Product Datasheet

53BP1 Antibody - BSA Free
NB100-305

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Reviews: 6 Publications: 144

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
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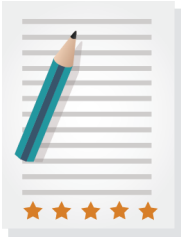
Updated 12/20/2023 v.20.1



NB100-305

53BP1 Antibody - BSA Free

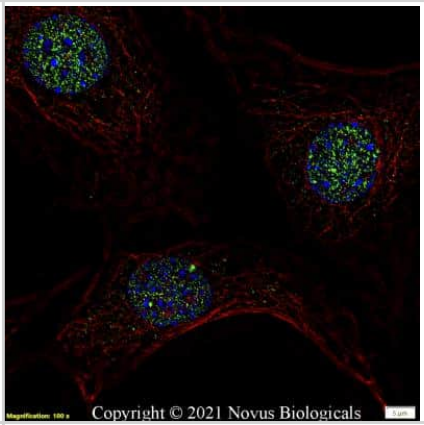
Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	7158
Gene Symbol	TP53BP1
Species	Human, Mouse, Rat, Bat, Bovine, Canine, Naked mole-rat
Reactivity Notes	Use in Naked mole-rat reported in scientific literature (PMID: 33608273). Human has been tested in both WB and ICC/IF, mouse has only been tested in ICC/IF. Feedback on bovine has been negative. Rat reactivity reported in scientific literature (PMID: 24244353). Bat, canine, and bovine reactivity reported in scientific literature (PMID: 27573809). Predicted cross-reactivity based on sequence identity: Chimpazee (100%), Equine (100%), Feline (100%), Gibbon (100%), Gorilla (100%), Hamster (100%), Marmoset (100%), Orangutan (100%), Panda (100%), Porcine (100%), Rabbit (100%), Sheep (100%).
Marker	DNA Double Strand Break Marker
Immunogen	The epitope recognized by 53BP1 Antibody maps to a region between residues 1925 and the C-terminus (residue 1972) of human 53BP1 (NP_005648.1).

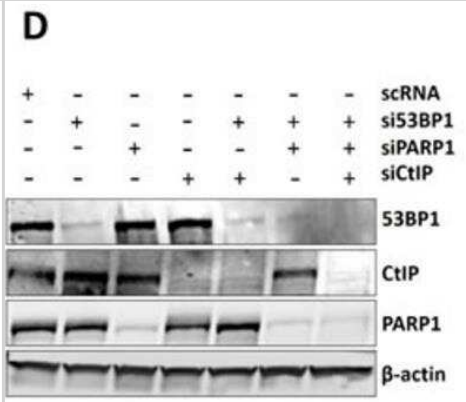
Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:2000-1:10000, Chromatin Immunoprecipitation reported in scientific literature, Flow Cytometry 2-5 ug/million cells, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:50-1:1000, Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen reported in scientific literature, Chromatin Immunoprecipitation (ChIP), Knockdown Validated

Images

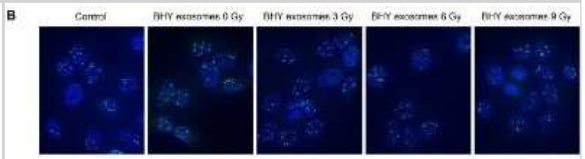
Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NB100-305 at 2 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



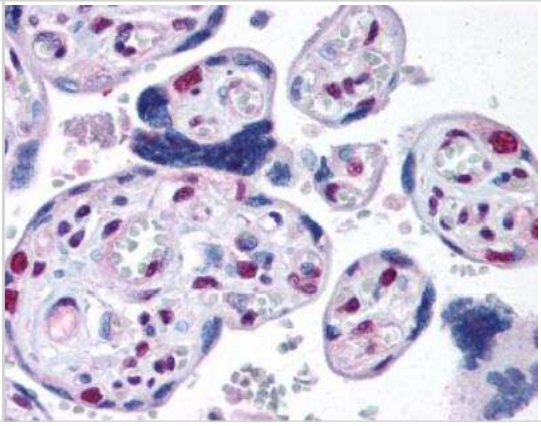
Knockdown Validated: 53BP1 Antibody [NB100-305] - Western blot showing an efficient siRNA-mediated knockdown of the indicated individual or combined proteins. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.11023) licensed under a CC-BY license.

