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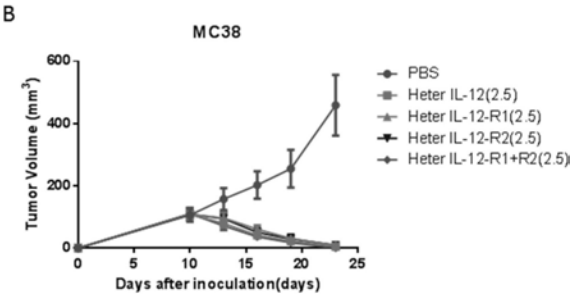
(54)发明名称

一种以白介素12为活性成分的融合蛋白型药物前体

(57)摘要

本发明涉及一种以白介素12为活性成分的融合蛋白型药物前体。所述融合蛋白型药物前体为同源或异源二聚体，二聚体中包括如下结构单元：(1)位于所述融合蛋白N端的第一结构单元：白介素12(IL-12)的一个或两个亚基，所述的白介素12的亚基为白介素12的P35亚基或P40亚基；(2)位于所述融合蛋白C端的第二结构单元：抗体Fc片段；(3)连接片段1：连接第一结构单元和第二结构单元或连接第一结构单元内部的两个亚基；以及可选的(4)通过能够被肿瘤微环境中特异性表达的蛋白水解酶识别并水解连接片段2连接的白介素12的受体结构单元。



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1.一种融合蛋白,其特征在于,所述融合蛋白包括:

(1)位于所述融合蛋白N端的第一结构单元:白介素12(IL-12)的一个或两个亚基,所述的白介素12的亚基为白介素12的P35亚基或P40亚基;

(2)位于所述融合蛋白C端的第二结构单元:抗体Fc片段;

(3)连接片段1:连接第一结构单元和第二结构单元或连接第一结构单元内部的两个亚基。

2.根据权利要求1所述的融合蛋白,其特征在于

所述的抗体Fc片段为人源IgG1,优选为人源Fc-knob或人源Fc-hole;

所述的P35亚基的氨基酸序列如Seq ID No.2所示,所述的P40亚基的氨基酸序列如Seq ID No.1所示;

所述的连接片段1的氨基酸序列如Seq ID No.9所示。

3.根据权利要求1或2所述的融合蛋白,其特征在于,所述的融合蛋白还包括修饰在位于融合蛋白的N端的白介素12亚基的N端的信号肽,所述的信号肽为:

(1)修饰在P35亚基N端的信号肽1,其氨基酸序列如Seq ID No.11所示;

或(2)修饰在P40亚基N端的信号肽2,其氨基酸序列如Seq ID No.12所示。

4.根据权利要求1或2所述的融合蛋白,其特征在于,所述的融合蛋白还包括:

(4)连接在第一结构单元N端的白介素12受体;

(5)连接白介素12受体和第一结构单元的连接片段2。

5.根据权利要求4所述的融合蛋白,其特征在于,

所述的白介素12受体为:IL-12Rβ1或IL-12Rβ2;

所述连接片段2能够被肿瘤微环境中特异性表达的蛋白水解酶识别并水解。

6.根据权利要求5所述的融合蛋白,其特征在于:

所述的IL-12Rβ1的氨基酸序列如Seq ID No.6所示,所述的IL-12Rβ2的氨基酸序列如Seq ID No.7所示;

所述的肿瘤微环境中特异性表达的蛋白水解酶为基质金属蛋白酶,优选为基质金属蛋白酶14(MMP14)。

7.根据权利要求6所述的融合蛋白,其特征在于,

所述的连接片段2的氨基酸序列如Seq ID No.10所示,其被基质金属蛋白酶14识别的氨基酸的结构的序列如Seq ID No.8所示。

8.根据权利要求1～7任一所述的融合蛋白,其特征在于,所述的融合蛋白中各个结构单元的排列方式为:

(1)白介素12受体的C端与第一结构单元的N端通过连接片段2相连;

(2)第一结构单元的C端与第二结构单元的N端通过连接片段1相连;

(3)如第一结构单元内部有两个亚基,则第一亚基的C端和第二亚基的N端通过连接片段1相连。

9.一种药物前体,所述的药物前体为同源或异源二聚体,其特征在于,构成所述的二聚体的单体任选自权利要求1～8任一所述的融合蛋白。

10.根据权利要求9所述的药物前体,其特征在于,药物前体为如下药物前体1～7:

(1)药物前体1(Homo IL-12)为同源二聚体,

组成的单体为:含有信号肽2的P40亚基、连接片段1、P35亚基、连接片段1、人源IgG1连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.13所示;

(2)药物前体2(Heter IL-12)为异源二聚体,

单体1为:含有信号肽2的P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.14所示;

单体2为:含有信号肽1的P35亚基、连接片段1、人源Fc-hole连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.15所示;

(3)药物前体3(Homo-R1)为同源二聚体,

组成的单体为:IL-12的受体Rβ1片段、连接片段2、P40亚基、连接片段1、P35亚基、连接片段1、人源IgG1依次连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.16所示;

(4)药物前体4(Homo-R2)为同源二聚体,

组成的单体为:IL-12的受体Rβ2片段、连接片段2、P40亚基、连接片段1、P35亚基、连接片段1、人源IgG1连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.17所示;

(5)药物前体5(Heter-R1/R2)为异源二聚体,

单体1为:IL-12的受体Rβ1片段、连接片段2、P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.18所示;

单体2为:IL-12的受体Rβ2片段、连接片段2、P35亚基、连接片段1、人源Fc-hole依次连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.19所示;

(6)药物前体6(Heter-R1)为异源二聚体,

单体1为:IL-12的受体Rβ1片段、连接片段2、P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.20所示;

单体2为:含有信号肽1的P35亚基、连接片段1、人源Fc-hole依次连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.21所示;

(7)药物前体7(Heter-R2)为异源二聚体,

单体1为:含有信号肽2的P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.22所示;

单体2为:IL-12的受体Rβ2片段、连接片段2、P35亚基、连接片段1、人源Fc-hole连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.23所示。

11.编码权利要求1～9任一所述的融合蛋白或药物前体的核苷酸片段。

12.权利要求1～9任一所述的融合蛋白或药物前体在制备药物中的应用;优选的,所述的药物为抗肿瘤药物,最优选的,所述药物为抗结直肠癌的药物。

13.权利要求1～9任一所述的融合蛋白、药物前体的制备方法,所述的制备方法包括如下步骤:

(1)构建包含所述编码所述融合蛋白的编码基因的表达载体,优选的,所述的表达载体是pEE12.4表达载体;

(2)通过瞬时转染宿主细胞的方法构建包含所述表达载体的宿主细胞,优选的,所述的宿主细胞是293F细胞;

(3)培养所述宿主细胞并收集细胞上清;

(4)通过ProteinA/G的亲和层析柱纯化蛋白纯化所述融合蛋白或药物前体。

一种以白介素12为活性成分的融合蛋白型药物前体

技术领域

[0001] 本发明属于医药生物领域，具体而言，涉及一种以白介素12为活性成分的融合蛋白型药物前体。

背景技术

[0002] 白细胞介素12(interleukin-12,IL-12)又名细胞毒淋巴细胞成熟因子(cytotoxic lymphocyte maturation factor,CLMF)，也称为自然杀伤细胞刺激因子(natural killer cell stimulation factor,NKSF)，是1989年发现的白细胞介素家族的成员。IL-12是一种异源二聚体细胞因子，由p35和p40两个亚基通过二硫键组成，主要由树突状细胞(DC)、巨噬细胞、单核细胞、B淋巴细胞以及其他抗原递呈细胞(APC)产生，能促进T辅助细胞1(Th1)的增殖；诱导NK细胞和T细胞产生γ干扰素；提高NK细胞的细胞毒作用；促进细胞毒性T细胞的形成等。

[0003] IL-12最初在1989年被鉴定为对外周血淋巴细胞具有多重生物学作用的天然杀伤(NK)细胞刺激因子【1】。IL-12主要由抗原呈递细胞(APC)如树突状细胞(DC)，单核细胞，巨噬细胞和B细胞通过在Toll样受体相互作用下产生【2】因此，IL-12作为早期促炎细胞因子分泌以响应感染【3】。IL-12是分子量为70kDa的异二聚体，其由重(p40)和轻(p35)链亚基组成，其通过二硫键共价连接。p40可以通过吞噬细胞丰富地产生，但p35仅在低水平上普遍和组成型表达，需要与p40共表达才能分泌生物活性IL-12。在IL-12产生生物学效应的过程中p40仅发挥了媒介的作用，p35通过p40结合到细胞膜受体，两者通过由IL-12Rb1和IL-12Rb2组成的异二聚体IL-12受体(IL-12R)介导IL-12的信号传导【4】其中p35与IL-12Rb2的相互作用对向下传导信号至关重要，两个受体亚基的共表达是产生IL-12的高亲和力结合位点所必需的。p40亚基单独和受体结合可以竞争抑制IL-12的活性。在NK细胞，NK T细胞和活化的T细胞上发现IL-12R复合物【5】，同时也在髓系细胞【6】和扁桃体B细胞【7】上检测到。天然T细胞表达IL-12Rb1而不表达IL-12Rb2。在通过T细胞受体激活T细胞时，IL-12的两个受体都会被诱导，另外也会被IL-12本身，IFNγ，肿瘤坏死因子-α(TNF-α)和抗CD28共刺激物另外增强【8】【9】。受体的成功触发激活了Janus激酶STAT(信号转导和转录激活子)信号通路，主要导致STAT4磷酸化，其介导随后的细胞反应【10】【11】。

[0004] IL12在固有免疫和适应性免疫的作用，通过连接先天和适应性免疫应答，IL-12在调节炎症中起关键作用。炎症环境下APC释放IL-12，导致NK和T细胞的随后的激活和增殖，有这些亚类在刺激时增加其IFN-g分泌，从而诱导IL-12治疗后观察到的大部分肿瘤抑制途径。对于抗肿瘤作用，一方面IL-12和IFN-g通过诱导细胞因子和细胞溶解因子如穿孔素和颗粒酶B【12】的转录来促进其效应子功能。另一方面分泌的IFN-g参与直接肿瘤血管反应，例如ICAM-1和VCAM-1的上调和血管发生的抑制。粘附分子上调有助于白细胞募集到肿瘤组织。此外IL-12将T细胞极化为1型辅助T(Th1)效应细胞表型。IL-12进一步显著促进Th1极化通过抑制2型辅助T细胞的发育过程【13】诱导的调节性T细胞(Tregs)和Th17细胞的分化。此外IL-12可以编辑效应T细胞，以最佳产生效应记忆T细胞和T滤泡辅助细胞【14】【15】也报道

了IL-12对APC的直接作用,增加了APC呈现差的免疫原性肿瘤抗原肽的能力【16】【17】。

[0005] 在临床前模型的使用中,IL12单独使用对肿瘤控制也很有效。Brunda【18】等人早在1993年就报道了B16黑素瘤,M5975肉瘤和RENCA肾细胞癌时腹腔注射重组IL-12的抗肿瘤反应。当肿瘤接种后的后期开始治疗时,也观察到IL-12的抗肿瘤作用,发现部分依赖于CD8T细胞。尽管IL-12的全身性给药显示出其作为实验性抗癌剂的巨大潜力,但是在推注给药之后,这种细胞因子的不稳定性和短的半衰期导致了应该研发将其直接递送到肿瘤部位的新方法。遵循这一策略,Vom Berg等人【19】运用渗透微量泵将IL-12局部递送到含有GL-261胶质瘤的小鼠的脑中。值得注意的是,IL-12与共抑制性受体细胞毒性T淋巴细胞抗原4(CTLA-4)的全身阻断的联合治疗在T细胞依赖的方式下在晚期疾病阶段消除了甚至非常后期的肿瘤。在此证据的基础上,结合IL-12与调控途径的靶向治疗具有很大的潜力来克服肿瘤相关免疫抑制。

[0006] IL-12在临床前模型中的有效抗肿瘤作用证明了将这种方法转化为临床使用的良好前景。但不幸的是,在临床试验中系统的i.v重组IL-12的给药IL-12全身用药后不良反应较大,曾出现两例死于严重毒副作用的病人,且一般用药的病人会伴有如发热,胃肠道反应,淋巴细胞减少,肝功能异常等副作用,严重影响药物治疗效果【20】。

发明内容

[0007] 基于IL-12的上述问题和治疗潜力,在临床应用中,有必要对其进行更为有效和安全的进一步开发工作。

[0008] 本发明首先涉及一组融合蛋白,所述融合蛋白包括如下结构单元:

[0009] (1)位于所述融合蛋白N端的第一结构单元:白介素12(IL-12)的一个或两个亚基,所述的白介素12的亚基为白介素12的P35亚基或P40亚基;

[0010] (2)位于所述融合蛋白C端的第二结构单元:抗体Fc片段;

[0011] (3)连接片段1:连接第一结构单元和第二结构单元或连接第一结构单元内部的两个亚基。

[0012] 其中,

[0013] 所述的抗体Fc片段为人源IgG1,优选为人源Fc-knob或人源Fc-hole;

[0014] 所述的P35亚基的氨基酸序列如Seq ID No.2所示,所述的P40亚基的氨基酸序列如Seq ID No.1所示;

[0015] 所述的连接片段1的氨基酸序列如Seq ID No.9所示。

[0016] 根据需要,本发明所述的融合蛋白还可以包括修饰在位于融合蛋白的N端的白介素12亚基的N端的信号肽,所述的信号肽为:

[0017] (1)修饰在P35亚基N端的信号肽1,其氨基酸序列如Seq ID No.11所示;

[0018] 或(2)修饰在P40亚基N端的信号肽2,其氨基酸序列如Seq ID No.12所示。

[0019] 所述的融合蛋白中各个结构单元的排列方式为:

[0020] (1)第一结构单元的C端与第二结构单元的N端通过连接片段1相连;

