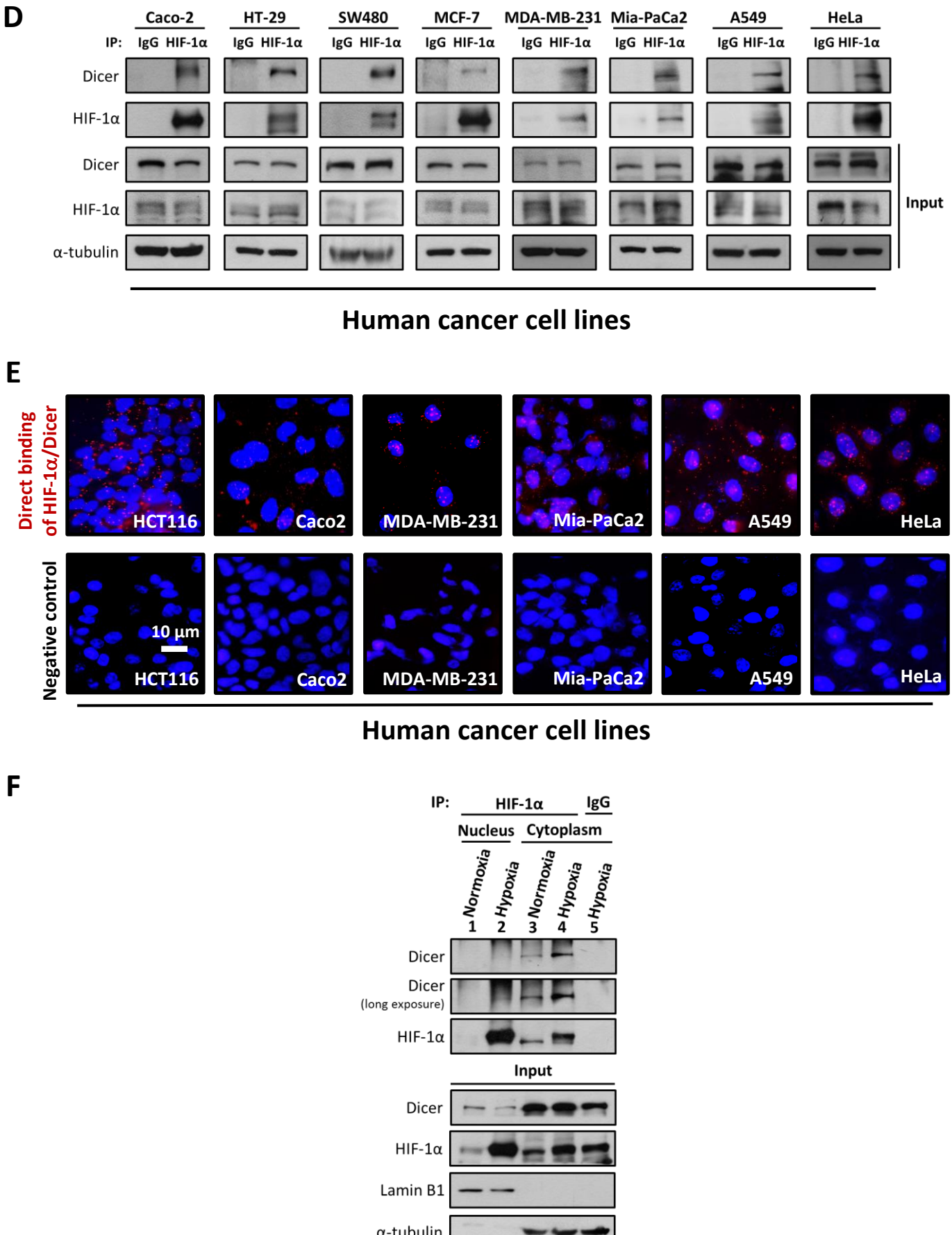
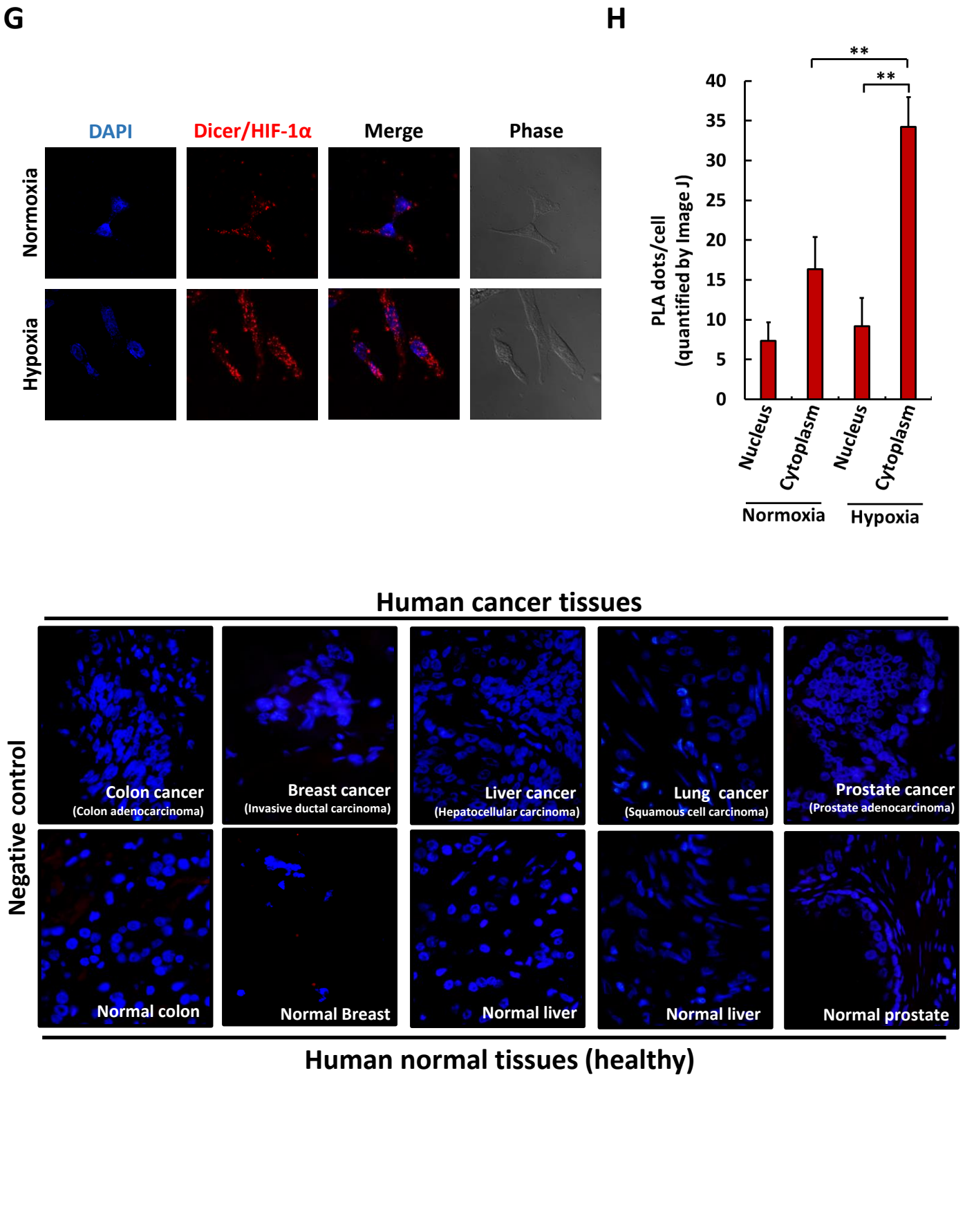
C