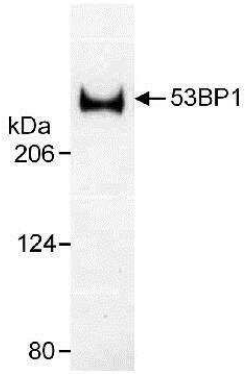
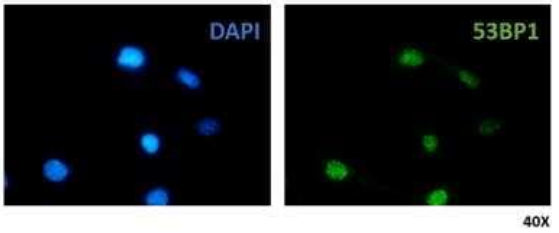
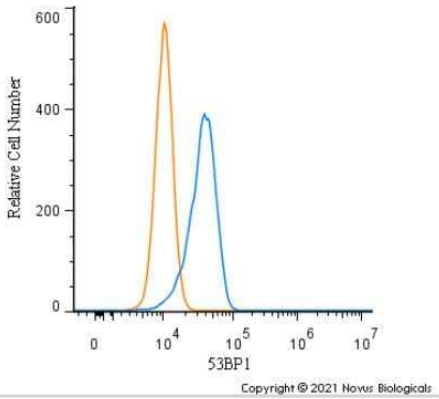
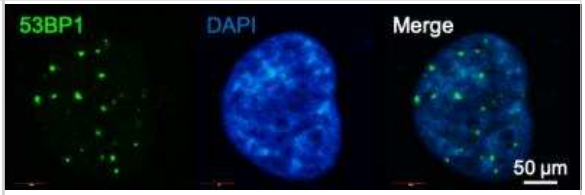


Immunocytochemistry/Immunofluorescence: 53BP1 Antibody - BSA Free [NB100-305] - 53BP1 Antibody [NB100-305] - Exosomes modulate the repair of DNA DSBs in irradiated recipient cells. Representative images of 53BP1 foci in BHY cells 6 hours after 2 Gy and transfer of BHY exosomes isolated 24 hours after irradiation with 0, 3, 6 or 9 Gy (53BP1 foci green, nuclei blue). Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0152213), licensed under a CC-BY license.



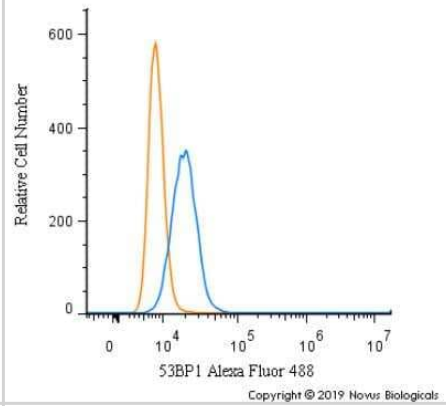
Immunohistochemistry: 53BP1 Antibody [NB100-305] - Immunohistochemical staining of placental villi with 53BP1 Antibody (Catalog #NB100-305) at 40X magnification.



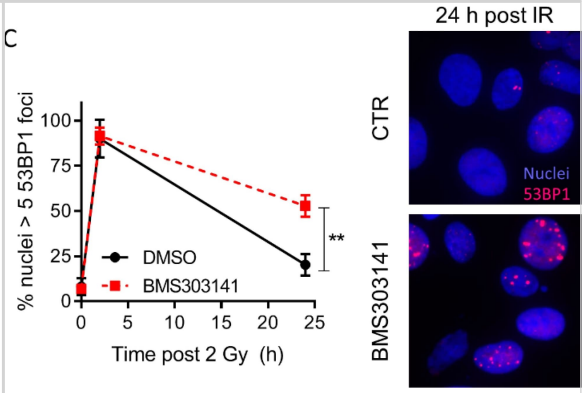
Western Blot: 53BP1 Antibody [NB100-305] - Western blot of whole cell lysate (20 ug/lane) from U2Os cells resolved on a 3 to 8 percent trisacetate gel. A band appears at approximately the theoretical molecular weight of 214 kDa.	
Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - HCC1937 cells (human) were treated with PARPi for 96 h. Nuclei are stained in blue (DAPI) and 53BP1 antibody (foci in green). ICC/IF image submitted by a verified customer review.	
Flow Cytometry: 53BP1 Antibody [NB100-305] - An intracellular stain was performed on Ntera2 cells with 53BP1 Antibody NB100-305 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - Paraformaldehyde (4%) fixed Saos-2 cells (human osteosarcoma cell line) were stained with anti-53BP1 antibody followed by Alexa Fluor 488 secondary antibody. ICC/IF image submitted by a verified customer review.	

