

Hypoxia-inducible miR-182 enhances HIF1 α signaling via targeting PHD2 and FIH1 in prostate cancer

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Supplementary Table 1. miRNA/siRNAs and oligonucleotide sequences

miRNA/siRNA	Sense Sequence (5'→3')	Antisense Sequence (5'→3')
miR-182	UUUGGCAAUGGUAGAACUCACACU	AGUGUGAGUUCUACCAUUGCCAAA
N.C.	UUCUCCGAACGUGUCACGU	ACGUGACACGUUCGGAGAA
miR-182 Inhibitor	AGUGUGAGUUCUACCAUUGCCAAA	
Inhibitor NC	CAGUACUUUUGUGUAGUACAA	
siHIF1α-854	AAGUCUGCAACAUGGAAGGUtt	ACCUUCCAUGUUGCAGACUtt
siHIF1α-1035	GACACAGCCUGGAUAUGAAtt	UUCAUAUCCAGGCUGUGUCtt
siHIF1α-1941	AGAACCCAUUUUCUACUCAGtt	CUGAGUAGAAAUGGGUUCUtt

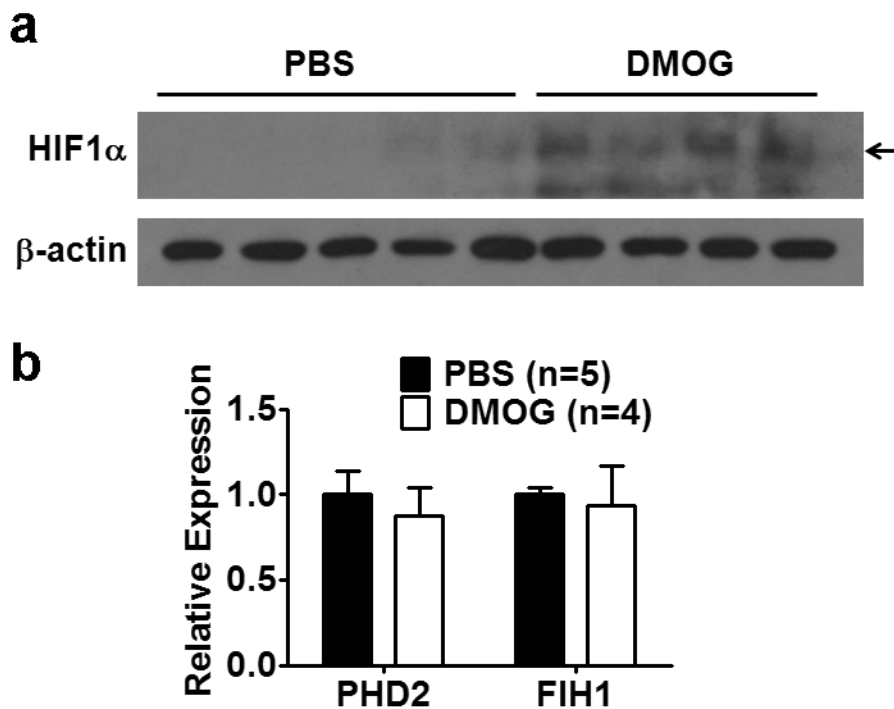
Supplementary Table 2. Primer sequences for RT-PCR

gene	Sense Sequence (5'→3')	Antisense Sequence (5'→3')
miR-182	TTTGGCAATGGTAGAACTCA	
miR-96	TTTGGCACTAGCACATTTTTGC	
miR-183	TATGGCACTGGTAGAATCACT	
miR-210	CTGTGCGTGTGACAGCGGCT	
U6	ATTCGTGAAGCGTTCCATAT	
Uni-primer	CGAATTCTAGAGCTCGAGGCAGG	
18S	ACCGCAGCTAGGAATAATGGA	CAAATGCTTTCGCTCTGGTC
PHD2	GTGCCGTGCATGAACAAGC	CAGGTGATCTTATCGCCTCGG
FIH1	GAGTGCCTCTACCCATACCCT	TCGTAGTCGGGATTGTCAAAGT
VEGF	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
HIF1 α	CTGATCATCTGACCAAACTC	GTTTCAACCCAGACATATCCAC
mCD31	CTCATTGCGGTGTTGTCAT	TTCTGTTT-GGCCTTGGCTTT
mVEGF	CTGCCGTCCGATTGAGACC	CCCCTCCTTGTACCACTGTC
mPHD2	AGCTGGTCAGCCAGAAGAGT	GCCCTCGATCCAGGTGATCT
mFIH1	CATTGGCATGGAAGGAAATGTG	GACAGGGTATGGATAGAGGCA

Supplementary Table 3. Primer sequences for ChIP assay

Name	Sense Sequence (5'→3')	Antisense Sequence (5'→3')
miR-182 HRE1	GCTTTTCGAGAGAGCTGGGA	TCGGCTGGGCCAGGTTCTC
miR-182 HRE2	TCTGTTCTCCAGCTCTAAGG	ATTCTTCTTTTGGGGACAGC
miR-210 HRE	GCAGACGTGCAGAAAAGAACG	CCGTATGCAAATGACCTGGTC
non-HRE	GCCTTCAGGTGGAGATAGGAGA	TTCAAGGAGACAGGGACAGTGC

Supplementary Fig. S1



Supplementary Figure. S1.

