abcam

ab45178

Anti-ASK1 antibody [EP553Y]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP553Y
Purification technique	Affinity purification Protein A
Concentration	1.005 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/50
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	For unpurified use at 1/2000

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/100 - 1/250
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information MAP3K5

Function	Serine/threonine kinase which acts as an essential component of the MAP kinase signal transduction pathway. Plays an important role in the cascades of cellular responses evoked by changes in the environment. Mediates signaling for determination of cell fate such as differentiation and survival. Plays a crucial role in the apoptosis signal transduction pathway through mitochondria-dependent caspase activation. MAP3K5/ASK1 is required for the innate immune response, which is essential for host defense against a wide range of pathogens. Mediates signal transduction of various stressors like oxidative stress as well as by receptor-mediated inflammatory signals, such as the tumor necrosis factor (TNF) or lipopolysaccharide (LPS). Once activated, acts as an upstream activator of the MKK/JNK signal transduction cascade and the p38 MAPK signal transduction cascade through the phosphorylation and activation of several MAP kinase kinases like MAP2K4/SEK1, MAP2K3/MKK3, MAP2K6/MKK6 and MAP2K7/MKK7. These MAP2Ks in turn activate p38 MAPKs and c-jun N-terminal kinases (JNKs). Both p38 MAPK and JNKs control the transcription factors activator protein-1 (AP-1).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Species reactivity
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The ASK1 protein also known as apoptosis signal-regulating kinase 1 is an important component in cellular stress response pathways. This 150 kDa protein is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family. ASK1 is expressed in several tissues including the heart brain liver and pancreas. Mechanically ASK1 gets activated in response to a variety of stress stimuli such as oxidative stress and endoplasmic reticulum stress which significantly impact the regulation of programmed cell death.
Biological function summary	In response to stressors ASK1 activates the c-Jun N-terminal kinase (JNK) and p38 MAPK signaling pathways which play roles in apoptosis and inflammation. ASK1 tends to form a complex with TRAF2 and TRAF6 key adapters in downstream signaling. This kinase acts as an important link bridging environmental stress signals to cellular responses. Through its function ASK1 influences cell fate decisions in response to various stress conditions.
Pathways	ASK1 is a central player in the MAPK signaling cascade. Within this cascade ASK1 regulates JNK and p38 MAPK pathways which are significant in stress adaptation and cellular responses. ASK1 interacts with proteins like MKK4 and MKK7 in the JNK pathway amplifying the stress signals. Through these interactions ASK1 ensures the propagation of signals important for adaptive responses or programmed cell death.
Associated diseases and disorders	Dysfunction in ASK1 activity associates with neurodegenerative diseases and cardiovascular disorders. In Alzheimer's disease for instance ASK1 contributes to neuronal apoptosis via its engagement with JNK signaling. In cardiovascular disorders ASK1 exacerbates heart failure by promoting cell death and

inflammation. ASK1's interaction with proteins such as TNF receptor-associated factor 2 (TRAF2) highlights its role in these pathological conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

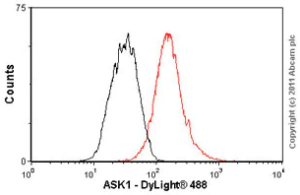
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

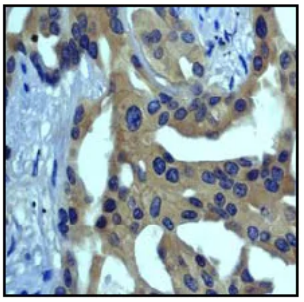
12 product images



Flow Cytometry (Intracellular) - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ASK1 antibody

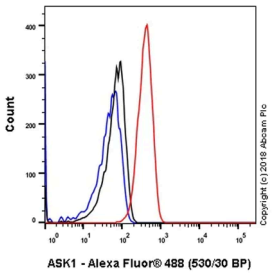
Overlay histogram showing HeLa cells stained with unpurified ab45178 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab45178, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit DyLight® 488 (IgG H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ASK1 antibody

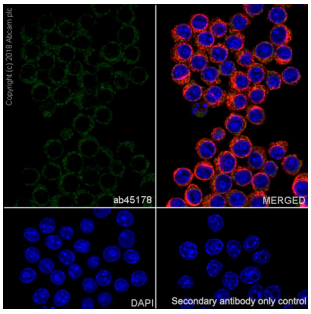
Unpurified ab45178 (1/10) staining human ASK1 in human lung carcinoma by immunohistochemistry using paraffin embedded tissue.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ASK1 antibody

