Product Datasheet

HIF-2 alpha/EPAS1 Antibody (ep190b)
NB100-132

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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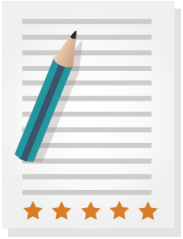
Updated 12/20/2023 v.20.1



NB100-132

HIF-2 alpha/EPAS1 Antibody (ep190b)

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	ep190b
Preservative	0.05% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS, 1% BSA
Target Molecular Weight	96.5 kDa

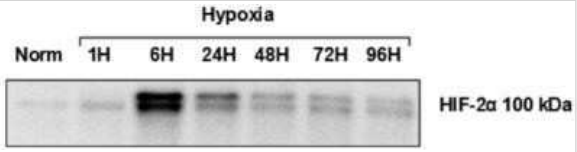
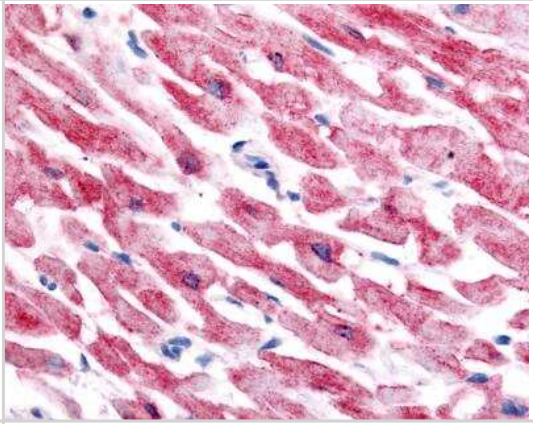
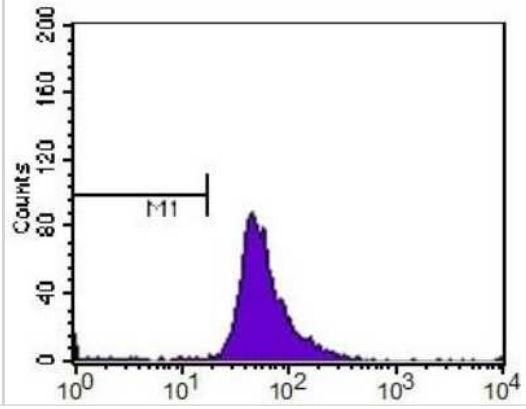
Product Description

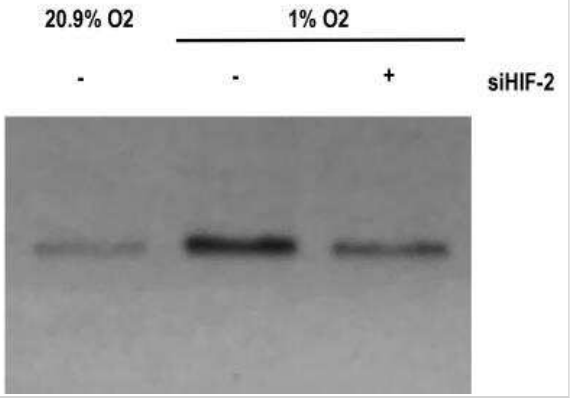
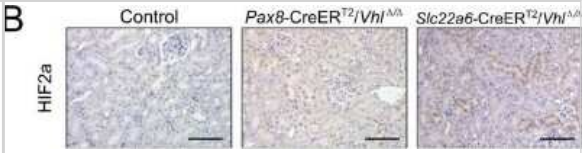
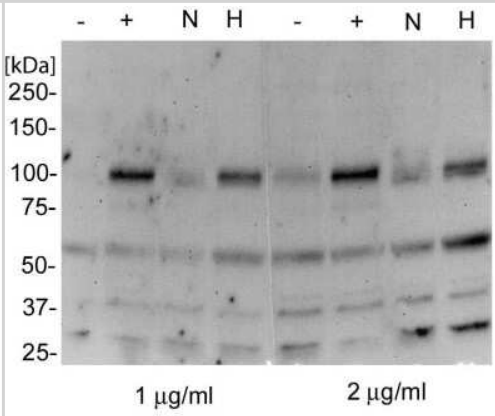
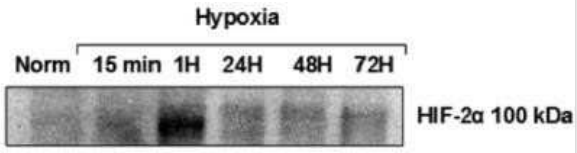
Host	Mouse
Gene ID	2034
Gene Symbol	EPAS1
Species	Human, Mouse, Rat, Bovine, Hamster
Reactivity Notes	Ability to use HIF-2 alpha/EPAS1 Antibody (ep190b) in mouse is mixed with some positive and some negative results. Use in Bovine reported in scientific literature (PMID:32054096).
Specificity/Sensitivity	This HIF-2 alpha/EPAS1 Antibody (ep190b) is specific for HIF-2 alpha/EPAS1, and does not cross-react with HIF-1 alpha.
Immunogen	The immunogen recognized by this HIF-2 alpha/EPAS1 Antibody (ep190b) maps to a region between amino acids 535-631. [UniProt# Q99814]

