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权利要求书3页 说明书50页
序列表170页 附图34页

(54)发明名称

细胞

(57)摘要

本发明提供了在细胞表面上共表达第一嵌合抗原受体(CAR)和第二CAR的细胞,每个CAR包含:(i)抗原结合域;(ii)间隔区;(iii)跨膜域;和(iv)内域,其中所述第一和第二CAR的抗原结合域结合不同抗原,且并且其中第一或第二CAR中的一个是包含活化性内域的活化性CAR,并且另一个CAR是包含连接关闭抑制性内域的抑制性CAR。

1.T细胞,其在细胞表面上共表达第一嵌合抗原受体(CAR)和第二CAR,每个CAR包含:

(i)抗原结合域;

(ii)间隔区;

(iii)跨膜域;和

(iv)内域;

其中所述第一和第二CAR的抗原结合域结合不同抗原,并且其中所述第一或第二CAR之一是包含激活性内域的激活性CAR,所述激活性内域含有基于免疫受体酪氨酸的活化基序(ITAM),并且另一个CAR是包含连接开启抑制性内域的抑制性CAR,所述抑制性内域包含来自含Src同源(SH2)域的蛋白质酪氨酸磷酸酶的酪氨酸磷酸酶域,其被磷酸化的基于免疫受体酪氨酸的抑制基序(ITIM)募集;并且其中在所述第一CAR和所述第二CAR连接后,所述第一CAR的间隔区和所述第二CAR的间隔区共定位。

2.根据权利要求1的T细胞,其中所述第一和第二CAR的间隔区是足够不同的,从而防止交叉配对。

3.根据权利要求2的T细胞,其中所述第一和第二CAR的间隔区是直系同源的。

4.根据权利要求1-3中任一项的T细胞,其中所述连接开启抑制性内域包含PTPN6的全部或部分。

5.根据权利要求1-3中任一项的T细胞,其中所述包含激活性内域的CAR包含结合CD33的抗原结合域,并且包含所述连接开启抑制性内域的CAR包含结合CD34的抗原结合域。

6.根据权利要求4的T细胞,其中所述包含激活性内域的CAR包含结合CD33的抗原结合域,并且包含所述连接开启抑制性内域的CAR包含结合CD34的抗原结合域。

7.核酸序列,其编码如权利要求1-6中任一项所定义的第一和第二嵌合抗原受体(CAR)。

8.根据权利要求7的核酸序列,其具有以下结构:

AgB1-间隔区1-TM1-endo1-coexpr-AgB2-间隔区2-TM2-endo2

其中,

AgB1是编码所述第一CAR的抗原结合域的核酸序列;

间隔区1是编码所述第一CAR的间隔区的核酸序列;

TM1是编码所述第一CAR的跨膜域的核酸序列;

endo 1是编码所述第一CAR的内域的核酸序列;

coexpr是使两个CAR能够共表达的核酸序列;

AgB2是编码所述第二CAR的抗原结合域的核酸序列;

间隔区2是编码所述第二CAR的间隔区的核酸序列;

TM2是编码所述第二CAR的跨膜域的核酸序列;

endo 2是编码所述第二CAR的内域的核酸序列;

其中当在T细胞中表达时,核酸序列编码多肽,所述多肽在切割位点处被切割,使得所述第一和第二CAR共表达在T细胞的表面上。

9.根据权利要求8的核酸序列,其中所述coexpr编码包含自我切割肽的序列。

10.根据权利要求8或9的核酸序列,其中在编码相同或相似的氨基酸序列的序列区中使用备选密码子,以避免同源重组。

11.试剂盒,其包含

(i)第一核酸序列,其编码权利要求1-6中任一项所定义的第一嵌合抗原受体(CAR),所述核酸序列具有以下结构:

AgB1-间隔区1-TM1-endo1

其中,

AgB1是编码所述第一CAR的抗原结合域的核酸序列;

间隔区1是编码所述第一CAR的间隔区的核酸序列;

TM1是编码所述第一CAR的跨膜域的核酸序列;

endo 1是编码所述第一CAR的内域的核酸序列;和

(ii)第二核酸序列,其编码权利要求1-6中任一项所定义的第二嵌合抗原受体(CAR),所述核酸序列具有以下结构:

AgB2-间隔区2-TM2-endo2

其中,

AgB2是编码所述第二CAR的抗原结合域的核酸序列;

间隔区2是编码所述第二CAR的间隔区的核酸序列;

TM2是编码所述第二CAR的跨膜域的核酸序列;

endo 2是编码所述第二CAR的内域的核酸序列。

12.试剂盒,其包含:第一载体,其包含如权利要求11定义的第一核酸序列;和第二载体,其包含如权利要求11定义的第一核酸序列。

13.根据权利要求12的试剂盒,其中所述载体是整合型病毒载体或转座子。

14.载体,其包含根据权利要求7-10中任一项所定义的核酸序列。

15.根据权利要求14的逆转录病毒载体或慢病毒载体或转座子。

16.制备根据权利要求1-6中任一项的T细胞的方法,其包括将根据权利要求7-10中任一项的核酸序列;如权利要求11中定义的第一核酸序列和第二核酸序列;和/或如权利要求12中定义的第一载体和第二载体或根据权利要求13-15中任一项的载体导入T细胞的步骤。

17.根据权利要求16的方法,其中所述T细胞来自从受试者分离的样品。

18.药物组合物,其包含根据权利要求1-6中任一项的多种T细胞。

19.根据权利要求18的药物组合物,其用于治疗疾病。

20.根据权利要求1-6中任一项的T细胞在制备用于治疗疾病的药物中的用途。

21.根据权利要求20的用途,其中治疗方法包括以下步骤:

(i)从受试者分离包含T细胞的样品;

(ii)用根据权利要求7-10中任一项的核酸序列;如权利要求11中定义的第一核酸序列和第二核酸序列;如权利要求12中定义的第一载体和第二载体或根据权利要求13-15中任一项的载体转导或转染所述T细胞;并

(iii)将来自(ii)的T细胞施用于所述受试者。

22.根据权利要求20或21的用途,其中所述疾病是癌症。

23.天然杀伤(NK)细胞,其在细胞表面上共表达第一嵌合抗原受体(CAR)和第二CAR,每个CAR包含:

(i)抗原结合域;

(ii)间隔区；

(iii)跨膜域；和

(iv)内域

其中所述第一和第二CAR的抗原结合域结合不同抗原，并且其中所述第一或第二CAR之一是包含激活性内域的激活性CAR，所述激活性内域含有基于免疫受体酪氨酸的活化基序(ITAM)，并且另一个CAR是包含连接开启抑制性内域的抑制性CAR，所述抑制性内域包含来自含Src同源(SH2)域的蛋白质酪氨酸磷酸酶的酪氨酸磷酸酶域，其被磷酸化的基于免疫受体酪氨酸的抑制基序(ITIM)募集；并且其中在所述第一CAR和所述第二CAR连接后，所述第一CAR的间隔区和所述第二CAR的间隔区共定位。

24.包含根据权利要求1的CAR表达性T细胞和/或根据权利要求23的CAR表达性NK细胞的细胞组合物，其通过用编码所述第一和第二CAR的核酸转导离体血液样品制备。

细胞

发明领域

[0001] 本发明涉及包含超过一种嵌合抗原受体(CAR)的细胞。由于靶细胞的两个或更多个抗原的表达(或非表达)的差异模式,所述细胞可以能够特异性识别靶细胞。

[0002] 发明背景

[0003] 已经描述了用于癌症治疗的许多免疫治疗剂,包括治疗性单克隆抗体(mAb),免疫缀合性mAb,放射缀合性mAb和双特异性T细胞结合剂(engager)。

[0004] 通常,这些免疫治疗剂靶向单一抗原:例如,利妥昔单抗(Rituximab)靶向CD20;Myelotarg靶向CD33;并且阿仑珠单抗(Alemtuzumab)靶向CD52。

[0005] 然而,单一抗原的存在(或缺乏)有效描述癌症是相对罕见的,这可以导致缺少特异性。靶向正常细胞上的抗原表达导致中靶脱瘤毒性(on-target off-tumour toxicity)。

[0006] 基于单一抗原不能将大多数癌症与正常组织区分。因此,发生相当大的“中靶脱瘤”毒性,由此正常组织被疗法损伤。例如,虽然用利妥昔单抗靶向CD20以治疗B细胞淋巴瘤,但消耗了整个正常B细胞区室,虽然靶向CD52以治疗慢性淋巴细胞性白血病,但消耗整个淋巴样区室,虽然靶向CD33以治疗急性髓样白血病,但损伤了整个髓样区室等。

[0007] 预测的“中靶脱瘤”毒性的问题已经通过临床试验证实。例如,靶向ERBB2的方法引起具有对肺和肝转移性的结肠癌的患者的死亡。ERBB2在某些患者的结肠癌中过表达,但它也在几种正常组织,包括心脏和正常血管系统上表达。

[0008] 在一些癌症中,肿瘤是通过一种抗原(通常是组织特异性抗原)的存在和存在于正常细胞上的另一种抗原的缺乏来最佳限定。例如,急性髓样白血病(AML)细胞表达CD33。正常干细胞表达CD33,但也表达CD34,而AML细胞通常是CD34阴性的。仅靶向CD33以治疗AML与显著毒性相关,因为它消耗正常干细胞。然而,特异性靶向CD33阳性,但非CD34阳性的细胞会避免这种相当大的中靶脱瘤毒性。

[0009] 因此,需要能够更为靶向以反映与许多癌症相关的标志物表达的复杂模式的免疫治疗剂。

[0010] 嵌合抗原受体(CAR)

[0011] 嵌合抗原受体是将单克隆抗体(mAb)的特异性嫁接到T细胞的效应子功能的蛋白质。它们通常的形式是I型跨膜域蛋白的形式,具有全部连接到复合内域(endodomain)的抗原识别氨基端,间隔区(spacer),跨膜域,所述内域传送T细胞存活和活化信号(参见图1A)。

[0012] 这些分子的最常见形式是来源于识别靶抗原的单克隆抗体的单链可变片段(scFv)的融合体,所述单链可变片段通过间隔区和跨膜域融合到信号传导内域。这类分子导致响应scFv对其靶标的识别的T细胞活化。当T细胞表达此种CAR时,它们识别并杀死表达靶抗原的靶细胞。已经开发了针对肿瘤相关抗原的几种CAR,并且使用这样的CAR表达T细胞进行的过继转移方法目前在临床试验中用于各种癌症的治疗中。

[0013] 然而,CAR表达性T细胞的使用也与中靶脱瘤毒性相关。例如,靶向羧基脱水酶-IX(carboxy anyhydrase-IX)(CAIX)以治疗肾细胞癌的基于CAR的方法导致肝毒性,其被认为是由于特异性攻击胆管上皮细胞引起(Lamers et al(2013)Mol.Ther.21:904-912)。

[0014] 因此,需要具有更大的选择性和降低的中靶脱瘤毒性的备选的基于CAR T细胞的方法。

[0015] 附图简述

[0016] 图1:(a)CAR的一般化结构:结合域识别抗原;间隔区从细胞表面抬高结合域;跨膜域将蛋白质锚定到膜上,并且内域传递信号。(b)至(d):CAR内域的不同代和排列:(b)起始设计经由FcεR1-γ或CD3ζ内域传递ITAM信号,而后来的设计顺式传递额外的(c)一个或(d)两个共刺激信号。

[0017] 图2:显示本发明的示意图

[0018] 本发明涉及工程化T细胞以响应靶细胞抗原表达的逻辑规则。这用设想的FACS散点图最好地例示。靶细胞群表达抗原“A”和“B”中的两种或者一种,或均不表达这两种抗原。不同的靶标群(红色标记)通过用不同门(gate)连接的一对CAR转导的T细胞杀死。用或(OR)门控的受体,将会杀死单阳性和双重阳性细胞两者。用和(AND)门控受体,仅杀死双重阳性靶细胞。用和非(和非)门孔,在单阳性靶标的情况下,双重阳性靶标得到保持。

[0019] 图3:靶细胞群的产生

[0020] SupT1细胞被用作靶细胞。转导这些细胞以表达CD19,CD33或CD19和CD33两者。用适宜的抗体染色靶细胞,并通过流式细胞术分析。

[0021] 图4:或门的盒设计

[0022] 单个可读框给这两个CAR提供具有符合读码框的FMD-2A序列,导致两种蛋白质。信号1是来源于IgG1的信号肽(但可以是任何有效的信号肽)。scFv1是识别CD19的单链可变区段(但可以是scFv或肽环或配体或实际上是识别任何期望的任意靶标的任何域)。STK是CD8茎,但可以是任何合适的胞外域。CD28tm是CD28跨膜域,但可以是任何合适的I型蛋白跨膜域,并且CD3Z是CD3Zeta内域,但可以是包含ITAM的任何内域。信号2是来源于CD8的信号肽,但可以是在DNA序列上与信号1不同的任何有效的信号肽。scFv识别CD33,但就scFv1而言是任意的。HC2CH3是人IgG1的铰链-CH2-CH3,但可以是不与第一CAR中使用的隔离区交叉配对的任何胞外域。CD28tm'和CD3Z'与CD28tm和CD3Z编码相同的蛋白质序列但密码子摆动,以防止同源重组。

[0023] 图5:用于或门的嵌合抗原受体(CAR)的示意图

[0024] 构建刺激性CAR,其由下列各项组成:N端A)抗CD19scFv域,紧接着是人CD8的胞外铰链区,或B)抗CD33的scFv域,紧接着是人IgG1的胞外铰链区,CH2和CH3区(含有pvaa突变以降低FcR结合)。这两种受体包含人CD28跨膜域和人CD3Zeta(CD247)内域。“S”描述了二硫键的存在。

[0025] 图6:显示两种CAR在一种T细胞表面上共表达的表达数据。

[0026] 图7:或门的功能分析

[0027] 表达或门构建体的效应细胞(5×10^4细胞)与可变数量的靶细胞共温育,并且在16小时后通过ELISA分析IL-2。图显示来自单独的效应细胞的化学刺激(PMA和离子霉素(lonomycin))的平均最大IL-2分泌和来自三个重复的无任何刺激的效应细胞的平均背景IL-2。

[0028] 图8:显示用于表达两种和门的盒的两种形式的草图

[0029] 再一次使用FMD-2A序列共表达活化性和抑制性CAR。信号1是来源于IgG1的信号肽

(但可以是任何有效的信号肽)。scFv1是识别CD19的单链可变区段(但可以是scFv或肽环或配体或实际上是识别任何期望的任意靶标的任何域)。STK是CD8茎,但可以是任何不庞大(non-bulky)的胞外域。CD28tm是CD28跨膜域,但可以是任何稳定的I型蛋白跨膜域,并且CD3Z是CD3Zeta内域,但可以是包含ITAM的任何内域。信号2是来源于CD8的信号肽,但可以是DNA序列上与信号1不同的任何有效的信号肽。scFv识别CD33,但就scFv1而言是任意的。HC2CH3是人IgG1的铰链-CH2-CH3,但可以是任意庞大的胞外域。CD45和CD148分别是CD45和CD148的跨膜和内域,但可以来源于任何此类蛋白质。

[0030] 图9:针对和门的嵌合抗原受体(CAR)的蛋白质结构的示意图。

[0031] 刺激性CAR由N端抗CD19的scFv域,紧接着是人CD8的胞外茎区,人CD28的跨膜域和人CD3Zeta(CD247)胞内域组成。测试了两种抑制性CAR。这些由以下组成:N端抗CD33的scFv域,紧接着是人IgG1的胞外铰链区,CH2和CH3区(含有pvaa突变以降低FcR结合),紧接着是人CD148或CD45的跨膜域和胞内域。“S”描述了二硫键的存在。

[0032] 图10:活化和抑制性CAR的共表达

[0033] BW5147细胞被用作效应细胞,并被转导以表达活化抗CD19CAR和抑制性抗CD33CAR之一两者。用CD19-小鼠-Fc和CD33-兔-Fc与合适的二抗染色效应细胞,并通过流式细胞术分析。

[0034] 图11:和门的功能分析

[0035] 表达活化抗CD19CAR和具有A)CD148或B)CXD45胞内域的抑制性抗CD33CAR的效应细胞(5×10^4细胞)与可变数量的靶细胞共温育,并且在16小时后通过ELISA分析IL-2。图显示来自单独的效应细胞的化学刺激(PMA和离子霉素)的最大IL-2分泌和来自三个重复的无任何刺激的效应细胞的背景IL-2。

[0036] 图12:显示用于产生和非门的三种形式的盒的草图

[0037] 再一次使用FMD-2A序列共表达活化性和抑制性CAR。信号1是来源于IgG1的信号肽(但可以是任何有效的信号肽)。scFv1是识别CD19的单链可变区段(但可以是scFv或肽环或配体或实际上是识别任何期望的任意靶标的任何域)。STK是人CD8茎,但可以是任何不庞大的胞外域。CD28tm是CD28跨膜域,但可以是任何稳定的I型蛋白跨膜域,并且CD3Z是CD3Zeta内域,但可以是包含ITAM的任何内域。信号2是来源于CD8的信号肽,但可以是DNA序列上与信号1不同的任何有效的信号肽。scFv识别CD33,但就scFv1而言是任意的。muSTK是小鼠CD8茎,但也可以是与活化性CAR的间隔物共定位但不交叉配对的任何间隔区。dPTPN6是PTPN6的磷酸酶域。LAIR1是LAIR1的跨膜域和内域。2Aw是FMD-2A序列的密码子摆动的形式。SH2-CD148是与CD148的磷酸酶域融合的PTPN6的SH2域。

[0038] 图13:针对非和(NOT AND)门的嵌合抗原受体(CAR)的示意图。

[0039] A)刺激性CAR由N端抗CD19的scFv域,紧接着是人CD8的茎区,人CD28的跨膜域和人CD247的胞内域组成。B)抑制性CAR由N端抗CD33的scFv域,紧接着是小鼠CD8的茎区,小鼠CD8的跨膜区和PTPN6的磷酸酶域组成。C)抑制性CAR由N端抗CD33的scFv域,紧接着是小鼠CD8的茎区,和LAIR1的跨膜和胞内区段组成。D)抑制性CAR与前面的CAR相同,只是它与PTPN6 SH2域和CD148磷酸酶域的融合蛋白共表达。

[0040] 图14:非和门的功能分析

[0041] 表达A)全长SHP-1或B)SHP-1的截短形式的效应细胞(5×10^4细胞)与可变数量的

靶细胞共温育，并且在16小时后通过ELISA分析IL-2。图显示来自单独的效应细胞的化学刺激(PMA和离子霉素)的平均最大的IL-2分泌和来自三个重复的无任何刺激的效应细胞的平均背景IL-2。

[0042] 图15:或门的氨基酸序列

[0043] 图16:基于CD148和CD145的和门的氨基酸序列

[0044] 图17:两种和非(AND NOT)门的氨基酸序列

[0045] 图18:和门功能的剖析

[0046] A.和门原型显示于右侧，并且其响应于CD19，CD33单阳性靶标和CD19，CD33双重阳性靶标的函数显示于左侧。B.交换scFv，以便活化性内域被CD33触发，并且抑制性内域通过CD19活化。尽管此scFv交换，这种和门仍保持功能性。C.在抑制性CAR的间隔区中，CD8小鼠茎替换了Fc。通过此种修饰，门不能响应CD19单阳性或CD19，CD33双重阳性靶标。

[0047] 图19:靶抗原在人工靶细胞上的表达

[0048] A.显示原始组的来源于SupT1细胞的人工靶细胞的CD19相对于CD33的流式细胞术散点图。从左至右:双阴性SupT1细胞，CD19阳性的SupT1细胞，CD33阳性的SupT1细胞，以及CD19和CD33阳性的SupT1细胞。B.显示产生以测试CD19和GD2门的人工靶细胞的CD19相比于GD2的流式细胞术散点图，从左至右依次为:阴性SupT1细胞，表达CD19的SupT1细胞，用GD2和GM3合酶载体转导从而成为GD2阳性的SupT1细胞，用CD19以及GD2和GM3合酶转导从而成为GD2和CD19阳性的SupT1细胞。C.显示产生以测试CD19和EGFRvIII门的人工靶标的CD19相比于EGFRvIII的流式细胞术散点图。从左至右为:阴性SupT1细胞，表达CD19的SupT1细胞，用EGFRvIII转导的SupT1细胞，和用CD19以及EGFRvIII转导的SupT1细胞。D.显示产生以测试CD19和CD5门的人工靶标的CD19相比于CD5的流式细胞术散点图。从左至右为:阴性293T细胞，用CD19转导的293T细胞，用CD5转导的293T细胞，和用CD5以及CD19载体转导的293T细胞。

[0049] 图20:和门的一般化(generalizability)

[0050] A.和门的草图，其修改为使得第二CAR的特异性从CD33的原始特异性改变以产生3个新的CAR:CD19和GD2，CD19和EGFRvIII，CD19和CD5。B.CD19和GD2AND门:左:显示和门的表达，CD19CAR的重组CD19-Fc染色(x轴)对GD2CAR的抗人Fc染色(Y轴)。右:响应于单阳性和双重阳性靶标的函数。C.CD19和EGFRvIII和门:左:显示了和门的表达，CD19CAR的重组CD19-Fc的染色(x轴)，对针对EGFRvIII CAR的抗人Fc染色(Y轴)。右:响应于单阳性和双重阳性靶标的函数。D.CD19和CD5和门:左:显示了和门的表达，针对CD19CAR的重组CD19-Fc的染色(x轴)对针对CD5CAR的抗人Fc染色(Y轴)。右:响应于单阳性和双重阳性靶标的函数。

[0051] 图21:和非门的功能

[0052] 显示了和非门的三种实施方式的功能。经测试的门的草图显示于右侧，并且响应于单阳性和双重阳性靶标的函数显示于左侧。A.基于PTPN6的和非门，其中第一CAR识别CD19，具有人CD8茎间隔区和包含ITAM的活化性内域；与第二CAR共表达，所述第二CAR识别CD33，具有小鼠CD8茎间隔区并具有由PTPN6磷酸酶域构成的内域。B.基于ITIM的和非门与PTPN6门是相同的，只是内域被来自LAIR1的内域取代。C.CD148加强的和非门与基于ITIM的门是相同的，只是表达PTPN6SH2和CD148的内域之间的额外的融合物。所有这三种门如预期地工作，响应CD19而活化，但不响应一起的CD19和CD33。

[0053] 图22:基于PTPN6的和非门功能的剖析

[0054] 将原始的基于PTPN6的和非门与几种对照比较来证明该模型。经测试的门的图显示于右侧,响应于单阳性和双重阳性靶标的函数显示于左侧。A.原始的和非门,其中第一CAR识别CD19,具有人CD8茎间隔区和包含ITAM的活化性内域;与第二CAR的共表达,所述第二CAR识别CD33,具有小鼠CD8茎间隔区并具有由PTPN6磷酸酶域构成的内域。B.和非门,其修饰为使得小鼠CD8茎间隔区被替换为Fc间隔区。C.和非门,其修饰为使得PTPN6磷酸酶域被替换为CD148内域。原始的和非门(A.),功能如预期那样,响应CD19而触发,但不响应CD19和CD33两者。B中的门响应单独的CD19或一起的CD19和CD33而触发。C中的门控不响应一种或两种靶标而触发。

[0055] 图23:基于LAIR1的和非门的剖析

[0056] 显示了针对CD19阳性,CD33阳性和CD19,CD33双重阳性靶标的功能活性。A.原始的基于ITIM的和非门的结构和活性。此门由两个CAR组成:第一CAR识别CD19,具有人CD8茎间隔区和含有ITAM的内域;第二CAR识别CD33,具有小鼠CD8茎间隔区和含有ITAM的内域。B基于ITIM的对照门的结构和活性,其中小鼠CD8茎间隔区已经替换成Fc域。此门由两个CAR组成:第一个识别CD19,具有人CD8茎间隔区和含有ITAM的内域;第二CAR识别CD33,具有Fc间隔区和含有ITAM的内域。这两种门响应CD19单阳性靶标,而仅原始门在对CD19和CD33双重阳性靶标的响应中是无活性的。

[0057] 图24:CAR逻辑门的动力学分离模型

[0058] 和门、非和门和对照的动力学分离和行为模型。CAR识别CD19或CD33。可以设想免疫突触在代表靶细胞膜的蓝线和代表T细胞膜的红线之间。'45'是存在于T细胞上的天然CD45蛋白质。'H8'是具有人CD8茎作为间隔区的CAR胞外域。'Fc'是具有人HCH2CH3作为间隔区的CAR胞外域。'M8'是具有鼠CD8茎作为间隔区的CAR胞外域。'19'表示靶细胞表面上的CD19。'33'表示在靶细胞表面上的CD33。符号'⊕'表示包含ITAMS的活化性内域。符号'⊖'代表具有慢动力学的磷酸酶-'连接开启'内域,诸如由PTPN6的催化域或ITIM构成的内域。符号'∅'表示具有快速动力学的磷酸酶-'连接关闭'内域,如CD45或CD148的内域。在图中这个符号被放大以强调其强大的活性。

[0059] (a)显示推测的功能性和门的行为,所述和门由一对CAR构成,其中第一CAR识别CD19,具有人CD8茎间隔区和活化性内域;并且第二CAR识别CD33,具有Fc间隔区和CD148内域。

[0060] (b)显示推测的对照和门的行为。此处,第一CAR识别CD19,具有人CD8茎间隔区和活化性内域;并且第二CAR识别CD33,但具有小鼠CD8茎间隔区和CD148内域。

[0061] (c)显示功能性和非门的行为,所述和非门由一对CAR构成,其中第一CAR识别CD19,具有人CD8茎间隔区和活化性内域;并且第二CAR识别CD33,具有小鼠CD8茎间隔区和PTPN6内域。

[0062] (d)显示推测的对照和非门的行为,所述对照和非门由一对CAR构成,其中第一CAR识别CD19,具有人CD8茎间隔区和活化性内域;并且第二CAR识别CD33,但具有Fc间隔区和PTPN6内域。

[0063] 在第一列中,靶细胞是CD19和CD33阴性的。在第二列中,靶标是CD19阴性和CD33阳

性的。在第三列中，靶细胞是CD19阳性和CD33阴性的。在第四列中，靶细胞是CD19和CD33阳性的。

[0064] 图25：基于APRIL的CAR的设计。

[0065] 修饰CAR设计，使得scFv被替换为增殖诱导配体(APRIL)的修饰形式，其与BCMA，TACI和蛋白聚糖相互作用以起抗原结合域作用：APRIL是截短的以使蛋白聚糖结合的氨基端不存在。然后，将信号肽连接至截短的APRIL氨基端，以将蛋白质导向到细胞表面。产生了三种具有这种基于APRIL的结合域的CAR：A.在第一CAR中，人类CD8茎域用作间隔域。B.在第二CAR中，来自IgG1的铰链用作间隔域。C.在第三CAR中，使用修饰为具有由Hombach et al (2010Gene Ther.17:1206-1213)描述的pva/a突变以降低Fc受体结合的人IgG1的铰链，CH2和CH3域作为间隔区(因此称作Fc-pvaa)。在全部CAR中，这些间隔区连接至CD28跨膜域，然后连接至含有CD28，OX40和CD3-Zeta内域的融合物的三部分内域(Pule et al，Molecular therapy，2005：Volume 12；Issue 5；Pages 933-41)。

[0066] 图26：注释的上述三种APRIL-CAR的氨基酸序列

[0067] A：显示注释的CD8茎APRIL CAR的氨基酸序列；B：显示注释的基于APRIL IgG1铰链的CAR的氨基酸序列；C：显示注释的基于APRIL Fc-pvaa的CAR的氨基酸序列。

[0068] 图27：基于APRIL的不同CAR的表达和配体结合

[0069] A.在逆转录病毒基因载体中受体与标记基因截短的CD34共表达。标记基因在经转导的细胞上的表达允许转导的确认。B.用具有CD8茎间隔区，IgG1的铰链或Fc间隔区的基于APRIL的CAR转导T细胞。为测试这些受体是否可以在细胞表面上稳定表达，然后用抗APRIL-生物素/链霉亲合素APC和抗CD34染色T细胞。进行流式细胞分析。在细胞表面上在三种CAR中同等地检测到APRIL，提示它们是同等稳定地表达。C.接着，测定了CAR识别TACI和BCMA的能力。用融合至小鼠IgG2a Fc融合物的重组BCMA或TACI以及抗小鼠二抗和抗CD34染色经转导的T细胞。所有三种受体形式显示结合BCMA和TACI两者。惊奇的发现是与BCMA的结合看起来强于与TACI的结合。另一个惊奇的发现是，虽然这三种CAR都同等地表达，CD8茎和IgG1铰链CAR显示比具有Fc间隔区的CAR更好地识别BCMA和TACI。

[0070] 图28：不同CAR构建体的功能

[0071] 用三种不同的基于APRIL的CAR进行了功能测定。正常供体外周血T细胞未转导(NT)，或经转导以表达不同的CAR。使用相同滴度的上清液进行转导。然后消耗这些T细胞的CD56以除去非特异性NK活性并用作效应物。将未转导的SupT1细胞(NT)或者经转导以表达BCMA或TACI的SupT1细胞用作靶标。显示的数据是来自5个独立实验的平均值和标准偏差。A.使用铬释放测定BCMA和TACI表达T细胞的特异性杀伤。B.也测定了干扰素μ的释放。以1:1的比例共培养靶标和效应物。24小时后，通过ELISA测定在上清液中的干扰素μ。CAR T细胞的增殖/存活也通过计数再温育6天的相同共培养物中的CAR的T细胞的数量确定。全部3种CAR直接响应表达BCMA和TACI的靶标。对BCMA的响应比对TACI的响应强。

[0072] 图29：和门在原代细胞中的功能性

[0073] 从血液中分离PBMC，并使用PHA和IL-2刺激。两天后，在Retronectin包被的板上用含有CD19:CD33和门构建体的逆转录病毒转导细胞。在第5天，通过流式细胞术评估了由和门构建体翻译的两种CAR的表达水平并且对细胞消耗了CD56+细胞(主要是NK细胞)。在第6天，以1:2的效应物比靶细胞的比例使PBMC与靶细胞共培养。在第8天收集上清液并通过

ELISA分析IFN-γ的分泌。

[0074] 图30：在和非门中的IgM和IgG

[0075] 为了测试和非门是否可以在延长的间隔区长度上发挥功能，将激活性CAR(抗CD19)和抑制性CAR(抗CD33)间隔区取代为较长间隔区。将人IgM和IgG的Fc区用于延长间隔区的长度。IgM的Fc含有相比于IgG的额外的Ig域，由于此原因将IgM的间隔区放置在已知具有膜近端结合表位的抗CD19 CAR上。相反，抗CD33结合表位位于该分子的远端末端，因此在此CAR上使用在相对较短的IgG间隔区。将延长间隔区的和非门构建体转导到小鼠的T细胞系中。然后将固定数量的经转导的T细胞与可变数量的靶细胞共培养16-24小时，在此之后通过ELISA测定在上清液中分泌的IL-2的量。

[0076] 图31：抗CD19/抗GD2和非门

[0077] 为了测试和非门平台的稳健性(robustness)，用两个其他的不相关的结合剂(抗GD2和抗EGFRvIII)取代来自抑制性CAR(抗CD33)的结合域。在具有截短SHP-1或LAIR胞质域的和非门平台中，将抗GD2或抗EGFRvIII的scFv片段取代为抗CD33。将这些构建体转导到小鼠T细胞系中，并且将固定数量的T细胞与可变数量的靶细胞共培养。在共培养16-24小时后，通过ELISA分析在上清液中分泌的IL-2的量。A)抗CD19/抗GD2和非门，B)抗CD19/抗EGFRvIII和非门。

[0078] 图32：用于构建逻辑门控CAR T细胞的设计规则。

[0079] 用草图形式显示或、和非及和门控的CAR，其中靶细胞在顶端，T细胞在底部，突触在中间。靶细胞表达任意的靶抗原A和B。

[0080] T细胞表达两种CAR，其由抗A和抗B识别域，间隔区和内域构成。或门要求(1)仅间隔区，其允许抗原识别和CAR活化，和(2)两种CAR以具有活化性内域；和非门需要(1)间隔区，其在识别两种抗原后导致两种CAR的共分离和(2)具有活化性内域的一种CAR，和其内域包含或募集弱磷酸酶的另一种CAR；和门需要(1)间隔区，其导致在识别两种抗原后两种CAR分离成免疫突触的不同部分，和(2)具有活化性内域的一种CAR，和其内域包含强的磷酸酶的另一种CAR。

[0081] 发明概述

[0082] 本发明人已经开发了一组“逻辑门控”嵌合抗原受体对，它们当通过细胞(如T细胞)表达时能够检测至少两个靶抗原表达的特定模式。如果至少两个靶抗原任意地表示为抗原A和抗原B，三个可能的选项如下：

[0083] “或门(OR GATE)”：当抗原A或抗原B存在于靶细胞上时T细胞触发

[0084] “和门(AND GATE)”：只有当两种抗原A和B都存在于靶细胞上时，T细胞触发

[0085] “和非门”：如果仅抗原A存在于靶细胞上，T细胞触发，但如果这两种抗原A和B都存在于靶细胞上，T细胞不会触发

[0086] 可以基于两个或更多个标记物的其特定的表达(或表达缺乏)，调整表达这些CAR组合的工程化改造的T细胞，以对癌细胞敏锐特异性的。

[0087] 因此，在第一方面，本发明提供细胞，其在细胞表面上共表达第一嵌合抗原受体(CAR)和第二CAR，每一CAR包含：

[0088] (i)抗原结合域；

[0089] (ii)间隔区；

[0090] (iii)跨膜域;和

[0091] (iv)胞内T细胞信号传导域(内域)

[0092] 其中第一和第二CAR的抗原结合域结合不同抗原,其中第一或第二CAR中的一个是包含活化性细胞内T细胞信号传导域的活化性CAR,并且另一个CAR是包含“连接开启”(如本文定义)抑制性细胞内T细胞信号传导域的抑制性CAR。

[0093] 细胞可以是免疫效应细胞,如T细胞或天然杀伤(NK)细胞。本文提及的与T细胞相关的特征同样适用于其它免疫效应细胞,如NK细胞。

[0094] 第一CAR的间隔区可以与第二CAR的间隔区不同。

[0095] 第一和第二CAR的间隔区可以足够不同以防止交叉配对,但是必须足够相似以导致CAR在T细胞膜上共定位。

[0096] 在第一和第二CAR的间隔区可以是直系同源的,如小鼠和人CD8茎。

[0097] 在涉及“和非”门的本发明中,第一CAR或第二CAR中的一个是包含活化性内域的活化性CAR,而另一个CAR是包含“连接开启”抑制性内域的抑制性CAR。在不存在抑制CAR连接的情况中,抑制性CAR不显著抑制通过激活性CAR的T-细胞活化,但是当连接抑制性CAR时,抑制通过激活性CAR的T-细胞活化。在这些实施方案中,第一和第二CAR间隔区是足够地不同以防止第一和第二CAR的交叉配对,但是足够相似以导致连接后的第一和第二CAR的共定位。

[0098] 抑制性内域可包含蛋白质-酪氨酸磷酸酶的至少一部分。

[0099] 抑制性内域可以包含PTPN6的全部或一部分。

[0100] 抑制性内域可以包含ITIM域。

[0101] 抑制性内域可以包含与融合物共表达相结合的ITIM域,所述融合物在蛋白质-酪氨酸磷酸酶的至少一部分和受体样酪氨酸磷酸酶的至少一部分之间。该融合物可以包含来自蛋白质-酪氨酸磷酸酶的一个或多个SH2域。例如,融合可以在PTPN6SH2域和CD45内域之间或PTPN6SH2域和CD148内域之间进行。

[0102] 如在导言中解释的,急性髓样白血病(AML)细胞表达CD33。正常干细胞表达CD33,但也表达CD34,而AML细胞通常是CD34阴性的。仅靶向CD33以治疗AML与显著毒性相关,因为它消耗正常干细胞。然而,特异性靶向CD33阳性,但非CD34阳性的细胞会避免这种相当大的脱靶毒性。因此,在本发明中,包含激活性内域的CAR可以包含结合CD33的抗原结合域,并且包含连接开启抑制性内域的CAR可以包含结合CD34的抗原结合域。

[0103] 在第二方面,本发明提供核酸序列,其编码在本发明的第一方面所定义的第一和第二嵌合抗原受体(CAR)两者。

[0104] 相应的核酸序列可以具有以下结构:AgB1-间隔区1-TM1-endo1-coexpr-AgB2-间隔区2-TM2-endo2

[0105] 其中,

[0106] AgB1是编码第一CAR的抗原结合域的核酸序列;

[0107] 间隔区1是编码第一CAR的间隔区的核酸序列;

[0108] TM1是编码第一CAR的跨膜域的核酸序列;

[0109] endo 1是编码第一CAR的内域的核酸序列;

[0110] coexpr是允许两个CAR共表达的核酸序列(例如,切割位点);

[0111] AgB2是编码第二CAR的抗原结合域的核酸序列；

[0112] 间隔区2是编码第二CAR的间隔区的核酸序列；

[0113] TM2是编码第二CAR的跨膜域的核酸序列；

[0114] endo 2是编码第二CAR的内域的核酸序列；

[0115] 其中当在T细胞中表达时，所述核酸序列编码的多肽在切割位点被切割，使得第一和第二CAR共表达在T细胞的表面上。

[0116] 允许两个CAR共表达的核酸序列可编码自我切割肽或序列(其是允许共表达两个CAR的备选手段)例如内部核糖体进入序列或2nd启动子或其他这样的手段，由此本领域技术人员可以从同一载体表达两种蛋白质。

[0117] 备选的密码子可以用于编码相同或相似的氨基酸序列的序列区，以避免同源重组。

[0118] 在第三方面，本发明提供试剂盒，其包含

[0119] (i)第一核酸序列，其编码在本发明的第一方面所定义的第一嵌合抗原受体(CAR)，所述核酸序列具有以下结构：

[0120] AgB1-间隔区1-TM1-endo1

[0121] 其中，

[0122] AgB1是编码第一CAR的抗原结合域的核酸序列；

[0123] 间隔区1是编码第一CAR的间隔区的核酸序列；

[0124] TM1是编码第一CAR的跨膜域的核酸序列；

[0125] endo 1是编码第一CAR的内域的核酸序列；和

[0126] (ii)第二核酸序列，其编码在本发明的第一方面所定义的第二嵌合抗原受体(CAR)，所述核酸序列具有以下结构：

[0127] AgB2-间隔区2-TM2-endo2

[0128] 其中，

[0129] AgB2是编码第二CAR的抗原结合域的核酸序列；

[0130] 间隔区2是编码第二CAR的间隔区的核酸序列；

[0131] TM2是编码第二CAR的跨膜域的核酸序列；

[0132] endo 2是编码第二CAR的内域的核酸序列。

[0133] 在第四方面，本发明提供试剂盒，其包含：第一载体，其包含如上定义的第一核酸序列；和第二载体，其包含如上定义的第一核酸序列。

[0134] 载体可以是质粒载体，逆转录病毒载体或转座子载体。载体可以是慢病毒载体。

[0135] 在第五方面，本发明提供载体，其包含根据本发明的第二个方面的核酸序列。载体可以是慢病毒载体。

[0136] 载体可以是质粒载体，逆转录病毒载体或转座子载体。

[0137] 在第六个方面，本发明涉及以下述方式共表达超过两个(more than two)CAR，使得在靶细胞上可以识别超过两种抗原的复杂模式。

[0138] 在第七方面，本发明提供了制备根据本发明的第一方面的T细胞的方法，其包括将编码第一和第二CAR的一个或多个核酸序列或如上定义的一种或多种载体导入T细胞的步骤。

[0139] T细胞可以来自从患者，相关的或不相关的造血移植供体，完全不相关的供体，从脐带血分离的样品，或从胚胎细胞系分化，从诱导的祖细胞系分化，或来源于经转化的T细胞系。

[0140] 在第八方面，本发明提供了包含根据本发明的第一方面的多个T细胞的药物组合物。

[0141] 在第九方面，本发明提供了用于治疗和/或预防疾病的方法，其包括将根据本发明的第八方面的药物组合物施用于受试者的步骤。

[0142] 该方法可以包括以下步骤：

[0143] (i)分离如上列出的T细胞。

[0144] (ii)用编码第一和第二CAR的一种或多种核酸序列或包含此种核酸序列的一种或多种载体转导或转染所述T细胞；和

[0145] (iii)将来自(ii)的T细胞施用于受试者。

[0146] 疾病可以是癌症。

[0147] 在第十个方面，本发明提供根据本发明的第八方面的药物组合物，其用于治疗和/或预防疾病。

[0148] 疾病可以是癌症。

[0149] 在第十一方面，本发明提供根据本发明第一方面的T细胞的用途，用于制备用于治疗和/或预防疾病的药物。

[0150] 疾病可以是癌症。

[0151] 本发明还提供了核酸序列，其包含：

[0152] a)编码第一嵌合抗原受体(CAR)的第一核苷酸序列；

[0153] b)编码第二CAR的第二核苷酸序列；

[0154] c)编码位于第一和第二核苷酸序列之间的自我切割肽的序列，从而使得两个CAR被表达为单独的实体。

[0155] 备选密码子可以用于编码相同或相似氨基酸序列的区域中的第一和第二核苷酸序列中的一个或多个部分。

[0156] 本发明还提供此类核酸的载体和细胞。

[0157] 本发明的“和非门”提供了相比于至今描述的涉及靶向单一肿瘤相关抗原的CAR的显著的优势。这里，当肿瘤细胞的特征在于存在一个(或多个)抗原和不存在另一个抗原时，这可以使用本发明的基于CAR的和非方法可以特异性靶定。将不会靶定表达这两种抗原的正常细胞，导致更大的选择性和降低的中靶脱瘤毒性。在这种情况下，针对单一抗原的CAR方法将同时靶向肿瘤细胞和正常细胞。

[0158] 本发明的其它方面

[0159] 本发明还涉及在下述编号的段落中列出的方面：

[0160] 1.T细胞，其在细胞表面上共表达第一嵌合抗原受体(CAR)和第二CAR，每一CAR包含：

[0161] (i)抗原结合域；

[0162] (ii)间隔区

[0163] (iii)跨膜域；和

[0164] (iv)内域

[0165] 其中所述第一和第二CAR的抗原结合域结合不同抗原，其中所述第一CAR的间隔区不同于所述第二CAR的间隔区，并且其中第一或第二CAR中的一个是包含活化内域的活化性CAR，并且另一个CAR是包含活化内域的活化性CAR或包含连接开启或连接关闭的抑制性内域的抑制性CAR。

[0166] 2.根据段1的T细胞，其中第一CAR的间隔区具有与第二CAR的间隔区不同的长度和/或电荷和/或大小和/或构造和/或糖基化，使得当第一CAR和第二CAR结合它们各自的靶抗原时，第一CAR和第二CAR在T细胞膜上是空间上分离的。

[0167] 3.根据段2的T细胞，其中第一间隔区或第二间隔区包含CD8茎，并且另一个间隔区包含IgG1的铰链，CH2和CH3域。

[0168] 4.根据段1的T细胞，其中第一CAR和第二CAR是活化性CAR。

[0169] 5.根据段4的T细胞，其中一种CAR结合CD19，并且另一种CAR结合CD20。

[0170] 6.根据段2或3的T细胞，其中第一CAR或第二CAR中的一个是包含活化内域的活化性CAR，和另一个CAR是包含连接关闭的抑制性内域的抑制性CAR，在不存在抑制性CAR连接时，所述抑制性CAR抑制通过活化性CAR的T-细胞活化，当连接抑制性CAR时，抑制性CAR不会显著地抑制通过活化性CAR的T-细胞活化。

[0171] 7.根据段6的T细胞，其中抑制性内域包含来自CD148或CD45的内域的全部或部分。

[0172] 8.根据段6或7的T细胞，其中第一CAR的抗原结合域结合CD5和第二CAR的抗原结合域结合CD19。

[0173] 9.根据段1的T细胞，其中第一和第二间隔区是足够不同的，从而防止第一和第二CAR的交叉配对，但足够相似以导致第一和第二CAR在连接后的共定位。

[0174] 10.根据段9的T细胞，其中第一CAR或第二CAR中的一个是包含活化内域的活化性CAR，和另一个CAR是包含连接开启的抑制性内域的抑制性CAR，在不存在抑制性CAR连接时，所述抑制性CAR不会显著地抑制通过活化性CAR的T-细胞活化，当连接抑制性CAR时，抑制性CAR抑制通过活化性CAR的T-细胞活化。