[0021] (2)如第一结构单元内部有两个亚基,则第一亚基的C端和第二亚基的N端通过连接片段1相连。

[0022] 本发明还涉及一种融合蛋白,包括如下结构单元:

[0023] (1)位于所述融合蛋白N端的第一结构单元:白介素12(IL-12)的一个或两个亚基,所述的白介素12的亚基为白介素12的P35亚基或P40亚基;

[0024] (2)位于所述融合蛋白C端的第二结构单元:抗体Fc片段;

[0025] (3)连接片段1:连接第一结构单元和第二结构单元或连接第一结构单元内部的两个亚基;

[0026] (4)连接在第一结构单元N端的白介素12受体;

[0027] (5)连接白介素12受体和第一结构单元的连接片段2。

[0028] 其中,

[0029] 所述的抗体Fc片段为人源IgG1,优选为人源Fc-knob或人源Fc-hole;

[0030] 所述的P35亚基的氨基酸序列如Seq ID No.2所示,所述的P40亚基的氨基酸序列如Seq ID No.1所示;

[0031] 所述的连接片段1的氨基酸序列如Seq ID No.9所示;

[0032] 所述的白介素12受体为:IL-12Rβ1或IL-12Rβ2,所述的IL-12Rβ1的氨基酸序列如Seq ID No.6所示,所述的IL-12Rβ2的氨基酸序列如Seq ID No.7所示;

[0033] 所述连接片段2能够被肿瘤微环境中特异性表达的蛋白水解酶识别并水解,所述的肿瘤微环境中特异性表达的蛋白水解酶为基质金属蛋白酶,优选为基质金属蛋白酶14(MMP14)。

[0034] 最优选的,所述的连接片段2的氨基酸序列如Seq ID No.10所示,其被基质金属蛋白酶14识别的氨基酸的结构的序列如Seq ID No.8所示。

[0035] 所述的融合蛋白中各个结构单元的排列方式为:

[0036] (1)白介素12受体的C端与第一结构单元的N端通过连接片段2相连;

[0037] (2)第一结构单元的C端与第二结构单元的N端通过连接片段1相连;

[0038] (3)如第一结构单元内部有两个亚基,则第一亚基的C端和第二亚基的N端通过连接片段1相连。

[0039] 本发明还涉及一种药物前体,所述的药物前体为同源或异源二聚体,构成所述的二聚体的单体为所述的融合蛋白。

[0040] 优选的,所述的药物前体为如下药物前体1～7:

[0041] (1)药物前体1(Homo IL-12)为同源二聚体,

[0042] 组成的单体为:含有信号肽2的P40亚基、连接片段1、P35亚基、连接片段1、人源IgG1连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.13所示;

[0043] (2)药物前体2(Heter IL-12)为异源二聚体,

[0044] 单体1为:含有信号肽2的P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.14所示;

[0045] 单体2为:含有信号肽1的P35亚基、连接片段1、人源Fc-hole连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.15所示;

[0046] (3)药物前体3(Homo-R1)为同源二聚体,

[0047] 组成的单体为:IL-12的受体Rβ1片段、连接片段2、P40亚基、连接片段1、P35亚基、连接片段1、人源IgG1依次连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.16所示;

[0048] (4)药物前体4(Homo-R2)为同源二聚体,

[0049] 组成的单体为:IL-12的受体Rβ2片段、连接片段2、P40亚基、连接片段1、P35亚基、连接片段1、人源IgG1连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.17所示;

[0050] (5)药物前体5(Heter-R1/R2)为异源二聚体,

[0051] 单体1为:IL-12的受体Rβ1片段、连接片段2、P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.18所示;

[0052] 单体2为:IL-12的受体Rβ2片段、连接片段2、P35亚基、连接片段1、人源Fc-hole依次连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.19所示;

[0053] (6)药物前体6(Heter-R1)为异源二聚体,

[0054] 单体1为:IL-12的受体Rβ1片段、连接片段2、P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.20所示;

[0055] 单体2为:含有信号肽1的P35亚基、连接片段1、人源Fc-hole依次连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.21所示;

[0056] (7)药物前体7(Heter-R2)为异源二聚体,

[0057] 单体1为:含有信号肽2的P40亚基、连接片段1、人源Fc-knob连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.22所示;

[0058] 单体2为:IL-12的受体Rβ2片段、连接片段2、P35亚基、连接片段1、人源Fc-hole连接成的融合蛋白,其氨基酸序列结构如SEQ ID No.23所示。

[0059] 本发明还涉及编码所述融合蛋白、所述药物前体的核苷酸片段。

[0060] 本发明还涉及所述融合蛋白、所述药物前体在制备药物中的应用;优选的,所述的药物为抗肿瘤药物,最优选的,所述药物为抗结直肠癌的药物。

[0061] 本发明还涉及所述的融合蛋白或药物前体的制备方法,所述的制备方法包括如下步骤:

[0062] (1)构建包含所述编码所述融合蛋白或融合蛋白2或药物前体的编码基因的表达载体,优选的,所述的表达载体是pEE12.4表达载体;

[0063] (2)通过瞬时转染宿主细胞的方法构建包含所述表达载体的宿主细胞,优选的,所述的宿主细胞是293F细胞;

[0064] (3)培养所述宿主细胞并收集细胞上清;

[0065] (4)通过ProteinA/G的亲和层析柱纯化蛋白纯化所述融合蛋白、融合蛋白2或药物前体。

附图说明

[0066] 图1、串联形式的IL-12-Fc二聚体produrg:Homodimer-IL-12-Fc(Homo IL-12)的结构示意图。

[0067] 图2、并联形式的IL-12-Fc二聚体produrg:Heterdimer-IL-12-Fc(Heter IL-12)的结构示意图,Fc-k是Fc-knob的简写,Fc-h是Fc-hole的简写。

[0068] 图3、Homodimer-IL-12-Rβ1二聚体prodrug(Homo-R1)结构示意图。

[0069] 图4、Homodimer-IL-12-Rβ2二聚体prodrug(Homo-R2)结构示意图。

[0070] 图5、Heterdimer-IL-12-Rβ1/Rβ2二聚体prodrug(Heter-R1/R2)结构示意图,Fc-k是Fc-knob的简写,Fc-h是Fc-hole的简写。

[0071] 图6、Heterdimer-IL-12-Rβ1二聚体prodrug(Heter-R1)结构示意图,Fc-k是Fc-knob的简写,Fc-h是Fc-hole的简写。

[0072] 图7、Heterdimer-IL-12-Rβ2二聚体prodrug(Heter-R2)结构示意图,Fc-k是Fc-knob的简写,Fc-h是Fc-hole的简写。

[0073] 图8、图1～7所示的7种融合蛋白表达后的SDS-PAGE电泳鉴定图谱。

[0074] 图9、注射给药未连接IL-12受体的IL-12-Fc可以完全清除MC38肿瘤,且Heter IL-12比Homo IL-12有更强的清除效果。

[0075] 图10、Heter IL-12的细胞毒性高于Homo IL-12。

[0076] 图11、Heter-R1,Heter-R2,Heter-R1/R2均可以有效地清除MC38肿瘤。

[0077] 图12、Heter IL-12连接IL-12受体的prodrug在系统性给药时有更小的毒副作用

具体实施方式

[0078] 实施例1、七种IL-12-Fc prodrug的设计

[0079] IL-12由p35和p40两个亚基组成,选用humanIgG1、humanFc-knob、humanFc-hole的Fc片段构建相应的prodrug;实际的prodrug的构建中,我们选择将IL-12的两个亚基串联或并联,具体形式如下:

[0080] 图1为,两个亚基串联的IL-12的produrg:Homodimer-IL-12-Fc(Homo IL-12)的结构示意图,二聚体的分子量为175KD(MW＝175KD);

[0081] 图2为:两个亚基并联的IL-12的produrg:Heterdimer-IL-12-Fc(Heter IL-12)的结构示意图,二聚体的分子量为115KD(MW＝115KD),此二聚体中,P35和P40亚基的C端分别添加P35信号肽和P40信号肽。

[0082] 接下来,我们通过阻断IL-12与其任意一受体的结合或者同时阻断其与两个受体的同时结合的方法来构建IL-12Prodrug;截取IL-12Rβ1与p40相互作用的N端两个CHR domain来阻断p40与IL-12Rβ1的结合;同时截取IL-12Rβ2与p35相互作用的N端的一个Ig domain以及两个CHR domain来阻断p35与IL-12Rβ2的结合。

[0083] 对于Homodimer-IL-12的prodrug构建如图3所示。

[0084] 图3为:Homodimer-IL-12-Rβ1的prodrug结构示意图(以下简称Homo-R1),二聚体MW＝232KD;

[0085] 图4为:Homodimer-IL-12-Rβ2的prodrug结构示意图(以下简称Homo-R2),二聚体MW＝250KD;

[0086] 图5为:Heterdimer-IL-12-Rβ1/Rβ2的prodrug结构示意图(以下简称Heter-R1/R2),二聚体MW＝182KD;

[0087] 图6为:Heterdimer-IL-12-Rβ1的prodrug结构示意图(以下简称Heter-R1),二聚体MW＝144KD,此二聚体中,P35-Fc-hole单体的融合片段P35的C端添加了P35信号肽;

[0088] 图7为:Heterdimer-IL-12-Rβ2的prodrug结构示意图(以下简称Heter-R2),二聚体MW＝153KD,此二聚体中,P40-Fc-knob单体的融合片段P40的C端添加了P40信号肽。

[0089] 实施例2、IL-12prodrug的构建、纯化与生产

[0090] 我们对于实施例1所述的7种蛋白进行表达、生产,首先在pEE12.4的表达载体上进行了融合蛋白的构建,然后通过瞬时转染293F细胞的方法构建包含所述载体的宿主细胞,

培养所述宿主细胞并收集细胞上清,最后通过ProteinA/G的亲和层析柱纯化蛋白。

[0091] 对于同源二聚体蛋白的表达只需转入一种构建的表达载体质粒,细胞内表达的单体可以自发形成同源二聚体蛋白。对于异源二聚体蛋白的表达需转入摩尔比相同的两种表达载体质粒,细胞内表达的单体同样可以自发形成异源二聚体。

[0092] SDS-PAGE电泳鉴定图谱如图8所示:

[0093] 1、载体的构建、宿主细胞的转染及诱导表达

[0094] 1.1、我们在PEE12.4的载体上构建了各种融合蛋白的表达质粒,然后通过瞬时转染293F的方法获得各个蛋白的表达上清,最后通过Protein A亲和层析柱纯化了各种蛋白。

[0095] 构建表达载体

[0096] (1)PEE12.4-HindⅢ-p40(signal)-NruI-p35(no signal)-BsiWI-hIgG1-EcoRI

[0097] (2)PEE12.4-HindⅢ-P35(signal)-BsiWI-Fch-EcoRI

[0098] (3)PEE12.4-HindⅢ-P40(signal)-NruI-Fck-EcoRI

[0099] (4)PEE12.4-HindⅢ-IL12Rb1/IL12Rb2-BsiWI-p40(no signal)-NruI-p35(no signal)-BsiWI

[0100] -hIgG1-EcoRI

[0101] (5)PEE12.4-HindⅢ-IL12Rb1-BsiWI-p40(no signal)-NruI-Fck-EcoRI

[0102] (6)PEE12.4-HindⅢ-IL12Rb2-BstBI-p35(no signal)-BsiWI-Fch-EcoRI

[0103] 其中,HindⅢ、NruI、BsiWI、EcoRI为酶切位点。

[0104] 各个融合蛋白片段间的连接序列为:

[0105] (1)homo IL-12:P40和P35之间为

[0106] (2)linker1,P35和Fc之间为linker1;

[0107] (2)heter IL-12:P40和Fc之间为linker1,P35和Fc之间为linker1;

[0108] (3)Rβ1与P40之间为linker2;相应的蛋白酶的酶切靶序列为SGRSENIRTA;

[0109] (4)Rβ2与P40之间为linker2;相应的蛋白酶的酶切靶序列为SGRSENIRTA;

[0110] (5)Rβ2与P35之间为linker2;相应的蛋白酶的酶切靶序列为SGRSENIRTA;

[0111] 1.2、瞬时转染快速表达目的蛋白:

[0112] (1)细胞复苏:Freestyle 293F细胞以3×10^7cells/ml的浓度于CD OptiCHO™ media(含10%DMSO)中冻存。从液氮中取出后在37℃水浴锅中快速溶化,加入到含有10ml OptiCHO™ media的15ml离心管中离心,1,000rpm,5min。弃上清,将细胞沉淀悬浮培养于30ml OptiCHO™ media中,37℃,8%CO_2,135rpm。4天后将细胞进行扩大培养,扩大培养时浓度不要超过3×10^6cells/ml。

[0113] (2)转染前两天,准备悬浮培养的293F细胞用于瞬时转染(200ml),接种密度为$0.6-0.8\times10^6$cells/ml。

[0114] (3)两天后对待转染细胞悬液进行计数,预计细胞密度$2.5-3.5\times10^6$cells/ml,随后取细胞悬液1,000rpm离心5min,弃上清。

[0115] (4)用50ml的新鲜的Freestyle 293media重新悬浮细胞,再次1,000rpm离心5min,弃上清液。

[0116] (5)用200ml Freestyle 293media重新悬浮293F细胞。

[0117] (6)用5ml Freestyle 293media培养基稀释600μg质粒,并利用0.22μM滤器过滤除

菌。

[0118] (7)用5ml Freestyle 293media培养基稀释1.8mg PEI并利用0.22μM滤器过滤除菌。随后立即将5ml质粒和5ml的PEI混匀,室温静置5分钟。

[0119] (8)将质粒/PEI混合物加入细胞悬液中,放置在37℃,8％CO_2,85rpm培养箱中培养,同时补加生长因子50ug/L LONG™R3IGF-1。

