Expanded View Figures

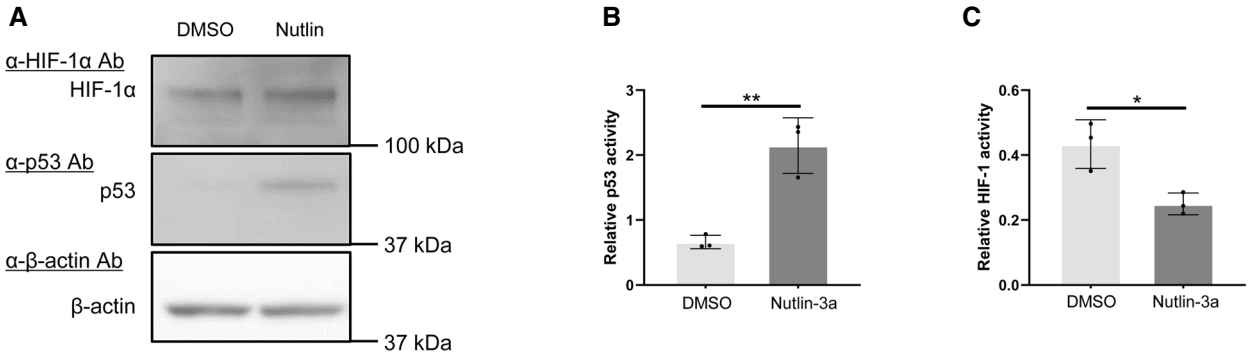

Figure EV1. Relationship between p53 and HIF-1.

A–C U2OS cells transfected with p53-Luc (B; #219083, Agilent Technologies) or p5HRE-Luc (C) were treated with DMSO (for control) or 2.5 μM of Nutlin-3a, cultured under < 0.1% oxygen conditions for 24 h, and subjected to western blotting using the indicated antibodies (A) and luciferase assays (B, C). pRL-CMV was used as an internal control (B, C).

Data information: Mean ± s.d. The number of technical replicates in all of the experimental groups was 3 (B, C), and reproducibility of the results was confirmed at least three times by biologically independent experiments (A–C) = 3, *$P < 0.05$, **$P < 0.01$, Student's t-test.
Source data are available online for this figure.

