Supplemental Materials

Molecular Biology of the Cell

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SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. A non-muscle actin peptide is hydroxylated on either a proline or methionine residue. (A) The hydroxylated peptide of non-muscle actin was identified by mass spectrometry. The hydroxyproline residue is underlined. (B) MS/MS spectrum of the hydroxylated non-muscle actin peptide is shown. The hydroxyproline residue is shown in lowercase.

FIGURE S2. PHD1 and PHD2 fail to interact with actin. (A) HeLa cells were transfected with vector encoding FLAG-PHD1 and exposed to 20% or 1% O_2 for 24 h. IP of whole cell lysates (WCLs) using IgG or anti-FLAG antibody was performed followed by immunoblot assays with antibodies against the indicated proteins. (B) HeLa cells were exposed to 20% or 1% O_2 for 24 h. IP of WCLs using IgG or anti-PHD2 antibody was performed followed by immunoblot assays with antibodies against the indicated proteins.

FIGURE S3. PHD3 knockdown increases actin polymerization in cancer cells. HeLa-shSC and HeLa-shPHD3-2244 cells were fixed, permeabilized, stained with Alexa Fluor 555-conjugated phalloidin, and imaged by fluorescence microscopy. The boxed areas are enlarged and shown at the bottom. Representative images from at least three independent experiments are shown. Scale bar, 100 μm.

FIGURE S4. PHD3 knockdown has no effect on HeLa cell proliferation. HeLa-shSC and HeLa-shPHD3 cells were cultured for 72 h. Viable cells were counted at the indicated time points by trypan blue staining using an automatic cell counter. The data are presented as mean ± SEM, n = 3.

FIGURE S5. PHD3 knockdown alters cell morphology. HeLa-shSC and HeLa-shPHD3 cells were imaged by brightfield microscopy. The boxed areas are enlarged and shown on the right side. Scale bar, 100 μm.

VIDEO 1. HeLa-shSC cell migration. Cells were seeded onto microfluidic chips and chemotaxis was driven by a serum gradient (0.5% to 10%). Images were acquired every 10 min with Zeiss microscopy.

VIDEO 2. HeLa-shPHD3 cell migration. Cells were seeded onto microfluidic chips and chemotaxis was driven by a serum gradient with (0.5% to 10%). Images were acquired every 10 min with Zeiss microscopy.

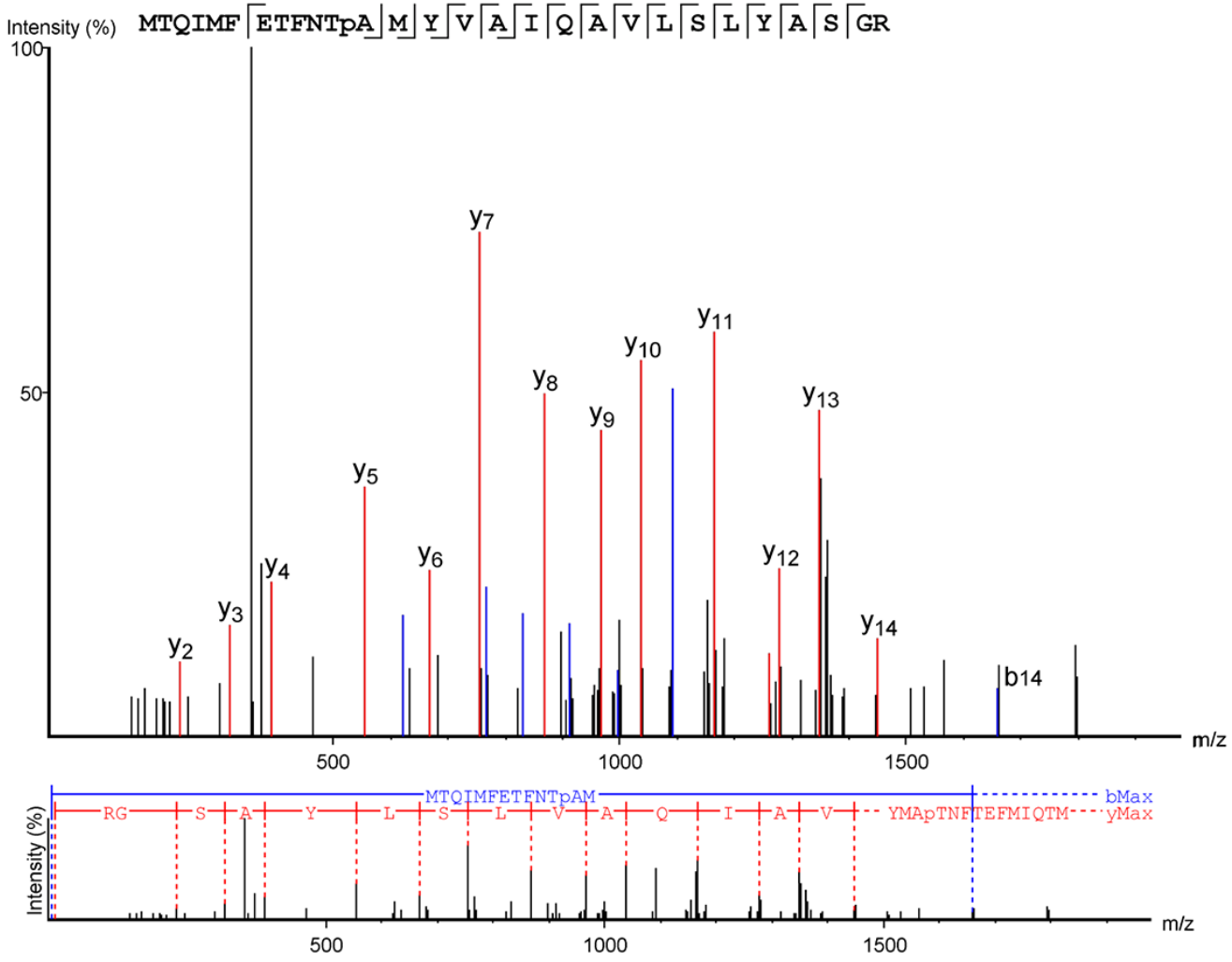
VIDEO 3. DMSO-treated HeLa cell migration. Cells were seeded onto microfluidic chips and chemotaxis was driven by a gradient (0.5% to 10%). Images were acquired every 10 min with Zeiss microscopy.

