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Supplementary Materials for

HILPS, a long noncoding RNA essential for global oxygen sensing in humans

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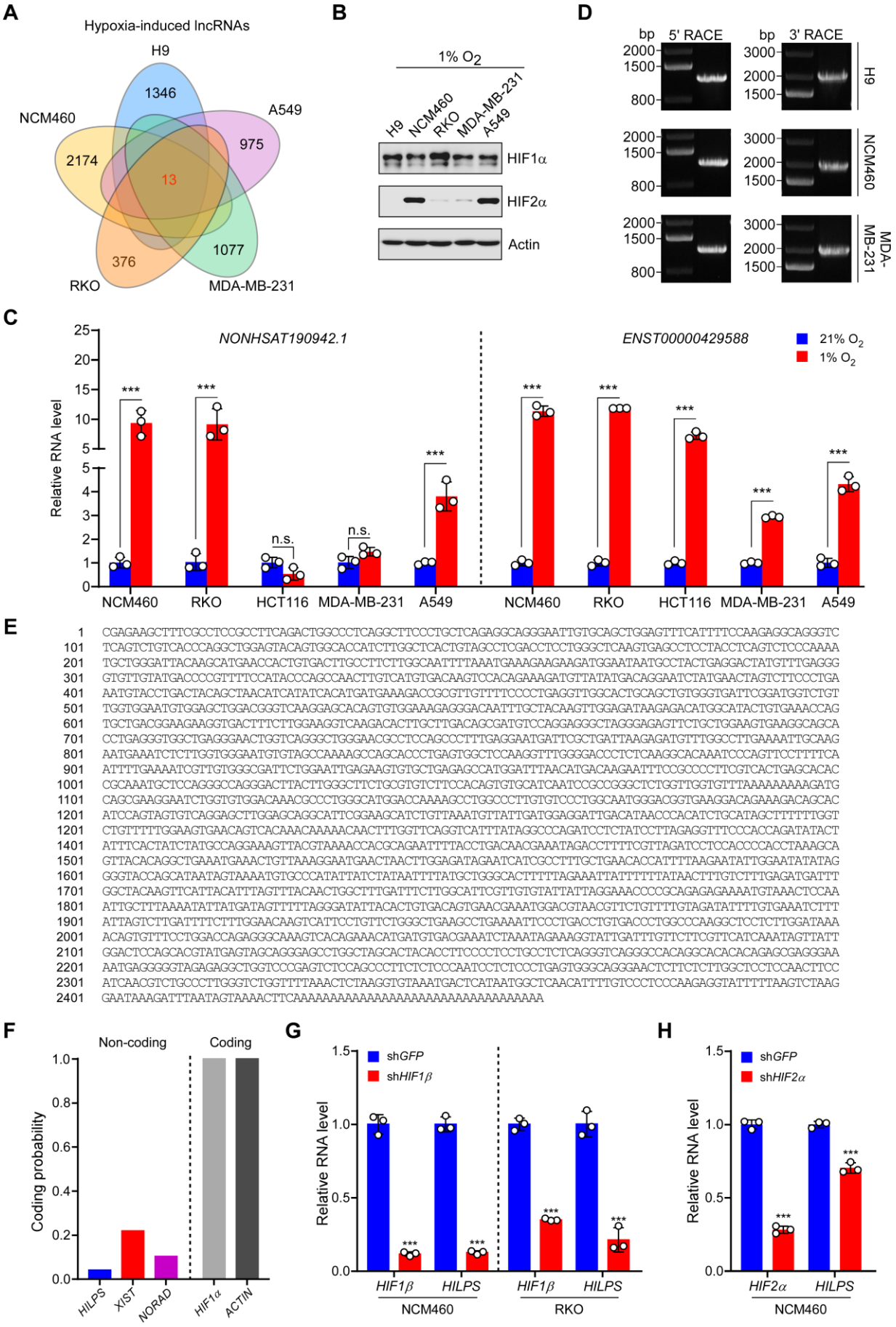
A
Hypoxia-induced lncRNAs
H9
NCM460
A549
RKO
MDA-MB-231
1346
2174
975
13
376
1077
B
1% O2
H9
NCM460
RKO
MDA-MB-231
A549
HIF1α
HIF2α
Actin
D
bp
5' RACE
3' RACE
2000
1500
800
3000
H9
NCM460
MDA-MB-231
C
NONHSAT190942.1
ENST00000429588
21% O2
1% O2
Relative RNA level
***
n.s.
NCM460
RKO
HCT116
MDA-MB-231
A549
E
1 CGAGAAGCTTTCGCCTCCGCCTTCAGACTGGCCCTCAGGCTTCCCTGCTCAGAGGCAGGGAATTGTGCAGCTGGAGTTTCATTTTCCAAGAGGCAGGGTC
101 TCAGTCTGTCACCCAGGCTGGAGTACAGTGGCACCATCTTGGCTCACTGTAGCCTCGACCTCCTGGGCTCAAGTGAGCCTCCTACCTCAGTCTCCCAAAA
201 TGCTGGGATTACAAGCATGAACCACTGTGACTTGCCTTCTTGGCAATTTTAAATGAAAGAAGAAGATGGAATAATGCCTACTGAGGACTATGTTTGAGGG
301 GTGTTGTATGACCCCGTTTTCCATACCCAGCCAACTTGTCATGTGACAAGTCCACAGAAAGATGTTATATGACAGGAATCTATGAACTAGTCTTCCCTGA
401 AATGTACCTGACTACAGCTAACATCATATCACATGATGAAAGACCGCGTTGTTTTCCCCTGAGGTTGGCACTGCAGCTGTGGGTGATTCGGATGGTCTGT
501 TGGTGGAATGTGGAGCTGGACGGGTCAAGGAGCACAGTGTGGAAAGAGGGACAATTTGCTACAAGTTGGAGATAAGAGACATGGCATACTGTGAAACCAG
601 TGCTGACGGAAGAAGGTGACTTTCTTGGAAGGTCAAGACACTTGCTTGACAGCGATGTCCAGGAGGGCTAGGGAGAGTTCTGCTGGAAGTGAAGGCAGCA
701 CCTGAGGGTGGCTGAGGGAACTGGTCAGGGCTGGGAACGCCTCCAGCCCTTTGAGGAATGATTCGCTGATTAAGAGATGTTTGGCCTTGAAAATTGCAAG
801 AATGAAATCTCTTGGTGGGAATGTGTAGCCAAAAGCCAGCACCCTGAGTGGCTCCAAGGTTTGGGGACCCTCTCAAGGCACAAATCCCAGTTCCTTTTCA
901 ATTTTGAAAATCGTTGTGGGCGATTCTGGAATTGAGAAGTGTGCTGAGAGCCATGGATTTAACATGACAAGAATTTCCGCCCCTTCGTCACTGAGCACAC
1001 CGCAAATGCTCCAGGGCCAGGGACTTACTTGGGCTTCTGCGTGTCTTCCACAGTGTGCATCAATCCGCCGGGCTCTGGTTGGTGTTTAAAAAAAAAGATG
1101 CAGCGAAGGAATCTGGTGTGGACAAACGCCCTGGGCATGGACCAAAAGCCTGGCCCTTGTGTCCCTGGCAATGGGACGGTGAAGGACAGAAAGACAGCAC
1201 ATCCAGTAGTGTCAGGAGCTTGGAGCAGGCATTCGGAAGCATCTGTTAAATGTTATTGATGGAGGATTGACATAACCCACATCTGCATAGCTTTTTTGGT
1201 CTGTTTTTGGAAGTGAACAGTCACAAACAAAAACAACTTTGGTTCAGGTCATTTATAGGCCCAGATCCTCTATCCTTAGAGGTTTCCCACCAGATATACT
1401 ATTTCACTATCTATGCCAGGAAAGTTACGTAAAACCACGCAGAATTTTACCTGACAACGAAATAGACCTTTTCGTTAGATCCTCCACCCCACCTAAAGCA
1501 GTTACACAGGCTGAAATGAAACTGTTAAAGGAATGAACTAACTTCGGAGATAGAATCATCGCCTTTGCTGAACACCATTTTAAGAATATTGGAATATATAG
1601 GGGTACCAGCATAATAGTAAAATGTGCCCATATTATCTATAATTTTATGCTGGGCACTTTTTAGAAATTATTTTTTATAACTTTGTCTTTGAGATGATTT
1701 GGCTACAAGTTCATTACATTTAGTTTACAACTGGCTTTGATTTCTTGGCATTCGTTGTGTATTATTAGGAAACCCCGCAGAGAGAAAATGTAAACTCCAA
1801 ATTGCTTTAAAATATTATGATAGTTTTTAGGGATATTACACTGTGACAGTGAACGAAATGGACGTAACGTTCTGTTTTGTAGATATTTTGTGAAATCTTT
1901 ATTAGTCTTGATTTTCTTTGGAACAAGTCATTCCTGTTCTGGGCTGAAGCCTGAAAATTCCCTGACCTGTGACCCTGGCCCAAGGCTCCTCTTGGATAAA
2001 ACAGTGTTTCCTGGACCAGAGGGCAAAGTCACAGAAACATGATGTGACGAAATCTAAATAGAAAGGTATTGATTTGTTCTTCGTTCATCAAATAGTTATT
2101 GGACTCCAGCACGTATGAGTAGCAGGGAGCCTGGCTAGCACTACACCTTCCCCTCCTGCCTCTCAGGGTCAGGGCCACAGGCACACACAGAGCGAGGGAA
2201 AATGAGGGGGTAGAGAGGCTGGTCCCGAGTCTCCAGCCCTTCTCTCCCAATCCTCTCCCTGAGTGGGCAGGGAACTCTTCTCTTGGCTCCTCCAACTTCC
2301 ATCAACGTCTGCCCTTGGGTCTGGTTTTAAACTCTAAGGTGTAAATGACTCATAATGGCTCAACATTTTGTCCCTCCCAAGAGGTATTTTTAAGTCTAAG
2401 GAATAAAGATTTAATAGTAAAACTTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
F
Non-coding
Coding
Coding probability
HILPS
XIST
NORAD
HIF1α
ACTIN
G
shGFP
shHIF1β
Relative RNA level
HIF1β
HILPS
NCM460
RKO
H
shGFP
shHIF2α
Relative RNA level
HIF2α
HILPS
NCM460