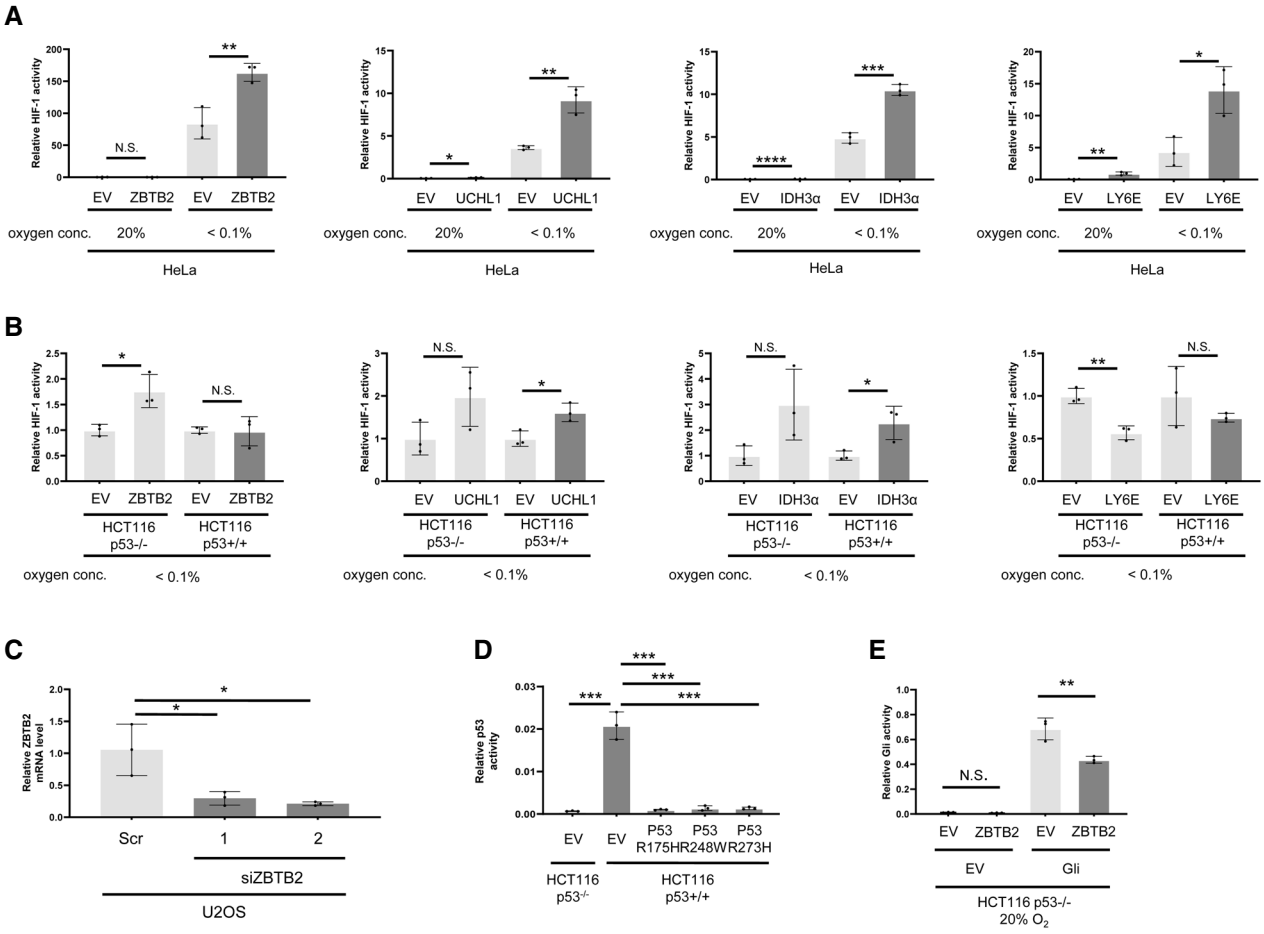

Figure EV2. Relationships among HIF-1, p53, and ZBTB2.

A, B The indicated cells transfected with p5HRE-Luc were transiently co-transfected with pEF6/ZBTB2 (ZBTB2), pcDNA4/UCHL1 (UCHL1), pcDNA4/IDH3α (IDH3α), pcDNA4/Ly6E (Ly6E), or each of their corresponding control vectors (EV), pEF6/myc-His B, pcDNA4/myc-His A, pcDNA4/myc-His A, or pcDNA4/myc-His A, respectively. The cells were then cultured under the indicated oxygen conditions for 24 h and subjected to the luciferase assay.

C The indicated cells were treated with scramble-siRNA (Scr) or ZBTB2-siRNA (siZBTB2), cultured under < 0.1% oxygen conditions for 24 h, and subjected to qRT–PCR for ZBTB2 mRNA levels.

D The indicated cells transiently transfected with p53-Luc (#219083, Agilent Technologies) were additionally transfected with pcDNA3/p53 R175H, R248W, R273H, or pcDNA3 (EV) as indicated, cultured under < 0.1% oxygen conditions for 24 h, and subjected to the luciferase assay.

E The indicated cells transiently transfected with either pGL3/(3′Gli-BS)4-Luc or its empty vector, pGL3-Luc (EV), were additionally transfected with either pEF6/ZBTB2 (ZBTB2) or pEF6/myc-His B (EV), cultured 20% oxygen conditions for 24 h, and subjected to the luciferase assay.

Data information: pRL-CMV was used as an internal control in every luciferase assay (A, B, D, E). Mean ± s.d. The number of technical replicates in all of the experimental groups was 3, and the reproducibility of the results was confirmed at least three times by biologically independent experiments (A–E). N.S., not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, Student's t-test.

