

## Anti-GPX4 Rabbit Monoclonal Antibody

Catalog Number: M02059

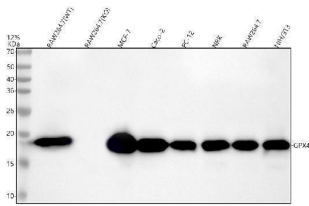
### Overview

Product Name	Anti-GPX4 Rabbit Monoclonal Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-GPX4 Rabbit Monoclonal Antibody catalog # M02059. Tested in WB, IHC, ICC/IF applications. This antibody reacts with Human, Mouse, Rat.
Application	IF, IHC, ICC, WB
Clonality	Monoclonal ACCO-7
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	P36969

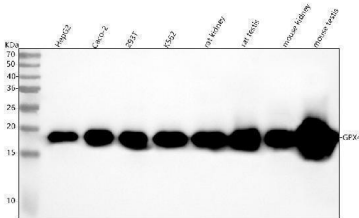
### Technical Details

Immunogen	A synthesized peptide derived from human GPX4
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

## Anti-GPX4 Rabbit Monoclonal Antibody (M02059) Images

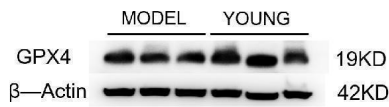


Western blot analysis of GPX4 using anti-GPX4 antibody (M02059). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: mouse RAW264.7(WT) whole cell lysates, Lane 2: mouse RAW264.7(KO) whole cell lysates, Lane 3: human MCF-7 whole cell lysates, Lane 4: human Caco-2 whole cell lysates, Lane 5: rat PC-12 whole cell lysates, Lane 6: rat NRK whole cell lysates, Lane 7: mouse RAW264.7 whole cell lysates, Lane 8: 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-GPX4 antigen affinity purified monoclonal antibody (M02059) 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 Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for GPX4 at approximately 19 kDa. The expected band size for GPX4 is at 22 kDa.

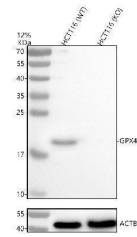


Western blot analysis of GPX4 using anti-GPX4 antibody (M02059). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HepG2 whole cell lysates, Lane 2: human CACO-2 whole cell lysates, Lane 3: human 293T whole cell lysates, Lane 4: human K562 whole cell lysates, Lane 5: rat kidney tissue lysates, Lane 6: rat testis tissue lysates, Lane 7: mouse kidney tissue lysates, Lane 8: mouse testis 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-GPX4 antigen affinity purified monoclonal antibody (Catalog # M02059) 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 1:1000 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 GPX4 at approximately 19 kDa. The expected band size for GPX4 is at 22 kDa.

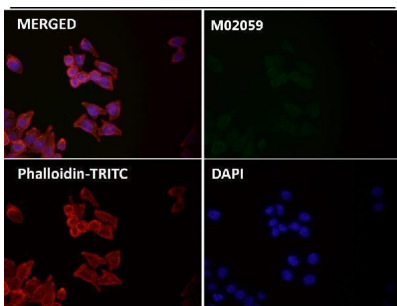
Western blot analysis of GPX4 using anti-GPX4 antibody (M02059). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug



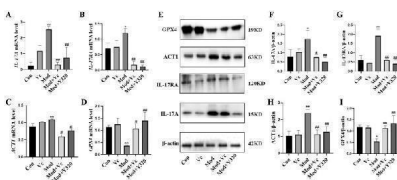
of sample under reducing conditions. Lane 1-3: model group-mouse uterine tissue lysates, Lane 4-6: young group-mouse uterine tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-GPX4 antigen affinity purified monoclonal antibody (M02059) 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 (Catalog # BA1054) at a dilution of 1:5000 for 1 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 GPX4 at approximately 19 kDa. The expected band size for GPX4 is at 19 kDa.



Western blot analysis of GPX4 using anti-GPX4 antibody (M02059). 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  $\mu$ g of sample under reducing conditions. Lane 1: human HCT116- WT whole cell lysates, Lane 2: human HCT116-GPX4 KO 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. Then the membrane was incubated with rabbit anti-GPX4 antigen affinity purified monoclonal antibody (M02059) at 0.5  $\mu$ g/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 (Catalog # BA1054) 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 GPX4 at approximately 19 kDa. The expected band size for GPX4 is at 22 kDa.

