

外泌体技术资料

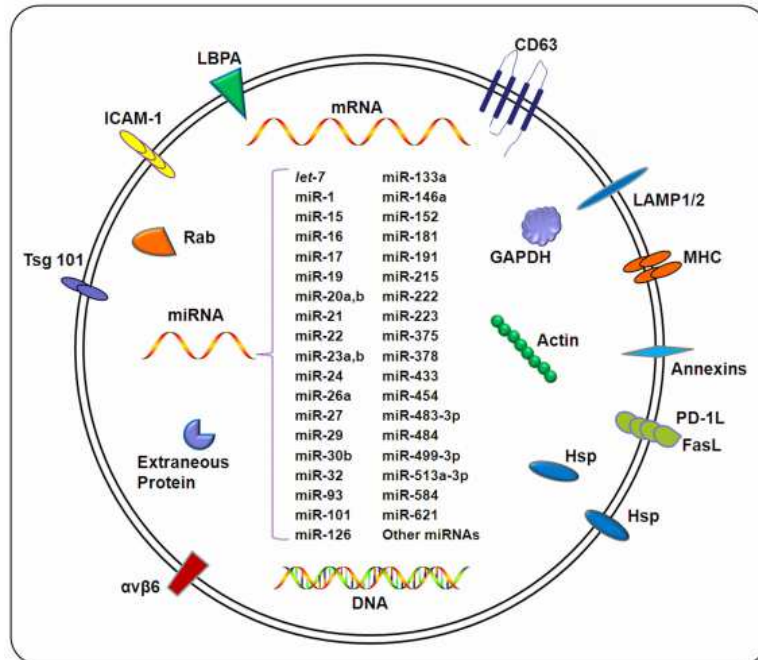
Western blot 结果文献数据汇总

宇玫博生物

2018-3

常见技术问题

1) 外泌体 Western blot 鉴定时通常会检测哪些指标蛋白?



根据 22 篇外泌体相关文献的统计, 排在前 4 位的检测指标为 CD63 (13/22)、Tsg101(8/22)、CD9 和 CD81 并列第三位(6/22); 接着检测较多的 4 个指标为 Alix(4/22)、HSP70(3/22)、flotillin (3/22)和 Syntenin (2/22); 此外还有一些指标仅在 1 篇文献中出现过, 例如 HSP90、LAMP2B、LMP1、ADAM10、nicastatin、AChE、AQP2、RPL5、a-1AT。

针对外泌体的定性检测至少选择两个指标就能满足文章发表需要了, 比如检测 CD63 和 Tsg101。

2, 外泌体 Western blot 鉴定时通常使用哪些公司的抗体?

CD63 抗体的供应商: 文献报道最多的两个公司 Abcam (ab68418 和 ab8219) 和 Santa Cruz (sc-5275), 其他公司有 Systems Biosciences Inc、Abnova (clone MEM-259)、BD Pharmingen (551458)。

Tsg101 抗体的供应商: 文献报道最多的两个公司 Abcam (ab125011) 和 Santa Cruz (sc-7964), 其他公司有 Thermo Pierce 和 Sigma-Aldrich。

CD9 抗体的供应商: 文献报道最多的是 Abcam (ab92726), 其他的公司有 Santa Cruz 和 Systems Biosciences Inc。

CD81 抗体的供应商: 文献报道最多的是 Santa Cruz (sc-9158 和 H-121), 其他的公司有 Novus Biologicals (Cat# NB100-65805)、ImmunoWay。

Alix 抗体的供应商: 文献报道的公司有 Cell Signaling (#2171)、Santa Cruz、Abcam。

HSP70 抗体的供应商: 文献报道的公司有 Abcam (ab19136)、Santa Cruz (sc-24)。

flotillin 抗体的供应商: 文献报道的公司有 BD Bioscience。

Syntenin 抗体的供应商: 文献报道的公司有 ThermoFisher Scientific (Cat# PA5-28826) 和 Abcam (ab133267)。

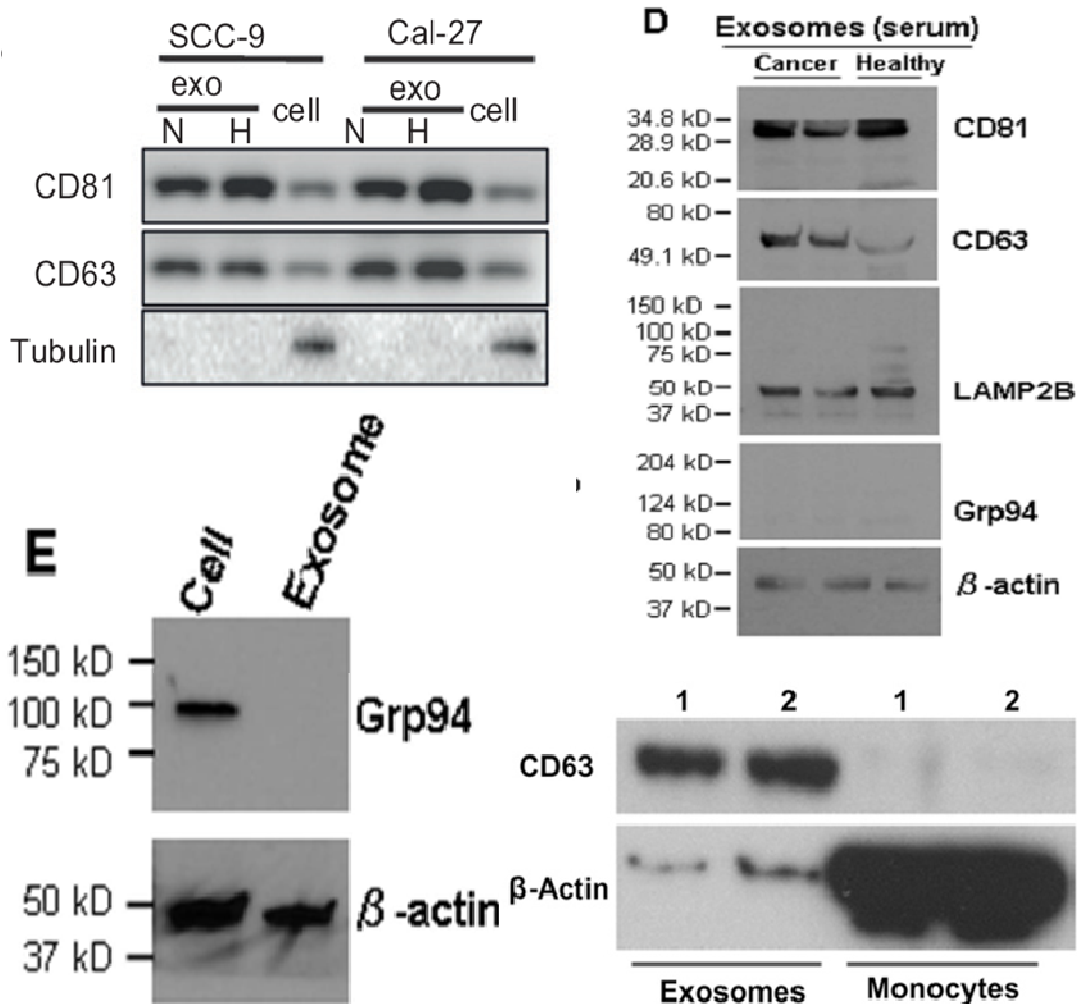
其他抗体信息请查阅附录整理的文献资料。

3, 外泌体 Western blot 定性检测中有没有阳性对照?

