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Anti-TNF alpha Antibody Picoband®

Catalog Number: PA1079

About TNF

TNF alpha (Tumor Necrosis Factor alpha) gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine.

Overview

Product Name	Anti-TNF alpha Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-TNF alpha Antibody catalog # PA1079. Tested in Flow Cytometry, 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, IHC, 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	P01375

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human TNF alpha, different from the related mouse sequence by five amino acids, and rat sequence by seven amino acids.
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).
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 Flow Cytometry (Fixed), 1-3 ug/1x10^6 cells, Human

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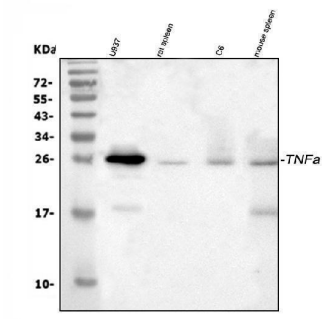
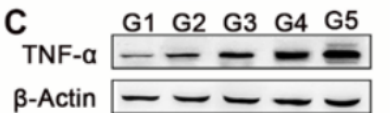
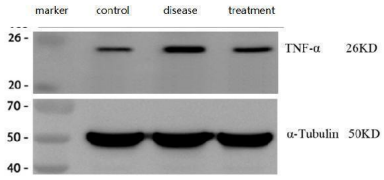
Anti-TNF alpha Antibody Picoband® (PA1079) Images



Western blot analysis of TNF alpha using anti-TNF alpha antibody (PA1079). 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 30 ug of sample under reducing conditions. Lane 1: human U937 whole cell lysates, Lane 2: rat spleen tissue lysates, Lane 3: rat C6 whole cell lysates, Lane 4: mouse spleen tissue 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-TNF alpha antigen affinity purified polyclonal antibody (Catalog # PA1079) 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 Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for TNF alpha at approximately 25 kDa. The expected band size for TNF alpha is at 26 kDa.



Western blot analysis of TNF alpha using anti-TNF alpha antibody (PA1079). Electrophoresis was performed on a 5-20% 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 1-5: mouse lung cancer tissue. 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-TNF alpha antigen affinity purified polyclonal antibody (PA1079) at 1:1000 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 for 1 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. The expected band size for TNF alpha is at 26 kDa.



Western blot analysis of TNF alpha using anti-TNF alpha antibody (PA1079). 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 30 ug of sample under reducing conditions. Lane 1: control group-normal mouse hippocampal tissue lysates, Lane 2: hippocampal tissue from Alzheimer's disease model mouse, Lane 3: hippocampal tissue from Alzheimer's disease model mouse treated with a self-developed drug. 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-TNF alpha antigen

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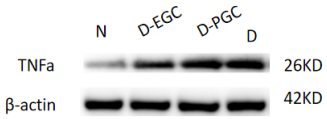
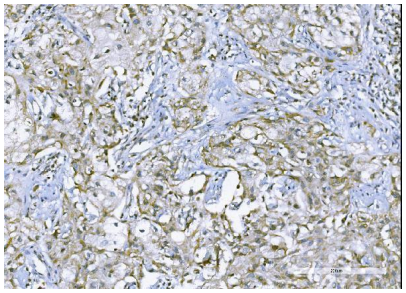
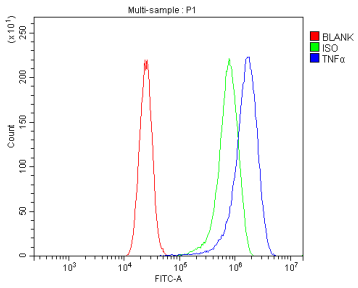
affinity purified polyclonal antibody (Catalog # PA1079) at 1:1000 overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with ChemiDoc MP system. A specific band was detected for TNF alpha at approximately 26 kDa. The expected band size for TNF alpha is at 26 kDa.



