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Anti-YTHDF2 Antibody Picoband®

Catalog Number: A02621-1

About YTHDF2

YTH N6-methyladenosine RNA binding protein 2 is a protein that in humans is encoded by the YTHDF2 gene. This gene encodes a member of the YTH (YT521-B homology) superfamily containing YTH domain. The YTH domain is typical for the eukaryotes and is particularly abundant in plants. The YTH domain is usually located in the middle of the protein sequence and may function in binding to RNA. In addition to a YTH domain, this protein has a proline rich region which may be involved in signal transduction. An Alu-rich domain has been identified in one of the introns of this gene, which is thought to be associated with human longevity. In addition, reciprocal translocations between this gene and the Runx1 (AML1) gene on chromosome 21 has been observed in patients with acute myeloid leukemia. This gene was initially mapped to chromosome 14, which was later turned out to be a pseudogene. Alternatively spliced transcript variants encoding different isoforms have been identified in this gene.

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Overview

Product Name	Anti-YTHDF2 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-YTHDF2 Antibody Picoband® catalog # A02621-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q9Y5A9

Technical Details

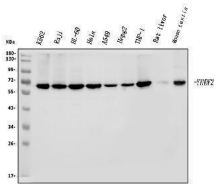
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human YTHDF2, identical to

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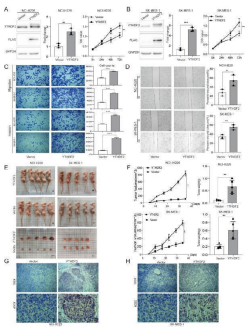
	the related mouse and rat sequences.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.25ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human, Mouse, Rat Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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Anti-YTHDF2 Antibody Picoband® (A02621-1) Images



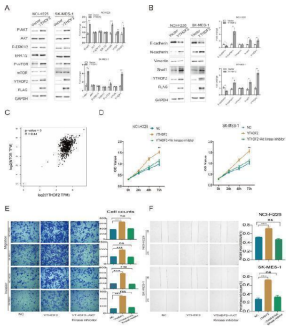
Western blot analysis of YTHDF2 using anti-YTHDF2 antibody (A02621-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: human K562 whole cell lysates, Lane 2: human Raji whole cell lysates, Lane 3: human HL-60 whole cell lysates, Lane 4: human Hela whole cell lysates, Lane 5: human A549 whole cell lysates, Lane 6: human Hepg2 whole cell lysates, Lane 7: human THP-1 whole cell lysates, Lane 8: rat liver tissue lysates, Lane 9: mouse testis tissue lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-YTHDF2 antigen affinity purified polyclonal antibody (Catalog # A02621-1) at 0.25 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for YTHDF2 at approximately 65KD. The expected band size for YTHDF2 is at 65KD.



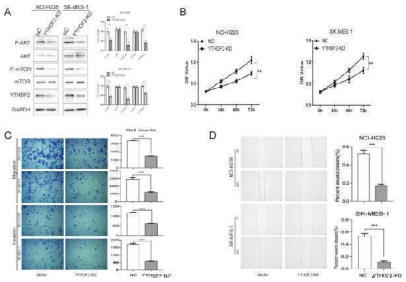
YTHDF2 overexpression promotes cell proliferation and invasion in LUSC. A and B Representative immunoblot showed that the protein level of YTHDF2 was steadily up-regulated in two LUSC cell lines studied. The CCK8 assay was used to assess cell viability in NCI-H226 and SK-MES-1 cells. C and D The transwell assay and the wound-healing assay were used to assess the invasion potential and migration ability of NCI-H226 and SK-MES-1 cells. E and F Tumor size was measured twice a week. After 5 weeks, we dissected tumors from nude mice which had been injected with the indicated stable cell, then measured the tumor size and weight of nude mice injected with the indicated stable cells. G and H Immunohistochemistry showed the expression level of YTHDF2 from tumors of nude mice injected with the indicated stable cells. Data are represented by the mean ± SD of three independent experiments. *P < 0.05 vs. the vector group Index in PubMed under a CC BY license. PMID: 34996459