从发表的文章来看,并没有一篇文章结果中提到了阳性对照样品,但是已经被文献报道的样品可以作为后人 Western blot 检测实验的阳性对照,通过这样阳性样品的检测来判断实验体系是否正常。罗列一下文献中的阳性对照样品:脂肪组织巨噬细胞外泌体、神经干细胞外泌体、大鼠皮层神经元外泌体、SCC-9 和 Cal-27 细胞外泌体、MDA-MB-231 外泌体、星型胶质细胞外泌体、中性白细胞外泌体等。

4, 外泌体 Western blot 定性检测中有没有内参?

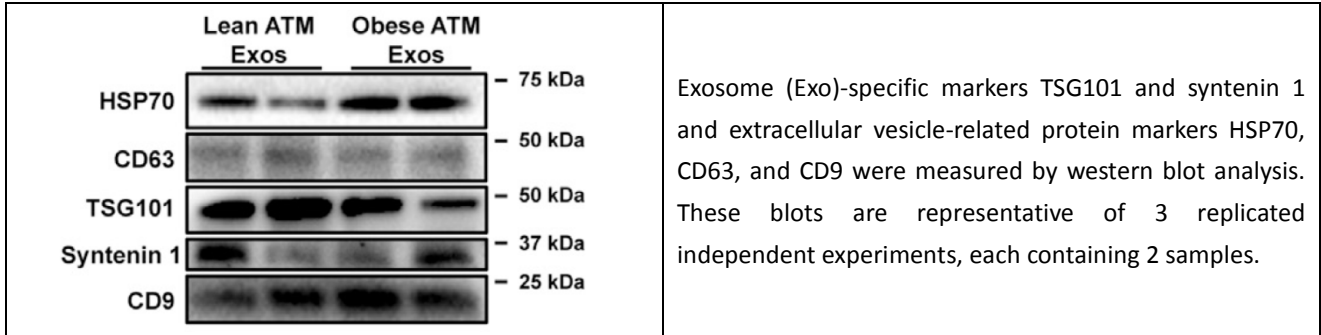
大部分文献中没有检测内参基因 (19/22),个别文章中使用了 Actin、GAPDH 和 Tubulin 作为内参进行检测 (见下图),从检测的结果来看,外泌体中的 Actin、GAPDH 和 Tubulin 的表达丰度相对于正常细胞样品中的较低,Western blot 检测时可能会检测不到任何条带或者条带微弱。



附录：文献资料

1, [Cell](#). 2017 Oct 5;171(2):372-384.e12. doi: 10.1016/j.cell.2017.08.035. Epub 2017 Sep 21.

Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity.



抗体信息：

Rabbit polyclonal anti-CD63 Abcam Cat# ab68418; RRID:AB_10563972

Rabbit monoclonal anti-CD9 Abcam Cat# ab92726; RID:AB_10561589

Rabbit monoclonal anti-TSG101 Abcam Cat# ab125011; RRID:AB_10974262

Mouse monoclonal anti-HSP70 Santa Cruz Cat# sc-24; RRID:AB_627760

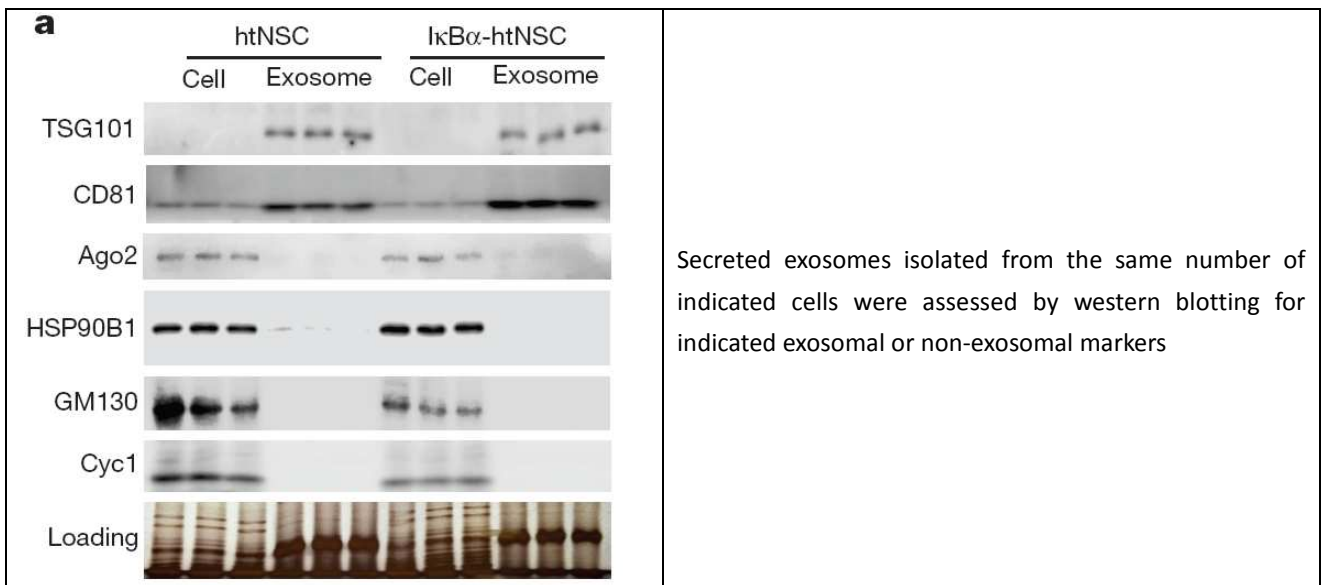
Rabbit polyclonal anti-Syntenin 1 ThermoFisher Scientific Cat# PA5-28826; RRID:AB_2546302

样品信息：

脂肪组织巨噬细胞及其外泌体；

2, [Nature](#). 2017 Aug 3;548(7665):52-57. doi: 10.1038/nature23282. Epub 2017 Jul 26.

Hypothalamic stem cells control ageing speed partly through exosomal miRNAs.

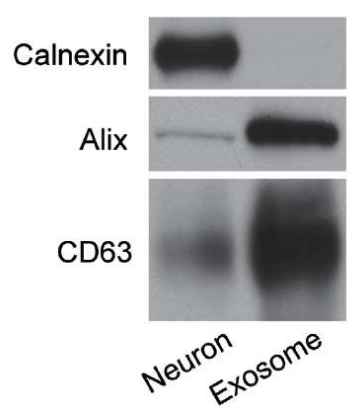


抗体信息： mouse anti-TSG101 (Santa Cruz, sc-7964), rabbit anti-CD81 (Santa Cruz, sc-9158), rabbit anti-AGO2 (Cell Signaling, 2897), rabbit anti-HSP90B1 (Cell signaling, 2104), mouse anti-GM130 (Santa Cruz, sc-55591), and rabbit anti-Cyc1 (Cell Signaling, 4272). Silver staining was performed using the ProteoSilver Silver Stain Kit (Sigma-Aldrich).

样品信息： 神经干细胞及其外泌体；

3, [Cell Res.](#) 2017 Jul;27(7):882-897. doi: 10.1038/cr.2017.62. Epub 2017 Apr 21.

Neurons secrete miR-132-containing exosomes to regulate brain vascular integrity.

