

Product Datasheet

TRF-2 Antibody - BSA Free

NB110-57130

Unit Size: 0.1 ml

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

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NB110-57130

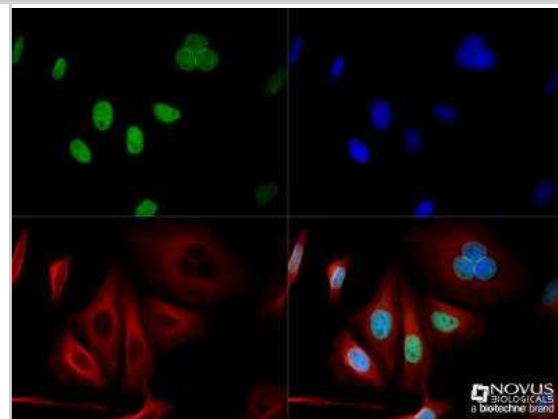
TRF-2 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 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
Target Molecular Weight	59.6 kDa
Product Description	
Host	Rabbit
Gene ID	7014
Gene Symbol	TERF2
Species	Human, Mouse, Rat, Chinese Hamster, Primate
Marker	Telomeres marker
Immunogen	This TRF-2 Antibody was developed against Baculovirus purified TRF2 protein.
Product Application Details	
Applications	Western Blot, Simple Western, Dot Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:2000 - 1:5000, Simple Western 1:25, Flow Cytometry 1-5 ug/ml, ELISA reported in scientific literature (PMID 31575660), Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50 - 1:200, Immunoprecipitation 1:10 - 1:500. Use reported in scientific literature, Immunohistochemistry-Paraffin 1:200, Dot Blot reported in scientific literature (PMID 31026066), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockdown Validated reported in scientific literature (PMID 31026066)
Application Notes	<p>In Western blot, a band at approx. 56 kDa is seen. In ICC/IF, nuclear staining was observed in HeLa cells. In IHC, nuclear staining was observed in xenografted human breast cancer tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</p> <p>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.</p>



Images

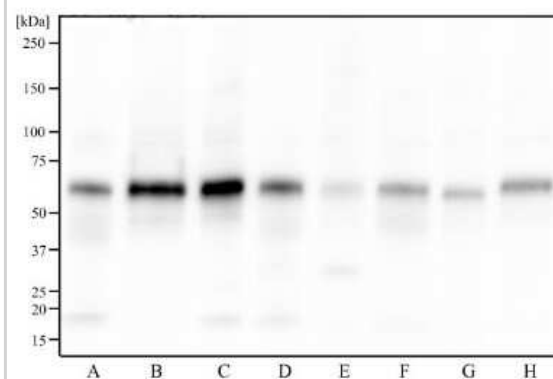
Immunocytochemistry/Immunofluorescence: TRF-2 Antibody [NB110-57130] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with antibody at a 1:200 dilution overnight at 4 degrees Celsius and detected with DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin was used as a co-stain at a 1:1000 dilution and detected with Dylight 550 (Red). Nuclei were detected with DAPI (Blue) at 2.0 ug/ml in 1X PBS. Cells were imaged using a 40X objective.



Simple Western: TRF-2 Antibody [NB110-57130] - Lane view shows a specific band for TRF2 in 1.0 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



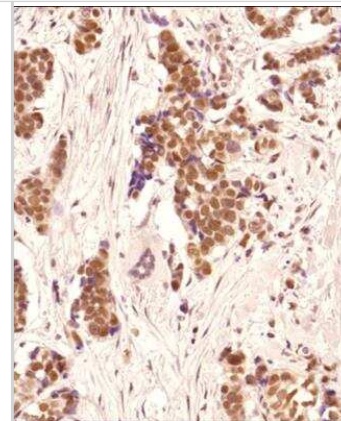
Western Blot: TRF-2 Antibody [NB110-57130] - Analysis of HeLa whole cell lysate (A), HeLa nuclear cell lysate (B), k562 cell lysate (C), HepG2 cell lysate (D), NIH/3T3 cell lysate (E), CHO cell lysate (F), PC12 cell lysate (G), and Cos7 cell lysate (H) using antibody at a concentration of 2 ug/mL.