C57BL/6 mice were treated with DMOG or vehicle as described in Materials and Methods. The prostate tissues were harvested and the HIF1 α protein expression (a) and PHD2 and FIH1 miRNA expression (b) were determined by western blot analysis and RT-PCR, respectively (n=4-5). Full-length blots are presented in Supplementary Fig. S6.

Supplementary Fig. S2

	HRE1		HRE2
human	AGCGG GCGT GGCACC-----		GTGCT GCGT GTTC
chimpanzee	AGCGG GCGT GGCACC-----		GTGCT GCGT GTTC
monkey	AGCAG GCCT GGCATC-----		GTGCT GCGT GTTC

Supplementary Figure. S2.

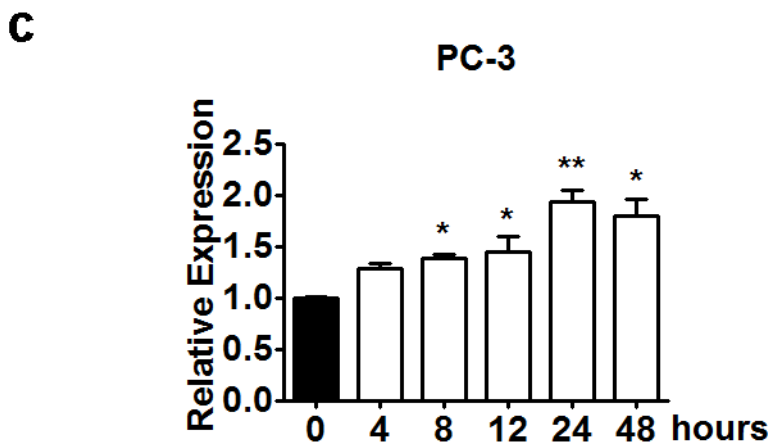
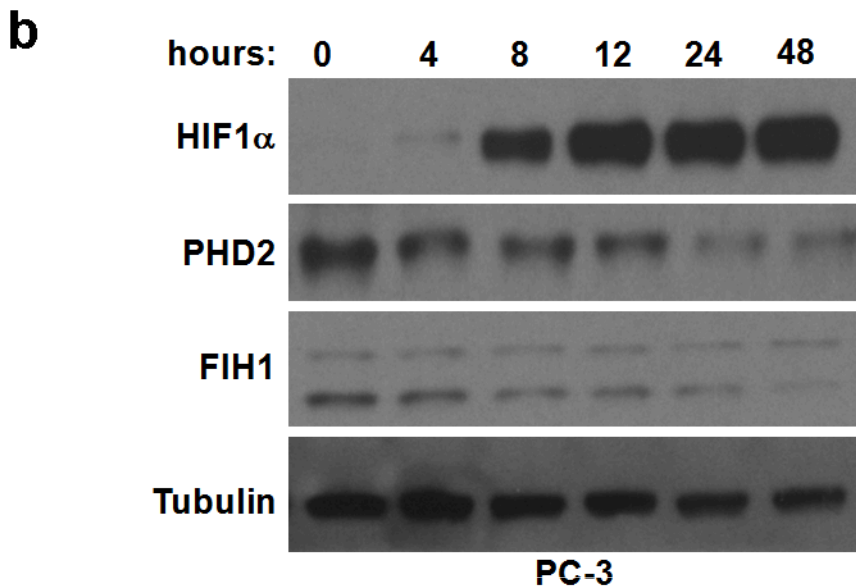
Sequence alignment of miR-182 promoter region containing hypoxia-responsive elements (HREs) from different species as indicated. The HRE1 and HRE2 are shown in red.

Supplementary Fig. S3

a

PHD2-3'UTR: 5'...GUCCUUGAUC----U--UCU**UUGCCAAA**AU...3'
MRE-mutant: 5'...GUCCUUGAUC----U--UCU**AACGGUUU**AU...3'