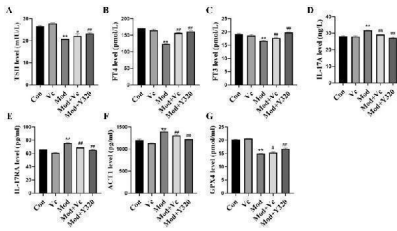


Immunofluorescent analysis using the Antibody at 1:500 dilution.

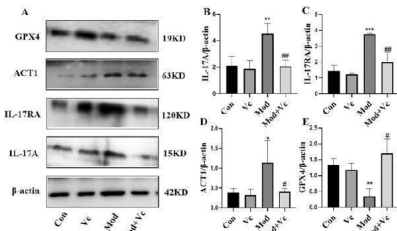


Effects of Vitamin C intervention on the IL-17A/IL-17RA/ACT1 signaling pathway and ferroptosis in FRTL5 cells stimulated by PM2.5. (A-D) qPCR was used to detect the transcription levels of IL-17A/IL-17RA/ACT1 signaling pathway-related factors. (E-I) The protein expression levels of the IL-17A/IL-17RA/ACT1 signaling pathway were detected by western blot. The data are presented as mean  $\pm$  SD; n = 3/group. Compared with the Con group, \* P<0.05 or \*\* P<0.01; Compared with the Mod group, # P<0.05 or ## P<0.01. Con: Control group, normal medium for 24 h; Vc:

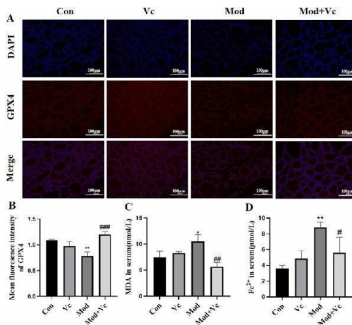
medium containing 50 umol/L Vc for 24 h; Mod: medium containing 400 ug/mL PM2.5 for 24 h; Mod + Vc: medium containing 400 ug/mL PM2.5 and 50 umol/L Vc for 24 h; Mod + Y320: medium containing 400 ug/mL PM2.5 and 0.08 umol/L Y320 for 24 h. Index in PubMed under a CC BY license. PMID: 40753780



Effect of Vitamin C on FRTL5 cell function and IL-17A signaling pathway and ferroptosis-related factors stimulated by PM2.5. (A-C) The levels of (A, TSH; B, FT4; and C, FT3) in the supernatant of FRTL5 cells were detected by ELISA. (D-G) ELISA was used to measure the levels of IL-17A (D), IL-17RA (E), ACT1 (F), and GPX4 (G) in the supernatant of FRTL5 cells. The data are presented as mean  $\pm$  SD; n = 5/group). Compared with the Con group, \*\* P<0.01; Compared with the Mod group, # P<0.05 or ## P<0.01. Con: Control group, normal medium for 24 h; Vc: medium containing 50 umol/L Vc for 24 h; Mod: medium containing 400 ug/mL PM2.5 for 24 h; Mod + Vc: medium containing 400 ug/mL PM2.5 and 50 umol/L Vc for 24 h; Mod + Y320: medium containing 400 ug/mL PM2.5 and 0.08 umol/L Y320 for 24 h. Index in PubMed under a CC BY license. PMID: 40753780

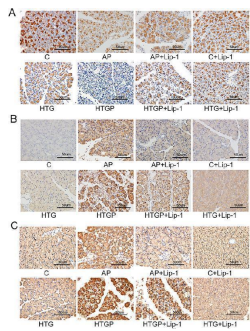


Vitamin C regulated the protein expression levels of IL-17A signaling pathway and ferroptosis-related factors. (A) The expression levels of the IL-17A pathway (B, IL-17A; C, IL-17RA; D, ACT1) and ferroptosis (E, GPX4)-related genes in the thyroid tissues were determined by western blot (n = 3/group). The data are presented as mean  $\pm$  SD. Compared with the Con group, \*P<0.05, \*\*P<0.01, or \*\*\*P<0.001; Compared with the Mod group, # P<0.05 or ## P<0.01. Con: Control group, normal environment for eight weeks; Vc: vitamin C was administered by gavage at 120 mg/kg for eight weeks; Mod: PM2.5 exposure for eight weeks; Mod + Vc: after PM2.5 exposure, vitamin C was administered by gavage at 120 mg/kg for eight weeks. Index in PubMed under a CC BY license. PMID: 40753780

