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Anti-APG5L/ATG5 Antibody Picoband®

Catalog Number: PA2260

About ATG5

Autophagy protein 5 is a protein that in humans is encoded by the ATG5 gene. It is also known as APG5 or ASP, and this gene is mapped to 6q21. It is found that knockdown of ATG5 in hepatocytes increased triglyceride levels with oleate or a second endogenous stimulus for triglyceride formation. These hepatocytes with ATG5 knockdown also had increased lipid droplet number and size. ATG5 is an E3 ubiquitin ligase which is necessary for autophagy due to its role in autophagosome elongation. It is activated by ATG7 and forms a complex with ATG12 and ATG16L1. This complex is necessary for LC3-1 conjugation to PE to form LC3-II.

Overview

Product Name	Anti-APG5L/ATG5 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-APG5L/ATG5 Antibody catalog # PA2260. Tested in Flow Cytometry, ICC/IF, IHC, 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 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
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	Q9H1Y0

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence in the middle region of human APG5L, identical to the related mouse sequence, and different from the related rat sequence by one amino acid.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized

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Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry(Fixed), 1-3 ug/1x10^6 cells, Human

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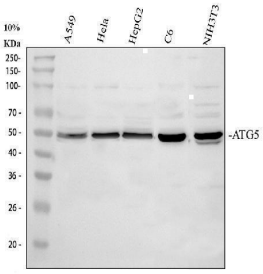
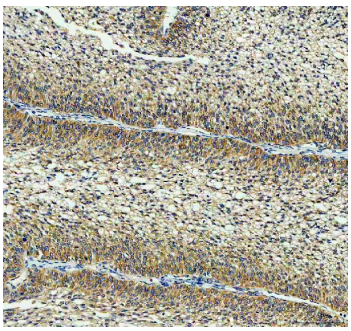
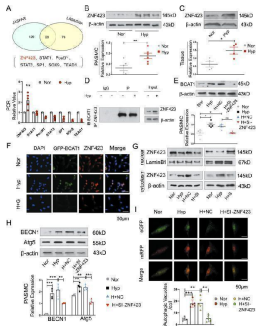
Anti-APG5L/ATG5 Antibody Picoband® (PA2260) Images



Western blot analysis of ATG5 using anti-ATG5 antibody (PA2260). Electrophoresis was performed on a 10% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: Lane 1: human A549 whole cell lysates, Lane 2: human Hela whole cell lysates, Lane 3: human HepG2 whole cell lysates, Lane 4: rat C6 whole cell lysates, Lane 5: mouse NIH/3T3 whole cell lysates.After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-ATG5 antigen affinity purified polyclonal antibody (PA2260) at 0.5 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 ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for ATG5 at approximately 50-52 kDa. The expected band size for ATG5 is at 32 kDa.



IHC analysis of ATG5 using anti-ATG5 antibody (PA2260). ATG5 was detected in a paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ATG5 Antibody (PA2260) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



Hypoxia leads to the transfer of ZNF423 from the nucleus to the cytoplasm, where it bound BCAT1 to promote autophagy activity. a Bioinformatics analysis of proteins associated with BCAT1. Upside: According to the JASPAR database and LASAGNA-Search 2.0 database, there was 28 genes that may bind to bcat1, and the binding ability of ZNF423, STAT1, Pou5f1, STAT3, SP1, SOX9, and TEAD1 was strong. Underside: RT-PCR analysis of the mRNA levels of ZNF423, STAT1, Pou5f1, STAT3, SP1, SOX9, and TEAD1 with rat beta-actin serving as the standard in PASMCs under NOR or HYP for 24 h (n = 5). b Western blot analysis of the expression of ZNF423 in PASMCs under NOR or HYP for 24 h (n = 6). c ZNF423 protein levels were assayed in pulmonary arterial tissues of hypoxic model rats (n = 4). d Coimmunoprecipitation of whole-cell lysates of PASMCs exposed to normoxia or hypoxia for 24 h with anti-ZNF423, followed by probing with anti-BCAT1 (n = 3). e Western blot analysis of BCAT1 expression in PASMCs transfected with