[0120] (9)4小时后补加200ml EX-CELL™ 293media培养基和2mM Glutamine,将转速调至135rpm继续培养。

[0121] (10)24小时后加入细胞增殖抑制剂3.8mM VPA,72小时后加入40ml medium D继续培养,转然后6-8天(细胞存活率低于70％)收集上清液进行下一步纯化。

[0122] 1.3、融合蛋白的收集、纯化和电泳验证

[0123] 利用Protein A进行目的蛋白纯化

[0124] (1)样品准备:将悬浮细胞培养液转至500ml离心桶中离心,8,000rpm,20min弃沉淀,将上清经0.45μM滤器过滤除去杂质,然后加入终浓度为0.05％NaN3防止纯化过程中细菌污染。

[0125] (2)组装层析柱:取适量Protein A Agarose(每1ml Protein A纯化20mg human Fc融合蛋白计算)混匀后加入层析柱,室温静置约10min,待Protein A与20％乙醇溶液分层后把底部的出口打开,让乙醇溶液通过重力作用缓慢流出。

[0126] (3)分别用10倍柱体积的蒸馏水和Binding buffer(20mM sodium phosphate+0.15M NaCl,pH 7.0)冲洗和平衡层析柱。

[0127] (4)利用恒流泵进行上样,流速为10倍柱体积/小时,收集流穿液,重复上样2次。

[0128] (5)用10倍柱体积以上的Binding buffer冲洗柱子,洗去杂蛋白,冲洗至流出液无蛋白检出。

[0129] (6)使用Elution Buffer(0.1M Glycine,pH 2.7)进行洗脱,洗脱液分管收集,每1ml收集1管,并采用蛋白指示液(Bio-Rad protein assay)观察洗脱峰。将洗脱峰的收集管混合后加入适量的1M Tris,pH 9.0中和(调节pH值至6-8,应于所纯化蛋白等电点相差0.5以上)。

[0130] (7)利用Zeba脱盐离心柱或浓缩离心柱将目的蛋白溶液置换到所需要的缓冲液中(注意调节缓冲液pH,避开蛋白的等电点)。利用BSA为标准品,通过SDS-PAGE电泳(每个样品的蛋白上样量为2.5ug)和NanoDrop2000确定蛋白浓度。

[0131] 洗脱结束后,依次使用20倍柱体积的蒸馏水冲洗柱子,再用10倍柱体积的20％乙醇冲洗柱子,最后乙醇溶液要浸没凝胶介质,4℃保存。

[0132] 实施例3、各融合蛋白的体内抗肿瘤活性

[0133] 1、系统性注射IL-12-Fc可以完全清除MC38肿瘤,并且Heter IL-12的治疗效果比Homo IL-12更好

[0134] 为了确定我们设计并生产的IL-12-Fc在系统性给药时是否能够有效地清除肿瘤并且比较两种形式的IL-12-Fc的治疗效果。我们在MC38模型中通过系统性给药的方式对小鼠进行不同剂量的治疗。试验结果如图9所示:

[0135] 图9A:WT C57BL/6小鼠(n＝5只/组)在第0天时皮下接种$5x10^5$个MC38细胞。第13,16,20天时腹腔注射PBS,0.5ug,1ug,5ug,10ug的Homo IL-12治疗,记录荷瘤小鼠的肿瘤体

积。

[0136] 图9B:WT C57BL/6小鼠(n＝5只/组)在第0天时皮下接种5x10^{5}个MC38细胞。第13,16,20天时腹腔注射PBS,0.5ug,1ug,5ug,10ug的Heter IL-12治疗,记录荷瘤小鼠的肿瘤体积。

[0137] 这项结果表明IL-12-Fc能够有效的清除肿瘤并且Heter IL-12比Homo IL-12对肿瘤有更好的清除。

[0138] 2、IL-12-Fc的系统性使用会引发严重的毒副作用且Heter IL-12毒性高于Homo IL-12

[0139] 由于IL-12的受体广泛存在于T,B,NK细胞,所以IL-12的使用常常伴随着很强的毒副作用,临床上病人主要表现为各种血液疾病和肝脏毒性等。我们进一步检测了IL-12-Fc的毒性,主要检测指标是小鼠血清中各种炎症细胞因子的含量。

[0140] 取WT C57BL/6小鼠(n＝5只/组),在第0天时皮下接种5x10^{5}个MC38细胞;第13,16,20天时腹腔注射PBS,5ug的Homo IL-12或Heter IL-12治疗,在第20天给药治疗后6h,眼静脉取血检测血清中炎性因子IL-12p70,IFNγ,TNF,MCP-1,IL-10,IL-6的含量。

[0141] 结果如图10所示,Homo IL-12与Heter IL-12均会引发强的细胞毒性,且Heter IL-12的细胞毒性高于Homo IL-12。

[0142] 3、Heter IL-12连接IL-12受体的prodrug可以有效地清除MC38肿瘤

[0143] 我们构建IL-12prodrug,通过肿瘤微环境中特异性表达的蛋白水解酶的底物来连接IL-12Receptor与IL-12,目的是通过肿瘤微环境中特异性的蛋白水解酶将IL-12prodrug中的底物特异性的在肿瘤微环境被切开,增加IL-12使用的靶向性也降低IL-12全身性使用时的毒性。通过之前体内实验探究我们确定当使用量小于5ug/小鼠时,Heter IL-12比Homo IL-12对MC38肿瘤有更好的清除效果,但同时有更强的毒性。所以我们使用实施例1所述的Heter IL-12以不同方式连接IL-12受体的前药(prodrug)即Heter-R1,Heter-R2,Heter-R1/R2;以及Homo IL-12以不同方式连接IL-12受体的前药(prodrug)即Homo-R1,Homo-R2,来测试prodrug的体内效果。

[0144] 图11A:WT C57BL/6小鼠(n＝5只/组)在第0天时皮下接种5x10^{5}个MC38细胞,第10,13,16天时腹腔注射5ug的Heter IL-12、Heter-R1、Heter-R2、Heter-R1/R2或Homo-R2治疗,对照组为PBS。

[0145] 图11B:WT C57BL/6小鼠(n＝5只/组)在第0天时皮下接种5x10^{5}个MC38细胞。第10,13,16天时腹腔注射2.5ug的Heter IL-12、Heter-R1、Heter-R2或Heter-R1/R2治疗,对照组为PBS。

[0146] 实验结果表明,三种形式的Heter IL-12的前药(prodrug)均可以有效地清除肿瘤,给药剂量为5ug/小鼠时,Heter IL-12的三种形式的前药比Homo IL-12的前药对MC38有更好的清除效果,将剂量降低一倍即2.5ug/小鼠,Heter IL-12prodrug(Heter R1、Heter R2或Heter-R1/R2)仍然可以有效的清除肿瘤。

[0147] 4、Heter IL-12连接IL-12受体的prodrug在系统性给药时有更小的毒副作用

[0148] 根据前述实验结果,已知未偶联IL-12受体的IL-12-Fc在系统性给药时可以引发严重的毒副作用,因此我们记录了连接IL-12受体的不同形式的Heter IL-12prodrug在系统性给药后小鼠体重的波动情况,并且通过小鼠的眼静脉取血检测了血清中炎症因子的表

达水平。

[0149] 图12A:WT C57BL/6小鼠(n＝5只/组)在第0天时皮下接种5x10^{5}个MC38细胞,第10,13,16天时腹腔注射2.5ug的Heter IL-12、Heter R1、Heter R2、Heter R1/R2治疗,对照组为PBS,在治疗的同时测量小鼠体重。

[0150] 图12B-G:治疗同时进行小鼠眼静脉取血,检测血清中炎性因子IL-12p70,TNF,IFNγ,MCP-1,IL-10,IL-6的含量。

[0151] 实验结果表明,在剂量为2.5ug/小鼠系统性给药时,Heter IL-12以不同形式连接IL-12受体的prodrug(Heter R1、Heter R2)相较于未连接IL-12受体的Heter IL-12而言,有更小的毒副作用,并且Heter-R2相较于其他类型的prodrug有更小的毒性。

[0152] 综合上述实施例,在mouse MC38模型中,连接IL-12受体的IL-12-Fc的prodrug可以保持抗肿瘤的有效性和并且提高IL-12-Fc使用过程的安全性。低剂量(2.5ug)的连接了IL-12受体的Heter IL-12前药可以完全清除肿瘤体积为130-150mm^{3}的Mc38肿瘤,并且肿瘤不会复发。当对比同样剂量的未连接IL-12受体的IL-12-Fc时,前者在系统性注射的过程中产生更小的毒副作用,一方面体现在小鼠体重不会明显降低,另一方面体现在连接了IL-12受体的Heter IL-12引发的血液中炎性因子表达水平更低,尤其是Heter R2的应用更加安全。

[0153] 最后需要说明的是,以上实施例仅用作帮助本领域技术人员理解本发明的实质,不用做对本发明保护范围的限定。

SEQUENCE LISTING

<110> 中国科学院生物物理研究所

<120> 一种以白介素12为活性成分的融合蛋白型药物前体

<130> CP11802086C

<160> 23

<170> PatentIn version 3.3

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Gln Tyr Thr Cys His Lys Gly Gly Glu Thr Leu Ser His Ser His Leu
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Asn Phe Lys Asn Lys Thr Phe Leu Lys Cys Glu Ala Pro Asn Tyr Ser
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Asp Tyr Glu Lys Tyr Ser Val Ser Cys Gln Glu Asp Val Thr Cys Pro
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Thr Ala Glu Glu Thr Leu Pro Ile Glu Leu Ala Leu Glu Ala Arg Gln
180 185 190
Gln Asn Lys Tyr Glu Asn Tyr Ser Thr Ser Phe Phe Ile Arg Asp Ile
195 200 205
Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Met Lys Pro Leu Lys Asn

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Ser Gln Val Glu Val Ser Trp Glu Tyr Pro Asp Ser Trp Ser Thr Pro
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His Ser Tyr Phe Ser Leu Lys Phe Phe Val Arg Ile Gln Arg Lys Lys
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Glu Lys Met Lys Glu Thr Glu Glu Gly Cys Asn Gln Lys Gly Ala Phe
260 265 270
Leu Val Glu Lys Thr Ser Thr Glu Val Gln Cys Lys Gly Gly Asn Val
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Ala Cys Val Pro Cys Arg Val Arg Ser
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Leu Lys His Tyr Ser Cys Thr Ala Glu Asp Ile Asp His Glu Asp Ile
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His Lys Asn Glu Ser Cys Leu Ala Thr Arg Glu Thr Ser Ser Thr Thr
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Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
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Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
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Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
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Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125
Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130 135 140
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145 150 155 160
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
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Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
180 185 190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
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Pro Gly Lys
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Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
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Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
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Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
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Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
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Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
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Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
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Pro Gly Lys
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Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
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His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
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Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
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Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
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Val Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp
180 185 190
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Leu His
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Gly Lys
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Trp Gln Tyr Asp Gly Pro Glu Asp Asn Val Ser His Val Leu Trp Cys
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Cys Phe Val Pro Pro Asn His Thr His Thr Gly Gln Glu Arg Cys Arg
85 90 95

Tyr Phe Ser Ser Gly Pro Asp Arg Thr Val Gln Phe Trp Glu Gln Asp
100 105 110

Gly Ile Pro Val Leu Ser Lys Val Asn Phe Trp Val Glu Ser Arg Leu
115 120 125

Gly Asn Arg Thr Met Lys Ser Gln Lys Ile Ser Gln Tyr Leu Tyr Asn
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Trp Thr Lys Thr Thr Pro Pro Leu Gly His Ile Lys Val Ser Gln Ser
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His Arg Gln Leu Arg Met Asp Trp Asn Val Ser Glu Glu Ala Gly Ala
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290 295 300
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35 40 45
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50 55 60
Arg His Gly Val Ile Gly Ser Gly Lys Thr Leu Thr Ile Thr Val Lys
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Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Thr
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Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys Gln
180 185 190
Glu Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu Leu
195 200 205
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Asn Gly Glu Thr Leu Arg Gln Lys Pro Pro Val Gly Glu Ala Asp Pro
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Tyr Arg Val Lys Met Lys Leu Cys Ile Leu Leu His Ala Phe Ser Thr
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530 535 540
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys
545 550 555 560
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro

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Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
610 615 620
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
625 630 635 640
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
645 650 655
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
660 665 670
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
675 680 685
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
690 695 700
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
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Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
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Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
740 745 750
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Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile Thr Trp Thr Ser Asp Gln

50 55 60

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Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Thr
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Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn Lys Thr Phe Leu Lys Cys
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Asp Ser Arg Ala Val Thr Cys Gly Met Ala Ser Leu Ser Ala Glu Lys
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Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys Gln
180 185 190

Glu Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu Leu
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Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln
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Met Lys Pro Leu Lys Asn Ser Gln Val Glu Val Ser Trp Glu Tyr Pro
245 250 255

Asp Ser Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Lys Phe Phe Val
260 265 270

Arg Ile Gln Arg Lys Lys Glu Lys Met Lys Glu Thr Glu Glu Gly Cys
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Asn Gln Lys Gly Ala Phe Leu Val Glu Lys Thr Ser Thr Glu Val Gln
290 295 300

Cys Lys Gly Gly Asn Val Cys Val Gln Ala Gln Asp Arg Tyr Tyr Asn
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Ser Ser Cys Ser Lys Trp Ala Cys Val Pro Cys Arg Val Arg Ser Gly
325 330 335

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys
340 345 350

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
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Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
370 375 380
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
385 390 395 400
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
405 410 415
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
420 425 430
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
435 440 445
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
450 455 460
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
465 470 475 480
Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp
485 490 495
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
500 505 510
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
515 520 525
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
530 535 540
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Lys

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Lys Thr Ala Arg Glu Lys Leu Lys His Tyr Ser Cys Thr Ala Glu Asp

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Ile Asp His Glu Asp Ile Thr Arg Asp Gln Thr Ser Thr Leu Lys Thr
65 70 75 80

