NK细胞在HIV感染中的作用和机制研究

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NK细胞的发现

1975年R. Kiessling等人在《欧洲免疫学杂志》上首次报道，正常小鼠的脾脏中存在一群淋巴细胞，可对**小鼠莫罗尼白血病细胞发生**自然并特异性地**杀伤**，并将其命名为 “Natural killer cells”----自然杀伤细胞，简称NK细胞。

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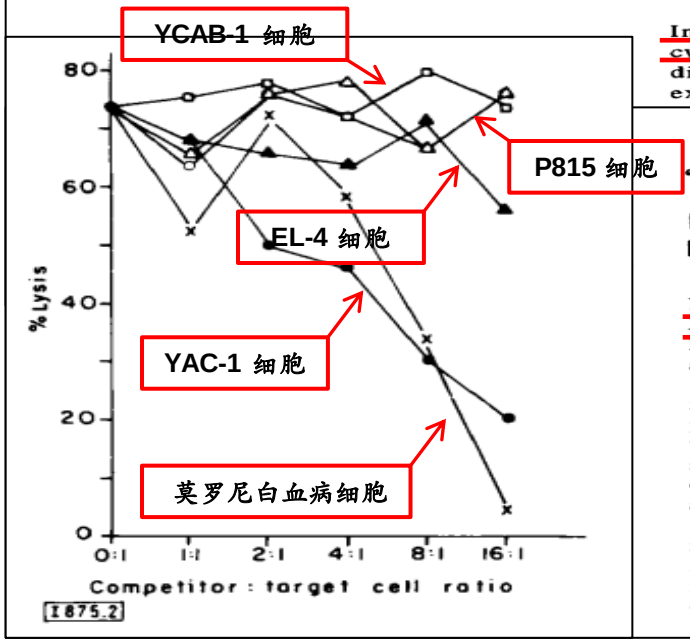
Department of Tumor Biology, Karolinska Institute, Stockholm+ and Department of Immunology, Uppsala University, Uppsala°

“Natural” killer cells in the mouse

I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype*

In the spleens of young, adult mice there exist naturally occurring killer lymphocytes with specificity for mouse Moloney leukemia cells. The lytic activity was directed against syngeneic or allogeneic Moloney leukemia cells to a similar extent, but was primarily expressed when tested against *in vitro* grown leukemia



“Natural” killer cells in the mouse

II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell*

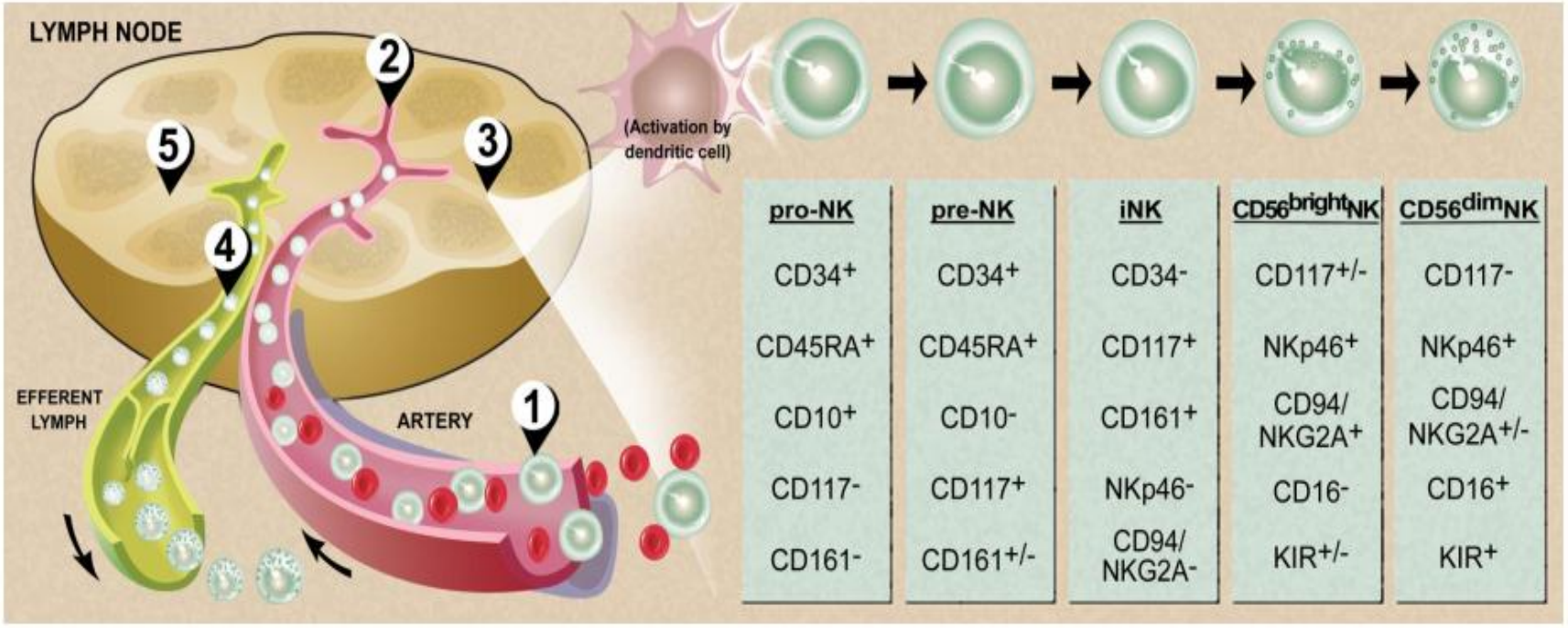
Normal mice contain cytolytic cells with specificity for *in vitro* grown mouse Moloney leukemia cells. Such killer cells are most frequent in the spleens; lymph node and bone marrow contain less and thymus virtually no killer activity. Peak activity is found around one to three months of age. Spleen cells from genetically athymic mice are as active killer cells as those from normal mice of the same strain. Treatment with anti-theta serum plus complement followed by removal of adherent and surface Ig positive cells by filtration through anti-Ig columns will leave between 1–5 % of the original spleen cell population from a normal mouse. These cells have the morphology of small lymphocytes and perhaps contain all of the total original killer activity of the spleen against the Moloney leukemia cells. Such killer enriched cells are devoid of T and B lymphocytes and largely fail to function in antibody induced, cell-mediated lysis against antibody-coated chicken erythrocytes. It is concluded that the spontaneous selective cytotoxic activity of normal mouse spleen cells against Moloney leukemia cells is exerted by small lymphocytes of yet undefined nature.

1975. ***Eur. J . Immunol.*** R. Kiessling, E. Klein, H. Pross and H. Wigzell

NK 细胞的定义及细胞标志

定义： NK细胞占循环淋巴细胞的15%左右，是固有免疫系统的重要组成部分，可杀伤肿瘤和病毒感染细胞，是机体免疫防御第一道防线。

表面标志： CD3-CD56+CD16+/-，CD14-CD19-



Caligiuri MA,Blood 2008 Aug.

发挥功能的机制

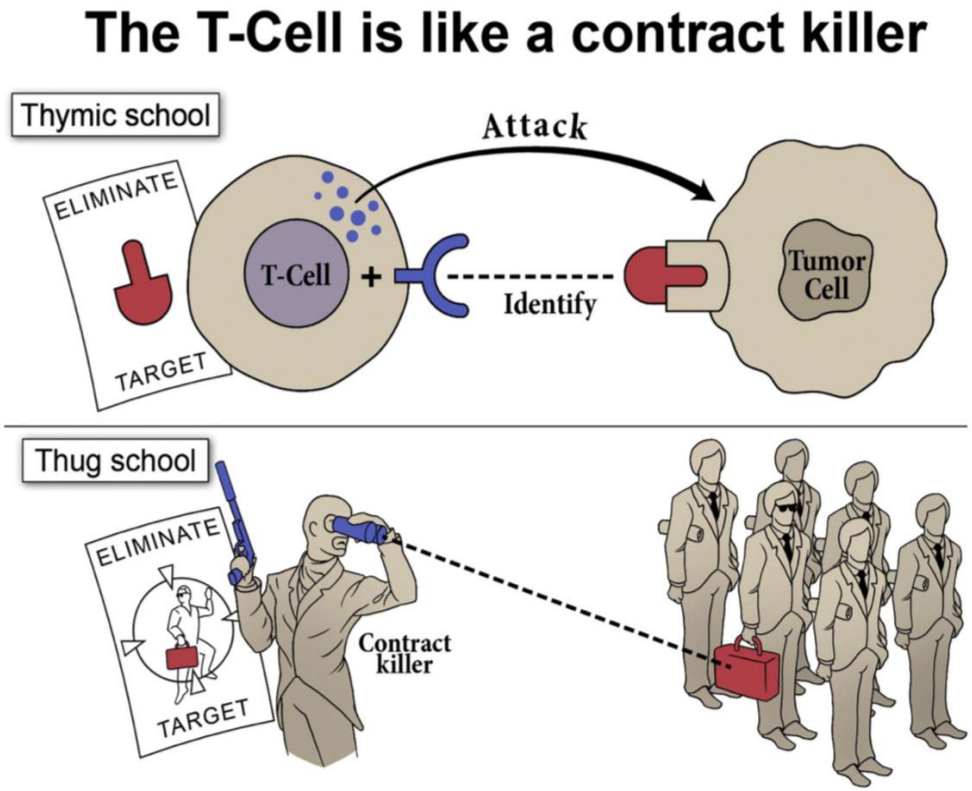


Fig. 1. T cells as contract killers. T cells undergo extensive education in the thymus where T cells that potentially recognize self (normal citizens) are eliminated through negative selection. During periods of inflammation (conflict) the T cell receptor (TCR) goes through rearrangement in order to become exquisitely specific to a single target. Recognition of targets must be done through presentation on self-MHC (e.g., a photo in official documents such as a passport). Through this high degree of specificity, T cells are able to pick targets out of a crowd of normal cells.

Please cite this article as: Kannan GS, et al, Natural killer cells in malignant hematology: A primer for the non-immunologist, Blood Rev (2016),

Blood Reviews,2016

发挥功能的机制

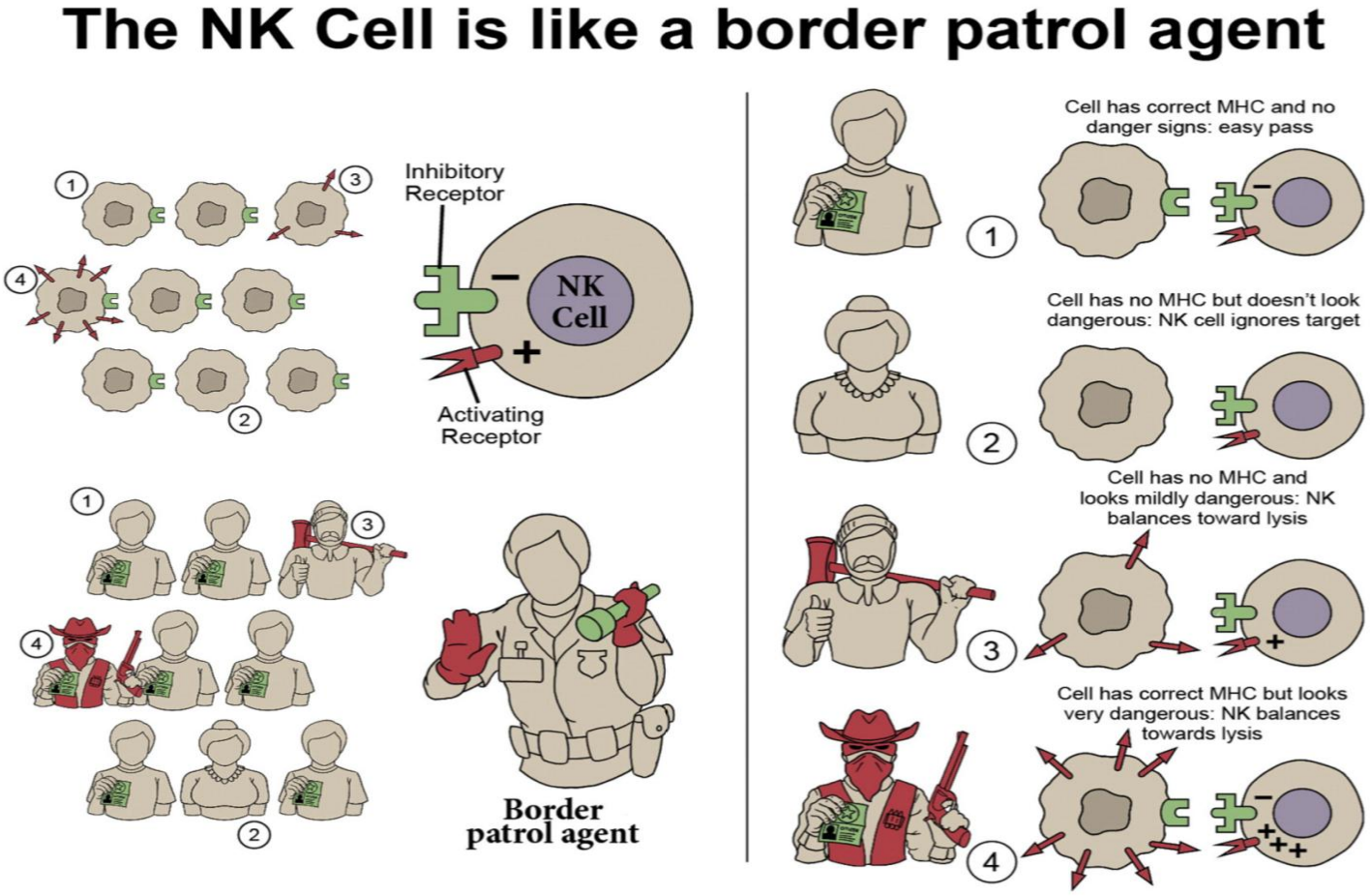
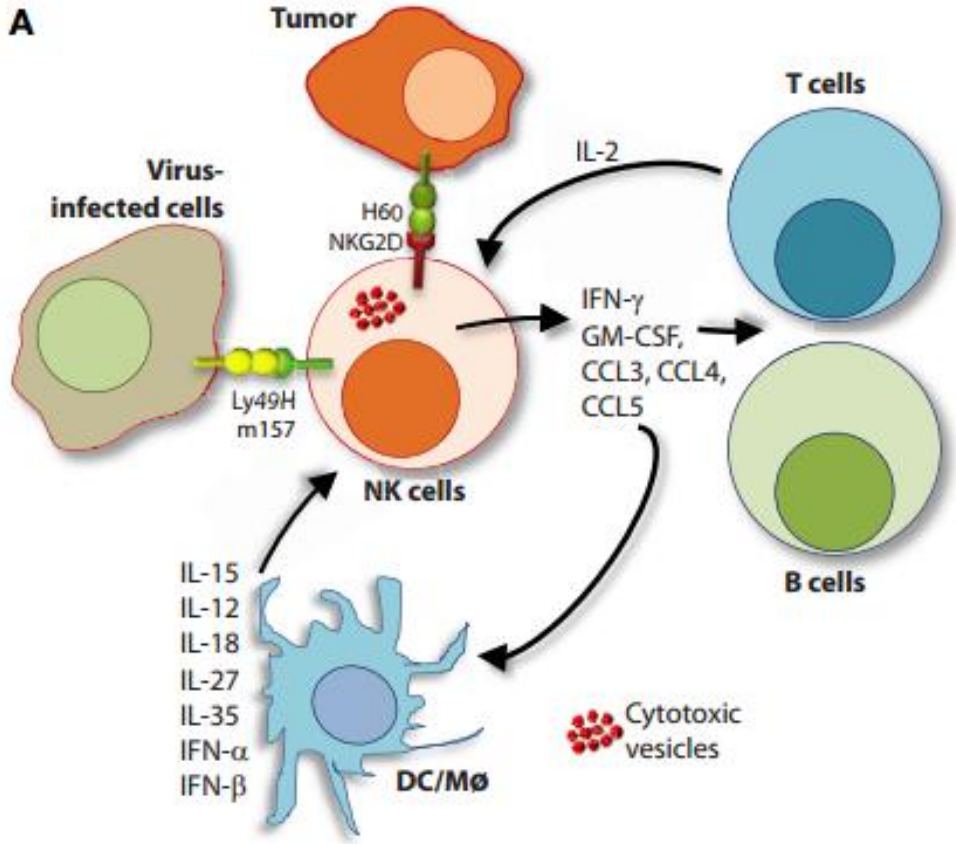


Fig. 2. NK cells as border patrol. NK cells use inhibitory receptors to recognize MHC (proper identification documents such as passports) as evidence of self and receive negative signals preventing self-attack. They also have a wide range of surface activating receptors to identify activating ligands which are elements associated with danger on target cells (weapons). Recognition is determined by the balance between the severity or number of danger signals and the number of documents supporting self. Through broad assessment of danger and self on every cell, NK cells pick out potentially dangerous cells independent of knowing their specific identity.