YTHDF2 overexpression upregulates the protein levels of P-AKT and P-mTOR and induces EMT in LUSC cells. A The western blot detected the expression of mTOR/AKT-related proteins in NCI-H226 and SK-MES-1 cells. B The western blot detected the expression of EMT-related proteins in NCI-H226 and SK-MES-1 cells. C Using the Pearson correlation statistics, we examine the pairwise gene correlation analysis between YTHDF2 and mTOR in LUSC through GEPIA' TCGA and GTEx expression data. D and E and F YTHDF2

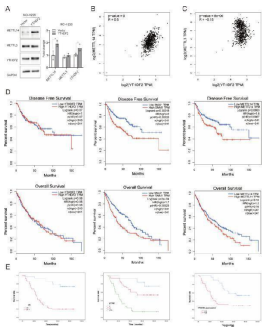
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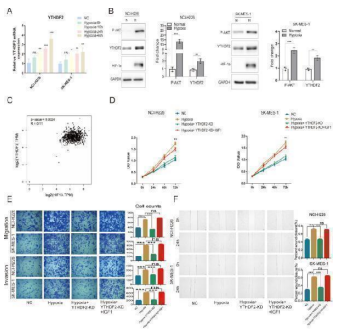
overexpression resulted in significant promotion of cellular proliferation, invasion and migration in NCI-H226 and SK-MES-1 cells, while AKT Kinase inhibitor eliminated the promotion of YTHDF2 overexpression. Data are represented by the mean ± SD of three independent experiments. *P < 0.05 vs. the vector group Index in PubMed under a CC BY license. PMID: 34996459



YTHDF2 knockdown inhibits cell proliferation and invasion in LUSC. A The western blot analyzed the expression of mTOR/AKT-related proteins in NCI-H226 and SK-MES-1 cells. B and C NCI-H226 and SK-MES-1 had YTHDF2 knocked out, resulting in significant inhibition of cell proliferation, migration and invasion. D YTHDF2 knockdown significantly inhibits cell migration viability in NCI-H226 and SK-MES-1 cells by the wound-healing assay. Data are represented by the mean ± SD of three independent experiments. *P < 0.05 vs. the vector group Index in PubMed under a CC BY license. PMID: 34996459



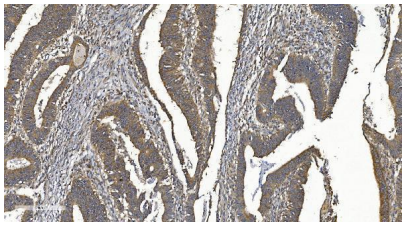
YTHDF2, as a tumor promoter, may lead to a poor prognosis for LUSC patients. A Representative immunoblot showed YTHDF2 overexpression to promote METTL14 upregulation in NCI-H226, not of METTL3. Data are represented by the mean ± SD of three independent experiments. *P < 0.05 vs. the vector group. B and C Using the Pearson correlation statistics, we examine the pairwise gene correlation analysis between YTHDF2 and METTL14, YTHDF2 and METTL3 by TCGA and GTEx expression data of GEPIA. D By a log-rank test for the overall survival (OS) and disease-free survival (DFS) analysis in LUSC, we respectively investigate gene YTHDF2, METTL14, and Snail by the ‘Survival’ tab of GEPIA. E In the immunohistochemical staining results, the overall survival of LUSC patients with pN, pTNM, and YTHDF2 expression was analyzed by A Kaplan–Meier analysis Index in PubMed under a CC BY license. PMID: 34996459



