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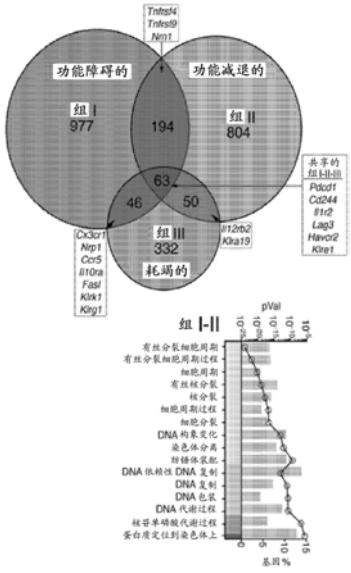
(54)发明名称

肿瘤微环境中功能障碍的抗原特异性CD8$^+$T细胞

(57)摘要

本文提供了用于检测和/或靶向肿瘤微环境中功能障碍的肿瘤抗原特异性CD8$^+$T细胞以用于诊断、治疗和/或研究应用的组合物和方法。具体地，功能障碍的肿瘤抗原特异性CD8$^+$T细胞通过它们的本文所述的细胞表面受体(诸如4-1BB、LAG-3)的表达或与4-1BB和LAG-3表达相关的另外标志物(诸如在T细胞表面上差异表达的标志物)而被检测和/或靶向。



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1.一种治疗患有癌症的受试者的方法，所述方法包括施用特异性靶向功能障碍的肿瘤抗原特异性$CD8^+$T细胞的剂。

2.如权利要求1所述的方法，其中所述受试者患有实体肿瘤癌症。

3.如权利要求2所述的方法，其中所述肿瘤允许T细胞浸润，但对免疫疗法具有抗性。

4.如权利要求2所述的方法，其中所述肿瘤环境包含功能障碍的肿瘤抗原特异性$CD8^+$T细胞。

5.如权利要求1的方法，其包括使所述功能障碍的肿瘤抗原特异性$CD8^+$T细胞与抗4-1BB和/或抗LAG3剂接触。

6.如权利要求5所述的方法，其中所述抗4-1BB和/或抗LAG3剂是抗体、抗体片段或抗体模拟分子。

7.如权利要求1所述的方法，其还包括共施用另外的治疗剂。

8.如权利要求7所述的方法，其中所述另外的治疗剂是化学治疗剂或免疫治疗剂。

9.如权利要求8所述的方法，其中所述另外的治疗剂是选自由以下项组成的列表的免疫治疗剂：基于细胞的疗法、单克隆抗体(mAb)疗法、细胞因子疗法以及辅助治疗。

10.如权利要求9所述的方法，其中所述免疫治疗剂是选自由以下项组成的列表的mAb疗法：抗CTLA-4单克隆抗体和/或抗PD-L1单克隆抗体。

11.如权利要求9所述的方法，其中所述免疫治疗剂是选自由以下项组成的列表的基于细胞的疗法：树突细胞疗法和T细胞疗法。

12.如权利要求7所述的方法，其中所述另外的治疗剂靶向表2中列出的受体中的一种。

13.如权利要求7所述的方法，其中所述另外的治疗剂靶向PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205、TMEM126A、CRTAM和/或Sema7a。

14.如权利要求1所述的方法，其包括使所述功能障碍的肿瘤抗原特异性$CD8^+$T细胞与靶向表2中列出的所述受体中的一种的治疗剂接触。

15.如权利要求14所述的方法，其中所述治疗剂靶向PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205、TMEM126A、CRTAM和/或Sema7a。

16.如权利要求15所述的方法，其中所述治疗剂是抗Nrn1抗体、抗体片段或抗体模拟分子。

17.如权利要求15所述的方法，其中所述治疗剂是抗Sema7a抗体、抗体片段或抗体模拟分子。

18.如权利要求15所述的方法，其中所述治疗剂是抗CRTAM抗体、抗体片段或抗体模拟分子。

19.一种组合物，其包含：(a)抗4-1BB剂、抗LAG-3剂、抗Nrn1剂、抗Sema7a剂和抗CRTAM剂中的一种或多种；和(b)免疫治疗剂，所述组合物被配制成用于向受试者治疗递送。

20.如权利要求19所述的组合物，其中所述抗4-1BB剂、抗LAG-3剂、抗Nrn1剂、抗Sema7a剂和/或抗CRTAM剂是抗体、抗体片段或抗体模拟分子。

21.一种方法，其包括：(a)测试来自细胞群体的$CD8^+$T细胞以确定它们是否共表达LAG-3和4-1BB；和(b)施用抗Nrn1剂、抗Sema7a剂和抗CRTAM剂。

22.如权利要求21所述的方法，其中所述抗Nrn1剂、抗Sema7a剂和/或抗CRTAM剂是抗体、抗体片段或抗体模拟分子。

23.如权利要求21所述的方法，其中体外执行所述测试。

24.一种鉴定功能障碍的T细胞的方法，所述方法通过测试所述细胞共表达4-1BB和LAG-3来进行。

肿瘤微环境中功能障碍的抗原特异性CD8$^+$T细胞

[0001] 相关申请的交叉引用

[0002] 本发明要求2017年1月17日提交的美国临时专利申请序列号62/447,199的优先权,该美国临时专利申请的全文以引用方式并入。

[0003] 政府支持声明

[0004] 本发明根据美国国立卫生研究院颁发的第R01 CA161005号拨款在政府支持下进行。政府拥有本发明的某些权利。

技术领域

[0005] 本文提供了用于检测和/或靶向肿瘤微环境中功能障碍的肿瘤抗原特异性CD8$^+$ T细胞以用于诊断、治疗和/或研究应用的组合物和方法。具体地,功能障碍的肿瘤抗原特异性CD8$^+$ T细胞通过它们的本文所述的细胞表面受体(诸如4-1BB、LAG-3)的表达或与4-1BB和LAG-3表达相关的另外标志物(诸如在T细胞表面上差异表达的标志物)而被检测和/或靶向。

背景技术

[0006] 免疫系统在保护宿主免于癌症中起关键作用(Vesely等人,2011;该文献全文以引用方式并入)。肿瘤的先天感知通过呈递肿瘤相关抗原(TAA)而导致适应性T细胞应答,所述肿瘤相关抗原(TAA)源自促成癌变的突变和表观遗传改变(Gajewski等人,2013;该文献全文以引用方式并入)。自发致敏的CD8+ T细胞归巢于小鼠肿瘤模型中的肿瘤部位(Harlin等人,2009;Fuertes等人,2011;该文献全文以引用方式并入)和一部分晚期癌症患者中的肿瘤部位(Harlin等人,2006;该文献全文以引用方式并入)。这些肿瘤浸润淋巴细胞(TIL)具有识别肿瘤抗原的能力,并且基于活化的CD8+ T细胞浸润与改善的预后和对免疫疗法的响应之间的相关性,而被认为有助于癌症患者的肿瘤控制(Fridman等人,2012;Tumeh等人,2014;这些文献全文以引用方式并入本文)。然而,在没有额外操纵的情况下,该内源性抗肿瘤响应通常不足以介导对已建立肿瘤的完全排斥(Gajewski,2007b;Pardoll,2012;Baitsch等人,2011;Gajewski等人,2006;Larkin等人,2015)。过去几年积累的数据表明,具有自发抗肿瘤T细胞应答的肿瘤具有破坏应答的效应阶段的免疫抑制途径的高度表达。这些免疫抑制途径包括PD-L1/PD-1相互作用(Pardoll,2012;该文献全文以引用方式并入)、CD4+Foxp3+调节性T(Treg)细胞的募集(Gajewski,2007a;该文献全文以引用方式并入),以及通过吲哚胺-2,3-双加氧酶(IDO)使代谢调节异常(Spranger等人,2013;该文献全文以引用方式并入)。然而,即使当远离这些外在免疫抑制因子从肿瘤中分离出肿瘤抗原特异性CD8+ T细胞时,它们仍然在体外显示出改变的功能特性(Harlin等人,2006;Baitsch等人,2011;这些文献全文以引用方式并入)。

[0007] PD-1的表达已经被描述为用以鉴定肿瘤特异性耗竭的T细胞(Ahmadzadeh等人,2009;Fourcade等人,2012;Wu等人,2014;Gros等人,2014;这些文献全文以引用方式并入)。然而,在慢性感染的情况下表达PD-1的T细胞仍然可以保留效应功能(Wherry和Kurachi,

2015;该文献全文以引用方式并入),并且PD-1不是诱导T细胞耗竭所必需的(Odorizzi等人,2015;该文献全文以引用方式并入)。除PD-1外,还有一些其他共抑制受体,包括CD223(LAG-3)、CD244(2B4)、具有Ig和ITIM结构域的T细胞免疫受体(TIGIT)、甲型肝炎病毒细胞受体2(TIM-3)和细胞毒性T淋巴细胞相关蛋白4(CTLA-4)也在功能障碍的T细胞上表达,并且较多数量的抑制性受体的表达与细胞因子(特别是IFN-g和TNF-a)分泌和增殖能力减少有关(Blackburn等人,2009;该文献全文以引用方式并入)。已经在病毒和癌症模型中观察到这些受体的表达,然而,缺乏在肿瘤情形中对相同群体上的共抑制和共刺激受体的完整分析。

发明内容

[0008] 本文提供了用于检测和/或靶向肿瘤微环境中功能障碍的肿瘤抗原特异性$CD8^+$ T细胞以用于诊断、治疗和/或研究应用的组合物和方法。具体地,功能障碍的肿瘤抗原特异性$CD8^+$ T细胞通过它们的本文所述的细胞表面受体(诸如4-1BB、LAG-3)的表达或与4-1BB和LAG-3表达相关的另外标志物(诸如在T细胞表面上差异表达的标志物(例如,PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205,以及TMEM126A,CRTAM和Sema7a))而被检测和/或靶向。

[0009] 在一些实施方案中,本文提供了治疗患有癌症的受试者的方法,所述方法包括施用特异性靶向功能障碍的肿瘤抗原特异性$CD8^+$ T细胞的剂。在一些实施方案中,受试者患有实体肿瘤癌症。在一些实施方案中,肿瘤允许T细胞浸润,但对免疫疗法具有抗性。在一些实施方案中,肿瘤环境包含功能障碍的肿瘤抗原特异性$CD8^+$ T细胞。在一些实施方案中,使功能障碍的肿瘤抗原特异性$CD8^+$ T细胞与抗4-1BB和/或抗LAG3剂接触。在一些实施方案中,抗4-1BB和/或抗LAG3剂是抗体、抗体片段,或抗体模拟分子。在一些实施方案中,方法还包括共施用另外的治疗剂。在一些实施方案中,所述另外的治疗剂是化学治疗剂或免疫治疗剂。在一些实施方案中,另外的治疗剂是选自由以下项组成的列表的免疫治疗剂:基于细胞的疗法、单克隆抗体(mAb)疗法、细胞因子疗法以及辅助治疗。在一些实施方案中,免疫治疗剂是选自由以下项组成的列表的mAb疗法:抗CTLA-4单克隆抗体和/或抗PD-L1单克隆抗体。在一些实施方案中,免疫治疗剂是选自由以下项组成的列表的基于细胞的疗法:树突细胞疗法和T细胞疗法。在一些实施方案中,另外的治疗剂靶向表2中列出的标志物/受体中的一种。在一些实施方案中,另外的治疗剂靶向在T细胞表面上表达的标志物/受体。在一些实施方案中,另外的治疗剂靶向PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205和TMEM126A、CRTAM或Sema7a。在一些实施方案中,另外的治疗剂靶向Nrn1、Sema7a或CRTAM。

[0010] 在一些实施方案中,本文提供了治疗患有癌症的受试者的方法,所述方法包括施用特异性靶向功能障碍的肿瘤抗原特异性$CD8^+$ T细胞的治疗剂,其中所述剂靶向表2中列出的受体中的一种受体。在一些实施方案中,治疗剂靶向在T细胞表面上表达的标志物/受体。在一些实施方案中,治疗剂靶向PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205和TMEM126A、CRTAM或Sema7a。在一些实施方案中,治疗剂靶向Nrn1、Sema7a或CRTAM。在一些实施方案中,治疗剂是结合靶标志物/受体的抗体、抗体片段或抗体模拟分子。在一些实施方案中,治疗剂是抗Nrn的抗体、抗体片段或抗

体模拟分子。在一些实施方案中，治疗剂是抗Sema7a抗体、抗体片段或抗体模拟分子。在一些实施方案中，治疗剂是抗CRTAM的抗体、抗体片段或抗体模拟分子。

[0011] 在一些实施方案中，本文提供的组合物包含：(a)抗4-1BB剂、抗LAG-3剂、抗Nrn1剂、抗Sema7a剂和抗CRTAM剂中的一种或多种；以及(b)免疫治疗剂，所述组合物被配制成用于向受试者治疗递送。在一些实施方案中，抗-4-1BB剂，抗LAG-3剂，抗-Nrn1剂，抗Sema7a剂和/或抗CRTAM剂是抗体，抗体片段或抗体模拟分子。

[0012] 在一些实施方案中，本文提供的组合物包含：(a)靶向和/或结合PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205和TMEM126A中的一种的剂；和(b)免疫治疗剂，所述组合物经被配制成用于向受试者治疗递送。

[0013] 在一些实施方案中，本文提供的方法包括：(a)测试来自细胞群体的$CD8^+$ T细胞以确定CD8+ T细胞是否共表达LAG-3和4-1BB；和(b)施用靶向和/或结合PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205和TMEM126A中的一种的一种或多种剂。在一些实施方案中，所述剂是抗Nrn1剂，抗Sema7a剂和抗CRTAM剂。在一些实施方案中，抗Nrn1剂、抗Sema7a剂和/或抗CRTAM剂是抗体、抗体片段或抗体模拟分子。在一些实施方案中，体外执行测试。

[0014] 在一些实施方案中，本文提供了通过测试功能障碍的T细胞共表达4-1BB和LAG-3来鉴定所述细胞的方法。在一些实施方案中，本文提供通过测试功能障碍的T细胞表达表2中的标志物/受体(例如，T细胞表面标志物/受体(例如，PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205、TMEM126A))中的一种或多种来鉴定所述细胞的方法。

附图说明

[0015] 图1A至图1J.4-1BB和LAG-3的共表达鉴定了在进展中的肿瘤中存在很大一部分的$CD8^+$ TIL隔室。(A)对来自肿瘤皮下接种后第7天、第14天和第21天的荷瘤小鼠的B16.SIY肿瘤、脾和TdLN的$CD8^+$ T细胞上4-1BB和LAG-3表达的代表性分析。(B-D)组合物的纵向总结，n＝5；每个时间点进行四至五次独立实验，(C)绝对细胞数，n＝5；每个时间点进行七至九次独立实验，以及(D)$CD8^+$ 4-1BB/LAG-3TIL亚群的细胞密度，n＝5；每个时间点进行两至五次独立实验。通过用流式细胞术获得完整的肿瘤样品来确定绝对细胞数。(E)第14天对$CD8^+$ 4-1BB/LAG-3TIL亚群为$Ki67^+$的比例的总结。n＝3-5；两次独立实验。(F)对24小时BrdU脉冲后$CD8^+$ 4-1BB/LAG-3TIL亚群中第13天的BrdU摄取的总结。n＝5；三次独立实验。(G-I)其他肿瘤模型中4-1BB/LAG-3群体的代表性流式图(G和H)和总结(I)。将小鼠用2×10^6的C1498.SIY、MC38.SIY、EL4.SIY、B16亲本、MC57.SIY或1969.SIY皮下接种，并在肿瘤接种后第14天分析4-1BB和LAG-3表达。n＝3-5；每个时间点进行2至5次独立实验。(J)将小鼠用2×10^6的MC57.SIY或B16.SIY在两侧腹上接种，在指定的时间点合并来自每只小鼠的肿瘤并分析$CD8^+$ TIL隔室中4-1BB和LAG-3的共表达，n＝3-5；每个时间点进行两次独立实验。所有的误差条指示±SEM。*:P<0.05，**:P<0.01，***:P<0.001。使用双因素方差分析和Bonferroni事后检验来进行(B、C、D、H)纵向研究，并使用Kruskal-Wallis(非参数)检验来进行一个时间点处的(E和F)分析。

[0016] 图2A至图2G.Egr2和Egr2转录网络的组分在4-1BB^{+}LAG-3^{+}CD8^{+} TIL中富集。(A)Egr2^{EGFP}表达的代表性流式图和总结。将Egr2^{EGFP}小鼠皮下接种2×10^{6}的B16.SIY肿瘤。在第7天和第14天分析来自肿瘤、TdLN和脾的CD8^{+} T细胞的Egr2^{EGFP}表达。n=4-5;两次独立实验。(B)Egr2靶基因的表达(Zheng等人,2013)。将来自第14天荷瘤小鼠的CD8^{+} TIL基于Egr2^{EGFP}的高或低表达对进行分选,并通过qRT-PCR直接分析Egr2靶标的表达。合并每只小鼠的相对侧腹上的两个肿瘤。n=3;两次独立实验。(C)在第7天和第14天对CD8^{+}Egr2GFP^{高}和Egr2GFP^{低}TIL中4-1BB/LAG-3亚群的代表性流式图和总结。n=4-5。每个时间点进行两次独立实验。(D)4-1BB^{+}LAG-3^{+}和4-1BB^{-}LAG-3^{-}亚群中Egr2靶标的表达。将亚群分选并通过qRT-PCR直接分析靶表达。合并每只小鼠的相对侧腹上的两个肿瘤。n=4;两次独立实验。(E)将Egr2$^{flox/flox}$ x pLCKCreERT2 x YFP-Rosa26小鼠通过灌胃给予5剂量的它莫西芬,并在3天后接种2×10^{6}个B16.SIY细胞。将YFP^{+}或YFP^{-}CD8^{+} TIL分选,并直接和在体外刺激后进行Egr2转录物分析。合并每只小鼠的相对侧腹上的两个肿瘤。n=3;两次独立实验。(F)在第7天和第14天YFP^{+}或YFP^{-}CD8^{+} TIL中的4-1BB/LAG-3共表达的代表性流式图和总结。n=3;两次独立实验。(G)在来自第7天从Egr2GFP小鼠分离的CD8^{+} TIL的Egr2GFP^{高}和Egr2GFP^{低}中Egr3和Hif1α的表达。n=5;两次独立实验。误差条指示±SEM。*:P<0.05,**:P<0.01,***:P<0.001。使用双因素方差分析和Bonferroni事后检验进行纵向研究(A和C),并使用Mann-Whitney检验计算(B、D、E、F和G)的显著性。

[0017] 图3A至图3H.4-1BB和LAG-3的共表达鉴定进展中肿瘤中的肿瘤抗原特异性TIL。(A)来自不同4-1BB/LAG-3亚群和从脾分离出的CD8^{+} T细胞的代表性CDR3β分布。加框区域代表4-1BB^{+}LAG-3^{+}CD8^{+} TIL亚群中的优势峰。(B)作为偏斜的度量,计算在同一小鼠内的每个TIL亚群与CD8^{+} T细胞脾群体之间的每个Vβ谱型的汉明距离(Hamming Distance,HD)。作为对照,计算小鼠之间来自CD8^{+}脾细胞群体的HD(灰色条)。n=3;一次独立实验。(C-D)在B16.SIY和MC38.SIY或(D)MC57.SIY和1969.SIY肿瘤接种后第14天对H-2K^{b}/SIY^{+}和H-2K^{b}/SIY^{-}CD8^{+} TIL中4-1BB/LAG-3亚群的代表性流式分析。n=3-4;三至五次独立实验。(E)在肿瘤接种后第14天对共表达4-1BB和LAG-3的H-2K^{b}/SIY^{+}和H-2K^{b}/SIY^{-}CD8^{+} TIL的组成进行总结,从而比较B16.SIY、MC38.SIY、MC57.SIY和1969.SIY肿瘤。n=5;三至四次独立实验。(F-H)在肿瘤接种后第7天,将1×10^{6}个P14/CD45.2和2C/CD45.1/2Tg T细胞通过尾静脉过继转移到荷有CD45.1同系肿瘤的宿主中,并分析(F)肿瘤中回收细胞的总数、(G和H)2C、P14和宿主CD8^{+} TIL中4-1BB和LAG-3表达的分布。n=5;两次独立实验。所有的误差条指示±SEM。*:P<0.05,**:P<0.01,***:P<0.001。使用Kruskal-Wallis(非参数)检验来进行(B)谱型分析和(E和F)H-2K^{b}/SIY分析。使用双因素方差分析和Bonferroni事后检验进行(H)2C、宿主和P14组成分析。

[0018] 图4A至图4G.4-1BB和LAG-3但不是PD-1的共表达定义功能障碍的CD8^{+} TIL,具有减少的IL-2。(A和B)将从第14天B16.SIY荷瘤小鼠分选的细胞用抗CD3ε和抗CD28进行体外刺激12小时,并(A)通过qRT-PCR分析Il-2转录物和(B)通过ELISA分析IL-2蛋白。合并每只小鼠的相对侧腹上的两个肿瘤。n=4-5;三次独立实验。(C)从第14天荷有B16.SIY肿瘤的Egr2^{GFP}小鼠中分选Egr2GFP^{高}和Egr2GFP^{低}TIL,并且体外刺激12小时,并通过qRT-PCR分析Il-2转录物。合并每只小鼠的相对侧腹上的两个肿瘤。n=5;两次独立实验。(D)在肿瘤接种后第7天将1×10^{6}个2C/CD45.1/2Tg T细胞转移到小鼠中,7天后对从肿瘤分选的宿主4-1BB^{+}

LAG-3$^+$ T细胞和从肿瘤或TdLN分选的2C T细胞进行体外刺激，并通过qRT-PCR分析Il-2转录物的表达。合并每只小鼠的相对侧腹上的两个肿瘤。n＝3；两次独立实验。(E和F)对4-1BB/LAG-3 CD8$^+$ TIL亚群上的PD-1表达的代表性流式分析，以及(F)对第14天和第21天在CD8$^+$ TIL隔室中的4-1BB$^-$LAG-3$^-$PD-1$^+$亚群的组成的总结。n＝5；三次独立实验。(G)从第14天荷瘤小鼠中分选4-1BB$^-$LAG-3$^-$PD-1$^+$和LAG-3$^+$4-1BB$^+$ CD8$^+$ TIL，进行体外刺激，并通过qRT-PCR分析Il-2转录物。合并每只小鼠的相对侧腹上的两个肿瘤。n＝3；两次独立实验。所有的误差条指示±SEM。*:P<0.05，**:P<0.01，***:P<0.001****:P<0.0001。使用Kruskal-Wallis(非参数)检验进行多重比较分析(A、B和D)，并且使用Mann-Whitney检验进行成对比较(C和G)。

[0019] 图5A至图5E.功能障碍的CD8$^+$ TIL保持IFN-γ产生、细胞溶解能力并产生Treg募集趋化因子。(A)纵向分析CD8$^+$ TIL亚群的细胞因子产生能力。分选出CD8$^+$ TIL亚群，并用抗CD3ε和抗CD28刺激10-12小时，并测量IL-2、IFN-γ和TNF-α的浓度。将浓度归一化为细胞数。在第7天和第14天合并相对侧腹上的两个肿瘤。n＝4-5；两次独立实验。(B)直接离体分析4-1BB/LAG-3亚群中的Ifn-γ Tnf-α和Gzmb转录物水平。合并每只小鼠的相对侧腹上的两个肿瘤。n＝3-5；三次独立实验。(C)直接离体分析的IFN-γ产生的代表性流式图和总结。简言之，在肿瘤接种后第14天，在肿瘤内注射100μl含有2mg/mL GolgiPlug的PBS。8小时后，分离出TIL。所有步骤均用含有1mg/mL的GolgiStop的培养基在冰上执行，直至固定。n＝5；两次独立实验。(D)将指定时间点的CD8$^+$ TIL亚群进行分选，并与50,000个P815靶细胞和1μg/mL抗CD3ε一起铺板。通过碘化丙锭和/或活/死可固定的活力染料的阳性染色来测量裂解的靶细胞。将在没有CTL的情况下铺板的P815靶细胞用作阴性对照(黑色条)。将致敏的OTI细胞用作阳性对照。将来自在相对侧腹上具有2个肿瘤的10只小鼠的肿瘤合并，以获得足够量的CD8$^+$ TIL。数据代表3次独立实验。(E)通过qRT-PCR直接离体分析的4-1BB/LAG-3亚群中的Ccl1和Ccl22转录物水平。n＝4；两次独立实验。*:P<0.05，**:P<0.01，***:P<0.001，****:P<0.0001。使用Kruskal-Wallis(非参数)测试进行(A-C、E)细胞因子/趋化因子分析，并使用双因素方差分析和Bonferroni事后检验来进行(D)细胞溶解测定。

[0020] 图6A至图6D.功能障碍的CD8$^+$ TIL表达广泛的共抑制和共刺激受体。(B)4-1BB/LAG-3 CD8$^+$ TIL亚群中的细胞表面受体的基因表达谱。显示了这样的探针组，所述探针组揭示4-1BB$^+$LAG-3$^+$群体中相对于4-1BB$^-$LAG-3$^-$PD-1$^-$群体的1.5倍增长。列显示经$\log_2$转换的信号强度。(C)对所选上调的细胞表面受体的纵向研究。流式图表示第14天的CD8$^+$ TIL子集。n＝5；每个时间点进行两至五次独立实验。(D)肿瘤接种后第14天4-1BB/LAG-3亚群中KLRG-1和IL-7Rα表达的代表性流式图和总结。n＝5；两次独立实验。*:P<0.05，**:P<0.01，***:P<0.001，****:P<0.0001。将双因素方差分析和Bonferroni事后检验用于所有分析。

[0021] 图7A至图7G.抗4-1BB和抗LAG-3协同作用以控制肿瘤生长并恢复TIL功能。(A)以mm^2测量的肿瘤生长。箭头指示小鼠在哪些天接受抗体治疗。将指示时间点的统计学显著性与抗4-1BB+抗LAG-3治疗进行比较。n＝5；两次独立实验。(B)在第14天H-2K^b/SIY$^+$CD8$^+$ TIL的组成。小鼠在第7天、第10天、第13天和第16天接受抗体剂量(每次100μg)。n＝5；两次独立实验。(C-F)在肿瘤接种后第14天在没有FTY720(C和D)和具有FTY720(E和F)的情况下H-2K^b/SIY$^+$ CD8$^+$ TIL中的NRP1/2B4(C和E)和KLRG-1/IL-7Rα(D和F)表达的代表性流式图和总

结。小鼠接受如(A和B)中的抗体治疗,并且在治疗前一天开始通过灌胃以25μg/小鼠的剂量施用FTY720,并且每天继续一个剂量直至分析(第6天至第13天)。n＝5;两次独立实验。(G)治疗后的IL-2产生。对从治疗或未治疗的第14天B16.SIY荷瘤小鼠分选出的细胞进行体外刺激12小时,并通过qRT-PCR分析Il-2转录物。通过基于珠粒的LEGENDplex免疫测定法测定蛋白质浓度,并将所述蛋白质浓度归一化为细胞数。合并每只小鼠的相对侧腹上的两个肿瘤。n＝2-3;两次独立实验。将双因素方差分析和Bonferroni事后检验用于所有分析。*:P<0.05,**:P<0.01,***:P<0.001。

[0022] 图8.用于图3B中的分析的光谱型图。

[0023] 图9.施用FTY720后第14天的CD3+ T细胞。

[0024] 图10A至10B.基因表达谱的交叉研究比较的统计分析。(A)每次成对比较的等级间超几何图。(B)每个数据集之间的表达值的成对相关性。Rho(ρ)是斯皮尔曼等级相关系数。

[0025] 图11A至11E。Nrn1,CRTAM和Sema7a是抗肿瘤免疫的调节剂。(A)以mm2测量的肿瘤生长。对$Nrn1^{-/-}$或$Sema7a^{-/-}$和同窝对照小鼠皮下植入2×106个B16.SIY细胞。(B)对脾、TdLN和肿瘤的T细胞亚群中的Nrn1的基因表达分析。(C)第7天WT、$Nrn1^{-/-}$或(D)$CRTAM^{-/-}$2C T细胞的IFN-g产生的代表性流式图和总结。简而言之,在肿瘤接种的同一天,通过尾静脉注射将1×10^6个Cell Trace Violet标记的2C T细胞转移至小鼠中。在第7天,用SIY肽重新刺激整个TdLN悬浮液12小时,并分析细胞痕量稀释和IFN-g产量。(E)接受1×10^6个$Nrn1^{-/-}$ 2C T细胞的小鼠与接受相同数量的WT 2C T细胞的小鼠相比,更可能表现出完全的肿瘤控制。以与(C)中相同的方式执行T细胞的过继转移。

[0026] 图12.示例性实验方案和数据。

[0027] 定义

[0028] 尽管与本文中描述的那些方法和材料类似或等效的任何方法和材料均可用于本文描述的实施方案的实践或测试,但本文描述了一些优选的方法、组合物、装置和材料。然而,在描述本发明的材料和方法之前,应理解本发明不限于本文所述的特定分子、组合物、方法或方案,因为这些可根据常规实验和优化而变化。还应当理解,在描述中使用的术语仅用于描述特定版本或实施方案的目的,并不意欲限制本文描述的实施方案的范围。

[0029] 除非另外定义,否则本文所用的所有技术和科学术语均具有与由本发明所属领域的普通技术人员通常所理解的相同的含义。然而,在发生冲突的情况下,以本说明书(包括其中定义)为准。因此,在本文描述的实施方案的上下文中,以下定义适用。

[0030] 如本文中和所附权利要求书中使用的,单数形式“一”、“一个”和“所述”包括多个指代物,除非上下文另外明确指出。因此,例如,提及“一种抗体”是提及一种或多种抗体及本领域中技术人员已知的其等同物,等等。

[0031] 如本文所用,术语“包括”及其语言变型表示存在所述一种或多种特征、一种或多种元素、一种或多种方法步骤等,但不排除存在附加一种或多种特征、一种或多种元素、一种或多种方法步骤等。相反地,术语“由.....组成”及其语言变型表示存在所述一种或多种特征、一种或多种元素、一种或多种方法步骤等,并且排除了除了通常相关的杂质之外的任何未叙述的一种或多种特征、一种或多种元素、一种或多种方法步骤等。短语“基本上由.....组成”表示所述的一种或多种特征、一种或多种元素、一种或多种方法步骤等,以及任何附加的一种或多种特征、一种或多种元素、一种或多种方法步骤等,所述任何附加的

特征、元素、方法步骤等不会实质影响组合物、系统或方法的基本性质。本文的许多实施方案使用开放的“包含”语言来描述。此类实施方案包括多个封闭的“由.....组成”和/或“基本上由.....组成”实施方案，其可替代地使用这种语言来要求保护或描述。

[0032] 如本文所用，术语“受试者”广泛地指任何动物，包括但不限于人和非人动物(例如，狗、猫、牛、马、绵羊、家禽、鱼、甲壳类动物等)。如本文所用，术语“患者”通常是指正在针对疾病或病症(例如，癌症、实体肿瘤癌症等)进行治疗的受试者。

[0033] 如本文所用，“免疫应答”是指免疫系统的细胞(例如，T淋巴细胞、B淋巴细胞、自然杀伤(NK)细胞、巨噬细胞、嗜酸性粒细胞、肥大细胞、树突细胞、嗜中性粒细胞等)和由这些细胞或肝脏中的任一者产生的可溶性大分子(包括抗体、细胞因子和补体)的作用，所述作用导致从受试者中选择性靶向、结合、损伤、破坏和/或消除入侵病原体、被病原体感染的细胞或组织、或癌症细胞或其他异常细胞。

[0034] 如本文所用，术语“免疫调节剂”是指调节免疫应答的物质、剂、信号传导途径或它们的组分。“调节”、“修饰”或“调控”免疫应答是指免疫系统的细胞或此类细胞的活性的任何改变。这种调节包括刺激或抑制免疫系统，所述调节可以通过各种细胞类型的数量的增加或减少、这些细胞的活性增强或减弱，或免疫系统内可能发生的任何其他变化来表现。已经鉴定了抑制性和刺激性免疫调节剂，所述抑制性和刺激性免疫调节剂中一些可能在癌症微环境中具有增强的功能。

[0035] 如本文所用，术语“免疫疗法”是指通过包括诱导、增强、抑制或以其他方式改变免疫应答的方法来治疗或预防疾病或病症。

[0036] 如本文所用，“增强内源性免疫应答”意指增加受试者中现有免疫应答的有效性或效力。例如通过克服抑制内源性宿主免疫应答的机制或通过刺激增强内源性宿主免疫应答的机制，可以实现有效性和效力的这种增加。

[0037] 如本文所用，术语“抗体”是指完整抗体分子或其片段(例如，片段，诸如Fab、Fab’和$F(ab')_2$)，除非另外指明(例如，“完整抗体”、“抗体片段”)。抗体可以是多克隆或单克隆抗体、嵌合抗体、人源化抗体、人抗体等。

[0038] 天然抗体通常具有四聚体结构。四聚体通常包含两对相同的多肽链，每对多肽链具有一条轻链(在某些实施方案中，约25kDa)和一条重链(在某些实施方案中，约50-70kDa)。在天然抗体中，重链包含可变区V_H，以及三个恒定区C_{H1}、C_{H2}和C_{H3}。V_H结构域位于重链的氨基末端，而C_{H3}结构域位于羧基末端。在天然抗体中，轻链包含可变区V_L，以及恒定区C_L。轻链的可变区位于轻链的氨基末端。在天然抗体中，每个轻/重链对的可变区通常形成抗原结合位点。恒定区通常负责效应功能。

[0039] 在天然抗体中，可变区通常表现出相同的一般结构，其中相对保守的骨架区(FR)通过三个高变区接合，该三个高变区也称为互补决定区(CDR)。每对的两条链的CDR通常通过骨架区对齐，该骨架区可允许与特定表位结合。从N末端至C末端，轻链和重链可变区二者通常包含结构域FR1、CDR1、FR2、CDR2、FR3、CDR3和FR4。因此，重链上的CDR称为H1、H2和H3，而轻链上的CDR称为L1、L2和L3。通常，CDR3为抗原结合位点内分子多样性的最大来源。例如，在某些情况下，H3可短到两个氨基酸残基或超过26个氨基酸残基。氨基酸到每个结构域的分配通常符合Kabat等人(1991)Sequences of Proteins of Immunological Interest(美国国立卫生研究院，公开号91-3242，第1-3卷，Bethesda,Md.)；Chothia,C.和Lesk,A.M.

(1987)J.Mol.Biol.196:901-917;或者Chothia,C.等人,Nature 342:878-883(1989)的定义。在本申请中,除非另有说明,否则术语“CDR”是指来自轻链或重链的CDR。

[0040] 如本文所用,术语“重链”是指这样的多肽,所述多肽包含足够的重链可变区序列,以单独或与轻链组合赋予抗原特异性。

[0041] 如本文所用,术语“轻链”是指这样的多肽,所述多肽包含足够的轻链可变区序列,以单独或与重链组合赋予抗原特异性。

[0042] 如本文所用,当抗体或其他实体“特异性识别”或“特异性结合”抗原或表位时,其优先识别蛋白质和/或大分子的复杂混合物中的抗原,并以亲和力结合该抗原或表位,所述亲和力显著高于不展示该抗原或表位的其他实体。在这方面,“显著高于……的亲和力”是指亲和力高到足以使得能够使用所需测定或测量装置来检测与实体不同的抗原或表位。通常,这意味着结合亲和力具有的结合常数(K_a)为至少10^7M^{-1}(例如,$>10^7M^{-1}$、$>10^8M^{-1}$、$>10^9M^{-1}$、$>10^{10}M^{-1}$、$>10^{11}M^{-1}$、$>10^{12}M^{-1}$、$>10^{13}M^{-1}$等)。在某些此类实施方案中,抗体能够结合不同的抗原,只要不同的抗原包含该特定的表位即可。在某些情况下,例如,来自不同物种的同源蛋白质可包含相同的表位。

[0043] 如本文所用,术语“抗4-1BB抗体”或“4-1BB抗体”是指特异性识别由4-1BB呈递的抗原和/或表位的抗体。类似地,术语“抗LAG-3抗体”和“LAG-3抗体”是指特异性识别由LAG-3呈递的抗原和/或表位的抗体,术语“抗Nrn1抗体”和“Nrn1抗体”是指特异性识别由Nrn1呈递的抗原和/或表位的抗体,术语“抗CRTAM抗体”和“CRTAM抗体”是指特异性识别由CRTAM呈递的抗原和/或表位的抗体,并且术语“抗Sema7a抗体”和“Sema7a抗体”是指特异性识别由Sema7a呈递的抗原和/或表位的抗体。识别其他分子实体上的表位的抗体可以根据类似的方案来提及(例如,抗CTLA-4、抗PD-L1等)。

[0044] 如本文所用,术语“单克隆抗体”是指这样的抗体,所述抗体是特异性结合相同表位的基本上同质的抗体群体的成员。在某些实施方案中,单克隆抗体由杂交瘤分泌。在某些此类实施方案中,根据本领域技术人员已知的某些方法来产生杂交瘤。参见例如Kohler和Milstein(1975)Nature 256:495-499;该文献全文以引用方式并入本文。在某些实施方案中,使用重组DNA方法产生单克隆抗体(参见例如美国专利号4,816,567)。在某些实施方案中,单克隆抗体是指从噬菌体展示文库中分离的抗体片段。参见例如Clackson等人,(1991)Nature 352:624-628;和Marks等人,(1991)J.Mol.Biol.222:581-597;这些文献全文以引用方式并入本文。修饰词“单克隆”表示从基本上同质的抗体群体中获得的抗体的性质,而不将产生抗体的方法限制于特定方法。关于各种其它单克隆抗体生产技术,参见例如Harlow和Lane(1988)Antibodies:A Laboratory Manual(Cold Spring Harbor Laboratory,Cold Spring Harbor,N.Y.);该文献全文以引用方式并入本文。

[0045] 如本文所用,术语“抗体片段”是指全长抗体的一部分,该部分包括至少一部分抗原结合区或可变区。抗体片段包括但不限于Fab、Fab'、$F(ab')_2$、Fv、scFv、Fd、双体抗体,以及保留完整抗体可变区的至少一部分的其它抗体片段。参见例如Hudson等人,(2003)Nat.Med.9:129-134;该文献全文以引用方式并入本文。在某些实施方案中,通过对由重组DNA技术或化学多肽合成产生的完整抗体进行酶促或化学切割(例如,对抗体进行木瓜蛋白酶消化和胃蛋白酶消化)来产生抗体片段。

[0046] 例如,“Fab”片段包括一条轻链,以及一条重链的C_{H1}和可变区。Fab分子的重链不能

与另一个重链分子形成二硫键。“Fab’”片段包含一条轻链和一条重链，所述重链包含在C_{H1}结构域与C_{H2}结构域之间延伸的另外的恒定区。可以在Fab’片段的两条重链之间形成链间二硫键以形成“$F(ab')_2$”分子。

[0047] “Fv”片段包含来自重链和轻链两者的可变区，但缺少恒定区。单链Fv(scFv)片段包含通过柔性接头连接的重链和轻链可变区，以形成具有抗原结合区的单一多肽链。示例性的单链抗体在WO 88/01649和美国专利号4,946,778和5,260,203中详细讨论；这些专利全文以引用方式并入本文。在某些情况下，单个可变区(例如，重链可变区或轻链可变区)可具有识别和结合抗原的能力。

[0048] 技术人员将理解其他抗体片段。

[0049] 如本文所用，术语“嵌合抗体”是指由来自至少两种不同来源的组分组成的抗体。在某些实施方案中，嵌合抗体包含来源于第一物种的抗体的一部分，该部分与另一分子(例如，来源于第二物种的抗体的一部分)融合。在某些此类实施方案中，嵌合抗体包含来源于非人动物的抗体的一部分，该部分与来源于人的抗体的一部分融合。在某些此类实施方案中，嵌合抗体包含来源于非人动物的抗体的可变区的全部或一部分，该全部或一部分与来源于人的抗体的恒定区融合。

[0050] “人源化”抗体是指这样的非人抗体，所述非人抗体经修饰而使得其与人抗体更密切地匹配(在氨基酸序列方面)。因此，人源化抗体是一种嵌合抗体。在某些实施方案中，对非人抗体可变区的抗原结合残基之外的氨基酸残基进行修饰。在某些实施方案中，通过以下方式来构建人源化抗体：用来自具有所需抗原结合特异性的另一抗体(诸如非人抗体)的CDR的全部或一部分替换人抗体的互补决定区(CDR)的全部或一部分。在某些实施方案中，人源化抗体包含可变区，在所述可变区中所有或基本上所有CDR对应于非人抗体的CDR，并且所有或基本上所有骨架区(FR)对应于人抗体的FR。在某些此类实施方案中，人源化抗体还包含人抗体的恒定区(Fc)。

[0051] 术语“人抗体”是指含有人抗体序列并且不含来自非人动物的抗体序列的单克隆抗体。在某些实施方案中，人抗体可含有天然抗体中未发现的合成序列。该术语不受制备抗体的方式的限制。例如，在各种实施方案中，可以通过噬菌体展示、通过人B淋巴细胞或通过重组方法在转基因小鼠中制备人抗体。

[0052] 如本文所用，术语“天然抗体”是指这样的抗体，在所述抗体中所述抗体的重链和轻链已由多细胞生物的免疫系统制备和配对。例如，由从用抗原免疫的第一动物分离的抗体产生细胞产生的抗体是天然抗体。天然抗体含有天然成对的重链和轻链。术语“天然人抗体”是指这样的抗体，在所述抗体中所述抗体的重链和轻链已由人受试者的免疫系统制备和配对。

[0053] 天然人轻链通常被分类为κ轻链和λ轻链。天然人重链通常被分类为μ、δ、γ、α或ε，并且分别将抗体的同种型定义为IgM、IgD、IgG、IgA和IgE。IgG具有亚类，包括但不限于IgG1、IgG2、IgG3和IgG4。IgM具有亚类，包括但不限于IgM1和IgM2。IgA具有亚类，包括但不限于IgA1和IgA2。在天然人轻链和重链内，可变区和恒定区通常由具有约12个或更多个氨基酸的“J”区接合，其中重链也包括具有约10个或更多个氨基酸的“D”区。参见例如Fundamental Immunology(1989)第7章(Paul,W.编辑，第2版，Raven Press,N.Y.)；该文献全文以引用方式并入本文。

[0054] 术语“中和抗体”或“中和……的抗体”是指降低包含抗体特异性结合的表位的多肽的至少一种活性的抗体。在某些实施方案中，中和抗体降低了体外和/或体内活性。在一些实施方案中，通过中和包含表位的多肽，中和抗体抑制展示表位的细胞的能力。

[0055] 如本文所用，如本文所用的术语“糖基改造的”对包括天然存在的或重组的蛋白质、多肽或其片段的糖基化模式的任何操纵。

[0056] 术语“抗原结合位点”是指抗体的一部分，该部分能够特异性结合抗原。在某些实施方案中，抗原结合位点由一个或多个抗体可变区提供。

[0057] 术语“表位”是指能够特异性结合免疫球蛋白或T细胞受体或B细胞受体的任何多肽决定簇。在某些实施方案中，表位是抗原中由抗体特异性结合的区域。在某些实施方案中，表位可包括分子的化学活性表面基团，诸如氨基酸、糖侧链、磷酰基或磺酰基。在某些实施方案中，表位可具有特定的三维结构特征(例如，“构象”表位)和/或特定的电荷特征。

[0058] 如果特定抗体特异性结合一个表位和另一个表位，则这两个表位被定义为“相同”。在某些实施方案中，具有不同一级氨基酸序列的多肽可包含相同的表位。在某些实施方案中，相同的表位可具有不同的一级氨基酸序列。如果不同的抗体竞争与相同表位的特异性结合，则认为它们与该表位结合。

[0059] “保守”氨基酸取代是指多肽中的氨基酸被具有相似性质(诸如大小或电荷)的另一种氨基酸取代。在某些实施方案中，包含保守氨基酸取代的多肽保持未取代多肽的至少一种活性。保守氨基酸取代可以包括非天然存在的氨基酸残基，所述非天然存在的氨基酸残基通常通过化学肽合成而不是通过生物系统中的合成来掺入。这些非天然存在的氨基酸残基包括但不限于肽模拟物和氨基酸部分的其他反向或倒置形式。天然存在的残基可以基于常见的侧链特性进行分类，例如：疏水性的：正亮氨酸、Met、Ala、Val、Leu和Ile；中性亲水性的：Cys、Ser、Thr、Asn和Gln；酸性的：Asp和Glu；碱性的：His、Lys和Arg；影响链取向的残基：Gly和Pro；以及芳香族的：Trp、Tyr，以及Phe。非保守取代可涉及将这些类别中的一个类别的成员交换为来自另一个类别的成员；而保守取代可涉及将这些类别中的一个类别的成员交换为同一类别中的另一个成员。

[0060] 如本文所用，术语“序列同一性”是指两个聚合物序列(例如，肽、多肽、核酸等)具有相同的单体亚基序列组成的程度。术语“序列相似性”是指两个聚合物序列(例如，肽、多肽、核酸等)具有相似聚合物序列的程度。例如，类似的氨基酸是这样的氨基酸，所述氨基酸共享相同生物物理特征并且可以分组成家族(参见上文)。“序列同一性百分比”(或“序列相似性百分比”)通过以下方法计算：(1)在比较窗口(例如，较长序列的长度、较短序列的长度、指定窗口等)上比较两个最佳比对的序列，(2)确定含有相同(或相似)单体(例如，两个序列中出现的相同氨基酸，两个序列中出现的相似氨基酸)的位置数以产生匹配位置的数目，(3)将匹配的位置数除以比较窗口(例如，较长序列的长度、较短序列的长度、指定窗口)中的位置总数，以及(4)将结果乘以100以产生序列同一性百分比或序列相似性百分比。例如，如果肽A和B的长度均为20个氨基酸并且除了1个位置以外具有完全相同的氨基酸，则肽A和肽B具有95％序列同一性。如果非相同位置处的氨基酸共享相同的生物物理特征(例如，两者都是酸性的)，则肽A和肽B将具有100％序列相似性。作为另一个实例，如果肽C的长度为20个氨基酸且肽D的长度为15个氨基酸，并且肽D中的15个氨基酸中的14个与肽C的一部分的氨基酸相同，则肽C和D具有70％序列同一性，但肽D与肽C的最佳比较窗口具有93.3％

序列同一性。为了计算本文中的“序列同一性百分比”(或“序列相似性百分比”),将比对序列中的任何空位视为该位置处不匹配。

[0061] 术语“有效剂量”或“有效量”是指引起患者症状减轻或引起所需生物学结局的剂(例如,抗体)的量。在某些实施方案中,有效剂量或有效量足以治疗或减轻疾病或病症的症状。

[0062] 如本文所用,术语“施用”和“向……施用”是指向受试者或体内、体外或离体细胞、组织和器官给予药物、前药或其它剂或治疗剂的行为。向人体施用的示例性途径可以通过脑或脊髓的蛛网膜下的空间(鞘内)、眼部(眼用)、口腔(口服)、皮肤(局部或经皮)、鼻(经鼻)、肺部(吸入)、口腔粘膜(经颊)、耳、直肠、阴道、通过注射(例如,静脉内、皮下、瘤内、腹膜内等),等等。

[0063] 除非另有说明,否则术语“治疗”包括治疗和预防/防范性措施。需要治疗的人包括但不限于已经患有特定病症的个体,以及有风险获得特定病症或疾患的个体(例如,基于年龄、性别、生活方式等具有遗传或表观遗传易感性的个体)。术语“治疗”是指将剂施用于受试者以用于治疗和/或预防/防范性目的。

[0064] “治疗剂”是指可以体内施用以产生治疗和/或预防/防范性效果的剂。

[0065] “治疗性抗体”是指可以体内施用以产生治疗和/或预防/防范性效果的抗体。

[0066] 如本文用,术语“共同施用”是指向受试者施用至少两种剂或疗法。在一些实施方案中,两种或更多种剂或疗法的共同施用是同时的。在其它实施方案中,第一剂/疗法在第二剂/疗法之前施用。本领域的技术人员理解,所用的各种剂或疗法的配制和/或施用途径可以变化。用于共同施用的适当剂量可由本领域的技术人员容易地确定。在一些实施方案中,当剂或疗法共同施用时,各剂或疗法以低于适合其单独施用的剂量施用。因此,在其中共同施用剂或疗法降低潜在有害(例如,毒性)剂的必需剂量的实施方案中和/或当共同施用两种或更多种剂导致受试者通过所述剂中的一种剂的共同施用而对另一种剂的有益效应敏感时,共同施用是尤其期望的。

[0067] 如本文所用,术语“药物组合物”是指活性剂(例如,结合剂)与惰性或活性载体组合,使得该组合物特别适用于体外、体内或离体诊断或治疗用途。

[0068] 如本文所用的术语“药学上可接受的”或“药理学上可接受的”是指当施用于受试者时,基本上不产生不良反应,例如毒性反应、变态反应或免疫反应的组合物。

[0069] 如本文中所用,术语“药学上可接受的载体”是指任何标准药物载体,包括但不限于磷酸盐缓冲盐水溶液、水、乳液(例如,油/水或水/油乳液)和各种类型的润湿剂,任何和所有溶剂、分散介质、包衣、硫酸月桂酯钠、等渗和吸收延迟剂、崩解剂(例如,马铃薯淀粉或羧基乙酸淀粉钠)等。所述组合物还可包括稳定剂和防腐剂。对于载体、稳定剂和佐剂的实例,参见例如Martin,Remington’s Pharmaceutical Sciences,第15版,Mack Publ.Co.,Easton,Pa.(1975),该文献全文以引用方式并入本文。

[0070] 如本文所用,“诊断”或“诊断测试”包括检测、鉴定或表征受试者的疾病状态或状况。例如,可以表征疾病或病症以确定患有疾病或病症的受试者将对特定疗法作出响应的可能性,确定患有疾病或病症的受试者的预后(或其可能的进展或消退),确定治疗对患有疾病或病症的受试者的影响,或确定未来的治疗过程。

具体实施方式

[0071] 本文提供了用于检测和/或靶向肿瘤微环境中功能障碍的肿瘤抗原特异性$CD8^+$ T细胞以用于诊断、治疗和/或研究应用的组合物和方法。具体地，功能障碍的肿瘤抗原特异性$CD8^+$ T细胞通过它们的本文所述的细胞表面受体(诸如4-1BB、LAG-3)的表达或与4-1BB和LAG-3表达相关的另外标志物(诸如在T细胞表面上差异表达的标志物(例如，PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205，以及TMEM126A，CRTAM和Sema7a))而被检测和/或靶向。

[0072] 在本文的实施方案的开发过程中进行的实验鉴定了与肿瘤抗原特异性$CD8^+$ T细胞功能障碍相关和/或负责其的标志物/受体。在一些实施方案中，标志物/受体在功能障碍的肿瘤抗原特异性$CD8^+$ T细胞中过表达。在此类实施方案中，检测此类标志物的水平(例如，高于阈值)提供了用于检测肿瘤抗原特异性$CD8^+$ T细胞功能障碍的诊断。此外，在此类实施方案中，靶向此类标志物/受体(例如，抑制(例如，表达和/或活性))提供了治疗剂。在其他实施方案中，标志物/受体在功能障碍的肿瘤抗原特异性$CD8^+$ T细胞中表达不足。在此类实施方案中，检测此类标志物的水平(例如，低于阈值)提供了用于检测肿瘤抗原特异性$CD8^+$ T细胞功能障碍的诊断。此外，在此类实施方案中，靶向此类标志物/受体(例如，增强(例如，表达和/或活性))提供了治疗剂。

[0073] 转录因子Egr2是体外操纵的$CD4^+$ T细胞克隆中的无反应状态的关键调节因子(Zheng等人，2013;2012;该文献全文以引用方式并入)。Egr2还已显示为参与几种体内模型系统中T细胞活化的负调节(Sumitomo等人，2013;该文献全文以引用方式并入)。Egr2有助于DGKα和-z的上调，所述上调起到钝化TCR介导的Ras途径活化的作用(Zha等人，2006;该文献全文以引用方式并入)。通过比较无反应性细胞的基因表达谱以及进行Egr2 ChIP-Seq分析，鉴定出了多个额外的Egr2驱动的基因靶标(Zheng等人，2013;该文献全文以引用方式并入)。这些基因靶包括4-1BB(Tnfrsf9或CD137)、Lag3、Nrn1、Sema7a、Crtam和Rank1，它们编码细胞表面蛋白。

[0074] 4-1BB是在TCR接合后瞬时表达的共刺激分子。Lag3(淋巴细胞活化基因3或CD223)是CD4同源物并且起抑制性受体的作用。4-1BB和Lag3的表达在TCR接合后被调节，并在整个分化过程中继续。在人中，4-1BB和LAG-3在来自人黑素瘤肿瘤的CD8+ TIL上表达(Gros等人，2014;Baitsch等人，2012;这些文献全文以引用方式并入)。在小鼠和人中，任一种单独分子都在活化的T细胞群上表达。然而，共表达更受限制并且在循环T细胞中很少观察到。共表达这些标志物的CD8+ TIL的功能是未知的。

[0075] 在开发本文的实施方案期间进行实验以使用小鼠肿瘤模型研究表达4-1BB和LAG-3的CD8+ TIL的详细特征。发现4-1BB和LAG-3的共表达足以鉴定富含Egr2靶基因表达的肿瘤抗原特异性功能障碍CD8+ TIL。这些CD8+ TIL在体外刺激后未能产生IL-2，但仍然产生IFN-g和Treg募集趋化因子，并离体裂解靶细胞，表明它们不是完全功能惰性的。用抗LAG-3/抗4-1BB的组合治疗恢复了该群体的功能并促进了KLRG-1hi效应细胞的原位获得。另外的基因表达谱分析提供了该T细胞亚群的完整表型分析，这揭示了广泛的抑制性受体和共刺激受体(例如，表2的受体(例如Nrn1、Sema7a、CRTAM等))的表达。在T细胞表面上展示的在该谱分析中鉴定出的抑制性受体和共刺激受体包括PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205，以及TMEM126A。因此，这些方法

使得能够表征在具有改变的功能特性的肿瘤微环境中特异性产生的肿瘤抗原特异性CD8+ T细胞群。在一些实施方案中，该群体是用于恢复所需功能和促进肿瘤消退的免疫治疗方法的靶标。在一些实施方案中，本文鉴定的受体/标志物(例如，4-1BB、LAG-3、表2的受体/标志物(例如，表面标志物/受体(例如Nrn1、Sema7a、CRTAM等)等)等)被靶向(例如，通过免疫治疗方法)，以恢复所需的免疫应答性，促进肿瘤消退和/或用于治疗癌症。

[0076] 在本文的实施方案的开发期间进行的实验应用了Egr2靶标的知识来评估这些标志物对于理解体内肿瘤内的功能障碍T细胞的适用性。数据确实证实LAG-3和4-1BB的共表达足以鉴定肿瘤微环境内的大多数肿瘤抗原特异性$CD8^+$ T细胞。在荷瘤小鼠的外周淋巴器官中未观察到这些标志物的共表达，表明肿瘤情形特有的性质驱动4-1BB和LAG-3表达。此外，在经历成功排斥的肿瘤中未观察到LAG-3和4-1BB表达的获得，表明在不完全抗原清除的条件下发生该表型的获得。

[0077] 在一些实施方案中，本文所述的癌症治疗方法包括施用(或与一种或多种另外的疗法/治疗剂共同施用)一种或多种抗4-1BB和/或抗LAG-3试剂(例如，抗体、抗体片段、抗体模拟分子(例如，DARPin、亲和体、适体、纳米抗体等)等)。在一些实施方案中，施用抗4-1BB和/或抗LAG-3剂以使癌细胞、肿瘤和/或肿瘤微环境易接近或易感于用其他疗法/治疗剂(例如，免疫治疗剂)进行治疗。可用于本文所述实施方案的抗4-1BB和/或抗LAG-3剂不受它们的作用机制的限制。剂可以是小分子、肽、多肽、蛋白质、核酸(例如，反义、RNAi等)、抗体、抗体片段等。

[0078] 在一些实施方案中，本文所述的癌症治疗方法包括增强本文鉴定的与肿瘤抗原特异性$CD8^+$ T细胞功能障碍负相关的标志物/受体的活性或表达。

[0079] 在本文的实施方案的开发期间进行的实验鉴定了在功能障碍的CD8+ TIL中差异表达的受体/标志物(参见表2)。对在该筛选中鉴定出的感兴趣的靶标的测试表明，至少神经突蛋白1(Nrn1)、细胞毒性和调节性t细胞分子(CRTAM)和脑信号蛋白7A(Sema7a)是抗肿瘤免疫的调节剂，其中Nrn1和CRTAM阻断与肿瘤面积增大相关，并且Sema7a阻断与肿瘤面积减小相关。

[0080] 在一些实施方案中，本文所述的癌症治疗方法包括施用(或与一种或多种另外的疗法/治疗剂共同施用)靶向表2的一种或多种受体/标志物(例如PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205，以及TMEM126A、Nrn1、CRTAM、Sema7a等)的剂(例如，抗体、抗体片段、抗体模拟分子(例如，DARPin、亲和体、适体、纳米抗体等)等)。在一些实施方案中，施用剂以使癌细胞、肿瘤和/或肿瘤微环境易接近或易感于用另外的疗法/治疗剂(例如，免疫治疗剂)治疗。可用于本文所述实施方案中的靶向表2的一种或多种受体/标志物(例如PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205，以及TMEM126A、Nrn1、CRTAM、Sema7a等)的剂不受它们的作用机制的限制。剂可以是小分子、肽、多肽、蛋白质、核酸(例如，反义、RNAi等)、抗体、抗体片段等。在一些实施方案中，施用Nrn1的拮抗剂。在一些实施方案中，施用CRTAM的拮抗剂。在一些实施方案中，施用Sema7a的激动剂。

[0081] 在一些实施方案中，提供了靶向4-1BB、LAG-3和/或表2的一种或多种受体/标志物(例如PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205，以及TMEM126A、CRTAM、Sema7a等)的抗体、抗体片段、抗体模拟分子(例如，

DARPin、亲和体、适体、纳米抗体等)，或它们的片段。这些剂可以是裸露的，通过靶结合(例如，中和靶标)来获得它们的作用，或者可以与功能部分(例如，药物、毒素、效应部分等)缀合。

[0082] 在一些实施方案中，用(i)靶向4-1BB、LAG-3和/或表2的一种或多种受体/标志物(例如PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205，以及TMEM126A、CRTAM、Sema7a等)的一种或多种剂(例如，抗体、抗体片段、抗体模拟分子(例如，DARPin、亲和体、适体、纳米抗体等)等)以及(ii)一种或多种另外的癌症疗法来治疗受试者。此类疗法包括化疗、免疫疗法、放射、手术等。在一些实施方案中，将靶向本文所述受体/标志物的剂与一种或多种另外的剂共同施用以治疗癌症。

[0083] 在一些实施方案中，适用于本文所述的组合物和方法的示例性抗癌剂包括但不限于：1)生物碱，包括微管抑制剂(例如，长春新碱、长春碱和长春地辛等)、微管稳定剂(例如，紫杉醇(红豆杉醇)和多西紫杉醇等)，以及染色质功能抑制剂，包括拓扑异构酶抑制剂，诸如表鬼臼毒素(例如，依托泊苷(VP-16)和替尼泊苷(VM-26)等)，以及靶向拓扑异构酶I的剂(例如，喜树碱和伊立替康(isirinotecan)(CPT-11)等)；2)共价DNA结合剂(烷化剂)，包括氮芥(例如，二氯甲基二乙胺、苯丁酸氮芥、环磷酰氮芥、异环磷酰胺和白消安(MYLERAN)等)、亚硝基脲(例如，卡莫司汀、洛莫司汀和司莫司汀等)和其他烷化剂(例如，达卡巴嗪、羟甲基三聚氰胺、噻替派和丝裂霉素等)；3)非共价DNA结合剂(抗肿瘤抗生素)，包括核酸抑制剂(例如，放线菌素(放线菌素D)等)、蒽环霉素(例如，柔红霉素(道诺霉素和盐酸佐柔比星)、阿霉素(亚德里亚霉素)和伊达比星(伊达霉素)等)、蒽二酮类(例如，蒽环霉素类似物，诸如米托蒽醌类等)、博来霉素(BLENOXANE)等，以及普卡霉素(光神霉素)等；4)抗代谢物，包括抗叶酸剂(例如，甲氨蝶呤、FOLEX和MEXATE，等等)、嘌呤抗代谢物(例如，6-巯基嘌呤(6-MP,PURINETHOL)、6-硫鸟嘌呤(6-TG)、硫唑嘌呤、阿昔洛韦、更昔洛韦、氯脱氧腺苷、2-氯脱氧腺苷(CdA)，以及2'-脱氧助间型霉素(喷司他丁)等)、嘧啶拮抗剂(例如，氟嘧啶(例如，5-氟尿嘧啶(ADRUCIL)、5-氟脱氧尿苷(FdUrd)(氟尿苷))等)，以及胞嘧啶阿拉伯糖苷(例如，CYTOSAR(ara-C)和氟达拉滨等)；5)酶，包括L-天冬酰胺酶和羟基脲等；6)激素，包括糖皮质激素、抗雌激素(例如，它莫西芬等)、非甾体抗雄激素(例如，氟他胺等)，以及芳香酶抑制剂(例如，阿那曲唑(ARIMIDEX)等)；7)铂化合物(例如，顺铂和卡铂等)；8)单克隆抗体(例如，与抗癌药物、毒素和/或放射性核素等缀合；中和抗体；等)；9)生物反应调节剂(例如，干扰素(例如，IFN-α等)和白细胞介素(例如，IL-2等)等)；10)过继免疫疗法；11)造血生长因子；12)诱导肿瘤细胞分化的剂(例如，全反式维甲酸等)；13)基因治疗技术；14)反义治疗技术；15)肿瘤疫苗；16)针对肿瘤转移的疗法(例如，(巴马司他等)；17)血管生成抑制剂；18)蛋白体抑制剂(例如，VELCADE)；19)乙酰化和/或甲基化的抑制剂(例如，HDAC抑制剂)；20)NFκB的调控剂；21)细胞周期调节的抑制剂(例如，CDK抑制剂)；以及22)p53蛋白功能的调节剂。

