Product Datasheet

SR-BI Antibody - BSA Free
NB400-104

Unit Size: 0.1 ml

Store at 4C short term. Store at -20C long term. Avoid freeze-thaw cycles.

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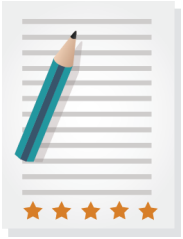
Updated 3/24/2024 v.20.1



NB400-104

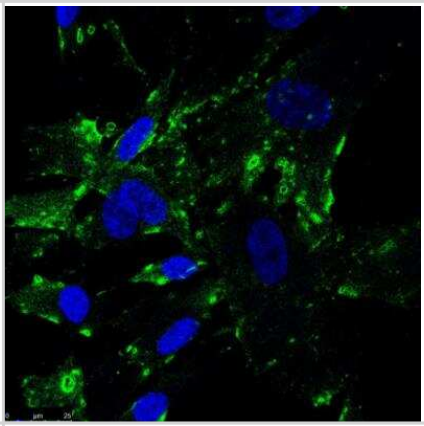
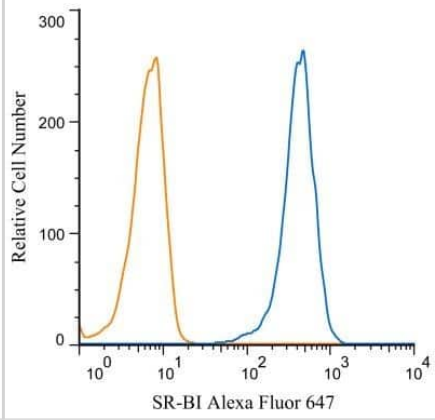
SR-BI Antibody - BSA Free

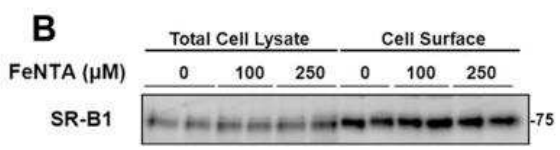
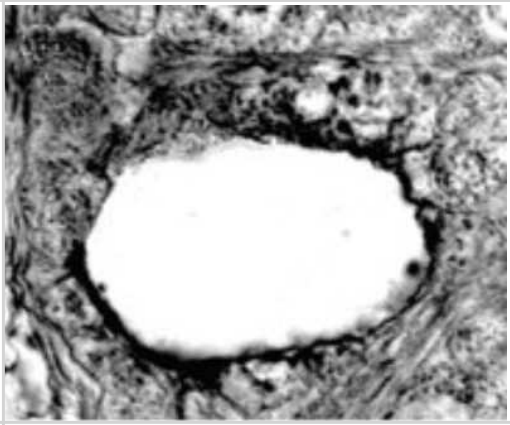
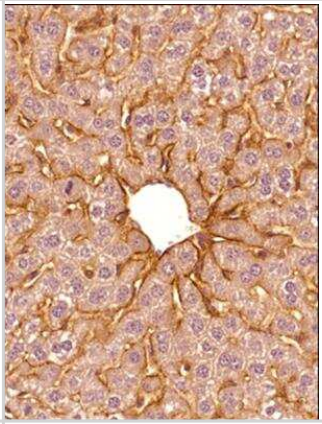
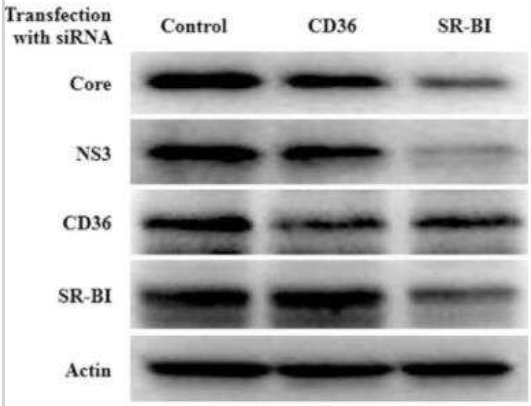
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	82 kDa