Figure S1. *HILPS* is a long non-coding RNA regulated by hypoxia-inducible factors, related to Figure 1.

(**A**) Venn diagram of overlapping lncRNAs induced by hypoxia (1% O_2) in H9, NCM460, RKO, MDA-MB-231 and A549 cells.

(**B**) Immunoblots of HIF1α and HIF2α in H9, NCM460, RKO, MDA-MB-231 and A549 cells exposed to 1% O_2 for 24 h.

(**C**) qPCR analysis of *NONHSAT190942.1* and *ENST00000429588* in various cells exposed to 21% or 1% O_2 for 24 h.

(**D**) 5′ and 3′ RACE amplicons of *HILPS* using total RNA isolated from H9, NCM460 and MDA-MB-231 cells exposed to 1% O_2 for 24 h as a template. nt, nucleotide; bp, base pair.

(**E**) The nucleotide sequence of *HILPS* identified by 5′ and 3′ RACE in RKO cells, which has been deposited in the GenBank with the accession number OQ550038.

(**F**) Coding probability of *HILPS* as well as other known coding and non-coding RNAs determined by Coding Potential Calculator 2 (http://cpc2.gao-lab.org/).

(**G**) qPCR analysis of *HILPS* in *HIF1β*-depleted NCM460 and RKO cells exposed to 1% O_2 for 24 h.

(**H**) qPCR analysis of *HILPS* in *HIF2α*-depleted NCM460 cells exposed to 1% O_2 for 24 h.

Data shown are mean ± SD from biological triplicates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n.s., no significance, unpaired two-tailed Student's t test.