Immunohistochemistry-Paraffin: TRF-2 Antibody [NB110-57130] - Analysis in xenografted human breast cancer tissue using DAB with hematoxylin counterstain.



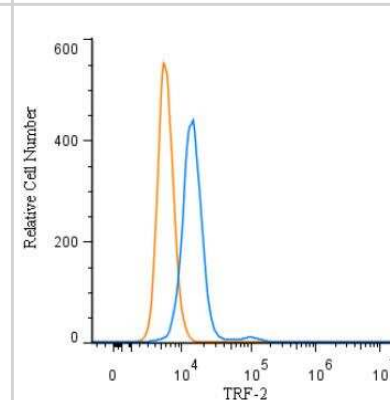
Immunohistochemistry-Paraffin: TRF-2 Antibody [NB110-57130] - Analysis of FFPE human breast cancer tissue with rabbit polyclonal TRF2 antibody at a dilution of 1:200. The staining was developed with HRP-DAB detection method and the counterstaining was performed using hematoxylin. This TRF2 antibody generated an expected nuclear signal in all the cancer cells and the stromal cells. In the tested section, only a subset of myoepithelial cells showed positivity for this protein.



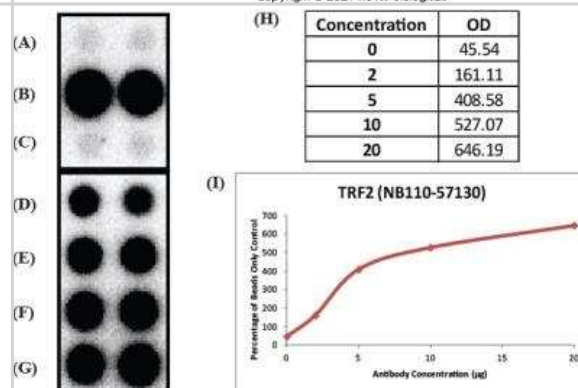
Immunocytochemistry/Immunofluorescence: TRF-2 Antibody [NB110-57130] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-TRF-2 Antibody NB110-57130 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



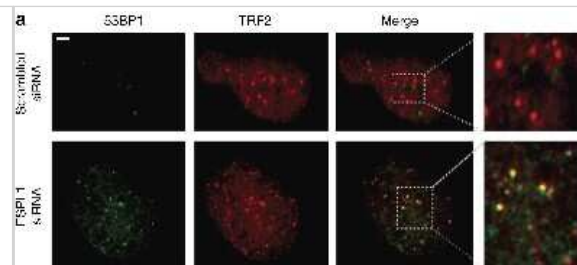
Flow Cytometry: TRF-2 Antibody [NB110-57130] - An intracellular stain was performed on HeLa cells with TRF-2 Antibody NB110-57130 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution 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).



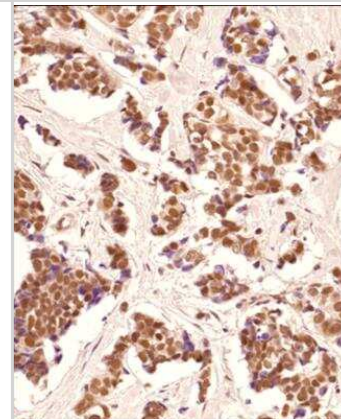
Chromatin Immunoprecipitation: TRF-2 Antibody [NB110-57130] - Analysis in mouse. Titrated TRF2 antibody to determine concentration required for ChIP experiment. ChIP image submitted by a verified customer review.