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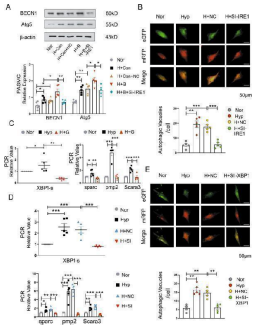
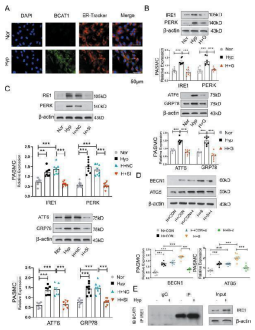
ZNF423 siRNA under NOR or HYP for 24 h (n = 4). f PASMCs were exposed to HYP for 24 h, and the colocalization between BCAT1 and ZNF423 was determined by immunofluorescence. GFP-BCAT1 (green), ZNF423 (red), and DAPI (blue). Scale bar = 50 um (n = 3). g The translocation of ZNF423 between the nucleus and cytoplasm in PASMCs transfected with BCAT1 siRNA or gabapentin (n = 3). h Western blot analysis of the expression of BECN1 and Atg5 in PASMCs transfected with ZNF423 siRNA under HYP for 24 h (n = 4). i Autophagic flux of PASMCs cotransfected with eGFP-mRFP-LC3 plasmid and control siRNA or ZNF423 siRNA under HYP for 24 h. Scale bar = 50 um (n = 5). Nor normoxia, Hyp hypoxia, H + G hypoxia plus gabapentin, H + NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, H + si-ZNF423 hypoxia plus ZNF423 siRNA, IP immunoprecipitation, IB immunoblotting. Statistical analysis was performed with one-way ANOVA or the Student’s t test. All values are presented as the mean ± SEM. * p



BCAT1 regulates autophagy during hypoxia by activating ERs via the IRE1-XBP1-RIDD axis. a Western blot analysis of BECN1 and Atg5 in PASMCs cotransfected with BCAT1 and IRE1 siRNA (n = 5). b Autophagic flux was monitored in PASMCs cotransfected with eGFP-mRFP-LC3 plasmid and control siRNA or IRE1 siRNA that were then exposed to HYP for 24 h. Scale bar = 50 um (n = 3). c , d RT-PCR analysis of the mRNA levels of XBP1-s, sparc, pmp2, and Scara3 with rat beta-actin serving as the standard (n = 5). e The formation of autophagosomes was detected, and autophagic activity was estimated in cells in which the expression of XBP1 was knocked down with XBP1 siRNA under HYP for 24 h. Scale bar = 50 μm (n = 5). Nor normoxia, Hyp hypoxia, H + G hypoxia plus gabapentin, H + NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, H + SI-IRE1 hypoxia plus IRE1 siRNA, H + SI-XBP1 hypoxia plus XBP1 siRNA, H + Con hypoxia plus control vector, H + B hypoxia plus BCAT1 plasmid, H + Con+NC hypoxia plus control vector plus control siRNA, H + B + Si-IRE hypoxia plus BCAT1 plasmid plus IRE1 siRNA. Statistical analysis was performed with one-way ANOVA. All values are presented as the mean ± SEM. * p



BCAT1 regulates autophagy through the endoplasmic reticulum stress pathway. a Expression of BCAT1 and ER-Tracker Red staining in PASMCs exposed to NOR or HYP for 24 h. Scale bar = 50 um (n = 3). b Western blot analysis of PERK, IRE1, ATF6, and GRP78 protein expression in the ERs pathway in PASMCs treated with gabapentin (n = 8). c Western blot analysis of IRE1, PERK, ATF6, and GRP78 expression in PASMCs transfected with BCAT1 siRNA (n = 8). d Western blot analysis of BECN1 and Atg5 in PASMCs treated with the ERs pathway inhibitor 4-PBA and BCAT1 plasmid (n = 8). e Coimmunoprecipitation of the whole-cell lysates of PASMCs exposed to normoxia or hypoxia for 24 h with anti-IRE1, followed by probing with anti-BCAT1 (n = 3). Nor normoxia, Hyp hypoxia, H + G hypoxia plus gabapentin, H + NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, N + Con normoxia plus control vector, H +

