

MULTI-SPECIFIC BINDING PROTEINS FOR CANCER TREATMENT

BACKGROUND OF THE INVENTION

5 TECHNICAL FIELD

The present invention relates to multi-specific binding proteins comprising a first antigen binding unit specific for B7H6 (also referred to herein as “B7-H6”) and a second antigen binding unit specific for CD3. The invention also relates to nucleic acids encoding such binding proteins, to methods for preparing such binding proteins; host cells expressing or
10 capable of expressing such binding proteins, compositions comprising such binding proteins and to uses of such binding proteins or such compositions, in particular for therapeutic purposes in the field of cancer diseases.

BACKGROUND INFORMATION

15 B7H6 is a tumor-selective B7 family member that has been described to attract innate immunity to target cells and shares a similar function as other B7 family members with two Ig-like domains in the extracellular domain, an N-terminal IgV-like domain and a C-terminal IgC1-like domain. B7H6 triggers NKp30 mediated activation of human natural killer (NK) cells leading to degranulation and IFN γ secretion. (Brandt et al., J. Exp. Med. 2009 206(7); 1495-1503). Currently available data suggest a role of B7H6 in inflammatory responses to infectious conditions as well as in solid tumors.

B7H6 has been shown to be expressed on CD14⁺CD16⁺ cells isolated from the peripheral blood of sepsis patients as a result of the inflammatory process in this acute disease state. These findings have been confirmed by *in vitro* analysis of the upregulation of B7H6 on
25 the cell surface of CD14⁺CD16⁺ proinflammatory monocytes and neutrophils upon stimulation by IL-1 β and TNF α . (Matta et al., Blood 2013 122(3)), suggesting a role of B7H6 in inflammatory responses to sepsis conditions.

With the exception of the aforementioned sepsis conditions, B7H6 is otherwise selectively expressed in tumor cells and could not be detected in normal human tissues at steady state.
30 For example, expression of B7H6 has been described for T cell lymphoma, myeloid leukemia, colon carcinoma, breast cancer and ovarian carcinoma cell lines (Brandt et al., J. Exp. Med. 2009 206(7); 1495-1503; Li et al., J. Exp. Med. 2011 208(4); Greaves et al.,

Blood 2013 121(5); Zhang et al. Oncology Letters 2018 16:91-96), non-small cell lung cancer tissues (Zhang et al., Int J clin Exp Pathol 2014;7(10):6936-6942), gastro-intestinal tumor tissues (Chen et al., Pathol. Oncol. Res.2014 20:203-207; Zhao et al., Cell Proliferation 2018; e12468), ovarian carcinoma tissues (Zhou et al., Int clin Exp Pathol 5 2015 8(8), oral squamous carcinoma tissues (Wang et al., J Oral Pathol Med. 2017;46:766-772), and hepatocellular carcinoma tissues (Li et al., Int. J. Mol. Sci. 2019, 20, 156), however, the function of B7H6 in tumors is not fully understood.

Therapeutic applications including the treatment of cancer using anti-B7H6 antibodies which engage the ADCC/CDC pathway or anti-B7H6 antibody-drug conjugates are 10 described in WO2009/046407A2 and WO2011/07044A2.

However, B7H6 targeted therapy based on ADCC/CDC activity is not an optimal mode of action because of the low cell surface expression of B7H6 and low success rates using conventional antibodies with ADCC/CDC activity in solid tumors.

Targeted therapy based on B7H6-specific antibody drug conjugates (ADCs) might have 15 limitations as well, since the majority of patients relapse after chemotherapy treatment and due to the low expression of B7H6 on the cell surface. In addition, ADC approaches often have off-target toxicities caused by free drug as a result of linker instability or degradation.

CAR-T-cells and T cell engaging antibodies are further approaches for targeted therapy of B7H6-expressing solid tumors (Wu et al., Gene 2015 22, 675-684; Hua et al. Protein 20 Engineering, Design & Selection 201730(10), 713-721; WO2017/181001). For example, 20 Wu et al. (J Immunol. 2015 Jun 1;194(11):5305-11) describes preclinical data with a B7H6-specific-BiTE, BiTE standing for Bi-specific T cell Engager which is an approximately 55 Kda fusion protein consisting of two single-chain variable fragments (scFvs)). In that case the B7H6-specific-BiTE was engineered based on the OKT3-CD3- 25 binder and a previously published B7H6 antibody (Zhang et al., J Immunol. 2012 Sep 1;189(5):2290-9; WO 2013/169691). However, the OKT3 antibody is not cross-reactive with cynomolgus monkey CD3 and therefore does not allow preclinical toxicological testing in cynomolgus monkeys which is the preferred testing species for preparation of clinical trials (Chatenoud et al., The Rev Diabet Stud 2012;9(4):372-381). An additional 30 challenge is the short half-life of the relatively small, easily degraded BiTE molecules, which requires continuous intravenous dosing in the clinic. Therefore, it is unproven whether this approach will be successful. To date, no targeted therapies for B7-H6-

expressing tumors are available, and there remains an unmet need unaddressed by current approaches.

For example, colorectal cancer (CRC) shows a high prevalence and predictable expression of B7-H6. It is one of the leading causes of cancer morbidity and mortality worldwide.

5 Approximately 25 % of CRC patients initially present with overt metastasis and metastatic disease develops in 40 – 50 % of newly diagnosed patients. Although recent improvements in chemotherapy and targeted therapies have extended survival durations of metastatic CRC, most patients will succumb to their disease.

In view of the poor outlook for cancer patients with advanced disease, there is a need to
10 identify more efficacious therapies, particularly efficacious therapies with improved tolerability.

Thus, it is an object of the invention to provide pharmacologically active agents, compositions and/or methods of treatment that provide certain advantages compared to the agents, compositions and/or methods currently used and/or known in the art. These
15 advantages include improved therapeutic and pharmacological properties, such as *in vivo* efficacy, less side effects, reduced immunogenicity, improved therapeutic window, reduced administration (e.g. infusion) times, lower dosage, extended half-life to allow less frequent dosing and other advantageous properties such as improved ease of preparation, stability, compatibility with conventional antibody processes or reduced costs of goods,
20 especially as compared to candidate drugs already known in the art.

BRIEF SUMMARY OF THE INVENTION

The present invention is based on a bispecific T cell engaging approach employing multi-specific binding proteins with a binding arm to CD3, which is expressed on T cells, and a
25 binding arm to B7H6, which is expressed on the cell surface of tumor cells. Through simultaneously binding to T cells and tumors cells, the T cell engagers of the present invention force the formation of a cytolytic synapse between the two cells and thereby redirect the T cell activity selectively to the targeted tumor cells.

In one aspect, the invention provides a multi-specific binding protein comprising a first
30 antigen binding unit specifically binding to B7H6 and a second antigen binding unit specifically binding to CD3, wherein said first antigen binding unit specifically binding to B7H6 is selected from the group consisting of i) to xxiv):

- i) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:1 (CDR1), SEQ ID NO:2 (CDR2) and SEQ ID NO:3 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:4 (CDR1), SEQ ID NO:5 (CDR2) and SEQ ID NO:6 (CDR3);
- 5 ii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:7 (CDR1), SEQ ID NO:8 (CDR2) and SEQ ID NO:9 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:10 (CDR1), SEQ ID NO:11 (CDR2) and SEQ ID NO:12 (CDR3);
- 10 iii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:13 (CDR1), SEQ ID NO:14 (CDR2) and SEQ ID NO:15 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:16 (CDR1), SEQ ID NO:17 (CDR2) and SEQ ID NO:18 (CDR3);
- 15 iv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:19 (CDR1), SEQ ID NO:20 (CDR2) and SEQ ID NO:21 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:22 (CDR1), SEQ ID NO:23 (CDR2) and SEQ ID NO:24 (CDR3);
- 20 v) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:25 (CDR1), SEQ ID NO:26 (CDR2) and SEQ ID NO:27 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:28 (CDR1), SEQ ID NO:29 (CDR2) and SEQ ID NO:30 (CDR3);
- vi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:31 (CDR1), SEQ ID NO:32 (CDR2) and SEQ ID NO:33 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:34 (CDR1), SEQ ID NO:35 (CDR2) and SEQ ID NO:36 (CDR3);
- 25 vii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:37 (CDR1), SEQ ID NO:38 (CDR2) and SEQ ID NO:39 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:40 (CDR1), SEQ ID NO:41 (CDR2) and SEQ ID NO:42 (CDR3);
- 30 viii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:43 (CDR1), SEQ ID NO:44 (CDR2) and SEQ ID NO:45

- (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:46 (CDR1), SEQ ID NO:47 (CDR2) and SEQ ID NO:48 (CDR3);
- ix) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:49 (CDR1), SEQ ID NO:50 (CDR2) and SEQ ID NO:51 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:52 (CDR1), SEQ ID NO:53 (CDR2) and SEQ ID NO:54 (CDR3);
- x) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:55 (CDR1), SEQ ID NO:56 (CDR2) and SEQ ID NO:57 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:58 (CDR1), SEQ ID NO:59 (CDR2) and SEQ ID NO:60 (CDR3);
- xi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:61 (CDR1), SEQ ID NO:62 (CDR2) and SEQ ID NO:63 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:64 (CDR1), SEQ ID NO:65 (CDR2) and SEQ ID NO:66 (CDR3);
- xii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:67 (CDR1), SEQ ID NO:68 (CDR2) and SEQ ID NO:69 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:70 (CDR1), SEQ ID NO:71 (CDR2) and SEQ ID NO:72 (CDR3);
- xiii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:73 (CDR1), SEQ ID NO:74 (CDR2) and SEQ ID NO:75 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:76 (CDR1), SEQ ID NO:77 (CDR2) and SEQ ID NO:78 (CDR3);
- xiv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3);
- xv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3);

- xvi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3);
- 5 xvii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:97 (CDR1), SEQ ID NO:98(CDR2) and SEQ ID NO:99 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:100 (CDR1), SEQ ID NO:101 (CDR2) and SEQ ID NO:102 (CDR3);
- 10 xviii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:103 (CDR1), SEQ ID NO:104 (CDR2) and SEQ ID NO:105 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:106 (CDR1), SEQ ID NO:107 (CDR2) and SEQ ID NO:108 (CDR3);
- 15 xix) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:109 (CDR1), SEQ ID NO:110 (CDR2) and SEQ ID NO:111 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:112 (CDR1), SEQ ID NO:113 (CDR2) and SEQ ID NO:114 (CDR3);
- 20 xx) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:115 (CDR1), SEQ ID NO:116 (CDR2) and SEQ ID NO:117 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:118 (CDR1), SEQ ID NO:119 (CDR2) and SEQ ID NO:120 (CDR3);
- 25 xxi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:121 (CDR1), SEQ ID NO:122 (CDR2) and SEQ ID NO:123 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:124 (CDR1), SEQ ID NO:125 (CDR2) and SEQ ID NO:126 (CDR3);
- 30 xxii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:127 (CDR1), SEQ ID NO:128 (CDR2) and SEQ ID NO:129 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:130 (CDR1), SEQ ID NO:131 (CDR2) and SEQ ID NO:132 (CDR3);
- 30 xxiii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:133 (CDR1), SEQ ID NO:134 (CDR2) and SEQ ID NO:135 (CDR3) and heavy chain CDRs comprising the amino acid sequences of

SEQ ID NO:136 (CDR1), SEQ ID NO:137 (CDR2) and SEQ ID NO:138 (CDR3);
and

- xxiv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:139 (CDR1), SEQ ID NO:140 (CDR2) and SEQ ID NO:141 (CDR3) and heavy chain CDRs comprising the amino acid sequences of
5 SEQ ID NO:142 (CDR1), SEQ ID NO:143 (CDR2) and SEQ ID NO:144 (CDR3).

In some embodiments of the binding protein of the invention, the first antigen binding unit specifically binding to B7H6 is selected from the group consisting of i) to xxiv):

- i) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:145 and heavy chain variable domain comprising the amino acid sequence of
10 SEQ ID NO:146;
- ii) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:147 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:148;
- 15 iii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:149 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:150;
- iv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:151 and heavy chain variable domain comprising the amino acid sequence of
20 SEQ ID NO:152;
- v) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:153 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:154;
- vi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:155 and heavy chain variable domain comprising the amino acid sequence of
25 SEQ ID NO:156;
- vii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:157 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:158;

- viii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:159 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:160;
- 5 ix) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:161 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:162;
- x) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:163 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:164;
- 10 xi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:165 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:166;
- xii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:167 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168;
- 15 xiii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:169 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:170;
- xiv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:171 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172;
- 20 xv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:173 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:174;
- xvi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:175 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:176;
- 25 xvii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:177 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:178;
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- xviii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:179 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:180;
- xix) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:181 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:182;
- xx) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:183 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:184;
- xxi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:185 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:186;
- xxii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:187 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:188;
- xxiii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:189 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:190; and
- xxiv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:191 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:192.

In some embodiments of the binding protein of the invention, the second antigen binding unit specifically binding to CD3 is selected from the group consisting of i)-vi):

- i) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3);
- ii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:263 (CDR1), SEQ ID NO:264 (CDR2) and SEQ ID NO:265 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:266 (CDR1), SEQ ID NO:267 (CDR2) and SEQ ID NO:268 (CDR3);

- iii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:269 (CDR1), SEQ ID NO:270 (CDR2) and SEQ ID NO:271 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:272 (CDR1), SEQ ID NO:273 (CDR2) and SEQ ID NO:274 (CDR3);
- 5 iv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:275 (CDR1), SEQ ID NO:276 (CDR2) and SEQ ID NO:277 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:278 (CDR1), SEQ ID NO:279 (CDR2) and SEQ ID NO:280 (CDR3);
- v) an antigen binding unit comprising light chain CDRs comprising the amino acid
10 sequences of SEQ ID NO:281 (CDR1), SEQ ID NO:282 (CDR2) and SEQ ID NO:283 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:284 (CDR1), SEQ ID NO:285 (CDR2) and SEQ ID NO:286 (CDR3);
and
- vi) an antigen binding unit comprising light chain CDRs comprising the amino acid
15 sequences of SEQ ID NO:287 (CDR1), SEQ ID NO:288 (CDR2) and SEQ ID NO:289 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:290 (CDR1), SEQ ID NO:291 (CDR2) and SEQ ID NO:292 (CDR3).

In some embodiments of the binding protein of the invention, the second antigen binding unit specifically binding to CD3 is selected from the group consisting of i) to vi):

- 20 i) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294;
- ii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:295 and a heavy chain variable domain comprising the amino acid sequence of
25 SEQ ID NO:296;
- iii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:297 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:298;
- iv) a light chain variable domain comprising the amino acid sequence of SEQ ID
30 NO:299 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:300;

- v) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:301 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:302; and
- vi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:303 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:304.

In some embodiments of the binding protein of the invention, the first antigen binding unit specifically binding to B7H6 comprises from its N- to C-terminus a first light chain variable domain, a first light chain constant domain, a first peptide linker, a first heavy chain variable domain and a first heavy chain constant CH1 domain; and the second antigen binding unit specifically binding to CD3 comprises from its N- to C-terminus a second light chain variable domain, a second light chain constant domain, a second peptide linker, a second heavy chain variable domain and a second heavy chain constant CH1 domain. In some embodiments of the binding protein of the invention, the first and/or second peptide linker comprises 26 to 42 amino acids, preferably any one of 30 to 40 amino acids, 34 to 40 amino acids, or 36 to 39 amino acids, more preferably 38 amino acids. In some embodiments of the invention, the first linker and/or second linker is a Gly-Ser linker, preferably comprising the amino acid sequence of SEQ ID NO:250, more preferably said first and second peptide linker comprise the same sequence (e.g. SEQ ID NO:250). In some embodiments of the invention, the first light chain constant domain and the second light chain constant domain comprise independently a human kappa or lambda domain.

In some embodiments, the first antigen binding unit specific for B7H6 of the binding protein of the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196 SEQ ID NO:197 SEQ ID NO:198 SEQ ID NO:199 SEQ ID NO:200 SEQ ID NO:201 SEQ ID NO:202 SEQ ID NO:203 SEQ ID NO:204 SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, and SEQ ID NO:216 and the second antigen binding unit specific for CD3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:305, SEQ ID NO:306, SEQ ID NO:307, SEQ ID NO:308, SEQ ID NO:309, and SEQ ID NO:310, preferably SEQ ID NO:305.

In some embodiments, the binding protein of the invention further comprises a first and a second Fc domain, wherein said first Fc domain is covalently linked to said first antigen binding unit, preferably to the C-terminus of said first antigen binding unit, and said second Fc domain is covalently linked to said second antigen binding unit, preferably to the C-terminus of said second antigen binding unit.

In some embodiments of the invention,

- i) the first Fc domain comprises a tyrosine (Y) at position 366 [T366Y], and the second Fc domain comprises a threonine (T) at position 407 [Y407T], or
- ii) the first Fc domain comprises a tryptophan (W) at position 366 [T366W], and the second Fc domain comprises a serine (S) at position 366 [T366S], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V], or
- iii) the second Fc domain comprises a tyrosine (Y) at position 366 [T366Y], and the first Fc domain comprises a threonine (T) at position 407 [Y407T], or
- iv) the second Fc domain comprises a tryptophan (W) at position 366 [T366W], and the first Fc domain comprises a serine (S) at position 366 [T366S], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V],

preferably wherein the first or the second Fc domain further comprises an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F]. In some embodiments, the first and/or second Fc domain comprises an alanine at position 234 [L234A] and at position 235 [L235A].

In some embodiments, the binding protein of the invention comprises a first polypeptide chain specifically binding to B7H6 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO: 224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, and SEQ ID NO:240 and a second polypeptide chain specifically binding to CD3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:311, SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, and SEQ ID NO:316, preferably SEQ ID NO:311.

In a further aspect, the invention provides an isolated nucleic acid molecule i) encoding a first antigen binding unit and/or a second antigen binding unit of a binding protein of the invention, optionally further encoding a first and/or a second Fc domain, or ii) encoding the first and/or the second polypeptide chain of binding protein of the invention. In further
5 aspects provided herein are expression vectors comprising the nucleic acid molecule of the invention, host cells transfected with such expression vectors, and methods of manufacturing a protein of the invention.

In a further aspect of the invention, provided herein is a multi-specific binding protein comprising a first polypeptide chain specifically binding to B7H6 and a second polypeptide
10 chain specifically binding to CD3, where the first polypeptide chain comprises a first light chain, a first linker, and a first heavy chain and the second polypeptide chain comprises a second light chain, a second linker, and a second heavy chain, preferably the C-terminus of the first light chain is covalently bound to the N-terminus of the first heavy chain via the first peptide linker and the C-terminus of the second light chain is covalently bound to the N-
15 terminus of the second heavy chain via the second peptide linker. The skilled person would understand that any reference herein to a "light chain" or "heavy chain" refers to an antibody light chain or antibody heavy chain, respectively.

In some embodiments of the protein of the invention, the first polypeptide chain specifically binding to B7H6 comprises a light chain variable and heavy chain variable domain
20 comprising CDR sequences, VH/VL sequences and/or single chain Fab sequences as defined for the antigen binding units of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23 and B7H6#24 described herein. In some
25 embodiments, the second polypeptide chain specifically binding to CD3 comprises a light chain variable and heavy chain variable domain comprising CDR sequences, VH/VL sequences and/or scFab sequences as defined for the antigen binding units of CD3#1, as described herein.

Further aspects, embodiments, uses and methods involving the binding proteins of the invention will become clear from the following detailed description of the invention and
30 from the appended claims.

The invention provides for novel binding proteins that allow a more efficient treatment of B7H6 expressing cancers, such as (metastatic) colorectal cancer ((m)CRC), non-small cell lung cancer (NSCLC), or head and neck squamous cell carcinoma (HNSCC).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Schematic representation of a bispecific binding protein of the invention

Figure 2: Schematic representation of the extracellular B7H6 protein expressed on the cell surface of CHO-K1 cells.

Figure 3: Binding of 34 exemplary B7H6/CD3 binding proteins to recombinant human B7H6 extracellular protein.

Figure 4: Binding of 34 exemplary B7H6/CD3 binding proteins to recombinant human Alanine-mutated B7H6 extracellular protein.

Figure 5: Binding of 34 exemplary B7H6/CD3 binding proteins to HCT-15 cells expressing endogenous human B7H6.

Figure 6: Binding of 23 exemplary B7H6/CD3 binding proteins to recombinant CHO-K1 cells expressing cynomolgus monkey B7H6.

Figure 7: Binding of 23 exemplary B7H6/CD3 binding proteins to human T-cells expressing CD3.

Figure 8: Binding of 23 exemplary B7H6/CD3 binding proteins to B7H6-negative CHO-K1 cells.

Figure 9: Inhibitory activity of 17 exemplary B7H6/CD3 binding proteins of B7H6-dependent IFN γ secretion by NK-92MI cells.

Figure 10: Potency in lysing target cells of 11 exemplary B7H6/CD3 binding proteins redirecting non-stimulated T-cells towards human HCT-15 cells.

Figure 11: Potency in lysing target cells of 23 exemplary B7H6/CD3 binding proteins redirecting non-stimulated T-cells towards human HCT-15 cells.

Figure 12: Potency in lysing target cells of an exemplary B7H6/CD3 binding protein at various effector to target (E:T) cell ratios.

Figure 13: Potency in lysis cells of 23 exemplary B7H6/CD3 binding proteins redirecting non-stimulated T-cells towards recombinant CHO cells transfected with B7-H6 and Cho wt cells.

Figure 14: Potency in upregulation of CD25 expression on T-cells in presence of HCT-15
5 cells of 6 exemplary B7H6/CD3 binding proteins.

Figure 15: Potency in upregulation of intracellular Perforin expression in T-cells in presence of HCT-15 cells of 6 exemplary B7H6/CD3 binding proteins.

Figure 16: Potency in upregulation of intracellular Granzyme B expression in T-cells in presence of HCT-15 cells of 6 exemplary B7H6/CD3 binding proteins.

Figure 17: Potency in proliferation of T-cells in presence of HCT-15 cells of 6 exemplary
10 B7H6/CD3 binding proteins.

Figure 18: Potency in secretion of IFN γ by T-cells in presence of HCT-15 cells of 5 exemplary B7H6/CD3 binding proteins.

Figure 19: Pharmacokinetic profile of one exemplary B7H6/CD3 binding protein.

Figure 20: Anti-tumor activity of one exemplary B7H6/CD3 binding protein in a T-cell
15 engrafted mouse xenograft model.

Figure 21: T cell infiltration in NCI-H716 xenograft tumor tissue with an exemplary B7H6/CD3 binding protein.

Figure 22: Pharmacokinetic profile of four exemplary B7H6 binding protein.

Figure 23: Anti-tumor activity of four exemplary B7H6/CD3 binding proteins in a T-cell
20 engrafted mouse xenograft model.

Figure 24: Anti-tumor activity of an exemplary B7H6/CD3 binding protein in a T-cell engrafted mouse xenograft model administered q7d or as one single dose.

25 DETAILED DESCRIPTION OF THE INVENTION

USED TERMS AND DEFINITIONS

The above and other aspects and embodiments of the invention will become clear from the further description herein, in which:

30 Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Sambrook et al, "Molecular Cloning: A Laboratory Manual" (2nd Ed.),

VoIs. 1-3, Cold Spring Harbor Laboratory Press (1989); Lewin, "Genes IV", Oxford University Press, New York, (1990), and Roitt *et al.*, "Immunology" (2nd Ed.), Gower Medical Publishing, London, New York (1989), as well as to the general background art cited herein. Furthermore, unless indicated otherwise, all methods, steps, techniques and
5 manipulations that are not specifically described in detail can be performed and have been performed in a manner known *per se*, as will be clear to the skilled person. Reference is for example again made to the standard handbooks, to the general background art referred to above and to the further references cited therein.

When used herein the term "comprising" and variations thereof such as "comprises" and
10 "comprise" can be substituted with the term "containing" or "including" or "having."

The term "sequence" as used herein (for example in terms like "heavy/light chain sequence", "antibody sequence", "variable domain sequence", "constant domain sequence" or "protein sequence"), should generally be understood to include both the relevant amino acid sequence as well as nucleic acid sequences or nucleotide sequences encoding the
15 same, unless the context requires a more limited interpretation.

The term "antigen binding unit" as used herein comprises the minimal structural requirements derived from an antibody (i.e., the minimal structural requirements typically present in an antibody) which allow for binding to its specific target or antigen. Thus, an antigen binding unit comprises at least the three light chain and three heavy chain CDR
20 sequences; preferably it comprises at least a light chain variable domain and a heavy chain variable domain.

The generalized structure of an antibody or immunoglobulin is well known to those of skill in the art. These molecules are heterotetrameric glycoproteins, typically of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains and
25 are typically referred to as full length antibodies. Each light chain is covalently linked to a heavy chain by one disulfide bond to form a heterodimer, and the heterotetrameric molecule is formed through a covalent disulfide linkage between the two identical heavy chains of the heterodimers. Although the light and heavy chains are linked together by one disulfide bond, the number of disulfide linkages between the two heavy chains varies by immunoglobulin
30 isotype. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at the N-terminus a variable domain (VH), followed by three or four (in case of IgE) constant domains (CH1, CH2, CH3, and CH4), as well as a hinge region

between CH1 and CH2. Each light chain has two domains, an N-terminal variable domain (VL) and a C-terminal constant domain (CL). The VL domain associates non-covalently with the VH domain, whereas the CL domain is commonly covalently linked to the CH1 domain via a disulfide bond. Particular amino acid residues are believed to form an interface
5 between the light and heavy chain variable domains (Chothia et al., 1985, J. Mol. Biol. 186:651-663). Variable domains are also referred to herein as variable regions or Fv and denote the part that confers specificity to an antibody for the antigen by carrying the antigen-binding site.

The "light chain variable domain" (or "light chain variable region") and "heavy chain
10 variable domain" (or "heavy chain variable region") as used herein have the same general structure and each domain essentially consists of four framework (FR) regions whose sequences are widely conserved, which are referred to in the art and herein below as "framework region 1" or "FR1"; as "framework region 2" or "FR2"; as "framework region 3" or "FR3"; and as "framework region 4" or "FR4", respectively; which framework
15 regions are interrupted by three hypervariable regions, HVRs (or CDRs), which are referred to in the art and herein below as "complementarity determining region 1" or "CDR1"; as "complementarity determining region 2" or "CDR2"; and as "complementarity determining region 3" or "CDR3", respectively. Thus, the general structure or sequence of an immunoglobulin variable domain can be indicated as follows: FR1 - CDR1 - FR2 -
20 CDR2 - FR3 - CDR3 - FR4. The framework regions adopt a beta-sheet conformation and the CDRs may form loops connecting the beta-sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site.

Various definitions of CDRs are known in the art, for example, the definition based on CCG, also referred to as IMGT (Lefranc MP, Pommié C, Ruiz M, Giudicelli V, Foulquier E, Truong L, Thouvenin-Contet V, Lefranc G. "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains." Dev Comp Immunol. 2003 Jan;27(1):55-77; Giudicelli V, Brochet X, Lefranc MP. "IMGT/V-QUEST: IMGT standardized analysis of the immunoglobulin (IG) and T cell receptor (TR) nucleotide
30 sequences". Cold Spring Harb Protoc. 2011;2011(6):695-715) or the definition based on Chothia (Chothia and Lesk, J. Mol. Biol. 1987, 196: 901-917), together with Kabat (E.A. Kabat, T.T. Wu, H. Bilofsky, M. Reid-Miller and H. Perry, Sequence of Proteins of

Immunological Interest, National Institutes of Health, Bethesda (1983)). Within the context of this invention, reference to CDR's is based on the definition of CCG (IMGT).

The term "constant domains" or "constant region" as used within the current application denotes the sum of the domains of an antibody other than the variable region. Such
5 constant domains and regions are well known in the state of the art and e.g. described by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91-3242 (1991)). Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins are divided in the classes: IgA, IgD, IgE, IgG and IgM. According to the heavy chain constant
10 regions the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ respectively. Several of these may be further divided into subclasses (isotypes), e.g. IgG1, IgG2, IgG3, and IgG4, IgA1, and IgA2.

The "Fc part" or "Fc domain" of an antibody is not involved directly in binding of an antibody to an antigen, but exhibits various effector functions. An "Fc part/domain of an
15 antibody" is a term well known to the skilled artisan and defined on the basis of papain cleavage of antibodies. The Fc part of an antibody is directly involved in ADCC (antibody dependent cell-mediated cytotoxicity) and CDC (complement-dependent cytotoxicity) based on complement activation, Clq binding and Fc receptor binding. Complement activation (CDC) is initiated by binding of complement factor Clq to the Fc part of most
20 IgG antibody subclasses. While the influence of an antibody on the complement system is dependent on certain conditions, binding to Clq is caused by defined binding sites in the Fc part. Such binding sites are e.g. L234, L235, D270, N297, E318, K320, K322, P331 and P329 (numbering according to Eu numbering (Edelman et al, Proc Natl Acad Sci U S A. 1969 May;63(1):78-85)). Most crucial among these residues in mediating Clq and
25 Fc γ receptor binding in IgG1 are L234 and L235 (Hezareh et al., J. Virology 75 (2001) 12161- 12168, Shields et al (2001) JBC, 276 (9): 6591–6604). Antibodies of subclass IgG1 and IgG3 usually show complement activation and Clq and C3 binding, whereas IgG2 and IgG4 do not activate the complement system and do not bind Clq and C3.

30 The term "antibody" or "antibody molecule" (used synonymously herein) encompasses a monoclonal antibody, a polyclonal antibody, a human antibody, a humanized antibody, a sequence-optimized antibody, a chimeric antibody, multispecific antibodies (e.g.,

bispecific antibodies), a fragment of an antibody, in particular a Fv, Fab, Fab', or F(ab')₂ fragment, a single chain antibody, in particular a single chain variable fragment (scFv), a single chain Fab fragment (scFab), a Small Modular Immunopharmaceutical (SMIP), a domain antibody, a nanobody®, a diabody. The antibody may have an effector function, such as ADCC or CDC, that is usually mediated by the Fc part of the antibody, or it may have no effector function, e.g. by lacking a Fc part or having a blocked, masked Fc part, in essence a Fc part that is not or insufficiently recognized by immune cells or immune system components, like the complement system.

Monoclonal antibodies (mAb) are monospecific antibodies that are identical in amino acid sequence. They may be produced by hybridoma technology from a hybrid cell line (called hybridoma) representing a clone of a fusion of a specific antibody-producing B cell with a myeloma (B cell cancer) cell (Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495-7.). Alternatively, monoclonal antibodies may be produced by recombinant expression in host cells (Norderhaug L, Olafsen T, Michaelsen TE, Sandlie I. (May 1997). "Versatile vectors for transient and stable expression of recombinant antibody molecules in mammalian cells." *J Immunol Methods* 204 (1): 77-87; see also below). A "recombinant antibody" or "recombinant binding protein" is an antibody or binding protein which has been produced by a recombinantly engineered host cell. It is optionally isolated or purified.

Antibody molecules according to the present invention also include fragments of immunoglobulins which retain antigen binding properties, like Fab, Fab', or F(ab')₂ fragments. Such fragments may be obtained by fragmentation of immunoglobulins e.g. by proteolytic digestion, or by recombinant expression of such fragments. For example, immunoglobulin digestion can be accomplished by means of routine techniques, e.g. using papain or pepsin (WO 94/29348). Papain digestion of antibodies typically produces two identical antigen binding fragments (Fabs). A Fab fragment is composed of one constant and one variable domain of each of the heavy and the light chain. Pepsin treatment yields an F(ab')₂. In Fab fragments, the variable domains are each fused to an immunoglobulin constant domain, preferably of human origin. Thus, the heavy chain variable domain is fused to a CH1 domain (a so-called Fd fragment), and the light chain variable domain is fused to a CL domain. Fab fragments may be produced by recombinant expression of respective nucleic acids in host cells, see below.

A number of technologies have been developed for placing variable domains of immunoglobulins, or molecules derived from such variable domains, in a different molecular context. Those should also be considered as “antibodies” or “antibody molecules” in accordance with the present invention. In general, these antibody molecules are smaller in size compared to immunoglobulins, and may comprise a single amino acid chain or several amino acid chains. For example, a “single-chain variable fragment (scFv)” is a fusion of the variable regions of the heavy and light chains of immunoglobulins, linked together with a short linker, usually serine (S) or glycine (G) (WO 88/01649; WO 91/17271; Huston et al; International Reviews of Immunology, Volume 10, 1993, 195 - 217). “Single domain antibodies” or a “nanobody ®” harbour an antigen-binding site in a single Ig-like domain (WO 94/04678; WO 03/050531, Ward et al., Nature. 1989 Oct 12;341(6242):544-6; Revets et al., Expert Opin Biol Ther. 5(1):111-24, 2005). One or more single domain antibodies with binding specificity for the same or a different antigen may be linked together. “Diabodies” are bivalent antibody molecules consisting of two amino acid chains comprising two variable domains (WO 94/13804, Holliger et al., Proc Natl Acad Sci U S A. 1993 Jul 15;90(14):6444-8). Other examples of antibody-like molecules are “immunoglobulin super family antibodies” (IgSF; Srinivasan and Roeske, Current Protein Pept. Sci. 2005, 6(2): 185-96). A different concept leads to the so-called “Small Modular Immunopharmaceutical (SMIP)” which comprises a Fv domain linked to single-chain hinge and effector domains devoid of the constant domain CH1 (WO 02/056910). A “single-chain Fab” or “scFab” is a fusion of a light chain Fab domain (i.e. a light chain variable domain (VL) that is linked to one light chain constant domain (CL)) with a heavy chain Fab domain (i.e. a heavy chain variable domain (VH) that is linked to one heavy chain constant domain (CH1)). The single chain Fab is capable of recognizing and binding an antigen. The scFab may optionally also contain a linker (e.g., a peptide linker) positioned between the CL and VH domain (Hust et al. BMC Biotechnology 2007, 7:14).

For application in man, it is often desirable to reduce immunogenicity of therapeutic molecules, such as antibodies or binding proteins comprising an antigen binding unit as described herein, originally derived from other species, like mouse. This can be done by construction of chimeric antibodies/binding proteins, or by a process called “humanization”. In this context, a “chimeric antibody”, or “chimeric antigen binding unit” is understood to be an antibody or an antigen binding unit comprising a sequence part (e.g.

a variable domain) derived from one species (e.g. mouse) fused to a sequence part (e.g. the constant domains) derived from a different species (e.g. human). In this context, a “humanized antibody”, “humanized antigen binding unit” or a “a humanized VL/VH domain” is an antibody, antigen binding unit or VH/VL domain comprising a variable domain originally derived from a non-human species, wherein certain amino acids have been mutated to make the overall sequence of that variable domain more closely resemble a sequence of a human variable domain. Methods of humanization of antibodies are well-known in the art (Billetta R, Lobuglio AF. “Chimeric antibodies”. *Int Rev Immunol.* 1993;10(2-3):165-76; Riechmann L, Clark M, Waldmann H, Winter G (1988). "Reshaping human antibodies for therapy". *Nature*: 332:323).

The terms "human antibody", “human antigen binding unit”, or “ human VH/VL domain” as used herein, include antibodies, antigen binding units or VH/VL domains having variable (and constant, if applicable) regions derived from human germline immunoglobulin sequences. The term "human antibody", “human antigen binding unit”, or “ human VH/VL domain” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another (mammalian) species, such as a mouse, rat or rabbit, have been grafted onto human framework sequences. Thus, as used herein, the terms "human antibody", “human antigen binding unit”, or “human VH/VL domain” refer to an antibody, antigen binding unit or VH/VL domain in which every part of the protein (e.g., CDR, framework, CL, CH domains (e.g., CH1 , CH2, CH3), hinge, VL, VH) is substantially non- immunogenic in humans, with only minor sequence changes or variations as further described herein below.

Technologies for creating such "human antibodies", “human antigen binding units”, or “human VH/VL domains” have been described and include without being limiting phage display or use of transgenic animals (WWW. Ablexis.com/technology-alivamab.php; WO 90/05144; D. Marks, H.R. Hoogenboom, T.P. Bonnert, J. McCafferty, A.D. Griffiths and G. Winter (1991) "By-passing immunisation. Human antibodies from V-gene libraries displayed on phage." *J.Mol.Biol.*, 222, 581-597; Knappik et al., *J. Mol. Biol.* 296: 57-86, 2000; S. Carmen and L. Jermutus, "Concepts in antibody phage display". *Briefings in Functional Genomics and Proteomics* 2002 1(2):189-203; Lonberg N, Huszar D. "Human antibodies from transgenic mice". *Int Rev Immunol.* 1995;13(1):65-93.; Brüggemann M, Taussig MJ. "Production of human antibody repertoires in transgenic mice". *Curr Opin Biotechnol.* 1997 Aug;8(4):455-8.).

Thus, a human antibody, human antigen binding unit or human VH/VL domain is distinct from e.g., a chimeric or humanized antibody. It is pointed out that a human antibody, human antigen binding unit or human VH/VL domain can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes.

The chimeric, humanized or human antibodies, antigen binding units, or VH/VL domains of the present invention may further be optimized; also referred to herein as “optimized” or “sequence-optimized” antibodies, antigen binding units, or VH/VL domains. Such optimization includes without limitation the removal or exchange of undesired amino acids, for example to reduce immunogenicity in humans, or to avoid deamidation, undesirable charges or lipophilicity or non-specific binding. Such removal or exchange of undesired amino acids can, for example, be introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo. Moreover, in connection with chimeric or humanized antibodies, antigen binding units or VH/VL domains, it will be understood that certain mouse FR residues may be important to the function of the optimized antibodies, antigen binding units and VH/VL domains. Therefore these important amino acid residues may be retained in an optimized antibody, antigen binding unit and VH/VL domain.