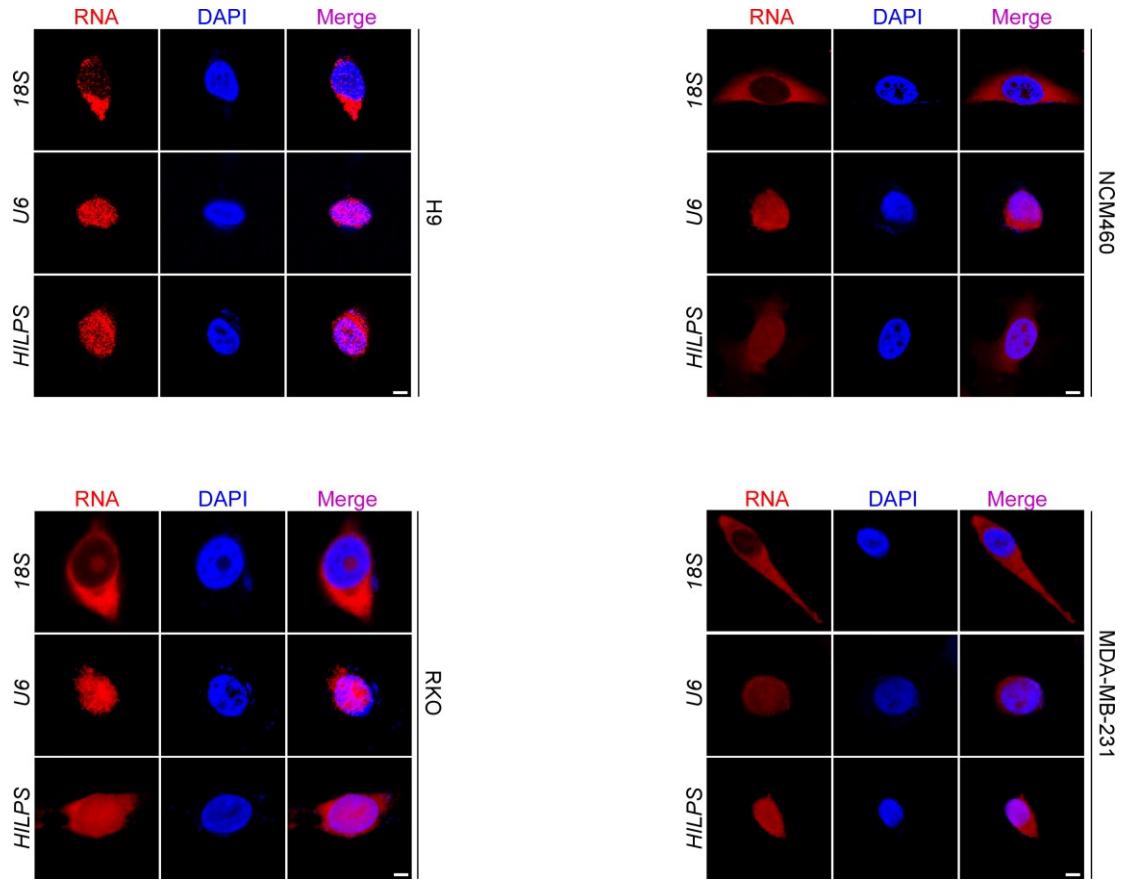

Figure S2. Subcellular localization of *HILPS* detected by FISH in H9, NCM460, RKO and MDA-MB-231 cells, related to Figure 1. Scale bar, 10 μm.

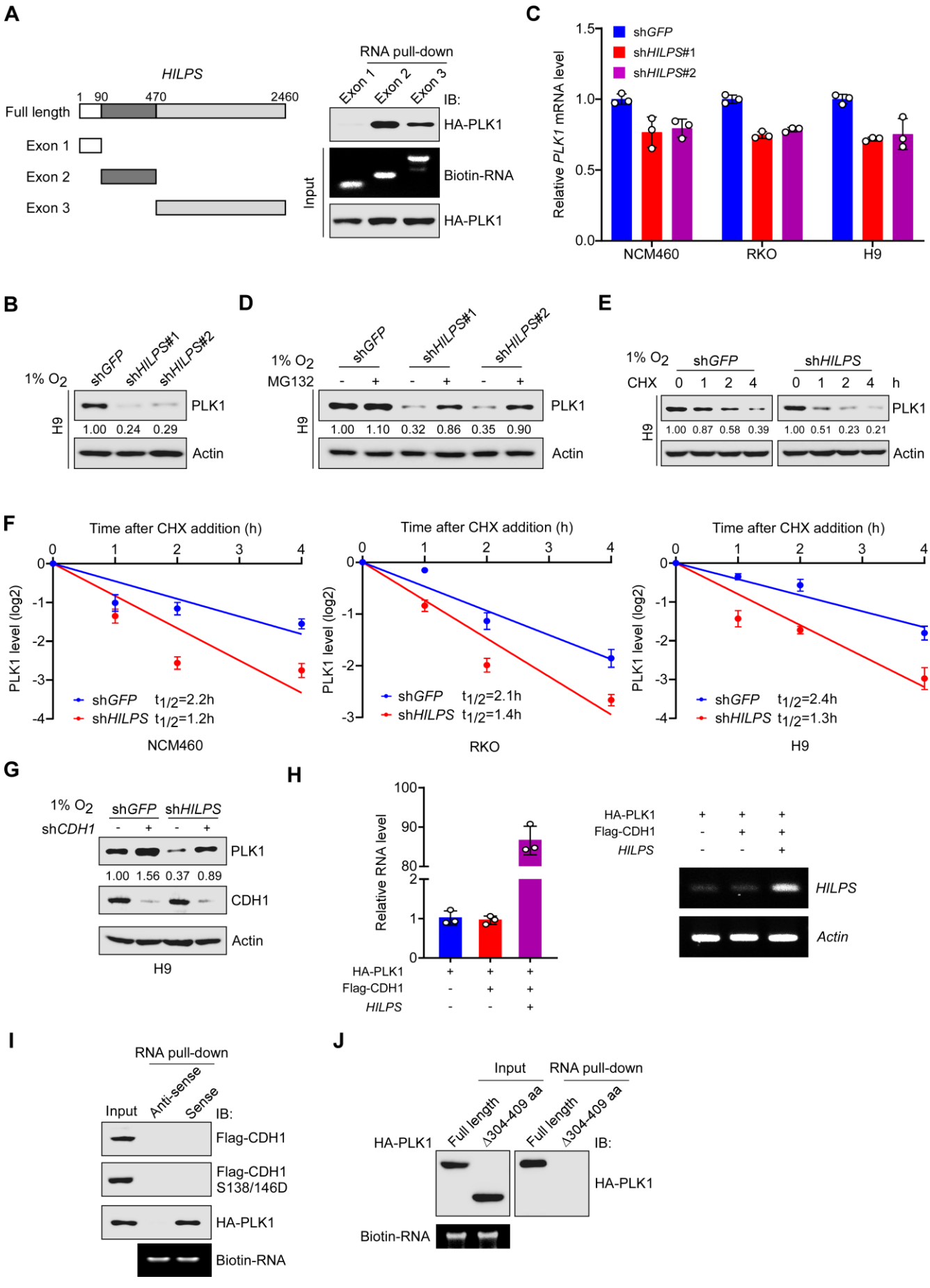
A
HILPS
1 90 470 2460
Full length
Exon 1
Exon 2
Exon 3
RNA pull-down
Exon 1
Exon 2
Exon 3
IB:
HA-PLK1
Input
Biotin-RNA
HA-PLK1
C
shGFP
shHILPS#1
shHILPS#2
Relative PLK1 mRNA level
1.5
1.0
0.5
0.0
NCM460
RKO
H9
B
1% O2
shGFP
shHILPS#1
shHILPS#2
H9
PLK1
1.00 0.24 0.29
Actin
D
1% O2
shGFP
shHILPS#1
shHILPS#2
MG132 - + - + - +
H9
PLK1
1.00 1.10 0.32 0.86 0.35 0.90
Actin
E
1% O2
shGFP
shHILPS
CHX 0 1 2 4 0 1 2 4 h
H9
PLK1
1.00 0.87 0.58 0.39 1.00 0.51 0.23 0.21
Actin
F
Time after CHX addition (h)
PLK1 level (log2)
shGFP t1/2=2.2h
shHILPS t1/2=1.2h
NCM460
Time after CHX addition (h)
PLK1 level (log2)
shGFP t1/2=2.1h
shHILPS t1/2=1.4h
RKO
Time after CHX addition (h)
PLK1 level (log2)
shGFP t1/2=2.4h
shHILPS t1/2=1.3h
H9
G
1% O2
shGFP
shHILPS
shCDH1 - + - +
PLK1
1.00 1.56 0.37 0.89
CDH1
Actin
H9
H
Relative RNA level
HA-PLK1 + + +
Flag-CDH1 - + +
HILPS - - +
HA-PLK1 + + +
Flag-CDH1 - + +
HILPS - - +
HILPS
Actin
I
RNA pull-down
Input
Anti-sense
Sense
IB:
Flag-CDH1
Flag-CDH1 S138/146D
HA-PLK1
Biotin-RNA
J
Input
RNA pull-down
HA-PLK1
Full length
Δ304-409 aa
Full length
Δ304-409 aa
IB:
HA-PLK1
Biotin-RNA