Supplemental Figure S1 (continued)



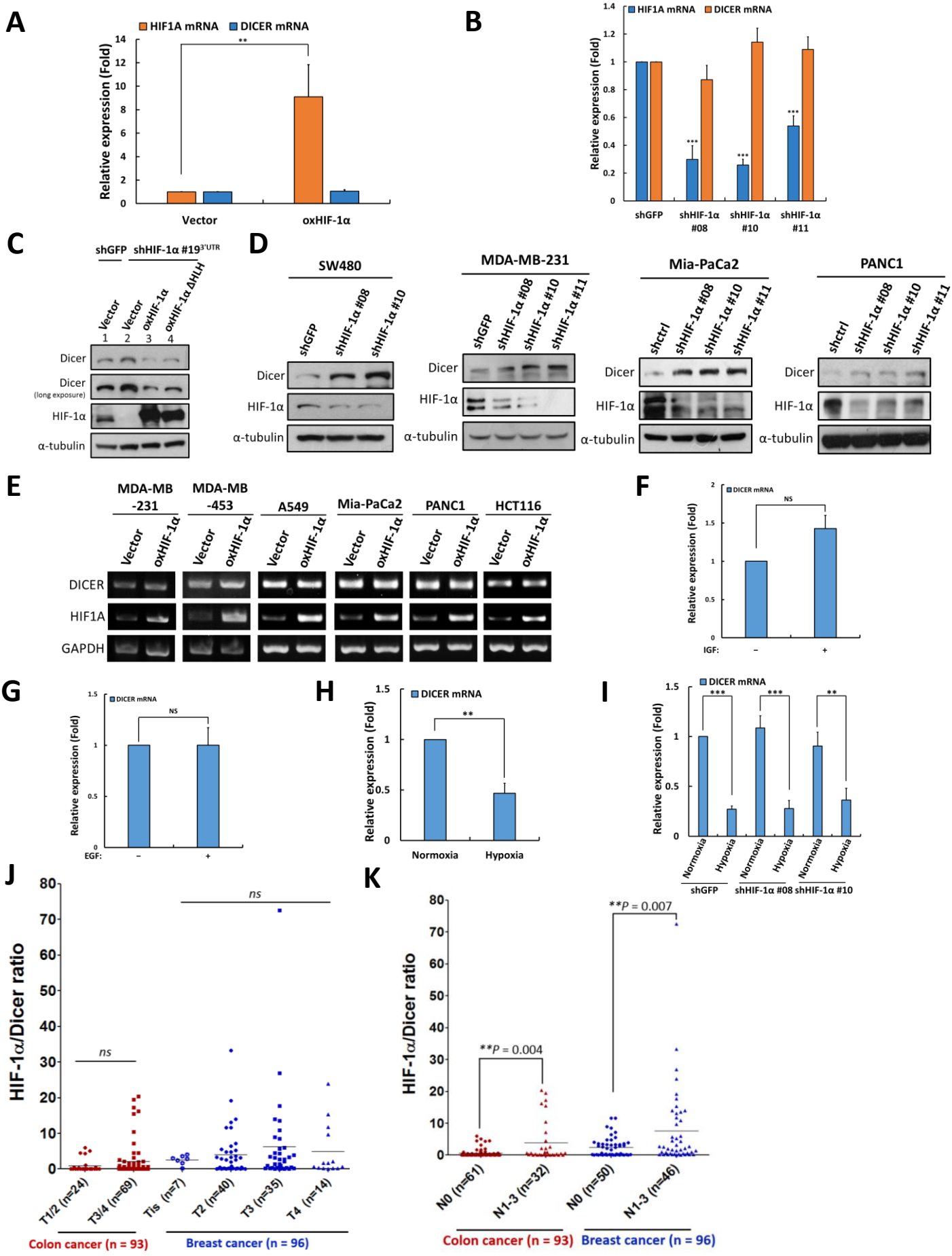
Supplemental Figure S1 (continued)



Supplemental Figure S1. Identification of Dicer as a component of the HIF-1α complex.

