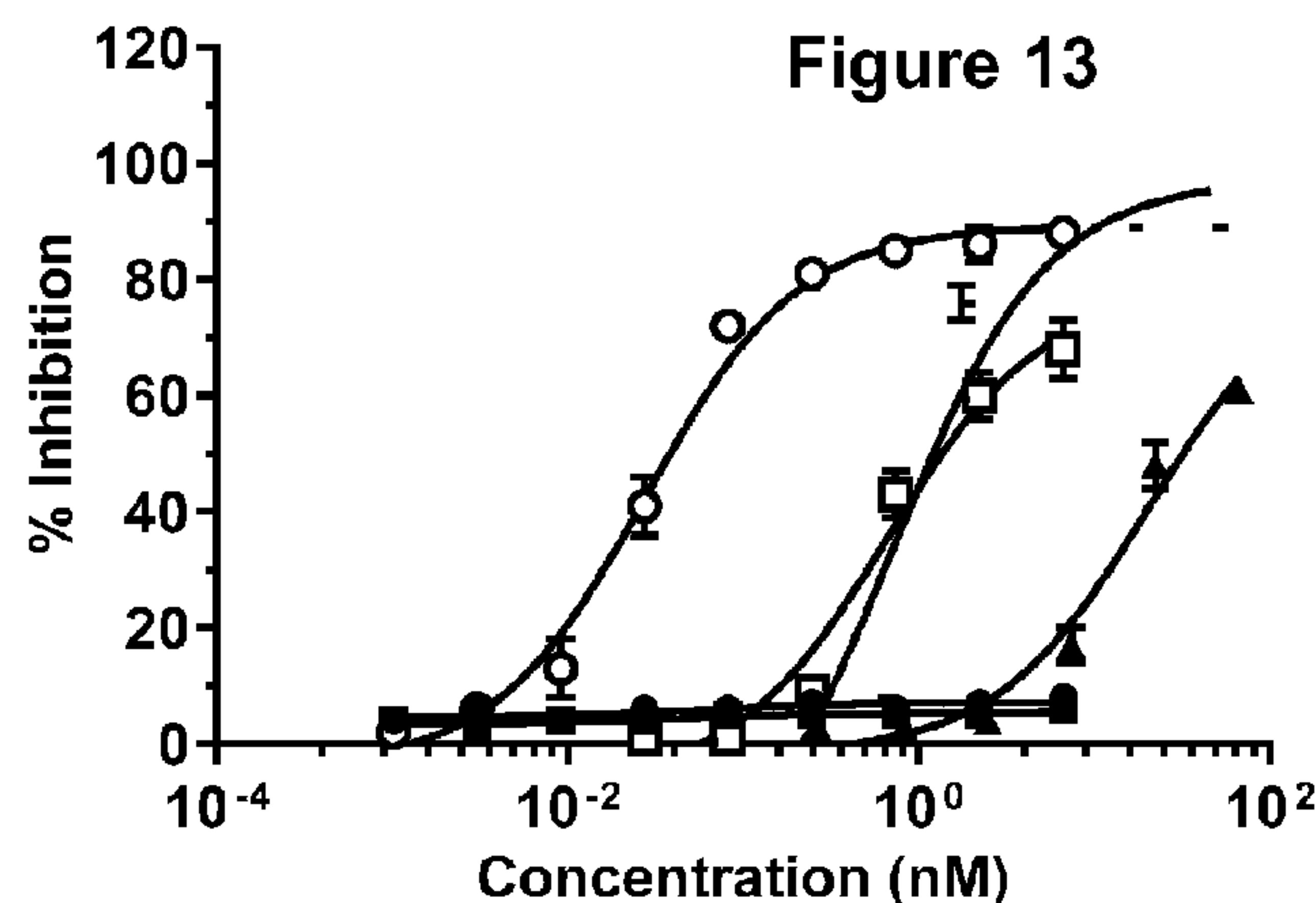




(86) Date de dépôt PCT/PCT Filing Date: 2015/05/29
(87) Date publication PCT/PCT Publication Date: 2016/08/04
(85) Entrée phase nationale/National Entry: 2017/07/24
(86) N° demande PCT/PCT Application No.: US 2015/033099
(87) N° publication PCT/PCT Publication No.: 2016/122702
(30) Priorités/Priorities: 2015/01/26 (US62/107,871);
2015/04/17 (US62/149,139)

(51) Cl.Int./Int.Cl. *C07K 16/28* (2006.01)
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(54) Titre : MOLECULES MULTIVALENTES COMPRENANT DES DOMAINES DE LIAISON AU DR5
(54) Title: MULTIVALENT MOLECULES COMPRISING DR5-BINDING DOMAINS



- DR5 mAb 1
- DR5 mAb 1 + αmFc
- DR5 mAb 2
- DR5 mAb 2 + αmFc
- ▲ DR5 mAb 1 + DR5 mAb 2
- R&D TRAIL/His

(57) **Abrégé/Abstract:**

The present invention is directed to multivalent DR5 -Binding Molecules that comprise Binding Domain(s) of anti-DR5 antibodies, and particularly Binding Domain(s) of anti-human DR5 antibodies. The DR5 -Binding Molecules of the present invention include bivalent and tetravalent molecules having two, three or four DR5-Binding Domains each capable of binding human DR5. In particular, the present invention is directed to multivalent DR5 -Binding Molecules that comprise diabodies, and more particularly, diabodies that comprise a covalently bonded complex of two or more polypeptide chains. The invention particularly pertains to such multivalent DR5 -Binding Molecules that comprise fragments of the anti-DR5 antibodies DR5 mAb 1 and/or DR5 mAb 2, and/or humanized and chimeric versions of such antibodies.



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

(19) World Intellectual Property
Organization
International Bureau(10) International Publication Number
WO 2016/122702 A1(43) International Publication Date
4 August 2016 (04.08.2016)(51) International Patent Classification:
C07K 16/28 (2006.01)

(21) International Application Number:

PCT/US2015/033099

(22) International Filing Date:

29 May 2015 (29.05.2015)

(25) Filing Language:

English

(26) Publication Language:

English

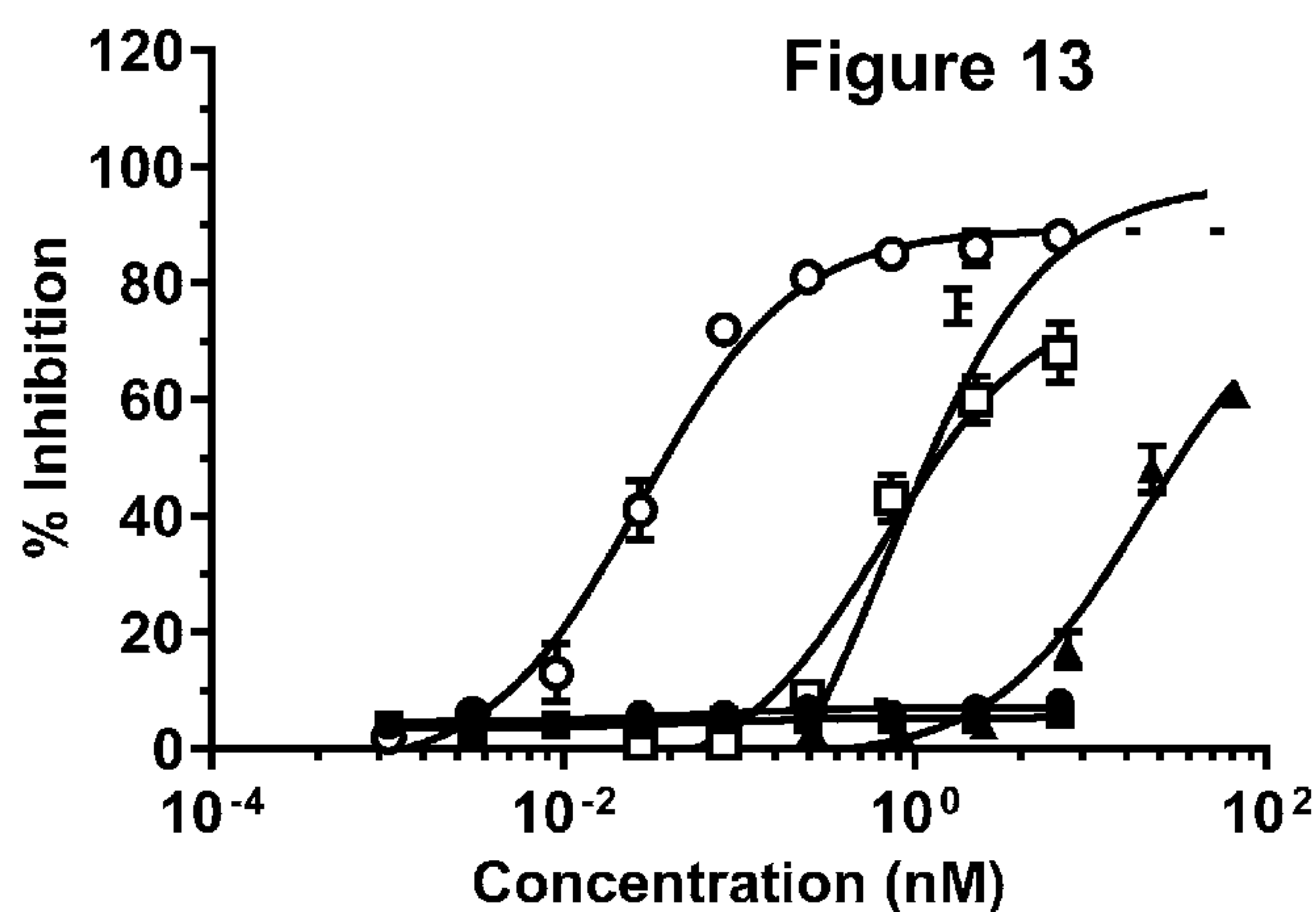
(30) Priority Data:

62/107,871	26 January 2015 (26.01.2015)	US
62/149,139	17 April 2015 (17.04.2015)	US

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[Continued on next page]

(54) Title: MULTIVALENT MOLECULES COMPRISING DR5-BINDING DOMAINS



- DR5 mAb 1
- DR5 mAb 1 + αmFc
- DR5 mAb 2
- DR5 mAb 2 + αmFc
- ▲ DR5 mAb 1 + DR5 mAb 2
- R&D TRAIL/His

(57) **Abstract:** The present invention is directed to multivalent DR5 -Binding Molecules that comprise Binding Domain(s) of anti-DR5 antibodies, and particularly Binding Domain(s) of anti-human DR5 antibodies. The DR5 -Binding Molecules of the present invention include bivalent and tetravalent molecules having two, three or four DR5-Binding Domains each capable of binding human DR5. In particular, the present invention is directed to multivalent DR5 -Binding Molecules that comprise diabodies, and more particularly, diabodies that comprise a covalently bonded complex of two or more polypeptide chains. The invention particularly pertains to such multivalent DR5 -Binding Molecules that comprise fragments of the anti-DR5 antibodies DR5 mAb 1 and/or DR5 mAb 2, and/or humanized and chimeric versions of such antibodies.

WO 2016/122702 A1

SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, — *with sequence listing part of description (Rule 5.2(a))*
GW, KM, ML, MR, NE, SN, TD, TG).

Published:

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

Title of the Invention:**Multivalent Molecules Comprising DR5-Binding Domains****Cross-Reference to Related Applications:**

[0001] This application claims priority to United States Patent Applications No. 62/149,139 (filed April 17, 2015; pending) and 62/107,871 (filed January 26, 2015; pending), each of which applications is herein incorporated by reference in its entirety.

Reference to Sequence Listing:

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 et seq., which are disclosed in computer-readable media (file name: 1301_0118PCT_Sequence_Listing_ST25.txt, created on 18 May 2015, and having a size of 215,084 bytes), which files is herein incorporated by reference in its entirety.

Background of the Invention:**Field of the Invention:**

[0003] The present invention is directed to multivalent DR5-Binding Molecules that comprise Binding Domain(s) of anti-DR5 antibodies, and particularly Binding Domain(s) of anti-human DR5 antibodies. The DR5-Binding Molecules of the present invention include bivalent and tetravalent molecules having two, three or four DR5-Binding Domains each capable of binding human DR5. In particular, the present invention is directed to multivalent DR5-Binding Molecules that comprise diabodies, and more particularly, diabodies that comprise a covalently bonded complex of two or more polypeptide chains. The invention particularly pertains to such multivalent DR5-Binding Molecules that comprise fragments of the anti-DR5 antibodies DR5 mAb 1 and/or DR5 mAb 2, and/or humanized and chimeric versions of such antibodies.

Description of Related Art:**I. Death Receptor 5 (“DR5”)**

[0004] Healthy animals maintain a continuous immune surveillance against tumor cells. Through the interplay of various growth factors, cytokines and hormones, such

animals can mediate the programmed death (**apoptosis**) of encountered damaged cells. Damaged cells that acquire resistance to this cell death process can and which acquire the ability to replicate in an uncontrolled fashion can become tumor cells and lead to cancer (Abdulghani, J. *et al.* (2010) “*TRAIL Receptor Signaling And Therapeutics*,” Expert Opin. Ther. Targets 14(10):1091-1108; Andera, L. (2009) “*Signaling Activated By The Death Receptors Of The TNFR Family*,” Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 153(3):173-180; Carlo-Stella, C. *et al.* (2007) “*Targeting TRAIL Agonistic Receptors for Cancer Therapy*,” Clin. Cancer 13(8):2313-2317; Chaudhari, B.R. *et al.* (2006) “*Following the TRAIL to Apoptosis*,” Immunologic Res. 35(3):249-262).

[0005] Methods that are capable of selectively targeting the cell death pathways so as to spare normal cells while increasing the effectiveness of such pathways in killing cancer cells are of particular interest in cancer therapy. Members of the Tumor Necrosis Factor (**TNF**) superfamily including Fas ligand, TNF and the TNF-related apoptosis-inducing ligand (**TRAIL**) have been identified as targets for cancer biotherapy (Walczak, H. (2013) “*Death Receptor – Ligand Systems in Cancer, Cell Death, and Inflammation*,” Cold Spring Harb. Perspect. Biol. 2013;5:a008698; pp. 1-19; Falschlehner, C. *et al.* (2007) “*TRAIL Signalling: Decisions Between Life And Death*,” Intl. J. Biochem. Cell Biol. 39:1462-1475; Abdulghani, J. *et al.* (2010) “*TRAIL Receptor Signaling And Therapeutics*,” Expert Opin. Ther. Targets 14(10):1091-1108). TRAIL is a cytokine that is expressed by effector lymphocytes. TRAIL is expressed on the surface of immune effector cells such as natural killer cells, macrophages, dendritic cells and cytotoxic T cells in response to cytokines, particularly interferon-gamma that possesses a response element in the TRAIL gene promoter (Allen, J.E. *et al.* (2012) “*Regulation Of The Human TRAIL Gene*,” Cancer Biol. Ther. 13(12):1143-1151). Its expression level is extremely low in freshly-isolated lymphocytes, and only a small fraction of natural killer (**NK**) cells express detectable TRAIL. TRAIL is believed to play a role in regulating the innate immune response involving the interferons, boosting host responses to tumor cells and changing the tumor microenvironment to enhance antigen presentation and promote tissue infiltration by NK cells and other immune system cells.

[0006] One important distinction between TRAIL-induced apoptosis and apoptosis induced by conventional chemotherapy and radiotherapy is that the latter is largely dependent on cellular damage recognition by, for example, the p53 tumor suppressor protein (Dimberg, L.Y. *et al.* (2013) “*On The TRAIL To Successful Cancer Therapy? Predicting And Counteracting Resistance Against TRAIL-Based Therapeutics,*” *Oncogene* 32:1341-1350). The dependence on p53 to elicit an apoptotic response poses a problem in cancer therapy, as loss of p53 occurs in more than half of all cancers cells because of inactivating mutations (Hollstein, M. *et al.* (1994) “*Database Of p53 Gene Somatic Mutations In Human Tumors And Cell Lines,*” *Nucleic Acids Res.* 22:3551-3555).

[0007] TRAIL is a type II protein with 281 amino acid residues and has homology with TNF- α and FasL (**CD95L**) (Chaudhari, B.R. *et al.* (2006) “*Following the TRAIL to Apoptosis,*” *Immunologic Res.* 35(3):249-262). TRAIL consists of an extracellular TNF-like Domain, an extracellular stalk, a transmembrane helix, and a Cytoplasmic Domain. TRAIL binds to two different types of receptors: death receptors (**DR**) that trigger TRAIL-induced apoptosis and decoy receptors inhibit this pathway. To date, two human death receptors specific for TRAIL have been recognized: **TRAIL-R1** (also known as **DR4**) and **TRAIL-R2** (also known as **DR5**). Additionally, three putative decoy receptors have been identified: **TRAIL-R3 (DcR1)**, **TRAIL-R4 (DcR2)** and osteoprotegerin (Chaudhari, B.R. *et al.* (2006) “*Following the TRAIL to Apoptosis,*” *Immunologic Res.* 35(3):249-262; Carlo-Stella, C. *et al.* (2007) “*Targeting TRAIL Agonistic Receptors for Cancer Therapy,*” *Clin, Cancer* 13(8):2313-2317; Allen, J.E. *et al.* (2012) “*Regulation Of The Human TRAIL Gene,*” *Cancer Biol. Ther.* 13(12):1143-1151). TRAIL-R1 (DR4) is expressed at very low levels in most human tissues including the spleen, thymus, liver, peripheral blood leukocytes, activated T cells, small intestine and some tumor cell lines. In contrast, TRAIL-R2 (DR5) is ubiquitously distributed both in normal and tumor cell lines but is more abundant in spleen, peripheral blood leukocytes, activated lymphocytes and hepatocytes (Abdulghani, J. *et al.* (2010) “*TRAIL Receptor Signaling And Therapeutics,*” *Expert Opin. Ther. Targets* 14(10):1091-1108).

[0008] DR4 and DR5 are single-pass type-I membrane proteins and are encoded by two genes located on chromosome 8p. DR4 and DR5 each contain extracellular regions that comprise Cysteine-Rich Domains (**CRDs**), a Transmembrane Domain, and a Death Domain located within the cytoplasmic portion of the receptors. Two splice variants of DR5 have been identified, long DR5 (**DR5(L)**) and short DR5 (**DR5(S)**). These variants differ in a stretch of 29 amino acids located between the receptors' CRDs and their Transmembrane Domain. DR4 and DR5 are able to transduce an apoptosis signal following TRAIL binding (van Roosmalen, I.A.M. *et al.* (2014) "*Two Death-Inducing Human TRAIL Receptors To Target In Cancer: Similar Or Distinct Regulation And Function?*," Biochem. Pharmacol. 91:447-456).

[0009] When TRAIL binds to DR4 or DR5, the receptors homotrimerize, enabling the receptor's Death Domain to recruit the adaptor protein Fas-Associated Death Domain and the inactive, uncleaved form of caspase 8 (**pro-caspase 8**) or the uncleaved form of caspase 10 (**pro-caspase 10**). The receptors, Fas-associated protein with Death Domain, and pro-caspase 8 or pro-caspase 10 together form the Death-Inducing Signaling Complex, (**DISC**). At the DISC, pro-caspase 8 is activated, in a process that is dependent on both dimerization and cleavage. Activated caspase 8 then cleaves downstream substrates ultimately resulting in the cleavage and activation of effector caspase 3. Activation of caspase 3 initiates a cascade of molecular activation events that ultimately leads to the production of death substrates (Schneider-Brachert, W. *et al.* (2013) "*Membrane Trafficking of Death Receptors: Implications on Signalling*," Int. J. Mol. Sci. 14:14475-14503; Falschlehner, C. *et al.* (2009) "*TRAIL and Other TRAIL Receptor Agonists as Novel Cancer Therapeutics*," In: THERAPEUTIC TARGETS OF THE TNF SUPERFAMILY (Grewal, I.S., Ed.) Landes Bioscience and Springer Science+Business Media, NY; pp. 195-206; Falschlehner, C. *et al.* (2007) "*TRAIL Signalling: Decisions Between Life And Death*," Intl. J. Biochem. Cell Biol. 39:1462-1475; Guicciardi, M.E. *et al.* (2009) "*Life And Death By Death Receptors*," FASEB J. 23:1625-1637; Kischkel, F.C. *et al.* (2000) "*Apo2L/TRAIL-Dependent Recruitment of Endogenous FADD and Caspase-8 to Death Receptors 4 and 5*," Immunity 12:611-620; Dimberg, L.Y. *et al.* (2013) "*On The TRAIL To Successful Cancer Therapy? Predicting And Counteracting Resistance Against TRAIL-Based Therapeutics*," Oncogene 32:1341-1350; Buchsbaum, D.J. *et al.* (2007) "*TRAIL-Receptor-Antibodies*

as a Potential Cancer Treatment,” *Future Oncol.* 3(4):405-409; Buchsbaum, D.J. *et al.* (2006) “*TRAIL Receptor-Targeted Therapy*,” *Future Oncol.* 2(4):493-508; Andera, L. (2009) “*Signaling Activated By The Death Receptors Of The TNFR Family*,” *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub.* 153(3):173-180; Chan, F.K.-M. (2007) “*Three is Better Than One: Pre-Ligand Receptor Assembly in the Regulation of TNF Receptor Signaling*,” *Cytokine* 37(2):101-107). The three decoy receptors either act as decoys or transduce antiapoptotic signals (Carlo-Stella, C. *et al.* (2007) “*Targeting TRAIL Agonistic Receptors for Cancer Therapy*,” *Clin. Cancer* 13(8):2313-2317; Mahmood, Z. *et al.* (2010) “*Death Receptors: Targets For Cancer Therapy*,” *Exper. Cell. Res.* 316:887-899; Oikonomou, E. *et al.* (2013) “*The TRAIL Of Oncogenes To Apoptosis*,” *Intl. J Union Biochem. Molec. Biol.* 39(4):343-354).

[0010] In addition to such an “**extrinsic**” pathway, TRAIL may mediate cell death via an “**intrinsic**” pathway (Carlo-Stella, C. *et al.* (2007) “*Targeting TRAIL Agonistic Receptors for Cancer Therapy*,” *Clin. Cancer* 13(8):2313-2317; Buchsbaum, D.J. *et al.* (2006) “*TRAIL Receptor-Targeted Therapy*,” *Future Oncol.* 2(4):493-508; Buchsbaum, D.J. *et al.* (2007) “*TRAIL-Receptor-Antibodies as a Potential Cancer Treatment*,” *Future Oncol.* 3(4):405-409). The intrinsic pathway is mediated by the cleavage activation of the pro-apoptotic protein **Bid**, which then binds with other pro-apoptotic proteins to form a complex that mediates the release of cytochrome c from mitochondria. Such release triggers a cascade of caspase release and activation leading to cell death (Kandasamy, K. *et al.* (2003) “*Involvement Of Proapoptotic Molecules Bax And Bak In Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL)-Induced Mitochondrial Disruption And Apoptosis: Differential Regulation Of Cytochrome C And Smac/DIABLO Release*,” *Cancer Res.* 63:1712-1721; Rudner, J. *et al.* (2005) “*Type I And Type II Reactions In TRAIL-Induced Apoptosis – Results From Dose-Response Studies*,” *Oncogene* 24:130-140).

[0011] The molecular pathways are, however, complex. Depending on the cell type, the relative strength and duration of the ligand signal, and either the presence, absence or activation state of the intracellular proteins that signal downstream of TRAIL receptors, treatment with TRAIL may stimulate either apoptosis or in rare instances cell proliferation (Abdulghani, J. *et al.* (2010) “*TRAIL Receptor Signaling*

And Therapeutics,” Expert Opin. Ther. Targets 14(10):1091-1108; Andera, L. (2009) “*Signaling Activated By The Death Receptors Of The TNFR Family*,” Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 153(3):173-180). Moreover, certain cancers have a DR preference (*i.e.*, DR4 or DR5) for inducing apoptosis, whereas other tumor types do not (van Roosmalen, I.A.M. *et al.* (2014) “*Two Death-Inducing Human TRAIL Receptors To Target In Cancer: Similar Or Distinct Regulation And Function?*,” Biochem. Pharmacol. 91:447-456).

II. Therapeutic Uses of TRAIL Proteins and Anti-DR Antibodies

[0012] Because TRAIL is highly selective in its ability to recognize and kill damaged cells, while sparing normal cells, soluble recombinant TRAIL has been stated to have potential utility in the treatment of cancer (*e.g.*, colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin’s lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer and rectal cancer (see, Micheau, O. *et al.* (2013) “*Death Receptors As Targets In Cancer*,” Br. J. Pharmacol. 169:1723-1744); Falschlehner, C. *et al.* (2009) “*TRAIL and Other TRAIL Receptor Agonists as Novel Cancer Therapeutics*,” In: THERAPEUTIC TARGETS OF THE TNF SUPERFAMILY (Grewal, I.S., Ed.) Landes Bioscience and Springer Science+Business Media, NY; pp. 195-206; Buchsbaum, D.J. *et al.* (2006) “*TRAIL Receptor-Targeted Therapy*,” Future Oncol. 2(4):493-508; Wajant, H. *et al.* (2013) “*Engineering Death Receptor Ligands For Cancer Therapy*,” Canc. Lett. 332:163-174; Buchsbaum, D.J. *et al.* (2007) “*TRAIL-Receptor-Antibodies as a Potential Cancer Treatment*,” Future Oncol. 3(4):405-409; Abdulghani, J. *et al.* (2010) (“*TRAIL Receptor Signaling And Therapeutics*,” Expert Opin. Ther. Targets 14(10):1091-1108; Finnberg, N. *et al.* (2008) “*TRAIL Death Receptors As Tumor Suppressors And Drug Targets*,” Cell Cycle 7(11):1525-1528; Hellwig, C.T. *et al.* (2012) “*TRAIL Signaling and Synergy Mechanisms Used in TRAIL-Based Combination Therapies*,” Molec. Cancer Ther. 11(1):3-13; Henson, E.S. *et al.* (2008) “*The Role Of TRAIL Death Receptors In The Treatment Of Hematological Malignancies*,” Leukemia & Lymphoma 49(1):27-35; Huang, Y. *et al.* (2007) “*TRAIL Death Receptors And Cancer Therapeutics*,” Toxicol. Appl. Pharmacol. 224:284-289; Humphreys, R.C. *et al.* (2008) “*Trail Receptors: Targets for Cancer Therapy*,” In:

PROGRAMMED CELL DEATH IN CANCER PROGRESSION AND THERAPY Khosravi-Far, R. and White, E. (Eds.) Springer, NY; pp. 127-158; Koschny, R. *et al.* (2007) “*The Promise Of TRAIL – Potential And Risks Of A Novel Anticancer Therapy*,” J. Molec. Med. 85:923-935; Kruyt, F.A.E. (2008) “*TRAIL and Cancer Therapy*,” Cancer Lett. 263:14-25; Kuijlen, J.M.A. *et al.* (2010) “*Review: On TRAIL For Malignant Glioma Therapy?*,” Neuropathol. Appl. Neurobiol. 36:168-182; Mellier, G. *et al.* (2010) (“*TRAILing Death in Cancer*,” Molec. Aspects Med. 31:93-112; Rahman, M. *et al.* (2009) “*The TRAIL To Targeted Therapy Of Breast Cancer*,” Adv. Cancer Res. 103:43-73; Voelkel-Johnson, C.(2011) “*TRAIL-Mediated Signaling In Prostate, Bladder And Renal Cancer*,” Nat. Rev. Urol. 8:417-427).

[0013] Anti-DR4 and anti-DR5 monoclonal antibodies that might be capable of mimicking the signaling of TRAIL have been proposed as providing greater selectivity (Buchsbaum, D.J. *et al.* (2006) “*TRAIL Receptor-Targeted Therapy*,” Future Oncol. 2:493-508; Kelley, S.K. *et al.* (2004) “*Targeting Death Receptors In Cancer With Apo2L/TRAIL*,” Curr. Opin. Pharmacol. 4:333-339; Papenfuss, K. *et al.* (2008) “*Death Receptors As Targets For Anti-Cancer Therapy*,” J. Cell. Mol. Med. 12:2566-2585; de Bruyn, M. *et al.* (2013) “*Antibody-Based Fusion Proteins To Target Death Receptors In Cancer*,” Cancer Lett. 332:175-183).

[0014] Three Phase II clinical studies of **mapatumumab**, an anti-DR4 agonist antibody (Human Genome Sciences) have been reported to show a therapeutic effect in patients suffering from non-Hodgkin’s lymphoma (**NHL**), colorectal cancer (**CRC**) and non-small cell lung cancer (**NSCLC**) (Greco, F.A. *et al.* (2008) “*Phase 2 Study Of Mapatumumab, A Fully Human Agonistic Monoclonal Antibody Which Targets And Activates The TRAIL Receptor-1, In Patients With Advanced Non-Small Cell Lung Cancer*,” Lung Cancer 61:82-90; Trarbach, T. *et al.* (2010) “*Phase II Trial Of Mapatumumab, A Fully Human Agonistic Monoclonal Antibody That Targets And Activates The Tumour Necrosis Factor Apoptosis-Inducing Ligand Receptor-1 (TRAIL-R1), In Patients With Refractory Colorectal Cancer*,” Br. J. Cancer 102:506-512; Falschlehner, C. *et al.* (2009) “*TRAIL and Other TRAIL Receptor Agonists as Novel Cancer Therapeutics*,” In: THERAPEUTIC TARGETS OF THE TNF SUPERFAMILY (Grewal, I.S., Ed.) Landes Bioscience and Springer Science+Business Media, NY; pp. 195-206).

TRA-8/CS-1008, a humanized anti-DR5 antibody (Daiichi Sankyo (Tokyo, Japan)) is reported to have exhibited high antitumor activity against astrocytoma and leukemia cells in vitro and engrafted breast cancer cells *in vivo* (Buchsbaum, D.J. *et al.* (2003) “*Antitumor Efficacy Of TRA-8 Anti-DR5 Monoclonal Antibody Alone Or In Combination With Chemotherapy And/Or Radiation Therapy In A Human Breast Cancer Model*,” Clin. Cancer Res. 9:3731-3741; Ichikawa, K. *et al.* (2001) “*Tumoricidal Activity Of A Novel Anti-Human DR5 Monoclonal Antibody Without Hepatocyte Cytotoxicity*,” Nat. Med. 7:954-960; Saleh, M.N. *et al.* (2008) “*A Phase I Study Of CS-1008 (Humanized Monoclonal Antibody Targeting Death Receptor 5 Or DR5), Administered Weekly To Patients With Advanced Solid Tumors Or Lymphomas*,” 2008 ASCO Annual Meeting Proceedings, J. Clin. Oncol. 26(20S): Abstract 3537).

mDRA-6 (IgG1-k), a murine anti-human anti-DR5 monoclonal antibody (Henan University) has been reported to be able to induce the apoptosis of Jurkat cells via the TRAIL extrinsic pathway (Du, Y.-W. *et al.* (2011) “*A Novel Agonistic Anti-Human Death Receptor 5 Monoclonal Antibody With Tumoricidal Activity Induces Caspase-And Mitochondrial-Dependent Apoptosis In Human Leukemia Jurkat Cells*,” Cancer Biother. Radiopharmaceut. 26(2):143-152). The chimeric DR-5-targeting antibody **LBY135** (Novartis) has been reported to have induced apoptosis in 50% of a panel of 40 human colon cancer cell lines with an IC50 of 10 nM or less and to have verified *in vivo* antitumor activity in human colorectal xenograft models in mice (Li, J. *et al.* (2008) “*LBY135, A Novel Anti-DR5 Agonistic Antibody Induces Tumor Cell-Specific Cytotoxic Activity In Human Colon Tumor Cell Lines And Xenografts*,” Drug Dev. Res. 69:69-82; Sharma, S. *et al.* (2008) “*Phase I Trial Of LBY135, A Monoclonal Antibody Agonist To DR5, Alone And In Combination With Capecitabine In Advanced Solid Tumors*,” 2008 ASCO Annual Meeting Proceedings, J. Clin. Oncol. 26(15S):3538). Additional anti-DR antibodies in clinical development include: **ApomAb** (Camidge, D. *et al.* (2007) “*A Phase I Safety And Pharmacokinetic Study Of Apomab, A Human DR5 Agonist Antibody, In Patients With Advanced Cancer*,” 2007 ASCO Annual Meeting Proceedings (Post-Meeting Edition), J. Clin. Oncol. 25(18S):3582; Johnstone, R.W. *et al.* (2008) “*The TRAIL Apoptotic Pathway In Cancer Onset, Progression And Therapy*,” Nat. Rev. Cancer 8:782-798); **AMG655** (LoRusso, P. *et al.* (2007) “*First-In-Human Study Of AMG 655, A Pro-Apoptotic TRAIL Receptor-2 Agonist, In Adult*

Patients With Advanced Solid Tumors,” 2007 ASCO Annual Meeting Proceedings Part I. J. Clin. Oncol. 25(18S):3534); **conatumumab** (Bajaj, M. *et al.* (2011) “*Conatumumab: A Novel Monoclonal Antibody Against Death Receptor 5 For The Treatment Of Advanced Malignancies In Adults*,” Expert Opin. Biol. Ther. 11(11):1519-1524); **lexatumumab**, an anti-DR5 agonist antibody (Human Genome Sciences) (Plummer, R. *et al.* (2007) “*Phase 1 And Pharmacokinetic Study Of LEXATUMUMAB In Patients With Advanced Cancers*,” Clin. Cancer Res. 13:6187-6194); **drozitumab** (Kang, Z. *et al.* (2011) “*Drozitumab, A Human Antibody To Death Receptor 5, Has Potent Antitumor Activity Against Rhabdomyosarcoma With The Expression Of Caspase-8 Predictive Of Response*,” Clin. Cancer Res. 17(10):3181-3192; Zinonos, I. *et al.* (2014) “*Doxorubicin Overcomes Resistance to Drozitumab by Antagonizing Inhibitor of Apoptosis Proteins (IAPs)*,” Anticancer Res. 34(12):7007-7020; Xiang, H. *et al.* (2013) “*Death Receptor 5 Agonistic Antibody PRO95780: Preclinical Pharmacokinetics And Concentration-Effect Relationship Support Clinical Dose And Regimen Selection*,” Cancer Chemother. Pharmacol. 72(2):405-415; Stern, H.M. *et al.* (2010) “*Development Of Immunohistochemistry Assays To Assess GALNT14 And FUT3/6 In Clinical Trials Of Dulanermin And Drozitumab*,” Clin. Cancer Res. 16(5):1587-1596) and **KMTR2** (Nagane, M. *et al.* (2010) “*Predominant Antitumor Effects By Fully Human Anti-TRAIL-Receptor 2 (DR5) Monoclonal Antibodies In Human Glioma Cells In Vitro And In Vivo*,” Neuro. Oncol. 12(7):687-700; Motoki, K. *et al.* (2005) “*Enhanced Apoptosis And Tumor Regression Induced By A Direct Agonist Antibody To Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor 2*,” Clin. Cancer Res. 11(8):3126-3135).

[0015] The use of anti-DR antibodies is reviewed in: Falschlehner, C. *et al.* (2009) (“*TRAIL and Other TRAIL Receptor Agonists as Novel Cancer Therapeutics*,” In: THERAPEUTIC TARGETS of THE TNF SUPERFAMILY (Grewal, I.S., Ed.) Landes Bioscience and Springer Science+Business Media, NY; pp. 195-206); Hellwig, C.T. *et al.* (2012) (“*TRAIL Signaling and Synergy Mechanisms Used in TRAIL-Based Combination Therapies*,” Molec. Cancer Ther. 11(1):3-13); Huang, Y. *et al.* (2007) (“*TRAIL Death Receptors And Cancer Therapeutics*,” Toxicol. Appl. Pharmacol. 224:284-289); Humphreys, R.C. *et al.* (2008) (“*Trail Receptors: Targets for Cancer Therapy*,” In: PROGRAMMED CELL DEATH IN CANCER PROGRESSION AND THERAPY

Khosravi-Far, R. and White, E. (Eds.) Springer, NY; pp. 127-158); Kruyt, F.A.E. (2008) (“*TRAIL and Cancer Therapy*,” *Cancer Lett.* 263:14-25); Mellier, G. *et al.* (2010) (“*TRAILing Death in Cancer*,” *Molec. Aspects Med.* 31:93-112); Oldenhuis, C.N.A.M. *et al.* (2008) (“*Targeting TRAIL Death Receptors*,” *Curr. Opin. Pharmacol.* 8:433-439); Papenfuss, K. *et al.* (2008) (“*Death Receptors As Targets For Anti-Cancer Therapy*,” *J. Cell. Mol. Med.* 12(6B):2566-2585); Micheau, O. *et al.* (2013) (“*Death Receptors As Targets In Cancer*,” *Br. J. Pharmacol.* 169:1723-1744; and in van Roosmalen, I.A.M. *et al.* (2014) (“*Two Death-Inducing Human TRAIL Receptors To Target In Cancer: Similar Or Distinct Regulation And Function?*,” *Biochem. Pharmacol.* 91:447-456).

[0016] Present data suggests that such agents are well-tolerated and have plasma half-lives of less than 12 days, however, the potential application of this therapy is limited by the fact that some primary cancer cells are resistant to TRAIL apoptosis, even after combination treatment with chemotherapy (Buchsbaum, D.J. *et al.* (2007) “*TRAIL-Receptor-Antibodies as a Potential Cancer Treatment*,” *Future Oncol.* 3(4):405-409; see also, Dimberg, L.Y. *et al.* (2013) “*On The TRAIL To Successful Cancer Therapy? Predicting And Counteracting Resistance Against TRAIL-Based Therapeutics*,” *Oncogene* 32:1341-1350; Falschlehner, C. *et al.* (2009) “*TRAIL and Other TRAIL Receptor Agonists as Novel Cancer Therapeutics*,” In: *THERAPEUTIC TARGETS OF THE TNF SUPERFAMILY* (Grewal, I.S., Ed.) Landes Bioscience and Springer Science+Business Media, NY; pp. 195-206; Maksimovic-Ivanic, D. *et al.* (2012) “*Resistance To TRAIL And How To Surmount It*,” *Immunol. Res.* 52:157-168).