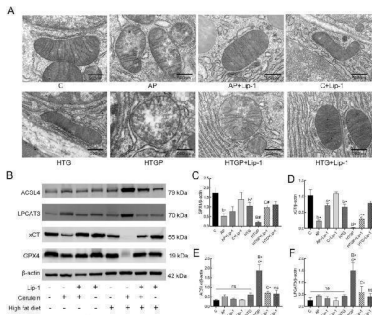


Vitamin C improved PM2.5-induced female rats' thyroid ferroptosis. (A) Immunofluorescence assay was used to detect the expression of GPX4 protein in thyroid tissues. (B) Quantitative immunofluorescence analysis (n = 3/group). (C) Serum malondialdehyde (MDA) content. (D) Serum ferrous ion (Fe<sup>2+</sup>) content (n = 10/group). The data are presented as mean  $\pm$  SD. Compared with the Con group, \*P<0.05 or \*\* P<0.01; Compared with the Mod group, # P<0.05, ## P<0.01, or ### P<0.001. Con: Control group, normal environment for eight weeks; Vc: vitamin C was administered by gavage at 120 mg/kg for eight weeks; Mod: PM2.5 exposure for eight weeks; Mod + Vc: after PM2.5 exposure, vitamin C was administered by gavage at 120 mg/kg for eight weeks. Index in PubMed under a CC BY license. PMID: 40753780

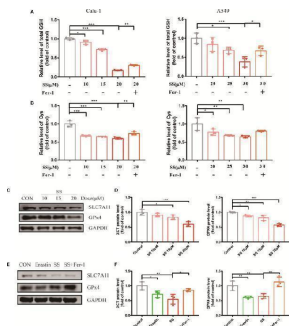
Immunohistochemically stained pancreas tissue ( n = 6).



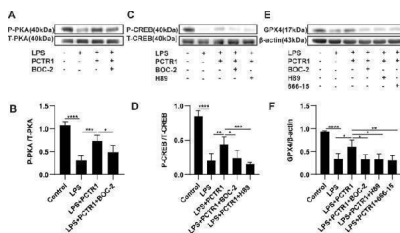
Protein levels of ( A ) GPX4, ( B ) ACSL4, and ( C ) LPCAT3 in rats (×400). GPX4, glutathione peroxidase 4; xCT, cysteine/glutamate transporter; ACSL4, acyl-CoA synthetase long-chain family member 4. Index in PubMed under a CC BY license. PMID: 38664508



Ferroptosis was observed and alleviated in the pancreas of rats ( n = 6 ). ( A ) Ultrastructure of the mitochondria in the pancreas obtained by TEM (×20,000); ( B - F ) Western blot analysis of ferroptosis-related proteins, GPX4, xCT, ACSL4, and LPCAT3. GPX4, glutathione peroxidase 4; xCT, cysteine/glutamate transporter; ACSL4, acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; C, control; AP, acute pancreatitis; HTG, hypertriglyceridemic; HTGP, HTG pancreatitis; \*b vs. the C group, \*c vs. the AP group, \*B vs. the HTG group, \*C vs. the HTGP group, \* P

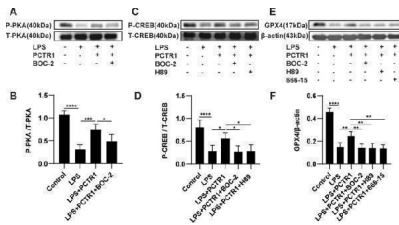


SS attenuated the oxidation resistance of LUAD cells. ( A, B ) GSH or Cys was detected in Calu-1 or A549 cells, which were respectively treated with 10, 15, 20 uM or 20, 25, 30 uM SS for 6 h, and pretreated with or without Fer-1 (1 uM), the data statistic was shown in a histogram (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001). ( C ) Western blot analysis was used to detect the expressions of SLC7A11 and GPX4 in Calu-1 cells, which were treated with 10, 15, 20 uM SS for 6 h. ( D ) Quantitative analysis of gray value of the SLC7A11 and GPX4 blots. ( E ) Western blotting analysis was used to detect the expressions of SLC7A11 and GPX4 in Calu-1 cells, which were treated with 20 uM SS or 4 uM erastin, with or without Fer-1 (1 uM) for 6 h. ( F ) Quantitative analysis of gray value of the SLC7A11 and GPX4 blots. Index in PubMed under a CC BY license. PMID: 35664792

