Product Datasheet

LC3A Antibody - BSA Free
NB100-2331

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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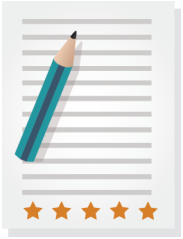
Updated 12/20/2023 v.20.1



NB100-2331

LC3A Antibody - BSA Free

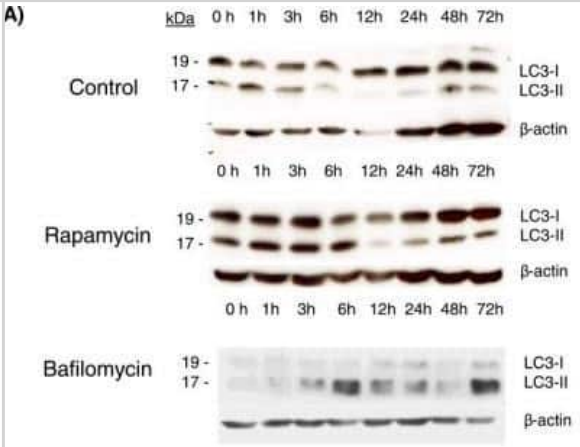
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	84557
Gene Symbol	MAP1LC3A
Species	Human, Mouse, Rat, Amphibian, Canine, Fish, Plant, Zebrafish
Reactivity Notes	Use in Rat reported in scientific literature (PMID:33678798). Use in Amphibian reported in scientific literature (PMID:29777142).
Marker	Autophagosome Marker
Specificity/Sensitivity	This LC3A Antibody detects both LC3A and LC3B.
Immunogen	This LC3A Antibody was prepared from a synthetic peptide made to an internal portion of the human LC3 protein sequence (between residues 25-121). [Uniprot: Q9H492].

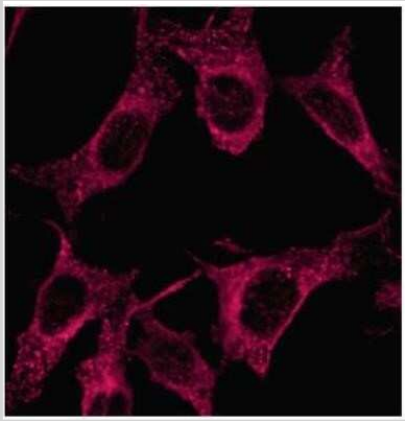
Product Application Details	
Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Southern Blot, Chromatin Immunoprecipitation (ChIP), Immunohistochemistry Whole-Mount
Recommended Dilutions	Western Blot 2.0 ug/ml, Simple Western 1:50, Chromatin Immunoprecipitation reported in scientific literature (PMID 33035707), Flow Cytometry reported in scientific literature (PMID 24419333), ELISA reported in scientific literature (PMID 20930550), Immunohistochemistry 1:200-1:400, Immunocytochemistry/ Immunofluorescence 1:100-1:300. Use reported in scientific literature (PMID 21545732), Immunoprecipitation 20 ug / 500 ug of lysate, Immunohistochemistry-Paraffin 1:200-1:400. Use reported in scientific literature (PMID 26571030), Immunohistochemistry-Frozen, Immunoblotting reported in scientific literature (PMID 28253371), Southern Blot, Immunohistochemistry Whole-Mount reported in scientific literature (PMID 31783118), Chromatin Immunoprecipitation (ChIP)
Application Notes	Western blot bands are seen at ~19 kDa, representing LC3-I, and ~17 kDa, representing LC3-II. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors. Use in Southern blot reported in scientific literature (PMID: 21262964). In ICC, cytoplasmic staining was observed in HeLa cells. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.

Images

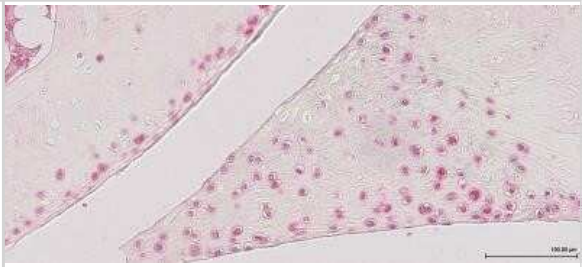
Western Blot: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - High autophagosome concentration is consumed during early immortalized human mesenchymal stem cell differentiation. Immortalized human mesenchymal stem cells were differentiated under osteogenic conditions (see Materials and methods) and assayed for changes in LC3I and LC3II during a 72-hour window. Cells were differentiated under standard conditions (top) or with addition of 5 uM rapamycin (middle) or 5 nM bafilomycin (bottom) for the first 3 hours of differentiation to modulate autophagy. Immunoblots were performed for LC3 at the indicated time points to assess autophagosome degradation via relative changes in LC3II (lower band; 17 kDa). Studies were repeated three times with similar trends seen consistently. Image collected and cropped by CiteAb from the following publication (https://stemcellres.com/content/5/6/140), licensed under a CC-BY license.



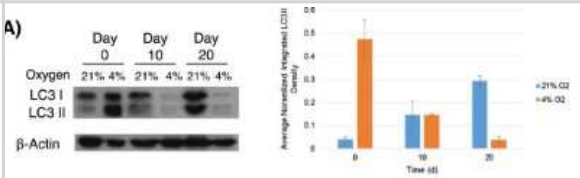
Immunocytochemistry/Immunofluorescence: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Analysis in PFA fixed NIH/3T3 cells using anti-LC3A antibody. Image from verified customer review.

