Product Datasheet

HIF-1 alpha Antibody - BSA Free
NB100-479

Unit Size: 0.1 ml

Store at -20C.

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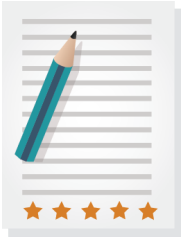
Updated 10/23/2024 v.20.1



NB100-479

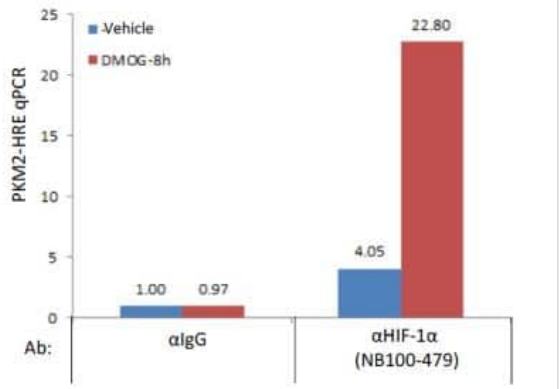
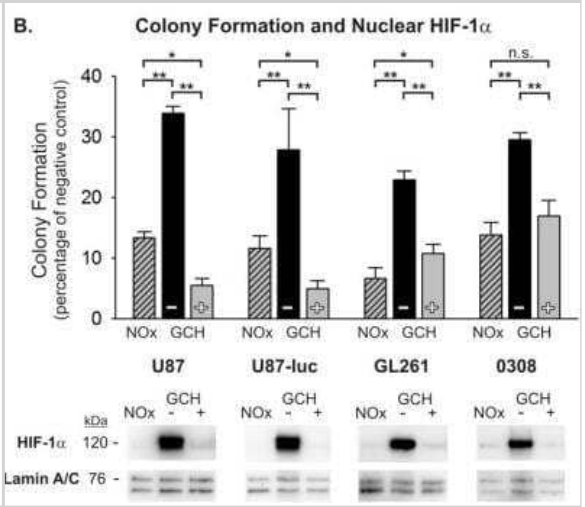
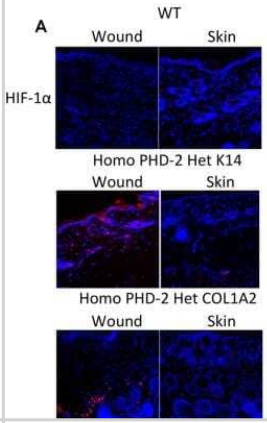
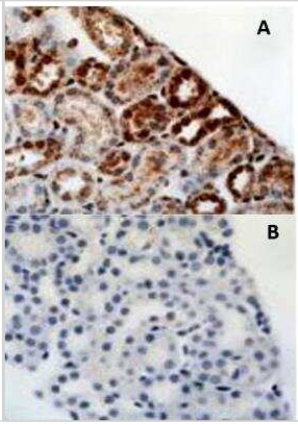
HIF-1 alpha Antibody - BSA Free

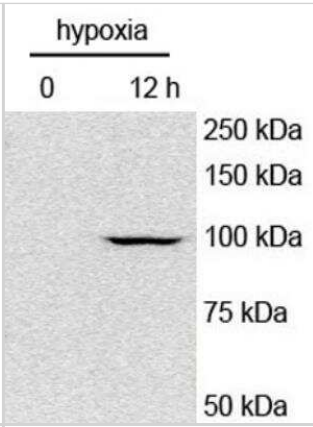
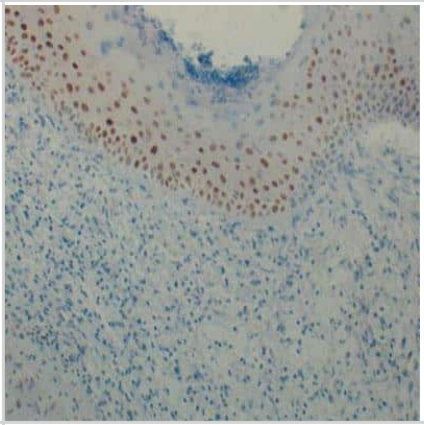
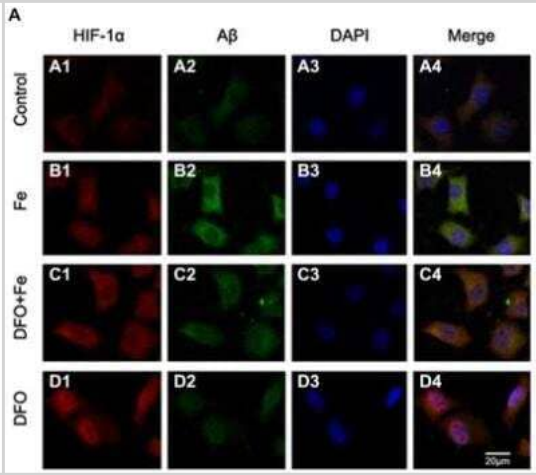
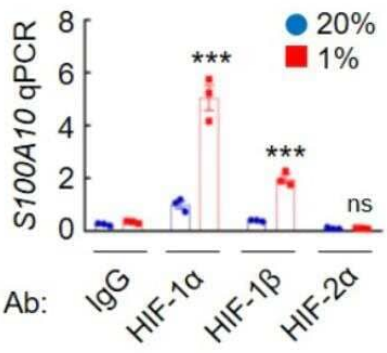
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at -20C.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	93 kDa

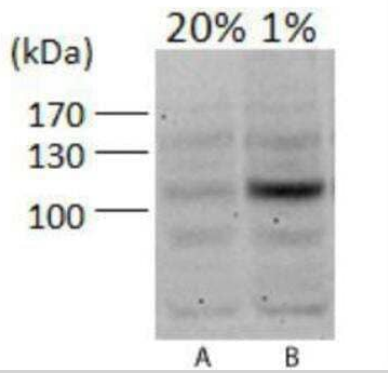
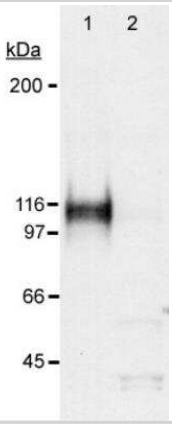
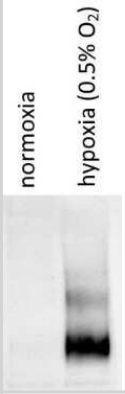
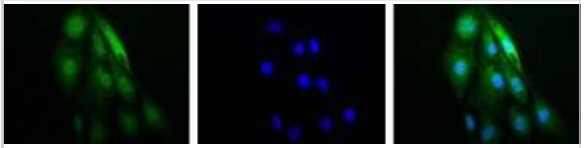
Product Description	
Host	Rabbit
Gene ID	3091
Gene Symbol	HIF1A
Species	Human, Mouse, Rat, Porcine, Canine, Fish, Goat, Hamster, Primate, Rabbit, Zebrafish
Reactivity Notes	Use in Zebrafish reported in scientific literature (PMID:35457018).Use in Mouse reported in scientific literature (PMID:33727588). Use in Goat reported in scientific literature (PMID:21599540).
Immunogen	This HIF-1 alpha antibody was developed against a fusion protein including amino acids 530 - 825 of the mouse HIF-1 alpha protein [Uniprot# Q61221].

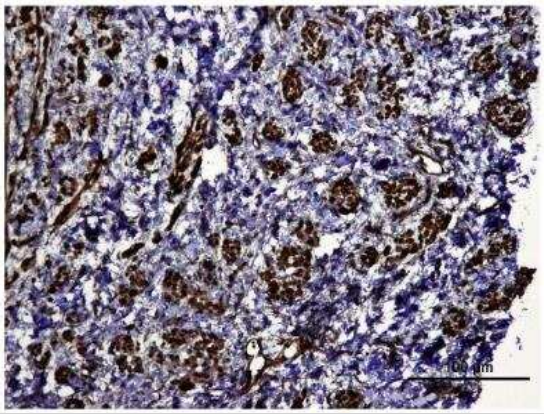
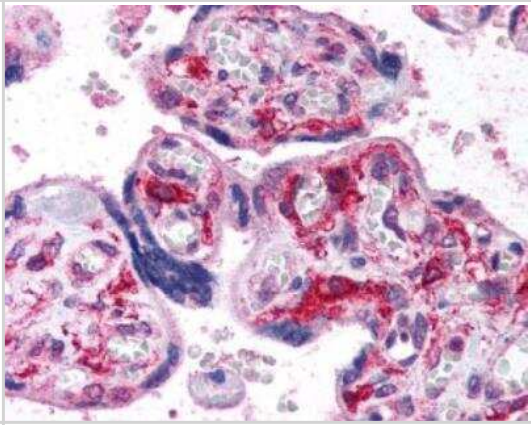
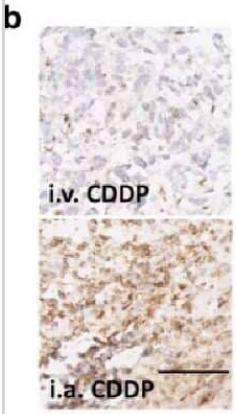
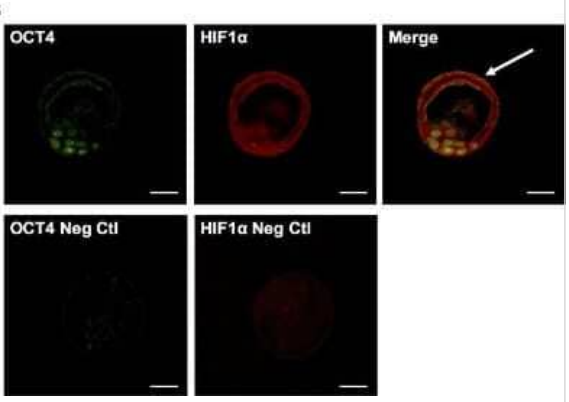
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Immunohistochemistry Whole-Mount, Knockdown Validated
Recommended Dilutions	Western Blot 1-2ug/ml, Simple Western, Flow Cytometry 1:10 - 1:1000. Use reported in scientific literature (PMID 21917971), Immunohistochemistry 5 - 10 ug/mL, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 5 - 10 ug/mL, Immunohistochemistry-Frozen 5 - 10 ug/mL, Immunoblotting reported in scientific literature (PMID 28506759), Immunohistochemistry Whole-Mount reported in scientific literature (PMID 27148974), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockdown Validated
Application Notes	WB recognizes a band in hypoxic samples at ~115 kDa. HIF-1 alpha protein (NBC1-18422) has been successfully used for antibody preadsorption/blocking assay in IHC-P with HIF-1 alpha antibody NB100-479.

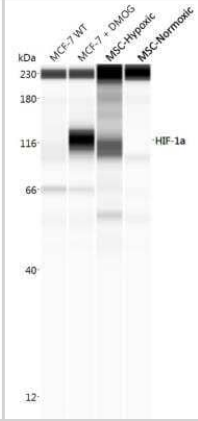
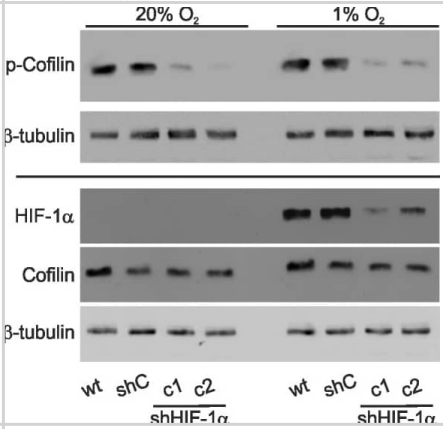
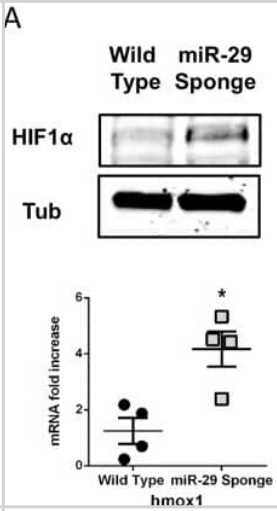
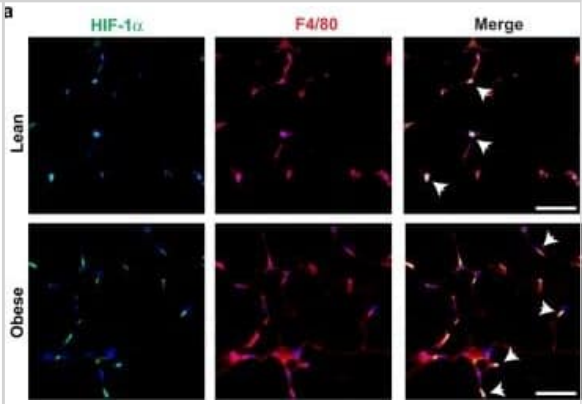
Images

ChIP-qPCR assay of HIF-1 binding to hypoxia response element (HRE) of the PKM2 gene in human MDA-MB-231 breast cancer cells treated with vehicle or DMOG (HIF inducer) for 8 hours. Negative control: immunoprecipitation was performed with IgG. Image courtesy of Gregg Semenza, Johns Hopkins University School of Medicine.	
Normoxic pre-treatment sensitizes glioma cells to radiation after graded chronic hypoxic (GCH) exposure. The results of anchorage-independent colony forming assays are shown for U87, U87-luc, GL261 glioma cells and 0308 GSCs after 5 Gy radiation exposure under varying O2 conditions. For easier comparisons among cell types, raw values are expressed as a % of the cell type's negative (non-irradiated) control and the means & SEMs are plotted. Each result represents at least 3 independent samples, plated in triplicate. Holm-Sidak comparisons for multiple groups were used for statistical comparisons of raw values (*p<0.05, **p<0.01). Also shown are Western blots of nuclear HIF-1alpha at the time of irradiation. Corresponding Western blots of lamin A/C are shown as a loading control. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0111199) licensed under a CC-BY license.	
Immunofluorescence of wounded tissue and normal unwounded skin. Representative images for immunofluorescent staining of HIF-1 alpha on healed wounded skin and adjacent normal unwounded skin of wild type, heterozygous K14-Cre/homozygous floxed PHD-2, and heterozygous Col1 alpha2-Cre-ER/homozygous floxed PHD-2 mice at 400x magnification. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0093373), licensed under a CC-BY license.	
Staining in normal mouse kidney showing (A) staining with HIF-1 alpha antibody and (B) blocking peptide used at 10-fold excess concentration. Images provided by Dr Yves Heremans. Please see the product review of NBC1-18422 for additional details.	

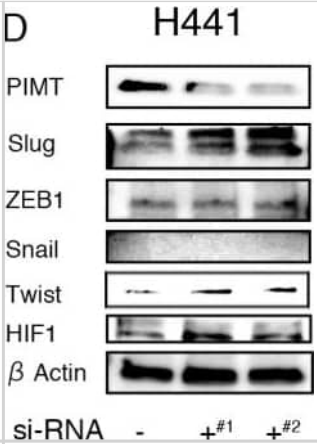
Analysis of HIF-1 alpha in mouse hepatocytes (left lane: normoxic and right lane: hypoxic) using HIF-1 alpha antibody. Western blot image submitted by a verified customer review.	
Analysis in paraffin section of mouse skin wound using NB100-479 at 1:6000. Image courtesy of product review by Ian Darby of RMIT University.	
Immunofluorescence (IF) labeling and confocal microscopy analysis of the distribution of HIF-1alpha (A1-D1) and Abeta (A2-D2) in APPsw cells. DFO treatment significantly increased HIF-1alpha IF (D1) and reduced the Abeta IF (D2) in APPsw cells compared with controls (A1,A2). (A3-D3) DAPI was used to detect the nucleus (blue). (A4-D4) Merged images from the double channels indicated that DFO has an inhibitory effect on Abeta formation, and is involved in HIF-1alpha stabilization in APPsw cells. Fe treatment (100 uM) decreased HIF-1alpha (B1) and increased the Abeta levels (B2). DFO treatment reversed the reduction of HIF-1alpha (C1) and the increase of Abeta (C2) in cultures pretreated with 100 uM Fe. Scale bar = 20 um. Image collected and cropped by CiteAb from the following publication (https://journal.frontiersin.org/Article/10.3389/fnagi.2015.00104/abstract) licensed under a CC-BY license.	
MDA-MB-231 cells were exposed to 20% or 1% O2 for 16 hours, and chromatin immunoprecipitation (ChIP) was performed with the indicated antibody (Ab). Primers flanking the HIF binding site in the S100A10 gene were used for qPCR. ChIP image submitted by a verified customer review.	

Analysis in A. PC12 (rat) cells, 20% oxygen and B. PC12 (rat) cells, 1% oxygen.	
Analysis in a hypoxic sample. Lane 1: CoCl2 treated Cos-7 nuclear extract (50 ug, hypoxic). Lane 2: Untreated Cos-7 nuclear extract (50 ug, normoxic). 10 second ECL exposure.	
U251 cells exposed to 4 hour hypoxia (0.5% oxygen) incubation. Western blot image submitted by a verified customer review.	
Detection of HIF-1 Alpha (green) in RCC4 cells using NB100-479. Nuclei (Blue) were counterstained using Hoechst 33258.	

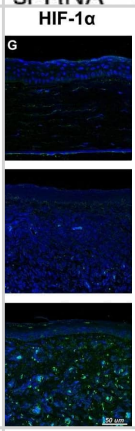
Analysis of HIF-1 alpha in human kidney tumor.	
Staining of human placenta, villi.	
Effects of different routes of CDDP administration on remaining viable tumor tissue. Representative images of tumor tissue (i.v. CDDP, i.a. CDDP) stained for HIF-1alpha (20X). Scale bar corresponds to 100 um. Image collected and cropped by CiteAb from the following publication (https://jeccr.biomedcentral.com/articles/10.1186/s13046-016-0392-1), licensed under a CC-BY license.	
Hif1alpha expression in preimplantation embryos. Co-staining of OCT4 and Hif1alpha in inner cell mass of blastocyst (top) and negative control images without primary antibodies (bottom). Scale bar: 30um. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0154309) licensed under a CC-BY license.	

