读 书 报 告

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Exosomes derived from alcohol-treated hepatocytes horizontally transfer liver specific miRNA-122 and sensitize monocytes to LPS

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目录

1 研究背景

2 结果分析

3 总结讨论

4 心得体会

01 研究背景

研究背景

- 外泌体在细胞之间和各种组织间通过水平转移大分子作为信息的天然输送机。外泌体在不同疾病的发病机理中起作用，并且还可以通过脂质，蛋白质或遗传物质的转移来影响细胞。然而，外泌体在酒精性肝炎（AH）中的作用尚待探究。

Exosome-mediated transfe **Codon-optimized Gaussia luciferase cDNA for mammalian**
novel mechanism of geneti **gene expression in culture and in vivo.**

来自Oxford Univ Press

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作者 H Valadi，K Ekström，A Bossios，M Sjöstra

摘要 ABSTRACT Exosomes are vesicles of endoc communication between cells, facilitating pro from a mouse and a human mast cell line (M derived mouse mast cells, contain RNA. Mici approximately 1300 genes, many of which ar

出版源 《Nature Cell Biology》, 2007, 9(9):654-659

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来自Wiley

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作者 BA Tannous，DE Kim，JL Fernandez，R Weissleder，XO Breakefield

摘要 Photoproteins have played a major role in advancing our understanding of biological processes. A broader array of biocompatible, nontoxic, and novel reporters can serve to expand this potential. Here we describe the properties of a luciferase from the copepod marine organism Gaussia princeps. It is a monomeric protein composed of 185 aa (19.9 kDa) with a short coding sequence (555 bp) making it suitable for viral vectors. The humanized form of Gaussia luciferase (hGLuc) was efficiently expressed in mammalian cells following de... 更多

出版源 《Molecular Therapy the Journal of the American ..., 2005, 11(3):435-43

被引量 503

源自肥大细胞的外泌体可以转移RNA到小鼠和人类肥大细胞

荧光标记的成胶质细胞瘤衍生的外泌体向人脑微血管内皮细胞的水平转移

研究背景

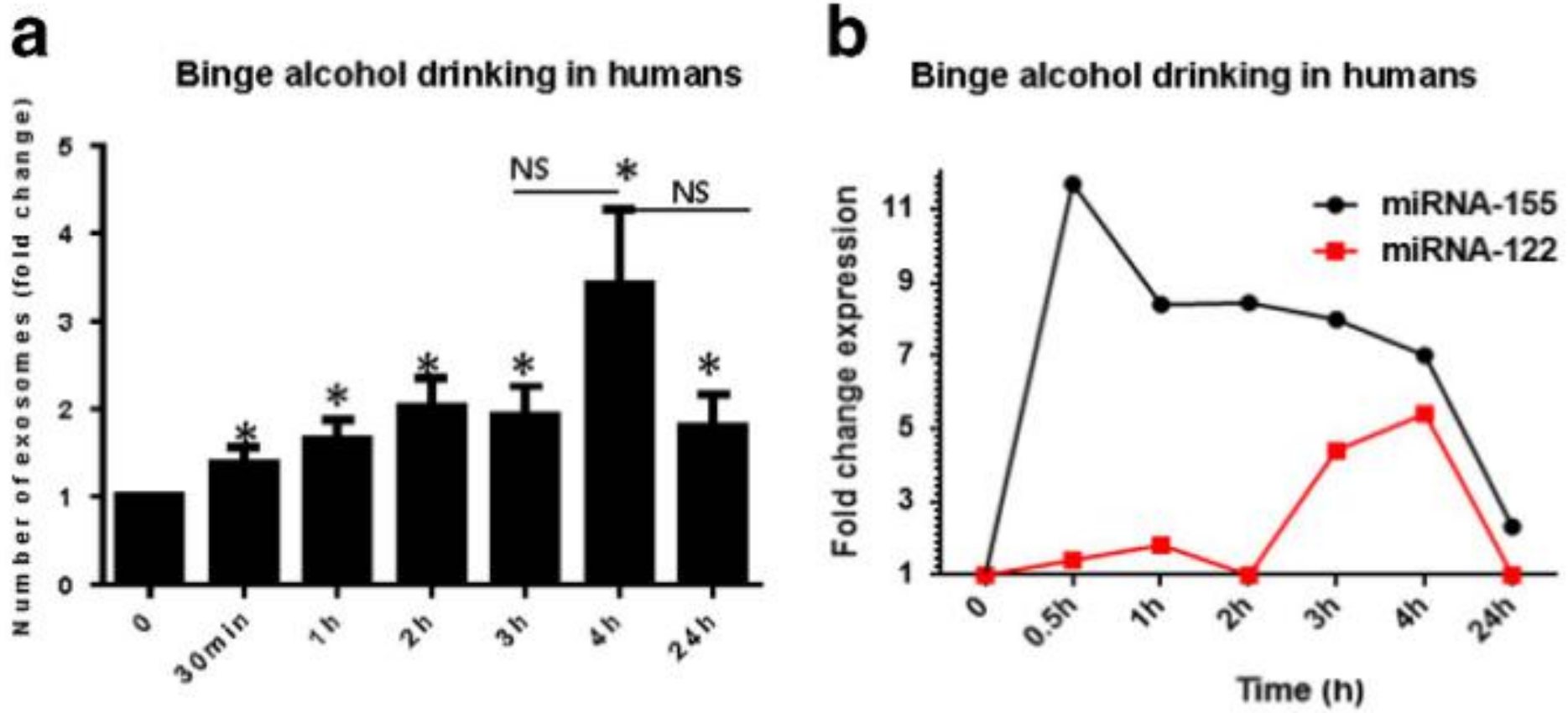
- 研究显示肠道微生物衍生的脂多糖（LPS）和促炎细胞因子例如TNFα和IL-1β在动物模型中在酒精性肝炎（AH）中起关键作用，表明酒精相关炎症有助于AH的发展 。AH的功能研究表明炎症介质、促炎细胞因子、抗炎细胞因子和肝保护细胞因子在AH的发病机制中的作用。 在AH发病机理中的多种炎性诱导物，包括酒精，其代谢物和随后的细胞变化已经被充分记录。最近研究显示肝细胞损伤是酒精诱导肝炎的先决条件。然而，外泌体在肝细胞和免疫细胞之间可能的作用还有待探索。