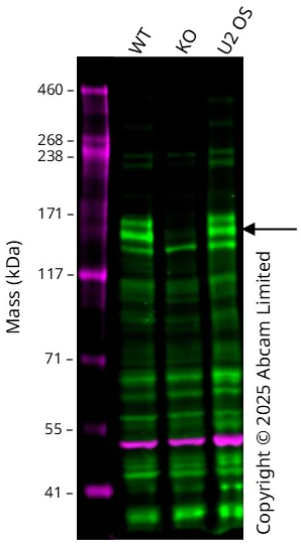
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling ASK1 with purified ab45178 at 1/100 dilution (10 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ASK1 antibody

Immunocytochemistry/ Immunofluorescence analysis of RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling ASK1 with Purified ab45178 at 1:100 (9.9 µg/ml). Cells were fixed in 100% Methanol. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Western blot staining using rabbit Anti-ASK1 antibody

Western blot: Rabbit Monoclonal [EP553Y] to ASK1 ab45178 staining at 1/2000 dilution, shown in green; Mouse anti alpha Tubulin (ab7291) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 150-170 kDa in Wild-type A549 cell lysates with no signal observed at this size in MAP3K5 knockout A549 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-ASK1 antibody [EP553Y] (ab45178) at 1/2000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:

Western blot - Human MAP3K5 knockout A549 cell line (ab301060) at 20 µg

Lane 3:
U-2 OS at 20 µg

Secondary

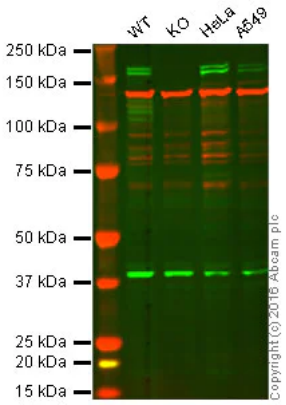
Lanes 1 - 3:
Goat anti-Rabbit 800CW at 1/20000 dilution

Lanes 1 - 3:
Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 155 kDa

Observed band size: 150-170 kDa, 50 kDa



Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

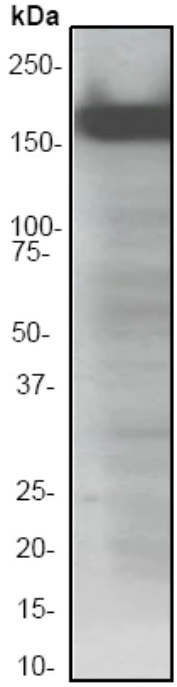
ASK1 Western blot staining using rabbit Anti-ASK1 antibody

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: ASK1 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: A549 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab45178 observed at 155, 160 kDa. Red - loading control, ab1240, observed at 124 kDa.

Unpurified ab45178 was shown to recognize ASK1 when ASK1 knockout samples were used, along with additional cross-reactive bands. Wild-type and ASK1 knockout samples were subjected to SDS-PAGE. ab45178 and ab1240 (loading control to Vinculin) were diluted to 1/2000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

Predicted band size: 155 kDa



Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

All lanes:
Western blot - Anti-ASK1 antibody [EP553Y] (ab45178) at 1/2000 dilution

All lanes:
HeLa cell lysate at 10 µg

Secondary

All lanes:
Goat anti-rabbit, HRP labelled at 1/2000 dilution

Predicted band size: 155 kDa

Observed band size: 155 kDa

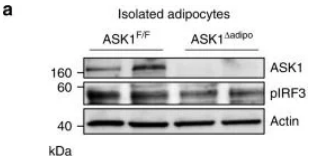


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Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Western blot staining using rabbit Anti-ASK1 antibody

Western Blotting using Anti-ASK1 antibody [EP553Y], ab45178. Publication image from Lucchini, F. C. et al., 2020, Nat Commun, 32242025. Legend direct from paper.

ASK1 phosphorylates IRF3.a, b Representative western blot and quantification of pIRF3 protein levels in isolated adipocytes of HFD-fed ASK1F/F and ASK1δadipo mice. n = 6 mice per group. *p = 0.048. c, d Representative western blot and quantification of pIRF3 upon incubation of recombinant IRF3 with or without active ASK1. n = 6 biological replicates. ***p = 0.0006. e, f Western blot and quantification of pIRF3 protein levels in subcutaneous adipocytes transfected with lentivirus expressing wild-type (pLV-CMV-ASK1; ASK1 wt) or kinase-negative (pLV-CMV-ASK1-K716R; ASK1 mut) ASK1. n = 3 biological replicates. #p = 0.070. g, h Representative western blot and quantification of pIRF3 protein levels in subcutaneous adipocytes transfected with control siRNA (Co siRNA) or siRNA targeting ASK1 (ASK1 siRNA) treated with or without 100 ng/ml LPS for 6 h. n = 4 biological replicates. ****p < 0.0001. iUcp1 mRNA expression in subcutaneous adipocytes transfected with control shRNA lentivirus (shLuc) or shRNA lentivirus targeting IRF3 (shIRF3) pre-treated with 100 ng/ml LPS for 24 h followed by stimulation with 0.1 µM isoproterenol for 6 h. n = 6 biological replicates. *p = 0.019. Values are expressed as mean ± SEM. Statistical tests used: two-sided t-tests for (b, d, f, i), two-sided one sample t-test for (h). Source data are provided as a Source Data file.

