

# Supplementary Materials for

## **TiPARP Forms Nuclear Condensates to Degrade HIF-1 $\alpha$ and Suppress Tumorigenesis**

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### **This PDF file includes:**

Supplementary materials and methods  
Figs. S1 to S6

**Cell Culture, hypoxic incubation and transfection.** MCF-7, RCC4 and HEK293T cells were obtained from the American Type Culture Collection (Manassas, VA), and were cultured in Dulbecco's modified Eagle's medium (Gibco, 11965–092) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, 26140079). HCT116 cells were cultured in McCoy's 5A medium (16600082) with 10% FBS. Cells were grown in a humidified atmosphere at 37 °C at gas tensions of 20% O<sub>2</sub> / 5% CO<sub>2</sub> for normoxic incubation and 1% O<sub>2</sub> / 5% CO<sub>2</sub> for hypoxic incubation.

For transient overexpression of proteins of interest, cells were transfected with expression vectors using FuGene 6 according to the manufacturer's protocol. To stably knock down TIPARP or PML proteins, lentivirus was produced by transfecting HEK 293T cells with pCMV-ΔR8.2 (packaging vector), pM2D.G (envelope vector) and TIPARP or PML shRNAs (Sigma). Virus-containing medium was harvested 24 h after transfection. Target cells were seeded in a 6-well plate 20 h prior to transduction ( $3 \times 10^5$  cells per well). At the confluence of 50%, target cells were transduced with virus particles in medium supplemented with 5 μg/ml polybrene (Sigma), and incubated for 6 h. The medium was removed and fresh medium was added to the cells. After 72 h, 2 μg/mL of puromycin was added to the cell culture media for 10 days to select stably transduced target cells. 5 nM of siRNA was transfected with Lipofectamine RNAiMAX according to the manufacturer's instructions. Nontarget siRNA was used in control transfections.

**Plasmids.** Human TIPARP cDNAs was amplified by PCR using pCMV6-XL4-hTiPARP as templates (Origene, Rockville, MD, USA. Cat: RC230398). Following amplification, the CDS of TIPARP was cloned into EcoRI and SalI sites in pAcGFP-C1 vector to generate GFP-TiPARP expression vector (forward primer: 5'-AGTCAGGAATTCTATGGAAATGGAAACCACCGAACCTGAGCCAGA-3'; reverse primer: 5'-AGTCAGGGATCCCTACTTATCGTCGTCATCCTTGTAATCCAGGATATCATTGTC-3'). Expression vectors for TiPARP H532A catalytic mutant was generated by QuikChange site-directed mutagenesis (forward primer: 5'-AAATGAGAGACATTTATTTGCTGGAACATCCCAGGATGTGGT-3'; reverse primer: 5'-AAATAAATGCTCTCATTATTATCCTGTCACGGCCAAACATTTTCC-3'). TiPARP W347A mutant was generated with forward primer: 5'-TATTTACCACACAGTCGCGAAATTCTTCTGTAGGG-3' and reverse primer: 5'-CGCGACTGTGTGGTAAATAGAGTTGACATTGCTA-3'. TiPARP W357A mutant was generated with forward primer: 5'-

AGGGACCACTTTGGAGCGAGAGAGTATCCCGAG-3' and reverse primer: 5'-CGCTCCAAAGTGGTCCCTACAGAAGAATTT CCAGACTGT-3'. Luciferase reporter construct with *TiPARP* promoter was obtained by PCR amplification of a 1.2kb proximal promoter fragment from human cDNA library and inserting this fragment into the XhoI and HindIII restriction sites in the pGL4.14 vector (Promega) (forward primer: 5'-AGTCAGCTCGAGAGATCTTGTCTCAATAAGATTTTAAATGTAAAGATTTTC A-3'; reverse primer: 5'-AGTCAGAAGCTT GTGCGGTGGACTTATGCTCCC-3'). Mutant promoter-luciferase construct was obtained by QuikChange site-directed mutagenesis on core HRE sequence (forward primer: 5'-TCCTTCCT CACAGCCTTGTGTAGACGCGGACCC-3'; reverse primer: 5'-GGCTGTGAGGAAGGAAGG CGCGTGCCGCGTGGG-3'). pcDNA-HA-HIF-1 $\alpha$  plasmid was a gift from Dr. M. Celeste Simon. Truncations of N-terminal HA tagged HIF-1 $\alpha$  was obtained by introducing stop codons at truncated sites via mutagenesis. Primers used for generating HIF-1 $\alpha$  truncations are listed in Table S1. Human HIF-1 $\alpha$  cDNA ORF clone with C-GFPspark® tag was purchased from Sino Biological (Cat# HG11977-ACG). Via Addgene, HRE-luciferase construct was obtained from Navdeep Chandel (# 26731); pUltra-dox (# 58749) and pUltra-puro-RTTA3(# 58750) constructs were obtained from Yildirim Dogan and Kitai Kim; HA-HIF2 $\alpha$ -pcDNA3(# 18950) was obtained from William Kaelin; pBV-Luc wt MBS1-4 (# 16564) was obtained from Bert Vogelstein; pcDNA-HA-ERapha WT(# 49498) was obtained from Sarat Chandarlapaty; pcDNA-Flag-ERbeta (# 35562) was obtained from Harish Srinivas; 3X ERE-TATA-luc (luciferase reporter containing three copies of estrogen response elements) (# 11354) construct was obtained from Donald McDonnell. Human c-Myc expression construct with N-terminal Flag-tag was obtained by PCR amplification from cDNA library and subcloning into pCMV-tag-4a vector through BamHI and XhoI sites. Human cMyc cDNA with N-terminal Flag-tag was obtained by PCR amplification of Flag-c-Myc and subcloning via BamHI and XhoI sites into pCMV-tag-4a vector. shRNA plasmids targeting human *TiPARP* and *PML* mRNAs were purchased from Sigma. Sequences for human *TiPARP* shRNAs and human *PML* shRNAs are listed in Table S2. pcDNA3.1-HUWE1-V5-His plasmid was a gift from Dr. Wei Gu (accession: AY772009). HUWE1 W1617/1619A mutant was generated by QuikChange site-directed mutagenesis with (forward primer: 5'- AACAACGCGCGCGCGTTTGATGATCGCTCTG-3'; reverse primer: 5'- ATCAAACGCGCGCGCGTTGTTGCTGTTGGATTGC-3'). Phusion® High-

Fidelity DNA Polymerase from NEB was used in PCR reactions following the manufacturer's instructions.