[0175] 11.根据段10的T细胞，其中所述连接开启的抑制性内域包含磷酸酶的至少一部分。

[0176] 12.根据段11的T细胞，其中所述连接开启的抑制性内域包含PTPN6的全部或部分。

[0177] 13.根据段10的T细胞，其中所述连接开启的抑制性内域包含至少一种ITIM域。

[0178] 14.根据段13的T细胞，其中所述连接开启的抑制性内域的活性通过PTPN6-CD45或CD148融合蛋白的共表达而增强。

[0179] 15.根据段10-14中任一项的T细胞，其中所述包含活化内域的CAR包含结合CD33的抗原结合域，并且包含连接开启的抑制性内域的CAR包含结合CD34的抗原结合域。

[0180] 16.T细胞，其包含如前述段落中定义的两种以上的CAR，使得所述CAR被具有两种以上抗原的不同模式的细胞，如T细胞特异性刺激。

[0181] 17.核酸序列，其编码如段1-16中之一所定义的第一和第二嵌合抗原受体(CAR)。

[0182] 18.根据段7的核酸序列，其具有以下结构：

[0183] AgB1-间隔区1-TM1-endo1-coexpr-AgB2-间隔区2-TM2-endo2

[0184] 其中，

[0185] AgB1是编码第一CAR的抗原结合域的核酸序列；

[0186] 间隔区1是编码第一CAR的间隔区的核酸序列；

[0187] TM1是编码第一CAR的跨膜域的核酸序列；

[0188] endo 1是编码第一CAR的内域的核酸序列；

[0189] coexpr是使两个CAR共表达的核酸序列；

[0190] AgB2是编码第二CAR的抗原结合域的核酸序列；

[0191] 间隔区2是编码第二CAR的间隔区的核酸序列；

[0192] TM2是编码第二CAR的跨膜域的核酸序列；

[0193] endo 2是编码第二CAR的内域的核酸序列；

[0194] 其中当在T细胞中表达时，所述核酸序列编码的多肽在切割位点被切割，使得第一和第二CAR共表达在T细胞的表面上。

[0195] 19.根据段18的核酸序列，其中所述coexpr编码包含自我切割肽的序列。

[0196] 20.根据段18或19的核酸序列，其中备选密码子用于编码相同或相似的氨基酸序列的序列区，以避免同源重组。

[0197] 21.试剂盒，其包含

[0198] (i)第一核酸序列，其编码段1-16中之一所定义的第一嵌合抗原受体(CAR)，所述核酸序列具有以下结构：

[0199] AgB1-间隔区1-TM1-endo1

[0200] 其中，

[0201] AgB1是编码第一CAR的抗原结合域的核酸序列；

[0202] 间隔区1是编码第一CAR的间隔区的核酸序列；

[0203] TM1是编码第一CAR的跨膜域的核酸序列；

[0204] endo 1是编码第一CAR的内域的核酸序列；和

[0205] (ii)第二核酸序列，其编码段1-16中之一所定义的第二嵌合抗原受体(CAR)，所述核酸序列具有以下结构：

[0206] AgB2-间隔区2-TM2-endo2

[0207] 其中，

[0208] AgB2是编码第二CAR的抗原结合域的核酸序列；

[0209] 间隔区2是编码第二CAR的间隔区的核酸序列；

[0210] TM2是编码第二CAR的跨膜域的核酸序列；

[0211] endo 2是编码第二CAR的内域的核酸序列。

[0212] 22.试剂盒，其包含：第一载体，其包含如段21定义的第一核酸序列；和第二载体，其包含如段21定义的第一核酸序列。

[0213] 23.根据段22的试剂盒，其中所述载体是整合型病毒载体或转座子。

[0214] 24.载体，其包含根据段17-20中任一项所定义的的核酸序列。

[0215] 25.根据段24的逆转录病毒载体或慢病毒载体或转座子。

[0216] 26.制备根据段1-16中任一项的T细胞的方法，其包括将根据段17-20中任一项的核酸序列，如段21中定义的第一核酸序列和第二核酸序列；和/或如段22中定义的第一载体和第二载体或根据段24或25的载体导入T细胞的步骤。

[0217] 27.根据段24的方法,其中所述T细胞来自从受试者分离的样品。

[0218] 28.药物组合物,其包含根据段1-16中任一项的多种T细胞。

[0219] 29.用于治疗和/或预防疾病的方法,其包括将根据段28的药物组合物施用于受试者的步骤。

[0220] 30.根据段29的方法,其包括以下步骤:

[0221] (i)从受试者分离包含T细胞的样品。

[0222] (ii)用根据段17-20中任一项的核酸序列,如段21中定义的第一核酸序列和第二核酸序列;和/或如段22或23中定义的第一载体和第二载体或根据段24或25的载体转导或转染所述T细胞;和

[0223] (iii)将来自(ii)的T细胞施用于受试者。

[0224] 31.根据段29或30的方法,其中所述疾病是癌症。

[0225] 32.根据段28的药物组合物,其用于治疗和/或预防疾病。

[0226] 33.根据段1-16中任一项的T细胞用于制备用于治疗和/或预防疾病的药物的用途。

[0227] 发明详述

[0228] 嵌合抗原受体(CAR)

[0229] 在图1中示意性显示的CAR是嵌合的I型跨膜蛋白质,其将胞外抗原识别域(结合剂)连接至胞内信号传导域(内域)。结合剂通常为来源于单克隆抗体(mAb)的单链可变片段(scFv),但它可以是基于包含抗体样抗原结合位点的其他形式。为将结合剂与膜分离并允许它具有合适的方向,间隔域通常是必需的。常用的间隔域是IgG1的Fc。取决于抗原,更紧凑的间隔区可以足够,例如来自CD8α的茎和甚至是仅IgG1的铰链。跨膜域将蛋白质锚定在细胞膜中,并将间隔区连接至内域。

[0230] 早期的CAR设计具有来源于FcεR1或CD3ζ的γ链的细胞内部分的内域。因此,这些第一代受体传输免疫信号1,其足以触发相关靶细胞的T细胞杀死,但未能充分活化T细胞以增殖和存活。为了克服此限制,已构建了复合内域:T细胞共刺激分子的胞内部分与CD3ζ的胞内部分的融合导致了可在抗原识别后同时传输活化和共刺激信号的第二代受体。最常使用的共刺激域是CD28的共刺激域。这提供最有效的共刺激信号-即触发T细胞增殖的免疫信号2。也已经描述了包括TNF受体家族内域的一些受体,例如密切相关的传输存活信号的OX40和41BB。甚至现在已经描述了更强大的第三代CAR,其具有能够传输活化,增殖和存活信号的内域。

[0231] 使用例如逆转录病毒载体可以将CAR编码核酸转移到T细胞。可使用慢病毒载体。通过这种方式可产生用于过继性细胞转移的大量的癌特异性T细胞。当CAR结合靶抗原时,这导致活化信号传输到表达所述CAR的T细胞上。因此,CAR指导T细胞的针对表达靶抗原的肿瘤细胞的特异性和细胞毒性。

[0232] 本发明的第一方面涉及共表达第一CAR和第二CAR的T细胞,使得T细胞可以以在真值表(truth table)(表1,2和3)中详细描述的逻辑门的方式识别靶细胞上表达的所希望的模式。

[0233] 第一CAR和第二CAR两者(和任选地后来的CAR)包含:

[0234] (i)抗原结合域;

[0235] (ii)间隔区

[0236] (iii)跨膜域;和

[0237] (iv)胞内域。

[0238] 表1:CAR或门的真值表

[0239]

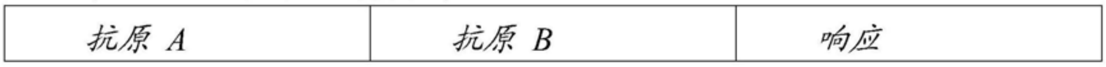
抗原A	抗原B	响应
不存在	不存在	未活化
不存在	存在	活化
存在	不存在	活化
存在	存在	活化

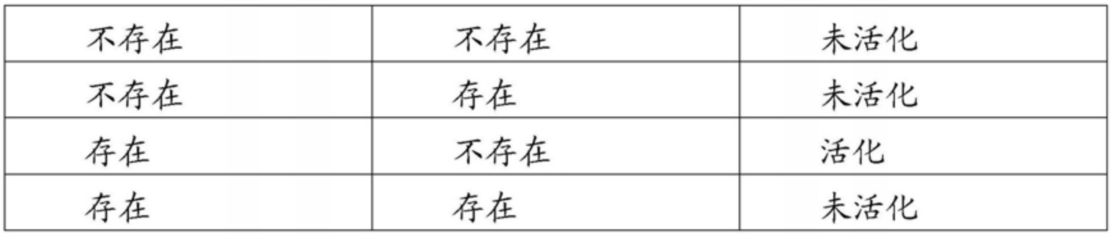
[0240] 表2:CAR和门的真值表

[0241]

抗原A	抗原B	响应
不存在	不存在	未活化
不存在	存在	未活化
存在	不存在	未活化
存在	存在	活化

[0242] 表3:CAR和非门的真值表

[0243] 

[0244] 

抗原 A	抗原 B	响应
不存在	不存在	未活化
不存在	存在	未活化
存在	不存在	活化
存在	存在	未活化

[0245] 本发明的T细胞的第一和第二CAR可以产生为包含两个CAR和切割位点的多肽。

[0246] SEQ ID No.1-5给出了这样的多肽的实例,其中每一个包含两个CAR。因此,CAR可包含对应于单个CAR的以下氨基酸序列中的一个或其它部分。

[0247] SEQ ID No 1是识别CD19或CD33的CAR或门。

[0248] SEQ ID No 2是使用CD148磷酸酶识别CD19和CD33的CAR和门。

[0249] SEQ ID No 3是使用CD45磷酸酶识别CD19和CD33的CAR和门的备选实施方式。

[0250] SEQ ID No 4是基于PTPN6磷酸酶识别CD19和非CD33的CAR和非门。

[0251] SEQ ID No 5是识别CD19和非CD33的CAR和非门的备选实施方式并且基于来自LAIR1的含有ITIM的内域。

[0252] SEQ ID No 6是识别CD19和非CD33的CAR和非门的其他备选实施方式并且募集PTPN6-CD148融合蛋白至含有ITIM的内域。

[0253] SEQ ID No.1

[0254] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDN

SKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREE
YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIYFNL
VWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSG
GGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGG
STYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSSMDPAEPKSPDKTH
TCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLV
VVGGVLACYSLLVTVAFIIFWVRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0255] SEQ ID No.2

[0256] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI
YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSG
GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDN
SKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREE
YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIYFNL
VWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSG
GGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGG
STYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSSMDPAEPKSPDKTH
TCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKAVFGC
IFGALVIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPK
YAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYA
IIMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDT
TDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQY
VFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIA

[0257] SEQ ID No.3

[0258] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI
YHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSG
GGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDN
SKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREE
YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIYFNL

VWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSSMDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKALIAFLAFLIIVTSIALLVVLYKIYDLHKKRSCNLDEQQELVERDDEKQLMNVEPIHADILLETYKRKIADEGRLFLAEFQSIPRVFSKFPIKEARKPFNQNKNRYVDILPYDYNRVELSEINGDAGSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMVTRCEEGNRNKCAEYWPSMEEGTRAFGDVVVKINQHKRCPDYIIQKLNIVNKKEKATGREVTHIQFTSWPDHGVPEDPHLLLKLRRRVNAFSNFFSGPIVVHCSAGVGRTGTYIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEAQYILIHQALVEYNQFGETEVNLSELHPYLHNMKKRDPPSEPSPLEAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVIPYDYNRVPLKHELEMSKESEHDSDESSDDDSDSEEPSKYINASFIMSYWKPEVMIAAQGPLKETIGDFWQMIFQRKVKVIVMLTELKHGDQEICAQYWGEGKQTYGDIEVDLKDTDKSSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQVVKQKLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIFCALLNLLESAETEEVVDIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKKNNHQEDKIEFDNEVDKVKQDANCVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEHSVNGPASPALNQGS

[0259] SEQ ID No.4

[0260] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSSMDPATTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDIYWAPLAGICVALLLSLIITLICYHRSRKRVCKSGGGSFWEEFESLQKQEVKNLHQRLEGQRPENKGKNRYKNILPFDHSRVILQGRDSNIPGSDYINANYIKNQLLGPDENAKTYIASQGCLEATVNDFWQMAWQENSRVIVMTTREVEKGRNKCVPYWPEVGMQRAYGPYSVTNCGEHDTTEYKLRTLQVSPLDNGDLIREIWHYQYLSWPDHGVPSEPGGVLSFLDQINQRQESLPHAGPIIVHCSAGIGRTGTIIVIDMLMENISTKGLDCDIDIQKTIQMVRAQRSGMVQTEAQYKFIYVAIAQFIETTKKKL

[0261] SEQ ID No.5

[0262] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA

LPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSSMDPATTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDILIGVSVVFLFCLLLLVLFCLHRQNQIKQGPPRSKDEEQKPQQRPDLAVDVLERTADKATVNGLPEKDRETDTSALAAGSSQEVTYAQLDHWALTQRTARAVSPQSTKPMAESITYAAVARH

[0263] SEQ ID No.6

[0264] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSSMDPATTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDILIGVSVVFLFCLLLLVLFCLHRQNQIKQGPPRSKDEEQKPQQRPDLAVDVLERTADKATVNGLPEKDRETDTSALAAGSSQEVTYAQLDHWALTQRTARAVSPQSTKPMAESITYAAVARHRAEGRGSLLTCGDVEENPGPWYHGHMSGGQAETLLQAKGEPWTFLVRESLSQPGDFVLSVLSDQPKAGPGSPLRVTHIKVMCEGGRYTVGGLETFDSLTDLVEHFKKTGIEEASGAFVYLRQPYSGGGGSFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIASGS

[0265] CAR可以包含如SEQ ID No.1,2,3,4,5或6中所示序列的CAR编码部分的变体,其具有至少80％,85％,90％,95％,98％或99％的序列同一性,条件是变体序列是具有所需性质的CAR。

[0266] 序列比对的方法是本领域是公知的,并且使用合适的比对程序来完成。％序列同一性指的是当在最佳比对两个序列时,它们中相同的氨基酸或核苷酸残基的百分比。可使用标准算法诸如BLAST程序(在国家生物技术信息中心基本局部比对搜索工具(Basic Local Alignment Search Tool at the National Center for Biotechnology Information))使用默认参数确定核苷酸和蛋白质序列的同源性或同一性,这在http://blast.ncbi.nlm.nih.gov是可公开获得的。用于确定序列同一性或同源性的其它算法包括:LALIGN(http://www.ebi.ac.uk/Tools/psa/lalign/和http://www.ebi.ac.uk/Tools/psa/lalign/nucleotide.html),AMAS(多比对序列分析(Analysis of Multiply Aligned Sequences),在http://www.compbio.dundee.ac.uk/Software/Amas/amas.html),FASTA(http://www.ebi.ac.uk/Tools/sss/fasta/),Clustal Omega(http://www.ebi.ac.uk/

Tools/msa/clustalo/),SIM(http://web.expasy.org/sim/),and EMBOSS Needle(http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html)。

[0267] CAR逻辑或门

[0268] 在本实施方案中,本发明的第一和第二CAR的抗原结合域结合不同的抗原并且两个CAR均包含活化内域。两个CAR具有不同的间隔区域以防止两个不同受体的交叉配对。因此可以工程化改造T细胞以在识别任一个或两个抗原后活化。如Goldie-Coldman假说所指示的,这在肿瘤学领域是有用的:由于在大多数癌症中所固有的高突变率,单一抗原的单一靶向可以导致通过调制所述抗原进行肿瘤逃逸。通过同时靶向两种抗原,此种逃逸的概率可以成指数地减少。

[0269] 如下表4中所示,各种肿瘤相关抗原是已知的。对于给定的疾病,第一CAR和第二CAR可以结合到与该疾病相关的两个不同的TAA。以这种方式,由于第二抗原也被靶向,防止了通过调控单一抗原进行的肿瘤逃逸。例如,当靶向B细胞恶性肿瘤时,可同时靶向CD19和CD20两者。在本实施方案中,这两个CAR不异二聚体化是重要的。

[0270] 表4

[0271]

癌症类型	TAA
弥漫性大B细胞淋巴瘤	CD19,CD20
乳腺癌	ErbB2,MUC1
AML	CD13,CD33
神经母细胞瘤	GD2,NCAM
B-CLL	CD19,CD52
大肠癌	叶酸结合蛋白,CA-125

[0272] 动力学分离模型

[0273] 产生和门及和非门的CAR的随后配对是基于T细胞活化的动力学分离模型(KS)。这是由实验数据支持的功能性模型,其解释了如何将通过T细胞受体进行的抗原识别转换成下游活化信号。简要地:在基态下,在T细胞膜上的信号传导组分处于动态平衡,由此相比于磷酸化ITAM,有利于脱磷酸化ITAM。这是由于跨膜CD45/CD148磷酸酶比膜栓系(membrane-tethered)激酶例如lck更大的活性。当T细胞通过相关抗原的T细胞受体(或CAR)识别而衔接靶细胞时,形成了紧密的免疫突触。T细胞和靶标膜的这种紧密并置排除了CD45/CD148,这是因为它们的不能装配到突触中的较大胞外域。在无磷酸酶的存在下,高浓度的T细胞受体相关ITAM和激酶在突触中的分离导致了有利于磷酸化ITAM的状态。ZAP70识别磷酸化ITAM的阈值并且传播T细胞活化信号。本发明利用了T细胞活化的这种先进的见解。具体而言,本发明是基于这样的见解:不同长度和/或体积和/或电荷和/或构造和/或糖基化的胞外域如何在突触形成后导致差别分离。

[0274] CAR逻辑和门

[0275] 在本实施方案中,一个CAR包含活化性内域,并且一个CAR包含抑制性内域,其中抑制性CAR组成性抑制第一活化性CAR,但在识别其相关抗原后解除其对活化性CAR的抑制。以这种方式,可以工程化改造T细胞,从而仅当靶细胞表达两种相关抗原时触发。此行为是由包含含有ITAM域的活化性内域(例如CD3Zeta内域)的活化性CAR和包含来自能够使ITAM脱磷酸的磷酸酶的内域(例如CD45或CD148)的抑制性CAR实现的。关键的是,两个CAR的间隔区

域在大小和/或形状和/或电荷等方面显著地不同。当仅连接活化性CAR时，抑制性CAR在T细胞表面上在溶液中(in solution)，并可以在抑制活化性CAR的突触中扩散并且扩散出抑制活化性CAR的突触。当连接这两个CAR时，由于间隔区性质的差异，活化和抑制性CAR被差异地分开，从而允许活化性CAR触发T细胞活化而不会受抑制性CAR的阻碍。

[0276] 这是在癌症治疗领域相当有用的。目前，免疫疗法一般靶向单一抗原。大多数癌症基于单一抗原不能与正常组织区分。因此，发生大量的“中靶脱瘤”毒性，由此使正常组织被治疗损伤。例如，虽然用利妥昔单抗靶向CD20以治疗B细胞淋巴瘤，但消耗了整个正常B细胞区室。例如，虽然靶向CD52以治疗慢性淋巴细胞性白血病，但消耗整个淋巴样区室。例如，虽然靶向CD33以治疗急性髓样白血病，但损伤了整个髓样区室等。通过将活性限制到一对抗原上，可以开发精细多得多的靶向和因此毒性较低的治疗。实际的实例是靶向同时表达CD5和CD19的CLL。仅正常B细胞的一小部分表达这两种抗原，因此用逻辑和门靶向这两种抗原的脱靶毒性实质性小于单独靶向每种抗原。

[0277] 本发明的设计相对于Wilkie et al.((2012).J.Clin.Immunol.32,1059-1070)描述和之后在体内测试的(Kloss et al(2013)Nat.Biotechnol.31,71-75)的先前的实施方式是相当大的改进。在本实施方式中，第一CAR包含(comprises of)活化性内域，并且第二CAR包含共刺激域。以此方式，当两种抗原都存在时，T细胞只接收活化和共刺激信号。然而，在仅存在第一抗原的情况下，T细胞将仍然活化，这导致潜在的脱靶毒性。另外，本发明的实施方式允许多个复合连接门(compound linked gate)，由此细胞可以解读抗原的复杂模式。

[0278] 表5

[0279]

癌症类型	抗原
慢性淋巴细胞性白血病	CD5，CD19
神经母细胞瘤	ALK，GD2
神经胶质瘤	EGFR，波形蛋白
多发性骨髓瘤	BCMA，CD138

[0280]

肾细胞癌	碳酸酐酶IX，G250
T-ALL	CD2，N-钙粘蛋白
前列腺癌	PSMA, hepsin(或其他)

[0281] CAR逻辑和非门

[0282] 在本实施方案中，一个CAR包含活化性内域，并且一个CAR包含抑制性内域，使得此抑制性CAR仅在它识别其相关抗原时活化。因此，以这种方式工程化改造的T细胞响应第一抗原的单独存在而活化，但在两种抗原均存在时不被活化。本发明是通过具有与第一CAR共定位的间隔区的抑制性CAR实施的，但抑制性CAR的磷酸酶活性应当不是如此有力的，以致于它在溶液中抑制，或仅当抑制性CAR识别其相关靶标时，抑制内域实际上募集磷酸。这种内域在本文被称为“连接开启”或半抑制。

[0283] 当通过肿瘤相关抗原的存在和在正常组织表达的抗原的丢失可以区分肿瘤和正常组织时，本发明在改善靶向中是有用的。和非门在肿瘤学领域中是相当有用的，因为它允许靶向由正常细胞表达的抗原，所述正常细胞也表达包含活化性内域的CAR识别的抗原。这

种抗原的实例是CD33，其是由正常干细胞和急性髓样白血病(AML)细胞表达。CD34在干细胞上表达，但通常不在AML细胞上表达。识别CD33和非CD34的T细胞将导致白血病细胞的破坏，但节约了(spare of)正常干细胞。

[0284] 用于与和非门一起使用的潜在抗原对显示于表6。

[0285] 表6

[0286]

疾病	TAA	表达TAA的正常组织	由正常细胞而非癌细胞表达的抗原
AML	CD33	干细胞	CD34
骨髓瘤	BCMA	树突细胞	CD1c
B-CLL	CD160	天然杀伤细胞	CD56
前列腺癌	PSMA	神经组织	NCAM
肠癌	A33	正常肠上皮	HLA-Ⅰ类

[0287] 复合门(compound gate)

[0288] 具有上述组份的动力学分离模型允许制备复合门，例如响应超过两个靶抗原的模式而触发的T细胞。例如，有可能制备仅当3种抗原存在(A和B和C)时触发的T细胞。此处，细胞表达3个CAR，各自识别抗原A，B和C。一个CAR是兴奋性的(excitatory)，并且两个是抑制性的，其中的每个CAR具有导致差别分离的间隔区域。只有当连接所有三个时，T细胞会活化。另一个实例：(A或B)和C：此处，识别抗原A和B的CAR在活化，并具有共定位的间隔区，而识别抗原C的CAR是抑制性的并且具有导致差别共分离的间隔区。另一个实例(A和非B)和C：此处，针对抗原A的CAR具有活化性内域并且与针对抗原B的具有条件性抑制性内域的CAR共定位。针对抗原C的CAR具有与A或B差别分离的间隔区并且是抑制性的。事实上，甚至更复杂的布尔(boolean)逻辑可以用本发明的这些简单的组分与具有任何数量的CAR和间隔区一起进行编程。

[0289] 信号肽

[0290] 本发明的T细胞的CAR可以包含信号肽使得当CAR在细胞，如T细胞内表达时，初生蛋白被引导至内质网并随后引导至其表达的细胞表面上。

[0291] 信号肽的核心可含有一段长的疏水性氨基酸，其具有形成单个α-螺旋的倾向。信号肽可以起始于短的带正电的一段氨基酸，这有助于在移位期间实施多肽的合适的拓扑学。在信号肽的末端通常有被信号肽酶识别和切割的一段氨基酸。信号肽酶可以在移位期间或完成移位之后切割以产生游离的信号肽和成熟蛋白。然后游离的信号肽被特定的蛋白酶消化。

[0292] 信号肽可以是在分子的氨基末端。

[0293] 信号肽可包含SEQ ID No.7，8或9或其变体，所述变体具有5，4，3，2或1个氨基酸突变(插入，取代或添加)，条件是信号肽仍然行使导致CAR的细胞表面表达的功能。

[0294] SEQ ID No.7：MGTSLLCWMALCLLGADHADG

[0295] SEQ ID No.7的信号肽是紧凑和高效的。预测通过信号肽酶在末端甘氨酸后产生约95％的切割，产生有效的移除。

[0296] SEQ ID No.8：MSLPVTALLLPLALLLHAARP

[0297] SEQ ID No.8的信号肽来源于IgG1。

[0298] SEQ ID No.9:MAVPTQVLGLLLLWLTDARC

[0299] SEQ ID No.9的信号肽来源于CD8。

[0300] 第一CAR的信号肽可以具有与第二CAR(和第三CAR和第四CAR等)的信号肽不同的序列。

[0301] 抗原结合域

[0302] 抗原结合域是CAR的识别抗原的部分。众多的抗原结合域是本领域公知的,包括基于抗体,抗体模拟物和T细胞受体的抗原结合位点的那些抗原结合域。例如,抗原结合域可以包含:由单克隆抗体衍生的单链可变片段(scFv);靶抗原的天然配体;具有针对靶标的足够亲和性的肽;单域抗体;人工单结合剂诸如Darpin(设计的锚蛋白重复蛋白);或T细胞受体衍生的单链。

[0303] 抗原结合域可包含不基于抗体的抗原结合位点的域。例如,抗原结合域可包含基于蛋白质/肽的域,所述蛋白质/肽为肿瘤细胞表面受体的可溶性配体(例如可溶性肽,如细胞因子或趋化因子);或膜锚定配体或者受体的胞外域,对于所述配体或者受体,结合对对应物表达在肿瘤细胞上。

[0304] 实施例11-13涉及结合BCMA的CAR,在其中抗原结合域包含包括APRIL,即BCMA的配体。

[0305] 抗原结合域可基于抗原的天然配体。

[0306] 抗原结合域可包含来自组合文库的亲和肽或从头设计的亲和蛋白质/肽。

[0307] 间隔区域

[0308] CAR包含间隔区序列以连接抗原结合域与跨膜域并且在空间上分离来自内域的抗原结合域。柔性间隔区允许抗原结合域在不同方向上定向以促进结合。

[0309] 在本发明的T细胞中,第一和第二CAR可以包含不同的间隔区分子。例如,间隔区序列可以例如包含IgG1Fc区,IgG1铰链或人CD8茎或小鼠CD8茎。间隔区可以备选地包含备选的接头序列,其具有与IgG1Fc区,IgG1铰链或CD8茎相似的长度和/或域间距性质。可以改变人IgG1间隔区以除去Fc结合基序。

[0310] 这些间隔区的氨基酸序列的实例如下:

[0311] SEQ ID No.10(人IgG1的铰链CH2CH3)

[0312] AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKD

[0313] SEQ ID No.11(人CD8茎):

[0314] TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

[0315] SEQ ID No.12(人IgG1的铰链):

[0316] AEPKSPDKTHTCPPCPKDPK

[0317] SEQ ID No.13(CD2胞外域)

[0318] KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSDKKKIAQFRKEKETFKEKDTYKLFKNGTLKIKHLKTDDQDIYKVSIYDTKGKNVLEKIFDLKIQERVSKPKISWTCINTTLTCEVMNGTDPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKVSKESSVEPVSCPEKGLD

[0319] SEQ ID no.14(CD34胞外域)

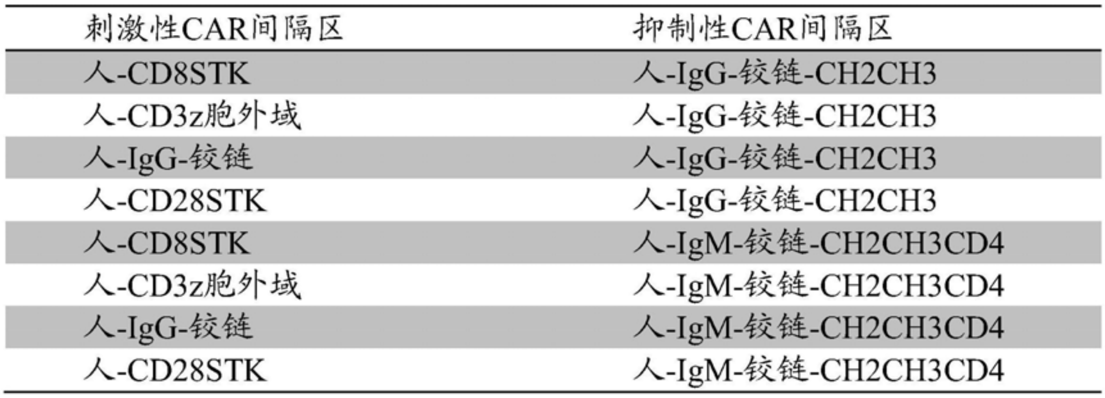
[0320] SLDNNGTATPELPTQGTFSNVSTNVSYQETTTPSTLGSTSLHPVSQHGNEATTNITETTVKFTSTSVITSVYGNTNSSVQSQTSVISTVFTTPANVSTPETTLKPSLSPGNVSDLSTTSTSLATSPTKPYTSSSPILSDIKAEIKCSGIREVKLTQGICLEQNKTSSCAEFKKDRGEGLARVLCGEEQADADAGAQVCSLLLAQSEVRPQCLLLVLANRTEISSKLQLMKKHQSDLKKLGILDFTEQDVASHQSYSQKT

[0321] 因为CAR通常是同二聚体(见图1a)，所以交叉配对可能导致异二聚体嵌合抗原受体。基于各种原因，这是不希望的，例如：(1)表位可能不是处在靶细胞的相同“水平”上，使得交叉配对的CAR可能只能够结合一种抗原；(2)来自两个不同的scFv的VH和VL可以交换并且不能识别靶标或者更坏识别意外的和不可预测的抗原。对于“或”门和“和非”门，第一CAR的间隔区可以与第二CAR的间隔区是足够地不同的，以避免交叉配对。第一间隔区的氨基酸序列可以与第二间隔区在氨基酸水平上共享小于50％，40％，30％或20％的同一性。

[0322] 在本发明的其他方面(例如和门)，重要的是，第一CAR的间隔区具有不同的长度和/或电荷和/或形状和/或构造和/或糖基化，使得当第一CAR和第二CAR两者结合它们的靶抗原时，间隔区电荷或尺寸的不同导致两种类型的CAR的空间分离至膜的不同部分，从而导致活化，如通过动力学分离模型所预测的。在这些方面，牢记大小和靶抗原上的结合表位，仔细地选择间隔区的不同长度和/或形状和/或构造，以允许在相关靶标识别后的差别分离。例如，预期IgG1铰链，CD8茎，IgG1Fc，CD34的胞外域，CD45胞外域差别分离。

[0323] 差别分离并且因此适用于与和门一起使用的间隔区对的实例在下表中显示：

[0324]

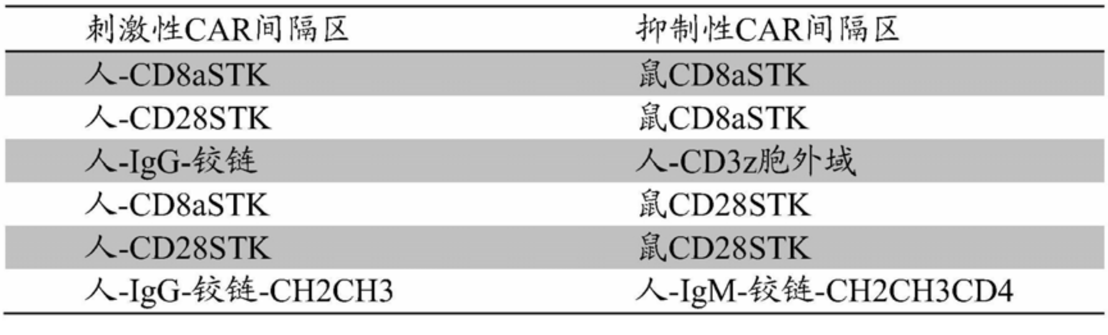


刺激性CAR间隔区	抑制性CAR间隔区
人-CD8STK	人-IgG-铰链-CH2CH3
人-CD3z胞外域	人-IgG-铰链-CH2CH3
人-IgG-铰链	人-IgG-铰链-CH2CH3
人-CD28STK	人-IgG-铰链-CH2CH3
人-CD8STK	人-IgM-铰链-CH2CH3CD4
人-CD3z胞外域	人-IgM-铰链-CH2CH3CD4
人-IgG-铰链	人-IgM-铰链-CH2CH3CD4
人-CD28STK	人-IgM-铰链-CH2CH3CD4

[0325] 在本发明的其他方面(例如和非门)，重要的是间隔区足够不同以防止交叉配对，但要足够相似以共定位。可采用直系同源间隔区序列对。实例是鼠和人CD8茎，或备选地单体的间隔区域-例如CD2的胞外域。

[0326] 共定位并因此适用于与和非门一起使用的间隔区对的实例在下表中显示：

[0327]



刺激性CAR间隔区	抑制性CAR间隔区
人-CD8aSTK	鼠CD8aSTK
人-CD28STK	鼠CD8aSTK
人-IgG-铰链	人-CD3z胞外域
人-CD8aSTK	鼠CD28STK
人-CD28STK	鼠CD28STK
人-IgG-铰链-CH2CH3	人-IgM-铰链-CH2CH3CD4

[0328] 上述提到的所有间隔区域形成同二聚体。然而，该机制不限于使用同二聚体受体，

并且应该与单体受体一起使用，只要间隔区具有足够的刚性。这样的间隔区的实例是CD2或截短的CD22。

[0329] 跨膜域

[0330] 跨膜域是CAR的跨越膜的序列。

[0331] 跨膜域可以是在膜中热力学稳定的任何蛋白质结构。这通常是由几个疏水性残基构成的α-螺旋。可以将任何跨膜蛋白质的跨膜域用于提供本发明的跨膜部分。蛋白质的跨膜域的存在和跨度可以通过本领域的技术人员使用TMHMM算法(http://www.cbs.dtu.dk/services/TMHMM-2.0/)来确定。此外，考虑到蛋白质的跨膜域是相对简单的结构，即预测形成足够长的疏水α螺旋以跨越膜的多肽序列，也可以使用人工设计的TM域(US7052906B1描述了合成的跨膜组分)。

[0332] 跨膜域可以来源于CD28，其提供了良好的受体稳定性。

[0333] 活化性内域

[0334] 内域是CAR的信号传输部分。在抗原识别后，受体聚簇，天然CD45和CD148被排除在突触外，并且将信号传输到细胞。最常用的内域组分是含有3个ITAM的CD3-zeta的内域。这在结合抗原后将活化信号传输到T细胞。CD3-zeta可能不能提供完全有能力(competent)的活化信号，并且可能需要额外的共刺激信号。例如，可以将嵌合CD28和OX40与CD3-zeta一起用于传输增殖/存活信号，或全部三种可以一起使用。

[0335] 在本发明的T细胞包含具有活化性内域的CAR的情况中，它可以仅包含CD3-Zeta内域，CD3-Zeta内域与CD28或OX40的内域，或CD28内域和OX40和CD3-Zeta内域。

[0336] 包含ITAM基序的任何内域可以作为本发明的活化性内域起作用。已知几种蛋白质含有内域，所述内域具有一个或更多个ITAM基序。这种蛋白质的实例包括CD3ε链、CD3γ链和CD3δ链等等。考虑标签YxxL/l，任意两个其他氨基酸可以容易地将ITAM基序识别为与亮氨酸或异亮氨酸分开的酪氨酸。通常但不总是，这些基序中的两个是由分子尾部的6-8个氨基酸分隔(YxxL/IX(6-8)YxxL/I)。因此，本领域技术人员可以容易地找到含有一个或更多ITAM的现存蛋白质以传输活化信号。此外，考虑到基序是简单的，并且不需要复杂的二级结构，本领域的技术人员可以设计含有人工ITAM的多肽以传输活化信号(参见WO2000063372，其涉及合成的信号分子)。

[0337] 具有活化性内域的CAR的跨膜和胞内T细胞传递域(内域)可以包含如SEQ ID No.15,16中显示的序列或其变体，所述变体具有至少80％序列同一性。

[0338] SEQ ID No.15，包含CD28跨膜域和CD3Z内域

[0339] FWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0340] SEQ ID No.16，包含CD28跨膜域，CD28和CD3Zeta内域

[0341] FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0342] SEQ ID No.17，包含CD28跨膜域，CD28，OX40和CD3Zeta内域

[0343] FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD

PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0344] 变体序列可具有与SEQ ID No.15，16或17至少80％，85％，90％，95％，98％或99％的序列同一性，条件是该序列提供了有效的跨膜域和有效的细胞内T细胞信号传导域。

[0345] “连接关闭”抑制性内域

[0346] 在上面称为和门的实施方案中，CAR中的一个包含抑制性内域，使得在不存在抑制性CAR连接的情况下，抑制性CAR抑制通过活化性CAR导致的T细胞活化，当连接抑制性CAR时，不显著抑制通过活化性CAR导致的T细胞活化。这被称为“连接关闭”抑制性内域。

[0347] 在这种情况下，抑制性CAR的间隔区具有与活化性CAR不同的长度，电荷，形状和/或构造和/或糖基化，使得当连接两个受体时，间隔区尺寸的差异导致抑制性CAR和活化性CAR在免疫突触的不同膜区室中分离，使得活化性内域从由抑制性内域导致的抑制中解除出来。

[0348] 因此，用于连接关闭抑制性CAR的抑制性内域可以包含任何序列，当其在相同膜区室中时(在不存在抑制性CAR的抗原的情况中)其抑制通过活化性CAR的T细胞信号传导，但当它与抑制性CAR在膜的不同部分中分离时不显著抑制T细胞传递。

[0349] 连接关闭抑制性内域可以是或包含酪氨酸磷酸酶，例如受体样酪氨酸磷酸酶。抑制性内域可以是或包含任何酪氨酸磷酸酶，当仅连接刺激性受体时，所述酪氨酸磷酸酶能够抑制TCR信号传导。抑制性内域可以是或包含具有针对磷酸化的ITAM的足够快的催化速率的任何酪氨酸磷酸酶，当仅连接刺激性受体时，所述酪氨酸磷酸酶能够抑制TCR信号传导。

[0350] 例如，和门的抑制性内域可以包含CD148或CD45的内域。CD148和CD45已显示天然地作用于TCR信号传导的磷酸化的酪氨酸上游。

[0351] CD148是受体样蛋白质酪氨酸磷酸酶，其通过干扰PLCγ1和LAT的磷酸化和功能负调节TCR信号传导。

[0352] CD45存在于所有造血细胞中，是一种蛋白酪氨酸磷酸酶，它能够再次通过磷酸化PLCγ1调节信号转导和功能性响应。

[0353] 抑制性内域可包含受体样酪氨酸磷酸酶的全部或一部分。磷酸酶可以干扰参与T细胞信号传导的元件如PLCγ1和/或LAT的磷酸化和/或功能。

[0354] CD45和CD148的跨膜和内域分别显示为SEQ ID No.18和No.19。

[0355] SEQ ID 18-CD45跨膜和内域序列

[0356] ALIAFLAFLIIVTSIALLVVLYKIYDLHKKRSCNLDEQQELVERDDEKQLMNVEPIHADILLETYKRKIADEGRLFLAEFQSIPRVFSKFPIKEARKPFNQNKNRYVDILPYDYNRVELSEINGDAGSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMVTRCEEGNRNKCAEYWPSMEEGTRAFGDVVVKINQHKRCPDYIIQKLNIVNKKEKATGREVTHIQFTSWPDHGVPEDPHLLLKLRRRVNAFSNFFSGPIVVHCSAGVGRTGTYIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEAQYILIHQALVEYNQFGETEVNLSELHPYLHNMKKRDPPSEPSPLEAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVIPYDYNRVPLKHELEMSKESEHDSDESSDDDSDSEEPSKYINASFIMSYWKPEVMIAAQGPLKETIGDFWQMIFQRKVKVIVMLTELKHGDQEICAQYWGEGKQTYGDIEVDLKDTDKSSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQVVKQKLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIFCALLNLLESAETEEVVDIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKKNNHQEDKIEFDNEVDKVKQDANCVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEHSVNGPASPALNQGS

[0357]　SEQ ID 19-CD148跨膜和内域序列

[0358]　AVFGCIFGALVIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIA

[0359]　抑制性CAR可以包含SEQ ID No 18或19的全部或部分(例如,它可以包含内域的磷酸酶功能)。它可以包含该序列或其部分的具有至少80％的序列同一性的变体,只要所述变体保留了基本上抑制由活化性CAR导致的T细胞信号传导的能力。

[0360]　其他间隔区和内域可以例如使用本文列举的模型系统来进行测试。可以通过单独或双重转导合适的细胞系诸如SupT1细胞系产生靶细胞群以建立针对两种抗原呈阴性的细胞(野生型),针对任一种抗原呈阳性的细胞和针对两种抗原呈阳性的细胞(如CD19-CD33-,CD19+CD33-,CD19,CD33+和CD19+CD33+)。可以用一对CAR转导T细胞如在活化后释放IL-2的小鼠T细胞系BW5147,并且通过测量IL-2的释放(例如通过ELISA)测量它们在逻辑门中发挥作用的能力。例如,在实施例4中显示CD148和CD45内域两者可以作为抑制性CAR与含有CD3Zeta内域的活化性CAR组合行使功能。这些CAR依靠一个CAR上的短/不庞大的CD8茎间隔区和另一个CAR上庞大的Fc区实现和门控。当连接了两个受体时,间隔区尺寸的差异导致不同受体在不同膜区室中的分离,从而将CD3Zeta受体从由CD148或CD45内域导致的抑制中解除出来。以这种方式,仅当两种受体被活化时才发生活化。可以容易地看出,这种模块系统可用于测试备选的间隔区对和抑制性内域。如果在两种受体的连接之后,间隔区没有实现分离,那么抑制将不会被释放,所以不会发生活化。如果所测试的抑制性内域是无效的,那么在活化性CAR的连接的存在下将预期活化,与抑制性CAR的连接状态无关。

[0361]　“连接开启”内域

[0362]　在上述称为和非门的实施方案中,CAR中的一个包含”连接开启”抑制性内域,使得在缺乏抑制性CAR连接的情况下,抑制性CAR不显著抑制由活化性CAR导致的T细胞活化,当连接抑制性CAR时,其抑制由由活化性CAR导致的T细胞活化。

[0363]　“连接开启”抑制性内域可以是或包含酪氨酸磷酸酶,当仅连接刺激性受体时,所述酪氨酸磷酸酶不能抑制TCR信号传导。

[0364]　“连接开启”抑制性内域可以是或包含具有针对磷酸化的ITAM的足够慢的催化速率的酪氨酸磷酸酶,当仅连接刺激性受体时,所述酪氨酸磷酸酶不能够抑制TCR信号传导,但当在突触处集中时,能抑制TCR信号传导响应。通过抑制性受体连接实现了突触处的富集。

[0365]　如果酪氨酸磷酸酶具有针对”连接开启”抑制性内域太快的催化速率,则有可能通过修饰如点突变和短接头(其导致位阻)来下调(tune-down)磷酸酶的催化速率,以使其适合于“连接开启”抑制性内域。

[0366]　在第一实施方案中,内域可以是或包含具有比CD45或CD148具有少得相当多的活性的磷酸酶,使得ITAMS的显著脱磷酸化仅在活化和抑制性内域共定位时发生。许多合适的序列是本领域已知的。例如,非和门的抑制性内域可包含蛋白质-酪氨酸磷酸酶如PTPN6的全部或部分。