Blood Reviews,2016

NK细胞功能(1)



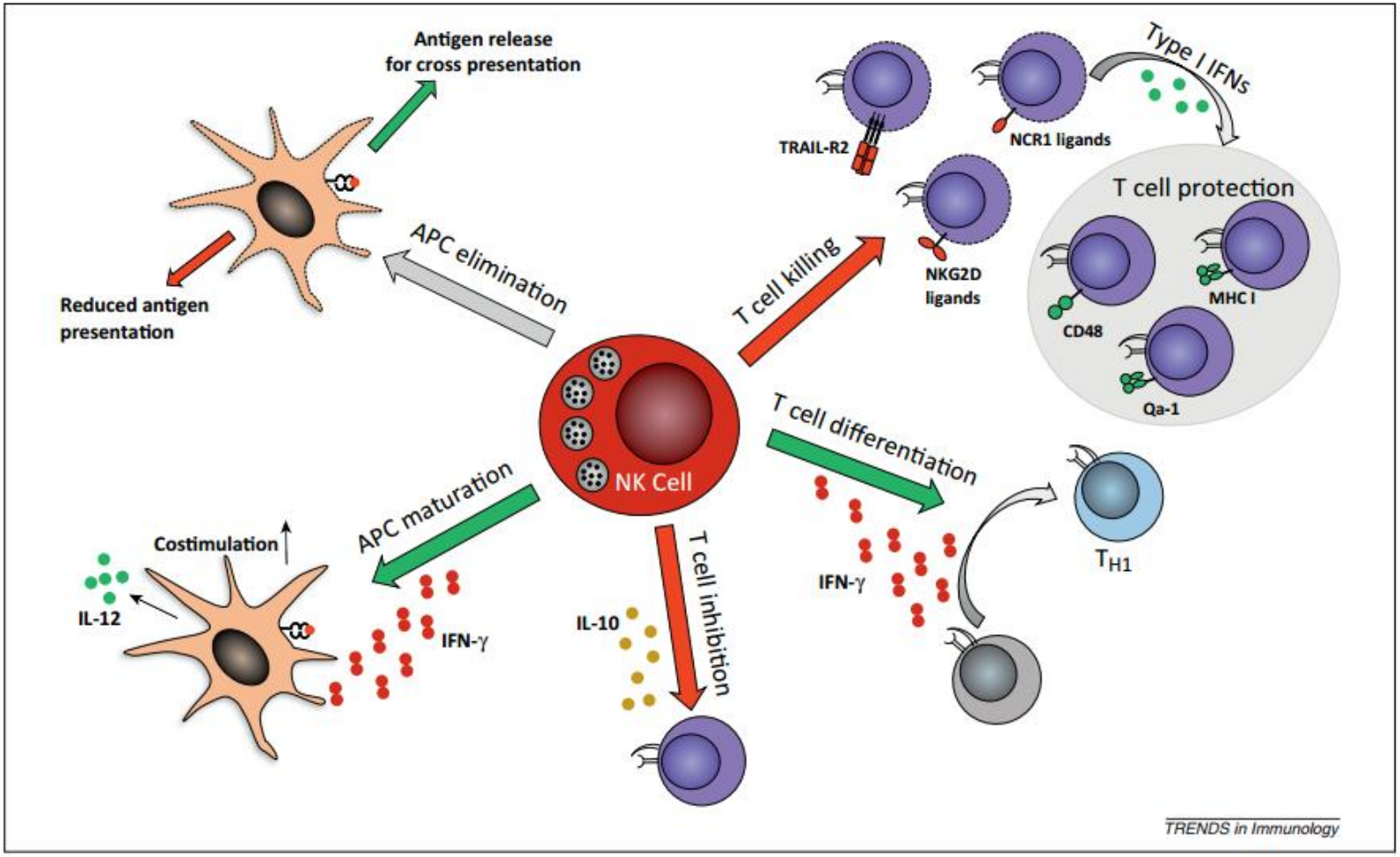
B

Gzms, Prfs, IFN-γ, FasL, TNF-α	Anti-tumor cytotoxicity
Gzms, Prfs, IFN-γ, FasL, TNF-α	Anti-viral and bacterial
GM-CSF, CCL3, CCL4, CCL5, XCL1	Helping adaptive immunity
IL-17, IL-22	Epithelial regeneration
IL-10	Regulatory functions
Gzms, Prfs, IFN-γ, FasL, TNF-α	Adoptive cellular therapy

- NK细胞具有介导细胞毒性并产生炎性细胞因子和趋化因子的固有能力。

Front Immunol. 2018

NK细胞功能(2)