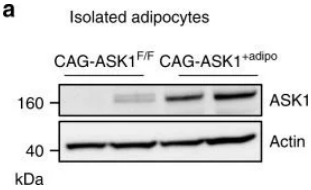


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Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Western blot staining using rabbit Anti-ASK1 antibody

Western Blotting using Anti-ASK1 antibody [EP553Y], ab45178. Publication image from Lucchini, F. C. et al., 2020, Nat Commun, 32242025. Legend direct from paper.

Adipocyte-specific ASK1 overexpression decreases adipose tissue browning.a Protein levels of ASK1 in lysate of isolated adipocytes harvested from CAG-ASK1F/F and CAG-ASK1+adipo mice (n = 2 mice per group). b mRNA expression of respective genes in inguinal adipose tissue of cold-exposed CAG-ASK1F/F (n = 5) and CAG-ASK1+adipo (n = 6) mice. #p = 0.094, *p = 0.042, *p = 0.017. c, d Representative western blot and quantification of UCP1 protein levels in inguinal adipose tissue harvested from cold-exposed CAG-ASK1F/F (n = 7) and CAG-ASK1+adipo (n = 8) mice. *p =

0.037. Values are expressed as mean ± SEM. Statistical tests used: two-sided t-tests for (b) (Ucp1 and Pgc1α), d two-sided Mann–Whitney for (b) (Cidea). Source data are provided as a Source Data file.

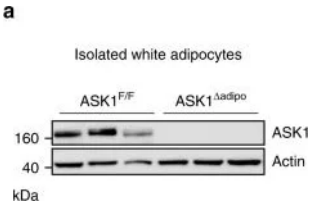


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Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Western blot staining using rabbit Anti-ASK1 antibody

Western Blotting using Anti-ASK1 antibody [EP553Y], ab45178. Publication image from Lucchini, F. C. et al., 2020, Nat Commun, 32242025. Legend direct from paper.

Reduced body weight and improved glucose tolerance in HFD-fed ASK1δadipo mice.a Protein levels of ASK1 in lysate of isolated epididymal adipocytes harvested from ASK1F/F and ASK1δadipo mice (n = 3 mice per group). b, c Body weight and body weight gain during 12 weeks of chow (ASK1F/F, n = 20 mice; ASK1δadipo, n = 13 mice) or HFD feeding (ASK1F/F, n = 52 mice; ASK1δadipo, n = 58 mice). **p = 0.02, ***p < 0.001. d, e Intraperitoneal glucose-tolerance test (chow-fed: ASK1F/F, n = 10 mice; ASK1δadipo, n = 8 mice; HFD-fed: ASK1F/F, n = 13 mice; ASK1δadipo, n = 14 mice) at 18 weeks of age. **p = 0.03, ***p < 0.001. f Glucose infusion rate (GIR) during hyperinsulinemic-euglycemic clamps in HFD-fed ASK1F/F (n = 6) and ASK1δadipo (n = 8) mice. *p = 0.011. g Glucose uptake into respective tissues during hyperinsulinemic-euglycemic clamps in HFD-fed ASK1F/F (n = 5 (ing WAT, epiWAT and skeletal muscle) or n = 6 (BAT)) and ASK1δadipo (n = 4 (BAT), n = 6 (ingWAT) or n = 7 (epiWAT and skeletal muscle)) mice. *p = 0.034, #p = 0.062. h Endogenous glucose production (EGP) during hyperinsulinemic-euglycemic clamps in HFD-fed ASK1F/F (n = 6) and ASK1δadipo (n = 8) mice. *p = 0.040. i Liver triglyceride levels determined in HFD-fed ASK1F/F (n = 6) and ASK1δadipo (n = 6) mice. *p = 0.012. j Mesenteric adipose tissue mRNA expression of respective targets determined in HFD-fed ASK1F/F (n = 5 (Il-10, F4/80) or n = 8 (TNFα, Il-6, Mcp1, Il-1β) and ASK1δadipo (n = 7 (Il-10, F4/80) or n = 9 (TNFα, Il-6, Mcp1, Il-1β)) mice. Values are expressed as mean ± SEM. Statistical tests used: two-sided t-tests for (f, g) (without adjustments for multiple comparisons), h, i ANOVA for (b–e). AUC: area under the curve. Source data are provided as a Source Data file.