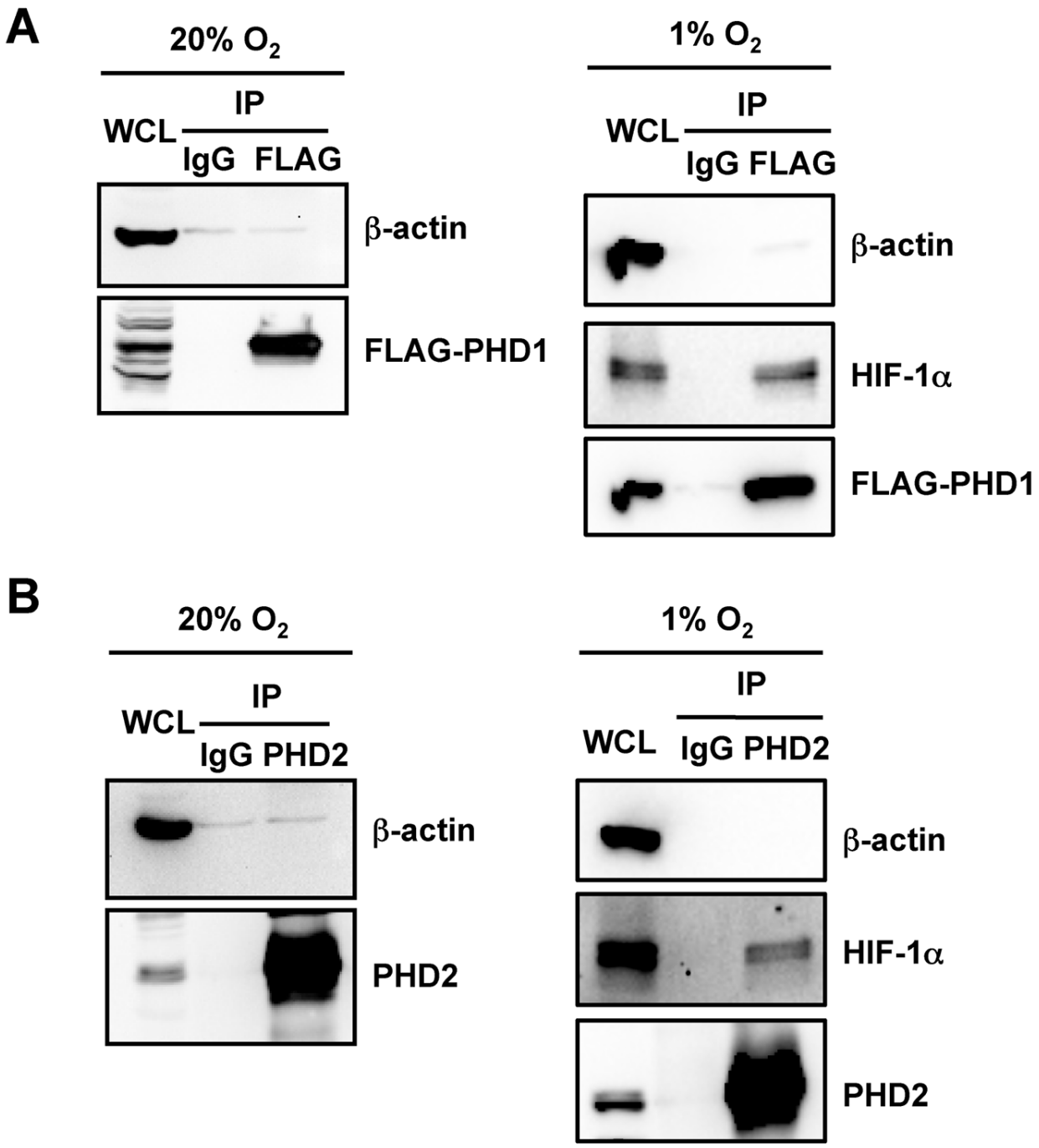
VIDEO 4. DMOG-treated HeLa cell migration. Cells were seeded onto microfluidic chips and chemotaxis was driven by a serum gradient (0.5% to 10%). Images were acquired every 10 min with Zeiss microscopy.