**Western Blot.** Western blot analysis was performed following previously published methods (38). The proteins of interest were detected and visualized using a Typhoon FLA 7000 scanner (GE Healthcare) and ChemiDoc XRS System (Bio-rad).  $\beta$ -actin antibody (C4) and ER $\alpha$  antibody (F-10) were purchased from Santa Cruz. HIF-1 $\alpha$  antibody was purchased from BD Biosciences. c-Myc (D84C12), ubiquitin (P4D1) and PARP1 (9542) antibodies were purchased from Cell Signaling. TipARP antibody was purchased from Abcam (ab84664). Antibody against poly(ADP-ribose) (PAR) polymer was purchased from Trevigen (4335-MC-100). Antibodies used in western blotting were diluted 1:1000.

**Quantitative real time PCR (RT-qPCR).** Total RNA was isolated using RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. Total RNA (0.2  $\mu$ g) was employed for cDNA synthesis using the SuperScript III First-Strand Synthesis kit (Invitrogen) following the manufacturer's instructions. For quantitative real-time PCR analysis, iTaq Universal SYBR Green Supermix (Bio-Rad) was used following the manufacturer's instructions. The reaction was performed with QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems™). In each run, melt curve analysis was performed to ensure the amplification of a single product. The relative expression of each gene, normalized to actin, was calculated using the  $2^{-\Delta\Delta C_t}$  method. Primers used for RT-qPCR are listed in Table S3.

**Generation of inducible TIPARP overexpression cells.** FLAG-TIPARP gene was cloned into lentiviral pUltra-dox vector (doxycycline inducible multicistronic lentiviral gene expression system). Tet-on inducible HCT116 stable cell lines were generated as previously reported (38) with slight modifications. Briefly, HEK 293T cells were transfected with pUltra-puro-rtTA3 (rtTA3, reverse tetracycline-controlled transactivator 3) and pUltra-dox-TIPARP plasmid to produce lentivirus. HCT116 cells were first transduced with viral particles carrying rtTA3 construct and selected with 2  $\mu$ g/ml puromycin for 10 days. Cells stably expressing rtTA3 were further infected with virus carrying pUltra-dox-TIPARP. Expression of FLAG-TIPARP is induced by the treatment of 10 $\mu$ g/ml doxycycline for 24 hr. Successful induction is confirmed by performing anti-flag immunofluorescence confocal imaging and western blot.

**Cell proliferation assay.** HCT116 and MCF-7 cells stably expressing luciferase (Ctrl) shRNA or TIPARP shRNA were seeded in 24-well plate at a density of 3,000 cells/well. After 24 h cells in each well were washed with PBS, fixed with ice-cold methanol for 10 min and stained with 0.5% crystal violet (m/v, in 25% methanol). Stained cells were then washed with water and air-dried. Crystal violet stain in each well was eluted with 500  $\mu$ l of 10% acetic acid in water. After transferring 100  $\mu$ l of sample from each well to a 96-well plate, the 550 nm absorption for each sample was measured. From day 0 to day 5, cells were fixed every 24 h to monitor the growth rate.

**Soft agar colony formation assay.** In a 6-well plate, 2 ml of 0.6% base low-melting point agarose (LMP) in complete medium supplemented with 10% FBS was added to each well, and allowed to solidify for 30 min at room temperature. For each well,  $5 \times 10^3$  HCT116 cells stably expressing luciferase (Ctrl) shRNA or TIPARP shRNA were resuspended in 1 ml of 0.3% agarose in 10% FBS McCoy's 5A medium, and plated on top of the 0.6% agarose base. Every two days, 200  $\mu$ l of fresh medium was added to the cells. After 2-3 weeks of culture, colonies were stained with 0.1% crystal violet (m/v in 25% methanol) for 20 min at room temperature, rinsed with 50% methanol, and counted with ImageJ.

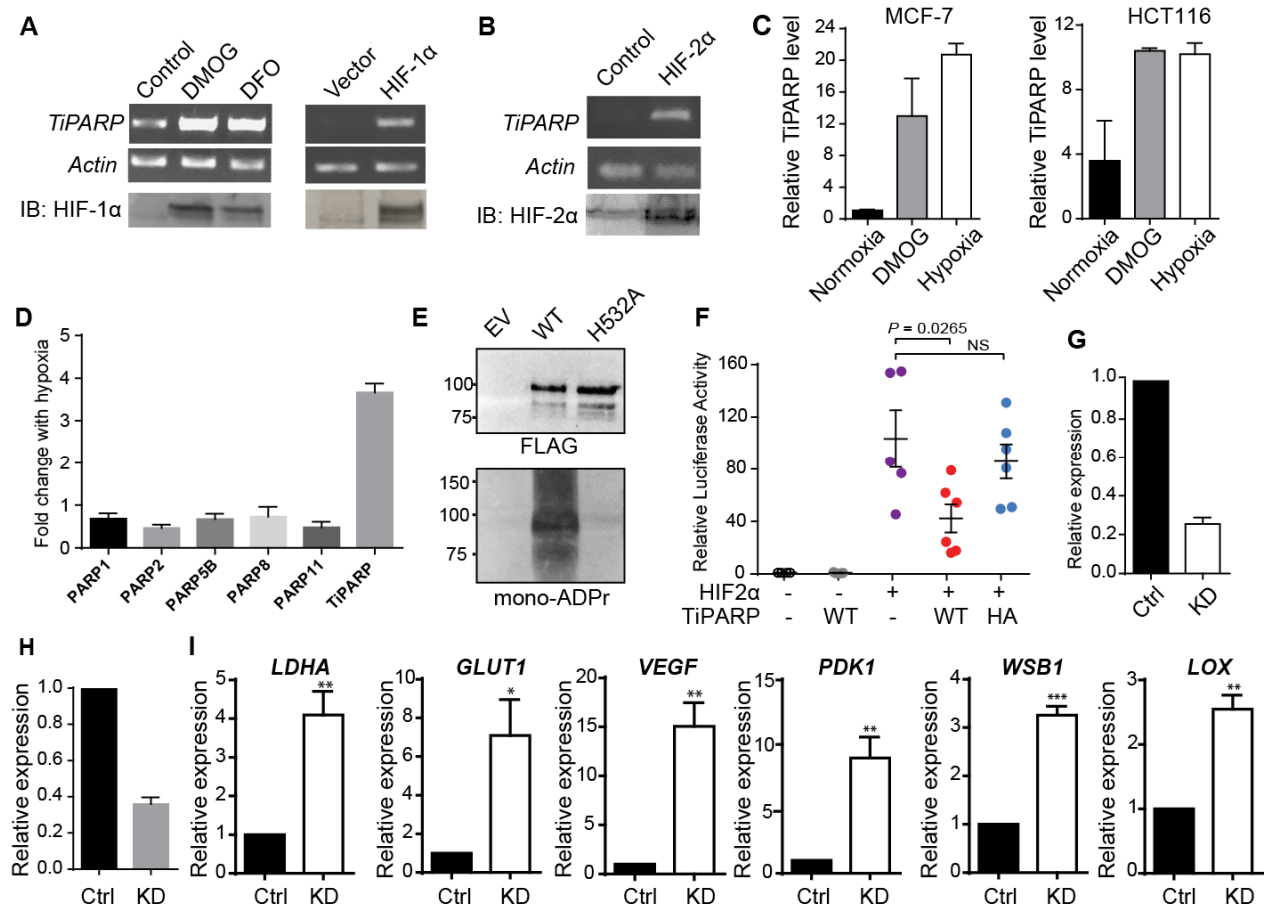
**Lactate and Glucose Measurements.** Glucose and lactate levels in culture media were measured using the Glucose Assay Kit and Lactate Assay Kit (Biomedical Research Service Centre, SUNY Buffalo). Fresh media were added to subconfluent cells in a 24-well plate, and extracellular glucose/lactate concentration were measured 24 hours later and normalized to the number of cells in each well.