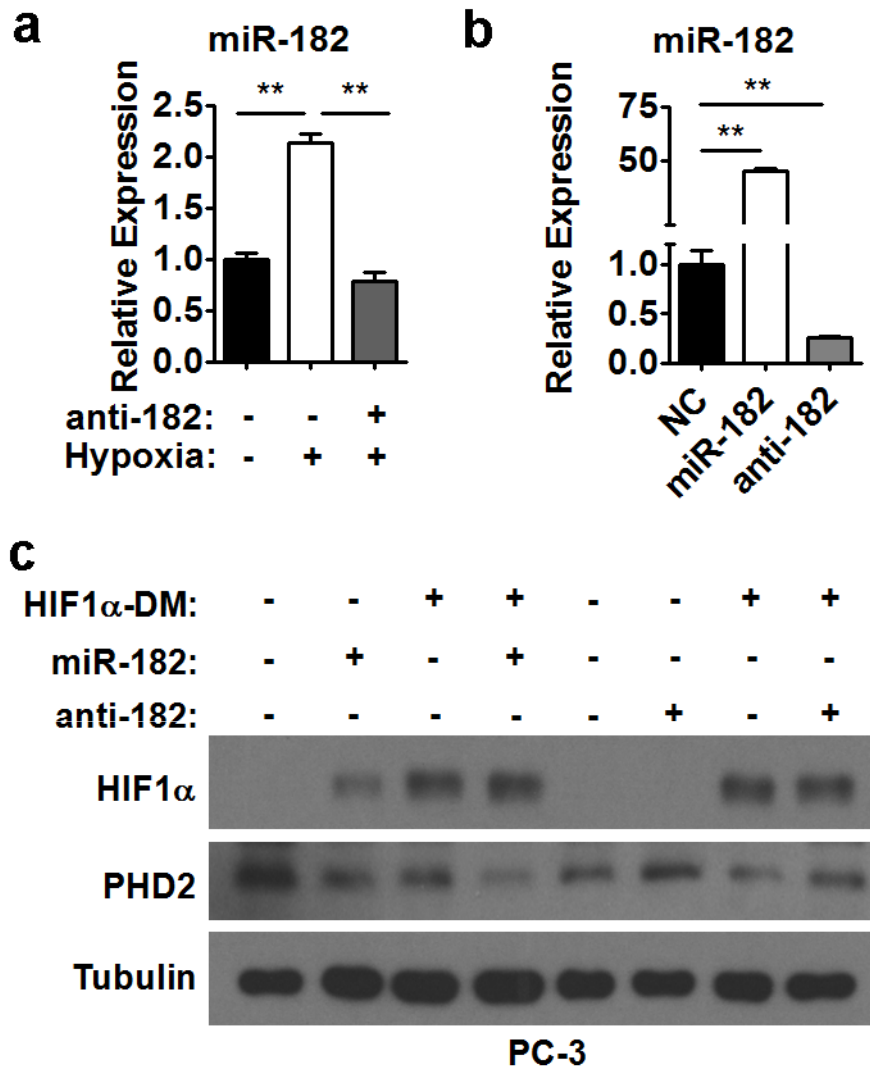
FIH1-3'UTR: 5'...UUUUUUUUUUU-----**UUGCCAAA**AU...3'
MRE-mutant: 5'...UUUUUUUUUUU-----**UAACGGUUU**AU...3'



Supplementary Figure. S3.

(a) Mutation (blue character) were introduced into miR-182 regulatory elements in PHD2 and FIH1 3'-UTR. (b, c) Cellular hypoxia was induced in PC-3 cells for different times as indicated. HIF1 α , PHD2, and FIH1 protein levels were determined by western blot analysis (b). miR-182 expression was analyzed by RT-PCR (c). Data are mean \pm SEM (n=3). *p<0.05, **p<0.01. Full-length blots are presented in Supplementary Fig. S6.