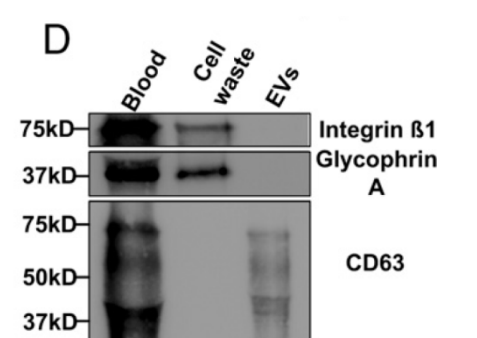
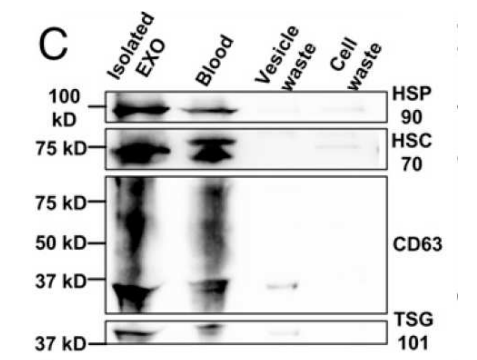
	The exosomes were isolated from the conditioned medium of cultured primary rat cortical neurons. Both Alix and CD63 are exosomal markers, and Calnexin is an endoplasmic reticulum marker.
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抗体信息: anti-CD63 (BD Pharmingen,551458); anti-Calnexin (Abcam, ab22595), anti-Alix (Cell Signaling, 2171).

样品信息: 大鼠皮层神经元及其外泌体;

4, [Proc Natl Acad Sci U S A.](#) 2017 Oct 3;114(40):10584-10589. doi: 10.1073/pnas.1709210114. Epub 2017 Sep 18.

Isolation of exosomes from whole blood by integrating acoustics and microfluidics.

D 	(D) Western blot with expression of RBC marker (GYPA), PLT marker (integrin β1), and EV markers (CD63). The proteins from blood, cell waste sample, and isolated EVs were extracted and prepared for electrophoresis.
C 	(C) Western blot of exosome markers, showing a prominent expression in the isolated exosome and blood samples, while the other samples (vesicle waste and cell waste) exhibited low expression level of exosomal proteins.

抗性信息: The membranes were incubated separately with mouse anti-CD63 (sc-5275, 1 μ g/mL, Santa Cruz), mouse anti-HSP90 (ab13492, 1 μ g/mL, Abcam), rabbit anti-TSG101 (ab30871, 1 μ g/mL, Abcam), rat anti-HSC70 (ab19136, 1 μ g/mL, Abcam),

样品信息: 细胞上清外泌体;

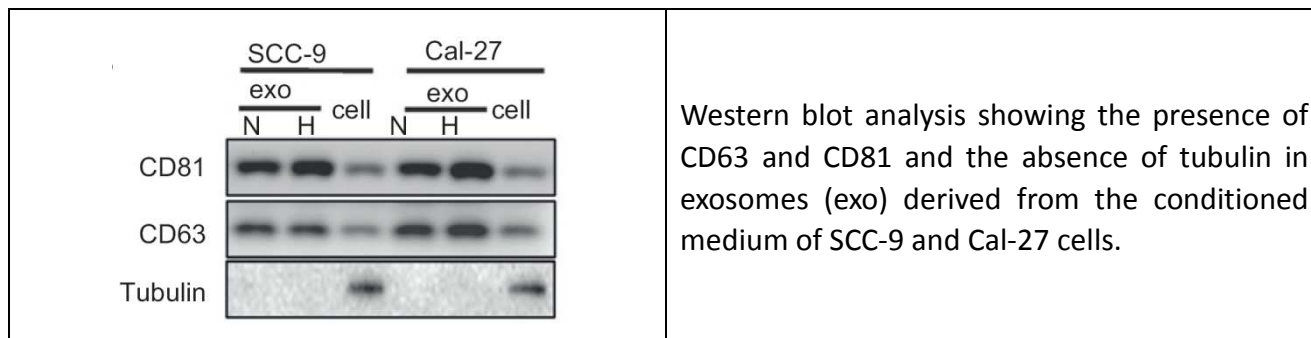
上海宇玫博生物科技有限公司 Umibio (Shanghai) Co.Ltd

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5, [Cancer Res.](#) 2016 Apr 1;76(7):1770-80. doi: 10.1158/0008-5472.CAN-15-1625. Epub 2016 Mar 18.

Exosomes Derived from Hypoxic Oral Squamous Cell Carcinoma Cells Deliver miR-21 to Normoxic Cells to Elicit a Prometastatic Phenotype.

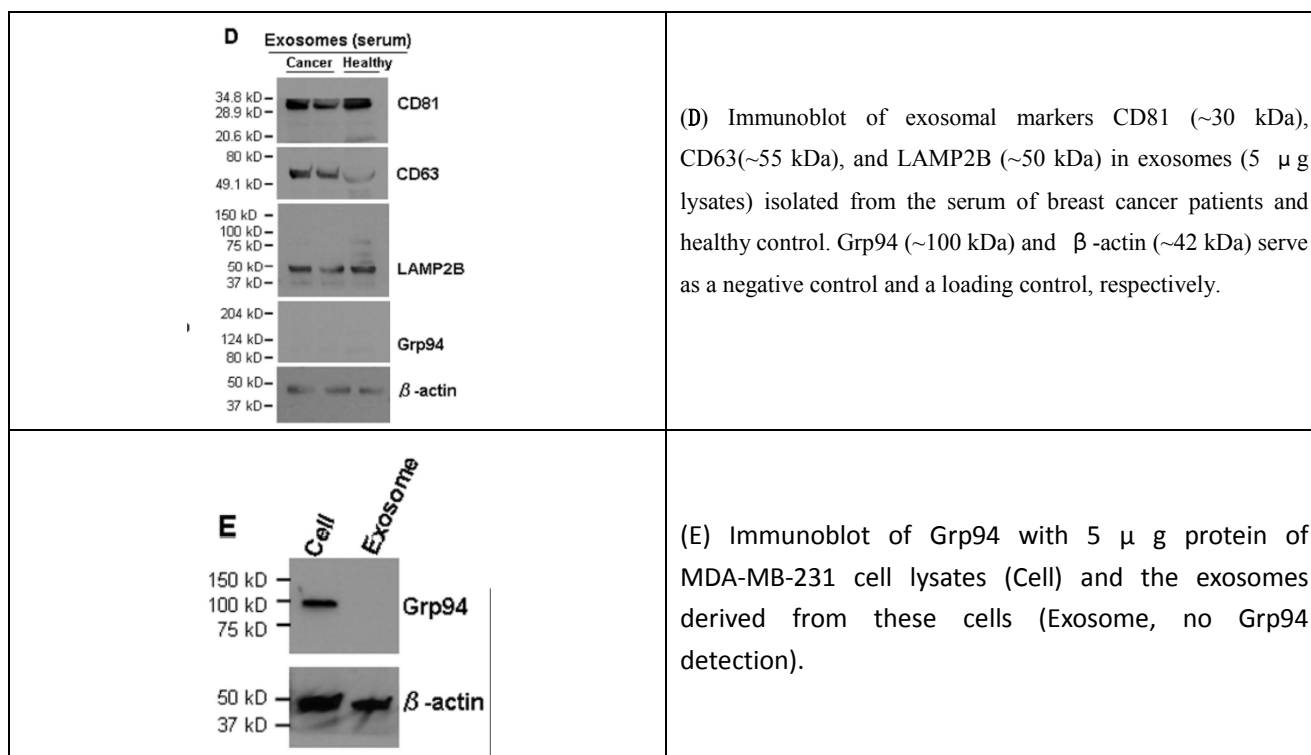


抗性信息: mouse anti-CD81 (1:1,000, Santa Cruz Biotechnology), mouse anti-CD63 (1:1,000, Santa Cruz Biotechnology), rabbit antitubulin (1:1,000 Novus).