Cys Leu Pro Leu Glu Leu His Lys Asn Glu Ser Cys Leu Ala Thr Arg
85 90 95

Glu Thr Ser Ser Thr Thr Arg Gly Ser Cys Leu Pro Pro Gln Lys Thr
100 105 110

Ser Leu Met Met Thr Leu Cys Leu Gly Ser Ile Tyr Glu Asp Leu Lys
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Met Tyr Gln Thr Glu Phe Gln Ala Ile Asn Ala Ala Leu Gln Asn His
130 135 140

Asn His Gln Gln Ile Ile Leu Asp Lys Gly Met Leu Val Ala Ile Asp
145 150 155 160

Glu Leu Met Gln Ser Leu Asn His Asn Gly Glu Thr Leu Arg Gln Lys
165 170 175

Pro Pro Val Gly Glu Ala Asp Pro Tyr Arg Val Lys Met Lys Leu Cys
180 185 190

Ile Leu Leu His Ala Phe Ser Thr Arg Val Val Thr Ile Asn Arg Val
195 200 205

Met Gly Tyr Leu Ser Ser Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly
210 215 220

Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
225 230 235 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
245 250 255

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
260 265 270

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
275 280 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
290 295 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305 310 315 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
325 330 335

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
340 345 350

Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu
355 360 365

Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro
370 375 380
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
385 390 395 400
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
405 410 415
Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
420 425 430
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
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Lys Ser Leu Ser Leu Ser Pro Gly Lys
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Leu Leu Cys Gln Leu Gly Ala Ser Gly Pro Gly Asp Gly Cys Cys Val
20 25 30
Glu Lys Thr Ser Phe Pro Glu Gly Ala Ser Gly Ser Pro Leu Gly Pro
35 40 45
Arg Asn Leu Ser Cys Tyr Arg Val Ser Lys Thr Asp Tyr Glu Cys Ser
50 55 60
Trp Gln Tyr Asp Gly Pro Glu Asp Asn Val Ser His Val Leu Trp Cys
65 70 75 80
Cys Phe Val Pro Pro Asn His Thr His Thr Gly Gln Glu Arg Cys Arg
85 90 95
Tyr Phe Ser Ser Gly Pro Asp Arg Thr Val Gln Phe Trp Glu Gln Asp
100 105 110
Gly Ile Pro Val Leu Ser Lys Val Asn Phe Trp Val Glu Ser Arg Leu
115 120 125
Gly Asn Arg Thr Met Lys Ser Gln Lys Ile Ser Gln Tyr Leu Tyr Asn
130 135 140
Trp Thr Lys Thr Thr Pro Pro Leu Gly His Ile Lys Val Ser Gln Ser
145 150 155 160
His Arg Gln Leu Arg Met Asp Trp Asn Val Ser Glu Glu Ala Gly Ala
165 170 175

Glu Val Gln Phe Arg Arg Arg Met Pro Thr Thr Asn Trp Thr Leu Gly
180 185 190
Asp Cys Gly Pro Gln Val Asn Ser Gly Ser Gly Val Leu Gly Asp Ile
195 200 205
Arg Gly Ser Met Ser Glu Ser Cys Leu Cys Pro Ser Glu Asn Met Ala
210 215 220
Gln Glu Ile Gln Ile Arg Arg Arg Arg Arg Leu Ser Ser Gly Ala Pro
225 230 235 240
Gly Gly Pro Trp Ser Asp Trp Ser Met Pro Val Cys Val Pro Pro Glu
245 250 255
Val Leu Pro Gly Gly Gly Gly Ser Ser Gly Arg Ser Glu Asn Ile Arg
260 265 270
Thr Ala Gly Gly Gly Gly Ser Met Trp Glu Leu Glu Lys Asp Val Tyr
275 280 285
Val Val Glu Val Asp Trp Thr Pro Asp Ala Pro Gly Glu Thr Val Asn
290 295 300
Leu Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile Thr Trp Thr Ser Asp
305 310 315 320
Gln Arg His Gly Val Ile Gly Ser Gly Lys Thr Leu Thr Ile Thr Val
325 330 335
Lys Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu
340 345 350
Thr Leu Ser His Ser His Leu Leu Leu His Lys Lys Glu Asn Gly Ile
355 360 365
Trp Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn Lys Thr Phe Leu Lys
370 375 380
Cys Glu Ala Pro Asn Tyr Ser Gly Arg Phe Thr Cys Ser Trp Leu Val
385 390 395 400
Gln Arg Asn Met Asp Leu Lys Phe Asn Ile Lys Ser Ser Ser Ser Ser
405 410 415
Pro Asp Ser Arg Ala Val Thr Cys Gly Met Ala Ser Leu Ser Ala Glu
420 425 430
Lys Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys
435 440 445
Gln Glu Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu
450 455 460
Leu Ala Leu Glu Ala Arg Gln Gln Asn Lys Tyr Glu Asn Tyr Ser Thr
465 470 475 480
Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu

485 490 495

Gln Met Lys Pro Leu Lys Asn Ser Gln Val Glu Val Ser Trp Glu Tyr
500 505 510

Pro Asp Ser Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Lys Phe Phe
515 520 525

Val Arg Ile Gln Arg Lys Lys Glu Lys Met Lys Glu Thr Glu Glu Gly
530 535 540

Cys Asn Gln Lys Gly Ala Phe Leu Val Glu Lys Thr Ser Thr Glu Val
545 550 555 560

Gln Cys Lys Gly Gly Asn Val Cys Val Gln Ala Gln Asp Arg Tyr Tyr
565 570 575

Asn Ser Ser Cys Ser Lys Trp Ala Cys Val Pro Cys Arg Val Arg Ser
580 585 590

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg
595 600 605

Val Ile Pro Val Ser Gly Pro Ala Arg Cys Leu Ser Gln Ser Arg Asn
610 615 620

Leu Leu Lys Thr Thr Asp Asp Met Val Lys Thr Ala Arg Glu Lys Leu
625 630 635 640

Lys His Tyr Ser Cys Thr Ala Glu Asp Ile Asp His Glu Asp Ile Thr
645 650 655

Arg Asp Gln Thr Ser Thr Leu Lys Thr Cys Leu Pro Leu Glu Leu His
660 665 670

Lys Asn Glu Ser Cys Leu Ala Thr Arg Glu Thr Ser Ser Thr Thr Arg
675 680 685

Gly Ser Cys Leu Pro Pro Gln Lys Thr Ser Leu Met Met Thr Leu Cys
690 695 700

Leu Gly Ser Ile Tyr Glu Asp Leu Lys Met Tyr Gln Thr Glu Phe Gln
705 710 715 720

Ala Ile Asn Ala Ala Leu Gln Asn His Asn His Gln Gln Ile Ile Leu
725 730 735

Asp Lys Gly Met Leu Val Ala Ile Asp Glu Leu Met Gln Ser Leu Asn
740 745 750

His Asn Gly Glu Thr Leu Arg Gln Lys Pro Pro Val Gly Glu Ala Asp
755 760 765

Pro Tyr Arg Val Lys Met Lys Leu Cys Ile Leu Leu His Ala Phe Ser
770 775 780

Thr Arg Val Val Thr Ile Asn Arg Val Met Gly Tyr Leu Ser Ser Ala
785 790 795 800

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
805 810 815
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
820 825 830
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu Met Ile
835 840 845
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
850 855 860
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
865 870 875 880
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
885 890 895
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
900 905 910
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
915 920 925
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
930 935 940
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
945 950 955 960
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
965 970 975
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
980 985 990
Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
995 1000 1005
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Leu His
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Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
1025 1030 1035
Pro Gly Lys
1040

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<211> 1119
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<400> 17

Met Ala Gln Thr Val Arg Glu Cys Ser Leu Ala Leu Leu Phe Leu Phe
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Met Trp Leu Leu Ile Lys Ala Asn Ile Asp Val Cys Lys Leu Gly Thr
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Val Thr Val Gln Pro Ala Pro Val Ile Pro Leu Gly Ser Ala Ala Asn
35 40 45
Ile Ser Cys Ser Leu Asn Pro Lys Gln Gly Cys Ser His Tyr Pro Ser
50 55 60
Ser Asn Glu Leu Ile Leu Leu Lys Phe Val Asn Asp Val Leu Val Glu
65 70 75 80
Asn Leu His Gly Lys Lys Val His Asp His Thr Gly His Ser Ser Thr
85 90 95
Phe Gln Val Thr Asn Leu Ser Leu Gly Met Thr Leu Phe Val Cys Lys
100 105 110
Leu Asn Cys Ser Asn Ser Gln Lys Lys Pro Pro Val Pro Val Cys Gly
115 120 125
Val Glu Ile Ser Val Gly Val Ala Pro Glu Pro Pro Gln Asn Ile Ser
130 135 140
Cys Val Gln Glu Gly Glu Asn Gly Thr Val Ala Cys Ser Trp Asn Ser
145 150 155 160
Gly Lys Val Thr Tyr Leu Lys Thr Asn Tyr Thr Leu Gln Leu Ser Gly
165 170 175
Pro Asn Asn Leu Thr Cys Gln Lys Gln Cys Phe Ser Asp Asn Arg Gln
180 185 190
Asn Cys Asn Arg Leu Asp Leu Gly Ile Asn Leu Ser Pro Asp Leu Ala
195 200 205
Glu Ser Arg Phe Ile Val Arg Val Thr Ala Ile Asn Asp Leu Gly Asn
210 215 220
Ser Ser Ser Leu Pro His Thr Phe Thr Phe Leu Asp Ile Val Ile Pro
225 230 235 240
Leu Pro Pro Trp Asp Ile Arg Ile Asn Phe Leu Asn Ala Ser Gly Ser
245 250 255
Arg Gly Thr Leu Gln Trp Glu Asp Glu Gly Gln Val Val Leu Asn Gln
260 265 270
Leu Arg Tyr Gln Pro Leu Asn Ser Thr Ser Trp Asn Met Val Asn Ala
275 280 285
Thr Asn Ala Lys Gly Lys Tyr Asp Leu Arg Asp Leu Arg Pro Phe Thr
290 295 300
Glu Tyr Glu Phe Gln Ile Ser Ser Lys Leu His Leu Ser Gly Gly Ser
305 310 315 320
Trp Ser Asn Trp Ser Glu Ser Leu Arg Thr Arg Thr Pro Glu Glu Glu

325 330 335
Pro Gly Gly Gly Gly Ser Ser Gly Arg Ser Glu Asn Ile Arg Thr Ala
340 345 350
Gly Gly Gly Gly Ser Met Trp Glu Leu Glu Lys Asp Val Tyr Val Val
355 360 365
Glu Val Asp Trp Thr Pro Asp Ala Pro Gly Glu Thr Val Asn Leu Thr
370 375 380
Cys Asp Thr Pro Glu Glu Asp Asp Ile Thr Trp Thr Ser Asp Gln Arg
385 390 395 400
His Gly Val Ile Gly Ser Gly Lys Thr Leu Thr Ile Thr Val Lys Glu
405 410 415
Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Thr Leu
420 425 430
Ser His Ser His Leu Leu Leu His Lys Lys Glu Asn Gly Ile Trp Ser
435 440 445
Thr Glu Ile Leu Lys Asn Phe Lys Asn Lys Thr Phe Leu Lys Cys Glu
450 455 460
Ala Pro Asn Tyr Ser Gly Arg Phe Thr Cys Ser Trp Leu Val Gln Arg
465 470 475 480
Asn Met Asp Leu Lys Phe Asn Ile Lys Ser Ser Ser Ser Ser Pro Asp
485 490 495
Ser Arg Ala Val Thr Cys Gly Met Ala Ser Leu Ser Ala Glu Lys Val
500 505 510
Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys Gln Glu
515 520 525
Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu Leu Ala
530 535 540
Leu Glu Ala Arg Gln Gln Asn Lys Tyr Glu Asn Tyr Ser Thr Ser Phe
545 550 555 560
Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Met
565 570 575
Lys Pro Leu Lys Asn Ser Gln Val Glu Val Ser Trp Glu Tyr Pro Asp
580 585 590
Ser Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Lys Phe Phe Val Arg
595 600 605
Ile Gln Arg Lys Lys Glu Lys Met Lys Glu Thr Glu Glu Gly Cys Asn
610 615 620
Gln Lys Gly Ala Phe Leu Val Glu Lys Thr Ser Thr Glu Val Gln Cys
625 630 635 640

Lys Gly Gly Asn Val Cys Val Gln Ala Gln Asp Arg Tyr Tyr Asn Ser
645 650 655
Ser Cys Ser Lys Trp Ala Cys Val Pro Cys Arg Val Arg Ser Gly Gly
660 665 670
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg Val Ile
675 680 685
Pro Val Ser Gly Pro Ala Arg Cys Leu Ser Gln Ser Arg Asn Leu Leu
690 695 700
Lys Thr Thr Asp Asp Met Val Lys Thr Ala Arg Glu Lys Leu Lys His
705 710 715 720
Tyr Ser Cys Thr Ala Glu Asp Ile Asp His Glu Asp Ile Thr Arg Asp
725 730 735
Gln Thr Ser Thr Leu Lys Thr Cys Leu Pro Leu Glu Leu His Lys Asn
740 745 750
Glu Ser Cys Leu Ala Thr Arg Glu Thr Ser Ser Thr Thr Arg Gly Ser
755 760 765
Cys Leu Pro Pro Gln Lys Thr Ser Leu Met Met Thr Leu Cys Leu Gly
770 775 780
Ser Ile Tyr Glu Asp Leu Lys Met Tyr Gln Thr Glu Phe Gln Ala Ile
785 790 795 800
Asn Ala Ala Leu Gln Asn His Asn His Gln Gln Ile Ile Leu Asp Lys
805 810 815
Gly Met Leu Val Ala Ile Asp Glu Leu Met Gln Ser Leu Asn His Asn
820 825 830
Gly Glu Thr Leu Arg Gln Lys Pro Pro Val Gly Glu Ala Asp Pro Tyr
835 840 845
Arg Val Lys Met Lys Leu Cys Ile Leu Leu His Ala Phe Ser Thr Arg
850 855 860
Val Val Thr Ile Asn Arg Val Met Gly Tyr Leu Ser Ser Ala Gly Gly
865 870 875 880
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr
885 890 895
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
900 905 910
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Gln Leu Met Ile Ser Arg
915 920 925
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
930 935 940
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala