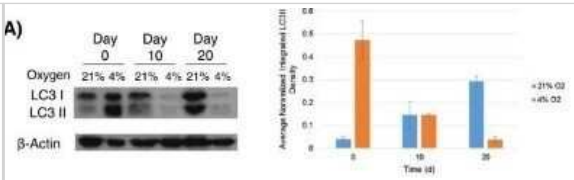
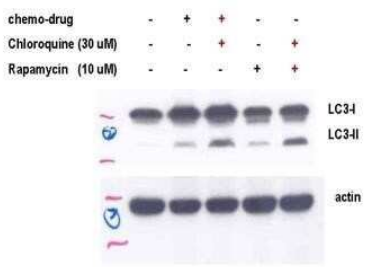
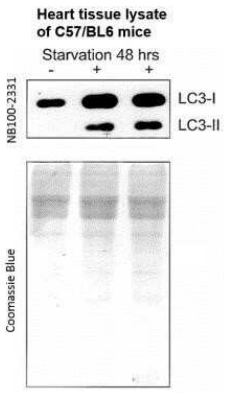
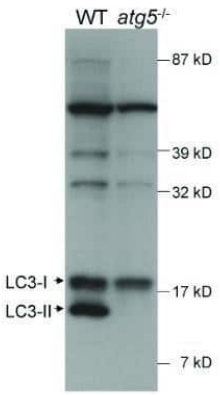


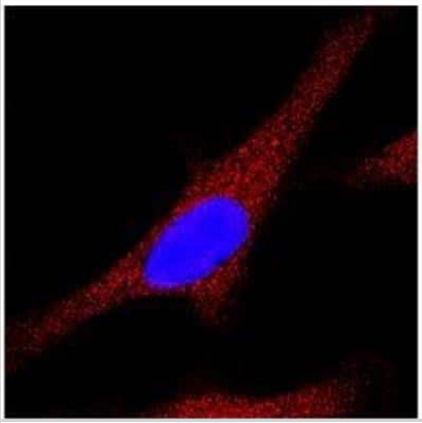
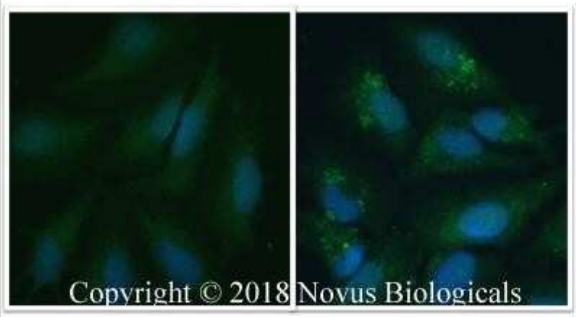
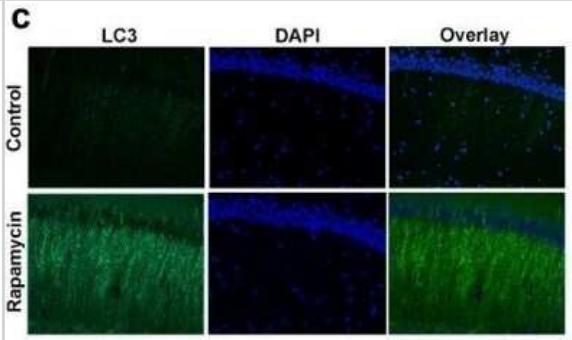
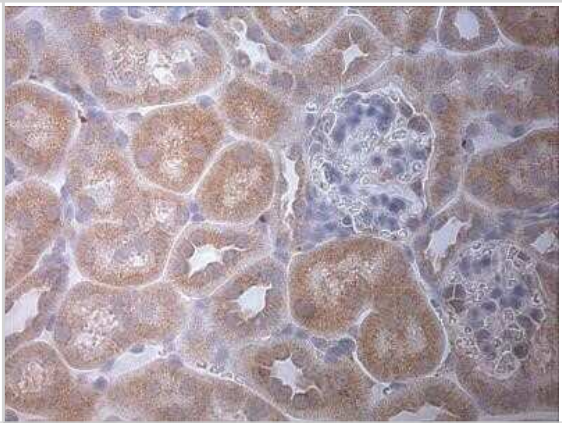
Immunohistochemistry: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Staining in mouse meniscus and cartilage. Image from verified customer review.

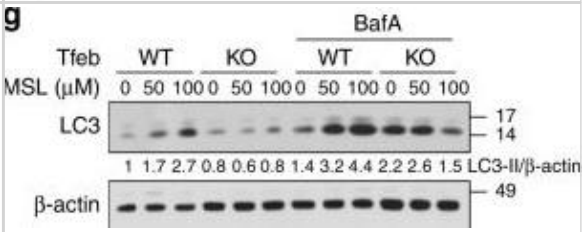
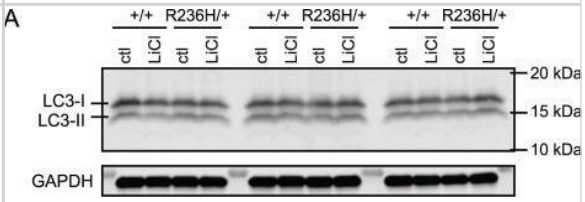
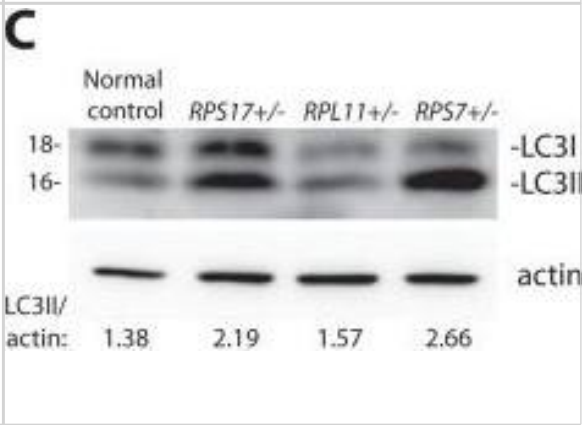


Western Blot: LC3A Antibody - BSA Free [NB100-2331] - Autophagy mobilized during differentiation of immortalized human mesenchymal stem cells (ihMSCs). Cultured ihMSCs were stimulated to undergo osteogenesis to form osteoblasts for 30 days at 21% and 4% oxygen. LC3I and LC3II levels were assessed via immunoblot every 10 days and normalized to beta-actin to derive average LC3II band densities. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/25523618/) licensed under a CC-BY license.