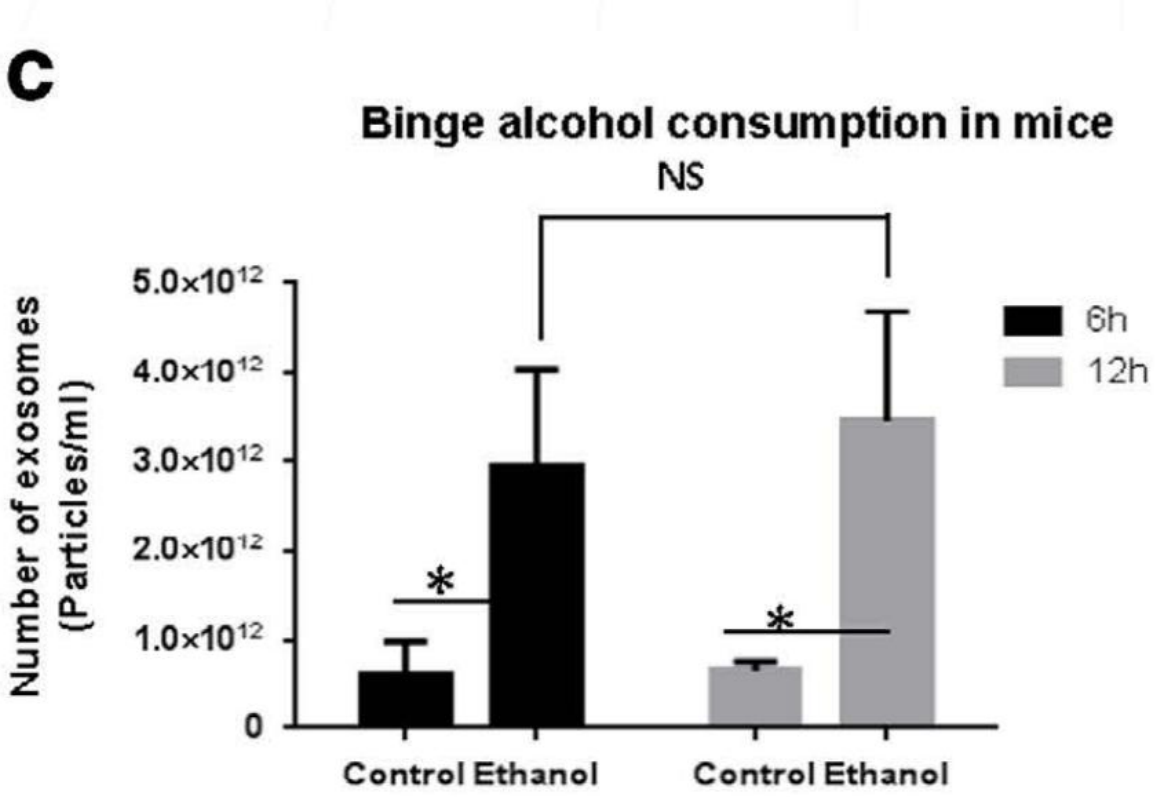
研究背景

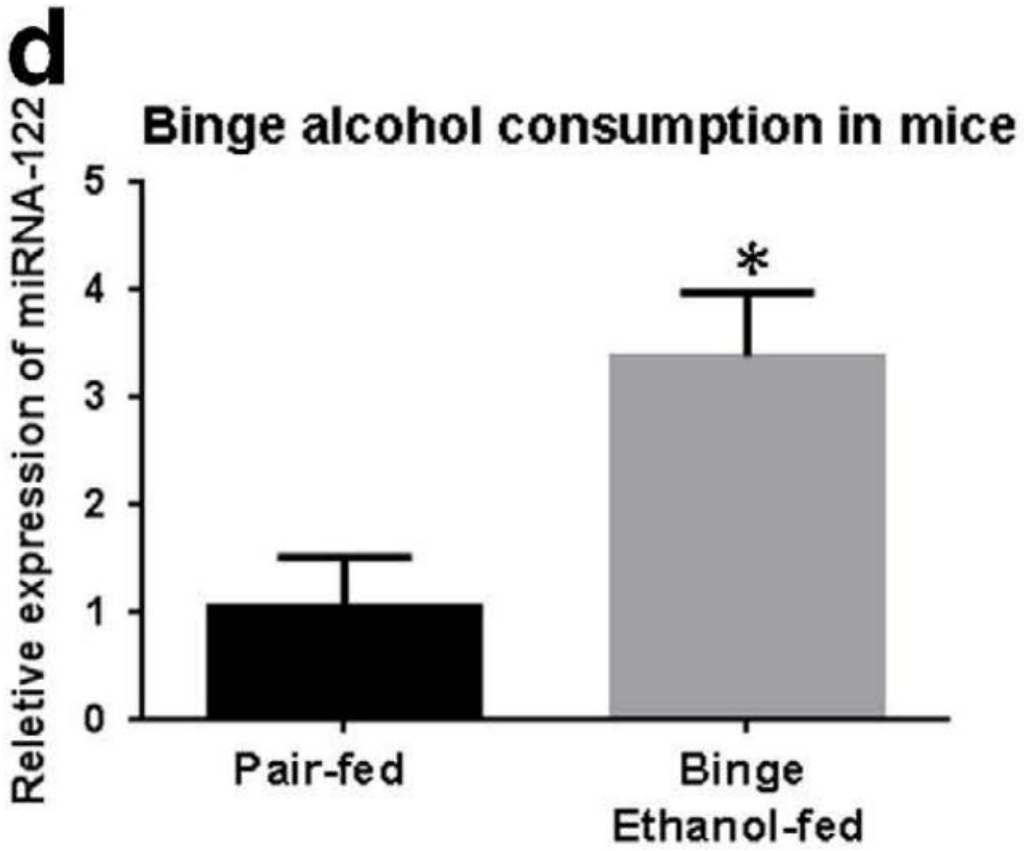
- miRNA-122是肝特异性miRNA并且在肝细胞中丰富。miRNA-122是第一个鉴定的组织特异性miRNA之一，并且在肝脏中高度富集，但在其他组织和其他细胞类型（如免疫细胞）中没有或非常低的表达其功能未知。

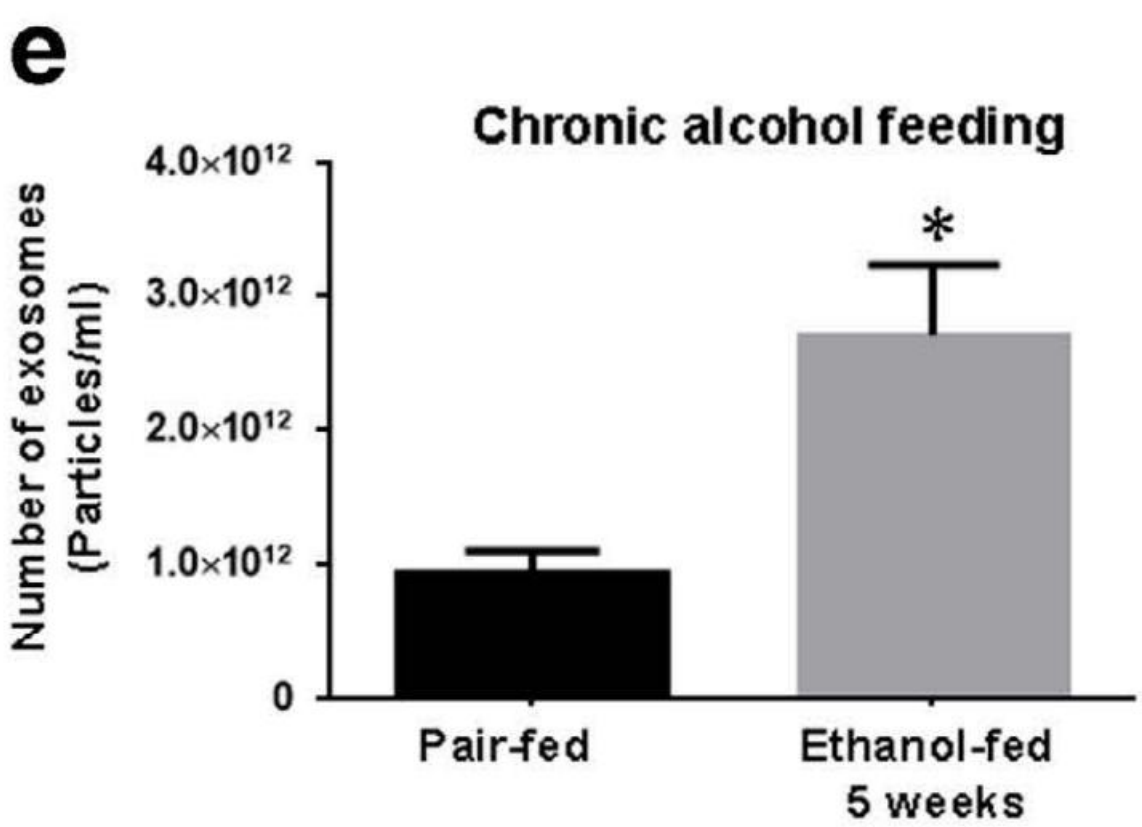
02 结果分析

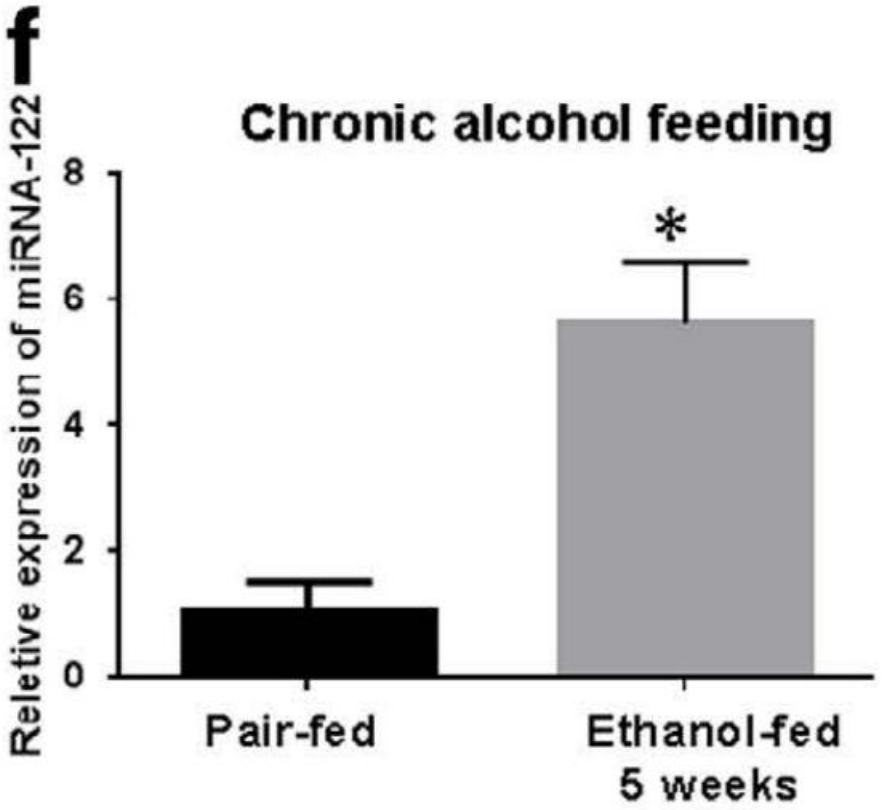
1、酒精处理后，外泌体数量显著增加



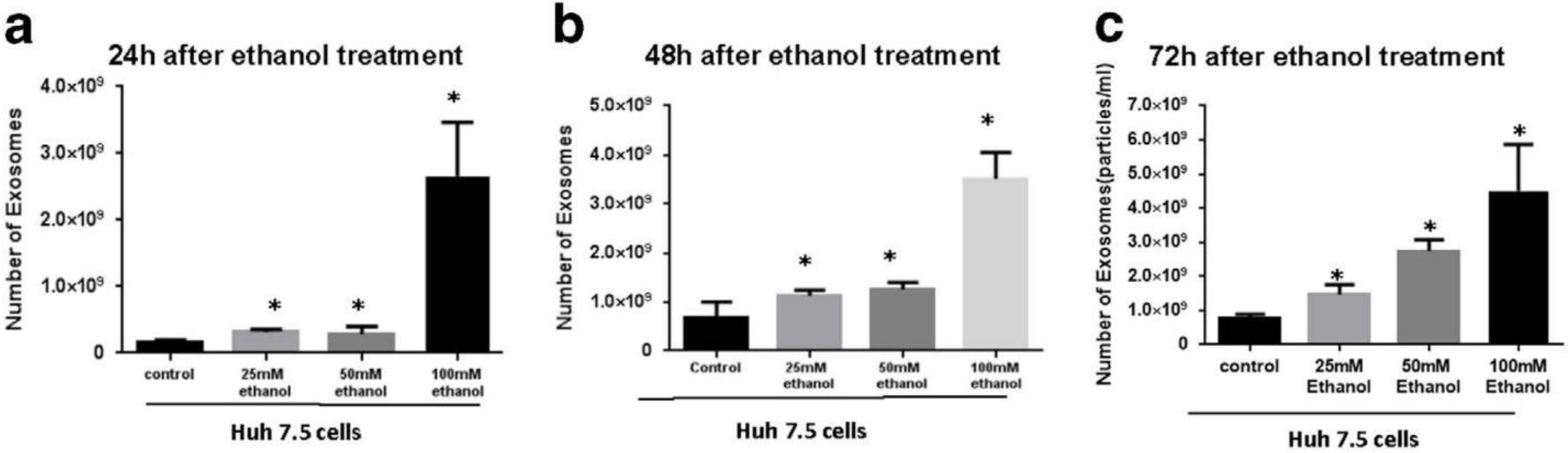

c
Binge alcohol consumption in mice
NS
Number of exosomes (Particles/ml)
5.0×10^12
4.0×10^12
3.0×10^12
2.0×10^12
1.0×10^12
0
*
*
6h
12h
Control Ethanol
Control Ethanol


d
Binge alcohol consumption in mice
Reletive expression of miRNA-122
5
4
3
2
1
0
*
Pair-fed
Binge Ethanol-fed


e
Chronic alcohol feeding
Number of exosomes (Particles/ml)
4.0×10¹²
3.0×10¹²
2.0×10¹²
1.0×10¹²
0
*
Pair-fed
Ethanol-fed 5 weeks