The term “monomer” refers to a homogenous form of an antibody or a multispecific protein as described herein. For example, for a full-length antibody, monomer means a monomeric antibody having two identical heavy chains and two identical light chains. In the context of the present invention, a monomer means a protein of the present invention having a single antigen binding unit specific for B7H6, and a single antigen binding unit specific for CD3 as described herein. For example, a monomer of a binding protein described herein may have two polypeptide chains, a first polypeptide chain comprising a single chain Fab specific for B7H6 and a first Fc domain and a second polypeptide chain comprising a single chain Fab specific for CD3 and a second Fc domain.

An epitope is a region of an antigen that is bound by an antibody or antigen binding moiety (e.g. the antigen binding unit of the proteins described herein). The term “epitope” includes any polypeptide determinant capable of specific binding to an antibody or antigen binding moiety. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, glycan side chains, phosphoryl, or sulfonyl,

and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

- 5 An antigen binding molecule/protein (such as an immunoglobulin, an antibody, an antigen binding unit, or a fragment of such antigen binding molecule/protein) that can “*bind*”, “*bind to*”, “*specifically bind*”, or “*specifically bind to*”, is “*binding (to)*” or “*specifically binding to*” that “*has affinity for*”, “*is specific for*” and/or that “*has specificity for*” a certain epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be “*against*” or “*directed against*” said epitope, antigen or protein or is a “*binding*”
10 molecule/protein with respect to such epitope, antigen or protein. These terms are used herein interchangeably.

- As used herein, the terms “binding” and “specific binding” refer to the binding of an antigen binding molecule/protein (such as an immunoglobulin, an antibody, an antigen binding unit,
15 or a fragment of such antigen binding molecule/protein) to an epitope of the antigen in an in vitro assay, preferably in a plasmon resonance assay ((Malmqvist M., "Surface plasmon resonance for detection and measurement of antibody-antigen affinity and kinetics.", Curr Opin Immunol. 1993 Apr;5(2):282-6.)) with purified wild-type antigen. Antibody affinity can also be measured using kinetic exclusion assay (KinExA) technology (Darling, R.J., and
20 Brault P-A., “Kinetic exclusion assay technology: Characterization of Molecular Interactions.” ASSAY and Drug Development Technologies. 2004, Dec 2(6): 647-657). For example, a binding protein or protein of the invention binds to an epitope of B7H6 with its first antigen binding unit/first polypeptide chain and to an epitope of CD3 with its second antigen binding unit/second polypeptide chain.

- 25 Generally, the term “*specificity*” refers to the number of different types of antigens or epitopes to which a particular antigen binding molecule/protein (such as an immunoglobulin, an antibody, an antigen binding unit, or a fragment of such antigen binding molecule/protein) can bind. Binding specificity for B7H6 means that the antigen binding protein/molecule of the invention (e.g. the first antigen binding unit of such
30 binding protein) has a significantly higher binding affinity to B7H6 than to structurally unrelated molecules. Binding specificity for CD3 means that the antigen binding protein/molecule of the invention (e.g. the second antigen binding unit of such binding

protein) has a significantly higher binding affinity to CD3 than to structurally unrelated molecules. The specificity of an antigen-binding molecule/protein can be determined based on its affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (K_D), is a measure for the
5 binding strength between an epitope and an antigen-binding site on the antigen-binding molecule/protein: the lesser the value of the K_D , the stronger the binding strength between an epitope and the antigen-binding site (alternatively, the affinity can also be expressed as the affinity constant (K_A), which is $1/K_D$). As will be clear to the skilled person (for example on the basis of the further disclosure herein), affinity can be determined in a
10 manner known *per se*, depending on the specific antigen of interest. Avidity is the measure of the strength of binding between an antigen-binding molecule/protein (such as an immunoglobulin, an antibody, an antigen binding unit, or fragment of such antigen binding molecule/protein) and the pertinent antigen. Avidity is related to both the affinity between an epitope and its antigen binding site on the antigen-binding molecule/protein and the
15 number of pertinent binding sites present on the antigen-binding molecule/protein.

When referring to an antigen binding unit/antigen, ligand/receptor, or other binding pair, the term "specifically binds" or "selectively binds" indicates a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified antigen binding unit binds
20 to a particular antigen and does not bind in a significant amount to other proteins present in the sample. The antigen binding unit binds to its antigen with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater under the designated conditions than the affinity with unrelated antigens.

25 The term "isolated," as used herein, refers to material that is removed from its original or native environment (e.g. the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated by human intervention from some or all of the co-existing materials in the natural system, is isolated. Such
30 polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature. For example, a nucleic acid, protein/polypeptide molecule is considered to be "(in) essentially isolated (form)" - when

compared to its native biological source and/or the reaction medium or cultivation medium from which it has been obtained - when it has been separated from at least one other component with which it is usually associated in said source or medium, such as another nucleic acid, another protein/polypeptide, another biological component or macromolecule or at least one contaminant, impurity or minor component. In particular, a nucleic acid or protein/polypeptide molecule is considered "essentially isolated" when it has been purified at least 2-fold, in particular at least 10-fold, more in particular at least 100-fold, and up to 1000-fold or more. A nucleic acid or protein/polypeptide molecule that is "in essentially isolated form" is preferably essentially homogeneous, as determined using a suitable technique, such as a suitable chromatographical technique, e.g., polyacrylamide-gelelectrophoresis.

As used herein, the terms "identical" or "percent identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence. To determine the percent identity, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions / total # of positions (e.g., overlapping positions) x 100). In some embodiments, the two sequences that are compared are the same length after gaps are introduced within the sequences, as appropriate (e.g., excluding additional sequence extending beyond the sequences being compared). For example, when variable region sequences are compared, the leader (signal peptide) and/or constant domain sequences are not considered. For sequence comparisons between two sequences, a "corresponding" CDR refers to a CDR in the same location in both sequences (e.g., CDR-H1 of each sequence).

The determination of percent identity or percent similarity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a

mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12, to obtain nucleotide sequences homologous to a nucleic acid encoding a protein of interest. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3, to obtain amino acid sequences homologous to a protein of interest. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, 1994, Comput. Appl. Biosci. 10:3-5; and FASTA described in Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup=2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA. Alternatively, protein sequence alignment may be carried out using the CLUSTAL W algorithm, as described by Higgins et al., 1996, Methods Enzymol. 266:383-402.

The term “covalently linked” or “covalently bound” as used herein means either a direct covalent bond between residues, or an indirect link/bond where two residues are not directly bonded but are both covalently bonded to an intermediate molecule or domain, e.g. an intermediate domain of an immunoglobulin or a linker.

MULTI-SPECIFIC BINDING PROTEINS OF THE INVENTION

The present invention provides multi-specific binding proteins comprising at least one antigen binding unit specifically binding to B7H6 (a first antigen binding unit), and at least
5 one antigen binding unit specifically binding to CD3 (a second antigen binding unit).

Through simultaneous binding to a tumor cell antigen and CD3 on a T cell, the binding proteins act as T cell activating proteins and are also referred to herein as T cell engagers. The term “(multi-specific) binding protein” is used herein interchangeably with the term “(multi-specific) binding molecule”. Further terms used herein to refer to the multi-specific
10 binding protein of the invention are “protein of the invention”, “binding protein of the invention”, “antigen-binding protein” as well as “multi-specific protein”.

The inventors have surprisingly found that multi-specific binding proteins of the invention induce potent and selective lysis of B7H6-positive colorectal cancer cell lines in the presence of T cells and are already active at low effector to target cell ratios. Importantly,
15 the binding proteins of the invention do not lyse B7H6-negative cells and do not cause T cell activation, T cell proliferation, and cytokine secretion in the absence of B7H6-positive cells. Notably, proteins of the invention that do not inhibit B7H6-dependent NK cell activation via NKp30 in vitro, are more potent in lysing B7H6-positive tumor cells. This activity is described, for example, in the in vitro assay in Example 11.

20 For avoidance of doubt, B7H6 as used herein refers to human B7H6 of UniProt Q68D85 and the nucleic acid sequence encoding that protein. CD3 as used herein refers to human CD3epsilon (UniProt P07766) and CD3 gamma (Uniprot: P09693) complexes, (human CD3εγ complexes). The skilled person would appreciate that the terms B7H6 and B7-H6 are used interchangeably herein.

25 In one aspect, the multi-specific binding protein of the invention comprises a first antigen binding unit specifically binding to B7H6 and a second antigen binding unit specifically binding to CD3, wherein said first binding unit is selected from the group consisting of i) to xxiv):

- 30 i) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:1 (CDR1), SEQ ID NO:2 (CDR2) and SEQ ID NO:3 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ

ID NO:4 (CDR1), SEQ ID NO:5 (CDR2) and SEQ ID NO:6 (CDR3) (antigen binding unit B7H6#1);

ii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:7 (CDR1), SEQ ID NO:8 (CDR2) and SEQ ID NO:9 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:10 (CDR1), SEQ ID NO:11 (CDR2) and SEQ ID NO:12 (CDR3) (antigen binding unit B7H6#2);

iii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:13 (CDR1), SEQ ID NO:14 (CDR2) and SEQ ID NO:15 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:16 (CDR1), SEQ ID NO:17 (CDR2) and SEQ ID NO:18 (CDR3) (antigen binding unit B7H6#3);

iv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:19 (CDR1), SEQ ID NO:20 (CDR2) and SEQ ID NO:21 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:22 (CDR1), SEQ ID NO:23 (CDR2) and SEQ ID NO:24 (CDR3) (antigen binding unit B7H6#4);

v) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:25 (CDR1), SEQ ID NO:26 (CDR2) and SEQ ID NO:27 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:28 (CDR1), SEQ ID NO:29 (CDR2) and SEQ ID NO:30 (CDR3) (antigen binding unit B7H6#5);

vi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:31 (CDR1), SEQ ID NO:32 (CDR2) and SEQ ID NO:33 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:34 (CDR1), SEQ ID NO:35 (CDR2) and SEQ ID NO:36 (CDR3) (antigen binding unit B7H6#6);

vii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:37 (CDR1), SEQ ID NO:38 (CDR2) and SEQ ID NO:39 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:40 (CDR1), SEQ ID NO:41 (CDR2) and SEQ ID NO:42 (CDR3) (antigen binding unit B7H6#7);

- viii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:43 (CDR1), SEQ ID NO:44 (CDR2) and SEQ ID NO:45 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:46 (CDR1), SEQ ID NO:47 (CDR2) and SEQ ID NO:48 (CDR3) (antigen binding unit B7H6#8);
- ix) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:49 (CDR1), SEQ ID NO:50 (CDR2) and SEQ ID NO:51 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:52 (CDR1), SEQ ID NO:53 (CDR2) and SEQ ID NO:54 (CDR3) (antigen binding unit B7H6#9);
- x) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:55 (CDR1), SEQ ID NO:56 (CDR2) and SEQ ID NO:57 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:58 (CDR1), SEQ ID NO:59 (CDR2) and SEQ ID NO:60 (CDR3) (antigen binding unit B7H6#10);
- xi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:61 (CDR1), SEQ ID NO:62 (CDR2) and SEQ ID NO:63 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:64 (CDR1), SEQ ID NO:65 (CDR2) and SEQ ID NO:66 (CDR3) (antigen binding unit B7H6#11);
- xii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:67 (CDR1), SEQ ID NO:68 (CDR2) and SEQ ID NO:69 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:70 (CDR1), SEQ ID NO:71 (CDR2) and SEQ ID NO:72 (CDR3) (antigen binding unit B7H6#12));
- xiii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:73 (CDR1), SEQ ID NO:74 (CDR2) and SEQ ID NO:75 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:76 (CDR1), SEQ ID NO:77 (CDR2) and SEQ ID NO:78 (CDR3) (antigen binding unit B7H6#13);
- xiv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID

NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3) (antigen binding unit B7H6#14);

xv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3) (antigen binding unit B7H6#15);

xvi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3) (antigen binding unit B7H6#16);

xvii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:97 (CDR1), SEQ ID NO:98 (CDR2) and SEQ ID NO:99 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:100 (CDR1), SEQ ID NO:101 (CDR2) and SEQ ID NO:102 (CDR3) (antigen binding unit B7H6#17);

xviii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:103 (CDR1), SEQ ID NO:104 (CDR2) and SEQ ID NO:105 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:106 (CDR1), SEQ ID NO:107 (CDR2) and SEQ ID NO:108 (CDR3) (antigen binding unit B7H6#18);

xix) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:109 (CDR1), SEQ ID NO:110 (CDR2) and SEQ ID NO:111 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:112 (CDR1), SEQ ID NO:113 (CDR2) and SEQ ID NO:114 (CDR3) (antigen binding unit B7H6#19);

xx) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:115 (CDR1), SEQ ID NO:116 (CDR2) and SEQ ID NO:117 (CDR3) and heavy chain CDRs comprising the amino acid sequences

of SEQ ID NO:118 (CDR1), SEQ ID NO:119 (CDR2) and SEQ ID NO:120 (CDR3) (antigen binding unit B7H6#20);

xxi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:121 (CDR1), SEQ ID NO:122 (CDR2) and SEQ ID NO:123 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:124 (CDR1), SEQ ID NO:125 (CDR2) and SEQ ID NO:126 (CDR3) (antigen binding unit B7H6#21);

xxii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:127 (CDR1), SEQ ID NO:128 (CDR2) and SEQ ID NO:129 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:130 (CDR1), SEQ ID NO:131 (CDR2) and SEQ ID NO:132 (CDR3) (antigen binding unit B7H6#22);

xxiii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:133 (CDR1), SEQ ID NO:134 (CDR2) and SEQ ID NO:135 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:136 (CDR1), SEQ ID NO:137 (CDR2) and SEQ ID NO:138 (CDR3) (antigen binding unit B7H6#23); and

xxiv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:139 (CDR1), SEQ ID NO:140 (CDR2) and SEQ ID NO:141 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:142 (CDR1), SEQ ID NO:143 (CDR2) and SEQ ID NO:144 (CDR3) (antigen binding unit B7H6#24).

In some embodiments of the binding protein of the invention, said second antigen binding unit specifically binding to CD3 is selected from the group consisting of i)-vi):

i) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3) (antigen binding unit CD3#1);

ii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:263 (CDR1), SEQ ID NO:264 (CDR2) and SEQ ID NO:265 (CDR3) and heavy chain CDRs comprising the amino acid sequences

of SEQ ID NO:266 (CDR1), SEQ ID NO:267 (CDR2) and SEQ ID NO:268 (CDR3) (antigen binding unit CD3#2);

iii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:269 (CDR1), SEQ ID NO:270 (CDR2) and SEQ ID NO:271 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:272 (CDR1), SEQ ID NO:273 (CDR2) and SEQ ID NO:274 (CDR3) (antigen binding unit CD3#3);

iv) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:275 (CDR1), SEQ ID NO:276 (CDR2) and SEQ ID NO:277 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:278 (CDR1), SEQ ID NO:279 (CDR2) and SEQ ID NO:280 (CDR3) (antigen binding unit CD3#4);

v) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:281 (CDR1), SEQ ID NO:282 (CDR2) and SEQ ID NO:283 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:284 (CDR1), SEQ ID NO:285 (CDR2) and SEQ ID NO:286 (CDR3) (antigen binding unit CD3#5); and

vi) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:287 (CDR1), SEQ ID NO:288 (CDR2) and SEQ ID NO:289 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:290 (CDR1), SEQ ID NO:291 (CDR2) and SEQ ID NO:292 (CDR3) (antigen binding unit CD3#6).

The first antigen binding units i) to xxiv) as outlined above are termed B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, and B7H6#24, respectively and the second antigen binding units i) to vi) as outlined above are termed CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 and CD3#6, respectively. Provided herein is a sequence table which readily allows identification of individual amino acid sequences to specific antigen binding units and full length binding proteins of the present invention. A summary is provided in Table 1 in Example 2.

The terms “first” and “second” with respect to antigen binding units in general, as used herein, is solely intended to indicate that these units are two different units (as they bind to different target antigens). Thus, these terms shall not be understood to refer to the exact order or sequence of the units within the binding protein of the invention.

- 5 In some embodiments, the binding protein of the invention comprises a first antigen binding unit selected from the group consisting of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, and B7H6#24 as defined by the respective CDR sequences shown in Table 1 and a second antigen binding unit selected from the group consisting of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 and CD3#6 as defined by the respective CDR sequences shown in Table 1.

- In some embodiments, the binding protein of the invention comprises a first antigen binding unit selected from the group consisting of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, and B7H6#24 as defined by the respective CDR sequences shown in Table 1 and a second antigen binding unit of CD3#1 as defined by the respective CDR sequences shown in Table 1. In preferred embodiments, the binding protein of the invention comprises a first antigen binding unit selected from the group consisting of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, and B7H6#24 as defined by the respective CDR sequences shown in Table 1 and a second antigen binding unit of CD3#1 as defined by the respective CDR sequences shown in Table 1. In preferred embodiments, the binding protein of the invention comprises a first antigen binding unit selected from the group consisting of B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, and B7H6#24 as defined by the respective CDR sequences shown in Table 1 and a second antigen binding unit of CD3#1 as defined by the respective CDR sequences shown in Table 1. In preferred embodiments, the binding protein of the invention comprises a first antigen binding unit selected from the group consisting of B7H6#12, B7H6#14, B7H6#15, B7H6#16, and B7H6#23 as defined by the

respective CDR sequences shown in Table 1 and a second antigen binding unit of CD3#1 as defined by the respective CDR sequences shown in Table 1.

In addition to the CDR sequences as set out herein, the antigen binding units of the binding proteins of the invention include immunoglobulin framework region (FR) sequences.

- 5 These sequences are preferably not immunogenic in humans, and are therefore preferably human, humanized or optimized FR sequences. Suitable human, humanized or optimized FR sequences are known in the art. Specifically preferred FR sequences can be taken from the embodiments shown herein, disclosing the complete antigen binding units and thereby CDR sequences as well as FR sequences. In one preferred embodiment, the binding protein
- 10 of the invention comprises a first antigen binding unit specifically binding to B7H6, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:67 (CDR1), SEQ ID NO:68 (CDR2) and SEQ ID NO:69 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:70 (CDR1), SEQ ID NO:71 (CDR2) and SEQ ID NO:72 (CDR3) and a second antigen binding unit specifically binding to
- 15 CD3, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3). Such antigen binding protein is referred to herein as B7H6#14/CD3#1. In a particularly preferred embodiment, the antigen binding units
- 20 specifically binding to B7H6 and CD3, respectively, each comprise CDRs as defined above (B7H6#12/CD3#1) within a VL/VH domain, e.g. a sequence optimized VL/VH domain. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively (B7H6#12/CD3#1), are each formed by a scFab and are optionally each linked to an Fc domain.
- 25 In one preferred embodiment, the binding protein of the invention comprises a first antigen binding unit specifically binding to B7H6, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3) and a second antigen
- 30 binding unit specifically binding to CD3, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3). Such antigen

binding protein is referred to herein as B7H6#14/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, each comprise CDRs as defined above (B7H6#14/CD#1) within a VL/VH domain, e.g. a sequence optimized VL/VH domain. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively (B7H6#14/CD3#1), are each formed by a scFab and are optionally each linked to an Fc domain.

In one preferred embodiment, the binding protein of the invention comprises a first antigen binding unit specifically binding to B7H6, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3) and a second antigen binding unit specifically binding to CD3, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3). Such antigen binding protein is referred to herein as B7H6#15/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, each comprises CDRs as defined above (B7H6#15/CD3#1) within a VL/VH domain, e.g. a sequence optimized VL/VH domain. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively (B7H6#15/CD3#1), are each formed by a scFab and are optionally each linked to an Fc domain.

In one preferred embodiment, the binding protein of the invention comprises a first antigen binding unit specifically binding to B7H6, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3) and a second antigen binding unit specifically binding to CD3, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3). Such antigen binding protein is referred to herein as B7H6#16/CD3#1. In a particularly preferred

embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, each comprise CDRs as defined above (B7H6#16/CD3#1) within a VL/VH domain, e.g. a sequence optimized VL/VH domain. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively (B7H6#16/CD3#1), are each formed by a scFab and are optionally each linked to an Fc domain.

In one preferred embodiment, the binding protein of the invention comprises a first antigen binding unit specifically binding to B7H6, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:133 (CDR1), SEQ ID NO:134 (CDR2) and SEQ ID NO:135 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:136 (CDR1), SEQ ID NO:137 (CDR2) and SEQ ID NO:138 (CDR3) and a second antigen binding unit specifically binding to CD3, comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3). Such antigen binding protein is referred to herein as B7H6#14/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, each comprise CDRs as defined above (B7H6#23/CD3#1) within a VL/VH domain, e.g. a sequence optimized VL/VH domain. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively (B7H6#23/CD3#1), are each formed by a scFab and are optionally each linked to an Fc domain.

In preferred embodiments of the binding proteins of the invention, the first and the second binding unit each comprise a light chain variable domain and a heavy chain variable domain said light/heavy chain variable domains defined by the CDR sequences of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, or B7H6#24 for the first antigen binding unit and said light/heavy chain variable domains defined by the CDR sequences of any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 for the second antigen binding unit. In some embodiments of the binding protein of the invention, the VH and/or VL domain of the antigen binding units of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11,

B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, B7H6#24, CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 is a human, humanized or optimized VH and/or VL domain.

In preferred embodiments of the binding protein of the invention, the light/heavy chain
5 variable domains of the first antigen binding unit specifically binding to B7H6 are further defined as follows

- i) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:145 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:146 (antigen binding unit B7H6#1); or
- 10 ii) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:147 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:148 (antigen binding unit B7H6#2); or
- iii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:149 and heavy chain variable domain comprising the amino acid sequence
15 of SEQ ID NO:150 (antigen binding unit B7H6#3); or
- iv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:151 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:152 (antigen binding unit B7H6#4); or
- v) a light chain variable domain comprising the amino acid sequence of SEQ ID
20 NO:153 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:154 (antigen binding unit B7H6#5); or
- vi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:155 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:156 (antigen binding unit B7H6#6); or
- 25 vii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:157 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:158 (antigen binding unit B7H6#7); or
- viii) a light chain variable domain comprising the amino acid sequence of SEQ ID
30 NO:159 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:160 (antigen binding unit B7H6#8); or

- ix) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:161 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:162 (antigen binding unit B7H6#9); or
- 5 x) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:163 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:164 (antigen binding unit B7H6#10); or
- xi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:165 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:166 (antigen binding unit B7H6#11); or
- 10 xii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:167 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168 (antigen binding unit B7H6#12); or
- xiii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:169 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:170 (antigen binding unit B7H6#13); or
- 15 xiv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:171 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172 (antigen binding unit B7H6#14); or
- xv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:173 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:174 (antigen binding unit B7H6#15); or
- 20 xvi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:175 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:176 (antigen binding unit B7H6#16); or
- xvii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:177 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:178 (antigen binding unit B7H6#17); or
- 25 xviii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:179 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:180 (antigen binding unit B7H6#18); or
- 30

- xix) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:181 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:182 (antigen binding unit B7H6#19); or
- 5 xx) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:183 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:184 (antigen binding unit B7H6#20); or
- xxi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:185 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:186 (antigen binding unit B7H6#21); or
- 10 xxii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:187 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:188 (antigen binding unit B7H6#22); or
- xxiii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:189 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:190 (antigen binding unit B7H6#23); or
- 15 xxiv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:191 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:192 (antigen binding unit B7H6#24).

In preferred embodiments of the binding protein of the invention, the light/heavy chain variable domains of the second antigen binding unit specifically binding to CD3 are further defined as follows

20

- i) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294) (antigen binding unit CD3#1); or
- 25 ii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:295 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:296 (antigen binding unit CD3#2); or
- iii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:297 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:298 (antigen binding unit CD3#3); or
- 30

- iv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:299 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:300 (antigen binding unit CD3#4); or
 - v) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:301 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:302 (antigen binding unit CD3#5); or
 - vi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:303 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:304 (antigen binding unit CD3#6).
- 10 In preferred embodiments, the binding protein of the invention comprises a combination of a first and a second antigen binding unit selected from the group consisting of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#6/CD3#1, B7H6#7/CD3#1, B7H6#8/CD3#1, B7H6#9/CD3#1, B7H6#10/CD3#1, B7H6#11/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1,
- 15 B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, and B7H6#24/CD3#1, the first and second antigen binding unit being defined by the CDR and/or VH and VL sequences of the antigen binding units as shown in Table 1.
- 20 In preferred embodiments, the binding protein of the invention comprises a combination of a first and a second antigen binding unit selected from the group consisting of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1,
- 25 B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, and B7H6#24/CD3#1, the first and second antigen binding unit being defined by the CDR and/or VH and VL sequences of the antigen binding units as shown in Table 1.
- In preferred embodiments, the binding protein of the invention comprises a combination of a first and a second antigen binding unit selected from the group consisting of
- 30 B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, and

B7H6#24/CD3#1, the first and second antigen binding unit being defined by the CDR and/or VH and VL sequences of the antigen binding units as shown in Table 1.

In preferred embodiments, the binding protein of the invention comprises a combination of a first and a second antigen binding unit selected from the group consisting of
5 B7H6#12/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, and B7H6#23/CD3#1, the first and second antigen binding unit being defined by the CDR and/or VH and VL sequences of the antigen binding units as shown in Table 1.

In one preferred embodiment, the binding protein of the invention comprises (i) a first antigen binding unit specifically binding to B7H6 comprising a light chain variable domain
10 comprising the amino acid sequences of SEQ ID NO:167 and a heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:168 and (ii) a second antigen binding unit specifically binding to CD3 comprising a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294. Such binding protein is
15 referred to herein as B7H6#12/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, as defined above (B7H6#12/CD3#1) are each formed by a scFab, optionally covalently linked to an Fc domain.

In one preferred embodiment, the binding protein of the invention comprises (i) a first
20 antigen binding unit specifically binding to B7H6 comprising a light chain variable domain comprising the amino acid sequences of SEQ ID NO:171 and a heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:172 and (ii) a second antigen binding unit specifically binding to CD3 comprising a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable
25 domain comprising the amino acid sequence of SEQ ID NO:294. Such binding protein is referred to herein as B7H6#14/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, as defined above (B7H6#14/CD3#1) are each formed by a scFab, optionally covalently linked to an Fc domain.

30 In one preferred embodiment, the binding protein of the invention comprises (i) a first antigen binding unit specifically binding to B7H6 comprising a light chain variable domain comprising the amino acid sequences of SEQ ID NO:173 and a heavy chain variable

domain comprising the amino acid sequences of SEQ ID NO:174 and (ii) a second antigen binding unit specifically binding to CD3 comprising a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294. Such binding protein is
5 referred to herein as B7H6#15/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, as defined above (B7H6#15/CD3#1) are each formed by a scFab, optionally covalently linked to an Fc domain.

In one preferred embodiment, the binding protein of the invention comprises (i) a first
10 antigen binding unit specifically binding to B7H6 comprising a light chain variable domain comprising the amino acid sequences of SEQ ID NO:175 and a heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:176 and (ii) a second antigen binding unit specifically binding to CD3 comprising a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable
15 domain comprising the amino acid sequence of SEQ ID NO:294. Such binding protein is referred to herein as B7H6#16/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, as defined above (B7H6#16/CD3#1) are each formed by a scFab, optionally covalently linked to an Fc domain.

In one preferred embodiment, the binding protein of the invention comprises (i) a first
20 antigen binding unit specifically binding to B7H6 comprising a light chain variable domain comprising the amino acid sequences of SEQ ID NO:189 and a heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:190 and (ii) a second antigen binding unit specifically binding to CD3 comprising a light chain variable domain
25 comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294. Such binding protein is referred to herein as B7H6#23/CD3#1. In a particularly preferred embodiment, the antigen binding units specifically binding to B7H6 and CD3, respectively, as defined above (B7H6#23/CD3#1) are each formed by a scFab, optionally covalently linked to an Fc
30 domain.

In some embodiments, the binding protein of the invention comprises i) a first antigen binding unit specifically binding to B7H6 (e.g. any one of B7H6#1, B7H6#2, B7H6#3,

B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, B7H6#24 as defined by the respective CDR or VH/VL sequences shown in Table 1) which comprises a first light chain variable domain covalently
 5 linked to a first heavy chain variable domain with a first peptide linker and/or ii) a second antigen binding unit specifically binding to CD3 (e.g. any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 as defined by the respective CDR or VH/VL sequences shown in Table 1) which comprises a second light chain variable domain covalently linked to a second heavy chain variable domain with a second peptide linker. Optionally, the first and the
 10 second antigen binding units are covalently linked to each other with a peptide linker.

In some embodiments of the binding proteins of the invention, the first and/or the second antigen binding unit further comprises a CL and a CH1 domain like in a light/heavy Fab fragment of a conventional antibody molecule, thus said first binding unit comprises a) a VL domain (e.g., defined by the light chain CDR (LCCDR) or VL sequences of any one of
 15 B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, or B7H6#24) covalently linked (preferably directly bound) to a first CL domain and b) a VH domain (e.g., defined by the heavy chain CDR (HCCDR) or VH sequences of any one of B7H6#1, B7H6#2,
 20 B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, or B7H6#24) covalently linked (preferably directly bound) to a first CH1 domain and/or said second antigen binding unit comprises a) a VL domain (e.g., defined by the LCCDR or VL sequences of any one of CD3#1, CD3#2,
 25 CD3#3, CD3#4, CD3#5 or CD3#6) covalently linked (preferably directly bound) to a second CL domain and b) a VH domain (e.g., defined by HCCDR or VH sequences of any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6) covalently linked (preferably directly bound) to a second CH1 domain.

In the context of the present invention, a CL domain is the constant domain of an antibody
 30 light chain, for example either a kappa (κ) or a lambda (λ) light chain. An example of a constant region of a kappa light chain is shown in SEQ ID NO:247. An example of a constant region of a lambda light chain is shown in SEQ ID NO:248. In some embodiments, the first and the second CL domain are the same, e.g. the first and the

second CL domain are both a kappa light chain constant domain or the first and the second CL domain are both a lambda light chain constant domain. In preferred embodiments, the first and the second CL domain are different, e.g., the first CL domain is a constant kappa domain and the second CL domain is a constant lambda domain or vice versa.

- 5 In the context of the present invention, a CH1 domain is the first constant domain of an antibody heavy chain. An example of a constant CH1 domain is shown in SEQ ID NO:249.

In preferred embodiments of the binding proteins of the invention, the first antigen binding unit specific for B7H6 (e.g., any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, or B7H6#24 defined by the CDR and/or VH/VL sequences shown in Table 1) comprises from N- to C-terminus: a first light chain variable domain, a first CL domain, a first linker peptide, a first VH domain and a first CH1 domain, and/or the second binding unit (e.g., CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 defined by the CDR and/or VH/VL sequences shown in Table 1) of the binding proteins of the invention comprises from N- to C-terminus: a second light chain variable domain, a second CL domain, a second linker peptide, a second VH domain and a second CH1 domain. In these embodiments, the first and/or the second binding unit have the structure of a single chain Fab. For both, the first and/or the second antigen binding unit, when forming a single chain Fab, the order can be reversed such that from N- to C-terminus the antigen binding unit comprises: VH-CH1-[linker peptide]-VL-CL. In some embodiments of the protein of the invention when the first and/or second antigen binding unit comprise a Fab or a single chain Fab, the constant domains can be of the same type (e.g., both CL domains are kappa or lambda light chain constant domains) or of different types (the first CL domain is a kappa and the second CL domain is a lambda light chain constant domain or vice versa), preferably the first and the second CL domain are of different types. In preferred embodiments, the first antigen binding unit consists of a first single chain Fab specific for B7H6 (preferably any one of B7H6#12, B7H6#14, B7H6#15, B7H6#16 or B7H6#23 as defined by the CDR and or VH/VL sequences as shown in Table 1) and the second antigen binding unit consists of a second single chain Fab specific for CD3 (e.g., CD3#1 as defined by the CDR and or VH/VL sequences as shown in Table 1).

The linker sequence of the B7H6/CD3 binding proteins (e.g., B7H6/CD3 scFabs described above) may be a naturally occurring sequence or a non-naturally occurring sequence. If used for therapeutic purposes, the linker is preferably non-immunogenic in the subject to which the binding protein of the invention is administered. Preferably, the linker comprises
5 26 to 42 amino acids, for example 30 to 40 amino acids. In a further aspect, a linker used in a protein of the present invention comprises 34 to 40 amino acids, for example 36 to 39 amino acids, for example 38 amino acids.

One useful group of linker sequences are linkers derived from the hinge region of heavy chain antibodies as described in WO1996/34103 and WO1994/04678. Other examples are
10 poly-alanine linker sequences such as Ala-Ala-Ala.

Further preferred examples of linker sequences are Gly/Ser linkers of different length such as (glyxsery)z linkers, including e.g. (gly4ser)3, (gly4ser)5, (gly4ser)7, (gly3ser)3, (gly3ser)5, (gly3ser)7, (gly3ser2)3, (gly3ser2)5, and (gly3ser2)7 or a linker of any one of SEQ ID NOs: 250, 251, 252, 253, 254, 255 or 256, preferably SEQ ID NO: 250.

15 In some embodiments of the binding proteins of the invention, the VL domain of the first antigen binding unit (e.g., defined by the light chain CDR (LCCDR) or VL sequences of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18 B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, B7H6#24 as
20 shown in Table 1) is covalently linked via a first Gly/Ser linker (e.g., Gly/Ser linker of any one of 26 to 42 amino acids, 30 to 40 amino acids, 34 to 40 amino acids, or 36 to 39 amino acids, preferably 38 amino acids) to the VH domain of the first antigen binding unit (e.g., defined by the heavy chain CDR (HCCDR) or VH sequences of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18
25 B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, B7H6#24 as shown in Table 1); and the VL domain of the second antigen binding unit (e.g., defined by the light chain CDR (LCCDR) or VL sequences of any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 as shown in Table 1) is covalently linked via a second Gly/Ser
30 linker of any one of 26 to 42 amino acids, 30 to 40 amino acids, 34 to 40 amino acids, or 36 to 39 amino acids, preferably 38 amino acids) to the VH domain of the second antigen binding unit (e.g., defined by the heavy chain CDR (HCCDR) or VH sequences of any one

of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 as shown in Table 1). More preferably, the first and the second linker are the same. Even more preferably, the first and the second linker each comprise the amino acid sequence of SEQ ID NO:250.

In preferred embodiments of the binding proteins of the invention, the first antigen binding unit specifically binding to B7H6 comprises from N to C-terminus i) a VL domain (e.g.,
 5 defined by the light chain CDR (LCCDR) or VL sequences of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, B7H6#24 as shown in Table 1), ii) a
 10 first CL domain, iii) a via a first Gly/Ser linker (e.g., Gly/Ser linker of any one of 26 to 42 amino acids, 30 to 40 amino acids, 34 to 40 amino acids, or 36 to 39 amino acids, preferably 38 amino acids), iv) a VH domain (e.g., defined by the heavy chain CDR (HCCDR) or VH sequences of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13,
 15 B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, B7H6#24 as shown in Table 1), and v) a first CH1 domain and/or the second antigen binding unit specifically binding to CD3 comprises from N to C terminus i) a VL domain (e.g., defined by the light chain CDR (LCCDR) or VL sequences of any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 as shown in Table 1), ii)
 20 a second CL domain, iii) a second Gly/Ser linker (e.g., Gly/Ser linker of any one of 26 to 42 amino acids, 30 to 40 amino acids, 34 to 40 amino acids, or 36 to 39 amino acids, preferably 38 amino acids), iv) a VH domain of the second antigen binding unit (e.g., defined by the heavy chain CDR (HCCDR) or VH sequences of any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 as shown in Table 1) and v) a second CH1
 25 domain. Preferably, i) to v) are each linked via a direct covalent bond in the order i) to v) from the N to the C terminus of the antigen binding unit (each antigen binding unit thus having the structure of a scFab). More preferably, the first and the second linker are the same. Even more preferably, the first and the second linker each comprise the amino acid sequence of SEQ ID NO:250.

30 In preferred embodiments, the binding protein of the invention comprises a first single chain Fab forming a first antigen binding unit specific for B7H6 and comprising a sequence selected from the group consisting of SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID

NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215 and SEQ ID NO:216 and a second single chain Fab forming a second antigen binding unit specific for CD3 and comprising the sequence of SEQ ID NO:305.

In preferred embodiments, the binding protein of the invention comprises a first single chain Fab forming a first antigen binding unit specific for B7H6 and comprising a sequence selected from the group consisting of SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215 and SEQ ID NO:216 and a second single chain Fab forming a second antigen binding unit specific for CD3 and comprising the sequence of SEQ ID NO:305.

In preferred embodiments, the binding protein of the invention comprises a first single chain Fab forming a first antigen binding unit specific for B7H6 and comprising a sequence selected from the group consisting of SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215 and SEQ ID NO:216 and a second single chain Fab forming a second antigen binding unit specific for CD3 and comprising the sequence of SEQ ID NO:305.