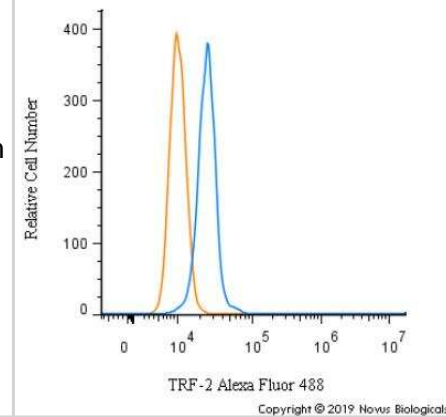
Immunocytochemistry/Immunofluorescence: TRF-2 Antibody [NB110-57130] - RNAi-mediated depletion of human separase (ESPL1) induces TIFs. Control scrambled siRNA- (control) and ESPL1 siRNA-treated fibroblasts stained with anti-p53-binding protein 1 (53BP1; green) and anti-TRF2 (red). It is noteworthy that in ESPL1 siRNA-treated cells, 53BP1 signals frequently overlap with TRF2 signals marking the TIFs. Scale bar, 5 μ m. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/doi/10.1038/ncomms10405>), licensed under a CC-BY license.



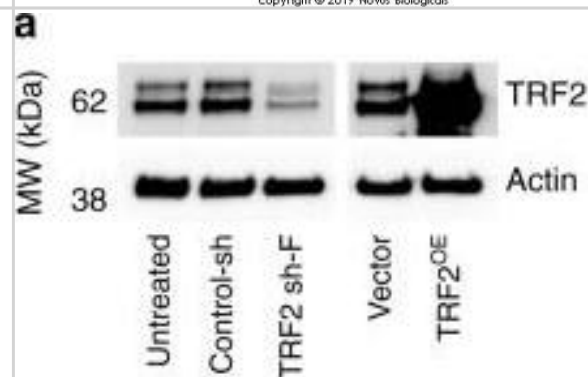
Immunohistochemistry-Paraffin: TRF-2 Antibody [NB110-57130] - Analysis of FFPE human breast cancer tissue with rabbit polyclonal TRF2 antibody at 1:200 dilution. The staining was developed with HRP-DAB detection method and the counterstaining was performed using hematoxylin. This TRF2 antibody generated an expected nuclear signal in all the cancer cells and the stromal cells. In the tested section, only a subset of myoepithelial cells showed positivity for this protein.



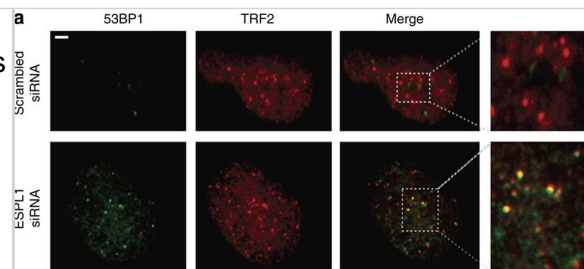
Flow Cytometry: TRF-2 Antibody [NB110-57130] - An intracellular stain was performed on HeLa cells with TRF-2 Antibody NB110-57130AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 μ g/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



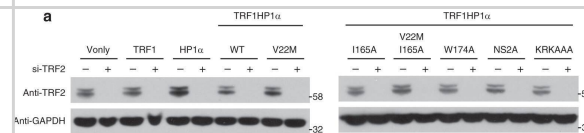
Telomere deprotection contributes to replication stress lethality. A) Western blots of whole cell extracts from HT1080 6TG cells stably transduced with control, TRF2 shRNA (TRF sh-F) or TRF2 over expression (TRF2OE) vectors. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31530811>), licensed under a CC-BY licence.