Simple Western lane view shows lysates of MCF +/- DMOG, BioSpherix MSCs in hypoxic conditions, and BioSpherix MSCs in normoxic conditions loaded at 0.5 mg/ml. A band was detected for HIF-1 alpha at approximately 116 kDa (as indicated) using NB100-479 (1:100 dilution) followed by Anti-Rabbit Secondary Antibody (042-206, ProteinSimple). This experiment was conducted under standard assay conditions, and using the 12-230 kDa separation module (SW-W004). Non-specific interaction with the 230 kDa Simple Western standard seen with this antibody. Image from an internal validation.	
Cofilin phosphorylation is HIF-1α dependent.Wt, shC, and the HIF-1α knock down cell clones c1 and c2 cells were lysed after 24 hrs of normoxia (20% O2) or hypoxia (1% O2). Cell extracts were analysed by Western blots. Note that p-cofilin levels are reduced in c2 and c2 cells compared to wt and shC cells at 20% O2 and 1% O2.	
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxic markers accumulate in miR-29-sponge Zebrafish hearts. (A) Representative western blot analysis of hypoxia inducible factor 1α (HIF1α) expression in Wild Type & miR-29-sponge Zebrafish heart. In each condition, α-tubulin was used as loading control. Three independent experiments were performed. Full-length blot is presented in Supplementary Figure 6.(B) qRT-PCR mRNA analysis of hypoxia associated genes: erythropoietin alpha (epoa); hexokinase2 (hk2); heme oxygenase1a (hmox1a); lactate dehydrogenase A (ldha); cyclin-dependent kinase inhibitor 1B (p27) in Wild Type (black circles; n = 4) & miR-29-sponge (gray squares; n = 4) Zebrafish hearts expressed as fold-change versus Wild Type samples. *p < 0.05 Vs Wild Type. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-017-16829-w), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - (a,b) Immunofluorescence staining of (a) HIF-1α (green), the macrophage marker F4/80 (red) & DAPI nuclear stain (blue), & (b) lactate dehydrogenase (LDH, green), F4/80 (red) & DAPI (blue) in WAT of lean & obese mice. Colocalization is shown in the merged image (arrows). Scale bar = 100 µm. (c,d) Relative mRNA expression of (c) Hif1a & Hif2a, & the HIF target genes (d) Vegfa & Glut1 in ATM isolated from lean & obese mice (n = 3). (e) Levels of succinate in ATM of lean & obese mice (n = 6). (f) Relative mRNA levels of the glutamate transporter Slc3a2 in ATM from lean & obese (n = 3). Data is expressed as mean ± s.e.m. *p < 0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32221369), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Increased expression of HIFα and/or Twist in A549 & H441 cells induced by the inhibition of PIMT & Thapsigargin(A) Immunoblotting of Slug, ZEB1, Snail1, Twist, & HIF1α in A549 sh-PIMT & sh-control cells. (B, C) Immunoblotting & relative intensity of HIF1α in A549 cells treated with Tg. (D) Immunoblotting of Slug, ZEB1, Snail1, Twist, & HIF1α in si-control cells & si-PIMT H441 cells. (E, F) Immunoblotting & relative intensity of HIF1α in H441 cells treated with Tg. #1 & #2 indicates si-RNA of J-010000-05-0002 & J-010000-07-0002, respectively. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.24324), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - Histology & immunofluorescence in rat corneal sections. a Hematoxylin & Eosin (H&E), b VEGF-A (green); c CCL2 (green); d TNF-α (green); e CXCL5 (green); f CD45 (green) & g HIF-1α (green) staining in rat cornea tissue. Nuclear counterstaining by DAPI (blue) in fluorescent images Image collected & cropped by CiteAb from the following publication (http://link.springer.com/10.1007/s10456-018-9594-9), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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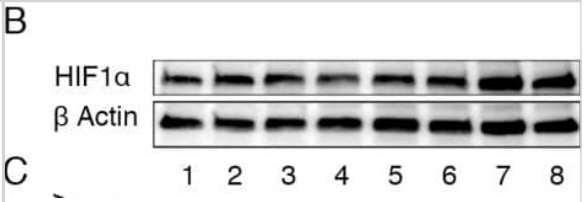
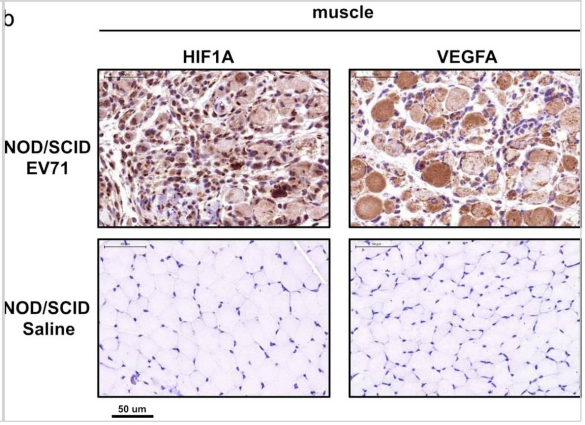
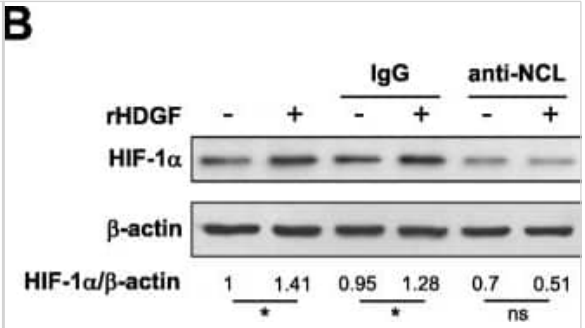


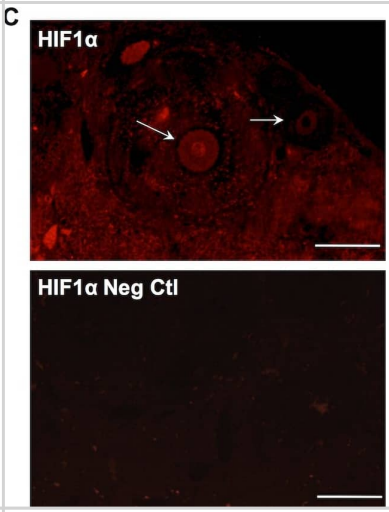
Immunohistochemistry: HIF-1 alpha Antibody - BSA Free [NB100-479] - White-jaded spleen & muscle in another NOD/SCID mouse model. One-week-old NOD/SCID mice were i.p. infected with EV-A71, & scarified after disease manifestation. aUpper panel: An atrophic & discolored spleen was identified in EV-A71-infected mice. Viral protein (VP1) & HIF1A were both expressed in the infected spleen. Lower panel: A saline control showed a normal-sized spleen with no VP1 & less expression of HIF1A. b Both HIF1A & VEGFA were strongly expressed in the whitened muscles infected with EV-A71, but not detectable in the saline control Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31711481), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



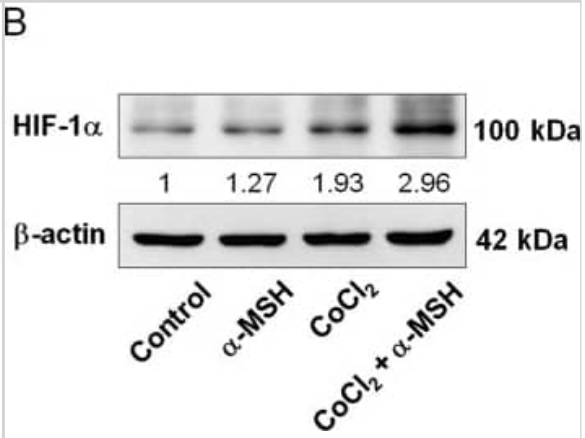
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - The Neutralizing antibody against nucleolin eliminate HDGF-stimulated AKT/HIF-1α/NF-κB/VEGF signaling in SCC4 oral cancer cells. a-d SCC4 cells were treated with recombinant HDGF protein (100 ng/ml) in the presence of anti-NCL or anti-IgG antibody (5 μg/ml) for 24 h before total protein extraction. Cell lysates were subjected to Western blotting with the indicated antibodies. β-actin was used as an internal control for loading & transfer. Data were mean of three experiments. *, P < 0.05; **, P < 0.01; ns, not statistically significant Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31711427), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



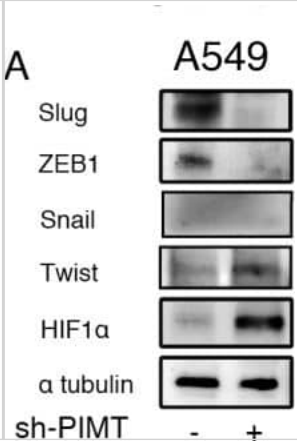
Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α expression in neonatal & adult ovary.(A) Section of P5 ovary showing MVH expression in all oocytes & Hif1α expression only in small oocytes (primary follicles; top). Negative control images without primary antibodies (bottom). Scale bar: 50μm. (B) Section of adult ovary showing absence of Hif1α expression in primary follicles detected with MVH staining (top). Negative control without primary antibody (bottom). Scale bar: 50μm. (C) Section of adult ovary showing Hif1α expression in mature primary oocyte (arrow). Negative control without primary antibody (bottom). Scale bar: 100μm. (D) Image of a Metaphase II oocyte from superovulated three-month-old female showing Hif1α expression. Scale bar: 50μm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



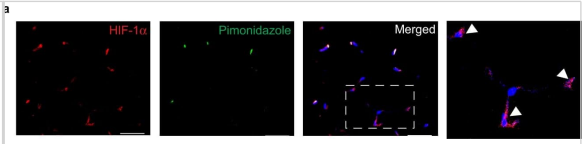
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of α-MSH on HIF-1α expression & activity in B16-F10 melanoma cells during hypoxia.a, b α-MSH increased HIF-1α mRNA & protein expression levels during hypoxia. c Cells were transfected with luciferase reporter driven by a promoter region containing HIF-1α-specific binding sites before α-MSH treatment at the indicated time & concentration. The luciferase activities were measured immediately & expressed as means ± SD from triplicate experiments. d Relative mRNA expression levels were analyzed by real-time PCR. Data are expressed as fold change compared with control (means ± SD of triplicate experiments). *p < 0.05, **p < 0.01. α-MSH elevated the Bcl-2 family gene expression levels by HIF-1α activation Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30062060), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



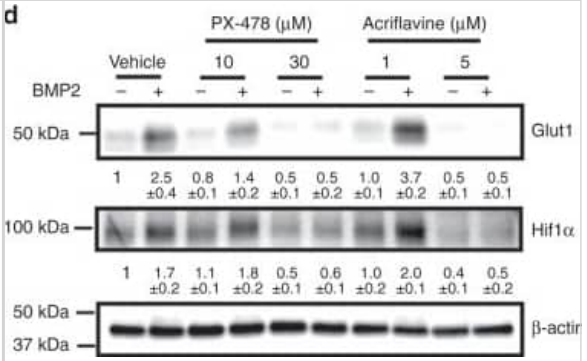
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Increased expression of HIFα and/or Twist in A549 & H441 cells induced by the inhibition of PIMT & Thapsigargin(A) Immunoblotting of Slug, ZEB1, Snail1, Twist, & HIF1α in A549 sh-PIMT & sh-control cells. (B, C) Immunoblotting & relative intensity of HIF1α in A549 cells treated with Tg. (D) Immunoblotting of Slug, ZEB1, Snail1, Twist, & HIF1α in si-control cells & si-PIMT H441 cells. (E, F) Immunoblotting & relative intensity of HIF1α in H441 cells treated with Tg. #1 & #2 indicates si-RNA of J-010000-05-0002 & J-010000-07-0002, respectively. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.24324), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



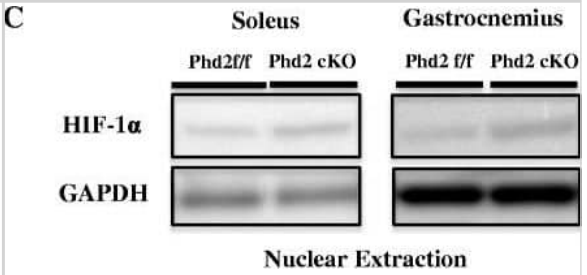
Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - (a) Immunofluorescence staining for HIF-1α (red), hypoxia probe pimonidazole (green) & DAPI nuclei (blue) in WAT of obese mice. Boxed region in the merged image shows HIF-1α-positive cells in crown-like structures that are Pimo− (arrows). (b) HIF-1α protein levels in BMDM treated with BSA (control) or BSA-conjugated palmitate (Palm) for 6, 12 & 24 hours. Tubulin is shown as an internal loading control. (c) Fold change in mRNA expression of Hif1a, Vegfa & Il1b in BMDM treated with palmitate or BSA. (d) Relative mRNA expression in BMDM treated as indicated in the presence or absence of a HIF-1α inhibitor. Data are representative of 3 independent experiments & are expressed as mean ± s.e.m. *p < 0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32221369), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



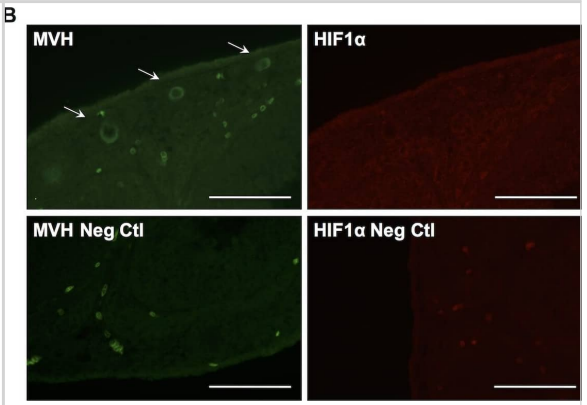
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α mediates Glut1 induction downstream of Bmp-mTORC1 signaling. a–c Western blot (a, c) or RT-qPCR (b) analyses in primary chondrocytes after 24 h of BMP2 treatment with or without mTOR inhibitors. Rapa: rapamycin. d Western blot analyses in primary chondrocytes in response to BMP2 with or without the Hif1α inhibitors for 24 h. Quantification of all western blots denotes average fold change over vehicle control after normalization to β-actin (±SD, n = 3). *p < 0.05, n = 3, error bar indicates SD, two-tailed Student's t-test Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30446646), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



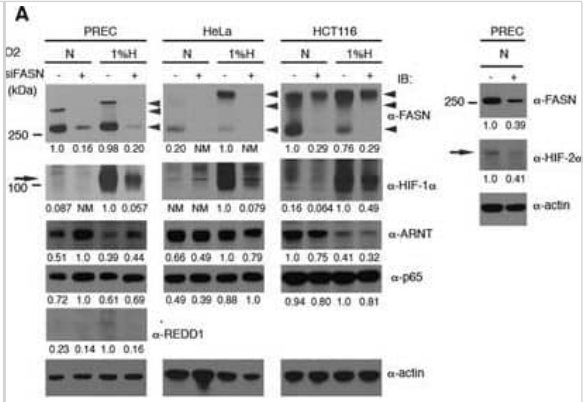
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Tamoxifen administration-induced PHD2 deletion in skeletal muscle in Phd2f/f/Rosa26CreERT2 mice. a. PHD1, 2, & 3 deletion efficiency in the gastrocnemius muscles of tamoxifen-treated Phd2f/f/Rosa26CreERT2 mice was determined using qRT-PCR. Relative gene expression was determined using gastrocnemius muscle tissue cDNA (n = 3–4 mice per group). b. Anti-PHD2 & PHD3 Western blotting of gastrocnemius & soleus muscles at 6 weeks after tamoxifen administration. c. The expression of HIF-1α in gastrocnemius & soleus at 5 weeks after tamoxifen administration. d. The level of hif-1α mRNA in gastrocnemius at 5 weeks after tamoxifen administration. *p < 0.05; **p < 0.01 compared to control. Values are means ± SEM Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26949511), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



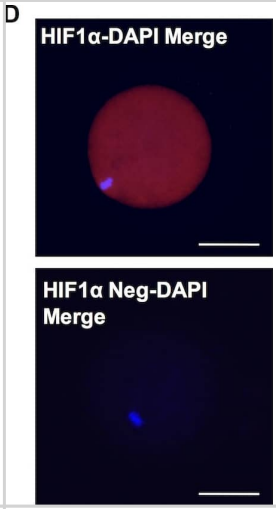
Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α expression in neonatal & adult ovary.(A) Section of P5 ovary showing MVH expression in all oocytes & Hif1α expression only in small oocytes (primary follicles; top). Negative control images without primary antibodies (bottom). Scale bar: 50μm. (B) Section of adult ovary showing absence of Hif1α expression in primary follicles detected with MVH staining (top). Negative control without primary antibody (bottom). Scale bar: 50μm. (C) Section of adult ovary showing Hif1α expression in mature primary oocyte (arrow). Negative control without primary antibody (bottom). Scale bar: 100μm. (D) Image of a Metaphase II oocyte from superovulated three-month-old female showing Hif1α expression. Scale bar: 50μm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



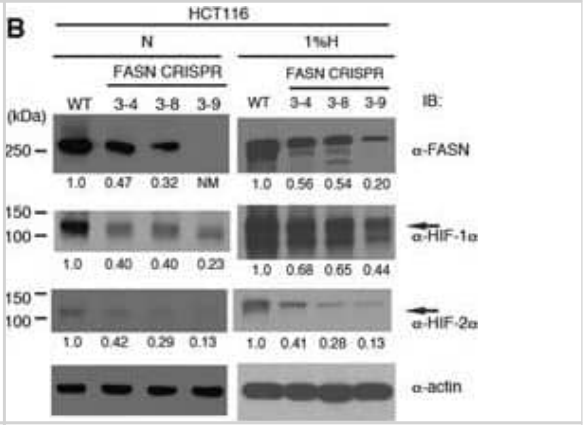
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - FASN Regulates HIF-α Protein Stability & HIF Target Gene Expression. (A) FASN siRNA downregulates HIF-α protein levels. PREC, HeLa & HCT116 cells treated with FASN (+) or control (−) siRNA & harvested after 72 h. Cells exposed to hypoxia (1% oxygen) or maintained in normoxia (21% oxygen) 4 h before harvesting & subjecting WCEs to WB analysis. Unmodified (250 kDa) & modified (larger) FASN bands indicated by large arrowheads, REDD1 by a small arrowhead, & HIF-1α & HIF-2α by arrows. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28775317), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