Immunocytochemistry/Immunofluorescence: 53BP1 Antibody [NB100-305] - Ntera2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NB100-305 at 2 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

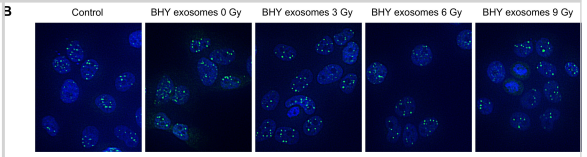
Flow Cytometry: 53BP1 Antibody [NB100-305] - An intracellular stain was performed on HeLa cells with 53BP1 Antibody (Catalog #NB100-305AF488) (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



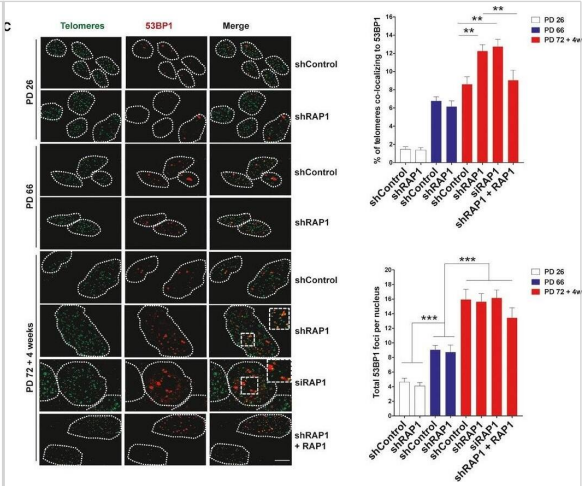
Inhibition of ACLY exacerbates radiation induced DNA damage in HNSCC cells and impairs homologous recombination. (C,D) Quantification of cells positive for 53BP1 foci (>5 53BP1 foci/nucleus in a single plane of view) in UM-SCC-6 (C) and UT-SCC-5 (D) cells. Shown are mean percentages of three biological replicates. Immunofluorescent representative images of examples of UM-SCC-6 or UT-SCC-5 cells, 24 h post irradiation, with or without 5 uM BMS303141 pre-treatment. Nuclei in blue, 53BP1 foci in red. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31817870), licensed under a CC-BY licence.



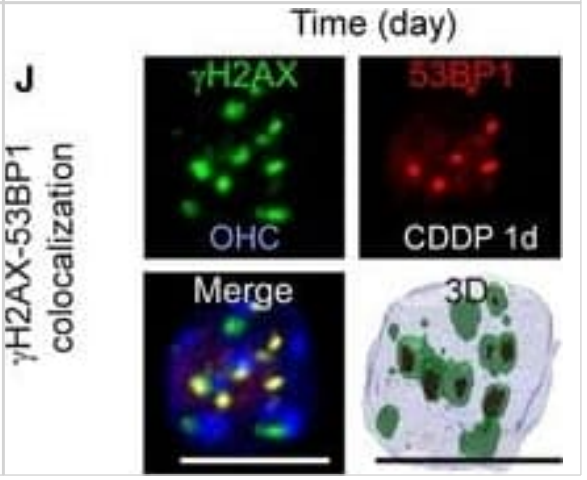
Exosomes modulate the repair of DNA DSBs in irradiated recipient cells. (B) Representative images of 53BP1 foci in BHY cells 6 hours after 2 Gy and transfer of BHY exosomes isolated 24 hours after irradiation with 0, 3, 6 or 9 Gy (53BP1 foci green, nuclei blue). Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0152213), licensed under a CC-BY licence.