Product Description	
Host	Rabbit
Gene Symbol	SCARB1
Species	Human, Mouse, Rat, Porcine, Chinese Hamster, Hamster, Mustelid, Primate, Rabbit, Golden Syrian Hamster
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:33719499).
Immunogen	A C-terminal peptide containing residues from mouse SR-BI (within residues 450 -509). [UniProt Q61009]

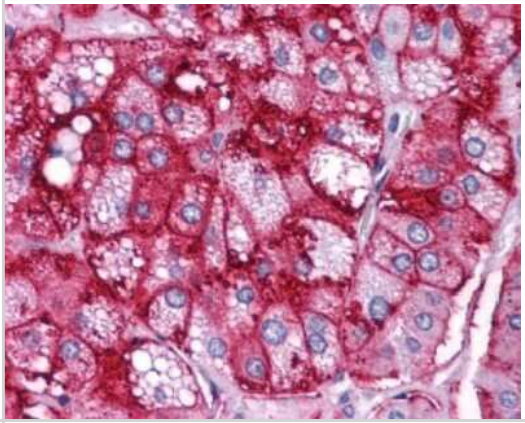
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay, Block/Neutralize, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000 - 1:5000, Simple Western 1:100, Flow Cytometry 1:10 - 1:1000, Immunohistochemistry 2.5 - 5 ug/mL, Immunocytochemistry/ Immunofluorescence 1:50 - 1:1000, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 2.5 - 5 ug/mL, Immunohistochemistry-Frozen reported in scientific literature (PMID 26865459), Proximity Ligation Assay reported in scientific literature (10.1016/j.jbc.2021.100828), Knockout Validated reported in scientific literature (PMID 31462534), Knockdown Validated, Block/Neutralize reported in scientific literature (PMID 24859737)
Application Notes	In Western blot a band is observed at approx. 82 kDa in tissues that express SR-BI such as liver, ovary and adrenals and to a lesser extent testis, heart and mammary gland. In Simple Western only 10-15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

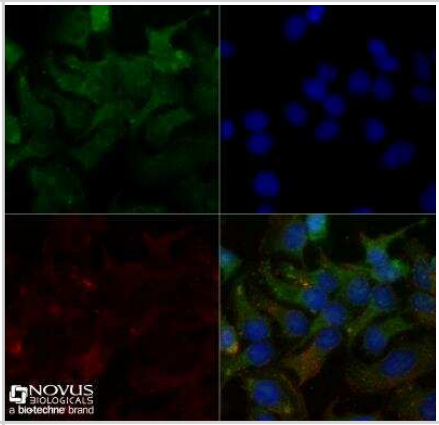
Simple Western: SR-BI Antibody [NB400-104] - Image shows a specific band for SR-BI in 0.5 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: SR-BI Antibody [NB400-104] - SR-BI antibody was tested in human adrenal cell lysate.	
Immunocytochemistry/Immunofluorescence: SR-BI Antibody [NB400-104] - SR-BI antibody was tested in human fibroblast samples fixed in 4% PFA and permeabilized in PBS (0.2% Tween). Primary incubation overnight at 4C using a 1:100 dilution in PBS (0.1% Tween) with 1% BSA. Secondary antibody is anti-rabbit conjugate to Alexa Fluor 488. SR-BI is shown in green and nucleus in blue (Hoescht 33342 stain).	
Flow Cytometry: SR-BI Antibody [NB400-104] - An intracellular stain was performed on HeLa cells with SR-BI antibody NB400-104AF488 (blue) and a matched isotype control NBP2-24893AF488 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.	

Western Blot: SR-BI Antibody [NB400-104] - Detection of SR-BI in rat H4IIE total cell lysates and plasma membrane proteins. Photo courtesy of product review by verified customer.	
Immunohistochemistry: SR-BI Antibody [NB400-104] - Immunolocalization of SR-BI in adult mink testis using NB400-104. SR-BI labeling is visible at the surface and along the outline of the large vacuole. Photo courtesy of R.M. Pelletier, University of Montreal.	
Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-104] - FFPE tissue section of mouse liver using SR-BI antibody (Lot 8310) at 1:300 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody generated a specific membrane signal of SR-BI protein in the murine hepatocytes.	
Knockdown Validated: SR-BI Antibody [NB400-104] - HCV replication was increased by CD36. Silence of CD36 decreased HCV infection and replication (n = 3). Huh7.5 cells were transfected with 0.2 u g CD36-HA plasmid or with 150 pmol siRNA for CD36 (B) for 48 hrs, and then were washed and infected with HCV (150 IU/cell) for 2 hrs, followed by washing and continuously incubating. Intracellular HCV RNA and proteins were detected in 96 hrs, and the cytotoxicity of siRNAs was measured in 96-wells plate with a MTT assay in B. Image collected and cropped by CiteAb from the following publication (http://www.nature.com/articles/srep21808) licensed under a CC-BY license.	