(**A**) Dicer was identified in whole cell extracts immunoprecipitated with anti-HIF-1α antibodies. A band corresponding to a protein having a molecular weight of about 220-240 kDa was cut from a Coomassie blue-stained gel. (**B**) The peptides indicated in red covering 15.83 % (266/1680 residues) of the entire sequence of Dicer were identified by LC-MS/MS. (**C**) Immunofluorescence staining was performed using anti-HIF-1α, anti-Dicer antibodies, and DAPI for nuclear staining in HCT116 cells. Images were obtained by using confocal microscope, as indicated. Dicer (Red); HIF-1α (Green); DAPI (Blue). (**D** and **E**) HIF-1α interacts with Dicer in multiple human cancer cell lines including Caco-2, HT29, SW480 (colon), MCF-7, MDA-MB-231 (breast), Mia-PaCa-2 (pancreas), A549 (lung) and HeLa (cervix) cancer cells. Interaction between HIF-1α and Dicer was detected by using immunoprecipitation (**D**) and *in situ* PLA (**E**). PLA signals are shown in red along with the DAPI nuclear staining (Blue). Each red fluorescent dot indicates the direct binding of the HIF-1α/Dicer complex in close distance (<40 nm) and was quantified by direct counting. (**E**, top). Cells were stained with only anti-HIF-1α antibodies were also analyzed as negative controls (**E**, bottom). (**F-H**) HCT116 cells were exposed under hypoxic conditions. The nuclear and cytoplasmic fractions were isolated from cell lysates and incubated with anti-Dicer antibody for immunoprecipitation. Lamin B1 was used as a nuclear loading control (**F**). The distribution of binding between endogenous Dicer and HIF-1α was determined by staining with *in situ* PLA and detected with a confocal microscope (**G**). The PLA dots were quantified with ImageJ software (**H**). (**I**) Human normal and cancer tissues used in Figure 1J were stained with only anti-HIF-1α antibodies as negative controls. The experiments in **F** were performed in the presence of CQ and NH_4Cl to block autophagy-lysosomal degradation. Mean ± S.D. (at least n = 3 per group) are shown. Multigroup comparisons were analyzed by one way ANOVA with Turkey's post hoc test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

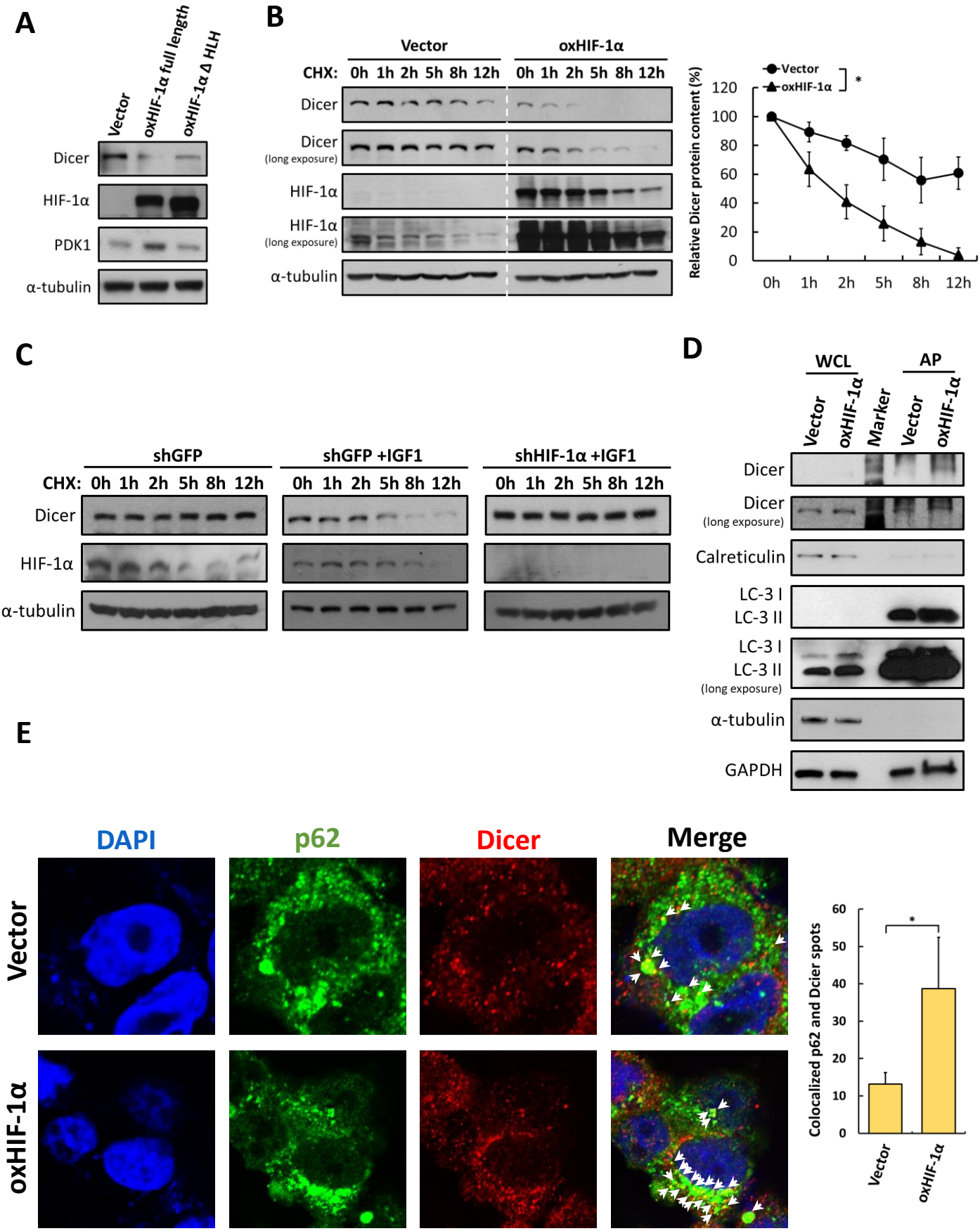
Supplemental Figure S2



Supplemental Figure S2. HIF-1α post-transcriptionally downregulates Dicer expression.