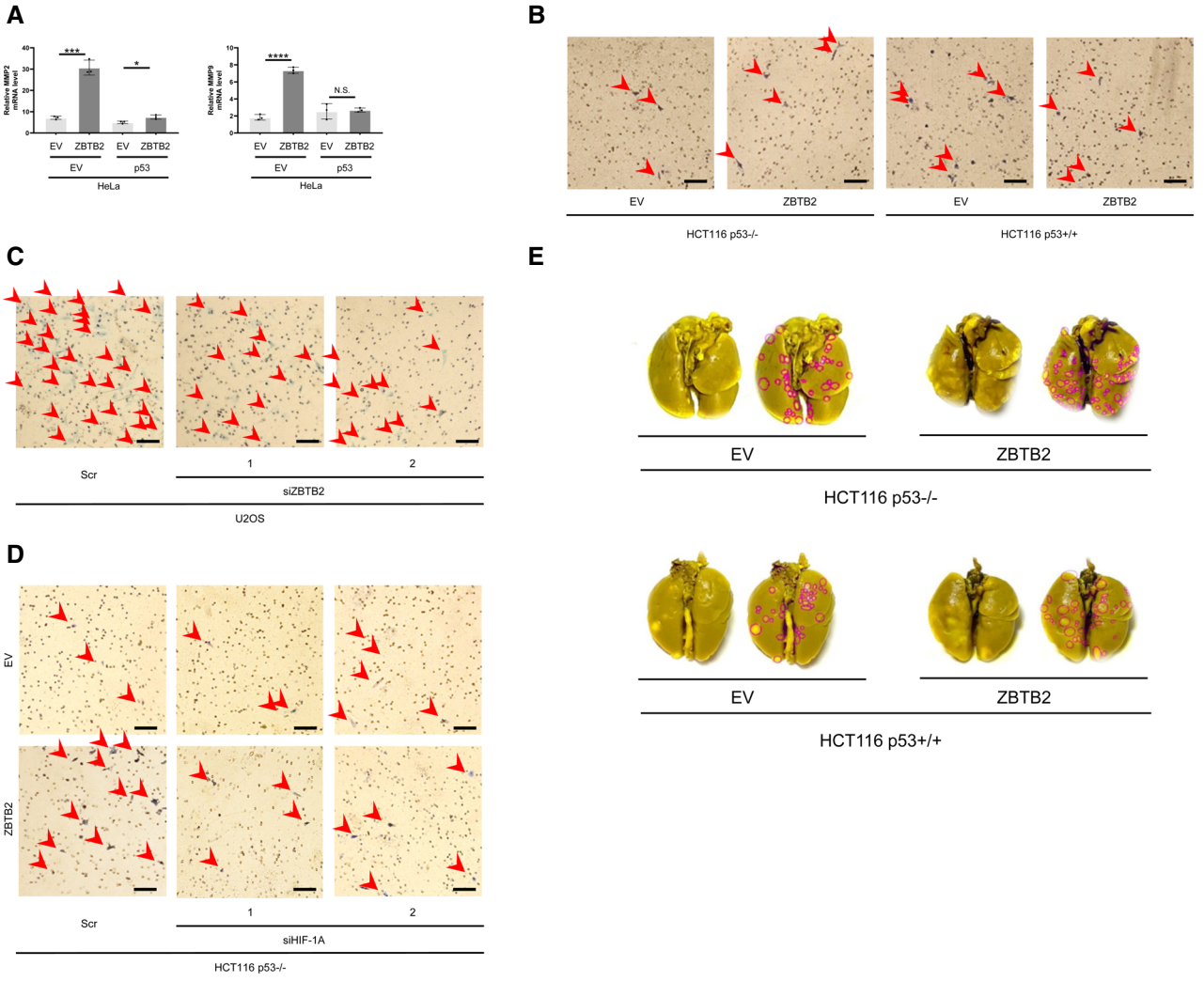

Figure EV3. The ZBTB2–HIF-1 axis promotes invasion and metastasis of p53-deficient cancers.