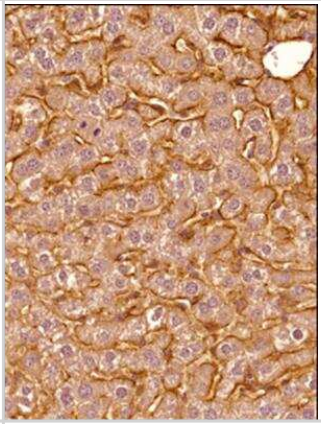
Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-104] - Staining of human adrenal cortex.



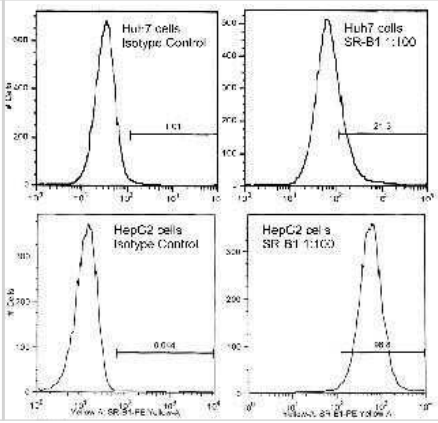
Immunocytochemistry/Immunofluorescence: SR-BI Antibody [NB400-104] - HeLa cells were fixed and permeabilized for 10 minutes using -20C MeOH. The cells were incubated with anti-SR-BI at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



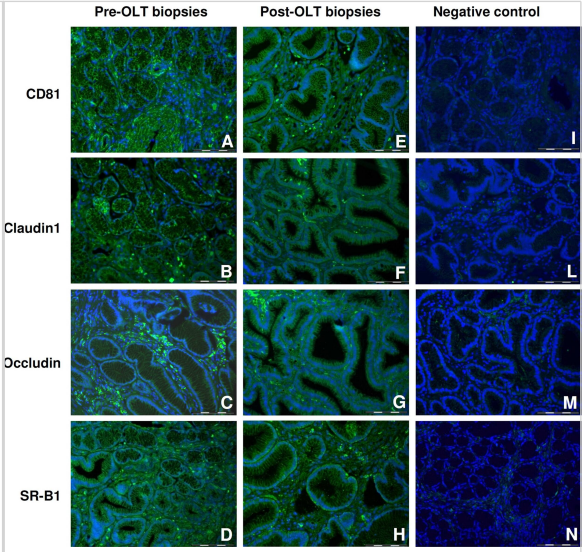
Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-104] - FFPE tissue section of mouse liver using SR-BI antibody (Lot R-4) at 1:300 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody generated mainly a membranous signal of SR-BI protein in the murine hepatocytes.



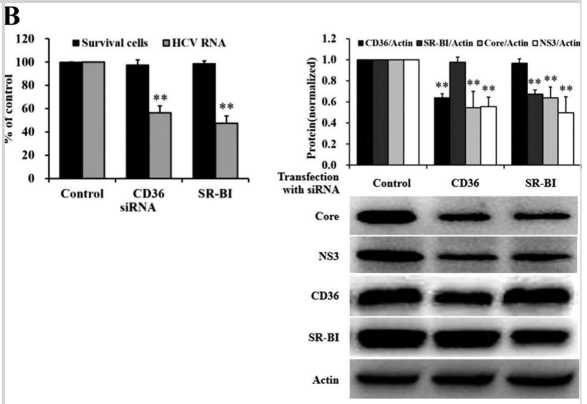
Flow Cytometry: SR-BI Antibody [NB400-104] - Analysis of Huh7 and HepG2 cells using SR-B1 antibody NB400-104. Courtesy of Bruno Sainz, Jr., PhD, University of Illinois at Chicago.