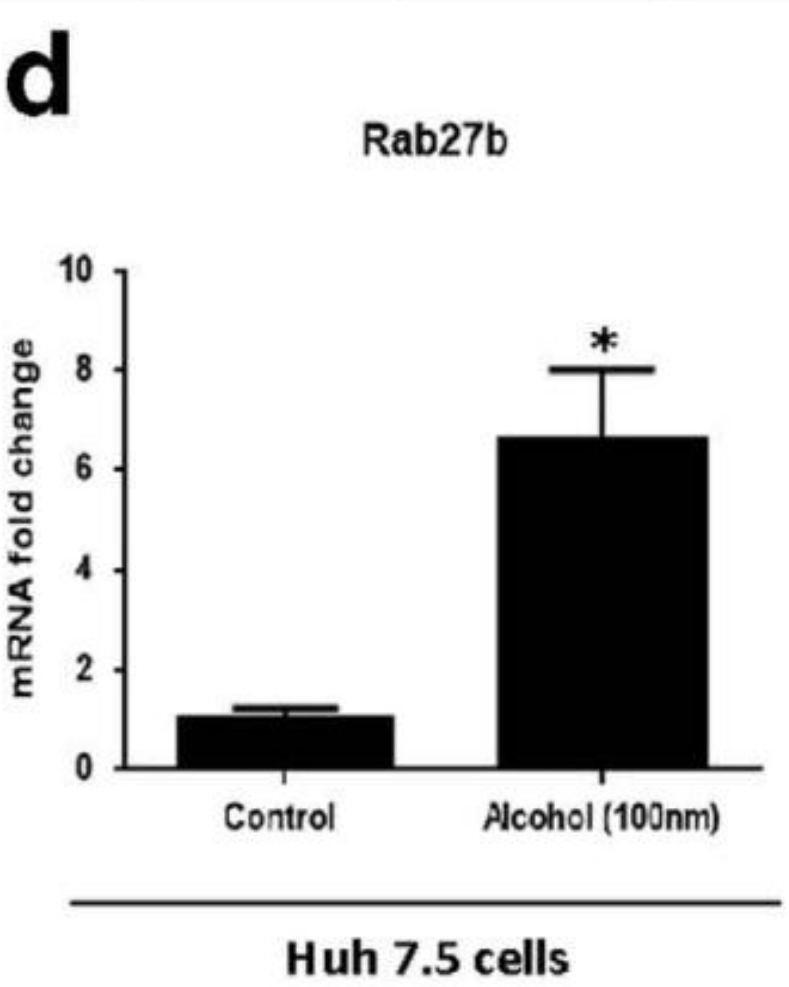

f
Chronic alcohol feeding
Reletive expression of miRNA-122
8
6
4
2
0
*
Pair-fed
Ethanol-fed 5 weeks