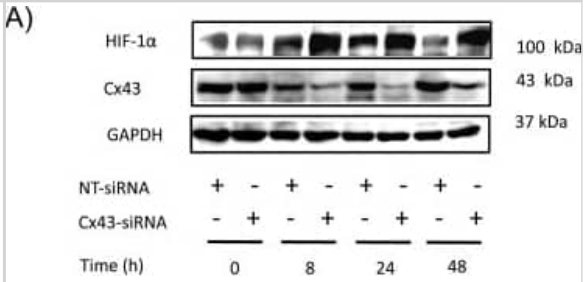
Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α expression in neonatal & adult ovary.(A) Section of P5 ovary showing MVH expression in all oocytes & Hif1α expression only in small oocytes (primary follicles; top). Negative control images without primary antibodies (bottom). Scale bar: 50µm. (B) Section of adult ovary showing absence of Hif1α expression in primary follicles detected with MVH staining (top). Negative control without primary antibody (bottom). Scale bar: 50µm. (C) Section of adult ovary showing Hif1α expression in mature primary oocyte (arrow). Negative control without primary antibody (bottom). Scale bar: 100µm. (D) Image of a Metaphase II oocyte from superovulated three-month-old female showing Hif1α expression. Scale bar: 50µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



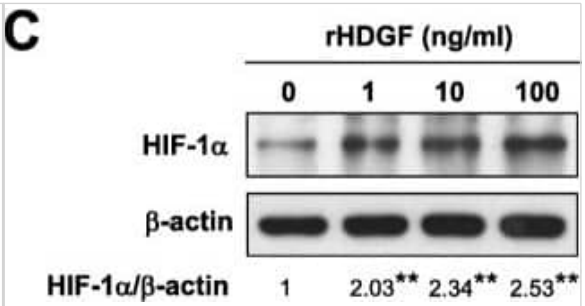
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - FASN Regulates HIF-α Protein Stability & HIF Target Gene Expression. (B) Downregulation of HIF-1α & HIF-2α in HCT116 cell clones carrying the FASN gene mutagenized using CRISPR-Cas9 (See Supplementary Fig. S3 for genomic DNA information). Protein samples prepared in normoxia (left column) or hypoxia (right column) for WB analysis. Asterisk indicates a non-specific band. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28775317), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



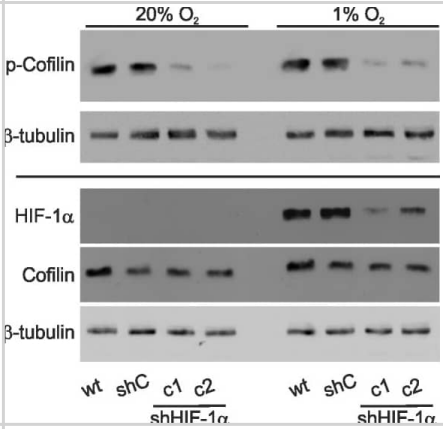
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of silencing Cx43 on the expression of HIF-1α in astrocytes.Astrocytes were transfected with NT-siRNA or with Cx43-siRNA. At the indicated times the expression of HIF-1α & Cx43 was analysed by Western blot. A) Representative Western blot of HIF-1α, Cx43 & GAPDH showing that the decrease in Cx43 expression was concomitant with HIF-1α up-regulation. B) HIF-1α quantification. C) Cx43 quantification. The results are expressed as percentages of the level found in the NT-siRNA condition at time 0. ***p<0.001 versus the corresponding NT-siRNA values. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0032448), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



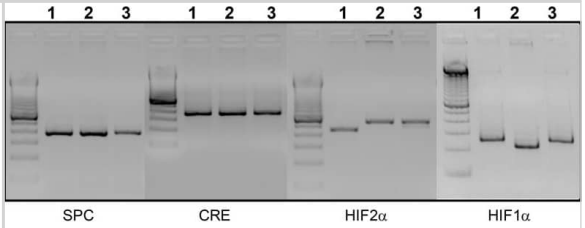
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - HDGF triggered AKT/HIF-1α/NF-κB signaling in SCC4 oral cancer cells. a-d Cells were treated with recombinant HDGF (1–100 ng/ml) for 24 h & then harvested for total protein extraction. The cell lysates were separated by SDS-PAGE & detected by Western blotting with the indicated primary antibodies. β-actin was used as an internal control for loading & transfer. Data were mean of three experiments. *, P < 0.05; **, P < 0.01; ns, not statistically significant Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31711427), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



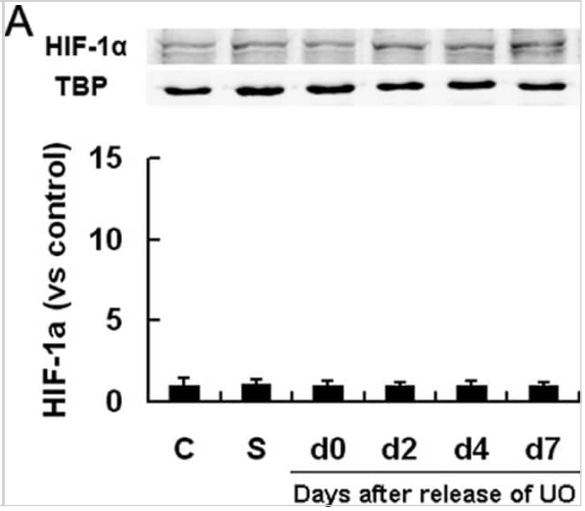
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Cofilin phosphorylation is HIF-1α dependent.Wt, shC, & the HIF-1α knock down cell clones c1 & c2 cells were lysed after 24 hrs of normoxia (20% O2) or hypoxia (1% O2). Cell extracts were analysed by Western blots. Note that p-cofilin levels are reduced in c2 & c2 cells compared to wt & shC cells at 20% O2 & 1% O2. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0069128), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



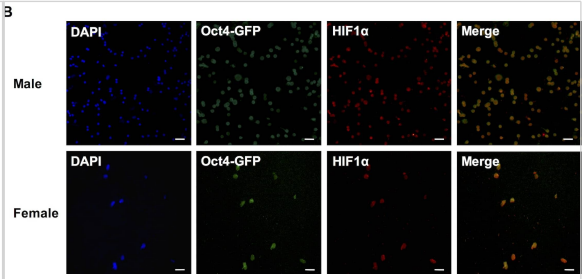
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Genotyping of transgenes.PCR genotyping was performed for all four transgenes as described in materials & methods. Sizes of the amplified products obtained are: 240 bp for Hif-1α (wild type); 274 bp for Hif-1αflox/flox; 410 bp for Hif-2α (wild type); 444bp for Hif-2αflox/flox; 370 bpfor Cre transgene; 350 bpfor rtTA transgene. One representative sample was genotyped for the four transgenes from each of three generated mouse strains: SPC-rtTA-/tg/(tetO)7-Cre-/tg/Hif-1αfl/fl (Lane 1), SPC-rtTA-/tg/(tetO)7-Cre-/tg/Hif-2αfl/fl mouse (Lane 2), & SPC-rtTA-/tg/(tetO)7-Cre-/tg/Hif-1α/2αfl/fl (Lane 3)). Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0139270), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



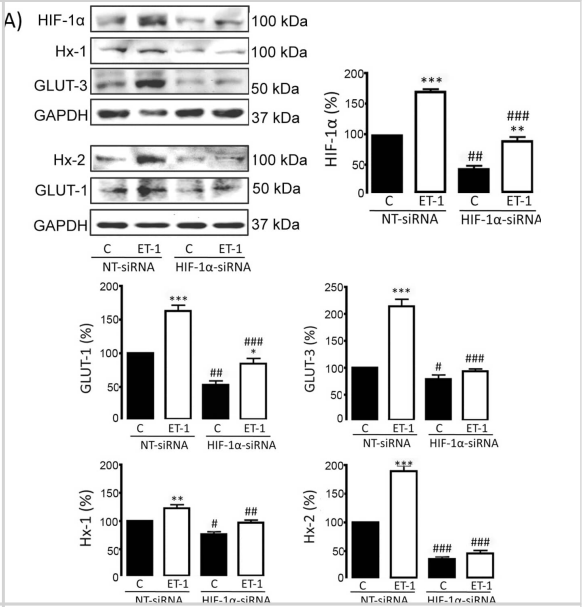
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of 24 h UO on renal HIF-1α/2α expression.WT mice were exposed to either sham-operation or left UO, which continued for 24 hours, & then was released. The left kidneys were harvested immediately (d0), 2, 4 or 7 days after release of obstruction (n=4 at each time point). Immunoblot analyses of HIF-1α & HIF-2α in left kidneys were then performed & co-detection of TBP was performed to assess equal loading. HIF protein bands were quantified & normalized to TBP. Data were expressed as mean ± SD, & the mean value obtained from non-operated control mice was arbitrarily defined as 1. *, p<0.05 versus sham-operated controls; **, p<0.05 versus all the other groups. C, non-operated controls; S, sham-operated controls. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/22295069), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



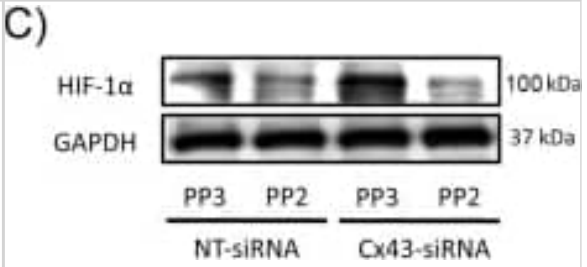
Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α expression in 15.5dpc germ cells.(A) Sections of male (top) & female (bottom) gonads from 15.5 dpc Oct4-GFP embryos showing Oct4-GFP & Hif1α expressions in germ cells. Scale bars: 50µm (male) & 20µm (female). (B) FACS-sorted male (top) & female (bottom) 15.5dpc germ cells showing Oct4-GFP & Hif1α expressions. Scale bars: 100µm (male) & 50µm (female). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



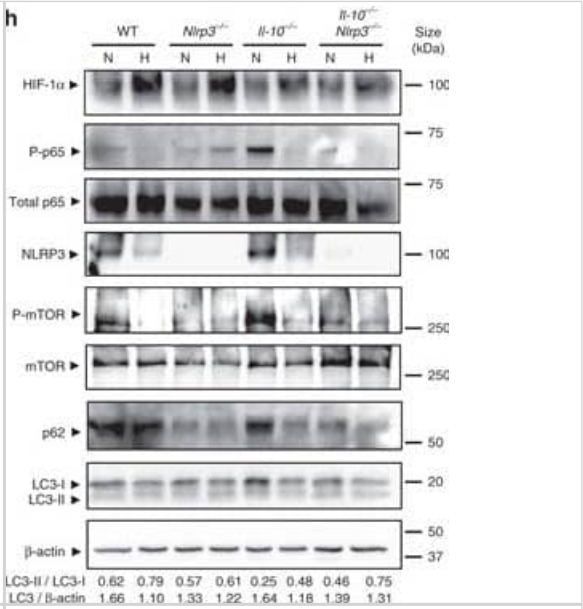
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of ET-1 on glucose uptake in HIF-1α-silenced astrocytes.Astrocytes were transfected with NT-siRNA or with HIF-1α-siRNA. After 48 h, astrocytes were incubated in the absence (control, C) or presence of 0.1 µM ET-1 for 24 h. A) HIF-1α, Hx-1, GLUT-3, Hx-2, GLUT-1 & GAPDH Western blots & quantification. The results are the means ± SEM of at least three independent experiments & they are expressed as percentages of the level found in the control NT-siRNA. B) Glucose uptake expressed as pmol of 2-deoxyglucose (2-DG) taken up per hour & per milligram of protein. The results show that the down-regulation of HIF-1α levels promoted by HIF-1α-siRNA decreased the rate of glucose uptake & the expression of GLUT-1, GLUT-3, Hx-1 & Hx-2, both in the control & in the ET-1 treated astrocytes. ***p<0.001, **p<0.01 & *p<0.05 versus the corresponding controls (C); ###p<0.001, ##p<0.01 & #p<0.05 versus the corresponding NT-siRNA. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0032448), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



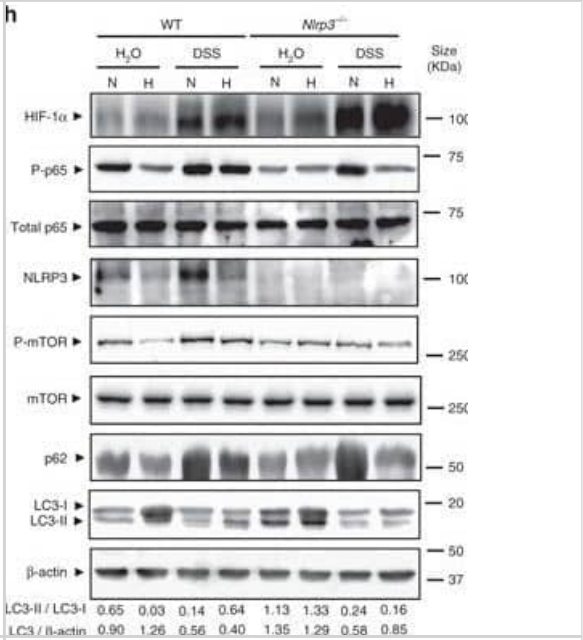
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of ET-1 & Cx43 on HIF-1α expression & glucose uptake when c-Src is inhibited.Astrocytes were preincubated with 100 ng/µL PP2 (c-Src inhibitor) or 100 ng/µL PP3 (inactive analogue) for 1 h. Then, cells were incubated in the absence (control) or presence of 0.1 µM ET-1 for 24 h. A) HIF-1α Western blot & quantification. The results are expressed as percentages of the level found in the controls treated with PP3 & they show that the inhibitor of c-Src PP2 prevented the up-regulation of HIF-1α promoted by ET-1. ***p<0.001 versus the absence of ET-1. B) Glucose uptake expressed as pmol of 2-deoxyglucose taken up per hour & per milligram of protein. The results show that the inhibitor of c-Src PP2 prevented the increase in the rate of glucose uptake promoted by ET-1. ***p<0.001 versus the absence of ET-1. C) Astrocytes were preincubated with 100 ng/µL PP2 or 100 ng/µL PP3 for 1 h. Then, cells were transfected with NT-siRNA or with Cx43-siRNA & after 48 h HIF-1α was analysed by Western blot. The results are expressed as percentages of the level found in the PP3 NT-siRNA & they show that the inhibitor of c-Src PP2 prevented the up-regulation of HIF-1α promoted by silencing Cx43. ***p<0.001 versus the corresponding NT-siRNA. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0032448), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



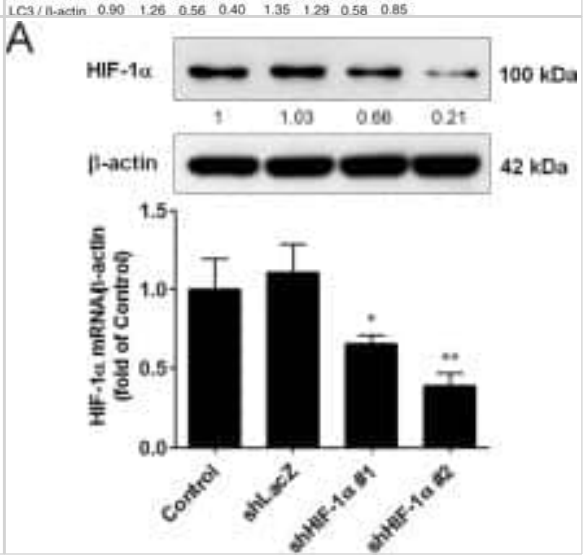
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxia reduces inflammatory processes & induces autophagy in Il-10−/− mice. a, b, c, d, e, f & g WT, Nrlp3−/−,Il-10−/−, & Il-10−/−Nrlp3−/−double knockout mice were subjected to normoxia (N, 21% O2) or hypoxia (H, 8% O2). After 18 h, mice were killed & colon biopsies were collected. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Results represent mean + s.e.m., WT mice under normoxia: n = 5, WT mice under hypoxia: n = 5; Nrlp3−/− mice under normoxia: n = 4; Nrlp3−/− mice under hypoxia: n = 5; Il-10−/− mice under hypoxia: n = 4; Il-10−/− mice under hypoxia: n = 4; Il-10−/−Nrlp3−/− mice under normoxia: n = 7; Il-10−/−Nrlp3−/− mice under hypoxia: n = 9, *P < 0.05; **P < 0.01; ***P < 0.001. h Total protein was isolated & western blot performed. LC3-I & LC3-II bands were quantified, & autophagy was measured by variations in the ratio of LC3-II/LC3-I & the total amount of LC3 (LC3-I plus LC3-II) relative to β-actin Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28740109), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



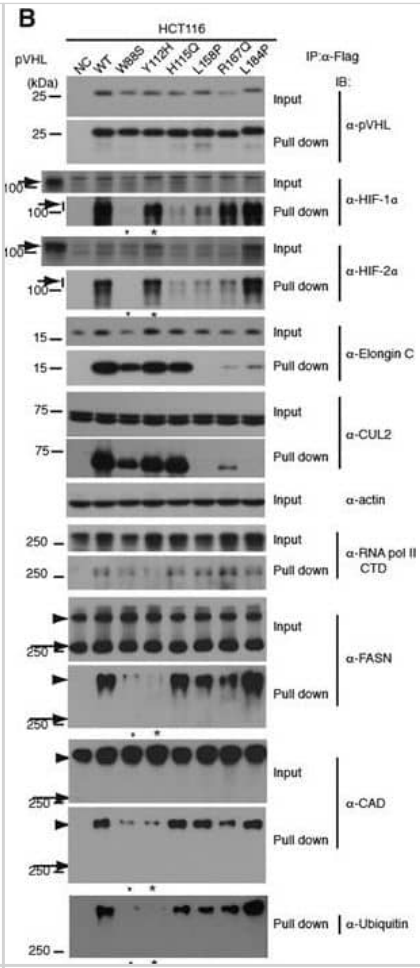
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxia reduces inflammatory processes & induces autophagy in the DSS mouse model of colitis. a, b, c, d, e, f & g WT & Nrlp3−/− mice were administered with DSS or DSS-free water & subjected to normoxia (N, 21% O2) or hypoxia (H, 8% O2). After 18 h, mice were killed & colon biopsies were collected. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Results represent mean + s.e.m., WT mice under normoxia: n = 5, WT mice under normoxia: n = 5, DSS-treated WT mice under normoxia: n = 6; Nrlp3−/− mice under normoxia: n = 5; DSS-treated Nrlp3−/− mice under normoxia: n = 6; WT mice under hypoxia: n = 4, DSS-treated WT mice under hypoxia: n = 5; Nrlp3−/− mice under hypoxia: n = 3; DSS-treated Nrlp3−/− mice under hypoxia: n = 3, *P < 0.05; **P < 0.01; ***P < 0.001. h Total protein was isolated & western blot performed. LC3-I & LC3-II bands were quantified, & autophagy was measured by variations in the ratio of LC3-II/LC3-I & the total amount of LC3 (LC3-I plus LC3-II) relative to β-actin Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28740109), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