[0017] Despite the promise of such antibody therapy, studies have shown that some anti-DR monoclonal antibodies have not exhibited sufficient selectivity for clinical use. This may reflect the fact that only one specific isoform of TRAIL among the nine reported variants exhibit such selectivity (Allen, J.E. *et al.* (2012) “*Regulation Of The Human TRAIL Gene*,” *Cancer Biol. Ther.* 13(12):1143-1151). Induction of apoptosis in normal human cells, such as hepatocytes or keratinocytes by some rTRAIL and anti-DR monoclonal antibodies have been observed *in vitro* (Jo, M. *et al.* (2000) “*Apoptosis Induced In Normal Human Hepatocytes By Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand*,” *Nat. Med.* 6:564-567; Lawrence, D. *et al.* (2001) “*Differential*

*Hepatocyte Toxicity Of Recombinant Apo2L/TRAIL Versions,” Nat. Med. 7:383-385; Mori, E. et al. (2004) “Human Normal Hepatocytes Are Susceptible To Apoptosis Signal Mediated By Both TRAIL-R1 And TRAIL-R2,” Cell. Death Differ. 11:203-207; Qin, J. et al. (2001) “Avoiding Premature Apoptosis Of Normal Epidermal Cells,” Nat. Med. 7:385-386). Hepatotoxicity with increased serum alanine aminotransferase, aspartate aminotransferase and bilirubin was reported in a few patients when treated with higher doses (20 mg per kg) of **lexatumumab** anti-DR5 agonist antibody from Human Genome Sciences) (Plummer, R. et al. (2007) “Phase I And Pharmacokinetic Study Of LEXATUMUMAB In Patients With Advanced Cancers,” Clin. Cancer Res. 13:6187-6194).*

[0018] Anti-DR antibodies are disclosed in United States Patents No. 8,790,663; 8,715,668; 8,703,712; 8,461,311; 8,409,570; 8,372,396; 8,329,180; 8,173,128; 8,097,704; 8,067,001; 8,030,023; 8,029,783; 7,981,421; 7,897,730; 7,893,216; 7,704,502 and 7,476,383; in United States Patent Publications No. 2014/0370019; 2014/0308288; 2014/0105898; 2014/0004120; 2014/0010812; 2013/0324433; 2013/0280282; 2013/0243780; 2013/0064838; 2012/0184718; 2012/0087922; 2012/0070432; 2011/0070248; 2010/0080806; 2009/0317384; 2009/0317396; 2009/0208483; 2009/0175854 and 2009/0136503; in European Patent Publications No. EP 2021370; EP 1790663; EP 2059533; EP 1506285; EP 1576179; EP 2636736; EP 2684896; EP 2636736; EP 2569336; EP 2046836; EP 2480230; EP 2368910; EP 2350641; EP 2292794; EP 2287285; EP 2292794 and EP 2021370; and in WIPO Patent Publications No. WO 2014/159562; WO 2014/161845; WO 2014/050779; WO 2014/035474; WO 2014/009358; WO 2013/163229 and WO 2013/148877.

[0019] Bispecific antibody molecules, having an scFv Domain capable of binding to a tumor antigen and a soluble TRAIL (**sTRAIL**) or Fas (**CD95**) Ligand (**FasL**) Domain capable of binding to a death receptor or to Fas, have also been proposed (see, Wajant, H. et al. (2013) “Engineering Death Receptor Ligands For Cancer Therapy,” Canc. Lett. 332:163-174). Such genetic fusion of a tumor-selective antibody fragment to sTRAIL and sFasL yielded highly selective anticancer therapeutics with favorable anticancer features. However, the employed fusion proteins were twice the size of non-targeted soluble ligands. Thus, the approach appears to be limited by the relative

difficulty of the fusion protein diffusing through multiple cellular in order to penetrate into solid tumors (de Bruyn, M. *et al.* (2013) “*Antibody-Based Fusion Proteins To Target Death Receptors In Cancer*,” *Cancer Lett.* 332:175-183). Bispecific antibody molecules capable of binding to DR5 are disclosed in United States Patent Publications No. 2014/0370019; 2014/0308288; 2013/0243780; 2012/0184718 and 2009/0175854; in European Patent Publication Nos. EP 1790663; EP 2059533; EP 2684896 and EP 2350641; and in WIPO Publications No. WO 2014/159562; WO 2014/161845; WO 2014/050779; WO 2014/009358 and WO 2013/148877.

[0020] In addition to its potential in the treatment of cancer, TRAIL has been proposed as a potential therapeutic for the treatment of bacterial pathogens (Benedict, C.A. *et al.* (2012) “*TRAIL: Not Just For Tumors Anymore?*,” *J. Exp. Med.* 209(11):1903-1906). TRAIL may also have a role in the structural changes in asthmatic airways because it is expressed by various inflammatory cells including eosinophils (Chaudhari, B.R. *et al.* (2006) “*Following the TRAIL to Apoptosis*,” *Immunologic Res.* 35(3):249-262). One drawback of the use of soluble TRAIL preparations has been its relatively short *in vivo* half-life (approximately 30 minutes; Walczak, H. *et al.* (1999) “*Tumoricidal Activity Of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand In Vivo*,” *Nat. Med.* 5:157-163). Additionally, soluble recombinant TRAIL is capable of binding to TRAIL receptors (thus promoting cancer treatment) and to TRAIL decoy receptors (thus putatively providing no therapeutic benefit). TRAIL may also have a role in cardiovascular disease (Martin-Ventura, J.L. *et al.* (2007) “*TRAIL and Vascular Injury*,” *Frontiers in Bioscience* 12:3656-3667) and in inflammation (Walczak, H. (2013) “*Death Receptor – Ligand Systems in Cancer, Cell Death, and Inflammation*,” *Cold Spring Harb. Perspect. Biol.* 2013;5:a008698; pp. 1-19).

[0021] Whereas clinical trials using TRAIL therapies have shown low toxicity in patients, disappointingly small therapeutic effects have been observed when TRAIL agonists are used as a monotherapy (Dimberg, L.Y. *et al.* (2013) “*On The TRAIL To Successful Cancer Therapy? Predicting And Counteracting Resistance Against TRAIL-Based Therapeutics*,” *Oncogene* 32:1341-1350). This conclusion reflects the observation that a substantial proportion of damaged cells that have evolved into tumor cells are found to be TRAIL-resistant. Such experiences have led to the conclusion that

TRAIL therapy may be very beneficial, but only for a small subset of patients (Dimberg, L.Y. *et al.* (2013) “*On The TRAIL To Successful Cancer Therapy? Predicting And Counteracting Resistance Against TRAIL-Based Therapeutics,*” *Oncogene* 32:1341-1350).

[0022] Multiple mechanisms of TRAIL resistance have been identified (Maksimovic-Ivanic, D. *et al.* (2012) “*Resistance To TRAIL And How To Surmount It,*” *Immunol. Res.* 52:157-168; Dimberg, L.Y. *et al.* (2013) “*On The TRAIL To Successful Cancer Therapy? Predicting And Counteracting Resistance Against TRAIL-Based Therapeutics,*” *Oncogene* 32:1341-1350; Thorburn, A. *et al.* (2008) “*TRAIL Receptor-Targeted Therapeutics: Resistance Mechanisms And Strategies To Avoid Them,*” *Drug Resist. Updat.* 11(1-2):17-24; Whiteside, T.L. (2007) “*The Role of Death Receptor Ligands in Shaping Tumor Microenvironment,*” *Immunol. Investig.* 36:25-46). Among the hypothesized explanations are the possibility of decreased expression of certain caspases (*e.g.*, caspase 8) by TRAIL-resistant tumor cells, or the increased expression of caspase inhibitors (*e.g.*, XIAP, cIAP) by such cells, or the increased expression of inhibitors of apoptosis (*e.g.*, Bcl-2, Mcl-1, *etc.*) by such cells (Abdulghani, J. *et al.* (2010) “*TRAIL Receptor Signaling And Therapeutics,*” *Expert Opin. Ther. Targets* 14(10):1091-1108; Buchsbaum, D.J. *et al.* (2006) “*TRAIL Receptor-Targeted Therapy,*” *Future Oncol.* 2(4):493-508). Alternatively, TRAIL resistance may reflect the presence of defects in the TRAIL receptors of the tumor cells, or increased expression of inhibitors that are very selective for death receptors such as FLIP or the decoy receptors TRAIL-R3 and TRAIL-R4. See, Abdulghani, J. *et al.* (2010) (“*TRAIL Receptor Signaling And Therapeutics,*” *Expert Opin. Ther. Targets* 14(10):1091-1108). In light of such resistance, TRAIL-based therapeutics have typically been proposed only as agents to be provided in concert with other chemotherapeutic agents (Buchsbaum, D.J. *et al.* (2006) “*TRAIL Receptor-Targeted Therapy,*” *Future Oncol.* 2(4):493-508).

[0023] Thus, despite all prior advances, a need remains for anti-DR5 antibodies and molecules comprising DR5-binding domains that could provide improved therapeutic value to patients suffering from cancer or other diseases and conditions. The present invention is directed to this and other goals.

Summary of the Invention:

[0024] The present invention is directed to multivalent DR5-Binding Molecules that comprise Binding Domain(s) of anti-DR5 antibodies, and particularly Binding Domain(s) of anti-human DR5 antibodies. The DR5-Binding Molecules of the present invention include bivalent and tetravalent molecules having two, three or four DR5-Binding Domains each capable of binding human DR5. In particular, the present invention is directed to multivalent DR5-Binding Molecules that comprise diabodies, and more particularly, diabodies that comprise a covalently bonded complex of two or more polypeptide chains. The invention particularly pertains to such multivalent DR5-Binding Molecules that comprise fragments of the anti-DR5 antibodies DR5 mAb 1 and/or DR5 mAb 2, and/or humanized and chimeric versions of such antibodies.

[0025] In detail, the invention provides a multivalent DR5-Binding Molecule that is a bispecific binding molecule, capable of simultaneously binding to two different epitopes of human Death Receptor 5 (DR5), wherein the multivalent DR5-Binding Molecule comprises four antigen-binding domains each capable of binding human DR5. The invention also provides a multivalent DR5-Binding Molecule that is a monospecific binding molecule, capable of binding to an epitope of human DR5, wherein the multivalent DR5-Binding Molecule comprises four antigen-binding domains each capable of binding human DR5. The invention particularly concerns the embodiment of all such multivalent DR5-Binding Molecules capable of simultaneously binding to two, three, or four human DR5 polypeptides.

[0026] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the multivalent DR5-Binding Molecule is an Fc Region-containing diabody, the diabody being a covalently bonded complex that comprises two pairs of polypeptides, wherein each pair comprises a first polypeptide chain and a second polypeptide chain.

[0027] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein:

- (A) the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

- (i) a variable light chain (VL) Domain of a monoclonal antibody capable of binding to a first DR5 epitope (VL1);
 - (ii) a first peptide linker (Linker 1);
 - (iii) a variable heavy chain (VH) Domain of a monoclonal capable of binding to a second DR5 epitope (VH2);
 - (iv) a second peptide linker (Linker 2);
 - (v) a Heterodimer-Promoting Domain comprising a E-coil Domain or a K-coil Domain;
 - (vi) a third peptide linker (Linker 3); and
 - (vii) a polypeptide portion of an IgG Fc Region having CH2 and CH3 domains of an IgG immunoglobulin Fc Region; and
- (B) the second polypeptide chain comprises, in the N-terminal to C-terminal direction:
- (i) a VL Domain of a monoclonal antibody capable of binding to the second DR5 epitope (VL2);
 - (ii) a first peptide linker (Linker 1);
 - (iii) a VH Domain of a monoclonal capable of binding to the first DR5 epitope (VH1);
 - (iv) a second peptide linker (Linker 2); and
 - (v) a Heterodimer-Promoting Domain comprising a E-coil Domain or a K-coil Domain, wherein the Heterodimer-Promoting Domain of the first polypeptide chain and the Heterodimer-Promoting Domain of the second polypeptide chain are not both E-coil Domains or both K-coil Domains;

and wherein:

- (a) the VL1 Domain of the first polypeptide chain and the VH1 Domain of the second polypeptide chain form an Antigen-Binding Domain capable of specific binding to a first epitope of DR5;
- (b) the VH2 Domain of the first polypeptide chain and the VL1 Domain of the second polypeptide chain form an Antigen-Binding Domain capable of specific binding to a second epitope of DR5; and

- (c) the CH2-CH3 portions of the pair of first polypeptide chains form an IgG Fc Region.

[0028] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein:

- (i) the Linker 1 has the amino acid sequence of **SEQ ID NO:33**,
- (ii) the Linker 1 has the amino acid sequence of **SEQ ID NO:47**,
- (iii) the E-coil Domain has the amino acid sequence of **SEQ ID NO:41**,
- (iv) the K-coil Domain has the amino acid sequence of **SEQ ID NO:42**,
- (v) the Linker 3 has the amino acid sequence of **SEQ ID NO:51**, and
- (vi) the CH2-CH3 domain has the amino acid sequence of **SEQ ID NO:1 or SEQ ID NO:102**, wherein the C-terminal residue is optionally included.

[0029] The invention further concerns the embodiments of all such multivalent DR5-Binding Molecules, wherein the Fc Region comprises one or more amino acid modifications that reduce the affinity of the variant Fc Region for an FcγR or stabilizes the Fc Region. The invention further concerns the embodiments of all such DR5-Binding Molecule, wherein the modifications comprise the substitution of L234A; L235A; or L234A and L235A.

[0030] The invention particularly concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the VL1 comprises a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} Domain, and the VH1 comprises a CDR_{H1} Domain, a CDR_{H2} Domain and a CDR_{H3} Domain, wherein:

- (i) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy

- Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**; or
- (ii) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
- (iii) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hDR5 mAb 2 VL-3, and, respectively have the amino acid sequences: **SEQ ID NO:162**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of hDR5 mAb 2 VH-3, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
- (iv) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:56**, and **SEQ ID NO:57**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:59**, **SEQ ID NO:60**, and **SEQ ID NO:61**; or
- (v) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:63**, **SEQ ID NO:64**, and **SEQ ID NO:65**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:67**, **SEQ ID NO:68**, and **SEQ ID NO:69**; or
- (vi) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid

sequences: **SEQ ID NO:71**, **SEQ ID NO:72**, and **SEQ ID NO:73**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:75**, **SEQ ID NO:76**, and **SEQ ID NO:77**; or

(vii) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:79**, **SEQ ID NO:80**, and **SEQ ID NO:81**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:83**, **SEQ ID NO:84**, and **SEQ ID NO:85**; or

(viii) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:87**, **SEQ ID NO:88**, and **SEQ ID NO:89**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:91**, **SEQ ID NO:92**, and **SEQ ID NO:93**; or

(ix) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:95**, **SEQ ID NO:96**, and **SEQ ID NO:97**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:99**, **SEQ ID NO:100**, and **SEQ ID NO:101**.

[0031] The invention particularly concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the VL2 comprises a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} Domain, and the VH2 comprises a CDR_{H1} Domain, a CDR_{H2} Domain and a CDR_{H3} Domain, wherein:

(i) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid

- sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**; or
- (ii) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
- (iii) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:162**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
- (iv) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:56**, and **SEQ ID NO:57**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:59**, **SEQ ID NO:60**, and **SEQ ID NO:61**; or
- (v) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:63**, **SEQ ID NO:64**, and **SEQ ID NO:65**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:67**, **SEQ ID NO:68**, and **SEQ ID NO:69**; or

- (vi) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:71**, **SEQ ID NO:72**, and **SEQ ID NO:73**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:75**, **SEQ ID NO:76**, and **SEQ ID NO:77**; or
- (vii) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:79**, **SEQ ID NO:80**, and **SEQ ID NO:81**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:83**, **SEQ ID NO:84**, and **SEQ ID NO:85**; or
- (viii) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:87**, **SEQ ID NO:88**, and **SEQ ID NO:89**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:91**, **SEQ ID NO:92**, and **SEQ ID NO:93**; or
- (ix) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:95**, **SEQ ID NO:96**, and **SEQ ID NO:97**, and the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:99**, **SEQ ID NO:100**, and **SEQ ID NO:101**.

[0032] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the VL1 and the VL2 comprise the same CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain, and wherein the VH1 and the VH2 comprise the

same CDR_{H1} Domain, CDR_{H2} Domain and CDR_{H3} Domain, and particularly concerns the embodiment of such multivalent DR5-Binding Molecules, wherein:

- (i) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**; or
- (ii) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
- (iii) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:162**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**.

[0033] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the VL1 and the VL2 do not comprise the same CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain, and wherein the VH1 and the VH2 do not comprise the same CDR_{H1} Domain, CDR_{H2} Domain and CDR_{H3} Domain, and particularly concerns the embodiment of such multivalent DR5-Binding Molecules, wherein:

- (i) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL1 are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino

acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of VH1 are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**; and the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL2 are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of VH2 are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or

- (ii) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL1 are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of VH1 are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; and the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL2 are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of VH2 are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**.

[0034] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein:

- (A) (i) the VL1 has the amino acid sequence of **SEQ ID NO:3**, and the VH1 has the amino acid sequence of **SEQ ID NO:8**; or
- (ii) the VL1 has the amino acid sequence of **SEQ ID NO:13**, and the VH1 has the amino acid sequence of **SEQ ID NO:18**; or
- (iii) the VL1 has the amino acid sequence of **SEQ ID NO:23**, and the VH1 has the amino acid sequence of **SEQ ID NO:31**; or

- (iv) the VL1 has the amino acid sequence of **SEQ ID NO:25**, and the VH1 has the amino acid sequence of **SEQ ID NO:31**; or
- (vi) the VL1 has the amino acid sequence of **SEQ ID NO:27**, and the VH1 has the amino acid sequence of **SEQ ID NO:31**; or
- (vii) the VL1 has the amino acid sequence of **SEQ ID NO:29**, and the VH1 has the amino acid sequence of **SEQ ID NO:31**; or
- (viii) the VL1 has the amino acid sequence of **SEQ ID NO:54**, and the VH1 has the amino acid sequence of **SEQ ID NO:58**; or
- (ix) the VL1 has the amino acid sequence of **SEQ ID NO:62**, and the VH1 has the amino acid sequence of **SEQ ID NO:66**; or
- (x) the VL1 has the amino acid sequence of **SEQ ID NO:70**, and the VH1 has the amino acid sequence of **SEQ ID NO:74**; or
- (xi) the VL1 has the amino acid sequence of **SEQ ID NO:78**, and the VH1 has the amino acid sequence of **SEQ ID NO:82**; or
- (xii) the VL1 has the amino acid sequence of **SEQ ID NO:86**, and the VH1 has the amino acid sequence of **SEQ ID NO:90**; or
- (xiii) the VL1 has the amino acid sequence of **SEQ ID NO:94**, and the VH1 has the amino acid sequence of **SEQ ID NO:98**;

and wherein:

- (B) (i) the VL2 has the amino acid sequence of **SEQ ID NO:3**, and the VH2 has the amino acid sequence of **SEQ ID NO:8**; or
- (ii) the VL2 has the amino acid sequence of **SEQ ID NO:13**, and the VH2 has the amino acid sequence of **SEQ ID NO:18**; or
- (iii) the VL2 has the amino acid sequence of **SEQ ID NO:23**, and the VH2 has the amino acid sequence of **SEQ ID NO:31**; or
- (iv) the VL2 has the amino acid sequence of **SEQ ID NO:25**, and the VH2 has the amino acid sequence of **SEQ ID NO:31**; or
- (vi) the VL2 has the amino acid sequence of **SEQ ID NO:27**, and the VH2 has the amino acid sequence of **SEQ ID NO:31**; or
- (vii) the VL2 has the amino acid sequence of **SEQ ID NO:29**, and the VH2 has the amino acid sequence of **SEQ ID NO:31**; or

- (viii) the VL2 has the amino acid sequence of **SEQ ID NO:54**, and the VH2 has the amino acid sequence of **SEQ ID NO:58**; or
- (ix) the VL2 has the amino acid sequence of **SEQ ID NO:62**, and the VH2 has the amino acid sequence of **SEQ ID NO:66**; or
- (x) the VL2 has the amino acid sequence of **SEQ ID NO:70**, and the VH1 has the amino acid sequence of **SEQ ID NO:74**; or
- (xi) the VL2 has the amino acid sequence of **SEQ ID NO:78**, and the VH1 has the amino acid sequence of **SEQ ID NO:82**; or
- (xii) the VL2 has the amino acid sequence of **SEQ ID NO:86**, and the VH1 has the amino acid sequence of **SEQ ID NO:90**; or
- (xiii) the VL2 has the amino acid sequence of **SEQ ID NO:94**, and the VH2 has the amino acid sequence of **SEQ ID NO:98**.

[0035] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the VL1 and the VL2 have the same amino acid sequence, and wherein the VH1 and the VH2 have the same amino acid sequence.

[0036] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the VL1 and the VL2 do not have the same amino acid sequence, and wherein the VH1 and the VH2 do not have the same amino acid sequence.

[0037] The invention further concerns the embodiments of such multivalent DR5-Binding Molecules, wherein the multivalent DR5-Binding Molecule is an Fc Region-containing diabody, the diabody being a covalently bonded complex that comprises two pairs of polypeptides wherein:

- (i) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:116** or **SEQ ID NO:120**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:118**; or
- (ii) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:122** or **SEQ ID NO:126**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:124**; or

- (iii) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:128** or **SEQ ID NO:132**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:130**; or
- (iv) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:134** or **SEQ ID NO:138**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:136**; or
- (v) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:140** or **SEQ ID NO:144**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:142**; or
- (vi) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:146** or **SEQ ID NO:150**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:148**; or
- (vii) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:152** or **SEQ ID NO:156**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:154**; or
- (vi) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:158** or **SEQ ID NO:162**, and the second polypeptide chain has the amino acid sequence of **SEQ ID NO:160**.

[0038] The invention further concerns compositions comprising any of the above described multivalent DR5-Binding Molecules and an excipient. The invention further concerns such compositions further comprising a histone deacetylase inhibitor.

[0039] The invention further concerns methods of promoting cell death comprising exposing a cell to any of the above described multivalent DR5-Binding Molecules. In particular, where the cell is a tumor cell. The invention further concerns such methods of promoting cell death further comprising exposing the cell to a histone deacetylase inhibitor.

[0040] The invention further concerns the embodiments in which any of the above-described multivalent DR5-Binding Molecules is used in the treatment of cancer. The invention further concerns the embodiments in which any of the above-described

multivalent DR5-binding molecules is used in combination with a histone deacetylase inhibitor in the treatment of cancer.

[0041] The invention further concerns the embodiments in which any of the above-described multivalent DR5-Binding Molecules is detectably labeled and is used in the diagnosis or prognosis of cancer.

[0042] The invention particularly concerns such use of any of the above described multivalent DR5-Binding Molecules in the treatment or diagnosis or prognosis of cancer, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocyoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0043] The invention particularly concerns such use of any of the above described multivalent DR5-Binding Molecules in the treatment or diagnosis or prognosis of cancer, wherein the cancer is colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer or a rectal cancer.

[0044] The invention particularly concerns such use of any of the above described multivalent DR5-Binding Molecules in the treatment or diagnosis or prognosis of cancer, wherein the cancer is acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantle cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

Brief Description of the Drawings:

[0045] **Figure 1** provides a schematic of a representative covalently bonded diabody molecule having two epitope binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading. In certain embodiments, the epitopes are different epitopes of the same antigen resulting in a bispecific molecule that is monovalent for each epitope, but is bivalent with respect to the antigen (*e.g.*, DR5). In certain embodiments the epitopes are the same epitope (*e.g.*, the same VL Domain CDRs and VH Domain CDRs are used on each chain) resulting in a monospecific molecule that is bivalent.

[0046] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of a naturally occurring Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading. In certain embodiments, the epitopes are different epitopes of the same antigen resulting in a

bispecific molecule that is monovalent for each epitope, but is bivalent with respect to the antigen (*e.g.*, DR5). In certain embodiments the epitopes are the same epitope (*e.g.*, the same VL Domain CDRs and VH Domain CDRs are used on each chain) resulting in a monospecific molecule that is bivalent.

[0047] **Figure 3** provides a schematic showing a representative tetravalent diabody molecule composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of a naturally occurring Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading. The two pairs of polypeptide chains may be same. In such embodiments wherein the VL and VH Domains recognize different epitopes (as shown), the resulting molecule is bispecific and bivalent with respect to each bound epitope. In such embodiments wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains) the resulting molecule is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments wherein the VL and VH Domains of each pair of polypeptides recognize different epitopes (as shown), the resulting molecule is tetraspecific and monovalent with respect to each bound epitope. In embodiments wherein the epitopes are all epitopes of the same antigen, the resulting molecule is tetravalent with respect to the antigen (*e.g.*, DR5). In certain embodiments the epitopes are the same epitope (*e.g.*, the same 3 CDR_{LS} and the same 3 CDR_{HS} domains are used on each chain), the resulting molecule is monospecific and tetravalent.

[0048] **Figures 4A and 4B** provide schematics of alternative tetravalent diabody molecules that are also composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of a naturally occurring Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading. The two pairs of polypeptide chains may be the same. In such embodiments, wherein the VL and VH Domains recognize different epitopes (as shown) the resulting molecule is bispecific and bivalent with

respect to each bound epitope. In such embodiments, wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains) the resulting molecule is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments, wherein the VL and VH Domains recognize different epitopes, the resulting molecule is tetraspecific and monovalent with respect to each bound epitope. In embodiments wherein the epitopes are all epitopes of the same antigen, the resulting molecule is tetravalent with respect to the antigen (*e.g.*, DR5). The diabody portion of the construct in **Figure 4A** shows an Fc-containing diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 4B** shown and Fc-containing diabody which contains E-coil and K-coil Heterodimer-Promoting Domains comprising a cysteine residue and a linker (with an optional cysteine residue).

[0049] **Figure 5** shows the ability of anti-human DR5 monoclonal antibodies DR5 mAb 1 and DR5 mAb 2 to bind to human DR5 and to the DR5 of cynomolgus monkey.

[0050] **Figure 6, Panels A-H**, show the kinetics of binding of DR5 mAb 1 (**Panels A and E**), DR5 mAb 2 (**Panels B and F**), DR5 mAb 3 (**Panels C and G**) and DR5 mAb 4 (**Panels D and H**) for human DR 5 (**Panels A-D**) and for cynomolgus monkey DR5 (**Panels E-H**).

[0051] **Figures 7A-7B** show the ability of DR5 mAb 1 to differentially bind to tumor cells. **Figure 7A** shows histological stains of normal colon (**Panels A and G**), liver (**Panels B and H**), lung (**Panels C and I**), pancreas (**Panels D and J**), kidney (**Panels E and K**) and heart (**Panels F and L**) tissue. **Figure 7A, Panels A-F** show the results of tissue incubated with labeled DR5 mAb 1 (5 µg/mL). **Figure 7A, Panels G-L** show the results of tissue incubated with labeled isotype control mAb (5 µg/mL). **Figure 7B** shows histological stains of tumorous colon (**Panels A and C**) and tumorous lung (**Panels B and D**). **Figure 7B, Panels A-B** show the results of tissue incubated with labeled DR5 mAb 1 (5 µg/mL). **Figure 7B, Panels C-D** show the results of tissue incubated with labeled isotype control mAb (5 µg/mL).

[0052] **Figures 8A-8B** show the ability of DR5 mAb 2 to differentially bind to tumor cells. **Figure 8A** shows histological stains of normal colon (**Panel A**), kidney (**Panel B**), lung (**Panel C**), heart (**Panel D**), liver (**Panel E**) and pancreas (**Panel F**) tissue incubated with labeled DR5 mAb 2 (5 µg/mL). **Figure 8B** shows histological stains of tumorous colon (**Panels A and B**) and tumorous lung (**Panels C and D**). **Figure 8B, Panels A and C** show the results of tissue incubated with labeled DR5 mAb 2 (5 µg/mL). **Figure 8B, Panels B and D** show the results of tissue incubated with labeled isotype control mAb (5 µg/mL).

[0053] **Figures 9A-9K** show the ability of the DR5 mAb 2 x CD3 mAb 2 diabody to mediate the cytotoxicity of 786O renal cell adenocarcinoma cells (**Figure 9A**), A498 kidney carcinoma cells (**Figure 9B**), AsPC1 pancreatic adenocarcinoma cells (**Figure 9C**), LNCap androgen-sensitive human prostate adenocarcinoma cells (**Figure 9D**), SW48 colorectal adenocarcinoma cells (**Figure 9E**), A549 adenocarcinomic human alveolar basal epithelial cells (**Figure 9F**), SKMES human lung cancer cells (**Figure 9G**), DU145 human prostate cancer cells (**Figure 9H**), A375 human malignant melanoma cells (**Figure 9I**), SKBR3 human HER2-overexpressing breast carcinoma cells (**Figure 9J**) and JIMT human breast carcinoma cells (**Figure 9K**). Such target cells were incubated in the presence of peripheral blood mononuclear cells (PBMC) for 24 hours at an effector to target cell ratio of 20:1 or 30:1. The percentage cytotoxicity of the target cells was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells.

[0054] **Figures 10A-10F** show the unexpected superiority of DR5 mAb 1 and DR5 mAb 2. Superiority was assessed by comparing the ability of DR5 x CD3 diabodies having the VL and VH Domains of DR5 mAb 1, DR5 mAb 2, DR5 mAb 3, or DR5 mAb 4, to mediate the cytotoxicity of tumor cells. The employed target tumor cells were: A549 adenocarcinomic human alveolar basal epithelial cells (**Figure 10A**), SKMES human lung cancer cells (**Figure 10B**), DU145 human prostate cancer cells (**Figure 10C**), A375 human malignant melanoma cells (**Figure 10D**), and SKBR3 human HER2-overexpressing breast carcinoma cells (**Figure 10E**) and JIMT human breast carcinoma cells (**Figure 10F**).

[0055] **Figure 11** shows the ability of DR5 mAb 2 x CD3 mAb 2 diabody and its humanized derivatives: hDR5 mAb 2 (2.2) x CD3 mAb 2, hDR5 mAb 2 (2.3) x CD3 mAb 2, hDR5 mAb 2 (2.4) x CD3 mAb 2, or hDR5 mAb 2 (2.5) x CD3 mAb 2 to simultaneously bind to DR5 and to CD3.

[0056] **Figure 12** shows the ability of DR5 mAb 2 x CD3 mAb 2 diabody and its humanized derivatives: hDR5 mAb 2 (2.2) x CD3 mAb 2, hDR5 mAb 2 (2.3) x CD3 mAb 2, hDR5 mAb 2 (2.4) x CD3 mAb 2, or hDR5 mAb 2 (2.5) x CD3 mAb 2 to mediate the cytotoxicity of Colo205 colorectal carcinoma cells.

[0057] **Figure 13** shows the growth inhibition curves of COLO205 cells treated with DR5 mAb 1, DR5 mAb 2, cross-linked DR5 mAb 1, cross-linked DR5 mAb 2, or the combination of DR5 mAb 1 and DR5 mAb 2 without cross-linking. Cross-linked DR5 mAb 1, cross-linked DR5 mAb 2, and the combination of DR5 mAb 1 and DR5 mAb 2 without cross-linking are able to inhibit the growth of COLO205 cells.

[0058] **Figure 14A-14C** show that both cross-linked DR5 mAb 1 and cross-linked DR5 mAb 2 induce apoptosis as measured by increased production of nucleosomes (**Figure 14A**), increased cleaved PARP (**Figure 14B**), and increased active caspase 3 (**Figure 14C**).

[0059] **Figure 15A-15B** show that a representative tetravalent DR5-Binding Molecule (a bispecific E-coil/K-coil-Fc Region-containing diabody tetravalent for DR5 designated “**DR5 mAb 2 x DR5 mAb 1 Fc diabody**”) does not bind normal tissues. **Figure 15A** shows histological stains of normal colon (**Panels A and G**), liver (**Panels B and H**), lung (**Panels C and I**), pancreas (**Panels D and J**), kidney (**Panels E and K**) and heart (**Panels F and L**) tissue. **Figure 15A, Panels A-F** show the results of tissue incubated with labeled with the tetravalent DR5-Binding Molecule (DR5 mAb 2 x DR5 mAb 1 Fc diabody at 0.625 µg/mL). **Figure 15A, Panels G-L** show the results of tissue incubated with labeled a control diabody (4-4-20 x CD3 mAb 2 at 0.625 µg/mL). **Figure 15B** shows representative histological stains of additional normal liver samples. **Figure 15B, Top Panel** shows the results of tissue incubated with a labeled tetravalent DR5-Binding Molecule (DR5 mAb 2 x DR5 mAb 1 Fc diabody at 0.625 µg/mL).

Figure 15 B, Bottom Panel show the results of tissue incubated with labeled control diabody (4-4-20 x CD3 mAb 2 at 0.625 µg/mL).

[0060] **Figure 16** show that a representative tetravalent DR5-Binding Molecule (a bispecific E-coil/K-coil-Fc Region-containing diabody tetravalent for DR5) strongly binds tumorous tissues. **Figure 16** shows histological stains of tumorous breast (**Panels A and E**), tumorous colon (**Panels B and F**), tumorous lung (**Panels C and G**), and tumorous prostate tissue (**Panels D and H**). **Figure 16, Panels A-D** show the results of tissue incubated with a labeled tetravalent DR5-Binding Molecule (DR5 mAb 2 x DR5 mAb 2 Fc diabody at 0.625 µg/mL). **Figure 16, Panels E-H** show the results of tissue incubated with labeled control diabody (4-4-20 x CD3 mAb 2 at 0.625 µg/mL).

[0061] **Figure 17A-17C** shows the growth inhibition curves of COLO205 (**Figure 17A**), A498 (**Figure 17B**) and SKMES (**Figure 17C**) cells treated with six different representative tetravalent DR5-Binding Molecules (monospecific or bispecific E-coil/K-coil-Fc Region-containing diabodies tetravalent for DR5) including two comprising Fc Region variants with reduce binding to FcγRs and reduced effector function activity. All the tetravalent DR5-Binding Molecules have potent cytotoxicity in these cells and were more potent than the TRAIL-His positive control.

[0062] **Figure 18** shows that representative tetravalent DR5-Binding Molecules (monospecific or bispecific E-coil/K-coil-Fc Region-containing diabodies tetravalent for DR5) induce apoptosis as measured by increased production of nucleosomes.

[0063] **Figure 19** shows the growth inhibition curves of COLO205 cells treated with different DR5-Binding Molecules including three different representative tetravalent DR5-Binding Molecules (monospecific or bispecific E-coil/K-coil-Fc Region-containing diabodies tetravalent for DR5); and two different anti-DR5 antibodies (DR5 mAb 8 (KMTR2); and DR5 mAb 4 (conatumumab) with and without cross-linking). Each DR5-Binding Molecule tested comprised an Fc Region variant with reduce binding to FcγRs and reduced effector function activity. The cytotoxic activity of the tetravalent DR5-Binding Molecules is independent of cross-linking and more potent than the anti-DR5 antibodies DR5 mAb 4 and DR5 mAb 8.

[0064] **Figure 20** shows the cytotoxicity activity of several different DR5-Binding Molecules including three different representative tetravalent DR5-Binding Molecules (monospecific or bispecific E-coil/K-coil-Fc Region-containing diabodies tetravalent for DR5); and two different anti-DR5 antibodies (DR5 mAb 8 (KMTR2), and DR5 mAb 4 (conatumumab)) on cancer stem cell-like (CSLC) RECA0201 cells. Each DR5-Binding Molecule tested comprised an Fc Region variant with reduced binding to FcγRs and reduced effector function activity. All the tetravalent DR5-Binding Molecules have potent cytotoxicity in these cells and were more potent than the anti-DR5 antibodies DR5 mAb 4 and DR5 mAb 8.

[0065] **Figure 21** shows the change in tumor volume over time in mice implanted with COLO205 cells. Female hCD16A FOX N1 mice (n=7/group) were implanted subcutaneously (SC) with COLO205 cells Day 0. The mice were then treated twice a week with a representative tetravalent DR5-Binding Molecule (monospecific tetravalent DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA) at 0.5, 0.05, 0.005 mg/kg); two different DR5 antibodies (DR5 mAb 4 (AA) at 5 mg/kg; DR5 mAb 8 (AA) at 0.5, 0.05, 0.005 mg/kg), or vehicle. Tumor volume is shown as a group mean ± SEM.

Detailed Description of the Invention:

[0066] The present invention is directed to multivalent DR5-Binding Molecules that comprise Binding Domain(s) of anti-DR5 antibodies, and particularly Binding Domain(s) of anti-human DR5 antibodies. The DR5-Binding Molecules of the present invention include bivalent and tetravalent molecules having two, three or four DR5-Binding Domains each capable of binding human DR5. In particular, the present invention is directed to multivalent DR5-Binding Molecules that comprise diabodies, and more particularly, diabodies that comprise a covalently bonded complex of two or more polypeptide chains. The invention particularly pertains to such multivalent DR5-Binding Molecules that comprise fragments of the anti-DR5 antibodies DR5 mAb 1 and/or DR5 mAb 2, and/or humanized and chimeric versions of such antibodies.

