**HIF-transcribed p53 chaperones HIF-1α**

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**SUPPLEMENTARY FIGURES**

**Figure S1. HIF-1α and p53 protein expression in hypoxic zones of pancreatic and kidney cancers**

IHC for observing the expression of p53 and HIF-1α is performed in kidney and pancreatic cancer samples. To observe the impact of HIF-1α on p53 protein expression, 10 diferent areas with variable HIF-1α expression are chosen and the expression of p53 in these areas is observed. The plot shows the correlation between the expression of HIF-1α and p53 in these 10 different areas of both cancers. The analysis shows that the correlation of the expression between p53 and HIF-1α is weaker at the protein level than at the mRNA level. This is owing to differential protein degradation rates of HIF-1α and p53. All R2 values are labled on the figure, n=3.

**MATERIALS AND METHODS**

**Putative transcription-factor-binding-site (TFBS) analysis**

TFBSs specific to HIF-1α in *p53* promoter; HIF-1α in *VEGF* promoter and p53 in *Bax* promoter; were analyzed using MatInspector (Genomatix, Munich, Germany) with MatBase matrix library 8.0. MatInspector can be used online at (http://www.genomatix.de/en/index.html), and details regarding the weight matrices used to identify potential TFBSs have been described previously (1). Heat maps generated using Morpheus Tool (Broad Institute).

**Generation of *p53* and *HIF-1α* knockout (KO) cells**

All guide RNAs targeting *p53* and *HIF-1α* [*p53* CRISPR Guide RNA (crRNA 1): CCGGTTCATGCCGCCCATGC; crRNA 2: CGCTATCTGAGCAGCGCTCA; crRNA 3: CCCCGGACGATATTGAACAA; crRNA 4: GAGCGCTGCTCAGATAGCGA] and [*HIF-1α* CRISPR Guide RNA (crRNA 1): TTCTTTACTTCGCCGAGATC; crRNA 2: CCTCACACGCAAATAGCTGA; crRNA 3: TGTTTACAGTTTGAACTAAC; crRNA 4: TACTCATCCATGTGACCATG] were cloned into the plentiCRISPR v2 (Addgene plasmid 49535) by Genscript. HEK293FT cells were transfected with each pLentiCRISPR v2 using Lipofectamine® 3000 and the appropriate packaging mix (2). Target cells, MFC-7, and PSN1 cells were infected with lentiviral supernatants containing 5 ug/ml polybrene for 24 h. After 48 h, the media was replaced with selection media (growth media containing 2 ug/ml puromycin). And after 2 weeks of puromycin selection, single-cell colonies were isolated via ring cloning, and DNA from each colony was extracted using QuickExtract (Epicentre, WI, USA) and confirmed for the *HIF-1α* and *p53* gene knockout.

**Luciferase assay**

5 × 104 cells per well were seeded in 24-well plates 24 h prior to transfection. Cells were transfected with reporter plasmids (1.0-1.5mg/well) using Lipofectamine® 3000 as per the manufacturer's protocol. Renilla luciferase expression plasmid with empty vector control (0.5 μg) and β-galactosidase construct (0.2 μg) were co-transfected as internal controls. After the desired incubation, the cells were washed in cold PBS three times and lysed with 200 μl of the lysis buffer by a freeze-thaw cycle, and lysates were collected by centrifugation at 14,000 rpm for 2 min in a bench-top centrifuge. 20 μl of the supernatant was used for the assay of luciferase activity using a kit (Promega) as previously described (3). Each transfected well was assayed in triplicates, and the firefly luciferase activity was normalized to β-galactosidase activity. All luciferase constructs were cloned into PGL4 vector.