Figure S3. *HILPS* binds to and stabilizes PLK1, related to Figure 4.

(**A**) Schematic presentation of HILPS exons used in biotin-labeled RNA pull-down assay (left). Immunoblots of PLK1 from biotin-labeled *HILPS* exon pull-down assay using 293T cell lysates with HA-PLK1 expression (right).

(**B**) Immunoblots of PLK1 in *HILPS*-depleted H9 cells exposed to 1% O_2. Actin is shown as a loading control.

(**C**) qPCR analysis of *PLK1* mRNA expression in *HILPS*-depleted NCM460, RKO and H9 cells exposed to 1% O_2.

(**D**) Immunoblots of PLK1 in *HILPS*-depleted H9 cells treated with MG132 (10 μM) for 6 h before harvest. Cells were cultured in 1% O_2.

(**E**) Time-course analysis of PLK1 degradation. *HILPS*-depleted H9 cells were cultured in 1% O_2, treated with cycloheximide (CHX, 100 μg/mL) and harvested at the indicated time points followed by immunoblotting of PLK1.

(**F**) Quantification of PLK1 protein degradation. PLK1 band density was normalized to Actin and relative to t = 0 control.

(**G**) Immunoblots of PLK1 and CDH1 in 1% O_2-cultured H9 cells expressing shRNA targeting *HILPS* and/or *CDH1*.

(**H**) qPCR analysis (left) and PCR amplicons (right) of *HILPS* in 293T cells expressing HA-PLK1, Flag-CDH1, and/or *HILPS* as indicated.

(**I**) RNA pull-down assay using biotin-labeled *HILPS* and lysates from 293T cells expressing Flag-CDH1, Flag-CDH1 mutant (S138/146D) or HA-PLK1.

(**J**) RNA pull-down assay using biotin-labeled *HILPS* and lysates from 293T cells expressing full-length HA-PLK1 or HA-PLK1 with amino acids 304-409 depletion.

Data shown are mean ± SD from biological triplicates.

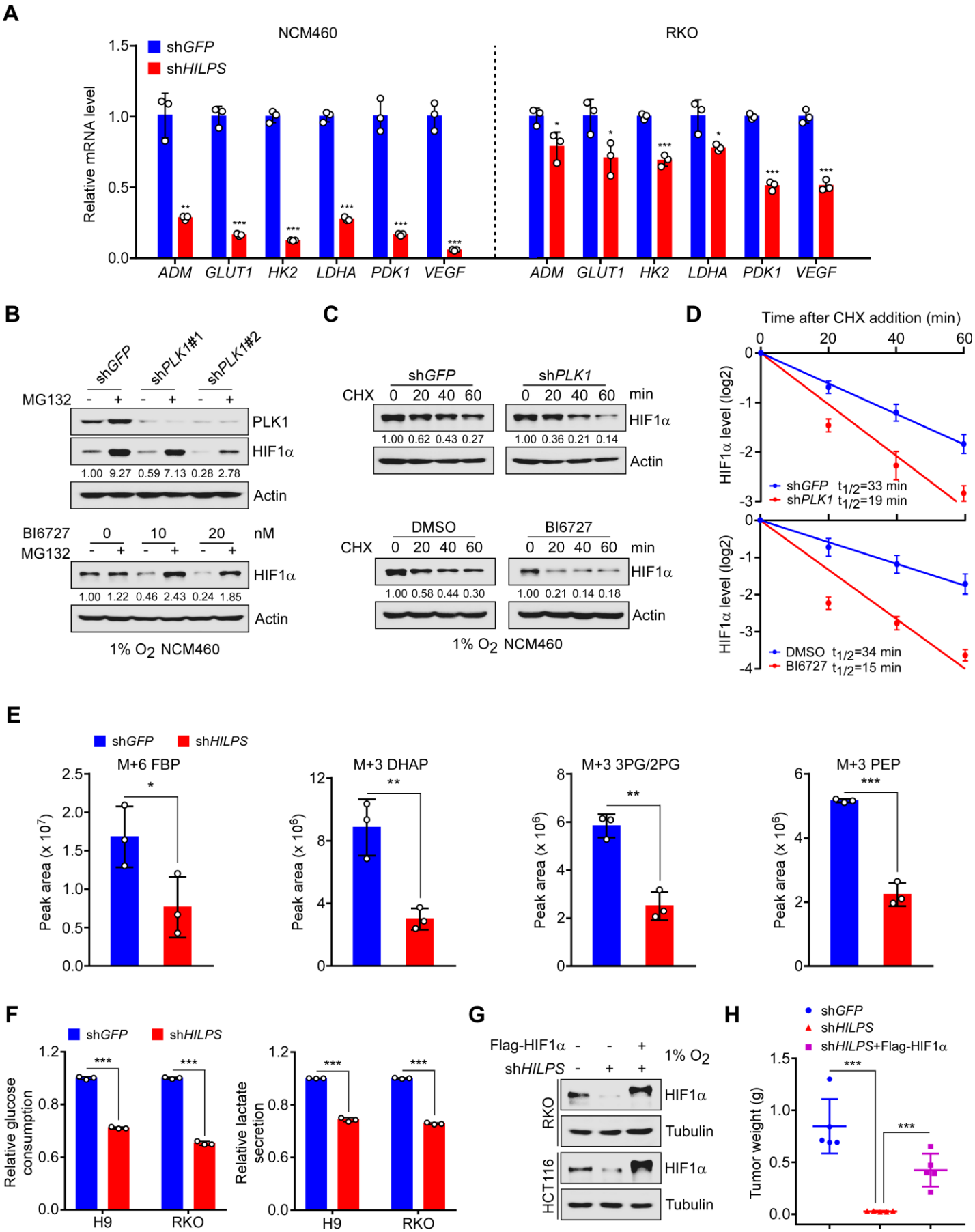
A
NCM460
RKO
shGFP
shHILPS
Relative mRNA level
ADM GLUT1 HK2 LDHA PDK1 VEGF
B
shGFP
shPLK1#1
shPLK1#2
MG132
PLK1
HIF1α
1.00 9.27 0.59 7.13 0.28 2.78
Actin
BI6727 0 10 20 nM
MG132
HIF1α
1.00 1.22 0.46 2.43 0.24 1.85
Actin
1% O2 NCM460
C
shGFP
shPLK1
CHX 0 20 40 60 min
HIF1α
1.00 0.62 0.43 0.27
1.00 0.36 0.21 0.14
Actin
DMSO
BI6727
CHX 0 20 40 60 min
HIF1α
1.00 0.58 0.44 0.30
1.00 0.21 0.14 0.18
Actin
1% O2 NCM460
D
Time after CHX addition (min)
HIF1α level (log2)
shGFP t1/2=33 min
shPLK1 t1/2=19 min
DMSO t1/2=34 min
BI6727 t1/2=15 min
E
shGFP
shHILPS
M+6 FBP
M+3 DHAP
M+3 3PG/2PG
M+3 PEP
Peak area (x 10^7)
Peak area (x 10^6)
F
shGFP
shHILPS
Relative glucose consumption
Relative lactate secretion
H9
RKO
G
Flag-HIF1α
shHILPS
1% O2
RKO
HCT116
HIF1α
Tubulin
H
shGFP
shHILPS
shHILPS+Flag-HIF1α
Tumor weight (g)