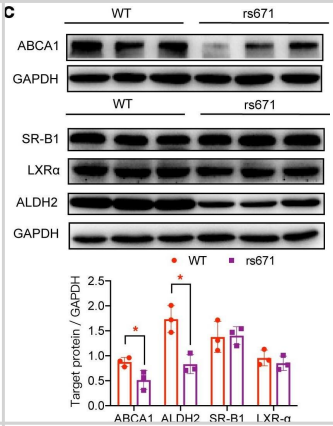
Immunofluorescence assay on GIM biopsies of HCV-transplanted patients.Immunofluorescence assay on GIM polyp biopsies of HCV-transplanted patients, using antibodies against HCV receptors: CD81, SR-B1, Claudin-1, Occludin (Original magnification X400). (A, E) CD81 in colon (patient 26); (B, F) Claudin-1 in colon (patient 26); (C, G) Occludin in colon (C patient 28, G patient 26); (D) SR-B1 in antrum (patient 28); (H) SR-B1 in colon (patient 26). All the sections were clearly positive for the analyzed HCV receptors before (A-D) and after transplantation (E-H). Negative controls (I-N) were performed on GIM biopsies of HCV positive patients by omitting the primary antibodies, and by using polyclonal FITC-conjugated Donkey anti-mouse (I) and anti-rabbit (L, M, N) as secondary antibodies. (I CD81 in antrum; L Claudin-1 in polyp colon; M Occludin in antrum; N SR-B1 in polyp colon. Nuclei were counterstained with DAPI (blue). Scale bar 50 um. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0181683), licensed under a CC-BY licence.



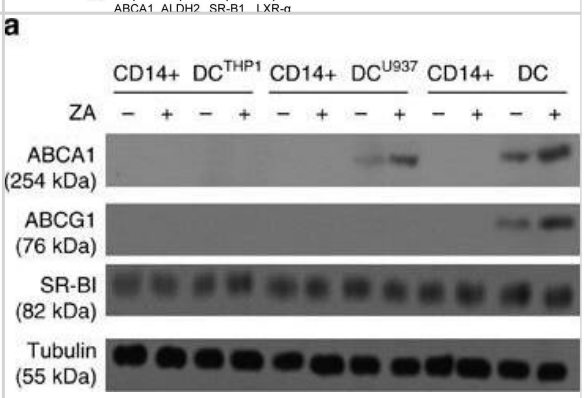
HCV replication was increased by CD36. (B) Silence of CD36 decreased HCV infection and replication (n = 3). Huh7.5 cells were transfected with 0.2 ug CD36-HA plasmid (A) or with 150 pmol siRNA for CD36 (B) for 48 hrs, and then were washed and infected with HCV (150 IU/cell) for 2 hrs, followed by washing and continuously incubating. Intracellular HCV RNA and proteins were detected in 96 hrs, and the cytotoxicity of siRNAs was measured in 96-wells plate with a MTT assay in B. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep21808), licensed under a CC-BY licence.



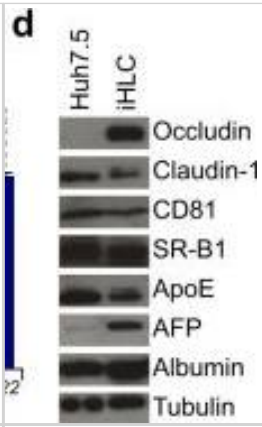
ALDH2 rs671 modulates HDL-C levels in mice and human liver through increasing poly(ADP-ribosyl)ation of LXRalpha due to attenuated ALDH2/PARP1 interaction. (C) Western blotting analysis of ABCA1, ALDH2, LXRalpha, and SR-B1 expression in mouse liver tissue. WT, n = 3; rs671, n = 3. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35393951), licensed under a CC-BY licence.