样品信息: SCC-9 and Cal-27 cells 及其对应外泌体

6, [Sci Rep.](#) 2016 Nov 7;6:36502. doi: 10.1038/srep36502.

A rapid, automated surface protein profiling of single circulating exosomes in human blood.



抗体信息:

mouse anti-human CD63 antibody (Cat# ab8219, Abcam, Cambridge, MA, USA);
mouse monoclonal anti-human CD81 (Cat# NB100-65805, Novus Biologicals, Littleton, CO, USA)
rabbit polyclonal anti-human LAMP2B (Cat# ab18529, Abcam, Cambridge, MA, USA);
rabbit polyclonal anti-human Grp94 (Cat# 2104P, Cell Signaling Technology, Danvers, MA, USA)

样品信息:

人血清外泌体; MDA-MB-231外泌体;

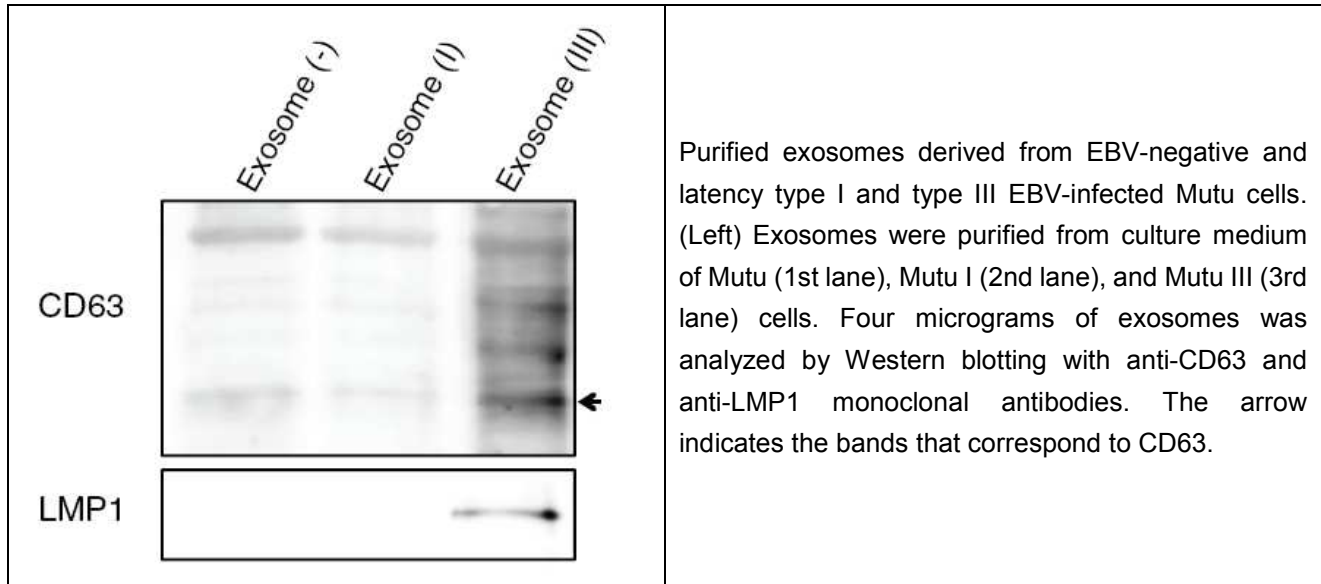
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Add: Rm 303, 1st building, No.88 Cailun Rd, Shanghai, China Tel: (86)21 60521508

7, [J Virol](#). 2013 Sep;87(18):10334-47. doi: 10.1128/JVI.01310-13. Epub 2013 Jul 17.

Exosomes derived from Epstein-Barr virus-infected cells are internalized via caveola-dependent endocytosis and promote phenotypic modulation in target cells.



抗体信息:

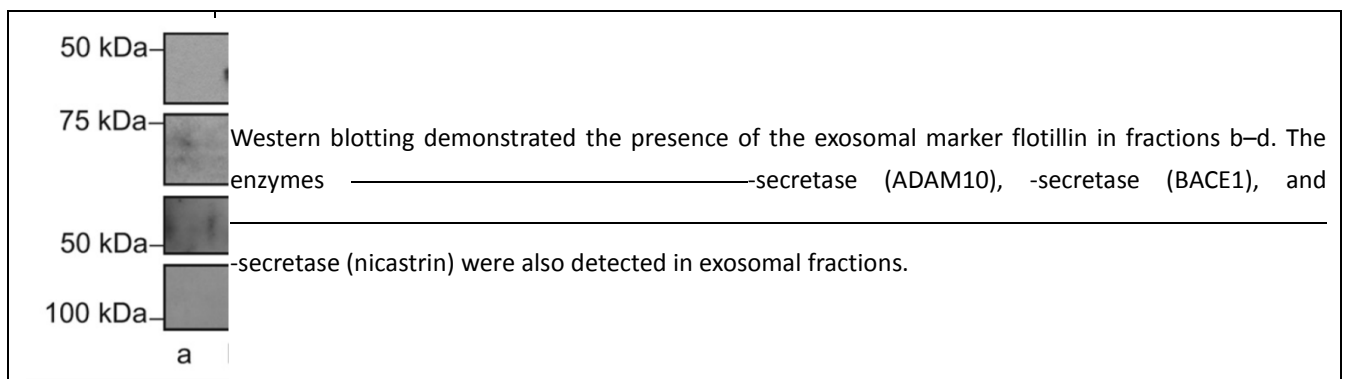
anti-CD63 monoclonal antibody (clone MEM-259, 1:1,000 dilution; Abnova, Taipei, Taiwan)

anti-LMP1 monoclonal antibody (clone S12, 1:10,000 dilution; kindly provided by Teruhito Yasui, Osaka University)

样品信息: 细胞上清外泌体;

8, [J Biol Chem](#). 2012 Dec 14;287(51):43108-15. doi: 10.1074/jbc.M112.404467. Epub 2012 Nov 5.

The exosome secretory pathway transports amyloid precursor protein carboxyl-terminal fragments from the cell into the brain extracellular space.



抗体信息:

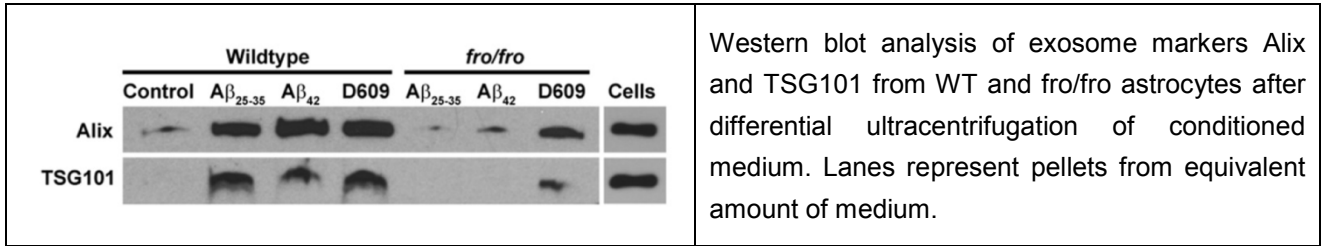
anti-flotillin (1:1000; BD Transduction Laboratories), anti-ADAM10 (1:2000; Millipore),

anti-BACE1 (1:1000; Millipore), and anti-nicastrin (1:1000; Millipore).