(**A** and **B**) Effects of HIF-1α on Dicer mRNA expression. HIF-1α was overexpressed (**A**) or knocked down (**B**) in HCT116 cells. HIF1A and DICER1 mRNA levels were assayed and were normalized to GAPDH. (**C**) Effects of HIF-1α restoration on regulating Dicer expression. Restoration of HIF-1α was performed with wild-type or HLH-truncated HIF-1α in HCT116 cells expressing shRNAs which target to 3'UTR of HIF1A mRNA (shHIF-1α #19$^{3'UTR}$). The cell lysates were collected and assayed by western blot for Dicer expression. (**D** and **E**) HIF-1α suppresses the protein expression of Dicer in multiple cancer cell lines. The cell lysates SW480, MDA-MB-231, Mia-PaCa-2, PANC-1 cells with knockdown of HIF-1α (**D**) were subjected to western blot analysis. The immunoblots presented were derived from replicate samples run on parallel gels (**D**, PANC1). (**E**) MDA-MB-231, MDA-MB-453, A549, Mia-PaCa-2, PANC-1, HCT116 cells with HIF-1α overexpression were subjected to RNA isolation and analyzed by RT-PCR for the expression of DICER1 and HIF1A mRNAs. (**F**-**H**) DICER1 mRNA is not affected by growth factor stimulation. IGF- (**F**), EGF-treated (**G**) HCT116 cells, and cells cultured under hypoxia (**H**) were analyzed by qRT-PCR for the expression of DICER1 mRNA. The level of DICER1 mRNA was normalized to GAPDH. (**I**) The hypoxia-induced downregulation of DICER1 mRNA is HIF-1α independent. HCT116 cells with HIF-1α knockdown were cultured under hypoxia and analyzed by qRT-PCR for the expression of DICER1 mRNA. The level of DICER1 was normalized to GAPDH. (**J** and **K**) Dicer and HIF-1α scores were counted and calculated as PLA dots/cell for analyzing their expression. The HIF-1α/Dicer ratio were used to study clinical associations with T (**J**), and N (**K**) in both colon and breast cancers. Mean ± S.D. (at least n = 3 per group) are shown. Unpaired two independent group were analyzed by two-tailed Student's *t* test. Multigroup comparisons were analyzed by one way ANOVA with Turkey's post hoc test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

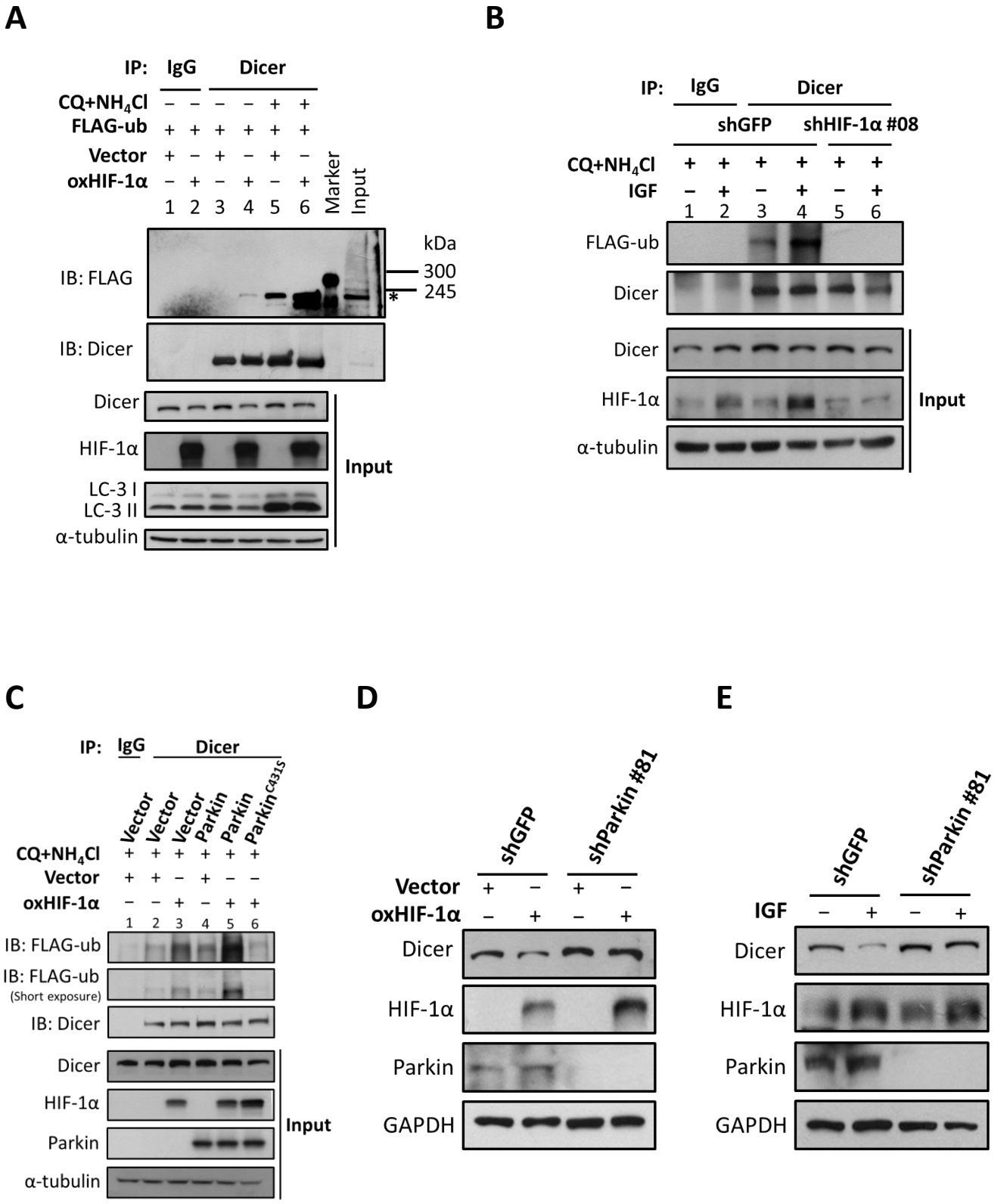
Supplemental Figure S3



Supplemental Figure S3. HIF-1α facilitates autophagic degradation of Dicer.