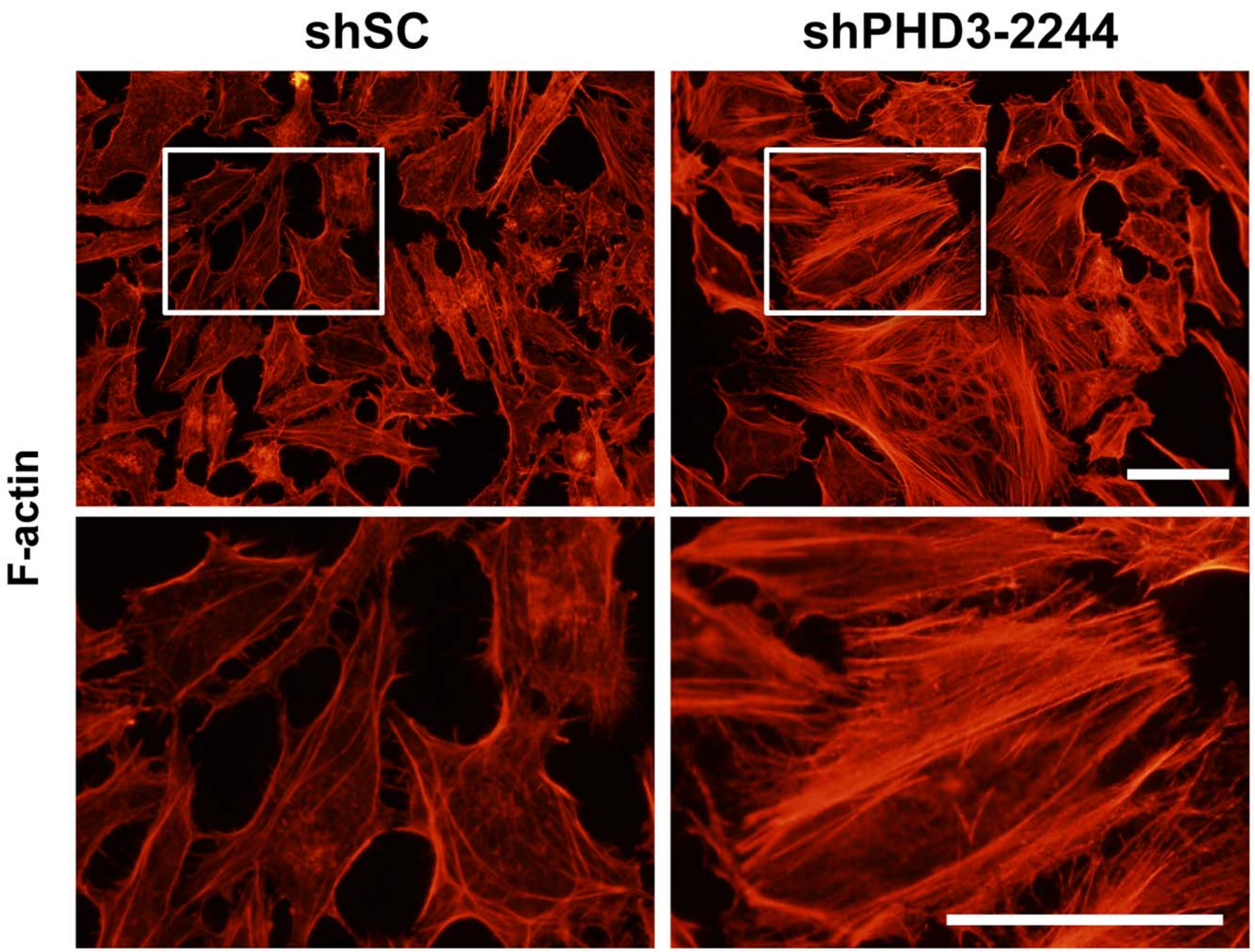
A

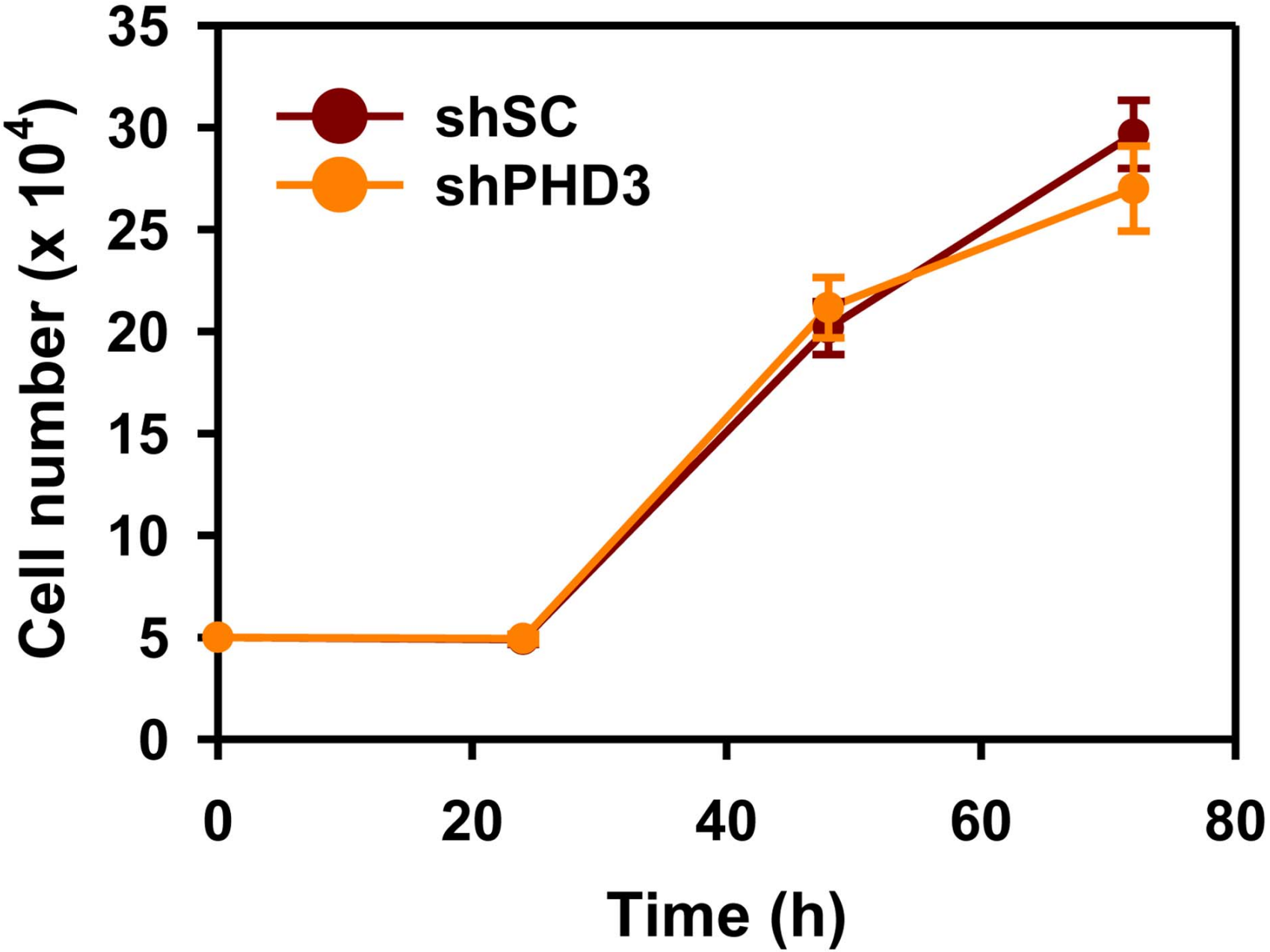
Protein	Peptide	Heavy/ Light	Heavy peptide mass shift
Non-muscle actin	MTQIMFETFNTPAMYVAIQAVLSLYASGR	0.783	3.33 Da