样品信息: 小鼠脑浆组织外泌体 (a-g为密度梯度离心中的不同密度样品)

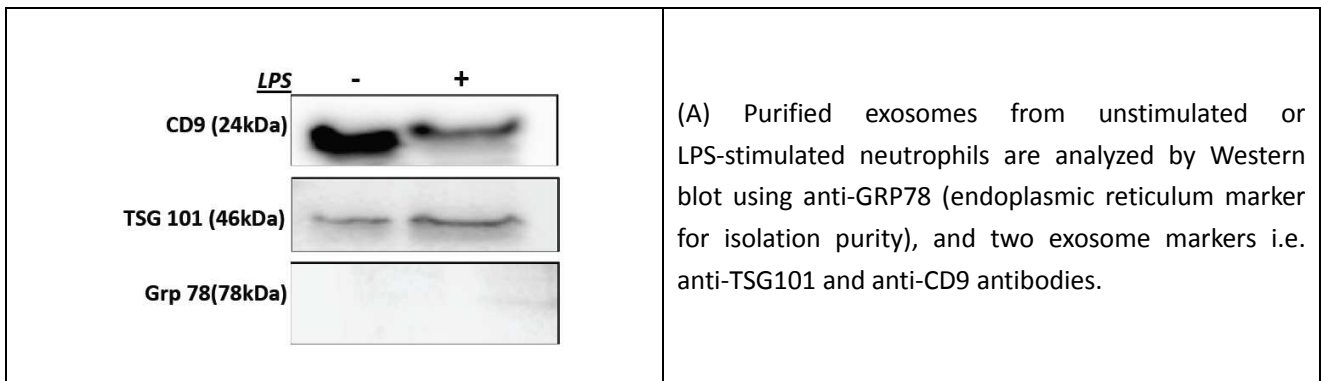
9, [J Neurosci](#). 2016 Aug 17;36(33):8653-67. doi: 10.1523/JNEUROSCI.1429-16.2016.

Neutral Sphingomyelinase-2 Deficiency Ameliorates Alzheimer's Disease Pathology and Improves Cognition in the 5XFAD Mouse.



抗体信息：. Antibodies against TSG101 (C-2), Alix (1A12), were from Santa Cruz Biotechnology

样品信息：astrocytes Exosome（星型胶质细胞外泌体）

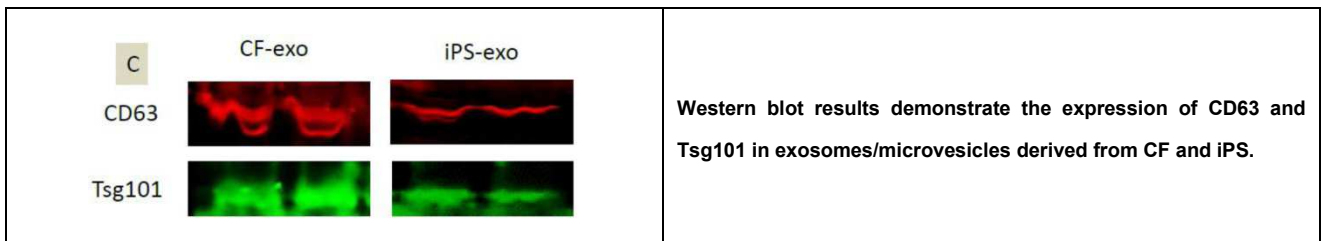


抗体信息：anti-TSG101 (1:300; #ARP38773; Cederlane), anti-CD9 (1:200; #312102, Biolegend), and anti-Grp78 (1:250; #LC-C638; Cederlane) antibodies.

样品信息：neutrophils exosome（中性白细胞外泌体）

10, [Int J Cardiol](#). 2015 Aug 1;192:61-9. doi: 10.1016/j.ijcard.2015.05.020. Epub 2015 May 8.

Exosomes/microvesicles from induced pluripotent stem cells deliver cardioprotective miRNAs and prevent cardiomyocyte apoptosis in the ischemic myocardium.

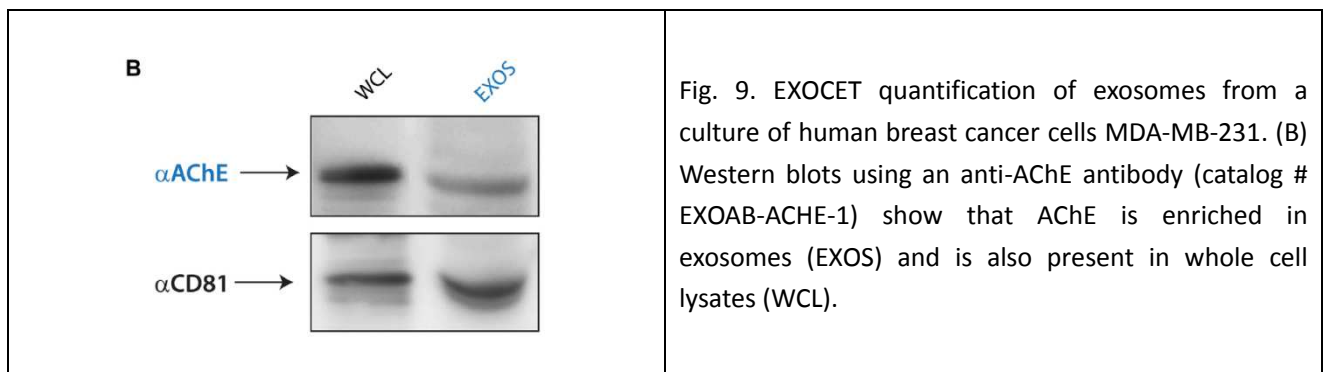


抗体信息： rabbit anti-CD63 (1:250, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), mouse anti-TSG101 (1:250, Thermo Pierce),

样品信息：cardiac fibroblast exosome(CF-exo), iPS cells exosome (iPS-exo)

11, [Methods](#). 2015 Oct 1;87:31-45. doi: 10.1016/j.ymeth.2015.04.015. Epub 2015 Apr 24.

Integrated systems for exosome investigation.