[0367] 蛋白酪氨酸磷酸酶(PTP)是调节多种细胞过程,包括细胞生长,分化,有丝分裂周期和致癌性转化的信号传导分子。此PTP的N末端部分含有两个串联的Src同源(SH2)域,它作为蛋白质磷酸酪氨酸结合域起作用,并介导此PTP与其底物的相互作用。此PTP主要在造血细胞中表达,并在造血细胞中作为多种信号传导途径的重要调节剂发挥作用。

[0368] 抑制剂域可包含整个PTPN6(SEQ ID No.20)或仅磷酸酶域(SEQ ID No.21)。

[0369] SEQ ID No.20-PTPN6序列

[0370] MVRWFHRDLSGLDAETLLKGRGVHGSFLARPSRKNQGDFSLSVRVGDQVTHIRIQNSGDFYDLYGGEKFATLTELVEYYTQQQGVLQDRDGTIIHLKYPLNCSDPTSERWYHGHMSGGQAETLLQAKGEPWTFLVRESLSQPGDFVLSVLSDQPKAGPGSPLRVTHIKVMCEGGRYTVGGLETFDSLTDLVEHFKKTGIEEASGAFVYLRQPYYATRVNAADIENRVLELNKKQESEDTAKAGFWEEFESLQKQEVKNLHQRLEGQRPENKGKNRYKNILPFDHSRVILQGRDSNIPGSDYINANYIKNQLLGPDENAKTYIASQGCLEATVNDFWQMAWQENSRVIVMTTREVEKGRNKCVPYWPEVGMQRAYGPYSVTNCGEHDTTEYKLRTLQVSPLDNGDLIREIWHYQYLSWPDHGVPSEPGGVLSFLDQINQRQESLPHAGPIIVHCSAGIGRTGTIIVIDMLMENISTKGLDCDIDIQKTIQMVRAQRSGMVQTEAQYKFIYVAIAQFIETTKKKLEVLQSQKGQESEYGNITYPPAMKNAHAKASRTSSKHKEDVYENLHTKNKREEKVKKQRSADKEKSKGSLKRK

[0371] SEQ ID No.21-PTPN6磷酸酶域序列

[0372] FWEEFESLQKQEVKNLHQRLEGQRPENKGKNRYKNILPFDHSRVILQGRDSNIPGSDYINANYIKNQLLGPDENAKTYIASQGCLEATVNDFWQMAWQENSRVIVMTTREVEKGRNKCVPYWPEVGMQRAYGPYSVTNCGEHDTTEYKLRTLQVSPLDNGDLIREIWHYQYLSWPDHGVPSEPGGVLSFLDQINQRQESLPHAGPIIVHCSAGIGRTGTIIVIDMLMENISTKGLDCDIDIQKTIQMVRAQRSGMVQTEAQYKFIYVAIAQF

[0373] 连接开启抑制性内域的第二种实施方案是含有ITIM(基于免疫受体酪氨酸的抑制基序)的内域,如来自CD22,LAIR-1,杀手(killer)抑制性受体家族(KIR),LILRB1,CTLA4,PD-1,BTLA等的内域。当被磷酸化时,ITIM通过其SH2域募集内源PTPN6。如果与含ITAM的内域共定位,那么发生脱磷酸化,并且抑制了活化性CAR。

[0374] ITIM是在免疫系统的许多抑制性受体的胞质尾部中发现的氨基酸的保守序列(S/I/V/LxYxxI/V/L)。本领域技术人员可以很容易地发现含ITIM的蛋白质域。已经通过蛋白质组全扫描(Staub,et al(2004)Cell.Signal.16,435-456)产生含ITIM的人类候选蛋白质的列表。此外,由于共有序列是公知的并且小的二级结构似乎是需要的,本领域技术人员可以产生人工ITIM。

[0375] 来自PDCD1,BTLA4,LILRB1,LAIR1,CTLA4,KIR2DL1,KIR2DL4,KIR2DL5,KIR3DL1和KIR3DL3的ITIM内域分别显示于SEQ ID 22至31中。

[0376] SEQ ID 22PDCD1内域

[0377] CSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

[0378] SEQ ID 23BTLA4

[0379] KLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPMMEDGISYTTLRFPEMNIPRTGDAESSEMQRPPPDCDDTVTYSALHKRQVGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENVDYVILKH

[0380] SEQ ID 24LILRB1

[0381] LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATE

PPPSQEGPSPAVPSIYATLAIH

[0382] SEQ ID 25LAIR1

[0383] HRQNQIKQGPPRSKDEEQKPQQRPDLAVDVLERTADKATVNGLPEKDRETDTSALAAGSSQEVTYAQLDHWALTQRTARAVSPQSTKPMAESITYAAVARH

[0384] SEQ ID 26CTLA4

[0385] FLLWILAAVSSGLFFYSFLLTAVSLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPYFIPIN

[0386] SEQ ID 27KIR2DL1

[0387] GNSRHLHVLIGTSVVIIPFAILLFFLLHRWCANKKNAVVMDQEPAGNRTVNREDSDEQDPQEVTYTQLNHCVFTQRKITRPSQRPKTPPTDIIVYTELPNAESRSKVVSCP

[0388] SEQ ID 28KIR2DL4

[0389] GIARHLHAVIRYSVAIILFTILPFFLLHRWCSKKKENAAVMNQEPAGHRTVNREDSDEQDPQEVTYAQLDHCIFTQRKITGPSQRSKRPSTDTSVCIELPNAEPRALSPAHEHHSQALMGSSRETTALSQTQLASSNVPAAGI

[0390] SEQ ID 29KIR2DL5

[0391] TGIRRHLHILIGTSVAIILFIILFFFLLHCCCSNKKNAAVMDQEPAGDRTVNREDSDDQDPQEVTYAQLDHCVFTQTKITSPSQRPKTPPTDTTMYMELPNAKPRSLSPAHKHHSQALRGSSRETTALSQNRVASSHVPAAGI

[0392] SEQ ID 30KIR3DL1

[0393] KDPRHLHILIGTSVVIILFILLLFFLLHLWCSNKKNAAVMDQEPAGNRTANSEDSDEQDPEEVTYAQLDHCVFTQRKITRPSQRPKTPPTDTILYTELPNAKPRSKVVSCP

[0394] SEQ ID 31KIR3DL3

[0395] KDPGNSRHLHVLIGTSVVIIPFAILLFFLLHRWCANKKNAVVMDQEPAGNRTVNREDSDEQDPQEVTYAQLNHCVFTQRKITRPSQRPKTPPTDTSV

[0396] 连接开启抑制性内域的第三个实施方案是含有ITIM的与融合蛋白共表达的内域。融合蛋白可以包含蛋白质-酪氨酸磷酸酶的至少一部分和受体样酪氨酸磷酸酶的至少一部分。该融合物可以包含来自蛋白质-酪氨酸磷酸酶的一个或多个SH2域。例如，融合可以是在PTPN6SH2域和CD45内域之间或PTPN6SH2域和CD148内域之间进行。当被磷酸化时，ITIM域募集融合蛋白，使高度有力的CD45或CD148磷酸酶接近活化性内域以阻断活化。

[0397] 融合蛋白的序列列在32和33

[0398] SEQID 32PTPN6-CD45融合蛋白

[0399] WYHGHMSGGQAETLLQAKGEPWTFLVRESLSQPGDFVLSVLSDQPKAGPGSPLRVTHIKVMCEGGRYTVGGLETFDSLTDLVEHFKKTGIEEASGAFVYLRQPYKIYDLHKKRSCNLDEQQELVERDDEKQLMNVEPIHADILLETYKRKIADEGRLFLAEFQSIPRVFSKFPIKEARKPFNQNKNRYVDILPYDYNRVELSEINGDAGSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMVTRCEEGNRNKCAEYWPSMEEGTRAFGDVVVKINQHKRCPDYIIQKLNIVNKKEKATGREVTHIQFTSWPDHGVPEDPHLLLKLRRRVNAFSNFFSGPIVVHCSAGVGRTGTYIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEAQYILIHQALVEYNQFGETEVNLSELHPYLHNMKKRDPPSEPSPLEAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVIPYDYNRVLKHELEMSKESEHDSDESSDDDSDSEEPSKYINASFIMSYWKPEVMIAAQGPLKETIGDFMIQRKVKVIVMLTELKHGDQEICAQYWGEGKQTYGDIEVDLKDTDKSSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQVVKQKLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIFCALLNLLESAETEEVVDIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKKNNHQEDKIEFDNEVDKVKQDANCVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEHSVNGPASPALNQGS

[0400] SEQ ID 33PTPN6-CD148融合蛋白

[0401] ETLLQAKGEPWTFLVRESLSQPGDFVLSVLSDQPKAGPGSPLRVTHIKVMCEGGRYTVGGLETFDSLTDLVEHFKKTGIEEASGAFVYLRQPYRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIA

[0402] 连接开启抑制性CAR可以包含SEQ ID No 20或21的全部或部分。它可以包含SEQ ID No 20至31的全部或部分。它可以包含与SEQ ID 32或33共表达的SEQ ID 22至31的全部或部分。它可以包含具有至少80％序列同一性的序列或其部分的变体，只要所述变体保留了在抑制性CAR连接后抑制由活化性CAR导致的T细胞信号传导的能力。

[0403] 如上述，可以例如使用本文列举的模型系统测试备选的间隔区和内域。在实施例5中显示，PTPN6内域可以作为半抑制性CAR与含有CD3Zeta内域的活化性CAR组合发挥功能。这些CAR依靠一个CAR上的人CD8茎间隔区和另一CAR上的小鼠CD8茎间隔区。直系同源序列防止交叉配对。然而，当连接两种受体时，间隔区的相似性导致在相同的膜区室中的不同受体的共分离。这导致了PTPN6内域抑制CD3Zeta受体。如果只是活化性CAR连接，那么PTPN6内域不是足够有活性以防止T细胞的活化。以这种方式，仅当活化性CAR连接并且抑制性CAR不连接时(和非门)时发生活化。可以容易地看出，这种模块系统可用于测试备选的间隔区对和抑制性域。如果在两种受体连接后，间隔区没有实现共分离，那么抑制不会是有效的，并且因此不会发生活化。如果所测试的半抑制性内域是无效的，将预期在活化性CAR连接的存在下活化，与半抑制性CAR的连接状态无关。

[0404] 共表达位点

[0405] 本发明的第二方面涉及编码第一CAR和第二CAR的核酸。

[0406] 核酸可以产生多肽，其包含通过切割位点连接的两个CAR分子。切割位点可以自我切割，使得当多肽产生时，它立即被切割成第一CAR和第二CAR而不需要任何外部切割活性。

[0407] 多种自我切割位点是已知的，包括口蹄疫病毒(FMDV)2a自我切割肽，其具有如SEQ ID No.34中所示的序列：

[0408] SEQ ID No.34

[0409] RAEGRGSLLTCGDVEENPGP

[0410] 共表达序列可以是内部核糖体进入序列(IRES)。共表达序列可以是一个内部启动子。

[0411] 细胞

[0412] 本发明的第一方面涉及在细胞表面共表达第一CAR和第二CAR的细胞。

[0413] 细胞可以是能够在细胞表面表达CAR的任何真核细胞如免疫细胞。

[0414] 具体地，细胞可以是免疫效应细胞，如T细胞或天然杀伤(NK)细胞。

[0415] T细胞或T淋巴细胞是在细胞介导的免疫中起中心作用的一类淋巴细胞。可以通过细胞表面上存在的T细胞受体(TCR)将它们与其他的淋巴细胞，如B细胞和天然杀伤细胞(NK细胞)区分开来。有多种类型的T细胞的，总结如下。

[0416] 辅助性T辅助细胞(TH细胞)在免疫学过程中协助其他的白细胞，所述免疫学过程

包括B细胞成熟为浆细胞和记忆B细胞，细胞毒性T细胞和巨噬细胞的活化。TH细胞在其表面表达CD4。TH细胞当它们通过在抗原呈递细胞(APC)表面上的MHC II类分子而呈递有肽抗原时被活化。这些细胞能够分化成几种亚型之一，包括TH1，TH2，TH3，TH17，TH9，或TFH，其分泌不同细胞因子以促进不同类型的免疫应答。

[0417] 细胞毒性T细胞(TC细胞，或CTL)破坏病毒感染的细胞和肿瘤细胞，并且也涉及移植排斥。CTL在其表面表达CD8。这些细胞通过结合与存在于所有有核细胞的表面上的MHC I类结合的抗原识别它们的靶标。通过调节性T细胞分泌的IL-10，腺苷和其它分子，可以使CD8+细胞失活至无反应性状态，这防止自身免疫疾病如实验性自身免疫性脑脊髓炎。

[0418] 记忆T细胞是感染已经结束后长期存在的抗原特异性T细胞的亚组。在重新暴露于它们的相关抗原后，它们很快扩展成大量的效应T细胞，从而给免疫系统提供针对过去感染的“记忆”。记忆T细胞包括三个亚型：中枢记忆T细胞(TCM细胞)和两种类型的效应子记忆T细胞(TEM细胞和TEMRA细胞)。记忆细胞可以是CD4+或CD8+。记忆T细胞通常表达细胞表面蛋白CD45RO。

[0419] 调节性T细胞(Treg细胞)，以前称作抑制性T细胞，对于维持免疫耐受是关键的。它们的主要作用是朝向免疫反应结束关闭T细胞介导的的免疫并抑制逃脱了胸腺中的负选择过程的自身反应性T细胞。

[0420] 已经描述了两种主要类型的CD4+Treg细胞-天然存在的Treg细胞和适应性Treg细胞。

[0421] 天然存在的Treg细胞(也称为CD4+CD25+FoxP3+Treg细胞)在胸腺中出现并且已经与发展中的T细胞与用TSLP活化的两种髓样(CD11c+)以及浆细胞样(CD123+)树突细胞之间的相互作用相关。通过称为FoxP3的胞内分子的存在，可以将天然存在的Treg细胞与其他T细胞区分开来。FOXP3基因突变可以阻止调节性T细胞发育，导致致命的自身免疫性疾病IPEX。

[0422] 适应性Treg细胞(也称为Tr1细胞或Th3的细胞)可以在正常免疫应答过程中起源。

[0423] 本发明的T细胞可以是上文提到的任何T细胞类型，特别是CTL。

[0424] 自然杀伤(NK)细胞是一类形成先天性免疫系统的一部分的细胞溶解性细胞。NK细胞以独立于MHC的方式，提供对来自病毒感染细胞的先天信号的快速应答。

[0425] NK细胞(属于先天淋巴样细胞组)被定义为大颗粒淋巴细胞(LGL)，并构成了区分于通常的产生B和T淋巴细胞的淋巴样前体的第三种细胞。已知NK细胞在骨髓、淋巴结，脾，扁桃体和胸腺中分化和成熟，在那里它们进入中循环。

[0426] 本发明的CAR细胞可以是上文提到的任何细胞类型。

[0427] CAR表达细胞，如CAR表达T或NK细胞可以从患者自身的外周血(第一方)，或在来自供体外周血(第二方)的造血干细胞移植物的背景中，或者从无关供体(第三方)的外周血离体产生。

[0428] 本发明还提供细胞组合物，其包含根据本发明的CAR表达性T细胞和/或CAR表达性NK细胞。细胞组合物可以通过用根据本发明的核酸离体转导或转染血液样品来制备。

[0429] 备选地，CAR表达细胞可以来源于诱导祖细胞或胚胎祖细胞到相关的细胞类型，如T细胞的离体分化。备选地，可以使用永生化细胞系，如保留其裂解功能并能用作治疗剂的T细胞系。

[0430] 在所有这些实施方案中，通过许多方式之一，包括用病毒载体转导，用DNA或RNA转染导入编码CAR的DNA或RNA来产生CAR细胞。

[0431] 本发明的CAR T细胞可以是来自受试者的离体T细胞。T细胞可以来自外周血单核细胞(PBMC)样品。在用CAR编码核酸转导之前，可以例如通过用抗CD3单克隆抗体处理来活化和/或扩增T细胞。

[0432] 本发明的CAR T细胞可以通过以下制备：

[0433] (i)从受试者或上面列出的其它来源分离含T细胞的样品；和

[0434] (ii)用编码第一和第二CAR的一种或多种核酸序列转导或转染T细胞。

[0435] 然后可以纯化T细胞，例如基于第一和第二CAR的共表达来选择。

[0436] 核酸序列

[0437] 本发明的第二方面涉及编码如在本发明的第一方面所定义的第一CAR和第二CAR的一个或多个核酸序列。

[0438] 核酸序列可包含以下序列之一或其变体：

[0439] SEQ ID 35或门

[0440] 使用CD45的SEQ ID 36和门

[0441] 使用CD148的SEQ ID 37和门

[0442] 使用PTPN6作为内域的SEQ ID 38和非门

[0443] 使用LAIR1内域的SEQ ID 39和非门

[0444] 使用具有CD148磷酸酶的LAIR1和PTPN6SH2融合物的SEQ ID 40和非门

[0445] SEQ ID No.35：

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GTGGCAATGACATCAGAAATTGTTCTTCCGGAATGGACCATCAGAGATTTCACAGTGAAAAATATCCAGACAAGTGA
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[0454] SEQ ID No.38

[0455] >16076.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-tm-dPTPN6

[0456] ATGAGCCTGCCCGTGACCGCCCTGCTGCTGCCCCTGGCCCTGCTGCTGCACGCCGCCAGACCAGACATCCAGATGACCCAGACCACCAGCAGCCTGAGCGCCAGCCTGGGCGACCGGGTGACCATCAGCTGCAGAGCCAGCCAGGACATCAGCAAGTACCTGAACTGGTACCAGCAGAAGCCCGACGGCACCGTGAAGCTGCTGATCTACCACACCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTCAGCGGCAGCGGCAGCGGCACCGACTACAGCCTGACCATCAGCAACCTGGAGCAGGAGGACATCGCCACCTACTTCTGCCAGCAGGGCAACACCCTGCCCTACACCTTCGGAGGCGGCACCAAGCTGGAGATCACCAAGGCCGGAGGCGGAGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCGAGGTGAAGCTGCAGGAGTCTGGCCCAGGCCTGGTGGCCCCAAGCCAGAGCCTGAGCGTGACCTGCACCGTGAGCGGCGTGAGCCTGCCCGACTACGGCGTGAGCTGGATCAGGCAGCCCCCACGGAAGGGCCTGGAGTGGCTGGGCGTGATCTGGGGCAGCGAGACCACCTACTACAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGCAAGAGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTACTACTGCGCCAAGCACTACTACTATGGCGGCAGCTACGCTATGGACTACTGGGGCCAGGGCACCAGCGTGACCGTGAGCTCAGATCCCACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCTCCTCGCAGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGCCGTGCCCACTCAGGTCCTGGGGTTGTTGCTACTGTGGCTTACAGATGCCAGATGTGACATCCAGATGACACAGTCTCCATCTTCCCTGTCTGCATCTGTCGGAGATCGCGTCACCATCACCTGTCGAGCAAGTGAGGACATTTATTTTAATTTAGTGTGGTATCAGCAGAAACCAGGAAAGGCCCCTAAGCTCCTGATCTATGATACAAATCGCTTGGCAGATGGGGTCCCATCACGGTTCAGTGGCTCTGGATCTGGCACACAGTATACTCTAACCATAAGTAGCCTGCAACCCGAAGATTTCGCAACCTATTATTGTCAACACTATAAGAATTATCCGCTCACGTTCGGTCAGGGGACCAAGCTGGAAATCAAAAGATCTGGTGGCGGAGGGTCAGGAGGCGGAGGCAGCGGAGGCGGTGGCTCGGGAGGCGGAGGCTCGAGATCTGAGGTGCAGTTGGTGGAGTCTGGGGGCGGCTTGGTGCAGCCTGGAGGGTCCCTGAGGCTCTCCTGTGCAGCCTCAGGATTCACTCTCAGTAATTATGGCATGCACTGGATCAGGCAGGCTCCAGGGAAGGGTCTGGAGTGGGTCTCGTCTATTAGTCTTAATGGTGGTAGCACTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGGGACAATGCAAAAAGCACCCTCTACCTTCAAATGAATAGTCTGAGGGCCGAGGACACGGCCGTCTATTACTGTGCAGCACAGGACGCTTATACGGGAGGTTACTTTGATTACTGGGGCCAAGGAACGCTGGTCACAGTCTCGTCTATGGATCCCGCCACCACAACCAAGCCCGTGCTGCGGACCCCAAGCCCTGTGCACCCTACCGGCACCAGCCAGCCTCAGAGACCCGAGGACTGCCGGCCTCGGGGCAGCGTGAAGGGCACCGGCCTGGACTTCGCCTGCGACATCTACTGGGCACCTCTGGCCGGAATATGCGTGGCACTGCTGCTGAGCCTCATCATCACCCTGATCTGTTATCACCGAAGCCGCAAGCGGGTGTGTAAAAGTGGAGGCGGAAGCTTCTGGGAGGAGTTTGAGAGTTTGCAGAAGCAGGAGGTGAAGAACTTGCACCAGCGTCTGGAAGGGCAGCGGCCAGAGAACAAGGGCAAGAACCGCTACAAGAACATTCTCCCCTTTGACCACAGCCGAGTGATCCTGCAGGGACGGGACAGTAACATCCCCGGGTCCGACTACATCAATGCCAACTACATCAAGAACCAGCTGCTAGGCCCTGATGAGAACGCTAAGACCTACATCGCCAGCCAGGGCTGTCTGGAGGCCACGGTCAATGACTTCTGGCAGATGGCGTGGCAGGAGAACAGCCGTGTCATCGTCATGACCACCCGAGAGGTGGAGAAAGGCCGGAACAAATGCGTCCCATACTGGCCCGAGGTGGGCATGCAGCGTGCTTATGGGCCCTACTCTGTGACCAACTG

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[0457] SEQ ID No.39

[0458] >MP16091.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1tm-endo

[0459] ATGAGCCTGCCCGTGACCGCCCTGCTGCTGCCCCTGGCCCTGCTGCTGCACGCCGCCAGACCAGACATCCAGATGACCCAGACCACCAGCAGCCTGAGCGCCAGCCTGGGCGACCGGGTGACCATCAGCTGCAGAGCCAGCCAGGACATCAGCAAGTACCTGAACTGGTACCAGCAGAAGCCCGACGGCACCGTGAAGCTGCTGATCTACCACACCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTCAGCGGCAGCGGCAGCGGCACCGACTACAGCCTGACCATCAGCAACCTGGAGCAGGAGGACATCGCCACCTACTTCTGCCAGCAGGGCAACACCCTGCCCTACACCTTCGGAGGCGGCACCAAGCTGGAGATCACCAAGGCCGGAGGCGGAGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCGAGGTGAAGCTGCAGGAGTCTGGCCCAGGCCTGGTGGCCCCAAGCCAGAGCCTGAGCGTGACCTGCACCGTGAGCGGCGTGAGCCTGCCCGACTACGGCGTGAGCTGGATCAGGCAGCCCCCACGGAAGGGCCTGGAGTGGCTGGGCGTGATCTGGGGCAGCGAGACCACCTACTACAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGCAAGAGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTACTACTGCGCCAAGCACTACTACTATGGCGGCAGCTACGCTATGGACTACTGGGGCCAGGGCACCAGCGTGACCGTGAGCTCAGATCCCACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCTCCTCGCAGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGCCGTGCCCACTCAGGTCCTGGGGTTGTTGCTACTGTGGCTTACAGATGCCAGATGTGACATCCAGATGACACAGTCTCCATCTTCCCTGTCTGCATCTGTCGGAGATCGCGTCACCATCACCTGTCGAGCAAGTGAGGACATTTATTTTAATTTAGTGTGGTATCAGCAGAAACCAGGAAAGGCCCCTAAGCTCCTGATCTATGATACAAATCGCTTGGCAGATGGGGTCCCATCACGGTTCAGTGGCTCTGGATCTGGCACACAGTATACTCTAACCATAAGTAGCCTGCAACCCGAAGATTTCGCAACCTATTATTGTCAACACTATAAGAATTATCCGCTCACGTTCGGTCAGGGGACCAAGCTGGAAATCAAAAGATCTGGTGGCGGAGGGTCAGGAGGCGGAGGCAGCGGAGGCGGTGGCTCGGGAGGCGGAGGCTCGAGATCTGAGGTGCAGTTGGTGGAGTCTGGGGGCGGCTTGGTGCAGCCTGGAGGGTCCCTGAGGCTCTCCTGTGCAGCCTCAGGATTCACTCTCAGTAATTATGGCATGCACTGGATCAGGCAGGCTCCAGGGAAGGGTCTGGAGTGGGTCTCGTCTATTAGTCTTAATGGTGGTAGCACTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGGGACAATGCAAAAAGCACCCTCTACCTTCAAATGAATAGTCTGAGGGCCGAGGACACGGCCGTCTATTACTGTGCAGCACAGGACGCTTATACGGGAGGTTACTTTGATTACTGGGGCCAAGGAACGCTGGTCACAGTCTCGTCTATGGATCCCGCCACCACAACCAAGCCCGTGCTGCGGACCCCAAGCCCTGTGCACC

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[0460] SEQ ID no.40

[0461] >MP16092.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1tm-endo-2A-PTPN6_SH2-dCD148

[0462] ATGAGCCTGCCCGTGACCGCCCTGCTGCTGCCCCTGGCCCTGCTGCTGCACGCCGCCAGACCAGACATCCAGATGACCCAGACCACCAGCAGCCTGAGCGCCAGCCTGGGCGACCGGGTGACCATCAGCTGCAGAGCCAGCCAGGACATCAGCAAGTACCTGAACTGGTACCAGCAGAAGCCCGACGGCACCGTGAAGCTGCTGATCTACCACACCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTCAGCGGCAGCGGCAGCGGCACCGACTACAGCCTGACCATCAGCAACCTGGAGCAGGAGGACATCGCCACCTACTTCTGCCAGCAGGGCAACACCCTGCCCTACACCTTCGGAGGCGGCACCAAGCTGGAGATCACCAAGGCCGGAGGCGGAGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCGAGGTGAAGCTGCAGGAGTCTGGCCCAGGCCTGGTGGCCCCAAGCCAGAGCCTGAGCGTGACCTGCACCGTGAGCGGCGTGAGCCTGCCCGACTACGGCGTGAGCTGGATCAGGCAGCCCCCACGGAAGGGCCTGGAGTGGCTGGGCGTGATCTGGGGCAGCGAGACCACCTACTACAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGCAAGAGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTACTACTGCGCCAAGCACTACTACTATGGCGGCAGCTACGCTATGGACTACTGGGGCCAGGGCACCAGCGTGACCGTGAGCTCAGATCCCACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCTCCTCGCAGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGCCGTGCCCACTCAGGTCCTGGGGTTGTTGCTACTGTGGCTTACAGATGCCAGATGTGACATCCAGATGACACAGTCTCCATCTTCCCTGTCTGCATCTGTCGGAGATCGCGTCACCATCACCTGTCGAGCAAGTGAGGACATTTATTTTAATTTAGTGTGGTATCAGCAGAAACCAGGAAAGGCCCCTAAGCTCCTGATCTATGATACAAATCGCTTGGCAGATGGGGTCCCATCACGGTTCAGTGGCTCTGGATCTGGCACACAGTATACTCTAACCATAAGTAGCCTGCAACCCGAAGATTTCGCAACCTATTATTGTCAACACTATAAGAATTATCCGCTCACGTTCGGTCAGGGGACCAAGCTGGAAATCAAAAGATCTGGTGGCGGAGGGTCAGGAGGCGGAGGCAGCGGAGGCGGTGGCTCGGGAGGCGGAGGCTCGAGATCTGAGGTGCAGTTGGTGGAGTCTGGGGGCGGCTTGGTGCAGCCTGGAGGGTCCCTGAGGCTCTCCTGTGCAGCCTCAGGATTCACTCTCAGTAATTATGGCATGCACTGGATCAGGCAGGCTCCAGGGAAGGGTCTGGAGTGGGTCTCGTCTATTAGTCTTAATGGTGGTAGCACTTACTATCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGGGACAATGCAAAAAGCACCCTCTACCTTCAAATGAATAGTCTGAGGGCCGAGGACACGGCCGTCTATTACTGTGCAGCACAGGACGCTTATACGGGAGGTTACTTTGATTACTGGGGCCAAGGAACGCTGGTCACAGTCTCGTCTATGGATCCCGCCACCACAACCAAGCCCGTGCTGCGGACCCCAAGCCCTGTGCACC

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CATCGCCAGCGGTAGCTAA

[0463] 核酸序列编码的氨基酸序列可以与SEQ ID No.35,36,37,38,39或40编码的氨基酸序列相同,但由于遗传密码的简并性可以具有不同的核酸序列。核酸序列可具有与SEQ ID No.35,36,37,38,39或40中显示的序列至少80％,85,90,95,98或99％的同一性,条件是它编码如在本发明的第一方面限定的第一CAR和第二CAR。

[0464] 载体

[0465] 本发明还提供包含一个或多个CAR编码核酸序列的载体或载体试剂盒。这样的载体可用于将核酸序列导入宿主细胞中,使得其表达第一和第二CAR。

[0466] 载体可以是例如质粒或病毒载体,例如逆转录病毒载体或慢病毒载体,或基于转座子的载体或合成的mRNA。

[0467] 载体可以能够转染或转导的T细胞。

[0468] 药物组合物

[0469] 本发明还涉及含有根据本发明的第一方面的多个CAR表达细胞,例如T细胞或NK细胞的药物组合物。药物组合物可另外包含可药用的载体,稀释剂或赋形剂。药物组合物可任选包含一种或多种其它药物活性多肽和/或化合物。这样的制剂可以例如是适合于静脉内输注的形式。

[0470] 治疗方法

[0471] 本发明的T细胞可以能够杀死靶细胞，例如癌细胞。靶细胞可以通过限定的抗原表达模式识别，例如抗原A和抗原B的表达；抗原A或抗原B的表达；或抗原A和非抗原B的表达或这些门的复杂迭代。

[0472] 本发明的T细胞可用于感染如病毒感染的治疗。

[0473] 本发明的T细胞也可以用于控制病原性免疫应答，例如，自身免疫性疾病，变态反应和移植抗宿主排斥。

[0474] 本发明的T细胞可以用于治疗癌性疾病，如膀胱癌，乳腺癌，结肠癌，子宫内膜癌，肾癌(肾细胞)，白血病，肺癌，黑素瘤，非霍奇金淋巴瘤，胰腺癌，前列腺癌和甲状腺癌。

[0475] 它特别适合于治疗实体瘤，其中良好选择性的单一靶标的利用度是有限的。

[0476] 本发明的T细胞可用于治疗：口腔和咽的癌症，包括舌癌，口腔癌和咽癌；消化系统的癌症，其包括食管癌，胃癌和结肠直肠癌；肝脏和胆道系统的癌症，其包括肝细胞癌和胆管上皮癌(cholangiocarcinoma)；呼吸系统的癌症，其包括支气管癌和喉癌；骨和关节的癌症，包括骨肉瘤；皮肤癌，其包括黑素瘤；乳腺癌；生殖道的癌症，其包括在女性中的子宫癌，卵巢癌和宫颈癌，以及在男性中的前列腺癌和睾丸癌；肾道的癌症，其包括肾细胞癌，尿道或膀胱的移行细胞癌；脑癌，其包括胶质瘤，多行性胶质母细胞瘤和髓母细胞瘤(medullobastomas)；内分泌系统的癌症，包括甲状腺癌，肾上腺癌和与多发性内分泌腺新生物综合征相关的癌症；淋巴瘤，其包括霍奇金淋巴瘤和非霍奇金淋巴瘤；多发性骨髓瘤和浆细胞瘤；急性和慢性白血病，髓样或淋巴性白血病；以及其他的未指明位点的癌症，包括神经母细胞瘤。

[0477] 用本发明的T细胞进行的治疗可以帮助防止在标准方法中经常发生的肿瘤细胞的逃逸或释放。

[0478] 本发明现在将通过实施例进一步描述，所述实施例意在用于协助本领域普通技术人员中实施本发明，并且不是意在以任何方式限制本发明的范围。

实施例

[0479] 实施例1：靶细胞群的产生

[0480] 为了证明本发明的原理的目的，任意地选择了基于抗CD19和抗CD33的受体。使用逆转录病毒载体，克隆了CD19和CD33。将这些蛋白质截短，以便它们不能发信号并且可以长时段稳定地表达。接着，单一或双重使用这些载体来转导SupT1细胞系以建立针对两种抗原呈阴性(野生型)，对任一种抗原呈阳性和对两种抗原呈阳性的细胞。表达数据显示于图3。

[0481] 实施例2：或门的设计和功能

[0482] 为了构建或门，共表达识别CD19和CD33的一对受体。将不同的间隔区用于防止交叉配对。这两种受体具有来源于CD28的用于改善表面稳定性的跨膜域和来源于CD3Zeta的内域的用于提供简单的活化信号的内域。以这种方式，共表达了一对独立的第1代CAR。用于共表达序列的逆转录病毒载体盒利用口蹄疫病毒2A自我切割肽，以允许共表达1:1的两种受体。盒设计显示于图4，并且蛋白质结构显示于图5中。同源区的核苷酸序列是密码子摆动的，以防止在逆转录病毒载体逆转录过程中的重组。

[0483] 实施例3：测试或门

[0484] 通过用融合至Fc的相关抗原染色在T细胞表面上测试两种CAR的表达。通过使用不同种类的Fc域(对于CD19为小鼠并且对于CD33为兔),通过用缀合有不同荧光团的不同二抗染色在细胞表面上确定两种CAR的共表达。这显示于图6。

[0485] 然后使用小鼠T细胞系BW5147进行功能测试。这种细胞系在活化后释放IL2,以允许简单的定量读数。这些T细胞与增加量的上述人工靶细胞共培养。如通过ELISA测量的IL2释放显示的,T细胞响应表达任一抗原的靶细胞。显示两种CAR在细胞表面上表达,并且显示T细胞响应任一种或两种抗原。这些数据显示在图7中。

[0486] 实施例4:和门的设计和功能

[0487] 和门组合简单活化受体与基本上抑制活性但其活性在连接受体后被关闭的受体。这通过组合标准的第一代CAR与第二受体实现,所述第一代CAR具有短/非庞大的CD8茎间隔区和CD3Zeta内域,所述第二受体具有庞大的Fc间隔区,它的内域包含CD148或CD45内域。当连接两个受体时,间隔区尺寸的差异导致不同受体在不同膜区室中的分离,从而使CD3Zeta受体从由CD148或CD45内域导致的抑制中解除出来。以这种方式,仅在两种受体被活化时发生活化。对此选择了CD148和CD45,因为它们天然地在这种方式发挥功能:例如,非常庞大的CD45胞外域将整个受体排除在免疫突触外。表达盒描述在图8中并且在图9中描述了后续蛋白质。

[0488] 针对不同的特异性的表面染色显示,两种受体对可以在图10中显示的细胞表面上有效地表达。在BW5147中的功能显示T细胞仅在两种抗原的存在下才被活化(图11)。

[0489] 实施例5:和门的一般化的证明

[0490] 为了确保观察结果不是CD19/CD33和已经使用的其结合剂的一些特定特征的表现,交换了两个靶向性scFv,使得现在活化(ITAM)信号在识别CD33,而不是CD19后传输;并且抑制信号(CD148)在识别CD19而不是CD33后传输。因为认为CD45和CD148内域在功能上类似,所以将实验限制到具有CD148内域的和门。这应当仍然导致功能性和门。用具有单独的CD19或CD33或两者的靶标攻击表达新逻辑门的T细胞。T细胞响应同时表达CD19和CD33的靶标,但不响应只表达这些抗原中的一种或不表达这些抗原的靶标。这表明,和门在此形式中仍是有功能的(图18B)。

[0491] 以同样的思路,试图确立如何一般化我们的和门:和门应当在不同的靶标间一般化。虽然门给出的相对抗原密度,相关scFv结合动力学和scFv结合表位的精确距离可能具有更低或更高的保真度,但将预期用一大批靶物和结合剂看到一些和门的表现。为了测试这点,产生了三种另外的和门。再一次,将实验限制到CD148形式的和门。将来自原始的CD148和门的第二scFv替换成抗GD2scFv huK666(SEQ ID41和SEQ ID42),或抗CD5scFv(SEQ ID43和SEQ ID44),或抗EGFRvIII scFv MR1.1(SEQ ID45和SEQ ID46),以产生以下CAR和门:CD19和GD2;CD19和CD5;CD19和EGFRvIII。也产生了下面的人工抗原表达细胞系:通过用GM3和GD2合酶转导SupT1和我们的SupT1.CD19,产生了SupT1.GD2和SupT1.CD19.GD2。通过用编码EGFRvIII的逆转录病毒载体转导SupT1和SupT1.CD19产生了SupT1.EGFRvIII和SupT1.CD19.EGFRvIII。因为CD5在SupT1细胞上表达,所以将不同的细胞系用于产生靶细胞:产生了表达单独的CD19,单独的CD5和CD5和CD19两者的293T细胞。表达通过流式细胞术确认(图19)。用SupT1.CD19和各自的相关双重阳性和单阳性靶细胞攻击表达三种新CAR和门的T细胞。所有三种和门显示与单阳性靶标相比通过双重阳性细胞系导致的活化降低(图

20)。这显示了和门设计至任意靶标和相关结合剂的一般化。

[0492] 实施例6:CAR和门的动力学分离模型的实验证据

[0493] 目的是要证明下述模型,即通过不同间隔区引起的差别分离是产生这些逻辑CAR门的能力背后的中心机制。该模型是,如果仅连接活化性CAR,那么有力的的抑制性“连接关闭”型CAR在膜中在溶液中,并且可以抑制活化性CAR。一旦两个CAR均连接,若两个CDR间隔区充分不同,那么它们将在突触内分离并且不会共定位。因此,关键的需求是间隔区是充分不同的。如果模型是正确的,如果这两个间隔物充分相似,从而当连接两个受体时它们共定位,那么门将不能发挥功能。为了验证这一点,我们用鼠CD8间隔区替换了原始CAR中的庞大Fc间隔区。预测这具有与人CD8类似的长度,体积和电荷,但因此不应与它交叉配对。因此,新的门具有识别CD19的第一CAR,人CD8茎间隔区和活化性内域;而第二CAR识别CD33,具有小鼠CD8茎间隔区和CD148内域(图18C)。转导T细胞以表达这种新的CAR门。然后用表达单独的CD19,单独的CD33或一起的CD19和CD33的SupT1细胞攻击这些T细胞。按照原始的和门,T细胞不响应单独表达任一种抗原的SupT1细胞。然而,CAR T细胞不能响应表达两种抗原的SupT1细胞,从而证实了模型(图18C)。功能性和门需要两种CAR具有充分不同的间隔区,使得它们不在免疫突触内共定位(图23A和B)。

[0494] 实施例7:和非门的设计和功能

[0495] 磷酸酶如CD45和CD148是如此有力的,以致少量进入了免疫突触的磷酸酶能抑制ITAM活化。这是逻辑和门抑制的基础。其他类别的磷酸酶不是如此有力,例如PTPN6及相关磷酸酶。据预测,通过扩散进入突触的少量PTPN6不会抑制活化。此外,据预测,如果抑制性CAR与活化性CAR具有充分相似的间隔区,那么若连接这两个CAR,则它可以在突触内共定位。在这种情况下,当两种抗原均存在时,大量的抑制性内域将足以停止ITAMS活化。以这种方式,可以创建和非门。

[0496] 对于非和门,第二信号需要“否决(veto)”活化。这是通过使抑制性信号进入免疫突触中完成的,例如通过引入酶的磷酸酶,如PTPN6完成。我们因此产生如下的和非门:两个CAR共表达,其中第一个识别CD19,具有人CD8茎间隔区和活化性内域;与抗CD33CAR共表达,所述抗CD33CAR具有小鼠CD8茎间隔区和由PTPN6的催化域构成的内域(SEQ ID 38,图13A和B)。合适的盒显示于图12,并且初步功能数据显示于图14。

[0497] 此外,发展了一种用于产生和非门的备选策略。免疫酪氨酸酶抑制基序(ITIM)以与ITAMS类似的方式活化,因为它们在聚簇和磷酸酶排斥后通过lck磷酸化。代替通过结合ZAP70触发活化,磷酸化的ITIM通过它们的相关SH2域募集磷酸酶,如PTPN6。ITIM可以作为抑制性内域发挥功能,只要活化和抑制性CAR上的间隔区可以共定位。为了产生这种构建体,如下产生了和非门:两个CAR共表达,其中第一个识别CD19,具有人CD8茎间隔区和活化内域;与抗CD33CAR共表达,所述抗CD33CAR具有小鼠CD8茎间隔区和来源于LAIR1的内源的含有ITIM的内域(SEQ ID 39,图13A和C)。

[0498] 也开发了另外的更复杂的和非门,其中ITIM通过额外的嵌合蛋白质的存在而增强:PTPN6的SH2域和CD148的内域的胞内融合物。在这种设计中表达三种蛋白质-第一种识别CD19,具有人CD8茎间隔区和活化内域;与抗CD33CAR共表达,所述抗CD33CAR具有鼠CD8茎间隔区和来源于LAIR1的内域的含有ITIM的内域。另外的2A肽允许PTPN6-CD148融合物的共表达(SEQ ID40,图13A和D)。预测这些和非门将具有不同程度的抑制:PTPN6-CD148>PTPN6>

>ITIM。

[0499] 用这些门转导T细胞并用表达单独的任一种CD19或CD33或一起的CD19和CD33两者的靶标攻击T细胞。所有三个门响应仅表达CD19的靶标，但不响应表达一起的CD19和CD33两者的靶标(图21)，证实全部三种和非门均是有功能的。

[0500] 实施例8：基于PTPN6的和非门的动力学分离模型的实验证据

[0501] 和非门的模型集中于这样的事实，即在两个CAR中使用的间隔区的性质对于门的正确功能是关键的。在具有PTPN6的功能性和非门中，两个CAR间隔区充分相似，使得当两个CAR连接时，这两者在突触内以如此高的浓度共定位，即使弱PTPN6足以抑制活化。如果间隔区是不同的，那么在突触中的分离会使PTPN6与ITAM分离，从而允许扰乱和非门的活化。为了测试这一点，产生用Fc间隔区替换鼠CD8茎间隔区的对照。在这种情况下，测试门由两个CAR组成：第一识别CD19，具有人CD8茎间隔区和ITAM内域；而第二CAR识别CD33，具有Fc间隔区和由来自PTPN6的磷酸酶构成的内域。这种门响应CD19而活化，但也响应一起的CD19和CD33而活化(图22B，其中这种门的功能与原始和非，和在实施例6中描述的对照和门变体比较)。这种实验数据证明了以下模型：对于具有PTPN6的功能性和非门，需要共定位的间隔区。

[0502] 实施例9：基于ITIM的和非门的动力学分离模型的实验证据

[0503] 与基于PTPN6的和非门相似，基于ITIM的门也需要在免疫突触中共定位以作为和非门发挥功能。为了证明这一假设，如下产生了基于ITIM的对照门：两个CAR共表达-第一种识别CD19，具有人CD8茎间隔区和活化性内域；与抗CD33CAR共表达，所述抗CD33CAR具有Fc间隔区和来源于LAIR1的内域的含有ITIM的内域。将这种门的活性与原始的基于ITTM的和非门的活性相比较。在这种情况下，经修饰的门响应于表达CD19的靶标而活化，但也响应于表达CD19和CD33两者的细胞而活化。这些数据表明，基于ITIM的和非门遵循基于动力学分离的模型，并且必须选择正确的间隔区以创建功能性门(图23B)。