945 950 955 960
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
965 970 975
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
980 985 990
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
995 1000 1005
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
1010 1015 1020
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
1025 1030 1035
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
1040 1045 1050
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
1055 1060 1065
Pro Val Leu Asp Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr
1070 1075 1080
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
1085 1090 1095
Val Leu His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
1100 1105 1110
Ser Leu Ser Pro Gly Lys
1115

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<211> 834
<212> PRT
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<400> 18

Met Asp Met Met Gly Leu Ala Gly Thr Ser Lys His Ile Thr Phe Leu
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20 25 30
Glu Lys Thr Ser Phe Pro Glu Gly Ala Ser Gly Ser Pro Leu Gly Pro
35 40 45
Arg Asn Leu Ser Cys Tyr Arg Val Ser Lys Thr Asp Tyr Glu Cys Ser
50 55 60
Trp Gln Tyr Asp Gly Pro Glu Asp Asn Val Ser His Val Leu Trp Cys
65 70 75 80
Cys Phe Val Pro Pro Asn His Thr His Thr Gly Gln Glu Arg Cys Arg

85 90 95

Tyr Phe Ser Ser Gly Pro Asp Arg Thr Val Gln Phe Trp Glu Gln Asp
100 105 110

Gly Ile Pro Val Leu Ser Lys Val Asn Phe Trp Val Glu Ser Arg Leu
115 120 125

Gly Asn Arg Thr Met Lys Ser Gln Lys Ile Ser Gln Tyr Leu Tyr Asn
130 135 140

Trp Thr Lys Thr Thr Pro Pro Leu Gly His Ile Lys Val Ser Gln Ser
145 150 155 160

His Arg Gln Leu Arg Met Asp Trp Asn Val Ser Glu Glu Ala Gly Ala
165 170 175

Glu Val Gln Phe Arg Arg Arg Met Pro Thr Thr Asn Trp Thr Leu Gly
180 185 190

Asp Cys Gly Pro Gln Val Asn Ser Gly Ser Gly Val Leu Gly Asp Ile
195 200 205

Arg Gly Ser Met Ser Glu Ser Cys Leu Cys Pro Ser Glu Asn Met Ala
210 215 220

Gln Glu Ile Gln Ile Arg Arg Arg Arg Arg Leu Ser Ser Gly Ala Pro
225 230 235 240

Gly Gly Pro Trp Ser Asp Trp Ser Met Pro Val Cys Val Pro Pro Glu
245 250 255

Val Leu Pro Gly Gly Gly Gly Ser Ser Gly Arg Ser Glu Asn Ile Arg
260 265 270

Thr Ala Gly Gly Gly Gly Ser Met Trp Glu Leu Glu Lys Asp Val Tyr
275 280 285

Val Val Glu Val Asp Trp Thr Pro Asp Ala Pro Gly Glu Thr Val Asn
290 295 300

Leu Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile Thr Trp Thr Ser Asp
305 310 315 320

Gln Arg His Gly Val Ile Gly Ser Gly Lys Thr Leu Thr Ile Thr Val
325 330 335

Lys Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu
340 345 350

Thr Leu Ser His Ser His Leu Leu Leu His Lys Lys Glu Asn Gly Ile
355 360 365

Trp Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn Lys Thr Phe Leu Lys
370 375 380

Cys Glu Ala Pro Asn Tyr Ser Gly Arg Phe Thr Cys Ser Trp Leu Val
385 390 395 400

Gln Arg Asn Met Asp Leu Lys Phe Asn Ile Lys Ser Ser Ser Ser Ser
405 410 415
Pro Asp Ser Arg Ala Val Thr Cys Gly Met Ala Ser Leu Ser Ala Glu
420 425 430
Lys Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys
435 440 445
Gln Glu Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu
450 455 460
Leu Ala Leu Glu Ala Arg Gln Gln Asn Lys Tyr Glu Asn Tyr Ser Thr
465 470 475 480
Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu
485 490 495
Gln Met Lys Pro Leu Lys Asn Ser Gln Val Glu Val Ser Trp Glu Tyr
500 505 510
Pro Asp Ser Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Lys Phe Phe
515 520 525
Val Arg Ile Gln Arg Lys Lys Glu Lys Met Lys Glu Thr Glu Glu Gly
530 535 540
Cys Asn Gln Lys Gly Ala Phe Leu Val Glu Lys Thr Ser Thr Glu Val
545 550 555 560
Gln Cys Lys Gly Gly Asn Val Cys Val Gln Ala Gln Asp Arg Tyr Tyr
565 570 575
Asn Ser Ser Cys Ser Lys Trp Ala Cys Val Pro Cys Arg Val Arg Ser
580 585 590
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
595 600 605
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
610 615 620
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
625 630 635 640
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
645 650 655
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
660 665 670
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
675 680 685
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
690 695 700
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu

705 710 715 720
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
725 730 735
Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
740 745 750
Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
755 760 765
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
770 775 780
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
785 790 795 800
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
805 810 815
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
820 825 830
Gly Lys

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<211> 792
<212> PRT
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<400> 19

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Met Trp Leu Leu Ile Lys Ala Asn Ile Asp Val Cys Lys Leu Gly Thr
20 25 30
Val Thr Val Gln Pro Ala Pro Val Ile Pro Leu Gly Ser Ala Ala Asn
35 40 45
Ile Ser Cys Ser Leu Asn Pro Lys Gln Gly Cys Ser His Tyr Pro Ser
50 55 60
Ser Asn Glu Leu Ile Leu Leu Lys Phe Val Asn Asp Val Leu Val Glu
65 70 75 80
Asn Leu His Gly Lys Lys Val His Asp His Thr Gly His Ser Ser Thr
85 90 95
Phe Gln Val Thr Asn Leu Ser Leu Gly Met Thr Leu Phe Val Cys Lys
100 105 110
Leu Asn Cys Ser Asn Ser Gln Lys Lys Pro Pro Val Pro Val Cys Gly
115 120 125
Val Glu Ile Ser Val Gly Val Ala Pro Glu Pro Pro Gln Asn Ile Ser
130 135 140

Cys Val Gln Glu Gly Glu Asn Gly Thr Val Ala Cys Ser Trp Asn Ser
145 150 155 160
Gly Lys Val Thr Tyr Leu Lys Thr Asn Tyr Thr Leu Gln Leu Ser Gly
165 170 175
Pro Asn Asn Leu Thr Cys Gln Lys Gln Cys Phe Ser Asp Asn Arg Gln
180 185 190
Asn Cys Asn Arg Leu Asp Leu Gly Ile Asn Leu Ser Pro Asp Leu Ala
195 200 205
Glu Ser Arg Phe Ile Val Arg Val Thr Ala Ile Asn Asp Leu Gly Asn
210 215 220
Ser Ser Ser Leu Pro His Thr Phe Thr Phe Leu Asp Ile Val Ile Pro
225 230 235 240
Leu Pro Pro Trp Asp Ile Arg Ile Asn Phe Leu Asn Ala Ser Gly Ser
245 250 255
Arg Gly Thr Leu Gln Trp Glu Asp Glu Gly Gln Val Val Leu Asn Gln
260 265 270
Leu Arg Tyr Gln Pro Leu Asn Ser Thr Ser Trp Asn Met Val Asn Ala
275 280 285
Thr Asn Ala Lys Gly Lys Tyr Asp Leu Arg Asp Leu Arg Pro Phe Thr
290 295 300
Glu Tyr Glu Phe Gln Ile Ser Ser Lys Leu His Leu Ser Gly Gly Ser
305 310 315 320
Trp Ser Asn Trp Ser Glu Ser Leu Arg Thr Arg Thr Pro Glu Glu Glu
325 330 335
Pro Gly Gly Gly Gly Ser Ser Gly Arg Ser Glu Asn Ile Arg Thr Ala
340 345 350
Gly Gly Gly Gly Ser Arg Val Ile Pro Val Ser Gly Pro Ala Arg Cys
355 360 365
Leu Ser Gln Ser Arg Asn Leu Leu Lys Thr Thr Asp Asp Met Val Lys
370 375 380
Thr Ala Arg Glu Lys Leu Lys His Tyr Ser Cys Thr Ala Glu Asp Ile
385 390 395 400
Asp His Glu Asp Ile Thr Arg Asp Gln Thr Ser Thr Leu Lys Thr Cys
405 410 415
Leu Pro Leu Glu Leu His Lys Asn Glu Ser Cys Leu Ala Thr Arg Glu
420 425 430
Thr Ser Ser Thr Thr Arg Gly Ser Cys Leu Pro Pro Gln Lys Thr Ser
435 440 445
Leu Met Met Thr Leu Cys Leu Gly Ser Ile Tyr Glu Asp Leu Lys Met

450 455 460
Tyr Gln Thr Glu Phe Gln Ala Ile Asn Ala Ala Leu Gln Asn His Asn
465 470 475 480
His Gln Gln Ile Ile Leu Asp Lys Gly Met Leu Val Ala Ile Asp Glu
485 490 495
Leu Met Gln Ser Leu Asn His Asn Gly Glu Thr Leu Arg Gln Lys Pro
500 505 510
Pro Val Gly Glu Ala Asp Pro Tyr Arg Val Lys Met Lys Leu Cys Ile
515 520 525
Leu Leu His Ala Phe Ser Thr Arg Val Val Thr Ile Asn Arg Val Met
530 535 540
Gly Tyr Leu Ser Ser Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
545 550 555 560
Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
565 570 575
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
580 585 590
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
595 600 605
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
610 615 620
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
625 630 635 640
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
645 650 655
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
660 665 670
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
675 680 685
Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
690 695 700
Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser
705 710 715 720
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
725 730 735
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val
740 745 750
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
755 760 765

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
770 775 780
Ser Leu Ser Leu Ser Pro Gly Lys
785 790

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<400> 20

Met Asp Met Met Gly Leu Ala Gly Thr Ser Lys His Ile Thr Phe Leu
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20 25 30
Glu Lys Thr Ser Phe Pro Glu Gly Ala Ser Gly Ser Pro Leu Gly Pro
35 40 45
Arg Asn Leu Ser Cys Tyr Arg Val Ser Lys Thr Asp Tyr Glu Cys Ser
50 55 60
Trp Gln Tyr Asp Gly Pro Glu Asp Asn Val Ser His Val Leu Trp Cys
65 70 75 80
Cys Phe Val Pro Pro Asn His Thr His Thr Gly Gln Glu Arg Cys Arg
85 90 95
Tyr Phe Ser Ser Gly Pro Asp Arg Thr Val Gln Phe Trp Glu Gln Asp
100 105 110
Gly Ile Pro Val Leu Ser Lys Val Asn Phe Trp Val Glu Ser Arg Leu
115 120 125
Gly Asn Arg Thr Met Lys Ser Gln Lys Ile Ser Gln Tyr Leu Tyr Asn
130 135 140
Trp Thr Lys Thr Thr Pro Pro Leu Gly His Ile Lys Val Ser Gln Ser
145 150 155 160
His Arg Gln Leu Arg Met Asp Trp Asn Val Ser Glu Glu Ala Gly Ala
165 170 175
Glu Val Gln Phe Arg Arg Arg Met Pro Thr Thr Asn Trp Thr Leu Gly
180 185 190
Asp Cys Gly Pro Gln Val Asn Ser Gly Ser Gly Val Leu Gly Asp Ile
195 200 205
Arg Gly Ser Met Ser Glu Ser Cys Leu Cys Pro Ser Glu Asn Met Ala
210 215 220
Gln Glu Ile Gln Ile Arg Arg Arg Arg Arg Leu Ser Ser Gly Ala Pro
225 230 235 240

Gly Gly Pro Trp Ser Asp Trp Ser Met Pro Val Cys Val Pro Pro Glu
245 250 255
Val Leu Pro Gly Gly Gly Gly Ser Ser Gly Arg Ser Glu Asn Ile Arg
260 265 270
Thr Ala Gly Gly Gly Gly Ser Met Trp Glu Leu Glu Lys Asp Val Tyr
275 280 285
Val Val Glu Val Asp Trp Thr Pro Asp Ala Pro Gly Glu Thr Val Asn
290 295 300
Leu Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile Thr Trp Thr Ser Asp
305 310 315 320
Gln Arg His Gly Val Ile Gly Ser Gly Lys Thr Leu Thr Ile Thr Val
325 330 335
Lys Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu
340 345 350
Thr Leu Ser His Ser His Leu Leu Leu His Lys Lys Glu Asn Gly Ile
355 360 365
Trp Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn Lys Thr Phe Leu Lys
370 375 380
Cys Glu Ala Pro Asn Tyr Ser Gly Arg Phe Thr Cys Ser Trp Leu Val
385 390 395 400
Gln Arg Asn Met Asp Leu Lys Phe Asn Ile Lys Ser Ser Ser Ser Ser
405 410 415
Pro Asp Ser Arg Ala Val Thr Cys Gly Met Ala Ser Leu Ser Ala Glu
420 425 430
Lys Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys
435 440 445
Gln Glu Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu
450 455 460
Leu Ala Leu Glu Ala Arg Gln Gln Asn Lys Tyr Glu Asn Tyr Ser Thr
465 470 475 480
Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu
485 490 495
Gln Met Lys Pro Leu Lys Asn Ser Gln Val Glu Val Ser Trp Glu Tyr
500 505 510
Pro Asp Ser Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Lys Phe Phe
515 520 525
Val Arg Ile Gln Arg Lys Lys Glu Lys Met Lys Glu Thr Glu Glu Gly
530 535 540
Cys Asn Gln Lys Gly Ala Phe Leu Val Glu Lys Thr Ser Thr Glu Val

