Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2985698 A1 2016/11/17

(21) **2 985 698**

(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2016/05/13
(87) **Date publication PCT/PCT Publication Date:** 2016/11/17
(85) **Entrée phase nationale/National Entry:** 2017/11/10
(86) **N° demande PCT/PCT Application No.:** EP 2016/060859
(87) **N° publication PCT/PCT Publication No.:** 2016/180969
(30) **Priorités/Priorities:** 2015/05/13 (US62/160,757); 2016/04/07 (US62/319,486)

(51) **Cl.Int./Int.Cl.** *C07K 16/28* (2006.01), *C07K 16/30* (2006.01), *C07K 16/46* (2006.01)

(71) **Demandeur/Applicant:**
ABLYNX N.V., BE

(72) **Inventeurs/Inventors:**
ROOBROUCK, ANNELIES, BE;
VAN HOORICK, DIANE, BE;
VIEIRA, JOAO, GB

(74) **Agent:** BORDEN LADNER GERVAIS LLP

(54) **Titre :** POLYPEPTIDES DE RECRUTEMENT DE LYMPHOCYTES T SUR LA BASE DE LA REACTIVITE DU TCR ALPHA/BETA
(54) **Title:** T CELL RECRUITING POLYPEPTIDES BASED ON TCR ALPHA/BETA REACTIVITY

(57) **Abrégé/Abstract:**
T cell recruiting polypeptides are provided that bind the constant domain of TCR on a T cell. The polypeptides can be used in methods for treatment of cancers.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 November 2016 (17.11.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/180969 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *C07K 16/46* (2006.01)
C07K 16/30 (2006.01)

(21) International Application Number:
PCT/EP2016/060859

(22) International Filing Date:
13 May 2016 (13.05.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/160,757 13 May 2015 (13.05.2015) US
62/319,486 7 April 2016 (07.04.2016) US

(71) Applicant: **ABLYNX N.V.** [BE/BE]; Technologiepark 21, 9052 Ghent-Zwijnaarde (BE).

(72) Inventors: **ROOBROUCK, Annelies**; Noenendal 25, 8700 Oudenaarde (BE). **VAN HOORICK, Diane**; Warande 118, 9270 Laarne (BE). **VIEIRA, Joao**; 12 Greenfich Road, Didcot Oxfordshire OX11 6BG (GB).

(74) Agents: **STEFFERL Andreas** et al.; HOFFMANN EITLE PATENT- UND RECHTSANWÄLTE PARTMBB, Arabellastraße 30, 81925 Munich (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2016/180969 A1

(54) **Title**: T CELL RECRUITING POLYPEPTIDES BASED ON TCR ALPHA/BETA REACTIVITY

(57) **Abstract**: T cell recruiting polypeptides are provided that bind the constant domain of TCR on a T cell. The polypeptides can be used in methods for treatment of cancers.

T CELL RECRUITING POLYPEPTIDES BASED ON TCR ALPHA/BETA REACTIVITY

FIELD OF THE INVENTION

The present invention provides multispecific T cell recruiting polypeptides binding the constant domain of TCR on a T cell and at least one antigen on a target cell. The present invention also relates to the monovalent T cell recruiting polypeptides for use in these multispecific polypeptides. The invention also provides methods for treatment and kits providing the same.

BACKGROUND

Cancer takes an enormous human toll around the world. It is nowadays the world's leading cause of death, followed by heart disease and stroke. Cancers figure among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012. The number of new cases is expected to rise by about 70% over the next 2 decades (source: WHO Cancer). The total economic impact of premature death and disability from cancer worldwide was about $900 billion in 2008, representing 1.5% of the world's gross domestic product.

Available treatment regimens for solid tumours typically include a combination of surgical resection chemotherapy and radiotherapy. In 40 years of clinical experience little progress has been achieved, especially in advanced stages of cancer.

New therapies combatting cancer are eagerly awaited.

Antibody therapy is now an important part of the physician's armamentarium to battle diseases and especially cancer. Monoclonal antibodies have been established as a key therapeutic approach for a range of diseases already for several years. All of the contemporaneously approved antibody therapies rely on monospecific monoclonal antibodies (mAbs). Until today, most of the targets of the mAbs require either an agonistic or an antagonistic approach. Whereas targeting of cell-surface antigens themselves can mediate antitumour activity through the induction of apoptosis, most mAb-based activity against hematologic malignancies is reliant on either Fc-mediated effector functions such as complement dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC).

Immunotherapy has emerged as a rapidly growing area of cancer research. Immunotherapy is directing the body’s immune surveillance system, and in particular T cells, to cancer cells.

Cytotoxic T cells (CTL) are T lymphocytes that kill cancer cells, cells that are infected (particularly with viruses), or cells that are damaged in other ways. T lymphocytes (or T cells) express the T cell receptor or TCR molecule and the CD3 receptor on the cell surface. The αβ TCR–CD3 complex (or "TCR complex") is composed of six different type I single-spanning transmembrane proteins: the TCRα and TCRβ chains that form the TCR heterodimer responsible for ligand recognition, and the non-covalently associated CD3γ, CD3δ, CD3ε and ζ chains, which bear cytoplasmic sequence motifs that are phosphorylated upon receptor activation and recruit a large number of signalling components (Call et al. 2004, Molecular Immunology 40: 1295–1305).

Both α and β chains of the T cell receptor consist of a constant domain and a variable domain. Physiologically, the αβ chains of the T cell receptor recognize the peptide loaded MHC complex and couple upon engagement to the CD3 chains. These CD3 chains subsequently transduce the engagement signal to the intracellular environment.

Considering the potential of naturally occurring cytotoxic T lymphocytes (CTLs) to mediate cell lysis, various strategies have been explored to recruit immune cells to mediate tumour cell killing. Since T lymphocytes lack the expression of Fc receptors, they are not recruited to a tumour site through the Fc tail of an anti-tumour monoclonal. As an alternative, the patient's T cells were modified with a second TCR of known specificity for a defined tumour antigen. This adoptive cell transfer is by nature highly personalized and labour intensive. However, the main problem of T cell therapies remains the large number of immune escape mechanisms known to occur in cancer patients (Nagorsen et al. 2012, Pharmacology & Therapeutics 136: 334–342).

Rather than eliciting specific T cell responses, which rely on expression by cancer cells of MHC molecules and the presence, generation, transport and display of specific peptide antigens, more recent developments have attempted to combine the advantages of immunotherapy with antibody therapy by engaging all T cells of a patient in a polyclonal fashion via recombinant antibody based technologies: "bispecifics".

Bispecific antibodies have been engineered that have a tumour recognition part on the one arm (target-binding arm) whereas the other arm of the molecule has specificity for a T cell antigen (effector-binding arm), mostly CD3. Through the simultaneous binding of the two arms to their respective antigens, T lymphocytes are directed towards and activated at the tumour cell where they can exert their cytolytic function.

The concept of using bispecific antibodies to activate T cells against tumour cells was described more than 20 years ago, but manufacturing problems and clinical failures sent the field into stagnation. Smaller format bispecifics were developed, which more easily penetrate tissues and tumours than conventional antibodies. In addition, the smaller format is better at creating the cytolytic synapses, which kill the target cell. It was thought that the smaller format bispecifics would be easier to manufacture and less immunogenic than conventional antibodies. However, the smaller bispecific BiTE molecules, consisting of two single chain variable fragments (scFvs) joined by a 5 amino acid peptide linker, presented a lack of stability (scFvs tend to aggregate), low expression titres and poor solubility. Moreover, the first clinical trials of Blinatumomab (a BiTE molecule), which recognizes CD3 chains, were prematurely stopped due to neurologic adverse events, cytokine release syndrome and infections on the one hand and the absence of objective clinical responses or robust signs of biological activity on the other hand. Efficacy aside, BiTEs must be continuously infused -- probably due to the lack of an Fc domain -- which does not contribute to patient compliance. The same problem holds true for DARTs (dual affinity retargeting molecules developed by MacroGenics), in which the heavy chain variable domain from one antibody (Ab) is linked with the light chain variable domain of another Ab. MacroGenics now attempts to solve this problem by fusing an Fc domain onto its next generation DARTs, which makes the molecule not only bigger, but also results in manufacturing problems and importation of other Fc functions. The larger format with Fc is expected to have a better PK, but re-introduces the risk of off-target activity. (Garber 2014, Nature reviews 13: 799-801)

Hence, there remains a need for alternative bispecific formats.

SUMMARY OF THE INVENTION

The invention solves this problem by providing multispecific polypeptides comprising a first and at least one further immunoglobulin single variable domain (ISV), wherein said first ISV has a high affinity for / binds to a constant domain of the T cell receptor (TCR); said at least one second ISV has a high affinity for / binds to an antigen present on a target cell. In a particular aspect, the binding of the first ISV will activate the inherent cytolytic potential of the T cell against a target cell independently of MHC.

Thus, in a first aspect the present invention provides a polypeptide comprising a first and a second immunoglobulin single variable domain (ISV), wherein

- said first ISV has high affinity for/binds to the constant domain of the T cell receptor (TCR) present on a T cell;
- said second ISV has high affinity for/binds to a first antigen on a target cell;

wherein said first antigen is different from said TCR; and

wherein said target cell is different from said T cell.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said polypeptide directs the T cell to the target cell.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said polypeptide induces T cell activation.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said T cell activation is independent from MHC recognition.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said T cell activation depends on presenting said polypeptide bound to said first antigen on a target cell to a T cell.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said T cell activation causes one or more cellular response of said T cell, wherein said cellular response is selected from the group consisting of proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, expression of activation markers and redirected target cell lysis.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said T cell activation causes inhibition of an activity of said target cell by more than about 10%, such as 20%, 30%, or 40% or even more than 50%, such as more than 60%, such as 70%, 80%, or even more than 90% , such as 100%.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first ISV binds to the constant domain of a T cell receptor α (TCR-α) (SEQ ID NO: 348) and/or the constant domain of the T cell receptor β (TCR-β) (SEQ ID NO: 349), or polymorphic variants or isoforms thereof.

Alternatively, the present invention provides a polypeptide as described herein, wherein said first ISV binds to the constant domain of a T cell receptor α (TCR-α) (SEQ ID NO: 484) and/or the constant domain of the T cell receptor β (TCR-β) (SEQ ID NO: 485), or polymorphic variants or isoforms thereof.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said polypeptide and/or first ISV has an on rate constant (Kon) for binding to said TCR selected from the group consisting of at least about 10^2 $M^{-1}s^{-1}$, at least about 10^3 $M^{-1}s^{-1}$, at least about 10^4 $M^{-1}s^{-1}$, at least about 10^5 $M^{-1}s^{-1}$, at least about 10^6 $M^{-1}s^{-1}$, 10^7 $M^{-1}s^{-1}$, at least about 10^8 $M^{-1}s^{-1}$, at least about 10^9 $M^{-1}s^{-1}$, and at least about 10^{10} $M^{-1}s^{-1}$, preferably as measured by surface plasmon resonance or BLI.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said polypeptide and/or first ISV has an off rate constant (Koff) for binding to said TCR selected from the group consisting of at most about $10^{-3}s^{-1}$, at most about $10^{-4}s^{-1}$, at most about $10^{-5}s^{-1}$, at most about $10^{-6}s^{-1}$, at most about $10^{-7}s^{-1}$, at most about $10^{-8}s^{-1}$, at most about $10^{-9}s^{-1}$, and at most about $10^{-10}s^{-1}$, preferably as measured by surface plasmon resonance or BLI.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first ISV binds to said TCR with an EC50 value of between 100 nM and 1 pM, such as at an average EC50 value of 100 nM or less, even more preferably at an average EC50 value of 90 nM or less, such as less than 80, 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM or even less, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5 pM, or even less, such as less than 4 pM, preferably as measured by flow cytometry.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first ISV binds to said TCR with an average KD value of between 100 nM and 10 pM, such as at an average KD value of 90 nM or less, even more preferably at an average KD value of 80 nM or less, such as less than 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20 pM, or even less, such as less than 10 pM. Preferably, the KD is determined by Kinexa, BLI or SPR, for instance as determined by Proteon.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NOs: 119-133; or (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123 or with any of SEQ ID NOs: 199-133, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 134-163; or (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153 or with any of SEQ ID NOs: 134-163, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 164-174; or (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170 or with any of SEQ ID NOs: 164-174, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 123; and

(b) amino acid sequences that have 1, 2 or 3 amino acid difference(s) with SEQ ID NO: 123, wherein

- at position 2 the D has been changed into A, S, E or G;
- at position 4 the H has been changed into Y;
- at position 5 the K has been changed into L;
- at position 6 the I has been changed into L;
- at position 8 the F has been changed into I or V; and/or
- at position 10 the G has been changed into S.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 153; and

(b) amino acid sequences that have 1, 2, 3, 4 or 5 amino acid difference(s) with SEQ ID NO: 153, wherein

- at position 1 the H has been changed into T or R;
- at position 3 the S has been changed into T or A;
- at position 5 the G has been changed into S or A;
- at position 7 the Q has been changed into D, E, T, A or V;
- at position 8 the T has been changed into A or V; and/or

- at position 9 the D has been changed into A, Q, N, V or S.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 170; and
(b) amino acid sequences that have 1, 2 or 3 amino acid difference(s) with SEQ ID NO: 170, wherein
    - at position 1 the F has been changed into Y, L or G;
    - at position 4 the I has been changed into L;
    - at position 5 the Y has been changed into W; and/or
    - at position 8 the D has been changed into N or S.

Preferably, the polypeptide comprising the one or more CDRs with 5, 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDRs without the 5, 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which

(i) CDR1 is chosen from the group consisting of
    (a) SEQ ID NO: 123; and
    (b) amino acid sequences that have 1, 2, or 3 amino acid difference(s) with SEQ ID NO: 123, wherein
        - at position 2 the D has been changed into A, S, E or G;
        - at position 4 the H has been changed into Y;
        - at position 5 the K has been changed into L;
        - at position 6 the I has been changed into L;
        - at position 8 the F has been changed into I or V; and/or
        - at position 10 the G has been changed into S,

    provided that the polypeptide comprising the CDR1 with 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and in which

(ii) CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 153; and

(b) amino acid sequences that have 1, 2, 3, 4, or 5 amino acid difference(s) with SEQ ID NO: 153, wherein

- at position 1 the H has been changed into T or R;
- at position 3 the S has been changed into T or A;
- at position 5 the G has been changed into S or A;
- at position 7 the Q has been changed into D, E, T, A or V;
- at position 8 the T has been changed into A or V; and/or
- at position 9 the D has been changed into A, Q, N, V or S,

provided that the polypeptide comprising the CDR2 with 5, 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 5, 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and in which

(iii) CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 170; and

(b) amino acid sequences that have 1, 2, or 3 amino acid difference(s) with SEQ ID NO: 170, wherein

- at position 1 the F has been changed into Y, L or G;
- at position 4 the I has been changed into L;
- at position 5 the Y has been changed into W; and/or
- at position 8 the D has been changed into N or S,

provided that the polypeptide comprising the CDR3 with 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 124; and

(b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein

- at position 2 the E has been changed into Q; and/or
- at position 6 the I has been changed into V.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 145; and
(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein
    - at position 9 the N has been changed into D.

In a further aspect the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 167; and
(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein
    - at position 4 the L has been changed into I.

Preferably, the polypeptide comprising the one or more CDRs with 2 or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDRs without the 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which

(i) CDR1 is chosen from the group consisting of
    (a) SEQ ID NO: 124; and
    (b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein
        - at position 2 the E has been changed into Q; and/or
        - at position 6 the I has been changed into V,

    provided that the polypeptide comprising the CDR1 with 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and in which

(ii) CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 145; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein

- at position 9 the N has been changed into D,

provided that the polypeptide comprising the CDR2 with 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and in which

(iii) CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 167; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein

- at position 4 the L has been changed into I,

provided that the polypeptide comprising the CDR3 with 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is SEQ ID NO: 130.

In a further, aspect the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 157; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein

- at position 8 the T has been changed into I.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is SEQ ID NO: 172.

Preferably, the polypeptide comprising the CDR with 1 amino acid difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDRs without the 1 amino acid difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which

(i) CDR1 is SEQ ID NO: 130;

and in which

(ii) CDR2 is chosen from the group consisting of

 (a) SEQ ID NO: 157; and

 (b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein

  - at position 8 the T has been changed into I,

 provided that the polypeptide comprising the CDR2 with 1 amino acid difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 1 amino acid difference, said affinity as measured by surface plasmon resonance;

and in which

(iii) CDR3 is SEQ ID NO: 172.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:

 (a) SEQ ID NOs: 119-123, 125-127, 129, 132 and 133; and

 (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123; and/or

(ii) CDR2 is chosen from the group consisting of:

 (c) SEQ ID NOs: 134-141, 143-144, 146-156, 159-163; and

 (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153; and/or

(iii) CDR3 is chosen from the group consisting of:

 (e) SEQ ID NOs: 164-166, 169-171, 173-174; and

 (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NOs: 119-123, 125-127, 129, 132 and 133; and

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 134-141, 143-144, 146-156, 159-163; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 164-166, 169-171, 173-174; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 123, CDR2 is represented by SEQ ID NO: 153, and CDR3 is represented by SEQ ID NO: 170.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV is chosen from the group consisting of SEQ ID NOs: 1-104.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV cross-blocks the binding to the constant domain of the T cell receptor (TCR) by at least one of the polypeptides with SEQ ID NOs: 1-104.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV is cross-blocked from binding to the constant domain of the T cell receptor (TCR) by at least one of the polypeptides with SEQ ID NOs: 1-104.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

- (i) CDR1 is chosen from the group consisting of:
  - (a) SEQ ID NOs: 124, 128 and 131; and
  - (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 124; and/or
- (ii) CDR2 is chosen from the group consisting of:
  - (c) SEQ ID NOs: 142 and 145; and
  - (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 145; and/or
- (iii) CDR3 is chosen from the group consisting of:
  - (e) SEQ ID NOs: 167 and 168; and
  - (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 167.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

- (i) CDR1 is chosen from the group consisting of:
  - (a) SEQ ID NOs: 124, 128 and 131; and
  - (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 124, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
- (ii) CDR2 is chosen from the group consisting of:
  - (c) SEQ ID NOs: 142 and 145; and
  - (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 145, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher

affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 167 and 168; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 167, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 124, CDR2 is represented by SEQ ID NO: 145, and CDR3 is represented by SEQ ID NO: 167.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV is chosen from the group consisting of SEQ ID NOs: 105-115.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV cross-blocks the binding to the constant domain of the T cell receptor (TCR) by at least one of the polypeptides with SEQ ID NOs: 105-115.

In a further aspect, the present invention provides a polypeptide as described herein, in which the first ISV is cross-blocked from binding to the constant domain of the T cell receptor (TCR) by at least one of the polypeptides with SEQ ID NOs: 105-115.

In a further aspect the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 130; and

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 130; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 157-158; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 157; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NO: 172; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 172.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 130; and

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 130, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 157-158; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 157, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NO: 172; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 172, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 130, CDR2 is represented by SEQ ID NO: 157, and CDR3 is represented by SEQ ID NO: 172.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV is chosen from the group consisting of SEQ ID NOs: 116-118.

In a further aspect, the present invention provides a polypeptide as described herein, in which said first ISV cross-blocks the binding to the constant domain of the T cell receptor (TCR) by at least one of the polypeptides with SEQ ID NOs: 116-118.

In a further aspect, the present invention provides a polypeptide as described herein in which said first ISV is cross-blocked from binding to the constant domain of the T cell receptor (TCR) by at least one of the polypeptides with SEQ ID NOs: 116-118.

In a further aspect, the present invention provides a polypeptide as described herein, further comprising a third ISV, which has high affinity for/binds to a second antigen on a target cell, wherein said second antigen is different from said first antigen.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first antigen on a target cell is a tumour antigen, preferably a tumour associated antigen (TAA).

In a further aspect, the present invention provides a polypeptide as described herein, wherein said second antigen on a target cell is a tumour antigen, preferably a tumour associated antigen (TAA).

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first antigen and said second antigen are present on the same target cell.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first antigen and said second antigen are present on different target cells.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said TAA's are independently chosen from the group consisting of Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Fibroblast Activation Protein (FAP), MART-1, carcinoembryonic antigen (CEA), gp100, MAGE-1, HER-2, Lewis^{Y} antigens, CD123, CD44, CLL-1, CD96, CD47, CD32, CXCR4, Tim-3, CD25, TAG-72, Ep-CAM, PSMA, PSA, GD2, GD3, CD4, CD5, CD19, CD20, CD22, CD33, CD36, CD45, CD52, CD147, growth factor receptors including ErbB3 and ErbB4, Cytokine receptors including Interleukin-2 receptor gamma chain (CD132 antigen), Interleukin-10 receptor alpha chain (IL-10R-A), Interleukin-10 receptor beta chain (IL-10R-B), Interleukin-12 receptor beta-1 chain (IL-12R-beta1), Interleukin-12 receptor beta-2 chain (IL-12 receptor beta-2), Interleukin-13 receptor alpha-1 chain (IL-13R-alpha-1) (CD213a1 antigen), Interleukin-13 receptor alpha-2 chain (Interleukin-13 binding protein), Interleukin-17 receptor (IL-17 receptor), Interleukin-17B receptor (IL-17B receptor), Interleukin 21 receptor precursor (IL-21R), Interleukin-1 receptor type I (IL-1R-1) (CD121a); Interleukin-1 receptor type II (IL-1R-beta) (CDw121b), Interleukin-1 receptor antagonist protein (IL-1ra), Interleukin-2 receptor alpha chain (CD25 antigen, Interleukin-2 receptor beta chain (CD122 antigen), Interleukin-3 receptor alpha chain (IL-3R-alpha) (CD123 antigen), CD30, IL23R, IGF-1R, IL5R, IgE, CD248 (endosialin), CD44v6, gpA33, Ron, Trop2, PSCA, claudin 6, claudin 18.2, CLEC12A, CD38, ephA2, c-Met, CD56, MUC16, EGFRvIII, AGS-16, CD27L, Nectin-4, SLITRK6, mesothelin, folate receptor, tissue factor, axl, glypican-3, CA9, Cripto, CD138, CD37, MUC1, CD70, gastrin releasing peptide receptor, PAP, CEACAM5, CEACAM6, CXCR7, N-cadherin, FXYD2 gamma a, CD21, CD133, Na/K-ATPase, mIgM (membrane-bound IgM), mIgA (membrane-bound IgA), Mer, Tyro2, CD120, CD95, CA 195, DR5, DR6, DcR3 and CAIX, including related polymorphic variants and isoforms.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said TAA is CD20 (UniProt 11836), HER2 (Uniprot P04626), EGFR, CEA, polymorphic variants or isoforms thereof.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first antigen and said second antigen are chosen from the group consisting of:

- EGFR as a first antigen and CEA as a second antigen;
- CD19 as a first antigen and CD20 as a second antigen;
- CD19 as a first antigen and CD22 as a second antigen;
- CD123 as a first antigen and Tim-3 as a second antigen;
- CD123 as a first antigen and CD69 as a second antigen.

In a further aspect, the present invention provides a polypeptide as described herein, further comprising a serum protein binding moiety.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said serum protein binding moiety binds serum albumin.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said serum protein binding moiety is an ISV binding serum albumin.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said ISV binding serum albumin essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which CDR1 is SFGMS (SEQ ID NO: 481), CDR2 is SISGSGSDTLYADSVKG (SEQ ID NO: 482) and CDR3 is GGSLSR (SEQ ID NO: 475), CDR determined according to Kabat definition; and/or in which CDR1 is GFTFSSFGMS (SEQ ID NO: 472) or GFTFRSFGMS (SEQ ID NO: 473), CDR2 is SISGSGSDTL (SEQ ID NO: 474) and CDR3 is GGSLSR (SEQ ID NO: 475), CDR determined according to Kontermann 2010.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said ISV binding serum albumin is selected from Alb8, Alb23, Alb129, Alb132, Alb11, Alb11 (S112K)-A, Alb82, Alb82-A, Alb82-AA, Alb82-AAA, Alb82-G, Alb82-GG and Alb82-GGG (SEQ ID NOs: 400 to 412).

In a further aspect, the present invention provides a polypeptide as described herein, wherein said ISVs are directly linked to each other or are linked via a linker.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first ISV and/or said second ISV and/or possibly said third ISV and/or possibly said ISV binding serum albumin are linked via a linker.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said linker is chosen from the group consisting of linkers of 5GS, 7GS, 9GS, 10GS, 15GS, 18GS, 20GS, 25GS, 30GS and 35GS (SEQ ID NOs: 376 to 385).

In a further aspect, the present invention provides a polypeptide as described herein, wherein said serum protein binding moiety is a non-antibody based polypeptide.

In a further aspect, the present invention provides a polypeptide as described herein, further comprising PEG.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said ISV is a Nanobody, a V_{HH}, a humanized V_{HH}, or a camelized V_H.

In a further aspect, the present invention provides a polypeptide wherein said first ISV is chosen from the group consisting of SEQ ID NOs: 1 to 118.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said first ISV is chosen from the group consisting of SEQ ID NOs: 1 to 118, and wherein said second ISV is chosen from the group consisting of SEQ ID NOs: 350-358.

In a further aspect, the present invention provides a polypeptide chosen from the group consisting of SEQ ID NOs: 292, 295-296, 299-300, 303, 306-343, 387-388, 390, 414, 417-418, 421-422, 425, 428-464, 467-468, 470-471 and 486-487.

In a further aspect, the present invention provides a polypeptide that specifically binds the constant domain of the T cell receptor (TCR) and that comprises or essentially consists of 4 framework regions

(FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

- (i) CDR1 is chosen from the group consisting of:
  - (a) SEQ ID NOs: 119-133; or
  - (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 119-133, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
- (ii) CDR2 is chosen from the group consisting of:
  - (c) SEQ ID NOs: 134-163; or
  - (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 134-163, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
- (iii) CDR3 is chosen from the group consisting of:
  - (e) SEQ ID NOs: 164-174; or
  - (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 164-174, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

The present invention also provides a polypeptide as described herein, in which:

- (i) CDR1 is chosen from the group consisting of:
  - (a) SEQ ID NOs: 119-123, 125-127, 129, 132 and 133; and
  - (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the

4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 134-141, 143-144, 146-156, 159-163; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 164-166, 169-171, 173-174; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 123; and

(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 123, wherein

- at position 2 the D has been changed into A, S, E or G;
- at position 4 the H has been changed into Y;
- at position 5 the K has been changed into L;
- at position 6 the I has been changed into L;
- at position 8 the F has been changed into I or V; and/or
- at position 10 the G has been changed into S.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 153; and

(b) amino acid sequences that have 1, 2, 3, 4 or 5 amino acid difference(s) with SEQ ID NO: 153, wherein

- at position 1 the H has been changed into T or R;
- at position 3 the S has been changed into T or A;
- at position 5 the G has been changed into S or A;
- at position 7 the Q has been changed into D, E, T, A or V;
- at position 8 the T has been changed into A or V; and/or
- at position 9 the D has been changed into A, Q, N, V or S.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 170; and

(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 170, wherein
- at position 1 the F has been changed into Y, L or G;
- at position 4 the I has been changed into L;
- at position 5 the Y has been changed into W; and/or
- at position 8 the D has been changed into N or S.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is represented by SEQ ID NO: 123, CDR2 is represented by SEQ ID NO: 153, and CDR3 is represented by SEQ ID NO: 170.

In a further aspect, the present invention provides a polypeptide as described herein, in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NOs: 124, 128 and 131; and

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 124, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 142 and 145; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 145, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the

4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 167 and 168; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 167, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 124; and

(b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein

- at position 2 the E has been changed into Q; and/or
- at position 6 the I has been changed into V.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 145; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein

- at position 9 the N has been changed into D.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 167; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein

- at position 4 the L has been changed into I.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is represented by SEQ ID NO: 124, CDR2 is represented by SEQ ID NO: 145, and CDR3 is represented by SEQ ID NO: 167.

In a further aspect, the present invention provides a polypeptide as described herein, in which:

(i) CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 130; and

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 130, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 157-158; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 157, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NO: 172; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 172, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is chosen from SEQ ID NO: 130.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 157; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein

- at position 8 the T has been changed into I.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR3 is chosen from SEQ ID NO: 172.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is represented by SEQ ID NO: 130, CDR2 is represented by SEQ ID NO: 157, and CDR3 is represented by SEQ ID NO: 172.

In another aspect, the invention provides a polypeptide that specifically binds carcinoembryonic antigen (CEA) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NO: 361 (GDTYGSYWMG); or

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 361, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NO: 363 (AINRGGGYTV); or

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 363, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NO: 365 (SGVLGGLHEDWFNY); or

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 365, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is represented by SEQ ID NO: 361, CDR2 is represented by SEQ ID NO: 363, and CDR3 is represented by SEQ ID NO: 365.

In another aspect, the invention provides a polypeptide that specifically binds CD20 and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
  (a) SEQ ID NO: 362 (GGTFSSYTMG); or
  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 362, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:
  (c) SEQ ID NO: 364 (EVRWGGVTT); or
  (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 364, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:
  (e) SEQ ID NO: 366 (VRQMYMTVVPDY); or
  (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 366, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In a further aspect, the present invention provides a polypeptide as described herein, in which CDR1 is represented by SEQ ID NO: 362, CDR2 is represented by SEQ ID NO: 364, and CDR3 is represented by SEQ ID NO: 366.

In a further aspect, the present invention provides a polypeptide as described herein, which is a Nanobody, a V_{HH}, a humanized V_{HH}, or a camelized V_H.

In a further aspect, the present invention provides a polypeptide as described herein, further comprising a serum protein binding moiety.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said serum protein binding moiety binds serum albumin.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said serum protein binding moiety is an ISV that binds serum albumin.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said ISV that binds serum albumin essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which CDR1 is SFGMS (SEQ ID NO: 481), CDR2 is SISGSGSDTLYADSVKG (SEQ ID NO: 482) and CDR3 is GGSLSR (SEQ ID NO: 475), CDR as determined according to Kabat definition; and/or in which CDR1 is GFTFSSFGMS (SEQ ID NO: 472) or GFTFRSFGMS (SEQ ID NO: 473), CDR2 is SISGSGSDTL (SEQ ID NO: 474) and CDR3 is GGSLSR (SEQ ID NO: 475); CDR as determined according to Kontermann 2010.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said ISV that binds serum albumin is selected from Alb8, Alb23, Alb129, Alb132, Alb11, Alb11 (S112K)-A, Alb82, Alb82-A, Alb82-AA, Alb82-AAA, Alb82-G, Alb82-GG, and Alb82-GGG (SEQ ID NOs: 400 to 412).

In a further aspect, the present invention provides a polypeptide as described herein, wherein said ISV is directly linked or is linked via a linker.

In a further aspect, the present invention provides a polypeptide as described herein, wherein said linker is chosen from the group consisting of linkers of 5GS, 7GS, 9GS, 10GS, 15GS, 18GS, 20GS, 25GS, 30GS and 35GS (SEQ ID NOs:376 to 385).

In a further aspect, the present invention provides a polypeptide as described herein, further comprising a PEG moiety.

In a further aspect, the present invention provides a nucleic acid or nucleic acid sequence encoding a polypeptide as defined herein.

In a further aspect, the present invention provides a vector comprising a nucleic acid or nucleic acid sequence as defined herein.

In a further aspect, the present invention provides a host cell transformed or transfected with the nucleic acid or nucleic acid sequence as defined herein or with the vector as defined herein.

In a further aspect, the present invention provides a process for the production of the polypeptide as described herein, said process comprising culturing a host cell as defined herein under conditions allowing the expression of the polypeptide as defined herein and recovering the produced polypeptide from the culture.

In a further aspect, the present invention provides a pharmaceutical composition comprising the polypeptide as described herein, or, the polypeptide produced according to the process as described herein.

In a further aspect, the present invention provides a polypeptide as described herein, or produced according to the process as described herein, for use in treating a subject in need thereof.

In a further aspect, the present invention provides a method for delivering a prophylactic or therapeutic polypeptide to a specific location, tissue or cell type in the body, the method comprising the steps of administering to a subject a polypeptide as described herein, or produced according to the process as described herein.

In a further aspect, the present invention provides a polypeptide as described herein, or produced according to the process as described herein, for use in the prevention, treatment or amelioration of a disease selected from the group consisting of a proliferative disease, an inflammatory disease, an infectious disease and an autoimmune disease.

In a further aspect, the present invention provides a method for the prevention, treatment or amelioration of a disease selected from the group consisting of a proliferative disease, an inflammatory disease, an infectious disease and an autoimmune disease, comprising the step of administering to a subject in need thereof the polypeptide as described herein, or produced according to a process as described herein.

In a further aspect, the present invention provides a polypeptide for use in or a method for the prevention, treatment or amelioration of a disease as described herein, wherein said proliferative disease is cancer.

In a further aspect, the present invention provides a polypeptide for use in or a method for the prevention, treatment or amelioration of a disease as described herein, wherein said cancer is chosen from the group consisting of carcinomas, gliomas, mesotheliomas, melanomas, lymphomas, leukemias, adenocarcinomas: breast cancer, ovarian cancer, cervical cancer, glioblastoma, multiple myeloma (including monoclonal gammopathy of undetermined significance, asymptomatic and symptomatic myeloma), prostate cancer, and Burkitt's lymphoma, head and neck cancer, colon cancer, colorectal cancer, non-small cell lung cancer, small cell lung cancer, cancer of the esophagus, stomach cancer, pancreatic cancer, hepatobiliary cancer, cancer of the gallbladder, cancer of the small intestine, rectal cancer, kidney cancer, bladder cancer, prostate cancer, penile cancer, urethral cancer, testicular cancer, vaginal cancer, uterine cancer, thyroid cancer, parathyroid cancer, adrenal cancer, pancreatic endocrine cancer, carcinoid cancer, bone cancer, skin cancer, retinoblastomas, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Kaposi's sarcoma, multicentric Castleman's disease or AIDS-associated primary effusion lymphoma, neuroectodermal tumors, rhabdomyosarcoma; as well as any metastasis of any of the above cancers, as well as non-cancer indications such as nasal polyposis.

In a further aspect, the present invention provides a polypeptide for use in or a method for the prevention, treatment or amelioration of a disease as described herein, wherein the treatment is a combination treatment.

In a further aspect, the present invention provides a kit comprising a polypeptide as defined herein, a nucleic acid or nucleic acid sequence as defined herein, a vector as defined herein, or a host cell as defined herein.

FIGURE LEGENDS

Figure 1: Assessment of the expression of human TCR/CD3 and human CD3 on transfected CHO, HEK293 and Llana cell lines using 100nM anti-human TCR α/β antibody (clone BW242/412) (black) and 100nM anti-human CD3 antibody (clone OKT-3) (grey). The MCF value (mean channel fluorescence) was plotted for each cell line. The X-axis depicts the cell type and the transfected genes; CD3 indicates transfection with the CD3 complex (epsilon, delta, gamma and zeta chains), huTCR indicates transfection with the TCR α/β chains, wherein the variable domain used is between brackets.

Figure 2: Quality assessment of soluble recombinant cynomolgus TCR α/β proteins using anti-non-human primate/Rat TCRα/β antibody clone R73; anti-human TCR α/β antibodies (solid circles) and an irrelevant anti-egg lysozyme Nanobody (cAblys) (open circles). The OD value was plotted against the concentration of the Nanobody.

Figures 3A and 3B: Dose dependent binding of monovalent anti-TCR Nanobodies to human TCR/CD3 expressed on CHO-K1 cells **(Figure 3A)** and to primary human T cells **(Figure 3B)**. The MCF value (mean channel fluorescence) was plotted against the concentration of the Nanobody.

Figure 4: Dose dependent binding of monovalent anti-TCR Nanobodies to HEK293H human TCR(2IAN)/CD3 (closed circle), HEK293H human CD3 (cross) and to HEK293H reference cell line (open circles). The MCF value (mean channel fluorescence) was plotted against the concentration of the Nanobody.

Figure 5: Dose dependent binding of monovalent anti-TCR Nanobodies (closed circles) and an irrelevant Nanobody (open circles) to soluble recombinant human TCR α/β(2XN9)-zipper protein. The OD at 450nm was plotted against the concentration of the Nanobody.

Figures 6A and 6B: Kinetic analysis of T01700055A02 **(Figure 6A)** and T01700056G05 **(Figure 6B)** on soluble recombinant human TCR α/β (2XN9)-zipper protein interaction via BioLayer Interferometry on an Octet RED384 instrument. Applied analyte concentrations were: 1000, 333, 111, 37, 12.3, 4.1 and 1.4 nM. Langmuir fits to the kinetic data are indicated with the black lines, whereas sensorgrams are presented by the grey lines.

Figures 7A and 7B: T cell activation data of bead coupled monovalent anti-TCR Nanobodies **(Figure 7A)**. T cell activation data of monovalent anti-TCR Nanobodies presented in solution **(Figure 7B)**. Activation was measured by monitoring the CD69 upregulation on primary human T cells. The MCF value (mean channel fluorescence) was plotted for each Nanobody.

Figure 8: Binding of a dilution series of CD20xTCR (full line) and TCRxCD20 (dotted line) multispecific polypeptides to human TCR/CD3 expressed on CHO-K1 cells (A), primary human T cells (B) and Ramos cells (C). The MCF value (mean channel fluorescence) was plotted against the concentration of the polypeptides.

Figures 9A and 9B: Dose-dependent killing effect of CD20xTCR (full line) binding and TCRxCD20 (dotted line) binding bispecific polypeptides in a flow cytometry based human T cell mediated Ramos B cell killing assay. The % cell death (% of TOPRO positive cells) is plotted against the concentration of the polypeptides **(Figure 9A)**. Dose-dependent killing effect of CD20xTCR (full line) binding bispecific polypeptides in a flow cytometry based human T cell mediated Raji B cell killing assay. The % cell death (% of TOPRO positive cells) was plotted against the concentration of the polypeptides **(Figure 9B)**.

Figure 10: Dose-dependent binding of the anti-CD20 Nanobody on human CD20 Ramos (open symbols) and Raji (closed symbols) cells. The MCF value (mean channel fluorescence) was plotted against the concentration of the Nanobody.

Figures 11A and 11B: Dose-dependent killing effect of CD20xTCR binding (full line) and TCRxCD20 binding (dotted line) bispecific polypeptides in the xCELLigence based assay using CHO-K1 human CD20 transfected cells **(Figure 11A).** Dose-dependent killing effect of T017000055 in the xCELLigence based assay using CHO-K1 human CD20 transfected cells (full line, closed symbol) and CHO-K1 parental cell line (open circles) to illustrate TAA (CD20) dependent killing **(Figure 11B).** The cell index (CI) was plotted against the concentration of polypeptides.

Figure 12: Dose-dependent killing effect of CD20 x TCR polypeptides using a 5GS linker (open squares), 9GS linker (open circles) and a 35GS linker (closed symbols) in a xCELLigence based killing assay using CHO-K1 human TCR(2XN9)/CD3 cells. The cell index (CI) was plotted against the concentration of polypeptides.

Figures 13A and 13B: Dose-dependent killing effect of T017000055 **(Figure 13A)** and T017000076 **(Figure 13B)** in a flow cytometry based human T cell mediated Ramos B cell killing assay using different effector (E) to target (T) ratio's (E:T ratio 10:1 – closed circles, E:T ratio 5:1 – open squares, E:T ratio 2:1 – closed triangles and E:T ratio 1:1 – open diamonds). The % cell death (% of TOPRO positive cells) was plotted against the concentration of the polypeptides.

Figure 14: Time-dependent cytolytic activity of CD20/TCR binding polypeptides in the purified primary human T cell mediated killing assay in xCELLigence using CHO-K1 human CD20 target cells. The % specific lysis was plotted against the concentration of the construct. The different curves represent the analysis time after addition of the T cells.

Figures 15A-15C: Binding of a serial dilution of half-life extended polypeptides to human TCR/CD3 expressed on CHO-K1 cells **(Figure 15A)**, primary human T cells **(Figure 15B)** and Ramos cells **(Figure 15C)**. The MCF value (mean channel fluorescence) was plotted against the concentration of the polypeptides.

Figures 16A-16D: Dose-dependent killing effect of CD20xTCR binding multispecific polypeptides versus CD20xTCRxALB11 binding polypeptides in a flow cytometry based human T cell mediated Ramos B cell killing assay **(Figure 16A, Figure 16C).** Dose-dependent killing effect of CD20xTCRxALB11 binding polypeptides in the absence or presence of 30µM HSA in a flow cytometry based human T cell mediated Ramos B cell killing assay **(Figure 16B, Figure 16D)**. The % cell death (% of TOPRO positive cells) was plotted against the concentration of the polypeptides.

Figure 17: Binding of 100nM monovalent anti-HER2 Nanobody (5F07) to SKBR3, MCF-7 and MDA-MB-468 cell lines in flow cytometry to assess HER2 expression levels. The MCF value (mean channel fluorescence) was plotted for each cell line.

Figures 18A-18C: Dose-dependent killing effect of multispecific TCRxHER2 binding polypeptides (dotted line) and multispecific HER2xTCR binding polypeptides (full line) in an xCELLigence based human T cell mediated killing assay using SKBR-3 cells **(Figure 18A)**, MCF-7 cells **(Figure 18B)** and MDA-MB-468 cells **(Figure 18C)**. Data were analysed after 18h. The cell index (CI) was plotted against the concentration of the polypeptides.

Figure 19: Dose-dependent INF-γ production by human T cells after incubation of HER2-positive cells with multispecific TCRxHER2 binding polypeptides (dotted line) and multispecific HER2xTCR binding polypeptides (full line) in an xCELLigence based assay. Data were analysed after 72h incubation. The OD was plotted against the concentration of the polypeptides.

Figure 20: Dose-dependent killing effect of CD20xTCR binding (full line) and TCRxCD20 binding (dotted line) multispecific polypeptides in a flow cytometry based cynomolgus T cell mediated Ramos B cell killing assay. The % cell death (% of TOPRO positive cells) was plotted against the concentration of the polypeptides.

Figure 21: Dose-dependent killing effect of CD20xTCR binding (full line) and TCRxCD20 binding (dotted line) bispecific polypeptides in the xCELLigence based cynomolgus T cell mediated CHO-K1 human CD20 killing assay. The CI was plotted against the concentration of polypeptides.

Figure 22: Dose dependent binding of monovalent anti-TCR Nanobodies (closed circles) and an irrelevant Nanobody (open circles) to soluble recombinant cynomolgus TCR α/β-zipper protein. The OD at 450nm was plotted against the concentration of the Nanobody.

Figures 23A and 23B: Kinetic analysis of T0170055A02 **(Figure 23A)** and T0170056G05 **(Figure 23B)** on soluble recombinant cynomolgus TCRα/β-zipper protein interaction via BioLayer Interferometry on an Octet RED384 instrument. Applied analyte concentrations were: 1000, 333, 111, 37, 12.3, 4.1 and 1.4 nM. Langmuir fits to the kinetic data are indicated with the black lines, whereas sensorgrams are presented by the grey lines.

Figures 24A and 24B: Dose-dependent killing effect of T017000076 (CD20xTCR binding multispecific construct) versus T017000093 (CD20xTCRxALB11 binding construct) in a flow cytometry based cynomolgus T cell mediated Ramos B cell killing assay **(Figure 24A)**. Dose-dependent killing of T017000093 in the presence of 30µM HSA in a flow cytometry based cynomolgus T cell mediated Ramos B cell killing assay **(Figure 24B)**. The % cell death (% of TOPRO positive cells) was plotted against the concentration of the Nanobody.

Figure 25: Dose-dependent killing effect of CD20xTCR binding multispecific construct versus CD20xTCRxALB11 binding construct and versus CD20xTCRxALB11 binding construct in the presence of 30µM HSA in a cynomolgus T cell mediated CHO-K1 human CD20 transfected cell xCELLigence based assay. The cell index (CI) ws plotted against the concentration of Nanobody.

Figure 26: Dose-dependent killing effect of multispecific TCRxHER2 binding polypeptides (dotted line) and multispecific HER2xTCR binding polypeptides (full line) in an xCELLigence based cynomolgus T cell mediated HER2-positive SKBR-3 tumour killing assay. Data were analysed after 40h incubation. The cell index (CI) was plotted against the concentration of the Nanobody.

Figure 27: Study design for Ramos model as described in Example 19. Ramos cells were injected intravenously in to mice on day 1 (D1). PBMCs were injected intraperitoneally in to animals on day

3(D3). Mice were treated from D3 to day 7 (D7) with T017000083 (TCR/CD20) IV once daily during 5 days (Q1Dx5) or T017000088 (irrelevant multispecific polypeptide) IV Q1Dx5.

<u>Figure 28:</u> Absolute Ramos B cell count on log scale in bone marrow **(Figure 28A**) and spleen **(Figure 28B)**. Absolute PBMC derived B cell count on log scale in bone marrow (**Figure 28C**) and spleen (**Figure 28D**). Individual animal results are depicted. The number of B cells is shown in function of the different treatment groups. The open circles on top of the graphs show which active doses were statistically significant different from the irrelevant polypeptide (T017000088) based on the F-tests from the mixed-effects ANOVA analysis. All effects in the spleen are statistically significant at the 5% level of significance. Notably, the Y-axis value of 0.001 signifies no cells counted.

<u>Figure 29</u>**:** Study design for PBMC B cell depletion model as described in Example 20. PBMCs were injected intraperitoneally to animals on day 3 (D3). Mice were treated from D3 to day 7 (D7) with T017000083 (TCR/CD20) IV once daily during 5 days (Q1Dx5) or T017000088 (irrelevant polypeptide) IV Q1Dx5.

<u>Figure 30</u>: Absolute PBMC-derived B cell count on log scale. Individual animal results are depicted. The number of B cells is shown in function of the different treatment groups. Notably, Y-axis value of 0.001 signifies no cells counted.

<u>Figures 31A and 31B</u>: Determination of EGFR (**Figure 31A**; with anti-EGFR antibody; Santa Cruz, sc-120 PE) or CEACAM5 (**Figure 31B**; with anti-CEACAM antibody; Sino Biological, 11077-MM02-P) expression level on HER14, Hela, LoVo and LS174-T cell lines in flow cytometry. The MCF value (mean channel fluorescence) was plotted for each cell line.

<u>Figure 32</u>: Dose-dependent binding of the monovalent Nanobodies and multispecific polypeptides to HER14 (A), LS174T (B), primary human T cells (C) and LoVo cells (D). The mean channel fluorescence intensity was plotted against the concentration.

<u>Figure 33</u>: Determination of EGFR and CEACAM5 expression levels of HER14, LoVo and LS174T cell lines using 100nM of the EGFR and CEACAM Nanobodies in flow cytometry. The MFI value (mean fluorescence intensity) was plotted for each cell line.

<u>Figure 34</u>: Dose-dependent redirected LoVo(A, B), LST174T (C, D) and HER14 (E) target cell killing of multispecific polypeptides by human effector T cells in the xCELLigence based assay using an effector to target ratio of 15. The CI after an incubation time of 30h-40h (A, C) and 50h-60h (B, D, E) was plotted against the concentration of the multispecific polypeptides. (T017000107 = circle, T017000109 = square, T017000110 = triangle).

Figure 35: Study design of the *in vivo* Ramos model used in Example 26. Ramos cells were injected intravenously into mice on day 1 (D1). PBMCs were injected intraperitoneally to animals on day 3 (D3). Mice were treated from D3 to day 7 (D7) with T017000088, T017000106, T017000083, T017000104 or T017000105.

Figure 36: Absolute Ramos B cell count on log scale in bone marrow (A) and spleen (B). Individual animal results are depicted. The number of Ramos B cells are shown in function of the different treatment groups. The stars on top of the graph show which active doses were statistically significant different from the control Nanobody (T017000088 or T017000106) based on the F-tests from the mixed-effects ANOVA analysis. All effects are statistically significant at the 5% level of significance.

Figure 37: Absolute PBMC derived B cell count on log scale in bone marrow (A) and spleen (B). Individual animal results are depicted. The number of PBMC derived B cells are shown in function of the different treatment groups. The stars on top of the graph show which active doses were statistically significant different from the control Nanobody (T017000088 or T017000106) based on the F-tests from the mixed-effects ANOVA analysis. All effects are statistically significant at the 5% level of significance.

Figure 38: Dose-dependent redirected SKBR3 target cell killing of T017000102 by human effector T cells isolated from donor 932 (A), human CD4+ T cells isolated from donor 941 (B) and human CD8+ T cells isolated from donor 941 (C) in the xCELLigence based assay using an effector to target ratio of 15**.** The CI after an incubation time of 40h was plotted against the concentration of the multispecific polypeptide.

Figure 39: Dose-dependent T cell activation of human effector T cells isolated from donor 932 (A, D), human CD4+ T cells isolated from donor 941 (B, E) and human CD8+ T cells isolated from donor 941 (C, F) by T017000102 in a redirected SKBR3 target cell killing setting. Activation was measured by monitoring the CD69 (A, B, C) upregulation and CD25 (D, E, F) upregulation. The mean fluorescence intensity (MFI) was plotted against the concentration.

Figure 40: Dose-dependent cytokine production by human effector T cells isolated from donor 932(A, D), human CD4+ T cells isolated from donor 941 (B, E) and human CD8+ T cells isolated from donor 941 (C, F) by T017000102 in a redirected SKBR3 target cell killing setting. Human INF-γ production (A, B, C) and human IL-6 (D, E, F) was measured after 72h of incubation. The measured concentration human INF-γ and human IL-6 (in pg/ml) was plotted against the concentration of the Nanobody.

Figure 41: Dose-dependent binding of HLE construct T017000108 to HER14 (A), LS174T (B), primary human T cells (C) and LoVo cells (D). The mean fluorescence intensity (MFI) was plotted against the concentration.

Figure 42: Dose-dependent redirected LS174T target cell killing of T017000107 (solid line) and T017000108 (dotted line) by human effector T cells in the xCELLigence based assay using an effector to target ratio of 15. The CI after an incubation time of 48h was plotted against the concentration of the multispecific polypeptides.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors realized that formats bringing T cells and tumour cells together to induce an immune response should comply with various and frequently opposing requirements. The format should be broadly applicable. In particular, the format should preferably be useful in a broad range of patients and preferably also against a broad range of tumours. The format should preferably be safe and only target the intended cells. In addition, the format should preferably be small enough to easily penetrate tissues and tumours, while on the other hand the format should be patient friendly. For instance, the format should have an extended half-life, such that the format is not removed instantaneous upon administration by renal clearance. However, extending the half-life should preferably not introduce off-target activity and side effects or limit the penetration into tissues and tumours. Additionally, it was recognized that tumour cells often create escape mechanisms by the down-regulation of targeted antigens within a therapy. Accordingly, in a further preferred version, the format should simultaneously target multiple antigens.

The present invention realizes at least one of these requirements.

In particular, it was hypothesized that immunoglobulin single variable domains (ISVs) would in principle be ideal candidates, since they are small enough to easily penetrate (tumour) tissue and can be combined with other ISVs as building blocks. Next, ISVs directed against the constant TCR domains should have broad applicability. In contrast to the variable TCR domains, these constant TCR domains display less sequence variability, and consequently should be useful in a broad range of patients.

Unexpectedly, it turned out to be extremely difficult to generate ISVs via immunization in llamas against the constant domains of TCR. Either no significant immune response was mounted, or the generated ISVs were directed against the variable TCR domains. Only by implementing a rigorously carried out immunization and screening method using different cells and sequences for immunization and boosting as well as using different screening proteins, the inventors were able to isolate ISVs against the constant TCR domains. Although only three clusters of related ISVs were identified, these ISVs had an unexpected range of advantageous features. First, the ISVs were unexpectedly broadly applicable, *i.e.* the TCR ISVs were able to bind to T cells from different donors with high affinity. Formatted in a multispecific polypeptide, the TCR ISVs enabled tumour cell killing with different tumour associated antigens. Hence, the TCR ISVs can be used against a multitude of cancers. In addition, the multispecific polypeptides comprising the TCR ISVs remained active when bound to albumin. This contributes to a favourable PK profile and patient compliance, while minimizing side effects. The polypeptides of the invention only showed effects when bound both to the T cell and the target cell, which is indicative of its safety.

The present inventors considered that the simultaneous targeting of multiple antigens reduces the probability of generating tumour escape variants, because of which the therapeutic activity of T cell engaging strategy is improved. Multispecific polypeptides are provided which comprise a TCR ISV combined with immunoglobulin single variable domains against different target antigens and/or different epitopes on a particular antigen (biparatopic).

Immunoglobulin sequences, such as antibodies and antigen binding fragments derived there from (*e.g.*, immunoglobulin single variable domains or ISVs) are used to specifically target their respective antigens in research and therapeutic applications. The generation of immunoglobulin single variable domains such as *e.g.*, VHHs or Nanobodies may involve the immunization of an experimental animal such as a Llama, construction of phage libraries from immune tissue, selection of phage displaying antigen binding immunoglobulin single variable domains and screening of said domains and engineered constructs thereof for the desired specificities (WO 94/04678). Alternatively, similar immunoglobulin single variable domains such as *e.g.*, dAbs can be generated by selecting phage displaying antigen binding immunoglobulin single variable domains directly from naive or synthetic libraries and subsequent screening of said domains and engineered constructs thereof for the desired specificities (Ward *et al.*, Nature, 1989, 341: 544-546; Holt *et al.*, Trends Biotechnol., 2003, 21(11):484-490; as well as for example WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd.). Unfortunately, the use of monoclonal and/or heavily engineered antibodies also carries a high manufacturing cost and may result in suboptimal tumor penetration compared to other strategies.

The present invention provides multispecific polypeptides that specifically bind to the T cell receptor (TCR), with an unexpected range of advantageous features. First, the polypeptides are easy to manufacture. Moreover, the ISVs are unexpectedly broadly applicable, *i.e.* the TCR ISVs were able to bind to T cells from different donors with high affinity. Formatted in a multispecific polypeptide, the TCR ISVs enabled tumour cell killing with different tumour associated antigens. In contrast, no killing was observed when the polypeptides were not bound to T cells and target cell which underscores the safety of the polypeptides of the invention. Hence, the TCR ISVs can be used against a multitude of cancers. Moreover, the TCR ISVs can be considered as safe. In addition, the multispecific polypeptides comprising the TCR ISVs remained active when bound to albumin. This will contribute to a favourable PK profile and patient compliance, while minimizing side effects.

Accordingly, the present invention relates to a polypeptide comprising a first and a second immunoglobulin single variable domain (ISV), wherein the first ISV has high affinity for / binds to the constant domain of the T cell receptor (TCR) and the second ISV has high affinity for / binds to an antigen on a cell (target cell), preferably a tumour cell. The antigen is preferably specific for said target cell, such as e.g. a tumour associated antigen (TAA). The multispecific polypeptide of the invention directs the T cell to the cell, e.g. a tumour cell and induces T cell activation in order to allow said T cell to inhibit or kill said target cell, e.g. said tumour cell.

Definitions:

a) 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 mentioned in paragraph a) on page 46 of WO 08/020079.

b) The term “immunoglobulin single variable domain”, interchangeably used with “single variable domain” and “ISV”, defines molecules wherein the antigen binding site is present on, and formed by, a single immunoglobulin domain. This sets immunoglobulin single variable domains apart from “conventional” immunoglobulins or their fragments (such as Fabs, scFvs, etc.), wherein two immunoglobulin domains, in particular two variable domains, interact to form an antigen binding site. Typically, in conventional immunoglobulins, a heavy chain variable domain (VH) and a light chain variable domain (VL) interact to form an antigen binding site. In this case, the complementarity determining regions (CDRs) of both VH and VL will contribute to the antigen binding site, *i.e.* a total of 6 CDRs will be involved in antigen binding site formation. In contrast, the binding site of an immunoglobulin single variable domain is formed by a single VH or VL domain. Hence, the antigen binding site of an immunoglobulin single variable domain is formed by no more than three CDRs.

The terms “immunoglobulin single variable domain”, “single variable domain”, and “ISV” hence do not comprise conventional immunoglobulins or their fragments which require interaction of at least two variable domains for the formation of an antigen binding site. However, these terms do comprise fragments of conventional immunoglobulins wherein the antigen binding site is formed by a single variable domain.

The term “immunoglobulin single variable domain” or "ISV" includes (without being limiting) antigen-binding domains or fragments such as V_{HH} domains or V_H or V_L domains, respectively.

The terms antigen-binding molecules or antigen-binding protein are used interchangeably and include also the term Nanobodies. The immunoglobulin single variable domains can be light chain variable domain sequences (*e.g.*, a V_L-sequence), or heavy chain variable domain sequences (*e.g.*, a V_H-sequence); more specifically, they can be heavy chain variable domain sequences that are derived from a conventional four-chain antibody or heavy chain variable domain sequences that are derived from a heavy chain antibody. Accordingly, the immunoglobulin single variable domains can be domain antibodies, or immunoglobulin sequences that are suitable for use as domain antibodies, single domain antibodies, or immunoglobulin sequences that are suitable for use as single domain antibodies, "dAbs", or immunoglobulin sequences that are suitable for use as dAbs, or Nanobodies, including but not limited to V_{HH} sequences, humanized VHH sequences or camelized VH sequences. The invention includes immunoglobulin sequences of different origin, comprising mouse, rat, rabbit, donkey, human and camelid immunoglobulin sequences. The immunoglobulin single variable domain includes fully human, humanized, otherwise sequence optimized or chimeric immunoglobulin sequences. The immunoglobulin single variable domain and structure of an immunoglobulin single variable domain can be considered - without however being limited thereto - to be comprised of four framework regions or “FR’s”, which are referred to in the art and herein 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 regions are interrupted by three complementary determining regions or “CDR’s”, which are referred to in the art as “Complementarity Determining Region 1” or “CDR1”; as “Complementarity Determining Region 2” or “CDR2”; and as “Complementarity Determining Region 3” or “CDR3”, respectively. It is noted that the terms Nanobody or Nanobodies are registered trademarks of Ablynx N.V. and thus may also be referred to as Nanobody or Nanobodies, respectively.

c) Unless indicated otherwise, the terms “immunoglobulin sequence”, “sequence”, “nucleotide sequence” and “nucleic acid” are as described in paragraph b) on page 46 of WO 08/020079.

d) Unless indicated otherwise, all methods, steps, techniques and 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 and the general background art mentioned herein and to the further references cited therein; as well as to for example the following reviews Presta 2006 (Adv. Drug Deliv. Rev. 58 (5-6):640-656), Levin and Weiss 2006 (Mol. Biosyst. 2(1):49-57), Irving et al. 2005 (J. Immunol. Methods 248(1-2):31-45), Schmitz et al. 2000 (Placenta 21 Suppl. A: S106-

112, Gonzales et al. 2005 (Tumour Biol. 26(1):31-43), which describe techniques for protein engineering, such as affinity maturation and other techniques for improving the specificity and other desired properties of proteins such as immunoglobulins.

e) Amino acid residues will be indicated according to the standard three-letter or one-letter amino acid code. Reference is made to Table A-2 on page 48 of the International application WO 08/020079 of Ablynx N.V. entitled "*Immunoglobulin single variable domains directed against IL-6R and polypeptides comprising the same for the treatment of diseases and disorders associated with Il-6 mediated signalling*".

f) For the purposes of comparing two or more nucleotide sequences, the percentage of "*sequence identity*" between a first nucleotide sequence and a second nucleotide sequence may be calculated or determined as described in paragraph e) on page 49 of WO 08/020079 (incorporated herein by reference), such as by dividing [*the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence*] by [*the total number of nucleotides in the first nucleotide sequence*] and multiplying by [*100%*], in which each deletion, insertion, substitution or addition of a nucleotide in the second nucleotide sequence - compared to the first nucleotide sequence - is considered as a difference at a single nucleotide (position); or using a suitable computer algorithm or technique, again as described in paragraph e) on pages 49 of WO 08/020079 (incorporated herein by reference).

g) For the purposes of comparing two or more immunoglobulin single variable domains or other amino acid sequences such e.g. the polypeptides of the invention etc., the percentage of "*sequence identity*" between a first amino acid sequence and a second amino acid sequence (also referred to herein as "*amino acid identity*") may be calculated or determined as described in paragraph f) on pages 49 and 50 of WO 08/020079 (incorporated herein by reference), such as by dividing [*the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence*] by [*the total number of amino acid residues in the first amino acid sequence*] and multiplying by [*100%*], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence - compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), *i.e.,* as an "amino acid difference" as defined herein; or using a suitable computer algorithm or technique, again as described in paragraph f) on pages 49 and 50 of WO 08/020079 (incorporated herein by reference).

Also, in determining the degree of sequence identity between two immunoglobulin single variable domains, the skilled person may take into account so-called "conservative" amino acid substitutions, as described on page 50 of WO 08/020079.

Any amino acid substitutions applied to the polypeptides described herein may also be based on the analysis of the frequencies of amino acid variations between homologous proteins of different species developed by Schulz et al. 1978 (Principles of Protein Structure, Springer-Verlag), on the analyses of structure forming potentials developed by Chou and Fasman 1975 (Biochemistry 13: 211) and 1978 (Adv. Enzymol. 47: 45-149), and on the analysis of hydrophobicity patterns in proteins developed by Eisenberg et al. 1984 (Proc. Natl. Acad. Sci. USA 81: 140-144), Kyte & Doolittle 1981 (J Molec. Biol. 157: 105-132), and Goldman et al. 1986 (Ann. Rev. Biophys. Chem. 15: 321-353), all incorporated herein in their entirety by reference. Information on the primary, secondary and tertiary structure of Nanobodies is given in the description herein and in the general background art cited above. Also, for this purpose, the crystal structure of a V_{HH} domain from a llama is for example given by Desmyter et al. 1996 (Nature Structural Biology, 3: 803), Spinelli et al. 1996 (Natural Structural Biology 3: 752-757), and Decanniere et al. 1999 (Structure, 7: 361). Further information about some of the amino acid residues that in conventional V_H domains form the V_H/V_L interface and potential camelizing substitutions on these positions can be found in the prior art cited above.

h) Immunoglobulin single variable domains and nucleic acid sequences are said to be "*exactly the same*" if they have 100% sequence identity (as defined herein) over their entire length.

i) When comparing two immunoglobulin single variable domains, the term "*amino acid difference*" refers to an insertion, deletion or substitution of a single amino acid residue on a position of the first sequence, compared to the second sequence; it being understood that two immunoglobulin single variable domains can contain one, two or more such amino acid differences.

j) When a nucleotide sequence or amino acid sequence is said to "comprise" another nucleotide sequence or amino acid sequence, respectively, or to "essentially consist of" another nucleotide sequence or amino acid sequence, this has the meaning given in paragraph i) on pages 51-52 of WO 08/020079.

k) The term "in essentially isolated form" has the meaning given to it in paragraph j) on pages 52 and 53 of WO 08/020079.

l) The terms "domain" and "binding domain" have the meanings given to it in paragraph k) on page 53 of WO 08/020079.

m) The terms "antigenic determinant" and "epitope", which may also be used interchangeably herein, have the meanings given to it in paragraph l) on page 53 of WO 08/020079.

n) As further described in paragraph m) on page 53 of WO 08/020079, an amino acid sequence (such as an antibody, a polypeptide of the invention, or generally an antigen-binding protein or polypeptide or a fragment thereof) that can (specifically) bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be "*against*" or "*directed against*" said antigenic determinant, epitope, antigen or protein.

o) The term "*specificity*" refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein (such as an ISV, Nanobody or a polypeptide of the invention) can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity.

The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (K_D or KD), is a measure for the binding strength between an antigenic determinant, i.e. the target, and an antigen-binding site on the antigen-binding protein, i.e. the ISV or Nanobody: the lesser the value of the K_D, the stronger the binding strength between an antigenic determinant and the antigen-binding molecule (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 manner known per se, depending on the specific antigen of interest.

Avidity is the affinity of the polypeptide, i.e. the ligand is able to bind via two (or more) pharmacophores (ISV) in which the multiple interactions synergize to enhance the "apparent" affinity. Avidity is the measure of the strength of binding between the polypeptide of the invention and the pertinent antigens. The polypeptide of the invention is able to bind via its two (or more) building blocks, such as ISVs or Nanobodies, to the at least two targets, in which the multiple interactions, e.g. the first building block, ISV or Nanobody binding to the first target and the second building block, ISV, or Nanobody binding to the second target, synergize to enhance the "apparent" affinity. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antigen-binding molecule and the number of pertinent binding sites present on the antigen-binding molecules. For example, and without limitation, polypeptides that contain two or more building blocks, such as ISVs or Nanobodies directed against different targets on a cell may (and usually will) bind with higher avidity than each of the individual monomers or individual building blocks, such as, for instance, the monovalent ISVs or Nanobodies, comprised in the polypeptides of the invention.

Any K_D value greater than 10^{-4} mol/liter (or any K_A value lower than 10^4 M^{-1}) liters/mol is generally considered to indicate non-specific binding.

The polypeptides of the invention comprise a first and a second building block, e.g. a first and a second ISV, or a first and a second Nanobody. Preferably the affinity of each building block, e.g. ISV or Nanobody, is determined individually. In other words, the affinity is determined for the monovalent building block, ISV or Nanobody, independent of avidity effects due to the other building block, ISV or Nanobody, which might or might not be present. The affinity for a monovalent building block, ISV or Nanobody can be determined on the monovalent building block, ISV or Nanobody per se, i.e. when said monovalent building block, ISV or Nanobody is not comprised in the polypeptide of the invention. In the alternative or in addition, the affinity for a monovalent building block, ISV or Nanobody can be determined on one target while the other target is absent.

The binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art; as well as the other techniques mentioned herein.

The dissociation constant may be the actual or apparent dissociation constant, as will be clear to the skilled person. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned herein. In this respect, it will also be clear that it may not be possible to measure dissociation constants of more than 10^{-4} moles/liter or 10^{-3} moles/liter (e.g., of 10^{-2} moles/liter). Optionally, as will also be clear to the skilled person, the (actual or apparent) dissociation constant may be calculated on the basis of the (actual or apparent) association constant (K_A), by means of the relationship [$K_D = 1/K_A$].

The affinity denotes the strength or stability of a molecular interaction. The affinity is commonly given as by the K_D, or dissociation constant, which has units of mol/liter (or M). The affinity can also be expressed as an association constant, K_A, which equals $1/K_D$ and has units of $(\text{mol/liter})^{-1}$ (or M^{-1}). In the present specification, the stability of the interaction between two molecules (such as an amino acid sequence, Nanobody or polypeptide of the invention and its intended target) will mainly be expressed in terms of the K_D value of their interaction; it being clear to the skilled person that in view of the relation $K_A = 1/K_D$, specifying the strength of molecular interaction by its K_D value can also be used to calculate the corresponding K_A value. The K_D value characterizes the strength of a molecular interaction also in a thermodynamic sense as it is related to the free energy (DG) of binding by the well known relation DG=RT.ln(K_D) (equivalently DG=-RT.ln(K_A)), where R equals the gas constant, T equals the absolute temperature and ln denotes the natural logarithm.

The K_D for biological interactions which are considered meaningful (e.g. specific) are typically in the range of 10^{-10}M (0.1 nM) to 10^{-5}M (10000 nM). The stronger an interaction is, the lower is its K_D.

The K_D can also be expressed as the ratio of the dissociation rate constant of a complex, denoted as k_{off}, to the rate of its association, denoted k_{on} (so that $K_D = k_{off}/k_{on}$ and $K_A = k_{on}/k_{off}$). The off-rate k_{off} has units s^{-1} (where s is the SI unit notation of second). The on-rate k_{on} has units $M^{-1}s^{-1}$. The on-rate may vary between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, approaching the diffusion-limited association rate constant for biomolecular interactions. The off-rate is related to the half-life of a given molecular interaction by the relation $t_{1/2}=\ln(2)/k_{off}$. The off-rate may vary between $10^{-6}s^{-1}$ (near irreversible complex with a $t_{1/2}$ of multiple days) to $1s^{-1}$ ($t_{1/2}$ = 0.69s).

The affinity of a molecular interaction between two molecules can be measured via different techniques known per se, such as the well-known surface plasmon resonance (SPR) biosensor technique (see for example Ober et al. 2001, Intern. Immunology, 13: 1551-1559). The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, where one molecule is immobilized on the biosensor chip and the other molecule is passed over the immobilized molecule under flow conditions yielding k_{on}, k_{off} measurements and hence K_D (or K_A) values. This can for example be performed using the well-known BIAcore® system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jonsson et al. 1993 (Ann. Biol. Clin. 51: 19-26), Jonsson et al. 1991 (Biotechniques 11: 620-627), Johnsson, et al. 1995 (J. Mol. Recognit. 8: 125-131), and Johnnson, et al. 1991 (Anal. Biochem. 198: 268-277).

Another well-known biosensor technique to determine affinities of biomolecular interactions is bio-layer interferometry (BLI) (see for example Abdiche et al. 2008, Anal. Biochem. 377: 209-217). The term “bio-layer Interferometry” or “BLI”, as used herein, refers to a label-free optical technique that analyzes the interference pattern of light reflected from two surfaces: an internal reference layer (reference beam) and a layer of immobilized protein on the biosensor tip (signal beam). A change in the number of molecules bound to the tip of the biosensor causes a shift in the interference pattern, reported as a wavelength shift (nm), the magnitude of which is a direct measure of the number of molecules bound to the biosensor tip surface. Since the interactions can be measured in real-time, association and dissociation rates and affinities can be determined. BLI can for example be performed using the well-known Octet® Systems (ForteBio, a division of Pall Life Sciences, Menlo Park, USA).

Alternatively, affinities can be measured in Kinetic Exclusion Assay (KinExA) (see for example Drake et al. 2004, Anal. Biochem., 328: 35-43), using the KinExA® platform (Sapidyne Instruments Inc, Boise, USA). The term "KinExA", as used herein, refers to a solution-based method to measure true equilibrium binding affinity and kinetics of unmodified molecules. Equilibrated solutions of an antibody/antigen complex are passed over a column with beads precoated with antigen (or antibody), allowing the free antibody (or antigen) to bind to the coated molecule. Detection of the antibody (or antigen) thus captured is accomplished with a fluorescently labeled protein binding the antibody (or antigen).

It will also be clear to the skilled person that the measured K_D may correspond to the apparent K_D if the measuring process somehow influences the intrinsic binding affinity of the implied molecules for example by artefacts related to the coating on the biosensor of one molecule. Also, an apparent K_D may be measured if one molecule contains more than one recognition site for the other molecule. In such situation the measured affinity may be affected by the avidity of the interaction by the two molecules.

Another approach that may be used to assess affinity is the 2-step ELISA (Enzyme-Linked Immunosorbent Assay) procedure of Friguet et al. 1985 (J. Immunol. Methods, 77: 305-19). This method establishes a solution phase binding equilibrium measurement and avoids possible artefacts relating to adsorption of one of the molecules on a support such as plastic.

However, the accurate measurement of K_D may be quite labour-intensive, and as consequence, often apparent K_D values are determined to assess the binding strength of two molecules. It should be noted that as long as all measurements are made in a consistent way (e.g. keeping the assay conditions unchanged) apparent K_D measurements can be used as an approximation of the true K_D and hence, in the present document, K_D and apparent K_D should be treated with equal importance or relevance.

Finally, it should be noted that in many situations the experienced scientist may judge it to be convenient to determine the binding affinity relative to some reference molecule. For example, to assess the binding strength between molecules A and B, one may e.g. use a reference molecule C that is known to bind to B and that is suitably labelled with a fluorophore or chromophore group or other chemical moiety, such as biotin for easy detection in an ELISA or FACS (Fluorescent activated cell sorting) or other format (the fluorophore for fluorescence detection, the chromophore for light absorption detection, the biotin for streptavidin-mediated ELISA detection). Typically, the reference molecule C is kept at a fixed concentration and the concentration of A is varied for a given concentration or amount of B. As a result an IC50 value is obtained corresponding to the concentration of A at which the signal measured for C in absence of A is halved. Provided $K_{D\,ref}$, the K_D of the reference molecule, is known, as well as the total concentration c_{ref} of the reference molecule, the apparent K_D for the interaction A-B can be obtained from following formula: $K_D = IC50/(1+c_{ref}/K_{D\,ref})$. Note that if $c_{ref} << K_{D\,ref}$, $K_D \approx IC50$. Provided the measurement of the IC50 is performed in a consistent way (e.g. keeping c_{ref} fixed) for the binders that are compared, the strength or stability of a molecular interaction can be assessed by the IC50 and this measurement is judged as equivalent to K_D or to apparent K_D throughout this text.

p) The half-life of an amino acid sequence, compound or polypeptide of the invention can generally be defined as described in paragraph o) on page 57 of WO 08/020079 and as mentioned therein refers to the time taken for the serum concentration of the amino acid sequence, compound or polypeptide to be reduced by 50%, in vivo, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The *in vivo* half-life of an amino acid sequence, compound or polypeptide of the invention can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally be as described in paragraph o) on page 57 of WO 08/020079. As also mentioned in paragraph o) on page 57 of WO 08/020079, the half-life can be expressed using parameters such as the t1/2-alpha, t1/2-beta and the area under the curve (AUC). Reference is for example made to the Experimental Part below, as well as to the standard handbooks, such as Kenneth et al. 1996 (Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists) and Peters et al. 1996 (Pharmacokinete analysis: A Practical Approach). Reference is also made to Gibaldi & Perron 1982 (Pharmacokinetics, Dekker M, 2nd Rev. edition). The terms “increase in half-life” or “increased half-life” are as defined in paragraph o) on page 57 of WO 08/020079 and in particular refer to an increase in the t1/2-beta, either with or without an increase in the t1/2-alpha and/or the AUC or both.

q) In respect of a target or antigen, the term “interaction site” on the target or antigen means a site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is a site for binding to a ligand, receptor or other binding partner, a catalytic site, a cleavage site, a site for allosteric interaction, a site involved in multimerisation (such as homomerization or heterodimerization) of the target or antigen; or any other site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is involved in a biological action or mechanism of the target or antigen. More generally, an “interaction site” can be any site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen to which an amino acid sequence or polypeptide of the invention can bind such that the target or antigen (and/or any pathway, interaction, signalling, biological mechanism or biological effect in which the target or antigen is involved) is modulated.

r) An immunoglobulin single variable domain or polypeptide is said to be “*specific for*” a first target or antigen compared to a second target or antigen when it binds to the first antigen with an affinity/avidity (as described above, and suitably expressed as a K_D value, K_A value, K_{off} rate and/or K_{on} rate) that is at least 10 times, such as at least 100 times, and preferably at least 1000 times, and up to 10.000 times or more better than the affinity with which said amino acid sequence or polypeptide binds to the second target or polypeptide. For example, the first antigen may bind to the target or antigen with a K_D value that is at least 10 times less, such as at least 100 times less, and preferably at least 1000 times less, such as 10.000 times less or even less than that, than the K_D with which said amino acid sequence or polypeptide binds to the second target or polypeptide. Preferably, when an immunoglobulin single variable domain or polypeptide is “specific for” a first target or antigen compared to a second target or antigen, it is directed against (as defined herein) said first target or antigen, but not directed against said second target or antigen.

s) The terms “*cross-block*”, “*cross-blocked*” and “*cross-blocking*” are used interchangeably herein to mean the ability of an immunoglobulin single variable domain or polypeptide to interfere with the binding of the natural ligand to its receptor(s). The extent to which an immunoglobulin single variable domain or polypeptide of the invention is able to interfere with the binding of another compound such as the natural ligand to its target and therefore whether it can be said to cross-block according to the invention, can be determined using competition binding assays. One particularly suitable quantitative cross-blocking assay uses a FACS- or an ELISA-based approach or Alphascreen to measure competition between the labelled (*e.g.*, His tagged or biotinylated) immunoglobulin single variable domain or polypeptide according to the invention and the other binding agent in terms of their binding to the target. The experimental part generally describes suitable FACS-, ELISA- or Alphascreen-displacement-based assays for determining whether a binding molecule cross-blocks or is capable of cross-blocking an immunoglobulin single variable domain or polypeptide according to the invention. It will be appreciated that the assay can be used with any of the immunoglobulin single variable domains or other binding agents described herein. Thus, in general, a cross-blocking amino acid sequence or other binding agent according to the invention is for example one which will bind to the target in the above cross-blocking assay such that, during the assay and in the presence of a second amino acid sequence or other binding agent of the invention, the recorded displacement of the immunoglobulin single variable domain or polypeptide according to the invention is between 60% and 100% (*e.g.*, in ELISA/Alphascreen based competition assay) or between 80% to 100% (*e.g.*, in FACS based competition assay) of the maximum theoretical displacement (e.g. displacement by cold (*e.g.*, unlabeled) immunoglobulin single variable domain or polypeptide that needs to be cross-blocked) by the to be tested potentially cross-blocking agent that is present in an amount of 0.01 mM or less (cross-blocking agent may be another conventional monoclonal antibody such as IgG, classic monovalent antibody fragments (Fab, scFv) and engineered variants (*e.g.*, diabodies, triabodies, minibodies, VHHs, dAbs, VHs, VLs)).

t) An amino acid sequence such as e.g. an immunoglobulin single variable domain or polypeptide according to the invention is said to be a "*VHH1 type immunoglobulin single variable domain*" or "*VHH type 1 sequence*", if said VHH1 type immunoglobulin single variable domain or VHH type 1 sequence has 85% identity (using the VHH1 consensus sequence as the query sequence and use the blast algorithm with standard setting*, i.e.*, blosom62 scoring matrix) to the VHH1 consensus sequence (QVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREGVSCISSSD GSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA) (SEQ ID NO: 483), and mandatorily has a cysteine in position 50, *i.e.,* C50 (using Kabat numbering).

u) An amino acid sequence such as *e.g.,* an immunoglobulin single variable domain or polypeptide according to the invention is said to be "*cross-reactive*" for two different antigens or antigenic determinants (such as serum albumin from two different species of mammal, such as human serum albumin and cynomolgus monkey serum albumin) if it is specific for (as defined herein) both these different antigens or antigenic determinants.

v) As further described in paragraph q) on pages 58 and 59 of WO 08/020079 (incorporated herein by reference), the amino acid residues of an immunoglobulin single variable domain are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muyldermans, 2000 (J. Immunol. Methods 240 (1-2): 185-195; see for example Figure 2 of this publication). It should be noted that - as is well known in the art for V_H domains and for VHH domains - the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering). This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence. The total number of amino acid residues in a VH domain and a VHH domain will usually be in the range of from 110 to 120, often between 112 and 115. It should however be noted that smaller and longer sequences may also be suitable for the purposes described herein.

Determination of CDR regions may also be done according to different methods. In the CDR determination according to Kabat, FR1 of an immunoglobulin single variable domain comprises the amino acid residues at positions 1-30, CDR1 of an immunoglobulin single variable domain comprises the amino acid residues at positions 31-35, FR2 of an immunoglobulin single variable domain comprises the amino acids at positions 36-49, CDR2 of an immunoglobulin single variable domain comprises the amino acid residues at positions 50-65, FR3 of an immunoglobulin single variable domain comprises the amino acid residues at positions 66-94, CDR3 of an immunoglobulin single variable domain comprises the amino acid residues at positions 95-102, and FR4 of an immunoglobulin single variable domain comprises the amino acid residues at positions 103-113.

In the present application, however, unless indicated otherwise, CDR sequences were determined according to Kontermann and Dübel (Eds. 2010, Antibody Engineering, vol 2, Springer Verlag Heidelberg Berlin, Martin, Chapter 3, pp. 33-51). According to this method, FR1 comprises the amino acid residues at positions 1-25, CDR1 comprises the amino acid residues at positions 26-35, FR2 comprises the amino acids at positions 36-49, CDR2 comprises the amino acid residues at positions 50-58, FR3 comprises the amino acid residues at positions 59-94, CDR3 comprises the amino acid residues at positions 95-102, and FR4 comprises the amino acid residues at positions 103-113.

w) The Figures, Sequence Listing and the Experimental Part/Examples are only given to further illustrate the invention and should not be interpreted or construed as limiting the scope of the invention and/or of the appended claims in any way, unless explicitly indicated otherwise herein.

x) The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting a biological or biochemical function, e.g. a pharmacological effect. This quantitative measure indicates how much of the ISV or Nanobody (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor, chemotaxis, anaplasia, metastasis, invasiveness, etc) by half. In other words, it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC50). The IC50 of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist such as the ISV or Nanobody of the invention on reversing agonist activity. IC50 values can be calculated for a given antagonist such as the ISV or Nanobody of the invention by determining the concentration needed to inhibit half of the maximum biological response of the agonist.

The term half maximal effective concentration (EC50) refers to the concentration of a compound which induces a response halfway between the baseline and maximum after a specified exposure time. In the present context it is used as a measure of a polypeptide's, ISV's or Nanobody's potency. The EC50 of a graded dose response curve represents the concentration of a compound where 50% of its maximal effect is observed. Concentration is preferably expressed in molar units.

In biological systems, small changes in ligand concentration typically result in rapid changes in response, following a sigmoidal function. The inflection point at which the increase in response with increasing ligand concentration begins to slow is the EC50. This can be determined mathematically by derivation of the best-fit line. Relying on a graph for estimation is convenient in most cases. In case the EC50 is provided in the examples section, the experiments were designed to reflect the KD as accurate as possible. In other words, the EC50 values may then be considered as KD values. The term "average KD" relates to the average KD value obtained in at least 1, but preferably more than 1, such as at least 2 experiments. The term "average" refers to the mathematical term "average" (sums of data divided by the number of items in the data).

It is also related to IC50 which is a measure of a compound's inhibition (50% inhibition). For competition binding assays and functional antagonist assays, IC50 is the most common summary measure of the dose-response curve. For agonist/stimulator assays the most common summary measure is the EC50.

y) It must be noted that as used herein, the singular forms "a", "an", and "the", include plural references unless the context clearly indicates otherwise. Thus, for example, reference to "a reagent" includes one or more of such different reagents and reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention.

The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within 20%, preferably within 15%, more preferably within 10%, and most preferably within 5% of a given value or range.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term "comprising" can be substituted with the term "containing" or "including" or sometimes when used herein with the term "having".

The present invention relates to a polypeptide comprising at least a first and at least one further immunoglobulin single variable domain (ISV), wherein said at least first ISV has high affinity for / binds to the constant domain of the T cell receptor (TCR) and said at least one further ISV has high affinity for / binds to an antigen on a target cell.

Typically, the multispecific polypeptides of the invention combine high affinity antigen recognition on the target cell with T cell activation, resulting in an activation that is independent of the T cells' natural specificity. The mode of action of the binding molecules that bind both to a cell surface molecule on a target cell such as a tumour antigen and to the T cell TCR is commonly known. Bringing a T cell in close vicinity to a target cell, *i.e.*, engaging said T cell and clustering of the TCR complex results in killing of the target cell by the T cell. In the present invention this process is exploited in fighting against proliferative disease, inflammatory disease, infectious disease and autoimmune disease. Generally T cells are equipped with granules containing a deadly combination of pore-forming proteins, called perforins, and cell death-inducing proteases, called granzymes. Preferably, these proteins are delivered into target cells via a cytolytic synapse that forms if T cells are in close vicinity with a target cell that is aimed to be killed. Normally, close vicinity between a T cell and a target cell is achieved by the T cell binding to an MHC/peptide complex using its matching T cell receptor. The polypeptides of the invention bring a T cell into such close vicinity to a target cell in the absence of T cell receptor/MHC interaction.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said polypeptide directs the T cell to the target cell.

With one arm (first ISV), the multispecific polypeptide has high affinity for / binds to the constant domain of the TCR subunit, a protein component of the signal-transducing complex of the T cell receptor on T cells. With another arm (second ISV and/or third ISV, etc.), the multispecific polypeptide recognizes, has high affinity for / binds an antigen(s) on target cells. Preferably, T cell activation is only seen when the multispecific polypeptides are presented to T cells on the surface of target cells. Antigen dependence on target cells for activation results in a favourable safety profile. In an embodiment, the multispecific polypeptides transiently tether T cells and target cells. Preferably, the multispecific polypeptide can induce resting polyclonal T cells, such as $CD4^+$ and/or $CD8^+$ T cells into activation, for highly potent redirected lysis of target cells. Preferably, the T cell is directed to a next target cell after lysis of the first target cell.

Proteins and polypeptides that comprise or essentially consist of two or more immunoglobulin single variable domains (such as at least two immunoglobulin single variable domains of the invention) will be referred to herein as “multivalent” proteins or polypeptides or as “multivalent constructs”. Some non-limiting examples of such multivalent constructs will become clear from the further description herein. The polypeptides of the invention are "multivalent", *i.e.* comprising two or more building blocks or ISVs of which at least the first building block, ISV or Nanobody and the second building block, ISV or Nanobody are different, and directed against different targets, such as antigens or antigenic determinants. Polypeptides of the invention that contain at least two building blocks, ISVs or Nanobodies, in which at least one building block, ISV or Nanobody is directed against a first antigen (*i.e.,* against the first target, such as e.g. the constant domain of a TCR) and at least one building block, ISV or Nanobody is directed against a second antigen (*i.e.,* against the second target which is different from the first target, such as e.g. a TAA, such as CD20 or HER2), will also be referred to as “multispecific” polypeptides of the invention, and the building blocks, ISVs or Nanobodies present in such polypeptides will also be referred to herein as being in a “multivalent format” or “multispecifc format”. Thus, for example, a “bispecific” polypeptide of the invention is a polypeptide that comprises at least one building block, ISV or Nanobody directed against a first target (e.g. TCR) and at least one further building block, ISV or Nanobody directed against a second target (*i.e.,* directed against a second target different from said first target, such as e.g. a TAA, e.g. CD20 or

HER2), whereas a “trispecific” polypeptide of the invention is a polypeptide that comprises at least one building block, ISV or Nanobody directed against a first target (*e.g.,* TCR), a second building block, ISV or Nanobody directed against a second target different from said first target (e.g. a TAA, e.g. CD20 or HER2) and at least one further building block, ISV or Nanobody directed against a third antigen (*i.e.,* different from both the first and the second target, such as another TAA); etc. As will be clear from the description, the invention is not limited to bispecific polypeptides, in the sense that a multispecific polypeptide of the invention may comprise at least a first building block, ISV or Nanobody against a first target, a second building block, ISV or Nanobody against a second target and any number of building blocks, ISVs or Nanobodies directed against one or more targets, which may be the same or different from the first and/or second target, respectively. The building blocks, ISVs or Nanobodies can optionally be linked via linker sequences.

The terms bispecific polypeptide, bispecific format, bispecific construct, bispecific Nanobody construct, bispecific and bispecific antibody are used interchangeably herein.

As will be clear from the further description above and herein, the immunoglobulin single variable domains of the invention can be used as “building blocks” to form polypeptides of the invention, *e.g.,* by suitably combining them with other groups, residues, moieties or binding units, in order to form compounds or constructs as described herein (such as, without limitations, the bi-/tri-/tetra-/ multivalent and bi-/tri-/tetra-/multispecific polypeptides of the invention described herein) which combine within one molecule one or more desired properties or biological functions.

It will be appreciated (as is also demonstrated in the Example section) that the ISV binding TCR and the ISV binding the antigen on a target cell can be positioned in any order in the polypeptide of the invention. More particularly, in one embodiment, the ISV binding TCR is positioned N-terminally and the ISV binding the antigen on a target cell is positioned C-terminally. In another embodiment, the ISV binding the antigen on a target cell is positioned N-terminally and the ISV binding TCR is positioned C-terminally.

In a preferred aspect, the polypeptide of the invention comprises at least a first, at least a second and at least a third immunoglobulin single variable domain (ISV), wherein said at least a first ISV has high affinity for / binds to the constant domain of the T cell receptor (TCR); said at least a second ISV has high affinity for / binds to a first antigen on a target cell, and said at least a third ISV has high affinity for / binds to a second antigen on a target cell, wherein said second antigen is different from said first antigen. Said first antigen and said second antigen can be on the same or on different target cells.

It will be appreciated (as is also demonstrated in the Example section) that the ISV binding TCR and the ISVs binding the first and second antigen on a target cell can be positioned in any order in the polypeptide of the invention. More particularly, in one embodiment, the ISV binding TCR is positioned N-terminally, the ISV binding the first antigen on a target cell is positioned centrally and the ISV binding the second antigen on a target cell is positioned C-terminally. In another embodiment, the ISV binding TCR is positioned N-terminally, the ISV binding the second antigen on a target cell is positioned centrally and the ISV binding the first antigen on a target cell is positioned C-terminally. In another embodiment, the ISV binding the first antigen on a target cell is positioned N-terminally, the ISV binding the second antigen on a target cell is positioned centrally and the ISV binding the TCR is positioned C-terminally. In another embodiment, the ISV binding the first antigen on a target cell is positioned N-terminally, the ISV binding the TCR is positioned centrally and the ISV binding the second antigen on a target cell is positioned C-terminally. In another embodiment, the ISV binding the second antigen on a target cell is positioned N-terminally, the ISV binding the TCR is positioned centrally and the ISV binding the first antigen on a target cell is positioned C-terminally. In another embodiment, the ISV binding the second antigen on a target cell is positioned N-terminally, the ISV binding the first antigen on a target cell is positioned centrally and the ISV binding the TCR is positioned C-terminally.