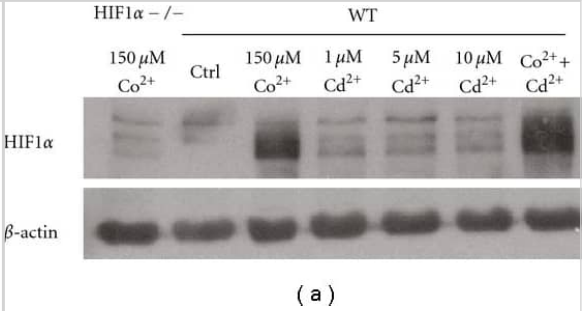
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of HIF-1α silencing on α-MSH-induced apoptosis in B16-F10 melanoma cells during hypoxia.a Cells were transfected with shLacZ or shHIF-1α plasmids for 48 h before harvest. HIF-1α shRNA reduced the basal HIF-1α mRNA & protein expression levels. b, c Relative mRNA expression levels were analyzed by real-time PCR. Data are expressed as fold change compared with control (means ± SD of triplicate experiments). d Cell lysates were analyzed by immunoblot using the indicated antibodies. β-Actin was used as an internal control for loading & transfer. e The population of apoptotic cells was analyzed by flow cytometry & qualified as mean ± SD from triplicate experiments. *p < 0.05, **p < 0.01. Knockdown of HIF-1α diminished α-MSH-induced apoptosis in B16-F10 melanoma cells during hypoxia Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30062060), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



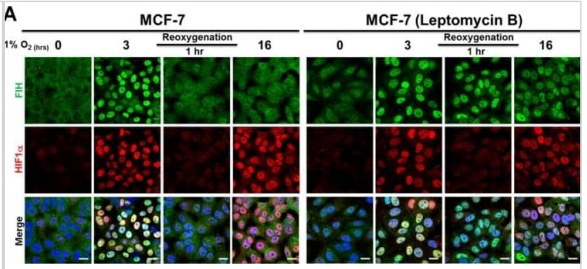
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - FASN & CAD Binding Specificity of pVHL Mutants Derived from VHL Patients. (A) Schematic representation of pVHL domains. Dotted lines indicate the approximate positions of the HIF-1α & Elongin C binding domains. The α & β structural domains are also indicated. (B) Effects of pVHL mutations on interactions with E3 ligase components, HIF-α & other interacting proteins including FASN. pVHL mutants were stably expressed by lentiviral vectors in HCT116 cells, & WCEs were tested in precipitation assays. Note that the mutant pVHL-W88S almost completely lost the ability to bind to HIF-1α & HIF-2α, whereas pVHL-Y112H retains this ability almost completely. However, both pVHL-W88S & pVHL-Y112H show significantly reduced binding to FASN & CAD, as indicated by small & large asterisks, respectively. Arrows indicate HIF-1α & HIF-2α bands. Long arrows & arrowheads indicate unmodified & modified forms of FASN, respectively. Protein samples prepared from hypoxia (1% oxygen)-exposed cells were added as positive control in left-end extra lanes. (C) The same samples as used in B were analyzed by silver staining. The E3 ligase components appear as major bands as indicated. (D) Subcellular localization of stably expressed pVHL mutants in 786-O. pVHL-Y112H & pVHL-W88S showed preferential nuclear localization, whereas pVHL-WT & pVHL-H115Q which both strongly bind to FASN & CAD, showed cytoplasmic localization. Cells showing clear nuclear (C < N) or cytoplasmic (C > N) localization in three microscopic viewing areas were counted as shown in the lower panel. The total counted cell numbers ranged from 35 to 64. The experiments were repeated more than twice, except for the cell localization count, & the results were reproduced. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28775317), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



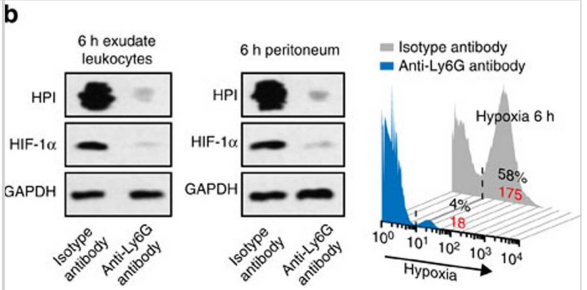
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxia signaling & cytotoxicity to CdCl2. (a) Wild-type (WT) cells were left untreated (Ctrl) or exposed to 150 µM CoCl2 (Co2+), 1, 5 or 10 µM CdCl2 (Cd2+) or 150 µM CoCl2 & 10 µM CdCl2 for 24 hours. HIF1α −/− cell extract treated with 150 µM CoCl2 was used as a negative control. Nuclear protein was extracted & separated by SDS-PAGE, transferred to nitrocellulose membrane & probed with a HIF1α (upper panel) or β-actin (lower panel) specific antibody. The bands observed in the cadmium only WT cells are nonspecific as they are also observed in the HIF1α −/− cells. (b) BNip3 mRNA expression levels were analyzed by qRT-PCR in wild type (WT, white bars) & HIF1α −/− cells (black bars). Cells were left untreated (0), or exposed to 5 µM CdCl2 (Cd2+) or 150 µM CoCl2 (Co2+) for 24 hours. Each value was normalized to the control level in the corresponding cell line. *P < 0.05 compared to the corresponding controls, n = 4. (c) BNIP3 protein levels were determined in wild type & HIF1α −/− cells after treatment with 150 µM CoCl2 (Co2+) or 5 µM CdCl2 (Cd2+) for 24 hours using a BNIP3 specific antibody & β-actin was used as a loading control (lower Panel). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/21811500), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



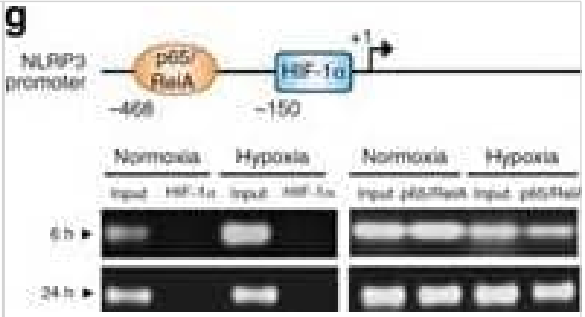
Immunocytochemistry/ Immunofluorescence: HIF-1 alpha Antibody - BSA Free [NB100-479] - FIH exits the nucleus via a Leptomycin B-sensitive exportin1-dependent pathway. (A) Immunofluorescence staining of FIH (green) & HIF1α (red) in MCF7 cells after the indicated hypoxia (0.5% O2) & re-oxygenation treatments. TO-PRO-3 (blue) was used to stain nuclei. (B) Total cell lysates from U2OS cells were immunoprecipitated with an anti-exportin1 antibody or control IgG. FIH, exportin1 & β-tubulin levels are indicated. (C) Immunofluorescence staining of FIH (green) in FIH-null mouse embryonic fibroblasts (MEFs) transfected with HA-FIH 1–349 or HA-FIH ΔNES followed by normoxia, hypoxia (1% O2, 3 h) or 3 h of hypoxia followed by re-oxygenation for 1 h. TO-PRO-3 (blue) was used to stain nuclei. Arrows indicate nuclear localization of signal. (D) Total cell lysates from U2OS cells transfected with control vector, HA-FIH 1–349 or HA-FIH ΔNES were immunoprecipitated with an anti-exportin 1 antibody. HA-FIH, exportin1 & β-tubulin levels are indicated. FL, full length; IgGL, IgG light chain. Scale bars: 20 μm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30333145), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



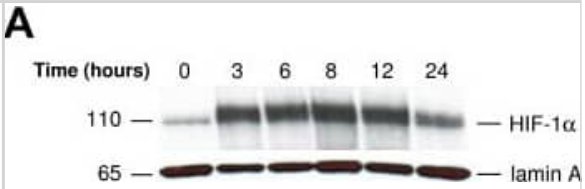
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Phagocyte infiltration contributes to the respiratory burst & consequent EPO signalling activation during inflammation resolution.ZymA (i.p., 1 mg per mouse) was applied to induce peritonitis in male WT mice. (a–c) Mice were treated with either 0.5 mg per mice i.p. of anti-Ly6G antibody or control (isotype control rat IgG2a) 1.5 day before zymA injection, & exudate leucocytes, peritoneum & peritoneal fluid were collected for analysis of ROS (a, n=3), hypoxia, HIF-1α (b, n=3) & EPO (c, n=3) at 6 24 h. (d–f) Mice were treated with either anti-Ly6G antibody (0.5 mg per mice) plus clodronate liposomes (0.2 ml per 10 g) or control (isotype antibody+empty liposome) before zymA injection, & exudate leucocytes, peritoneum & peritoneal fluid were collected for analysis of ROS (d, n=3), hypoxia, HIF-1α (e, n=3) & EPO (f, n=3) at 24 h. Representative data from two independent experiments are shown. For flow cytometry data, black numbers refer to the percentage of positive cells & red numbers refer to the mean fluorescent intensity. Error bars represent the s.e.m. *P<0.05, two-tailed unpaired Student's t-test. Full-size images for b & e are shown in Supplementary Fig. 12. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27397585), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



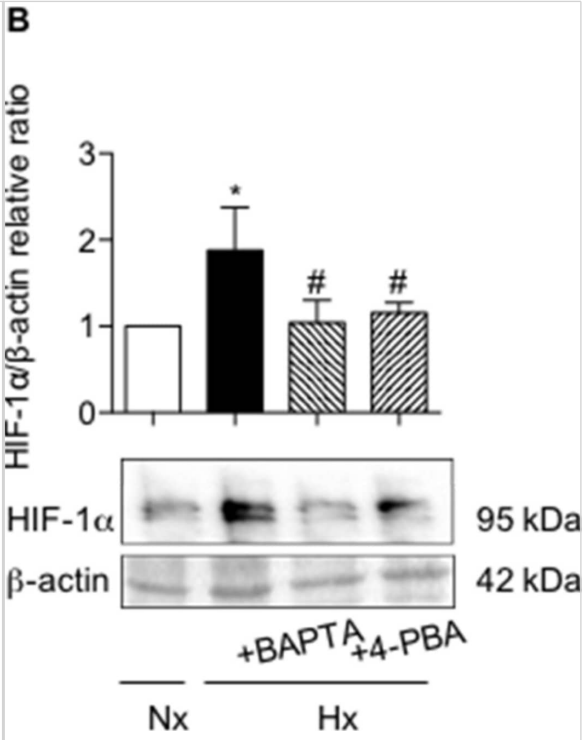
Chromatin Immunoprecipitation: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxia reduces inflammatory signaling pathways & NLRP3 expression & induces autophagy in IECs. a HT-29 cells were subjected to normoxia & hypoxia at the indicated times in the absence or presence of 10 µg/ml LPS. Autophagy was measured by variations in the ratio of LC3-II/LC3-I & the total amount of LC3 (LC3-I plus LC3-II) relative to β-actin. Results are representative of two independent experiments. b, c & d HT-29 cells were subjected to normoxia & hypoxia for the indicated periods in the absence or presence of 10 µg/ml LPS, followed by transcript analysis. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Results represent mean + s.e.m. of two independent experiments done in triplicate, *P < 0.05; **P < 0.01; ***P < 0.001; ns not significant. e HT-29 cells were subjected to normoxia or hypoxia in the presence & absence of 10 µg/ml LPS & 20 µM of MG132. Results are representative of two independent experiments. f & g Putative binding sites for HIF-1α & NF-κB were found in the p62 f & NLRP3 g promoters using Genomatix software tools. Numbers under the boxes indicate the distance from the transcription start site. HT-29 cells were subjected to normoxia (21% O2) or hypoxia (0.2% O2) for 6 h & 24 h. ChIP analysis was performed using antibodies against HIF-1α & NF-κB for immunoprecipitation. PCR was performed using the promoter-specific primers for the p62 f & NLRP3 g promoter binding sites of HIF-1α & NF-κB. Aliquots taken prior to immunoprecipitation were used as input control. PCR products were run on 2% agarose gel. The results are representative of three independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28740109), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



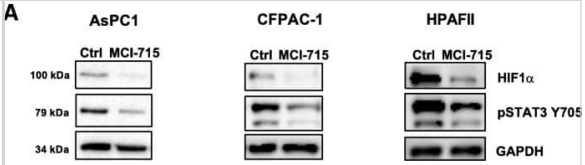
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of Hif1a deletion upon growth & invasion. (A) Wild-type (WT) mammary tumor epithelial cells (MTECs) grown to 80% confluence subjected to hypoxic culture for the indicated times up to 24 hours, or cells continued to be cultured under normoxic conditions such that the time t = 0 sample harvested on the same day as the time t = 24 hours hypoxic condition sample. High-salt enriched whole-cell extracts resolved on 3% to 8% Tris-acetate gels & blotted onto polyvinylidene fluoride membrane, which divided horizontally at approximately 60 kDa. The top half of the blot used to detect HIF-1α, & the lower portion used to detect lamin (loading control) to avoid the need to strip & reprobe the blot. (B) Growth curve of WT & knockout (KO) MTECs cultured at normoxia (Nor) or hypoxia (Hyp) in growth medium supplemented w/ 5% fetal bovine serum (FBS) + epidermal growth factor (EGF) (left) or w/ 2% FBS (right). For cells grown in 5% FBS + EGF, a representative graph is shown in which the mean ± SEM of cell number per time point of quadruplicate wells per genotype/oxygen tension is plotted per time point. For cells grown in 2% FBS, the grand mean ± SEM of cell number is presented, which calculated as an average of the mean cell number observed per replicates per time point as observed in three replicate experiments. All data analyzed by two-way analysis of variance (ANOVA, *P < 0.05). (C) The mean fold change (FC) in invasion normalized to invasion index observed for WT cells cultured at normoxia (FC = 1.0). Data represent the mean FC in invasion observed in three independent experiments. All columns compared using one-way ANOVA w/ a Bonferroni posttest. *P < 0.05. Image collected & cropped by CiteAb from the following publication (http://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr3087), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



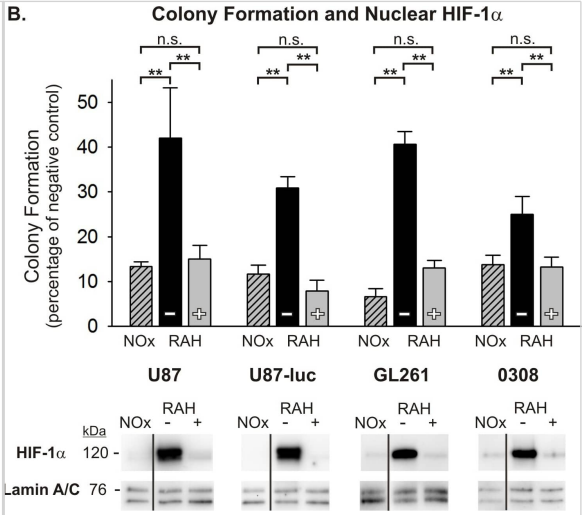
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Involvement of HIF-1α in alveolar epithelial cell phenotypic changes induced by hypoxia. Primary rat AECs were cultured in normoxia (Nx) (21% O2) or hypoxia (Hx) (1.5% O2) during 6 days in presence or absence of 10 μM YC-1. (A) Immunostaining of ZO-1 (magenta) & TTF1 (cyan) were performed. n = 4 experiments were performed. Isolated primary rat AECs were cultured in normoxia (Nx) (21% O2) or hypoxia (Hx) (1.5% O2) during 6 h in the presence or absence of 100 mM 4-phenylbutyrate (4-PBA) or pre-treated or not with 1 μM BAPTA-AM 90 min before exposition to hypoxia. A representative picture of at least n = 4 independent experiments for each condition has been presented & scale bar represents 50 μm. (B) Western blot of HIF-1α protein levels was performed. Representative blot of n = 5 experiments is shown. Expression levels of HIF-1α were quantified & reported to β-actin expression for each condition. Primary rat AECs were transfected with plasmid coding for luciferase reporter activity of hypoxia responsive element (HRE: i.e., HIF-luc), & cultured as described. (C) Luciferase activity corresponding to the transcriptional capacity of HIF was reported (n = 4 experiments). Raw data were submitted a Kruskal-Wallis test. * & ** indicate a significant difference as compared with normoxic value with $p < 0.05$ & $p < 0.01$ respectively. # & ## indicate a significant difference as compared with value in untreated hypoxic cells with $p < 0.05$ & $p < 0.01$, respectively. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30875855), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Pharmacological inhibition of ABCC3 reduces cell proliferation through STAT3 & HIF1α dysregulation & induction of apoptosis. a Representative Western blot images show the effects of pharmacological inhibition of ABCC3 with MCI-715 on the expression of pSTAT3 Y705 & HIF1α in three PDAC cell lines (AsPC1, HPAFII, CFPAC-1). Cells were treated with MCI-715 at the concentration of 10 μM & collected after 24 h (CFPAC-1) or 48 h (AsPC1, HPAFII). The quantitative analysis of n = 3 separate experiments is presented in Additional file 1: Figure S2; b The effects of the treatment of AsPC1, HPAFII & CFPAC-1 cell lines with 10 μM MCI-715 on the Caspase 3/7 activity (72 h post treatment) measured with Caspase 3/7 fluorigenic probe; c Representative Western blotting images & quantitative analysis of cleaved caspase 3 expression following treatment of indicated PDAC cell lines with 10 μM MCI-715. All results are presented as mean ± SEM of 3 independent experiments. The quantitative analysis was performed with the use of ImageJ & Image Lab software, unpaired Student's t-test was performed for statistical analysis, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31378204), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