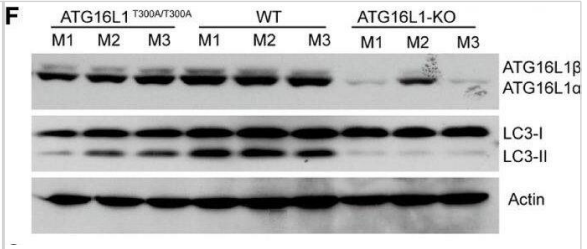


Western Blot: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Autophagy mobilized during differentiation of immortalized human mesenchymal stem cells (ihMSCs). Cultured ihMSCs were stimulated to undergo osteogenesis to form osteoblasts for 30 days at 21% and 4% oxygen. LC3I and LC3II levels were assessed via immunoblot every 10 days and normalized to beta-actin to derive average LC3II band densities. Image collected and cropped by CiteAb from the following publication (https://stemcellres.com/content/5/6/140), licensed under a CC-BY license.	
Western Blot: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - This LC3A antibody Image shows Analysis in human cell lysates.	
Western Blot: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - This LC3A antibody Image shows analysis of heart tissue lysates from mice which were subjected or not to 48 hours of starvation. The signal was developed using ECL method and this LC3 antibody was found to detect both forms of LC3, i.e. LC3A and LC3B. As expected, the levels of LC3B form were higher in the heart tissue lysates from starved mice.	
Western Blot: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Detection of HRP conjugated autophagic LC3 in mouse ES cell lysate. The atg5-/- lane (ES cells, cultured to form embryonic bodies, that are deficient in conversion of LC3-1 to LC3-11) demonstrates the specificity of NB 100-2331, as there is no detection of LC3-11. Photo courtesy of Dr. Beth Levine, UT Southwestern Medical Center.	

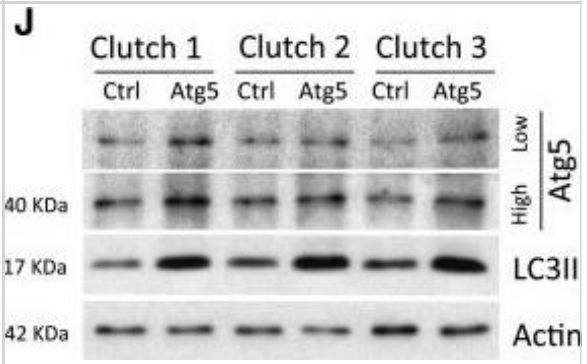
Immunocytochemistry/Immunofluorescence: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - This LC3A antibody Image shows an analysis in HeLa cells using anti-LC3 antibody (red). Nuclei were counterstained with DAPI (blue).	
Immunocytochemistry/Immunofluorescence: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Left panel shows untreated HeLa cells. Right panel shows HeLa cells that were treated with 50 uM CQ overnight. Cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-LC3A antibody at 5 ug/mL overnight at 4C and detected with an anti-mouse DyLight 488 (Green) at a 1:500. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Immunohistochemistry: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Rapamycin increases autophagy in brains of PDAPP mice. Representative epifluorescent (c200x) image of hippocampal CA1 in control- and rapamycin-fed transgenic PDAPP mice stained with an anti-LC3 antibody. An increase in LC3-immunoreactive puncta was observed in CA1 projections of transgenic PDAPP mice following rapamycin administration. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0009979) licensed under a CC-BY license.	
Immunohistochemistry: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Analysis in mouse renal tissue. Image from verifed customer review.	

Simple Western: LC3A Antibody - BSA Free [NB100-2331] - LC3A Antibody [NB100-2331] - Image shows a specific band for LC3 in 0.5 mg/mL of Neuro2A lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Identification of an autophagy enhancer small-molecule (MSL) inducing nuclear translocation of TFEB. g) WT and Tfeb knockout HeLa cells were treated with MSL in the presence or absence of bafilomycin A1 (BafA), and cell extract was subjected to Western blot analysis using the indicated antibodies. Numbers below LC3 immunoblot bands indicate fold changes of LC3-II normalized to beta-actin bands. All data in this figure are the means +/- s.e.m. from >=3 independent experiments performed in triplicate (scale bar, 20 um). **P < 0.01 and ***P < 0.001 by one-way ANOVA with Tukey's post-hoc test (f) and two-tailed Student's t-test (b-d) Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-018-03939-w), licensed under a CC-BY licence.	
LiCl administered via 0.5% LiCl food pellets for 4 wks does not increase markers for autophagy in Gfap-R236H/+ mice.Each lane of the immunoblots is tissue from one mouse. Immunoblots for LC3-I and LC3-II in (A) did not detect a change in parietal cortex (and underlying white matter) with LiCl treatment. LC3-II bands normalized to LC3-I are quantified in B (N = 3-4 mice from 3-4 cages per genotype, and is representative of 3 blots). LC3-II normalized to GAPDH gave similar results and is not shown. P62 was increased in control diet R236H/+ mouse olfactory bulb compared with control diet +/+, but LiCl did not change P62 levels in GFAP+/+ or R236H/+ mice (C-D). P62 was normalized to total protein loaded. Error bars are SEM. ****P < 0.0001. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26378915), licensed under a CC-BY licence.	
DBA mutations induce autophagy. (C) Western blot analysis of LC3 in DBA LCLs compared to normal controls. The LC3II/actin ratio is determined by densitometer analysis. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pgen.1004371), licensed under a CC-BY licence.	

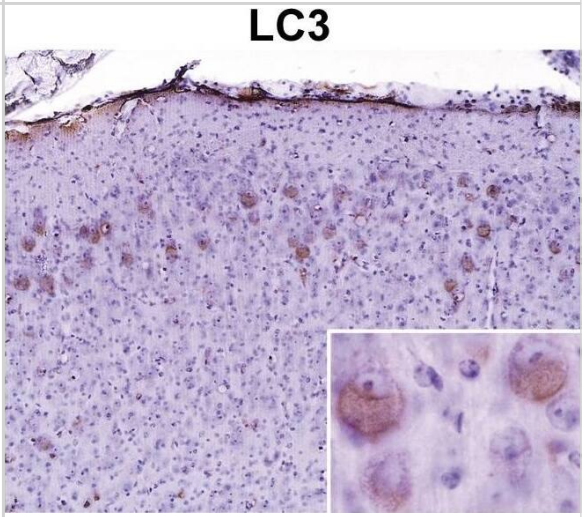
Impaired function of mucin secretion in Atg16L1T300A/T300A goblet cells is autophagy-dependent. Isolated colon or distal ileum tissues from cohoused WT and Atg16L1T300A/T300A mice stained with Helix pomatia lectin for mucus secretion by whole-mount staining, and microbiota localization in the colon was detected by FISH staining. n= 6-8 mice/group. (F) Colonic epithelial cells were isolated from the colon of cohoused WT, Atg16L1T300A/T300A, and Atg16L1-KO mice, and Western blot analysis was used to detect Atg16L1 and LC3 changes from whole-cell lysates. Actin was used as loading control. n = 3 mice/group. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35154071), licensed under a CC-BY licence.