In one preferred embodiment, the binding protein of the invention comprises a first single chain Fab comprising the sequence of SEQ ID NO:204 and a second single chain Fab comprising the sequence of SEQ ID NO:305, optionally each single chain Fab is further linked to an Fc domain and thereby forming a first polypeptide chain (a “B7H6 chain”) and a second polypeptide chain (a “CD3 chain”) In one preferred embodiment, the binding protein of the invention comprises a first single chain Fab comprising the sequence of SEQ ID NO:206 and a second single chain Fab comprising the sequence of SEQ ID NO:305, optionally each single chain Fab is further linked to an Fc domain and thereby forming a first polypeptide chain (a B7H6 chain) and a second polypeptide chain (a CD3 chain). In one preferred embodiment, the binding protein of the invention comprises a first single chain Fab comprising the sequence of SEQ ID NO:207 and a second single chain Fab comprising the sequence of SEQ ID NO:305, optionally each single chain Fab is further

linked to an Fc domain and thereby forming a first polypeptide chain (a B7H6 chain) and a second polypeptide chain (a CD3 chain). In one preferred embodiment, the binding protein of the invention comprises a first single chain Fab comprising the sequence of SEQ ID NO:208 and a second single chain Fab comprising the sequence of SEQ ID NO:305,
 5 optionally each single chain Fab is further linked to an Fc domain and thereby forming a first polypeptide chain (a B7H6 chain) and a second polypeptide chain (a CD3 chain). In one preferred embodiment, the binding protein of the invention comprises a first single chain Fab comprising the sequence of SEQ ID NO:215 and a second single chain Fab comprising the sequence of SEQ ID NO:305, optionally each single chain Fab is further
 10 linked to an Fc domain and thereby forming a first polypeptide chain (a B7H6 chain) and a second polypeptide chain (a CD3 chain).

In some embodiments, the first antigen binding unit (e.g., any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18 B7H6#19,
 15 B7H6#20, B7H6#21, B7H6#22, B7H6#23, or B7H6#24 as defined by the CDR and/or VH/VL sequences shown in Table 1) and/or the second antigen binding unit (e.g., any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 or CD3#6 as defined by the CDR and/or VH/VL sequences shown in Table 1) comprises a VL domain covalently linked (preferably directly bound) to a CL domain and a VH domain linked to a CH1 domain
 20 (together forming a Fab fragment), and said CH1 domain is further covalently linked (e.g., directly bound) to an Fc domain thereby forming an arm of a conventional Y shaped antibody molecule with one light and one heavy chain. In some embodiments, the first and the second antigen binding unit each form a Fab fragment, i.e. a first and a second Fab fragment, which is each covalently linked (preferably directly bound) to a first and a
 25 second Fc domain, respectively, thereby forming a conventional heterotetrameric bispecific and bivalent (monovalent for B7H6 and CD3, respectively) antibody molecule.

In preferred embodiments, the binding protein of the invention comprises (i) a first antigen binding unit comprising a first single chain Fab specifically binding to B7H6, i.e. an antibody light chain (VL-CL) covalently linked to the VH-CH1 domains of a heavy chain
 30 (the VL and VH domain of any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18 B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, or B7H6#24 as defined by the CDR and/or VH/VL sequences

shown in Table 1) via a peptide linker (e.g., Gly/Ser linker of any one of 26 to 42 amino acids, 30 to 40 amino acids, 34 to 40 amino acids, or 36 to 39 amino acids, preferably 38 amino acids, even more preferably a linker of SEQ ID NO:250), which first antigen binding unit is covalently linked (e.g., directly bound) to a first Fc domain and (ii) a second antigen binding unit comprising a second single chain Fab specifically binding to CD3, i.e. an antibody light chain (VL-CL) covalently linked to the VH-CH1 domains of a heavy chain (the VL and VH domain of any one of CD3#1 CD3#2, CD3#3, CD3#4, CD3#5, CD3#6 as defined by the respective CDR or VH/VL sequences shown in Table 1), which second antigen binding unit is covalently linked (e.g., directly bound) to a second Fc domain. Thus, in preferred embodiments, the binding protein of the invention comprises (i) a first polypeptide chain comprising (a) a first antigen binding unit specific for B7H6, said first antigen binding unit comprising a first single chain Fab specific for B7H6 (preferably any one of B7H6#12, B7H6#14, B7H6#15, B7H6#16 or B7H6#23 as defined by the CDR and or VH/VL sequences as shown in Table 1) and (b) a first Fc domain (this first polypeptide chain herein referred to also as “B7H6 chain”) and (ii) a second polypeptide chain specific for CD3 comprising (a) a second antigen binding unit comprising a second single chain Fab specific for CD3 (preferably CD3#1 as defined by the CDR and/or VL/VH sequences shown in Table 1) and (b) a second Fc domain (this second polypeptide chain also herein referred to as “CD3 chain”). Accordingly, the term “polypeptide chain” as used herein comprises at least a scFab and an Fc domain. In some embodiments, the first and the second Fc domain are the same. In preferred embodiments, the first and the second Fc domains are different. The resulting binding proteins of the invention comprise two different polypeptide chains bearing a full Fc and having two independent binding sites, a first antigen binding unit, formed by a first scFab specific for B7H6 and a second binding unit, formed by a second scFab specific for CD3.

In preferred embodiments, the binding protein of the invention comprises two different polypeptide chains, each comprising an antigen binding unit, formed by a scFab, with different specificity each covalently linked to an Fc domain, the polypeptide chains covalently linked to each other, either via disulfide bonds or potentially via a peptide linker. In preferred embodiments, the binding protein of the invention is a bispecific, bivalent (monovalent for B7H6 and CD3, respectively) heterodimeric protein comprising two polypeptide chains, one polypeptide chain (a first polypeptide chain or B7H6 chain) comprising an antigen binding unit formed by a scFab specifically binding to B7H6 (e.g.,

any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18 B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, or B7H6#24 as defined by the CDR and/or VH/VL sequences shown in Table 1) and an Fc domain
5 (preferably an Fc domain of SEQ ID NO:242) and another polypeptide chain (a second polypeptide chain or CD3 chain) comprising an antigen binding unit formed by a scFab specifically binding to CD3 (e.g. any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5, or CD3#6) and an Fc domain (preferably an Fc domain of SEQ ID NO:243). In some embodiments, the first antigen binding unit consists of a first single chain Fab and the
10 second antigen binding unit consists of a second single chain Fab. In some embodiments of the binding protein, the first polypeptide chain specific for B7H6 (a B7H6 chain) consists of a) a first antigen binding unit consisting of a scFab (preferably any one of B7H6#12, B7H6#14, B7H6#15, B7H6#16 or B7H6#23 as defined by the CDR, VH/VL and/or scFab sequences as shown in Table 1) and b) a first Fc domain and the second polypeptide chain
15 specific for CD3 (a CD3 chain) consists of a) a second antigen binding unit consisting of a scFab (preferably CD3#1 as defined by the CDR, VH/VL and/or scFab sequences as shown in Table 1) and b) a second Fc domain. Preferably, the C-terminus of the scFab is linked to the N-terminus of the Fc domain via a direct covalent bond. Preferably, the first and the second polypeptide chain are covalently linked to each other via disulfide bonds,
20 and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In the context of the present invention, an Fc domain is for example derived from the heavy chain of an IgG, for example an IgG₁, IgG₂ or IgG₄. For example, an Fc domain of the present invention is a Fc domain of a heavy chain of an IgG₁ or IgG₄ and comprises a
25 hinge region and two constant domains (C_{H2} and C_{H3}). Examples of Fc domains (including a hinge region) are shown in SEQ ID NOs:241 and 244.

The numbering of the amino acids in the amino acid chains of a protein of the present invention is herein according to the Eu numbering system (Edelman et al, PNAS USA 1969 May, 63(1):78-85; Cunningham et al. PNAS USA 1969, Nov, 64(3):997-1003),
30 unless otherwise specified. This means that the amino acid numbers indicated herein correspond to the positions in a heavy chain of the corresponding sub-type (e.g. IgG₁ or IgG₄), according to the Eu numbering system, unless otherwise specified.

In some embodiments, the first Fc domain and the second Fc domain in a protein of the present invention each comprises one or more amino acid changes which reduce the formation of homodimers of the first or second polypeptide chains instead of heterodimers of a first and a second polypeptide chain. Through these changes, a "protrusion" is
5 generated in one of the Fc domains by replacing one or more, small amino acid side chains from the interface of one of the heavy chains with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size are created on the interface of the other Fc domain by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the
10 heterodimer over other unwanted end-products such as homodimers, in particular homodimers of the Fc domain with the "protrusion" (see for example Ridgway et al. Protein Eng, 1996. 9(7): p. 617-21; Atwell et al, JMB, 1997, 270, 26-35). In some embodiments, such amino acid changes are a tyrosine (Y) at position 366 [T366Y] of the first Fc domain and a threonine (T) at position 407 [Y407T] of the second Fc domain. In
15 some embodiments, the first Fc domain comprises a serine (S) at position 366 [T366S] and the second Fc domain comprises a tryptophan (W) at position 366 [T366W], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V]. In preferred embodiments, the first Fc domain comprises a tryptophan (W) at position 366 [T366W] and the second Fc domain comprises a serine (S) at position 366 [T366S], an alanine (A) at
20 position 368 [L368A] and a valine (V) at position 407 [Y407V]. For example, position 366 of the Fc domain according to Eu numbering, corresponding to the amino acid position 146 in the human IgG1 Fc sequence of SEQ ID NO:241, is changed from T at position 146 in SEQ ID NO:241 to W at position 146 in SEQ ID NO:242; and positions 366, 368 and 407 according to Eu numbering, corresponding to the amino acid positions 146, 148 and 187,
25 respectively, in SEQ ID NO:241, are changed from T, L and Y at these positions in SEQ ID NO:241 to S, A and V at these positions in SEQ ID NO:243. In any of these embodiments, the amino acid changes described for the first Fc domain may be located in the second Fc domain and the respective amino acid changes for the second Fc domain may be located in the first Fc domain. In other words, the term "first" and "second" can be
30 exchanged in these embodiments. In some embodiments, such a Fc domain is an Fc domain derived from the heavy chain of an IgG₁ or IgG₄.

In some embodiments, the first Fc domain comprises a cysteine (C) at position 354 [S354C] in addition to the tryptophan (W) at position 366 [T366W] and the second Fc

domain comprises a cysteine (C) at position 349 [Y349C] in addition to the serine (S) at position 366 [T366S], the alanine (A) at position 368 [L368A] and the valine (V) at position 407 [Y407V]. In one aspect, such Fc domain is an Fc domain derived from the heavy chain of an IgG₄.

- 5 In some embodiments, the first Fc domain or the second Fc domain in a binding protein of the present invention further comprises one or more amino acid changes which reduce the binding of the Fc domain to protein A. In some embodiments, such amino acid changes are an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F] of one of the Fc domains. Both changes are derived from the sequence of human IgG3 (IgG3 does
10 not bind to protein A). These two mutations are located in the CH3 domain and are incorporated in one of the Fc domains to reduce binding to Protein A (see for example Jendeborg et al. J Immunol Methods, 1997. 201(1): p. 25-34). These two changes facilitate the removal of homodimers of heavy chains comprising these changes during protein purification.
- 15 In some embodiments, in a binding protein of the present invention, the Fc domain, which comprises a threonine (T) at position 407 [Y407T], further comprises an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F]. In this case, the other heavy chain comprises a tyrosine (Y) at position 366 [T366Y], but does not include the two changes at positions 435 and 436. Alternatively, in some embodiments, in a protein of
20 the present invention, the Fc domain, which comprises a serine (S) at position 366 [T366S], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V], further comprises an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F]. In this case, the other Fc domain comprises a tryptophan (W) at position 366 [T366W], but does not include the two changes at positions 435 and 436. Thus, the Fc
25 domain comprising the amino acid change resulting in a “cavity” as described above also comprises the amino acid changes, which reduce binding to Protein A. Homodimers comprising this Fc domain are removed through reduced binding to Protein A. The production of homodimers of the other Fc domain, which comprises the “protrusion”, is reduced by the presence of the “protrusion”.
- 30 In some embodiments, the Fc domain of a protein of the present invention may or may not further comprises YTE mutations (M252Y/S254T/T256E, Eu numbering (Dall'Acqua et al. J. Biol. Chem. 2006, 281(33):23514-24). These mutations have been shown to improve the

pharmacokinetic properties of Fc domains through preferential enhancement of binding affinity for neonatal FcRn receptor at pH 6.0.

In some embodiments, the first and/or the second Fc domain of the present invention derived from an IgG1 also includes the “KO” mutations (L234A, L235A) (Xu et al, Cellular Immunology 2000 Feb 25, 200(1):16-26). In a further aspect, the first and/or the second Fc domain of the present invention derived from an IgG4 also includes the Pro hinge mutation (S228P) (Angal et al, Molecular Immunology 1993, 30(1):105-108; Labrijn et al, Nature Biotechnology 2009, 27:767-771).

In preferred embodiments of the binding protein of the invention, the first Fc domain comprises an amino acid sequence of SEQ ID NO:242 and the second Fc domain comprises an amino acid sequence of SEQ ID NO:243.

In preferred embodiments of the invention, the binding protein comprises i) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:217 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#1/CD3#1), or ii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:218 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#2/CD3#1), or iii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:219 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#3/CD3#1), or iv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:220 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#4/CD3#1), or v) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:221 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#5/CD3#1), or vi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:222 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#6/CD3#1); or vii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:223 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#7/CD3#1); or viii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:224 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#8/CD3#1); or ix) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:225 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#9/CD3#1); or x) a first polypeptide chain

comprising the amino acid sequence of SEQ ID NO:226 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#10/CD3#1); or xi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:227 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#11/CD3#1); or xii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:228 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#12/CD3#1); or xiii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:229 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#13/CD3#1); or xiv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:230 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#14/CD3#1); or xv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:231 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#15/CD3#1), or xvi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:232 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#16/CD3#1); or xvii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:233 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#17/CD3#1); or xviii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:234 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#18/CD3#1); or xix) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:235 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#19/CD3#1); or xx) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:236 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#20/CD3#1); or xxi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:237 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#21/CD3#1); or xxii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:238 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#22/CD3#1); or xxiii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:239 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#23/CD3#1); or xxiv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:240 and a second polypeptide chain comprising the amino acid sequence of

SEQ ID NO:311 (B7H6#24/CD3#1). Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In preferred embodiments, the first polypeptide chain comprises an amino acid sequence
5 from the group consisting of any one of SEQ ID NOs:217, 218, 219, 220, 221, 228, 229,
230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 240 and the second polypeptide chain
comprises the amino acid sequence of SEQ ID NO:311. Even more preferably, the first
polypeptide chain comprises an amino acid sequence of any one of SEQ ID NOs: 228, 229,
230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 240 and the second polypeptide chain
10 comprises the amino acid sequence of SEQ ID NO:311, the first and second polypeptide
chain are linked via one or more disulfide bonds and form an antibody like structure (Figure
1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain
specific for B7H6 comprising an amino acid sequence of SEQ ID NO:228 and a second
15 polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311.

In one preferred embodiment, the binding protein comprises a first polypeptide chain
specific for B7H6 comprising an amino acid sequence of SEQ ID NO:230 and a second
polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311.

In one preferred embodiment, the binding protein comprises a first polypeptide chain
20 specific for B7H6 comprising an amino acid sequence of SEQ ID NO:231 and a second
polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311.

In one preferred embodiment, the binding protein comprises a first polypeptide chain
specific for B7H6 comprising an amino acid sequence of SEQ ID NO:232 and a second
polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311.

25 In one preferred embodiment, the binding protein comprises a first polypeptide chain
specific for B7H6 comprising an amino acid sequence of SEQ ID NO:239 and a second
polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311.

For all of the embodiments described herein it shall be understood that, by using the term
"comprising", it is intended to also include an embodiment in which the respective protein,
30 molecule, antigen binding unit or polypeptide chain "consists of" the amino acid sequence
as indicated:

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 consisting of an amino acid sequence of SEQ ID NO:228 and a second polypeptide chain specific for CD3 consisting of the amino acid sequence of SEQ ID NO:311. Preferably, the first and second polypeptide chain are linked via one or more
5 disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 consisting of an amino acid sequence of SEQ ID NO:230 and a second polypeptide chain specific for CD3 consisting of the amino acid sequence of SEQ ID
10 NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 consisting of an amino acid sequence of SEQ ID NO:231 and a second
15 polypeptide chain specific for CD3 consisting of the amino acid sequence of SEQ ID NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 consisting of an amino acid sequence of SEQ ID NO:232 and a second polypeptide chain specific for CD3 consisting of the amino acid sequence of SEQ ID
20 NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 consisting of an amino acid sequence of SEQ ID NO:239 and a second polypeptide chain specific for CD3 consisting of the amino acid sequence of SEQ ID
25 NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.
30

In a further aspect, the present invention provides a binding protein comprising a first polypeptide chain specifically binding to B7H6 (a B7H6 chain) and a second polypeptide

chain specifically binding to CD3 (a CD3 chain), wherein the first polypeptide chain specifically binding to B7H6 comprises a first light chain covalently linked (preferably directly bound) to a first linker, which is itself covalently linked (e.g., directly bound) to a first heavy chain, and wherein the second polypeptide chain specifically binding to CD3
5 comprises a second light chain covalently linked (preferably directly bound) to a second linker, which is itself covalently linked (e.g., directly bound) to a second heavy chain.

All definitions and preferred embodiments provided herein above with regard to the binding protein of the invention having the specifically recited antigen-binding units apply *mutatis mutandis* also to this binding proteins of the invention comprising a first and
10 second polypeptide chain, unless otherwise defined herein.

In some embodiments, the first polypeptide chain (also referred to herein as B7H6 chain), starting from its N-terminus, comprises a first light chain variable domain specifically binding to B7H6, a first light chain constant domain, a first linker, a first heavy chain variable domain specific for B7H6 and a first heavy chain constant region. In some
15 embodiments, the second polypeptide chain (also referred to herein as CD3 chain) starting from its N-terminus, comprises a second light chain variable domain specifically binding to CD3, a second light chain constant domain, a second linker, a second heavy chain variable domain specific for CD3 and a second heavy chain constant domain.

The resulting proteins bear a full Fc, and is larger than an IgG (due to the presence of the linker between the light chain and the heavy chain) and has two independent binding sites
20 (e.g., each binding site being monovalent for the respective antigen), a first binding site for B7H6 and a second binding site for CD3. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds. As such, the proteins of the invention are antibody-like structures, having the Y shaped structure of a conventional full length antibody
25 (see Figure 1), comprising two polypeptide chains, each comprising a scFab and a Fc domain. In preferred embodiments, the proteins of the invention comprise (i) a first polypeptide chain specific for B7H6 (a B7H6 chain) consisting of a first scFab specific for B7H6 and a first Fc domain and (ii) a second polypeptide chain specific for CD3 (a CD3 chain) consisting of a second single chain Fab specific for CD3 and a second Fc domain.

30 Preferably, the first scFab is linked to the first Fc domain via a direct covalent bond and the second scFab is linked to the second Fc domain via a direct covalent bond. This bispecific format greatly reduces heterogeneity after expression and purification (e.g. by avoiding

mispairing of light and heavy variable domains with different binding specificities), while maintaining the functional properties of the binding moieties within a structure less likely to generate unwanted immunogenic reactions. This also enables good expression of heterodimeric proteins, e.g. in mammalian cells.

- 5 In preferred embodiments of the protein of the invention, the first polypeptide chain specifically binding to B7H6 (B7H6 chain) comprises a first light chain variable domain and a first heavy chain variable domain, which comprise CDR sequences selected from the group consisting of i) to xxiv):
- 10 i) light chain CDRs comprising the amino acid sequences of SEQ ID NO:1 (CDR1), SEQ ID NO:2 (CDR2) and SEQ ID NO:3 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:4 (CDR1), SEQ ID NO:5 (CDR2) and SEQ ID NO:6 (CDR3);
 - 15 ii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:7 (CDR1), SEQ ID NO:8 (CDR2) and SEQ ID NO:9 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:10 (CDR1), SEQ ID NO:11 (CDR2) and SEQ ID NO:12 (CDR3);
 - 20 iii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:13 (CDR1), SEQ ID NO:14 (CDR2) and SEQ ID NO:15 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:16 (CDR1), SEQ ID NO:17 (CDR2) and SEQ ID NO:18 (CDR3);
 - 25 iv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:19 (CDR1), SEQ ID NO:20 (CDR2) and SEQ ID NO:21 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:22 (CDR1), SEQ ID NO:23 (CDR2) and SEQ ID NO:24 (CDR3);
 - 30 v) light chain CDRs comprising the amino acid sequences of SEQ ID NO:25 (CDR1), SEQ ID NO:26 (CDR2) and SEQ ID NO:27 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:28 (CDR1), SEQ ID NO:29 (CDR2) and SEQ ID NO:30 (CDR3);
 - vi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:31 (CDR1), SEQ ID NO:32 (CDR2) and SEQ ID NO:33 (CDR3) and heavy chain CDRs

- comprising the amino acid sequences of SEQ ID NO:34 (CDR1), SEQ ID NO:35 (CDR2) and SEQ ID NO:36 (CDR3);
- vii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:37 (CDR1), SEQ ID NO:38 (CDR2) and SEQ ID NO:39 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:40 (CDR1), SEQ ID NO:41 (CDR2) and SEQ ID NO:42 (CDR3);
- viii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:43 (CDR1), SEQ ID NO:44 (CDR2) and SEQ ID NO:45 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:46 (CDR1), SEQ ID NO:47 (CDR2) and SEQ ID NO:48 (CDR3);
- ix) light chain CDRs comprising the amino acid sequences of SEQ ID NO:49 (CDR1), SEQ ID NO:50 (CDR2) and SEQ ID NO:51 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:52 (CDR1), SEQ ID NO:53 (CDR2) and SEQ ID NO:54 (CDR3);
- x) light chain CDRs comprising the amino acid sequences of SEQ ID NO:55 (CDR1), SEQ ID NO:56 (CDR2) and SEQ ID NO:57 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:58 (CDR1), SEQ ID NO:59 (CDR2) and SEQ ID NO:60 (CDR3);
- xi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:61 (CDR1), SEQ ID NO:62 (CDR2) and SEQ ID NO:63 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:64 (CDR1), SEQ ID NO:65 (CDR2) and SEQ ID NO:66 (CDR3);
- xii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:67 (CDR1), SEQ ID NO:68 (CDR2) and SEQ ID NO:69 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:70 (CDR1), SEQ ID NO:71 (CDR2) and SEQ ID NO:72 (CDR3);
- xiii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:73 (CDR1), SEQ ID NO:74 (CDR2) and SEQ ID NO:75 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:76 (CDR1), SEQ ID NO:77 (CDR2) and SEQ ID NO:78 (CDR3);

- xiv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3);
- 5 xv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3);
- 10 xvi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3);
- 15 xvii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:97 (CDR1), SEQ ID NO:98(CDR2) and SEQ ID NO:99 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:100 (CDR1), SEQ ID NO:101 (CDR2) and SEQ ID NO:102 (CDR3);
- 20 xviii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:103 (CDR1), SEQ ID NO:104 (CDR2) and SEQ ID NO:105 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:106 (CDR1), SEQ ID NO:107 (CDR2) and SEQ ID NO:108 (CDR3);
- 25 xix) light chain CDRs comprising the amino acid sequences of SEQ ID NO:109 (CDR1), SEQ ID NO:110 (CDR2) and SEQ ID NO:111 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:112 (CDR1), SEQ ID NO:113 (CDR2) and SEQ ID NO:114 (CDR3);
- 30 xx) light chain CDRs comprising the amino acid sequences of SEQ ID NO:115 (CDR1), SEQ ID NO:116 (CDR2) and SEQ ID NO:117 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:118 (CDR1), SEQ ID NO:119 (CDR2) and SEQ ID NO:120 (CDR3);
- 30 xxi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:121 (CDR1), SEQ ID NO:122 (CDR2) and SEQ ID NO:123 (CDR3) and heavy chain

CDRs comprising the amino acid sequences of SEQ ID NO:124 (CDR1), SEQ ID NO:125 (CDR2) and SEQ ID NO:126 (CDR3);

xxii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:127 (CDR1), SEQ ID NO:128 (CDR2) and SEQ ID NO:129 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:130 (CDR1), SEQ ID NO:131 (CDR2) and SEQ ID NO:132 (CDR3);

xxiii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:133 (CDR1), SEQ ID NO:134 (CDR2) and SEQ ID NO:135 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:136 (CDR1), SEQ ID NO:137 (CDR2) and SEQ ID NO:138 (CDR3); and

xxiv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:139 (CDR1), SEQ ID NO:140 (CDR2) and SEQ ID NO:141 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:142 (CDR1), SEQ ID NO:143 (CDR2) and SEQ ID NO:144 (CDR3).

The respective light/heavy chain variable domains defined by these CDR sequences are termed B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23 and B7H6#24, respectively.

In preferred embodiments of the binding protein of the invention, said second polypeptide chain specifically binding to CD3 (CD3 chain) comprises a second light chain variable domain and second heavy chain variable domain, which comprises CDR sequences selected from the group consisting of:

i) light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3);

ii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:263 (CDR1), SEQ ID NO:264 (CDR2) and SEQ ID NO:265 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:266 (CDR1), SEQ ID NO:267 (CDR2) and SEQ ID NO:268 (CDR3);

- iii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:269 (CDR1), SEQ ID NO:270 (CDR2) and SEQ ID NO:271 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:272 (CDR1), SEQ ID NO:273 (CDR2) and SEQ ID NO:274 (CDR3);
- 5 iv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:275 (CDR1), SEQ ID NO:276 (CDR2) and SEQ ID NO:277 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:278 (CDR1), SEQ ID NO:279 (CDR2) and SEQ ID NO:280 (CDR3);
- v) light chain CDRs comprising the amino acid sequences of SEQ ID NO:281 (CDR1), SEQ ID NO:282 (CDR2) and SEQ ID NO:283 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:284 (CDR1), SEQ ID NO:285 (CDR2) and SEQ ID NO:286 (CDR3); and
- 10 vi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:287 (CDR1), SEQ ID NO:288 (CDR2) and SEQ ID NO:289 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:290 (CDR1), SEQ ID NO:291 (CDR2) and SEQ ID NO:292 (CDR3).

The respective light/heavy chain variable domains defined by these CDR sequences are termed CD3#1, CD3#2, CD3#3, CD3#4, CD3#5 and CD3#6, respectively.

Preferably, the light chain and heavy chain CDR sequences are selected from the group
 20 consisting of B7H6#1, B7H6#2, B7H6#, B7H6#4, B7H6#5, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23 and B7H6#24 as defined above.

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6 (B7H6 chain) , comprising a first light
 25 chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:67 (CDR1), SEQ ID NO:68 (CDR2) and SEQ ID NO:69 (CDR3) and a first heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:70 (CDR1), SEQ ID NO:71 (CDR2) and SEQ ID NO:72 (CDR3); and (ii) a second polypeptide chain specifically binding to CD3, comprising a second light chain
 30 variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and a second

heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6 (B7H6 chain) , comprising a first light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and a first heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3); and (ii) a second polypeptide chain specifically binding to CD3, comprising a second light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and a second heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6, comprising a first light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and a first heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3); and (ii) a second polypeptide chain specifically binding to CD3, comprising a second light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and a second heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6, comprising a first light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and a first heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3); and (ii) a second polypeptide chain specifically binding to CD3, comprising a second light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:257

(CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and a second heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6, comprising a first light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:133 (CDR1), SEQ ID NO:134 (CDR2) and SEQ ID NO:135 (CDR3) and a first heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:136 (CDR1), SEQ ID NO:137 (CDR2) and SEQ ID NO:138 (CDR3); and (ii) a second polypeptide chain specifically binding to CD3, comprising a second light chain variable domain with light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and a second heavy chain variable domain with heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).

In preferred embodiments of the protein of the invention, said first polypeptide chain specifically binding to B7H6 (B7H6 chain) comprises a light chain variable domain (a first light chain variable domain) and a heavy chain variable domain (a first heavy chain variable domain) selected from the group consisting of i) to xiv):

- i) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:145 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:146 (B7H6#1);
- ii) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:147 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:148 (B7H6#2);
- iii) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:149 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:150 (B7H6#3);
- iv) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:151 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:152 (B7H6#4);

- v) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:153 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:154 (B7H6#5);
- 5 vi) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:155 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:156 (B7H6#6);
- vii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:157 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:158 (B7H6#7);
- 10 viii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:159 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:160 (B7H6#8);
- ix) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:161 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:162 (B7H6#9);
- 15 x) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:163 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:164 (B7H6#10);
- xi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:165 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:166 (B7H6#11);
- 20 xii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:167 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168 (B7H6#12);
- 25 xiii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:169 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:170 (B7H6#13);
- xiv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:171 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172 (B7H6#14);
- 30

- xv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:173 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:174 (B7H6#15);
- 5 xvi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:175 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:176 (B7H6#16);
- xvii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:177 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:178 (B7H6#17);
- 10 xviii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:179 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:180 (B7H6#18);
- xix) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:181 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:182 (B7H6#19);
- 15 xx) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:183 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:184 (B7H6#20);
- xxi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:185 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:186 (B7H6#21);
- 20 xxii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:187 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:188 (B7H6#22);
- xxiii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:189 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:190 (B7H6#23); and
- 25 xxiv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:191 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:192 (B7H6#24).
- 30

Preferably, the light chain variable and heavy chain variable domain sequences are selected from the group consisting of B7H6#1, B7H6#2, B7H6#, B7H6#4, B7H6#5, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23 and B7H6#24 as defined above.

- 5 In preferred embodiments of the protein of the invention, said second polypeptide chain specifically binding to CD3 (CD3 chain) comprises a light chain variable domain (a second light chain variable domain) and a heavy chain variable domain (a second heavy chain variable domain) selected from the group consisting of:
- 10 i) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294 (CD3#1);
 - ii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:295 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:296 (CD3#2);
 - 15 iii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:297 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:298 (CD3#3)
 - iv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:299 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:300 (CD3#4)
 - 20 v) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:301 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:302 (CD3#5)
 - vi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:303 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:304 (CD3#6).
 - 25

In some embodiments, the binding protein of the invention comprises a first and a second polypeptide chain comprising CDR and/or VH and VL sequences of the light/heavy chain variable domains selected from the list consisting of B7H6#1/CD3#1, B7H6#2/CD3#1, 30 B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#6/CD3#1, B7H6#7/CD3#1, B7H6#8/CD3#1, B7H6#9/CD3#1, B7H6#10/CD3#1, B7H6#11/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1,

B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1, In preferred embodiments, the binding protein of the invention comprises a first and a second polypeptide chain comprising CDR and/or VH and VL sequences of the light/heavy chain variable domains selected from the list consisting of B7H6#12/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#23/CD3#1. Even more preferably, the first polypeptide chain comprises an Fc domain comprising an amino acid sequence of SEQ ID NO:242 and said second polypeptide chain comprises an Fc domain comprising an amino acid sequence of SEQ ID NO:243.

10 In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6 (B7H6 chain) comprising a light chain variable domain of SEQ ID NO:167 and heavy chain variable domain of SEQ ID NO:168; and (ii) a second polypeptide chain specifically binding to CD3 (CD3 chain), comprising a light chain variable domain of SEQ ID NO:293 and a heavy chain variable domain of SEQ
15 ID NO:294.

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6 (B7H6 chain), comprising a light chain variable domain of SEQ ID NO:171 and heavy chain variable domain of SEQ ID NO:172; and (ii) a second polypeptide chain specifically binding to CD3 (CD3 chain), comprising a
20 light chain variable domain of SEQ ID NO:293 and a heavy chain variable domain of SEQ ID NO:294.

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6 (B7H6 chain), comprising a light chain variable domain of SEQ ID NO:173 and heavy chain variable domain of SEQ ID NO:174; and (ii) a second polypeptide chain specifically binding to CD3 (CD3 chain), comprising a
25 light chain variable domain of SEQ ID NO:293 and a heavy chain variable domain of SEQ ID NO:294.

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6 (B7H6 chain), comprising a light chain
30 variable domain of SEQ ID NO:175 and heavy chain variable domain of SEQ ID NO:176; and (ii) a second polypeptide chain specifically binding to CD3 (CD3 chain), comprising a

light chain variable domain of SEQ ID NO:293 and a heavy chain variable domain of SEQ ID NO:294.

In one preferred embodiment, the binding protein of the invention comprises (i) a first polypeptide chain specifically binding to B7H6 (B7H6 chain), comprising a light chain variable domain of SEQ ID NO:189 and heavy chain variable domain of SEQ ID NO:190;
5 and (ii) a second polypeptide chain specifically binding to CD3 (CD3 chain), comprising a light chain variable domain of SEQ ID NO:293 and a heavy chain variable domain of SEQ ID NO:294.

In preferred embodiments, the first polypeptide chain specific for B7H6 comprises a single
10 chain Fab with the amino acid sequence of any one of SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214,
15 SEQ ID NO:215 or SEQ ID NO:216 and the second polypeptide chain specific for CD3 comprises a single chain Fab with the amino acid sequence of SEQ ID NO:305.

Preferably, the first polypeptide chain comprises a single chain Fab comprising an amino acid sequence selected from the group consisting of SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:204, SEQ ID NO:205,
20 SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215 and SEQ ID NO:216, more preferably an amino acid sequence selected from the group consisting of SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ
25 ID NO:213, SEQ ID NO:214, SEQ ID NO:215 and SEQ ID NO:216, and the second polypeptide chain comprises a single chain Fab comprising an amino acid sequence of SEQ ID NO:305.

In one preferred embodiment, the first polypeptide chain specific for B7H6 (B7H6 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:204 and
30 the second polypeptide chain specific for CD3 (CD3 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:305.

In one preferred embodiment, the first polypeptide chain specific for B7H6 (B7H6 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:206 and the second polypeptide chain specific for CD3 (CD3 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:305.

- 5 In one preferred embodiment, the first polypeptide chain specific for B7H6 (B7H6 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:207 and the second polypeptide chain specific for CD3 (CD3 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:305.

- 10 In one preferred embodiment, the first polypeptide chain specific for B7H6 (B7H6 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:208 and the second polypeptide chain specific for CD3 (CD3 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:305.

- 15 In one preferred embodiment, the first polypeptide chain specific for B7H6 (B7H6 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:215 and the second polypeptide chain specific for CD3 (CD3 chain) comprises a single chain Fab comprising the amino acid sequence of SEQ ID NO:305.

Also with regard to this specific embodiment directed to scFabs, it is intended that the term comprising also includes "consisting of" the amino acid sequence as defined herein above in more general terms.

- 20 In some embodiments of binding protein of the invention, the first and second polypeptide chain comprises an Fc domain derived from the heavy chain of an IgG, for example an IgG1, IgG2 or IgG4. For example, an Fc domain of the present invention is a Fc domain of a heavy chain of an IgG1 or IgG4 and comprises a hinge region and two constant domains (CH2 and CH3). Examples of Fc domains of human IgGs are shown in SEQ ID NO:241
25 and SEQ ID NO:244.

- In some embodiments of the binding protein of the invention, the heavy chain comprises one or more amino acid changes. For example, such amino acid changes are a tyrosine (Y) at position 366 [T366Y] of the first heavy chain and a threonine (T) at position 407 [Y407T] of the second heavy chain. In some embodiments, the first heavy chain comprises
30 a serine (S) at position 366 [T366S] and the second heavy chain comprises a tryptophan (W) at position 366 [T366W], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V]. In preferred embodiments, the first heavy chain comprises a

- tryptophan (W) at position 366 [T366W] and the second heavy chain comprises a serine (S) at position 366 [T366S], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V]. For example, position 366 of the Fc domain according to Eu numbering, corresponding to the amino acid position 146 in the human IgG1 Fc sequence of SEQ ID NO:241, is changed from T at position 146 in SEQ ID NO:241 to W at position 146 in SEQ ID NO:242; and positions 366, 368 and 407 according to Eu numbering, corresponding to the amino acid positions 146, 148 and 187, respectively, in SEQ ID NO:241, are changed from T, L and Y at these positions in SEQ ID NO:241 to S, A and V at these positions in SEQ ID NO:243. In any of these embodiments, the amino acid changes described for the first heavy chain may be located in the second heavy chain and the respective amino acid changes for the second heavy chain may be located in the first heavy chain. In other words, the term “first” and “second” can be exchanged in these embodiments. In some embodiments, the heavy chain is derived from the heavy chain of an IgG₁ or IgG₄.
- 15 In some embodiments, the first heavy chain or the second heavy chain in a protein of the present invention further comprises one or more amino acid changes which reduce the binding of the heavy chain to protein A. In some embodiments, such amino acid changes are an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F] of one of the heavy chains.
- 20 In some embodiments, in a protein of the present invention, the heavy chain, which comprises a threonine (T) at position 407 [Y407T], further comprises an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F]. In this case, the other heavy chain comprises a tyrosine (Y) at position 366 [T366Y], but does not include the two changes at positions 435 and 436. Alternatively, in some embodiments, in a protein of the present invention, the heavy chain, which comprises a serine (S) at position 366 [T366S], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V], further comprises an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F]. In this case, the other heavy chain comprises a tryptophan (W) at position 366 [T366W], but does not include the two changes at positions 435 and 436. Thus, the heavy chain comprising the amino acid change resulting in a “cavity” as described above also comprises the amino acid changes, which reduce binding to Protein A. Homodimers comprising these heavy chains are removed through reduced binding to Protein A. The
- 30

production of homodimers of the other heavy chain, which comprises the “protrusion”, is reduced by the presence of the “protrusion”.