(**A**) HIF-1α downregulates Dicer in a manner independent of HIF-1α transcriptional activity. Effects of the non-transcriptional activity of HIF-1α on Dicer. Wild-type HIF-1α or HLH-truncated HIF-1α was overexpressed in HEK293T cells for western blot analysis for the expression of Dicer and PDK1. (**B**) HIF-1α destabilizes Dicer protein in Mia-PaCa2 cells. Mia-PaCa-2 cells were treated with cycloheximide (CHX) to block de novo protein synthesis. The cell lysates were collected and assayed by western blot for Dicer expression. Mean ± S.D. (n = 3) are shown. $^{*}P<0.05$, by two way ANOVA. All of the cell lysates were run on the same gel. (**C**) IGF-induced HIF-1α destabilizes Dicer protein. HCT116 cells were pre-treated with IGF for 16 hours. The cells were then treated with cycloheximide (CHX) to block de novo protein synthesis. The cell lysates were collected and assayed by western blot for Dicer expression. The immunoblots presented were derived from replicate samples run on parallel gels. (**D** and **E**) HIF-1α induces translocation of Dicer into autophagosomes. (**D**) Vector- and HIF-1α-transfected HCT116 cells were harvested and subjected to autophagosome (AP) isolation. The immunoblots presented were derived from replicate samples run on parallel gels. (**E**) Fluorescence immunostaining analysis was performed using anti-p62, anti-Dicer antibodies, and DAPI for nuclear staining. Images were obtained by using confocal microscope, as indicated. Dicer (Red); p62 (Green); DAPI (Blue) (**E**, left). The yellow spots of co-localization were isolated from the original images and calculated with ImageJ software (**E**, right). The experiments in **D** and **E** were performed in the presence of CQ and NH_4Cl to block autophagy-lysosomal degradation.

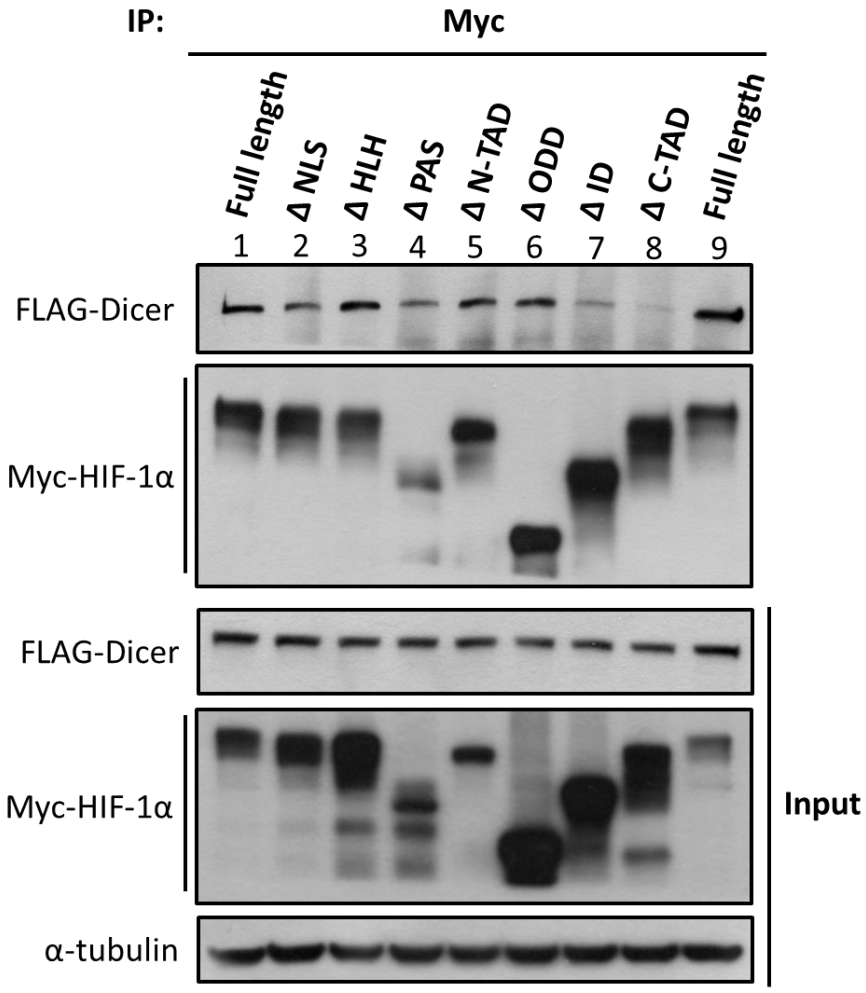
Supplemental Figure S4



Supplemental Figure S4. Ectopically-expressed and IGF-induced HIF-1α enhances Parkin-mediated ubiquitination of Dicer.

(**A**) HIF-1α induces ubiquitination of Dicer. FLAG-ubiquitin was expressed in HCT116 cells for Dicer ubiquitination assays. The immunoprecipitates isolated by anti-Dicer antibodies were subjected to western blot for determining the expression of FLAG-Ubiquitin, indicating the ubiquitination levels of Dicer. LC3 was detected for autophagosome accumulation after treatment with CQ and NH_4Cl. **(B**) HIF-1α is required for IGF to enhance ubiquitination of Dicer. FLAG-Ubiquitin-expressing HCT116 cells were treated with IGF for 24 hours. The immunoprecipitates were isolated by anti-Dicer antibodies and were subjected to western blot for determining the ubiquitination levels of Dicer. The experiments were performed in the presence of CQ and NH_4Cl to block autophagy-lysosomal degradation. (**C**) HIF-1α and Parkin or $Parkin^{C431S}$ with a mutated catalytic domain were co-expressed in HCT116 cells to determine the effects of Parkin on the ubiquitination of Dicer. (**D** and **E**) Role of Parkin on HIF-1α-mediated down-regulation of Dicer. HIF-1α was induced by HIF-1α overexpression (**D**) or IGF treatment (**E**) in HCT116 cells with shGFP or shParkin. The experiments in **B** and **C** were performed in the presence of CQ and NH_4Cl to block autophagy-lysosomal degradation.