Product Application Details

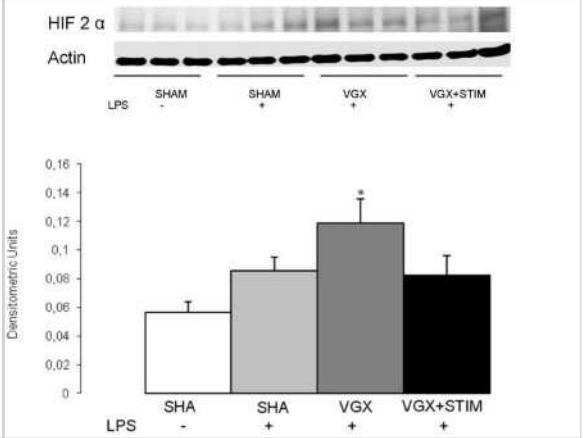
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vivo assay, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Gel Supershift Assay, Knockdown Validated
Recommended Dilutions	Western Blot 1 - 2 ug/mL, Simple Western 1:100, Flow Cytometry 1:400, ELISA 1:100-1:2000, Immunohistochemistry 1:150 - 1:300, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Immunohistochemistry-Paraffin 1:150 - 1:300, Immunohistochemistry-Frozen reported in scientific literature (PMID 24973414), Gel Super Shift Assays reported in scientific literature (PMID 17404621), In vivo assay reported in scientific literature (PMID 23857308), Gel Supershift Assay, Chromatin Immunoprecipitation (ChIP), Knockdown Validated reported in scientific literature (PMID 32054096)
Application Notes	In WB, it recognizes a band at approx. 118 kDa representing HIF-2 alpha. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

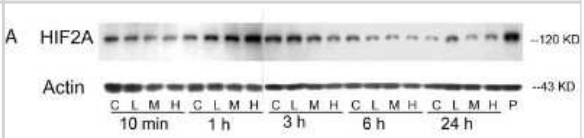
Simple Western: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Lane view shows a specific band for HIF-2 alpha in 0.5 mg/mL of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 alpha stabilization over time in 791T cells following exposure to hypoxia. Image using the HRP form of this antibody (NB100-132H). Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/23785417/) licensed under a CC-BY license.	
Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 in human cardiac myocytes using HIF-2 alpha/EPAS1 Antibody (ep190b).	
Flow Cytometry: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - HIF-2 alpha antibody was tested at 1:400 in HepG2 cells using an Alexa Fluor 488 secondary (shown in purple). M1 is defined by unstained cells.	

Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Mouse aortic endothelial cells treated (1%) or not treated (20.9%) in hypoxia for 3 hrs. Cells where also transfected with a specific siRNA against (siHIF-2) or a control siRNA (-). Western blot image submitted by a verified customer review.	
Immunohistochemistry-Paraffin: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Renal tubule specific models of Vhl deletion. Histological images of representative renal sections from 12 month old control, Pax8-CreERT2/Vhldelta/delta and Slc22a6-CreERT2/Vhldelta/delta mice (stains and antibodies as indicated, arrowheads indicate abnormal vascularization). Scale bars, 100 um. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0148055), licensed under a CC-BY license.	
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HepG2 without Cobalt (II) Chloride (1), HepG2 with Cobalt (II) Chloride (2), HepG2 normoxic (3), HepG2 hypoxic (4), HepG2 without Cobalt (II) Chloride (5), HepG2 with Cobalt (II) Chloride (6), HepG2 normoxic (7), and HepG2 hypoxic (8) using this antibody (NB100-132) at 1 - 2 ug/mL.	
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - [HRP] [NB100-132H] - Analysis of HIF-2 alpha stabilization over time in HOS cells following exposure to hypoxia. Image using the HRP form of this antibody (NB100-132H). Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/23785417/) licensed under a CC-BY license.	

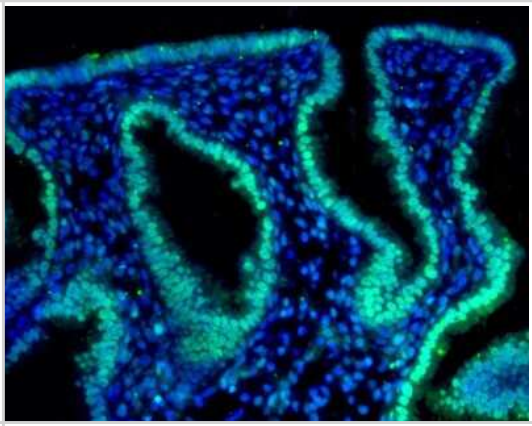
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Western blot analysis and quantification of HIF-2 alpha expression in the cortex 4.5 hours after lipopolysaccharide (LPS) administration for all LPS groups and the control group (SHAM, white bar). With the exception of the vagotomy group (VGX LPS, gray bar), no significant differences to the SHAM group were found in the LPS-treated and sham-operated (SHAM+LPS, light gray bar) or vagus nerve-stimulated groups (VGX LPS+STIM, black bar). The significant increase in the VGX LPS (gray bar) group is an indicator of a hypoxic condition; * P<0.05 compared to SHAM; n=6 rats each. Data are given as the mean+/-SEM. Image collected and cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-9-183), licensed under a CC-BY license.