In some embodiments, the heavy chain of a protein of the present invention may or may not further comprise YTE mutations (M252Y/S254T/T256E, Eu numbering (Dall'Acqua et al., J. Biol. Chem. 2006, 281(33):23514-24)). These mutations have been shown to improve
5 the pharmacokinetic properties of heavy chain through preferential enhancement of binding affinity for neonatal FcRn receptor at pH 6.0.

In some embodiments, the first and/or the second heavy chain of the present invention derived from an IgG1 also includes the “KO” mutations (L234A, L235A) (Xu et al,
10 Cellular Immunology 2000 Feb 25, 200(1):16-26). In a further aspect, the first and/or the second heavy chain of the present invention derived from an IgG4 also includes the Pro hinge mutation (S228P) (Angal et al, Molecular Immunology 1993, 30(1):105-108; Labrijn et al, Nature Biotechnology 2009, 27:767-771).

In preferred embodiments of the binding protein of the invention, the first polypeptide
15 chain comprises an Fc domain comprising an amino acid sequence of SEQ ID NO:242 and the second polypeptide chain comprises an Fc domain comprising an amino acid sequence of SEQ ID NO:243.

In preferred embodiments of the invention, the binding protein comprises i) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:217 and a second
20 polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#1/CD3#1), or ii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:218 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#2/CD3#1), or iii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:219 and a second polypeptide chain comprising the amino
25 acid sequence of SEQ ID NO:311 (B7H6#3/CD3#1), or iv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:220 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#4/CD3#1), or v) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:221 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311
30 (B7H6#5/CD3#1), or vi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:222 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#6/CD3#1); or vii) a first polypeptide chain comprising the amino

acid sequence of SEQ ID NO:223 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#7/CD3#1); or viii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:224 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#8/CD3#1); or ix) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:225 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#9/CD3#1); or x) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:226 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#10/CD3#1); or xi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:227 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#11/CD3#1); or xii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:228 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#12/CD3#1); or xiii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:229 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#13/CD3#1); or xiv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:230 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#14/CD3#1); or xv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:231 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#15/CD3#1), or xvi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:232 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#16/CD3#1); or xvii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:233 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#17/CD3#1); or xviii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:234 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#18/CD3#1); or xix) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:235 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#19/CD3#1); or xx) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:236 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#20/CD3#1); or xxi) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:237 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311

(B7H6#21/CD3#1); or xxii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:238 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#22/CD3#1); or xxiii) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:239 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#23/CD3#1); or xxiv) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO:240 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO:311 (B7H6#24/CD3#1). Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 comprising an amino acid sequence of SEQ ID NO:228 and a second polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 comprising an amino acid sequence of SEQ ID NO:230 and a second polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 comprising an amino acid sequence of SEQ ID NO:231 and a second polypeptide chain specific for CD3 comprising of the amino acid sequence of SEQ ID NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In one preferred embodiment, the binding protein comprises a first polypeptide chain specific for B7H6 comprising an amino acid sequence of SEQ ID NO:232 and a second polypeptide chain specific for CD3 comprising the amino acid sequence of SEQ ID NO:311. Preferably, the first and second polypeptide chain are linked via one or more disulfide bonds

and form an antibody like structure (Figure 1) similar to a conventional Y-shaped antibody molecule.

In a further aspect, the proteins of the invention comprise a first antigen binding unit or polypeptide chain specific for B7H6 with an affinity of preferably $\leq 10\text{nM}$, more preferably $\leq 1\text{nM}$, even more preferable $\leq 0.1\text{nM}$, to human and cynomolgus monkey B7H6. The affinity can be measured in a SPR (BIAcore® SPR system (GE Healthcare Life Sciences)) assay using recombinant B7H6-protein, as described, e.g. in the examples or other methods that are well known for the skilled person. The proteins comprise a second antigen binding unit or polypeptide chain with an affinity of preferably $\leq 500\text{ nM}$, more preferably $\leq 100\text{ nM}$, even more preferably $\leq 10\text{ nM}$ to human and cynomolgus monkey CD3 ϵ complex.

In a further aspect, the B7H6/CD3 binding proteins of the invention do not bind to B7H6-negative cells and do not cross-react with B7H1 (see e.g. example 10 and example 4, respectively).

In preferred embodiments, the B7H6/CD3 binding proteins of the present invention (e.g. any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) do not inhibit activation of natural killer cells. Notably, B7H6/CD3 binding proteins of the invention, which do not inhibit activation of natural killer cells *in vitro*, bind to B7H6 in which the NKp30 interaction sites were substituted with alanine.

B7H6 on the cell surface binds to NKp30 on the cell surface of NK cells, which triggers NKp30-mediated activation of NK cells, NK cell cytotoxicity and cytokine secretion (Brandt et al, J.Exp. Med.2009, 206(7):1495-503). This situation can be mimicked *in vitro* by cultivation of NK cell lines (e.g. NK92MI) or primary NK cells on plates coated with recombinant B7H6 extracellular domain protein with subsequent analysis of upregulation of activation markers such as CD25 or CD69 or cytokine secretion by NK cells. This assay setting was used to assess whether our B7H6/CD3 binding proteins inhibit the interaction of B7H6 and NKp30 resulting in inhibition of IFN γ secretion (Example 11).

Using recombinant Ala-mutated B7H6 extracellular proteins; in which the NKp30 interaction sites were substituted with alanine, it was seen that there are two groups of binding proteins: 1) binding proteins that potently bind to wild-type B7H6 but which do not or only weakly bind to recombinant Ala-mutated B7H6 extracellular proteins were found to inhibit the B7H6 dependent secretion of IFN γ by NK cells in vitro (“inhibitors of B7H6 dependent NK cell activation”) and 2) binding proteins that potently bind to wild-type B7H6 and maintain the ability to bind also to the recombinant Ala-mutated B7H6 extracellular proteins were found to not inhibit the B7H6 dependent activation of NK cells and associated IFN γ secretion in vitro (“non-inhibitors of B7H6 dependent NK cell activation”) (See examples 6 and 11, Figures 4 and 9). Surprisingly binding proteins of the invention which are non-inhibitors of B7H6 dependent NK cell activation are more potent in T cell redirected lysis of B7H6 expressing tumor cells (see example 12, Figures 10 and 11). Without being bound by theory, it is likely that non-inhibitors of B7H6 dependent NK cell activation allow B7H6 NKp30 interaction without impacting the natural role of B7H6 in mediating innate immunity.

In a further aspect, the B7H6/CD3 binding proteins of the present invention are capable of mediating T cell redirected cytotoxicity against tumor cells independent of NK cell activity (as shown in the mouse xenograft model where no NK cells are present, see Example 19, Figures 20 and 23, as well as in the cell lysis assay in the absence of NK cells, see Example 12, Figures 10 and 11).

Various methods can be used to measure the cytotoxicity mediated by the B7H6/CD3 binding proteins of the present invention. For example, cytotoxicity can be measured using the method described in example 12. Effector cells can be e.g. stimulated or unstimulated (human or cynomolgus monkey) T cells or their subsets (e.g. CD4, CD8) or unstimulated (human or cynomolgus monkey) peripheral blood mononuclear cells (PBMCs). The target cells should express at least the extracellular domain of (human or cynomolgus monkey) B7H6 and can be cells with endogenous (natural) B7H6 expression, such as human small cell lung carcinoma cell lines SHP77, NCI-H82, alternatively also recombinant cells that express either the full-length B7H6 or the extracellular domain of B7H6. The effector to target cell ratio (E:T) is usually about 10:1 but can vary. Cytotoxic activity of B7H6/CD3 binding molecules can be determined e.g. in a LDH-release assay after 48 or 72 hours of incubation. Modifications in incubation time and read-out used for determination of cytotoxicity are possible and known to the skilled person. Read-out systems for

cytotoxicity can comprise MTT/MTS assays, ATP-based assays, FACS-based assays, 51-Chromium release assays, sulforhodamine B (SRB) assays, colorimetric (WST) assays, clonogenic assays, ECIS technology and bioluminescent assays.

The cytotoxic activity mediated by B7H6/CD3 binding proteins of the present invention is preferably measured in a cell-based cytotoxicity assay. The cytotoxicity is represented by the EC₉₀ values measured in the cytotoxicity assay. The skilled person is aware that an EC₉₀ can be expected to be lower when purified T cells are used as effector cells, compared with PBMCs, the skilled person is also aware that the EC₉₀ can be even lower when stimulated T cells are used. It can furthermore expected that the EC₉₀ values are lower when the target cells express a high number of B7H6 on the cell surface compared to cell expressing a low number of B7H6 molecules on the cell surface. The EC₉₀ of the B7H6/CD3 binding protein is preferably ≤ 10 nM, more preferably ≤ 5 nM and even more preferably ≤ 1 nM.

Preferably, the multi-specific binding proteins of the invention do not induce/mediate lysis of B7H6 negative cells. The term “do not induce/mediate lysis” of B7H6-negative cells means that an B7H6/CD3 binding molecule does not induce or mediate lysis of more than 30%, preferably not more than 20%, more preferably not more than 10% and particular not more than 5% of B7H6-negative cells, whereas lysis of the B7H6-positive colorectal cell line is set to be 100%. This usually applies for concentrations of the binding protein of up to 1000 nM.

Furthermore, the B7H6/CD3 binding proteins of the invention are shown to reach a monomer content of above 95% in a two-step purification process (see example 20), have favorable pharmacokinetic properties and good downstream manufacturability and are further expected to have good bio-distribution (see e.g., example 18). The proteins of the present invention furthermore have a favorable immunogenicity profile (see example 22) and have good stability in-vitro and in-vivo (see e.g., examples 21 and 18). Furthermore, the B7H6/CD3 binding proteins of the invention show favorable efficacy in a humanized *in vivo* xenograft mouse model. B7H6/CD3 binding proteins induced strong tumor regression starting already after the first dose of B7H6/CD3 binding proteins (see e.g., examples 19). Furthermore the B7H6/CD3 binding proteins of the invention induce tumor regression at very low doses of 0.05 mg/kg administered once weekly (q7d), further supporting their therapeutic applicability. In particular, the B7H6/CD3 binding proteins of the invention

induce selective T cell proliferation, T cell activation, T cell degranulation and cytokine secretion (see examples 16, 14, 15, 17, respectively) only in the presence of B7H6-positive target cells and not in the presence of B7H6-negative target cells, and further significantly increase T cell infiltration into tumor tissue (see example 24).

- 5 A further aspect of the present invention provides isolated nucleic acid molecules encoding the first and/or the second antigen binding unit (any one of the antigen binding units B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22, B7H6#23, and B7H6#24 and/or any
- 10 one of the antigen binding units CD3#1, CD3#2, CD3#3, CD3#4, CD3#5, and CD3#6 as defined by CDR, VH/VL or scFab sequences as shown in Table 1, respectively) of a multi-specific binding protein of the invention. In some embodiments, the nucleic acid molecules further encode a first and/or a second Fc domain as described herein, the first and/or
- 15 second Fc domain linked to the 3' end of the nucleic acid molecule encoding the first and/or second antigen binding unit, respectively. In some embodiments, the nucleic acid molecule encodes i) a first polypeptide chain comprising a first single chain Fab specific for B7H6 (e.g., any one of B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20, B7H6#21, B7H6#22,
- 20 B7H6#23, and B7H6#24), and a first Fc domain and/or ii) a second polypeptide chain comprising a second single chain Fab specific for CD3 (e.g., any one of CD3#1, CD3#2, CD3#3, CD3#4, CD3#5, and CD3#6, preferably CD3#1) and a second Fc domain.

Preferably the nucleic acid molecule comprises a nucleotide sequence encoding a first single chain Fab specific for B7H6 of any one of SEQ ID NO:193, SEQ ID NO:194, SEQ

25 ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215 or SEQ ID NO:216 and/or a second single chain Fab of SEQ ID NO:305. In

30 preferred embodiments, the nucleic acid molecule comprises the nucleotide sequence encoding a first scFab specific for B7H6 of any one of SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, or SEQ ID NO:215, and/or the nucleotide sequence

encoding a second scFab specific for CD3 comprising the amino acid sequence of SEQ ID NO:305.

A further aspect of the invention provides an expression vector containing a DNA molecule comprising the nucleotide sequence encoding the first and/or second antigen binding domain (e.g. a first and/or second single chain Fab of the invention). Preferably the expression vector comprises, in addition, a nucleic acid molecule, preferably a DNA molecule, encoding a first and/or second Fc domain, linked to the nucleic acid molecule, preferably the DNA molecule, encoding the first and/or second antigen binding domain (e.g. first and/or second single chain Fab) respectively. As such, the expression vector comprises a nucleotide sequence encoding a polypeptide chain comprising a first single chain Fab linked to a first Fc domain and/or a nucleotide sequence encoding a polypeptide chain comprising a second single chain Fab linked to a second Fc domain.

In a preferred embodiment, the expression vector contains a DNA molecule comprising the nucleotide sequence encoding the first polypeptide chain specific for B7H6 and/or the second polypeptide chain specific for CD3 of the invention. In a preferred embodiment, the expression vector comprises the nucleotide sequence encoding a first polypeptide chain of any one of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO: 224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239 or SEQ ID NO: 240 and/or the nucleotide sequence encoding a second polypeptide chain comprising SEQ ID NO:311.

In further preferred embodiments, the expression vector comprises the nucleotide sequence encoding a first polypeptide chain of any one of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, and SEQ ID NO:239 and/or the nucleotide sequence encoding a second polypeptide chain comprising SEQ ID NO:311.

In a specifically preferred embodiment, two expression vectors may be used, one of them for expression of the first polypeptide chain specific for B7H6, the other one for expression of the second polypeptide chain specific for CD3, which two expression vectors may then both be transfected into a host cell for recombinant protein expression.

Preferably, the expression vector will be a vector comprising said nucleic acid molecule or molecules, operably linked to at least one regulatory sequence, wherein such regulatory

sequence may be a promoter, enhancer, or terminator sequence, and most preferably a heterologous promoter, enhancer, or terminator sequence.

In another aspect, the invention relates to a host cell having an expression vector encoding a first polypeptide chain specific for B7H6 of the invention and an expression vector
5 encoding a second polypeptide chain specific for CD3 of the invention.

According to a particularly preferred embodiment, said host cells are eukaryotic cells such as mammalian cells. In another embodiment, such host cells are bacterial cells. Other useful cells are yeast cells or other fungal cells.

Suitable mammalian cells include for example CHO cells, BHK cells, HeLa cells, COS
10 cells, and the like. However, amphibian cells, insect cells, plant cells, and any other cells used in the art for the expression of heterologous proteins can be used as well.

ANTI-B7H6 ANTIBODIES

A further aspect of the invention provides anti-B7H6 antibody molecules comprising

- 15 i) light chain CDRs comprising the amino acid sequences of SEQ ID NO:1 (CDR1), SEQ ID NO:2 (CDR2) and SEQ ID NO:3 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:4 (CDR1), SEQ ID NO:5 (CDR2) and SEQ ID NO:6 (CDR3); or
- 20 ii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:7 (CDR1), SEQ ID NO:8 (CDR2) and SEQ ID NO:9 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:10 (CDR1), SEQ ID NO:11 (CDR2) and SEQ ID NO:12 (CDR3); or
- 25 iii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:13 (CDR1), SEQ ID NO:14 (CDR2) and SEQ ID NO:15 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:16 (CDR1), SEQ ID NO:17 (CDR2) and SEQ ID NO:18 (CDR3); or
- 30 iv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:19 (CDR1), SEQ ID NO:20 (CDR2) and SEQ ID NO:21 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:22 (CDR1), SEQ ID NO:23 (CDR2) and SEQ ID NO:24 (CDR3); or

- v) light chain CDRs comprising the amino acid sequences of SEQ ID NO:25 (CDR1), SEQ ID NO:26 (CDR2) and SEQ ID NO:27 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:28 (CDR1), SEQ ID NO:29 (CDR2) and SEQ ID NO:30 (CDR3); or
- 5 vi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:31 (CDR1), SEQ ID NO:32 (CDR2) and SEQ ID NO:33 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:34 (CDR1), SEQ ID NO:35 (CDR2) and SEQ ID NO:36 (CDR3); or
- vii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:37 (CDR1), SEQ ID NO:38 (CDR2) and SEQ ID NO:39 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:40 (CDR1), SEQ ID NO:41 (CDR2) and SEQ ID NO:42 (CDR3); or
- 10 viii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:43 (CDR1), SEQ ID NO:44 (CDR2) and SEQ ID NO:45 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:46 (CDR1), SEQ ID NO:47 (CDR2) and SEQ ID NO:48 (CDR3); or
- 15 ix) light chain CDRs comprising the amino acid sequences of SEQ ID NO:49 (CDR1), SEQ ID NO:50 (CDR2) and SEQ ID NO:51 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:52 (CDR1), SEQ ID NO:53 (CDR2) and SEQ ID NO:54 (CDR3); or
- 20 x) light chain CDRs comprising the amino acid sequences of SEQ ID NO:55 (CDR1), SEQ ID NO:56 (CDR2) and SEQ ID NO:57 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:58 (CDR1), SEQ ID NO:59 (CDR2) and SEQ ID NO:60 (CDR3); or
- 25 xi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:61 (CDR1), SEQ ID NO:62 (CDR2) and SEQ ID NO:63 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:64 (CDR1), SEQ ID NO:65 (CDR2) and SEQ ID NO:66 (CDR3); or
- 30 xii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:67 (CDR1), SEQ ID NO:68 (CDR2) and SEQ ID NO:69 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:70 (CDR1), SEQ ID NO:71 (CDR2) and SEQ ID NO:72 (CDR3); or

- xiii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:73 (CDR1), SEQ ID NO:74 (CDR2) and SEQ ID NO:75 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:76 (CDR1), SEQ ID NO:77 (CDR2) and SEQ ID NO:78 (CDR3); or
- 5 xiv) comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3); or
- xv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3); or
- 10 xvi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3); or
- 15 xvii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:97 (CDR1), SEQ ID NO:98 (CDR2) and SEQ ID NO:99 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:100 (CDR1), SEQ ID NO:101 (CDR2) and SEQ ID NO:102 (CDR3); or
- 20 xviii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:103 (CDR1), SEQ ID NO:104 (CDR2) and SEQ ID NO:105 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:106 (CDR1), SEQ ID NO:107 (CDR2) and SEQ ID NO:108 (CDR3); or
- 25 xix) light chain CDRs comprising the amino acid sequences of SEQ ID NO:109 (CDR1), SEQ ID NO:110 (CDR2) and SEQ ID NO:111 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:112 (CDR1), SEQ ID NO:113 (CDR2) and SEQ ID NO:114 (CDR3); or
- 30 xx) light chain CDRs comprising the amino acid sequences of SEQ ID NO:115 (CDR1), SEQ ID NO:116 (CDR2) and SEQ ID NO:117 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:118 (CDR1), SEQ ID NO:119 (CDR2) and SEQ ID NO:120 (CDR3); or

- xxi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:121 (CDR1), SEQ ID NO:122 (CDR2) and SEQ ID NO:123 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:124 (CDR1), SEQ ID NO:125 (CDR2) and SEQ ID NO:126 (CDR3); or
- 5 xxi) light chain CDRs comprising the amino acid sequences of SEQ ID NO:127 (CDR1), SEQ ID NO:128 (CDR2) and SEQ ID NO:129 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:130 (CDR1), SEQ ID NO:131 (CDR2) and SEQ ID NO:132 (CDR3); or
- xxiii) light chain CDRs comprising the amino acid sequences of SEQ ID NO:133 (CDR1),
 10 SEQ ID NO:134 (CDR2) and SEQ ID NO:135 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:136 (CDR1), SEQ ID NO:137 (CDR2) and SEQ ID NO:138 (CDR3); or
- xxiv) light chain CDRs comprising the amino acid sequences of SEQ ID NO:139 (CDR1), SEQ ID NO:140 (CDR2) and SEQ ID NO:141 (CDR3) and heavy chain CDRs
 15 comprising the amino acid sequences of SEQ ID NO:142 (CDR1), SEQ ID NO:143 (CDR2) and SEQ ID NO:144 (CDR3).

The antibodies i) to xxiv) as outlined above are termed B7H6#1, B7H6#2, B7H6#3, B7H6#4, B7H6#5, B7H6#6, B7H6#7, B7H6#8, B7H6#9, B7H6#10, B7H6#11, B7H6#12, B7H6#13, B7H6#14, B7H6#15, B7H6#16, B7H6#17, B7H6#18, B7H6#19, B7H6#20,
 20 B7H6#21, B7H6#22, B7H6#23, and B7H6#24, respectively. Provided herein is a sequence table which readily allows identification of individual amino acid sequences to specific antibodies of the present invention.

In some embodiments, the anti-B7H6 antibody of the invention is a chimeric, a humanized a human or an optimized antibody molecule. In some embodiments, the antibody molecule
 25 is a monoclonal antibody Fab, F(ab)₂, Fv or scFv. In some embodiments, the anti-B7H6 antibody molecule of the invention comprises a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions. In some embodiments, the light chain constant region of the anti-B7H6 antibody molecule of the invention is kappa or lambda.

30 In some embodiments, the anti-B7H6 antibody of the invention has a heavy chain variable domain comprising an amino acid sequence at least 85% identical to any one of SEQ ID NOs:146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178,

180, 182, 184, 186, 188, 190, and 192. Preferably, the antibody molecule has a heavy chain variable domain comprising an amino acid sequence of SEQ ID NOs: 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190 or 192.

- 5 In some embodiments, the anti-B7H6 antibody molecule has a light chain variable domain comprising an amino acid sequence at least 85% identical to any one of SEQ ID NOs: 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, and 191. Preferably, the antibody molecule has a light chain variable domain comprising an amino acid sequence of SEQ ID NOs: 145, 147, 149, 151, 153, 155,
10 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, and 191.

Methods of calculating amino acid sequence identities are well known in the art and further discussed herein in the Definitions section of the specification.

- In some embodiments, the anti-B7H6 antibody molecule has i) a heavy chain variable
15 domain comprising the amino acid sequence of SEQ ID NO:146 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:145 (B7H6#1), or ii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 148 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 147 (B7H6#2); or iii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID
20 NO:150 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:149 (B7H6#3), or iv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:152 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:151 (B7H6#4); or v) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:154 and a light chain variable domain comprising
25 the amino acid sequence of SEQ ID NO:153 (B7H6#5); or vi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:156 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:155 (B7H6#6); or vii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:158 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:157 (B7H6#7);
30 or viii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:160 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:159 (B7H6#8); or ix) a heavy chain variable domain comprising the amino acid

sequence of SEQ ID NO:162 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:161 (B7H6#9); or x) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:164 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:163 (B7H6#10); or xi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:166 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:165 (B7H6#11); or xii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:167 (B7H6#12); or xiii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:170 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:169 (B7H6#13); or xiv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:171 (B7H6#14); or xv) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:174 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:173 (B7H6#15); or xvi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:176 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:175 (B7H6#16); or xvii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:178 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:177 (B7H6#17); or xviii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:180 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:179 (B7H6#18); or xix) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:182 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:181 (B7H6#19); or xx) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:184 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:183 (B7H6#20); or xxi) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:186 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:185 (B7H6#21); or xxii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:188 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:187 (B7H6#22); or xxiii) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:190 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:189 (B7H6#23); or xxiv) a heavy

chain variable domain comprising the amino acid sequence of SEQ ID NO:192 and light chain variable domain comprising the amino acid sequence of SEQ ID NO:191 (B7H6#24).

In some embodiments, the anti-B7H6 antibody of the invention is a mouse monoclonal antibody. In the context of this invention a mouse monoclonal antibody includes an antibody where the VH and VL are obtained from immunization of mice with human B7H6 protein, subsequent selection of suitable VH and VL sequences binding with certain affinity to human B7H6, and then further joining such VH and VL sequences to constant domains which are derived from mouse (e.g., from mouse IgG2a) by recombinant techniques; and which are produced by recombinant expression in host cells. Further encompassed by the invention are chimeric antibodies, e.g., comprising variable and constant regions from different species. In some embodiments, the antibody molecule of the invention is a chimeric antibody comprising VH and VL domains derived from mouse as described above and further comprising constant domains derived from another species such as human, rabbit, rat, goat, donkey. In some embodiments, the chimeric antibody comprises VH and VL domains derived from mouse and further humanized or sequence optimized as defined above and further comprises constant domains derived from another species. In some embodiments, the chimeric antibody comprises VH and VL domains derived from a transgenic animal (e.g. a mouse) comprising human IgG sequences, thus comprises human VH and VL sequences, and further comprises constant domains derived from another species. In any of the embodiments of chimeric antibodies as outlined above, the heavy chain constant region is a mouse, human, rabbit, rat, goat or donkey heavy chain region.

In some embodiments, the anti-B7H6 antibody molecule of the invention has a constant domain selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant domains. In a preferred embodiment, the anti-B7H6 antibody has a constant domain of IgG2a. In some embodiments, the anti-B7H6 antibody molecule has a light chain constant domain which is kappa or lambda, preferably the light chain constant domain is a kappa light chain constant domain, preferably comprising the sequence of SEQ ID NO:247.

The B7H6 specific antibodies provided herein may be used for labelling, localizing, identifying or targeting cells expressing B7H6 (e.g. in ELISA assays, FACS analysis,

immunohistology or the like) by attaching a dye, a drug or another molecule with binding specificity for a different antigen.

Another aspect of the present invention provides isolated nucleic acid molecules encoding the heavy chain variable domain and/or the light chain variable domain of an anti-B7H6
5 antibody molecule of the invention.

Preferably the nucleic acid molecule comprises a nucleotide sequence encoding the heavy chain variable domain of any one of SEQ ID NOs: 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, or 192.

Preferably the nucleic acid molecule comprises a nucleotide sequence encoding the light
10 chain variable domain of any one of SEQ ID NOs: 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, or 191.

A further aspect of the invention provides an expression vector containing a DNA molecule comprising the nucleotide sequence encoding the heavy chain variable domain and/or the light chain variable domain of an anti-B7H6 antibody molecule of the invention.

15 Preferably the expression vector comprises, in addition, a nucleic acid molecule, preferably a DNA molecule, encoding the constant domains of a heavy chain and/or the constant domain of a light chain, respectively, linked to the nucleic acid molecule, preferably the DNA molecule, encoding the heavy chain variable domain and/or the light chain variable domain, respectively.

20 In a specifically preferred embodiment, two expression vectors may be used, one of them for expression of the heavy chain, the other one for expression of the light chain, which two expression vectors may then both be transfected into a host cell for recombinant protein expression.

Preferably, the expression vector will be a vector comprising said nucleic acid molecule or
25 molecules, operably linked to at least one regulatory sequence, wherein such regulatory sequence may be a promoter, enhancer, or terminator sequence, and most preferably a heterologous promoter, enhancer, or terminator sequence.

In another aspect, the invention relates to a host cell having an expression vector encoding a heavy chain of an anti-B7H6 antibody molecule of the invention and an expression
30 vector encoding a light chain of an anti-B7H6 antibody molecule of the invention.

According to a particularly preferred embodiment, said host cells are eukaryotic cells such as mammalian cells. In another embodiment, such host cells are bacterial cells. Other useful cells are yeast cells or other fungal cells.

Suitable mammalian cells include for example CHO cells, BHK cells, HeLa cells, COS
5 cells, and the like. However, amphibian cells, insect cells, plant cells, and any other cells used in the art for the expression of heterologous proteins can be used as well.

METHODS OF MANUFACTURE AND PURIFICATION

The invention further provides methods of manufacturing a multi-specific binding protein
10 of the invention, such methods generally comprising the steps of:

- culturing host cells comprising an expression vector comprising a nucleic acid encoding a binding protein of the invention under conditions that allow formation of the binding protein of the invention; and,
- recovering the binding protein expressed by the host cells from the culture; and
- 15 - optionally further purifying and/or modifying and/or formulating the binding protein of the invention.

The invention further provides methods of manufacturing an anti-B7H6 antibody of the invention, such methods generally comprising the steps of:

- culturing host cells comprising an expression vector comprising a nucleic acid encoding
20 an antibody molecule of the invention under conditions that allow formation of the antibody molecule; and,
- recovering the antibody molecule expressed by the host cells from the culture; and
- optionally further purifying and/or modifying and/or formulating the antibody molecule of the invention.

25 A nucleic acid of the invention can e.g. be a DNA molecule comprising coding sequences as well as regulatory sequences and optionally natural or artificial introns, or can be a cDNA molecule. It may have its original codons or may have an optimized codon usage that has been specifically adapted for expression in the intended host cell or host organism. According to one embodiment of the invention, the nucleic acid of the invention is in
30 essentially isolated form, as defined above.

The nucleic acids of the invention may be prepared or obtained in a manner known per se (e.g. by automated DNA synthesis and/or recombinant DNA technology), based on the information on the amino acid sequences for the proteins of the invention given herein.

The nucleic acid of the invention will typically be incorporated into an expression vector, i.e. a vector that can provide for expression of the protein when transfected into a suitable host cell or other expression system.

For manufacturing the binding proteins or antibodies of the invention, the skilled artisan may choose from a great variety of expression systems well known in the art, e.g. those reviewed by Kipriyanow and Le Gall, 2004.

Expression vectors include plasmids, retroviruses, cosmids, EBV derived episomes, and the like. The expression vector and expression control sequences are selected to be compatible with the host cell. The nucleotide sequence encoding the first antigen binding unit (e.g. the B7H6 specific single chain Fab or the full length B7H6 chain of the binding protein of the invention) and the nucleotide sequence encoding the second antigen binding unit (e.g. CD3 specific single chain Fab or the full length CD3 chain of the binding protein of the invention) of the B7H6/CD3 binding protein can be inserted into separate vectors. In certain embodiments, both DNA sequences are inserted into the same expression vector. The nucleotide sequence encoding the light chain of a B7H6 antibody and the nucleotide sequence encoding the heavy chain of a B7H6 antibody can be inserted into separate vectors. In certain embodiments, both DNA sequences are inserted into the same expression vector.

Convenient vectors are those that encode a functionally complete human CH (constant heavy) immunoglobulin sequence, with appropriate restriction sites engineered so that any antigen binding unit such as a single chain Fab sequence or any heavy/light chain variable domain can be easily inserted and expressed, as described above. For the antibody heavy chain, it can be, without limitation, any IgG isotype (IgG1, IgG2, IgG3, IgG4) or other immunoglobulins, including allelic variants.

The recombinant expression vector may also encode a signal peptide that facilitates secretion of the full length CD3 or B7H6 chain from a host cell or of the light/heavy chain of an anti-B7H6 antibody. The DNA encoding the protein chain may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the mature full length chain DNA. The signal peptide may be an immunoglobulin signal peptide or a

heterologous peptide from a non-immunoglobulin protein. Alternatively, the DNA sequence encoding the full length chains of the protein of the invention may already contain a signal peptide sequence.

- In addition to the B7H6/CD3 chain encoding DNA sequences or the heavy/light chain of a B7H6 antibody encoding DNA sequences, the recombinant expression vectors typically carries regulatory sequences, optionally heterologous regulatory sequences, including promoters, enhancers, termination and polyadenylation signals and other expression control elements that control the expression of the protein chains in a host cell. Examples for promoter sequences (exemplified for expression in mammalian cells) are promoters and/or enhancers derived from CMV (such as the CMV Simian Virus 40 (SV40) promoter/enhancer), adenovirus, (e. g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. Examples for polyadenylation signals are BGH polyA, SV40 late or early polyA; alternatively, 3'UTRs of immunoglobulin genes etc. can be used.
- The recombinant expression vectors may also carry sequences that regulate replication of the vector in host cells (e. g. origins of replication) and selectable marker genes. Nucleic acid molecules encoding the full length chain with the first antigen binding unit (single chain Fab and Fc domain) or an antigen-binding portion thereof and/or the full length chain with the second antigen binding unit (single chain Fab and Fc domain) or an antigen-binding portion thereof, and vectors comprising these DNA molecules can be introduced into host cells, e.g. bacterial cells or higher eukaryotic cells, e.g. mammalian cells, according to transfection methods well known in the art, including liposome-mediated transfection, polycation-mediated transfection, protoplast fusion, microinjections, calcium phosphate precipitation, electroporation or transfer by viral vectors.
- Preferably, the DNA molecules encoding the B7H6 and CD3 chain of the protein of the invention are present on two expression vectors which are co-transfected into the host cell, preferably a mammalian cell.

Mammalian cell lines available as hosts for expression are well known in the art and include, inter alia, Chinese hamster ovary (CHO) cells, NS0, SP2/0 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human carcinoma cells (e. g., Hep G2 and A-549 cells), 3T3 cells or the derivatives/progenies of any such cell line. Other mammalian cells, including but not limited to human, mice, rat, monkey and rodent

cells lines, or other eukaryotic cells, including but not limited to yeast, insect and plant cells, or prokaryotic cells such as bacteria may be used.

The proteins of the invention are produced by culturing the host cells for a period of time sufficient to allow for expression of the protein in the host cells. Protein molecules are preferably recovered from the culture medium as a secreted polypeptide or it can be recovered from host cell lysates if for example expressed without a secretory signal. It is necessary to purify the protein molecules using standard protein purification methods used for recombinant proteins and host cell proteins in a way that substantially homogenous preparations of the protein are obtained. By way of example, state-of-the art purification methods useful for obtaining protein molecules of the invention include, as a first step, removal of cells and/or particulate cell debris from the culture medium or lysate. The protein is then purified from contaminant soluble proteins, polypeptides and nucleic acids, for example, by fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, Sephadex chromatography, chromatography on silica or on a cation exchange resin. As a final step in the process for obtaining a protein molecule preparation, the purified protein molecule may be dried, e.g. lyophilized, as described below for therapeutic applications.

The present invention relates to binding proteins that have binding specificities for at least two different targets. In relation to the present invention, the binding molecules are derived from antibodies. Techniques for making binding molecules include, but are not limited to, recombinant co-expression of two immunoglobulin chains having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731, 168; Atwell et al, *JMB*, 1997, 270, 26-35). Binding proteins of the invention may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross- linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific proteins (see, e.g., Kostelny et al., *Immunol.*, 148(5): 1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444- 6448 (1993)); and using single-chain Fv (sFv) dimers (see, e.g. Gruber et al., *Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al., *Immunol.* 147: 60 (1991).

The compositions (e.g., multi-specific binding proteins and anti-B7H6 antibodies) and methods disclosed herein encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, e.g., sequences at least 85%, 90%, 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid sequence that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain have at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, e.g., a sequence provided herein. In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity, for example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence.

The nucleic acid molecules of the invention include, but are not limited to, the DNA molecules encoding the polypeptide sequences shown in the sequence listing. Also, the present invention also relates to nucleic acid molecules that hybridize to the DNA molecules encoding the polypeptide sequences shown in the sequence listing under high stringency binding and washing conditions, as defined in WO 2007/042309. Preferred molecules (from an mRNA perspective) are those that have at least 75% or 80% (preferably at least 85%, more preferably at least 90% and most preferably at least 95%) homology or sequence identity with one of the DNA molecules described herein. By way of example, in view of expressing the antibodies in eukaryotic cells, the DNA sequences shown in the sequence listing have been designed to match codon usage in eukaryotic cells. If it is desired to express the antibodies in *E. coli*, these sequences can be changed to match *E. coli* codon usage. Variants of DNA molecules of the invention can be constructed in several different ways, as described e.g. in WO 2007/042309.

The proteins of the invention may have a modified N-terminal sequence, e.g. a deletion of one or more of the N-terminal amino acids, or an exchange of e.g. the first, N-terminal amino acid (e.g. glutamate to alanine), to optimize the molecule for being expressed by using certain expression systems (such as specific vectors or host cells), or for being
5 expressed as inclusion bodies or in soluble form, or for being secreted into the medium or the periplasmic space or for being contained within the cell, or for yielding a more homogenous product. The polypeptides of the invention may have a modified C-terminal sequence, such as an additional alanine, and/or further amino acid exchanges in the C-terminal part or at other defined positions within any of the framework regions, as
10 explained e.g. in WO2012/175741, WO2011/075861, or WO2013/024059, in order to e.g. further enhance stability or reduce immunogenicity of such polypeptides.

For the avoidance of doubt, all of the embodiments relating to pharmaceutical compositions, kits, treatment methods, medical uses, combinations, methods of administration and dosages as described herein are contemplated for any of the multi-
15 specific binding proteins described herein, either alone or in combination with further therapeutic agents (as specified in more detail below).

PHARMACEUTICAL COMPOSITIONS, METHODS OF ADMINISTRATION, DOSAGES

20 The invention further relates to pharmaceutical compositions for the treatment of a disease (as specified in more detail below), wherein such compositions comprise at least one multi-specific binding protein of the invention. The invention further encompasses methods of treating a disease (as specified in more detail below) using at least one multi-specific binding protein of the invention or pharmaceutical composition as set out below, and
25 further encompasses the preparation of a medicament for the treatment of such disease by using such binding protein of the invention or pharmaceutical composition.