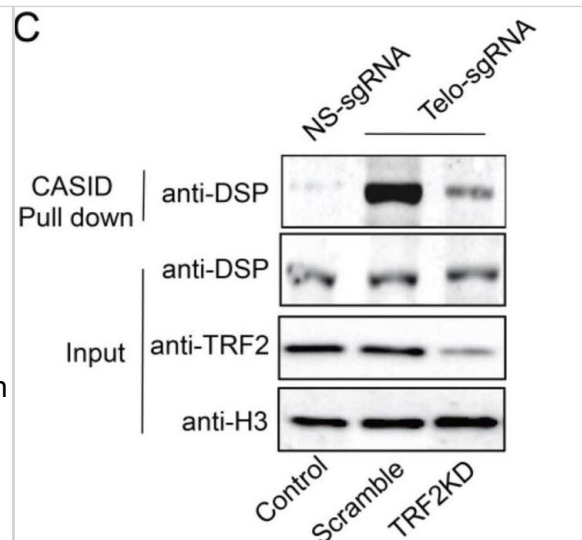
RNAi-mediated depletion of human separase (ESPL1) induces TIFs. (a) Control scrambled siRNA- (control) and ESPL1 siRNA-treated fibroblasts stained with anti-p53-binding protein 1 (53BP1; green) and anti-TRF2 (red). It is noteworthy that in ESPL1 siRNA-treated cells, 53BP1 signals frequently overlap with TRF2 signals marking the TIFs. Scale bar, 5 μ m. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/26778495>), licensed under a CC-BY licence.



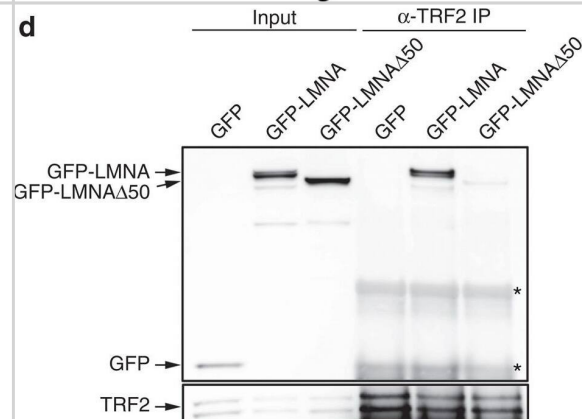
Polyphyllin I triggers PINK1/PARK2-dependent mitophagy. MDA-MB-231 cells were treated with 8 μ M PPI for different periods of time as indicated, and PARK2, P62, and ubiquitin (UB) levels in mitochondrial fractions were determined by western blot. B. Cells were cotransfected with GFP-UB and RFP-mito and treated with 8 μ M PPI for 9 h, after which PARK2 (Alexa Fluor 405, pink) and P62 (Alexa Fluor 647, blue) immunostaining was detected using confocal microscopy. C. Cells cotransfected with GFP-UB and RFP-LC3 were treated with 8 μ M PPI for 9 h, after which PARK2 (Alexa Fluor 405, pink) and TOMM20 (Alexa Fluor 647, blue) immunostaining was detected using confocal microscopy. Scale bars: 10 μ m. D-E. MDA-MB-231 cells were treated with 8 μ M PPI for different periods of time as indicated; whole-cell lysates were then separated on 8% SDS-PAGE gels and analyzed by western blot using the anti-PINK1 antibody. Relative full-length (\square 63 kDa) and cleaved (\square 52 kDa) PINK1 levels were quantified by densitometry and normalized to Tubulin. The results were expressed as a percentage of control, which was set at 100%. Data are presented as mean \pm SD (* P < 0.01 vs. the control). F. Cells were treated with 8 μ M PPI for different periods of time as indicated, and whole-cell lysates were then subjected to western blot analysis. G. Cells were treated with 8 μ M PPI for 9 h, after which mitochondrial fractions were prepared and subjected to immunoprecipitation using anti-PINK1 antibody; associated PARK2 was detected using immunoblotting. H. RFP-mito-expressing MDA-MB-231 cells were treated with 8 μ M PPI for 9 h, and PINK1 (Alexa Fluor 488, green) and PARK2 (Alexa Fluor 405, pink) immunostaining were evaluated using confocal microscopy. Scale bars: 10 μ m. Image collected and cropped by CiteAb from the following open publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.14413>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