NK细胞可正向或负向调节免疫细胞应答

Trends Immunol. 2015

HIV感染者NK功能研究

近两年本团队研究

😊 *正向作用*

a. 急性期ADCC效应

b. NKG2C^{+} NK 细胞起保护作用

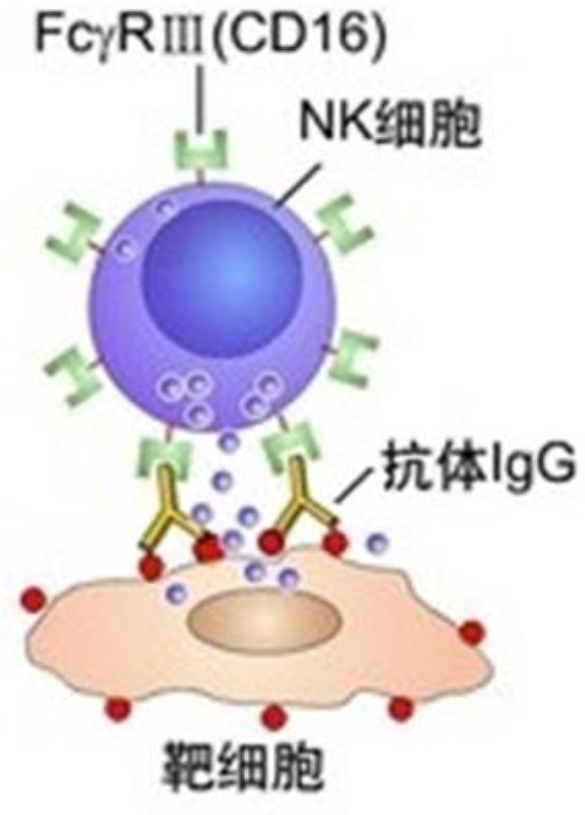
😭 *负向作用*

a. Tigit^{+} NK 细胞功能受损

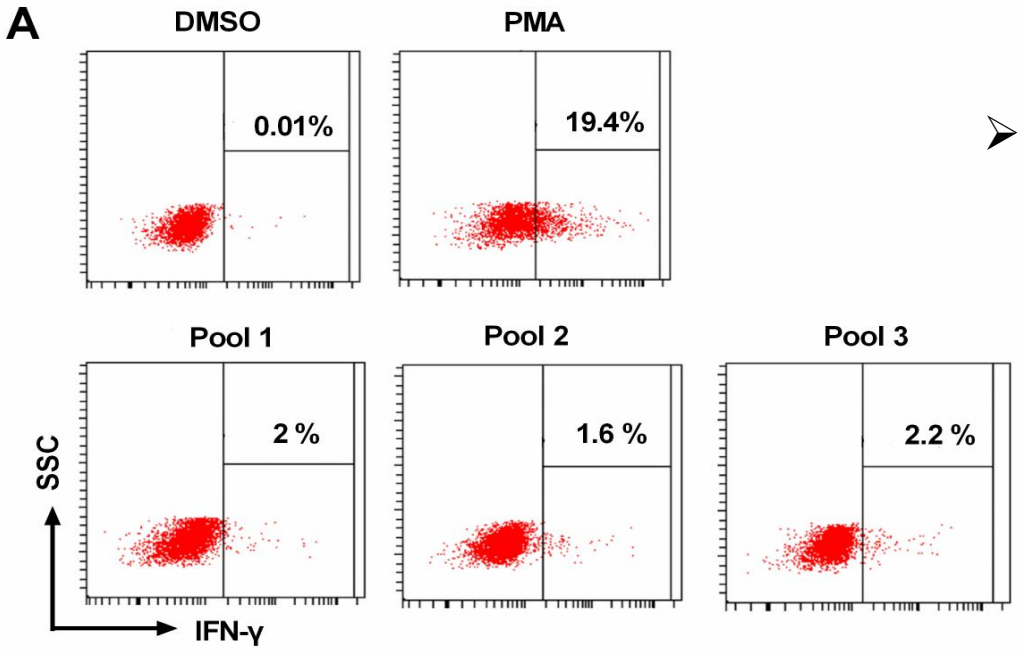
b. CD56^{-}CD16^{+} NK细胞负向调节作用

c. 通过CD54分子杀伤自体CD4^{+}T细胞

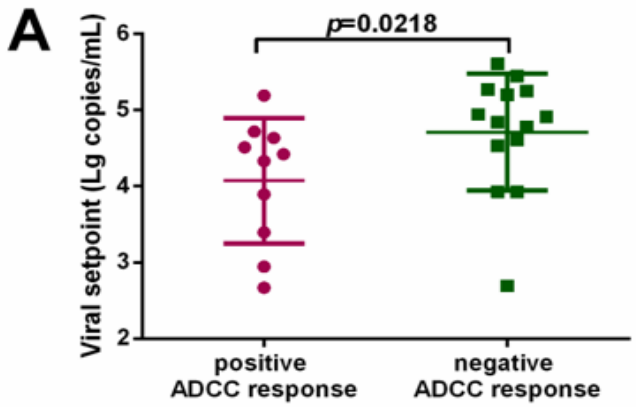
HIV感染者NK细胞ADCC效应

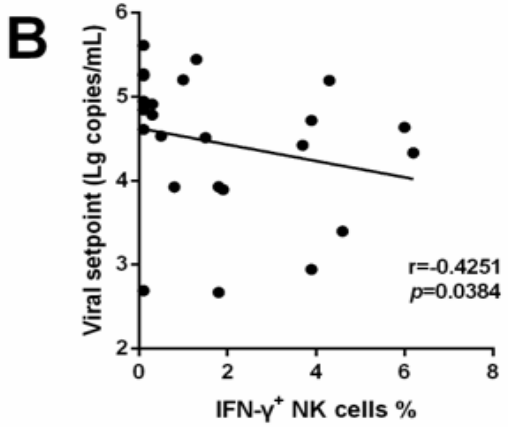


HIV感染急性期即出现ADCC 应答



- 感染52天出现ADCC应答

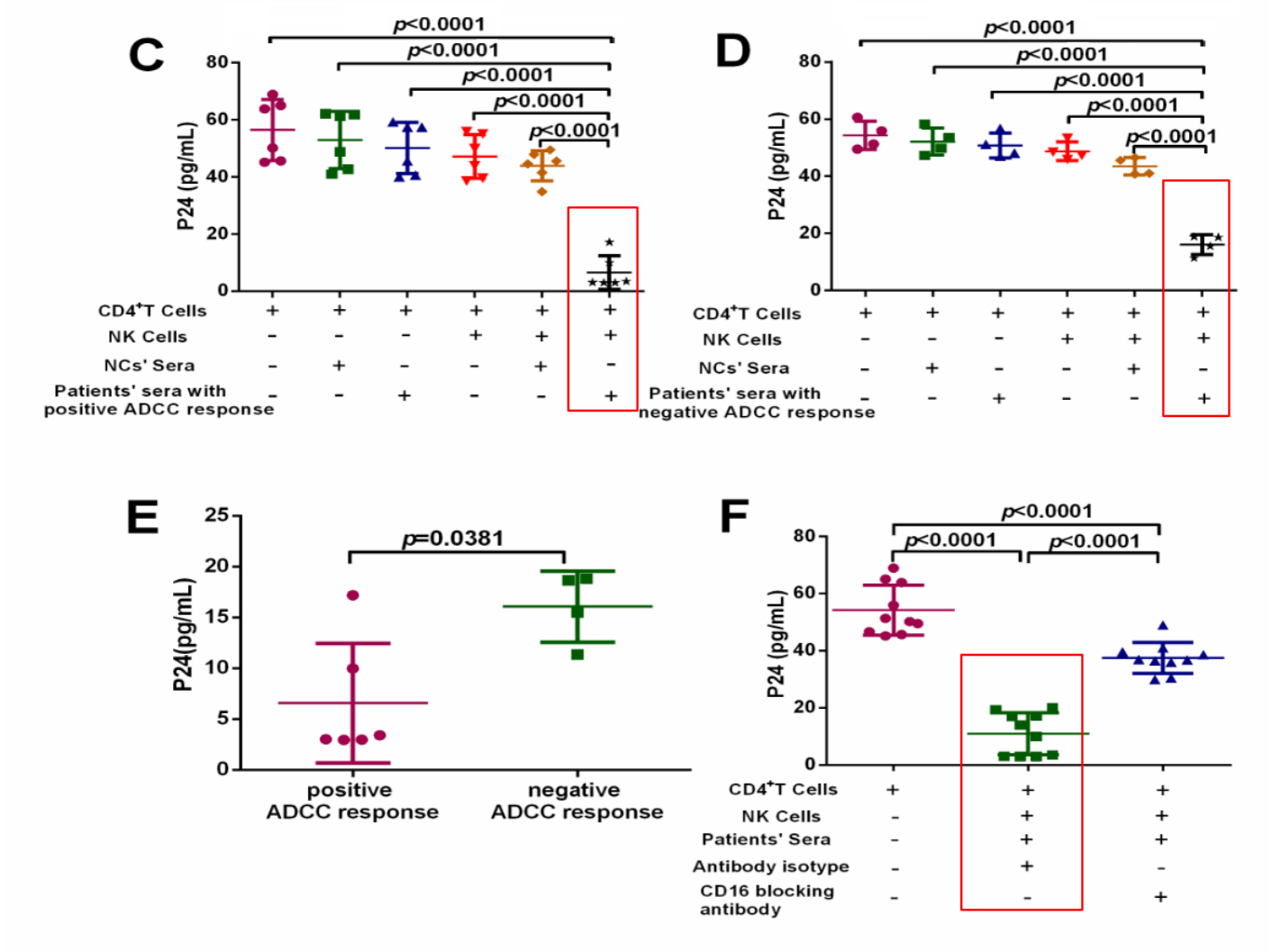




- 急性期有ADCC应答者，病毒调定点低

*Chen X，… **Jiang Y.** Front Immunol 2018;9*

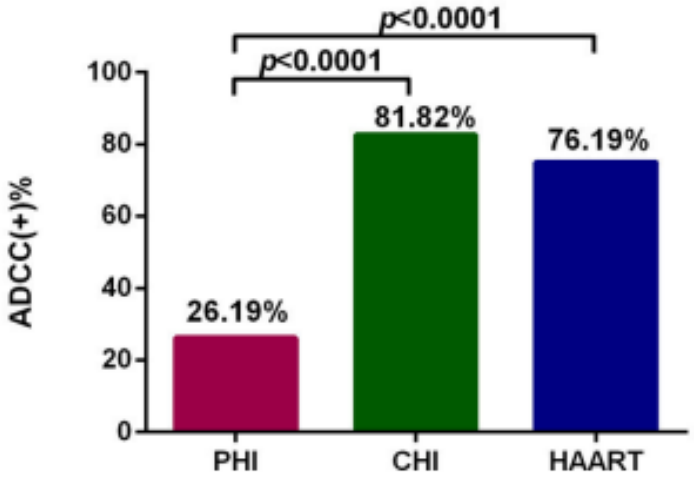
体外实验证实急性期ADCC 可抑制病毒

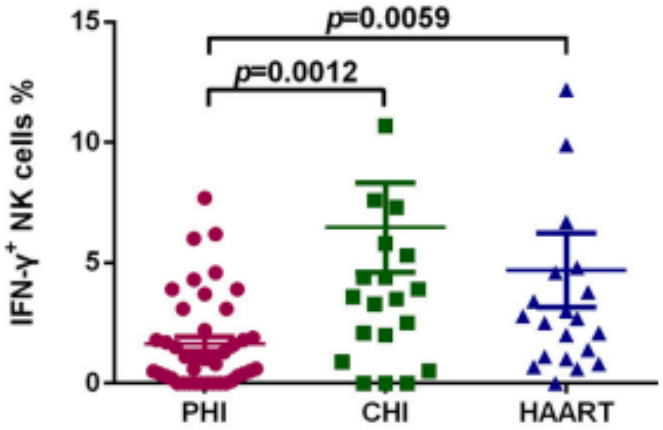


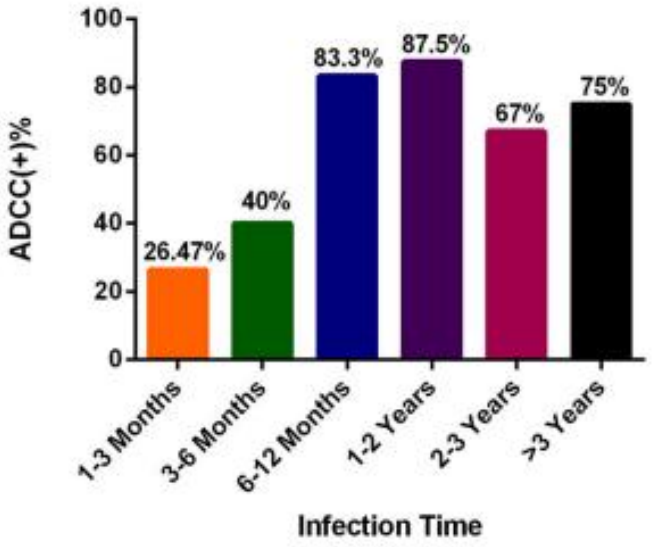
- 体外构建完整的ADCC模型证实：急性期ADCC应答可抑制病毒感染

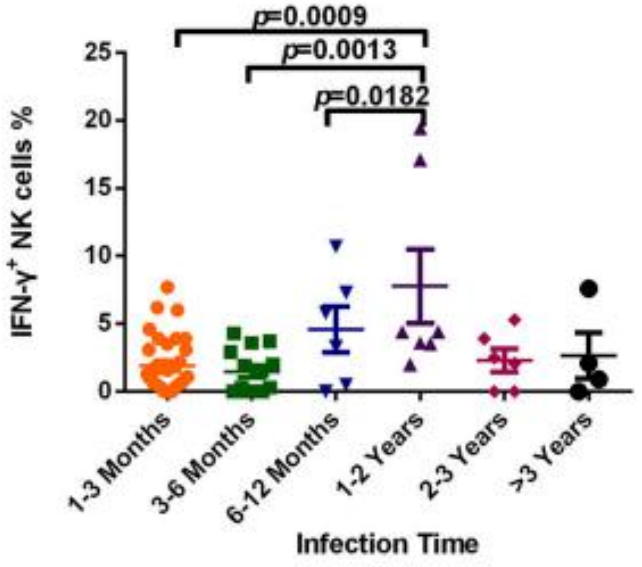
Chen X，…Jiang Y. Front Immunol 2018;9

ADCC动态变化



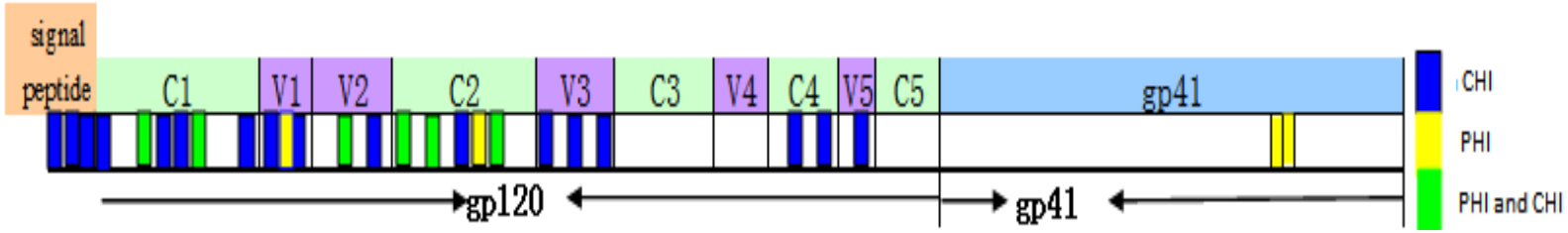






*Chen X，… **Jiang Y.** Front Immunol 2018;9*

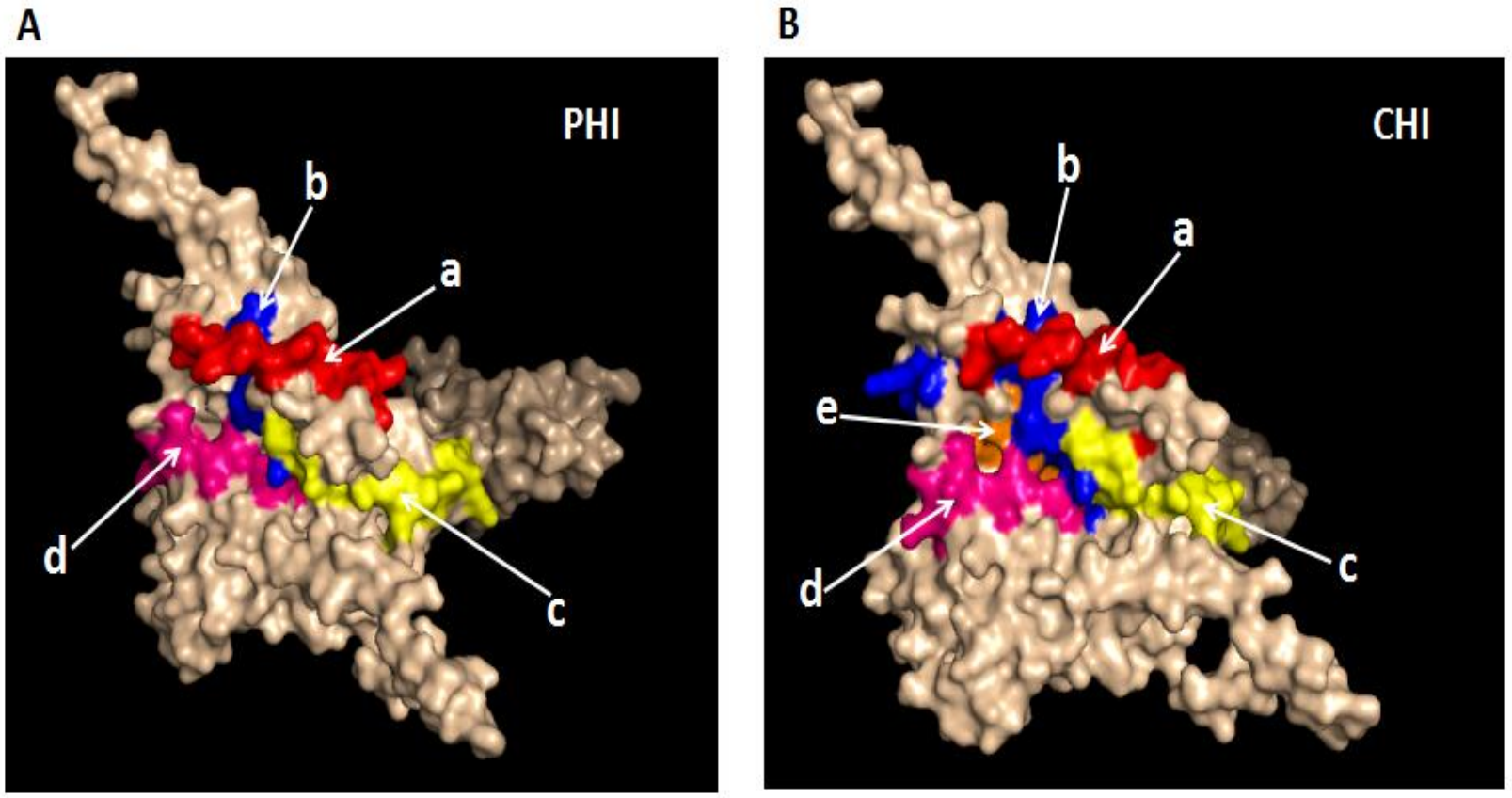
ADCC识别的HIV env相对保守表位



sequence	Epitope prevalence scores	Shannon entropy scores
PHI group		
HNVWATYACVPTDPNPQE	0.5946	0.5807
TSVIKQACPKISFDPIPI	0.5946	0.5915
VVSTQLLLNGSLAEEEII	0.8378	0.5471
QCTHGIKPVVSTQLLLNG	0.9459	0.5287
CHI group		
HNVWATYACVPTDPNPQE	0.6	0.59
TSVIKQACPKISFDPIPI	0.6	0.59
VVSTQLLLNGSLAEEEII	0.84	0.53
RPGGGNIKDNWRSELYKY	0.68	0.59
PCKNVSSVQCTHGIKPVV	0.65	0.57