[0084] 在一些实施方案中，施用靶向4-1BB、LAG-3和/或表2的一种或多种受体/标志物(例如Nrn1、Sema7a、CRTAM等)的试剂以克服癌细胞、肿瘤、肿瘤微环境等的免疫侵袭。在一些实施方案中，采用一种或多种另外的癌症免疫疗法(例如，同时或连续)以利用经治疗的细胞/肿瘤的免疫应答性。合适的免疫疗法可包括但不限于：基于细胞的疗法(例如，树突细胞或T细胞疗法等)、单克隆抗体(mAb)疗法(例如，裸mAb、缀合的mAb)、细胞因子疗法(例如，

干扰素、白细胞介素等)、辅助治疗(例如,多糖K)等。

[0085] 在一些实施方案中,将靶向4-1BB、LAG-3和/或表2的一种或多种受体/标志物(例如PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205,以及TMEM126A、CRTAM、Sema7a等)的剂与靶向一种或多种癌细胞或肿瘤)标志物或组分的剂(例如,小分子、肽、抗体、抗体片段等)共同施用。在一些实施方案中,此种共同施用使得癌细胞、肿瘤和/或肿瘤微环境易感于和/或易接近用另外的试剂治疗。

[0086] 在一些实施方案中,用于本文所述方法和组合物的剂靶向和/或结合癌症或肿瘤细胞标志物或组分,所述癌症或肿瘤细胞标志物或组分选自包括但不限于以下的组:表皮生长因子受体(EGFR、EGFR1、ErbB-1、HER1)。ErbB-2(HER2/neu)、ErbB-3/HER3、ErbB-4/HER4、EGFR配体家族;胰岛素样生长因子受体(IGFR)家族、IGF结合蛋白(IGFBP)、IGFR配体家族(IGF-1R);血小板来源的生长因子受体(PDGFR)家族、PDGFR配体家族;成纤维细胞生长因子受体(FGFR)家族、FGFR配体家族、血管内皮生长因子受体(VEGFR)家族、VEGF家族;HGF受体家族:TRK受体家族;肝配蛋白(EPH)受体家族:AXL受体家族;白细胞酪氨酸激酶(LTK)受体家族;TIE受体家族,血管生成素1、2;受体酪氨酸激酶样孤儿受体(ROR)受体家族;盘状结构域受体(DDR)家族;RET受体家族;KLG受体家族;RYK受体家族;MuSK受体家族;转化生长因子α(TGF-α)、TGF-α受体;转化生长因子-β(TGF-β)、TGF-β受体;白细胞介素β受体α2链(IL13Rα2)、白细胞介素-6(IL-6)、1L-6受体、白细胞介素-4、IL-4受体、细胞因子受体、I类(血细胞生成素家族)和II类(干扰素/1L-10)家族)受体、肿瘤坏死因子(TNF)家族、TNF-α、肿瘤坏死因子(TNF)受体超家族(TNTRSF)、死亡受体家族、TRAIL受体;癌症-睾丸(CT)抗原、谱系特异性抗原、分化抗原、α-辅肌动蛋白-4、ARTC1、断点簇区域-Abelson(Bcr-abl)融合产物、B-RAF、半胱天冬酶-5(CASP-5)、半胱天冬酶-8(CASP-8)、β-连环蛋白(CTNNB1)、细胞分裂周期27(CDC27)、细胞周期蛋白依赖性激酶4(CDK4)、CDKN2A、COA-1、dek-can融合蛋白、EFTUD-2、延长因子2(ELF2)、Ets变体基因6/急性髓细胞白血病1基因ETS(ETC6-AML1)融合蛋白、纤连蛋白(FN)、GPNMB、低密度脂质受体/GDP-L岩藻糖:β-D-半乳糖2-α-L岩藻糖转移酶(LDLR/FUT)融合蛋白、HLA-A2、MLA-A11、热休克蛋白70-2突变(HSP70-2M)、KIAA0205、MART2、黑色素瘤泛在突变1、2、3(MUM-1、MUM-2、MUM-3)、前列腺酸性磷酸酶(PAP)、neo-PAP、肌球蛋白1类、NFYC、OGT、OS-9、pml-RARα融合蛋白、PRDX5、PTPRK、K-ras(KRAS2)、N-ras(NRAS)、HRAS、RBAF600、SIRT12、SNRPD1、SYT-SSX1或-SSX2融合蛋白、磷酸丙糖异构酶、BAGE、BAGE-1、BAGE-2、BAGE-3、BAGE-4、BAGE-5、GAGE-1、GAGE-2、GAGE-3、GAGE-4、GAGE-5、GAGE-6、GAGE-7、GAGE-8,GnT-V(异常N-乙酰葡糖胺基转移酶V,MGAT5)、HERV-K MEL、KK-LC、KM-HN-1、LAGE、LAGE-1,CTL识别的黑素瘤抗原(CAMEL),MAGE-A1(MAGE-1)。MAGE-A2、MAGE-A3、MAGE-A4、MAGE-AS、MAGE-A6、MAGE-A8、MAGE-A9、MAGE-A10。MAGE-A11、MAGE-A12、MAGE-3、MAGE-B1、MAGE-B2、MAGE-B5。MAGE-B6、MAGE-C1、MAGE-C2、粘蛋白1(MUC1)、MART-1/Melan-A(MLANA)、gp100、gp100/Pme117(S1LV)、酪氨酸酶(TYR)、TRP-1、HAGE、NA-88、NY-ESO-1、NY-ESO-1/LAGE-2、SAGE、Sp17。SSX-1、SSX-2、SSX-3、SSX-4、TRP2-1NT2、癌胚抗原(CEA)、激肽释放酶4、乳腺珠蛋白-A、OA1、前列腺特异性抗原(PSA)、前列腺特异性膜抗原,TRP-1/,75。TRP-2脂肪分化相关蛋白、在黑素瘤2中不存在的干扰素诱导蛋白(AIM-2)。BING-4、CPSF、细胞周期蛋白D1、上皮细胞粘附分子(Ep-CAM)、EpbA3、成纤维细胞生长因子-5(FGF-5)、糖蛋白250(gp250肠羧酸酯酶(iCE)、α-胎蛋白(AFP)、M-CSF、mdm-2、MUCI、p53(TP53)、PBF、

PRAME、PSMA、RAGE-1、RNF43、RU2AS、SOX10、STEAP1、存活蛋白(BIRCS)、人端粒酶逆转录酶(hTERT)、端粒酶、Wilms的肿瘤基因(WT1)、SYCP1、BRDT、SPANX、XAGE、ADAM2、PAGE-5、LIP1、CTAGE-1、CSAGE、MMA1、CAGE、BORIS、HOM-TES-85、AF15q14、HCA66I、LDHC、MORC、SGY-1、SPO11、TPX1、NY-SAR-35、FTHLI7、NXF2 TDRD1、TEX 15、FATE、TPTE、免疫球蛋白个体基因型、Bence-Jones蛋白、雌激素受体(ER)、雄激素受体(AR)、CD40、CD30、CD20、CD19、CD33、CD4、CD25、CD3、癌抗原72-4(CA 72-4)、癌抗原15-3(CA 15-3)、癌抗原27-29(CA 27-29)、癌抗原125(CA 125)、癌抗原19-9(CA 19-9)、β-人绒毛膜促性腺激素、1-2微球蛋白、鳞状细胞癌抗原、神经元特异性烯醇化酶、热休克蛋白gp96。GM2、沙莫司亭、CTLA-4、707丙氨酸脯氨酸(707-AP)、T细胞识别的腺癌抗原4(ART-4)、癌胚抗原肽1(CAP-1)、钙激活氯通道2(CLCA2)、亲环素B(Cyp-B)、人印戒指肿瘤-2(HST-2)等。

[0087] 可掺入本文公开的组合物和方法中的抗体的实例包括但不限于诸如以下抗体:曲妥单抗(抗HER2/neu抗体);帕妥珠单抗(抗HER2mAb);西妥昔单抗(表皮生长因子受体EGFR的嵌合单克隆抗体);帕尼单抗(抗EGFR抗体);尼妥珠单抗(抗EGFR抗体);扎鲁妥木单抗(抗EGFR mAb);耐昔妥珠单抗(抗EGFR mAb);MDX-210(人源化抗HER-2双特异性抗体);MDX-210(人源化抗HER-2双特异性抗体);MDX-447(人源化抗EGF受体双特异性抗体);利妥昔单抗(嵌合鼠/人抗CD20 mAb);阿妥珠单抗(抗CD20 mAb);奥法木单抗(抗CD20 mAb);托西莫单抗-1131(抗CD20 mAb);替坦异贝莫单抗(抗CD20 mAb);贝伐单抗(抗VEGF mAb);雷莫卢单抗(抗VEGFR2 mAb);兰尼单抗(抗VEGF mAb);阿柏西普(与IgG1 Fc融合的VEGFR1和VEGFR2的细胞外结构域);AMG386(与IgG1 Fc融合的血管生成素-1和血管生成素-2结合肽);戴妥珠单抗(抗IGF-1R mAb);吉妥珠单抗奥唑米星(抗CD33 mAb);阿仑单抗(抗Campath-1/CD52 mAb);本妥昔单抗(抗CD30 mAb);卡妥索单抗(靶向上皮细胞粘附分子和CD3的双特异性mAb);他那莫单抗(抗5T4 mAb);吉瑞妥昔单抗(抗碳酸酐酶ix);或法勒珠单抗(抗叶酸受体)。其他实例包括抗体,诸如Panorex™(17-1A)(鼠单克隆抗体);Panorex(@(17-1A))(嵌合鼠单克隆抗体);BEC2(抗独特性mAb,模拟GD表位)(具有BCG);Oncolym(Lym-1单克隆抗体);SMART M195 Ab,人源化13'1 LYM-1(Oncolym)。Ovarex(B43.13,抗独特性小鼠mAb);3622W94 mAb与腺癌上的EGP40(17-1A)泛癌抗原(pancarcinoma antigen)结合;Zenapax(SMART抗Tac(IL-2受体);SMART M195 Ab,人源化Ab,人源化);NovoMAb-G2(泛癌特异性Ab);TNT(组蛋白抗原的嵌合mAb);TNT(组蛋白抗原的嵌合mAb);胶质瘤-H(单克隆-人源化Ab);GNI-250 Mab;EMD-72000(嵌合-EGF拮抗剂);LymphoCide(人源化IL.L.2抗体);以及MDX-260双特异性,靶向GD-2、ANA Ab、SMART IDIO Ab、SMART ABL 364 Ab或ImmuRAIT-CEA。

[0088] 在一些实施方案中,可用于本文实施方案中的剂特异性结合调节性T细胞、骨髓抑制细胞或树突细胞的组分。在另一个方面,靶向部分特异性结合以下分子中的一种:CD4;CD25(IL-2α受体;IL-2αR);细胞毒性T淋巴细胞抗原-4(CTLA-4;CD152);白细胞介素-10(IL-10);转化生长因子-β受体(TGF-βR);转化生长因子-β(TGF-β);程序性死亡-1(PD-1);程序性死亡-1配体(PD-L1或PD-L2);核因子-κB的受体激活剂(RANK);核因子-κB受体激活剂(RANK)的配体(RANKL);LAG-3;糖皮质激素诱导的肿瘤坏死因子受体家族相关基因(GITR;TNFRSF18);或白细胞介素-4受体(IL-4R)。在一些实施方案中,所述剂是增强靶向分子功能的激动剂。在其他实施方案中,所述剂是抑制靶向分子功能的拮抗剂。

[0089] 在一些实施方案中,可用于本文实施方案的剂结合调节免疫系统的特定细胞因

子、细胞因子受体、共刺激分子、共抑制分子或免疫调节受体。在另一方面，靶向部分特异性结合以下分子中的一种：肿瘤坏死因子(TNF)超家族；肿瘤坏死因子-α(TNF-α)；肿瘤坏死因子受体(TNFR)超家族；白细胞介素-12(IL-12)；IL-12受体；4-1BB(CD137)；4-1BB配体(4-1BBL；CD137L)；OX40(CD134；TNR4)；OX40配体(OX40L；CD40；CD40配体(CD40L)；CTLA-4；程序性死亡-1(PD-1)；PD-1配体I(PD-L1：B7-H1)；或PD-1配体2(PD-L2；B7-DC)；B7家族；B7-1(CD80)；B7-2(CD86)；B7-H3；B7-H4；GITR/AITR：GITRL/AITRL；BTLA；CD70；CD27；LIGHT；HVEM：Toll样受体(TLR)(TLR 1、TLR 2、TLR 3、TLR 4、TLR 5，、TLR 6、TLR 7、TLR 8、TLR 9、TLR 10)。在一些实施方案中，所述剂是增强靶向分子功能的激动剂。在其他实施方案中，所述剂是抑制靶向分子功能的拮抗剂。

[0090] 在一些实施方案中，将靶向4-1BB、LAG-3和/或表2的一种或多种受体/标志物(例如PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205，以及TMEM126A、CRTAM、Sema7a等)的剂(例如，免疫治疗剂)与一种或多种佐剂共同施用(例如，连续或顺序地)。合适的佐剂包括但不限于以下项中的一种或多种：油乳剂(例如，弗氏佐剂)；皂苷配方；病毒体和病毒样颗粒；细菌和微生物衍生物；免疫刺激性寡核苷酸；ADP核糖基化毒素和解毒衍生物；明矾；BCG；含矿物质的组合物(例如，矿物盐，诸如铝盐和钙盐、氢氧化物、磷酸盐、硫酸盐等)；生物粘合剂和/或粘膜粘合剂；微粒；脂质体；聚氧乙烯醚和聚氧乙烯酯配方；聚磷腈；胞壁酰肽；咪唑喹诺酮类化合物；表面活性物质(例如溶血卵磷脂、普朗尼克多元醇(pluronic polyol)、聚阴离子、肽、油乳剂、钥孔戚血蓝蛋白和二硝基苯酚)。

[0091] 佐剂还可包括免疫调节剂，诸如细胞因子、白细胞介素(例如，IL-1、IL-2、IL-4、IL-5、IL-6、IL-7、IL-12等)、干扰素(例如，干扰素γ)、巨噬细胞集落刺激因子和肿瘤坏死因子。除了变体B7-DC多肽之外，还可以施用其他共刺激分子，包括B7家族的其他多肽。蛋白质佐剂可以作为全长多肽或其活性片段提供，或以DNA的形式提供，诸如质粒DNA。

[0092] 本文所述的药物和免疫治疗组合物可通过任何合适的施用途径(例如，口服递送、肠胃外递送、粘膜递送、肺部递送、静脉内递送等)来递送。用于此类递送途径的合适制剂在本领域中是可理解的。

[0093] 可以用本文所述的组合物和方法治疗的癌症的非限制性实例包括但不限于：黑素瘤(例如，转移性恶性黑素瘤)、肾癌(例如，透明细胞癌)、前列腺癌(例如，激素难治性前列腺腺癌)、胰腺癌(例如，腺瘤)、乳癌、结肠癌、肺癌(例如，非小细胞肺癌)、食道癌、头颈鳞状细胞癌、肝癌、卵巢癌、宫颈癌、甲状腺癌、成胶质细胞瘤、神经胶质瘤、白血病、淋巴瘤及其它赘生性恶性疾病。在一些实施方案中，癌症是实体肿瘤癌症。

[0094] 本文描述的一些实施方案特别可用于治疗对免疫治疗方法无响应的肿瘤。在一些实施方案中，本文提供了对T细胞或抗原呈递细胞(例如，树突细胞(例如，$CD103^+$DC等)，等等)没有响应(或具有降低的响应)的癌症的治疗。在一些实施方案中，本文提供的是治疗尽管T细胞浸润，但对治疗无响应的癌症。在一些实施方案中，本文所述的组合物和方法可用于治疗这样的癌症，在所述癌症中T细胞未针对肿瘤相关抗原进行适当的致敏。在一些实施方案中，本文所述的组合物和方法可用于治疗包含在树突细胞(例如，$CD103^+$DC等)募集方面有缺陷的肿瘤或细胞的癌症。在一些实施方案中，本文所述的组合物和方法可用于治疗包含在趋化因子CCL4产生方面有缺陷的肿瘤或细胞的癌症。

[0095] 在一些实施方案中，本文的治疗组合物和方法可用于例如WO2016/141312中描述的那些；该专利全文以引用方式并入。

[0096] 在一些实施方案中，提供了用于测试来自受试者的样品(例如，细胞、组织、细胞群、肿瘤、血液、尿液、唾液等)的一种或多种生物标志物(例如，功能障碍的肿瘤抗原特异性$CD8^+$ T细胞的生物标志物)的方法。此类生物标志物可包括核酸、小分子、蛋白质、肽等，并且可使用任何合适的技术测定法进行检测。在一些实施方案中，本文提供基于DNA-、RNA-、小分子和/或基于蛋白质的诊断方法，所述诊断方法直接或间接检测癌细胞或肿瘤逃避免疫应答或免疫疗法的生物标志物。本发明还提供用于此类诊断目的的组合物、试剂和试剂盒。

[0097] 在一些实施方案中，在核酸(例如，RNA)水平上检测生物标志物。例如，测定样品中生物标志物核酸(例如，mRNA)的存在或量(例如，以测定生物标志物表达的存在或水平)。可以使用本领域普通技术人员已知的多种核酸技术来检测/定量生物标志物核酸(例如，RNA、扩增的cDNA等)，所述核酸技术包括但不限于核酸测序、核酸杂交、核酸扩增(例如，通过PCR、RT-PCR、qPCR等)、微阵列、Southern和Northern印迹、测序等。可以通过任何常规手段检测未扩增或扩增的核酸。例如，在一些实施方案中，通过与可检测标记的探针杂交并测量所得杂交体来检测核酸。核酸检测试剂可以是标记的(例如，荧光的)或未标记的，并且可以在溶液中游离或固定(例如，在珠粒、孔、表面、芯片等上)。

[0098] 在一些实施方案中，在蛋白质水平上检测生物标志物。例如，测定样品中生物标志物蛋白的存在或量(例如，以测定生物标志物表达的存在或水平或定位)。在一些实施方案中，提供试剂以检测和/或定量生物标志物蛋白。合适的试剂包括一抗(例如，结合生物标志物)、二抗(例如，结合一抗)、抗体片段、适体等。蛋白质检测试剂可以被标记(例如，荧光)或未标记，并且可以在溶液中游离或固定(例如，在珠粒、孔、表面、芯片等上)。

[0099] 在一些实施方案中，提供生物标志物捕获试剂以对生物标志物进行定位、浓缩、聚集等。例如，在一些实施方案中，与生物标志物相互作用的生物标志物捕获试剂与固体支持物(例如，珠粒、表面、树脂、柱等)连接，这允许使用者在宏观尺度上进行操纵。通常，固体支持物允许使用机械手段从异质溶液中分离和纯化生物标志物。例如，当与珠粒连接时，通过例如通过物理移动从异质溶液中去除珠粒来实现分离。在珠粒是磁性或顺磁性的实施方案中，使用磁场来实现捕获试剂(并因此靶标)与异质溶液的物理分离。用于分离靶标的磁珠在本领域中描述于例如欧洲专利申请号87309308中，该专利申请出于所有目整体并入本文。

[0100] 用于本文所述的诊断方法或测试步骤的组合物包括但不限于探针、扩增寡核苷酸和抗体。本文描述的实施方案中使用的任何检测和/或诊断试剂可以单独提供或与试剂盒形式的其他组合物组合提供。试剂盒可以包括测定所必需或足够的任何和所有组分，包括但不限于检测试剂、缓冲液、对照试剂(例如，组织样品、阳性和阴性对照样品等)、固体支持物、标签、书写和/或图示说明和产品信息、抑制剂、标记和/或检测试剂、包装环境控制(例如，冰、干燥剂等)等。在一些实施例中，试剂盒提供所需组分的子组，其中预期使用者将提供剩余的组分。在一些实施例中，试剂盒包括两个或更多个单独的容器，其中每个容器容纳待递送的组分的2。

[0101] 在一些实施方案中，使用基于计算机的分析程序来将通过检测测定生成的原始数

据(例如,生物标志物的存在、不存在或表达量)转化成临床医生的预测值数据。在一些实施方案中,计算机分析将各种数据组合成单个分数或值,该单个分数或值为预测性的和/或诊断性的。临床医生可以使用任何合适的手段访问预测性数据。因此,在一些优选的实施方案中,本发明提供了以下进一步的益处:不太可能接受遗传学或分子生物学训练的临床医生不需要理解原始数据。数据以最有用的形式直接呈现给临床医生。然后,临床医生能够立即利用该信息以优化受试者的护理。本文考虑的是能够从进行测定的实验室、信息提供者,医疗人员和受试者接收、处理信息并向所述实验室、信息提供者,医疗人员和受试者传送信息的任何方法。例如,在本发明的一些实施方案中,从受试者获得样品((例如,活检,细胞或血液样品),并提交给谱分析服务(例如,医疗机构的临床实验室、第三方检测服务、基因组分析业务等,以生成原始数据。在样品包括组织或其他生物样品的情况下,受试者可以访问医疗中心以获得样品并将其发送到分析中心,或者受试者可以自己收集样品并直接将其发送到分析中心。在一些实施方案中,生成报告(例如,由临床医生、由测试中心、由计算机或其他自动分析系统等)。报告可包含测试结果、诊断和/或治疗建议。

[0102] 实验

[0103] 材料和方法

[0104] 小鼠和肿瘤接种

[0105] 从Taconic Farms购买6至8周范围内的雌性C57BL/6小鼠。从Taconic Farms获得处于C57BL/6背景的CD45.1和$Rag2^{-/-}$小鼠,并在芝加哥大学繁殖。先前已经描述了2C/$Rag2^{-/-}$和P14/$Rag2^{-/-}$小鼠(Brown等人,2006;该文献全文以引用方式并入本文)。产生pLCK-CreERT2 x ROSA-YFP小鼠并且已经进行了描述(Evaristo等人,2016;该文献全文以引用方式并入本文)。将B16.SIY.dsRed(Kline等人,2012;该文献全文以引用方式并入本文)、C1498.SIY.GFP(Zhang等人,2009;该文献全文以引用方式并入本文)和MC57.SIY.GFP(Spiotto等人,2002;该文献全文以引用方式并入本文)肿瘤细胞工程改造以使dsRed或GFP与$H2\text{-}K^{b}$限制模型抗原SIYRYYGL同框表达。通过使用表达SIYRYYGL的cDNA的pLEGFP质粒(Spiotto等人,2002;该文献全文以引用方式并入)对1969细胞系进行逆转录病毒转导(Diamond等人,2011;该文献全文以引用方式并入),而将1969.SIY.GFP细胞系工程改造。对于实验,使6至9周龄小鼠在左侧腹上或左侧腹和右侧腹两侧上皮下接受2×10^{6}个肿瘤细胞。根据国立卫生研究院动物护理指南维护所有小鼠,并根据IACUC批准的方案进行研究。

[0106] 为了产生$Egr2^{EGFP}$敲入报告小鼠的靶向构造,将包含egr2基因的12.6kb小鼠基因组DNA片段用SacII切下并克隆到pEasy-Flox载体中与胸苷激酶(TK)选择标记相邻。将含有IRES2-eGFP和LoxP侧翼新霉素选择标记的盒插入egr2基因的翻译终止密码子(TGA)与聚腺苷酸化信号之间的NheI位点。对来自129只小鼠的ES细胞克隆进行电穿孔并选择新霉素抗性。通过PCR和使用5'和3'探针进行southern印迹,验证了ES细胞克隆在内源基因座中的同源插入。将小鼠与C57BL/6回交超过8代。

[0107] TIL分离

[0108] 在指定的时间点从小鼠收获肿瘤。将肿瘤通过50μm过滤器解离并用PBS洗涤。通过在细胞悬浮液下层叠泛影葡胺(Ficoll-Hypaque),然后以400×g无间断地离心30min,来进一步富集TIL。分离血沉棕黄层(buffy-layer)并用PBS洗涤两次,然后染色。为了通过FACS分离特定细胞群,在指示时合并肿瘤,并通过泛影葡胺离心两次来重新纯化细胞层。对于第

28天肿瘤，在泛影葡胺分离后，根据制造商的说明书(MAGNISORT,eBiosciences)通过负性珠粒选择来进一步纯化T细胞。然后用PBS洗涤细胞，在4℃下染色15分钟，然后重悬于完全DMEM(cDMEM:10％FBS、100U/mL青霉素-链霉素、1％MEM非必需氨基酸、50μMβ-ME、0.01M MOPS)中，并根据实验测定分选到RLT裂解缓冲液(QIAGEN)或cDMEM中。一旦分选完成，就将分选到RLT缓冲液中的细胞直接放在干冰上。

[0109] 流式细胞术和抗体

[0110] 将细胞悬浮液在PBS中洗涤两次，然后在FACS缓冲液(10％FBS、2mM EDTA、0.001％NaN_3)中染色。将细胞在冰上染色30min并在1％PFA中固定。使用针对以下分子的抗体:CD3(17A2、AX700)、2B4(2B4、FITC)、CD127(A7R34、PE)、OX-40(OX-86、PE)、4-1BB(17B5、生物素、APC)、CD160(7H1、PE-Cy7)、LAG-3(C9B7W、PerCPeFluor710)、PD-1(RMP1-30、PE-Cy7)、NRP1(3E12、BV421)、GITR(DTA-1、FITC)、ICOS(7E.17G9、BV421)、KLRG-1(2F1、eF450、BV605)、TIGIT(1G9、APC)、TIM-3(RMT3-23、PE)、CD4(RM4-5、BV605)、CD45.1(A20、FITC)、CD45.2(104、PE)、CD8α(53-6.7、BV711)。使用可固定活力染料506(eBioscience)进行活/死辨别。利用SIYRYYGL五聚体(PE)(Proimmune)执行SIY特异性T细胞的染色;将SIINFEKL五聚体(PE)用作非特异性对照。所有流式细胞术分析均在LSRFortessa(BD)上进行，并使用FlowJo软件(Tree Star)进行分析。

[0111] 定量实时PCR

[0112] 使用RNEasy Micro Kit(QIAGEN)按照制造商的方案从分选的细胞群体中提取总RNA。使用高容量cDNA逆转录试剂盒(Applied Biosystems)根据制造商的说明书来合成cDNA。使用引物-探针组(表1a和表1b)测定转录物水平，所述引物-探针组是通过在线ProbeFinder软件和通用探针库(Roche)开发的，IL-2(Mm00434256_m1)和18S(Hs99999901_s1)除外。为了使批次影响最小化，在可能的情况下，将探测的基因的所有样品都在相同的96孔qRT-PCR板上运行。所有引物-探针组均含有跨越外显子-外显子边界的引物或跨越内含子的引物。将转录物的表达水平归一化为18S表达。

[0113] 表1a.引物序列

[0114]

编号	Wilson	IMGT	序列	(SEQ ID NO:
0	Cβ1.1	TRBC1	CTCAAACAAGGAGACCTTGGGTGG	1
1	Vβ1	TRVB5	CAGACAGCTCCAAGCTACTTTTAC	2
2	Vβ2	TRVB1	ATGAGCCAGGGCAGAACCTTGTAC	3
3	Vβ3	TRVB26	GAAATTCAGTCCTCTGAGGCAGGA	4
4	Vβ4	TRVB2	CTAAAGCCTGATGACTCGGCCACA	5
5	Vβ5.1	TRVB12-2	CTTTGGAGCTAGAGGACTCTGCCG	6
6	Vβ5.2	TRVB12-1	CCTTGGAACTGGAGGACTCTGCTA	7
7	Vβ6	TRVB19	GCCCAGAAGAACGAGATGGCCGTT	8
8	Vβ7	TRVB29	GGATTCTGCTAAAACAAACCAGAC ATCTGT	9
9	Vβ8.1	TRVB13-3	GCTTCCCTTTCTCAGACAGCTGTA	10
10	Vβ8.2	TRVB13-2	GCTACCCCCTCTCAGACATCAGTG	11
11	Vβ8.3	TRVB13-3	GGCTTCTCCCTCTCAGACATCTT	12
12	Vβ9	TRVB17	CTCTCTCTACATTGGCTCTGCAGG	13
13	Vβ10	TRVB4	CTTCGAATCAAGTCTGTAGAGCCG	14
14	Vβ11	TRVB16	TGAAGATCCAGAGCAGCGGGCCCC	15
15	Vβ12	TRVB15	CCACTCTGAAGATTCAACCTACAG AACCC	16
16	Vβ13	TRVB14	CAAGATCCAGTCTGCAAAGCAGGG	17
17	Vβ14	TRVB31	GCACGGAGAAGCTGCTTCTCAGCC	18
18	Vβ15	TRVB20	GCATATCTTGAAGACAGAGGC	19
19	Vβ16	TRVB3	CTCTGAAAATCCAACCCACAGCAC TGG	20
20	Vβ17	TRVB24	TCTGAAGAAGACGACTCAGCACTG	21
21	Vβ18	TRVB30	GCAAGGCCTGGAGACAGCAGTATC	22

[0115] 表1b.引物/探针

[0116]

基因	SEQ ID NO:	引物 1	引物 2	SEQ ID NO:	罗氏探针编号
Lag3	23	tgctttgggaagctccagt	gctgcagggaagatggac	42	79
Tnfrsf9	24	ccggtcttaagcacagacct	gaacggtactggcgtctgtc	43	108
Egr2	25	ctacccggtggaagacctc	aatgttgatcatgccatctcc	44	60
Sema7a	26	tcaatcggctgcaagatgt	cgcagacagctgagtagttcc	45	15
Crtam	27	agatccaacaacgaggagaca	tcatgcaacgcttagactgg	46	71
Ccl1	28	tcaccatgaaacccactgc	agcagcagctattggagacc	47	71
Ngn	29	caccctagcctaacctcaacc	tgaaaacctcctcccctctt	48	45
Arl3	30	ctggcagatccagtcctgtt	acccagttcatgccatcct	49.	100

[0117]

Exph5	31	atgagggaggagagcggtat	cagcttgttgtccaaatcgtc	50	67
Fhl2	32	agaaaaccatcatgccaggt	acaggtgaagcaggtctcgt	51	74
Nrn1	33	atcctcgcggtgcaaata	gcccttaaagactgcatcaca	52	108
Ptgfrn	34	ccggggagatctcatcaaa	tcgaaggccatgtcatctg	53	12
Rankl	35	tgaagacacactacctgactcctg	cccacaatgtgttgcagttc	54	88
Hif1a	36	gctgctcactgtgaaggaagt	tggggaatgcattttaccat	55	2
Egr3	37	caatctgtaccccgaggaga	ccgatgtccatcacattctct	56	74
Tnfa	38	ctgtagcccacgtcgtagc	ttgagatccatgccgttg	57	25
Gzmb	39	gctgctcactgtgaaggaagt	tggggaatgcattttaccat	58	2
Ccl1	40	tcaccatgaaacccactgc	agcagcagctattggagacc	59	71
Ccl22	41	tcttgctgtggcaattcaga	gcagagggtgacggatgtag	60	74

[0118] 体内增殖测定

[0119] 在流式细胞术分析之前24小时通过BrdU脉冲测量体内增殖。在肿瘤接种后第12天,每只小鼠i.p.(腹膜内)注射0.8mg BrdU。分离TIL并如上所述执行表面染色。表面染色后,根据制造商的方案,使用Foxp3染色试剂盒(BD)固定并透化细胞,并在37℃下用100μl的PBS/DNA酶溶液(300μg/ml)孵育30分钟。洗涤细胞并在室温下用抗BrdU(FITC,Bu20a)孵育30分钟,然后用PBS洗涤并重悬于PBS中。

[0120] 体外刺激测定

[0121] 将经组织培养处理的96孔圆底板在DPBS中用抗CD3ε(1μg/ml;2C11)在4℃下包被过夜或在37℃下包被2小时。将细胞分选到冷的cDMEM培养基中,并在分选完成后立即放到冰上。然后沉淀细胞,重悬于50μl的cDMEM中,并与可溶性抗CD28(2μg/ml;PV-1)一起孵育10-12小时,最终体积为100μl。刺激后,取出上清液用于ELISA或基于珠粒的免疫测定(LegendPlex),并将细胞用DPBS洗涤一次并重悬于15μl的RNAlater稳定溶液(QIAGEN)或300μl的RLT缓冲液中。将细胞保存在-80℃直至执行RNA分离。

[0122] 蛋白质定量

[0123] 通过标准ELISA或基于珠粒的免疫测定(LEGENDplex,BioLegend)来测定蛋白质浓度的测量值。根据制造商的方案(Ready-SET-Go ELISA;eBioscience)对来自体外刺激的上清液执行ELISA。使用Emax酶标仪(Molecular Devices)获得在450nm处的吸光度值,并通过标准曲线确定IL-2浓度。将蛋白质浓度值归一化为铺板的分选细胞的数目。根据制造商的方案进行LEGENDplex测定。在单独的实验中通过两种方法来确认IL-2浓度(图4B),其中该两种方法之间无显著的IL-2浓度差异。

[0124] 谱型分析和测序

[0125] 对小鼠注射2×10^6个B16.SIY.dsRed肿瘤细胞。14天后,收获肿瘤,并将特异性$CD8^+$ TIL亚群分选到RLT缓冲液(QIAGEN)中并立即冷冻。从分选的细胞群合成cDNA,并用与FAM-Cβ1.1引物配对的21种不同Vβ-5'引物,通过PCR扩增CDR3区(表1)。三次VβPCR反应未达到用于分析的显著扩增,所以被从分析中去除。为了测序,使用QIAquick PCR纯化试剂盒(QIAGEN)纯化Cβ-VβPCR产物,并在芝加哥大学基因组学核心设施(University of Chicago Genomics Core Facility上进行测序。在芝加哥大学基因组学核心(the University of Chicago Genomics core)通过毛细管电泳分析Cβ-VβPCR产物,并使用Liz500梯比对CDR3峰。使用GeneiousR9软件(Kearse等,2012)显示光谱型图。为了生成每个Vβ谱型的频率谱,

使用peak studio(fodorlab.uncc.edu/software/peakstudio)测量每个峰下的面积。计算给定小鼠内来自每个$CD8^+$脾和TIL群体的各个Vβ谱型之间的汉明距离(Currier和Robinson,2001;该文献全文以引用方式并入)。为了确定来自每次比较的HD之间的显著性,对来自小鼠的各个Vβ的HD进行平均,并且执行具有Dunn校正的单因素方差分析以进行多重比较。

[0126] TCR转基因T细胞转移实验

[0127] 从来自同源2C/$Rag2^{-/-}$/CD45.1/2和/或P14/$Rag2^{-/-}$/CD45.2小鼠的脾和淋巴结产生细胞悬浮液,并通过根据制造商的方案在磁柱上进行$CD8^+$阴性选择(Miltenyi Biotechnologies)来纯化T细胞。用PBS洗涤TCR转基因(Tg)T细胞,以10×10^6/ml的浓度重悬,并将1×10^6个TCR Tg细胞通过尾静脉转移以0.1mL的体积过继转移到荷有CD45.1肿瘤的小鼠中。在指定的时间后,对2C T细胞和相应的宿主$CD8^+$ T细胞进行分选和刺激,如上所述。

[0128] 体外细胞毒性测定

[0129] 每次单独实验,对10只C57BL/6小鼠在左侧腹和右侧腹s.c.(皮下地)注射2×10^6个B16.SIY细胞。在第14天,合并所有20个肿瘤,并按照制造商的方案使用肿瘤解离试剂盒(Miltenyi Biotec)进行解离。用PBS洗涤肿瘤细胞悬浮液3-5次,并通过Ficoll-Hypaque梯度离心富集TIL。将TIL染色,分选并直接置于冰上。滴定TIL并直接加入含有50,000个P815肥大细胞瘤细胞和1μg/mL抗CD3的96孔板中。对于阳性对照,从OT-I/$Rag2^{-/-}$小鼠分离OT-I细胞,并用板结合的抗CD3(0.25μg/mL)、抗CD28(2μg/mL)和100U/mL的IL-2刺激2-3天。对于阴性对照,将P815细胞单独培养,或与从淋巴结分离的初始$CD8^+$ T细胞一起培养。温育12小时后,对细胞进行Thy1、CD45、CD8α、可固定活力染料450(eBioscience)和/或碘化丙啶的染色。

[0130] 基因表达分析

[0131] 将$CD8^+$ TIL亚群的总RNA按照制造商的方案(RNEasy Micro Kit:QIAGEN),从自10只小鼠合并的分选细胞中分离出来。使用Illumina MouseRef8微阵列芯片通过芝加哥大学基因组学设施(University of Chicago Genomics Facility)分析样品。进行两次实验重复,并且结果为经过$\log_2$转化和平均的。鉴定出揭示相对于$CD8^+4\text{-}1BB^-LAG\text{-}3^-PD\text{-}1^-$细胞的1.5倍差异abs($\log_2$(比率)>1.5))的探针组并用于后续分析。微阵列数据可在Gene Expression Omnibus数据库(ncbi.nlm.nih.gov/gds)中以登录号GSE79919获得。对于交叉研究比较,使用GEO2R在线软件从功能减退的$CD8^+$ TIL数据集、GSE79858((GSM2107353、GSM2107353和GSM2107355)对比(GSM2107350、GSM2107351、GSM210732))和$CD8^+$ T细胞耗竭数据集、GSE41870((GSM1026819、GSM1026820、GSM1026821)对比(GSM1026786、GSM1026787、GSM1026788、GSM1026789))提取log2倍数变化值。将显示2倍差异的上调基因用于分析。鉴定来自GEO2R提取数据的多个基因名称,并与来自Illumina数据集的基因名称进行匹配。在systems.crump.ucla.edu/rankrank/index.php和相关的生物导体包"RRHO"(Rosenblatt和Stein,2014;该文献全文以引用方式并入本文)处进行秩-秩超几何重叠分析(Plaisier等人,2010;该文献全文以引用方式并入本文)。

[0132] 基因本体富集分析

[0133] 以成对方式将共享的上调基因用作具有Cytoscape应用程序的ClueGO软件的输入(Shannon等人,2003;该文献全文以引用方式并入本文)。生物过程和免疫系统过程基因本体注释都用于分析。当产生途径节点时,仅考虑Bonferroni逐步减低校正p值>0.01的途径。

将在每个节点内发现具有最多基因数的非冗余途径用作图6A中的示例。

[0134] 抗体和FTY720治疗

[0135] 用100μg/小鼠的抗4-1BB(Bio-X-Cell;LOB12.3)抗体和/或100μg/小鼠抗LAG-3(Bio-X-Cell;C9B7W)腹膜内治疗小鼠。对于肿瘤生长实验,在肿瘤接种后第7天、第10天、第13天和第16天治疗小鼠。对于离体功能实验,将小鼠在第7天、第10天和第13天进行治疗,并在第14天对细胞进行分选。对于阻断淋巴结出口的实验,在第一抗体治疗前一天(第6天)通过灌胃给予25μg的FTY720,并且每天持续直至第14天的终点。

[0136] 结果

[0137] 4-1BB和LAG-3鉴定了$CD8^+$ TIL的主要群体

[0138] 为了确定4-1BB和LAG-3是否能鉴定功能障碍的$CD8^+$ TIL,使用充分表征的B16.SIY黑素瘤模型来检查LAG-3和4-1BB的表达模式。在肿瘤接种后第7天,$4\text{-}1BB^+LAG\text{-}3^+$群体占所有$CD8^+$ TIL的15.8%。到第21天,该群体的频率显著增长到44%。从第7天到第14天,$4\text{-}1BB^-LAG\text{-}3^+(4^-L^+)$群体的频率也增加1.9倍,变成占$CD8^+$TIL隔室的25%。相比之下,到第21天,$4\text{-}1BB^-LAG\text{-}3^-(4^-L^-)$群体的频率降低2.7倍。在实验的时间范围内,$4\text{-}1BB^+LAG\text{-}3^-CD8^+$ TIL的比例或数量没有显著的增长(图1A和图1B)。在分析细胞亚群的绝对数量时,可以看到类似的模式(图1C和图1D)。获得这些表型对于肿瘤微环境是特异性的,因为它们在脾或肿瘤引流淋巴结(TdLN)中未观察到(图1A)。这些数据表明肿瘤微环境优先支持诱导的LAG-3和4-1BB的共表达。

[0139] 肿瘤进展期间朝向$4\text{-}1BB^-LAG\text{-}3^+$和$4\text{-}1BB^+LAG\text{-}3^+$群体的细胞数量选择性增加和比例偏移表明这些群体的扩增发生在肿瘤微环境中。在肿瘤接种后第14天对$CD8^+$ TIL进行Ki67染色,并通过流式细胞术分析。81%的$4\text{-}1BB^-LAG\text{-}3^+$细胞和85%的$4\text{-}1BB^+LAG\text{-}3^+$细胞是$Ki67^+$,相比之下$4\text{-}1BB^-LAG\text{-}3^-$TIL的仅为32%(图1E)。在第12天用BrdU脉冲小鼠,并在24小时后分析$CD8^+$ TIL亚群的BrdU掺入。的确,$4\text{-}1BB^-LAG\text{-}3^+$和$4\text{-}1BB^+LAG\text{-}3^+$群体与$4\text{-}1BB^-LAG\text{-}3^-$群体相比掺入了更多的BrdU(图1F)。这些数据表明曾一旦$CD8^+$ T细胞到达肿瘤部位,一部分TIL就在肿瘤内扩增,并且这些扩增的TIL通过4-1BB和LAG-3的表达增加来鉴定。

[0140] 为了确定LAG-3和4-1BB的上调是否仅仅是B16.SIY肿瘤模型的产物,或者它是否是肿瘤内的$CD8^+$ T细胞的更一般特征,分析来自另外三个逐渐生长的肿瘤模型C1498.SIY、MC38.SIY、EL4.SIY和B16F10亲本的T细胞。在第14天分析TIL的4-1BB和LAG-3的表达。发现表达模式与在从B16.SIY肿瘤分离出的$CD8^+$ TIL中观察到的相似(图1G和图1I)、来自B16F10亲本肿瘤的结果证实,不需要SIY的存在来观察到4-1BB和LAG-3的共表达。为了确定$4\text{-}1BB^+LAG\text{-}3^+$ TIL亚群是仅在进展中的肿瘤中产生还是也在被排斥的肿瘤中产生,分析了1969.SIY和MC57.SIY纤维肉瘤肿瘤模型中的T细胞表型,所述T细胞表型更具免疫原性并且经历自发排斥。明显更少的$4\text{-}1BB^+LAG\text{-}3^+$细胞在1969.SIY和MC57.SIY肿瘤中的$CD8^+$ TIL隔室中被发现(图H和图I)。随着时间的推移,4-1BB和LAG-3的共表达在B16.SIY肿瘤中维持,而不在MC57.SIY肿瘤中维持(图1J)。这些数据表明,$LAG\text{-}3^+4\text{-}1BB^+$TIL表型的获得优先发生在肿瘤微环境内,并且仅在肿瘤进展而不是消退的条件下发生。

[0141] $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TIL表达Egr2和多个Egr2基因靶标

[0142] 在本文的实施方案的开发期间进行实验以确定Egr2表达本身是否也是$CD8^+$ TIL隔室内的T细胞的特征;利用Egr2-IRES-GFP($Egr2^{GFP}$)敲入报告小鼠。在第7天和第14天,所

有$CD8^+$ TIL的约14％是GFP^+(图2A)。为了确认Egr2被忠实地报告,对表达高水平和低水平EGFP的$CD8^+$ TIL进行分选,并通过qRT-PCR筛选Egr2和几个Egr2靶标。Egr2-$GFP^{高}$群体表达更高水平的Egr2和许多先前使用体外无反应性模型定义的Egr2靶基因。这些靶基因包括Tnfrsf9、Lag3、Ngn、Sema7a、Crtam、Ccl1和Nrn1(图2B)。通过流式细胞术确认4-1BB和LAG-3在Egr2-$GFP^{高}CD8^+$ TIL中的表达。大部分Egr2-$GFP^{高}$细胞表达LAG-3和/或4-1BB。在第14天,在亚群上,Egr2$GFP^{低}$细胞也显示为表达4-1BB和LAG-3(图2C)。该结果表明表达Egr2的$CD8^+$ TIL仅包含表达LAG-3和/或4-1BB的TIL的子集,或者Egr2被瞬时表达并且随后在诱导LAG-3和4-1BB后下调。

[0143] 使用来自体外无反应性$CD4^+$ T细胞克隆(Zheng等人,2013;该文献全文以引用方式并入)的Egr2靶基因,在分选的4-1BB^-LAG-3^-和4-1BB^+LAG-3^+细胞中通过qRT-PCR来检查Egr2驱动的转录程序。在所检查的43个Egr2靶基因中,10个在4-1BB^+LAG-3^+群体中显示出可检测的增加的表达,同时相似基因子集的表达在4-1BB^-LAG-3^+群体中增长(图2D)。总的来说,这些数据表明Egr2在表达LAG-3和/或4-1BB的$CD8^+$ TIL亚群中表达,并且在这些较大的T细胞群体中检测到了作为整体的已知Egr2靶的子集。

[0144] 接下来检查在体内$CD8^+$ TIL中表达LAG-3和4-1BB是否需要Egr2。为此目的,利用$Egr2^{flox/flox}$ X pLCK-CreERT2 x ROSA-YFP小鼠,其中口服他莫昔芬施用导致一部分$CD8^+$ T细胞缺失Egr2并表达YFP(图2E)。这允许比较同一肿瘤内的Egr2足量(YFP^-)和Egr2缺陷(YFP^+)$CD8^+$。为了确定Egr2事实上从YFP^+部分中缺失,分选出YFP^+和YFP^-CD8^+ TIL两者,并直接离体测量并且在离体刺激后测量Egr2转录物。与YFP^-对应物相比,YFP^+ $CD8^+$ TIL表达明显较少的Egr2转录物(图2E)。为了确定4-1BB和LAG-3表达是否需要Egr2,在肿瘤接种后第7天和第14天分析$CD8^+$ TIL,并比较YFP^+和YFP^-群体与没有用他莫昔芬治疗的小鼠。在第7天,YFP^+部分与YFP^-群体和WT $CD8^+$ TIL相比表达较少的4-1BB和LAG-3。然而,在第14天,4-1BB和LAG-3的表达没有显著差异(图2F)。这表明其他转录调节因子补偿并促成LAG-3和4-1BB的表达,尤其是在稍后的时间点。

[0145] Egr3已显示与Egr2具有重叠功能(Safford等人,2005;该文献全文以引用方式并入)并且HIF1α可有助于4-1BB表达(Palazón等人,2012)。为了研究这些转录因子是否可以补偿4-1BB和/或LAG-3表达,我们在第7天对表达4-1BB和LAG-3的Egr2$GFP^{高}$和Egr2$GFP^{低}CD8^+$ TIL进行分选,并通过qRT-PCR分析Egr3和HIF1α的表达。Egr3和HIF1α确实在Egr2$GFP^{高}$和Egr2$GFP^{低}$群体中都表达。确认Egr2和CCL1在Egr2$GFP^{高}$和Egr2$GFP^{低}$群体之间的差异表达以确保分选纯度(图2G)。总之,这些数据表明Egr2有助于在早期时间点上调4-1BB和LAG-3表达,但随着T细胞-肿瘤相互作用的进展,其他转录调节因子补偿并驱动LAG-3和4-1BB的表达。

[0146] $CD8^+$4-1BB^+LAG-3^+ TIL是寡克隆的并且富含肿瘤抗原特异性

[0147] 并非肿瘤微环境中的所有T细胞都对肿瘤相关抗原具有特异性,因为在TIL中经常发现对无关抗原特异的记忆T细胞,并且已经记录了体内非特异性T细胞运输(Harlin等人,2006;该文献全文以引用方式并入)。在本文的实施方案的开发期间进行实验以确定4-1BB^+ LAG-3^+ $CD8^+$ TIL是否为肿瘤抗原特异性的。TCR刺激后LAG-3、4-1BB和Egr2上调,并且实验表明该群体在肿瘤微环境中原位扩增。采用了三种互补技术。首先,基于LAG-3和4-1BB表达,通过细胞分选来分离$CD8^+$ TIL,并执行TCRβ谱型分析。相比于4-1BB^-LAG-3^-TIL和$CD8^+$脾细胞,4-1BB^+LAG-3^+ TIL具有非高斯分布并共享一个或两个主峰(图3A)。对显示一个主导峰

的几个Vβ的分析显示，Vβ7含有在4-1BB$^-$LAG-3$^+$与4-1BB$^+$LAG-3$^+$群体之间共享的单个CDR3β序列，表明克隆关系(图3A)。为了测量CDR3β库的寡克隆性，计算三只独立小鼠中在CD8$^+$ TIL亚群与脾CD8$^+$群体之间的各个Vβ的汉明距离(HD)(图8)。通过将每个谱型转换为曲线频率谱下的面积，汉明距离计算频率的变化并报告0和1之间的比较值，其中0表示完全相同的频率谱，1表示完全不一致的谱。作为对照，计算不同小鼠之间的脾CD8$^+$群体的HD(图3B，黑色条)。因为脾CD8$^+$谱型主要是高斯分布，所以该值代表两个相似分布之间的HD。对CD8$^+$ TIL亚群之间的HD的分析揭示，4-1BB$^+$LAG-3$^+$和4-1BB$^-$LAG-3$^+$，而不是4-1BB$^-$LAG-3$^-$CDR3β的分布与脾CD8$^+$群体显著不同(较低的高斯分布)(图3B)。这些数据表明4-1BB$^+$LAG-3$^+$和4-1BB$^-$LAG-3$^+$群体是TIL的寡克隆扩增子集，表明了这些亚群中的抗原特异性。

[0148] 作为第二种方法，利用B16.SIY黑素瘤和MC38.SIY腺癌模型。对特异于H-2K^{b}受限的SIY表位(SIYRYYGL)的CD8$^+$ T细胞进行监测。在肿瘤接种后第14天，发现SIYRYYGL/K^{b}五聚体$^+$(H-2K^b/SIY)细胞在B16.SIY和MC38.SIY肿瘤内为扩增的数目(图3C)。几乎47％的H-2K^b/SIY$^+$细胞表达4-1BB和LAG-3，相比之下32％的H-2K^b/SIY$^-$群体表达4-1BB和LAG-3(图3C和图3E)。抗原特异性CD8$^+$ TIL在4-1BB$^+$LAG-3$^+$群体中的这种富集指示这些标志物鉴别肿瘤抗原特异性TIL。H-2K^b/SIY$^-$细胞也含有大量的4-1BB$^+$LAG-3$^+$细胞，这与SIY以外的肿瘤抗原也被体内CD8$^+$ TIL亚群识别的观点一致(图3C)。脾或TdLN中的H-2K^b/SIY$^+$细胞不共表达4-1BB和LAG-3，表明该表型是在肿瘤微环境中获得的。

[0149] 还在肿瘤抗原特异性CD8$^+$ TIL的情形中在两个自发排斥的肿瘤模型中分析了这些特征。为此，评估来自MC57.SIY和1969.SIY肿瘤的H-2K^b/SIY特异性CD8$^+$ TIL细胞。在肿瘤接种后第14天，在4-1BB$^+$LAG-3$^+$部分中发现约5％的H-2K^b/SIY特异性CD8$^+$ TIL。与B16.SIY肿瘤一样，在TdLN或脾(未示出)中没有H-2K^b/SIY特异性CD8 T细胞共表达4-1BB和LAG-3(未示出)(图3D)。与B16.SIY和MC38.SIY肿瘤不同，没有观察到4-1BB$^+$LAG-3$^+$H-2K^b/SIY特异性CD8$^+$ TIL的明显富集(图3D和图3E)。这些数据表明肿瘤抗原特异性本身不能确定功能障碍，并且这是进展性肿瘤的微环境所特有的特征。

[0150] 作为确定肿瘤抗原特异性CD8$^+$ T细胞需要4-1BB$^+$LAG-3$^+$表型的第三种措施，将从2C/Rag2$^{-/-}$和P14/Rag2$^{-/-}$小鼠分离出的先天性标记的2C和P14转基因(Tg)T细胞转移到荷有肿瘤的宿主中。2C TCR特异于B16.SIY肿瘤细胞表达的SIY模型抗原，而P14是特异于LCMV来源的gp$_{33-41}$表位的无关TCR；两种TCR都是H-2K^{b}受限的。在肿瘤接种后7天通过尾静脉将2C和P14 Tg CD8$^+$ T细胞进行转移。转移后7天，提取肿瘤和TdLN，并分析转移群体的表型谱。该系统允许分析在相同肿瘤微环境中具有确定的抗原特异性的两个T细胞群体，以及多克隆宿主CD8$^+$ T细胞。与P14 T细胞相比，2C T细胞在肿瘤微环境中更有效地募集和扩增，并且包含较大部分的总CD8$^+$TIL群体(图3F)。在2C T细胞中，几乎所有细胞均表达LAG-3和/或4-1BB，而这仅适用于较少百分比的P14细胞(图3G和图3H)。与SIY-K^{b}五聚体分析一致，在TdLN中未观察到LAG-3和4-1BB在2C T细胞上的共表达。总之，这些结果证明了4-1BB$^+$LAG-3$^+$表型是肿瘤抗原特异性TIL在肿瘤进展条件下的特性。

[0151] 表达LAG-3和4-1BB的CD8$^+$ TIL表现出IL-2产生缺陷但产生IFN-γ和Treg-募集趋化因子

[0152] 基于体外T细胞无反应性模型导致鉴定Egr2作为重要调节因子的特征，在本文实施方案的开发过程中进行实验以确定肿瘤抗原特异性4-1BB$^+$LAG-3$^+$ CD8$^+$ TIL群体在其产

生IL-2的能力方面是否为功能障碍的。为此，将每个亚群分选，并用抗CD3和抗CD28 mAb刺激，并通过qRT-PCR和ELISA分析IL-2产量。因为几乎所有的$CD8^+$TIL显示激活的表型，所以将$CD8^+CD44^+$脾细胞用作阳性对照。的确，$4\text{-}1BB^+LAG\text{-}3^+$细胞显示为与$4\text{-}1BB^-LAG\text{-}3^-$群体相比降低100倍的Il-2mRNA，以及降低多至40倍的IL-2蛋白水平(图4A和图4B)。作为第二种方法，通过利用Egr2-GFP报告小鼠来检查$Egr2^{高}$TIL(其也大部分为$4\text{-}1BB^+LAG\text{-}3^+$)。确实，与$Egr2\text{-}GFP^{低}$细胞相比，离体刺激的$Egr2\text{-}GFP^{高}CD8^+$ TIL也表现出减少的Il-2转录物(图4C)。作为最终方法，将先天性标记的2C T细胞静脉内过继转移到荷瘤宿主中，并在7天后从肿瘤和TdLN中回收2C T细胞。与从TdLN分离的2C $CD44^+$ T细胞相比，从肿瘤中分离的2C T细胞表现出降低的产生Il-2转录物的能力，该能力的水平与$4\text{-}1BB^+LAG\text{-}3^+$ TIL的水平相当(图4D)。在慢性感染模型中，已经提出PD-1的表达以鉴定本质上功能障碍或“耗尽”的$CD8^+$ T细胞。为了确定单独的PD-1是否足以鉴定缺乏产生IL-2的能力的细胞，分离出缺乏LAG-3和4-1BB表达的$CD8^+$TIL，并测试$PD\text{-}1^+$部分产生IL-2的能力。在第14天和第21天，约10％的$CD8^+$ TIL为$4\text{-}1BB^-LAG\text{-}3^-PD\text{-}1^+$(图4E和图4F)。在离体刺激后，该群体保留了以与$4\text{-}1BB^-LAG\text{-}3^-$细胞相当的水平产生Il-2mRNA的能力(图4G)。这些结果表明单独的PD-1表达不足以鉴定肿瘤微环境中功能障碍的TIL。

[0153] 为了进一步检查肿瘤进展期间的功能改变，在TCR刺激后测试IL-2、IFN-γ和TNF-α的蛋白质水平。由于$CD8^+$ TIL丧失产生细胞因子的能力被认为是被报道为在进入肿瘤微环境后起始的时域过程(Waugh等人，2016；Schietinger等人，2016；这些文献全文以引用方式并入本文)或者在慢性LCMV模型中30天后逐渐发生的(Wherry等人，2007；该文献全文以引用方式并入本文)，所以在第7天、第14天、第21天和第28天测试细胞因子产生。$4\text{-}1BB^+LAG\text{-}3^+$群体早在第7天就丧失了产生IL-2的能力，而$4\text{-}1BB^-LAG\text{-}3^+$群体在在第7天与第14天之间丧失IL-2产生(图5A)。$4\text{-}1BB^-LAG\text{-}3^-$群体在任何测试的时间点都没有丧失产生IL-2的能力(图5A)，支持该群体不具有肿瘤抗原特异性并且分化为功能障碍状态是抗原依赖性过程的观点(Schietinger等人，2016；该文献全文以引用方式并入本文)。$4\text{-}1BB^+LAG\text{-}3^+$群体与其阴性对应物相比，在第7天后的所有时间点产生更多的IFN-γ，尽管随着时间推移IFN-γ产量略微减少。虽然IFN-γ的增加维持到稍后的时间点，但到第28天TNF-α的产生丧失(图5A)。

[0154] 在本文的实施方案的开发过程中进行实验以在不进行体外重新刺激的情况下直接评估肿瘤中细胞因子的产生，这可以更接近地反映哪些T细胞正在接受原位TCR刺激。每个T细胞群体在不进行任何培养的情况下直接离体分选，并通过qRT-PCR测量mRNA水平。与$4\text{-}1BB^-LAG\text{-}3^-$细胞相比，从$4\text{-}1BB^+LAG\text{-}3^+$亚群中观察到了Ifn-γ和Gzmb转录物的增多，以及略有下降的Tnf-α水平(图5B)。在通过细胞内细胞因子染色分析之前，通过用布雷菲德菌素A注射肿瘤来确认原代TIL中IFN-γ的产生。与mRNA表达一致，$4\text{-}1BB^+LAG\text{-}3^+$群体产生显著更大量的IFN-γ蛋白质(图5C)。因此，$4\text{-}1BB^+LAG\text{-}3^+$ TIL并非完全缺乏功能，因为尽管缺乏IL-2的产生，它们仍继续产生IFN-γ。该表型与体外T细胞无反应性模型(Jenkins等人，1987；该文献全文以引用方式并入)一致。

[0155] 为了测试$4\text{-}1BB^+LAG\text{-}3^+$是群体是否仍然保留细胞毒性能力，通过在分选后直接将抗CD3结合的P815肥大细胞瘤靶细胞与不同的$CD8^+$ TIL亚群共培养来执行重定向裂解。从第14天肿瘤分离的$4\text{-}1BB^+LAG\text{-}3^+$ $CD8^+$ TIL能够以与体外致敏的OT-I细胞相当的功效来裂解靶细胞。从第21天肿瘤分离的$4\text{-}1BB^+LAG\text{-}3^+$ TIL仍然能够裂解靶细胞，尽管与致敏的OT-I细胞相比程度较小(图5D)。

[0156] 肿瘤中的$CD8^+$ T细胞可以是将$FoxP3^+$调节性T细胞(Treg)募集到肿瘤微环境中的趋化因子CCL22的来源(Spranger等人,2013;该文献全文以引用方式并入)。此外,趋化因子Ccl1是无反应性的T细胞中的Egr2靶标(Zheng等人,2013;该文献全文以引用方式并入),并且已经认为CCL1也有助于体内肿瘤环境中的Treg募集(Hoelzinger等人,2010;该文献全文以引用方式并入)。然而,是否肿瘤中的所有$CD8^+$ T细胞都产生这些趋化因子,或者所述趋化因子是否仅由T细胞亚群产生尚未确定。为了解决这个问题,通过qRT-PCR直接离体分析$CD8^+$ TIL表型亚群的Ccl1和Ccl22 mRNA表达。的确,$4\text{-}1BB^+LAG\text{-}3^+$ TIL群体与其阴性对应物或脾$CD8^+CD44^+$ T细胞相比产生显著更多的Ccl1和Ccl22(图4K)。作为对照,发现不同趋化因子Ccl5的表达没有差异表达。

[0157] 总之,这些数据显示4-1BB和LAG-3的共表达描绘了肿瘤抗原特异性$CD8^+$ TIL缺乏产生IL-2的能力,但仍保留产生IFN-γ、体外杀伤靶细胞和分泌能够进行Treg募集的趋化因子的能力。鉴于IFN-γ负责肿瘤微环境中PD-L1和IDO的上调,并且由$CD8^+$ TIL产生的趋化因子有助于Treg募集的事实(Spranger等人,2013;该文献全文以引用方式并入),这些数据表明$4\text{-}1BB^+LAG\text{-}3^+$群体有助于肿瘤微环境中限制抗肿瘤免疫功效的免疫抑制机制的网络。

[0158] 基因表达谱分析显示$CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TIL表达广泛的额外共刺激和共抑制受体