The invention further relates to compounds or constructs, and in particular proteins or polypeptides that comprise or essentially consist of one or more ISVs or polypeptides of the invention, and optionally further comprise one or more other groups, residues, moieties or binding units. As will become clear to the skilled person from the further disclosure herein, such further groups, residues, moieties, binding units or amino acid sequences may or may not provide further functionality to the polypeptide of the invention (and/or to the compound or construct in which it is present) and may or may not modify the properties of the polypeptide of the invention.

The compounds, constructs or polypeptides of the invention can generally be prepared by a method which comprises at least one step of suitably linking the one or more immunoglobulin single variable domains of the invention to the one or more further groups, residues, moieties or binding units, optionally via one or more suitable linkers, so as to provide the compound, construct or polypeptide of the invention. Polypeptides of the invention can also be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a polypeptide of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide of the invention. Such methods can be performed in a manner known *per se*, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

The process of designing/selecting and/or preparing a compound, construct or polypeptide of the invention, starting from an amino acid sequence of the invention, is also referred to herein as "*formatting*" said amino acid sequence of the invention; and an amino acid of the invention that is made part of a compound, construct or polypeptide of the invention is said to be "*formatted*" or to be "*in the format of*" said compound, construct or polypeptide of the invention. Examples of ways in which an amino acid sequence of the invention can be formatted and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted immunoglobulin single variable domains or polypeptides form a further aspect of the invention.

For example, such further groups, residues, moieties or binding units may be one or more additional immunoglobulin single variable domains, such that the compound or construct is a (fusion) protein or (fusion) polypeptide. In a preferred but non-limiting aspect, said one or more other groups, residues, moieties or binding units are immunoglobulin sequences. Even more preferably, said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, immunoglobulin single variable domains that are suitable for use as a domain antibody, single domain antibodies, immunoglobulin single variable domains (ISVs) that are suitable for use as a single domain antibody, "dAb"'s, immunoglobulin single variable domains that are suitable for use as a dAb, or Nanobodies. Alternatively, such groups, residues, moieties or binding units may for example be chemical groups, residues, moieties, which may or may not by themselves be biologically and/or pharmacologically active. For example, and without limitation, such groups may be linked to the one or more immunoglobulin single variable domains or polypeptides of the invention so as to provide a "derivative" of an ISV or polypeptide of the invention, as further described herein.

Also within the scope of the present invention are compounds or constructs, which comprise or essentially consist of one or more derivatives as described herein, and optionally further comprise one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers. Preferably, said one or more other groups, residues, moieties or binding units are immunoglobulin single variable domains. In the compounds or constructs described above, the one or more immunoglobulin single variable domains of the invention and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, moieties or binding units are immunoglobulin single variable domains, the linkers may also be immunoglobulin single variable domains, so that the resulting compound or construct is a fusion protein or fusion polypeptide.

In some embodiments, the polypeptides comprise at least two or more immunoglobulin single variable domains disclosed herein. In some embodiments, the polypeptides essentially consist of two or more immunoglobulin single variable domains disclosed herein. A polypeptide that "essentially consists of" two or more immunoglobulin single variable domains, is a polypeptide that in addition to the two or more immunoglobulin single variable domains disclosed herein does not have additional immunoglobulin single variable domains. For instance, a polypeptide that essentially consists of two immunoglobulin single variable domains does not include any additional immunoglobulin single variable domains. However, it should be appreciated that a polypeptide that essentially consists of two or more immunoglobulin single variable domains may include additional functionalities, such as a label, a toxin, one or more linkers, a binding sequence, etc. These additional functionalities include both amino acid based and non-amino acid based groups. In some embodiments, the polypeptides consist of one or more immunoglobulin single variable domains disclosed herein. It should be appreciated that the terms "polypeptide construct" and "polypeptide" can be used interchangeably herein (unless the context clearly dictates otherwise).

In some embodiments, the polypeptides include multivalent or multispecific constructs comprising immunoglobulin single variable domains disclosed herein. In some embodiments, the polypeptides comprise one or more antibody based-scaffolds and/or non-antibody based scaffolds disclosed herein. In some embodiments, the polypeptides comprise a serum binding protein moiety. In some embodiments, the serum binding protein moiety is an immunoglobulin single variable domain. In some embodiments, the immunoglobulin single variable domain is a Nanobody.

It will be appreciated that the order of the building blocks, such as e.g. a first building block, a second building block, a third building block etc., on the polypeptide (orientation) can be chosen according to the needs of the person skilled in the art, as well as the relative affinities which may depend on the location of these building blocks in the polypeptide. Whether the polypeptide comprises a linker, is a matter of design choice. However, some orientations, with or without linkers, may provide preferred binding characteristics in comparison to other orientations. For instance, the order of a first and a second building block in the polypeptide of the invention can be (from N-terminus to C-terminus): (i) first building block (e.g. a first ISV such as a first Nanobody) - [linker] - second building block (e.g. a second ISV such as a second Nanobody); or (ii) second building block (e.g. a second ISV such as a second Nanobody) - [linker] - first building block (e.g. a first ISV such as a first Nanobody); (wherein the linker is optional). All orientations are encompassed by the invention. Polypeptides that contain an orientation of building blocks that provides desired (binding) characteristics can be easily identified by routine screening, for instance as exemplified in the experimental section.

The first immunoglobulin single variable domain (ISV) of the polypeptide of the invention has high affinity for / binds to an effector cell, preferably the TCR of said effector cell, and even more preferably the constant domain of the T cell receptor (TCR).

An effector cell is a cell comprising a TCR complex, preferably an immune cell, such as a T cell, preferably a $CD4^+$ T-helper cell (also known as CD4 cell, T-helper cell or T4 cell), more preferably a Cytotoxic T cell (also known as T_C cell, CTL or $CD8^+$ T cells) or Natural Killer T cells (NKT cells). In some embodiments, the cell is present *in vivo*. In some embodiments, the cell is present *in vitro*. The effector cell of the invention relates in particular to mammalian cells, preferably to primate cells, and even more preferably to human cells.

As used herein, the terms "TCR complex" or "αβ TCR–CD3 complex" refers to the T cell receptor complex presented on the surface of T cells (see Kuhns et al. 2006, Immunity 24: 133-139). The TCR complex is composed of six different type I single-spanning transmembrane proteins: the TCRα and TCRβ chains that form the TCR heterodimer responsible for ligand recognition, and the non-covalently associated CD3γ, CD3δ, CD3ε and ζ chains, which bear cytoplasmic sequence motifs that are phosphorylated upon receptor activation and recruit a large number of signalling components. Both α and β chains of the T cell receptor consist of a constant domain and a variable domain. The sequences for the human CD3 and the human TCRα/β constant domains are provided in Table A-6 (SEQ ID NOs: 344-349; cf. UniProt identifiers: CD3 delta: P04234, CD3 gamma: P09693, CD3 epsilon: P07766, CD3 zeta: P20963, TCR alpha: P01848 and TCR beta: related to P01850).

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said first ISV binds to the constant domain of a T cell receptor α (TCR-α) (SEQ ID NO: 348) and/or the constant domain of the T cell receptor β (TCR-β) (SEQ ID NO: 349), or polymorphic variants or isoforms thereof.

Alternatively, the present invention relates to a polypeptide as described herein, wherein said first ISV binds to the constant domain of a T cell receptor α (TCR-α) (SEQ ID NO: 484) and/or the constant domain of the T cell receptor β (TCR-β) (SEQ ID NO: 485), or polymorphic variants or isoforms thereof.

Isoforms are alternative protein sequences that can be generated from the same gene by a single or by the combination of biological events such as alternative promoter usage, alternative splicing, alternative initiation and ribosomal frameshifting, all as known in the art.

"T cell activation" as used herein refers to one or more cellular response(s) of a T cell, e.g. a cytotoxic T cell, such as selected from: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, expression of activation markers, and redirected target cell lysis.

The polypeptides of the invention are capable of inducing T cell activation. Suitable assays to measure T cell activation are known in the art described herein, for instance as described in WO 99/54440 or by Schlereth et al. 2005 (Cancer Immunol. Immunother. 20: 1-12), or as exemplified in the examples or below.

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said polypeptide induces T cell activation. Preferably, the polypeptide of the invention induces T cell activation only when said second and/or further ISV is bound to an antigen on a target cell.

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said T cell activation depends on presenting said polypeptide bound to said first antigen on a target cell to a T cell.

T cell activation by the polypeptides of the invention can be monitored by upregulation of CD69, CD25 and various cell adhesion molecules, de novo expression and/or release of cytokines (e.g., IFN-γ, TNF-α, IL-6, IL-2, IL-4 and IL-10), upregulation of granzyme and perforin expression, and/or cell proliferation, membrane blebbing, activation of procaspases 3 and/or 7, fragmentation of nuclear DNA and/or cleavage of caspase substrate poly (ADPribose) polymerase. Preferably, redirected lysis of target cells by multispecific polypeptides is independent of T cell receptor specificity, presence of MHC class I and/or β2 microglobulin, and/or of any co-stimulatory stimuli.

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said T cell activation is independent from MHC recognition.

The polypeptides of the invention show redirected lysis *in vitro* with previously unstimulated peripheral polyclonal $CD8^+$- and $CD4^+$-positive T cells. The redirected lysis of target cells via the recruitment of T cells by the polypeptides of the invention involves cytolytic synapse formation and delivery of perforin and granzymes. Cell lysis by T cells has been described, e.g. by Atkinson and Bleackley 1995 (Crit. Rev. Immunol 15(3-4):359-384). Preferably, the engaged T cells are capable of serial target cell lysis, and are not affected by immune escape mechanisms interfering with peptide antigen processing and presentation, or clonal T cell differentiation (see, for example, WO 2007/042261). *In vitro*, redirected lysis is seen at low picomolar concentrations, suggesting that very low numbers of the polypeptides of the invention need to be bound to target cells for triggering T cells. As demonstrated in the examples, the low effector to target ratio might be indicative for serial target cell lysis. Accordingly, the present invention relates to potent polypeptides. Preferably, the polypeptide of the invention mediates killing of target cells, e.g. cancer cells, such as stimulating T cells in pore formation and delivering pro-apoptotic components of cytotoxic T cell granules.

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said T cell activation causes one or more cellular response of said T cell, wherein said cellular response is selected from the group consisting of proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, expression of activation markers and redirected target cell lysis.

As used herein, the term "potency" is a measure of the biological activity of an agent, such as a polypeptide, ISV or Nanobody. Potency of an agent can be determined by any suitable method known in the art, such as for instance as described in the experimental section. Cell culture based potency assays are often the preferred format for determining biological activity since they measure the physiological response elicited by the agent and can generate results within a relatively short period of time. Various types of cell based assays, based on the mechanism of action of the product, can be used, including but not limited to proliferation assays, cytotoxicity assays, cell killing assays, reporter gene assays, cell surface receptor binding assays, and assays to measure induction/inhibition of functionally essential proteins or other signal molecules (such as phosphorylated proteins, enzymes, cytokines, cAMP and the like), Ramos B cell depletion model, T cell mediated tumour cell killing assay (for instance as set out in the Examples section), all well known in the art. Results from cell based potency assays can be expressed as "relative potency" as determined by comparison of the multispecific polypeptide of the invention to the response obtained for the corresponding reference monovalent ISV, e.g. a polypeptide comprising only one ISV or one Nanobody, optionally further comprising an irrelevant Nanobody (cf. experimental section).

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said T cell activation causes inhibition of an activity of said target cell, such as to delay or minimize the spread of the target cell, to inhibit or delay growth and/or proliferation of the target cell, and/or to kill the target cell (e.g., cause regression of the disorder and/or symptoms) by more than about 10%, such as 20%, 30%, or 40% or even more than 50%, such as more than 60%, such as 70%, 80%, or even more than 90%, such as 100%.

The first building block, ISV, Nanobody or VHH of the invention has a high affinity for its -- the constant domain of TCR -- target. The first building block, ISV or Nanobody of the invention may for example be directed against an antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of said first target. The first building block, e.g. the first ISV, Nanobody or VHH, is preferably chosen for its high affinity for its target per se, disregarding the influence of any avidity effects.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said first ISV binds to the constant domain of the T cell receptor (TCR) with an average KD value of between 100 nM and 10 pM, such as at an average KD value of 90 nM or less, even more preferably at an average KD value of 80 nM or less, such as less than 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20 pM, or even less, such as less than 10 pM. Preferably, the KD is determined by Kinexa, BLI or SPR, for instance as determined by Proteon. For instance, said KD is determined as set out in the Examples section.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said first ISV has a high affinity when measured as a monovalent. Preferably said average KD is measured by surface plasmon resonance (SPR) on recombinant protein.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said polypeptide has a dissociation constant (K_D) to (or for binding) said TCR selected from the group consisting of: at most about 10^{-5} M, at most about 10^{-6} M, at most about 10^{-7} M, at most about 10^{-8} M, at most about 10^{-9} M, at most about 10^{-10} M, at most about 10^{-11} M, and at most about 10^{-12} M, preferably as measured by surface plasmon resonance.

The present invention also relates to a polypeptide as described herein, wherein said first ISV binds to said TCR with an EC50 value of between 100 nM and 1 pM, such as at an average EC50 value of 100 nM or less, even more preferably at an average EC50 value of 90 nM or less, such as less than 80, 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM or even less, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5 pM, or even less, such as less than 4 pM.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said average KD is determined by FACS, Biacore, ELISA, on a monovalent first ISV, such as a Nanobody, or a polypeptide comprising a monovalent first ISV, such as a Nanobody, for instance said EC50 is determined as set out in the Examples section.

It has been shown in the examples that the KD correlates well with the EC50.

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said polypeptide has an on rate constant (Kon) to (or for binding) said TCR selected from the group consisting of at least about 10^2 $M^{-1}s^{-1}$, at least about 10^3 $M^{-1}s^{-1}$, at least about 10^4 $M^{-1}s^{-1}$, at least about 10^5 $M^{-1}s^{-1}$, at least about 10^6 $M^{-1}s^{-1}$, 10^7 $M^{-1}s^{-1}$, at least about 10^8 $M^{-1}s^{-1}$, at least about 10^9 $M^{-1}s^{-1}$, and at least about 10^{10} $M^{-1}s^{-1}$, preferably as measured by surface plasmon resonance or as performed in the examples section.

In an embodiment, the present invention relates to a polypeptide as described herein, wherein said polypeptide has an off rate constant (Koff) to (or for binding) said TCR selected from the group consisting of at most about $10^{-3}s^{-1}$, at most about $10^{-4}s^{-1}$, at most about $10^{-5}s^{-1}$, at most about $10^{-6}s^{-1}$, at most about $10^{-7}s^{-1}$, at most about $10^{-8}s^{-1}$, at most about $10^{-9}s^{-1}$, and at most about $10^{-10}s^{-1}$, preferably as measured by surface plasmon resonance or as performed in the examples section.

Amino acid sequence modifications of the binding molecules, ISVs, or polypeptides described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody or ISV. Amino acid sequence variants of the binding molecules, ISVs, or polypeptides are prepared by introducing appropriate nucleotide changes into the binding molecules, ISVs, or polypeptides nucleic acid, or by peptide synthesis.

Such modifications include, for example, deletions from, and/or insertions into, and/or substitutions of, residues within the amino acid sequences of the binding molecules, ISVs or polypeptides. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the binding molecules, such as changing the number or position of glycosylation sites. Preferably, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids may be substituted in a CDR, while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be substituted in the framework regions (FRs). The substitutions are preferably conservative substitutions as described herein. Additionally or alternatively, 1, 2, 3, 4, 5, or 6 amino acids may be inserted or deleted in each of the CDRs (of course, dependent on their length), while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be inserted or deleted in each of the FRs.

A useful method for identification of certain residues or regions of the binding molecules, ISVs or polypeptides, that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells 1989 (Science 244: 1081-1085). Here, a residue or group of target residues within the binding molecule is/are identified (e.g. charged residues such as Arg, Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the epitope. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se needs not to be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at a target codon or region and the expressed binding molecule variants are screened for the desired activity.

Preferably, amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues to polypeptides containing a hundred or more residues.

Another type of variant is an amino acid substitution variant. These variants have preferably at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues in the binding molecule, ISV or polyptide replaced by a different residue. The sites of greatest interest for substitution mutagenesis include the CDRs, in particular the hypervariable regions, but FR alterations are also contemplated. For example, if a CDR sequence encompasses 6 amino acids, it is envisaged that one, two or three of these amino acids are substituted. Similarly, if a CDR sequence encompasses 15 amino acids it is envisaged that one, two, three, four, five or six of these amino acids are substituted.

Generally, if amino acids are substituted in one or more or all of the CDRs, it is preferred that the then-obtained "substituted" sequence is at least 60%, more preferably 65%, even more preferably 70%, particularly preferably 75%, more particularly preferably 80% or even more than 90% identical to the "original" CDR sequence. This means that it is dependent of the length of the CDR to which degree it is identical to the "substituted" sequence. For example, a CDR having 5 amino acids is preferably 80% identical to its substituted sequence in order to have at least one amino acid substituted. Accordingly, the CDRs of the binding molecule may have different degrees of identity to their substituted sequences, e.g., CDR1 may have 80%, while CDR3 may have 90%.

Preferred substitutions (or replacements) are conservative substitutions. However, any substitution (including non-conservative substitution or one or more from the "exemplary substitutions" listed in Table B-1 below) is envisaged as long as the polypeptide retains its capability to bind to the constant domain of the T cell receptor (TCR) present on a T cell via the first ISV and to a first antigen on a target cell via the second ISV and/or its CDRs have an identity to the then substituted sequence (at least 60%, more preferably 65%, even more preferably 70%, particularly preferably 75%, more particularly preferably 80% identical to the "original" CDR sequence).

Conservative substitutions are shown in Table B-1 below.

As indicated before, only after rigorous immunization and screening and selection methods, the present inventors were able to identify ISVs binding to the constant domains of TCR. Accordingly, the present invention relates to polypeptides comprising a first ISV chosen from the group consisting of SEQ ID NOs: 1-118 (cf. Table A-4). Sequence analysis further demonstrated that all ISVs binding to TCR comprised a very similar CDR3. Accordingly, the present invention relates to a polypeptide according to the invention in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which

CDR3 has the amino acid sequence $X_1SR X_2X_3PYX_4Y$, in which X_1 is F, Y, G, L or K, X_2 is I or L, X_3 is Y or W, and X_4 is D, N or S.

Sequence analysis further revealed that there are only a limited number of sequence variations in the CDRs (cf. Example 4.2 and Tables A-1 to A-3).

Table B-1: Amino Acid Substitutions

Original	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val, leu, ile	val
Arg (R)	lys, gln, asn	lys
Asn (N)	gln, his, asp, lys, arg	gln
Asp (D)	glu, asn	glu
Cys (C)	ser, ala	ser
Gln (Q)	asn, glu	asn
Glu (E)	asp, gln	asp
Gly (G)	ala	ala
His (H)	asn, gln, lys, arg	arg
Ile (I)	leu, val, met, ala, phe	leu
Leu (L)	norleucine, ile, val, met, ala	ile
Lys (K)	arg, gln, asn	arg
Met (M)	leu, phe, ile	leu
Phe (F)	leu, val, ile, ala, tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr, phe	tyr
Tyr (Y)	trp, phe, thr, ser	phe
Val (V)	ile, leu, met, phe, ala	leu

Accordingly, the present invention relates to a polypeptide as described herein, wherein said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
   (a) SEQ ID NOs: 119-133; or
   (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123 or with any of SEQ ID NOs: 119-133; and/or
(ii) CDR2 is chosen from the group consisting of:
   (c) SEQ ID NOs: 134-163; or
   (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153 or with any of SEQ ID NOs: 134-163; and/or
(iii) CDR3 is chosen from the group consisting of:
   (e) SEQ ID NOs: 164-174; or
   (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170 or with any of SEQ ID NOs: 164-174.

Further preferred CDR sequences are depicted in Table A-4.

Generally, the combinations of CDR's listed in Table A-4 (i.e. those mentioned on the same line in Table A-4) are preferred. Thus, it is generally preferred that, when a CDR in an ISV is a CDR sequence mentioned in Table A-4 or suitably chosen from the group consisting of CDR sequences that have 4, 3, 2 or only 1 amino acid difference(s) with a CDR sequence listed in Table A-4, that at least one and preferably both of the other CDR's are suitably chosen from the CDR sequences that belong to the same combination in Table A-4 (i.e. mentioned on the same line in Table A-4) or are suitably chosen from the group consisting of CDR sequences that have 4, 3, 2 or only 1 amino acid difference(s) with the CDR sequence(s) belonging to the same combination.

Sequence analysis of the resulting binders further resulted in the identification of 3 distinct clusters. Corresponding alignments are provided (see Table A-1, Table A-2 and Table A-3). Clustering was based on sequence similarities and differences in CDR2 and CDR3. Cluster A is the most prominent comprising 104 clones (SEQ ID NOs: 1-104), cluster B comprises 11 clones (SEQ ID NOs: 105-115), and cluster C is represented by only 3 clones (SEQ ID NOs: 116-118). The clustering based on the structural similarities and differences in the amino acid sequence translated into functional similarities and differences as revealed by the examples. Representatives of all clusters were isolated based on high affinity binding to the constant domain of the TCR (Examples 3 & 4) and human T cell activation (Example 4.2). In general cluster A representatives demonstrated the best EC50 values. In addition, cluster A representatives were cross-reactive with the constant domain of cynomolgus TCR (cf. Example 18). Although cluster C representatives had somewhat less favourable EC50 values than cluster B representatives, cluster C representatives had lower IC50 values in a flow cytometry based T cell mediated Ramos killing assay (cf. Example 10).

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 123; and
(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 123, wherein
    - at position 2 the D has been changed into A, S, E or G;
    - at position 4 the H has been changed into Y;
    - at position 5 the K has been changed into L;
    - at position 6 the I has been changed into L;
    - at position 8 the F has been changed into I or V; and/or
    - at position 10 the G has been changed into S.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 153; and
(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 153, wherein
    - at position 1 the H has been changed into T or R;
    - at position 3 the S has been changed into T or A;
    - at position 5 the G has been changed into S or A;
    - at position 7 the Q has been changed into D, E, T, A or V;
    - at position 8 the T has been changed into A or V; and/or
    - at position 9 the D has been changed into A, Q, N, V or S.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 170; and

(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 170, wherein
  - at position 1 the F has been changed into Y, L or G;
  - at position 4 the I has been changed into L;
  - at position 5 the Y has been changed into W; and/or
  - at position 8 the D has been changed into N or S.

In an embodiment, the invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
  (a) SEQ ID NOs: 119-123, 125-127, 129, 132 and 133; and
  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123; and/or

(ii) CDR2 is chosen from the group consisting of:
  (c) SEQ ID NOs: 134-141, 143-144, 146-156, 159-163; and
  (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153; and/or

(iii) CDR3 is chosen from the group consisting of:
  (e) SEQ ID NOs: 164-166, 169-171, 173-174; and
  (f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170.

In an embodiment, the invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 123, CDR2 is represented by SEQ ID NO: 153, and CDR3 is represented by SEQ ID NO: 170.

Nanobodies of cluster B show relatively limited sequence variability in the CDRs.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 124; and
(b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein

- at position 2 the E has been changed into Q; and/or
- at position 6 the I has been changed into V.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 145; and
(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein
   - at position 9 the N has been changed into D.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 167; and
(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein
   - at position 4 the L has been changed into I.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
   (a) SEQ ID NOs: 124, 128 and 131; and
   (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 124; and/or
(ii) CDR2 is chosen from the group consisting of:
   (c) SEQ ID NOs: 142 and 145; and
   (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 145; and/or
(iii) CDR3 is chosen from the group consisting of:
   (e) SEQ ID NOs: 167 and 168; and
   (f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 167.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 124, CDR2 is represented by SEQ ID NO: 145, and CDR3 is represented by SEQ ID NO: 167.

In Cluster C, the sequence variation is even more limited than within the other clusters.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 157; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein

- at position 8 the T has been changed into I.

Accordingly, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of

  (a) SEQ ID NO: 130; and

  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 130; and/or

(ii) CDR2 is chosen from the group consisting of:

  (c) SEQ ID NOs: 157-158; and

  (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 157; and/or

(iii) CDR3 is chosen from the group consisting of:

  (e) SEQ ID NO: 172; and

  (f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 172.

In an aspect, the present invention relates to a polypeptide as described herein, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 130, CDR2 is represented by SEQ ID NO: 157, and CDR3 is represented by SEQ ID NO: 172.

The second immunoglobulin single variable domain (ISV) of the polypeptide of the invention has a high affinity for / binds to an antigen on a target cell, preferably a cancer cell. A “target cell” as referred to herein, is a cell that presents a particular antigen on its surface. In a preferred aspect, the "target cell" is a cancer cell.

The membrane (also called plasma membrane or phospholipid bilayer) surrounds the cytoplasm of a cell, which is the outer boundary of the cell, i.e. the membrane is the surface of the cell. This membrane serves to separate and protect a cell from its surrounding environment and is made mostly from a double layer of phospholipids. Embedded within this membrane is a variety of protein molecules, such as channels, pumps and cellular receptors. Since the membrane is fluid, the protein molecules can travel within the membrane. The term "antigen on a target cell" as used herein denotes a molecule, which is displayed on the surface of a cell. In most cases, this molecule will be located in or on the plasma membrane of the cell such that at least part of this molecule remains accessible from outside the cell in tertiary form. A non-limiting example of a cell surface molecule, which is located in the plasma membrane, is a transmembrane protein comprising, in its tertiary conformation, regions of hydrophilicity and hydrophobicity. Here, at least one hydrophobic region allows the cell surface molecule to be embedded, or inserted in the hydrophobic plasma membrane of the cell while the hydrophilic regions extend on either side of the plasma membrane into the cytoplasm and extracellular space, respectively.

Said antigen can be any target on a cell, e.g. a tumour antigen. In a preferred embodiment, said antigen is specific for said target cell, e.g. cancer cell, such as a tumour associated antigen (TAA) on said cancer cell.

The term "tumour antigen" as used herein may be understood as those antigens that are presented on tumour cells. These antigens can be presented on the cell surface with an extracellular part, which is often combined with a transmembrane and cytoplasmic part of the molecule. These antigens can sometimes be presented only by tumour cells and never by a normal or healthy cell. Tumour antigens can be exclusively expressed on tumour cells or might represent a tumour specific mutation compared to normal cells. In this case, they are called tumour-specific antigens. However, this will not be the case generally. More common are antigens that are presented by tumour cells and normal cells, and they are called "tumour-associated antigens (TAA)". These tumour-associated antigens can be overexpressed on tumour cells compared to normal cells or are better accessible for antibody binding in tumour cells due to the less compact structure of the tumour tissue compared to normal tissue. TAA are preferably antigens that are expressed on cells of particular tumours, but that are preferably not expressed in normal cells. Often, TAA are antigens that are normally expressed in cells only at particular points in an organism's development (such as during fetal development) and that are being inappropriately expressed in the organism at the present point of development, or are antigens not expressed in normal tissues or cells of an organ now expressing the antigen.

In an embodiment, said first antigen on a target cell is a tumour antigen, preferably a tumour associated antigen (TAA).

In an embodiment, said second antigen on a target cell is a tumour antigen, preferably a tumour associated antigen (TAA).

In an embodiment, said antigen is present more abundantly on a cancer cell than on a normal cell. The antigen on a target cell is preferably a tumor-associated antigen (TAA). Preferred TAAs include MART-1, carcinoembryonic antigen ("CEA"), gp100, MAGE-1, HER-2, CD20, Lewis^{Y} antigens, Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Fibroblast Activation Protein (FAP), CD19 and CD33.

Cell surface antigens that are preferentially expressed on AML LSC compared with normal hematopoietic stem cells, and thus preferred as TAA, include CD123, CD44, CLL-1, CD96, CD47, CD32, CXCR4, Tim-3 and CD25.

Other tumor-associated antigens suitable as an antigen on a target cell for binding by the second ISV within the polypeptides of the invention include: TAG-72, Ep-CAM, PSMA, PSA, glycolipids such as GD2 and GD3.

The TAA of the invention include also hematopoietic differentiation antigens, *i.e.* glycoproteins usually associated with cluster differentiation (CD) grouping, such as CD4, CD5, CD19, CD20, CD22, CD33, CD36, CD45, CD52, CD69 and CD147; growth factor receptors, including HER2, ErbB3 and ErbB4; Cytokine receptors, including Interleukin-2 receptor gamma chain (CD132 antigen), Interleukin-10 receptor alpha chain (IL-10R-A), Interleukin-10 receptor beta chain (IL-10R-B), Interleukin-12 receptor beta-1 chain (IL-12R-beta1), Interleukin-12 receptor beta-2 chain (IL-12 receptor beta-2), Interleukin-13 receptor alpha-1 chain (IL-13R-alpha-1) (CD213a1 antigen), Interleukin-13 receptor alpha-2 chain (Interleukin-13 binding protein), Interleukin-17 receptor (IL-17 receptor), Interleukin-17B receptor (IL-17B receptor), Interleukin 21 receptor precursor (IL-21R), Interleukin-1 receptor type I (IL-1R-1) (CD121a), Interleukin-1 receptor type II (IL-1R-beta) (CDw121b), Interleukin-1 receptor antagonist protein (IL-1ra), Interleukin-2 receptor alpha chain (CD25 antigen), Interleukin-2 receptor beta chain (CD122 antigen), Interleukin-3 receptor alpha chain (IL-3R-alpha) (CD123 antigen); as well as others, such as CD30, IL23R, IGF-1R, IL5R, IgE, CD248 (endosialin), CD44v6, gpA33, Ron, Trop2, PSCA, claudin 6, claudin 18.2, CLEC12A, CD38, ephA2, c-Met, CD56, MUC16, EGFRvIII, AGS-16, CD27L, Nectin-4, SLITRK6, mesothelin, folate receptor, tissue factor, axl, glypican-3, CA9, Cripto, CD138, CD37, MUC1, CD70, gastrin releasing peptide receptor, PAP, CEACAM5, CEACAM6, CXCR7, N-cadherin, FXYD2 gamma a, CD21, CD133, Na/K-ATPase, mIgM (membrane-bound IgM), mIgA (membrane-bound IgA), Mer, Tyro2, CD120, CD95, CA 195, DR5, DR6, DcR3 and CAIX.

Accordingly the present invention relates to a polypeptide as described herein, wherein said TAA is chosen from the group consisting of Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Fibroblast Activation Protein (FAP), MART-1, carcinoembryonic antigen ("CEA"), gp100, MAGE-1, HER-2, Lewis^{Y} antigens, CD123, CD44, CLL-1, CD96, CD47, CD32, CXCR4, Tim-3, CD25, TAG-72, Ep-CAM, PSMA, PSA, GD2, GD3, CD4, CD5, CD19, CD20, CD22, CD33, CD36, CD45, CD52, CD147; growth factor receptors, including ErbB3 and ErbB4; Cytokine receptors, including Interleukin-2 receptor gamma chain (CD132 antigen), Interleukin-10 receptor alpha chain (IL-10R-A), Interleukin-10 receptor beta chain (IL-10R-B), Interleukin-12 receptor beta-1 chain (IL-12R-beta1), Interleukin-12 receptor beta-2 chain (IL-12 receptor beta-2), Interleukin-13 receptor alpha-1 chain (IL-13R-alpha-1) (CD213a1 antigen), Interleukin-13 receptor alpha-2 chain (Interleukin-13 binding protein), Interleukin-17 receptor (IL-17 receptor), Interleukin-17B receptor (IL-17B receptor), Interleukin 21 receptor precursor (IL-21R), Interleukin-1 receptor type I (IL-1R-1) (CD121a), Interleukin-1 receptor type II (IL-1R-beta) (CDw121b), Interleukin-1 receptor antagonist protein (IL-1ra), Interleukin-2 receptor alpha chain (CD25 antigen), Interleukin-2 receptor beta chain (CD122 antigen), Interleukin-3 receptor alpha chain (IL-3R-alpha) (CD123 antigen), CD30, IL23R, IGF-1R, IL5R, IgE, CD248 (endosialin), CD44v6, gpA33, Ron, Trop2, PSCA, claudin 6, claudin 18.2, CLEC12A, CD38, ephA2, c-Met, CD56, MUC16, EGFRvIII, AGS-16, CD27L, Nectin-4, SLITRK6, mesothelin, folate receptor, tissue factor, axl, glypican-3, CA9, Cripto, CD138, CD37, MUC1, CD70, gastrin releasing peptide receptor, PAP, CEACAM5, CEACAM6, CXCR7, N-cadherin, FXYD2 gamma a, CD21, CD133, Na/K-ATPase, mIgM (membrane-bound IgM), mIgA (membrane-bound IgA), Mer, Tyro2, CD120, CD95, CA 195, DR5, DR6, DcR3 and CAIX, and related polymorphic variants and isoforms, preferably said TAA is CD20 (UniProt 11836), HER2 (Uniprot P04626), EGFR, or CEACAM, polymorphic variants and/or isoforms thereof.

The second building block, ISV, Nanobody or VHH of the invention has a high affinity for its antigen. The second building block, ISV or Nanobody of the invention may, for example, be directed against an antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of said antigen on a target cell.

The target cell of the invention relates in particular to mammalian cells, preferably to primate cells, and even more preferably to human cells. The target cell is preferably a hyperproliferative cell such as e.g. a cancer cell.

The present invention relates to a polypeptide as described herein, wherein said second or further ISV binds to an antigen on a target cell with an average KD value of between 100 nM and 10 pM, such as at an average KD value of 90 nM or less, even more preferably at an average KD value of 80 nM or less, such as less than 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20 pM, or even less, such as less than 10 pM. Preferably, the KD is determined by Kinexa, BLI or SPR, for instance as determined by a Proteon.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said second or further ISV has a high affinity for its antigen when measured as a monovalent.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said average KD is measured by surface plasmon resonance (SPR) and/or KinExA or Proteon, for instance on recombinant protein, such as described in the Examples section.

The present invention also relates to a polypeptide as described herein, wherein said second or further ISV binds to an antigen on a target cell with an EC50 value of between 100 nM and 1 pM, such as at an average EC50 value of 100 nM or less, even more preferably at an average EC50 value of 90 nM or less, such as less than 80, 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM or even less, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5 pM, or even less, such as less than 4 pM.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said average EC50 is determined by FACS or ELISA, on a monovalent second ISV, such as a Nanobody, or a polypeptide comprising a monovalent second ISV, such as a Nanobody.

It has been shown in the examples that the KD correlates well with the EC50.

Simultaneous targeting of multiple antigens can reduce the probability of generating tumour escape variants, because of which the therapeutic activity of T cell engaging strategy is improved. The present invention provides multispecific polypeptides which comprise a TCR ISV combined with immunoglobulin single variable domains against different (target) antigens (on a target cell). Preferred combinations of first and second antigens are provided below (it will be appreciated that the ISVs binding said antigens can be positioned in any order in the polypeptide of the invention):

first antigen	second antigen
EGFR (OMIM: 131550)	CD20 (OMIM: 112210)
EGFR (OMIM:131550)	CEA (OMIM: 114890)

EGFR (OMIM:131550)	HER2 (OMIM: 164870)
HER2 (OMIM: 164870)	CD20 (OMIM: 112210)
HER2 (OMIM: 164870)	CEA (OMIM: 114890)
CD20 (OMIM: 112210)	CEA (OMIM: 114890)

Similarly, simultaneous targeting of multiple epitopes, antigenic determinants, parts, domains, subunits or conformations of a protein or antigen on a target cell can reduce the probability of generating tumour escape variants, because of which the therapeutic activity of T cell engaging strategy is improved (cf. Example 22). The present invention provides polypeptides which comprise an anti-TCR ISV combined with immunoglobulin single variable domains against different epitopes, antigenic determinants, parts, domains, subunits or conformations of an antigen on a target cell (also referred to as biparatopic constructs). Preferred combinations of first and second TAA ISVs are provided below (it will be appreciated that the ISVs binding said antigens can be positioned in any order in the polypeptide of the invention):

TAA1 ISV	name	SEQ ID NO:	TAA2 ISV	name	SEQ ID NO:
EGFR-1	7D12	355	EGFR-2	9G08	352
HER2-1	5F07	350	HER2-2	47D05	351
CEA-1	CEA#1	353	CEA-2	CEA#5	354

The polypeptides and compositions of the present invention can be used for the prevention and/or treatment of diseases and disorders of the present invention (herein also “diseases and disorders of the present invention”) which include, but are not limited to cancer. The term "cancer" refers to the pathological condition in mammals that is typically characterized by dysregulated cellular proliferation or survival. Examples of cancer include, but are not limited to, carcinomas, gliomas, mesotheliomas, melanomas, lymphomas, leukemias, adenocarcinomas: breast cancer, ovarian cancer, cervical cancer, glioblastoma, multiple myeloma (including monoclonal gammopathy of undetermined significance, asymptomatic and symptomatic myeloma), prostate cancer, and Burkitt's lymphoma, head and neck cancer, colon cancer, colorectal cancer, non-small cell lung cancer, small cell lung cancer, cancer of the esophagus, stomach cancer, pancreatic cancer, hepatobiliary cancer, cancer of the gallbladder, cancer of the small intestine, rectal cancer, kidney cancer, bladder cancer, prostate cancer, penile cancer, urethral cancer, testicular cancer, vaginal cancer, uterine cancer, thyroid cancer, parathyroid cancer, adrenal cancer, pancreatic endocrine cancer, carcinoid cancer, bone cancer, skin cancer, retinoblastomas, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Kaposi's sarcoma, multicentric Castleman's disease or AIDS-associated primary effusion lymphoma, neuroectodermal tumors, rhabdomyosarcoma (see *e.g.*, Cancer, Principles and practice (DeVita et al.

eds 1997) for additional cancers); as well as any metastasis of any of the above cancers, as well as non-cancer indications such as nasal polyposis, as well as other disorders and diseases described herein.

For a general description of immunoglobulin single variable domains, reference is made to the further description below, as well as to the prior art cited herein. In this respect, it should however be noted that this description and the prior art mainly describes immunoglobulin single variable domains of the so-called "V_H3 class" (*i.e.,* immunoglobulin single variable domains with a high degree of sequence homology to human germline sequences of the V_H3 class such as DP-47, DP-51 or DP-29), which form a preferred aspect of this invention. It should, however, be noted that the invention in its broadest sense generally covers any type of immunoglobulin single variable domains and for example also covers the immunoglobulin single variable domains belonging to the so-called "V_H4 class" (*i.e.,* immunoglobulin single variable domains with a high degree of sequence homology to human germline sequences of the V_H4 class such as DP-78), as for example described in WO 07/118670.

Generally, immunoglobulin single variable domains (in particular V_{HH} sequences and sequence optimized immunoglobulin single variable domains) can in particular be characterized by the presence of one or more "*Hallmark residues*" (as described for example in Table B2) in one or more of the framework sequences (again as further described herein).

Table B-2: Hallmark Residues in VHHs

Position	Human V_H3	Hallmark Residues
11	L, V; predominantly L	L, S, V, M, W, F, T, Q, E, A, R, G, K, Y, N, P, I; preferably L
37	V, I, F; usually V	F[1], Y, V, L, A, H, S, I, W, C, N, G, D, T, P, preferably F[1] or Y
44[8]	G	E[3], Q[3], G[2], D, A, K, R, L, P, S, V, H, T, N, W, M, I; preferably G[2], E[3] or Q[3];most preferably G[2] or Q[3].
45[8]	L	L[2], R[3], P, H, F, G, Q, S, E, T, Y, C, I, D, V; preferably L[2] or R[3]
47[8]	W, Y	F[1], L[1] or W[2] G, I, S, A, V, M, R, Y, E, P, T, C, H, K, Q, N, D; preferably W[2] , L[1] or F[1]
83	R or K; usually R	R, K[5], T, E[5], Q, N, S, I, V, G, M, L, A, D, Y, H; preferably K or R; most preferably K

84	A, T, D; predominantly A	P[5], S, H, L, A, V, I, T, F, D, R, Y, N, Q, G, E; preferably P
103	W	W[4], R[6], G, S, K, A, M, Y, L, F, T, N, V, Q, P[6], E, C; preferably W
104	G	G, A, S, T, D, P, N, E, C, L; preferably G
108	L, M or T; predominantly L	Q, L[7], R, P, E, K, S, T, M, A, H; preferably Q or L[7]

Notes:

(1) In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.

(2) Usually as GLEW at positions 44-47.

(3) Usually as KERE or KQRE at positions 43-46, e.g. as KEREL, KEREF, KQREL, KQREF, KEREG, KQREW or KQREG at positions 43-47. Alternatively, also sequences such as TERE (for example TEREL), TQRE (for example TQREL), KECE (for example KECEL or KECER), KQCE (for example KQCEL), RERE (for example REREG), RQRE (for example RQREL, RQREF or RQREW), QERE (for example QEREG), QQRE, (for example QQREW, QQREL or QQREF), KGRE (for example KGREG), KDRE (for example KDREV) are possible. Some other possible, but less preferred sequences include for example DECKL and NVCEL.

(4) With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.

(5) Often as KP or EP at positions 83-84 of naturally occurring V_{HH} domains.

(6) In particular, but not exclusively, in combination with GLEW at positions 44-47.

(7) With the proviso that when positions 44-47 are GLEW, position 108 is always Q in (non-humanized) V_{HH} sequences that also contain a W at 103.

(8) The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEW, DLEW, GIEW, ELEW, GPEW, EWLP, and GPER.

The immunoglobulins of the invention may also contain a C-terminal extension (X)n (in which n is 1 to 10, preferably 1 to 5, such as 1, 2, 3, 4 or 5 (and preferably 1 or 2, such as 1); and each X is an (preferably naturally occurring) amino acid residue that is independently chosen, and preferably independently chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L) or isoleucine (I)), for which reference is made to WO 12/175741 and WO 15/060643.

Apart from this and/or in addition, the immunoglobulin of the invention may have certain preferred amino acid residues at positions 11, 89, 110 and/or 112 as is described in further detail in WO 15/060643 (which is incorporated herein as reference).

Again, such immunoglobulin single variable domains may be derived in any suitable manner and from any suitable source, and may for example be naturally occurring V_{HH} sequences (*i.e.*, from a suitable species of Camelid, *e.g.*, llama) or synthetic or semi-synthetic VHs or VLs (*e.g.*, from human). Such immunoglobulin single variable domains may include “humanized” or otherwise “sequence optimized” VHHs, “camelized” immunoglobulin sequences (and in particular camelized heavy chain variable domain sequences, *i.e.,* camelized VHs), as well as human VHs, human VLs, camelid VHHs that have been altered by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing as further described herein. As mentioned herein, a particularly preferred class of immunoglobulin single variable domains of the invention comprises immunoglobulin single variable domains with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_{HH} domain, but that has been “humanized”, i.e. by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring V_{HH} sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a V_H domain from a conventional 4-chain antibody from a human being (e.g. indicated above). This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the further description herein and the prior art on humanization referred to herein. Again, it should be noted that such humanized immunoglobulin single variable domains of the invention can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring V_{HH} domain as a starting material.

Another particularly preferred class of immunoglobulin single variable domains of the invention comprises immunoglobulin single variable domains with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_H domain, but that has been “camelized”, i.e. by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_{HH} domain of a heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the description herein. Such “camelizing” substitutions are preferably inserted at amino acid positions that form and/or are present at the V_H-V_L interface, and/or at the so-called Camelidae hallmark residues, as defined herein (see also for example WO 94/04678 and Davies and Riechmann 1994 (FEBS letters 339: 285-290) and 1996 (Protein Engineering 9: 531-537)). Preferably, the V_H sequence that is used as a starting material or starting point for generating or designing the camelized immunoglobulin single variable domains is preferably a V_H sequence from a mammal, more preferably the V_H sequence of a human being, such as a V_H3 sequence. However, it should be noted that such camelized immunoglobulin single variable domains of the invention can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring V_H domain as a starting material.

For example, again as further described herein, both "humanization" and "camelization" can be performed by providing a nucleotide sequence that encodes a naturally occurring V_{HH} domain or V_H domain, respectively, and then changing, in a manner known per se, one or more codons in said nucleotide sequence in such a way that the new nucleotide sequence encodes a "humanized" or "camelized" immunoglobulin single variable domain of the invention, respectively. This nucleic acid can then be expressed in a manner known per se, so as to provide the desired immunoglobulin single variable domains of the invention. Alternatively, based on the amino acid sequence of a naturally occurring V_{HH} domain or V_H domain, respectively, the amino acid sequence of the desired humanized or camelized immunoglobulin single variable domains of the invention, respectively, can be designed and then synthesized *de novo* using techniques for peptide synthesis known per se. Also, based on the amino acid sequence or nucleotide sequence of a naturally occurring V_{HH} domain or V_H domain, respectively, a nucleotide sequence encoding the desired humanized or camelized immunoglobulin single variable domains of the invention, respectively, can be designed and then synthesized *de novo* using techniques for nucleic acid synthesis known per se, after which the nucleic acid thus obtained can be expressed in a manner known per se, so as to provide the desired immunoglobulin single variable domains of the invention.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said ISV is a Nanobody, a V_{HH}, a humanized V_{HH}, or a camelized V_H.

Generally, proteins or polypeptides that comprise or essentially consist of a single building block, single immunoglobulin single variable domain or single Nanobody will be referred to herein as "monovalent" proteins or polypeptides, as "monovalent constructs", as "monovalent building block", as "monovalent immunoglobulin single variable domain", or as "monovalent Nanobody", respectively.

In this respect, the present invention also relates to the monovalent building blocks that make up the polypeptides of the invention.

Accordingly, the present invention relates to an ISV or polypeptide that specifically binds the constant domain of the T cell receptor (TCR) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NOs: 119-133; or

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 119-133, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 134-163; or

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 134-163, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 164-174; or

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 164-174, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

As discussed above, ISVs were isolated that belong to different clusters, based on structural similarities and differences in CDR2 and CDR3.

Immunoglobulin single variable domains belonging to cluster A are represented by polypeptides according in which:

(i) CDR1 is chosen from the group consisting of:

  (a) SEQ ID NOs: 119-123, 125-127, 129, 132 and 133; and

  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(ii) CDR2 is chosen from the group consisting of:

  (c) SEQ ID NOs: 134-141, 143-144, 146-156, 159-163; and

  (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(iii) CDR3 is chosen from the group consisting of:

  (e) SEQ ID NOs: 164-166, 169-171, 173-174; and

  (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In another aspect, in the polypeptides belonging to cluster A, CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 123; and

(b) amino acid sequences that have 1, 2, or 3 amino acid difference(s) with SEQ ID NO: 123, wherein

  - at position 2 the D has been changed into A, S, E or G;

- at position 4 the H has been changed into Y;
- at position 5 the K has been changed into L;
- at position 6 the I has been changed into L;
- at position 8 the F has been changed into I or V; and/or
- at position 10 the G has been changed into S.

In another aspect, in the polypeptides belonging to cluster A, CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 153; and
(b) amino acid sequences that have 1, 2, 3, 4 or 5 amino acid difference(s) with SEQ ID NO: 153, wherein
    - at position 1 the H has been changed into T or R;
    - at position 3 the S has been changed into T or A;
    - at position 5 the G has been changed into S or A;
    - at position 7 the Q has been changed into D, E, T, A or V;
    - at position 8 the T has been changed into A or V; and/or
    - at position 9 the D has been changed into A, Q, N, V or S.

In another aspect, in the polypeptides belonging to cluster A, CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 170; and
(b) amino acid sequences that have 1, 2, or 3 amino acid difference(s) with SEQ ID NO: 170, wherein
    - at position 1 the F has been changed into Y, L or G;
    - at position 4 the I has been changed into L;
    - at position 5 the Y has been changed into W; and/or
    - at position 8 the D has been changed into N or S.

Accordingly, the present invention relates to an ISV or polypeptide that specifically binds the constant domain of the T cell receptor (TCR) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
    (a) SEQ ID NO: 123; and
    (b) amino acid sequences that have 1, 2, or 3 amino acid difference(s) with SEQ ID NO: 123, wherein
        - at position 2 the D has been changed into A, S, E or G;

- at position 4 the H has been changed into Y;
- at position 5 the K has been changed into L;
- at position 6 the I has been changed into L;
- at position 8 the F has been changed into I or V; and/or
- at position 10 the G has been changed into S;

and

(ii) CDR2 is chosen from the group consisting of:

(a) SEQ ID NO: 153; and

(b) amino acid sequences that have 1, 2, 3, 4 or 5 amino acid difference(s) with SEQ ID NO: 153, wherein

- at position 1 the H has been changed into T or R;
- at position 3 the S has been changed into T or A;
- at position 5 the G has been changed into S or A;
- at position 7 the Q has been changed into D, E, T, A or V;
- at position 8 the T has been changed into A or V; and/or
- at position 9 the D has been changed into A, Q, N, V or S;

and

(iii) CDR3 is chosen from the group consisting of:

(a) SEQ ID NO: 170; and

(b) amino acid sequences that have 1, 2, or 3 amino acid difference(s) with SEQ ID NO: 170, wherein

- at position 1 the F has been changed into Y, L or G;
- at position 4 the I has been changed into L;
- at position 5 the Y has been changed into W; and/or
- at position 8 the D has been changed into N or S.

In another aspect, the invention relates to a polypeptide in which CDR1 is represented by SEQ ID NO: 123, CDR2 is represented by SEQ ID NO: 153, and CDR3 is represented by SEQ ID NO: 170. Preferably the polypeptide is selected from any of SEQ ID NOs: 1 to 104.

Immunoglobulin single variable domains belonging to cluster B are represented by polypeptides according in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NOs: 124, 128 and 131; and

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 124, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 142 and 145; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 145, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 167 and 168; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 167, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In another aspect, in the polypeptides belonging to cluster B, CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 124; and

(b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein

- at position 2 the E has been changed into Q; and/or
- at position 6 the I has been changed into V.

In another aspect, in the polypeptides belonging to cluster B, CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 145; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein

- at position 9 the N has been changed into D.

In another aspect, in the polypeptides belonging to cluster B, CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 167; and
(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein
  - at position 4 the L has been changed into I.

Accordingly, the present invention relates to an ISV or polypeptide that specifically binds the constant domain of the T cell receptor (TCR) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
  (a) SEQ ID NO: 124; and
  (b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein
    - at position 2 the E has been changed into Q; and/or
    - at position 6 the I has been changed into V;

and

(ii) CDR2 is chosen from the group consisting of:
  (a) SEQ ID NO: 145; and
  (b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein
    - at position 9 the N has been changed into D;

and

(iii) CDR3 is chosen from the group consisting of:
  (a) SEQ ID NO: 167; and
  (b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein
    - at position 4 the L has been changed into I.

In another aspect, the invention relates to a polypeptide in which CDR1 is represented by SEQ ID NO: 124, CDR2 is represented by SEQ ID NO: 145, and CDR3 is represented by SEQ ID NO: 167. Preferably the polypeptide is selected from any of SEQ ID NOs: 105-115.

Immunoglobulin single variable domains belonging to cluster C are represented by polypeptides according in which:

(i) CDR1 is chosen from the group consisting of:
  (a) SEQ ID NO: 130; and
  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 130, provided that the polypeptide comprising the CDR1

with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 157-158; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 157, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NO: 172; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 172, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In another aspect, in the polypeptides belonging to cluster C, CDR1 is chosen from SEQ ID NO: 130.

In another aspect, in the polypeptides belonging to cluster C, CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 157; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein

- at position 8 the T has been changed into I.

In another aspect, in the polypeptides belonging to cluster C, CDR3 is chosen from SEQ ID NO: 172.

Accordingly, the present invention relates to an ISV or polypeptide that specifically binds the constant domain of the T cell receptor (TCR) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from SEQ ID NO: 130;

and

(ii) CDR2 is chosen from the group consisting of:

(a) SEQ ID NO: 157; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein

- at position 8 the T has been changed into I;

and

(iii) CDR3 is chosen from SEQ ID NO: 172.

In another aspect, the invention relates to a polypeptide in which CDR1 is represented by SEQ ID NO: 130, CDR2 is represented by SEQ ID NO: 157, and CDR3 is represented by SEQ ID NO: 172. Preferably the polypeptide is selected from any of SEQ ID NOs: 116-118.

In a further aspect, the invention relates to polypeptides that cross-block the binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides belonging to Cluster A, B or C.

Accordingly, the present invention relates to polypeptides that cross-block the binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides with SEQ ID NOs: 1-104.

In another aspect, the present invention relates to ISVs or polypeptides that cross-block the binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides with SEQ ID NOs: 105-115.

In yet another aspect, the present invention relates to ISVs or polypeptides that cross-block the binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides with SEQ ID NOs: 116-118.

In a further aspect, the invention relates to polypeptides that are cross-blocked from binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides belonging to Cluster A, B or C.

Accordingly, the present invention relates to polypeptides that are cross-blocked from binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides belonging to SEQ ID NOs: 1-104.

In another aspect, the present invention relates to polypeptides that are cross-blocked from binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides belonging to SEQ ID NOs: 105-115.

In another aspect, the present invention relates to polypeptides that are cross-blocked from binding to the constant domain of the T cell receptor (TCR) by at least one of the ISVs or polypeptides belonging to SEQ ID NOs: 116-118.

The present invention also relates to an ISV or polypeptide that specifically binds carcinoembryonic antigen (CEA) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
   (a) SEQ ID NO: 361 (GDTYGSYWMG); or
   (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 361, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(ii) CDR2 is chosen from the group consisting of:
   (c) SEQ ID NO: 363 (AINRGGGYTV); or
   (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NO: 363, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(iii) CDR3 is chosen from the group consisting of:
   (e) SEQ ID NO: 365 (SGVLGGLHEDWFNY); or
   (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NO: 365, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3

without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In another aspect, the invention relates to a polypeptide in which CDR1 is represented by SEQ ID NO: 361, CDR2 is represented by SEQ ID NO: 363, and CDR3 is represented by SEQ ID NO: 365. Preferred polypeptides include SEQ ID NOs: 353 and 354.

In a further aspect, the invention relates to polypeptides that cross-block the binding to carcinoembryonic antigen (CEA) by at least one of the ISVs or polypeptides with SEQ ID NOs: 353 or 354.

In a further aspect, the invention relates to polypeptides that are cross-blocked from binding to carcinoembryonic antigen (CEA) by at least one of the ISVs or polypeptides with SEQ ID NOs: 353 or 354.

The present invention also relates to an ISV or polypeptide that specifically binds CD20 and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
  (a) SEQ ID NO: 362 (GGTFSSYTMG); or
  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 362, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(ii) CDR2 is chosen from the group consisting of:
  (c) SEQ ID NO: 364 (EVRWGGVTT); or
  (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NO: 364, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance;

and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NO: 366 (VRQMYMTVVPDY); or

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NO: 366, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

In another aspect, the invention relates to a polypeptide in which CDR1 is represented by SEQ ID NO: 362, CDR2 is represented by SEQ ID NO: 364, and CDR3 is represented by SEQ ID NO: 366. A preferred polypeptide includes SEQ ID NO: 357.

In a further aspect, the invention relates to polypeptides that cross-block the binding to CD20 by the ISV or polypeptide with SEQ ID NO: 357.

In a further aspect, the invention relates to polypeptides that are cross-blocked from binding to CD20 by the ISV or polypeptide with SEQ ID NO: 357.

The invention further relates to compounds or constructs, and in particular proteins or polypeptides that comprise or essentially consist of one or more ISVs or polypeptides of the invention, and optionally further comprise one or more other groups, residues, moieties or binding units. As will become clear to the skilled person from the further disclosure herein, such further groups, residues, moieties, binding units or amino acid sequences may or may not provide further functionality to the polypeptide of the invention (and/or to the compound or construct in which it is present) and may or may not modify the properties of the polypeptide of the invention.

In a specific, but non-limiting aspect of the invention, which will be further described herein, the ISVs and polypeptides of the invention may have an increased half-life in serum (as further described herein) compared to the immunoglobulin single variable domain or polypeptide from which they have been derived. For example, an immunoglobulin single variable domain or polypeptide of the invention may be linked (chemically or otherwise) to one or more groups or moieties that extend the half-life (such as PEG), so as to provide a derivative of the ISV or polypeptide of the invention with increased half-life.

In a specific aspect of the invention, a compound or construct of the invention or a polypeptide of the invention may have an increased half-life, compared to the corresponding ISV or polypeptide of the invention. Some preferred, but non-limiting examples of such compounds, constructs and polypeptides will become clear to the skilled person based on the further disclosure herein, and for example comprise immunoglobulin single variable domains or polypeptides of the invention that have been chemically modified to increase the half-life thereof (for example, by means of pegylation); immunoglobulin single variable domains or polypeptides of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin); or constructs or polypeptides of the invention which comprise at least ISV or polypeptide of the invention that is linked to at least one moiety (and in particular at least one amino acid sequence) which increases the half-life of the ISV or polypeptide of the invention. Examples of ISVs or polypeptides of the invention which comprise such half-life extending moieties or immunoglobulin single variable domains will become clear to the skilled person based on the further disclosure herein; and for example include, without limitation, polypeptides in which the one or more immunoglobulin single variable domains or polypeptide of the invention are suitably linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, domain antibodies, immunoglobulin single variable domains that are suitable for use as a domain antibody, single domain antibodies, immunoglobulin single variable domains that are suitable for use as a single domain antibody, "dAb"'s, immunoglobulin single variable domains that are suitable for use as a dAb, or Nanobodies that can bind to serum proteins such as serum albumin (such as human serum albumin), serum immunoglobulins such as IgG, or transferrin; reference is made to the further description and references mentioned herein); ISVs or polypeptides in which an ISV or polypeptide of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more immunoglobulin single variable domains or polypeptide of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins, such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489, WO 08/068280, WO 09/127691 and WO 11/095545.

Generally, the compounds, constructs or polypeptides of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding ISV or polypeptide of the invention per se. For example, the compounds, constructs or polypeptides of the invention with increased half-life may have a half-life *e.g.*, in humans that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding ISV or polypeptide of the invention per se.

In a preferred, but non-limiting aspect of the invention, such compounds, constructs or polypeptides of the invention have a serum half-life e.g. in humans that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding ISV or polypeptide of the invention per se.

In another preferred, but non-limiting aspect of the invention, such compounds, constructs or polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds, constructs or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

In the present invention, it was demonstrated that the inclusion of an albumin targeting binding unit in the construct as such did not have an essential impact on the obtained potency or efficacy. Although a minor loss of efficacy/potency was observed in the presence of HSA, the half-life extended TCR binding multispecific polypeptides were still potent in tumour cell killing. Albumin-based drug delivery has been demonstrated to be useful for achieving improved cancer therapy, largely due to its passive target toward tumour via the enhanced permeability and retention effect and the increased demand for albumin by tumour cells as source of energy and amino acids. However, albumin lacks not only the active mechanism to overcome the cell membrane barrier, but also the ability to penetrate into tumour tissues (Qianqian Guo et al. 2013, Polym. Chem. 4: 4584-4587).

In a particularly preferred but non-limiting aspect of the invention, the invention provides a polypeptide of the invention comprising a first and a second immunoglobulin single variable domain (ISV); and further comprising one or more (preferably one) serum albumin binding immunoglobulin single variable domain as described herein, e.g. the serum albumin binding immunoglobulin single variable domain referred to as Alb8, Alb23, Alb129, Alb132, Alb11, Alb11 (S112K)-A, Alb82, Alb82-A, Alb82-AA, Alb82-AAA, Alb82-G, Alb82-GG, Alb82-GGG (Table B-3).

Table B-3: Immunoglobulin single variable domains for use in HLE of the ISVs and polypeptides of the invention

ISV	Sequence	SEQ ID NO
Alb8	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS	400
Alb23	EVQLLESGGGLVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDT LYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS	401
Alb129	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT	402

ISV	Sequence	SEQ ID NO
	LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTATYYCTIGGSLSRSSQGTLVTVSSA	
Alb132	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSD TLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTATYYCTIGGSLSRSSQGTLVTVSSA	403
Alb11	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS	404
Alb11 (S112K)-A	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVKVSSA	405
Alb82	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS	406
Alb82-A	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSSA	407
Alb82-AA	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSSAA	408
Alb82-AAA	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSSAAA	409
Alb82-G	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSSG	410
Alb82-GG	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSSGG	411
Alb82-GGG	EVQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSSGGG	412

Accordingly, the present invention relates to a polypeptide as described herein, further comprising a serum protein binding moiety.

The present invention relates to a polypeptide as described herein, wherein said serum protein binding moiety binds serum albumin.

The present invention relates to a polypeptide as described herein, wherein said serum protein binding moiety is an immunoglobulin single variable domain binding serum albumin.

The present invention relates to a polypeptide as described herein, wherein said ISV binding serum albumin essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which CDR1 is SFGMS (SEQ ID NO: 481), CDR2 is SISGSGSDTLYADSVKG (SEQ ID NO: 482), and in which CDR3 is GGSLSR (SEQ ID NO: 475), CDR determined according to Kabat definition; and/or in which CDR1 is GFTFSSFGMS (SEQ ID NO: 472) or GFTFRSFGMS (SEQ ID NO: 473), CDR2 is SISGSGSDTL (SEQ ID NO: 474) and CDR3 is GGSLSR (SEQ ID NO: 475), CDR determined according to Kontermann 2010.

The present invention relates to a polypeptide as described herein, wherein said ISV binding serum albumin comprises Alb8, Alb23, Alb129, Alb132, Alb11, Alb11 (S112K)-A, Alb82, Alb82-A, Alb82-AA, Alb82-AAA, Alb82-G, Alb82-GG, Alb82-GGG (Table -B3).

In the polypeptides of the invention, the two or more building blocks, ISVs or Nanobodies and the optionally one or more polypeptides, one or more other groups, drugs, agents, residues, moieties or binding units may be directly linked to each other (as for example described in WO 99/23221) and/or may be linked to each other via one or more suitable spacers or linkers, or any combination thereof.

Suitable spacers or linkers for use in multivalent and multispecific polypeptides will be clear to the skilled person, and may generally be any linker or spacer used in the art to link amino acid sequences. Preferably, said linker or spacer is suitable for use in constructing proteins or polypeptides that are intended for pharmaceutical use.

Some particularly preferred spacers include the spacers and linkers that are used in the art to link antibody fragments or antibody domains. These include the linkers mentioned in the general background art cited above, as well as for example linkers that are used in the art to construct diabodies or ScFv fragments (in this respect, however, it should be noted that, whereas in diabodies and in ScFv fragments, the linker sequence used should have a length, a degree of flexibility and other properties that allow the pertinent V_H and V_L domains to come together to form the complete antigen-binding site, there is no particular limitation on the length or the flexibility of the linker used in the polypeptide of the invention, since each ISV or Nanobody by itself forms a complete antigen-binding site).

For example, a linker may be a suitable amino acid sequence, and in particular amino acid sequences of between 1 and 50, preferably between 1 and 30, such as between 1 and 10 amino acid residues. Some preferred examples of such amino acid sequences include gly-ser linkers, for example of the type $(gly_xser_y)_z$, such as (for example $(gly_4ser)_3$ or $(gly_3ser_2)_3$, as described in WO 99/42077, and the GS30, GS15, GS9 and GS7 linkers described in the applications by Ablynx mentioned herein (see for example WO 06/040153 and WO 06/122825), as well as hinge-like regions, such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences (such as described in WO 94/04678). Preferred linkers are depicted in Table B-4.

Table B-4: Linkers

Linker	Sequence	SEQ ID NO
5GS	GGGGS	376
7GS	SGGSGGS	377
9GS	GGGGSGGGS	378
10GS	GGGGSGGGGS	379
15GS	GGGGSGGGGSGGGGS	380
18GS	GGGGSGGGGSGGGGGGGS	381
20GS	GGGGSGGGGSGGGGSGGGGS	382

Linker	Sequence	SEQ ID NO
25GS	GGGGSGGGGSGGGGSGGGGSGGGGS	383
30GS	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS	384
35GS	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS	385
Poly-A	AAA	386

Some other particularly preferred linkers are poly-alanine (such as AAA), as well as the linkers GS30 (SEQ ID NO: 85 in WO 06/122825) and GS9 (SEQ ID NO: 84 in WO 06/122825).

Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in proteins for pharmaceutical use. For instance, poly(ethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026.

It is encompassed within the scope of the invention that the length, the degree of flexibility and/or other properties of the linker(s) used (although not critical, as it usually is for linkers used in ScFv fragments) may have some influence on the properties of the final polypeptide of the invention, including but not limited to the affinity, specificity or avidity for the TCR, or for one or more of the other antigens. Based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

For example, in multivalent polypeptides of the invention that comprise building blocks, ISVs or Nanobodies directed against a first and second target, the length and flexibility of the linker are preferably such that it allows each building block, ISV or Nanobody of the invention present in the polypeptide to bind to its cognate target, e.g. the antigenic determinant on each of the targets. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

It is also within the scope of the invention that the linker(s) used confer one or more other favourable properties or functionality to the polypeptides of the invention, and/or provide one or more sites for the formation of derivatives and/or for the attachment of functional groups (e.g. as described herein for the derivatives of the ISVs, Nanobodies, or polypeptides of the invention). For example, linkers containing one or more charged amino acid residues can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

Finally, when two or more linkers are used in the polypeptides of the invention, these linkers may be the same or different. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

Usually, for ease of expression and production, a polypeptide of the invention will be a linear polypeptide. However, the invention in its broadest sense is not limited thereto. For example, when a polypeptide of the invention comprises three or more building blocks, ISVs or Nanobodies, it is possible to link them by use of a linker with three or more "arms", with each "arm" being linked to a building block, ISV or Nanobody, so as to provide a "star-shaped" construct. It is also possible, although usually less preferred, to use circular constructs.

Accordingly, the present invention relates to a polypeptide as described herein, wherein said first ISV and said second ISV and possibly said third ISV and/or said ISV binding serum albumin are directly linked to each other or are linked via a linker.

The present invention relates to a polypeptide as described herein, wherein said linker is chosen from the group consisting of linkers of 5GS, 7GS, 9GS, 10GS, 15GS, 18GS, 20GS, 25GS, 30GS and 35GS.

The present invention relates to a polypeptide as described herein, wherein said serum protein binding moiety is a non-antibody based polypeptide (e.g. PEG).

The invention also relates to methods for preparing the ISVs, polypeptides and constructs described herein. The ISVs, polypeptides and constructs of the invention can be prepared in a manner known per se, as will be clear to the skilled person from the further description herein. For example, the ISVs, polypeptides and constructs of the invention can be prepared in any manner known per se for the preparation of antibodies and in particular for the preparation of antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments). Some preferred, but non-limiting methods for preparing the polypeptides and constructs include the methods and techniques described herein.

The method for producing an ISV, polypeptide or protein construct of the invention may comprise the following steps:

- the expression, in a suitable host cell or host organism (also referred to herein as a "host of the invention") or in another suitable expression system of a nucleic acid that encodes said ISV, polypeptide or protein construct of the invention,

optionally followed by:

- isolating and/or purifying the ISV, polypeptide or protein construct of the invention thus obtained.

In particular, such a method may comprise the steps of:

– cultivating and/or maintaining a host of the invention under conditions that are such that said host of the invention expresses and/or produces at least one ISV, polypeptide or protein construct of the invention;

optionally followed by:

– isolating and/or purifying the ISV, polypeptide or protein construct of the invention thus obtained.

Accordingly, the present invention also relates to a nucleic acid or nucleotide sequence that encodes an ISV, polypeptide or protein construct of the invention (also referred to as "*nucleic acid of the invention*" or "*nucleotide sequence of the invention*"). A nucleic acid of the invention can be in the form of single or double stranded DNA or RNA, and is preferably in the form of double stranded DNA. For example, the nucleotide sequences of the invention may be genomic DNA, cDNA or synthetic DNA (such as DNA with a 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 essentially isolated from, as defined herein. The nucleic acid of the invention may also be in the form of, be present in and/or be part of a vector, such as for example a plasmid, cosmid or YAC, which again may be in essentially isolated form.

The nucleic acids of the invention can be prepared or obtained in a manner known per se, based on the information on the polypeptides or protein constructs of the invention given herein, and/or can be isolated from a suitable natural source. Also, as will be clear to the skilled person, to prepare a nucleic acid of the invention, also several nucleotide sequences, such as at least one nucleotide sequence encoding an immunoglobulin single variable domain of the invention and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (*e.g.* to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned herein, as well as the Examples below.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a genetic construct, as will be clear to the person skilled in the art. Such genetic constructs generally comprise at least one nucleic acid of the invention that is optionally linked to one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) and the further elements of genetic constructs referred to herein. Such genetic constructs comprising at least one nucleic acid of the invention will also be referred to herein as "*genetic constructs of the invention*".

The genetic constructs of the invention may be DNA or RNA, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable for independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector, i.e. a vector that can provide for expression *in vitro* and/or *in vivo* (*e.g.* in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting embodiment, a genetic construct of the invention comprises

a) at least one nucleic acid of the invention; operably connected to

b) one or more regulatory elements, such as a promoter and optionally a suitable terminator;

and optionally also

c) one or more further elements of genetic constructs known per se;

in which the terms "regulatory element", "promoter", "terminator" and "operably connected" have their usual meaning in the art (as further described herein); and in which said "further elements" present in the genetic constructs may for example be 3'- or 5'-UTR sequences, leader sequences, selection markers, expression markers/reporter genes, and/or elements that may facilitate or increase (the efficiency of) transformation or integration. These and other suitable elements for such genetic constructs will be clear to the skilled person, and may for instance depend upon the type of construct used; the intended host cell or host organism; the manner in which the nucleotide sequences of the invention of interest are to be expressed (*e.g.* via constitutive, transient or inducible expression); and/or the transformation technique to be used. For example, regulatory sequences, promoters and terminators known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments) may be used in an essentially analogous manner.

Preferably, in the genetic constructs of the invention, said at least one nucleic acid of the invention and said regulatory elements, and optionally said one or more further elements, are "operably linked" to each other, by which is generally meant that they are in a functional relationship with each other. For instance, a promoter is considered "operably linked" to a coding sequence if said promoter is able to initiate or otherwise control/regulate the transcription and/or the expression of a coding sequence (in which said coding sequence should be understood as being "under the control of" said promoter). Generally, when two nucleotide sequences are operably linked, they will be in the same orientation and usually also in the same reading frame. They will usually also be essentially contiguous, although this may also not be required.

The nucleic acids of the invention and/or the genetic constructs of the invention may be used to transform a host cell or host organism, i.e. for expression and/or production of the polypeptide or protein construct of the invention. The host is preferably a non-human host. Suitable hosts or host cells will be clear to the skilled person, and may for example be any suitable fungal, prokaryotic or eukaryotic cell or cell line or any suitable fungal, prokaryotic or eukaryotic organism, for example:

- a bacterial strain, including but not limited to gram-negative strains such as strains of *Escherichia coli*; of *Proteus*, for example of *Proteus mirabilis*; of *Pseudomonas*, for example of *Pseudomonas fluorescens*; and gram-positive strains such as strains of *Bacillus*, for example of *Bacillus subtilis* or of *Bacillus brevis*; of *Streptomyces*, for example of *Streptomyces lividans*; of *Staphylococcus*, for example of *Staphylococcus carnosus*; and of *Lactococcus*, for example of *Lactococcus lactis*;

- a fungal cell, including but not limited to cells from species of *Trichoderma*, for example from *Trichoderma reesei*; of *Neurospora*, for example from *Neurospora crassa*; of *Sordaria*, for example from *Sordaria macrospora*; of *Aspergillus*, for example from *Aspergillus niger* or from *Aspergillus sojae*; or from other filamentous fungi;

- a yeast cell, including but not limited to cells from species of *Saccharomyces*, for example of *Saccharomyces cerevisiae*; of *Schizosaccharomyces*, for example of *Schizosaccharomyces pombe*; of *Pichia*, for example of *Pichia pastoris* or of *Pichia methanolica*; of *Hansenula*, for example of *Hansenula polymorpha*; of *Kluyveromyces*, for example of *Kluyveromyces lactis*; of *Arxula*, for example of *Arxula adeninivorans*; of *Yarrowia*, for example of *Yarrowia lipolytica*;

- an amphibian cell or cell line, such as *Xenopus oocytes*;

- an insect-derived cell or cell line, such as cells/cell lines derived from lepidoptera, including but not limited to *Spodoptera* SF9 and Sf21 cells or cells/cell lines derived from *Drosophila*, such as Schneider and Kc cells;
- a plant or plant cell, for example in tobacco plants; and/or
- a mammalian cell or cell line, for example a cell or cell line derived from a human, a cell or a cell line from mammals including but not limited to CHO-cells, BHK-cells (for example BHK-21 cells) and human cells or cell lines such as HeLa, COS (for example COS-7) and PER.C6 cells;

as well as all other hosts or host cells known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments), which will be clear to the skilled person. Reference is also made to the general background art cited hereinabove, as well as to for example WO 94/29457; WO 96/34103; WO 99/42077; Frenken et al. 1998 (Res. Immunol. 149: 589-99); Riechmann and Muyldermans 1999 (J. Immunol. Met. 231: 25-38); van der Linden 2000 (J. Biotechnol. 80: 261-70); Joosten et al. 2003 (Microb. Cell Fact. 2: 1); Joosten et al. 2005 (Appl. Microbiol. Biotechnol. 66: 384-92); and the further references cited herein.

For expression of the ISVs, polypeptides or constructs in a cell, they may also be expressed as so-called “intrabodies”, as for example described in WO 94/02610, WO 95/22618 and US 7004940; WO 03/014960; in Cattaneo and Biocca 1997 (Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag); and in Kontermann 2004 (Methods 34: 163-170).

According to one preferred, but non-limiting embodiment of the invention, the ISV, polypeptide or protein construct of the invention is produced in a bacterial cell, in particular a bacterial cell suitable for large scale pharmaceutical production, such as cells of the strains mentioned above.

According to another preferred, but non-limiting embodiment of the invention, the ISV, polypeptide or protein construct of the invention is produced in a yeast cell, in particular a yeast cell suitable for large scale pharmaceutical production, such as cells of the species mentioned above.

According to yet another preferred, but non-limiting embodiment of the invention, the ISV, polypeptide or construct of the invention is produced in a mammalian cell, in particular in a human cell or in a cell of a human cell line, and more in particular in a human cell or in a cell of a human cell line that is suitable for large scale pharmaceutical production, such as the cell lines mentioned hereinabove.

Suitable techniques for transforming a host or host cell of the invention will be clear to the skilled person and may depend on the intended host cell/host organism and the genetic construct to be used. Reference is again made to the handbooks and patent applications mentioned above.

After transformation, a step for detecting and selecting those host cells or host organisms that have been successfully transformed with the nucleotide sequence/genetic construct of the invention may be performed. This may for instance be a selection step based on a selectable marker present in the genetic construct of the invention or a step involving the detection of the polypeptide of the invention, *e.g.* using specific antibodies.

The transformed host cell (which may be in the form or a stable cell line) or host organisms (which may be in the form of a stable mutant line or strain) form further aspects of the present invention.

Preferably, these host cells or host organisms are such that they express, or are (at least) capable of expressing (*e.g.* under suitable conditions), an ISV, polypeptide or protein construct of the invention (and in case of a host organism: in at least one cell, part, tissue or organ thereof). The invention also includes further generations, progeny and/or offspring of the host cell or host organism of the invention, for instance obtained by cell division or by sexual or asexual reproduction.

Accordingly, in another aspect, the invention relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) an ISV, polypeptide or protein construct of the invention; and/or that contains a nucleic acid encoding the same. Some preferred but non-limiting examples of such hosts or host cells can be as generally described in WO 04/041867, WO 04/041865 or WO 09/068627. For example, ISVs, polypeptides and protein constructs of the invention may with advantage be expressed, produced or manufactured in a yeast strain, such as a strain of *Pichia pastoris*. Reference is also made to WO 04/25591, WO 10/125187, WO 11/003622, and WO 12/056000 which also describes the expression/production in *Pichia* and other hosts/host cells of immunoglobulin single variable domains and polypeptides comprising the same.

To produce/obtain expression of the ISVs, polypeptides or protein constructs of the invention, the transformed host cell or transformed host organism may generally be kept, maintained and/or cultured under conditions such that the (desired) ISV, polypeptide or protein construct of the invention is expressed/produced. Suitable conditions will be clear to the skilled person and will usually depend upon the host cell/host organism used, as well as on the regulatory elements that control the expression of the (relevant) nucleotide sequence of the invention. Again, reference is made to the handbooks and patent applications mentioned above in the paragraphs on the genetic constructs of the invention.

Generally, suitable conditions may include the use of a suitable medium, the presence of a suitable source of food and/or suitable nutrients, the use of a suitable temperature, and optionally the presence of a suitable inducing factor or compound (*e.g.* when the nucleotide sequences of the invention are under the control of an inducible promoter); all of which may be selected by the skilled person. Again, under such conditions, the ISVs, polypeptides or protein constructs of the invention may be expressed in a constitutive manner, in a transient manner, or only when suitably induced.

It will also be clear to the skilled person that the ISV, polypeptide or protein construct of the invention may (first) be generated in an immature form (as mentioned above), which may then be subjected to post-translational modification, depending on the host cell/host organism used. Also, the ISV, polypeptide or protein construct of the invention may be glycosylated, again depending on the host cell/host organism used.

The ISV, polypeptide or protein construct of the invention may then be isolated from the host cell/host organism and/or from the medium in which said host cell or host organism was cultivated, using protein isolation and/or purification techniques known per se, such as (preparative) chromatography and/or electrophoresis techniques, differential precipitation techniques, affinity techniques (*e.g.* using a specific, cleavable amino acid sequence fused with the polypeptide or construct of the invention) and/or preparative immunological techniques (i.e. using antibodies against the amino acid sequence to be isolated).

The constructs of the invention can generally be prepared by a method which comprises at least the step of suitably linking ISVs or polypeptides of the invention to the one or more further groups, residues, moieties or binding units, optionally via the one or more suitable linkers, so as to provide the constructs of the invention. The ISVs, polypeptides and constructs of the invention can then further be modified, and in particular by chemical and/or biological (*e.g.* enzymatical) modification, of one or more of the amino acid residues that form the polypeptides or constructs of the invention, to obtain derivatives of the polypeptides or constructs of the invention.

The invention also relates to a pharmaceutical composition comprising the ISV, polypeptide, compound or construct of the invention.

In the above methods, the amino acid sequences, ISVs, Nanobodies, polypeptides, compounds or constructs of the invention and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the amino acid sequences, ISVs, Nanobodies, polypeptides, compounds or constructs of the invention and/or the compositions comprising the same can for example be administered orally, intraperitoneally (e.g. intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease or disorder to be prevented or treated and other factors well known to the clinician.

As used herein, the term "therapeutic agent" refers to any agent that can be used in the treatment and/or management of a hyperproliferative cell disorder, e.g., cancer, or one or more symptoms thereof. In certain embodiments, the term "therapeutic agent" refers to a multispecific polypeptide of the invention. Preferably, a therapeutic agent is an agent which is known to be useful for, or has been or is currently being used for the treatment, prevention and/or management of a hyperproliferative cell disorder, e.g., cancer, or one or more symptoms thereof.

As used herein, a "therapeutically effective amount" in the context of cancer refers to the amount of a therapy alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment and/or management of cancers. In one aspect, a therapeutically effective amount refers to the amount of a therapy sufficient to destroy, modify, control or remove primary, regional or metastatic cancer tissue. In another aspect, a therapeutically effective amount refers to the amount of a therapy sufficient to reduce the symptoms of a cancer. In another aspect, a therapeutically effective amount refers to the amount of a therapy sufficient to delay or minimize the spread of cancer. In a specific embodiment, a therapeutically effective amount of a therapy is an amount of a therapy sufficient to inhibit growth or proliferation of cancer cells, kill existing cancer cells (e.g., cause regression of the cancer), and/or prevent the spread of cancer cells to other tissues or areas (e.g., prevent metastasis). In another specific embodiment, a therapeutically effective amount of a therapy is the amount of a therapy sufficient to inhibit the growth of a tumor by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% as measured by a standard method known in the art. Used in connection with an amount of a multispecific polypeptide of the invention, the term can encompass an amount that improves overall therapy, reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with another therapy. In one embodiment, a therapeutically effective amount of a therapy reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with another therapy by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% relative to a control (e.g., a negative control such as phosphate buffered saline) in an assay known in the art or described herein.

As used herein, a "therapeutically effective amount" in the context of a non-cancer hyperproliferative cell disorder refers to the amount of a therapy alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment and/or management of said disorder. In one aspect, a therapeutically effective amount refers to the amount of a therapy sufficient to destroy, modify, control or remove cells affected by a non-cancer hyperproliferative cell disorder. In another aspect, a therapeutically effective amount refers to the amount of a therapy sufficient to reduce the symptoms of a non-cancer hyperproliferative cell disorder. In another aspect, a therapeutically effective amount refers to the amount of a therapy sufficient to delay or minimize the spread of the non-cancer hyperproliferative cell disorder. In a specific embodiment, a therapeutically effective amount of a therapy is an amount of a therapy sufficient to inhibit growth or proliferation of the non-cancer hyperproliferative cell disorder, kill existing non-cancer hyperproliferative cells (e.g., cause regression of the disorder). In another specific embodiment, a therapeutically effective amount of a therapy is the amount of a therapy sufficient to inhibit the growth of the non-cancer hyperproliferative cells by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% as measured by a standard method known in the art. Used in connection with an amount of a multispecific polypeptide of the invention, the term can encompass an amount that improves overall therapy, reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with another therapy. In one embodiment, a therapeutically effective amount of a therapy reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with another therapy by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% relative to a control (e.g., a negative control such as phosphate buffered saline) in an assay known in the art.

As used herein, the term "therapy" refers to any protocol, method and/or agent that can be used in the treatment, prevention and/or management of a hyperproliferative cell disorder, e.g., cancer. In certain embodiments, the terms "therapies" and "therapy" refer to a biological therapy, supportive therapy, and/or other therapies useful in the treatment, prevention and/or management of a hyperproliferative cell disorder, e.g., cancer, or one or more symptoms thereof known to one of skill in the art such as medical personnel.

As used herein, the terms "treat", "treatment" and "treating" in the context of administering (a) therapy(ies) to a subject refer to the reduction or amelioration of the progression, severity, and/or duration of a disorder associated with a hyperproliferative cell disorder, e.g., cancer, and/or the amelioration of one or more symptoms thereof resulting from the administration of one or more therapies (including, but not limited to, the administration of one or more prophylactic or therapeutic agents). In specific embodiments, the terms "treat", "treatment" and "treating" in the context of administering (a) therapy(ies) to a subject refer to the reduction or amelioration of the progression, severity, and/or duration of a hyperproliferative cell disorder, e.g., cancer, refers to a reduction in cancer cells by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% relative to a control (e.g., a negative control such as phosphate buffered saline). In other embodiments, the terms "treat", "treatment" and "treating" in the context of administering (a) therapy(ies) to a subject refer to the reduction or amelioration of the progression, severity, and/or duration of a hyperproliferative cell disorder, e.g., cancer, refers to no change in cancer cell number, a reduction in hospitalization time, a reduction in mortality, or an increase in survival time of the subject with cancer.

The amino acid sequences, ISVs, Nanobodies, polypeptides, compounds and/or constructs of the invention and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the hyperproliferative cell disorder, e.g., cancer, to be prevented or treated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the stage of the hyperproliferative cell disorder, e.g., cancer, to be treated, the severity of the hyperproliferative cell disorder, e.g., cancer, to be treated and/or the severity of the symptoms thereof, the specific amino acid sequence, ISV, Nanobody, polypeptide, compound and/or construct of the invention to be used, the specific route of administration and pharmaceutical formulation or composition to be 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 amino acid sequences, ISVs, Nanobodies, polypeptides, compounds and/or constructs of the invention, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amount(s) or doses to be administered can be determined by the clinician, again based on the factors cited above.

Generally, for the prevention and/or treatment of a hyperproliferative cell disorder, e.g., cancer, mentioned herein and depending on the type of hyperproliferative cell disorder, e.g., cancer, and stage of the disease to be treated, the potency of the specific amino acid sequence, ISV, Nanobody, polypeptide, compound or construct of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the amino acid sequences, ISVs, Nanobodies, polypeptides, compounds or constructs of the invention will generally be administered in an amount between 1 gram and 0.01 milligram per kg body weight per day, preferably between

0.1 gram and 0.01 milligram per kg body weight per day, such as about 0.1, 1, 10, 100 or 1000 milligram per kg body weight per day, e.g. from 0.1 mg per kg to 25 mg per kg of the subject's body weight; either continuously (e.g. by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from these amounts, for example on the basis of the factors cited above and his expert judgment. Generally, some guidance on the amounts to be administered can be obtained from the amounts usually administered for comparable conventional antibodies or antibody fragments against the same target administered via essentially the same route, taking into account however differences in affinity/avidity, efficacy, biodistribution, half-life and similar factors well known to the skilled person.

Usually, in the above method, a single amino acid sequence, ISV, Nanobody, polypeptide, compound or construct of the invention will be used. It is however within the scope of the invention to use two or more amino acid sequences, ISVs, Nanobodies, polypeptides compounds and/or constructs of the invention in combination.

The ISVs, Nanobodies, amino acid sequences, polypeptides, compounds and/or constructs of the invention may also be used in combination with one or more further pharmaceutically active compounds or principles, *i.e.* as a combined treatment regimen, which may or may not lead to a synergistic effect. Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgement.

In particular, the amino acid sequences, ISVs, Nanobodies, polypeptides, compounds and/or constructs of the invention may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the hyperproliferative cell disorder, e.g., cancer, disease and/or disorder cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or compositions for administering them will be clear to the clinician.

When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of administration, at essentially the same time or at different times (e.g. essentially simultaneously, consecutively, or according to an alternating regime). When the substances or principles are to be administered simultaneously via the same route of administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

In one aspect, the disclosure provides methods for the administration of immunoglobulin single variable domains and polypeptide constructs thereof comprising one or more immunoglobulin single variable domains, polypeptides, compounds and/or constructs. In some embodiments, the immunoglobulin single variable domain, polypeptide, compound and/or construct is administered as a pharmaceutical composition. The pharmaceutical composition, in addition to the immunoglobulin single variable domains and polypeptide constructs thereof includes a pharmaceutically-acceptable carrier.

As described in detail, the pharmaceutical compositions of the disclosure may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream or foam; sublingually; ocularly; transdermally; or nasally, pulmonary and to other mucosal surfaces.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

Formulations of the disclosure include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient (*e.g.,* immunoglobulin single variable domain or polypeptide constructs thereof) which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, and the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, this amount will range from about 1% to about 99% of active ingredient, preferably from about 5% to about 70%, most preferably from about 10% to about 30%.

In certain embodiments, a formulation comprises an excipient selected from the group consisting of cyclodextrins, liposomes, micelle forming agents, *e.g.*, bile acids, and polymeric carriers, *e.g.*, polyesters and polyanhydrides. In certain embodiments, an aforementioned formulation renders orally bioavailable an immunoglobulin single variable domain or polypeptide construct.

Methods of preparing these formulations or compositions include the step of bringing into association an immunoglobulin single variable domain or polypeptide construct with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association an immunoglobulin single variable domain or polypeptide construct with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of an immunoglobulin single variable domain or polypeptide construct as an active ingredient. An immunoglobulin single variable domain or polypeptide construct invention may also be administered as a bolus, electuary or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and non-ionic surfactants; absorbents, such as kaolin and bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxy-propylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made in a suitable machine in which a mixture of the powdered compound is moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, *e.g.*, freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing an immunoglobulin single variable domain or polypeptide construct with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of an immunoglobulin single variable domain or polypeptide construct include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of an immunoglobulin single variable domain or polypeptide construct to the body. Dissolving or dispersing the compound in the proper medium can make such dosage forms. Absorption enhancers can also be used to increase the flux of the compound across the skin. Either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel can control the rate of such flux.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this disclosure.

Pharmaceutical compositions suitable for parenteral administration comprise one or more an immunoglobulin single variable domains or polypeptide constructs in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and non-aqueous carriers, which may be employed in the pharmaceutical compositions include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject compounds may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly-(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions, which are compatible with body tissue.

In another aspect, kits are provided comprising a binding molecule of the invention, a nucleic acid molecule of the invention, a vector of the invention, or a host cell of the invention. The kit may comprise one or more vials containing the binding molecule and instructions for use. The kit may also contain means for administering the binding molecule of the present invention such as a syringe, pump, infuser or the like.

The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Modifications and variation of the above-described embodiments of the invention are possible without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.

The invention will now be further described by means of the following non-limiting preferred aspects, examples and figures.

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove.