*Chen X，… **Jiang Y.** Front Immunol 2018;9*

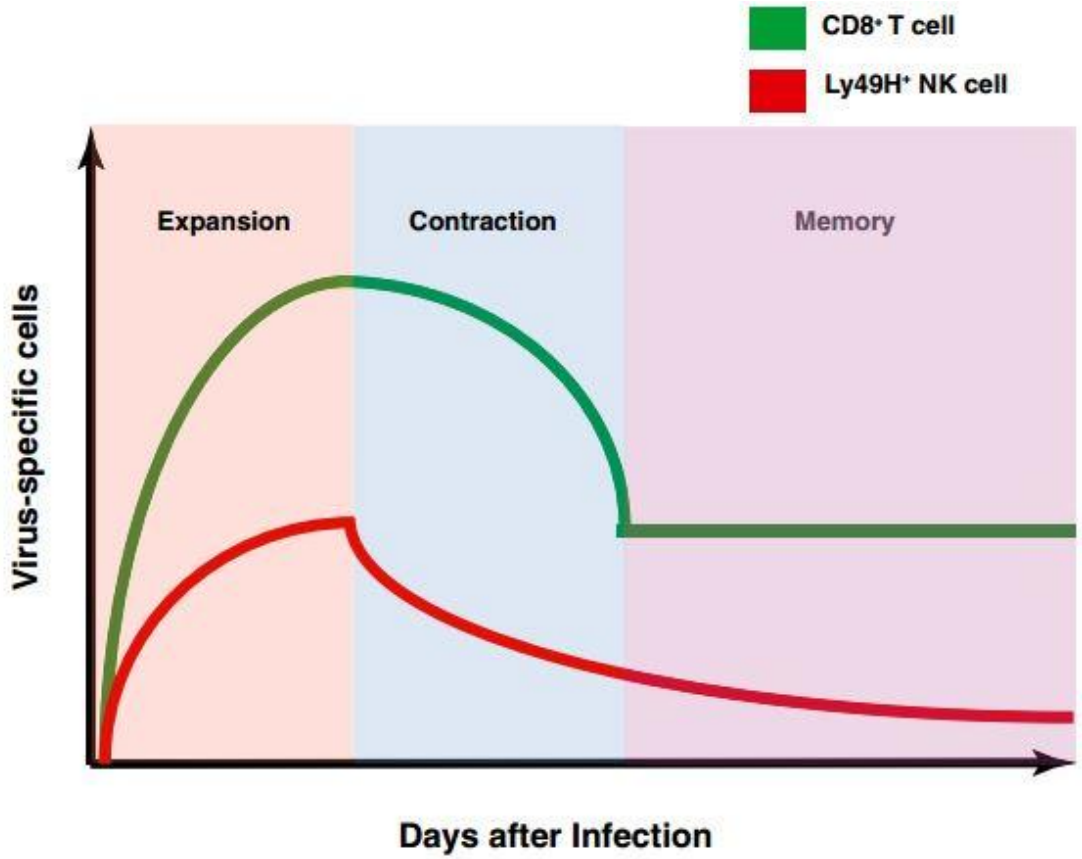
ADCC识别相对保守表位的空间构象



获2018年F1000推荐

Chen X，…Jiang Y. Front Immunol 2018;9

NKG2C+NK细胞功能强

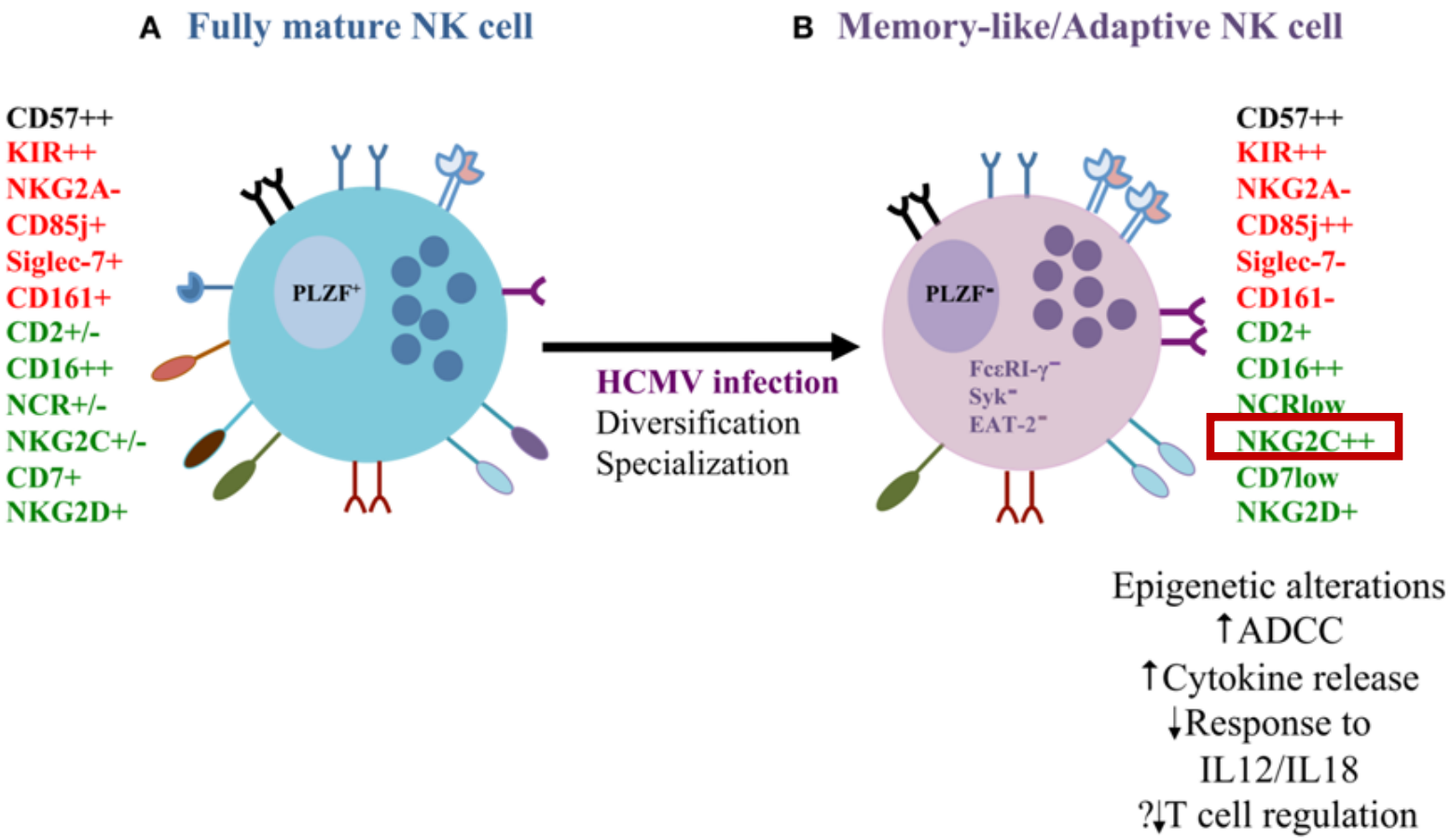


- NK细胞特异性应答 ：

在某种刺激物二次刺激活化后，产生更快、更强的免疫反应，

Immunity.2015

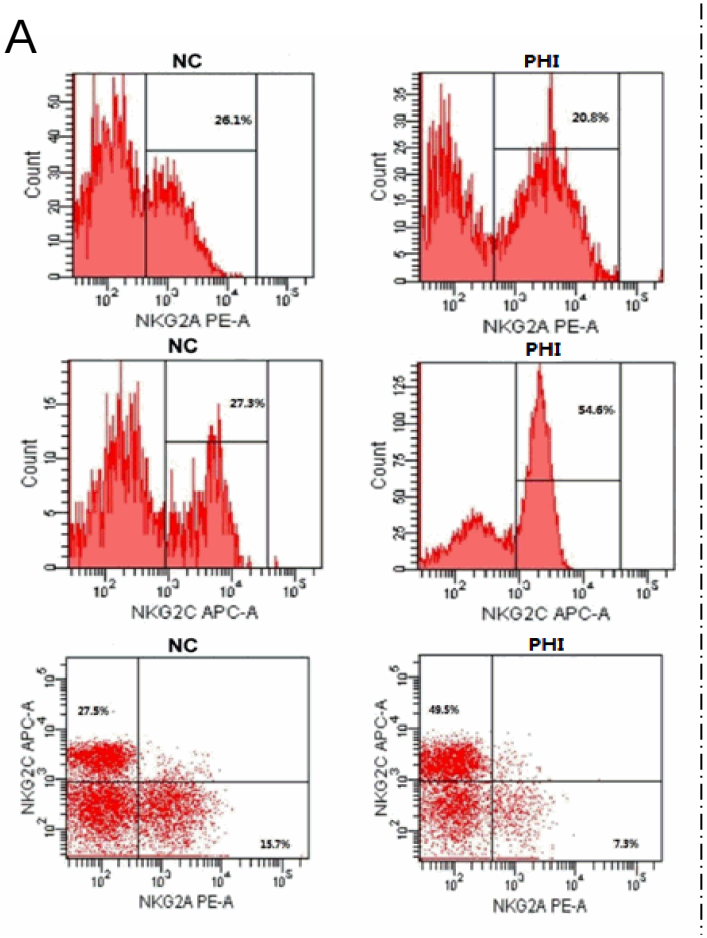
记忆样NK细胞的特征

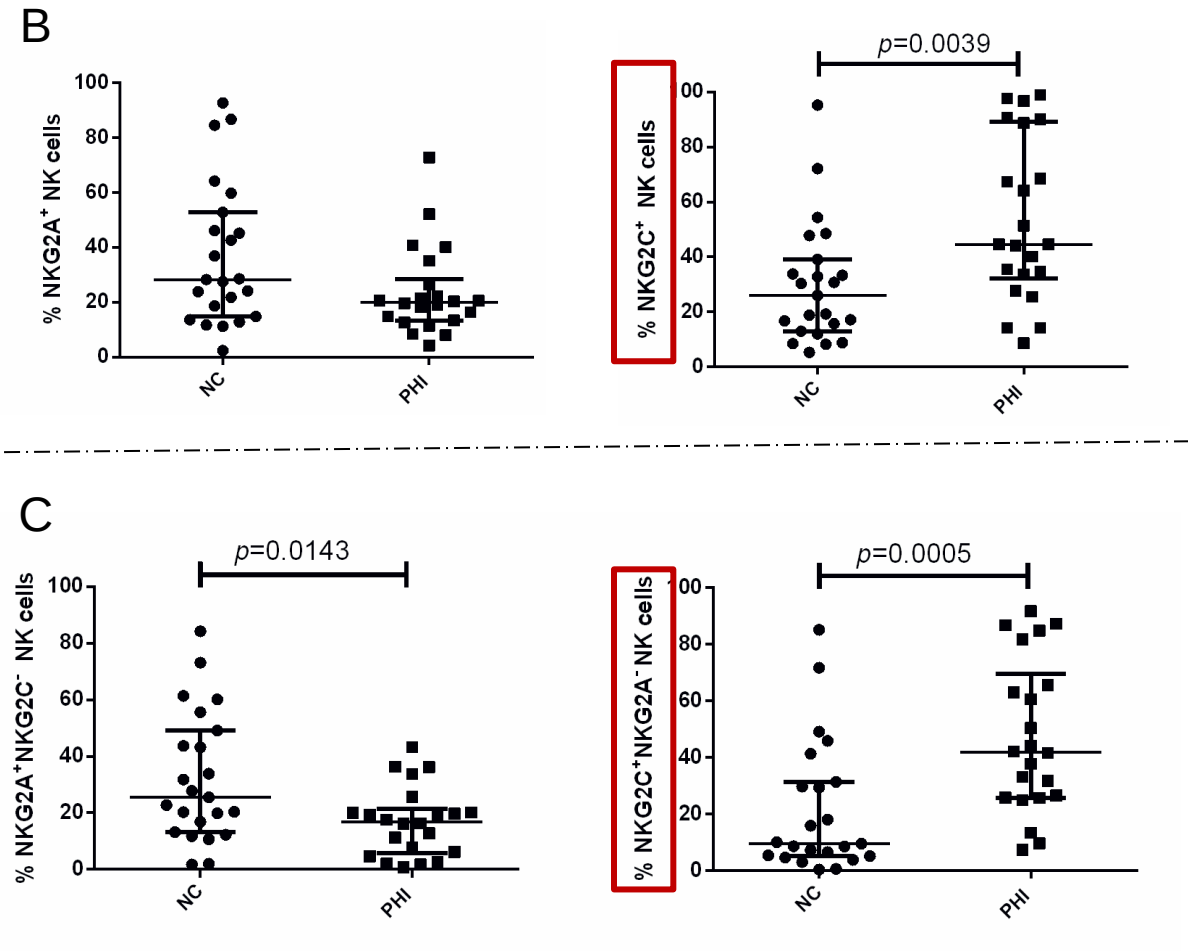


- CMV感染时记忆性NK细胞会发生表型变化、表观修饰变化、功能变化

Peppa et al. Frontiers in Immunology, 2017 Oct 17;8:1322

HIV感染后NKG2C⁺NK细胞增加

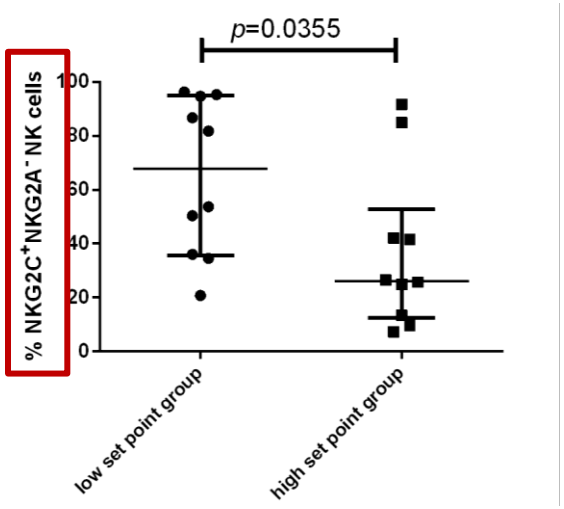


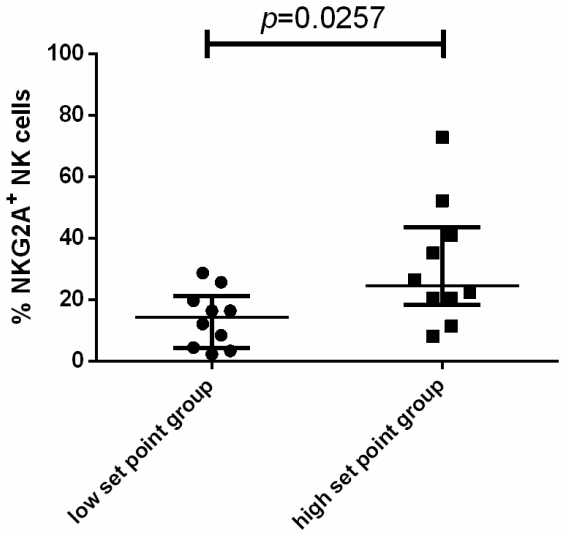


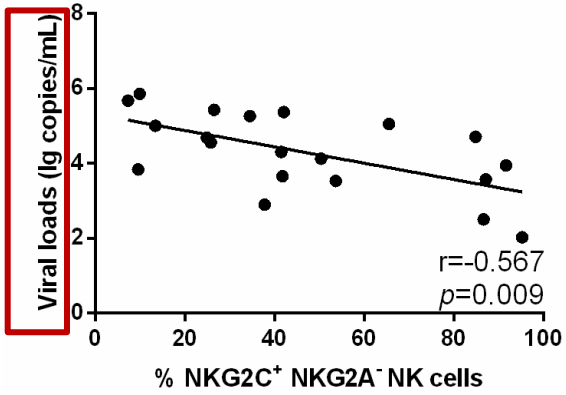
➢ PHI感染者NKG2C⁺ NK cell，NKG2C⁺NKG2A⁻ NK cell百分比增加

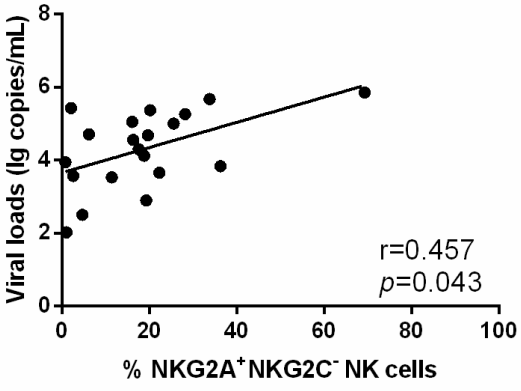
Ma M... Jiang Y. Front Immunol 2017;8

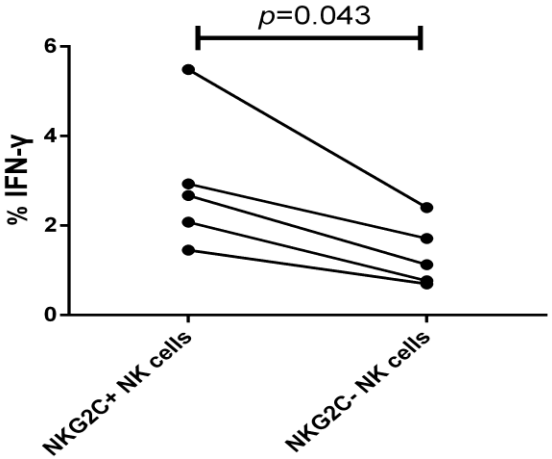
NKG2C⁺NK细胞与疾病进展负相关

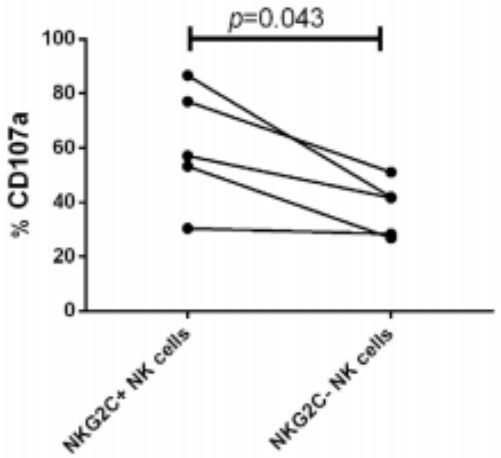








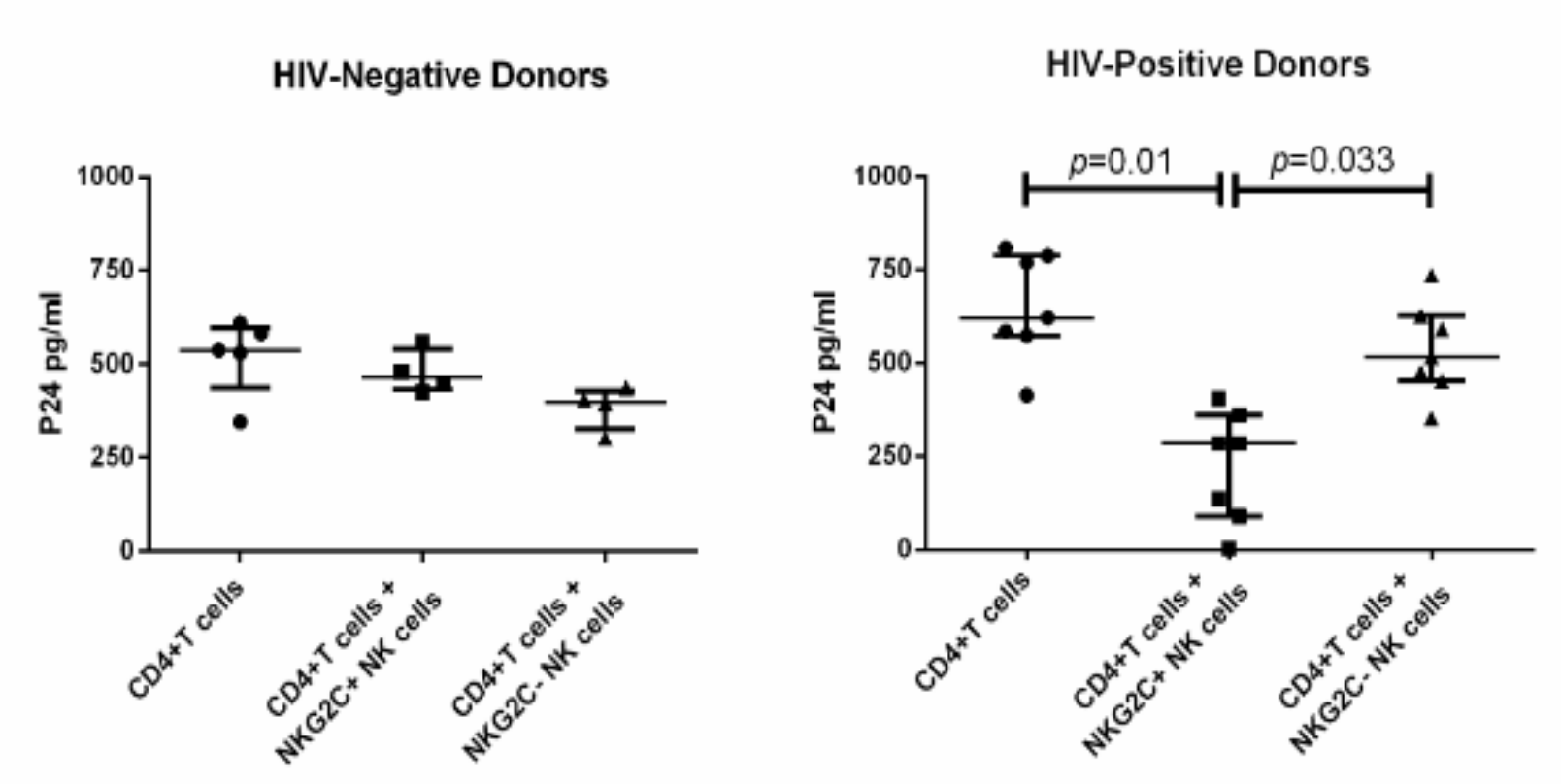




- 低病毒载量HIV感染者，NKG2C⁺NKG2A⁻ NK细胞百分比较高，且与病毒载量负相关；NKG2C⁺ NK细胞功能强

Ma M... Jiang Y. Front Immunol 2017;8

HIV感染时NKG2C+NK细胞参与特异性应答

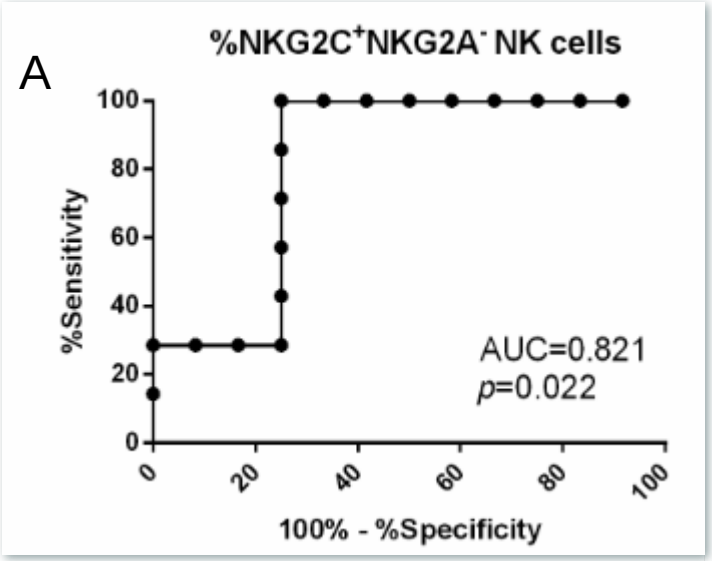


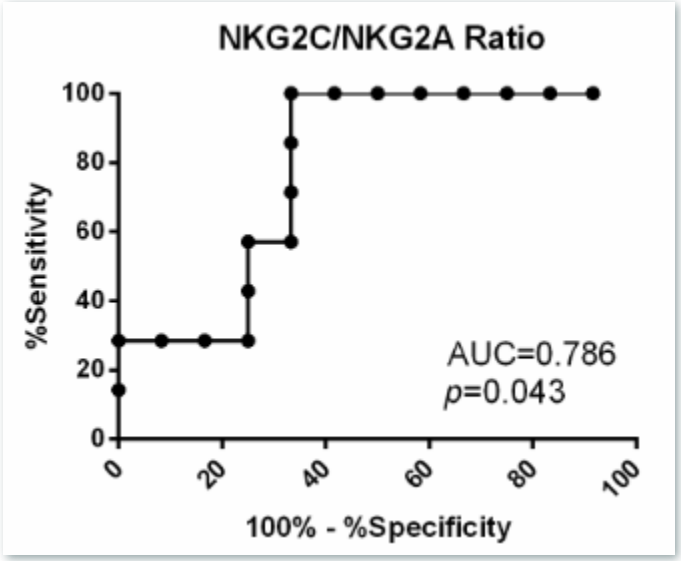
- 用HIV分别感染正常人、HIV感染者CD4+T细胞，发现HIV感染者的NKG2C+NK细胞抗病毒能力更强，说明NKG2C+NK细胞在遇到HIV时反应更强。