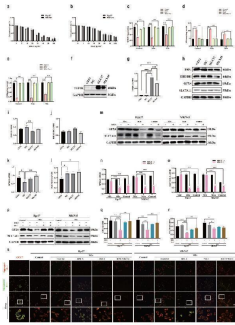


PCTR1 activates CREB by the ALX/PKA pathway to increase GPX4 expression in vitro. BOC-2 (ALX receptor inhibitor, 10 uM), H89 (PKA inhibitor, 10 uM), 666-15 (CREB inhibitor, 1 uM) or an equivalent volume of DMSO was administered to H1299 cells for 30 min in advance, and then LPS and PCTR1 were co-administered for 48 h. A , B The protein level of P- PKA was measured by western blot. C , D The protein level of P-CREB was measured by western blot. E , F The protein level of GPX4 was measured by western blot. Data are presented as the mean ± SD, n = 5-6. \* p

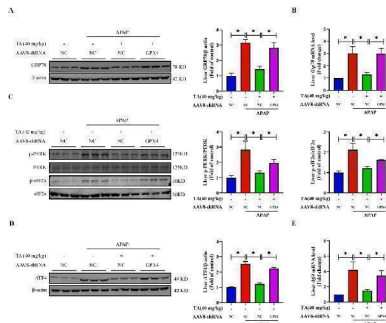
PCTR1 activates CREB via the ALX/PKA pathway to increase GPX4 expression in vivo. PCTR1 at a dose of 200 ng was injected into each mouse via the caudal vein 6 h after LPS (15 mg/kg, ip) administration. BOC-2 (ALX receptor inhibitor, 600 ng/kg), H89 (PKA inhibitor, 10 mg/kg), 666-15 (CREB



inhibitor, 10 mg/kg) or an equivalent volume of DMSO was injected into the caudal vein 1 h before PCTR1 treatment. The mice were sacrificed 24 h after LPS stimulation. **A**, **B** The protein expression level of P-PKA was determined by western blotting. **C**, **D** The protein expression level of P-CREB was determined by western blotting. **E**, **F** The protein expression level of GPX4 was determined by western blotting. Data are presented as the mean  $\pm$  SD, n = 4-6. \* p

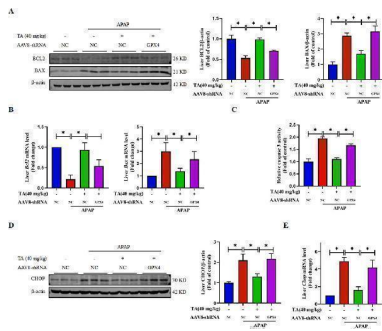


M2c macrophages increase ferroptosis resistance in gastric cancer cells. **a** The CCK-8 method was used to detect the survival of gastric cancer cells (Hgc27 and MKN45) intervened with RSL3 for 24 h. **b** The CCK-8 method was used to detect the survival of gastric cancer cells (Hgc27 and MKN45) intervened with Fer-1 for 24 h. **c** The expression of SOD in different intervention groups. **d** The expression of MDA in different intervention groups. **e** The expression of GSH in different intervention groups. **f** The expression of TGFβ1 protein WB in different cell lines. **g** The expression results of TGFβ1 protein. **h** The expression of key ferroptosis proteins WB in different cell lines. **i** The expression results of FSP1 protein. **j** Expression results of DHODH protein. **k** Expression results of GPX4 protein. **l** SLC7A11 protein expression results. **m** The intervention of RSL3 on the expression of key ferroptosis protein WB in different co culture groups. **n** The expression results of GPX4 protein. **o** SLC7A11 protein expression results. **p** The WB expression of key proteins involved in ferroptosis in different intervention groups. **q** The expression results of GPX4 protein. **r** The expression results of SLC7A11 protein. **s** Fluorescence results of mitochondrial membrane potential in different intervention groups. Scale bar=50 um. \*p