Western blot analysis of TNF alpha using anti-TNF alpha antibody (PA1079). 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 30 ug of sample under reducing conditions. Lane 1: normal rat alveolar bone tissue lysates, Lane 2: alveolar bone from a diabetic bone defect model treated with EGCG, Lane 3: alveolar bone from a diabetic bone defect model treated with PGC-1alpha, Lane 4: alveolar bone from a diabetic bone defect model. 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-TNF alpha antigen affinity purified polyclonal antibody (Catalog # PA1079) at 1:1000 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 1:10000 for 1 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with ChemiDoc MP system. A specific band was detected for TNF alpha at approximately 26 kDa. The expected band size for TNF alpha is at 26 kDa.



IHC analysis of TNF alpha using anti-TNF alpha antibody (PA1079). TNF alpha was detected in a paraffin-embedded section of human B lymphocytic tumor 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-TNF alpha Antibody (PA1079) 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.



Flow Cytometry analysis of CACO-2 cells using anti-TNF alpha antibody (PA1079). Overlay histogram showing CACO-2 cells stained with PA1079 (Blue line). The cells were fixed with 4% paraformaldehyde and blocked with 10% normal goat serum. And then incubated with rabbit anti-TNF alpha Antibody (PA1079, 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

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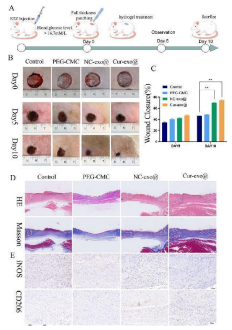
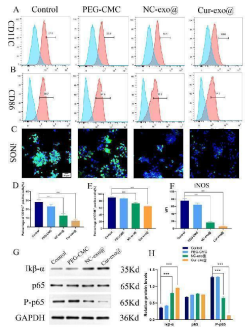
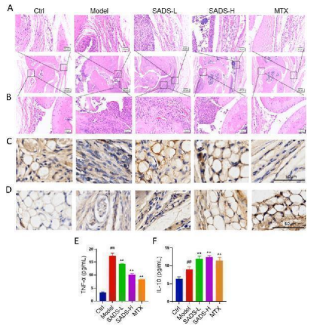
same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



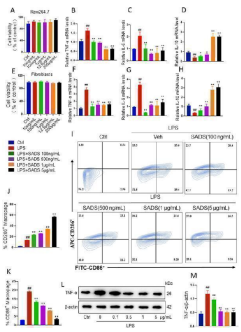
Wound healing with different hydrogels in vivo . (A) Preparation and observation of diabetic chronic wounds related to orthopedics. (B) Photographs of the wound treated with the hydrogel in different hydrogel groups. (C) Wound recovery curve of different hydrogel groups. (D) H&E staining and Masson staining of wound tissue on day 10 in different hydrogel groups. (E) IHC staining of wound tissue in iNOS and CD206.Index in PubMed under a CC BY license. PMID: 41190287



Immunomodulatory properties pathway of the hydrogel. (A, B) Flow cytometry analysis of the macrophage surface markers CD11C and CD86. (C) Representative immunofluorescence images of iNOS in RAW264.7 cells. (D, E) Quantitative analysis of flow cytometry in CD11C and CD86. (F) Quantitative analysis of the mean fluorescence intensity of iNOS. (G) Western blot of IkappaB-alpha, p65, and p-p65. (H) Quantitative analysis of the relative expression of IkappaB-alpha, p65, and p-p65 in different hydrogels.Index in PubMed under a CC BY license. PMID: 41190287



Pathological evaluation of IL-1RA−/− mice synovial tissue. HE staining showed synovial hyperplasia (A) and inflammation (B). (C) IHC staining of TNF-alpha in synovial tissue. (D) IHC staining of IL-10 in synovial tissue.(E and F) Levels of TNF-alpha and IL-10 in the blood of IL1RA−/ -deficient mice. ##P < 0.01 versus Ctrl; ∗∗P < 0.01versus Model. n = 6 mice for each group. Control is wild-type mice, and the model is IL1RA−/− mice.Index in PubMed under a CC BY license. PMID: 40688514