[0504] 实施例10：通过动力学分离产生的CAR逻辑门的模型的总结

[0505] 基于动力学分离模型和本文所描述的实验数据的正确理解，在图24中呈现了两个CAR门模型的总结。此图显示表达两种CAR的细胞，每种CAR识别不同抗原。当任一或两个CAR识别细胞上的靶抗原时，形成了突触，并且天然CD45和CD148由于其庞大的胞外域而被排除在突触之外。这设置T细胞活化阶段。在靶细胞仅具有一种相关抗原的情况下，相关CAR连接，并且相关CAR分离到突触中。未连接的CAR仍然保留在T细胞膜上的溶液中，并且可以扩散进出突触，使得形成了连接的CAR的高局部浓度区域与未连接的CAR的低浓度的区域。在这种情况下，如果连接的CAR具有ITAM，并且未连接的CAR具有“连接关闭”类型的抑制性内域如CD148的内域，那么未连接的CAR的量足以抑制活化并且门是关闭的。与此相反，在此情况下，如果连接的CAR具有ITAM，并且未连接的CAR具有“连接开启”类型的抑制内域如PTPN6，那么未连接的CAR的量不足以抑制活化并且门是开启的。当受到具有两种相关抗原的靶细胞攻击时，连接了这两个相关的CAR并且形成免疫突触的一部分。重要的是，如果CAR间隔区足够相似，那么CAR在突触内共定位，但如果CAR间隔区足够不同，CAR在突触内分离。在后一种情况下，形成了膜区域，其中存在高浓度的一种CAR，但是另一种CAR是不存在的。在这种情况下，由于分离完成，即使抑制性内域是“连接关闭”类型，门仍是开启的。在前者的情况下，形成了高浓度的两种CAR混合在一起的膜区域。在这种情况下，由于两个内域都

被浓缩，即使抑制内域是“连接开启”类型，门仍关闭。通过选择间隔区和内域的正确组合，可将逻辑编程到CAR T细胞中。

[0506] 基于我们的以上工作，我们已经建立了一系列的设计规则，以允许产生逻辑门控的CAR（如图32所示）。为产生“抗原A或抗原B”门控的CAR T细胞，必须产生抗A和抗B CAR，使得(1)每一CAR具有仅允许抗原访问和突触形成的间隔区，使得CAR发挥功能，和(2)每一CAR具有活化性内域；为了产生“抗原A和非B”门控的CAR T细胞，必须产生抗A和抗B CAR，使得(1)这两个CAR具有间隔区，所述间隔物不交叉配对，但会允许CAR在识别靶细胞上的两个相关抗原后共分离，(2)并且一个CAR具有活化性内域，而另一个CAR具有包含或募集弱磷酸酶(例如PTPN6)的内域；(3)为了产生“抗原A和抗原B”门控的CAR T细胞，必须产生抗A和抗B CAR，使得(1)一个CAR具有与另一个CAR足够不同的间隔区，使得在识别靶细胞上的两个相关抗原后，两个CAR不会共分离，(2)一个CAR具有活化性内域，而另一个CAR具有由有力的磷酸酶构成的内域(例如，CD45或CD148的内域)。实现期望的效应的正确的间隔区可以从一组具有已知尺寸/形状等的间隔区选择，同时考虑靶抗原的尺寸/形状等和靶抗原上相关表位的位置。

[0507] SEQ ID No 41:

[0508] SFG.aCD19-CD8STK-CD28tmZ-2A-aGD2-HCH2CH3pvaa-dCD148

[0509] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMETDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGSTNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKAVFGCIFGALVIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIA

[0510] SEQ ID No.42:

[0511] SFG.aCD19-CD8STK-CD28tmZ-2A-aGD2-HCH2CH3pvaa-dCD148

[0512] ATGAGCCTGCCCGTGACCGCCCTGCTGCTGCCCCTGGCCCTGCTGCTGCACGCCGCCAGACCAGACATCCAGATGACCCAGACCACCAGCAGCCTGAGCGCCAGCCTGGGCGACCGGGTGACCATCAGCTGCAGAGCCAGCCAGGACATCAGCAAGTACCTGAACTGGTACCAGCAGAAGCCCGACGGCACCGTGAAGCTGCTGATCTACCACACCAGCCGGC

TGCACAGCGGCGTGCCCAGCCGGTTCAGCGGCAGCGGCAGCGGCACCGACTACAGCCTGACCATCAGCAACCTGGAG
CAGGAGGACATCGCCACCTACTTCTGCCAGCAGGGCAACACCCTGCCCTACACCTTCGGAGGCGGCACCAAGCTGGA
GATCACCAAGGCCGGAGGCGGAGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCGAGG
TGAAGCTGCAGGAGTCTGGCCCAGGCCTGGTGGCCCCAAGCCAGAGCCTGAGCGTGACCTGCACCGTGAGCGGCGTG
AGCCTGCCCGACTACGGCGTGAGCTGGATCAGGCAGCCCCCACGGAAGGGCCTGGAGTGGCTGGGCGTGATCTGGGG
CAGCGAGACCACCTACTACAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGCAAGAGCCAGGTGT
TCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTACTACTGCGCCAAGCACTACTACTATGGCGGCAGC
TACGCTATGGACTACTGGGGCCAGGGCACCAGCGTGACCGTGAGCTCAGATCCCACCACGACGCCAGCGCCGCGACC
ACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCG
CAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGC
TATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCC
CGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACA
AGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTG
CAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGG
CCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCTCCTCGCAGAG
CCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGAGACCGACACCCTGCTG
CTGTGGGTGCTGCTGCTGTGGGTGCCAGGCAGCACCGGCCAGGTGCAGCTGCAGGAGTCTGGCCCAGGCCTGGTGAA
GCCCAGCCAGACCCTGAGCATCACCTGCACCGTGAGCGGCTTCAGCCTGGCCAGCTACAACATCCACTGGGTGCGGC
AGCCCCCAGGCAAGGGCCTGGAGTGGCTGGGCGTGATCTGGGCTGGCGGCAGCACCAACTACAACAGCGCCCTGATG
AGCCGGCTGACCATCAGCAAGGACAACAGCAAGAACCAGGTGTTCCTGAAGATGAGCAGCCTGACAGCCGCCGACAC
CGCCGTGTACTACTGCGCCAAGCGGAGCGACGACTACAGCTGGTTCGCCTACTGGGGCCAGGGCACCCTGGTGACCG
TGAGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCTCTGGCGGAGGCGGCAGCGAGAACCAGATGACCCAGAGCCCC
AGCAGCTTGAGCGCCAGCGTGGGCGACCGGGTGACCATGACCTGCAGAGCCAGCAGCAGCGTGAGCAGCAGCTACCT
GCACTGGTACCAGCAGAAGAGCGGCAAGGCCCCAAAGGTGTGGATCTACAGCACCAGCAACCTGGCCAGCGGCGTGC
CCAGCCGGTTCAGCGGCAGCGGCAGCGGCACCGACTACACCCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCC
ACCTACTACTGCCAGCAGTACAGCGGCTACCCCATCACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGCGGTCGGA
TCCCGCCGAGCCCAAATCTCCTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTCCCGTGGCCGGCCCGTCAG
TCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCGCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCC
GCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
CCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCT
GGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAACCGGAGAACAACTACAAGACCA
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[0513] SEQ ID No.43:

[0514] SFG.aCD19-CD8STK-CD28tmZ-2A-aCD5-HCH2CH3pvaa-dCD148

[0515] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLI
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VAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
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[0516] SEQ ID No.44:

[0517] SFG.aCD19-CD8STK-CD28tmZ-2A-aCD5-HCH2CH3pvaa-dCD148

[0518] ATGAGCCTGCCCGTGACCGCCCTGCTGCTGCCCCTGGCCCTGCTGCTGCACGCCGCCAGACCAGACATC
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[0519] SEQ ID No.45:

[0520] SFG.aCD19-CD8STK-CD28tmZ-2A-aEGFRvIII-HCH2CH3pvaa-dCD148

[0521] MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMETDTLLLWVLLLWVPGSTGQVKLQQSGGGLVKPGASLKLSCVTSGFTFRKFGMSWVRQTSDKRLEWVASISTGGYNTYYSDNVKGRFTISRENAKNTLYLQMSSLKSEDTALYYCTRGYSSTSYAMDYWGQGTTVTVSSGGGGSGGGGSGGGGSDIELTQSPASLSVATGEKVTIRCMTSTDIDDDMNWYQQKPGEPPKFLISEGNTLRPGVPSRFSSSGTGTDFVFTIENTLSEDVGDYYCLQSFNVPLTFGDGTKLEIKRSDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKAVFGCIFGALVIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIA

[0522] SEQ ID No.46:

[0523] SFG.aCD19-CD8STK-CD28tmZ-2A-aEGFRvIII-HCH2CH3pvaa-dCD148

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[0525] 实施例11:基于APRIL的CAR的设计和构建

[0526] 天然形式的APRIL是一种分泌性II型蛋白。将APRIL用作CAR的BCMA结合域需要将这种II型分泌蛋白转化成I型膜结合蛋白,并且对于这种蛋白质是稳定的,并在这种形式中保留对BCMA的结合。为了产生候选分子,将APRIL的最终氨基末端删除以除去与蛋白聚糖的结合。接着,加入信号肽以将新生蛋白质引导至内质网,从而引导至细胞表面。此外,由于使用的间隔区的性质可以改变CAR的功能,测试了三种不同的间隔区域:产生了基于APRIL的CAR,其包含(i)经改变以除去Fc结合基序的人IgG1间隔区;(ii)CD8茎;和(iii)仅IgG1铰链(草图25中的图和图26中的氨基酸序列)。这些CAR在双顺反子逆转录病毒载体中表达(图27A),使得标记蛋白—截短的CD34可共表达为一种方便的标记基因。

[0527] 实施例12:基于APRIL的CAR的表达和功能

[0528] 本研究的目的是测试已经构建的基于APRIL的CAR是否在细胞表面上表达和APRIL是否已经折叠以形成天然蛋白。用这些不同的CAR构建体转导T细胞,并使用市售的抗APRIL mAb染色以及对标记基因染色,并通过流式细胞术分析。实验结果显示于图27B,其中将APRIL结合对标记基因荧光作图。这些数据显示,在这种形式中,基于APRIL的CAR是细胞表面上表达,并且APRIL充分折叠从而通过抗APRIL mAb识别。

[0529] 接着,确定这种形式中的APRIL是否可识别BCMA和TACI。以与小鼠IgG2a-Fc的融合物产生了重组BCMA和TACI。将这些重组蛋白与经转导的T细胞一起温育。在此之后,洗涤细胞并用抗小鼠荧光团缀合的抗体和检测缀合到不同荧光团的标记基因的抗体对细胞染色。通过流式细胞术分析细胞,结果在图27C中呈现。不同的CAR能够同时结合BCMA和TACI。出人意料地,相比于TACI,CAR能够更好地结合BCMA。此外,出人意料地,相比于具有Fc间隔区的CAR,具有CD8茎或IgG1铰链间隔区的CAR能够更好地结合BCMA和TACI。

[0530] 实施例13:基于APRIL的嵌合抗原受体针对BCMA表达性细胞是有活性的

[0531] 用不同APRIL CAR转导来自正常供体的T细胞,并且针对野生型或经工程化改造表达BCMA和TACI的SupT1细胞进行测试。使用几种不同的测定法来确定功能。进行了经典的铬释放测定法。这里,用51Cr标记靶细胞(SupT1细胞)并以不同比例与效应物(经转导的T细胞)混合。靶细胞的裂解通过计数共培养物上清液中的51Cr确定(图28A显示了累积数据)。

[0532] 此外,通过ELISA测定了来自以1:1与SupT1细胞一起培养的T细胞的上清液中的γ干扰素(图28B显示出了累积数据)。也进行了与SupT1细胞共培养一周后的T细胞扩增的测量(图28C)。通过用计数珠校准的流式细胞术对T细胞计数。这些实验数据显示,基于APRIL的CAR可以杀死表达BCMA的靶标。此外,这些数据显示,基于CD8茎或IgG1铰链的CAR比基于Fc-pvaa的CAR表现得更好。

[0533] 实施例14:和门在原代细胞中的功能分析

[0534] 从血液中分离PBMC,并使用PHA和IL-2刺激。两天后,在Retronectin包被的板上用含有CD19:CD33和门构建体的逆转录病毒转导细胞。在第5天,通过流式细胞术评估了通过

和门构建体翻译的两种CAR的表达水平并且细胞消耗了CD56+的细胞(主要是NK细胞)。在第6天,以1:2的效应物比靶细胞的比例使PBMC与靶细胞共培养。在第8天收集上清液并通过ELISA分析IFN-γ的分泌(图29)。

[0535] 这些数据证明和门在原代细胞中发挥功能。

[0536] 实施例15:测试具有延长的间隔区的和非门

[0537] 为了测试和非门是否可以在延长的间隔区长度上发挥功能,将激活性CAR(抗CD19)和抑制性CAR(抗CD33)间隔区两者取代为较长的间隔区。将人IgM和IgG的Fc区用于延长间隔区的长度。IgM的Fc含有相比于IgG的额外的Ig域,由于此原因将IgM的间隔区放置在已知具有膜近端结合表位的抗CD19CAR上。相反,抗CD33结合表位位于该分子的远端末端,因此在此CAR上使用在相对较短的IgG间隔区(参见图30)。将延长间隔区的和非门构建体转导到小鼠的T细胞系中。然后将固定数量的经转导的T细胞与可变数量的靶细胞共培养16-24小时,在此之后通过ELISA测定在上清液中分泌的IL-2的量。

[0538] 结果显示于图30中。具有IgG/IgM间隔区对的和非门运行良好。

[0539] 实施例16:测试和非门平台的稳健性(robustness)

[0540] 为了测试和非门平台的稳健性,用两个其他的不相关的结合剂(抗GD2和抗EGFRvIII)取代来自抑制性CAR(抗CD33)的结合域。在具有截短SHP-1或LAIR胞质域的和非门平台中,将抗GD2或抗EGFRvIII的scFv片段取代为抗CD33。将这些构建体转导到小鼠T细胞系中,并且将固定数量的T细胞与可变数量的靶细胞共培养。在共培养16-24小时后,通过ELISA分析在上清液中分泌的IL-2的量。

[0541] 结果显示于图31中。具有截短的SHP-1或LAIR胞质域的和非门与抗CD19/抗GD2结合剂和抗CD19/抗EGFRvIII结合剂一起运行良好。

[0542] 将上述说明书中提及的所有出版物并入本文作为参考。对描述的本发明的方法和系统的多种修改和变化在不背离本发明的范围和精神的情况下对于本领域技术人员是显而易见的。尽管已经结合具体优选的实施方案描述了本发明,但应当理解,要求保护的本发明不应当不适当地受限于这些具体实施方案。实际上,对描述的用于实施本发明的模式的多种修改对于分子生物学,细胞生物学或相关领域的技术人员是显而易见的,预期落在所附权利要求书的范围之内。

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<120> T细胞

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Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

[0002] Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

[0003] Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

```
Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
        435                 440                 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
    450                 455                 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465                 470                 475                 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
                485                 490                 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
            500                 505                 510

[0004] Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
        515                 520                 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
    530                 535                 540

Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
545                 550                 555                 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
                565                 570                 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
            580                 585                 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
        595                 600                 605
```

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

[0005] Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
755 760 765

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
770 775 780

Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
785 790 795 800

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
805 810 815

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
820 825 830

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
835 840 845

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
850 855 860

[0006] Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
865 870 875 880

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
885 890 895

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
900 905 910

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
915 920 925

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
930 935 940

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
945 950 955 960

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
965 970 975

Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Phe Trp Val Leu
980 985 990

Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val
995 1000 1005

Ala Phe Ile Ile Phe Trp Val Arg Ser Arg Val Lys Phe Ser Arg
1010 1015 1020

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr
1025 1030 1035

[0007] Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
1040 1045 1050

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
1055 1060 1065

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys
1070 1075 1080

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
1085 1090 1095

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
1100 1105 1110

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
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His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30

[0008]

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr

115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

[0009]

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly

290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

[0010]

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala

465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
545 550 555 560

[0011]

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
580 585 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe

645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
725 730 735

[0012]

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
755 760 765

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
770 775 780

Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
785 790 795 800

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
805 810 815

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln

820 825 830

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
835 840 845

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
850 855 860

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
865 870 875 880

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
885 890 895

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
900 905 910

[0013]

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
915 920 925

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
930 935 940

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
945 950 955 960

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
965 970 975

Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Ala Val Phe Gly
980 985 990

Cys Ile Phe Gly Ala Leu Val Ile Val Thr Val Gly Gly Phe Ile Phe

995 1000 1005

Trp Arg Lys Lys Arg Lys Asp Ala Lys Asn Asn Glu Val Ser Phe
1010 1015 1020

Ser Gln Ile Lys Pro Lys Lys Ser Lys Leu Ile Arg Val Glu Asn
1025 1030 1035

Phe Glu Ala Tyr Phe Lys Lys Gln Gln Ala Asp Ser Asn Cys Gly
1040 1045 1050

Phe Ala Glu Glu Tyr Glu Asp Leu Lys Leu Val Gly Ile Ser Gln
1055 1060 1065

Pro Lys Tyr Ala Ala Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg
1070 1075 1080

[0014]

Tyr Asn Asn Val Leu Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser
1085 1090 1095

Val Gln Thr His Ser Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met
1100 1105 1110

Pro Gly Tyr His Ser Lys Lys Asp Phe Ile Ala Thr Gln Gly Pro
1115 1120 1125

Leu Pro Asn Thr Leu Lys Asp Phe Trp Arg Met Val Trp Glu Lys
1130 1135 1140

Asn Val Tyr Ala Ile Ile Met Leu Thr Lys Cys Val Glu Gln Gly
1145 1150 1155

Arg Thr Lys Cys Glu Glu Tyr Trp Pro Ser Lys Gln Ala Gln Asp

1160 1165 1170

Tyr Gly Asp Ile Thr Val Ala Met Thr Ser Glu Ile Val Leu Pro
1175 1180 1185

Glu Trp Thr Ile Arg Asp Phe Thr Val Lys Asn Ile Gln Thr Ser
1190 1195 1200

Glu Ser His Pro Leu Arg Gln Phe His Phe Thr Ser Trp Pro Asp
1205 1210 1215

His Gly Val Pro Asp Thr Thr Asp Leu Leu Ile Asn Phe Arg Tyr
1220 1225 1230

Leu Val Arg Asp Tyr Met Lys Gln Ser Pro Pro Glu Ser Pro Ile
1235 1240 1245

[0015]

Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr Phe Ile
1250 1255 1260

Ala Ile Asp Arg Leu Ile Tyr Gln Ile Glu Asn Glu Asn Thr Val
1265 1270 1275

Asp Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro Leu
1280 1285 1290

Met Val Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val
1295 1300 1305

Leu Asp Ile Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile
1310 1315 1320

Tyr Gln Asn Thr Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro

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Val Thr Thr Phe Gly Lys Thr Asn Gly Tyr Ile Ala
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Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

[0017] Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

[0018] Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

[0019] Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
545 550 555 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
580 585 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

[0020] Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
755 760 765

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
770 775 780

Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
785 790 795 800

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
805 810 815

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
820 825 830

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
835 840 845

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
850 855 860

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
865 870 875 880

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
885 890 895

[0021] Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
900 905 910

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
915 920 925

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
930 935 940

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
945 950 955 960

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
965 970 975

Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Ala Leu Ile Ala
980 985 990

Phe Leu Ala Phe Leu Ile Ile Val Thr Ser Ile Ala Leu Leu Val Val
995 1000 1005

Leu Tyr Lys Ile Tyr Asp Leu His Lys Lys Arg Ser Cys Asn Leu
1010 1015 1020

Asp Glu Gln Gln Glu Leu Val Glu Arg Asp Asp Glu Lys Gln Leu
1025 1030 1035

Met Asn Val Glu Pro Ile His Ala Asp Ile Leu Leu Glu Thr Tyr
1040 1045 1050

Lys Arg Lys Ile Ala Asp Glu Gly Arg Leu Phe Leu Ala Glu Phe
1055 1060 1065

[0022] Gln Ser Ile Pro Arg Val Phe Ser Lys Phe Pro Ile Lys Glu Ala
1070 1075 1080

Arg Lys Pro Phe Asn Gln Asn Lys Asn Arg Tyr Val Asp Ile Leu
1085 1090 1095

Pro Tyr Asp Tyr Asn Arg Val Glu Leu Ser Glu Ile Asn Gly Asp
1100 1105 1110

Ala Gly Ser Asn Tyr Ile Asn Ala Ser Tyr Ile Asp Gly Phe Lys
1115 1120 1125

Glu Pro Arg Lys Tyr Ile Ala Ala Gln Gly Pro Arg Asp Glu Thr
1130 1135 1140

Val Asp Asp Phe Trp Arg Met Ile Trp Glu Gln Lys Ala Thr Val
1145 1150 1155

Ile Val Met Val Thr Arg Cys Glu Glu Gly Asn Arg Asn Lys Cys
1160 1165 1170

Ala Glu Tyr Trp Pro Ser Met Glu Glu Gly Thr Arg Ala Phe Gly
1175 1180 1185

Asp Val Val Val Lys Ile Asn Gln His Lys Arg Cys Pro Asp Tyr
1190 1195 1200

Ile Ile Gln Lys Leu Asn Ile Val Asn Lys Lys Glu Lys Ala Thr
1205 1210 1215

Gly Arg Glu Val Thr His Ile Gln Phe Thr Ser Trp Pro Asp His
1220 1225 1230

[0023] Gly Val Pro Glu Asp Pro His Leu Leu Leu Lys Leu Arg Arg Arg
1235 1240 1245

Val Asn Ala Phe Ser Asn Phe Phe Ser Gly Pro Ile Val Val His
1250 1255 1260

Cys Ser Ala Gly Val Gly Arg Thr Gly Thr Tyr Ile Gly Ile Asp
1265 1270 1275

Ala Met Leu Glu Gly Leu Glu Ala Glu Asn Lys Val Asp Val Tyr
1280 1285 1290

Gly Tyr Val Val Lys Leu Arg Arg Gln Arg Cys Leu Met Val Gln
1295 1300 1305

Val Glu Ala Gln Tyr Ile Leu Ile His Gln Ala Leu Val Glu Tyr
1310 1315 1320

Asn Gln Phe Gly Glu Thr Glu Val Asn Leu Ser Glu Leu His Pro
1325 1330 1335

Tyr Leu His Asn Met Lys Lys Arg Asp Pro Pro Ser Glu Pro Ser
1340 1345 1350

Pro Leu Glu Ala Glu Phe Gln Arg Leu Pro Ser Tyr Arg Ser Trp
1355 1360 1365

Arg Thr Gln His Ile Gly Asn Gln Glu Glu Asn Lys Ser Lys Asn
1370 1375 1380

Arg Asn Ser Asn Val Ile Pro Tyr Asp Tyr Asn Arg Val Pro Leu
1385 1390 1395

[0024] Lys His Glu Leu Glu Met Ser Lys Glu Ser Glu His Asp Ser Asp
1400 1405 1410

Glu Ser Ser Asp Asp Asp Ser Asp Ser Glu Glu Pro Ser Lys Tyr
1415 1420 1425

Ile Asn Ala Ser Phe Ile Met Ser Tyr Trp Lys Pro Glu Val Met
1430 1435 1440

Ile Ala Ala Gln Gly Pro Leu Lys Glu Thr Ile Gly Asp Phe Trp
1445 1450 1455

Gln Met Ile Phe Gln Arg Lys Val Lys Val Ile Val Met Leu Thr
1460 1465 1470

Glu Leu Lys His Gly Asp Gln Glu Ile Cys Ala Gln Tyr Trp Gly
1475 1480 1485

Glu Gly Lys Gln Thr Tyr Gly Asp Ile Glu Val Asp Leu Lys Asp
1490 1495 1500

Thr Asp Lys Ser Ser Thr Tyr Thr Leu Arg Val Phe Glu Leu Arg
1505 1510 1515

His Ser Lys Arg Lys Asp Ser Arg Thr Val Tyr Gln Tyr Gln Tyr
1520 1525 1530

Thr Asn Trp Ser Val Glu Gln Leu Pro Ala Glu Pro Lys Glu Leu
1535 1540 1545

Ile Ser Met Ile Gln Val Val Lys Gln Lys Leu Pro Gln Lys Asn
1550 1555 1560

[0025] Ser Ser Glu Gly Asn Lys His His Lys Ser Thr Pro Leu Leu Ile
1565 1570 1575

His Cys Arg Asp Gly Ser Gln Gln Thr Gly Ile Phe Cys Ala Leu
1580 1585 1590

Leu Asn Leu Leu Glu Ser Ala Glu Thr Glu Glu Val Val Asp Ile
1595 1600 1605

Phe Gln Val Val Lys Ala Leu Arg Lys Ala Arg Pro Gly Met Val
1610 1615 1620

Ser Thr Phe Glu Gln Tyr Gln Phe Leu Tyr Asp Val Ile Ala Ser
1625 1630 1635

Thr Tyr Pro Ala Gln Asn Gly Gln Val Lys Lys Asn Asn His Gln
1640 1645 1650

Glu Asp Lys Ile Glu Phe Asp Asn Glu Val Asp Lys Val Lys Gln
1655 1660 1665

Asp Ala Asn Cys Val Asn Pro Leu Gly Ala Pro Glu Lys Leu Pro
1670 1675 1680

Glu Ala Lys Glu Gln Ala Glu Gly Ser Glu Pro Thr Ser Gly Thr
1685 1690 1695

Glu Gly Pro Glu His Ser Val Asn Gly Pro Ala Ser Pro Ala Leu
1700 1705 1710

Asn Gln Gly Ser
1715

<210> 4
[0026] <211> 1114
<212> PRT
<213> 人工序列

<220>
<223> 嵌合抗原受体(CAR)

<400> 4

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr

50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

[0027]

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr

225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

[0028]

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala

405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

[0029]

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
545 550 555 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr

580 585 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

[0030]

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro

755 760 765

Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
770 775 780

Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Tyr Trp Ala
785 790 795 800

Pro Leu Ala Gly Ile Cys Val Ala Leu Leu Leu Ser Leu Ile Ile Thr
805 810 815

Leu Ile Cys Tyr His Arg Ser Arg Lys Arg Val Cys Lys Ser Gly Gly
820 825 830

Gly Ser Phe Trp Glu Glu Phe Glu Ser Leu Gln Lys Gln Glu Val Lys
835 840 845

[0031]

Asn Leu His Gln Arg Leu Glu Gly Gln Arg Pro Glu Asn Lys Gly Lys
850 855 860

Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Ser Arg Val Ile Leu
865 870 875 880

Gln Gly Arg Asp Ser Asn Ile Pro Gly Ser Asp Tyr Ile Asn Ala Asn
885 890 895

Tyr Ile Lys Asn Gln Leu Leu Gly Pro Asp Glu Asn Ala Lys Thr Tyr
900 905 910

Ile Ala Ser Gln Gly Cys Leu Glu Ala Thr Val Asn Asp Phe Trp Gln
915 920 925

Met Ala Trp Gln Glu Asn Ser Arg Val Ile Val Met Thr Thr Arg Glu

930 935 940

Val Glu Lys Gly Arg Asn Lys Cys Val Pro Tyr Trp Pro Glu Val Gly
945 950 955 960

Met Gln Arg Ala Tyr Gly Pro Tyr Ser Val Thr Asn Cys Gly Glu His
965 970 975

Asp Thr Thr Glu Tyr Lys Leu Arg Thr Leu Gln Val Ser Pro Leu Asp
980 985 990

Asn Gly Asp Leu Ile Arg Glu Ile Trp His Tyr Gln Tyr Leu Ser Trp
995 1000 1005

Pro Asp His Gly Val Pro Ser Glu Pro Gly Gly Val Leu Ser Phe
1010 1015 1020

[0032]

Leu Asp Gln Ile Asn Gln Arg Gln Glu Ser Leu Pro His Ala Gly
1025 1030 1035

Pro Ile Ile Val His Cys Ser Ala Gly Ile Gly Arg Thr Gly Thr
1040 1045 1050

Ile Ile Val Ile Asp Met Leu Met Glu Asn Ile Ser Thr Lys Gly
1055 1060 1065

Leu Asp Cys Asp Ile Asp Ile Gln Lys Thr Ile Gln Met Val Arg
1070 1075 1080

Ala Gln Arg Ser Gly Met Val Gln Thr Glu Ala Gln Tyr Lys Phe
1085 1090 1095

Ile Tyr Val Ala Ile Ala Gln Phe Ile Glu Thr Thr Lys Lys Lys

1100 1105 1110

Leu

<210> 5
<211> 918
<212> PRT
<213> 人工序列

<220>
<223> 嵌合抗原受体(CAR)

<400> 5

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

[0033] His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

[0034] Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

[0035] Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

[0036] Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
545 550 555 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
580 585 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

[0037] Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro
755 760 765

Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
770 775 780

Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Leu Ile Gly
785 790 795 800

Val Ser Val Val Phe Leu Phe Cys Leu Leu Leu Leu Val Leu Phe Cys
805 810 815

Leu His Arg Gln Asn Gln Ile Lys Gln Gly Pro Pro Arg Ser Lys Asp
820 825 830

Glu Glu Gln Lys Pro Gln Gln Arg Pro Asp Leu Ala Val Asp Val Leu
835 840 845

Glu Arg Thr Ala Asp Lys Ala Thr Val Asn Gly Leu Pro Glu Lys Asp
850 855 860

Arg Glu Thr Asp Thr Ser Ala Leu Ala Ala Gly Ser Ser Gln Glu Val
865 870 875 880

Thr Tyr Ala Gln Leu Asp His Trp Ala Leu Thr Gln Arg Thr Ala Arg
885 890 895

Ala Val Ser Pro Gln Ser Thr Lys Pro Met Ala Glu Ser Ile Thr Tyr
[0038] 900 905 910

Ala Ala Val Ala Arg His
915

<210> 6
<211> 1363
<212> PRT
<213> 人工序列

<220>
<223> 嵌合抗原受体(CAR)

<400> 6

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu

20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

[0039]

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr

195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

[0040]

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg

370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

[0041]

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe

545 550 555 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
580 585 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

[0042]

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe

725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro
755 760 765

Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
770 775 780

Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Leu Ile Gly
785 790 795 800

Val Ser Val Val Phe Leu Phe Cys Leu Leu Leu Leu Val Leu Phe Cys
805 810 815

[0043]

Leu His Arg Gln Asn Gln Ile Lys Gln Gly Pro Pro Arg Ser Lys Asp
820 825 830

Glu Glu Gln Lys Pro Gln Gln Arg Pro Asp Leu Ala Val Asp Val Leu
835 840 845

Glu Arg Thr Ala Asp Lys Ala Thr Val Asn Gly Leu Pro Glu Lys Asp
850 855 860

Arg Glu Thr Asp Thr Ser Ala Leu Ala Ala Gly Ser Ser Gln Glu Val
865 870 875 880

Thr Tyr Ala Gln Leu Asp His Trp Ala Leu Thr Gln Arg Thr Ala Arg
885 890 895

Ala Val Ser Pro Gln Ser Thr Lys Pro Met Ala Glu Ser Ile Thr Tyr

900 905 910

Ala Ala Val Ala Arg His Arg Ala Glu Gly Arg Gly Ser Leu Leu Thr
915 920 925

Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Trp Tyr His Gly His Met
930 935 940

Ser Gly Gly Gln Ala Glu Thr Leu Leu Gln Ala Lys Gly Glu Pro Trp
945 950 955 960

Thr Phe Leu Val Arg Glu Ser Leu Ser Gln Pro Gly Asp Phe Val Leu
965 970 975

Ser Val Leu Ser Asp Gln Pro Lys Ala Gly Pro Gly Ser Pro Leu Arg
980 985 990

[0044]

Val Thr His Ile Lys Val Met Cys Glu Gly Gly Arg Tyr Thr Val Gly
995 1000 1005

Gly Leu Glu Thr Phe Asp Ser Leu Thr Asp Leu Val Glu His Phe
1010 1015 1020

Lys Lys Thr Gly Ile Glu Glu Ala Ser Gly Ala Phe Val Tyr Leu
1025 1030 1035

Arg Gln Pro Tyr Ser Gly Gly Gly Gly Ser Phe Glu Ala Tyr Phe
1040 1045 1050

Lys Lys Gln Gln Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr
1055 1060 1065

Glu Asp Leu Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala

1070 1075 1080

Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu
1085 1090 1095

Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln Thr His Ser
1100 1105 1110

Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser
1115 1120 1125

Lys Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu
1130 1135 1140

Lys Asp Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile
1145 1150 1155

[0045]

Ile Met Leu Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu
1160 1165 1170

Glu Tyr Trp Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr
1175 1180 1185

Val Ala Met Thr Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg
1190 1195 1200

Asp Phe Thr Val Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu
1205 1210 1215

Arg Gln Phe His Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp
1220 1225 1230

Thr Thr Asp Leu Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr

1235 1240 1245

Met Lys Gln Ser Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser
1250 1255 1260

Ala Gly Val Gly Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu
1265 1270 1275

Ile Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile
1280 1285 1290

Val Tyr Asp Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu
1295 1300 1305

Asp Gln Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg
1310 1315 1320

[0046]

Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr
1325 1330 1335

Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly
1340 1345 1350

Lys Thr Asn Gly Tyr Ile Ala Ser Gly Ser
1355 1360

<210> 7
<211> 21
<212> PRT
<213> 人工序列

<220>
<223> 信号肽

<400> 7

Met Gly Thr Ser Leu Leu Cys Trp Met Ala Leu Cys Leu Leu Gly Ala
1   5   10   15

Asp His Ala Asp Gly
            20

<210> 8
<211> 21
<212> PRT
<213> 人工序列

<220>
<223> 信号肽

<400> 8

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1   5   10   15

[0047]

His Ala Ala Arg Pro
            20

<210> 9
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> 信号肽

<400> 9

Met Ala Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr
1   5   10   15

Asp Ala Arg Cys
            20

<210> 10
<211> 234
<212> PRT
<213> 人工序列

<220>
<223> 间隔区 (hinge-CH2CH3 of human IgG1)

<400> 10

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

[0048]

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr

130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

[0049]

Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp
225 230

<210> 11
<211> 46
<212> PRT
<213> 人工序列

<220>
<223> 间隔区（人类 CD8 茎）

<400> 11

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
1 5 10 15

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
20 25 30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
35 40 45

<210> 12
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> 间隔区（人类 IgG1 铰链）

<400> 12

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

Lys Asp Pro Lys
20

[0050]

<210> 13
<211> 185
<212> PRT
<213> 人工序列

<220>
<223> 间隔区 (CD2 胞外域)

<400> 13

Lys Glu Ile Thr Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp
1 5 10 15

Ile Asn Leu Asp Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp
20 25 30

Ile Lys Trp Glu Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg
35 40 45

Lys Glu Lys Glu Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys
50 55 60

Asn Gly Thr Leu Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile
65 70 75 80

Tyr Lys Val Ser Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys
85 90 95

Ile Phe Asp Leu Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser
100 105 110

Trp Thr Cys Ile Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr
115 120 125

[0051] Asp Pro Glu Leu Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser
130 135 140

Gln Arg Val Ile Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe
145 150 155 160

Lys Cys Thr Ala Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro
165 170 175

Val Ser Cys Pro Glu Lys Gly Leu Asp
180 185

<210> 14
<211> 259
<212> PRT
<213> 人工序列

<220>
<223> 间隔区（CD34 胞外域）

<400> 14

Ser Leu Asp Asn Asn Gly Thr Ala Thr Pro Glu Leu Pro Thr Gln Gly
1   5   10   15

Thr Phe Ser Asn Val Ser Thr Asn Val Ser Tyr Gln Glu Thr Thr Thr
            20   25   30

Pro Ser Thr Leu Gly Ser Thr Ser Leu His Pro Val Ser Gln His Gly
        35   40   45

Asn Glu Ala Thr Thr Asn Ile Thr Glu Thr Thr Val Lys Phe Thr Ser
    50   55   60

Thr Ser Val Ile Thr Ser Val Tyr Gly Asn Thr Asn Ser Ser Val Gln
65   70   75   80

[0052]

Ser Gln Thr Ser Val Ile Ser Thr Val Phe Thr Thr Pro Ala Asn Val
                85   90   95

Ser Thr Pro Glu Thr Thr Leu Lys Pro Ser Leu Ser Pro Gly Asn Val
            100   105   110

Ser Asp Leu Ser Thr Thr Ser Thr Ser Leu Ala Thr Ser Pro Thr Lys
        115   120   125

Pro Tyr Thr Ser Ser Ser Pro Ile Leu Ser Asp Ile Lys Ala Glu Ile
    130   135   140

Lys Cys Ser Gly Ile Arg Glu Val Lys Leu Thr Gln Gly Ile Cys Leu
145   150   155   160

Glu Gln Asn Lys Thr Ser Ser Cys Ala Glu Phe Lys Lys Asp Arg Gly

165 170 175

Glu Gly Leu Ala Arg Val Leu Cys Gly Glu Glu Gln Ala Asp Ala Asp
180 185 190

Ala Gly Ala Gln Val Cys Ser Leu Leu Leu Ala Gln Ser Glu Val Arg
195 200 205

Pro Gln Cys Leu Leu Leu Val Leu Ala Asn Arg Thr Glu Ile Ser Ser
210 215 220

Lys Leu Gln Leu Met Lys Lys His Gln Ser Asp Leu Lys Lys Leu Gly
225 230 235 240

Ile Leu Asp Phe Thr Glu Gln Asp Val Ala Ser His Gln Ser Tyr Ser
245 250 255

[0053]

Gln Lys Thr

<210> 15
<211> 140
<212> PRT
<213> 人工序列

<220>
<223> CD28 跨膜域 和 CD3 Z 内域

<400> 15

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe
20 25 30

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
35 40 45

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
50 55 60

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
65 70 75 80

Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
85 90 95

Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
100 105 110

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
[0054] 115 120 125

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
130 135 140

<210> 16
<211> 180
<212> PRT
<213> 人工序列

<220>
<223> CD28 跨膜域 和 CD28 和 CD3 Zeta 内域

<400> 16

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser

20 25 30

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
35 40 45

Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
50 55 60

Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
65 70 75 80

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
85 90 95

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
100 105 110

[0055]

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
115 120 125

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
130 135 140

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
145 150 155 160

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
165 170 175

Leu Pro Pro Arg
180

<210> 17

<211> 216
<212> PRT
<213> 人工序列

<220>
<223> CD28 跨膜域 和 CD28, OX40 和 CD3 Zeta 内域

<400> 17

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
20 25 30

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
35 40 45

[0056] Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
50 55 60

Ala Tyr Arg Ser Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro
65 70 75 80

Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp
85 90 95

Ala His Ser Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg Ser Ala
100 105 110

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
115 120 125

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
130 135 140

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
145 150 155 160

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
165 170 175

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
180 185 190

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
195 200 205

His Met Gln Ala Leu Pro Pro Arg
210 215

<210> 18
[0057] <211> 729
<212> PRT
<213> 人工序列

<220>
<223> CD45 跨膜和内域

<400> 18

Ala Leu Ile Ala Phe Leu Ala Phe Leu Ile Ile Val Thr Ser Ile Ala
1 5 10 15

Leu Leu Val Val Leu Tyr Lys Ile Tyr Asp Leu His Lys Lys Arg Ser
20 25 30

Cys Asn Leu Asp Glu Gln Gln Glu Leu Val Glu Arg Asp Asp Glu Lys
35 40 45

Gln Leu Met Asn Val Glu Pro Ile His Ala Asp Ile Leu Leu Glu Thr

50 55 60

Tyr Lys Arg Lys Ile Ala Asp Glu Gly Arg Leu Phe Leu Ala Glu Phe
65 70 75 80

Gln Ser Ile Pro Arg Val Phe Ser Lys Phe Pro Ile Lys Glu Ala Arg
85 90 95

Lys Pro Phe Asn Gln Asn Lys Asn Arg Tyr Val Asp Ile Leu Pro Tyr
100 105 110

Asp Tyr Asn Arg Val Glu Leu Ser Glu Ile Asn Gly Asp Ala Gly Ser
115 120 125

Asn Tyr Ile Asn Ala Ser Tyr Ile Asp Gly Phe Lys Glu Pro Arg Lys
130 135 140

[0058]

Tyr Ile Ala Ala Gln Gly Pro Arg Asp Glu Thr Val Asp Asp Phe Trp
145 150 155 160

Arg Met Ile Trp Glu Gln Lys Ala Thr Val Ile Val Met Val Thr Arg
165 170 175

Cys Glu Glu Gly Asn Arg Asn Lys Cys Ala Glu Tyr Trp Pro Ser Met
180 185 190

Glu Glu Gly Thr Arg Ala Phe Gly Asp Val Val Val Lys Ile Asn Gln
195 200 205

His Lys Arg Cys Pro Asp Tyr Ile Ile Gln Lys Leu Asn Ile Val Asn
210 215 220

Lys Lys Glu Lys Ala Thr Gly Arg Glu Val Thr His Ile Gln Phe Thr

225 230 235 240

Ser Trp Pro Asp His Gly Val Pro Glu Asp Pro His Leu Leu Leu Lys
245 250 255

Leu Arg Arg Arg Val Asn Ala Phe Ser Asn Phe Phe Ser Gly Pro Ile
260 265 270

Val Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr Tyr Ile Gly
275 280 285

Ile Asp Ala Met Leu Glu Gly Leu Glu Ala Glu Asn Lys Val Asp Val
290 295 300

Tyr Gly Tyr Val Val Lys Leu Arg Arg Gln Arg Cys Leu Met Val Gln
305 310 315 320

[0059]

Val Glu Ala Gln Tyr Ile Leu Ile His Gln Ala Leu Val Glu Tyr Asn
325 330 335

Gln Phe Gly Glu Thr Glu Val Asn Leu Ser Glu Leu His Pro Tyr Leu
340 345 350

His Asn Met Lys Lys Arg Asp Pro Pro Ser Glu Pro Ser Pro Leu Glu
355 360 365

Ala Glu Phe Gln Arg Leu Pro Ser Tyr Arg Ser Trp Arg Thr Gln His
370 375 380

Ile Gly Asn Gln Glu Glu Asn Lys Ser Lys Asn Arg Asn Ser Asn Val
385 390 395 400

Ile Pro Tyr Asp Tyr Asn Arg Val Pro Leu Lys His Glu Leu Glu Met

405 410 415

Ser Lys Glu Ser Glu His Asp Ser Asp Glu Ser Ser Asp Asp Asp Ser
420 425 430

Asp Ser Glu Glu Pro Ser Lys Tyr Ile Asn Ala Ser Phe Ile Met Ser
435 440 445

Tyr Trp Lys Pro Glu Val Met Ile Ala Ala Gln Gly Pro Leu Lys Glu
450 455 460

Thr Ile Gly Asp Phe Trp Gln Met Ile Phe Gln Arg Lys Val Lys Val
465 470 475 480

Ile Val Met Leu Thr Glu Leu Lys His Gly Asp Gln Glu Ile Cys Ala
485 490 495

[0060]

Gln Tyr Trp Gly Glu Gly Lys Gln Thr Tyr Gly Asp Ile Glu Val Asp
500 505 510

Leu Lys Asp Thr Asp Lys Ser Ser Thr Tyr Thr Leu Arg Val Phe Glu
515 520 525

Leu Arg His Ser Lys Arg Lys Asp Ser Arg Thr Val Tyr Gln Tyr Gln
530 535 540

Tyr Thr Asn Trp Ser Val Glu Gln Leu Pro Ala Glu Pro Lys Glu Leu
545 550 555 560

Ile Ser Met Ile Gln Val Val Lys Gln Lys Leu Pro Gln Lys Asn Ser
565 570 575

Ser Glu Gly Asn Lys His His Lys Ser Thr Pro Leu Leu Ile His Cys

580 585 590

Arg Asp Gly Ser Gln Gln Thr Gly Ile Phe Cys Ala Leu Leu Asn Leu
595 600 605

Leu Glu Ser Ala Glu Thr Glu Glu Val Val Asp Ile Phe Gln Val Val
610 615 620

Lys Ala Leu Arg Lys Ala Arg Pro Gly Met Val Ser Thr Phe Glu Gln
625 630 635 640

Tyr Gln Phe Leu Tyr Asp Val Ile Ala Ser Thr Tyr Pro Ala Gln Asn
645 650 655

Gly Gln Val Lys Lys Asn Asn His Gln Glu Asp Lys Ile Glu Phe Asp
660 665 670

[0061]

Asn Glu Val Asp Lys Val Lys Gln Asp Ala Asn Cys Val Asn Pro Leu
675 680 685

Gly Ala Pro Glu Lys Leu Pro Glu Ala Lys Glu Gln Ala Glu Gly Ser
690 695 700

Glu Pro Thr Ser Gly Thr Glu Gly Pro Glu His Ser Val Asn Gly Pro
705 710 715 720

Ala Ser Pro Ala Leu Asn Gln Gly Ser
725

<210> 19
<211> 362
<212> PRT
<213> 人工序列

<220>
<223> CD148 跨膜和内域

<400> 19

Ala Val Phe Gly Cys Ile Phe Gly Ala Leu Val Ile Val Thr Val Gly
1 5 10 15

Gly Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala Lys Asn Asn Glu
20 25 30

