abcam

ab140601

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589]

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) is a rabbit monoclonal antibody detecting Ubiquitin in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat,**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 120 publications

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	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	EP8589
Purification technique	Affinity purification Protein A
Specificity	This antibody only recognizes polyubiquitin chains formed by Lys-48 (K48) residue linkage. This antibody can detect the target in mouse and rat cell lines and induced tissues.
Concentration	1.454 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/200 - 1/10000
Notes	-

Species	Mouse
Dilution info	1/200 - 1/10000
Notes	-

Species	Rat
Dilution info	1/200 - 1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20 - 1/1000

Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

IHC-P

Tested

Species	Human
Dilution info	1/250
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information UBC Linkage-specific K48

Function

Ubiquitin. Exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains) or a linear polymer linked via the initiator Met of the ubiquitin (linear polyubiquitin chains). Polyubiquitin chains, when attached to a target protein, have different functions depending on the Lys residue of the ubiquitin that is linked: Lys-6-linked may be involved in DNA repair; Lys-11-linked is involved in ERAD (endoplasmic reticulum-associated degradation) and in cell-cycle regulation; Lys-29-linked is involved in proteotoxic stress response and cell cycle; Lys-33-linked is involved in kinase modification; Lys-48-linked is involved in protein degradation via the proteasome; Lys-63-linked is involved in endocytosis, DNA-damage responses as well as in signaling processes leading to activation of the transcription factor NF-kappa-B. Linear polymer chains formed via attachment by the initiator Met lead to cell

signaling. Ubiquitin is usually conjugated to Lys residues of target proteins, however, in rare cases, conjugation to Cys or Ser residues has been observed. When polyubiquitin is free (unanchored-polyubiquitin), it also has distinct roles, such as in activation of protein kinases, and in signaling. During ubiquitination, the acceptor ubiquitin is positioned in the active site via direct interaction with the E2 ubiquitin-conjugating enzymes such as UBE2R2 (PubMed:38326650). As a monoubiquitin, its C-terminal glycine is recognized as a C-degron by Cul2-RING (CRL2) E3 ubiquitin-protein ligase complexes (PubMed:39548056).

Storage

Shipped at conditions	Conditional Ambient
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

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, WB in human, mouse, rat, recombinant fragment samples.

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) has been cited over 129 times in peer reviewed journals and is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) has 10 independent reviews from customers.

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) specifically detects Ubiquitin -K48 (UniProt ID: P0CG48; Molecular weight: 9kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone EP8589 - ab271911.

Antibody clone EP8589 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 594, PE, APC, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 750 (ab204274, ab204476, ab206366, ab303017, ab303018, ab312065, ab312542, ab321118).

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For

details on our patents, please refer to RabMAb® patents.

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.

Supplementary info

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

Activity summary	Ubiquitin is a small regulatory protein found in almost all tissues of eukaryotic organisms. It has a molecular weight of approximately 8.6 kilodaltons. It functions mechanically by attaching to proteins through a process called ubiquitination which involves the formation of an isopeptide bond. Ubiquitin molecules can form polyubiquitin chains through different lysine residues such as K48 and K63 that determine their function. These chains label substrate proteins for various fates including degradation. Ubiquitin is expressed ubiquitously in cells reflecting its essential role in maintaining protein homeostasis.
Biological function summary	The ubiquitin system plays a critical role in regulating protein turnover and quality control within cells. It is part of a larger complex known as the ubiquitin-proteasome system (UPS) which is responsible for degrading proteins that need to be turned over. This process is essential for cell cycle control response to oxidative stress and DNA repair. Ubiquitin's role in tagging proteins for degradation or signaling allows cells to respond quickly to changes in their environment and maintain balance.
Pathways	Ubiquitin functions in several important biological pathways including the Wnt and NF-kB pathways. In the Wnt signaling pathway ubiquitination modulates the stability of key components thereby affecting the pathway's overall activity. In the NF-kB signaling pathway ubiquitin labels inhibitor proteins for degradation which releases and activates NF-kB. These pathways highlight ubiquitin's interaction with proteins such as beta-catenin in Wnt and IkB in NF-kB illustrating how it regulates diverse cellular processes.
Associated diseases and disorders	The dysfunction of the ubiquitin system is linked to neurodegenerative diseases and cancers. Ubiquitin-related defects in protein degradation can lead to the buildup of unwanted proteins contributing to conditions like Parkinson's disease. Connections with cancer are evident as ubiquitin controls cell cycle proteins and aberrant ubiquitination may drive tumor growth and progression. The protein p53 known to be controlled by ubiquitination plays a significant role in cancer related mechanisms when dysregulated. Understanding and targeting ubiquitin-related pathways may provide new therapeutic opportunities for treating these 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.