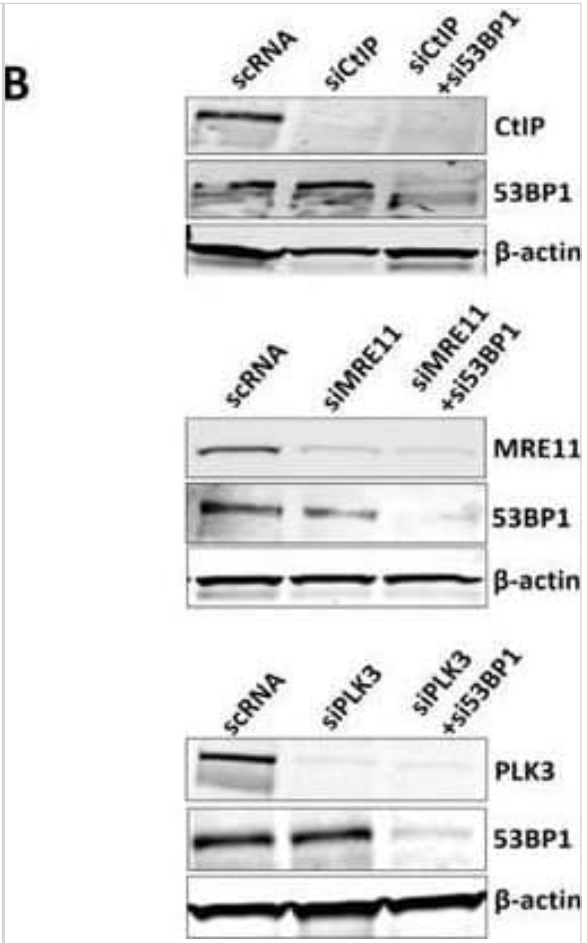
RAP1 protects telomeres against DNA damageWestern blotting showing the expression of RAP1 and TRF2 in MRC-5 cells of different population doublings (PD). Senescent cells (PD 72) were left in culture for at least 4 weeks before harvesting for analysis.ChIP analysis of young (PD 30) and senescent (PD 72) MRC-5 cells performed with either anti-RAP1, anti-TRF2, or an IgG antibody. The immunoprecipitated and input products were loaded into a slot blot membrane and hybridized with a telomere probe, and stripped and hybridized to an Alu probe in order to determine unspecific binding. Quantification was performed by normalizing the telomere signal of the immunoprecipitated to that of the input. Data represent mean +/- SD of three biological replicates.Immunofluorescence detection of 53BP1 (red) and a FISH probe staining telomeres (green) in young (PD 26), pre-senescent (PD 66), and senescent (PD 72 + 4 weeks) MRC-5 fibroblasts. The upper graph shows the percentage of telomere co-localizing with 53BP1 (TIFs). Total DNA damage was measured by immunofluorescence detection of 53BP1. The lower graph shows the total number of 53BP1 foci per nucleus. Approximately 40-50 cells were analyzed per replicate and per condition. Bars represent SEM of two biological replicates.Data information: Statistical analyses were performed using Mann-Whitney U-test (*P < 0.05; **P < 0.001; ***P < 0.0001). Scale bar = 10 um.Source data are available online for this figure. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32096305), licensed under a CC-BY licence.



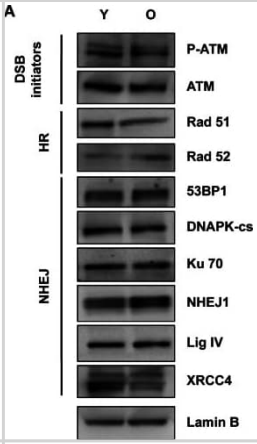
SNX9 colocalizes with FCHSD1 in the cuticular plate of mouse cochlear hair cells.Shown are single confocal sections. SNX9 immunoreactivity visualized with FITC-conjugated secondary antibody was distinctly associated with the cuticular plate, which was visualized with rhodamine-conjugated phalloidin. (A) SNX9 immunoreactivity in the cuticular plate of 2-week old mouse cochlear hair cells. (B) SNX9 immunoreactivity in the cuticular plate of 3-week old mouse outer hair cells. (C) and (D) SNX9 immunoreactivity colocalized with FCHSD1 immunoreactivity, which was visualized with Cy5-conjugated secondary antibody, in the cuticular plate of 3 week-old mouse cochlear hair cells. The Novus FCHSD1 antibody was used in (C) and (D). Scale bars: 10 µm in (A–C), 5 µm in (D). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/23437151), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