Val Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser Lys Leu Ile Arg Val
35 40 45

Glu Asn Phe Glu Ala Tyr Phe Lys Lys Gln Gln Ala Asp Ser Asn Cys
50 55 60

[0062] Gly Phe Ala Glu Glu Tyr Glu Asp Leu Lys Leu Val Gly Ile Ser Gln
65 70 75 80

Pro Lys Tyr Ala Ala Glu Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr
85 90 95

Asn Asn Val Leu Pro Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln
100 105 110

Thr His Ser Thr Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr
115 120 125

His Ser Lys Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr
130 135 140

Leu Lys Asp Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile
145 150 155 160

Ile Met Leu Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu
165 170 175

Tyr Trp Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala
180 185 190

Met Thr Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr
195 200 205

Val Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His
210 215 220

Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu Leu
225 230 235 240

[0063] Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser Pro Pro
245 250 255

Glu Ser Pro Ile Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly
260 265 270

Thr Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln Ile Glu Asn Glu Asn
275 280 285

Thr Val Asp Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro
290 295 300

Leu Met Val Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val
305 310 315 320

Leu Asp Ile Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr
325 330 335

Gln Asn Thr Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr
340 345 350

Thr Phe Gly Lys Thr Asn Gly Tyr Ile Ala
355 360

<210> 20
<211> 595
<212> PRT
<213> 人工序列

<220>
<223> PTPN6 序列

<400> 20

Met Val Arg Trp Phe His Arg Asp Leu Ser Gly Leu Asp Ala Glu Thr
1 5 10 15

[0064]

Leu Leu Lys Gly Arg Gly Val His Gly Ser Phe Leu Ala Arg Pro Ser
20 25 30

Arg Lys Asn Gln Gly Asp Phe Ser Leu Ser Val Arg Val Gly Asp Gln
35 40 45

Val Thr His Ile Arg Ile Gln Asn Ser Gly Asp Phe Tyr Asp Leu Tyr
50 55 60

Gly Gly Glu Lys Phe Ala Thr Leu Thr Glu Leu Val Glu Tyr Tyr Thr
65 70 75 80

Gln Gln Gln Gly Val Leu Gln Asp Arg Asp Gly Thr Ile Ile His Leu
85 90 95

Lys Tyr Pro Leu Asn Cys Ser Asp Pro Thr Ser Glu Arg Trp Tyr His

100 105 110

Gly His Met Ser Gly Gly Gln Ala Glu Thr Leu Leu Gln Ala Lys Gly
115 120 125

Glu Pro Trp Thr Phe Leu Val Arg Glu Ser Leu Ser Gln Pro Gly Asp
130 135 140

Phe Val Leu Ser Val Leu Ser Asp Gln Pro Lys Ala Gly Pro Gly Ser
145 150 155 160

Pro Leu Arg Val Thr His Ile Lys Val Met Cys Glu Gly Gly Arg Tyr
165 170 175

Thr Val Gly Gly Leu Glu Thr Phe Asp Ser Leu Thr Asp Leu Val Glu
180 185 190

[0065]

His Phe Lys Lys Thr Gly Ile Glu Glu Ala Ser Gly Ala Phe Val Tyr
195 200 205

Leu Arg Gln Pro Tyr Tyr Ala Thr Arg Val Asn Ala Ala Asp Ile Glu
210 215 220

Asn Arg Val Leu Glu Leu Asn Lys Lys Gln Glu Ser Glu Asp Thr Ala
225 230 235 240

Lys Ala Gly Phe Trp Glu Glu Phe Glu Ser Leu Gln Lys Gln Glu Val
245 250 255

Lys Asn Leu His Gln Arg Leu Glu Gly Gln Arg Pro Glu Asn Lys Gly
260 265 270

Lys Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Ser Arg Val Ile

275 280 285

Leu Gln Gly Arg Asp Ser Asn Ile Pro Gly Ser Asp Tyr Ile Asn Ala
290 295 300

Asn Tyr Ile Lys Asn Gln Leu Leu Gly Pro Asp Glu Asn Ala Lys Thr
305 310 315 320

Tyr Ile Ala Ser Gln Gly Cys Leu Glu Ala Thr Val Asn Asp Phe Trp
325 330 335

Gln Met Ala Trp Gln Glu Asn Ser Arg Val Ile Val Met Thr Thr Arg
340 345 350

Glu Val Glu Lys Gly Arg Asn Lys Cys Val Pro Tyr Trp Pro Glu Val
355 360 365

[0066]

Gly Met Gln Arg Ala Tyr Gly Pro Tyr Ser Val Thr Asn Cys Gly Glu
370 375 380

His Asp Thr Thr Glu Tyr Lys Leu Arg Thr Leu Gln Val Ser Pro Leu
385 390 395 400

Asp Asn Gly Asp Leu Ile Arg Glu Ile Trp His Tyr Gln Tyr Leu Ser
405 410 415

Trp Pro Asp His Gly Val Pro Ser Glu Pro Gly Gly Val Leu Ser Phe
420 425 430

Leu Asp Gln Ile Asn Gln Arg Gln Glu Ser Leu Pro His Ala Gly Pro
435 440 445

Ile Ile Val His Cys Ser Ala Gly Ile Gly Arg Thr Gly Thr Ile Ile

450 455 460

Val Ile Asp Met Leu Met Glu Asn Ile Ser Thr Lys Gly Leu Asp Cys
465 470 475 480

Asp Ile Asp Ile Gln Lys Thr Ile Gln Met Val Arg Ala Gln Arg Ser
485 490 495

Gly Met Val Gln Thr Glu Ala Gln Tyr Lys Phe Ile Tyr Val Ala Ile
500 505 510

Ala Gln Phe Ile Glu Thr Thr Lys Lys Lys Leu Glu Val Leu Gln Ser
515 520 525

Gln Lys Gly Gln Glu Ser Glu Tyr Gly Asn Ile Thr Tyr Pro Pro Ala
530 535 540

[0067]

Met Lys Asn Ala His Ala Lys Ala Ser Arg Thr Ser Ser Lys His Lys
545 550 555 560

Glu Asp Val Tyr Glu Asn Leu His Thr Lys Asn Lys Arg Glu Glu Lys
565 570 575

Val Lys Lys Gln Arg Ser Ala Asp Lys Glu Lys Ser Lys Gly Ser Leu
580 585 590

Lys Arg Lys
595

<210> 21
<211> 272
<212> PRT
<213> 人工序列

<220>
<223> PTPN6 的磷酸酶域的序列

<400> 21

Phe Trp Glu Glu Phe Glu Ser Leu Gln Lys Gln Glu Val Lys Asn Leu
1 5 10 15

His Gln Arg Leu Glu Gly Gln Arg Pro Glu Asn Lys Gly Lys Asn Arg
20 25 30

Tyr Lys Asn Ile Leu Pro Phe Asp His Ser Arg Val Ile Leu Gln Gly
35 40 45

Arg Asp Ser Asn Ile Pro Gly Ser Asp Tyr Ile Asn Ala Asn Tyr Ile
50 55 60

[0068] Lys Asn Gln Leu Leu Gly Pro Asp Glu Asn Ala Lys Thr Tyr Ile Ala
65 70 75 80

Ser Gln Gly Cys Leu Glu Ala Thr Val Asn Asp Phe Trp Gln Met Ala
85 90 95

Trp Gln Glu Asn Ser Arg Val Ile Val Met Thr Thr Arg Glu Val Glu
100 105 110

Lys Gly Arg Asn Lys Cys Val Pro Tyr Trp Pro Glu Val Gly Met Gln
115 120 125

Arg Ala Tyr Gly Pro Tyr Ser Val Thr Asn Cys Gly Glu His Asp Thr
130 135 140

Thr Glu Tyr Lys Leu Arg Thr Leu Gln Val Ser Pro Leu Asp Asn Gly
145 150 155 160

Asp Leu Ile Arg Glu Ile Trp His Tyr Gln Tyr Leu Ser Trp Pro Asp
165 170 175

His Gly Val Pro Ser Glu Pro Gly Gly Val Leu Ser Phe Leu Asp Gln
180 185 190

Ile Asn Gln Arg Gln Glu Ser Leu Pro His Ala Gly Pro Ile Ile Val
195 200 205

His Cys Ser Ala Gly Ile Gly Arg Thr Gly Thr Ile Ile Val Ile Asp
210 215 220

Met Leu Met Glu Asn Ile Ser Thr Lys Gly Leu Asp Cys Asp Ile Asp
225 230 235 240

[0069] Ile Gln Lys Thr Ile Gln Met Val Arg Ala Gln Arg Ser Gly Met Val
245 250 255

Gln Thr Glu Ala Gln Tyr Lys Phe Ile Tyr Val Ala Ile Ala Gln Phe
260 265 270

<210> 22
<211> 97
<212> PRT
<213> 人工序列

<220>
<223> PDCD1 内域

<400> 22

Cys Ser Arg Ala Ala Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly Gln
1 5 10 15

Pro Leu Lys Glu Asp Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr

20 25 30

Gly Glu Leu Asp Phe Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val
35 40 45

Pro Cys Val Pro Glu Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro Ser
50 55 60

Gly Met Gly Thr Ser Ser Pro Ala Arg Arg Gly Ser Ala Asp Gly Pro
65 70 75 80

Arg Ser Ala Gln Pro Leu Arg Pro Glu Asp Gly His Cys Ser Trp Pro
85 90 95

Leu

[0070]

<210> 23
<211> 141
<212> PRT
<213> 人工序列

<220>
<223> BTLA4 内域

<400> 23

Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly Leu Gln
1 5 10 15

Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys Lys Val Arg
20 25 30

Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly Cys Tyr Asn Pro
35 40 45

Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu Arg Phe Pro Glu Met
50 55 60

Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser Ser Glu Met Gln Arg Pro
65 70 75 80

Pro Pro Asp Cys Asp Asp Thr Val Thr Tyr Ser Ala Leu His Lys Arg
85 90 95

Gln Val Gly Asp Tyr Glu Asn Val Ile Pro Asp Phe Pro Glu Asp Glu
100 105 110

Gly Ile His Tyr Ser Glu Leu Ile Gln Phe Gly Val Gly Glu Arg Pro
115 120 125

Gln Ala Gln Glu Asn Val Asp Tyr Val Ile Leu Lys His
[0071] 130 135 140

<210> 24
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> LILRB1 内域

<400> 24

Leu Arg His Arg Arg Gln Gly Lys His Trp Thr Ser Thr Gln Arg Lys
1 5 10 15

Ala Asp Phe Gln His Pro Ala Gly Ala Val Gly Pro Glu Pro Thr Asp
20 25 30

Arg Gly Leu Gln Trp Arg Ser Ser Pro Ala Ala Asp Ala Gln Glu Glu

35 40 45

Asn Leu Tyr Ala Ala Val Lys His Thr Gln Pro Glu Asp Gly Val Glu
50 55 60

Met Asp Thr Arg Ser Pro His Asp Glu Asp Pro Gln Ala Val Thr Tyr
65 70 75 80

Ala Glu Val Lys His Ser Arg Pro Arg Arg Glu Met Ala Ser Pro Pro
85 90 95

Ser Pro Leu Ser Gly Glu Phe Leu Asp Thr Lys Asp Arg Gln Ala Glu
100 105 110

Glu Asp Arg Gln Met Asp Thr Glu Ala Ala Ala Ser Glu Ala Pro Gln
115 120 125

[0072]

Asp Val Thr Tyr Ala Gln Leu His Ser Leu Thr Leu Arg Arg Glu Ala
130 135 140

Thr Glu Pro Pro Pro Ser Gln Glu Gly Pro Ser Pro Ala Val Pro Ser
145 150 155 160

Ile Tyr Ala Thr Leu Ala Ile His
165

<210> 25
<211> 101
<212> PRT
<213> 人工序列

<220>
<223> LAIR1 内域

<400> 25

His Arg Gln Asn Gln Ile Lys Gln Gly Pro Pro Arg Ser Lys Asp Glu
1   5   10   15

Glu Gln Lys Pro Gln Gln Arg Pro Asp Leu Ala Val Asp Val Leu Glu
            20   25   30

Arg Thr Ala Asp Lys Ala Thr Val Asn Gly Leu Pro Glu Lys Asp Arg
        35   40   45

Glu Thr Asp Thr Ser Ala Leu Ala Ala Gly Ser Ser Gln Glu Val Thr
    50   55   60

Tyr Ala Gln Leu Asp His Trp Ala Leu Thr Gln Arg Thr Ala Arg Ala
65   70   75   80

[0073] Val Ser Pro Gln Ser Thr Lys Pro Met Ala Glu Ser Ile Thr Tyr Ala
                85   90   95

Ala Val Ala Arg His
            100

<210> 26
<211> 62
<212> PRT
<213> 人工序列

<220>
<223> CTLA4 内域

<400> 26

Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu Phe Phe Tyr
1   5   10   15

Ser Phe Leu Leu Thr Ala Val Ser Leu Ser Lys Met Leu Lys Lys Arg

20 25 30

Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu Pro
35 40 45

Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
50 55 60

<210> 27
<211> 111
<212> PRT
<213> 人工序列

<220>
<223> KIR2DL1 内域

<400> 27

[0074] Gly Asn Ser Arg His Leu His Val Leu Ile Gly Thr Ser Val Val Ile
1 5 10 15

Ile Pro Phe Ala Ile Leu Leu Phe Phe Leu Leu His Arg Trp Cys Ala
20 25 30

Asn Lys Lys Asn Ala Val Val Met Asp Gln Glu Pro Ala Gly Asn Arg
35 40 45

Thr Val Asn Arg Glu Asp Ser Asp Glu Gln Asp Pro Gln Glu Val Thr
50 55 60

Tyr Thr Gln Leu Asn His Cys Val Phe Thr Gln Arg Lys Ile Thr Arg
65 70 75 80

Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Ile Ile Val Tyr Thr
85 90 95

Glu Leu Pro Asn Ala Glu Ser Arg Ser Lys Val Val Ser Cys Pro
100 105 110

<210> 28
<211> 143
<212> PRT
<213> 人工序列

<220>
<223> KIR2DL4 内域

<400> 28

Gly Ile Ala Arg His Leu His Ala Val Ile Arg Tyr Ser Val Ala Ile
1 5 10 15

Ile Leu Phe Thr Ile Leu Pro Phe Phe Leu Leu His Arg Trp Cys Ser
20 25 30

[0075]

Lys Lys Lys Glu Asn Ala Ala Val Met Asn Gln Glu Pro Ala Gly His
35 40 45

Arg Thr Val Asn Arg Glu Asp Ser Asp Glu Gln Asp Pro Gln Glu Val
50 55 60

Thr Tyr Ala Gln Leu Asp His Cys Ile Phe Thr Gln Arg Lys Ile Thr
65 70 75 80

Gly Pro Ser Gln Arg Ser Lys Arg Pro Ser Thr Asp Thr Ser Val Cys
85 90 95

Ile Glu Leu Pro Asn Ala Glu Pro Arg Ala Leu Ser Pro Ala His Glu
100 105 110

His His Ser Gln Ala Leu Met Gly Ser Ser Arg Glu Thr Thr Ala Leu

115 120 125

Ser Gln Thr Gln Leu Ala Ser Ser Asn Val Pro Ala Ala Gly Ile
130 135 140

<210> 29
<211> 143
<212> PRT
<213> 人工序列

<220>
<223> KIR2DL5 内域

<400> 29

Thr Gly Ile Arg Arg His Leu His Ile Leu Ile Gly Thr Ser Val Ala
1 5 10 15

[0076] Ile Ile Leu Phe Ile Ile Leu Phe Phe Phe Leu Leu His Cys Cys Cys
20 25 30

Ser Asn Lys Lys Asn Ala Ala Val Met Asp Gln Glu Pro Ala Gly Asp
35 40 45

Arg Thr Val Asn Arg Glu Asp Ser Asp Asp Gln Asp Pro Gln Glu Val
50 55 60

Thr Tyr Ala Gln Leu Asp His Cys Val Phe Thr Gln Thr Lys Ile Thr
65 70 75 80

Ser Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Thr Thr Met Tyr
85 90 95

Met Glu Leu Pro Asn Ala Lys Pro Arg Ser Leu Ser Pro Ala His Lys
100 105 110

His His Ser Gln Ala Leu Arg Gly Ser Ser Arg Glu Thr Thr Ala Leu
115 120 125

Ser Gln Asn Arg Val Ala Ser Ser His Val Pro Ala Ala Gly Ile
130 135 140

<210> 30
<211> 111
<212> PRT
<213> 人工序列

<220>
<223> KIR3DL1 内域

<400> 30

Lys Asp Pro Arg His Leu His Ile Leu Ile Gly Thr Ser Val Val Ile
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[0077]

Ile Leu Phe Ile Leu Leu Leu Phe Phe Leu Leu His Leu Trp Cys Ser
20 25 30

Asn Lys Lys Asn Ala Ala Val Met Asp Gln Glu Pro Ala Gly Asn Arg
35 40 45

Thr Ala Asn Ser Glu Asp Ser Asp Glu Gln Asp Pro Glu Glu Val Thr
50 55 60

Tyr Ala Gln Leu Asp His Cys Val Phe Thr Gln Arg Lys Ile Thr Arg
65 70 75 80

Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Thr Ile Leu Tyr Thr
85 90 95

Glu Leu Pro Asn Ala Lys Pro Arg Ser Lys Val Val Ser Cys Pro

100 105 110

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<213> 人工序列

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<400> 31

Lys Asp Pro Gly Asn Ser Arg His Leu His Val Leu Ile Gly Thr Ser
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Val Val Ile Ile Pro Phe Ala Ile Leu Leu Phe Phe Leu Leu His Arg
20 25 30

[0078] Trp Cys Ala Asn Lys Lys Asn Ala Val Val Met Asp Gln Glu Pro Ala
35 40 45

Gly Asn Arg Thr Val Asn Arg Glu Asp Ser Asp Glu Gln Asp Pro Gln
50 55 60

Glu Val Thr Tyr Ala Gln Leu Asn His Cys Val Phe Thr Gln Arg Lys
65 70 75 80

Ile Thr Arg Pro Ser Gln Arg Pro Lys Thr Pro Pro Thr Asp Thr Ser
85 90 95

Val

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Ala Lys Gly Glu Pro Trp Thr Phe Leu Val Arg Glu Ser Leu Ser Gln
20 25 30

Pro Gly Asp Phe Val Leu Ser Val Leu Ser Asp Gln Pro Lys Ala Gly
35 40 45

Pro Gly Ser Pro Leu Arg Val Thr His Ile Lys Val Met Cys Glu Gly
50 55 60

[0079]

Gly Arg Tyr Thr Val Gly Gly Leu Glu Thr Phe Asp Ser Leu Thr Asp
65 70 75 80

Leu Val Glu His Phe Lys Lys Thr Gly Ile Glu Glu Ala Ser Gly Ala
85 90 95

Phe Val Tyr Leu Arg Gln Pro Tyr Lys Ile Tyr Asp Leu His Lys Lys
100 105 110

Arg Ser Cys Asn Leu Asp Glu Gln Gln Glu Leu Val Glu Arg Asp Asp
115 120 125

Glu Lys Gln Leu Met Asn Val Glu Pro Ile His Ala Asp Ile Leu Leu
130 135 140

Glu Thr Tyr Lys Arg Lys Ile Ala Asp Glu Gly Arg Leu Phe Leu Ala

145 150 155 160

Glu Phe Gln Ser Ile Pro Arg Val Phe Ser Lys Phe Pro Ile Lys Glu
165 170 175

Ala Arg Lys Pro Phe Asn Gln Asn Lys Asn Arg Tyr Val Asp Ile Leu
180 185 190

Pro Tyr Asp Tyr Asn Arg Val Glu Leu Ser Glu Ile Asn Gly Asp Ala
195 200 205

Gly Ser Asn Tyr Ile Asn Ala Ser Tyr Ile Asp Gly Phe Lys Glu Pro
210 215 220

Arg Lys Tyr Ile Ala Ala Gln Gly Pro Arg Asp Glu Thr Val Asp Asp
225 230 235 240

[0080]

Phe Trp Arg Met Ile Trp Glu Gln Lys Ala Thr Val Ile Val Met Val
245 250 255

Thr Arg Cys Glu Glu Gly Asn Arg Asn Lys Cys Ala Glu Tyr Trp Pro
260 265 270

Ser Met Glu Glu Gly Thr Arg Ala Phe Gly Asp Val Val Val Lys Ile
275 280 285

Asn Gln His Lys Arg Cys Pro Asp Tyr Ile Ile Gln Lys Leu Asn Ile
290 295 300

Val Asn Lys Lys Glu Lys Ala Thr Gly Arg Glu Val Thr His Ile Gln
305 310 315 320

Phe Thr Ser Trp Pro Asp His Gly Val Pro Glu Asp Pro His Leu Leu

325 330 335

Leu Lys Leu Arg Arg Arg Val Asn Ala Phe Ser Asn Phe Phe Ser Gly
340 345 350

Pro Ile Val Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr Tyr
355 360 365

Ile Gly Ile Asp Ala Met Leu Glu Gly Leu Glu Ala Glu Asn Lys Val
370 375 380

Asp Val Tyr Gly Tyr Val Val Lys Leu Arg Arg Gln Arg Cys Leu Met
385 390 395 400

Val Gln Val Glu Ala Gln Tyr Ile Leu Ile His Gln Ala Leu Val Glu
405 410 415

[0081]

Tyr Asn Gln Phe Gly Glu Thr Glu Val Asn Leu Ser Glu Leu His Pro
420 425 430

Tyr Leu His Asn Met Lys Lys Arg Asp Pro Pro Ser Glu Pro Ser Pro
435 440 445

Leu Glu Ala Glu Phe Gln Arg Leu Pro Ser Tyr Arg Ser Trp Arg Thr
450 455 460

Gln His Ile Gly Asn Gln Glu Glu Asn Lys Ser Lys Asn Arg Asn Ser
465 470 475 480

Asn Val Ile Pro Tyr Asp Tyr Asn Arg Val Leu Lys His Glu Leu Glu
485 490 495

Met Ser Lys Glu Ser Glu His Asp Ser Asp Glu Ser Ser Asp Asp Asp

500 505 510

Ser Asp Ser Glu Glu Pro Ser Lys Tyr Ile Asn Ala Ser Phe Ile Met
515 520 525

Ser Tyr Trp Lys Pro Glu Val Met Ile Ala Ala Gln Gly Pro Leu Lys
530 535 540

Glu Thr Ile Gly Asp Phe Met Ile Gln Arg Lys Val Lys Val Ile Val
545 550 555 560

Met Leu Thr Glu Leu Lys His Gly Asp Gln Glu Ile Cys Ala Gln Tyr
565 570 575

Trp Gly Glu Gly Lys Gln Thr Tyr Gly Asp Ile Glu Val Asp Leu Lys
580 585 590

[0082]

Asp Thr Asp Lys Ser Ser Thr Tyr Thr Leu Arg Val Phe Glu Leu Arg
595 600 605

His Ser Lys Arg Lys Asp Ser Arg Thr Val Tyr Gln Tyr Gln Tyr Thr
610 615 620

Asn Trp Ser Val Glu Gln Leu Pro Ala Glu Pro Lys Glu Leu Ile Ser
625 630 635 640

Met Ile Gln Val Val Lys Gln Lys Leu Pro Gln Lys Asn Ser Ser Glu
645 650 655

Gly Asn Lys His His Lys Ser Thr Pro Leu Leu Ile His Cys Arg Asp
660 665 670

Gly Ser Gln Gln Thr Gly Ile Phe Cys Ala Leu Leu Asn Leu Leu Glu

675 680 685

Ser Ala Glu Thr Glu Glu Val Val Asp Ile Phe Gln Val Val Lys Ala
690 695 700

Leu Arg Lys Ala Arg Pro Gly Met Val Ser Thr Phe Glu Gln Tyr Gln
705 710 715 720

Phe Leu Tyr Asp Val Ile Ala Ser Thr Tyr Pro Ala Gln Asn Gly Gln
725 730 735

Val Lys Lys Asn Asn His Gln Glu Asp Lys Ile Glu Phe Asp Asn Glu
740 745 750

Val Asp Lys Val Lys Gln Asp Ala Asn Cys Val Asn Pro Leu Gly Ala
755 760 765

[0083]

Pro Glu Lys Leu Pro Glu Ala Lys Glu Gln Ala Glu Gly Ser Glu Pro
770 775 780

Thr Ser Gly Thr Glu Gly Pro Glu His Ser Val Asn Gly Pro Ala Ser
785 790 795 800

Pro Ala Leu Asn Gln Gly Ser
805

<210> 33
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<223> PTPN6-CD148 融合蛋白

<400> 33

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Glu Ser Leu Ser Gln Pro Gly Asp Phe Val Leu Ser Val Leu Ser Asp
20 25 30

Gln Pro Lys Ala Gly Pro Gly Ser Pro Leu Arg Val Thr His Ile Lys
35 40 45

Val Met Cys Glu Gly Gly Arg Tyr Thr Val Gly Gly Leu Glu Thr Phe
50 55 60

Asp Ser Leu Thr Asp Leu Val Glu His Phe Lys Lys Thr Gly Ile Glu
65 70 75 80

[0084] Glu Ala Ser Gly Ala Phe Val Tyr Leu Arg Gln Pro Tyr Arg Lys Lys
85 90 95

Arg Lys Asp Ala Lys Asn Asn Glu Val Ser Phe Ser Gln Ile Lys Pro
100 105 110

Lys Lys Ser Lys Leu Ile Arg Val Glu Asn Phe Glu Ala Tyr Phe Lys
115 120 125

Lys Gln Gln Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr Glu Asp
130 135 140

Leu Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala Glu Leu Ala
145 150 155 160

Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu Pro Tyr Asp Ile
165 170 175

Ser Arg Val Lys Leu Ser Val Gln Thr His Ser Thr Asp Asp Tyr Ile
180 185 190

Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys Lys Asp Phe Ile Ala
195 200 205

Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp Phe Trp Arg Met Val
210 215 220

Trp Glu Lys Asn Val Tyr Ala Ile Ile Met Leu Thr Lys Cys Val Glu
225 230 235 240

Gln Gly Arg Thr Lys Cys Glu Glu Tyr Trp Pro Ser Lys Gln Ala Gln
245 250 255

[0085] Asp Tyr Gly Asp Ile Thr Val Ala Met Thr Ser Glu Ile Val Leu Pro
260 265 270

Glu Trp Thr Ile Arg Asp Phe Thr Val Lys Asn Ile Gln Thr Ser Glu
275 280 285

Ser His Pro Leu Arg Gln Phe His Phe Thr Ser Trp Pro Asp His Gly
290 295 300

Val Pro Asp Thr Thr Asp Leu Leu Ile Asn Phe Arg Tyr Leu Val Arg
305 310 315 320

Asp Tyr Met Lys Gln Ser Pro Pro Glu Ser Pro Ile Leu Val His Cys
325 330 335

Ser Ala Gly Val Gly Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu
340 345 350

Ile Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val
355 360 365

Tyr Asp Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp Gln
370 375 380

Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser Gln Lys
385 390 395 400

Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala Met Thr Ile
405 410 415

Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly Lys Thr Asn Gly Tyr
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Ile Ala

[0086]

<210> 34
<211> 20
<212> PRT
<213> 口蹄疫病毒

<400> 34

Arg Ala Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu
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Asn Pro Gly Pro
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<210> 35
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cagtccacaa agcccatggc cgagtccatc acgtatgcag ccgttgccag acacagggca 2760

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gatatttccc gtgtcaaact ttcggtccag acccattcaa cggatgacta catcaatgcc 3360

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gctcaggact atggagacat aactgtggca atgacatcag aaattgttct tccggaatgg 3600

accatcagag atttcacagt gaaaaatatc cagacaagtg agagtcaccc tctgagacag 3660

[0105]

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cattgcagtg ctggggtcgg aaggacgggc actttcattg ccattgatcg tctcatctac 3840

cagatagaga atgagaacac cgtggatgtg tatgggattg tgtatgacct tcgaatgcat 3900

aggcctttaa tggtgcagac agaggaccag tatgttttcc tcaatcagtg tgttttggat 3960

attgtcagat cccagaaaga ctcaaaagta gatcttatct accagaacac aactgcaatg 4020

acaatctatg aaaaccttgc gcccgtgacc acatttggaa agaccaatgg ttacatcgcc 4080

agcggtagct aa 4092

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His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

[0106]

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly

145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

[0107]

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val

325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

[0108]

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu
465 470 475 480

Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser
485 490 495

Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro

500 505 510

Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ala
515 520 525

Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu
530 535 540

Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala
545 550 555 560

Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Gln Val
565 570 575

Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val Tyr Tyr
580 585 590

[0109]

Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp Gly Gln
595 600 605

Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
610 615 620

Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser Pro Ser
625 630 635 640

Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys Arg Ala
645 650 655

Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln Lys Ser
660 665 670

Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser

675 680 685

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr
690 695 700

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
705 710 715 720

Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr Lys Val
725 730 735

Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr
740 745 750

His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
755 760 765

[0110]

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr
770 775 780

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
785 790 795 800

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
805 810 815

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
820 825 830

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
835 840 845

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile

850 855 860

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
865 870 875 880

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
885 890 895

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
900 905 910

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
915 920 925

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
930 935 940

[0111]

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
945 950 955 960

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys
965 970 975

Asp Pro Lys Ala Val Phe Gly Cys Ile Phe Gly Ala Leu Val Ile Val
980 985 990

Thr Val Gly Gly Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala Lys
995 1000 1005

Asn Asn Glu Val Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser Lys
1010 1015 1020

Leu Ile Arg Val Glu Asn Phe Glu Ala Tyr Phe Lys Lys Gln Gln

1025 1030 1035

Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr Glu Asp Leu Lys
1040 1045 1050

Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala Glu Leu Ala Glu
1055 1060 1065

Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu Pro Tyr Asp Ile
1070 1075 1080

Ser Arg Val Lys Leu Ser Val Gln Thr His Ser Thr Asp Asp Tyr
1085 1090 1095

Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys Lys Asp Phe
1100 1105 1110

[0112]

Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp Phe Trp
1115 1120 1125

Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile Met Leu Thr
1130 1135 1140

Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu Tyr Trp Pro
1145 1150 1155

Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met Thr
1160 1165 1170

Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp Phe Thr Val
1175 1180 1185

Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His

1190 1195 1200

Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp Leu
1205 1210 1215

Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser
1220 1225 1230

Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser Ala Gly Val Gly
1235 1240 1245

Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln Ile
1250 1255 1260

Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val Tyr Asp Leu
1265 1270 1275

[0113]

Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp Gln Tyr Val
1280 1285 1290

Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser Gln Lys Asp
1295 1300 1305

Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala Met Thr Ile
1310 1315 1320

Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly Lys Thr Asn Gly
1325 1330 1335

Tyr Ile Ala
1340

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<211> 4026
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accatcagct gcagagccag ccaggacatc agcaagtacc tgaactggta ccagcagaag 180

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caggaggaca tcgccaccta cttctgccag cagggcaaca ccctgcccta caccttcgga 360

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atctggggca gcgagaccac ctactacaac agcgccctga agagccggct gaccatcatc 660

aaggacaaca gcaagagcca ggtgttcctg aagatgaaca gcctgcagac cgacgacacc 720

gccatctact actgcgccaa gcactactac tatggcggca gctacgctat ggactactgg 780

ggccagggca ccagcgtgac cgtgagctca gatcccacca cgacgccagc gccgcgacca 840

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caggaaggcc tgtacaatga actgcagaaa gataagatgg cggaggccta cagtgagatt 1260

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[0115]

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[0117]

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His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

[0118] Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

[0119] Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu
465 470 475 480

Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser
485 490 495

Thr Gly Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro
500 505 510

[0120] Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser
515 520 525

Thr Ser Gly Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly
530 535 540

Leu Glu Trp Leu Ala His Ile Trp Trp Asp Asp Asp Val Tyr Tyr Asn
545 550 555 560

Pro Ser Leu Lys Asn Gln Leu Thr Ile Ser Lys Asp Ala Ser Arg Asp
565 570 575

Gln Val Phe Leu Lys Ile Thr Asn Leu Asp Thr Ala Asp Thr Ala Thr
580 585 590

Tyr Tyr Cys Val Arg Arg Arg Ala Thr Gly Thr Gly Phe Asp Tyr Trp
595 600 605

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
610 615 620

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Val Met Thr Gln Ser
625 630 635 640

His Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Ile Ala Cys
645 650 655

Lys Ala Ser Gln Asp Val Gly Thr Ala Val Ala Trp Tyr Gln Gln Lys
660 665 670

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg His
675 680 685

[0121] Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
690 695 700

Thr Leu Thr Ile Thr Asn Val Gln Ser Glu Asp Leu Ala Asp Tyr Phe
705 710 715 720

Cys His Gln Tyr Asn Ser Tyr Asn Thr Phe Gly Ser Gly Thr Arg Leu
725 730 735

Glu Leu Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr
740 745 750

His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
755 760 765

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr
770 775 780

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
785 790 795 800

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
805 810 815

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
820 825 830

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
835 840 845

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
850 855 860

[0122] Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
865 870 875 880

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
885 890 895

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
900 905 910

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
915 920 925

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
930 935 940

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
945 950 955 960

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys
965 970 975

Asp Pro Lys Ala Val Phe Gly Cys Ile Phe Gly Ala Leu Val Ile Val
980 985 990

Thr Val Gly Gly Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala Lys
995 1000 1005

Asn Asn Glu Val Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser Lys
1010 1015 1020

Leu Ile Arg Val Glu Asn Phe Glu Ala Tyr Phe Lys Lys Gln Gln
1025 1030 1035

[0123] Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr Glu Asp Leu Lys
1040 1045 1050

Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala Glu Leu Ala Glu
1055 1060 1065

Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu Pro Tyr Asp Ile
1070 1075 1080

Ser Arg Val Lys Leu Ser Val Gln Thr His Ser Thr Asp Asp Tyr
1085 1090 1095

Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys Lys Asp Phe
1100 1105 1110

Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp Phe Trp
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Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met Thr
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Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe His
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Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln Ser
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Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser Ala Gly Val Gly
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Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln Ile
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Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val Tyr Asp Leu
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Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp Gln Tyr Val
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Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser Gln Lys Asp
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Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala Met Thr Ile
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Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
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Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
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Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

[0129]

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Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
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Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg

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Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
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Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
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Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
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Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
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[0130]

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305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn

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Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
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Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
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Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
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Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
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[0131]

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485 490 495

Thr Gly Gln Val Lys Leu Gln Gln Ser Gly Gly Gly Leu Val Lys Pro
500 505 510

Gly Ala Ser Leu Lys Leu Ser Cys Val Thr Ser Gly Phe Thr Phe Arg
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530 535 540

Trp Val Ala Ser Ile Ser Thr Gly Gly Tyr Asn Thr Tyr Tyr Ser Asp
545 550 555 560

Asn Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Thr
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Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Leu Tyr
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Tyr Cys Thr Arg Gly Tyr Ser Ser Thr Ser Tyr Ala Met Asp Tyr Trp
595 600 605

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
610 615 620

[0132]

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser
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Pro Ala Ser Leu Ser Val Ala Thr Gly Glu Lys Val Thr Ile Arg Cys
645 650 655

Met Thr Ser Thr Asp Ile Asp Asp Asp Met Asn Trp Tyr Gln Gln Lys
660 665 670

Pro Gly Glu Pro Pro Lys Phe Leu Ile Ser Glu Gly Asn Thr Leu Arg
675 680 685

Pro Gly Val Pro Ser Arg Phe Ser Ser Ser Gly Thr Gly Thr Asp Phe
690 695 700

Val Phe Thr Ile Glu Asn Thr Leu Ser Glu Asp Val Gly Asp Tyr Tyr

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Cys Leu Gln Ser Phe Asn Val Pro Leu Thr Phe Gly Asp Gly Thr Lys
725 730 735

Leu Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys
740 745 750

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
755 760 765

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg
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Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
785 790 795 800

[0133]

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
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Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
820 825 830

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
835 840 845

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
850 855 860

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
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Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys

885 890 895

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
900 905 910

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
915 920 925

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
930 935 940

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
945 950 955 960

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
965 970 975

[0134]

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980 985 990

Val Thr Val Gly Gly Phe Ile Phe Trp Arg Lys Lys Arg Lys Asp Ala
995 1000 1005

Lys Asn Asn Glu Val Ser Phe Ser Gln Ile Lys Pro Lys Lys Ser
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Lys Leu Ile Arg Val Glu Asn Phe Glu Ala Tyr Phe Lys Lys Gln
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Gln Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr Glu Asp Leu
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Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala Glu Leu Ala

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Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu Pro Tyr Asp
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Ile Ser Arg Val Lys Leu Ser Val Gln Thr His Ser Thr Asp Asp
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Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys Lys Asp
1100 1105 1110

Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys Asp Phe
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Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile Met Leu
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[0135]

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Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val Ala Met
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1175 1180 1185

Val Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg Gln Phe
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His Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr Thr Asp
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Leu Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met Lys Gln

1220 1225 1230

Ser Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser Ala Gly Val
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Gly Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu Ile Tyr Gln
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Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val Tyr Asp
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Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp Gln Tyr
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Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser Gln Lys
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[0136]

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<221> MISC_FEATURE
<222> (1)..(1)
<223> Xaa 可以是 Ser, Ile, Val 或 Leu

<220>
[0140] <221> misc_feature
<222> (2)..(2)
<223> Xaa 可以是任何天然存在的氨基酸
<220>
<221> misc_feature
<222> (4)..(5)
<223> Xaa 可以是任何天然存在的氨基酸
<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> Xaa 可以是 Ile, Val or Leu

<400> 47

Xaa Xaa Tyr Xaa Xaa Xaa
1 5

<210> 48
<211> 1114
<212> PRT
<213> 人工序列

<220>
<223> AND NOT gate 门控的氨基酸序列
(16076.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-tm-dPTP
N6)

<400> 48

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

[0141]

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly

145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

[0142]

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val

325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

[0143]

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser

500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
545 550 555 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
580 585 590

[0144]

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr

675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro
755 760 765

[0145]

Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
770 775 780

Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Tyr Trp Ala
785 790 795 800

Pro Leu Ala Gly Ile Cys Val Ala Leu Leu Leu Ser Leu Ile Ile Thr
805 810 815

Leu Ile Cys Tyr His Arg Ser Arg Lys Arg Val Cys Lys Ser Gly Gly
820 825 830

Gly Ser Phe Trp Glu Glu Phe Glu Ser Leu Gln Lys Gln Glu Val Lys
835 840 845

Asn Leu His Gln Arg Leu Glu Gly Gln Arg Pro Glu Asn Lys Gly Lys

850 855 860

Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Ser Arg Val Ile Leu
865 870 875 880

Gln Gly Arg Asp Ser Asn Ile Pro Gly Ser Asp Tyr Ile Asn Ala Asn
885 890 895

Tyr Ile Lys Asn Gln Leu Leu Gly Pro Asp Glu Asn Ala Lys Thr Tyr
900 905 910

Ile Ala Ser Gln Gly Cys Leu Glu Ala Thr Val Asn Asp Phe Trp Gln
915 920 925

Met Ala Trp Gln Glu Asn Ser Arg Val Ile Val Met Thr Thr Arg Glu
930 935 940

[0146]

Val Glu Lys Gly Arg Asn Lys Cys Val Pro Tyr Trp Pro Glu Val Gly
945 950 955 960

Met Gln Arg Ala Tyr Gly Pro Tyr Ser Val Thr Asn Cys Gly Glu His
965 970 975

Asp Thr Thr Glu Tyr Lys Leu Arg Thr Leu Gln Val Ser Pro Leu Asp
980 985 990

Asn Gly Asp Leu Ile Arg Glu Ile Trp His Tyr Gln Tyr Leu Ser Trp
995 1000 1005

Pro Asp His Gly Val Pro Ser Glu Pro Gly Gly Val Leu Ser Phe
1010 1015 1020

Leu Asp Gln Ile Asn Gln Arg Gln Glu Ser Leu Pro His Ala Gly

1025 1030 1035

Pro Ile Ile Val His Cys Ser Ala Gly Ile Gly Arg Thr Gly Thr
1040 1045 1050

Ile Ile Val Ile Asp Met Leu Met Glu Asn Ile Ser Thr Lys Gly
1055 1060 1065

Leu Asp Cys Asp Ile Asp Ile Gln Lys Thr Ile Gln Met Val Arg
1070 1075 1080

Ala Gln Arg Ser Gly Met Val Gln Thr Glu Ala Gln Tyr Lys Phe
1085 1090 1095

Ile Tyr Val Ala Ile Ala Gln Phe Ile Glu Thr Thr Lys Lys Lys
1100 1105 1110

[0147]

Leu

<210> 49
<211> 918
<212> PRT
<213> 人工序列

<220>
<223> AND NOT 门控的氨基酸序列
(MP16091.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1
tm-endo)

<400> 49

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu

20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110

[0148]

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr

195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
275 280 285

[0149]

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg

370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly
450 455 460

[0150]

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe

545 550 555 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
580 585 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
625 630 635 640

[0151]

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe

725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Ala Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro
755 760 765

Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly
770 775 780

Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Leu Ile Gly
785 790 795 800

Val Ser Val Val Phe Leu Phe Cys Leu Leu Leu Leu Val Leu Phe Cys
805 810 815

[0152]

Leu His Arg Gln Asn Gln Ile Lys Gln Gly Pro Pro Arg Ser Lys Asp
820 825 830

Glu Glu Gln Lys Pro Gln Gln Arg Pro Asp Leu Ala Val Asp Val Leu
835 840 845

Glu Arg Thr Ala Asp Lys Ala Thr Val Asn Gly Leu Pro Glu Lys Asp
850 855 860

Arg Glu Thr Asp Thr Ser Ala Leu Ala Ala Gly Ser Ser Gln Glu Val
865 870 875 880

Thr Tyr Ala Gln Leu Asp His Trp Ala Leu Thr Gln Arg Thr Ala Arg
885 890 895

Ala Val Ser Pro Gln Ser Thr Lys Pro Met Ala Glu Ser Ile Thr Tyr

900 905 910

Ala Ala Val Ala Arg His
915

<210> 50
<211> 1362
<212> PRT
<213> 人工序列

<220>
<223> AND NOT 门控的氨基酸序列
(MP16092.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1
tm-endo-2A-PTPN6_SH2-dCD148)

<400> 50

Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

[0153]

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly

100 105 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125

Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
145 150 155 160

Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly
165 170 175

Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg
180 185 190

[0154]

Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr
195 200 205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser
210 215 220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
225 230 235 240

Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala
245 250 255

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp Pro
260 265 270

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala

275 280 285

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
290 295 300

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Phe Trp
305 310 315 320

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
325 330 335

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

[0155]

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg Arg Ala Glu Gly Arg Gly

450 455 460

Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ala
465 470 475 480

Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr Asp Ala
485 490 495

Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
500 505 510

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asp Ile Tyr
515 520 525

Phe Asn Leu Val Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
530 535 540

[0156]

Leu Ile Tyr Asp Thr Asn Arg Leu Ala Asp Gly Val Pro Ser Arg Phe
545 550 555 560

Ser Gly Ser Gly Ser Gly Thr Gln Tyr Thr Leu Thr Ile Ser Ser Leu
565 570 575

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Tyr Lys Asn Tyr
580 585 590

Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ser Gly
595 600 605

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
610 615 620

Gly Gly Ser Arg Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu

625 630 635 640

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
645 650 655

Thr Leu Ser Asn Tyr Gly Met His Trp Ile Arg Gln Ala Pro Gly Lys
660 665 670

Gly Leu Glu Trp Val Ser Ser Ile Ser Leu Asn Gly Gly Ser Thr Tyr
675 680 685

Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
690 695 700

Lys Ser Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
705 710 715 720

[0157]

Ala Val Tyr Tyr Cys Ala Ala Gln Asp Ala Tyr Thr Gly Gly Tyr Phe
725 730 735

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Met Asp Pro
740 745 750

Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro Thr
755 760 765

Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly Ser
770 775 780

Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Leu Ile Gly Val
785 790 795 800