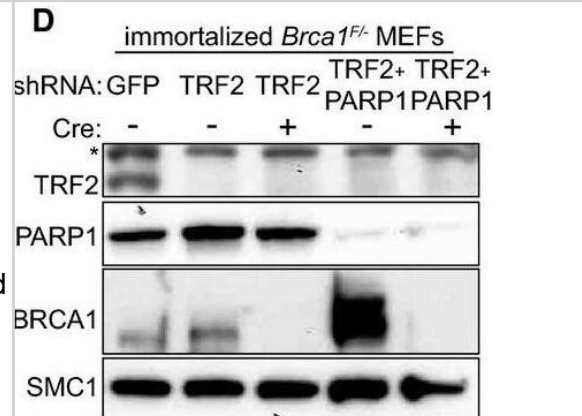
PHD3 depletion stabilizes hypoxic p27 expression by increasing p27 half-life. a Cell cycle arrest at G0 and subsequent release shows an increase of p27 expression in siPHD3 exposed cells. b Quantification for p27 expression under PHD3 depletion at indicated time points after cell cycle release in HeLa and 786-O cells. Asterisk indicates significant difference ($p < 0,05$; $n = 3$). c Cell cycle arrest at G0 and inhibition of protein synthesis with cycloheximide indicate increased p27 stability in PHD3 depleted HeLa cells. d Quantification of p27 expression using siPHD3 or control at indicated time points. Four independent experiments (\pm SEM) are shown ($p < 0,05$; $n = 4$). e Analysis of p27 stability in 786-O cells by cycloheximide chase during reoxygenation after 24 h hypoxia demonstrates markedly increased half-life of p27 upon PHD3 depletion Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/26223520>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



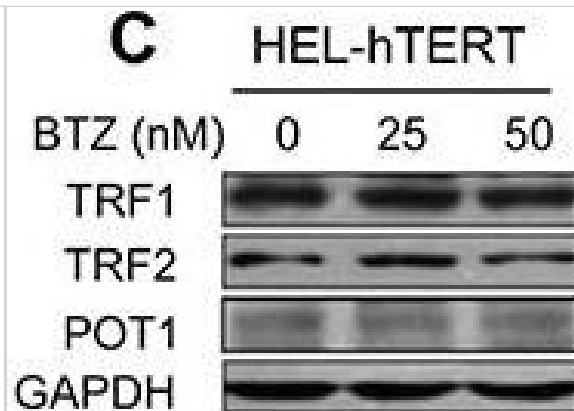
Immunohistochemical staining of HIF-1 α , VEGF-A and VEGF-C in normal renal tissue (A-C) and clear cell renal cell carcinoma (CCRCC) (D-F). A homogeneous cytoplasmic staining of tubular cells and weak staining in glomerules was observed with HIF-1 α (A), while VEGF-A and VEGF-C were positive in tubular cells, glomerular mesangium and interstitial macrophages (B and C). In CCRCC, HIF-1 α immunoreactivity was nuclear and/or cytoplasmic (D), while it was perimembranous and/or diffuse cytoplasmic for VEGF-A and VEGF-C (E and F). (magnification $\times 200$). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/19302703>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