A HeLa cells, whose p53 activity is usually suppressed, were transiently transfected with either pEF6/ZBTB2 (ZBTB2) or pEF6/myc-His B (empty vector: EV) and with either pcDNA3/p53 (p53) or pcDNA (EV), cultured under < 0.1% oxygen conditions for 24 h, and subjected to qRT–PCR to quantify the mRNA levels of the indicated genes. Mean ± s.d. The number of technical replicates in all of the experimental groups was 3. N.S., not significant, *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$, Student's *t*-test.

B–D Representative image of the invasion assay using the matrigel-coated transwell chamber in Fig 2D (B), Fig 2E (C), and Fig 2F (D). Arrowheads indicate invading cells. Scale bar, 100 μm.

E Representative images of lungs with metastatic tumors in Fig 2G. Red ROIs represent metastatic colonies in each lung.

Data information: Reproducibility of the result was confirmed at least three times by biologically independent experiments (A–E).
Source data are available online for this figure.

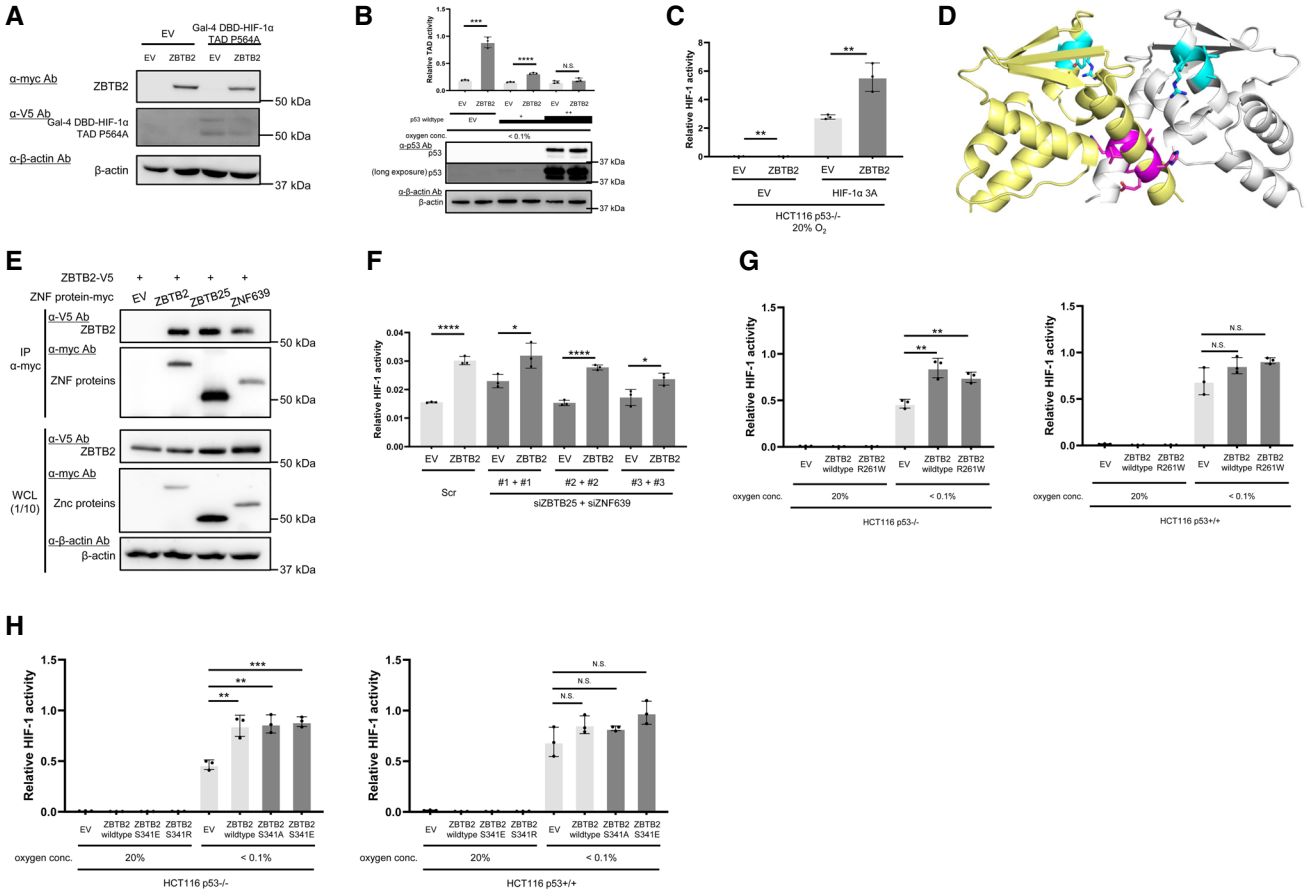

Figure EV4. Mechanistic relationships among HIF-1, p53, and ZBTB2.

A Western blotting to confirm the expression of the indicated proteins under < 0.1% oxygen conditions in the luciferase assay in order to evaluate the HIF-1α TAD activity in Fig 4B.

B Luciferase assay for HIF-1α TAD activity was conducted using HCT116 $p53^{-/-}$ cells after transient transfection of low (+) or high (++) concentrations of the p53 expression vector or its empty vector (EV). Western blotting to confirm the forced expression of p53 was also conducted.

C Cells transiently co-transfected with p5HRE-Luc, either pEF6/ZBTB2 (ZBTB2) or pEF6/myc-His B (empty vector: EV), and either pcDNA4A/ HIF-1α 3A (HIF-1α 3A) or pcDNA4A (EV) were cultured under 20% oxygen conditions for 24 h and subjected to the luciferase assay.

D Crystal structure of the hMN homodimer (PDB: 2VPK). The 8–11 a.a. residues (EHLL) and 51–54 a.a. residues (AIYR) are highlighted in pink and blue, respectively.