Ser Val Val Phe Leu Phe Cys Leu Leu Leu Leu Val Leu Phe Cys Leu

805 810 815

His Arg Gln Asn Gln Ile Lys Gln Gly Pro Pro Arg Ser Lys Asp Glu
820 825 830

Glu Gln Lys Pro Gln Gln Arg Pro Asp Leu Ala Val Asp Val Leu Glu
835 840 845

Arg Thr Ala Asp Lys Ala Thr Val Asn Gly Leu Pro Glu Lys Asp Arg
850 855 860

Glu Thr Asp Thr Ser Ala Leu Ala Ala Gly Ser Ser Gln Glu Val Thr
865 870 875 880

Tyr Ala Gln Leu Asp His Trp Ala Leu Thr Gln Arg Thr Ala Arg Ala
885 890 895

[0158]

Val Ser Pro Gln Ser Thr Lys Pro Met Ala Glu Ser Ile Thr Tyr Ala
900 905 910

Ala Val Ala Arg His Arg Ala Glu Gly Arg Gly Ser Leu Leu Thr Cys
915 920 925

Gly Asp Val Glu Glu Asn Pro Gly Pro Trp Tyr His Gly His Met Ser
930 935 940

Gly Gly Gln Ala Glu Thr Leu Leu Gln Ala Lys Gly Glu Pro Trp Thr
945 950 955 960

Phe Leu Val Arg Glu Ser Leu Ser Gln Pro Gly Asp Phe Val Leu Ser
965 970 975

Val Leu Ser Asp Gln Pro Lys Ala Gly Pro Gly Ser Pro Leu Arg Val

980 985 990

Thr His Ile Lys Val Met Cys Glu Gly Gly Arg Tyr Thr Val Gly Gly
995 1000 1005

Leu Glu Thr Phe Asp Ser Leu Thr Asp Leu Val Glu His Phe Lys
1010 1015 1020

Lys Thr Gly Ile Glu Glu Ala Ser Gly Ala Phe Val Tyr Leu Arg
1025 1030 1035

Gln Pro Tyr Ser Gly Gly Gly Gly Ser Phe Glu Ala Tyr Phe Lys
1040 1045 1050

Lys Gln Gln Ala Asp Ser Asn Cys Gly Phe Ala Glu Glu Tyr Glu
1055 1060 1065

[0159]

Asp Leu Lys Leu Val Gly Ile Ser Gln Pro Lys Tyr Ala Ala Glu
1070 1075 1080

Leu Ala Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Val Leu Pro
1085 1090 1095

Tyr Asp Ile Ser Arg Val Lys Leu Ser Val Gln Thr His Ser Thr
1100 1105 1110

Asp Asp Tyr Ile Asn Ala Asn Tyr Met Pro Gly Tyr His Ser Lys
1115 1120 1125

Lys Asp Phe Ile Ala Thr Gln Gly Pro Leu Pro Asn Thr Leu Lys
1130 1135 1140

Asp Phe Trp Arg Met Val Trp Glu Lys Asn Val Tyr Ala Ile Ile

1145 1150 1155

Met Leu Thr Lys Cys Val Glu Gln Gly Arg Thr Lys Cys Glu Glu
1160 1165 1170

Tyr Trp Pro Ser Lys Gln Ala Gln Asp Tyr Gly Asp Ile Thr Val
1175 1180 1185

Ala Met Thr Ser Glu Ile Val Leu Pro Glu Trp Thr Ile Arg Asp
1190 1195 1200

Phe Thr Val Lys Asn Ile Gln Thr Ser Glu Ser His Pro Leu Arg
1205 1210 1215

Gln Phe His Phe Thr Ser Trp Pro Asp His Gly Val Pro Asp Thr
1220 1225 1230

[0160]

Thr Asp Leu Leu Ile Asn Phe Arg Tyr Leu Val Arg Asp Tyr Met
1235 1240 1245

Lys Gln Ser Pro Pro Glu Ser Pro Ile Leu Val His Cys Ser Ala
1250 1255 1260

Gly Val Gly Arg Thr Gly Thr Phe Ile Ala Ile Asp Arg Leu Ile
1265 1270 1275

Tyr Gln Ile Glu Asn Glu Asn Thr Val Asp Val Tyr Gly Ile Val
1280 1285 1290

Tyr Asp Leu Arg Met His Arg Pro Leu Met Val Gln Thr Glu Asp
1295 1300 1305

Gln Tyr Val Phe Leu Asn Gln Cys Val Leu Asp Ile Val Arg Ser

1310 1315 1320

Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln Asn Thr Thr Ala
1325 1330 1335

Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr Phe Gly Lys
1340 1345 1350

Thr Asn Gly Tyr Ile Ala Ser Gly Ser
1355 1360

<210> 51
<211> 424
<212> PRT
<213> 人工序列

<220>
[0161] <223> 基于APRIL（基于增殖诱导配体）的CAR，CD8
茎 APRIL CAR

<400> 51

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser
20 25 30

Lys Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg
35 40 45

Arg Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp
50 55 60

Ala Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr
65 70 75 80

Phe Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu
85 90 95

Thr Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala
100 105 110

Tyr Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp
115 120 125

Ile Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser
130 135 140

Pro His Gly Thr Phe Leu Gly Phe Val Lys Leu Ser Gly Gly Gly Ser
145 150 155 160

[0162] Asp Pro Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
165 170 175

Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
180 185 190

Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
195 200 205

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
210 215 220

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
225 230 235 240

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
245 250 255

Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
260 265 270

Ala Tyr Arg Ser Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro
275 280 285

Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp
290 295 300

Ala His Ser Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg Ser Ala
305 310 315 320

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
325 330 335

[0163] Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
340 345 350

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
355 360 365

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
370 375 380

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
385 390 395 400

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
405 410 415

His Met Gln Ala Leu Pro Pro Arg
420

<210> 52
<211> 398
<212> PRT
<213> 人工序列

<220>
<223> 基于APRIL（基于增殖诱导配体）的CAR，基于APRIL
IgG1 铰链的CAR

<400> 52

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser
20 25 30

Lys Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg
[0164] 35 40 45

Arg Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp
50 55 60

Ala Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr
65 70 75 80

Phe Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu
85 90 95

Thr Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala
100 105 110

Tyr Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp
115 120 125

Ile Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser
130 135 140

Pro His Gly Thr Phe Leu Gly Phe Val Lys Leu Ser Gly Gly Gly Ser
145 150 155 160

Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro
165 170 175

Cys Pro Lys Asp Pro Lys Phe Trp Val Leu Val Val Val Gly Gly Val
180 185 190

Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp
195 200 205

[0165] Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met
210 215 220

Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala
225 230 235 240

Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Asp Gln Arg Leu Pro
245 250 255

Pro Asp Ala His Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile
260 265 270

Gln Glu Glu Gln Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg Val
275 280 285

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn
290 295 300

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val
305 310 315 320

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg
325 330 335

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys
340 345 350

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg
355 360 365

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys
370 375 380

[0166] Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
385 390 395

<210> 53
<211> 614
<212> PRT
<213> 人工序列

<220>
<223> 基于APRIL（基于增殖诱导配体）的CAR，基于APRIL
Fc-pvaa 的 CAR

<400> 53

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser
20 25 30

Lys Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg
35 40 45

Arg Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp
50 55 60

Ala Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr
65 70 75 80

Phe Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu
85 90 95

Thr Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala
100 105 110

[0167] Tyr Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp
115 120 125

Ile Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser
130 135 140

Pro His Gly Thr Phe Leu Gly Phe Val Lys Leu Ser Gly Gly Gly Ser
145 150 155 160

Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro
165 170 175

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
180 185 190

Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys
195 200 205

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
210 215 220

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
225 230 235 240

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
245 250 255

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
260 265 270

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
275 280 285

[0168] Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
290 295 300

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
305 310 315 320

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
325 330 335

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
340 345 350

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
355 360 365

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
370 375 380

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Phe Trp
385 390 395 400

Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val
405 410 415

Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu
420 425 430

Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr
435 440 445

Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr
450 455 460

[0169] Arg Ser Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly
465 470 475 480

Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His
485 490 495

Ser Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
500 505 510

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
515 520 525

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
530 535 540

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
545 550 555 560

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
565 570 575

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
580 585 590

[0170]

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
595 600 605

Gln Ala Leu Pro Pro Arg
610

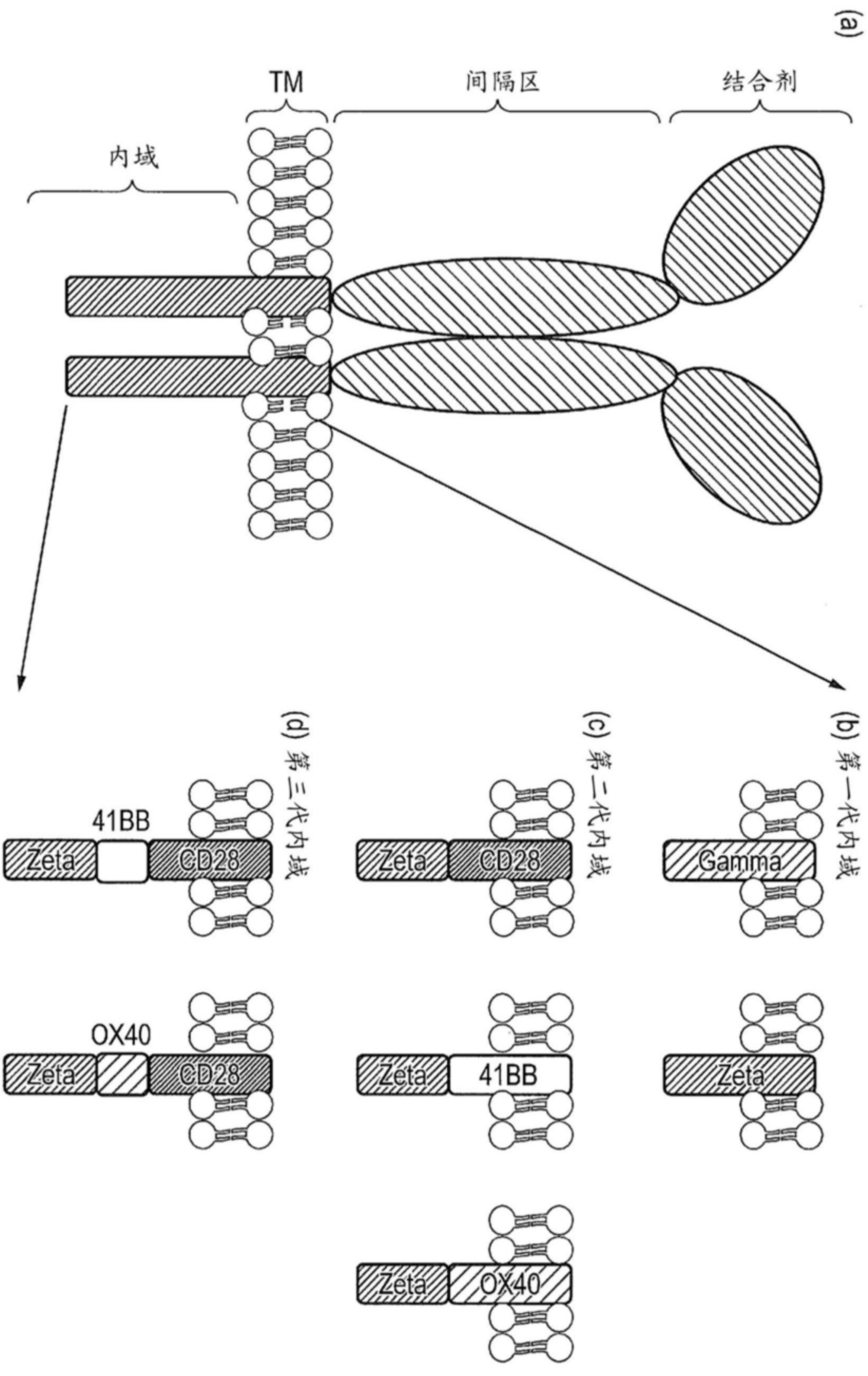

(a)
TM
间隔区
结合剂
内域
(b) 第一代内域
Gamma
Zeta
(c) 第二代内域
Zeta
CD28
41BB
OX40
(d) 第三代内域
41BB
OX40
CD28

图1

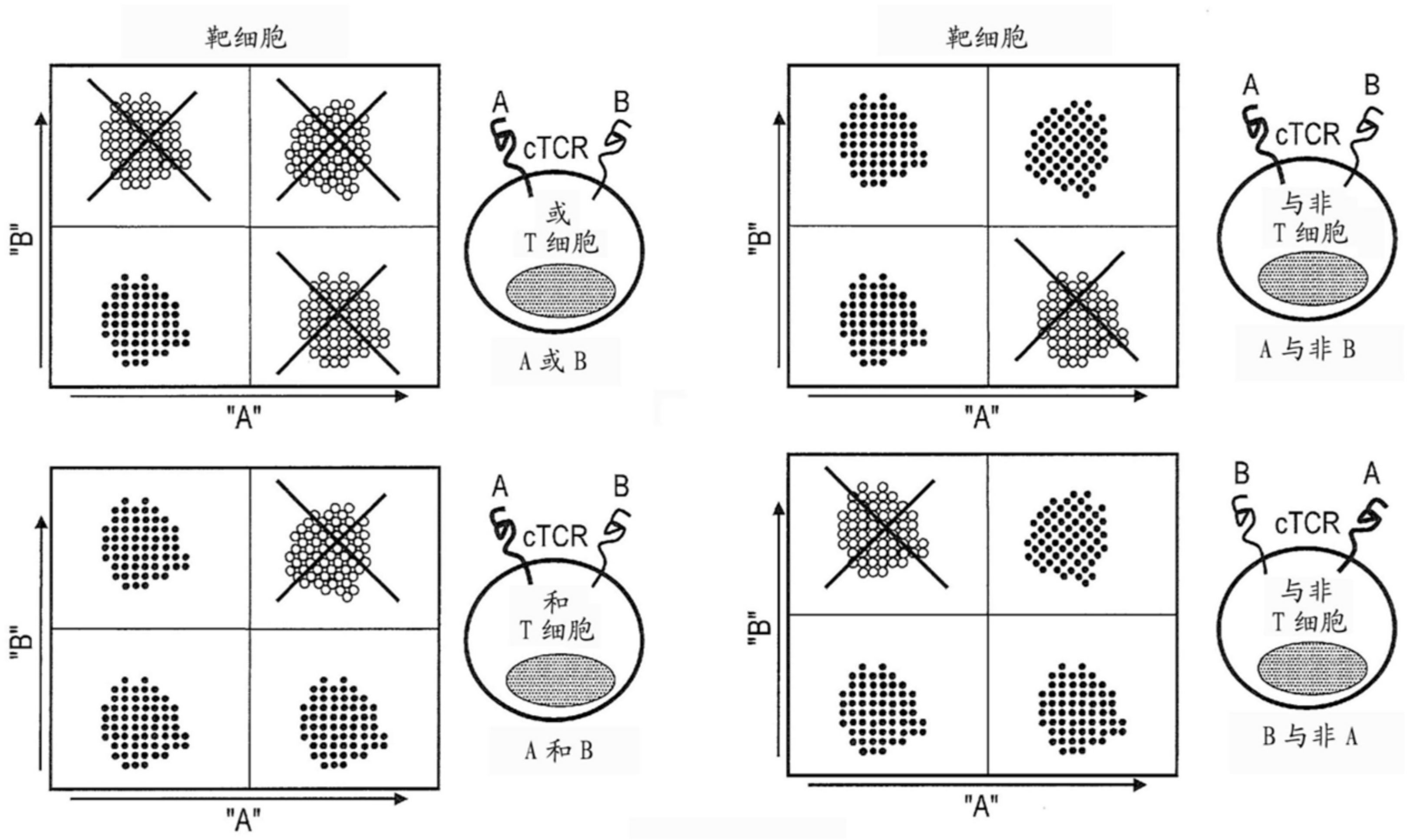

靶细胞
"B"
"A"
A
B
cTCR
或
T 细胞
A 或 B
靶细胞
"B"
"A"
A
B
cTCR
与非
T 细胞
A 与非 B
"B"
"A"
A
B
cTCR
和
T 细胞
A 和 B
"B"
"A"
B
A
cTCR
与非
T 细胞
B 与非 A

图2

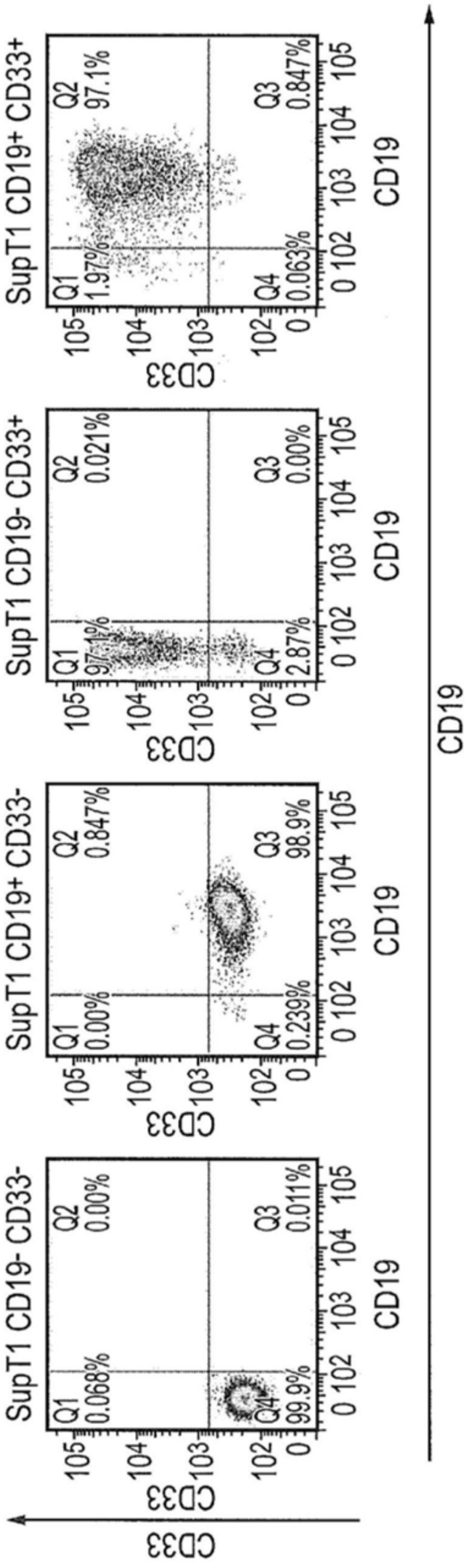

SupT1 CD19- CD33-
Q1 0.068%
Q2 0.00%
Q3 0.011%
Q4 99.9%
SupT1 CD19+ CD33-
Q1 0.00%
Q2 0.847%
Q3 98.9%
Q4 0.239%
SupT1 CD19- CD33+
Q1 97.1%
Q2 0.021%
Q3 0.00%
Q4 2.87%
SupT1 CD19+ CD33+
Q1 1.97%
Q2 97.1%
Q3 0.847%
Q4 0.063%
CD33
CD19
0
102
103
104
105

图3

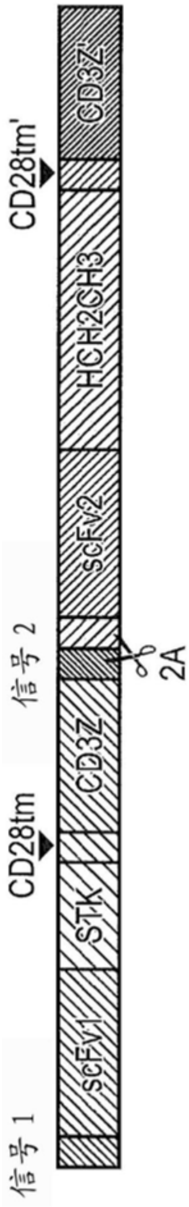


图4

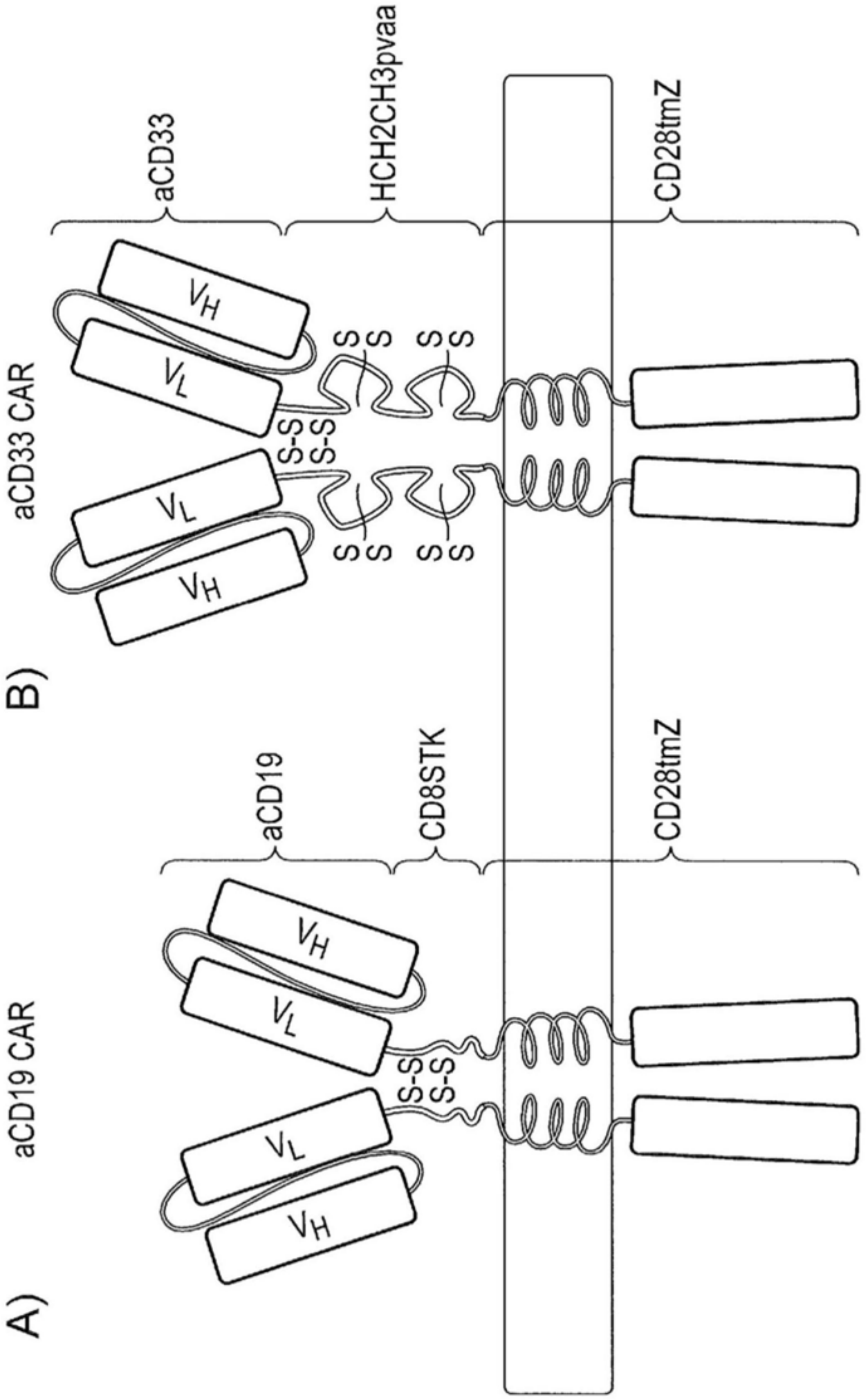

A)
aCD19 CAR
aCD19
CD8STK
CD28tmZ
V_H
V_L
S-S
S-S
B)
aCD33 CAR
aCD33
HCH2CH3pvaa
CD28tmZ
V_H
V_L
S-S
S-S
S S
S S

图5

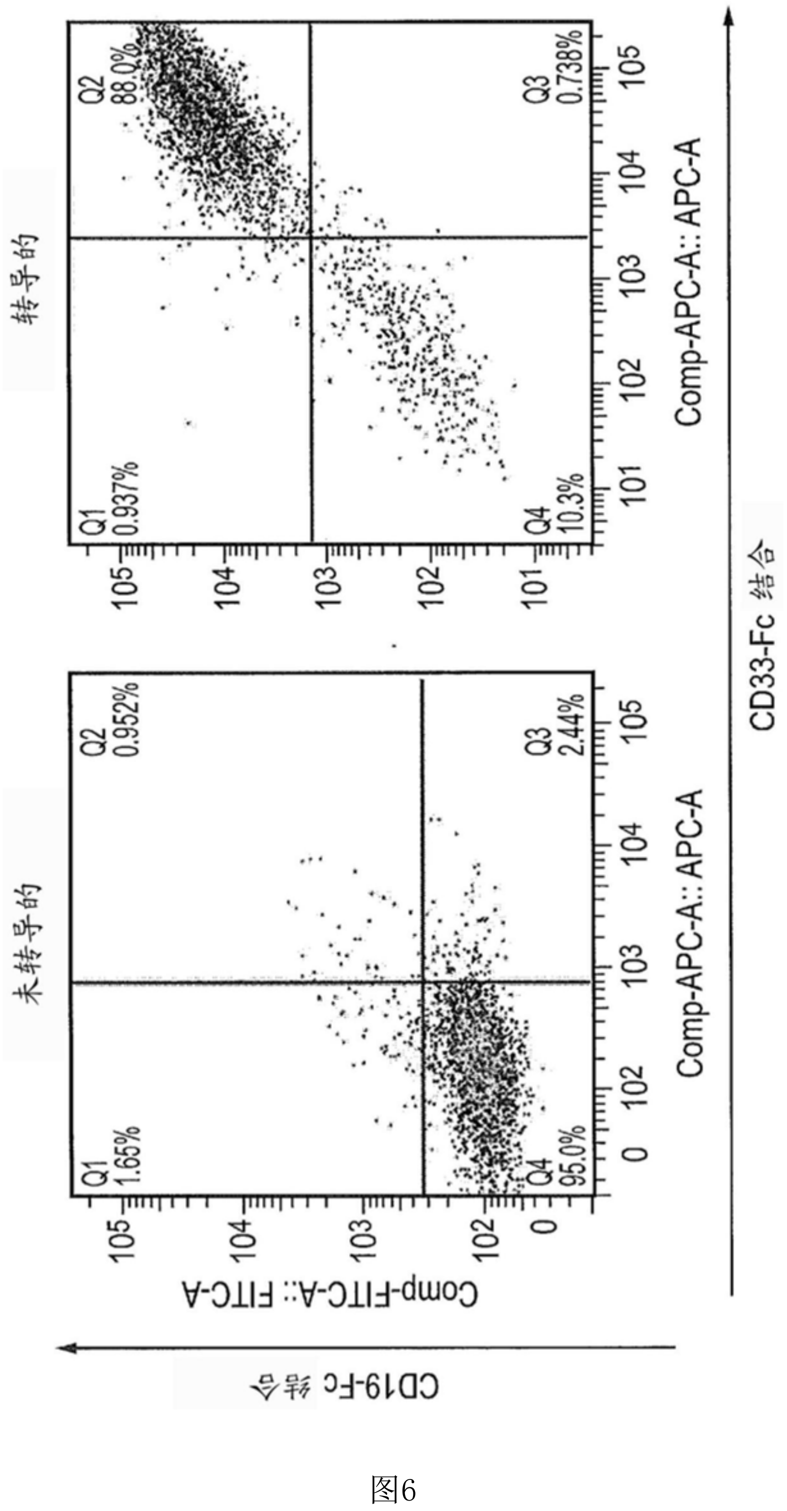

未转导的
Q1 1.65%
Q2 0.952%
Q3 2.44%
Q4 95.0%
转导的
Q1 0.937%
Q2 88.0%
Q3 0.738%
Q4 10.3%
Comp-APC-A:: APC-A
Comp-FITC-A:: FITC-A
0
10^1
10^2
10^3
10^4
10^5
CD33-Fc 结合
CD19-Fc 结合

图6

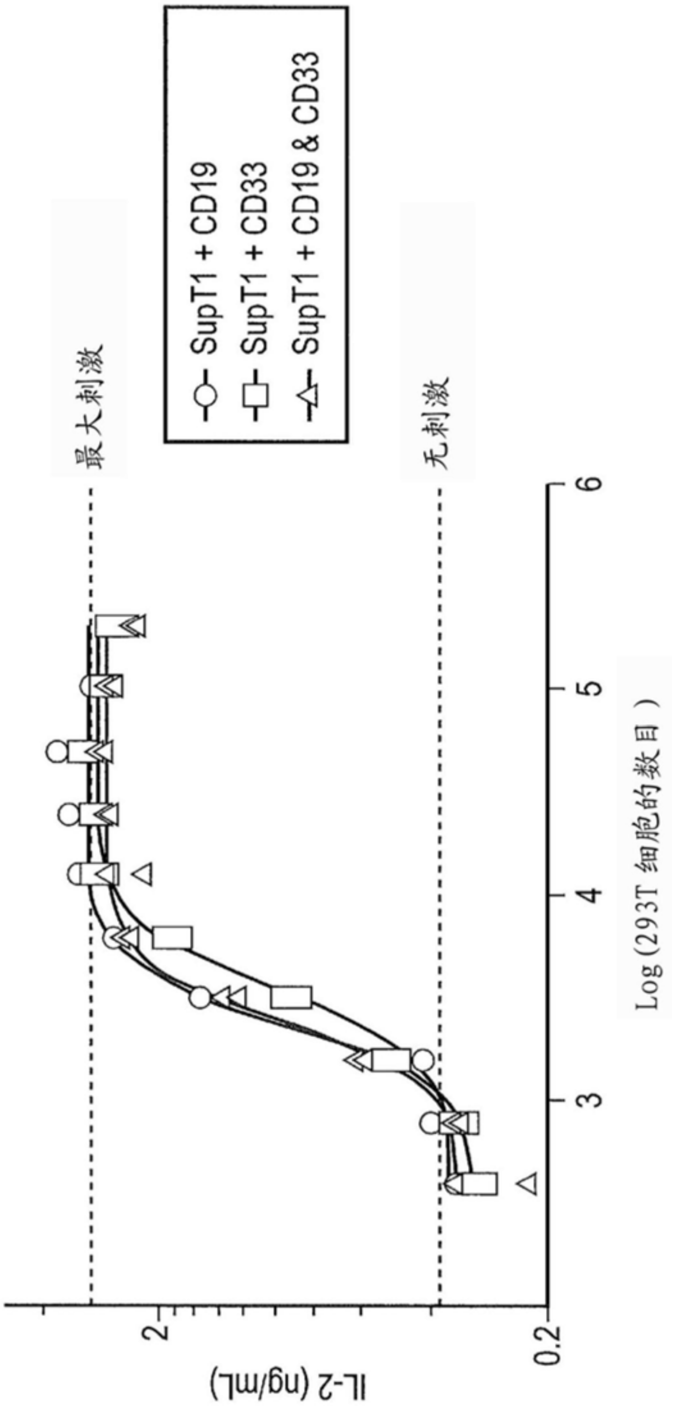

SupT1 + CD19
SupT1 + CD33
SupT1 + CD19 & CD33
最大刺激
无刺激
IL-2 (ng/mL)
0.2
2
3
4
5
6
Log(293T 细胞的数目)

图7

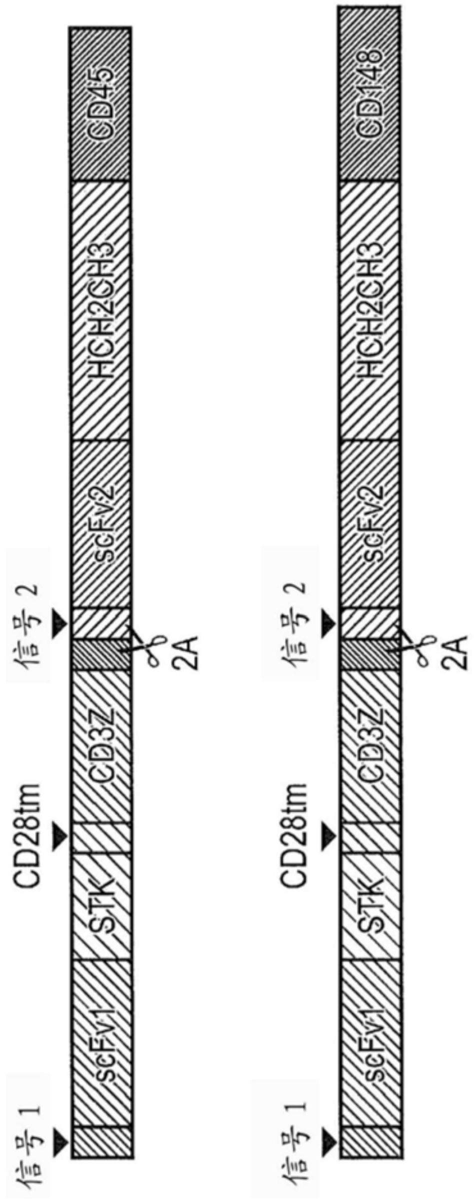

信号 1
scFV1
STK
CD28tm
CD3Z
2A
信号 2
scFV2
HCH2CH3
CD45
信号 1
scFV1
STK
CD28tm
CD3Z
2A
信号 2
scFV2
HCH2CH3
CD148

图8

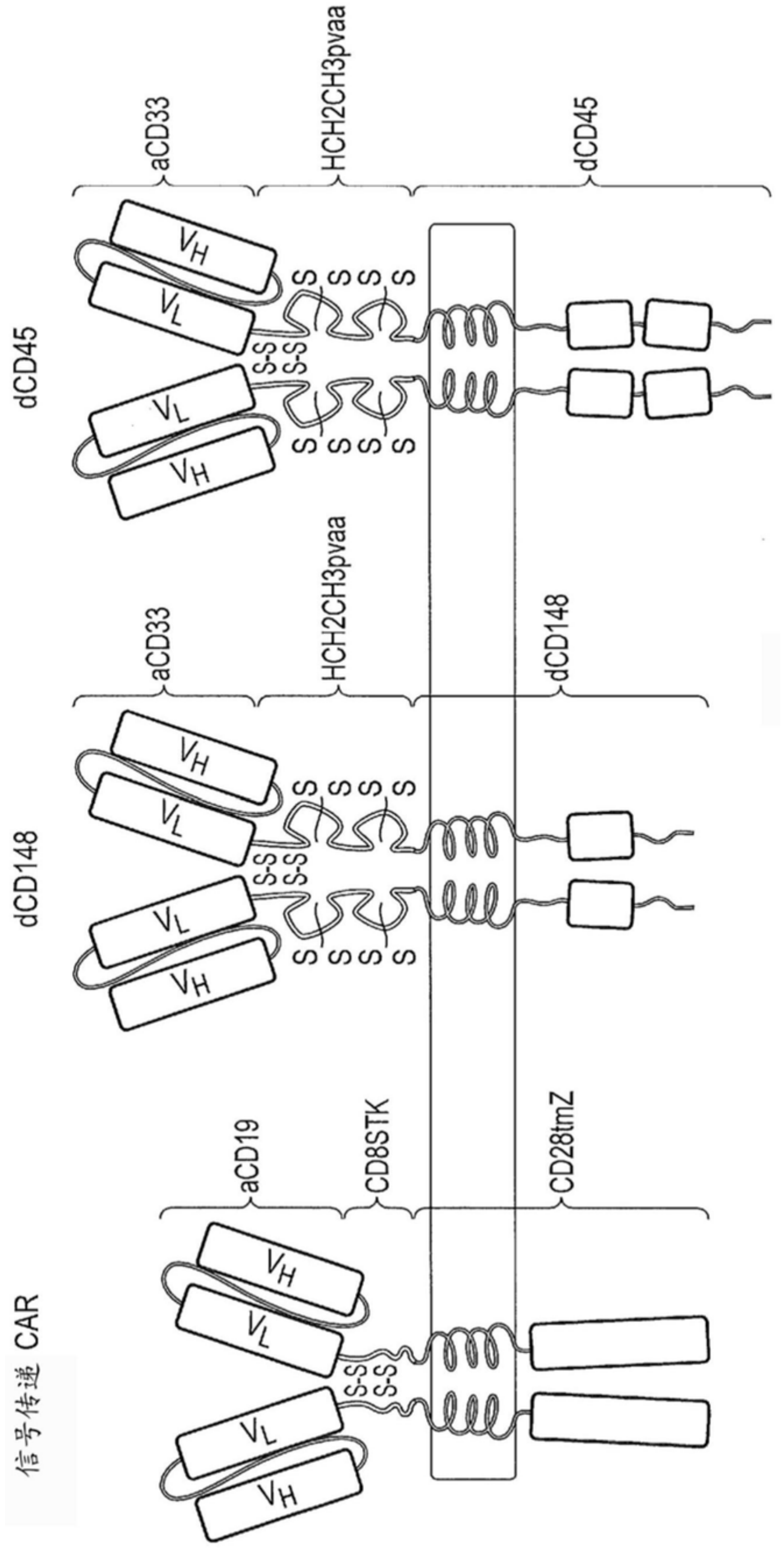

dCD45
aCD33
HCH2CH3pvaa
dCD45
V_H
V_L
S-S
S-S
S
dCD148
aCD33
HCH2CH3pvaa
dCD148
V_H
V_L
S-S
S-S
S
信号传递 CAR
aCD19
CD8STK
CD28tmZ
V_H
V_L
S-S
S-S

图9

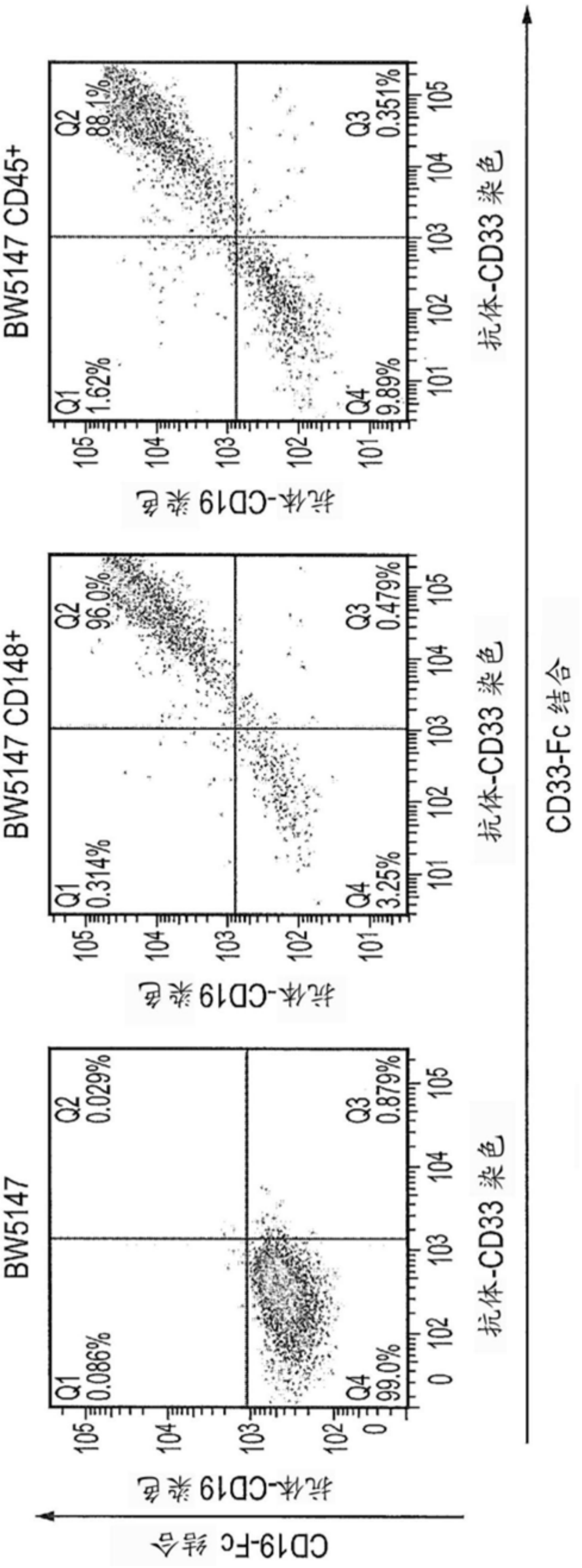

BW5147
Q1 0.086%
Q2 0.029%
Q3 0.879%
Q4 99.0%
BW5147 CD148+
Q1 0.314%
Q2 96.0%
Q3 0.479%
Q4 3.25%
BW5147 CD45+
Q1 1.62%
Q2 88.1%
Q3 0.351%
Q4 9.89%
抗体-CD19 染色
抗体-CD33 染色
CD33-Fc 结合
CD19-Fc 结合
10^5
10^4
10^3
10^2
10^1
0

图10

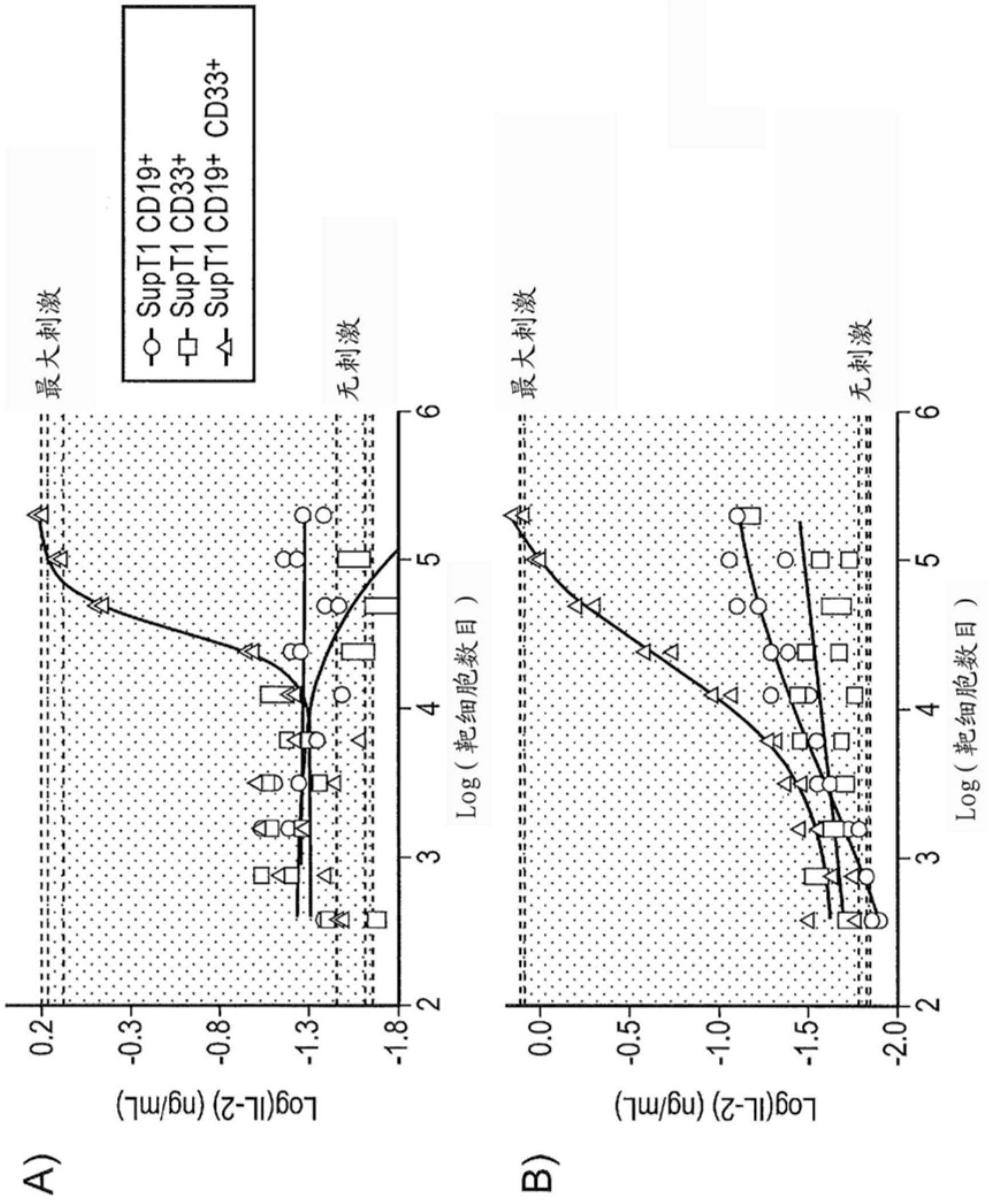

A)
Log(IL-2) (ng/mL)
0.2
-0.3
-0.8
-1.3
-1.8
2
3
4
5
6
Log（靶细胞数目）
最大刺激
无刺激
SupT1 CD19+
SupT1 CD33+
SupT1 CD19+ CD33+
B)
Log(IL-2) (ng/mL)
0.0
-0.5
-1.0
-1.5
-2.0
2
3
4
5
6
Log（靶细胞数目）
最大刺激
无刺激