[0159] 在具有定义肿瘤抗原特异性功能障碍的$CD8^+$ TIL的表面标记物的情况下,在本文的实施方案的开发期间进行实验以比较该群体的基因表达谱与功能障碍的$CD8^+$ T细胞的其他公开的表达谱,以确定在这种功能障碍状态下的细胞中调节或差异表达的基因。为此目的,对来自利用鼠CT26肿瘤模型(Waugh等人,2016;该文献全文以引用方式并入)和LCMV“耗竭的”GP33特异性$CD8^+$ T细胞(Doering等人,2012;该文献全文以引用方式并入)的研究的“功能障碍的”$4\text{-}1BB^+LAG\text{-}3^+$ $CD8^+$ TIL、“功能减退的”$CD8^+$ TIL的转录谱进行交叉研究比较。结果描绘于表2中。仅考虑每个研究中相对于对照独立地增加2倍的基因。发现与耗尽的T细胞谱相比,在功能障碍的TIL数据集与之前公开的功能减退的$CD8^+$ TIL数据之间共享超过2倍数目的基因(图6A)。此外,秩-秩超几何重叠(RRHO)分析表明,与病毒诱导耗竭的$CD8^+$ T细胞的基因表达谱相比,当前功能障碍的TIL的基因表达谱与公布的功能减退的$CD8^+$ TIL的基因表达谱之间具有更大的统计学上显著的重叠(图10A)和更大的相关性(图10B),表明相较于慢性病毒感染,从肿瘤中分离的$CD8^+$ T细胞之间存在更相似的分子程序。

[0160] 表2.$CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TIL中差异调节的基因

[0161]

基因	基因描述	Log2-倍数变化	基因符号	基因描述	Log2-倍数变化
GLDC	glycine decarboxylase	11.25109772	CRY2	cryptochrome circadian clock 2	-1.546648257
GZMD	Granzyme D	10.66720027	KCMF1	potassium channel modulatory factor 1	-1.546835341
SLC17A6	solute carrier family 17 member 6	8.946467699	RHOB	ras homolog family member B	-1.548813112
IL1R2	interleukin 1 receptor type 2	7.595353131	KRT15	keratin 15	-1.549018071
LTF	lactotransferrin	7.530211233	RRAD	RRAD, Ras related glycolysis inhibitor and calcium channel regulator	-1.549530357
NRGN	neurogranin	7.334049768	C3	complement component 3	-1.54996

[0162]

						0037
GZME	granzyme E	7.16037 5687		ITFG3	Description Not Found	-1.55016 2812
RPL6	ribosomal protein L6	7.14210 7057		HAAO	3-hydroxyanthranilate 3,4-dioxygenase	-1.55055 3207
NRN1	neuritin 1	7.08799 3146		RNF138	ring finger protein 138	-1.55144 9524
LPL	lipoprotein lipase	7.00450 1392		UNC93B1	unc-93 homolog B1 (C. elegans)	-1.55176 7491
CLGN	calmegin	6.93369 0655		ANKZF1	ankyrin repeat and zinc finger domain containing 1	-1.55221 4097
CD70	CD70 molecule	6.90689 0596		IFITM3	interferon induced transmembrane protein 3	-1.55264 4542
AREG	amphiregulin	6.71287 0868		TXNIP	thioredoxin interacting protein	-1.55278 5452
ZRANB3	zinc finger RANBP2-type containing 3	6.59544 3985		LMAN1L	lectin, mannose binding 1 like	-1.55458 8852
ASNS	asparagine synthetase (glutamine-hydrolyzing)	6.59496 878		ALDH3B1	aldehyde dehydrogenase 3 family member B1	-1.55471 1558
FANCD2	Fanconi anemia complementation group D2	6.35314 6826		GIP	gastric inhibitory polypeptide	-1.55551 1104
GM156	predicted gene 156(Gm156)	6.29370 1542		COX7A2L	cytochrome c oxidase subunit 7A2 like	-1.55557 2553
ACAA1B	acetyl-Coenzyme A acyltransferase 1B(Acaa1b)	6.29370 1542		APPL2	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2	-1.55559 8704
IGF2BP3	insulin like growth factor 2 mRNA binding protein 3	6.18685 7067		KLHL22	kelch like family member 22	-1.55592 9583
GZMG	granzyme G	6.09381 3673		OLFR272	olfactory receptor 272(Olfr272)	-1.55748 2156
CIB2	calcium and integrin binding family member 2	6.00786 8243		LRRC29	leucine rich repeat containing 29	-1.55936 6716
ATG9B	autophagy related 9B	5.98641 0935		A630095E1 3RIK	Description Not Found	-1.56071 4954
XKR8	XK related 8	5.97727 9924		OLFR194	olfactory receptor 194(Olfr194)	-1.56071 4954
EPDR1	ependymin related 1	5.95652 1363		OLFR1013	olfactory receptor 1013(Olfr1013)	-1.56071 4954
SPP1	secreted phosphoprotein 1	5.79776 9502		GLRA4	glycine receptor alpha 4	-1.56071

[0163]

					4954
RGS8	regulator of G-protein signaling 8	5.75380 5672	P2RY6	pyrimidinergic receptor P2Y6	-1.56071 4954
MDFIC	MyoD family inhibitor domain containing	5.73063 9956	RASGEF1B	RasGEF domain family member 1B	-1.56071 4954
DMWD	dystrophia myotonica, WD repeat containing	5.68720 0695	IL22RA2	interleukin 22 receptor subunit alpha 2	-1.56071 4954
KIF11	kinesin family member 11	5.66959 3751	LIN7C	lin-7 homolog C, crumbs cell polarity complex component	-1.56071 4954
LGI2	leucine rich repeat LGI family member 2	5.65535 1829	DMRT1	doublesex and mab-3 related transcription factor 1	-1.56071 4954
ZFP41	ZFP41 zinc finger protein	5.61544 5725	TSPAN12	tetraspanin 12	-1.56071 4954
MLKL	mixed lineage kinase domain-like	5.60584 9867	PAK3	p21 (RAC1) activated kinase 3	-1.56071 4954
CENPH	centromere protein H	5.56376 8278	COL2A1	collagen type II alpha 1 chain	-1.56071 4954
SERPINF1	serpin family F member 1	5.53605 29	SLC37A1	solute carrier family 37 member 1	-1.56071 4954
UNC13B	unc-13 homolog B (C. elegans)	5.50303 0646	PSD3	pleckstrin and Sec7 domain containing 3	-1.56071 4954
MLANA	melan-A	5.49665 4083	RDH5	retinol dehydrogenase 5	-1.56071 4954
PES1	pescadillo ribosomal biogenesis factor 1	5.48437 6709	ABCA3	ATP binding cassette subfamily A member 3	-1.56126 3453
2900026A02RIK	Description Not Found	5.47735 3527	PLA2G4E	phospholipase A2 group IVE	-1.56165 0879
OSR2	odd-skipped related transciption factor 2	5.41616 4165	DDIT3	DNA damage inducible transcript 3	-1.56356 6526
MPP6	membrane palmitoylated protein 6	5.40850 6442	ZFP12	zinc finger protein 12(Zfp12)	-1.56430 8646
HIST1H3C	histone cluster 1, H3c	5.39746 0726	PIGYL	phosphatidylinositol glycan anchor biosynthesis, class Y-like(Pigyl)	-1.56458 5219
PI4K2B	phosphatidylinositol 4-kinase type 2 beta	5.37503 9431	CCDC97	coiled-coil domain containing 97	-1.56535 5117
SH3YL1	SH3 and SYLF domain containing 1	5.37503 9431	OLFR1112	olfactory receptor 1112(Olfr1112)	-1.56589 319
RAD51	RAD51 recombinase	5.37155 8863	ACTN2	actinin alpha 2	-1.56693 1646

[0164]

ZBTB32	zinc finger and BTB domain containing 32	5.31831 6841		POLG	polymerase (DNA) gamma, catalytic subunit	-1.56726 5595
MSC	musculin	5.28540 2219		FBXO32	F-box protein 32	-1.56728 1905
TG	thyroglobulin	5.25927 2487		MRPL15	mitochondrial ribosomal protein L15	-1.57072 2678
RSPH1	radial spoke head 1 homolog	5.23649 2618		FCHSD2	FCH and double SH3 domains 2	-1.57182 1211
ARL11	ADP ribosylation factor like GTPase 11	5.21916 852		RECQL	RecQ like helicase	-1.57288 9668
NUDT11	nudix hydrolase 11	5.21529 0306		NDUFB11	NADH:ubiquinone oxidoreductase subunit B11	-1.57288 9668
APBB1	amyloid beta precursor protein binding family B member 1	5.19770 8158		SOX8	SRY-box 8	-1.57334 1535
SPINK2	serine peptidase inhibitor, Kazal type 2	5.18982 4559		1700030J22 RIK	Description Not Found	-1.57662 394
HMGN3	high mobility group nucleosomal binding domain 3	5.16892 2782		EMB	embigin	-1.57789 0585
FAM20B	family with sequence similarity 20 member B	5.12722 055		CELSR1	cadherin EGF LAG seven-pass G-type receptor 1	-1.57820 1987
CDC25C	cell division cycle 25C	5.11997 861		COL1A2	collagen type I alpha 2 chain	-1.58068 2782
FAM20A	family with sequence similarity 20 member A	5.10852 4457		1700080E1 1RIK	Description Not Found	-1.58104 6002
PPP1R16B	protein phosphatase 1 regulatory subunit 16B	5.09592 442		GALNT12	polypeptide N-acetylgalactosaminyltransferase 12	-1.58136 3645
SBNO1	strawberry notch homolog 1 (Drosophila)	5.05093 6965		RMND5B	required for meiotic nuclear division 5 homolog B	-1.58396 0816
ST14	suppression of tumorigenicity 14	5.02680 0059		LRRC28	leucine rich repeat containing 28	-1.58398 7499
LRRC49	leucine rich repeat containing 49	5.02470 4311		OLFR622	olfactory receptor 622(Olfr622)	-1.58496 2501
TIAM1	T-cell lymphoma invasion and metastasis 1	5.00450 1392		OLFR339	olfactory receptor 339(Olfr339)	-1.58496 2501
APLF	aprataxin and PNKP like factor	4.95186 7504		NEIL3	nei like DNA glycosylase 3	-1.58496 2501
PGPEP1	pyroglutamyl-peptidase I	4.92718 5358		SNX24	sorting nexin 24	-1.58496 2501
ALCAM	activated leukocyte cell adhesion molecule	4.90929 3086		SLC7A11	solute carrier family 7 member 11	-1.58496

[0165]

					2501
B9D1	B9 domain containing 1	4.90689 0596	FOXJ1	forkhead box J1	-1.58496 2501
SCIN	scinderin	4.87282 876	TAF3	TATA-box binding protein associated factor 3	-1.58496 2501
EXOC3L	exocyst complex component 3-like(Exoc3l)	4.84401 3973	MATN2	matrilin 2	-1.58496 2501
SLC35D3	solute carrier family 35 member D3	4.84046 3234	ADHFE1	alcohol dehydrogenase, iron containing 1	-1.58628 0668
ALDOC	aldolase, fructose-bisphosphate C	4.83289 0014	NANOS1	nanos C2HC-type zinc finger 1	-1.58691 4831
TMEM205	transmembrane protein 205	4.83018 2468	PPP2R5B	protein phosphatase 2 regulatory subunit B'beta	-1.58691 4831
PLEKHA8	pleckstrin homology domain containing A8	4.82017 8962	USP22	ubiquitin specific peptidase 22	-1.58870 3598
SPC25	SPC25, NDC80 kinetochore complex component	4.81762 3258	DAGLB	diacylglycerol lipase beta	-1.58881 7933
PCYT1B	phosphate cytidylyltransferase 1, choline, beta	4.74953 4268	KCTD6	potassium channel tetramerization domain containing 6	-1.58969 0033
SLC6A8	solute carrier family 6 member 8	4.74953 4268	ACTL6B	actin like 6B	-1.59135 1555
TUBB6	tubulin beta 6 class V	4.74924 1128	FAM129B	family with sequence similarity 129 member B	-1.59150 39
BSPRY	B-box and SPRY domain containing	4.71149 4907	APOE	apolipoprotein E	-1.59168 3393
ICA1	islet cell autoantigen 1	4.70873 9041	GPR18	G protein-coupled receptor 18	-1.59238 4168
TNFSF13B	tumor necrosis factor superfamily member 13b	4.70321 1467	GSTP2	glutathione S-transferase, pi 2(Gstp2)	-1.59255 9885
GSTCD	glutathione S-transferase C-terminal domain containing	4.70043 9718	GPR114	Description Not Found	-1.59382 9527
CCNB1	cyclin B1	4.69905 1844	CHUK	conserved helix-loop-helix ubiquitous kinase	-1.59482 3937
4930539E08RIK	Description Not Found	4.69321 1287	TAS1R3	taste 1 receptor member 3	-1.59659 5048
SRXN1	sulfiredoxin 1	4.66106 548	SLC7A7	solute carrier family 7 member 7	-1.59693 5142
SERF1	small EDRK-rich factor 1(Serf1)	4.63226 8216	SPIB	Spi-B transcription factor	-1.59767 7703
CCDC77	coiled-coil domain	4.62935	POLR3A	polymerase (RNA) III	-

	containing 77	662			subunit A	1.59958 8488
RHBDF1	rhomboid 5 homolog 1	4.62643 9137		OLFR952	olfactory receptor 952(Olfr952)	- 1.59967 9175
REEP3	receptor accessory protein 3	4.59991 2842		1700021F05 RIK	Description Not Found	- 1.60162 3253
ITGA3	integrin subunit alpha 3	4.59096 1241		CCDC79	Description Not Found	- 1.60219 5565
SCCPDH	saccharopine dehydrogenase (putative)	4.59096 1241		FAM134B	family with sequence similarity 134 member B	- 1.60271 5966
MYADM	myeloid associated differentiation marker	4.58796 4989		SEMA3B	semaphorin 3B	- 1.60288 4409
FAM132A	family with sequence similarity 132 member A	4.58195 3751		FA2H	fatty acid 2-hydroxylase	- 1.60449 4406
FOXRED2	FAD dependent oxidoreductase domain containing 2	4.57288 9668		ULK1	unc-51 like autophagy activating kinase 1	- 1.60465 3903
CENPK	centromere protein K	4.56985 5608		MCOLN1	mucolipin 1	- 1.60624 2992
DCXR	dicarbonyl and L-xylulose reductase	4.56224 2424		BMP5	bone morphogenetic protein 5	- 1.60676 0033
TSPAN6	tetraspanin 6	4.54225 805		ANKRD50	ankyrin repeat domain 50	- 1.60713 7028
UPP1	uridine phosphorylase 1	4.53838 296		OLFR560	olfactory receptor 560(Olfr560)	- 1.60880 9243
DOK4	docking protein 4	4.52042 2249		OLFR366	olfactory receptor 366(Olfr366)	- 1.60880 9243
ELOVL4	ELOVL fatty acid elongase 4	4.50143 9145		OLFR273	olfactory receptor 273(Olfr273)	- 1.60880 9243
KNDC1	kinase non-catalytic C-lobe domain containing 1	4.49979 0117		FHIT	fragile histidine triad	- 1.60880 9243
KRT17	keratin 17	4.49185 3096		AQP11	aquaporin 11	- 1.60880 9243
CHST2	carbohydrate sulfotransferase 2	4.48731 5031		TMEM176 A	transmembrane protein 176A	- 1.60880 9243
TPX2	TPX2, microtubule nucleation factor	4.47573 3431		ENAH	enabled homolog (Drosophila)	- 1.60880 9243
DUSP14	dual specificity phosphatase 14	4.45614 9035		CLDN6	claudin 6	- 1.60880 9243
BGN	biglycan	4.44956 1375		SP1	Sp1 transcription factor	- 1.60880 9243

[0166]

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FKBP9	FK506 binding protein 9	4.44294 3496		SP140	SP140 nuclear body protein	-1.60880 9243
CAPN5	calpain 5	4.38543 1037		RASGRP3	RAS guanyl releasing protein 3	-1.60880 9243
SLC1A4	solute carrier family 1 member 4	4.37503 9431		HIF3A	hypoxia inducible factor 3 alpha subunit	-1.60942 2664
IDI2	isopentenyl-diphosphate delta isomerase 2	4.35755 2005		FYCO1	FYVE and coiled-coil domain containing 1	-1.61122 0598
AKR1E1	aldo-keto reductase family 1, member E1(Akr1e1)	4.34659 6388		FBXL12	F-box and leucine rich repeat protein 12	-1.61193 68
GNB4	G protein subunit beta 4	4.33608 8936		KLRA10	killer cell lectin-like receptor subfamily A, member 10(Klra10)	-1.61848 4777
CPNE2	copine 2	4.31864 0898		ABAT	4-aminobutyrate aminotransferase	-1.62058 641
FAM132B	family with sequence similarity 132, member B(Fam132b)	4.25927 2487		AMHR2	anti-Mullerian hormone receptor type 2	-1.62058 641
SLC6A12	solute carrier family 6 member 12	4.25927 2487		DDX3Y	DEAD-box helicase 3, Y-linked	-1.62064 9859
CPLX1	complexin 1	4.24031 4329		LGALS4	galectin 4	-1.62155 0215
PDCD1	programmed cell death 1	4.22110 3725		SPG20	spastic paraplegia 20 (Troyer syndrome)	-1.62165 3602
UTF1	undifferentiated embryonic cell transcription factor 1	4.20163 3861		CTRL	chymotrypsin like	-1.62729 369
WDR60	WD repeat domain 60	4.14974 712		GREM2	gremlin 2, DAN family BMP antagonist	-1.62792 7342
EGFL7	EGF like domain multiple 7	4.13750 3524		ZMAT3	zinc finger matrin-type 3	-1.62836 2075
ASPM	abnormal spindle microtubule assembly	4.13339 9125		AP4M1	adaptor related protein complex 4 mu 1 subunit	-1.62889 8157
TMBIM1	transmembrane BAX inhibitor motif containing 1	4.10462 8811		NT5C2	5'-nucleotidase, cytosolic II	-1.63059 747
KNTC1	kinetochore associated 1	4.09395 2772		TMIE	transmembrane inner ear	-1.63160 6148
1700019D0 3RIK	Description Not Found	4.08746 2841		OLFR556	olfactory receptor 556(Olfr556)	-1.63226 8216
TM4SF5	transmembrane 4 L six family member 5	4.08746 2841		OLFR463	olfactory receptor 463(Olfr463)	-1.63226 8216
BIRC5	baculoviral IAP repeat containing 5	4.02790 5997		CTS3	cathepsin 3(Cts3)	-1.63226

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						8216
SYNGR3	synaptogyrin 3	4.02236 7813		OAS1B	2'-5' oligoadenylate synthetase 1B(Oas1b)	-1.63226 8216
PLSCR4	phospholipid scramblase 4	4		KCNF1	potassium voltage-gated channel modifier subfamily F member 1	-1.63226 8216
KIF15	kinesin family member 15	3.96237 6898		GCGR	glucagon receptor	-1.63226 8216
TICAM2	toll like receptor adaptor molecule 2	3.95884 2675		NR1I3	nuclear receptor subfamily 1 group I member 3	-1.63226 8216
CENPM	centromere protein M	3.95768 2486		FSTL1	follistatin like 1	-1.63226 8216
KIF4	kinesin family member 4(Kif4)	3.95609 7191		ASAP3	ArfGAP with SH3 domain, ankyrin repeat and PH domain 3	-1.63226 8216
E2F2	E2F transcription factor 2	3.93191 939		IHH	indian hedgehog	-1.63226 8216
MSN	moesin	3.93073 7338		SEMA3A	semaphorin 3A	-1.63226 8216
PTPRA	protein tyrosine phosphatase, receptor type A	3.92898 9949		RAMP1	receptor activity modifying protein 1	-1.63257 5446
BC026585	cDNA sequence BC026585(BC026585)	3.88264 3049		NFKBID	NFKB inhibitor delta	-1.63315 8642
IQGAP3	IQ motif containing GTPase activating protein 3	3.86789 6464		KLK15	kallikrein related peptidase 15	-1.63377 3522
CD244	CD244 molecule	3.86789 6464		CYP1B1	cytochrome P450 family 1 subfamily B member 1	-1.63468 4534
HIST1H3G	histone cluster 1, H3g	3.83794 3242		DNAJA1	DnaJ heat shock protein family (Hsp40) member A1	-1.63511 1002
SLC15A3	solute carrier family 15 member 3	3.83289 0014		SDSL	serine dehydratase like	-1.63580 7742
GIPC2	GIPC PDZ domain containing family member 2	3.81762 3258		CCDC137	coiled-coil domain containing 137	-1.63683 8653
UTP15	UTP15, small subunit processome component	3.81249 8225		ZSWIM4	zinc finger SWIM-type containing 4	-1.63815 2805
PDIA6	protein disulfide isomerase family A member 6	3.81249 8225		BBC3	BCL2 binding component 3	-1.63833 6813
JDP2	Jun dimerization protein 2	3.80735 4922		SOCS3	suppressor of cytokine signaling 3	-1.63887 6738
MESDC1	mesoderm development candidate 1	3.80672 3946		2900092C05RIK	Description Not Found	-1.63915 7339
GAS2	growth arrest specific 2	3.80219		CSRNP2	cysteine and serine rich	-

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		3217			nuclear protein 2	1.63938 3642
IL4I1	interleukin 4 induced 1	3.80219 3217		BLOC1S3	biogenesis of lysosomal organelles complex 1 subunit 3	-1.63958 5785
PHF19	PHD finger protein 19	3.80219 3217		ELL	elongation factor for RNA polymerase II	-1.64021 945
CKAP2L	cytoskeleton associated protein 2 like	3.79701 2978		GTF3C4	general transcription factor IIIC subunit 4	-1.64065 8029
GSTT1	glutathione S-transferase theta 1	3.79181 4071		MYLPF	myosin light chain, phosphorylatable, fast skeletal muscle	-1.64066 0074
ADAM3	a disintegrin and metallopeptidase domain 3 (cyritestin)(Adam3)	3.78135 9714		CYP2A12	cytochrome P450, family 2, subfamily a, polypeptide 12(Cyp2a12)	-1.64194 7141
SLAMF7	SLAM family member 7	3.78135 9714		RNF139	ring finger protein 139	-1.64201 0395
MCPT8	mast cell protease 8(Mcpt8)	3.77082 9046		C78339	Description Not Found	-1.64357 3868
DGKG	diacylglycerol kinase gamma	3.76553 4746		EDEM1	ER degradation enhancing alpha-mannosidase like protein 1	-1.64385 619
NLGN2	neuroligin 2	3.71699 0894		UBE2E1	ubiquitin conjugating enzyme E2 E1	-1.64585 9791
SERPINE2	serpin family E member 2	3.69488 0193		PALMD	palmdelphin	-1.64632 2067
IL10	interleukin 10	3.68929 9161		AMICA1	adhesion molecule, interacts with CXADR antigen 1(Amica1)	-1.64747 8619
SLC6A13	solute carrier family 6 member 13	3.68929 9161		KLHL11	kelch like family member 11	-1.65061 1828
STAU2	staufen double-stranded RNA binding protein 2	3.66675 6592		IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	-1.65105 0175
ARHGDIG	Rho GDP dissociation inhibitor gamma	3.65535 1829		DECR1	2,4-dienoyl-CoA reductase 1, mitochondrial	-1.65140 6438
TK1	thymidine kinase 1	3.63747 7097		SAMD3	sterile alpha motif domain containing 3	-1.65321 3853
PCYT1A	phosphate cytidylyltransferase 1, choline, alpha	3.61772 8231		9130409I23 RIK	Description Not Found	-1.65535 1829
LAMB3	laminin subunit beta 3	3.60880 9243		2010107G1 2RIK	Description Not Found	-1.65535 1829
UBE2N	ubiquitin conjugating enzyme E2 N	3.59096 1241		ZFP354B	zinc finger protein 354B(Zfp354b)	-1.65535 1829
STARD8	StAR related lipid transfer domain containing 8	3.57893 8713		TAS2R143	taste receptor, type 2, member 143(Tas2r143)	-1.65535 1829

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PRR5	proline rich 5	3.57893 8713		OLFR65	olfactory receptor 65(Olfr65)	-1.65535 1829
BDH2	3-hydroxybutyrate dehydrogenase, type 2	3.55458 8852		NRP	neural regeneration protein(Nrp)	-1.65535 1829
FAM124B	family with sequence similarity 124 member B	3.54843 6625		DOK3	docking protein 3	-1.65535 1829
MGAT3	mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	3.54843 6625		HIGD1A	HIG1 hypoxia inducible domain family member 1A	-1.65535 1829
LAG3	lymphocyte activating 3	3.54234 6309		CCDC13	coiled-coil domain containing 13	-1.65535 1829
GDPD5	glycerophosphodiester phosphodiesterase domain containing 5	3.53881 2733		ANGPTL2	angiopoietin like 2	-1.65535 1829
RNF168	ring finger protein 168	3.53605 29		CNGB3	cyclic nucleotide gated channel beta 3	-1.65535 1829
LYPLA1	lysophospholipase I	3.52982 0947		HOXD4	homeobox D4	-1.65535 1829
TUBGCP4	tubulin gamma complex associated protein 4	3.52356 1956		KIFC3	kinesin family member C3	-1.65535 1829
PYGL	phosphorylase, glycogen, liver	3.51412 226		AMACR	alpha-methylacyl-CoA racemase	-1.65535 1829
CCL3	C-C motif chemokine ligand 3	3.51028 1539		2310014L17RIK	Description Not Found	-1.65570 7015
BCAT1	branched chain amino acid transaminase 1	3.50816 3667		BRAP	BRCA1 associated protein	-1.65709 0723
ATP6V0A1	ATPase H+ transporting V0 subunit a1	3.50143 9145		SLC39A1	solute carrier family 39 member 1	-1.65763 1089
EIF4E	eukaryotic translation initiation factor 4E	3.49825 0868		OLFR419	olfactory receptor 419(Olfr419)	-1.65813 796
HIST1H4B	histone cluster 1, H4b	3.49185 3096		NHP2L1	NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae)(Nhp2l1)	-1.65829 8045
LAD1	ladinin 1	3.49085 426		STOML2	stomatin like 2	-1.65935 7735
ITGAV	integrin subunit alpha V	3.48542 6827		SAMM50	SAMM50 sorting and assembly machinery component	-1.66240 0762
MRPL47	mitochondrial ribosomal protein L47	3.48542 6827		CCDC91	coiled-coil domain containing 91	-1.66322 99
CAMK2N1	calcium/calmodulin dependent protein kinase II inhibitor 1	3.48446 0783		ATF3	activating transcription factor 3	-1.66348 3642
UEVLD	UEV and lactate/malate	3.46597		RAI1	retinoic acid induced 1	-

	dehyrogenase domains	4465				1.66388 5989
SFXN4	sideroflexin 4	3.46270 6751		RRAS2	related RAS viral (r-ras) oncogene homolog 2	-1.66582 6896
2810417H13RIK	Description Not Found	3.46163 4298		UROS	uroporphyrinogen III synthase	-1.66592 3156
RAD51AP1	RAD51 associated protein 1	3.45943 1619		SCOC	short coiled-coil protein	-1.66627 2349
FUT4	fucosyltransferase 4	3.45285 8965		DUSP10	dual specificity phosphatase 10	-1.66648 5948
CTNNBIP1	catenin beta interacting protein 1	3.44625 623		CYB5R4	cytochrome b5 reductase 4	-1.66675 6592
ZBTB8OS	zinc finger and BTB domain containing 8 opposite strand	3.42626 4755		9930104L06RIK	Description Not Found	-1.66715 0978
LYSMD4	LysM domain containing 4	3.42259 008		ZFP579	zinc finger protein 579(Zfp579)	-1.66902 3741
DIAP3	Description Not Found	3.40599 236		RGP1	RGP1 homolog, RAB6A GEF complex partner 1	-1.66939 3721
PTGIS	prostaglandin I2 (prostacyclin) synthase	3.39917 1094		PIAS2	protein inhibitor of activated STAT 2	-1.67213 7196
MOAP1	modulator of apoptosis 1	3.39231 7423		METTL1	methyltransferase like 1	-1.67242 5342
SLC27A3	solute carrier family 27 member 3	3.39231 7423		POU5F1	POU class 5 homeobox 1	-1.67385 4965
MRPL39	mitochondrial ribosomal protein L39	3.37149 2175		SERPINB6C	serine (or cysteine) peptidase inhibitor, clade B, member 6c(Serpinb6c)	-1.67393 2658
WTAP	Wilms tumor 1 associated protein	3.36457 2432		STXBP4	syntaxin binding protein 4	-1.67555 2278
RAD54L	RAD54-like (S. cerevisiae)	3.35658 9854		RIMS3	regulating synaptic membrane exocytosis 3	-1.67612 0648
CETN4	centrin 4(Cetn4)	3.33628 3388		XYLT2	xylosyltransferase 2	-1.67697 6793
CEP55	centrosomal protein 55	3.32912 3596		TAS2R107	taste receptor, type 2, member 107(Tas2r107)	-1.67807 1905
CYP4F39	cytochrome P450, family 4, subfamily f, polypeptide 39(Cyp4f39)	3.32192 8095		SKP1A	S-phase kinase-associated protein 1A(Skp1a)	-1.67807 1905
PTPN5	protein tyrosine phosphatase, non-receptor type 5	3.31469 6526		OLFR165	olfactory receptor 165(Olfr165)	-1.67807 1905
TUBE1	tubulin epsilon 1	3.29278 1749		OLFR111	olfactory receptor 111(Olfr111)	-1.67807 1905

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TCAM1	testicular cell adhesion molecule 1(Tcam1)	3.28540 2219		CYP4A12A	cytochrome P450, family 4, subfamily a, polypeptide 12a(Cyp4a12a)	-1.67807 1905
MID1IP1	MID1 interacting protein 1	3.26303 4406		TLR6	toll like receptor 6	-1.67807 1905
ABHD6	abhydrolase domain containing 6	3.26068 2276		KCNS3	potassium voltage-gated channel modifier subfamily S member 3	-1.67807 1905
ZCCHC4	zinc finger CCHC-type containing 4	3.25550 0733		FARSA	phenylalanyl-tRNA synthetase alpha subunit	-1.67807 1905
MGST3	microsomal glutathione S-transferase 3	3.25353 624		SLC2A4	solute carrier family 2 member 4	-1.67807 1905
BC022687	cDNA sequence BC022687(BC022687)	3.24792 7513		GDPD4	glycerophosphodiester phosphodiesterase domain containing 4	-1.67807 1905
ACSF3	acyl-CoA synthetase family member 3	3.24325 855		RCAN1	regulator of calcineurin 1	-1.67807 1905
ADAM8	ADAM metallopeptidase domain 8	3.24031 4329		CCDC82	coiled-coil domain containing 82	-1.67807 1905
SGCB	sarcoglycan beta	3.23703 4772		CDYL2	chromodomain protein, Y-like 2	-1.67807 1905
SOCS2	suppressor of cytokine signaling 2	3.23266 0757		MBD5	methyl-CpG binding domain protein 5	-1.67807 1905
HIST1H2AG	histone cluster 1, H2ag	3.22300 0387		ACSL1	acyl-CoA synthetase long-chain family member 1	-1.67807 1905
CRMP1	collapsin response mediator protein 1	3.20163 3861		OTUB2	OTU deubiquitinase, ubiquitin aldehyde binding 2	-1.67807 1905
RPS19BP1	ribosomal protein S19 binding protein 1	3.20163 3861		NPPA	natriuretic peptide A	-1.67807 1905
1700020L24RIK	Description Not Found	3.19377 1743		LY96	lymphocyte antigen 96	-1.67959 4789
CCDC109B	coiled-coil domain containing 109B(Ccdc109b)	3.18127 6986		OLFR351	olfactory receptor 351(Olfr351)	-1.68073 0557
UBE2C	ubiquitin conjugating enzyme E2 C	3.17791 7792		TGFBR1	transforming growth factor beta receptor 1	-1.68106 8055
SLC25A16	solute carrier family 25 member 16	3.17791 7792		KLHL6	kelch like family member 6	-1.68353 1539
ARHGAP19	Rho GTPase activating protein 19	3.16770 5534		ELMO2	engulfment and cell motility 2	-1.68369 6454
TYMS-PS	thymidylate synthase, pseudogene(Tyms-ps)	3.16636 2514		POLR3D	polymerase (RNA) III subunit D	-1.68394 2043
IL3RA	interleukin 3 receptor	3.14579		RALGPS1	Ral GEF with PH domain	-

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	subunit alpha	3675			and SH3 binding motif 1	1.68552 4532
TMEM53	transmembrane protein 53	3.14159 6278		ATL2	atlastin GTPase 2	-1.68573 1341
THNSL2	threonine synthase like 2	3.14159 6278		RAD52	RAD52 homolog, DNA repair protein	-1.68952 3672
2810408M09RIK	Description Not Found	3.12928 3017		GPC1	glypican 1	-1.68964 6894
ADAMDEC1	ADAM like decysin 1	3.12101 5401		ARHGAP15	Rho GTPase activating protein 15	-1.69080 4518
ASB2	ankyrin repeat and SOCS box containing 2	3.11879 2343		GPRC5B	G protein-coupled receptor class C group 5 member B	-1.69399 9744
SLC37A4	solute carrier family 37 member 4	3.11270 0133		ZBTB1	zinc finger and BTB domain containing 1	-1.69404 6727
NICN1	nicolin 1	3.10847 8268		NARFL	nuclear prelamin A recognition factor like	-1.69488 0193
2310067B10RIK	Description Not Found	3.08746 2841		SLC26A6	solute carrier family 26 member 6	-1.69525 2347
PIGL	phosphatidylinositol glycan anchor biosynthesis class L	3.07723 9787		MAPKBP1	mitogen-activated protein kinase binding protein 1	-1.69590 8738
1190005I06RIK	Description Not Found	3.07038 9328		RAB6B	RAB6B, member RAS oncogene family	-1.69754 1036
DHFR	dihydrofolate reductase	3.07038 9328		ARL2	ADP ribosylation factor like GTPase 2	-1.70034 9879
FABP5	fatty acid binding protein 5	3.06608 919		ZFP646	zinc finger protein 646(Zfp646)	-1.70043 9718
POMT2	protein O-mannosyltransferase 2	3.05579 4286		SELENBP2	selenium binding protein 2(Selenbp2)	-1.70043 9718
F2RL2	coagulation factor II thrombin receptor like 2	3.05311 1336		ACOT3	acyl-CoA thioesterase 3(Acot3)	-1.70043 9718
GRB7	growth factor receptor bound protein 7	3.04885 2907		REG3G	regenerating family member 3 gamma	-1.70043 9718
SNX21	sorting nexin family member 21	3.04439 4119		GAB1	GRB2 associated binding protein 1	-1.70043 9718
SUFU	SUFU negative regulator of hedgehog signaling	3.04439 4119		LCN10	lipocalin 10	-1.70043 9718
RFC3	replication factor C subunit 3	3.02928 8361		MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like	-1.70043 9718
CLDN12	claudin 12	3.01792 1908		PTCD3	pentatricopeptide repeat domain 3	-1.70043 9718

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C1QTNF6	C1q and tumor necrosis factor related protein 6	3.014450679		NTHL1	nth-like DNA glycosylase 1	-1.700439718
PLCXD1	phosphatidylinositol specific phospholipase C X domain containing 1	2.990954 86		NUDT3	nudix hydrolase 3	-1.700439718
SULT4A1	sulfotransferase family 4A member 1	2.990954 86		CLEC12A	C-type lectin domain family 12 member A	-1.700439718
CTTNBP2NL	CTTNBP2 N-terminal like	2.981852653		ZBTB3	zinc finger and BTB domain containing 3	-1.700439718
SNX5	sorting nexin 5	2.977279924		AMT	aminomethyltransferase	-1.700439718
HPS5	HPS5, biogenesis of lysosomal organelles complex 2 subunit 2	2.972692654		ZDHHC14	zinc finger DHHC-type containing 14	-1.700439718
WISP1	WNT1 inducible signaling pathway protein 1	2.968090752		NKX2-5	NK2 homeobox 5	-1.700491519
PTPN9	protein tyrosine phosphatase, non-receptor type 9	2.963474124		FOXA3	forkhead box A3	-1.702815694
USP37	ubiquitin specific peptidase 37	2.95419631		WASF1	WAS protein family member 1	-1.706412734
SH3BGRL	SH3 domain binding glutamate rich protein like	2.935459748		OLFR690	olfactory receptor 690(Olfr690)	-1.707192688
NCALD	neurocalcin delta	2.935459748		ENTPD5	ectonucleoside triphosphate diphosphohydrolase 5	-1.707764551
CDC42EP4	CDC42 effector protein 4	2.916476644		PCDHGA4	protocadherin gamma subfamily A, 4	-1.709042655
IGFBP7	insulin like growth factor binding protein 7	2.910553168		TCF12	transcription factor 12	-1.710308209
ABHD4	abhydrolase domain containing 4	2.908868748		MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	-1.711494907
CSF1	colony stimulating factor 1	2.906890596		CDKN1C	cyclin dependent kinase inhibitor 1C	-1.711690028
COX7A1	cytochrome c oxidase subunit 7A1	2.897240426		PRICKLE1	prickle planar cell polarity protein 1	-1.713410822
TTYH2	tweety family member 2	2.892391026		ATXN7L1	ataxin 7 like 1	-1.71669984
ACO1	aconitase 1	2.87774425		SLCO3A1	solute carrier organic anion transporter family member 3A1	-1.719235762
BARD1	BRCA1 associated RING domain 1	2.867896464		TMEM110	transmembrane protein 110	-1.720046704
GPN1	GPN-loop GTPase 1	2.86789		KLF2	Kruppel like factor 2	-

		6464			1.72137 4729
PTTG1	pituitary tumor-transforming 1	2.86789 6464	FGG	fibrinogen gamma chain	-1.72246 6024
2810408A11RIK	Description Not Found	2.85798 0995	ASAH2	N-acylsphingosine amidohydrolase 2	-1.72246 6024
BBX	BBX, HMG-box containing	2.85798 0995	LAP3	leucine aminopeptidase 3	-1.72246 6024
LTBP3	latent transforming growth factor beta binding protein 3	2.83794 3242	STAB2	stabilin 2	-1.72246 6024
ACTG2	actin, gamma 2, smooth muscle, enteric	2.82781 9025	IL22RA1	interleukin 22 receptor subunit alpha 1	-1.72246 6024
ISLR	immunoglobulin superfamily containing leucine rich repeat	2.82781 9025	SERINC4	serine incorporator 4	-1.72246 6024
NARS2	asparaginyl-tRNA synthetase 2, mitochondrial (putative)	2.82308 7408	GPR180	G protein-coupled receptor 180	-1.72246 6024
ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	2.81452 379	TIPARP	TCDD inducible poly(ADP-ribose) polymerase	-1.72246 6024
ABCB8	ATP binding cassette subfamily B member 8	2.81335 8991	USP11	ubiquitin specific peptidase 11	-1.72246 6024
IDI1	isopentenyl-diphosphate delta isomerase 1	2.81178 2922	TRIP6	thyroid hormone receptor interactor 6	-1.72246 6024
GLS2	glutaminase 2	2.79701 2978	KCNH2	potassium voltage-gated channel subfamily H member 2	-1.72246 6024
HDAC8	histone deacetylase 8	2.79701 2978	ESR2	estrogen receptor 2	-1.72246 6024
BRIP1	BRCA1 interacting protein C-terminal helicase 1	2.79701 2978	FGF13	fibroblast growth factor 13	-1.72263 9247
USP6NL	USP6 N-terminal like	2.79441 5866	KBTBD7	kelch repeat and BTB domain containing 7	-1.72423 7927
TLCD2	TLC domain containing 2	2.79181 4071	UHRF1BP1	UHRF1 binding protein 1	-1.72583 5292
GUCY1A3	guanylate cyclase 1 soluble subunit alpha	2.78750 2763	BCAM	basal cell adhesion molecule (Lutheran blood group)	-1.72650 9704
OCA2	OCA2 melanosomal transmembrane protein	2.78659 6362	ELOVL6	ELOVL fatty acid elongase 6	-1.72656 5554
VAT1	vesicle amine transport 1	2.77250 2543	PPM1K	protein phosphatase, Mg2+/Mn2+ dependent 1K	-1.72664 3643
HIST1H2AB	histone cluster 1, H2ab	2.76791 4142	SPATA6	spermatogenesis associated 6	-1.72767 3077

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PIGC	phosphatidylinositol glycan anchor biosynthesis class C	2.76022 0946		NAV1	neuron navigator 1	-1.72792 0455
PARG	poly(ADP-ribose) glycohydrolase	2.75655 8208		ANK3	ankyrin 3, node of Ranvier (ankyrin G)	-1.72792 0455
ESCO2	establishment of sister chromatid cohesion N-acetyltransferase 2	2.75488 7502		KCNAB1	potassium voltage-gated channel subfamily A member regulatory beta subunit 1	-1.72792 0455
HIPK2	homeodomain interacting protein kinase 2	2.75488 7502		CYP27A1	cytochrome P450 family 27 subfamily A member 1	-1.72792 0455
IMPA1	inositol monophosphatase 1	2.75294 5007		MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	-1.72975 6006
COQ4	coenzyme Q4	2.74416 1096		ANKRD7	ankyrin repeat domain 7	-1.73064 6873
ZBTB7A	zinc finger and BTB domain containing 7A	2.74416 1096		IFRD1	interferon related developmental regulator 1	-1.73244 7522
GAMT	guanidinoacetate N-methyltransferase	2.74416 1096		ALX3	ALX homeobox 3	-1.73335 4341
BIK	BCL2 interacting killer	2.74416 1096		SNURF	SNRPN upstream reading frame	-1.73335 4341
PMS1	PMS1 homolog 1, mismatch repair system component	2.73335 4341		AMZ2	archaelysin family metallopeptidase 2	-1.73350 053
HAVCR2	hepatitis A virus cellular receptor 2	2.72976 9667		ROGDI	rogdi homolog	-1.73419 198
FHL2	four and a half LIM domains 2	2.72725 4747		DAGLA	diacylglycerol lipase alpha	-1.73447 1203
CHAF1A	chromatin assembly factor 1 subunit A	2.72524 8783		4930432K2 1RIK	Description Not Found	-1.73624 3886
2810004N2 3RIK	Description Not Found	2.72246 6024		KRCC1	lysine rich coiled-coil 1	-1.73665 741
TBC1D14	TBC1 domain family member 14	2.72246 6024		OLFR1331	olfactory receptor 1331(Olfr1331)	-1.73682 6447
EHD2	EH domain containing 2	2.71149 4907		SLC25A25	solute carrier family 25 member 25	-1.73690 749
APH1A	aph-1 homolog A, gamma-secretase subunit	2.70597 7902		CXCR4	C-X-C motif chemokine receptor 4	-1.73777 9353
TMEM2	transmembrane protein 2	2.70321 1467		EPB41L3	Description Not Found	-1.73876 7837
LCAT	lecithin-cholesterol acyltransferase	2.70043 9718		CEP164	centrosomal protein 164	-1.73879 5736
FBXO15	F-box protein 15	2.68929		AGER	advanced glycosylation	-

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		9161			end product-specific receptor	1.73961488
ADAP1	ArfGAP with dual PH domains 1	2.67439 1397		B3GALT5	beta-1,3-galactosyltransferase 5	-1.74021 5306
PPAPDC1B	Description Not Found	2.66675 6592		OLFR450	olfactory receptor 450(Olfr450)	-1.74228 265
CD48	CD48 molecule	2.66675 6592		ZFP780B	zinc finger protein 780B(Zfp780b)	-1.74416 1096
CAMK4	calcium/calmodulin dependent protein kinase IV	2.65535 1829		OLFR485	olfactory receptor 485(Olfr485)	-1.74416 1096
SAC3D1	SAC3 domain containing 1	2.64385 619		OLFR47	olfactory receptor 47(Olfr47)	-1.74416 1096
ECHDC2	enoyl-CoA hydratase domain containing 2	2.64072 5033		CYP4F18	cytochrome P450, family 4, subfamily f, polypeptide 18(Cyp4f18)	-1.74416 1096
INCENP	inner centromere protein	2.63846 0117		PLOD2	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	-1.74416 1096
INTS9	integrator complex subunit 9	2.63492 0268		OSBPL1A	oxysterol binding protein like 1A	-1.74416 1096
KLRA17	killer cell lectin-like receptor, subfamily A, member 17(Klra17)	2.63226 8216		CHRNA5	cholinergic receptor nicotinic alpha 5 subunit	-1.74416 1096
MAN2B2	mannosidase alpha class 2B member 2	2.63226 8216		TSSK4	testis specific serine kinase 4	-1.74416 1096
DOLK	dolichol kinase	2.63226 8216		ALKBH8	alkB homolog 8, tRNA methyltransferase	-1.74416 1096
SAP30BP	SAP30 binding protein	2.63226 8216		GPX2	glutathione peroxidase 2	-1.74416 1096
RTN1	reticulon 1	2.62789 8616		ATG4D	autophagy related 4D cysteine peptidase	-1.74416 1096
ADAM15	ADAM metallopeptidase domain 15	2.62643 9137		SCRN3	secernin 3	-1.74416 1096
STAG3	stromal antigen 3	2.62058 641		NOTCH3	notch 3	-1.74416 1096
NUDT2	nudix hydrolase 2	2.61077 5705		OLFR113	olfactory receptor 113(Olfr113)	-1.74435 7436
GLT8D2	glycosyltransferase 8 domain containing 2	2.60998 8757		CD28	CD28 molecule	-1.74460 5653
CAPSL	calcyphosine like	2.60880 9243		SAG	S-antigen; retina and pineal gland (arrestin)	-1.74522 4161
CALR	calreticulin	2.60880 9243		AGTRAP	angiotensin II receptor associated protein	-1.74910 7415

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CRYBG3	crystallin beta-gamma domain containing 3	2.60539 3551		BLK	BLK proto-oncogene, Src family tyrosine kinase	-1.74953 4268
DIXDC1	DIX domain containing 1	2.59694 0379		MGAT5	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase	-1.74953 4268
TACSTD2	tumor-associated calcium signal transducer 2	2.59392 6161		RNF2	ring finger protein 2	-1.75089 0228
TRP53RK	Description Not Found	2.58806 6506		COL14A1	collagen type XIV alpha 1 chain	-1.75209 3722
PDCD1LG2	programmed cell death 1 ligand 2	2.58496 2501		PLEKHG3	pleckstrin homology and RhoGEF domain containing G3	-1.75210 9698
SEC23IP	SEC23 interacting protein	2.58496 2501		ARHGEF18	Rho/Rac guanine nucleotide exchange factor 18	-1.75410 0479
ORM1	orosomucoid 1	2.58496 2501		LEF1	lymphoid enhancer binding factor 1	-1.75488 7502
ZFP322A	zinc finger protein 322A(Zfp322a)	2.57502 4164		COMMD9	COMM domain containing 9	-1.75490 709
4931406C0 7RIK	Description Not Found	2.56071 4954		SLC20A1	solute carrier family 20 member 1	-1.75863 7847
ZFP382	zinc finger protein 382(Zfp382)	2.56071 4954		ACTR5	ARP5 actin-related protein 5 homolog	-1.75924 4091
CLIP2	CAP-Gly domain containing linker protein 2	2.56071 4954		UBQLN3	ubiquilin 3	-1.76510 9548
TNFAIP8L 1	TNF alpha induced protein 8 like 1	2.56071 4954		ZFP770	zinc finger protein 770(Zfp770)	-1.76553 4746
NRCAM	neuronal cell adhesion molecule	2.56071 4954		PCDHB18	protocadherin beta 18(Pcdhb18)	-1.76553 4746
HPSE	heparanase	2.56071 4954		OLFR700	olfactory receptor 700(Olfr700)	-1.76553 4746
RTKN	rhotekin	2.55898 5655		FOXP4	forkhead box P4	-1.76553 4746
DLGAP5	DLG associated protein 5	2.55012 5328		CDC34	cell division cycle 34	-1.76553 4746
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	2.54843 6625		HIST1H1E	histone cluster 1, H1e	-1.76553 4746
GCNT1	glucosaminyl (N-acetyl) transferase 1, core 2	2.54843 6625		G6PC2	glucose-6-phosphatase catalytic subunit 2	-1.76553 4746
SASS6	SAS-6 centriolar assembly protein	2.54843 6625		FUT1	fucosyltransferase 1 (H blood group)	-1.76553 4746
AMIGO3	adhesion molecule with	2.54843		ZFP69	ZFP69 zinc finger protein	-

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	Ig-like domain 3	6625				1.76553 4746
APH1B	aph-1 homolog B, gamma-secretase subunit	2.54843 6625		WBSCR27	Williams Beuren syndrome chromosome region 27	- 1.76553 4746
ABCC5	ATP binding cassette subfamily C member 5	2.54784 6505		METTL8	methyltransferase like 8	- 1.76688 0868
YIPF6	Yip1 domain family member 6	2.54380 5176		TMEM170	transmembrane protein 170(Tmem170)	- 1.76746 2508
FFAR1	free fatty acid receptor 1	2.53605 29		TRP53INP1	transformation related protein 53 inducible nuclear protein 1(Trp53inp1)	- 1.76751 8474
TSSK6	testis specific serine kinase 6	2.53605 29		H2-Q5	histocompatibility 2, Q region locus 5(H2-Q5)	- 1.76967 6967
ETV6	ETS variant 6	2.53538 5323		ADCK1	aarF domain containing kinase 1	- 1.77003 3995
PTGDS	prostaglandin D2 synthase	2.52983 8423		IMPAD1	inositol monophosphatase domain containing 1	- 1.77143 4505
SH3D19	SH3 domain containing 19	2.52356 1956		E4F1	E4F transcription factor 1	- 1.77242 7885
KIF5C	kinesin family member 5C	2.51829 8014		ZFYVE20	Description Not Found	- 1.77294 2676
PTGER2	prostaglandin E receptor 2	2.51727 5693		PNPLA6	patatin like phospholipase domain containing 6	- 1.77507 4114
INSR	insulin receptor	2.51096 1919		TRIB3	tribbles pseudokinase 3	- 1.77521 5233
MAPK6	mitogen-activated protein kinase 6	2.50462 0392		GM614	predicted gene 614(Gm614)	- 1.77610 3988
OXSR1	oxidative stress responsive 1	2.50221 1192		D5ERTD57 9E	DNA segment, Chr 5, ERATO Doi 579, expressed(D5Ertd579e)	- 1.77630 6798
EZH2	enhancer of zeste 2 polycomb repressive complex 2 subunit	2.50143 9145		SCAND1	SCAN domain containing 1	- 1.77785 827
BNIP1	BCL2 interacting protein 1	2.49825 0868		ASB13	ankyrin repeat and SOCS box containing 13	- 1.78220 5107
LPCAT4	lysophosphatidylcholine acyltransferase 4	2.49528 5165		ARHGEF4	Rho guanine nucleotide exchange factor 4	- 1.78407 2601
PPAP2C	Description Not Found	2.48542 6827		H1FNT	H1 histone family member N, testis specific	- 1.78485 543
IFNA12	interferon alpha 12(Ifna12)	2.48542 6827		BLOC1S1	biogenesis of lysosomal organelles complex 1 subunit 1	- 1.78491 1393
DCLK1	doublecortin like kinase 1	2.48542 6827		ZFYVE27	zinc finger FYVE-type containing 27	- 1.78510

						13
MX1	MX dynamin like GTPase 1	2.48542 6827		RHOX4B	reproductive homeobox 4B(Rhox4b)	-1.78659 6362
SMTN	smoothelin	2.48542 6827		OLFR1134	olfactory receptor 1134(Olfr1134)	-1.78659 6362
PLA2G15	phospholipase A2 group XV	2.48194 563		CAR11	carbonic anhydrase 11(Car11)	-1.78659 6362
OLFR192	olfactory receptor 192(Olfr192)	2.47248 7771		LRRIQ4	leucine rich repeats and IQ motif containing 4	-1.78659 6362
ITGB5	integrin subunit beta 5	2.47248 7771		CASP12	caspase 12 (gene/pseudogene)	-1.78659 6362
RAPSN	receptor associated protein of the synapse	2.46597 4465		ODF3L1	outer dense fiber of sperm tails 3 like 1	-1.78659 6362
SNX3	sorting nexin 3	2.45943 1619		CCDC3	coiled-coil domain containing 3	-1.78659 6362
FERMT2	fermitin family member 2	2.45943 1619		SSPN	sarcospan	-1.78659 6362
CCR5	C-C motif chemokine receptor 5 (gene/pseudogene)	2.44441 0478		KLK1	kallikrein 1	-1.78659 6362
UPK1A	uroplakin 1A	2.43962 3138		SENP7	SUMO1/sentrin specific peptidase 7	-1.78689 7131
BCL2L2	BCL2 like 2	2.43629 512		CAML	calcium modulating ligand(Caml)	-1.78773 5284
2610002M06RIK	Description Not Found	2.43295 9407		YEATS2	YEATS domain containing 2	-1.78862 7083
CENPN	centromere protein N	2.43295 9407		SERPINF2	serpin family F member 2	-1.79181 4071
HBEGF	heparin binding EGF like growth factor	2.43096 254		KCNMB1	potassium calcium-activated channel subfamily M regulatory beta subunit 1	-1.79259 7191
TYMS	thymidylate synthetase	2.42710 3287		FCHO2	FCH domain only 2	-1.79266 6489
MGA	MGA, MAX dimerization protein	2.42693 9834		BBS9	Bardet-Biedl syndrome 9	-1.79273 4984
RAI14	retinoic acid induced 14	2.42626 4755		OLFR323	olfactory receptor 323(Olfr323)	-1.79460 9131
CFI	complement factor I	2.41953 8892		CD247	CD247 molecule	-1.79608 1585
PLK4	polo like kinase 4	2.41953 8892		HIST2H2AA1	histone cluster 2, H2aa1(Hist2h2aa1)	-1.79684 7743

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SLC6A9	solute carrier family 6 member 9	2.41953 8892		PDK1	pyruvate dehydrogenase kinase 1	-1.80056 3818
TMED2	transmembrane p24 trafficking protein 2	2.41953 8892		NRARP	NOTCH-regulated ankyrin repeat protein	-1.80304 9246
TMEM120 B	transmembrane protein 120B	2.41857 423		BTBD11	BTB domain containing 11	-1.80479 3263
TRIM36	tripartite motif containing 36	2.41785 2515		CSF2RA	colony stimulating factor 2 receptor alpha subunit	-1.80508 9518
CCDC93	coiled-coil domain containing 93	2.41616 4165		DEXI	Dexi homolog	-1.80699 8156
SLC25A35	solute carrier family 25 member 35	2.40936 7225		OLFR1276	olfactory receptor 1276(Olfr1276)	-1.80735 4922
BNC1	basonuclin 1	2.40599 236		TCSTV3	2-cell-stage, variable group, member 3(Tcstv3)	-1.80735 4922
FOXL2	forkhead box L2	2.40599 236		SPRR2D	small proline rich protein 2D	-1.80735 4922
TFPI2	tissue factor pathway inhibitor 2	2.40599 236		SEMA4G	semaphorin 4G	-1.80735 4922
NET1	neuroepithelial cell transforming 1	2.40599 236		KCNK9	potassium two pore domain channel subfamily K member 9	-1.80735 4922
SLCO2A1	solute carrier organic anion transporter family member 2A1	2.40599 236		SNAPC3	small nuclear RNA activating complex polypeptide 3	-1.80738 5513
A730008H2 3RIK	Description Not Found	2.39927 5037		AXIN2	axin 2	-1.80842 9403
CDKN2B	cyclin dependent kinase inhibitor 2B	2.39726 4578		PCNXL3	Description Not Found	-1.80899 5133
ZFP532	zinc finger protein 532(Zfp532)	2.39313 8801		KLHL7	kelch like family member 7	-1.80901 6035
GTSE1	G2 and S-phase expressed 1	2.39242 8431		ZFP281	zinc finger protein 281(Zfp281)	-1.81155 6991
CCDC14	coiled-coil domain containing 14	2.39231 7423		CHRNB2	cholinergic receptor nicotinic beta 2 subunit	-1.81249 8225
ADAT1	adenosine deaminase, tRNA specific 1	2.39231 7423		TBC1D15	TBC1 domain family member 15	-1.81290 9044
DGKH	diacylglycerol kinase eta	2.39231 7423		GALNT9	polypeptide N-acetylgalactosaminyltransferase 9	-1.81340 7449
ZRSR1	zinc finger CCCH-type, RNA binding motif and serine/arginine rich 1	2.39231 7423		DYNC1I1	dynein cytoplasmic 1 intermediate chain 1	-1.81343 4179
NFE2	nuclear factor, erythroid 2	2.39152 9377		MYH8	myosin heavy chain 8	-1.81403

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					224
CD63	CD63 molecule	2.38785 3137	CEP57	centrosomal protein 57	-1.81568 4972
MIB1	mindbomb E3 ubiquitin protein ligase 1	2.38645 559	LTK	leukocyte receptor tyrosine kinase	-1.81762 3258
TSN	translin	2.38234 9023	COMMD2	COMM domain containing 2	-1.81762 3258
2510003E0 4RIK	Description Not Found	2.37851 1623	MEF2C	myocyte enhancer factor 2C	-1.81762 3258
BC043934	cDNA sequence BC043934(BC043934)	2.37851 1623	LONRF2	LON peptidase N-terminal domain and ring finger 2	-1.81794 1412
AHCYL1	adenosylhomocysteinase like 1	2.36673 4247	PDCD6IP	programmed cell death 6 interacting protein	-1.82057 5529
OLFR731	olfactory receptor 731(Olfr731)	2.36457 2432	DHX16	DEAH-box helicase 16	-1.82066 1084
CDKN2A	cyclin dependent kinase inhibitor 2A	2.36457 2432	ZFYVE19	zinc finger FYVE-type containing 19	-1.82528 1028
SLC29A4	solute carrier family 29 member 4	2.36457 2432	H2-T10	histocompatibility 2, T region locus 10(H2-T10)	-1.82621 8639
SLC4A10	solute carrier family 4 member 10	2.36457 2432	ARID1A	AT-rich interaction domain 1A	-1.82704 3205
CYCS	cytochrome c, somatic	2.35187 2866	NOD1	nucleotide binding oligomerization domain containing 1	-1.82718 5706
COL5A1	collagen type V alpha 1	2.35049 7247	2610318N0 2RIK	Description Not Found	-1.82781 9025
UTRN	utrophin	2.35049 7247	BC048644	cDNA sequence BC048644(BC048644)	-1.82781 9025
AURKA	aurora kinase A	2.34967 8136	CDC42EP2	CDC42 effector protein 2	-1.82781 9025
KREMEN2	kringle containing transmembrane protein 2	2.34943 1709	CCL25	C-C motif chemokine ligand 25	-1.82781 9025
FGL2	fibrinogen like 2	2.34640 9407	TBX6	T-box 6	-1.82781 9025
NCAM1	neural cell adhesion molecule 1	2.34340 7822	PLEKHG4	pleckstrin homology and RhoGEF domain containing G4	-1.82781 9025
ALG8	ALG8, alpha-1,3-glucosyltransferase	2.34340 7822	RAD18	RAD18, E3 ubiquitin protein ligase	-1.83064 2494
OLFR703	olfactory receptor 703(Olfr703)	2.33628 3388	SLC12A9	solute carrier family 12 member 9	-1.83080 7586
SLC39A10	solute carrier family 39	2.33628	NR1D2	nuclear receptor subfamily	-

[0183]

	member 10	3388			1 group D member 2	1.83794 3242
HIST1H2A H	histone cluster 1, H2ah	2.32214 1712		NLK	nemo like kinase	-1.84017 0811
TSGA8	testis specific gene A8(Tsga8)	2.32192 8095		TTC37	tetratricopeptide repeat domain 37	-1.84046 2743
ELOVL2	ELOVL fatty acid elongase 2	2.32192 8095		DLG3	discs large MAGUK scaffold protein 3	-1.84150 7525
MLF1	myeloid leukemia factor 1	2.32192 8095		PCF11	PCF11 cleavage and polyadenylation factor subunit	-1.84334 9827
FZD6	frizzled class receptor 6	2.32192 8095		HIST1H4D	histone cluster 1, H4d	-1.84638 6944
PLD1	phospholipase D1	2.32192 8095		PEX26	peroxisomal biogenesis factor 26	-1.84744 0096
IFRD2	interferon-related developmental regulator 2	2.32192 8095		CYP2B10	cytochrome P450, family 2, subfamily b, polypeptide 10(Cyp2b10)	-1.84799 6907
OLA1	Obg-like ATPase 1	2.32192 8095		GDF3	growth differentiation factor 3	-1.84799 6907
ASPA	aspartoacylase	2.32192 8095		GPR33	G protein-coupled receptor 33 (gene/pseudogene)	-1.84799 6907
TGFB3	transforming growth factor beta 3	2.32192 8095		TDG	thymine DNA glycosylase	-1.84799 6907
PKIG	protein kinase (cAMP-dependent, catalytic) inhibitor gamma	2.31469 6526		HIPK3	homeodomain interacting protein kinase 3	-1.84799 6907
TNFRSF4	tumor necrosis factor receptor superfamily member 4	2.30883 2886		PAPOLA	poly(A) polymerase alpha	-1.84799 6907
IQCB1	IQ motif containing B1	2.30798 4443		MAPK4	mitogen-activated protein kinase 4	-1.84799 6907
SLC16A11	solute carrier family 16 member 11	2.30766 2797		FRAT2	frequently rearranged in advanced T-cell lymphomas 2	-1.84969 115
1190002N1 5RIK	Description Not Found	2.30742 8525		HEXIM1	hexamethylene bisacetamide inducible 1	-1.85103 5845
LCE1L	late cornified envelope 1L(Lce1l)	2.30742 8525		TATDN2	TatD DNase domain containing 2	-1.85143 3223
RGS13	regulator of G-protein signaling 13	2.30742 8525		KLRB1C	killer cell lectin-like receptor subfamily B member 1C(Klrb1c)	-1.85425 3843
FBXW8	F-box and WD repeat domain containing 8	2.29998 7517		SLC16A9	solute carrier family 16 member 9	-1.85508 3462
SNCA	synuclein alpha	2.29645 7407		ACBD4	acyl-CoA binding domain containing 4	-1.85573 9032