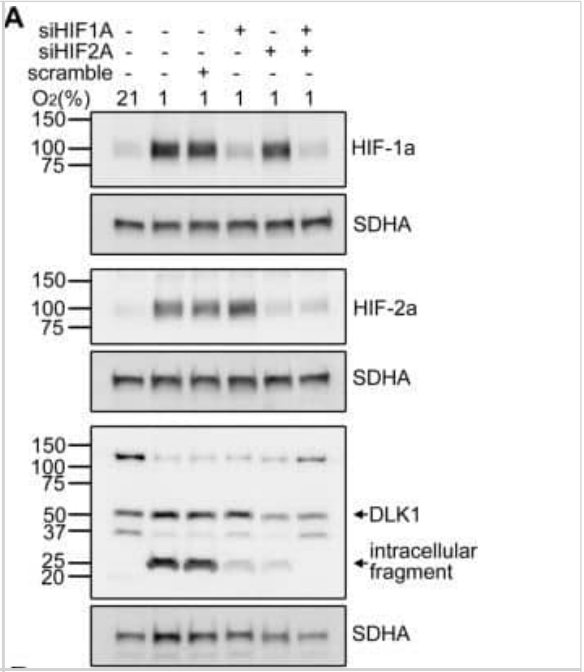


Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Normoxic pretreatment sensitizes glioma cells to radiation after rapid acute hypoxic (RAH) exposure.(A) The rapid acute hypoxia (RAH) protocol is shown depicting the timing & severity of hypoxic exposure. Cells either remain in a continuous hypoxic environment (–) or are transiently (25 min) exposed to normoxia 25 min prior to radiation (+). Continuously normoxic cells (NOx) were irradiated as a positive control. (B) The results of anchorage-independent colony forming assays are shown for U87, U87-luc, GL261 glioma cells & 0308 GSCs after 5 Gy radiation exposure under varying oxygen conditions. To allow for ease of comparisons among cell types, raw values are expressed as a percentage of the corresponding cell type's negative (non-irradiated) control & the means & SEMs are plotted. Each result represents at least three independent samples, plated in triplicate. Holm-Sidak comparisons for multiple groups were used for statistical comparisons of raw values (**p<0.01). Also shown are Western blots of nuclear HIF-1α at the time of irradiation for each cell type. Corresponding Western blots of lamin A/C are shown as a loading control. All lanes shown that are non-adjacent to the negative control (NOx) are denoted with a separating black line. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25350400), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

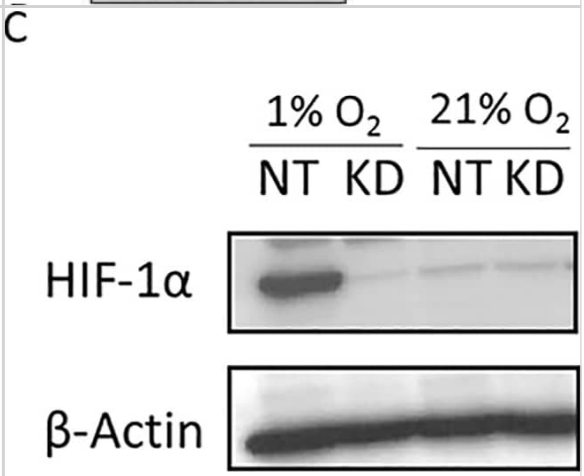


Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Multiple distinct mechanisms for downregulation of AKT activity in TSC1/2-deficient SCs. A-F: Western blots of sciatic nerve lysates from control & TSC2-SCKO/TSC1-SCKO mice at age P28 (A-E) or P14 (F) (3 mice per group), probed with the indicated antibodies. G: Immunofluorescence of longitudinal frozen sciatic nerve sections from control & TSC2-SCKO mice (age P28) showing greatly increased immunoreactivity of PTEN in the mutant (cytoplasmic signals surrounding DAPI+ nuclei). Scale bars: 50 µm H: Schematic summarizing distinct mechanisms underlying the downregulation of AKT activity in SCs from TSC1/2-SCKO nerves. Components highlighted in red are upregulated while blue highlighting depicts downregulation in mutant nerves. Note downregulation of insulin receptor substrate 1 (IRS-1) by constitutively active S6 kinases downstream of mTORC1. This leads to attenuated activation of PI3K/AKT. Lack of mTORC2 stimulation occurs through abolished TSC1/2 which results in reduced AKT phosphorylation in position Ser473 as well as through feedback inhibition by S6 kinases. Lastly, hyperactive mTORC1 promotes Hif1α transcription factor expression which results in increased PTEN transcription, & thus attenuated PI3K/AKT signaling. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29497474), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

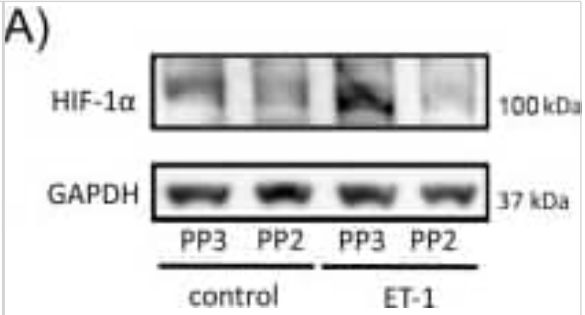
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - DLK1 cleavage is dependent on HIFs & ADAM 17 activity.a, b Representative images & densitometric analysis of western blots showing HIF-1α, HIF-2α, & DLK1 expression & cleavage in U3082MG cells after siRNA targeting of HIF1A & HIF2A in hypoxia. c, d Representative images & densitometric analysis of western blots showing the effects of ADAM inhibition by pre-treatment with 20 μM TAPI-2 on DLK1 cleavage in U3082MG cells grown at 21% or 1% O2 for 48 h. SDHA was used as loading control. e, f Representative images & densitometric analysis of western blots showing the effects of ADAM17 inhibition by pre-treatment with 0.5 μM TMI-1 on DLK1 cleavage in U3082MG cells grown at 21% or 1% O2 for 48 h. SDHA was used as loading control. Statistical analysis: b has 3 independent experiments while d & f have four independent experiments, all data are expressed as mean ± SEM. Statistical significance was determined by one-way ANOVA, followed by Bonferroni post hoc test. In the whole figure significance is represented as *p < 0.05, **p < 0.01, & ***p < 0.001 vs. respective 21% O2 controls or as indicated by straight lines. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32205867), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



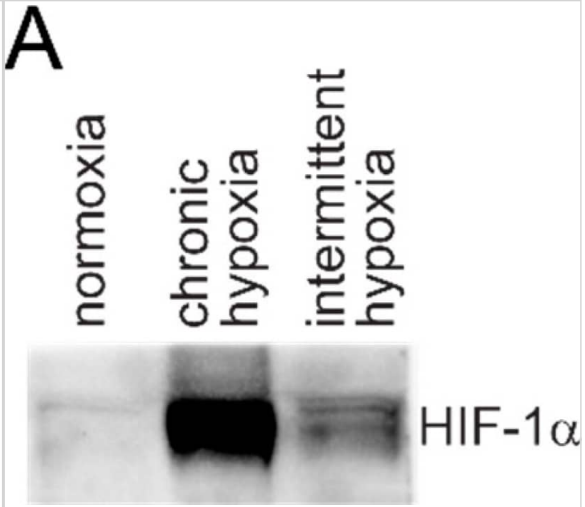
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxia downregulates MHC class I expression via HIF transcription factors.(A-C): siRNA mediated knockdown of HIF-1α reversed hypoxic downregulation of MHC class I expression as compared with the scrambled, non-targeting (NT) siRNA control. MCA205 tumor cells were reverse transfected with scrambled siRNA (NT; red histogram) or with HIF-1α specific siRNA (blue histogram) & cultured as 3D spheroids under 1% (A) or 21% (B) oxygen for 48h. Levels of MHC class I surface expression was determined using flow cytometry. Efficacy of gene knockdown was assessed using Western blot (C). β-Actin was used as the loading control. Representative data of 3 independent experiments shown. (D-F): Flow cytometry assessment of surface expression of HLA-ABC on paired isogenic renal cell carcinoma cell lines RCC4 (D), UMRC2 (E) & CAKI2 (F). Each pair had the parental cell line that lacked endogenous wild-type VHL (VHL null, transfected with empty vector) & one with vector stably expressing functional VHL (VHL restored). Restoring VHL function & thereby reducing HIF expression, significantly increased HLA-ABC expression on the cells. Representative histograms of 4 independent experiments are shown. Grey filled: unstained control; red: VHL null genotype; blue: VHL restored genotype. (D1-F1): Inactivation of HIF-1α by restoring VHL expression was verified by Western blotting for RCC4 (D1), UMRC2 (E1) & CAKI2 (F1) cells. β-Actin was used as the loading control. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0187314), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



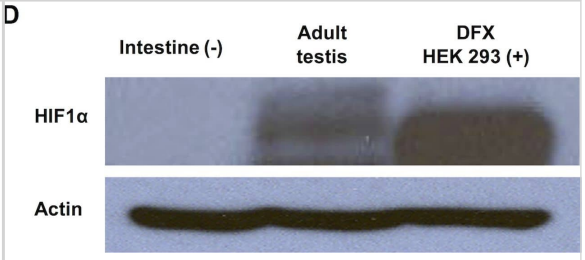
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of ET-1 & Cx43 on HIF-1α expression & glucose uptake when c-Src is inhibited.Astrocytes were preincubated with 100 ng/µL PP2 (c-Src inhibitor) or 100 ng/µL PP3 (inactive analogue) for 1 h. Then, cells were incubated in the absence (control) or presence of 0.1 µM ET-1 for 24 h. A) HIF-1α Western blot & quantification. The results are expressed as percentages of the level found in the controls treated with PP3 & they show that the inhibitor of c-Src PP2 prevented the up-regulation of HIF-1α promoted by ET-1. ***p<0.001 versus the absence of ET-1. B) Glucose uptake expressed as pmol of 2-deoxyglucose taken up per hour & per milligram of protein. The results show that the inhibitor of c-Src PP2 prevented the increase in the rate of glucose uptake promoted by ET-1. ***p<0.001 versus the absence of ET-1. C) Astrocytes were preincubated with 100 ng/µL PP2 or 100 ng/µL PP3 for 1 h. Then, cells were transfected with NT-siRNA or with Cx43-siRNA & after 48 h HIF-1α was analysed by Western blot. The results are expressed as percentages of the level found in the PP3 NT-siRNA & they show that the inhibitor of c-Src PP2 prevented the up-regulation of HIF-1α promoted by silencing Cx43. ***p<0.001 versus the corresponding NT-siRNA. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0032448), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



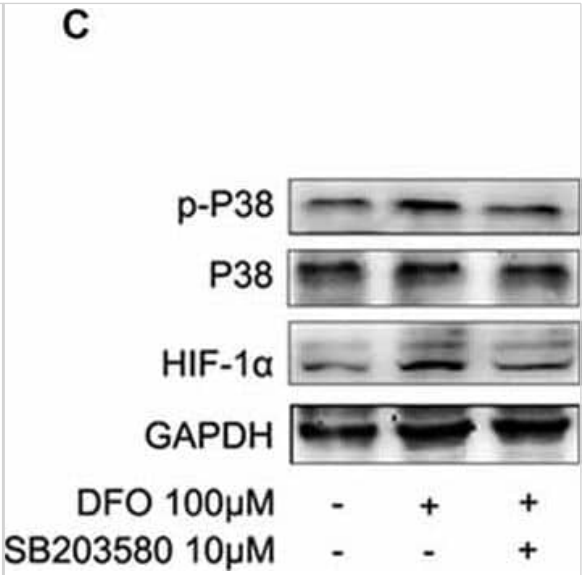
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - HIF-1α protein increases in HCT116 cells exposed to rapid intermittent hypoxia. Increased HIF-1α increases HIF-1 transactivation, leading to increased expression of HIF-1 target genes at both the mRNA & protein level. (A) Rapid intermittent hypoxia increases HIF-1α protein after 6 h of exposure. (B) Rapid intermittent hypoxia increases the mRNA expression of genes involved in glycolysis, extracellular matrix remodeling & the HIF pathway after 18 h of exposure. (C) Additional genes with known hypoxia response elements (HRE) binding sites for HIF-1 were measured & all tested genes showed an increase in both chronic hypoxia & intermittent hypoxia after 18 h. All values were normalized to normoxic expression levels, which is equivalent to 0 on the Log2 scale. Results are the mean ± SEM of independent experiments run in duplicate (n ≥ 3). (D) The increase in mRNAs is also reflected in an increase in the protein levels of Glut1, HK2, LDHA & PHD2. * p < 0.05, ** p < 0.01, *** p < 0.001 & **** p < 0.0001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30669593), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



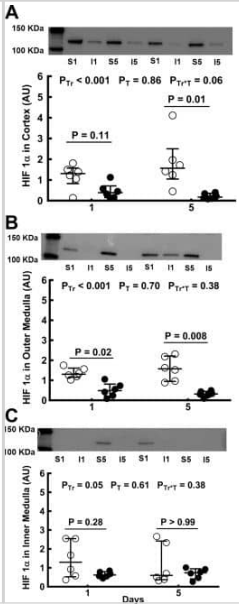
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α expression in neonatal & adult testis.(A) Section of testis from 5-day old (P5) male new born pups showing Hif1α expression in MVH+ gonocytes within the seminiferous tubules (top) & negative control images without primary antibodies (bottom). Scale bar: 50µm. (B) Western blot analysis of Hif1α expression in P5 testes (left) compared to extract of the adult brain sub-ventricular zone (SVZ) (right). Loading control (β-Actin) is shown below. (C) Section of adult (3 month old) testis showing Hif1α expression in spermatogonia. Scale bar: 30µm. (D) Western blot analysis of whole adult testis. HEK293 cells treated with DFX were used as a positive control & intestinal tissue was used as a negative control. Loading control (β-Actin) is shown below. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



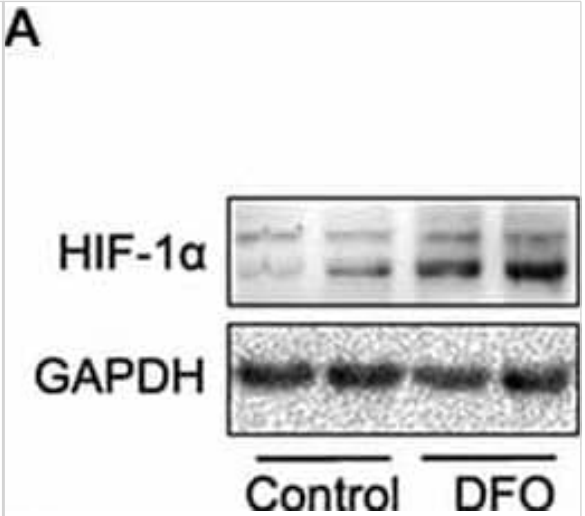
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Roles of P38 mitogen-activated protein kinase (MAPK) in the DFO-mediated up-regulate of HIF-1α protein in APPsw cells. (A) APPsw were exposed to 100 μM Fe, 100 μM DFO, or both combined for 24 h. Whole cell lysates were prepared & subjected to analysis for P38 or GAPDH protein. (B) Western blot analysis revealed that strong phosphorylation of P38 MAPK was detected in the DFO-treated cells, whereas no significant changes were observed in cells treated Fe alone compared with control. There were not statistical changes in the levels of P38 MAPK among the groups. The data represent the mean ± S.E. of three independent experiments. *p < 0.05, **p < 0.01 compared with the control; #p < 0.05, ##p < 0.01 with respect to the Fe treatment group. (C–E) APPsw cells were pretreated without or with SB203580 (10 μM) for 2 h & then treated without or with DFO for additional 22 h in the absence or presence of the inhibitor. Immunoblotting showed that DFO treatment significantly increased the levels of p-P38 MAPK compared with the control group, whereas the increase was significantly ameliorated by SB203580. Furthermore, the DFO-mediated up-regulation of HIF-1α protein was greatly inhibited by SB203580. The data represent the mean ± S.E. (n = 3). **p < 0.01 compared with the control; ##p < 0.01 compared to the values of DFO treatment group (two-way ANOVA with Post hoc Fisher's PLSD). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26082716), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



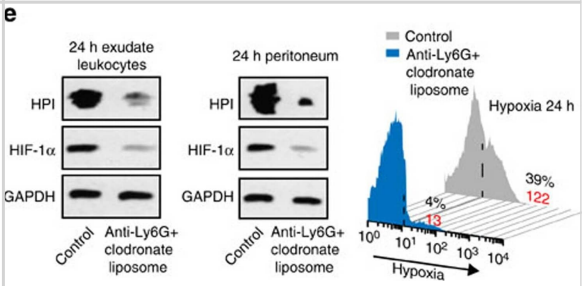
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Expression of hypoxia-inducible factor (HIF) proteins after bilateral renal ischemia or sham ischemia. Immunoblots for HIF-1α (A–C) & HIF-2α (D–F) of tissue extracts from the cortex & outer & inner medulla of the left kidneys of rats 24 h & 5 days following recovery from either sham ischemia (○) or bilateral renal ischemia (●); n = 6 per group. G: typical image of the gel following electrophoresis. H: typical image of the nitrocellulose membrane following transfer. Values are expressed as medians (25th percentile, 75th percentile). Paired comparisons were performed using the Mann-Whitney U-test. Because paired comparisons were made at two time points, P values were conservatively adjusted using the Dunn-Sidak method with k = 2. PTr, PT, & PTr*T are the outcomes of two-way analysis of variance on ranking with the factors treatment (Tr) & time (T). AU, arbitrary unit; I1, 24 h after ischemia; I5, 5 days after ischemia; S1, 24 h after sham ischemia; S5, 5 days after sham ischemia. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30110566), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



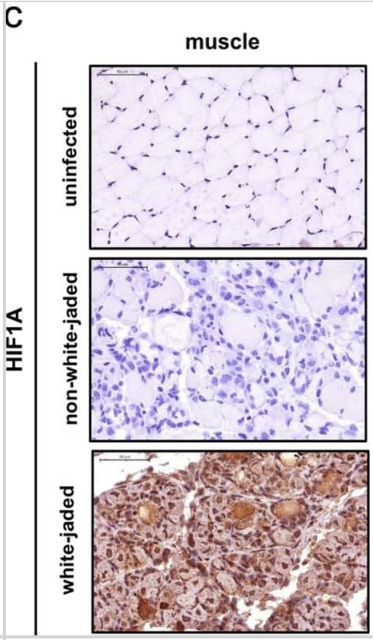
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - DFO regulates the expression of HIF-1α & HIF-1-related protein in the brains of APP/PS1 mice. (A) Western blots showing the expression levels of HIF-1α protein in control & DFO-treated transgenic mice brains. (B) Immunoblot showed that DFO treatment significantly increased HIF-1α protein levels, compared with controls. (C) The expression levels of HIF-1α mRNA were detected by RT-PCR in the brains of APP/PS1 transgenic mice treated with DFO. (D) RT-PCR analysis showed that DFO treatment significantly increased the HIF-1α mRNA levels in the transgenic mouse brain. GAPDH served as the internal control. (E) Immunohistochemically stains showed the distribution of HIF-1α in the cortical & hippocampus sections of APP/PS1 mouse brain (Scale bar = 100 μm). (F) Western blots showing the expression levels of brain-derived neurotrophic factor (BDNF), DMT1, & TFR proteins in APP/PS1 transgenic mice brains 3 months after DFO administration. GAPDH was used as a loading control. (G–J) DFO treatment led to a marked increase in the BDNF, DMT1 + IRE, & TFR protein levels in the brains of transgenic mice compared with the control. There was no significant change in the expression levels of DMT1-IRE protein between the groups, compared with the control. Data represent the mean ± S.E. of 6 independent experiments. **P < 0.01 vs. control group. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26082716), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