图11

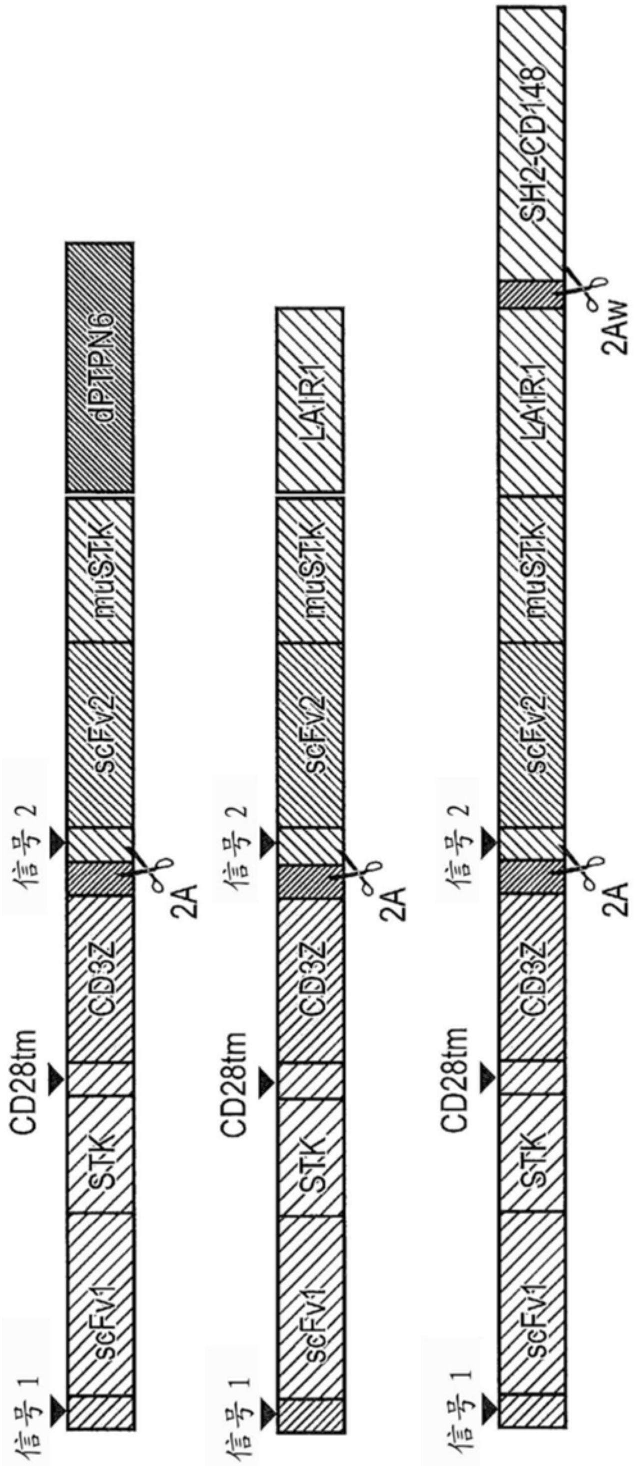

信号 1
scFv1
STK
CD28tm
CD3Z
2A
信号 2
scFv2
muSTK
dPTPN6
信号 1
scFv1
STK
CD28tm
CD3Z
2A
信号 2
scFv2
muSTK
LAIR1
信号 1
scFv1
STK
CD28tm
CD3Z
2A
信号 2
scFv2
muSTK
LAIR1
2Aw
SH2-CD148

图12

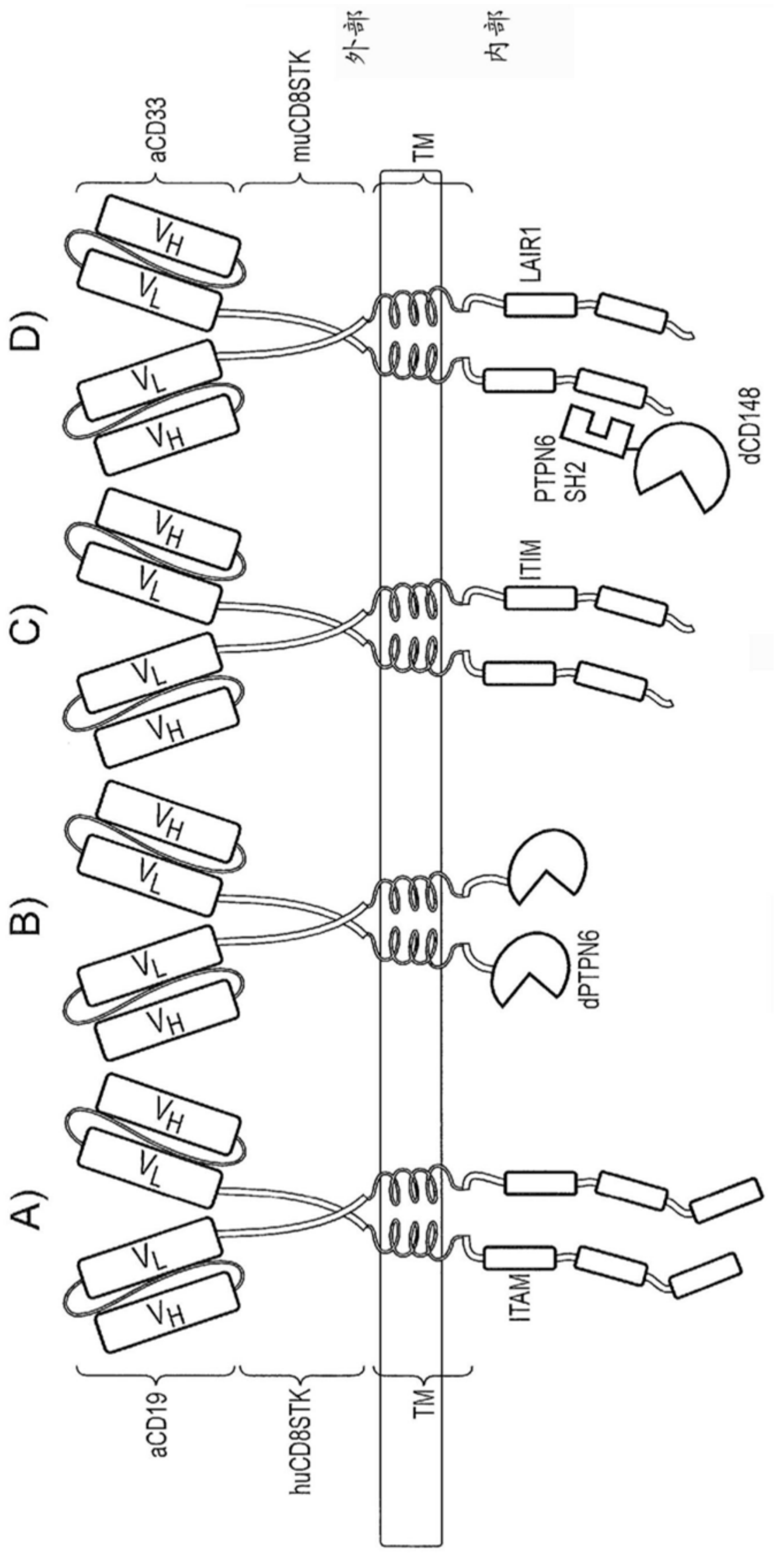

外部
内部
aCD33
muCD8STK
TM
D)
C)
B)
A)
VH
VL
LAIR1
PTPN6
SH2
dCD148
ITIM
dPTPN6
ITAM
aCD19
huCD8STK
TM

图13

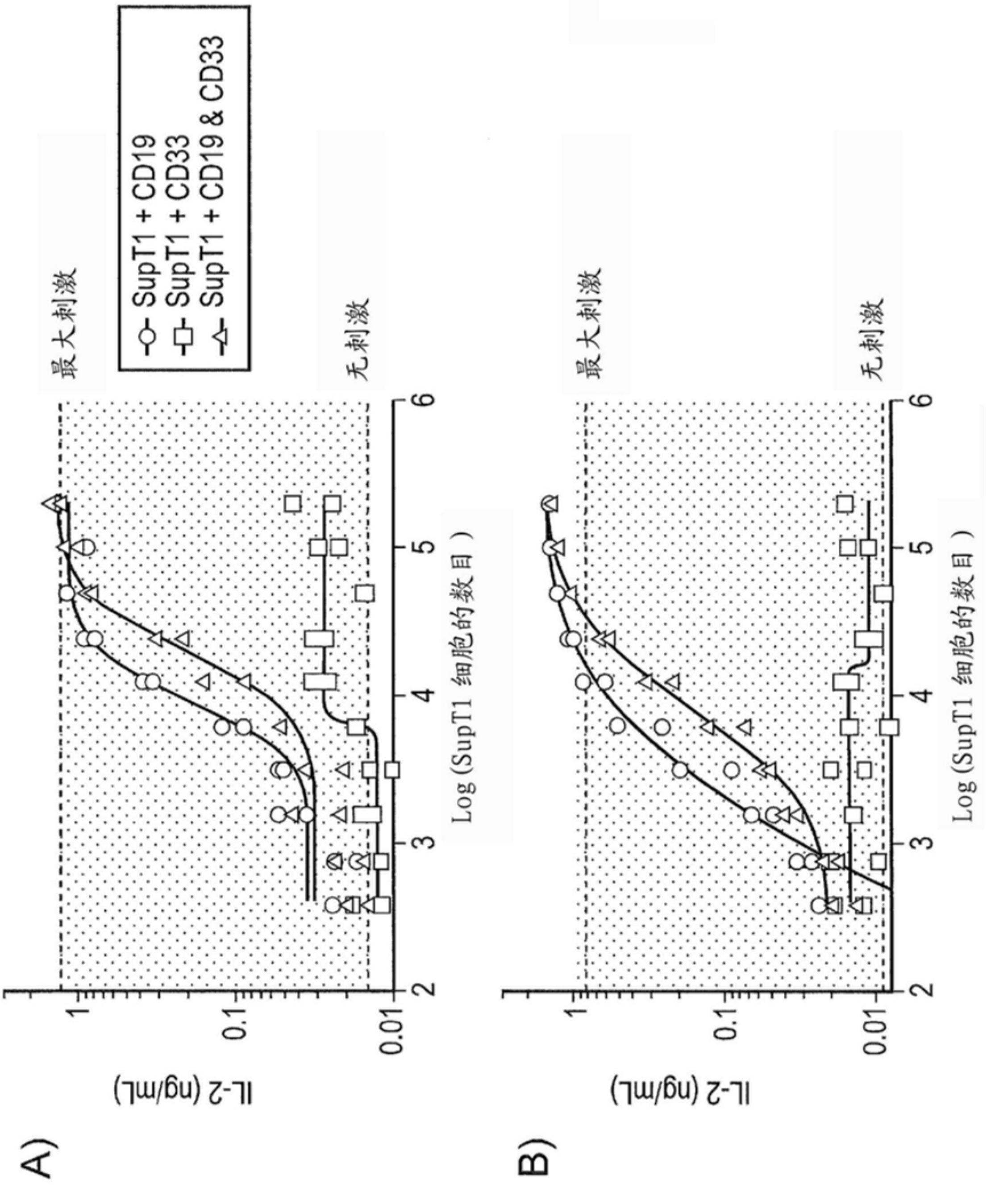

A)
IL-2 (ng/mL)
1
0.1
0.01
2
3
4
5
6
Log (SupT1 细胞的数目)
最大刺激
无刺激
SupT1 + CD19
SupT1 + CD33
SupT1 + CD19 & CD33
B)
IL-2 (ng/mL)
1
0.1
0.01
2
3
4
5
6
Log (SupT1 细胞的数目)
最大刺激
无刺激

图14

>MP13974.SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3pvaa-CD28tmZw

MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRF
SGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQ
SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY
YYGGSYAMDYWGQGTSVTVS***SDP***TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACY
SLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARC
DIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDF
ATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMH
WIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTV
SS***MDP***AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVG
GVLACYSLLVTVAFIIFWVRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

区域	描述
信号1	信号肽 1
scFv1	scFv 1－抗-CD19
SDP	接头和链中断
STK	CD8 α 茎
CD28tmZ	CD28 跨膜域和 CD3 Zeta 内域
FMD-2A	口蹄疫 2A 肽
信号2	信号肽 2
scFv2	scFv 2－抗-CD33
MDP	接头和链中断
HCH2CH3	人类 IgG1 的铰链、CH2 和 CH3
CD28tmZ'	CD28 跨膜域和 CD Zeta 内域

图15

>MP14801.SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3pvaa-dCD148

```
MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRF
SGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQ
SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY
YYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACY
SLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARC
DIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDF
ATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMH
WIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTV
SSMDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKAVFGCIFG
ALVIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENR
GKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTKCVEQGRTKC
EEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPE
SPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTT
AMTIYENLAPVTTFGKTNGYIA
```

MP14802. SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3pvaa-dCD45

```
MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRF
SGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQ
SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY
YYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACY
SLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARC
DIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDF
ATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMH
WIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTV
SSMDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKALIAFLAF
LIIVTSIALLVVLYKIYDLHKKRSCNLDEQQELVERDDEKQLMNVEPIHADILLETYKRKIADEGRLFLAEFQSIPRVFSKFP
IKEARKPFNQNKNRYVDILPYDYNRVELSEINGDAGSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMV
TRCEEGNRNKCAEYWPSMEEGTRAFGDVVVKINQHKRCPDYIIQKLNIVNKKEKATGREVTHIQFTSWPDHGVPEDPHLLLKL
RRRVNAFSNFFSGPIVVHCSAGVGRTGTYIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEAQYILIHQALVEYNQFGE
TEVNLSELHPYLHNMKKRDPPSEPSPLEAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVIPYDYNRVPLKHELEMSKESEHD
SDESSDDDSDSEEPSKYINASFIMSYWKPEVMIAAQGPLKETIGDFWQMIFQRKVKVIVMLTELKHGDQEICAQYWGEGKQTY
GDIEVDLKDTDKSSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQVVKQKLPQKNSSEGNKHHKSTPLL
IHCRDGSQQTGIFCALLNLLESAETEEVVDIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKKNNHQEDKIEFD
NEVDKVKQDANCVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEHSVNGPASPALNQGS
```

区域	描述
信号1	信号肽 1
scFv1	scFv 1 - 抗 -CD19
SDP	接头和铰链中断
STK	CD8 α 茎
CD28tmZ	CD28 跨膜域和 CD3 Zeta 内域
FMD-2A	口蹄疫 2A 肽
信号2	信号肽 2
scFv2	scFv 2 - 抗 -CD33
MDP	接头和铰链中断
HCH2CH3	人类 IgG1 的铰链、CH2 和 CH3
dCD148 / dCD45	CD148 和 CD45 的跨膜域和内域

图16

>16076.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-tm-dPTPN6

MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGS
GTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGT
SVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIY
FNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGG
SGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNA
KSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSSMDPATTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLD
FACDIYWAPLAGICVALLLSLIITLICYHRSRKRVCKSGGGSFWEEFESLQKQEVKNLHQRLEGQRPENKGKNRYKNILPFDHSRVIL
QGRDSNIPGSDYINANYIKNQLLGPDENAKTYIASQGCLEATVNDFWQMAWQENSRVIVMTTREVEKGRNKCVPYWPEVGMQRAYGPY
SVTNCGEHDTTEYKLRTLQVSPLDNGDLIREIWHYQYLSWPDHGVPSEPGGVLSFLDQINQRQESLPHAGPIIVHCSAGIGRTGTIIV
IDMLMENISTKGLDCDIDIQKTIQMVRAQRSGMVQTEAQYKFIYVAIAQFIETTKKKL

>MP16091.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1tm-endo

MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGS
GTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGT
SVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIY
FNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGG
SGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNA
KSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSS*MDP*ATTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLD
FACDILIGVSVVFLFCLLLLVLFCLHRQNQIKQGPPRSKDEEQKPQQRPDLAVDVLERTADKATVNGLPEKDRETDTSALAAGSSQEV
TYAQLDHWALTQRTARAVSPQSTKPMAESITYAAVARH

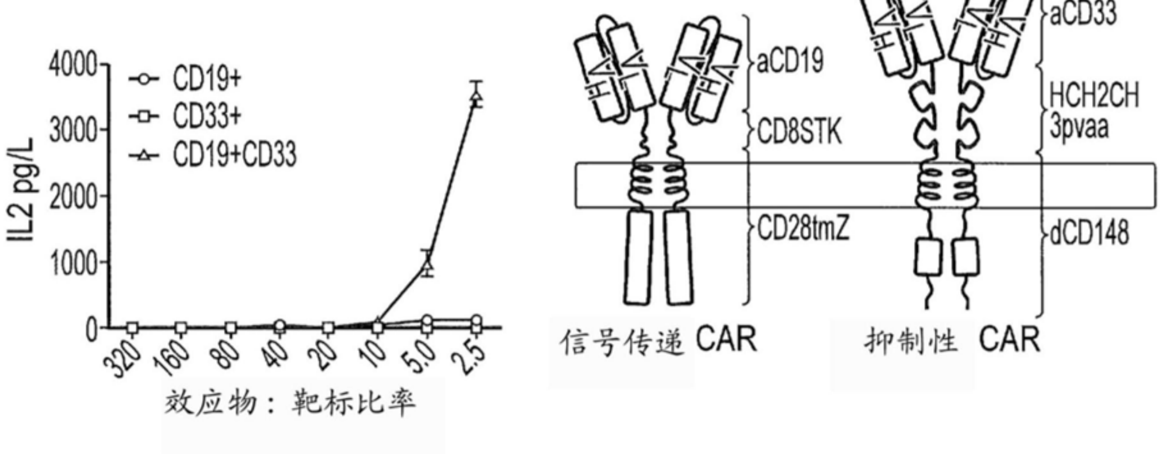
>MP16092.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1tm-endo-2A-PTPN6_SH2-dCD148

MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGS
GTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGT
SVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIY
FNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGG
SGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNA
KSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTLVTVSS*MDP*TTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDF
ACDILIGVSVVFLFCLLLLVLFCLHRQNQIKQGPPRSKDEEQKPQQRPDLAVDVLERTADKATVNGLPEKDRETDTSALAAGSSQEVT
YAQLDHWALTQRTARAVSPQSTKPMAESITYAAVARHRAEGRGSLLTCGDVEENPGPWYHGHMSGGQAETLLQAKGEPWTFLVRESLS
QPGDFVLSVLSDQPKAGPGSPLRVTHIKVMCEGGRYTVGGLETFDSLTDLVEHFKKTGIEEASGAFVYLRQPY*SGGGGS*FEAYFKKQQ
ADSNCGFAEEYEDLKLVGISQPKYAAELAENRGKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKD
FWRMVWEKNVYAIIMLTKCVEQGRTKCEEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPD
TTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDI
VRSQKDSKVDLIYQNTTAMTIYENLAPVTTFGKTNGYIASGS

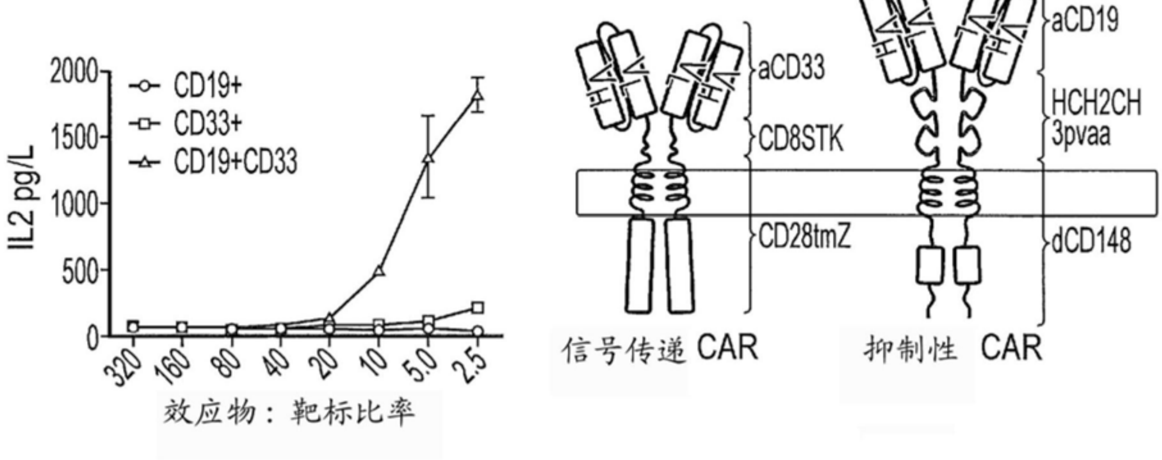
区域	描述
信号1	信号肽 1
scFv1	scFv 1－抗-CD19
SDP	接头和链中断
STK	人类 CD8α茎
CD28tmZ	CD28 跨膜域和 CD3 Zeta 内域
FMD-2A	口蹄疫 2A 肽
信号2	信号肽 2
scFv2	scFv 2－抗-CD33
MDP	接头和链中断
STK	鼠 CD8α茎
dLAIR1	人类 IgG1 的铰链、CH2 和 CH3
dPTPN6	PTPN6 的磷酸酶域
FMD-2A'	口蹄疫 2A 肽密码子摇摆
PTPN6 SH2	PTPN6 的 SH2 域
SGGGGS	丝氨酸甘氨酸接头和链中断
dCD148	CD148 的磷酸酶域

图17

A 原始



B scFv 交换



C 相似的间隔区

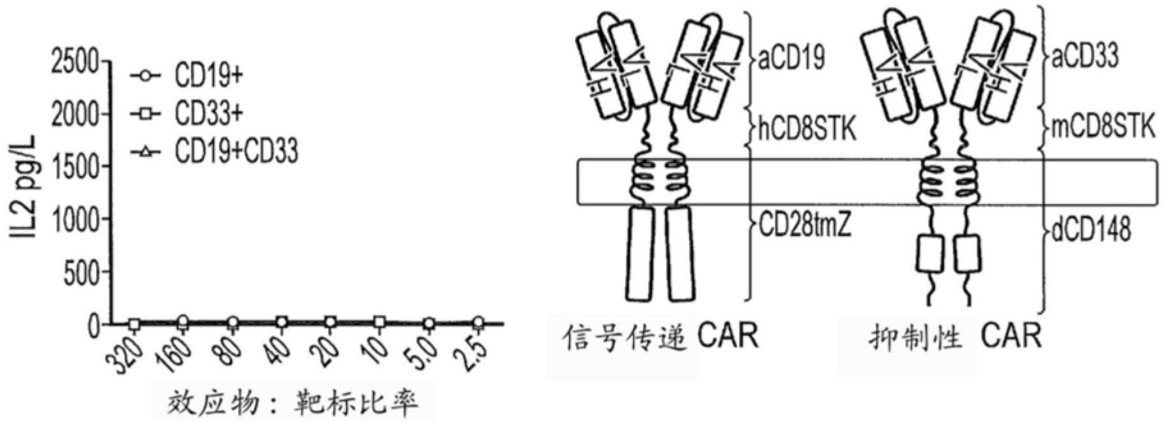


图18

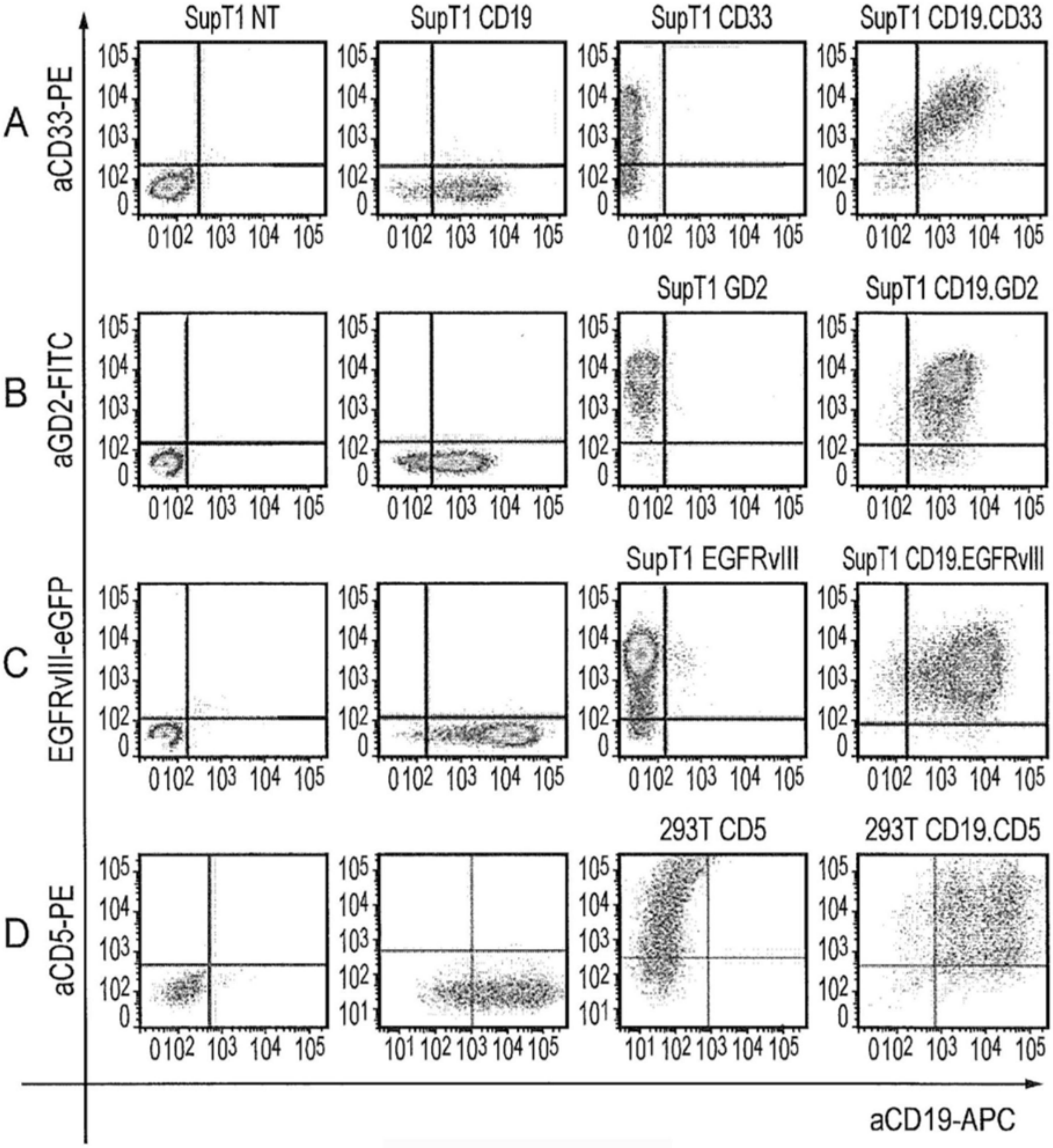

SupT1 NT
SupT1 CD19
SupT1 CD33
SupT1 CD19.CD33
A
aCD33-PE
SupT1 GD2
SupT1 CD19.GD2
B
aGD2-FITC
SupT1 EGFRvIII
SupT1 CD19.EGFRvIII
C
EGFRvIII-eGFP
293T CD5
293T CD19.CD5
D
aCD5-PE
aCD19-APC

图19

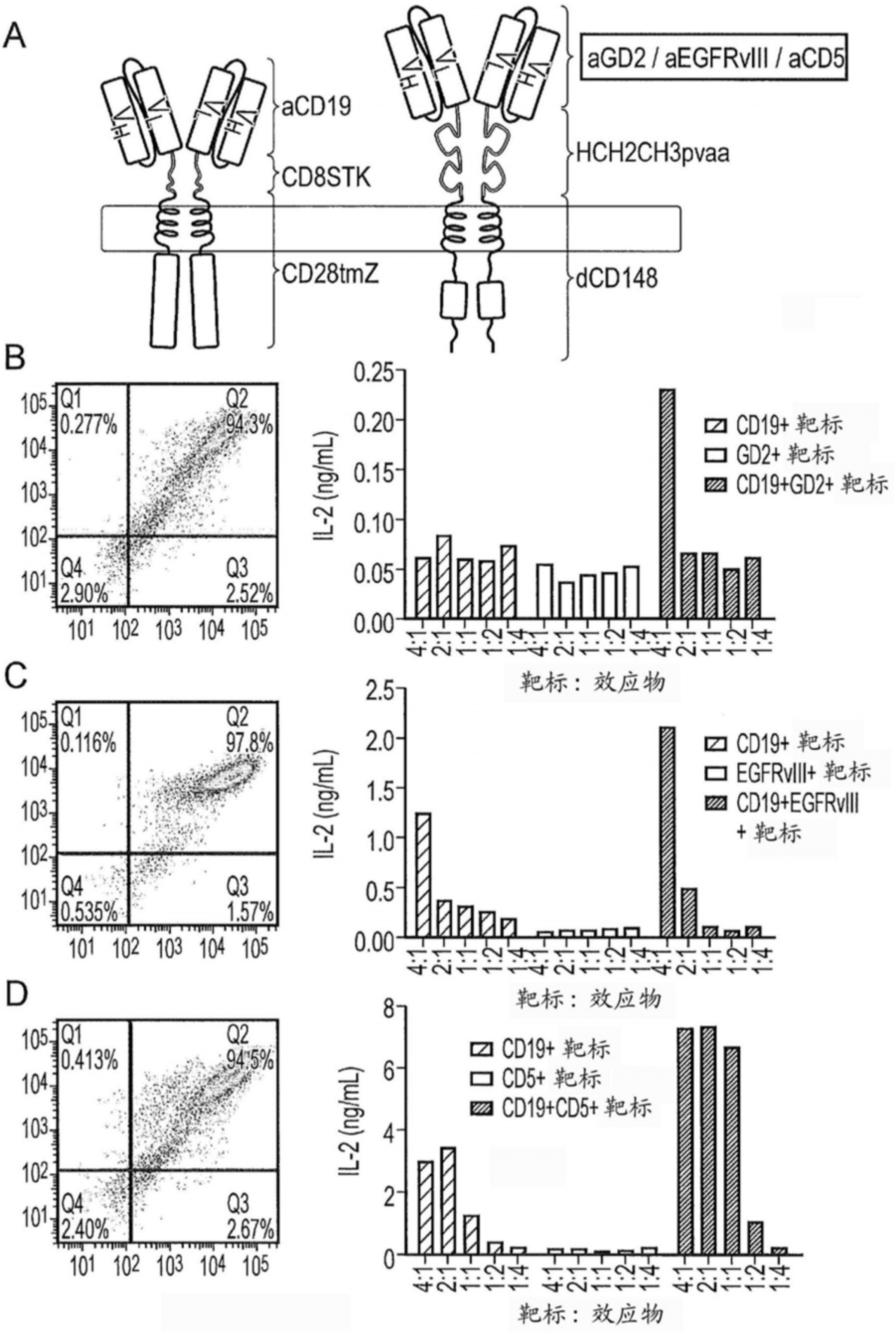

A
aCD19
CD8STK
CD28tmZ
VH
VL
aGD2 / aEGFRvIII / aCD5
HCH2CH3pvaa
dCD148
B
Q1
0.277%
Q2
94.3%
Q4
2.90%
Q3
2.52%
10^1
10^2
10^3
10^4
10^5
IL-2 (ng/mL)
0.25
0.20
0.15
0.10
0.05
0.00
▨ CD19+ 靶标
☐ GD2+ 靶标
▨ CD19+GD2+ 靶标
4:1
2:1
1:1
1:2
1:4
靶标：效应物
C
Q1
0.116%
Q2
97.8%
Q4
0.535%
Q3
1.57%
IL-2 (ng/mL)
2.5
2.0
1.5
1.0
0.5
0.00
▨ CD19+ 靶标
☐ EGFRvIII+ 靶标
▨ CD19+EGFRvIII + 靶标
靶标：效应物
D
Q1
0.413%
Q2
94.5%
Q4
2.40%
Q3
2.67%
IL-2 (ng/mL)
8
6
4
2
0
▨ CD19+ 靶标
☐ CD5+ 靶标
▨ CD19+CD5+ 靶标
靶标：效应物

图20

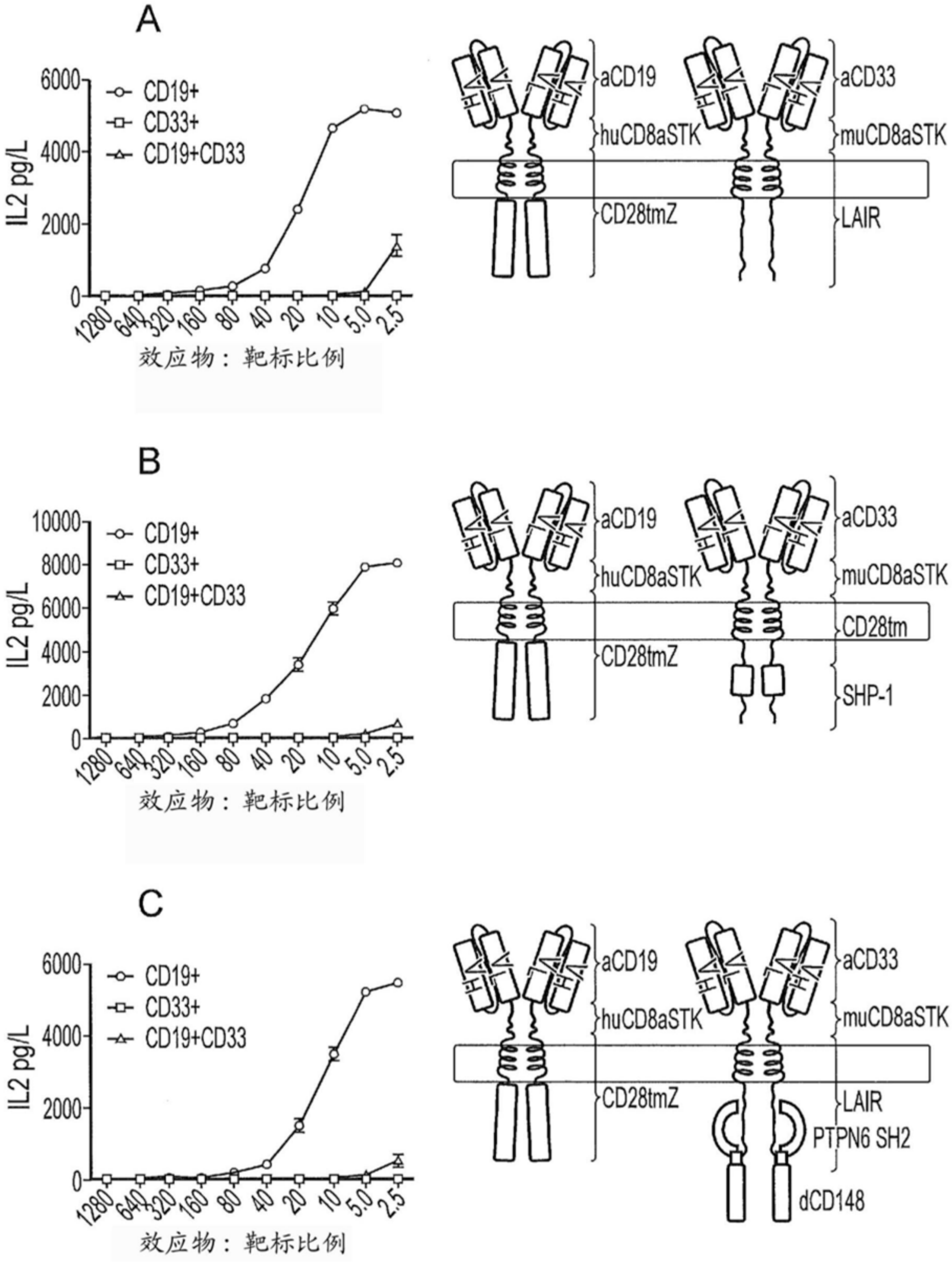

A
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
LAIR
B
10000
8000
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
CD28tm
SHP-1
C
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
LAIR
PTPN6 SH2
dCD148

图21

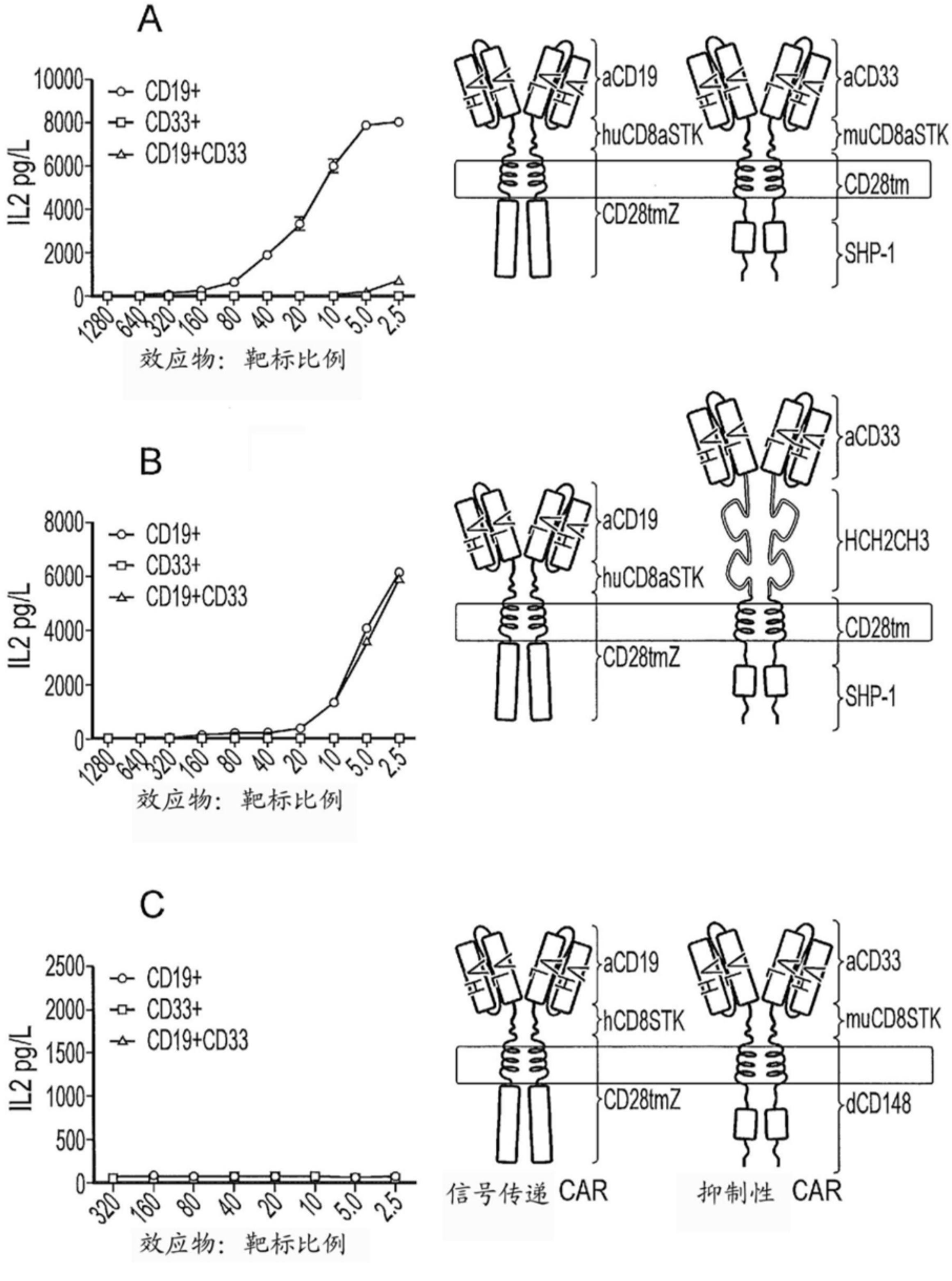

A
IL2 pg/L
10000
8000
6000
4000
2000
0
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
CD28tm
SHP-1
B
IL2 pg/L
8000
6000
4000
2000
0
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
huCD8aSTK
CD28tmZ
aCD33
HCH2CH3
CD28tm
SHP-1
C
IL2 pg/L
2500
2000
1500
1000
500
0
CD19+
CD33+
CD19+CD33
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
hCD8STK
CD28tmZ
aCD33
muCD8STK
dCD148
信号传递 CAR
抑制性 CAR

图22

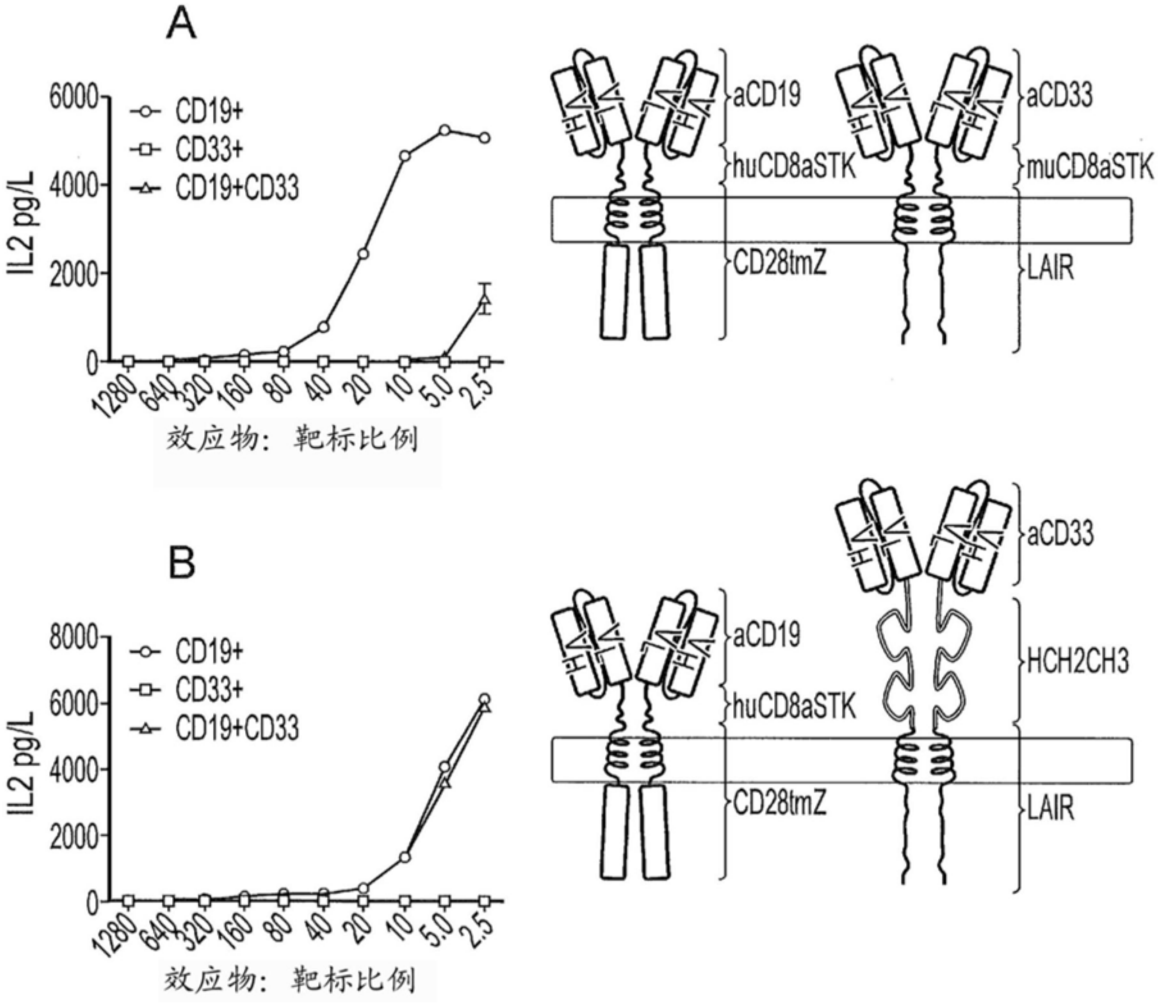

A
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
LAIR
B
8000
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
效应物：靶标比例
aCD19
huCD8aSTK
CD28tmZ
aCD33
HCH2CH3
LAIR