Ma M... Jiang Y. Front Immunol 2017;8

NKG2C⁺NKG2A⁻NK%预测HIV疾病进展

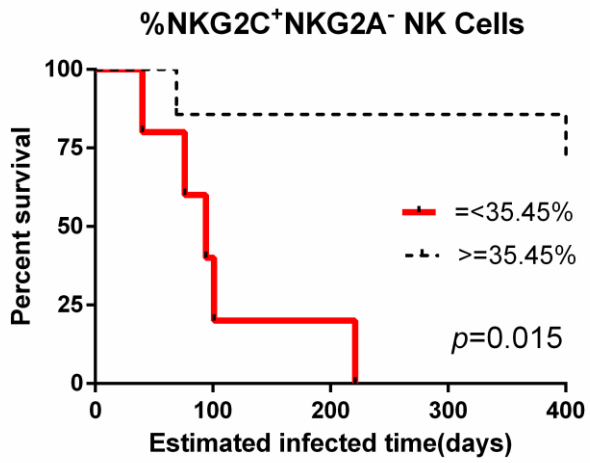
A

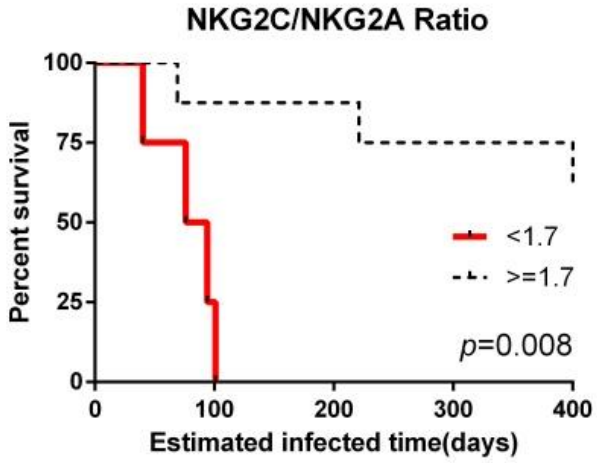




- NKG2C⁺NKG2A⁻细胞升高预后较好

B

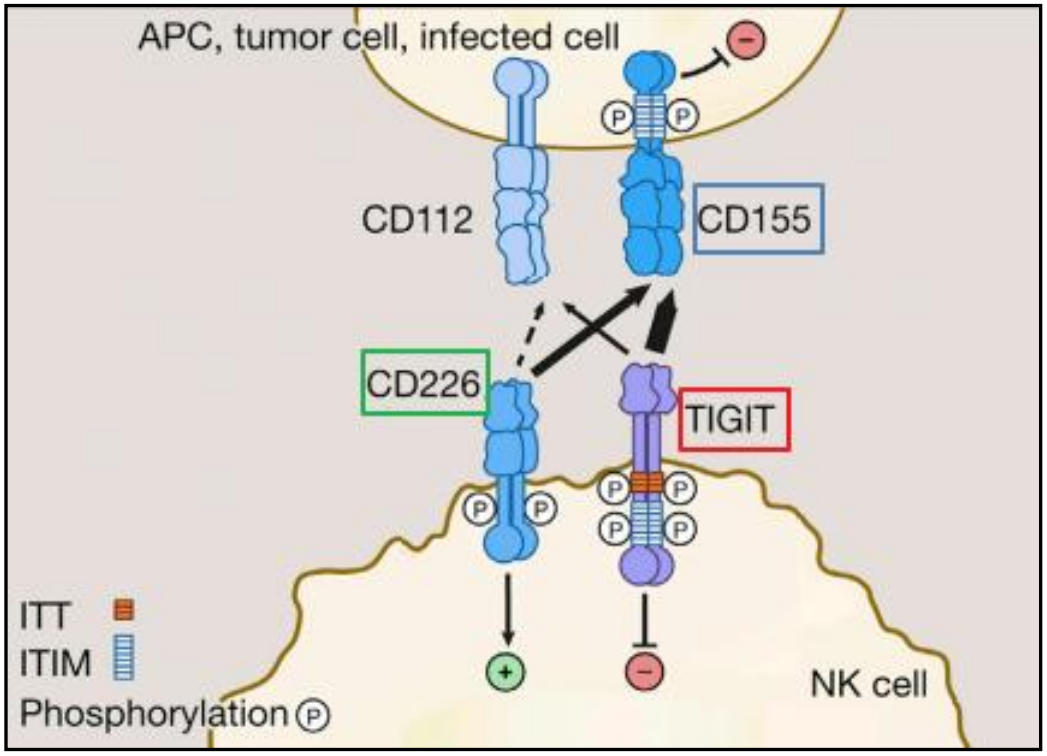




- NKG2C⁺NKG2A⁻ NK 细胞百分比≤35.45%；NKG2C/NKG2A<1.7患者预后差

Ma M... Jiang Y. Front Immunol 2017;8

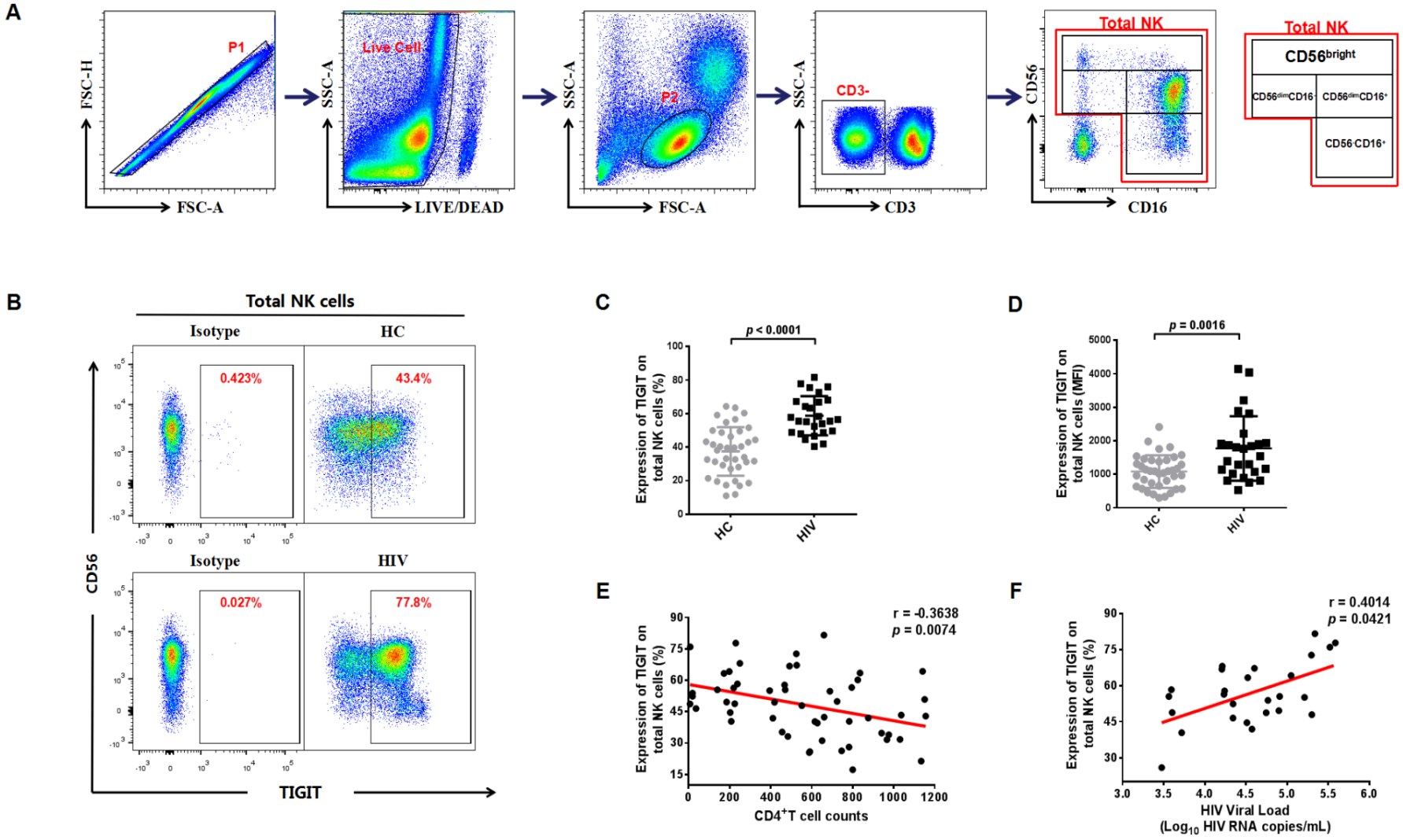
NK细胞高表达TIGIT，功能被抑制



TIGIT>PD-1

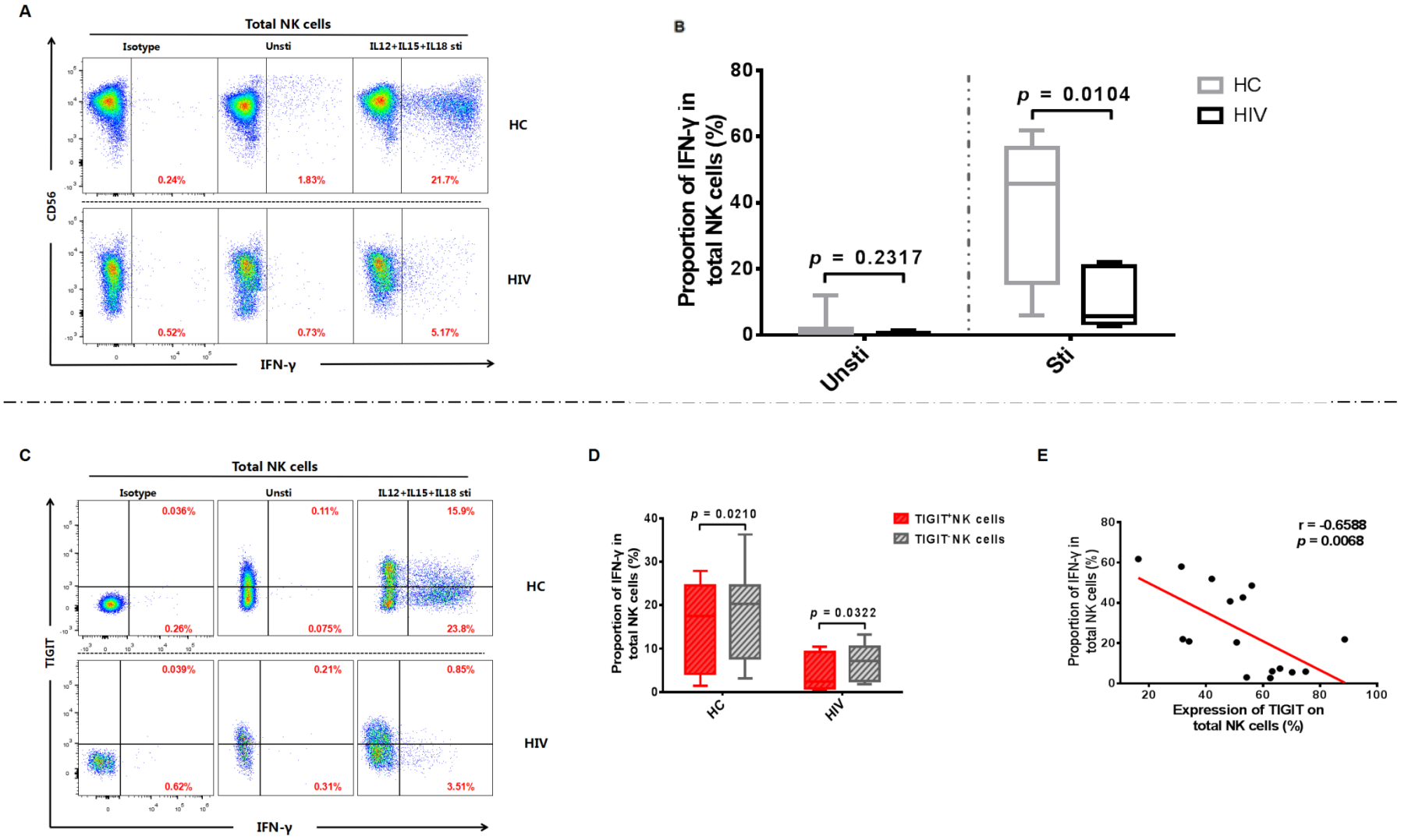
Immunity. 2016.