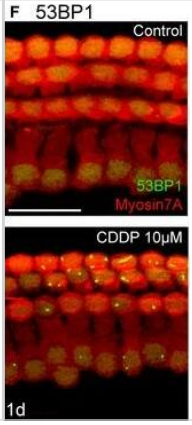
Aβ facilitates HIF1α synthesis and autophagy inhibition via mTOR activation. (A) SK-N-MC cells were exposed to Aβ (5 μM) for 0–48 h. HIF1α and β-actin expression was analyzed by western blot. n = 3. (B) Cells were pretreated with NAC (1 mM) for 30 min prior to Aβ treatment for 24 h. HIF1α and β-actin expression were analyzed by western blot. n = 3. (C,E) Cells were incubated with rapamycin (10 nM) for 30 min prior to Aβ treatment for 24 h. Phosphorylation of 4EBP1 (Thr 37/46) and 4EBP1, phosphorylation of p70S6K1 (Thr 389), HIF1α and β-actin were analyzed by western blot. n = 6. (D) Protein samples were immunoprecipitated by eukaryotic translation initiation factor 4E (eIF4E) antibody-conjugated protein A/G agarose beads. Samples were blotted with 4EBP1 and eIF4E-specific antibodies. n = 3. (F) Cells were exposed to PF4708671 (10 μM) for 30 min prior to Aβ treatment for 24 h. HIF1α and β-actin expression was detected by western blot. n = 6. (G) Cells were exposed to cycloheximide (4 μM) for 30 min prior to Aβ treatment for 24 h. HIF1α and β-actin expressions were detected by western blot. n = 6. (H) Cells were pretreated with rapamycin (10 nM) for 30 min, incubated with Aβ for 24 h and analyzed by western blotting with LC3, p62 and β-actin specific antibodies. n = 3–6. (I) LC3 puncta was visualized by confocal microscopy. Presented results are merged images. Green and red fluorescents indicate LC3 and PI respectively. Scale bars, 50 μm (magnification × 600). (J) Cells were pretreated with trehalose (10 μM) for 30 min prior to Aβ treatment for 24 h. Cytotoxicity was measured by MTT assay at an absorbance of 545 nm using a microplate reader. Data present the mean ± SE. n = 6. (K) Cell viability was measured by trypan blue exclusion assay. Data are presented as a mean ± SE. n = 6. Each blot image was presented as representative image. *p < 0.05 vs. control, #p < 0.05 vs. Aβ treatment. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/28790888), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



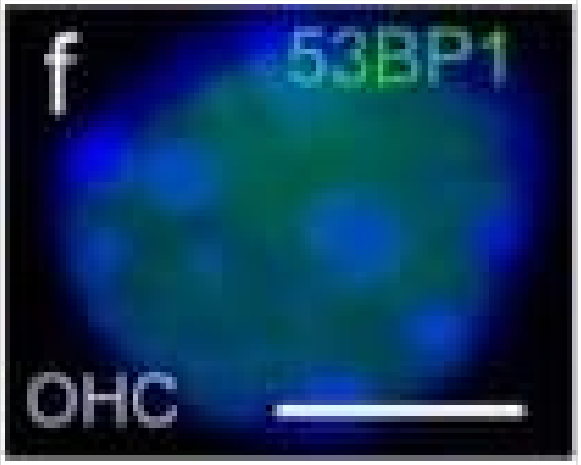
Expression analysis of mouse Fchsd1 and Fchsd2 in different tissues.(A) Total RNA from postnatal day 5 mouse tissues was extracted and used as template for reverse transcription. PCR was performed using this cDNA as template. Upper panel, Fchsd1 mRNA is expressed more abundantly in nervous tissues. Middle panel, Fchsd2 mRNA is expressed ubiquitously in all tissues examined. Lower panel, β-actin specific primers were used as the RT-PCR template control. (B) Total proteins of postnatal day 5 mouse cochlea and vestibula were extracted and separated by PAGE and detected with antibodies against FCHSD1 (Novus), FCHSD2, or SNX9. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/23437151), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