EXAMPLES

Example 1: Material and methods

1.1 TCR αβ /CD3 transfected cell lines

Transient and stable CHO-K1 (ATCC: CCL-61), HEK293H (Life technologies 11631-017), Llana (Fibroblast cells from llama Navel cord cells) cell lines with recombinant overexpression of all 6 chains of the full human T cell Receptor complex were generated. For this, the coding sequences of the TCR alpha (α) and TCR beta (β) chain were cloned in a pcDNA3.1-derived vector, downstream of a CMV promotor and a 2A-like viral peptide sequence was inserted between both chains to induce ribosomal skipping during translation of the polyprotein. In the same vector, the coding sequences of the epsilon, delta, gamma and zeta chains of the CD3 complex were cloned downstream of an additional CMV promotor, also using 2A-like viral peptide sequences between the respective chains. In addition, a stable HEK293H clone with recombinant overexpression of the 4 chains of the human CD3 was generated as described above using a single gene vector.

The used sequences for the human CD3 and the human TCRα/β constant domains were derived from UniProtKB (CD3 delta: P04234, CD3 gamma: P09693, CD3 epsilon: P07766, CD3 zeta: P20963, TCR α: P01848 and TCR β: P01850; SEQ ID NOs: 344 to 349, respectively). The sequences for the human TCRα/β variable domains were derived from crystal structure sequences (PDB codes: 2IAN, 2XN9 and 3TOE) (human TCR α variable domains derived from 2IAN, 2XN9 and 3TOE with SEQ ID NOs: 393 to 395, respectively; human TCR β variable domains derived from 2IAN, 2XN9 and 3TOE with SEQ ID NOs: 476 to 478, respectively).

The cell surface expression of the human T cell receptor complex was confirmed by flow cytometry using a functional mouse IgG2b anti-human TCRα/β antibody, clone BW242/412 (Miltenyi 130-098-219) and a functional mouse IgG2a anti-CD3 PE labelled antibody, clone OKT-3 (eBioscience 12-0037) (Figure 1).

1.2 Soluble recombinant TCR α/β proteins

Soluble human and cynomolgus/rhesus monkey TCR α/β proteins were generated in house. The sequences for the extracellular part of the human TCRα/β constant domain were derived from UniProtKB (TCR α: P01848 and TCR β: P01850; SEQ ID NOs: 479 and 480, respectively). The human TCR α/β variable domains were derived from crystal structure sequence (PDB code : 2XN9, SEQ ID NOs: 394 and 477, respectively for α and β chain).

The sequences for the extracellular part of the cynomolgus/rhesus monkey TCR α/β constant domains were derived from GenBank files EHH63463 and AEA41868 respectively (SEQ ID NOs: 396

and 397). The sequences for the cynomolgus/rhesus monkey TCR α/β variable domains were derived from AEA41865 and AEA41866 (SEQ ID NOs: 398 and 399, respectively for α and β chain).

The extracellular domains of human TCR α/β (2XN9) or cynomolgus/rhesus monkey TCR α/β were fused to a zipper protein coding sequence (O'Shea et al. 1993 Curr. Biol. 3(10): 658-667), produced by CHOK1SV cells (Lonza) using Lonza's GS Gene Expression System™ and subsequently purified.

Quality of the TCR α/β zipper proteins was assessed in an ELISA binding assay. Maxisorp 96-well ELISA plates (Nunc) were coated with 2µg/mL soluble recombinant human TCR α/β (2XN9)-zipper protein or soluble recombinant cynomolgus TCR α/β-zipper protein. After an overnight incubation, plates were washed and blocked with PBS + 1% casein for 1h at room temperature. Next, plates were incubated with serial dilutions of either a functional flag tagged Nanobody or the functional mouse IgG anti-non-human primate/Rat TCRα/β antibody, clone R73 (eBioscience 16-5960) for 1h at room temperature while shaking, washed again and incubated with mouse anti-flag-HRP (Sigma, #A8592) respectively rabbit anti-mouse-HRP (Dako, # P0260) . After 1h, TMB One Solution (Promega #G7431) was added. The reaction was stopped with 2M H_2SO_4 and the dose dependent binding was determined by measuring the OD at 450nm using the Tecan sunrise 4 (Figure 2).

Example 2: Immunization of llamas with TCR/CD3, cloning of the heavy chain-only antibody fragment repertoires and preparation of phages

2.1 Immunization

It was set out to generate heavy chain only antibodies in camelidae (e.g. llama and alpaca) against T cell receptor (TCR) α and/or β constant chains. Although the native T cell receptor complex consists of both CD3 (gamma, delta, epsilon and zeta) chains, as well as TCR α- and β-chains, it was hypothesized that the absence of CD3 chains would facilitate access to the constant domains of the TCR. Especially since the CD3 chains laterally surround, and limit access to the constant domains of the TCR α- and β-chains. Contrary to our experience with other targets, the obtaining of an immune response against TCR α- or β-chains was not as straight forward as expected.

In a final approach, after approval of the Ethical Committee (CRIA, LA1400575, Belgium- EC2012#1), the inventors attempted a complex immunization protocol with DNA encoding for T cell complex. In short, 3 additional llamas were immunized with a pVAX1-human TCR(2IAN)/CD3 (described in Example 1.2) plasmid vector (Invitrogen, Carlsbad, CA, USA) and with a pVAX1-human TCR(2XN9)/CD3 (described in Example 1.2) plasmid vector (Invitrogen, Carlsbad, CA, USA) according to standard protocols. Two llamas received additionally 1 subcutaneous injection of primary human T cells. Human T cells were collected from Buffy Coat blood, from healthy volunteers (Blood bank Gent) using RosetteSep (StemCell Technologies, #15061) followed by enriching on Ficoll-Paque™ PLUS (GE Healthcare #17-1440-03) according to manufactures instructions and stored in liquid nitrogen. After thawing, cells were washed, and re-suspended in D-PBS from Gibco and kept on ice prior to injection.

2.2 Cloning of the heavy chain-only antibody fragment repertoires and preparation of phages

Per animal, blood samples were collected after the injection of one type of immunization antigen. From these blood samples, PBMC were prepared using Ficoll-Hypaque according to the manufacturer's instructions (Amersham Biosciences, Piscataway, NJ, USA). For each immunized llama, libraries were constructed by pooling the total RNA isolated from samples originating from a certain subset of the immunization schedule, *i.e.* after one type of immunization antigen.

In short, the PCR-amplified VHH repertoire was cloned via specific restriction sites into a vector designed to facilitate phage display of the VHH library. The vector was derived from pUC119. In frame with the VHH coding sequence, the vector encodes a C-terminal 3xFLAG and His6 tag. Phages were prepared according to standard protocols (see for example WO 04/041865, WO 04/041863, WO 04/062551, WO 05/044858 and other prior art and applications filed by Ablynx N.V. cited herein).

Example 3: Selection of TCR/CD3 specific VHHs via phage display

The vast majority of selected VHHs were directed against the variable regions of either the TCR α or TCR β chain. Therefore different selection and counter-selection strategies had to be devised by the inventors.

In short, VHH repertoires obtained from all llamas and cloned as phage library were used in different selection strategies, applying a multiplicity of selection conditions. Selections using human TCR/CD3 transfected cell lines with the same variable domain as used during immunization resulted in only variable domain binders. Therefore, tools containing a different variable TCRα/β domain (transfected cells (described in Example 1.1), soluble protein (described in Example 1.2), or human primary T cells (isolated as described in Example 2.1)) were used during selections and proved to be crucial in identification of constant domain binders. Additional variables during selections included the antigen presentation method (in solution when using cells or coated onto plates when proteins), the antigen concentration, the orthologue used (human or cynomolgus recombinant TCR α/β protein), and the number of selection rounds. All solid coated phase selections were done in Maxisorp 96-well plates (Nunc, Wiesbaden, Germany).

Selections were performed as follows: TCRα/β-CD3 antigen preparations for solid and solution phase selection formats were presented as described above at multiple concentrations. After 2h incubation with the phage libraries, followed by extensive washing, bound phages were eluted with trypsin (1 mg/mL) for 15 minutes. The trypsin protease activity was immediately neutralized by applying 0.8 mM protease inhibitor ABSF. As control, selections without antigen were performed in parallel.

Phage outputs were used to infect *E. coli* for analysis of individual VHH clones. Periplasmic extracts were prepared according to standard protocols (see for example WO 03/035694, WO 04/041865, WO 04/041863, WO 04/062551 and other prior art and applications filed by Ablynx N.V. cited herein).

Example 4: Screening, sequence analysis and purification

4.1 Screening for TCR/CD3 binding Nanobodies in a flow cytometry assay

Periplasmic extracts were screened for cell expressed TCR/CD3 binding using human TCR/CD3 transfected CHO-K1 or HEK293H cells and the respective CHO-K1 or HEK293H reference cell line in a mixed cell line setup. To this end, a large batch of the reference cell lines were labelled with 8 µM PKH26 and frozen. $5x10^4$ PKH labelled reference cells were mixed with $5x10^4$ target cells and incubated with periplasmic extracts for 30min at 4°C, and washed 3 times. Next, cells were incubated with 1 µg/ml monoclonal ANTI-FLAG® M2 antibody (Sigma-Aldrich, cat# F1804) for 30 min at 4°C, washed again, and incubated for 30 min at 4°C with goat anti-mouse APC labelled antibody (Jackson Immunoresearch 115-135-164, 1:100). Samples were washed, resuspended in FACS Buffer (D-PBS from Gibco, with 10% FBS from Sigma and 0.05% sodium azide from Merck) and then analysed via a BD FACSArray. First a P1 population which represented more than 80% of the total cell population was selected based on FSC-SSC distribution. In this gate, 20,000 cells were counted during acquisition. Based on PKH26-SSC distribution, the PKH labelled parental population and the human TCR/CD3 unlabelled target population was selected. For these 2 populations the mean APC value was calculated.

4.2 Screening for TCR/CD3 binding Nanobodies in a human T cell activation assay

After several attempts, it turned out that activation of purified human T cells by antibodies or Nanobodies according to standard protocols, *i.e.* coated onto a 96 well plate, was not sensitive enough (data not shown).

In order to assess activity, a different assay was developed, based on bead coupled T cell activation. In short, goat anti-mouse IgG dynabeads (Life technologies #11033) were coated with mouse anti-flag IgG antibodies (Sigma F1804), (15µg/1E7beads). After an incubation period of 2h at 4°C, beads were washed and incubated with 80µl periplasmic extract for 20 min at 4°C while shaking. Non-coupled Nanobodies were washed away before adding the bead complex together with soluble mouse anti-CD28 antibody (Pelicluster CD28 - Sanquin #M1650) to purified primary human T cells (isolated as described in Example 2.1). As control condition, non-stimulated human T cells were used. In brief, goat anti-mouse IgG dynabeads coupled to mouse anti-flag IgG were incubated in 80µl periplasmic extract containing irrelevant Nanobodies. After removal of the non-coupled Nanobodies during a wash step, the irrelevant Nanobody-bead complex was added to purified primary human T cells.

After an incubation of 24h at 37° and 5% CO_2 the activation status of the human T cells was determined by measuring the CD69 expression level in flow cytometry using monoclonal mouse anti-human CD69PE (BD Biosciences # 557050).

4.3 Sequence analysis of the obtained Nanobodies

Nanobodies which scored positive in the flow cytometric binding screen and the T cell activation assay were sequenced.

The sequence analysis demonstrated that all anti-TCR ISVs comprised a very similar CDR3. In particular, the CDR3 has the amino acid sequence X_1SR X_2X_3PYX_4Y, in which X_1 is F, Y, G, L or K, X_2 is I or L, X_3 is Y or W, and X_4 is D, N or S.

The sequence analysis further resulted in the identification of 3 distinct clusters. Corresponding alignments are provided (Table A-1, Table A-2, Table A-3)**.** Clustering was based on sequence similarities and differences in CDR2 and CDR3. Cluster A is the most prominent comprising 104 clones (SEQ ID NOs: 1-104), cluster B comprises 11 clones (SEQ ID NOs: 105-115), and cluster C is represented by only 3 clones (SEQ ID NOs: 116-118).

Sequence variability of the CDRs was determined for the different clusters. For **cluster A,** the amino acid sequence of the CDRs of clone 56G05 was used as a reference, against which the CDRs of all other cluster A clones were compared. The sequence variability against 56G05 is depicted in the tables below.

56G05	CDR1									
Kabat numbering	26	27	28	29	30	31	32	33	34	35
absolute numbering	1	2	3	4	5*	6	7	8	9	10
56G05 sequence	G	D	V	H	K	I	N	F	L	G
variations		A		Y	L	L		I		S
variations		S						V		
variations		E								
variations		G								

* in case position 5 is an L, then position 6 is also L

56G05	CDR2								
Kabat numbering	50	51	52	53	54	55	56	57	58
absolute numbering	1	2	3	4	5	6	7	8	9
56G05 sequence	H	I	S	I	G	D	Q	T	D
variations	T		T		S		D	V	A
variations	R		A		A		E	A	Q
variations							T		N
variations							A		V
variations							V		S

56G05	CDR3								
Kabat numbering	95	96	97	98	99	100	100a	101	102
absolute numbering	1	2	3	4	5	6	7	8	9
56G05 sequence	F	S	R	I	Y	P	Y	D	Y
variations	Y			L	W			N	
variations	G							S	
variations	L								

For **cluster B,** the amino acid sequence of the CDRs of clone 55C07 was used as a reference, against which the CDRs of all other cluster B clones were compared. The sequence variability against 55C07 is depicted in the tables below.

55C07	CDR1									
Kabat numbering	26	27	28	29	30	31	32	33	34	35
absolute numbering	1	2	3	4	5	6	7	8	9	10
55C07 sequence variations	G	E Q	T	F	K	I V	N	I	W	G

55C07	CDR2								
Kabat numbering	50	51	52	53	54	55	56	57	58
absolute numbering	1	2	3	4	5	6	7	8	9
55C07 sequence variations	S	L	T	I	G	G	A	T	N D

55C07	CDR3								
Kabat numbering	95	96	97	98	99	100	100a	101	102
absolute numbering	1	2	3	4	5	6	7	8	9
55C07 sequence variations	K	S	R	L I	Y	P	Y	D	Y

For **cluster C,** the amino acid sequence of the CDRs of clone 61G01 was used as a reference, against which the CDRs of all other cluster C clones were compared. The sequence variability against 61G01 is depicted in the tables below.

61G01	CDR1									
Kabat numbering	26	27	28	29	30	31	32	33	34	35
absolute numbering	1	2	3	4	5	6	7	8	9	10
61G01 sequence variations	G	E	I	G	R	I	N	F	Y	R

61G01	CDR2

Kabat numbering	50	51	52	53	54	55	56	57	58
absolute numbering	1	2	3	4	5	6	7	8	9
61G01 sequence variations	T	I	T	I	A	D	K	T I	D

61G01	CDR3								
Kabat numbering	95	96	97	98	99	100	100a	101	102
absolute numbering	1	2	3	4	5	6	7	8	9
61G01 sequence variations	G	S	R	L	Y	P	Y	D	Y

The clustering based on the sequence transmuted into functional differences (see *infra*).

4.4 Purification of monovalent Nanobodies

Representative Nanobodies for each cluster were selected and expressed in *E. coli* TG1 as triple Flag, His6-tagged proteins. Expression was induced by addition of 1 mM IPTG and allowed to continue for 4 hours at 37°C. After spinning the cell cultures, periplasmic extracts were prepared by freeze-thawing the pellets. These extracts were used as starting material and Nanobodies were purified via IMAC and size exclusion chromatography (SEC).

The Nanobodies were purified to 95% purity as assessed via SDS-PAGE (data not shown).

Example 5: Binding of anti-TCR Nanobodies to human TCR/CD3 expressed on CHO-K1 cells and to purified primary human T cells

Binding of purified monovalent anti-TCR Nanobodies to human TCR(2XN9)/CD3 expressed on CHO-K1 cells and to purified primary human T cells was evaluated in flow cytometry as outlined in Example 4.1. Dilution series of Nanobodies 55A02 (cluster A), 56G05 (cluster A), 68G05 (cluster B) and 61G01 (cluster C) starting from 1 µM were applied to the cells.

The results are shown in Figure 3.

Nanobodies clearly bound to human TCR/CD3 expressed on CHO-K1 cells. The cluster A representatives showed the best affinity, followed by the cluster B representative and the cluster C representative. Nanobodies bound to purified primary human T cells, although with slightly lower potency compared to the CHO-K1 human TCR(2XN9)/CD3 cells. The representatives of cluster A showed the best affinity for binding human primary T cells, in line with the data on the CHO-K1 (2XN9)/CD3. The EC50 values obtained from the dose response curve are represented in Table C-1.

Table C-1: EC50 (M) of anti-TCR monovalent Nanobodies for binding CHO-K1 human TCR(2XN9)/CD3 cells and for binding purified primary T cells as determined in flow cytometry.

		CHO-K1TCR(2XN9)/CD3			Primary human T cells		
Cluster	**sample ID**	**EC50 (M)**	**95% LCI**	**95% UCI**	**EC50 (M)**	**95% LCI**	**95% UCI**
A	T0170055A02	8.4E-09	7.2E-09	9.7E-09	9.1E-08	8.1E-08	1.0E-07
A	T0170056G05	8.9E-09	8.3E-09	9.4E-09	9.1E-08	8.3E-08	9.9E-08
B	T0170068G05	1.2E-08	1.0E-08	1.3E-08	>1E-07	/	/
C	T0170061G01	3.1E-08	2.8E-08	3.4E-08	>1E-07	/	/

Example 6: Determination of binding epitope

Binding of purified monovalent anti-TCR Nanobodies to human TCR(2IAN)/CD3 expressed on HEK293H cells was evaluated and compared with the binding to HEK293H cells transfected with human CD3 in flow cytometry, as outlined in Example 5. Dilution series of anti-TCR Nanobodies starting from 1 µM were applied to the cells. The parental HEK293H cell line was included as TCR/CD3 negative cell line.

The results are shown in Figure 4.

Nanobodies clearly bound to human TCR(2IAN)/CD3 expressed on HEK293H but not to the HEK293H cells transfected with human CD3 only, nor to the HEK293H parental cell line. The EC50 values obtained from the dose response curve are depicted in Table C-2.

Table C-2: EC50 (M) of anti-TCR monovalent Nanobodies for binding human TCR(2IAN)/CD3 or human CD3 expressed on HEK293H cells, as determined in flow cytometry.

		HEK293H wt		HEK293H CD3		HEK293H TCR/CD3	
cluster	**Sample ID**	**EC50**	**MCF at 1µM**	**EC50**	**MCF at 1µM**	**EC50**	**MCF at 1µM**
A	T0170055A02	No fit	246	No fit	1194	5.5E-08	91229
A	T0170056G05	No fit	299	No fit	352	8.4E-08	86510
B	T0170068G05	No fit	206	No fit	240	>1E-07	31202
C	T0170061G01	No fit	374	No fit	495	>1E-07	10032

In conclusion, the clones were specific for binding to human TCR α/β. No binding was observed to human CD3.

Example 7: Binding of anti-TCR Nanobodies to soluble recombinant human TCR α/β protein

7.1 Binding of anti-TCR Nanobodies to human T cell receptor protein in ELISA

Binding of purified monovalent TCR Nanobodies to soluble recombinant human TCR α/β protein was evaluated in ELISA (as described in Example 1.2) using 2µg/ml directly coated soluble recombinant human TCR α/β protein.

The results are shown in Figure 5. The EC50 values obtained from the dose response curve are depicted in Table C-3.

Table C-3: EC50 (M) of anti-TCR monovalent Nanobodies for binding soluble recombinant human TCR(2XN9) protein, as determined in ELISA.

Cluster	sample ID	EC50 (M)	95% LCI	95% UCI
A	T0170055A02	1.9E-09	1.7E-09	2.2E-09
A	T0170056G05	4.0E-09	3.5E-09	4.6E-09
B	T0170068G05	1.6E-08	1.3E-08	1.9E-08
C	T0170061G01	5.2E-08	4.2E-08	6.5E-08

In conclusion, representative clones of all clusters bind to soluble recombinant human TCR α/β protein.

7.2 Binding of anti-TCR Nanobodies to human T cell receptor protein in BLI

Binding affinities were measured using Bio-Layer Interferometry (BLI) on an Octet RED384 instrument (Pall ForteBio Corp.). Recombinant human soluble TCR(2XN9)-zipper protein was covalently immobilized on amine-reactive sensors (ForteBio) via NHS/EDC coupling chemistry. For kinetic analysis, sensors were first dipped into running buffer (10mM Hepes, 150mM NaCl, 0.05% p20, pH7.4 from GE Healthcare Life Sciences) to determine baseline setting. Subsequently, sensors were dipped into wells containing different concentrations of purified Nanobodies (range between 1.4 nM and 1 mM) for the association step (180s) and transferred to wells containing running buffer for the dissociation (15 min) step. Affinity constants (KD) were calculated applying a 1:1 interaction model using the ForteBio Data Analysis software.

The results are depicted in Figure 6. The binding characteristics are listed in Table C-4.

Table C-4: Kinetic analysis of anti-TCR monovalent Nanobodies for binding soluble recombinant human TCR(2XN9) protein as determined with the Octet RED384 instrument.

		Human sTCR (2XN9)-zipper

Cluster	sample ID	kon(1/Ms)	koff(1/s)	KD (M)
A	T0170055A02	4.9E+04	8.4E-04	1.7E-08
A	T0170056G05	5.0E+04	1.2E-03	2.4E-08

In conclusion, the binding affinity for cluster A representatives determined using BLI on human soluble 2XN9 showed correlation with the affinities determined on CHO-K1(2XN9)/CD3 cells in flow cytometry (cf. Example 5).

Example 8: Determination of purified primary human T cell activation capacity

Functionality of purified monovalent anti-TCR Nanobodies was evaluated in the human T cell activation assay. Goat anti-mouse IgG dynabeads (Life technologies #11033) were coated with mouse anti-Flag IgG antibodies (Sigma F1804), (15µg/1E7beads). After an incubation period of 2h at 4°C, beads were washed and incubated with a fixed (1µg) amount of purified Flag tagged Nanobody for 20 min at 4°C while shaking. Non-coupled Nanobodies were washed away before adding the bead complex together with soluble mouse anti-CD28 antibody (Pelicluster CD28 - Sanquin #M1650) to purified primary human T cells isolated (isolated as described in Example 2.1) from distinct healthy donors.

In addition, the effect of monovalent TCR binding by the Nanobodies was evaluated by the incubation of the Nanobody with the purified primary human T cells without prior capture onto anti-mouse IgG dynabeads, in the presence of anti-CD28 antibody.

The activation status of the purified primary human T cells was monitored by measuring the CD69 expression in flow cytometry using monoclonal mouse anti-human CD69PE (BD Biosciences # 557050) after an incubation of 24h at 37°C and 5% CO_2, as described in Example 4.2.

In conclusion, anti-TCR Nanobodies of all clusters showed clear CD69 upregulation after capturing onto anti-mouse IgG dynabeads. The irrelevant Nanobody did not show any CD69 upregulation (Figure 7A). In addition, none of the Nanobodies presented in solution were able to activate purified primary human T cells as measured by increased expression of CD69 (Figure 7B).

Example 9: Binding of multispecific TCR binding polypeptides to human T cell receptor complex expressed on cells

To demonstrate that redirection of engaged T cells to tumour cells is possible by the Nanobodies, the CD20 antigen was chosen as exemplary tumour target.

Different TCR binding building blocks (*i.e.* Nanobodies) were formatted into a multispecific construct with a human CD20 targeting Nanobody (see Table C-5). The effector and tumour Nanobodies were genetically linked with a 35GS linker and subsequently expressed in the yeast Pichia according to standard protocols (multispecific polypeptides).

Irrelevant constructs were generated by replacing the effector or tumour Nanobody with an irrelevant anti-egg lysozyme (cAblys) Nanobody (Table C-5)

Table C-5: Sample ID and description of multispecific constructs.

	Target Nanobody x Effector Nanobody		
Cluster	**Sample ID**	**SEQ ID NO**	**Description**
A	T017000014	300	20CD019C07-35GS-T0170028B01-FLAG3-HIS6
A	T017000015	301	cAbLys3(D1E)-35GS-T0170028B01-FLAG3-HIS6
ctrl	T017000018	302	20CD019C07-35GS-cAbLys3-FLAG3-HIS6
A	T017000054	321	20CD019C07-35GS-T0170055A03-FLAG3-HIS6
A	T017000055	322	20CD019C07-35GS-T0170055A02-FLAG3-HIS6
A	T017000058	323	20CD019C07-35GS-T0170040C01-FLAG3-HIS6
A	T017000060	324	20CD019C07-35GS-T0170028B01-FLAG3-HIS6
A	T017000076	334	20CD019C07-35GS-T0170056G05-FLAG3-HIS6
A	T017000063	325	20CD019C07-35GS-T0170069B08-FLAG3-HIS6
A	T017000064	326	20CD019C07-35GS-T0170068E08-FLAG3-HIS6
B	T017000068	328	20CD019C07-35GS-T0170055C07-FLAG3-HIS6
B	T017000070	330	20CD019C07-35GS-T0170055B06-FLAG3-HIS6
A	T017000069	329	20CD019C07-35GS-T0170055B11-FLAG3-HIS6
A	T017000050	319	20CD019C07-35GS-T0170069C08-FLAG3-HIS6
A	T017000065	327	20CD019C07-35GS-T0170067E06-FLAG3-HIS6
A	T017000078	336	20CD019C07-35GS-T0170069F05-FLAG3-HIS6
A	T017000079	337	20CD019C07-35GS-T0170067D01-FLAG3-HIS6
C	T017000051	320	20CD019C07-35GS-T0170061G01-FLAG3-HIS6
A	T017000075	333	20CD019C07-35GS-T0170067E03-FLAG3-HIS6
	Effector Nb x Target Nb		
Cluster	**Sample ID**	**SEQ ID NO**	**Description**
A	T017000019	303	T0170028B01-35GS-20CD019C07-FLAG3-HIS6
A	T017000025	305	T0170028B01-35GS-cAbLys3-FLAG3-HIS6
ctrl	T017000023	304	cAbLys3(D1E)-35GS-20CD019C07-FLAG3-HIS6
A	T017000041	314	T0170055A03-35GS-20CD019C07-FLAG3-HIS6
A	T017000042	315	T0170055A02-35GS-20CD019C07-FLAG3-HIS6
A	T017000044	316	T0170040C01-35GS-20CD019C07-FLAG3-HIS6
A	T017000046	317	T0170028B01-35GS-20CD019C07-FLAG3-HIS6
A	T017000074	332	T0170056G05-35GS-20CD019C07-FLAG3-HIS6

A	T017000029	306	T0170069B08-35GS-20CD019C07-FLAG3-HIS6
A	T017000035	311	T0170068E08-35GS-20CD019C07-FLAG3-HIS6
B	T017000031	308	T0170055C07-35GS-20CD019C07-FLAG3-HIS6
B	T017000033	310	T0170055B06-35GS-20CD019C07-FLAG3-HIS6
A	T017000032	309	T0170055B11-35GS-20CD019C07-FLAG3-HIS6
A	T017000037	312	T0170069C08-35GS-20CD019C07-FLAG3-HIS6
A	T017000077	335	T0170067E06-35GS-20CD019C07-FLAG3-HIS6
A	T017000049	318	T0170069F05-35GS-20CD019C07-FLAG3-HIS6
A	T017000030	307	T0170067D01-35GS-20CD019C07-FLAG3-HIS6
C	T017000038	313	T0170061G01-35GS-20CD019C07-FLAG3-HIS6
A	T017000073	331	T0170067E03-35GS-20CD019C07-FLAG3-HIS6

Binding of the multispecific constructs to human TCR/CD3 expressed on CHO-K1 cells, purified primary human T cells and CD20 positive Ramos cells (ATCC: CRL-1596) was evaluated in flow cytometry as outlined in Example 5. The results are presented in Figure 8**.**

The EC50 values obtained from the dose response curve are depicted in Table C-6**.**

The data indicate similar binding of the TCRxCD20 multispecific polypeptides compared to their monovalent counterparts. However, a reduced binding of the CD20XTCR multispecific polypeptides to CHO-K1 human TCR(2XN9)/CD3 cells and purified primary human T cells was detected compared to their monovalent counterparts. On the human CD20 positive Ramos cell line, the multispecific polypeptide with the CD20 at the C terminus showed reduced binding in comparison to the polypeptides with the CD20 at the N terminus.

Example 10: Functional characterization of multispecific CD20xTCR binding polypeptides in a flow cytometry based killing assay

In order to assess whether multispecific polypeptides were able to kill tumour cells, cytotoxicity assays were performed with isolated human T cells as effector cells.

Human T cells were isolated as described in Example 2.1. The quality and purity of the purified human T cells was checked with anti-CD3 (eBioscience # 12-0037-73), anti-CD8 (BD Biosciences #345775), anti-CD4 (BD Biosciences #345771), anti-CD45RO (BD Biosciences #555493), anti-CD45RA (BD Biosciences # 550855), anti-CD19 (BD Biosciences #555413), anti-CD25 (BD Pharmigen #557138) and anti-CD69 (BD Pharmigen #557050) fluorescently labelled antibodies in a flow cytometric assay. Human CD20 expressing Ramos cells and human CD20 expressing Raji cells (ECACC: 85011429), labelled with the PKH-26 membrane dye as described above were used as target cells. $2.5x10^5$

effector and $2.5x10^4$ target cells were co-incubated in 96-well V-bottom plates at an effector versus target ratio of 10:1. For measurement of the concentration-dependent cell lysis, serial dilutions of multispecific polypeptides (Table C-5) were added to the samples and incubated for 18 h in a 5% CO_2 atmosphere at 37°C. After incubation, cells were pelleted by centrifugation and washed with FACS buffer. Subsequently, cells were resuspended in FACS buffer supplemented with 5 nM TOPRO3 (Molecular Probes cat# T3605) to distinguish live from dead cells. Cells were analysed using a FACS Array flow cytometer (BD Biosciences). Per sample, a total sample volume of 80µl was acquired. Gating was set on PKH26 positive cells, and within this population the TOPRO3 positive cells were determined.

The CD20xTCR binding multispecific polypeptides showed dose dependent killing of the Ramos cells (Figure 9A). T017000014 (cluster A, 20CD019C07-35GS-T0170028B01-FLAG3-HIS6) showed a dose dependent killing on both Ramos (Figure 9A) and Raji (Figure 9B) cells confirming that the observed cytotoxic effect was not restricted to a single tumour cell line. The expression level of the tumour antigen CD20 was determined for both cell lines (Figure 10).

The IC50 values and the % lysis obtained from the dose response curve are depicted in Table C-7 (% lysis = % death cells at 500nM of Nanobody minus % dead cells of the no Nanobody control).

These results demonstrate that the TCR multispecific polypeptides can induce T cell mediated killing of tumour target positive cell lines. When either the targeting Nanobody or the effector Nanobody was replaced by an irrelevant Nanobody, no effect on the viability of the Ramos cells could be observed. There was no clear preference of the orientation between the individual binding blocks in the multispecific polypeptide.

Example 11: Functional characterization of multispecific CD20xTCR binding polypeptides in an xCELLigence based killing assay

The TCR binding multispecific polypeptides were also tested for their cell toxicity on human CD20 transfected adherent target cells in the presence of human effector T cells using real-time electrical impedance based technique. Here, fluctuations in impedance induced by the adherence of cells to the surface of an electrode were measured. T cells are non-adherent and therefore do not impact the impedance measurements.

In brief, the xCELLigence station was placed in a 37°C incubator at 5% CO_2. 50 µl of assay medium was added to each well of E-plate 96 (ACEA Biosciences; cat#05 232 368 001) and a blank reading on the xCELLigence system was performed to measure background impedance in absence of cells. Subsequently, human CD20 transfected CHO-K1 or CHO-K1 reference cells ($1x10^4$) were seeded onto the E-plates 96, and 50µl of a serial dilution of multispecific polypeptide was added. After 30 min at RT, 50µl of human T cells were added per well ($3x10^5$) to have an effector to target ratio of 30:1. The plate was placed in the xCELLigence station and impedance was measured every 15 min during 3 days. The data were analysed using a fixed time point indicated in the results.

The IC50 values are depicted in Table C-8.

Table C-8: IC50 (M) of the multispecific polypeptides in the xCELLigence based human T cell mediated CHO-K1 CD20 killing assay using an effector to target ratio of 30 to 1, analysed at 44h after seeding.

Cluster	ID monovalent Nanobody	sample ID (CD20xTCR)	n	IC50 (M)	sample ID (TCRxCD20)	n	IC50 (M)
A	T0170055A02	T017000055	2	1.4E-09	T017000042	2	4.2E-09
A	T0170056G05	T017000076	3	3.6E-10	T017000074	3	1.5E-09
B	T0170055C07	T017000068	1	1.3E-08	T017000031	1	2.1E-09
C	T0170061G01	T017000051	1	2.7E-08	T017000038	1	4.1E-09

The multispecific polypeptides showed tumour antigen dependent killing. The multispecific polypeptides were not able to induce T cell mediated killing of CHO-K1 reference cells, but induced dose dependent human T cell mediated killing of the CD20 transfected CHO-K1 cells. An example is shown in Figure 11.

These results confirm the outcome obtained in the flow cytometry based killing assay of Example 10. In addition, only when the tumour target antigen is present T cell mediated killing was observed, indicating that the multispecific polypeptides are critically dependent on their target for induction of cytotoxicity.

Example 12: Linker length evaluation of the multispecific polypeptides

To evaluate the impact of the linker length used in the CD20/TCR binding multispecific polypeptides on the cytotoxic capacity, the effector and tumour building blocks were genetically linked with a 5GS (SEQ ID NO: 376), 9GS (SEQ ID NO: 378) or 35GS (SEQ ID NO: 385) linker and subsequently expressed in Pichia according to standard protocols (see Table C-9).

Table C-9: Sample ID and description of multispecific construct to evaluate impact of linker length.

	Target Nanobody x Effector Nanobody		
Cluster	Sample ID	SEQ ID NO	Description
A	T017000002	292	20CD019C07-5GS-T0170028B01-FLAG3-HIS6
A	T017000008	296	20CD019C07-9GS-T0170028B01-FLAG3-HIS6

A	T017000014	300	20CD019C07-35GS-T0170028B01-FLAG3-HIS6
A	T017000060	324	20CD019C07-35GS-T0170028B01-FLAG3-HIS6
	Effector Nanobody x Target Nanobody		
Cluster	**Sample ID**	**SEQ ID NO**	**Description**
A	T017000013	299	T0170028B01-9GS-20CD019C07-FLAG3-HIS6
A	T017000019	303	T0170028B01-35GS-20CD019C07-FLAG3-HIS6
A	T017000046	317	T0170028B01-35GS-20CD019C07-FLAG3-HIS6
	Control Polypeptides		
Cluster	**Sample ID**	**SEQ ID NO**	**Description**
ctrl	T017000003	293	cAbLys3(D1E)-5GS-T0170028B01-FLAG3-HIS6
ctrl	T017000006	294	20CD019C07-5GS-cAbLys3-FLAG3-HIS6
ctrl	T017000009	297	cAbLys3(D1E)-9GS-T0170028B01-FLAG3-HIS6
ctrl	T017000012	298	20CD019C07-9GS-cAbLys3-FLAG3-HIS6
ctrl	T017000015	301	cAbLys3(D1E)-35GS-T0170028B01-FLAG3-HIS6
ctrl	T017000018	302	20CD019C07-35GS-cAbLys3-FLAG3-HIS6
ctrl	T017000023	304	cAbLys3(D1E)-35GS-20CD019C07-FLAG3-HIS6
ctrl	T017000025	305	T0170028B01-35GS-cAbLys3-FLAG3-HIS6

The impact of the linker length used in the CD20/TCR binding multispecific polypeptides on the human primary effector T cell induced cellular toxicity on the adherent CHO-K1 human CD20 transfectged target cells was evaluated using real-time electrical impedance based technique as described Example 11.

The results are summarized in Figure 12.

All multispecific polypeptides, *i.e.* all linker lengths demonstrated specific cell killing. Unexpectedly, the TCR multispecific polypeptides with the longest linker (35GS linker) showed the best potency. In view of these results, further experiments were performed with multispecific polypeptides comprising the 35GS linker.

Example 13: Influence of effector to target ratio on the killing effect of the multispecific polypeptides

To evaluate the effect of different effector to target (E:T) ratios on the killing properties of the polypeptides, CD20xTCR binding multispecific polypeptides were incubated with $2.5x10^4$ PKH labelled Ramos cells in the presence of respectively $2.5x10^5$ (E:T=10:1), $1.25x10^5$ (E:T=5:1), $5x10^4$ (E:T=2:1) and $2.5x10^4$ (E:T=1:1) human primary T cells as described in Example 10**.**

Exemplary results are shown in Figure 13. The IC50 values are depicted in Table C-10.

Table C-10: IC50 (M) of the multispecific polypeptides in the flow cytometry based T cell mediated Ramos killing assay using different effector to target ratios.

Clus-ter	ID monovalent Nanobody	sample ID (CD20xTCR)	E:T	n	IC50 (M)	95% LCI	95% UCI	% lysis
A	T0170056G05	T017000076	10	1	7.5E-10	5.2E-10	1.1E-09	25
A	T0170056G05	T017000076	5	1	8.9E-10	5.2E-10	1.5E-09	17
A	T0170056G05	T017000076	2	1	1.6E-09	6.1E-10	4.1E-09	10
A	T0170056G05	T017000076	1	1	4.6E-09	3.0E-10	6.9E-08	4
A	T0170055A02	T017000055	10	1	4.1E-09	3.1E-09	5.3E-09	28
A	T0170055A02	T017000055	5	1	2.5E-09	1.6E-09	4.0E-09	15
A	T0170055A02	T017000055	2	1	7.4E-10	3.4E-10	1.6E-09	9
A	T0170055A02	T017000055	1	1	3.4E-09	1.8E-10	6.4E-08	3

Both constructs were able to kill the human CD20 target cells at different E:T ratios, even at a ratio of 1:1, after an incubation time of 18h with little difference in potency. Although there was an impact of the E:T ratio on the % lysis, this might also be linked to the incubation time (see below).

Example 14: Time dependent cytolytic activity of CD20/TCR binding multispecific constructs in the purified primary human T cell mediated assay in xCELLigence

To evaluate the impact of incubation time on the killing properties of the CD20xTCR binding multispecific constructs, specific lysis of target cells was calculated for different time-points in xCELLigence. In brief, the xCELLigence station was placed in a 37°C incubator at 5% CO_2. 50 µl of assay medium was added to each well of E-pate 96 (ACEA Biosciences; cat#05 232 368 001) and a blank reading on the xCELLigence system was performed to measure background impedance in absence of cells. Subsequently, human CD20 transfected CHO-K1 or CHO-K1 reference cells ($1x10^4$) were seeded onto the E-plates 96. After 20h, $3x10^5$ purified primary human T cells (described *supra*) and 100nM or 1.5nM multispecific constructs were added, respectively. The cell index (CI) was measured every 15 min during 5 days. Using the normalized CI (the normalized cell index – NCI, is calculated by dividing the cell index value at a particular time point by the cell index value of the time-point when purified primary human T cells were added) specific lysis at different time points of the condition with constructs was calculated in relation to the condition lacking construct. (% specific lysis = $((NCI_{no\ construct} - NCI_{with\ construct})/NCI_{no\ construct}))x100$.

The results are depicted in Figure 14.

Already one hour after the addition of human primary T cells and the multispecific construct, an increase of cell lysis can be observed which clearly increased further upon longer incubation times. The maximal effect was clearly dependent on the incubation time but the obtained IC50 value did not change with increased incubation times. The irrelevant construct did not show any killing of the human CD20 transfected CHO-K1 cells.

Example 15: Exploration of half-life extension (HLE)

It was hypothesized that HLE via albumin binding might be suitable to comply with various requirements, including (i) half-life extension (HLE) of the moiety; and (ii) efficacy of the multispecific polypeptide. Preferably, the HLE function would not impair the penetration of tumours and tissues.

Alb11 (SEQ ID NO: 404), a Nanobody binding to human serum albumin (HSA) was linked to the multispecific CD20xTCR binding polypeptides to increase the in vivo half-life of the formatted molecules (WO 06/122787). A number of formats were generated based on the CD20 tumour targeting building block at the N-terminus, the TCRα/β recruiting building blocks in the middle and the albumin targeting Nanobody at the C-terminus using a 35GS linker and expressed as indicated above. An overview of the explored formats is shown in Table C-11.

Table C-11: Sample ID and description of HLE constructs.

Cluster	Sample ID	SEQ ID NO	Description
A	T017000093	340	20CD019C07-35GS-T0170056G05-35GS-ALB11-FLAG3-HIS6
B	T017000095	341	20CD019C07-35GS-T0170055C07-35GS-ALB11-FLAG3-HIS6

As the addition of the Alb11 Nanobody might influence the affinity or potency of the construct and the binding of HSA to the Alb11 Nanobody might have an impact on the affinity or potency of the half-life extended constructs, the half-life extended constructs were characterized for binding to TCR overexpressing CHO-K1 and primary human T cells. In addition, the potency in the functional T cell dependent Ramos B cell killing assay was evaluated in the presence and absence of HSA (described in 15.1 and 15.2 below).

15.1 Impact of Alb11 building block on the binding properties

Analogous to the experiments described in Example 5, binding of half-life extended anti-TCR polypeptides to CHO-K1 human TCR(2XN9)/CD3 cells, primary human T cells and Ramos cells was evaluated in a flow cytometric assay in the absence of HSA.

The results are provided in Figure 15. The EC50 values obtained in this assay are listed in Table C-12.

Comparison of the CD20-35GS-TCR HLE construct with the non-HLE constructs showed similar binding on all three cell lines tested. The data presented showed that coupling of the Alb11 building block did not influence the binding properties.

15.2 Impact of human serum albumin on potency in human T cell mediated B cell killing Assay

The functionality of half-life extended anti-TCR polypeptides was evaluated in the human T cell mediated Ramos killing assay as described in Example 10 in the presence and absence of 30µM HSA and compared with the functionality of the non-HLE multispecific constructs.

The results are depicted in Figure 16. The IC50 values obtained in this assay are listed in Table C-13.

Table C-13: IC50 (M) of and % lysis by the HLE polypeptides in the T cell dependent B cell (Ramos) killing assay to evaluate the effect of HLE.

Cluster	sample ID	IC50 (M)	95% LCI	95% UCI	% lysis
A	T017000076	1.1E-10	7.6E-11	1.5E-10	25
A	T017000093	3.4E-10	2.4E-10	4.9E-10	28
A	T017000093	2.7E-10	2.1E-10	3.5E-10	26
A	T017000093 +HSA	1.6E-09	1.1E-09	2.2E-09	21
B	T017000068	5.6E-10	2.7E-10	1.2E-09	12
B	T017000095	3.3E-09	1.8E-09	5.8E-09	16
B	T017000095	1.1E-09	6.5E-10	2.0E-09	16
B	T017000095 +HSA	5.7E-09	2.0E-09	1.6E-08	9

The results indicate that the inclusion of the albumin targeting Nanobody in the construct as such did not have an essential impact on the obtained potency or efficacy. Although a minor loss of efficacy/potency was observed in the presence of HSA, the half-life extended TCR multispecific polypeptides were still potent in tumour cell killing.

Example 16: Functional characterization of multispecific polypeptides in an xCELLigence based human T cell mediated HER2-positive tumour killing assay

In order to assess the general applicability of the TCR building blocks in directing T cells to tumour cells, TCR binding building blocks were combined with building block that binds a different TAA, in this case a Nanobody binding to HER2.

The anti-TCR building block was combined with a Nanobody that binds the HER2 solid tumour antigen in two orientations (Table C-14) and characterized in the xCELLigence based human T cell mediated HER2-positive tumour killing assay as described in Example 11 using two HER2 expressing cell lines (SKBR3 (ATCC: HTB-30), MCF-7 (ATCC: HTB-22)) and a HER2 negative reference cell line (MDA-MB-468 (ATCC HTB-132)) as target cell population. Human HER2 expression levels were confirmed using 100nM of the monovalent anti-HER2 Nanobody HER2005F07 (SEQ ID NO: 350) in flow cytometry as described in Example 5. Results are shown in Figure 17.

Table C-14: Sample ID and description of HER2/TCR binding polypeptides.

	Target Nb x Effector Nb		
Cluster	**Sample ID**	**SEQ ID NO**	**Description**
A	T017000102	342	HER2005F07(Q108L)-35GS-T0170056G05-FLAG3-HIS6
	Effector Nb x Target Nb		
Cluster	**Sample ID**	**SEQ ID NO**	**Description**
A	T017000103	343	T0170056G05-35GS-HER2005F07(Q108L)-FLAG3-HIS6

In brief, SKBR3 ($4x10^4$ cells/well), MDA-MB-468 ($4x10^4$ cells/well) or MCF-7 ($2x10^4$ cells/well) were seeded in 96well E-plates and incubated with $6x10^5$ cells or $3x10^5$ cells human primary T cells (effector target ratio of 15 to 1) in the presence or absence of the multispecific constructs and followed over time. Data were analysed after 18h and are shown in Figure 18.

The IC50 values obtained in this assay are listed in Table C-15**.**

The data indicate specific killing of HER2-positive tumour cell lines by directing human primary T cells to the tumour cells via the anti-TCR Nanobody. Hence, the TCR binding building blocks are broadly applicable for directing cytotoxic T cells to tumours. Despite the large difference in tumour antigen density on SKBR3 and MCF-7 cells, both were efficiently killed by the addition of multispecific polypeptide constructs.

Example 17: Effect of HER2/TCR binding polypeptides on IFN-γ release by human T cells in the HER2-positive tumour cell killing assay

To further evaluate the broad applicability of the TCR binding building blocks, the induction of cytokine release was monitored during the human T cell mediated SKBR3 killing assay based on xCELLigence. The release of the cytokine IFN-γ was measured by ELISA. Briefly, SKBR3 cells were seeded in 96 E-plate in the presence of purified human primary T cells with or without multispecific HER2/TCR binding or irrelevant polypeptides as described in Example 16. 72h after the addition of the human primary T cells/polypeptides to the E-plates, IFN-γ production by the human primary T cells was measured. Maxisorp 96-well ELISA plates (Nunc) were coated with anti-human IFN-γ antibody (BD Biosciences #551221). After overnight incubation, plates were washed and blocked with PBS + 2% BSA for 1h at room temperature. Next, plates were incubated with 100µl of the supernatants (2 fold diluted) and 1µg/ml biotinylated anti-human IFN-γ antibody (BD Biosciences, #554550) for 2h 30min while shaking, washed again and incubated with streptavidin-HRP (Dakocytomation #P0397). After 30 min, TMB One Solution (Promega #G7431) was added. The reaction was stopped with 2M H_2SO_4 and the polypeptide dose dependent production of IFN-γ was determined by measurement of the OD at 405nm using the Tecan sunrise 4.

The results are shown in Figure 19. The EC50 values obtained in this assay are listed in Table C-16.

The multispecific HER2/TCR binding polypeptides induced a dose dependent production of the cytokine IFN-γ, indicating that the human T cells were activated only in presence of the relevant polypeptide.

Example 18: Cynomolgus cross-reactivity of anti-TCR Nanobodies

The cross-reactivity of the TCR binding building blocks with cynomolgus monkey TCR was evaluated.

18.1 Functional characterization of the multispecific polypeptides in a cynomolgus T cell mediated Ramos CD20 positive tumour killing assay

In a first experiment, a flow cytometric killing assay was set up, essentially as described in Example 10, using 2.5×10^5 primary cynomolgus T cells (isolated using Pan T Cell Isolation Kit MACS#130-091-993) as effector cells and 2.5×10^4 human CD20 positive Ramos cells as target cells.

The IC50 values and the % lysis obtained from the dose response curve are depicted in Table C-17. The results are shown in Figure 20.

The TCR binding multispecific polypeptides that contained a TCR binding building block belonging to cluster A showed dose dependent killing of the Ramos cells using cynomolgus T cells.

18.2 Functional characterization of the multispecific polypeptides in a cynomolgus T cell mediated CHO-K1 human CD20 positive cell killing assay

To further assess the cross-reactivity of the TCR binding building blocks in the TCR/CD20 binding multispecific constructs, the xCELLigence based killing assay using purified primary cynomolgus T cells essentially as described in Example 11 was used.

The assay used an effector to target ratio of 30 to 1, i.e. $3x10^5$ effector cynomolgus T cells (isolated using Pan T Cell Isolation Kit MACS#130-091-993) and $1x10^4$ target CHO-K1 human CD20 cells.

The IC50 values obtained in this assay are listed in Table C-18. The results are summarized in Figure 21.

It can be concluded that the TCR binding multispecific polypeptides that contain a TCR binding building block belonging to cluster A showed dose dependent killing of the CHO-K1 CD20 transfected cells using cynomolgus T cells. Hence, cluster A Nanobodies cross-react with primary cynomolgus T cells and can elicit potent killing based on these cynomolgus T cells.

18.3 Binding of anti-TCR Nanobodies to cynomolgusT cell receptor protein (ELISA)

Binding of purified monovalent anti-TCR Nanobodies to soluble recombinant cynomolgus TCR α/β protein was evaluated in ELISA (as described in Example 1.2) using 2µg/ml directly coated recombinant soluble cynomolgus TCR-α/β zipper protein.

The EC50 values obtained from the dose response curve are depicted in Table C-19.

An exemplary result is shown in Figure 22.

Table C-19: EC50 (M) of anti-TCR monovalent Nanobodies for binding to soluble recombinant cynomolgus TCR/CD3 protein as determined in ELISA.

Cluster	sample ID	EC50 (M)	95% LCI	95% UCI
A	T0170055A02	1.6E-07	1.5E-07	1.7E-07
A	T0170056G05	7.7E-08	6.6E-08	9.1E-08
B	T0170068G05	8.7E-08	8.5E-08	8.9E-08
C	T0170061G01	>1E7		

The results indicate that the anti-TCR Nanobodies from cluster A and cluster B bind to the recombinant soluble cynomolgus TCR-α/β zipper protein.

18.4 Evaluation of cynomolgus cross-reactivity in Bio-Layer Interferometry

Binding affinities of the monovalent anti-TCR Nanobodies were measured using Bio-Layer Interferometry (BLI) on an Octet RED384 instrument (Pall ForteBio Corp.) essentially as described in Example 7.1 using cynomolgus TCR α/β zipper. The results are depicted in Figure 23, the binding characteristics of the anti-TCR Nanobodies are listed in Table C-20.

Table C-20: Binding characteristics of monovalent anti-TCR Nanobodies determined in Octet using directly coated cynoTCR-zipper protein.

Cluster	sample ID	kon(1/Ms)	koff(1/s)	KD (M)
A	T0170055A02	1.1E+05	2.4E-02	2.1E-07
A	T0170056G05	1.1E+05	1.6E-02	1.5E-07

The cluster A Nanobodies bind to the soluble recombinant cynomolgus TCR α/β zipper with a 10 fold lower affinity compared to soluble recombinant human TCR α/β zipper.

18.5 Functional characterization of half-life extended multispecific polypeptides in a cynomolgus T cell mediated Ramos CD20 positive tumour killing assay

Analogous to the set up described in Example 18.1, the half-life extended TCR binding polypeptides were evaluated in a cynomolgus T cell mediated Ramos killing assay.

The IC50 values obtained in this assay are listed in Table C-21. The results are depicted in Figure 24.

Table C-21: IC50 of and % lysis by HLE multispecific polypeptides in the cynomolgus T cell dependent B cell (Ramos) killing assay to evaluate the effect of HLE.

Cluster	sample ID	IC50 (M)	95% LCI	95% UCI	% lysis
A	T017000076	5.2E-10	2.9E-10	9.5E-10	29
A	T017000093	1.0E-09	5.5E-10	2.0E-09	28
A	T017000093 +HSA	8.2E-10	4.7E-10	1.4E-09	18

The HLE extended TCR binding multispecific polypeptides that contain a TCR binding building block belonging to cluster A showed dose dependent killing of the Ramos cells using purified primary cynomolgus T cells. The inclusion of the ALB11 in the construct as such did not impact the potency (overlapping CI). Upon addition of HSA, a small drop in efficacy was observed while the potency was not affected.

18.6 Functional characterization of half-life extended CD20xTCR binding multispecific polypeptides in a cynomolgus T cell mediated CHO-CD20 positive tumour cell killing assay

To confirm the data in the flow cytometry based assay, the HLE constructs were tested in the xCELLigence based CHO-K1 human CD20 killing using purified primary cynomolgus T cells as described in Example 11.

The results are shown in Figure 25. The IC50 values obtained from the dose response curve are depicted in Table C-22.

Table C-22: IC50 (M) of the TCR/CD20 binding multispecific and HLE constructs in the cynomolgus T cell mediated CHO-K1 human CD20 tumour killing assay to evaluate the effect of ALB11 and HSA.

sample ID	IC50 (M)	95% LCI	95% UCI
T017000076	1.4E-10	9.5E-11	2.1E-10
T017000093	1.9E-10	1.3E-10	2.8E-10
T017000093 +HSA	7.4E-10	5.0E-10	1.1E-09

The HLE TCR binding multispecific polypeptides showed dose dependent killing of CHO-K1 human CD20 tranfected cells using cynomolgus T cells, confirming that the ALB11 has no impact on the cynomolgus cross-reactivity of the TCR binding building block.

18.7 Functional characterization of multispecific polypeptides in a cynomolgus T cell mediated HER2 positive tumour cell killing assay

The multispecific polypeptides were functionally characterized in a cynomolgus T cell mediated HER2 positive tumour cell killing assay. In short, the TCR/HER2 binding multispecific polypeptides were evaluated in a xCELLigence based killing assay essentially as described in Example 16, using $6x10^5$ cynomolgus T cells as effector cells and $4x10^4$ SKBR3 as target cells (effector to target of 30 to 1). Data were analysed after 18h.

The results are depicted in Figure 26. The IC50 values obtained in this assay are listed in Table C-23.

The cynomolgus cross-reactivity of the TCR binding building block was confirmed using the HER2/TCR binding multispecific constructs.

Example 19: *In vivo* proof-of-concept in a Ramos B cell depletion model

In this B cell depletion model, Ramos cells (a Burkitt's lymphoma cell line) and human PBMC were injected respectively intravenously and intraperitoneally in to NOG mice. Ramos B cell and PBMC-derived B cell killing by Nanobody-mediated recruitment of T cells present in the PBMC population was evaluated reflecting the potential of multispecific polypeptides to activate T cells by direct linkage of T cells via TCR to target B cells via CD20, resulting in target cell killing.

The *in vivo* efficacy of the bi-specific polypeptide T017000083 (CD20xTCR binding) on B cell depletion in a Ramos NOG mouse model was evaluated and compared with the irrelevant multispecific polypeptide T017000088 (irrelevant Nanobody + TCR binding Nanobody). The study demonstrated a statistically significant effect in bone marrow and spleen on Ramos B cell depletion and on PBMC derived B cell depletion in spleen.

In detail, the B cell depletion was evaluated in mice, intravenously injected with 10^6 Ramos cells in 200 µL of Roswell Park Memorial Institute (RPMI) medium 1640 at day one (D1). This injection took place 24 hours after a whole body irradiation of mice with a ɣ-source (1.44 Gy, 60Co) (D0). 10^7 PBMCs (500 µL in PBS) were injected on D3 (*i.e.* two days after tumor cell injection) after randomization of the mice into groups each of 24 animals. The treatment started on D3 one hour after PBMC injection and was repeated for 5 consecutive days in total until D7 (Figure 27). Three dose levels of the TCR/CD20 binding polypeptides were tested (0.5mg/kg, 5mg/kg and 23mg/kg).

On D20 or on D21, mice were sacrificed and spleen and bone marrow (femur) were collected for FACS analysis (mCD45, hCD45, hCD19, hCD20, hCD10) to analyze and quantify the presence of Ramos B cells (hCD19+ hCD20+ hCD45+ mCD45- hCD10+) and PBMC-derived B cells (hCD19+ hCD20+ hCD45+ mCD45- hCD10-).

Results for Ramos B cell depletion are represented in Figure 28A and Figure 28B. Mice treated with an irrelevant multispecific polypeptide were considered as control group for analyses. From Figure 28A, a dose response pattern was seen in the bone marrow for T017000083 (CD20/TCR) versus the irrelevant multispecific polypeptide for reducing Ramos cell numbers. The 2 highest dose levels for the CD20/TCR binding multispecific polypeptide were statistically significantly different from the irrelevant multispecific polypeptide. Statistical analysis has been performed with F-tests from the mixed-effects ANOVA analysis. For the spleen, all tested dose levels of T017000083 were statistically significantly different from the irrelevant multispecific polypeptide, as depicted in Figure 28B. The dose response pattern in spleen was less pronounced since all doses were close to or estimated to be on the maximum effect and thus very similar.

Results for PBMC-derived B cell depletion are represented in Figure 28C and Figure 28D. In bone marrow, a dose response pattern was seen for T017000083 as depicted in Figure 28C. The estimated difference in human B cell numbers for T017000083 versus the irrelevant multispecific polypeptide for the 2 highest dose levels was statistically significantly different at the 5% level of significance. In the spleen, B cell counts were statistically significantly different from the irrelevant multispecific polypeptide-treated group in all the groups treated with T017000083, and this at all tested dose levels. The dose response pattern was less pronounced since all doses were close to or estimated to be on the maximum effect and thus very similar, although an increase in estimated difference with increasing dose was observed.

In conclusion, these results demonstrate that CD20/TCR multispecific polypeptides are able to significantly decrease Ramos B cells and PBMC-derived B cells in spleen and Ramos B cells in bone marrow in this model. This confirms the polypeptide-induced T cell activation by cross-linking T cells to target B cells and killing of the latter.

Example 20: *In vivo* proof-of-concept in a PBMC B cell depletion model

In this B cell depletion model, human PBMC were injected intraperitoneally in to NOG mice. PBMC-derived B cell killing by polypeptide-mediated recruitment of T cells present in the PBMC population was evaluated reflecting the potential of the polypeptides of the invention to activate T cells by direct linkage of T cells via TCR to target B cells via CD20, resulting in target cell killing.

The *in vivo* efficacy of the multispecific polypeptide T017000083 (CD20xTCR binding) on B cell depletion in a PBMC NOG mouse model was evaluated and compared with the irrelevant polypeptide T017000088. The study demonstrated a clear effect on PBMC derived B cell depletion in spleen.

In detail, the B cell depletion was evaluated in mice, intraperitoneally injected with $3x10^7$ PBMCs in 500 µL of PBS at day three (D3) after a whole body irradiation of mice with a ɣ-source (1.44 Gy, 60Co) (D0) and randomization of the mice into groups each of 12 animals. The treatment started on D3 one hour after PBMC injection and was repeated for 5 consecutive days, in total until day 7 (D7) (Figure 29). Three dose levels of the CD20/TCR binding polypeptide were tested (0.5mg/kg, 5mg/kg and 23mg/kg).

On day 18 (D18), mice were sacrificed and the spleen was collected for FACS analysis (mCD45, hCD45, hCD19, hCD20) to analyze and quantify the presence of PBMC-derived human B cells (hCD19+ hCD20+ hCD45+ mCD45-).

Results for PBMC-derived B cell depletion are represented in Figure 30. In the spleen, B cell counts were clearly different from the irrelevant polypeptide-treated group in all the groups treated with T017000083 and this at all tested dose levels. The dose response pattern is not pronounced since all doses are close to or estimated to be on the maximum effect and thus very similar.

In conclusion, these results demonstrate that a CD20/TCR binding multispecific polypeptide is able to significantly decrease PBMC-derived B cells in spleen in this model. This confirms the polypeptide-induced T cell activation by cross-linking T cells to target B cells and killing of the latter.

Example 21: Targeting of tumour cells with multispecific T cell engaging polypeptides

The therapeutic activity of T cell engaging strategy can be improved by the simultaneous targeting of multiple tumour associated antigens. Often tumour cells create an escape mechanism by the down-regulation of targeted antigens within a therapy. The simultaneous targeting of multiple antigens is likely to reduce the probability of generating tumour escape variants. The individual affinity of the respective tumour targeting Nanobodies may be varied such that preferable binding to either a single marker or simultaneous binding to both tumour markers is achieved. Antigens present on different cell populations can be combined or even soluble proteins can be targeted in combination with a tumour associated antigen.

As the Nanobody platform is ideally suited to combine different specificities into a multispecific format, the anti-TCR Nanobodies of the invention are combined into formats illustrating these concepts, *i.e.* with different tumour antigen binding Nanobodies in a multispecific polypeptide.

For the double tumour antigen targeting concept, a Nanobody reactive towards a first tumour antigen (TA1, e.g. CEA) is linked to a second Nanobody with different specificity (TA2, e.g. EGFR), different from TA1, in combination with a TCR reactive Nanobody. The specific order of the building blocks is varied within the format as well as the applied linker lengths in between the different building blocks. Combinations of TA1 and TA2 which are tested are depicted in Table C-24.

Table C-24: Combination of TCR, TA1, TA2 and Alb binding building blocks in multispecific polypeptides.

T cell ISV	TA1 ISV	TA2 ISV	ALB-ISV
TCR	CEA	Irr	+
TCR	CEA	Irr	-
TCR	CEA	EGFR	+
TCR	CEA	EGFR	-
TCR	Irr	EGFR	+

TCR	Irr	EGFR	-

In order to test half-life extension, an Alb Nanobody is included as well in the polypeptides as set out in Table C-24.

To demonstrate the specific killing, a mixed cell culture assay system is used where TA1 single positive (e.g. MC38-huCEA or MKN45) and TA2 single positive tumour cells (e.g. Hela or Her14) are co-incubated. The expression level of the respective tumour antigens was determined in different cell lines and is represented in Figure 31. Upon addition of the polypeptides of the invention, primary human T cells and albumin if required, the T cell mediated cytotoxicity is monitored based on a cytometric read out. A comparison is made with respect to double negative cells or formats containing one or more irrelevant Nanobodies.

In order to verify the specific killing, the induced killing of double positive tumour (for TA1 and TA2, e.g. LS174T or LoVo) cells is compared with the induced killing of single positive tumour cells. For this, a T cell mediated cytoxicity assay is used as described above with a single type of tumour cells positive for both markers (cf. Example 19).

Example 22: Targeting of tumour cells with multispecific T cell engaging polypeptides

As mentioned above, the therapeutic activity of T cell engaging strategy can be improved by the simultaneous targeting of multiple tumour associated antigens. Not only do tumour cells create an escape mechanism by the down-regulation of targeted antigens within a therapy, but also by introducing (point-)mutations. Also in this case, simultaneous targeting of multiple epitopes on an antigen is likely to reduce the probability of generating tumour escape variants. Moreover, targeting multiple epitopes on a single antigen can increase the affinity of binding (avidity effect).

As the Nanobody platform is ideally suited to combine different specificities into a multivalent format, the anti-TCR Nanobodies of the invention are combined into formats illustrating these concepts, *i.e.* with different tumour antigen binding Nanobodies in a multispecific polypeptide.

For the multivalent tumour antigen targeting concept, two Nanobodies reactive towards an antigen are linked (TA1 and TA2, respectively), followed by a TCR reactive Nanobody. The specific order of the building blocks is varied within the format as well as the applied linker lengths in between the different building blocks. Combinations of TA1 and TA2 which are tested are depicted in Table C-25.

Table C-25: Combination of TCR, TA1, TA2 and Alb binding building blocks in multispecific polypeptides.

T cell ISV	TA1 ISV	TA2 ISV	ALB-ISV
TCR	EGFR-1 (7D12)	EGFR-2 (9G08)	+
TCR	EGFR-1 (7D12)	EGFR-2 (9G08)	-
TCR	Her2-1 (5F07)	Her2-2 (47D05)	+
TCR	Her2-1 (5F07)	Her2-2 (47D05)	-

In order to test half-life extension, an albumin binding Nanobody is included as well in the polypeptides as set out in Table C-25.

The potency and efficacy of these multivalent formats is evaluated and compared with the respective bispecific formats in an in vitro tumour cell killing assay comparable to the assay described in Example 10 but with the relevant cell lines (e.g. Hela, Her14, Ls174T, SKBR3, MCF7). Additionally, the effector-target ratio is varied such that an estimate is made whether a multivalent/multispecific polypeptide has a higher efficacy with lower effector target ratios.

Example 23: **Binding of monovalent Nanobodies and multispecific polypeptides to cells in flow cytometry**

As described earlier, the therapeutic activity of T cell engaging strategy can be improved by the simultaneous targeting of multiple tumour associated antigens, as tumour cells often create an escape mechanism by the down-regulation of targeted antigens within a therapy. The simultaneous targeting of multiple antigens is likely to reduce the generation of tumour escape variants.

For this double tumour antigen targeting concept, a Nanobody reactive towards a first tumour antigen (EGFR) was linked to a second Nanobody with different specificity (CEACAM5), in combination with a TCR reactive Nanobody. The specific order of the building blocks was varied within the format. The effector and tumour Nanobodies were genetically linked with 35GS linker and subsequently expressed in the yeast *Pichia* according to standard protocols. Irrelevant constructs were generated by replacing the tumour Nanobody with an irrelevant anti-egg lysozyme (cAblys) Nanobody (Table C-26).

Table C-26: Sample ID and description of multispecific polypeptides.

Sample ID	SEQ ID NO	TAA1	TAA2	T cell ISV	Description
T017000107	390	EGFR	CEACAM5	TCR	EGFR038G07-35GS-NbCEA5-35GS-T0170056G05-FLAG3-HIS6
T017000109	391	Irrelevant	CEACAM5	TCR	cAbLys3(D1E,Q5V,A6E,Q108L)-35GS-NbCEA5-35GS-T0170056G05-FLAG3-HIS6
T017000110	392	EGFR	Irr	TCR	EGFR038G07-35GS-cAbLys3(D1E,Q5V,A6E,Q108L)-35GS-

					T0170056G05-FLAG3-HIS6

Dose-dependent binding of the monovalent Nanobodies and multispecific polypeptides to cancer cell lines expressing CEACAM5 and EGFR (LoVo; ATCC CCL-229 and LS174T; ECACC 87060401), a cell line expressing EGFR (HER14; NIH3T3 (ATCC CRL-1658) transfected with EGFR), and to purified primary human T cells (isolated as described in Example 2.1) was evaluated in flow cytometry as outlined in Example 5. The results are presented in Figure 32.

The expression level of the respective tumour antigens on the different cells was determined in flow cytometry using 100nM of a monovalent anti-EGFR Nanobody (EGFR038G07) and a monovalent anti-CEA Nanobody, as described in Example 5. Results are shown in Figure 33.

The EC50 values obtained from the dose response curve for binding HER14 cells are depicted in Table C-27. The EC50 values obtained from the dose response curve for binding LS174T and LoVo cells are depicted in Table C-28.

Table C-27: EC50 (M) of monovalent Nanobodies and multispecific polypeptides for binding HER14 cells as determined in flow cytometry.

	HER14			
sample ID	**EC50 (M)**	**95% LCI**	**95% UCI**	**Top**
T017000107	1.6E-09	1.4E-09	1.9E-09	146111
T017000109	/	/	/	/
T017000110	7.3E-09	6.4E-09	8.3E-09	164582
EGFR038G07	1.8E-09	1.6E-09	2.1E-09	166237
NbCEA5	/	/	/	

Table C-28: EC50 (M) of monovalent Nanobodies and multispecific polypeptides for binding LoVo and LS174T cells as determined in flow cytometry.

	LS174T				LoVo			
sample ID	**EC50 (M)**	**95% LCI**	**95% UCI**	**Top**	**EC50 (M)**	**95% LCI**	**95% UCI**	**Top**
T017000107	1.1E-08	9.7E-09	1.3E-08	64605	3.2E-09	2.7E-09	3.7E-09	88604
T017000109	2.5E-08	2.2E-08	2.9E-08	50982	3.6E-08	1.4E-08	9.1E-08	18514
T017000110	6.3E-09	5.5E-09	7.2E-09	40779	2.4E-09	2.1E-09	2.8E-09	95866
EGFR038G07	9.3E-10	8.1E-10	1.1E-09	32191	5.4E-10	4.8E-10	6.2E-10	52268
NbCEA5	5.9E-10	5.3E-10	6.6E-10	42978	1.2E-09	6.4E-10	2.4E-09	11238

The data showed binding of the EGFR monovalent Nanobody and of the multispecific polypeptides containing the EGFR building block to the HER14 cells, expressing only EGFR. No binding of the

CEACAM monovalent Nanobody and the multispecific polypeptides containing only the CEACAM5 tumour anchor building block was observed. All monovalent and multispecific polypeptides showed binding to the EGFR, CEACAM5 double positive cell lines LS174T and LoVo, as expected. There was also binding of the multispecific polypeptides to the human primary T cells. A drop in affinity of the multispecific polypeptides versus the monovalent TCR building block was observed due to the C-terminal position of the TCR building block.

Example 24: Binding of monovalent Nanobodies to human EGFR and CEACAM5 protein (SPR)

Binding affinity of the purified EGFR monovalent Nanobody was evaluated by means of a surface plasmon resonance (SPR) based affinity determination on a Biacore T100 instrument. Thereto, hEGFR (Sino Biological, #10001-H08H) was immobilized onto a CM5 chip via amine coupling, using EDC and NHS chemistry. Purified Nanobodies were injected for 2 minutes at different concentrations (between 1.37 and 3000 nM) and allowed to dissociate for 15 min at a flow rate of 45 µl/min. In between sample injections, the surfaces were regenerated with 50mM NaOH. HBS-EP+ (Hepes buffer pH7.4) was used as running buffer.

Binding affinityies of the purified CEACAM5 monovalent Nanobody was evaluated by means of an SPR based affinity determination on a Biacore T100 instrument. Thereto, hCEACAM-5 (R&D Systems, # 4128-CM) was immobilized onto a CM5 chip via amine coupling, using EDC and NHS chemistry. Purified Nanobodies were injected for 2 minutes at different concentrations (between 0.31 and 2000 nM) and allowed to dissociate for 15 min at a flow rate of 45 µl/min. In between sample injections, the surfaces were regenerated with 10 mM Glycine pH 1.5. HBS-EP+ (Hepes buffer pH7.4) was used as running buffer.

The kinetic constants were calculated from the sensorgrams using the BIAEvaluation software (1:1 interaction). The affinity constants (KD) were calculated from resulting association and dissociation rate constants kon and koff, and are shown in Table C-29.

Table C-29: Affinity constant of monovalent Nanobodies for binding hEGFR and hCEACAM5, determined in Biacore using directly coated proteins.

	hCEACAM5			hEGFR ECD		
	kon (1/Ms)	Koff (1/s)	KD (M)	kon (1/Ms)	koff (1/s)	KD (M)
EGFR038G07	/	/	/	5.58E+05	4.26E-04	7.63E-10
NbCEA5	9.9E+05	5.1E-04	5.1E-10	/	/	/

Example 25: Redirected cell killing of multispecific polypeptides by human effector T cells in the xCELLigence based assay

The multispecific polypeptides were functionally characterized in a human T cell mediated EGFR/CEACAM positive tumour cell killing assay. In short, the multispecific constructs were evaluated in a xCELLigence based killing assay essentially as described in Example 16, using 6x10^5 human T cells as effector cells and 4x10^4 LS174T cells (ECACC 87060401) or LoVo cells (ATCC CCL-229) respectively as target cells (effector to target of 15 to 1). Data were analysed after 30-40h and after 50-60h.

The results are depicted in Figure 34. The IC50 values obtained in this assay are listed in Tables 30 and 31.

Table 30: IC50 (M) of the multispecific polypeptides in the human T cell mediated xCELLigence based killing assay using an effector to target ratio of 15 .Data were analysed after 30-40h.

	LoVo				LS174T			
ID construct	**n**	**IC50 (M)**	**95% LCI**	**95% UCI**	**n**	**IC50 (M)**	**95% LCI**	**95% UCI**
T017000107	1	3.0E-10	2.6E-10	3.6E-10	1	1.0E-08	6.1E-09	1.7E-08
T017000109	1	9.3E-09	6.7E-09	1.3E-08	1	1.0E-07	4.9E-08	2.2E-07
T017000110	1	1.5E-09	1.3E-09	1.9E-09	1	2.6E-08	1.1E-08	6.0E-08

The data on the EGFR+/CEA+ LoVo cells showed a ~28 fold difference in potency between the CEACAM5 only and the EGFR-CEA multispecific constructs and a ~8 fold difference in potency between the EGFR only constructs and the EGFR-CEA multispecific constructs. On EGFR+/CEA+ LS174T cells, a ~7 fold difference in potency between the CEACAM5 only and the EGFR-CEA multispecific constructs was observed and ~2 fold difference between the EGFR only constructs and the EGFR-CEA multispecific constructs. These results showed that potency enhancements were obtained with multispecific constructs reactive against two different antigens present on a cell.

Example 26: In vivo B cell depletion by half-life extended (HLE) polypeptides in a Ramos B cell depletion model

In this B cell depletion model, Burkitt's lymphoma Ramos cells and human PBMC were injected respectively intravenously and intraperitoneally in to mice after which Ramos B cell and PBMC-derived B cell killing by Nanobody-mediated recruitment of T cells present in PBMC population was evaluated: i.e. the potential of Nanobodies, HLE and non-HLE (NHLE), to activate T cells by direct linking of T cells via TCR to target B cells via CD20, resulting in target cell killing.

The polypeptides used in this study are described in Table 32:

Table 32: Sample ID and description of multispecific polypeptides used in in vivo study.

Sample ID	SEQ ID NO	TAA	TCR ISV	HLE	Description
T017000083	338	CD20	TCR	NHLE	20CD019C07(E1D)-35GS-T0170055A02-A
T017000088	339	Irr	TCR	NHLE	RSV007B02(E1D)-35GS-T0170055A02-A
T017000104	387	CD20	TCR	HLE	20CD019C07(E1D)-35GS-T0170055A02-35GS-ALB11-A
T017000105	388	CD20	TCR	NHLE	20CD019C07(E1D)-35GS-T0170056G05-A
T017000106	389	Irr	TCR	HLE	RSV007B02(E1D)-35GS-T0170055A02-35GS-ALB11-A

The *in vivo* efficacy of a HLE bispecific Nanobody, T017000104 (TCR and CD20-specific coupled to an albumin targeting building block) in a Ramos NOG mouse model was evaluated and compared with the irrelevant Nanobody T017000106. The non-half-life extended (NHLE) Nanobody T017000105 (TCR and CD20-specific) and T0107000083 (TCR and CD20-specific) were compared to the NHLE irrelevant Nanobody T017000088 (irrelevant and TCR-specific). The study demonstrated a statistically significant effect in bone marrow and spleen on PBMC derived B cell depletion for T017000104 and T017000105 compared to their respective irrelevant control NBs. The Ramos cells were significantly reduced in T017000104 and T017000105 treated mice, both in spleen and bone marrow.

In detail, the B cell depletion was evaluated in the mice, intravenously injected with Ramos cells at day one (D1) and intraperitoneally with PBMCs at D3. Mice were treated from D3 to D7 (Figure 35). Tumors were induced by intravenous injection of 10^6 Ramos cells in 200 µL of Roswell Park Memorial Institute (RPMI) 1640 medium (D1) 24 hours after a whole body irradiation of mice with a ɣ-source (1.44 Gy, 60Co) (D0). PBMCs were injected on D3 (i.e. two days after tumor cell injection) after randomization of the mice into groups each of 12 animals (11 animals for group 6) based on body weight. The animals received one single intraperitoneal (IP) injection of 10^7 PBMCs (500 µL in PBS). The treatment started on D3 one hour after PBMC injection and was repeated for 5 consecutive days in total for groups 1-4 and 6, whereas group 5 received only two injections (D3 and D5).

On D20 or on D21, the mice were sacrificed and spleen and bone marrow (femur) were collected for FACS analysis (mCD45, hCD45, hCD19, hCD20, hCD10) and analyzed for presence of Ramos B cells and PBMC-derived B cells.

Results for Ramos B cell depletion are represented in Figure 36. Test items T017000083 and T017000105, and T017000104 were compared to their respective control groups (groups 1 and 2) based on presence/absence of HLE. In bone marrow, a statistically significant difference in Ramos B cell number between the control Nanobodies and the NHLE Nanobodies T017000083 and T017000105 was observed (Figure 36A). In spleen the difference was significant only for T017000105 (Figure 36B). The HLE Nanobody T017000104 significantly reduced the Ramos cells in spleen and bone marrow irrespective of the dosing frequency applied (Figures 36A and B). Statistical analysis has been performed with F-tests from the mixed-effects ANOVA analysis.

Results for PBMC-derived B cell depletion are represented in Figure 37. In bone marrow, a reduction was observed in absolute B cell count in all treatment groups, compared to the irrelevant control Nanobody and this effect was significant for Nanobodies T017000083 and T017000104 (Figure 37 A). In the spleen, the effect was more pronounced and significant for all treatment groups (Figure 37 B).

In conclusion, these results demonstrate that both the anti-CD20/anti-TCR polypeptide and the HLE anti-CD20/anti-TCR polypeptide are able to significantly decrease Ramos B cells and PBMC-derived B cells in spleen and bone marrow in this B cell depletion model. This confirms the Nanobody-induced T cell activation by cross-linking T cells to target B cells and killing of the latter.

Example 27: Role of human effector CD4+ and CD8+ T cells in T cell activation

To investigate the role of CD4+, respectively CD8+ human T cells in the redirected killing, targeting of tumour cells with multispecific T cell engaging polypeptides was performed using T cell subsets. In this case, the HER2-positive cell line SKBR3 and Nanobody T017000102 (targetting HER2 and TCR) were used.

27.1 Redirected cell killing of multispecific polypeptides by human effector CD4+ and CD8+ T cells in the xCELLigence based assay

Human T cells were collected as described in Example 2.1. After thawing, human CD4+, respectively CD8+ T cells were isolated using the CD4+ (Miltenyi Biotec #130-096-533), respectively CD8+ (Miltenyi Biotech #130-096-495) T cell isolation kit. SKBR3 cells, were seeded in 96well E-plates ($2x10^4$ cells/well) and incubated with $3x10^5$ human primary effector T cells, human primary effector CD4+ T cells or human primary effector CD8+ T cells (effector target ratio of 15:1) in the presence or absence of multispecific constructs and followed over time, as described in Example16. Data were analysed after 40h.

Results are shown in Figure 38. The IC50 values are depicted in Table 33.

Table 33: IC50 (M) of the multispecific polypeptides in the xCELLigence based human T cell mediated SKBR3 killing assay using an effector to target ratio of 15 to 1.

	IC50 (M)	95% LCI	95% UCI
T cells	2.4E-11	1.9E-11	3.0E-11
CD8+ T cells	2.9E-11	2.1E-11	4.1E-11
CD4+ T cells	1.2E-11	1.1E-11	1.3E-11

The data showed dose dependent specific killing of HER2-positive tumour cell lines by directing human primary T cells, human CD4+ or human CD8+ T cells to the tumour cells via the TCR polypeptide.

27.2 Effect of HER2/TCR binding polypeptides on CD69 and CD25 expression on human effector CD4+ and CD8+ T cells in a HER2-positive tumour cell killing assay

Primary human T cells and CD4+ and CD8+ T cell subpopulations were isolated and a redirected HER2-positive tumour cell killing assay using SKBR3 cells was performed as described in Example 27.1. T cell activation was determined by measuring CD69 and CD25 upregulation after 24h and 72h of incubation respectively on the human primary T cells and on the CD4+ and CD8+ human T cell populations. CD69 and CD25 expression was measured in flow cytometry, using monoclonal mouse anti-human CD69PE (BD Biosciences # 557050) and mouse anti-human CD25PE (BD Pharmigen #557138) antibody, for CD69 and CD25 measurement respectively.

Exemplary results are shown in Figure 39.

The data showed dose dependent upregulation of CD69 and CD25 on human primary T cells, human CD4+ and human CD8+ T cells.

27.3 Effect of HER2/TCR binding polypeptides on IFN-γ and IL-6 release by human effector CD4+ and CD8+ T cells in a HER2-positive tumour cell killing assay

Primary human T cells and CD4+ and CD8+ T cell subpopulations were isolated and a redirected HER2-positive tumour cell killing assay using SKBR3 cells was performed as described in Example 27.1. The release of the cytokine IFN−γ was measured by ELISA as described in Example 17 and the release of IL-6 was measured in ELISA using the human IL-6 Quantikine ELISA Kit (R&D Systems, #D6050) according to manufactures instructions.

Exemplary results are shown in Figure 40.

The data showed dose dependent IFN-γ and IL-6 release by human primary T cells, human CD4+ and human CD8+ T cells.

Example 28: Exploration of half-life extension (HLE)

It was hypothesized that HLE via albumin binding might be suitable to comply with various requirements, including (i) half-life extension (HLE) of the moiety; and (ii) efficacy of the multispecific polypeptide. Preferably, the HLE function would not impair the penetration of tumours and tissues.

Alb11 (SEQ ID NO: 404), a Nanobody binding to human serum albumin (HSA) was linked to the multispecific EGFRxCEAxTCR binding polypeptide to increase the in vivo half-life of the formatted molecule (WO 06/122787). A format was generated with the albumin targeting Nanobody at the C-terminus using a 35GS linker and expressed as indicated above. The explored format is shown in Table 34.

Table 34: Sample ID and description of the multispecific HLE polypeptide.

Sample ID	SEQ ID NO	Description
T017000108	486	EGFR038G07-35GS-NbCEA5-35GS-T0170056G05-35GS-ALB11-FLAG3-HIS6

As the addition of the Alb11 Nanobody might influence the affinity or potency of the construct, the half-life extended multispecific polypeptide was characterized for binding to EGFR and CEA expressing cell lines and primary human T cells. In addition, the potency in the functional T cell dependent LS174T killing assay was evaluated (described in 28.1 and 28.2 below).

28.1 Impact of the Alb11 building block on the binding properties

Dose-dependent binding of the HLE multispecific polypeptide to cancer cell lines expressing CEACAM5 and EGFR (LS174T and LoVo), a cell line expressing EGFR (HER14; NIH3T3 transfected with EGFR), and to purified primary human T cells (isolated as described in Example 2.1) was evaluated in flow cytometry as outlined in Example 5, in the absence of HSA.

The results are presented in Figure 41**.** The EC50 value obtained from the dose response curve for binding to HER14 cells is depicted in Table 35. The EC50 values obtained from the dose response curves for binding to LS174T and LoVo cells are depicted in Table 36.

Table 35: EC50 (M) of T017000108 for binding to HER14 cells as determined in flow cytometry.

	HER14			
sample ID	EC50 (M)	95% LCI	95% UCI	Top
T017000108	1.9E-09	1.7E-09	2.2E-09	158884

Table 36: EC50 (M) of T017000108 for binding to LoVo and LS174T as determined in flow cytometry.

	LS174T				LoVo			
sample ID	EC50 (M)	95% LCI	95% UCI	Top	EC50 (M)	95% LCI	95% UCI	Top
T017000108	1.4E-08	1.2E-08	1.6E-08	70202	3.3E-09	2.9E-09	3.9E-09	92787

Comparison of the HLE construct with the non-HLE construct showed similar binding to all three cell lines tested. The data presented showed that coupling of the Alb11 building block did not influence the binding properties.

28.2 Impact of the Alb11 Nanobody in the redirected cell killing by human effector T cells in the xCELLigence based assay

The functionality of the half-life extended multispecific polypeptide was evaluated in the absence of HSA in the human T cell mediated LS174T killing assay as described in Example 10 and compared with the functionality of the non-HLE multispecific constructs.

The results are depicted in Figure 42. The obtained IC50 values are listed in Table 37.

Table 37: IC50 (M) of the multispecific polypeptides in the human T cell mediated xCELLigence based killing assay using an effector to target ratio of 15. Data were analysed after 40-50h.

sample ID	IC50 (M)	95% LCI	95% UCI
T017000107	7.9E-10	5.9E-10	1.1E-09
T017000108	5.5E-09	4.1E-09	7.3E-09

The results indicate that the inclusion of the albumin targeting Nanobody in the construct as such did not have an essential impact on the obtained potency or efficacy.