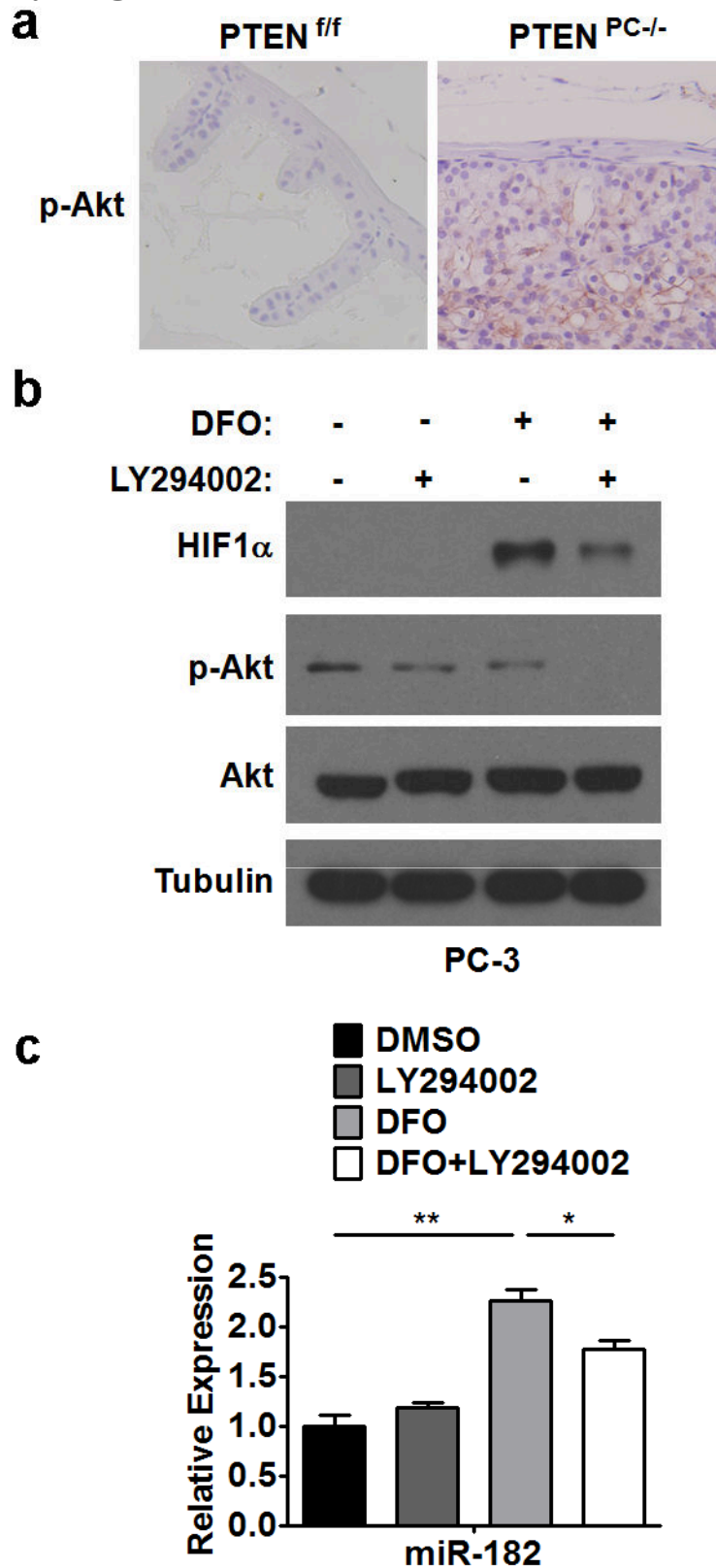
Supplementary Fig. S4



Supplementary Figure. S4.

(a) RT-PCR analysis of miR-182 expression in PC-3 cells transfected with miR-182 antagomir (anti-182) after the induction of cellular hypoxia. (b) RT-PCR analysis of miR-182 expression in PC-3 cells transfected with miR-182 mimics or miR-182 antagomir (anti-182) in a normoxic environment. Data are mean \pm SEM (n=3). **p<0.01. (c) PC-3 cells were co-transfected with HIF1 α -DM plasmid and miR-182 mimics or miR-182 antagomir (anti-182). 48 hours after transfection, the protein expression of HIF1 α and PHD2 were determined by western blot analysis. Full-length blots are presented in Supplementary Fig. S6.

Supplementary Fig. S5



Supplementary Figure. S5.

(a) Immunostaining of p-Akt in the prostate tissues from PTEN^{f/f} and PTEN^{PC-/-} mice. (b, c) Western blot analysis of HIF1 α , p-Akt, and total Akt protein expression (b) and RT-PCR analysis of miR-182 expression (c) in PC-3 cells treated with hypoxia inducer DFO and/or PI3K inhibitor LY294002 as indicated for 12 hours. Data are mean \pm SEM (n=3). *p<0.05, **p<0.01. Full-length blots are presented in Supplementary Fig. S6.