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OSGIN1	oxidative stress induced growth inhibitor 1	2.29449 1702		REXO1	RNA exonuclease 1 homolog	-1.85798 0995
BC004004	cDNA sequence BC004004(BC004004)	2.29278 1749		OLFR1442	olfactory receptor 1442(Olfr1442)	-1.85928 6959
WNT10A	Wnt family member 10A	2.29278 1749		PHOSPHO1	phosphoethanolamine/phosphocholine phosphatase	-1.85974 7926
THG1L	tRNA-histidine guanylyltransferase 1 like	2.29278 1749		ITPKA	inositol-trisphosphate 3-kinase A	-1.85988 1803
MLH1	mutL homolog 1	2.29278 1749		ZFHX2	zinc finger homeobox 2	-1.86051 3882
RRM2	ribonucleotide reductase regulatory subunit M2	2.28943 5485		TOR1A	torsin family 1 member A	-1.86094 9348
SHISA4	shisa family member 4	2.27798 4747		CDKAL1	CDK5 regulatory subunit associated protein 1 like 1	-1.86279 4137
DDAH2	dimethylarginine dimethylaminohydrolase 2	2.27798 4747		SMAD1	SMAD family member 1	-1.86346 2947
APBA1	amyloid beta precursor protein binding family A member 1	2.26908 5766		ZC3H13	zinc finger CCCH-type containing 13	-1.86353 5399
MMAB	methylmalonic aciduria (cobalamin deficiency) cblB type	2.26491 1693		ZSCAN20	zinc finger and SCAN domain containing 20	-1.86396 2106
DIAP1	Description Not Found	2.26303 4406		EPB41L4A	Description Not Found	-1.86789 6464
CAR14	carbonic anhydrase 14(Car14)	2.26303 4406		ZFP280C	zinc finger protein 280C(Zfp280c)	-1.86789 6464
C2	complement component 2	2.26303 4406		GM1322	predicted gene 1322(Gm1322)	-1.86789 6464
MAG	myelin associated glycoprotein	2.26303 4406		OLFR472	olfactory receptor 472(Olfr472)	-1.86789 6464
KCNIP3	potassium voltage-gated channel interacting protein 3	2.26303 4406		OLFR171	olfactory receptor 171(Olfr171)	-1.86789 6464
CFD	complement factor D	2.26303 4406		OLFR1249	olfactory receptor 1249(Olfr1249)	-1.86789 6464
CCNE1	cyclin E1	2.26272 3645		PRH1	proline rich protein HaeIII subfamily 1	-1.86789 6464
RYR1	ryanodine receptor 1	2.26130 5322		ARSI	arylsulfatase family member I	-1.86789 6464
PROC	protein C, inactivator of coagulation factors Va and VIIIa	2.25550 0733		KRT7	keratin 7	-1.86789 6464
ZFP27	zinc finger protein 27(Zfp27)	2.24792 7513		PCGF3	polycomb group ring finger 3	-1.86789

						6464
TBX1	T-box 1	2.24792 7513		PCTP	phosphatidylcholine transfer protein	-1.86789 6464
DHRS13	dehydrogenase/reductase 13	2.24792 7513		CALD1	caldesmon 1	-1.86789 6464
HSPG2	heparan sulfate proteoglycan 2	2.24792 7513		TREML2	triggering receptor expressed on myeloid cells like 2	-1.86789 6464
FRMD8	FERM domain containing 8	2.24777 312		RTN4RL1	reticulon 4 receptor like 1	-1.86789 6464
MIOX	myo-inositol oxygenase	2.24057 9987		PARVA	parvin alpha	-1.86847 9018
LYRM1	LYR motif containing 1	2.23266 0757		NPCD	neuronal pentraxin chromo domain(Npcd)	-1.87190 2039
STAP1	signal transducing adaptor family member 1	2.23266 0757		RFXANK	regulatory factor X associated ankyrin containing protein	-1.87206 109
NAT2	N-acetyltransferase 2	2.23266 0757		MAP3K14	mitogen-activated protein kinase kinase kinase 14	-1.87229 1304
SRGAP3	SLIT-ROBO Rho GTPase activating protein 3	2.23266 0757		KLHL9	kelch like family member 9	-1.87452 8943
NXT2	nuclear transport factor 2 like export factor 2	2.23266 0757		SESN1	sestrin 1	-1.87526 0951
RCOR1	REST corepressor 1	2.23266 0757		ADAMTS7	ADAM metallopeptidase with thrombospondin type 1 motif 7	-1.87940 4807
SRR	serine racemase	2.23083 6503		SNAPC1	small nuclear RNA activating complex polypeptide 1	-1.88488 993
IKBKAP	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	2.22617 7109		ADAR	adenosine deaminase, RNA specific	-1.88529 9379
AI597479	expressed sequence AI597479(AI597479)	2.22581 9675		LCE1C	late cornified envelope 1C	-1.88562 6461
POP1	POP1 homolog, ribonuclease P/MRP subunit	2.22496 6365		FBXO21	F-box protein 21	-1.88615 5099
SLC35E4	solute carrier family 35 member E4	2.21723 0716		2610524H0 6RIK	Description Not Found	-1.88752 5271
XAB2	XPA binding protein 2	2.21723 0716		1700016K1 9RIK	Description Not Found	-1.88752 5271
MREG	melanoregulin	2.21292 58		ZFP715	zinc finger protein 715(Zfp715)	-1.88752 5271
FKBP11	FK506 binding protein 11	2.21072 1954		OLFR446	olfactory receptor 446(Olfr446)	-1.88752

[0185]

[0186]

						5271
IGF2BP2	insulin like growth factor 2 mRNA binding protein 2	2.20778 9851		PTK7	protein tyrosine kinase 7 (inactive)	- 1.88752 5271
NUP133	nucleoporin 133	2.20744 7199		TMEM117	transmembrane protein 117	- 1.88752 5271
OLFR1183	olfactory receptor 1183(Olfr1183)	2.20163 3861		ITIH2	inter-alpha-trypsin inhibitor heavy chain 2	- 1.88752 5271
IL1F6	interleukin 1 family, member 6(Il1f6)	2.20163 3861		TAGLN3	transgelin 3	- 1.88752 5271
OTX1	orthodenticle homeobox 1	2.20163 3861		IFI203	interferon activated gene 203(Ifi203)	- 1.88764 4112
MSH3	mutS homolog 3	2.20163 3861		ATP1B1	ATPase Na+/K+ transporting subunit beta 1	- 1.88766 4186
SCN4B	sodium voltage-gated channel beta subunit 4	2.20163 3861		BLCAP	bladder cancer associated protein	- 1.88859 6201
CROCC	ciliary rootlet coiled-coil, rootletin	2.20163 3861		IGF1R	insulin like growth factor 1 receptor	- 1.89024 137
NSUN2	NOP2/Sun RNA methyltransferase family member 2	2.19434 9986		HMG20A	high mobility group 20A	- 1.89057 9593
GAS2L1	growth arrest specific 2 like 1	2.19377 1743		WDR24	WD repeat domain 24	- 1.89152 7175
3110007F17 RIK	Description Not Found	2.19074 0399		CDX4	caudal type homeobox 4	- 1.89265 5439
DEFB15	defensin beta 15(Defb15)	2.18586 6545		CLDN18	claudin 18	- 1.89344 9375
C1QTNF2	C1q and tumor necrosis factor related protein 2	2.18586 6545		IL4RA	interleukin 4 receptor, alpha(Il4ra)	- 1.89536 9594
RAP1GAP	RAP1 GTPase activating protein	2.18586 6545		RETNLA	resistin like alpha(Retnla)	- 1.89573 9477
SNTB1	syntrophin beta 1	2.18586 6545		AA388235	expressed sequence AA388235(AA388235)	- 1.89573 9477
FAH	fumarylacetoacetate hydrolase	2.18292 5576		ZC3H6	zinc finger CCCH-type containing 6	- 1.89612 7489
AVPI1	arginine vasopressin induced 1	2.17439 3775		D930015E0 6RIK	RIKEN cDNA D930015E06 gene(D930015E06Rik)	- 1.89965 6973
RPA2	replication protein A2	2.17275 1912		NPFFR2	neuropeptide FF receptor 2	- 1.90207 3579
BRCA2	BRCA2, DNA repair associated	2.16873 2488		IRAK1	interleukin 1 receptor associated kinase 1	- 1.90243 374
RBM47	RNA binding motif	2.16591		CWF19L2	CWF19-like 2, cell cycle	-

	protein 47	1939			control (S. pombe)	1.90370 4505
MSL3L2	male-specific lethal 3-like 2 (Drosophila)(Msl3l2)	2.15906 1455		STK40	serine/threonine kinase 40	-1.90396 4448
TNFRSF9	tumor necrosis factor receptor superfamily member 9	2.15607 1704		MARS2	methionyl-tRNA synthetase 2, mitochondrial	-1.90457 1951
TRF	transferrin(Trf)	2.15458 8207		RAB5A	RAB5A, member RAS oncogene family	-1.90635 0687
ZDHHC15	zinc finger DHHC-type containing 15	2.15437 2546		OLFR1037	olfactory receptor 1037(Olfr1037)	-1.90689 0596
IGJ	Description Not Found	2.15380 5336		ARHGAP22	Rho GTPase activating protein 22	-1.90689 0596
FBXO27	F-box protein 27	2.15380 5336		DENND1B	DENN domain containing 1B	-1.90689 0596
ZDHHC24	zinc finger DHHC-type containing 24	2.15380 5336		EAPP	E2F associated phosphoprotein	-1.90689 0596
SPCS2	signal peptidase complex subunit 2	2.15380 5336		ANKRD13D	ankyrin repeat domain 13D	-1.90689 0596
UCN3	urocortin 3	2.15380 5336		EFCAB2	EF-hand calcium binding domain 2	-1.90689 0596
SLC35A1	solute carrier family 35 member A1	2.15380 5336		HOXC9	homeobox C9	-1.90689 0596
PODXL	podocalyxin like	2.15380 5336		SENP6	SUMO1/sentrin specific peptidase 6	-1.90795 6932
FAM154B	Description Not Found	2.15379 2145		SIDT1	SID1 transmembrane family member 1	-1.90828 6674
NRP1	neuropilin 1	2.14747 0553		2310057J18RIK	Description Not Found	-1.91647 6644
ERGIC1	endoplasmic reticulum-golgi intermediate compartment 1	2.14710 4727		SPRYD4	SPRY domain containing 4	-1.91647 6644
RNF26	ring finger protein 26	2.14681 0011		LY6D	lymphocyte antigen 6 complex, locus D	-1.91647 6644
LCN3	lipocalin 3(Lcn3)	2.13750 3524		PPARGC1B	PPARG coactivator 1 beta	-1.91729 1956
FMO1	flavin containing monooxygenase 1	2.13750 3524		SH3TC1	SH3 domain and tetratricopeptide repeats 1	-1.91790 6346
RAB20	RAB20, member RAS oncogene family	2.13750 3524		FOXO1	forkhead box O1	-1.92020 9106
KATNAL1	katanin catalytic subunit A1 like 1	2.13750 3524		DHX40	DEAH-box helicase 40	-1.92062 3917

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GPR107	G protein-coupled receptor 107	2.13642 4717	RECQL5	RecQ like helicase 5	-1.92066 4575
MELK	maternal embryonic leucine zipper kinase	2.13339 9125	RBM15	RNA binding motif protein 15	-1.92261 6041
KCTD9	potassium channel tetramerization domain containing 9	2.13207 329	EGLN2	egl-9 family hypoxia inducible factor 2	-1.92407 9933
PBK	PDZ binding kinase	2.13041 7144	GPR112	Description Not Found	-1.92599 9419
ENPP5	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative)	2.12411 2676	OLFR829	olfactory receptor 829(Olfr829)	-1.92599 9419
ZDHHC16	zinc finger DHHC-type containing 16	2.12361 008	OLFR684	olfactory receptor 684(Olfr684)	-1.92599 9419
OLFR1346	olfactory receptor 1346(Olfr1346)	2.12101 5401	RETN	resistin	-1.92599 9419
MILL1	MHC I like leukocyte 1(Mill1)	2.12101 5401	ST6GALNAC2	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	-1.92599 9419
RHCG	Rh family C glycoprotein	2.12101 5401	FES	FES proto-oncogene, tyrosine kinase	-1.92599 9419
CLDN1	claudin 1	2.12101 5401	KIF13A	kinesin family member 13A	-1.92599 9419
LHX3	LIM homeobox 3	2.12101 5401	TRPT1	tRNA phosphotransferase 1	-1.92645 7816
TUBB2A	tubulin beta 2A class IIa	2.12101 5401	PLCB2	phospholipase C beta 2	-1.92734 3833
GSG2	germ cell associated 2, haspin	2.11941 2265	NADSYN1	NAD synthetase 1	-1.92967 4394
HYAL2	hyaluronoglucosaminidase 2	2.10734 5942	4833420G17RIK	Description Not Found	-1.93060 469
1700003F12RIK	Description Not Found	2.10433 666	P2RY10	purinergic receptor P2Y10	-1.93073 7338
RUSC2	RUN and SH3 domain containing 2	2.10433 666	PPAPDC3	Description Not Found	-1.93545 9748
LRRIQ3	leucine rich repeats and IQ motif containing 3	2.10433 666	DIP2B	disco interacting protein 2 homolog B	-1.93545 9748
CHSY1	chondroitin sulfate synthase 1	2.10433 666	RHAG	Rh-associated glycoprotein	-1.93545 9748
DUSP23	dual specificity phosphatase 23	2.10433 666	EMID1	EMI domain containing 1	-1.93545 9748
RRAGB	Ras related GTP binding	2.10433	RNF4	ring finger protein 4	-

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	B	666			1.93883 4579
KCNAB3	potassium voltage-gated channel subfamily A regulatory beta subunit 3	2.10433 666	UBL5	ubiquitin like 5	- 1.93895 2478
GRPEL2	GrpE like 2, mitochondrial	2.10312 9681	PROSC	proline synthetase cotranscribed homolog (bacterial)	- 1.94016 675
TRAF2	TNF receptor associated factor 2	2.10202 9095	FZD5	frizzled class receptor 5	- 1.94250 3137
COQ7	coenzyme Q7, hydroxylase	2.10020 5246	UBE2D1	ubiquitin conjugating enzyme E2 D1	- 1.94277 5467
TMEM126 B	transmembrane protein 126B	2.09918 7297	KLRA7	killer cell lectin-like receptor, subfamily A, member 7(Klra7)	- 1.94351 0757
SGPL1	sphingosine-1-phosphate lyase 1	2.09711 2667	TMEM63C	transmembrane protein 63C	- 1.94425 562
CAPN2	calpain 2	2.09644 7979	2810006K2 3RIK	Description Not Found	- 1.94485 8446
CHEK2	checkpoint kinase 2	2.08845 7439	OLFR672	olfactory receptor 672(Olfr672)	- 1.94485 8446
GLRP1	glutamine repeat protein 1(Glrp1)	2.08746 2841	OLFR1347	olfactory receptor 1347(Olfr1347)	- 1.94485 8446
RTN4R	reticulon 4 receptor	2.08746 2841	MTTP	microsomal triglyceride transfer protein	- 1.94485 8446
TRIM37	tripartite motif containing 37	2.08746 2841	MSX1	msh homeobox 1	- 1.94485 8446
NUCB2	nucleobindin 2	2.08746 2841	BSND	barttin CLCNK type accessory beta subunit	- 1.94485 8446
UBE2T	ubiquitin conjugating enzyme E2 T	2.07361 6696	MARK1	microtubule affinity regulating kinase 1	- 1.94485 8446
CREB3L3	cAMP responsive element binding protein 3 like 3	2.07038 9328	CHRNB1	cholinergic receptor nicotinic beta 1 subunit	- 1.94485 8446
CHRM4	cholinergic receptor muscarinic 4	2.07038 9328	CRYL1	crystallin lambda 1	- 1.94641 9425
SLC16A13	solute carrier family 16 member 13	2.07038 9328	TEC	tec protein tyrosine kinase	- 1.94733 0641
OLFML2B	olfactomedin like 2B	2.07038 9328	XKR6	XK related 6	- 1.95031 589
CSNK1G1	casein kinase 1 gamma 1	2.07038 9328	ARC	activity-regulated cytoskeleton-associated protein	- 1.95363 6949
S100A14	S100 calcium binding protein A14	2.07038 9328	WFDC10	WAP four-disulfide core domain 10(Wfdc10)	- 1.95419 631

[0190]

SMYD4	SET and MYND domain containing 4	2.07038 9328		OLFR866	olfactory receptor 866(Olfr866)	- 1.95976 8144
CH25H	cholesterol 25-hydroxylase	2.07038 9328		WIPI2	WD repeat domain, phosphoinositide interacting 2	- 1.96017 1668
TEX2	testis expressed 2	2.06787 5748		OLFR948	olfactory receptor 948(Olfr948)	- 1.96347 4124
SYN1	synapsin I	2.06342 9187		CRTAM	cytotoxic and regulatory T-cell molecule	- 1.96347 4124
CYP3A13	cytochrome P450, family 3, subfamily a, polypeptide 13(Cyp3a13)	2.06058 1758		CCDC116	coiled-coil domain containing 116	- 1.96347 4124
CBX8	chromobox 8	2.06029 7534		ALAS2	5'-aminolevulinate synthase 2	- 1.96347 4124
TOR2A	torsin family 2 member A	2.05653 5553		SDC4	syndecan 4	- 1.96347 4124
E230025N2 2RIK	Riken cDNA E230025N22 gene(E230025N22Rik)	2.05311 1336		LENG1	leukocyte receptor cluster member 1	- 1.96347 4124
OLFR963	olfactory receptor 963(Olfr963)	2.05311 1336		TRIM65	tripartite motif containing 65	- 1.96347 4124
OLFR694	olfactory receptor 694(Olfr694)	2.05311 1336		ADRA2B	adrenoceptor alpha 2B	- 1.96347 4124
AKR1B8	aldo-keto reductase family 1, member B8(Akr1b8)	2.05311 1336		CPSF4	cleavage and polyadenylation specific factor 4	- 1.96401 6356
UGDH	UDP-glucose 6-dehydrogenase	2.05311 1336		LRCH1	leucine rich repeats and calponin homology domain containing 1	- 1.96606 8313
CLPB	ClpB homolog, mitochondrial AAA ATPase chaperonin	2.05311 1336		CPXM1	carboxypeptidase X (M14 family), member 1	- 1.96782 195
KLHDC9	kelch domain containing 9	2.05311 1336		PARP6	poly(ADP-ribose) polymerase family member 6	- 1.96836 2498
MCPH1	microcephalin 1	2.05121 1057		GTF3C2	general transcription factor IIIC subunit 2	- 1.97568 7807
IL2RA	interleukin 2 receptor subunit alpha	2.04922 5103		NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase	- 1.97851 8523
CAR9	carbonic anhydrase 9(Car9)	2.04439 4119		DICER1	dicer 1, ribonuclease III	- 1.97959 126
USP10	ubiquitin specific peptidase 10	2.04439 4119		GBA2	glucosylceramidase beta 2	- 1.98038 7638
FASTKD2	FAST kinase domains 2	2.04439 4119		OLFR1269	olfactory receptor 1269(Olfr1269)	- 1.98185

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						2653
STRA13	stimulated by retinoic acid 13	2.04439 4119		EAR10	eosinophil-associated, ribonuclease A family, member 10(Ear10)	-1.98185 2653
HIST1H2AD	histone cluster 1, H2ad	2.04411 1161		ADAM5	ADAM metallopeptidase domain 5 (pseudogene)	-1.98185 2653
PLA1A	phospholipase A1 member A	2.03715 7781		MED1	mediator complex subunit 1	-1.98185 2653
MCM3	minichromosome maintenance complex component 3	2.03646 2274		FGFRL1	fibroblast growth factor receptor-like 1	-1.98185 2653
PIF1	PIF1 5'-to-3' DNA helicase	2.03609 4966		EXTL1	exostosin like glycosyltransferase 1	-1.98185 2653
GALR1	galanin receptor 1	2.03562 391		ZFHX3	zinc finger homeobox 3	-1.98185 2653
DLD	dihydrolipoamide dehydrogenase	2.03562 391		FBXO30	F-box protein 30	-1.98185 2653
GGCX	gamma-glutamyl carboxylase	2.03562 391		RNF112	ring finger protein 112	-1.98468 1148
CEP68	centrosomal protein 68	2.03562 391		PARP3	poly(ADP-ribose) polymerase family member 3	-1.98599 548
MMP11	matrix metallopeptidase 11	2.03562 391		AIRE	autoimmune regulator	-1.98641 0935
STMN1	stathmin 1	2.03331 6653		CYB561D1	cytochrome b561 family member D1	-1.98710 7951
SLCO4A1	solute carrier organic anion transporter family member 4A1	2.03217 627		TRAPPC5	trafficking protein particle complex 5	-1.98726 9174
TIAL1	TIA1 cytotoxic granule-associated RNA binding protein-like 1	2.02888 965		RFTN2	raftlin family member 2	-1.98749 308
0610009B22RIK	Description Not Found	2.01792 1908		FRAT1	frequently rearranged in advanced T-cell lymphomas 1	-1.99989 4159
GM1673	predicted gene 1673(Gm1673)	2.01792 1908		DMC1	DNA meiotic recombinase 1	-2
CCL26	C-C motif chemokine ligand 26	2.01792 1908		RIPK4	receptor interacting serine/threonine kinase 4	-2
ZWILCH	zwilch kinetochore protein	2.01792 1908		PVR	poliovirus receptor	-2
GABRA1	gamma-aminobutyric acid type A receptor alpha1 subunit	2.01792 1908		LPIN2	lipin 2	-2
ACP2	acid phosphatase 2, lysosomal	2.01714 3376		THAP2	THAP domain containing 2	-2
FAM131A	family with sequence similarity 131 member A	2.01321 9985		SHE	Src homology 2 domain containing E	-2
PXMP4	peroxisomal membrane protein 4	2.01249 7517		ARHGAP25	Rho GTPase activating protein 25	-2.00561 8551

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CDC6	cell division cycle 6	2.011166077		CSF1R	colony stimulating factor 1 receptor	-2.006350699
AXL	AXL receptor tyrosine kinase	2.008131619		ZFP1	ZFP1 zinc finger protein	-2.007904843
RBBP7	RB binding protein 7, chromatin remodeling factor	2.006746832		SFN	stratifin	-2.008988783
PABPC4	poly(A) binding protein cytoplasmic 4	2.005260152		COL17A1	collagen type XVII alpha 1	-2.010386372
HIST1H2AK	histone cluster 1, H2ak	2.003307679		XKRX	XK related, X-linked	-2.0105696
MTFMT	mitochondrial methionyl-tRNA formyltransferase	2.001754595		BRD8	bromodomain containing 8	-2.01346226
ZFP449	zinc finger protein 449(Zfp449)	2		ZFP213	zinc finger protein 213(Zfp213)	-2.013532276
D930020B18RIK	RIKEN cDNA D930020B18 gene(D930020B18Rik)	2		ZFY2	zinc finger protein 2, Y-linked(Zfy2)	-2.015657249
LCE1D	late cornified envelope 1D	2		MAP3K3	mitogen-activated protein kinase kinase kinase 3	-2.01612652
UCN	urocortin	2		ZFP445	zinc finger protein 445(Zfp445)	-2.017921908
SYT4	synaptotagmin 4	2		MTAP7D3	MAP7 domain containing 3(Mtap7d3)	-2.017921908
GPR132	G protein-coupled receptor 132	2		TMPRSS11A	transmembrane protease, serine 11A	-2.017921908
SDHD	succinate dehydrogenase complex subunit D	2		OLFM2	olfactomedin 2	-2.017921908
PANK3	pantothenate kinase 3	2		GRM4	glutamate metabotropic receptor 4	-2.017921908
SBSN	suprabasin	1.990954866		ONECUT2	one cut homeobox 2	-2.017921908
WDR59	WD repeat domain 59	1.989976974		HNRNPH3	heterogeneous nuclear ribonucleoprotein H3	-2.017921908
MTMR9	myotubularin related protein 9	1.987844644		ZMYM5	zinc finger MYM-type containing 5	-2.020204421
IL15RA	interleukin 15 receptor subunit alpha	1.985628881		RAPGEF6	Rap guanine nucleotide exchange factor 6	-2.020953989
RHBDF2	rhomboid 5 homolog 2	1.984681148		CD34	CD34 molecule	-2.026714044
NHLRC2	NHL repeat containing 2	1.98375117		ACVR2B	activin A receptor type 2B	-2.02671

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						4044
NMRAL1	NmrA-like family domain containing 1	1.98337 0163		RILP	Rab interacting lysosomal protein	- 2.02680 0059
OLFR120	olfactory receptor 120(Olfr120)	1.98185 2653		EMR1	Description Not Found	- 2.03121 8731
OLFR1051	olfactory receptor 1051(Olfr1051)	1.98185 2653		DNAJA2	DnaJ heat shock protein family (Hsp40) member A2	- 2.03129 1874
PCDHGA9	protocadherin gamma subfamily A, 9	1.98185 2653		SEMA4B	semaphorin 4B	- 2.03198 5281
FST	follistatin	1.98185 2653		1700015E1 3RIK	Description Not Found	- 2.03562 391
RECQL4	RecQ like helicase 4	1.97661 1605		RHOX1	reproductive homeobox 1(Rhox1)	- 2.03562 391
NFKBIL1	NFKB inhibitor like 1	1.97096 9489		TCP11	t-complex 11	- 2.03562 391
TUBD1	tubulin delta 1	1.96436 7355		FBXW11	F-box and WD repeat domain containing 11	- 2.03562 391
FSD1	fibronectin type III and SPRY domain containing 1	1.96347 4124		ALX1	ALX homeobox 1	- 2.03562 391
GDF5	growth differentiation factor 5	1.96347 4124		BST1	bone marrow stromal cell antigen 1	- 2.03562 391
TREML4	triggering receptor expressed on myeloid cells like 4	1.96347 4124		GPR83	G protein-coupled receptor 83	- 2.03562 391
SORD	sorbitol dehydrogenase	1.96347 4124		RECK	reversion inducing cysteine rich protein with kazal motifs	- 2.03611 2118
HEBP1	heme binding protein 1	1.96347 4124		ABHD14B	ablhydrolase domain containing 14B	- 2.04046 0993
KDELR2	KDEL endoplasmic reticulum protein retention receptor 2	1.96155 465		GPRC6A	G protein-coupled receptor class C group 6 member A	- 2.04212 2888
TRPV4	transient receptor potential cation channel subfamily V member 4	1.95884 2675		GRAMD3	GRAM domain containing 3	- 2.04229 6131
ABHD5	abhydrolase domain containing 5	1.95738 9419		IMPACT	impact RWD domain protein	- 2.04243 6285
YOD1	YOD1 deubiquitinase	1.95419 631		TOP1	topoisomerase (DNA) I	- 2.04439 4119
MAGOHB	mago homolog B, exon junction complex core component	1.95293 2368		NACC2	NACC family member 2	- 2.04439 4119
TSPAN2	tetraspanin 2	1.95176 103		PKNOX1	PBX/knotted 1 homeobox 1	- 2.04579 7958
LDB3	LIM domain binding 3	1.94850		TMEM79	transmembrane protein 79	-

		842			2.04662 8729
1700067P10 RIK	Description Not Found	1.94485 8446	MYCBP2	MYC binding protein 2, E3 ubiquitin protein ligase	- 2.04736 8853
9530091C0 8RIK	Description Not Found	1.94485 8446	MAS1	MAS1 proto-oncogene, G protein-coupled receptor	- 2.04805 5651
RHOJ	ras homolog family member J	1.94485 8446	GEMIN6	gem nuclear organelle associated protein 6	- 2.05311 1336
SFRP1	secreted frizzled related protein 1	1.94485 8446	TMEM100	transmembrane protein 100	- 2.05311 1336
XPNPEP2	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	1.94485 8446	FOXI1	forkhead box I1	- 2.05311 1336
RNASE4	ribonuclease A family member 4	1.93545 9748	OPLAH	5-oxoprolinase (ATP-hydrolysing)	- 2.05311 1336
NAPSA	napsin A aspartic peptidase	1.93158 6931	BC094916	Description Not Found	- 2.05833 7935
TIMM22	translocase of inner mitochondrial membrane 22 homolog (yeast)	1.93120 2999	GZMM	granzyme M	- 2.06119 3332
MTCH2	mitochondrial carrier 2	1.92977 4464	RCOR2	REST corepressor 2	- 2.06280 495
ADCK4	aarF domain containing kinase 4	1.92792 1426	NR2E1	nuclear receptor subfamily 2 group E member 1	- 2.06366 268
PDSS1	prenyl (decaprenyl) diphosphate synthase, subunit 1	1.92624 5513	NT5DC1	5'-nucleotidase domain containing 1	- 2.06599 4119
ZFP94	zinc finger protein 94(Zfp94)	1.92599 9419	SCN8A	sodium voltage-gated channel alpha subunit 8	- 2.06750 099
FABP9	fatty acid binding protein 9	1.92599 9419	CBX7	chromobox 7	- 2.06750 099
RNF170	ring finger protein 170	1.92599 9419	FHAD1	forkhead associated phosphopeptide binding domain 1	- 2.06811 4527
TLR3	toll like receptor 3	1.92599 9419	KCNQ3	potassium voltage-gated channel subfamily Q member 3	- 2.06888 5643
LIPH	lipase H	1.92599 9419	BC025920	zinc finger protein pseudogene(BC025920)	- 2.07038 9328
PLEKHA7	pleckstrin homology domain containing A7	1.92599 9419	FCGR1	Fc receptor, IgG, high affinity I(Fcgr1)	- 2.07038 9328
LXN	latexin	1.92446 06	SYN3	synapsin III	- 2.07038 9328
PPCS	phosphopantothenoylcys teine synthetase	1.92294 738	KLHL5	kelch like family member 5	- 2.07038 9328

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[0195]

BTRC	beta-transducin repeat containing E3 ubiquitin protein ligase	1.92065845		EDA2R	ectodysplasin A2 receptor	-2.070389328
APIP	APAF1 interacting protein	1.92032 6443		STK38	serine/threonine kinase 38	-2.070389328
ANK1	ankyrin 1	1.91647 6644		CDKN2D	cyclin dependent kinase inhibitor 2D	-2.072205467
TOMM70A	translocase of outer mitochondrial membrane 70 homolog A (yeast)(Tomm70a)	1.91310 7017		IL6ST	interleukin 6 signal transducer	-2.072660321
ABCB1B	ATP-binding cassette, sub-family B (MDR/TAP), member 1B(Abcb1b)	1.90803 3945		OLFR427	olfactory receptor 427(Olfr427)	-2.074318985
ACN9	Description Not Found	1.90689 0596		BAIAP2	BAI1 associated protein 2	-2.078951341
DLX1AS	distal-less homeobox 1, antisense(Dlx1as)	1.90689 0596		TIMP2	TIMP metallopeptidase inhibitor 2	-2.079805224
MRGPRD	MAS related GPR family member D	1.90689 0596		CDCP1	CUB domain containing protein 1	-2.083991945
WDHD1	WD repeat and HMG-box DNA binding protein 1	1.90689 0596		RGS14	regulator of G-protein signaling 14	-2.084198537
USP46	ubiquitin specific peptidase 46	1.90689 0596		VASP	vasodilator-stimulated phosphoprotein	-2.086359868
PKN3	protein kinase N3	1.90689 0596		ZFP318	zinc finger protein 318(Zfp318)	-2.087462841
OSCAR	osteoclast associated, immunoglobulin-like receptor	1.90689 0596		PSG25	pregnancy-specific glycoprotein 25(Psg25)	-2.087462841
CDK2	cyclin dependent kinase 2	1.90674 6727		PDZD8	PDZ domain containing 8	-2.087462841
TRIM62	tripartite motif containing 62	1.90552 0967		DET1	de-etiolated homolog 1 (Arabidopsis)	-2.087462841
SQLE	squalene epoxidase	1.90376 7694		CHST3	carbohydrate sulfotransferase 3	-2.087462841
MCM10	minichromosome maintenance 10 replication initiation factor	1.89598 378		EHHADH	enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	-2.087462841
CCDC90B	coiled-coil domain containing 90B	1.89480 3124		FCGRT	Fc fragment of IgG receptor and transporter	-2.090735607
SPATS1	spermatogenesis associated serine rich 1	1.89284 8083		CFP	complement factor properdin	-2.09437407
GPNMB	glycoprotein nmb	1.89142 7809		SOCS6	suppressor of cytokine signaling 6	-2.09463

[0196]

						8136
MST1	macrophage stimulating 1	1.88993148		SYT11	synaptotagmin 11	-2.09592442
LTB4R1	leukotriene B4 receptor 1(Ltb4r1)	1.887644112		MBTPS2	membrane bound transcription factor peptidase, site 2	-2.09592442
DNAJC5B	DnaJ heat shock protein family (Hsp40) member C5 beta	1.887525271		MEFV	Mediterranean fever	-2.097059135
PCDHGC4	protocadherin gamma subfamily C, 4	1.887525271		SRPK2	SRSF protein kinase 2	-2.10044313
HMX2	H6 family homeobox 2	1.887525271		DUSP16	dual specificity phosphatase 16	-2.102740277
NDUFAB1	NADH:ubiquinone oxidoreductase subunit AB1	1.887525271		SLC6A7	solute carrier family 6 member 7	-2.103129681
MGP	matrix Gla protein	1.887525271		HBB-B1	hemoglobin, beta adult major chain(Hbb-b1)	-2.10433666
ZKSCAN2	zinc finger with KRAB and SCAN domains 2	1.887525271		TNPO3	transportin 3	-2.10433666
CCDC51	coiled-coil domain containing 51	1.887525271		CSNK2B	casein kinase 2 beta	-2.10433666
CTSK	cathepsin K	1.887525271		BCAS1	breast carcinoma amplified sequence 1	-2.10433666
PRDM9	PR domain 9	1.887525271		INO80	INO80 complex subunit	-2.10433666
C8A	complement component 8 alpha subunit	1.887525271		MPG	N-methylpurine DNA glycosylase	-2.10433666
NEUROG1	neurogenin 1	1.887082413		FOXP1	forkhead box P1	-2.107557734
NUSAP1	nucleolar and spindle associated protein 1	1.886951242		USP21	ubiquitin specific peptidase 21	-2.107658353
LZIC	leucine zipper and CTNNBIP1 domain containing	1.877899051		LIMS1	LIM zinc finger domain containing 1	-2.112700133
ZFP609	zinc finger protein 609(Zfp609)	1.87774425		FXYD1	FXYD domain containing ion transport regulator 1	-2.112700133
GPR87	G protein-coupled receptor 87	1.87774425		POU3F1	POU class 3 homeobox 1	-2.113574207
GMPPB	GDP-mannose pyrophosphorylase B	1.871523637		OLFR591	olfactory receptor 591(Olfr591)	-2.114494844
TMEM115	transmembrane protein 115	1.870364796		GRAMD4	GRAM domain containing 4	-2.114673101
DSN1	DSN1 homolog, MIS12	1.86847		BCL2	BCL2, apoptosis regulator	-

[0197]

	kinetochore complex component	9018				2.11587 8669
A530099J1 9RIK	Description Not Found	1.86789 6464		PELI3	pellino E3 ubiquitin protein ligase family member 3	- 2.11891 5146
1700007K0 9RIK	Description Not Found	1.86789 6464		PPP1CB	protein phosphatase 1 catalytic subunit beta	- 2.11923 6221
1810043G0 2RIK	Description Not Found	1.86789 6464		TFF2	trefoil factor 2	- 2.12101 5401
UCHL1	ubiquitin C-terminal hydrolase L1	1.86789 6464		GCA	grancalcin	- 2.12101 5401
PTCH2	patched 2	1.86789 6464		LYL1	LYL1, basic helix-loop-helix family member	- 2.12101 5401
APBB3	amyloid beta precursor protein binding family B member 3	1.86789 6464		ATG4B	autophagy related 4B cysteine peptidase	- 2.12101 5401
PTER	phosphotriesterase related	1.86789 6464		CCDC102A	coiled-coil domain containing 102A	- 2.12101 5401
PRKCE	protein kinase C epsilon	1.86789 6464		ATP2A1	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 1	- 2.12101 5401
PLEKHM3	pleckstrin homology domain containing M3	1.86789 6464		TERF2	telomeric repeat binding factor 2	- 2.12358 5568
HIST1H4C	histone cluster 1, H4c	1.86789 6464		LCN5	lipocalin 5(Lcn5)	- 2.12443 2612
PLS3	plastin 3	1.86789 6464		TM6SF1	transmembrane 6 superfamily member 1	- 2.12453 3495
DUSP4	dual specificity phosphatase 4	1.86768 6654		SSBP2	single stranded DNA binding protein 2	- 2.12928 3017
SCLY	selenocysteine lyase	1.86280 2277		KRTAP6-2	keratin associated protein 6-2	- 2.13750 3524
RPRD1A	regulation of nuclear pre-mRNA domain containing 1A	1.86177 7838		CRHBP	corticotropin releasing hormone binding protein	- 2.13750 3524
CCRL2	C-C motif chemokine receptor like 2	1.86175 579		TOPBP1	topoisomerase (DNA) II binding protein 1	- 2.13750 3524
CCT7	chaperonin containing TCP1 subunit 7	1.86163 6037		SLC35A3	solute carrier family 35 member A3	- 2.13750 3524
ZFP217	zinc finger protein 217(Zfp217)	1.86109 7096		CACNB4	calcium voltage-gated channel auxiliary subunit beta 4	- 2.13750 3524
ACTN4	actinin alpha 4	1.85968 9938		TASP1	taspase 1	- 2.13750 3524
KCNA3	potassium voltage-gated channel subfamily A	1.85913 5363		HMBOX1	homeobox containing 1	- 2.14531

[0198]

	member 3					3833
CUL7	cullin 7	1.85859 7911		ZFP62	ZFP62 zinc finger protein	-2.14567 7455
LRRC59	leucine rich repeat containing 59	1.85754 3219		PCDHB4	protocadherin beta 4	-2.14866 6128
PHTF2	putative homeodomain transcription factor 2	1.85560 2651		SLC35F3	solute carrier family 35 member F3	-2.15120 644
KDELC1	KDEL motif containing 1	1.85255 6218		AW549877	expressed sequence AW549877(AW549877)	-2.15132 4826
SEC24D	SEC24 homolog D, COPII coat complex component	1.84838 41		GIMAP9	GTPase, IMAP family member 9(Gimap9)	-2.15240 0921
OLFR222	olfactory receptor 222(Olfr222)	1.84799 6907		ZFP329	zinc finger protein 329(Zfp329)	-2.15380 5336
OLFR118	olfactory receptor 118(Olfr118)	1.84799 6907		KRT74	keratin 74	-2.15380 5336
CASKIN2	CASK interacting protein 2	1.84799 6907		REG3A	regenerating family member 3 alpha	-2.15380 5336
TPK1	thiamin pyrophosphokinase 1	1.84799 6907		RAB4A	RAB4A, member RAS oncogene family	-2.15430 8231
NOL3	nucleolar protein 3	1.84799 6907		CECR5	cat eye syndrome chromosome region, candidate 5	-2.15568 2653
UBA6	ubiquitin like modifier activating enzyme 6	1.84738 8943		ESM1	endothelial cell specific molecule 1	-2.15715 6463
RAVER1	ribonucleoprotein, PTB binding 1	1.84615 1947		HS6ST1	heparan sulfate 6-O-sulfotransferase 1	-2.16482 0712
NAT10	N-acetyltransferase 10	1.84330 0131		DDB2	damage specific DNA binding protein 2	-2.16833 8824
HIST1H3H	histone cluster 1, H3h	1.84205 5889		5430435G2 2RIK	Description Not Found	-2.16992 5001
SNX8	sorting nexin 8	1.84098 5134		ALOX12B	arachidonate 12-lipoxygenase, 12R type	-2.16992 5001
POLR3K	polymerase (RNA) III subunit K	1.83953 8616		SLC34A3	solute carrier family 34 member 3	-2.16992 5001
WDR55	WD repeat domain 55	1.83595 7408		TNS4	tensin 4	-2.16992 5001
WDR93	WD repeat domain 93	1.83054 1464		CANX	calnexin	-2.16992 5001
PLSCR1	phospholipid scramblase 1	1.82863 5636		BET1	Bet1 golgi vesicular membrane trafficking protein	-2.16992 5001
ARL6	ADP ribosylation factor	1.82781		BEST2	bestrophin 2	-

	like GTPase 6	9025			2.16992 5001
NOL9	nucleolar protein 9	1.82781 9025	USP28	ubiquitin specific peptidase 28	-2.17299 8154
PNKD	paroxysmal nonkinesigenic dyskinesia	1.82781 9025	PDE4B	phosphodiesterase 4B	-2.17361 4018
TMEM139	transmembrane protein 139	1.82781 9025	CNOT4	CCR4-NOT transcription complex subunit 4	-2.17791 7792
ASPH	aspartate beta-hydroxylase	1.82781 9025	NECAP1	NECAP endocytosis associated 1	-2.17804 3245
LZTFL1	leucine zipper transcription factor like 1	1.82781 9025	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	-2.17856 5309
RHEBL1	Ras homolog enriched in brain like 1	1.82781 9025	SLC10A7	solute carrier family 10 member 7	-2.17990 909
CHCHD5	coiled-coil-helix-coiled-coil-helix domain containing 5	1.82552 849	IL17A	interleukin 17A	-2.18170 2586
GPD2	glycerol-3-phosphate dehydrogenase 2	1.82414 8697	ERICH1	glutamate rich 1	-2.18228 6216
STK39	serine/threonine kinase 39	1.82360 8879	HN1L	hematological and neurological expressed 1-like	-2.18586 6545
MAGED2	MAGE family member D2	1.82086 3253	SLFNL1	schlafen like 1	-2.18586 6545
TBC1D9B	TBC1 domain family member 9B	1.81321 9568	MYOD1	myogenic differentiation 1	-2.18586 6545
LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	1.80954 0228	TRIM35	tripartite motif containing 35	-2.18586 6545
OLFR859	olfactory receptor 859(Olfr859)	1.80735 4922	CHRNE	cholinergic receptor nicotinic epsilon subunit	-2.18639 7884
OLFR1225	olfactory receptor 1225(Olfr1225)	1.80735 4922	PHF21A	PHD finger protein 21A	-2.19094 3197
IFNA11	interferon alpha 11(Ifna11)	1.80735 4922	HIST1H2AE	histone cluster 1, H2ae	-2.19669 8179
ARG1	arginase 1	1.80735 4922	SATB1	SATB homeobox 1	-2.19865 9952
ASCL3	achaete-scute family bHLH transcription factor 3	1.80735 4922	LCN8	lipocalin 8	-2.20163 3861
AGA	aspartylglucosaminidase	1.80735 4922	ABCG5	ATP binding cassette subfamily G member 5	-2.20163 3861
MAP3K12	mitogen-activated protein kinase kinase kinase 12	1.80653 0545	KRBA1	KRAB-A domain containing 1	-2.20295 9029

[0199]

[0200]

COMMD10	COMM domain containing 10	1.80277 1724		CD274	CD274 molecule	-2.20608 1393
STYX	serine/threonine/tyrosine interacting protein	1.80125 1483		DYRK2	dual specificity tyrosine phosphorylation regulated kinase 2	-2.20673 0511
EPHA6	EPH receptor A6	1.79758 3147		ZFP292	zinc finger protein 292(Zfp292)	-2.20945 3366
SERPINA3F	serine (or cysteine) peptidase inhibitor, clade A, member 3F(Serpina3f)	1.79444 5043		PRX	periaxin	-2.20945 3366
PUS10	pseudouridylate synthase 10	1.79181 4071		SPAG1	sperm associated antigen 1	-2.20945 3366
RASL12	RAS like family 12	1.79165 2715		ASGR2	asialoglycoprotein receptor 2	-2.20978 4456
MRPL51	mitochondrial ribosomal protein L51	1.78763 1232		PTEN	phosphatase and tensin homolog	-2.21501 3513
OLFR1306	olfactory receptor 1306(Olfr1306)	1.78659 6362		IL1A	interleukin 1 alpha	-2.21723 0716
BCL2A1C	B cell leukemia/lymphoma 2 related protein A1c(Bcl2a1c)	1.78659 6362		TPCN2	two pore segment channel 2	-2.21723 0716
HOXD1	homeobox D1	1.78659 6362		IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	-2.21723 0716
MEMO1	mediator of cell motility 1	1.78659 6362		ST6GAL1	ST6 beta-galactoside alpha-2,6-sialyltransferase 1	-2.21834 2351
ARCN1	archain 1	1.78659 6362		TMEM161A	transmembrane protein 161A	-2.23266 0757
NUDT10	nudix hydrolase 10	1.78659 6362		STK32B	serine/threonine kinase 32B	-2.23266 0757
SLC4A4	solute carrier family 4 member 4	1.78659 6362		CHST14	carbohydrate sulfotransferase 14	-2.23266 0757
DHRS4	dehydrogenase/reductase 4	1.78659 6362		AQP3	aquaporin 3 (Gill blood group)	-2.23266 0757
TOM1	target of myb1 membrane trafficking protein	1.78659 6362		RASSF3	Ras association domain family member 3	-2.23350 5898
TST	thiosulfate sulfurtransferase	1.78659 6362		OTUD7B	OTU deubiquitinase 7B	-2.24292 3867
RIPK2	receptor interacting serine/threonine kinase 2	1.78442 8584		AP3M2	adaptor related protein complex 3 mu 2 subunit	-2.24748 1244
NAIP2	NLR family, apoptosis inhibitory protein 2(Naip2)	1.78035 1745		PSMA6	proteasome subunit alpha 6	-2.24792 7513

[0201]

OLFR133	olfactory receptor 133(Olfr133)	1.77946628		PRCC	papillary renal cell carcinoma (translocation-associated)	-2.247927513
NBR1	NBR1, autophagy cargo receptor	1.77699 5396		ZFP688	zinc finger protein 688(Zfp688)	-2.262218541
GLIS1	GLIS family zinc finger 1	1.77651 2203		DOCK11	dedicator of cytokinesis 11	-2.262218541
SLC35A2	solute carrier family 35 member A2	1.77623 2819		PLA2G4F	phospholipase A2 group IVF	-2.263034406
AU022252	expressed sequence AU022252(AU022252)	1.77455 9318		MYPN	myopalladin	-2.263034406
OLFR64	olfactory receptor 64(Olfr64)	1.77399 1786		FRS2	fibroblast growth factor receptor substrate 2	-2.263034406
PPAPDC2	Description Not Found	1.77198 3065		STARD6	StAR related lipid transfer domain containing 6	-2.263034406
DIS3	DIS3 homolog, exosome endoribonuclease and 3'-5' exoribonuclease	1.77137 5295		WSCD2	WSC domain containing 2	-2.270653766
4931440F15RIK	Description Not Found	1.77082 9046		TLE1	transducin like enhancer of split 1	-2.272631746
ZFP771	zinc finger protein 771(Zfp771)	1.77019 569		HDHD3	haloacid dehalogenase like hydrolase domain containing 3	-2.272966802
HMBS	hydroxymethylbilane synthase	1.76967 6967		1700029J07RIK	Description Not Found	-2.277984747
RCC1	regulator of chromosome condensation 1	1.76826 7605		CLEC2D	C-type lectin domain family 2 member D	-2.277984747
SPAG5	sperm associated antigen 5	1.76798 0257		PPM1G	protein phosphatase, Mg2+/Mn2+ dependent 1G	-2.277984747
TSPAN31	tetraspanin 31	1.76762 6782		CDKN1B	cyclin dependent kinase inhibitor 1B	-2.280970508
PCDHGB8	protocadherin gamma subfamily B, 8(Pcdhgb8)	1.76553 4746		OASL1	2'-5' oligoadenylate synthetase-like 1(Oasl1)	-2.28169825
PRL2B1	prolactin family 2, subfamily b, member 1(Prl2b1)	1.76553 4746		G0S2	G0/G1 switch 2	-2.282045463
OBOX5	oocyte specific homeobox 5(Obox5)	1.76553 4746		TMEM17	transmembrane protein 17	-2.285402219
PIK3R3	phosphoinositide-3-kinase regulatory subunit 3	1.76553 4746		BLVRB	biliverdin reductase B	-2.290619427
MAP3K4	mitogen-activated protein kinase kinase kinase 4	1.76553 4746		GOSR1	golgi SNAP receptor complex member 1	-2.290897209
LRRC30	leucine rich repeat containing 30	1.76553 4746		ZFP26	zinc finger protein 26(Zfp26)	-2.29278

[0202]

						1749
EN2	engrailed homeobox 2	1.76553 4746		CXCL2	C-X-C motif chemokine ligand 2	-2.29278 1749
HOOK3	hook microtubule-tethering protein 3	1.76553 4746		SNX7	sorting nexin 7	-2.29278 1749
MYO9A	myosin IXA	1.76553 4746		ZDHHC23	zinc finger DHHC-type containing 23	-2.29278 1749
STX7	syntaxin 7	1.76506 0364		GALNT6	polypeptide N-acetylgalactosaminyltransferase 6	-2.29278 1749
ATM	ATM serine/threonine kinase	1.76350 4031		AMPD1	adenosine monophosphate deaminase 1	-2.29784 4157
KCNK6	potassium two pore domain channel subfamily K member 6	1.76338 5753		GIMAP5	GTPase, IMAP family member 5	-2.30324 6615
PQLC3	PQ loop repeat containing 3	1.75995 4577		ATP5F1	ATP synthase, H+ transporting, mitochondrial Fo complex subunit B1	-2.30539 9163
KIFAP3	kinesin associated protein 3	1.75884 3168		LHFPL2	lipoma HMGIC fusion partner-like 2	-2.30742 8525
E2F4	E2F transcription factor 4	1.75775 2886		KIF1B	kinesin family member 1B	-2.31323 1129
ETV5	ETS variant 5	1.75770 9335		TLE6	transducin like enhancer of split 6	-2.32192 8095
GTF2E2	general transcription factor IIE subunit 2	1.75666 387		SHF	Src homology 2 domain containing F	-2.33069 1998
GPR150	G protein-coupled receptor 150	1.75547 927		NGFR	nerve growth factor receptor	-2.33143 8521
E130308A19RIK	RIKEN cDNA E130308A19 gene(E130308A19Rik)	1.75488 7502		KLRA4	killer cell lectin-like receptor, subfamily A, member 4(Klra4)	-2.33448 5632
DPYSL4	dihydropyrimidinase like 4	1.75488 7502		ITGAE	integrin subunit alpha E	-2.33594 8972
FNBP1	formin binding protein 1	1.75468 902		PQLC2	PQ loop repeat containing 2	-2.33614 1568
TMOD4	tropomodulin 4	1.75406 4107		KLRB1A	killer cell lectin-like receptor subfamily B member 1A(Klrb1a)	-2.33628 3388
ERLIN1	ER lipid raft associated 1	1.75115 4691		IRF9	interferon regulatory factor 9	-2.33630 8285
ENOPH1	enolase-phosphatase 1	1.74844 7442		GATA3	GATA binding protein 3	-2.33897 1433
RAB31	RAB31, member RAS oncogene family	1.74621 5332		RSAD2	radical S-adenosyl methionine domain containing 2	-2.33997 952

[0203]

HOXA6	homeobox A6	1.745184623	RNF215	ring finger protein 215	-2.341976415
TAS2R126	taste receptor, type 2, member 126(Tas2r126)	1.744161096	IL7R	interleukin 7 receptor	-2.343395577
AGXT2	alanine--glyoxylate aminotransferase 2	1.744161096	ACP5	acid phosphatase 5, tartrate resistant	-2.345270806
STK32C	serine/threonine kinase 32C	1.744161096	STYXL1	serine/threonine/tyrosine interacting-like 1	-2.346956889
P2RY2	purinergic receptor P2Y2	1.744161096	NOXO1	NADPH oxidase organizer 1	-2.35030956
NWD1	NACHT and WD repeat domain containing 1	1.744161096	IGFALS	insulin like growth factor binding protein acid labile subunit	-2.358664554
UQCRQ	ubiquinol-cytochrome c reductase complex III subunit VII	1.744161096	STIM1	stromal interaction molecule 1	-2.359335599
PPP1R3A	protein phosphatase 1 regulatory subunit 3A	1.744161096	TMEM186	transmembrane protein 186	-2.361030771
GOLT1A	golgi transport 1A	1.744161096	OLFR1043	olfactory receptor 1043(Olfr1043)	-2.364572432
EZH1	enhancer of zeste 1 polycomb repressive complex 2 subunit	1.744161096	D8ERTD82E	DNA segment, Chr 8, ERATO Doi 82, expressed(D8Ertd82e)	-2.364572432
MTHFD2	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	1.744154314	MYOG	myogenin	-2.364572432
PGRMC1	progesterone receptor membrane component 1	1.742545062	NCLN	nicalin	-2.364572432
DNAJB12	DnaJ heat shock protein family (Hsp40) member B12	1.741863621	MTSS1	metastasis suppressor 1	-2.364572432
DNAJC11	DnaJ heat shock protein family (Hsp40) member C11	1.738767837	TRMU	tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase	-2.364572432
TOMM6	translocase of outer mitochondrial membrane 6	1.738448709	EMILIN2	elastin microfibril interfacer 2	-2.369119767
RPS6KL1	ribosomal protein S6 kinase like 1	1.738393453	MPV17L	MPV17 mitochondrial inner membrane protein like	-2.371558863
CDC73	cell division cycle 73	1.736657741	WWC2	WW and C2 domain containing 2	-2.371558863
NDC80	NDC80, kinetochore complex component	1.732078892	TMEM178	transmembrane protein 178(Tmem178)	-2.374005585
TACC3	transforming acidic coiled-coil containing	1.731372884	TPCN1	two pore segment channel 1	-2.37523

[0204]

	protein 3				2208
CPSF3	cleavage and polyadenylation specific factor 3	1.72792 6568	LRRC45	leucine rich repeat containing 45	-2.37720 7351
ARID3A	AT-rich interaction domain 3A	1.72647 1722	1110059G1 0RIK	Description Not Found	-2.37791 5929
LLPH	LLP homolog, long-term synaptic facilitation	1.72610 7859	MCOLN2	mucolipin 2	-2.37851 1623
PCNA	proliferating cell nuclear antigen	1.72544 1599	DDX58	DEXD/H-box helicase 58	-2.37851 1623
GJC2	gap junction protein gamma 2	1.72297 8517	H2-OA	histocompatibility 2, O region alpha locus(H2-Oa)	-2.38232 9516
OLFR373	olfactory receptor 373(Olfr373)	1.72246 6024	RARG	retinoic acid receptor gamma	-2.38882 7772
H2-T24	histocompatibility 2, T region locus 24(H2-T24)	1.72246 6024	SERPINB1 A	serine (or cysteine) peptidase inhibitor, clade B, member 1a(Serpinb1a)	-2.39231 7423
AKAP7	A-kinase anchoring protein 7	1.72246 6024	GHRL	ghrelin/obestatin prepropeptide	-2.39231 7423
NDUFB7	NADH:ubiquinone oxidoreductase subunit B7	1.72246 6024	ZMAT4	zinc finger matrin-type 4	-2.39231 7423
PRR11	proline rich 11	1.72246 6024	BTBD6	BTB domain containing 6	-2.39289 7478
TJP1	tight junction protein 1	1.72246 6024	KLRA16	killer cell lectin-like receptor, subfamily A, member 16(Klra16)	-2.39453 4969
S100A3	S100 calcium binding protein A3	1.72246 6024	EPS15L1	epidermal growth factor receptor pathway substrate 15 like 1	-2.39701 2831
KRT78	keratin 78	1.71872 9711	VCPIP1	valosin containing protein interacting protein 1	-2.39730 3585
GMDS	GDP-mannose 4,6-dehydratase	1.71790 4741	RRP7A	ribosomal RNA processing 7 homolog A	-2.40499 2223
PDGFB	platelet derived growth factor subunit B	1.71440 0534	IL1B	interleukin 1 beta	-2.40599 236
SLC36A1	solute carrier family 36 member 1	1.71429 7338	NAT14	N-acetyltransferase 14 (putative)	-2.40599 236
RSU1	Ras suppressor protein 1	1.71264 7036	SLC40A1	solute carrier family 40 member 1	-2.40599 236
STX12	syntaxin 12	1.71191 1478	RAB37	RAB37, member RAS oncogene family	-2.40599 236
SLC25A34	solute carrier family 25 member 34	1.71149 4907	IL17RA	interleukin 17 receptor A	-2.40599 236
AFG3L2	AFG3 like matrix AAA	1.71105	BACE1	beta-secretase 1	-

[0205]

	peptidase subunit 2	7				2.40599236
RPL24	ribosomal protein L24	1.709193708		CTNS	cystinosin, lysosomal cystine transporter	-2.40599236
UBE3C	ubiquitin protein ligase E3C	1.708789682		IFIT3	interferon induced protein with tetratricopeptide repeats 3	-2.411404504
CAR12	carbonic anhydrase 12(Car12)	1.70867626		ZFYVE21	zinc finger FYVE-type containing 21	-2.412378292
ZFP207	zinc finger protein 207(Zfp207)	1.707603009		1700016D06RIK	Description Not Found	-2.419538892
XIST	X inactive specific transcript (non-protein coding)	1.706065607		STK25	serine/threonine kinase 25	-2.419538892
NCAPD2	non-SMC condensin I complex subunit D2	1.705012178		PLEKHJ1	pleckstrin homology domain containing J1	-2.419538892
ZSWIM2	zinc finger SWIM-type containing 2	1.704802998		TGIF2	TGFB induced factor homeobox 2	-2.419538892
CASP1	caspase 1	1.70065942		SLC25A29	solute carrier family 25 member 29	-2.419538892
OLFR701	olfactory receptor 701(Olfr701)	1.700439718		DAPL1	death associated protein like 1	-2.419661316
CBLC	Cbl proto-oncogene C	1.700439718		P2RX4	purinergic receptor P2X 4	-2.425748008
HIST1H2AC	histone cluster 1, H2ac	1.700439718		1700001O22RIK	Description Not Found	-2.426264755
EPHA10	EPH receptor A10	1.700439718		C9	complement component 9	-2.429615964
NDUFC2	NADH:ubiquinone oxidoreductase subunit C2	1.700439718		KLF13	Kruppel like factor 13	-2.430628023
DLG1	discs large MAGUK scaffold protein 1	1.700439718		GADD45A	growth arrest and DNA damage inducible alpha	-2.432591239
SCN10A	sodium voltage-gated channel alpha subunit 10	1.700439718		OLFR788	olfactory receptor 788(Olfr788)	-2.432959407
RGL3	ral guanine nucleotide dissociation stimulator like 3	1.700439718		FADS6	fatty acid desaturase 6	-2.432959407
TMCO3	transmembrane and coiled-coil domains 3	1.700439718		CHCHD2	coiled-coil-helix-coiled-coil-helix domain containing 2	-2.432959407
BCL2L14	BCL2 like 14	1.700439718		MPPE1	metallophosphoesterase 1	-2.432959407
THOP1	thimet oligopeptidase 1	1.700290033		CHAC1	ChaC glutathione specific gamma-glutamylcyclotransferase 1	-2.432959407

[0206]

MTIF3	mitochondrial translational initiation factor 3	1.69830 5331		2310011J03 RIK	Description Not Found	-2.43501 7448
XDH	xanthine dehydrogenase	1.69771 7724		LRSAM1	leucine rich repeat and sterile alpha motif containing 1	-2.43747 3925
ANXA9	annexin A9	1.69718 4071		SIRPA	signal regulatory protein alpha	-2.44312 5132
OLFR1502	olfactory receptor 1502(Olfr1502)	1.69404 6727		CYP24A1	cytochrome P450 family 24 subfamily A member 1	-2.44625 623
HCFC2	host cell factor C2	1.69378 0609		NQO1	NAD(P)H quinone dehydrogenase 1	-2.44625 623
DIDO1	death inducer-obliterator 1	1.69359 6948		HRH4	histamine receptor H4	-2.44625 623
PGAM1	phosphoglycerate mutase 1	1.68984 6917		NUDCD1	NudC domain containing 1	-2.44625 623
RASGEF1C	RasGEF domain family member 1C	1.68929 9161		CCND1	cyclin D1	-2.44792 4527
SLC25A42	solute carrier family 25 member 42	1.68677 4817		ADAM22	ADAM metallopeptidase domain 22	-2.45285 8965
CPT2	carnitine palmitoyltransferase 2	1.68636 4794		MDK	midkine (neurite growth-promoting factor 2)	-2.45614 9035
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	1.68616 1103		STX1A	syntaxin 1A	-2.45672 9828
NQO2	NAD(P)H quinone dehydrogenase 2	1.68555 8757		HEMK1	HemK methyltransferase family member 1	-2.45943 1619
HIP1R	huntingtin interacting protein 1 related	1.68547 3307		B4GALT7	beta-1,4-galactosyltransferase 7	-2.45943 1619
ALOX12E	arachidonate lipoxygenase, epidermal(Alox12e)	1.68437 3244		ASXL2	additional sex combs like 2, transcriptional regulator	-2.45943 1619
LMAN1	lectin, mannose binding 1	1.68351 4205		TLR7	toll like receptor 7	-2.46052 038
ASB3	ankyrin repeat and SOCS box containing 3	1.68014 2991		TDP1	tyrosyl-DNA phosphodiesterase 1	-2.46446 1869
XKR5	XK related 5	1.67925 4438		1700025G0 4RIK	Description Not Found	-2.46930 3076
ZFP235	zinc finger protein 235(Zfp235)	1.67807 1905		SLC16A6	solute carrier family 16 member 6	-2.47104 5434
OLFR971	olfactory receptor 971(Olfr971)	1.67807 1905		DOXL2	diamine oxidase-like protein 2(Doxl2)	-2.47248 7771
OLFR374	olfactory receptor 374(Olfr374)	1.67807 1905		PKD1L3	polycystin 1 like 3, transient receptor potential	-2.47248