SADS inhibited Raw264.7 and fibroblast-like synoviocyte inflammation in vitro. (A) The effect of SADS on the viability of Raw264.7. (B–D) RT-PCR analysis of TNF-alpha, IL-6 and IL-10 in Raw264.7 treated with SADS.(E) The effect of SADS on the viability of fibroblast-like synoviocytes. (F–H) RT-PCR analysis of TNF-alpha, IL-6 and IL-10 in fibroblast-like synoviocytes treated with SADS.(I) The phenotype of Raw264.7 was analyzed by flow cytometry.(J and K) Statistics of the proportion of M2 and M1 macrophages. (L and M) TNF-alpha protein expression level detection. ##P < 0.01 versus Ctrl; ∗∗P < 0.01versus LPS. n = 6.Index in PubMed under a CC BY license. PMID: 40688514

Influence of the invasion of pannus in the cartilage area on cartilaginous structures and related inflammation expression (10 × 20 magnification). a - e In sacroiliitis, abundant fibrovascular tissue formed and invaded into cartilage,

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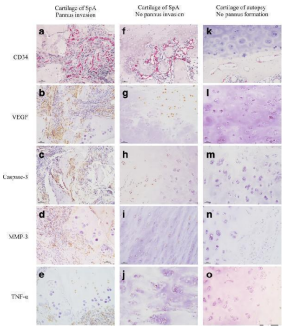
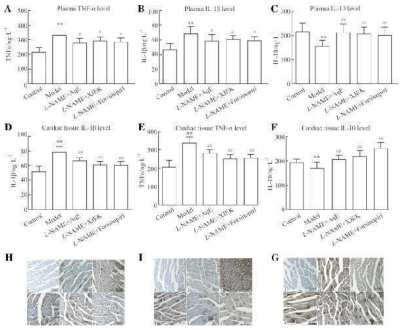
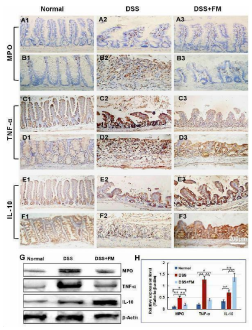


chondrocytes and matrix degenerated (a), accompanied by high levels of vascular endothelial growth factor (VEGF) (b), caspase-3 (c), matrix metalloproteinase-3 MMP-3 (d) and TNF-alpha (e) expressed in the cartilage. f - j In sacroiliitis, fibrovascular tissue formed in the subchondral area without invading into cartilage (f), accompanied by significantly lower levels of VEGF (g), caspase-3 (h), MMP-3 (i) and TNF-alpha (j) expressed in the cartilage. k - o In autopsy controls, there was no fibrovascular tissue formation and only partial superficial chondrocytes expressed low levels of VEGF (l), caspase-3 (m) and TNF-alpha (o). a , f , k , AP-Red staining; b - e , g - j , l - o , 3,3-diaminobenzidine staining. SpA, spondyloarthritis Index in PubMed under a CC BY license. PMID: 29884210



Effects of polysaccharide extract from XJEK on TNF-alpha, IL-1beta and IL-10 in L -NAME-induced hypertensive mice. (a) TNF-alpha expression level in plasma. (b) IL-1beta expression level in plasma. (c) IL-10 expression level in plasma. (d) IL-1beta expression level in cardiac tissues. (e) TNF-alpha expression level in cardiac tissues. (f) IL-10 expression level in cardiac tissues. (g) Representative image of IL-1beta immunocytochemistry. (h) Representative image of TNF-alpha immunocytochemistry.(i) Representative image of IL-10 immunocytochemistry.1,negative group; 2,control group; 3, model group; 4, L -NAME+AqE group; 5, L -NAME+XJEK group; 6, L -NAME+fosinopril group. Data are presented as the mean ± SD (n = 10). ** P