545 550 555 560
Gln Cys Lys Gly Gly Asn Val Cys Val Gln Ala Gln Asp Arg Tyr Tyr
565 570 575
Asn Ser Ser Cys Ser Lys Trp Ala Cys Val Pro Cys Arg Val Arg Ser
580 585 590
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
595 600 605
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
610 615 620
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
625 630 635 640
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
645 650 655
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
660 665 670
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
675 680 685
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
690 695 700
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
705 710 715 720
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
725 730 735
Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
740 745 750
Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
755 760 765
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
770 775 780
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
785 790 795 800
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
805 810 815
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
820 825 830
Gly Lys

<210> 21
<211> 457
<212> PRT

<213> 人工序列

<400> 21

Met Cys Gln Ser Arg Tyr Leu Leu Phe Leu Ala Thr Leu Ala Leu Leu
1 5 10 15
Asn His Leu Ser Leu Ala Arg Val Ile Pro Val Ser Gly Pro Ala Arg
20 25 30
Cys Leu Ser Gln Ser Arg Asn Leu Leu Lys Thr Thr Asp Asp Met Val
35 40 45
Lys Thr Ala Arg Glu Lys Leu Lys His Tyr Ser Cys Thr Ala Glu Asp
50 55 60
Ile Asp His Glu Asp Ile Thr Arg Asp Gln Thr Ser Thr Leu Lys Thr
65 70 75 80
Cys Leu Pro Leu Glu Leu His Lys Asn Glu Ser Cys Leu Ala Thr Arg
85 90 95
Glu Thr Ser Ser Thr Thr Arg Gly Ser Cys Leu Pro Pro Gln Lys Thr
100 105 110
Ser Leu Met Met Thr Leu Cys Leu Gly Ser Ile Tyr Glu Asp Leu Lys
115 120 125
Met Tyr Gln Thr Glu Phe Gln Ala Ile Asn Ala Ala Leu Gln Asn His
130 135 140
Asn His Gln Gln Ile Ile Leu Asp Lys Gly Met Leu Val Ala Ile Asp
145 150 155 160
Glu Leu Met Gln Ser Leu Asn His Asn Gly Glu Thr Leu Arg Gln Lys
165 170 175
Pro Pro Val Gly Glu Ala Asp Pro Tyr Arg Val Lys Met Lys Leu Cys
180 185 190
Ile Leu Leu His Ala Phe Ser Thr Arg Val Val Thr Ile Asn Arg Val
195 200 205
Met Gly Tyr Leu Ser Ser Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly
210 215 220
Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
225 230 235 240
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
245 250 255
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
260 265 270
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
275 280 285
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu

290 295 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305 310 315 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
325 330 335

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
340 345 350

Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu
355 360 365

Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro
370 375 380

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
385 390 395 400

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
405 410 415

Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
420 425 430

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
435 440 445

Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 22

<211> 577

<212> PRT

<213> 人工序列

<400> 22

Met Cys Pro Gln Lys Leu Thr Ile Ser Trp Phe Ala Ile Val Leu Leu
1 5 10 15

Val Ser Pro Leu Met Ala Met Trp Glu Leu Glu Lys Asp Val Tyr Val
20 25 30

Val Glu Val Asp Trp Thr Pro Asp Ala Pro Gly Glu Thr Val Asn Leu
35 40 45

Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile Thr Trp Thr Ser Asp Gln
50 55 60

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

Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Thr
85 90 95

Leu Ser His Ser His Leu Leu Leu His Lys Lys Glu Asn Gly Ile Trp

100 105 110

Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn Lys Thr Phe Leu Lys Cys

115 120 125

Glu Ala Pro Asn Tyr Ser Gly Arg Phe Thr Cys Ser Trp Leu Val Gln

130 135 140

Arg Asn Met Asp Leu Lys Phe Asn Ile Lys Ser Ser Ser Ser Ser Pro

145 150 155 160

Asp Ser Arg Ala Val Thr Cys Gly Met Ala Ser Leu Ser Ala Glu Lys

165 170 175

Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys Gln

180 185 190

Glu Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu Leu

195 200 205

Ala Leu Glu Ala Arg Gln Gln Asn Lys Tyr Glu Asn Tyr Ser Thr Ser

210 215 220

Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln

225 230 235 240

Met Lys Pro Leu Lys Asn Ser Gln Val Glu Val Ser Trp Glu Tyr Pro

245 250 255

Asp Ser Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Lys Phe Phe Val

260 265 270

Arg Ile Gln Arg Lys Lys Glu Lys Met Lys Glu Thr Glu Glu Gly Cys

275 280 285

Asn Gln Lys Gly Ala Phe Leu Val Glu Lys Thr Ser Thr Glu Val Gln

290 295 300

Cys Lys Gly Gly Asn Val Cys Val Gln Ala Gln Asp Arg Tyr Tyr Asn

305 310 315 320

Ser Ser Cys Ser Lys Trp Ala Cys Val Pro Cys Arg Val Arg Ser Gly

325 330 335

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys

340 345 350

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro

355 360 365

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser

370 375 380

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp

385 390 395 400

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn

405 410 415

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
420 425 430
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
435 440 445
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
450 455 460
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
465 470 475 480
Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp
485 490 495
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
500 505 510
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
515 520 525
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
530 535 540
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
545 550 555 560
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
565 570 575
Lys

<210> 23
<211> 792
<212> PRT
<213> 人工序列
<400> 23

Met Ala Gln Thr Val Arg Glu Cys Ser Leu Ala Leu Leu Phe Leu Phe
1 5 10 15
Met Trp Leu Leu Ile Lys Ala Asn Ile Asp Val Cys Lys Leu Gly Thr
20 25 30
Val Thr Val Gln Pro Ala Pro Val Ile Pro Leu Gly Ser Ala Ala Asn
35 40 45
Ile Ser Cys Ser Leu Asn Pro Lys Gln Gly Cys Ser His Tyr Pro Ser
50 55 60
Ser Asn Glu Leu Ile Leu Leu Lys Phe Val Asn Asp Val Leu Val Glu
65 70 75 80
Asn Leu His Gly Lys Lys Val His Asp His Thr Gly His Ser Ser Thr
85 90 95
Phe Gln Val Thr Asn Leu Ser Leu Gly Met Thr Leu Phe Val Cys Lys

100 105 110

Leu Asn Cys Ser Asn Ser Gln Lys Lys Pro Pro Val Pro Val Cys Gly
115 120 125

Val Glu Ile Ser Val Gly Val Ala Pro Glu Pro Pro Gln Asn Ile Ser
130 135 140

Cys Val Gln Glu Gly Glu Asn Gly Thr Val Ala Cys Ser Trp Asn Ser
145 150 155 160

Gly Lys Val Thr Tyr Leu Lys Thr Asn Tyr Thr Leu Gln Leu Ser Gly
165 170 175

Pro Asn Asn Leu Thr Cys Gln Lys Gln Cys Phe Ser Asp Asn Arg Gln
180 185 190

Asn Cys Asn Arg Leu Asp Leu Gly Ile Asn Leu Ser Pro Asp Leu Ala
195 200 205

Glu Ser Arg Phe Ile Val Arg Val Thr Ala Ile Asn Asp Leu Gly Asn
210 215 220

Ser Ser Ser Leu Pro His Thr Phe Thr Phe Leu Asp Ile Val Ile Pro
225 230 235 240

Leu Pro Pro Trp Asp Ile Arg Ile Asn Phe Leu Asn Ala Ser Gly Ser
245 250 255

Arg Gly Thr Leu Gln Trp Glu Asp Glu Gly Gln Val Val Leu Asn Gln
260 265 270

Leu Arg Tyr Gln Pro Leu Asn Ser Thr Ser Trp Asn Met Val Asn Ala
275 280 285

Thr Asn Ala Lys Gly Lys Tyr Asp Leu Arg Asp Leu Arg Pro Phe Thr
290 295 300

Glu Tyr Glu Phe Gln Ile Ser Ser Lys Leu His Leu Ser Gly Gly Ser
305 310 315 320

Trp Ser Asn Trp Ser Glu Ser Leu Arg Thr Arg Thr Pro Glu Glu Glu
325 330 335

Pro Gly Gly Gly Gly Ser Ser Gly Arg Ser Glu Asn Ile Arg Thr Ala
340 345 350

Gly Gly Gly Gly Ser Arg Val Ile Pro Val Ser Gly Pro Ala Arg Cys
355 360 365

Leu Ser Gln Ser Arg Asn Leu Leu Lys Thr Thr Asp Asp Met Val Lys
370 375 380

Thr Ala Arg Glu Lys Leu Lys His Tyr Ser Cys Thr Ala Glu Asp Ile
385 390 395 400

Asp His Glu Asp Ile Thr Arg Asp Gln Thr Ser Thr Leu Lys Thr Cys
405 410 415

Leu Pro Leu Glu Leu His Lys Asn Glu Ser Cys Leu Ala Thr Arg Glu
420 425 430
Thr Ser Ser Thr Thr Arg Gly Ser Cys Leu Pro Pro Gln Lys Thr Ser
435 440 445
Leu Met Met Thr Leu Cys Leu Gly Ser Ile Tyr Glu Asp Leu Lys Met
450 455 460
Tyr Gln Thr Glu Phe Gln Ala Ile Asn Ala Ala Leu Gln Asn His Asn
465 470 475 480
His Gln Gln Ile Ile Leu Asp Lys Gly Met Leu Val Ala Ile Asp Glu
485 490 495
Leu Met Gln Ser Leu Asn His Asn Gly Glu Thr Leu Arg Gln Lys Pro
500 505 510
Pro Val Gly Glu Ala Asp Pro Tyr Arg Val Lys Met Lys Leu Cys Ile
515 520 525
Leu Leu His Ala Phe Ser Thr Arg Val Val Thr Ile Asn Arg Val Met
530 535 540
Gly Tyr Leu Ser Ser Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
545 550 555 560
Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
565 570 575
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
580 585 590
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
595 600 605
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
610 615 620
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
625 630 635 640
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
645 650 655
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
660 665 670
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
675 680 685
Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
690 695 700
Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser
705 710 715 720
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr