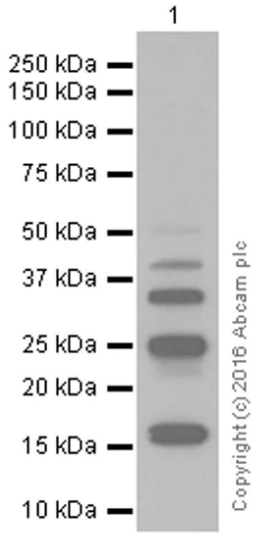
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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11 product images



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Blocking and diluting buffer: 5% NFDM/TBST. This image is produced useing purified ab140601.
All lanes:
Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/1000 dilution

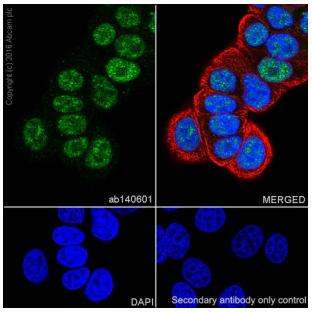
All lanes:
K48-linked-Ub2-7recombinant protein lysate at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 26 kDa, 35 kDa, 38 kDa, 40 kDa, 58 kDa, 79 kDa

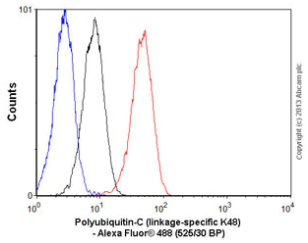
Observed band size: 26 kDa, 38 kDa, 39 kDa, 42 kDa, 78 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Ubiquitin (linkage-specific K48) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

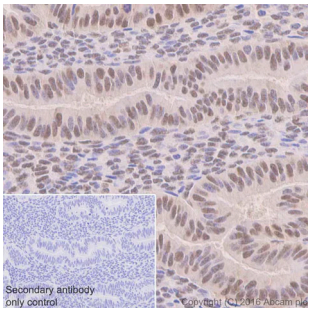
Purified ab140601 staining Ubiquitin (linkage-specific K48) in MCF7 (Human breast adenocarcinoma cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. ab195889 was used as a counterstain for primary antibody ab133645 at 1/2000. DAPI was used as a nuclear counterstain and PBS as a negative control.



Flow Cytometry (Intracellular) - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Ubiquitin (linkage-specific K48) Flow Cytometry (Intracellular) staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

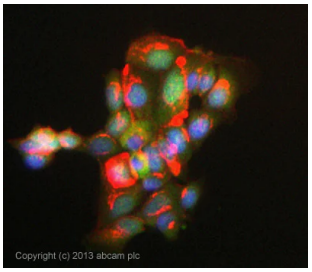
Overlay histogram showing HeLa cells stained with ab140601 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab140601, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 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. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Ubiquitin (linkage-specific K48) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

Purified ab140601 staining Ubiquitin (linkage-specific K48) in human endometrium carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody at a dilution of 1/500.
Negative control 1: PBS in place of primary antibody.