首次发现HIV感染者NK细胞TIGIT表达升高，与HIV疾病进展相关



Yin X... Jiang Y. Front Immunol 2018;10

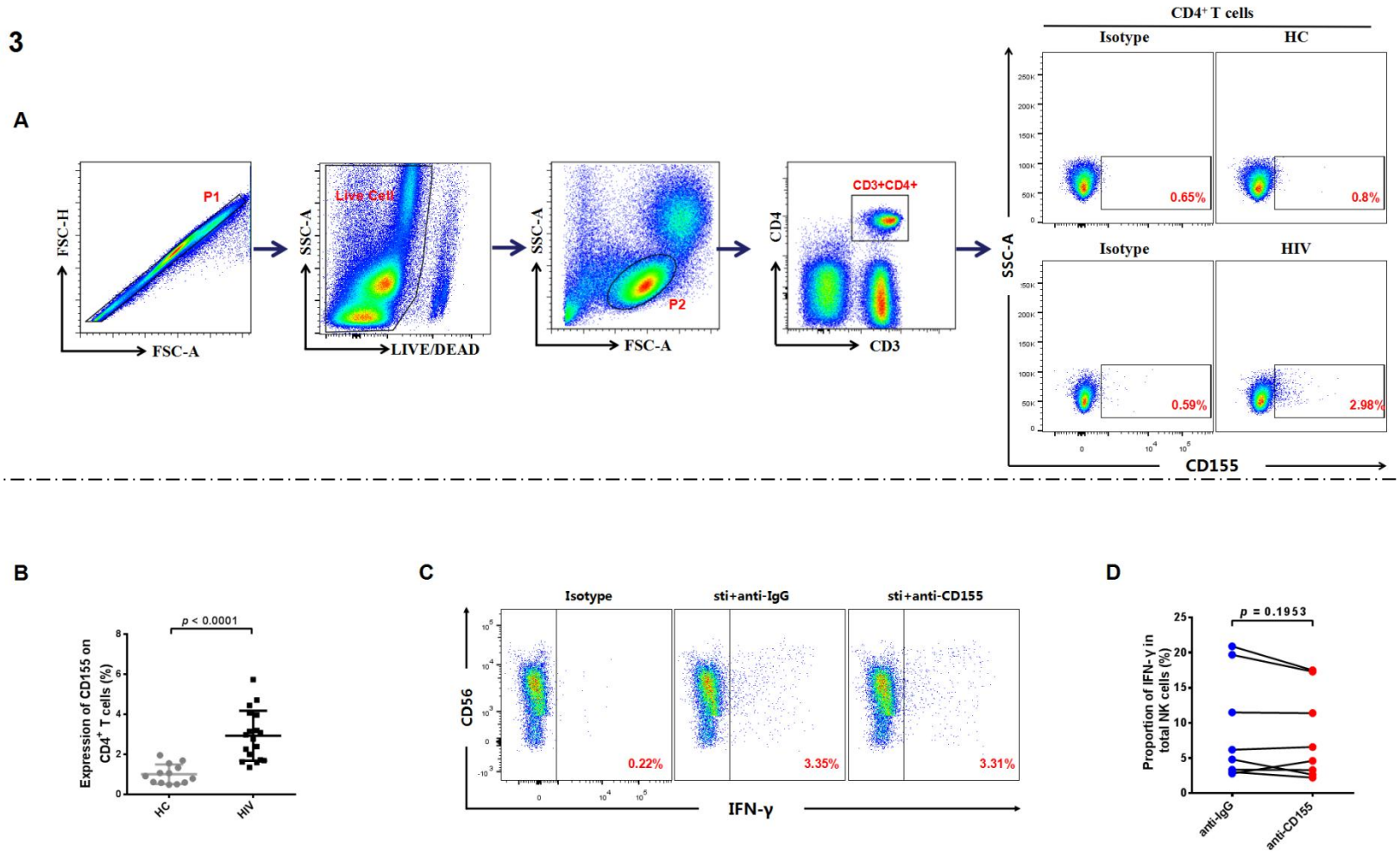
TIGIT抑制NK细胞IFN-γ分泌，与IFN-γ分泌呈负相关



Yin X... Jiang Y. Front Immunol 2018;10

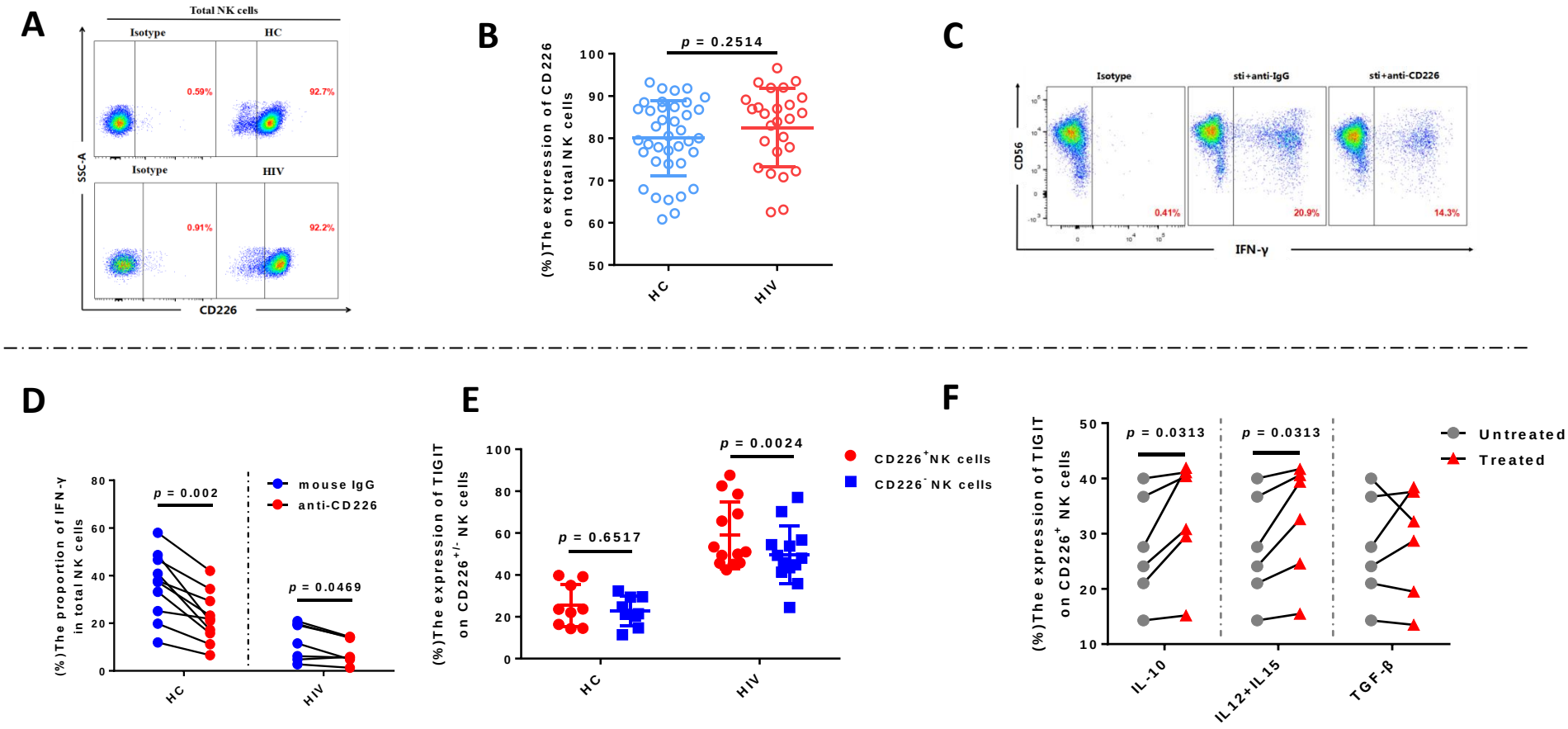
TIGIT配体CD155表达升高

Fig 3



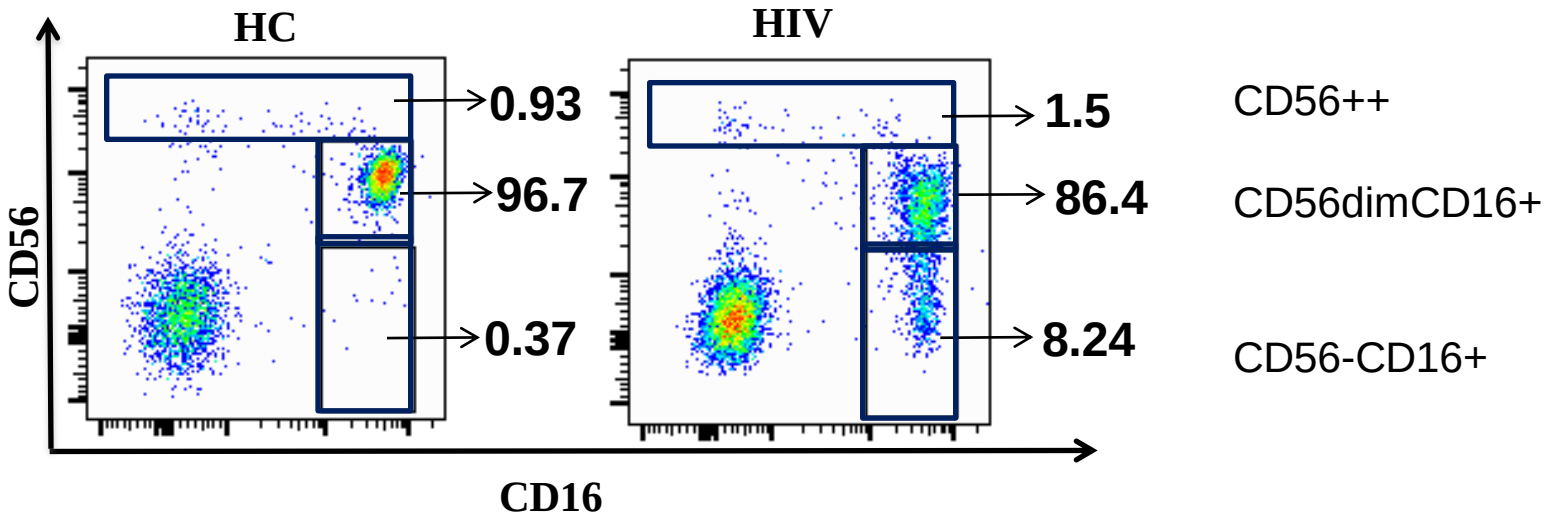
Yin X... Jiang Y. Front Immunol 2018;10

TIGIT表达在具有活化功能的CD226+ NK细胞表面



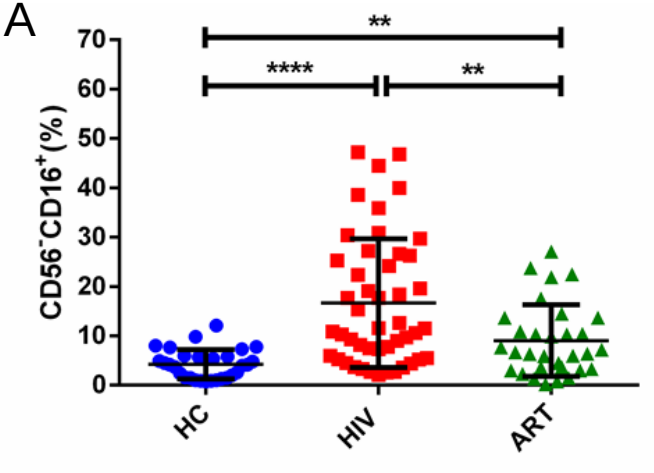
Yin X... Jiang Y. Front Immunol 2018;10

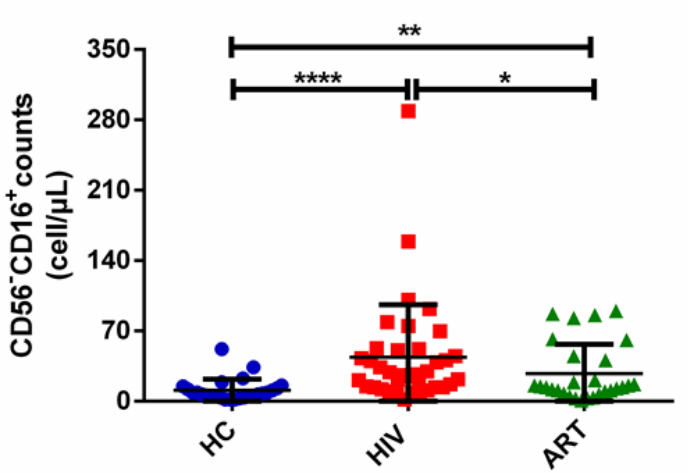
CD56^{-}CD16^{+}NK亚群作用



HIV感染后CD56⁻CD16⁺NK细胞增加

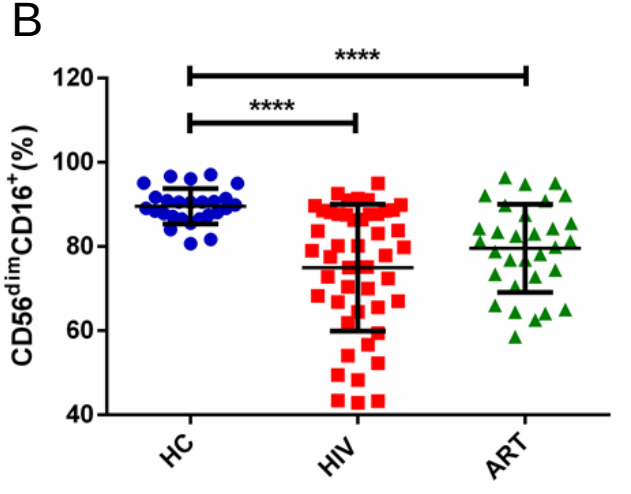
A

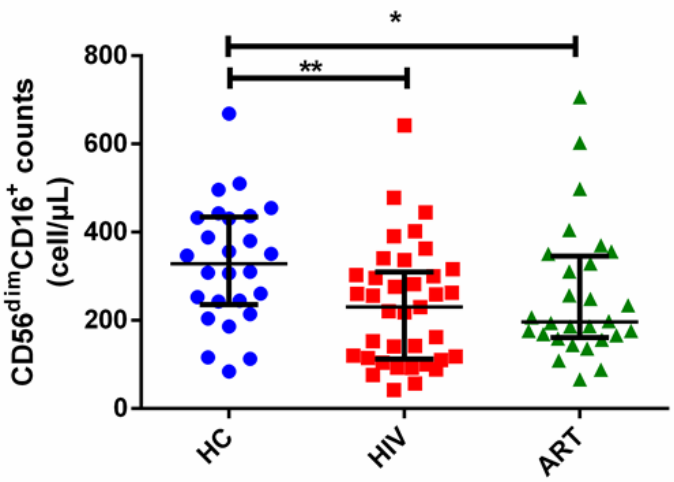




- HIV感染后CD56⁻CD16⁺NK细胞百分比及绝对数增加
- ART治疗者降低

B

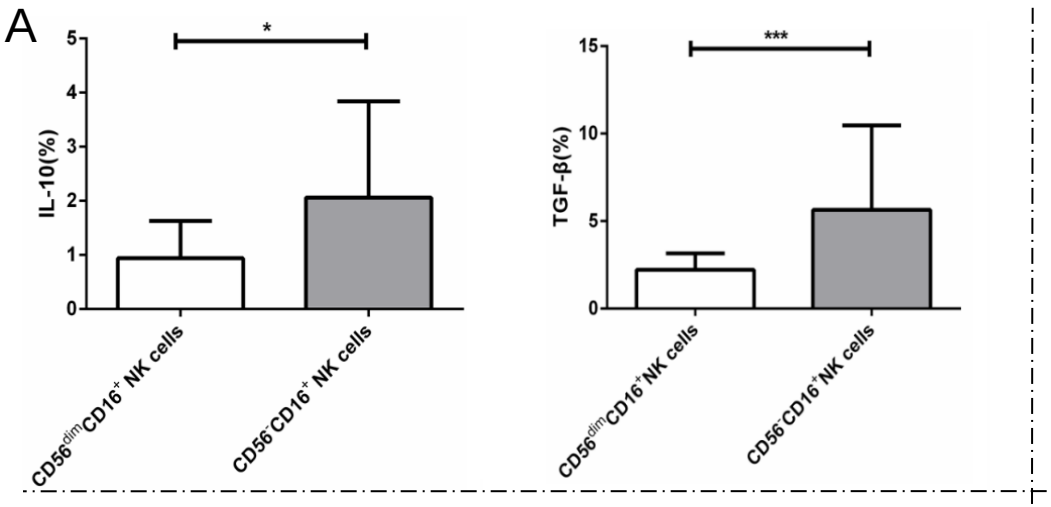


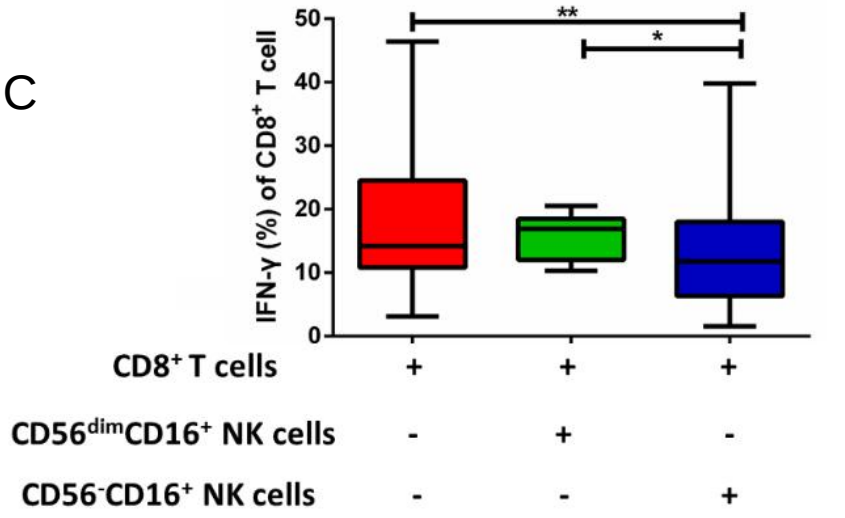


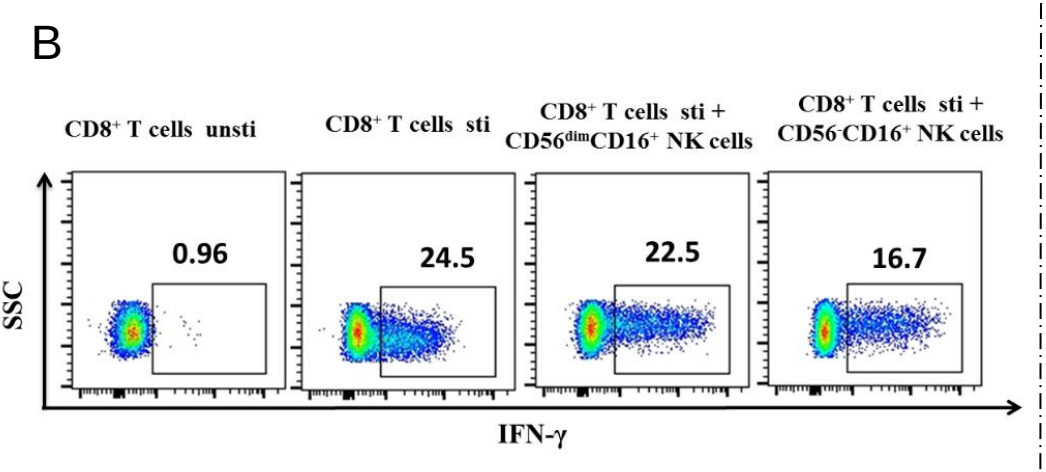
- CD56ᵈⁱᵐCD16⁺NK细胞百分比及绝对数减少

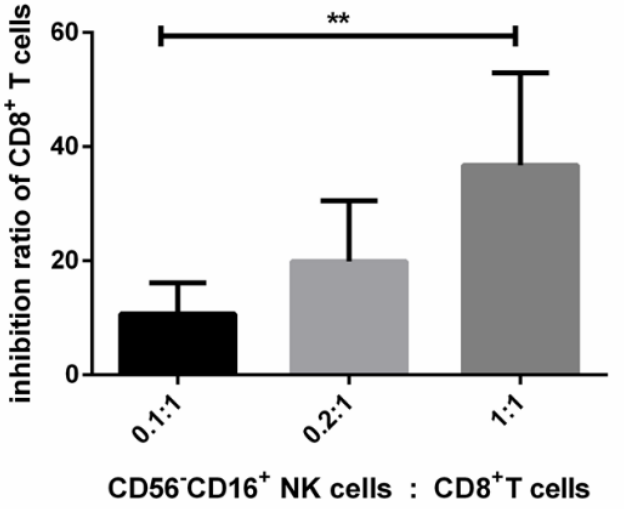
Ma M... Jiang Y.. J. Leukoc. Biol. 2019;8