图23

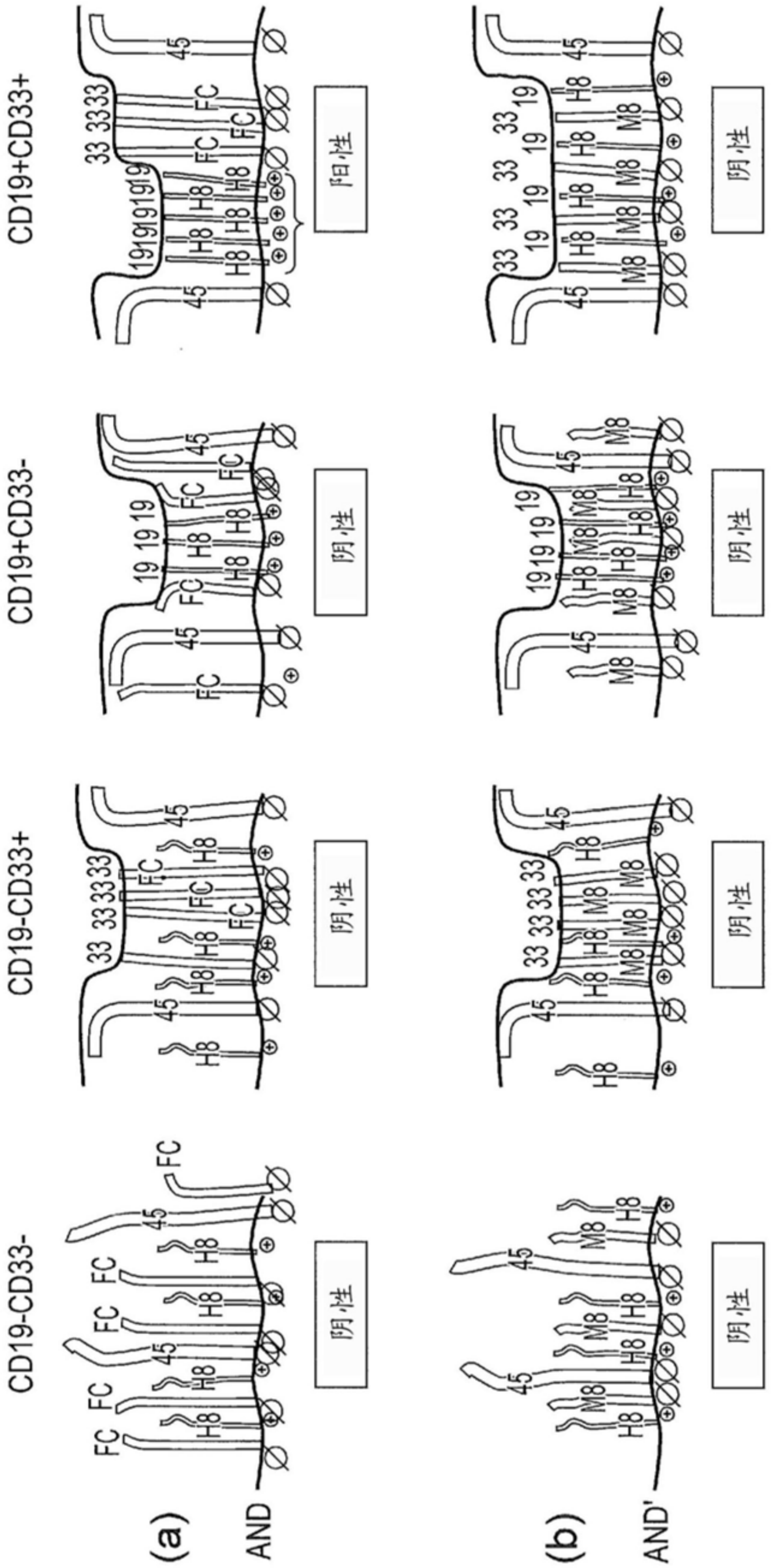

CD19-CD33-
CD19-CD33+
CD19+CD33-
CD19+CD33+
(a)
AND
(b)
AND'
FC
H8
45
33
19
M8
阴性
阳性

图24

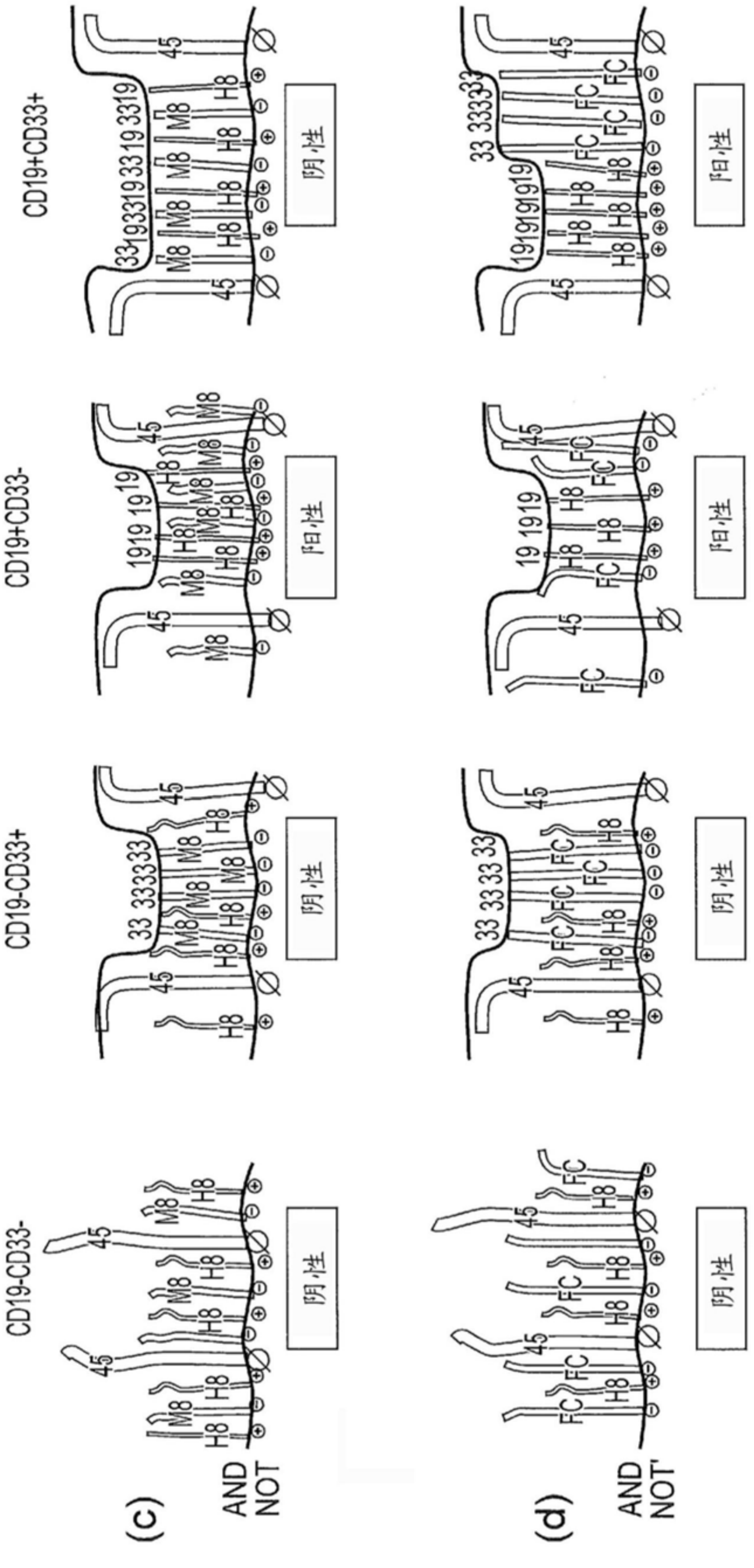

(c)
AND
NOT
CD19-CD33-
阴性
CD19-CD33+
阴性
CD19+CD33-
阳性
CD19+CD33+
阴性
(d)
AND
NOT'
阴性
阴性
阳性
阳性
H8
M8
FC
45
19
33

图24(续)

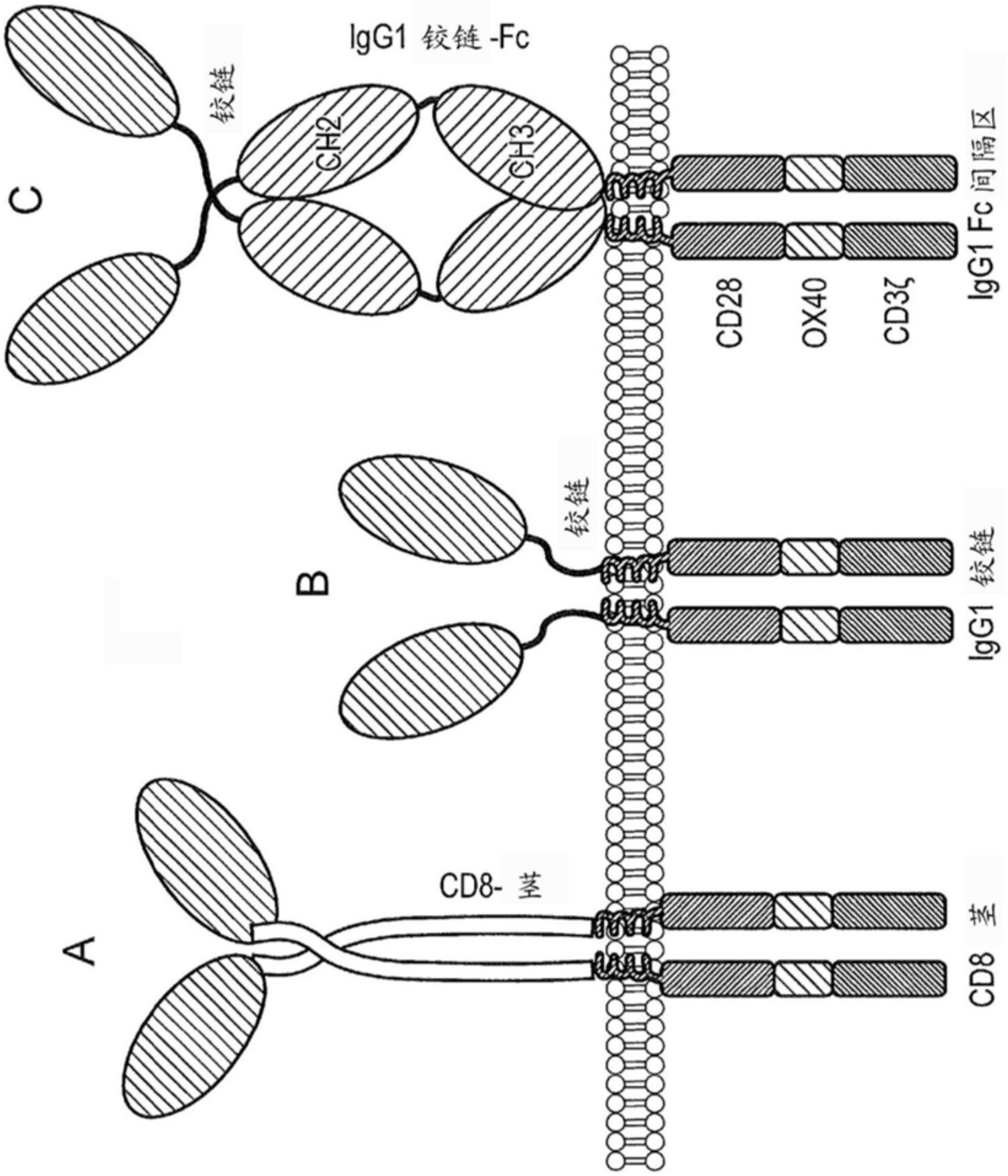
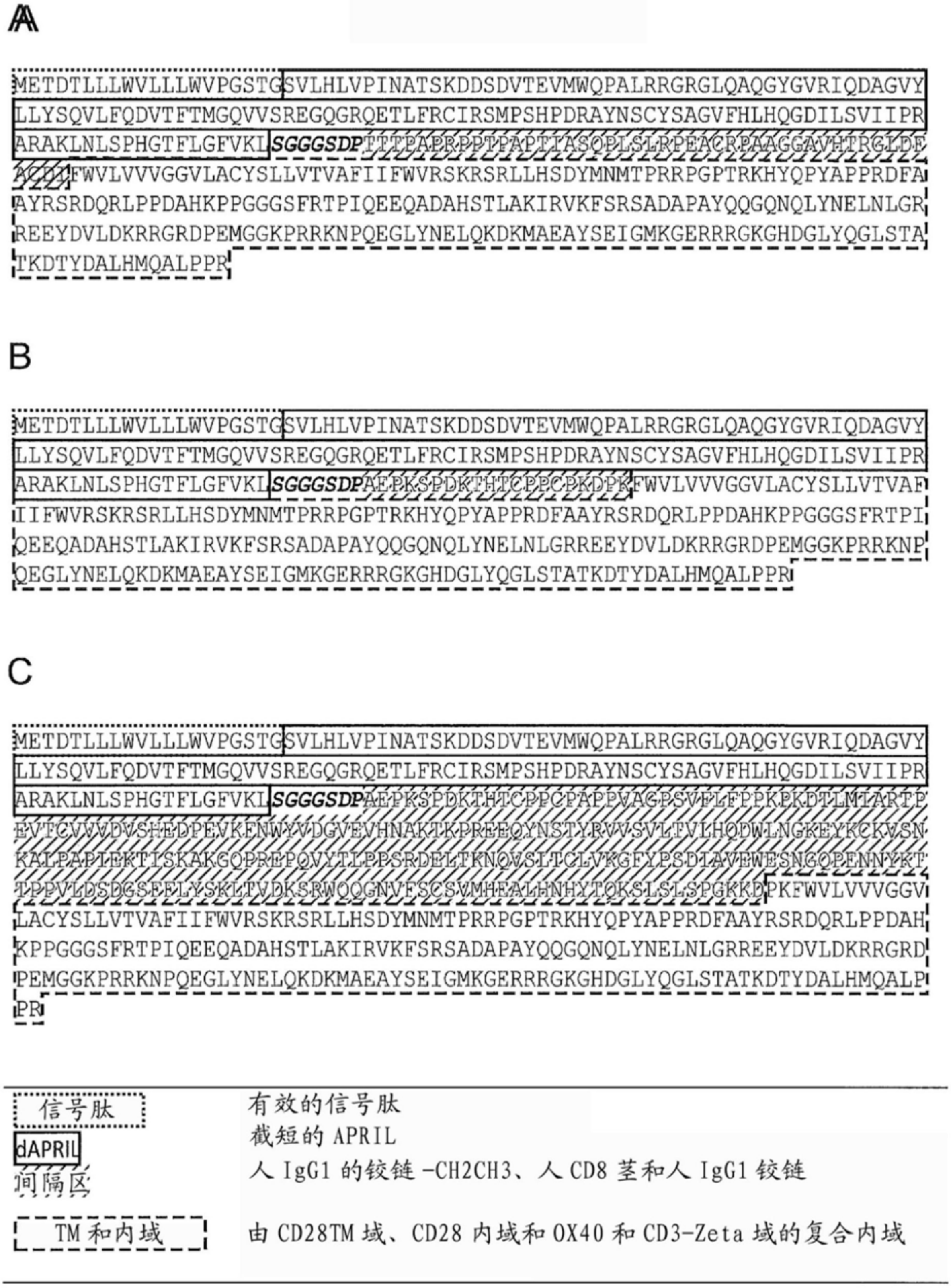

C
IgG1 铰链-Fc
铰链
CH2
CH3
CD28
OX40
CD3ζ
IgG1 Fc 间隔区
B
铰链
IgG1 铰链
A
CD8-茎
CD8 茎

图25



A

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVY
LLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPR
ARAKLNLSPHGTFLGFVKL***SGGGSDP***TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
ACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFA
AYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGR
REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA
TKDTYDALHMQALPPR

B

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVY
LLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPR
ARAKLNLSPHGTFLGFVKL***SGGGSDP***AEPKSPDKTHTCPPCPKDPKFWVLVVVGGVLACYSLLVTVAF
IIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPI
QEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

C

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVY
LLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPR
ARAKLNLSPHGTFLGFVKL***SGGGSDP***AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGV
LACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAH
KPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR

信号肽	有效的信号肽
dAPRIL	截短的 APRIL
间隔区	人 IgG1 的铰链 -CH2CH3、人 CD8 茎和人 IgG1 铰链
TM 和内域	由 CD28TM 域、CD28 内域和 OX40 和 CD3-Zeta 域的复合内域

图26

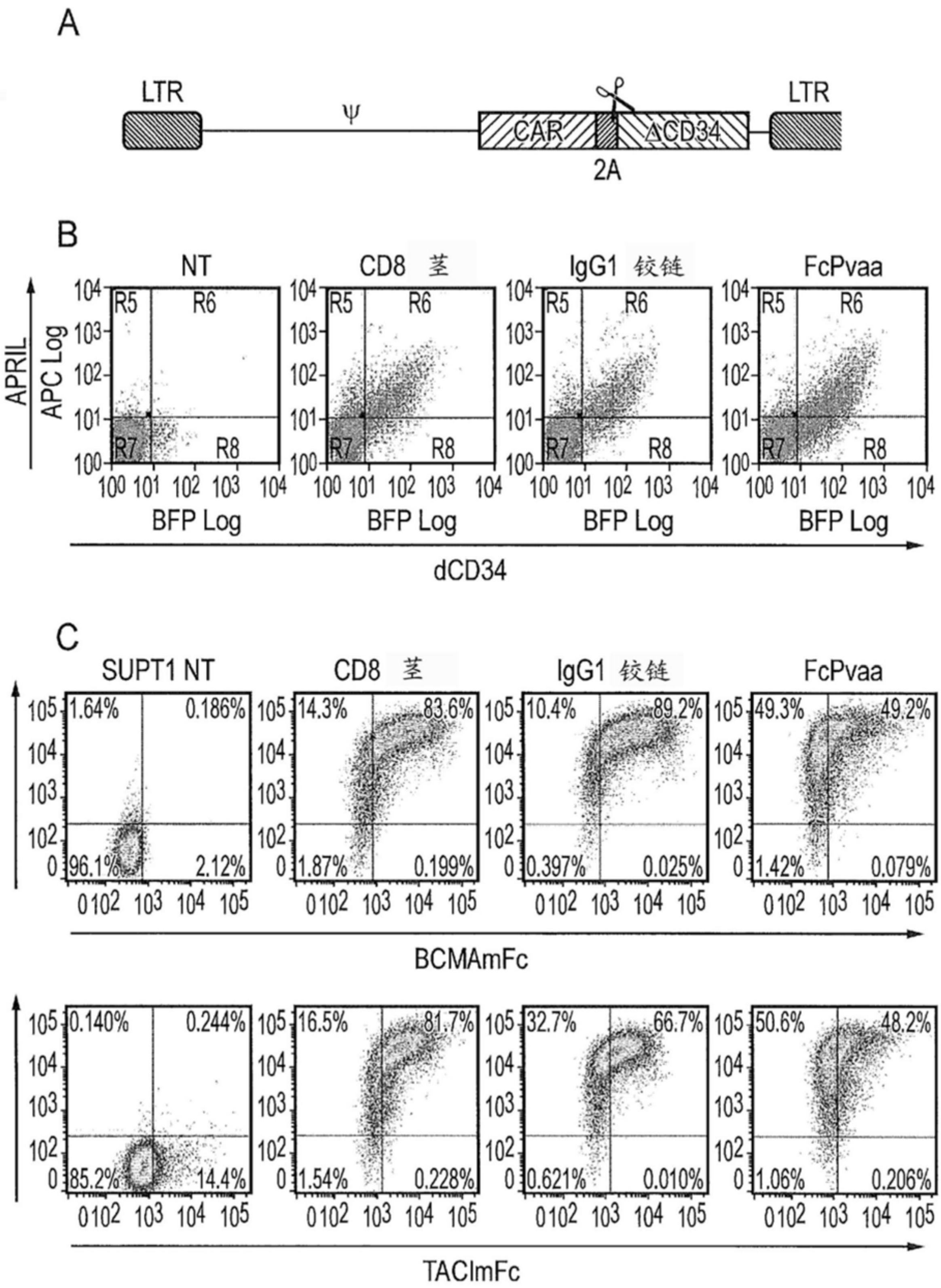

A
LTR
Ψ
CAR
2A
ΔCD34
LTR
B
NT
CD8 茎
IgG1 铰链
FcPvaa
APRIL
APC Log
R5
R6
R7
R8
10^0 10^1 10^2 10^3 10^4
BFP Log
dCD34
C
SUPT1 NT
CD8 茎
IgG1 铰链
FcPvaa
1.64% 0.186% 96.1% 2.12%
14.3% 83.6% 1.87% 0.199%
10.4% 89.2% 0.397% 0.025%
49.3% 49.2% 1.42% 0.079%
0 10^2 10^3 10^4 10^5
BCMAmFc
0.140% 0.244% 85.2% 14.4%
16.5% 81.7% 1.54% 0.228%
32.7% 66.7% 0.621% 0.010%
50.6% 48.2% 1.06% 0.206%
TACImFc

图27

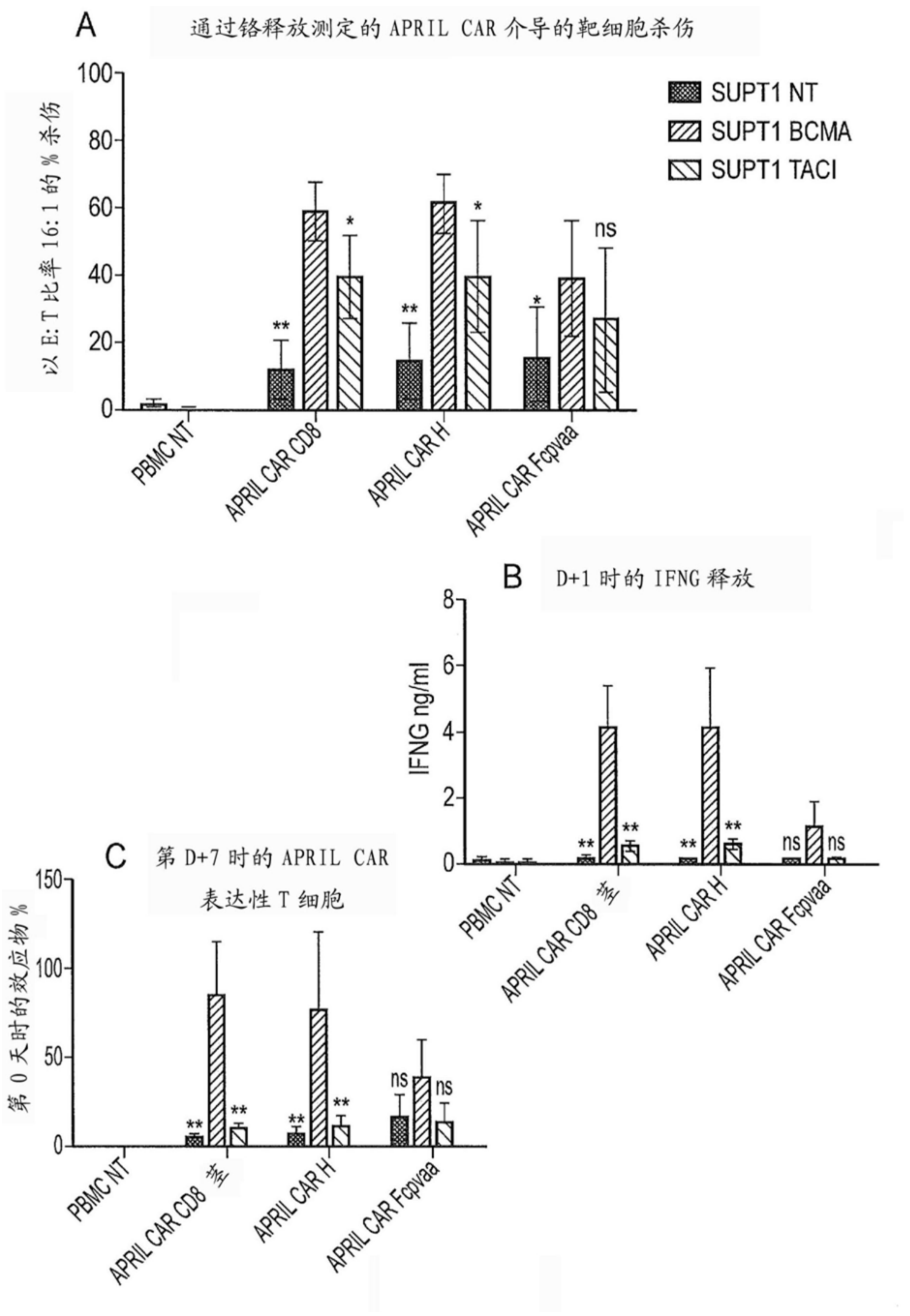

A
通过铬释放测定的 APRIL CAR 介导的靶细胞杀伤
以 E: T 比率 16: 1 的 % 杀伤
100
80
60
40
20
0
SUPT1 NT
SUPT1 BCMA
SUPT1 TACI
**
*
**
*
*
ns
PBMC NT
APRIL CAR CD8
APRIL CAR H
APRIL CAR Fcpvaa
B
D+1 时的 IFNG 释放
IFNG ng/ml
8
6
4
2
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 茎
APRIL CAR H
APRIL CAR Fcpvaa
C
第 D+7 时的 APRIL CAR
表达性 T 细胞
第 0 天时的效应物 %
150
100
50
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 茎
APRIL CAR H
APRIL CAR Fcpvaa

图28

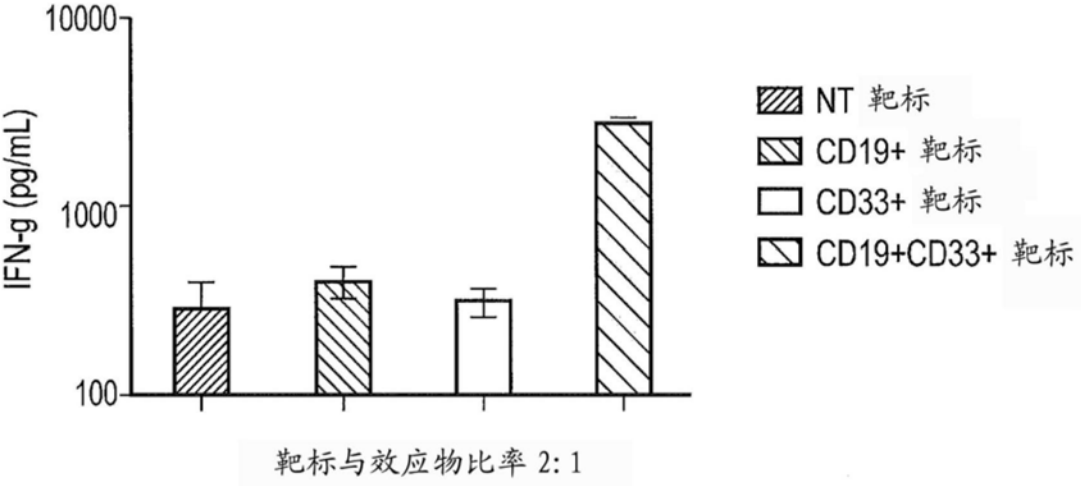

10000
1000
100
IFN-g (pg/mL)
NT 靶标
CD19+ 靶标
CD33+ 靶标
CD19+CD33+ 靶标
靶标与效应物比率 2:1

图29

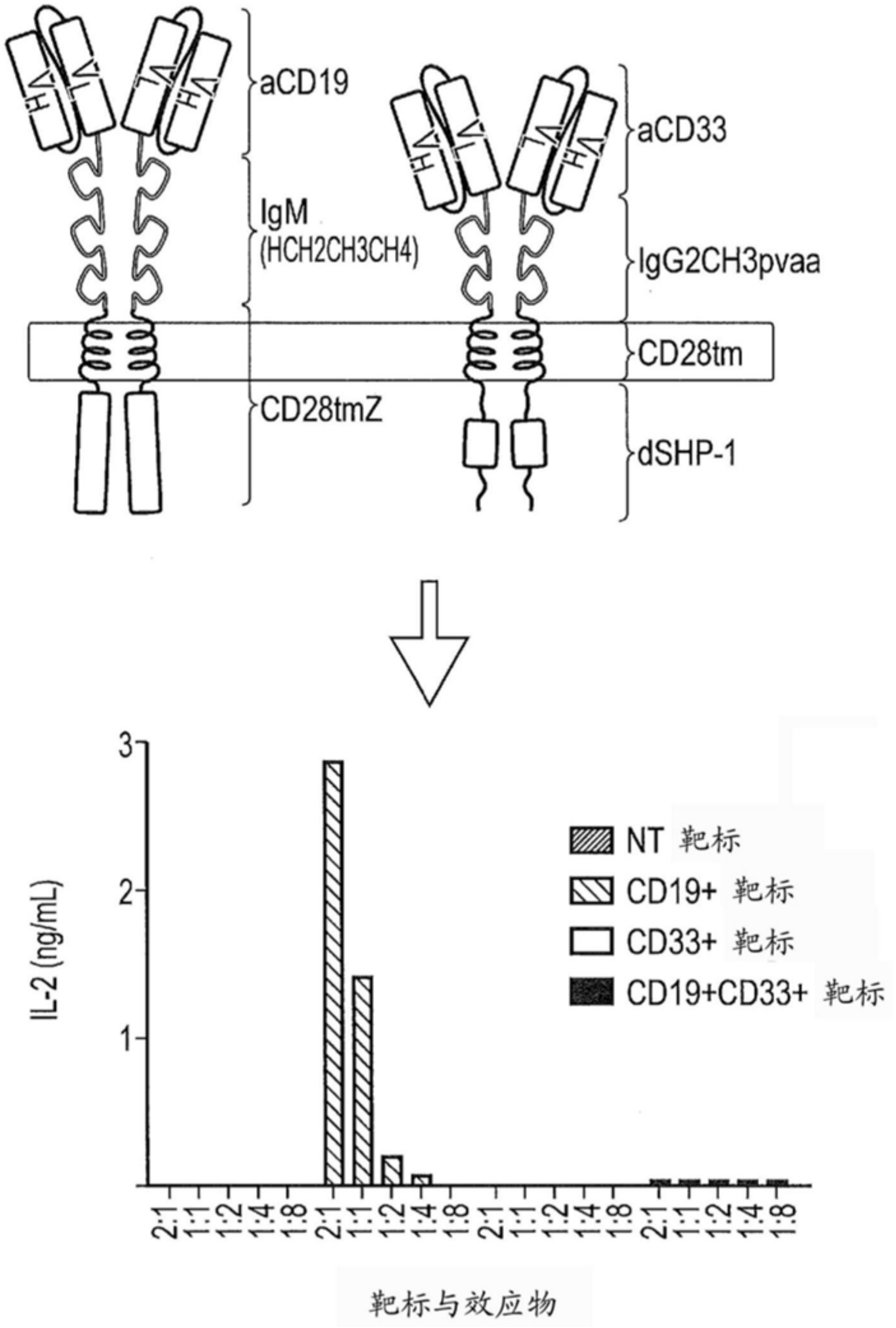

VH
VL
VL
VH
aCD19
IgM
(HCH2CH3CH4)
CD28tmZ
VH
VL
VL
VH
aCD33
IgG2CH3pvaa
CD28tm
dSHP-1
IL-2 (ng/mL)
3
2
1
NT 靶标
CD19+ 靶标
CD33+ 靶标
CD19+CD33+ 靶标
2:1
1:1
1:2
1:4
1:8
2:1
1:1
1:2
1:4
1:8
2:1
1:1
1:2
1:4
1:8
2:1
1:1
1:2
1:4
1:8
靶标与效应物

图30

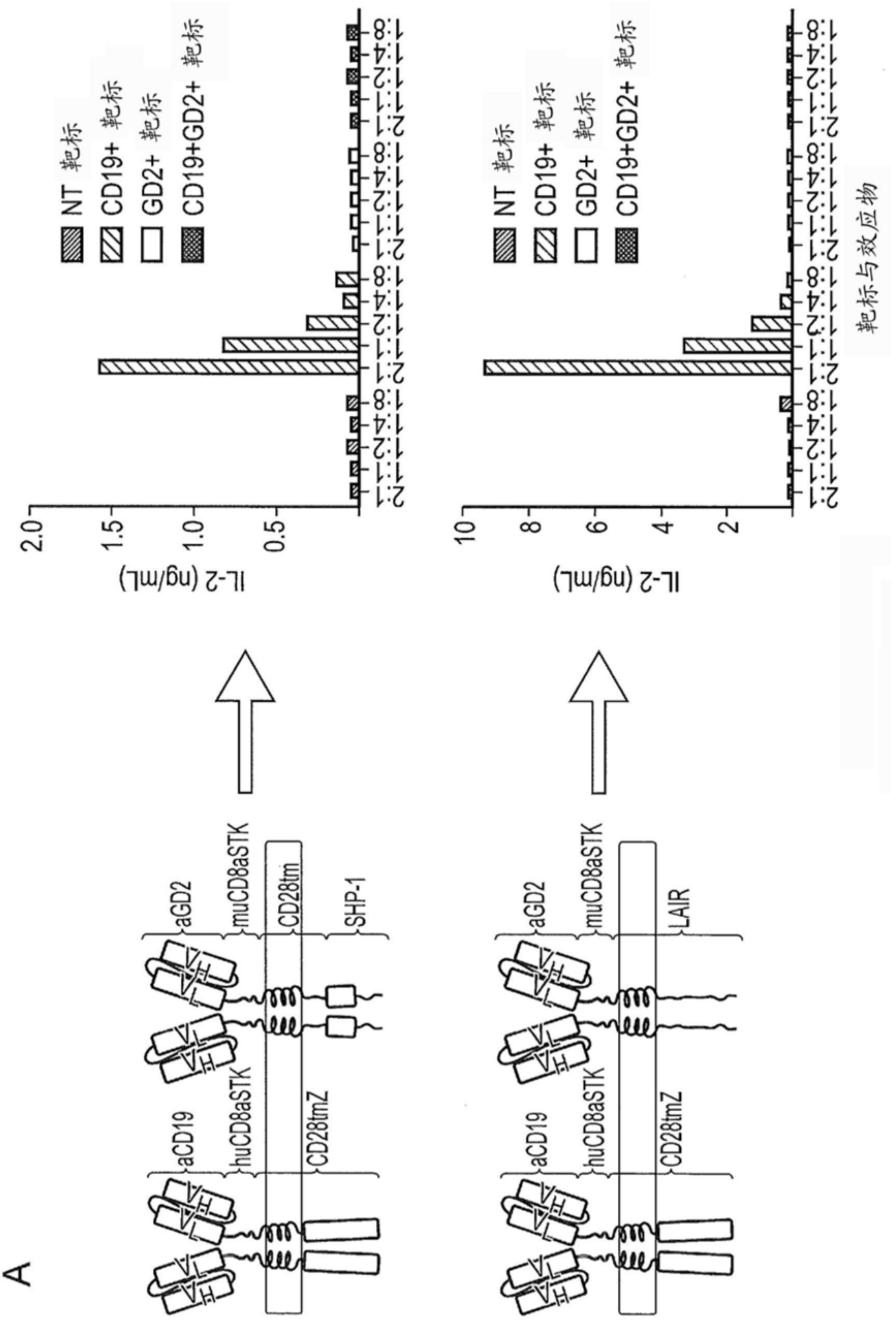

A
aCD19
huCD8aSTK
CD28tmZ
aGD2
muCD8aSTK
CD28tm
SHP-1
LAIR
VL
VH
IL-2 (ng/mL)
2.0
1.5
1.0
0.5
10
8
6
4
2
2:1
1:1
1:2
1:4
1:8
NT 靶标
CD19+ 靶标
GD2+ 靶标
CD19+GD2+ 靶标
靶标与效应物

图31

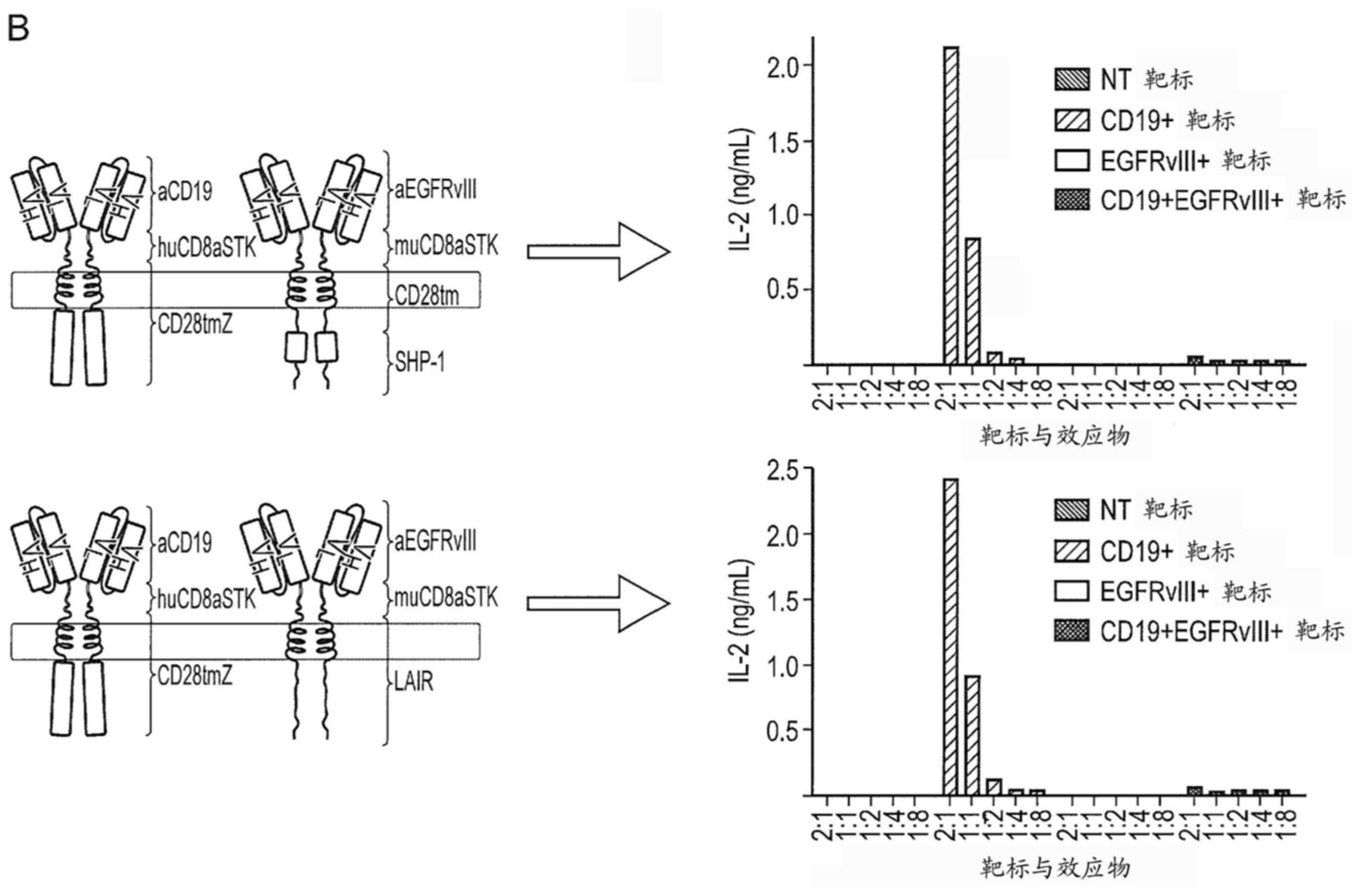

B
aCD19
huCD8aSTK
CD28tmZ
aEGFRvIII
muCD8aSTK
CD28tm
SHP-1
LAIR
IL-2 (ng/mL)
2.5
2.0
1.5
1.0
0.5
2:1
1:1
1:2
1:4
1:8
靶标与效应物
▨ NT 靶标
▨ CD19+ 靶标
☐ EGFRvIII+ 靶标
▩ CD19+EGFRvIII+ 靶标

图31(续)

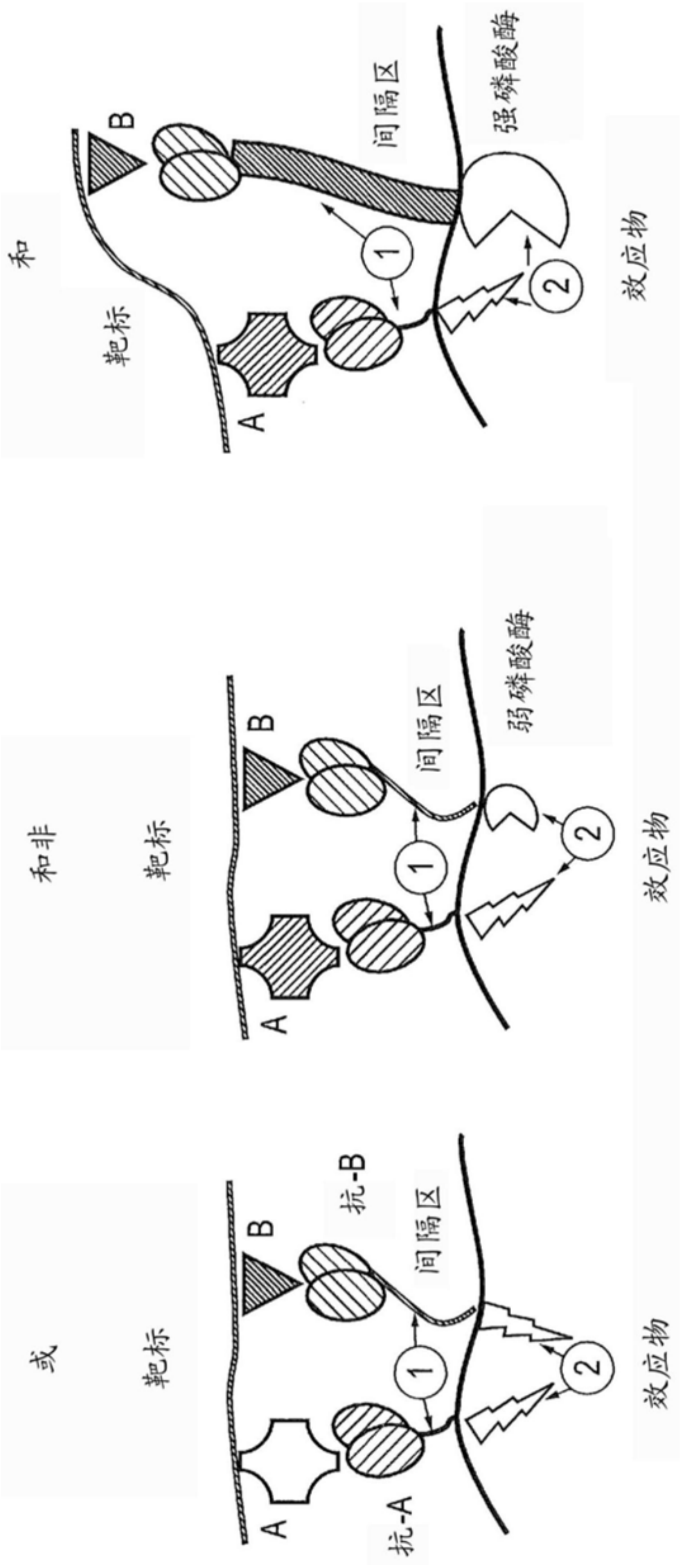

或
靶标
A
B
抗-A
抗-B
间隔区
①
②
效应物
和非
靶标
A
B
间隔区
弱磷酸酶
①
②
效应物
和
靶标
A
B
间隔区
强磷酸酶
①
②
效应物

图32