### **Chromatin immunoprecipitation (ChIP)-qPCR**

ChIP experiments were performed as described previously (3-5). Cells were fixed with 1% formaldehyde for 10 min at 22 °C and then quenched with 0.125 M glycine (Sigma). The cells were scraped in cold PBS and collected by centrifugation and rinsed in cold PBS. The cell pellets were re-suspended in swelling buffer (10 mM potassium acetate, 15 mM magnesium acetate, 0.1 M Tris; pH 7.6, 0.5 mM phenylmethylsulfonyl fluoride, and 100 ng of leupeptin and aprotinin/ml), incubated on ice for 20 min, and then dounce-homogenized. The nuclei were collected by micro-centrifugation and then resuspended in sonication buffer (1% sodium dodecyl sulfate, 10 mM EDTA, 50 mM Tris–HCl; pH 8.1, 0.5 mM phenylmethylsulfonyl fluoride, and 100 ng of leupeptin and aprotinin/ml) and incubated on ice for 10 min. Chromatin was sonicated using an ultrasonics sonicator to achieve an average length of approximately 1000 bps. 20% of total chromatin was saved as total input chromatin. Samples were immunoprecipitated overnight at 4 °C with antibody-conjugated beads or no antibody-bound beads as control. Cross-links were reversed by the addition of NaCl to a final concentration of 200 mM, and RNA was removed by the addition of 10 µg of RNase A per sample for 4–5 h at 65 °C. The samples were then precipitated at 20 °C overnight by the addition of 2.5 volumes of ethanol and then pelleted by microcentrifugation. The samples were re-suspended in 100 µl of Tris–EDTA; pH 7.5, 25 µl of 5X proteinase K buffer (1.25% sodium dodecyl sulfate, 50 mM Tris; pH 7.5, and 25 mM EDTA), and 1.5 µl of proteinase K (Boehringer Mannheim, Stuttgart, Germany) and incubated at 45 °C for 2 h. Samples were extracted with phenol-chloroform-isoamyl alcohol (25:24:1) followed by extraction with chloroform-isoamyl alcohol and then precipitated with 1/10 volume of 3 M NaOAc (pH 5.3), 5 µg of glycogen, and 2.5 volumes of ethanol. The pellets were collected by micro-centrifugation, resuspended in 30 µl of water, and analyzed by PCR and qPCR. The primers used are as follows: HRE1 forward, 5’- TGAGTATATCTCATCTTCCCGGAG-3’ and reverse, 5’- ACGACCCATCATTGCACTCT-3’; HRE2 forward, 5’- TTTGGGCTAGGCCATTCCAG-3’ and reverse, 5’- CTGCACGGAGGACCACAC-3’; HRE3 forward, 5’‑GTTCTTCCTGGTAGGAGGCG-3’ and reverse, 5’-CGCTGTGTGTAAATGCCACC-3’; HRE4 forward, 5’‑CTCCGTGATGCCTACCAAGT-3’ and reverse, 5’-AAATAAAGGTGGGGTCGGGA-3’; HRE5 forward, 5’‑CAGACTGCCTTCCGGGTCA-3’ and reverse, 5’-TGGATCCACTCACAGTTTCCAT-3’; HRE5 forward, 5’‑CAGACTGCCTTCCGGGTCA-3’ and reverse, 5’-TGGATCCACTCACAGTTTCCAT-3’; EPO forward, 5’‑TTTCTGGGAACCTCCAAATCCC-3’ and reverse, 5’-AGGCCTTGAATGGAGCCACCTTAT-3’; GPI forward, 5’‑ACTGGGAGGGGATTAGCACT-3’ and reverse, 5’-TTTATTTGAGGCCTGCTTGG-3’; SERPINE1 forward, 5’‑ACAGACACAGGCAGAGGGCAGAAA-3’ and reverse, 5’-TCTGGGACTTGCTGAGGCATGTGT-3’; VEGFA forward, 5’‑TCAGTTCCCTGGCAACATCTGG-3’ and reverse, 5’-AGTTTGTGGAGCTGAGAACGGGAA-3’; NDRG1 forward, 5’‑AACACGTGAGCTAAGCTGTCCGA-3’ and reverse, 5’-ATGGAGGCAGAAGGAACATGTGAG-3’; DDIT4 forward, 5’‑CGCGCTAGTCCTTATAGGCTGCT-3’ and reverse, 5’-AACTGCTAAGACAAGTGCGTCCTG-3’; ENO1 forward, 5’‑AGATAGGACCGGTGAGCCGAACT-3’ and reverse, 5’-AAAGTTGTCAGCAAGGTCGAGGG-3’; HK2 forward, 5’‑ATGGGCCGACTCTTGTATTG-3’ and reverse, 5’-GGAAAGTGAGCCGCCTTAG-3’; LDHA forward, 5’‑TTGGAGGGCAGCACCTTACTTAGA-3’ and reverse, 5’-GCCTTAAGTGGAACAGCTATGCTGAC-3’; PDK1 forward, 5’‑ACGTCCCTCACGTACCACT-3’ and reverse, 5’-AGCTGAAGCTGCGGCTGA-3’; PFKFB4 forward, 5’‑AACCTGCCCGCGCTGATTTGCATAG-3’ and reverse, 5’-TAGGCCTCGTCCCACTGCATGAAA-3’; PGK1 forward, 5’‑TCTCGCACATTCTTCACGTCCGTT-3’ and reverse, 5’-TAGTGAGACGTGCGGCTTCCGTTT-3’; BNIP3L forward, 5’‑TGTGCCTTGCTTCTCCTTTT-3’ and reverse, 5’-AAATCACTTGGGCAACTTCG-3’; IER3 forward, 5’‑AGAGTGACACATGGTGAGCCGA-3’ and reverse, 5’-TTAATCGTCGGAATTTCCAGCCCG-3’; and NOS3 forward, 5’‑GCATGGATATATGTGTAAGAAAGTGTGC-3’ and reverse, 5’-AGATTGGGACAAATCTGCGCCT -3’.