**Mice study.** Tumors were established by subcutaneously injection of lentivirus-infected MCF-7 and HCT116 cells ( $5 \times 10^6$  cells/animal) into 4- to 6-week-old NOD scid gamma mice (NSG mice) (Jackson Laboratory, Bar Harbor, US). For TiPARP expression xenografts, HCT116 Tet-on TiPARP stable cell lines were treated with or without 20  $\mu$ M doxycycline for 36 hr. Cells were then injected subcutaneously into mice. In control group, mice were fed with control diet; in Dox group, mice were fed with food containing doxycycline hyclate (625 mg per kg diet). Tumor volume (TV) was monitored daily and was calculated as  $V = \frac{1}{2} \times \text{length} \times \text{width}^2$ . At the termination of experiment, tumor tissues were harvested, weighted, and immunohistochemistry was further conducted. All animal experiments were carried out in accordance with the guidelines of the

National Advisory Committee on Laboratory Animal Research and the Cornell University Institutional Animal Care and Use Committee.

**Immunohistochemistry.** Tumors were fixed in 4% paraformaldehyde, embedded in paraffin and cut into 4- $\mu$ m thick sections for immunohistochemical analysis. After deparaffinization in xylene and rehydration in graded ethanol, the sections were subjected to antigen retrieval. For TIPARP, antigen retrieval was performed by steaming slides in Tris-EDTA buffer (pH 9.0) for 20 min. For CD31 and HIF-1, antigen retrieval was performed by steaming in citrate buffer (10 mM, pH 6.0) for 20 min. Then, the endogenous peroxidase activity was quenched by 0.3% hydrogen peroxide in distilled water for 10 minutes. For TIPARP and CD31, IHC analysis was performed by using ImmPRESS HRP Anti-Rabbit Ig (Peroxidase) Polymer Detection Kit (Vector Laboratories) following the kit instruction. The tissue section was incubated with primary antibody for 1.5 h at room temperature with the following dilutions: rabbit polyclonal anti-TIPARP IgG (Sigma-Aldrich) at 1:50; rabbit polyclonal anti-CD31 IgG (Abcam, ab28364) at 1:50. Negative controls were run in parallel by replacing the primary antibody with rabbit IgG at the equivalent final concentration. As for HIF-1, M.O.M Immunodetection Kit (Vector Laboratories) was used to eliminate background staining of mouse primary antibody on mouse tissue. IHC was performed by following the kit instruction with slight modification. The section was incubated with mouse monoclonal anti-HIF alpha IgG2b (Clone H1alpha67, Novas Biologicals, 1:400 dilution) for 1 h at room temperature. Mouse IgG2b at equivalent concentration was used as isotype negative control to validate epitope-specific staining. For all three antibodies, Nova Red (Vector Laboratories) was used as chromogen to visualize antigen localization, and the sections were lightly counterstained with hematoxylin. IHC results were examined by Olympus AX 70 compound microscope equipped with MicroFire camera and PictureFrame for image processing and capture (Optronics).