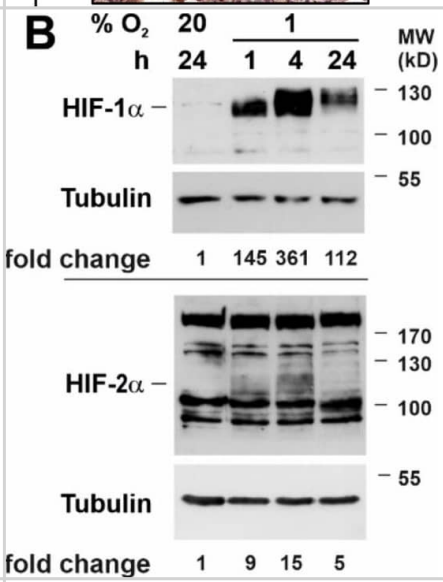
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Phagocyte infiltration contributes to the respiratory burst & consequent EPO signalling activation during inflammation resolution.ZymA (i.p., 1 mg per mouse) was applied to induce peritonitis in male WT mice. (a–c) Mice were treated with either 0.5 mg per mice i.p. of anti-Ly6G antibody or control (isotype control rat IgG2a) 1.5 day before zymA injection, & exudate leucocytes, peritoneum & peritoneal fluid were collected for analysis of ROS (a, n=3), hypoxia, HIF-1α (b, n=3) & EPO (c, n=3) at 6 24 h. (d–f) Mice were treated with either anti-Ly6G antibody (0.5 mg per mice) plus clodronate liposomes (0.2 ml per 10 g) or control (isotype antibody+empty liposome) before zymA injection, & exudate leucocytes, peritoneum & peritoneal fluid were collected for analysis of ROS (d, n=3), hypoxia, HIF-1α (e, n=3) & EPO (f, n=3) at 24 h. Representative data from two independent experiments are shown. For flow cytometry data, black numbers refer to the percentage of positive cells & red numbers refer to the mean fluorescent intensity. Error bars represent the s.e.m. *P<0.05, two-tailed unpaired Student's t-test. Full-size images for b & e are shown in Supplementary Fig. 12. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27397585), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



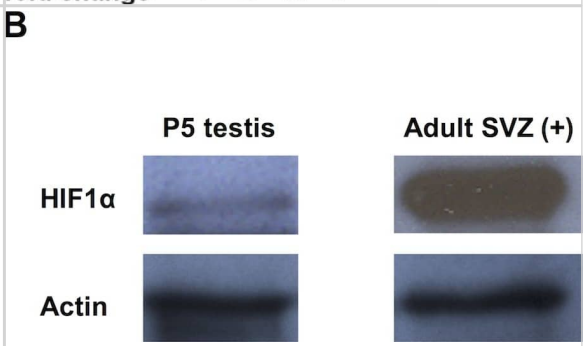
Immunohistochemistry: HIF-1 alpha Antibody - BSA Free [NB100-479] - A “white-jade” muscle phenotype in EV-A71-infected mouse strain 129. aLeft panel: We coined a “white-jade” phenotype to describe the pathological changes in the color of the hindlimb muscle in EV-A71-infected mice (indicated by arrow). This phenotype showed characteristic muscle with locally whitened color in appearance & hardened tissue mass. In the saline control, tendons (not muscle) can be seen in white color. Right panel: An anatomical sketch of the hindlimb muscle. GM: gluteal muscle, QF: quadratus femoris muscle, BF: Biceps femoris muscle. b Both VP1 protein (upper) & myogenin protein (lower) were detected by IHC staining in the muscles, indicating EV-A71 infection, muscle injury & regeneration. The magnification is 200X. c Striking expression of HIF1A (hypoxia inducible factor 1-α) protein (brown color) was detected by IHC staining in the white-jaded tissue, but not in the non-white-jaded tissue from the same mouse. d Specific association between HIF1A expression & white-jaded muscle Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31711481), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



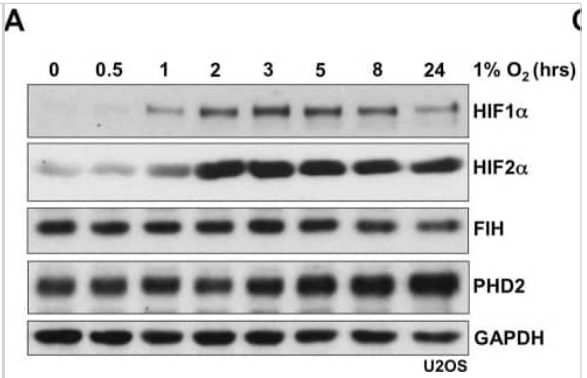
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Kidney-derived Sca-1+ cells produce Epo. (A) Epo protein levels in supernatants obtained from Sca-1+ cells cultured in normoxia or hypoxia (n = 3 biological replicates). (B) HIF-1α & HIF-2α protein levels in Sca-1+ kidney-derived cells after exposure to normoxia (20% O2) or hypoxia (1% O2). Numbers below the blots indicate the fold change of the ratio of HIF-1α/Tubulin & HIF-2α/Tubulin in 1% O2 to the respective 20% O2 control. (C) Epo RNA levels in Sca-1+ cells that were incubated either at 20% O2 of 1% O2 as indicated (n = 4 biological replicates). (D) Mesenchymal stem cell markers in renal Sca-1+ cells on day 21 of culture; reads per kilobase million (RPKM). (E) Volcano plot of 4592 significantly up- & 5376 significantly downregulated mRNAs in kidney-derived Sca-1+ cells on day 21 in culture compared to day 0. Mean values ± SEM are shown. * p < 0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35203399), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



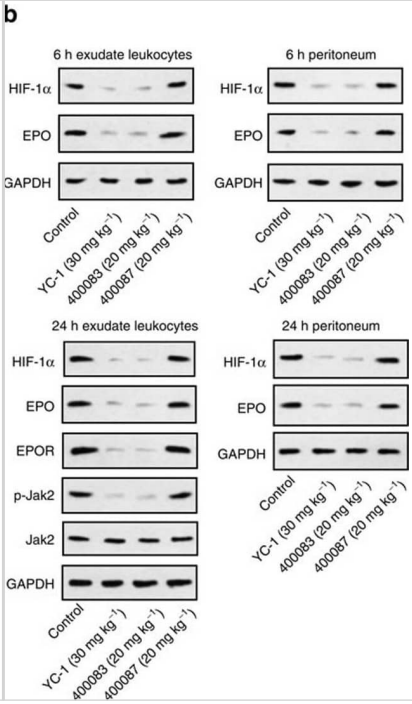
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α expression in neonatal & adult testis.(A) Section of testis from 5-day old (P5) male new born pups showing Hif1α expression in MVH+ gonocytes within the seminiferous tubules (top) & negative control images without primary antibodies (bottom). Scale bar: 50μm. (B) Western blot analysis of Hif1α expression in P5 testes (left) compared to extract of the adult brain sub-ventricular zone (SVZ) (right). Loading control (β-Actin) is shown below. (C) Section of adult (3 month old) testis showing Hif1α expression in spermatogonia. Scale bar: 30μm. (D) Western blot analysis of whole adult testis. HEK293 cells treated with DFX were used as a positive control & intestinal tissue was used as a negative control. Loading control (β-Actin) is shown below. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



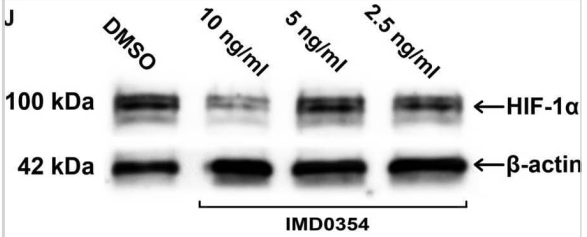
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Evidence that hypoxia induces nuclear entry of FIH. (A) Protein levels of HIF1α, HIF2α, FIH & PHD2 in U2OS cells during hypoxia (1% O2) treatment at the indicated time points. GAPDH was used as a loading control. (B) Immunofluorescence staining of FIH (green) in U2OS cells under hypoxic conditions (1% O2) at the indicated time points. TO-PRO-3 (blue) was used to stain nuclei. (C) Protein levels of FIH & HIF1α from cytoplasmic or nuclear fractions in U2OS cells in normoxia or hypoxia (1% O2, 3 h). β-tubulin & PARP were used as loading controls for the cytoplasmic & nuclear fractions, respectively. Figures beneath lanes 2 & 4 indicate relative intensities of nuclear FIH in normoxia & hypoxia. Note that different quantities of cytoplasmic & nuclear extracts were loaded. Scale bars: 20 μm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30333145), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



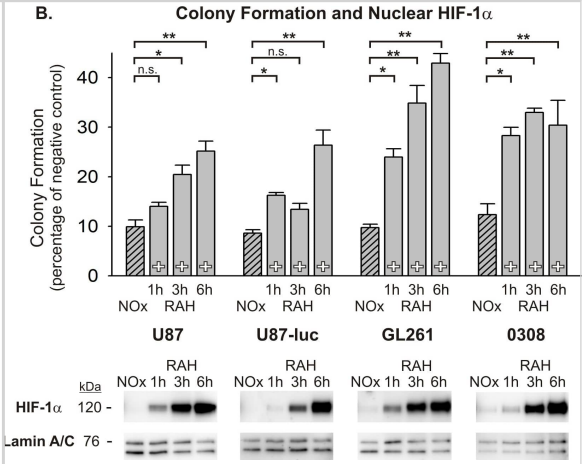
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Respiratory burst contributes to the EPO signalling activation during inflammation resolution.ZymA (i.p., 1 mg per mouse) was applied to induce peritonitis in male WT mice or CGD mice. (a) Exudate leucocytes & peritoneum were collected at indicated intervals, & protein levels of HIF-1α & HIF-2α were measured by WB (n=3). (b) YC-1, 400083 or 400087 were intraperitoneally given to WT mice 3 h before zymA injection & the protein levels of related molecules were analysed by WB (n=2). (c) Mice were killed 1 h after injection of pimonidazole at indicated intervals & the protein adducts of reductively-activated pimonidazole were detected by WB with Hypoxiprobe-1-Mab-1 (HPI) (n=2). (d) Flow cytometry for ROS at indicated intervals (n=3). (e) Flow cytometry for cellular sources of ROS at indicated intervals (n=3). Representative data from at least two independent experiments are shown. For flow cytometry data from d, black numbers refer to the percentage of positive cells & red numbers refer to the mean fluorescent intensity. Error bars represent the s.e.m. *P<0.05, two-tailed unpaired Student's t-test. Full-size images for a–c are shown in Supplementary Figs 8–10, respectively. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27397585), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



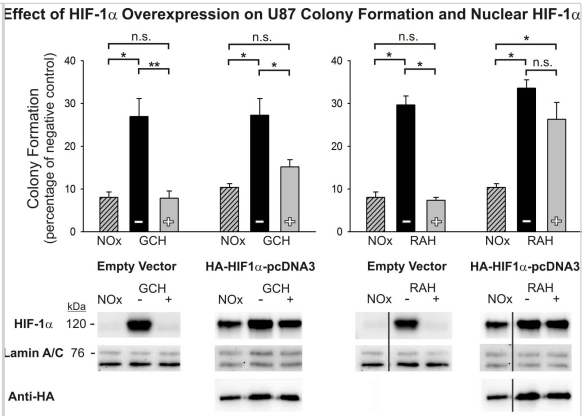
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of IKK2 inhibition on HUVEC, microvessel outgrowth from aortic rings, & VEGF-A expression. a Dose-dependent (10, 5 & 2.5 ng/ml) inhibitory effect of IMD0354 on HUVEC migration relative to control (DMSO 1 µl/ml). Living HUVEC were visualized with Calcein-AM (green). b Quantification of HUVEC migration distance (n = 8). One-way ANOVA test with Tukey multiple comparison was used to determine statistical significance. c HUVEC has grown on Geltrex to evaluate tube formation in the presence of IMD0354 compared to drug-free vehicle (DMSO). Vital HUVEC are stained with calcein-AM (green), & dead HUVEC are displayed in red (propidium iodide). Quantitative analysis of a number of junctions (d), & tubules (e) formed by HUVEC, & total tubule length (f) (n = 8). g Quantitative analysis of cell death induced by IMD0354 treatment (n = 8). h Effect of IMD0354 on cell proliferation in the aortic ring assay. Student t test was used to determine statistical significance. i Western blot analysis of VEGF-A expression in HUVEC treated with IMD0354 (10, 5, 2.5 ng/ml), with β-actin as a loading control. j Western blot analysis of HIF-1α expression in HUVEC treated with IMD0354 (10, 5, 2.5 ng/ml), with β-actin as a loading control. k Immunofluorescent detection of VEGF-A (green) in HUVEC treated with IMD0354 (10, 5, 2.5 ng/ml). Cell nuclei visualized with DAPI staining (blue). n.s. $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ Image collected & cropped by CiteAb from the following publication (http://link.springer.com/10.1007/s10456-018-9594-9), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



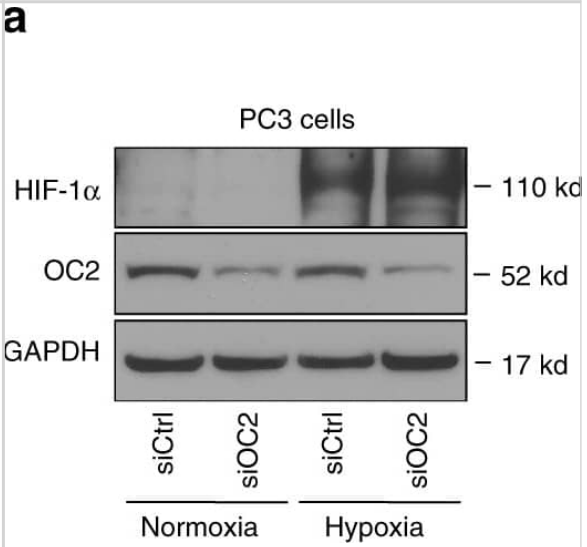
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - The duration of enhanced radiosensitivity after normoxic pretreatment of cells undergoing Rapid Acute Hypoxia.(A) The RAH protocol is shown with variable delays to radiation after normoxic pretreatment. In this protocol all cells are transiently (25 min) exposed to normoxia for 25 min (+) & then returned to severe hypoxia (1% O2) for 1, 3, or 6 hours prior to radiation. Continuously normoxic cells (NOx) were irradiated as a positive control. (B) Results from anchorage-independent colony forming assays indicate that the decay of enhanced radiosensitivity for cells in the RAH protocol is generally more rapid than that observed for cells in the GCH protocol. To allow for ease of comparisons among cell types, raw values are expressed as a percentage of the corresponding cell type's negative (non-irradiated) control & the means & SEMs are plotted. Each result represents at least three independent samples, plated in triplicate. Holm-Sidak comparisons for multiple groups were used for statistical comparisons of raw values (*p<0.05, **p<0.01). Also shown are Western blots of nuclear HIF-1α at the time of irradiation for each cell line. Corresponding Western blots of lamin A/C are shown as a loading control. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25350400), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



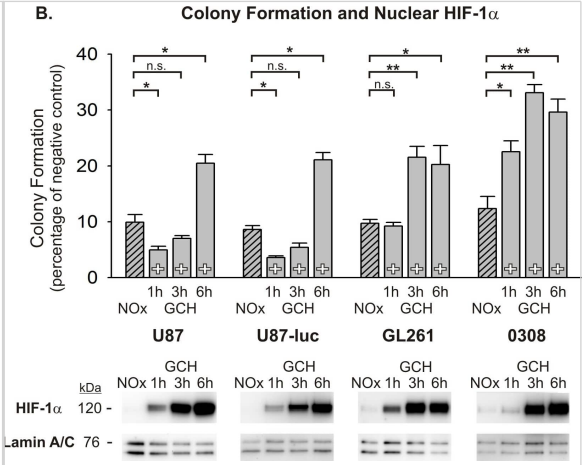
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - HIF-1α overexpression rescues oxygen-induced radioresistance in RAH-treated cells, but not GCH-treated cells.Results are shown for the anchorage-independent colony forming assays for U87 cells transfected with either an empty vector or HIF-1α expression vector & then exposed to GCH or RAH protocols without (–) or with (+) reoxygenation. Continuously normoxic cells (NOx) were irradiated as a positive control. To allow for ease of comparisons among conditions, raw values are presented as a percentage of that cell type's negative (non-irradiated) control & the means & SEMs are plotted. Each result represents at least three independent samples, plated in triplicate. Holm-Sidak comparisons for multiple groups were used for statistical comparisons of raw values (*p<0.05, **p<0.01). Western blotting analysis of nuclear HIF-1α at the time of irradiation is shown for each cell type below clonogenic results. Corresponding Western blots of lamin A/C are shown as a loading control & blots for hemagglutinin (HA) are shown below HIF-1α overexpression vector results to demonstrate transfection efficacy. All lanes shown that are non-adjacent to the negative control (NOx) are denoted with a separating black line. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25350400), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