Supplementary Fig. S6

Fig.1c

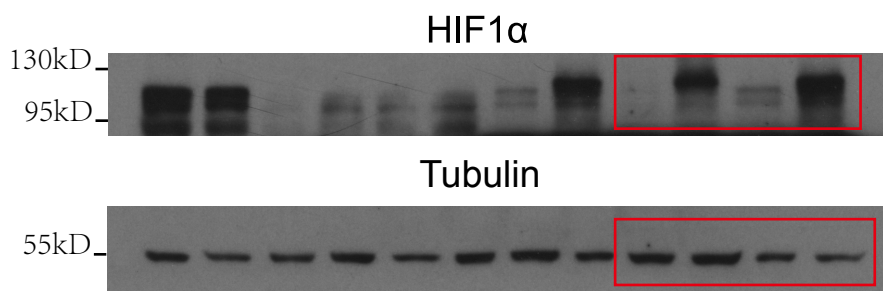


Fig.1g

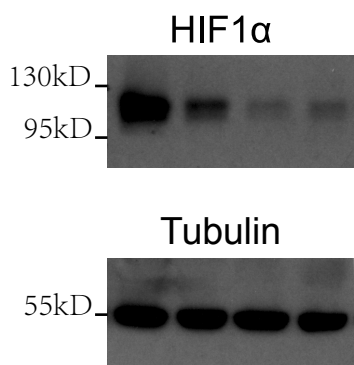


Fig.S1a

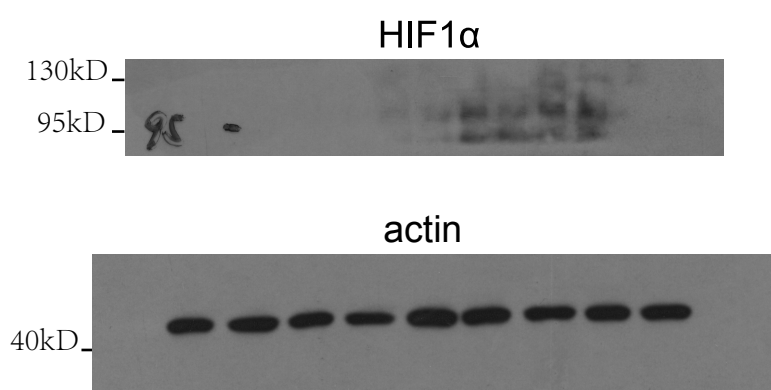
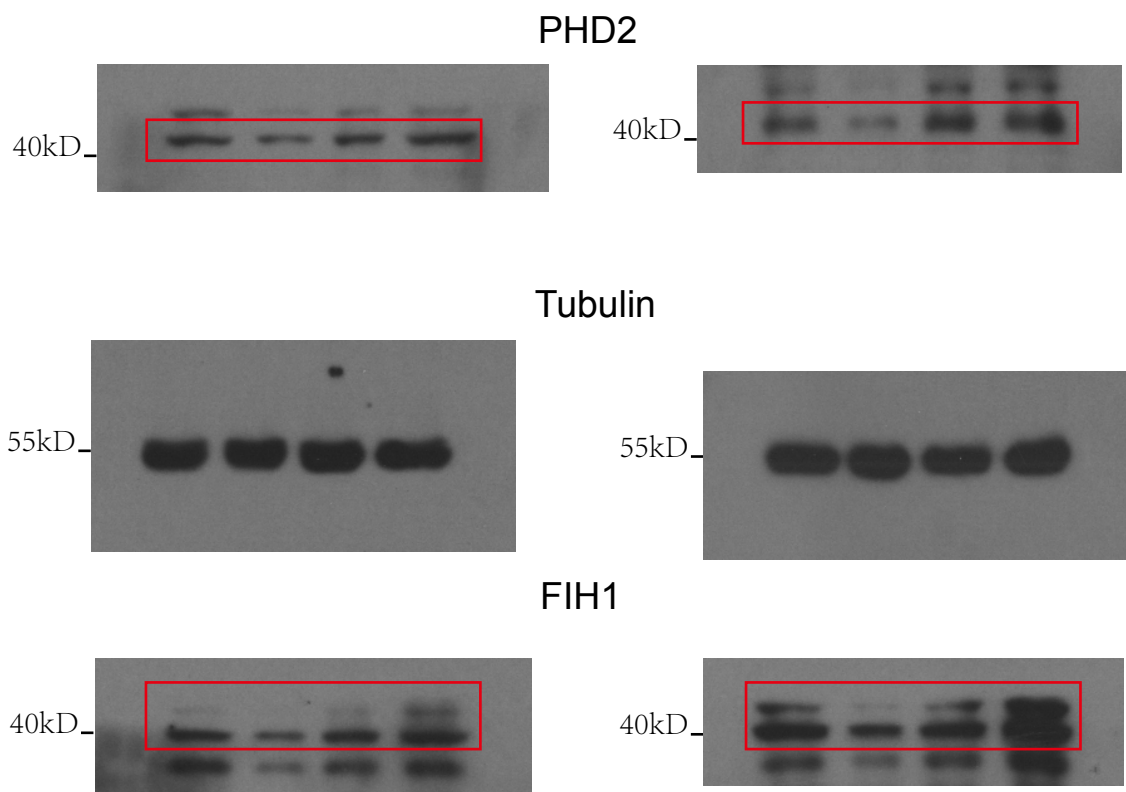