[0207]

					channel interacting	7771
NOS1AP	nitric oxide synthase 1 adaptor protein	1.67807 1905		ZC3H11A	zinc finger CCCH-type containing 11A	-2.47248 7771
GALM	galactose mutarotase	1.67807 1905		LY6K	lymphocyte antigen 6 complex, locus K	-2.47248 7771
MEGF9	multiple EGF like domains 9	1.67807 1905		KLF7	Kruppel like factor 7	-2.47475 5307
CCDC66	coiled-coil domain containing 66	1.67807 1905		BTLA	B and T lymphocyte associated	-2.47560 4026
LRRC40	leucine rich repeat containing 40	1.67807 1905		CDON	cell adhesion associated, oncogene regulated	-2.48542 6827
RALA	RALA Ras like proto-oncogene A	1.67807 1905		DDC	dopa decarboxylase	-2.48542 6827
YIPF4	Yip1 domain family member 4	1.67807 1905		GTF2A2	general transcription factor IIA subunit 2	-2.48542 6827
TAL2	T-cell acute lymphocytic leukemia 2	1.67807 1905		DTX4	deltex E3 ubiquitin ligase 4	-2.48542 6827
LRRC8A	leucine rich repeat containing 8 family member A	1.67807 1905		GSTK1	glutathione S-transferase kappa 1	-2.48619 5934
APOM	apolipoprotein M	1.67807 1905		OLFR213	olfactory receptor 213(Olfr213)	-2.48912 5048
KCNG3	potassium voltage-gated channel modifier subfamily G member 3	1.67807 1905		PDE5A	phosphodiesterase 5A	-2.49057 1469
CNN1	calponin 1	1.67807 1905		TOB1	transducer of ERBB2, 1	-2.49676 3907
STAC2	SH3 and cysteine rich domain 2	1.67807 1905		1700109H0 8RIK	Description Not Found	-2.49825 0868
SFRP2	secreted frizzled related protein 2	1.67807 1905		LEFTY1	left-right determination factor 1	-2.49825 0868
SERPINB9 E	serine (or cysteine) peptidase inhibitor, clade B, member 9e(Serpinb9e)	1.67016 9131		SNAPC4	small nuclear RNA activating complex polypeptide 4	-2.50087 8922
TFB1M	transcription factor B1, mitochondrial	1.66894 6692		RNF41	ring finger protein 41	-2.50355 1585
SLC25A10	solute carrier family 25 member 10	1.66885 6925		KLHL34	kelch like family member 34	-2.50462 0392
BID	BH3 interacting domain death agonist	1.66799 2567		SSH2	slingshot protein phosphatase 2	-2.50549 2762
MRPS27	mitochondrial ribosomal protein S27	1.66729 5766		CAMK2B	calcium/calmodulin dependent protein kinase II beta	-2.50704 7355

[0208]

NEDD4	neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase	1.66675 6592		IRF7	interferon regulatory factor 7	-2.50759 0939
VANGL2	VANGL planar cell polarity protein 2	1.66675 6592		SCML4	sex comb on midleg-like 4 (Drosophila)	-2.52311 8672
UBE2R2	ubiquitin conjugating enzyme E2 R2	1.66664 1116		EPB4.1	Description Not Found	-2.52356 1956
KLHL30	kelch like family member 30	1.66651 9523		PARP12	poly(ADP-ribose) polymerase family member 12	-2.52356 1956
FBXO36	F-box protein 36	1.66558 8375		CACNB3	calcium voltage-gated channel auxiliary subunit beta 3	-2.52987 7218
DCT	dopachrome tautomerase	1.66401 6818		NRG4	neuregulin 4	-2.53318 567
CCDC120	coiled-coil domain containing 120	1.66393 1727		OLFR1383	olfactory receptor 1383(Olfr1383)	-2.53605 29
TMEM38B	transmembrane protein 38B	1.66345 5268		PTGR1	prostaglandin reductase 1	-2.53605 29
ENDOD1	endonuclease domain containing 1	1.66332 7923		NFAM1	NFAT activating protein with ITAM motif 1	-2.53605 29
PTPRD	protein tyrosine phosphatase, receptor type D	1.66321 5776		ARL4C	ADP ribosylation factor like GTPase 4C	-2.53605 29
ARL3	ADP ribosylation factor like GTPase 3	1.66169 0196		LACE1	lactation elevated 1	-2.53605 29
CDC37	cell division cycle 37	1.66156 7827		CDC14B	cell division cycle 14B	-2.54535 0645
MKKS	McKusick-Kaufman syndrome	1.66106 548		GUCA1A	guanylate cyclase activator 1A	-2.54843 6625
CHN2	chimerin 2	1.66099 8764		KIF21B	kinesin family member 21B	-2.55458 8852
CRTAP	cartilage associated protein	1.65943 1912		ARID3B	AT-rich interaction domain 3B	-2.55808 7884
CXCR6	C-X-C motif chemokine receptor 6	1.65751 5938		HBA-A1	hemoglobin alpha, adult chain 1(Hba-a1)	-2.56071 4954
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	1.65691 495		CSF2RB2	colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage)(Csf2rb2)	-2.56071 4954
B430306N0 3RIK	RIKEN cDNA B430306N03 gene(B430306N03Rik)	1.65535 1829		ATP6V1B1	ATPase H+ transporting V1 subunit B1	-2.56071 4954
OLFR1262	olfactory receptor 1262(Olfr1262)	1.65535 1829		PCSK1N	proprotein convertase subtilisin/kexin type 1	-2.56071

[0209]

					inhibitor	4954
SLC38A5	solute carrier family 38 member 5	1.65535 1829		ZFP667	zinc finger protein 667(Zfp667)	-2.56667 0372
VAT1L	vesicle amine transport 1-like	1.65535 1829		SH3BP1	SH3 domain binding protein 1	-2.56673 4604
HOXB7	homeobox B7	1.65535 1829		FFAR2	free fatty acid receptor 2	-2.57288 9668
GAN	gigaxonin	1.65535 1829		EEF2K	eukaryotic elongation factor 2 kinase	-2.57288 9668
MMP28	matrix metallopeptidase 28	1.65535 1829		SLPI	secretory leukocyte peptidase inhibitor	-2.57472 1828
METTL10	methyltransferase like 10	1.65535 1829		CMA1	chymase 1	-2.58496 2501
SIX4	SIX homeobox 4	1.65535 1829		ASCL1	achaete-scute family bHLH transcription factor 1	-2.58496 2501
TDRD6	tudor domain containing 6	1.65535 1829		ACPP	acid phosphatase, prostate	-2.58496 2501
COMMD5	COMM domain containing 5	1.65460 4999		CLCNKB	chloride voltage-gated channel Kb	-2.59693 5142
PRDX4	peroxiredoxin 4	1.65192 3925		FBXW7	F-box and WD repeat domain containing 7	-2.59693 5142
HS3ST3A1	heparan sulfate-glucosamine 3-sulfotransferase 3A1	1.64929 8274		OLIG3	oligodendrocyte transcription factor 3	-2.59693 5142
CALCA	calcitonin related polypeptide alpha	1.64906 7786		WHRN	whirlin	-2.60678 9951
SLC12A2	solute carrier family 12 member 2	1.64844 9243		DNAJC14	DnaJ heat shock protein family (Hsp40) member C14	-2.60880 9243
TJP2	tight junction protein 2	1.64414 5647		PIGT	phosphatidylinositol glycan anchor biosynthesis class T	-2.61103 1218
LRRC16B	Description Not Found	1.64385 619		AP1G2	adaptor related protein complex 1 gamma 2 subunit	-2.61470 9844
AP3S2	adaptor related protein complex 3 sigma 2 subunit	1.64385 619		SAA2	serum amyloid A2	-2.62058 641
PSMD9	proteasome 26S subunit, non-ATPase 9	1.64385 619		USP30	ubiquitin specific peptidase 30	-2.62058 641
PARD6G	par-6 family cell polarity regulator gamma	1.64337 9419		RPE65	retinal pigment epithelium specific protein 65	-2.63226 8216
CIAPIN1	cytokine induced apoptosis inhibitor 1	1.64321 9709		CML1	Description Not Found	-2.63489 1632
CKAP5	cytoskeleton associated	1.64274		SLC6A19	solute carrier family 6	-

[0210]

	protein 5	7156			member 19	2.64093 0751
E430025E2 1RIK	RIKEN cDNA E430025E21 gene(E430025E21Rik)	1.64190 2626		FGF15	fibroblast growth factor 15(Fgf15)	- 2.64385 619
PIAS3	protein inhibitor of activated STAT 3	1.64188 4484		HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3	- 2.64385 619
USP1	ubiquitin specific peptidase 1	1.64023 3791		ADAMTSL 4	ADAMTS like 4	- 2.64385 619
RAB3GAP2	RAB3 GTPase activating non-catalytic protein subunit 2	1.63959 2623		HYAL3	hyaluronoglucosaminidase 3	- 2.64385 619
CSRP2	cysteine and glycine rich protein 2	1.63904 6229		SLC15A2	solute carrier family 15 member 2	- 2.64821 7996
MOV10	Mov10 RISC complex RNA helicase	1.63807 3837		UFSP1	UFM1-specific peptidase 1 (inactive)	- 2.64955 3823
GM1965	predicted gene 1965(Gm1965)	1.63788 1562		6430573F11 RIK	Description Not Found	- 2.65535 1829
POMGNT1	protein O-linked mannose N-acetylglucosaminyltransf erase 1 (beta 1,2-)	1.63623 7884		DNM3OS	DNM3 opposite strand/antisense RNA	- 2.65535 1829
FIGNL1	fidgetin like 1	1.63395 0492		F2RL1	F2R like trypsin receptor 1	- 2.65535 1829
TMEM177	transmembrane protein 177	1.63347 5547		SNX33	sorting nexin 33	- 2.66665 4581
ALX4	ALX homeobox 4	1.63286 4872		CXCL9	C-X-C motif chemokine ligand 9	- 2.66675 6592
OLFR533	olfactory receptor 533(Olfr533)	1.63226 8216		TEAD2	TEA domain transcription factor 2	- 2.66675 6592
H2-M10.3	histocompatibility 2, M region locus 10.3(H2-M10.3)	1.63226 8216		QSOX1	quiescin sulfhydryl oxidase 1	- 2.66675 6592
GPX7	glutathione peroxidase 7	1.63226 8216		TLR13	toll-like receptor 13(Tlr13)	- 2.67807 1905
STXBP6	syntaxin binding protein 6	1.63226 8216		SCD3	stearoyl-coenzyme A desaturase 3(Scd3)	- 2.67807 1905
RAB33A	RAB33A, member RAS oncogene family	1.63226 8216		SDC3	syndecan 3	- 2.67807 1905
PDCL3	phosducin like 3	1.63226 8216		GRPR	gastrin releasing peptide receptor	- 2.67807 1905
GPR20	G protein-coupled receptor 20	1.63226 8216		MAFK	MAF bZIP transcription factor K	- 2.67807 1905
GSTA2	glutathione S-transferase alpha 2	1.63226 8216		DIRC2	disrupted in renal carcinoma 2	- 2.67807

						1905
ADCY10	adenylate cyclase 10 (soluble)	1.63226 8216		ZCCHC12	zinc finger CCHC-type containing 12	-2.67833 354
PEX12	peroxisomal biogenesis factor 12	1.63226 8216		ADCY6	adenylate cyclase 6	-2.68088 6921
IQCC	IQ motif containing C	1.63226 8216		ECM1	extracellular matrix protein 1	-2.68345 512
ENPP1	ectonucleotide pyrophosphatase/phosph odiesterase 1	1.63208 6279		AFP	alpha fetoprotein	-2.68929 9161
ADAL	adenosine deaminase-like	1.63066 4126		GP5	glycoprotein V platelet	-2.68929 9161
SCRN2	secernin 2	1.63056 6247		GAB3	GRB2 associated binding protein 3	-2.69140 5681
CEP78	centrosomal protein 78	1.62985 1642		USP2	ubiquitin specific peptidase 2	-2.69333 4369
SLC25A15	solute carrier family 25 member 15	1.62979 8606		PLXNB1	plexin B1	-2.70043 9718
ADSSL1	adenylosuccinate synthase like 1	1.62827 2149		PODXL2	podocalyxin like 2	-2.70079 9925
TM6SF2	transmembrane 6 superfamily member 2	1.62775 8638		RAD9B	RAD9 checkpoint clamp component B	-2.70103 836
TUBG1	tubulin gamma 1	1.62451 1879		AKAP10	A-kinase anchoring protein 10	-2.70597 7902
FASTK	Fas activated serine/threonine kinase	1.62333 6662		PIGW	phosphatidylinositol glycan anchor biosynthesis class W	-2.71699 0894
RBBP5	RB binding protein 5, histone lysine methyltransferase complex subunit	1.62216 3711		COL12A1	collagen type XII alpha 1 chain	-2.72246 6024
1700071K0 1RIK	Description Not Found	1.62146 5074		GPR137B	G protein-coupled receptor 137B	-2.73335 4341
SLC25A33	solute carrier family 25 member 33	1.62128 2718		IMMP2L	inner mitochondrial membrane peptidase subunit 2	-2.73335 4341
MDM4	MDM4, p53 regulator	1.62058 641		PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta	-2.73732 0423
TOP2A	topoisomerase (DNA) II alpha	1.62037 4948		TGFBI	transforming growth factor beta induced	-2.74027 6443
OLFR139	olfactory receptor 139(Olfr139)	1.61973 1323		ZFP106	zinc finger protein 106(Zfp106)	-2.74416 1096
PAPLN	papilin, proteoglycan like sulfated glycoprotein	1.61876 2248		ARNTL	aryl hydrocarbon receptor nuclear translocator like	-2.74416 1096

[0211]

[0212]

PACSIN2	protein kinase C and casein kinase substrate in neurons 2	1.61740 1771		HS3ST3B1	heparan sulfate-glucosamine 3-sulfotransferase 3B1	-2.74416 1096
TRDMT1	tRNA aspartic acid methyltransferase 1	1.61523 9219		OASL2	2'-5' oligoadenylate synthetase-like 2(Oasl2)	-2.74489 2108
4932438H2 3RIK	Description Not Found	1.61468 1809		PRDX6	peroxiredoxin 6	-2.75084 462
SPAG9	sperm associated antigen 9	1.61456 7709		RASA2	RAS p21 protein activator 2	-2.75120 3108
RPA3	replication protein A3	1.61436 984		HOXB2	homeobox B2	-2.75488 7502
GNPTAB	N-acetylglucosamine-1-phosphate transferase alpha and beta subunits	1.61329 8199		TULP3	tubby like protein 3	-2.75488 7502
SNX9	sorting nexin 9	1.60925 1493		MFRP	membrane frizzled-related protein	-2.75488 7502
OLFR550	olfactory receptor 550(Olfr550)	1.60919 5813		MEN1	menin 1	-2.75755 6689
ZFP160	zinc finger protein 160(Zfp160)	1.60880 9243		C330021F2 3RIK	RIKEN cDNA C330021F23 gene(C330021F23Rik)	-2.76219 9201
TAS2R129	taste receptor, type 2, member 129(Tas2r129)	1.60880 9243		CSTAD	CSA-conditional, T cell activation-dependent protein(Cstad)	-2.76553 4746
OLFR371	olfactory receptor 371(Olfr371)	1.60880 9243		ALDH5A1	aldehyde dehydrogenase 5 family member A1	-2.77302 2439
OLFR281	olfactory receptor 281(Olfr281)	1.60880 9243		EPM2AIP1	EPM2A interacting protein 1	-2.77346 8928
OLFR195	olfactory receptor 195(Olfr195)	1.60880 9243		PDE8B	phosphodiesterase 8B	-2.77610 3988
OLFR142	olfactory receptor 142(Olfr142)	1.60880 9243		DMRTA1	DMRT like family A1	-2.77618 4379
PRSS3	protease, serine 3	1.60880 9243		LYPD6B	LY6/PLAUR domain containing 6B	-2.78004 8768
CX3CL1	C-X3-C motif chemokine ligand 1	1.60880 9243		CD300E	CD300e molecule	-2.78659 6362
TMPRSS6	transmembrane protease, serine 6	1.60880 9243		NPFF	neuropeptide FF-amide peptide precursor	-2.78659 6362
ALK	anaplastic lymphoma receptor tyrosine kinase	1.60880 9243		FASTKD1	FAST kinase domains 1	-2.79376 5229
ITGA9	integrin subunit alpha 9	1.60880 9243		OLFR802	olfactory receptor 802(Olfr802)	-2.79701 2978
TIMM13	translocase of inner mitochondrial membrane	1.60880 9243		HIVEP1	human immunodeficiency virus type I enhancer	-2.79701

[0213]

	13				binding protein 1	2978
MSH5	mutS homolog 5	1.60880 9243		HIC1	hypermethylated in cancer 1	-2.79701 2978
XPO4	exportin 4	1.60581 8241		TRIM33	tripartite motif containing 33	-2.80200 9226
MED21	mediator complex subunit 21	1.60330 9406		SELL	selectin L	-2.80327 4253
CHST12	carbohydrate sulfotransferase 12	1.60261 2589		EPHX1	epoxide hydrolase 1	-2.80375 8579
6030408B1 6RIK	Description Not Found	1.60219 5565		BCL9	B-cell CLL/lymphoma 9	-2.80735 4922
SLU7	SLU7 homolog, splicing factor	1.60154 8066		STAT2	signal transducer and activator of transcription 2	-2.80852 1822
CDK5RAP2	CDK5 regulatory subunit associated protein 2	1.60112 0229		ELMO3	engulfment and cell motility 3	-2.81249 8225
CASP7	caspase 7	1.60024 02		HDC	histidine decarboxylase	-2.81516 7456
KIF22	kinesin family member 22	1.59901 1705		AI317395	Description Not Found	-2.81762 3258
E2F1	E2F transcription factor 1	1.59844 9678		RPL14	ribosomal protein L14	-2.81762 3258
MXI1	MAX interactor 1, dimerization protein	1.59769 0116		SNAI1	snail family transcriptional repressor 1	-2.81825 6244
DONSON	downstream neighbor of SON	1.59693 5142		NUPR1	nuclear protein 1, transcriptional regulator	-2.82781 9025
TBX22	T-box 22	1.59693 5142		IGSF8	immunoglobulin superfamily member 8	-2.82781 9025
INPPL1	inositol polyphosphate phosphatase like 1	1.59630 0192		SLC12A7	solute carrier family 12 member 7	-2.82781 9025
CSE1L	chromosome segregation 1 like	1.59586 273		RENBP	renin binding protein	-2.83743 1463
NDFIP2	Nedd4 family interacting protein 2	1.59470 9608		ZFP553	zinc finger protein 553(Zfp553)	-2.83794 3242
LYPD6	LY6/PLAUR domain containing 6	1.59296 2293		LRFN2	leucine rich repeat and fibronectin type III domain containing 2	-2.83794 3242
DDX49	DEAD-box helicase 49	1.59219 0323		HP	haptoglobin	-2.83973 7506
MGLL	monoglyceride lipase	1.59094 8822		TOMM40	translocase of outer mitochondrial membrane 40	-2.84799 6907
NR4A3	nuclear receptor	1.59092		GABARAP	GABA type A receptor	-

[0214]

	subfamily 4 group A member 3	994		L2	associated protein like 2	2.847996907
LRRN3	leucine rich repeat neuronal 3	1.590360181		TMEM86A	transmembrane protein 86A	-2.855497819
PTPRK	protein tyrosine phosphatase, receptor type K	1.587927102		LRP1	LDL receptor related protein 1	-2.857980995
OLFR1212	olfactory receptor 1212(Olfr1212)	1.584962501		ATXN1	ataxin 1	-2.857980995
KLHL2	kelch like family member 2	1.584962501		FAS	Fas cell surface death receptor	-2.861524641
UBE2G2	ubiquitin conjugating enzyme E2 G2	1.584962501		ZDHHC18	zinc finger DHHC-type containing 18	-2.882740655
GRIN2A	glutamate ionotropic receptor NMDA type subunit 2A	1.584962501		LARGE	Description Not Found	-2.887525271
INHA	inhibin alpha subunit	1.584962501		SP5	Sp5 transcription factor	-2.887525271
RNPC3	RNA binding region (RNP1, RRM) containing 3	1.584962501		ATG7	autophagy related 7	-2.895440528
XKR7	XK related 7	1.584962501		DNAJC27	DnaJ heat shock protein family (Hsp40) member C27	-2.897240426
STX19	syntaxin 19	1.584962501		PCSK4	proprotein convertase subtilisin/kexin type 4	-2.900866808
SLC5A5	solute carrier family 5 member 5	1.584962501		RNF141	ring finger protein 141	-2.902073579
VPS37C	VPS37C, ESCRT-I subunit	1.584022655		GRAP2	GRB2-related adaptor protein 2	-2.904150467
ERMP1	endoplasmic reticulum metallopeptidase 1	1.582531434		VIPR1	vasoactive intestinal peptide receptor 1	-2.904484098
ZFP790	zinc finger protein 790(Zfp790)	1.581046002		CAR15	carbonic anhydrase 15(Car15)	-2.906890596
AA467197	expressed sequence AA467197(AA467197)	1.579947242		RELL2	RELT like 2	-2.906890596
UBE2Z	ubiquitin conjugating enzyme E2 Z	1.57976541		HECA	hdc homolog, cell cycle regulator	-2.916545968
SOAT2	sterol O-acyltransferase 2	1.577460518		DPM1	dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit	-2.933100475
ZMAT5	zinc finger matrin-type 5	1.576986214		AOC2	amine oxidase, copper containing 2	-2.936235748
CDCA3	cell division cycle associated 3	1.576323153		HIST2H2BE	histone cluster 2, H2be	-2.93632

[0215]

						0631
NEUROD2	neuronal differentiation 2	1.57626 6476		ACAD10	acyl-CoA dehydrogenase family member 10	-2.94251 4505
WDR35	WD repeat domain 35	1.57612 0636		NT5E	5'-nucleotidase ecto	-2.94403 9663
TWSG1	twisted gastrulation BMP signaling modulator 1	1.57587 28		SSH1	slingshot protein phosphatase 1	-2.94485 8446
PPT1	palmitoyl-protein thioesterase 1	1.57532 1868		SEMA4F	ssemaphorin 4F	-2.94832 9995
IRF8	interferon regulatory factor 8	1.57448 9283		NKD2	naked cuticle homolog 2	-2.95396 0396
PLEKHG5	pleckstrin homology and RhoGEF domain containing G5	1.57406 6379		TCEB3	transcription elongation factor B subunit 3	-2.95419 631
CDC20	cell division cycle 20	1.57371 8243		HDAC4	histone deacetylase 4	-2.95419 631
MFI2	antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5(Mfi2)	1.57288 9668		PCNX	pecanex homolog (Drosophila)(Pcnx)	-2.97269 2654
HDAC9	histone deacetylase 9	1.57162 5208		ARL5C	ADP ribosylation factor like GTPase 5C	-2.97269 2654
ASF1B	anti-silencing function 1B histone chaperone	1.57054 4039		1600014C1 0RIK	Description Not Found	-2.98185 2653
B3GNT1	Description Not Found	1.56917 1715		ANKRD23	ankyrin repeat domain 23	-2.98185 2653
SLC25A14	solute carrier family 25 member 14	1.56912 7395		CLOCK	clock circadian regulator	-2.98554 3793
FYN	FYN proto-oncogene, Src family tyrosine kinase	1.56746 2919		SFI1	SFI1 centrin binding protein	-2.98641 0935
SERPINB6 B	serine (or cysteine) peptidase inhibitor, clade B, member 6b(Serpinb6b)	1.56734 8435		HEY1	hes related family bHLH transcription factor with YRPW motif 1	-2.98763 2559
TOP1MT	topoisomerase (DNA) I, mitochondrial	1.56718 0597		ATP11C	ATPase phospholipid transporting 11C	-2.99095 486
CCDC50	coiled-coil domain containing 50	1.56627 3906		NUDCD3	NudC domain containing 3	-3
ZFP414	zinc finger protein 414(Zfp414)	1.56577 6574		CDC25A	cell division cycle 25A	-3.00023 8201
OGFOD2	2-oxoglutarate and iron dependent oxygenase domain containing 2	1.56551 2016		OLFR135	olfactory receptor 135(Olfr135)	-3.01792 1908
CTNNAL1	catenin alpha like 1	1.56358 6461		RC3H1	ring finger and CCCH-type domains 1	-3.01962 1529

[0216]

CREB3L2	cAMP responsive element binding protein 3 like 2	1.561361122	NSG2	neuron specific gene family member 2(Nsg2)	-3.020466888
OLFR492	olfactory receptor 492(Olfr492)	1.560714954	ID1	inhibitor of DNA binding 1, HLH protein	-3.026800059
OLFR1312	olfactory receptor 1312(Olfr1312)	1.560714954	CYP2D22	cytochrome P450, family 2, subfamily d, polypeptide 22(Cyp2d22)	-3.044282215
UPK2	uroplakin 2	1.560714954	H2AFJ	H2A histone family member J	-3.044297135
RESP18	regulated endocrine specific protein 18	1.560714954	TGFBR3	transforming growth factor beta receptor 3	-3.053111336
CRCT1	cysteine rich C-terminal 1	1.560714954	IRS2	insulin receptor substrate 2	-3.061776198
NEUROD4	neuronal differentiation 4	1.560714954	ADCY7	adenylate cyclase 7	-3.06608919
SENP1	SUMO1/sentrin specific peptidase 1	1.560714954	HYI	hydroxypyruvate isomerase (putative)	-3.072315809
MR1	major histocompatibility complex, class I-related	1.560714954	TRIP4	thyroid hormone receptor interactor 4	-3.078951341
BIVM	basic, immunoglobulin-like variable motif containing	1.560714954	D730001G18RIK	RIKEN cDNA D730001G18 gene(D730001G18Rik)	-3.087462841
KPNA2	karyopherin subunit alpha 2	1.560714954	PRR7	proline rich 7 (synaptic)	-3.087462841
BAG2	BCL2 associated athanogene 2	1.560714954	GFPT2	glutamine-fructose-6-phosphate transaminase 2	-3.09592442
SLC12A8	solute carrier family 12 member 8	1.560714954	SCMH1	sex comb on midleg homolog 1 (Drosophila)	-3.100136671
SCN7A	sodium voltage-gated channel alpha subunit 7	1.560714954	ANKRD12	ankyrin repeat domain 12	-3.107456458
SLC5A7	solute carrier family 5 member 7	1.560714954	PTPRV	protein tyrosine phosphatase, receptor type, V(Ptprv)	-3.112700133
ENPEP	glutamyl aminopeptidase	1.560714954	TMEM135	transmembrane protein 135	-3.112700133
ANGPTL4	angiopoietin like 4	1.560607777	AKAP3	A-kinase anchoring protein 3	-3.114600665
OSBPL3	oxysterol binding protein like 3	1.559778376	CBR2	carbonyl reductase 2(Cbr2)	-3.129283017
MCFD2	multiple coagulation factor deficiency 2	1.559617874	CXCL16	C-X-C motif chemokine ligand 16	-3.129283017
MAP2K1	mitogen-activated protein kinase kinase 1	1.558556708	MBTD1	mbt domain containing 1	-3.14567

						7455
ING2	inhibitor of growth family member 2	1.55722 3521		UBE2J2	ubiquitin conjugating enzyme E2 J2	-3.16188 7682
CDCA5	cell division cycle associated 5	1.55643 411		STK36	serine/threonine kinase 36	-3.16188 7682
MAP3K7	mitogen-activated protein kinase kinase kinase 7	1.55446 3905		SLC14A1	solute carrier family 14 member 1 (Kidd blood group)	-3.16922 072
GSTT3	glutathione S-transferase, theta 3(Gstt3)	1.55048 277		CTSE	cathepsin E	-3.17791 7792
PFN2	profilin 2	1.54969 0793		HSD3B7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	-3.17791 7792
HPS4	HPS4, biogenesis of lysosomal organelles complex 3 subunit 2	1.54911 5647		3010003L2 1RIK	Description Not Found	-3.17924 9632
CAPN8	calpain 8	1.54843 6625		BAI1	Description Not Found	-3.18646 1055
RAB11FIP5	RAB11 family interacting protein 5	1.54843 6625		ZFP451	zinc finger protein 451(Zfp451)	-3.18771 1618
CD9	CD9 molecule	1.54842 9184		CCDC28B	coiled-coil domain containing 28B	-3.19220 7249
CCR6	C-C motif chemokine receptor 6	1.54825 0633		MCF2L	MCF.2 cell line derived transforming sequence like	-3.19967 2345
ALG2	ALG2, alpha-1,3/1,6-mannosyltransferase	1.54799 2668		BCL6	B-cell CLL/lymphoma 6	-3.20102 4389
BCDIN3D	BCDIN3 domain containing RNA methyltransferase	1.54604 6129		PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	-3.20493 5584
NT5DC3	5'-nucleotidase domain containing 3	1.54522 349		PROS1	protein S (alpha)	-3.20945 3366
DNAJC18	DnaJ heat shock protein family (Hsp40) member C18	1.54462 6916		CTSH	cathepsin H	-3.21628 737
SH3RF1	SH3 domain containing ring finger 1	1.54415 6019		CRTC3	CREB regulated transcription coactivator 3	-3.21723 0716
RGS16	regulator of G-protein signaling 16	1.54138 2294		TNKS	tankyrase	-3.21723 0716
NCAPH	non-SMC condensin I complex subunit H	1.54078 8228		GRM6	glutamate metabotropic receptor 6	-3.22496 6365
USP14	ubiquitin specific peptidase 14	1.54033 3713		SPSB1	splA/ryanodine receptor domain and SOCS box containing 1	-3.25550 0733
RFT1	RFT1 homolog	1.54031 759		PARP8	poly(ADP-ribose) polymerase family member 8	-3.26303 4406

[0217]

[0218]

SLC31A1	solute carrier family 31 member 1	1.54027 5536		KCNRG	potassium channel regulator	-3.26303 4406
TCTEX1D2	Tctex1 domain containing 2	1.53833 2378		POU6F1	POU class 6 homeobox 1	-3.26851 7714
TTF2	transcription termination factor 2	1.53787 1953		REV3L	REV3 like, DNA directed polymerase zeta catalytic subunit	-3.27052 8942
ZFP7	zinc finger protein 7(Zfp7)	1.53605 29		TCF7	transcription factor 7 (T-cell specific, HMG-box)	-3.27241 9178
G6PD2	glucose-6-phosphate dehydrogenase 2(G6pd2)	1.53605 29		NME4	NME/NM23 nucleoside diphosphate kinase 4	-3.28355 1423
DEFB14	defensin beta 14(Defb14)	1.53605 29		PLAUR	plasminogen activator, urokinase receptor	-3.28540 2219
SLC18A3	solute carrier family 18 member A3	1.53605 29		CD4	CD4 molecule	-3.28540 2219
AHNAK2	AHNAK nucleoprotein 2	1.53605 29		ZMYND11	zinc finger MYND-type containing 11	-3.29318 6363
HOXC12	homeobox C12	1.53605 29		ARMCX5	armadillo repeat containing, X-linked 5	-3.29840 4158
CEACAM16	carcinoembryonic antigen related cell adhesion molecule 16	1.53605 29		LPHN1	Description Not Found	-3.30012 3725
MOSPD3	motile sperm domain containing 3	1.53605 29		PIK3IP1	phosphoinositide-3-kinase interacting protein 1	-3.30742 8525
DCTN1	dynactin subunit 1	1.53605 29		ERDR1	erythroid differentiation regulator 1(Erdr1)	-3.31765 1188
MYB	MYB proto-oncogene, transcription factor	1.53605 29		PLD4	phospholipase D family member 4	-3.32844 4792
GLIPR1L2	GLI pathogenesis-related 1 like 2	1.53605 29		BMF	Bcl2 modifying factor	-3.33628 3388
ALDH1A3	aldehyde dehydrogenase 1 family member A3	1.53605 29		GALNT11	polypeptide N-acetylgalactosaminyltransferase 11	-3.34511 8795
SLC2A8	solute carrier family 2 member 8	1.53605 29		LCN2	lipocalin 2	-3.37851 1623
SRC	SRC proto-oncogene, non-receptor tyrosine kinase	1.53605 29		PAG1	phosphoprotein membrane anchor with glycosphingolipid microdomains 1	-3.38543 1037
ZCCHC17	zinc finger CCHC-type containing 17	1.53561 8518		DTX1	deltex E3 ubiquitin ligase 1	-3.42557 6064
HNRNPUL1	heterogeneous nuclear ribonucleoprotein U like 1	1.53442 0207		RFFL	ring finger and FYVE-like domain containing E3 ubiquitin protein ligase	-3.42668 4082
TRIM68	tripartite motif	1.53305		MAFF	MAF bZIP transcription	-

	containing 68	7052			factor F	3.42961 5964
TPST1	tyrosylprotein sulfotransferase 1	1.53140 111		TOR1AIP2	torsin 1A interacting protein 2	- 3.43231 6325
OLFR922	olfactory receptor 922(Olfr922)	1.53126 0941		SNN	stannin	- 3.43231 6325
FIG4	FIG4 phosphoinositide 5-phosphatase	1.53044 2167		CLEC4N	C-type lectin domain family 4, member n(Clec4n)	- 3.43356 7144
SETMAR	SET domain and mariner transposase fusion gene	1.53044 2167		RREB1	ras responsive element binding protein 1	- 3.44378 0274
GSTM5	glutathione S-transferase mu 5	1.53005 3218		CCDC84	coiled-coil domain containing 84	- 3.44518 8687
TUBA3B	tubulin, alpha 3B(Tuba3b)	1.52798 6221		ID3	inhibitor of DNA binding 3, HLH protein	- 3.46350 285
PDCL	phosducin like	1.52780 7072		BC065397	cDNA sequence BC065397(BC065397)	- 3.46597 4465
SMPDL3B	sphingomyelin phosphodiesterase acid like 3B	1.52724 3888		VRK1	vaccinia related kinase 1	- 3.46760 555
ABHD14A	abhydrolase domain containing 14A	1.52721 3882		HOXD13	homeobox D13	- 3.49185 3096
TIPIN	TIMELESS interacting protein	1.52697 2991		MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2	- 3.49185 3096
DSCC1	DNA replication and sister chromatid cohesion 1	1.52598 6429		HOXA5	homeobox A5	- 3.51727 5693
PSMD1	proteasome 26S subunit, non-ATPase 1	1.52557 4957		HIST1H1A	histone cluster 1, H1a	- 3.52356 1956
BZRAP1	benzodiazepine receptor associated protein 1(Bzrap1)	1.52416 6255		MAML1	mastermind like transcriptional coactivator 1	- 3.52360 3553
ENO3	enolase 3	1.52377 8831		PTPDC1	protein tyrosine phosphatase domain containing 1	- 3.52669 4846
E330034G1 9RIK	RIKEN cDNA E330034G19 gene(E330034G19Rik)	1.52356 1956		TNFRSF12 A	tumor necrosis factor receptor superfamily member 12A	- 3.52872 5998
GABRP	gamma-aminobutyric acid type A receptor pi subunit	1.52356 1956		TNIP2	TNFAIP3 interacting protein 2	- 3.53915 8811
SLC14A2	solute carrier family 14 member 2	1.52356 1956		HIST2H4	histone cluster 2, H4(Hist2h4)	- 3.54077 3411
YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon	1.52247 8712		PIM2	Pim-2 proto-oncogene, serine/threonine kinase	- 3.55765 5155
EHBP1L1	EH domain binding	1.52228		DOK7	docking protein 7	-

[0219]

	protein 1 like 1	2169				3.56778 1854
CHGB	chromogranin B	1.51924 262		TNFSF14	tumor necrosis factor superfamily member 14	- 3.58889 5735
TXNRD2	thioredoxin reductase 2	1.51900 8256		TDRKH	tudor and KH domain containing	- 3.59096 1241
NCF1	neutrophil cytosolic factor 1	1.51887 3761		FIBCD1	fibrinogen C domain containing 1	- 3.60865 6121
OAF	out at first homolog	1.51743 1856		RBBP9	RB binding protein 9, serine hydrolase	- 3.60880 9243
FAM110A	family with sequence similarity 110 member A	1.51726 3583		DERL1	derlin 1	- 3.61765 1119
ANGEL1	angel homolog 1 (Drosophila)	1.51583 2566		LENG9	leukocyte receptor cluster member 9	- 3.62058 641
RTN4IP1	reticulon 4 interacting protein 1	1.51576 0776		TRPC2	transient receptor potential cation channel subfamily C member 2, pseudogene	- 3.62058 641
LAMP2	lysosomal associated membrane protein 2	1.51570 9038		CCDC134	coiled-coil domain containing 134	- 3.63226 8216
KRT4	keratin 4	1.51429 9789		OAS2	2'-5'-oligoadenylate synthetase 2	- 3.63226 8216
PAFAH1B3	platelet activating factor acetylhydrolase 1b catalytic subunit 3	1.51429 35		2410127L1 7RIK	Description Not Found	- 3.64673 8698
STT3A	STT3A, catalytic subunit of the oligosaccharyltransferase complex	1.51353 7695		RSAD1	radical S-adenosyl methionine domain containing 1	- 3.64922 0471
PRKAR1B	protein kinase cAMP-dependent type I regulatory subunit beta	1.51340 003		H2-DMB1	histocompatibility 2, class II, locus Mb1(H2-DMb1)	- 3.64961 5459
HIST1H2BB	histone cluster 1, H2bb	1.51294 1595		IFT81	intraflagellar transport 81	- 3.67383 9056
ZFP39	zinc finger protein 39(Zfp39)	1.51138 5424		MID1	midline 1	- 3.68369 6454
PLK1	polo like kinase 1	1.51115 1166		DEPDC1B	DEP domain containing 1B	- 3.68369 6454
1700028P14RIK	Description Not Found	1.51096 1919		SMAD3	SMAD family member 3	- 3.71629 6166
D10BWG1379E	Description Not Found	1.51096 1919		UBTD1	ubiquitin domain containing 1	- 3.71699 0894
TREM3	triggering receptor expressed on myeloid cells 3(Trem3)	1.51096 1919		FBXO44	F-box protein 44	- 3.73876 7837
GM128	predicted gene 128(Gm128)	1.51096 1919		KCNMB4	potassium calcium-activated channel	- 3.74195

[0220]

[0221]

					subfamily M regulatory beta subunit 4	1111
OLFR741	olfactory receptor 741(Olfr741)	1.510961919		FAIM3	Description Not Found	-3.754887502
OLFR523	olfactory receptor 523(Olfr523)	1.510961919		CCM2	CCM2 scaffolding protein	-3.754887502
DCPP1	demilune cell and parotid protein 1(Dcpp1)	1.510961919		DAG1	dystroglycan 1	-3.760220946
RPRML	reprimo like	1.510961919		FCGR3	Fc receptor, IgG, low affinity III(Fcgr3)	-3.776103988
CHRD	chordin	1.510961919		ZNRF1	zinc and ring finger 1, E3 ubiquitin protein ligase	-3.776103988
C5AR1	complement component 5a receptor 1	1.510961919		TLR1	toll like receptor 1	-3.786596362
APOA2	apolipoprotein A2	1.510961919		HSD17B11	hydroxysteroid 17-beta dehydrogenase 11	-3.789207575
PRG2	proteoglycan 2, pro eosinophil major basic protein	1.510961919		ZPBP	zona pellucida binding protein	-3.887525271
VCAM1	vascular cell adhesion molecule 1	1.510961919		ZSWIM3	zinc finger SWIM-type containing 3	-3.892391026
LY6G5B	lymphocyte antigen 6 complex, locus G5B	1.510961919		SOCS1	suppressor of cytokine signaling 1	-3.892391026
AIM2	absent in melanoma 2	1.510961919		KLF9	Kruppel like factor 9	-3.902021342
DMBX1	diencephalon/mesencephalon homeobox 1	1.510961919		AHSA2	AHA1, activator of heat shock 90kDa protein ATPase homolog 2 (yeast)	-3.904760449
HCN2	hyperpolarization activated cyclic nucleotide gated potassium channel 2	1.510961919		DDHD1	DDHD domain containing 1	-3.914086097
MRGPRF	MAS related GPR family member F	1.510961919		CNKSR3	CNKSR family member 3	-3.930737338
CYTH4	cytohesin 4	1.510961919		CPEB2	cytoplasmic polyadenylation element binding protein 2	-4.017516295
ANGPTL3	angiopoietin like 3	1.510961919		TRP53BP2	transformation related protein 53 binding protein 2(Trp53bp2)	-4.021932279
DHX29	DEAH-box helicase 29	1.510667738		FAM178A	family with sequence similarity 178, member A(Fam178a)	-4.03562391
PMPCB	peptidase, mitochondrial processing beta subunit	1.509477625		RCN3	reticulocalbin 3	-4.03562391
HRH3	histamine receptor H3	1.508554002		SPTLC2	serine palmitoyltransferase long chain base subunit 2	-4.04001

[0222]

						5679
ZFP282	zinc finger protein 282(Zfp282)	1.507419453		ZFP810	zinc finger protein 810(Zfp810)	-4.070389328
TBC1D7	TBC1 domain family member 7	1.504847821		NAGA	alpha-N-acetylgalactosaminidase	-4.074676686
ARSB	arylsulfatase B	1.504845728		KLRA20	killer cell lectin-like receptor subfamily A, member 20(Klra20)	-4.078951341
RAD17	RAD17 checkpoint clamp loader component	1.504177542		STK11IP	serine/threonine kinase 11 interacting protein	-4.083213368
CMTM7	CKLF like MARVEL transmembrane domain containing 7	1.503297831		KLF4	Kruppel like factor 4	-4.084306687
NFKB2	nuclear factor kappa B subunit 2	1.500363085		INADL	Description Not Found	-4.086667018
TOP3A	topoisomerase (DNA) III alpha	-1.50007357		URM1	ubiquitin related modifier 1	-4.0907078
RAB33B	RAB33B, member RAS oncogene family	-1.50054042		PELI1	pellino E3 ubiquitin protein ligase 1	-4.093813673
LYSMD1	LysM domain containing 1	-1.500614885		FBLN1	fibulin 1	-4.098032083
POLG2	polymerase (DNA) gamma 2, accessory subunit	-1.500707646		HR	hair growth associated	-4.135452784
TGIF1	TGFB induced factor homeobox 1	-1.501196523		ASB6	ankyrin repeat and SOCS box containing 6	-4.137503524
RELL1	RELT like 1	-1.50300255		SLC27A5	solute carrier family 27 member 5	-4.141596278
CYP26B1	cytochrome P450 family 26 subfamily B member 1	-1.50439813		PPP1R3F	protein phosphatase 1 regulatory subunit 3F	-4.14974712
PTRH2	peptidyl-tRNA hydrolase 2	-1.504678598		AB124611	cDNA sequence AB124611(AB124611)	-4.173373402
ZKSCAN3	zinc finger with KRAB and SCAN domains 3	-1.504916722		CD40	CD40 molecule	-4.181897643
SP8	Sp8 transcription factor	-1.505999092		SMAD5	SMAD family member 5	-4.183883459
SAMD14	sterile alpha motif domain containing 14	-1.506272343		COL23A1	collagen type XXIII alpha 1 chain	-4.221103725
MX2	MX dynamin like GTPase 2	-1.507268463		ZFP595	zinc finger protein 595(Zfp595)	-4.228818691
OCRL	OCRL, inositol polyphosphate-5-phosphatase	-1.507638755		PECAM1	platelet and endothelial cell adhesion molecule 1	-4.232789973
SYNJ2BP	synaptojanin 2 binding	-		TMEM138	transmembrane protein	-

[0223]

	protein	1.507669173			138	4.241228289
CPLX4	complexin 4	-1.508554002		RFX2	regulatory factor X2	-4.244125943
LGALS9	galectin 9	-1.509246723		KCTD12	potassium channel tetramerization domain containing 12	-4.247846204
TAZ	tafazzin	-1.509269953		TRIM56	tripartite motif containing 56	-4.262008929
2310002L09RIK	Description Not Found	-1.510961919		EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2	-4.263034406
ZFP97	zinc finger protein 97(Zfp97)	-1.510961919		RALGPS2	Ral GEF with PH domain and SH3 binding motif 2	-4.279842694
OLFR1494	olfactory receptor 1494(Olfr1494)	-1.510961919		TGM2	transglutaminase 2	-4.293161941
BC030867	cDNA sequence BC030867(BC030867)	-1.510961919		ENC1	ectodermal-neural cortex 1	-4.311067102
CEACAM9	carcinoembryonic antigen-related cell adhesion molecule 9(Ceacam9)	-1.510961919		LRIG1	leucine rich repeats and immunoglobulin like domains 1	-4.375039431
LRIT1	leucine rich repeat, Ig-like and transmembrane domains 1	-1.510961919		PRM1	protamine 1	-4.375039431
KLK5	kallikrein related peptidase 5	-1.510961919		DUSP7	dual specificity phosphatase 7	-4.383538076
KRT27	keratin 27	-1.510961919		SERTAD3	SERTA domain containing 3	-4.399171094
CACNG4	calcium voltage-gated channel auxiliary subunit gamma 4	-1.510961919		KCNC1	potassium voltage-gated channel subfamily C member 1	-4.409390936
IL13RA1	interleukin 13 receptor subunit alpha 1	-1.510961919		UBE2D3	ubiquitin conjugating enzyme E2 D3	-4.462706751
TMEM121	transmembrane protein 121	-1.510961919		SEPP1	selenoprotein P, plasma, 1	-4.463383458
HIST1H2AA	histone cluster 1, H2aa	-1.510961919		ADRB2	adrenoceptor beta 2	-4.463910999
MPZL3	myelin protein zero like 3	-1.510961919		PPP1R13B	protein phosphatase 1 regulatory subunit 13B	-4.471417658
TGFB2	transforming growth factor beta 2	-1.510961919		ARRDC3	arrestin domain containing 3	-4.504620392
IFT74	intraflagellar transport 74	-1.510961919		GNGT2	G protein subunit gamma transducin 2	-4.531381461
FCRL1	Fc receptor like 1	-1.51096		SIAH1A	seven in absentia 1A(Siah1a)	-4.53915

[0224]

		1919				8811
ADRB1	adrenoceptor beta 1	-1.510961919		XPC	XPC complex subunit, DNA damage recognition and repair factor	-4.563768278
MAGI2	membrane associated guanylate kinase, WW and PDZ domain containing 2	-1.510961919		HIPK1	homeodomain interacting protein kinase 1	-4.683696454
SCG5	secretogranin V	-1.510961919		H2-OB	histocompatibility 2, O region beta locus(H2-Ob)	-4.700439718
GCK	glucokinase	-1.510961919		BACH2	BTB domain and CNC homolog 2	-4.716990894
ASB10	ankyrin repeat and SOCS box containing 10	-1.510961919		MAP1LC3A	microtubule associated protein 1 light chain 3 alpha	-4.722466024
SELE	selectin E	-1.510961919		LRRFIP1	LRR binding FLII interacting protein 1	-4.761551232
IGFBP3	insulin like growth factor binding protein 3	-1.510961919		ATP10D	ATPase phospholipid transporting 10D (putative)	-4.766581958
TPT1	tumor protein, translationally-controlled 1	-1.510961919		IGFBP4	insulin like growth factor binding protein 4	-4.790993785
ROCK1	Rho associated coiled-coil containing protein kinase 1	-1.510961919		TMEM108	transmembrane protein 108	-4.865423978
OGFRL1	opioid growth factor receptor-like 1	-1.510961919		PTK2	protein tyrosine kinase 2	-4.875719796
TMEM38A	transmembrane protein 38A	-1.510961919		CLEC11A	C-type lectin domain family 11 member A	-4.897240426
RLTPR	Description Not Found	-1.51227339		LRP12	LDL receptor related protein 12	-4.955029571
ITPKC	inositol-trisphosphate 3-kinase C	-1.512389725		GCNT2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)	-4.958842675
TLE4	transducin like enhancer of split 4	-1.51341989		F10	coagulation factor X	-4.965784285
PDE4D	phosphodiesterase 4D	-1.513667908		DBP	D-box binding PAR bZIP transcription factor	-4.966549451
A130010J15RIK	Description Not Found	-1.514296211		ABCG1	ATP binding cassette subfamily G member 1	-5.002252452
RNF167	ring finger protein 167	-1.514765492		WDR78	WD repeat domain 78	-5.017921908
CCBL1	Description Not Found	-1.515626494		DNAJC6	DnaJ heat shock protein family (Hsp40) member C6	-5.017921908
HSD17B1	hydroxysteroid 17-beta dehydrogenase 1	-1.516875069		AFF4	AF4/FMR2 family member 4	-5.033423002

[0225]

OSM	oncostatin M	-1.517234668		TNFRSF26	tumor necrosis factor receptor superfamily, member 26(Tnfrsf26)	-5.040015679
RHPN1	rhophilin, Rho GTPase binding protein 1	-1.517275693		GFOD2	glucose-fructose oxidoreductase domain containing 2	-5.070389328
TAS2R105	taste receptor, type 2, member 105(Tas2r105)	-1.517431856		TYROBP	TYRO protein tyrosine kinase binding protein	-5.114783447
NIPBL	NIPBL, cohesin loading factor	-1.517569618		TMEM176B	transmembrane protein 176B	-5.118941073
CXCR3	C-X-C motif chemokine receptor 3	-1.519325267		ZFP710	zinc finger protein 710(Zfp710)	-5.159871337
SMURF1	SMAD specific E3 ubiquitin protein ligase 1	-1.520263252		ENPP4	ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative)	-5.181897643
RNF208	ring finger protein 208	-1.52126647		MAPK8	mitogen-activated protein kinase 8	-5.259272487
ITGA5	integrin subunit alpha 5	-1.523517983		TNFRSF25	tumor necrosis factor receptor superfamily member 25	-5.289096702
USP18	ubiquitin specific peptidase 18	-1.524814077		LCN4	lipocalin 4(Lcn4)	-5.366322214
PIP5K1A	phosphatidylinositol-4-phosphate 5-kinase type 1 alpha	-1.525074369		CRIM1	cysteine rich transmembrane BMP regulator 1	-5.369815424
STRBP	spermatid perinuclear RNA binding protein	-1.52561213		RTP4	receptor transporter protein 4	-5.444600814
GRAMD2	GRAM domain containing 2	-1.52652805		PRNP	prion protein	-5.495055528
ZFP101	zinc finger protein 101(Zfp101)	-1.526555668		ZFP747	zinc finger protein 747(Zfp747)	-5.496654083
RUNDC1	RUN domain containing 1	-1.526563287		CD7	CD7 molecule	-5.504620392
SLC13A3	solute carrier family 13 member 3	-1.528487927		ARHGAP26	Rho GTPase activating protein 26	-5.548436625
CCDC94	coiled-coil domain containing 94	-1.528487927		S100A9	S100 calcium binding protein A9	-5.557655155
MRPS14	mitochondrial ribosomal protein S14	-1.528962318		AQP9	aquaporin 9	-5.572889668
NEU4	neuraminidase 4 (sialidase)	-1.529820947		CXCR5	C-X-C motif chemokine receptor 5	-5.573647187
PCGF1	polycomb group ring finger 1	-1.53059536		CCNO	cyclin O	-5.574404309
PNPLA7	patatin like phospholipase domain	-1.53207		LYNX1	Ly6/neurotoxin 1	-5.66675

[0226]

	containing 7	883				6592
SPATA19	spermatogenesis associated 19	-1.533014103		CLDN10	claudin 10	-5.782015335
AP4B1	adaptor related protein complex 4 beta 1 subunit	-1.533821865		AMIGO2	adhesion molecule with Ig-like domain 2	-5.83541884
BC068281	cDNA sequence BC068281(BC068281)	-1.5360529		CD79B	CD79b molecule	-5.94016675
GK2	glycerol kinase 2	-1.5360529		USP53	ubiquitin specific peptidase 53	-5.980710829
PIGM	phosphatidylinositol glycan anchor biosynthesis class M	-1.5360529		IKBKE	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	-6.005624549
FKBP6	FK506 binding protein 6	-1.5360529		ALOX5AP	arachidonate 5-lipoxygenase activating protein	-6.008988783
EVI5	ecotropic viral integration site 5	-1.5360529		GGT1	gamma-glutamyltransferase 1	-6.010108453
BCL11A	B-cell CLL/lymphoma 11A	-1.5360529		CAMK2D	calcium/calmodulin dependent protein kinase II delta	-6.047669251
PER1	period circadian clock 1	-1.537278499		RAB3D	RAB3D, member RAS oncogene family	-6.156841525
BTBD9	BTB domain containing 9	-1.537451456		MAP3K8	mitogen-activated protein kinase kinase kinase 8	-6.376776572
USP38	ubiquitin specific peptidase 38	-1.537763627		NOTCH4	notch 4	-6.495055528
LRRC57	leucine rich repeat containing 57	-1.538083341		MACROD1	MACRO domain containing 1	-6.581200582
5830415F09RIK	Description Not Found	-1.53855912		RNF144A	ring finger protein 144A	-6.632268216
EGR2	early growth response 2	-1.540038325		PDE2A	phosphodiesterase 2A	-6.86913112
GMEB2	glucocorticoid modulatory element binding protein 2	-1.541122795		THA1	threonine aldolase 1(Tha1)	-6.885086225
PIK3R4	phosphoinositide-3-kinase regulatory subunit 4	-1.541975323		APP	amyloid beta precursor protein	-6.940754047
KRR1	KRR1, small subunit processome component homolog	-1.54225805		FAM109A	family with sequence similarity 109 member A	-6.968666793
COL9A1	collagen type IX alpha 1	-1.54225805		LRG1	leucine rich alpha-2-glycoprotein 1	-6.995484519
POLD4	polymerase (DNA) delta 4, accessory subunit	-1.542654605		IL11RA1	interleukin 11 receptor, alpha chain 1(Il11ra1)	-7.016251155
ACSS2	acyl-CoA synthetase	-		CNR2	cannabinoid receptor 2	-

[0227]

	short-chain family member 2	1.54404 5378			7.21334 7282
PDLIM1	PDZ and LIM domain 1	- 1.54478 5186	NUAK2	NUAK family kinase 2	- 7.36981 5424
A430107P0 9RIK	Description Not Found	- 1.54492 1568	GPR146	G protein-coupled receptor 146	- 7.57780 6447
SLC38A11	solute carrier family 38 member 11	- 1.54622 2547			

[0228] *Log2倍数变化＝log2(4+L+/4-L-)

[0229] 为了研究这三个群体之间的分子途径,将基因本体网络分组成节点并确定每个节点内最重要的途径(图6A)。在我们的功能障碍的T细胞数据集与已公开的功能减退的T细胞数据集之间共享的基因本体(GO)项在细胞周期基因中极其富含的,与观察到功能障碍的群体主要是$Ki67^+$是一致的。在功能障碍和耗竭的基因集之间共享的GO项包括效应程序,诸如对细胞杀伤的调节、趋化、干扰素-γ产生。在功能减退和耗竭的基因集之间共享的GO项由细胞周期途径、对淋巴细胞的负性调节和干扰素-γ产生组成。这些数据表明,虽然一些保守的分子程序可能存在于这些功能障碍的分化状态中,但许多途径可在慢性病毒感染与肿瘤环境中之间差异调节。

[0230] 虽然许多抑制性受体,包括Pdcd1(PD-1)、Havcr2(TIM-3)、Cd244(2B4)、Klre1和Lag3在内,在所有数据集之间共享;但是共刺激受体Tnfrsf4(OX-40)和Tnfrsf9(4-1BB)在功能障碍和功能减退的$CD8^+$TIL数据集中上调。因此,为了富集肿瘤特异性$CD8^+$ TIL上的潜在标志物和治疗靶标,对$4\text{-}1BB^+LAG\text{-}3^+$ CD8 TIL群体的完整细胞表面表型进行了表征。通过比较不同的$CD8^+$ TIL亚群,发现了几种另外的上调共刺激受体:Tnfrsf18(GITR)、Nkg2d(KLRK1)和Cd27。Nrp1(神经纤毛蛋白-1)的转录物也被高度表达,该转录物编码参与$CD4^+$ Treg功能(Sarris等人,2008;该文献全文以引用方式并入)的细胞表面受体蛋白。在肿瘤接种后第7天、第14天和第21天时通过流式细胞术证实了许多这些分子的表达(图6C)。将所述分析扩展为包括共刺激分子ICOS和CD160和具有Ig和ITIM结构域(TIGIT)的抑制性受体T细胞免疫受体,这是因为ICOS和CD160接近截止值并且基因阵列中没有针对TIGIT的探针存在。此外,最近的报道表明,靶向这些受体可以在癌症鼠模型中为治疗性的(Johnston等人,2014;Fan等人,2014;该文献全文以引用方式并入)。PD-1、TIGIT、TIM-3、CD27和NRP1在$4\text{-}1BB^+LAG\text{-}3^+$ TIL群体中的大部分中表达,并且表达随着时间推移被维持。2B4、CD160、CTLA4、OX-40和GITR细分了$4\text{-}1BB^+LAG\text{-}3^+$群体中的较小部分。在该3周的时间范围内,几种抑制性受体2B4、TIM3和CD160的表达增加,而共刺激受体ICOS和OX-40的表达降低(图6C)。

[0231] 为了解决功能障碍的$CD8^+$ TIL是终末分化的短期效应细胞还是记忆样细胞,表达KLRG-1和IL-7Rα(Joshi等人,2007)。$CD8^+$ TIL中的大部分是对KLRG-1表达呈阴性的,并且在$4\text{-}1BB^+LAG\text{-}3^+$和$4\text{-}1BB^-LAG\text{-}3^-$群体之间没有差异。然而,$4\text{-}1BB^+LAG\text{-}3^+$ TIL中的大部分与它们的阴性对应部分相比不表达IL-7受体(IL-7Rα)(图6D)。这些结果表明,对肿瘤微环境中表达的抗原没有明显特异性的$4\text{-}1BB^-LAG\text{-}3^-$TIL是更记忆样的,但同时肿瘤抗原特异性$LAG\text{-}3^+4\text{-}1BB^+$子集尚未完全获得末端效应表型。

[0232] $CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TIL中差异调节的基因的功能相关性

[0233] 表2中的基因阵列结果提供了表征$CD8^+4\text{-}1BB^+LAG\text{-}3^+$ TIL的基因的列表。该列表包

括抗肿瘤免疫的治疗靶标和其他标志物。在本文的实施方案的开发期间进行实验以测试这些另外的靶标/标记物的功能相关性(图11)。数据表明阵列已经使用敲除小鼠(例如,PD-1、TIM-3、OX-40ICOS、TIGIT、CD244、TNFRSF18、Nrn1、Nrp1、KLRG1、GM156、GPNMB、GPR65、TMEM205和TMEM126A、CRTAM、Sema7a等)鉴定了免疫疗法的靶标。实验证明Nrn1和CRTAM是抗肿瘤免疫应答的负调节因子,因为缺乏这些分子中的任一种的敲除小鼠在体内显示出改善的免疫介导的肿瘤控制。相比之下,Sema7a是抗肿瘤免疫应答的正调节因子,因为缺乏该分子的敲除小鼠在体内显示出减少的免疫介导的肿瘤控制(图11)。这些实验表明,Sema7a信号传导的激动剂和Nrn1和/或CRTAM的拮抗剂应该是治疗癌症的有用治疗剂。

[0234] 靶向4-1BB和LAG-3在体内发挥抗肿瘤活性并使$CD8^+$ TIL的功能和表型组成归一化

[0235] 在开发本文的实施方案期间进行实验以评估靶向这些受体是否具有治疗效用。为此,在荷有已建立的B16.SIY肿瘤的小鼠中单独或与阻断性抗LAG-3 mAb组合地施用激动性抗4-1BB mAb。虽然单独的每种抗体治疗具有如由较慢的肿瘤生长所反映的一些治疗效果,但该组合为特别有效的(图7A)。对肿瘤微环境的分析表明,使用联合治疗改善的肿瘤控制伴随着特异于SIY抗原的$CD8^+$ TIL的数量增加(图7B),这与先前报道的使用抗PD-L1+抗CTLA-4 mAb的结果一致(Spranger等人,2014b;Twyman-Saint Victor等人,2015;这些文献的全文以引用方式并入)。

[0236] 接下来检查抗4-1BB+抗LAG-3 mAb的治疗效果是否与稳定状态下限定功能障碍的T细胞的表型标志物的丧失相关。由于担心重新分析T细胞的LAG-3和4-1BB表达可能是有问题的,因为施用的Ab在理论上可以调控来自细胞表面的靶受体,所以利用如上所述通过基因表达谱来鉴定的另外受体的协调表达。对大量TIL亚群的初步分析显示,在抗LAG-3+抗4-1BB治疗后NRP1和2B4的表达降低(数据未显示)。分析通过五聚体染色鉴定的2B4和NRP1在SIY反应性$CD8^+$TIL上的共表达。在抗4-1BB+和抗LAG-3 mAb治疗后观察到了2B4和NRP1的共表达的2.7倍降低(图7C),表明与T细胞功能障碍相关的表面表型的丧失。为了确定这种变化是否伴随向效应表型的转变,检查了KLGR-1的表达。实际上,治疗后在SIY反应性TIL上观察到KLGR-1表达显著增加,并且观察到在KLRG-$1^{高}$IL-7R$A^{低}$群体中增加了3.7倍(图7D)。