I. Antibodies and Their Binding Domains

[0067] The antibodies of the present invention are immunoglobulin molecules capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid,

polypeptide, *etc.*, through at least one antigen recognition site, located in the Variable Region of the immunoglobulin molecule. As used herein, the terms “**antibody**” and “**antibodies**” refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, polyclonal antibodies, camelized antibodies, single-chain Fvs (scFv), single-chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked bispecific Fvs (sdFv), intrabodies, and and epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen-binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass. In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents. The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs (Chan, C.E. *et al.* (2009) “*The Use Of Antibodies In The Treatment Of Infectious Diseases*,” Singapore Med. J. 50(7):663-666). Nearly 200 antibody-based drugs have been approved for use or are under development.

[0068] The term “**monoclonal antibody**” refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term “monoclonal antibody” encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂ Fv), single-chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of “antibody.” Methods of making monoclonal antibodies are known in the art. One

method which may be employed is the method of Kohler, G. *et al.* (1975) “*Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity*,” *Nature* 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) “*Review of Selected Adjuvants Used in Antibody Production*,” *ILAR J.* 37(3):119-125). In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freud's adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the multispecific (*e.g.*, bispecific, trispecific and tetraspecific) molecules of the invention as well as an affinity optimized antibody, a chimeric antibody, a humanized antibody, or a caninized antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences.

[0069] Natural antibodies (such as IgG antibodies) are composed of two **Light Chains** complexed with two **Heavy Chains**. Each light chain contains a Variable Domain (**VL**) and a Constant Domain (**CL**). Each heavy chain contains a Variable Domain (**VH**), and three Constant Domains (**CH1**, **CH2** and **CH3**), and a Hinge Domain located between the **CH1** and **CH2** Domains. The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is thus a tetramer having two light chains and two heavy chains, usually expressed as a glycoprotein of about 150,000 Da. The amino-terminal (“N”) portion of each chain includes a Variable Domain of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal (“C”) portion of each chain defines a constant region, with light chains having a single constant domain and heavy chains usually having three constant domains and a hinge region. Thus, the structure of the light chains of an IgG molecule is n-VL-CL-c and the structure of the IgG heavy chains is n-VH-CH1-H-CH2-CH3-c (where H is the hinge region, and n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide). The Variable Domains of an IgG molecule consist of the complementarity determining regions (**CDR**), which contain the residues in contact with epitope, and non-CDR segments, referred to as framework segments (**FR**), which in general maintain the structure and determine the positioning of the CDR loops so as to permit such contacting (although certain framework residues may also contact antigen). Thus, the **V_L** and **V_H** Domains have the structure n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c. Polypeptides that are (or may serve as) the first, second and third CDR of an antibody Light Chain are herein respectively designated **CDR_{L1} Domain**, **CDR_{L2} Domain**, and **CDR_{L3} Domain**. Similarly, polypeptides that are (or may serve as) the first, second and third CDR of an antibody Heavy Chain are herein respectively designated **CDR_{H1} Domain**, **CDR_{H2} Domain**, and **CDR_{H3} Domain**. Thus, the terms CDR_{L1} Domain, CDR_{L2} Domain, CDR_{L3} Domain, CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are directed to polypeptides that when incorporated into a protein cause that protein to be able to bind to a specific epitope regardless of whether such protein is an antibody having light and heavy chains or a diabody or a single-chain binding molecule (*e.g.*, an scFv, a BiTe, *etc.*), or is another type of protein.

[0070] The invention also encompasses multivalent DR5-Binding Molecules comprising single-chain Variable Domain fragments (“**scFv**”) of the anti-DR5

antibodies of this invention. Single-chain Variable Domain fragments are made by linking Light and/ or Heavy chain Variable Domain by using a short linking peptide. Bird *et al.* (1988) (“*Single-Chain Antigen-Binding Proteins*,” Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) “*Single-Chain Antigen-Binding Proteins*,” Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0071] The invention also particularly encompasses multivalent DR5-Binding Molecules comprising humanized variants of the anti-DR5 antibodies of the invention. The term “**humanized**” antibody refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and /or sequence of a human immunoglobulin.

[0072] The anti-human DR5 antibodies of the present invention include humanized, chimeric or caninized derivatives of antibodies DR5 mAb 1 or DR5 mAb 2. The polynucleotide sequence of the variable domains of such antibodies may be used for genetic manipulation to generate such derivatives and to improve the affinity, or other characteristics of such antibodies. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These

are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing process (3) the actual humanizing methodologies /techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[0073] The antigen-binding site may comprise either complete variable domains fused onto constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the Variable Domains. Antigen-binding sites may be wild-type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign Variable Domains remains (LoBuglio, A.F. *et al.* (1989) “*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*,” Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the Variable Domains as well so as to reshape them as closely as possible to human form. It is known that the Variable Domains of both heavy and light chains contain three complementarity determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the Variable Domains can be “reshaped” or “humanized” by grafting CDRs derived from non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) “*Reshaping Human Antibodies for Therapy*,” Nature 332:323-327; Verhoeyen, M. *et al.* (1988) “*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*,” Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) “*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*,” Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) “*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*,” Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) “*Reshaping A*

Therapeutic CD4 Antibody,” Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) “*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*,” Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) “*Humanized Antibodies For Antiviral Therapy*,” Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) “*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*,” Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) “*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*,” J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which are altered with respect to the original antibody, which differ in sequence relative to the original antibody.

[0074] A number of “humanized” antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent V regions and their associated complementarity determining regions (CDRs) fused to human constant domains (see, for example, Winter *et al.* (1991) “*Man-made Antibodies*,” Nature 349:293-299; Lobuglio *et al.* (1989) “*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*,” Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224 (1989), Shaw *et al.* (1987) “*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*,” J. Immunol. 138:4534-4538, and Brown *et al.* (1987) “*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*,” Cancer Res. 47:3577-3583). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody constant domain (see, for example, Riechmann, L. *et al.* (1988) “*Reshaping Human Antibodies for Therapy*,” Nature 332:323-327; Verhoeyen, M. *et al.* (1988) “*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*,” Science 239:1534-1536; and Jones *et al.* (1986) “*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*,” Nature 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for

example, European Patent Publication No. 519,596. These “humanized” molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) “*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins,*” Nucl. Acids Res. 19:2471-2476 and in U.S. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

II. Fc Receptors (FcγRs)

[0075] The CH2 and CH3 Domains of the two heavy chains interact to form the **Fc Region**, which is a domain that is recognized by cellular **Fc Receptors (FcγRs)**. As used herein, the term “Fc Region” is used to define a C-terminal region of an IgG heavy chain. The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (**SEQ ID NO:1**):

231	240	250	260	270	280
APELLGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	
	290	300	310	320	330
GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	
	340	350	360	370	380
PIEKTISKAK	GQPREPQVYT	LPPSREEMTK	NQVSLTCLVK	GFYPSDIAVE	
	390	400	410	420	430
WESNGQPENN	YKTTTPVLDS	DGSFFLYSKL	TVDKSRWQQG	NVFSCSVMHE	
	440	447			
ALHNHYTQKS	LSLSPG	K			

[0076] The amino acid sequence of the CH2-CH3 domain of an exemplary human IgG2 is (SEQ ID NO:164):

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231      240      250      260      270      280
APPVA-GPSV  FLFPPKPKDT  LMISRTPEVT  CVVVDVSHED  PEVQFNWYVD

      290      300      310      320      330
GVEVHNAKTK  PREEQFNSTF  RVVSVLTVVH  QDWLNGKEYK  CKVSNKGLPA

      340      350      360      370      380
PIEKTISKTK  GQPREPQVYT  LPPSREEMTK  NQVSLTCLVK  GFYPSDISVE

      390      400      410      420      430
WESNGQPENN  YKTTTPMLDS  DGSFFLYSKL  TVDKSRWQQG  NVFSCSVMHE

      440      447
ALHNHYTQKS  LSLSPGK

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[0077] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (SEQ ID NO:168):

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231      240      250      260      270      280
APELLGGPSV  FLFPPKPKDT  LMISRTPEVT  CVVVDVSHED  PEVQFKWYVD

      290      300      310      320      330
GVEVHNAKTK  PREEQYNSTF  RVVSVLTVLH  QDWLNGKEYK  CKVSNKALPA

      340      350      360      370      380
PIEKTISKTK  GQPREPQVYT  LPPSREEMTK  NQVSLTCLVK  GFYPSDIAVE

      390      400      410      420      430
WESSGQPENN  YNTTPMLDS  DGSFFLYSKL  TVDKSRWQQG  NIFSCSVMHE

      440      447
ALHNRFTQKS  LSLSPGK

```

[0078] The amino acid sequence of the CH2-CH3 domain of an exemplary human IgG4 is (SEQ ID NO:103):

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231      240      250      260      270      280
APEFLGGPSV  FLFPPKPKDT  LMISRTPEVT  CVVVDVSQED  PEVQFNWYVD

      290      300      310      320      330
GVEVHNAKTK  PREEQFNSTY  RVVSVLTVLH  QDWLNGKEYK  CKVSNKGLPS

      340      350      360      370      380
SIEKTISKAK  GQPREPQVYT  LPPSQEEMTK  NQVSLTCLVK  GFYPSDIAVE

      390      400      410      420      430
WESNGQPENN  YKTTTPVLDS  DGSFFLYSRL  TVDKSRWQEG  NVFSCSVMHE

      440      447
ALHNHYTQKS  LSLSLGK

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[0079] Throughout the present specification, the numbering of the residues in an IgG heavy chain is that of the EU index as in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, NH1, MD (1991), expressly incorporated herein by references. The “EU index as in Kabat” refers to the numbering of the human IgG1 EU antibody. Amino acids from the Variable Domains of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid. Kabat’s numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody light chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody light chain.

[0080] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, “*The Human IgG Subclasses: Molecular Analysis Of Structure, Function And Regulation*,” Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet. 50:199-211). It is specifically contemplated that the antibodies of the present invention may incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and are not limited to the allotype, isoallotype or haplotype of the sequences provided herein. Furthermore, in some expression systems the C-terminal amino acid residue (bolded above) of the CH3 Domain may be post-translationally removed. Accordingly, the C-terminal residue of the CH3 Domain is an optional amino acid residue in the Multivalent DR5-

Binding Molecules of the invention. Exemplary Multivalent DR5-Binding Molecules lacking the C-terminal residue of SEQ ID NO:1 are provided below. Also specifically encompassed by the instant invention are such constructs comprising the C-terminal residue.

[0081] Activating and inhibitory signals are transduced through the Fc Receptors (FcγRs) following their ligation to an Fc Region. These diametrically opposing functions result from structural differences among the different receptor isoforms. Two distinct domains within the cytoplasmic signaling domains of the receptor called immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs) account for the different responses. The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγR complexes include FcγRI, FcγRIIA, FcγRIIIA, whereas ITIM-containing complexes only include FcγRIIB. Human neutrophils express the FcγRIIA gene. FcγRIIA clustering via immune complexes or specific antibody cross-linking serves to aggregate ITAMs along with receptor-associated kinases which facilitate ITAM phosphorylation. ITAM phosphorylation serves as a docking site for Syk kinase, activation of which results in activation of downstream substrates (*e.g.*, PI₃K). Cellular activation leads to release of proinflammatory mediators. The FcγRIIB gene is expressed on B lymphocytes; its extracellular domain is 96% identical to FcγRIIA and binds IgG complexes in an indistinguishable manner. The presence of an ITIM in the cytoplasmic domain of FcγRIIB defines this inhibitory subclass of FcγR. Recently the molecular basis of this inhibition was established. When co-ligated along with an activating FcγR, the ITIM in FcγRIIB becomes phosphorylated and attracts the SH2 domain of the inositol polyphosphate 5'-phosphatase (SHIP), which hydrolyzes phosphoinositol messengers released as a consequence of ITAM-containing FcγR-mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca⁺⁺. Thus, cross-linking of FcγRIIB dampens the activating response to FcγR ligation and inhibits cellular responsiveness. B cell activation, B cell proliferation and antibody secretion is thus aborted.

III. Multivalent Antibodies, Multivalent Diabodies and DART[®] Diabodies

[0082] The ability of an antibody to bind an epitope of an antigen depends upon the presence and amino acid sequence of the antibody's VL and VH Domains. Interaction of an antibody light chain and an antibody heavy chain and, in particular, interaction of its VL and VH Domains forms one of the two epitope-binding sites of a natural antibody. Natural antibodies are capable of binding to only one epitope species (*i.e.*, they are monospecific), although they can bind multiple copies of that species (*i.e.*, exhibiting bivalency or multivalency).

[0083] The binding domains of the present invention bind to epitopes in an **"immunospecific"** manner. As used herein, an antibody, diabody or other epitope binding molecule is said to **"immunospecifically"** bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that immunospecifically binds to a viral epitope is an antibody that binds this viral epitope with greater affinity, avidity, more readily, and /or with greater duration than it immunospecifically binds to other viral epitopes or non-viral epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that immunospecifically binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means "specific" binding. Two molecules are said to be capable of binding to one another in a **"physiospecific"** manner, if such binding exhibits the specificity with which receptors bind to their respective ligands.

[0084] The functionality of antibodies can be enhanced by generating multispecific antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen) and/or by generating antibody-based molecule having higher valency (*i.e.*, more than two binding sites) for the same epitope and/or antigen.

[0085] In order to provide molecules having greater capability than natural antibodies, a wide variety of recombinant bispecific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use linker peptides either to fuse a further binding protein (*e.g.*, an scFv, VL, VH, *etc.*) to, or within the antibody core (IgA, IgD, IgE, IgG or IgM, or to fuse multiple antibody binding portions *e.g.*, two Fab fragments or scFvs. Alternative formats use linker peptides to fuse a binding protein (*e.g.*, an scFv, VL, VH, *etc.*) to an a dimerization domain such as the CH2-CH3 Domain or alternative polypeptides (WO 2005/070966, WO 2006/107786A WO 2006/107617A, WO 2007/046893). Typically, such approaches involve compromises and trade-offs. For example, PCT Publications Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose that the use of linkers may cause problems in therapeutic settings, and teaches a trispecific antibody in which the CL and CH1 Domains are switched from their respective natural positions and the VL and VH Domains have been diversified (WO 2008/027236; WO 2010/108127) to allow them to bind to more than one antigen. Thus, the molecules disclosed in these documents trade binding specificity for the ability to bind additional antigen species. PCT Publications Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. The document notes that the CH2 Domain likely plays only a minimal role in mediating effector function. PCT Publications Nos. WO 2010/028797, WO2010028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Regions have been replaced with additional VL and VH Domains, so as to form trivalent binding molecules. PCT Publications Nos. WO 2003/025018 and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv Domains. PCT Publications No. WO 2013/006544 discloses multivalent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. Thus, the molecules disclosed in these documents trade all or some of the capability of mediating effector function for the ability to bind additional antigen species. PCT Publications Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715, WO 2007/075270, WO 1998/002463, WO

1992/022583 and WO 1991/003493 disclose adding additional binding domains or functional groups to an antibody or an antibody portion (*e.g.*, adding a diabody to the antibody's light chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another). Thus, the molecules disclosed in these documents trade native antibody structure for the ability to bind additional antigen species.

[0086] The art has additionally noted the capability to produce **diabodies** that differ from such natural antibodies in being capable of binding two or more different epitope species (*i.e.*, exhibiting bispecificity or multispecificity in addition to bivalency or multivalency) (see, *e.g.*, Holliger *et al.* (1993) “*Diabodies’: Small Bivalent And Bispecific Antibody Fragments*,” Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*,” J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) “*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*,” Protein Eng. Des. Sel. 17(1):21-27; Wu, A. *et al.* (2001) “*Multimerization Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange*,” Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) “*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*,” Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) “*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*,” Cancer Res. 69(12):4941-4944).

[0087] The design of a diabody is based on the antibody derivative known as a single-chain Variable Domain fragment (**scFv**). Such molecules are made by linking Light and/ or Heavy chain Variable Domain by using a short linking peptide. Bird *et al.* (1988) (“*Single-Chain Antigen-Binding Proteins*,” Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy

terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) “*Single-Chain Antigen-Binding Proteins*,” Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0088] The provision of non-monospecific **diabodies** provides significant advantages over antibodies, including but not limited to, the capacity to co-ligate and co-localize cells that express different epitopes and the capacity to form inter- and/or intra molecular interactions by binding different epitopes of the same antigen. Bivalent diabodies thus have wide-ranging applications including therapy and immunodiagnosis. Bispecificity allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) “*Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris*,” Protein Eng. 10:1221). Of particular importance is the co-ligating of differing cells, for example, the cross-linking of cytotoxic T cells to tumor cells (Staerz *et al.* (1985) “*Hybrid Antibodies Can Target Sites For Attack By T Cells*,” Nature 314:628-631, and Holliger *et al.* (1996) “*Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody*,” Protein Eng. 9:299-305; Marvin *et al.* (2005) “*Recombinant Approaches To IgG-Like Bispecific Antibodies*,” Acta Pharmacol. Sin. 26:649-658).

[0089] However, the above advantages come at a salient cost. The formation of such non-monospecific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to monospecific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-monospecific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” *Protein Eng.* 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” *Protein Eng.* 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) “*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*,” *Prot. Engr. Des. Sel.* 17:21-27; Asano *et al.* (2004) “*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*,” Abstract 3P-683, *J. Biochem.* 76(8):992; Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*,” *Protein Eng.* 13(8):583-588; Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*,” *J. Biol. Chem.* 280(20):19665-19672).

[0090] However, the art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*,” *J. Biol. Chem.* 280(20):19665-19672).

[0091] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-monospecific diabodies, termed **DART[®] (Dual Affinity Re-Targeting Reagents)** diabodies; see, *e.g.*, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538; and Moore, P.A. *et al.* (2011) “*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*,” *Blood* 117(17):4542-4551; Veri, M.C. *et al.* (2010) “*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*,” *Arthritis Rheum.* 62(7):1933-1943; Johnson, S. *et al.* (2010) “*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*,” *J. Mol. Biol.* 399(3):436-449). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species that permit disulfide bonds to form and thereby covalently bond two polypeptide chains. For example, the addition of a cysteine residue to the c-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[0092] Each of the two polypeptides of the simplest bispecific **DART[®]** diabody comprises three Domains. The first polypeptide comprises (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of a first immunoglobulin (VL1), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), and (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a Heterodimer-Promoting Domain that serves to promote heterodimerization with the second polypeptide of the diabody and to covalently bond the diabody's first and second polypeptides to one another. The second polypeptide contains (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of the second immunoglobulin (VL2), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable

Domain of the first immunoglobulin (VH1), and (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and, a complementary Heterodimerization-Promoting Domain that complexes with the Heterodimerization-Promoting Domain of the first polypeptide chain in order to promote heterodimerization with the first polypeptide chain. The cysteine residue (or cysteine-containing domain) of the third domain of the second polypeptide serves to promote the covalent bonding of the second polypeptide chain to the first polypeptide chain of the diabody. Such molecules are stable, potent and have the ability to simultaneously bind two or more different antigens or two different epitopes on the same antigen. In one embodiment, the Third Domains of the first and second polypeptides each contain a cysteine residue, which serves to bind the polypeptides together via a disulfide bond. **Figure 1** provides a schematic of such a diabody, which utilizes E-coil/K-coil heterodimerization domains and a cysteine containing linker for covalent bonding. As provided in **Figures 2-4**, one or both of the polypeptides may additionally possess the sequence of a CH2-CH3 Domain, such that complexing between the two diabody polypeptides forms an Fc Region that is capable of binding to the Fc receptor of cells (such as B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells). As provided in more detail below, the CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains.

[0093] Many variations of such molecules have been described (see, *e.g.*, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538). These Fc Region-containing **DART®** diabodies may comprise two pairs of polypeptide chains. The first polypeptide comprises (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of a first immunoglobulin (VL1), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), (iii) a Third Domain that contains a cysteine residue (or a cysteine containing domain) and a Heterodimerization-Promoting Domain that serves to promote heterodimerization with the second polypeptide of the diabody and to covalently bond the diabody's first and

second polypeptides to one another, and (iv) a CH2-CH3 Domain. The second polypeptide contains (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of the second immunoglobulin (VL2), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of the first immunoglobulin (VH1), and (iii) a Third Domain that contains a cysteine residue (or a cysteine containing domain) and a Heterodimerization-Promoting Domain capable of interacting with the Third Domain of the first polypeptide chain in order to promote heterodimerization and covalent bonding between the two polypeptide chains. Here two first polypeptides complex with each other to form an Fc Region. **Figures 3 and 4A-4B** provide schematics of three variations of such diabodies utilizing different heterodimer-promoting domains. Other Fc Region-containing DART® diabodies may comprise three polypeptide chains. The first polypeptide of such DART® diabodies contains three Domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second polypeptide of such DART® diabodies contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such DART® diabodies comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such a diabody associate together to form a VL1/VH1 binding site that is capable of binding to the epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. Such more complex diabodies also possess cysteine-containing domains which function to form a covalently bonded complex. Thus, the first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Region that is stabilized via a disulfide bond.

[0094] Alternative constructs are known in the art for applications where a tetravalent molecule is desirable but an Fc is not required including, but not limited to, tetravalent tandem antibodies, also referred to as “**TandAbs**” (see, *e.g.* United States Patent Publications Nos. 2005-0079170, 2007-0031436, 2010-0099853, 2011-020667 2013-0189263; European Patent Publication Nos. EP 1078004, EP 2371866, EP 2361936 and EP 1293514; PCT Publications Nos. WO 1999/057150, WO

2003/025018, and WO 2013/013700) which are formed by the homo-dimerization of two identical chains each possessing a VH1, VL2, VH2, and VL2 Domain.

IV. Anti-Human DR5-Binding Molecules of the Present Invention

[0095] The preferred multivalent DR5-Binding Molecules of the present invention are capable of binding to a continuous or discontinuous (*e.g.*, conformational) portion (**epitope**) of human DR5 . The DR5-Binding Molecules of the present invention will preferably also exhibit the ability to bind to the DR5 molecules of one or more non-human species, especially, murine, rodent, canine, and primate species. The amino acid sequence of human DR5 precursor (NCBI Sequence NP_003833.4) (**SEQ ID NO:2**) is:

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MEQRGQNAPA ASGARKRHGP GPREARGARP GLRVPKTLVL VVAVLLLLVS
AESALITQQD LAPQQRVAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG
QDYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP
EMCRKCRTGC PRGMVKVGDC TPWSDIECVH KESGTKHSGE APAVEETVTS
SPGTPASPCS LSGIIIGVTV AAVVLIVAVF VCKSLWKKV LPYLKGICSG
GGDPERVDR SSQRPGAEDN VLNEIVSILQ PTQVPEQEME VQEPAEPTGV
NMLSPGESEH LLEPAEAERS QRRLLVPAN EGDPTETLRQ CFDDFADLVP
FDSWEPLMRK LGLMDNEIKV AKAEAAGHRD TLYTMLIKWV NKTGRDASVH
TLLEDALETLG ERLAKQKIED HLLSSGKFMY LEGNADSAMS
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[0096] Of the 440 amino acid residues of DR5 (**SEQ ID NO:2**), residues 1-55 are a signal sequence, residues 57-94 are a first Cysteine-Rich Domain (**CRD**), residues 97-137 are a second Cysteine-Rich Domain (**CRD**), residues 138-178 are a third Cysteine-Rich Domain (**CRD**), residues 211-231 are the Transmembrane Domain, and residues 232-440 are the Cytoplasmic Domain (containing the receptor's Death Domain).

[0097] The present invention includes multivalent DR5-Binding Molecules possessing at least two, and preferably, at least four DR5 binding sites. The DR5 binding sites may bind the same DR5 epitope or different DR5 epitopes. Accordingly, the multivalent DR5-Binding Molecules of the invention may be monospecific, binding just one epitope of DR5, or they may be multispecific, binding different epitopes of DR5.

[0098] Exemplary multivalent DR5-Binding Molecules of the present invention includes bispecific molecules (*e.g.*, bispecific antibodies, non-monospecific diabodies,

etc.) possessing at least one “**First Binding Site**” which binds a “**First DR5 Epitope**” and at least one “**Second Binding Site**” which binds a “**Second DR5 Epitope**.” Such molecules are bispecific with respect to said first DR5 epitope and second DR5 epitope and are at least bivalent with respect to DR5. Bispecific multivalent DR5-Binding Molecules exhibiting a higher valency for DR5 may be generated by the addition of one or more additional First Binding Sites and/or Second Binding Sites. Exemplary multivalent DR5-Binding Molecules of the present invention also include multispecific molecules possessing at least three, or at least four, or more different binding sites each of which binds a different DR5 epitope. Such molecules are multispecific with respect to said DR5 epitopes and are multivalent with respect to DR5.

[0099] One exemplary bispecific multivalent DR5-Binding Molecule of the present invention possesses two First Binding Sites which bind a first DR5 epitope, and two Second Binding Sites which bind a second DR5 epitope. Such a DR5-Binding Molecule is bispecific with respect to said first and second DR5 epitopes and tetravalent with respect to DR5.

[00100] Preferably, the multispecific multivalent (*e.g.*, bispecific, bivalent) DR5-Binding Molecules of the invention are capable of simultaneously binding to the different DR5 epitopes. Such binding may be intramolecular (*i.e.*, to the different DR5 epitopes on a single DR5 polypeptide) and/or intermolecular (*i.e.*, to the different DR5 epitopes on separate DR5 polypeptides).

[00101] Exemplary multivalent DR5-Binding Molecules of the present invention also includes monospecific molecules (*e.g.*, bispecific antibodies, monospecific diabodies, *etc.*) possessing at least two, preferably at least four, binding sites which bind the same DR5 epitope. Such molecules are monospecific with respect to said DR5 epitope, and are at least bivalent, preferably tetravalent, with respect to DR5. It will be noted that where more than two binding sites that bind the same DR5 epitope are present, the multivalent DR5-Binding Molecule will remain monospecific with respect to the epitope but will exhibit a higher valency for DR5.

[00102] One exemplary monospecific multivalent DR5-Binding Molecule of the present invention possesses four binding sites which bind the same DR5 epitope, and is monospecific with respect to said DR5 epitope and tetravalent with respect to DR5.

[00103] Preferably, the monospecific multivalent (*e.g.*, monospecific, bivalent) DR5-Binding Molecules of the invention are capable of simultaneously binding to the DR5 epitope. Such binding is intermolecular (*i.e.*, to the same DR5 epitope on separate DR5 polypeptides).

[00104] The multivalent DR5-Binding Molecules of the present invention may possess the VL and/or VH Domains of one or more of the anti-DR5 antibodies disclosed herein. The preferred multivalent DR5-Binding Molecules of the present invention possess the VL and/or VH Domains of anti-human DR5 monoclonal antibodies “**DR5 mAb 1**” and/or “**DR5 mAb 2**,” and/or “**hDR5 mAb2**,” and more preferably possess 1, 2 or all 3 of the CDR_{LS} of the VL Domain and/or 1, 2 or all 3 of the CDR_{HS} of the VH Domain of such anti-human DR5 monoclonal antibodies. The amino acid sequences of particular anti-DR5-Binding Molecules, and polynucleotides encoding the same, are provided below. The present invention also encompasses minor variations of these sequences including, for example amino acid substitutions of the C-terminal and/or N-terminal amino acid residues which may be introduced to facilitate subcloning.

A. The Anti-Human DR5 Antibody DR5 mAb 1

[00105] The amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**) is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**RASKSVS** **SSGYSYMH**WY QQKPGQPPKV
LIF**LSSNLDS** GVPARFSGSG SGTDFTLNIH PVEDGDAATY YC**QHSRDLPP**
TFGGGTKLEI K

CDR_{L1} of DR5 mAb 1 (**SEQ ID NO:4**): **RASKSVSSSGYSYMH**

CDR_{L2} of DR5 mAb 1 (**SEQ ID NO:5**): **LSSNLDS**

CDR_{L3} of DR5 mAb 1 (**SEQ ID NO:6**): **QHSRDLPP**

[00106] The VL Domain of DR5 mAb 1 is preferably encoded by a polynucleotide (**SEQ ID NO:7**) having the sequence shown below (polynucleotides encoding the CDR_L residues are shown in underline):

gacattgtgc tgacacagtc tectgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcactggtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccagggttcag
tggcagtggg tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgt cagc acagtaggga tcttcctccg
acgttcgggtg gaggcaccaa gctggaaatc aaa

[00107] The amino acid sequence of the VH Domain of DR5 mAb 1 (SEQ ID NO:8) is shown below (CDR_H residues are shown underlined), the C-terminal amino acid may be substituted with alanine to facilitate subcloning of this VH Domain:

EVKFLES GGG LVQPGGSLKL SCVAS GFDFS RYWMSWVRQA PGKGLEWIGE
INPDSNTINY TPSLKDKFII SRDNAKNTLY LQMTKVRSED TALYYCTRRA
YYGNPAWFAY WGQGTILVTVSS

CDR_H1 of DR5 mAb 1 (SEQ ID NO:9): **GFDFSRYWMS**

CDR_H2 of DR5 mAb 1 (SEQ ID NO:10): **EINPDSNTINYTPSLKD**

CDR_H3 of DR5 mAb 1 (SEQ ID NO:11): **RAYYGNPAWFAY**

[00108] The VH Domain of DR5 mAb 1 is preferably encoded by a polynucleotide (SEQ ID NO:12) having the sequence shown below (polynucleotides encoding the CDR_H residues are shown in underline):

gaggtgaagt ttctcgagtc tggaggtggc ctggtgcagc ctggaggatc
cctgaaactc tctgtgttag cctcag ggatt cgatttttagt agatactgga
tgagttgggt ccggcaggct ccagggaag ggctagaatg gattggag gaa
attaatccag atagcaatac gataaactat acgccatctc taaaggataa
attcatcatc tccagagaca acgcaaaaaa tacgctgtat ctgcaaataga
ccaaagtgag atctgaggac acagcccttt attattgtac aaga agggcc
tactatggta acccggcctg gtttgcttac tggggccaag ggactctggt
cactgtctct tcc

B. The Anti-Human DR5 Antibody DR5 mAb 2

1. Murine Anti-Human Antibody DR5 mAb 2

[00109] The amino acid sequence of the VL Domain of DR5 mAb 2 (SEQ ID NO:13) is shown below (CDR_L residues are shown underlined):

DIVMTQSHKF MSTSVGDRVS ITC KASQDVN TAVAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTIKSVQA EDLTLYYC QQ HYITPWTFGG
GTKLEIK

CDR_L1 of DR5 mAb 2 (SEQ ID NO:14): **KASQDVNTAVA**

CDR_L2 of DR5 mAb 2 (SEQ ID NO:15): **WASTRHT**

CDR_L3 of DR5 mAb 2 (SEQ ID NO:16): **QQHYITPWT**

[00110] The VL Domain of DR5 mAb 2 is preferably encoded by a polynucleotide (SEQ ID NO:17) having the sequence shown below (polynucleotides encoding the CDR_L residues are shown in underline):

gacattgtga tgacccagtc tcacaaattc atgtccactt cagtaggaga
cagggtcagc atcacctgca a aggccagtca ggatgtgaat actgctgtag
cctggtatca acaaaaacca gggcaatctc ctaaactact gatttacttgg
gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
tgggacagat tatacactca ccatacaaaag tgtgcaggct gaagacctga
cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga
ggcaccaagc tggaaatcaaa

[00111] The amino acid sequence of the VH Domain of DR5 mAb 2 (SEQ ID NO:18) is shown below (CDR_H residues are shown underlined):

KVQLQQSGAE LVKPGASVKL SCKASGYTFT EYILHWVKQK SGQGLEWIGW
FYPGNNNIKY NEKFKDKATL TADKSSSTVY MELSRLTSED SAVYFCARHE
QGPGYFDYWG QGTTLTVSS

CDR_H1 of DR5 mAb 2 (SEQ ID NO:19): **GYTFTEYILH**

CDR_H2 of DR5 mAb 2 (SEQ ID NO:20): **WFYPGNNNIKYNEKFKD**

CDR_H3 of DR5 mAb 2 (SEQ ID NO:21): **HEQGPGYFDY**

[00112] The VH Domain of DR5 mAb 2 is preferably encoded by a polynucleotide (SEQ ID NO:22) having the sequence shown below (polynucleotides encoding the CDR_H residues are shown in underline):

aaggtccagc tgcagcagtc tggagctgaa ctggtgaaac ccgggggcac
agtgaagctg tcctgcaagg cttctgggta caccttcact gagtatatatt
tacactgggt aaagcagaag tctggacagg gtcttgagtg gattgggttgg
ttttatcctg gaaataataa tataaagtac aatgagaaat tcaaggacaa
ggccacactg actgcggaca aatcctccag cacagtctat atggaactta
gtagattgac atctgaagac tctgcggtct atttctgtgc aagacacgaa
caaggaccag gttactttga ctactggggc caaggcacca ctctcacagt
ctcctcc

2. Humanization of the Anti-Human DR5 Antibody DR5 mAb 2 to Form “hDR5 mAb 2”

[00113] The above-described murine anti-human DR5 antibody DR5 mAb 2 was humanized in order to demonstrate the capability of humanizing an anti-human DR5 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded four humanized VL Domains designated herein as “hDR5

mAb 2 VL-2,” “hDR5 mAb 2 VL-3,” “hDR5 mAb 2 VL-4,” and “hDR5 mAb 2 VL-5,” and one humanized VH Domain, designated herein as “hDR5 mAb 2 VH-2.” Any of the humanized VL Domains may be paired with the humanized VH Domain. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as “hDR5 mAb 2,” and particular combinations of humanized VL/VH Domains are referred to by reference to the VL Domain.

[00114] The amino acid sequence of the VL Domain of hDR5 mAb 2 VL-2 (SEQ ID NO:23) is shown below (CDRL residues are shown underlined):

DIQMTQSPSF LSASVGDRVIT ITCKASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
 GTKLEIK

[00115] hDR5 mAb 2 VL-2 is preferably encoded by a polynucleotide (SEQ ID NO:24) having the sequence shown below:

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
 ccgcgtgact attacttgta aagcttctca ggatgtcaac accgccgtgg
 cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
 gccagcactc ggcacaccgg agtcccattc aggttctctg gcagtggatc
 agggacagac ttaccctga caattagctc cctgcagccc gaggatgtgg
 ctacttacta ttgtcagcag cactacatca ctccttggac cttcggcggg
 ggcacaaaac tggaaatcaa a

[00116] The amino acid sequence of the VL Domain of hDR5 mAb 2 VL-3 (SEQ ID NO:25) is shown below (CDRL residues are shown underlined):

DIQMTQSPSF LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPD RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
 GTKLEIK

[00117] hDR5 mAb 2 VL-3 is preferably encoded by a polynucleotide (SEQ ID NO:26) having the sequence shown below:

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
 ccgcgtgact attacttgta ggccttctca ggatgtcaac accgccgtgg
 cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
 gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
 agggacagac ttaccctga caattagctc cctgcagccc gaggatgtgg
 ctacttacta ttgtcagcag cactacatca ctccttggac cttcggcggg
 ggcacaaaac tggaaatcaa a

[00118] The amino acid sequence of the VL Domain of **hDR5 mAb 2 VL-4** (SEQ ID NO:27) is shown below (CDRL residues are shown underlined):

DIQMTQSPSF LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
 GTKLEIK

[00119] **hDR5 mAb 2 VL-4** is preferably encoded by a polynucleotide (SEQ ID NO:28) having the sequence shown below:

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
 ccgcgtgact attacttgct gggcttctca ggatgtcaac accgccgtgg
 cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
 gccagcactc ggcacaccgg agtcccattt aggttctctg gcagtggatc
 agggacagac ttaccctga caattagctc cctgcagcca gaggatatcg
 ctacatacta ttgtcagcag cactacatca ctccttggac ctcggcgagg
 ggcacaaaac tggaaatcaa a

[00120] The amino acid sequence of the VL Domain of **hDR5 mAb 2 VL-5** (SEQ ID NO:29) is shown below (CDRL residues are shown underlined):

DIQMTQSPSF LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPD RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
 GTKLEIK

[00121] **hDR5 mAb 2 VL-5** is preferably encoded by a polynucleotide (SEQ ID NO:30) having the sequence shown below:

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
 ccgcgtgact attacttgct gggcttctca ggatgtcaac accgccgtgg
 cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
 gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
 agggacagac ttaccctga caattagctc cctgcagccc gaggatatcg
 ctacttacta ttgtcagcag cactacatca ctccttggac ctcggcgagg
 ggcacaaaac tggaaatcaa a

[00122] The CDRL1 of the VL Domain of **hDR5 mAb 2 VL-3**, **hDR5 mAb 2 VL-4** and **hDR5 mAb VL-5** has the amino acid sequence RASQDVNTAVA (SEQ ID NO:165).

[00123] The amino acid sequence of the VH Domain of **hDR5 mAb 2 VH-2** (SEQ ID NO:31) is shown below (CDRH residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT EYILHWVRQA PGQGLEWMGW
FYPGNNIKY NEKFKDRVTI TADKSTSTVY MELSSLRSED TAVYYCARHE
QGPGYFDYWG QGTLVTVSS

[00124] **hDR5 mAb 2 VH-2** is preferably encoded by a polynucleotide (**SEQ ID NO:32**) having the sequence shown below:

```
caggtccagc tgggtgcagag tgggggcagag gtgaaaaagc cagggggcatc
agtgaaagtg tcttgtaaag catcagggtta tacatttact gagtacatcc
tgcactgggt gcgacaggca ccaggacagg gactggaatg gatgggggtgg
ttctaccctg gcaacaacaa cattaagtac aacgagaagt ttaaagaccg
ggtgaccatc acagcggata agtctaccag tacagtctat atggagctga
gctccctgag aagcgaagac accgccgtct actattgcgc tcgccacgaa
caggggtccag gttactttga ttattggggg caggggaactc tggtcacagt
cagctcc
```

C. Additional Anti-Human DR5 Antibodies

[00125] In addition to the novel anti-human DR5 antibodies DR5 mAb 1 and DR5 mAb 2, a number of additional anti-human DR5 antibodies are known in the art including: **drozitumab** (designated herein as “**DR5 mAb 3**”), **conatumumab** (designated herein as “**DR5 mAb 4**”), **tigatumumab** (designated herein as “**DR5 mAb 5**”), **LBY135-1** (designated herein as “**DR5 mAb 6**”), **LBY135-2** (designated herein as “**DR5 mAb 7**”) and **KMTR2** (designated herein as “**DR5 mAb 8**”). It is specifically contemplated that the multivalent DR5-Binding Molecules of the instant invention may comprise the CDRs of the VL and/or VH Domains from one or more of DR5 mAb 1, DR5 mAb 2, hDR5 mAb2, DR5 mAb 3, DR5 mAb 4, DR5 mAb 5, DR5 mAb 6, DR5 mAb 7, and DR5 mAb 8. Alternatively, or optionally, the multivalent DR5-Binding Molecules of the instant invention may comprise at least one antigen-binding portion from one or more of DR5 mAb 1, DR5 mAb 2, hDR5 mAb2, DR5 mAb 3, DR5 mAb 4, DR5 mAb 5, DR5 mAb 6, DR5 mAb 7, and DR5 mAb 8. In one embodiment, the multivalent DR5-Binding Molecules of the instant invention comprise at least one antigen-binding portion from DR5 mAb 1 and/or DR5 mAb 2.

1. Drozitumab (“DR5 mAb 3”)

[00126] The amino acid sequence of the VL Domain of **drozitumab** (“**DR5 mAb 3**”) (**SEQ ID NO:54**) is shown below (CDRL residues are shown underlined):

```
SELTQDPAVS VALGQTVRIT CSGDSLRSYY ASWYQQKPG QAPVLVIYGA
NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSA DSSGNHVVFG
GGTKLTVLG
```

CDRL1 of DR5 mAb 3 (**SEQ ID NO:55**): **SGDSLRSYYAS**

CDRL2 of DR5 mAb 3 (**SEQ ID NO:56**): **GANNRPS**

CDR_L3 of DR5 mAb 3 (SEQ ID NO:57): **NSADSSGNHVV**

[00127] The amino acid sequence of the VH Domain of **drozitumab** (“DR5 mAb 3”) (SEQ ID NO:58) is shown below (CDR_H residues are shown underlined):

EVQLVQSGGG VERPGGSLRL SCAAS**GFTFD** **DYAMS**WVRQA PGKGLEWVSG
INWQGGSTGY **ADSVKGRVTI** SRDNAKNSLY LQMNSLRAED TAVYYCAK**IL**
GAGRGWYFDY WGKGITVTVS S

CDR_H1 of DR5 mAb 3 (SEQ ID NO:59): **GFTFDDYAMS**

CDR_H2 of DR5 mAb 3 (SEQ ID NO:60): **INWQGGSTGYADSVKG**

CDR_H3 of DR5 mAb 3 (SEQ ID NO:61): **ILGAGRGWYFDY**

2. Conatumumab (“DR5 mAb 4”)

[00128] The amino acid sequence of the VL Domain of **conatumumab** (“DR5 mAb 4”) (SEQ ID NO:62) is shown below (CDR_L residues are shown underlined):

EIVLTQSPGT LSLSPGERAT LSC**RASQGIS** **RSYLA**WYQQK PGQAPSLLIY
GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYC**Q** **QFGSSPWT**FG
 QGTKVEIK

CDR_L1 of DR5 mAb 4 (SEQ ID NO:63): **RASQGISRSYLA**

CDR_L2 of DR5 mAb 4 (SEQ ID NO:64): **GASSRAT**

CDR_L3 of DR5 mAb 4 (SEQ ID NO:65): **QQFGSSPWT**

[00129] The amino acid sequence of the VH Domain of **conatumumab** (“DR5 mAb 4”) (SEQ ID NO:66) is shown below (CDR_H residues are shown underlined):

QVQLQESGPG LVKPSQTLST TCTVS**GGGIS** **SGDYFWS**WIR QLPKGLEWI
 G**HIHNSGTTY** **YNPSLKS**RVT ISVDTSKKQF SLRLSSVTAA DTAVYYCARD
RGGDYYYGMD **VWGQGT**TVTV SS

CDR_H1 of DR5 mAb 4 (SEQ ID NO:67): **GGSISSGDYFWS**

CDR_H2 of DR5 mAb 4 (SEQ ID NO:68): **HIHNSGTTYYNPSLKS**

CDR_H3 of DR5 mAb 4 (SEQ ID NO:69): **DRGGDYYYGMDV**

3. Tigatumumab (“DR5 mAb 5”)

[00130] The amino acid sequence of the VL Domain of **tigatumumab** (“DR5 mAb 5”) (SEQ ID NO:70) is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVIT ITC**KASQDVG** **TAVAWYQQKP** GKAPKLLIY**W**
ASTRHTGVPS RFSGSGSGTD FTLTISLQPF EDFATYYC**QQ** **YSSYRT**FGQG
 TKVEIK

CDR_L1 of DR5 mAb 5 (SEQ ID NO:71): **KASQDVGTAVA**

CDR_L2 of DR5 mAb 5 (SEQ ID NO:72): **WASTRHT**

CDR_L3 of DR5 mAb 5 (SEQ ID NO:73): **QQYSSYRT**

[00131] The amino acid sequence of the VH Domain of **tigatumumab** (“**DR5 mAb 5**”) (SEQ ID NO:74) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAAS**GFTFS** **SYVMS**WVRQA PGKGLEWVAT
ISSGGSYTTY **PDSVKGR**FTI SRDNAKNTLY LQMNSLRAED TAVYYCAR**RG**
DSMITTDYWG QGTLVTVSS

CDR_H1 of DR5 mAb 5 (SEQ ID NO:75): **GFTFSSYVMS**

CDR_H2 of DR5 mAb 5 (SEQ ID NO:76): **TISSGGSYTYYPDSVKG**

CDR_H3 of DR5 mAb 5 (SEQ ID NO:77): **RGDSMITTDY**

4. LBY135-1 (“DR5 mAb 6”)

[00132] The amino acid sequence of the VL Domain of **LBY135-1** (“**DR5 mAb 6**”) (SEQ ID NO:78) is shown below (CDR_L residues are shown underlined):

DIAMTQSHKF MSTLVGDRVS ITCKAS**QDVN** **TAIA**WYQQKP GQSPKLLIY**W**
ASTRHTGVPD RFYGS GSGTD YTLTISSMEA EDAATYYC**QQ** **WSSNPLT**FGA
 GTKLELKRA

CDR_L1 of DR5 mAb 6 (SEQ ID NO:79): **QDVNTAIA**

CDR_L2 of DR5 mAb 6 (SEQ ID NO:80): **WASTRHT**

CDR_L3 of DR5 mAb 6 (SEQ ID NO:81): **QQWSSNPLT**

[00133] The amino acid sequence of the VH Domain of **LBY135-1** (“**DR5 mAb 6**”) (SEQ ID NO:82) is shown below (CDR_H residues are shown underlined):

KVQLQQSGAE LVKPGASVKL SCKAS**GYTFT** **DYTIH**WVKQR SGQGLEWIG**W**
FYPGGGYIKY **NEKFKD**RATL TADKSSNTVY MELSRLTSEG SAVYFCAR**HE**
EGIFYFDYWGQ GTTLTVSS

CDR_H1 of DR5 mAb 6 (SEQ ID NO:83): **GYTFTDYTIH**

CDR_H2 of DR5 mAb 6 (SEQ ID NO:84): **WFYPGGGYIKYNEKFKD**

CDR_H3 of DR5 mAb 6 (SEQ ID NO:85): **HEEGIFYFDY**

5. LBY135-2 (“DR5 mAb 7”)

[00134] The amino acid sequence of the VL Domain of **LBY135-2** (“**DR5 mAb 7**”) (SEQ ID NO:86) is shown below (CDR_L residues are shown underlined):

DIVMTQSHKF MSTISVGDRVS ITCKASQDVN TAIAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTISSVQA EDLALYYCQQ HYTTPFTFGS
 GTKL

CDR_L1 of DR5 mAb 7 (SEQ ID NO:87): **KASQDVNTAIA**

CDR_L2 of DR5 mAb 7 (SEQ ID NO:88): **WASTRHT**

CDR_L3 of DR5 mAb 7 (SEQ ID NO:89): **QQHYTTPFT**

[00135] The amino acid sequence of the VH Domain of **LBY135-2** (“DR5 mAb 7”) (SEQ ID NO:90) is shown below (CDR_H residues are shown underlined):

KVQLQQSGAE LVKPGASVKL SCKASGYTFT DYTIHWVKQR SGQGLEWIGW
FYPGGGYIKY NEKFKDRATL TADKSSNTVY MELSRLTSED SAVYFCARHE
EGIFYFDYWGQ GTTLTVSS

CDR_H1 of DR5 mAb 7 (SEQ ID NO:91): **GYTFTDYTIH**

CDR_H2 of DR5 mAb 7 (SEQ ID NO:92): **WFYPGGGYIKYNEKFKD**

CDR_H3 of DR5 mAb 7 (SEQ ID NO:93): **HEEGIFYFDY**

6. **KMTR2** (“DR5 mAb 8”)

[00136] The amino acid sequence of the VL Domain of **KMTR2** (“DR5 mAb 8”) (SEQ ID NO:94) is shown below (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD
ASNRATGIPA RFSGSGSGTD FTLTISSELP EDFAVYYCQQ RSNWPLTFGG
 GTKVEIKR

CDR_L1 of DR5 mAb 8 (SEQ ID NO:95): **RASQSVSSYLA**

CDR_L2 of DR5 mAb 8 (SEQ ID NO:96): **DASNRAT**

CDR_L3 of DR5 mAb 8 (SEQ ID NO:97): **QQRSNWPLT**

[00137] The amino acid sequence of the VH Domain of **KMTR2** (“DR5 mAb 8”) (SEQ ID NO:98) is shown below (CDR_H residues are shown underlined):

QVQLVQSGAE MKKPGASVKV SCKTISGYTFT NYKINWVRQA PGQGLEWMGW
MNPDTDSTGY PQKFQGRVTM TRNTSISTAY MELSSLRSED TAVYYCARSY
SGGSYYRDYY YGMDVWGQGT TTVTVSS

CDR_H1 of DR5 mAb 8 (SEQ ID NO:99): **GYTFTNYKIN**

CDR_H2 of DR5 mAb 8 (SEQ ID NO:100): **WMNPDTDSTGY PQKFQ**

CDR_H3 of DR5 mAb 8 (SEQ ID NO:101): **SYSGGSYYRDYYYGMDV**

D. Multivalent DR5-Binding Molecules Having an Engineered Fc Region

[00138] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc Region of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: Fc γ RI (CD64), Fc γ RII (CD32), and Fc γ RIII (CD16). Fc γ RI (CD64), Fc γ RIIA (CD32A) and Fc γ RIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; Fc γ RIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. The amino acid sequence of an exemplary IgG1 Fc Region (**SEQ ID NO:1**) is presented above.

[00139] Modification of the Fc Region normally leads to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating cancer, for example. Reduction or elimination of effector function is desirable in certain cases, for example in the case of antibodies whose mechanism of action involves blocking or antagonism, but not killing of the cells bearing a target antigen. Increased effector function is generally desirable when directed to undesirable cells, such as tumor and foreign cells, where the Fc γ Rs are expressed at low levels, for example, tumor specific B cells with low levels of Fc γ RIIB (*e.g.*, non-Hodgkins lymphoma, CLL, and Burkitt's lymphoma). In said embodiments, molecules of the invention with conferred or altered effector function activity are useful for the treatment and/or prevention of a disease, disorder or infection where an enhanced efficacy of effector function activity is desired.

[00140] In certain embodiments, the multivalent DR5-Binding Molecules of the present invention comprise an Fc Region that possesses one or more modifications (*e.g.*,

substitutions, deletions, or insertions) to the sequence of amino acids of a wild-type Fc Region (**SEQ ID NO:1**), which reduce the affinity and avidity of the Fc Region and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the multivalent DR5-Binding Molecules of the invention comprise an Fc Region that possesses one or more modifications to the amino acids of the wild-type Fc Region, which increase the affinity and avidity of the Fc Region and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the multivalent DR5-Binding Molecules comprise a variant Fc Region wherein said variant confers or mediates increased ADCC activity and/or an increased binding to FcγRIIA, relative to a molecule comprising no Fc Region or comprising a wild-type Fc Region. In alternate embodiments, the molecules comprise a variant Fc Region wherein said variant confers or mediates decreased ADCC activity (or other effector function) and/or an increased binding to FcγRIIB, relative to a molecule comprising no Fc Region or comprising a wild-type Fc Region. In some embodiments, the invention encompasses multivalent DR5-Binding Molecules comprising a variant Fc Region, which variant Fc Region does not show a detectable binding to any FcγR, relative to a comparable molecule comprising the wild-type Fc Region. In other embodiments, the invention encompasses multivalent DR5-Binding Molecules comprising a variant Fc Region, which variant Fc Region only binds a single FcγR, preferably one of FcγRIIA, FcγRIIB, or FcγRIIA. Alternatively, the multivalent DR5-Binding Molecules of the invention comprise a Fc Region which inherently exhibits reduce affinity and/or avidity to FcγRs and/or reduced ADCC activity (relative to the binding exhibited by the wild-type IgG1 Fc Region is utilized, *e.g.*, an Fc Region from IgG2 (**SEQ ID NO:154**) or IgG4 (**SEQ ID NO:103**)). Any such change in affinity and/or avidity is preferably assessed by measuring *in vitro* the extent of detectable binding to the FcγR or FcγR-related activity in cells that express low levels of the FcγR when binding activity of the parent molecule (without the modified Fc Region) cannot be detected in the cells. In other embodiments, the modified molecule exhibits detectable binding in cells which express non-FcγR receptor target antigens at a density of 30,000 to 20,000 molecules/cell, at a density of 20,000 to 10,000 molecules/cell, at a density of 10,000 to 5,000 molecules/cell, at a density of 5,000 to 1,000 molecules/cell, at a density of 1,000 to

200 molecules/cell or at a density of 200 molecules/cell or less (but at least 10, 50, 100 or 150 molecules/cell).

[00141] The multivalent DR5-Binding Molecules of the present invention may comprise altered affinities for an activating and/or inhibitory Fc γ receptor. In one embodiment, the multivalent DR5-Binding Molecule comprises a variant Fc Region that has increased affinity for Fc γ RIIB and decreased affinity for Fc γ RIIA and/or Fc γ RIIA, relative to a comparable molecule with a wild-type Fc Region. In another embodiment, the multivalent DR5-Binding Molecule of the present invention comprise a variant Fc Region, which has decreased affinity for Fc γ RIIB and increased affinity for Fc γ RIIA and/or Fc γ RIIA, relative to a comparable molecule with a wild-type Fc Region. In yet another embodiment, the multivalent DR5-Binding Molecules of the present invention comprise a variant Fc Region that has decreased affinity for Fc γ RIIB and decreased affinity for Fc γ RIIA and/or Fc γ RIIA, relative to a comparable molecule with a wild-type Fc Region. In still another embodiment, the multivalent DR5-Binding Molecules of the present invention comprise a variant Fc Region, which has unchanged affinity for Fc γ RIIB and decreased (or increased) affinity for Fc γ RIIA and/or Fc γ RIIA, relative to a comparable molecule with a wild-type Fc Region.

[00142] In certain embodiments, the multivalent DR5-Binding Molecules of the present invention comprise a variant Fc Region having an altered affinity for Fc γ RIIA and/or Fc γ RIIA such that the immunoglobulin has an enhanced effector function, *e.g.*, antibody dependent cell mediated cytotoxicity. Non-limiting examples of effector cell functions include antibody dependent cell mediated cytotoxicity (ADCC), antibody dependent phagocytosis, phagocytosis, opsonization, opsonophagocytosis, cell binding, rosetting, C1q binding, and complement dependent cell mediated cytotoxicity.

[00143] In a preferred embodiment, the alteration in affinity or effector function is at least 2-fold, preferably at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 50-fold, or at least 100-fold, relative to a comparable molecule comprising a wild-type Fc Region. In other embodiments of the invention, the variant Fc Region immunospecifically binds one or more FcRs with at least 65%, preferably at least 70%, 75%, 80%, 85%, 90%, 95%,

100%, 125%, 150%, 175%, 200%, 225%, or 250% greater affinity relative to a molecule comprising a wild-type Fc Region. Such measurements can be in vivo or in vitro assays, and in a preferred embodiment are in vitro assays such as ELISA or surface plasmon resonance assays.

[00144] In different embodiments, the multivalent DR5-Binding Molecules of the present invention comprise a variant Fc Region that agonizes at least one activity of an FcγR receptor, or antagonizes at least one activity of an FcγR receptor. In a preferred embodiment, the molecules comprise a variant Fc Region that antagonizes one or more activities of FcγRIIB, for example, B cell receptor-mediated signaling, activation of B cells, B cell proliferation, antibody production, intracellular calcium influx of B cells, cell cycle progression, FcγRIIB-mediated inhibition of FcεRI signaling, phosphorylation of FcγRIIB, SHIP recruitment, SHIP phosphorylation and association with Shc, or activity of one or more downstream molecules (*e.g.*, MAP kinase, JNK, p38, or Akt) in the FcγRIIB signal transduction pathway. In another embodiment, the multivalent DR5-Binding Molecules of the present invention comprise a variant Fc Region that agonizes one or more activities of FcεRI, for example, mast cell activation, calcium mobilization, degranulation, cytokine production, or serotonin release.

[00145] In certain embodiments, the molecules comprise an Fc Region comprising regions from two or more IgG isotypes (*e.g.*, IgG1, IgG2, IgG3 and IgG4). The various IgG isotypes exhibit differing physical and functional properties including serum half-life, complement fixation, FcγR binding affinities and effector function activities (*e.g.*, ADCC, CDC, *etc.*) due to differences in the amino acid sequences of their hinge and/or Fc Regions, for example as described in Flesch and Neppert (1999) J. Clin. Lab. Anal. 14:141-156; Chappel *et al.* (1993) J. Biol. Chem. 33:25124-25131; Chappel *et al.* (1991) Proc. Natl. Acad. Sci. (U.S.A.) 88:9036-9040; or Brüggemann *et al.* (1987) J. Exp. Med 166:1351-1361. This type of variant Fc Region may be used alone, or in combination with an amino acid modification, to affect Fc-mediated effector function and/or binding activity. In combination, the amino acid modification and IgG hinge/Fc Region may display similar functionality (*e.g.*, increased affinity for FcγRIIA) and may act additively or, more preferably, synergistically to modify the effector functionality in the molecule of the invention, relative to a molecule of the invention comprising a

wild-type Fc Region. In other embodiments, the amino acid modification and IgG Fc Region may display opposite functionality (*e.g.*, increased and decreased affinity for FcγRIIA, respectively) and may act to selectively temper or reduce a specific functionality in the molecule of the invention, relative to a molecule of the invention not comprising an Fc Region or comprising a wild-type Fc Region of the same isotype.

[00146] In a preferred specific embodiment, the multivalent DR5-Binding Molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises at least one amino acid modification relative to a wild-type Fc Region, such that said molecule has an altered affinity for an FcR, provided that said variant Fc Region does not have a substitution at positions that make a direct contact with FcγR based on crystallographic and structural analysis of Fc-FcR interactions such as those disclosed by Sondermann *et al.* (2000) Nature 406:267-73. Examples of positions within the Fc Region that make a direct contact with FcγR are amino acid residues 234-239 (hinge region), amino acid residues 265-269 (B/C loop), amino acid residues 297-299 (C'/E loop), and amino acid residues 327-332 (F/G loop). In some embodiments, the molecules of the invention comprise variant Fc Regions comprise modification of at least one residue that does not make a direct contact with an FcγR based on structural and crystallographic analysis, *e.g.*, is not within the Fc-FcγR binding site.

[00147] Variant Fc Regions are well known in the art, and any known Fc variant may be used in the present invention to confer or modify the effector function exhibited by a molecule of the invention comprising an Fc Region (or portion thereof) as functionally assayed, *e.g.*, in an NK dependent or macrophage dependent assay. For example, Fc Region variants identified as altering effector function are disclosed in the Antibody Engineering Technology Art, and any suitable variant disclosed therein may be used in the present molecules.

[00148] In certain embodiments, the multivalent DR5-Binding Molecules of the present invention comprise a variant Fc Region, having one or more amino acid modifications in one or more regions, which modification(s) alter (relative to a wild-

type Fc Region) the **Ratio of Affinities** of the variant Fc Region to an activating FcγR (such as FcγRIIA or FcγRIIIA) relative to an inhibiting FcγR (such as FcγRIIB):

$$\text{Ratio of Affinities} = \frac{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Activating}}}{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Inhibiting}}}$$

[00149] Particularly preferred are multivalent DR5-Binding Molecules of the present invention that possess a variant Fc Region (relative to the wild-type Fc Region) in which the Fc variant has a Ratio of Affinities greater than 1. Such molecules have particular use in providing a therapeutic or prophylactic treatment of a disease, disorder, or infection, or the amelioration of a symptom thereof, where an enhanced efficacy of effector cell function (*e.g.*, ADCC) mediated by FcγR is desired, *e.g.*, cancer or infectious disease. In contrast, an Fc variant having a Ratio of Affinities less than 1 mediates decreased efficacy of effector cell function. **Table 1** lists exemplary single, double, triple, quadruple and quintuple mutations by whether their Ratio of Affinities is greater than or less than 1.

Table 1 Exemplary Single and Multiple Mutations Listed by Ratio of Affinities				
Single	Double	Triple	Quadruple	Quintuple
Ratio of Affinities > 1				
F243L D270E R292G R292P	F243L & R292P	F243L, P247L & N421K	L234F, F243L, R292P & Y300L	L235V, F243L, R292P, Y300L & P396L
	F243L & Y300L	F243L, R292P & Y300L	L235I, F243L, R292P & Y300L	L235P, F243L, R292P, Y300L & P396L
	F243L & P396L	F243L, R292P & V305I	L235Q, F243L, R292P & Y300L	F243L, R292P, V305I, Y300L & P396L
	D270E & P396L	F243L, R292P & P396L	F243L, P247L, D270E & N421K	
	R292P & Y300L	F243L, Y300L & P396L	F243L, R255L, D270E & P396L	
	R292P & V305I	P247L, D270E & N421K	F243L, D270E, G316D & R416G	
	R292P & P396L	R255L, D270E & P396L	F243L, D270E, K392T & P396L	
	Y300L & P396L	D270E, G316D & R416G	F243L, D270E, P396L & Q419H	
	P396L & Q419H	D270E, K392T & P396L	F243L, R292P, Y300L, & P396L	
		D270E, P396L & Q419H	F243L, R292P, V305I & P396L	
Ratio of Affinities < 1				
Y300L P396L	F243L & P396L	F243L, R292P & V305I		
	P247L & N421K			
	R255L & P396L			
	R292P & V305I			
	K392T & P396L			
	P396L & Q419H			

[00150] In a specific embodiment, in variant Fc Regions, any amino acid modifications (*e.g.*, substitutions) at any of positions 235, 240, 241, 243, 244, 247, 262, 263, 269, 298, 328, or 330 and preferably one or more of the following residues: A240, I240, L241, L243, H244, N298, I328 or V330. In a different specific embodiment, in variant Fc Regions, any amino acid modifications (*e.g.*, substitutions) at any of positions 268, 269, 270, 272, 276, 278, 283, 285, 286, 289, 292, 293, 301, 303, 305, 307, 309, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439 and preferably one or more of the following residues: H280, Q280, Y280, G290, S290, T290, Y290, N294, K295, P296, D298, N298, P298, V298, I300 or L300.

[00151] In a preferred embodiment, in variant Fc Regions that bind an FcγR with an altered affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 309, 312, 320, 322, 326, 329, 330, 332, 331, 333, 334, 335, 337, 338, 339, 340, 359, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439. Preferably, the variant Fc Region has any of the following residues: A256, N268, Q272, D286, Q286, S286, A290, S290, A298, M301, A312, E320, M320, Q320, R320, E322, A326, D326, E326, N326, S326, K330, T339, A333, A334, E334, H334, L334, M334, Q334, V334, K335, Q335, A359, A360 or A430.

[00152] In a different embodiment, in variant Fc Regions that bind an FcγR (via its Fc Region) with a reduced affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 252, 254, 265, 268, 269, 270, 278, 289, 292, 293, 294, 295, 296, 298, 300, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, or 439.

[00153] In a different embodiment, in variant Fc Regions that bind an FcγR (via its Fc Region) with an enhanced affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 280, 283, 285, 286, 290, 294, 295, 298, 300, 301, 305, 307, 309, 312, 315, 331, 333, 334, 337, 340, 360, 378, 398, or 430. In a different embodiment, in variant Fc Regions that binds FcγRIIA with an enhanced affinity, any of the following residues: A255, A256, A258, A267, A268, N268, A272, Q272, A276, A280,

A283, A285, A286, D286, Q286, S286, A290, S290, M301, E320, M320, Q320, R320, E322, A326, D326, E326, S326, K330, A331, Q335, A337 or A430.

[00154] Preferred variants include one or more modifications at any of positions: 228, 230, 231, 232, 233, 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 271, 273, 275, 281, 284, 291, 296, 297, 298, 299, 302, 304, 305, 313, 323, 325, 326, 328, 330 or 332.

[00155] Particularly preferred variants include one or more modifications selected from groups A-AI:

A	228E, 228K, 228Y or 228G;
B	230A, 230E, 230Y or 230G;
C	231E, 231K, 231Y, 231P or 231G;
D	232E, 232K, 232Y, 232G;
E	233D;
F	234I or 234F;
G	235D, 235Q, 235P, 235I or 235V;
H	239D, 239E, 239N or 239Q;
I	240A, 240I, 240M or 240T;
J	243R, 243, 243Y, 243L, 243Q, 243W, 243H or 243I;
K	244H;
L	245A;
M	247G, 247V or 247L;
N	262A, 262E, 262I, 262T, 262E or 262F;
O	263A, 263I, 263M or 263T;
P	264F, 264E, 264R, 264I, 264A, 264T or 264W;
Q	265F, 265Y, 265H, 265I, 265L, 265T, 265V, 265N or 265Q;
R	266A, 266I, 266M or 266T;
S	271D, 271E, 271N, 271Q, 271K, 271R, 271S, 271T, 271H, 271A, 271V, 271L, 271I, 271F, 271M, 271Y, 271W or 271G;
T	273I;
U	275L or 275W;
V	281D, 281K, 281Y or 281P;
W	284E, 284N, 284T, 284L, 284Y or 284M;
X	291D, 291E, 291Q, 291T, 291H, 291I or 291G;
Y	299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W or 299Y;
Z	302I;
AA	304D, 304N, 304T, 304H or 304L
AB	305I;
AC	313F;
AD	323I;
AE	325A, 325D, 325E, 325G, 325H, 325I, 325L, 325K, 325R, 325S, 325F, 325M, 325T, 325V, 325Y, 325W or 325P;
AF	328D, 328Q, 328K, 328R, 328S, 328T, 328V, 328I, 328Y, 328W, 328P, 328G, 328A, 328E, 328F, 328H, 328M or 328N;

AG	330L, 330Y, 330I or 330V;
AH	332A, 332D, 332E, 332H, 332N, 332Q, 332T, 332K, 332R, 332S, 332V, 332L, 332F, 332M, 332W, 332P, 332G or 332Y; and
AI	336E, 336K or 336Y

[00156] Still more particularly preferred variants include one or more modifications selected from Groups 1-105:

Group	Variant	Group	Variant
1	A330L / I332E	54	S239D / D265L / N297D / I332E
2	D265F / N297E / I332E	55	S239D / D265T / N297D / I332E
3	D265Y / N297D / I332E	56	S239D / D265V / N297D / I332E
4	D265Y / N297D / T299L / I332E	57	S239D / D265Y / N297D / I332E
5	F241E / F243Q / V262T / V264F	58	S239D / I332D
6	F241E / F243Q / V262T / V264E / I332E	59	S239D / I332E
7	F241E / F243R / V262E / V264R	60	S239D / I332E / A330I
8	F241E / F243R / V262E / V264R / I332E	61	S239D / I332N
9	F241E / F243Y / V262T / V264R	62	S239D / I332Q
10	F241E / F243Y / V262T / V264R / I332E	63	S239D / N297D / I332E
11	F241L / F243L / V262I / V264I	64	S239D / N297D / I332E / A330Y
12	F241L / V262I	65	S239D / N297D / I332E / A330Y / F241S / F243H / V262T / V264T
13	F241R / F243Q / V262T / V264R	66	S239D / N297D / I332E / K326E
14	F241R / F243Q / V262T / V264R / I332E	67	S239D / N297D / I332E / L235D
15	F241W / F243W / V262A / V264A	68	S239D / S298A / I332E
16	F241Y / F243Y / V262T / V264T	69	S239D / V264I / A330L / I332E
17	F241Y / F243Y / V262T / V264T / N297D / I332E	70	S239D / V264I / I332E
18	F243L / V262I / V264W	71	S239D / V264I / S298A / I332E
19	P243L / V264I	72	S239E / D265N
20	L328D / I332E	73	S239E / D265Q
21	L328E / I332E	74	S239E / I332D
22	L328H / I332E	75	S239E / I332E
23	L328I / I332E	76	S239E / I332N
24	L328M / I332E	77	S239E / I332Q
25	L328N / I332E	78	S239E / N297D / I332E
26	L328Q / I332E	79	S239E / V264I / A330Y / I332 E
27	L328T / I332E	80	S239E / V264I / I332 E
28	L328V / I332E	81	S239E / V264I / S298A / A330Y / I332E
29	N297D / A330Y / I332E	82	S239N / A330L / I332E

30	N297D / I332E	83	S239N / A330Y / I332E
31	N297D / I332E / S239D / A330L	84	S239N / I332D
32	N297D / S298A / A330Y / I332E	85	S239N / I332E
33	N297D / T299L / I332E	86	S239N / I332N
34	N297D / T299F / I332E / N297D / T299H / I332E	87	S239N / I332Q
35	N297D / T299I / I332E	88	S239N1S298A / I332E
36	N297D / T299L / I332E	89	S239Q / I332D
37	N297D / T299V / I332E	90	S239Q / I332E
38	N297E / I332E	91	S239Q / I332N
39	N297S / I332E	92	S239Q / I332Q
40	P230A / E233D / I332E	93	S239Q / V264I / I332E
41	P244H / P245A / P247V	94	S298A / I332E
42	S239D / A330L / I332E	95	V264E / N297D / I332E
43	S239D / A330Y / I332E	96	V264I / A330L / I332E
44	S239D / A330Y / I332E / K326E	97	V264I / A330Y / I332E
45	S239D / A330Y / I332E / K326T	98	V264I / I332E
46	S239D / A330Y / I332E / L234I	99	V264I / S298A / I332E
47	S239D / A330Y / I332E / L235D	100	Y296D / N297D / I332E
48	S239D / A330Y / I332E / V240I	101	Y296E / N297D / I332E
49	S239D / A330Y / I332E / V264T	102	Y296H / N297D / I332E
50	S239D / A330Y / I332E / V266I	103	Y296N / N297D / I332E
51	S239D / D265F / N297D / I332E	104	Y296Q / N297I / I332E
52	S239D / D265H / N297D / I332E	105	Y296T / N297D / I332E.
53	S239D / D265I / N297D / I332E		

[00157] In one embodiment, a multivalent DR5-Binding Molecule of the invention will comprise a variant Fc Region having at least one modification in the Fc Region. In certain embodiments, the variant Fc Region comprises at least one substitution selected from the group consisting of L235V, F243L, R292P, Y300L, V305I, and P396L, wherein said numbering is that of the EU index as in Kabat.

[00158] In a specific embodiment, the variant Fc Region comprises:

A) at least one substitution selected from the group consisting of F243L, R292P, Y300L, V305I, and P396L;

(B) at least two substitutions selected from the group consisting of:

(1) F243L and P396L;

(2) F243L and R292P; and

(3) R292P and V305I;

(C) at least three substitutions selected from the group consisting of:

(1) F243L, R292P and Y300L;

(2) F243L, R292P and V305I;

(3) F243L, R292P and P396L; and

(4) R292P, V305I and P396L;

(D) at least four substitutions selected from the group consisting of:

(1) F243L, R292P, Y300L and P396L; and

(2) F243L, R292P, V305I and P396L; or

(E) at least the five substitutions selected from the group consisting of:

(1) F243L, R292P, Y300L, V305I and P396L; and

(2) L235V, F243L, R292P, Y300L and P396L.

[00159] In another specific embodiment, the variant Fc Region comprises substitutions of:

(A) F243L, R292P, and Y300L;

(B) L235V, F243L, R292P, Y300L, and P396L; or

(C) F243L, R292P, Y300L, V305I, and P396L.

[00160] In other embodiments, the invention encompasses the use of any Fc variant known in the art, such as those disclosed in Jefferis, B.J. *et al.* (2002) “*Interaction Sites On Human IgG-Fc For FcγR: Current Models*,” *Immunol. Lett.* 82:57-65; Presta, L.G. *et al.* (2002) “*Engineering Therapeutic Antibodies For Improved Function*,” *Biochem. Soc. Trans.* 30:487-90; Idusogie, E.E. *et al.* (2001) “*Engineered Antibodies With Increased Activity To Recruit Complement*,” *J. Immunol.* 166:2571-75; Shields, R.L. *et al.* (2001) “*High Resolution Mapping Of The Binding Site On Human IgG1 For Fc γRI, Fc γRII, Fc γRIII, And FcRn And Design Of IgG1 Variants With Improved Binding To The Fc γR*,” *J. Biol. Chem.* 276:6591-6604; Idusogie, E.E. *et al.* (2000) “*Mapping Of The C1q Binding Site On Rituxan, A Chimeric Antibody With A Human IgG Fc*,” *J. Immunol.* 164:4178-84; Reddy, M.P. *et al.* (2000) “*Elimination Of Fc Receptor-Dependent Effector Functions Of A Modified IgG4 Monoclonal Antibody To Human CD4*,” *J. Immunol.* 164:1925-1933; Xu, D. *et al.* (2000) “*In Vitro Characterization of Five Humanized OKT3 Effector Function Variant Antibodies*,” *Cell. Immunol.* 200:16-26; Armour, K.L. *et al.* (1999) “*Recombinant human IgG Molecules Lacking FcγR Binding And Monocyte Triggering Activities*,” *Eur. J. Immunol.* 29:2613-24; Jefferis, R. *et al.* (1996) “*Modulation Of Fc(γ)R And Human Complement Activation By IgG3-Core Oligosaccharide*

Interactions,” Immunol. Lett. 54:101-04; Lund, J. *et al.* (1996) “*Multiple Interactions Of IgG With Its Core Oligosaccharide Can Modulate Recognition By Complement And Human Fc Gamma Receptor I And Influence The Synthesis Of Its Oligosaccharide Chains*,” J. Immunol. 157:4963-4969; Hutchins *et al.* (1995) “*Improved Biodistribution, Tumor Targeting, And Reduced Immunogenicity In Mice With A Gamma 4 Variant Of Campath-1H*,” Proc. Natl. Acad. Sci. (U.S.A.) 92:11980-84; Jefferis, R. *et al.* (1995) “*Recognition Sites On Human IgG For Fc Gamma Receptors: The Role Of Glycosylation*,” Immunol. Lett. 44:111-17; Lund, J. *et al.* (1995) “*Oligosaccharide-Protein Interactions In IgG Can Modulate Recognition By Fc Gamma Receptors*,” FASEB J. 9:115-19; Alegre, M.L. *et al.* (1994) “*A Non-Activating "Humanized" Anti-CD3 Monoclonal Antibody Retains Immunosuppressive Properties In Vivo*,” Transplantation 57:1537-1543; Lund *et al.* (1992) “*Multiple Binding Sites On The CH2 Domain Of IgG For Mouse Fc Gamma R11*,” Mol. Immunol. 29:53-59; Lund *et al.* (1991) “*Human Fc Gamma RI And Fc Gamma RII Interact With Distinct But Overlapping Sites On Human IgG*,” J. Immunol. 147:2657-2662; Duncan, A.R. *et al.* (1988) “*Localization Of The Binding Site For The Human High-Affinity Fc Receptor On IgG*,” Nature 332:563-564; US Patent Nos. 5,624,821; 5,885,573; 6,194,551; 7,276,586; and 7,317,091; and PCT Publications WO 00/42072 and PCT WO 99/58572.

[00161] In some embodiments, the molecules of the invention further comprise one or more glycosylation sites, so that one or more carbohydrate moieties are covalently attached to the molecule. Preferably, the molecules of the invention with one or more glycosylation sites and/or one or more modifications in the Fc Region confer or have an enhanced antibody mediated effector function, *e.g.*, enhanced ADCC activity, compared to to the unmodified molecule. In some embodiments, the invention further comprises molecules comprising one or more modifications of amino acids that are directly or indirectly known to interact with a carbohydrate moiety of the Fc Region, including but not limited to amino acids at positions 241, 243, 244, 245, 245, 249, 256, 258, 260, 262, 264, 265, 296, 299, and 301. Amino acids that directly or indirectly interact with a carbohydrate moiety of an Fc Region are known in the art, *see, e.g.*, Jefferis *et al.*, 1995 *Immunology Letters*, 44: 111-7, which is incorporated herein by reference in its entirety.

[00162] In another embodiment, the invention encompasses molecules that have been modified by introducing one or more glycosylation sites into one or more sites of the molecules, preferably without altering the functionality of the molecules, *e.g.*, binding activity to target antigen or FcγR. Glycosylation sites may be introduced into the variable and/or constant region of the molecules of the invention. As used herein, “glycosylation sites” include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach. Oligosaccharide side chains are typically linked to the backbone of an antibody via either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. The molecules of the invention may comprise one or more glycosylation sites, including N-linked and O-linked glycosylation sites. Any glycosylation site for N-linked or O-linked glycosylation known in the art may be used in accordance with the instant invention. An exemplary N-linked glycosylation site that is useful in accordance with the methods of the present invention is the amino acid sequence: Asn-X-Thr/Ser, wherein X may be any amino acid and Thr/Ser indicates a threonine or a serine. Such a site or sites may be introduced into a molecule of the invention using methods well known in the art to which this invention pertains (see for example, *IN VITRO* MUTAGENESIS, RECOMBINANT DNA: A SHORT COURSE, J. D. Watson, *et al.* W.H. Freeman and Company, New York, 1983, chapter 8, pp. 106-116, which is incorporated herein by reference in its entirety. An exemplary method for introducing a glycosylation site into a molecule of the invention may comprise: modifying or mutating an amino acid sequence of the molecule so that the desired Asn-X-Thr/Ser sequence is obtained.

[00163] In some embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies (and molecules comprising antibody domains, *e.g.*, Fc Domain) are well known in the art and encompassed within the invention, *see, e.g.*, U.S. Patent No. 6,218,149; EP 0 359 096 B1; U.S. Publication No. US 2002/0028486; WO 03/035835; U.S. Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are

incorporated herein by reference in their entirety. In other embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by deleting one or more endogenous carbohydrate moieties of the molecule. In a specific embodiment, the invention encompasses shifting the glycosylation site of the Fc Region of an antibody, by modifying positions adjacent to 297. In a specific embodiment, the invention encompasses modifying position 296 so that position 296 and not position 297 is glycosylated.

[00164] Effector function can also be modified by techniques such as by introducing one or more cysteine residues into the Fc Region, thereby allowing interchain disulfide bond formation in this region to occur, resulting in the generation of a homodimeric antibody that may have improved internalization capability and/or increased complement-mediated cell killing and ADCC (Caron, P.C. *et al.* (1992) “*Engineered Humanized Dimeric Forms Of IgG Are More Effective Antibodies*,” J. Exp. Med. 176:1191-1195; Shopes, B. (1992) “*A Genetically Engineered Human IgG Mutant With Enhanced Cytolytic Activity*,” J. Immunol. 148(9):2918-2922. Homodimeric antibodies with enhanced antitumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff, E.A. *et al.* (1993) “*Monoclonal Antibody Homodimers: Enhanced Antitumor Activity In Nude Mice*,” Cancer Research 53:2560-2565. Alternatively, an antibody can be engineered which has dual Fc Regions and may thereby have enhanced complement lysis and ADCC capabilities (Stevenson, G.T. *et al.* (1989) “*A Chimeric Antibody With Dual Fc Regions (bisFabFc) Prepared By Manipulations At The IgG Hinge*,” Anti-Cancer Drug Design 3:219-230).

E. Multivalent DR5-Binding Molecules Comprising Diabodies

1. Multivalent DR5-Binding Molecules Comprising Diabodies Lacking Fc Regions

[00165] One embodiment of the present invention relates to multivalent DR5-Binding Molecules comprising or consisting of bispecific diabodies that are capable of binding to a first epitope (“**Epitope 1**”) and a second epitope (“**Epitope 2**”), wherein the first epitope is an epitope of human DR5 and the second epitope is a different epitope of DR5. Preferably, such diabodies comprise, and most preferably are composed of, a first polypeptide chain and a second polypeptide chain, whose

sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated complex that is capable of simultaneously binding to a first DR5 epitope and the second DR5 epitope. Accordingly, such diabodies may bind the first and second epitope on a single DR5 polypeptide (*i.e.*, bind intramolecularly), or they may bind the first epitope on a one DR5 polypeptide and the second epitope on another DR5 polypeptide (*i.e.*, bind intermolecularly). Preferably, such diabodies cross-link DR5 molecules that are arrayed on the surface of a cell.

[00166] In one embodiment, the first polypeptide chain of such bispecific diabodies comprises, in the N-terminal to C-terminal direction, an N-terminus, the VL Domain of a first monoclonal antibody capable of binding to either the first or second epitope (*i.e.*, either VL_{Epitope 1} or VL_{Epitope 2}), a first intervening spacer peptide (Linker 1), a VH Domain of a second monoclonal antibody capable of binding to either the second epitope (if such first polypeptide chain contains VL_{Epitope 1}) or the first epitope (if such first polypeptide chain contains VL_{Epitope 2}), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a heterodimer-promoting Domain and a C-terminus (**Figure 1**). The notation “VL1” and “VH1” denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain of the first monoclonal antibody. Similarly, the notation “VL2” and “VH2” denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain of the second antibody.

[00167] The second polypeptide chain of this embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL Domain of a monoclonal antibody capable of binding to either the first or the second epitope (*i.e.*, either VL_{Epitope 1} or VL_{Epitope 2}, and being the VL Domain not selected for inclusion in the first polypeptide chain of the diabody), an intervening linker peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to either the second epitope (if such second polypeptide chain contains VL_{Epitope 1}) or to the first epitope (if such second polypeptide chain contains VL_{Epitope 2}), a spacer peptide (Linker 2) optionally containing a cysteine residue, a heterodimer-promoting Domain, and a C-terminus (**Figure 1**).

[00168] The VL Domain of the first polypeptide chain interacts with the VH Domain of the second polypeptide chain to form a first functional antigen-binding site that is specific for DR5 (*i.e.*, either the first or the second epitope). Likewise, the VL Domain of the second polypeptide chain interacts with the VH Domain of the first polypeptide chain in order to form a second functional antigen-binding site that is also specific for DR5 (*i.e.*, either the second epitope or the first epitope). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains is coordinated, such that the two polypeptide chains of the diabody collectively comprise VL and VH Domains capable of binding to both a first epitope of DR5 and to a second epitope of DR5 (*i.e.*, they comprise VL_{Epitope 1}/ VL_{Epitope 1} and VL_{Epitope 2}/VH_{Epitope 2}).

[00169] Most preferably, the length of the intervening linker peptide (Linker 1, which separates such VL and VH Domains) is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding to one another. Thus the VL and VH Domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL and VH Domains of the second polypeptide chain are substantially or completely incapable of binding to one another. A preferred intervening spacer peptide (Linker 1) has the sequence (**SEQ ID NO:33**): GGGSGGGG.

[00170] The second intervening spacer peptide (Linker 2) will optionally contain 1, 2, 3 or more cysteines. A preferred cysteine-containing spacer peptide (Linker 2) has the sequence is **SEQ ID NO:34**: GGCGGG. Alternatively, Linker 2 does not comprise a cysteine and a Cysteine-Containing Heterodimer-Promoting Domain, as described below is used. Optionally, both a cysteine-containing Linker 2 and a cysteine-containing Heterodimer-Promoting Domain are used.

[00171] The Heterodimer-Promoting Domains may be GVEPKSC (**SEQ ID NO:35**) VEPKSC (**SEQ ID NO:36**) or AEPKSC (**SEQ ID NO:169**) on one polypeptide chain and GFNRGEC (**SEQ ID NO:37**) or FNRGEC (**SEQ ID NO:38**) on the other polypeptide chain (US2007/0004909).

[00172] More preferably, however, the Heterodimer-Promoting Domains of such diabodies are formed from one, two, three or four tandemly repeated coil domains of

opposing charge that comprise a sequence of at least six, at least seven or at least eight charged amino acid residues (Apostolovic, B. *et al.* (2008) “*pH-Sensitivity of the E3/K3 Heterodimeric Coiled Coil*,” *Biomacromolecules* 9:3173–3180; Arndt, K.M. *et al.* (2001) “*Helix-stabilized Fv (hsFv) Antibody Fragments: Substituting the Constant Domains of a Fab Fragment for a Heterodimeric Coiled-coil Domain*,” *J. Molec. Biol.* 312:221-228; Arndt, K.M. *et al.* (2002) “*Comparison of In Vivo Selection and Rational Design of Heterodimeric Coiled Coils*,” *Structure* 10:1235-1248; Boucher, C. *et al.* (2010) “*Protein Detection By Western Blot Via Coiled–Coil Interactions*,” *Analytical Biochemistry* 399:138-140; Cachia, P.J. *et al.* (2004) “*Synthetic Peptide Vaccine Development: Measurement Of Polyclonal Antibody Affinity And Cross-Reactivity Using A New Peptide Capture And Release System For Surface Plasmon Resonance Spectroscopy*,” *J. Mol. Recognit.* 17:540-557; De Crescenzo, G.D. *et al.* (2003) “*Real-Time Monitoring of the Interactions of Two-Stranded de novo Designed Coiled-Coils: Effect of Chain Length on the Kinetic and Thermodynamic Constants of Binding*,” *Biochemistry* 42:1754-1763; Fernandez-Rodriquez, J. *et al.* (2012) “*Induced Heterodimerization And Purification Of Two Target Proteins By A Synthetic Coiled-Coil Tag*,” *Protein Science* 21:511-519; Ghosh, T.S. *et al.* (2009) “*End-To-End And End-To-Middle Interhelical Interactions: New Classes Of Interacting Helix Pairs In Protein Structures*,” *Acta Crystallographica D* 65:1032-1041; Grigoryan, G. *et al.* (2008) “*Structural Specificity In Coiled-Coil Interactions*,” *Curr. Opin. Struc. Biol.* 18:477-483; Litowski, J.R. *et al.* (2002) “*Designing Heterodimeric Two-Stranded α -Helical Coiled-Coils: The Effects Of Hydrophobicity And α -Helical Propensity On Protein Folding, Stability, And Specificity*,” *J. Biol. Chem.* 277:37272-37279; Steinkruger, J.D. *et al.* (2012) “*The d'--d--d' Vertical Triad is Less Discriminating Than the a'--a--a' Vertical Triad in the Antiparallel Coiled-coil Dimer Motif*,” *J. Amer. Chem. Soc.* 134(5):2626–2633; Straussman, R. *et al.* (2007) “*Kinking the Coiled Coil – Negatively Charged Residues at the Coiled-coil Interface*,” *J. Molec. Biol.* 366:1232-1242; Tripet, B. *et al.* (2002) “*Kinetic Analysis of the Interactions between Troponin C and the C-terminal Troponin I Regulatory Region and Validation of a New Peptide Delivery/Capture System used for Surface Plasmon Resonance*,” *J. Molec. Biol.* 323:345–362; Woolfson, D.N. (2005) “*The Design Of Coiled-Coil Structures And Assemblies*,” *Adv. Prot. Chem.* 70:79-112; Zeng, Y. *et al.* (2008) “*A Ligand-*

Pseudoreceptor System Based On de novo Designed Peptides For The Generation Of Adenoviral Vectors With Altered Tropism,” J. Gene Med. 10:355-367).

[00173] Such repeated coil domains may be exact repeats or may have substitutions. For example, the Heterodimer-Promoting Domain of the first polypeptide chain may comprise a sequence of eight negatively charged amino acid residues and the Heterodimer-Promoting Domain of the second polypeptide chain may comprise a sequence of eight positively charged amino acid residues. It is immaterial which coil is provided to the first or second polypeptide chains, provided that a coil of opposite charge is used for the other polypeptide chain. The positively charged amino acid may be lysine, arginine, histidine, *etc.* and/or the negatively charged amino acid may be glutamic acid, aspartic acid, *etc.* The positively charged amino acid is preferably lysine and/or the negatively charged amino acid is preferably glutamic acid. It is possible for only a single Heterodimer-Promoting Domain to be employed (since such domain will inhibit homodimerization and thereby promote heterodimerization), however, it is preferred for both the first and second polypeptide chains of the diabodies of the present invention to contain Heterodimer-Promoting Domains.

[00174] In a preferred embodiment, one of the Heterodimer-Promoting Domains will comprise four tandem “E-coil” helical domains (SEQ ID NO:39: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, while the other of the Heterodimer-Promoting Domains will comprise four tandem “K-coil” domains (SEQ ID NO:40: KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimer formation. Especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem “E-coil” helical domains of SEQ ID NO:39 has been modified to contain a cysteine residue: EVAACEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:41). Likewise, especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem “K-coil” helical domains of SEQ ID NO:40 has been modified to contain a cysteine residue: KVAACKE-KVAALKE-KVAALKE-KVAALKE (SEQ ID NO:42).

[00175] As disclosed in WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabodies, a diabody may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of the diabody. Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind to other proteins and thereby extend their serum half-lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) “Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Modules,” J. Biol. Chem. 277(10):8114-8120. Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 (**SEQ ID NO:43**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00176] As disclosed in WO 2012/162068 (herein incorporated by reference), “**deimmunized**” variants of **SEQ ID NO:43** have the ability to attenuate or eliminate MHC class II binding. Based on combinational mutation results, the following combinations of substitutions are considered to be preferred substitutions for forming such a deimmunized albumin-binding domain: 66S/70S +71A; 66S/70S +79A; 64A/65A/71A+66S; 64A/65A/71A+66D; 64A/65A/71A+66E; 64A/65A/79A+66S; 64A/65A/79A+66D; 64A/65A/79A+66E. Variant ABDs having the modifications L64A, I65A and D79A or the modifications N66S, T70S and D79A. Variant deimmunized ABD having the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNA₆₄A₆₅NNAKT VEGVKALIAA₇₉E ILAALP
(**SEQ ID NO:44**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLIS₆₆NAKS₇₀ VEGVKALIAA₇₉E ILAALP
(**SEQ ID NO:45**),

are particularly preferred as such deimmunized Albumin-Binding Domains exhibit substantially wild-type binding while providing attenuated MHC class II binding. Thus, the first polypeptide chain of such a diabody having an Albumin-Binding Domain contains a third linker (Linker 3) preferably positioned C-terminally to the E-coil (or K-coil) Domain of such polypeptide chain so as to intervene between the E-coil (or K-coil) Domain and the Albumin-Binding Domain (which is preferably a deimmunized Albumin-Binding Domain). A preferred sequence for such Linker 3 is **SEQ ID NO:46**: GGGG.

[00177] Another embodiment of the present invention relates to multivalent DR5-Binding Molecules comprising or consisting of monospecific diabodies capable of binding to one epitope of DR5. Preferably, such diabodies comprise, and most preferably are composed of, a first polypeptide chain and a second polypeptide chain, whose sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated complex having two binding domains, each capable of binding to the same DR5 epitope. Preferably, such diabodies are capable of simultaneously binding to the same DR5 epitope on two separate DR5 polypeptides. Preferably, such diabodies cross-link DR5 on the surface of a cell.

[00178] Monospecific diabodies may readily be generated from homodimerization of polypeptide chains comprising, in the N-terminal to C-terminal direction, an N-terminus, the VL Domain of a monoclonal antibody capable of binding to an epitope of DR5 a first intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to the epitope of DR5. As detailed above, the length of the intervening linker peptide (Linker 1, which separates such VL and VH Domains) is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding to one another. The polypeptide chains may optionally comprise a cysteine-containing peptide which can form a covalent disulfide linkage between the pair of polypeptides.

[00179] Alternatively, monospecific bivalent diabodies may readily be generated by heterodimerization of a first and second polypeptide as detailed above, for example, if the first monoclonal antibody and the second monoclonal antibody recognize the same

epitope, or the same VL and VH Domains are used on both the first and the second polypeptide chains.

2. Fc Region-Containing, Multivalent DR5-Binding Molecules

[00180] One embodiment of the present invention relates to Fc Region-containing, multivalent, DR5-Binding Molecules. The addition of IgG CH2-CH3 Domains to one or both of the diabody polypeptide chains, such that the complexing of the diabody chains results in the formation of an Fc Region, increases the biological half-life and/or alters the valency of the diabody.

[00181] Incorporating IgG CH2-CH3 Domains onto both of the diabody polypeptides will permit a two-chain Fc-containing diabody to form (**Figure 2**). As noted above, such a diabody will be bispecific or monospecific for DR5 epitopes depending on the selection of the VL and VH Domains, and will be bivalent with respect to the DR5 antigen.

[00182] Alternatively, incorporating an IgG CH2-CH3 domain onto only one of the diabody polypeptides will permit a four-chain Fc Region-containing diabody to form (**Figure 3** and **Figure 4**). Where each diabody portion is bispecific and monovalent for different DR5 epitopes the resulting four-chain molecule will be bispecific and bivalent with respect to each of two different DR5 epitopes, and tetravalent with respect to the DR5 antigen (**Figure 4**). Alternatively, if two different bispecific monovalent diabodies are combined, the resulting four-chain molecule will be tetraspecific and monovalent with respect to four different DR5 epitopes and tetravalent with respect to the DR5 antigen (**Figure 3**). Where each diabody portion is monospecific and bivalent for one DR5 epitope the resulting four-chain molecule will be monospecific and tetravalent with respect to one DR5 epitope and with respect to the DR5 antigen. Although **Figure 3** shows diabodies possessing the Constant Light (CL) Domain and the Constant Heavy-1 (CH1) Domain, fragments of such domains as well as other polypeptides may alternatively be employed as Heterodimer-Promoting Domains as shown schematically in **Figure 4** (see., e.g., United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO

2012/162068; WO 2012/018687; WO 2010/080538). Thus, for example, in lieu of the CH1 domain, one may employ a peptide having the amino acid sequence GVEPKSC (SEQ ID NO:35) or VEPKSC (SEQ ID NO:36), derived from the hinge domain of a human IgG, and in lieu of the CL domain, one may employ the C-terminal 6 amino acids of the human kappa light chain, GFNRGEC (SEQ ID NO:37) or FNRGEC (SEQ ID NO:38). A representative peptide containing four-chain diabody is shown in **Figure 4A**. Alternatively, or in addition, one may employ a peptide comprising tandem coil domains of opposing charge such as the “E-coil” helical domains (SEQ ID NO:39: EVAALEK-EVAALEK-EVAALEK-EVAALEK or SEQ ID NO:41: EVAACEK-EVAALEK-EVAALEK-EVAALEK); and the “K-coil” domains (SEQ ID NO:40: KVAALKE-KVAALKE-KVAALKE-KVAALKE or SEQ ID NO:42: KVAACKE-KVAALKE-KVAALKE-KVAALKE). A representative coil domain containing four-chain diabody is shown in **Figure 4B**.

[00183] Additional or alternative linkers that may be employed in the Fc Region-containing diabody molecules of the present invention include: ASTKG (SEQ ID NO:47), DKTHTCPPCP (SEQ ID NO:48), LEPKSS (SEQ ID NO:49), and APSSSPME (SEQ ID NO:50), GGC, and GGG. SEQ ID NO:49 may be used in lieu of GGG or GGC for ease of cloning. Additionally, SEQ ID NO:49 may be immediately followed by SEQ ID NO:47 to form an alternate linker (LEPKSSDKTHTCPPCP; SEQ ID NO:51).

[00184] As provided in Figure 3, and Figure 4A-4B, diabodies of the invention may comprise four different chains. The first and third polypeptide chains of such a diabody contain three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) Heterodimer-Promoting Domain and (iv) a Domain containing a CH2-CH3 sequence. The second and fourth polypeptide contain: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the first/third chains with the second/fourth chains. The VL and/or VH Domains of the third and fourth polypeptide chains, and VL and/or VH Domains of the first and second polypeptide chains may be the same or different so as to permit tetravalent binding that is either

monospecific, bispecific or tetraspecific. The general structure of the polypeptide chains of a representative four-chain Fc Region-containing diabodies of invention is provided in **Table 2**:

Table 2		
Bispecific	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer-Promoting Domain-COOH
	1 st Chain	NH ₂ -VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	1 st Chain	NH ₂ -VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer-Promoting Domain-COOH
Tetraspecific	2 nd Chain	NH ₂ -VL2-VH1-Heterodimer-Promoting Domain-COOH
	1 st Chain	NH ₂ -VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VL3-VH4-Heterodimer-Promoting Domain-CH2-CH3-COOH
	4 th Chain	NH ₂ -VL4-VH3-Heterodimer-Promoting Domain-COOH

[00185] The structure of the first and second polypeptide chains of representative Fc Region-containing diabodies of invention tetravalent for DR5 (*i.e.*, having four antigen-binding domains each capable of binding human DR5) are provided in **Table 3**. Each Fc Region-containing diabody comprises two pairs of covalently bonded first and second polypeptide chains such that:

- (a) the VL1 Domain of said first polypeptide chain and the VH1 Domain of said second polypeptide chain form an Antigen-Binding Domain capable of specific binding to a first epitope of DR5;
- (b) said VH2 Domain of said first polypeptide chain and said VL1 Domain of said second polypeptide chain form an Antigen-Binding Domain capable of specific binding to a second epitope of DR5; and
- (c) the CH2-CH3 portions of the pair of first polypeptide chains form an IgG Fc Region.

[00186] As described herein, the Fc Region (*i.e.*, CH2-CH3 domains of an IgG heavy chain) may be a variant Fc Region having altered affinity for an FcγR and/or altered effector function and/or altered serum half-life. In some embodiments, the Fc Region is a variant lacking the C-terminal residue.

Table 3	
Diabody DR5 Binding Molecules Comprising E/K-Coil Heterodimer-Promoting Domains	
1 st Polypeptide Chain	NH₂—[VL1 Domain]—[Linker 1]—[VH2 Domain]—[Linker 2] — [Heterodimer-Promoting domain]—[Linker 3]—[CH2-CH3 Domain]—COOH Wherein: [VL1 Domain] comprises the VL Domain from an anti-DR5 antibody binding to a first DR5 epitope; [Linker 1] is SEQ ID NO:33; [VH2 Domain] comprises the VH Domain from an anti-DR5 antibody binding to a second DR5 epitope; [Linker 2] comprises the amino acids “GGG,” or “GGC,” or is selected from ASTKG (SEQ ID NO:47) and GGCGGG (SEQ ID NO:34); [Heterodimer-Promoting Domain] is an E-coil Domain (SEQ ID NO:39 or 41), or a K-coil Domain (SEQ ID NO:40 or 42); [Linker 3] comprises the amino acids “GGG,” or “GGC,” or is selected from DKTHTCPPCP (SEQ ID NO:48), LEPKSS (SEQ ID NO:49), APSSSPME (SEQ ID NO:50) and LEPKSSDKTHTCPPCP (SEQ ID NO:51); and [CH2-CH3 Domain] comprises the CH2-CH3 domains of an IgG starting from residue 231 according to EU numbering, optionally lacking the C-terminal amino acid residue.
2 nd Polypeptide Chain	NH₂—[VL1 Domain]—[Linker 1]—[VH2 Domain]—[Linker 2] — [Heterodimer-Promoting Domain]—COOH Wherein: [VL1 Domain] comprises the VL Domain from the anti-DR5 antibody binding to the first DR5 epitope; [Linker 1] is SEQ ID NO:33; [VH2 Domain] comprises the VH Domain from the anti-DR5 antibody binding to the second DR5 epitope; [Linker 2] comprises the amino acids “GGG,” or “GGC,” or is selected from ASTKG (SEQ ID NO:47) and GGCGGG (SEQ ID NO:34); and [Heterodimer-Promoting Domain] is an E-coil Domain (SEQ ID NO:39 or 41), or a K-coil Domain (SEQ ID NO:40 or 42), wherein the [Heterodimer-Promoting Domain] of the first polypeptide chain and the [Heterodimer-Promoting Domain] of the second polypeptide chain are not both E-coil Domains or both K-coil Domains.

Table 3	
Diabody DR5 Binding Molecules Comprising Peptide Heterodimer-Promoting Domains	
1 st Polypeptide Chain	NH₂—[VL1 Domain]—[Linker 1]—[VH2 Domain]—[Linker 2] — [Heterodimer-Promoting Domain]—[Linker 3]—[CH2-CH3 Domain]—COOH Wherein: [VL1 Domain] comprises the VL Domain from an anti-DR5 antibody binding to a first DR5 epitope; [Linker 1] is SEQ ID NO:33; [VH2 Domain] comprises the VH Domain from an anti-DR5 antibody binding to a second DR5 epitope; [Linker 2] comprises the amino acids “GGG,” or “GGC,” or is selected from ASTKG (SEQ ID NO:47) and GGCGGG (SEQ ID NO:34); [Heterodimer-Promoting Domain] is] is (i) GVEPKSC (SEQ ID NO:35) or VEPKSC (SEQ ID NO:36); or (ii) GFNRGEC (SEQ ID NO:37) or FNRGEC (SEQ ID NO:38); [Linker 3] comprises the amino acids “GGG,” or “GGC,” or is selected from DKTHTCPPCP (SEQ ID NO:48), LEPKSS (SEQ ID NO:49), APSSSPME (SEQ ID NO:50) and LEPKSSDKTHTCPPCP (SEQ ID NO:51); and [CH2-CH3 Domain] comprises the CH2-CH3 domains of an IgG starting from reside 231 according to EU numbering, optionally lacking the C-terminal amino acid residue.
2 nd Polypeptide Chain	NH₂—[VL1 Domain]—[Linker 1]—[VH2 Domain]—[Linker 2] —[Heterodimer-Promoting Domain]—COOH Wherein: [VL1 Domain] comprises the VL Domain from the anti-DR5 antibody binding to the first DR5 epitope; [Linker 1] is SEQ ID NO:33; [VH2 Domain] comprises the VH Domain from the anti-DR5 antibody binding to the second DR5 epitope; [Linker 2] comprises the amino acids “GGG,” or “GGC,” or is selected from ASTKG (SEQ ID NO:47) and GGCGGG (SEQ ID NO:34); and [Heterodimer-Promoting Domain] is (i) GVEPKSC (SEQ ID NO:35) or VEPKSC (SEQ ID NO:36); or (ii) GFNRGEC (SEQ ID NO:37) or FNRGEC (SEQ ID NO:38), wherein the [Heterodimer-Promoting Domain] of the first polypeptide chain and the [Heterodimer-Promoting Domain] of the second polypeptide chain are not both selected from (i) or (ii).

Table 3	
Diabody DR5 Binding Molecules Comprising IgG Constant Domains	
1 st Polypeptide Chain	NH₂—[VL1 Domain]—[Linker 1]—[VH2 Domain]—[Linker 2] —[CH1-H-CH2-CH3 Domain]—COOH Wherein: [VL1 Domain] comprises the VL Domain from an anti-DR5 antibody binding to a first DR5 epitope; [Linker 1] is SEQ ID NO:33; [VH2 Domain] comprises the VH Domain from an anti-DR5 antibody binding to a second DR5 epitope; [Linker 2] comprises the amino acids “GGG,” or “GGC,” or is selected from ASTKG (SEQ ID NO:47) and GGCGGG (SEQ ID NO:34); [CH1-H-CH2-CH3 Domain] comprises the constant domains (CH1 to CH3) of an IgG heavy chain, optionally lacking the C-terminal amino acid residue.
2 nd Polypeptide Chain	NH₂—[VL1 Domain]—[Linker 1]—[VH2 Domain]—[Linker 2] —[CL Domain]—COOH Wherein: [VL1 Domain] comprises the VL Domain from the anti-DR5 antibody binding to the first DR5 epitope; [Linker 1] is SEQ ID NO:33; [VH2 Domain] comprises the VH Domain from the anti-DR5 antibody binding to the second DR5 epitope; [Linker 2] comprises the amino acids “GGG,” or “GGC,” or is selected from ASTKG (SEQ ID NO:47) and GGCGGG (SEQ ID NO:34); and [CL Domain] comprises the CL domain of an IgG light chain.

[00187] The Fc Region of the Fc Region-containing diabodies of the present invention may be either a complete Fc Region (*e.g.*, a complete IgG Fc Region) or only a fragment of a complete Fc Region. Although the Fc Region of the Fc Region-containing diabodies of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), more preferably such Fc Region will cause altered binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc Region) or will substantially eliminate the ability of such Fc Region to bind to inhibitory receptor(s). Thus, the Fc Region of the Fc Region-containing diabodies of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Region, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more

deletions with respect to the CH2 or CH3 Domains of a complete Fc Region). Such Fc Regions may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Regions, or may comprise non-naturally occurring orientations of CH2 and/or CH3 Domains (such as, for example, two CH2 Domains or two CH3 Domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, *etc.*).

[00188] In particular, it is preferred for the CH2-CH3 domains of the polypeptide chains of the Fc Region-containing diabodies of the present invention to exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**)). Fc variants and mutant forms capable of mediating such altered binding are described above. In a specific embodiment, the CH2-CH3 domains of the polypeptide chains of the Fc Region-containing diabodies of the present invention comprise an IgG Fc Region that mediates little or no ADCC effector function. In a preferred embodiment the CH2-CH3 Domain of the first and/or third polypeptide chains of such diabodies include any 1, 2, or 3, of the substitutions: L234A, L235A, D265A, N297Q, and N297G. In another embodiment, the human IgG1 Fc Region variant contains an N297Q substitution, an N297G substitution, L234A and L235A substitutions or a D265A substitution, as these mutations abolish FcR binding.

[00189] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 comprising the L234A/L235A substitutions is (**SEQ ID NO:102**):

