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Anti-Ubiquitin D UBD Rabbit Monoclonal Antibody

Catalog Number: M01970

Overview

Product Name	Anti-Ubiquitin D UBD Rabbit Monoclonal Antibody
Reactive Species	Human, Mouse
Description	Boster Bio Anti-Ubiquitin D UBD Rabbit Monoclonal Antibody catalog # M01970. Tested in WB, IHC, ICC/IF applications. This antibody reacts with Human, Mouse.
Application	IF, IHC, ICC, WB
Clonality	Monoclonal HID-21
Formulation	Rabbit IgG in stabilizing components, phosphate buffered saline, pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
Storage Instructions	Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	O15205

Technical Details

Immunogen	A synthesized peptide derived from human Ubiquitin D
Isotype	Rabbit IgG
Form	Liquid
Concentration	0.5mg/ml
Purification	Affinity-chromatography
Suggested Dilutions	WB 1:500-2000 IHC 1:50-200 ICC/IF 1:50-200

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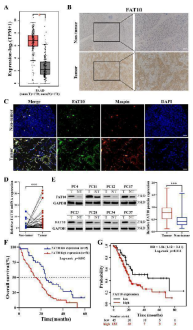
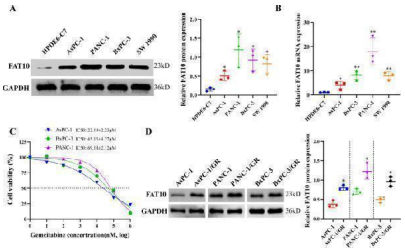
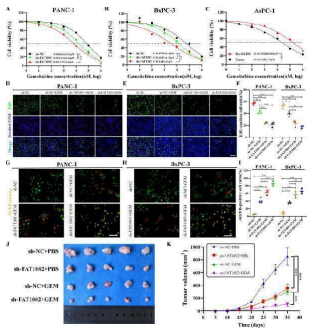
Anti-Ubiquitin D UBD Rabbit Monoclonal Antibody (M01970) Images



High FAT10 expression is associated with poor prognosis in patients with PC. A The GEPIA2 server was used to analyze the expression of FAT10 in PAAD (T, tumor; N, nontumorous tissues; * p



FAT10 expression is correlated with chemoresistance of PC cells to GEM. A Protein expression of FAT10 in noncancerous pancreatic ductal epithelial cells and PC cell lines was analyzed by western blotting. B The mRNA expression of FAT10 in normal pancreatic ductal epithelial cells and PC cell lines was analyzed by qRT–PCR. C Viabilities of PC cells were determined in response to different concentrations of GEM. Inhibition curves were fitted by nonlinear regression, and GEM IC50s were calculated using GraphPad Prism 8 software. D Western blotting was used to analyze expression levels of FAT10 in GEM-resistant (GR) PC cells. Data represent the mean ± SD of triplicate experiments and were statistically analyzed with Student’s t-test, * p



Inhibition of FAT10 increases the chemotherapeutic sensitivity of PC to GEM. A – C The IC50 of PC cells (FAT10 knockdown or overexpression) exposed to different concentrations of GEM was determined by cell viability experiments. D – F The effect of knocking down FAT10 on the proliferation rate of PC cells treated with GEM was detected by EdU staining. G – I The apoptosis rate of PC cells with FAT10 knockdown was detected by AO/EB staining after GEM treatment. Data represent the mean ± SD of triplicate experiments and were statistically analyzed by one-way ANOVA. J Representative images of different groups of tumors removed from mice are shown. K Growth curve showing changes in tumor volume in mice from different groups. Growth was assessed every 5 days beginning from the day of injection and during GEM treatment. At the end of the experiment, the tumor was dissected and photographed, and the tumor volume (V) was calculated as follows: V = 0.52 × length × width 2 and analyzed by one-way ANOVA. * p

FAT10 regulates EMT to promote chemotherapeutic resistance in PC cells. A Spearman correlation analysis of the correlation between FAT10 (UBD) and EMT pathway score. FAT10 expression is represented by the abscissa, and the EMT pathway score is represented by the ordinate. A density curve to the right represents the trend in the distribution of pathway scores, a density curve to the upper part

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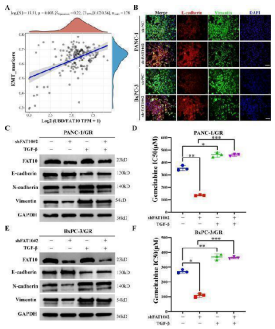
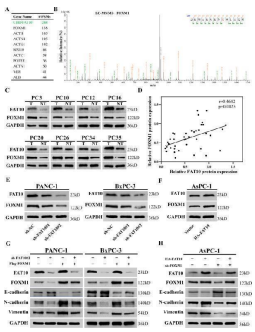
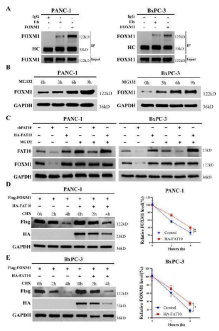


represents the trend in the distribution of gene expression. The top part shows the p -value, correlation coefficient, and correlation calculation method. B Immunofluorescence analysis of the effect of inhibiting FAT10 on the expression of EMT-related proteins (E-cadherin and Vimentin) in PC cells. Scale bar, 50 um. C – F GEM-resistant PC cell lines (PANC-1/GR and BxPC-3/GR) were transfected with interfering plasmid sh-FAT10#2 and or EMT activator (TGF-beta, 10 ng/mL) for 48 h. C , E Western blot analysis was used to observe the expression of EMT-related proteins in each treatment group. D , F Cell viability experiments were used to calculate the half-inhibitory concentration (IC50) of GEM-treated PC-resistant cells in each treatment group. Data represent the mean ± SD of triplicate experiments and were statistically analyzed by one-way ANOVA. * p