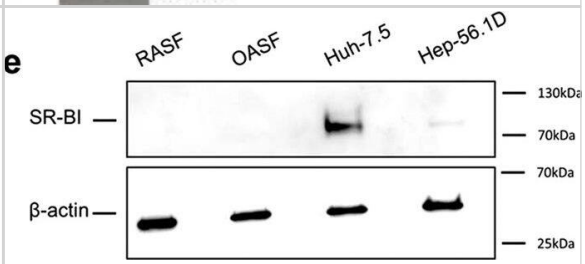
ZA treatment increases ABCA1 and apoA-1 expression.(a) Western blot analysis of ABCA1, ABCG1 and SR-BI expression in untreated (ZA-) and ZA-treated (ZA+) THP-1 cells, U937 cells, CD14+ cells and corresponding DC subsets (DCTHP1, DCU937, DC). ABCA1 was already detectable in DCU937 and DC and its expression was upregulated by ZA. beta-tubulin was employed to check the equal protein loading per lane. The results are obtained from one representative experiment of three experiments. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms15663), licensed under a CC-BY licence.



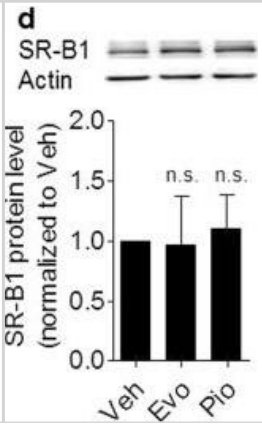
Differentiation and characteristics of iHLCs. (d) Western blot analysis of protein expression in HCV-infected iHLCs compared to Huh7.5 cells of different host factors crucial for HCV infection. Tubulin served as loading control. Full-length blots are presented in Supplementary Figure 1. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29497123), licensed under a CC-BY licence.



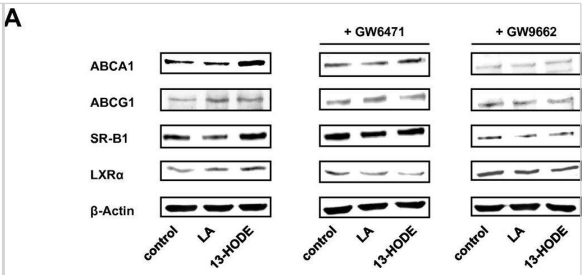
Expression of cellular HCV receptors at the mRNA and protein levels.(a) mRNA copy numbers of CD81, occluding (OCLN), claudin-1 (CLDN1) and SR-BI were quantified in OASF, RASF and Huh-7.5 cells, normalized to the reference gene GAPDH. (b) Detection of CD81 on Huh-7.5 cells, OASF, and RASF by FACS analysis. (c-d) Detection of OCLN, CLDN1 and SR-BI in OASF and RASF by immunoblot analysis. Huh-7.5 cells were used as positive control and Hep-56.1D as negative control. In both the real-time PCR and the FACS analysis experiments the number of biological replicates was 3 and the number of experimental replicates was 2 (total n = 6). In immunoblotting detection, the number of biological replicates was 2 and the number of experimental replicates was 2 (n = 4). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26643193), licensed under a CC-BY licence.



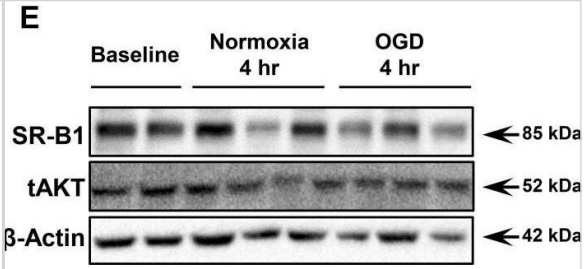
miR-27a/b inhibit PINK1 accumulation upon mitochondrial damage. a, b Overexpression of miR-27a/b inhibited PINK1 accumulation upon mitochondrial damage. 48 h post-transfection, HeLa cells were treated with 10 μM CCCP or combination of 10 μM oligomycin and 4 μM antimycin as indicated for 2 h (n = 4, two-way ANOVA). c, d Inhibition of endogenous miR-27a/b increased PINK1 accumulation upon CCCP treatment. (n = 4, two-way ANOVA). PINK1 levels were normalized to corresponding GAPDH level and quantified as a percentage of control. Values are mean ± SEM (n.s. = non-significant, *p < 0.05, **p < 0.01, ***p < 0.001) Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27456084), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