The infiltration of MPO + neutrophils, and the cellular distribution and relative expression level detection of the TNF and IL-10 in the small intestinal and colonic mucosa at 7 days after the termination of DSS administration. (A) The MPO immunohistochemistry staining of the small intestinal mucosa: (A1) the normal group: few neutrophils were observed in the small intestinal mucosa; (A2) the DSS group: a number of accumulative MPO + neutrophils (brown) infiltrated into the mucosa epithelium; (A3) the DSS + B. subtilis- fermented milk group: only limited neutrophil infiltration could be observed in the small intestinal mucosa. (B) The MPO immunohistochemistry staining of the colonic mucosa: (B1) the normal group: few neutrophils were observed in the colonic mucosa; (B2) the DSS group: colonic epithelium and the glands disappeared, and the ulcer was locally replaced by scars and a number of accumulative MPO + neutrophils (brown) were observed in the scars; (B3) the DSS + B. subtilis -fermented milk group: only limited MPO + neutrophils observed in the colonic mucosa. (C) The TNF immunohistochemistry staining of the small intestinal mucosa: (C1) the normal group: the epithelium was integrated with faint yellow staining, suggesting low expression of TNF; (C2) the DSS group: the villus structure is not integrated, and the epithelial cells showed black brown, suggesting overexpression of TNF; (C3) the DSS + B. subtilis -fermented milk group: the villus and the glands were almost integrated, and the staining of epithelial cells was similar to that of the normal group (C1) , suggesting low

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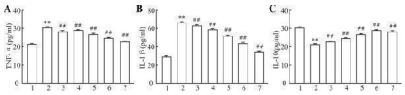
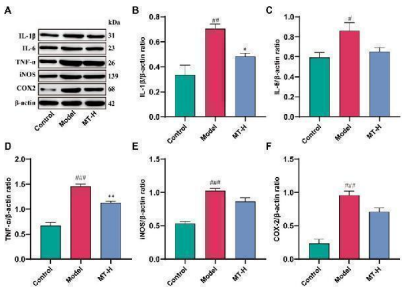
expression of TNF. (D) The TNF immunohistochemistry staining of the colonic mucosa: (D1) the normal colonic mucosa: the epithelium was integrated with low TNF expression (faint yellow); (D2) the DSS group: the epithelium structure and the glands were destroyed and replaced by a scar, and there were a number of TNF + inflammatory cells (black brown) in the scar; (D3) the DSS + B. subtilis -fermented milk group: the recovered epithelium showed faint yellow, suggesting low TNF expression. (E) The IL-10 immunohistochemistry staining of the small intestinal mucosa: (E1) the normal small intestinal mucosa: the IL-10 staining dispersed in the villi and the crypts with faint yellow, suggesting low-level expression of IL-10; (E2) the DSS group, the residual epithelium and the crypts were light brown, suggesting mid-level of IL-10 expression; (E3) the DSS + B. subtilis -fermented milk group: the dark brown staining of the regenerative epithelium represented high-level expression of IL-10. (F) The IL-10 immunohistochemistry staining of the colonic mucosa: (F1) the normal group: the IL-10 staining dispersed in the glands with bright yellow, suggesting low-level expression of IL-10; (F2) the DSS group: there were few IL-10 + cells in the scars; (F3) the DSS + B. subtilis -fermented milk group, the dark brown staining of the epithelial cells represented high-level expression of IL-10. (G,H) Western blotting analysis for the expression of MPO, TNF, and IL-10 in the samples containing equivalent ileum and colon. The expression level of MPO, TNF, and IL-10 in the DSS group was significantly higher than that of the normal (control) group. The expression level of MPO and TNF in the DSS + B. subtilis -fermented milk (FM) group was significantly lower than that of the DSS group, while the expression level of IL-10 in the DSS + B. subtilis -fermented milk (FM) group was significantly higher than that of the DSS group (n = 5, * represents p < 0.05, ** represents p < 0.01).Index in PubMed under a CC BY license. PMID: 33519783



Effects of polysaccharide extract from XJEK on TNF-alpha, IL-1beta and IL-10 of HUVECs induced by Ang II. (a) TNF-alpha level in supernatants of HUVECs; (b) IL-1beta level in supernatants of HUVECs; (c) IL-10 level in supernatants of HUVECs. 1, blank control group; 2, Ang II (10 − 5 mol/L) group; 3, Ang II (10 − 5 mol/L) + AqE (0.15 mg/ml) group; 4, Ang II (10 − 5 mol/L) + AqE (0.3 mg/ml) group; 5, Ang II (10 − 5 mol/L) + AqE (0.6 mg/ml) group; 6, Ang II (10 − 5 mol/L) + AqE (1.2 mg/ml) group; 7, Ang II (10 − 5 mol/L) + XJEK (1.6 mg/ml) group. Data are expressed as mean ± SD, n = 6. ** P