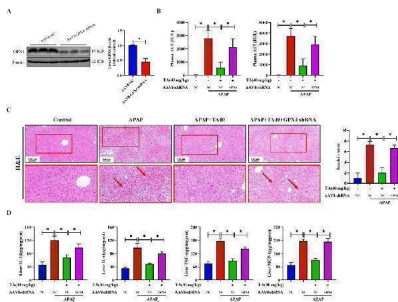


Liver-specific GPX4 knockdown inhibits TA-regulated ER stress in APAP-induced hepatotoxicity. **(A)** Western blotting assessed hepatic GRP78 expression, with beta-actin as a loading control. Band intensities were quantified using ImageJ. **(B)** q-PCR was conducted to evaluate the transcript levels of the Grp78 gene. **(C)** Western blotting assessed hepatic phosphorylated-PERK, phosphorylated-eIF2alpha, PERK, and eIF2alpha protein expressions. Band intensities were quantified using ImageJ. **(D)** Western blotting assessed hepatic ATF4 expression, with beta-actin as a loading control. Band intensities were quantified using ImageJ. **(E)** q-PCR was conducted to evaluate the transcript levels of the Atf4 gene. All data were presented as mean  $\pm$  SD, n = 3 for Western blotting, n = 5 for others. \* P < 0.05 vs. corresponding control. Index in PubMed under a CC BY license. PMID: 40051561

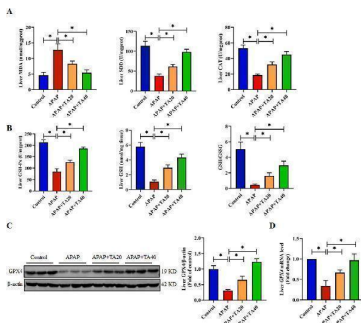
Liver-specific GPX4 knockdown abrogates the protective effect of TA against APAP-induced hepatocyte apoptosis. **(A)** Western blotting assessed hepatic BCL2 and BAX expression, with beta-actin as a loading control. Band intensities were quantified using ImageJ. **(B)** q-PCR was conducted to evaluate the transcript levels of BCL2 and BAX-related genes. **(C)** Hepatic caspase 3 activity was quantified



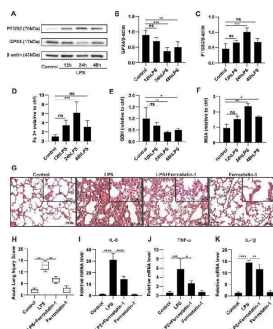
using a commercially available kit (fold of control). (D) Western blotting assessed hepatic CHOP expression, with beta-actin as a loading control. Band intensities were quantified using ImageJ. (E) q-PCR was conducted to evaluate the transcript levels of the Chop gene. All data were presented as mean  $\pm$  SD, n = 3 for Western blotting, n = 5 for others. \* P < 0.05 vs. corresponding control. Index in PubMed under a CC BY license. PMID: 40051561



Liver-specific GPX4 knockdown abrogates the hepatoprotective effects of TA against APAP-induced hepatotoxicity. (A) Western blotting assessed the detected hepatic GPX4 knockdown efficiency in protein levels. Hepatocyte-specific GPX4 knockdown mice were created by AAV8-mediated delivery of a TBG promoter-driven shRNA targeting GPX4. Null-vector-injected mice served as control. (B) Plasma levels of ALT and AST. (C) Liver tissues were subjected to H&E staining for histological examination. (D) Concentrations of hepatic IL-1beta, IL-6, TNF-alpha, and MCP-1 were quantified using commercially available ELISA kits. All data were presented as mean  $\pm$  SD, n = 3 for Western blotting, n = 5 for others. \* P < 0.05 vs. corresponding control. Index in PubMed under a CC BY license. PMID: 40051561

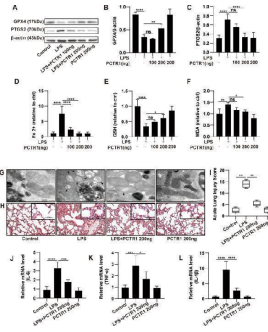


TA mediates the enhancement of hepatic antioxidant function by GPX4. (A) Hepatic concentrations of MDA, alongside the enzymatic activities of SOD and CAT. (B) Hepatic concentrations of GSH-Px, GSH, and the GSH redox ratio (GSH/GSSG) were determined. (C) Western blotting assessed hepatic GPX4 expression, with beta-actin as a loading control. Band intensities were quantified using ImageJ. (D) q-PCR was conducted to evaluate the transcript levels of the Gpx4 gene. All data were presented as mean  $\pm$  SD, n = 3 for Western blotting, n = 6-8 for others. \* P < 0.05 vs. corresponding control. Index in PubMed under a CC BY license. PMID: 40051561