2、酒精诱导肝细胞产生外泌体

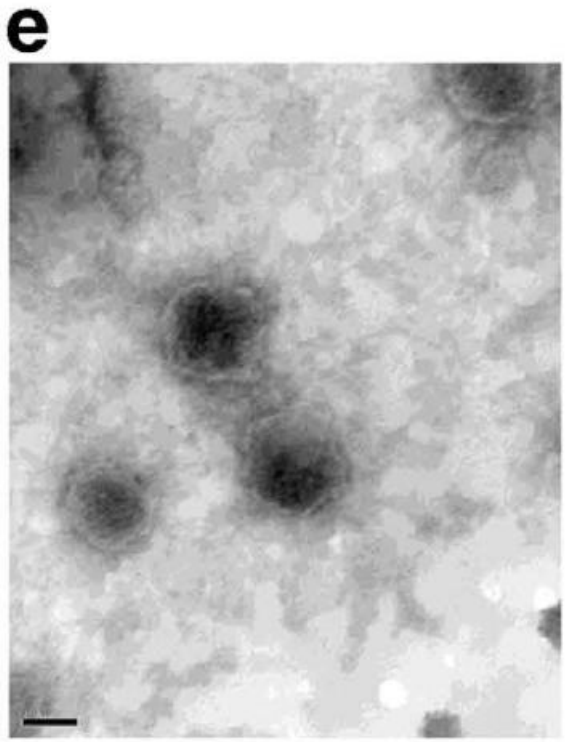


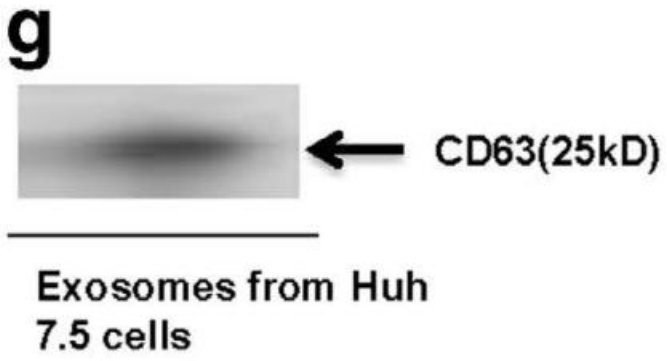
外泌体增加是时间和剂量依赖的

通过酒精处理，激活外泌体发生途径。Rab27b主要参与大多数分泌性细胞的胞吐作用及多泡体的运输过程。



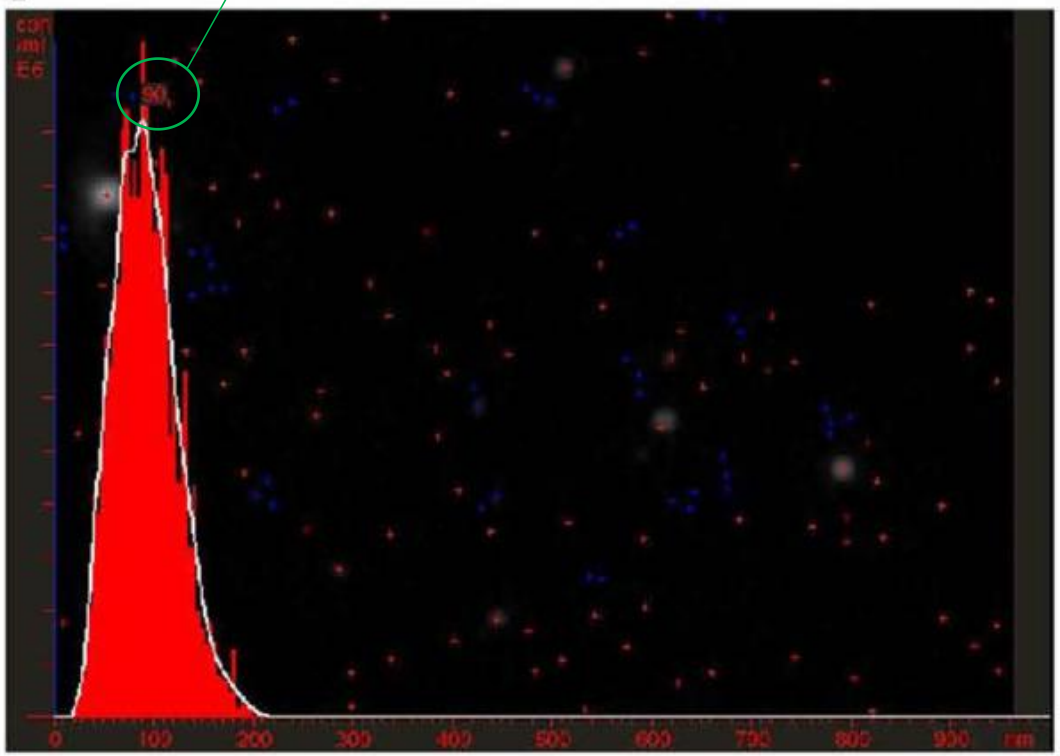
3、肝细胞（Huh7.5）衍生的外泌体特征及其货物

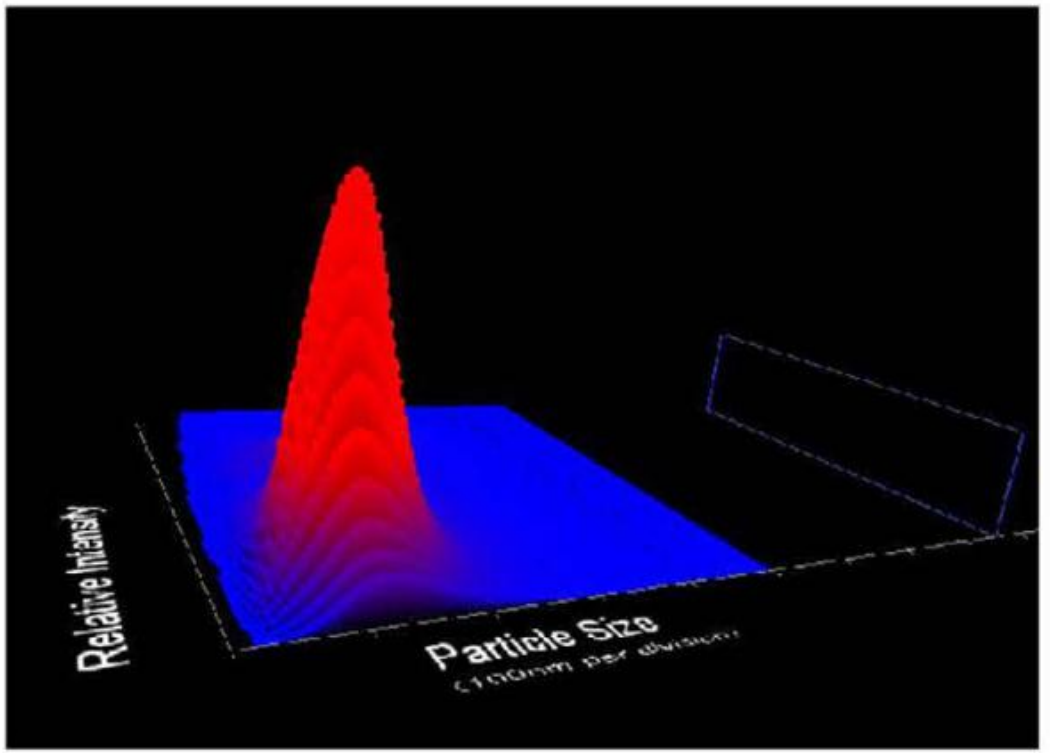




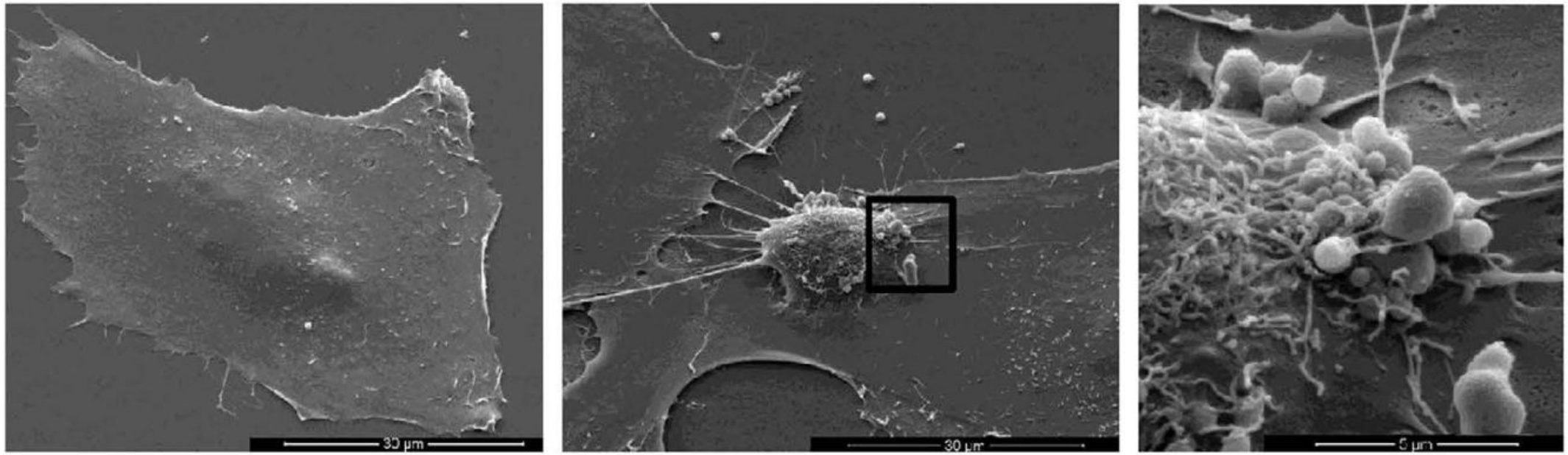
f

90


con
/ml
E6
90
0
100
200
300
400
500
600
700
800
900
nm


Relative Intensity
Particle Size

h



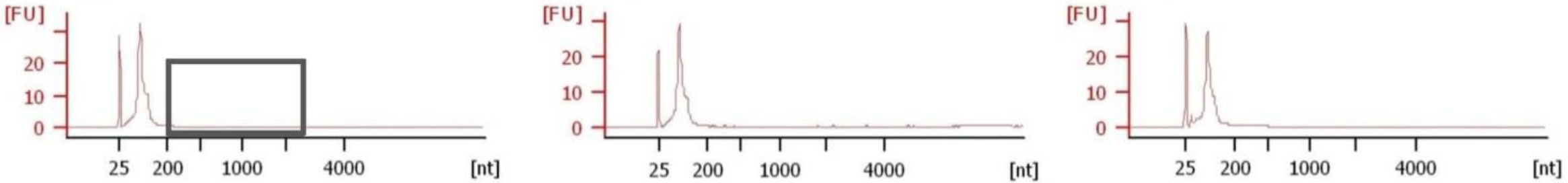
Control Huh 7.5 cells

Ethanol-treated Huh 7.5 cells

脱落的外泌体/微泡的数量增加

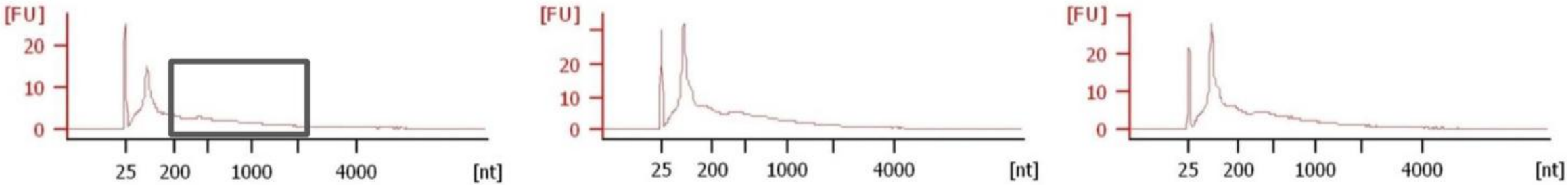
a

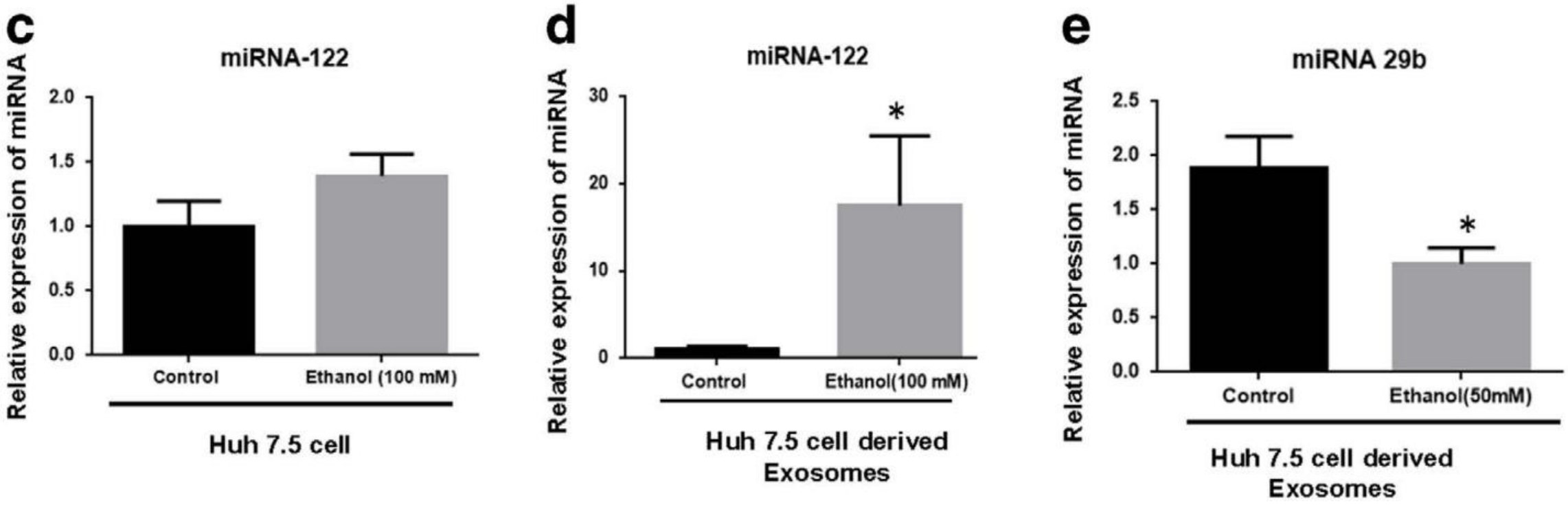
Exosomes derived from control Huh 7.5 cells



b

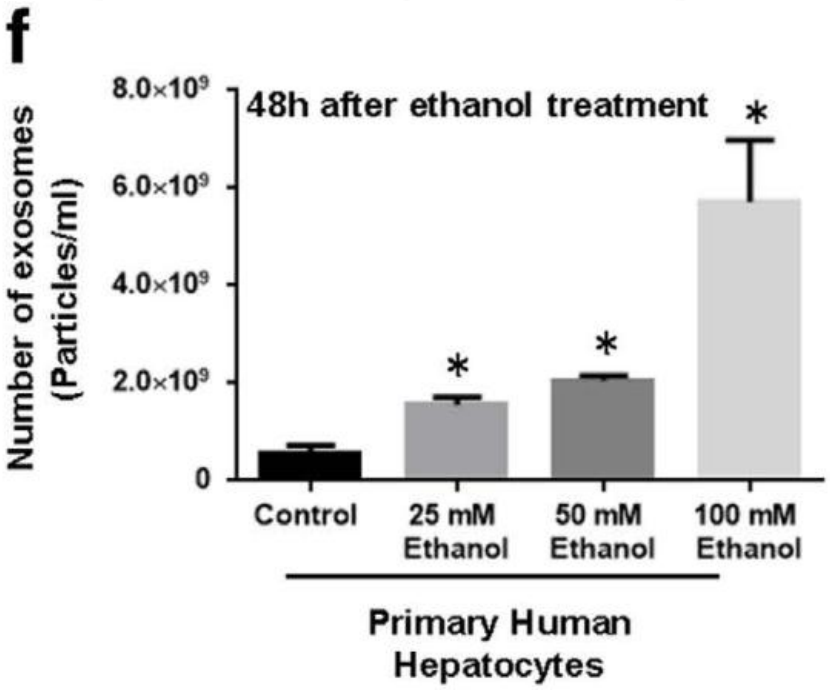
Exosomes derived from ethanol treated Huh 7.5 cells