Fig.3c

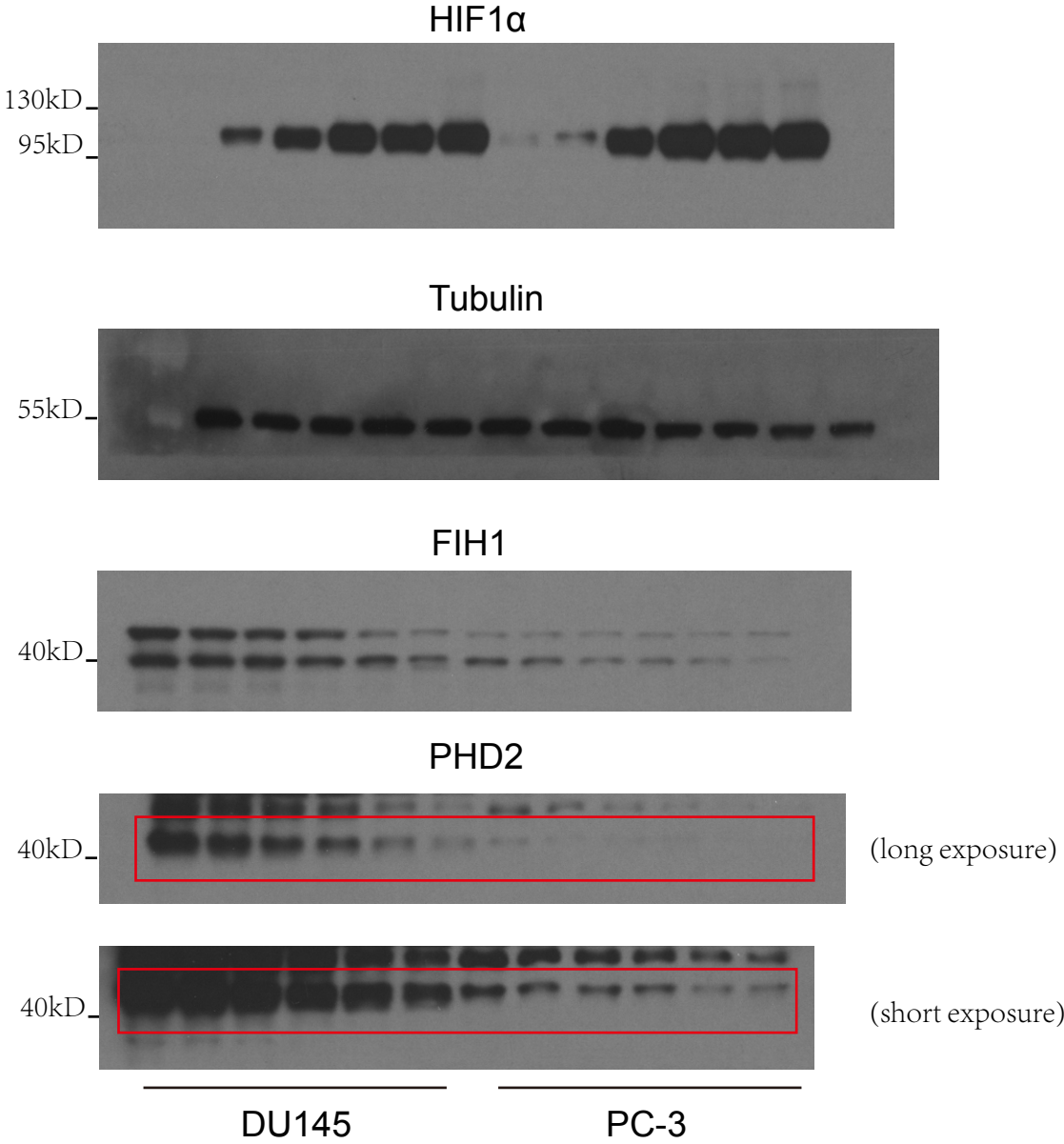


Supplementary Fig. S6

Full-length blots are shown.

Supplementary Fig. S6

Fig.3i & S3b



Supplementary Fig. S6

Full-length blots are shown.

Supplementary Fig. S6

Fig.4a

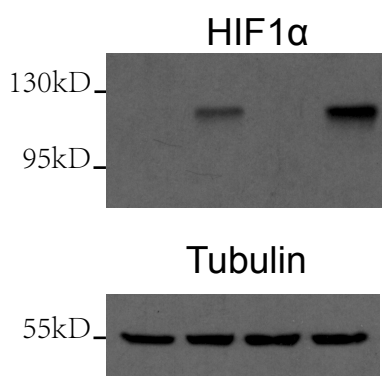
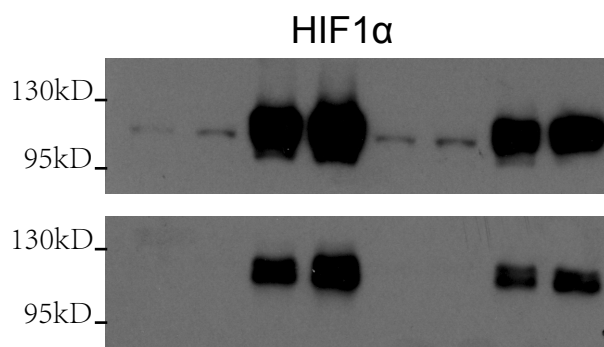
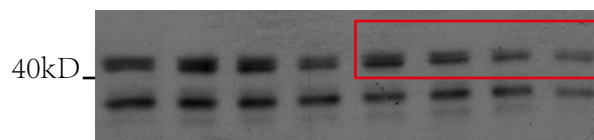


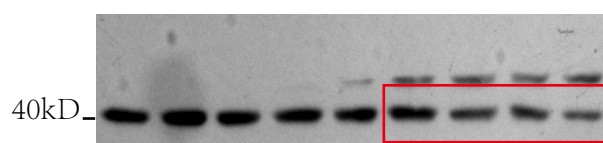
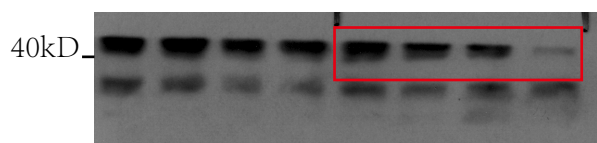
Fig.4b



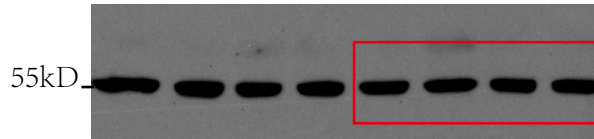
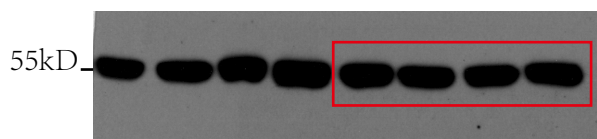
FIH1



PHD2



Tubulin



Supplementary Fig. S6

Full-length blots are shown.