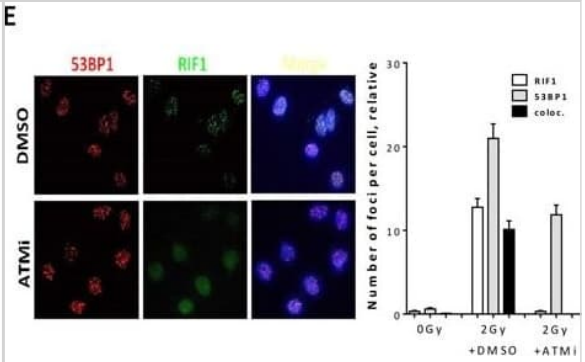
Genotyping of transgenes.PCR genotyping was performed for all four transgenes as described in materials and methods. Sizes of the amplified products obtained are: 240 bp for Hif-1α (wild type); 274 bp for Hif-1αflox/flox; 410 bp for Hif-2α (wild type); 444bp for Hif-2αflox/flox; 370 bpfor Cre transgene; 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), and SPC-rtTA-/tg/(tetO)7-Cre-/tg/Hif-1α/2αfl/fl (Lane 3)). Image collected and cropped by CiteAb from the following open publication (https://dx.plos.org/10.1371/journal.pone.0139270), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



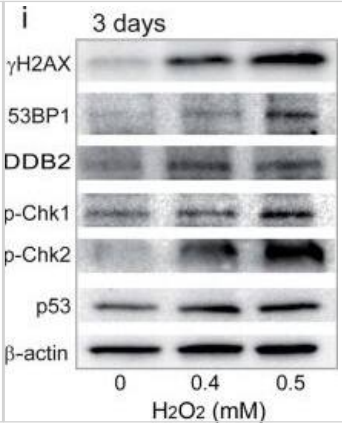
GPRC5A promotes hypoxic cell survival via YAPAHypoxia induced increased total YAP expression in a panel of colorectal cancer cell lines. Actin confirmed equal loading; blots are representative of at least two independent experiments. Related to Fig 4.B–EqRT–PCR analysis revealed that hypoxia increased the expression of known YAP target genes AREG, BCL2L1 (BCL□XL), CTGF and CYR61. Representative experiments are shown. Representative examples of n = 3 independent experiments are shown; data are presented as mean ± SD.FIncreased expression of YAP Ser397 in GPRC5A□depleted cells was overridden by expression of constitutively active RhoA (G14V) in hypoxia. Expression of dominant negative RhoA (T19N) did not further increase YAP Ser397 phosphorylation in response to GPRC5A depletion.GDarker exposure of the GPRC5A blot in Fig 4I, confirming expression of 30–40 kDa and 80 kDa species. Note that YAP siRNA partially diminishes GPRC5A in line with the existence of positive HIF□GPRC5A□YAP feedback loop. Asterisk (*) indicates non□specific band.Source data are available online for this figure. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/30143543), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Depletion of TPX2 causes defects in 53BP1 ionizing radiation-induced foci formation.(A) The protein level of 53BP1 is not affected by miRNA-mediated depletion of TPX2. Levels of actin were used as loading controls. (B) Doxycycline-induced expression of TPX2 miRNA significantly decreases the percentage of HeLa cells with more than five 53BP1 ionizing radiation-induced foci 15 min to 2 h after 2 Gy when compared to non-induced controls (Ctrl). Representative images of cells with 53BP1 ionizing radiation-induced foci 2 h after irradiation are shown (left). Doxycycline also induces expression of GFP reporter. Three independent experiments were performed and 18–20 pictures with an average of 16 cells per picture were analyzed per condition for each time point. Data are compiled in bar chart (right): [15 min: control (89.1+/−4.0) vs. TPX2 miRNA (42.6+/−3.6), p<0.001, n=18; 1 h: control (79.1+/−3.3) vs. TPX2 miRNA (47.6+/−5.3), p<0.001, n=20; 2 h: control (76.9+/−3.6) vs. TPX2 miRNA (37.2+/−4.5), p<0.001, n=20; 4 h: control (57.5+/−5.0) vs. TPX2 miRNA (41.0+/−4.2), p<0.05, n=20; 6 h: control (34.2+/−4.3) vs. TPX2 miRNA (26.7+/−3.9), p>0.05, n=18; group (mean % of cells with more than five 53BP1 ionizing radiation-induced foci +/−SE); unpaired t test]. See text for details. (C) 53BP1 accumulates at infrequent endogenous chromosomal breaks (indicated by asterisks; no ionizing radiation treatment) in presence or absence of TPX2. (D) Cell cycle profiles of control and TPX2 miRNA expressing HeLa cell cultures obtained via flow cytometry (n=2). Note that the slight □5% increase in the G2/M fraction upon TPX2 depletion can not account for the defect in 53BP1 ionizing radiation-induced foci formation exhibited by □55% of TPX2 miRNA expressing cells. See text for details. Error bars represent SE in (B) and SDEV in (D). n= # of independent experiments; NS: non-significant, * p<0.05, ** p<0.01, *** p<0.001; IR: ionizing radiation. Image collected and cropped by CiteAb from the following open publication (https://dx.plos.org/10.1371/journal.pone.0110994), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Hypoxia, blood vessel and proliferation staining. IHC staining in the Biopsy 23 and Biopsy 3 respectively for CA-9, EF5, CD31, Ki67 and LYVE1. 15× Magnification. Image collected and cropped by CiteAb from the following open publication (https://www.mdpi.com/2072-6694/4/3/821), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