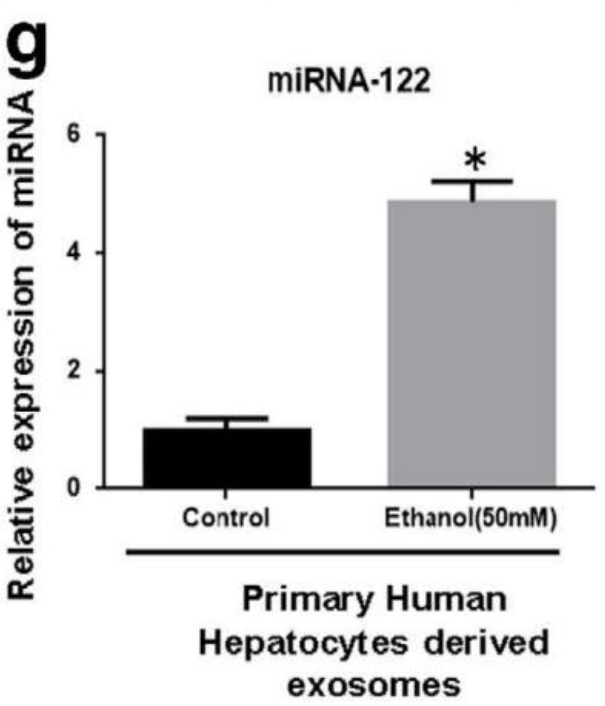


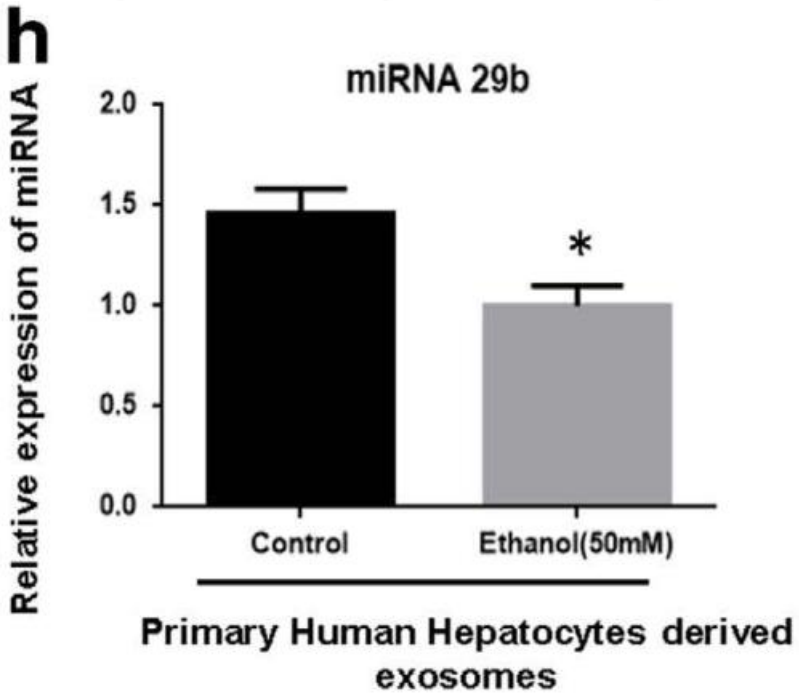


miRNA进入外泌体是一个受调节的过程，
外泌体对每个miRNA是特异性的。

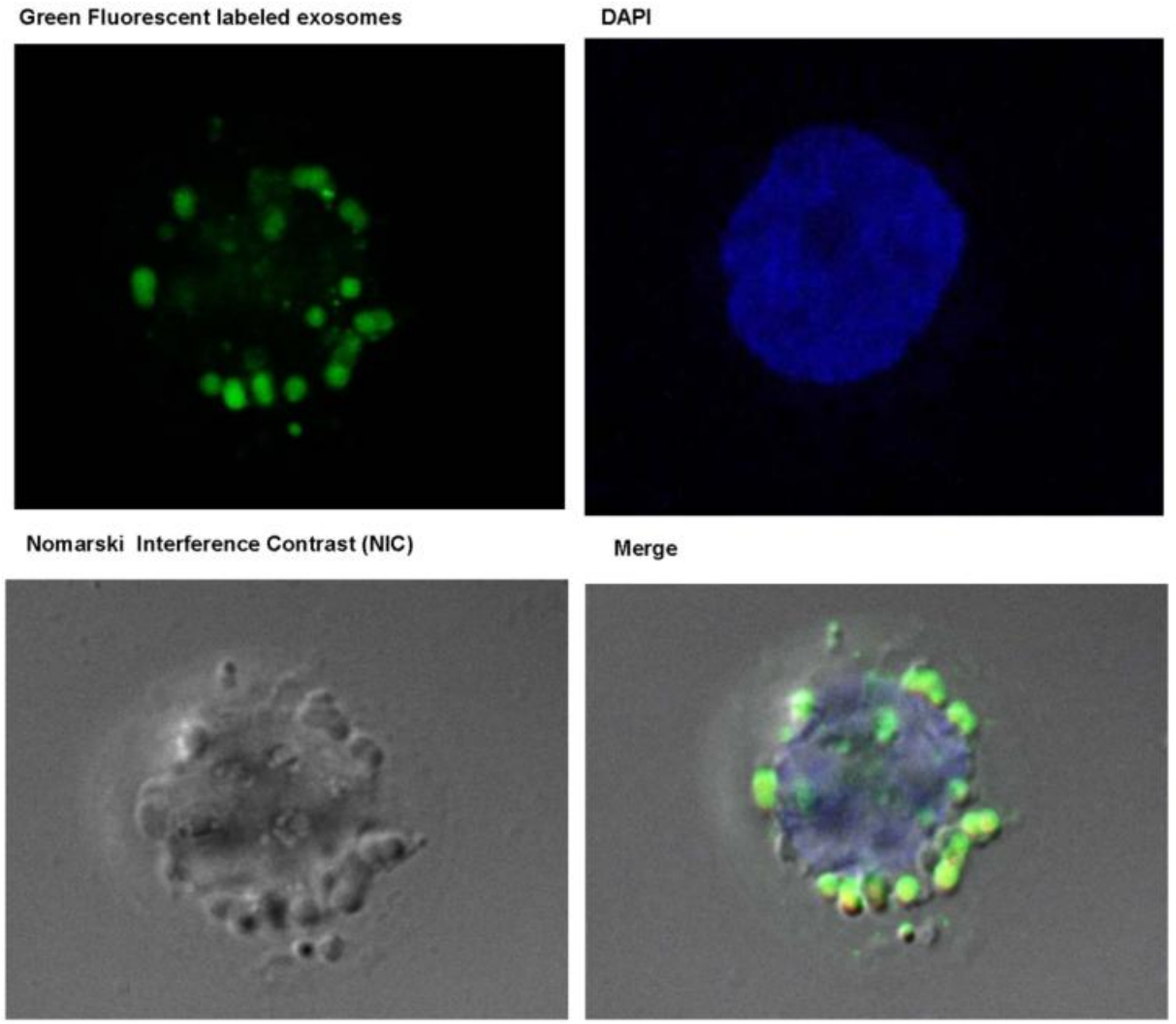
4、酒精处理后，原代人肝细胞分泌外泌体

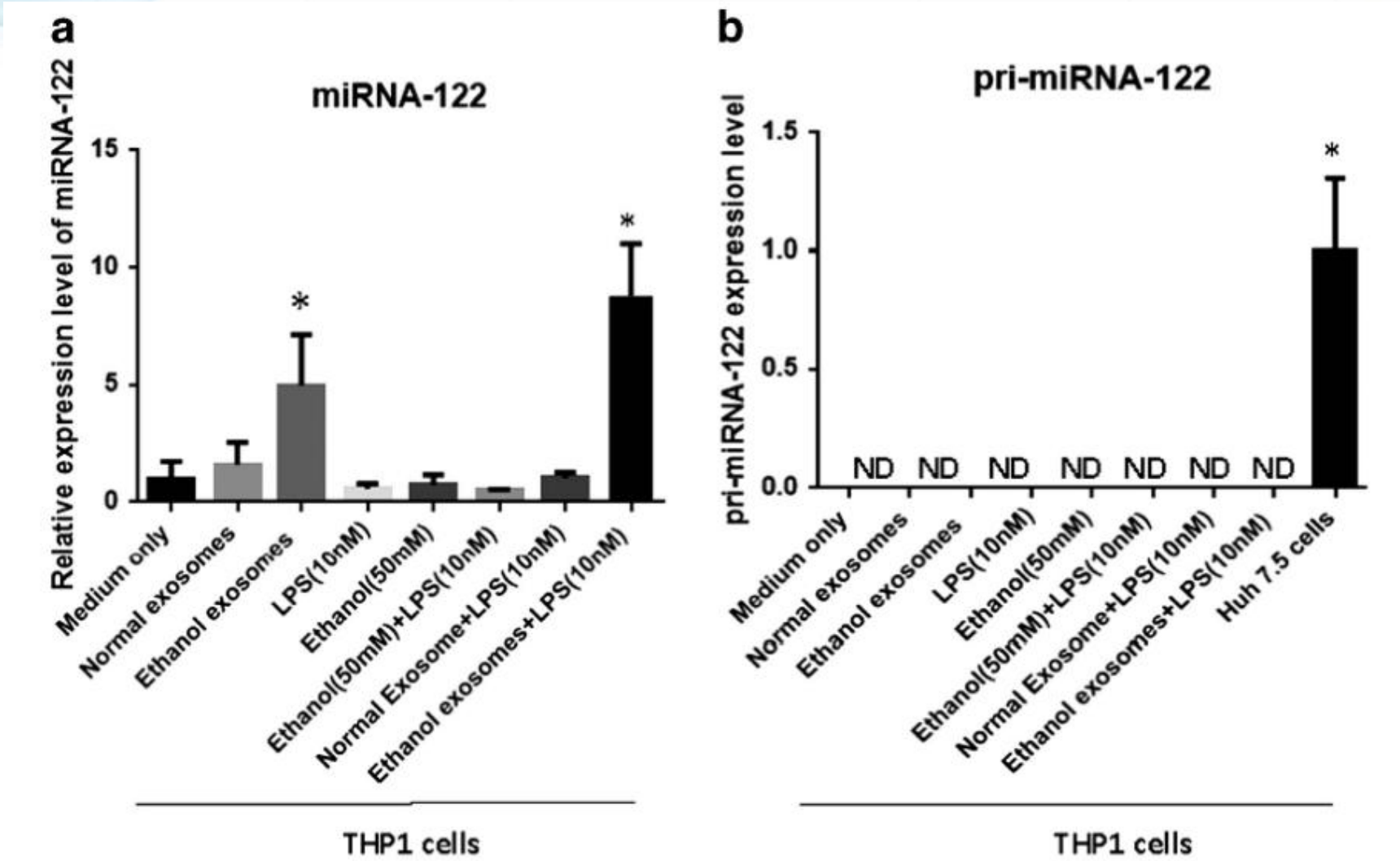


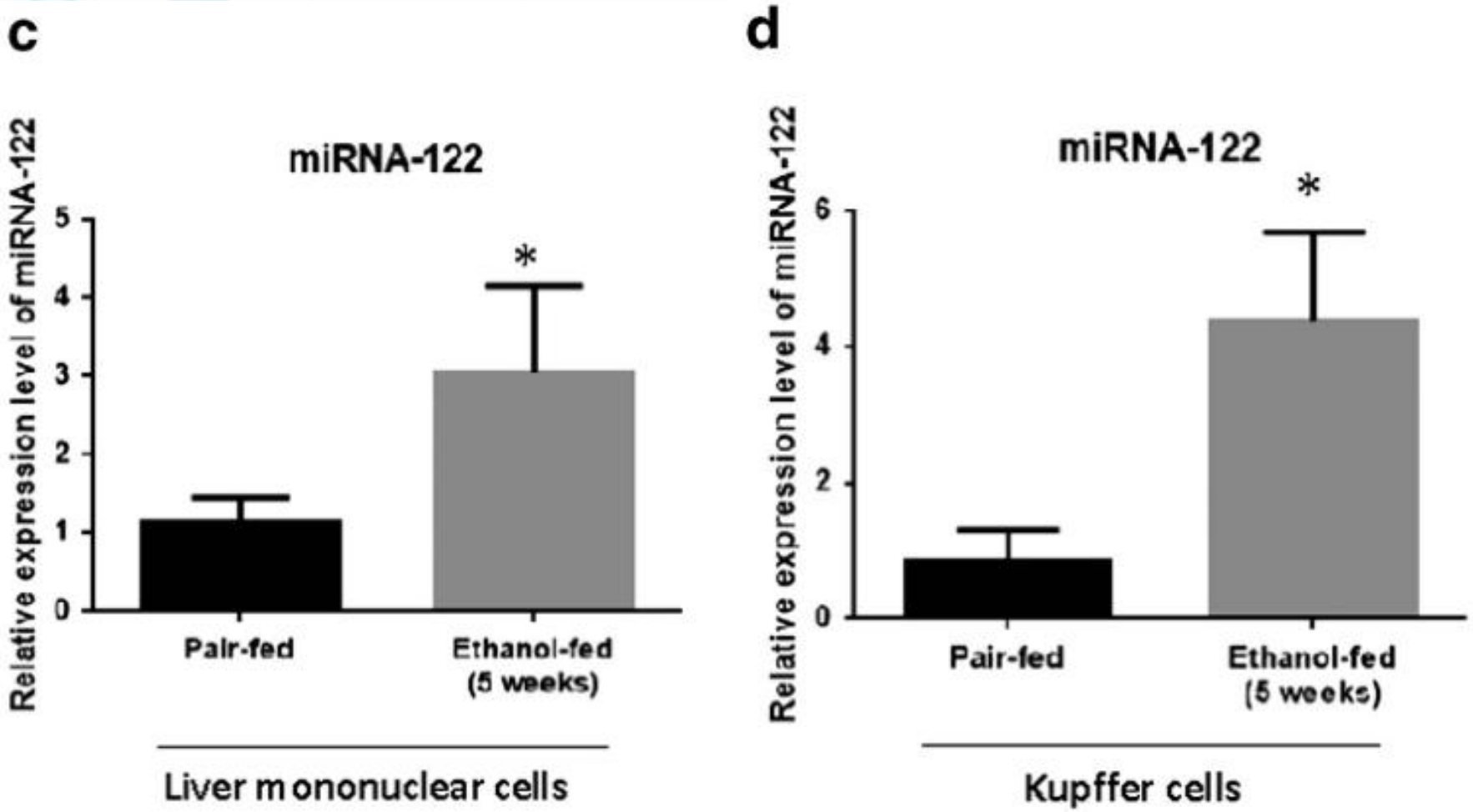




5、乙醇处理的肝细胞衍生的外泌体将成熟的miR-122转移到单核细胞

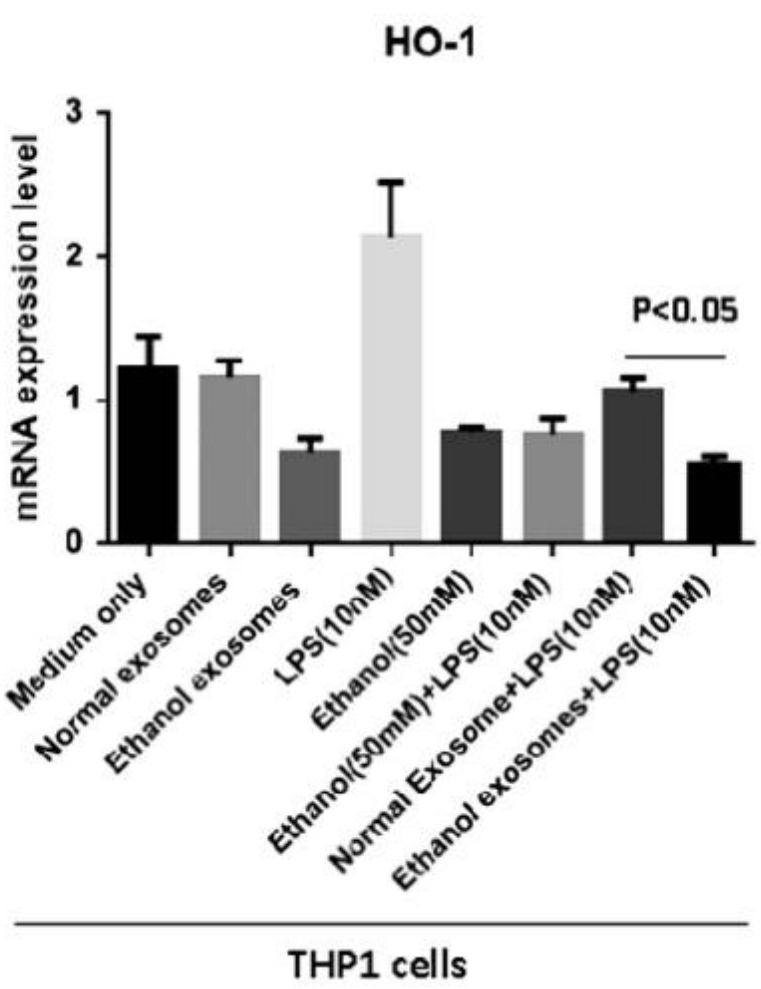


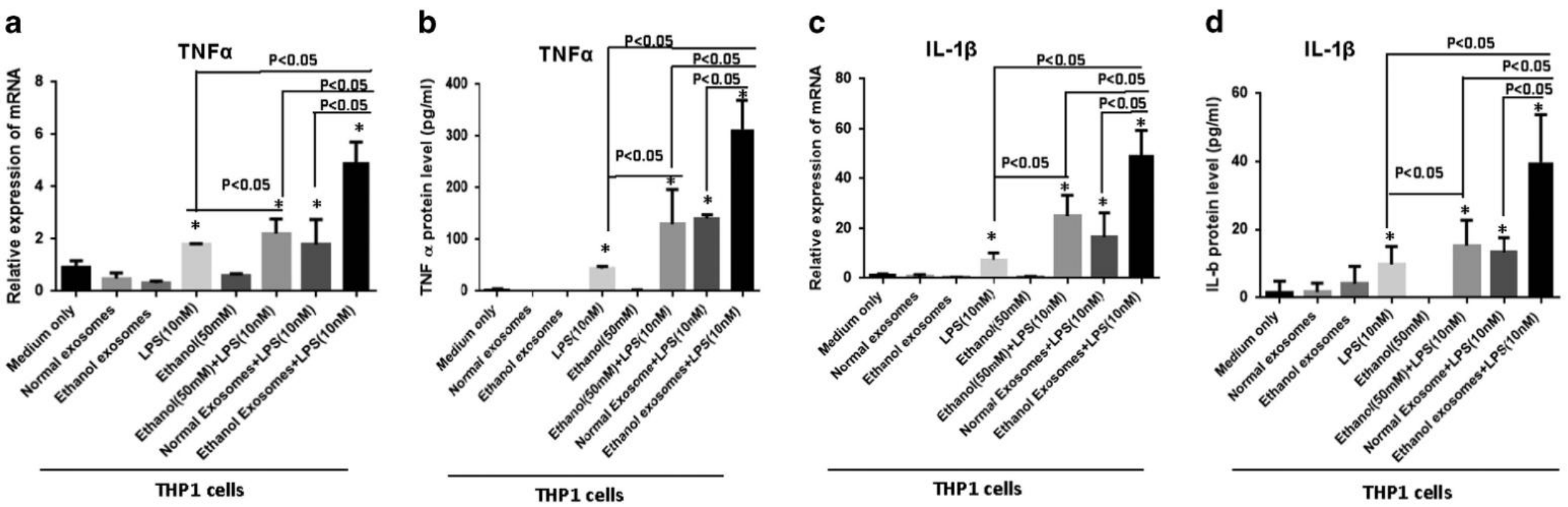

a
miRNA-122
Relative expression level of miRNA-122
15
10
5
0
*
*
Medium only
Normal exosomes
Ethanol exosomes
LPS(10nM)
Ethanol(50mM)
Ethanol(50mM)+LPS(10nM)
Normal Exosome+LPS(10nM)
Ethanol exosomes+LPS(10nM)
THP1 cells
b
pri-miRNA-122
pri-miRNA-122 expression level
1.5
1.0
0.5
0.0
ND
ND
ND
ND
ND
ND
ND
*
Medium only
Normal exosomes
Ethanol exosomes
LPS(10nM)
Ethanol(50mM)
Ethanol(50mM)+LPS(10nM)
Normal Exosome+LPS(10nM)
Ethanol exosomes+LPS(10nM)
Huh 7.5 cells
THP1 cells


c
miRNA-122
Relative expression level of miRNA-122
0
1
2
3
4
5
*
Pair-fed
Ethanol-fed
(5 weeks)
Liver mononuclear cells
d
miRNA-122
Relative expression level of miRNA-122
0
2
4
6
*
Pair-fed
Ethanol-fed
(5 weeks)
Kupffer cells