The binding proteins of the invention (e.g., any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#6/CD3#1, B7H6#7/CD3#1, B7H6#8/CD3#1, B7H6#9/CD3#1, B7H6#10/CD3#1, B7H6#11/CD3#1, B7H6#12/CD3#1,
30 B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1 as defined by

the sequences shown in Table 1) and/or the compositions comprising the same can be administered to a patient in need thereof in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the binding proteins of the invention and/or the compositions comprising the same can for example be administered
5 intravenously (i.v.), subcutaneously (s.c.), intramuscularly (i.m.), intraperitoneally (i.p.), transdermally, orally, sublingually (e.g. in the form of a sublingual tablet, spray or drop placed under the tongue and adsorbed through the mucus membranes into the capillary network under the tongue), (intra-)nasally (e.g. in the form of a nasal spray and/or as an aerosol), topically, by means of a suppository, by inhalation, or any other suitable manner
10 in an effective amount or dose. The binding protein can be administered by infusion, bolus or injection. In preferred embodiments, the administration is by intravenous infusion or subcutaneous injection.

The binding proteins of the invention and/or the compositions comprising the same are administered according to a regimen of treatment that is suitable for treating and/or
15 alleviating the disease, disorder or condition to be treated or alleviated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease, disorder or condition to be treated or alleviated, the severity of the disease, the severity of the symptoms thereof, the specific binding protein of the invention to be used, the specific route of administration and pharmaceutical formulation or composition to be
20 used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician. Generally, the treatment regimen will comprise the administration of one or more binding proteins of the invention, or of one or more compositions comprising the same, in therapeutically effective amounts or doses.

Generally, for the treatment and/or alleviation of the diseases, disorders and conditions
25 mentioned herein and depending on the specific disease, disorder or condition to be treated, the potency of the specific binding protein of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the binding proteins of the invention will generally be administered in an amount between 0.005 and 20.0 mg per kilogram of body weight and dose, preferably between 0.05 and
30 10.0 mg/kg/dose, either continuously (e.g. by infusion) or more preferably as single doses (such as e.g. twice a week, weekly, once every two or three weeks or monthly doses; cf. below), but can significantly vary, especially, depending on the before-mentioned parameters. Thus, in some cases it may be sufficient to use less than the minimum dose

given above, whereas in other cases the upper limit may have to be exceeded. When administering large amounts it may be advisable to divide them up into a number of smaller doses spread over a certain period, e.g. two or more days.

Depending on the specific binding protein of the invention and its specific pharmacokinetic and other properties, it may be administered daily, every second, third, fourth, fifth or sixth
5 day, weekly, once every two or three weeks, monthly, and the like. An administration regimen could include long-term treatment. By "long-term" is meant at least two weeks and preferably months, or years of duration.

The efficacy of the multi-specific binding protein of the invention, and of compositions
10 comprising the same, can be tested using any suitable in vitro assay, cell-based assay, in vivo assay and/or animal model known per se, or any combination thereof, depending on the specific disease involved. Suitable assays and animal models will be clear to the skilled person, and for example include the assays and animal models used in the Examples below.

15

FORMULATIONS

For pharmaceutical use, the binding proteins of the invention may be formulated as a pharmaceutical preparation comprising (i) at least one binding protein of the invention (e.g., any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1,
20 B7H6#5/CD3#1, B7H6#6/CD3#1, B7H6#7/CD3#1, B7H6#8/CD3#1, B7H6#9/CD3#1, B7H6#10/CD3#1, B7H6#11/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) and (ii) at least one
25 pharmaceutically acceptable carrier, diluent, excipient, adjuvant, and/or stabilizer, and (iii) optionally one or more further pharmacologically active polypeptides and/or compounds.

By "pharmaceutically acceptable" is meant that the respective material does not show any biological or otherwise undesirable effects when administered to an individual and does not interact in a deleterious manner with any of the other components of the
30 pharmaceutical composition (such as e.g. the pharmaceutically active ingredient) in which it is contained. Specific examples can be found in standard handbooks, such as e.g. Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, USA (1990).

For example, the binding proteins of the invention may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments and other pharmaceutically active proteins. Thus, according to a further embodiment, the invention relates to a pharmaceutical composition or preparation that contains at least one binding
5 protein of the invention and at least one pharmaceutically acceptable carrier, diluent, excipient, adjuvant and/or stabilizer, and optionally one or more further pharmacologically active substances, in the form of lyophilized or otherwise dried formulations or aqueous or non-aqueous solutions or suspensions.

Pharmaceutical preparations for parenteral administration, such as intravenous,
10 intramuscular, subcutaneous injection or intravenous infusion may for example be sterile solutions, suspensions, dispersions, emulsions, or powders which comprise the active ingredient and which are suitable, optionally after a further dissolution or dilution step, for infusion or injection. Suitable carriers or diluents for such preparations for example include, without limitation, sterile water and pharmaceutically acceptable aqueous buffers
15 and solutions such as physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution; water oils; glycerol; ethanol; glycols such as propylene glycol, as well as mineral oils, animal oils and vegetable oils, for example peanut oil, soybean oil, as well as suitable mixtures thereof.

Solutions of the binding proteins of the invention may also contain a preservative to
20 prevent the growth of microorganisms, such as antibacterial and antifungal agents, for example, p-hydroxybenzoates, parabens, chlorobutanol, phenol, sorbic acid, thiomersal, (alkali metal salts of) ethylenediamine tetraacetic acid, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Optionally, emulsifiers and/or dispersants may be used. The proper fluidity can be
25 maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. Other agents delaying absorption, for example, aluminum monostearate and gelatin, may also be added. The solutions may be filled into injection vials, ampoules, infusion bottles, and the like.

In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions
30 of manufacture and storage. Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case

of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

- 5 Usually, aqueous solutions or suspensions will be preferred. Generally, suitable formulations for therapeutic proteins such as the binding proteins of the invention are buffered solutions, such as solutions including the protein in a suitable concentration (such as from 0.001 to 400 mg/ml, preferably from 0.005 to 200 mg/ml, more preferably 0.01 to 200 mg/ml, more preferably 1.0 - 100 mg/ml, such as 1.0 mg/ml (*i.v.* administration) or 10 100 mg/ml (*s.c.* administration) and an aqueous buffer such as:

- phosphate buffered saline, pH 7.4,
 - other phosphate buffers, pH 6.2 to 8.2,
 - acetate buffers, pH 3.2 to 7.5, preferably pH 4.8 to 5.5
 - histidine buffers, pH 5.5 to 7.0,
 - 15 - succinate buffers, pH 3.2 to 6.6, and
 - citrate buffers, pH 2.1 to 6.2,
- and, optionally, salts (e.g. NaCl) and/or sugars (such as e.g. sucrose and trehalose) and/or other polyalcohols (such as e.g. mannitol and glycerol) for providing isotonicity of the solution.

- 20 In addition, other agents such as a detergent, e.g. 0.02 % TWEEN™ 20 or TWEEN™-80, may be included in such solutions. Formulations for subcutaneous application may include significantly higher concentrations of the antibody of the invention, such as up to 100 mg/ml or even above 100 mg/ml. However, it will be clear to the person skilled in the art that the ingredients and the amounts thereof as given above do only represent one, 25 preferred option. Alternatives and variations thereof will be immediately apparent to the skilled person, or can easily be conceived starting from the above disclosure. The above described formulations can optionally be provided as lyophilized formulation that is to be reconstituted in a solution, e.g. in water for injection (WFI).

- According to a further aspect of the invention, a binding protein of the invention may be 30 used in combination with a device useful for the administration of protein, such as a syringe, injector pen, micropump, or other device.

METHOD OF TREATMENT

A further aspect of the invention provides a method of treating cancer comprising administering to a patient in need thereof a therapeutically effective amount of the binding protein of the invention.

- 5 A further aspect of the invention provides a binding protein of the invention for use in a method of treating cancer.

A further aspect of the invention is the use of the binding protein of the invention for preparing a pharmaceutical composition for treating cancer.

- For the avoidance of doubt, the medical use aspects of the invention may comprise any of
10 the specific binding proteins of the invention as described above (e.g., any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#6/CD3#1, B7H6#7/CD3#1, B7H6#8/CD3#1, B7H6#9/CD3#1, B7H6#10/CD3#1, B7H6#11/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1,
15 B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1).

As used herein, the term "cancer" is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness.

- 20 Exemplary cancers whose growth can be inhibited using the multi-specific binding proteins described herein are any B7H6 expressing tumors, preferably colorectal cancer (e.g. metastatic colorectal cancer, mCRC), non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC).

- Cancers whose growth can be inhibited using the multi-specific binding proteins described
25 herein are any B7H6 expressing tumors including but not limited to T cell lymphoma, myeloid leukemia, breast cancer; ovarian cancer, oral squamous carcinoma and gastro-intestinal cancers. Gastro-intestinal cancers include but are not limited to esophageal cancer (e.g., gastroesophageal junction cancer), stomach (gastric) cancer, hepatocellular carcinoma, biliary tract cancer (e.g., cholangiocarcinoma), gallbladder
30 cancer, pancreatic cancer or colorectal cancer (CRC).

In some embodiments, the following cancers, tumors, and other proliferative diseases may be treated with multi-specific binding proteins of the invention: head and neck cancer, preferably HNSCC ; lung cancer; preferably NSCLC; breast cancer; thyroid cancer; cervical cancer; ovarian cancer; endometrial cancer; liver cancer (hepatoblastoma or hepatocellular carcinoma); pancreatic cancer; prostate cancer; gastric sarcoma;
5 gastrointestinal stromal tumor, esophageal cancer; colon cancer; colorectal cancer; renal cancer; skin cancer; brain tumor; glioblastoma; Non-Hodgkin lymphomas (T or B cell lymphoma); leukemia (chronic or acute myeloid leukemias, nonlymphocytic leukemia), or multiple myeloma.

10 In a preferred embodiment of the invention the cancer is mCRC.

All cancers, tumors, neoplasms, etc., mentioned above which are characterized by their specific location/origin in the body are meant to include both the primary tumors and the metastatic tumors derived therefrom.

It is possible that a patient is more likely to respond to treatment with a binding protein of the invention (as described herein) if that patient has a cancer which is characterized by
15 having a high expression of B7H6. Thus, in some embodiments, the cancer to be treated with the binding proteins of the invention is a cancer with high expression of B7H6, e.g., B7H6 expression is higher than the average expression in cancer cells of a population of patients suffering from the same type of a B7H6 expressing cancer.

20 The binding proteins of the invention may be used in therapeutic regimens in the context of first line, second line, or any further line treatments and maintenance treatment.

The binding proteins of the invention may be used for the prevention, short-term or long-term treatment of the above-mentioned diseases, optionally also in combination with radiotherapy, one or more additional therapeutic agents and/or surgery.

25 In preferred embodiments, the protein of the invention is used for the treatment of cancer in combination with a PD-1 antagonist, such as an anti-PD-1 antibody or an anti-PDL-1 antibody. Preferably said anti-PD-1 antibody is selected from the group consisting of pembrolizumab, nivolumab, pidilizumab, PD1-1, PD1-2, PD1-3, PD1-4, and PD1-5 as described herein (as defined by the sequences in Table A below) and in WO2017/198741
30 (incorporated herein by reference). Preferably said anti-PDL-1 antibody is selected from the group consisting of atezolizumab, avelumab and durvalumab. In particular preferred embodiments, the binding protein of the invention (preferably any one of B7H6#1/CD3#1,

B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) is used for the treatment of cancer in combination with PD1-1. In particular preferred embodiments, the binding protein of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) is used for the treatment of cancer in combination with PD1-2. In particular preferred embodiments, the binding protein of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) is used for the treatment of cancer in combination with PD1-3. In particular preferred embodiments, the binding protein of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) is used for the treatment of cancer in combination with PD1-4. In particular preferred embodiments, the binding protein of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1):.

Table A: Amino acid sequences and SEQ ID NOs of heavy chain and light chain sequences of anti-PD1 antibodies PD1-1, PD1-2, PD1-3, PD1-4, PD1-5.

SEQ ID Number:	Brief description of sequence	Sequence
SEQ ID NO:331	PD1-1 HC	EVMLVESGGGLVQPGGSLRLSCTASGFTFSASAMSWVRQAPGKGLEWV AYISGGGGDTYYSSSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARH SNVNYAMDYWGQGLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKY TCNVDHKPSNTKVDKRVESKYGPPCPPCAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSIVTLVHLDWLNGLKEYKCKVSNKGLPSSIEKISKAKGQPREPQVYTL PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLG
SEQ ID NO:332	PD1-1 LC	EIVLTQSPATLSLSPGERATMSCRASENIDTSGISFMNWWYQQKPGQAPKLL IYVASNQGSVIPARFSGSGSGTDFTLTISRLEPEDFAVYYCQQSKEVPWTFG QGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
SEQ ID NO:333	PD1-2 HC	EVMLVESGGGLVQPGGSLRLSCTASGFTFSASAMSWVRQAPGKGLEWV AYISGGGGDTYYSSSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARH SNPNYYAMDYWGQGLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKY TCNVDHKPSNTKVDKRVESKYGPPCPPCAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSIVTLVHLDWLNGLKEYKCKVSNKGLPSSIEKISKAKGQPREPQVYTL PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLG
SEQ ID NO:334	PD1-2 LC	EIVLTQSPATLSLSPGERATMSCRASENIDTSGISFMNWWYQQKPGQAPKLL IYVASNQGSVIPARFSGSGSGTDFTLTISRLEPEDFAVYYCQQSKEVPWTFG QGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

SEQ ID Number:	Brief description of sequence	Sequence
		DNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
SEQ ID NO:335	PD1-3 HC	EVMLVESGGGLVQPGGSLRLSCTASGFTFSKSAMSWVRQAPGKGLEWV AYISGGGGDTYYSSSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARH SNVNYAMDYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTKTY TCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTPETCVVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLG
SEQ ID NO:336	PD1-3 LC	EIVLTQSPATLSLSPGERATMSCRASENIDVSGISFMNWWYQQKPGQAPKLL IYVASNQSGGIPARFSGSGSGTDFTLTISRLEPEDFAVYYCQQSKVEPWTFG QGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
SEQ ID NO:337	PD1-4 HC	EVMLVESGGGLVQPGGSLRLSCTASGFTFSKSAMSWVRQAPGKGLEWV AYISGGGGDTYYSSSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARH SNVNYAMDYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTKTY TCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTPETCVVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLG
SEQ ID NO:338	PD1-4 LC	EIVLTQSPATLSLSPGERATMSCRASENIDVSGISFMNWWYQQKPGQAPKLL IYVASNQSGGIPARFSGSGSGTDFTLTISRLEPEDFAVYYCQQSKVEPWTFG QGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

SEQ ID Number:	Brief description of sequence	Sequence
		DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
SEQ ID NO:339	PD1-5 HC	EVMLVESGGGLVQPGGSLRLSCTASGFTFSKSAMSWVRQAPGKGLEWV AYISGGGGDTYYSSSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARH SNVNYAMDYWGQGLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKY TCNVDPKPSNTKVDKRVESEKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTEPVTCTVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSVELTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLG
SEQ ID NO:340	PD1-5 LC	EIVLTQSPATLSLSPGERATMSCRASENIDVSGISFMNWWYQQKPGQAPKLL IYVASNQSGGIPARFSGSGSGTDFTLTISRLEPEDFAVYYCQQSKVEPWTFG QGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC

According to these preferred embodiments and any other of the aspects of the present invention, antibodies PD1-1, PD1-2, PD1-3, PD1-4 and PD1-5 are antibody molecules as disclosed in WO2017/198741, and are defined by the sequences as shown in Table A above.

- 5 Accordingly, PD1-1 has a heavy chain comprising the amino acid sequence of SEQ ID NO:331 and a light chain comprising the amino acid sequence of SEQ ID NO:332;

PD1-2 has a heavy chain comprising the amino acid sequence of SEQ ID NO:333 and a light chain comprising the amino acid sequence of SEQ ID NO:334;

- PD1-3 has a heavy chain comprising the amino acid sequence of SEQ ID NO:335 and a light
10 chain comprising the amino acid sequence of SEQ ID NO:336;

PD1-4 has a heavy chain comprising the amino acid sequence of SEQ ID NO:337 and a light chain comprising the amino acid sequence of SEQ ID NO:338; and

PD1-5 has a heavy chain comprising the amino acid sequence of SEQ ID NO:339 and a light chain comprising the amino acid sequence of SEQ ID NO:340.

The above also includes the use of the binding proteins of the invention in various methods of treating the above diseases by administering a therapeutically effective dose to a patient in need thereof, as well as the use of these binding proteins for the manufacture of medicaments for the treatment of such diseases, as well as pharmaceutical compositions including such binding proteins of the invention, as well as the preparation and/or manufacture of medicaments including such binding proteins of the invention, and the like.

10 COMBINATIONS WITH OTHER ACTIVE SUBSTANCES OR TREATMENTS

A binding protein of the invention (e.g., any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#6/CD3#1, B7H6#7/CD3#1, B7H6#8/CD3#1, B7H6#9/CD3#1, B7H6#10/CD3#1, B7H6#11/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) may be used on its own or in combination with other cancer therapies, e.g. surgery, radiotherapy, chemotherapy, targeted therapies, immunotherapies or combinations thereof. For example, a binding protein of the invention may be used for the treatment of cancer in combination with one or more additional therapeutic agents, in particular in combination with a cytotoxic or cytostatic chemotherapeutic agent, a therapeutically active compound that inhibits angiogenesis, a signal transduction pathway inhibitor, e.g., an EGFR inhibitor, an immune modulator, an immune checkpoint inhibitor, a mitotic checkpoint inhibitor or a hormonal therapy agent.

The additional therapeutic agent may be administered simultaneously with, optionally as a component of the same pharmaceutical preparation, or before or after administration of the B7H6/CD3 binding protein.

Cytostatic and/or cytotoxic active substances which may be administered in combination with binding molecules of the invention include, without being restricted thereto, hormones, hormone analogues and antihormones, aromatase inhibitors, LHRH agonists and antagonists, inhibitors of growth factors (growth factors such as for example platelet derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth

factor (VEGF), epidermal growth factor (EGF), insuline-like growth factors (IGF), human epidermal growth factor (HER, e.g. HER2, HER3, HER4) and hepatocyte growth factor (HGF)), inhibitors are for example (anti-)growth factor antibodies, (anti-)growth factor receptor antibodies and tyrosine kinase inhibitors, such as for example cetuximab,

5 gefitinib, afatinib, nintedanib, imatinib, lapatinib, bosutinib and trastuzumab; antimetabolites (e.g. antifolates such as methotrexate, raltitrexed, pyrimidine analogues such as 5-fluorouracil (5-FU), FOLFOX (combination regimen of folinic acid, 5-FU and oxaliplatin), FOLFIRI (combination regimen of folinic acid, 5-FU and irinotecan), gemcitabine, irinotecan, doxorubicin, TAS-102, capecitabine and gemcitabine, purine and

10 adenosine analogues such as mercaptopurine, thioguanine, cladribine and pentostatin, cytarabine (ara C), fludarabine); antitumor antibiotics (e.g. anthracyclins); platinum derivatives (e.g. cisplatin, oxaliplatin, carboplatin); alkylation agents (e.g. estramustin, meclorethamine, melphalan, chlorambucil, busulphan, dacarbazine, cyclophosphamide, ifosfamide, temozolomide, nitrosoureas such as for example carmustine and lomustine,

15 thiotepa); antimitotic agents (e.g. Vinca alkaloids such as for example vinblastine, vindesine, vinorelbine and vincristine; and taxanes such as paclitaxel, docetaxel); angiogenesis inhibitors, including bevacizumab, ramucirumab and aflibercept, tubuline inhibitors; DNA synthesis inhibitors, PARP inhibitors, topoisomerase inhibitors (e.g. epipodophyllotoxins such as for example etoposide and etopophos, teniposide, amsacrin,

20 topotecan, irinotecan, mitoxantrone), serine/threonine kinase inhibitors (e.g. PDK1 inhibitors, Raf inhibitors, A-Raf inhibitors, B-Raf inhibitors, C-Raf inhibitors, mTOR inhibitors, mTORC1/2 inhibitors, PI3K inhibitors, PI3K α inhibitors, dual mTOR/PI3K inhibitors, STK33 inhibitors, AKT inhibitors, PLK1 inhibitors (such as volasertib), inhibitors of CDKs, including CDK9 inhibitors, Aurora kinase inhibitors), tyrosine kinase

25 inhibitors (e.g. PTK2/FAK inhibitors), protein protein interaction inhibitors, MEK inhibitors, ERK inhibitors, FLT3 inhibitors, BRD4 inhibitors, IGF-1R inhibitors, Bcl-xL inhibitors, Bcl-2 inhibitors, Bcl-2/Bcl-xL inhibitors, ErbB receptor inhibitors, BCR-ABL inhibitors, ABL inhibitors, Src inhibitors, rapamycin analogs (e.g. everolimus, temsirolimus, ridaforolimus, sirolimus), androgen synthesis inhibitors, androgen receptor

30 inhibitors, DNMT inhibitors, HDAC inhibitors, ANG1/2 inhibitors, CYP17 inhibitors, radiopharmaceuticals, immunotherapeutic agents such as immune checkpoint inhibitors (e.g. CTLA4, PD1, PD-L1, LAG3, and TIM3 binding molecules / immunoglobulins, such as ipilimumab, nivolumab, pembrolizumab) and various chemotherapeutic agents such as

amifostin, anagrelid, clodronat, filgrastin, interferon, interferon alpha, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer; proteasome inhibitors (such as Bortezomib); Smac and BH3 mimetics; agents restoring p53 functionality including mdm2-p53 antagonist; inhibitors of the Wnt/beta-catenin signaling pathway; and/or cyclin-dependent kinase 9 inhibitors.

Particularly preferred are treatments with the binding molecules of the invention in combination with one or more immunotherapeutic agents, including anti-PD-1 and anti-PD-L1 agents and anti LAG3 agents. Exemplary anti-PD1 agents include but are not limited to anti-PD-1 antibody PDR-001, pembrolizumab, nivolumab, pidilizumab and PD1-1, PD1-2, PD1-3, PD1-4 and PD1-5 as disclosed herein (Table A) and in WO2017/198741. Exemplary anti- PDL-1 agents include but are not limited to atezolizumab, avelumab and durvalumab. In preferred embodiments, the binding molecule of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) is combined with PD1-1. In preferred embodiments, the binding molecule of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) is combined with PD1-2. In preferred embodiments, the binding molecule of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) is combined with PD1-3. In preferred embodiments, the binding molecule of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1,

B7H6#24/CD3#1) is combined with PD1-4. In preferred embodiments, the binding molecule of the invention (preferably any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, 5 B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1)) is combined with PD1-5.

In certain embodiments, the additional therapeutic agent may be a further immunotherapeutic agent, such as modulators of: TIM-1, TIM-3, TIM-4, PD-L2, LAG3, CTLA-4, Galectin 9, Galectin-1, CD69, CD113, GPR56, CD48, GARP, CAECAM-1, 10 BTLA, TIGIT, CD160, LAIR1, 2B4, CEACAM, CD39, TGF β , IL-10, Fas ligand, ICOS, B7 family (B7-1, B7-2, B7-H1 (PDL-1), B7-DC (PD-L2), B7-H2 (ICOS-L), B7-H3, B7-H4, B7-H5 (VISTA)), gp49B, PIR-B, KIR family receptors, SIRPalpha (CD47), ILT-2, ILT-4, IDO, CD39, arginase, CD73 HHLA2, butyrophilins, or A2aR.

In some embodiments, the additional immunotherapeutic agent is a member of the TNF 15 family of molecules that bind to cognate TNF receptor family members, which include CD40 and CD40L, OX-40, OX-40L, CD70, CD27L, CD30, CD30L, 4-1BBL, CD137, CD137/FAP, GITR, TRAIL/Apo2-L, TRAILR1/DR4, TRAILR2/DR5, TRAILR3, TRAILR4, OPG, RANK, RANKL, TWEAKR/Fn14, TWEAK, BAFFR, EDAR, XEDAR, TACI, APRIL, BCMA, LIGHT, DcR3, HVEM, VEGI/TL1A, TRAMP/DR3, EDAR, EDAI, 20 XEDAR, EDA2, TNFR1, Lymphotoxin α /TNF β , TNFR2, TNF α , LT β R, Lymphotoxin α 1 β 2, FAS, FASL, RELT, DR6, TROY, NGFR. Preferably, the additional immunotherapeutic agent is CD137/FAP.

In some embodiments, the additional immunotherapeutic agent is selected from (i) antagonists of cytokines that inhibit T cell activation (e.g., IL-6, IL-10, TGF-B, VEGF; 25 "immunosuppressive cytokines") and/or (ii) agonists of cytokines that stimulate T cell activation and/or cytokines such as IL2, for stimulating an immune response, e.g., for treating proliferative diseases, such as cancer.

In some embodiments, the additional immunotherapeutic agent is an agonist of a protein that stimulates T cell activation, such as CD28, GITRL, OX40L, CD27, and CD28H or a STING 30 agonist.

In some embodiments, the additional therapeutic agent is an oncolytic virus including but not limited to an oncolytic virus derived from vaccinia virus, adenovirus, (AdV), herpes

simplex virus (HSV1 or HSV2), reovirus, myxoma virus (MYXV), poliovirus, vesicular stomatitis virus (VSV), Maraba virus, varicella virus, measles virus (MV), or Newcastle disease virus (NDV).

5 KITS

The invention also encompasses kits comprising at least a multi-specific binding protein of the invention (e.g., any one any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#6/CD3#1, B7H6#7/CD3#1, B7H6#8/CD3#1, B7H6#9/CD3#1, B7H6#10/CD3#1, B7H6#11/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) and optionally one or more other components selected from the group consisting of other drugs used for the treatment of the diseases and disorders as described above.

15 In one embodiment, the kit includes a composition containing an effective amount of a binding protein of the invention in unit dosage form.

The invention also encompasses kits comprising at least a multi-specific binding protein of the invention, and one or more other components selected from the group consisting of other drugs used for the treatment of the diseases and disorders as described above.

20 In one embodiment, the kit includes a composition containing an effective amount of a multi-specific binding protein of the invention in unit dosage form (preferably any one of any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1). In a further embodiment the kit includes both a composition containing an effective amount of a multi-specific binding protein of the invention in unit dosage form (preferably any one of any one of B7H6#1/CD3#1, B7H6#2/CD3#1, B7H6#3/CD3#1, B7H6#4/CD3#1, B7H6#5/CD3#1, B7H6#12/CD3#1, B7H6#13/CD3#1, B7H6#14/CD3#1, B7H6#15/CD3#1, B7H6#16/CD3#1, B7H6#17/CD3#1, B7H6#18/CD3#1, B7H6#19/CD3#1, B7H6#20/CD3#1, B7H6#21/CD3#1, B7H6#22/CD3#1, B7H6#23/CD3#1, B7H6#24/CD3#1) and a

composition containing an effective amount of a PD-1 antagonist in unit dosage form, such as an anti PD-1 antibody, most preferably PD1-1, PD1-2, PD1-3, PD1-4, and PD1-5 as described herein (e.g. Table A) and in WO2017/198741.

In some embodiments, the kit comprises a sterile container which contains such a composition; such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. Further, the kit may comprise the pharmaceutical composition in a first container with the binding protein of the invention in lyophilized form and a second container with a pharmaceutically acceptable diluent (e.g., sterile water) for injection. The pharmaceutically acceptable diluent can be used for reconstitution or dilution of the binding protein.

If desired, a multi-specific binding protein of the invention, is provided together with instructions for administering the multi-specific binding proteins to a subject having cancer. The instructions will generally include information about the use of the composition for the treatment or prevention of a cancer. In other embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of cancer or symptoms thereof; precautions; warnings; indications; counter-indications; overdosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

As amenable, these suggested kit components may be packaged in a manner customary for use by those of skill in the art. For example, these suggested kit components may be provided in solution or as a liquid dispersion or the like.

EXAMPLES

The following examples illustrate the invention. These examples should not be construed as to limit the scope of this invention.

Example 1: Design and construction of B7H6/CD3 binding proteins

- The present inventors have developed multi-specific binding proteins that bind B7H6 and CD3 and that induce T-cell activation leading to lysis of B7H6-expressing tumor cells. The molecular design used has an IgG antibody scaffold and an IgG-like structure. It features
- 5 knob-in-hole technology in the Fc for hetero-dimerization of the Knob and Hole arms. In addition, the binding protein has flexible peptide sequences between the light and the corresponding heavy chain in each arm. Thus, the binding protein comprises two arms, one binding to CD3, the other one binding to B7H6, each arm comprising a single chain Fab and an Fc region (see Figure 1).
- 10 Preferably the binding molecule is bispecific and bivalent (monovalent for each of the two targets).

Preparation of binding domains that recognize B7H6 and CD3 using high throughput V gene recovery from hybridomas and cultured single B cells.

- 15 To obtain anti-B7H6 binders, hybridomas or single B cells derived from B7H6 immunized wild-type and ALIVAMAB™ humanized mice (Ablexis, San Francisco, CA, USA: ALIVAMAB MOUSE™ transgenic mouse platform with human immunoglobulin loci) were cultured in vitro. Supernatants were screened for binding to recombinant human B7H6, by ALPHALISA® Immunoassay kit (PerkinElmer, Waltham, MA, USA), to NCI-
- 20 H716 cells (ATCC®, CCL-251™) expressing human B7H6, and also binding to recombinantly expressed cynomolgus B7-H6 on CHO cells, by Flow Cytometry.
- Immunoglobulin (Ig) VH and VL genes were then amplified from identified positive clones. To isolate RNA from hybridomas, about 2×10^6 cells from single clones were pelleted and used as source material. For single B cells, 100 to 500 cells expanded from
- 25 singularly isolated B cells were used as source material. RNA was isolated using RNeasy® Plus mini RNA extraction kit (Qiagen, Hilden, Germany). cDNA was then synthesized using SMARTer® cDNA synthesis kit (Clontech, Mountain View, CA) according to manufacturer's instructions. To facilitate cDNA synthesis, oligodT was used to prime reverse transcription of all messenger RNAs followed by "5' capping" with a SMARTer
- 30 IIA oligonucleotide. Subsequent amplification of the VH and VL fragments was performed using a 2-step PCR amplification using 5' primers targeting the SMARTer IIA cap and 3' primers targeting consensus regions in CH1. Briefly, each 50µl PCR reaction

consists of 20 μ M of forward and reverse primer mixes, 25 μ l of PrimeSTAR® Max DNA polymerase premix (Clontech), 2 μ l of unpurified cDNA, and 21 μ l of double-distilled H₂O. The cycling program starts at 94 °C for 3 min, followed by 35 cycles (94°C for 30 Sec, 50°C for 1 min, 68 °C for 1 min), and ends at 72 °C for 7 min. The second round PCR was performed with VL and VH 2nd round primers containing 15bp complementary extensions that “overlap” respective regions in their respective pTT5 mother vector (VH and VL). A second round PCR was performed with the same PCR cycling program.

In-Fusion® HD Cloning Kit (Clontech, U.S.A.) was used for directional cloning of VL gene into a pTT5 huIgK vector and VH gene into a pTT5 huIgG1KO vector. To facilitate In-Fusion® HD Cloning, PCR products were purified and treated with Cloning Enhancer before In-Fusion HD Cloning. Cloning and transformation were performed according to manufacturer’s protocol (Clontech, U.S.A.). Mini-prep DNAs were subjected to Sanger sequencing to confirm that complete V-gene fragments were obtained.

Using this methodology, pairs of Ig VH and VL genes encoding binding domains with specificity for B7H6 were prepared. Recombinant antibodies were produced by transient transfection of CHO-E37 cells with the corresponding heavy and light chain-encoding plasmids.

To obtain additional anti-CD3 binders, immunization of WT mice was carried out using a huCD3 ϵ peptide1-27 construct. Hybridoma supernatants were screened for binding to recombinant huCD3 ϵ +G-Fc protein and to recombinant cyCD3 ϵ +G-Fc protein, as well as for binding to huCD3-positive and cyCD3-positive cells. The variable regions of positive clones were recovered and cloned as IgG or IgG-like bispecific construct for further evaluation.

Humanization/Optimization of B7H6 and CD3 binders

Sequences of B7H6 or CD3 binders as described above as well as CD3 binders described in the literature (Pessano et al., EMBO J. 1985 Feb; 4(2): 337-44; Salmerón A et al., J Immunol. 1991 Nov 1;147(9):3047-52) were humanized and/or optimized. Sequence optimization/humanization of antibodies is a methodology to engineer antibodies raised in non-human species (against a specific antigen/ epitope) for use as therapeutics that resemble antibodies produced in humans and thereby eliminating potential adverse effects such as immunogenicity while retaining the specificity. The sequence

optimization/humanization approach utilized here was as described by Singh et al, 2015 (Singh S et al., mAbs 2015: 7(4):778-91). In brief, closely matching human germlines were identified *in silico*, and optimization/humanization variants were evaluated using a phage screening method. Final lead candidate sequences were selected based on binding, percent human score and EpiVax® (in silico predictive tool for potential immunogenicity) score.

Construction of bispecific proteins binding B7H6 and CD3

The variable regions of the B7H6 and CD3 binders were cloned into the expression vector pTT5 (National Research Council, Canada), using common molecular biology techniques to form bispecific binding proteins with one B7H6 specific binding arm comprising a single chain Fab binding to B7H6 and an Fc region (such binding unit also referred to herein as “B7H6 arm” or “B7H6 chain”) and a CD3 specific binding arm comprising a single chain Fab binding to CD3 and an Fc region (such binding unit also referred to herein as “CD3 arm” or “CD3 chain”). The Fc regions of the B7H6 and CD3 arms include either “W” or “SAV” mutations (Atwell et al, JMB, 1997, 270, 26-35) and the respective chains are referred to as W or SAV chains. For multi-fragment DNA assembly, a Gibson-assembly and NEBuilder® HiFi DNA Assembly approaches were used, following manufacturer’s protocols (New England Biolabs, Ipswich, MA, USA). DNA mini-preps were sequenced.

Each expression vector contains eukaryotic promoter elements for the chain-encoding gene (B7H6 or CD3 arm/chain), i.e., the gene encoding the signal sequence and the light and heavy chain, an expression cassette for a prokaryotic selection marker gene such as ampicillin, and an origin of replication. These DNA plasmids were propagated in ampicillin resistant *E. coli* colonies and cultures and were purified.

Example 2: Expression and purification of bispecific binding proteins binding B7H6 and CD3

Bispecific molecules binding B7H6 and CD3 were produced by transient transfection of CHO-E cells with the pTT5 vectors carrying the B7H6/CD3-chain-encoding genes (one chain as W chain and the other as SAV chain). Briefly, transfected CHO-E cells growing in suspension in serum-free media were cultivated in shake flasks under agitation at 140 rpm, 37°C and 5% CO₂ and kept at conditions of exponential growth. On the day of

transfection, cells were chemically transfected with W-chain plasmid and SAV-chain plasmid in 1:3 mass ratio, using Mirus Bio TransIT Pro® transfection reagent. Cells were then seeded at 1 to 2×10^6 cells/ml in 1 L of Gibco® FreeStyle™ CHO expression medium (Life Technologies, NY, US). Cells were then incubated under orbital shaking for
5 10 days with one-time feed at day7 with 200 ml commercial feed solution to optimize expression of the proteins. Antibody titers in the cell culture supernatants were determined using an Octet® instrument (Pall ForteBio, CA, US) and protA biosensor tips according to manufacturer's instructions.

Recombinant B7H6/CD3 binding proteins were purified from culture supernatant in a two-
10 step process, using a GE Healthcare Life Sciences ÄKTA™ Pure protein purification system. First, the sample was captured from the harvested cell culture fluid by Protein-A affinity chromatography using MabSelect™ column (GE Healthcare). Protein binds to Protein A at neutral pH and was washed with high salt (1M NaCl) to remove cell culture media components and any proteins or components which non-specifically bind to Protein
15 A. The antibody or antibody-like construct sample was eluted in an isocratic mode using 30 mM sodium acetate, pH 3.5. Eluted sample was neutralized to pH 5.0 using 1% solution of 3M sodium acetate, pH 9.0. Neutralized protein was sterile filtered with 0.22 µm filtration system. The concentration was measured by UV280 by nanodrop 8000 spectrophotometer. In a second purification, Cation exchange chromatography was
20 applied using a POROS™50 HS cation exchange resin column (Applied Biosystems, Carlsbad, CA, USA) or Size-exclusion chromatography using a HiLoad® 26/600 Superdex® 200 pg column (GE Healthcare). The two-step purified material was stored in final buffer of 50mM Sodium Acetate and 100mM NaCl, pH 5.0 Purity and degree of heterogeneity of the samples were assessed by analytical size-exclusion chromatography,
25 mass spectrometry and analytical ultracentrifugation. Samples that were advanced for functional testing comprised two-step purified material, with about 95 to 99% monomer content.