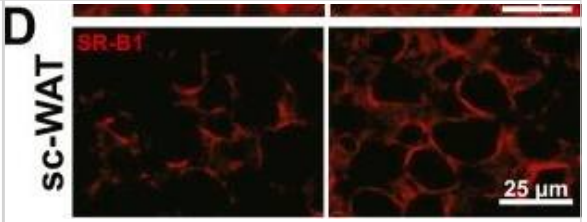
Aβ induces the expression of cell cycle regulatory proteins. (A) SK-N-MC cells were exposed to Aβ (5 μM) for 24 h. The mRNA expression levels of CDK5, P35 and P39 were analyzed by real-time PCR. The mRNA expression level was normalized by β-actin mRNA expression level. Data represent the mean ± SE. n = 4. (B)hif1α specific- and non-targeting (NT) siRNA were transfected to the cells for 24 h prior to Aβ treatment. Cyclin D1, CDK4, cyclin E, CDK2, HIF1α and β-actin was detected by western blot. n = 3. (C–F) Cells were pretreated with trehalose (10 μM), rapamycin (10 nM), PF4708671 (10 μM) and cycloheximide (4 μM) for 30 min prior to Aβ treatment for 24 h. Cyclin D1, CDK4, cyclin E, CDK2 and β-actin were detected by western blot. n = 3–6. (G) Mouse hippocampal neurons were transfected with hif1α specific- and NT siRNAs for 24 h prior to Aβ treatment for 24 h. Samples were blotted with Cyclin D1, CDK4, cyclin E, CDK2 and β-actin specific antibodies. n = 3–6. (H) Mouse hippocampal neurons were pretreated with trehalose (10 μM) for 30 min and incubated with Aβ for 24 h. cyclin D1, CDK4, cyclin E, CDK2, HIF1α and β-actin were analyzed by western blot. n = 3–6. Data are presented as a mean ± SE. *p < 0.05 vs. control, #p < 0.05 vs. Aβ treatment. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/28790888), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



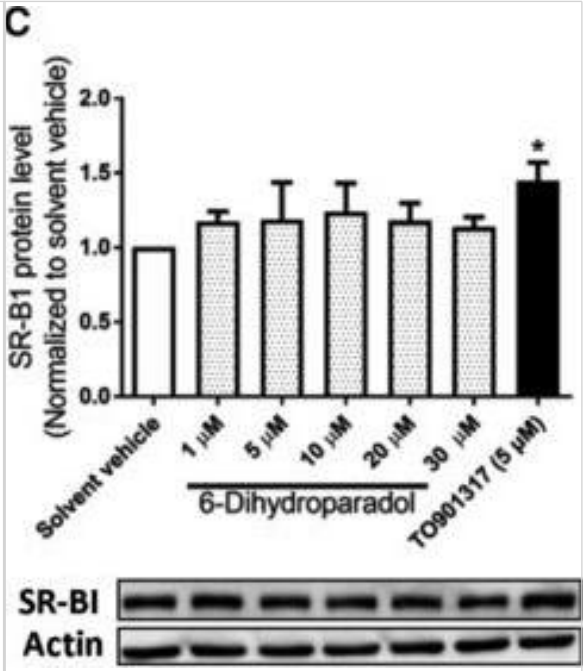
Yap deficiency suppresses cell proliferation in vivo and in vitro. (A-F) The Ki67 positive ratio of lens epithelial cells decreased in Yap-deficient mice at different stages (arrowheads indicate Ki67 positive cells). (G) The relative number of Ki67 positive lens epithelial cells (number of Ki67 positive lens epithelial cells / lens epithelium area). The data are shown as mean ± S.E.M. (Student's t-test, *P<0.05, **P<0.01, n=10). (H-I) Knockdown efficiency of Yap in αTN4 cell using siRNA. (J-K) Cell viability and growth assay revealed that proliferation was downregulated in Yap knockdown αTN4 cells. The data are shown as mean ± S.E.M. (Two-way RM ANOVA, **P<0.01, n=5). Scale bars: 50 μm. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31011480), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Knockdown of CIP2A inhibited cell proliferation and DNA synthesis of HPV-16E7-expressing cells(A) Western blot analysis of protein level of 16E7 and CIP2A in RPE1-16E7 cells and (B) with CIP2A siRNA for 48 hr. (C) CCK8 assay of cell proliferation of RPE1-16E7 cells with CIP2A siRNA. (D) Flow cytometry of cells with CIP2A siRNA and labeled with BrdU for 2 hr, then stained with PI and BrdU; and (E), Quantification. Babe, vector control. **, P < 0.01. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25650660), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