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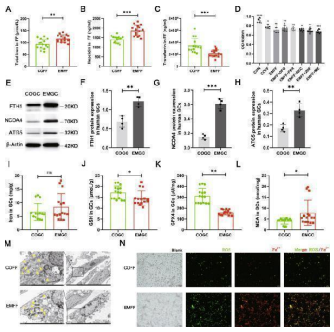
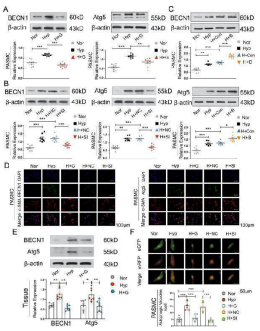
Con hypoxia plus control vector, H + B hypoxia plus BCAT1 plasmid, H + Con+4 hypoxia plus control vector plus 4-phenylbutyric acid, H + B + 4 hypoxia plus BCAT1 plasmid plus 4-phenylbutyric acid, IP immunoprecipitation, IB immunoblotting. Statistical analysis was performed with one-way ANOVA. All values are presented as the mean ± SEM. ** p



Iron-overloaded EMFF induced ferritinophagy-dependent ferroptosis in granulosa cells. A – C Levels of total iron, hepcidin, and transferrin in EMFF (n = 15) and COFF (n = 15). Data are expressed as means ± SD and analyzed by Student’s t test. D Results of mouse granulosa cells proliferation under different intervention conditions (each group in the figure is compared with COFF group). DFO, iron chelators; FER, ferroptosis inhibitor; NEC, necrosis inhibitor; ZDF, apoptosis inhibitor; ME, autophagy inhibitor. Data are expressed as means ± SD and analyzed by one-way ANOVA. E – H Comparison of ferritinophagy-related proteins FTH1, NCOA4, and ATG5 between human granulosa cells of infertile patients with EMs (EMGC) and of control group (COGC). The expression of beta-actin was used as an internal control. Data are expressed as means ± SD and analyzed by Student’s t test. I – L Detection of ferroptosis-related indicators iron, GSH, GPX4, and MDA in COGC and EMGC. Data are expressed as means ± SD and analyzed by Student’s t test. M Representative images of the mitochondrial morphology of mouse granulosa cells intervened by COFF and EMFF were observed under TEM. Yellow arrows indicate mitochondrion. Scale bar = 1.0 μm. Scale bar = 5.0 μm. N Representative images of ROS and ferrous ion fluorescence staining after COFF and EMFF intervention in mouse granulosa cells. Scale bar = 100 μm. * P



Upregulation of BCAT1 expression induced by hypoxia leads to PASMC autophagy. a Western blot analysis of BECN1 and Atg5 protein expression in PASMCs treated with the inhibitor gabapentin (20 μM) (n = 8). b Western blot analysis of BECN1 and Atg5 protein expression in PASMCs transfected with BCAT1 siRNA or BCAT1 plasmid (n = 8). c , d Immunofluorescence staining for BECN1 and Atg5 in PASMCs. BECN1 and Atg5 (green), alpha-SMA (red), and DAPI (blue). Scale bar = 50 um (n = 3). e Western blot analysis of BECN1 and Atg5 expression in the pulmonary arterial tissues of hypoxia model rats treated with gabapentin (n = 7). f Measurement of autophagic flux in PASMCs transfected with eGFP-mRFP-LC3 plasmid and exposed under NOR or HYP for 24 h treated with BCAT1 siRNA or the BCAT1 inhibitor gabapentin. Yellow and red dots indicate autolysosomes and autophagosomes, respectively. Scale bar = 50 um (n = 6). Nor normoxia, Hyp hypoxia, Mct monocrotaline, H + G hypoxia plus gabapentin, M + G monocrotaline plus gabapentin, H + NC hypoxia plus control siRNA, H + SI hypoxia plus BCAT1 siRNA, H + Con hypoxia plus control vector, H + B hypoxia plus BCAT1 plasmid. Statistical analysis was performed with one-way ANOVA. All values are presented as the mean ± SEM. * p