```
APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGK
```

[00190] Alternatively, a CH2-CH3 domain which inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**)) is utilized. In a specific embodiment, the Fc Region-containing diabodies of the present invention comprise an IgG2 Fc Region (**SEQ ID NO:164**) or an IgG4 Fc Region (**SEQ**

ID NO:103), optionally lacking the C-terminal amino acid residues. Where an IgG4 Fc Region is utilized the instant invention also encompasses the introduction of a stabilizing mutation such as S228P, as numbered by the EU index as set forth in Kabat (Lu *et al.*, (2008) “*The Effect Of A Point Mutation On The Stability Of IgG4 As Monitored By Analytical Ultracentrifugation*,” J Pharmaceutical Sciences 97:960-969) to reduce the incidence of strand exchange. Other stabilizing mutations known in the art may be introduced into an IgG4 Fc Region (Peters, P *et al.*, (2012) “*Engineering an Improved IgG4 Molecule with Reduced Disulfide Bond Heterogeneity and Increased Fab Domain Thermal Stability*,” J. Biol. Chem. 287:24525-24533; PCT Patent Publication No: WO 2008/145142). Since the N297G, N297Q, L234A, L235A and D265A substitutions abolish effector function, in circumstances in which effector function is desired, these substitutions would preferably not be employed. As described above, in some embodiments, the Fc Region lacks the C-terminal amino acid residue.

[00191] The CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a “knob”, *e.g.*, tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, “the hole” (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising CH2-CH3 Domains that form an Fc Region. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.*, Ridgway *et al.* (1996) “*‘Knobs-Into-Holes’ Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization*,” Protein Engr. 9:617-621, Atwell *et al.* (1997) “*Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library*,” J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) “*A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis*,” J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety). Preferably the “knob” is

engineered into the CH2-CH3 Domains of one first polypeptide chain and the “hole” is engineered into the CH2-CH3 Domains of the third polypeptide chain. Thus, the “knob” will help in preventing the first polypeptide chains from homodimerizing via its CH2 and/or CH3 Domains. As the third polypeptide chain preferably contains the “hole” substitution it will heterodimerize with the first polypeptide chain comprising the “knob” as well as homodimerize with itself.

[00192] A preferred knob is created by modifying a native IgG Fc Region to contain the modification T366W. A preferred hole is created by modifying a native IgG Fc Region to contain the modification T366S, L368A and Y407V. To aid in purifying the homodimers from the final heterodimer Fc Region-containing diabody, the protein A binding site of the CH2 and CH3 Domains of one chain is preferably mutated by amino acid substitution at position 435 (H435R) on the third polypeptide containing the “hole” substitutions. Thus, the homodimer of third polypeptide chains containing the “hole” substitutions will not bind to protein A, whereas the heterodimer will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

[00193] A preferred sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Region-containing diabody of the present invention will have the “**knob-bearing**” sequence (SEQ ID NO:52):

```
APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGK
```

[00194] A preferred sequence for the CH2 and CH3 Domains of the second polypeptide chain of an Fc Region-containing diabody of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Region-containing diabody having three polypeptide chains) will have the “**hole-bearing**” sequence (SEQ ID NO:53):

```
APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLVSKL TVDKSRWQQG NVFSCSVMHE
ALHNRYTQKS LSLSPGK
```

[00195] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:52** and **SEQ ID NO:53** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Region exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Region (**SEQ ID NO:1**)). The C-terminal residue is optionally included.

[00196] It is preferred that the first polypeptide chain will have a “knob-bearing” CH2-CH3 sequence, such as that of **SEQ ID NO:52**. However, as will be recognized, a “hole-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:53**) could be employed in the first polypeptide chain, in which case, a “knob-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:52**) would be employed in the second polypeptide chain of an Fc Region-containing diabody of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Region-containing diabody having three or four polypeptide chains). The C-terminal residue of **SEQ ID NO: 52** and/or **SEQ ID NO:53**, is optionally included.

V. Reference Antibodies

A. Reference Anti-Human CD3 Antibodies

[00197] CD3 is a T cell co-receptor composed of four distinct chains (Wucherpfennig, K.W. *et al.* (2010) “*Structural Biology Of The T-Cell Receptor: Insights Into Receptor Assembly, Ligand Recognition, And Initiation Of Signaling*,” Cold Spring Harb. Perspect. Biol. 2(4):a005140; pages 1-14). In mammals, the complex contains a CD3γ chain, a CD3δ chain, and two CD3ε chains. These chains associate with a molecule known as the T cell receptor (TCR) in order to generate an activation signal in T lymphocytes. In the absence of CD3, TCRs do not assemble properly and are degraded (Thomas, S. *et al.* (2010) “*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*,” Immunology 129(2):170–177). CD3 is found bound to the membranes of all mature T cells, and in virtually no other cell type (see, Janeway, C.A. *et al.* (2005) In: IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE,” 6th ed. Garland Science Publishing, NY, pp. 214- 216; Sun, Z. J. *et al.* (2001) “*Mechanisms Contributing To T Cell Receptor Signaling And Assembly Revealed By The Solution Structure Of An Ectodomain Fragment Of The*

CD3ε:γ Heterodimer,” Cell 105(7):913-923; Kuhns, M.S. *et al.* (2006) “*Deconstructing The Form And Function Of The TCR/CD3 Complex*,” Immunity. 2006 Feb;24(2):133-139).

[00198] As discussed below, in order to illustrate the present invention, bispecific anti-human CD3 x anti-human DR5-Binding Molecules were produced. An anti-human CD3 antibody used for such constructs is designated herein as “**CD3 mAb 2**.” The amino acid sequence of the VL Domain of **CD3 mAb 2** (SEQ ID NO:104) is shown below (CDRL residues are shown underlined):

QAVVTQEPSL TVSPGGTVTL TC**RSSTGAVT TSNYAN**WVQQ KPGQAPRGLI
G**GTNKRAP**WT PARFSGSLLG GKAALTITGA QAEDEADYYC **ALWYSNLWVF**
GGGTKLTVLG

CDRL1 of CD3 mAb 2 (SEQ ID NO:105): **RSSTGAVTTSNYAN**

CDRL2 of CD3 mAb 2 (SEQ ID NO:106): **GTNKRAP**

CDRL3 of CD3 mAb 2 (SEQ ID NO:107): **ALWYSNLWV**

[00199] The amino acid sequence of the VH Domain of **CD3 mAb 2** (SEQ ID NO:108) is shown below (CDRH residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKDRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTIL VTVSS

CDRH1 of CD3 mAb 2 (SEQ ID NO:109): **TYAMN**

CDRH2 of CD3 mAb 2 (SEQ ID NO:110): **RIRSKYNNYATYYADSVKD**

CDRH3 of CD3 mAb 2 (SEQ ID NO:111): **HGNFGNSYVSWFAY**

[00200] In some of the CD3 constructs, a variant VH Domain was employed for **CD3 mAb 2**. The variant VH Domain possesses a D65G substitution, thus having the amino acid sequence shown below (SEQ ID NO:112) (CDRH residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKGRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTIL VTVSS

[00201] The substitution causes the CDRH2 to have the amino acid sequence (SEQ ID NO:113) RIRSKYNNYATYYADSVKG. The substituted position (D65G) is shown in double underline.

[00202] A second anti-CD3 antibody used herein is antibody Muromonab-CD3 “OKT3” (Xu *et al.* (2000) “*In Vitro Characterization Of Five Humanized OKT3 Effector Function Variant Antibodies*,” Cell. Immunol. 200:16-26); Norman, D.J. (1995) “*Mechanisms Of Action And Overview Of OKT3*,” Ther. Drug Monit. 17(6):615-620; Canafax, D.M. *et al.* (1987) “*Monoclonal Antilymphocyte Antibody (OKT3) Treatment Of Acute Renal Allograft Rejection*,” Pharmacotherapy 7(4):121-124; Swinnen, L.J. *et al.* (1993) “*OKT3 Monoclonal Antibodies Induce Interleukin-6 And Interleukin-10: A Possible Cause Of Lymphoproliferative Disorders Associated With Transplantation*,” Curr. Opin. Nephrol. Hypertens. 2(4):670-678). The amino acid sequence of the VL Domain of **OKT3 (SEQ ID NO:166)** is shown below (CDRL residues are shown underlined):

QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDT
SKLASGVPAH FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFTFGSG
 TKLEINR

[00203] The amino acid sequence of the VH Domain of **OKT3 (SEQ ID NO:167)** is shown below (CDRH residues are shown underlined):

QVQLQQSGAE LARPGASVKM SCKASGYTFT RYTMHWVKQR PGQGLEWIGY
INPSRGYTNY NQKFKDKATL TTDKSSSTAY MQLSSLTSED SAVYYCARYY
DDHYCLDYWG QGTTLTVSSA KTTAPSVYPL APVCGDTTGS SVTLGCLVKG
 YFPEPVTLTW NSGSLSSGVH TTPAVLQSDL YTLSSSVTVT SS

B. Reference Anti-Fluorescein Antibody

[00204] The anti-fluorescein antibody 4-4-20 (Gruber, M. *et al.* (1994) “*Efficient Tumor Cell Lysis Mediated By A Bispecific Single Chain Antibody Expressed In Escherichia coli*,” J. Immunol. 152(11):5368-5374; Bedzyk, W.D. *et al.* (1989) “*Comparison Of Variable Region Primary Structures Within An Anti-Fluorescein Idiotypic Family*,” J. Biol. Chem. 264(3): 1565-1569) was used in control diabodies. The amino acid sequences of the variable light and variable heavy Domains of anti-fluorescein antibody 4-4-20 are as follows:

[00205] Amino Acid Sequence Of The Variable Light Chain Domain Of Anti-Fluorescein Antibody 4-4-20 (**SEQ ID NO:114**) (CDRL residues are underlined):

DVVMTQTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK
 VLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
WTFGGGTKLE IK

[00206] Amino Acid Sequence Of The Variable Heavy Chain Domain Of Anti-Fluorescein Antibody 4-4-20 (SEQ ID NO:115) (CDR_H residues are underlined):

EVKLD~~ET~~GGG LVQPGRPMKL SCVASGFTFS DYWMNWVRQS PEKGLEWVAQ
IRNKPYNYET YYSDSVKGRF TISRDDSKSS VYLQMNNLRV EDMGIYYCTG
SYYGMDYWGQ GTSVTVSS

VI. Exemplary Multivalent DR5-Binding Molecules

[00207] As described above multivalent DR5-Binding Molecules possessing at least two, and preferably, at least four DR5 binding sites may have a variety of structures. In particular, structures comprising the antigen-binding portions of immunoglobulins, including, but not limited to, IgG-based bispecific antibodies, and molecules comprising diabodies are preferred. Specific, non-limiting, examples of multivalent DR5-Binding Molecules comprising diabodies are provided. However, alternative structures, including those disclosed above (see, *e.g.*, **Figures 1-4**) or otherwise apparent to one of skill in the art are encompassed by the instant invention.

A. DR5 x DR5 Bispecific Fc Region-Containing Diabodies Tetravalent For DR5

1. DR5 mAb 1 x DR5 mAb 2 Fc Region-Containing Diabodies

[00208] Exemplary bispecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 1 and the VL and VH Domains of DR5 mAb 2. One Fc Region-Containing diabody designated “**DR5 mAb 1 x DR5 mAb 2 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (SEQ ID NO:116):

DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
 LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
 TFGGGTKLEI KGGGSGGGGK VQLQQSGAEL VKPGASVKLS CKASGYTFTE
 YILHWVKQKS GQGLEWIGWF YPGNNNIKYN EKFKDKATLT ADKSSSTVYM
 ELSRLTSEDS AVYFCARHEQ GPGYFDYWGO GTTLTVSSAS TKGEVAACEK
 EVAALEKEVA ALEKEVAALE KLEPKSSDKT HTCPPCPAPE LLGGPSVFLF
 PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
 EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
 REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
 TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
 SPG

[00209] In **SEQ ID NO:116**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), residues 112-119 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 120-238 correspond to the amino acid sequence of the VH Domain of DR5 mAb 2 (**SEQ ID NO:18**), residues 239-243 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 244-271 correspond to a cysteine-containing E-coil Domain (**SEQ ID NO:41**), residues 272-277 correspond to a LEPKSS linker (**SEQ ID NO: 49**), residues 278-287 correspond to a linker (DKTHTCPPCP; **SEQ ID NO:48**) derived from an IgG1 hinge domain, and residues 288-503 correspond to a wild-type IgG1 Fc Region (**SEQ ID NO:1**, lacking the C-terminal amino acid residue). A polynucleotide that encodes **SEQ ID NO:116** is **SEQ ID NO:117**:

```

gacattgtgc  tgacacagtc  tcttgccttc  ttagctgtat  ctctcgggca
gagggccacc  atctcatgca  gggccagcaa  aagtgtcagt  tcctctggct
atagttatat  gcaactgtac  caacagaaac  caggacagcc  acccaaagtc
ctcatctttc  ttctcatcaa  cctagattct  ggggtccctg  ccagggttcag
tggcagtggtg  tctgggacag  acttcaccct  caacatccat  cctgtggagg
atggggatgc  tgcaacctat  tactgtcagc  acagtaggga  tcttcctccg
acgttcggtg  gaggcaccaa  gctggaaatc  aaaggaggcg  gatccggcgg
cggaggcaag  gtccagctgc  agcagtctgg  agctgaactg  gtgaaaccgg
gggcatcagt  gaagctgtcc  tgcaaggctt  ctgggtacac  cttcactgag
tatattttac  actgggtaaa  gcagaagtct  ggacagggtc  ttgagtggat
tgggtgggtt  tatcctggaa  ataataatat  aaagtacaat  gagaaattca
aggacaaggc  cacactgact  gcggacaaat  cctccagcac  agtctatatg
gaacttagta  gattgacatc  tgaagactct  gcggtctatt  tctgtgcaag
acacgaacaa  ggaccagggt  actttgacta  ctggggccaa  ggcaccactc
tcacagtctc  ctccgcctcc  accaaggggc  aagtggccgc  atgtgagaaa
gaggttgctg  ctttggagaa  ggaggtcgct  gcacttgaaa  aggaggtcgc
agccctggag  aaactggagc  ccaaattctc  tgacaaaact  cacacatgcc
caccgtgccc  agcacctgaa  ctccctgggg  gaccgtcagt  cttcctcttc
ccccaaaac  ccaaggacac  cctcatgata  tcccggaccc  ctgaggtcac
atgcgtggtg  gtggacgtga  gccacgaaga  ccctgaggtc  aagttcaact
ggtacgtgga  cggcgtggag  gtgcataatg  ccaagacaaa  gccgcgggag
gagcagtaca  acagcacgta  ccgtgtggtc  agcgtcctca  ccgtcctgca
ccaggactgg  ctgaatggca  aggagtacaa  gtgcaaggtc  tccaacaaag
ccctcccagc  ccccatcgag  aaaaccatct  ccaaagccaa  agggcagccc
cgagaaccac  aggtgtacac  cctgccccca  tcccgggagg  agatgaccaa
gaaccaggtc  agcctgacct  gcctgggtcaa  aggcttctat  ccagcgcaca
tcgccgtgga  gtgggagagc  aatgggcagc  cggagaacaa  ctacaagacc
acgcctcccg  tgctggactc  cgacggctcc  ttcttcctct  acagcaagct
caccgtggac  aagagcagg  ggcagcagg  gaacgtcttc  tcatgctccg
tgatgcatga  ggctctgcac  aaccactaca  cgcagaagag  cctctccctg
tctccgggt

```

[00210] The amino acid sequence of the second polypeptide chain of DR5 mAb 1 x DR5 mAb 2 Fc diabody is (**SEQ ID NO:118**):

```
DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGEVKFL ESGGGLVQPG GSLKLSCVAS GFDFSRYWMS
WVRQAPGKGL EWIGEINPDS NTINYTPSLK DKFIISRDN KNTLYLQMTK
VRSEDTALYY CTRRAYYGNP AWFAYWGQGT LVTVSAASTK GKVAACKEKV
AALKEKVAAL KEKVAALKE
```

[00211] In **SEQ ID NO:118**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of DR5 mAb 2 (**SEQ ID NO:13**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-236 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (**SEQ ID NO:8**), except that the C-terminal serine residue of **SEQ ID NO:8** has been replaced with an alanine residue, residues 237-241 correspond to an ASTKG linker (**SEQ ID NO:47**), and residues 242-269 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**). A polynucleotide that encodes **SEQ ID NO:118** is **SEQ ID NO:119**:

```
gacattgtga tgacccagtc tcacaaattc atgtccactt cagtaggaga
cagggtcagc atcacctgca aggccagtca ggatgtgaat actgctgtag
cctggtatca acaaaaacca gggcaatctc ctaaactact gatttactgg
gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
tgggacagat tatacactca ccatcaaaag tgtgcaggct gaagacctga
cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga
ggcaccaagc tggaaatcaa aggaggcgga tccggcgggc gaggcgaggt
gaagtttctc gagtctggag gtggcctggt gcagcctgga ggatccctga
aactctcctg tgtagcctca ggattcgatt ttagtagata ctggatgagt
tggttccggc aggctccagg gaaagggcta gaatggattg gagaaattaa
tccagatagc aatacgataa actatacgcc atctctaaag gataaattca
tcatctccag agacaacgcc aaaaatacgc tgtatctgca aatgacccaa
gtgagatctg aggacacagc cctttattat tgtacaagaa gggcctacta
tggttaacccg gcctgggttg cttactgggg ccaagggact ctggtcactg
tctctgcagc ctccaccaag ggcaaagtgg ccgcatgtaa ggagaaagtt
gctgctttga aagagaaggt cgccgcactt aaggaaaagg tcgcagccct
gaaagag
```

[00212] Another Fc Region-containing diabody, designated “**DR5 mAb 1 x DR5 mAb 2 Fc diabody (AA)**,” is identical to DR5 mAb 1 x DR5 mAb 2 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**underlined**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The

amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (SEQ ID NO:120):

```

DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI KGGGSGGGGK VQLQQSGAEL VKPGASVKLS CKASGYTFTE
YILHWVKQKS GQGLEWIGWF YPGNNNIKYN EKFKDKATLT ADKSSSTVYM
ELSRLTSEDS AVYFCARHEQ GPGYFDYWGQ GTTLTVSSAS TKGEVAACEK
EVAALEKEVA ALEKEVAALE KLEPKSSDKT HTCPPCPAPE AAGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPG

```

[00213] A polynucleotide that encodes SEQ ID NO:120 is SEQ ID NO:121:

```

gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcaactgtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccaggttcag
tggcagtggtg tctgggacag acttcaccct caacatccat cctgtggagg
atgggggatgc tgcaacctat tactgtcagc acagtaggga tcttcctccg
acgttcggtg gaggcaccaa gctggaaatc aaaggaggcg gatccggcgg
cggaggcaag gtccagctgc agcagtctgg agctgaactg gtgaaacccg
gggcatcagt gaagctgtcc tgcaaggctt ctgggtacac cttcactgag
tatattttac actgggtaaa gcagaagtct ggacagggtc ttgagtggat
tggttggttt tatcctggaa ataataatat aaagtacaat gagaaattca
aggacaaggc cacactgact gcggacaaat cctccagcac agtctatatg
gaacttagta gattgacatc tgaagactct gcggtctatt tctgtgcaag
acacgaacaa ggaccagggtt actttgacta ctggggccaa ggcaccactc
tcacagtctc ctccgcctcc accaaggggc aagtggccgc atgtgagaaa
gaggttgctg ctttgagaaa ggaggtcgct gcacttgaaa aggaggtcgc
agccctggag aaactggagc ccaaattctc tgacaaaact cacacatgcc
caccgtgccc agcacctgaa ctcttggggg gaccgtcagt cttcctcttc
ccccaaaac ccaaggacac cctcatgata tcccggaccc ctgagggtcac
atgcgtggtg gtggacgtga gccacgaaga ccctgagggtc aagttcaact
ggtacgtgga cggcgtggag gtgcataatg ccaagacaaa gccgcgggag
gagcagtaca acagcacgta ccgtgtggtc agcgtcctca ccgtcctgca
ccaggactgg ctgaatggca aggagtacaa gtgcaagggtc tccaacaaag
ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc
cgagaaccac aggtgtacac cctgccccca tcccgggagg agatgaccaa
gaaccagggtc agcctgacct gcctgggtcaa aggttcttat cccagcgaca
tcgccgtgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc
acgcctcccg tgctggactc cgacggctcc ttcttcctct acagcaagct
caccgtggac aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg
tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg
tctccgggt

```

[00214] The second polypeptide chain of DR5 mAb 1 x DR5 mAb 2 Fc diabody (AA) is also **SEQ ID NO:118** (encoded by **SEQ ID NO:119**), described in detail above.

[00215] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 288-504 of **SEQ ID NOs:116 or 120** will be replaced with **SEQ ID NO:164** (CH2-CH3 of IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

2. DR5 mAb 2 x DR5 mAb 1 Fc Region-Containing Diabodies

[00216] Exemplary bispecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 and the VL and VH Domains of DR5 mAb 1. One Fc Region-Containing diabody designated “**DR5 mAb 2 x DR5 mAb 1 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:122**):

```

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAWAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGEVKFL ESGGGLVQPG GSLKLSCVAS GFDFSRYWMS
WVRQAPGKGL EWIGEINPDS NTINYTPSLK DKFIISRDN KNTLYLQMTK
VRSEDTALYY CTRRAYYGNP AWFAYWGQGT LTVVSAASTK GEVAACEKEV
AALEKEVAAL EKEVAALEKL EPKSSDKTHT CPPCPAPELL GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
PVLDSGGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP
G

```

[00217] In **SEQ ID NO:122**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of DR5 mAb 2 (**SEQ ID NO:13**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-236 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (**SEQ ID NO:8**) except that the C-terminal serine residue of **SEQ ID NO:8** has been replaced with an alanine residue, residues 237-241 correspond to an ASTKG linker

(SEQ ID NO:47) residues 242-269 correspond to a cysteine-containing E-coil Domain (SEQ ID NO:41), residues 270-275 correspond to a LEPKSS linker (SEQ ID NO:49), residues 276-285 correspond to a linker (DKTHTCPPCP; SEQ ID NO:48) derived from an IgG1 hinge domain, and residues 286-501 correspond to a wild-type IgG1 Fc Region (SEQ ID NO:1, lacking the C-terminal amino acid residue). A polynucleotide that encodes SEQ ID NO:122 is SEQ ID NO:123:

```

gacattgtga  tgacccagtc  tcacaaattc  atgtccactt  cagtaggaga
cagggtcagc  atcacctgca  aggccagtc  ggatgtgaat  actgctgtag
cctggatatca  acaaaaacca  gggcaatctc  ctaaactact  gatttactgg
gcatccaccc  ggcacactgg  agtccctgat  cgcttcacag  gcagtggatc
tgggacagat  tatacactca  ccatcaaaag  tgtgcaggct  gaagacctga
cactttatta  ctgtcagcaa  cactatatca  ctccgtggac  gttcgggtga
ggcaccaagc  tggaaatcaa  aggaggcgga  tccggcgggc  gaggcgaggt
gaagtttctc  gagtctggag  gtggcctggt  gcagcctgga  ggatccctga
aactctcctg  tgtagcctca  ggattcgatt  ttagtagata  ctggatgagt
tgggtccggc  aggctccagg  gaaagggcta  gaatggattg  gagaaattaa
tccagatagc  aatacgataa  actatacgcc  atctctaaag  gataaattca
tcatctccag  agacaacgcc  aaaaatacgc  tgtatctgca  aatgaccaa
gtgagatctg  aggacacagc  cctttattat  tgtacaagaa  gggcctacta
tggttaaccg  gcctggtttg  cttactgggg  ccaagggact  ctggtcactg
tctctgcagc  ctccaccaag  ggcgaagtgg  ccgcatgtga  gaaagaggtt
gctgcttttg  agaaggaggt  cgctgcactt  gaaaaggagg  tcgcagccct
ggagaaactg  gagcccaaatt  cttctgacaa  aactcacaca  tgcccaccgt
gcccagcacc  tgaactcctg  gggggaccgt  cagtcttcct  cttcccccca
aaaccaag  acaccctcat  gatctcccg  acccctgagg  tcacatgcgt
ggtggtggac  gtgagccacg  aagaccctga  ggtcaagttc  aactggtacg
tggaaggcgt  ggaggtgcat  aatgccaaga  caaagccgcg  ggaggagcag
tacaacagca  cgtaccgtgt  ggtcagcgtc  ctaccgtcc  tgcaccagga
ctggctgaat  ggcaaggagt  acaagtgcaa  ggtctccaac  aaagccctcc
cagcccccat  cgagaaaacc  atctccaaag  ccaaagggca  gccccgagaa
ccacaggtgt  acaccctgcc  cccatcccgg  gaggagatga  ccaagaacca
ggtcagcctg  acctgcctgg  tcaaaggctt  ctatcccagc  gacatcgccg
tggaagtggga  gagcaatggg  cagccggaga  acaactacaa  gaccacgcct
cccgtgctgg  actccgacgg  ctcttcttc  ctctacagca  agctcaccgt
ggacaagagc  aggtggcagc  aggggaacgt  cttctcatgc  tccgtgatgc
atgaggctct  gcacaaccac  tacacgcaga  agagcctctc  cctgtctccg
ggt

```

[00218] The amino acid sequence of the second polypeptide chain of DR5 mAb 2 x DR5 mAb 1 Fc diabody is (SEQ ID NO:124):

```

DIVLTQSPAS  LAVSLGQRAT  ISCRASKSVS  SSGYSYMHY  QQKPGQPPKV
LIFLSSNLDS  GVPARFSGSG  SGTDFTLNIH  PVEDGDAATY  YCQHSRDLPP
TFGGGTKLEI  KGGGSGGGGK  VQLQQSGAEL  VKPGASVKLS  CKASGYTFTE
YILHWVKQKS  GQGLEWIGWF  YPGNNNIKYN  EKFKDKATLT  ADKSSSTVYM

```

ELSRLTSEDS AVYFCARHEQ GPGYFDYWGO GTTLTVSSAS TKGKVAACKE
KVAALKEKVA ALKEKVAALK E

[00219] In **SEQ ID NO:124**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), residues 112-119 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 120-238 correspond to the amino acid sequence of the VH Domain of DR5 mAb 2 (**SEQ ID NO:18**), residues 239-243 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 244-271 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**). A polynucleotide that encodes **SEQ ID NO:124** is **SEQ ID NO:125**:

```
gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctcgggca
gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
atagttatat gcaactgtac caacagaaac caggacagcc acccaaagtc
ctcatctttc tttcatccaa cctagattct ggggtccctg ccaggttcag
tggcagtggtg tctgggacag acttcaccct caacatccat cctgtggagg
atggggatgc tgcaacctat tactgtcagc acagtaggga tcttctccg
acgttcggtg gaggcaccaa gctggaaatc aaaggaggcg gatccggcgg
cggaggcaag gtccagctgc agcagtctgg agctgaactg gtgaaaccg
gggcatcagt gaagctgtcc tgcaaggctt ctgggtacac cttcactgag
tataattttac actgggtaaa gcagaagtct ggacagggtc ttgagtggat
tgggtggttt tatcctggaa ataataatat aaagtacaat gagaaattca
aggacaaggc cacactgact gcggacaaat cctccagcac agtctatatg
gaacttagta gattgacatc tgaagactct gcggtctatt tctgtgcaag
acacgaacaa ggaccagggt actttgacta ctggggccaa ggcaccactc
tcacagtctc ctccgcctcc accaagggca aagtggccgc atgtaaggag
aaagttgctg ctttgaaaga gaaggtcgcc gcacttaagg aaaaggtcgc
agccctgaaa gag
```

[00220] Another Fc Region-containing diabody, designated “**DR5 mAb 2 x DR5 mAb 1 Fc diabody (AA)**,” is identical to DR5 mAb 2 x DR5 mAb 1 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**bolded**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:126**):

```
DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGEVKFL ESGGGLVQPG GSLKLSCVAS GFDFSRYWMS
WVRQAPGKGL EWIGEINPDS NTINYTPSLK DKFIISRDNA KNTLYLQMTK
VRSEDALYY CTRRAYYGNP AWFAYWGQGT LVTVSAASTK GEVAACEKEV
AALEKEVAAL EKEVAALEKL EPKSSDKTHT CPPCPAPEAA GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
```

PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
 PVLDS DGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 G

[00221] A polynucleotide that encodes **SEQ ID NO:126** is **SEQ ID NO:127**:

gacattgtga tgacccagtc tcacaaattc atgtccactt cagtaggaga
 cagggtcagc atcacctgca aggccagtc g gatgtgaat actgctgtag
 cctggatatca acaaaaacca gggcaatctc ctaaactact gatttactgg
 gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
 tgggacagat tatacactca ccatcaaaag tgtgcaggct gaagacctga
 cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga
 ggcaccaagc tggaaatcaa aggaggcggg tccggcggcg gaggcgaggt
 gaagttttctc gagtctggag gtggcctggc gcagcctgga ggatccctga
 aactctcctg tgtagcctca ggattcgatt ttagtagata ctggatgagt
 tgggtccggc aggctccagg gaaagggcta gaatggattg gagaaattaa
 tccagatagc aatacgataa actatacgcc atctctaaag gataaattca
 tcatctccag agacaacgcc aaaaatacgc tgtatctgca aatgaccaa
 gtgagatctg aggacacagc cctttattat tgtacaagaa gggcctacta
 tggtaaccgg gcctgggtttg cttactgggg ccaagggact ctgggtcactg
 tctctgcagc ctccaccaag ggcgaagtgg ccgcatgtga gaaagaggtt
 gctgcttttg agaaggaggt cgctgcactt gaaaaggagg tcgcagccct
 ggagaaactg gagcccaaat cttctgacaa aactcacaca tgcccaccgt
 gccagcacc tgaactcctg gggggaccgt cagtcttcct cttcccccca
 aaaccaagg acaccctcat gatctcccg acccctgagg tcacatgcgt
 ggtggtggac gtgagccacg aagaccctga ggtcaagttc aactggtacg
 tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag
 tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga
 ctggctgaat ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc
 cagcccccat cgagaaaacc atctccaaag ccaaagggca gccccgagaa
 ccacaggtgt acaccctgcc cccatcccg gaggagatga ccaagaacca
 ggtcagcctg acctgcctgg tcaaaggctt ctatcccagc gacatcgccg
 tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct
 cccgtgctgg actccgacgg ctctctcttc ctctacagca agctcaccgt
 ggacaagagc aggtggcagc aggggaacgt cttctcatgc tccgtgatgc
 atgaggctct gcacaaccac tacacgcaga agagcctctc cctgtctccg
 ggt

[00222] The second polypeptide chain of DR5 mAb 1 x DR5 mAb 2 Fc diabody (AA) is also **SEQ ID NO:124** (encoded by **SEQ ID NO:125**), described in detail above.

[00223] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 286-502 of **SEQ ID NOs:122 or 126** will be replaced with **SEQ ID NO:164** (CH2-CH3 of

IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

B. DR5 x DR5 Bispecific Diabodies Bivalent For DR5

1. DR5 mAb 1 x DR5 mAb 2 Diabody

[00224] Exemplary bispecific diabodies bispecific for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 1 and the VL and VH Domains of DR5 mAb 2. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-271 of **SEQ ID NO:116** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:118** described above.

[00225] Other exemplary bispecific diabodies bispecific for DR5 comprising an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 1 and the VL and VH Domains of DR5 mAb 2. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:116** or **SEQ ID NO:120** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:118**, and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc Region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

2. DR5 mAb 2 x DR5 mAb 1 Diabody

[00226] Exemplary bispecific diabodies bivalent for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 and the VL and VH Domains of DR5 mAb 1. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-269 of **SEQ ID NO:122** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:124** described above.

[00227] Other exemplary bispecific diabodies bivalent for DR5 containing an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 and the VL and VH Domains of DR5 mAb 1. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:122** or **SEQ ID NO:126** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:124**, and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc Region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

C. **DR5 x DR5 Monospecific Fc Region-Containing Diabodies Tetravalent For DR5**

1. **DR5 mAb 1 x DR5 mAb 1 Fc Region-Containing Diabodies**

[00228] Exemplary monospecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 1. One Fc Region-Containing diabody designated “**DR5 mAb 1 x DR5 mAb 1 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:128**):

```
DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
TFGGGTKLEI KGGSGGGGGE VKFLESGGGL VQPGGSLKLS CVASGFDFSR
YWMSWVRQAP GKGLEWIGEI NPDSNTINYT PSLKDKFIIS RDNAKNTLYL
QMTKVRSEDY ALYYCTRRAY YGNPAWFAYW GQGTLVTVSA ASTKGEVAAC
EKEVAALEKE VAALEKEVAA LEKLEPKSSD KTHTCPPCPA PELLGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVS NKALPAP IEKTISKAKG
QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
SLSPG
```

[00229] In **SEQ ID NO:128**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), residues 112-119 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 120-240 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (**SEQ ID NO:8**) except that the C-terminal serine residue of **SEQ ID NO:8** has

been replaced with an alanine residue, residues 241-245 correspond to an ASTKG linker (SEQ ID NO:47) residues 246-273 correspond to a cysteine-containing E-coil Domain (SEQ ID NO:41), residues 274-279 correspond to a LEPKSS linker (SEQ ID NO:49), residues 280-289 correspond to a linker (DKTHTCPPCP; SEQ ID NO:48) derived from an IgG1 hinge domain, and residues 290-505 correspond to a wild-type IgG1 Fc Region (SEQ ID NO:1, lacking the C-terminal amino acid residue). A polynucleotide that encodes SEQ ID NO:128 is SEQ ID NO:129:

```

gacattgtgc  tgacacagtc  tcctgcttcc  ttagctgtat  ctctcgggca
gagggccacc  atctcatgca  gggccagcaa  aagtgtcagt  tcctctggct
atagttatat  gcaactgtac  caacagaaac  caggacagcc  acccaaagtc
ctcatctttc  tttcatccaa  cctagattct  ggggtccctg  ccaggttcag
tggcagtggg  tctgggacag  acttcaccct  caacatccat  cctgtggagg
atggggatgc  tgcaacctat  tactgtcagc  acagtaggga  tcttctccg
acgttcggtg  gaggcaccaa  gctggaaatc  aaaggaggcg  gatccggcgg
cggaggcgag  gtgaagtttc  tcgagtctgg  aggtggcctg  gtgcagcctg
gaggatccct  gaaactctcc  tgtgtagcct  caggattcga  ttttagtaga
tactggatga  gttgggtccg  gcaggctcca  gggaaagggc  tagaatggat
tggagaaatt  aatccagata  gcaatacgat  aaactatacg  ccatctctaa
aggataaatt  catcatctcc  agagacaacg  ccaaaaatac  gctgtatctg
caaatgacca  aagtgagatc  tgaggacaca  gccctttatt  attgtacaag
aagggcctac  tatggtaacc  cggcctgggt  tgcttactgg  ggccaaggga
ctctggtcac  tgtctctgca  gcctccacca  agggcgaagt  ggccgcatgt
gagaaagagg  ttgctgcttt  ggagaaggag  gtcgctgcac  ttgaaaagga
ggtcgcagcc  ctggagaaac  tggagcccaa  atcttctgac  aaaactcaca
catgcccacc  gtgcccagca  cctgaactcc  tggggggacc  gtcagtcttc
ctcttcccc  caaaacccaa  ggacaccctc  atgatctccc  ggaccctga
ggtcacatgc  gtggtggtgg  acgtgagcca  cgaagaccct  gaggtcaagt
tcaactggta  cgtggacggc  gtggagggtg  ataatgcca  gacaaagccg
cgggaggagc  agtacaacag  cacgtaccgt  gtggtcagcg  tcctcaccgt
cctgcaccag  gactggctga  atggcaagga  gtacaagtgc  aagggtctca
acaaagccct  cccagcccc  atcgagaaaa  ccatctccaa  agccaaaggg
cagccccgag  aaccacaggt  gtacaccctg  ccccatccc  gggaggagat
gaccaagaac  caggtcagcc  tgacctgcct  ggtcaaaggc  ttctatccca
gcgacatcgc  cgtggagtgg  gagagcaatg  ggcagccgga  gaacaactac
aagaccacgc  ctcccgtgct  ggactccgac  ggctccttct  tcctctacag
caagctcacc  gtggacaaga  gcagggtggc  gcaggggaac  gtcttctcat
gctccgtgat  gcatgaggct  ctgcacaacc  actacacgca  gaagagcctc
tcctgtctc  cgggt

```

[00230] The amino acid sequence of the second polypeptide chain of DR5 mAb 1 x DR5 mAb 1 Fc diabody is (SEQ ID NO:130):

```

DIVLTQSPAS  LAVSLGQRAT  ISCRASKSVS  SSGYSYMHWY  QQKPGQPPKV
LIFLSSNLDS  GVPARFSGSG  SGTDFTLNIH  PVEDGDAATY  YCQHSRDLPP
TFGGGTKLEI  KGGGSGGGGE  VKFLESGGGL  VQPGGSLKLS  CVASGFDFSR

```

YWMSWVRQAP GKGLEWIGEI NPDSNTINYT PSLKDKFIIS RDNAKNTLYL
 QMTKVRSEDY ALYYCTRRAY YGNPAWFAYW GQGTLLTVSA ASTKGKVAAC
 KEKVAALKEK VAALKEKVAA LKE

[00231] In **SEQ ID NO:130**, amino acid residues 1-111 correspond to the amino acid sequence of the VL Domain of DR5 mAb 1 (**SEQ ID NO:3**), residues 112-119 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 120-240 correspond to the amino acid sequence of the VH Domain of DR5 mAb 1 (**SEQ ID NO:8**) except that the C-terminal serine residue of **SEQ ID NO:8** has been replaced with an alanine residue, residues 241-245 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 246-273 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**). A polynucleotide that encodes **SEQ ID NO:130** is **SEQ ID NO:131**:

gacattgtgc tgacacagtc tcttgccttc ttagctgtat ctctcgggca
 gagggccacc atctcatgca gggccagcaa aagtgtcagt tcctctggct
 atagttatat gcaactggtac caacagaaac caggacagcc acccaaagtc
 ctcatctttc ttctcatcaa cctagattct ggggtccctg ccagggttcag
 tggcagtggtg tctgggacag acttcaccct caacatccat cctgtggagg
 atggggatgc tgcaacctat tactgtcagc acagtaggga tcttcctccg
 acgttcggtg gaggcaccaa gctggaaatc aaaggaggcg gatccggcgg
 cggaggcgag gtgaagtttc tcgagtcctg aggtggcctg gtgcagcctg
 gaggatccct gaaactctcc tgtgtagcct caggattcga ttttagtaga
 tactggatga gttgggtccg gcaggctcca gggaaagggc tagaatggat
 tggagaaatt aatccagata gcaatacgat aaactatacg ccatctctaa
 aggataaatt catcatctcc agagacaacg ccaaaaatac gctgtatctg
 caaatgacca aagtgaagtc tgaggacaca gccctttatt attgtacaag
 aagggcctac tatggtaacc cggcctggtt tgcttactgg ggccaaggga
 ctctggtcac tgtctctgca gcctccacca agggcaaagt ggccgcatgt
 aaggagaaag ttgctgcttt gaaagagaag gtcgccgcac ttaaggaaaa
 ggtcgcagcc ctgaaagag

[00232] Another Fc Region-containing diabody, designated “**DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA)**,” is identical DR5 mAb 1 x DR5 mAb 1 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**underlined**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:132**):

DIVLTQSPAS LAVSLGQRAT ISCRASKSVS SSGYSYMHWY QQKPGQPPKV
 LIFLSSNLDS GVPARFSGSG SGTDFTLNIH PVEDGDAATY YCQHSRDLPP
 TFGGGTKLEI KGGSGGGGGE VKFLESGGGL VQPGGSLKLS CVASGFDFSR
 YWMSWVRQAP GKGLEWIGEI NPDSNTINYT PSLKDKFIIS RDNAKNTLYL

QMTKVRSEDT	ALYYCTRRAY	YGNPAWFAYW	GQGTLLVTVSA	ASTKGEVAAC
EKEVAALEKE	VAALEKEVAA	LEKLEPKSSD	KTHTCPPCPA	PE ~~AA~~ GGPSVF
LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP
REEQYNSTYR	VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAP	IEKTISKAKG
QPREPQVYTL	PPSREEMTKN	QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY
KTTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN	VFSCSVMHEA	LHNHYTQKSL
SLSPG				

[00233] A polynucleotide that encodes **SEQ ID NO:132** is **SEQ ID NO:133**:

gacattgtgc	tgacacagtc	tcttgcttcc	ttagctgtat	ctctcgggca
gagggccacc	atctcatgca	gggccagcaa	aagtgtcagt	tcctctggct
atagttatat	gcactggtac	caacagaaac	caggacagcc	acccaaagtc
ctcatctttc	tttcatccaa	cctagattct	ggggtccctg	ccagggttcag
tggcagtggg	tctgggacag	acttcaccct	caacatccat	cctgtggagg
atggggatgc	tgcaacctat	tactgtcagc	acagtaggga	tcttcctccg
acgttcggtg	gaggcaccaa	gctggaaatc	aaaggaggcg	gatccggcgg
cggaggcgag	gtgaagtttc	tcgagtctgg	aggtggcctg	gtgcagcctg
gaggatccct	gaaactctcc	tgtgtagcct	caggattcga	ttttagtaga
tactggatga	gttgggtccg	gcaggctcca	gggaaagggc	tagaatggat
tggagaaatt	aatccagata	gcaatacgat	aaactatacg	ccatctctaa
aggataaatt	catcatctcc	agagacaacg	ccaaaaatac	gctgtatctg
caaatgacca	aagtgagatc	tgaggacaca	gccctttatt	attgtacaag
aagggcctac	tatggtaacc	cggcctgggt	tgcttactgg	ggccaaggga
ctctggtcac	tgtctctgca	gcctccacca	agggcgaagt	ggccgcatgt
gagaaagagg	ttgctgcttt	ggagaaggag	gtcgctgcac	ttgaaaagga
ggtcgcagcc	ctggagaaac	tggagcccaa	atcttctgac	aaaactcaca
catgcccacc	gtgcccagca	cctgaagccg	cggggggacc	gtcagtcttc
ctcttccccc	caaaacccaa	ggacaccctc	atgatctccc	ggacccttga
ggtcacatgc	gtggtggtgg	acgtgagcca	cgaagaccct	gaggtcaagt
tcaactggta	cgtggacggc	gtggaggtgc	ataatgccaa	gacaaagccg
cgggaggagc	agtacaacag	cacgtaccgt	gtggtcagcg	tcctcaccgt
cctgcaccag	gactggctga	atggcaagga	gtacaagtgc	aaggtctcca
acaaagccct	cccagccccc	atcgagaaaa	ccatctccaa	agccaaaggg
cagccccgag	aaccacaggt	gtacaccctg	cccccatccc	gggaggagat
gaccaagaac	caggtcagcc	tgacctgcct	ggtcaaaggc	ttctatccca
gcgacatcgc	cgtggagtgg	gagagcaatg	ggcagccgga	gaacaactac
aagaccacgc	ctcccgtgct	ggactccgac	ggctccttct	tcctctacag
caagctcacc	gtggacaaga	gcaggtggca	gcaggggaac	gtcttctcat
gctccgtgat	gcatgaggct	ctgcacaacc	actacacgca	gaagagcctc
tcctgtctc	cgggt			

[00234] The second polypeptide chain of DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA) is also **SEQ ID NO:130** (encoded by **SEQ ID NO:131**), described in detail above.

[00235] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 290-506 of **SEQ ID NOs:128 or 132** will be replaced with **SEQ ID NO:164** (CH2-CH3 of IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

2. DR5 mAb 2 x DR5 mAb 2 Fc Region-Containing Diabodies

[00236] Exemplary monospecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2. The first Fc Region-Containing diabody designated “**DR5 mAb 2 x DR5 mAb 2 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:134**):

```
DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GQSPKLLIYW
ASTRHTGVDP RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGKGVQLQ QSGAELVKPG ASVKLSCKAS GYTFTEYILH
WVKQKSGQGL EWIGWFYPGN NNIKYNEKFK DKATLTADKS SSTVYMELSR
LTSEDSAVYF CARHEQGPGY FDYWGQGTTL TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV
LDS DGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
```

[00237] In **SEQ ID NO:134**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of DR5 mAb 2 (**SEQ ID NO:13**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-237 correspond to the amino acid sequence of the VH Domain of DR5 mAb 2 (**SEQ ID NO:18**), residues 235-239 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 240-267 correspond to a cysteine-containing E-coil Domain (**SEQ ID NO:41**), residues 268-273 correspond to a LEPKSS linker (**SEQ ID NO: 49**), residues 274-283 correspond to a linker (DKTHTCPCP; **SEQ ID NO:48**) derived from an IgG1 hinge domain, and residues 284-499 correspond to a wild-type IgG1 Fc Region (**SEQ ID NO:1**, lacking the C-terminal amino acid residue). A polynucleotide that encodes **SEQ ID NO:134** is **SEQ ID NO:135**:

```

gacattgtga tgacccagtc tcacaaattc atgtccactt cagtaggaga
cagggtcagc atcacctgca aggccagtca ggatgtgaat actgctgtag
cctggtatca acaaaaacca gggcaatctc ctaaactact gatttactgg
gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
tgggacagat tatacactca ccatcaaaag tgtgcaggct gaagacctga
cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga
ggcaccaagc tggaaatcaa aggaggcgga tccggcgggcg gaggcaaggt
ccagctgcag cagtctggag ctgaactggg gaaacccggg gcatcagtga
agctgtcctg caaggcttct gggtagacct tcactgagta tattttacac
tgggtaaagc agaagtctgg acaggggtctt gagtggattg ggtgggttta
tcctggaaat aataatataa agtacaaatga gaaattcaag gacaaggcca
cactgactgc ggacaaatcc tccagcacag tctatatgga acttagtaga
ttgacatctg aagactctgc ggtctatttc tgtgcaagac acgaacaagg
accaggttac tttgactact gggggccaagg caccactctc acagtctcct
ccgcctccac caagggcgaa gtggcccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgtag ccctggagaa
actggagccc aaatcttctg acaaaactca cacatgcccac ccgtgcccag
cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgg
ggacgtgagc cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaagggtctc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accagggtcag
cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa
gagcagggtg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00238] The amino acid sequence of the second polypeptide chain of DR5 mAb 2 x DR5 mAb 2 Fc diabody is (SEQ ID NO:136):

```

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGKVQLQ QSGAELVKPG ASVKLSCKAS GYTFTEYILH
WVKQKSGQGL EWIGWFYPGN NNIKYNEKFK DKATLTADKS SSTVYMELSR
LTSEDSAVYF CARHEQGPGY FDYWGQGTTL TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