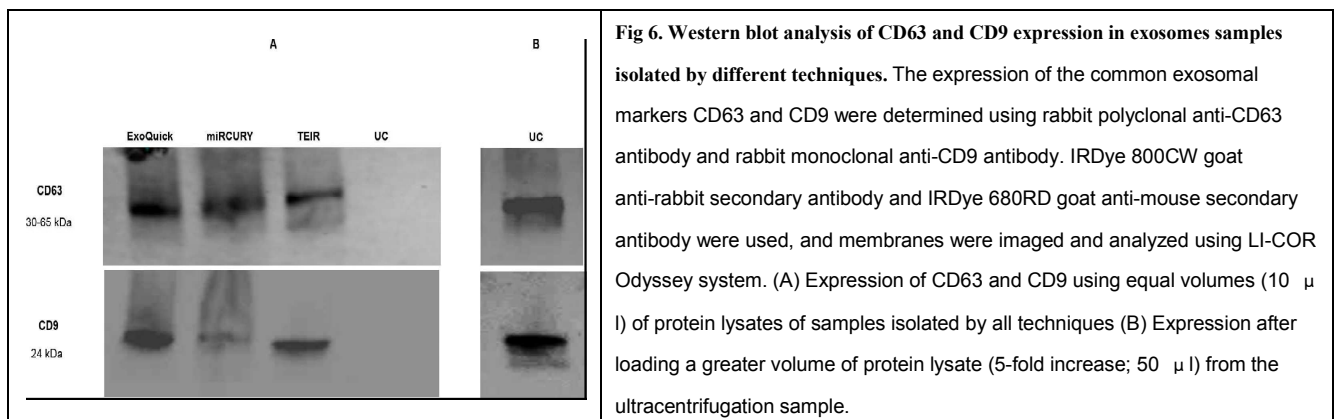


抗性信息：信息不全；

实验结果：CD81 分子在 MDA-MB-231.细胞裂解液和外泌体中都表达。

12, [PLoS One](#). 2017 Jan 23;12(1):e0170628. doi: 10.1371/journal.pone.0170628. eCollection 2017.

A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three Commercial Reagents.

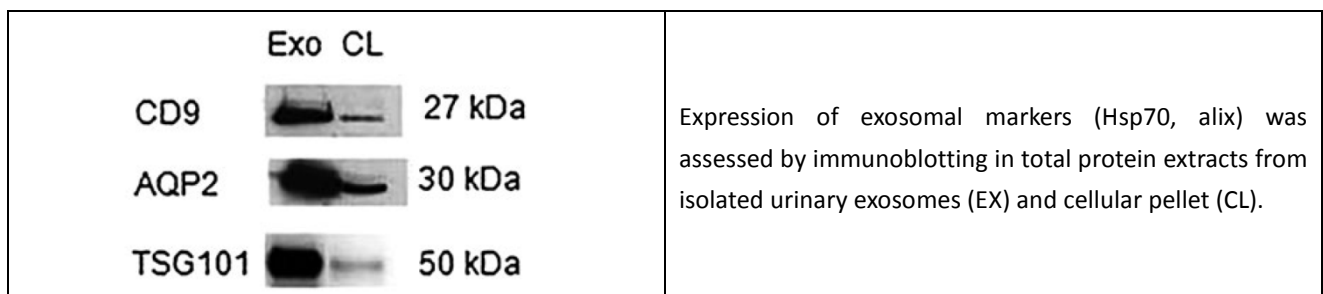


抗体信息：Rabbit monoclonal anti-CD63 antibody was from Abcam (Cambridge, MA), and rabbit polyclonal anti-CD9 antibody was purchased from Santa Cruz (Dallas, Texas).

样品信息：Pooled human serum (Valley Biomedical, Winchester, VA, catalog #HS1004P)

13, [Nephrol Dial Transplant](#). 2015 Sep;30(9):1488-96. doi: 10.1093/ndt/gfv128. Epub 2015 Jun 3.

miR-29c in urinary exosomes as predictor of early renal fibrosis in lupus nephritis.

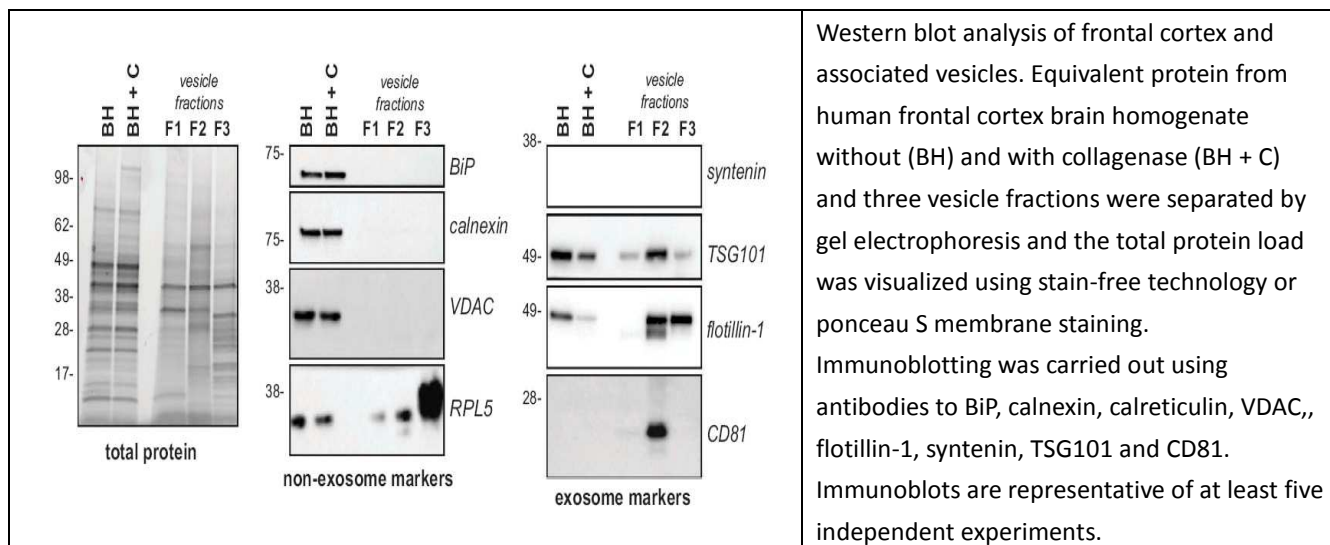


抗体信息：Different antibodies against exosomal markers (rabbit anti-CD9, rabbit anti-AQP2, rabbit anti-TSG101, dilutions 1:1000, Abcam)

样品信息：尿液细胞（CL）和尿液外泌体（Exo）

14, [J Extracell Vesicles](#). 2017 Jul 26;6(1):1348885. doi: 10.1080/20013078.2017.1348885. eCollection 2017.

A rigorous method to enrich for exosomes from brain tissue.



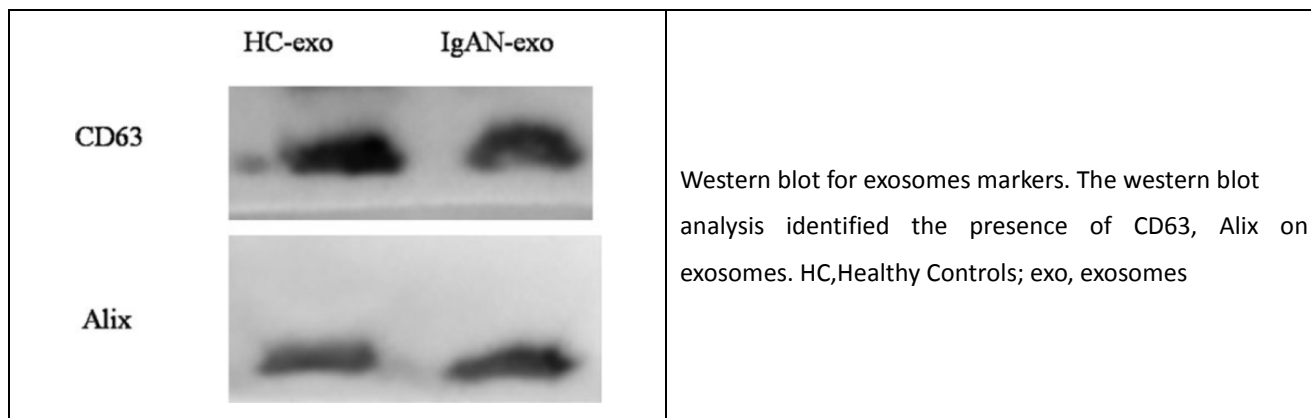
抗体信息:

The antibodies BiP and flotillin-1 were from BD Biosciences, syntenin (#ab133267) and calnexin (ab22595) from Abcam, calreticulin, VDAC, RPL5, anti-mouse and anti-rabbit HRP from Cell Signalling Technology, TSG-101 from Sigma-Aldrich and CD81 (H-121) from Santa-Cruz.