MT inhibited the production of pro-inflammatory proteins in sleep-deprived rats. (A) Western blot bands showing the protein expression levels of IL-1beta, IL-6, TNF-alpha, iNOS, and COX2 in the HP, respectively. (B–F) Relative protein expression level of IL-1beta, IL-6, TNF-alpha, iNOS, and COX2 in the HP, respectively. The data are expressed as the means ± SEM. # p < 0.05, ## p < 0.01, ### p < 0.001 vs Control group; * p < 0.05, ** p < 0.01 vs. Model group.Index in PubMed under a CC BY license. PMID: 39101143

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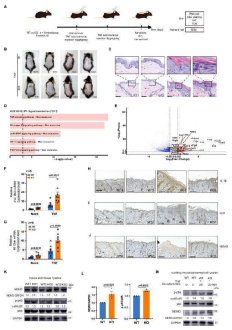
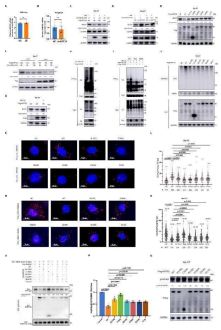


Skin phenotype in Krt32 KO mice induced by TNF. A Schematic representation of the experimental schedule. Eight-week-old Krt32 wildtype (WT) and knockout (KO) C57BL/6J mice (n = 8 mice/group) were subcutaneously injected with TNF at a dose of 6 ug/kg/day body weight into their shaved dorsal skin for a duration of 48 h. The dorsal skin of Krt32 (−/−) mice exhibited pronounced thickening and extensive yellow scaling, resembling the cutaneous manifestations observed in human patients with pityriasis rubra pilaris (PRP). Skin, hair, and nail samples were collected for RNA-seq, H&E staining, transmission electron microscopy (TEM), and scanning electron microscopy (SEM) analysis. B , C Two representative photos of the dorsal skin of WT and Krt32 KO mice (n = 8 mice/group) treated by TNF, along with H&E staining showing lymphocytes and hair follicular plugging indicated by red and black arrows respectively. Results from another six mice are presented in Supplementary Fig. A, B. D Selected KEGG signal transduction pathways identified for significant DEGs (P



The interaction of KRT32 with NEMO promotes NEMO degradation via K48-linked polyubiquitination modification and also inhibits the formation of the IKK complex. KRT32 expression level does not affect the transcription of IKBKG (the gene encoding NEMO) in Ker-CT cells. The mRNA level of NEMO in Ker-CT cells overexpressing KRT32 detected using RNA-seq (A) and in KRT32 knockdown cells detected by RT-qPCR analysis (B). Data are means ± SEM of three independent experiments in (A) and (B), and P value was calculated using a two-sided unpaired Student’s t test. C , D Immunoblotting of NEMO in Ker-CT cells with overexpressing KRT32 and knockdown with and without TNF treatment. E The impact of KRT32 wildtype and mutations overexpression on NEMO protein level in Ker-CT cells analyzed by Western blot. F Cycloheximide (CHX) chase assay to analyze the protein stability of NEMO in Ker-CT cells overexpressing KRT32. Cells were treated with 50 μg/ml CHX for indicated time. G The levels of NEMO were measured by western blotting with 5 uM MG132 in Ker-CT cells overexpressing KRT32 wildtype or negative control for 12 h. H Co-immunoprecipitation of HA-KRT32 and Flag-NEMO in HEK 293T cells, followed by detection of ubiquitin levels of NEMO by Western blotting. I Co-transfection of Flag-NEMO with HA-Ub-WT, HA-Ub-K48 or K48R (containing lysine at residue K48 or lysine to arginine mutation at residue K48) along with Myc-KRT32 in HEK 293 T cells, followed by immunoprecipitation and Western blotting analysis. J Immunoprecipitation of lysates with NEMO antibody from Ker-CT cells overexpressing KRT32 wildtype or mutations, and then detect the Ub-K48 modification of NEMO. PLA analysis in Ker-CT cells overexpression KRT32 wildtype and mutations to assess the interaction of Ub with NEMO (K) and IKKalpha with NEMO (M). Scale bars represent 10 um. L , N Quantification of the number of PLA foci per cell detected in (K) and (M), separately. Each dot on the graph corresponds to a specific analyzed cell. Red bars represent the mean ± SEM from the indicated number (N) of cells. The number of cells analyzed per group varies as follows: N