[0237] 为了消除用抗LAG-3+抗4-1BB mAb治疗不改变已经在肿瘤内的T细胞的表型,而是支持从次级淋巴器官中募集新致敏的功能性T细胞的可能性,利用S1PR抑制因子FTY720,该S1PR抑制因子FTY720防止T细胞从淋巴结中流出(Halin等人,2005;该文献全文以引用方式并入)。基于抗PD-L1的免疫疗法的功效在FTY720存在下被保留,支持TIL的再功能化是主要作用机制(Spranger等人,2014a;该文献全文以引用方式并入)。在肿瘤接种后第6天,在抗LAG-3+抗4-1BB治疗开始前24小时开始FTY720施用,并且每天持续直至在第14天进行TIL分析。在同一时间点分析的外周血显示出循环T细胞的显著耗尽(图9)。尽管有循环T细胞的该丧失,2B4和NRP1的下调以及向KLRG$1^{高}$IL-7R$A^{低}$表型的转变仍被保留(图7E和图7F)。

[0238] 为了检查TIL的功能恢复,在治疗后第14天从B16.SIY肿瘤中分选KLRG-$1^{低}$IL-7R$A^{低}$和KLRG-$1^{高}$IL-7R$A^{低}$$CD8^+$ TIL群体,并在体外重新刺激后分析IL-2。确实,KLRG-$1^{低}$IL-7R$A^{低}$和KLRG-$1^{高}$IL-7R$A^{低}$群体表现为在刺激后产生IL-2的能力增强(图7G)。Il-2mRNA的相对水平在两个$CD8^+$ TIL群体和对照$CD8^+CD44^+$ TdLN T细胞之间是相当的。总之,这些数据表明抗4-1BB/抗LAG-3组合治疗诱导表型谱的显著变化,并促进已经存在于肿瘤微环境中的肿

瘤抗原特异性$CD8^+$ T细胞的功能恢复。

[0239] 参考文献

[0240] 以下参考文献(所述参考文献中的一些参考文献在上面引用)全文以引用方式并入本文。

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<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 25
ctacccggtg gaagacctc 19
<210> 26
<211> 19
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 26
tcaatcggct gcaagatgt 19
<210> 27
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 27
agatccaaca acgaggagac a 21
<210> 28
<211> 19
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 28
tcaccatgaa acccactgc 19

<210> 29
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 29
caccctagcc taacctcaac c 21
<210> 30
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 30
ctggcagatc cagtcctgtt 20
<210> 31
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 31
atgagggagg agagcggtat 20
<210> 32
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 32
agaaaaccat catgccaggt 20
<210> 33
<211> 18
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 33

atcctcgcgg tgcaaata 18

<210> 34

<211> 19

<212> DNA

<213> 人工序列(Artificial sequence)

<220>

<223> 合成引物/探针

<400> 34

ccggggagat ctcatcaaa 19

<210> 35

<211> 24

<212> DNA

<213> 人工序列(Artificial sequence)

<220>

<223> 合成引物/探针

<400> 35

tgaagacaca ctacctgact cctg 24

<210> 36

<211> 21

<212> DNA

<213> 人工序列(Artificial sequence)

<220>

<223> 合成引物/探针

<400> 36

gctgctcact gtgaaggaag t 21

<210> 37

<211> 20

<212> DNA

<213> 人工序列(Artificial sequence)

<220>

<223> 合成引物/探针

<400> 37

caatctgtac cccgaggaga 20

<210> 38

<211> 19

<212> DNA

<213> 人工序列(Artificial sequence)

<220>

<223> 合成引物/探针

<400> 38
ctgtagccca cgtcgtagc 19
<210> 39
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 39
gctgctcact gtgaaggaag t 21
<210> 40
<211> 19
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 40
tcaccatgaa acccactgc 19
<210> 41
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 41
tcttgctgtg gcaattcaga 20
<210> 42
<211> 18
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 42
gctgcaggga agatggac 18
<210> 43
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>

<223> 合成引物/探针
<400> 43
gaacggtact ggcgtctgtc 20
<210> 44
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 44
aatgttgatc atgccatctc c 21
<210> 45
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 45
cgcagacagc tgagtagttc c 21
<210> 46
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 46
tcatgcaacg cttagactgg 20
<210> 47
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 47
agcagcagct attggagacc 20
<210> 48
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)

<220>
<223> 合成引物/探针
<400> 48
tgaaaacctc ctcccctctt 20
<210> 49
<211> 19
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 49
acccagttca tgccatcct 19
<210> 50
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 50
cagcttgttg tccaaatcgt c 21
<210> 51
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 51
acaggtgaag caggtctcgt 20
<210> 52
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 52
gcccttaaag actgcatcac a 21
<210> 53
<211> 19
<212> DNA

<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 53
tcgaaggcca tgtcatctg 19
<210> 54
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 54
cccacaatgt gttgcagttc 20
<210> 55
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 55
tggggaatgc attttaccat 20
<210> 56
<211> 21
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 56
ccgatgtcca tcacattctc t 21
<210> 57
<211> 18
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 57
ttgagatcca tgccgttg 18
<210> 58
<211> 20

<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 58
tggggaatgc attttaccat 20
<210> 59
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 59
agcagcagct attggagacc 20
<210> 60
<211> 20
<212> DNA
<213> 人工序列(Artificial sequence)
<220>
<223> 合成引物/探针
<400> 60
gcagagggtg acggatgtag 20

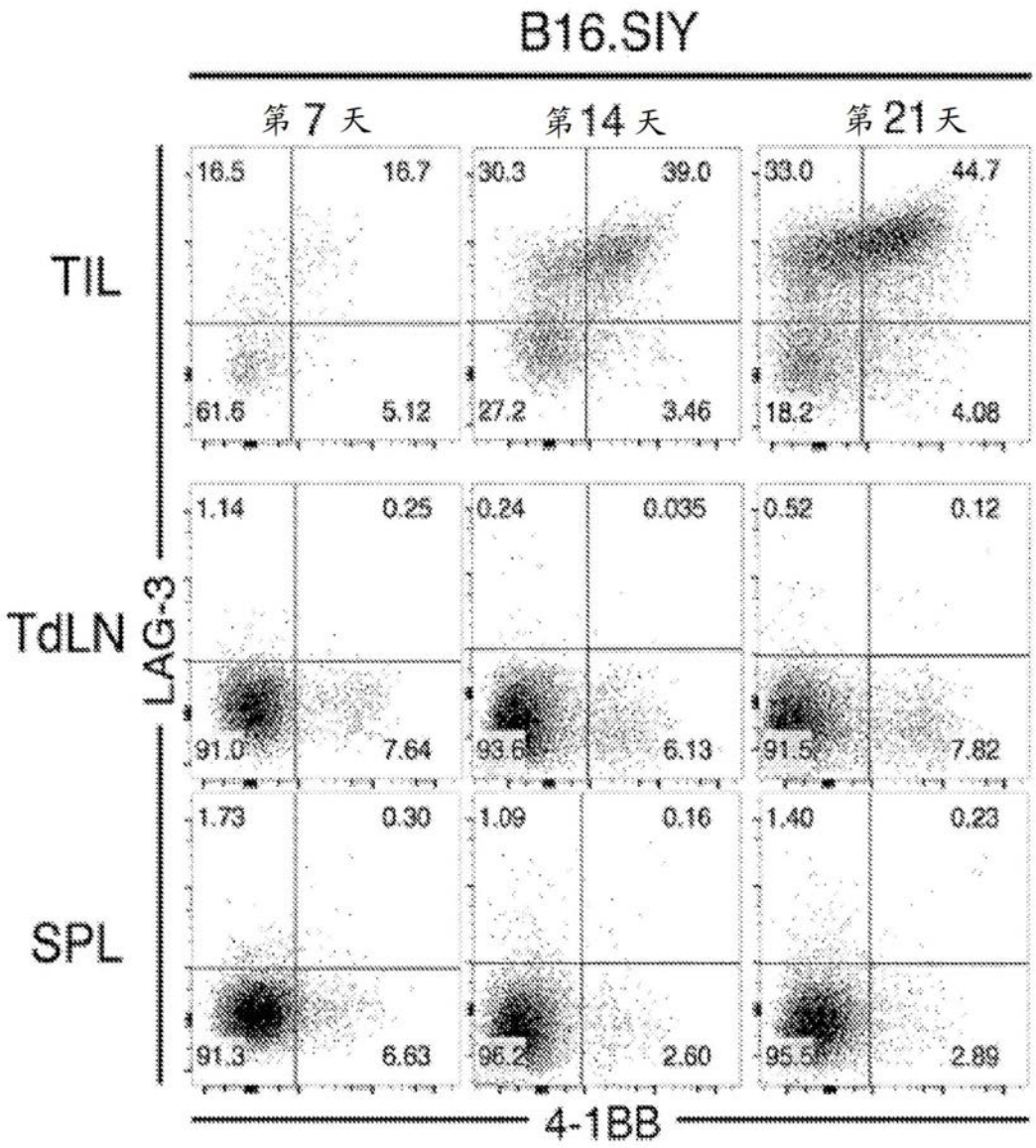

B16.SIY
第7天
第14天
第21天
TIL
TdLN
SPL
LAG-3
4-1BB
16.5
16.7
61.6
5.12
30.3
39.0
27.2
3.46
33.0
44.7
18.2
4.08
1.14
0.25
91.0
7.64
0.24
0.035
93.6
6.13
0.52
0.12
91.5
7.82
1.73
0.30
91.3
6.63
1.09
0.16
96.2
2.60
1.40
0.23
95.5
2.89

图1A

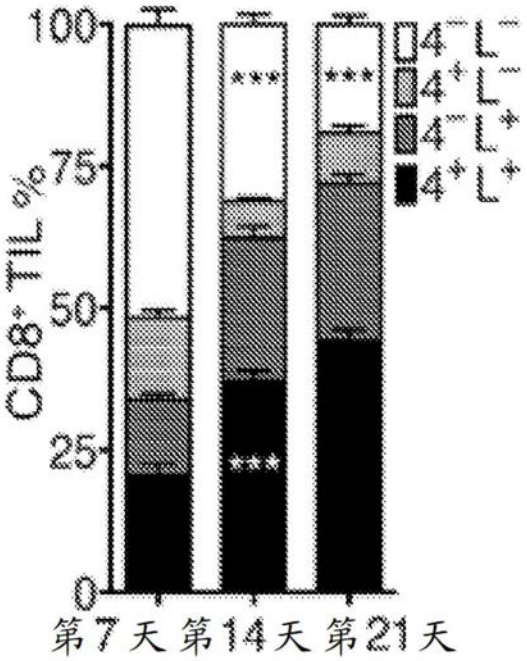

100
75
50
25
0
CD8+ TIL %
4⁻L⁻
4⁺L⁻
4⁻L⁺
4⁺L⁺
***
***
***
第7天 第14天 第21天

图1B

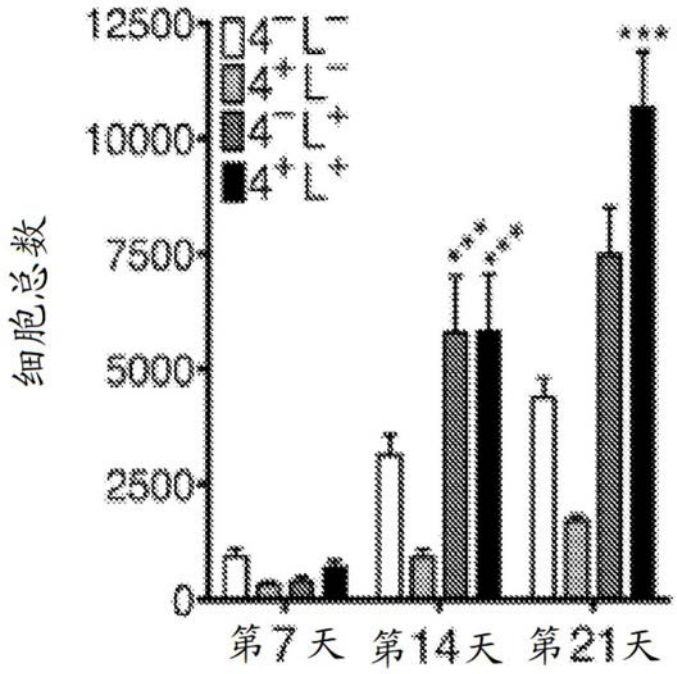

12500
10000
7500
5000
2500
0
细胞总数
4⁻L⁻
4⁺L⁻
4⁻L⁺
4⁺L⁺
***
***
***
第7天 第14天 第21天

图1C

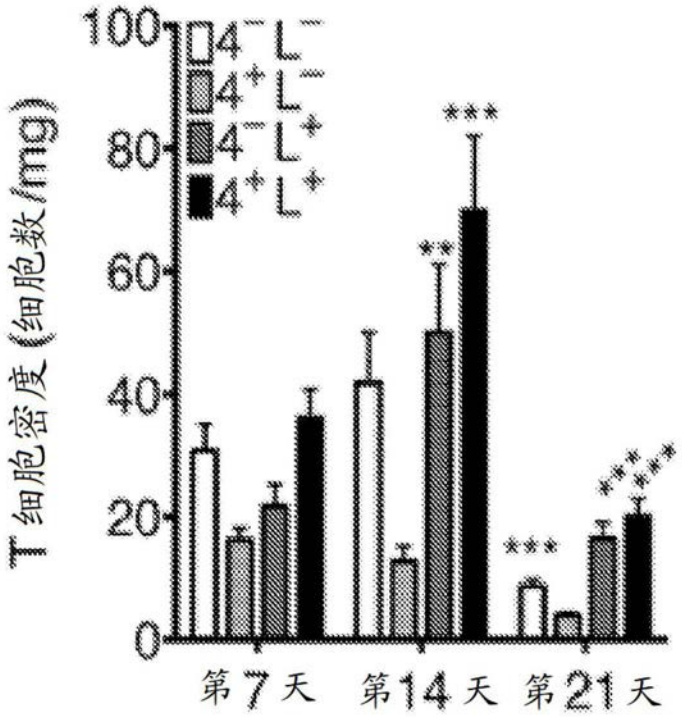

T细胞密度(细胞数/mg)
100
80
60
40
20
0
4⁻L⁻
4⁺L⁻
4⁻L⁺
4⁺L⁺
第7天
第14天
第21天

图1D

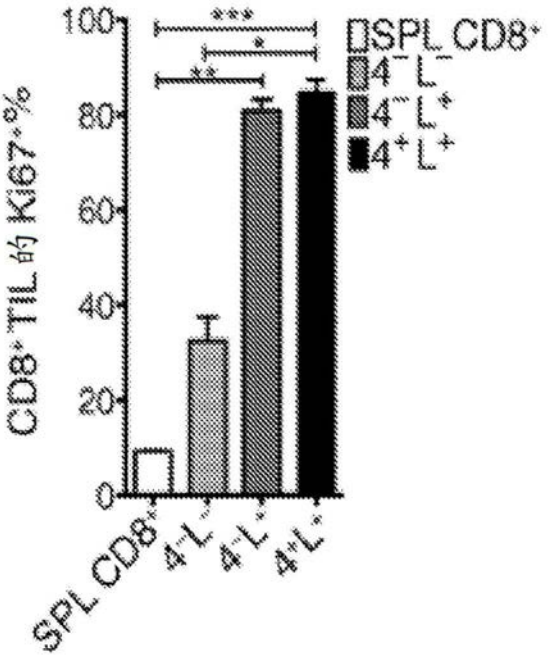

CD8⁺TIL的Ki67⁺%
100
80
60
40
20
0
SPL CD8⁺
4⁻L⁻
4⁻L⁺
4⁺L⁺

图1E

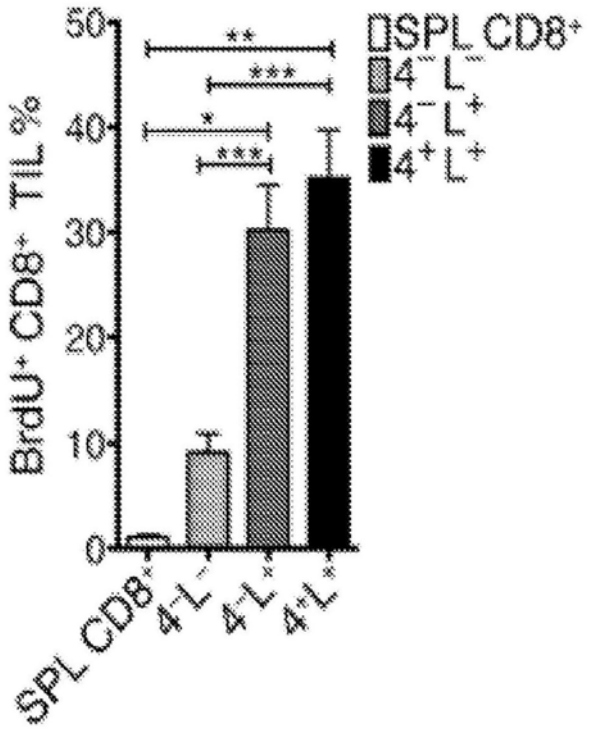

SPL CD8+
4-L-
4-L+
4+L+
BrdU+ CD8+ TIL %
0
10
20
30
40
50
*
**
***
***
SPL CD8+
4-L-
4-L+
4+L+

图1F

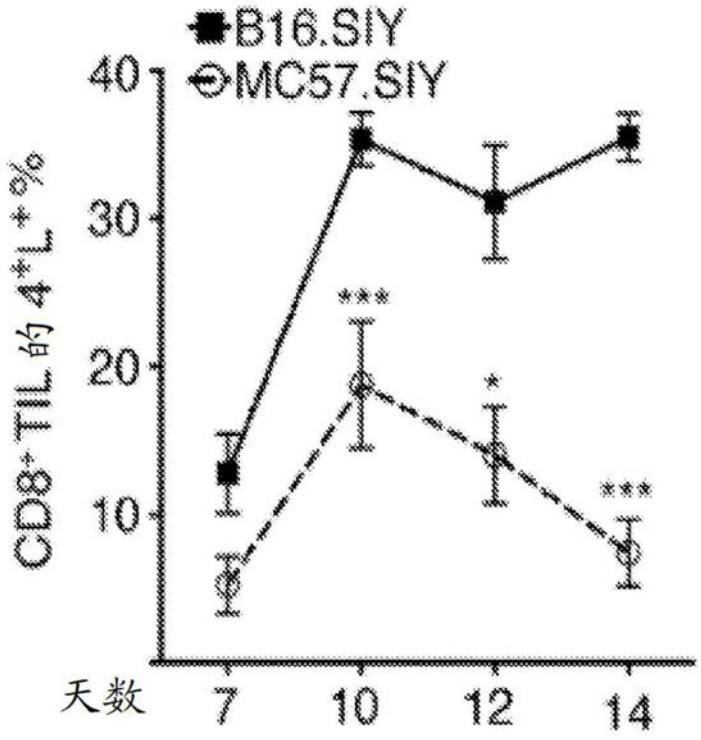

B16.SIY
MC57.SIY
CD8+ TIL 的 4+L+ %
10
20
30
40
***
*
***
天数
7
10
12
14

图1J

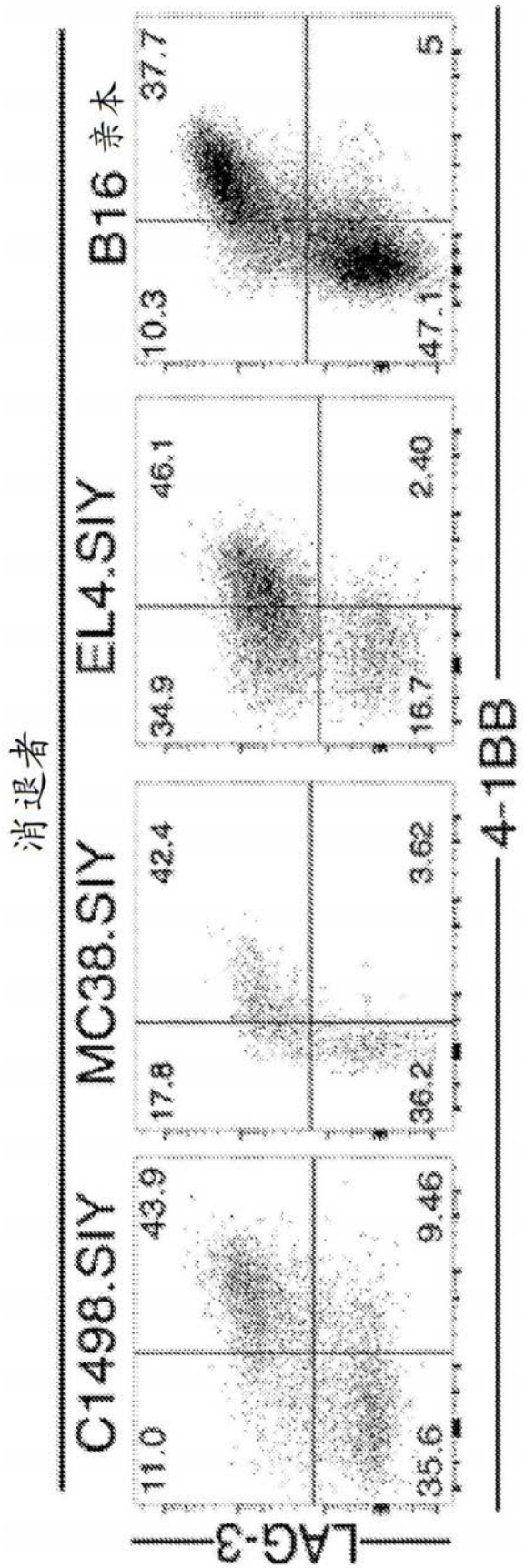

消退者
C1498.SIY
MC38.SIY
EL4.SIY
B16 亲本
LAG-3
4-1BB
11.0
43.9
35.6
9.46
17.8
42.4
36.2
3.62
34.9
46.1
16.7
2.40
10.3
37.7
47.1
5

图1G

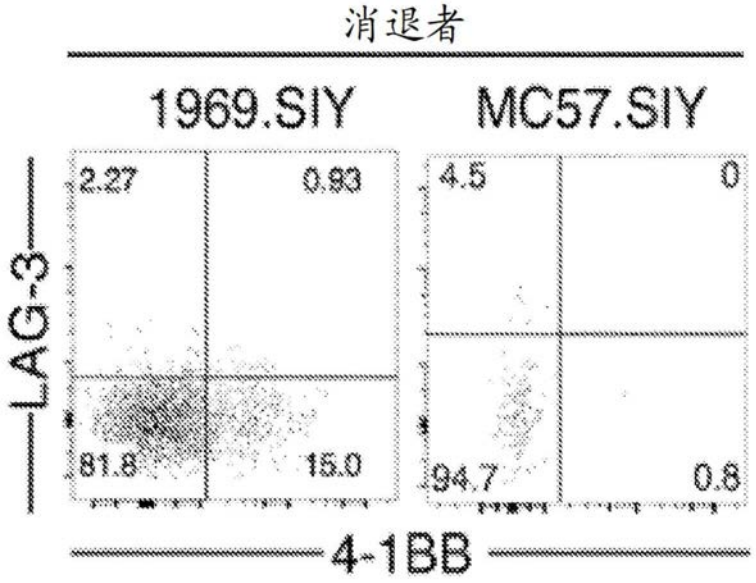

消退者
1969.SIY
MC57.SIY
LAG-3
2.27
0.93
81.8
15.0
4.5
0
94.7
0.8
4-1BB

图1H

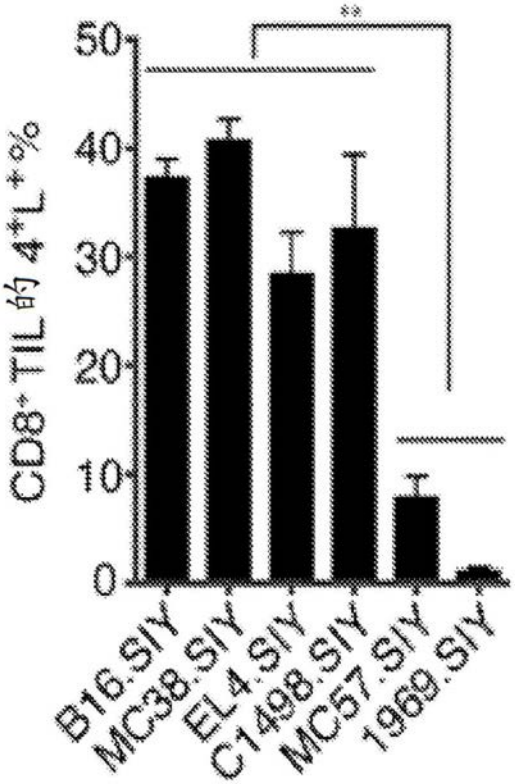

**
50
40
30
20
10
0
CD8+ TIL的4+L+%
B16.SIY
MC38.SIY
EL4.SIY
C1498.SIY
MC57.SIY
1969.SIY

图1I

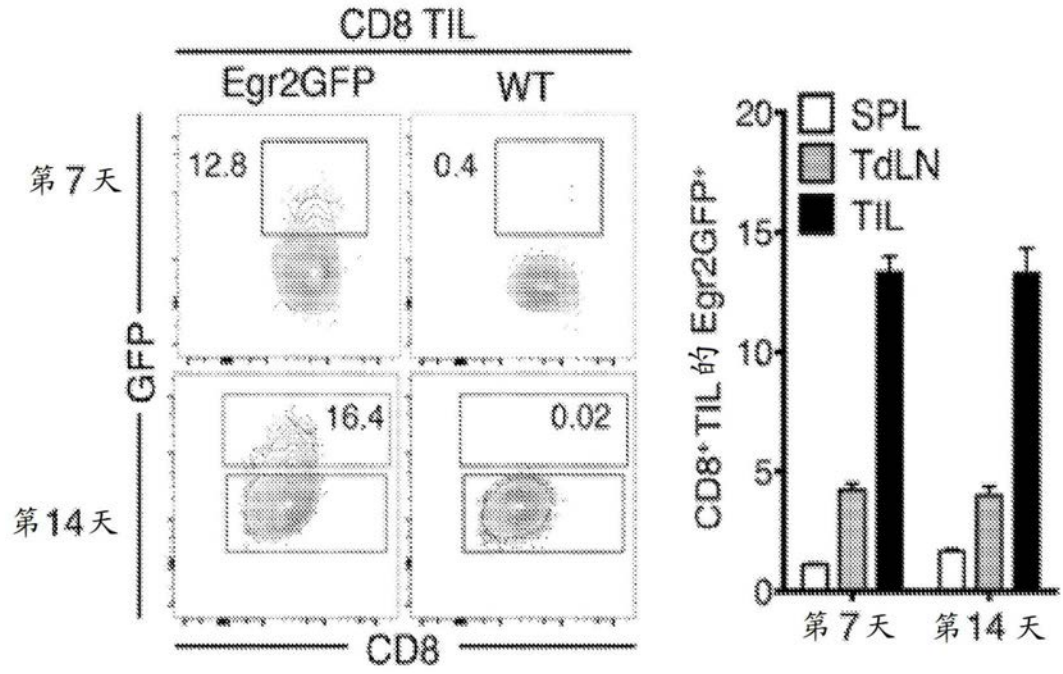

CD8 TIL
Egr2GFP
WT
第7天
第14天
GFP
12.8
0.4
16.4
0.02
CD8
SPL
TdLN
TIL
CD8+TIL的Egr2GFP+
第7天
第14天

图2A

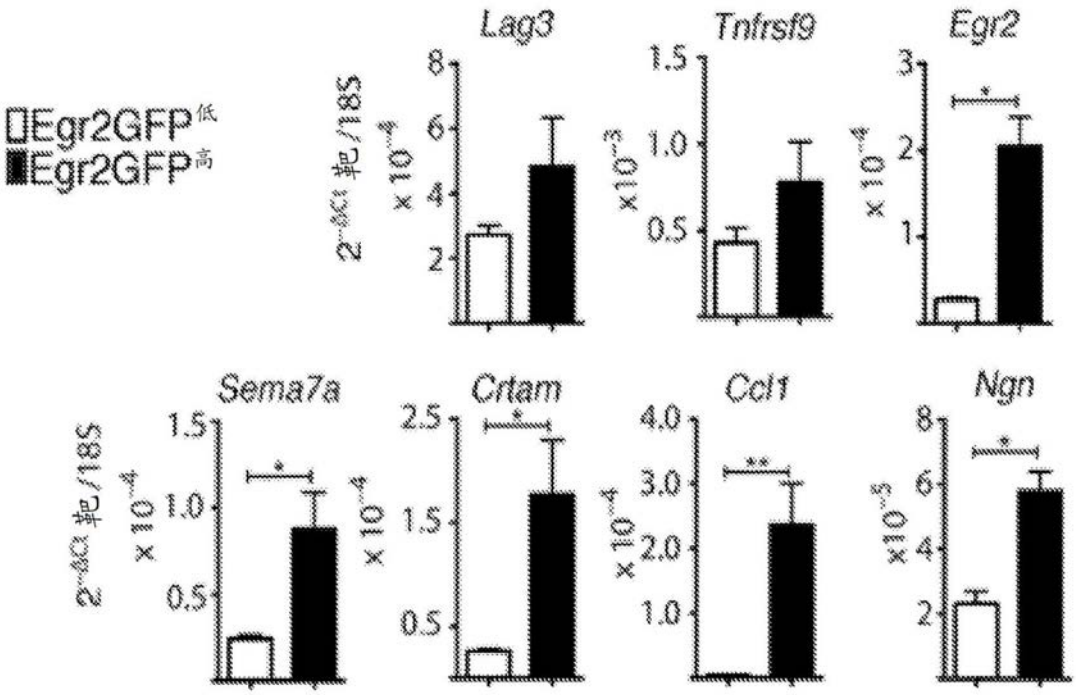

Egr2GFP低
Egr2GFP高
Lag3
Tnfrsf9
Egr2
Sema7a
Crtam
Ccl1
Ngn

图2B

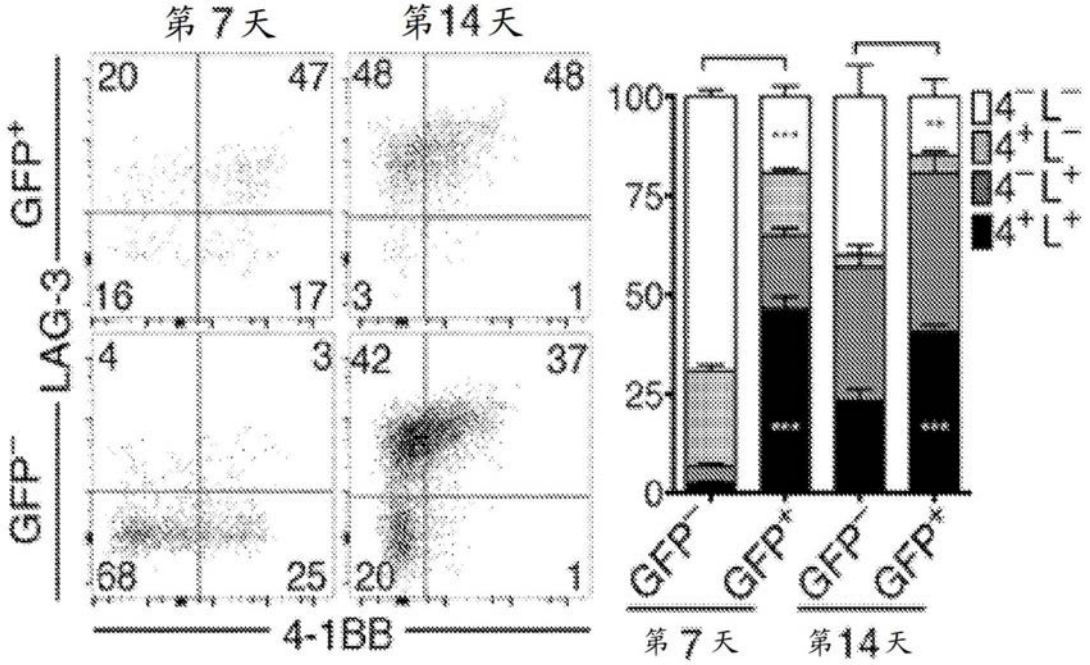

第7天
第14天
GFP+
GFP−
LAG-3
4-1BB
20
47
16
17
4
3
68
25
48
48
3
1
42
37
20
1
100
75
50
25
0
4− L−
4+ L−
4− L+
4+ L+
GFP−
GFP+
GFP−
GFP+
第7天
第14天

图2C

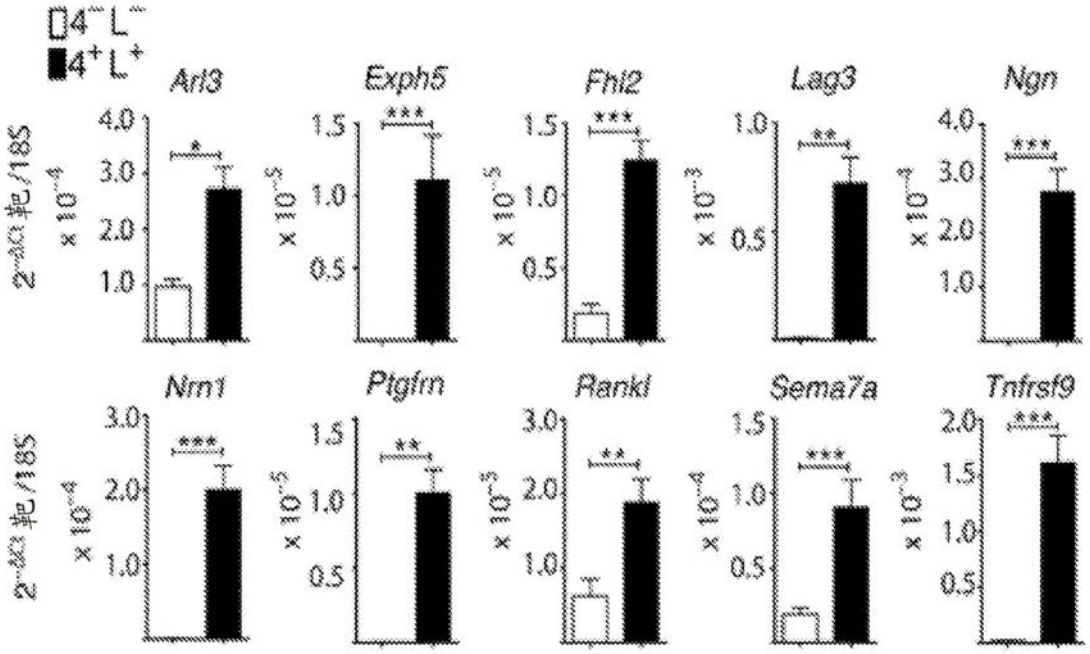

4− L−
4+ L+
Arl3
Exph5
Fhl2
Lag3
Ngn
Nrn1
Ptgfrn
Rankl
Sema7a
Tnfrsf9

图2D

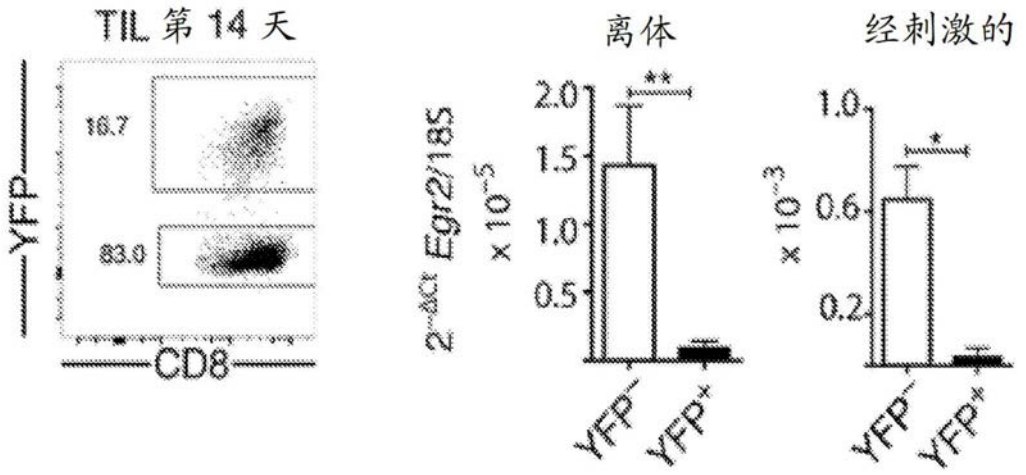

TIL 第 14 天
YFP
CD8
16.7
83.0
离体
经刺激的
$2^{-\Delta Ct}$ Egr2/18S
$\times 10^{-5}$
$\times 10^{-3}$
2.0
1.5
1.0
0.5
1.0
0.6
0.2
**
*
YFP⁻
YFP⁺

图2E

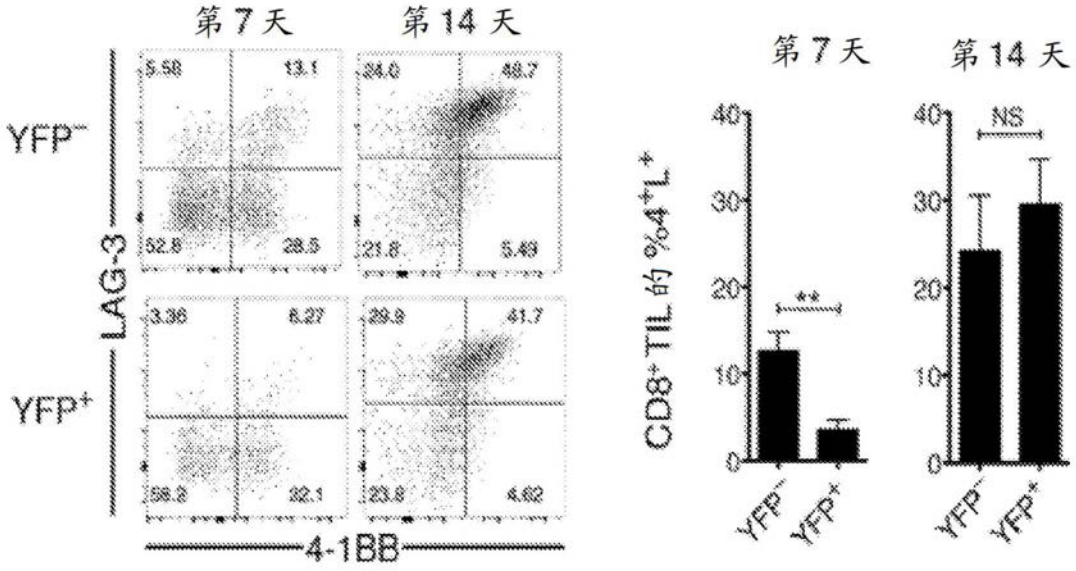

第 7 天
第 14 天
YFP⁻
YFP⁺
LAG-3
4-1BB
5.58
13.1
52.8
28.5
24.0
48.7
21.8
5.49
3.36
6.27
58.2
32.1
29.9
41.7
23.8
4.62
CD8⁺ TIL 的 %4⁺L⁺
40
30
20
10
0
**
NS

图2F

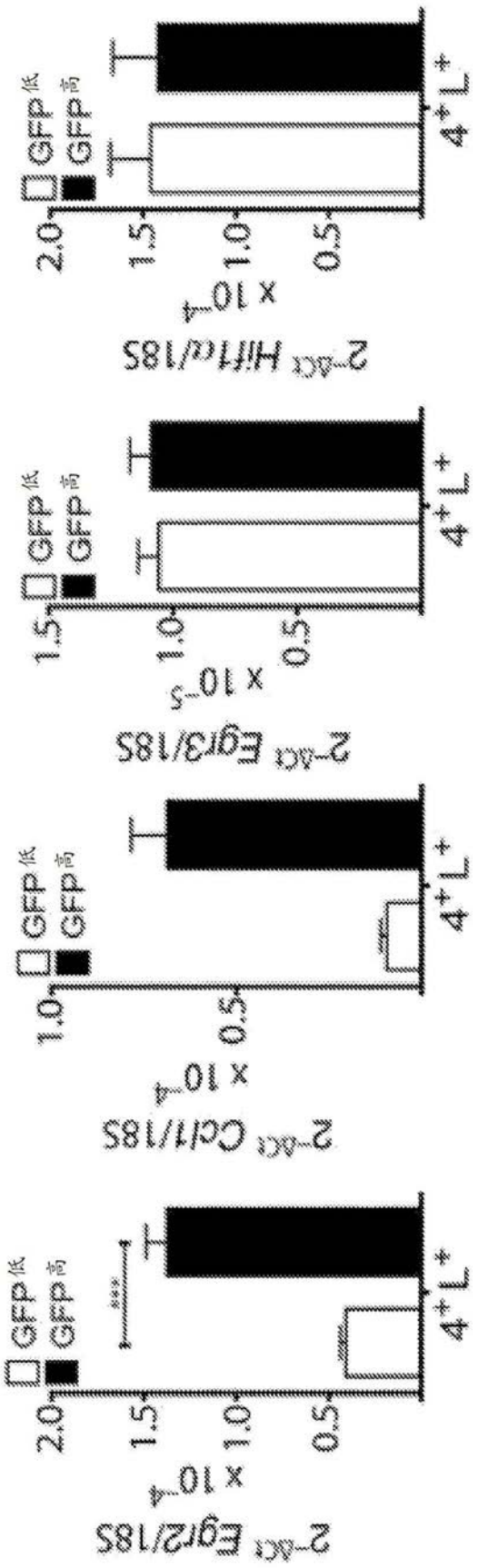

GFP低
GFP高
4+L+
2.0
1.5
1.0
0.5
x 10−4
2−ΔCt Hif1α/18S
GFP低
GFP高
4+L+
1.5
1.0
0.5
x 10−5
2−ΔCt Egr3/18S
GFP低
GFP高
4+L+
1.0
0.5
x 10−4
2−ΔCt Cd1/18S
GFP低
GFP高
**
4+L+
2.0
1.5
1.0
0.5
x 10−4
2−ΔCt Egr2/18S

图2G

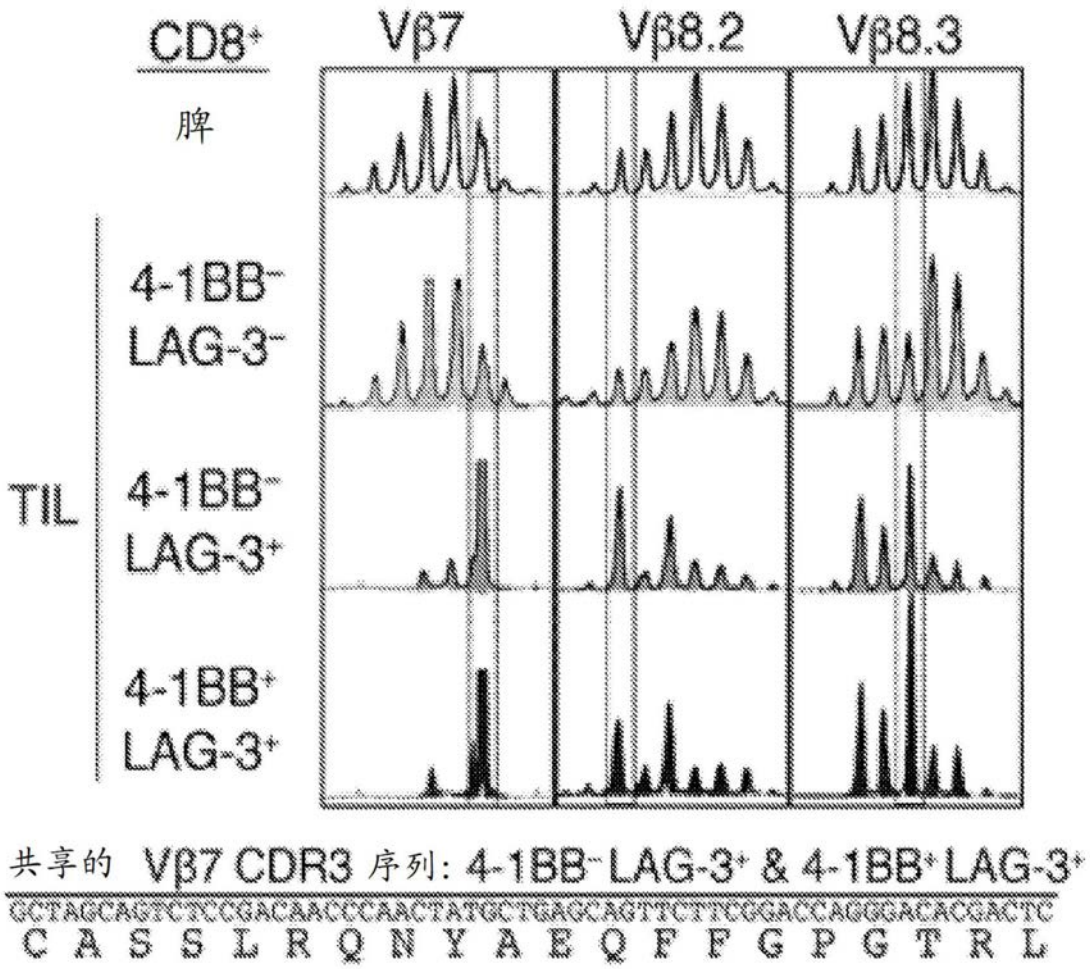

CD8+
Vβ7
Vβ8.2
Vβ8.3
脾
TIL
4-1BB−
LAG-3−
4-1BB−
LAG-3+
4-1BB+
LAG-3+
共享的 Vβ7 CDR3 序列: 4-1BB−LAG-3+ & 4-1BB+LAG-3+
GCTAGCAGTCTCCGACAACCCAACTATGCTGAGCAGTTCTTCGGACCAGGGACACGACTC
C A S S L R Q N Y A E Q F F G P G T R L

图3A

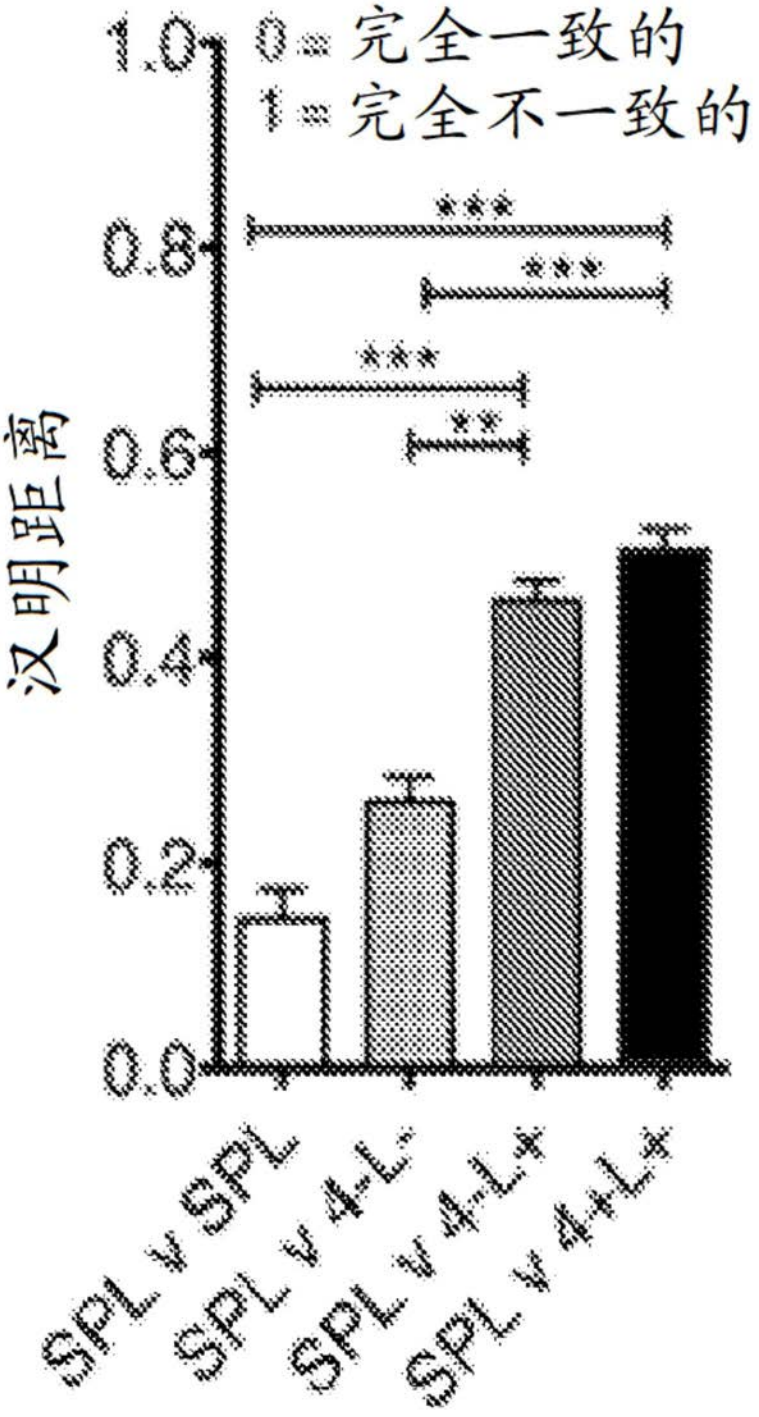

0=完全一致的
1=完全不一致的
汉明距离
1.0
0.8
0.6
0.4
0.2
0.0
***
***
***
**
SPL v SPL
SPL v 4-L
SPL v 4-L*
SPL v 4-L*

图3B

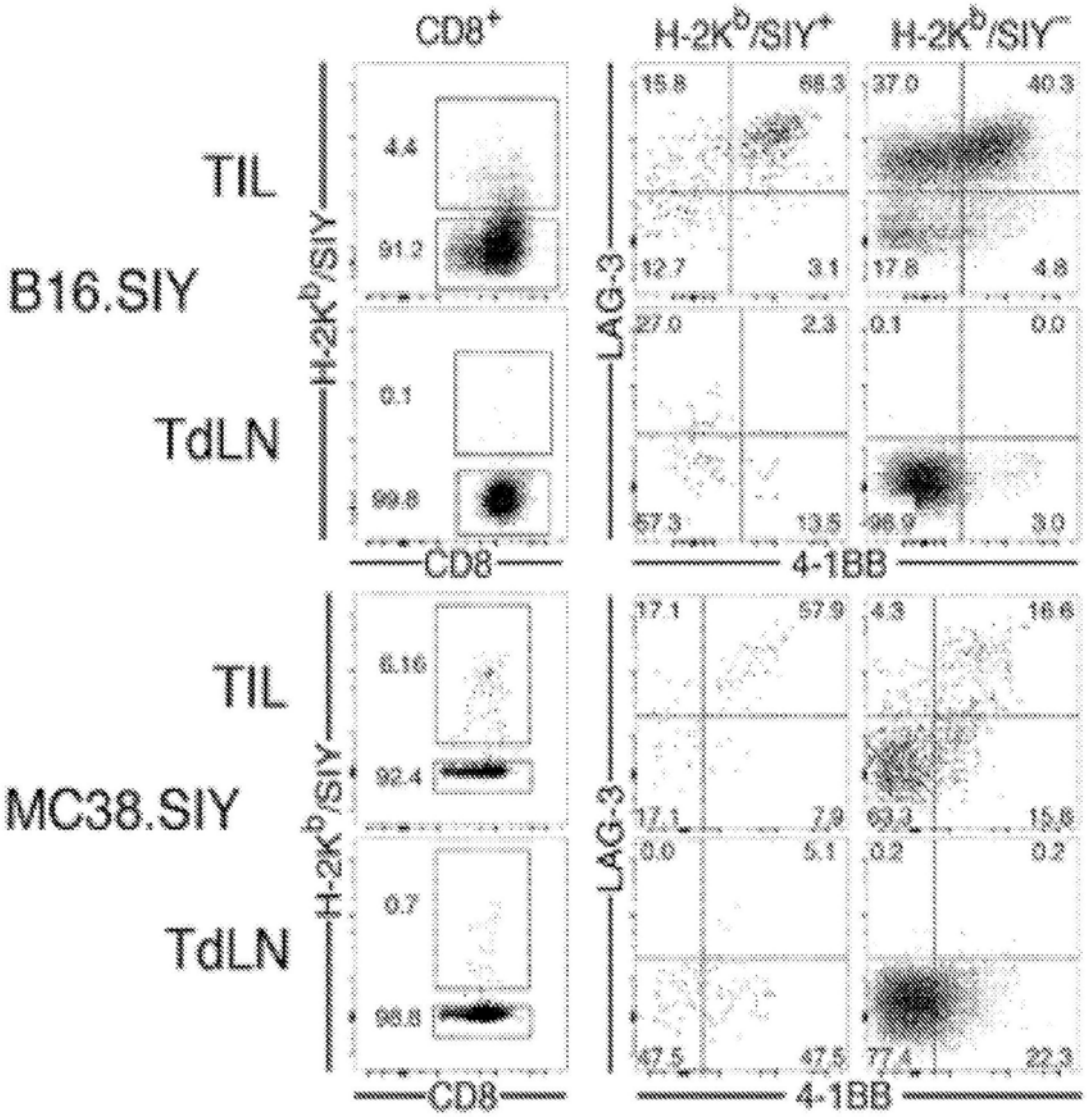

CD8+
H-2Kb/SIY+
H-2Kb/SIY−
TIL
B16.SIY
TdLN
TIL
MC38.SIY
TdLN
H-2Kb/SIY
CD8
LAG-3
4-1BB
4.4
91.2
0.1
99.8
15.8
68.3
12.7
3.1
37.0
40.3
17.8
4.8
27.0
2.3
57.3
13.5
0.1
0.0
99.9
3.0
6.16
92.4
0.7
98.8
17.1
57.9
7.9
4.3
16.6
63.2
15.6
0.0
5.1
47.5
47.5
0.2
0.2
77.4
22.3

图3C

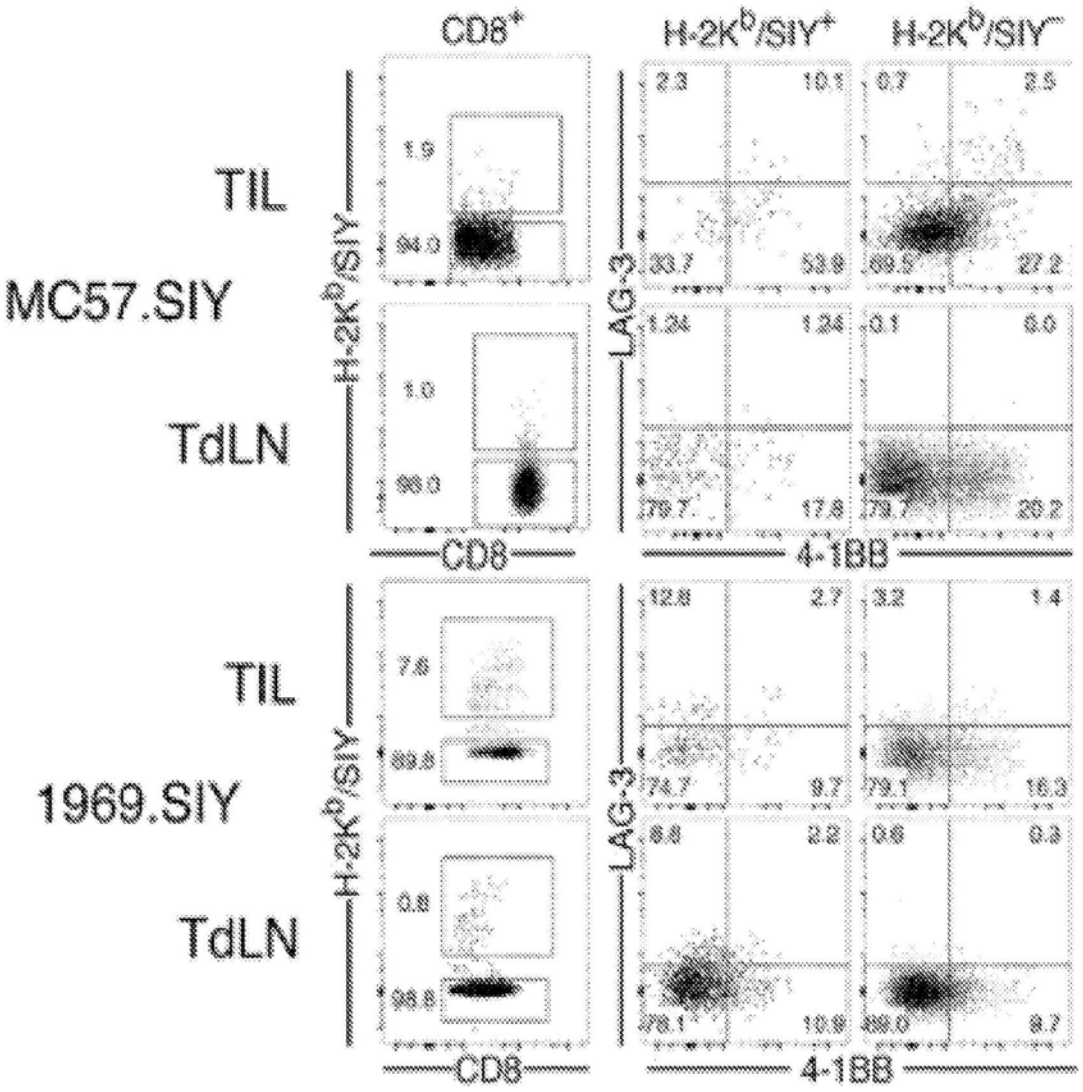

CD8+
H-2Kb/SIY+
H-2Kb/SIY−
TIL
MC57.SIY
TdLN
1969.SIY
H-2Kb/SIY
LAG-3
CD8
4-1BB
1.9
84.0
2.3
10.1
0.7
2.5
23.7
53.8
69.5
27.2
1.24
1.24
0.1
6.0
79.7
17.8
79.7
20.2
1.0
96.0
7.6
89.8
12.8
2.7
3.2
1.4
74.7
9.7
79.1
16.3
0.8
98.8
6.8
2.2
0.6
0.3
78.1
10.9
89.0
9.7