样品信息: 人源大脑皮层及其外泌体组分

15, [J Clin Lab Anal](#). 2018 Feb;32(2). doi: 10.1002/jcla.22226. Epub 2017 Apr 6.

Differential expression of urinary exosomal microRNAs in IgA nephropathy.



抗体信息: EV marker Alix (Anti-PDC6I antibody, 1:500 dilution; Abcam, Cambridge, UK)

样品信息: 尿液外泌体

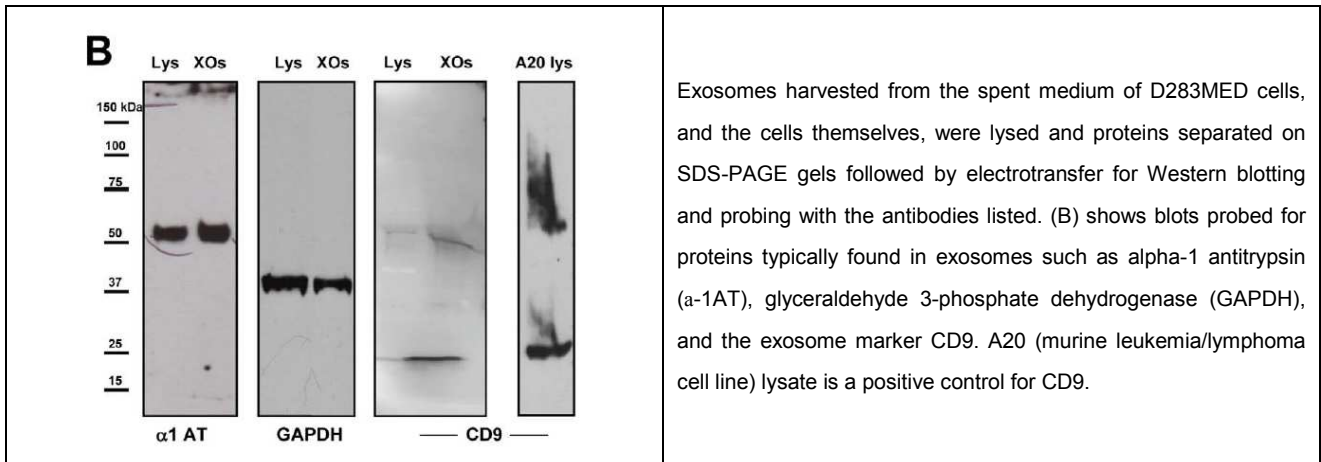
16, [PLoS One](#). 2012;7(7):e42064. doi: 10.1371/journal.pone.0042064. Epub 2012 Jul 27.

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Medulloblastoma exosome proteomics yield functional roles for extracellular vesicles.

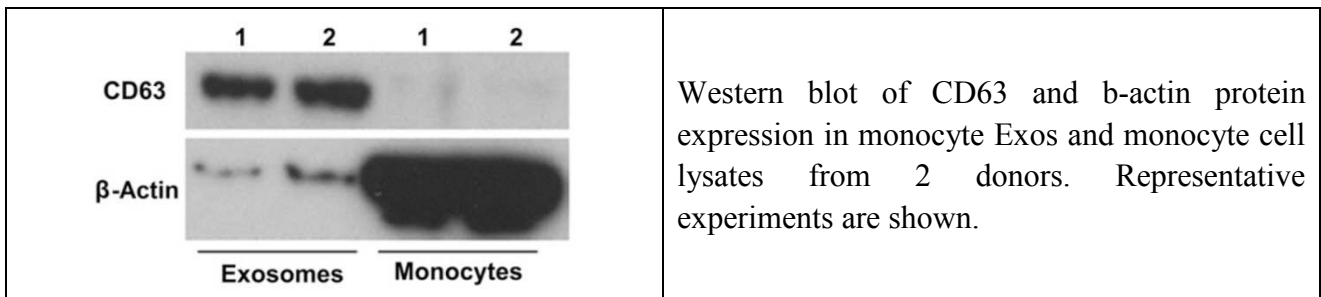


抗体信息: against CD9 and CD63, EXOAB-CD9A-1 and EXOABCD63A-1, respectively (Systems Biosciences Inc).

样品信息: D283MED cells (Lys) 和D283MED cells的外泌体 (XOs)

17, [FASEB J.](#) 2016 Sep;30(9):3097-106. doi: 10.1096/fj.201600368RR. Epub 2016 May 25.

Monocyte exosomes induce adhesion molecules and cytokines via activation of NF-κB in endothelial cells.

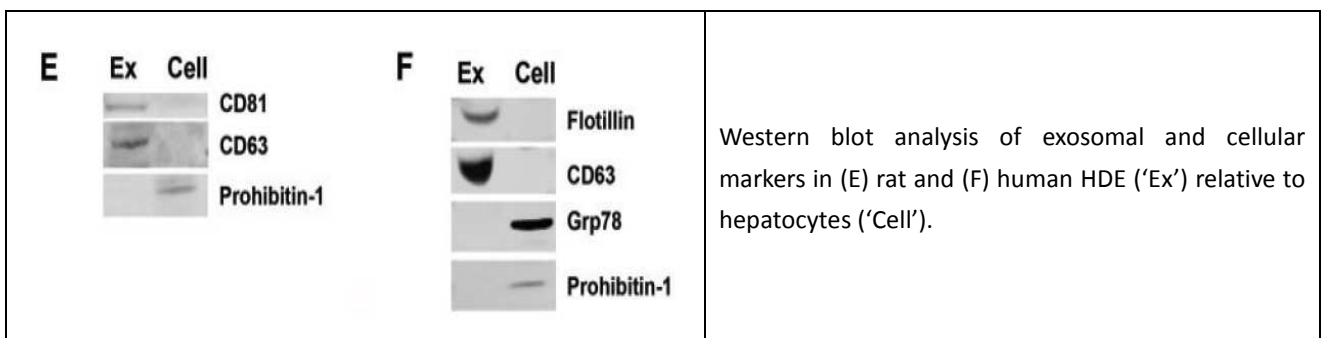


抗体信息: anti-CD63 antibody (System Biosciences Inc.)

样品信息: 人血液单核细胞及其外泌体

18, [Toxicol Sci.](#) 2016 Jun;151(2):365-75. doi: 10.1093/toxsci/kfw047. Epub 2016 Mar 8.