6、肝细胞衍生的外泌体转移的miR-122是功能性的，并调节单核细胞功能

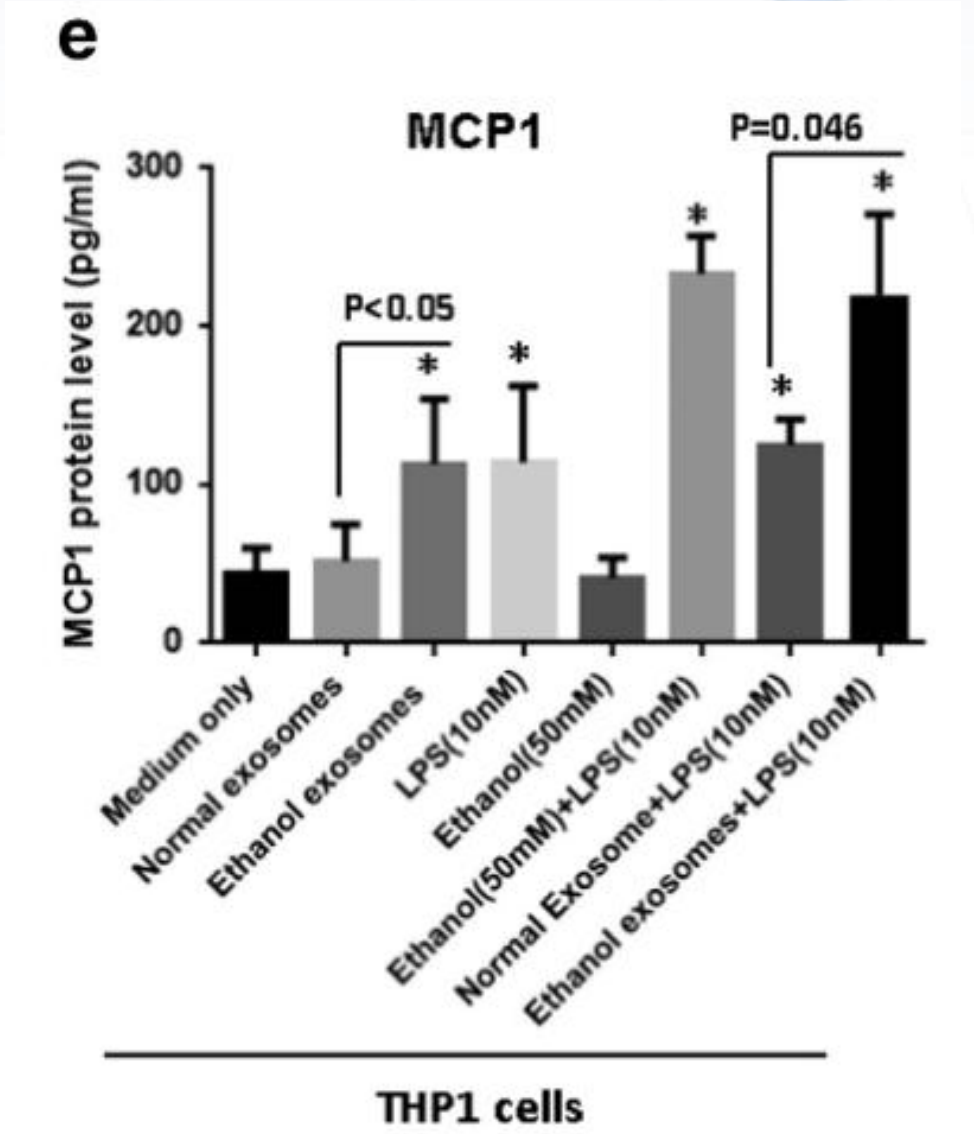
HO-1对细胞因子和活性氧化物介导的细胞损伤具有抑制作用。



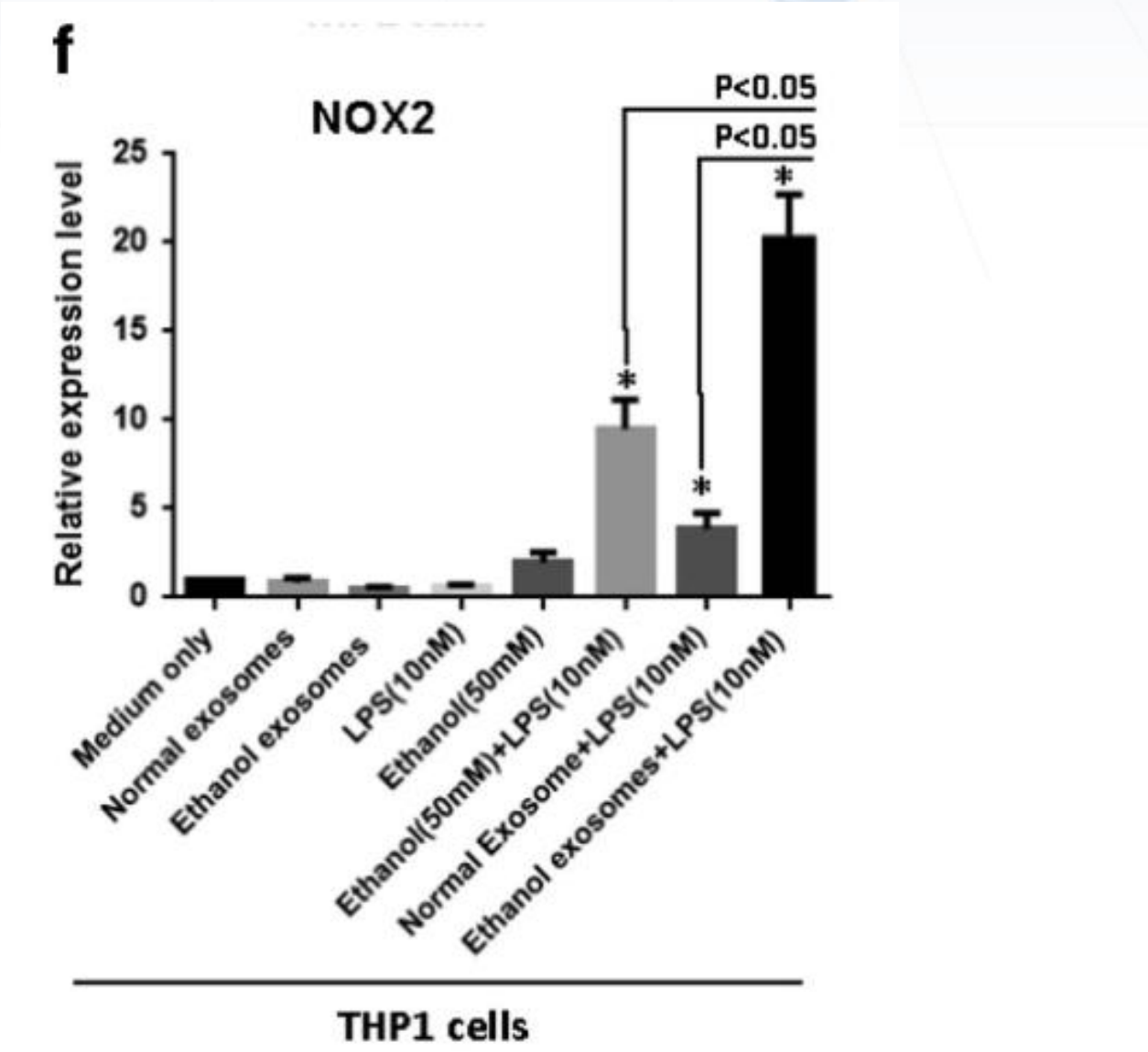


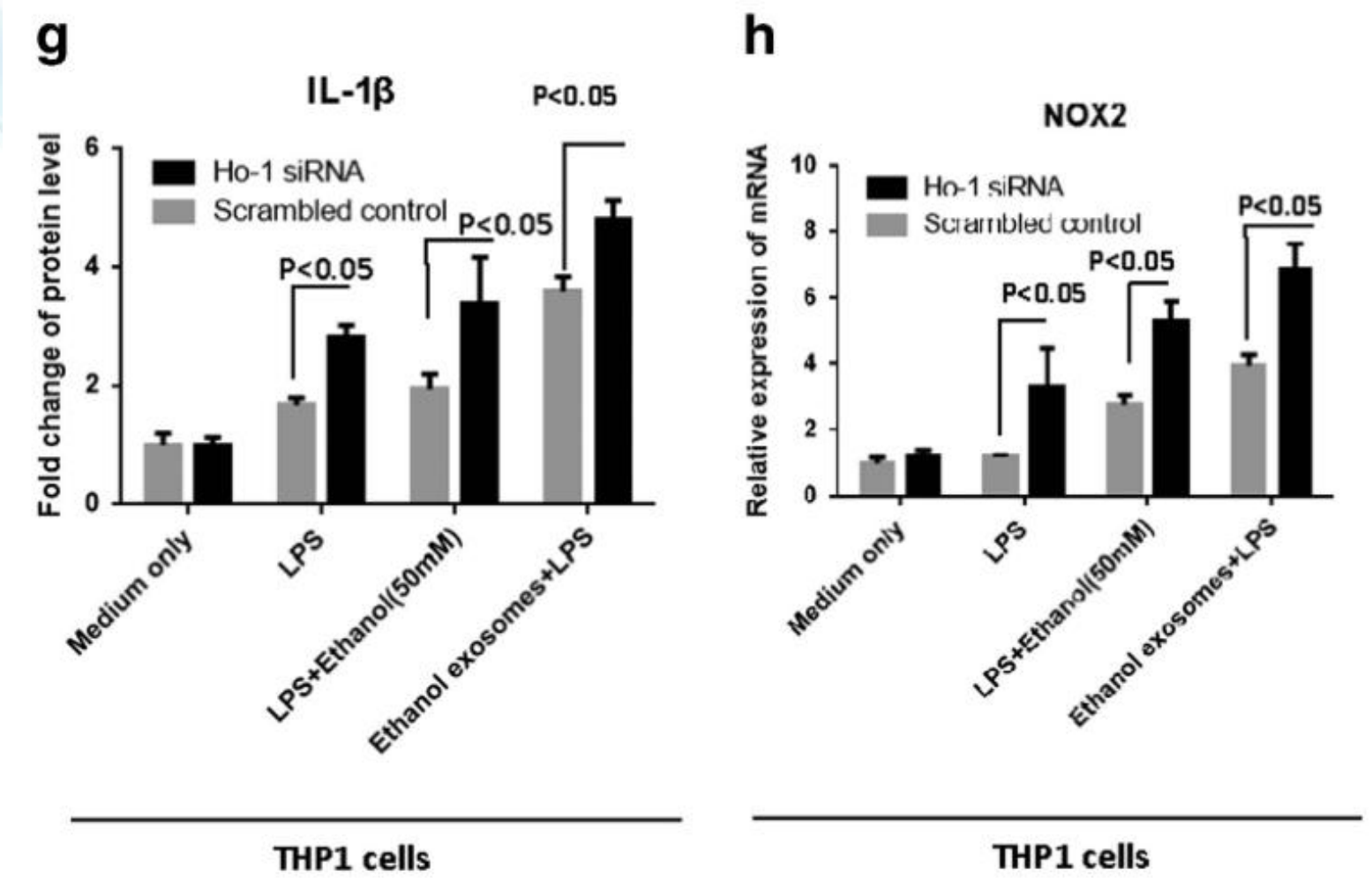
在LPS存在的情况下，酒精处理的肝细胞外泌体对THP1细胞有更加敏感的反应。

乙醇本身不诱导显著促炎细胞因子在单核细胞中的产生，但通过其对肝细胞中外泌体（携带miRNA-122）的数目的增加，间接诱导单核细胞促炎因子生成。

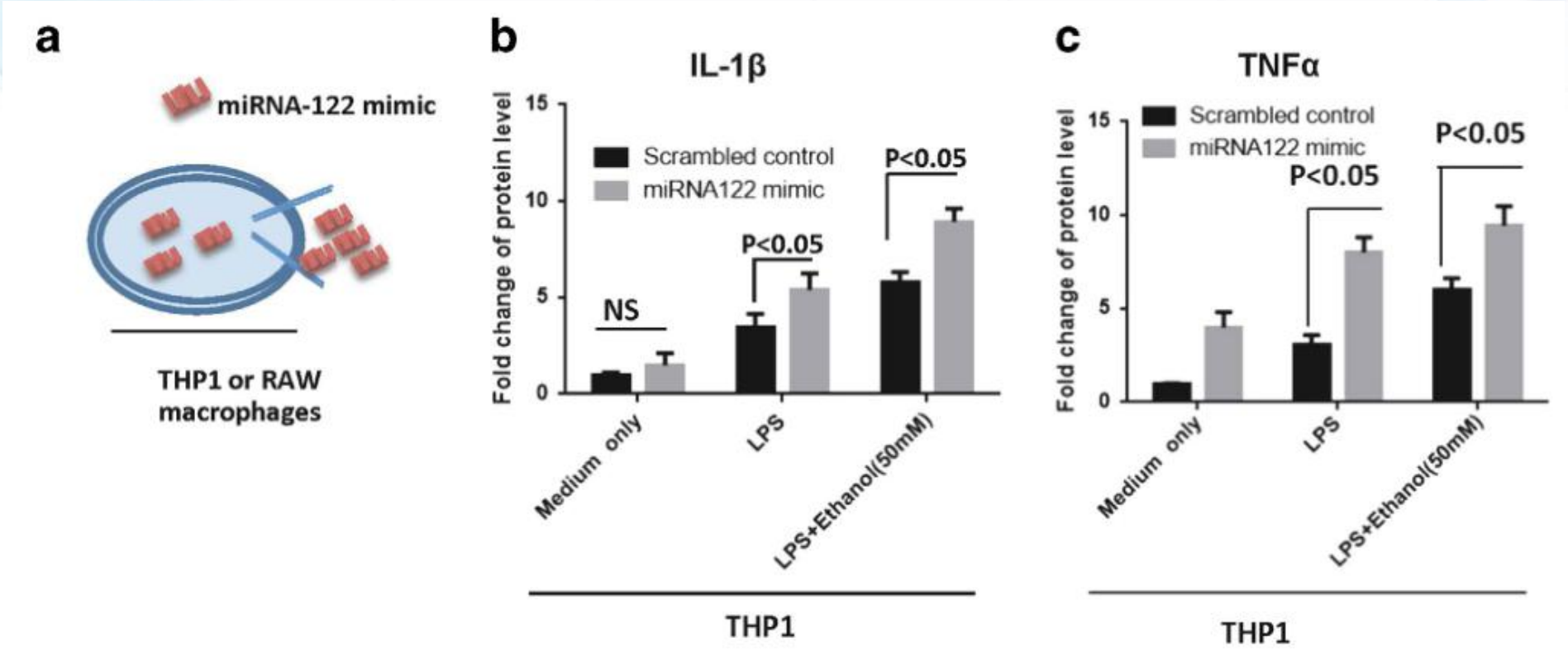


NOX2，一种HO-1调节基因，其细胞机制是产生活性氧，存在于免疫细胞中，并在机体防御中发挥关键作用。

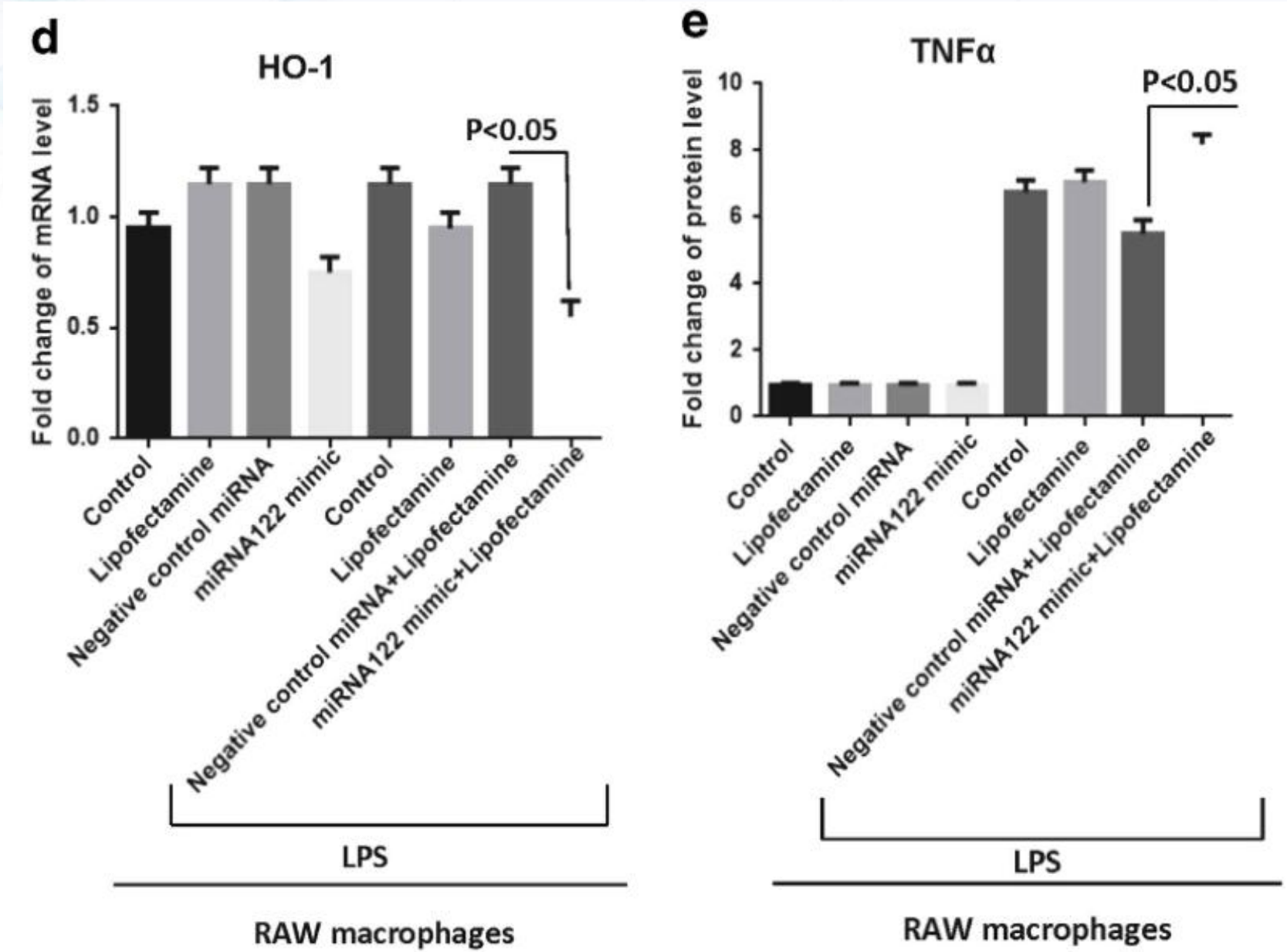




肝细胞特异性miR-122通过乙醇处理后的肝细胞外泌体成功地水平转移，其是功能性的并且通过调节HO-1途径来修饰THP1对LPS的反应。

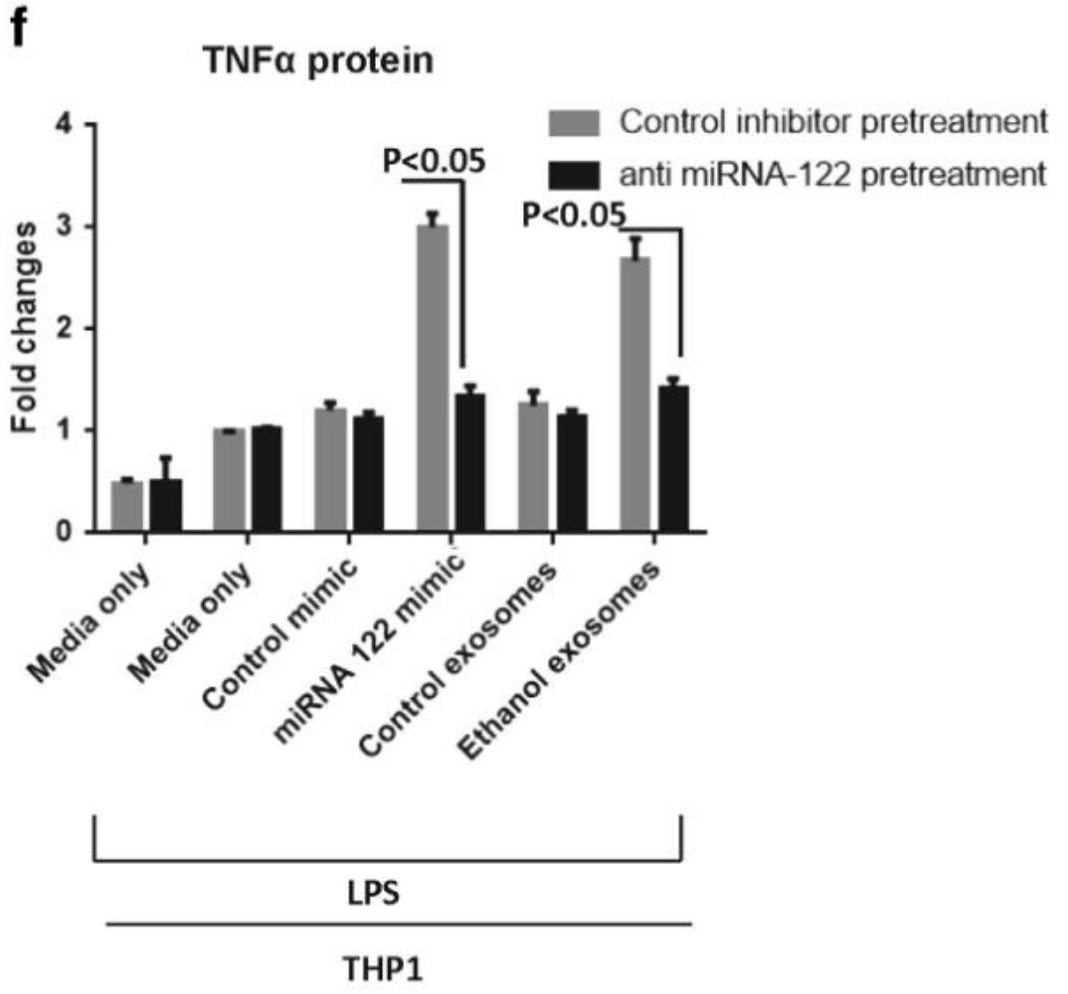


进一步证明miR-122引入THP1细胞使单核细胞对LPS敏感