725 730 735

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val

740 745 750

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

755 760 765

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

770 775 780

Ser Leu Ser Leu Ser Pro Gly Lys

785 790

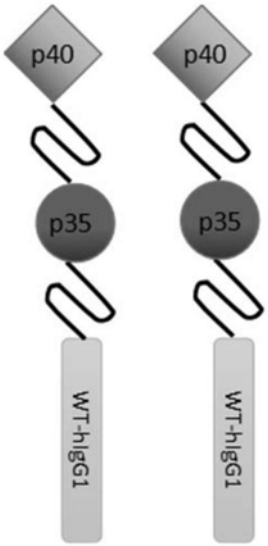


图1

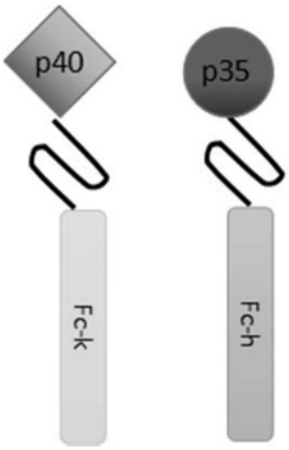


图2

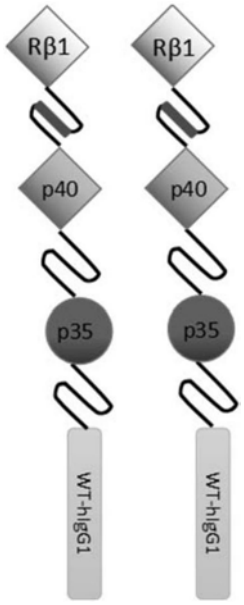

Rβ1
Rβ1
p40
p40
p35
p35
WT-hIgG1
WT-hIgG1

图3

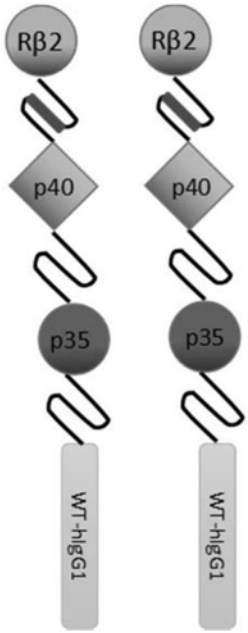

Rβ2
Rβ2
p40
p40
p35
p35
WT-hIgG1
WT-hIgG1

图4

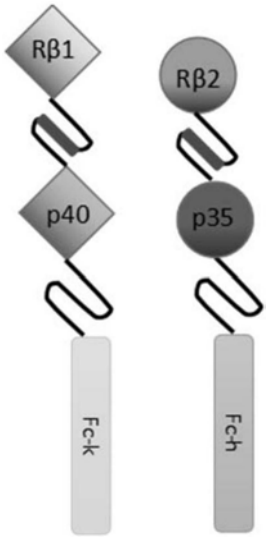


图5

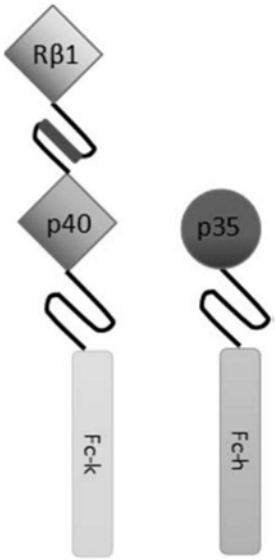


图6

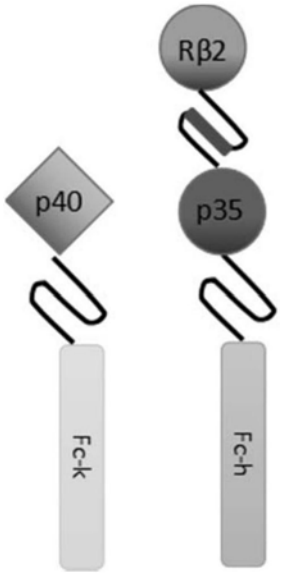


图7

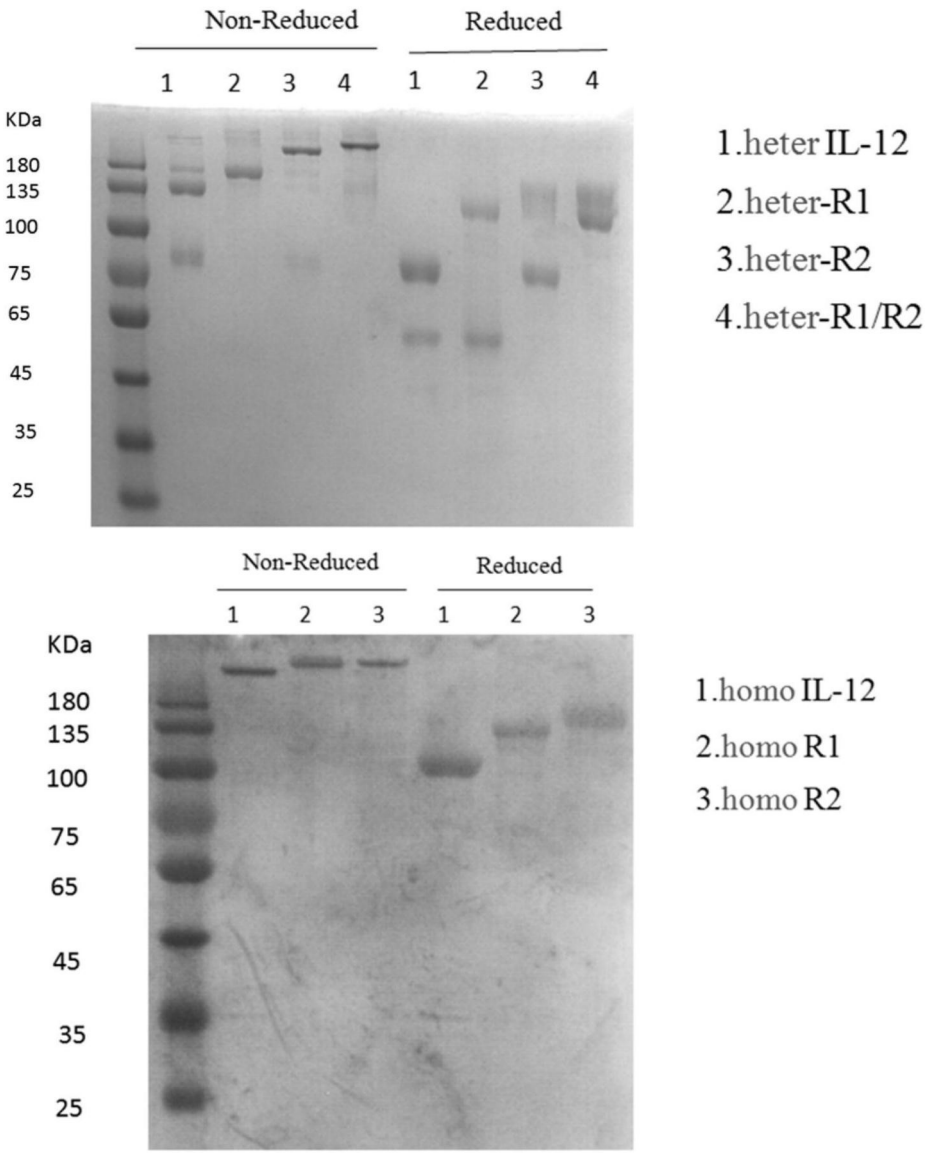
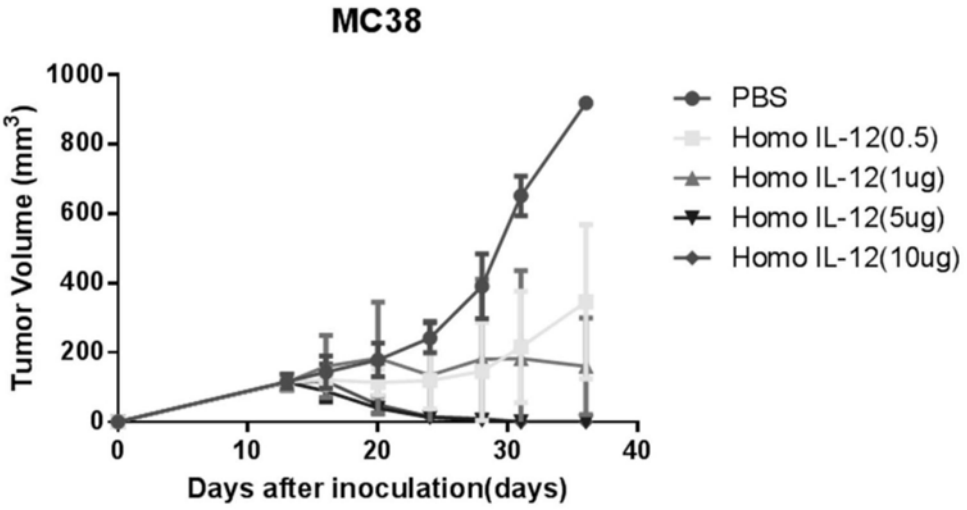

Non-Reduced
Reduced
1 2 3 4 1 2 3 4
KDa
180
135
100
75
65
45
35
25
1.heter IL-12
2.heter-R1
3.heter-R2
4.heter-R1/R2
Non-Reduced
Reduced
1 2 3 1 2 3
KDa
180
135
100
75
65
45
35
25
1.homo IL-12
2.homo R1
3.homo R2

图8

A


MC38
Tumor Volume (mm3)
1000
800
600
400
200
0
0
10
20
30
40
Days after inoculation(days)
PBS
Homo IL-12(0.5)
Homo IL-12(1ug)
Homo IL-12(5ug)
Homo IL-12(10ug)

B

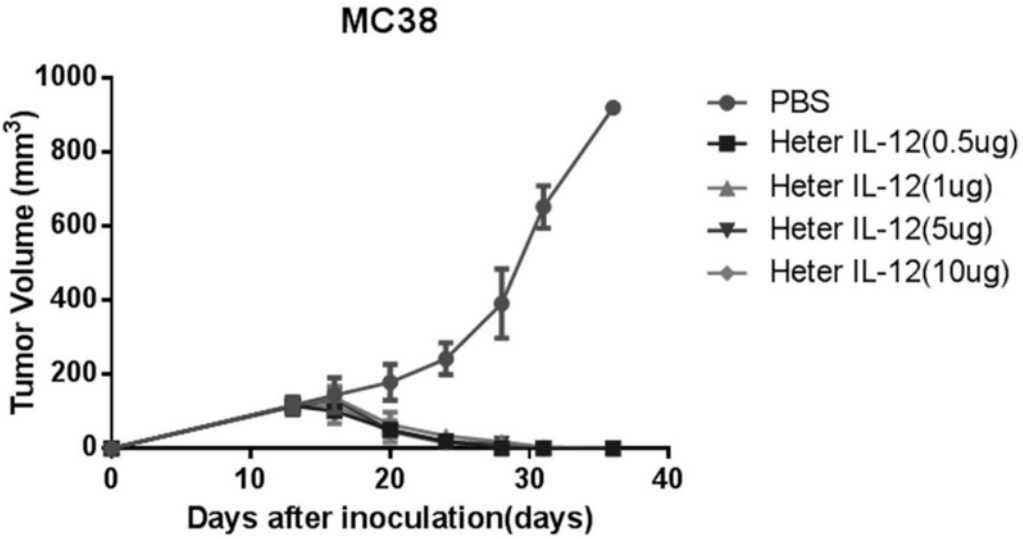
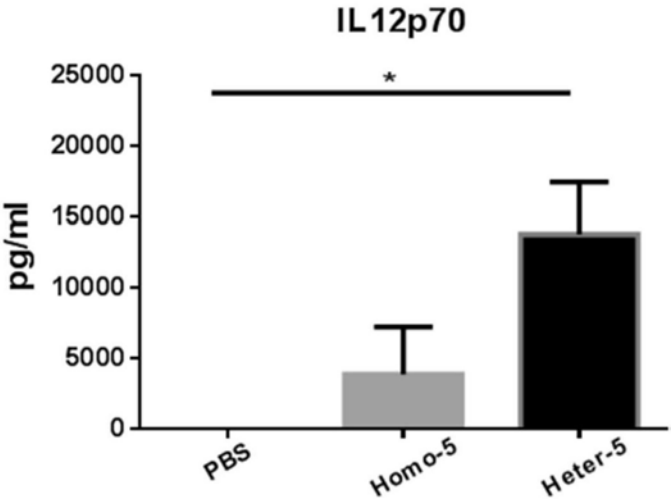
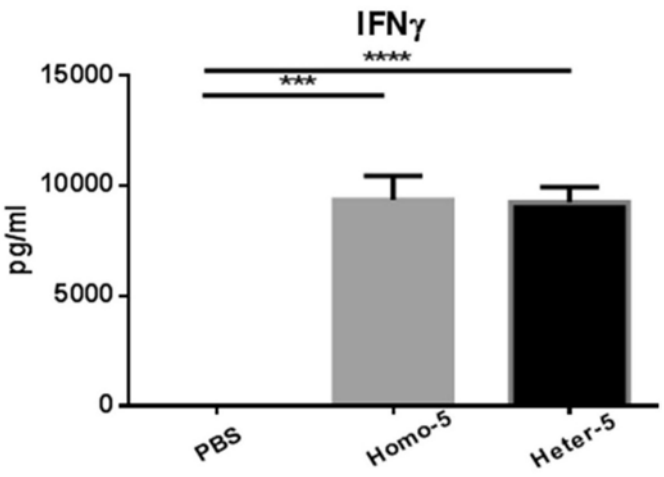

MC38
Tumor Volume (mm3)
1000
800
600
400
200
0
0
10
20
30
40
Days after inoculation(days)
PBS
Heter IL-12(0.5ug)
Heter IL-12(1ug)
Heter IL-12(5ug)
Heter IL-12(10ug)