图3D

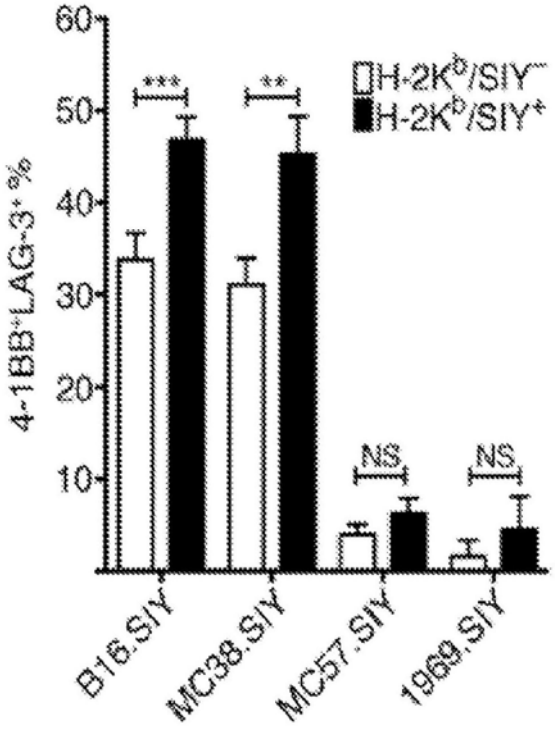

60
50
40
30
20
10
4-1BB+LAG-3+ %
H-2Kb/SIY−
H-2Kb/SIY+
***
**
NS
NS
B16.SIY
MC38.SIY
MC57.SIY
1969.SIY

图3E

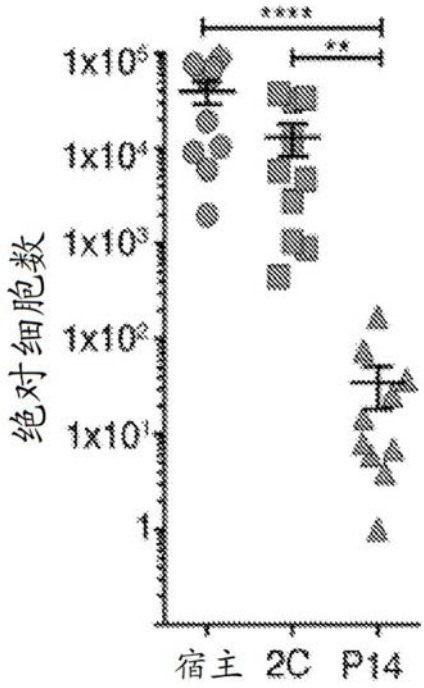

****
**
1x10⁵
1x10⁴
1x10³
1x10²
1x10¹
1
绝对细胞数
宿主
2C
P14

图3F

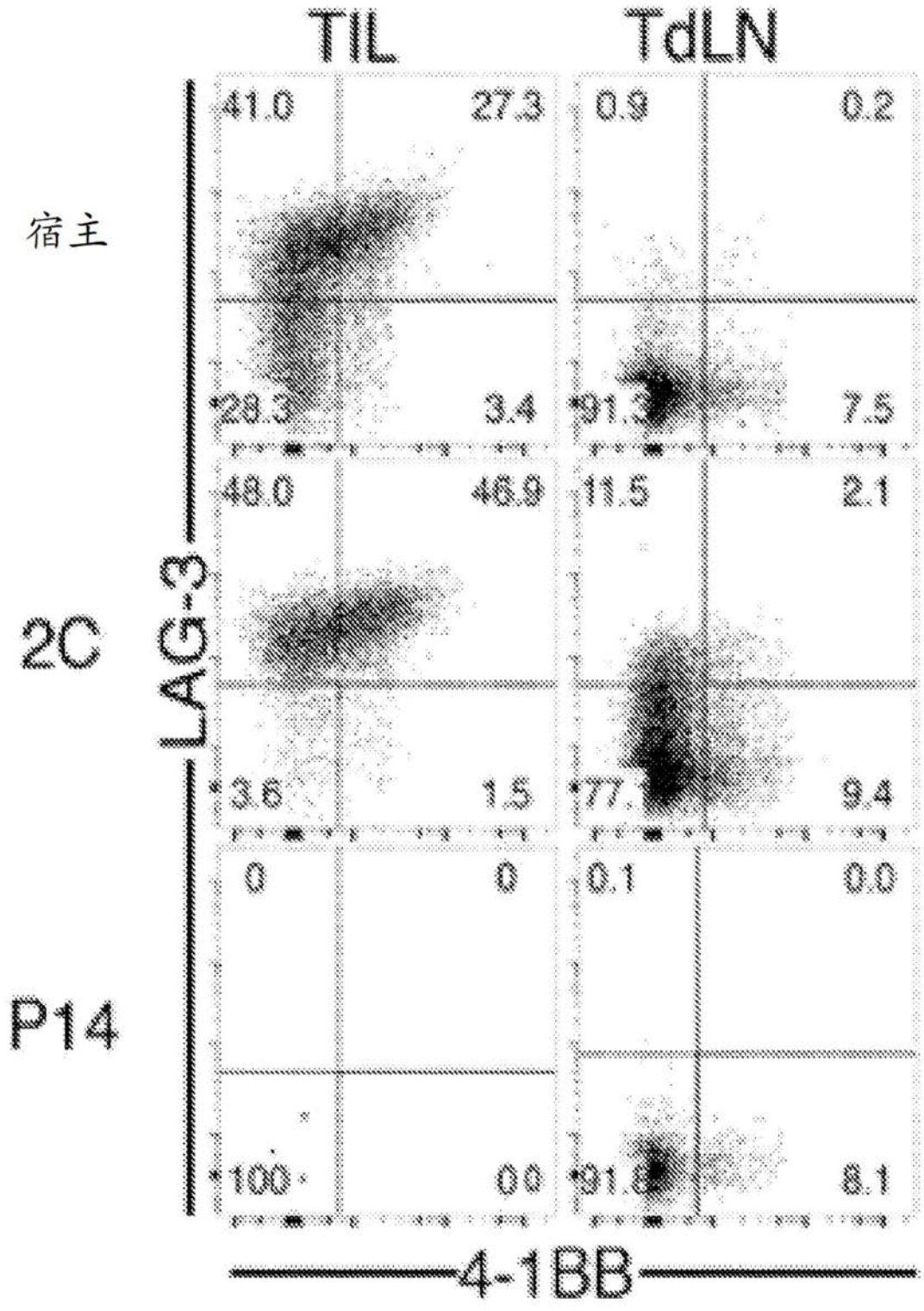

TIL
TdLN
宿主
2C
P14
LAG-3
4-1BB
41.0
27.3
28.3
3.4
0.9
0.2
91.3
7.5
48.0
46.9
3.6
1.5
11.5
2.1
77.1
9.4
0
0
100
0.0
0.1
0.0
91.8
8.1

图3G

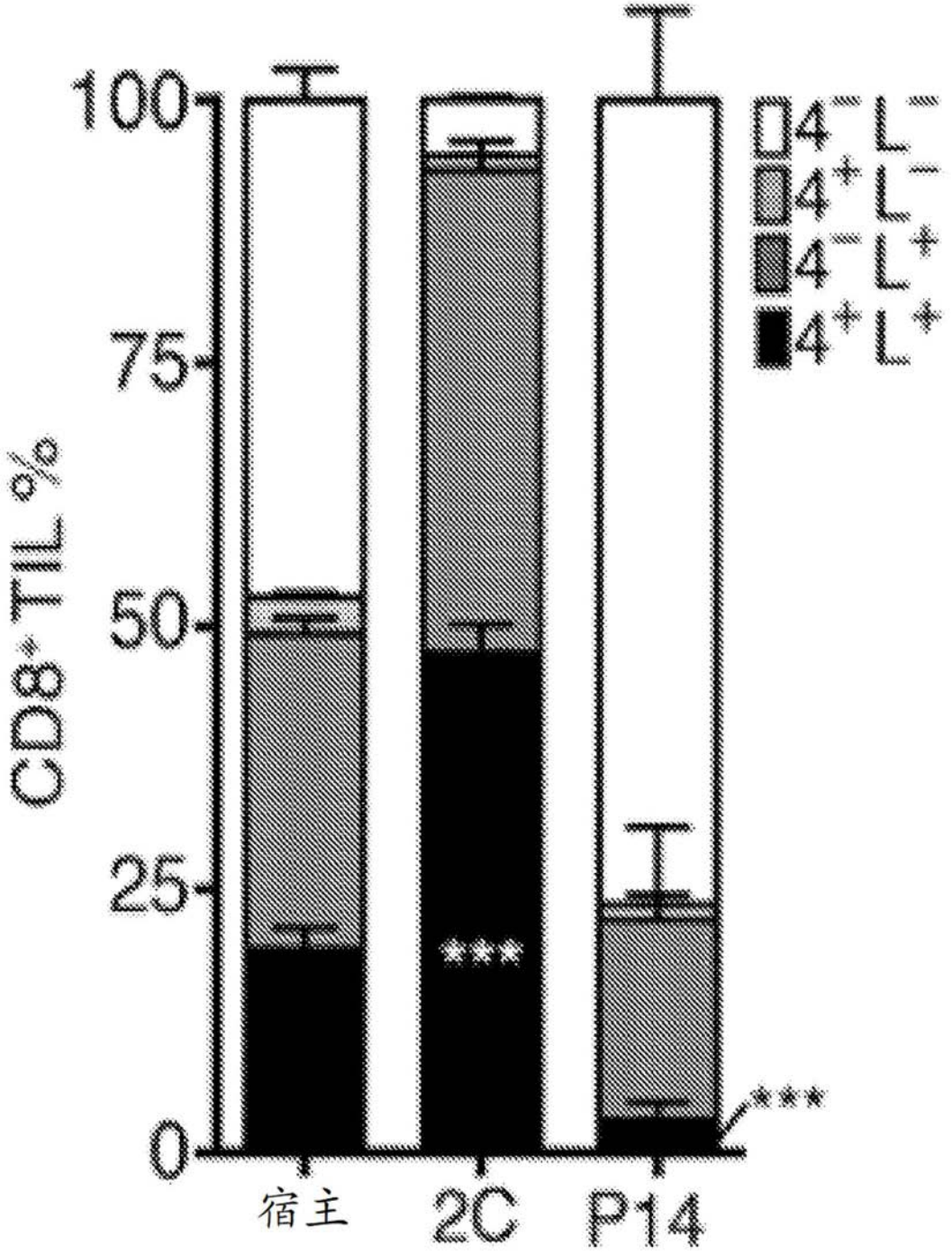


图3H

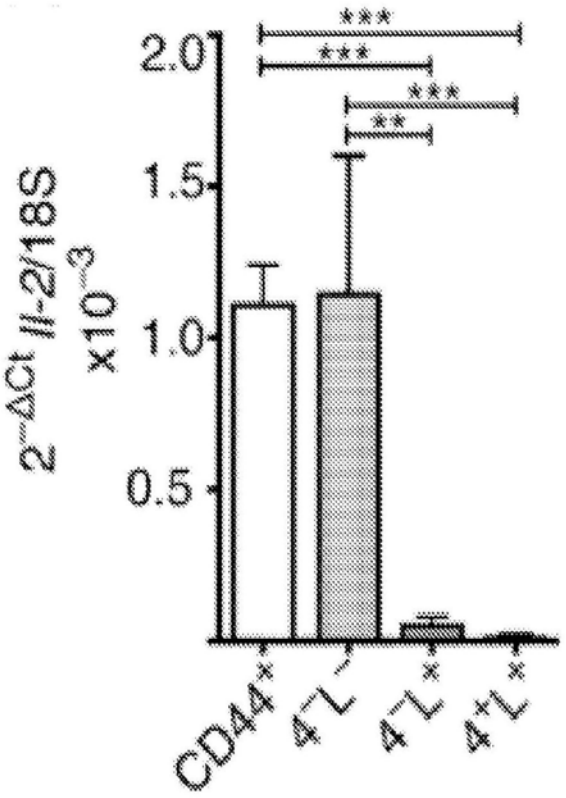

2.0
1.5
1.0
0.5
$2^{-\Delta Ct}$ Il-2/18S x10^{-3}
***
***
***
**
CD44+
4-L-
4-L+
4+L+

图4A

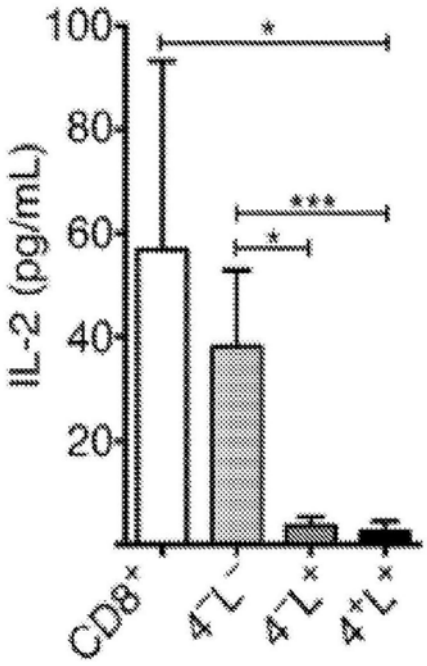

100
80
60
40
20
IL-2 (pg/mL)
*
***
*
CD8+
4-L-
4-L+
4+L+

图4B

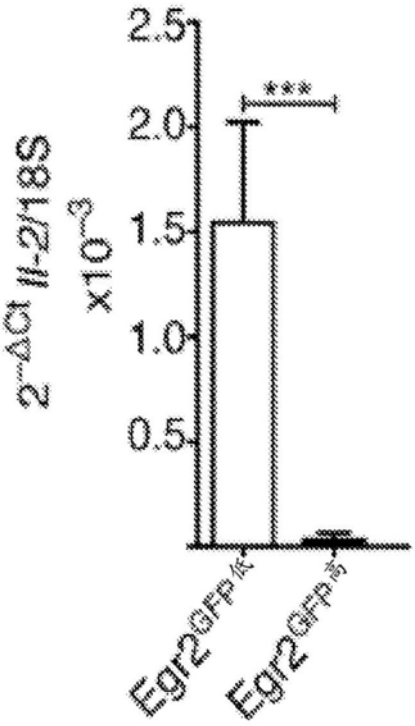

2.5
2.0
1.5
1.0
0.5
***
$2^{-\Delta Ct}$ Il-2/18S
x10^{-3}
Egr2$^{GFP^{低}}$
Egr2$^{GFP^{高}}$

图4C

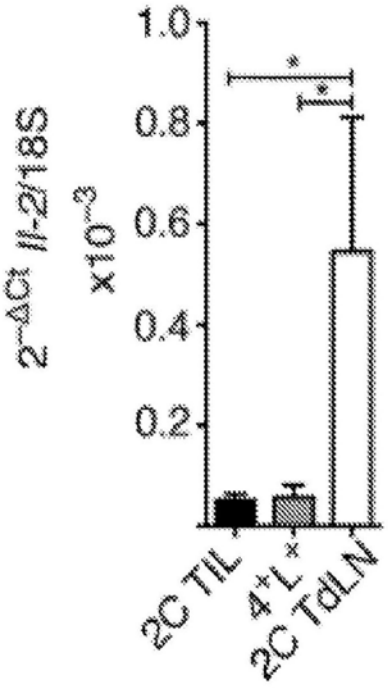

1.0
0.8
0.6
0.4
0.2
*
*
$2^{-\Delta Ct}$ Il-2/18S
x10^{-3}
2C TIL
4^{+}L^{+}
2C TdLN

图4D

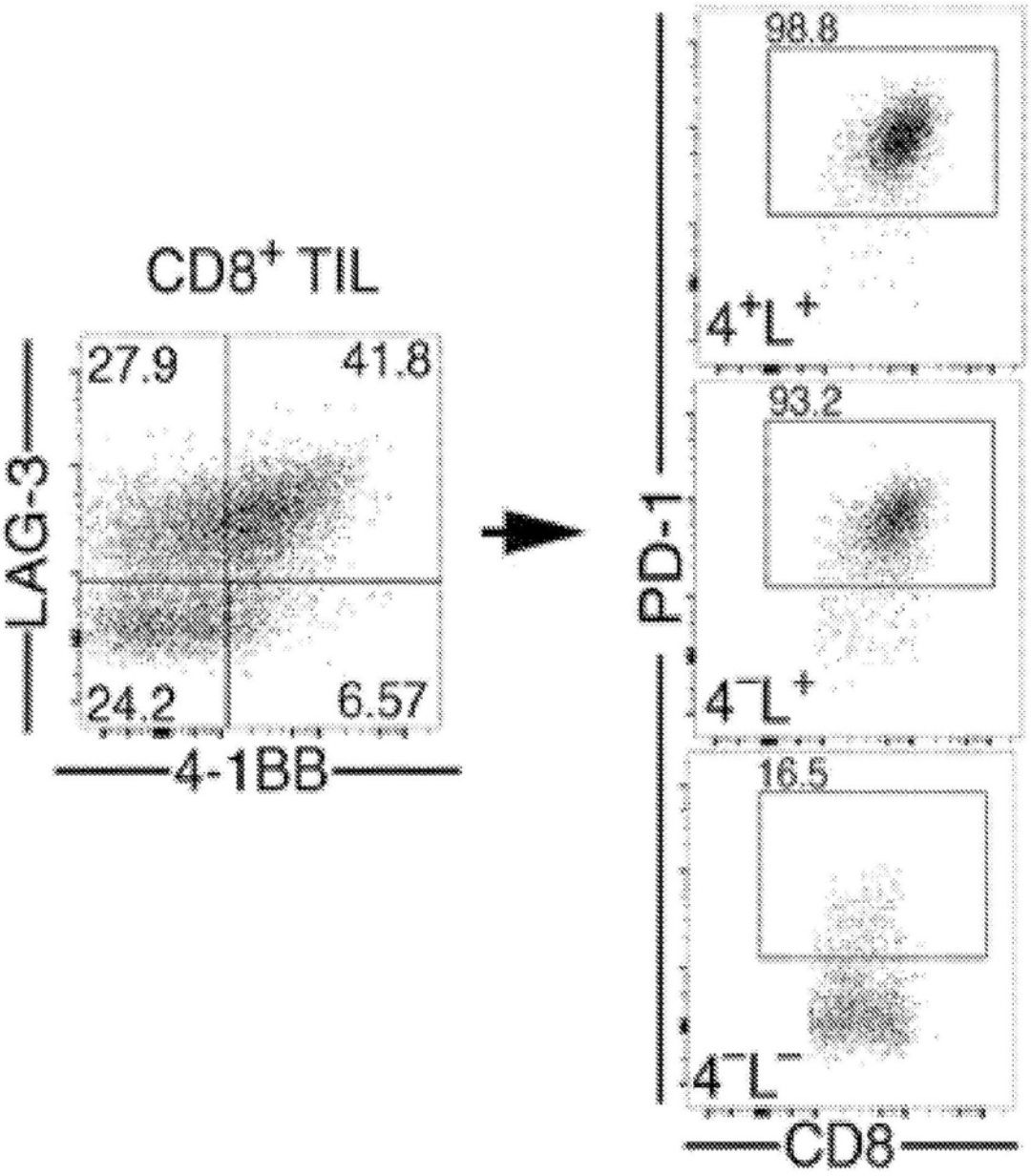


图4E

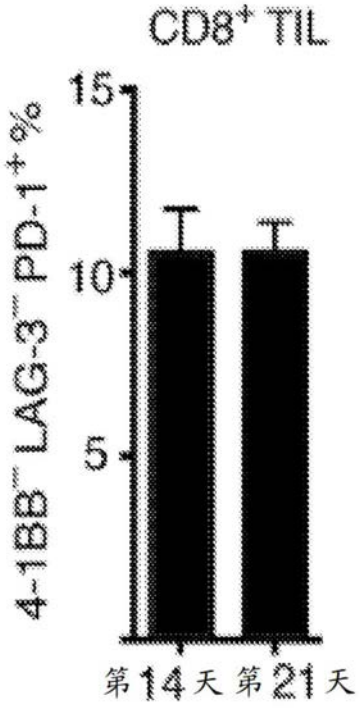

CD8+ TIL
4-1BB− LAG-3− PD-1+ %
15
10
5
第14天
第21天

图4F

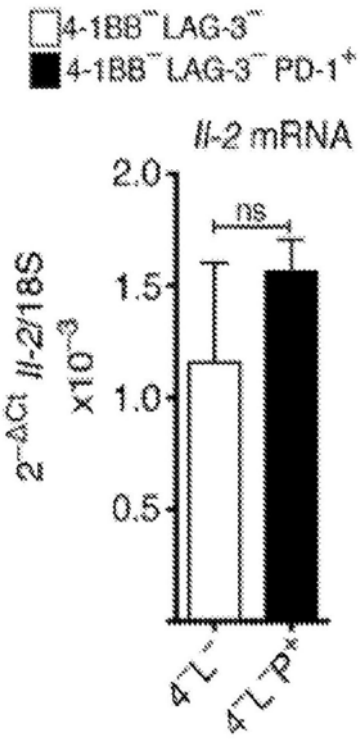

4-1BB− LAG-3−
4-1BB− LAG-3− PD-1+
Il-2 mRNA
2−ΔCt Il-2/18S ×10−3
2.0
1.5
1.0
0.5
ns
4−L−
4−L−P+

图4G

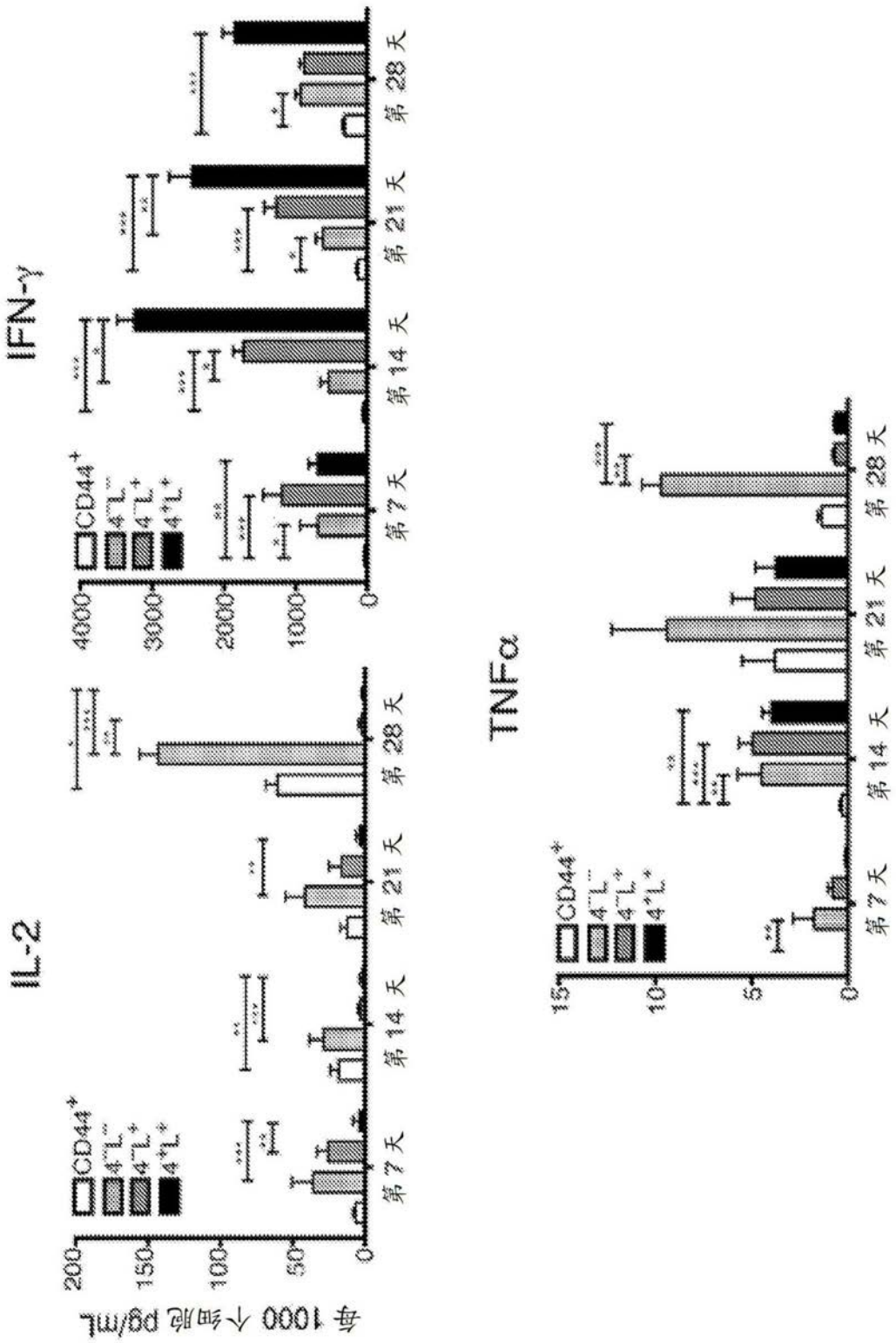

IFN-γ
IL-2
TNFα
每 1000 个细胞 pg/mL
CD44+
4-1L-
4-1L+
4+1L+
第 7 天
第 14 天
第 21 天
第 28 天

图5A

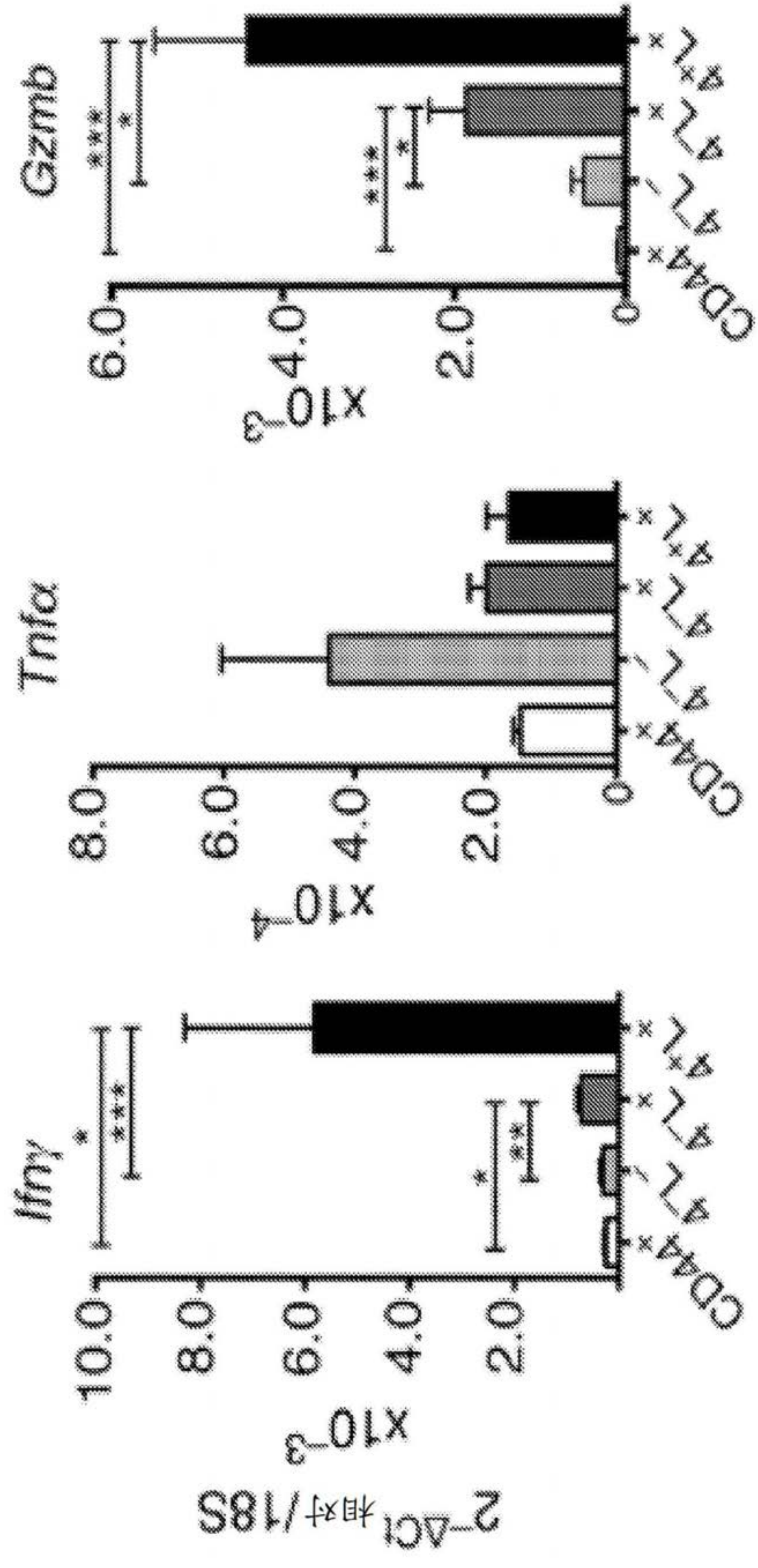


图5B

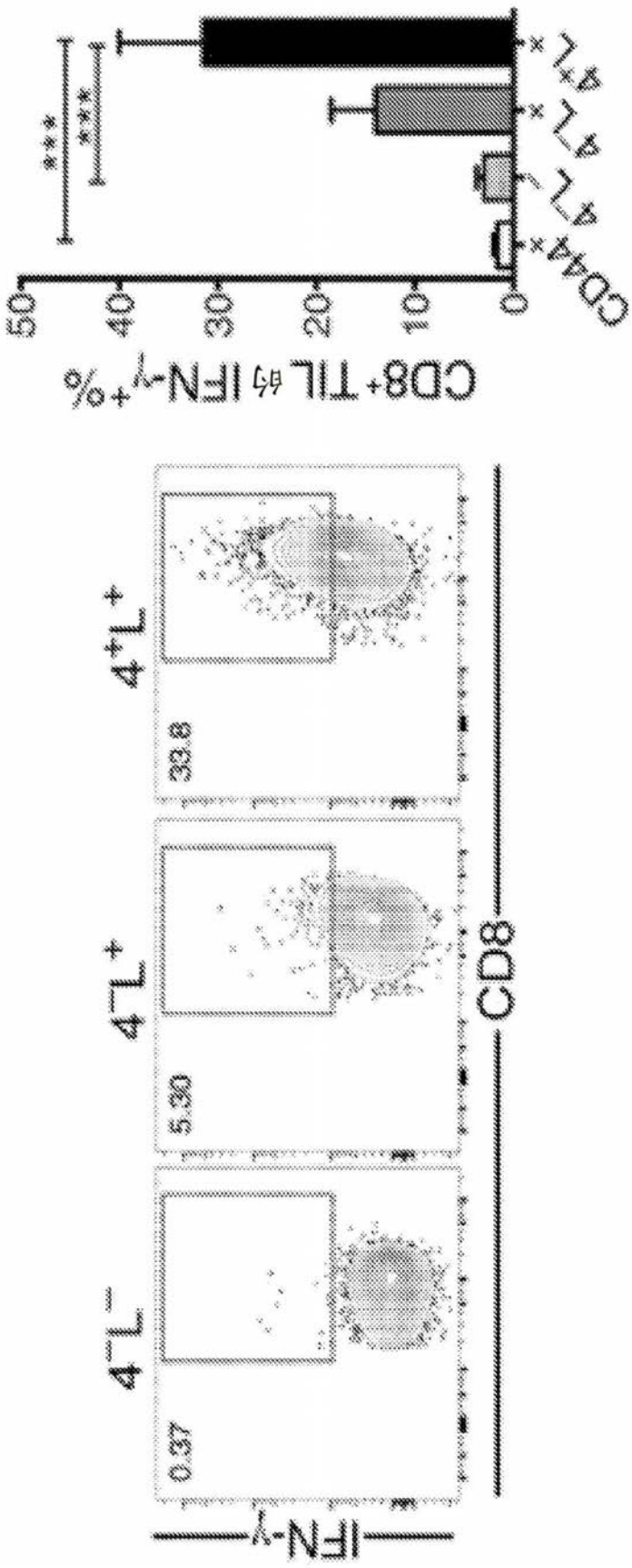
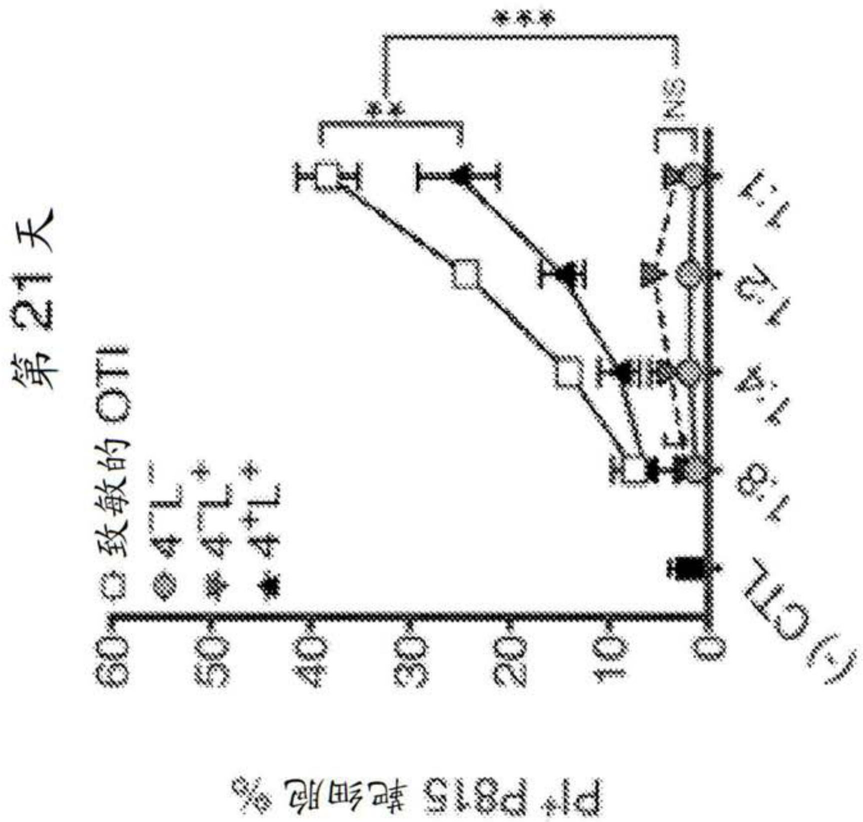


图5C


第21天
致敏的OTI
PI+ P815 靶细胞 %
(-) CTL
1:8
1:4
1:2
1:1
***
**
NS

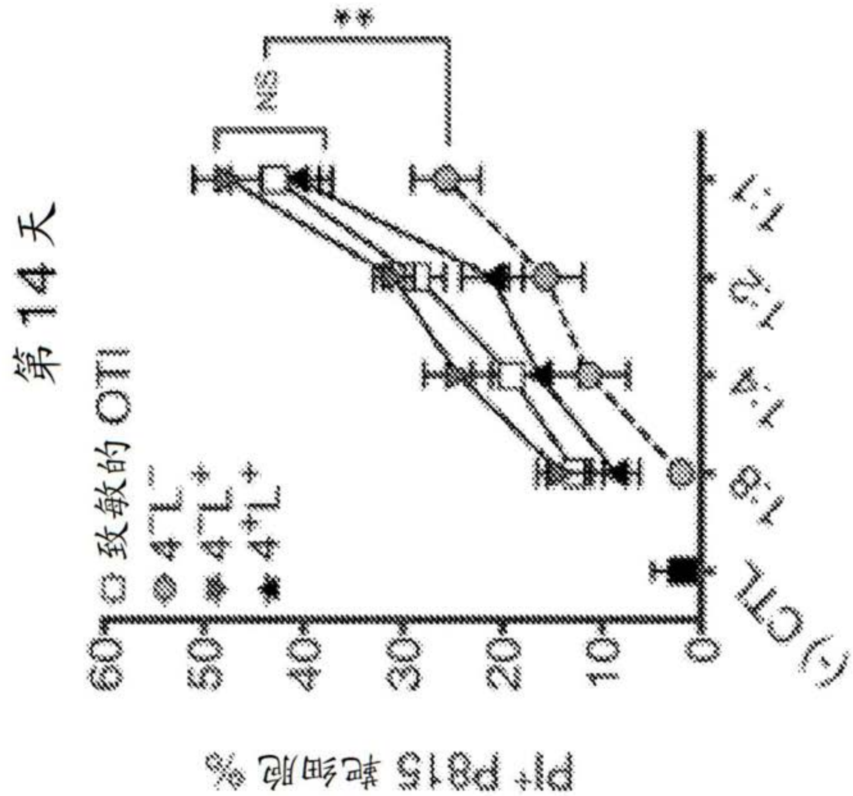

第14天
致敏的OTI
PI+ P815 靶细胞 %
(-) CTL
1:8
1:4
1:2
1:1
**
NS

图5D

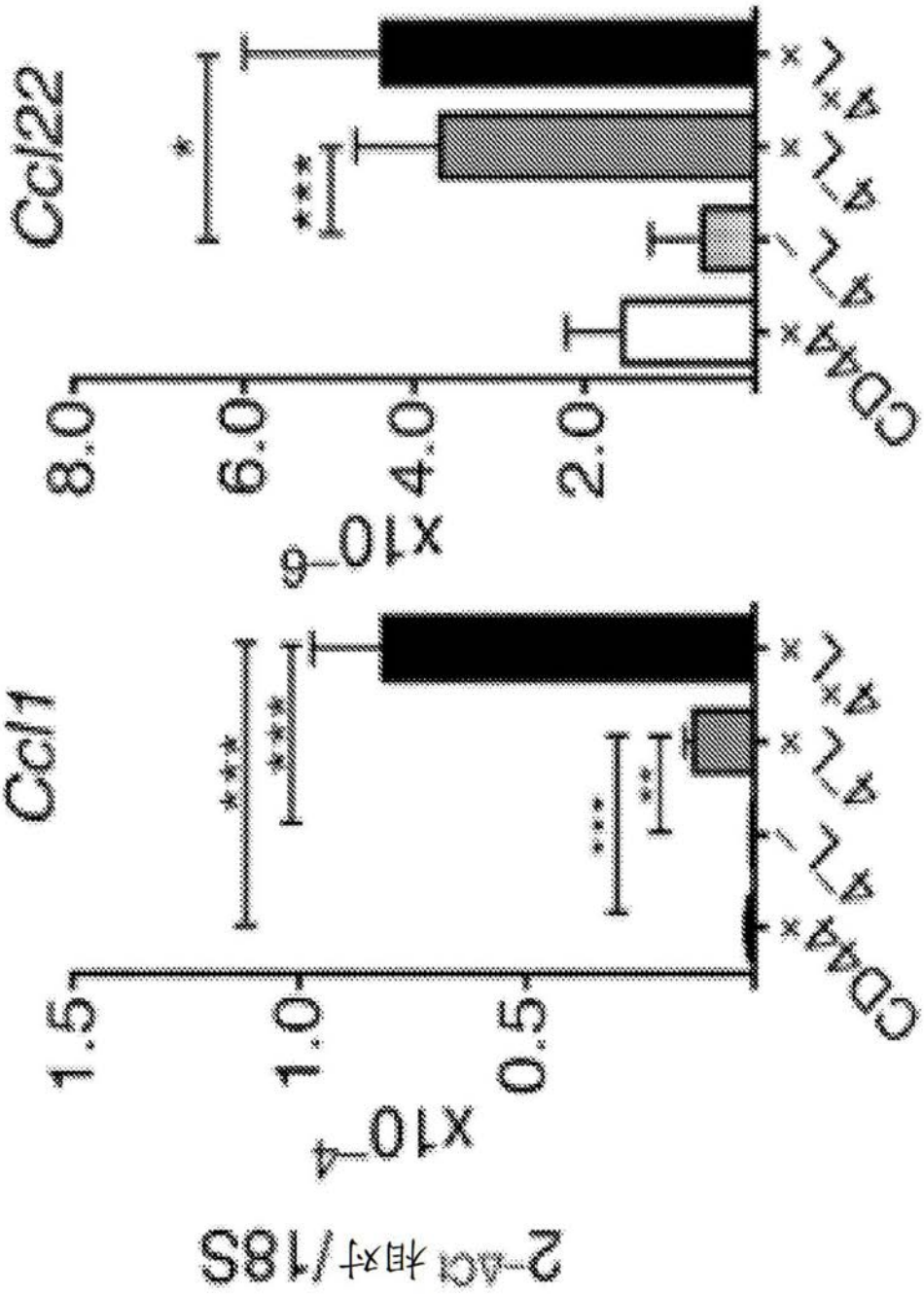

Ccl22
8.0
6.0
4.0
2.0
x10⁻⁶
Ccl1
1.5
1.0
0.5
x10⁻⁴
2⁻ΔΔCt 相对/18S
CD44+
CD44+
CD44+
CD44+

图5E

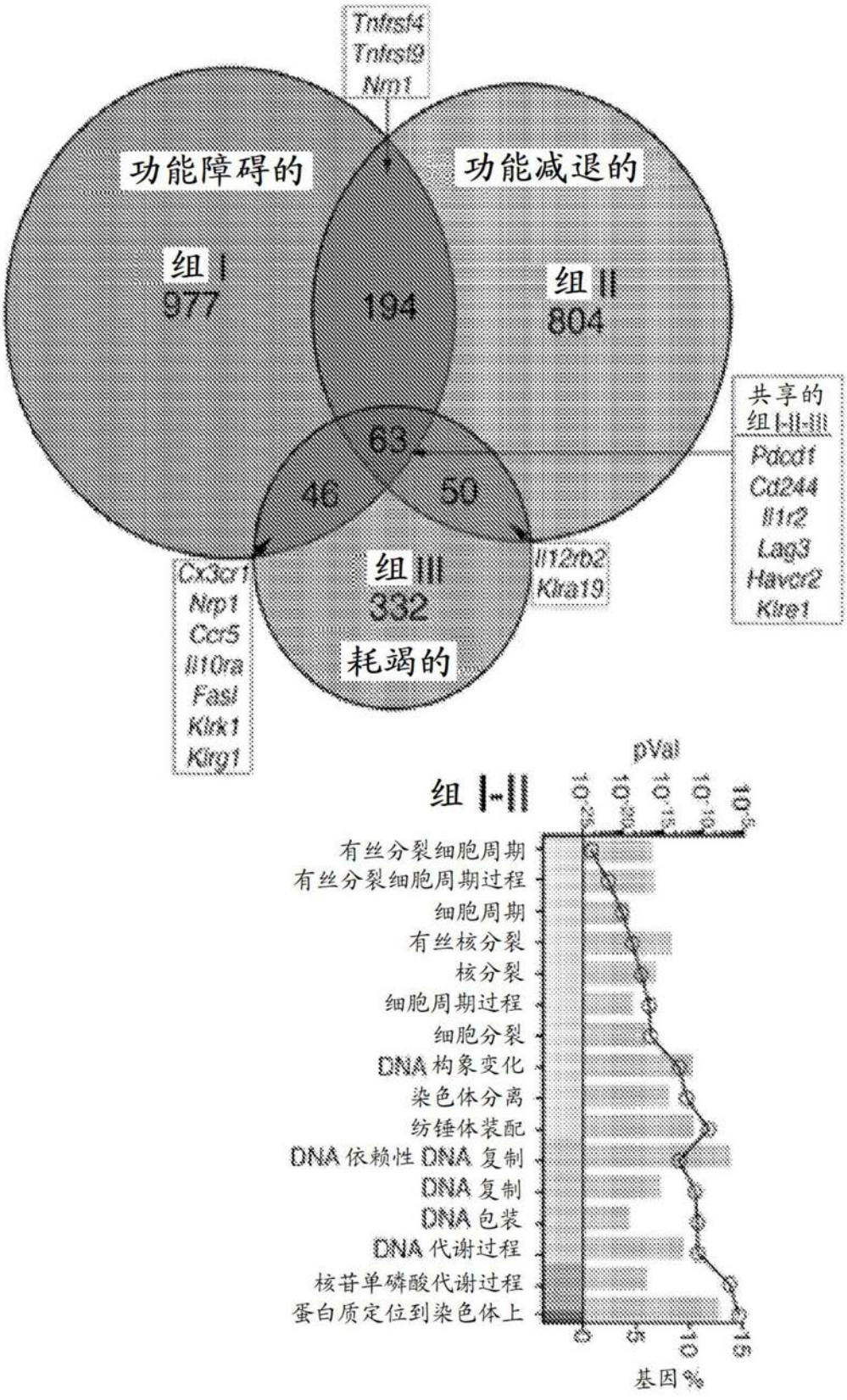

Tnfrsf4
Tnfrsf9
Nrn1
功能障碍的
功能减退的
组 I
977
194
组 II
804
63
46
50
共享的
组I-II-III
Pdcd1
Cd244
Il1r2
Lag3
Havcr2
Klre1
Cx3cr1
Nrp1
Ccr5
Il10ra
Fasl
Klrk1
Klrg1
组 III
332
耗竭的
Il12rb2
Klra19
pVal
组 I-II
有丝分裂细胞周期
有丝分裂细胞周期过程
细胞周期
有丝核分裂
核分裂
细胞周期过程
细胞分裂
DNA 构象变化
染色体分离
纺锤体装配
DNA 依赖性 DNA 复制
DNA 复制
DNA 包装
DNA 代谢过程
核苷单磷酸代谢过程
蛋白质定位到染色体上
基因%

图6A

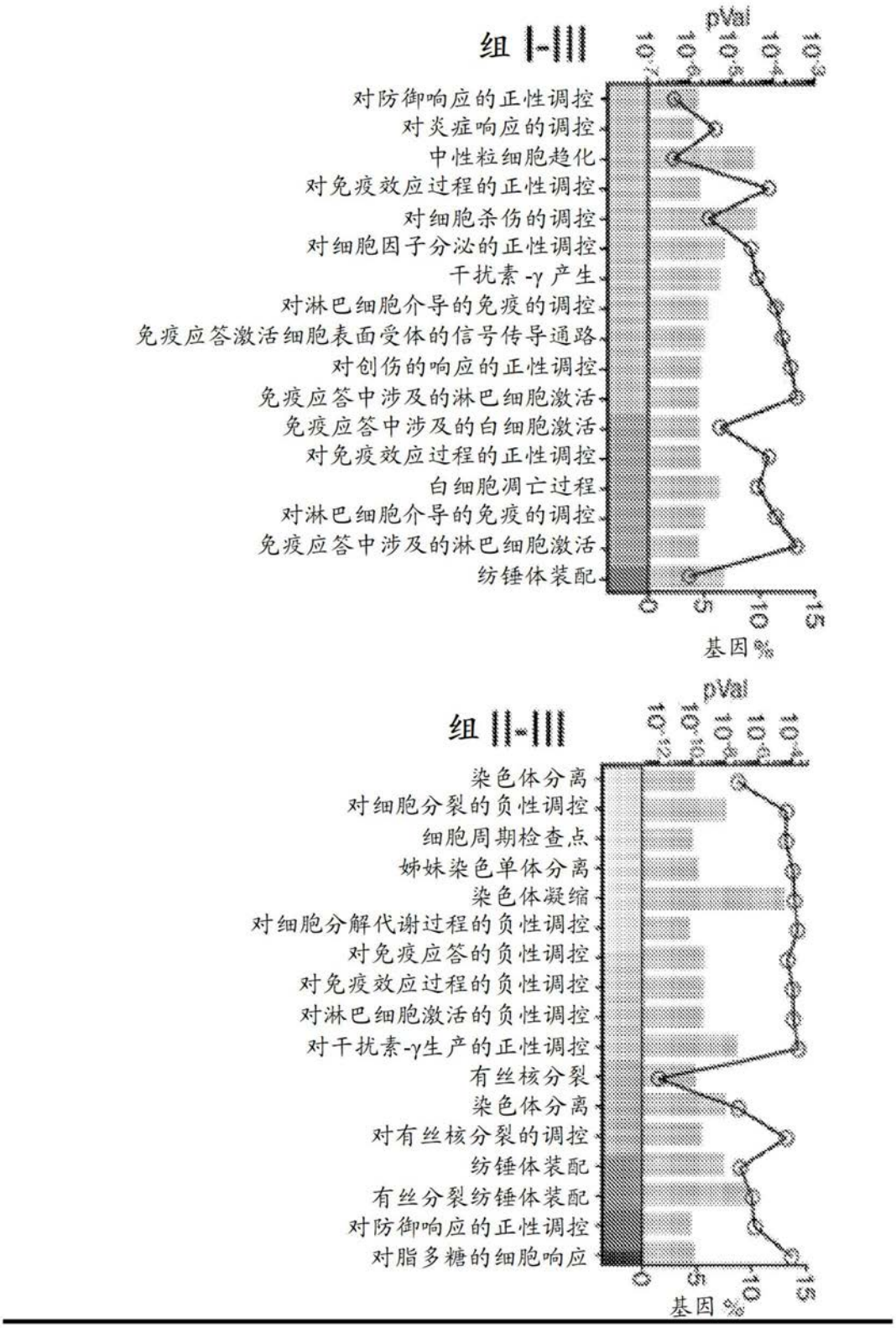

组 I-III
pVal
对防御响应的正性调控
对炎症响应的调控
中性粒细胞趋化
对免疫效应过程的正性调控
对细胞杀伤的调控
对细胞因子分泌的正性调控
干扰素-γ产生
对淋巴细胞介导的免疫的调控
免疫应答激活细胞表面受体的信号传导通路
对创伤的响应的正性调控
免疫应答中涉及的淋巴细胞激活
免疫应答中涉及的白细胞激活
对免疫效应过程的正性调控
白细胞凋亡过程
对淋巴细胞介导的免疫的调控
免疫应答中涉及的淋巴细胞激活
纺锤体装配
0 5 10 15
基因%
组 II-III
pVal
染色体分离
对细胞分裂的负性调控
细胞周期检查点
姊妹染色单体分离
染色体凝缩
对细胞分解代谢过程的负性调控
对免疫应答的负性调控
对免疫效应过程的负性调控
对淋巴细胞激活的负性调控
对干扰素-γ生产的正性调控
有丝核分裂
染色体分离
对有丝核分裂的调控
纺锤体装配
有丝分裂纺锤体装配
对防御响应的正性调控
对脂多糖的细胞响应
0 5 10 15
基因%

图6A(续)

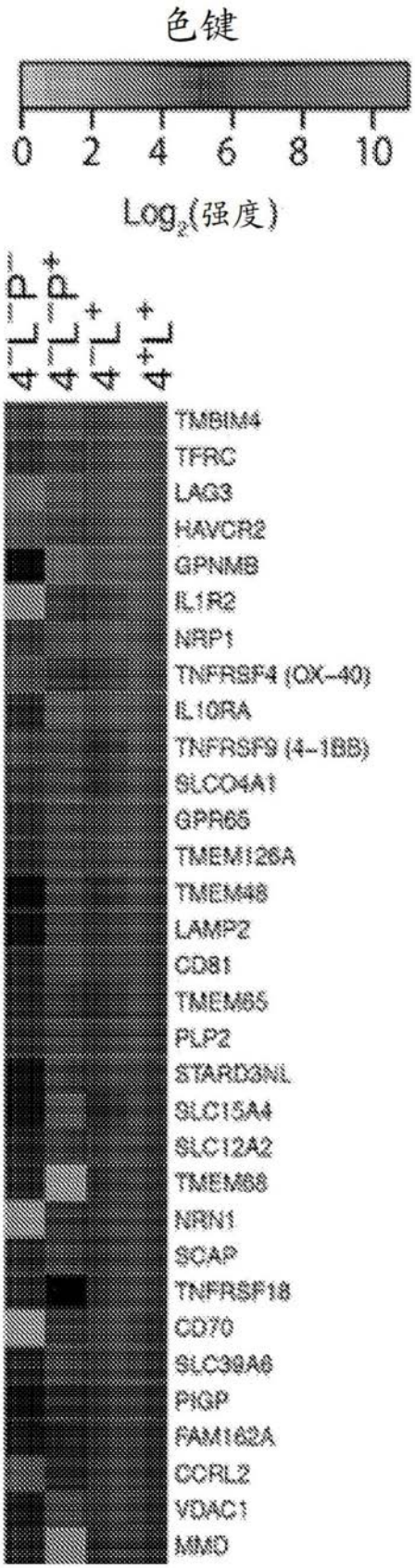

色键
0 2 4 6 8 10
Log2(强度)
4T1 p+
4T1 p-
4T1 +
4T1 +
TMBIM4
TFRC
LAG3
HAVCR2
GPNMB
IL1R2
NRP1
TNFRSF4 (OX-40)
IL10RA
TNFRSF9 (4-1BB)
SLCO4A1
GPR65
TMEM126A
TMEM48
LAMP2
CD81
TMEM65
PLP2
STARD3NL
SLC15A4
SLC12A2
TMEM68
NRN1
SCAP
TNFRSF18
CD70
SLC39A6
PIGP
FAM162A
CCRL2
VDAC1
MMD

图6B

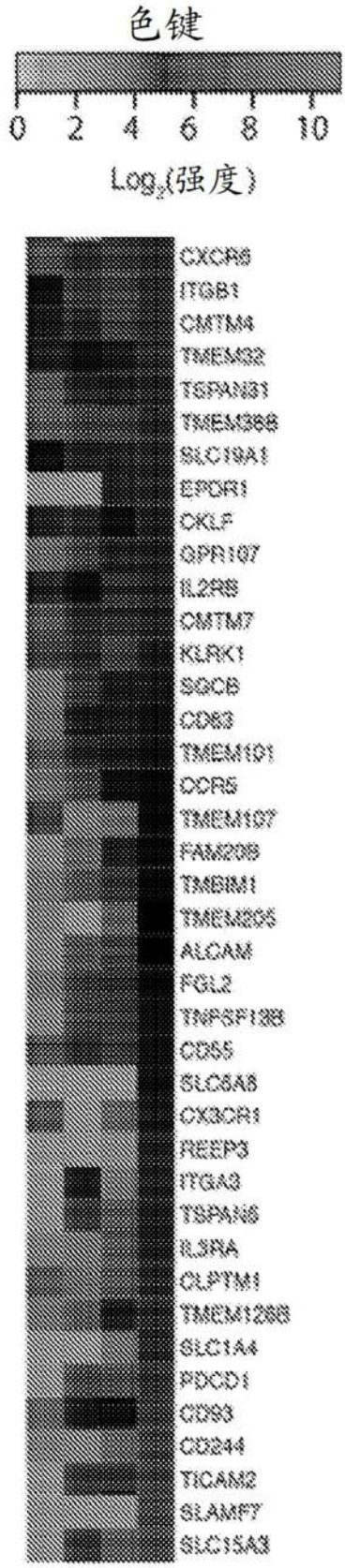

色键
0 2 4 6 8 10
Log2(强度)
CXCR6
ITGB1
CMTM4
TMEM32
TSPAN31
TMEM366
SLC19A1
EPOR1
CKLF
GPR107
IL2RB
CMTM7
KLRK1
SGCB
CD63
TMEM101
CCR5
TMEM107
FAM20B
TMBIM1
TMEM205
ALCAM
FGL2
TNFSF13B
CD55
SLC6A8
CX3CR1
REEP3
ITGA3
TSPAN8
IL3RA
CLPTM1
TMEM126B
SLC1A4
PDCD1
CD93
CD244
TICAM2
SLAMF7
SLC15A3

图6B(续)

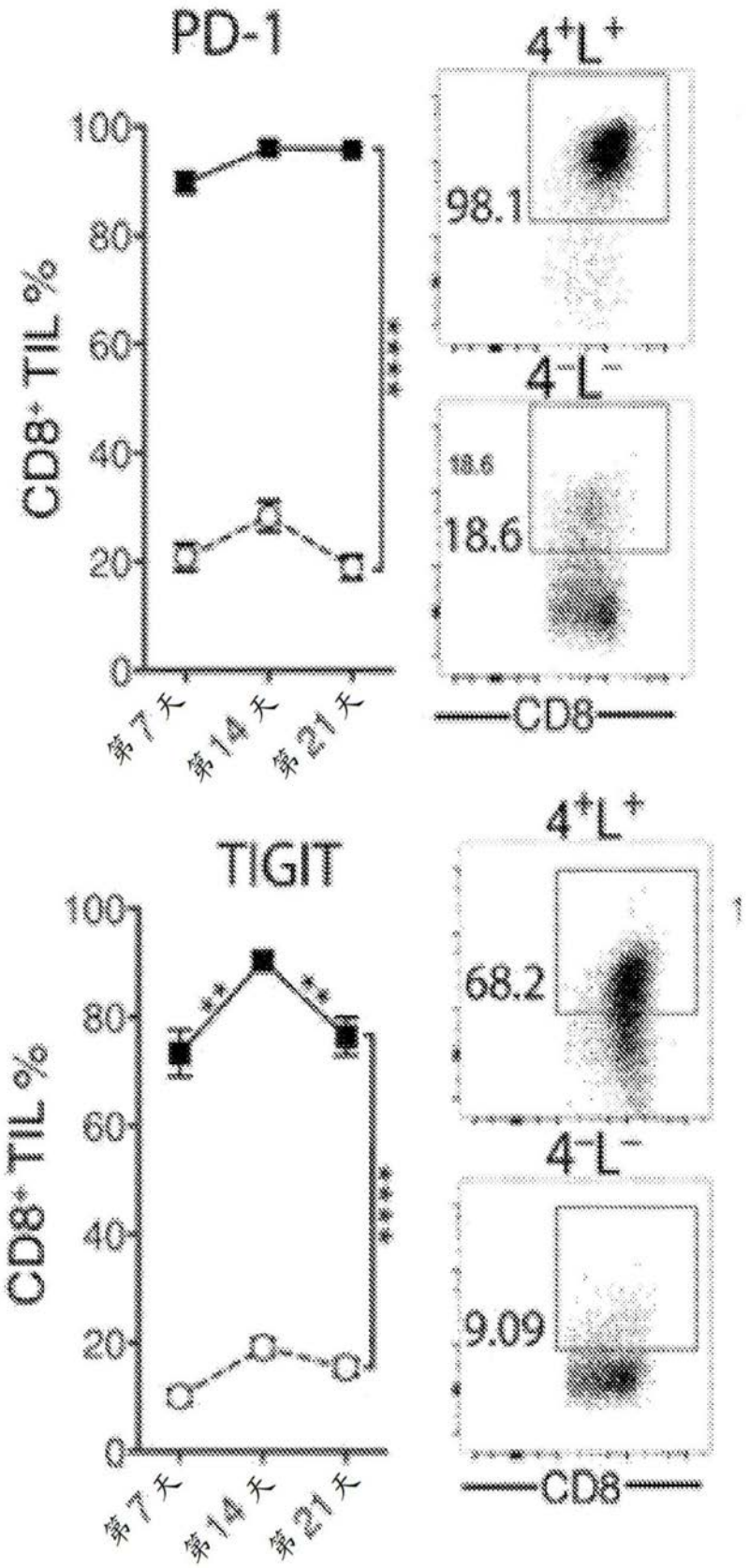

PD-1
CD8+ TIL %
100
80
60
40
20
0
第7天
第14天
第21天
****
4+L+
98.1
4-L-
18.6
CD8
TIGIT
CD8+ TIL %
100
80
60
40
20
0
第7天
第14天
第21天
**
**
****
4+L+
68.2
4-L-
9.09
CD8

图6C

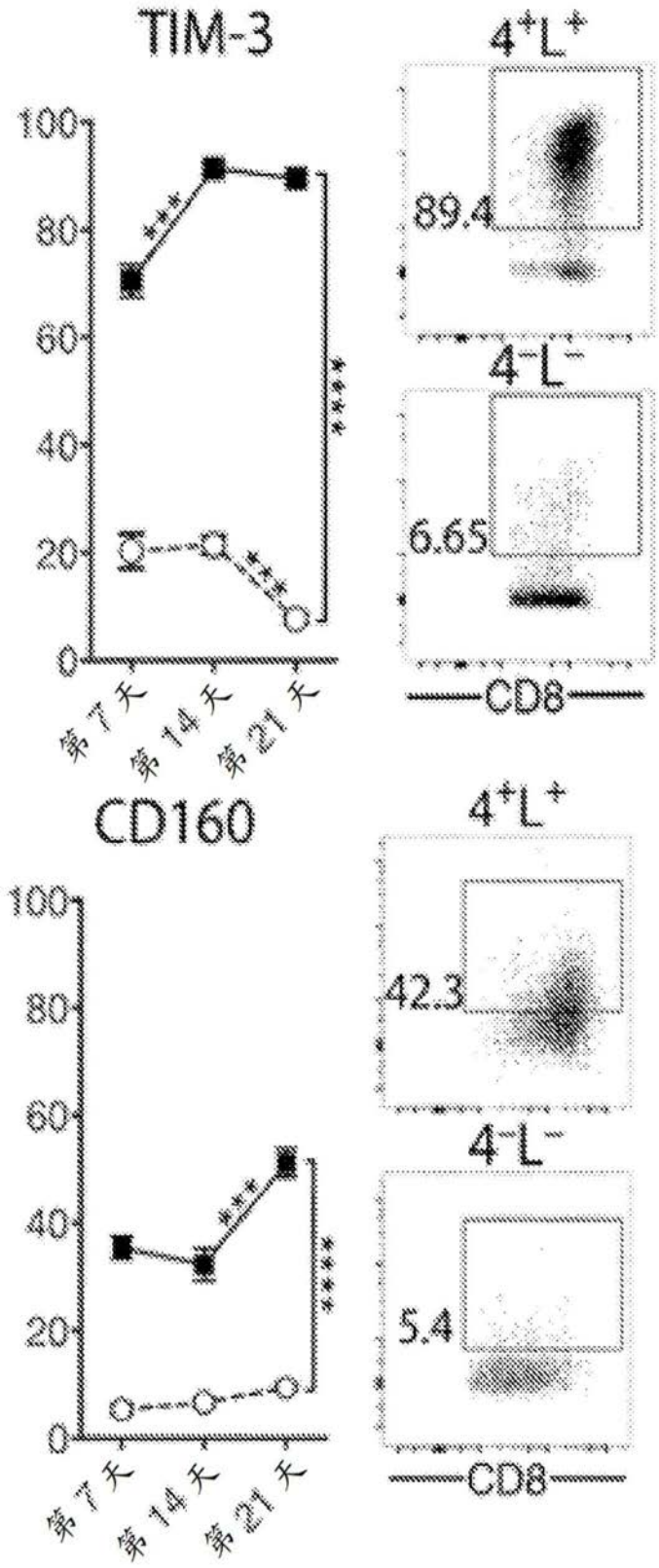

TIM-3
100
80
60
40
20
0
第7天
第14天
第21天
4+L+
89.4
4-L-
6.65
CD8
CD160
100
80
60
40
20
0
第7天
第14天
第21天
4+L+
42.3
4-L-
5.4
CD8

图6C(续)

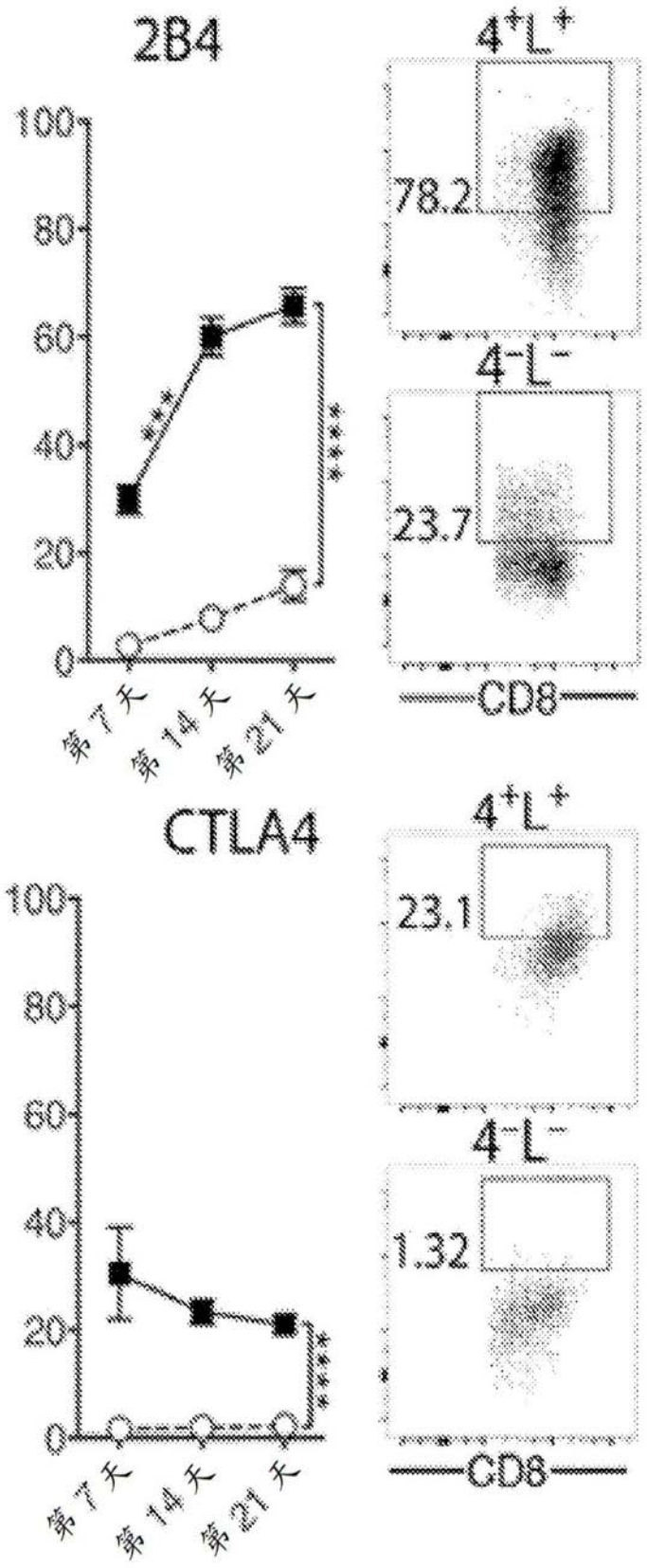

2B4
100
80
60
40
20
0
第7天
第14天
第21天
4+L+
78.2
4-L-
23.7
CD8
CTLA4
100
80
60
40
20
0
第7天
第14天
第21天
4+L+
23.1
4-L-
1.32
CD8