Figure S4. PLK1 mediates *HILPS* regulation of HIF1α, related to Figure 5.

(**A**) qPCR analysis of representative HIF1α target genes in *HILPS*-depleted NCM460 and RKO cells exposed to 1% O_2.

(**B**) Immunoblots of HIF1α and PLK1 in *PLK1*-depleted (upper) or PLK1 kinase inhibitor BI6727-treated (bottom) NCM460 cells exposed to 1% O_2. MG132 (10 μM) was added to cell culture for 6 h before harvest.

(**C** and **D**) Time-course analysis of HIF1α degradation in *PLK1*-depleted (upper) or BI6727-treated (bottom) NCM460 cells subjected to 1% O_2 exposure. HIF1α was analyzed by immunoblotting (C) and quantified as shown (D). HIF1α band density was normalized to Actin and relative to t = 0 control.

(**E**) Analysis of isotope-labeled glycolysis intermediates in *HILPS*-depleted cells labeled with $^{13}C_6$ glucose for 4 h during hypoxia culture (1% O_2). Peak area represents the abundance of labeled metabolites, normalized to the total protein. FBP: fructose-1,6-bisphosphate, DHAP: dihydroxyacetone phosphate, 3PG/2PG: 3-phosphoglycerate/2-phosphoglycerate, PEP: phosphoenolpyruvate.

(**F**) Assessment of glucose consumption (left) and lactate secretion (right) in *HILPS*-depleted H9 and RKO cells cultured in 1% O_2 for 24 h.

(**G**) Immunoblots of HIF1α in *HILPS*-depleted RKO and HCT116 cells with or without Flag-HIF1α overexpression.

(**H**) Tumor weight quantifications of *HILPS*-depleted HCT116 xenografts with or without ectopic expression of HIF1α (n=5).

Data shown are mean ± SD from biological triplicates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n.s., no significance, unpaired two-tailed Student's t test (**A**, **E**, and **F**) and one-way ANOVA (**H**).