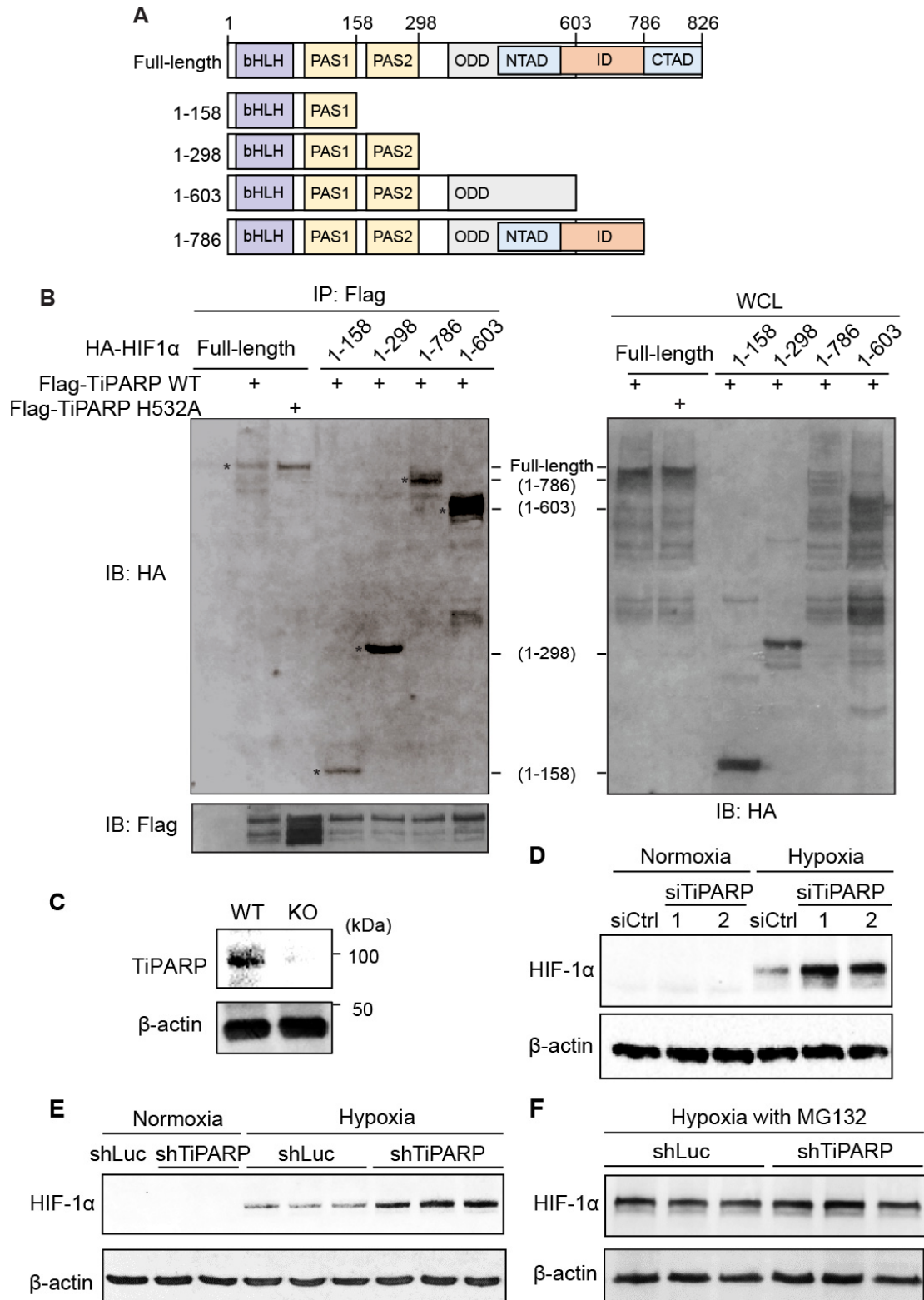
**Statistical analysis.** Means, standard deviation (sd), and standard error of the mean (sem) were analyzed using Prism (v6.0) or Microsoft Excel. *P* values were calculated based on two-tailed, unpaired Student's *t*-tests. Statistical significance was accepted for *P* values of < 0.05. All experiments were performed at least two to three times.



**Fig. S1: *TiPARP* is a target gene and a negative regulator of HIF.** (A) RT-PCR analysis of *TiPARP* mRNA level in HEK 293T treated with hypoxia-mimetic agent DMOG and DFO, or transfected with HA-tagged HIF-1 $\alpha$ . Expression of endogenous or HA-HIF-1 $\alpha$  was detected by HIF-1 $\alpha$  antibody on western blot (bottom). (B) Top: RT-PCR analysis of *TiPARP* mRNA with or without expression of HA-HIF-2 $\alpha$ . Bottom: western blot of HA-HIF-2 $\alpha$  in HEK 293T cell lysate with HA antibody. (C) Quantification of *TiPARP* protein levels upon normalization for  $\beta$ -actin in western blots of Fig. 1C. (D) HCT116 cells were cultured at normoxia or hypoxia (1% O<sub>2</sub>) for 16 h, followed by qRT-PCR analysis of the mRNA level of different PARPs. Fold changes in response to hypoxia were shown. Data are represented as means + sd,  $n = 5$ . (E) HEK293T cells were transfected with empty vector (EV), Flag-tagged WT or H532A mutant *TiPARP*, followed by immunoprecipitation with anti-FLAG resins. Mono-ADP-ribosylation of *TiPARP* was detected by western blot using anti-mono-ADP-ribose reagent to confirm the catalytic activity of WT *TiPARP*. (F) Luciferase reporter assay for HIF-2 $\alpha$  transactivation in HEK 293T cells co-transfected with HA-HIF-2 $\alpha$  and *TiPARP* WT or H532A mutant (HA). Luciferase reporter construct used in this