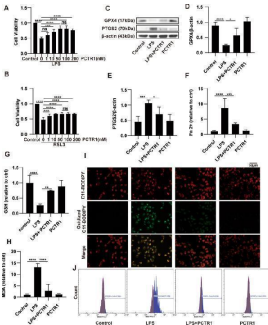


Ferroptosis is induced in LPS-induced ALI and associated with lung damage. LPS (15 mg/kg) in saline or an equivalent volume of saline was intraperitoneally injected into mice, and lung tissues were collected at 0, 12, 24, and 48 h, respectively. A-C Representative western blotting and quantification analysis of GPX4 and PTGS2. D-F Relative values of Fe<sup>2+</sup>, GSH and MDA concentrations. Mice were pretreated with ferrostatin-1 (10 mg/kg, ip) 1h before being injected with LPS (15 mg/kg, ip). Lung samples were collected 24 h after LPS injection. G Representative H&E staining of lung tissues (original magnification,  $\times$ 200; inset,  $\times$ 400). H Acute lung injury score of each group. I-K The relative mRNA expression levels of the inflammatory cytokines: IL-6, TNF-alpha and IL-1beta. The acute lung injury score data are presented as the median and range (25th-75th percentile), and other data are presented as the

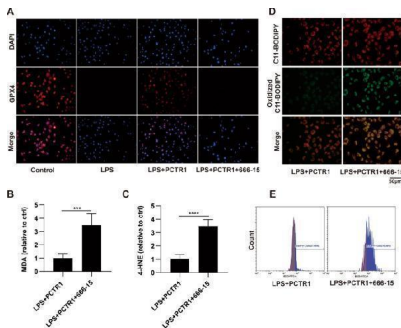
mean  $\pm$  SD. n = 4-6. \* p



Effects of PCTR1 on ferroptosis in LPS-induced ALI. PCTR1 (100 or 200 ng) was injected into the caudal vein of mice 6 h after LPS (15 mg/kg, ip) treatment. All lung specimens were harvested at 24 h after LPS stimulation. A-C Representative western blotting and quantification analysis of GPX4 and PTGS2. D-F Relative values of Fe<sup>2+</sup>, GSH and MDA concentrations. G Representative TEM images of each group. The black arrow indicates ferroptotic mitochondria. Magnification  $\times$ 30,000. H Representative H&E staining of lung tissues (original magnification,  $\times$ 200; inset,  $\times$ 400). I Acute lung injury score. J-L The relative mRNA expression levels of the inflammatory cytokines: IL-6, TNF- $\alpha$  and IL-1 $\beta$ . The acute lung injury score data are presented as the median and range (25th-75th percentile), and other data are presented as the mean  $\pm$  SD. n = 4-6. \* p



Effects of PCTR1 on LPS-induced ferroptosis in vitro. A H1299 cells were treated with LPS (10  $\mu$ g/mL) and different concentrations of PCTR1 for 48 h. Fold change in cell viability. B H1299 cells were stimulated with RSL3 (10 nM) and different concentrations of PCTR1 for 48 h. Fold change in cell viability. C-E Representative western blotting and quantification analysis of GPX4 and PTGS2. F-H Relative levels of Fe<sup>2+</sup>, GSH and MDA. I The level of lipid peroxidation was determined with the C11-BODIPY 581/591 fluorescent probe (original magnification  $\times$ 400). J Oxidized C11-BODIPY 581/591 probe was quantified by flow cytometry. Data are presented as the mean  $\pm$  SD, n = 4-6. \* p



CREB mediates the effect of PCTR1 on eliminating lipid peroxides in vitro. 666-15 (CREB inhibitor, 1  $\mu$ M) or an equivalent volume of DMSO was administered to H1299 cells for 30 min in advance, and then LPS and PCTR1 were co-administered for 48 h. A Immunofluorescence staining images of GPX4 (original magnification  $\times$ 400). B, C Relative expression levels of GSH, MDA and 4-HNE. D The level of lipid peroxidation was determined with the C11-BODIPY 581/591 fluorescent probe (original magnification  $\times$ 400). E Oxidized C11-BODIPY 581/591 probe was quantified by flow cytometry. Data are presented as the mean  $\pm$  SD, n = 5-6. \* p

## 1 Publications Citing This Product

1. PubMed ID: 33600944, Zhong B, Yu J, Hou Y, Ai N, Ge W, Lu JJ, Chen X. A novel strategy for glioblastoma treatment by induction of noptosis, an NQO1-dependent necrosis. *Free Radic Biol Med*. 2021 Feb 15;S0891-5849(21)00094-0. doi:10.1016/j.freeradbiomed.2021.02.014. Epub ahead of print. P

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### Anti-GPX4 Rabbit Monoclonal Antibody

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