Table 1: Amino acid sequences and SEQ ID NOs of CDRs, VH, VL, scFabs, B7H6-arm and CD3-arm sequences of the proteins/antibody constructs described herein:

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO: 1	B7H6#1 LCCDR1	KSSQSLFYSSNQKNYLA
SEQ ID NO:2	B7H6#1 LCCDR2	WASTRES
SEQ ID NO:3	B7H6#1 LCCDR3	QQYYNYPRT
SEQ ID NO:4	B7H6#1 HCCDR1	GYTFTDYMN
SEQ ID NO:5	B7H6#1 HCCDR2	YIYPKTGGNGYNQKFKD
SEQ ID NO:6	B7H6#1 HCCDR3	ENWDGYTMAY
SEQ ID NO:7	B7H6#2 LCCDR1	RATSSLYSMH
SEQ ID NO:8	B7H6#2 LCCDR2	ATFNLAS
SEQ ID NO:9	B7H6#2 LCCDR3	QQWSTNPPKLT
SEQ ID NO:10	B7H6#2 HCCDR1	GFNIKNTFIH
SEQ ID NO:11	B7H6#2 HCCDR2	RIDPANGNTIYASKFQG
SEQ ID NO:12	B7H6#2 HCCDR3	TYGGTNYFDY
SEQ ID NO:13	B7H6#3 LCCDR1	KASHNVGVYVA
SEQ ID NO:14	B7H6#3 LCCDR2	SASNRYS

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:15	B7H6#3 LCCDR3	QQYNSYPLT
SEQ ID NO:16	B7H6#3 HCCDR1	GFTFSDYYMT
SEQ ID NO:17	B7H6#3 HCCDR2	NIDYDGSRIYYLDSLKS
SEQ ID NO:18	B7H6#3 HCCDR3	DDPAWLAY
SEQ ID NO:19	B7H6#4 LCCDR1	KASQNVGKYVA
SEQ ID NO:20	B7H6#4 LCCDR2	SASNRYD
SEQ ID NO:21	B7H6#4 LCCDR3	QQYISYPLT
SEQ ID NO:22	B7H6#4 HCCDR1	GYTFTNYWMN
SEQ ID NO:23	B7H6#4 HCCDR2	GIYLNQDSTDYNEKFKG
SEQ ID NO:24	B7H6#4 HCCDR3	RGDYFGDF
SEQ ID NO:25	B7H6#5 LCCDR1	RASQDIRNDLG
SEQ ID NO:26	B7H6#5 LCCDR2	AASSLES
SEQ ID NO:27	B7H6#5 LCCDR3	LQYYNHPLT
SEQ ID NO:28	B7H6#5 HCCDR1	GYTFTGYIYH
SEQ ID NO:29	B7H6#5 HCCDR2	WINPHSGATNYAQNFQG

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:30	B7H6#5 HCCDR3	ERWGSGTFNI
SEQ ID NO:31	B7H6#6 LCCDR1	KASQSVSNDVV
SEQ ID NO:32	B7H6#6 LCCDR2	STSNRYI
SEQ ID NO:33	B7H6#6 LCCDR3	QQDYSSPYT
SEQ ID NO:34	B7H6#6 HCCDR1	GYTFTDYTMH
SEQ ID NO:35	B7H6#6 HCCDR2	GINPNYDNTGYSEKFKD
SEQ ID NO:36	B7H6#6 HCCDR3	SGSRRSFYFDY
SEQ ID NO: 37	B7H6#7 LCCDR1	RASQGISSWLA
SEQ ID NO:38	B7H6#7 LCCDR2	AASSLQS
SEQ ID NO:39	B7H6#7 LCCDR3	QQANSFPRT
SEQ ID NO:40	B7H6#7 HCCDR1	GGISYNYWS
SEQ ID NO:41	B7H6#7 HCCDR2	HIYSGSTNYPNPSLKS
SEQ ID NO:42	B7H6#7 HCCDR3	VGTWGSFDD
SEQ ID NO: 43	B7H6#8 LCCDR1	RSSQSLLYNNRYNYLD
SEQ ID NO:44	B7H6#8 LCCDR2	LGSNRAS

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:45	B7H6#8 LCCDR3	MQTLQIPIT
SEQ ID NO:46	B7H6#8 HCCDR1	GDTLNSYGIS
SEQ ID NO:47	B7H6#8 HCCDR2	GIIPFDTTKYAQKFQG
SEQ ID NO:48	B7H6#8 HCCDR3	ERGYRFSEDYFYGYGMDV
SEQ ID NO: 49	B7H6#9 LCCDR1	RASESVDNFGVFSMN
SEQ ID NO:50	B7H6#9 LCCDR2	AASNQGS
SEQ ID NO:51	B7H6#9 LCCDR3	QQSKEVPWT
SEQ ID NO:52	B7H6#9 HCCDR1	DYTFTHYWIH
SEQ ID NO:53	B7H6#9 HCCDR2	IIGPSDNEIHYNQDFKD
SEQ ID NO:54	B7H6#9 HCCDR3	QIISMVVGTEYFDV
SEQ ID NO: 55	B7H6#10 LCCDR1	RASQGISSWLA
SEQ ID NO:56	B7H6#10 LCCDR2	VASSLQR
SEQ ID NO:57	B7H6#10 LCCDR3	QQANSFPRT
SEQ ID NO:58	B7H6#10 HCCDR1	GDSISSYYWS
SEQ ID NO:59	B7H6#10 HCCDR2	HIYTSEKNNYNPSLKS

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:60	B7H6#10 HCCDR3	VGNWGSHTDA
SEQ ID NO: 61	B7H6#11 LCCDR1	RSSQSLHSNGYNYLD
SEQ ID NO:62	B7H6#11 LCCDR2	LGSNRAS
SEQ ID NO:63	B7H6#11 LCCDR3	MQALQTPLT
SEQ ID NO:64	B7H6#11 HCCDR1	GITFSYYTMN
SEQ ID NO:65	B7H6#11 HCCDR2	SISSRSSYIYYADSVKG
SEQ ID NO:66	B7H6#11 HCCDR3	DKGDYSKDIYYYYGMDV
SEQ ID NO: 67	B7H6#12 LCCDR1	KASQNVGKYVA
SEQ ID NO:68	B7H6#12 LCCDR2	SASNRVD
SEQ ID NO:69	B7H6#12 LCCDR3	QQYISYPLT
SEQ ID NO:70	B7H6#12 HCCDR1	GYTFTNYWMN
SEQ ID NO:71	B7H6#12 HCCDR2	GIYLNQDSTDYNEKFKG
SEQ ID NO:72	B7H6#12 HCCDR3	RGDYFGDF
SEQ ID NO: 73	B7H6#13 LCCDR1	KASQNVGKYVA
SEQ ID NO:74	B7H6#13 LCCDR2	SASNRVD

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:75	B7H6#13 LCCDR3	QQYISYPLT
SEQ ID NO:76	B7H6#13 HCCDR1	GYTFTSYWMN
SEQ ID NO:77	B7H6#13 HCCDR2	GIYLNQDSTDYNEKFKG
SEQ ID NO:78	B7H6#13 HCCDR3	RGDYFGDF
SEQ ID NO: 79	B7H6#14 LCCDR1	KASQNVGKYVA
SEQ ID NO:80	B7H6#14 LCCDR2	SASNRVD
SEQ ID NO:81	B7H6#14 LCCDR3	QQYISYPLT
SEQ ID NO:82	B7H6#14 HCCDR1	GYTFTNYWMN
SEQ ID NO:83	B7H6#14 HCCDR2	GIYLNQDSTDYNEKFKG
SEQ ID NO:84	B7H6#14 HCCDR3	RGDYFGDF
SEQ ID NO: 85	B7H6#15 LCCDR1	KASQNVGKYVA
SEQ ID NO:86	B7H6#15 LCCDR2	SASNRVD
SEQ ID NO:87	B7H6#15 LCCDR3	QQYISYPLT
SEQ ID NO:88	B7H6#15 HCCDR1	GYTFTNYWMN
SEQ ID NO:89	B7H6#15 HCCDR2	GIYLSGDSTDYNEKFKG

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:90	B7H6#15 HCCDR3	RGDYFGDF
SEQ ID NO: 91	B7H6#16 LCCDR1	KASQNVGKYVA
SEQ ID NO:92	B7H6#16 LCCDR2	SASNRVD
SEQ ID NO:93	B7H6#16 LCCDR3	QQYISYPLT
SEQ ID NO:94	B7H6#16 HCCDR1	GYTFTSYWMN
SEQ ID NO:95	B7H6#16 HCCDR2	GIYLSGESTDYNEKFKG
SEQ ID NO:96	B7H6#16 HCCDR3	RGDYFGDF
SEQ ID NO: 97	B7H6#17 LCCDR1	KASQNVGKYVA
SEQ ID NO:98	B7H6#17 LCCDR2	SASNRVD
SEQ ID NO:99	B7H6#17 LCCDR3	QQYISYPLT
SEQ ID NO:100	B7H6#17 HCCDR1	GYTFTSYWMN
SEQ ID NO:101	B7H6#17 HCCDR2	GIYLSGDSTDYNEKFKG
SEQ ID NO:102	B7H6#17 HCCDR3	RGDYFGDF
SEQ ID NO: 103	B7H6#18 LCCDR1	KASQNVGKYVA
SEQ ID NO:104	B7H6#18 LCCDR2	SASNRVD

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:105	B7H6#18 LCCDR3	QQYISYPLT
SEQ ID NO:106	B7H6#18 HCCDR1	GYTFTSYWMN
SEQ ID NO:107	B7H6#18 HCCDR2	GIYLSGDSTDYNEKFKG
SEQ ID NO:108	B7H6#18 HCCDR3	RGDYFGDF
SEQ ID NO: 109	B7H6#19 LCCDR1	KASQNVGKYVA
SEQ ID NO:110	B7H6#19 LCCDR2	SASNRYD
SEQ ID NO:111	B7H6#19 LCCDR3	QQYISYPLT
SEQ ID NO:112	B7H6#19 HCCDR1	GYTFTSYWMN
SEQ ID NO:113	B7H6#19 HCCDR2	GIYLSGESTDYNEKFKG
SEQ ID NO:114	B7H6#19 HCCDR3	RGDYFGDF
SEQ ID NO: 115	B7H6#20 LCCDR1	KASQNVGKYVA
SEQ ID NO:116	B7H6#20 LCCDR2	SASNRYD
SEQ ID NO:117	B7H6#20 LCCDR3	QQYISYPLT
SEQ ID NO:118	B7H6#20 HCCDR1	GYTFTSYWMN
SEQ ID NO:119	B7H6#20 HCCDR2	GIYLSGDSTDYNEKFKG

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:120	B7H6#20 HCCDR3	RGDYFGDF
SEQ ID NO: 121	B7H6#21 LCCDR1	KASQNVGKYVA
SEQ ID NO:122	B7H6#21 LCCDR2	SASNRVD
SEQ ID NO:123	B7H6#21 LCCDR3	QQYISYPLT
SEQ ID NO:124	B7H6#21 HCCDR1	GYTFTSYWMN
SEQ ID NO:125	B7H6#21 HCCDR2	GIYLSGESTDYNEKFKG
SEQ ID NO:126	B7H6#21 HCCDR3	RGDYFGDF
SEQ ID NO: 127	B7H6#22 LCCDR1	KASQNVGKYVA
SEQ ID NO:128	B7H6#22 LCCDR2	SASNRVD
SEQ ID NO:129	B7H6#22 LCCDR3	QQYISYPLT
SEQ ID NO:130	B7H6#22 HCCDR1	GYTFTSYWMN
SEQ ID NO:131	B7H6#22 HCCDR2	GIYLSGDSTDYNEKFKG
SEQ ID NO:132	B7H6#22 HCCDR3	RGDYFGDF
SEQ ID NO: 133	B7H6#23 LCCDR1	KASQNVGKYVA
SEQ ID NO:134	B7H6#23 LCCDR2	SASNRVD

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:135	B7H6#23 LCCDR3	QQYISYPLT
SEQ ID NO:136	B7H6#23 HCCDR1	GYTFTSYWMN
SEQ ID NO:137	B7H6#23 HCCDR2	GIYLSGESTDYNEKFKG
SEQ ID NO:138	B7H6#23 HCCDR3	RGDYFGDF
SEQ ID NO: 139	B7H6#24 LCCDR1	KASQNVGKYVA
SEQ ID NO:140	B7H6#24 LCCDR2	SASNRYD
SEQ ID NO:141	B7H6#24 LCCDR3	QQYISYPLT
SEQ ID NO:142	B7H6#24 HCCDR1	GYTFTNYWMN
SEQ ID NO:143	B7H6#24 HCCDR2	GIYLSGDSTDYNEKFKG
SEQ ID NO:144	B7H6#24 HCCDR3	RGDYFGDF
SEQ ID NO:145	B7H6#1 VL	DIVMSQSPSSLAHSVGEKVTMNCKSSQSLFYSSNQKNYLAWY QQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVKA EDLAVYYCQQYYNYPRTFGGGTKLEIK
SEQ ID NO:146	B7H6#1 VH	EVQLQQSGPELVKPGTSVKMSCKASGYTFTDYMNWVKQSQ GKNLEWIAIYIPKTGGNGYNQKFKDKATLTVDKSSNTAYMELR SLTSDDSAVYYCGRENWDGYTMAYWGQGTSTVTVSS
SEQ ID NO:147	B7H6#2 VL	EIVLTQSPDFLSASPGKVTMTCRATSSLYSMHWYQQKPGSSP KPWIYATFNLASGVPARFSGSGSGTSYSLTITRVEAEDAATYYC QQWSTNPPKLTFGAGTKLELK

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:148	B7H6#2 VH	EVQLQQSGAELVRPGASVKLSCTASGFNIKNTFIHWVNQRPEQ GLEWIGRIDPANGNTIYASKFQGRATITDTSSNTAYMHLSSLT SGDTAVYYCARTYGGTNYFDYWGQGTTTLTVSS
SEQ ID NO:149	B7H6#3 VL	DIVMTQSQKLLSTSVGDRISVTCKASHNVGVYVAWYQQKPGH SPKALIHASNRYSGVPDRFTGSGSGTDFTLTITNVQSEDLAEYF CQQYNSYPLTFGAGTKLELI
SEQ ID NO:150	B7H6#3 VH	EVKLVESEGLVQPGSSMKLSCTASGFTFSDYYMTWVRQVPE KGLEWVGNIDYDGSRIYYLDSLKSRFIISRDNAKNILYQMNSLK SEDTATYYCARDPAWLAYWGQGTTLTVSS
SEQ ID NO:151	B7H6#4 VL	DIVMTQSQKFMSTSVGDRVSVTCKASQNVGKYVAWYQQKP GQSPKALIYSASNRYDGVDPDRFTGSGSGTDFTLTITNVQSEDLT EYFCQQYISYPLTFGAGTKLELK
SEQ ID NO:152	B7H6#4 VH	QVQLQQPGSVLVRPGASVRLSCKASGYFTFTNYWMNWMKQR PGQGLEWIGGIYLNQDSTDYNEKFKGKATLTVDTSSSTTYMDL SSLTYEDSAVYYCTTRGDYFGDFWGQGTTTLTVSS
SEQ ID NO:153	B7H6#5 VL	AIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWFQQRPGK APNLLIYAASSLESQVPSRFSGRSGTDFTLTISLQPEDFATYYC LQYYNHPLTFGGGKVEIK
SEQ ID NO:154	B7H6#5 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTGYIHWVRQAPG QGLEWMGWINPHSGATNYAQNFQGRVTMTRDTSISTAYME LSRLRSDDAAVYYCARERWGSQGFNIWGQGTMTVTSS
SEQ ID NO:155	B7H6#6 VL	DIVMTQSPDSLPSAGDRVTITCKASQSVSNDVVWYQQKPGQ SPKLLMYSTSNRYIGVPDRFTGSGYGTDFFTISTVQAEDLAVYF CQQDYSSPYTFGGGKLEIK
SEQ ID NO:156	B7H6#6 VH	EVQLQQSGPELLKPGASVKISCKTSGYFTDYTMHWVKQSHG KSLEWIGGINPNYDNTGYSEKFKDKATLTVDKSSSTAYMELRSL TSEDSAVYYCTRSGSRRSFYFDYWGQGTTTLTVSS
SEQ ID NO:157	B7H6#7 VL	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGK APNLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC QQANSFPRTFGQGTKEIK

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:158	B7H6#7 VH	QVQLQESGPGLVKPSSETLSLTYSVSGGSISYNYWSWIRQPPEK GLEWIGHIYYSGSTNYPNPSLKSRTISVDTSKNQFSLKLSNVTA DTAVYYCARVGTWGSFDDWGQGLTVTVSS
SEQ ID NO:159	B7H6#8 VL	DIVMTQSPLSLPVTGPGEASISCRSSQSLLYNNRYNYLDWYLQK PGQSPQVLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDF GVYYCMQTLQIPITFGQGTREIK
SEQ ID NO:160	B7H6#8 VH	QVQVQSGAEVKKPGSSVKVSCKGSGDTLNSYGISWMRQAP GQGLEWMGGIIPFDITTKYAQKFQGRVTITADKSTTTVYMELS SLRFEDTAVYYCARERGRFSEDYFYGYMDVWGQGTITVTVS S
SEQ ID NO:161	B7H6#9 VL	DIVLTQSPVSLAVSLGQRATISCRASESVDNFGVSFMNWFQK PGQPPKLLIYAASNQSGVPSRFGSGSGTDFTSLNIHPLEEDT AMYFCQKSKEVPWTFGGGTREIK
SEQ ID NO:162	B7H6#9 VH	QVQLQQPGAEMVRPGSSVKLSCKASDYTFTHYWIHWVKQRP LEGLEWIGIIGPSDNEIHYNQDFKDKATLTVDKSSNTAYLHLNSL TSSESAVYYCARQIISMVVGTEYFDVWGQGTITVTVSS
SEQ ID NO:163	B7H6#10 VL	DIQMTQSPSSVSASVGDRTITCRASQGISSWLAWYQQKPGK APKLLIYVASSLQRGVPSRFGSGSGTDFTLTISNLQPEDFATYY CQQANSFPRTFGQGTKVEIK
SEQ ID NO:164	B7H6#10 VH	QVHLQESGPGLVKPSSETLSLTCTVSGDISSYWWSWIRQPAGK GLEWIGHIYTSEKNYPNPSLKSRTISVDTSKNQFSLNLSVTA ADTAVYYCARVGNWGSHTDAWGQGLTVTVSS
SEQ ID NO:165	B7H6#11 VL	DIVMTQSPLSLPVTGPGEASISCRSSQSLLHSNGYNYLDWYLQK PGQSPQVLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCMQALQTPITFGGGTKVEIK
SEQ ID NO:166	B7H6#11 VH	ELQLVNSGGGLVKSGGSLRLSCAASGITFSYTMNWRQAPG KGLEWVSSISSRSYIYADSVKGRFTISRDNALNSLYLQMNSLR AEDTAVYYCARDKGDYSKDIYIYGYMDVWGQGTITVTVSS
SEQ ID NO:167	B7H6#12 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFGSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:168	B7H6#12 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTFTNYWMNWKQA PGQGLEWMGGIYLNGDSTDYNEKFKGKATMTVDTSTSTVYM ELSSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:169	B7H6#13 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK
SEQ ID NO:170	B7H6#13 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYWMNWMKQA PGQGLEWMGGIYLNGDSTDYNEKFKGRVTMTVDTSTSTVYM ELSSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:171	B7H6#14 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIK
SEQ ID NO:172	B7H6#14 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTFTNYWMNWMKQA PGQGLEWIGGIYLNGDSTDYNEKFKGKVTMTVDTSTSTVYME LSSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:173	B7H6#15 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK
SEQ ID NO:174	B7H6#15 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTFTNYWMNWMRQA PGQGLEWMGGIYLSGDSTDYNEKFKGRVTMTVDTSTSTVYM ELSSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:175	B7H6#16 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK
SEQ ID NO:176	B7H6#16 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYWMNWMRQA PGQGLEWMGGIYLSGESTDYNEKFKGRVTMTVDTSTSTVYME LSSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:177	B7H6#17 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:178	B7H6#17 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYWMNWMRQA PGQGLEWMGGIYLSGDSTDYNEKFKGRVTMTVDSTSTVYM ELSSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:179	B7H6#18 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK
SEQ ID NO:180	B7H6#18 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYWMNWMRQA PGQGLEWMGGIYLSGDSTDYNEKFKGRVTMTVDSTSTVYM ELSSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:181	B7H6#19 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK
SEQ ID NO:182	B7H6#19 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYWMNWKQAP GQGLEWMGGIYLSGESTDYNEKFKGKATMTVDSTSTVYMEL SSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:183	B7H6#20 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK
SEQ ID NO:184	B7H6#20 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYWMNWKQAP GQGLEWMGGIYLSGDSTDYNEKFKGKATMTVDSTSTVYMEL SSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:185	B7H6#21 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIK
SEQ ID NO:186	B7H6#21 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTSYWMNWMKQA PGQGLEWIGGIYLSGESTDYNEKFKGKVTMTVDSTSTVYMEL SSLRSEDTAVYYCTRRGDYFGDFWGQGLTVTVSS
SEQ ID NO:187	B7H6#22 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIK

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:188	B7H6#22 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWMKQA PGQGLEWIGGIYLSGDSTDYNEKFKGKVTMTVDTSTSTVYMEL SSLRSEDATAVYYCTRRGDYFGDFWGGQGLTVTVSS
SEQ ID NO:189	B7H6#23 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIK
SEQ ID NO:190	B7H6#23 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMNWMKQA PGQGLEWIGGIYLSGESTDYNEKFKGKVTMTVDTSTSTVYMEL SSLRSEDATAVYYCTRRGDYFGDFWGGQGLTVTVSS
SEQ ID NO:191	B7H6#24 VL	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIK
SEQ ID NO:192	B7H6#24 VH	QVQLVQSGAEVKKPGASVKVSCKASGYFTFTNYWMNWMRQA PGQGLEWMGGIYLSGDSTDYNEKFKGRVTMTVDTSTSTVYM ELSSLRSEDATAVYYCTRRGDYFGDFWGGQGLTVTVSS
SEQ ID NO:193	B7H6#1 scFab	DIVMSQSPSSLAVSVGEKVTMNCKSSQSLFYSSNQKNYLAWY QQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVKA EDLAVYYCQQYINYPRTFGGGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR GECGGGGSEGKSSGSGSEKSTEGKSSGSGSEKSTGGGGSEV QLQQSGPELVKPGTSVKMSCKASGYFTDYMNWVKQSQGK NLEWIAIYPKTGGNGYNQKFKDKATLTVDKSSNTAYMELRSL TSDDSAVYYCGRENWDGYTMAYWGGQTSVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSC
SEQ ID NO:194	B7H6#2 scFab	EIVLTQSPDFLSASPGEKVTMTCRATSSLYSMHWYQQKPGSSP KPWIYATFNLASGVPARFSGSGSGTSYSLTITRVEAEDAATYYC QQWSTNPPKLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST

SEQ ID Number	Brief description of sequence	Sequence
		YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGG GGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLQQS GAELVRPGASVKLSCTASGFNIKNTFIHWVNQRPEQGLEWIGR IDPANGNTIYASKFQGRATITTTSSNTAYMHLSSLTSGDTAVY YCARTYGGTNYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSVSVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:195	B7H6#3 scFab	DIVMTQSQKLLSTSVGDRISVTCASHNVGVYVAWYQQKPGH SPKALIHASNRYSGVPDRFTGSGSGTDFTLTITNVQSEDLAEYF CQQYNSYPLTFGAGTKLELIRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVKLVESEGG LVQPGSSMKLSCTASGFTFSDYYMTWVRQVPEKGLEWVGNI DYDGSRIYYLDSLKSRIISRDNAKNILYQMNSLKSEDTATYYCA RDDPAWLAYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SVVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:196	B7H6#4 scFab	DIVMTQSQKFMSTSVGDRVSVTCASQNVGKYVAWYQQKP GQSPKALIYSASNRYDGVDPDRFTGSGSGTDFTLTITNVQSEDLT EYFCQQYISYPLTFGAGTKLELKRVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLQQ PGSVLVRPGASVRLSCKASGYFTFTNYWMNWMKQRPQGGL WIGGIYLNQDSTDYNEKFKGKATLTVDTSSSTTYMDLSSLTIED SAVYYCTTRGDYFGDFWGQGTTLTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYLSVSVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:197	B7H6#5 scFab	AIQMTQSPSSLSASVGDRVTITCRASQDIRNDLGWFQQRPGK APNLLIYAASSLESQVPSRFSGRSGTDFTLTISLQPEDFATYYC

SEQ ID Number	Brief description of sequence	Sequence
		LQYYNHPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGA EVKKPGASVKVCKASGYTFTGYYIHVVRQAPGQGLEWMG WINPHSGATNYAQNFQGRVTMTRDTSISTAYMELSLRSDDA AVYYCARERWGSQGFNIWGGQTMVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTWSNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SC
SEQ ID NO:198	B7H6#6 scFab	DIVMTQSPDSLPLVSAGDRVITITCKASQSVSNVWVYQQKPGQ SPKLLMYSTSNRYIGVPDRFTGSGYGTDFITISTVQAEDLAVYF CQQDYSSPYTFGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLQQSGPE LLKPGASVKISCKTSGYTFTDYMHWVKQSHGKSLWIGGINP NYDNTGYSEKFKDKATLTVDKSSSTAYMELRSLTSEDSAVYYCT RSGSRFSFYFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTWSNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:199	B7H6#7 scFab	DIQMTQSPSSVSASVGDRTITCRASQGISSWLAWYQQKPGK APNLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC QQANSFPRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLQESGP GLVKPSETLSLTYSVSGGSISYNYWSWIRQPPEKLEWIGHIYYS GSTNYPNLSKSRVTISVDTSKNQFSLKLNSTAAADTAVYYCARV GTWGSFDDWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTA

SEQ ID Number	Brief description of sequence	Sequence
		ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:200	B7H6#8 scFab	DIVMTQSPLSLPVTPGEPASISCRSSQSLLYNNRYNYLDWYLQK PGQSPEVLIYLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDF GVYYCMQTLQIPITFGQGRTRLEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSEGKSSGSGSEKSTEGKSSGSGSEKSTGGGGSQVQVQ SGAEVKKPGSSVKVCKGSGDTLNSYGISWMRQAPGQGLEW MGGIPIFDITTKYAQKFGQGRVTITADKSTTTVYMELSSLRFEDTA VYYCARERGRYFSEDYFFYYGMDVWGQGTITVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSC
SEQ ID NO:201	B7H6#9 scFab	DIVLTQSPVSLAVSLGQRATISCRASESVDNFGVSFMINWFQQK PGQPPKLLIYAASNQSGGVPARFSGSGSGTDFTSLNIHPLEDDT AMYFCQKSKEVPWTFGGGTRLEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE CGGGGSEGKSSGSGSEKSTEGKSSGSGSEKSTGGGGSQVQL QQPGAEMVRPGSSVKLSCKASDYTFTHYWIHWVKQRPLEGLE WIGIIGPSDNEIHYNQDFDKATLTVDKSSNTAYLHLNLTSEDS AVYYCARQIISMVVGTEYFDVWGTGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSC
SEQ ID NO:202	B7H6#10 scFab	DIQMTQSPSSVSASVGDRTITCRASQGISSWLAWYQQKPGK APKLLIYVASSLQRGVPSRFSGSGSGTDFTLTISNLQPEDFATYY CQQANSFPRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDY SLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG

SEQ ID Number	Brief description of sequence	Sequence
		GSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVHLQESG PGLVKPSETLSLTCTVSGDISSYYSWIRQPAGKGLEWIGHIY TSEKNNYNPSLKSRVIMSVDTSKNQFSLNLSSVTAADTAVYYCA RVGNWGSHTDAWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:203	B7H6#11 scFab	DIVMTQSPLSLPVTGPGEASISCRSSQSLHNSGYNLYDWYLQK PGQSPQVLIYLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDV GVYYCMQALQTPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVCLNNFYPRKAVQWVKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGSGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSELQLV NSGGGLVKSGSLRLSCLASGITFSYTMNWVRQAPGKGLEW VSSISSRSSYIYADSVKGRFTISRDNALQSLYQMNSLRVEDTA VYYCARDKGDYSDIYIYGGMDVWGQGTITVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSC
SEQ ID NO:204	B7H6#12 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTYYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV CLNNFYPRKAVQWVKVDNALQSGNSQESVTEQDSKSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYFTFTNYWMNWVKQAPGQGLEWMG GIYLNQDSTDYNEKFKGKATMTVDTSTSTVYMELSSLRSEDTA VYYCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:205	B7H6#13 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTYYC

SEQ ID Number	Brief description of sequence	Sequence
		QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTSYWMNWMKQAPGGGLEWMG GIYLNQDSTDYNEKFKGRVTMTVDTSTSTVYMELSSLRSEDTA VYYCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSG LYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:206	B7H6#14 scFab	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTNYWMNWMKQAPGGGLEWIGG IYLNQDSTDYNEKFKGKVTMTVDTSTSTVYMELSSLRSEDTAVY YCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:207	B7H6#15 scFab	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTNYWMNWMRQAPGGGLEWMG GIYLSGDSTDYNEKFKGRVTMTVDTSTSTVYMELSSLRSEDTAV YYCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGL YLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:208	B7H6#16 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISSLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCASGYTFTSYWMNWMRQAPGQGLEWMG GIYLSGESTDYNEFKGRVTMTVDTSTSTVYMELSSLRSEDVAV YYCTRRGDYFGDFWGQGLTVVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSC
SEQ ID NO:209	B7H6#17 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISSLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCASGYTFTSYWMNWMRQAPGQGLEWMG GIYLSGDSTDYNEFKGRVTMTVDTSTSTVYMELSSLRSEDVAV YYCTRRGDYFGDFWGQGLTVVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSC
SEQ ID NO:210	B7H6#18 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISSLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCASGYTFTSYWMNWMRQAPGQGLEWMG GIYLSGDSTDYNEFKGRVTMTVDTSTSTVYMELSSLRSEDVAV YYCTRRGDYFGDFWGQGLTVVSSASTKGPSVFPLAPSSKSTS

SEQ ID Number	Brief description of sequence	Sequence
		GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSVSVVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:211	B7H6#19 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGG EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTSYWMNWVKAPGQGLEWMG GIYLSGESTDYNEKFKGKATMTVDTSTSTVYMELSSLRSEDVAV YYCTRRGDYFGDFWGQGLTVVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSVSVVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:212	B7H6#20 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGG EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTSYWMNWVKAPGQGLEWMG GIYLSGDSTDYNEKFKGKATMTVDTSTSTVYMELSSLRSEDVAV YYCTRRGDYFGDFWGQGLTVVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSVSVVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:213	B7H6#21 scFab	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGG EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTSYWMNWMKAPGQGLEWIGG

SEQ ID Number	Brief description of sequence	Sequence
		IYLSGESTDYNEKFKGKVTMTVDTSTSTVYMELSSLRSEDNAVY YCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:214	B7H6#22 scFab	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTSYWMNWMKQAPGQGLEWIGG IYLSGDSTDYNEKFKGKVTMTVDTSTSTVYMELSSLRSEDNAVY YCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:215	B7H6#23 scFab	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTSYWMNWMKQAPGQGLEWIGG IYLSGESTDYNEKFKGKVTMTVDTSTSTVYMELSSLRSEDNAVY YCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:216	B7H6#24 scFab	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS

SEQ ID Number	Brief description of sequence	Sequence
		EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYFTFTNYWMNWMRQAPGQGLEWMG GIYLSGDSTDYNEKFKGRVTMTVDTSTSTVYMELSSLRSEDTAV YYCTRRGDYFGDFWGQGLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSVSVTPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSC
SEQ ID NO:217	B7H6#1 chain	DIVMSQSPSSLAHSVGEKVTMNCSSQSLFYSSNQKNYLAWY QQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVKA EDLAVYYCQQYYNYPRTFGGGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR GECGGGSGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEV QLQQSGPELVKPGTSVKMSCKASGYFTDYMNWVKQSQGK NLEWIAIYPKTGGNGYNQKFKDKATLTVDKSSNTAYMELRSL TSDDSAVYYCGRENWDGYTMAYWGQTSVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYLSVSVTPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT EVTCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSV MHEALHNHYTQKSLSLSPG
SEQ ID NO:218	B7H6#2 chain	EIVLTQSPDFLSASPGEKVTMTCRATSSLYSMHWYQQKPGSSP KPWIYATFNLASGVPARFSGSGSYSLTITRVEAEDAATYYC QQWSTNPPKLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA SVVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGECGG GGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLQQS GAELVRPGASVKLSCTASGFNIKNTFIHWVNQRPEQGLEWIGR IDPANGNTIYASKFQGRATITTTDSSNTAYMHLSSLTSGDTAVY

SEQ ID Number	Brief description of sequence	Sequence
		YCARTYGGTNYFDYWGQGTTTLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPL PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY TQKSLSLSPG
SEQ ID NO:219	B7H6#3 chain	DIVMTQSQKLLSTSVGDRISVTCKASHNVGVYVAWYQQKPGH SPKALIHASASNRYSGVPDRFTGSGSGTDFTLTITNVQSEDLAEYF CQQYNSYPLTFGAGTKLELIRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVKLVESEGG LVQPGSSMKLSCTASGFTFDYYMTWVRQVPEKGLEWVGNI DYDGSRIYYLDSLKSRIISRDNAKNILYLMNSLKSSEDATYCYA RDDPAWLAYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SVVTVPSLSLTQTYICNVNHKPSNTKVDKRVPEKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPG
SEQ ID NO:220	B7H6#4 chain	DIVMTQSQKFMSTSVGDRVSVTKASQNVGKYVAWYQQKP GQSPKALIYSASNRYDGVDPDRFTGSGSGTDFTLTITNVQSEDLT EYFCQQYISYPLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG

SEQ ID Number	Brief description of sequence	Sequence
		GGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLQQ PGSVLVRPGASVRLSCKASGYTFTNYWMNWMKQRPQGGL WIGGIYLNQDSTDYNEKFKGKATLTVDTSSTTYMDLSSLTIED SAVYYCTTRGDYFGDFWGQGTTLTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG
SEQ ID NO:221	B7H6#5 chain	AIQMTQSPSSLSASVGDRTITCRASQDIRNDLGWFQQRPGK APNLLIYAASSLESGVPSRFSGRGSGTDFTLTISLQPEDFATYYC LQYYNHPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGA EVKKPGASVKVSCKASGYTFTGYYIHWRQAPGQGLEWMG WINPHSGATNYAQNFQGRVTMTTRDTSISTAYMELSRLRSDDA AVYYCARERWGSQGFNIWGQGTMTVTSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPG
SEQ ID NO:222	B7H6#6 chain	DIVMTQSPDSLTPVSAGDRVTITCKASQSVSNDVVWYQQKPGQ SPKLLMYSTSNRYIGVPDRFTGSGYGTDFFTISTVQAEDLAVYF

SEQ ID Number	Brief description of sequence	Sequence
		CQQDYSSPYTFGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLQQSGPE LLKPGASVKISCKTSGYTFTDYMHWVKQSHGKSLEWIGGINP NYDNTGYSEKFKDKATLTVDKSSSTAYMELRSLTSEDAVYYCT RSGSRRSFYFDYWGGGTTLTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKHTHC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPG
SEQ ID NO:223	B7H6#7 chain	DIQMTQSPSSVSASVGDRVITTCRASQGISSWLAWYQQKPGK APNLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC QQANSFPRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG SEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLQESGP GLVKPSETLSLTYTVSGGSISYNYWSWIRQPPEKLEWIGHIYYS GSTNYPNPSLKSRTISVDTSKNQFSLKNSVTAADTAVYYCARV GTWGSFDDWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKHTHCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD

SEQ ID Number	Brief description of sequence	Sequence
		SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPG
SEQ ID NO:224	B7H6#8 chain	DIVMTQSPLSLPVTGPGEASISCRSSQSLLYNNRYNYLDWYLQK PGQSPEVLIYLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDF GVYYCMQTLQIPITFGQGRTRLEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQVWQ SGAEVKKPGSSVKVSCKSGDNLNSYGISWMRQAPGQGLEW MGGIIPFDITTKYAQKFQGRVTITADKSTTTVYMELSSLRFEDTA VYYCARERGRYFSEDYFFYYGMDVWGQGTITVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSKV HTFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTKAK GQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPG
SEQ ID NO:225	B7H6#9 chain	DIVLTQSPVSLAVSLGQRATISCRASESVDNFGVFSFMNWFQKQ PGQPPKLLIYAASNQSGGVPARFSGSGSGTDFTSLNIHPLEEDDT AMYFCQQSKVEPWTFGGGTRLEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE CGGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQL QQPGAEMVRPGSSVKLSCKASDYTFTHYWIHWVKQRPLEGLE WIGIIGPSDNEIHYNQDFKDKATLTVDKSSNTAYLHLNSLTSED AVYYCARQIISMVVGTEYFDVWGTGTTTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSKVHTFP AVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEV

SEQ ID Number	Brief description of sequence	Sequence
		TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG
SEQ ID NO:226	B7H6#10 chain	DIQMTQSPSSVSASVGDRTITCRASQGISSWLAWYQQKPGK APKLLIYVASSLQRGVPSRFSGSGSGTDFTLTISNLQPEDFATYY CQQANSFPRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDY SLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVHLQESG PGLVKPSETLSLTCTVSGDSISSYYWSWIRQPAGKGLEWIGHY TSEKNNYNPSLKS RVIMSVDTSKNQFSLNLSSVTAADTAVYYCA RVGNWGS HDAWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG
SEQ ID NO:227	B7H6#11 chain	DIVMTQSPSLPVTGPGEASISCRSSQSLHNSNGYNYLDWYLQK PGQSPQVLIYLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDV GVYYCMQALQTLPTFGGGTKEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSELQLV NSGGGLVKSGGSLRLSCAASGITFSYTMNWVRQAPGKGLEW VSSISSRSSYIYADSVKGRFTISRDAENSLYLQMNSLR AEDTA VYYCARDKGDYSKDIYYGMDVWGQGT VTVSSASTKGPSV