Supplemental Figure S5

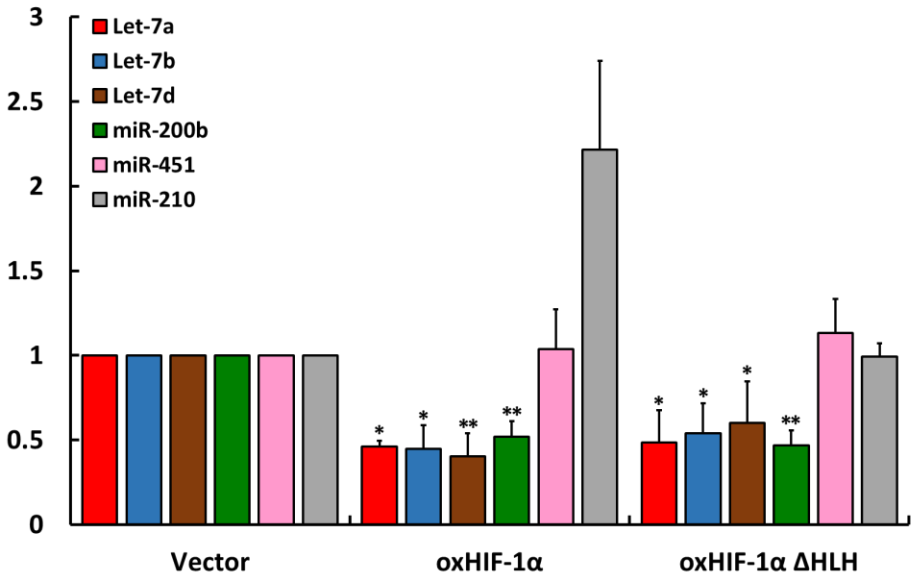


Supplemental Figure S5. The ID and C-TAD domains of HIF-1α are required for the protein-protein interaction with Dicer.

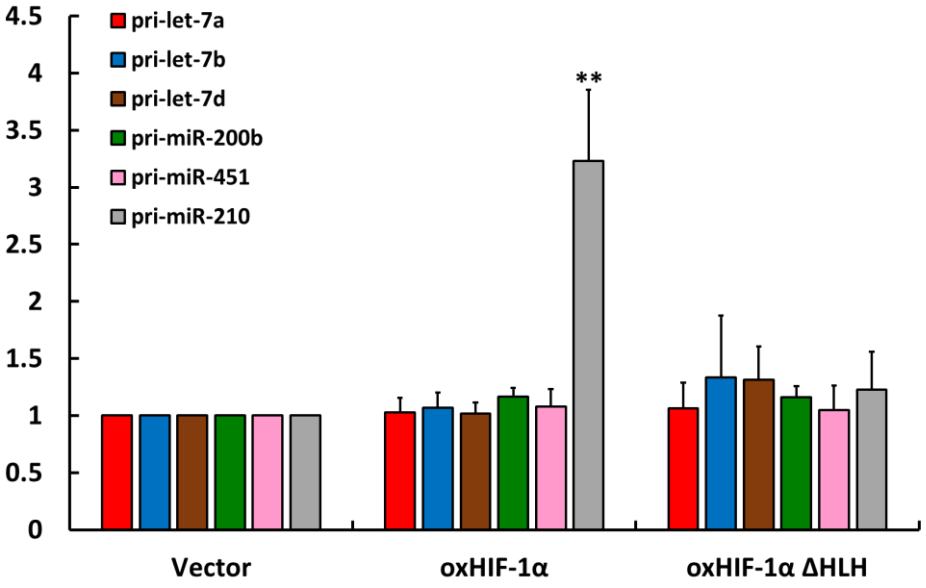
FLAG-Dicer and individual Myc-tagged HIF-1α truncations were co-expressed in HEK293T cells. The immunoprecipitates isolated by anti-Myc-tag abtibodies were subjected to western blot analysis to detect the interaction between truncated Myc-HIF-1α and FLAG-Dicer. This experiment was performed in the presence of CQ and NH_4Cl to block autophagy-lysosomal degradation.