CD56⁻CD16⁺NK细胞抑制CD8⁺T细胞功能





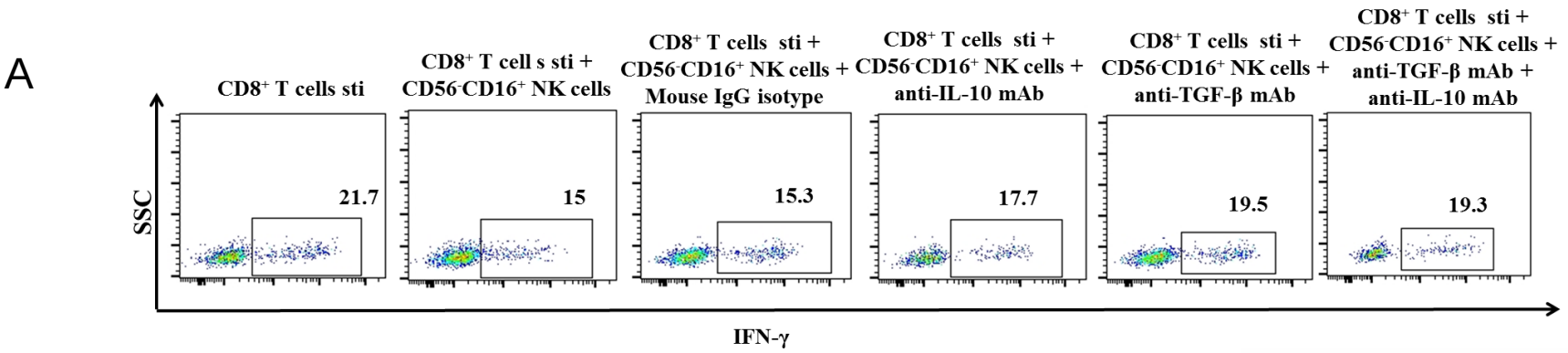


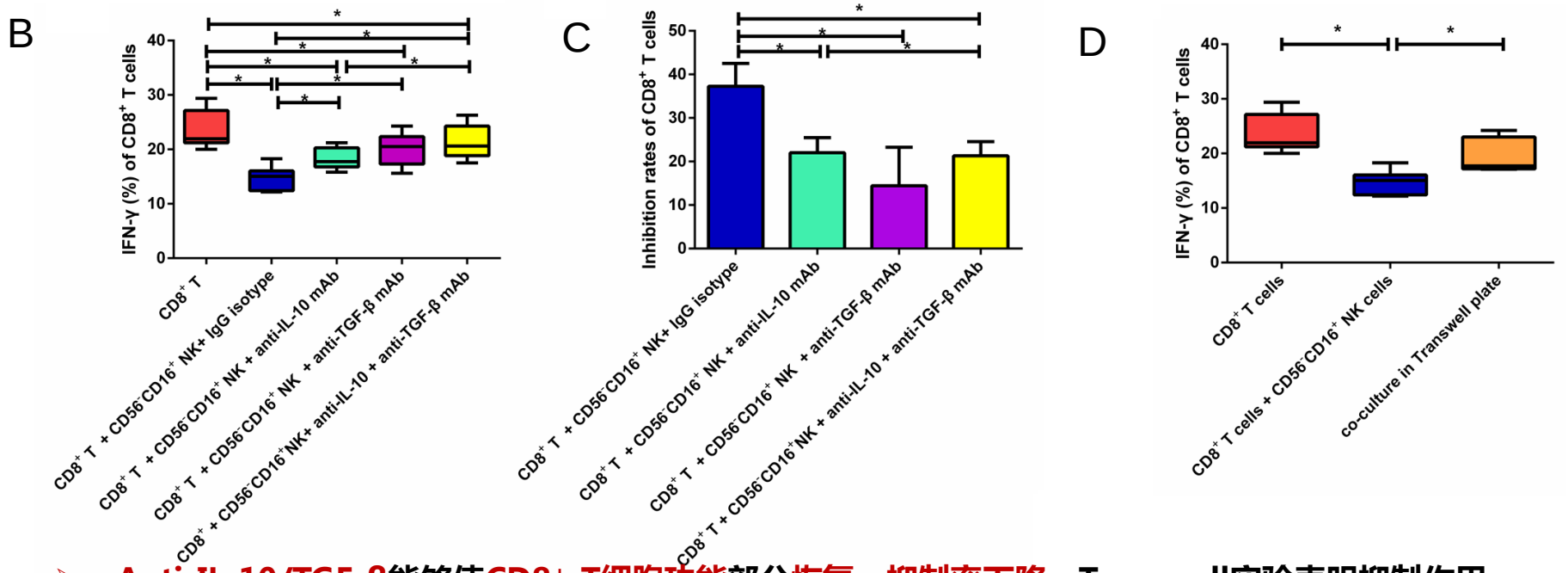


- CD56⁻CD16⁺NK细胞分泌IL-10, TGF-β,可抑制CD8⁺ T cells 分泌IFN-γ

Ma M... Jiang Y.. J. Leukoc. Biol. 2019;8

阻断细胞因子可恢复CD8⁺T细胞功能



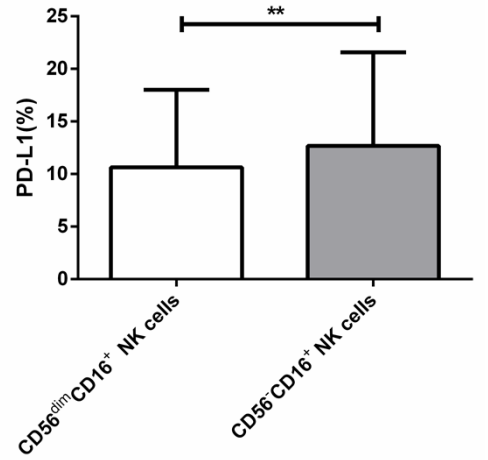


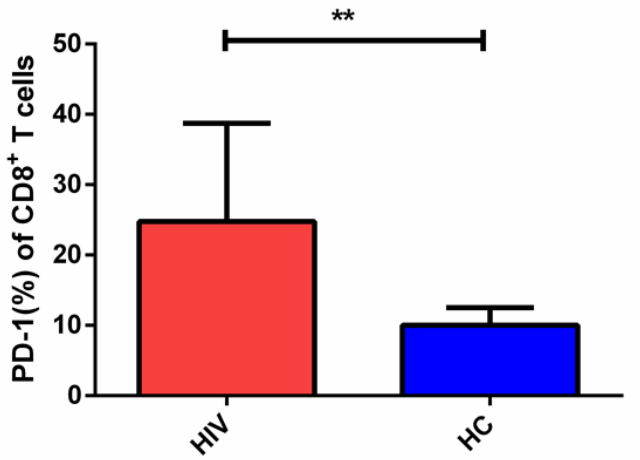
- **Anti-IL-10/TGF-β能够使CD8⁺ T细胞功能部分恢复，抑制率下降；Transwell实验表明抑制作用可能与细胞间直接接触有关**

Ma M... Jiang Y.. J. Leukoc. Biol. 2019;8

阻断PD-L1可恢复CD8⁺T细胞功能

A





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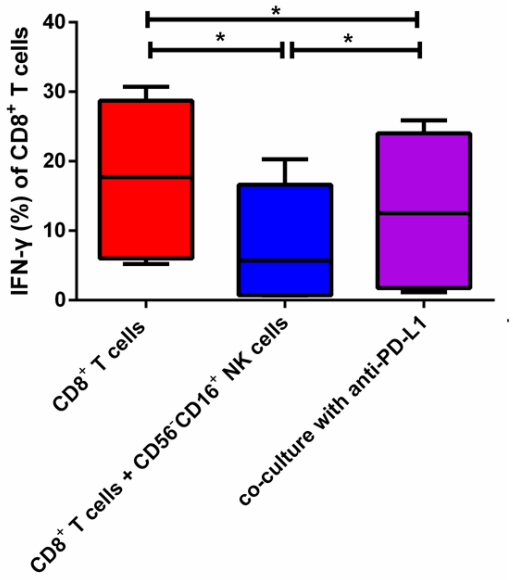
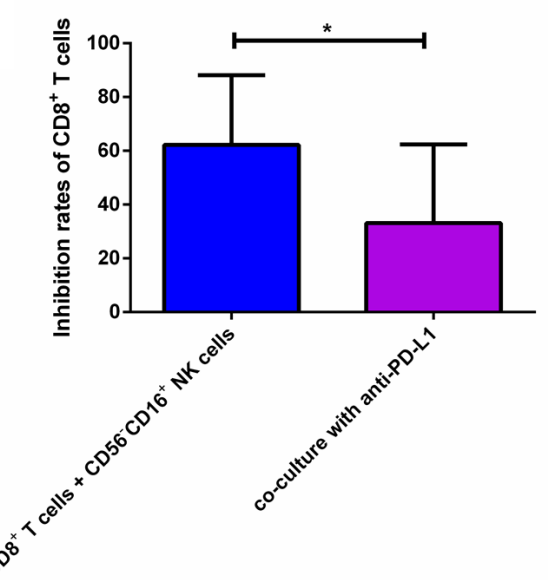


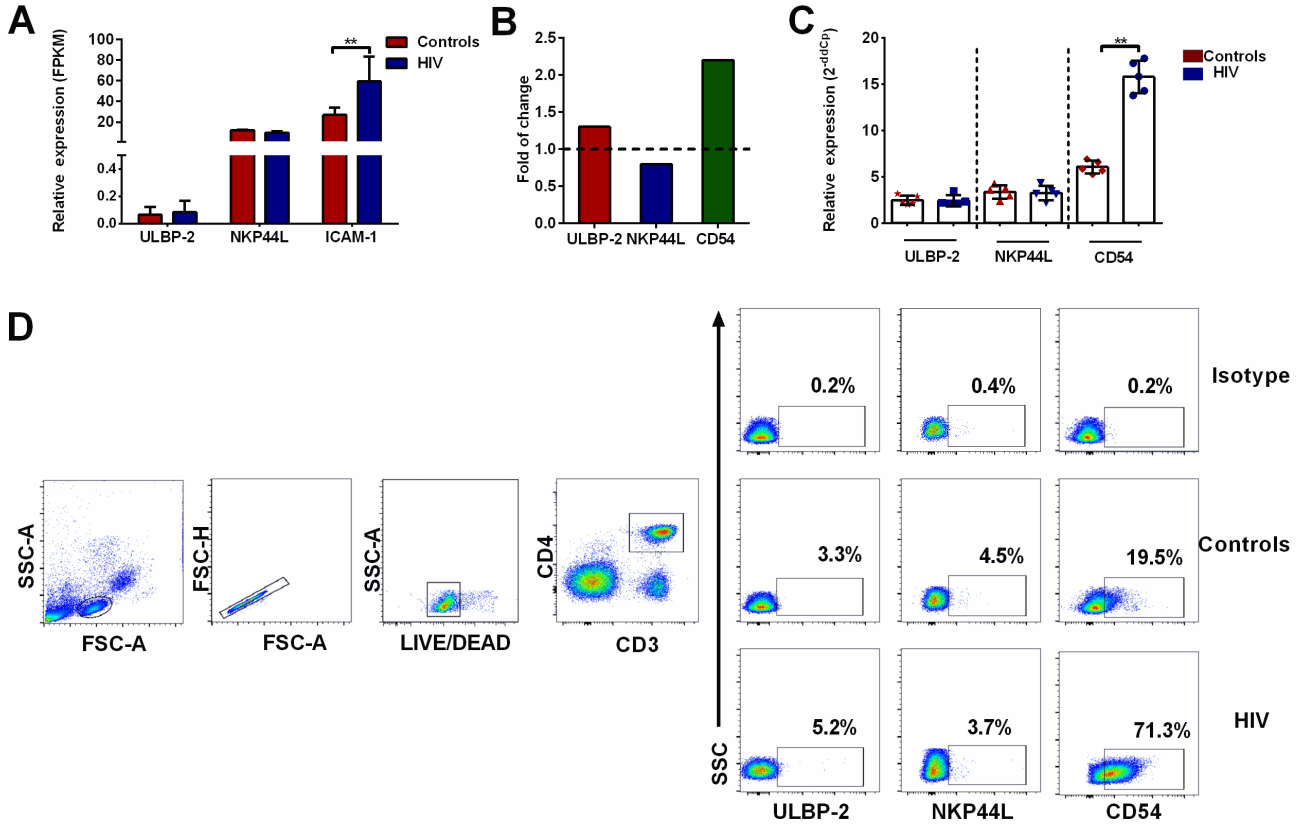


- CD56⁻CD16⁺NK细胞高表达PD-L1，Anti-PD-L1能够使CD8⁺ T细胞功能部分恢复，抑制率下降

Ma M… Jiang Y.. J. Leukoc. Biol. 2019;8

NK细胞通过升高的CD54杀伤自体$CD4^+$ T细胞

HIV感染者CD4+ T细胞上的高CD54表达



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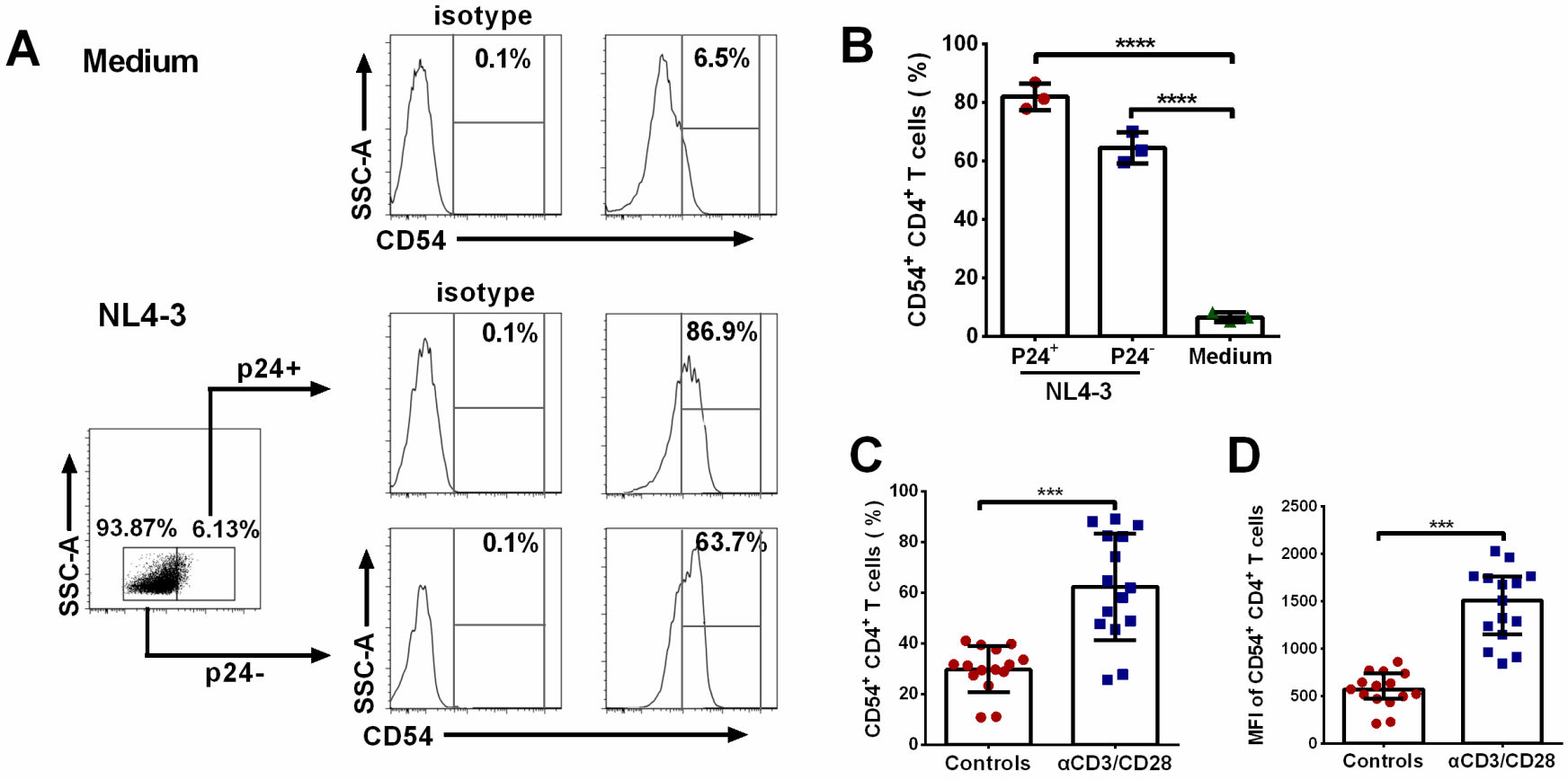
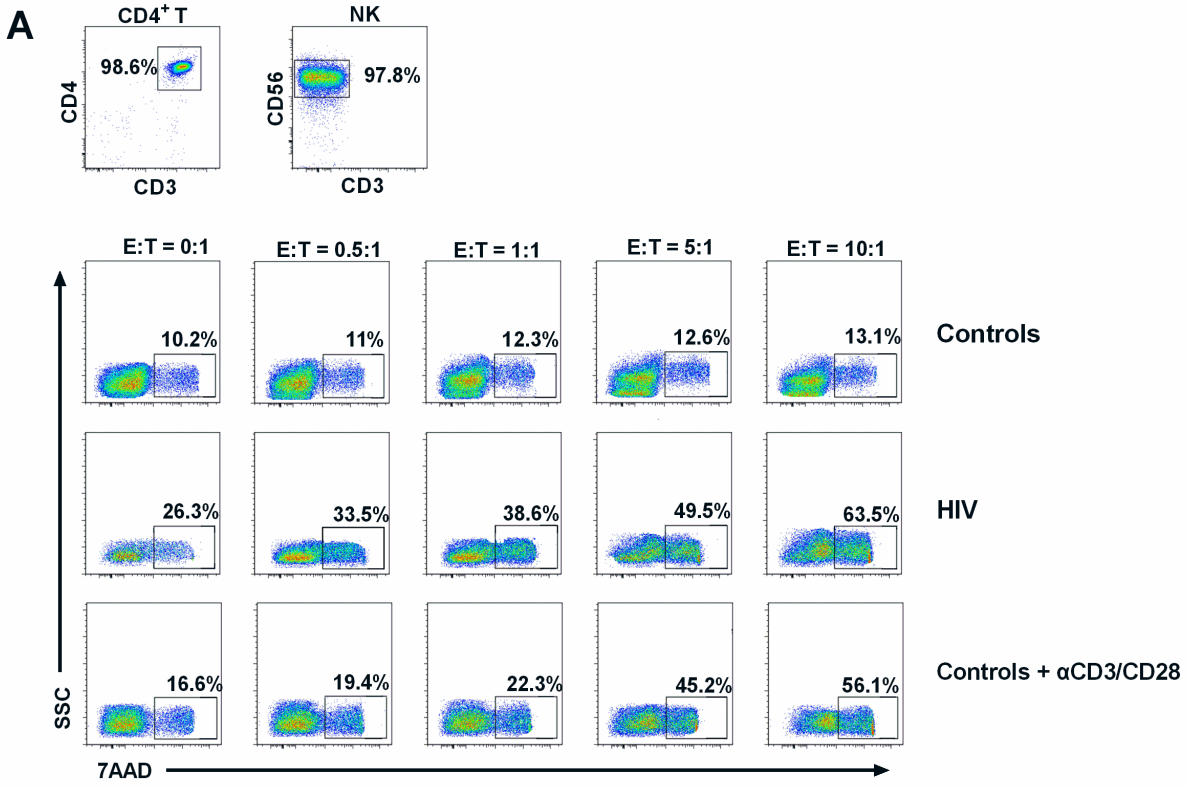
体外活化后CD4⁺ T细胞CD54表达升高