SEQ ID Number	Brief description of sequence	Sequence
		FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT EVTCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG
SEQ ID NO:228	B7H6#12 chain	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYFTFTNYWMNWVKQAPGQGLEWMG GIYLNQDSTDYNEKFKGKATMTVDSTSTVYMESSLRSEDTA VYYCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDV HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHY TQKSLSLSPG
SEQ ID NO:229	B7H6#13 chain	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE

SEQ ID Number	Brief description of sequence	Sequence
		VKKPGASVKVSCKASGYTFTSYWMNWMKQAPGQGLEWMG GIYLNQDSTDYNEKFKGRVTMTVDSTSTVYMELSSLRSEDTA VYYCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPL PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
SEQ ID NO:230	B7H6#14 chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCKASGYTFTNYWMNWMKQAPGQGLEWIGG IYLNQDSTDYNEKFKGKVTMTVDSTSTVYMELSSLRSEDTAVY YCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPG
SEQ ID NO:231	B7H6#15 chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV

SEQ ID Number	Brief description of sequence	Sequence
		CLLNNFYBPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCASGYFTFTNYWMNWMRQAPGGGLEWMG GIYLSGDSTDYNEKFKGRVTMTVDTSTSTVYMELSSLRSEDAV YYCTRRGDYFGDFWGQGLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTP PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY TQKSLSLSPG
SEQ ID NO:232	B7H6#16 chain	DIQMTQSPSSLSASVGRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYBPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCASGYFTTSYWMNWMRQAPGGGLEWMG GIYLSGESTDYNEKFKGRVTMTVDTSTSTVYMELSSLRSEDAV YYCTRRGDYFGDFWGQGLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPCPAPEAAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTP PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY TQKSLSLSPG

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:233	B7H6#17 chain	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISSLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCKASGYTFTSYWMNWMRQAPGQGLEWMG GIYLSGDSTDYNEKFKGRVTMTVDSTSTVYMELSSLRSEDAV YYCTRRGDYFGDFWGQGLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY TQKSLSLSPG
SEQ ID NO:234	B7H6#18 chain	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISSLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCKASGYTFTSYWMNWMRQAPGQGLEWMG GIYLSGDSTDYNEKFKGRVTMTVDSTSTVYMELSSLRSEDAV YYCTRRGDYFGDFWGQGLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL

SEQ ID Number	Brief description of sequence	Sequence
		PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
SEQ ID NO:235	B7H6#19 chain	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYFTSYWMNWVKQAPGQGLEWMG GIYLSGESTDYNEKFKGKATMTVDTSTSTVYMELSSLRSEDVAV YYCTRRGDYFGDFWGQGLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTP PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
SEQ ID NO:236	B7H6#20 chain	DIQMTQSPSSLSASVGDRTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYFTSYWMNWVKQAPGQGLEWMG GIYLSGDSTDYNEKFKGKATMTVDTSTSTVYMELSSLRSEDVAV YYCTRRGDYFGDFWGQGLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT

SEQ ID Number	Brief description of sequence	Sequence
		HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ KSLSLSPG
SEQ ID NO:237	B7H6#21 chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDGVPSRFSGSGSGTDFTLTISSLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKSTYSLS SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCASGYTFTSYWMNWMKQAPGQGLEWIGG IYLSGESTDYNEKFKGKVTMTVDTSTSTVYMELSSLRSEDYAVY YCTRGRDYGDFWGGQTLTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ KSLSLSPG
SEQ ID NO:238	B7H6#22 chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISSLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKSTYSLS SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVSCASGYTFTSYWMNWMKQAPGQGLEWIGG IYLSGDSTDYNEKFKGKVTMTVDTSTSTVYMELSSLRSEDYAVY

SEQ ID Number	Brief description of sequence	Sequence
		YCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPP VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ KSLSLSPG
SEQ ID NO:239	B7H6#23 chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFATYFC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYTFTSYWMNWMKQAPGQGLEWIGG IYLSGESTDYNEKFKGKVTMTVDTSTSTVYMELSSLRSEDAVY YCTRRGDYFGDFWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPP VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQ KSLSLSPG
SEQ ID NO:240	B7H6#24 chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGKYVAWYQQKPGK APKSLIYSASNRYDAVPSRFSGSGSGTDFTLTISLQPEDFTTYC QQYISYPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGS

SEQ ID Number	Brief description of sequence	Sequence
		EGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAE VKKPGASVKVCKASGYFTFTNYWMNWMRQAPGQGLEWMG GIYLSGDSTDYNEKFKGRVTMTVDTSTSTVYMELSSLRSEDTAV YYCTRRGDYFGDFWQQGTLTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGL YLSLVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
SEQ ID NO:241	Fc domain* (IgG1)	<u>DKTHTCPPCP</u> APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG
SEQ ID NO:242	Fc W domain (IgG1, LALA)	<u>DKTHTCPPCP</u> APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
SEQ ID NO:243	Fc, SAV domain (IgG1, RF/LALA)	<u>DKTHTCPPCP</u> APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN RFTQKSLSLSPG

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:244	Fc domain (IgG4Pro)	<u>ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV</u> VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLGLG
SEQ ID NO:245	Fc W domain (IgG4Pro)	<u>ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV</u> VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLGLG
SEQ ID NO:246	Fc SAV domain (IgG4Pro, RF)	<u>ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV</u> VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNRFTQKSLSLGLG
SEQ ID NO:247	constant region of a kappa light chain	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC
SEQ ID NO:248	constant region of a lambda light chain	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVKVAWK ADGSPVNTGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRYS CQVTHEGSTVEKTVAPAEC
SEQ ID NO:249	Constant region of heavy chain CH1	ASTKGPSVFPLAPSSKSTSGGTAAALGLVKDYFPEPVTWSNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKRVKPKSC

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:250	Linker	GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGS
SEQ ID NO:251	Linker	GGGGSGGGSGGSGGSGGGGS
SEQ ID NO:252	Linker	GGGGSGGGSGGGSGGGSGGGGS
SEQ ID NO:253	Linker	GGGGSGGGSGGGSGGGSGGGSGGGGS
SEQ ID NO:254	Linker	GGGGSGGGSGGGSGGGSGGGSGGGSGGGGS
SEQ ID NO:255	Linker	GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGS
SEQ ID NO:256	Linker	GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG GGS
SEQ ID NO:257	CD3#1 LCCDR1	RSSTGAVTTSNYAN
SEQ ID NO:258	CD3#1 LCCDR2	GTNKRAP
SEQ ID NO:259	CD3#1 LCCDR3	ALWYSNLWV
SEQ ID NO:260	CD3#1 HCCDR1	GFTFNTYAMN
SEQ ID NO:261	CD3#1 HCCDR2	RIRSKYNNYATYYADSVKD
SEQ ID NO:262	CD3#1 HCCDR3	HGNFGNSYVSWFAY
SEQ ID NO:263	CD3#2 LCCDR1	KSSQSLNSRTRKNYLA
SEQ ID NO:264	CD3#2 LCCDR2	WASTRES

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:265	CD3#2 LCCDR3	KQSFILRT
SEQ ID NO:266	CD3#2 HCCDR1	GYSFTDYYVH
SEQ ID NO:267	CD3#2 HCCDR2	WIYPGNGNIKYNERFRG
SEQ ID NO:268	CD3#2 HCCDR3	DNYSAYYFAY
SEQ ID NO:269	CD3#3 LCCDR1	KSSQSLNSRTRKVYLA
SEQ ID NO:270	CD3#3 LCCDR2	WASTRES
SEQ ID NO:271	CD3#3 LCCDR3	KQSFILRT
SEQ ID NO:272	CD3#3 HCCDR1	GYTFTSYVH
SEQ ID NO:273	CD3#3 HCCDR2	WIYPGGGNIKYAQKFQG
SEQ ID NO:274	CD3#3 HCCDR3	DQYSAYYFAY
SEQ ID NO:275	CD3#4 LCCDR1	KSSQSLNSRTRKVYLA
SEQ ID NO:276	CD3#4 LCCDR2	WASTRES
SEQ ID NO:277	CD3#4 LCCDR3	KQSFILRT
SEQ ID NO:278	CD3#4 HCCDR1	GYSFTSYVH
SEQ ID NO:279	CD3#4 HCCDR2	WIYPGGGNIKYNQKFQG

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:280	CD3#4 HCCDR3	DHYSAYYFAY
SEQ ID NO:281	CD3#5 LCCDR1	KSSQSLNSRTRKTYLA
SEQ ID NO:282	CD3#5 LCCDR2	WASTRES
SEQ ID NO:283	CD3#5 LCCDR3	KQSFILRT
SEQ ID NO:284	CD3#5 HCCDR1	GYTFTGYVH
SEQ ID NO:285	CD3#5 HCCDR2	WIYPGGGSTKYAQKFQG
SEQ ID NO:286	CD3#5 HCCDR3	DQYSAYYFAY
SEQ ID NO:287	CD3#6 LCCDR1	KSSQSLNSRTRKTYLA
SEQ ID NO:288	CD3#6 LCCDR2	WASTRES
SEQ ID NO:289	CD3#6 LCCDR3	KQSFILRT
SEQ ID NO:290	CD3#6 HCCDR1	GYTFTSYVH
SEQ ID NO:291	CD3#6 HCCDR2	WIYPGGGNIKYAQKFQG
SEQ ID NO:292	CD3#6 HCCDR3	DQYSAYYFAY
SEQ ID NO:293	CD3#1 VL	EAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPG QLPRGLIGGTNKRAPWVPARFSGSLLGGKAALTLSGAQPEDEA EYFCALWYSNLWVFGGGTKLTVL

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:294	CD3#1 VH	EVQLVESGGGLVQPGGSLKLSAASGFTFNTYAMNWVRQAP GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ MNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSA
SEQ ID NO:295	CD3#2 VL	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKNYLAWYQ QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAE DVAVYYCKQSFILRTFGQGTKLEIK
SEQ ID NO:296	CD3#2 VH	QVQLVQSGAEVKKPGASVKVSCKASGYSTDYVHWVRQAP GQGLEWMGWYIPGNGNIKYNERFRGRVTMTRDTSTSTVYME LSSLRSED TAVYYCARDNYSAYYFAYWGQGT TVTVSS
SEQ ID NO:297	CD3#3 VL	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKVLAWYQ QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAE DVAVYYCKQSFILRTFGQGTKLEIK
SEQ ID NO:298	CD3#3 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVHWVRQAPG QGLEWIGWIYPGGGNIKYAQKFQGRVTMTRDTSTSTVYMELS SLRSED TAVYYCARDQYSAYYFAYWGQGT TVTVSS
SEQ ID NO:299	CD3#4 VL	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKVLAWYQ QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAE DVAVYYCKQSFILRTFGQGTKLEIK
SEQ ID NO:300	CD3#4 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVHWVRQAPG QGLEWIGWIYPGGGNIKYNQKFQGRVTMTRDTSTSTVYMELS SLRSED TAVYYCARDHYSAYYFAYWGQGT TVTVSS
SEQ ID NO:301	CD3#5 VL	DIVMTQSPDSLAVSLGERATISCKSSQSLNSRTRKTYLAWYQQ KPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAE VAVYYCKQSFILRTFGQGTKLEIK
SEQ ID NO:302	CD3#5 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVHWVRQAP GQGLEWMGWYIPGGGSTKYAQKFQGRVTMTRDTSTSTVYM ELSSLRSED TAVYYCARDQYSAYYFAYWGQGT TVTVSS
SEQ ID NO:303	CD3#6 VL	DIVMTQSPDSLAVSLGERATISCKSSQSLNSRTRKTYLAWYQQ KPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAE VAVYYCKQSFILRTFGQGTKLEIK

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:304	CD3#6 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVHWVRQAPG QGLEWIGWIYPGGGNIKYAQKFQGRVTMTRDTSTSTVYMELS SLRSEDVAVYYCARDQYSAYYFAYWGQGTTVTVSS
SEQ ID NO:305	CD3#1 scFab	EAVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQEKPG QLPRGLIGGTNKRAPWVPARFSGSLLGGKAALTLSGAQPEDEA EYFCALWYSNLWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVKVAWKADGSPVNTGVETTTPSKQSN NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAEC SGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLV ESGGGLVQPGGSLKLSAASGFTFNTYAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTSAASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSC
SEQ ID NO:306	CD3#2 scFab	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKNYLAWYQ QKPGQPPKLLIWASTRESGVPRFSGSGSGTDFTLTISLQAE DVAVYYCKQSFILRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLV QSGAEVKKPGASVKVSCKASGYSTFYVHWVRQAPGQGLE WMGWYIPGNGNIKYNERFRGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCARDNYSAYYFAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSC
SEQ ID NO:307	CD3#3 scFab	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKVYLAWYQ QKPGQPPKLLIWASTRESGVPRFSGSGSGTDFTLTISLQAE DVAVYYCKQSFILRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSK

SEQ ID Number	Brief description of sequence	Sequence
		DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLV QSGAEVKKPGASVKVSCKASGYFTSYVHWVRQAPGQGLE WIGWIYPGGGNIKYAQKFQGRVTMTRDTSTSTVYMELSSLR EDTAVYYCARDQYSAYYFAYWGQGTITVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSC
SEQ ID NO:308	CD3#4 scFab	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKVLAWYQ QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAE DVAVYYCKQSFILRTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVCLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLV QSGAEVKKPGASVKVSCKASGYFTSYVHWVRQAPGQGLE WIGWIYPGGGNIKYNQKFQGRVTMTRDTSTSTVYMELSSLR EDTAVYYCARDHYSAYYFAYWGQGTITVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSC
SEQ ID NO:309	CD3#5 scFab	DIVMTQSPDSLAVSLGERATISCKSSQSLNSRTRKTYLAWYQQ KPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAE VAVYYCKQSFILRTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT ASVCLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQ SGAEVKKPGASVKVSCKASGYFTGYVHWVRQAPGQGLEW MGWIYPGGGSTKYAQKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCARDQYSAYYFAYWGQGTITVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV

SEQ ID Number	Brief description of sequence	Sequence
		LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SC
SEQ ID NO:310	CD3#6 scFab	DIVMTQSPDSLAVSLGERATISCKSSQSLNSRTRKTYLAWYQQ KPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAED VAVYYCKQSFILRTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQ SGAEVKKPGASVKVSCKASGYFTSYVHWVRQAPGQGLEWI GWIYPGGGNIKYAQKFQGRVTMTRDTSTSTVYMELSSLRSED AVYYCARDQYSAYYFAYWGQGTITVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
SEQ ID NO:311	CD3#1 chain	EAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQEKPG QLPRGLIGGTNKRAPWVPAFSGSLLGGKAALTLSGAQPEDEA EYFCALWYSNLWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVKVAWKADGSPVNTGVETTTPSKQSN NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLV ESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTSAASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSC SVMHEALHNRFTQKSLSLSPG

SEQ ID Number	Brief description of sequence	Sequence
SEQ ID NO:312	CD3#2 chain	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKNYLAWYQ QKPGQPPKLLIYWASTRESGVPDFRSGSGSGTDFTLTISLQAE DVAVYYCKQSFILRTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLV QSGAEVKKPGASVKVSCKASGYSFTDYVHWVRQAPGQGLE WMGWIYPGNGNIKYNERFRGMTMTTRDTSTSTVYMELSSLRS EDTAVYYCARDNYSAYYFAYWGQGTITVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSKVHTFPA VLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCAVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNRFTQKSLSLSPG
SEQ ID NO:313	CD3#3 chain	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKVYLAWYQ QKPGQPPKLLIYWASTRESGVPDFRSGSGSGTDFTLTISLQAE DVAVYYCKQSFILRTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLV QSGAEVKKPGASVKVSCKASGYFTSYVHWVRQAPGQGLE WIGWIYPGGNIKYAQKFQGRVTMTTRDTSTSTVYMELSSLRS EDTAVYYCARDQYSAYYFAYWGQGTITVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSKVHTFPA VLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

SEQ ID Number	Brief description of sequence	Sequence
		PQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGSEFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNRFTQKSLSLSPG
SEQ ID NO:314	CD3#4 chain	DIVMTQSPDSLAVSLGERATINCKSSQSLNSRTRKVLAWYQ QKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAE DVAVYYCKQSFILRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKS GTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLV QSGAEVKKPGASVKVSCKASGYSFTSYVHWVRQAPGQGLE WIGWIYPGGGNIKYNQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCARDHYSAYYFAYWGQTTTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSCDKHTCTPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGSEFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNRFTQKSLSLSPG
SEQ ID NO:315	CD3#5 chain	DIVMTQSPDSLAVSLGERATISCKSSQSLNSRTRKTYLAWYQQ KPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAE VAVYYCKQSFILRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGT ASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQ SGAEVKKPGASVKVSCKASGYFTGYVHWVRQAPGQGLEW MGWIYPGGGSTKYAQKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCARDQYSAYYFAYWGQTTTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK

SEQ ID Number	Brief description of sequence	Sequence
		SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGSEFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNRFTQKSLSPG
SEQ ID NO:316	CD3#6 chain	DIVMTQSPDSLAVSLGERATISCKSSQSLNSRTRKTYLAWYQQ KPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAE VAVYYCKQSFILRTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQ SGAEVKKPGASVKVSCKASGYFTSYVHWVRQAPGQGLEWI GWIYPGGGNIKYAQKFQGRVTMTRDTSTSTVYMELSSLRSED AVYYCARDQYSAYYFAYWGQGTITVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTWNSGALTSKVHTFPAVLQ SSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSEFLVSKLTVDKSRWQQGNVFSCSVMHEALHN RFTQKSLSPG

Example 3: Generation of recombinant proteins

- **Human B7H6-His**

- The full extracellular domain of Human-B7H6 was expressed with a His6-tag using a pTT vector (encoding human B7H6-His, SEQ ID NO: 317) by transient transfection using the Lenti-X™ Lentiviral System (Clontech). HEK 293F cells (Thermo Fisher) were used at 1.6×10^6 cells/ml at time of transfection, in Gibco™ Freestyle™ F17 expression medium

(Thermo Fisher Scientific). The DNA:PEI complex at 1:3 ratio and 1 mg/L of DNA was pre-incubated for 5 minutes, filtered, and pre-incubated at room temperature for another 15 minutes before adding to the cells. The cells were incubated at 37°C, 5% CO₂, and shaking at 140 rpm. Tryptone N1 was added to the cells 24 hours after transfection to a final concentration of 0.5%. The cells were fed again 48 hours after transfection with 2 mM glutamine and 2 g/L glucose. At the same time the temperature was lowered to 33°C. The final fed was added 120 hours after transfection with 2 mM glutamine and 1 g/L glucose. The cells were harvested 144 hours after transfection by centrifuging at 6000 rpm for 15 minutes. The supernatant was clarified using a G4 filter.

Protein purification was performed in two steps. First, a Ni-NTA column was used for affinity chromatography with Wash Buffer 1x PBS, pH 7.2 + 10 mM Imidazole for 10 CV, then Wash Buffer 1x PBS, pH 7.2 + 20 mM Imidazole for 10 CV, and elution gradient 4-60% of 1x PBS, pH 7.2 supplemented with 0.5M Imidazole. Fractions were collected and analyzed by SDS-PAGE prior to pooling, and concentrating. Second, a Superdex® 200, 16/600, 120 ml column was used for Gel Filtration chromatography (GE Healthcare Life Sciences). The concentrated pool after affinity chromatography, 5ml, was loaded on the column with flow rate of 0.5 ml/min. The formulation buffer was 20 mM HEPES, 100 mM NaCl, 5% sucrose, pH 7.4. Fractions were collected and analyzed by SDS-PAGE prior to pooling, and then sterilized using a 0.2 um filter.

• Cyno B7H6-His

The full extracellular domain of Cyno-B7H6 was expressed with a His6-tag using a pTT vector (encoding cyno B7H6-His, SEQ ID NO: 320) by transient transfection using the Lenti-X™ Lentiviral System (Clontech). HEK 293F cells (Thermo Fisher) were used at 1.6×10^6 cells/ml at time of transfection, in Gibco™ Freestyle™ F17 expression medium (Thermo Fisher Scientific). The DNA:PEI complex at 1:3 ratio and 1 mg/L of DNA was pre-incubated for 5 minutes, filtered, and pre-incubated at room temperature for another 15 minutes before adding to the cells. The cells were incubated at 37°C, 5% CO₂, and shaking at 140 rpm. Tryptone N1 was added to the cells 24 hours after transfection to a final concentration of 0.5%. The cells were fed again 48 hours after transfection with 2 mM glutamine and 2 g/L glucose. At the same time the temperature was lowered to 33°C. The final fed was added 120 hours after transfection with 2 mM glutamine and 1 g/L

glucose. The cells were harvested 144 hours after transfection by centrifuging at 6000 rpm for 15 minutes. The supernatant was clarified using a G4 filter.

Protein purification was performed in two steps. First, a Ni-NTA column was used for affinity chromatography with Wash Buffer 1x PBS, 0.2 M sucrose, 0.01% CHAPS, 5% glycerol, pH 7.2, + 10 mM Imidazole for 10 CV, then Wash Buffer 1x PBS, pH 7.2 + 20 mM Imidazole for 10 CV, and elution gradient 4-60% of 1x PBS, 0.2 M sucrose, 0.01% CHAPS, 5% glycerol, pH 7.2 supplemented with 0.5M Imidazole. Fractions were collected and analyzed by SDS-PAGE prior to pooling, and concentrating. Second, a Superdex® 200, 16/600, was used for Gel Filtration chromatography (GE Healthcare Life Sciences).

The concentrated pool after affinity chromatography, 10ml, was loaded on the column with flow rate of 1.0 ml/min. The formulation buffer was 1X PBS, 0.2M sucrose, 0.01% CHAPS, 5% glycerol, pH 7.2. Fractions were collected and analyzed by SDS-PAGE prior to pooling, and then sterilized using a 0.2 um filter.

• **Human CD3 E+G HuFc-6xHis (E+G indicates $\epsilon\gamma$ subunits)**

A cell line to produce Human CD3 E+G HuFc-6xHis was generated using HEK-293 cells (Thermo Fisher), the Lenti-X™ Lentiviral System (Clontech), and plasmid encoding Human CD3 E+G HuFc-6xHis (human CD3E Accession No: P07766; human CD3E+G-HuFc-His: SEQ ID NO:322). For expression, cells were cultured and expanded in Freestyle™ 293 media (Thermo Fisher Scientific), at 37C, humidified 8% CO2 environment, and shaking at 135 rpm. The conditioned culture supernatant was harvested at Day 6 by centrifugation for 30 minutes at 9300xg. Expression was monitored by SDS-PAGE and Western Blotting. The conditioned culture supernatant was adjusted with 0.2M Sucrose, 5% glycerol, 0.01% CHAPS, and 10mM Imidazole. The pH was then adjusted to 7.2. Purification was carried out in a two-step process: affinity purification using Ni/NTA resin (overnight incubation at 4C, and elution with 250mM Imidazole); followed by size-exclusion chromatography on a Superdex® 200 column (GE Healthcare Life Sciences) in destination buffer PBS with 0.2M Sucrose, 5% glycerol, 0.01% CHAPS, 1mM TCEP, pH7.2. The pooled material was concentrated using a 10K MWCO PES membrane Vivacell® 100 centrifugation device prior to final analysis and storage. The purified material was qualified by mass spectrometry and analytical ultra-centrifugation.

- **Cyno CD3 E+G HuFc-6xHis (E+G indicates $\epsilon\gamma$ subunits)**

A cell line to produce Cyno CD3 E+G HuFc-6xHis was generated using HEK-293 cells (Thermo Fisher), the Lenti-X™ Lentiviral System (Clontech), and plasmid encoding Cyno CD3 E+G HuFc-6xHis (Cynomolgus CD3E Accession No: Q95LI5 <, cyno CD3 E+G huFc-His: SEQ ID NO:323). For expression, cells were cultured and expanded in Freestyle™ 293 media (Thermo Fisher Scientific), at 37C, humidified 8% CO2 environment, and shaking at 135 rpm. The conditioned culture supernatant was harvested at Day 6 by centrifugation for 30 minutes at 9300xg. Expression was monitored by SDS-PAGE and Western Blotting. The conditioned culture supernatant was adjusted with 0.2M Sucrose, 5% glycerol, 0.01% CHAPS, and 10mM Imidazole. The pH was then adjusted to 7.2. Purification was carried out in a two-step process: affinity purification using Ni/NTA resin (overnight incubation at 4C, and elution with 250mM Imidazole); followed by size-exclusion chromatography on a Superdex® 200 column (GE Healthcare Life Sciences) in destination buffer PBS with 0.2M Sucrose, 5% glycerol, 0.01% CHAPS, 1mM TCEP, pH7.2. The pooled material was concentrated using a 10K MWCO PES membrane Vivacell® 100 centrifugation device prior to final analysis and storage. The purified material was qualified by mass spectrometry and analytical ultra-centrifugation.

- **Fc-His-tagged human B7H6 ECD**

In this construct, the huB7H6 ECD is followed by a GS linker, then by huIgG1-Fc domain and a C-terminal His6 tag (SEQ ID NO:318). The construct was expressed by transient transfection using HEK293-6E cells, with DNA:PEI ratio of 1:3 and 1mg DNA per L culture. The PEI reagent was linear PEI MAX [Mw 40,000] (Polysciences: Cat# 24765-2). Transfected cells were incubated at 37°C with 5% CO₂ and 130 rpm. 24-hr post transfection tryptone N1 (Organotechnie; Cat# 19553) and glucose were added to a final concentration of 0.5% and 1g/L respectively. Cells were harvested after 5 days. After centrifugation, the supernatant was filtered through 0.2 µm membrane filter. The huB7H6-ECD-Fc-His protein was purified in a two-step purification: first by affinity on Ni NTA Agarose matrix, and second by gel filtration using Superdex® 200, 26/600 column (GE Healthcare Life Sciences). The pooled fractions were filtered and stored in 1 x PBS, 0.2 M sucrose, 5% glycerol, 0.01% CHAPS, pH-7.2 formulation buffer

- **Fc-His-tagged human Ala-mutated B7H6 ECD (NKp30 interaction sites aa35-38 and aa102-105 substituted by Ala).**

This construct (SEQ ID NO:319) has huB7H6 with Ala substitutions at positions 35-38 and 102-105, so it does not bind NKp30. The huB7H6-Ala-ECD is followed by a GS linker, then by huIgG1Fc domain and a C-terminal His6 tag. This construct was expressed in HEK293-6E cells by transient transfection, purified in a two-step purification process, and stored as described above for the Fc-His-tagged huB7H6-ECD construct.

- **Human B7H1-Fc**

This construct (SEQ ID NO:324) contains huB7H1 with a cMyc tag, a Thrombin cleavage site and a huFc domain. The construct was expressed by transient transfection using HEK293f cells, DNA:PEI ratio of 1:1.5, and 1 mg of DNA per L culture. The flasks were incubated at 37°C in a humidified 8% CO₂ environment with shaking at 135 rpm. The cells were harvested after 3 days. After centrifugation of the cells, the protein was purified from the supernatant. First, affinity purification was performed using nProtein A Sepharose® 4 Fast Flow medium (GE Healthcare, #17-5280-03), and the eluate was dialyzed in 20 mM Tris, 100 mM NaCl, 10% glycerol, 1 mM TCEP, 3 mM CaCl₂, pH 8.0. Second, the sample was incubated with Thrombin CleanCleave™ resin (1 mL, Sigma). Third, the pool from the previous step was bound again to nProtein A Sepharose® 4 Fast Flow medium. The non-bound material was saved, further polished by gel filtration on Superdex® 75 (GE Healthcare) column equilibrated in PBS, 1 mM TCEP, pH 7.2 buffer, and concentrated.

Example 4: SPR based determination of affinities to recombinant B7H6 and CD3εγ subunits and interspecies cross-reactivity

To determine the affinity of human and cyno B7H6 and human B7H1 to B7H6/CD3 binding proteins, the experiment was performed on a Biacore™ 8K instrument (GE Healthcare Life Sciences). Briefly, the B7H6/CD3 binding protein was captured via Protein A/G. The running buffer for this experiment and all serial dilutions were prepared in HBS-EP+. The CM5 sensor chip was activated with an equal mixture of EDC/NHS across both flow cells for 420 s at a flow rate of 10 µL/min and immobilized with recombinant Protein A/G (50 µg/ml in 10 mM NaOAc, pH 4.5) across all flow cells for 420 s at a flow rate of 10 µL/min resulting in about 2500 RU of Protein A/G on the

surface. The sensor chip was deactivated with 1M ethanolamine-HCl across all flow cells for 420 s at a flow rate of 10 μ L/min.

About 700 RU of the B7H6/CD3 binding protein was captured on flow cell 2 of the Protein A/G surface for 60 s at a flow rate of 10 μ L/min. The analytes HuB7H6, CyB7H6 and HuB7H1 were injected across both flow cells over captured B7H6/CD3 binding protein for 300 s at a flow rate of 30 μ L/min with a dissociation of 1200 s. The concentrations of HuB7H6 and CyB7H6 were 0 nM, 6.25 nM, 12.5 nM, 25 nM, 50 nM and 100 nM. The concentrations of HuB7H1 were 0 nM and 1 μ M. The surface was regenerated by injecting 10 mM glycine-HCl, pH 1.5 for 20 s at a flow rate of 30 μ L/min across both flow cells.

The reference flow cell 1 (interactions with sensor surface) and blank (HBS-EP+ or 0 nM analyte) were subtracted from the raw data. Using the Biacore™ 8K Evaluation Software, sensorgrams were fit globally to 1:1 Langmuir binding to provide association rate constant (k_a), dissociation rate constant (k_d), and equilibrium dissociation constant (KD) values.

To determine the affinity of the B7H6/CD3 binding protein to human and cyno CD3E+G-hFc, the experiment was performed on a Bio-Rad ProteOn™ XPR36 instrument. Briefly, HuCD3E+G-hFc and CyCD3E+G were amine-coupled on a ProteOn™ GLM sensor chip (Bio-Rad) and the B7H6/CD3 binding protein was flowed over the immobilized surface. The running buffer for this experiment and all serial dilutions were prepared in HBS-EP+.

The GLM sensor chip was normalized according to Bio-Rad's recommendations. The sensor chip was activated with an equal mixture of EDC/s-NHS in the horizontal direction for 300 s at a flow rate of 30 μ L/min. HuCD3E+G-hFc was immobilized in the vertical direction at 0.4 μ g/mL, 0.2 μ g/mL and 0.1 μ g/mL in 10 mM acetate pH 4.5 to L1, L2 and L3, respectively, for 300 s at a flow rate of 30 μ L/min resulting in about 100 RU of HuCD3E+G-hFc on L1, 40 RU of HuCD3E+G-hFc on L2 and 0 RU of HuCD3E+G-hFc on L3. CyCD3E+G-hFc was immobilized in the vertical direction at 0.4 μ g/mL, 0.2 μ g/mL and 0.1 μ g/mL in 10 mM acetate pH 4.5 to L4, L5 and L6, respectively, for 300 s at a flow rate of 30 μ L/min resulting in about 385 RU of HuCD3E+G-hFc on L4, 170 RU of CyCD3E+G-hFc on L5 and 50 RU of CyCD3E+G-hFc on L6. The sensor chip was deactivated with 1M ethanolamine-HCl in the horizontal direction for 300 s at a flow rate of 30 μ L/min. The sensor chip was regenerated with 18 s of 0.85% phosphoric acid at a flow rate of 100 μ L/min 2 times horizontally and 2 times vertically.

The B7H6/CD3 binding protein analyte was injected horizontally over the immobilized surface for 300 s at a flow rate of 30 μ L/min with a dissociation of 600 s. The concentrations of the B7H6/CD3 binding protein used were 0 nM, 1.2 nM, 3.7 nM, 11.1 nM, 33.3 nM and 100 nM. The surface was regenerated by injecting 0.85% phosphoric acid for 18 s at a flow rate of 100 μ L/min 2 times horizontally.

The interspot (interactions with sensor surface) and blank (HBS-EP+ or 0 nM analyte) were subtracted from the raw data. Using the Bio-Rad ProteOn™ Manager software, sensorgrams were fit globally to 1:1 Langmuir binding to provide association rate constant (k_a), dissociation rate constant (k_d), and equilibrium dissociation constant (K_D) values.

Affinities determined as described above are shown for exemplary B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO: 228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) in Table 2.

Table 2: Affinities (K_D) of B7H6/CD3 binding proteins to humanB7H6, cynoB7H6, humanCD3 $\epsilon\gamma$ subunit, cynoCD3 $\epsilon\gamma$ subunit, and humanB7H1, as determined by SPR analysis. No detectable binding is denoted by 'nb'.

B7H6 binder	CD3 binder	huB7-H6 [nM]	cyB7-H6 [nM]	cy/hu B7-H6	huCD3 [nM]	cyCD3 [nM]	cy/hu CD3	huB7-H1
B7H6#12	CD3#1	1.1	2.3	2.1	3.6	2.5	0.7	nb at1 μ M
B7H6#13	CD3#1	3.0	4.6	1.5	4.1	2.8	0.7	nb at1 μ M
B7H6#14	CD3#1	0.1	0.6	5.9	4.2	2.7	0.6	nb at1 μ M
B7H6#15	CD3#1	0.8	2.2	2.8	3.8	2.6	0.7	nb at1 μ M
B7H6#16	CD3#1	3.1	7.2	2.3	3.7	2.6	0.7	nb at1 μ M
B7H6#17	CD3#1	8.7	8.4	1.0	13.5	3.7	0.3	nb at1 μ M
B7H6#18	CD3#1	1.4	3.3	2.3	3.9	2.8	0.7	nb at1 μ M
B7H6#19	CD3#1	1.6	5.1	3.1	4.0	2.4	0.6	nb at1 μ M
B7H6#20	CD3#1	1.1	4.9	4.6	4.0	2.6	0.7	nb at1 μ M
B7H6#21	CD3#1	3.3	1.4	0.4	4.4	2.7	0.6	nb at1 μ M
B7H6#22	CD3#1	1.5	1.4	1.0	5.1	2.8	0.5	nb at1 μ M
B7H6#23	CD3#1	1.3	1.2	0.9	5.5	2.7	0.5	nb at1 μ M

B7H6 binder	CD3 binder	huB7-H6 [nM]	cyB7-H6 [nM]	cy/hu B7-H6	huCD3 [nM]	cyCD3 [nM]	cy/hu CD3	huB7-H1
B7H6#24	CD3#1	1.8	3.1	1.7	7.8	3.3	0.4	nb at1μM
B7H6#14	CD3#2	1.2	1.0	0.9	16.9	17.7	1.0	nb at1μM
B7H6#14	CD3#3	1.1	1.1	0.9	4.8	7.1	1.5	nb at1μM
B7H6#14	CD3#4	1.3	1.1	0.8	7.6	8.5	1.1	nb at1μM
B7H6#14	CD3#5	0.9	1.0	1.1	4.3	5.2	1.2	nb at1μM
B7H6#14	CD3#6	0.4	0.9	2.1	4.1	5.7	1.4	nb at1μM
B7H6#23	CD3#2	1.9	2.0	1.1	10.4	10.0	1.0	nb at1μM
B7H6#23	CD3#3	2.2	1.9	0.9	13.8	11.9	0.9	nb at1μM
B7H6#23	CD3#4	1.7	1.7	1.0	3.8	3.6	0.9	nb at1μM
B7H6#23	CD3#5	2.2	1.9	0.9	2.8	3.7	1.3	nb at1μM
B7H6#23	CD3#6	1.9	2.0	1.1	3.4	3.9	1.2	nb at1μM

nb: no binding

Example 5: Generation of recombinant CHO-K1 cell lines expressing cynomolgus monkey B7H6 extracellular domain on the cell surface

- 5 For generation of stable CHO-K1 cells expressing the extracellular domain of cynomolgus monkey B7H6 (NCBI: XP_005578557) on the cell surface, the respective coding sequence (aa 25 to 262 of XP_005578557.1) was cloned into pcDNA3.1 (Thermo Fisher Scientific). The construct contains an N-terminal mouse IgG Vk-leading sequence, followed by a 6-His-myc-tag and the cynomolgus monkey B7H6 extracellular domain (aa25-262 of NCBI
- 10 XP_005578557.1). To ensure cell surface localization of the B7H6 extacellular domain, the construct was followed by a linker, and the transmembrane and intracellular domains of EpCAM (Uniprot P16422). The expression of the B7H6 domain on the cell surface was verified by flow cytometry using a mouse monoclonal anti-myc antibody (AbD Serotec). The used sequences are listed in Table 3, a schematic representation of the constructs is
- 15 shown in Figure 2.