图9

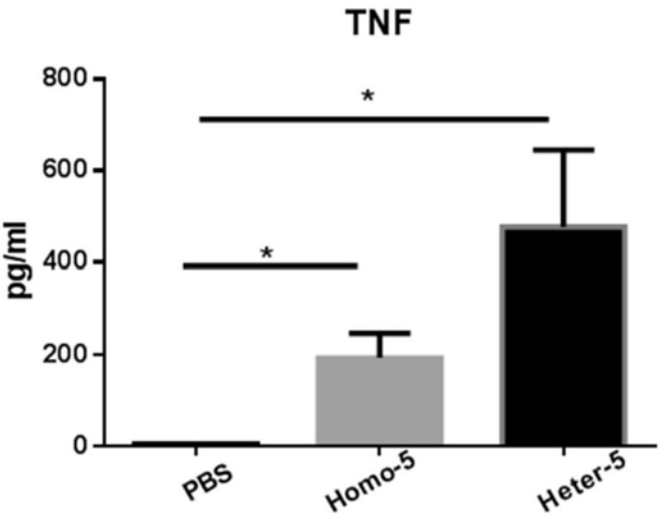
A


IL12p70
*
25000
20000
15000
10000
5000
0
pg/ml
PBS
Homo-5
Heter-5

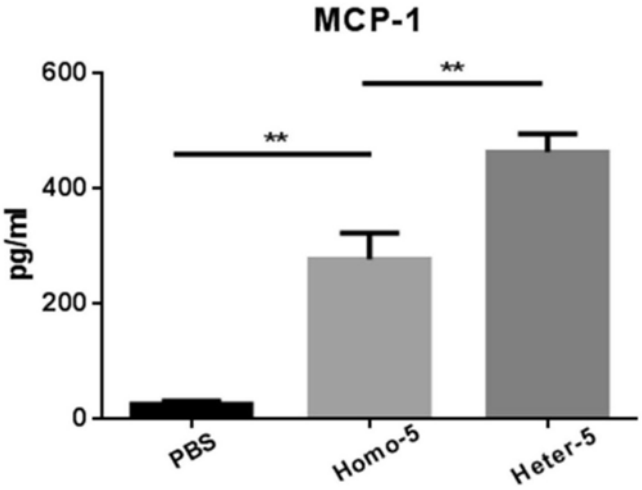
B


IFNγ
****
***
15000
10000
5000
0
pg/ml
PBS
Homo-5
Heter-5

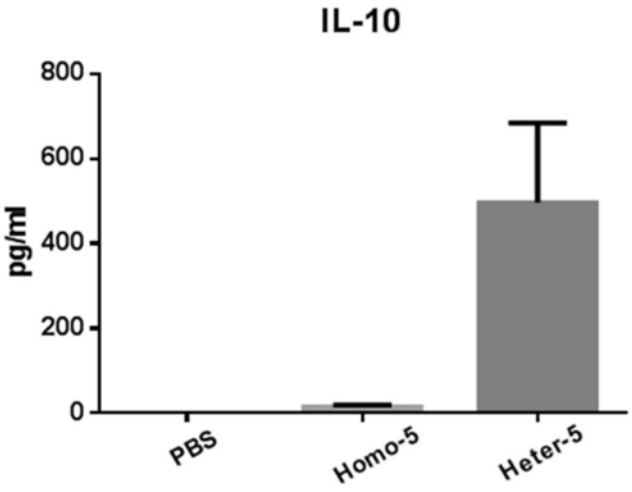
C


TNF
*
*
800
600
400
200
0
pg/ml
PBS
Homo-5
Heter-5

D


MCP-1
**
**
600
400
200
0
pg/ml
PBS
Homo-5
Heter-5

E


IL-10
800
600
400
200
0
pg/ml
PBS
Homo-5
Heter-5

F

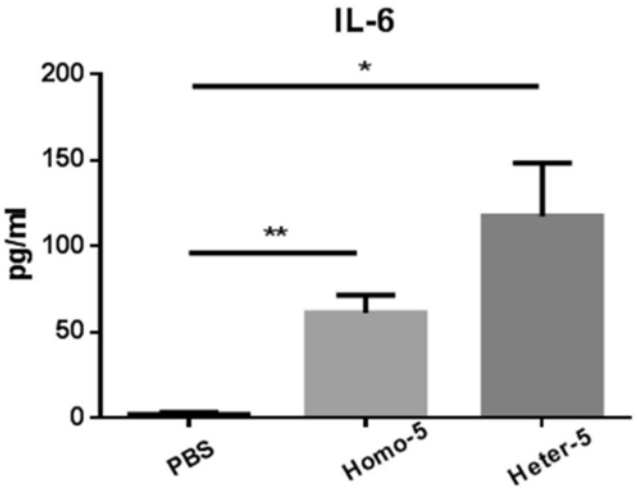
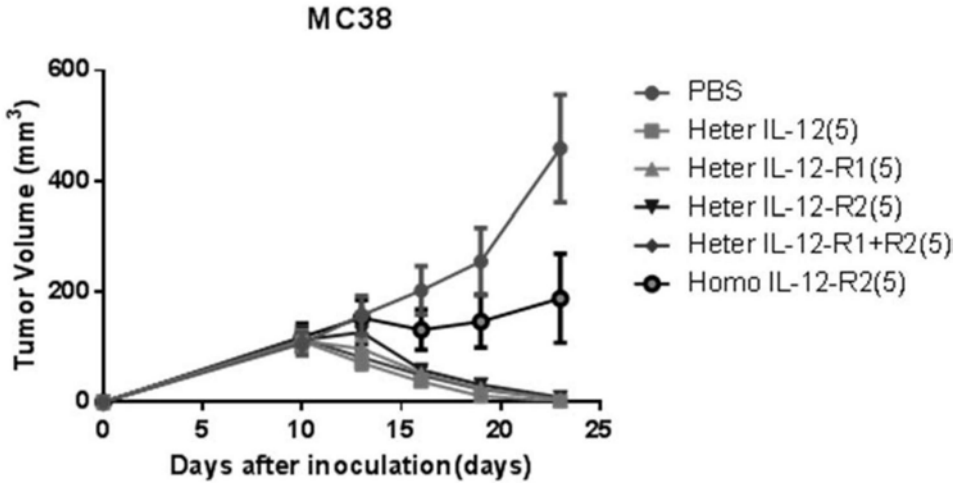

IL-6
200
150
100
50
0
pg/ml
*
**
PBS
Homo-5
Heter-5

图10

A


MC38
600
400
200
0
Tumor Volume (mm3)
0
5
10
15
20
25
Days after inoculation(days)
PBS
Heter IL-12(5)
Heter IL-12-R1(5)
Heter IL-12-R2(5)
Heter IL-12-R1+R2(5)
Homo IL-12-R2(5)

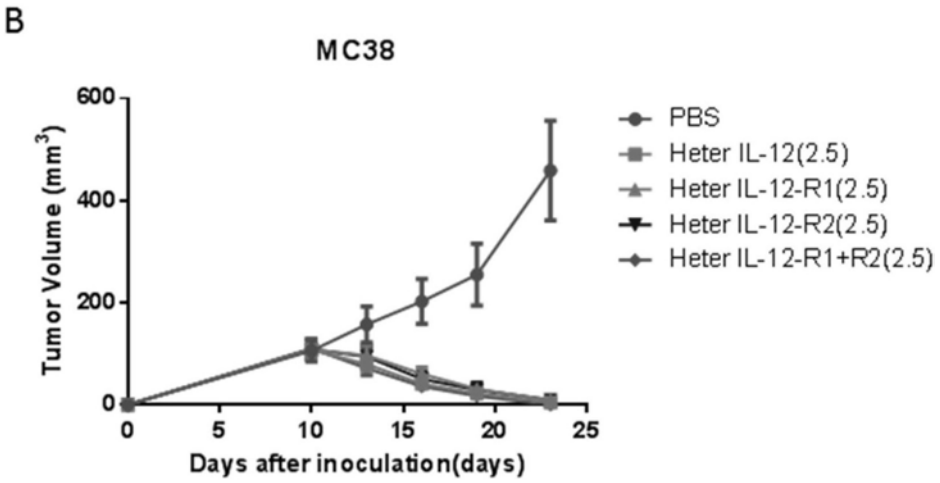
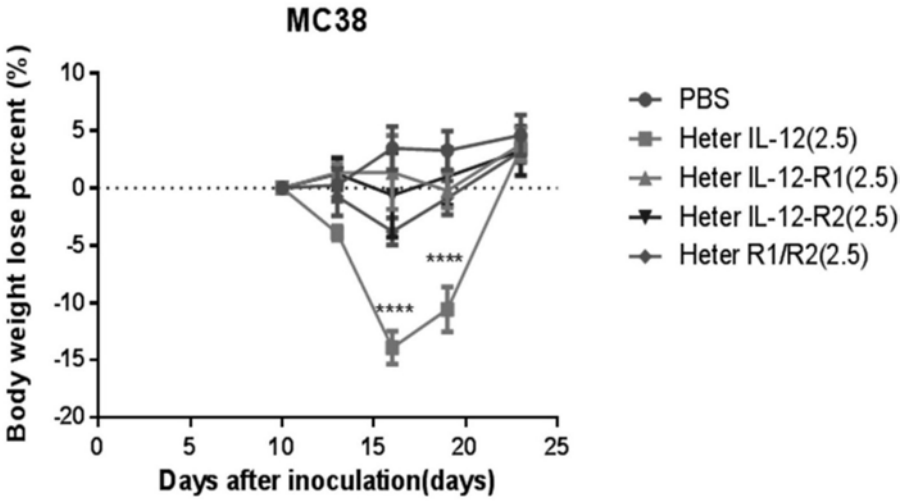
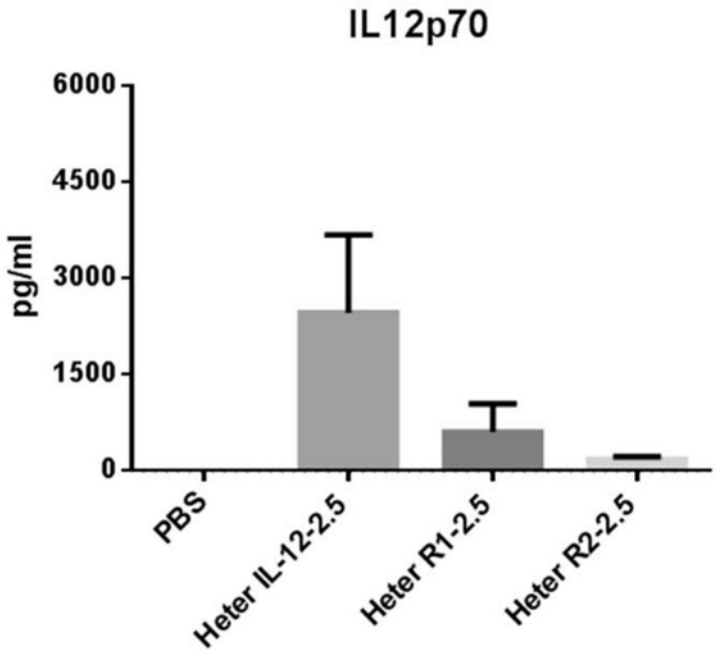

B
MC38
Tumor Volume (mm3)
600
400
200
0
0
5
10
15
20
25
Days after inoculation(days)
PBS
Heter IL-12(2.5)
Heter IL-12-R1(2.5)
Heter IL-12-R2(2.5)
Heter IL-12-R1+R2(2.5)

图11

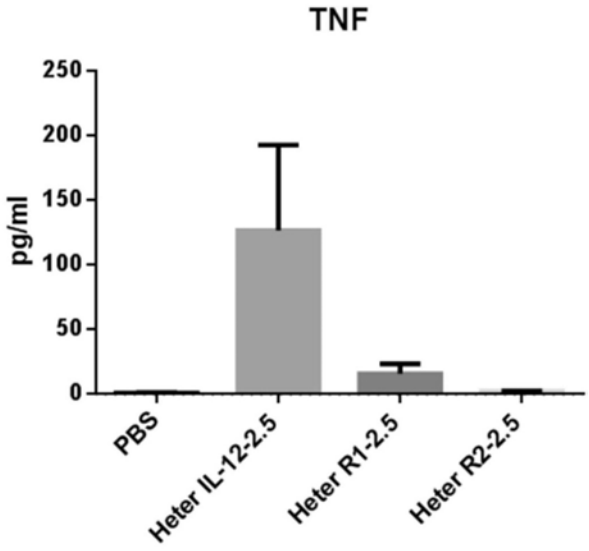
A


MC38
Body weight lose percent (%)
10
5
0
-5
-10
-15
-20
0
5
10
15
20
25
Days after inoculation(days)
****
****
PBS
Heter IL-12(2.5)
Heter IL-12-R1(2.5)
Heter IL-12-R2(2.5)
Heter R1/R2(2.5)

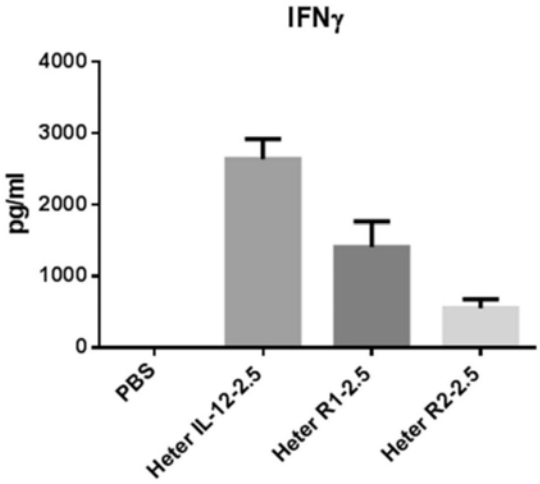
B


IL12p70
pg/ml
6000
4500
3000
1500
0
PBS
Heter IL-12-2.5
Heter R1-2.5
Heter R2-2.5

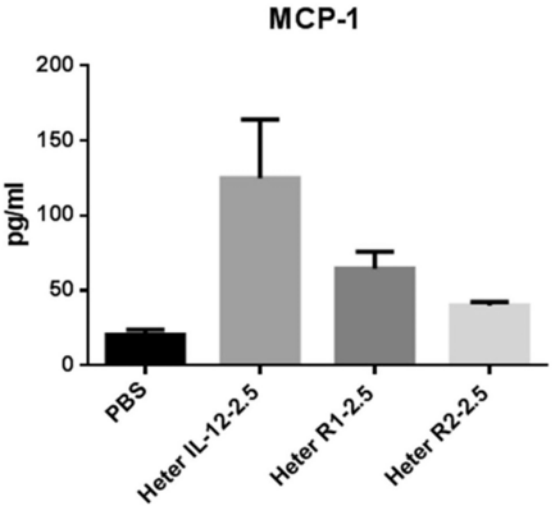
C


TNF
250
200
150
100
50
0
pg/ml
PBS
Heter IL-12-2.5
Heter R1-2.5
Heter R2-2.5

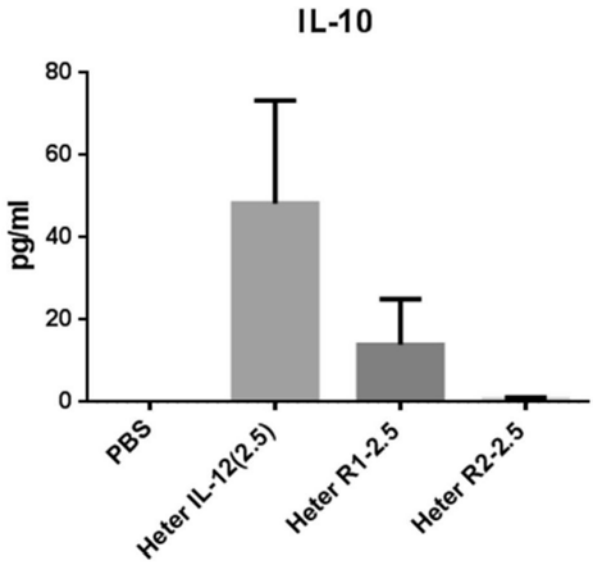
D


IFNγ
4000
3000
2000
1000
0
pg/ml
PBS
Heter IL-12-2.5
Heter R1-2.5
Heter R2-2.5

E


MCP-1
200
150
100
50
0
pg/ml
PBS
Heter IL-12-2.5
Heter R1-2.5
Heter R2-2.5

F


IL-10
pg/ml
80
60
40
20
0
PBS
Heter IL-12(2.5)
Heter R1-2.5
Heter R2-2.5

G

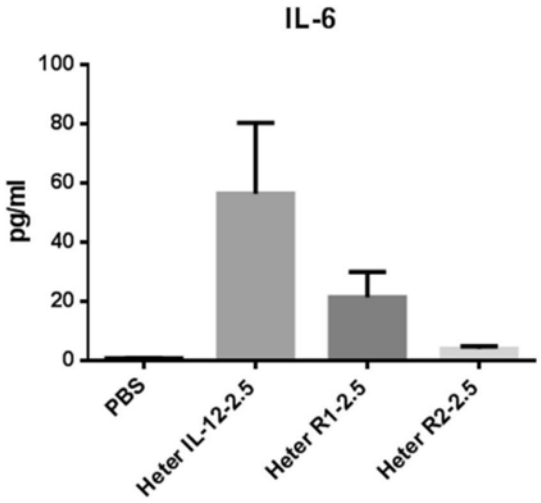

IL-6
pg/ml
100
80
60
40
20
0
PBS
Heter IL-12-2.5
Heter R1-2.5
Heter R2-2.5

图12