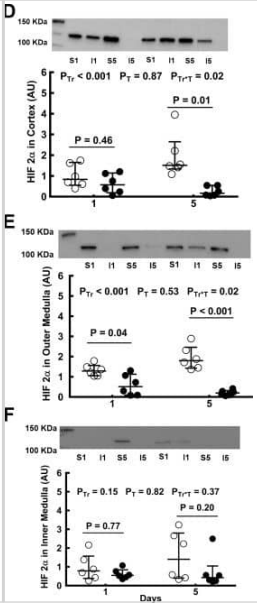
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - ONECUT2 modulates HIF1α binding to chromatin in NE-like PC3 cells. a Western blot of HIF1α & ONECUT2 in PC3 cells under normoxic & hypoxic conditions. Two different siRNAs targeting ONECUT2 were mixed together for knockdown experiments. b Left panel: heatmaps show HIF1α ChIP-Seq signal in PC3 cells under hypoxic conditions with & without knockdown of ONECUT2; right panel: pileup of HIF1α ChIP-Seq signals centered at HIF1α ChIP-Seq peaks center. c Expression of ANGPTL4 & ADM, two hypoxia-regulated genes, with & without knockdown of ONECUT2 in PC3 cells. d Schematic illustration of the analysis identifying SMAD3 as an ONECUT2 regulated HIF1α co-factor. Genes identified in motifs enriched in HIF1α binding sites & ONECUT2 target genes were further filtered by HIF1α interacting protein list from BioGRID. ONECUT2 target genes were defined as differentially expressed in ONECUT2 knockdown & control samples & with ONECUT2 binding sites nearby in PC3 cells under hypoxic conditions. e SMAD3 expression in response to ONECUT2 silencing in PC3 cells. f SMAD3 & HIF1α binding sites with & without silencing of SMAD3. g The overlap of SMAD3 ChIP-Seq, HIF1α ChIP-Seq & SMAD3-HIF1α ChIP-re-ChIP-Seq peaks. h SMAD3 ChIP-Seq, HIF1α ChIP-Seq & SMAD3-HIF1α ChIP-re-ChIP-Seq signal near the promoter regions of hypoxia-induced genes ANGPTL4 & ADM. Error bars indicate s.d. from at least two technical replicates. P-value is calculated by one-way ANOVA. **: P < 0.01. Source data are provided as a Source Data file Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30655535), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



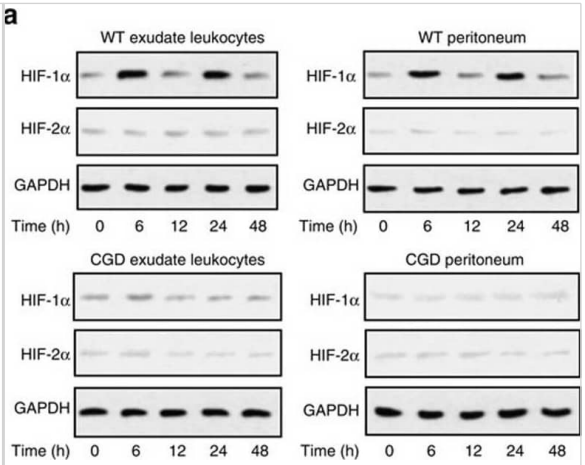
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - The duration of enhanced radiosensitivity after normoxic pretreatment differs among cell lines undergoing Graded Chronic Hypoxia.(A) The GCH protocol is shown with variable delays to radiation after normoxic pretreatment. After GCH, all cells are transiently (25 min) exposed to normoxia for 25 min (+) & are then returned to severe hypoxia (1% O2) for 1, 3, or 6 hours prior to radiation. Continuously normoxic cells (NOx) were irradiated as a positive control. (B) Results from anchorage-independent colony forming assays indicate that the decay of enhanced radiosensitivity differs among cell lines. To allow for ease of comparisons among cell types, raw values are expressed as a percentage of the corresponding cell type's negative (non-irradiated) control & the means & SEMs are plotted. Each result represents at least three independent samples, plated in triplicate. Holm-Sidak comparisons for multiple groups were used for statistical comparisons of raw values (*p<0.05, **p<0.01). Also shown are Western blots of nuclear HIF-1α at the time of irradiation for each cell line. Corresponding Western blots of lamin A/C are shown as a loading control. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25350400), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



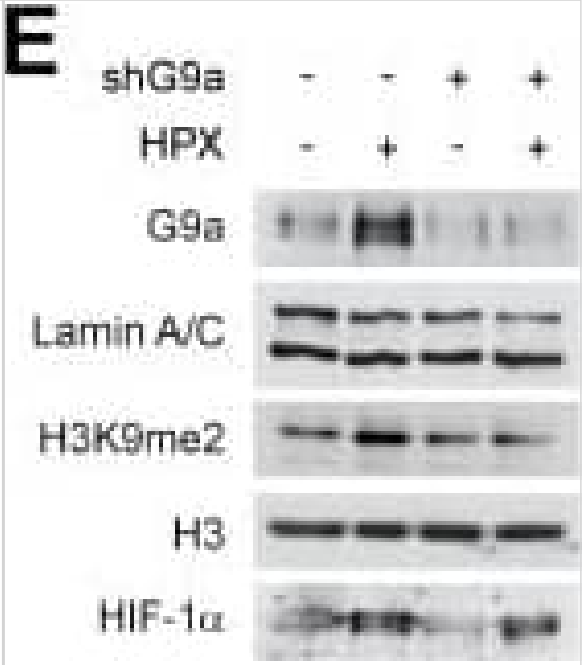
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Expression of hypoxia-inducible factor (HIF) proteins after bilateral renal ischemia or sham ischemia. Immunoblots for HIF-1α (A–C) & HIF-2α (D–F) of tissue extracts from the cortex & outer & inner medulla of the left kidneys of rats 24 h & 5 days following recovery from either sham ischemia (○) or bilateral renal ischemia (●); n = 6 per group. G: typical image of the gel following electrophoresis. H: typical image of the nitrocellulose membrane following transfer. Values are expressed as medians (25th percentile, 75th percentile). Paired comparisons were performed using the Mann-Whitney U-test. Because paired comparisons were made at two time points, P values were conservatively adjusted using the Dunn-Sidak method with k = 2. PTr, PT, & PTr*T are the outcomes of two-way analysis of variance on ranking with the factors treatment (Tr) & time (T). AU, arbitrary unit; I1, 24 h after ischemia; I5, 5 days after ischemia; S1, 24 h after sham ischemia; S5, 5 days after sham ischemia. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30110566), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



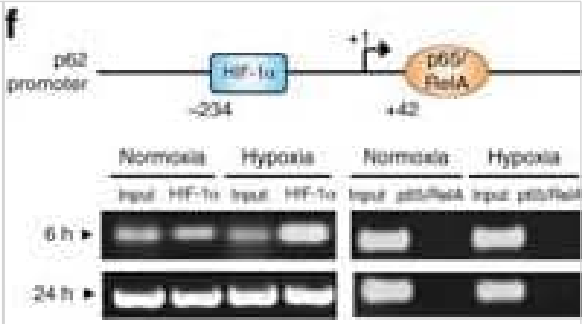
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Respiratory burst contributes to the EPO signalling activation during inflammation resolution.ZymA (i.p., 1 mg per mouse) was applied to induce peritonitis in male WT mice or CGD mice. (a) Exudate leucocytes & peritoneum were collected at indicated intervals, & protein levels of HIF-1α & HIF-2α were measured by WB (n=3). (b) YC-1, 400083 or 400087 were intraperitoneally given to WT mice 3 h before zymA injection & the protein levels of related molecules were analysed by WB (n=2). (c) Mice were killed 1 h after injection of pimonidazole at indicated intervals & the protein adducts of reductively-activated pimonidazole were detected by WB with Hypoxiprobe-1-Mab-1 (HPI) (n=2). (d) Flow cytometry for ROS at indicated intervals (n=3). (e) Flow cytometry for cellular sources of ROS at indicated intervals (n=3). Representative data from at least two independent experiments are shown. For flow cytometry data from d, black numbers refer to the percentage of positive cells & red numbers refer to the mean fluorescent intensity. Error bars represent the s.e.m. *P<0.05, two-tailed unpaired Student's t-test. Full-size images for a–c are shown in Supplementary Figs 8–10, respectively. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27397585), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - G9a inhibition reduces cell motility in breast cancer cell lines. G9a inhibition represses breast cancer cell motility in vitro. (A) Microarray analysis of differentially expressed genes comparing hypoxia-responsive genes from MCF7 cells expressing shNS & shG9a identifies a subset of 212 genes downregulated in hypoxia in a G9a-dependent manner. (B) Ingenuity Pathway Analysis results of the 212 G9a-dependent genes. (C) Evaluation of the migratory distance covered by MDA-MB-231 treated with UNC0642 (5 μM) or (D) transfected with either vector or shG9a. Results were evaluated through real-time imaging using the HoloMonitor M4, taking pictures every 10 minutes for 48 hours, in three independent experiments. An average of 20 cells per condition is shown for the representative circular displacement images. (E) Western blot analysis of G9a, H3K9me1 & H3K9me2 in MDA□MB-231 transfected with vector or shG9a & incubated in hypoxia for 24 hours. HIF1α was included as positive control for hypoxic conditions. H3 & Lamin A/C were used as loading control (F) Scratch wound assay for MDA-MB-231 breast cancer cells treated with 5 μM UNC0642, under both normoxic (21% O2) & hypoxic (1% O2) conditions. Results were evaluated by real-time imaging performed by the IncuCyte Zoom every 24 hours & wound closure was quantified using ImageJ. Scale bar represents 500 μm. (G) Scratch wound assay of MCF7 breast cancer cells following G9a KD & G9a reconstitution. Scale bar represents 500 μm. (H) Western blot analysis of MCF7 cells transfected with shG9a & reconstituted with WT G9a. Data are represented as mean ± SEM of three independent experiments (unpaired, non-parametric Student's t-test, **p<0.005, ****p<0.0001). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32292512), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

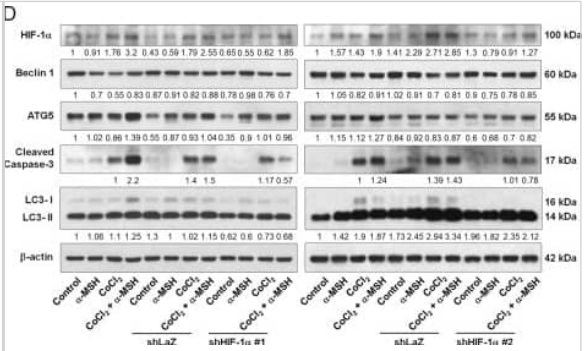


Chromatin Immunoprecipitation: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxia reduces inflammatory signaling pathways & NLRP3 expression & induces autophagy in IECs. a HT-29 cells were subjected to normoxia & hypoxia at the indicated times in the absence or presence of 10 µg/ml LPS. Autophagy was measured by variations in the ratio of LC3-II/LC3-I & the total amount of LC3 (LC3-I plus LC3-II) relative to β-actin. Results are representative of two independent experiments. b, c & d HT-29 cells were subjected to normoxia & hypoxia for the indicated periods in the absence or presence of 10 µg/ml LPS, followed by transcript analysis. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Results represent mean + s.e.m. of two independent experiments done in triplicate, *P < 0.05; **P < 0.01; ***P < 0.001; ns not significant. e HT-29 cells were subjected to normoxia or hypoxia in the presence & absence of 10 µg/ml LPS & 20 µM of MG132. Results are representative of two independent experiments. f & g Putative binding sites for HIF-1α & NF-κB were found in the p62 f & NLRP3 g promoters using Genomatix software tools. Numbers under the boxes indicate the distance from the transcription start site. HT-29 cells were subjected to normoxia (21% O2) or hypoxia (0.2% O2) for 6 h & 24 h. ChIP analysis was performed using antibodies against HIF-1α & NF-κB for immunoprecipitation. PCR was performed using the promoter-specific primers for the p62 f & NLRP3 g promoter binding sites of HIF-1α & NF-κB. Aliquots taken prior to immunoprecipitation were used as input control. PCR products were run on 2% agarose gel. The results are representative of three independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28740109), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

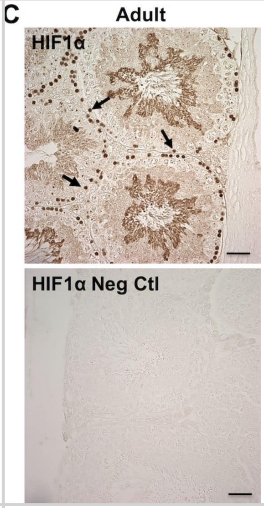


Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Deletion of Hif1α decreases primary tumor growth. (A) Wild-type (WT) or knockout (KO) cells (n = 50,000) were transplanted into FVB/Nj mice. All tumors were harvested at day 56 to evaluate tumor weight, volume & burden (percentage tumor weight/total body weight, BW) (n = 10 recipients/genotype, P < 0.05, unpaired Student's t-test). (B) The growth rate of WT & KO tumors following transplant of 50,000 cells. Best-fit curves were established based on a polynomial fit algorithm using GraphPad Prism 4.0 software. Data in (A) & (B) are representative of seven independent experiments (> 60 recipients/genotype). (C) When 500 WT & KO cells were implanted into the mammary fat pad, the median time until 50% of recipients developed tumors > 500 mm3 was 64 days for WT mice & 127 days for KO mice (n = 14 recipients/genotype, P < 0.001, logrank test). (D) Western blot for HIF-1α in three independent tumors (500 to 750 mm3) per genotype. WT & KO cells that were cultured for 6 hours under hypoxic conditions served as positive & negative controls, respectively. CRM, cross-reactive material. MTEC, mammary tumor epithelial cell. (E) Mean fold change ± SEM in expression of HIF-1 targets in KO tumors as determined by qRT-PCR (n = 5 tumors/genotype). Decreased gene expression in KO tumors is presented as a negative fold-change relative to WT tumors. (F) An increase in Ki67+ cells in KO tumors is balanced by an increase in caspase 3-positive cells (n = 5 tumors/genotype, *P < 0.05, Student's t-test). Representative immunostaining images are shown in Additional file 2 Figure S4. Image collected & cropped by CiteAb from the following publication (http://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr3087), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

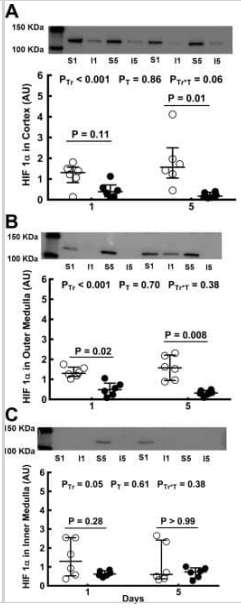
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Effect of HIF-1α silencing on α-MSH-induced apoptosis in B16-F10 melanoma cells during hypoxia.a Cells were transfected with shLacZ or shHIF-1α plasmids for 48 h before harvest. HIF-1α shRNA reduced the basal HIF-1α mRNA & protein expression levels. b, c Relative mRNA expression levels were analyzed by real-time PCR. Data are expressed as fold change compared with control (means ± SD of triplicate experiments). d Cell lysates were analyzed by immunoblot using the indicated antibodies. β-Actin was used as an internal control for loading & transfer. e The population of apoptotic cells was analyzed by flow cytometry & qualified as mean ± SD from triplicate experiments. *p < 0.05, **p < 0.01. Knockdown of HIF-1α diminished α-MSH-induced apoptosis in B16-F10 melanoma cells during hypoxia Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30062060), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



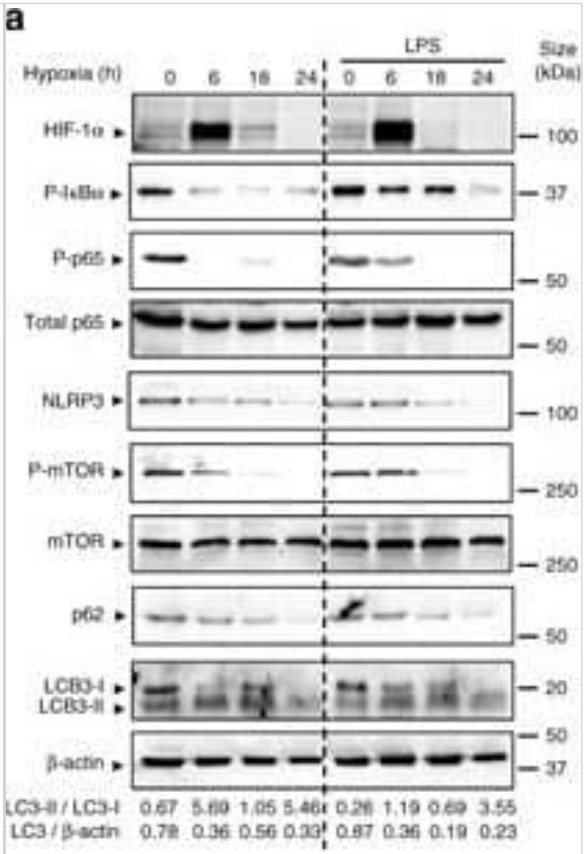
Immunohistochemistry: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hif1α expression in neonatal & adult testis.(A) Section of testis from 5-day old (P5) male new born pups showing Hif1α expression in MVH+ gonocytes within the seminiferous tubules (top) & negative control images without primary antibodies (bottom). Scale bar: 50µm. (B) Western blot analysis of Hif1α expression in P5 testes (left) compared to extract of the adult brain sub-ventricular zone (SVZ) (right). Loading control (β-Actin) is shown below. (C) Section of adult (3 month old) testis showing Hif1α expression in spermatogonia. Scale bar: 30µm. (D) Western blot analysis of whole adult testis. HEK293 cells treated with DFX were used as a positive control & intestinal tissue was used as a negative control. Loading control (β-Actin) is shown below. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



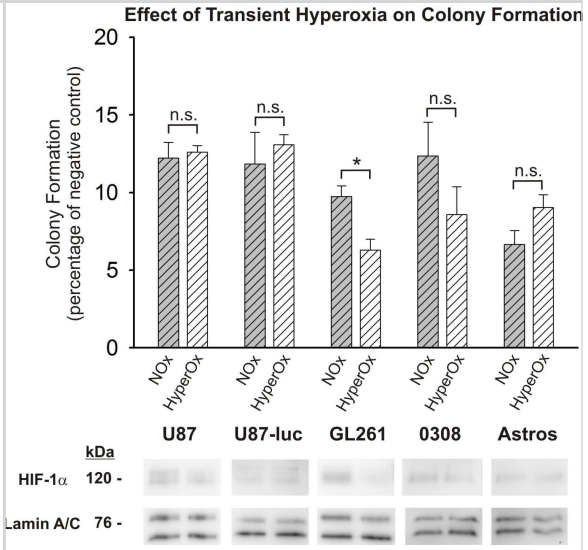
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Expression of hypoxia-inducible factor (HIF) proteins after bilateral renal ischemia or sham ischemia. Immunoblots for HIF-1α (A–C) & HIF-2α (D–F) of tissue extracts from the cortex & outer & inner medulla of the left kidneys of rats 24 h & 5 days following recovery from either sham ischemia (○) or bilateral renal ischemia (●); n = 6 per group. G: typical image of the gel following electrophoresis. H: typical image of the nitrocellulose membrane following transfer. Values are expressed as medians (25th percentile, 75th percentile). Paired comparisons were performed using the Mann-Whitney U-test. Because paired comparisons were made at two time points, P values were conservatively adjusted using the Dunn-Sidak method with k = 2. PTr, PT, & PTr*T are the outcomes of two-way analysis of variance on ranking with the factors treatment (Tr) & time (T). AU, arbitrary unit; I1, 24 h after ischemia; I5, 5 days after ischemia; S1, 24 h after sham ischemia; S5, 5 days after sham ischemia. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30110566), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