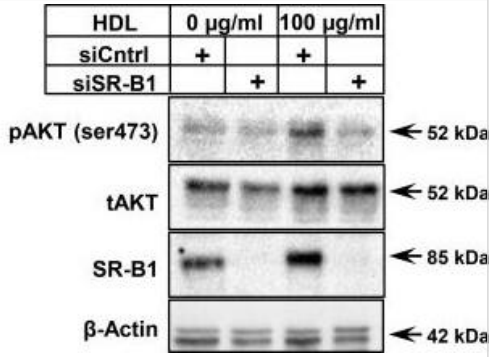


ATO interferes with efficient HAdV replication center formation and reorganization of PML□NBs. H1299 cells were infected with HAdV□C5 wt at a multiplicity of 20 FFU per cell, treated with the depicted concentrations of ATO at 2 h p.i., fixed 48 h p.i. with 4% PFA and double labeled with mAb B6□8 (α□E2A) and pAb NB100□59787 (α□PML). Primary antibodies were detected using Alexa488 (PML, green) and Alexa647 (E2A, red) conjugated secondary antibodies. A) Number of PML□NBs per cell in uninfected cells was determined using Volocity for at least n = 461 cells from two independent biological replicates. Statistically significant differences were determined using a one□way ANOVA and Dunnet's T3 test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. B) The proportion of infected cells showing track□like redistribution of PML□NBs was determined by counting for at least n = 212 cells and normalization to untreated infected cells. C) The proportion of infected cells showing formation of HAdV replication centers marked by the viral protein E2A (lower plot) was determined by counting for at least n = 212 cells and normalization to untreated infected cells. D) Cells showing either viral replication centers with PML track□like structures, replication centers without PML track□like structures, no replication centers but PML track□like structures or no replication centers, and no PML track□like structures were counted for at least n = 214 (virus infected cells due to E2A signal detected: either untreated/0 μm or treated with ATO/1 or 2 μm) and represented in pie charts. Statistically significant differences were determined using a one□way ANOVA and Dunnet's T3 test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. E) H1299 cells were infected with HAdV□C5 wt at a multiplicity of 20 FFU per cell, and treated with 0 or 2 μm of ATO at 2 h p.i. After 48 h, the cells were fixed with 4% PFA and stained using pAb NB100□59787 (α□PML) and mAb 6A□11 (α□E4orf3). Primary antibodies were detected using Alexa488 (PML, green) and Alexa647 (E4orf3, red) coupled secondary antibodies. Representative staining patterns for at least 30 uninfected cells treated with 0 or 2 μm ATO are shown in panels (a)–(d) and (m)–(p), infected cells treated with 0 or 2 μm ATO are shown in panels (e)–(l) and (q)–(x). Overlays of single fluorescence pictures (merge) are shown in panels (d), (h), (l), (p), (t), and (x). Data corresponds to two independent biological replicates performed and counted by different operators to avoid operator bias. Scale bar represents 10 μm. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32328411), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