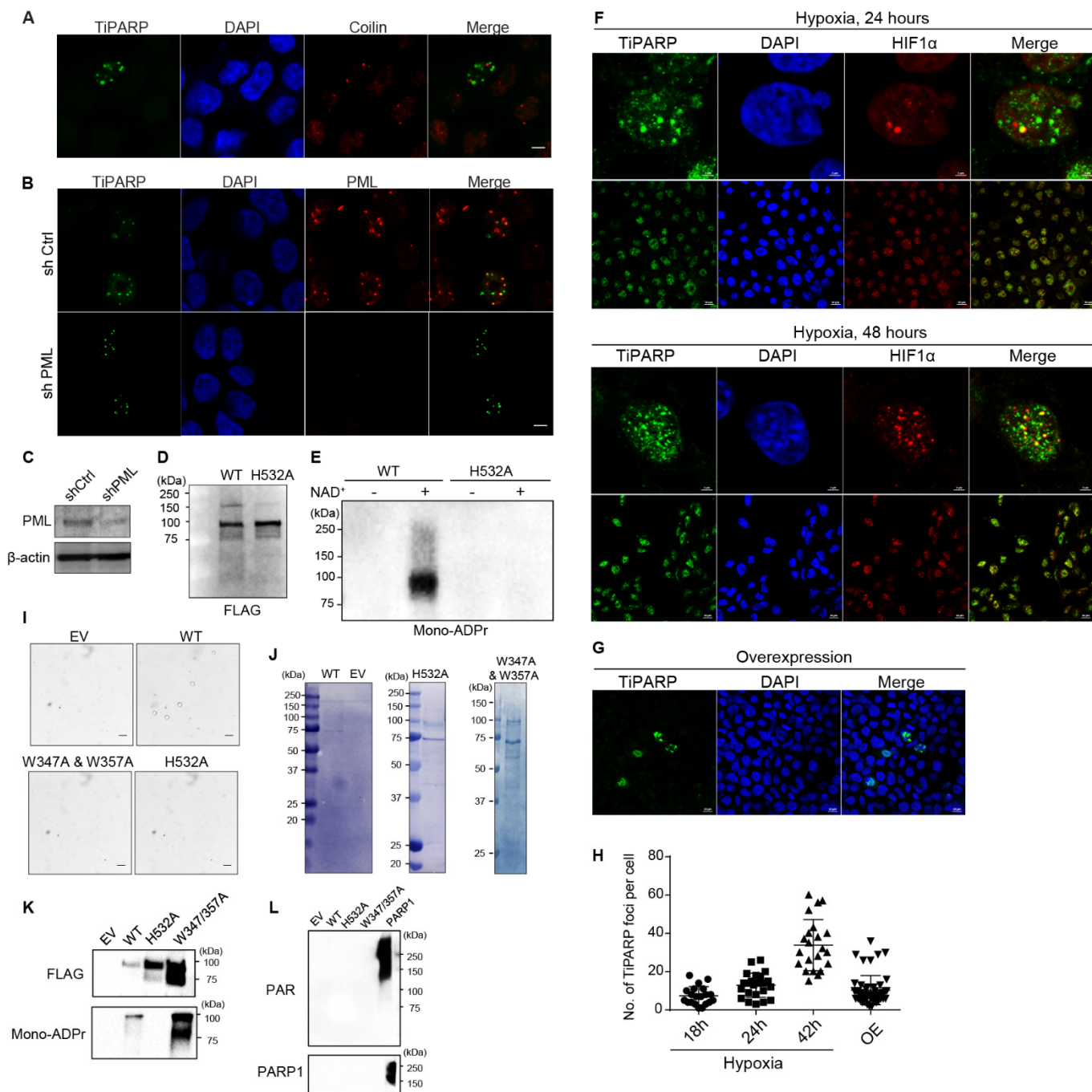
experiment contained three hypoxia response elements from the *Pgk-1* gene. Results are presented as mean  $\pm$  sd.,  $n = 6$ . **(G, H)** mRNA levels of *TiPAPR* in HCT116 **(G)** and RCC4 **(H)** cells stably expressing shRNA against luciferase (Ctrl) or TiPARP (KD) were measured by qRT-PCR to confirm the knockdown effect. Data are represented as means + sd.,  $n = 3$ . **(I)** Expression of HIF-1 $\alpha$  target genes in RCC4 cells measured by qRT-PCR. Data are represented as means + sd,  $n = 3$ . Statistical analyses were performed using unpaired two-tailed *t*-tests. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \* $p < 0.001$ ; NS, not significant.





**Fig. S2: TiPARP interacts with the N-terminal of HIF-1α and promotes its degradation. (A)** Schematic showing the full length and truncated HA-tagged HIF-1α constructs. Colored boxes

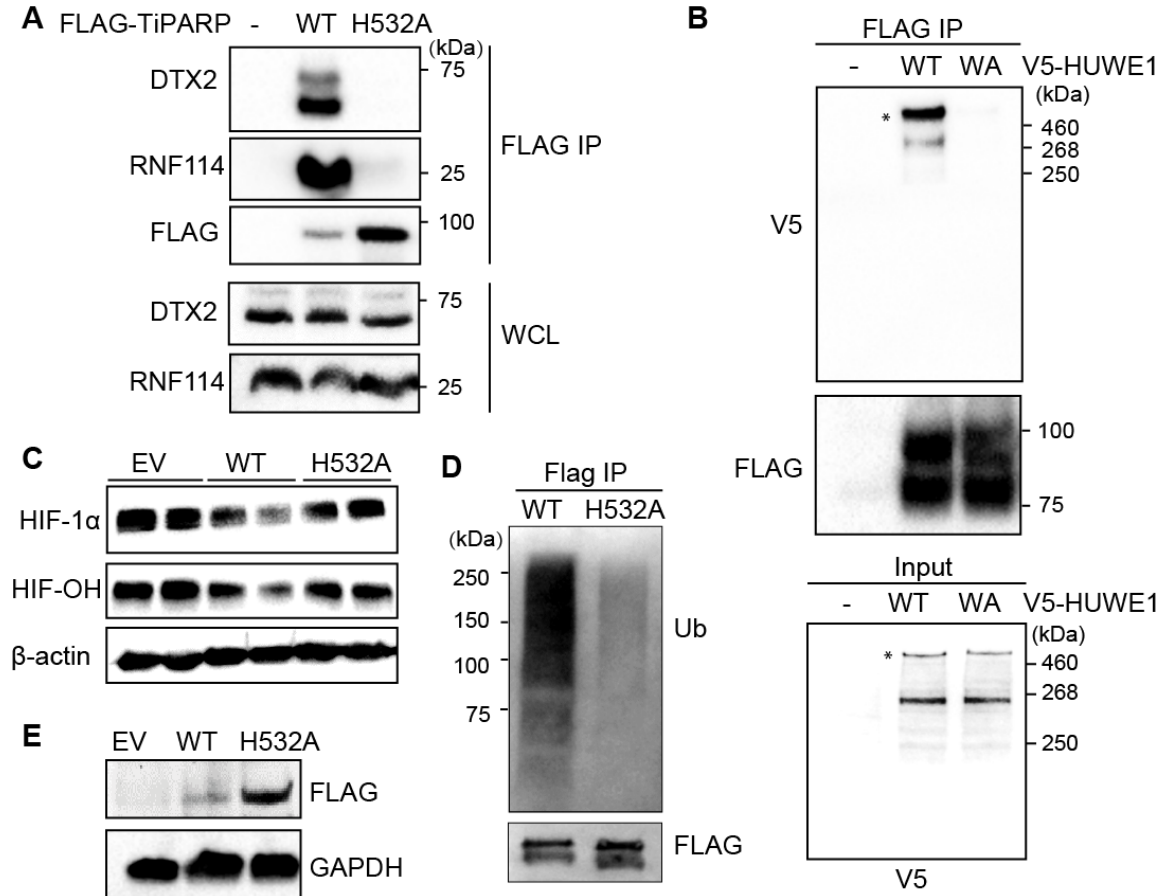
represent the functional domains of HIF-1 $\alpha$ . Numbers refer to the truncation positions. **(B)** Co-immunoprecipitation of Flag-TiPARP and truncated HA-HIF-1 $\alpha$  in HEK 293T cells. Expression of HA-tagged HIF-1 $\alpha$  truncations in whole cell lysates are shown in the right panel. **(C)** Knockout of TiPARP in HAP-1 cells was confirmed by western blot. **(D)** Western blot of HIF-1 $\alpha$  in HCT116 cells transfected with control or TiPARP siRNA for 24 h followed by 6 h of normoxia or hypoxia (1% O<sub>2</sub>) treatment. **(E)** Western blot of HIF-1 $\alpha$  in HCT116 cells stably expressing luciferase (control) or TiPARP shRNA, followed by 6 h of normoxia or hypoxia (1% O<sub>2</sub>) treatment. **(F)** HCT116 cells stably expressing luciferase (control) or TiPARP shRNA were treated with hypoxia (1% O<sub>2</sub>) and 20  $\mu$ M of MG132 for 6 h. HIF-1 $\alpha$  in cell lysates was analyzed by western blotting.