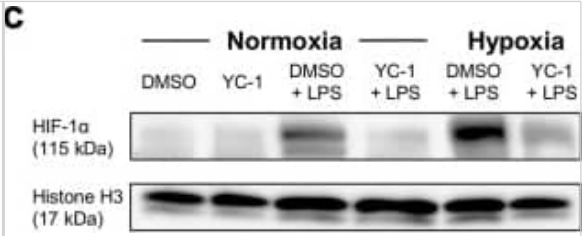
Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Hypoxia reduces inflammatory signaling pathways & NLRP3 expression & induces autophagy in IECs. a HT-29 cells were subjected to normoxia & hypoxia at the indicated times in the absence or presence of 10 µg/ml LPS. Autophagy was measured by variations in the ratio of LC3-II/LC3-I & the total amount of LC3 (LC3-I plus LC3-II) relative to β-actin. Results are representative of two independent experiments. b, c & d HT-29 cells were subjected to normoxia & hypoxia for the indicated periods in the absence or presence of 10 µg/ml LPS, followed by transcript analysis. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-test. Results represent mean + s.e.m. of two independent experiments done in triplicate, *P < 0.05; **P < 0.01; ***P < 0.001; ns not significant. e HT-29 cells were subjected to normoxia or hypoxia in the presence & absence of 10 µg/ml LPS & 20 µM of MG132. Results are representative of two independent experiments. f & g Putative binding sites for HIF-1α & NF-κB were found in the p62 f & NLRP3 g promoters using Genomatix software tools. Numbers under the boxes indicate the distance from the transcription start site. HT-29 cells were subjected to normoxia (21% O2) or hypoxia (0.2% O2) for 6 h & 24 h. ChIP analysis was performed using antibodies against HIF-1α & NF-κB for immunoprecipitation. PCR was performed using the promoter-specific primers for the p62 f & NLRP3 g promoter binding sites of HIF-1α & NF-κB. Aliquots taken prior to immunoprecipitation were used as input control. PCR products were run on 2% agarose gel. The results are representative of three independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28740109), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - Transient hyperoxia does not sensitize normal human astrocytes to radiation.Results are shown for the colony forming assays for all previously assayed cell lines & a normal human astrocyte cell line (Astro). Cells were continuously maintained under normoxic conditions (NOx) or exposed to 25 min of hyperoxia (50% O2) & then returned to normoxic conditions for 25 min (HyperOx) before being treated with a 5 Gy dose of radiation. To allow for ease of comparisons among cell types, raw values are expressed as a percentage of the corresponding cells type's negative (non-irradiated) control & the means & SEMs are plotted. Each result represents three independent samples, plated in triplicate (*p<0.05, Student's t-test). Also shown are Western blots of nuclear HIF-1α at the time of irradiation for each cell line. Corresponding Western blots of lamin A/C are shown as a loading control. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25350400), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: HIF-1 alpha Antibody - BSA Free [NB100-479] - YC-1 attenuates pro-inflammatory differentiation of macrophages. (a,b) qRT-PCR analyses, revealing mRNA expression of the pro-inflammatory M1-differentiation markers NOS2 (a) & IL-6 (b) in murine J774A.1 macrophages under normoxia (left bars) & hypoxia (right bars). LPS exposure was carried out to induce M1-polarization in presence of YC-1 or vehicle control (DMSO; **P ≤ 0.01, n = 9). (c,d) Western blots of nuclear extracts, revealing protein levels of HIF-1α (c) & HIF-2α (d) in J774.A1 macrophages treated with vehicle (DMSO) or YC-1 under normoxic (left) or hypoxic (right) culture conditions. Simultaneous LPS-treatment was performed to induce pro-inflammatory activation. Note LPS-induced stabilization of HIF-1α- (c), but not HIF-2α-levels (d), & reversal of LPS-induced HIF-1α-stabilization by YC-1 (c). Histone H3 was used as a loading control. (e,f) qRT-PCR analyses, revealing mRNA expression of the pro-inflammatory M1-differentiation markers NOS2 (e) & IL-6 (f) in BMDMs under normoxia (left bars) & hypoxia (right bars). LPS exposure was carried out to induce M1-polarization in presence of YC-1 or vehicle control (DMSO; ***P ≤ 0.001, **P ≤ 0.01, n = 6). Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-017-13638-z), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Shin J, Nunomiya A, Gonda K, Nagatomi R Specification of skeletal muscle fiber-type is determined by the calcineurin/NFATc1 signaling pathway during muscle regeneration Biochemical and Biophysical Research Communications 2023-03-01 [PMID: 37031590] (Western Blot, Mouse)

Bernice Woon Li Lee, You Heng Chuah, Jeehyun Yoon, Oleg V. Grinchuk, Yajing Liang, Jayshree L. Hirpara, Yating Shen, Loo Chien Wang, Yan Ting Lim, Tianyun Zhao, Radoslaw M. Sobota, Tseng Tsai Yeo, Andrea Li Ann Wong, Kejia Teo, Vincent Diong Weng Nga, Bryce Wei Quan Tan, Toshio Suda, Tan Boon Toh, Shazib Pervaiz, Zhewang Lin, Derrick Sek Tong Ong METTL8 links mt-tRNA m 3 C modification to the HIF1α/RTK/Akt axis to sustain GBM stemness and tumorigenicity Cell Death & Disease 2024-05-14 [PMID: 38744809]

Depanwita Saha, Debarpan Mitra, Neyaz Alam, Sagar Sen, Saunak Mitra Mustafi, Pradip K Majumder, Biswanath Majumder, Nabendu Murmu Lupeol and Paclitaxel cooperate in hindering hypoxia induced vasculogenic mimicry via suppression of HIF-1α-EphA2-Laminin-5γ2 network in human oral cancer. Journal of cell communication and signaling 2023-09-01 [PMID: 36063341]

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Xiaoyu Tang, Kaixuan Cui, Xi Lu, Peiqi Wu, Shanshan Yu, Boyu Yang, Yue Xu, Xiaoling Liang A Novel Hypoxia-inducible Factor 1α Inhibitor KC7F2 Attenuates Oxygen-induced Retinal Neovascularization Investigative Ophthalmology & Visual Science 2022-06-13 [PMID: 35695808]

Zheng X, Narayanan S, Xu C et al. Repression of hypoxia-inducible factor-1 contributes to increased mitochondrial reactive oxygen species production in diabetes eLife 2022-02-15 [PMID: 35164902]

Duc-Vinh Pham, Thi-Kem Nguyen, Bao-Loc Nguyen, Jong-Oh Kim, Jee-Heon Jeong, Inho Choi, Pil-Hoon Park Adiponectin restores the obesity-induced impaired immunomodulatory function of mesenchymal stromal cells via glycolytic reprogramming Acta Pharmaceutica Sinica. B 2023-10-28 [PMID: 38261813]

Romana Bohuslavova, Radka Cerychova, Frantisek Papousek, Veronika Olejnickova, Martin Bartos, Agnes Görlach, Frantisek Kolar, David Sedmera, Gregg L. Semenza, Gabriela Pavlinkova HIF-1α is required for development of the sympathetic nervous system Proceedings of the National Academy of Sciences of the United States of America 2019-07-02 [PMID: 31196952]

Ivan Menendez-Montes, Beatriz Escobar, Manuel J. Gomez, Teresa Albendea-Gomez, Beatriz Palacios, Elena Bonzon-Kulichenko, Jose Luis Izquierdo-Garcia, Ana Vanessa Alonso, Alessia Ferrarini, Luis Jesus Jimenez-Borreguero, Jesus Ruiz-Cabello, Jesus Vázquez, Silvia Martin-Puig Activation of amino acid metabolic program in cardiac HIF1-alpha-deficient mice iScience 2021-02-03 [PMID: 33665549]

Xun Wang, Yuemeng Jia, Jiawei Zhao, Nicholas P. Lesner, Cameron J. Menezes, Spencer D. Shelton, Siva Sai Krishna Venigalla, Jian Xu, Chunyu Cai, Prashant Mishra A mitofusin 2/HIF1α axis sets a maturation checkpoint in regenerating skeletal muscle The Journal of Clinical Investigation 2022-12-01 [PMID: 36125902]

Yong Hwan Kim, Na Yeon Park, Doo Sin Jo, Ji-Eun Bae, Joon Bum Kim, Kyuhee Park, Kwiwan Jeong, Pansoo Kim, Eunbyul Yeom, Dong-Hyung Cho Inhibition of VHL by VH298 Accelerates Pexophagy by Activation of HIF-1α in HeLa Cells. Molecules (Basel, Switzerland) 2024-01-24 [PMID: 38257395]

Ahmed A. Elbassiouny, Leslie T. Buck, Luis E. Abatti, Jennifer A. Mitchell, William G.R. Crampton, Nathan R. Lovejoy, Belinda S.W. Chang Evolution of a novel regulatory mechanism of hypoxia inducible factor in hypoxia-tolerant electric fishes The Journal of Biological Chemistry 2024-02-05 [PMID: 38325739]

More publications at http://www.novusbio.com/NB100-479

Procedures

Western Blot protocol for HIF-1 alpha Antibody (NB100-479)

Western Blot Protocol I

1. Perform SDS-PAGE on samples to be analyzed, loading 10ug of total protein per lane.
2. Transfer proteins to Nitrocellulose membrane according to the instructions provided by the manufacturer of the transfer apparatus.
3. Stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk in TBS for 1.5 hours.
6. Dilute the mouse anti-HIF-1 alpha primary antibody (NB 100-479) 1:500 in blocking buffer and incubate overnight at 4C.
7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1.5 hours at room temperature.
8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.
9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).
10. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Amersham ECL is the standard reagent used at Novus Biologicals).
Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Western Blot Protocol II (MEF Nuclear Extracts) Nuclear Extract Preparation for HIF 1 alpha

1. Wild type and HIF1a -/- MEF cells, (initially plated at 6X10 6 cells/plate), are grown to near confluence in two 15-cm plates in 20 ml of media appropriate to cell type.
2. For hypoxic induction, 20 ml of fresh medium is added to all plates. One plate is sealed in modulator incubator chamber, flushed with 1% 02 gas for 2-3 min. at 2 psi, and incubated at 37C for 6 hrs.
3. The cells are washed once with ice cold PBS (10 mls/plate) and scraped with a rubber policeman into 5 mls of PBS. Work as quickly as possible. Cells are collected by centrifugation at 4°C for 5 minutes at 1500 X g. Control cells were incubated at 37C at 20% 02 are harvested in the same way.
4. At this point, cells may be frozen at - 80C after removing PBS.
5. For nuclear extract preparation, the cells are resuspended in 4 PCV of Buffer A. Cells are kept on ice for 10 minutes.
6. Cell suspension is homogenized with 25 strokes in a glass douncer with type b pestle (loose) and transferred to 1.5 ml Eppendorf tube.
7. Nuclei are pelleted by centrifugation for 10 minutes at 12,000 X g and resuspended in 3.5 packed cell volumes of Buffer C, and rotated for 45 minutes in the cold room.
8. The samples are centrifuged for 10 minutes at 20,000 X g, (or full speed for 90 min. in a refrigerated tabletop microfuge) aliquotted, and stored at - 80C.
Buffer A: 10 mM Tris (pH 7.5) 1.5 mM MgCl2 10 mM KCl freshly supplemented with 1:500 (v/v) of 1M DTT, 0.2M PMSF, 1 mg/ml Leupeptin, 1 mg/ml Aprotinin, 1 mg/ml Pepstatin, and 0.5M Na3VO4. Buffer C: 0.42M KCl 20 mM Tris (pH 7.5) 20% glycerol 1.5 mM Mg Cl2 freshly supplemented with 1:500 (v/v) of 1M DTT, 0.2M PMSF, 1 mg/ml Leupeptin, 1 mg/ml Aprotinin, 1 mg/ml Pepstatin, and 0.5M Na3VO4. Buffer A and C may be supplemented with Complete Protease Inhibitor Tablets (Roche) in lieu of individual inhibitors. However, DTT and sodium vanadate must still be added separately

Western Blotting
1.Cast 7.5 % SDS-PAGE gel and separate 50 ug nuclear protein and transfer to nitrocellulose membrane.
2. Block the membrane with 5% skim milk for 1 hour at room temperature.
3. Incubate with HIF1a antibody (NB 100-479, Novus Biologicals) 1:2000 for 2 hours at RT.
4. Wash 3 times with 1X TBST (Tween 0.1%) for 15 min. each
5. Incubate with Rabbit secondary antibody (HRP) 1:10000 (sc-2004, Santacruz Biotech) for 1hour at RT.
6. Wash 3 times with 1X TBST (Tween 0.1%) for 15 min. each
7. Use Amersham ECL kit for detection.

I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:
A. Place slides in peroxidase quenching solution: 15-30 minutes.

To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol. Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96C.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution.
Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes.
Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes.
Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
-Prior to deparaffinization, heat slides overnight in a 60 degrees celcius oven.
-All steps in which Xylene is used should be performed in a fume hood.
-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.

-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining.
Counterstain for 1-1 ½ minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

HIF-1alpha blots with normox and hypoxed Hepa 1-6 cells
1. Urea buffer for Protocol I -
2. Homogenization Buffer A: 20 mM HEPES (pH 7.5), 1.5 mM MgCl , 0.2 mM EDTA, 100 mM NaCl
3. 4 M NaCl
4. Dilution buffer B: 20mM HEPES (pH 7.5), 1.5 mM MgCl , 0.2 mM EDTA, 0.45M NaCl, 40% vol/vol glycerol, 2mM DTT, 0.4mM PMSF and 1mM sodium vanadate
5. 1 M DTT
6. Protease inhibitor cocktail tablets (Complete Mini, EDTA free) from Roche
7. 250 mM sodium orthovanadate (45.98 mg/ml in water) - Sigma
8. 13 mM deferoxamine (8.54 mg/ml – freshly made) - Sigma (cat# D9533)
9. Nonfat dry milk
10. PBST
11. SDS-PAGE gel, running buffer, sample loading buffer, markers
12. Hybond-P membrane (Amersham)
13. Electrophoresis and transfer buffers (NuPAGE/Invitrogen
14. Primary antibodies HIF-1alpha (mouse and human Novus) and beta-actin (Sigma)
15. Secondary antibody HRP conjugated goat anti-rabbit IgG (Pierce)
16. Amersham ECL Plus reagent

Cell Culture:
1. Plate Hepa cells (4 X 100 mm plates) one day before the experiment.
2. Culture O/N at 37C in 20% O2+ 5% CO2 incubator.
3. On the morning of the experiment, aspirate media and add 10ml fresh media (DMEM-HG + 10% FCS) equilibrated O/N at 1% O2 to two plates. Add 100 ul of 13 mM deferoxamine freshly resuspended in sterile water (final conc = 130 uM)
4. Leave the other two plates at 20% O2+ 5% CO2 incubator with fresh media.
5. Make the urea and HEPES buffers 15 min before the 4 h are up.
6. After 4h, take the plates out and work quickly.
7. Aspirate media.
8. Wash plate with PBS and aspirate. Add the lysis buffers at per protocol.

Protein extraction - Protocol I (urea):
Buffer prep just prior to use:
1. To 10 ml urea buffer add 1 protease inhibitor tablet.
2. Remove 1.5 ml 8M urea, 10mM Tris pH 6.8, 1% SDS + protease cocktail into a new tube.
3. Add 7.5 ul of 1M solution - 5 mM DTT final

Processing of cells.

1. Process one plate at a time as quickly as possible.
2. Add 500 ul of the above buffer to hypoxed (1%) and normox (20%) plate.
3. Scrape cells, collect to end side of the plate by tilted and transfer to a 1.7 ml microfuge tube. Pass the lysate 6-7X through a 1 ml syringe with 27 gauge needle to shear genomic DNA.
4. Remove a 50 ul aliquot and dilute it 1:10 to measure protein by Bradford reagent (BioRad).
5. Freeze the extract on dry ice and keep at -80C till the following day

Protein extraction - Protocol II (HEPES buffer)

1. Add 1 protease inhibitor tablet to 10 ml of buffer A and 1 tablet to 10 ml Buffer B.
2. Add 20 ul of 1 M DTT (final conc 2 mM) to each tube.
3. Add 40 ul of 0.25M sodium vanadate (final 1 mM) to each tube.
4. Add 250 ul of buffer A (20 mM HEPES (pH 7.5), 1.5 mM MgCl2, 0.2 mM EDTA, 100 mM NaCl), 2 mM DTT, protease cocktail and 1 mM Na vanadate

5. Add 21.87 ul of 4M NaCl (final conc of 0.45M).
6. Spin at 10,000g for 30 min at 4C.
7. Mix supernatant with an equal volume (270 ul) of 20mM HEPES (pH 7.5), 1.5 mM MgCl2, 0.2 mM EDTA, 0.45M NaCl, 40% vol/vol glycerol, 2mM DTT, 0.4mM PMSF and 1mM sodium orthovanadate.
8. Remove a 50 ul aliquot and dilute it 1:10 to measure protein by Bradford reagent (BioRad).
9. Freeze 450 ul supernatant at -80C.
10. Run 50 ug of protein on a 7% Tris acetate gel (NuPAGE/Invitrogen).

Gel and Blot:

1. Run 50 ug of protein on a 7 % Tris acetate gel (NuPAGE/Invitrogen).
2. Transfer to Hybond-P membrane (Amersham).
3. Block with 5% non-fat milk in PBST for O/N at 4C.
4. Incubate with HIF1alpha antibody 1:1000 for 1 h at RT.
5. Wash 3X with 1X PBST (Tween 0.1%) for 15 min each.
6. Incubate with HRP coupled goat anti-rabbit secondary antibody (Pierce cat# 1858415) 1:10,000 for 1h at RT.
7. Wash 3X with 1X PBST (Tween 0.1%) for 15 min each.
8. Use Amersham ECL Plus kit for detection. (from Kong, et al. JBC. 282: 15498-15505, 2008; with modifications by Rashmi Chandra, Duke University)

Immunohistochemistry-Paraffin Protocol for HIF-1 alpha Antibody (NB100-479)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

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