TABLES

Table A-1: Sequence alignment of TCR cluster A binders – part 1

```
                                     1        10        20        30        40        50        60        70       78
                  Kabat #          : |        |         |         |         |         |         |         |        |
SEQ ID NO: 50     T0170PMP056G05 : EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMV
SEQ ID NO: 8      T0170PMP053D01 : ..................K...A...A............T.E....M..T.T...DV.............A....
SEQ ID NO: 60     T0170PMP067D01 : ..................K...A...A............T.E....M..T.T...EV.............A....
SEQ ID NO: 47     T0170PMP056F01 : ..................K.P.A...A............T.E....M..T.T...EV.............A....
SEQ ID NO: 76     T0170PMP069A06 : ..................K...A...A............T.E....M..T.T...EV..E..........A....
SEQ ID NO: 65     T0170PMP067F02 : ..................K...A...A............T.E....M..T.T...EV...H.........A....
SEQ ID NO: 66     T0170PMP068C03 : ..................K...A...A............T.E....M..T.T...EVA............A....
SEQ ID NO: 26     T0170PMP055E05 : ..................K...A...A..............E....M..T.T...EV.............A....
SEQ ID NO: 19     T0170PMP055C02 : ..................K...A...A............T.E....M....T...EV.............A....
SEQ ID NO: 61     T0170PMP067D06 : ..................K...A...A..............E....M..T.T...EV.............AT...
SEQ ID NO: 50     T0170PMP069C08 : ..............................................................................
SEQ ID NO: 28     T0170PMP055F03 : ................................I....................S......E.................
SEQ ID NO: 54     T0170PMP061A02 : ................................I..................A.S......E.................
SEQ ID NO: 45     T0170PMP056D11 : ................................I................R...S......E.................
SEQ ID NO: 77     T0170PMP069B02 : ............R...................I....................S......E.................
SEQ ID NO: 96     T0170PMP070G02 : ................................I....................S......E.................
SEQ ID NO: 2      T0170PMP028B01 : ......................A...S..................GL..T.T...T.....Y...........AR...
SEQ ID NO: 4      T0170PMP028G06 : ......................A...S...................L..T.T...T.....Y...........AR...
SEQ ID NO: 29     T0170PMP055F06 : ......................A...S...................L..T.T...A.....Y...........AR...
SEQ ID NO: 1      T0170PMP027A05 : ......................A...S...................L..T.T...T.................A....
SEQ ID NO: 6      T0170PMP040C01 : ......................A...S...................L..T.T...T.................A....
SEQ ID NO: 3      T0170PMP028F10 : ...............R....P.A...S...................M..T.T...A.................A....
SEQ ID NO: 5      T0170PMP029F08 : ......................A...S...................M..T.T...A.................A....
SEQ ID NO: 13     T0170PMP055A08 : ..........S...........A...S...................M..T.T...A.................A....
SEQ ID NO: 32     T0170PMP055G09 : ..........S...........A...S...................V..T.T...A.................A....
SEQ ID NO: 69     T0170PMP068D05 : ..........S........P..A...S...................M..T.T...A.................A....
SEQ ID NO: 75     T0170PMP068F08 : ..........S...........A...S..................GM..T.T...A.................A....
SEQ ID NO: 95     T0170PMP070F11 : ..........S...........A...S..........C........M..T.T...A.................A....
SEQ ID NO: 87     T0170PMP069E11 : ......................A...E.....I......C.....DM..T.T...A.................A....
SEQ ID NO: 99     T0170PMP084B07 : ......................A...E.....I............DM..T.T...E.Q...............A....
SEQ ID NO: 10     T0170PMP055A01 : ............R.........A.........I........A....M....T...A.................A....
SEQ ID NO: 11     T0170PMP055A02 : ......................A.........I........A....M....T...A.................A....
SEQ ID NO: 22     T0170PMP055D03 : ......................A.........I........A....M....T...A.N...............A....
SEQ ID NO: 24     T0170PMP055D10 : ......................A.........I........A....M....T...A....G............A....
```

Table A-2: Sequence alignment of TCR cluster A binders – part 1 continued

```
                                              79           90        100        110
                         Kabat #          : |   ab         |         |a         |
SEQ ID NO: 50            T0170PMP056G05 : YLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
SEQ ID NO: 8             T0170PMP053D01 : ....T.........V...G..L...N.............
SEQ ID NO: 60            T0170PMP067D01 : ....T.........V...G..L...N.............
SEQ ID NO: 47            T0170PMP056F01 : ....T.........V...G..L...N.............
SEQ ID NO: 76            T0170PMP069A06 : ....TG........V...G..L...N.............
SEQ ID NO: 65            T0170PMP067F02 : ....T.........V...G..L...N.......Q.....
SEQ ID NO: 66            T0170PMP068C03 : ....T.........V...G..L...N.......Q.....
SEQ ID NO: 26            T0170PMP055E05 : ....T.......T.V...G..L...N.......Q.....
SEQ ID NO: 19            T0170PMP055C02 : ....T..T......V...G..L...N.......Q.....
SEQ ID NO: 61            T0170PMP067D06 : ....T.............G......N.......Q.....
SEQ ID NO: 50            T0170PMP069C08 : .......................................
SEQ ID NO: 28            T0170PMP055F03 : ..............L........................
SEQ ID NO: 54            T0170PMP061A02 : ..............L........................
SEQ ID NO: 45            T0170PMP056D11 : ..............L........................
SEQ ID NO: 77            T0170PMP069B02 : ..............L..................Q.....
SEQ ID NO: 96            T0170PMP070G02 : ...........A..L..................Q.....
SEQ ID NO: 2             T0170PMP028B01 : ..................G..L...N.......Q.....
SEQ ID NO: 4             T0170PMP028G06 : ..................G..L...N.............
SEQ ID NO: 29            T0170PMP055F06 : ..................G..L...N.............
SEQ ID NO: 1             T0170PMP027A05 : ..............H...G..L...N.......Q.....
SEQ ID NO: 6             T0170PMP040C01 : ..................G..L...N.......Q.....
SEQ ID NO: 3             T0170PMP028F10 : ..................G..L...N.......Q.....
SEQ ID NO: 5             T0170PMP029F08 : ..................G..L...N.............
SEQ ID NO: 13            T0170PMP055A08 : .......S..........G..L...N.............
SEQ ID NO: 32            T0170PMP055G09 : .......S..........G..L...N.......Q.....
SEQ ID NO: 69            T0170PMP068D05 : .......S..........G..L...N.......Q.....
SEQ ID NO: 75            T0170PMP068F08 : .......S..........G..L...N.......Q.....
SEQ ID NO: 95            T0170PMP070F11 : .......S..........G..L...N.......Q.....
SEQ ID NO: 87            T0170PMP069E11 : ..................L..L...N.............
SEQ ID NO: 99            T0170PMP084B07 : ..................L..L...N.............
SEQ ID NO: 10            T0170PMP055A01 : ..................Y......N.............
SEQ ID NO: 11            T0170PMP055A02 : ..................Y......N.............
SEQ ID NO: 22            T0170PMP055D03 : ..................Y......N.............
SEQ ID NO: 24            T0170PMP055D10 : ..................Y......N.............
```

Table A-3: Sequence alignment of TCR cluster A binders – part 2

```
                                                1         10        20        30        40        50        60        70       78
                              Kabat #         : |         |         |         |         |         |         |         |        |
SEQ ID NO: 50     T0170PMP056G05 : EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMV
SEQ ID NO: 71     T0170PMP068E01 : ...............E......A.........I........A....M...T...A.................A....
SEQ ID NO: 101    T0170PMP084E03 : ......................A.........I........A....M...T...A.................A....
SEQ ID NO: 102    T0170PMP084E05 : ......................A.........I...H....A....M...T...A.................A....
SEQ ID NO: 30     T0170PMP055F08 : ......................A.........I........A....M...T...A...........A.....A....
SEQ ID NO: 37     T0170PMP056C01 : ......................A...G.....I........A....M...T...A.................A....
SEQ ID NO: 49     T0170PMP056G02 : ...........A..........A.........I........A....M...T...A.................A....
SEQ ID NO: 57     T0170PMP067A03 : ......................A.........I........A..H.M...T...A.................A....
SEQ ID NO: 59     T0170PMP067C09 : ......................A.........I........A....M...T...A.................AE...
SEQ ID NO: 74     T0170PMP068F06 : ......................A.........I........A....M...T...A.................A..V.
SEQ ID NO: 82     T0170PMP069D02 : ..........M...........A.........I........A....M...T...A.................A....
SEQ ID NO: 93     T0170PMP070D07 : ......................A.........I........A....M...T...A.................A....
SEQ ID NO: 100    T0170PMP084C02 : ......................A.........I........A....M...T...A.................A....
SEQ ID NO: 55     T0170PMP061B04 : ......................A.........I........A....M...T...A.................A....
SEQ ID NO: 83     T0170PMP069D07 : ......................A.........I......V.A....M...T...A.................A....
SEQ ID NO: 36     T0170PMP056B11 : .............A....T...A.........I........A....M...T...A.................A....
SEQ ID NO: 104    T0170PMP084F10 : ..........W..A........A.........I........A....M...T...A.................A....
SEQ ID NO: 39     T0170PMP056C03 : ......................A.........I........A....M...T...T.................A....
SEQ ID NO: 80     T0170PMP069C04 : ......................A.........I........A.G..M...T...A.................A....
SEQ ID NO: 44     T0170PMP056D02 : ......................A.........I........A....M...T...A.................A....
SEQ ID NO: 56     T0170PMP067A01 : ......................A.........I........A...GM...T...A.................A....
SEQ ID NO: 58     T0170PMP067B06 : ......................A.........I........AR...M...T...A.................A....
SEQ ID NO: 88     T0170PMP069F05 : ......................A.........I........A....M...T...A.................A....
SEQ ID NO: 103    T0170PMP084F04 : ......................A.........I...H....A....M...T...A.................A....
SEQ ID NO: 21     T0170PMP055C10 : ......................A.........V........A....M...T...A.................A....
SEQ ID NO: 9      T0170PMP053E10 : ......................R.........I........A....M...T...A....E............A....
SEQ ID NO: 15     T0170PMP055B01 : ......................R.........I........A....M...T...A.V..E............A....
SEQ ID NO: 79     T0170PMP069C01 : ......................R.........I........A....MI...T...A....E............A....
SEQ ID NO: 62     T0170PMP067D09 : ......................A.........I........A....M...T...A.S..G............A....
SEQ ID NO: 63     T0170PMP067E03 : ......................A.........I........A....M...T...A.S...............A....
SEQ ID NO: 43     T0170PMP056D01 : .........D............A.......................M...T.A.A....EF...........P....
SEQ ID NO: 64     T0170PMP067E06 : .........D............A.......................M...T.A.A....EF...........P....
SEQ ID NO: 86     T0170PMP069E09 : ......................A.......................M...T.A.A....EF...........P....
SEQ ID NO: 81     T0170PMP069C05 : .........D............A.......................M...T.A.A....EF...........P....
SEQ ID NO: 90     T0170PMP070B08 : .........D............A.......................M...T.A.A....EF...........P....
SEQ ID NO: 12     T0170PMP055A03 : ...................................................T....A................A....
```

Table A-4: Sequence alignment of TCR cluster A binders – part 2 continued

```
                                    79               90         100         110
                 Kabat #          : |   ab           |          |a          |
SEQ ID NO: 50    T0170PMP056G05 : YLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
SEQ ID NO: 71    T0170PMP068E01 : ...................Y......N............
SEQ ID NO: 101   T0170PMP084E03 : .........G.........Y......N............
SEQ ID NO: 102   T0170PMP084E05 : ...................Y......N............
SEQ ID NO: 30    T0170PMP055F08 : ...................Y......N............
SEQ ID NO: 37    T0170PMP056C01 : ...................Y......N............
SEQ ID NO: 49    T0170PMP056G02 : ...................Y......N............
SEQ ID NO: 57    T0170PMP067A03 : ...................Y......N............
SEQ ID NO: 59    T0170PMP067C09 : ...................Y......N............
SEQ ID NO: 74    T0170PMP068F06 : ...................Y......N............
SEQ ID NO: 82    T0170PMP069D02 : ...................Y......N............
SEQ ID NO: 93    T0170PMP070D07 : ...........A.......Y......N............
SEQ ID NO: 100   T0170PMP084C02 : ....D..............Y......N............
SEQ ID NO: 55    T0170PMP061B04 : .............A.....Y......N............
SEQ ID NO: 83    T0170PMP069D07 : .............A.....Y......N............
SEQ ID NO: 36    T0170PMP056B11 : ...................Y......N............
SEQ ID NO: 104   T0170PMP084F10 : ...................Y......N............
SEQ ID NO: 39    T0170PMP056C03 : ...................Y......N......Q.....
SEQ ID NO: 80    T0170PMP069C04 : ...................Y......N......Q.....
SEQ ID NO: 44    T0170PMP056D02 : ...................Y......N...R..Q.....
SEQ ID NO: 56    T0170PMP067A01 : ...................Y......N......Q.....
SEQ ID NO: 58    T0170PMP067B06 : ...................Y......N......Q.....
SEQ ID NO: 88    T0170PMP069F05 : ...............L...Y......N......Q.....
SEQ ID NO: 103   T0170PMP084F04 : .............A.....Y......N......Q.....
SEQ ID NO: 21    T0170PMP055C10 : H..................Y......N......Q.....
SEQ ID NO: 9     T0170PMP053E10 : ...................Y......N......Q.....
SEQ ID NO: 15    T0170PMP055B01 : ...................Y......N......Q.....
SEQ ID NO: 79    T0170PMP069C01 : ...................Y......N......Q.....
SEQ ID NO: 62    T0170PMP067D09 : ...L.N.............Y......N............
SEQ ID NO: 63    T0170PMP067E03 : ...L.N.............Y......N............
SEQ ID NO: 43    T0170PMP056D01 : H..............L...G......N......Q.....
SEQ ID NO: 64    T0170PMP067E06 : ...............L...G......N......Q.....
SEQ ID NO: 86    T0170PMP069E09 : ...............L...G......N......Q.....
SEQ ID NO: 81    T0170PMP069C05 : ...............L...G......S......Q.....
SEQ ID NO: 90    T0170PMP070B08 : .........V.....L...G......N............
SEQ ID NO: 12    T0170PMP055A03 : ...................G.............Q.....
```

Table A-5: Sequence alignment of TCR cluster A binders – part 3

```
                                   1         10        20        30        40        50        60        70      78
                   Kabat #       : |         |         |         |         |         |         |         |       |
SEQ ID NO: 50      T0170PMP056G05 : EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMV
SEQ ID NO: 41      T0170PMP056C07 : ...................................................T....A................A....
SEQ ID NO: 92      T0170PMP070C09 : .................P.................................T....A................A....
SEQ ID NO: 98      T0170PMP082B04 : ...................P...............................T.A..A................A....
SEQ ID NO: 33      T0170PMP056A02 : .................A.................................T.....................A....
SEQ ID NO: 31      T0170PMP055G05 : ......................A.............H..............T...A.................A....
SEQ ID NO: 78      T0170PMP069B08 : ......................A.....Y.......H..............T...A.................A....
SEQ ID NO: 20      T0170PMP055C06 : ............H.........A.............H..P...........T...V.................A....
SEQ ID NO: 23      T0170PMP055D06 : ......................A.............H..............T...V.................A....
SEQ ID NO: 25      T0170PMP055E01 : ......................A...E.Y......................T.A.VA....F...........A....
SEQ ID NO: 27      T0170PMP055F02 : ......................A...E.Y......................T.A.VA....F...........A....
SEQ ID NO: 38      T0170PMP056C02 : ......................A...E.Y......................T.A.VA....F.Q.........A....
SEQ ID NO: 97      T0170PMP070G06 : ...................P..A...E.Y......................T.A.VA....F...........A....
SEQ ID NO: 89      T0170PMP069G08 : ......................A...E.Y.......Q..............T.A.VA....F...........A....
SEQ ID NO: 94      T0170PMP070E07 : ......................A...E.Y......................T.A.VA....F..........GA....
SEQ ID NO: 70      T0170PMP068D07 : ......................A...E.Y......................T.A.VA....F...........V....
SEQ ID NO: 51      T0170PMP056G11 : ......................A...E.Y......................T.A.AA....F..........GA....
SEQ ID NO: 67      T0170PMP068C07 : ......................A...E.Y......................T.A.AA....F...........A....
SEQ ID NO: 84      T0170PMP069E02 : ......................A...E.Y......................T.A.AA....F...........A....
SEQ ID NO: 68      T0170PMP068C11 : ......................A...E.Y......................T.A.AA....F...........A....
SEQ ID NO: 72      T0170PMP068E08 : ......................A...E.Y......................T.A.AA....F...........A....
SEQ ID NO: 46      T0170PMP056E02 : ..............E.......A...E.Y......................T.A.VA....F....L......A....
SEQ ID NO: 35      T0170PMP056A10 : ......................AV..S..LL..............GV....T.A.A...SHF...........A....
SEQ ID NO: 42      T0170PMP056C10 : ......................AV..S..LL...............M.T..T.A.A...SHF...........A....
SEQ ID NO: 16      T0170PMP055B02 : ......................A...S..LL........C......M....T.A.A...SHF...........A....
SEQ ID NO: 34      T0170PMP056A08 : ......................A...S..LL...............M....T.A.A...SHF...........A....
SEQ ID NO: 40      T0170PMP056C04 : ......................A...S..LL...............M....T.A.A...SHF...........A....
SEQ ID NO: 53      T0170PMP057D06 : ......................A...S..LL...............M....T.A.A...SHF...........A....
SEQ ID NO: 7       T0170PMP053A03 : ......................AV..S..LL...............M....T.A.A...SHF...........A....
SEQ ID NO: 85      T0170PMP069E07 : ......................A...S..LL...............M....T.A.A...SHF...........A....
SEQ ID NO: 18      T0170PMP055B11 : ......................A...S..LL...............M....T.A.A....HF...........A....
SEQ ID NO: 48      T0170PMP056F08 : ..................G...A...S..LL...............M....T.A.A....HF...........A....
SEQ ID NO: 91      T0170PMP070B09 : ......................A...S..LL....................T.A.A...SHF...........A....
SEQ ID NO: 73      T0170PMP068F04 : ......................A...S..LL...............M....T.A.V...SYF...........A....
SEQ ID NO: 14      T0170PMP055A10 : ......................A...S..LL...............M......A.A....HF...........A....
SEQ ID NO: 17      T0170PMP055B03 : ...................P..A...S..LL...............M....T.A.A....HF...........A....
SEQ ID NO: 52      T0170PMP057B02 : ..........W...........A...S.Y.....S.......H...L..T.T...AA................AR...
```

Table A-6: Sequence alignment of TCR cluster A binders – part 3 continued

```
                                            79            90         100         110
                         Kabat #          : |   ab        |          |a          |
SEQ ID NO: 50            T0170PMP056G05 : YLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
SEQ ID NO: 41            T0170PMP056C07 : ...................G..........R........
SEQ ID NO: 92            T0170PMP070C09 : ...................G...................
SEQ ID NO: 98            T0170PMP082B04 : ...................G...................
SEQ ID NO: 33            T0170PMP056A02 : ...................G...W.........Q.....
SEQ ID NO: 31            T0170PMP055G05 : ...................G.............Q.....
SEQ ID NO: 78            T0170PMP069B08 : ...................G...................
SEQ ID NO: 20            T0170PMP055C06 : .....N.............G.............Q.....
SEQ ID NO: 23            T0170PMP055D06 : F....N.............G.............Q.....
SEQ ID NO: 25            T0170PMP055E01 : ...................G...W.........Q.....
SEQ ID NO: 27            T0170PMP055F02 : ...................G...W...............
SEQ ID NO: 38            T0170PMP056C02 : ...................G...W.........Q.....
SEQ ID NO: 97            T0170PMP070G06 : ...................G...W.........Q.....
SEQ ID NO: 89            T0170PMP069G08 : ...................G...W...............
SEQ ID NO: 94            T0170PMP070E07 : ...................G...W...............
SEQ ID NO: 70            T0170PMP068D07 : ...................G...W...............
SEQ ID NO: 51            T0170PMP056G11 : ...................G...W.........Q.....
SEQ ID NO: 67            T0170PMP068C07 : ...................G...W.........Q.....
SEQ ID NO: 84            T0170PMP069E02 : ...................G...W...............
SEQ ID NO: 68            T0170PMP068C11 : ...................G.............Q.....
SEQ ID NO: 72            T0170PMP068E08 : .......R...........G...W.........Q.....
SEQ ID NO: 46            T0170PMP056E02 : ...................G...W...............
SEQ ID NO: 35            T0170PMP056A10 : .......R...........G.............Q.....
SEQ ID NO: 42            T0170PMP056C10 : .......R...........G.............Q.....
SEQ ID NO: 16            T0170PMP055B02 : .......R...........G...................
SEQ ID NO: 34            T0170PMP056A08 : .......R...........G...................
SEQ ID NO: 40            T0170PMP056C04 : .......R...........G..........H........
SEQ ID NO: 53            T0170PMP057D06 : .......R...........G.............Q.....
SEQ ID NO: 7             T0170PMP053A03 : .......R...........G...................
SEQ ID NO: 85            T0170PMP069E07 : .....G.R...........G...................
SEQ ID NO: 18            T0170PMP055B11 : .......R...........G...................
SEQ ID NO: 48            T0170PMP056F08 : .......R...........G...................
SEQ ID NO: 91            T0170PMP070B09 : .....N.R...........G...................
SEQ ID NO: 73            T0170PMP068F04 : .......R...........G.............Q.....
SEQ ID NO: 14            T0170PMP055A10 : .......R...........G..........R..Q.....
SEQ ID NO: 17            T0170PMP055B03 : .......R...........G.............Q.....
SEQ ID NO: 52            T0170PMP057B02 : .............L...H.G..L...N......Q.....
```

Table A-7: Sequence alignment of TCR cluster B binders

```
                                    1       10        20        30        40        50        60        70     77
                  Kabat #         : |    a   |         |         |         |         |         |         |      |
SEQ ID NO: 106    T0170PMP055C07 : EVQLVE-SGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATNYADSVKGRFTISEDSAKNT
SEQ ID NO: 105    T0170PMP055B06 : ......-...................................................D...................
SEQ ID NO: 115    T0170PMP070C01 : ......-.....................................................................D.
SEQ ID NO: 108    T0170PMP056B02 : ......-.................................T.....................................
SEQ ID NO: 109    T0170PMP056D07 : ......-....................Q..................................................
SEQ ID NO: 112    T0170PMP069E03 : ......-.......................................................................
SEQ ID NO: 111    T0170PMP068G05 : ......E.......................................................................
SEQ ID NO: 114    T0170PMP070A09 : ......-.......................................................................
SEQ ID NO: 107    T0170PMP055D01 : ......-.....................................R.................................
SEQ ID NO: 110    T0170PMP068B03 : ......-........................V........G.....................................
SEQ ID NO: 113    T0170PMP069E06 : ......-................L.......V........G.....................................

                                    78            90        100       110
                  Kabat #         : |    abc       |         |a        |
SEQ ID NO: 106    T0170PMP055C07 : VYLQMNSLKPEDTAVYFCNAKSRLYPYDYWGQGTLVTVSS
SEQ ID NO: 105    T0170PMP055B06 : .........A........................Q.....
SEQ ID NO: 115    T0170PMP070C01 : ........................................
SEQ ID NO: 108    T0170PMP056B02 : ..................................Q.....
SEQ ID NO: 109    T0170PMP056D07 : ..................................Q.....
SEQ ID NO: 112    T0170PMP069E03 : ..............................D...Q.....
SEQ ID NO: 111    T0170PMP068G05 : .......................I.......R..Q.....
SEQ ID NO: 114    T0170PMP070A09 : .......................I.......R..Q.....
SEQ ID NO: 107    T0170PMP055D01 : .......................I..........Q.....
SEQ ID NO: 110    T0170PMP068B03 : ...............................R..Q.....
SEQ ID NO: 113    T0170PMP069E06 : .....S............................Q.....
```

Table A-8: Sequence alignment of TCR cluster C binders

```
                                        1        10        20        30        40        50        60        70      78
                    Kabat #           : |        |         |         |         |         |         |         |       |
SEQ ID NO: 116      T0170PMP061G01    : EVQLVESGGGLVQPGGSLRLSCAASGEIGRINFYRWYRQAPGNQREVVATITIADKTDYADSAKGRFTISRDESRNMV
SEQ ID NO: 118      T0170PMP075D02    : ..............................................................................
SEQ ID NO: 117      T0170PMP062D02    : .............................................G..........I.....................

                                        79           90       100        110
                                        |   abc      |        |a         |
SEQ ID NO: 116      T0170PMP061G01    : YLQMSSLKPEDTAVYFCHAGSRLYPYDYWGQGTQVTVSS
SEQ ID NO: 118      T0170PMP075D02    : ..........N............................
SEQ ID NO: 117      T0170PMP062D02    : ....G..................................
```

Table A-4: Sequences for CDRs and frameworks, plus preferred combinations as provided in formula I, namely FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. "SEQ" refers to the given SEQ ID NO. The first column refers to the SEQ ID NO of the complete ISV, i.e. FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. CDR1, CDR2 and CDR3 were determined according to Kontermann, 2010.

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
1	T0170PMP027A05	175	EVQLVESGGGLVQPGGSLRLSCAAS	119	GSVHKINFLG	203	WYRQAPGKERELVA	134	TITIGDTTD	235	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVHFCRA	164	GSRLYPYNY	285	WGQGTQVTVSS
2	T0170PMP028B01	175	EVQLVESGGGLVQPGGSLRLSCAAS	119	GSVHKINFLG	204	WYRQAPGKERGLVA	134	TITIGDTTD	236	YADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRA	164	GSRLYPYNY	285	WGQGTQVTVSS
3	T0170PMP028F10	176	EVQLVESGGGLVQPGRSLRLPCAAS	119	GSVHKINFLG	205	WYRQAPGKEREMVA	135	TITIGDATD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	164	GSRLYPYNY	285	WGQGTQVTVSS
4	T0170PMP028G06	175	EVQLVESGGGLVQPGGSLRLSCAAS	119	GSVHKINFLG	203	WYRQAPGKERELVA	134	TITIGDTTD	236	YADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRA	164	GSRLYPYNY	286	WGQGTLVTVSS
5	T0170PMP029F08	175	EVQLVESGGGLVQPGGSLRLSCAAS	119	GSVHKINFLG	205	WYRQAPGKEREMVA	135	TITIGDATD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	164	GSRLYPYNY	286	WGQGTLVTVSS
6	T0170PMP040C01	175	EVQLVESGGGLVQPGGSLRLSCAAS	119	GSVHKINFLG	203	WYRQAPGKERELVA	134	TITIGDTTD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	164	GSRLYPYNY	285	WGQGTQVTVSS
7	T0170PMP053A03	177	EVQLVESGGGLVQPGGSLRLSCAVS	120	GSVHLLNFLG	205	WYRQAPGKEREMVA	136	HITIADATD	238	YSHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTLVTVSS
8	T0170PMP053D01	178	EVQLVESGGGLVQPGGSLKLSCAAS	121	GAVHKINFLG	206	WYRQTPEKEREMVA	137	TITIGDDVD	239	YADSAKGRFTISRDEAKNMVYLQMTSLKPEDTAVYVCRA	164	GSRLYPYNY	286	WGQGTLVTVSS
9	T0170PMP053E10	179	EVQLVESGGGLVQPGGSLRLSCRAS	122	GDVHKINILG	207	WYRQAPAKEREMVA	138	HITIGDATD	240	YAESAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQVTVSS
10	T0170PMP055A01	180	EVQLVESGGGLVRPGGSLRLSCAAS	122	GDVHKINILG	207	WYRQAPAKEREMVA	138	HITIGDATD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTLVTVSS
11	T0170PMP055A02	175	EVQLVESGGGLVQPGGSLRLSCAAS	122	GDVHKINILG	207	WYRQAPAKEREMVA	138	HITIGDATD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTLVTVSS
12	T0170PMP055A03	181	EVQLVESGGGLVQPGGSLRLSCVAS	123	GDVHKINFLG	208	WYRQAPGKEREKVA	139	HITIGDQAD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQVTVSS
13	T0170PMP055A08	182	EVQLVESGGGSVQPGGSLRLSCAAS	119	GSVHKINFLG	205	WYRQAPGKEREMVA	135	TITIGDATD	241	YADSAKGRFTISRDEAKNMVYLQMNSLSPEDTAVYFCRA	164	GSRLYPYNY	286	WGQGTLVTVSS
14	T0170PMP055A10	175	EVQLVESGGGLVQPGGSLRLSCAAS	120	GSVHLLNFLG	205	WYRQAPGKEREMVA	140	HISIADATD	242	YAHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	287	WGRGTQVTVSS
15	T0170PMP055B01	179	EVQLVESGGGLVQPGGSLRLSCRAS	122	GDVHKINILG	207	WYRQAPAKEREMVA	141	HITIGDATV	240	YAESAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQVTVSS
16	T0170PMP055B02	175	EVQLVESGGGLVQPGGSLRLSCAAS	120	GSVHLLNFLG	209	WYRQCPGKEREMVA	136	HITIADATD	238	YSHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTLVTVSS

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
17	T0170PMP055B03	183	EVQLVESGGGLVQPGGSLRPSCAAS	120	GSVHLLNFLG	205	WYRQAPGKEREMVA	136	HITIADATD	242	YAHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQVTVSS
18	T0170PMP055B11	175	EVQLVESGGGLVQPGGSLRLSCAAS	120	GSVHLLNFLG	205	WYRQAPGKEREMVA	136	HITIADATD	242	YAHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTLVTVSS
19	T0170PMP055C02	178	EVQLVESGGGLVQPGGSLKLSCAAS	121	GAVHKINFLG	206	WYRQTPEKEREMVA	143	HITIGDEVD	244	YADSAKGRFTISRDEAKNMVYLQMTSLTPEDTAVYVCRA	164	GSRLYPYNY	285	WGQGTQVTVSS
20	T0170PMP055C06	185	EVQLVESGGGLVHPGGSLRLSCAAS	123	GDVHKINFLG	211	WHRQPPGKEREKVA	144	HITIGDVTD	245	YADSAKGRFTISRDEAKNMVYLQMNNLKPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQVTVSS
21	T0170PMP055C10	175	EVQLVESGGGLVQPGGSLRLSCAAS	125	GDVHKINVLG	207	WYRQAPAKEREMVA	138	HITIGDATD	247	YADSAKGRFTISRDEAKNMVHLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQVTVSS
22	T0170PMP055D03	175	EVQLVESGGGLVQPGGSLRLSCAAS	122	GDVHKINILG	207	WYRQAPAKEREMVA	146	HITIGDATN	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTLVTVSS
23	T0170PMP055D06	175	EVQLVESGGGLVQPGGSLRLSCAAS	123	GDVHKINFLG	213	WHRQAPGKEREKVA	144	HITIGDVTD	248	YADSAKGRFTISRDEAKNMVFLQMNNLKPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQVTVSS
24	T0170PMP055D10	175	EVQLVESGGGLVQPGGSLRLSCAAS	122	GDVHKINILG	207	WYRQAPAKEREMVA	138	HITIGDATD	249	YAGSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTLVTVSS
25	T0170PMP055E01	175	EVQLVESGGGLVQPGGSLRLSCAAS	126	GEVYKINFLG	208	WYRQAPGKEREKVA	147	HITIADVAD	250	YADFAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	285	WGQGTQVTVSS
26	T0170PMP055E05	178	EVQLVESGGGLVQPGGSLKLSCAAS	121	GAVHKINFLG	214	WYRQAPEKEREMVA	148	TITIGDEVD	251	YADSAKGRFTISRDEAKNMVYLQMTSLKPEDTTVYVCRA	164	GSRLYPYNY	285	WGQGTQVTVSS
27	T0170PMP055F02	175	EVQLVESGGGLVQPGGSLRLSCAAS	126	GEVYKINFLG	208	WYRQAPGKEREKVA	147	HITIADVAD	250	YADFAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	286	WGQGTLVTVSS
28	T0170PMP055F03	181	EVQLVESGGGLVQPGGSLRLSCVAS	122	GDVHKINILG	208	WYRQAPGKEREKVA	149	HISISDQTD	252	YAESAKGRFTISRDESKNMVYLQMNSLKPEDTAVYLCRA	170	FSRIYPYDY	286	WGQGTLVTVSS
29	T0170PMP055F06	175	EVQLVESGGGLVQPGGSLRLSCAAS	119	GSVHKINFLG	203	WYRQAPGKERELVA	135	TITIGDATD	236	YADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRA	164	GSRLYPYNY	286	WGQGTLVTVSS
30	T0170PMP055F08	175	EVQLVESGGGLVQPGGSLRLSCAAS	122	GDVHKINILG	207	WYRQAPAKEREMVA	138	HITIGDATD	253	YADSAKGRFAISRDEAKNMVYLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTLVTVSS
31	T0170PMP055G05	175	EVQLVESGGGLVQPGGSLRLSCAAS	123	GDVHKINFLG	213	WHRQAPGKEREKVA	138	HITIGDATD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQVTVSS
32	T0170PMP055G09	182	EVQLVESGGGSVQPGGSLRLSCAAS	119	GSVHKINFLG	215	WYRQAPGKEREVVA	135	TITIGDATD	241	YADSAKGRFTISRDEAKNMVYLQMNSLSPEDTAVYFCRA	164	GSRLYPYNY	285	WGQGTQVTVSS
33	T0170PMP056A02	186	EVQLVESGGGLVQPGGSARLSCVAS	123	GDVHKINFLG	208	WYRQAPGKEREKVA	150	HITIGDQTD	237	YADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	285	WGQGTQVTVSS
34	T0170PMP056A08	175	EVQLVESGGGLVQPGGSLRLSCAAS	120	GSVHLLNFLG	205	WYRQAPGKEREMVA	136	HITIADATD	238	YSHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTLVTVSS

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
35	T0170PMP 056A10	177	EVQLVESGGGLVQ PGGSLRLSCAVS	120	GSVHLLN FLG	216	WYRQAPGKE RGVVA	136	HITIAD ATD	238	YSHFAKGRFTISRDEAKNMV YLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQ VTVSS
36	T0170PMP 056B11	187	EVQLVESGGGLVQ AGGSLTLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
37	T0170PMP 056C01	175	EVQLVESGGGLVQ PGGSLRLSCAAS	127	GGVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
38	T0170PMP 056C02	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	147	HITIAD VAD	254	YADFAQGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	285	WGQGTQ VTVSS
39	T0170PMP 056C03	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	151	HITIGD TTD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQ VTVSS
40	T0170PMP 056C04	175	EVQLVESGGGLVQ PGGSLRLSCAAS	120	GSVHLLN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	238	YSHFAKGRFTISRDEAKNMV YLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	288	WGHGTL VTVSS
41	T0170PMP 056C07	181	EVQLVESGGGLVQ PGGSLRLSCVAS	123	GDVHKIN FLG	208	WYRQAPGKE REKVA	139	HITIGD QAD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	165	GSRIYPYDY	289	WGRGTL VTVSS
42	T0170PMP 056C10	177	EVQLVESGGGLVQ PGGSLRLSCAVS	120	GSVHLLN FLG	218	WYRQAPGKE REMVT	136	HITIAD ATD	238	YSHFAKGRFTISRDEAKNMV YLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQ VTVSS
43	T0170PMP 056D01	188	EVQLVESGGDLVQ PGGSLRLSCAAS	123	GDVHKIN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	255	YAEFAKGRFTISRDEPKNMV HLQMNSLKPEDTAVYLCRA	171	GSRIYPYNY	285	WGQGTQ VTVSS
44	T0170PMP 056D02	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	287	WGRGTQ VTVSS
45	T0170PMP 056D11	181	EVQLVESGGGLVQ PGGSLRLSCVAS	122	GDVHKIN ILG	208	WYRQAPGKE REKVA	152	RISISD QTD	252	YAESAKGRFTISRDESKNMV YLQMNSLKPEDTAVYLCRA	170	FSRIYPYDY	286	WGQGTL VTVSS
46	T0170PMP 056E02	189	EVQLVESGGGLVQ PEGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	147	HITIAD VAD	256	YADFAKGRLTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	286	WGQGTL VTVSS
47	T0170PMP 056F01	190	EVQLVESGGGLVQ PGGSLKLPCAAS	121	GAVHKIN FLG	206	WYRQTPEKE REMVA	148	TITIGD EVD	239	YADSAKGRFTISRDEAKNMV YLQMTSLKPEDTAVYVCRA	164	GSRLYPYNY	286	WGQGTL VTVSS
48	T0170PMP 056F08	191	EVQLVESGGGLVQ PGGSLGLSCAAS	120	GSVHLLN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	242	YAHFAKGRFTISRDEAKNMV YLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTL VTVSS
49	T0170PMP 056G02	192	EVQLVESGGGLAQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
50	T0170PMP 056G05 / T0170PMP 069C08	181	EVQLVESGGGLVQ PGGSLRLSCVAS	123	GDVHKIN FLG	208	WYRQAPGKE REKVA	153	HISIGD QTD	257	YADSAKGRFTISRDESKNMV YLQMNSLKPEDTAVYFCRA	170	FSRIYPYDY	286	WGQGTL VTVSS
51	T0170PMP 056G11	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	154	HITIAD AAD	258	YADFAKGRFTISRDGAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	285	WGQGTQ VTVSS

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
52	T0170PMP 057B02	193	EVQLVESGGGWVQ PGGSLRLSCAAS	129	GSVYKIN FLS	219	WYRQAPGHE RELVA	155	TITIGD AAD	259	YADSAKGRFTISRDEARNMV YLQMNSLKPEDTALYFCHA	164	GSRLYPYNY	285	WGQGTQ VTVSS
53	T0170PMP 057D06	175	EVQLVESGGGLVQ PGGSLRLSCAAS	120	GSVHLLN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	238	YSHFAKGRFTISRDEAKNMV YLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQ VTVSS
54	T0170PMP 061A02	181	EVQLVESGGGLVQ PGGSLRLSCVAS	122	GDVHKIN ILG	208	WYRQAPGKE REKVA	156	HIAISD QTD	252	YAESAKGRFTISRDESKNMV YLQMNSLKPEDTAVYLCRA	170	FSRIYPYDY	286	WGQGTL VTVSS
55	T0170PMP 061B04	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	260	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAAYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
56	T0170PMP 067A01	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	222	WYRQAPAKE RGMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQ VTVSS
57	T0170PMP 067A03	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	223	WYRQAPAKE HEMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
58	T0170PMP 067B06	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	224	WYRQAPARE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQ VTVSS
59	T0170PMP 067C09	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	263	YADSAKGRFTISRDEAENMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
60	T0170PMP 067D01	178	EVQLVESGGGLVQ PGGSLKLSCAAS	121	GAVHKIN FLG	206	WYRQTPEKE REMVA	148	TITIGD EVD	239	YADSAKGRFTISRDEAKNMV YLQMTSLKPEDTAVYVCRA	164	GSRLYPYNY	286	WGQGTL VTVSS
61	T0170PMP 067D06	178	EVQLVESGGGLVQ PGGSLKLSCAAS	121	GAVHKIN FLG	214	WYRQAPEKE REMVA	148	TITIGD EVD	264	YADSAKGRFTISRDEATNMV YLQMTSLKPEDTAVYFCRA	171	GSRIYPYNY	285	WGQGTQ VTVSS
62	T0170PMP 067D09	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	159	HITIGD ATS	265	YAGSAKGRFTISRDEAKNMV YLQLNNLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
63	T0170PMP 067E03	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	159	HITIGD ATS	266	YADSAKGRFTISRDEAKNMV YLQLNNLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
64	T0170PMP 067E06	188	EVQLVESGGDLVQ PGGSLRLSCAAS	123	GDVHKIN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	267	YAEFAKGRFTISRDEPKNMV YLQMNSLKPEDTAVYLCRA	171	GSRIYPYNY	285	WGQGTQ VTVSS
65	T0170PMP 067F02	178	EVQLVESGGGLVQ PGGSLKLSCAAS	121	GAVHKIN FLG	206	WYRQTPEKE REMVA	148	TITIGD EVD	268	YAHSAKGRFTISRDEAKNMV YLQMTSLKPEDTAVYVCRA	164	GSRLYPYNY	285	WGQGTQ VTVSS
66	T0170PMP 068C03	178	EVQLVESGGGLVQ PGGSLKLSCAAS	121	GAVHKIN FLG	206	WYRQTPEKE REMVA	160	TITIGD EVA	239	YADSAKGRFTISRDEAKNMV YLQMTSLKPEDTAVYVCRA	164	GSRLYPYNY	285	WGQGTQ VTVSS
67	T0170PMP 068C07	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	154	HITIAD AAD	250	YADFAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	285	WGQGTQ VTVSS
68	T0170PMP 068C11	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	154	HITIAD AAD	250	YADFAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQ VTVSS
69	T0170PMP 068D05	194	EVQLVESGGGSVQ PGGSLRPSCAAS	119	GSVHKIN FLG	205	WYRQAPGKE REMVA	135	TITIGD ATD	241	YADSAKGRFTISRDEAKNMV YLQMNSLSPEDTAVYFCRA	164	GSRLYPYNY	285	WGQGTQ VTVSS

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
70	T0170PMP 068D07	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	147	HITIAD VAD	269	YADFAKGRFTISRDEVKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	286	WGQGTL VTVSS
71	T0170PMP 068E01	195	EVQLVESGGGLVQ PGESLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
72	T0170PMP 068E08	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	154	HITIAD AAD	270	YADFAKGRFTISRDEAKNMV YLQMNSLRPEDTAVYFCRA	169	GSRIWPYDY	285	WGQGTQ VTVSS
73	T0170PMP 068F04	175	EVQLVESGGGLVQ PGGSLRLSCAAS	120	GSVHLLN FLG	205	WYRQAPGKE REMVA	161	HITIAD VTD	271	YSYFAKGRFTISRDEAKNMV YLQMNSLRPEDTAVYFCRA	165	GSRIYPYDY	285	WGQGTQ VTVSS
74	T0170PMP 068F06	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	272	YADSAKGRFTISRDEAKNVV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
75	T0170PMP 068F08	182	EVQLVESGGGSVQ PGGSLRLSCAAS	119	GSVHKIN FLG	226	WYRQAPGKE RGMVA	135	TITIGD ATD	241	YADSAKGRFTISRDEAKNMV YLQMNSLSPEDTAVYFCRA	164	GSRLYPYNY	285	WGQGTQ VTVSS
76	T0170PMP 069A06	178	EVQLVESGGGLVQ PGGSLKLSCAAS	121	GAVHKIN FLG	206	WYRQTPEKE REMVA	148	TITIGD EVD	273	YEDSAKGRFTISRDEAKNMV YLQMTGLKPEDTAVYVCRA	164	GSRLYPYNY	286	WGQGTL VTVSS
77	T0170PMP 069B02	197	EVQLVESGGGLVR PGGSLRLSCVAS	122	GDVHKIN ILG	208	WYRQAPGKE REKVA	149	HISISD QTD	252	YAESAKGRFTISRDESKNMV YLQMNSLKPEDTAVYLCRA	170	FSRIYPYDY	285	WGQGTQ VTVSS
78	T0170PMP 069B08	175	EVQLVESGGGLVQ PGGSLRLSCAAS	132	GDVYKIN FLG	213	WHRQAPGKE REKVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTL VTVSS
79	T0170PMP 069C01	179	EVQLVESGGGLVQ PGGSLRLSCRAS	122	GDVHKIN ILG	227	WYRQAPAKE REMIA	138	HITIGD ATD	240	YAESAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQ VTVSS
80	T0170PMP 069C04	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	228	WYRQAPAKG REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	285	WGQGTQ VTVSS
81	T0170PMP 069C05	188	EVQLVESGGDLVQ PGGSLRLSCAAS	123	GDVHKIN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	267	YAEFAKGRFTISRDEPKNMV YLQMNSLKPEDTAVYLCRA	173	GSRIYPYSY	285	WGQGTQ VTVSS
82	T0170PMP 069D02	198	EVQLVESGGGMVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
83	T0170PMP 069D07	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	229	WYRQVPAKE REMVA	138	HITIGD ATD	260	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAAYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
84	T0170PMP 069E02	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	154	HITIAD AAD	250	YADFAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	286	WGQGTL VTVSS
85	T0170PMP 069E07	175	EVQLVESGGGLVQ PGGSLRLSCAAS	120	GSVHLLN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	275	YSHFAKGRFTISRDEAKNMV YLQMNGLRPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTL VTVSS
86	T0170PMP 069E09	175	EVQLVESGGGLVQ PGGSLRLSCAAS	123	GDVHKIN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	267	YAEFAKGRFTISRDEPKNMV YLQMNSLKPEDTAVYLCRA	171	GSRIYPYNY	285	WGQGTQ VTVSS
87	T0170PMP 069E11	175	EVQLVESGGGLVQ PGGSLRLSCAAS	133	GEVHKIN ILG	230	WYRQCPGKE RDMVA	135	TITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	174	LSRLYPYNY	286	WGQGTL VTVSS

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
88	T0170PMP 069F05	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	276	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYLCRA	166	YSRIYPYNY	285	WGQGTQ VTVSS
89	T0170PMP 069G08	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	231	WQRQAPGKE REKVA	147	HITIAD VAD	250	YADFAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	286	WGQGTL VTVSS
90	T0170PMP 070B08	188	EVQLVESGGDLVQ PGGSLRLSCAAS	123	GDVHKIN FLG	205	WYRQAPGKE REMVA	136	HITIAD ATD	277	YAEFAKGRFTISRDEPKNMV YLQMNSLKPVDTAVYLCRA	171	GSRIYPYNY	286	WGQGTL VTVSS
91	T0170PMP 070B09	175	EVQLVESGGGLVQ PGGSLRLSCAAS	120	GSVHLLN FLG	208	WYRQAPGKE REKVA	136	HITIAD ATD	278	YSHFAKGRFTISRDEAKNMV YLQMNNLRPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTL VTVSS
92	T0170PMP 070C09	200	EVQLVESGGGLVQ PGGSPRLSCVAS	123	GDVHKIN FLG	208	WYRQAPGKE REKVA	139	HITIGD QAD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTL VTVSS
93	T0170PMP 070D07	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	280	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDAAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
94	T0170PMP 070E07	175	EVQLVESGGGLVQ PGGSLRLSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	147	HITIAD VAD	258	YADFAKGRFTISRDGAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	286	WGQGTL VTVSS
95	T0170PMP 070F11	182	EVQLVESGGGSVQ PGGSLRLSCAAS	119	GSVHKIN FLG	232	WYCQAPGKE REMVA	135	TITIGD ATD	241	YADSAKGRFTISRDEAKNMV YLQMNSLSPEDTAVYFCRA	164	GSRLYPYNY	285	WGQGTQ VTVSS
96	T0170PMP 070G02	181	EVQLVESGGGLVQ PGGSLRLSCVAS	122	GDVHKIN ILG	208	WYRQAPGKE REKVA	149	HISISD QTD	281	YAESAKGRFTISRDESKNMV YLQMNSLKPEDAAVYLCRA	170	FSRIYPYDY	285	WGQGTQ VTVSS
97	T0170PMP 070G06	183	EVQLVESGGGLVQ PGGSLRPSCAAS	126	GEVYKIN FLG	208	WYRQAPGKE REKVA	147	HITIAD VAD	250	YADFAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	169	GSRIWPYDY	285	WGQGTQ VTVSS
98	T0170PMP 082B04	201	EVQLVESGGGLVQ PGGSLRPSCVAS	123	GDVHKIN FLG	208	WYRQAPGKE REKVA	162	HITIAD QAD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	165	GSRIYPYDY	286	WGQGTL VTVSS
99	T0170PMP 084B07	175	EVQLVESGGGLVQ PGGSLRLSCAAS	133	GEVHKIN ILG	233	WYRQAPGKE RDMVA	163	TITIGD ETQ	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	174	LSRLYPYNY	286	WGQGTL VTVSS
100	T0170PMP 084C02	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	283	YADSAKGRFTISRDEAKNMV YLQMDSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
101	T0170PMP 084E03	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	284	YADSAKGRFTISRDEAKNMV YLQMNSLKPGDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
102	T0170PMP 084E05	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	234	WHRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
103	T0170PMP 084F04	175	EVQLVESGGGLVQ PGGSLRLSCAAS	122	GDVHKIN ILG	234	WHRQAPAKE REMVA	138	HITIGD ATD	260	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAAYFCRA	166	YSRIYPYNY	285	WGQGTQ VTVSS
104	T0170PMP 084F10	202	EVQLVESGGGWVQ AGGSLRLSCAAS	122	GDVHKIN ILG	207	WYRQAPAKE REMVA	138	HITIGD ATD	237	YADSAKGRFTISRDEAKNMV YLQMNSLKPEDTAVYFCRA	166	YSRIYPYNY	286	WGQGTL VTVSS
105	T0170PMP 055B06	184	EVQLVESGGGLVQ PGGSLRLSCITS	124	GETFKIN IWG	210	WYRQAPGKQ RELVA	142	SLTIGG ATD	243	YADSVKGRFTISEDSAKNTV YLQMNSLKAEDTAVYFCNA	167	KSRLYPYDY	285	WGQGTQ VTVSS

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
106	T0170PMP 055C07	184	EVQLVESGGGLVQ PGGSLRLSCITS	124	GETFKIN IWG	210	WYRQAPGKQ RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	167	KSRLYPYDY	286	WGQGTL VTVSS
107	T0170PMP 055D01	184	EVQLVESGGGLVQ PGGSLRLSCITS	124	GETFKIN IWG	212	WYRQAPGKR RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	168	KSRIYPYDY	285	WGQGTQ VTVSS
108	T0170PMP 056B02	184	EVQLVESGGGLVQ PGGSLRLSCITS	124	GETFKIN IWG	217	WYRQTPGKQ RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	167	KSRLYPYDY	285	WGQGTQ VTVSS
109	T0170PMP 056D07	184	EVQLVESGGGLVQ PGGSLRLSCITS	128	GQTFKIN IWG	210	WYRQAPGKQ RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	167	KSRLYPYDY	285	WGQGTQ VTVSS
110	T0170PMP 068B03	184	EVQLVESGGGLVQ PGGSLRLSCITS	131	GETFKVN IWG	225	WYRQGPGKQ RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	167	KSRLYPYDY	287	WGRGTQ VTVSS
111	T0170PMP 068G05	196	EVQLVEESGGGLV QPGGSLRLSCITS	124	GETFKIN IWG	210	WYRQAPGKQ RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	168	KSRIYPYDY	287	WGRGTQ VTVSS
112	T0170PMP 069E03	184	EVQLVESGGGLVQ PGGSLRLSCITS	124	GETFKIN IWG	210	WYRQAPGKQ RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	167	KSRLYPYDY	290	WDQGTQ VTVSS
113	T0170PMP 069E06	199	EVQLVESGGGLVQ PGGSLRLSCLTS	131	GETFKVN IWG	225	WYRQGPGKQ RELVA	145	SLTIGG ATN	274	YADSVKGRFTISEDSAKNTV YLQMSSLKPEDTAVYFCNA	167	KSRLYPYDY	285	WGQGTQ VTVSS
114	T0170PMP 070A09	184	EVQLVESGGGLVQ PGGSLRLSCITS	124	GETFKIN IWG	210	WYRQAPGKQ RELVA	145	SLTIGG ATN	246	YADSVKGRFTISEDSAKNTV YLQMNSLKPEDTAVYFCNA	168	KSRIYPYDY	287	WGRGTQ VTVSS
115	T0170PMP 070C01	184	EVQLVESGGGLVQ PGGSLRLSCITS	124	GETFKIN IWG	210	WYRQAPGKQ RELVA	145	SLTIGG ATN	279	YADSVKGRFTISEDSAKDTV YLQMNSLKPEDTAVYFCNA	167	KSRLYPYDY	286	WGQGTL VTVSS
116	T0170PMP 061G01	175	EVQLVESGGGLVQ PGGSLRLSCAAS	130	GEIGRIN FYR	220	WYRQAPGNQ REVVA	157	TITIAD KTD	261	YADSAKGRFTISRDESRNMV YLQMSSLKPEDTAVYFCHA	172	GSRLYPYDY	285	WGQGTQ VTVSS
117	T0170PMP 062D02	175	EVQLVESGGGLVQ PGGSLRLSCAAS	130	GEIGRIN FYR	221	WYRQAPGNQ RGVVA	158	TITIAD KID	262	YADSAKGRFTISRDESRNMV YLQMGSLKPEDTAVYFCHA	172	GSRLYPYDY	285	WGQGTQ VTVSS
118	T0170PMP 075D02	175	EVQLVESGGGLVQ PGGSLRLSCAAS	130	GEIGRIN FYR	220	WYRQAPGNQ REVVA	157	TITIAD KTD	282	YADSAKGRFTISRDESRNMV YLQMSSLKPENTAVYFCHA	172	GSRLYPYDY	285	WGQGTQ VTVSS

Table A-5: Sequences of multispecific polypeptides (with and without tags). "SEQ" refers to the given SEQ ID NO; "ID" refers to identification name; "Sequence" denotes amino acid sequence

SEQ	ID	Sequence
291	T017000001	EVQLVESGGGLVQGGGSLSLSCAASGRTFSSYAMAWFRQPPGKEREFVASISWSGENTNYRNSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAKIAKTYPDNWYWTKSNNYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQGGGSLSLSCAASGRTFSSYAMAWFRQPPGKEREFVASISWSGENTNYRNSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAKIAKTYPDNWYWTKSNNYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
292	T017000002	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
293	T017000003	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
294	T017000006	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
295	T017000007	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
296	T017000008	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
297	T017000009	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYY.CAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
298	T017000012	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
299	T017000013	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
300	T017000014	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH

SEQ	ID	Sequence
301	T017000015	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
302	T017000018	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
303	T017000019	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
304	T017000023	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
305	T017000025	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
306	T017000029	EVQLVESGGGLVQPGGSLRLSCAASGDVYKINFLGWHRQAPGKEREKVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
307	T017000030	EVQLVESGGGLVQPGGSLKLSCAASGAVHKINFLGWYRQTPEKEREMVATITIGDEVDYADSAKGRFTISRDEAKNMVYLQMTSLKPEDTAVYVCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
308	T017000031	EVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATNYADSVKGRFTISEDSAKNTVYLQMNSLKPEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
309	T017000032	EVQLVESGGGLVQPGGSLRLSCAASGSVHLLNFLGWYRQAPGKEREMVAHITIADATDYAHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH

CA 02985698 2017-11-10

SEQ	ID	Sequence
310	T017000033	EVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATDYADSVKGRFTISEDSAKNTVYLQMNSLKAEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
311	T017000035	EVQLVESGGGLVQPGGSLRLSCAASGEVYKINFLGWYRQAPGKEREKVAHITIADAADYADFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIWPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
312	T017000037	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
313	T017000038	EVQLVESGGGLVQPGGSLRLSCAASGEIGRINFYRWYRQAPGNQREVVATITIADKTDYADSAKGRFTISRDESRNMVYLQMSSLKPEDTAVYFCHAGSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
314	T017000041	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHITIGDQADYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
315	T017000042	EVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
316	T017000044	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERELVATITIGDTTDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
317	T017000046	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
318	T017000049	EVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYLCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH

SEQ	ID	Sequence
319	T017000050	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
320	T017000051	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGEIGRINFYRWYRQAPGNQREVVATITIADKTDYADSAKGRFTISRDESRNMVYLQMSSLKPEDTAVYFCHAGSRLYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
321	T017000054	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHITIGDQADYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
322	T017000055	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
323	T017000058	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERELVATITIGDTTDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
324	T017000060	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
325	T017000063	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVYKINFLGWHRQAPGKEREKVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
326	T017000064	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGEVYKINFLGWYRQAPGKEREKVAHITIADAADYADFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIWPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
327	T017000065	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGDLVQPGGSLRLSCAASGDVHKINFLGWYRQAPGKEREMVAHITIADATDYAEFAKGRFTISRDEPKNMVYLQMNSLKPEDTAVYLCRAGSRIYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH

SEQ	ID	Sequence
328	T017000068	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATNYADSVKGRFTISEDSAKNTVYLQMNSLKPEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
329	T017000069	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHLLNFLGWYRQAPGKEREMVAHITIADATDYAHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
330	T017000070	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATDYADSVKGRFTISEDSAKNTVYLQMNSLKAEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
331	T017000073	EVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATSYADSAKGRFTISRDEAKNMVYLQLNNLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
332	T017000074	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
333	T017000075	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATSYADSAKGRFTISRDEAKNMVYLQLNNLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
334	T017000076	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
335	T017000077	EVQLVESGGDLVQPGGSLRLSCAASGDVHKINFLGWYRQAPGKEREMVAHITIADATDYAEFAKGRFTISRDEPKNMVYLQMNSLKPEDTAVYLCRAGSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
336	T017000078	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYLCRAYSRIYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH

SEQ	ID	Sequence
337	T017000079	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGAVHKINFLGWYRQTPEKEREMVATITIGDEVDYADSAKGRFTISRDEAKNMVYLQMTSLKPEDTAVYVCRAGSRLYPYNYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
338	T017000083	DVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSA
339	T017000088	DVQLVESGGGLVQAGDSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAISWSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLTSTNPGSYIYIWAYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSA
340	T017000093	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
341	T017000095	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATNYADSVKGRFTISEDSAKNTVYLQMNSLKPEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
342	T017000102	EVQLVESGGGLVQAGGSLRLSCAASGITFSINTMGWYRQAPGKQRELVALISSIGDTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAAQGTDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
343	T017000103	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLRLSCAASGITFSINTMGWYRQAPGKQRELVALISSIGDTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAAQGTDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
387	T017000104	DVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSA
388	T017000105	DVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSA

SEQ	ID	Sequence
389	T017000106	DVQLVESGGGLVQAGDSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAISWSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLTSTNPGSYIYIWAYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSA
390	T017000107	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQATGKEREFVATIAWDSGSTYYADSVKGRFTISRDNAKNTVHLQMNSLKPEDTAVYYCAASYNVYYNNYYYPISRDEYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGDTYGSYWMGWFRQAPGKEREGVAAINRGGGYTVYADSVKGRFTISRDTAKNTVYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
391	T017000109	EVQLVESGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGDTYGSYWMGWFRQAPGKEREGVAAINRGGGYTVYADSVKGRFTISRDTAKNTVYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
392	T017000110	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQATGKEREFVATIAWDSGSTYYADSVKGRFTISRDNAKNTVHLQMNSLKPEDTAVYYCAASYNVYYNNYYYPISRDEYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH
413	T017000001	EVQLVESGGGLVQGGGSLSLSCAASGRTFSSYAMAWFRQPPGKEREFVASISWSGENTNYRNSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAKIAKTYPDNWYWTKSNNYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQGGGSLSLSCAASGRTFSSYAMAWFRQPPGKEREFVASISWSGENTNYRNSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAKIAKTYPDNWYWTKSNNYNYWGQGTLVTVSS
414	T017000002	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS
415	T017000003	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS
416	T017000006	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSS
417	T017000007	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
418	T017000008	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS

SEQ	ID	Sequence
419	T017000009	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS
420	T017000012	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSS
421	T017000013	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
422	T017000014	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS
423	T017000015	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS
424	T017000018	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSS
425	T017000019	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
426	T017000023	EVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
427	T017000025	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSS
428	T017000029	EVQLVESGGGLVQPGGSLRLSCAASGDVYKINFLGWHRQAPGKEREKVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
429	T017000030	EVQLVESGGGLVQPGGSLKLSCAASGAVHKINFLGWYRQTPEKEREMVATITIGDEVDYADSAKGRFTISRDEAKNMVYLQMTSLKPEDTAVYVCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
430	T017000031	EVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATNYADSVKGRFTISEDSAKNTVYLQMNSLKPEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS

SEQ	ID	Sequence
431	T017000032	EVQLVESGGGLVQPGGSLRLSCAASGSVHLLNFLGWYRQAPGKEREMVAHITIADATDYAHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
432	T017000033	EVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATDYADSVKGRFTISEDSAKNTVYLQMNSLKAEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
433	T017000035	EVQLVESGGGLVQPGGSLRLSCAASGEVYKINFLGWYRQAPGKEREKVAHITIADAADYADFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIWPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
434	T017000037	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
435	T017000038	EVQLVESGGGLVQPGGSLRLSCAASGEIGRINFYRWYRQAPGNQREVVATITIADKTDYADSAKGRFTISRDESRNMVYLQMSSLKPEDTAVYFCHAGSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
436	T017000041	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHITIGDQADYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
437	T017000042	EVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
438	T017000044	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERELVATITIGDTTDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
439	T017000046	EVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAPGKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
440	T017000049	EVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYLCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
441	T017000050	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
442	T017000051	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGEIGRINFYRWYRQAPGNQREVVATITIADKTDYADSAKGRFTISRDESRNMVYLQMSSLKPEDTAVYFCHAGSRLYPYDYWGQGTLVTVSS

SEQ	ID	Sequence
443	T017000054	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAP GKEREKVAHITIGDQADYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYPDYWGQGTLVTVSS
444	T017000055	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAP AKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSS
445	T017000058	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAP GKERELVATITIGDTTDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS
446	T017000060	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHKINFLGWYRQAP GKERGLVATITIGDTTDYADYAKGRFTISRDEARNMVYLQMNSLKPEDTAVYFCRAGSRLYPYNYWGQGTLVTVSS
447	T017000063	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVYKINFLGWHRQAP GKEREKVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSS
448	T017000064	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGEVYKINFLGWYRQAP GKEREKVAHITIADAADYADFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIWPYDYWGQGTLVTVSS
449	T017000065	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGDLVQPGGSLRLSCAASGDVHKINFLGWYRQAP GKEREMVAHITIADATDYAEFAKGRFTISRDEPKNMVYLQMNSLKPEDTAVYLCRAGSRIYPYNYWGQGTLVTVSS
450	T017000068	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAP GKQRELVASLTIGGATNYADSVKGRFTISEDSAKNTVYLQMNSLKPEDTAVYFCNAKSRLYPYDYWGQGTLVTVSS
451	T017000069	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSVHLLNFLGWYRQAP GKEREMVAHITIADATDYAHFAKGRFTISRDEAKNMVYLQMNSLRPEDTAVYFCRAGSRIYPYDYWGQGTLVTVSS
452	T017000070	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAV RQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAP GKQRELVASLTIGGATDYADSVKGRFTISEDSAKNTVYLQMNSLKAEDTAVYFCNAKSRLYPYDYWGQGTLVTVSS
453	T017000073	EVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATSYADSAKGRFTISRDEAKNMVYLQLNNLKPEDTAVYFCRAY SRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKE REFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
454	T017000074	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAF SRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKE REFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS

SEQ	ID	Sequence
455	T017000075	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATSYADSAKGRFTISRDEAKNMVYLQLNNLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSS
456	T017000076	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
457	T017000077	EVQLVESGGDLVQPGGSLRLSCAASGDVHKINFLGWYRQAPGKEREMVAHITIADATDYAEFAKGRFTISRDEPKNMVYLQMNSLKPEDTAVYLCRAGSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
458	T017000078	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYLCRAYSRIYPYNYWGQGTLVTVSS
459	T017000079	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGAVHKINFLGWYRQTPEKEREMVATITIGDEVDYADSAKGRFTISRDEAKNMVYLQMTSLKPEDTAVYVCRAGSRLYPYNYWGQGTLVTVSS
460	T017000093	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
461	T017000095	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCITSGETFKINIWGWYRQAPGKQRELVASLTIGGATNYADSVKGRFTISEDSAKNTVYLQMNSLKPEDTAVYFCNAKSRLYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
462	T017000102	EVQLVESGGGLVQAGGSLRLSCAASGITFSINTMGWYRQAPGKQRELVALISSIGDTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAAQGTDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
463	T017000103	EVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLRLSCAASGITFSINTMGWYRQAPGKQRELVALISSIGDTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAAQGTDYWGQGTLVTVSS
464	T017000107	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQATGKEREFVATIAWDSGSTYYADSVKGRFTISRDNAKNTVHLQMNSLKPEDTAVYYCAASYNVYYNNYYYPISRDEYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGSVQAGGSLRLSCAASGDTYGSYWMGWFRQAPGKEREGVAAINRGGGYTVYADSVKGRFTISRDTAKNTVYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS

SEQ	ID	Sequence
465	T017000109	EVQLVESGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGDTYGSYWMGWFRQAPGKEREGVAAINRGGGYTVYADSVKGRFTISRDTAKNTVYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
466	T017000110	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQATGKEREFVATIAWDSGSTYYADSVKGRFTISRDNAKNTVHLQMNSLKPEDTAVYYCAASYNVYYNNYYYPISRDEYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
467	T017000104	DVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
468	T017000105	DVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSS
469	T017000106	DVQLVESGGGLVQAGDSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAISWSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLTSTNPGSYIYIWAYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
470	T017000083	DVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSS
471	T017000088	DVQLVESGGGLVQAGDSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAISWSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLTSTNPGSYIYIWAYDYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGDVHKINILGWYRQAPAKEREMVAHITIGDATDYADSAKGRFTISRDEAKNMVYLQMNSLKPEDTAVYFCRAYSRIYPYNYWGQGTLVTVSS
486	T017000108	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQATGKEREFVATIAWDSGSTYYADSVKGRFTISRDNAKNTVHLQMNSLKPEDTAVYYCAASYNVYYNNYYYPISRDEYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGDTYGSYWMGWFRQAPGKEREGVAAINRGGGYTVYADSVKGRFTISRDTAKNTVYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTISRDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGAADYKDHDGDYKDHDIDYKDDDDKGAAHHHHHH

SEQ	ID	Sequence
487	T017000108	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQATGKEREFVATIAWDSGSTYYADSVKGRFTISRDNAKNTVHLQMNSLKPEDTAVYYCAA SYNVYYNNYYYPISRDEYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGDTYGSY WMGWFRQAPGKEREGVAAINRGGGYTVYADSVKGRFTISRDTAKNTVYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSSGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCVASGDVHKINFLGWYRQAPGKEREKVAHISIGDQTDYADSAKGRFTIS RDESKNMVYLQMNSLKPEDTAVYFCRAFSRIYPYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGN SLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVS S

Table A-6: Sequences of components of TCR complex. "SEQ" refers to the given SEQ ID NO; "ID" refers to identification name; "Sequence" denotes amino acid sequence

SEQ	ID	SEQUENCE
344	Human CD3 delta (P04234)	MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMCQSCVE LDPATVAGIIVTDVIATLLLALGVFCFAGHETGRLSGAADTQALLRNDQVYQPLRDRDDAQYSHLGGNWARNK
345	Human CD3 gamma (P09693)	MEQGKGLAVLILAIILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGFLTEDKKKWNLGSNAKDPRGMYQCKGSQNKSKPLQ VYYRMCQNCIELNAATISGFLFAEIVSIFVLAVGVYFIAGQDGVRQSRASDKQTLLPNDQLYQPLKDREDDQYSHLQGNQLRRN
346	Human CD3 epsilon(P0 7766)	MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVC YPRGSKPEDANFYLYLRARVCENCMEMDVMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVTRGAGAGGRQRGQNKERPPPVPNPDYEPIRKGQR DLYSGLNQRRI
347	Human CD3 zeta (P20963)	MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
348	Human TCR alpha constant domain (P01848)	PNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV KLVEKSFETDTNFRILLLKVAGFNLLMTLRLWSSLNFQNLSVIG
349	Human TCR beta constant domain (P01850)	EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQV QFYGLSENDEWTVSAEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDFQDRAKPVTQI
393	Human TCR alpha variable domain derived from 2IAN	IQVEQSPPDLILQEGANSTLRCNFSDSVNNLQWFHQNPWGQLINLFYIPSGTKQNGRLSATTVATERYSLLYISSSQTTDSGVYFCAALIQGAQKLVF GQGTRLTIN
476	Human TCR beta variable domain derived from 2IAN	NAGVTQTPKFRILKIGQSMTLQCTQDMNHNYMYWYRQDPGMGLKLIYYSVGAGITDKGEVPNGYNVSRSTTEDFPLRLELAAPSQTSVYFCASTYHGT GYFGEGSWLTVV

SEQ	ID	SEQUENCE
394	Human TCR alpha variable domain derived from 2XN9	QLLEQSPQFLSIQEGENLTVYCNSSSVFSSLQWYRQEPGEGPVLLVTVVTGGEVKKLKRLTFQFGDARKDSSLHITAAQPGDTGLYLCAGAGSQGNLI FGKGTKLSVK
477	Human TCR beta variable domain derived from 2XN9	DGGITQSPKYLFRKEGQNVTLSCEQNLNHDAMYWYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSSRSS YEQYFGPGTRLTVT
395	Human TCR alpha variable domain derived from 3TOE	GDAKTTQPNSMESNEEEPVHLPCNHSTISGTDYIHWYRQLPSQGPEYVIHGLTSNVNNRMASLAIAEDRKSSTLILHRATLRDAAVYYCTVYGGATNK LIFGTGTLLAVQ
478	Human TCR beta variable domain derived from 3TOE	VVSQHPSWVIAKSGTSVKIECRSLDFQATTMFWYRQFPKQSLMLMATSNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYICSARGGS YNSPLHFGNGTRLTVT
396	Cyno TCR alpha constant domain	PYIQNPDPAVYQLRGSKSNDTSVCLFTDFDSVMNVSQSKDSDVHITDKTVLDMRSMDFKSNGAVAWSNKSDFACTSAFKDSVIPADTFFPSPESSC
397	Rhesus TCR beta constant domain	EDLKKVFPPKVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALEDSRYSLSSRLRVSATFWHNPRNHFRCQV QFYGLSEDDEWTEDRDKPITQKISAEAWGRADC
398	Rhesus TCR alpha variable domain	QQIMQIPQYQHVQEGEDFTTYCNSSTTLSNIQWYKQRPGGHPVFLIMLVKSGEVKKQKRLIFQFGEAKKNSSLHITATQTTDVGTYFCATTGVNNLFF GTGTRLTVL

SEQ	ID	SEQUENCE
399	Rhesus TCR beta variable domain	AGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHDYMYWYRQDPGMGLRLIHYSVGEGSTEKGEVPDGYNVTRSNTEDFPLRLESAAPSQTSVYFCASS YWTGRSYEQYFGPGTRLTVI
479	Human TCR alpha constant domain	PNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKSVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSC
480	Human TCR beta constant domain	EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYSLSSRLRVSATFWQNPRNHFRCQV QFYGLSENDEWTQDRAKPVTQIVSAEAWGRADC
484	Human TCR alpha constant domain (P01848)	PNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV KLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
485	Human TCR beta constant domain	EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQV QFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF

Table A-7: Sequences of TAA binding building blocks and control Nanobodies. "SEQ" refers to a given SEQ ID NO; "ID" refers to identification name; "Sequence" denotes amino acid sequence

SEQ	ID	SEQUENCE
350	HER2005F07 (Q108L)	EVQLVESGGGLVQAGGSLRLSCAASGITFSINTMGWYRQAPGKQRELVALISSIGDTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCKRFRTAAQGTDYWGQGTLVTVSS
351	HER2047D05 (L108Q)	EVQLVESGGGLVQPGGSLRLSCAASGSIFGFNDMAWYRQAPGKQRELVALISRVGVTSSADSVKGRFTISRVNAKDTVYLQMNSLKPEDTAVYYCYMDQRLDGSTLAYWGQGTQVTVSS
352	EGFR009G08	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVVAINWSSGSTYYADSVKGRFTISRDNAKNTMYLQMNSLKPEDTAVYYCAAGYQINSGNYNFKDYEYDYWGQGTQVTVSS
353	NbCEA5 (CEA#1)	EVQLVESGGGSVQAGGSLRLSCAASGDTYGSYWMGWFRQAPGKEREGVAAINRGGGYTVYADSVKGRFTISRDTAKNTVYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSS
354	T023200005 (CEA#5)	EVQLVESGGGSVQAGGSLRLSCAASGDTYGSYWMGWFRQAPGQEREAVAAINRGGGYTVYADSVKGRFTISRDNAKNTLYLQMNSLRPDDTADYYCAASGVLGGLHEDWFNYWGQGTLVTVSS
355	7D12 (EGFR#1)	EVQLVESGGGSVQTGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSAWYGTLYEYDYWGQGTLVTVSS
356	T023200033 (EGFR#33)	EVQLVESGGGSVQAGGSLRLTCAASGSTSRSYGMGWFRQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWYGTLYEYDYWGQGTLVTVSS
357	20CD019C07	EVQLVESGGGLVQPGGSLRLSCTFSGGTFSSYTMGWFRQAPGKEREFVAEVRWGGVTTYSNSLKDRFSISEDSVKNAVYLQMNSLKPEDTAVYYCAAVRQMYMTVVPDYWGQGTLVTVSS
358	EGFR038G07	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYVMGWFRQATGKEREFVATIAWDSGSTYYADSVKGRFTISRDNAKNTVHLQMNSLKPEDTAVYYCAASYNVYYNNYYYPISRDEYDYWGQGTLVTVSS
359	cAblys3	DVQLQASGGGSVQAGGSLRLSCAASGYTIGPYCMGWFRQAPGKEREGVAAINMGGGITYYADSVKGRFTISQDNAKNTVYLLMNSLEPEDTAIYYCAADSTIYASYYECGHGLSTGGYGYDSWGQGTQVTVSS
360	RSV007B02	EVQLVESGGGLVQAGDSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAISWSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLTSTNPGSYIYIWAYDYWGQGTQVTVSS

Table A-8: Sequences for CDRs and frameworks of TAA binding building blocks, plus preferred combinations as provided in formula I, namely FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. "SEQ" refers to the given SEQ ID NO. The first column refers to the SEQ ID NO of the complete ISV, i.e. FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. CDR1, CDR2 and CDR3 were determined according to Kontermann, 2010.

SEQ	Nanobody	SEQ	FR1	SEQ	CDR1	SEQ	FR2	SEQ	CDR2	SEQ	FR3	SEQ	CDR3	SEQ	FR4
353	NbCEA5 (CEA#1)	367	EVQLVESGGGSVQ AGGSLRLSCAAS	361	GDTYGSY WMG	369	WFRQAPGKE REGVA	363	AINRGG GYTV	372	YADSVKGRFTISRDTAKNTV YLQMNSLRPDDTADYYCAA	365	SGVLGGLHED WFNY	375	WGQGTL VTVSS
354	T023200005 (CEA#5)	367	EVQLVESGGGSVQ AGGSLRLSCAAS	361	GDTYGSY WMG	370	WFRQAPGQE REAVA	363	AINRGG GYTV	373	YADSVKGRFTISRDNAKNTL YLQMNSLRPDDTADYYCAA	365	SGVLGGLHED WFNY	375	WGQGTL VTVSS
357	20CD019C07	368	EVQLVESGGGLVQ PGGSLRLSCTFS	362	GGTFSSY TMG	371	WFRQAPGKE REFVA	364	EVRWGG VTT	374	YSNSLKDRFSISEDSVKNAV YLQMNSLKPEDTAVYYCAA	366	VRQMYMTVVP DY	375	WGQGTL VTVSS

5

Table C-6: EC50 (M) of multispecific TCR binding polypeptides for binding to CHO-K1 human TCR(2XN9)/CD3, purified primary human T cells and Ramos cells as determined in flow cytometry.

		CHO-K1 huTCR(2XN9)/CD3			human T cells			Ramos cells		
Cluster	sample ID	EC50 (M)	95% LCI	95% UCI	EC50 (M)	95% LCI	95% UCI	EC50 (M)	95% LCI	95% UCI
A	T017000055	1.1E-07	9.8E-08	1.3E-07	1.3E-07	3.0E-08	5.6E-07	1.8E-09	1.6E-09	1.9E-09
A	T017000042	1.7E-08	1.4E-08	1.9E-08	2.3E-07	2.0E-07	2.6E-07	2.0E-08	1.8E-08	2.2E-08
A	T017000076	7.8E-08	7.1E-08	8.6E-08	1.3E-07	1.3E-08	1.3E-06	1.8E-09	1.7E-09	2.0E-09
A	T017000074	1.6E-08	1.5E-08	1.7E-08	1.9E-07	1.7E-07	2.1E-07	1.6E-08	1.4E-08	1.7E-08
B	T017000068	1.9E-07	1.5E-07	2.3E-07	6.2E-09	4.2E-09	8.9E-09	1.8E-09	1.7E-09	2.0E-09
B	T017000031	2.0E-08	1.8E-08	2.3E-08	3.5E-07	3.1E-07	4.0E-07	2.3E-08	2.0E-08	2.5E-08
C	T017000051	4.6E-07	4.1E-07	5.1E-07	>1E-07	/	/	2.7E-09	2.4E-09	2.9E-09
C	T017000038	1.7E-08	1.6E-08	1.9E-08	>1E-07	/	/	2.1E-08	1.9E-08	2.3E-08

Table C-7: IC50 (M) of the multispecific polypeptides in the flow cytometry based T cell mediated Ramos killing assay using an effector to target ratio of 10 to 1.

Cluster	ID monovalent Nanobody	ID construct (CD20xTCR)	n	IC50 (M)	% lysis	% lysis (stdev)	ID construct (TCRxCD20)	n	IC50 (M)	% lysis	% lysis (stdev)
A	T0170056G05	T017000076	8	3.0E-10	25	14	T017000074	6	9.2E-10	18	11
A	T0170055A02	T017000055	19	5.3E-10	29	13	T017000042	5	5.7E-09	17	11
B	T0170055C07	T017000068	7	1.0E-09	22	7	T017000031	3	1.1E-09	29	9
C	T0170061G01	T017000051	2	5.7E-10	16	2	T017000038	2	2.7E-09	27	4

Table C-12: EC50 values of the half-life extended polypeptides in cell based binding to CHO-K1 human TCR(2XN9)/CD3, primary human T cells and Ramos cells as determined in flow cytometry.

		CHO-K1-K1 huTCR (2XN9)/CD3			human T cells			Ramos cells		
Cluster	sample ID	EC50 (M)	95% LCI	95% UCI	EC50 (M)	95% LCI	95% UCI	EC50 (M)	95% LCI	95% UCI
A	T017000093	7.7E-08	7.0E-08	8.4E-08	4.8E-07	4.8E-08	4.8E-06	1.5E-09	1.3E-09	1.6E-09
B	T017000095	2.7E-07	2.2E-07	3.4E-07	2.2E-07	5.1E-08	9.8E-07	3.7E-09	3.3E-09	4.1E-09

Table C-15: IC50 values of the HER2/TCR binding polypeptides in the T cell mediated HER2-positive tumour killing assay.

	xCELLigence based cytotoxicity (readout 18h) - SKBR3 (E/T=15:1)											
Cluster	ID monovalent Nb	ID construct (HER2xTCR)	n	IC50 (M)	95% LCI	95% UCI	ID construct (TCRxHER2)	n	IC50 (M)	95% LCI	95% UCI
A	T0170056G05	T017000102	1	1.4E-11	1.1E-11	1.7E-11	T017000103	1	3.8E-12	3.2E-12	4.7E-12
	xCELLigence based cytotoxicity (readout 18h) - MCF-7 (E/T=15:1)										
Cluster	ID monovalent Nb	ID construct (HER2xTCR)	n	EC50 (M)	95% LCI2	95% UCI2	ID construct (TCRxHER2)	n	IC50 (M)	95% LCI	95% UCI
A	T0170056G05	T017000102	1	1.2E-10	9.0E-11	1.0E-10	T017000103	1	5.9E-11	5.0E-11	7.0E-11
	xCELLigence based cytotoxicity (readout 18h) - MDA-MB-468(E/T=15:1)										
Cluster	ID monovalent Nb	ID construct (HER2xTCR)	n	EC50 (M)	95% LCI2	95% UCI2	ID construct (TCRxHER2)	n	IC50 (M)	95% LCI	95% UCI
A	T0170056G05	T017000102	1	No fit			T017000103	1	No fit		

Table C-16: IC50 (M) of the HER2/TCR binding polypeptides for IFN-□ secretion by human T cells in the human T cell mediated xCELLigence based HER2 dependent killing assay.

ID monovalent Nb	ID construct (HER2xTCR)	n	IC50 (M)	95% LCI	95% UCI	ID construct (TCRxHER2)	n	IC50 (M)	95% LCI	95% UCI
T0170056G05	T017000102	1	2.8E-11	1.4E-11	5.6E-11	T017000103	1	1.1E-10	5.6E-11	2.2E-10

Table C-17: IC50 (M) of and % lysis by the TCR/CD20 binding multispecific constructs in the cynomolgus T cell mediated B cell (Ramos) killing assay to evaluate the cynomolgus functionality of the TCR building block.

Cluster	ID monovalent Nb	ID construct (CD20xTCR)	n	IC50 (M)	% lysis	% lysis (stdev)	ID construct (TCRxCD20)	n	IC50 (M)	% lysis	% lysis (stdev)
A	T0170055A02	T017000055	2	5.69E-10	17	6	T017000042	1	1.7E-10	18	
A	T0170056G05	T017000076	2	3.09E-10	20	1	T017000074	2	2.4E-10	31	2.21
B	T0170055C07	T017000068	1				T017000031	1	7.9E-10	10	

Table C-18: IC50 (M) of the TCR/CD20 binding multispecific polypeptides in the cynomolgus T cell mediated xCELLigence based CHO-K1 human CD20 killing assay.

Cluster	ID monovalent Nb	ID construct (CD20xTCR)	n	IC50 (M)	95% LCI	95% UCI	ID construct (TCRxCD20)	n	IC50 (M)	95% LCI	95% UCI
A	T0170055A02	T017000055	1	9.9E-10	4.2E-10	2.3E-09	T017000042	1	8.9E-10	6.0E-10	1.3E-09
A	T0170056G05	T017000076	1	6.7E-10	2.2E-10	2.0E-09	T017000074	1	1.2E-10	6.4E-11	2.3E-10
B	T0170055C07	T017000068		ND	ND	ND	T017000031	1	1.2E-09	7.2E-10	1.9E-09

Table C-23: IC50 (M) of the TCR/Her2 binding multispecific polypeptides in the cynomolgus T cell mediated xCELLigence based SKBR3 killing assay.

ID monovalent Nb	ID construct (HER2xTCR)	n	IC50 (M)	95% LCI	95% UCI	ID construct (TCRxHER2)	n	IC50 (M)	95% LCI	95% UCI
T0170056G05	T017000102	1	4.6E-11	1.3E-11	1.7E-10	T017000103	1	1.2E-11	7.3E-12	2.1E-11

Table 31: IC50 (M) of the multispecific polypeptides in the human T cell mediated xCELLigence based killing assay using an effector to target ratio of 15. Data were analysed after 50-60h.

	LoVo				LS174T				HER14			
ID construct	**n**	**IC50 (M)**	**95% LCI**	**95% UCI**	**n**	**IC50 (M)**	**95% LCI**	**95% UCI**	**n**	**IC50 (M)**	**95% LCI**	**95% UCI**
T017000107	1	6.1E-11	5.4E-11	7.0E-11	1	5.3E-09	4.2E-09	6.6E-09	1	1.4E-10	1.2E-10	1.6E-10
T017000109	1	2.0E-09	1.8E-09	2.2E-09	1	3.6E-08	2.7E-08	4.9E-08	1	/	/	/
T017000110	1	6.0E-10	5.3E-10	6.8E-10	1	8.9E-09	5.8E-09	1.4E-08	1	4.2E-10	3.7E-10	4.7E-10

CLAIMS

1. Polypeptide comprising a first and a second immunoglobulin single variable domain (ISV), wherein
   - said first ISV has high affinity for/binds to the constant domain of the T cell receptor (TCR) present on a T cell;
   - said second ISV has high affinity for/binds to a first antigen on a target cell;

   wherein said first antigen is different from said TCR; and
   wherein said target cell is different from said T cell.

2. The polypeptide according to claim 1, wherein said polypeptide directs the T cell to the target cell.

3. The polypeptide according to claim 1 or 2, wherein said polypeptide induces T cell activation.

4. The polypeptide according to claim 3, wherein said T cell activation is independent from MHC recognition.

5. The polypeptide according to claim 3 or 4, wherein said T cell activation depends on presenting said polypeptide bound to said first antigen on a target cell to a T cell.

6. The polypeptide according to any one of claims 3 to 5, wherein said T cell activation causes one or more cellular response of said T cell, wherein said cellular response is selected from the group consisting of proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, expression of activation markers and redirected target cell lysis.

7. The polypeptide according to any one of claims 3 to 5, wherein said T cell activation causes inhibition of an activity of said target cell by more than about 10%, such as 20%, 30%, or 40% or even more than 50%, such as more than 60%, such as 70%, 80%, or even more than 90%, such as 100%.

8. The polypeptide according to any of claims 1 to 7, wherein said first ISV binds to the constant domain of a T cell receptor α (TCR-α) (SEQ ID NO: 348 and/or SEQ ID NO: 484) and/or the

constant domain of the T cell receptor β (TCR-β) (SEQ ID NO: 349 and/or SEQ ID NO: 485), or polymorphic variants or isoforms thereof.

9. The polypeptide according to any of the preceding claims, wherein said polypeptide and/or first ISV has an on rate constant (Kon) for binding to said TCR selected from the group consisting of at least about 10^2 $M^{-1}s^{-1}$, at least about 10^3 $M^{-1}s^{-1}$, at least about 10^4 $M^{-1}s^{-1}$, at least about 10^5 $M^{-1}s^{-1}$, at least about 10^6 $M^{-1}s^{-1}$, 10^7 $M^{-1}s^{-1}$, at least about 10^8 $M^{-1}s^{-1}$, at least about 10^9 $M^{-1}s^{-1}$, and at least about 10^{10} $M^{-1}s^{-1}$, preferably as measured by surface plasmon resonance or BLI.

10. The polypeptide according to any of the preceding claims, wherein said polypeptide and/or first ISV has an off rate constant (Koff) for binding to said TCR selected from the group consisting of at most about $10^{-3}s^{-1}$, at most about $10^{-4}s^{-1}$, at most about $10^{-5}s^{-1}$, at most about $10^{-6}s^{-1}$, at most about $10^{-7}s^{-1}$, at most about $10^{-8}s^{-1}$, at most about $10^{-9}s^{-1}$, and at most about $10^{-10}s^{-1}$, preferably as measured by surface plasmon resonance or BLI.

11. The polypeptide according to any of the preceding claims, wherein said first ISV binds to said TCR with an EC50 value of between 100 nM and 1 pM, such as at an average EC50 value of 100 nM or less, even more preferably at an average EC50 value of 90 nM or less, such as less than 80, 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM or even less, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5 pM, or even less, such as less than 4 pM, preferably as measured by flow cytometry.

12. The polypeptide according to any of the preceding claims, wherein said first ISV binds to said TCR with an average KD value of between 100 nM and 10 pM, such as at an average KD value of 90 nM or less, even more preferably at an average KD value of 80 nM or less, such as less than 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20 pM, or even less, such as less than 10 pM, said KD preferably determined by Kinexa, BLI or SPR, for instance as determined by Proteon.

13. The polypeptide according to any of the preceding claims, wherein said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NOs: 119-133; or
(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123 or with any of SEQ ID NOs: 119-133; and/or

(ii) CDR2 is chosen from the group consisting of:
(c) SEQ ID NOs: 134-163; or
(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153 or with any of SEQ ID NOs: 134-163; and/or

(iii) CDR3 is chosen from the group consisting of:
(e) SEQ ID NOs: 164-174; or
(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170 or with any of SEQ ID NOs: 164-174.

14. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is chosen from the group consisting of
(a) SEQ ID NO: 123; and
(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 123, wherein
- at position 2 the D has been changed into A, S, E or G;
- at position 4 the H has been changed into Y;
- at position 5 the K has been changed into L;
- at position 6 the I has been changed into L;
- at position 8 the F has been changed into I or V; and/or
- at position 10 the G has been changed into S.

15. The polypeptide according to claim 13 or 14, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of
(a) SEQ ID NO: 153; and
(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 153, wherein
- at position 1 the H has been changed into T or R;
- at position 3 the S has been changed into T or A;
- at position 5 the G has been changed into S or A;
- at position 7 the Q has been changed into D, E, T, A or V;

- at position 8 the T has been changed into A or V; and/or
- at position 9 the D has been changed into A, Q, N, V or S.

16. The polypeptide according to any of claims 13 to 15, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is chosen from the group consisting of
    (a) SEQ ID NO: 170; and
    (b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 170, wherein
        - at position 1 the F has been changed into Y, L or G;
        - at position 4 the I has been changed into L;
        - at position 5 the Y has been changed into W; and/or
        - at position 8 the D has been changed into N or S.

17. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is chosen from the group consisting of
    (a) SEQ ID NO: 124; and
    (b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein
        - at position 2 the E has been changed into Q; and/or
        - at position 6 the I has been changed into V.

18. The polypeptide according to claim 13 or 17, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of
    (a) SEQ ID NO: 145; and
    (b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein
        - at position 9 the N has been changed into D.

19. The polypeptide according to any of claims 13, 17 or 18, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is chosen from the group consisting of
    (a) SEQ ID NO: 167; and
    (b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein

- at position 4 the L has been changed into I.

20. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR1 is SEQ ID NO: 130.

21. The polypeptide according to claim 13 or 20, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR2 is chosen from the group consisting of
    (a) SEQ ID NO: 157; and
    (b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein
        - at position 8 the T has been changed into I.

22. The polypeptide according to any of claims 13, 20 or 21, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which CDR3 is SEQ ID NO: 172.

23. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:
    (i) CDR1 is chosen from the group consisting of:
        (a) SEQ ID NOs: 119-123, 125-127, 129, 132 and 133; and
        (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123; and/or
    (ii) CDR2 is chosen from the group consisting of:
        (c) SEQ ID NOs: 134-141, 143-144, 146-156, 159-163; and
        (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153; and/or
    (iii) CDR3 is chosen from the group consisting of:
        (e) SEQ ID NOs: 164-166, 169-171, 173-174; and
        (f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170.

24. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3,

respectively), in which: CDR1 is represented by SEQ ID NO: 123, CDR2 is represented by SEQ ID NO: 153, and CDR3 is represented by SEQ ID NO: 170.

25. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:
  (a) SEQ ID NOs: 124, 128 and 131; and
  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 124; and/or
(ii) CDR2 is chosen from the group consisting of:
  (c) SEQ ID NOs: 142 and 145; and
  (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 145; and/or
(iii) CDR3 is chosen from the group consisting of:
  (e) SEQ ID NOs: 167 and 168; and
  (f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 167.

26. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 124, CDR2 is represented by SEQ ID NO: 145, and CDR3 is represented by SEQ ID NO: 167.

27. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of
  (a) SEQ ID NO: 130; and
  (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 130; and/or
(ii) CDR2 is chosen from the group consisting of:
  (c) SEQ ID NOs: 157-158; and
  (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 157; and/or

(iii) CDR3 is chosen from the group consisting of:
 (e) SEQ ID NO: 172; and
 (f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 172.

28. The polypeptide according to claim 13, in which said first ISV essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which: CDR1 is represented by SEQ ID NO: 130, CDR2 is represented by SEQ ID NO: 157, and CDR3 is represented by SEQ ID NO: 172.

29. The polypeptide according to any of the preceding claims, further comprising a third ISV, which has high affinity for/binds to a second antigen on a target cell, wherein said second antigen is different from said first antigen.

30. The polypeptide according to any of the preceding claims, wherein said first antigen on a target cell is a tumour antigen, preferably a tumour associated antigen (TAA).

31. The polypeptide according to claim 29 or 30, wherein said second antigen on a target cell is a tumour antigen, preferably a tumour associated antigen (TAA).

32. The polypeptide according to any of claims 29 or 31, wherein said first antigen and said second antigen are present on the same target cell.

33. The polypeptide according to any of claims 29 or 31, wherein said first antigen and said second antigen are present on different target cells.

34. The polypeptide according to any of claims 30 to 33, wherein said TAA (is) are independently chosen from the group consisting of Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Fibroblast Activation Protein (FAP), MART-1, carcinoembryonic antigen (CEA), gp100, MAGE-1, HER-2, Lewis^{Y} antigens, CD123, CD44, CLL-1, CD96, CD47, CD32, CXCR4, Tim-3, CD25, TAG-72, Ep-CAM, PSMA, PSA, GD2, GD3, CD4, CD5, CD19, CD20, CD22, CD33, CD36, CD45, CD52, CD147, growth factor receptors including ErbB3 and ErbB4, Cytokine receptors including Interleukin-2 receptor gamma chain (CD132 antigen), Interleukin-10 receptor alpha chain (IL-10R-A), Interleukin-10 receptor beta chain (IL-10R-B), Interleukin-12 receptor beta-1 chain (IL-12R-beta1), Interleukin-12 receptor beta-2 chain (IL-12

receptor beta-2), Interleukin-13 receptor alpha-1 chain (IL-13R-alpha-1) (CD213a1 antigen), Interleukin-13 receptor alpha-2 chain (Interleukin-13 binding protein), Interleukin-17 receptor (IL-17 receptor), Interleukin-17B receptor (IL-17B receptor), Interleukin 21 receptor precursor (IL-21R), Interleukin-1 receptor type I (IL-1R-1) (CD121a), Interleukin-1 receptor type II (IL-1R-beta) (CDw121b), Interleukin-1 receptor antagonist protein (IL-1ra), Interleukin-2 receptor alpha chain (CD25 antigen), Interleukin-2 receptor beta chain (CD122 antigen), Interleukin-3 receptor alpha chain (IL-3R-alpha) (CD123 antigen), CD30, IL23R, IGF-1R, IL5R, IgE, CD248 (endosialin), CD44v6, gpA33, Ron, Trop2, PSCA, claudin 6, claudin 18.2, CLEC12A, CD38, ephA2, c-Met, CD56, MUC16, EGFRvIII, AGS-16, CD27L, Nectin-4, SLITRK6, mesothelin, folate receptor, tissue factor, axl, glypican-3, CA9, Cripto, CD138, CD37, MUC1, CD70, gastrin releasing peptide receptor, PAP, CEACAM5, CEACAM6, CXCR7, N-cadherin, FXYD2 gamma a, CD21, CD133, Na/K-ATPase, mIgM (membrane-bound IgM), mIgA (membrane-bound IgA), Mer, Tyro2, CD120, CD95, CA 195, DR5, DR6, DcR3 and CAIX, and related polymorphic variants and isoforms.