```

[00239] In SEQ ID NO:136, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of DR5 mAb 2 (SEQ ID NO:13), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 116-237 correspond to the amino acid sequence of the VH Domain of DR5 mAb 2 (SEQ ID NO:18), residues 235-239 correspond to an ASTKG linker (SEQ ID

NO:47) residues 240-267 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**). A polynucleotide that encodes **SEQ ID NO:136** is **SEQ ID NO:137**:

```

gacattgtga tgacccagtc tcacaaattc atgtccactt cagtaggaga
cagggtcagc atcacctgca aggccagtca ggatgtgaat actgctgtag
cctggtatca acaaaaacca gggcaatctc ctaaactact gatttactgg
gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
tgggacagat tatacactca ccatcaaaag tgtgcaggct gaagacctga
cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga
ggcaccaagc tggaaatcaa aggaggcgga tccggcgggc gaggaagggt
ccagctgcag cagtctggag ctgaactggt gaaaccggg gcatcagtga
agctgtcctg caaggcttct gggtagacct tctactgagta tattttacac
tgggtaaagc agaagtctgg acagggtctt gagtggattg ggtggtttta
tcctggaaat aataatataa agtacaatga gaaattcaag gacaaggcca
cactgactgc ggacaaatcc tccagcacag tctatatgga acttagtaga
ttgacatctg aagactctgc ggtctatttc tgtgcaagac acgaacaagg
accaggttac tttgactact ggggccaagg caccactctc acagtctcct
ccgcctccac caagggcaaa gtggccgcat gtaaggagaa agttgctgct
ttgaaagaga aggtcgccgc acttaaggaa aaggtcgcag ccctgaaaga
g

```

[00240] Another Fc Region-containing diabody, designated “**DR5 mAb 2 x DR5 mAb 2 Fc diabody (AA)**,” is identical to DR5 mAb 2 x DR5 mAb 2 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**underlined**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:138**):

```

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GQSPKLLIYW
ASTRHTGVPD RFTGSGSGTD YTLTIKSVQA EDLTLYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGKVQLQ QSGAELVKPG ASVKLSCKAS GYTFTEYILH
WVKQKSGQGL EWIGWFYPGN NNIKYNEKFK DKATLTADKS SSTVYMELSR
LTSEDSAVYF CARHEQGPGY FDYWGQGTTL TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPEAAGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

```

[00241] A polynucleotide that encodes **SEQ ID NO:138** is **SEQ ID NO:139**:

```

gacattgtga tgacccagtc tcacaaattc atgtccactt cagtaggaga
cagggtcagc atcacctgca aggccagtca ggatgtgaat actgctgtag
cctggtatca acaaaaacca gggcaatctc ctaaactact gatttactgg
gcatccaccc ggcacactgg agtccctgat cgcttcacag gcagtggatc
tgggacagat tatacactca ccatcaaaag tgtgcaggct gaagacctga
cactttatta ctgtcagcaa cactatatca ctccgtggac gttcgggtgga

```

```

ggcaccaagc tggaaatcaa aggaggcgga tccggcgggc gaggcaaggt
ccagctgcag cagtctggag ctgaactggt gaaacccggg gcatcagtga
agctgtcctg caaggcttct gggtagacct tcactgagta tattttacac
tgggtaaagc agaagtctgg acaggggtctt gagtggattg ggtggtttta
tcctggaaat aataatataa agtacaatga gaaattcaag gacaaggcca
cactgactgc ggacaaatcc tccagcacag tctatatgga acttagtaga
ttgacatctg aagactctgc ggtctatttc tgtgcaagac acgaacaagg
accaggttac tttgactact ggggccaagg caccactctc acagtctcct
ccgcctccac caagggcgaa gtggccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgcag ccctggagaa
actggagccc aaatcttctg acaaaactca cacatgccca ccgtgcccag
cacctgaagc cgcgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgg
ggacgtgagc cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaagggtctc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag
cctgacctgc ctgggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa
gagcaggtgg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00242] The second polypeptide chain of DR5 mAb 2 x DR5 mAb 2 Fc diabody (AA) is also **SEQ ID NO:136** (encoded by **SEQ ID NO:137**), described in detail above.

[00243] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 284-500 of **SEQ ID NOs:134 or 138** will be replaced with **SEQ ID NO:164** (CH2-CH3 of IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

3. hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc Region-Containing Diabodies

[00244] Exemplary monospecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL Domain of anti-human DR5 antibody hDR5 mAb 2 VL-2 and the VH Domain of anti-human DR5 antibody hDR5 mAb 2 VH-2. The first Fc Region-Containing diabody

designated “**hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:140**):

```

DIQMTQSPSF LSASVGDRVT ITCKASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDTAVYY CARHEQGPGY FDYWGQGTLV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDS DGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

```

[00245] In **SEQ ID NO:140**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-2 (**SEQ ID NO:23**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (**SEQ ID NO:31**), residues 235-239 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 240-267 correspond to a cysteine-containing E-coil Domain (**SEQ ID NO:41**), residues 268-273 correspond to a LEPKSS linker (**SEQ ID NO: 49**), residues 274-283 correspond to a linker (DKTHTCPCP; **SEQ ID NO:48**) derived from an IgG1 hinge domain, and residues 284-499 correspond to a wild-type IgG1 Fc Region (**SEQ ID NO:1**, lacking the C-terminal amino acid residue). A polynucleotide that encodes **SEQ ID NO:140** is **SEQ ID NO:141**:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgta aagcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccatct aggttctctg gcagtggatc
agggacagac tttaacctga caattagctc cctgcagccc gaggatgtgg
ctacttacta ttgtcagcag cactacatca ctcttggtggac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcgggcg gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtggttcta
ccctggcaac aacaacatta agtacaacga gaagttttaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcgaa gtggccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgcag ccctggagaa

```

```

actggagccc aaatcttctg acaaaactca cacatgcccc ccgtgcccag
cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgt
ggacgtgagc cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaagggtct caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accagggtcag
cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa
gagcagggtg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00246] The amino acid sequence of the second polypeptide chain of hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc diabody is (SEQ ID NO:142):

```

DIQMTQSPSF LSASVGDRVIT ICKASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDITAVYY CARHEQGPGY FDYWGQGTILV TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

```

[00247] In SEQ ID NO:142, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-2 (SEQ ID NO:23), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (SEQ ID NO:31), residues 235-239 correspond to an ASTKG linker (SEQ ID NO:47) residues 240-267 correspond to a cysteine-containing K-coil Domain (SEQ ID NO:42). A polynucleotide that encodes SEQ ID NO:142 is SEQ ID NO:143:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgta aagcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccatct aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagccc gaggatgtgg
ctacttacta ttgtcagcag cactacatca ctcttggtgac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcgggc gaggccaggt
ccagctggtg cagagtgggg cagagggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tggtgctgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagtttaaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc

```

```

ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccagggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcaaa gtggccgcat gtaaggagaa agttgctgct
ttgaaagaga aggtcgccgc acttaaggaa aaggtcgag ccctgaaaga
g

```

[00248] Another Fc Region-containing diabody, designated “**hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc diabody (AA)**,” is identical to hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**underlined**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (SEQ ID NO:144):

```

DIQMTQSPSF LSASVGDRVIT ICKASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDVAVYY CARHEQGPGY FDYWGQGTILV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPEAAGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

```

[00249] A polynucleotide that encodes SEQ ID NO:144 is SEQ ID NO:145:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgta aagcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgc agtcccatct aggttctctg gcagtggatc
agggacagac tttaccctga caattagctc cctgcagccc gaggatgtgg
ctacttacta ttgtcagcag cactacatca ctcttggac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcggcg gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtggttcta
ccctggcaac aacaacatta agtacaacga gaagtttaaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccagggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcgaa gtggccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgag ccctggagaa
actggagccc aaatcttctg acaaaactca cacatgccc ccgtgcccag
cacctgaagc cgcgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccct gaggtcacat gcgtggtggt
ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac

```

```

agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaaggtctc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accagggtcag
cctgacctgc ctgggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa
gagcagggtg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00250] The second polypeptide chain of hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc diabody (AA) is also **SEQ ID NO:142** (encoded by **SEQ ID NO:143**), described in detail above.

[00251] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 284-500 of **SEQ ID NOs:140 or 144** will be replaced with **SEQ ID NO:164** (CH2-CH3 of IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

4. hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc Region-Containing Diabodies

[00252] Exemplary monospecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL Domain of anti-human DR5 antibody hDR5 mAb 2 VL-3 and the VH Domain of anti-human hDR5 antibody hDR5 mAb 2 VH-3. The first Fc Region-Containing diabody designated “**hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:146**):

```

DIQMTQSPSF LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVDP RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDVAVYY CARHEQGPY FDIWGQGTLV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV
LDS DGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

```

[00253] In **SEQ ID NO:146**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-3 (**SEQ ID NO:25**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (**SEQ ID NO:31**), residues 235-239 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 240-267 correspond to a cysteine-containing E-coil Domain (**SEQ ID NO:41**), residues 268-273 correspond to a LEPKSS linker (**SEQ ID NO: 49**), residues 274-283 correspond to a linker (DKTHTCPPCP; **SEQ ID NO:48**) derived from an IgG1 hinge domain, and residues 284-499 correspond to a wild-type IgG1 Fc Region (**SEQ ID NO:1**, lacking the C-terminal amino acid residue). A polynucleotide that encodes **SEQ ID NO:146** is **SEQ ID NO:147**:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgct gggcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagccc gaggatgtgg
ctacttacta ttgtcagcag cactacatca ctcttggtac ctccggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcgggc gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagttttaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcgaa gtggccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgag ccctggagaa
actggagccc aaatcttctg acaaaaactca cacatgccca ccgtgcccag
cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgg
ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaaggtctc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag
cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa
gagcaggtgg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00254] The amino acid sequence of the second polypeptide chain of hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc diabody is (**SEQ ID NO:148**):

```
DIQMTQSPSF LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPD RFSGSGSGTD FTLTISSLQP EDVATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDTAVYY CARHEQGPGY FDYWGQGTLV TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE
```

[00255] In **SEQ ID NO:148**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-3 (**SEQ ID NO:25**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (**SEQ ID NO:31**), residues 235-239 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 240-267 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**). A polynucleotide that encodes **SEQ ID NO:148** is **SEQ ID NO:149**:

```
gatatcaga tgaccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgtc gggcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
aggacagac ttaccctga caattagctc cctgcagccc gaggatgtgg
ctacttacta ttgtcagcag cactacatca ctccttgga cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcggcg gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccagg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tggtgtgcgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagtttaaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcaaa gtggccgcat gtaaggagaa agttgctgct
ttgaaagaga aggtcgccgc acttaaggaa aaggtcgag ccctgaaaga
g
```

[00256] Another Fc Region-containing diabody, designated “**hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc diabody (AA)**,” is identical to hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**underlined**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:150**):

DIQMTQSPSF	LSASVGDRVT	ITCRASQDVN	TAVAWYQQKP	GKAPKLLIYW
ASTRHTGVDP	RFSGSGSGTD	FTLTISLQP	EDVATYYCQQ	HYITPWTFGG
GTKLEIKGGG	SGGGGQVQLV	QSGAEVKKPG	ASVKVSCKAS	GYTFTEYILH
WVRQAPGQGL	EWMGWFYPGN	NNIKYNEKFK	DRVITITADKS	TSTVYMELSS
LRSEDTAVYY	CARHEQGPGY	FDYWGQGTLV	TVSSASTKGE	VAACEKEVAA
LEKEVAALEK	EVAALEKLEP	KSSDKTHTCP	PCPAPEAAAGG	PSVFLFPPKP
KDTLMISRTP	EVTCVVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN
STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ
VYTLPPSREE	MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV
LDSDGSFFLY	SKLTVDKSRW	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPG

[00257] A polynucleotide that encodes **SEQ ID NO:150** is **SEQ ID NO:151**:

gatattcaga	tgacccagag	tccctcattt	ctgtccgcct	ccgtcgggtga
ccgcgtgact	attacttgtc	gggcttctca	ggatgtcaac	accgccgtgg
cttggtacca	gcagaagccc	ggtaaagcac	ctaagctgct	gatctattgg
gccagcactc	ggcacaccgg	agtcccagat	aggttctctg	gcagtggatc
agggacagac	tttaccctga	caattagctc	cctgcagccc	gaggatgtgg
ctacttacta	ttgtcagcag	cactacatca	ctccttggac	cttcggcggg
ggcacaaaac	tggaaatcaa	aggaggcgga	tccggcgggc	gaggccaggt
ccagctggtg	cagagtgggg	cagaggtgaa	aaagccaggg	gcatcagtga
aagtgtcttg	taaagcatca	ggttatacat	ttactgagta	catcctgcac
tgggtgcgac	aggcaccagg	acagggactg	gaatggatgg	ggtgggttcta
ccctggcaac	aacaacatta	agtacaacga	gaagtttaaa	gaccgggtga
ccatcacagc	ggataagtct	accagtacag	tctatatgga	gctgagctcc
ctgagaagcg	aagacaccgc	cgtctactat	tgcgctcgcc	acgaacaggg
tccaggttac	tttgattatt	gggggcaggg	aactctggtc	acagtcagct
ccgcctccac	caagggcgaa	gtggccgcat	gtgagaaaga	ggttgctgct
ttggagaagg	aggtcgctgc	acttgaaaag	gaggtcgtag	ccctggagaa
actggagccc	aaatcttctg	acaaaactca	cacatgccc	ccgtgcccag
cacctgaagc	cgcgggggga	ccgtcagtct	tcctcttccc	cccaaaacc
aaggacaccc	tcatgatctc	ccggaccct	gaggtcacat	gcgtgggtgg
ggacgtgagc	cacgaagacc	ctgaggtcaa	gttcaactgg	tacgtggacg
gcgtggaggt	gcataatgcc	aagacaaagc	cgcgggagga	gcagtacaac
agcacgtacc	gtgtgggtcag	cgctctcacc	gtcctgcacc	aggactggct
gaatggcaag	gagtacaagt	gcaaggtctc	caacaaagcc	ctcccagccc
ccatcgagaa	aaccatctcc	aaagccaaag	ggcagccccg	agaaccacag
gtgtacaccc	tgcccccatc	ccgggaggag	atgaccaaga	accaggtcag
cctgacctgc	ctgggtcaaag	gcttctatcc	cagcgacatc	gccgtggagt
gggagagcaa	tgggcagccg	gagaacaact	acaagaccac	gcctcccgtg
ctggactccg	acggctcctt	cttctcttac	agcaagctca	ccgtggacaa
gagcaggtgg	cagcagggga	acgtcttctc	atgctccgtg	atgcatgagg
ctctgcacaa	ccactacacg	cagaagagcc	tctccctgtc	tccgggt

[00258] The second polypeptide chain of hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc diabody (AA) is also **SEQ ID NO:148** (encoded by **SEQ ID NO:149**), described in detail above.

[00259] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 284-500 of **SEQ ID NOs:146 or 150** will be replaced with **SEQ ID NO:164** (CH2-CH3 of IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

5. hDR5 mAb 2.4 x hDR5 mAb 2.4 Fc Region-Containing Diabodies

[00260] Exemplary monospecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL Domain of anti-human DR5 antibody hDR5 mAb 2 VL-4 and the VH Domain of anti-human hDR5 antibody hDR5 mAb 2 VH-4. The first Fc Region-Containing diabody designated “**hDR5 mAb 2.4 x hDR5 mAb 2.4 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:152**)

```
DIQMTQSPSF LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDTAVYY CARHEQGPGY FDYWGQGTLV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV
LDSGGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
```

[00261] In **SEQ ID NO:152**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-4 (**SEQ ID NO:27**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (**SEQ ID NO:31**), residues 235-239 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 240-267 correspond to a cysteine-containing E-coil Domain (**SEQ ID NO:41**), residues 268-273 correspond to a LEPKSS linker (**SEQ ID NO: 49**), residues 274-283 correspond to a linker (DKTHTCPPCP; **SEQ ID NO:48**) derived from an IgG1 hinge domain, and residues 284-499 correspond to a wild-type

IgG1 Fc Region (**SEQ ID NO:1**, lacking the C-terminal amino acid residue). A polynucleotide that encodes **SEQ ID NO:152** is **SEQ ID NO:153**:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgtc gggcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccattc aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagcca gaggatatcg
ctacatacta ttgtcagcag cactacatca ctccctggac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcgggc gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtggttcta
ccctggcaac aacaacatta agtacaacga gaagtttaaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcgaa gtggccgcac gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgag ccctggagaa
actggagccc aaatcttctg acaaaaactc cacatgccca ccgtgcccag
cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgg
ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaaggcttc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag
cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa
gagcaggtgg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00262] The amino acid sequence of the second polypeptide chain of hDR5 mAb 2 x hDR5 mAb 2 Fc diabody is (**SEQ ID NO:154**):

```

DIQMTQSPSF LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGQVQLV QSGAEVKKPG ASVKVSCAKS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVITADKS TSTVYMEISS
LRSEDVAVYY CARHEQGPGY FDYWGQGTILV TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

```

[00263] In **SEQ ID NO:154**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-4 (**SEQ ID NO:17**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID**

NO:33), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (**SEQ ID NO:31**), residues 235-239 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 240-267 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**). A polynucleotide that encodes **SEQ ID NO:154** is **SEQ ID NO:155**:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgtc gggcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccatct aggttctctg gcagtggatc
agggacagac tttaacctga caattagctc cctgcagcca gaggatatcg
ctacatacta ttgtcagcag cactacatca ctcttggac ctccggcggg
ggcacaaaac tggaaatcaa aggaggcggg tccggcggcg gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tggtgctgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagtttaaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctgggtc acagtcagct
ccgcctccac caagggcaaa gtggccgcat gtaaggagaa agttgctgct
ttgaaagaga aggtcgccgc acttaaggaa aaggtcgag ccctgaaaga
g

```

[00264] Another Fc Region-containing diabody, designated “**hDR5 mAb 2.4 x hDR5 mAb 2.4 Fc diabody (AA)**,” is identical to hDR5 mAb 2.4 x hDR5 mAb 2.4 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**underlined**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:156**):

```

DIQMTQSPSF LSASVGDRV T ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDVAVYY CARHEQGPGY FDYWGQGTLV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPEAAGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

```

[00265] A polynucleotide that encodes **SEQ ID NO:156** is **SEQ ID NO:157**:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgtc gggcttctca ggatgtcaac accgccgtgg

```

```

cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccattct aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagcca gaggatatcg
ctacatacta ttgtcagcag cactacatca ctcttggac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcggg tccggcggcg gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagttttaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcgaa gtggccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgag ccctggagaa
actggagccc aaatcttctg acaaaactca cacatgccc ccgtgcccag
cacctgaagc cgcgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccct gaggtcacat gcgtgggtgg
ggacgtgagc cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaagggtct caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accagggtcag
cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa
gagcagggtg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00266] The second polypeptide chain of hDR5 mAb 2 x hDR5 mAb 2 Fc diabody (AA) is also **SEQ ID NO:154** (encoded by **SEQ ID NO:155**), described in detail above.

[00267] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 284-500 of **SEQ ID NOs:152 or 156** will be replaced with **SEQ ID NO:164** (CH2-CH3 of IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

6. hDR5 mAb 2.5 x hDR5 mAb 2.5 Fc Region-Containing Diabodies

[00268] Exemplary monospecific Fc Region-Containing diabodies tetravalent for DR5 composed of two pairs of polypeptide chains are constructed having the VL Domain of anti-human DR5 antibody hDR5 mAb 2 VL-5 and the VH Domain of anti-human hDR5 antibody hDR5 mAb 2 VH-2. The first Fc Region-Containing diabody designated “**hDR5 mAb 2.5 x hDR5 mAb 2.5 Fc diabody**,” contains a wild-type IgG1 Fc Region. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (SEQ ID NO:158):

```
DIQMTQSPSF LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPD RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDTAVYY CARHEQGPGY FDYWGQGTLV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSGGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
```

[00269] In SEQ ID NO:158, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-5 (SEQ ID NO:29), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (SEQ ID NO:33), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (SEQ ID NO:31), residues 235-239 correspond to an ASTKG linker (SEQ ID NO:47) residues 240-267 correspond to a cysteine-containing E-coil Domain (SEQ ID NO:41), residues 268-273 correspond to a LEPKSS linker (SEQ ID NO:49), residues 274-283 correspond to a linker (DKTHTCPCP; SEQ ID NO:48) derived from an IgG1 hinge domain, and residues 284-499 correspond to a wild-type IgG1 Fc Region (SEQ ID NO:1, lacking the C-terminal amino acid residue). A polynucleotide that encodes SEQ ID NO:158 is SEQ ID NO:159:

```
gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgct gggcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
agggacagac tttaccctga caattagctc cctgcagccc gaggatatcg
ctacttacta ttgtcagcag cactacatca ctcttggtgac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcggcg gaggccaggt
```

```

ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagttttaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcgaa gtggccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgtag ccctggagaa
actggagccc aaatcttctg acaaaactca cacatgccca ccgtgcccag
cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgt
ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaaggtctc caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accagggtcag
cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa
gagcaggtgg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

```

[00270] The amino acid sequence of the second polypeptide chain of hDR5 mAb 2 x hDR5 mAb 2 Fc diabody is (**SEQ ID NO:160**):

```

DIQMTQSPSF LSASVGDRV TITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPD RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMELSS
LRSEDTAVYY CARHEQGPY FDYWGQGTLV TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

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[00271] In **SEQ ID NO:160**, amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of hDR5 mAb 2 VL-5 (**SEQ ID NO:29**), residues 108-115 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 116-237 correspond to the amino acid sequence of the VH Domain of hDR5 mAb 2 VH-2 (**SEQ ID NO:31**), residues 235-239 correspond to an ASTKG linker (**SEQ ID NO:47**) residues 240-267 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**). A polynucleotide that encodes **SEQ ID NO:160** is **SEQ ID NO:161**:

```

gatattcaga tgaccacagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgct gggcttctca ggatgtcaac accgccgtgg
cttggtagca gcagaagccc ggtaaagcac ctaagctgct gatctattgg

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gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagccc gaggatatcg
ctacttacta ttgtcagcag cactacatca ctccttggac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcgggc gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagtttaaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc
ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccaggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcaaa gtggccgcat gtaaggagaa agttgctgct
ttgaaagaga aggtcgccgc acttaaggaa aaggtcgag ccctgaaaga
g

```

[00272] Another Fc Region-containing diabody, designated “**hDR5 mAb 2.5 x hDR5 mAb 2.5 Fc diabody (AA)**,” is identical to hDR5 mAb 2.5 x hDR5 mAb 2.5 Fc diabody except the Fc Region is a variant having a L234A/L235A double mutation (**underlined**) which reduces/eliminates binding to FcγRIIIA and reduces/eliminates effector functions. The amino acid sequence of the first polypeptide chain of this Fc Region-Containing diabody is (**SEQ ID NO:162**):

```

DIQMTQSPSF LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYW
ASTRHTGVPD RFSGSGSGTD FTLTISSLQP EDIATYYCQQ HYITPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTEYILH
WVRQAPGQGL EWMGWFYPGN NNIKYNEKFK DRVTITADKS TSTVYMEISS
LRSEDTAVYY CARHEQGPGY FDYWGQGTLV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKLEP KSSDKTHTCP PCPAPEAAGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