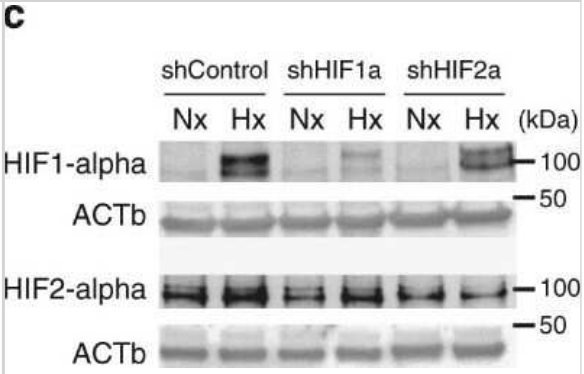
Western Blot: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - 4-OOHCPA exposure induced HIF-2 alpha/EPAS1. A: Western blot analysis of HIF-2 alpha/EPAS1 protein (118KD) and actin (43KD) in limbs at 10 min, 1, 3, 6, and 24 h after treatment with 4-OOHCPA at 0.3 ug/mL (L) 1.0 ug/mL (M) or 3.0 ug/mL (H). P represents the positive control. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0051937), licensed under a CC-BY license.



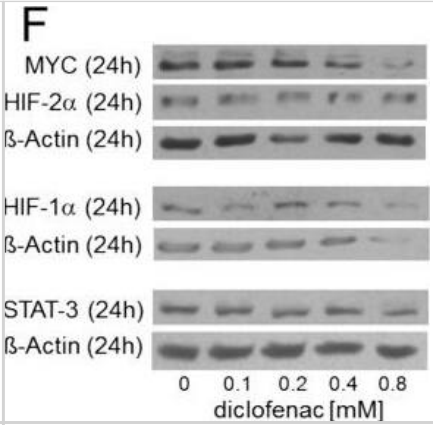
Immunohistochemistry: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Analysis of HIF-2 alpha in human endometrium using. Donkey anti-mouse Alexa Fluor 488 secondary antibody was used. IHC image submitted by a verified customer review.



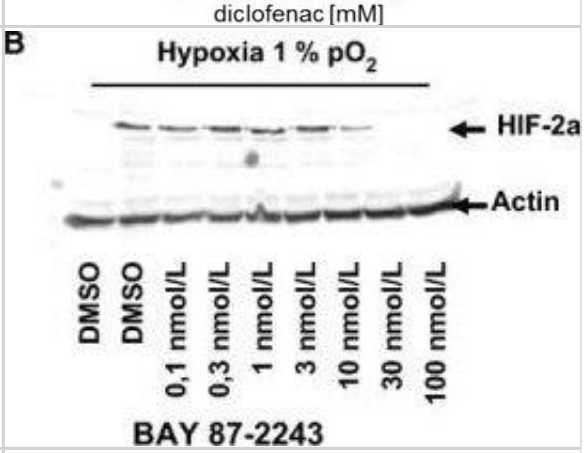
Knockdown Validated: HIF-2 alpha/EPAS1 Antibody (ep190b) [NB100-132] - Functional role of HIF2A in the transcriptional regulation of amphiregulin (AREG) in human cardiac myocytes. Immunoblot for HIF1A or HIF2A from shRNA-transfected normoxic or hypoxic HCM. Beta-Actin (ACTb) served as a loading control. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/29483579/) licensed under a CC-BY license.



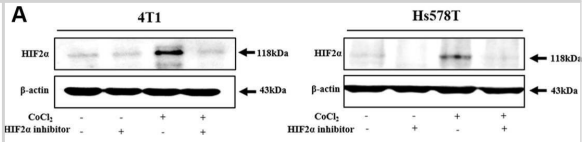
In vitro effects of diclofenac on proliferation and MYC expression in the human melanoma cell line MelIm. (E-G) MYC, STAT3, HIF1a and HIF2a protein expression were determined in cell lysates of MelIm incubated for 2 or 24 h with or without diclofenac (E,F) or ASA (G). The effect of diclofenac on MYC promoter activity was determined by transient transfection of a 2632-bp MYC promoter fragment (H). MelIm were transfected in 6-well-plates and diclofenac was added after 5 h. Luciferase activity was determined 24 h after transfection. Results represent the mean +/- standard deviation of 3 independent experiments. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0066987), licensed under a CC-BY licence.



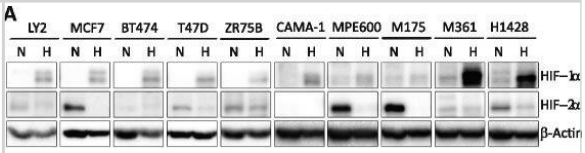
BAY 87-2243 inhibits hypoxia-inducible factor (HIF-1alpha) and HIF-2alpha protein accumulation in hypoxic H460 cells H460 under hypoxia but has no effect on HIF-1alpha protein levels induced by hypoxia mimetics and has no effect on prolyl hydroxylase 2 (PHD2) activity. (A, B) H460 cells were cultured for 16 h under normoxia or hypoxia (1% pO2) in the absence or presence of various concentrations of BAY 87-2243. HIF-1alpha (A) and HIF-2alpha (B) protein levels were assessed by Western Blot in whole cell extracts. beta-actin was used as a loading control. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24403227), licensed under a CC-BY licence.