Table 3: Amino acid sequences of B7H6 subdomains expressed on CHO-K1 cells

Name	Sequence
Vk leader	METDTLLLWVLLLWVPGSTGD (SEQ ID NO:325)
6-His-myc tag	HHHHHHEQKLISEEDL (SEQ ID NO:326)
Cynomolgus B7-H6 extracellular domain (NCBI XP_005578557.1, aa25-262)	DLKVEMMARGIQATRLNDSVTISCKVIYS QPLNITSMGITWFRKSLTDKEVKVFEFFG

Name	Sequence
	DHQKAFRPGANVSLWRLKSGDASLKLPG VQLEEAGEYRCEVVVTPKLAQGTVQLKVV ASPTSRLFQDQAVVKENEGKYMCESSRFY PKDINITWEKWTQKSPHHVVISENVITGP TIKNMDGTFNITSYLKLNSSQEDPGTVYRC VIRHESLHTPVSIDFILTPAQQSLSEPEKTDI FS (SEQ ID NO:327)
Linker	SGGGGS (SEQ ID NO:328)
Human EpCAM transmembrane domain (Uniprot P16422, aa266-288)	AGVIAVIVVVVIAVVAGIVVLVI (SEQ ID NO:329)
Human EpCAM intracellular domain (Uniprot P16422, aa289-314)	SRKKRMAKYEKAEIKEMGEMHRELNA (SEQ ID NO:330)

Example 6: Binding of exemplary B7H6 binding proteins to recombinant human B7H6 extracellular domain proteins

- 5 To assess binding of B7H6/CD3 binding proteins to recombinant human Fc-His-tagged B7H6 ECD and human Fc-His-tagged Ala-mutated B7H6 extracellular proteins as described in Example 3, MediSorp™ plates (Nunc, 467320) were coated with 2 µl/ml recombinant proteins overnight at 4 °C. The next day, plates were blocked with 0.5% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 1 hour at room temperature (RT).
- 10 Subsequently plates were washed with OBS containing 0.05% TWEEN®20 viscous liquid and B7H6/CD3 binding proteins were incubated at concentrations ranging from 0.00001 to 10 µg/ml. After an additional washing step, bound B7H6/CD3 binding proteins were detected by a peroxidase conjugated goat anti-human IgG F(ab')₂-specific secondary antibody (Jackson ImmunoResearch) and visualized by TMB substrate solution (Bender Med
- 15 Systems). Figures 3A+B and 4A+B show binding of exemplary B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID
- 20 NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and

B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) to recombinant human B7H6 ECD (Figure 3A+B) and human Ala-mutated B7H6 ECD (Figure 4A+B) proteins.

- 5 All tested exemplary B7H6/CD3 binding proteins show comparable binding to recombinant human B7H6 ECD (Figure 3A+B), whereas only B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively, show strong binding to human Fc-His-tagged Ala-mutated B7H6 extracellular protein, in which the NKp30 binding sites were mutated to Alanins. B7H6/CD3 binding proteins comprising a B7H6 chain or SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, and a CD3 chain of SEQ ID NO:311 show only weak binding at high concentrations or no binding to Fc-His-tagged Ala-mutated B7H6 extracellular protein.

20 **Example 7: Binding to B7H6-positive HCT cells**

- Binding of B7H6/CD3 binding proteins to HCT-15, a human (colo-rectal cancer) CRC cell line was tested by flow cytometry. In a previous experiment, it has been confirmed that HCT-15 cells expresses B7-H6 on the RNA levels as well as the protein level with approximately 8,000 B7-H6 receptors on the cell surface (data not shown). B7H6/CD3 binding proteins were produced, as described in Example 2. HCT-15 cells were stained with increasing concentrations of two-step purified B7H6/CD3 binding proteins in FACS buffer (PBS/0.5%BSA/0.05% sodium azide). Bound molecules were detected with PE-conjugated anti-human secondary antibody (Sigma-Aldrich, #P8047). Figures 5A+B show binding of exemplary B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID

NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) to human HCT-15
5 cells.

Example 8: Cross-reactivity to cynomolgus monkey B7H6

Binding of B7H6/CD3 binding proteins to recombinant CHO-K1 cells expressing cynomolgus B7H6 was tested by flow cytometry. B7H6/CD3 binding proteins were
10 produced, as described in Example 2. Recombinant cynomolgus B7H6-expressing cell lines were generated as described in Example 5. Cells were stained with increasing concentrations of two-step purified B7H6/CD3 binding proteins in FACS buffer (PBS/0.5%BSA/0.05% sodium azide). Bound molecules were detected with PE-conjugated anti-human secondary antibody (Sigma-Aldrich, #P8047). Figure 6 shows binding of
15 exemplary B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO: 228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or
20 SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) to recombinant cynomolgus monkey B7H6-expressing cells.

Example 9: Binding to human T-cells

25 Binding of B7H6/CD3 binding proteins to purified human T-cells was tested by flow cytometry. B7H6/CD3 binding proteins were produced, as described in Example 2. T-cells were isolated from buffy coats obtained from the Austrian Red Cross. Informed consent in accordance with the Declaration of Helsinki and with approval of the cantonal ethical committee in Austria was obtained for all buffy coats.
30 Human peripheral blood mononuclear cells (PBMCs) were prepared using Ficoll® Paque density gradient medium (GE Healthcare Lifesciences) followed by centrifugation. Human peripheral blood mononuclear cells (PBMCs) were derived from enriched lymphocyte preparations (buffy coats), a side product of blood banks collecting blood for

transfusions. Therefore, mononuclear cells were isolated by Ficoll® density gradient centrifugation (35 min without brake at 1400 rpm) and extensive washes with PBS. Remaining erythrocytes were removed by incubating for 3 minutes in ACK lysis buffer (Thermo Fisher Scientific, A1049201), followed by washing in PBS, before suspension in
 5 assay medium containing RPMI 1640 GlutaMAX™ supplement (Gibco #61870-010), 5% human AB serum AB (Gemini, GemCell cat # 100-512 LOT#H56500I) + 1% MEM-NEAA (Gibco #11140-035), 10mM HEPES (Affymetrix #7365-49-9), 10 µM beta-Mercaptoethanol (Gibco #21985-023) and sodium pyruvate (Gibco #11360-039).

T-cells were isolated by negative selection using the Pan T Cell Isolation Kit II (Miltenyi
 10 Biotec #130-091-156). In brief, cells were resuspended in 40 µl buffer PBS/0.5% BSA (Gibco ref#041-94553 M)/2mM EDTA (Invitrogen ref# 15575-038) per 10 Mio cells and incubated with 10 µl of Biotin-Antibody cocktail per 10 Mio cells for 5 min at 4 °C. Subsequently, 30 µl buffer and 20 µl anti-biotin MACS® MicroBeds/10 million cells were added and incubated for 10 min at 4°C. Subsequently the mixture was placed in a pre-rinsed
 15 25LS column (Miltenyi Biotec #130-042-401) in the magnetic field of suitable MACS® microbeads separator (Miltenyi Biotec). Flow-through was collected and washed in assay medium.

T-cells were stained with increasing concentrations of two-step purified B7H6/CD3 binding
 20 proteins in FACS buffer (PBS/0.5%BSA/0.05% sodium azide). Bound molecules were detected with PE-conjugated anti-human secondary antibody (Sigma-Aldrich, #P8047). Figure 7 shows binding of exemplary B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235,
 25 SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) to human T-cells.

30

Example 10: Binding selectivity

Binding of B7H6/CD3 binding proteins to B7H6- and CD3-negative CHO-K1 cells was tested by flow cytometry analysis. B7H6/CD3 binding proteins were produced, as described

in Example 2. CHO-K1 cells were stained with increasing concentrations of two-step purified B7H6/CD3 binding proteins in FACS buffer (PBS/0.5%BSA/0.05% sodium azide). Bound molecules were detected with PE-conjugated anti-human secondary antibody (Sigma-Aldrich, #P8047). Figure 8 shows binding of exemplary B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) to CHO-K1 cells .

Example 11: Inhibition of B7H6-dependent NK cell activity

Cell surface expressed B7H6 binds to NKp30 on NK cells, which triggers NKp30-mediated activation of NK cells and NK cell cytotoxicity and cytokine secretion (Brandt et al, J.Exp. Med. 2009;206(7):1495-1503). To assess B7H6-dependent activation of NK-cells, 96-well flat bottom cell culture plates were coated with 100 nM recombinant human B7H6 protein (R&DSYSTEMS#7144-B7-050) overnight at 4 °C. On the next day, plates were washed with PBS, subsequently increasing concentrations of B7H6/CD3 binding proteins or recombinant NKp30 protein (R&DSYSTEMS#1849-NK-025) were added and incubated for one hour at room temperature. B7H6/CD3 binding proteins were produced, as described in Example 2. After an additional washing step, 100,000 NK92MI (ATCC) cells in 100 µl medium (MEM alpha containing 12.5% fetal bovine serum, 12.5% horse serum, 0.2 mM D-Myo-Inositol, 0.02 mM folic acid and 0.1 mM β-mercaptoethanol) were added per well and incubated for 24 hours. On the next day, IFNγ concentration was quantified using the V-PLEX Human IFN-γ Kit (Meso Scal Discovery). Figures 9A+B show the ability of B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:235, SEQ ID NO:236, and a CD3 chain of SEQ ID NO:311) to inhibit B7-H6-dependent induction of IFNγ secretion by NK-92MI.

B7H6 binding proteins which do not or only weakly bind to Ala-mutated B7H6 extracellular proteins in which the NKp30 interaction sites were substituted with Alanine (B7-H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, and a CD3 chain of SEQ ID NO:311) (as shown in Figure 4, Example 6) inhibit the B7-H6-dependent induction of IFN γ secretion by NK-92MI cells, whereas binding protein that show strong binding to Ala-mutated B7H6 extracellular proteins comparable to wild type protein, (B7-H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:235, SEQ ID NO:236, and a CD3 chain of SEQ ID NO:311) (as shown in Figure 4, Example 6) do not influence the B7-H6-dependent induction of IFN γ secretion by NK-92MI cells.

Example 12: Potency of T-cell redirected lysis human HCT-15 cells

Potency of non-stimulated T-cells against HCT-15 cells was determined using lactate-dehydrogenase (LDH) release as readout for cell lysis. In this assay, the B7H6-positive CRC cell line HCT-15 was co-cultured with human T-cells as effector cells and increasing concentrations of B7H6/CD3 binding proteins for 72 hours at an effector to target cell ratio of 10:1. B7H6/CD3 binding proteins were produced, as described in Example 2. Purified T-cells were isolated as described in Example 9. Subsequently, HCT-15 cells and T-cells at a ratio of 1:10 were incubated with B7H6/CD3 binding proteins at concentrations from 0.00001 nM to 10 nM for 72 hours.

Cytotoxic activity was determined using the Cytotoxicity Detection Kit^{PLUS} (Roche), following the manufacturer's instructions. In brief, this method is based on the usage of the release of LDH from dead or plasma –membrane damaged cells. Cell culture supernatant is incubated with the reaction mixture from the kit for 30 minutes and the formation of Formazan, as a result of LDH activity is measured in a spectrophotometer at 500nm as surrogate for cell lysis. Percentage of cytotoxicity relative to the maximal lysis control was calculated according to the following formula:

$$\text{Cytotoxicity (relative to control)} = \frac{\text{measured value} - \text{background}}{\text{maximal lysis} - \text{minimal lysis}}$$

Background: Target cells + Effector cells

Maximal lysis: Target cells + 5% Triton X-100

5 Minimal lysis: Target cells

Using GraphPad®Prism® 5.0 software (GraphPad Software, Inc), the percentage of cytotoxicity relative to the maximal lysis control was plotted against the corresponding B7H6/CD3 binding protein concentrations. Dose response curves were analysed with the
 10 four-parameter logistic equation model for evaluation of sigmoidal dose-response curve and EC₅₀ values were calculated.

Figures 10A+B and 11A+B show examples of potency T-cell redirected lysis of HCT-15 cells mediated by exemplary B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID
 15 NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a
 20 B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively).

Figures 10 and 11 show that B7H6 binding proteins which bind to the recombinant Ala-mutated B7H6 extracellular proteins, in which the NKp30 interaction sites were substituted with Alanine, comparable as to wt protein, and do not inhibit the B7-H6-dependent
 25 secretion of IFN γ by NK92MI cells (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of
 30 SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) (as shown in, Example 6, 11)

show lower EC₅₀ values (Tables 4A+B) than B7H6/CD3 binding proteins which do not or only weakly bind to recombinant Ala-mutated B7H6 extracellular proteins and inhibit the B7-H6-dependent secretion of IFN γ by NK92MI cells (B7-H6/CD3 binding proteins comprising of a SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, or
 5 SEQ ID NO:221, and a CD3 chain of SEQ ID NO:CD3#1) (as shown in, Example 6, 11). Thus, binding proteins that do not inhibit B7H6 dependent NK cell activation surprisingly show a significantly higher T- cell mediated tumor cell lysis compared to binding proteins that do inhibit B7H6 dependent NK cell activation.

As shown in Figure 12 with an exemplary B7H6/CD3 binding protein, activity only
 10 requires low effector to target cell ratios.

Table 4A: EC₅₀ values [nM] of 11 exemplary B7H6/CD3 binding proteins as measured in 72 hour cytotoxicity assay with non-stimulated T-cells (from Donor #1) as effector cells and HCT-15 cells as target cells.

B7H6 /CD3	EC ₅₀ [nM]
B7H6#1/CD3#1	0.14
B7H6#2/CD3#1	0.22
B7H6#3/CD3#1	0.40
B7H6#4/CD3#1	0.23
B7H6#5/CD3#1	0.22
B7H6#6/CD3#1	0.46
B7H6#7/CD3#1	0.36
B7H6#8/CD3#1	0.62
B7H6#9/CD3#1	0.44
B7H6#10/CD3#1	0.53
B7H6#11/CD3#1	0.77

15

Table 4B: EC₅₀ values [nM] of 23 exemplary B7H6/CD3 binding proteins as measured in 72 hour cytotoxicity assay with non-stimulated T-cells (from Donor #2) as effector cells and HCT-15 cells as target cells.

B7H6 /CD3	EC₅₀ [nM]
B7H6#12/CD3#1	0.004
B7H6#13/CD3#1	0.008
B7H6#14/CD3#1	0.003
B7H6#15/CD3#1	0.004
B7H6#16/CD3#1	0.011
B7H6#17/CD3#1	0.014
B7H6#18/CD3#1	0.014
B7H6#19/CD3#1	0.011
B7H6#20/CD3#1	0.010
B7H6#21/CD3#1	0.010
B7H6#22/CD3#1	0.005
B7H6#23/CD3#1	0.005
B7H6#24/CD3#1	0.005
B7H6#14/CD3#2	0.118
B7H6#23/CD3#2	0.215
B7H6#14/CD3#3	0.069
B7H6#23/CD3#3	0.107
B7H6#14/CD3#4	0.292
B7H6#23/CD3#4	0.447
B7H6#14/CD3#5	0.005
B7H6#23/CD3#5	0.003
B7H6#14/CD3#6	0.081
B7H6#23/CD3#6	0.129

Example 13: Cross-reactivity and selectivity of T-cell redirected lysis

Potency of non-stimulated T-cells against HCT-15 cells was determined using lactate-dehydrogenase (LDH) release as readout for cell lysis as described in Example 12. B7H6/CD3 binding proteins were produced, as described in Example 2. Recombinant
5 cynomolgus B7H6-expressing cell lines were generated as described in Example 5.

Figure 13 shows examples of potency of T-cell redirected lysis of cyno B7H6 expressing cells (Figure 13A) and B7H6-negative parental CHO-K1 cells (Figure 13B) mediated by exemplary B7H6/CD3 binding proteins a B7H6 chain SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234,
10 SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively).

15

Example 14: Selectivity of activation of T cells

To determine activation of T-cells, a cytotoxicity assay with non-stimulated T-cells and B7H6-positive HCT-15 cells as target cells was setup as described in Example 12. B7H6/CD3 binding proteins were produced, as described in Example 2. To determine T cell
20 activation, cells were centrifuged and stained with antibodies against CD4 (BD #550630), CD8 (BD #557834), and CD25 (BD#340907) and measured by flow-cytometry. Figure 14 shows examples of potency of activation of CD4⁺ (Figure 14A) and CD8⁺ (Figure 14B) cells in presence and absence of B7H6-positive HCT-15 cells mediated by exemplary B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:235, or SEQ ID NO:236, and a CD3 chain of SEQ
25 ID NO:311.

Example 15: Selectivity of degranulation of T cells

To determine degranulation of T-cells via intracellular expression of Perforin and Granzyme B, a cytotoxicity assay with non-stimulated T-cells and B7H6-positive HCT-15 cells as target cells was setup as described in Example 12. B7H6/CD3 binding proteins were produced, as described in Example 2. To determine intracellular levels of Granzyme B and Perforin, cells were centrifuged and stained with antibodies against CD4 (BD #550630),
30

CD8 (BD #557834), subsequently the cells were permabilized using the Fixation/Permeabilization Solution (BD #554714) and stained with antibodies against Perforin (BioLegend #308120) and Granzyme B (BD #560221) and measured by flow-cytometry. Figure 15 shows examples of potency of upregulation of intracellular Perforin expression in CD4⁺ (Figure 15A) and CD8⁺ (Figure 15B) cells, and Figure 16 shows upregulation of intracellular Granzyme B expression in CD4⁺ (Figure 16A) and CD8⁺ (Figure 16B) cells in presence and absence of B7H6-positive HCT-15 cells mediated by exemplary B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:235, or SEQ ID NO:236, and a CD3 chain of SEQ ID NO:311.

Example 16: Induction of T-cell proliferation

A cytotoxicity assay with non-stimulated T-cells and B7H6-positive HCT-15 cells as target cells was setup as described in Example 12. B7H6/CD3 binding proteins were produced, as described in Example 2. To determine the proliferation of T cells, PBMCs were labeled with 5 µM Cell Trace™ CFSE (Invitrogen, C34554) and T cell stained with an anti-CD3 antibody (BioLegend cat#: 317336). Subsequently the labeled PBMCs were incubated with HCT-15 cells at a ratio of 10:1 and increasing concentrations of a B7H6/CD3 binding protein for 6 days. Figure 17 shows dose-dependent induction of proliferation of T-cells by B7H6-binding proteins by exemplary B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:235, or SEQ ID NO:236, and a CD3 chain of SEQ ID NO:311 in presence of B7H6-positive HCT-15 cells.

Example 17: Selectivity of IFN γ secretion

A cytotoxicity assay with non-stimulated T-cells and B7H6-positive HCT-15 cells as target cells was setup as described in Example 12. B7H6/CD3 binding proteins were produced, as described in Example 2. Cytokine levels in supernatants were determined by V-Plex Human IFN-gamma Kit (MSD, CAT: K151QOD-4). Figure 18 shows secretion of IFN γ induced by five exemplary B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:235, or SEQ ID NO:236, and a CD3 chain of SEQ ID NO:311.

Example 18: Mouse PK

B7H6/CD3 binding proteins were produced, as described in Example 2.

The PK of B7H6/CD3 binding proteins was evaluated in C57BL/6 mice following a single 1 mg/kg *i.v.* dose. Serum concentrations of B7H6/CD3 binding proteins were determined using a B7H6 capture/CD3 detection assay.

In brief, male C57BL/6 mice received a single 1 mg/kg intravenous (IV) dose ($n = 3$ per molecule). Blood samples were collected pre-dose and 0.25, 2, 6, 24, 48, 96, 168, 240 and 336 hours post-dose. Serum drug levels were measured with an MSD-based ligand binding assay, using biotinylated B7H6 as the capture reagent and sulfo-tagged CD3 ϵ as the detection reagent. Pharmacokinetic (PK) parameters were calculated from serum concentration time-profiles using non-compartmental analysis. The following PK parameters were assessed: AUC_{last} (area under the serum concentration-time curve from time zero to the last quantifiable time-point), AUC_{inf} (area under the serum concentration-time curve extrapolated to infinity), CL (systemic clearance), V_{ss} (steady-state volume of distribution) and T_{1/2} (terminal half-life).

Mean (SD) serum concentration time-profiles for an exemplary B7H6/CD3 binding protein are summarized in Figure 19. Mean (SD) PK parameters for these exemplary BH6/CD3 binding protein are summarized in Table 5.

Table 5: Mean (SD) PK parameters of an exemplary B7H6/CD3 binding protein in male C57BL/6 mice following a single 1 mg/kg intravenous dose

Dose (mg/kg)	C _{max} (ng/mL)	AUC _{last} (ng·hr/mL)	AUC _{inf} [*] (ng·hr/mL)	CL (mL/d/kg)	V _{ss} (mL/kg)	T _{1/2} (day)	MRT (day)
1	20500 (458)	1200000 (78100)	1840000 (112000)	13.1 (0.8)	173 (30)	10.2 (2.2)	13.3 (3.0)

*greater than 20% extrapolated AUC

Example 18A: Mouse PK

The PK of B7H6/CD3 binding proteins was evaluated as described in Example 18. Mean (SD) serum concentration time-profiles for four exemplary B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, or SEQ ID NO:232, and a CD3 chain of SEQ ID NO:311 are summarized in Figure 22.

Example 19: *In vivo* Efficacy

Efficacy studies were performed using a human xenograft mouse model reconstituted with human T cells. In detail, human NCI-H716 colorectal cancer cells (2.5×10^7) were injected subcutaneously (*s.c.*) into the right dorsal flank of sub-lethally irradiated (2 Gy, day -1) female NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac mice (Day -16). In parallel, human CD3 positive T cells (isolated from healthy human blood donor) were expanded *in vitro*.

Human peripheral blood mononuclear cells (PBMCs) were prepared as described in Example 9.

T-cells were isolated by negative selection using the Pan T Cell Isolation Kit II (Miltenyi Biotec #130-096-535). In brief, cells were resuspend in 40 µl buffer PBS/0.5% BSA (Gibco ref#041-94553 M)/2mM EDTA (Invitrogen ref# 15575-038) per 10×10^6 cells and incubated with 10 µl of Biotin-Antibody cocktail per 10×10^6 cells for 5 min at 4 °C. Subsequently, 30 µl buffer and 20 µl anti-biotin MACS® MicroBeads/ 10×10^6 cells were added and incubated for 10 min at 4°C. Subsequently the mixture was placed in a pre-rinsed 25LS column (Miltenyi Biotec #130-042-401) in the magnetic field of suitable MACS® microbead separator (Miltenyi Biotec). Flow-through was collected and washed in assay medium.

Subsequently T cells were expanded using the T Cell Activation/Expansion Kit human (Miltenyi Biotec Cat#130-091-441) for 17 days. In brief, anti-Biotin MACSiBead™ Particles are loaded with CD2-, CD3-, CD28 Biotin and are transferred to the purified T cells in a ratio of 2 cells per particle and incubated in presence of 20 Units recombinant IL-2 (R&D#202-IL-050/CF) at a density of 0.5×10^6 cells/ml for 14 days. Cells were supplemented with 20 Units fresh IL-2 every three days. Three days before injection into the animals, T cells were restimulated with anti-Biotin MACSiBead™ Particles are loaded with CD2-, CD3-, CD28 Biotin at a ratio of 1 bead per 4 cells for additional three days. Finally, beads were removed with a MACSiMAG™ Separator (Miltenyi Biotec) and T cells were washed in PBS.

On day -2, animals were randomized into treatment groups based on tumor volume and 2×10^7 human T cells were injected intra-peritoneally (*i.p.*). B7H6/CD3 binding proteins were produced, as described in Example 2. Treatment was started on day 1 and B7H6/CD3 binding protein or Vehicle buffer (50mM NaOAc, 100mM NaCl, pH 5.0) was administered in a q7d dosing regimen by intravenous (*i.v.*) bolus injections into the lateral tail vein. Tumor

growth was monitored by external caliper measurements and tumor volumes were calculated using a standard hemi-ellipsoid formula. Animals reaching sacrifice criteria were euthanized early during the studies for ethical reasons. Treatment of tumor-bearing mice with B7H6/CD3 binding proteins once weekly *i.v.* at 0.05 mg/kg induced significant tumor regression (Figure 20).

Example 19A: In vivo Efficacy

Efficacy studies were performed using a human NCI-H716 xenograft mouse model reconstituted with human CD3⁺ T cells as described in Example 19. Treatment of tumor-bearing mice with B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID No 230, SEQ ID NO:231, or SEQ ID NO:232, and a CD3 chain of SEQ ID NO:311 once weekly *i.v.* at 0.05 mg/kg induced significant tumor regression (Figure 23).

Example 19B: In vivo Efficacy

Efficacy studies were performed using a human NCI-H716 xenograft mouse model reconstituted with human CD3⁺ T cells as described in Example 19. Treatment of tumor-bearing mice with an exemplary B7H6/CD3 binding protein administered once weekly or as a single dose *i.v.* at 0.05 mg/kg induced significant tumor regression (Figure 24).

Example 20: Percent monomer content of B7H6/CD3 binding proteins

Percent monomer was determined for B7H6/CD3 binding proteins (B7H6/CD3 binding proteins comprising a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) by Analytical Size Exclusion Chromatography (aSEC) (shown in Table 6). aSEC was run on a Waters® (Milford, MA, USA) Acquity UPLC® system using a Protein BEH SEC column 200Å, 1.7µm, 4.6x150mm (Cat# 186005225). Running conditions were as follows: Mobile phase: 50mM Sodium Phosphate, 200mM Arginine

and 0.05% Sodium Azide; Flow rate: 0.5ml/min; Runtime: 5 minutes; Sample loading amount: 10µg; Peak detection: A280nm; Automated processing method of chromatograms.

Table 6: Percent monomer after first and second purification step

B7H6 binder	CD3 binder	Percent Monomer after 1st step	Percent Monomer after 2nd step
B7H6#1	CD3#1	70.5	98.5
B7H6#2	CD3#1	38.7	95.1
B7H6#3	CD3#1	50.1	96.6
B7H6#4	CD3#1	56.0	99.9
B7H6#5	CD3#1	66.5	99.3
B7H6#6	CD3#1	63.7	99.1
B7H6#7	CD3#1	43.4	97.7
B7H6#8	CD3#1	68.3	96.8
B7H6#9	CD3#1	61.3	95.7
B7H6#10	CD3#1	66.3	97.7
B7H6#11	CD3#1	38.2	95.3
B7H6#12	CD3#1	67.4	99.9
B7H6#13	CD3#1	61.9	99.9
B7H6#14	CD3#1	64.6	99.9
B7H6#15	CD3#1	53.8	99.9
B7H6#16	CD3#1	52.4	99.7
B7H6#17	CD3#1	46.8	99.9
B7H6#18	CD3#1	50.1	99.8
B7H6#19	CD3#1	64.1	99.9
B7H6#20	CD3#1	62.3	99.9
B7H6#21	CD3#1	60.4	99.9
B7H6#22	CD3#1	57.5	99.9
B7H6#23	CD3#1	59.5	99.8
B7H6#24	CD3#1	51.7	99.9
B7H6#14	CD3#2	71.9	99.9
B7H6#14	CD3#3	68.2	99.9
B7H6#14	CD3#4	62.2	99.6
B7H6#14	CD3#5	76.5	99.9
B7H6#14	CD3#6	70.5	99.9
B7H6#23	CD3#2	69.8	99.8

B7H6 binder	CD3 binder	Percent Monomer after 1st step	Percent Monomer after 2nd step
B7H6#23	CD3#3	67.3	99.6
B7H6#23	CD3#4	62.2	99.7
B7H6#23	CD3#5	75	99.9
B7H6#23	CD3#6	69.6	99.9

Example 21: Thermostability

Thermostability was determined by Thermal Shift Analysis (TSA) and results of the first melting transitions (T_{m1}) of B7H6/CD3 binding proteins (B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively) are shown in Table 7. The fluorescence intensity profile as a function of temperature was acquired using a QuantStudio™ 6 Flex real-time PCR system (Applied Biosystems, Waltham, MA) with SYPRO® Orange protein gel stain (Invitrogen, Carlsbad, CA) as the extrinsic fluorophore. Sample was diluted to 0.4 mg/ml in 10 mM histidine, pH 6.0 with 40 mM sodium chloride and 0.02% sodium azide. The melt curve was generated with a thermal ramp from 25°C to 95°C at a rate of 2°C/min, with data collected approximately every 0.4°C through the 'ROX' filter set (Ex: 580 ±10 nm, Em: 623 ±14 nm). Data were analyzed using Protein Thermal Shift™ Software Version v1.3 (ThermoFisher Scientific, Waltham, MA).

Table 7: Thermostability analysis

B7H6 binder	CD3 binder	T_{m1} (°C)
B7H6#12	CD3#1	65.3
B7H6#13	CD3#1	65.2
B7H6#14	CD3#1	65.7
B7H6#15	CD3#1	65.4

B7H6 binder	CD3 binder	Tm1 (°C)
B7H6#16	CD3#1	65.4
B7H6#17	CD3#1	65.4
B7H6#18	CD3#1	65.0
B7H6#19	CD3#1	64.0
B7H6#20	CD3#1	62.5
B7H6#21	CD3#1	64.9
B7H6#22	CD3#1	58.7
B7H6#23	CD3#1	64.7
B7H6#24	CD3#1	65.3
B7H6#14	CD3#2	61.7
B7H6#14	CD3#3	60.4
B7H6#14	CD3#4	60.3
B7H6#14	CD3#5	61.4
B7H6#14	CD3#6	61.4
B7H6#23	CD3#2	60.1
B7H6#23	CD3#3	64.9
B7H6#23	CD3#4	58.0
B7H6#23	CD3#5	57.9
B7H6#23	CD3#6	57.5

Example 22: Predicted Immunogenicity scores *in silico* by Epivax

Immunogenicity of sequences was evaluated *in silico* with a mathematical algorithm.

- Specifically, EpiMatrix® Treg-adjusted Scores (EpiVax Inc., Providence RI)) as a measure of immunogenicity scores, were determined for B7H6 chains (a B7H6 chain of SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240) and for CD3 chains (a CD3 chain of SEQ ID NO:311, SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316) and compared to the scores of various Fc sequences. These scores are taking T-cell epitopes and Treg epitopes into consideration. The lower the

immunogenicity score, the less likely a sequence to be immunogenic. In general, a negative score is considered low risk of immunogenicity, while a highly positive score is viewed as indication for potential immunogenicity. As shown in the table 8 and 9 below, exemplary B7H6/CD3 binding proteins described herein have very low immunogenicity scores, indicating that the risk of being immunogenic is low for these binding proteins.

Table 8: Adjusted Epivax® scores of B7H6/CD3 binding proteins

B7H6 or CD3 binding protein	VH	VL	Full polypeptide chain (VL-CL-linker-VH-CH1-hinge-CH2-CH3)
B7H6#1 B7H6 chain	-9.94	17.52	-29.07
B7H6#2 B7H6 chain	17.12	1.78	-27.07
B7H6#3 B7H6 chain	67.70	32.77	-11.61
B7H6#4 B7H6 chain	-20.24	-6.54	-34.34
B7H6#5 B7H6 chain	-58.29	-29.64	-43.99
B7H6#6 B7H6 chain	4.77	-9.90	-30.99
B7H6#7 B7H6 chain	16.81	-13.51	-28.20
B7H6#8 B7H6 chain	-31.42	35.40	-29.91
B7H6#9 B7H6 chain	-4.61	-4.44	-31.45
B7H6#10 B7H6 chain	10.78	3.40	-26.72
B7H6#11 B7H6 chain	4.49	47.21	-22.15
B7H6#12 B7H6 chain	-24.63	-48.50	-39.76
B7H6#13 B7H6 chain	-19.87	-48.50	-39.00
B7H6#14 B7H6 chain	-23.93	-48.50	-39.65
B7H6#15 B7H6 chain	-19.71	-48.50	-38.98
B7H6#16 B7H6 chain	-17.72	-48.50	-38.66
B7H6#17 B7H6 chain	-19.71	-48.50	-38.98
B7H6#18 B7H6 chain	-19.71	-50.41	-39.25
B7H6#19 B7H6 chain	-22.64	-48.50	-39.44
B7H6#20 B7H6 chain	-24.63	-50.41	-40.03
B7H6#21 B7H6 chain	-21.94	-48.50	-39.33
B7H6#22 B7H6 chain	-23.93	-50.41	-39.92
B7H6#23 B7H6 chain	-21.94	-50.41	-39.61
B7H6#24 B7H6 chain	-19.71	-50.41	-39.25
CD3#1 CD3 chain	-9.68	-50.52	-35.25
CD3#2 CD3 chain	-40.29	-8.95	-33.62
CD3#3 CD3 chain	-41.48	-4.54	-33.15
CD3#4 CD3 chain	-39.06	-4.54	-32.77
CD3#5 CD3 chain	-55.51	-14.24	-36.83
CD3#6 CD3 chain	-41.48	-14.24	-34.60

Table 9: Adjusted Epivax® scores of Fc domains

Fc Protein Chain	Adjusted Epivax score
Fc-IgG1-WT	-25.64
Fc-IgG1-LALA	-29.83
Fc-IgG1-LALA-KNOB	-31.76
Fc-IgG1-LALA-HOLE	-18.01

Example 23: Non-specific binding to surfaces

- 5 The specificity of the B7H6/CD3 binding proteins of the invention was further tested in an surface plasmon resonance (SPR)-based assay using highly charged proteins. A non-specific binding assay was developed using biosensor technology to determine if binding proteins have significant binding to unrelated charged proteins. In this assay, B7H6/CD3 binding proteins were passed over two SPR surfaces, one coated with a negatively charged protein (Trypsin Inhibitor) and one coated with a positively charged protein (Lysozyme).
10 When a protein displays significant non-specific binding to these surfaces, it is likely that the cause of binding is the presence of positive or negative charged surface patches on the candidate. Non-specific binding of proteins may translate to poor pharmacokinetics (PK) and biodistribution and may also have downstream manufacturability impacts.
- 15 The experiment was performed on a Biacore® T200 SPR system (GE Healthcare Life Sciences). The dilution, surface preparation, and binding experiments were performed at 25 °C in 1X HBS-EP buffer prepared from 10X HBS-EP. The flow rate for both the immobilization protocol and binding experiment was at 5 µL/min.
- To prepare the surface for the non-specific binding experiment, chicken egg white
20 lysozyme and trypsin inhibitor from glycine max soybean were coupled manually to a Biacore ® series S CM5 sensor chip (GE Healthcare sciences) with the surface density of 3000-5000 RU using the amine coupling kit according to the manufacture instructions. Samples were prepared at 1 µM in 1X HBS-EP buffer. The samples were injected over activated surfaces with a 10 min association and 15 min dissociation. The data was
25 collected using Biacore® T200 Control Software version 2.0.1 and analyzed using Biacore® T200 Evaluation Software version 3.0 (GE Healthcare Life Sciences).
- Table 10 shows no or very low binding to the two highly charged proteins, Trypsin Inhibitor and Lysozyme, of exemplary B7H6/CD3 binding proteins (B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:228, SEQ ID NO:229, SEQ ID NO:230,
30 SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235,

SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, or SEQ ID NO:240 and a CD3 chain of SEQ ID NO:311, and B7H6 binding proteins comprising a B7H6 chain of SEQ ID NO:230 or SEQ ID NO:239 and a CD3 chain of SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, or SEQ ID NO:316, respectively).

5

Table 10: Low RU numbers indicate no significant binding to unrelated charged proteins

B7H6 binder	CD3 binder	Non-specific binding (RU), Lysozyme (positive)	Non-specific binding (RU), Tryp Inhibitor (negative)
B7H6#12	CD3#1	9.2	35.4
B7H6#13	CD3#1	7.2	30.6
B7H6#14	CD3#1	8.1	32.7
B7H6#15	CD3#1	7.2	32.8
B7H6#16	CD3#1	8.2	42.6
B7H6#17	CD3#1	15.3	88.4
B7H6#18	CD3#1	6.6	32.8
B7H6#19	CD3#1	8.2	40.3
B7H6#20	CD3#1	9.8	48.4
B7H6#21	CD3#1	8.1	42
B7H6#22	CD3#1	7.8	39.5
B7H6#23	CD3#1	8.1	45.7
B7H6#24	CD3#1	7.9	43.5
B7H6#14	CD3#2	11.2	12.2
B7H6#14	CD3#3	12.4	26.4
B7H6#14	CD3#4	8.8	22.6
B7H6#14	CD3#5	16.2	109.6

B7H6 binder	CD3 binder	Non-specific binding (RU), Lysozyme (positive)	Non-specific binding (RU), Tryp Inhibitor (negative)
B7H6#14	CD3#6	15.7	34
B7H6#23	CD3#2	37.8	47.8
B7H6#23	CD3#3	18.9	38.7
B7H6#23	CD3#4	6.8	20.7
B7H6#23	CD3#5	15	114.7
B7H6#23	CD3#6	57.2	96.5

Example 24: T cell infiltration in SHP77 xenograft tumor tissue with an exemplary B7H6/CD3 binding protein.

Remaining tumor tissues from mice in the study described in Example 19 were prepared, fixed in formalin and embedded in paraffin. Subsequently tissue sections were prepared and stained for CD3 expression on T cells (anti-CD3 (2GV6), Ventana Medical Systems). T cell infiltration in NCI-H716 xenograft tumor tissue with an exemplary B7H6/CD3 binding protein is shown in Figure 21. The scoring in Table 11 was used to quantify CD3 expression in xenograft tumor tissues.

10

Table 11: Scoring for quantification of infiltrating CD3-positive T cells

Score	Description
0	< 5 CD3-positive T-cells per HPF (high-power field)
1	5-99 CD3-positive T-cells per HPF (high-power field)
2	100-200 CD3-positive T-cells per HPF (high-power field)
3	> 300 CD3-positive T-cells per HPF (high-power field)

Example 25: Pharmaceutical formulation for *i.v.* administration

Any of the above binding proteins/molecules of the invention can be selected for the manufacture of a pharmaceutical formulation for *i.v.* application. An example of a suitable pharmaceutical formulation for the antibody of the invention is as follows.

A 10 mL vial contains 10 mg/mL of the B7H6/CD3 binding molecule/protein of the invention, in a buffer comprising histidine, trehalose, polysorbate 20 and water for injection.