**Fig. S3: Condensates formation of TiPARP requires both catalytic activity and ADP-ribose binding ability.** (A) TiPARP nuclear bodies do not co-localize with Cajal bodies. HEK 293T cells were transfected with Flag-tagged TiPARP and stained with anti-FLAG (green) and anti-coilin antibody (red). (B) TiPARP nuclear bodies partially co-localizes with PML bodies but do not depend on PML. HEK 293T cells stably expressing shRNA targeting luciferase (sh Luc) or PML (sh PML) were transfected with Flag-TiPARP. Co-localization was analyzed by

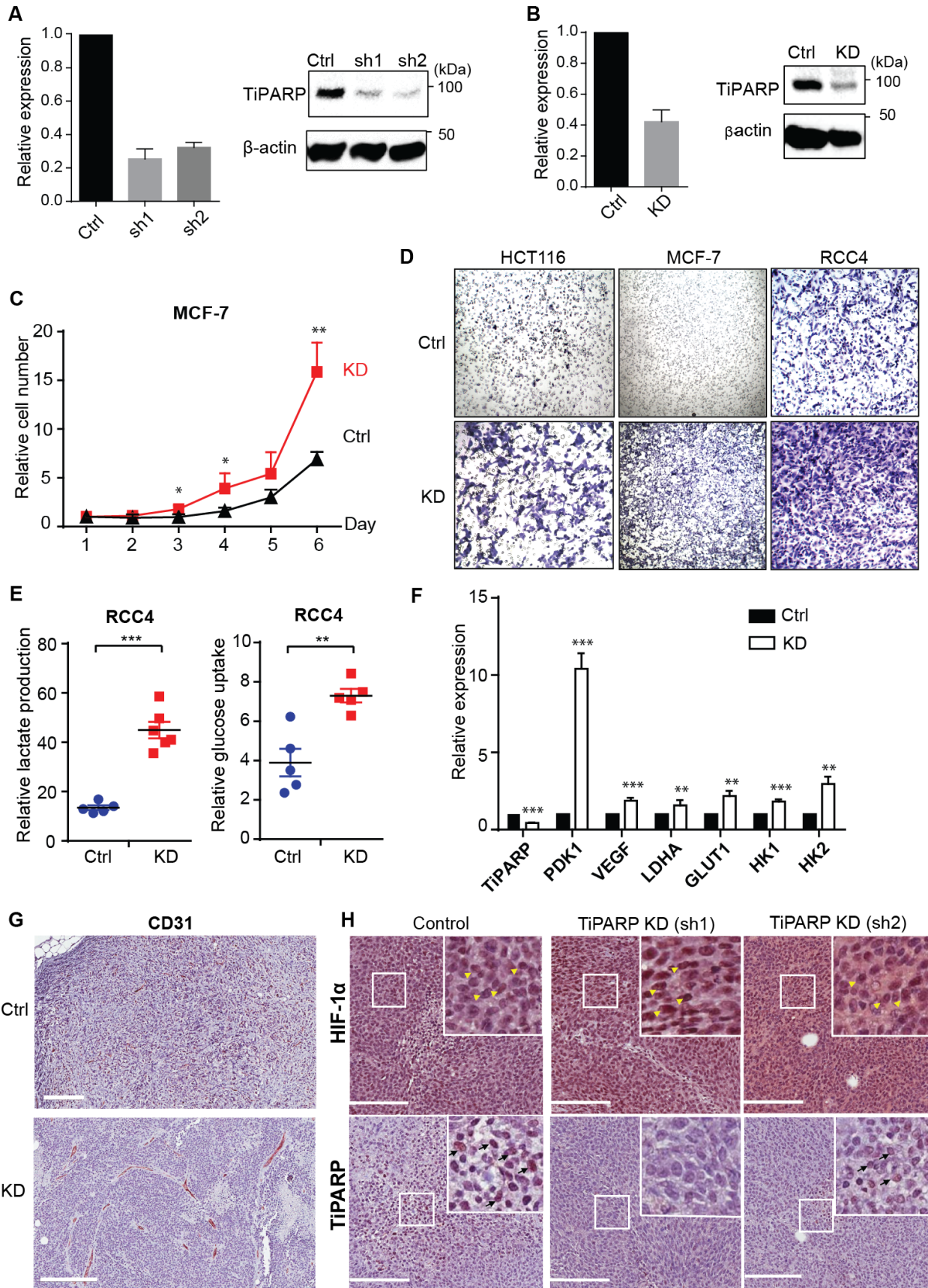
immunofluorescence microscopy with anti-FLAG (green) and anti-PML (red) antibodies. Scale bar, 5  $\mu\text{m}$ . **(C)** Western blot analysis of PML proteins in HEK 293T cells stably expressing shRNA targeting luciferase (shCtrl) or PML (shPML). **(D)** Western blot of Flag-tagged WT or inactive (H532A) TiPARP purified from HEK293T cells. For in vitro droplet formation assay, the amount of WT and mutant proteins are normalized to be equal. **(E)** Western blot analysis of *in vitro* auto-mono-ADP-ribosylation using mono-ADP-ribose binding agent. Purified Flag-TiPARP was incubated with or without 100  $\mu\text{M}$  of  $\text{NAD}^+$  at 37°C for 1 h. **(F)** Endogenous TiPARP forms nuclear condensates under hypoxia and partially co-localize with HIF-1 $\alpha$ . HEK 293T cells were incubated in 1%  $\text{O}_2$  (hypoxia) for 24 h (top section) and 42 h (bottom section), followed by fixation and immunofluorescent analysis using anti-TiPARP and anti-HIF-1 $\alpha$  antibodies. Scale bar of zoomed images: 2  $\mu\text{m}$ ; unzoomed images: 10  $\mu\text{m}$ . Representative images are shown. **(G)** Condensates formation by over-expressed Flag-TiPARP detected by immunofluorescence using TiPARP antibody. HEK 293T cells were cultured at normal condition and transfected with Flag-TiPARP plasmids for 24 h, followed by fixation and immunofluorescent analysis using anti-TiPARP antibody. Scale bar, 5  $\mu\text{m}$ . **(H)** Quantification of the number of TiPARP nuclear bodies per cell. For hypoxic samples,  $n=20$  in each group. For TiPARP overexpression sample (OE),  $n=52$ . **(I)** In vitro droplet formation by purified TiPARP proteins. Purified Flag-tagged WT TiPARP, inactive mutant (H532A), WWE domain mutant (W347A & W357A) were incubated in reaction buffer containing 100  $\mu\text{M}$   $\text{NAD}^+$  for 1 h at 30°C. As a negative control, cells expressing empty vector (EV) were also used for protein purification and same experiments were performed with the elution from control cells. Only WT, but not the inactive or WWE domain mutant formed droplets, suggesting that the interaction between ADP-ribosylated TiPARP was critical for droplet formation. Scale bar, 10  $\mu\text{m}$ . **(J)** Coomassie blue staining of the purified proteins. The concentration of purified proteins was adjusted to normalize the protein amount used for droplet formation assays and SDS-PAGE analysis. **(K)** ADP-ribosylation activity of purified TiPARP proteins. Purified proteins were incubated in reaction buffer containing 100  $\mu\text{M}$   $\text{NAD}^+$  for 1 h at 30°C. Western blot analysis were done using anti-Flag antibody (to detect the protein) and anti-mono-ADP-ribose binding agent (to confirm its catalytic activity). Both wild type and WWE domain mutant of TiPARP showed catalytic activity and auto-modified themselves. The expression level of H532A mutant is always higher than WT. **(L)** Western blot analysis using anti-poly(ADP-ribose) antibody and anti-PARP1 to detect potential formation of poly(ADP-ribose) and