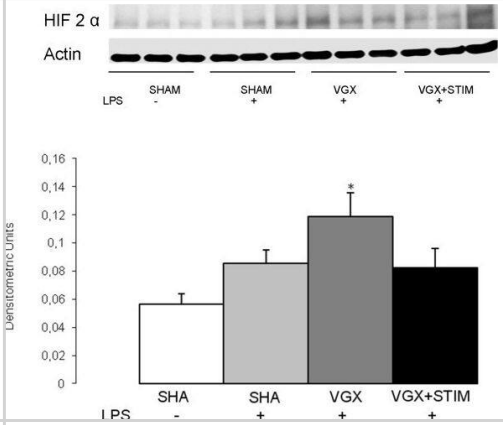
HIF2alpha inhibitor 76 suppressed CoCl2-induced immature phenotypic characteristics of BCSCs. (A) The inhibitory effect of small molecule HIF2alpha inhibitor 76 for 24h (10 uM for 4T1 cells; 25 uM for Hs578T cells) on CoCl2-induced expression of HIF2alpha was assessed in both 4T1 and Hs578T cells by western blot analysis. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.9846), licensed under a CC-BY licence.



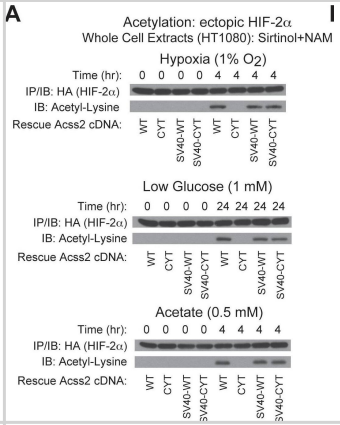
Hypoxic repression of ER-alpha is dependent on HIF-1alpha. a Representative western blots of HIF-1alpha, HIF-2alpha and beta-actin protein in ten ER-positive cell lines grown at normoxia or hypoxia (1% O2, 24 h). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28320353), licensed under a CC-BY licence.



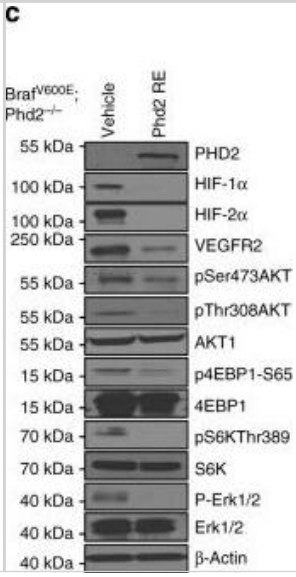
Western blot analysis and quantification of HIF-2alpha expression in the cortex 4.5 hours after lipopolysaccharide (LPS) administration for all LPS groups and the control group (SHAM, white bar). With the exception of the vagotomy group (VGX LPS, gray bar), no significant differences to the SHAM group were found in the LPS-treated and sham-operated (SHAM + LPS, light gray bar) or vagus nerve-stimulated groups (VGX LPS + STIM, black bar). The significant increase in the VGX LPS (gray bar) group is an indicator of a hypoxic condition; * P < 0.05 compared to SHAM; n = 6 rats each. Data are given as the mean +/- SEM. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/22830560), licensed under a CC-BY licence.



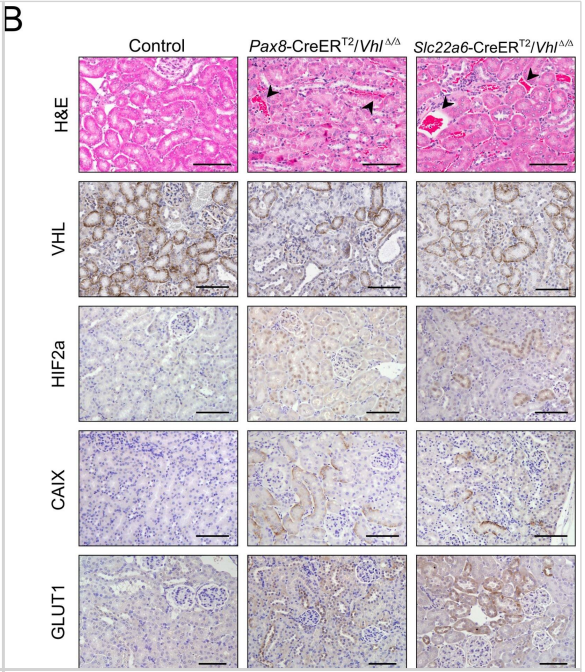
Cytosol-restricted Acss2 is enzymatically active.(A) Acetylation of ectopic HA-tagged HIF-2alpha detected by immunoblotting (IB) with anti-HA or anti- acetylated lysine antibodies following immunoprecipitation (IP) with anti-HA antibody in stably transformed HT1080 cells with knockdown of endogenous Acss2 and rescue with ectopic wild-type (WT) or cytosol-restricted mutant (CYT) Acss2 without or with an SV40 nuclear localization signal fused to the amino terminus. Studies were performed under hypoxia, low glucose, or acetate exposure for the indicated periods. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0190241), licensed under a CC-BY licence.