Supplementary Fig. S6

Fig.4f

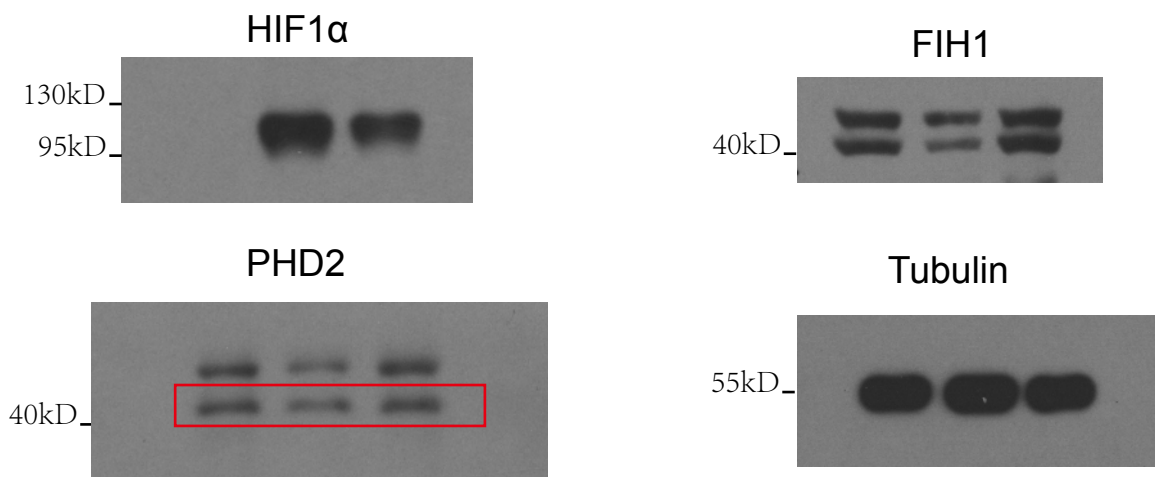


Fig.4g

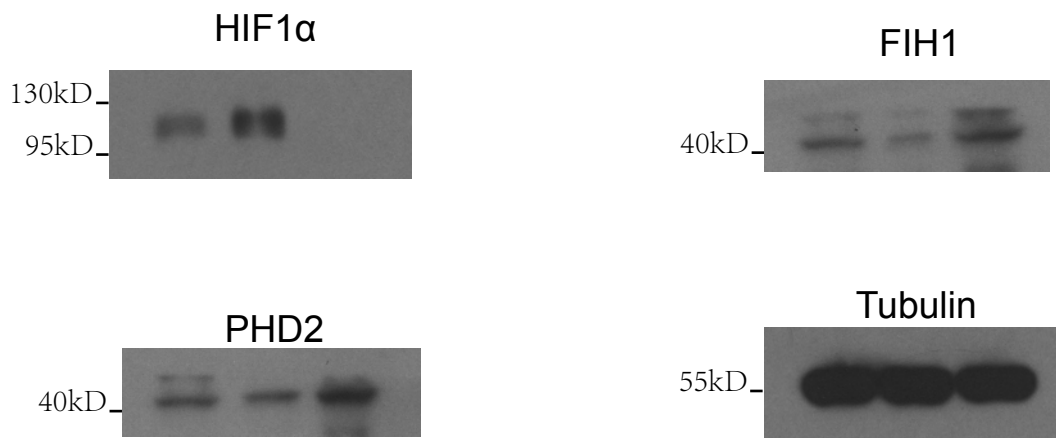
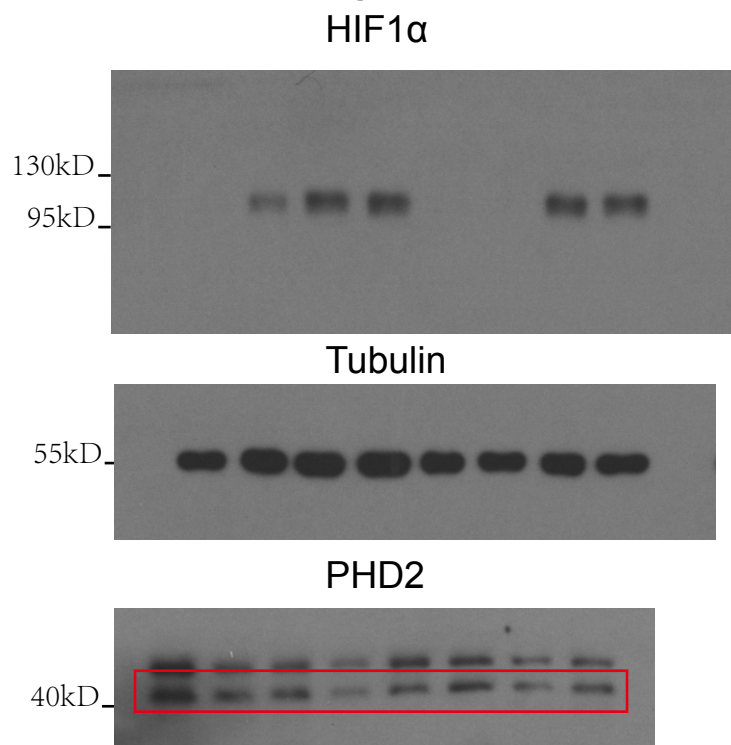