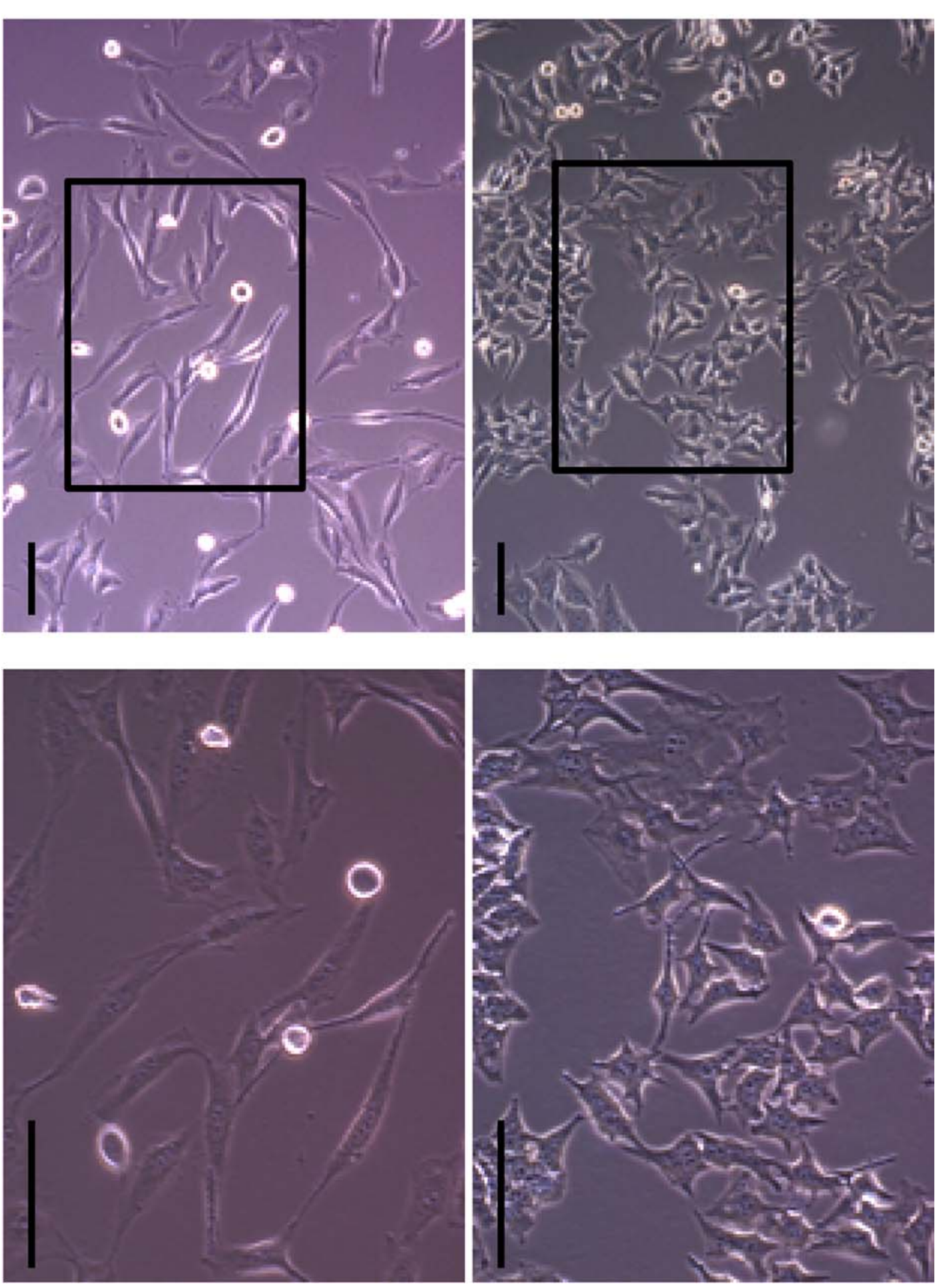
B




A
20% O2
1% O2
IP
WCL
IgG
FLAG
β-actin
FLAG-PHD1
HIF-1α
B
PHD2


shSC
shPHD3-2244
F-actin


shSC
shPHD3
Cell number (x 10^4)
35
30
25
20
15
10
5
0
0
20
40
60
80
Time (h)


HeLa-shPHD3
HeLa-shSC