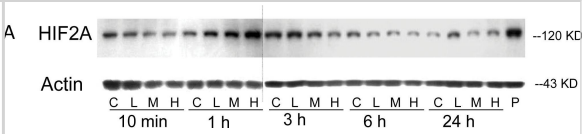
Localisation of CDS1 immunoreactivity and CDS activity to mitochondria. [A, B] Differentiated H9c2 cells fractionated and analysed for [A] CDS1 immunoreactivity by Western blot; [B] CDS activity. [C-E] Rat heart fractionated and analysed for [C] CDS1 immunoreactivity and markers, [D] CDS1 immunoreactivity (entire Western blot shown) and [E] CDS activity. [F–H] Rat heart fractionated in the presence of the protease, subtilisin and analysed for [F] CDS1 immunoreactivity by western blot, [G] CDS activity and [H] PI synthase (PIS) activity. CDS and PIS activity was monitored in triplicate and error bars denote ± S.E.M. COXIV, GRP75 and cyto c are markers for mitochondria, PITPNC1 and PITPα are cytosolic markers and calnexin is a marker for the ER. WCL, whole cell lysate; Micro, microsomes; C.Mito, crude mitochondria; Cyto, cytosol; P.Mito, pure mitochondria, MAMs, mitochondrial associated membranes; PIS, PI synthase; CDS, CDP-diacylglycerol synthase. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/29253589), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



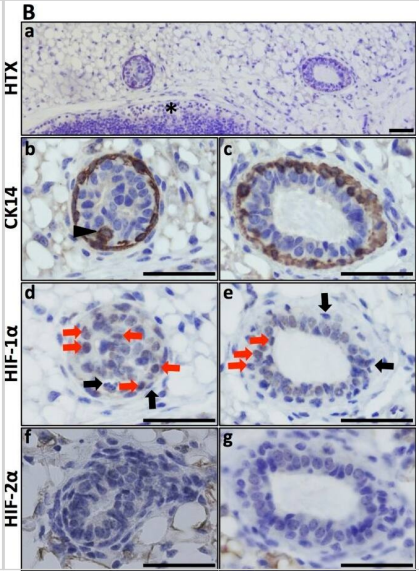
Effect of overexpression or knockdown of PAL31 in C6 on H2O2-induced toxicity. (A) Representative micrographs showing C6 overexpressing GFP or GFP-tagged PAL31 after being treated with H2O2 (0 ~ 1 mM) for 4 hours. (B) MTT assay in GFP- or GFP-PAL31-overexpressed C6 after H2O2 treatment showing significant difference (cytoprotective effect of PAL31) at 0.5 mM and 1 mM H2O2 treatment between C6/GFP and C6/PAL31 groups. The data in each dosage were analyzed by two-way ANOVA and Bonferroni post hoc test. *P < 0.05, GFP (+H2O2) compared to PAL31 (+H2O2), n = 4, at 0.5 mM and 1 mM. (C) Western blot analysis of pal31 siRNA-treated C6 showing knockdown of PAL31 expression by PAL31 silencer using 41.5 (lane1), 83 (lane2), and 166 (lane 3) picomole of pal31 siRNA or 332 picomole negative control. Actin works as a loading control. (D) MTT assay in Negative- or PAL31 silencer transfected C6 after H2O2 treatment showing significant difference at 1 mM H2O2 treatment between C6/Negative and C6/PAL31 siRNA groups. *P < 0.05, Negative (+H2O2) compared to PAL31 siRNA (+H2O2), n = 4. Magnification 100X (A). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25034417), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



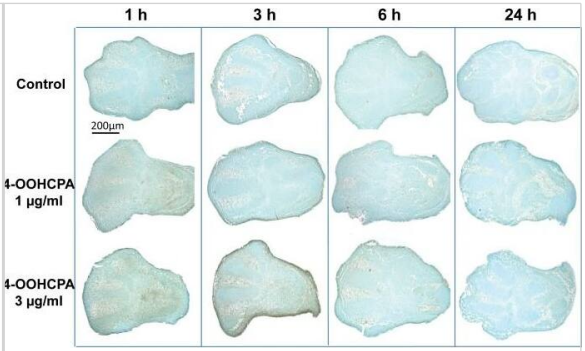
Expression of PRDX2 or PRDX4 does not affect HIF-1α or HIF-2α protein levelsA. and B. HeLa cells were transfected with EV or vector encoding PRDX2-V5 (A, P2) or PRDX4-V5 (B, P4), and exposed to 20% or 1% O2 for 24 h. WCL was subject to immunoblot assays with antibody against HIF-1α, HIF-2α, V5, or acin. C. HeLa-shSC (sc) and HeLa-shPRDX(2+4) (2+4) cells were exposed to 20% or 1% O2 for 24 h in the presence of doxycycline. WCL was subject to immunoblot assays with antibodies against HIF-1α, HIF-2α, PRDX2, PRDX4, and actin. Image collected and cropped by CiteAb from the following open publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.7142), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