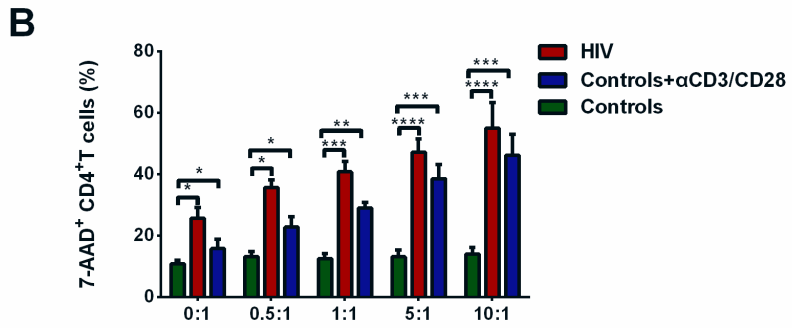


- 活化的CD4⁺T细胞上高表达CD54

*Chen X... **Jiang Y.** J. Infect. Dis. 2019*

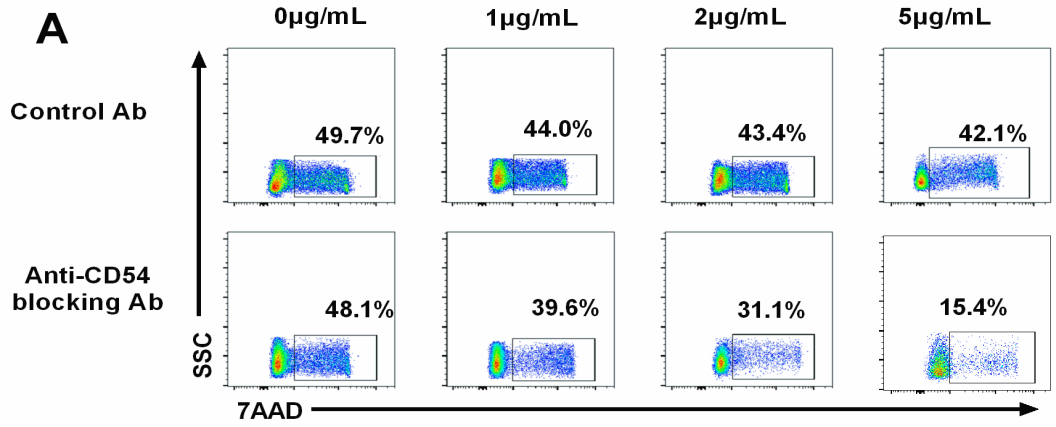
NK细胞可杀伤自体活化的CD4+ T细胞

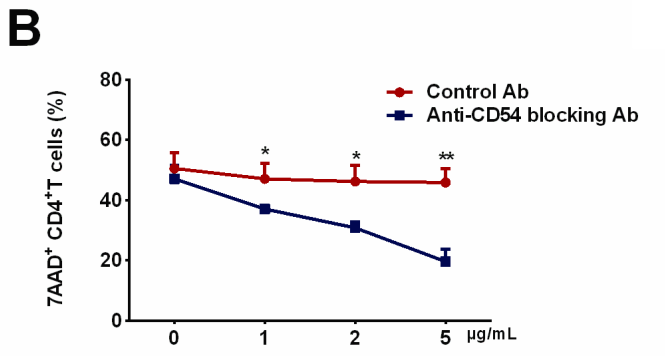


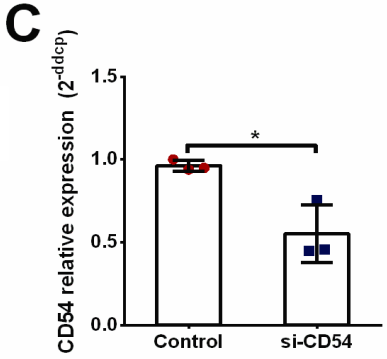


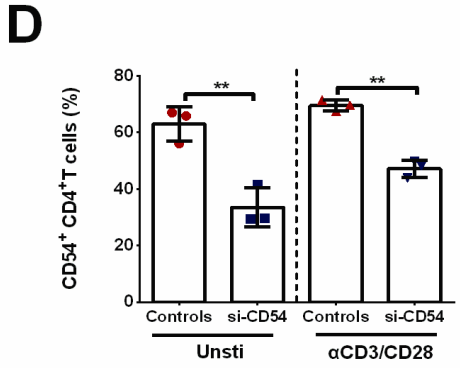
*Chen X... **Jiang Y.** J. Infect. Dis. 2019*

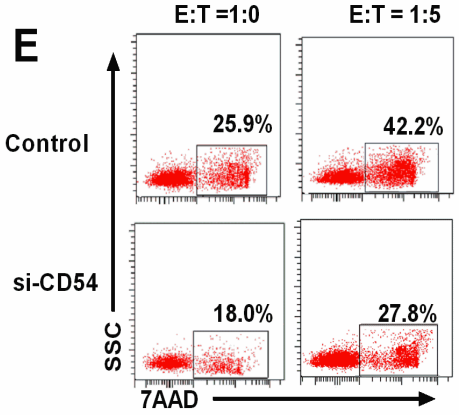
CD4+ T细胞上高表达CD54可介导NK细胞对自体CD4+ T细胞杀伤

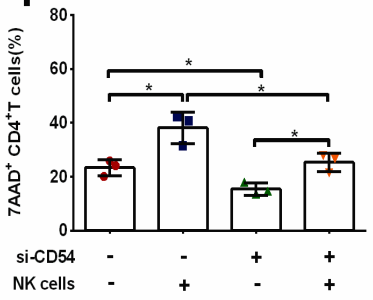












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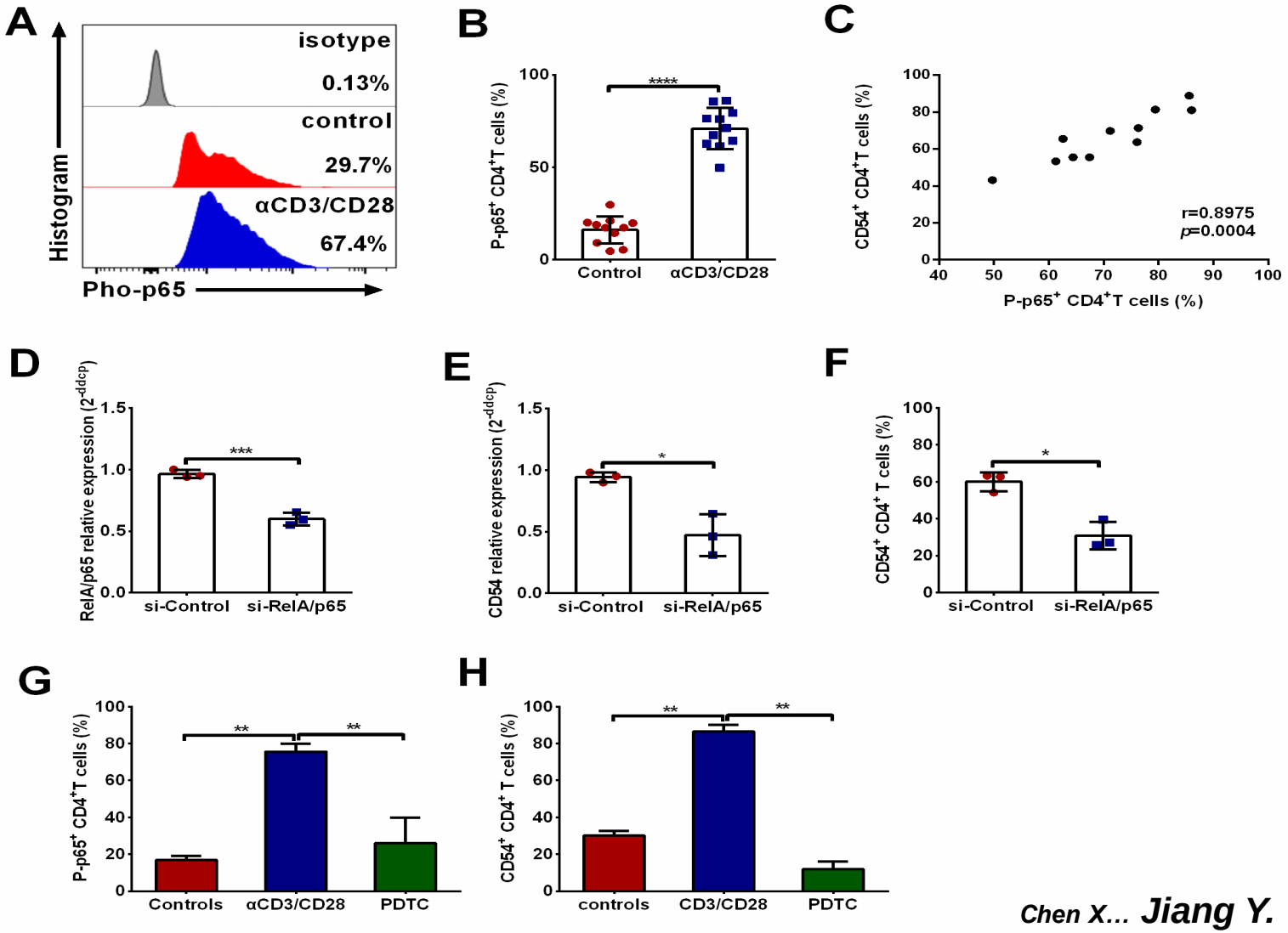
CD4+ T细胞CD54表达受NF-κB调节



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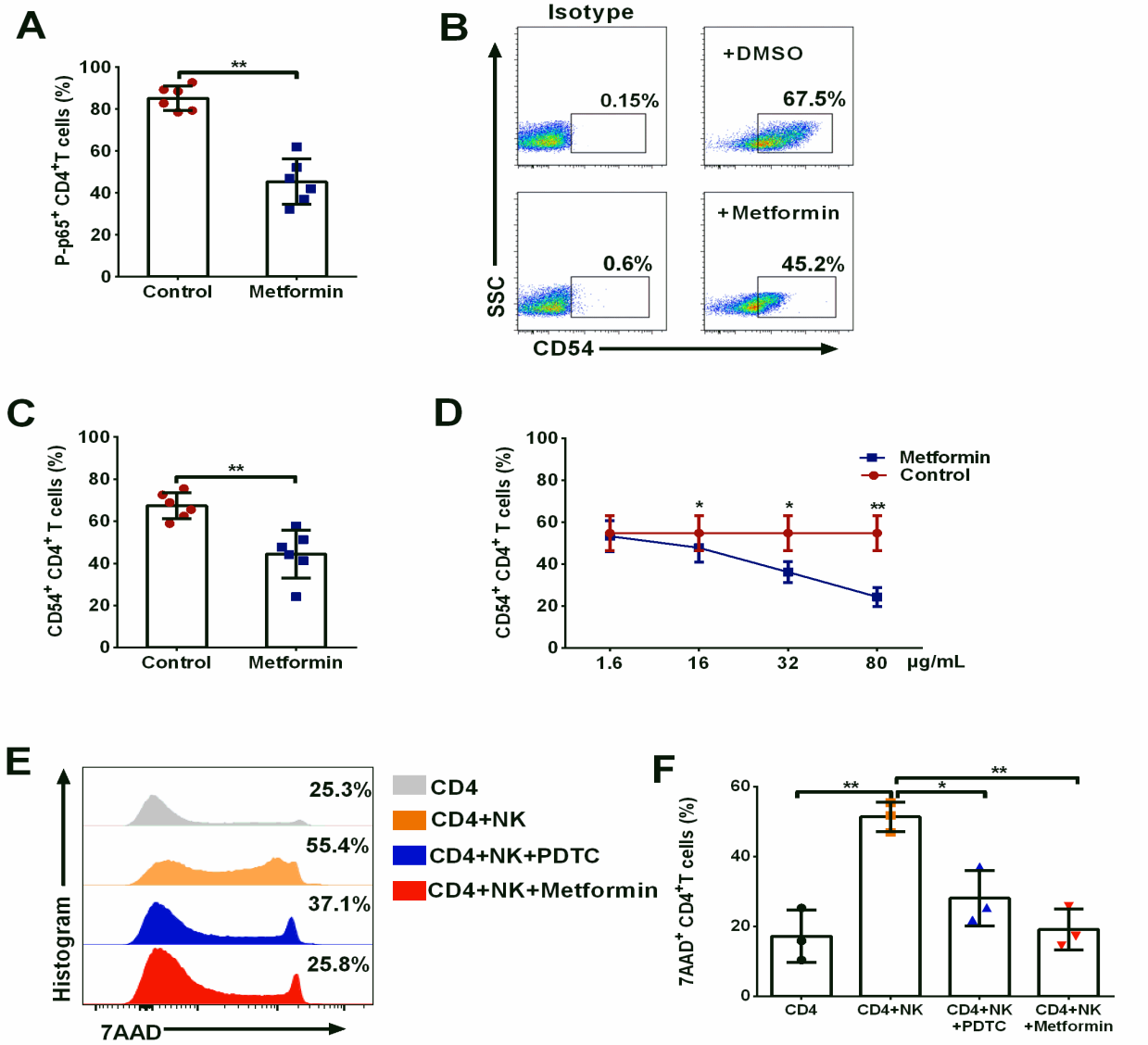
二甲双胍通过抑制NF-κB磷酸化来降低CD54表达



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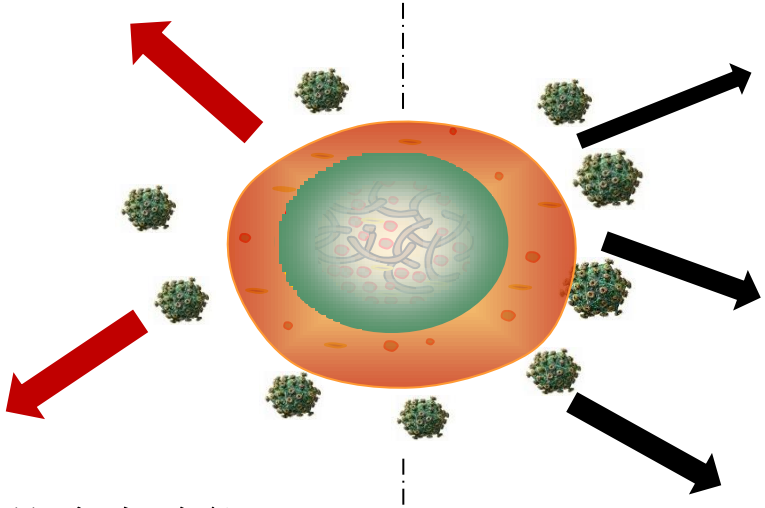
小 结

- 急性期即出现ADCC应答，
- 导致低病毒调定点，
- 确定了3个新的表面保守表位



$Tigit^+$ NK细胞增高
功能受损，

$CD56^-CD16^+$ NK cell增高
抑制$CD8^+$ T细胞反应

NK细胞杀伤
自体高表达CD54的 CD4+ T细胞

- $NKG2C^+$NK细胞抗病毒功能强
- $NKG2C^+NKG2A^-$Nk百分比
  NKG2C/NKG2A比值
  可预测疾病进展

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谢 谢!