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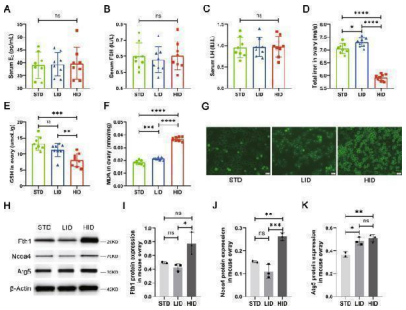
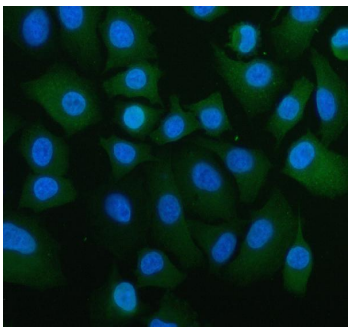
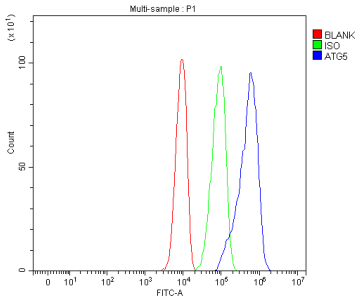


Construction of an iron overload mouse model. A – C Serum levels of E 2 , FSH, and LH in standard iron (STD), low iron (LID), and high iron (HID) diet feeding groups (n = 8). D – F Total iron, GSH, and MDA levels in the ovary tissues of mice in each group (n = 8). G Representative images of ROS fluorescence staining of ovarian mouse granulosa cells in three groups of mice. Scale bar = 20 μm. H – K Western blot analysis of ferritinophagy-related proteins, FTH1, NCOA4, and ATG5 in mouse ovary tissues in the STD, LID, and HID group. The expression of beta-actin was used as an internal control. All data are expressed as means ± SD and analyzed by one-way ANOVA. * P



IF analysis of ATG5 using anti-ATG5 antibody (PA2260). ATG5 was detected in an immunocytochemical section of A549 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 5 ug/mL rabbit anti-ATG5 Antibody (PA2260) overnight at 4°C. Fluoro488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:500 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 HepG2 cells using anti-ATG5 antibody (PA2260). Overlay histogram showing CACO-2 cells stained with PA2260 (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-ATG5 Antibody (PA2260, 1 ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

7 Publications Citing This Product

1. PubMed ID: -, Shen W,Jia N,Miao J,Chen S,Zhou S,Meng P,Zhou X,Tang L,Zhou L:Penicilliumin B Protects against Cisplatin-Induced Renal Tubular Cell Apoptosis through Activation of AMPK-Induced Autophagy and Mitochondrial Biogenesis.Kidney Dis 2021.doi:10.1159/000514657

2. PubMed ID: 29113167, Yang J, Sheng S, Yang Q, Li L, Qin S, Yu S, Zhang X. Oncol Lett. 2017 Nov;14(5):5333-5339. doi: 10.3892/ol.2017.6857. Epub 2017 Aug 31. Endocan silencing induces programmed cell death in hepatocarcinoma

3. PubMed ID: 26459718, Anti-autophagic and anti-apoptotic effects of memantine in a SH-SY5Y cell model of Alzheimer's disease via mammalian target of rapamycin-dependent and -independent pathways

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