John J. Krais, Yifan Wang, Pooja Patel, Jayati Basu, Andrea J. Bernhardy, Neil Johnson RNF168-mediated localization of BARD1 recruits the BRCA1-PALB2 complex to DNA damage Nature Communications 2021-08-18 [PMID: 34408138]

Kate M MacDonald, Shahbaz Khan, Brian Lin, Rose Hurren, Aaron D Schimmer, Thomas Kislinger, Shane M Harding The proteomic landscape of genotoxic stress-induced micronuclei. Molecular cell 2024-04-08 [PMID: 38423013]

Michelini F, Pitchiaya S, Vitelli V et al. Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks Nat. Cell Biol. 2017-12-01 [PMID: 29180822]

Christi Andrin, Darin McDonald, Kathleen M Attwood, Amélie Rodrigue, Sunita Ghosh, Razmik Mirzayans, Jean-Yves Masson, Graham Dellaire, Michael J Hendzel A requirement for polymerized actin in DNA double-strand break repair. Nucleus (Austin, Tex.) 2014-05-12 [PMID: 22688650]

Schwarz, B;Matejka, N;Rudigkeit, S;Sammer, M;Reindl, J; Chromatin Organization after High-LET Irradiation Revealed by Super-Resolution STED Microscopy International journal of molecular sciences 2024-01-03 [PMID: 38203799]

Krais JJ, Glass DJ, Chudoba I et al. Genetic separation of Brca1 functions reveal mutation-dependent Pol? vulnerabilities Nature communications 2023-11-24 [PMID: 38001070] (WB, Mouse)

Arnould C, Rocher V, Saur F et al. Chromatin compartmentalization regulates the response to DNA damage Nature 2023-11-01 [PMID: 37853125] (WB)

Details:
Dilution 1:1000

Wu W, Hill SE, Nathan WJ et al. Neuronal enhancers are hotspots for DNA single-strand break repair Nature 2021-05-20 [PMID: 33767446] (ICC/IF)

Kim JH, Kim SS, Byun SW et al. [Double strand break of DNA in gastric adenoma and adenocarcinoma] The Korean Journal of Gastroenterology 2010-01-26 [PMID: 20098063]

Capala ME, Pachler KS, Lauwers I et al. Ex Vivo Functional Assay for Evaluating Treatment Response in Tumor Tissue of Head and Neck Squamous Cell Carcinoma Cancers (Basel) 2023-01-12 [PMID: 36672427]

Sharma AB, Ramlee MK, Kosmin J et al. C16orf72/HAPSTR1/TAPR1 functions with BRCA1/Senataxin to modulate replication-associated R-loops and confer resistance to PARP disruption Nature Communications 2023-08-17 [PMID: 37591890] (WB)

Gatta AT, Olmos Y, Stoten CL et al. CDK1 controls CHMP7-dependent nuclear envelope reformation eLife 2021-07-21 [PMID: 34286694]

More publications at http://www.novusbio.com/NB100-305

Procedures

Immunohistochemistry protocol for 53BP1 Antibody (NB100-305)

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 style

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 Celcius.
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. 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 1-1.5 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).

Immunocytochemistry/Immunofluorescence protocol for 53BP1 Antibody (NB100-305)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NB100-305

NB820-59248	Human Placenta Whole Tissue Lysate (Adult Whole Normal)
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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