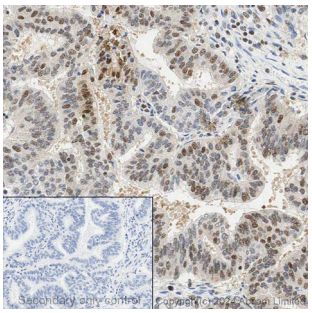


Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Ubiquitin (linkage-specific K48) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

ICC/IF image of ab140601 stained MCF-7 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then

incubated with the antibody ab140601 at 10µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

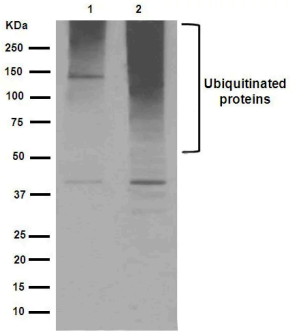


Immunohistochemistry - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Immunohistochemical analysis of formalin fixed paraffin embedded human endometrial carcinoma labelling Ubiquitin (linkage-specific K48) with ab140601 at a concentration of 1µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5 for 32 mins.

ab140601 anti-Ubiquitin (linkage-specific K48) [EP8589] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Observed band is above 60kDa
All lanes:
Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/200 dilution

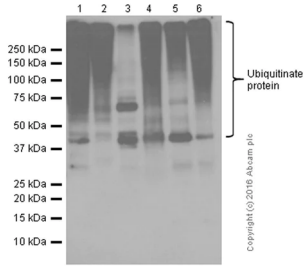
Lane 1:
HEK293 at 10 µg

Lane 2:
Jurkat at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 26 kDa



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Blocking and diluting buffer: 5% NFDM/TBST. This image is produced useing purified ab140601.
All lanes:
Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 200 µg

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Lane 2:
293 (Human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 3:
Mouse heart lysate at 20 µg

Lane 4:
Rat heart lysate at 20 µg

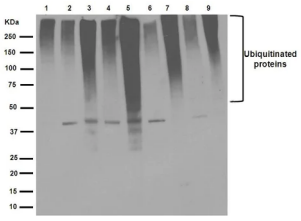
Lane 5:
C2C12 (Mouse myoblasts myoblast) whole cell lysate at 20 µg

Lane 6:
C6 (Rat glial tumor glial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 26 kDa



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Ubiquitin (linkage-specific K48) Western blot staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

Observed band is above 60kDa
All lanes:
Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/200 dilution

Lane 1:
PC-12 at 20 µg

Lane 2:
C6 at 20 µg

Lane 3:
L6 at 20 µg

Lane 4:
C2C12 at 20 µg

Lane 5:
Neuro-2a at 20 µg

Lane 6:
NIH3T3 at 20 µg

Lane 7:
SP2/0 at 20 µg

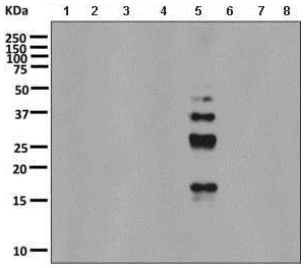
Lane 8:
Raw264.7 at 20 µg

Lane 9:
B16-F0 at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 26 kDa



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

All lanes:
Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/1000 dilution

Lane 1:
K6-linked-Ub2 recombinant protein at 0.01 µg

Lane 2:
K27-linked-Ub2 recombinant protein at 0.01 µg

Lane 3:
K29-linked-Ub2 recombinant protein at 0.01 µg

Lane 4:
K11-linked-Ub2 recombinant protein at 0.01 µg

Lane 5:
K48-linked-Ub2-7recombinant protein at 0.01 µg

Lane 6:
K63-linked-Ub2-7 recombinant protein at 0.01 µg

Lane 7:
K33-linked-Ub2 recombinant protein at 0.01 µg

Lane 8:
monoubiquitin recombinant protein at 0.01 µg

Secondary

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

Predicted band size: 26 kDa

Observed band size: 17-60 kDa

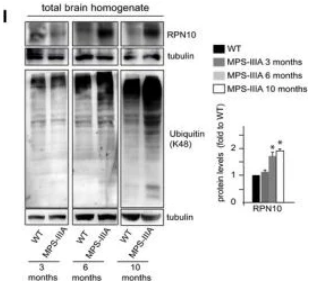


Image collected and cropped by CiteAb under a CC-BY license from the publication

Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

Ubiquitin (linkage-specific K48) Western blot staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