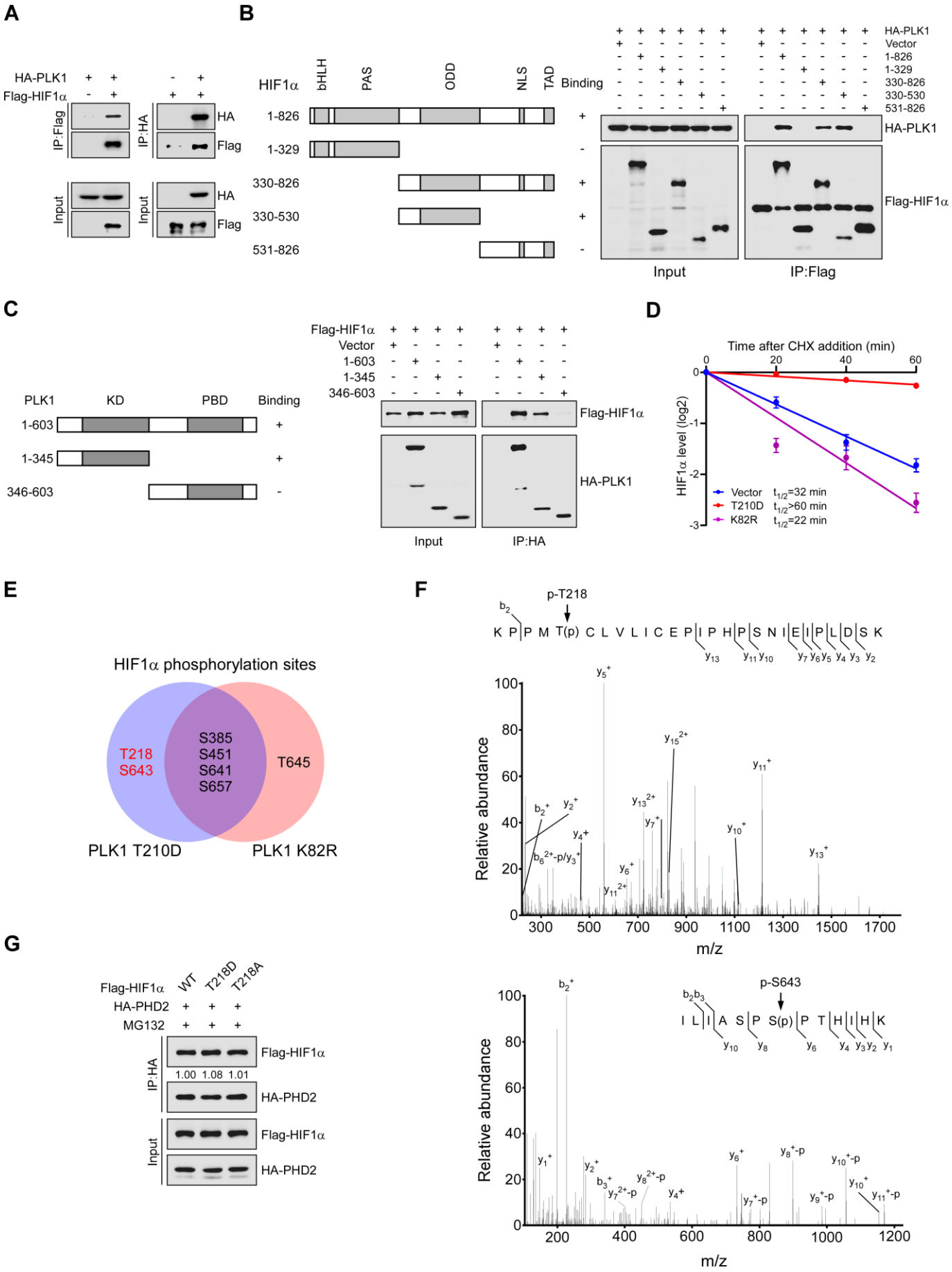
A
HA-PLK1
Flag-HIF1α
IP:Flag
IP:HA
Input
HA
Flag
B
HIF1α
bHLH
PAS
ODD
NLS
TAD
1-826
1-329
330-826
330-530
531-826
Binding
HA-PLK1
Vector
1-826
1-329
330-826
330-530
531-826
HA-PLK1
Flag-HIF1α
Input
IP:Flag
C
PLK1
KD
PBD
Binding
1-603
1-345
346-603
Flag-HIF1α
Vector
1-603
1-345
346-603
HA-PLK1
Input
IP:HA
D
Time after CHX addition (min)
HIF1α level (log2)
Vector t1/2=32 min
T210D t1/2>60 min
K82R t1/2=22 min
E
HIF1α phosphorylation sites
T218
S643
S385
S451
S641
S657
T645
PLK1 T210D
PLK1 K82R
F
p-T218
K P P M T(p) C L V L I C E P I P H P S N I E I P L D S K
Relative abundance
m/z
p-S643
I L I A S P S(p) P T H I H K
G
Flag-HIF1α
WT
T218D
T218A
HA-PHD2
MG132
IP:HA
1.00 1.08 1.01
Flag-HIF1α
HA-PHD2
Input
Flag-HIF1α
HA-PHD2

Figure S5. PLK1 phosphorylates HIF1α T218 and enhances HIF1α stability, related to Figure 6.

(**A**) Co-IP of exogenous HIF1α and PLK1 using lysates of 293T cells overexpressing epitope-tagged proteins.

(**B**) Schematic presentation of various human HIF1α truncations used in PLK1-binding assays (left). Co-IP using lysates of 293T cells overexpressing HA-PLK1 and/or various Flag-HIF1α truncations (right).

(**C**) Schematic presentation of various human PLK1 truncations used in HIF1α-binding assays (left). Co-IP using lysates of 293T cells overexpressing Flag-HIF1α and/or various HA-PLK1 truncations (right).

(**D**) Quantification of HIF1α protein degradation. HIF1α band density was normalized to Actin and relative to t = 0 control.

(**E**) Venn diagram of mass spectrometry analysis for HIF1α phosphorylation sites modified by PLK1 T210D or K82R.

(**F**) Mass spectrometry spectrum data mapping the HIF1α T218 and S643 phosphorylation status.

(**G**) Co-IP of Flag-HIF1α (WT or mutants) and HA-PHD2 in 293T cell lysate with epitope-tagged protein expression. MG132 (10 μM) was added to prevent HIF1α degradation.

Data shown are mean ± SD from biological triplicates.