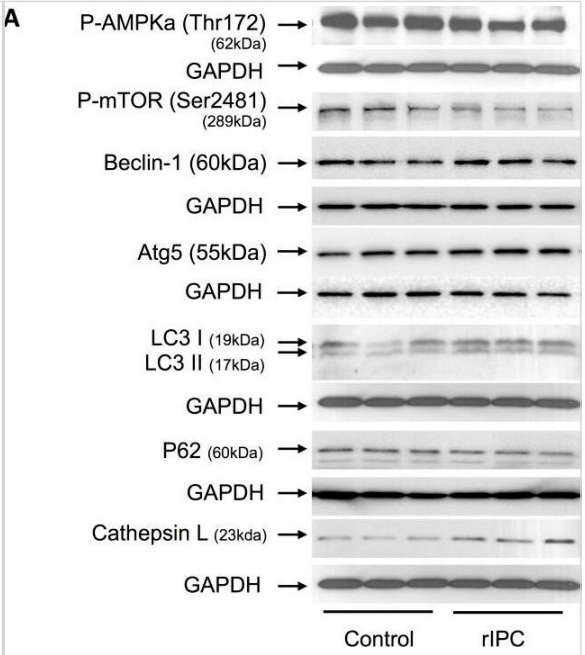
Localisation of CDS1 immunoreactivity and CDS activity to mitochondria. [A, B] Differentiated H9c2 cells fractionated and analysed for [A] CDS1 immunoreactivity by Western blot; [B] CDS activity. [C-E] Rat heart fractionated and analysed for [C] CDS1 immunoreactivity and markers, [D] CDS1 immunoreactivity (entire Western blot shown) and [E] CDS activity. [F–H] Rat heart fractionated in the presence of the protease, subtilisin and analysed for [F] CDS1 immunoreactivity by western blot, [G] CDS activity and [H] PI synthase (PIS) activity. CDS and PIS activity was monitored in triplicate and error bars denote ± S.E.M. COXIV, GRP75 and cyto c are markers for mitochondria, PITPNC1 and PITPα are cytosolic markers and calnexin is a marker for the ER. WCL, whole cell lysate; Micro, microsomes; C.Mito, crude mitochondria; Cyto, cytosol; P.Mito, pure mitochondria, MAMs, mitochondrial associated membranes; PIS, PI synthase; CDS, CDP-diacylglycerol synthase. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/29253589), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Localisation of CDS1 immunoreactivity and CDS activity to mitochondria. [A, B] Differentiated H9c2 cells fractionated and analysed for [A] CDS1 immunoreactivity by Western blot; [B] CDS activity. [C-E] Rat heart fractionated and analysed for [C] CDS1 immunoreactivity and markers, [D] CDS1 immunoreactivity (entire Western blot shown) and [E] CDS activity. [F–H] Rat heart fractionated in the presence of the protease, subtilisin and analysed for [F] CDS1 immunoreactivity by western blot, [G] CDS activity and [H] PI synthase (PIS) activity. CDS and PIS activity was monitored in triplicate and error bars denote ± S.E.M. COXIV, GRP75 and cyto c are markers for mitochondria, PITPNC1 and PITPα are cytosolic markers and calnexin is a marker for the ER. WCL, whole cell lysate; Micro, microsomes; C.Mito, crude mitochondria; Cyto, cytosol; P.Mito, pure mitochondria, MAMs, mitochondrial associated membranes; PIS, PI synthase; CDS, CDP-diacylglycerol synthase. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/29253589), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