35. The polypeptide according to claim 34, wherein said TAA is CD20 (UniProt 11836), HER2 (Uniprot P04626), EGFR, CEA, or a polymorphic variant or isoform thereof.

36. The polypeptide according to any one of the claims 29-34, wherein said first antigen and said second antigen are chosen from the group consisting of:
- EGFR as a first antigen and CEA as a second antigen;
- CD19 as a first antigen and CD20 as a second antigen;
- CD19 as a first antigen and CD22 as a second antigen;
- CD123 as a first antigen and Tim-3 as a second antigen; and
- CD123 as a first antigen and CD69 as a second antigen.

37. The polypeptide according to any of the preceding claims, further comprising a serum protein binding moiety.

38. The polypeptide according to claim 37, wherein said serum protein binding moiety binds serum albumin.

39. The polypeptide according to claim 37 or 38, wherein said serum protein binding moiety is an ISV binding serum albumin.

40. The polypeptide according to claim 39, wherein said ISV binding serum albumin essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which CDR1 is SFGMS (SEQ ID NO: 481), CDR2 is SISGSGSDTLYADSVKG (SEQ ID NO: 482) and CDR3 is GGSLSR (SEQ ID NO: 475), as determined by Kabat definition.

41. The polypeptide according to claim 40, wherein said ISV binding serum albumin is selected from Alb8, Alb23, Alb129, Alb132, Alb11, Alb11 (S112K)-A, Alb82, Alb82-A, Alb82-AA, Alb82-AAA, Alb82-G, Alb82-GG and Alb82-GGG (SEQ ID NOs: 400 to 412).

42. The polypeptide according to any one of the preceding claims, wherein said ISVs are directly linked to each other or are linked via a linker.

43. The polypeptide according to any one of the claims 1 to 41, wherein said first ISV and/or said second ISV and/or possibly said third ISV and/or possibly said ISV binding serum albumin are linked via a linker.

44. The polypeptide according to claim 43, wherein said linker is chosen from the group consisting of linkers of 5GS, 7GS, 9GS, 10GS, 15GS, 18GS, 20GS, 25GS, 30GS and 35GS (SEQ ID NOs: 376 to 385).

45. The polypeptide according to claim 37 or 38, wherein said serum protein binding moiety is a non-antibody based polypeptide.

46. The polypeptide according to any of the claims 1-36, further comprising PEG.

47. The polypeptide according to any of the preceding claims, wherein said ISV is a Nanobody, a V_{HH}, a humanized V_{HH}, or a camelized V_H.

48. The polypeptide according to any of the preceding claims, wherein said first ISV is chosen from the group consisting of SEQ ID NOs: 1 to 118, and wherein said second ISV is chosen from the group consisting of SEQ ID NOs: 350-358.

49. The polypeptide according to any of the preceding claims, chosen from the group consisting of SEQ ID NOs: 292, 295-296, 299-300, 303, 306-343, 387-388, 390, 414, 417-418, 421-422, 425, 428-464, 467-468, 470-471 and 486-487.

50. A polypeptide that specifically binds the constant domain of the T cell receptor (TCR) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:
   (i) CDR1 is chosen from the group consisting of:
      (a) SEQ ID NOs: 119-133; or
      (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 119-133, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
   (ii) CDR2 is chosen from the group consisting of:
      (c) SEQ ID NOs: 134-163; or
      (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 134-163, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
   (iii) CDR3 is chosen from the group consisting of:
      (e) SEQ ID NOs: 164-174; or
      (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of any of SEQ ID NOs: 164-174, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

51. The polypeptide according to claim 50, in which:
   (i) CDR1 is chosen from the group consisting of:
      (a) SEQ ID NOs: 119-123, 125-127, 129, 132 and 133; and

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 123, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 134-141, 143-144, 146-156, 159-163; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 153, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 164-166, 169-171, 173-174; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 170, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

52. The polypeptide according to any of claims 50 or 51, in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 123; and

(b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 123, wherein

- at position 2 the D has been changed into A, S, E or G;
- at position 4 the H has been changed into Y;
- at position 5 the K has been changed into L;
- at position 6 the I has been changed into L;
- at position 8 the F has been changed into I or V; and/or
- at position 10 the G has been changed into S.

53. The polypeptide according to any of claims 50 to 52, in which CDR2 is chosen from the group consisting of
    (a) SEQ ID NO: 153; and
    (b) amino acid sequences that have 1, 2, 3, 4 or 5 amino acid difference(s) with SEQ ID NO: 153, wherein
        - at position 1 the H has been changed into T or R;
        - at position 3 the S has been changed into T or A;
        - at position 5 the G has been changed into S or A;
        - at position 7 the Q has been changed into D, E, T, A or V;
        - at position 8 the T has been changed into A or V; and/or
        - at position 9 the D has been changed into A, Q, N, V or S.

54. The polypeptide according to any of claims 50 to 53, in which CDR3 is chosen from the group consisting of
    (a) SEQ ID NO: 170; and
    (b) amino acid sequences that have 1, 2, 3 or 4 amino acid difference(s) with SEQ ID NO: 170, wherein
        - at position 1 the F has been changed into Y, L or G;
        - at position 4 the I has been changed into L;
        - at position 5 the Y has been changed into W; and/or
        - at position 8 the D has been changed into N or S.

55. The polypeptide according to any of claims 50 to 54, in which CDR1 is represented by SEQ ID NO: 123, CDR2 is represented by SEQ ID NO: 153, and CDR3 is represented by SEQ ID NO: 170.

56. The polypeptide according to claim 50, in which:
    (i) CDR1 is chosen from the group consisting of:
        (a) SEQ ID NOs: 124, 128 and 131; and
        (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 124, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
    (ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NOs: 142 and 145; and

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 145, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NOs: 167 and 168; and

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 167, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

57. The polypeptide according to any of claims 50 or 56, in which CDR1 is chosen from the group consisting of

(a) SEQ ID NO: 124; and

(b) amino acid sequences that have 1, or 2 amino acid difference(s) with SEQ ID NO: 124, wherein

- at position 2 the E has been changed into Q; and/or
- at position 6 the I has been changed into V.

58. The polypeptide according to any of claims 50, 56 or 57, in which CDR2 is chosen from the group consisting of

(a) SEQ ID NO: 145; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 145, wherein

- at position 9 the N has been changed into D.

59. The polypeptide according to any of claims 50, 56 to 58, in which CDR3 is chosen from the group consisting of

(a) SEQ ID NO: 167; and

(b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 167, wherein

- at position 4 the L has been changed into I.

60. The polypeptide according to any of claims 50, 56 to 59, in which CDR1 is represented by SEQ ID NO: 124, CDR2 is represented by SEQ ID NO: 145, and CDR3 is represented by SEQ ID NO: 167.

61. The polypeptide according to claim 50, in which:
   (i) CDR1 is chosen from the group consisting of
      (a) SEQ ID NO: 130; and
      (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 130, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
   (ii) CDR2 is chosen from the group consisting of:
      (c) SEQ ID NOs: 157-158; and
      (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 157, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
   (iii) CDR3 is chosen from the group consisting of:
      (e) SEQ ID NO: 172; and
      (f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 172, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds TCR with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

62. The polypeptide according to any of claims 50 or 61, in which CDR1 is chosen from SEQ ID NO: 130.

63. The polypeptide according to any of claims 50, 61 or 62, in which CDR2 is chosen from the group consisting of
   (a) SEQ ID NO: 157; and
   (b) amino acid sequence that has 1 amino acid difference with SEQ ID NO: 157, wherein
      - at position 8 the T has been changed into I.

64. The polypeptide according to any of claims 50, 61 to 63, in which CDR3 is chosen from SEQ ID NO: 172.

65. The polypeptide according to any of claims 50, 61 to 64, in which CDR1 is represented by SEQ ID NO: 130, CDR2 is represented by SEQ ID NO: 157, and CDR3 is represented by SEQ ID NO: 172.

66. A polypeptide that specifically binds carcinoembryonic antigen (CEA) and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:
   (i) CDR1 is chosen from the group consisting of:
      (a) SEQ ID NO: 361 (GDTYGSYWMG); or
      (b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 361, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
   (ii) CDR2 is chosen from the group consisting of:
      (c) SEQ ID NO: 363 (AINRGGGYTV); or
      (d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 363, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or
   (iii) CDR3 is chosen from the group consisting of:
      (e) SEQ ID NO: 365 (SGVLGGLHEDWFNY); or

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 365, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds CEA with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

67. The polypeptide according to claim 66, in which CDR1 is represented by SEQ ID NO: 361, CDR2 is represented by SEQ ID NO: 363, and CDR3 is represented by SEQ ID NO: 365.

68. A polypeptide that specifically binds CD20 and that comprises or essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

(i) CDR1 is chosen from the group consisting of:

(a) SEQ ID NO: 362 (GGTFSSYTMG); or

(b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 362, provided that the polypeptide comprising the CDR1 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR1 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(ii) CDR2 is chosen from the group consisting of:

(c) SEQ ID NO: 364 (EVRWGGVTT); or

(d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 364, provided that the polypeptide comprising the CDR2 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR2 without the 4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance; and/or

(iii) CDR3 is chosen from the group consisting of:

(e) SEQ ID NO: 366 (VRQMYMTVVPDY); or

(f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 366, provided that the polypeptide comprising the CDR3 with 4, 3, 2, or 1 amino acid(s) difference binds CD20 with about the same or a higher affinity compared to the binding by the polypeptide comprising the CDR3 without the

4, 3, 2, or 1 amino acid(s) difference, said affinity as measured by surface plasmon resonance.

69. The polypeptide according to claim 68, in which CDR1 is represented by SEQ ID NO: 362, CDR2 is represented by SEQ ID NO: 364, and CDR3 is represented by SEQ ID NO: 366.

70. The polypeptide according to any of claims 50 to 69, which is a Nanobody, a V_{HH}, a humanized V_{HH}, or a camelized V_H.

71. The polypeptide according to any of claims 50 to 70, further comprising a serum protein binding moiety.

72. The polypeptide according to claim 71, wherein said serum protein binding moiety binds serum albumin.

73. The polypeptide according to any of claims 71 or 72, wherein said serum protein binding moiety is an ISV that binds serum albumin.

74. The polypeptide according to claim 73, wherein said ISV that binds serum albumin essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which CDR1 is SFGMS (SEQ ID NO: 481), CDR2 is SISGSGSDTLYADSVKG (SEQ ID NO: 482) and CDR3 is GGSLSR (SEQ ID NO: 475), according to Kabat definition.

75. The polypeptide according to claim 74, wherein said ISV that binds serum albumin is selected from Alb8, Alb23, Alb129, Alb132, Alb11, Alb11 (S112K)-A, Alb82, Alb82-A, Alb82-AA, Alb82-AAA, Alb82-G, Alb82-GG, and Alb82-GGG (SEQ ID NOs: 400 to 412).

76. The polypeptide according to any of claims 71 to 75, wherein said ISV is directly linked or is linked via a linker.

77. The polypeptide according to claim 76, wherein said linker is chosen from the group consisting of linkers of 5GS, 7GS, 9GS, 10GS, 15GS, 18GS, 20GS, 25GS, 30GS and 35GS (SEQ ID NOs: 376 to 385).

78. The polypeptide according to any of the claims 50 to 70, further comprising a PEG moiety.

79. A nucleic acid or nucleic acid sequence encoding the polypeptide as defined in any one of claims 1 to 78.

80. A vector comprising the nucleic acid or nucleic acid sequence as defined in claim 79.

81. A host cell transformed or transfected with the nucleic acid or nucleic acid sequence as defined in claim 79 or with the vector as defined in claim 80.

82. A process for the production of the polypeptide according to any one of claims 1 to 78, said process comprising culturing a host cell as defined in claim 81 under conditions allowing the expression of the polypeptide as defined in any one of claims 1 to 78 and recovering the produced polypeptide from the culture.

83. A pharmaceutical composition comprising the polypeptide according to any one of claims 1 to 78, or the polypeptide produced according to the process of claim 82.

84. The polypeptide according to any of claims 1-78, or produced according to the process of claim 82, for use in treating a subject in need thereof.

85. A method for delivering a prophylactic or therapeutic polypeptide to a specific location, tissue or cell type in the body, the method comprising the steps of administering to a subject a polypeptide according to any of the claims 1-78, or produced according to the process of claim 82.

86. The polypeptide according to any one of claims 1 to 78, or produced according to the process of claim 82, for use in the prevention, treatment or amelioration of a disease selected from the group consisting of a proliferative disease, an inflammatory disease, an infectious disease and an autoimmune disease.

87. A method for the treatment or amelioration of a disease selected from the group consisting of a proliferative disease, an inflammatory disease, an infectious disease and an autoimmune disease, comprising the step of administering to a subject in need thereof the polypeptide according to any one of claims 1 to 78, or produced according to the process of claim 82.

88. The polypeptide for use according to claim 86 or the method according to claim 87, wherein said proliferative disease is cancer.

89. The polypeptide for use or the method according to claim 88, wherein said cancer is chosen from the group consisting of carcinomas, gliomas, mesotheliomas, melanomas, lymphomas, leukemias, adenocarcinomas: breast cancer, ovarian cancer, cervical cancer, glioblastoma, multiple myeloma (including monoclonal gammopathy of undetermined significance, asymptomatic and symptomatic myeloma), prostate cancer, and Burkitt's lymphoma, head and neck cancer, colon cancer, colorectal cancer, non-small cell lung cancer, small cell lung cancer, cancer of the esophagus, stomach cancer, pancreatic cancer, hepatobiliary cancer, cancer of the gallbladder, cancer of the small intestine, rectal cancer, kidney cancer, bladder cancer, prostate cancer, penile cancer, urethral cancer, testicular cancer, vaginal cancer, uterine cancer, thyroid cancer, parathyroid cancer, adrenal cancer, pancreatic endocrine cancer, carcinoid cancer, bone cancer, skin cancer, retinoblastomas, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Kaposi's sarcoma, multicentric Castleman's disease or AIDS-associated primary effusion lymphoma, neuroectodermal tumors, rhabdomyosarcoma; as well as any metastasis of any of the above cancers, as well as non-cancer indications such as nasal polyposis.

90. The polypeptide for use or the method according to claim 88 or 89, wherein the treatment is a combination treatment.

91. A kit comprising a polypeptide as defined in any one of claims 1 to 78, a nucleic acid or nucleic acid sequence as defined in claim 79, a vector as defined in claim 80, or a host cell as defined in claim 81.

Figure 1:

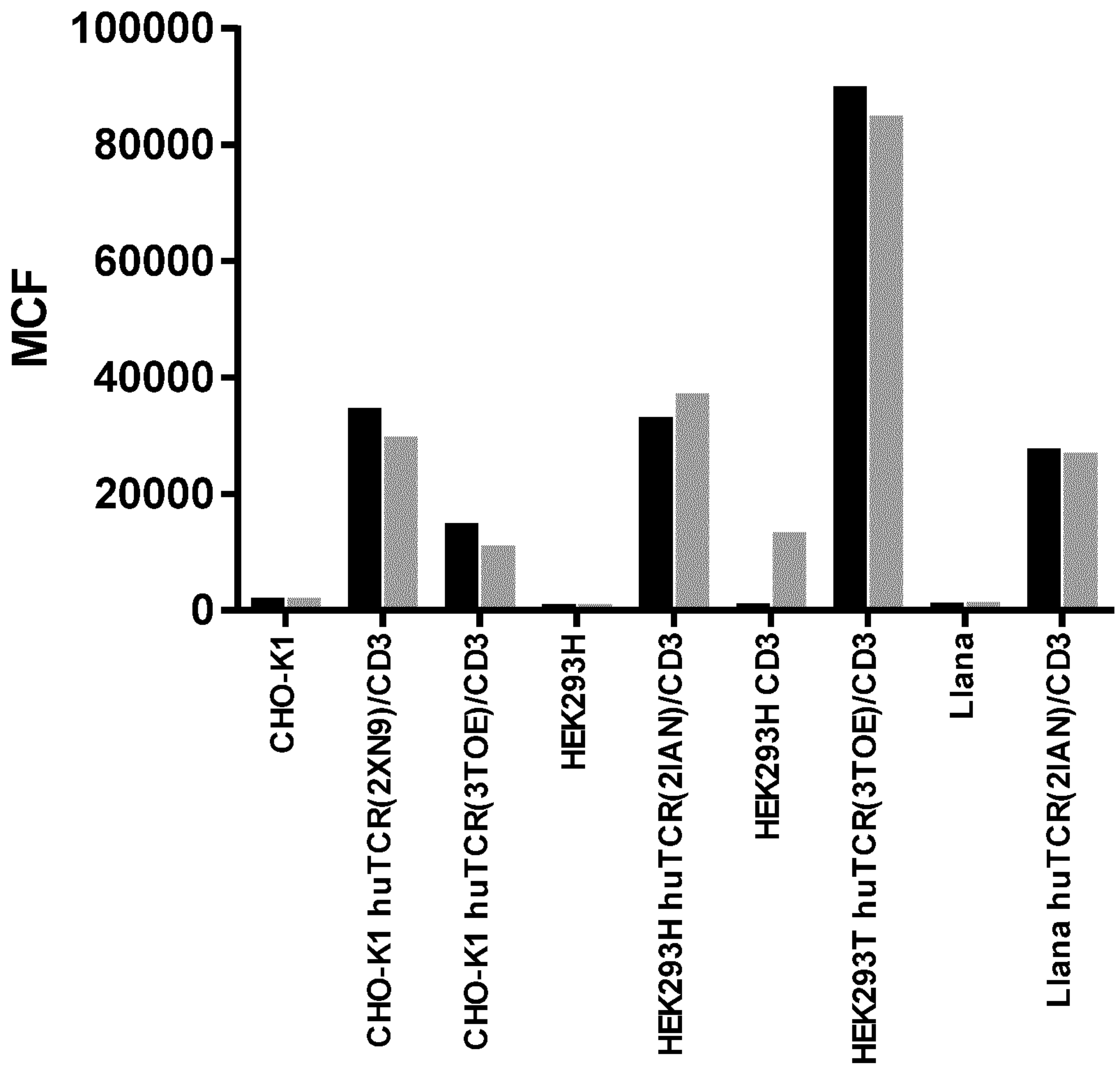


Figure 2:

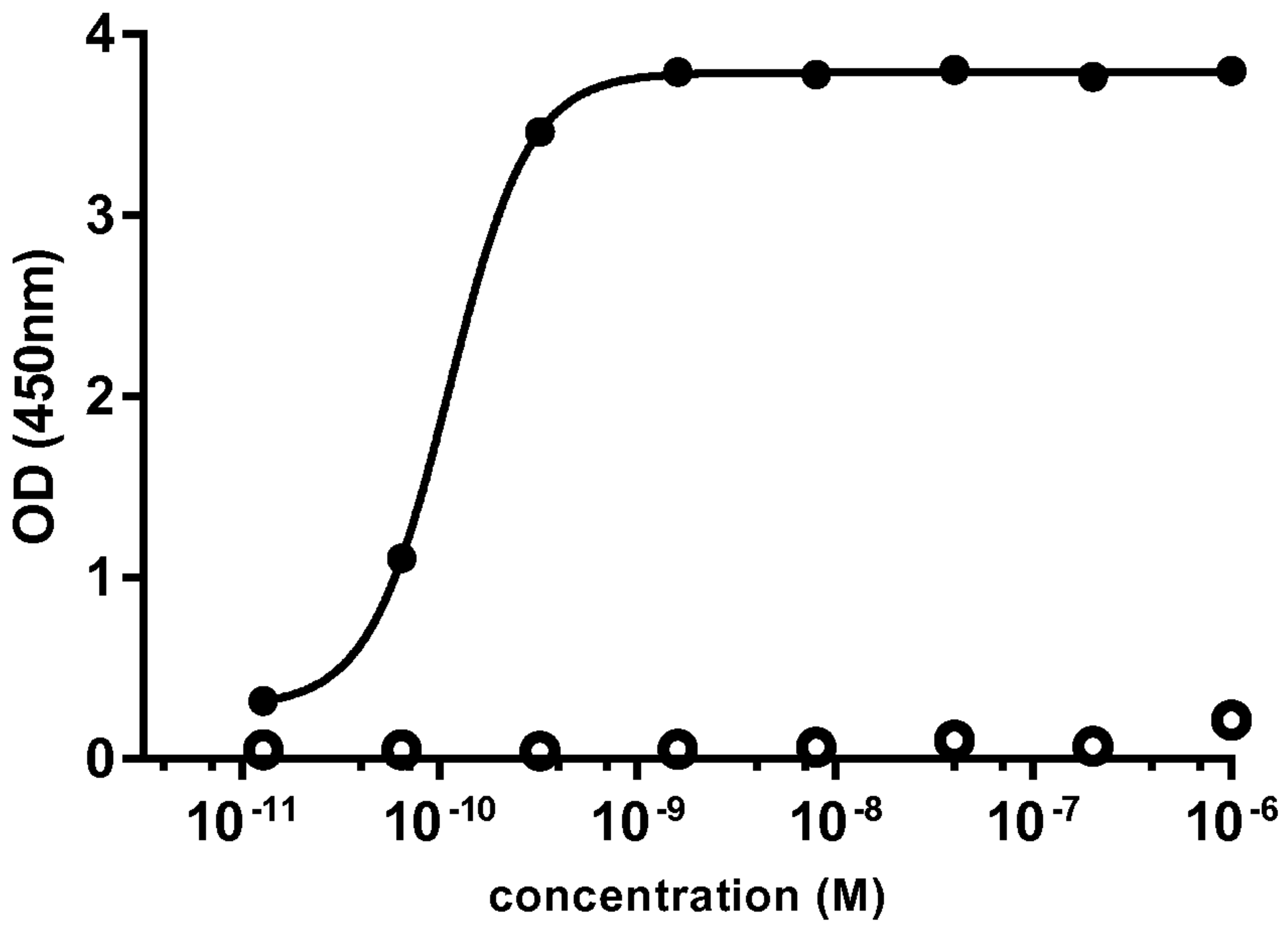


Figure 3:

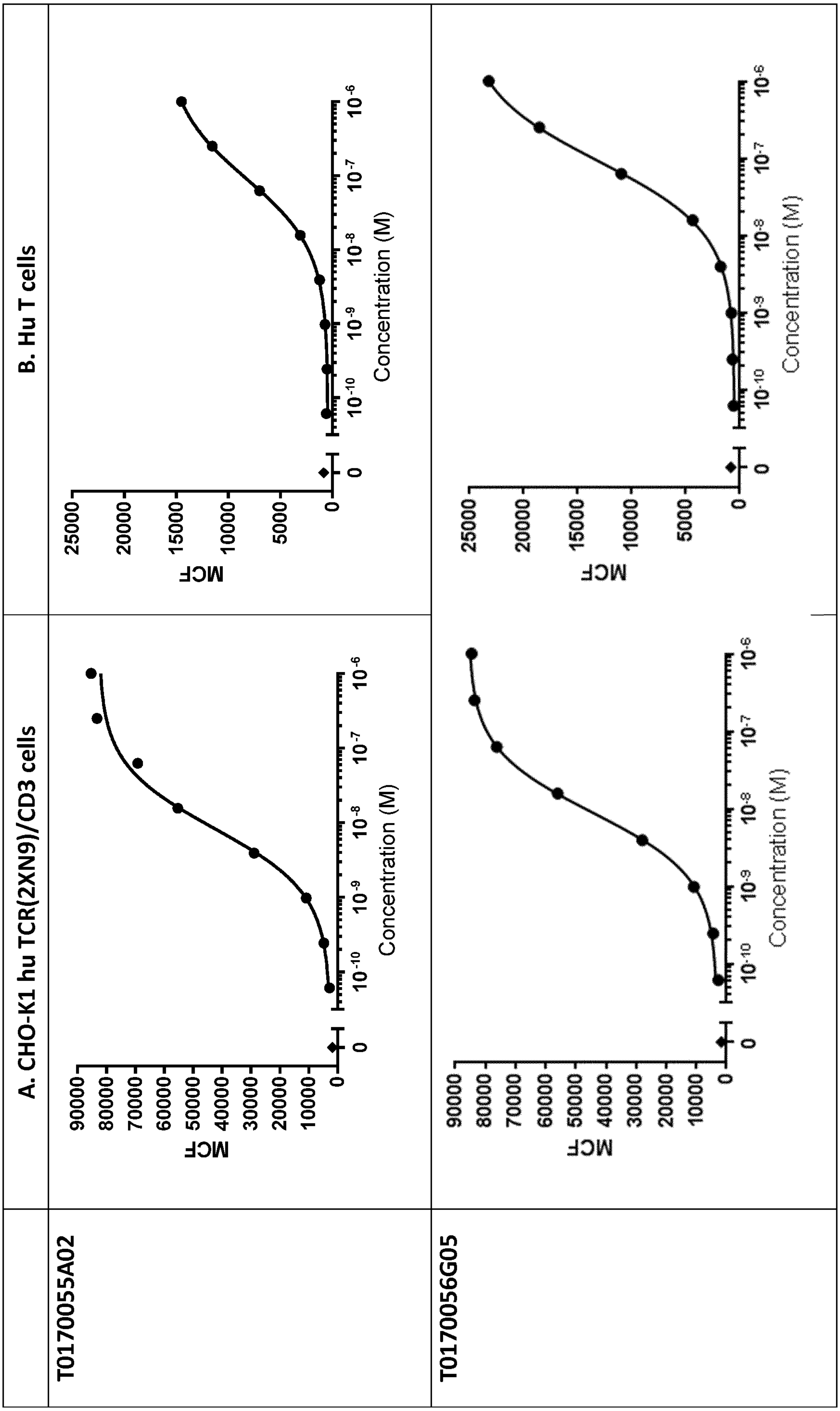

A. CHO-K1 hu TCR(2XN9)/CD3 cells
B. Hu T cells
T0170055A02
T0170056G05
MCF
Concentration (M)

Figure 4 cont':

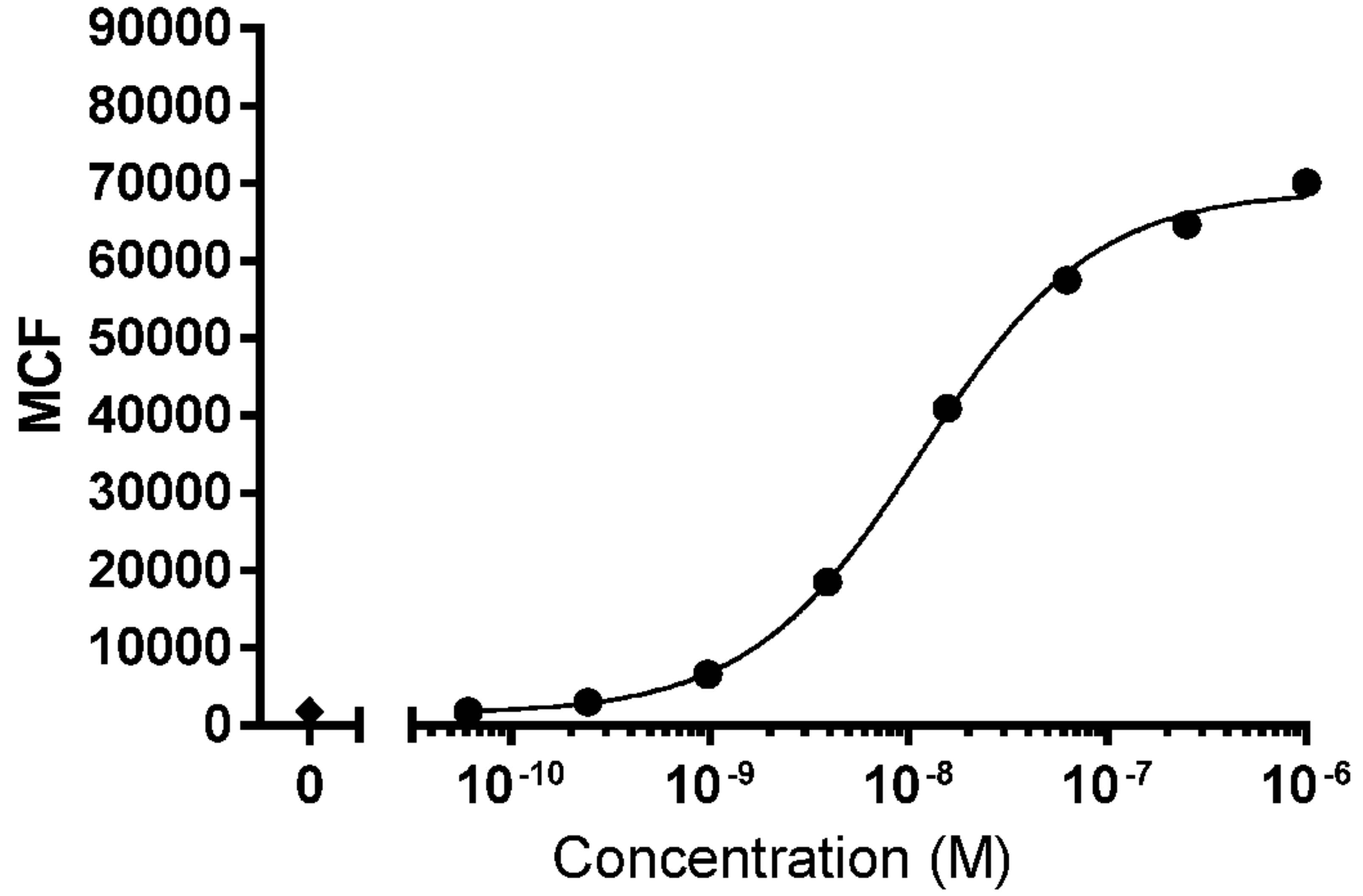
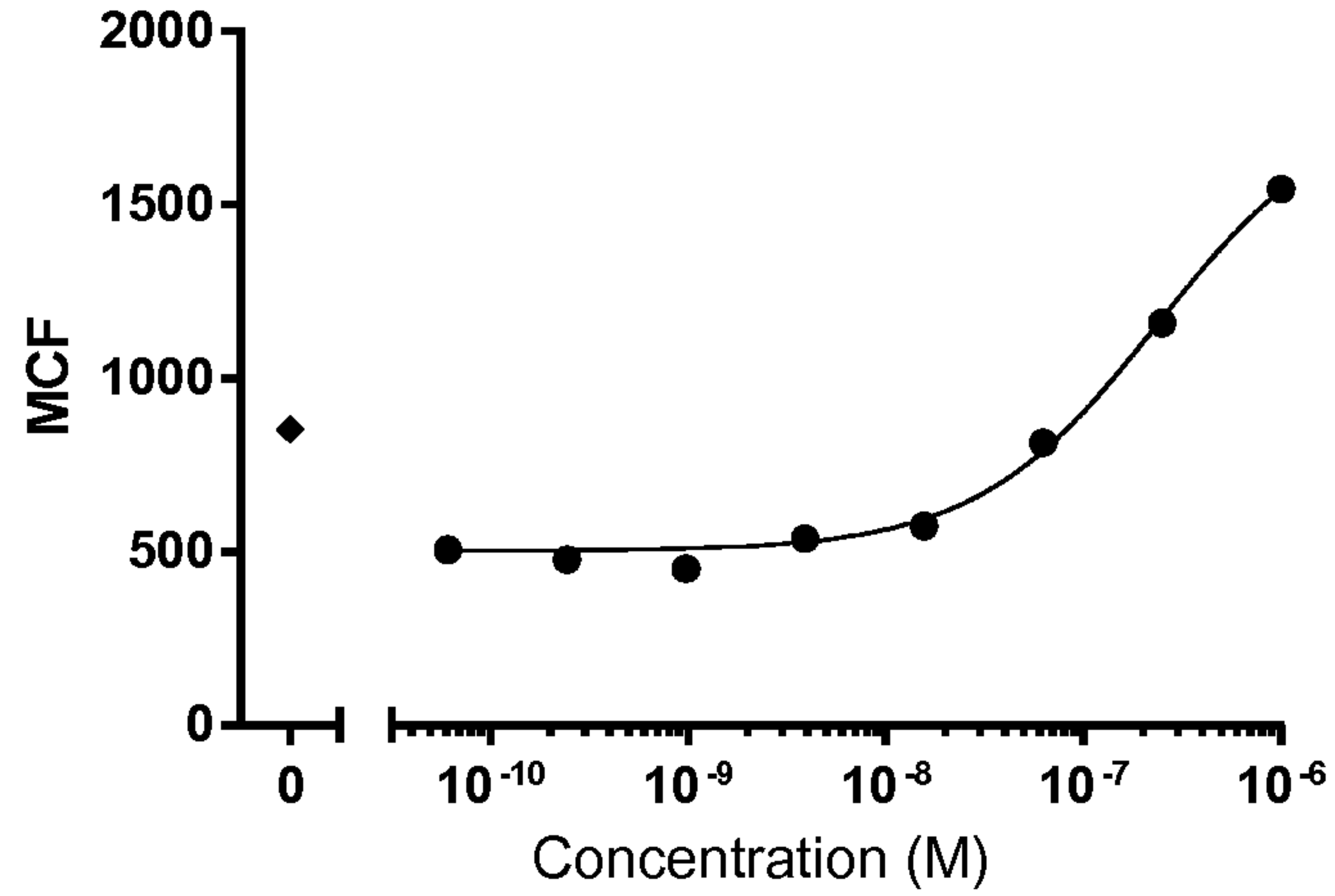
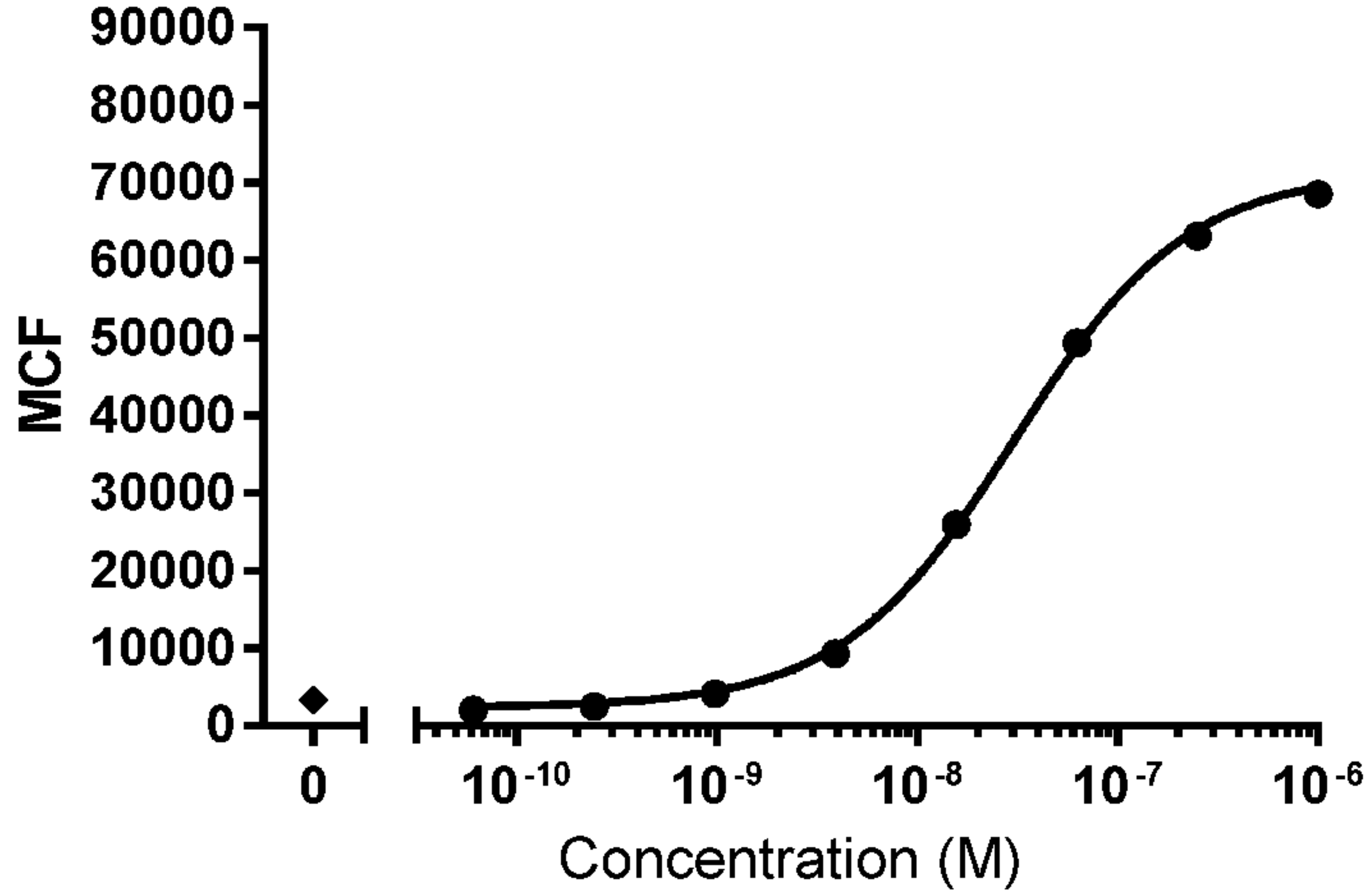
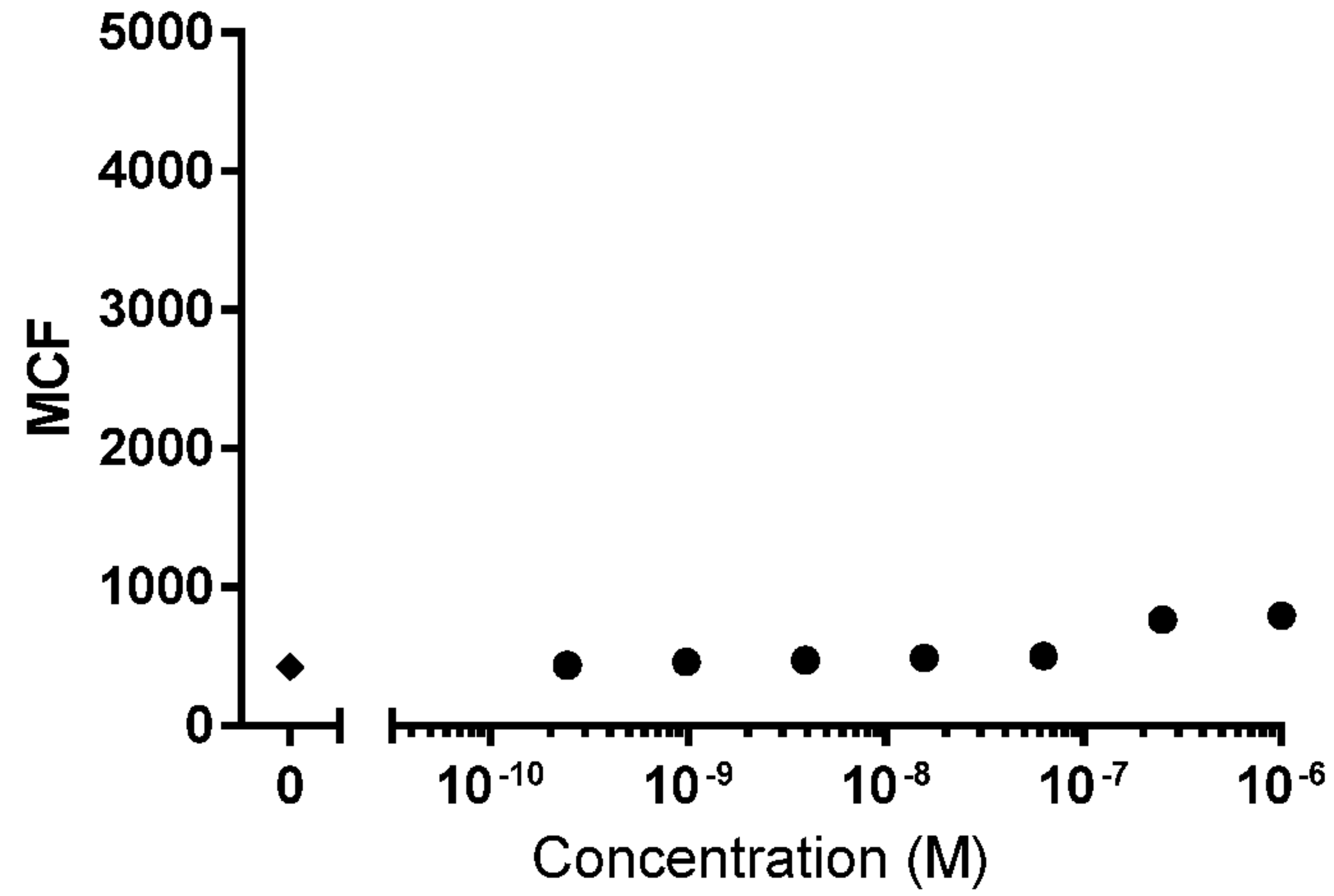
<table>
<tr><th></th><th>A. CHO-K1 hu TCR(2XN9)/CD3 cells</th><th>B. Hu T cells</th></tr>
<tr><td>T0170068G05</td><td>
</td><td>
</td></tr>
<tr><td>T0170061G01</td><td>
</td><td>
</td></tr>
</table>

Figure 5:

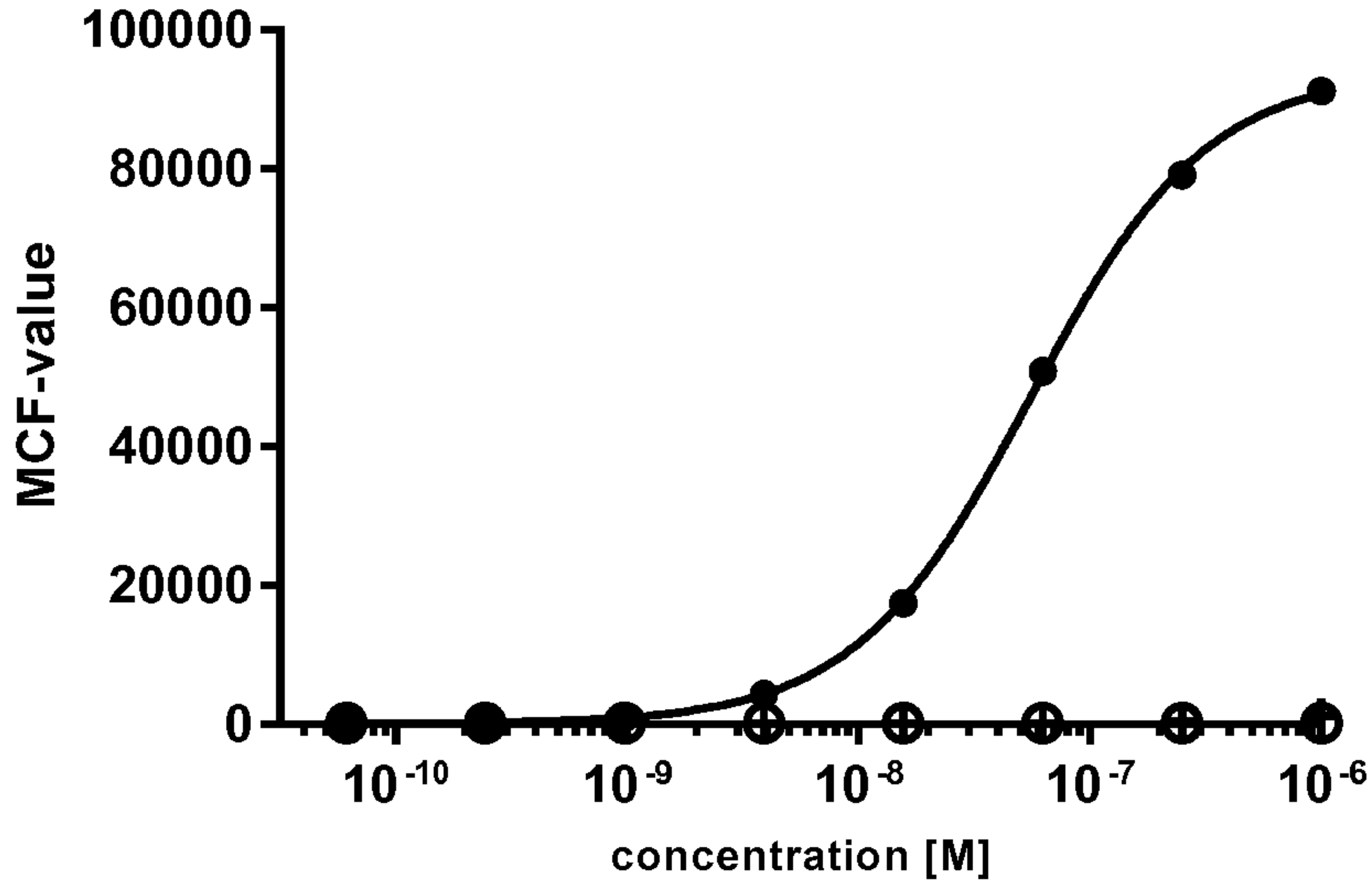
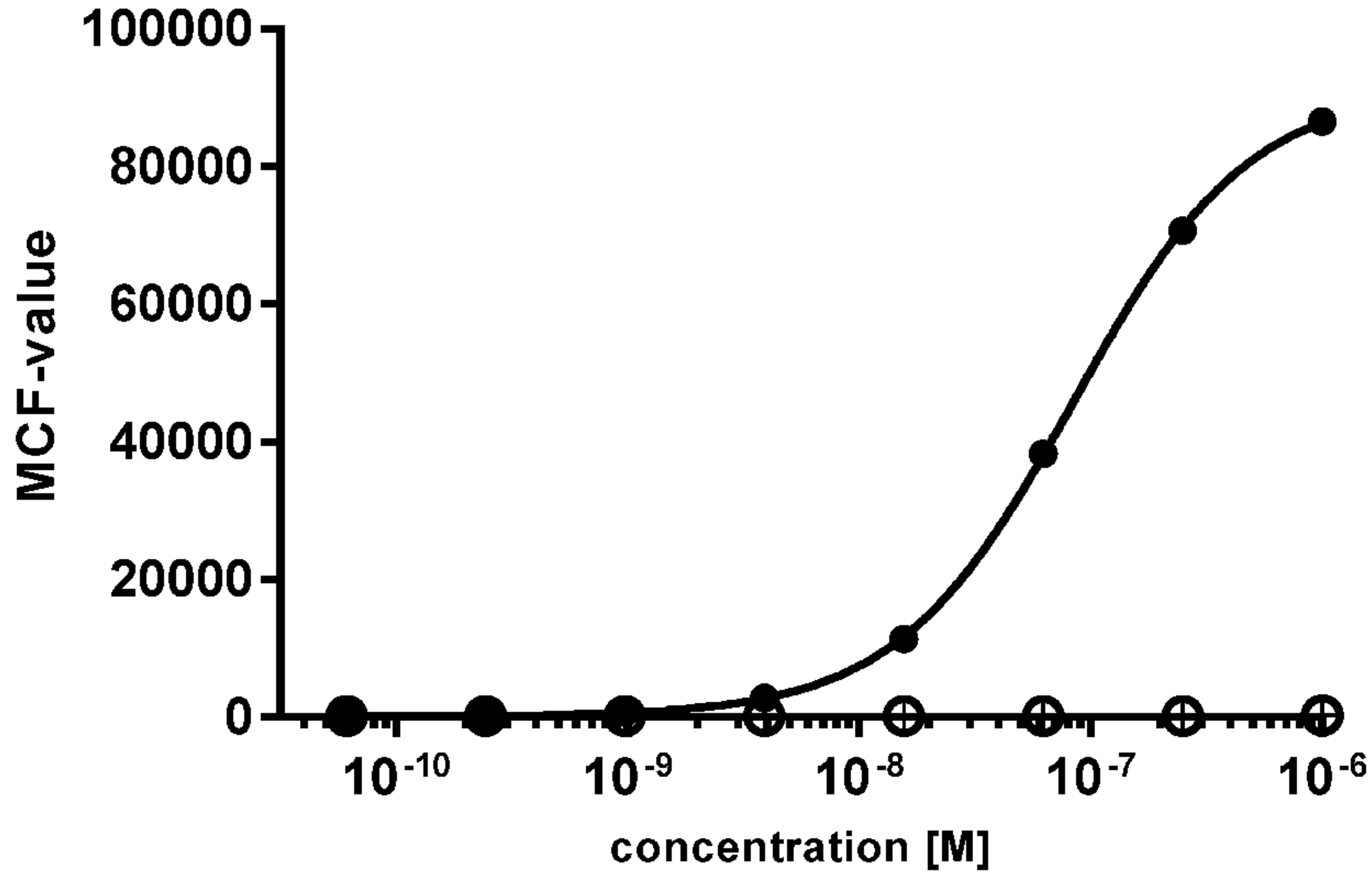
T0170055A02	
T0170056G05	

Figure 6 cont':

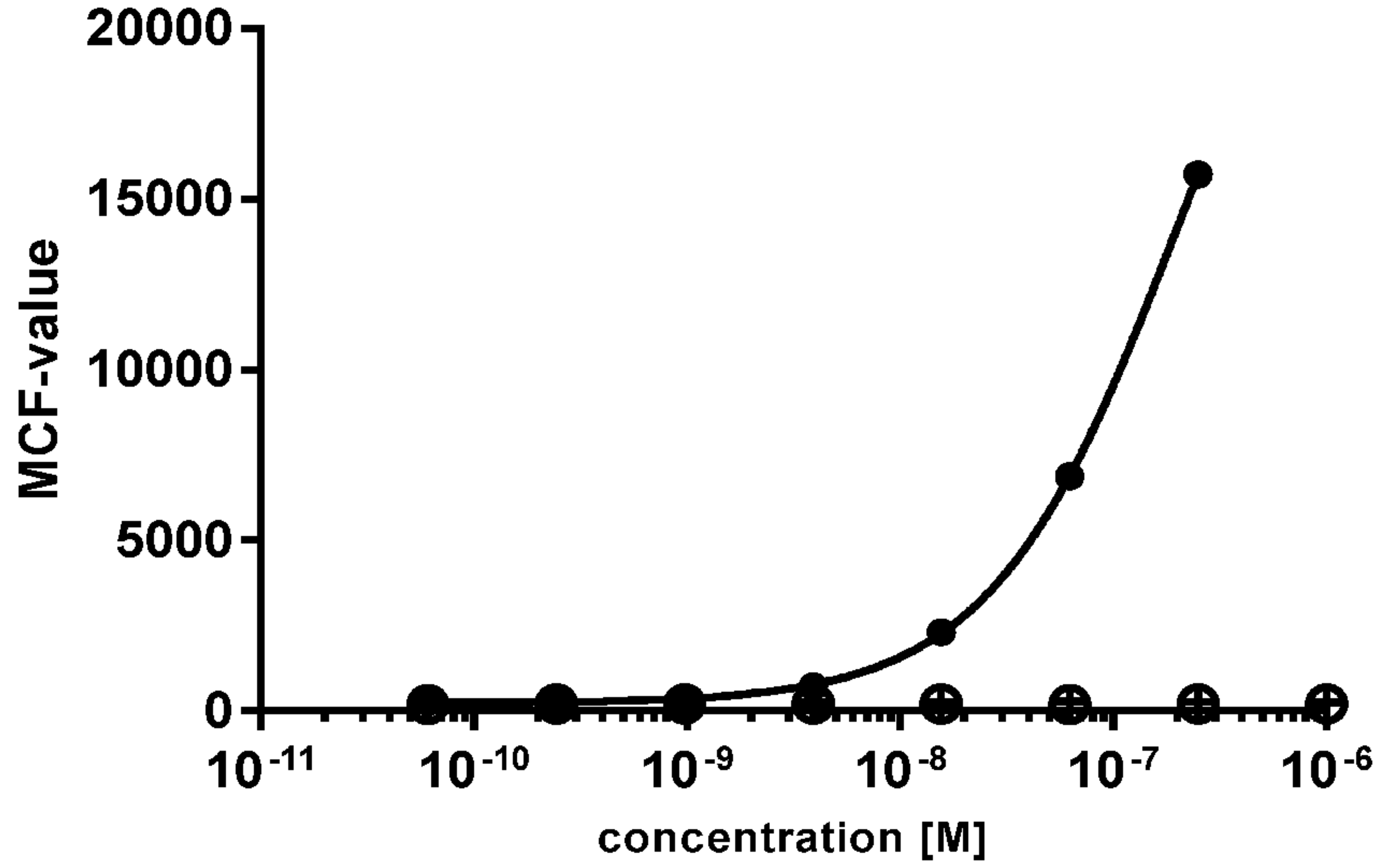
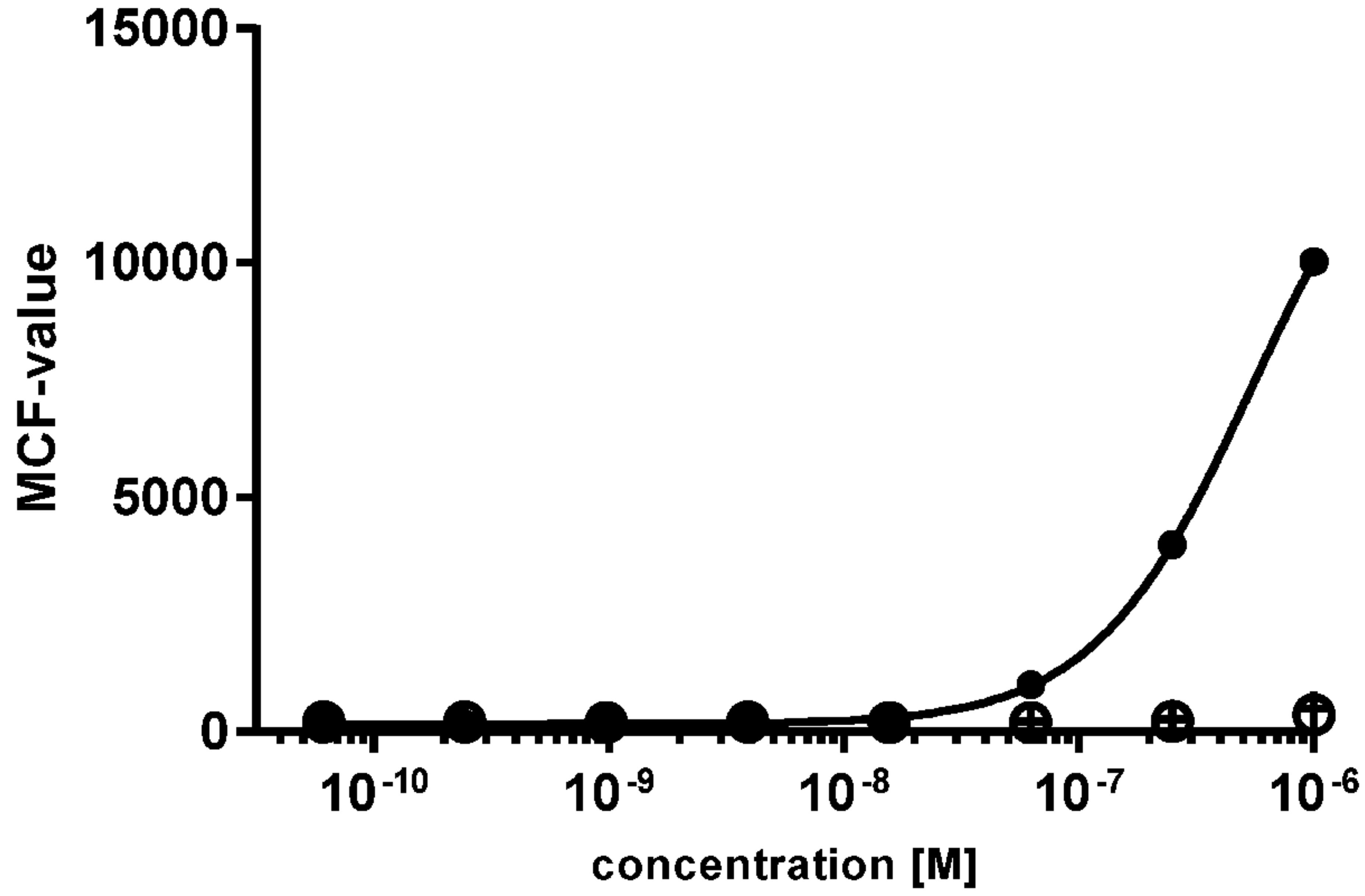
T0170068G05	
T0170061G01	

Figure 7:

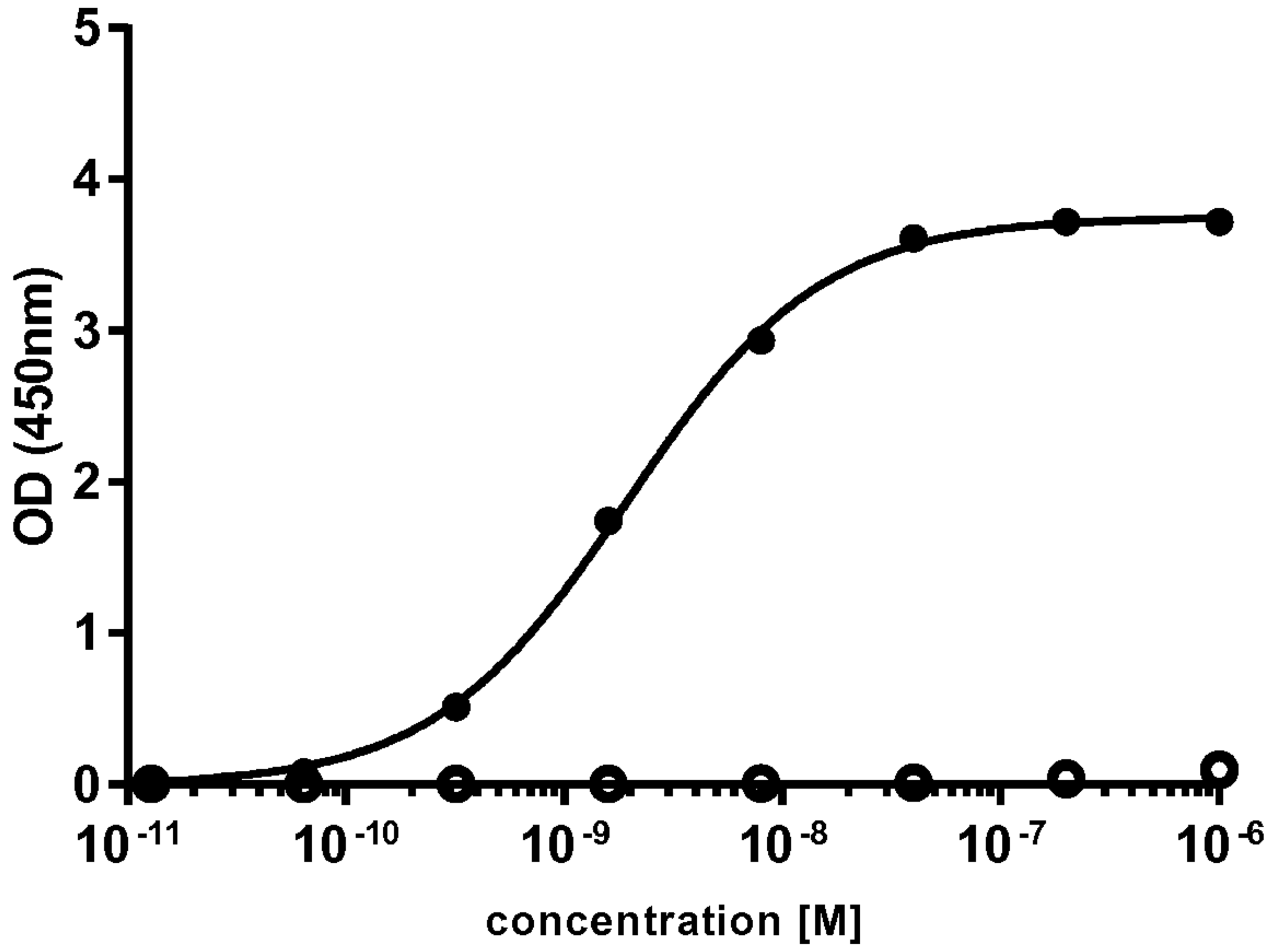
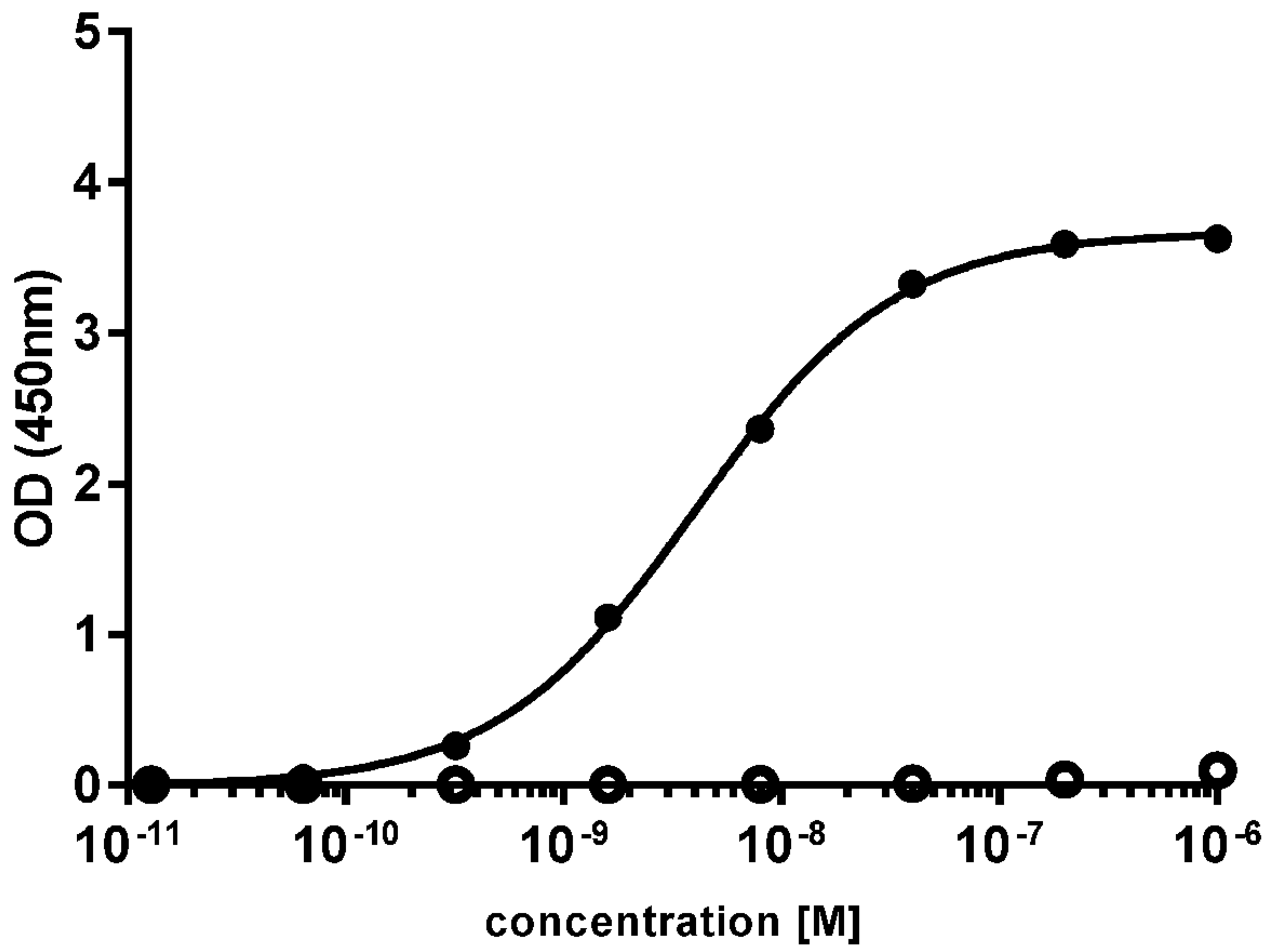
T0170055A02	
T0170056G05	

Figure 8 cont':

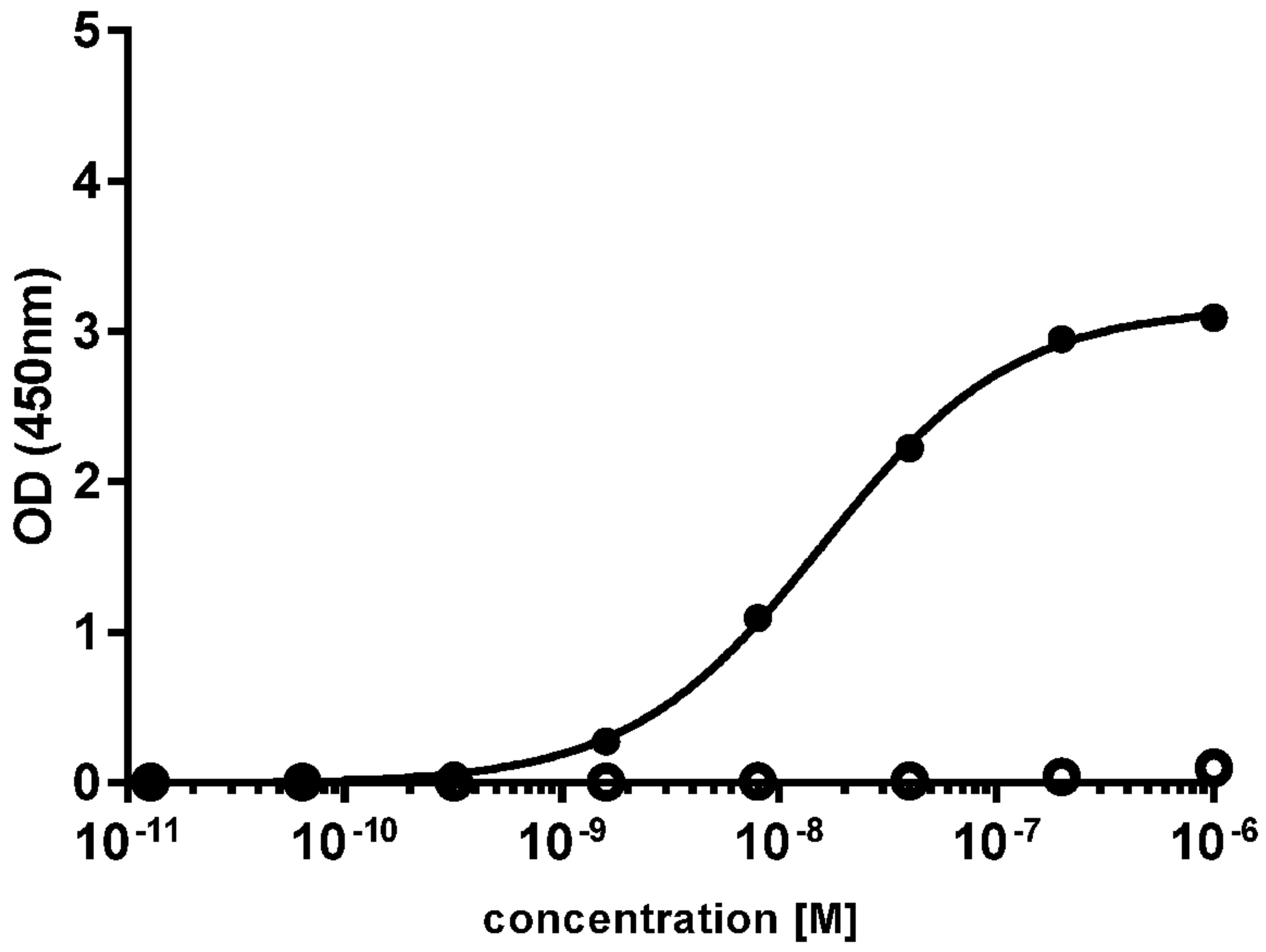
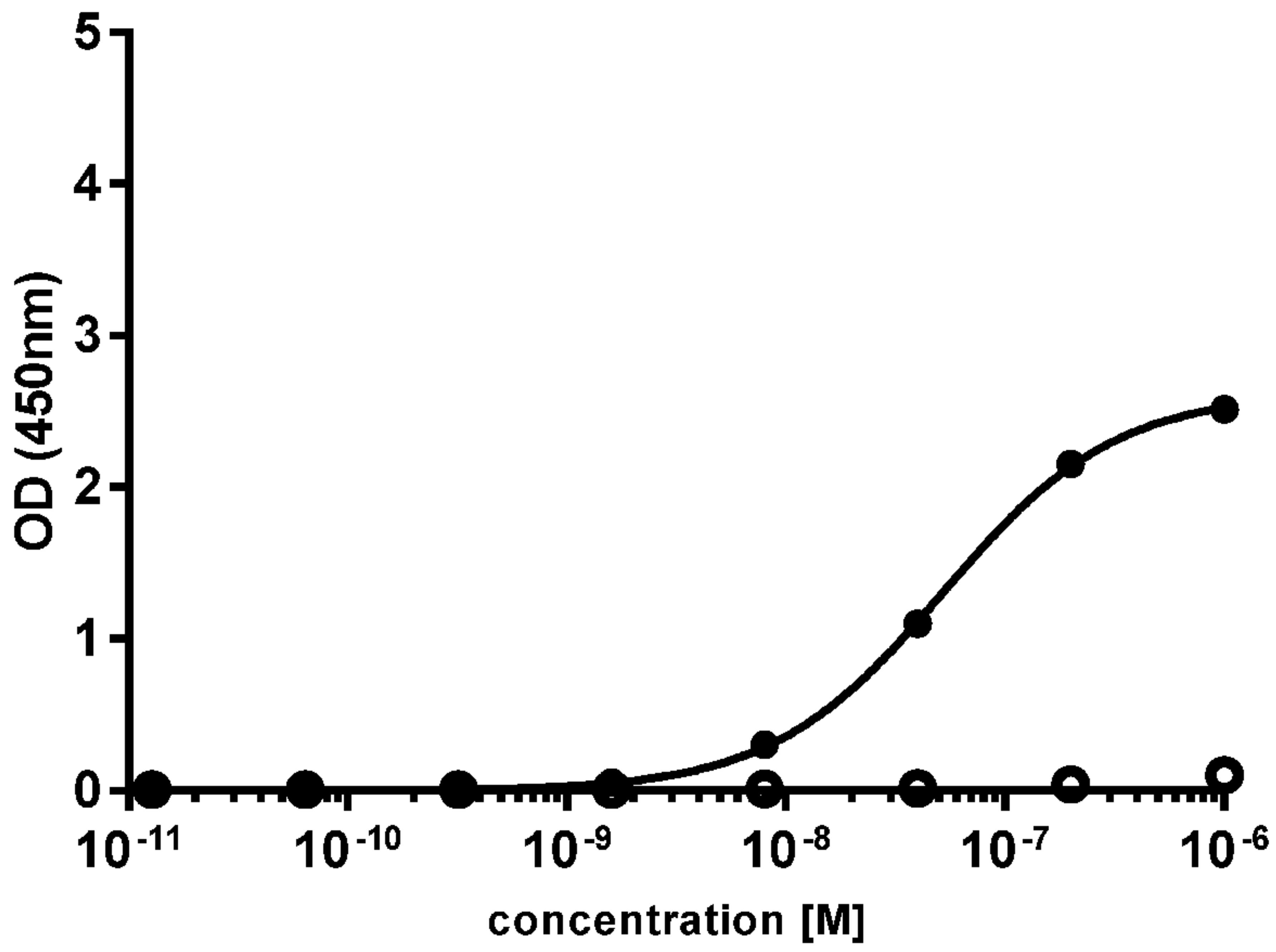
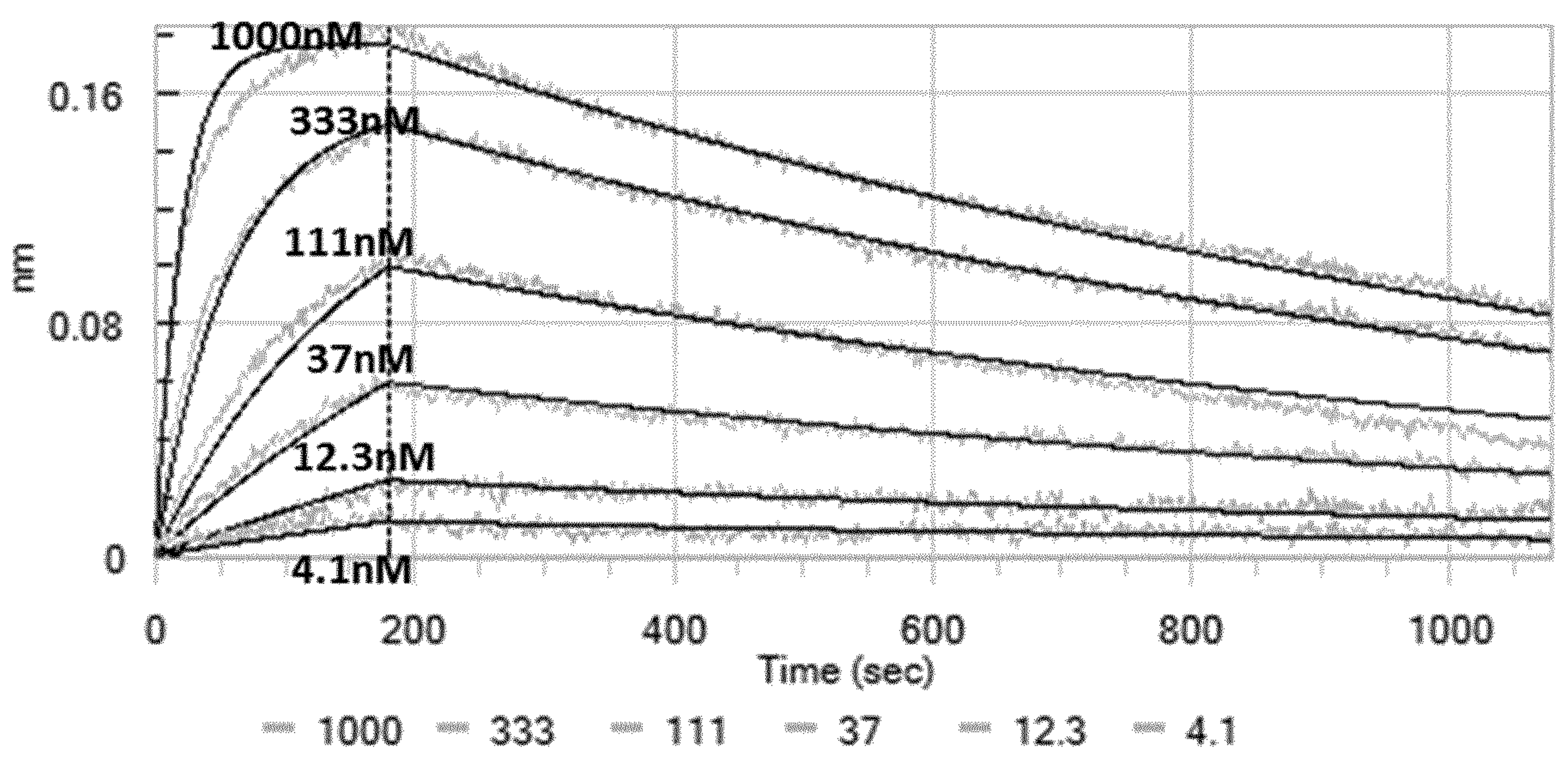
T0170068G05	
T0170061G01	

Figure 9:

A.


1000nM
333nM
111nM
37nM
12.3nM
4.1nM
0.16
0.08
0
nm
0
200
400
600
800
1000
Time (sec)
1000
333
111
37
12.3
4.1

B.

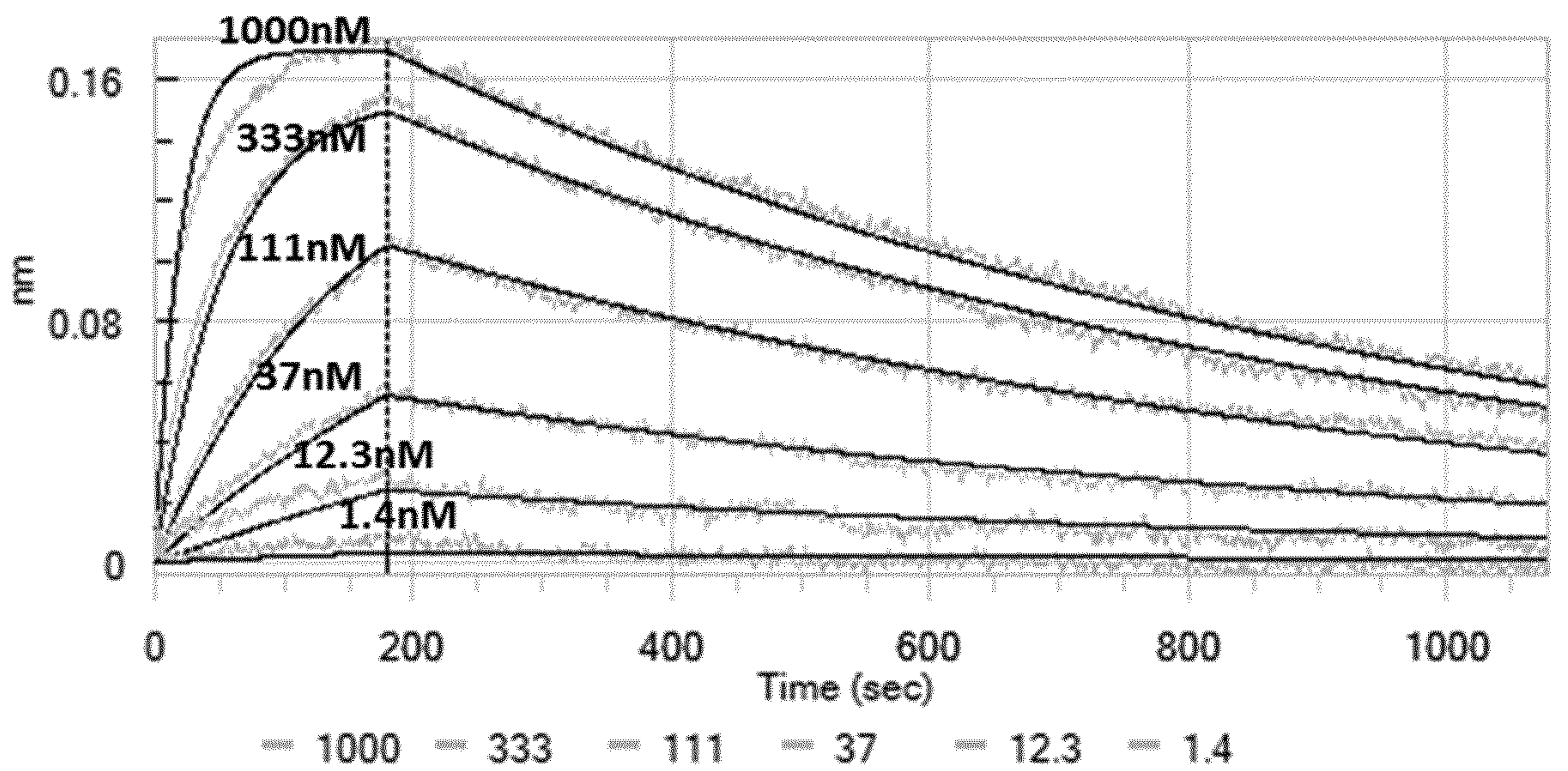
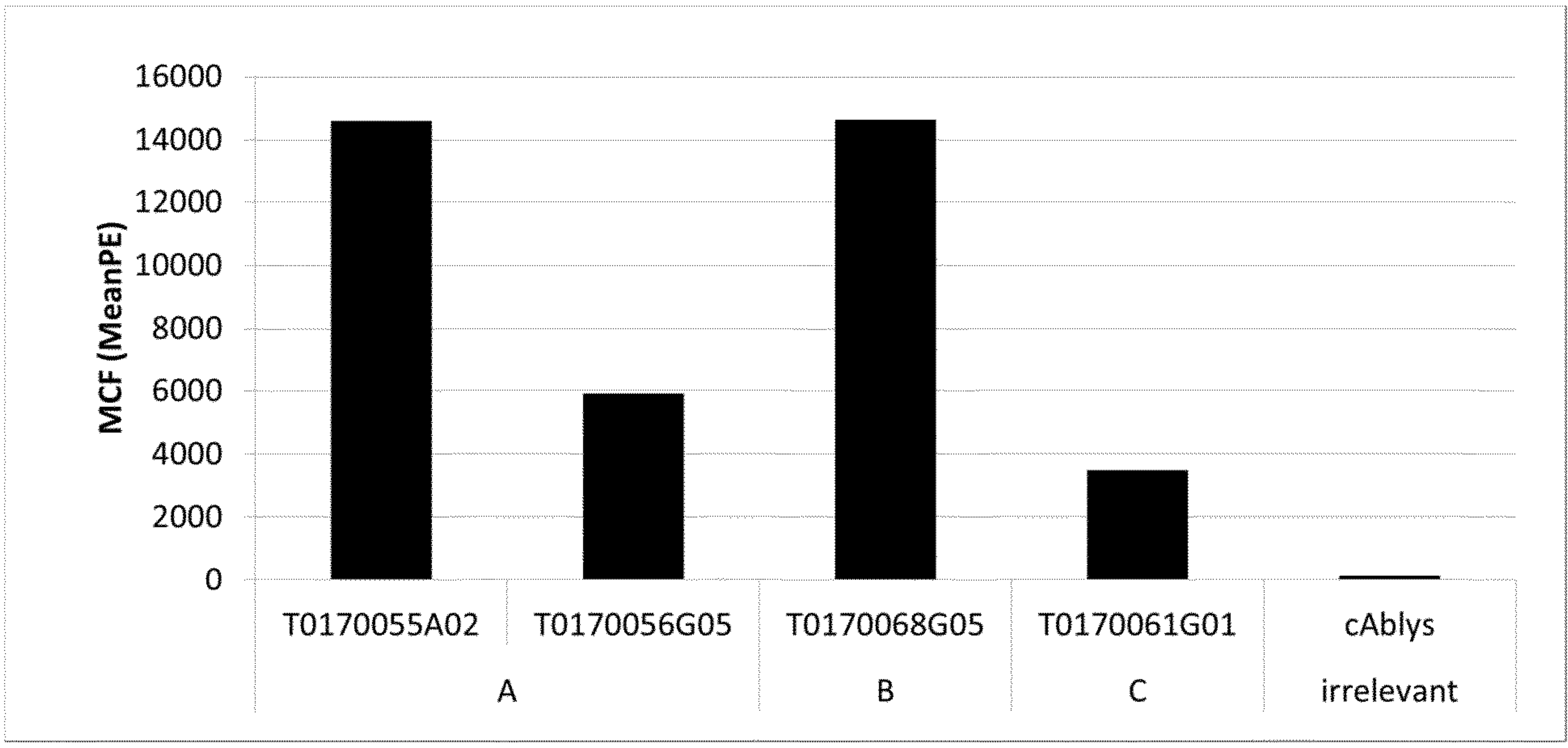

1000nM
333nM
111nM
37nM
12.3nM
1.4nM
0.16
0.08
0
nm
0
200
400
600
800
1000
Time (sec)
1000
333
111
37
12.3
1.4

Figure 10:

A.


16000
14000
12000
10000
8000
6000
4000
2000
0
MCF (MeanPE)
T0170055A02
T0170056G05
T0170068G05
T0170061G01
cAblys
A
B
C
irrelevant

B.