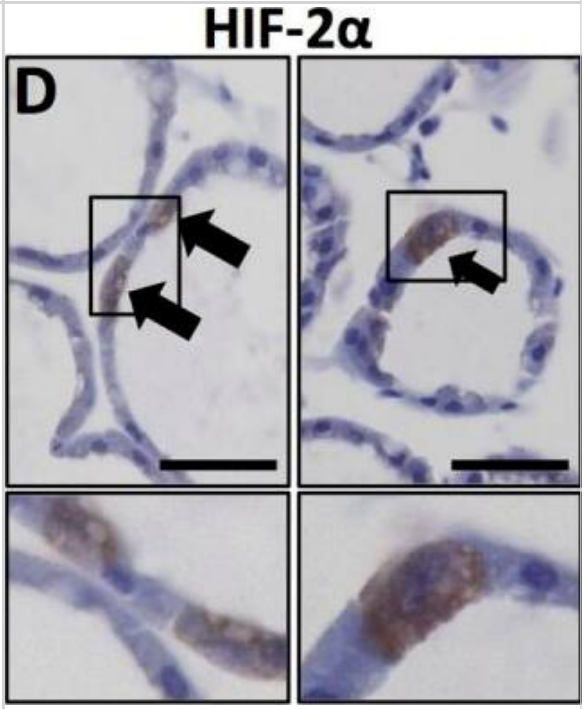
SAN1 functions independently of the FA pathway and does not affect FA pathway activation. a, b CSAs of HeLa WT and SAN1−/− cells treated with scrambled ctrl siRNA or FANCD2 siRNA, in response to Cisplatin and MMC (N = 3). Statistical significance determined by two-way ANOVA. Error bars denote s.e.m. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. c Immunoblot showing siRNA knockdown of FANCD2 in HeLa WT and SAN1−/− cells. d IF staining of FANCD2 foci in HeLa WT cells and SAN1−/− cells treated with 0.045 μM MMC. e Immunoblot of FANCD2 showing mono-ubiquitylation in HeLa WT and SAN1−/− cells treated with vehicle or 0.045 μM MMC. f, g CSAs of HeLa WT and SAN1−/− cells treated with ctrl or SNM1A siRNA and exposed to Cisplatin or MMC. Statistical significance was determined by two-way ANOVA test. h Immunoblot of SNM1A in HeLa WT and SAN1−/− cells treated with ctrl or SNM1A siRNA Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/29968717), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



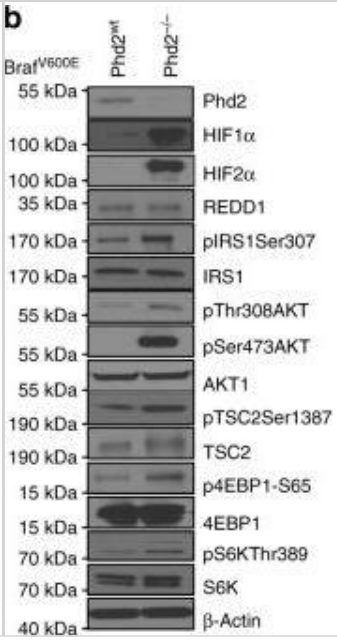
GAS6 triggers p-Axl, p-DAPK and CIP2A to form a protein complex in RSC96 cells(A-B) Co-immunoprecipitation between p-Axl, p-DAPK and CIP2A with GAS6 stimulation for 30 min in RSC96 cells. Total lysate indicates 1/10 input in each experiment. The relative quantification of protein expression was normalized with respect to β-actin expression. (C) Immunofluorescence image demonstrating co-localized CIP2A and p-Axl and co-localized p-Axl and p-DAPK (D) after GAS6 stimulation for 30 min in RSC96 cells. Image collected and cropped by CiteAb from the following open publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.23978), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



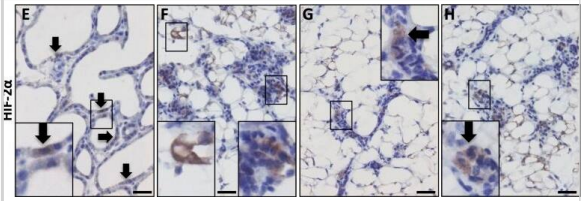
FKBP51 increases expression of melanoma CSC markers. (a) FKBP51 silencing decreases ABCB5 levels. (Left) Normalized expression rates (arbitrary units (AU)) (mean±S.D.) of FKBP51 (black) and ABCB5 mRNA (grey) (n=3). FKBP51-treated sample expression=1. (Right) Western blot assay of ABCB5 and FKBP51 levels. (b) FKBP51 overexpression enhances ABCB5 levels. (Left) Normalized expression of FKBP51 mRNA (black) and ABCB5 mRNA (grey) measured in WT, EV-, or FKBP51-stably transfected melanoma cells. WT sample expression=1; n=3. (Left) Western blot assay of ABCB5 and FKBP51 levels in the same cells. Anti-Flag-labelled exogenous FKBP51. (c) ABCG2+ melanoma cells increase in FKBP51-overexpressing cells. (Upper) Normalized expression of FKBP51 mRNA (black) and ABCG2 mRNA (grey) measured in WT, EV-, or FKBP51-stably transfected melanoma cells. WT sample expression=1; n=5. (Lower) Flow cytometric histograms of ABCG2 expression (green population); mean±S.D. of counts are shown. (d) Enhanced FKBP51 mRNA levels in sorted ABCG2+ melanoma cells (SAN, upper; A375, lower). Whole cell expression=1; (n=3). (e) Enhanced EMT gene transcripts in sorted ABCG2+ melanoma cells. ABCG2− sample expression=1; n=3. (f) Expression of ABCG2 transcript in 9 deparaffinized tumours, 4 primary melanoma (M) samples, and 5 metastases (MM). A naevus sample was arbitrarily chosen with expression=1 Image collected and cropped by CiteAb from the following open publication (https://www.nature.com/articles/cddis2013109), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