GPR50 interacts with TβRI and its expression is upregulated by TGFβ. a Tandem affinity purification of naive HEK293T cells stably expressing GPR50Δ4-TAP. After purification, mass spectrometry was employed for protein identification. b Left panel shows confocal images of GPR50 and TβRI staining in the lining of the third ventricle of brain slices of wt (top) and GPR50ko mice (bottom). Right panel visualizes TβRI/GPR50 interaction by proximity ligation assay (PLA) in the median eminence (ME) and third ventricle (3 V) of wt (top) and GPR50ko (bottom) mice (scale: 100 µm). White arrows depict immunoreactive (IR) regions. See also Supplementary Fig. 1a. c Confocal images of GPR50 (red) and TβRI (green) staining in primary rat tanycytes (scale: 10 µm). d Colocalization of GPR50 (red) and TβRI (green) in NCI-H520 cells. (scale: 10 µm). e Co-immunoprecipitation of GRP50 and TβRI in lysates of primary rat tanycyte cultures. Lysates with IgG served as negative control. f Co-immunoprecipitation of GRP50 and TβRI in the lysates of NCI-H520 after silencing either GPR50 (si-GPR50) or TβRI (si-TβRI). Control si-RNA (si-Ctrl) served as control. g, h Co-immunoprecipitation of GRP50 and TβRI in lysates of MDA-MB231 cells (g) and cortex (h) isolated from wild type (wt) or GPR50ko mice. IgG served as negative control. i Upper part depicts schematic representation of BRET assay to study the interaction between TβRI-Rluc8 and GPR50-YFP or TβRI-YFP (left and middle scheme) and right scheme between TβRI-Rluc8 and TβRII-YFP. Lower part shows BRET donor saturation curves in HEK293T cells (left: constant expression level of TβRI-Rluc8 and increasing levels of TβRI-YFP, GPR50Δ4-YFP or GPR50wt-YFP; right: constant expression level of TβRI-Rluc8 and increasing levels of GPR50Δ4-YFP or TβRII-YFP with TGFβ stimulation (0.6 nM, 30 min at 37 °C)). IR-YFP and OBRa-YFP served as negative control. BRET signals were normalized to BRETmax values. Curves are obtained from three independent experiments performed in triplicates. j NCI-H520 cells were starved and stimulated for 24 h with TGFβ (2 ng/mL). GPR50 expression was checked by Immunoblotting and Q-PCR. (Mean ± s.e.m., n = 3 independent experiments, *p < 0.05; **p < 0.01, two-tailed unpaired Student's t-test). Representative results are shown for e–h and i. See also Supplementary Fig. 1 Image collected and cropped by CiteAb from the following open publication (https://www.nature.com/articles/s41467-018-03609-x), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Disruption of the VANGL2–p62/SQSTM1 interaction in breast cancer cells.(a) Kaplan–Meier MFS curves of breast cancer patients according to concomitant VANGL2 and p62/SQSTM1 mRNA expression. The 5-year MFS are 49% (both upregulated; N=27), 64% (both not upregulated; N=833) and 56% (one upregulated, the other not upregulated; N=79 and N=269). (b) Soft agar colony formation of T47D cells overexpressing GFP, GFP–VANGL2 and GFP–p62/SQSTM1 (right). Protein expression was revealed with anti-p62/SQSTM1, anti-VANGL2 and anti-GFP antibodies by western blot analysis (right). In anti-GFP blot, the arrowhead indicates position of co-migrating GFP–VANGL2 and GFP–p62/SQSTM1 and the asterisk pinpoints GFP alone. Error bars represent mean±s.d. (n=3). (c) Protein levels of VANGL2, phosphorylated JNK, JNK (p54/p46), p62/SQSTM1 and tubulin assessed in eight breast cancer PDXs (PDX 1–8) by western blot analysis. (d) VANGL2, JNK, phosphorylated JNK and tubulin signals from 30 PDX protein extracts were quantified. VANGL2/tubulin ratios were plotted against pJNK/JNK ratios, arranged in ascending order into three equally sized groups (low, medium and high). High expression of VANGL2 protein is correlated to high levels of phosphorylated JNK. Box and whisker plots show the median value and interquartile ranges. The Kruskal–Wallis test was used for comparison of the median levels of expression. Statistically significant differences are indicated (*P≤0.05; **P≤0.01). (e) Treatment of the indicated PDX-derived cells (PDX. DC-2, −27, −13 and −26) with a Tat-conjugated JNK inhibitor (Tat-JIP at 10 μM) during 48 h led to greater reduction in cell proliferation of VANGL2high/pJNKhigh than VANGL2low/pJNKlow PDX-derived cells. Comparisons use Tukey's multiple comparisons test. NS, not significant. Data are representative of three independent experiments; *P≤0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001. (f) Treatment of the indicated PDX-derived cells (PDX. DC-2, −27, −13 and −26) with the p62DN peptide (225 μM) but not with control scrambled peptide (225 μM) during 48 h resulted in decreased cell proliferation of VANGL2high/pJNKhigh, but not VANGL2low/pJNKlow, PDX-derived cells. Data are representative of three independent experiments and statistical testing as stated in e. Image collected and cropped by CiteAb from the following open publication (https://www.nature.com/articles/ncomms10318), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

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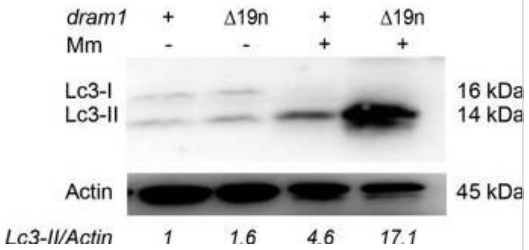
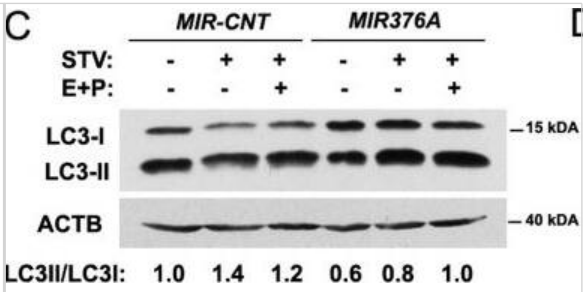
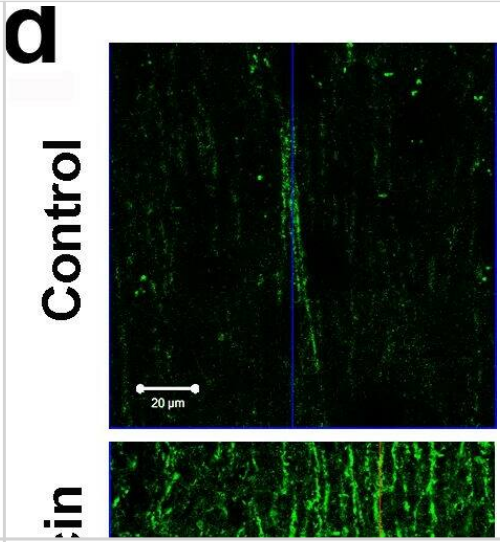