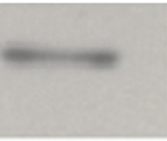
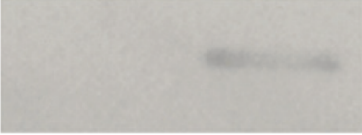
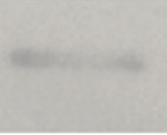
Subtoxic Alterations in Hepatocyte-Derived Exosomes: An Early Step in Drug-Induced Liver Injury?



抗体信息: The following primary antibodies were used for immunoblotting: Flotillin-1 (BD Transduction Laboratories), CD63 (Abcam), CD81 (AbD Serotech), Prohibitin-1 (Santa Cruz Biotechnology Inc.), and Grp78 (BD Transduction Laboratories).

样品信息: 大鼠和人源肝细胞及其外泌体

19, [Biomed Res Int.](#) 2017;2017:7298160. doi: 10.1155/2017/7298160. Epub 2017 Jan 29. **Urinary Exosomal miR-193a Can Be a Potential Biomarker for the Diagnosis of Primary Focal Segmental Glomerulosclerosis in Children.**

	CL	Exo	
HSP70			70 kDa
CD9			25 kDa

The detection of exosomal markers (Hsp70, CD9) by Western blotting. Data are representative images from the same FSGS patient. CL, urinary cellular lysates; Exo, urinary exosomes.

抗体信息:

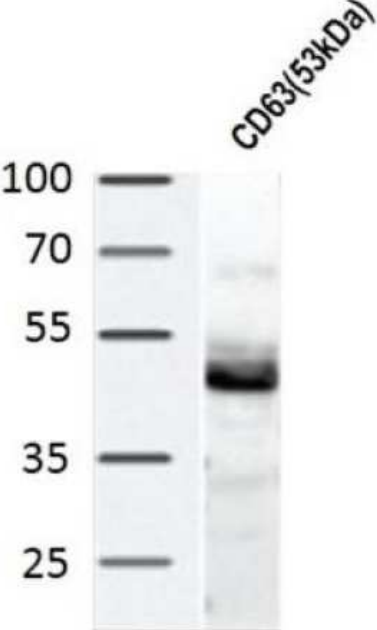
CD9 (dilution 1 : 1000, Abcam, Cambridge, MA, USA)

HSP70 (dilution 1 : 1000, Abcam, Cambridge, MA, USA)

样品信息: 尿液细胞CL及其外泌体Exo

20, [J Biotechnol.](#) 2017 Oct 10;259:135-139. doi: 10.1016/j.jbiotec.2017.07.029. Epub 2017 Jul 29.

Urinary-exosomal miR-2909: A novel pathognomonic trait of prostate cancer severity.

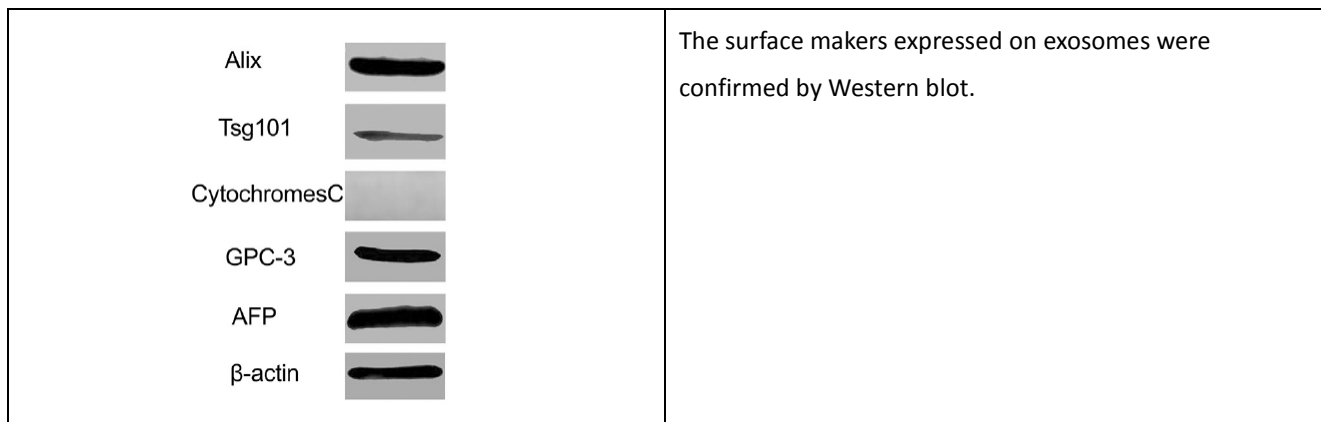
	Representative Immuno-blot depicting the characteristic marker (CD63) of exosomes isolated from either extracellular culture-media or human urine samples
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抗体信息: exosome-specific marker (CD63) antibody 未提供任何公司信息

样品信息: 尿液外泌体

21, [Transl Oncol](#). 2018 Jan 27;11(2):250-258. doi: 10.1016/j.tranon.2018.01.001. [Epub ahead of print]

Dendritic Cells Pulsed with Exosomes in Combination with PD-1 Antibody Increase the Efficacy of Sorafenib in Hepatocellular Carcinoma Model.

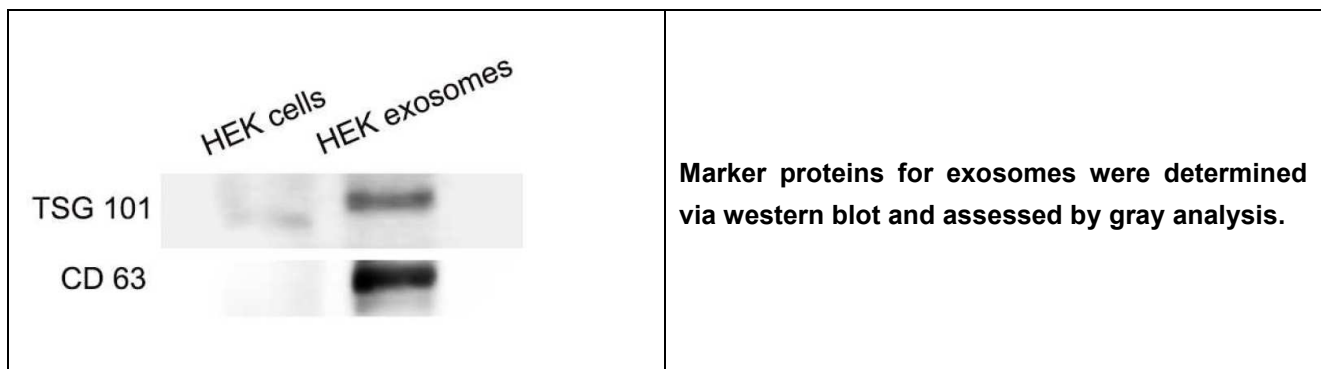


抗体信息：无任何信息；

样品信息：T cells外泌体

22, [Mol Ther](#). 2018 Jan 8. pii: S1525-0016(18)30008-X. doi: 10.1016/j.ymthe.2018.01.001. [Epub ahead of print]

Exosomes Serve as Nanoparticles to Deliver Anti-miR-214 to Reverse Chemoresistance to Cisplatin in Gastric Cancer.



抗体信息：anti-CD63 (1:200, Santa Cruz), anti-tsg 101 (1:200, Santa Cruz), anti-β-actin (1:500, Santa Cruz), CD81 antibody (1:500,

ImmunoWay),

样品信息：HEK293T及其外泌体



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