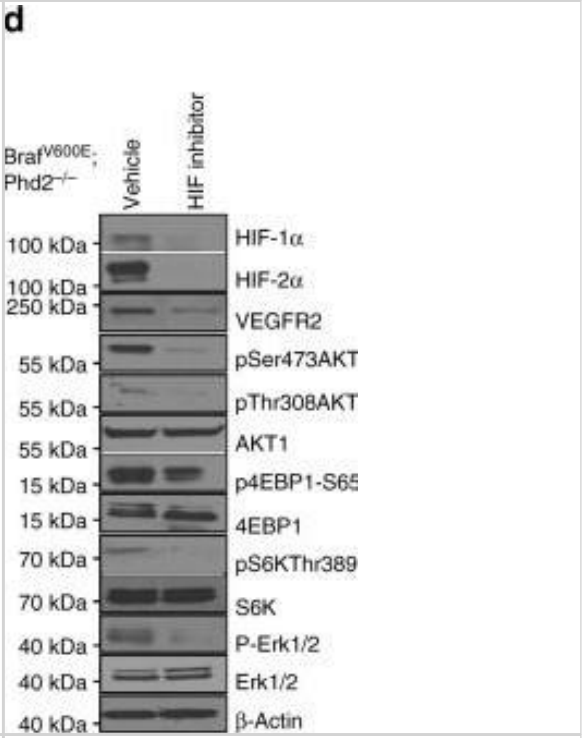
Spy1 stable protein accelerates tumorigenesis in vivo.(A) Percentage of mice presenting with palpable tumors from 0-19 days post-transplant. Each data point represents 4 mice per indicated construct. The entire experiment was repeated three times using three independently derived overexpressing cell lines for each construct. Mann-Whitney Test was performed ($p < 0.05$). (A; lower blot) Western blots were conducted to measure the stability of Myc-Spy1-WT (left) and Myc-Spy1-TST (right) from representative time points. Empty vector control (Cntl) cell expression levels of Spy1 are seen in lane 1 of each blot. (B) Total tumor volume was calculated for both Spy1-HC11 (Spy1-WT) and Spy1-TST-HC11 (Spy1-TST) transplanted glands. Results were taken from 45 transplants using cells from 3 separate transfections. Error bars reflect SE between transplants from different transfections. Left hand panel reflects overall volume, right hand panel reflects volumes of tissues taken over a month post-transplant. Image collected and cropped by CiteAb from the following open publication (https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-12-45), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



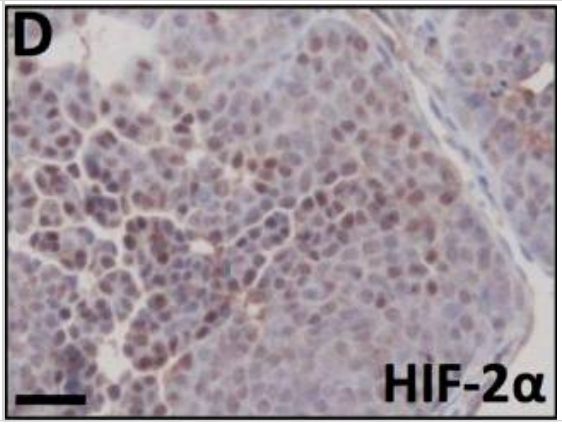
Livin staining in normal adrenal glandLivin staining in normal adrenal gland (A). It is possible to recognize the three different zone of the adrenal gland: the zona glomerulosa (ZG), the zona fasciculata (ZF) and the zona reticularis (ZR). The Figures (B) and (C) are 20× enlarged and detailed images of the 10× ZG, ZF and ZR, respectively. Scale bar: 100 µm. Image collected and cropped by CiteAb from the following open publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.14067), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Curcumin Analogs Induce Apoptosis in Cancerous Cells by Several Pathways. (a) E6-1 (Jurkat), dominant negative FADD (dnFADD) Jurkat, and overexpressing BCL-2 Jurkat were treated for 48 hours then stained for Annexin V and PI (b) E6-1 cells were plated and treated with or without the broad spectrum caspase inhibitor ZVAD(oMe)-FMK for 48 hours. Cells were stained for Annexin V and PI. Results were obtained using image-based cytometry with the Y-axis representative of percent of cells positive for Annexin V (green), PI (red), Annexin V and PI (yellow), or negative for both Annexin V and PI (blue). Values are expressed as a mean ± SD from three independent experiments. (c) E6-1 cells were treated for 24 hours with or without the broad spectrum caspase inhibitor ZVAD(oMe)-FMK and the studied compounds, lysed and subjected to Western blot analysis. (d) NHF and NCM460 cells were treated for 48 hours and 72 hours respectively, lysed and subjected to Western blot analysis. Bands were visualized with a chemiluminescence reagent. Images are representative of three independent experiments. Statistical calculations were performed using Two-Way ANOVA multiple comparison. *p < 0.05 vs % viable of Control (DMSO); #p < 0.05 vs % viable cells for groups without Z-VAD(oMe)FMK. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/28439094), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Effect of TSA treatment on the subcellular localization of Crm1.Control ES or Nup98-HoxA9 expressing ES cells were cultured in the presence or absence of 50 nM TSA for 24 hr. Then, the cells were fixed and stained with antibodies against FLAG (M2) and Crm1. Merged images of FLAG (green) and Crm1 (red) are shown. Nuclei were stained with DAPI. Bar, 10 µm. DAPI, 4',6-diamidino-2-phenylindole; ES, embryonic stem; TSA, trichostatin A.DOI:https://dx.doi.org/10.7554/eLife.09540.020 Image collected and cropped by CiteAb from the following open publication (https://elifesciences.org/articles/09540), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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Zhou X, Zheng Y, Sun W et al. D-mannose alleviates osteoarthritis progression by inhibiting chondrocyte ferroptosis in a HIF-2?-dependent manner Cell Proliferation 2021-11-01 [PMID: 34561933] (ICC/IF, WB, IHC)