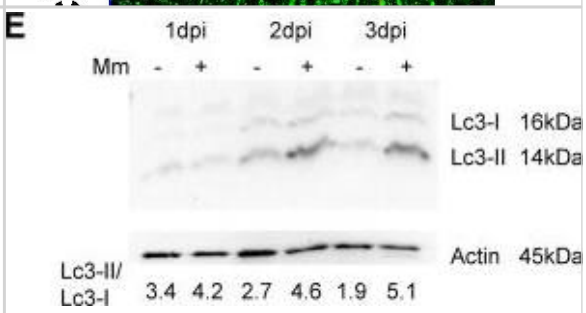
CENP-E Is Required for Tethering Specifically to Lateral Walls of Microtubules(A) Graph of percentage of detachment episodes observed in end-on or lateral kinetochores during the 5 min duration of time-lapse movies of HeLaYFP-Tub;Cen-Red cells transfected with control or CENP-E siRNA. Detachment episodes that lasted at least for three consecutive time frames alone were considered.(B) Immunofluorescence images of control or CENP-E siRNA-transfected cells treated with monastrol for 1 hr prior to fixation and immunostained with antibodies against Mad2 and tubulin and CREST antisera and stained with DAPI for DNA. Cropped images are 3× magnified. Scale bars represent 5 μm (insets, 2 μm).(C) Graph of percentage of lateral kinetochores with both, one, or none of the kinetochores displaying Mad2 in cells treated as in (B).(D and F) Immunofluorescence images of control or CENP-E siRNA-transfected cells treated with monastrol for 1 hr prior to fixation and immunostained with antibodies against astrin and tubulin and CREST antisera and stained with DAPI for DNA. Cropped images are 3× magnified showing lateral (D) and end-on (F) kinetochores. Scale bars represent 5 μm (insets, 2 μm).(E) Graph of percentage of lateral kinetochore pairs with both, one or none of the kinetochores displaying astrin in cells treated as in (D).(G) Representative still images from time-lapse movie of lateral kinetochore in cells treated or untreated with CENP-E inhibitor. Scale bars represent 5 μm.(H and I) Cumulative graph and T50 table showing the lifetime of lateral kinetochores (H) and the percentage of lateral kinetochores that detached or converted into end-on (I) in cells treated with monastrol for 1 hr prior to imaging in the presence or absence of the CENP-E inhibitor GSK923295. Detachment episodes that lasted at least for three consecutive time frames alone were considered.(J) Representative still images from a time-lapse movie of HeLa cells expressing siRNA-resistant HEC1Ndc80-Δloop-YFP (in green) and mKate-tubulin (in red) as indicated, treated with siRNA as indicated, and filmed in the presence of monastrol. Arrowheads mark lateral kinetochores. Scale bars represent 2 μm.(K) Illustration of a multistep end-on conversion process involving (1) kinetochore tethering to microtubule walls, (2) bringing kinetochore and microtubule plus end proximal to each other for removing wall contact, and (3) kinetochore tethering to dynamic microtubule ends. CENP-E's role in tethering kinetochores to lateral walls (4) and MCAK's role in eliminating microtubule walls interacting with kinetochore (5) and HEC1Ndc80-loop-dependent tethering of kinetochores to microtubule ends (6) have been assigned sequential steps.Error bars represent the SEM values across three experiments. p values representing significance levels were obtained with the proportion test, except in (H), where the paired sample t test was used. and # indicate significant and insignificant differenced, respectively. See also Figure S5. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/23891108), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



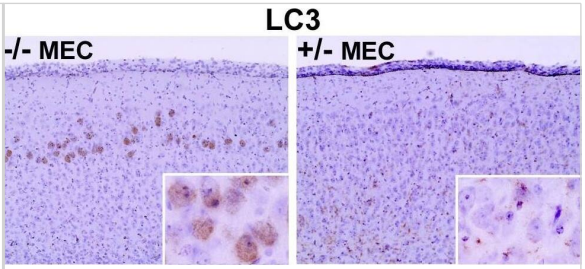
CtIP and MRE11 but not EXO1 are required for end resection in 53BP1-depleted G1 cellsA. A549 cells were treated as in Figure 4 with the indicated siRNAs and efficient knockdown of 53BP1 and/or EXO1 was confirmed by Western blot (upper panel). Lower panel: The focus intensity of RPA was measured in 53BP1- and/or EXO1- depleted cells at 2h post-2Gy in G1 and S/G2. B. Immunoblots showing efficient siRNA-mediated knockdown of CtIP and/or 53BP1 (upper panel), MRE11 and/or 53BP1 (middle panel), or PLK3 and/or 53BP1 (lower panel). (C & D) Quantitation of the percentage of G1 cells with more than three C. RPA or D. CtIP foci after the indicated treatments. At least 50 nuclei were counted. (**) indicates P < 0.001, and (ns) means P > 0.05. Shown are the mean ±SEM for three independent experiments. Image collected and cropped by CiteAb from the following open publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.11023), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



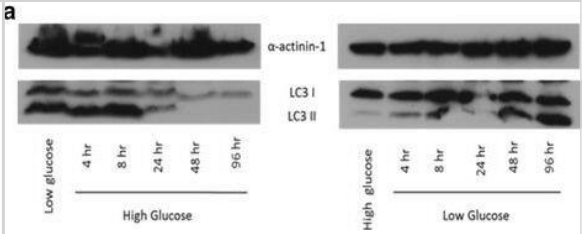
Truncated PPM1D impairs DNA damage response in mouse thymus. Expression of PPM1D mRNA was analyzed by RT-qPCR in the thymi of Ppm1d+/+, Ppm1dT/+ and Ppm1dT/T mice and was normalized to GAPDH (n = 3) (A). Thymi from mice of indicated genotypes were lysed and proteins were separated by SDS-PAGE. Samples were probed with antibody against PPM1D and importin-β as a loading control. The empty and full arrowheads indicate the position of the full-length and the C-terminally truncated PPM1D, respectively. (B). Cells from thymi from Ppm1d+/+ and Ppm1dT/+ mice were analyzed by flow cytometry. Plotted are the counts of the indicated populations as follows: double-negative T-cells (DN and DN1, DN2, DN3, DN4), double-positive T-cells (DP), CD8-positive T-cells (CD8+) and CD4-positive T-cells (CD4+) (n = 3) (C). The median size of the thymus was determined in Ppm1d +/+ (n = 11) and Ppm1dT/+ (n = 12) mice (D). A scheme of the experimental setup in F-I. Mice were exposed or not to a low dose of IR (3 Gy), sacrificed after 6 h and thymi and lymph nodes were collected (E). Proteins isolated from thymi from mice of indicated genotypes exposed to mock or to IR were probed with the indicated antibodies by immunoblotting (F). Proteins isolated from inguinal lymph nodes from mice of indicated genotypes exposed to mock or to IR were probed with the indicated antibodies by immunoblotting (G). RNA isolated from thymi from mice in E was analyzed by RT-qPCR. The expression of CDKN1Ap21 mRNA was normalized to GAPDH. Statistical significance was evaluated by two-tailed t-test, error bars indicate SD, n = 5 (H). RNA isolated from thymi from mice in D was analyzed by RT-qPCR. The expression of PUMA mRNA was normalized to GAPDH. Statistical significance was evaluated by two-tailed t-test, error bars indicate SD, n = 5. * p <0.05; *** p < 0.0005; **** p < 0.0001 (I). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32927737), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