FAT10 regulates EMT through FOXM1. A Top 10 proteins co-precipitated with FAT10 analyzed by LC-MS/MS. #PSMs, peptide spectrum matches. B Mass spectrum showing unique peptides of FOXM1 identified by 2D-LC-MS/MS from the protein lysates prepared from PANC-1 cells following immunoprecipitation with anti-FAT10. C Representative western blot analysis of FAT10 and FOXM1 protein expression in PC and paired paracancerous tissues (T, tumor; NT, non-tumor tissue). D Scatter plots showing a positive correlation between FAT10 and FOXM1 protein expression levels in 40 PC samples (n = 40, r = 0.4642, p = 0.0025, Pearson test). E , F Western blot analyses were used to detect FAT10 and FOXM1 protein expression in cells stably transfected with the shFAT10 or HA-FAT10 plasmid. G Western blot analysis confirming FAT10 silencing and FOXM1 restoration and their effects on EMT-related proteins. H Western blot analysis showing the levels of FAT10 overexpression and FOXM1 inhibition and their effects on EMT-related proteins. Index in PubMed under a CC BY license. PMID: 35614040



FAT10 increases FOXM1 protein levels by inhibiting the ubiquitination and degradation of FOXM1 in PC cells. A Co-IP was used to detect the interaction between FOXM1 and ubiquitin in PANC-1 and BxPC-3 cells. HC, heavy chain. B With MG132 (10 uM) added to PANC-1 and BxPC-3 cells, western blotting was used to detect protein levels of FOXM1 at different times. C MG132 (10 uM) was added to PANC-1 and BxPC-3 cells while the expression of FAT10 was altered. Western blotting was used to detect protein expression levels of FOXM1. D PANC-1 and BxPC-3 cells were treated with CHX (20 uM) for a specified time with or without the addition of the FAT10 overexpression plasmid, and FOXM1 protein levels were detected by western blotting. Data represent the mean ± SD of triplicate experiments and were statistically analyzed with Student’s t-test, * p

FAT10 competes with ubiquitin to bind and stabilize FOXM1. A Co-IP was used to detect the interaction between FOXM1 and FAT10 in PANC-1 and BxPC-3 cells. HC, heavy chain. B MG132 (10 uM) was added to PANC-1 and BxPC-3 cells,

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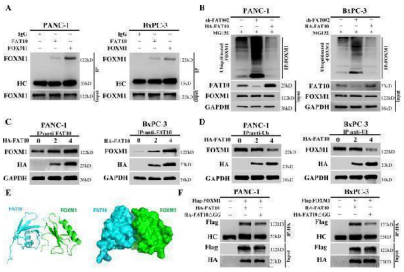
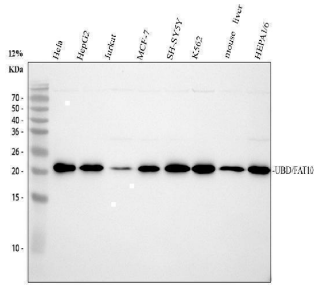


shFAT10 or HA-FAT10 plasmid was transfected at the same time, and then co-IP was used to detect the levels of ubiquitin bound to FOXM1 protein. C PANC-1 and BxPC-3 cells were transfected with different amounts of HA-FAT10 plasmid, and the level of FAT10 binding to FOXM1 protein was detected by co-IP. D PANC-1 and BxPC-3 cells were transfected with different amounts of HA-FAT10 plasmid, and the level of Ub binding to FOXM1 protein was detected by co-IP. E Docking conformation of the first ranking score. Three-dimensional structure of FAT10 and FOXM1. FAT10 is shown in green. FOXM1 is shown in cyan. F The Flag-FOXM1 plasmid was transfected into PANC-1 and BxPC-3 cells, the HA-FAT10 or HA-FAT10ΔGG plasmid was transfected at the same time, and then the protein level of Flag-FOXM1 bound to the HA-tagged protein was detected by co-IP. HC, heavy chain. Index in PubMed under a CC BY license. PMID: 35614040



Western blot analysis of Ubiquitin D using anti-Ubiquitin D antibody (M04905-1). Electrophoresis was performed on a 12% 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: human Hela whole cell lysates, Lane 2: human HepG2 whole cell lysates, Lane 3: human Jurkat whole cell lysates, Lane 4: human MCF-7 whole cell lysates, Lane 5: human SH-SY5Y whole cell lysates, Lane 6: human K562 whole cell lysates, Lane 7: mouse liver tissue lysates, Lane 8: mouse HEPA1/6 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-Ubiquitin D antigen affinity purified monoclonal antibody (M04905-1) at 1:500 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 1:500 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 Ubiquitin D at approximately 23 kDa. The expected band size for Ubiquitin D is at 18 kDa.

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