The samples were run on the QIAxcel Advanced instrument (Qiagen, Hilden, Germany) using QIAxcel DNA High-Resolution Kit (Qiagen) on the 0M500 method (sample injection voltage of 5 kV and a separation voltage of 5 kV), with a sample injection time of 15 seconds (6). During the run we used the QX DNA Size Marker 25 –500 bp v2.0 and the corresponding QX Alignment Marker 15 bp/600 bp (Qiagen). For the analysis of the results, we used the QIAxcel ScreenGel software. The analysis of samples is performed using a two-step approach. First, peaks are detected in the raw data. In a second step, the peak sizes and peak concentrations are determined by mapping the detected peaks to the peaks of the reference size marker.

**Western Blotting**

Total protein extracts from cells and human tissues were isolated using total protein extraction kit (Thermo Fisher Scientific) per manufacturer instructions. Lysates were incubated on ice for 60 min, followed by microcentrifugation at 10,000 g for 15 min at 4 °C. Nuclear extracts from cells and tissues were prepared using the NE-PER kit (Thermo Fisher Scientific) according to manufacturer instructions. 80 μg of protein prepared in 2X-SDS loading buffer were boiled at 95 °C for 10 min. The protein samples were separated on 4-20% polyacrylamide gels and transferred to nitrocellulose membranes (Sigma) using the iBlot system (Invitrogen). After blocking in 5% milk in TBST (Tris-buffered saline with Tween 20), the membrane was probed with primary antibody overnight at 4 °C. Membranes were further washed thrice with TBST for 10 min, incubated with appropriate HRP-conjugated-secondary antibodies (6).

**Cell line tumor xenografts**

Athymic nude mice used for the cell line tumor xenografts were purchased from Charles River. All animal protocols and procedures were approved by the Institutional Animal Care and Ethics committee of Champalimaud Foundation. The cell line tumor xenografts [MCF-7 *p53*+/+, HCT-116 *p53*+/+ (WT); MCF-7 *p53*+/-, HCT-116 *p53*+/- (MT) and MCF-7 *p53*-/-, HCT-116 *p53*-/- (null)] were established as described previously (7-9). Briefly, the desired cell lines were maintained in DMEM/F12 supplemented with 10% FBS and 1% antibiotics. For injection, cells at 70–90% confluency were trypsinized and re-suspended in serum-free media with 1:1 Matrigel at a concentration of 1 × 107 cells/ml on ice. Cell viability, required to be at least >95%, was determined by Trypan blue exclusion assay. For tumor induction, animals were first anesthetized with 1.5-3% isoflurane with 30% O2. An 80 μl cell suspension containing 1 x 107 cells was subcutaneously injected in the flank region of the mice. Vehicle control used for the study was DMSO. Tumor volumes were monitored weekly by caliper measurement and allowed to grow to a short diameter of 2 cm. The core hypoxic regions of these tumors were further extracted and used for subsequent qChIP studies. The hypoxic conditions were checked using HIF-1α western blotting.

**Laser-capture microdissection**

Demarcated tumor tissue regions were captured using a laser microdissection system (Leica Microsystems at UNMC; Zeiss Palm MicroBeam IV Laser Capture Microdissection system at Thornwood facility and Michigan University) and analyzed for gene expression of *HIF-1α* and *p53* by qPCR experiments.

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