Ubiquitin (linkage-specific K48) western blot using anti-Ubiquitin (linkage-specific K48) antibody [EP8589] ab140601. Publication image and figure legend from Sambri, I., D'Alessio, R., et al., 2017, EMBO Mol Med, PubMed 27881461.

ab140601 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab140601 please see the product overview.

Lysosomal-driven deregulation of α-synuclein and CSPα degradationWB analysis of LC3 and p62 (an autophagy substrate) was performed on WT and MPS-IIIA brain samples at the indicated ages. WB quantitation is shown.α-Synuclein was immunoblotted in WT and MPS-IIIA in both total and synaptosomal brain fractions at the indicated ages after sequential extraction with detergents with increased strength. Soluble (Sol.), lowly insoluble (L. Insol.), and highly insoluble (H. Insol.) forms correspond to the protein solubilized in Triton X-100, 10% SDS and 8 M urea, respectively.Co-immunofluorescence analysis of α-synuclein with SMI-32 in WT and MPS-IIIA hippocampal neurons (DIV14). α-Synuclein synaptic puncta present in a neurite tract of 10 μm is shown in a representative enlarged image. Quantification of α-synuclein synaptic puncta was calculated from 30 different enlarged images.Confocal analysis of α-synuclein (green) and LAMP1 (red) in WT and MPS-IIIA hippocampal neurons (DIV14). Enlarged merge images are also shown. Co-localization was quantified using the MCC (ImageJ) and displayed as percentage (MCC × 100) of α-synuclein co-localizing with LAMP1 (15 different images taken from 4 to 5 coverslips for each group).CSPα was immunoblotted in WT and MPS-IIIA total brain lysates at the indicated ages after sequential extraction with detergents with increased strength as in (B).Co-immunofluorescence analysis of CSPα and SMI-32 in DIV14 hippocampal neurons. CSPα synaptic puncta was quantified as in (C).CSPα protein levels were evaluated by immunoblot analysis in WT and MPS-IIIA hippocampal neurons (DIV14) at different times after cycloheximide treatment and expressed as percentage of remaining protein at T0 (100%). The proteasome was inhibited as indicated. WB quantification is shown.Palmitoylation-dependent shift in the molecular weight of CSPα was evaluated in WT and MPS-IIIA brain samples at the indicated ages by immunoblot CSPα in boiled samples prepared without exposure to sulfhydryl agents (β-mercaptoethanol or dithiothreitol).The protein levels of RPN10 (the regulatory subunit of 26S proteasome) and ubiquitinated proteins formed by Lys48 (K48) residue linkage (involved in protein degradation via the proteasome) were evaluated in WT and MPS-IIIA brain samples at the indicated ages by WB analysis. WB quantitation is shown.Proteasome activity was evaluated by measuring the chymotrypsin-like activity in WT and MPS-IIIA mouse brain samples at different ages. Proteasome activity was expressed as percentage of WT activity.Data information: Data are means ± s.e.m.; N = 3 (biological triplicate) in WB quantitations. *$p < 0.05$, **$p < 0.001$, Student's t-test: MPS-IIIA at each age vs. WT (A, I, J); MPS-IIIA vs. WT (C, D, F); either WT or MPS-IIIA + prot. inhib. (at each time point) vs. MPS-IIIA (G). Scale bars: 10 μm (C, D, F).Source data are available online for this figure.

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