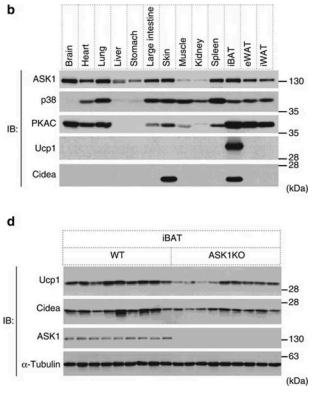


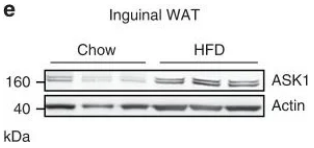
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Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

ASK1 Western blot staining using rabbit Anti-ASK1 antibody

Western Blotting using Anti-ASK1 antibody [EP553Y], ab45178. Publication image from Ichijo, H. et al., 2016, Nat Commun, 27045525. Legend direct from paper.

BAT function is impaired in ASK1-deficient mice.(a) qRT–PCR analysis of RNA isolated from tissues of adult WT mice. (b) Western blotting analysis of proteins isolated from tissues of adult WT mice. (c) qRT–PCR against indicated genes in iBAT (n=9). (d) Western blot against Ucp1 and Cidea in iBAT (n=9). (e,f) Band intensities of Ucp1 (e) and Cidea (f) were plotted (n=9). (g) VO2 of mice treated with CL316,243 (n=6). CL316,243 was injected i.p. at ☒1845 h. (h) Six-hour average of VO2 from 1900 h to 2400 h with or without CL316,243 injection (n=6). (i) VO2 of mice treated with CL316,243 (n=6, 8). CL316,243 was injected i.p. at ☒1845 h. (j) Six-hour average of VO2 from 1900 h to 2400 h with or without CL316,243 injection (n=6, 8). (b,d) The same amount of protein was loaded in each lane. (c,e,f) *P<0.05, **P<0.01, ***P<0.001 by unpaired two-tailed Student's t-test. (h,j) **P<0.01, ***P<0.001 by two-way ANOVA followed by Bonferroni's multiple comparisons test. All data are represented as the mean±s.e.m.



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Western Blotting using Anti-ASK1 antibody [EP553Y], ab45178. Publication image from Lucchini, F. C. et al., 2020, Nat Commun, 32242025. Legend direct from paper.

LPS treatment blunts UCP1 expression in subcutaneous adipocytes ASK1-dependently.a, b Western blot and quantified protein levels of UCP1 in total lysates of inguinal white adipose tissue harvested from cold-exposed C57BL/6J mice chronically treated with or without LPS. n = 3 mice per group. **p = 0.004. cUcp1 mRNA expression in inguinal fat explants harvested from C57BL/6J mice were treated with or without 100 ng/ml LPS and subsequently with 10 µM isoproterenol for 8 h. n = 3 biological replicates. *p = 0.036. dUcp1 mRNA expression in subcutaneous adipocytes pre-treated with or without 100 ng/ml LPS for 24 h followed by stimulation with 0.1 µM isoproterenol for 6 h. n = 7 (LPS) or n = 8 (control) biological replicates. **p = 0.007. e, f Western blot and quantified protein levels of ASK1 in total lysates of inguinal white adipose tissue harvested from C57BL/6J mice fed a chow or HFD for 20 weeks. n = 8 (chow) and n = 7 (HFD) mice per group. *p = 0.037. gAsk1 mRNA expression in subcutaneous adipocytes treated with or without 100 ng/ml LPS treatment for 24 h. n = 7 biological replicates. *p = 0.046. hUcp1 mRNA expression in subcutaneous adipocytes transfected with control shRNA lentivirus (shLuc) or shRNA lentivirus targeting ASK1 (shASK1) pre-treated with 100 ng/ml LPS for 24 h followed by stimulation with 0.1 µM isoproterenol for 6 h. n = 11 (shASK1 control and shLuc LPS) or n = 12 (shASK1 LPS and shLuc control) biological replicates. *p = 0.027. Values are expressed as mean ± SEM. Statistical tests used: two-sided t-tests for (b–d, f–h). Source data are provided as a Source Data file.

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