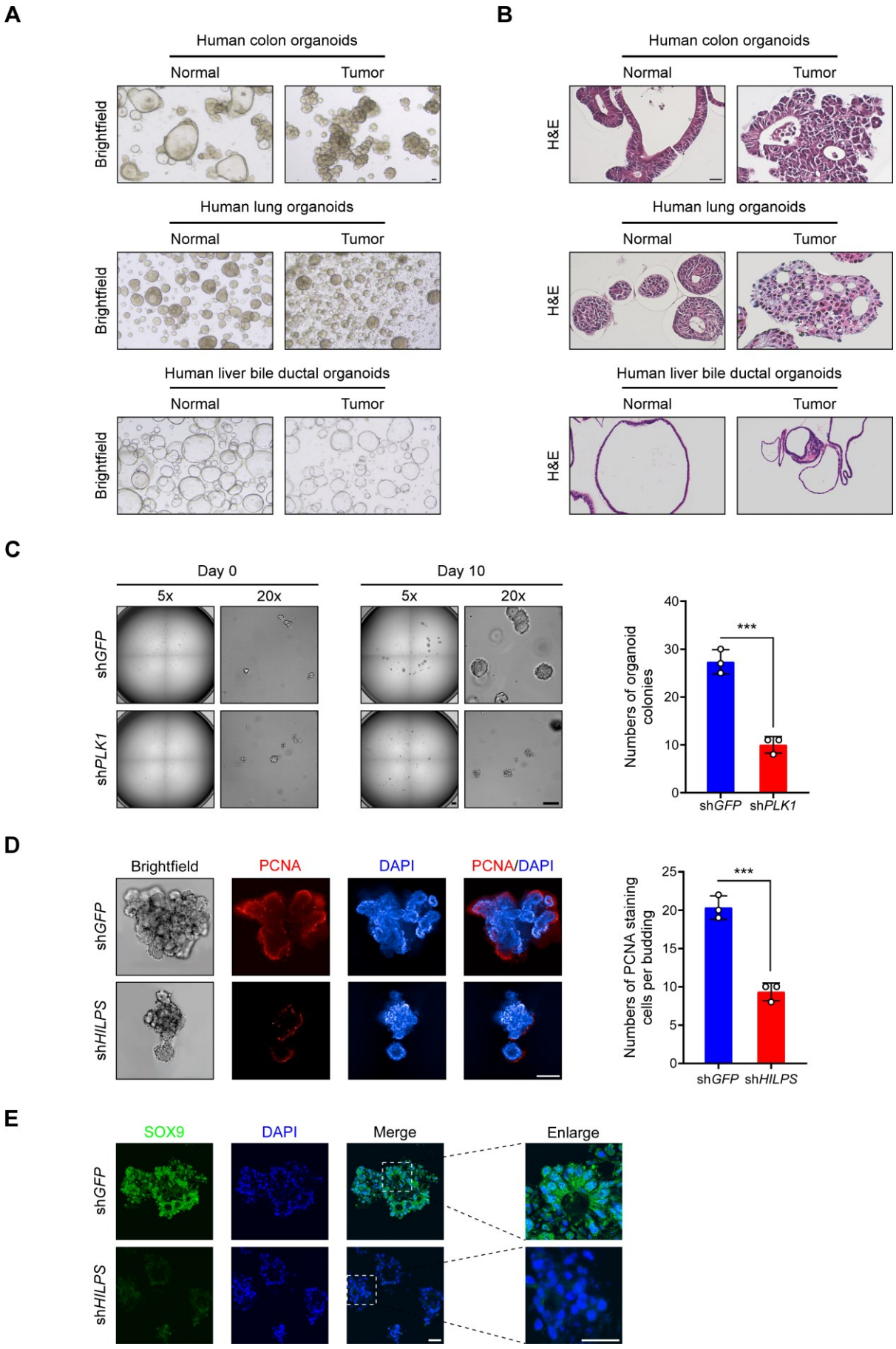
A
Human colon organoids
Normal
Tumor
Brightfield
Human lung organoids
Normal
Tumor
Brightfield
Human liver bile ductal organoids
Normal
Tumor
Brightfield
B
Human colon organoids
Normal
Tumor
H&E
Human lung organoids
Normal
Tumor
H&E
Human liver bile ductal organoids
Normal
Tumor
H&E
C
Day 0
5x
20x
Day 10
5x
20x
shGFP
shPLK1
Numbers of organoid colonies
40
30
20
10
0
***
shGFP
shPLK1
D
Brightfield
PCNA
DAPI
PCNA/DAPI
shGFP
shHILPS
Numbers of PCNA staining cells per budding
25
20
15
10
5
0
***
shGFP
shHILPS
E
SOX9
DAPI
Merge
Enlarge
shGFP
shHILPS

Figure S6. *HILPS* depletion impairs human colorectal cancer organoid oncogenicity, related to Figure 7.

(**A** and **B**) Representative images showing the morphology (A) and hematoxylin-eosin (H&E) staining (B) of indicated human organoids. Scale bar, 50 μm.

(**C**) Representative bright field images of *PLK1*-depleted colorectal cancer organoids cultured under 1% O_2 at indicated days (left). Scale bar, 200 μm. Quantitation of viable organoid colonies are shown on the right.

(**D**) Representative images showing brightfield view and immunofluorescence staining of PCNA in *HILPS*-depleted colorectal cancer organoids cultured under 1% O_2 (left). DAPI was stained to visualize the organoids in dark field. Scale bar, 200 μm. Quantitation of PCNA positive staining cells per budding are presented (right).

(**E**) Immunofluorescence staining of SOX9 of human colorectal cancer organoids with or without *HILPS* depletion and exposed to 1% O_2. Scale bar, 100 μm.

Data shown are mean ± SD from biological triplicates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n.s., no significance, unpaired two-tailed Student's t test.

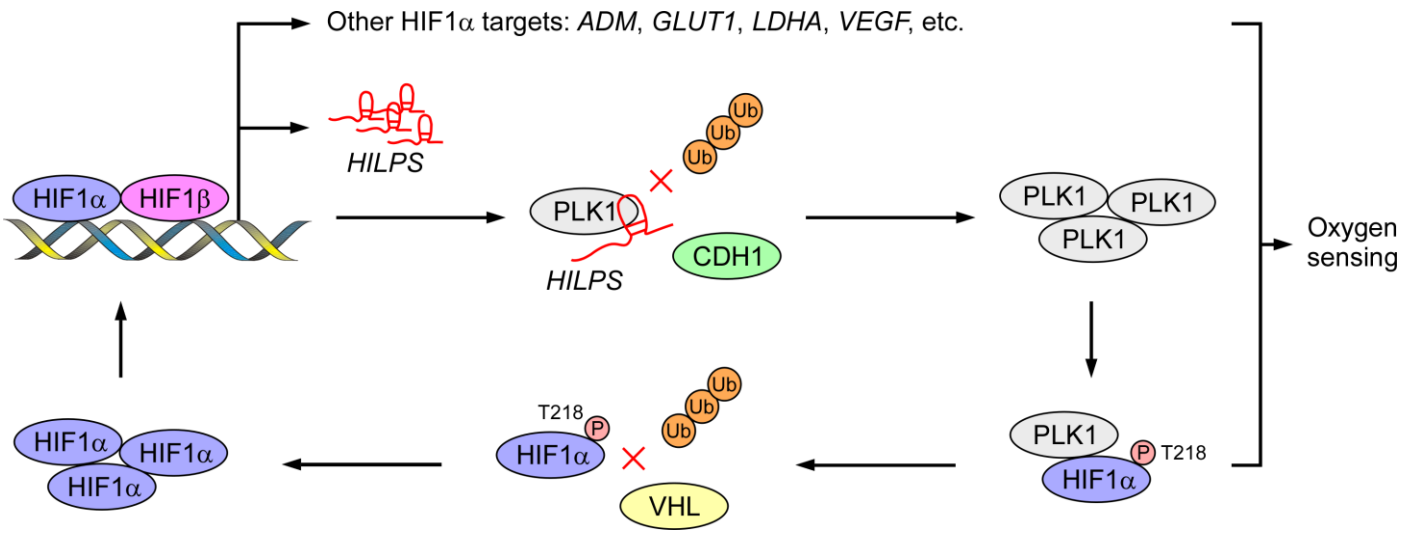

Figure S7. Model depicting a feed-forward circuit between *HILPS*, PLK1 and HIF1α for oxygen sensing in human.