E Twenty-four hours after co-transfection of the expression vector for ZBTB2-V5 (pcDNA4/ZBTB2) with that for either of zinc finger (ZNF) protein, ZBTB2-myc (pEF6/ZBTB2), ZBTB25-myc (pEF6/ZBTB25), or ZNF639-myc (pEF6/ZNF639), or with their empty vector (EV: pEF6/myc-His B), the myc-tagged proteins were immunoprecipitated using the anti-myc-tag antibody (middle) and co-precipitated ZBTB2-V5 was detected using anti-V5 tag antibody (upper). One-tenth of the whole-cell lysate (WCL) was subjected to immunoblotting with the indicated antibodies (lower).

F HCT116 $p53^{-/-}$ cells transfected with either pEF6/ZBTB2 (ZBTB2) or pEF6/myc-His B (EV) were treated with either scramble-siRNA (Scr) or both ZBTB25-siRNA (siZBTB25) and ZNF639-siRNA (siZNF639) as indicated, cultured under < 0.1% oxygen conditions for 24 h, and subjected to the luciferase assay.

G The indicated cells were transiently co-transfected with p5HRE-Luc and pEF6/ZBTB2 (ZBTB2 wild-type), pEF6/ZBTB2 R261W (ZBTB2 R261W), or pEF6/myc-His B (empty vector: EV), cultured under the indicated oxygen conditions for 24 h, and subjected to the luciferase assay.

H The indicated cells were transiently co-transfected with p5HRE-Luc and pEF6/ZBTB2 (ZBTB2 wild-type), pEF6/ZBTB2 S341E (ZBTB2 S341E), pEF6/ZBTB2 S341R (ZBTB2 S341R), or pEF6/myc-His B (empty vector: EV), cultured under the indicated oxygen conditions for 24 h, and subjected to the luciferase assay.

Data information: pRL-CMV was used as an internal control in every luciferase assay (B, C, F–H). Mean ± s.d. The number of technical replicates in all of the experimental groups was 3 (A–C, F–H), and reproducibility of the results was confirmed at least three times by biologically independent experiments (A–C, E–H). N.S., not significant, $*P < 0.05$, $*P < 0.01$, $***P < 0.001$, $****P < 0.0001$, Student's *t*-test.
Source data are available online for this figure.