contamination of PARP1. Purified PARP1 was used as a positive control, which catalyzed poly-ADP-ribosylation on itself. No poly(ADP-ribose) was formed from the *in vitro* reaction of TiPARP, confirming that TiPARP specifically catalyzed mono-ADP-ribosylation. No PARP1 was detected in our proteins. As a positive control, auto-poly-ADP-ribosylated PARP1 was detected by both antibodies.



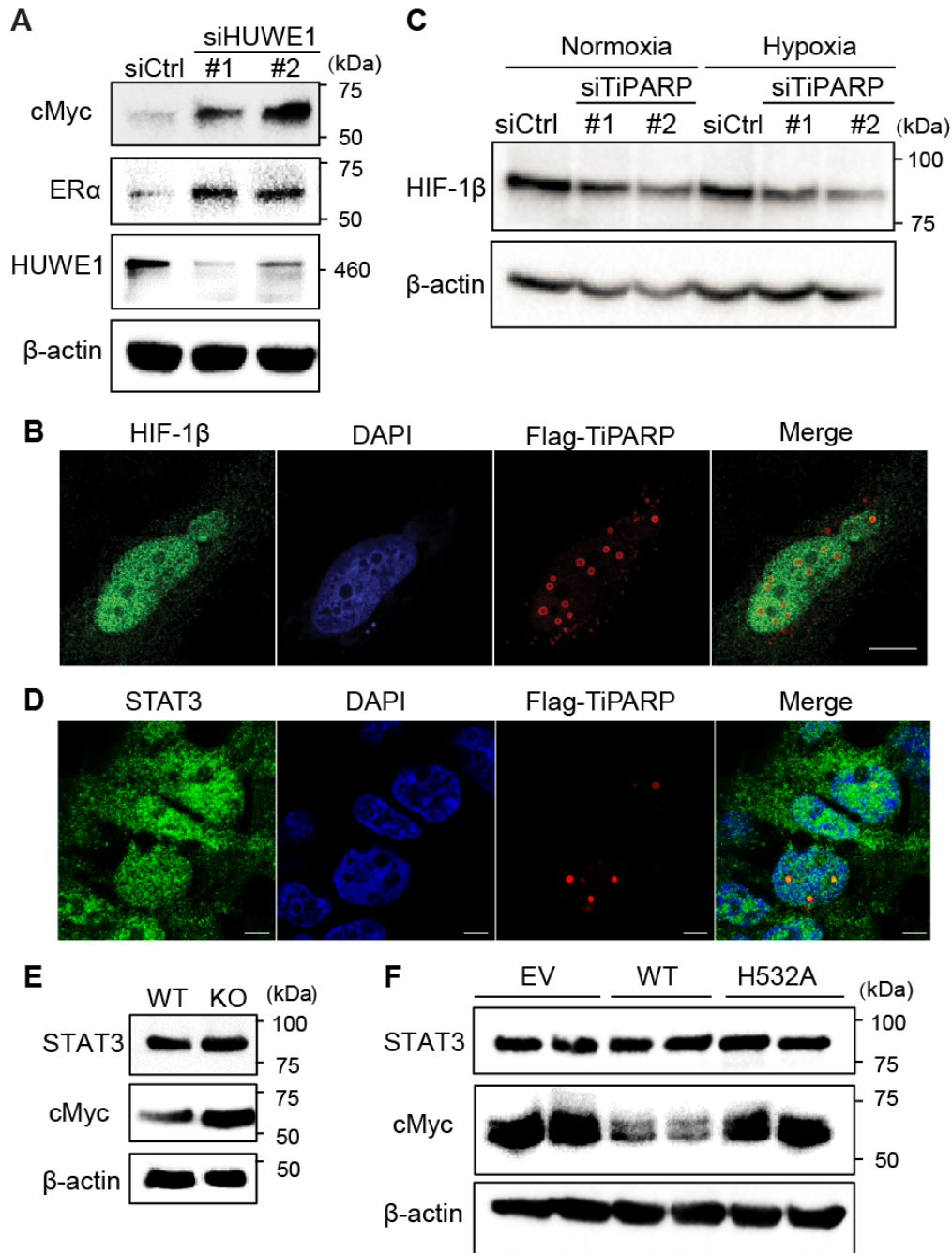
**Fig. S4: Interaction of TiPARP and E3 ubiquitin-protein ligase.** (A) DTX2 and RNF114 interact with TiPARP in an ADP-ribosylation dependent manner. HEK293T cells were transfected with empty vector, Flag-tagged WT or H532A mutant TiPARP. TiPARP was pulled down with anti-Flag resins and blotted for E3 ubiquitin-protein ligases DTX2 and RNF114. (B) HUWE1 interaction with TiPARP is dependent on the WWE domain of HUWE1. HEK293T cells were co-transfected with Flag-tagged TiPARP and empty vector, V5-tagged WT or W1617/1619A (WA) mutant HUWE1. TiPARP was pulled down with anti-Flag resins and blotted with an anti-V5 antibody to detect the interacting V5-HUWE1. The position of HUWE1 band is marked. (C) TiPARP does not affect HIF-1 $\alpha$  hydroxylation. HCT116 cells were treated with 1  $\mu$ g/ml of