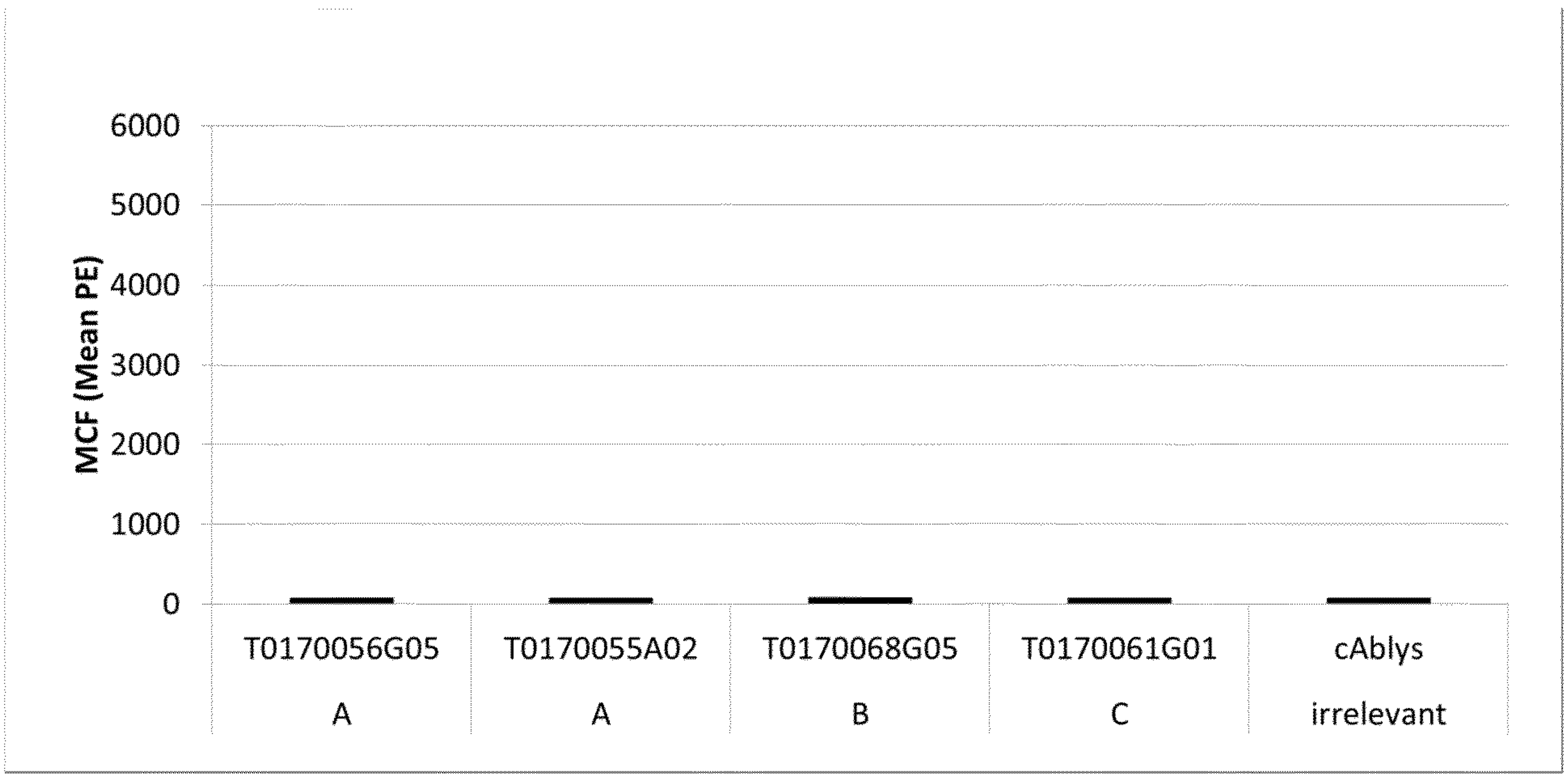

6000
5000
4000
3000
2000
1000
0
MCF (Mean PE)
T0170056G05
T0170055A02
T0170068G05
T0170061G01
cAblys
A
A
B
C
irrelevant

Figure 11:

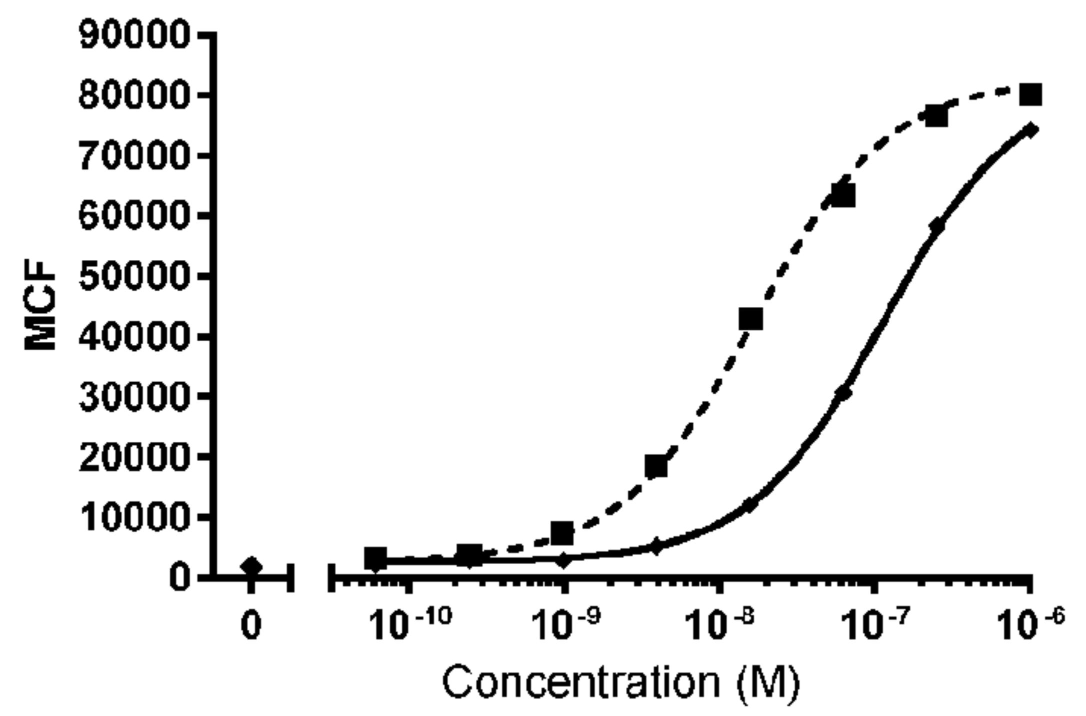
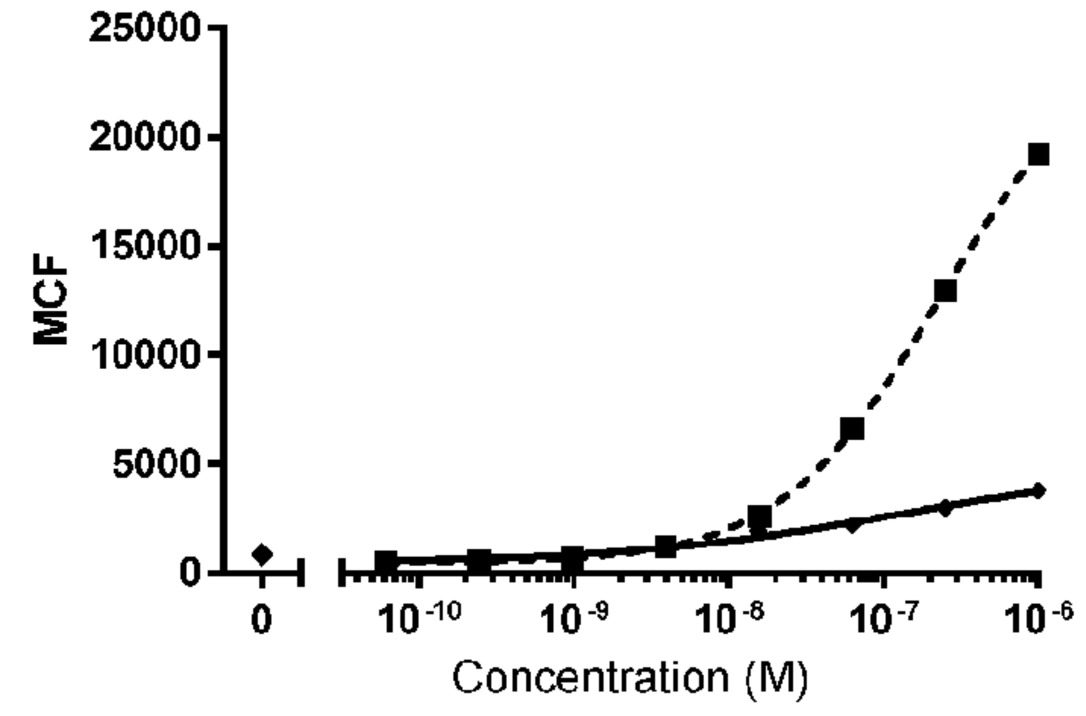
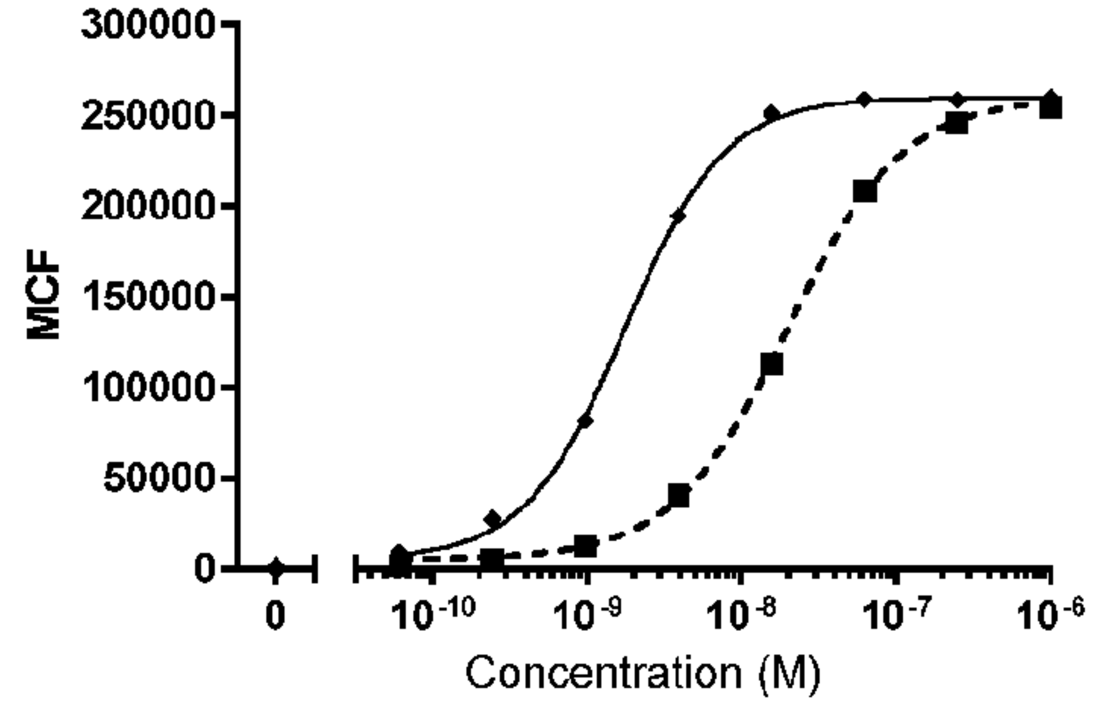
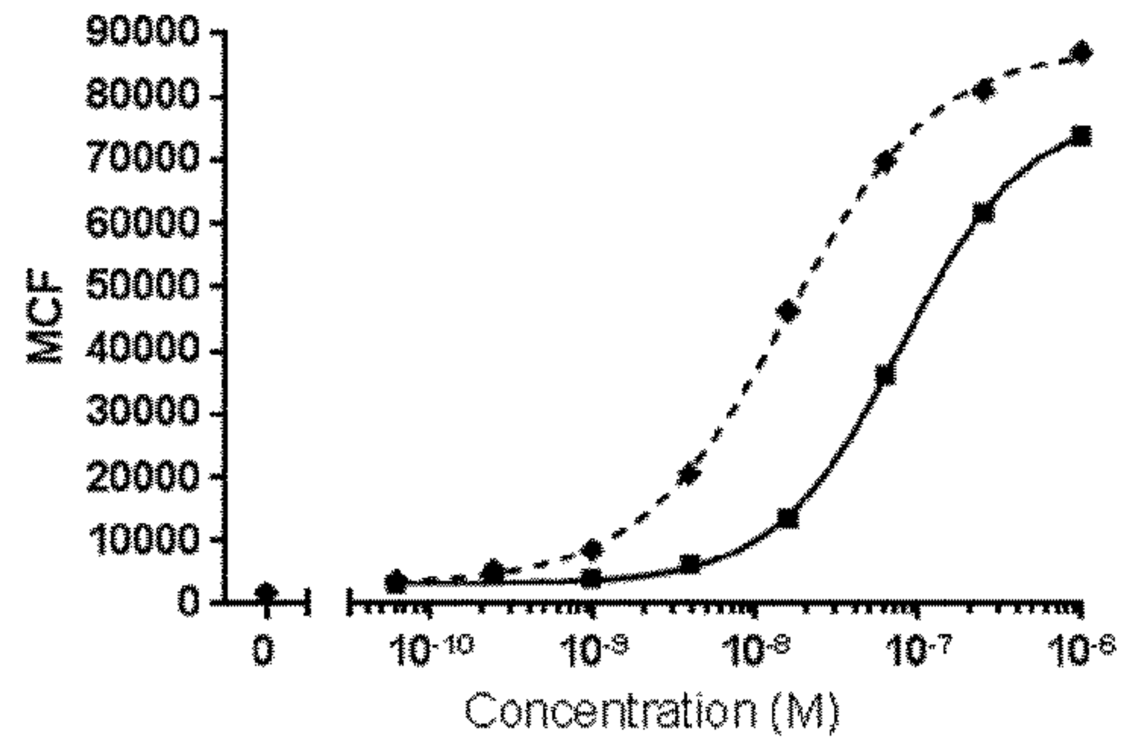
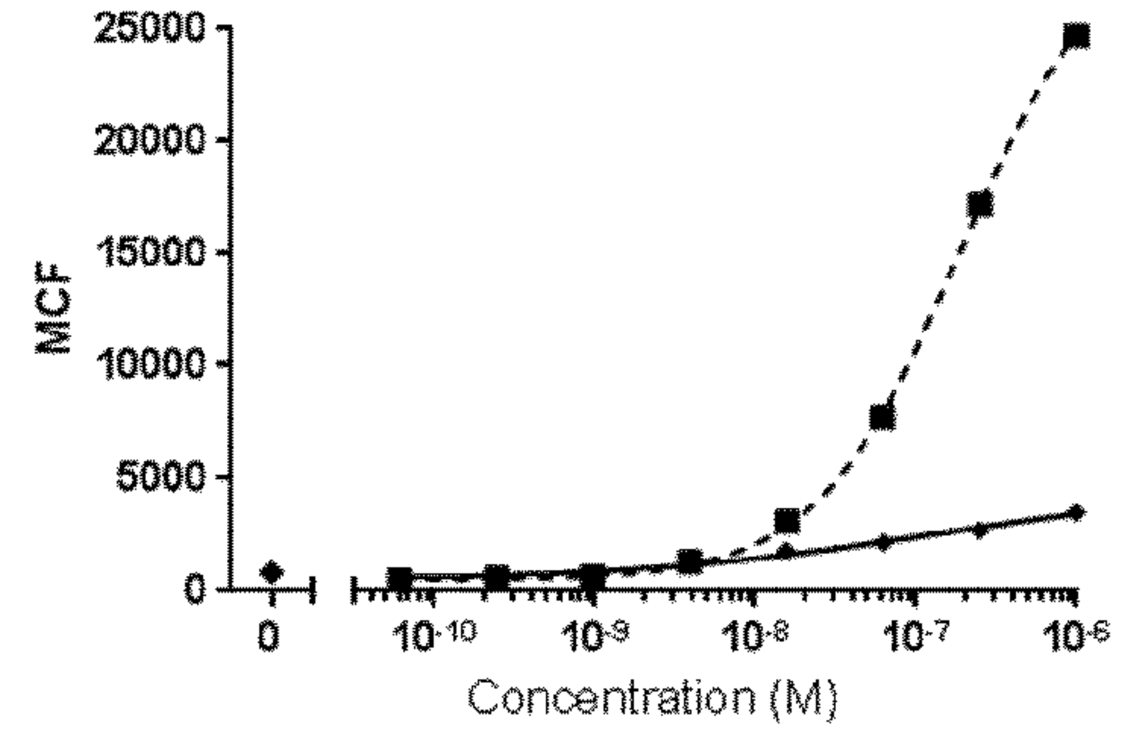
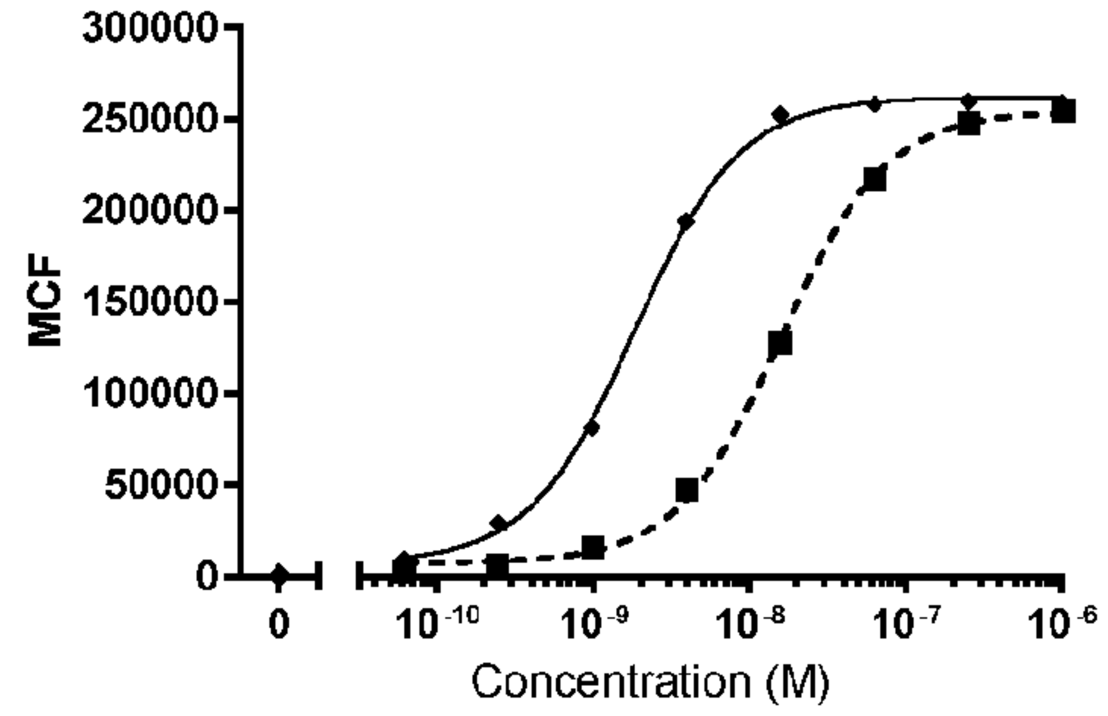
Sample ID	A. CHO-K1 huTCR (2XN9)/CD3 cells	B. Human T cells	C. Ramos cells
T017000055 (solid line) T017000042 (dotted line)			
T017000076 (solid line) T017000074 (dotted line)			

Figure 12 cont':

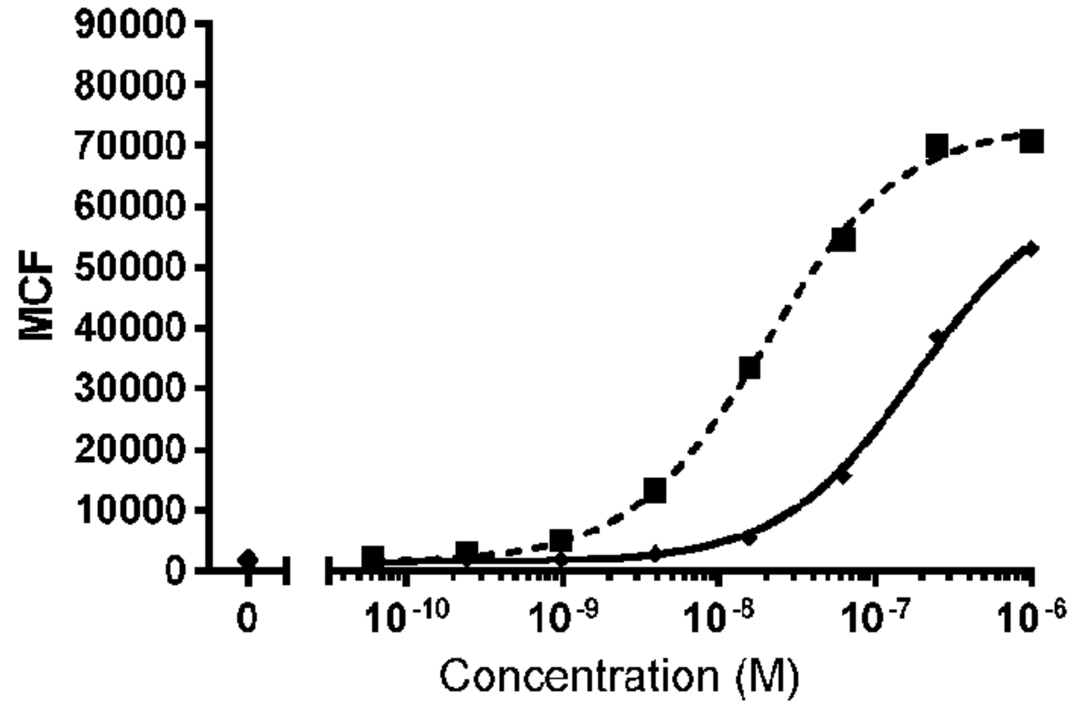
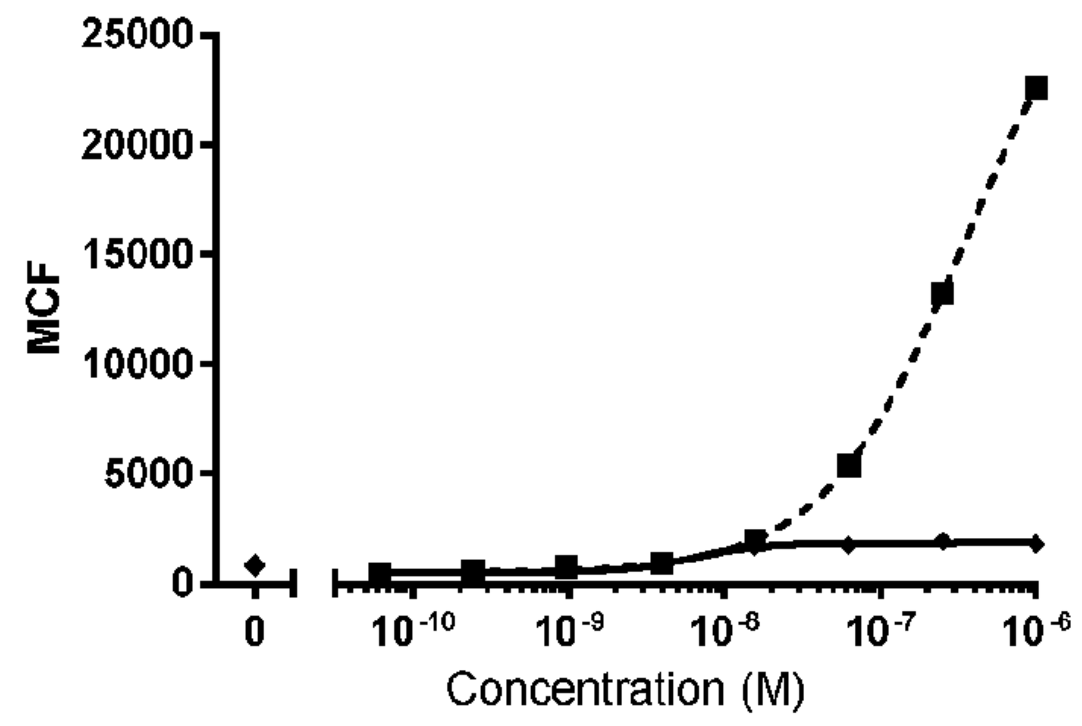
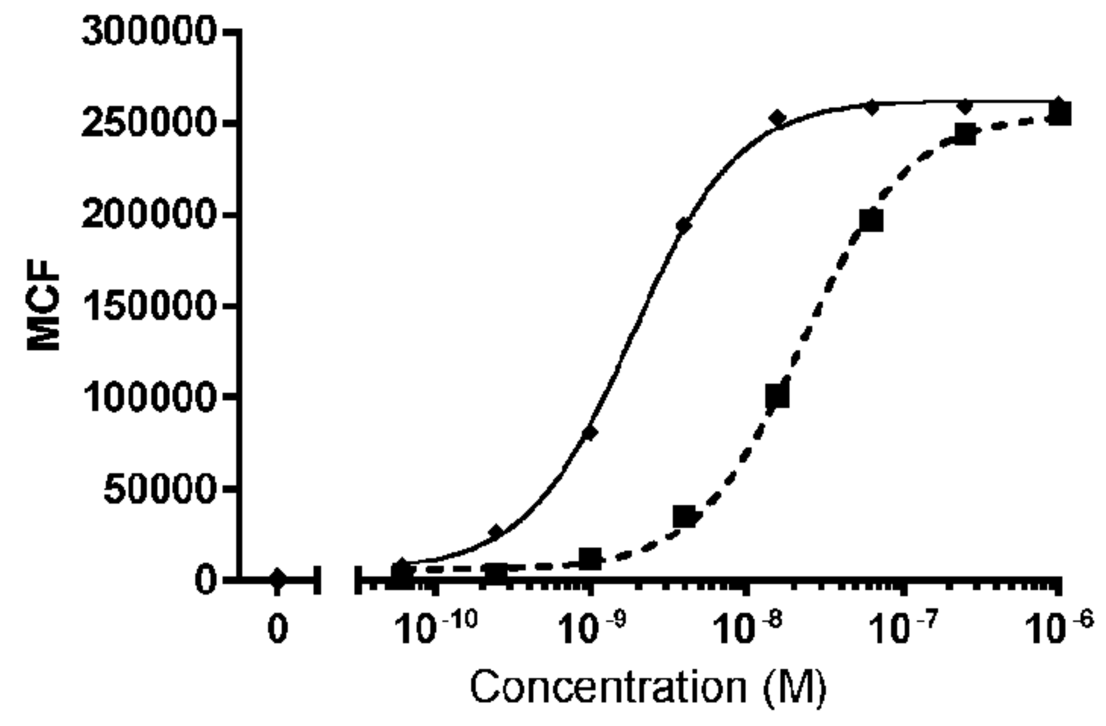
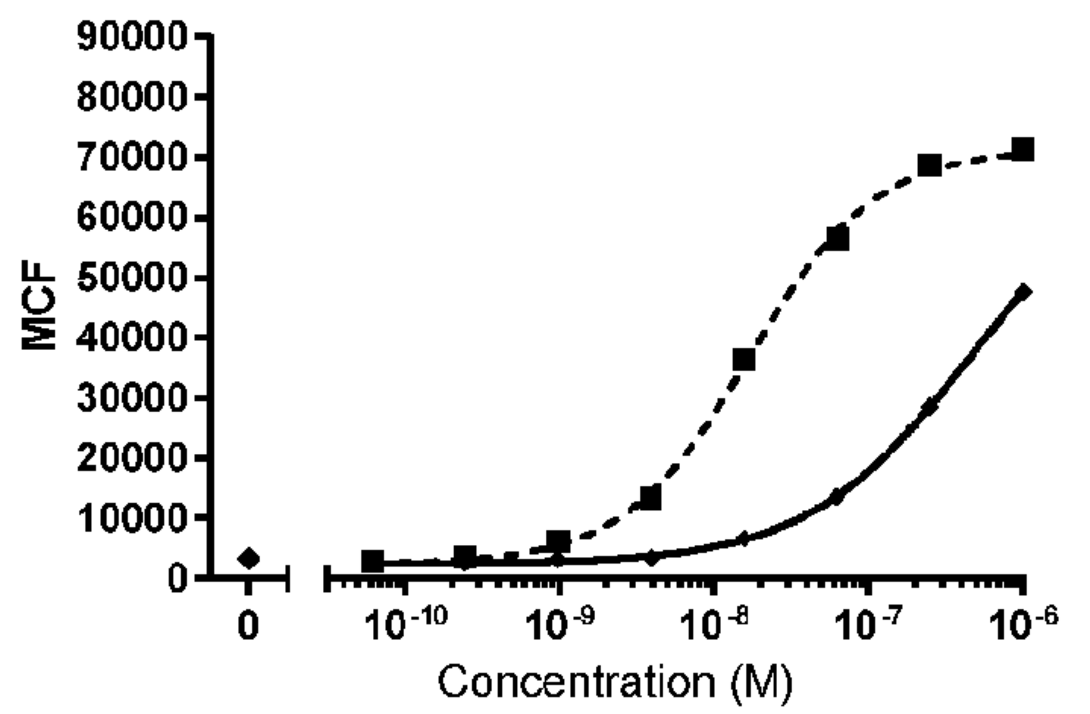
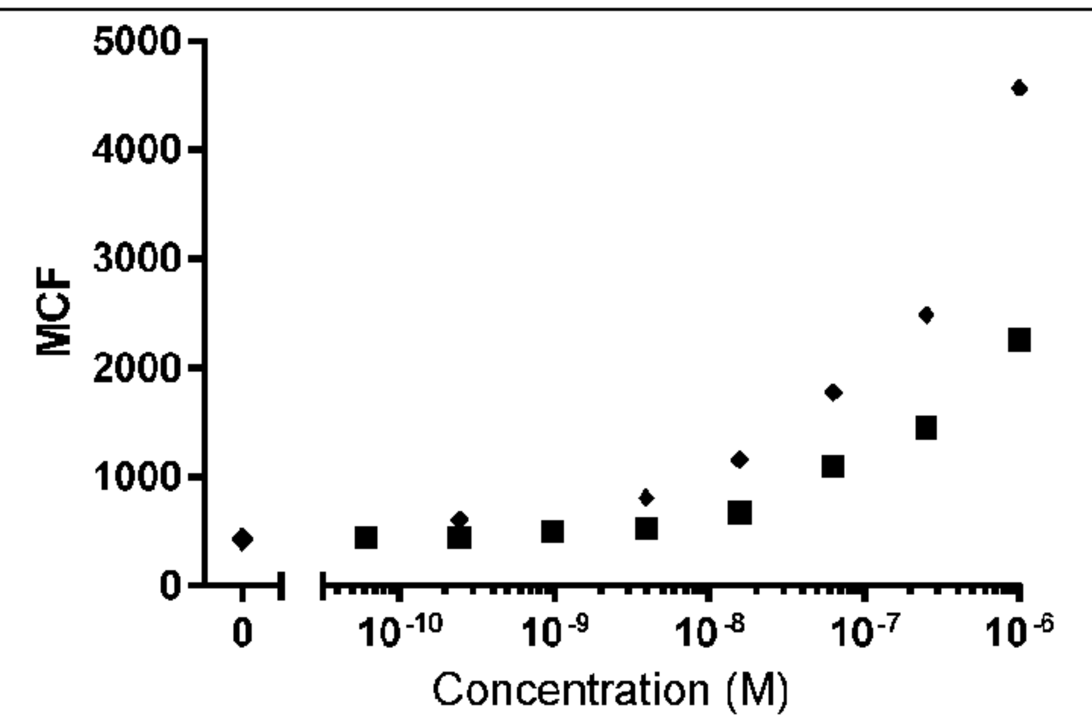
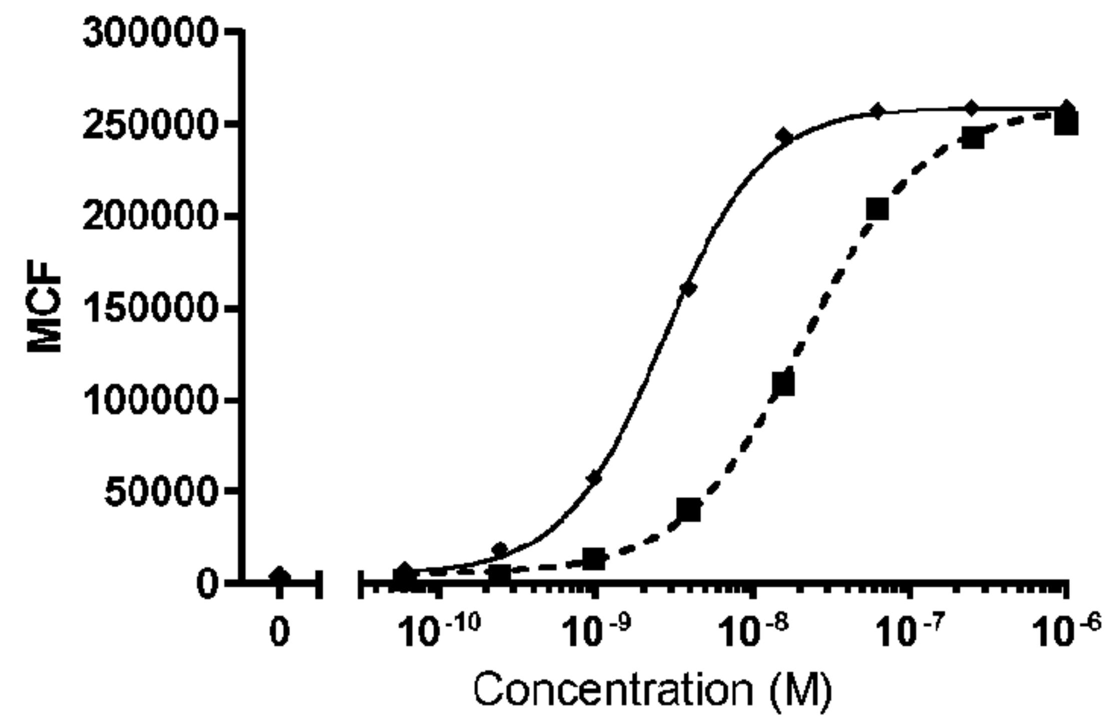
Sample ID	A. CHO-K1 huTCR (2XN9)/CD3 cells	B. Human T cells	C. Ramos cells
T017000068 (solid line) T017000031 (dotted line)			
T017000051 (solid line) T017000038 (dotted line)			

Figure 13:

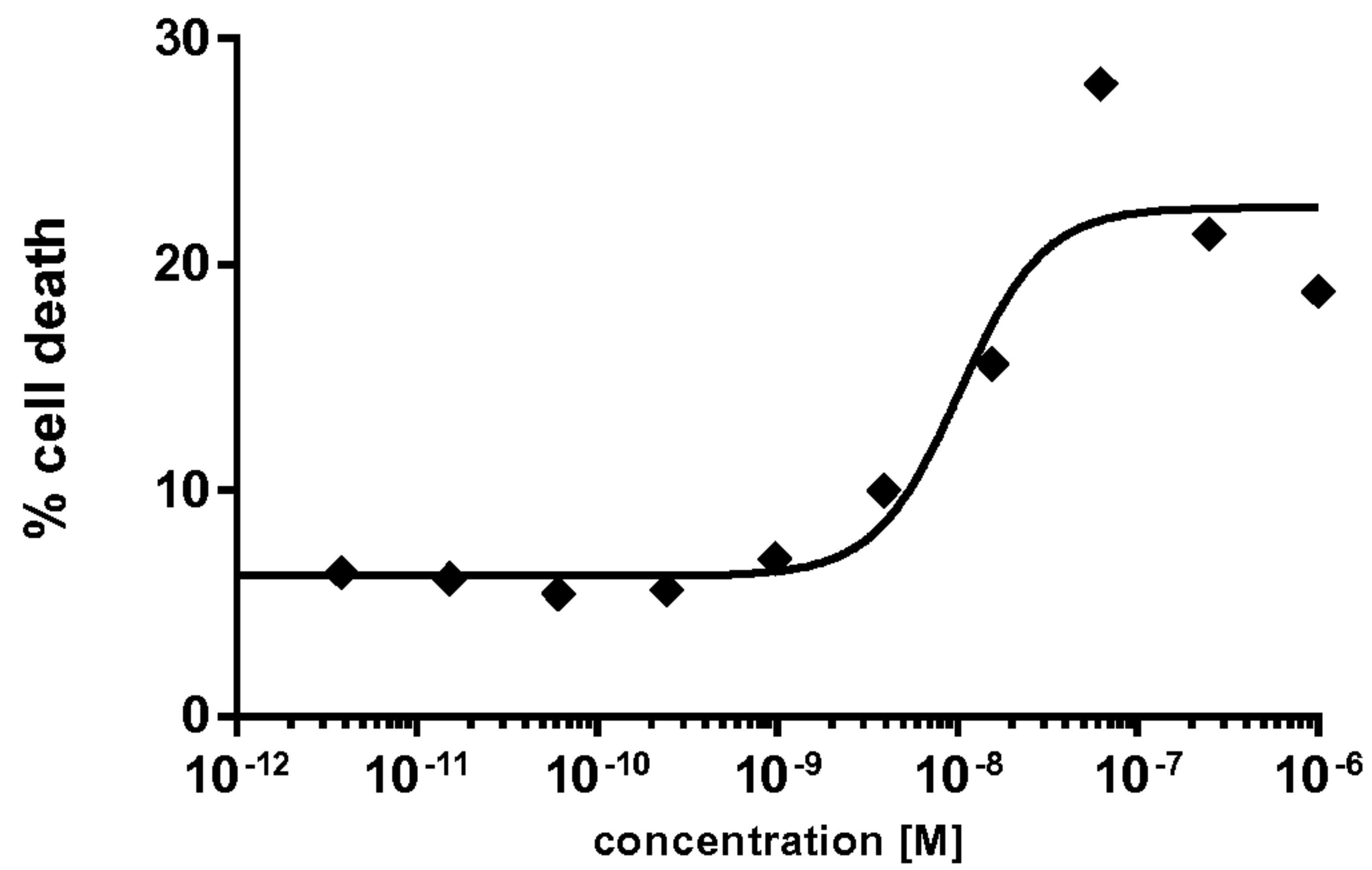
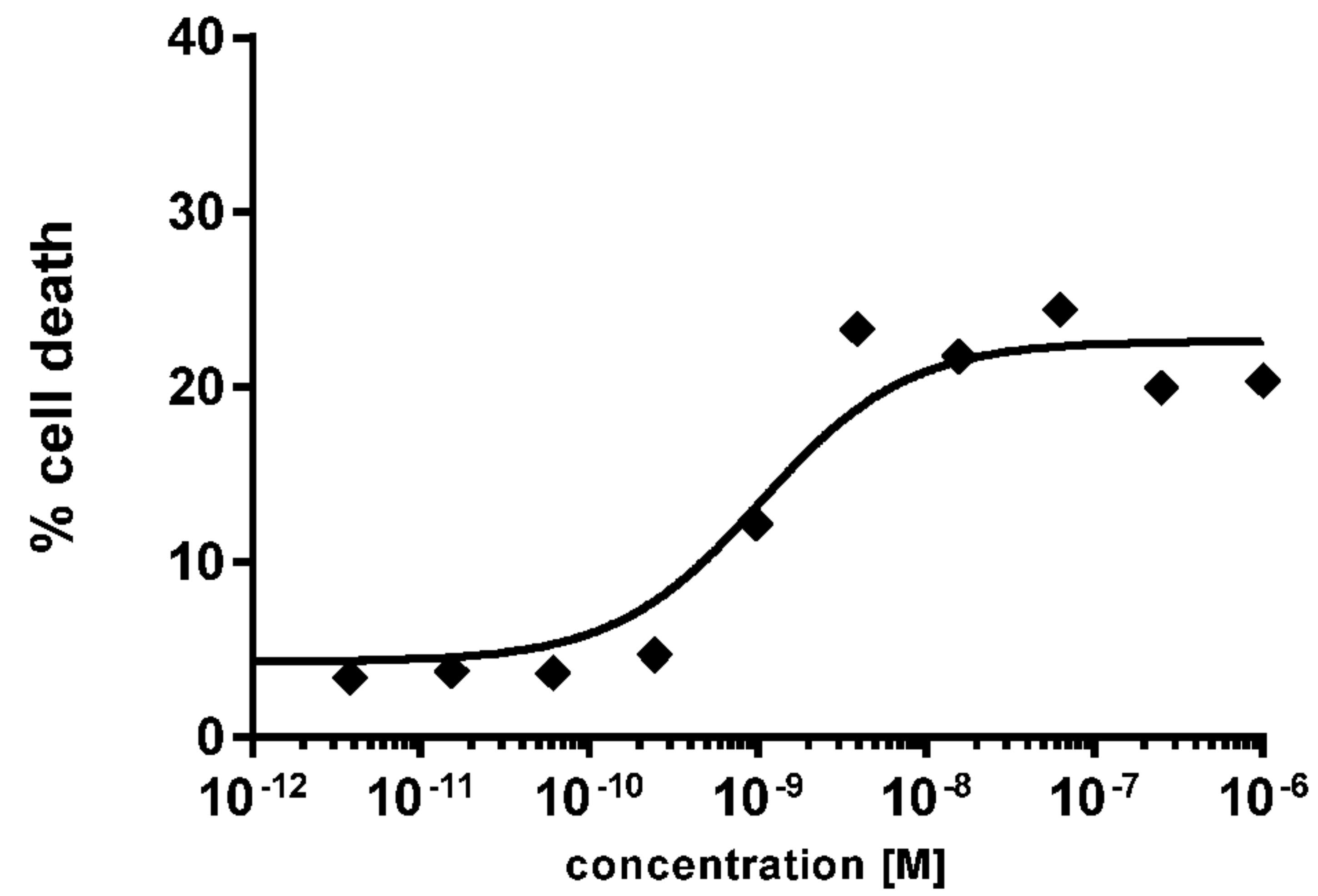
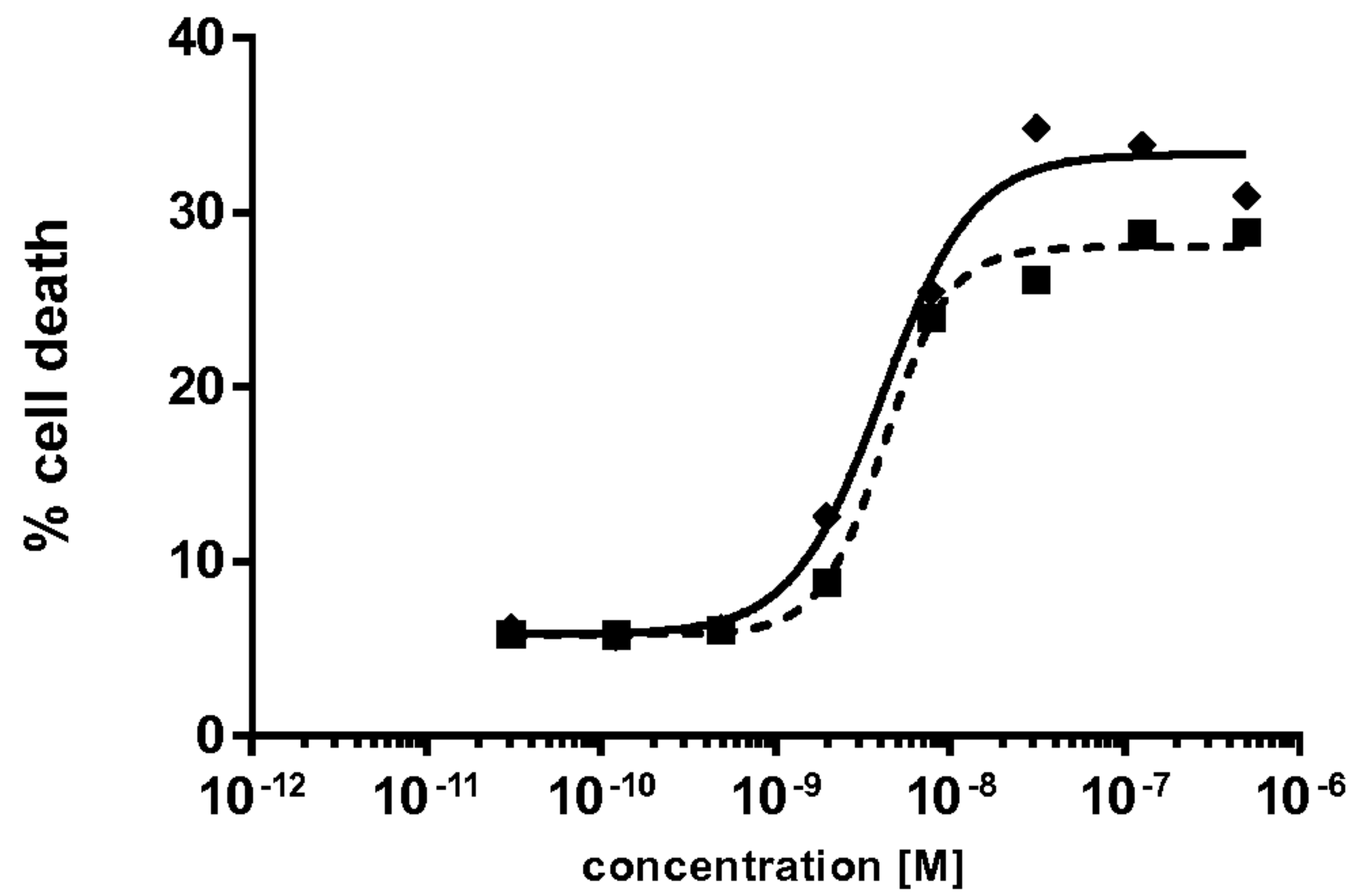
Sample ID	A. Ramos cells	B. Raji cells
T017000014 (solid line)		
T017000055 (solid line) T017000042 (dotted line)		

__Figure 14 cont':__

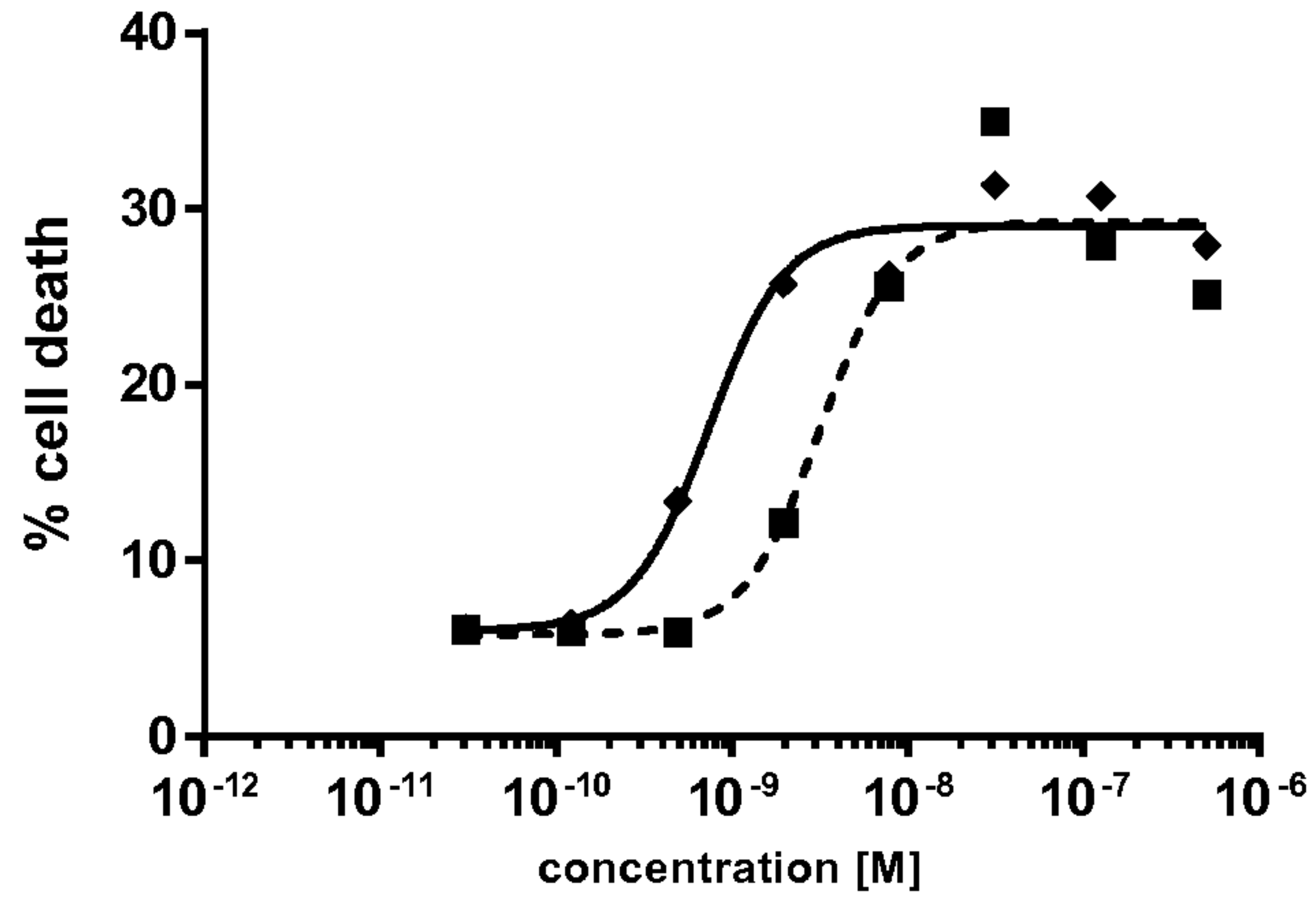
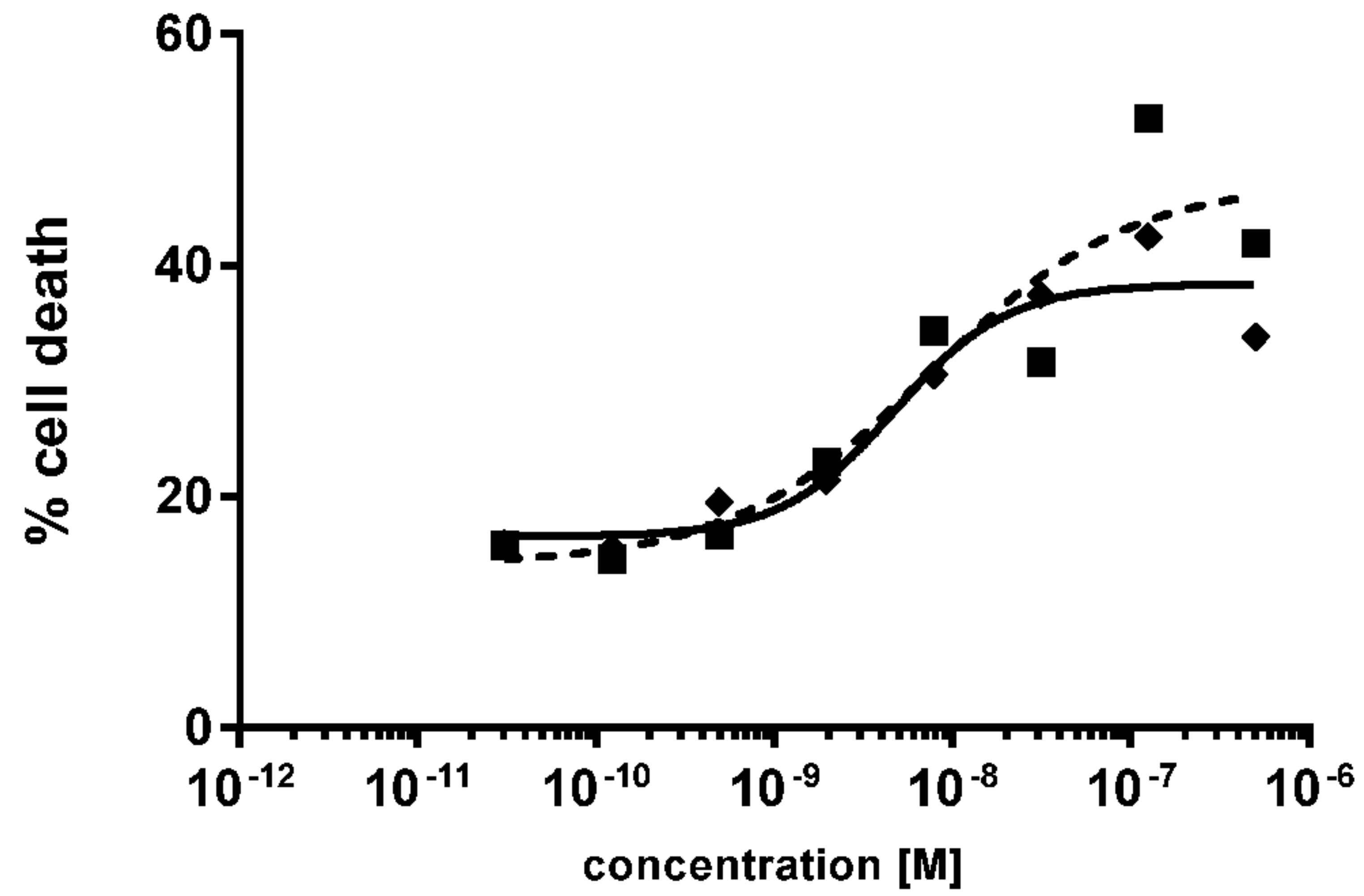
Sample ID	A. Ramos cells	B. Raji cells
T017000076 (solid line) **T017000074 (dotted line)**		
T017000068 (solid line) **T017000031 (dotted line)**		

__Figure 15 cont':__

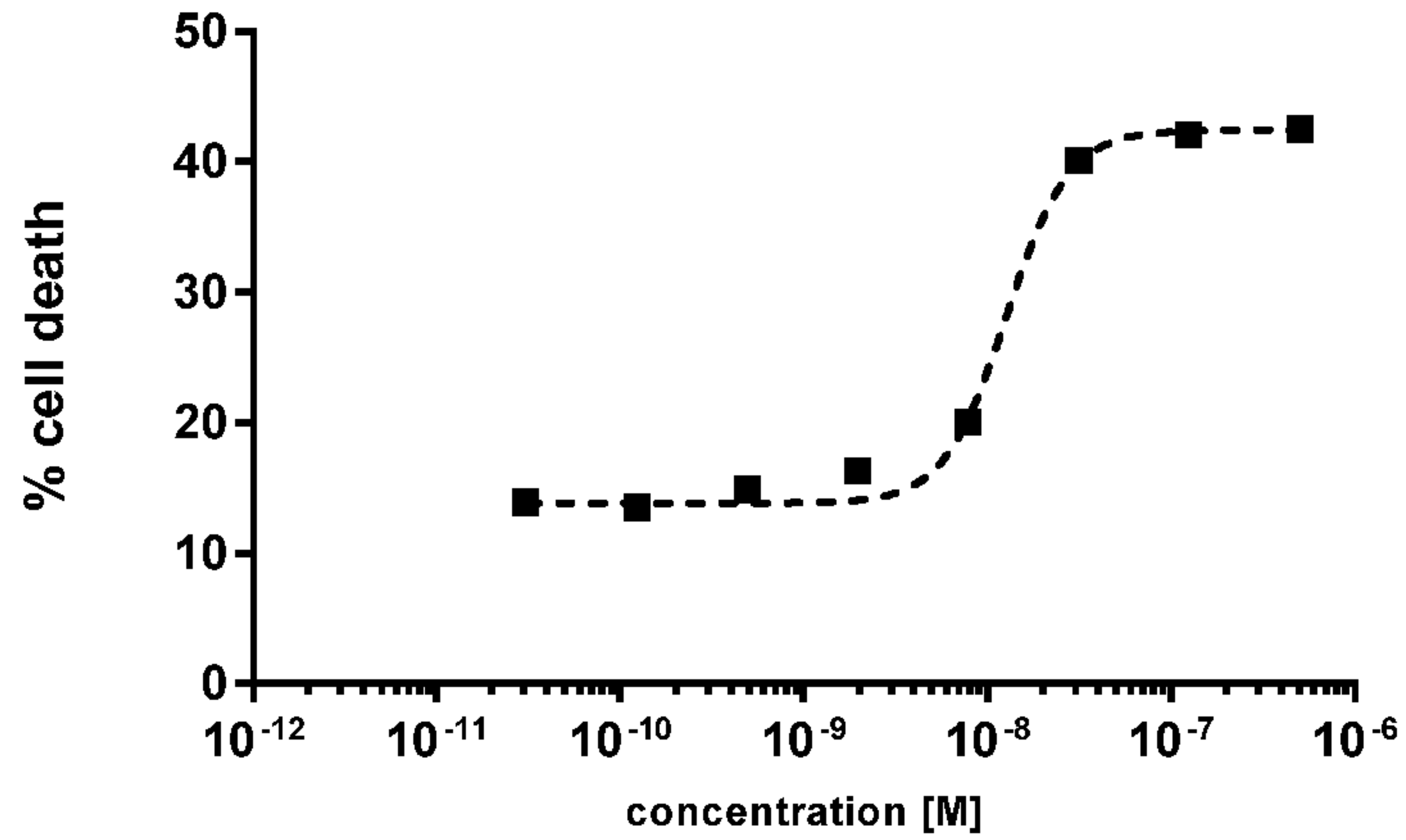
Sample ID	A. Ramos cells	B. Raji cells
T017000038 (dotted line)		

Figure 16:

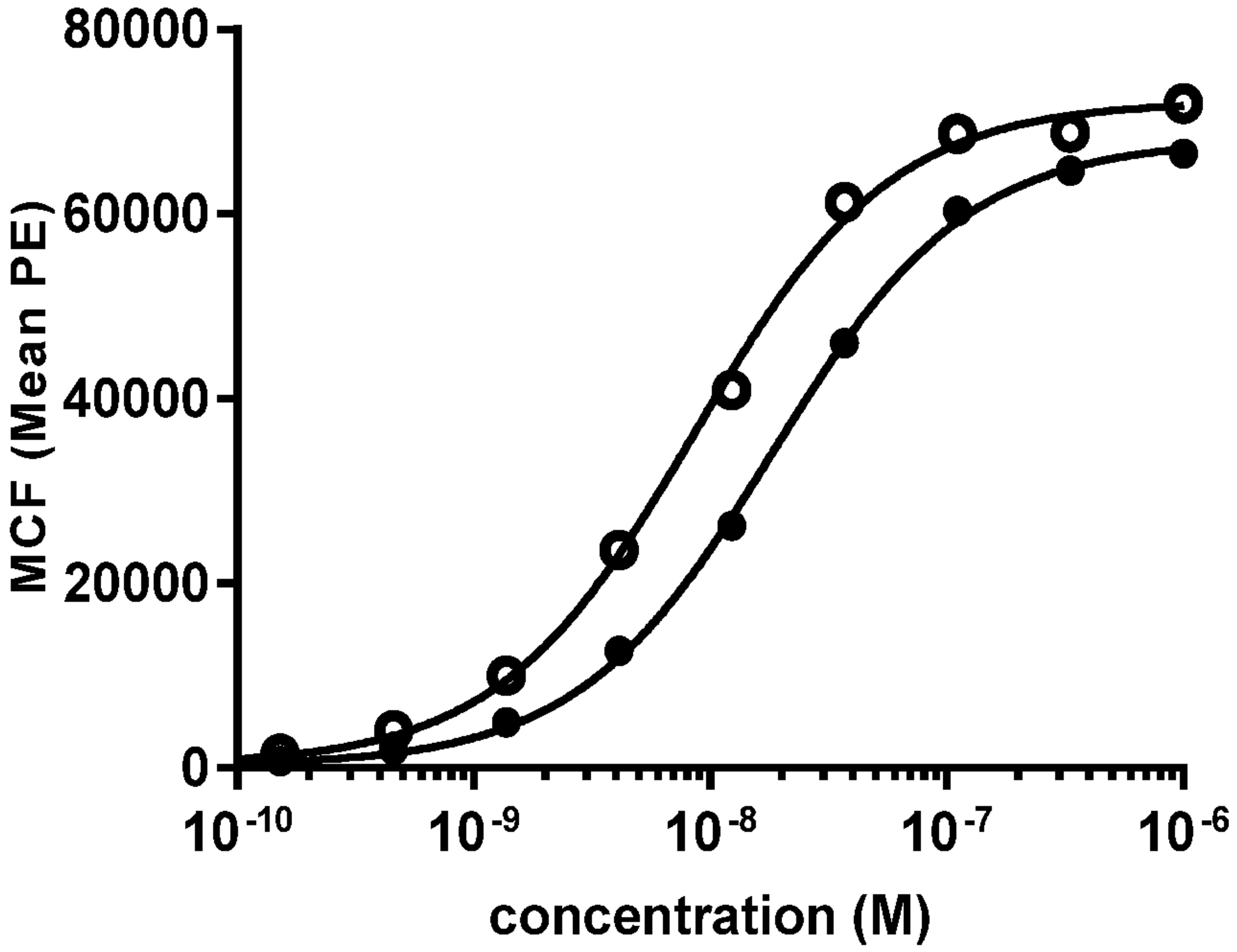

80000
60000
40000
20000
0
MCF (Mean PE)
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
concentration (M)

Figure 17:

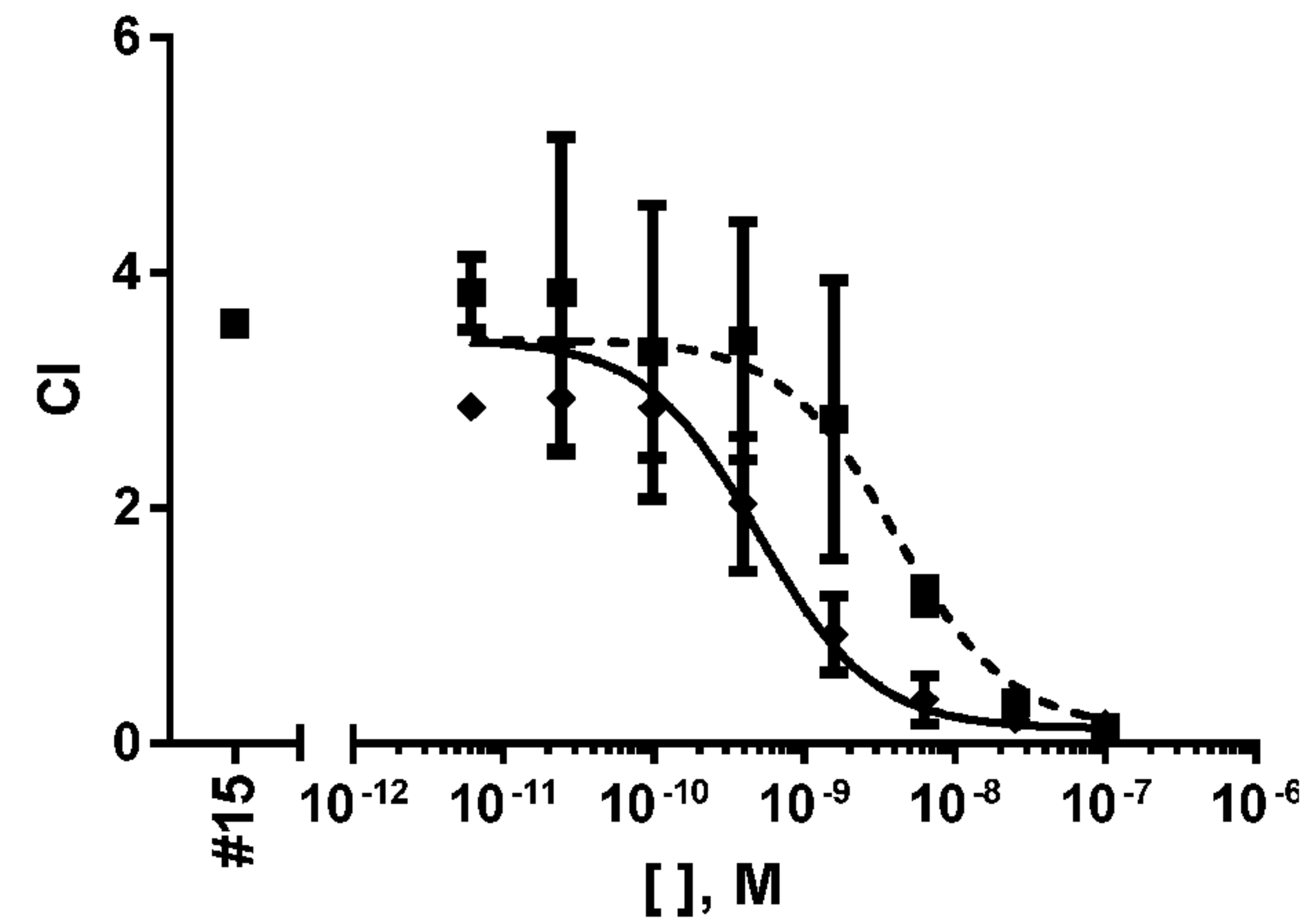
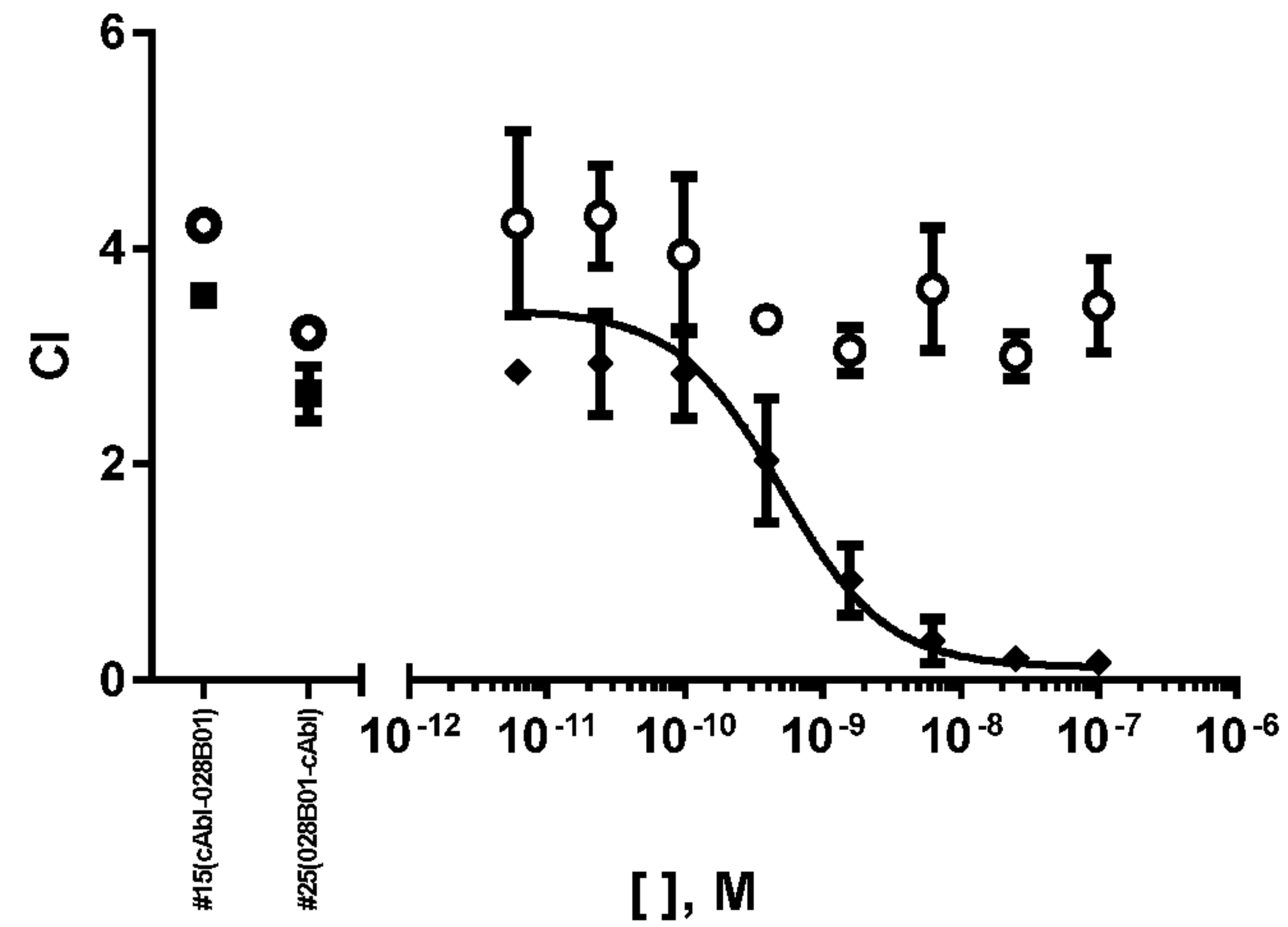
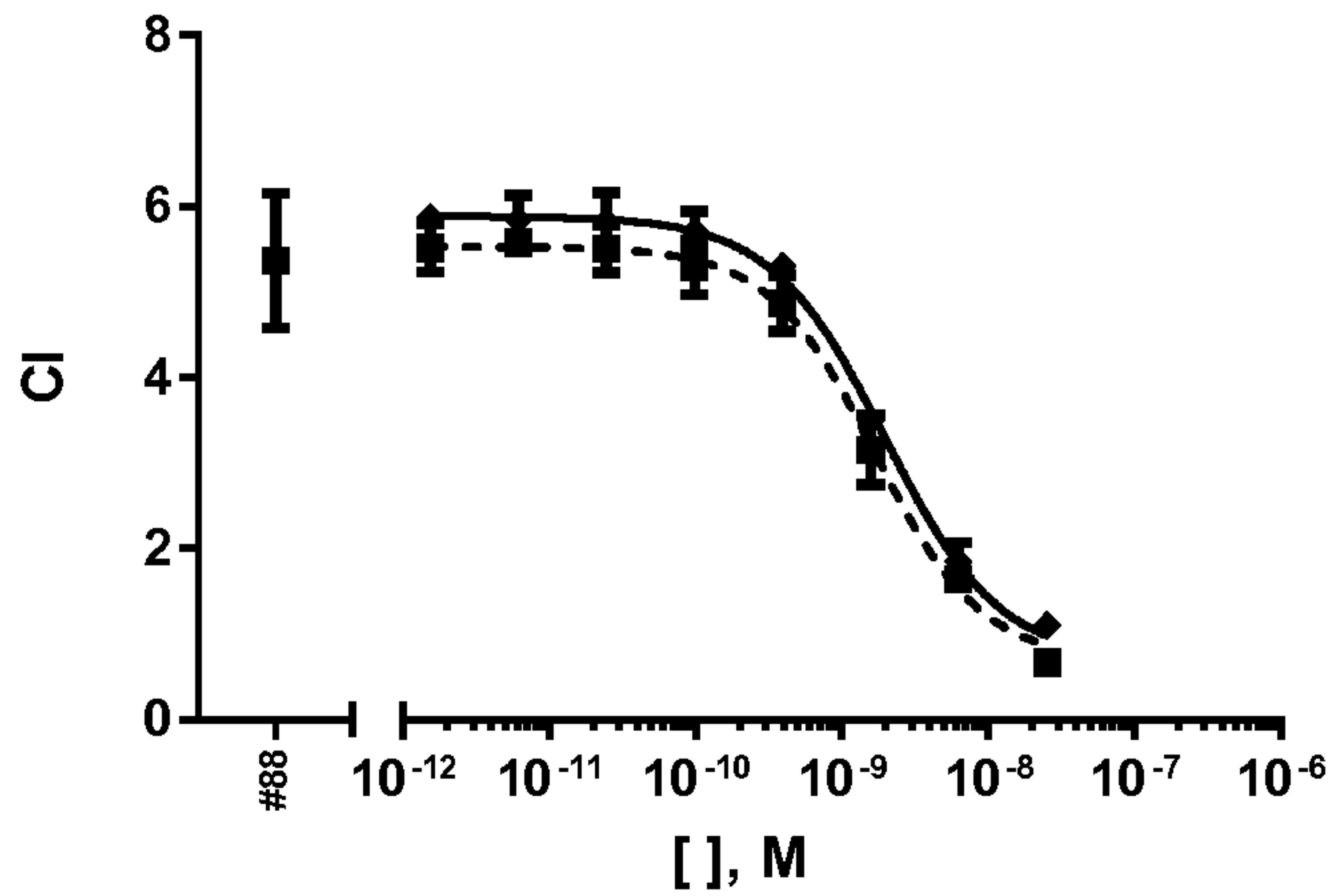
Sample ID	A. CHO-K1 hu CD20 cells	B. CHO-K1 hu CD20 cells and CHO-K1 cells
T017000055 (solid line) T017000042 (dotted line)		
T017000076 (solid line) T017000074 (dotted line)		

__Figure 18 cont':__

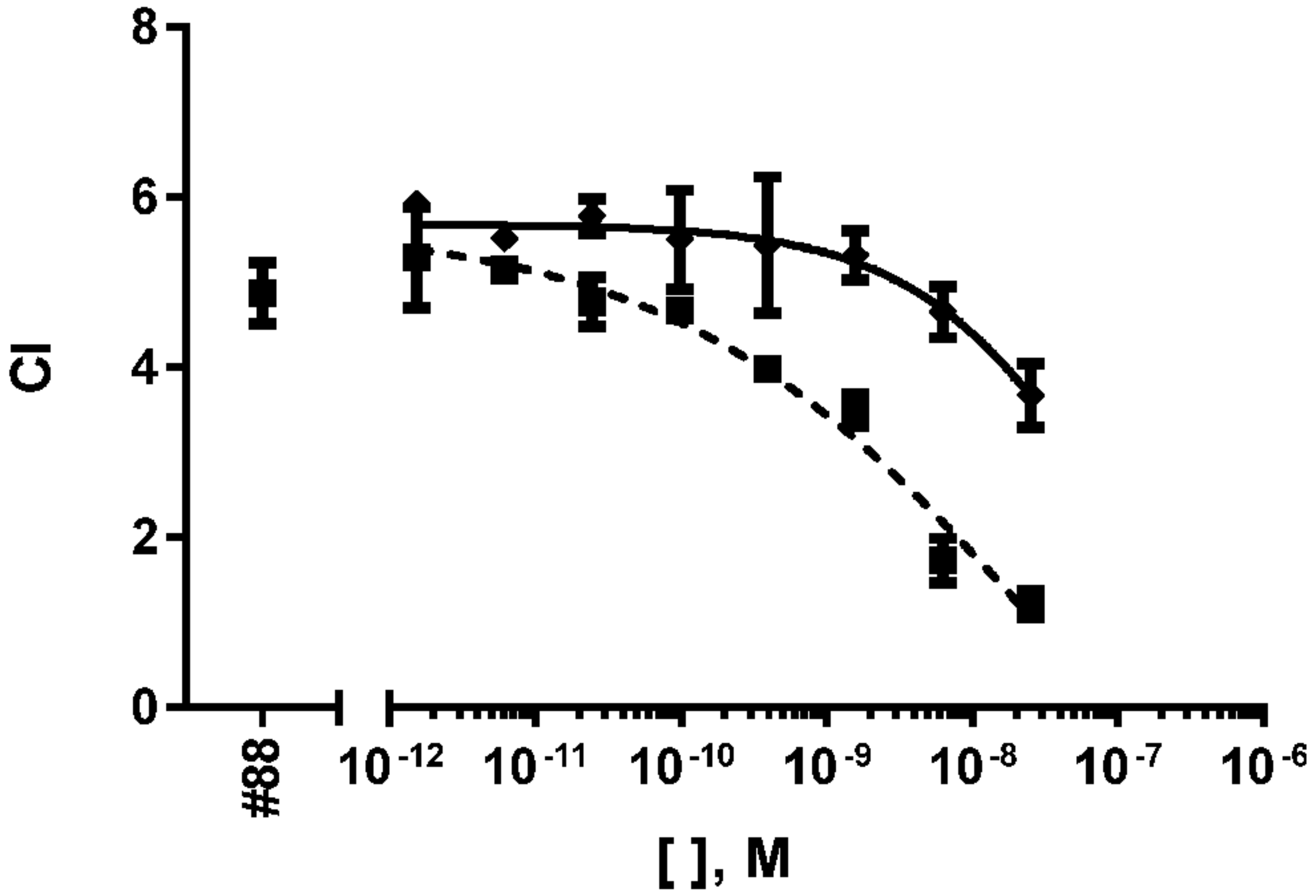
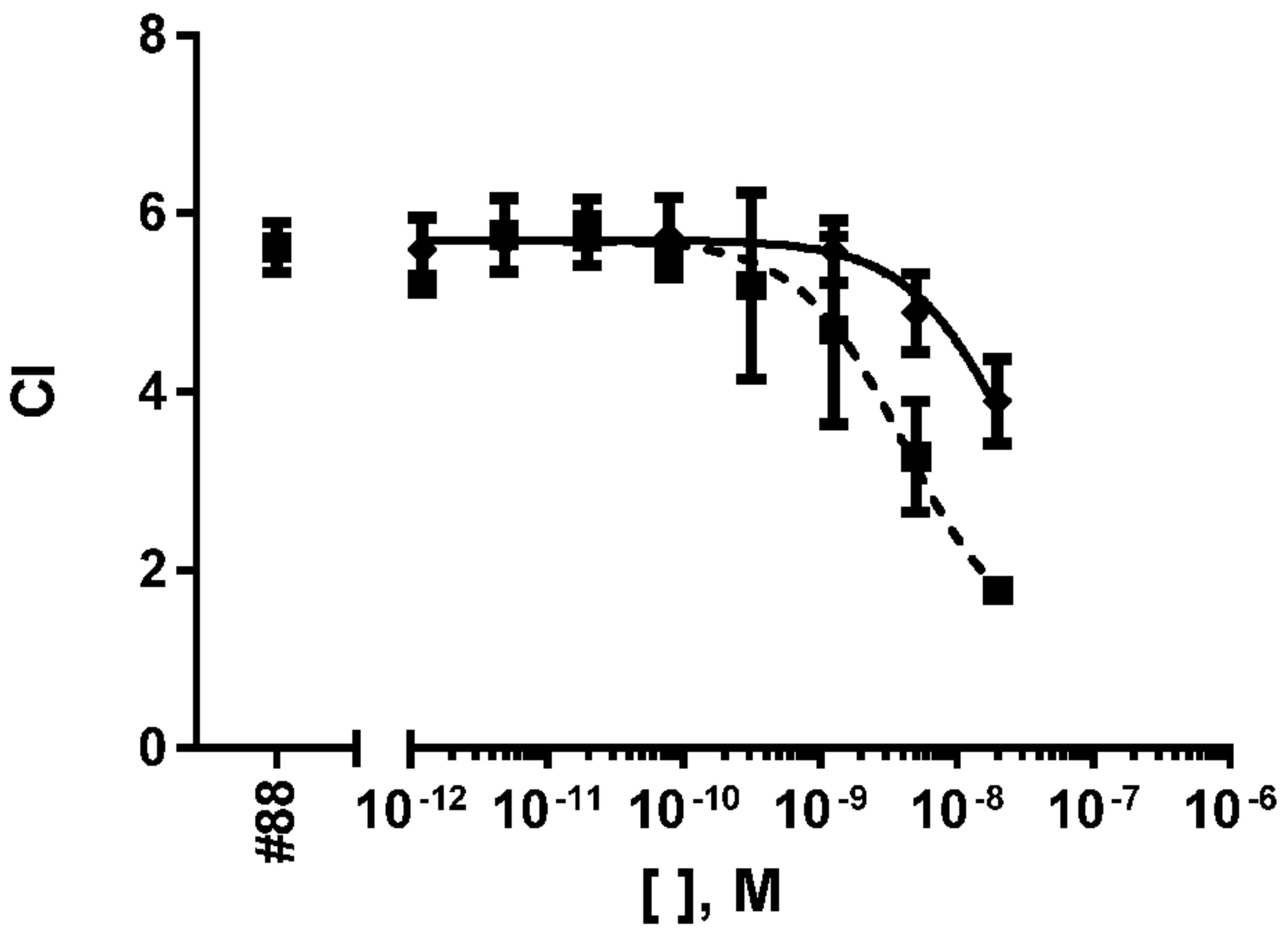
Sample ID	A. CHO-K1 hu CD20 cells	B. CHO-K1 hu CD20 cells and CHO-K1 cells
T017000068 (solid line) **T017000031 (dotted line)**		
T017000051 (solid line) **T017000038 (dotted line)**		

Figure 19:

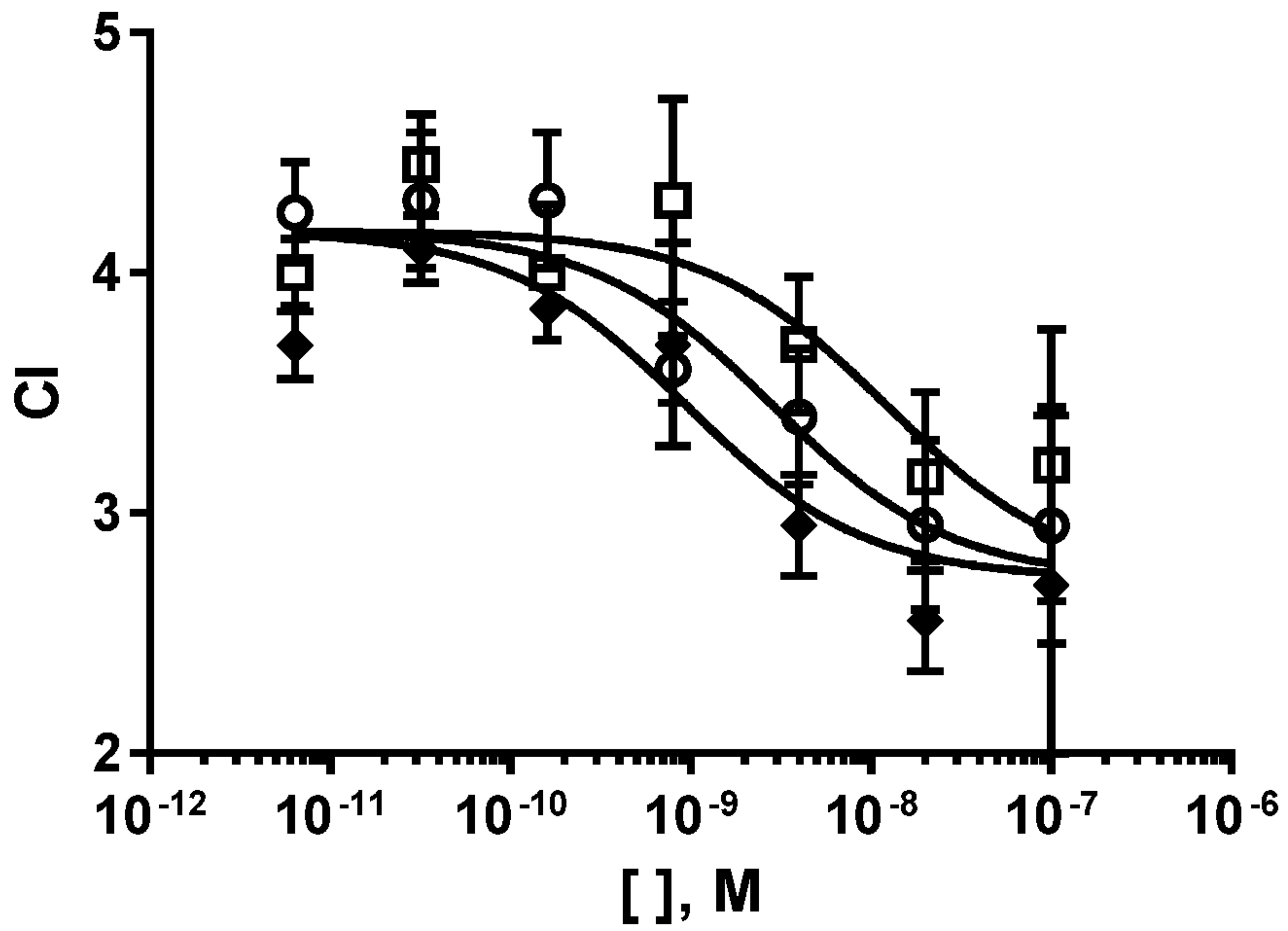


Figure 20:

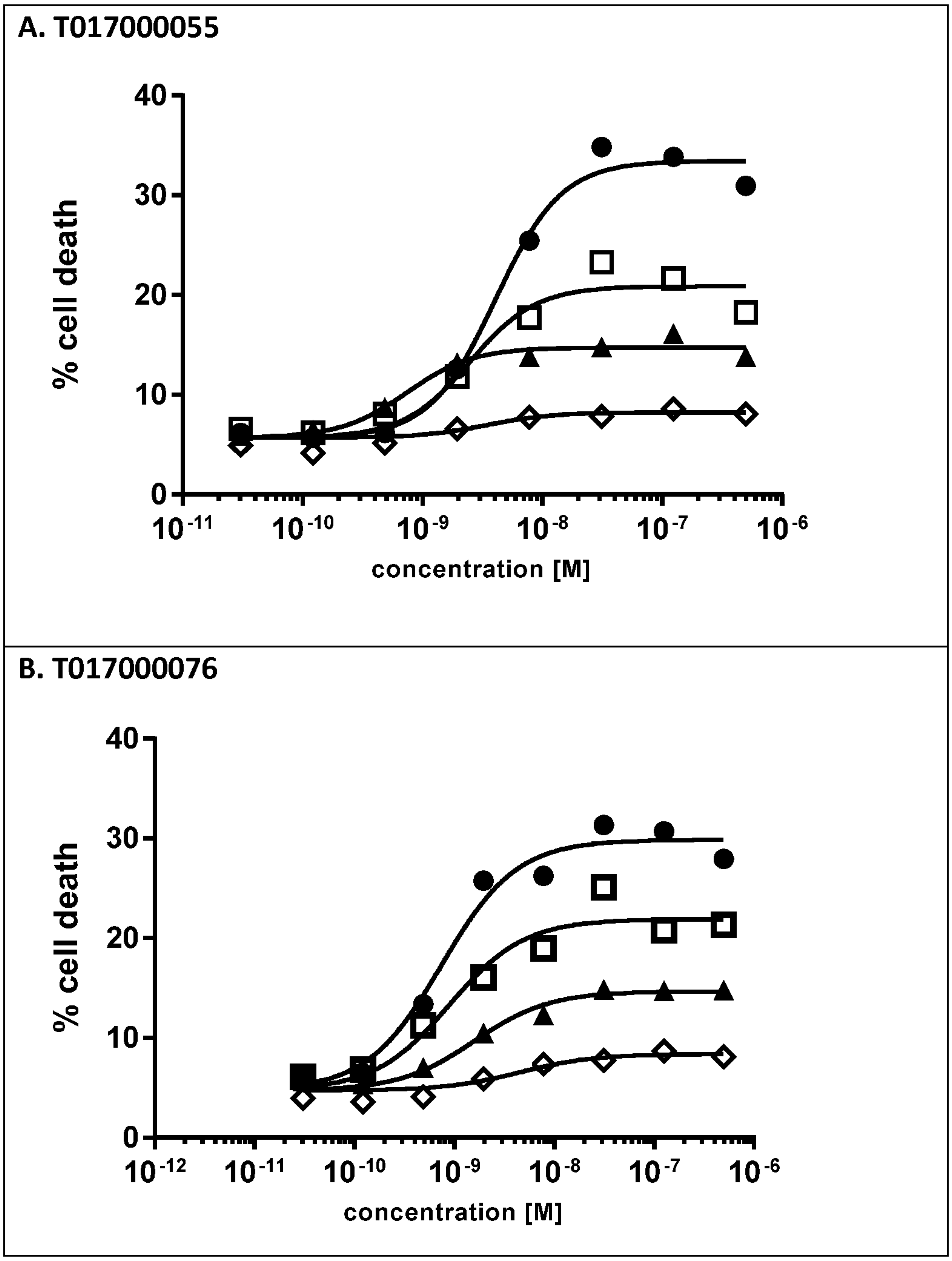

A. T017000055
% cell death
0
10
20
30
40
10-11
10-10
10-9
10-8
10-7
10-6
concentration [M]
B. T017000076
% cell death
0
10
20
30
40
10-12
10-11
10-10
10-9
10-8
10-7
10-6
concentration [M]

Figure 21:

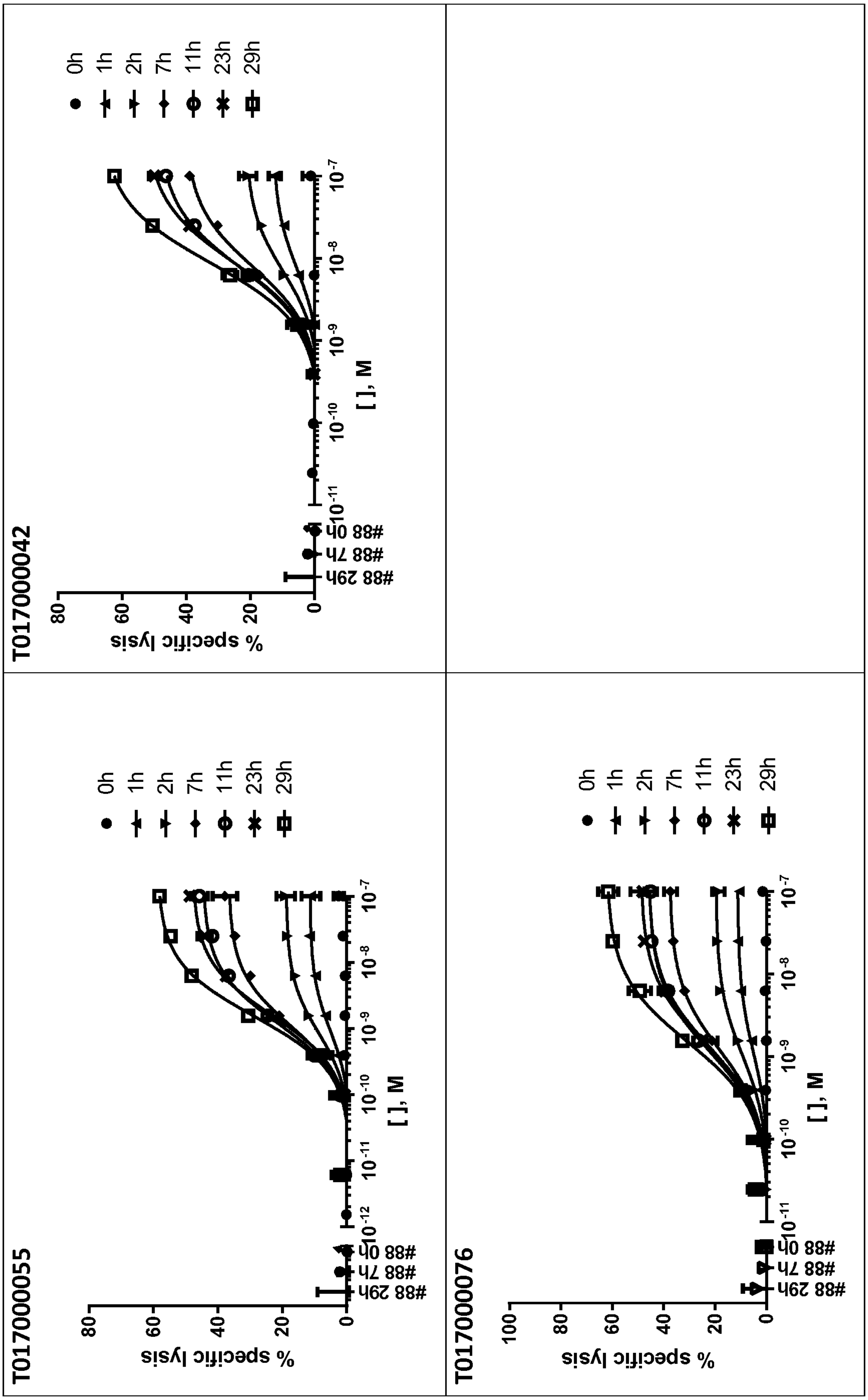

T017000042
T017000055
T017000076
% specific lysis
[ ], M
#88 0h
#88 7h
#88 29h
0h
1h
2h
7h
11h
23h
29h

Figure 22:

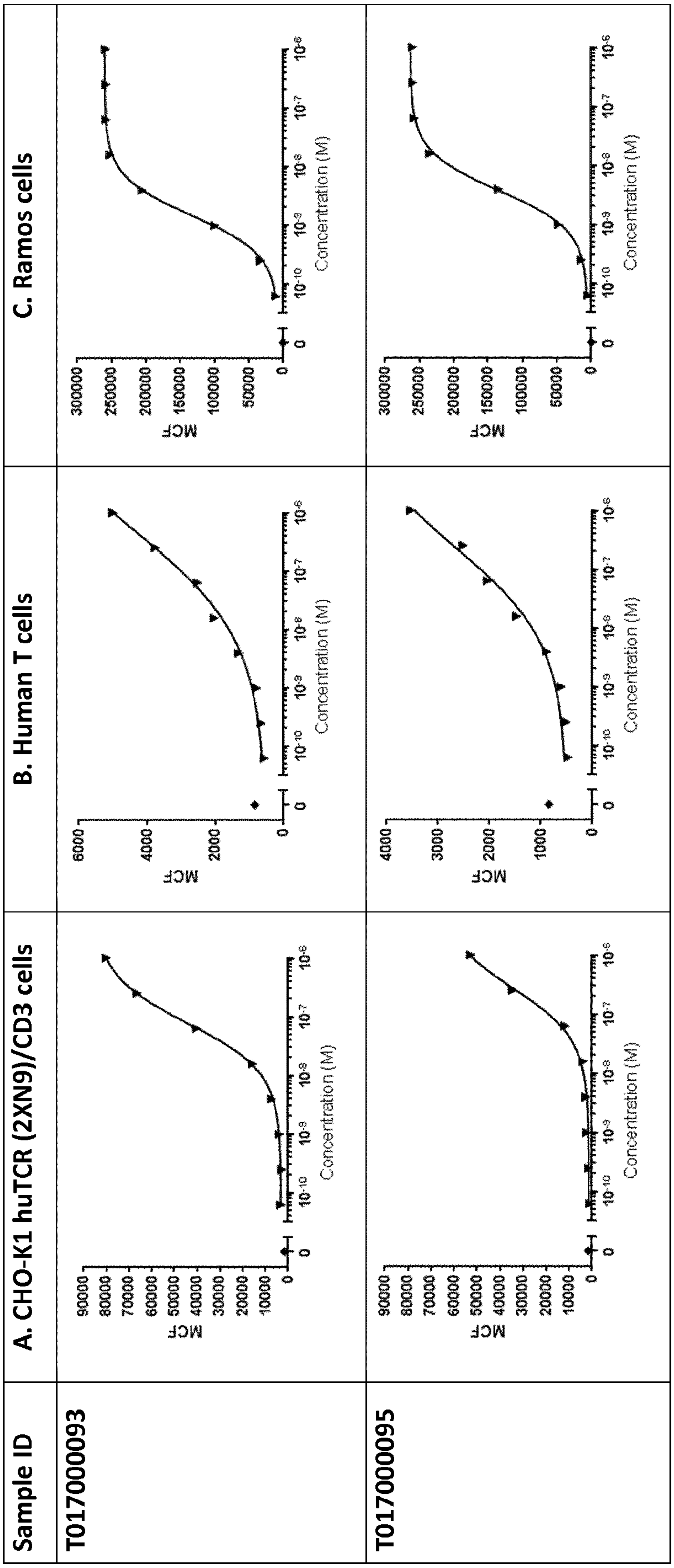
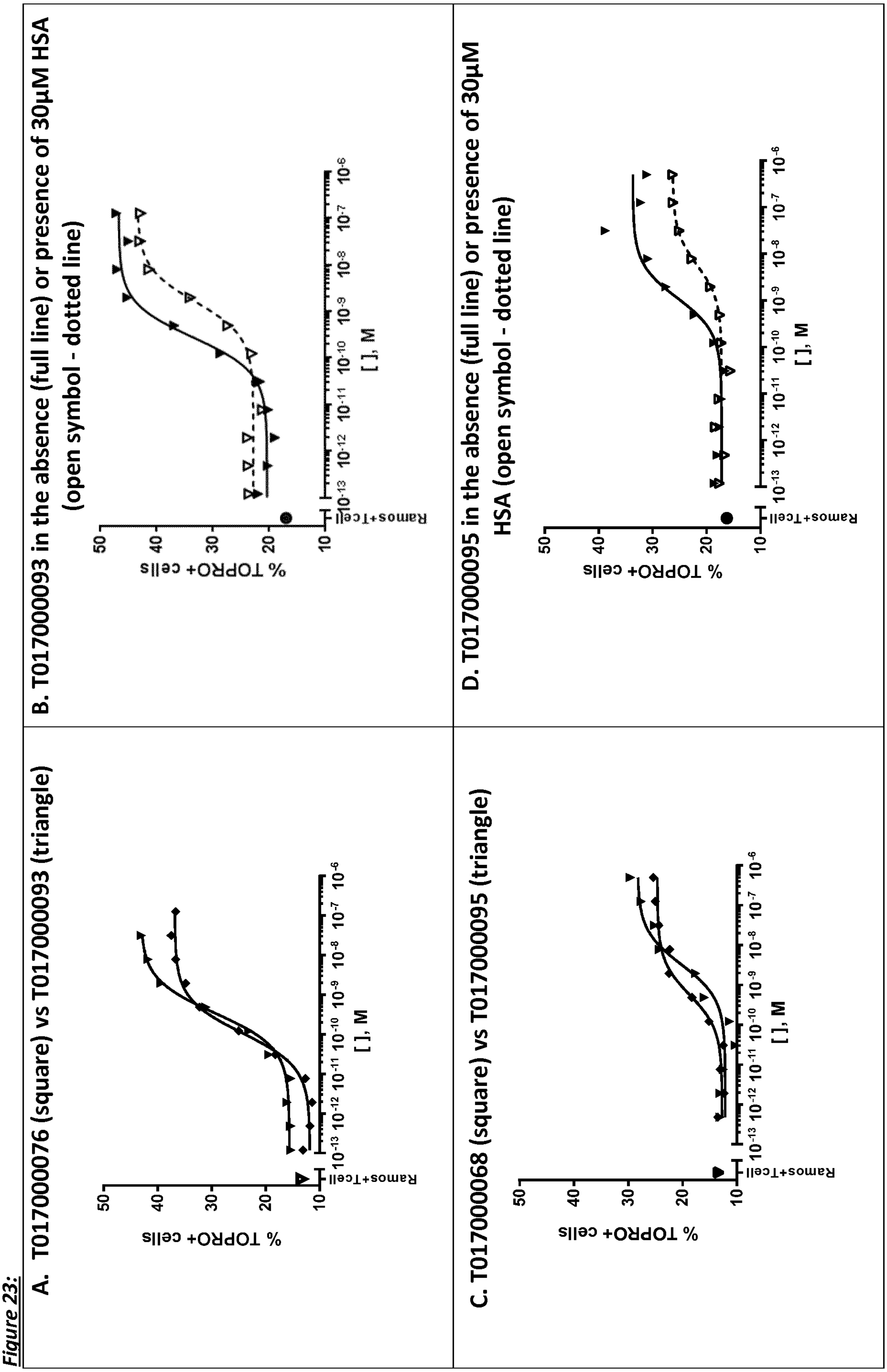

Sample ID
A. CHO-K1 huTCR (2XN9)/CD3 cells
B. Human T cells
C. Ramos cells
T017000093
T017000095
MCF
Concentration (M)

Figure 23:



A. T017000076 (square) vs T017000093 (triangle)

B. T017000093 in the absence (full line) or presence of 30µM HSA (open symbol - dotted line)

C. T017000068 (square) vs T017000095 (triangle)

D. T017000095 in the absence (full line) or presence of 30µM HSA (open symbol - dotted line)

Figure 24:

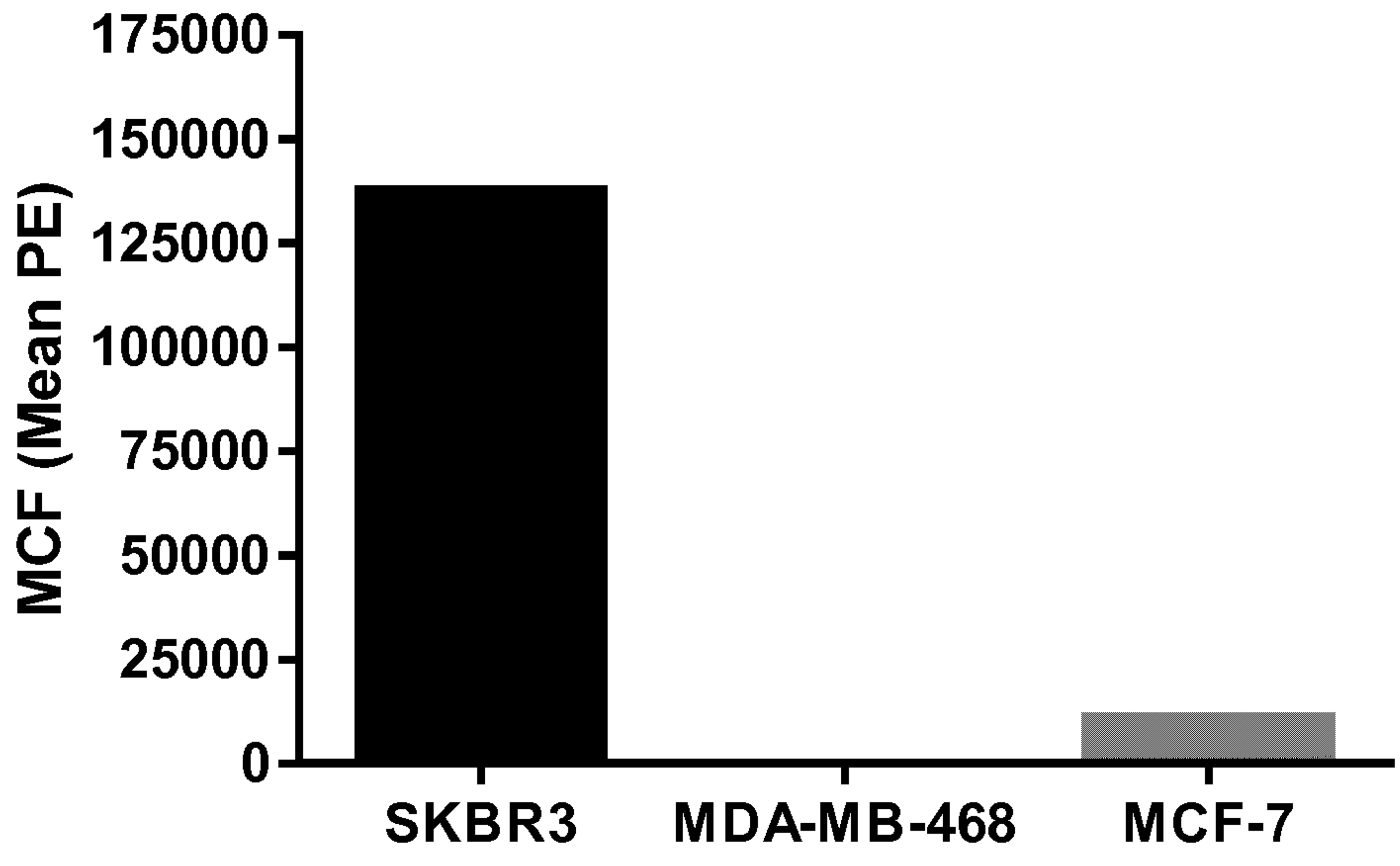


Figure 25:

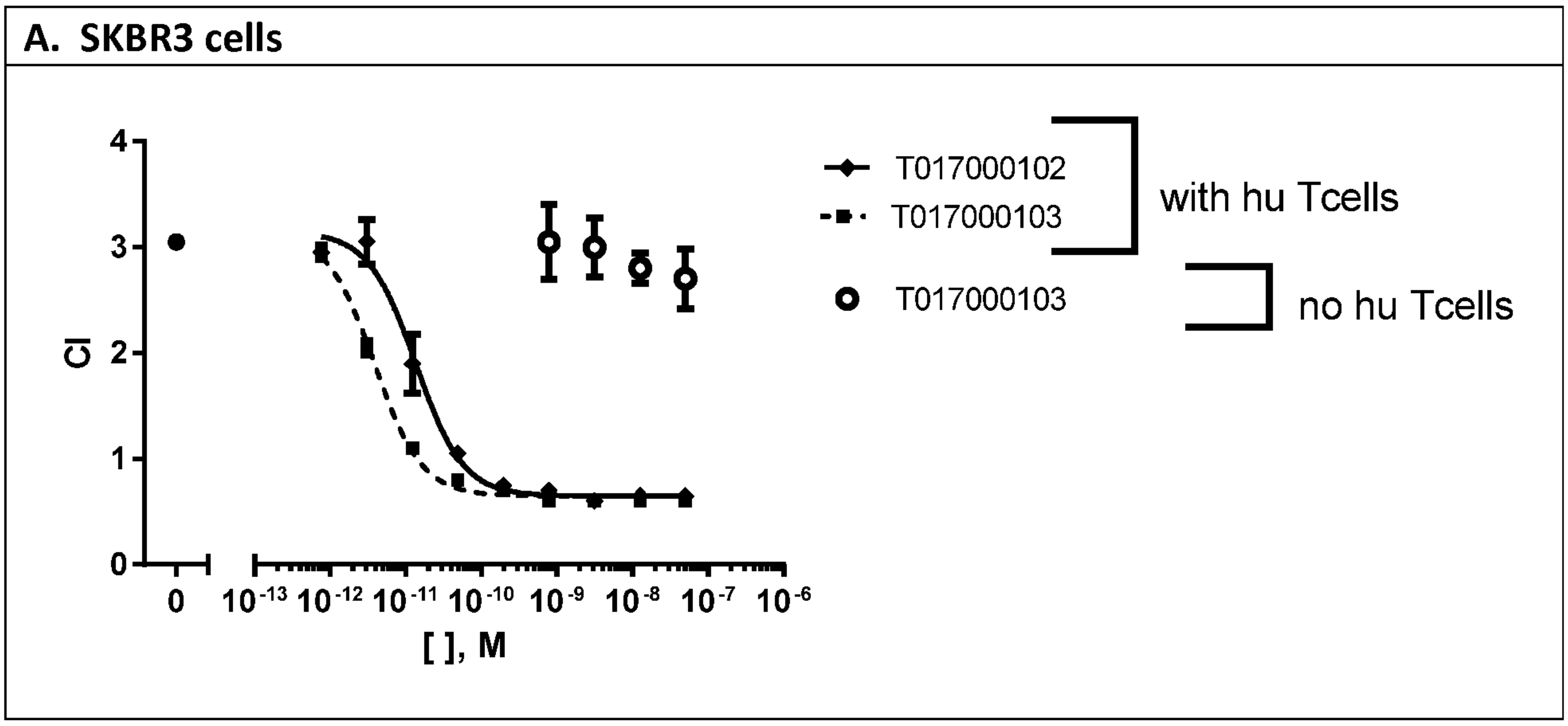
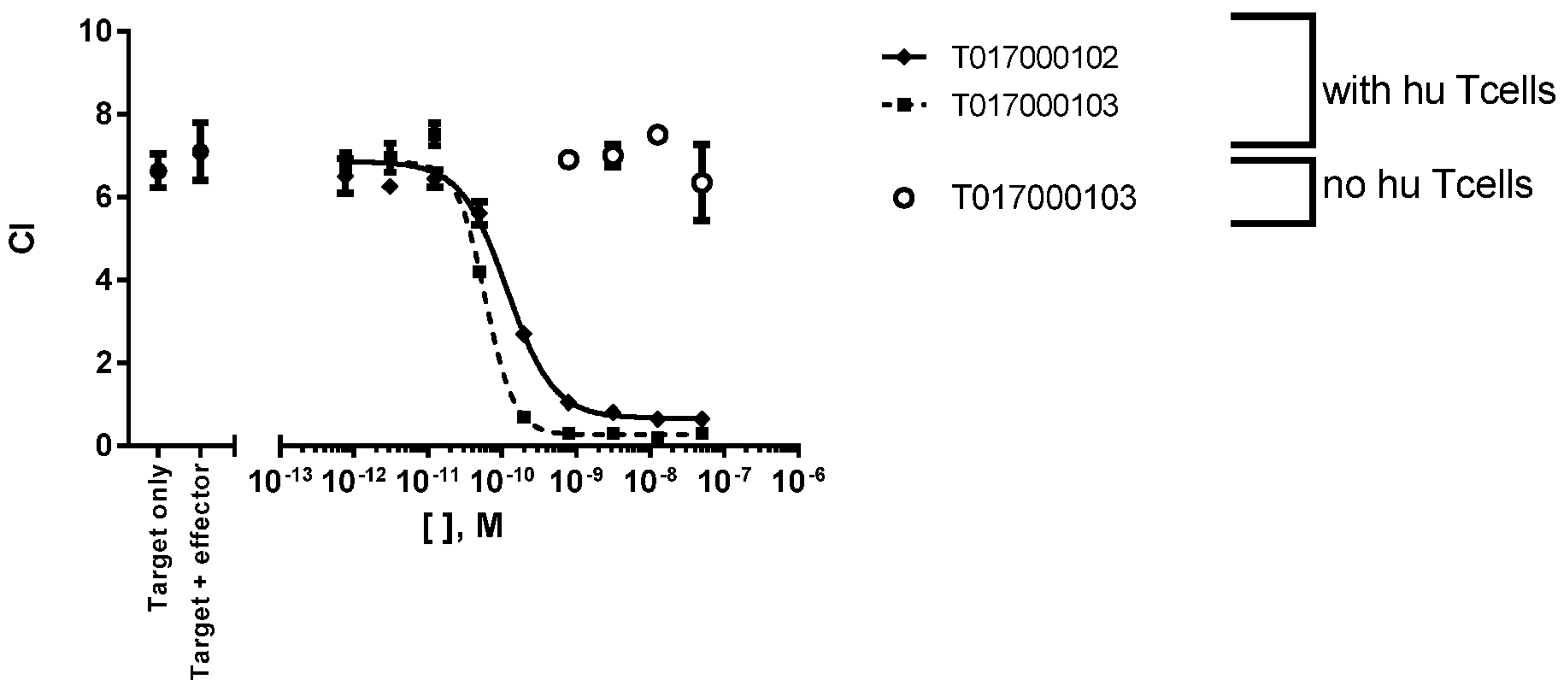


Figure 26 cont':

B. MCF-7 cells



C. MDA-MB-468 cells

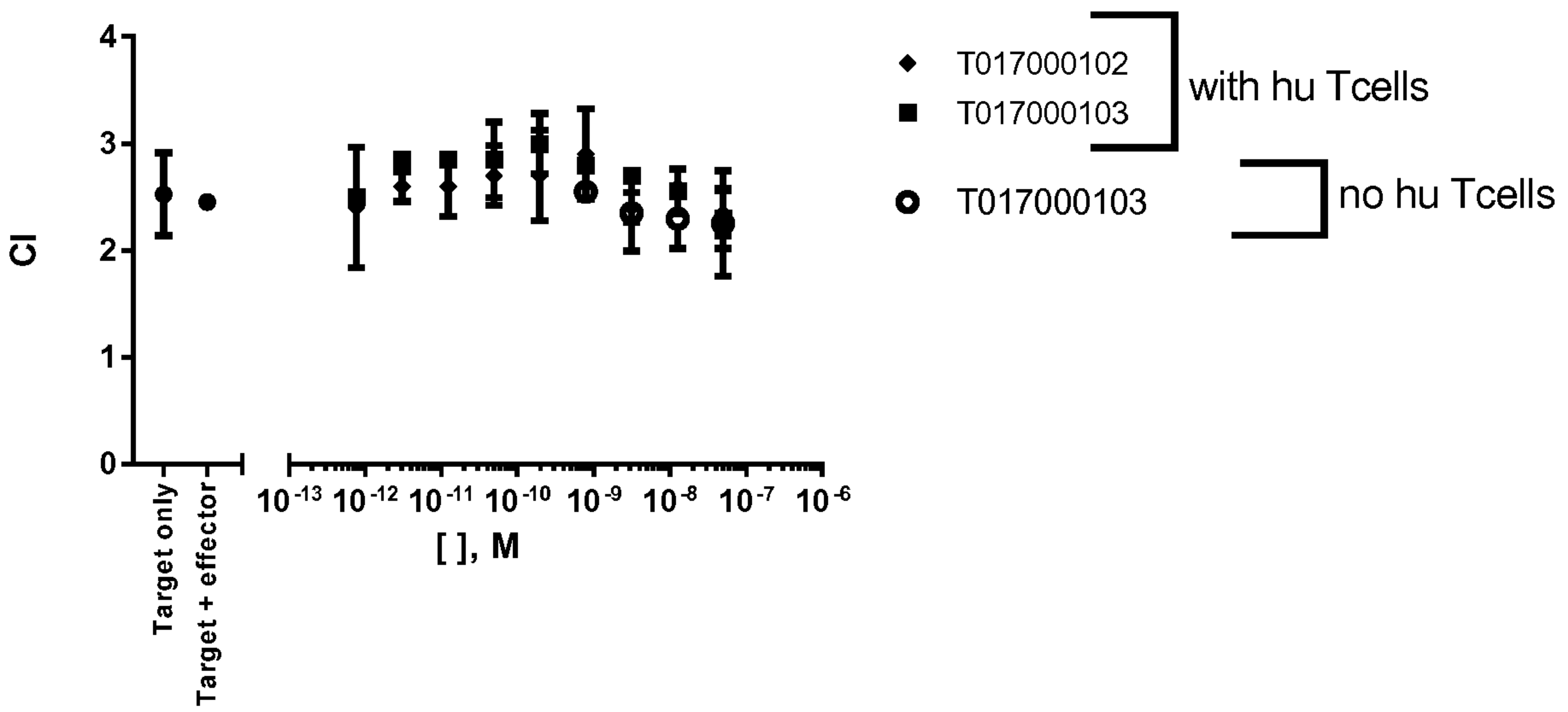


Figure 27:

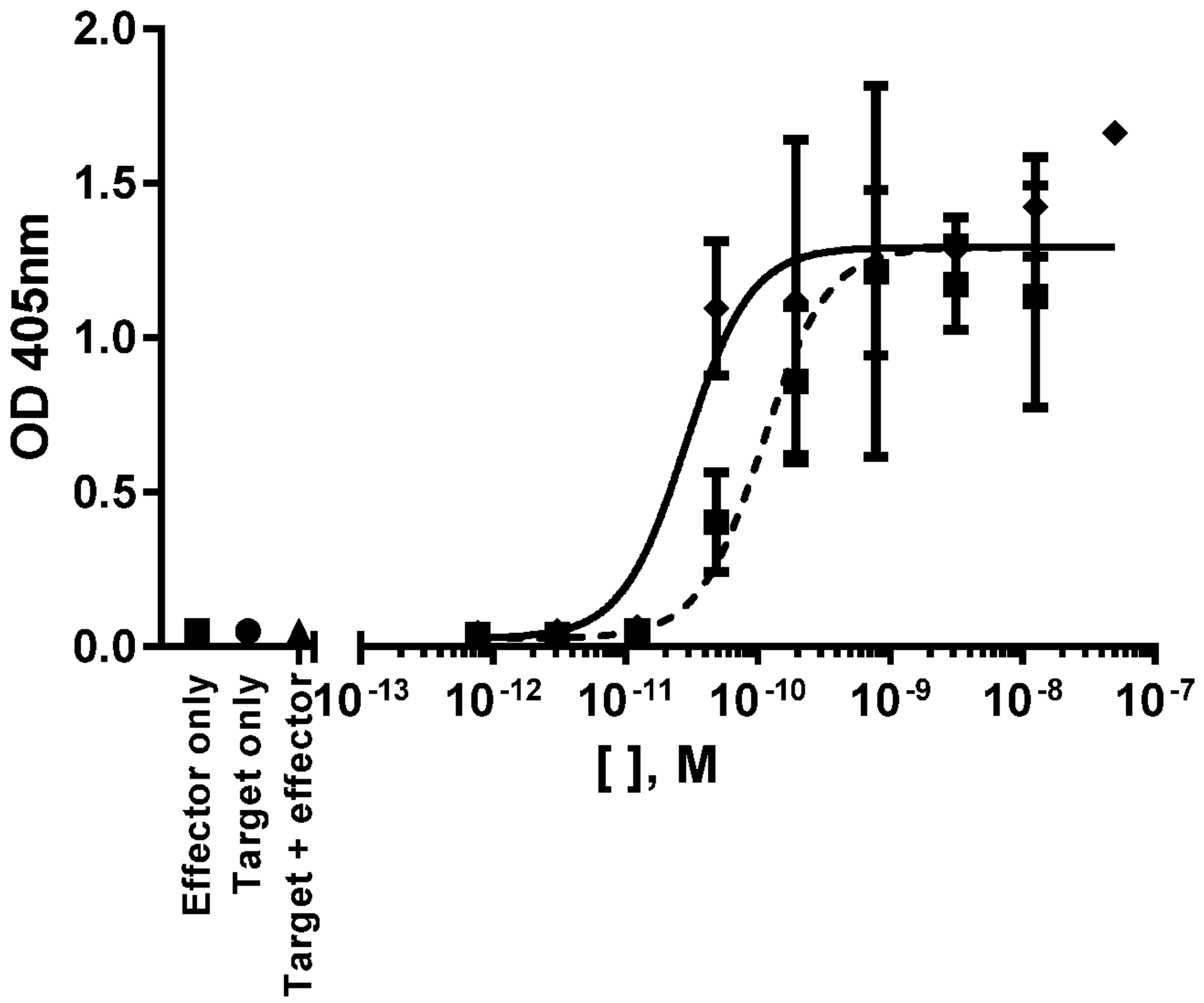


Figure 28:

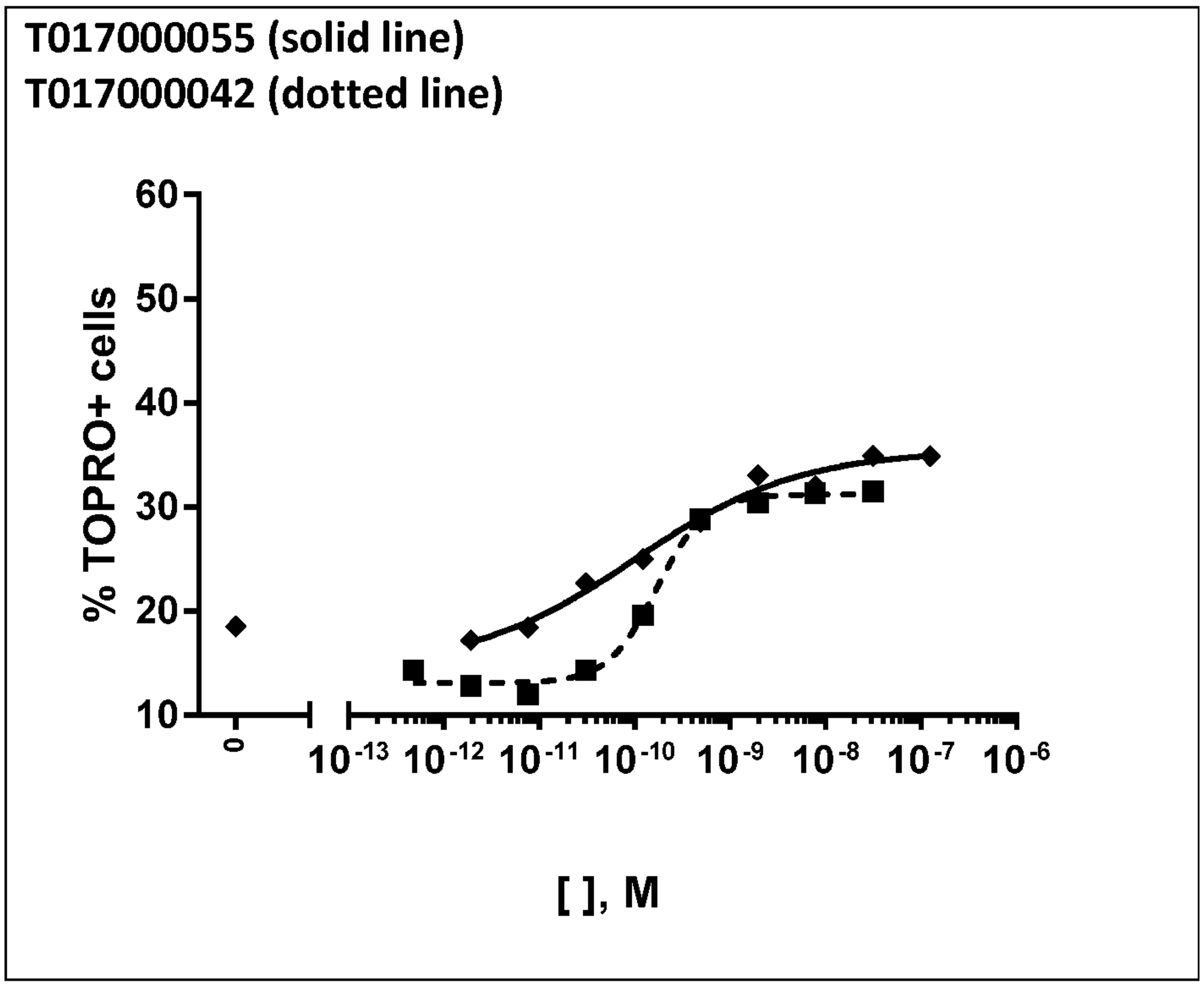
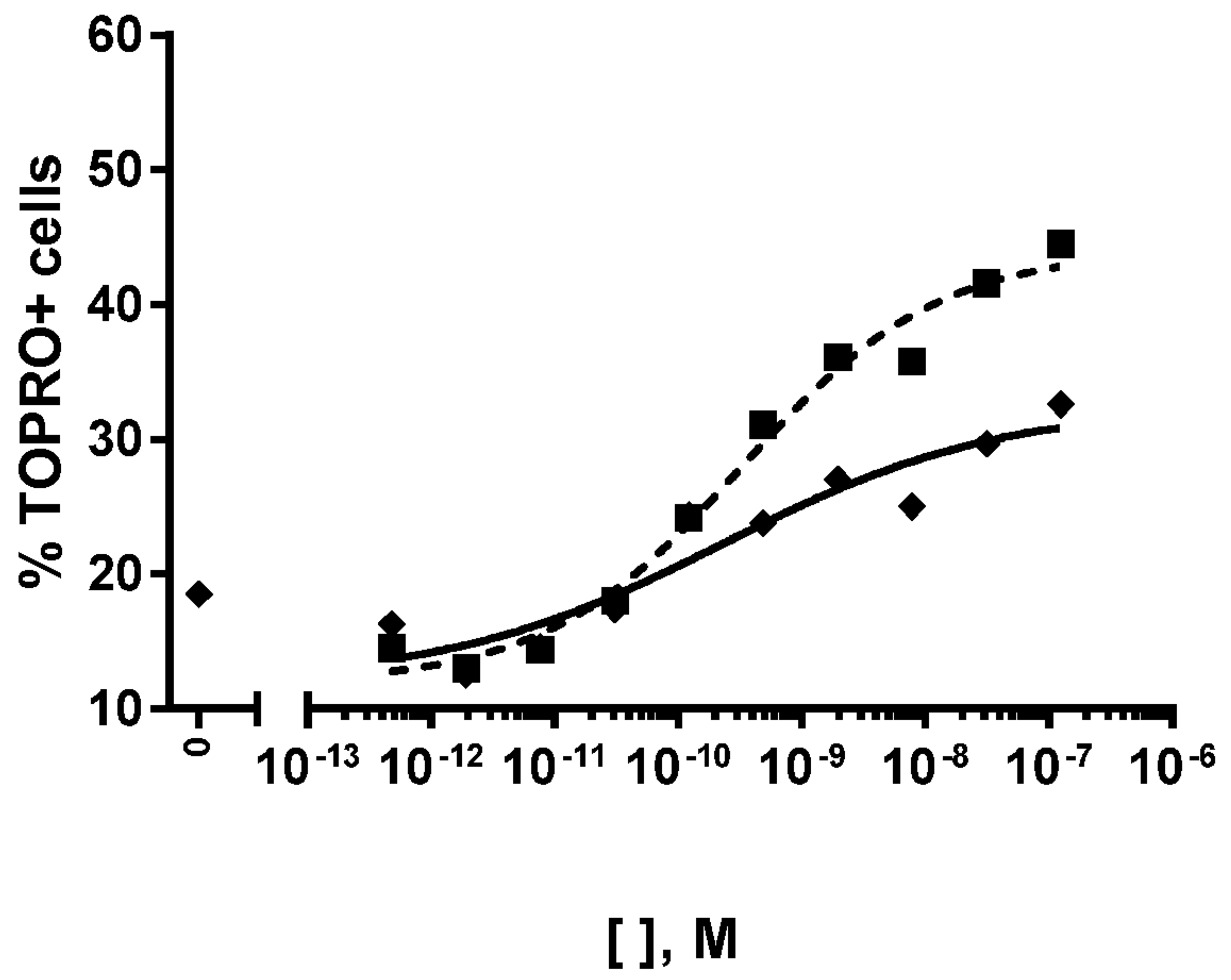


Figure 29 cont':

T017000076 (solid line)
T017000074 (dotted line)



T017000068 (solid line)
T017000031 (dotted line)

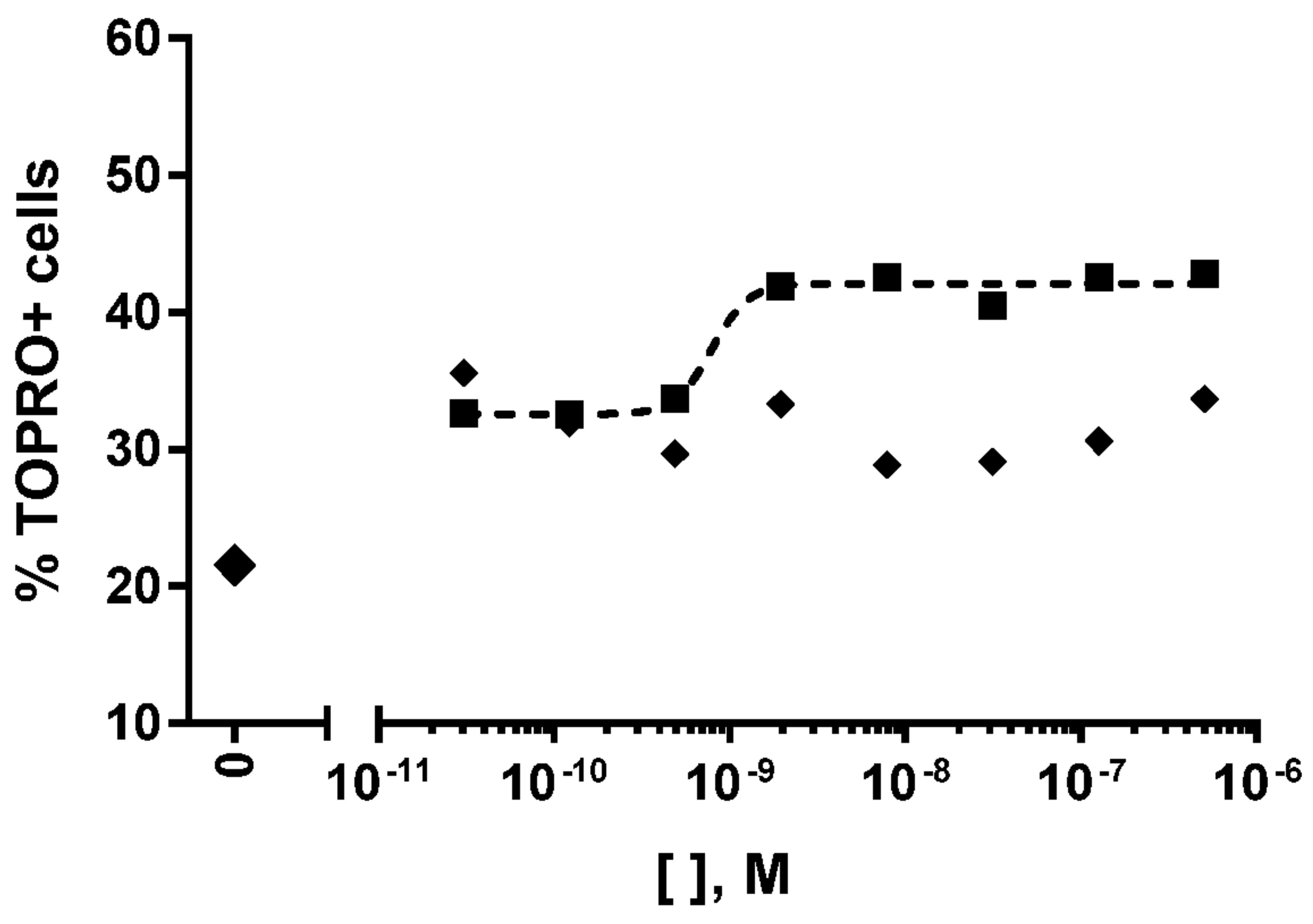
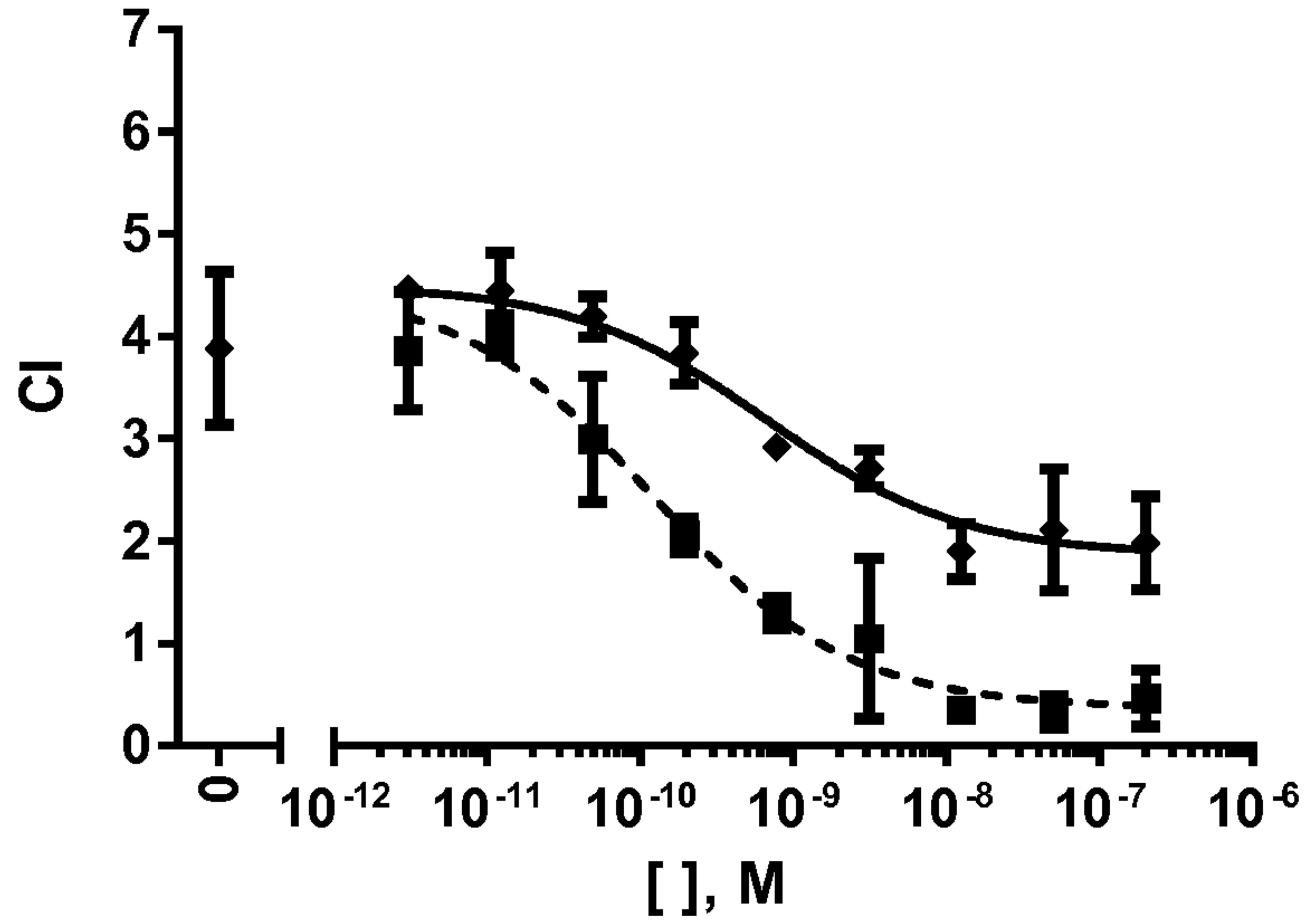


Figure 30:

T017000055 (solid line)
T017000042 (dotted line)



T017000076 (solid line)
T017000074 (dotted line)

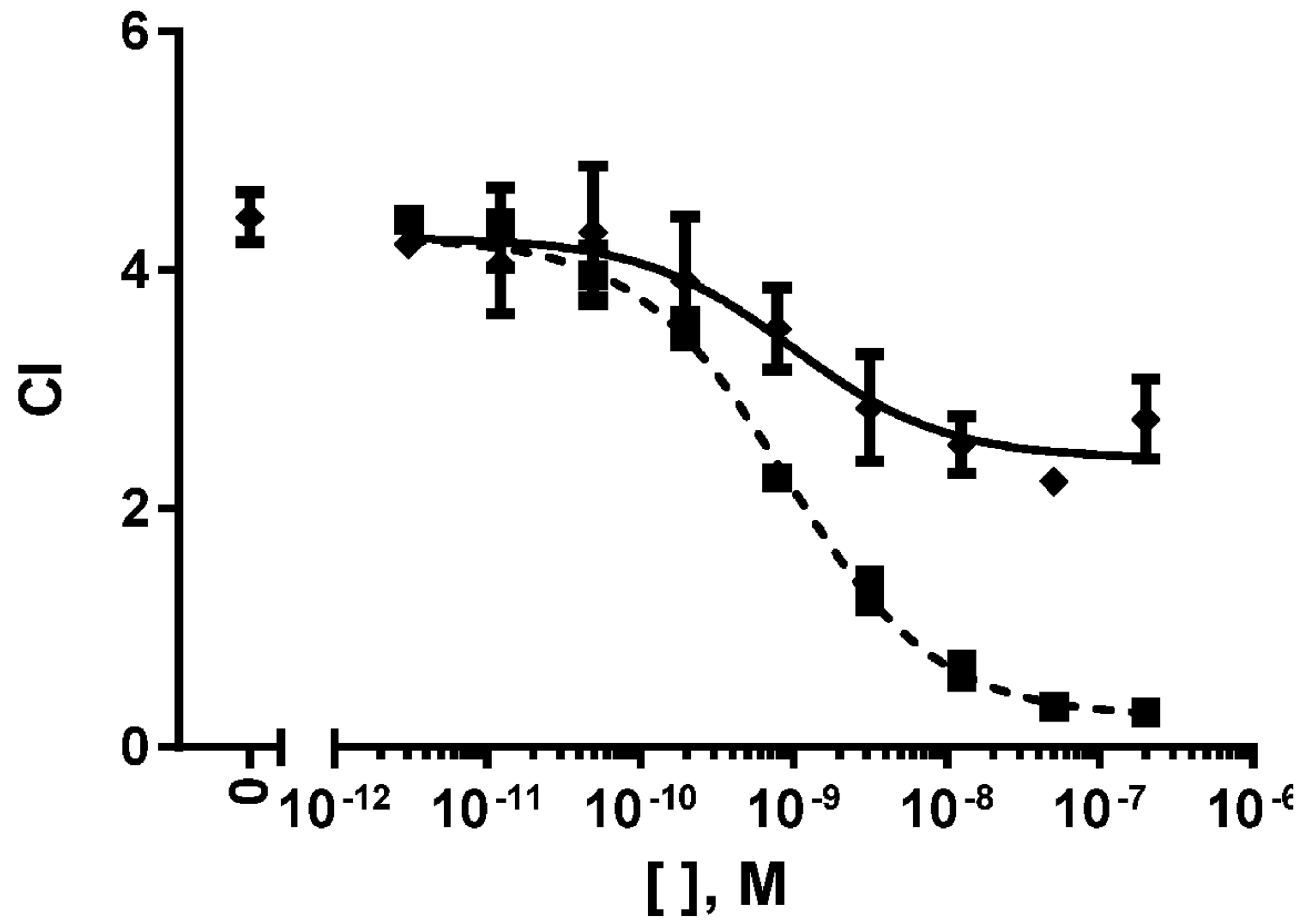


Figure 31 cont':

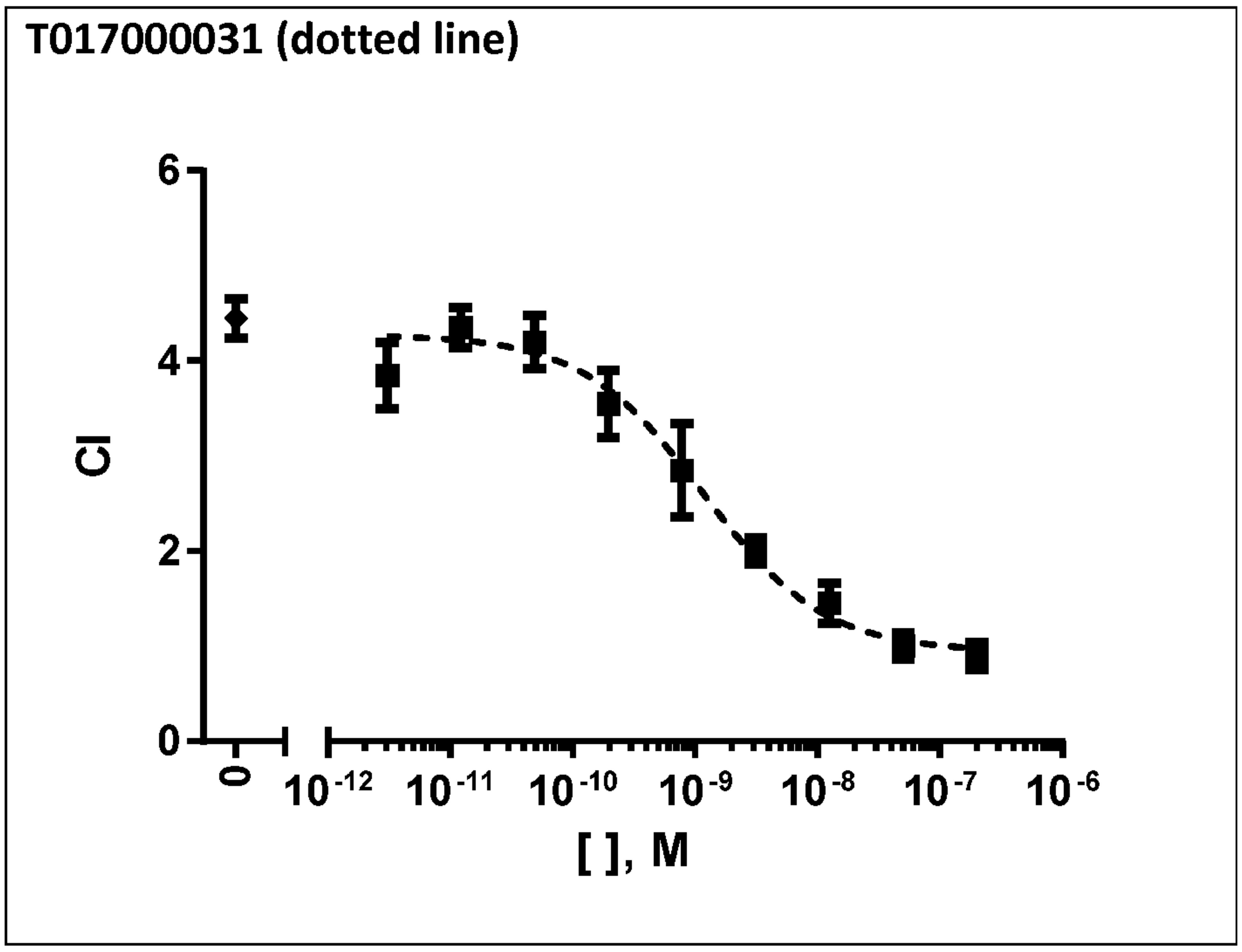


Figure 32:

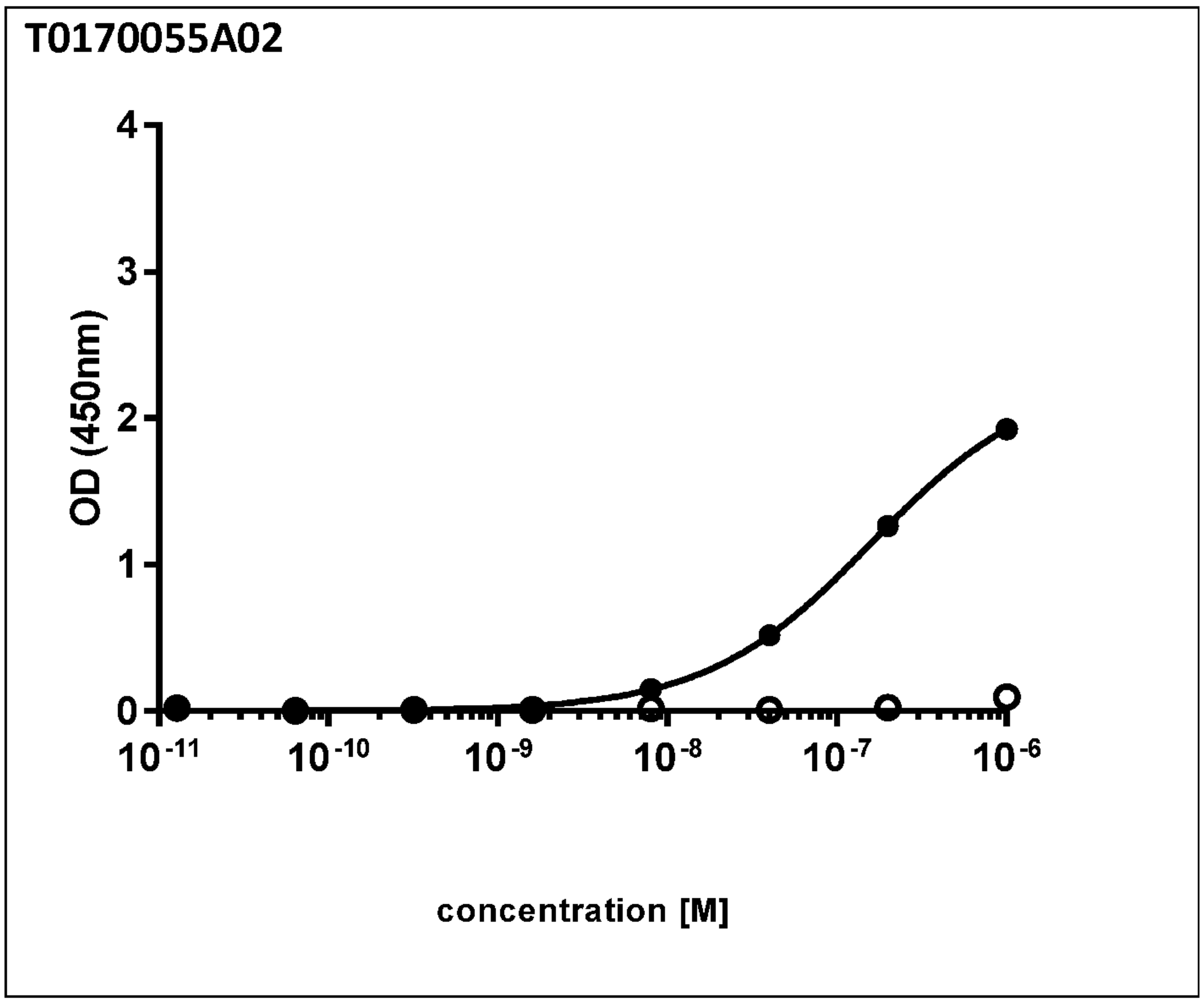
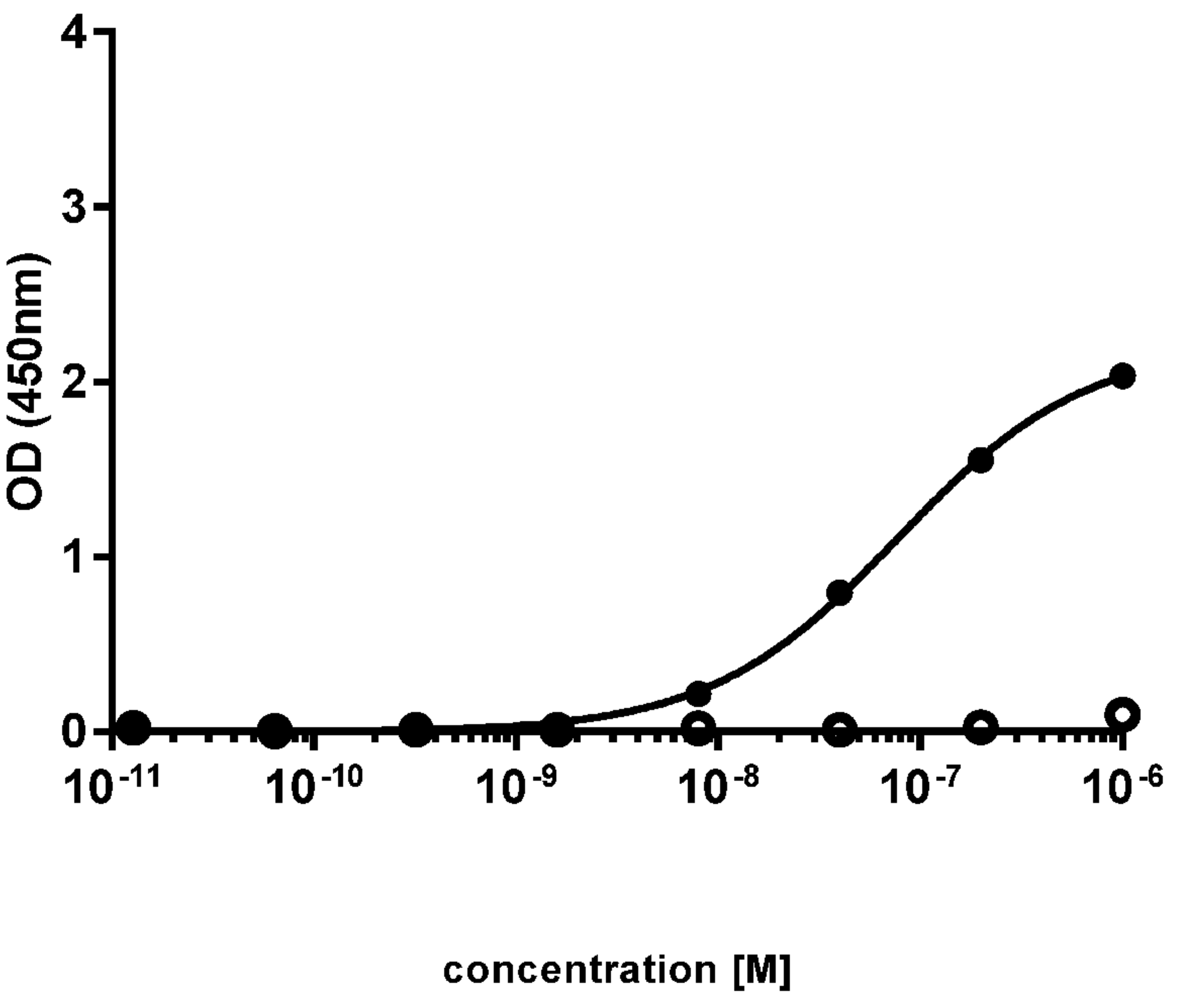


Figure 33 cont':

T0170056G05



T0170068G05

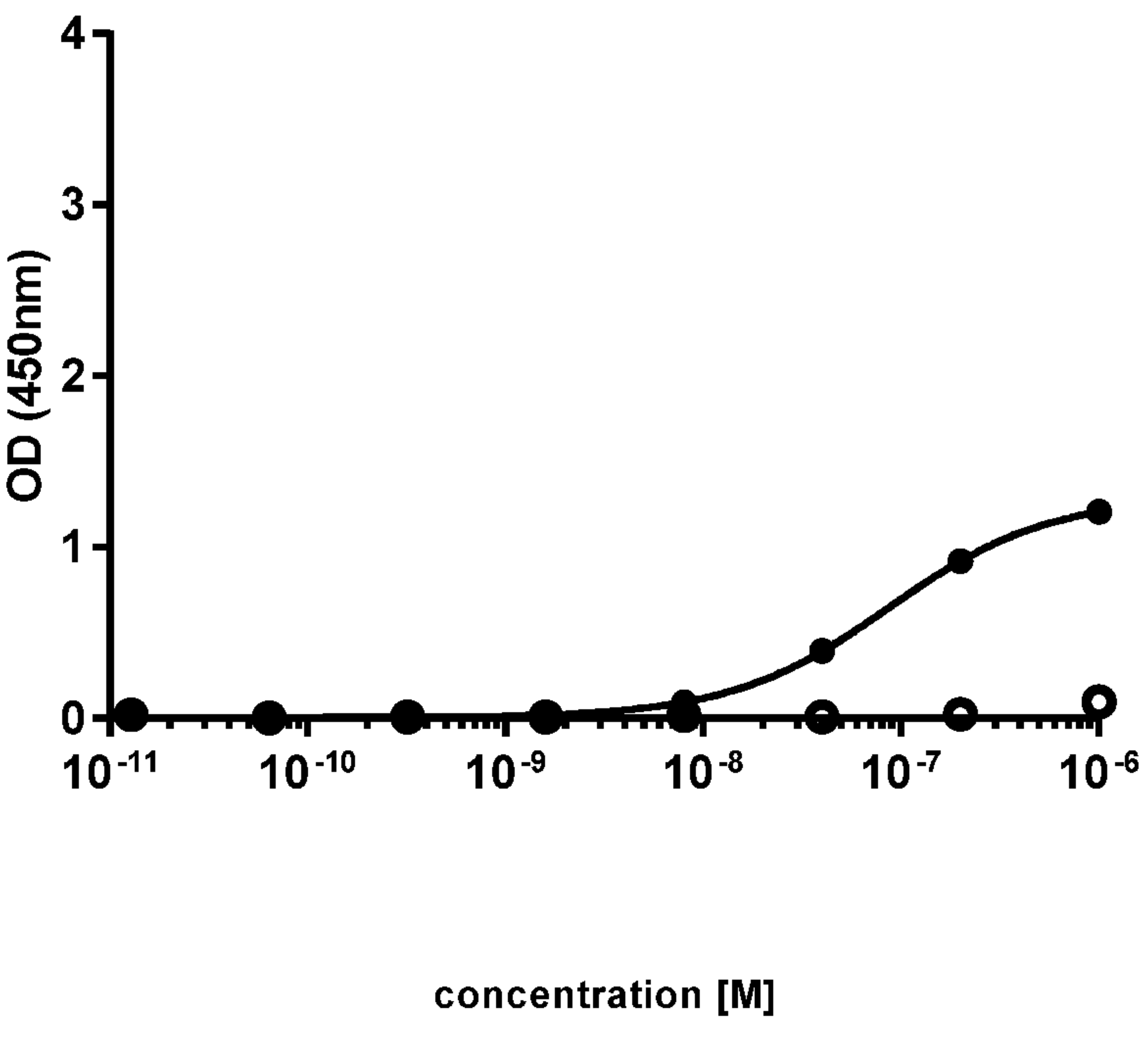


Figure 34 cont':

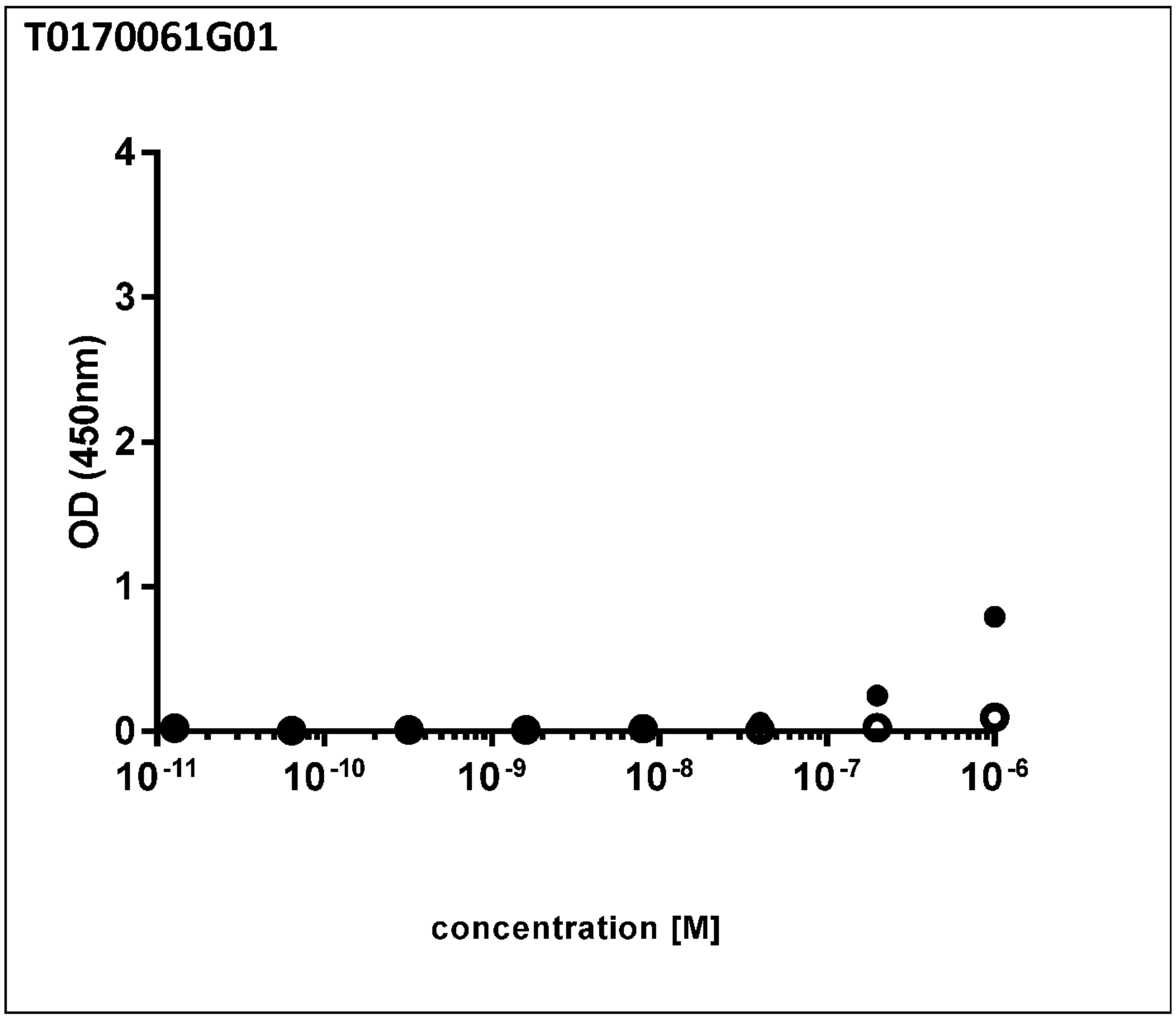
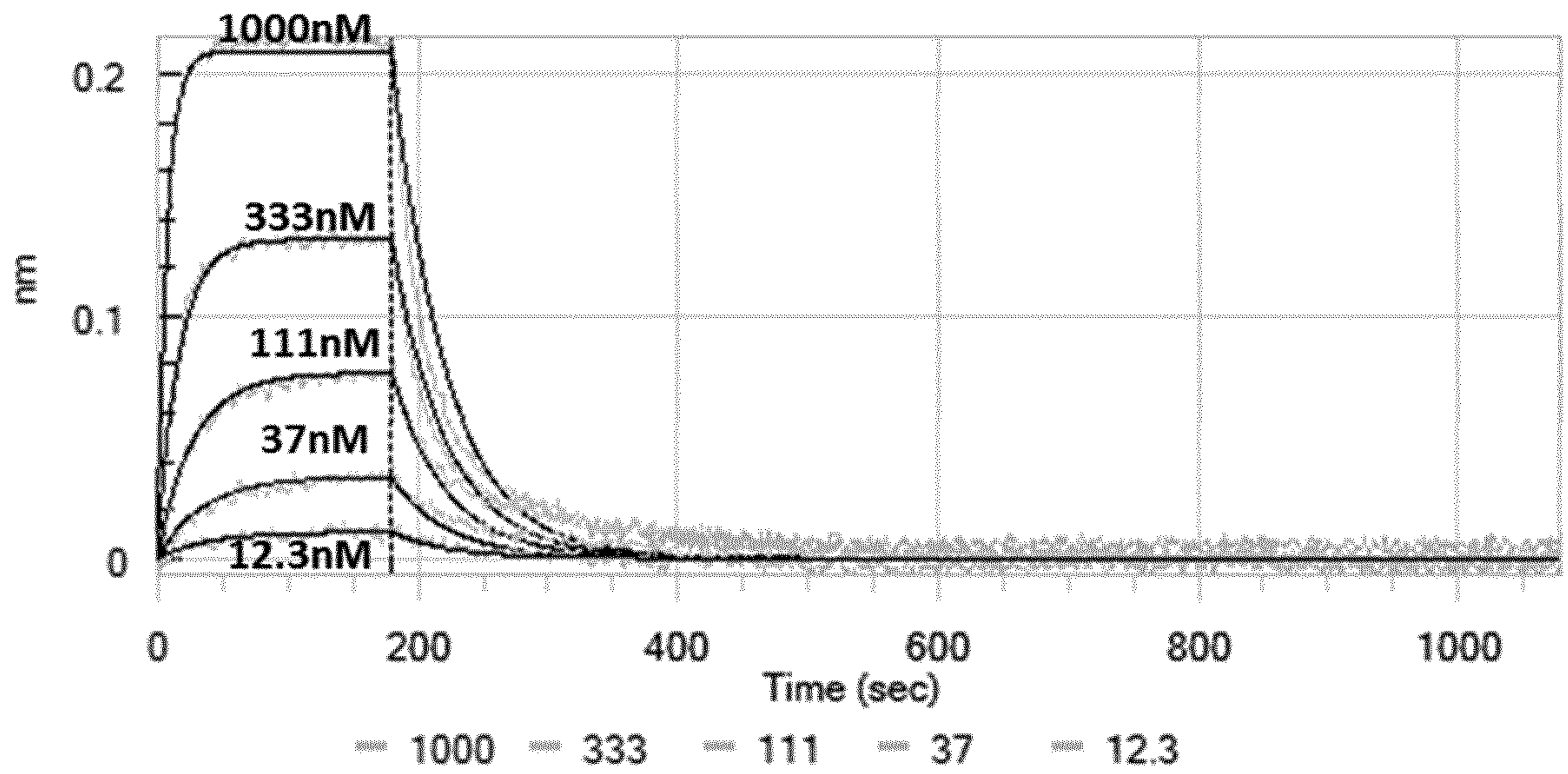


Figure 35:

A.


1000nM
333nM
111nM
37nM
12.3nM
0.2
0.1
0
nm
0
200
400
600
800
1000
Time (sec)
1000
333
111
37
12.3

B.

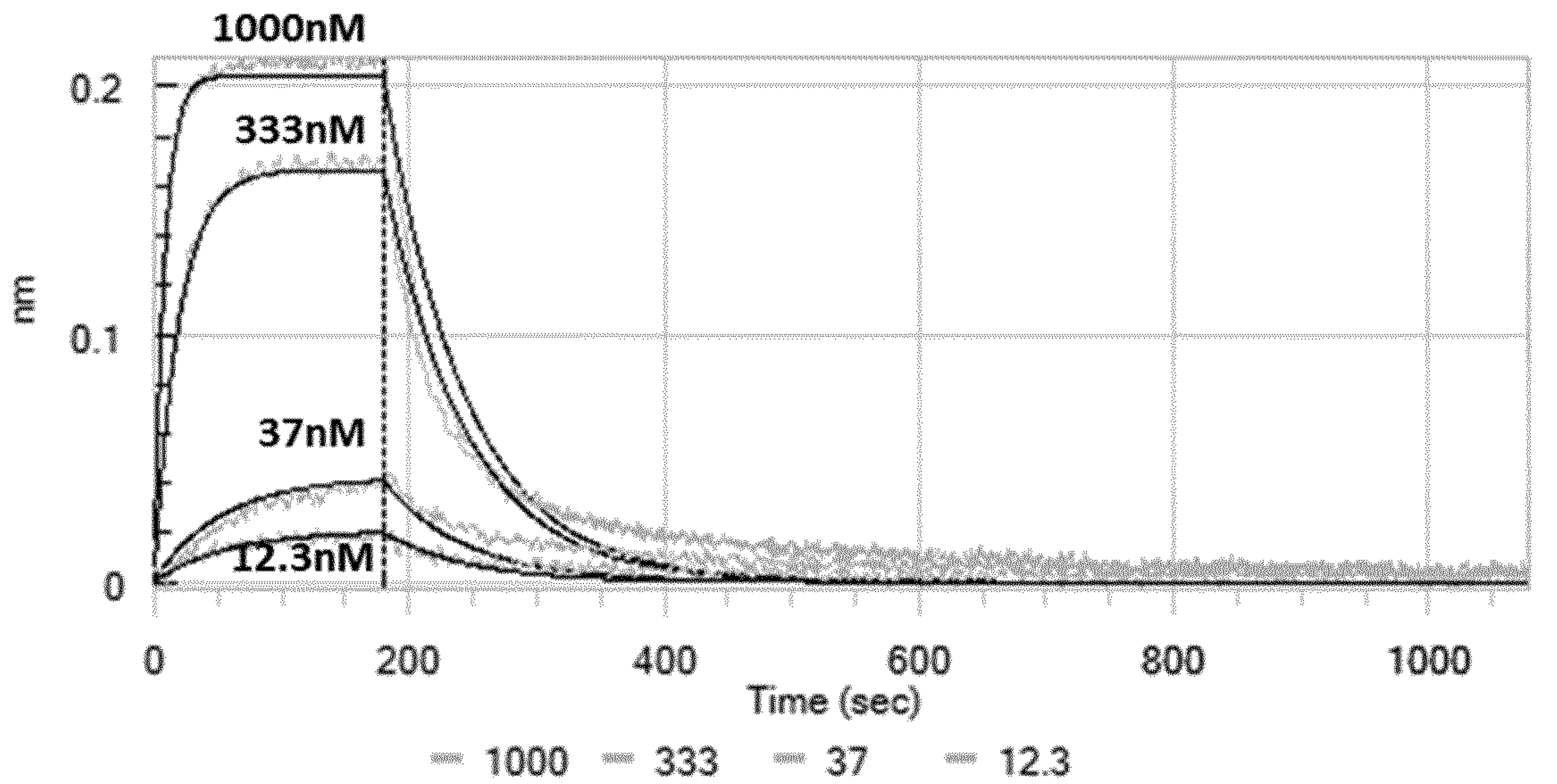

1000nM
333nM
37nM
12.3nM
0.2
0.1
0
nm
0
200
400
600
800
1000
Time (sec)
1000
333
37
12.3

Figure 36:

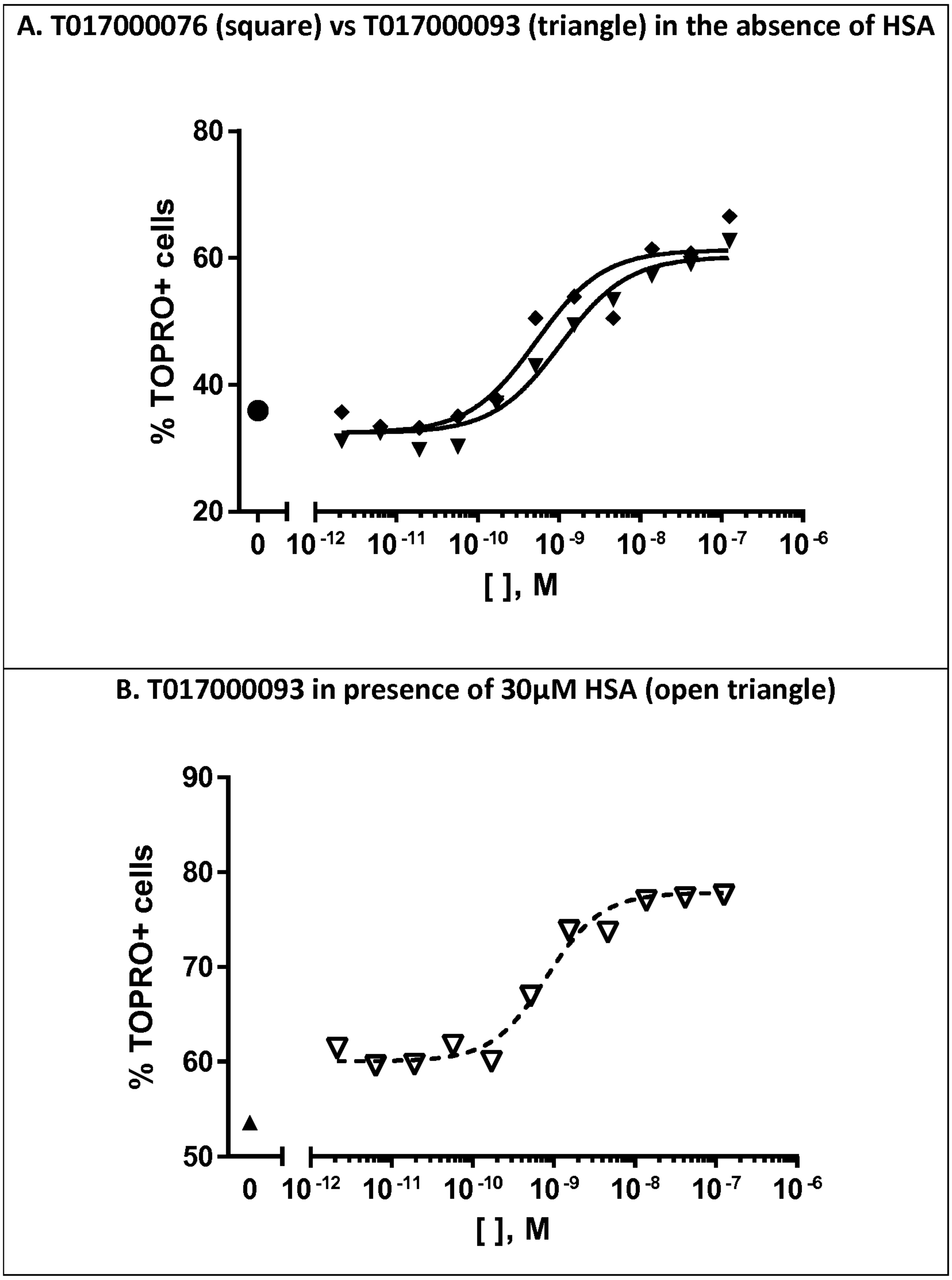

A. T017000076 (square) vs T017000093 (triangle) in the absence of HSA
80
60
40
20
% TOPRO+ cells
0
10 -12
10 -11
10 -10
10 -9
10 -8
10 -7
10 -6
[ ], M
B. T017000093 in presence of 30µM HSA (open triangle)
90
80
70
60
50
% TOPRO+ cells
0
10 -12
10 -11
10 -10
10 -9
10 -8
10 -7
10 -6
[ ], M

Figure 37:

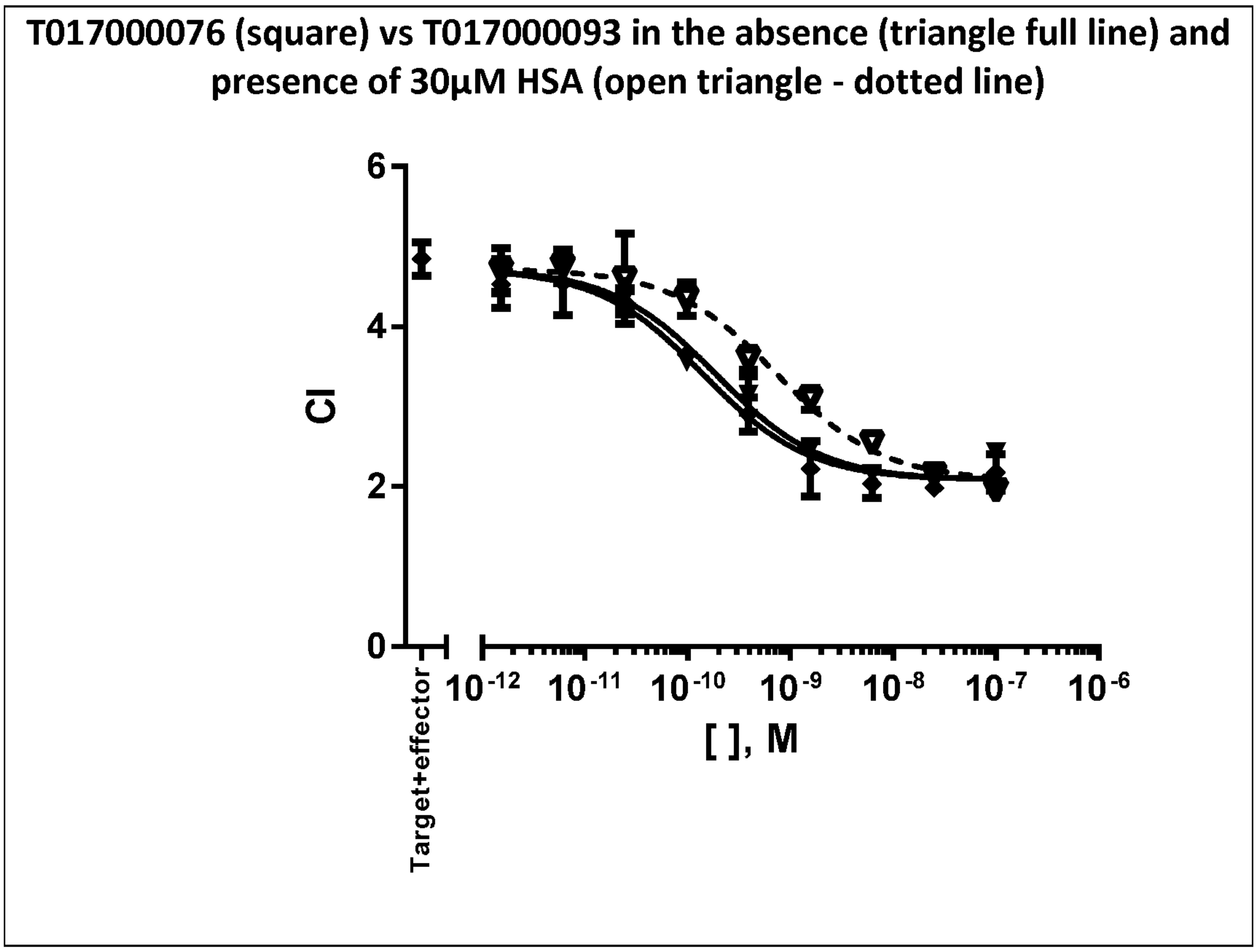

T017000076 (square) vs T017000093 in the absence (triangle full line) and presence of 30µM HSA (open triangle - dotted line)
CI
6
4
2
0
Target+effector
10-12
10-11
10-10
10-9
10-8
10-7
10-6
[ ], M

Figure 38:

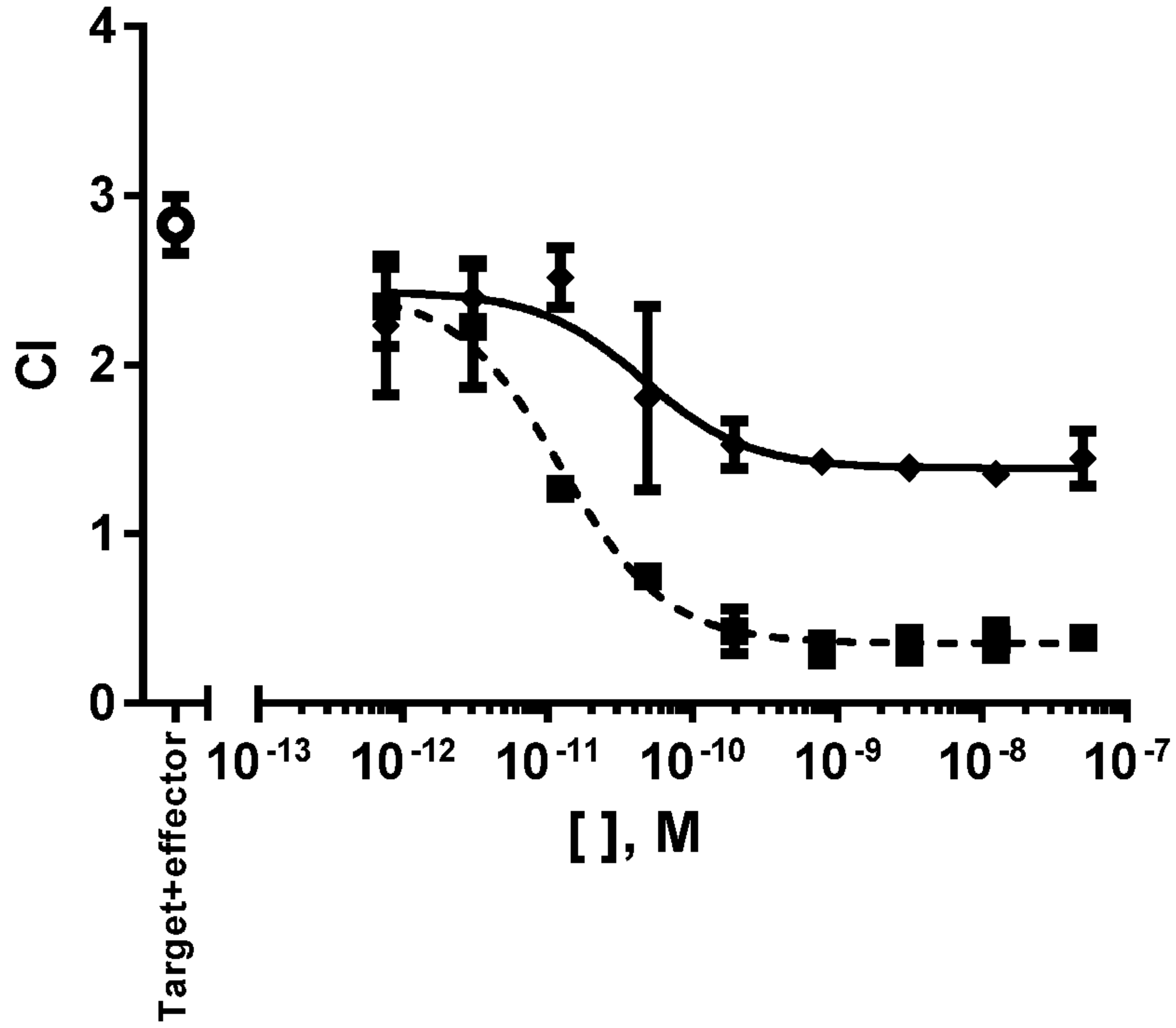


Figure 27:

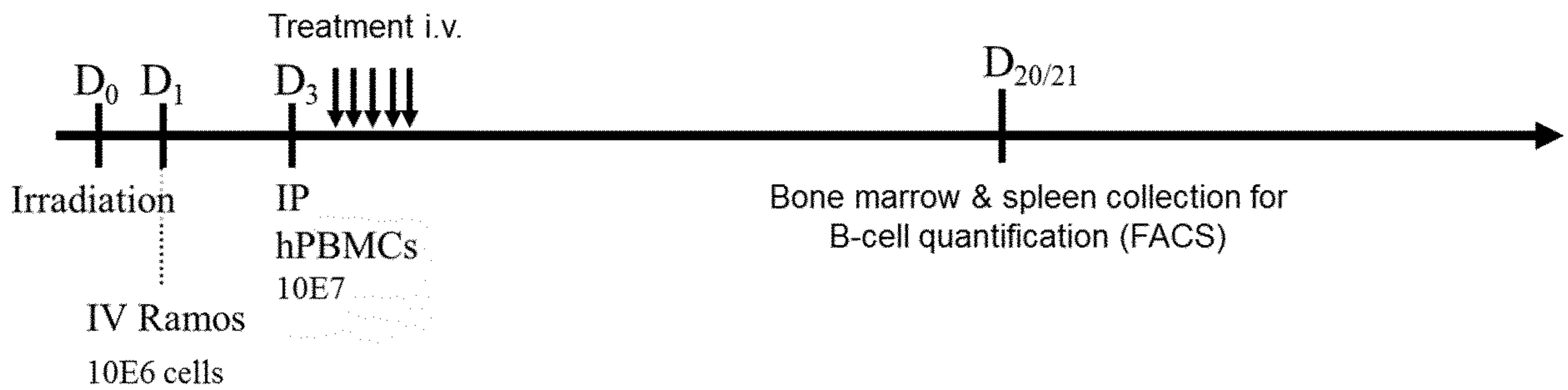


Figure 28A:

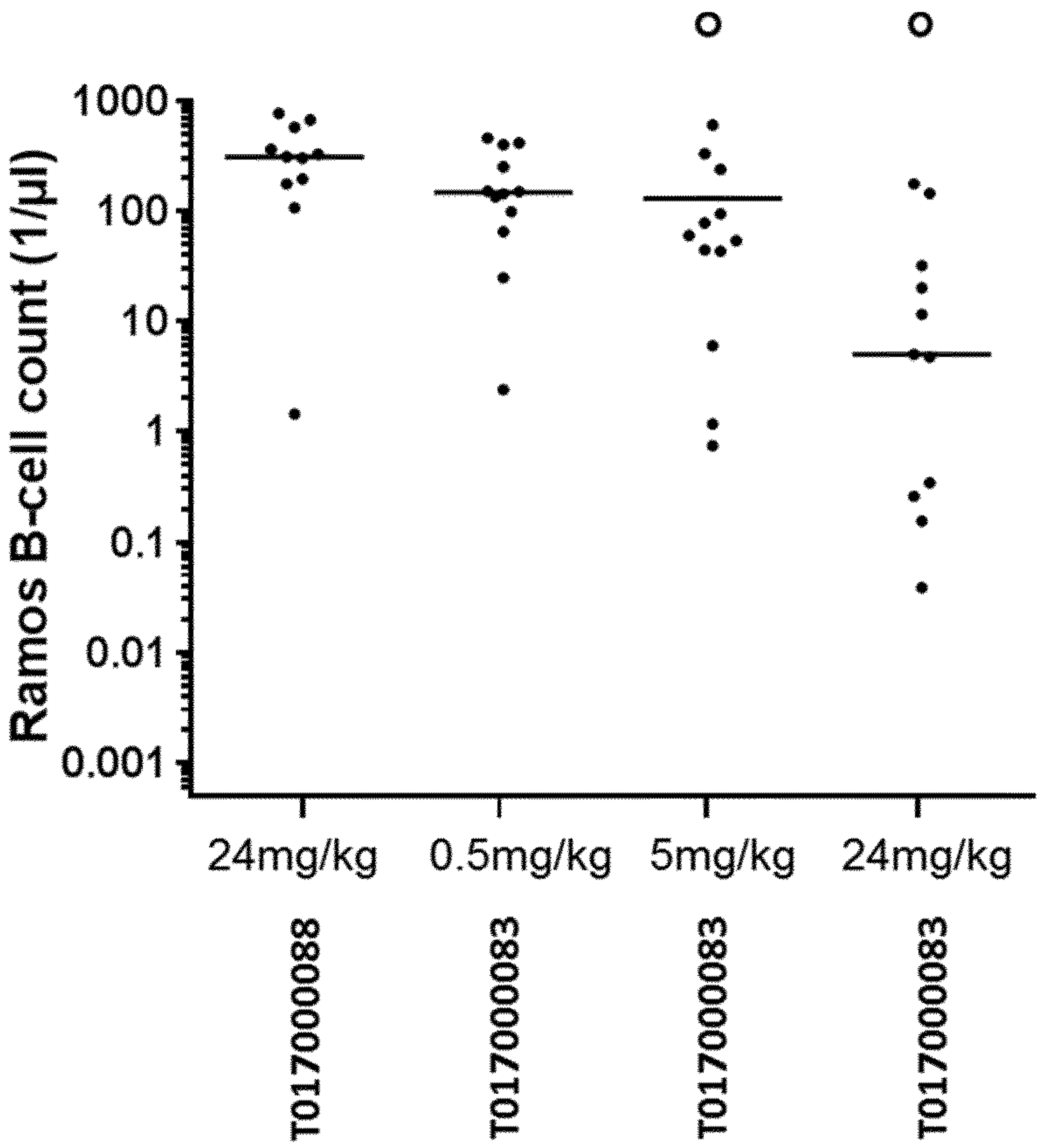


Figure 28B:

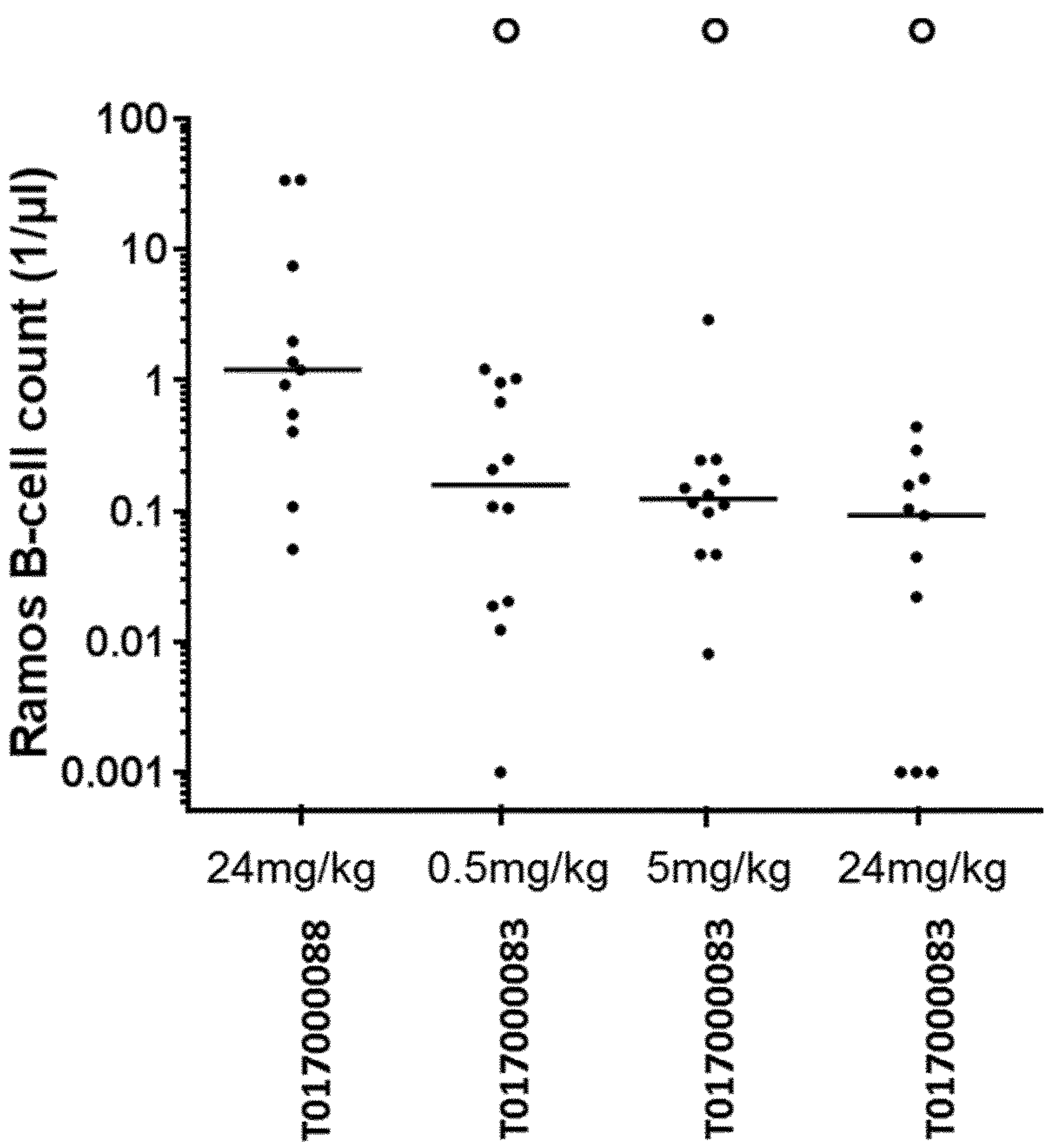


Figure 28C:

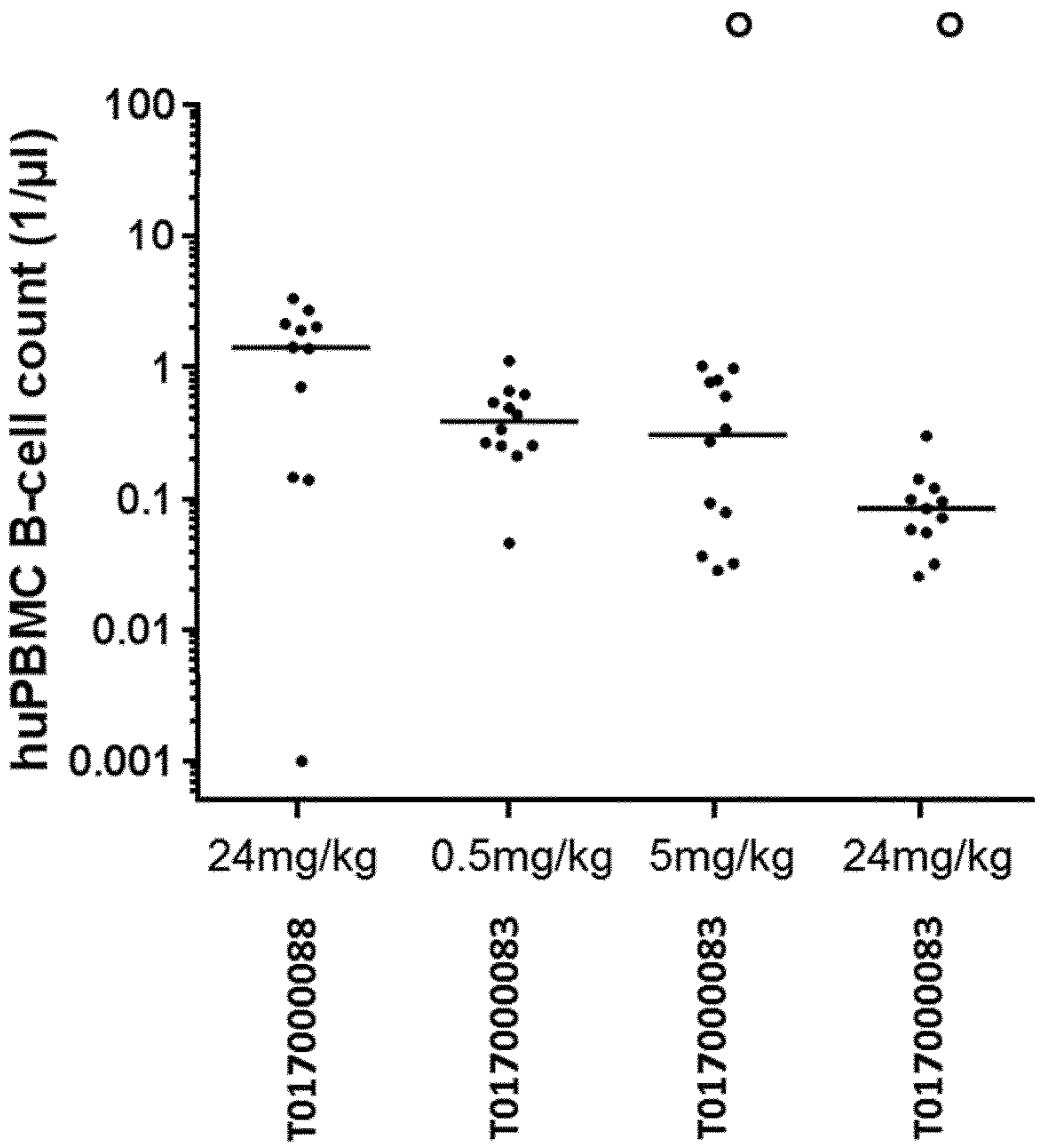


Figure 28D:

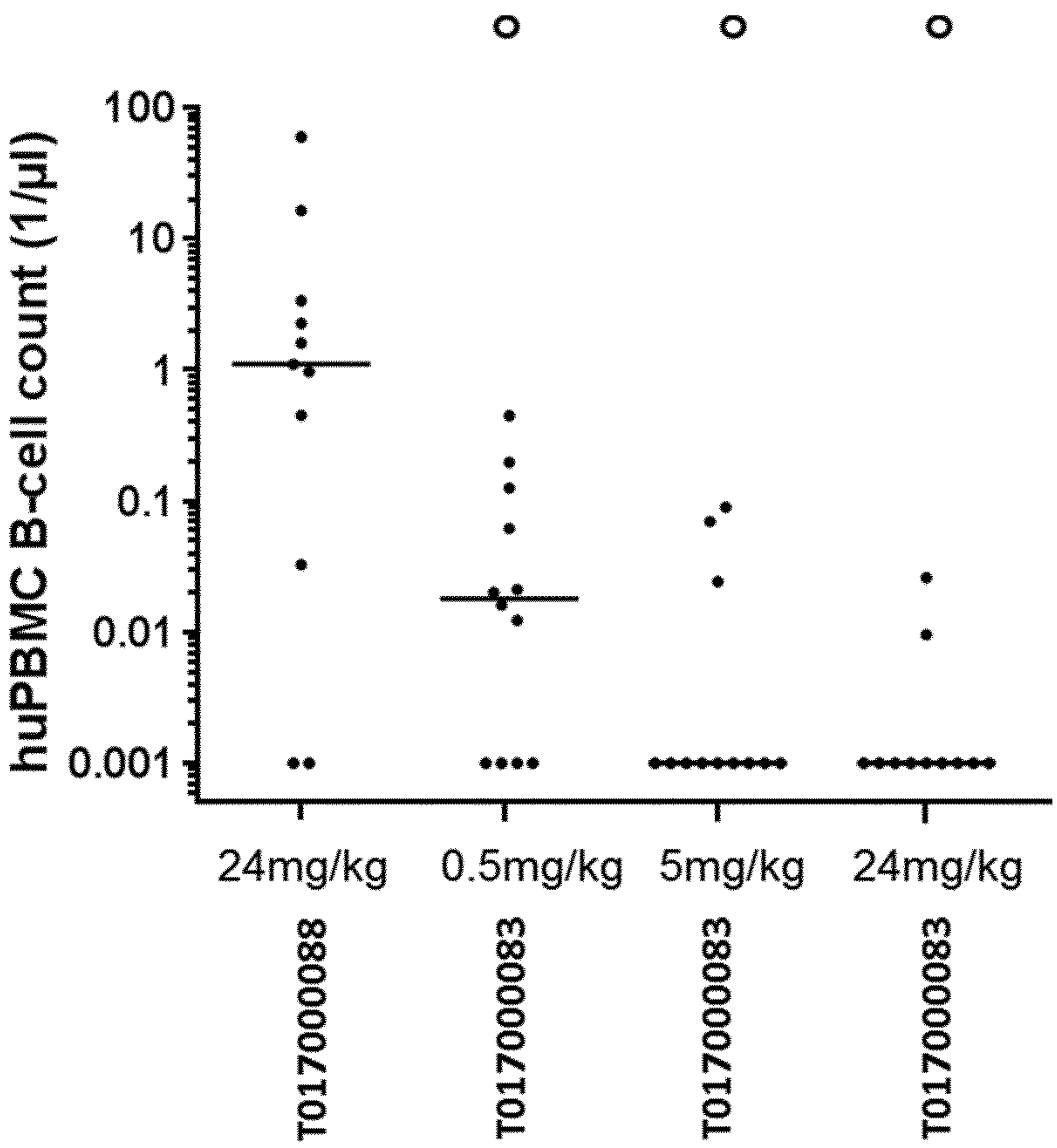


Figure 29:

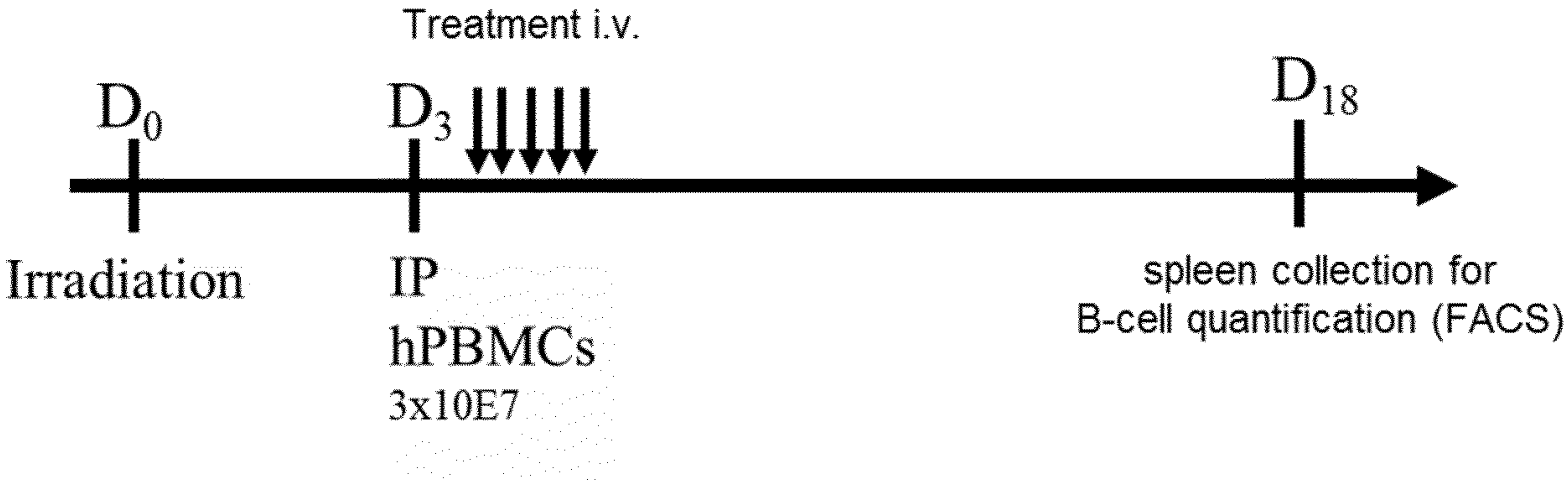


Figure 30:

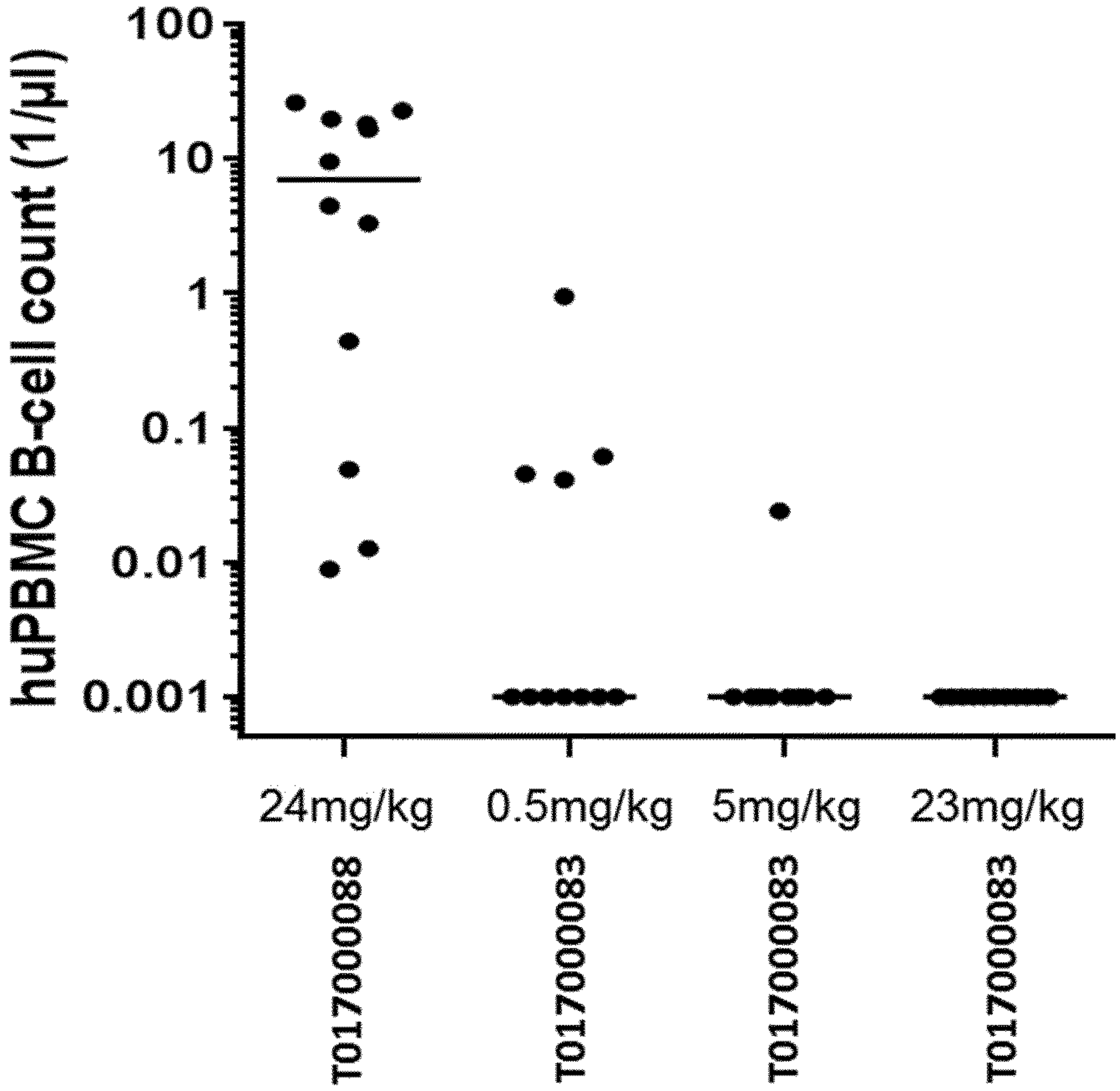
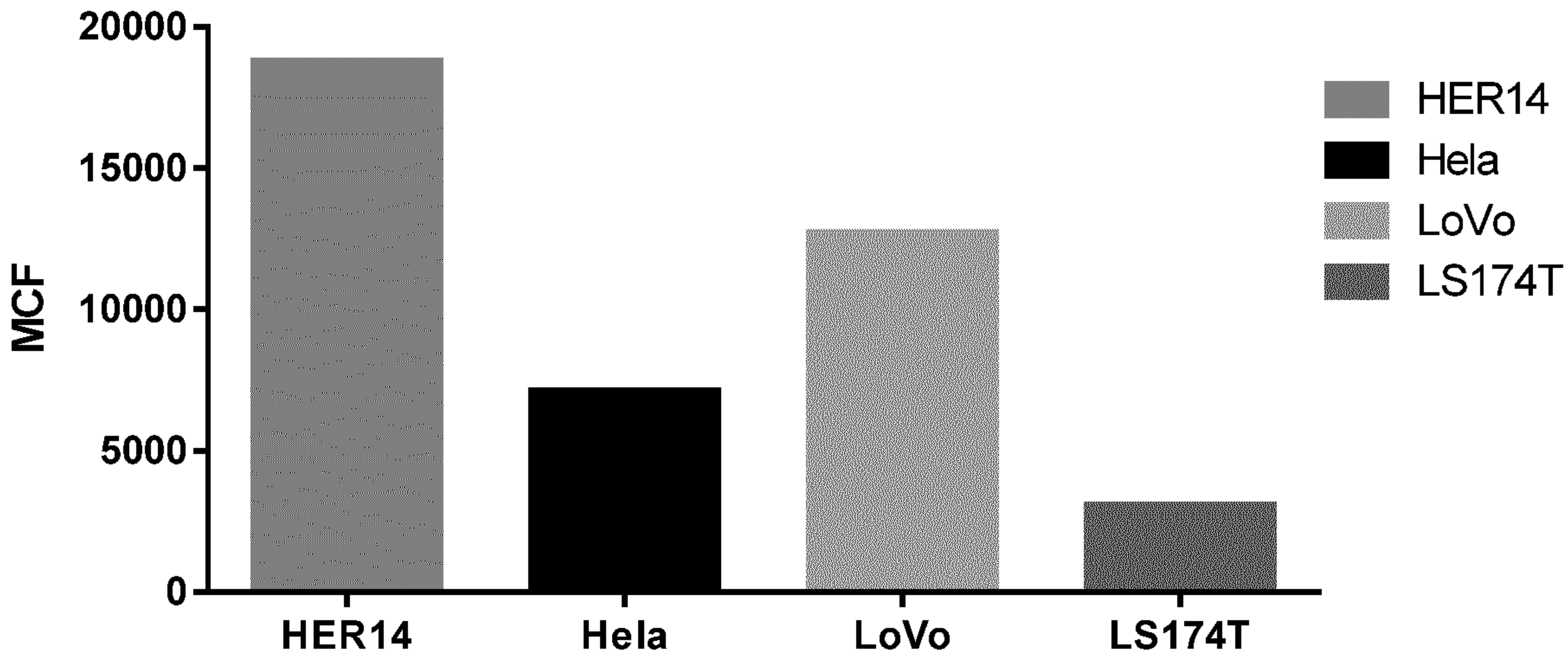


Figure 31:

A


20000
15000
10000
5000
0
MCF
HER14
Hela
LoVo
LS174T
HER14
Hela
LoVo
LS174T

B

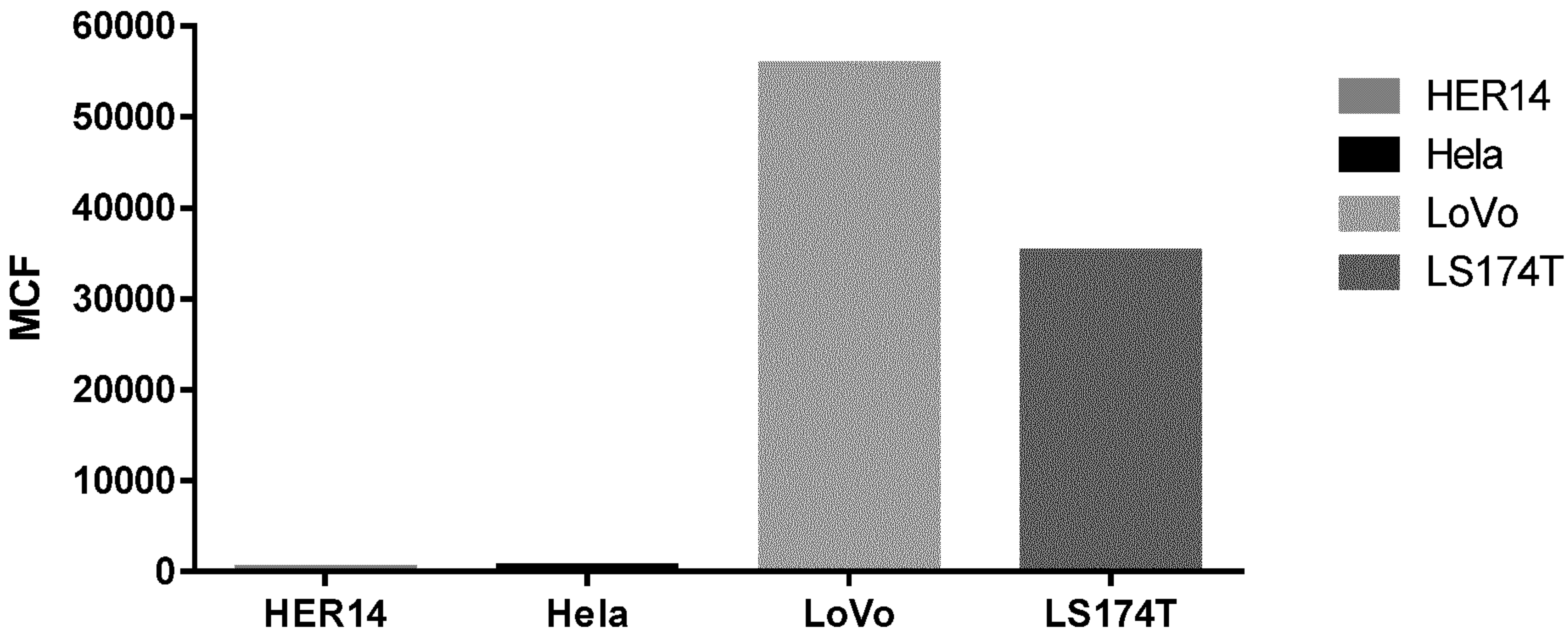

60000
50000
40000
30000
20000
10000
0
MCF
HER14
Hela
LoVo
LS174T
HER14
Hela
LoVo
LS174T

Figure 32:

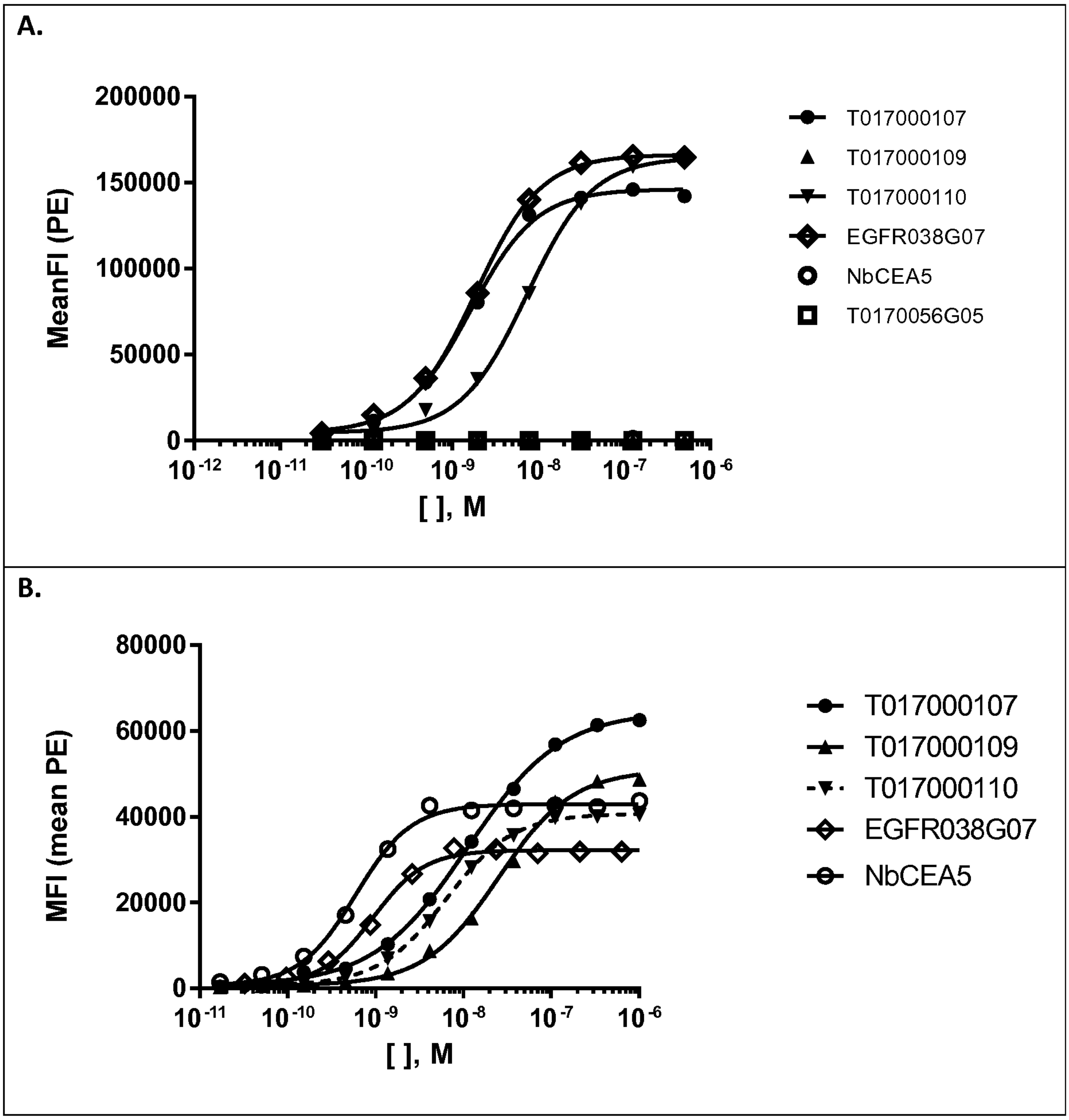

A.
200000
150000
100000
50000
0
MeanFI (PE)
10^{-12} 10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6}
[ ], M
T017000107
T017000109
T017000110
EGFR038G07
NbCEA5
T0170056G05
B.
80000
60000
40000
20000
0
MFI (mean PE)
10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6}
[ ], M
T017000107
T017000109
T017000110
EGFR038G07
NbCEA5

Figure 32 cont':

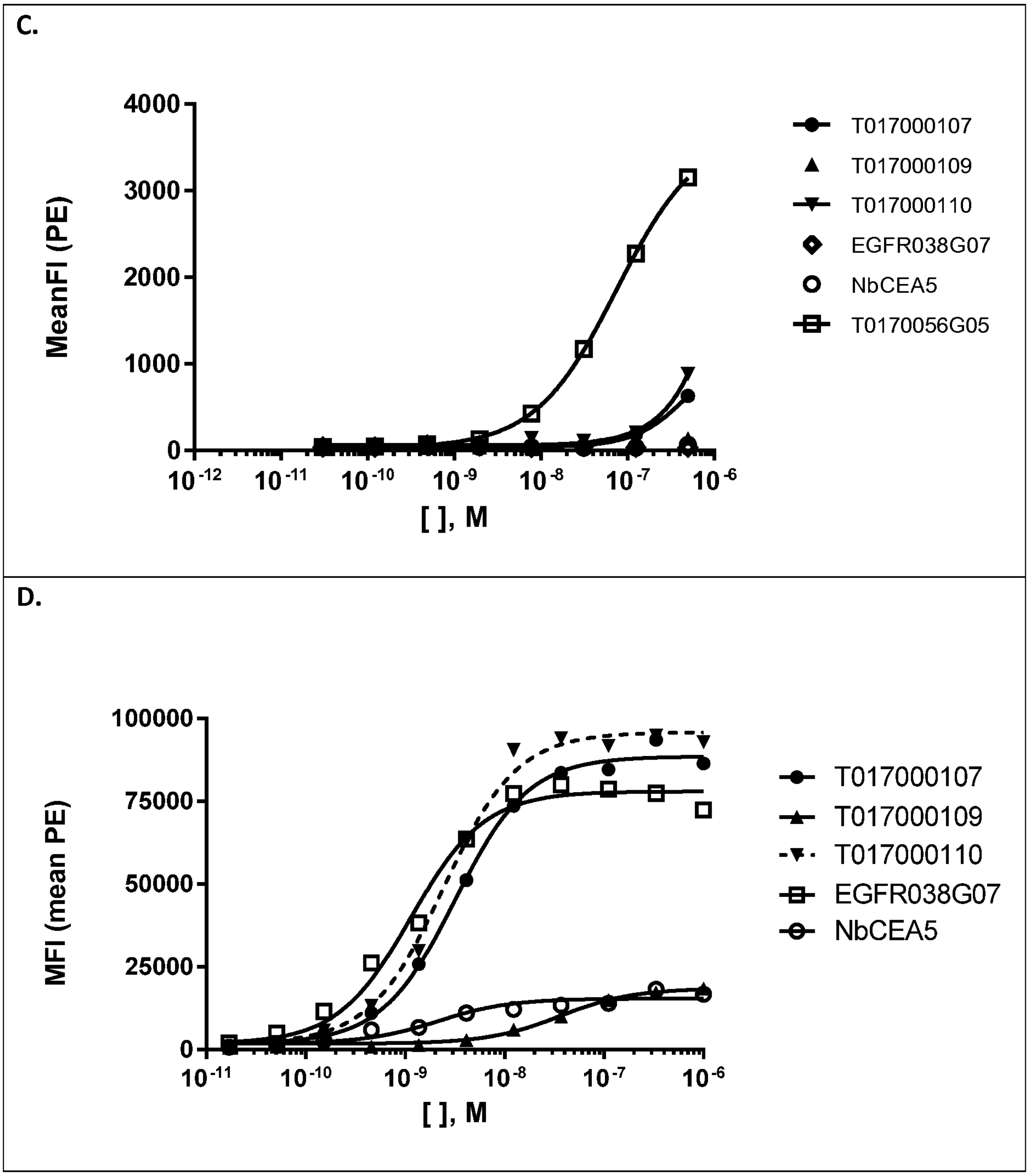


Figure 33:

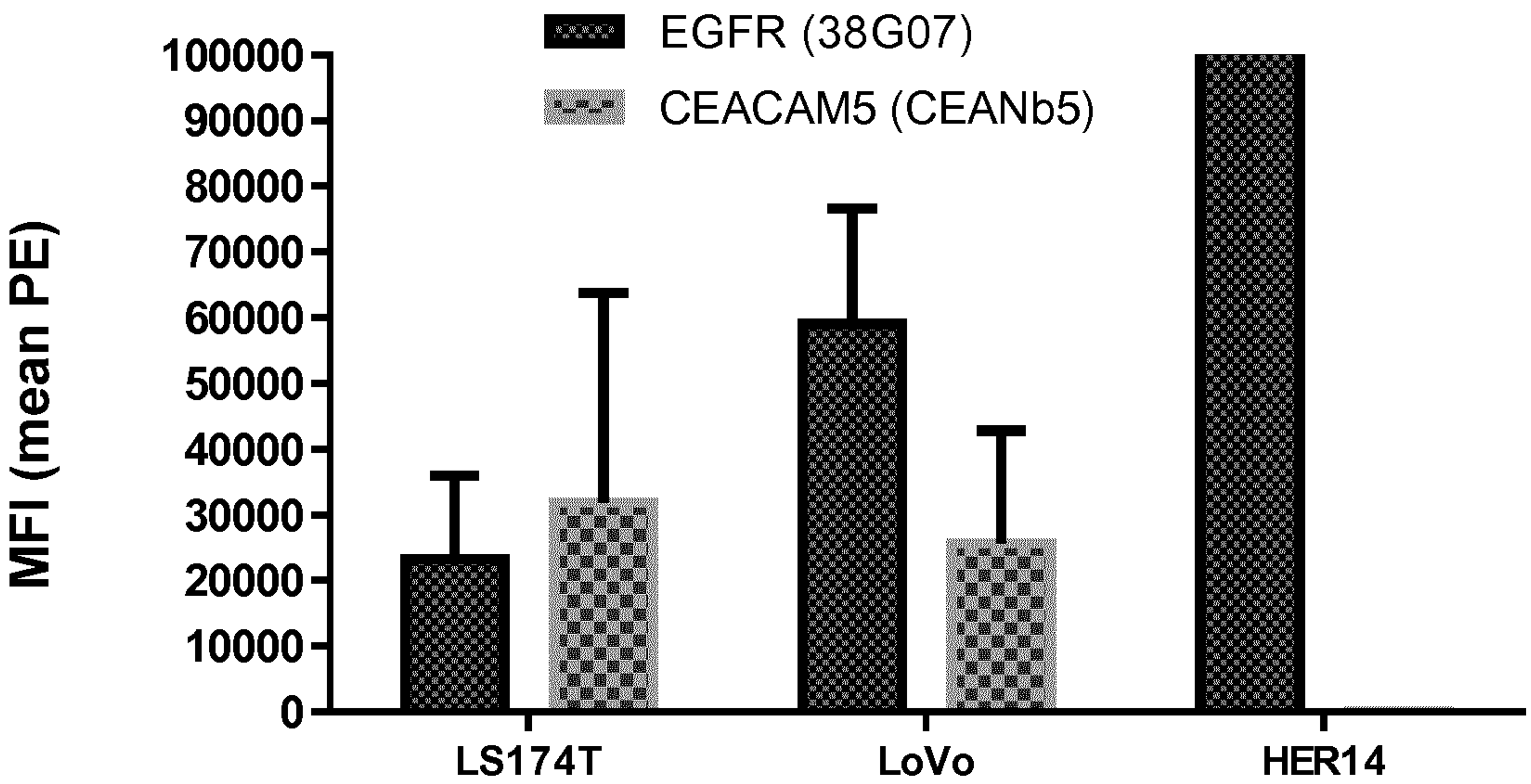

EGFR (38G07)
CEACAM5 (CEANb5)
MFI (mean PE)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
LS174T
LoVo
HER14

Figure 34:

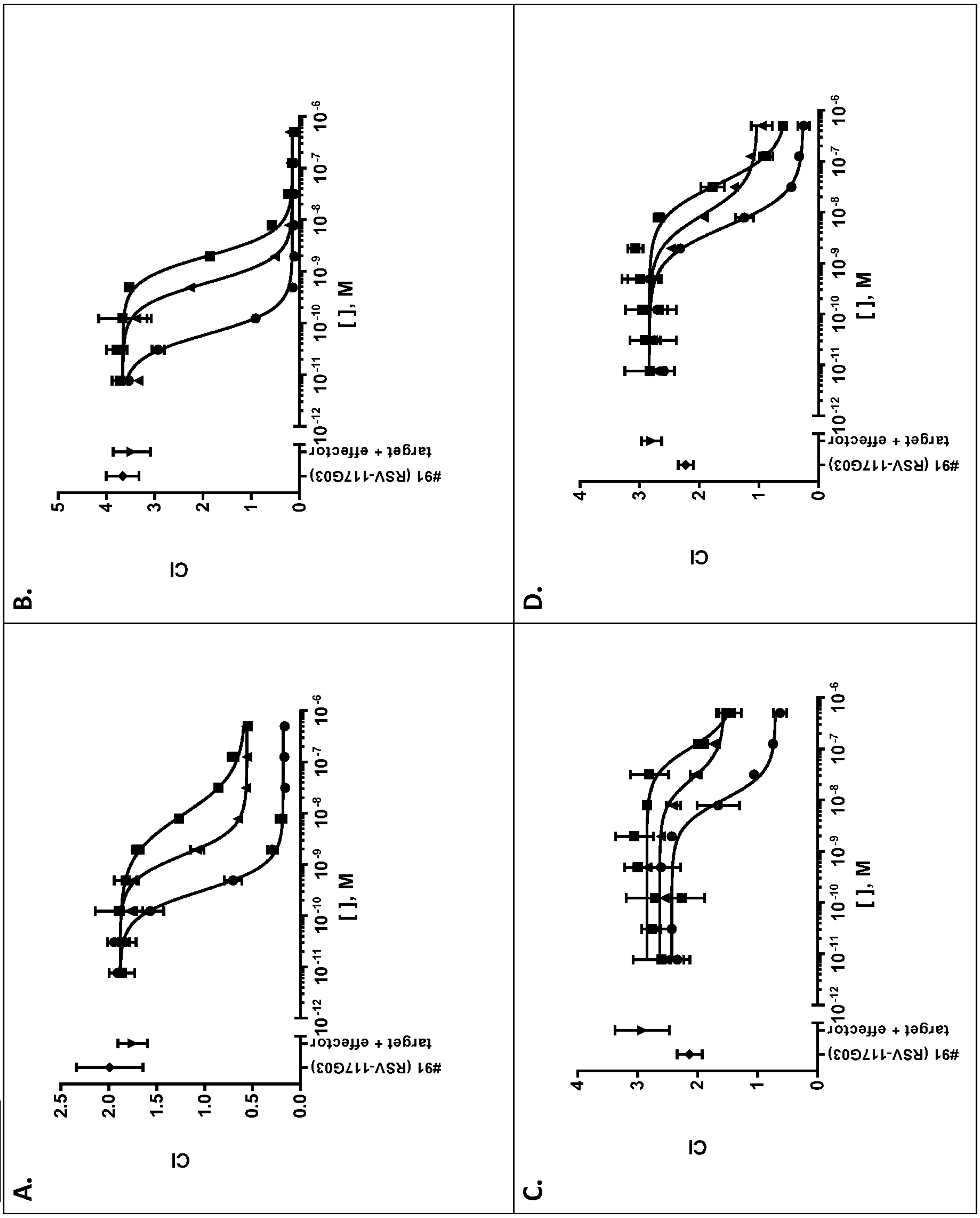


Figure 34 cont':

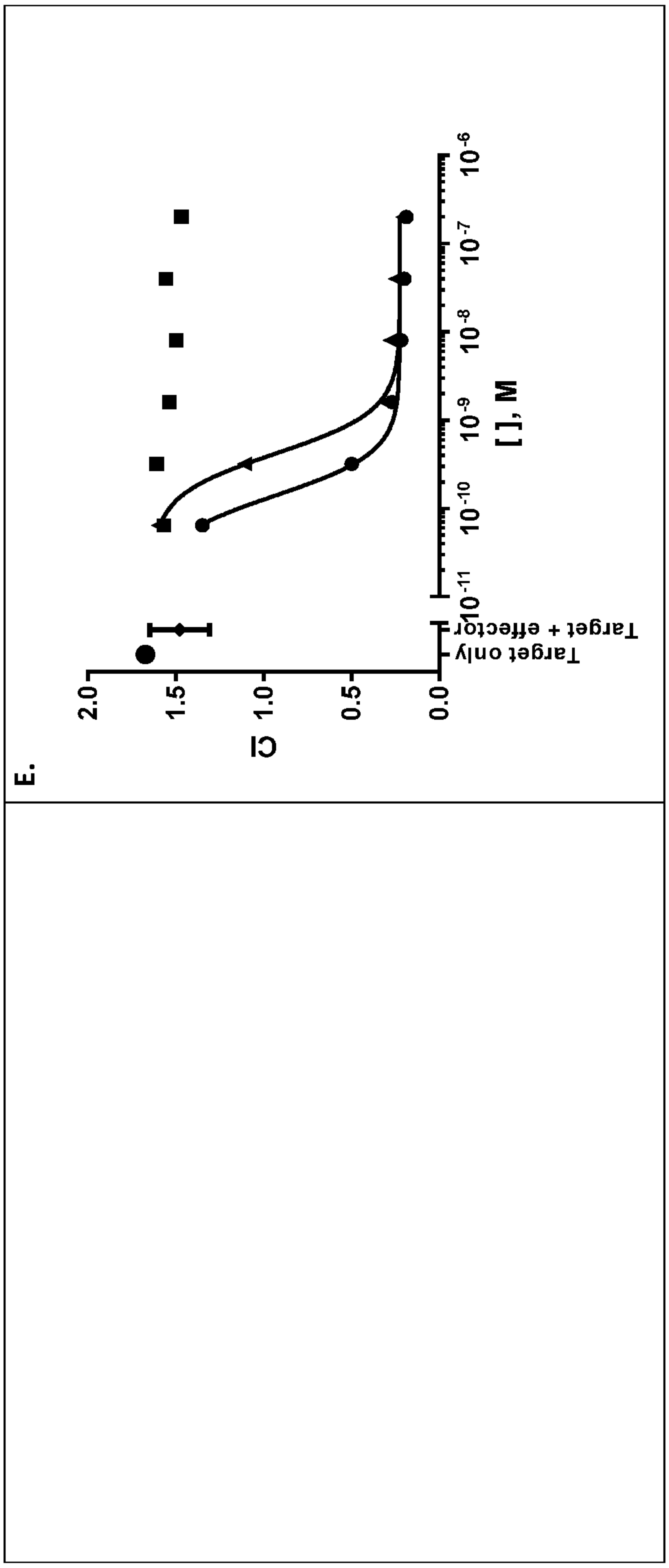
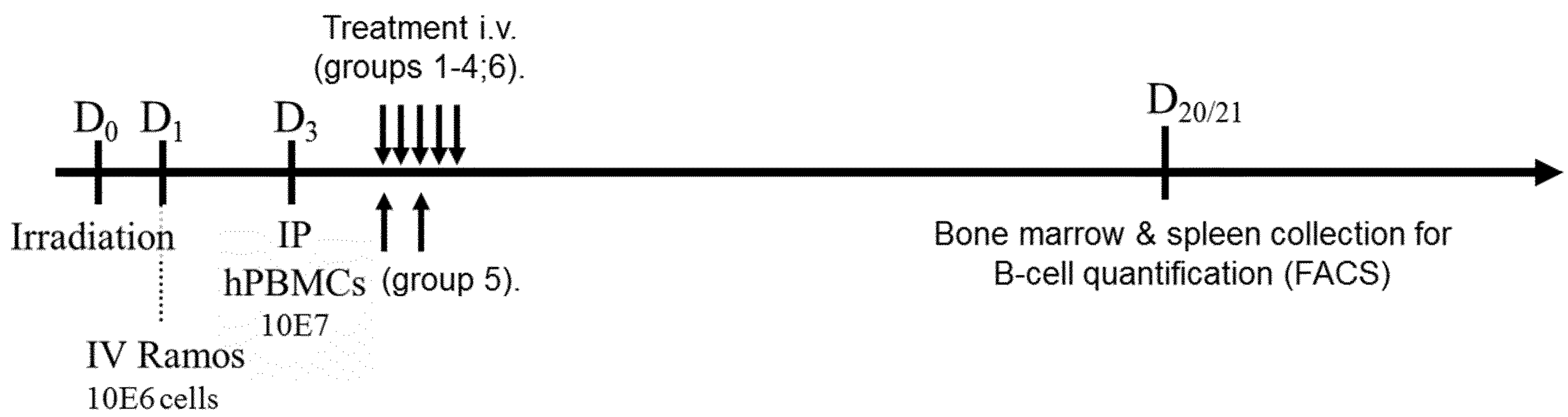


Figure 35:



(1) Irr-TCR (T017000088; Q1Dx5; 23,8 mg/kg; n=12)
(2) Irr-TCR-Alb (T017000106; Q1Dx5; 35,4 mg/kg; n=12)
(3) CD20-TCR (T017000083; Q1Dx5; 23,2 mg/kg; n=12)
(4) CD20-TCR-Alb (T017000104; Q1Dx5; 34,9 mg/kg; n=12)
(5) CD20-TCR-Alb (T017000104; D3+D5; 34,9 mg/kg; n=12)
(6) CD20-TCR (T017000105; Q1Dx5; 23,2 mg/kg; n=11)

Groups 3,4,5: building block 55A02
Group 6: building block 56G05

Figure 36A:

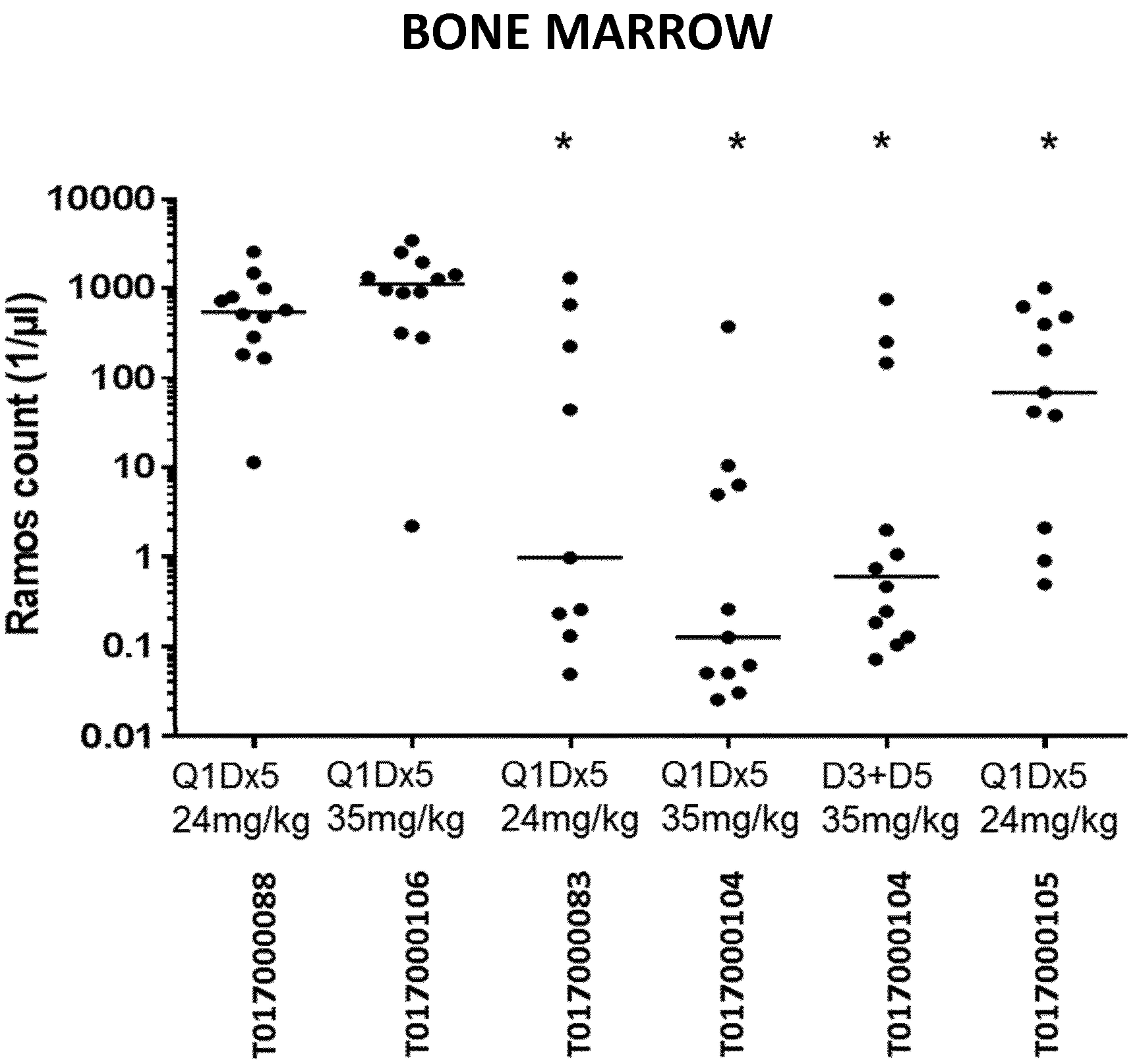

BONE MARROW
Ramos count (1/µl)
10000
1000
100
10
1
0.1
0.01
*
*
*
*
Q1Dx5 24mg/kg
Q1Dx5 35mg/kg
Q1Dx5 24mg/kg
Q1Dx5 35mg/kg
D3+D5 35mg/kg
Q1Dx5 24mg/kg
T017000088
T017000106
T017000083
T017000104
T017000104
T017000105

Figure 36B:

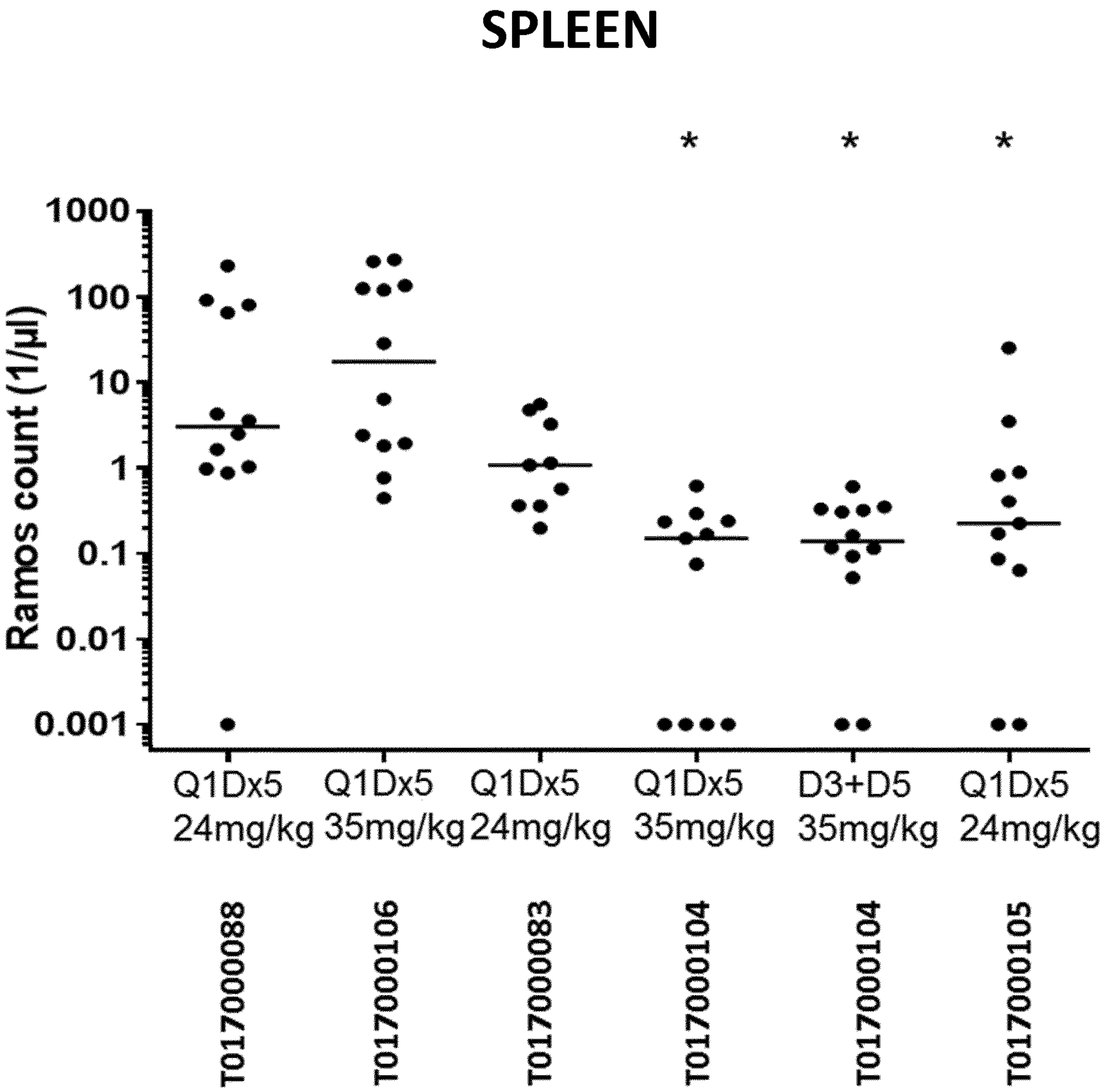

SPLEEN
*
*
*
1000
100
10
1
0.1
0.01
0.001
Ramos count (1/µl)
Q1Dx5 24mg/kg
Q1Dx5 35mg/kg
Q1Dx5 24mg/kg
Q1Dx5 35mg/kg
D3+D5 35mg/kg
Q1Dx5 24mg/kg
T0170000088
T0170000106
T0170000083
T0170000104
T0170000104
T0170000105

Figure 37A:

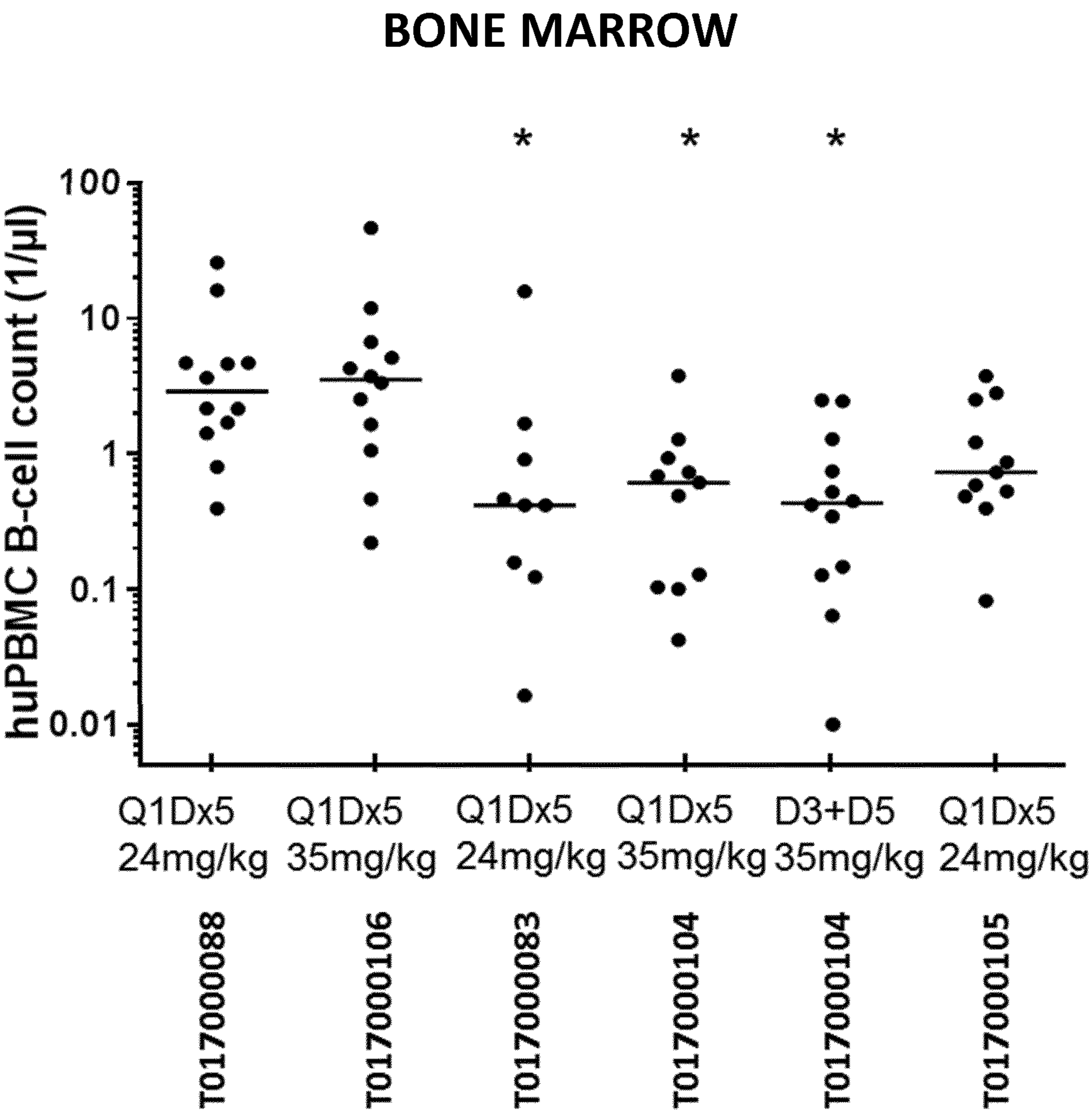

BONE MARROW
*
*
*
100
10
1
0.1
0.01
huPBMC B-cell count (1/µl)
Q1Dx5
24mg/kg
Q1Dx5
35mg/kg
Q1Dx5
24mg/kg
Q1Dx5
35mg/kg
D3+D5
35mg/kg
Q1Dx5
24mg/kg
T017000088
T017000106
T017000083
T017000104
T017000104
T017000105

Figure 37B:

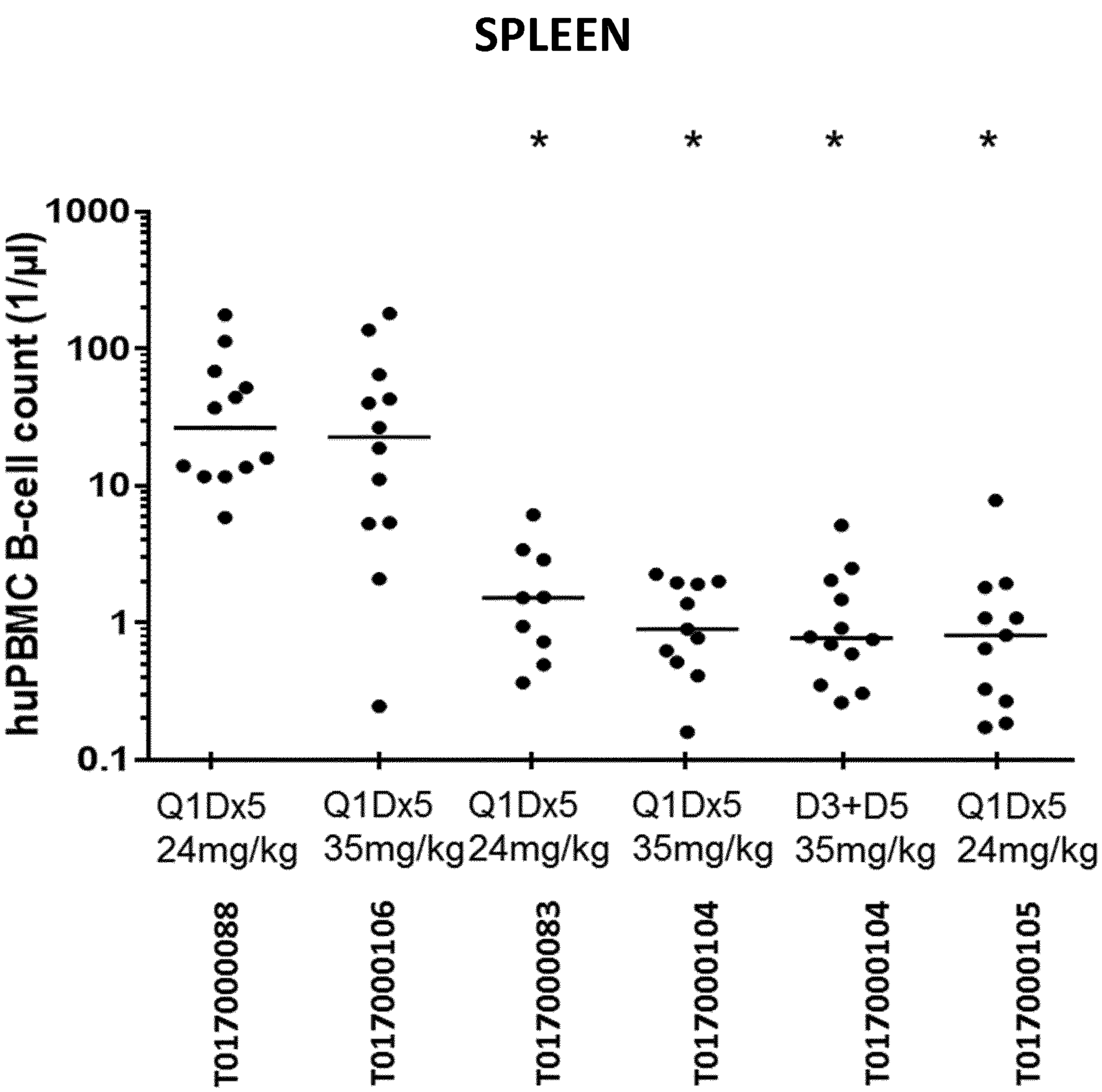

SPLEEN
*
*
*
*
hu PBMC B-cell count (1/µl)
1000
100
10
1
0.1
Q1Dx5
24mg/kg
Q1Dx5
35mg/kg
Q1Dx5
24mg/kg
Q1Dx5
35mg/kg
D3+D5
35mg/kg
Q1Dx5
24mg/kg
T017000088
T017000106
T017000083
T017000104
T017000104
T017000105

__Figure 38:__

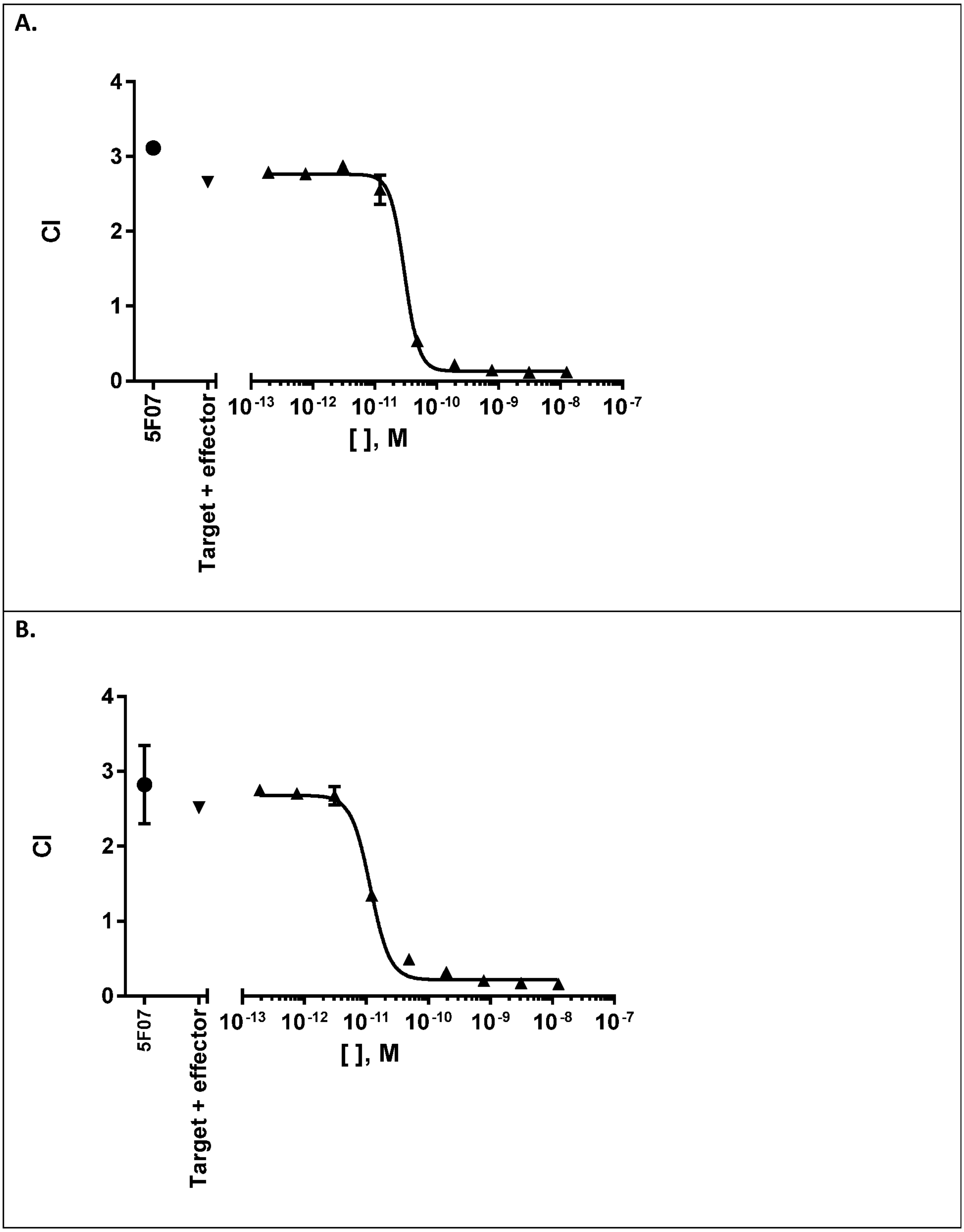

A.
CI
0
1
2
3
4
5F07
Target + effector
10^{-13}
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
[ ], M
B.
CI
0
1
2
3
4
5F07
Target + effector
10^{-13}
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
[ ], M

Figure 38 cont':

C.

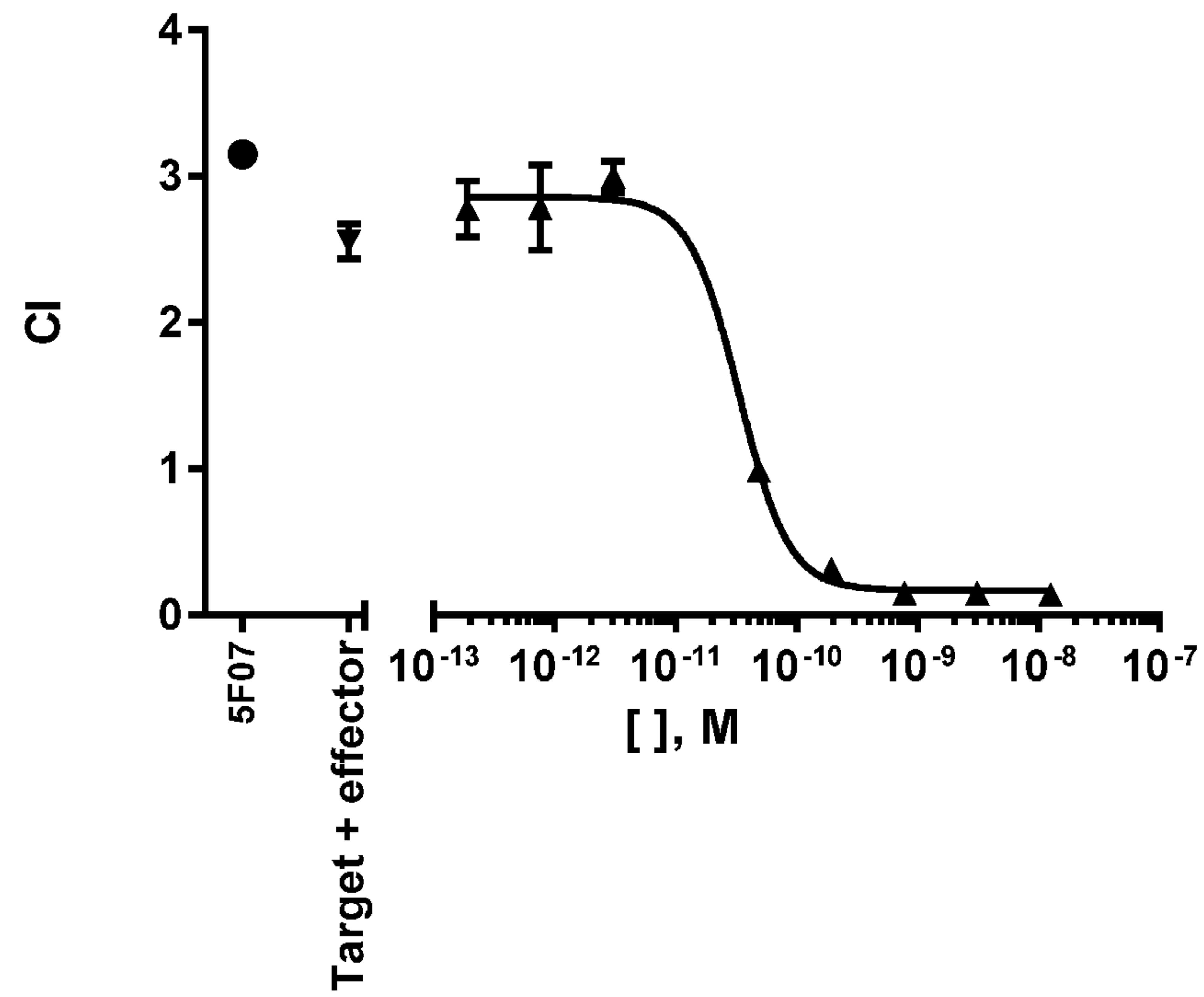


Figure 39:

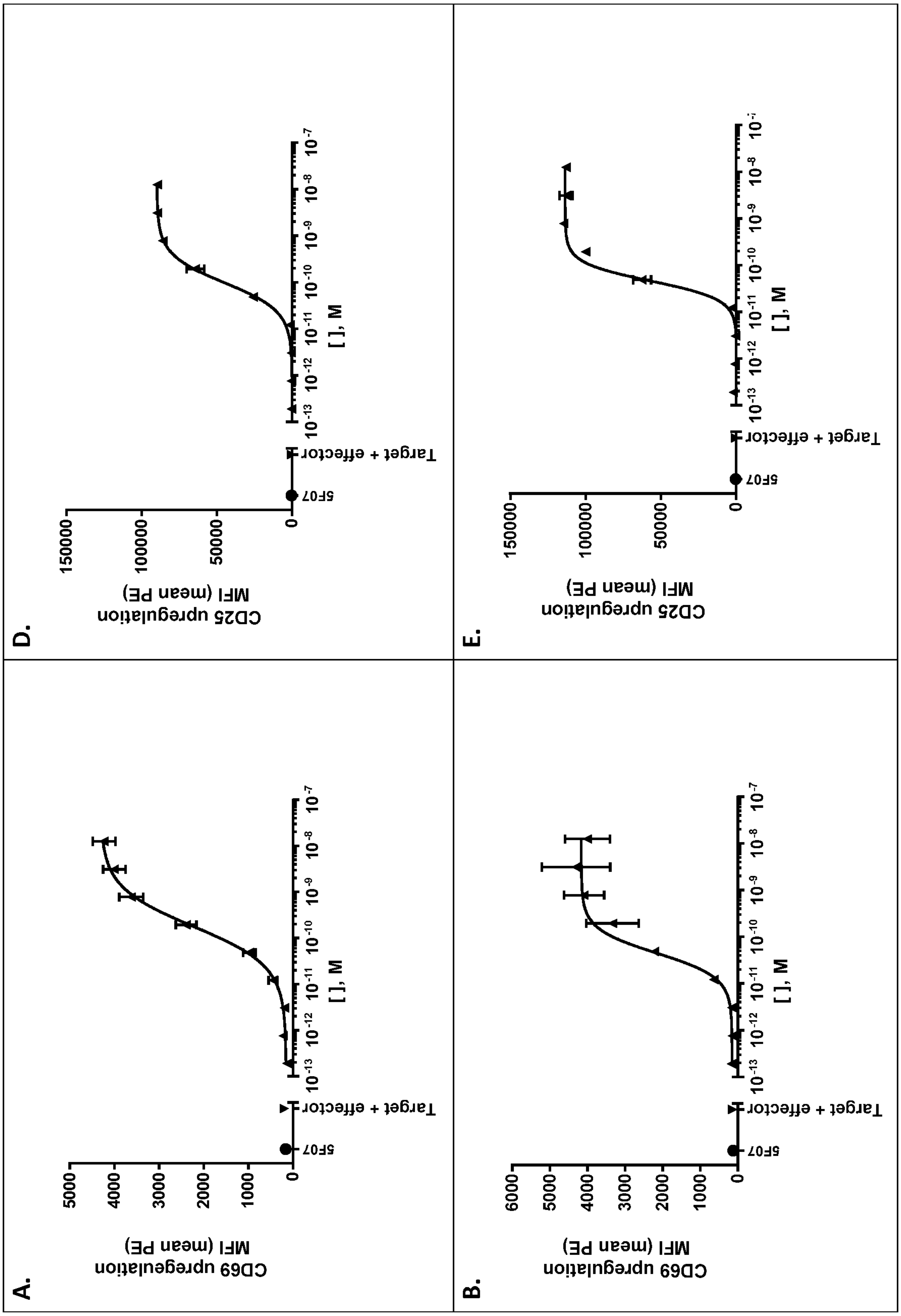

A.
CD69 upregulation
MFI (mean PE)
5F07
Target + effector
[ ], M
B.
CD69 upregulation
MFI (mean PE)
5F07
Target + effector
[ ], M
D.
CD25 upregulation
MFI (mean PE)
5F07
Target + effector
[ ], M
E.
CD25 upregulation
MFI (mean PE)
5F07
Target + effector
[ ], M

Figure 39 cont':

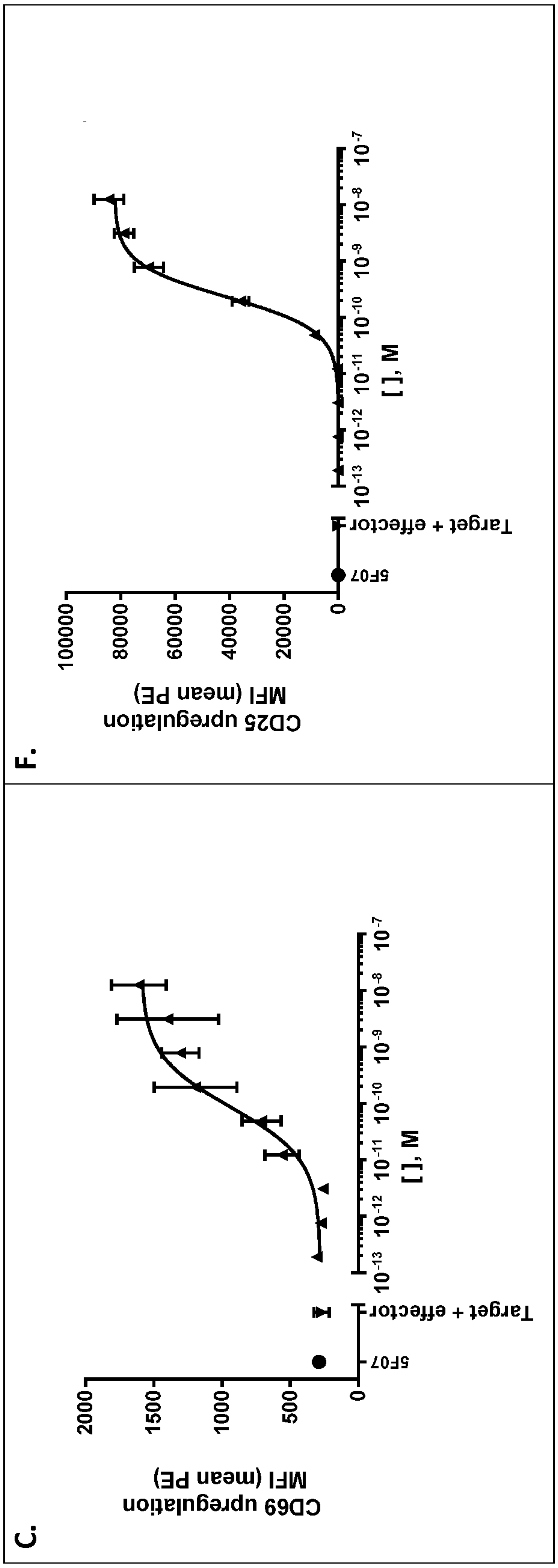


Figure 40:

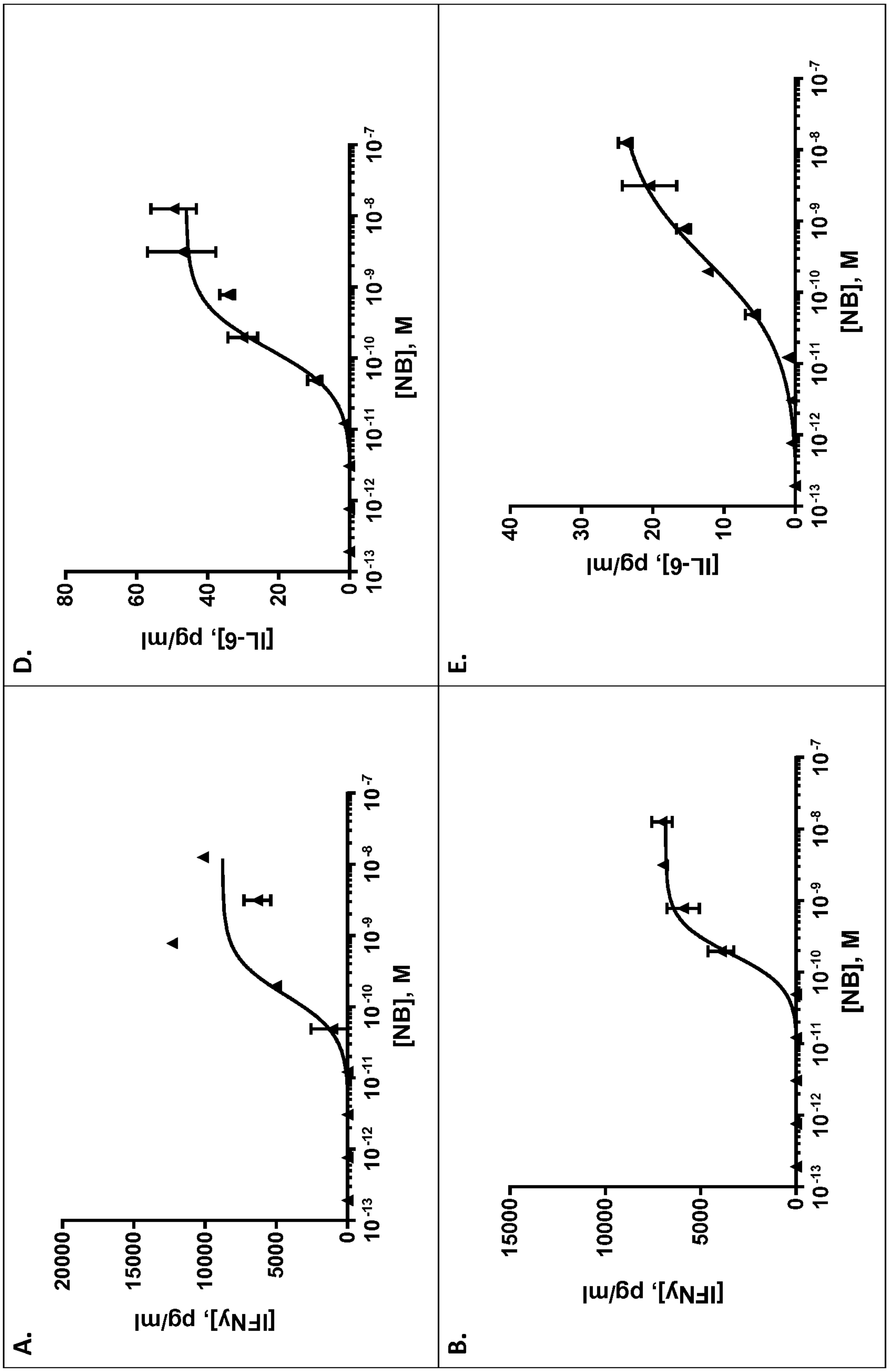
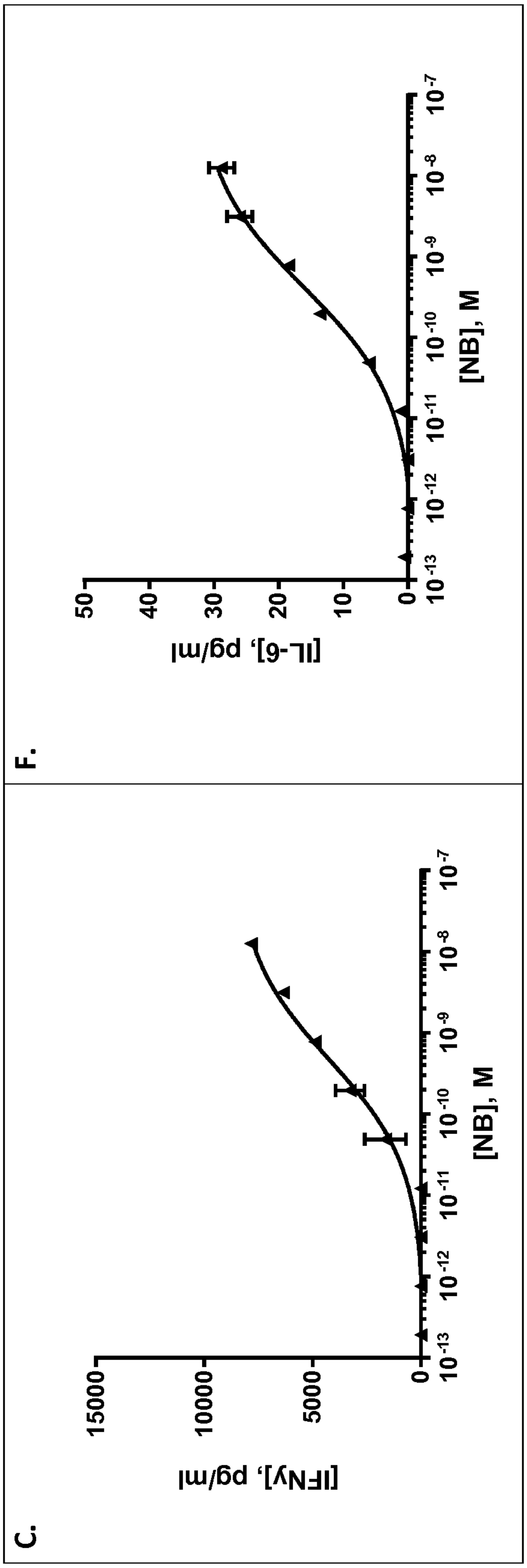

A.
[IFNγ], pg/ml
20000
15000
10000
5000
0
[NB], M
B.
[IFNγ], pg/ml
15000
10000
5000
0
[NB], M
D.
[IL-6], pg/ml
80
60
40
20
0
[NB], M
E.
[IL-6], pg/ml
40
30
20
10
0
[NB], M
10^{-13} 10^{-12} 10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7}

Figure 40 cont':



CA 02985698 2017-11-10

Figure 41:

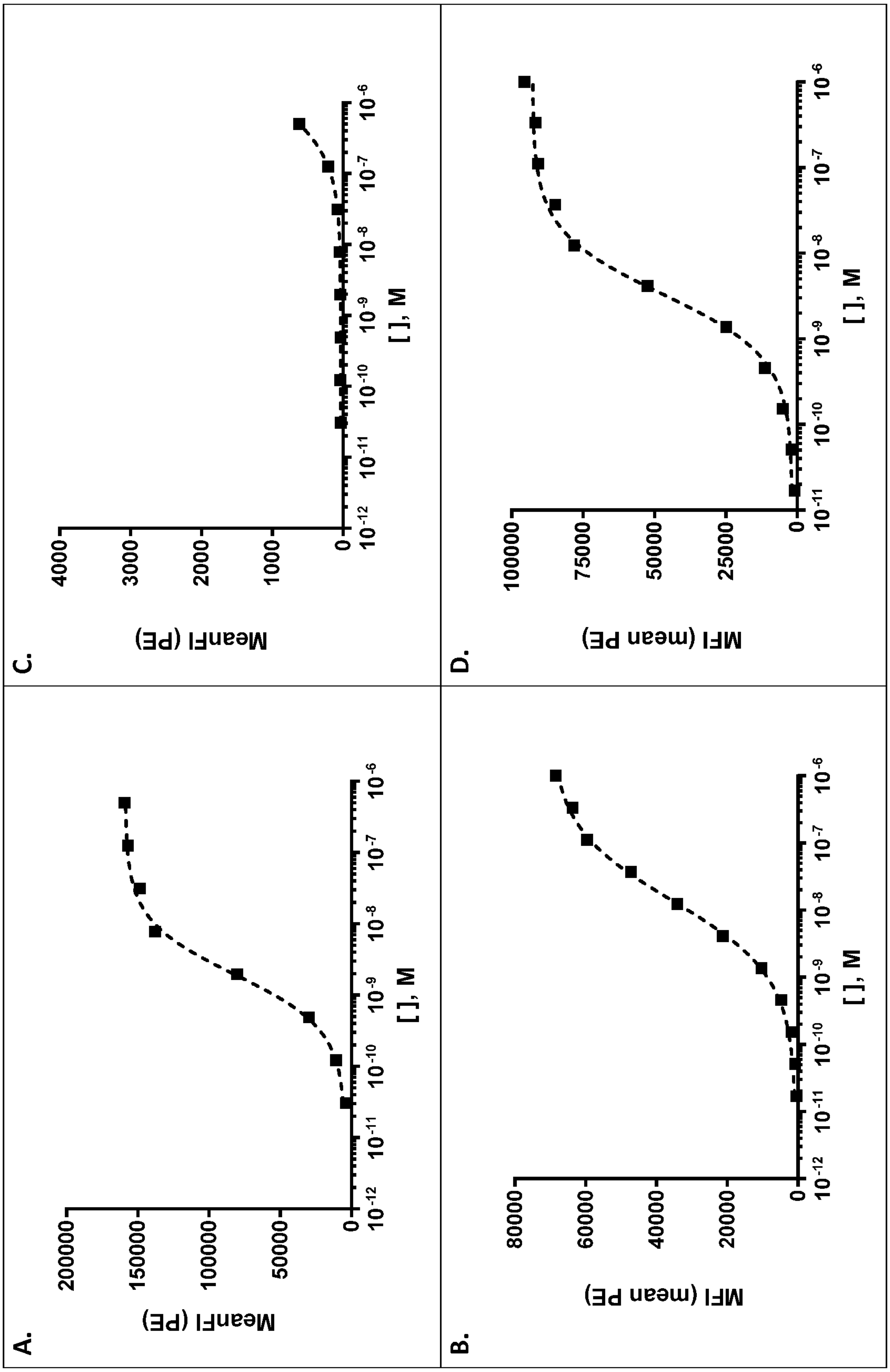
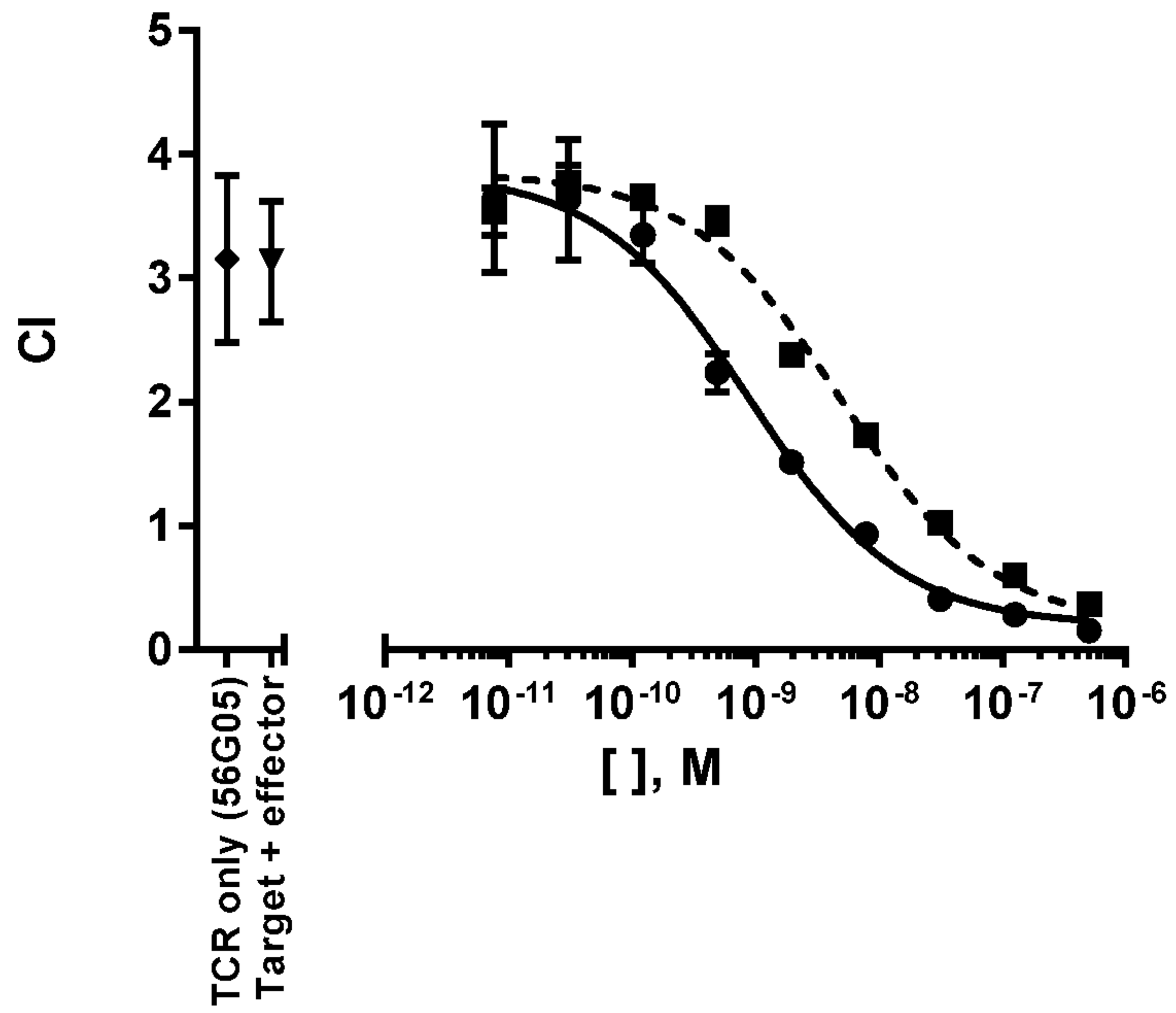


Figure 42:


CI
5
4
3
2
1
0
TCR only (56G05)
Target + effector
10^-12 10^-11 10^-10 10^-9 10^-8 10^-7 10^-6
[ ], M