乙醇处理的肝细胞外泌体可以转移miRNA-122到免疫细胞并且通过影响HO-1途径调节促炎细胞因子生成

7、外泌体介导的miR-122 RNAi传递阻止乙醇处理的肝细胞外泌体的促炎作用



03 总结讨论

在健康人类受试者和小鼠中以及慢性酒精饲喂小鼠后外泌体的总数显着增加

1

2

酗酒后，从健康受试者的血清中分离的外泌体含有丰富的miR-122

miRNA进入外泌体是一个特定的过程，衍生自乙醇处理的肝细胞的外泌体与控制外泌体相比携带不同的货物。

3

4

外泌体含有miR-122并且能水平转移miR-122到单核细胞

含有miR-122的外泌体能够使单核细胞产生炎症反应

5

6

转染miR-122抑制剂到THP1细胞，以阻断来自乙醇处理的肝细胞的外泌体对单核细胞的影响。

- 乙醇处理肝细胞衍生的外泌体能够使单核细胞产生促炎因子。
- 通过外泌体介导的miR-122的转移和HO-1途径的抑制对外泌体的免疫调节可以是单核细胞/巨噬细胞对AH中的LPS敏感的替代机制。
- 使用外泌体与miRNA-122抑制剂，并将它们作为一种治疗，减弱单核细胞中miRNA-122介导的免疫刺激，开辟了治疗潜力的新途径。

04

心得体会

- 学习文章的实验方法
- 学习文章的实验思路
- 拓展视野，丰富知识

Thank you