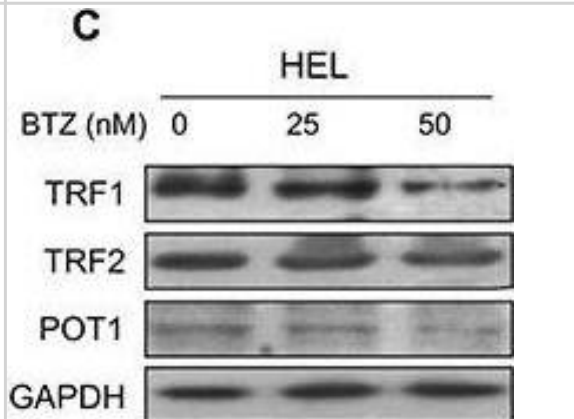
Resistance of FANCA-mutant cells with defective FANCD2 function to hydrogen peroxide (H₂O₂). (a) Representative images illustrating staining for subnuclear FANCD2 foci in isogenic fibroblast pairs, either deficient for FANCD2 (PD20) or FANCA (PD220) and their respective wild-type (wt) complemented counterparts. Foci were visualized three hours after treatment with H₂O₂ (25 μ M for 2 hours). (b) Clonogenic survival of cells with or without wild-type FANCA after treatment with varying concentrations of H₂O₂. Data represent logarithmic means \pm standard error from 15-repeat experiments. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/18483568>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



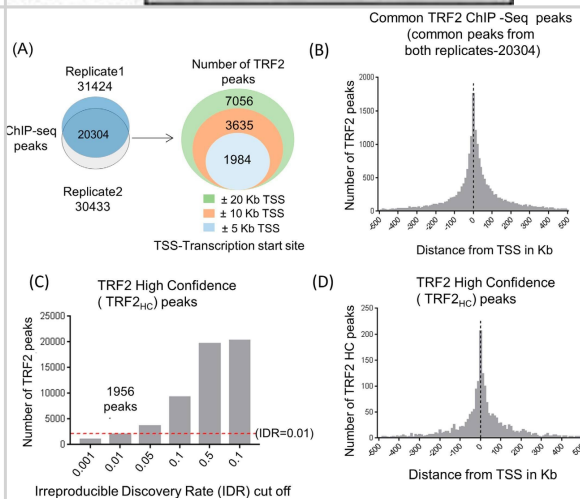
γ -Taxilin ablation causes ER stress responses and apoptosis in normoxic cells. (a) Knockdown of γ -taxilin expression by small interfering RNA (siRNA) in HeLa S3 cells. Two different siRNA constructs are equally effective. HeLa S3 cells were treated with solvent alone (Mock), control siRNA (Co), or γ -taxilin siRNA (γ -tax-1 or -2) for 96 h. (b) Induction of apoptosis by γ -taxilin ablation in HeLa S3 cells. γ -Taxilin ablation causes apoptosis in HeLa S3 cells, but Mock or Co treatment did not. Bar graph shows fractions of annexin-positive cells (means \pm S.D., n=3). *Significantly different from Mock- or Co treatment ($P < 0.001$, Tukey–Kramer test). (c) Confocal microscopy demonstrates coincidence of γ -taxilin depletion and apoptotic nuclei in HeLa S3 cells treated with γ -taxilin siRNA. Scale bar, 10 μ m. (d) γ -Taxilin depletion triggers ER stress responses in HeLa S3 cells. Upper panels, taxilin and NAC proteins; middle panels, UPR proteins; and lower panel, β -actin. (e) γ -Taxilin ablation induces accumulation of ubiquitinated proteins in HeLa S3 cells. Cell lysates were analyzed on 7.5% SDS-PAGE, followed by immunoblotting with antibodies specific for anti-mono- and polyubiquitinated conjugates (upper panel) and β -actin (lower panel) Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/25880086>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



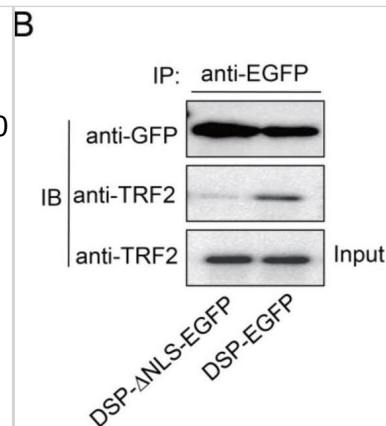
Damage response is restricted to MG. (A) RT-PCR analysis for Slc1a3, the gene encoding GLAST, at the indicated times after injury. (B) RT-PCR for MG and a photoreceptor-specific marker (Nrl) in GLAST-positive and negative fractions, after MACS in intact retinas. (C–F) qPCR quantification of Oct4, Nanog, Lin28, and Dnmt3b expression levels at the indicated time after injury in MACS GLAST-positive and negative fraction; C, intact retina as control (Student's t-test; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). Image collected and cropped by CiteAb from the following open publication (<https://journal.frontiersin.org/article/10.3389/fnins.2016.00523/full>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