Supplemental Figure S6

A



B

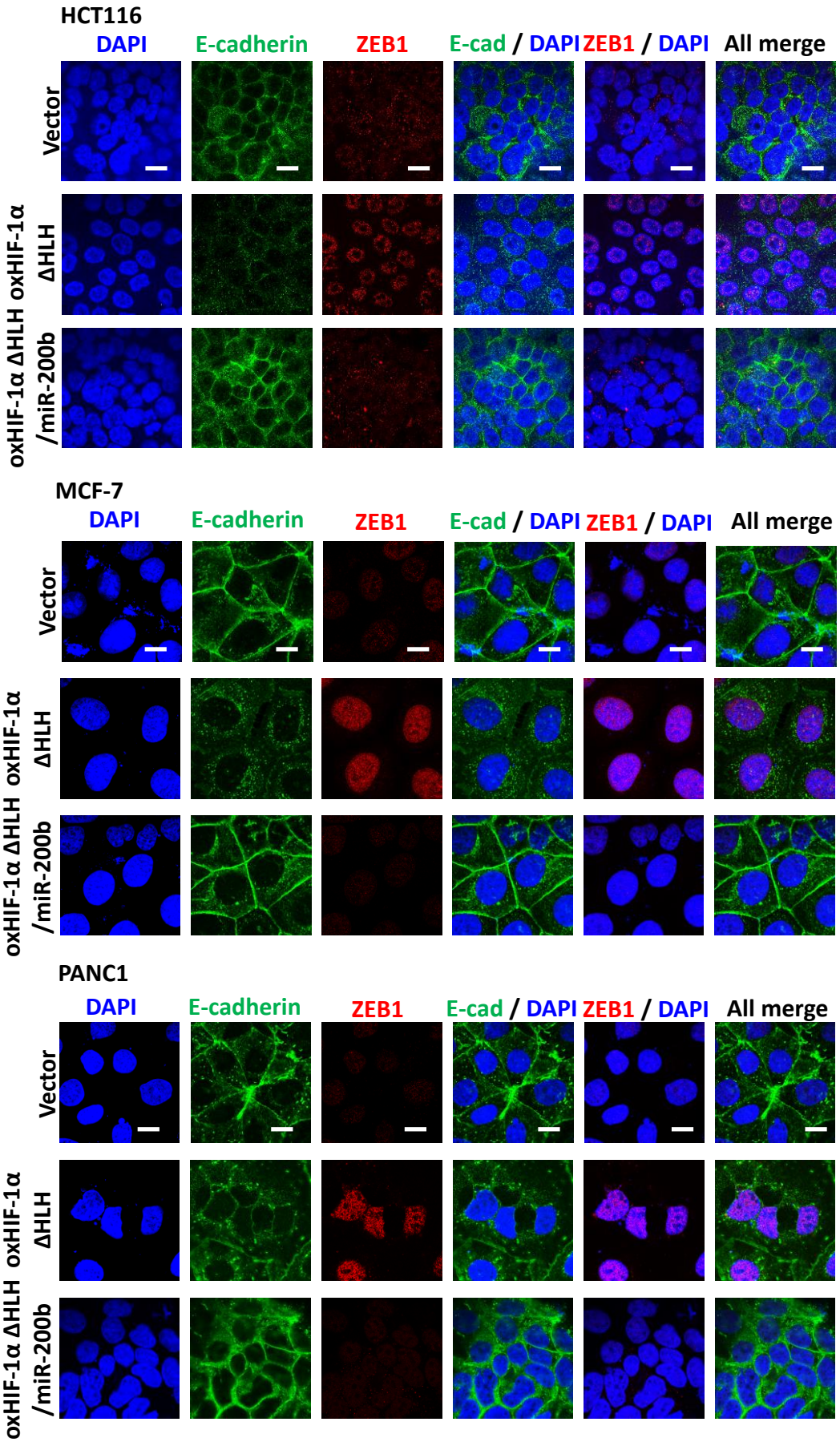


Supplemental Figure S6. Transcription-independent roles of HIF-1α in miRNA biogenesis.

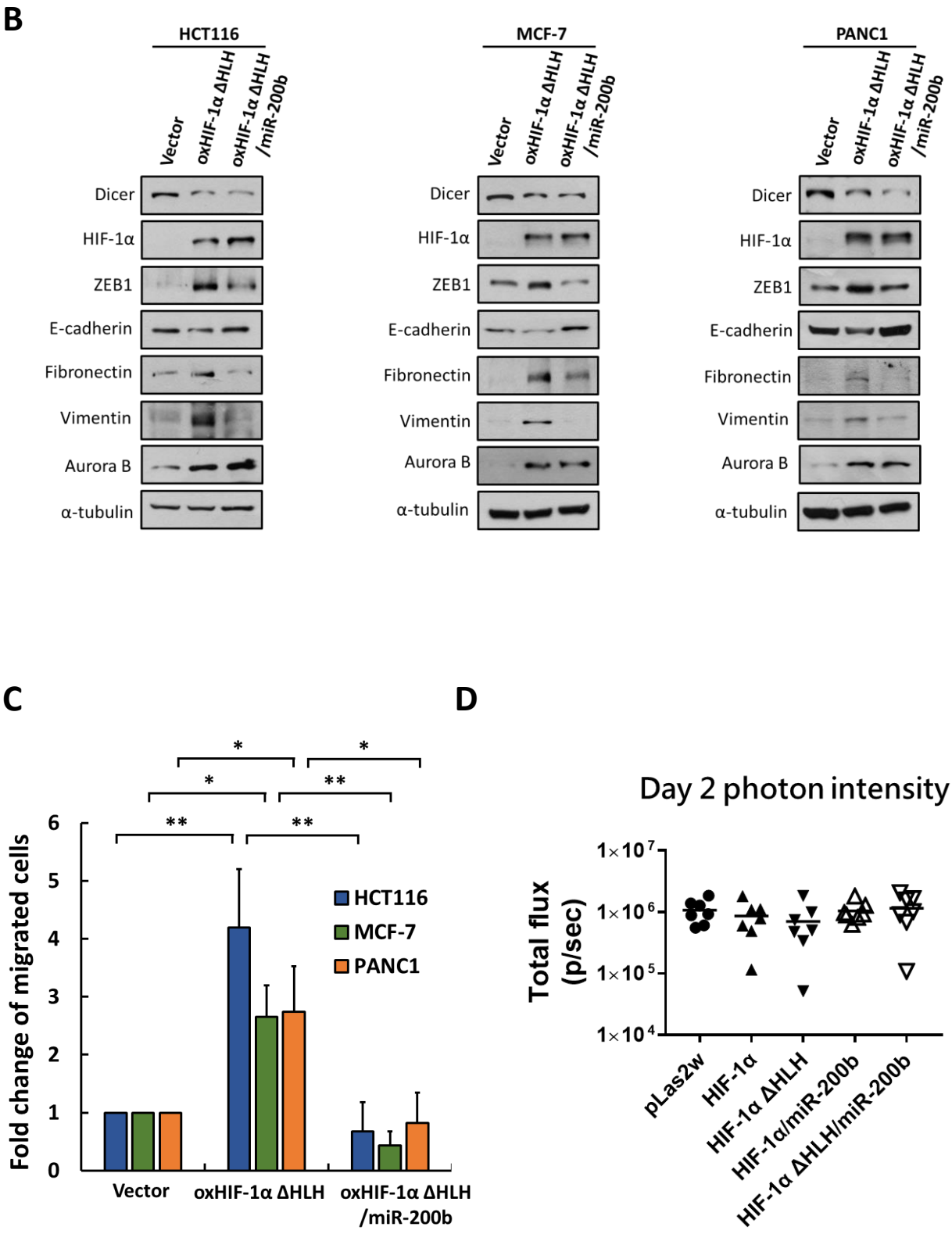
(**A** and **B**) HIF-1α-mediated effects on the expression of pri- and mature miRNAs. HCT116 cells overexpressing HIF-1α or HLH domain-truncated HIF-1α were subjected to qRT-PCR for analyzing the expression of both mature (**A**) and pri- (**B**) miRNAs, including let-7 family members (let-7a, b, d), miR-210, miR-200b, and miR-451. Mean ± S.D. (at least n = 3 per group) are shown. Multigroup comparisons were analyzed by one way ANOVA with Turkey's post hoc test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Supplemental Figure S7

A



Supplemental Figure S7 (continued)



Supplemental Figure S7. Transcription-independent roles of HIF-1α in EMT and cell migration.