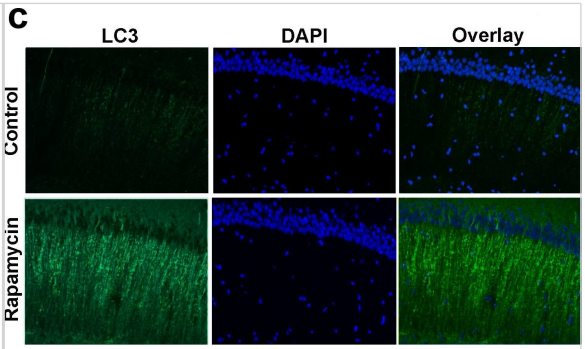


KDM1A inhibition impairs cell proliferation and migration and induces apoptosis in human medulloblastoma cell lines. a Bars represent KDM1A expression measured using real-time RT-PCR and normalized to the geometric mean of GAPDH, UBC and HPRT expression in DAOY and ONS-76 cell lines 72 h after KDM1A knockdown or mock transfection. ***p < 0.0001 b Knockdown of KDM1A protein was confirmed by western blotting of whole-cell lysates from DAOY and ONS-76 cells. β-actin served as loading control. c The DAOY and ONS-76 medulloblastoma cell lines were transfected with siRNA directed against KDMA1, and cell viability was measured using the MTT assay. Extinction relative to mock-transfected cultures at 72 h is shown. ***p < 0.0001 d Proliferation of DAOY and ONS-76 cells following mock transfection or transfection with siRNA directed against KDM1A was assessed by BrdU ELISA. Bars show extinction relative to mock-transfected cultures at 72 h. ***p < 0.0001 e Apoptosis in DAOY and ONS-76 cells was measured by Cell Death Detection ELISA™ 72 h after transfection with either siRNA directed against KDMA1 or mock transfection. Extinction is relative to mock-transfected cultures. ***p < 0.0001, *p < 0.05 f Migratory activity was assessed for the ONS-76 cell line 48 h after transfection with either siRNA directed against KDM1A or mock transfection in Boyden chamber assays. Representative images of DAPI-stained mock-transfected control cells (ONS-76 ctrl) and KDM1A-knockdown cells (ONS-76 siKDM1A) invading the membrane (scale bars = 100 μm). g Statistical analysis of results from Boyden chamber assays 24 h after DAOY and ONS-76 cells, either transfected with siRNA directed against KDM1A or mock-transfected, were plated in the upper chamber. Bars display quantity of cells per mm square which migrated through the membrane. **p < 0.01, *p < 0.05. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/24252778), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Expression of ER stress markers in mouse and human skeletal muscle (a) TA muscle lysates from high fat fed mice were used to determine the expression of ER stress markers by Western blot analysis (n=6). (b-g) Western blot analysis was done to measure proteins involved in ER stress in vastus lateralis muscle from lean, obese, or diabetic subjects (n=6-13). Data are the means ± S.E.M. * indicates p<0.05 and ** indicates p<0.01 vs. chow or lean controls, using Student's t-test (a) and One way ANOVA. Image collected and cropped by CiteAb from the following open publication (https://www.nature.com/articles/ncomms2851), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





The effect of ATG5 and ATG7 on autophagy, ER stress, apoptosis and cell cycle depends on PERK. a The ER stress proteins were analysed by western blotting in the GSK2606414 treated chondrocytes. b The levels of ER stress proteins were normalized to β-actin. c Determination of autophagy and apoptosis proteins expression by western blotting in the GSK2606414 treated chondrocytes. d The levels of related proteins were normalized to β-actin. e FCM analysis was used to calculate the percentage of apoptotic cells at the time point of 24 h. The apoptosis rate were increased when combined treated with GSK2606414. Experiments were repeated 3 times, Representative images are shown. f Analysis of cell apoptosis. Data come from 3 independent experiments. g FCM analysis indicated that the S phase percentage were decreased compared to that of the control groups when combine treated with GSK2606414 in C28I2 cells. Experiments were repeated 3 times, Representative images are shown. h Percentage of cells at each phase in different groups. *P < 0.05, **P < 0.01 compared with the controls. Values are means ± SD n = 3). (1:Ad-GFP, 2:Ad-ATG5 + Ad-ATG7, 3:Ad-ATG5 + Ad-ATG7 + DMSO, 4:Ad-ATG5 + Ad-ATG7+ GSK2606414) Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31060556), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Publications

Jussila M, Boswell CW, Griffiths NW et al. Live imaging and conditional disruption of native PCP activity using endogenously tagged zebrafish sfGFP-Vangl2 Nature Communications 2022-09-23 [PMID: 36151137] (ICC/IF)

Cui Y, Fang J, Guo H et al. Notch3-Mediated mTOR Signaling Pathway Is Involved in High Glucose-Induced Autophagy in Bovine Kidney Epithelial Cells Molecules 2022-05-13 [PMID: 35630598]

Kim M, Lu L, Dvornikov AV et al. TFEB Overexpression, Not mTOR Inhibition, Ameliorates RagC(S75Y) Cardiomyopathy International Journal of Molecular Sciences 2021-05-23 [PMID: 34071043] (WB, B/N)

Sasaki T, Yamada E, Uehara R et al. Role of Fyn and the interleukin-6-STAT-3-autophagy axis in sarcopenia iScience 2023-10-20 [PMID: 37744036]

Yamashita A, Ignatenko O, Nguyen M et al. Depletion of LONP2 unmasks differential requirements for peroxisomal function between cell types and in cholesterol metabolism Biol Direct 2023-09-22 [PMID: 37736739] (WB)