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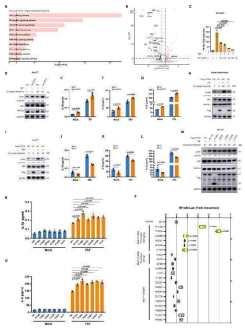
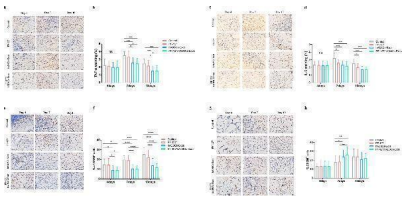
= 315, 302, 302, 310, 302, 315, 313, 311 in (L) and N = 243, 211, 214, 220, 217, 214, 205, 208 in (N), with each group consisting of three biological replicates. P value was calculated using a two-sided unpaired Student's t test. O GST-NEMO (120-419aa) fusion protein was incubated with excess E. coli extracts containing His-KRT32 (wildtype or six mutants), His-IKKalpha, and His-IKKbeta. GST complex was pulled down with glutathione-Sepharose beads, and the protein complexes were analyzed by western blotting. P Statistical analysis was performed on the binding ability of IKKalpha/beta and NEMO with wildtype KRT32 and its mutations addition from tree-independent experiments of (O). Data shown as means ± SEM of three independent experiments in (P). P value was calculated using a two-sided unpaired Student's t test. Q Immunoblotting assay of phosphorylated IKKalpha/beta in Ker-CT cells overexpressing KRT32 wildtype and mutations. One representative experiment from two independent experiments with similar results is shown in (C – G) and (J , Q). One representative experiment from three independent experiments with similar results is shown in (O). Source data are provided as file. Index in PubMed under a CC BY license. PMID: 39048559



KRT32 inhibits the activation of NF-kappaB signaling pathway. A Selected KEGG signal transduction pathway enrichment analysis with significant DEGs (FDR<0.05 and log 2 FC<−0.585) through RNA sequencing of Ker-CT cells with KRT32 overexpression. The statistical test was hypergeometric test, and the level of significance was set at a two-sided P



PF-127/hADSCs-Exos complex treatment inhibits inflammatory reaction. a Representative images of TNF-alpha immunostaining at 4, 7, and 10 days after treatment. Scale bar = 20 μm. b Quantification of TNF-alpha + IHC stained tissues. c Representative images illustrating IHC results of IL-6 at 4, 7, and 10 days after surgery. Scale bar = 20 μm. d Quantification of IL-6 + IHC stained tissues. e IHC images of wound sections stained with CD68 on days 4, 7, and 10 post-wounding. Scale bar = 20 μm. f Quantification of the number of CD68 positive cells in the wound area on days 4, 7, and 10. g IHC images of wound sections stained with CD206 at days 4, 7, and 10 post-wounding. Scale bar = 20 μm. h Quantification of the number of CD206 positive cells in the wound area on days 4, 7, and 10. In b, d, and f , data are shown as mean ± SEM; n = 6 for each group. * p

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3. PubMed ID: -, Yang Ping,Yingpeng Li,Shaowa Lü,Yali Sun,Wanmeng Zhang,Jialin Wu,Ting Liu,Yongji Li,A study of nanometre aggregates formation mechanism and antipyretic effect in Bai-Hu-Tang, an ancient Chinese herbal decoction,Biomedicine & Pharmacotherapy,Volume 124,202

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