(**A**-**C**) Transcription-independent roles of HIF-1α in miR-200b-mediated EMT and cell migration. HLH domain-truncated HIF-1α and miR-200b were co-expressed in HCT116 cells. Immunofluorescence staining was performed using anti-E-cadherin and anti-ZEB1 antibodies (A) or ZEB1, E-cadherin, Fibronectin and Vimentin were analyzed with western blot analysis (**B**) in HCT116, MCF-7 and PANC1 cells. Immunofluorescence images were obtained by using confocal microscope, as indicated: E-cadherin (Green); ZEB1 (Red); DAPI (Blue); scale bar: 10 μm. The effects on cell migration were measured using Boyden Chamber assays in HCT116, MCF-7 and PANC1 cells (**C**). The immunoblots presented were derived from replicate samples run on parallel gels (**B**). (**D**) The photon intensities of primary sites were monitored after intrasplenic injection of CT-26 cells for 2 days. Mean ± S.D. (at least n = 3 per group) are shown. Multigroup comparisons were analyzed by one way ANOVA with Turkey's post hoc test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Supplemental Table 1. Sequences and information of short hairpin RNA sequence

Target gene	shRNA sequence
GFP	shGFP: CCGGCCCGACCACATGAAGCAGCACCTCGAGGTGCTGCTTCATGTGGTCGGGTTTTTG
HIF1A	shHIF1A #08: CCGGCCGCTGGAGACACAATCATATCTCGAGATATGATTGTGTCTCCAGCG
	shHIF1A #10: CCGGGTGATGAAAGAATTACCGAATCTCGAGATTCGGTAATTCTTTCATCACTTTTT
	shHIF1A #11: CCGGCGGCGAAGTAAAGAATCTGAACTCGAGTTCAGATTCTTTACTTCGCCGTTTTT
	shHIF1A #19: CCGGTGCTCTTTGTGGTTGGATCTACTCGAGTAGATCCAACCACAAAGAGCATTTTT
DICER1	shDICER1 #58: CCGGGCTCGAAATCTTACGCAAATACTCGAGTATTTGCGTAAGATTTCGAGCTTTTTG
	shDICER1 #26: CCGGCCACACATCTTCAAGACTTAACTCGAGTTAAGTCTTGAAGATGTGTGGTTTTTG
PARK2	shPARK2 #81: CCGGCGTGAACATAACTGAGGGCATCTCGAGATGCCCTCAGTTATGTTCACGTTTTT
	shPARK2 #82:

	CCGGCGCAACAAATAGTCGGAACATCTCGAGATGTTCCGACTATTTGTTGCGTTTTT
STUB1 (CHIP)	shSTUB1 (CHIP) #25: CCGGCCCAAGTTCTGCTGTTGGACTCTCGAGAGTCCAACAGCAGAACTTGGGTTTTT
	shSTUB1 (CHIP) #26: CCGGGAAGAGGAAGAAGCGAGACATCTCGAGATGTCTCGCTTCTTCCTCTTCTTTTT
VHL	shVHL #60: CCGGTATCACACTGCCAGTGTATACCTCGAGGTATACACTGGCAGTGTGATATTTTTG
	shVHL #25: CCGGGATCTGGAAGACCACCCAAATCTCGAGATTTGGGTGGTCTTCCAGATCTTTTTG:
MDM2	shMDM2 #76: CCGGCTTTGGTAGTGGAATAGTGAACTCGAGTTCACTATTCCACTACCAAAGTTTTT
	shMDM2 #25 CCGGATTATCTGGTGAACGACAAAGCTCGAGCTTTGTCGTTCACCAGATAATTTTTTG
ATG5	shATG5 #63 CCGGCCTGAACAGAATCATCCTTAACTCGAGTTAAGGATGATTCTGTTCAGGTTTTTTG

Supplemental Table 2. Antibodies used in WB, IF, IP, and PLA

Target protein	Antibody	Application in analysis
FLAG epitope tag	*A2220* (Sigma)	WB, IP
α-tubulin	*T9026* (Sigma)	WB
Aurora B	*A5102* (Sigma)	WB
GAPDH	*MAB374* (Millipore)	WB
Lamin B1	*GTX103292* (GeneTex)	WB
Dicer	*sc-30226* (Santa Cruz)	WB, IP, PLA (For detection of HIF-1α/Dicer binding and Dicer expression)
	ab14601 (Abcam)	IF, PLA (For detection of Dicer expression)
Parkin	*sc-32282* (Santa Cruz)	WB, IP
HIF-1α	*GTX127309* (GeneTex)	Ab#1; WB, IP, IF, PLA (For detection of HIF-1α expression)
	GTX628480 (GeneTex)	Ab#2; IP, PLA (For detection of HIF-1α/Dicer binding)
	NB100-105 (Novus)	PLA (For detection of HIF-1α expression)
MDM2	*GTX100531* (GeneTex)	WB
VHL	*GTX101087* (GeneTex)	WB,
LC3B	*GTX127375* (GeneTex)	WB
STUB1 (CHIP)	*GTX109676* (GeneTex)	WB
Calreticulin	*GTX11627* (GeneTex)	WB
SQSTM1(p62)	*GTX100685* (GeneTex)	WB, IF
ZEB1	*#3396P* (Cell Signaling)	WB, IF
E-cadherin	*#3195* (Cell Signaling)	WB
	GTX629691 (GeneTex)	IF
Fibronectin	*ab32419* (Abcam)	WB
Vimentin	*#5741* (Cell signaling)	WB
PDK1	*ADI-KAP-PK112* (ENZO)	WB
PAI-1	*GTX100550* (GeneTex)	WB
ATG5	*ab108327* (Abcam)	WB
Myc epitope tag	*MA1-21316* (Pierce)	IP

Supplemental Table 3. Sequences of RT-PCR primers

Target gene	primer sequence
HIF1A forward	TTTCCTCAGTCGACACAGCC
HIF1A reverse	GGGGCCAGCAAAGTTAAAGC
DICER1 forward	TACGACGGGAAGGTCAGAGT
DICER1 reverse	ACAAAGCAGAAGTGAGGAAAGA
GAPDH forward	GTCAGTGGTGGACCTGACCT
GAPDH reverse	AGGGGTCTACATGGCAACTG