Dixon A, Shim MS, Nettesheim A et al. Autophagy deficiency protects against ocular hypertension and neurodegeneration in experimental and spontanous glaucoma mouse models Cell Death & Disease 2023-08-24 [PMID: 37620383]

Pedre□o-L□pez S, Garc□a E, Guerrero D et al. Modulation of the autophagic pathway inhibits HIV-1 infection in human lymphoid tissue cultured ex vivo Scientific Reports 2022-05-06 [PMID: 35523829] (WB, B/N)

Zheng H, Li Q, Li S et al. Loss of Ptpmt1 limits mitochondrial utilization of carbohydrates and leads to muscle atrophy and heart failure in tissue-specific knockout mice Elife 2023-09-06 [PMID: 37672386]

Liu H, Gao P, Jia B et al. IBD-Associated Atg16L1T300A Polymorphism Regulates Commensal Microbiota of the Intestine Frontiers in Immunology 2022-01-27 [PMID: 35154071] (WB, B/N)

Zhang H, Li X, Li Y et al. CREB Ameliorates Osteoarthritis Progression Through Regulating Chondrocytes Autophagy via the miR-373/METTL3/TFEB Axis Frontiers in Cell and Developmental Biology 2022-06-09 [PMID: 35756079] (WB)

Tshilenge KT, Aguirre CG, Bons J et al. Proteomic Analysis of Huntington's Disease Medium Spiny Neurons Identifies Alterations in Lipid Droplets Molecular & cellular proteomics : MCP 2023-03-21 [PMID: 36958627] (ICC/IF, Human)

Details:
Dilution used in ICC/IF 1:100

Banducci-Karp A, Xie J, Engels SAG et al. DRAM1 Promotes Lysosomal Delivery of Mycobacterium marinum in Macrophages Cells 2023-03-07 [PMID: 36980169] (WB, ICC/IF, Mouse)

Details:
Dilution used in WB 1:1000, ICC/IF 1:500

More publications at http://www.novusbio.com/NB100-2331

Procedures

Western Blot protocol specific for LC3 Antibody (NB100-2331)

Protocol: Inhibition of Autophagy and LC3 Antibody (NB100-2331) Western Blot

Materials

Chloroquine diphosphate (CQ) (10 mM) in dH2O
1X PBS
Sample buffer, 2X Laemmli buffer: 4% SDS, 5% 2-mercaptoethanol (BME), 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl, pH 6.8
RIPA buffer: 150 mM NaCl, 1% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, 20 mM Tris-HCl, pH 7.5
1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3
1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol, Adjust to pH 8.3
TBS
TBST, TBS and 0.1% Tween
Blocking solution: TBST, 5% non-fat dry milk
rabbit anti-LC3 primary antibody (NB100-2331) in blocking buffer (~2 ug/mL)

Methods

Tip: For more information on Western Blotting, see our Western Blot handbook.

1. Grow cells (e.g. HeLa or Neuro2A) in vitro to semi-confluency (70-75%).

2. Add CQ to culture dishes to a final concentration of 50 uM and incubate overnight (16 hours). Remember to include an untreated sample as a negative control.
Note: Validated autophagy inducers should be included as positive controls.

3. Rinse cells with ice-cold 1X PBS and lyse cells with sample buffer.
Note: LC3-I and LC3-II are sensitive to degradation, although LC3-I is more labile. These proteins are sensitive to freeze-thaw cycles and SDS sample buffers. Fresh samples should be analyzed quickly to prevent protein degradation.

4. Sonicate and incubate cells for 5 minutes at 95oC.
Tip: Cells are lysed directly in sample buffer or may be lysed in RIPA buffer.

5. Load samples of Chloroquine-treated and -untreated cell lysates 40 ug/lane on a 4-20% polyacrylamide gradient gel (SDS-PAGE).
Tip: For detection of LC3 it is particularly important to monitor the progress of the gel as this protein is relatively small (~14kDa).

Tip: Alternatively, for non-gradient gels, use a 20% polyacrylamide gel.

6. Transfer proteins to a 0.2 um PVDF membrane for 30 minutes at 100V.

7. After transfer, rinse the membrane with dH2O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.

8. Rinse the membrane in dH2O to remove excess stain and mark the loaded lanes and molecular weight markers using a pencil.

9. Block the membrane using blocking buffer solution (5% non-fat dry milk in TBST) for 1 hour at room temperature.

10.Rinse the membrane with TBST for 5 minutes.

11.Dilute the rabbit anti-LC3 primary antibody (NB100-2331) (~2 ug/mL) in blocking buffer and incubate the

membrane for 1 hour at room temperature.

12.Rinse the membrane with dH2O.

13.Rinse the membrane with TBST, 3 times for 10 minutes each.

14.Incubate the membrane with diluted secondary antibody, according with product's specifications, (e.g. anti-rabbit-IgG HRP-conjugated) in blocking buffer for 1 hour at room temperature.
Note: Tween-20 may be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

15.Rinse the membrane with TBST, 3 times for 10 minutes each.

16.Apply the detection reagent of choice (e.g. BioFX Super Plus ECL) in accordance with the manufacturer's instructions.

17.Image the blot.
Tip: LC3-I and it's lipidated form LC3-II have different electrophoretic mobility properties, with the lipidated form moving faster in an SDS-PAGE gel, albeit its larger molecular weight. LC3-II runs at 14-16 kDa while LC3-I runs at 16 -18kDa.

Note: This assay measures the difference in the LC3-II signal in the presence and absence of inhibitors (e.g., lysosomotropic agents). When autophagic flux is present or induced in a system an increase in the LC3-II signal should be observed with the inhibitor.

Immunohistochemistry-Paraffin protocol for LC3A Antibody (NB100-2331)
LC3A Antibody:
I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:
To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
**Use within 4 hours of preparation
A.Place slides in peroxidase quenching solution: 15-30 minutes.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96C.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution.

**Do not allow tissue sections to dry for the rest of the procedure.

C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes.
Check development with microscope.

I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes.
Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60C oven.
- All steps in which Xylene is used should be performed in a fume hood.
- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts.
- For small tissue sections less than 200 ul may be used.
- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining,
further dilution of the primary antibody may be necessary.
-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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