ABCC4 protein expression level in CRC cell lines. Western blot performed in standard reducing SDS PAGE conditions using goat anti ABCC4 (#PA5 18315, Thermo Scientific) and rabbit anti ABCG2 (#ORB 155559 Biorbyt). (A) Protein expression level of ABCC4 and ABCG2 in HT-29 stably overexpressing transcription factor Snail (HT29/Snail) and control HT-29. ABCC4 level in the membrane fraction (obtained by biotynylation using EZ-Link Sulfo -NHS-Biotin Thermo Scientific kit) of HT-29 control cells and HT-29 Snail n = 3. (B) ABCC4 protein expression level in CRC cells in different states of EMT: CCD841CoN (most epithelial), CaCo-2 (moderate EMT), and Colo-320 (most mesenchymal) n = 3. (C) ABCC4 protein abundance in Extracellular Vesicles (EVs) released from HT-29 control cells and two HT-29 stably overexpressing transcription factor Snail clones (HT-29/Snail and HT-29/Snail17), n = 2. (D) Intracellular cAMP level measurement. Accumulation of cAMP in HT29 cells was measured using a cAMP competitive kit (#581001 Cayman Chemical). Cells were incubated for 24 h with MK571 20 μM, or untreated ones were assayed according to the manufacturer's protocol. Calculation were conducted using the Cayman data sheet. cAMP concentration of HT29 was set as 100%. T-test performed, n = 5; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$. NS—not statistically significant. (E) PKA phosphorylation profile analysis. HT29 Snail cells were seeded on a 6-well plate. Then, 24 h after, full growth medium was changed into starving (FBS free) medium for 24 h. Next, 20uM of MK571 was added to cells for 60, 30, 5, and 1 min. Cells without the starving procedure were used as a positive control, and negative control cells were not treated with MK571. Phosphorylation profile analysis was performed using phospho-(ser/thr) PKA Substrate Antibody #9621 (Cell Signaling Technology). Significant time- (exposure) related impact on the phosphorylation profile was observed for 42 kDa and 95–100 kDa proteins in HT29 Snail cells compared to no time-related changes in control cells, n = 3. (F) HT-29/Snail PKA phosphorylation profile analyzed with densitometry; statistical significance estimated using T-test. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$. NS—not statistically significant. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/33261018), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

E



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Hu S, Zhu Y, Zhao X et al. Hepatocytic lipocalin-2 controls HDL metabolism and atherosclerosis via Nedd4-1-SR-BI axis in mice Developmental cell 2023-10-18 [PMID: 37863040] (PLA, ICC/IF, Human, Mouse)

Xu (???) Y, Liu (??) C, Han (???) X et al. E17241 as a Novel ABCA1 (ATP-Binding Cassette Transporter A1) Upregulator Ameliorates Atherosclerosis in Mice Arteriosclerosis, Thrombosis, and Vascular Biology 2021-06-01 [PMID: 33441025]

Wolfisberg R, Thorselius CE, Salinas E et al. Neutralization and receptor use of infectious culture-derived rat hepacivirus as a model for HCV Hepatology 2022-11-01 [PMID: 35445423] (WB)

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Wolfisberg R, Holmbeck K, Billerbeck E et al. Molecular Determinants of Mouse Adaptation of Rat Hepacivirus Journal of virology 2023-03-27 [PMID: 36971565]

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Lyu J, Imachi H, Fukunaga K et al. Exendin-4 Increases Scavenger Receptor Class BI Expression via Activation of AMPK/FoxO1 in Human Vascular Endothelial Cells Current issues in molecular biology 2022-11-03 [PMID: 36354682] (WB, Human)

Frey K, Goetze S, Rohrer L et al. Decoding Functional High-Density Lipoprotein Particle Surfaceome Interactions International journal of molecular sciences 2022-08-22 [PMID: 36012766] (KD, WB, Human)

Details:
Supplementary Figure 6

Traughber Ca, Opoku E, Brubaker G et Al. SR-B1 uptake of HDL promotes prostate cancer proliferation and tumor progression Front Pharmacol 2016-12-27 [PMID: 28018216]

Raith M Interactions Between Soft Nanoparticles and Mammalian Cells Thesis 2022-01-01 (ICC/IF, Mouse)

Li L, Zhong S, Li R et al. Aldehyde dehydrogenase 2 and PARP1 interaction modulates hepatic HDL biogenesis by LXRalpha-mediated ABCA1 expression JCI insight [PMID: 35393951]

More publications at http://www.novusbio.com/NB400-104

Procedures

Western Blot protocol for SR-BI Antibody (NB400-104)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunocytochemistry/ Immunofluorescence Protocol for SR-BI Antibody (NB400-104)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for SR-BI Antibody (NB400-104)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Flow (Intracellular) Protocol for SR-BI Antibody (NB400-104)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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

Limitations

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