doxycycline for 24 h to induce the expression of empty vector (EV), Flag-tagged WT or inactive H532A mutant TiPARP. Cells were treated with 20  $\mu$ M of MG-132 for 6 h to accumulate hydroxylated HIF-1 $\alpha$ . Cell extracts were blotted with antibodies specific to hydroxylated HIF-1 $\alpha$  (HIF-OH) or total HIF-1 $\alpha$ . **(D)** TiPARP is ubiquitinated in an ADP-ribosylation dependent manner. Flag-TiPARP was pulled down from HEK 293T cells using anti-FLAG resins. Ubiquitination and the loading of TiPARP were analyzed using anti-Ub and anti-FLAG antibodies. **(E)** Western blot analysis of Flag-tagged WT or H532A mutant TiPARP in the whole cell extracts of HCT116 cells.



**Fig. S5: TiPARP is a tumor suppressor.** (A, B) Knockdown efficiency in HCT116 (A) and MCF-7 (B) cells stably expressing shRNA against luciferase (Ctrl) or TiPARP (KD) was measured by qRT-PCR for mRNA levels (left), and western blots for protein levels (right). Data are represented as means + sd,  $n = 3$ . (C) Growth curve of control luciferase knockdown (Ctrl) and TiPARP knockdown (KD) MCF-7 cells. Data are represented as means + sd,  $n = 3$ . (D) Representative images of transwell migration assays in different cancer cell lines stably expressing luciferase (Ctrl) or TiPARP (KD) shRNA. (E) Lactate secretion and glucose uptake in control and TiPARP knockdown RCC4 cells. Data are represented as means + sd,  $n = 5$ . (F) mRNA levels of HIF-1 target genes in MCF-7 xenografts. Data are represented as means + sd,  $n = 3$ . (G) Representative images of CD31 immunohistochemical staining in MCF-7 xenograft tumors. Scale bar, 300  $\mu\text{m}$ . (H) Immunohistochemistry staining of HIF-1 $\alpha$  and TiPARP in HCT116 xenograft tumors. Arrows point to representative sites of HIF-1 $\alpha$  and TiPARP nuclear staining. Scale bar, 200  $\mu\text{m}$ . Statistical analyses were performed using unpaired two-tailed  $t$ -tests. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \* $p < 0.001$ .





**Fig. S6: TiPARP does not regulate HIF-1 $\beta$  degradation.** (A) HUWE1 knockdown increased c-MYC and ER $\alpha$  levels. HCT116 cells were transfected with 25 nM control (siCtrl) or HUWE1 siRNA for 24 h. Cell lysates were blotted against the indicated proteins. (B) HIF-1 $\beta$  is not recruited to TiPARP nuclear bodies. HEK 293T cells were transfected with Flag-TiPARP. TiPARP was stained with anti-FLAG antibody (red) and endogenous HIF-1 $\beta$  was stained with anti-HIF-1 $\beta$  antibody (green). Scale bar, 5  $\mu$ m. (C) HIF-1 $\beta$  level is not affected by TiPARP knockdown. Western blot analysis of endogenous HIF-1 $\beta$  in HCT116 cells transfected with control (siCtrl) or

TiPARP siRNA, followed by incubation at normoxia or hypoxia (1% O<sub>2</sub>) conditions for 6 hr. **(D)** STAT3 is not recruited to TiPARP nuclear bodies. HEK 293T cells were transfected with Flag-TiPARP. TiPARP was stained with anti-FLAG antibody (red) and endogenous STAT3 was stained with anti-STAT3 antibody (green). Scale bar, 5 μm. **(E)** Western blot of STAT3 and c-Myc in HAP-1 WT or *TiPARP* KO cells. STAT3 level was not affected by TiPARP knockout, but c-Myc level is. **(F)** STAT3 and c-Myc levels with WT or H332A mutant of TiPARP. HCT116 cells were treated with 1 μg/ml doxycycline for 24 h to induce the expression of empty vector (EV), Flag-tagged wild type TiPARP (WT), or inactive H532A mutant (HA). The protein levels of STAT3 and c-Myc were analyzed by western blot. c-Myc level was decreased by WT TiPARP, but STAT3 level was not.