Fig.S4



Supplementary Fig. S6

Full-length blots are shown.

Supplementary Fig. S6

Fig.6d

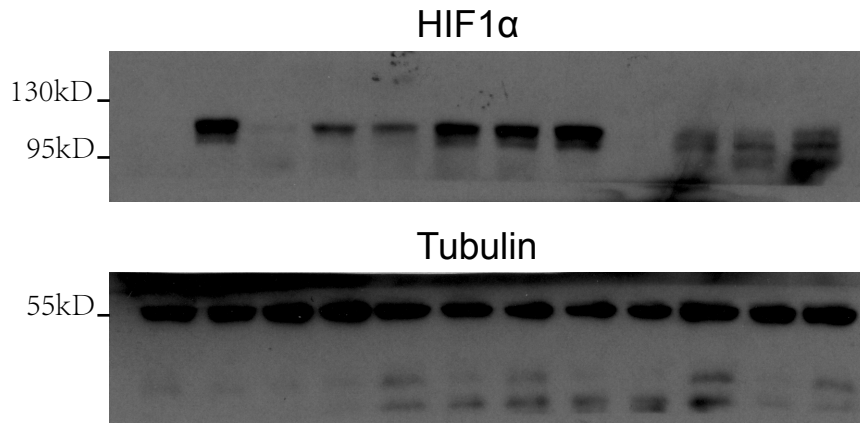
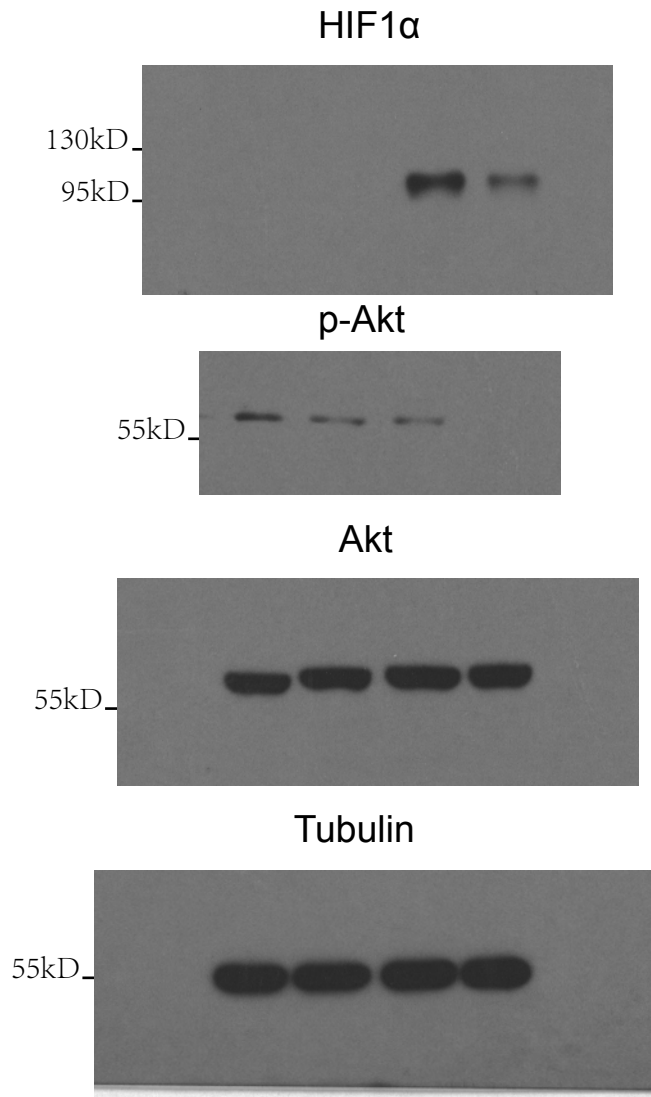


Fig.S5b



Supplementary Fig. S6

Full-length blots are shown.