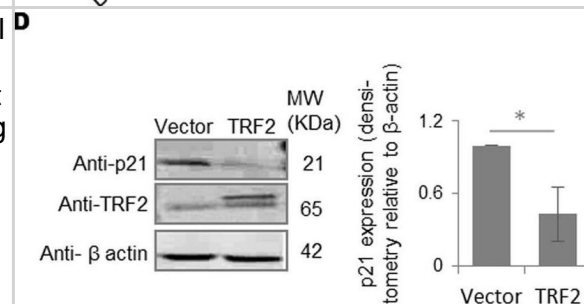
Effect of ET-1 and Cx43 on HIF-1 α expression and glucose uptake when c-Src is inhibited. Astrocytes were preincubated with 100 ng/ μ L PP2 (c-Src inhibitor) or 100 ng/ μ L PP3 (inactive analogue) for 1 h. Then, cells were incubated in the absence (control) or presence of 0.1 μ M ET-1 for 24 h. A) HIF-1 α Western blot and quantification. The results are expressed as percentages of the level found in the controls treated with PP3 and they show that the inhibitor of c-Src PP2 prevented the up-regulation of HIF-1 α 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 and 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/ μ L PP2 or 100 ng/ μ L PP3 for 1 h. Then, cells were transfected with NT-siRNA or with Cx43-siRNA and after 48 h HIF-1 α was analysed by Western blot. The results are expressed as percentages of the level found in the PP3 NT-siRNA and they show that the inhibitor of c-Src PP2 prevented the up-regulation of HIF-1 α promoted by silencing Cx43. *** $p < 0.001$ versus the corresponding NT-siRNA. Image collected and cropped by CiteAb from the following open publication (<https://dx.plos.org/10.1371/journal.pone.0032448>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



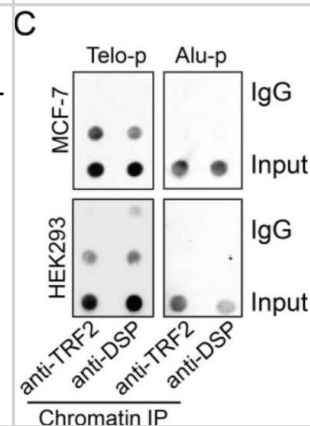
Genotyping of transgenes. PCR genotyping was performed for all four transgenes as described in materials and methods. Sizes of the amplified products obtained are: 240 bp for Hif-1 α (wild type); 274 bp for Hif-1 α flox/flox; 410 bp for Hif-2 α (wild type); 444bp for Hif-2 α flox/flox; 370 bp for Cre transgene; 350 bp for 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-1 α f1/fl (Lane 1), SPC-rtTA-/tg/(tetO)7-Cre-/tg/Hif-2 α f1/fl mouse (Lane 2), and SPC-rtTA-/tg/(tetO)7-Cre-/tg/Hif-1 α /2 α f1/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.