HIF1α directly activates *HILPS* transcription. The accumulated *HILPS* sequesters PLK1 from its E3 ligase CDH1, promoting PLK1 stabilization. Stabilized PLK1 phosphorylates HIF1α at T218, preventing it from VHL-mediated protein degradation. The regulatory circuit of *HILPS*-PLK1-HIF1α strengthens the oxygen-sensing mechanism mediated by HIF1α in human cells. See discussion for more details.

Table S1. List of primers and oligonucleotides used in this study.

Primers for Real-time qPCR	
ACTIN forward	CATGTACGTTGCTATCCAGGC
ACTIN reverse	CTCCTTAATGTCACGCACGAT
NONHSAT190942.1 forward	AGTCTTCCCTGAAATGTACCTGA
NONHSAT190942.1 reverse	GCACCAGAAGACGAAGGACA
ENST00000429588 forward	AGTCTTCCCTGAAATGTACCTGA
ENST00000429588 reverse	TTCCACCAACAGACCATCCG
HILPS forward	AAGAATTTCCGCCCCTTCGT
HILPS reverse	TCCACACCAGATTCCTTCGC
HIF1α forward	GAAGACATCGCGGGGAC
HIF1α reverse	TGGCTGCATCTCGAGACTTT
HIF1β forward	CTGCCAACCCCGAAATGACAT
HIF1β reverse	CGCCGCTTAATAGCCCTCTG
NANOG forward	CCCCAGCCTTTACTCTTCCTA
NANOG reverse	CCAGGTTGAATTGTTCCAGGTC
OCT4 forward	CAAAGCAGAAACCCTCGTGC
OCT4 reverse	TCTCACTCGGTTCTCGATACTG
SOX2 forward	GTCATTTGCTGTGGGTGATG
SOX2 reverse	AGAAAAACGAGGGAAATGGG
PAX6 forward	TCCGTTGGAACTGATGGAGT
PAX6 reverse	GTTGGTATCCGGGGACTTC
SOX1 forward	ATTATTTTGCCCGTTTTCCC
SOX1 reverse	TCAAGGAAACACAATCGCTG
FOX2A forward	GGAGCAGCTACTATGCAGAGC
FOX2A reverse	CGTGTTCATGCCGTTCATCC
SOX17 forward	GCATGACTCCGGTGTGAATCT
SOX17 reverse	TCACACGTCAGGATAGTTGCAGT
MIXL1 forward	GAGACTTGGCACGCCTGT
MIXL1 reverse	GGTACCCCGACATCCACTT
TBXT forward	GATGATCGTGACCAAGAACGG
TBXT reverse	CCACGAAGTCCAGCAGGAA
GATA6 forward	AGTTCCTACGCTTCGCATCCCTTC
GATA6 reverse	TGAACAGCAGCAAGTCCTCCCA
PLK1 forward	TGTTAGTGGGCAAACCACCT
PLK1 reverse	CAGCTCGTTAATGGTTGGGC
ADM forward	ATGAAGCTGGTTTCCGTCG
ADM reverse	GACATCCGCAGTTCCCTCTT
GLUT1 forward	GGCCAAGAGTGTGCTAAAGAA
GLUT1 reverse	ACAGCGTTGATGCCAGACAG
HK2 forward	GAGCCACCACTCACCCTACT
HK2 reverse	CCAGGCATTCGGCAATGTG
LDHA forward	ATGGCAACTCTAAAGGATCAGC

LDHA reverse	CCAACCCCAACAACTGTAATCT
PDK1 forward	CTGTGATACGGATCAGAAACCG
PDK1 reverse	TCCACCAAACAATAAAGAGTGCT
VEGF forward	AGGGCAGAATCATCACGAAGT
VEGF reverse	AGGGTCTCGATTGGATGGCA
Primers for ChIP-qPCR	
HRE1 forward	CTCAGCTCTGGCCTCTGAGT
HRE1 reverse	GCTGAGCTCCGTTAGTCACC
HRE2 forward	AAAACCCAGCTCTTTGGTCA
HRE2 reverse	ATGATCCTCCCACCTTAGCC
shRNA sequences	
sh*GFP*	TACAACAGCCACAACGTCTAT
sh*HILPS*#1	GGGCTCTGGTTGGTGTTTAAA
sh*HILPS*#2	ATTCGGAAGCATCTGTTAAAT
sh*HIF1α*	GTGATGAAAGAATTACCGAAT
sh*HIF1β*	GAGAAGTCAGATGGTTTATTT
sh*CDH1*	AGAAGGGTCTGTTCACGTATT
sh*PLK1*#1	CGATACTACCTACGGCAAATT
sh*PLK1*#2	CGCCTCATCCTCTACAATGAT
Primers for RACE	
5′ RACE GSP	TGTGCTGTCTTTCTGTCCTTCACCGTCC
3′ RACE GSP	TCGGATGGTCTGTTGGTGGAATGTGGAG
5′ RACE Nested PCR	TGTCCTTCACCGTCCCATTGCCAG
3′ RACE Nested PCR	GTGGAGCTGGACGGGTCAAGGAGCAC

Table S2. List of primary antibodies used in this study.

Antibodies	**Source**	**Identifier**
β-Actin	ABclonal	Cat# AC026; RRID: AB_2768234
NANOG	ABclonal	Cat# A3232; RRID: AB_2765000
OCT4	Cell Signaling Technology	Cat# 2750; RRID: AB_823583
SOX2	Cell Signaling Technology	Cat# 3579; RRID: AB_2195767
HA-tag	ABclonal	Cat# AE008; RRID: AB_2770404
HA-tag (peroxidase conjugate)	Roche	Cat# 12013819001; RRID: AB_390917
GST-tag	ABclonal	Cat# AE001; RRID: AB_2770403
PLK1	Cell Signaling Technology	Cat# 4513; RRID: AB_2167409
Myc-tag	ABclonal	Cat# AE010; RRID: AB_2770408
CDH1	CUSABIO	Cat# CSB-PA892473LA01HU
Flag-tag	Sigma-Aldrich	Cat# F1804; RRID: AB_262044

HIF1α	Cell Signaling Technology	Cat# 14179; RRID: AB_2622225
HIF2α	Cell Signaling Technology	Cat# 59973; RRID: AB_2799579
β-Tubulin	ABclonal	Cat# AC021; RRID: AB_2773004
Rabbit control IgG	ABclonal	Cat# AC005; RRID: AB_2771930
Ki67	BD Biosciences	Cat# 550609; RRID: AB_393778
PCNA	Abcam	Cat# ab29; RRID: AB_303394
SOX9	Millipore	Cat# AB5535; RRID: AB_2239761