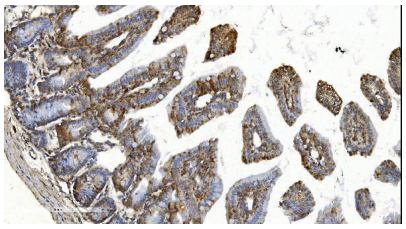
Hypoxia specifically induces YTHDF2 overexpression to activate the mTOR/AKT axis in LUSC cells. A RT-qPCR was used to investigate the alterations of mRNA levels of YTHDF2 when LUSC cells were exposed to 1% O2 for 6 h, 16 h, 24 h, 48 h, respectively. B NCI-H226 and SK-MES-1 cells were exposed with 20% O2 or 1% O2 (1% O2, 5%CO2, 94% N2) for 24 h. The protein levels of HIF-1alpha, YTHDF2, and P-AKT (be phosphorylated at serine 473) were analyzed by western blot. C Using the Pearson correlation statistics, we examine the pairwise gene correlation analysis between HIF-1alpha and YTHDF2 by TCGA and GTEx expression data of GEPIA. D The cellular growth was analyzed by CCK8 assay at different time points. E The cellular invasion and migration growths were analyzed by transwell assay. F The migration viability was analyzed by wound-healing assay. Data are represented by the mean ± SD of three

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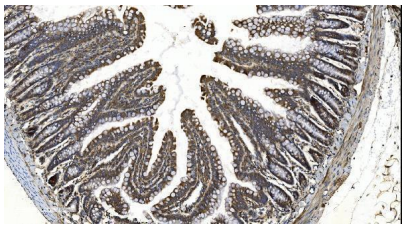
independent experiments. *P < 0.05 vs. the vector group
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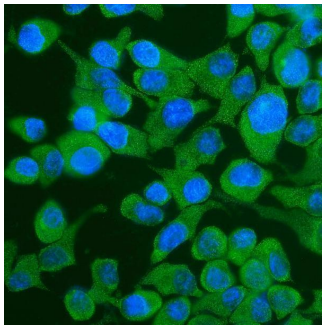
IHC analysis of YTHDF2 using anti-YTHDF2 antibody (A02621-1). YTHDF2 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-YTHDF2 Antibody (A02621-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



IHC analysis of YTHDF2 using anti-YTHDF2 antibody (A02621-1). YTHDF2 was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-YTHDF2 Antibody (A02621-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



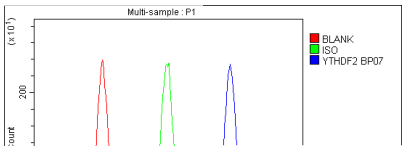
IHC analysis of YTHDF2 using anti-YTHDF2 antibody (A02621-1). YTHDF2 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-YTHDF2 Antibody (A02621-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.



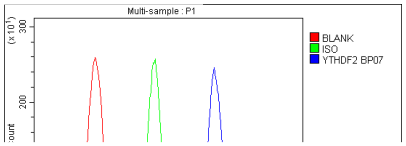
IF analysis of YTHDF2 using anti-YTHDF2 antibody (A02621-1). YTHDF2 was detected in immunocytochemical section of Hep cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti-YTHDF2 Antibody (A02621-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Flow Cytometry analysis of RAW264.7 cells using anti-

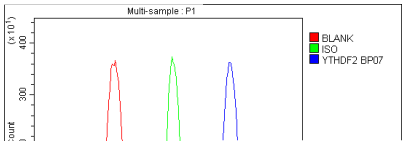
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YTHDF2 antibody (A02621-1). Overlay histogram showing RAW264.7 cells stained with A02621-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-YTHDF2 Antibody (A02621-1, 1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



Flow Cytometry analysis of RH-35 cells using anti-YTHDF2 antibody (A02621-1). Overlay histogram showing RH-35 cells stained with A02621-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-YTHDF2 Antibody (A02621-1, 1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



Flow Cytometry analysis of THP-1 cells using anti-YTHDF2 antibody (A02621-1). Overlay histogram showing THP-1 cells stained with A02621-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-YTHDF2 Antibody (A02621-1, 1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

Anti-YTHDF2 Antibody

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