The expression patterns of Yap and GFAP-Cre recombinase in postnatal mouse eyes. (A) Schematic of a transverse section of mouse eye. (B-C) Immunostaining with anti-Yap antibody (green) on frozen eye sections at different ages. Nuclei were counterstained with DAPI (blue). Yap staining was detected in scattered cells within the INL (arrowheads) and GCL of the retina and the lens epithelium (arrows). (D) Cre recombinase (red) was expressed in the lens epithelium and INL, GCL of retina in frozen eye sections of Tomatof/+; GFAP-Cre mice at P14. Nuclei were counterstained with DAPI (blue). LE, lens epithelium; TZ, transitional zone; RPE, retinal pigment epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars: 25 μ m (B-C), 100 μ m (D). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/31011480>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Representative picture of western blot in histopathologically unchanged tissue (N) and primary cancerous tissue (C) from patients with CRC. Immunodetection of bands was performed with Rp anti- PHD1, - PHD2, - PHD3 and - FIH Ab, followed by incubation with goat anti-rabbit HRP-conjugated Ab. The membrane was stripped and incubated with Rp anti-GAPDH Ab, followed by incubation with goat anti-rabbit HRP-conjugated Ab. Bands were revealed using SuperSignal West Femto Chemiluminescent Substrate, Thermo Fisher Scientific (Rockford, IL) and Biospectrum® Imaging System 500, UVP Ltd. (Upland, CA). Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/24195777>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Zhang K Preserving Genetic Integrity in Reproduction: Insights from Telomere Protection and Sperm Head-Tail Junctions Thesis 2023-01-01

Roy S, Bagri S, Sengupta A et al. Artificially inserted G-quadruplex DNA secondary structures induce long-distance chromatin activation bioRxiv 2023-11-29 (ChIP, Human)

Sengupta A, Vinayagamurthy S, Soni D et al. Telomeres control regulation of the human Telomerase (hTERT) gene through non-telomeric TRF2 and independent of Telomere looping bioRxiv 2023-10-11 (ChIP, Human)

Meng X, Yao D, Imaizumi K et al. Assembloid CRISPR screens reveal impact of disease genes in human neurodevelopment Nature 2023-10-01 [PMID: 37758944] (WB, Human)

Sullivan DI, Bello FM, Silva AG et al. Intact mitochondrial function in the setting of telomere-induced senescence Aging cell 2023-09-08 [PMID: 37688329] (WB, Mouse)

Marín-Gual L, González-Rodelas L, M Garcias M et al. Meiotic chromosome dynamics and double strand break formation in reptiles Frontiers in Cell and Developmental Biology 2022-10-12 [PMID: 36313577] (ICC/IF)

Barry RM, Sacco O, Mameri A et al. Rap1 regulates TIP60 function during fate transition between two-cell-like and pluripotent states Genes & Development 2022-03-01 [PMID: 35210222] (WB)

Xu Q, Mojiri A, Boulahouache L et al. Vascular senescence in progeria: role of endothelial dysfunction European Heart Journal Open 2022-07-28 [PMID: 36117952] (WB, B/N)

Silva B, Arora R, Bione S, Azzalin CM. TERRA transcription destabilizes telomere integrity to initiate break-induced replication in human ALT cells Nature Communications 2021-06-18 [PMID: 34145295] (B/N)

Jacome Burbano MS, Robin JD, Bauwens S et al. Non-canonical telomere protection role of FOXO3a of human skeletal muscle cells regulated by the TRF2-redox axis Communications biology 2023-05-25 [PMID: 37231173] (WB, B/N)

Details:

Dilution:1:200

Jahn A, Rane G, Paszkowski-Rogacz M et al. ZBTB48 is both a vertebrate telomere-binding protein and a transcriptional activator. EMBO Rep. 2017-05-12 [PMID: 28500257] (B/N)

Drosopoulos WC, Deng Z, Twayana S et al. TRF2 Mediates Replication Initiation within Human Telomeres to Prevent Telomere Dysfunction Cell Reports 2020-11-10 [PMID: 33176153]

More publications at <http://www.novusbio.com/NB110-57130>



Procedures

Western Blot protocol for TRF2 Antibody (NB110-57130)

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

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

Immunocytochemistry/Immunofluorescence Protocol for TRF2 Antibody (NB110-57130)

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.

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Novus Biologicals USA

10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada

21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd

19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information

www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

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