图6C(续)

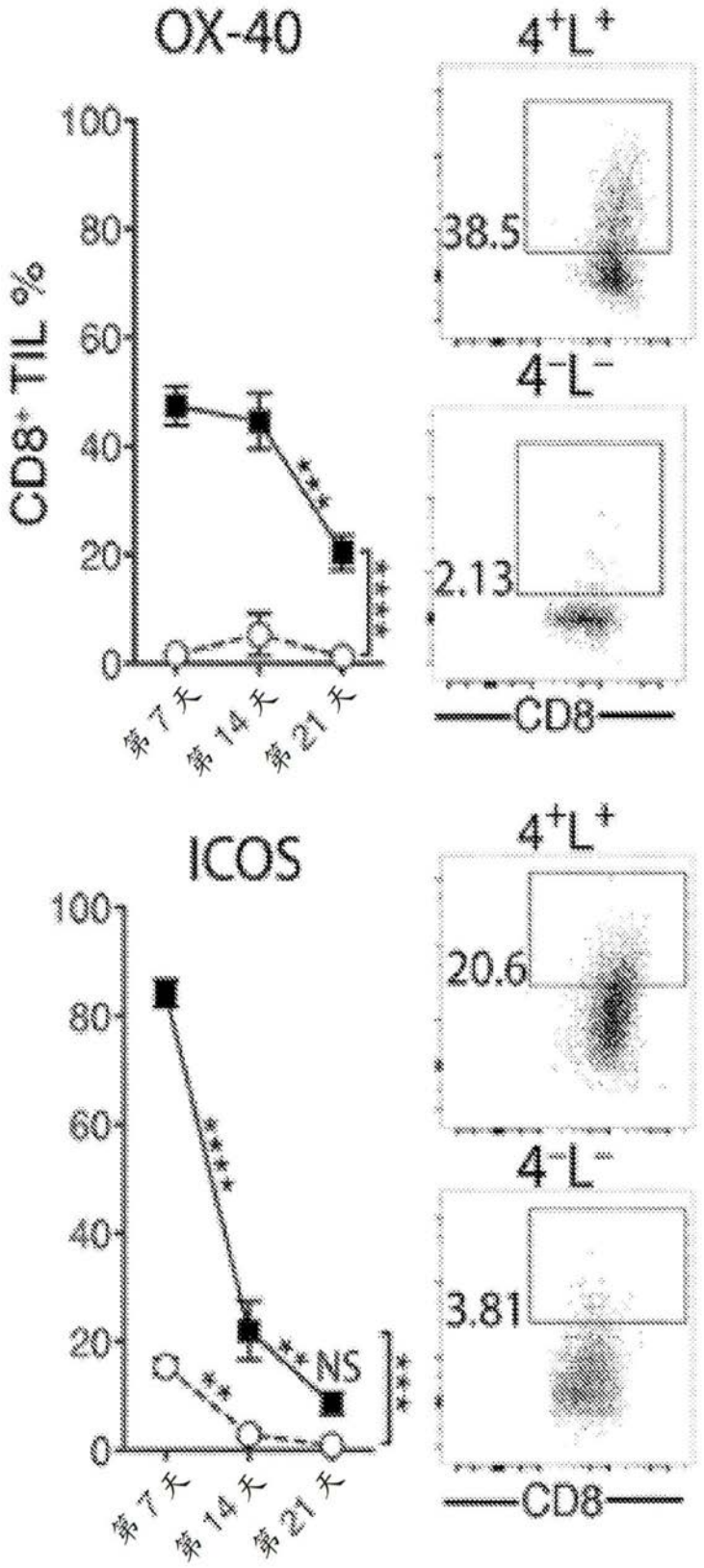

OX-40
CD8+ TIL %
100
80
60
40
20
0
第7天
第14天
第21天
4+L+
38.5
4-L-
2.13
CD8
ICOS
20.6
3.81
NS

图6C(续)

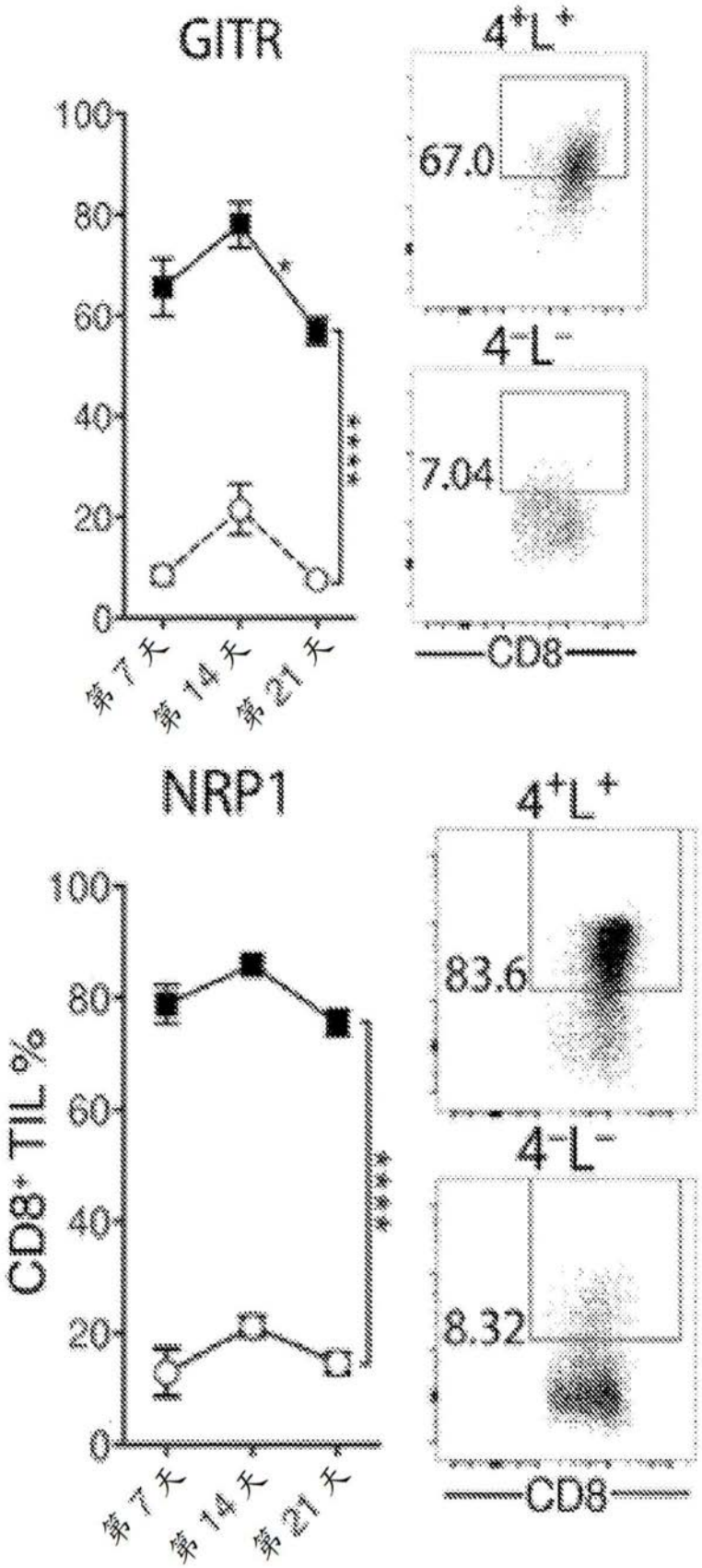

GITR
4+L+
67.0
4-L-
7.04
CD8
100
80
60
40
20
0
第7天
第14天
第21天
****
NRP1
4+L+
83.6
4-L-
8.32
CD8
CD8+ TIL %
100
80
60
40
20
0
第7天
第14天
第21天
****

图6C(续)

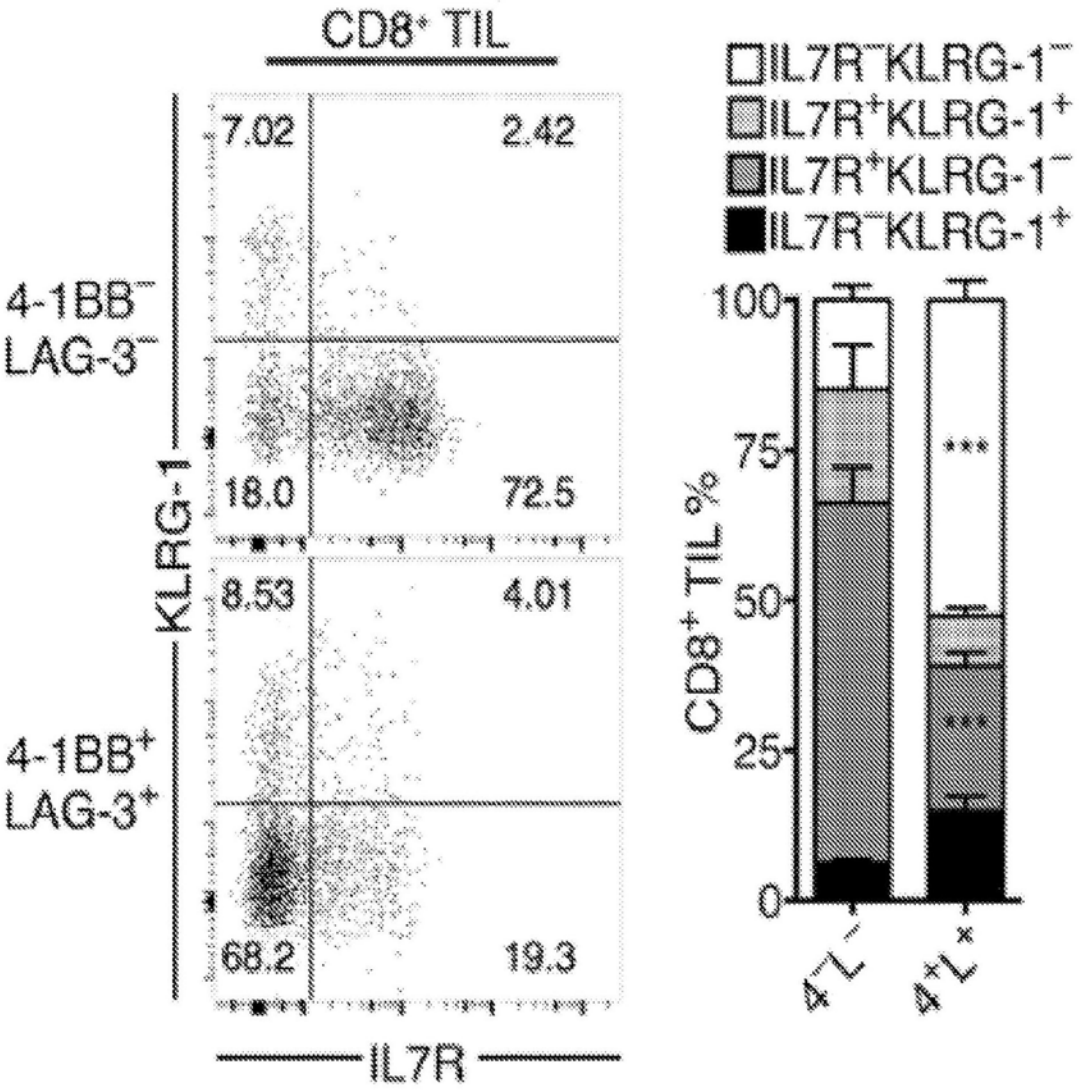

CD8+ TIL
7.02
2.42
18.0
72.5
8.53
4.01
68.2
19.3
4-1BB−
LAG-3−
4-1BB+
LAG-3+
KLRG-1
IL7R
IL7R−KLRG-1−
IL7R+KLRG-1+
IL7R+KLRG-1−
IL7R−KLRG-1+
CD8+ TIL %
100
75
50
25
0
***
***
Δ−L−
Δ+L+

图6D

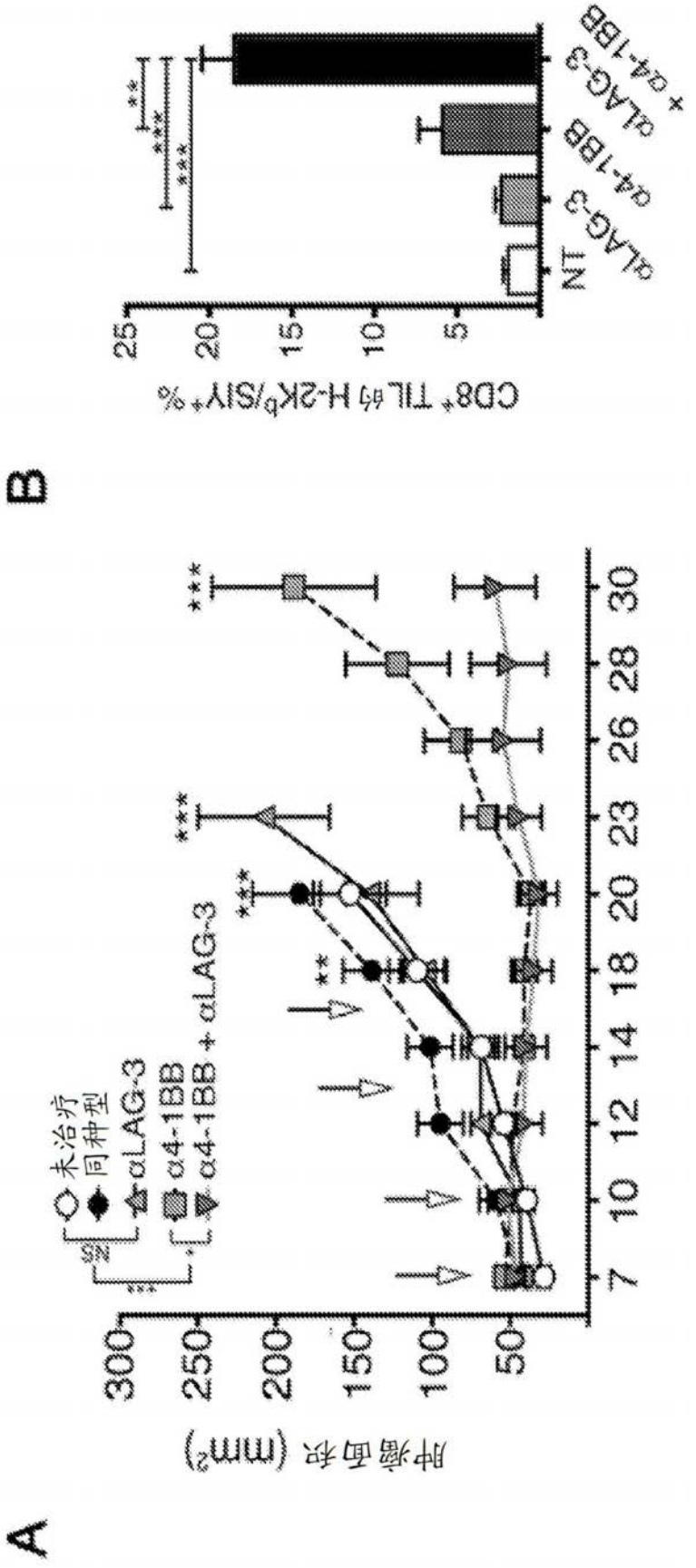

A
肿瘤面积 (mm²)
300
250
200
150
100
50
7
10
12
14
18
20
23
26
28
30
未治疗
同种型
αLAG-3
α4-1BB
α4-1BB + αLAG-3
B
CD8⁺ TIL 的 H-2Kᵇ/SIY⁺ %
25
20
15
10
5
NT
αLAG-3
α4-1BB
α4-1BB + αLAG-3

图7

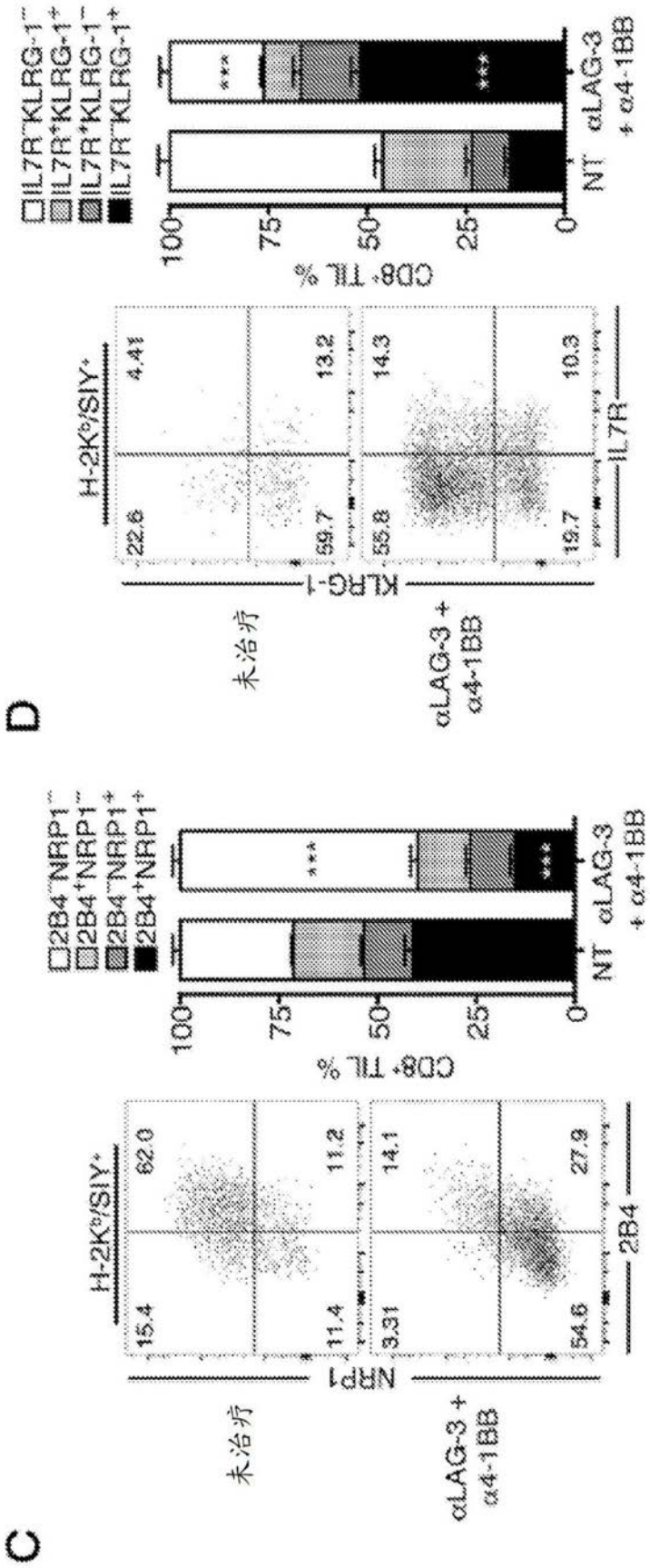

C
D
未治疗
αLAG-3 + α4-1BB
H-2Kb/SIY+
NRP1
2B4
KLRG-1
IL7R
CD8+ TIL %
NT
αLAG-3 + α4-1BB
2B4-NRP1-
2B4+NRP1-
2B4-NRP1+
2B4+NRP1+
IL7R-KLRG-1-
IL7R+KLRG-1-
IL7R-KLRG-1+
IL7R+KLRG-1+

图7(续)

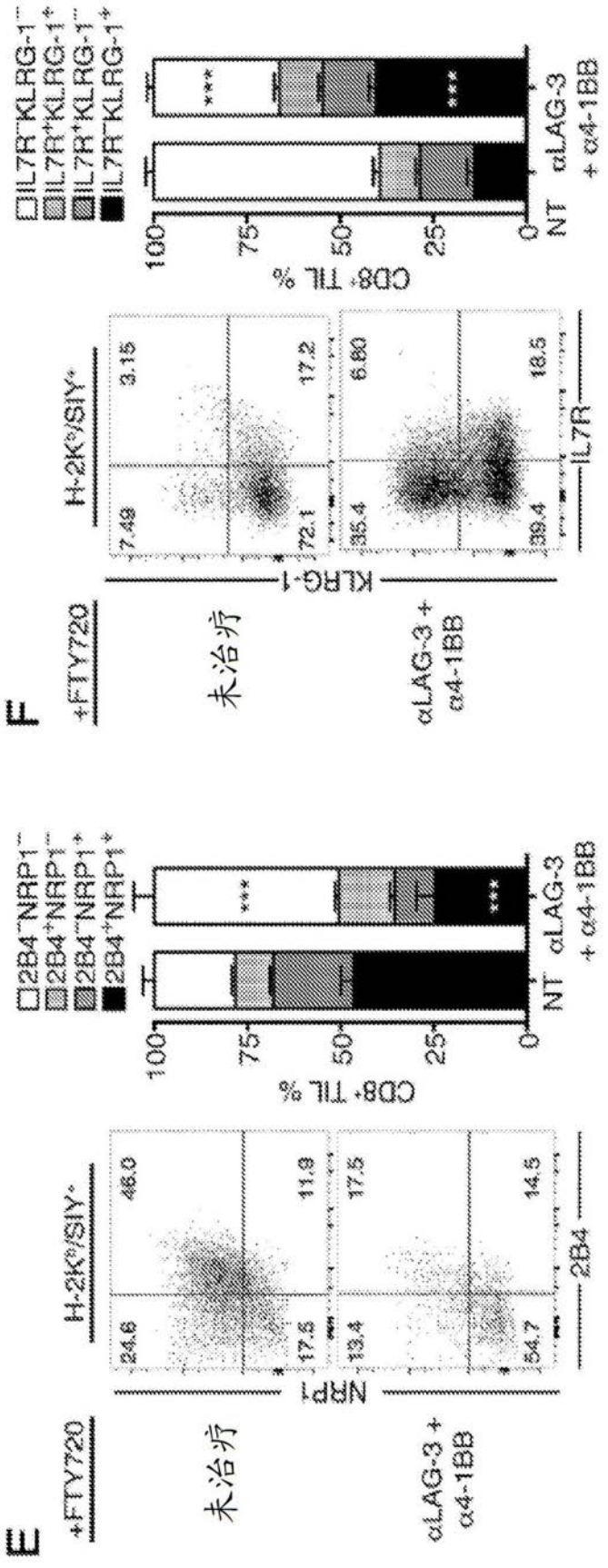
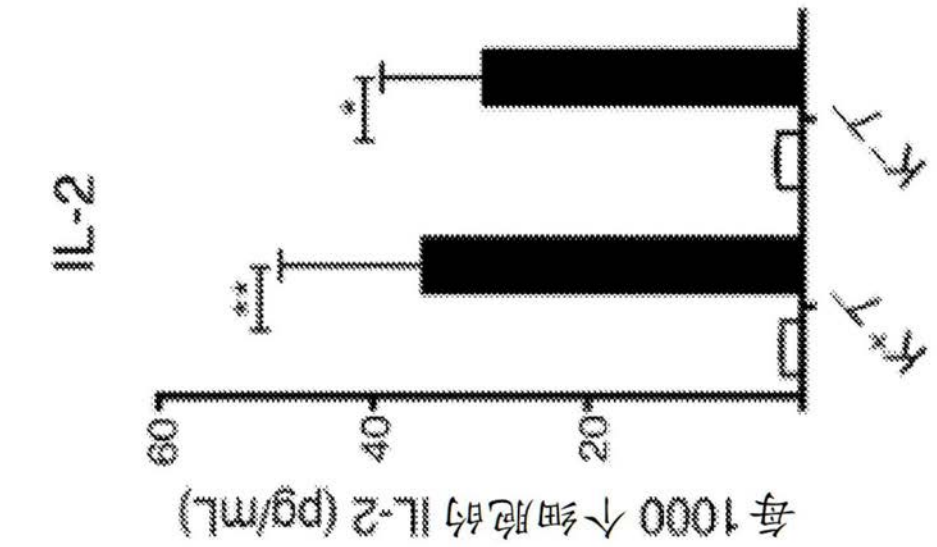

F
+FTY720
未治疗
αLAG-3 + α4-1BB
H-2Kb/SIY+
KLRG-1
IL7R
7.49
72.1
3.15
17.2
35.4
6.80
39.4
18.5
IL7R-KLRG-1-
IL7R+KLRG-1-
IL7R-KLRG-1+
IL7R+KLRG-1+
CD8+ TIL %
NT
αLAG-3 + α4-1BB
E
+FTY720
未治疗
αLAG-3 + α4-1BB
H-2Kb/SIY+
NRP1
2B4
24.6
17.5
46.0
11.9
13.4
54.7
17.5
14.5
2B4-NRP1-
2B4+NRP1-
2B4-NRP1+
2B4+NRP1+
CD8+ TIL %
NT
αLAG-3 + α4-1BB

图7(续)


IL-2
每1000个细胞的 IL-2 (pg/mL)
60
40
20
*
**
Tconv
Treg

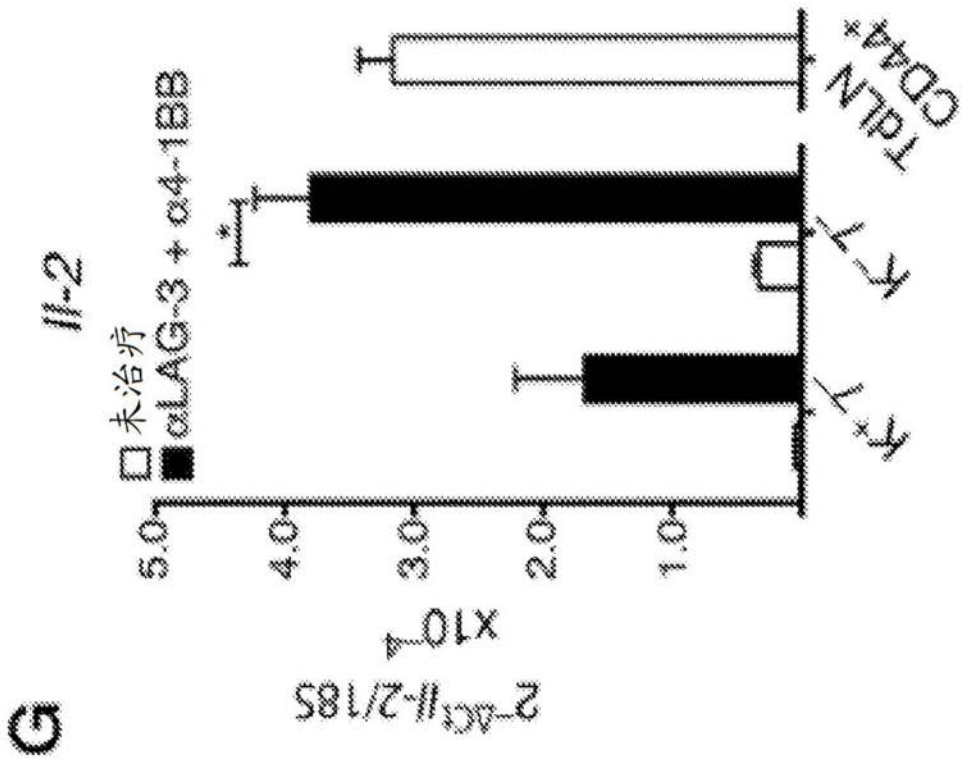

G
Il-2
2−ΔCtIl-2/18S
x10−4
6.0
5.0
4.0
3.0
2.0
1.0
□未治疗
■αLAG-3 + α4-1BB
*
Treg
Tconv
TdLN CD44+

图7(续)

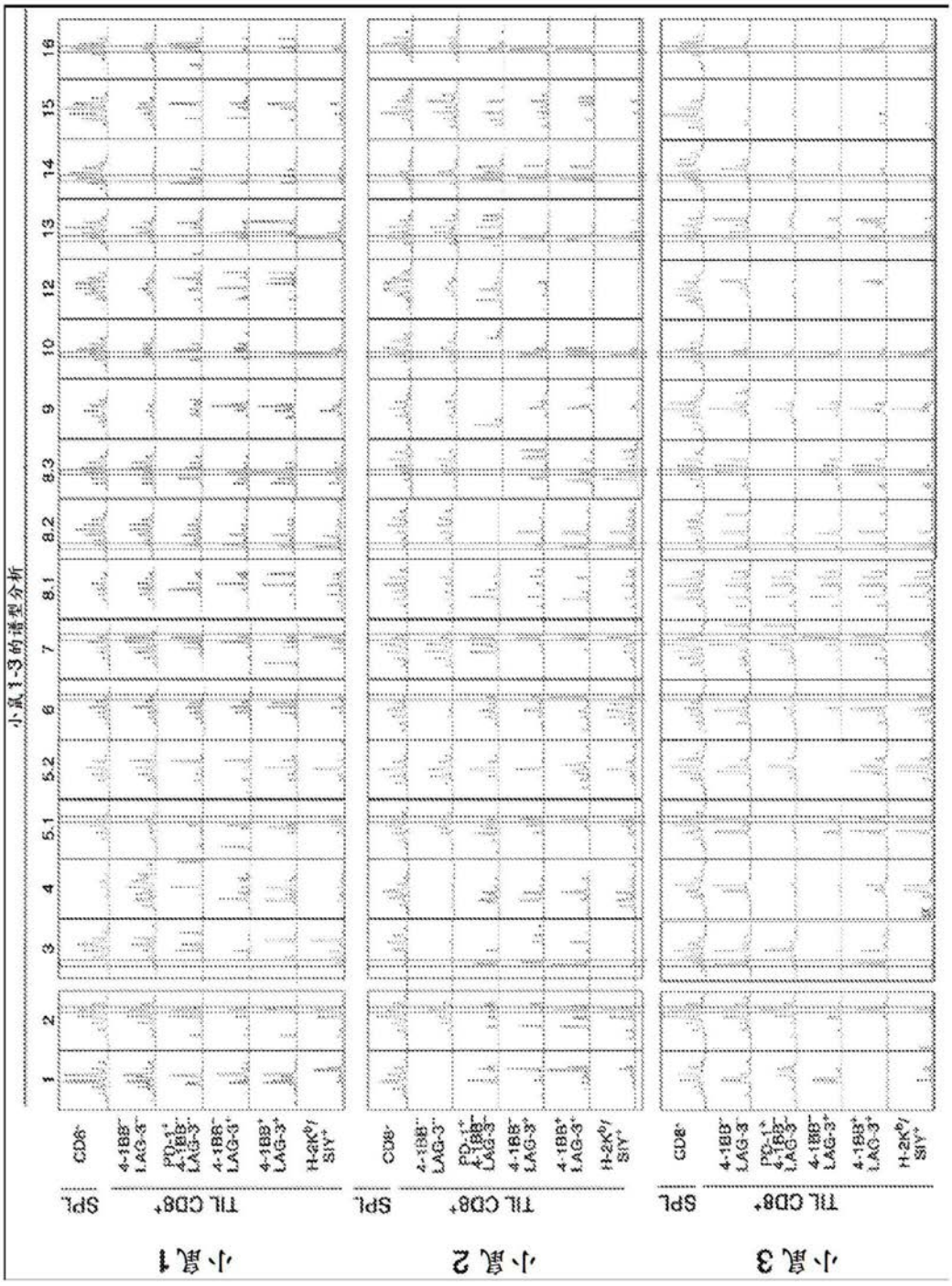

小鼠1-3的谱型分析
小鼠1
小鼠2
小鼠3
SPL
TIL CD8+
CD8+
4-1BB+ LAG-3+
PD-1+ 4-1BB+ LAG-3+
4-1BB- LAG-3+
4-1BB+ LAG-3+
H-2Kb/ SIY+
1
2
3
4
5.1
5.2
6
7
8.1
8.2
8.3
9
10
12
13
14
15
16

图8

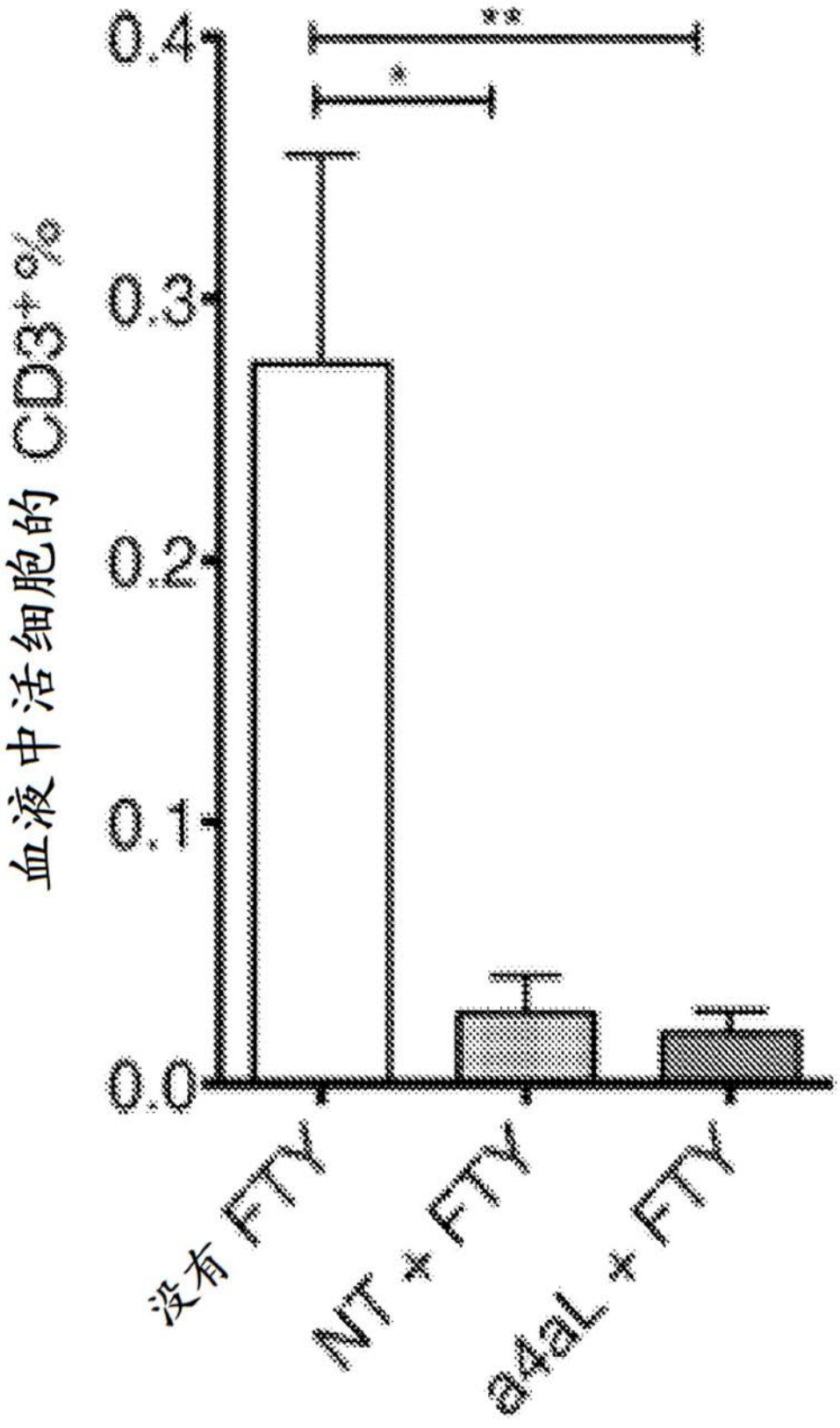


图9

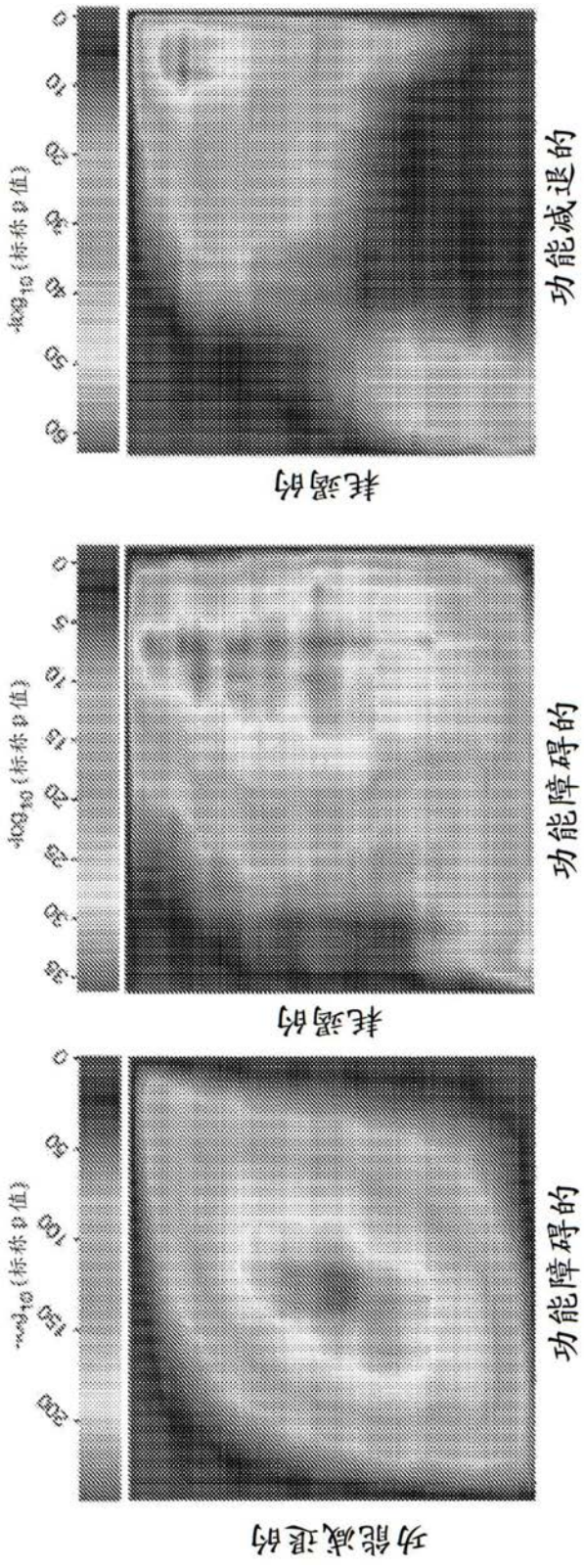


图10A

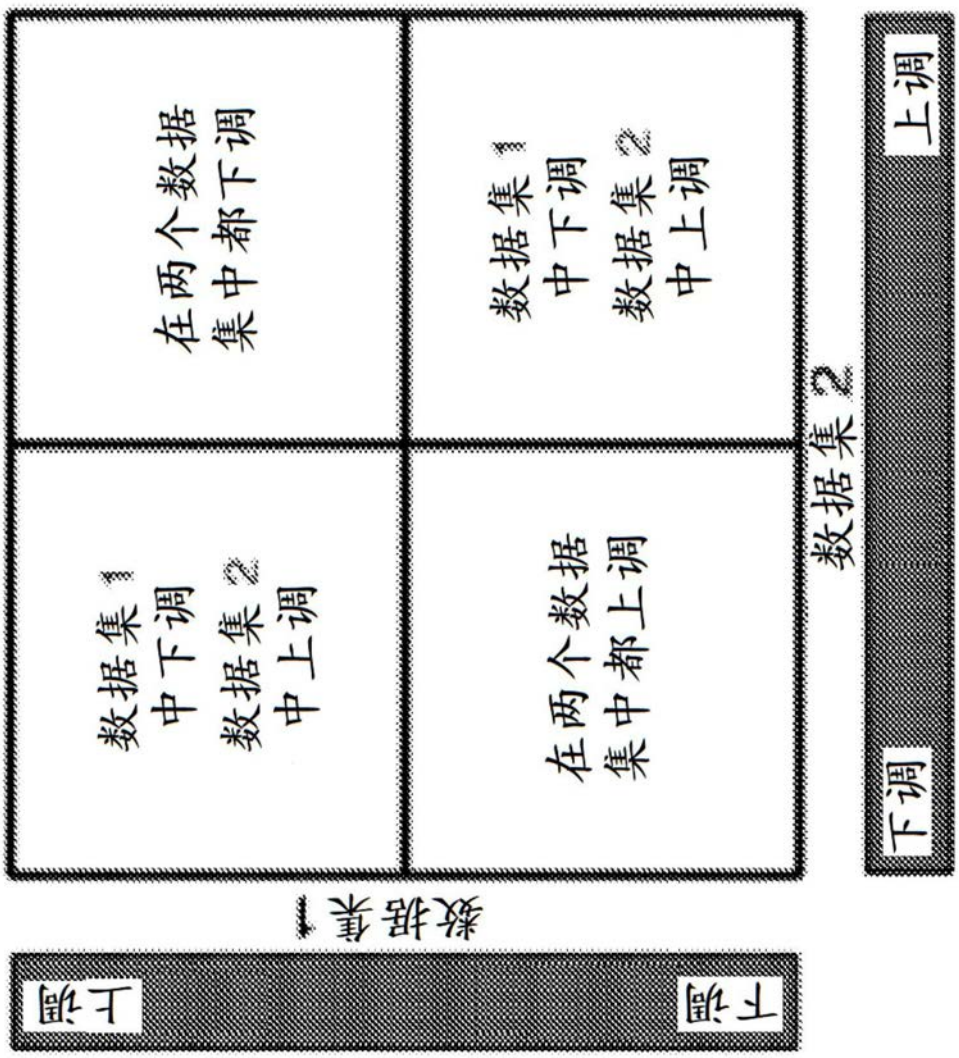

在两个数据集中都下调
数据集1中下调数据集2中上调
数据集1中下调数据集2中上调
在两个数据集中都上调
下调
上调
数据集2
下调
上调
数据集1

图10A(续)

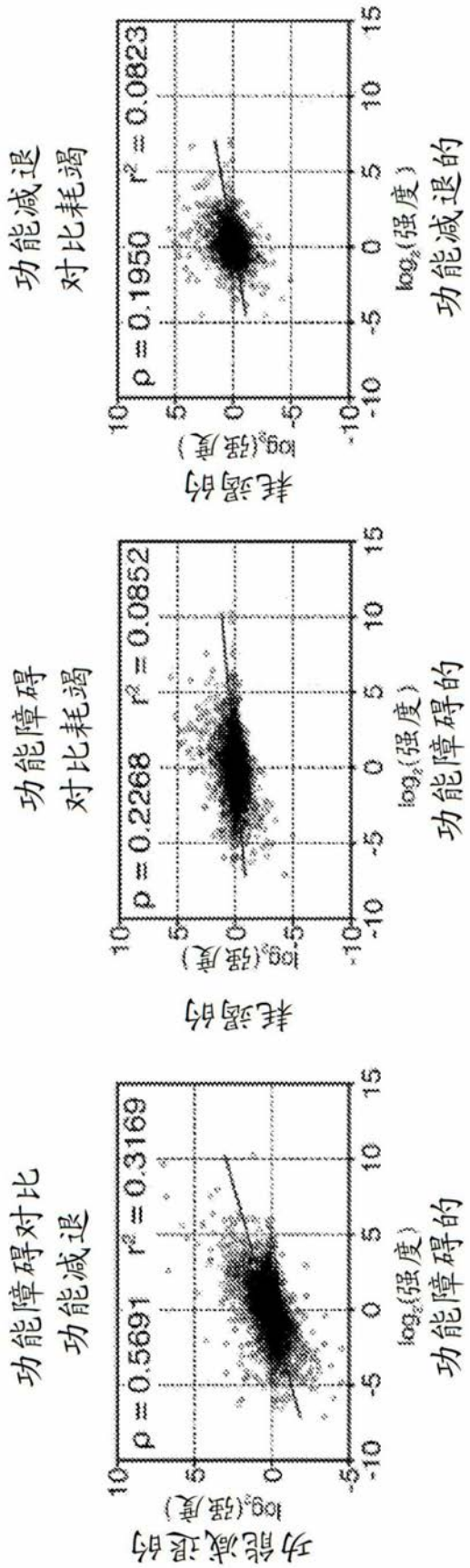

功能减退
对比耗竭
p = 0.1950 r² = 0.0823
耗竭的
log₂(强度)
log₂(强度)
功能减退的
功能障碍
对比耗竭
p = 0.2268 r² = 0.0852
耗竭的
log₂(强度)
log₂(强度)
功能障碍的
功能障碍对比
功能减退
p = 0.5691 r² = 0.3169
功能减退的
log₂(强度)
log₂(强度)
功能障碍的

图10B

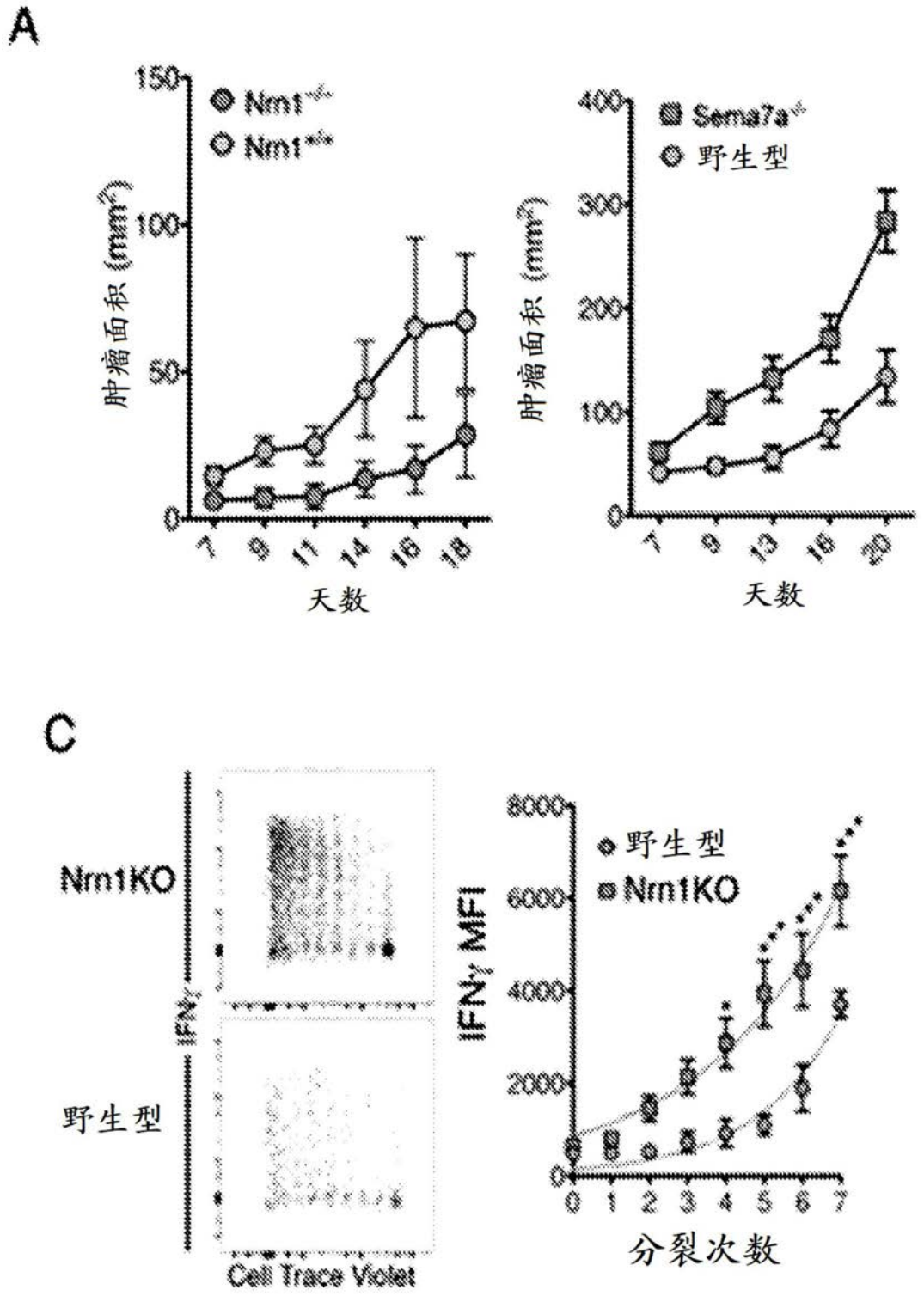

A
Nm1-/-
Nm1+/+
肿瘤面积 (mm²)
天数
Sema7a-/-
野生型
C
Nm1KO
野生型
IFNγ
Cell Trace Violet
IFNγ MFI
分裂次数

图11

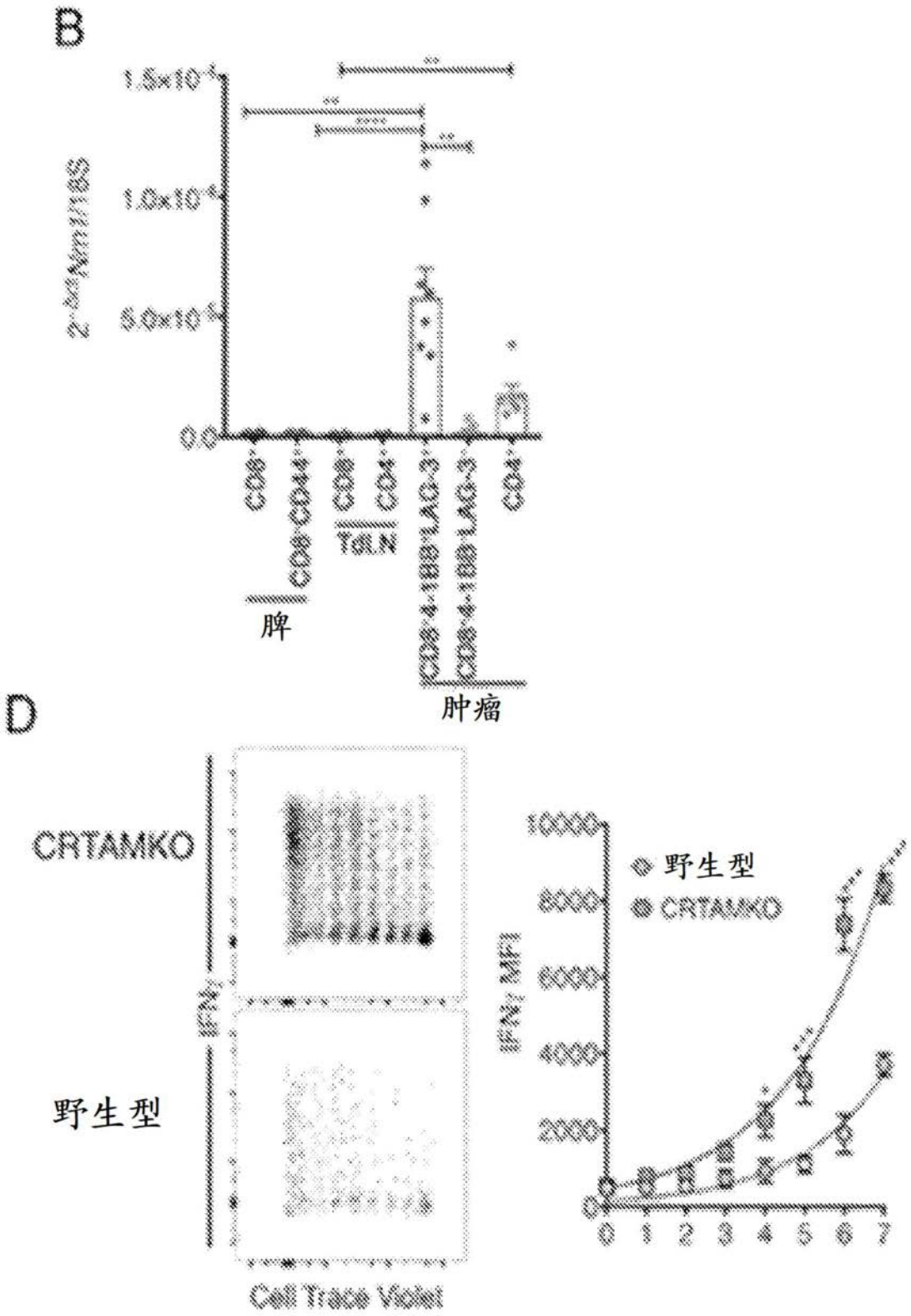


图11(续)

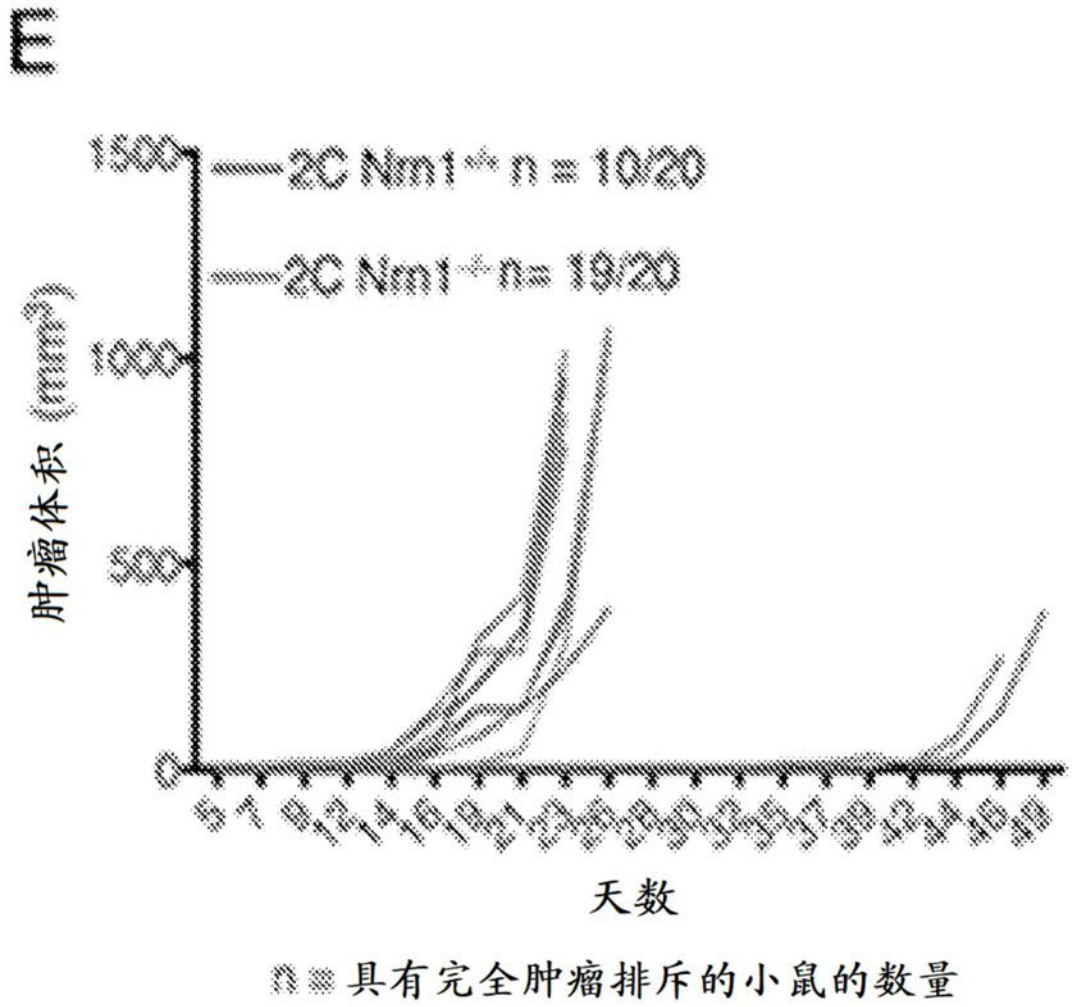

E
1500
1000
500
0
肿瘤体积 (mm³)
2C Nm1⁻/⁻ n = 10/20
2C Nm1⁺/⁻ n= 19/20
天数
n = 具有完全肿瘤排斥的小鼠的数量

图11(续)

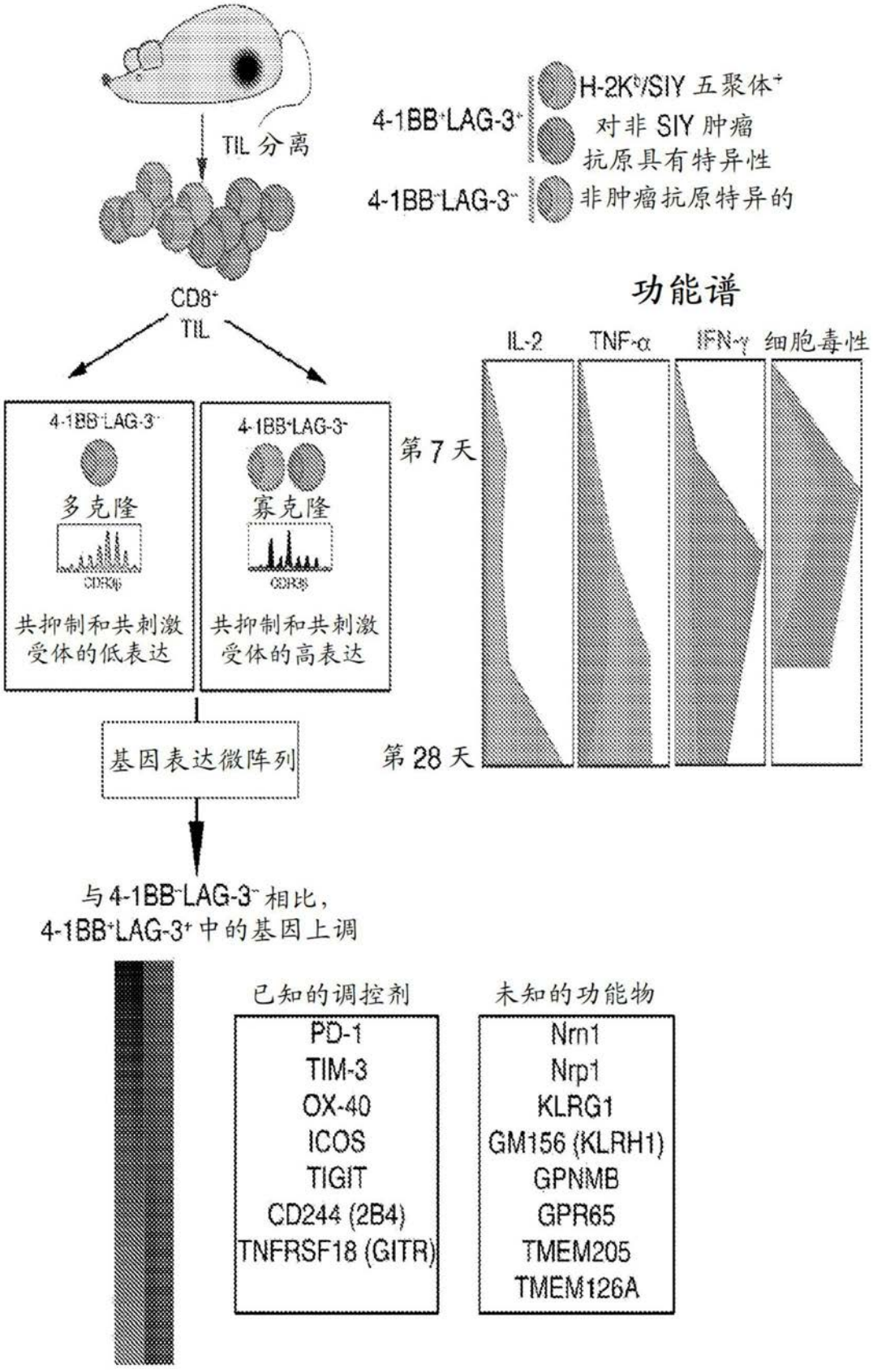

TIL 分离
4-1BB+LAG-3+
H-2Kb/SIY 五聚体+
对非 SIY 肿瘤
抗原具有特异性
4-1BB-LAG-3-
非肿瘤抗原特异的
CD8+
TIL
功能谱
IL-2
TNF-α
IFN-γ
细胞毒性
第 7 天
第 28 天
4-1BB+LAG-3-
多克隆
CDR3β
共抑制和共刺激
受体的低表达
4-1BB+LAG-3+
寡克隆
CDR3β
共抑制和共刺激
受体的高表达
基因表达微阵列
与 4-1BB-LAG-3- 相比，
4-1BB+LAG-3+ 中的基因上调
已知的调控剂
PD-1
TIM-3
OX-40
ICOS
TIGIT
CD244 (2B4)
TNFRSF18 (GITR)
未知的功能物
Nrn1
Nrp1
KLRG1
GM156 (KLRH1)
GPNMB
GPR65
TMEM205
TMEM126A

图12