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More publications at http://www.novusbio.com/NB100-132

Procedures

Immunohistochemistry Protocol for HIF-2 alpha Antibody (NB100-132)

Monoclonal Anti-HIF-2 alpha Western Blot Procedure
1. Resolve nuclear cell extracts (50-100 ug/lane) on a 6% SDS-polyacrylamide gel, under reducing conditions.
2. Transfer to a nitrocellulose membrane, overnight, or to a *PVDF membrane [*in 20 mM Tris/100 mM glycine/10% (v/v) methanol/0.05% SDS].
3. Block the membrane in TBS containing 5% non-fat dry milk and 0.1% Tween-20.
4. Rinse the membrane in TBST, twice.
5. Incubate the membrane in anti-HIF-2 alpha (NB 100-132), diluted 1:500 in TBS+1% BSA, overnight at 4C.
6. Wash membrane with TBST for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
7. Incubate the membrane with diluted HRP conjugated goat anti-mouse antibody.
8. Wash membrane with TBST for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
9. Use Amersham ECL Kit, as directed, to detect image.

Immunohistochemistry Procedure for Paraffin Sections
1. Prior to performing the IPOX experiment, dewax the paraffin sections by baking them at 60C for 30 minutes and then putting them through citroclear.
2. Hydrate the sections through the following series:
A. 3 X 5 minutes xylenes
B. 3 X 5 minutes 100% Etoh
C. 2 minutes 95% Etoh
D. 2 minutes 70% Etoh
E. 1 minute 50% Etoh
F. 1 minute ddH2O
G. 1 minute TBS
3. Block endogenous peroxidase with 0.5% hydrogen peroxide in water, for 30 minutes.
4. Antigen unmasking is performed by incubating at 60C for 16 hours, in 50mmol/L Tris and 0.2 mmol/L EDTA (pH 9.0), using a covered water bath.
5. Rinse slides with PBS and then incubate with PBS containing 0.2% Triton X-100 for 10 minutes.
6. Rinse slides with PBS.
7. Incubate sections with 1:1,000-1:3,000 dilution of anti-HIF-2 alpha (NB 100-132) for 90 minutes at RT.
8. Incubate sections in secondary HRP-conjugated goat anti-mouse serum for 30 minutes at RT.
9. Incubate sections in tertiary HRP-conjugated rabbit anti-goat serum for 30 minutes at RT.
10. Develop the peroxidase reaction using diaminobenzidine.
11. Wash slide and mount in aqueous mountant.

Substitution of the primary antibody with PBS can be used as a negative control.
1. Sub-confluent cells are grown on chamber slides and incubated for 16 hours either in air or under 0.1% hypoxia.
2. Wash cells in ice-cold PBS.
3. Fix cells in formaldehyde (3.7% in PBS) for 10 minutes at room temperature (RT).
4. Wash cells twice, in PBS, and permeabilize by incubating in 0.2% Triton X-100 in PBS for 10 minutes at RT.
5. Incubate the slides with 1:1,000-1:3,000 dilution of anti-HIF-2 alpha (NB 100-132) for 1 hour at RT.
6. Wash in PBS for 5 minutes.
7. Incubate with HRP-conjugated goat anti-mouse for 30 minutes at RT.
8. Detect binding using 3Prime-diaminobenzidine.
9. Counterstain with hematoxylin.
IHC-FFPE sections
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 90-96 degrees C.
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 C 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).

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