```

[00273] A polynucleotide that encodes **SEQ ID NO:162** is **SEQ ID NO:163**:

```

gatattcaga tgacccagag tccctcattt ctgtccgcct ccgtcgggtga
ccgcgtgact attacttgct gggcttctca ggatgtcaac accgccgtgg
cttggtacca gcagaagccc ggtaaagcac ctaagctgct gatctattgg
gccagcactc ggcacaccgg agtcccagat aggttctctg gcagtggatc
agggacagac ttaccctga caattagctc cctgcagccc gaggatatcg
ctacttacta ttgtcagcag cactacatca ctccttggac cttcggcggg
ggcacaaaac tggaaatcaa aggaggcgga tccggcgggc gaggccaggt
ccagctggtg cagagtgggg cagaggtgaa aaagccaggg gcatcagtga
aagtgtcttg taaagcatca gggtatacat ttactgagta catcctgcac
tgggtgcgac aggcaccagg acagggactg gaatggatgg ggtgggttcta
ccctggcaac aacaacatta agtacaacga gaagtttaaa gaccgggtga
ccatcacagc ggataagtct accagtacag tctatatgga gctgagctcc

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ctgagaagcg aagacaccgc cgtctactat tgcgctcgcc acgaacaggg
tccagggttac tttgattatt gggggcaggg aactctggtc acagtcagct
ccgcctccac caagggcgaa gtggccgcat gtgagaaaga ggttgctgct
ttggagaagg aggtcgctgc acttgaaaag gaggtcgag ccctggagaa
actggagccc aaatcttctg acaaaactca cacatgccc ccgtgcccag
cacctgaagc cgcgggggga ccgtcagtct tcctcttccc cccaaaaccc
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtggtggt
ggacgtgagc cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg
gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac
agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct
gaatggcaag gagtacaagt gcaagggtct caacaaagcc ctcccagccc
ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag
gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accagggtcag
cctgacctgc ctggtcaaag gcttctatcc cagcgacatc gccgtggagt
gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa
gagcagggtg cagcagggga acgtcttctc atgctccgtg atgcatgagg
ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggt

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[00274] The second polypeptide chain of hDR5 mAb 2 x hDR5 mAb 2 Fc diabody (AA) is also **SEQ ID NO:160** (encoded by **SEQ ID NO:161**), described in detail above.

[00275] Alternatively, where reduced/eliminated binding to FcγRIIIA and/or reduced/eliminated effector functions is desired, the CH2-CH3 region of IgG2 or IgG4 may be used. In such an Fc Region-Containing diabody, amino acid residues 284-500 of **SEQ ID NOs:158 or 162** will be replaced with **SEQ ID NO:164** (CH2-CH3 of IgG2) or **SEQ ID NO:103** (CH2-CH3 of IgG4), optionally lacking the C-terminal amino acid residue.

D. DR5 x DR5 Monospecific Diabodies Bivalent For DR5

1. DR5 mAb 1 x DR5 mAb 1 Diabody

[00276] Exemplary monospecific diabodies bivalent for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 1 and the VL and VH Domains of DR5 mAb 1. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-273 of **SEQ ID NO:128** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:130** described above.

[00277] Other exemplary monospecific diabodies bivalent for DR5 containing an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 1 and the VL and VH Domains of DR5 mAb 1. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:128** or **SEQ ID NO:132** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:130** and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

2. DR5 mAb 2 x DR5 mAb 2 Diabody

[00278] Exemplary monospecific diabodies bivalent for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 and the VL and VH Domains of DR5 mAb 2. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-267 of **SEQ ID NO:134** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:136** described above.

[00279] Other exemplary monospecific diabodies bivalent for DR5 containing an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 and the VL and VH Domains of DR5 mAb 2. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:134** or **SEQ ID NO:138**. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:136**, and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc Region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

3. DR5 mAb 2.2 x DR5 mAb 2.2 Diabody

[00280] Exemplary monospecific diabodies bivalent for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-2 and the VL and VH Domains of DR5

mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-267 of **SEQ ID NO:140**, described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:142**, described above.

[00281] Other exemplary monospecific diabodies bivalent for DR5 containing an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-2 and the VL and VH Domains of DR5 mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:140** or **SEQ ID NO:144**. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:142** and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc Region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

4. DR5 mAb 2.3 x DR5 mAb 2.3 Diabody

[00282] Exemplary monospecific diabodies bivalent for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-3 and the VL and VH Domains of DR5 mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-267 of **SEQ ID NO:146** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:148** described above.

[00283] Other exemplary monospecific diabodies bivalent for DR5 containing an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-3 and the VL and VH Domains of DR5 mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:146** or **SEQ ID NO:150**. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:148** and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc Region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

5. DR5 mAb 2.4 x DR5 mAb 2.4 Diabody

[00284] Exemplary monospecific diabodies bivalent for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-4 and the VL and VH Domains of DR5 mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-267 of **SEQ ID NO:152** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:154** described above.

[00285] Other exemplary monospecific diabodies bivalent for DR5 containing an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-4 and the VL and VH Domains of DR5 mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:152** or **SEQ ID NO:156**. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:154** and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc Region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

6. DR5 mAb 2.5 x DR5 mAb 2.5 Diabody

[00286] Exemplary monospecific diabodies bivalent for DR5 lacking an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-5 and the VL and VH Domains of DR5 mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises amino acid residues 1-267 of **SEQ ID NO:158** described above. The amino acid sequence of the second polypeptide chain of this diabody comprises **SEQ ID NO:160** described above.

[00287] Other exemplary monospecific diabodies bivalent for DR5 containing an Fc Region composed of two polypeptide chains are constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 VL-5 and the VL and VH Domains of DR5 mAb 2 VH-2. The amino acid sequence of the first polypeptide chain of this diabody comprises **SEQ ID NO:158** or **SEQ ID NO:162**. The amino acid sequence of

the second polypeptide chain of this diabody comprises **SEQ ID NO:160** and further comprises a linker having the amino acid residues LEPKSSDKTHTCPPCP; **SEQ ID NO:51**, and an IgG1 Fc Region have the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, optionally lacking the C-terminal amino acid residue.

E. Additional DR5 x DR5 Diabodies

[00288] In alternative embodiments, the DR5 x DR5 diabodies of the invention are constructed having the VL and VH Domains of humanized anti-human DR5 antibody DR5 mAb 1 and/or the VL and VH Domains of humanized DR5 mAb 2. In a specific embodiment, the VL Domain of hDR5 mAb2 VL VL-2 (**SEQ ID NO:23**), hDR5 mAb2 VL VL-3 (**SEQ ID NO:25**), hDR5 mAb2 VL VL-4 (**SEQ ID NO:27**), or hDR5 mAb2 VL VL-5 (**SEQ ID NO:29**) is incorporated into the above constructs in place of **SEQ ID NO:13**, and/or the VH Domain of hDR5 mAb2 VH-2 (**SEQ ID NO:31**) is incorporated into the above construct in place of **SEQ ID NO: 18**. Alternatively, or in addition, a humanized VL Domain of DR5 mAb 1 is incorporated into the above constructs in place of **SEQ ID NO:3** and/or a humanized VH Domain is incorporated into the above constructs in place of **SEQ ID NO:8**.

[00289] Although the exemplary multivalent DR5-Binding Molecules described above comprise three CDR_{LS} of the Light Chain (VL) and three CDR_{HS} of the Heavy Chain (VH) for each binding domain, it will be recognized that the invention also includes multivalent DR5-Binding Molecules that possess:

- (1) at least one of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 1;
- (2) at least two of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 1;
- (3) the three CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 1;
- (4) at least one of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 1;
- (5) at least two of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 1;

- (6) the three CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 1;
 - (7) at least one of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 1 and at least one of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 1;
 - (8) at least two of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 1 and at least two of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 1;
 - (9) the three CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 1 and the three CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 1;
 - (10) the VL Domain of the anti-human DR5 antibody DR5 mAb 1;
 - (11) the VH Domain of the anti-human DR5 antibody DR5 mAb 1;
 - (12) the VL and VH Domains of the anti-human DR5 antibody DR5 mAb 1;
 - (13) or may compete with anti-human DR5 antibody DR5 mAb 1 for binding to human DR5;
- or
- (14) compete with any of (1)-(13) for binding to human DR5.

[00290] Similarly, it will be recognized that the invention also includes multivalent DR5-Binding Molecules that possess:

- (15) at least one of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 2;
- (16) at least two of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 2;
- (17) the three CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 2;
- (18) at least one of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 2;
- (19) at least two of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 2;
- (20) the three CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 2;

- (21) at least one of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 2 and at least one of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 2 ;
 - (22) at least two of the CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 2 and at least two of the CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 2;
 - (23) the three CDR_{LS} of the VL Domain of the anti-human DR5 antibody DR5 mAb 2 and the three CDR_{HS} of the VH Domain of the anti-human DR5 antibody DR5 mAb 2;
 - (24) the VL Domain of the anti-human DR5 antibody DR5 mAb 2;
 - (25) the VH Domain of the anti-human DR5 antibody DR5 mAb 2;
 - (26) the VL and VH Domains of the anti-human DR5 antibody DR5 mAb 2;
 - (27) compete with anti-human DR5 antibody DR5 mAb 2 for binding to human DR5;
- or
- (28) or that compete with any of (15)-(27) for binding to human DR5.

VII. Methods of Production

[00291] A multivalent DR5-Binding Molecule, and other DR5 agonists, antagonists and modulators can be created from the polynucleotides and/or sequences of the DR5 mAb 1 or DR5 mAb 2 antibodies by methods known in the art, for example, synthetically or recombinantly. One method of producing such peptide agonists, antagonists and modulators involves chemical synthesis of the polypeptide, followed by treatment under oxidizing conditions appropriate to obtain the native conformation, that is, the correct disulfide bond linkages. This can be accomplished using methodologies well known to those skilled in the art (see, *e.g.*, Kelley, R. F. *et al.* (1990) In: GENETIC ENGINEERING PRINCIPLES AND METHODS, Setlow, J.K. Ed., Plenum Press, N.Y., vol. 12, pp 1-19; Stewart, J.M *et al.* (1984) SOLID PHASE PEPTIDE SYNTHESIS, Pierce Chemical Co., Rockford, IL; see also United States Patents Nos. 4,105,603; 3,972,859; 3,842,067; and 3,862,925).

[00292] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "Solid Phase Synthesis," Science

232(4748):341-347; Houghten, R.A. (1985) “*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*,” Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A. (2006) “*Solid-Phase Synthesis In The Twenty-First Century*,” Mini Rev. Med. Chem. 6(1):3-10).

[00293] In yet another alternative, fully human antibodies having one or more of the CDRs of DR5 mAb 1 or DR5 mAb 2 or which compete with DR5 mAb 1 or DR5 mAb 2 for binding to human DR5 or a soluble form thereof may be obtained through the use of commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (*e.g.*, fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XENOMOUSE™ (Abgenix, Inc., Fremont, CA) and HUMAB-MOUSE® and TC MOUSE™ (both from Medarex, Inc., Princeton, NJ).

[00294] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*, CHO cells). Another method that may be employed is to express the antibody sequence in plants (*e.g.*, tobacco) or transgenic milk. Suitable methods for expressing antibodies recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) “*Production Of Antibodies And Antibody Fragments In Plants*,” Vaccine 19:2756; Lonberg, N. *et al.* (1995) “*Human Antibodies From Transgenic Mice*,” Int. Rev. Immunol 13:65-93; and Pollock *et al.* (1999) “*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*,” J. Immunol Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*, humanized, single-chain, *etc.* are known in the art. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) “*Making Antibodies By Phage Display Technology*,” Annu. Rev. Immunol. 12:433-455).

[00295] The antibodies or protein of interest may be subjected to sequencing by Edman degradation, which is well known to those of skill in the art. The peptide information generated from mass spectrometry or Edman degradation can be used to design probes or primers that are used to clone the protein of interest.

[00296] An alternative method of cloning the protein of interest is by “panning” using purified DR5 or portions thereof for cells expressing an antibody or protein of interest that possesses one or more of the CDRs of DR5 mAb 1 or DR5 mAb 2 or that competes with DR5 mAb 1 or DR5 mAb 2 for binding to human DR5. The “panning” procedure may be conducted by obtaining a cDNA library from tissues or cells that express DR5, overexpressing the cDNAs in a second cell type, and screening the transfected cells of the second cell type for a specific binding to DR5 in the presence or absence of DR5 mAb 1 or DR5 mAb 2. Detailed descriptions of the methods used in cloning mammalian genes coding for cell surface proteins by “panning” can be found in the art (see, for example, Aruffo, A. *et al.* (1987) “*Molecular Cloning Of A CD28 cDNA By A High-Efficiency COS Cell Expression System*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 84:8573-8577 and Stephan, J. *et al.* (1999) “*Selective Cloning Of Cell Surface Proteins Involved In Organ Development: Epithelial Glycoprotein Is Involved In Normal Epithelial Differentiation*,” *Endocrinol.* 140:5841-5854).

[00297] Vectors containing polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE- dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00298] Any host cell capable of overexpressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells. Preferably, the host cells express the cDNAs at a level of about 5-fold higher, more preferably 10-fold higher, even more preferably

20-fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to DR5 is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

[00299] The invention includes polypeptides comprising an amino acid sequence of the antibodies of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (*i.e.*, single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an anti-DR5 polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase method.

[00300] The invention includes modifications to DR5 mAb 1 or DR5 mAb 2 antibodies and their polypeptide fragments that bind to DR5 and the agonists, antagonists, and modulators of such molecules, including functionally equivalent antibodies and fusion polypeptides that do not significantly affect the properties of such molecules as well as variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues that can be conservatively substituted for one another include but are not limited to: glycine/alanine; serine/threonine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; lysine/arginine; and phenylalanine/tyrosine. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, *i.e.*, the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known

in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00301] The invention encompasses fusion proteins comprising one or more of the polypeptides or DR5 mAb 1 or DR5 mAb 2 antibodies of this invention. In one embodiment, a fusion polypeptide is provided that comprises a light chain, a heavy chain or both a light and heavy chain. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another embodiment, the fusion polypeptide contains a Light Chain Variable Domain and a Heavy Chain Variable Domain of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody fusion protein contains one or more polypeptide domains that specifically bind to DR5 and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

VIII. Uses of the Multivalent DR5-Binding Molecules of the Present Invention

[00302] The present invention encompasses compositions, including pharmaceutical compositions, comprising the multivalent DR5-Binding Molecules of the present invention (*e.g.*, multivalent DR5-Binding Molecules comprising antigen-binding domains from anti-DR5 antibodies, such as DR5 mAb 1 and DR5 mAb 2, or their humanized derivatives), polypeptides derived from such molecules, polynucleotides comprising sequences encoding such molecules or polypeptides, and other agents as described herein.

[00303] As discussed above, activation of DR5 by the TRAIL cytokine results in the highly selective recognition and killing of tumor cells. The multivalent DR5-Binding

Molecules of the present invention have the ability to act as agonist agents, mimicking TRAIL, and thus leading to the activation of DR5. As such, the multivalent DR5-Binding Molecules comprising antigen-binding domains from anti-DR5 antibodies, such as DR5 mAb 1 and DR5 mAb 2, and their humanized derivatives, may be used as surrogates for TRAIL so as to promote the death of tumor cells that express DR5. Since DR5 is ubiquitously distributed in tumor cell lines, the multivalent DR5-Binding Molecules of the present invention provide a general therapy for cancer. The cancers that may be treated by such molecules include cancers characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocyoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[00304] In particular, the multivalent DR5-Binding Molecules of the present invention may be used in the treatment of colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer,

neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer and rectal cancer.

[00305] In some embodiments the multivalent DR5-Binding Molecules of the present invention may be used to promote the death of tumor cells which are human cancer stem cells. Cancer stem cells (CSCs) have been hypothesized to play a role in tumor growth and metastasis (Ghotra, V.P. *et al.* (2009) "*The Cancer Stem Cell Microenvironment And Anti-Cancer Therapy*," Int. J. Radiat. Biol. 85(11):955-962; Gupta, P.B. *et al.* (2009) "*Cancer Stem Cells: Mirage Or Reality?*" Nat. Med. 15(9):1010-1012; Lawson, J.C. *et al.* (2009) "*Cancer Stem Cells In Breast Cancer And Metastasis*," Breast Cancer Res. Treat. 118(2):241-254; Hermann, P.C. *et al.* (2009) "*Pancreatic Cancer Stem Cells--Insights And Perspectives*," Expert Opin. Biol. Ther. 9(10):1271-1278; Schatton, T. *et al.* (2009) "*Identification And Targeting Of Cancer Stem Cells*," Bioessays 31(10):1038-1049; Mittal, S. *et al.* (2009) "*Cancer Stem Cells: The Other Face Of Janus*," Amer. J. Med. Sci. 338(2):107-112; Alison, M.R. *et al.* (2009) "*Stem Cells And Lung Cancer: Future Therapeutic Targets?*" Expert Opin. Biol. Ther. 9(9):1127-1141; Charafe-Jauffret, E. *et al.* (2009) "*Breast Cancer Stem Cells: Tools And Models To Rely On*," BMC Cancer 9:202; Scopelliti, A. *et al.* (2009) "*Therapeutic Implications Of Cancer Initiating Cells*," Expert Opin. Biol. Ther. 9(8):1005-1016; PCT Publication WO 2008/091908). Under this hypothesis, the CSCs comprise a small, distinct subset of cells within each tumor that are capable of indefinite self-renewal and of developing into the more adult tumor cell(s) that are relatively limited in replication capacity. It has been hypothesized that cancer stem cells might be more resistant to chemotherapeutic agents, radiation or other toxic conditions, and thus, might persist after clinical therapies and later grow into secondary tumors, metastases or be responsible for relapse. It has been suggested that CSCs can arise either from 'normal' tissue stem cells or from more differentiated tissue progenitor cells. As demonstrated herein, the multivalent DR5-Binding Molecules of the present invention are cytotoxic to cells that appear like cancer stem cells (*i.e.* cancer stem cell-like (CSCL) cells). Accordingly, the multivalent DR5-Binding Molecules of the invention may be used to promote the death of human cancer stem cells.

[00306] In addition, Histone deacetylase (HDAC) inhibitors, such as vorinostat, have been reported to sensitize tumor cells to apoptosis induced via the DR5 pathway (Nakata *et al.* (2004) “*Histone deacetylase inhibitors upregulate death receptor 5/TRAIL-R2 and sensitize apoptosis induced by TRAIL/APO2-L in human malignant tumor cells*,” *Oncogene* 19:6261-71; Butler *et al.* (2006) “*The histone deacetylase inhibitor, suberoylanilide hydroxamic acid, overcomes resistance of human breast cancer cells to Apo2L/TRAIL*,” *Int J Cancer*. 15:944-54; Shankar *et al.* (2009) “*Suberoylanilide hydroxamic acid (Zolinza/vorinostat) sensitizes TRAIL-resistant breast cancer cells orthotopically implanted in BALB/c nude mice*,” *Mol Cancer Ther.* 8:1596-605). As demonstrated herein, the ability of the multivalent DR5-Binding Molecules of the present invention to promote cell death is augmented by treatment in combination with an HDAC inhibitor (*e.g.*, vorinostat). Accordingly, the use of an HDAC inhibitor in combination with multivalent DR5-Binding Molecules is particularly useful for the treatment of cancers expressing DR5 which are not sensitive to treatment with a multivalent DR5-Binding Molecule as a single agent..

[00307] In addition to their utility in therapy, the multivalent DR5-Binding Molecules of the present invention may be detectably labeled and used in the diagnosis of cancer or in the imaging of tumors and tumor cells.

IX. Pharmaceutical Compositions

[00308] The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of the multivalent DR5-Binding Molecules of the present invention, or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of the multivalent DR5-Binding Molecules of the present invention and a pharmaceutically acceptable carrier. The invention particularly encompasses such pharmaceutical compositions in which the multivalent DR5-Binding Molecule comprises antigen-binding domains from anti-DR5 antibodies, such as: DR5 mAb 1, a

DR5 mAb 2 antibody, a humanized DR5 mAb 1, a humanized DR5 mAb 2 antibody, or a DR5-binding fragment of any such antibody. Especially encompassed are such molecules that comprise: the 3 CDR_{LS} and the 3 CDR_{HS} of DR5 mAb 1; the 3 CDR_{LS} and the 3 CDR_{HS} of DR5 mAb 2; and/or the 3 CDR_{HS} and the 3 CDR_{HS} of hDR5 mAb 2 VL-3.

[00309] The invention encompasses compositions comprising a multivalent DR5-Binding Molecule of the present invention, and a pharmaceutically acceptable carrier. The invention also encompasses such pharmaceutical compositions that additionally include a second therapeutic antibody (*e.g.*, tumor specific monoclonal antibody) that is specific for a particular cancer antigen, and a pharmaceutically acceptable carrier.

[00310] In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, adjuvant (*e.g.*, Freund’s adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00311] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as

an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00312] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

[00313] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a multivalent DR5-Binding Molecule of the present invention (and more preferably, a tetravalent Fc Region-containing diabody comprising the CDRs of DR5 mAb 1, and/or DR5 mAb 2 antibody, and/or a humanized DR5 mAb 1, and/or humanized DR5 mAb 2 antibody (especially, a tetravalent E-coil/K-coil-Fc Region-containing diabody). Especially encompassed are such molecules that comprise: the 3 CDR_{LS} and the 3 CDR_{HS} of DR5 mAb 1; the 3 CDR_{LS} and the 3 CDR_{HS} of DR5 mAb 2; and/or the 3 CDR_{LS} and the 3 CDR_{HS} of hDR5 mAb 2 V-3, alone or with such pharmaceutically acceptable carrier. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00314] The present invention provides kits that can be used in the above methods. A kit can comprise any of the multivalent DR5-Binding Molecules of the present

invention. The kit can further comprise one or more other prophylactic and/or therapeutic agents useful for the treatment of cancer, in one or more containers; and/or the kit can further comprise one or more cytotoxic antibodies that bind one or more cancer antigens associated with cancer. In certain embodiments, the other prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

X. Methods of Administration

[00315] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a fusion protein or a conjugated molecule of the invention, or a pharmaceutical composition comprising a fusion protein or a conjugated molecule of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00316] Various delivery systems are known and can be used to administer the compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) "Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System," J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00317] Methods of administering a molecule of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the multivalent DR5-Binding Molecules of the present invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion

or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

[00318] The invention also provides that the multivalent DR5-Binding Molecules of the present invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the molecule. In one embodiment, such molecules are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the multivalent DR5-Binding Molecules of the present invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 µg, more preferably at least 10 µg, at least 15 µg, at least 25 µg, at least 50 µg, at least 100 µg, or at least 200 µg.

[00319] The lyophilized multivalent DR5-Binding Molecules of the present invention should be stored at between 2 and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, such multivalent DR5-Binding Molecules when provided in liquid form are supplied in a hermetically sealed container in which the molecules are present at a concentration of least 1 µg/ml, more preferably at least 2.5 µg/ml, at least 5 µg/ml, at least 10 µg/ml, at least 50 µg/ml, or at least 100 µg/ml.

[00320] The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a

disorder can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00321] For the multivalent DR5-Binding Molecules encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject. The dosage administered is typically from at least about 0.3 ng/kg per day to about 0.9 ng/kg per day, from at least about 1 ng/kg per day to about 3 ng/kg per day, from at least about 3 ng/kg per day to about 9 ng/kg per day, from at least about 10 ng/kg per day to about 30 ng/kg per day, from at least about 30 ng/kg per day to about 90 ng/kg per day, from at least about 100 ng/kg per day to about 300 ng/kg per day, from at least about 200 ng/kg per day to about 600 ng/kg per day, from at least about 300 ng/kg per day to about 900 ng/kg per day, from at least about 400 ng/kg per day to about 800 ng/kg per day, from at least about 500 ng/kg per day to about 1000 ng/kg per day, from at least about 600 ng/kg per day to about 1000 ng/kg per day, from at least about 700 ng/kg per day to about 1000 ng/kg per day, from at least about 800 ng/kg per day to about 1000 ng/kg per day, from at least about 900 ng/kg per day to about 1000 ng/kg per day, or at least about 1,000 ng/kg per day. The calculated dose will be administered based on the patient's body weight at baseline. Significant ($\geq 10\%$) change in body weight from baseline or established plateau weight should prompt recalculation of dose.

[00322] In another embodiment, the patient is administered a treatment regimen comprising one or more doses of such prophylactically or therapeutically effective amount of a multivalent DR5-Binding Molecule of the present invention, wherein the treatment regimen is administered over 2 days, 3 days, 4 days, 5 days, 6 days or 7 days. In certain embodiments, the treatment regimen comprises intermittently administering doses of the prophylactically or therapeutically effective amount of the multivalent DR5-Binding Molecules of the present invention (for example, administering a dose on day 1, day 2, day 3 and day 4 of a given week and not administering doses of the prophylactically or therapeutically effective amount of the multivalent DR5-Binding

Molecule (and particularly, a tetravalent Fc Region-containing diabody comprising the CDRs of DR5 mAb 1, and/or DR5 mAb 2 antibody, and/or a humanized DR5 mAb 1, and/or humanized DR5 mAb 2 antibody (especially, a tetravalent E-coil/K-coil-Fc Region-containing diabody). Especially encompassed is the administration (on day 5, day 6, and day 7 of the same week) of molecules that comprise: the 3 CDR_{LS} and the 3 CDR_{HS} of DR5 mAb 1; the 3 CDR_{LS} and the 3 CDR_{HS} of DR5 mAb 2; and/or the 3 CDR_{LS} and the 3 CDR_{HS} of hDR5 mAb 2 V-3. Typically, there are 1, 2, 3, 4, 5 or more courses of treatment. Each course may be the same regimen or a different regimen.

[00323] In another embodiment, the administered dose escalates over the first quarter, first half or first two-thirds or three-quarters of the regimen(s) (*e.g.*, over the first, second, or third regimens of a 4 course treatment) until the daily prophylactically or therapeutically effective amount of the multivalent DR5-Binding Molecule is achieved. **Table 4** provides 5 examples of different dosing regimens described above for a typical course of treatment.

Table 4						
Regimen	Day	Diabody Dosage (ng diabody per kg subject weight per day)				
1	1, 2, 3, 4	100	100	100	100	100
	5, 6, 7	none	none	none	none	none
2	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none
3	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none
4	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none

[00324] The dosage and frequency of administration of a multivalent DR5-Binding Molecule of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the molecule by modifications such as, for example, lipidation.

[00325] The dosage of a multivalent DR5-Binding Molecule of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the molecule may be used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when said molecules are used as a single agent therapy.

[00326] The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00327] The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) "New Methods Of Drug Delivery," Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 3 17-327).

[00328] The compositions of the invention can be delivered in a controlled-release or sustained-release system. Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more of the multivalent DR5-Binding Molecule(s) of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.* (1996) "Intratumoral Radioimmunotherapy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) "Antibody Mediated Lung Targeting Of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) "Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) "Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled-release system (*See* Langer, *supra*; Sefton, (1987) "Implantable Pumps," CRC Crit. Rev. Biomed. Eng. 14:201-240; Buchwald *et al.* (1980) "Long-Term, Continuous Intravenous Heparin Administration By An Implantable Infusion Pump In Ambulatory Patients With Recurrent Venous Thrombosis," Surgery 88:507-516; and Saudek *et al.* (1989) "A Preliminary Trial Of

The Programmable Implantable Medication System For Insulin Delivery,” N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled-release of the molecules (*see e.g.*, MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984); Levy *et al.* (1985) “*Inhibition Of Calcification Of Bioprosthetic Heart Valves By Local Controlled-Release Diphosphonate,*” Science 228:190-192; During *et al.* (1989) “*Controlled Release Of Dopamine From A Polymeric Brain Implant: In Vivo Characterization,*” Ann. Neurol. 25:351-356; Howard *et al.* (1989) “*Intracerebral Drug Delivery In Rats With Lesion-Induced Memory Deficits,*” J. Neurosurg. 7(1):105-112); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained-release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. A controlled-release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, *supra*, vol. 2, pp. 115-138 (1984)). Polymeric compositions useful as controlled-release implants can be used according to Dunn *et al.* (See U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled-release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. A non-polymeric sustained delivery system can be used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (See U.S. 5,888,533).

[00329] Controlled-release systems are discussed in the review by Langer (1990, *"New Methods Of Drug Delivery,"* Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotherapy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery,"* Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety.

[00330] Where the composition of the invention is a nucleic acid encoding a multivalent DR5-Binding Molecule of the present invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded multivalent DR5-Binding Molecule by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (See U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (See *e.g.*, Joliot *et al.* (1991) *"Antennapedia Homeobox Peptide Regulates Neural Morphogenesis,"* Proc. Natl. Acad. Sci. (U.S.A.) 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00331] Treatment of a subject with a therapeutically or prophylactically effective amount of a multivalent DR5-Binding Molecule of the present invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with such a diabody one time per week for between about

1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The pharmaceutical compositions of the invention can be administered once a day, twice a day, or three times a day. Alternatively, the pharmaceutical compositions can be administered once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year. It will also be appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

XI. Examples

[00332] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified. It will be apparent to those skilled in the art that many modifications, both to materials and methods, can be practiced without departing from the scope of the present disclosure.

A. Example 1: Characterization of Anti-Human DR5 Monoclonal Antibodies DR5 mAb 1 and DR5 mAb 2

[00333] Two monoclonal antibodies were isolated as being capable of immunospecifically binding to human DR5, and accorded the designations “DR5 mAb 1” and “DR5 mAb 2”. As discussed above, the CDRs of these antibodies were found to differ. In order to determine whether the antibodies bound to different DR5 epitopes, a human DR5-Fc fusion protein was prepared and was coated to an immobilized surface. DR5 mAb 1 (1 µg/mL) was biotinylated and incubated with either a control IgG or with DR5 mAb 2 (10 µg/mL), and the ability of the IgG or DR5 mAb 2 antibody to compete for binding (to human DR5-Fc fusion protein) with DR5 mAb 1 was assessed by measuring the amount of immobilized biotinylated antibody. Additionally, the ability of the IgG or DR5 mAb 1 antibody to compete for binding with biotinylated DR5 mAb 2 was assessed. The results of this experiment are shown in **Table 5**.

Table 5				
1 μ g/mL DR5-Fc Fusion coat		10 μ g/mL Competitor mAb		
		mIgG	DR5 mAb 1	DR5 mAb 2
1 μ g/mL biotinylated DR5 mAb	DR5 mAb 1	2.162	self	0.826
	DR5 mAb 2	2.102	2.377	self

[00334] The results of this experiment indicate that the biotinylated antibody was capable of binding to the DR5 protein even in the presence of excess amounts of the non-biotinylated antibody. Thus, the results show that DR5 mAb 1 and DR5 mAb 2 bind to different epitopes of DR5.

[00335] In order to further characterize the DR5 mAb 1 and DR mAb 2 antibodies, their ability to block binding between DR5 and the TRAIL ligand as assessed. Thus, biotinylated DR5 mAb 1, biotinylated DR5 mAb 2 or biotinylated DR5-Fc fusion (each at 2 μ g/mL) were separately incubated with immobilized DR5-Fc fusion (1 μ g/mL) in the presence of either buffer or histidine tagged TRAIL (20 μ g/mL). The amount of immobilized biotinylated antibody was assessed. The results of this experiment are shown in **Table 6**.

Table 6			
2 μ g/mL Biotinylated DR5 mAb	1 μ g/mL DR5-Fc fusion coat		1 μ g/mL TRAIL- His coat
	20 μ g/mL TRAIL- His	Buffer	
DR5 mAb 1	1.939	2.118	0.007
DR5 mAb 2	2.052	2.052	0.008
DR5-Fc fusion	--	--	0.288

[00336] The results show that the amount of DR5 mAb 1 or DR5 mAb 2 bound to the immobilized DR5-Fc was not affected by the presence of the histidine tagged TRAIL, thus indicating that neither DR5 mAb 1 nor DR5 mAb 2 block the TRAIL ligand binding site of DR5. Additionally, neither antibody was capable of binding to the histidine tagged TRAIL ligand.

B. Example 2: Species Specificity of Anti-Human DR5 Monoclonal Antibodies DR5 mAb 1 and DR5 mAb 2

[00337] In order to assess the species specificity of anti-human DR5 monoclonal antibodies DR5 mAb 1 and DR5 mAb 2, the ability of the antibodies to bind to human DR5 was compared with their ability to bind cynomolgus monkey (*Macaca fascicularis*) DR5. The results of this experiment are shown in **Figure 5**. The results show that both antibodies are capable of binding to cynomolgus monkey DR5, but that they each exhibit higher binding affinity for human DR5.

[00338] The kinetics of binding was investigated using Biacore Analysis, as shown in **Figure 6**. Bispecific DR5 x CD3 diabodies were incubated with His-tagged DR5 and the kinetics of binding was determined via Biacore analysis. The diabodies employed were DR5 mAb 1 x CD3 mAb 2 (**Figure 6, Panels A and E**), DR5 mAb 2 x CD3 mAb 2 (**Figure 6, Panels B and F**), DR5 mAb 3 x CD3 mAb 2 (**Figure 6, Panels C and G**), and DR5 mAb 4 x CD3 mAb 2 (**Figure 6, Panels D and H**). **Figure 6, Panels A-D** show the results for human DR5. **Figure 6, Panels E-H** show the results for cynomolgus monkey DR5. The calculated k_a , k_d and K_D are presented in **Table 7**.

Table 7						
Anti-DR Antibody	Human			Cynomolgus Monkey		
	k_a	k_d	K_D (nM)	k_a	k_d	K_D (nM)
DR mAb 1	8.5×10^6	1.2×10^{-3}	0.14	4.0×10^6	1.3×10^{-1}	32.5
DR mAb 2	3.4×10^5	2.1×10^{-4}	0.62	2.4×10^5	1.0×10^{-4}	0.42
DR mAb 3	4.2×10^6	3.7×10^{-2}	8.8	3.3×10^6	4.4×10^{-2}	13.3
DR mAb 4	5.4×10^6	1.7×10^{-2}	3.2	2.5×10^6	4.1×10^{-2}	16.4

[00339] The results demonstrate that DR5 mAb 1 and DR5 mAb 2 exhibit altered kinetics of binding relative to reference antibodies DR5 mAb 3 and DR5 mAb 4.

C. Example 3: Tumor Cell Specificity of Anti-Human DR5 Monoclonal Antibodies DR5 mAb 1 and DR5 mAb 2

[00340] The tumor cell specificity of anti-human DR5 monoclonal antibodies DR5 mAb 1 and DR5 mAb 2 were investigated. Normal tissue was contacted with DR5 mAb 1 or with an isotype control (5 μ g/mL) and the extent of staining was visualized. As shown in **Figure 7A, Panels A-L**, DR5 mAb 1 and the isotype control both failed

to label cells of the normal tissue. In contrast, DR5 mAb 1 was found to strongly label cells of colon cancer tissue (**Figure 7B, Panel A**) and lung cancer tissue (**Figure 7B, Panel B**). In contrast, the isotype control failed to label either tissue (**Figure 7B, Panels C-D**). The results presented in **Figures 7A-7B** thus indicate that DR5 mAb 1 was capable of specifically binding to cancer cells.

[00341] Similarly, normal tissue was contacted with DR5 mAb 2 (5 µg/mL) and the extent of staining was visualized. As shown in **Figure 8A, Panels A-F**, DR5 mAb 2 failed to label cells of the normal tissue. In contrast, DR5 mAb 2 was found to strongly label cells of colon cancer tissue (**Figure 8B, Panel A**) and lung cancer tissue (**Figure 8B, Panel C**). In contrast, the isotype control failed to label either tissue (**Figure 8B, Panels B and D**). The results presented in **Figures 8A-8B** thus indicate that DR5 mAb 2 was capable of specifically binding to cancer cells.

D. Example 4: Tumor Cell Cytotoxicity of DR5 mAb 2 x CD3 mAb 2 Diabody

[00342] The ability of DR5-Binding Molecules of the present invention to mediate cytotoxicity was assessed by incubating a bispecific DR5 x CD3 diabody or a control diabody in the presence of a target tumor cell and peripheral blood mononuclear cells (**PBMC**) for 24 hours at an effector to target cell ratio of 30:1 or 20:1. The percentage cytotoxicity was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells.

[00343] For this investigation, an exemplary bispecific diabody designated “**DR5 mAb 2 x CD3 mAb 2**” having the structure shown in Fig. 1 was constructed having the VL and VH Domains of anti-human DR5 antibody DR5 mAb 2 and the VL and VH Domains of CD3 mAb 2 (this diabody is monovalent for DR5 and CD3). The diabody was composed of two polypeptide chains. The first polypeptide chain of the diabody comprises amino acid residues 1-107 correspond to the amino acid sequence of the VL Domain of DR5 mAb 2 (**SEQ ID NO:13**), residues 108-115 correspond to intervening spacer peptide (Linker 1) (**SEQ ID NO:33**), residues 116-240 correspond to the amino acid sequence of the VH Domain of CD3 mAb 2 having the D65G substitution (**SEQ**

ID NO:112), residues 241-245 correspond to an ASTKG linker (**SEQ ID NO:47**) and residues 246-273 correspond to a cysteine-containing E-coil Domain (**SEQ ID NO:41**).

[00344] The second polypeptide chain of the DR5 mAb 2 x CD3 mAb 2 diabody comprises amino acid residues 1-110 correspond to the amino acid sequence of the VL Domain of CD3 mAb 2 (**SEQ ID NO:104**), residues 111-118 correspond to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-237 correspond to the amino acid sequence of the VH Domain of DR5 mAb 2 (**SEQ ID NO:18**), residues 238-242 correspond to an ASTKG linker (**SEQ ID NO:47**), and residues 243-270 correspond to a cysteine-containing K-coil Domain (**SEQ ID NO:42**).

[00345] The employed control diabody contained the VL and VH Domains of anti-fluorescein antibody 4-4-20 (respectively, **SEQ ID NOs:114 and 115**) and the VL and VH Domains of CD3 mAb 2 (respectively, **SEQ ID NOs:102 and 108**), and was designated as the anti-fluorescein x anti-CD3 control diabody “**4-4-20 x CD3 mAb 2.**” The diabody was composed of two polypeptide chains. The first polypeptide chain of the diabody

[00346] comprises amino acid residues 1-112 corresponding to the VL Domain of anti-fluorescein antibody 4-4-20 (**SEQ ID NO:114**), residues 113-120 corresponding to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 121-245 corresponding to the VH Domain of CD3 mAb 2 (**SEQ ID NO:108**), residues 246-251 are a cysteine-containing spacer peptide (GGCGGG) (**SEQ ID NO:34**), and residues 252-280 corresponding to an E-coil Domain (**SEQ ID NO:39**).

[00347] The second polypeptide chain of the **4-4-20 x CD3 mAb 2** diabody comprises amino acid residues 1-110 corresponding to the VL Domain of CD3 mAb 2 (**SEQ ID NO:114**), residues 111-118 corresponding to the intervening spacer peptide GGGSGGGG (Linker 1) (**SEQ ID NO:33**), residues 119-236 corresponding to the VH Domain of anti-fluorescein antibody 4-4-20 (**SEQ ID NO:115**), residues 237-242 are a cysteine-containing spacer peptide (GGCGGG) (**SEQ ID NO:34**), and residues 243-270 corresponding to a K-coil Domain (**SEQ ID NO:40**).

[00348] The results of this investigation are shown in **Figures 9A-9K**. The employed target tumor cells were: 786 O renal cell adenocarcinoma cells (**Figure 9A**), A498 kidney carcinoma cells (**Figure 9B**), AsPC1 pancreatic adenocarcinoma cells (**Figure 9C**), LNCap androgen-sensitive human prostate adenocarcinoma cells (**Figure 9D**), SW48 colorectal adenocarcinoma cells (**Figure 9E**), A549 adenocarcinomic human alveolar basal epithelial cells (**Figure 9F**), SKMES human lung cancer cells (**Figure 9G**), DU145 human prostate cancer cells (**Figure 9H**), A375 human malignant melanoma cells (**Figure 9I**), SKBR3 human HER2-overexpressing breast carcinoma cells (**Figure 9J**) and JIMT human breast carcinoma cells (**Figure 9K**). The results indicate that the DR5 mAb 2 x CD3 mAb 2 diabody was capable of mediating a potent cytotoxic attack on the cancer cells.

E. Example 5: Unexpected Superiority of DR5 mAb 1 and DR5 mAb 2

[00349] The ability of DR5-Binding Molecules DR5 mAb 1 and DR5 mAb 2 of the present invention to mediate cytotoxicity was compared with that of the reference anti-DR5 antibodies: DR5 mAb 3 and DR5 mAb 4. In order to make such a comparison, a bispecific DR5 x CD3 diabody having the structure shown in **Figure 1** containing the VL and VH Domains of these anti-DR5 antibodies and the VL and VH Domains of CD3 mAb 2 were prepared essentially as described above. The first and second The prepared diabodies were designated “**DR5 mAb 1 x CD3 mAb 2**”; “**DR5 mAb 2 x CD3 mAb 2**”; “**DR5 mAb 3 x CD3 mAb 2**”; and “**DR5 mAb 4 x CD3 mAb 2**”.

[00350] Target tumor cells were incubated with one of these diabodies or with the control diabody (4-4-20 x CD3 mAb 2) in the presence of peripheral blood mononuclear cells (**PBMC**) and target tumor cells for 24 hours at an effector to target cell ratio of 20:1. The percentage cytotoxicity was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells.

[00351] The results of this investigation are shown in **Figures 10A-10F**. The employed target tumor cells were: A549 adenocarcinomic human alveolar basal epithelial cells (**Figure 10A**), SKMES human lung cancer cells (**Figure 10B**), DU145 human prostate cancer cells (**Figure 10C**), A375 human malignant melanoma cells

(**Figure 10D**), SKBR3 human HER2-overexpressing breast carcinoma cells (**Figure 10E**), and JIMT human breast carcinoma cells (**Figure 10F**). The results indicate that the VL and VH Domains of DR5 mAb 1 and DR5 mAb 2 are significantly and unexpectedly more potent in inducing cytotoxicity than those of the reference DR5 mAbs.

F. Example 6: Dual and Simultaneous Binding of DR5 mAb 1 and DR5 mAb 2

[00352] In order to demonstrate the ability of the DR5 x CD3 diabodies of the present invention to simultaneously bind to DR5 and to CD3, soluble human DR5 (tagged with histidine) was coated to a support surface. The support was then incubated with DR5 mAb 2 x CD3 mAb 2 diabody or one of its humanized derivatives: hDR5 mAb 2 (2.2) x CD3 mAb 2, hDR5 mAb 2 (2.3) x CD3 mAb 2, hDR5 mAb 2 (2.4) x CD3 mAb 2, or hDR5 mAb 2 (2.5) x CD3 mAb 2. Thereafter, CD3, conjugated with biotin, was provided and the amount of CD3 immobilized to the support was measured.

[00353] The results of this experiment are shown in **Figure 11**. All of the diabodies were found to be capable of simultaneously binding to both DR5 and CD3.

G. Example 7: Cytotoxicity of Humanized Derivatives of DR5 mAb 2

[00354] In order to demonstrate the ability of the humanized DR5 mAb 2 x CD3 diabodies of the present invention to mediate cytotoxicity, DR5 mAb 2 x CD3 mAb 2 diabody or one of its humanized derivatives: hDR5 mAb 2 (2.2) x CD3 mAb 2, hDR5 mAb 2 (2.3) x CD3 mAb 2, hDR5 mAb 2 (2.4) x CD3 mAb 2, or hDR5 mAb 2 (2.5) x CD3 mAb 2 was incubated for 24 hours with pan T cells and target Colo206 colorectal carcinoma cells that had been engineered to express the luciferase (luc) reporter gene (Colo205-Luc cells) (effector to target ratio of 10:1). Cytotoxicity was measured by the increase in luminescence caused by the release of luciferase upon cell lysis.

[00355] The results of this investigation are shown in **Figure 12**. Each of the DR5 mAb 2 x CD3 diabodies was found to be capable of mediating the cytotoxicity of the colorectal carcinoma cells.

H. Example 8: Cytotoxicity of DR5 mAbs Alone, Cross-linked or in Combination

[00356] The cytotoxicity of the DR5-Binding Molecules DR5 mAb 1 and DR5 mAb 2 of the present invention was examined in a number of cell lines using a non-radioactive cell proliferation assay. The activity of DR5 mAb 1 and DR5 mAb 2 alone, cross-linked, or in combination was examined.

[00357] Cell lines obtained from ATCC were cultured under standard tissue culture conditions. Each cell line was plated at $\sim 2 \times 10^4$ cells/well (in 96-well plates), and incubated overnight in F12/DMEM media supplemented with 10% FBS. Separate wells (in triplicate) were treated with 0, or 1 $\mu\text{g/ml}$ of DR5 mAb 1 or DR5 mAb 2 $\pm$ 10 $\mu\text{g/ml}$ goat anti-mouse IgG Fc antibody (designated “ αmFc ”) added one hour after the DR5 antibody to cross-link the DR5 mAbs, or with 1 $\mu\text{g/ml}$ each DR5 mAb 1 and DR5 mAb 2 (total of 2 $\mu\text{g/ml}$ anti-DR5 antibody) and incubated for two days. Cell viability was determined using Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Cat # G5430) essentially as described in the manufacturer’s instructions to assay the amount of soluble formazan produced by cellular reduction of the MTS, which is a measure of the number of viable cells in the culture. Briefly, MTS/PMS reagent was added to the wells and the absorbance at 490 nm (referenced at 650 nm) was read in a Molecular Devices ThermoMax microplate reader.

[00358] Cell viability of cells treated with the test articles is normalized to the negative control (medium only) which is set to 100% to give the “% Medium Ctrl.” The % inhibition = 100% - % Medium Ctrl, and is provided in **Table 8**, where larger values indicate a greater inhibition of growth reflecting the cytotoxicity of the test article. Similar studies were performed over a range of anti-DR5 mAb concentrations from $\sim 10^{-3}$ nM to $\sim 10^2$ nM. The data for COLO205 cells is provided in **Figure 13**, and are representative of cell lines sensitive to the antibodies of the invention.

[00359] The results indicate that neither DR5 mAb 1, nor DR5 mAb 2 alone is capable of inhibiting cell growth in any of cell lines examined, suggesting that neither antibody alone is an agonist. However, each of DR5 mAb 1 and DR5 mAb 2 showed potent cytotoxicity in a number of cell lines when cross-linked by goat anti-mouse IgG Fc antibody. In particular, the growth of COLO205, SW48, SW948, A498 and SKMES

cell lines were dramatically reduced when treated with cross-linked DR5 mAbs of the invention.

[00360] Surprisingly, the combination of DR5 mAb 1 and DR5 mAb 2 was also seen to significantly inhibit the growth of several cell lines (*e.g.*, COLO205 and SW948) in the *absence* of cross-linking. Thus, the combination of DR5 mAb 1 and DR5 mAb 2 exhibits an agonist activity not seen with either antibody alone. These data indicate that a combination of anti-DR5 antibodies can be used to agonize DR5 in a therapeutic setting where a single antibody would be ineffective.

Table 8						
% Inhibition (Average)		DR5 mAb 1	DR5 mAb 1 + α mFc	DR5 mAb 2	DR5 mAb 2 + α mFc	DR5 mAb 1 + DR5 mAb 2
Breast	BT474	2.26	16.13	2.84	12.84	4.98
	MCF7	2.47	12.59	3.98	11.2	2.96
	MDA-MB- 175VII	1.6	10.83	1.09	10.99	2.78
	MDA-MB- 231	1.79	20.78	4.47	12.59	10.14
	MDA-MB- 361	1.54	16.8	1.98	11.51	3.39
	SKBR3	1.97	23.91	2.55	10.98	6.12
Stomach	NCI-N87	1.6	16.99	0.67	10.78	1.46
	Hs746T	2.83	11.02	1.25	10.6	4.62
Pancreas	AsPC1	1.73	18.38	0.13	10.65	2.87
	HPAFII	2.86	12.1	2.68	10.63	2.4
	Hs700T	1.21	20.54	0.99	16.12	7.14
Colon	COLO205	3.5	89.06	3.61	69.73	29.31
	HT29	0.68	22.47	0.87	14.58	4.15
	SW48	2.65	60.95	2.44	58.12	14.63
	SW948	3.53	79.23	1.76	71.26	50.48
Kidney	786O	0.91	11.09	1.79	10.54	9.03
	A498	0.89	78.9	1.92	50.35	10.4
	CaKi2	0.55	30.09	2.64	11.08	2.5
Lung	A549	2.79	10.89	4.25	10.71	0.79
	Calu3	2.86	12.79	2.06	10.02	5.46
	SKMES	1.75	77.59	1.28	69.93	18.67
Ovary	ES2	3.31	14.83	1.88	13.7	5.92
	SKOV3	3.16	19.28	2.02	15.11	3.35
Prostate	22RV1	3.03	10.92	3.89	10.8	1.64
	DU145	1.66	18.77	2.09	15.93	2.75
	LNCap	2.1	20.17	1.72	19.26	10.37
	PC3	2.79	16.1	2.19	13.84	5.16

I. Example 9: Cytotoxicity of DR5 mAb 1 and DR5 mAb 2 is Apoptosis

[00361] The cytotoxic mechanism of DR5 mAb 1 and DR5 mAb 2 was investigated. Specifically, three different measurements of apoptosis: (i) nucleosome enrichment, (ii) PARP cleavage, and (iii) active caspase 3, were employed on COLO205 cells treated with DR5 mAb 1 or DR5 mAb 2 alone or in the presence of α mFc to cross-link the DR5 mAbs.

[00362] For all assays COLO205 cells were plated at $\sim 10^4$ cells/well (in 96-well plates), and incubated overnight in F12/DMEM media supplemented with 10% FBS. Separate wells were treated (in triplicate) with 0, or 1 μ g/ml of DR5 mAb 1 or DR5 mAb 2 $\pm$ 10 μ g/ml α mFc (added one hour after the DR5 antibody) and incubated for four hours.

[00363] Nucleosome enrichment (**Figure 14A**) was determined using Roche Cell Death Detection ELISA^{PLUS} assay (Cat# 1774425) essentially as described in the manufacturer's instructions to assay. Briefly, after the incubation, cells were lysed in lysis buffer and cleared. The cleared lysates were incubated with anti-histone-biotin/anti-DNA-POD antibody cocktail in a streptavidin-coated ELISA plate, the plate was then washed and ABTS reagent was added and the absorbance at 405 nm (referenced at 490 nm) was read in a Molecular Devices ThermoMax microplate reader. The enrichment factor (a measure of nucleosomes released into the cytoplasm) calculated using the formula : enrichment factor = mU of the sample (dying/dead cells)/mU of the corresponding negative control (cells without antibody treatment), where mU = absorbance [10^{-3}] is plotted in **Figure 14A**.

[00364] PARP cleavage and Active Caspase 3 (**Figures 14B and 14C**, respectively) were determined using MILLIPLEX MAP Human Late Apoptosis Magnetic Bead 3-Plex Kit - Cell Signaling Multiplex Assay (Cat. # 48-670) essentially as described in the manufacturer's instructions to assay. Briefly, after the incubation, Briefly, after the incubation, cells were lysed in lysis buffer and cleared. The cleared lysates were incubated with the reference bead-conjugated primary then washed, and incubated with the respective biotinylated secondary antibodies. The plate was then incubated with streptavidin-PE (SAPE) then washed, assay buffer was added to each well and the plate

was read in a Luminex LX-100 system with XY platform. The results are plotted in **Figures 14B** (cleaved PARP) and **Figure 14A** (Active Caspase 3).

[00365] All three measurements of apoptosis are increased in cell cultures treated with cross-linked DR5 mAb 1 or DR5 mAb 2 demonstrating that the cytotoxicity seen is the result of apoptosis.

J. Example 10: Tetravalent DR5-Binding Molecules

[00366] To examine the impact of valency on DR5-Binding Molecules, molecule tetravalent for DR5 are generated. In this example E-coil/K-coil-Fc Region-containing diabodies are prepared. Several of these E-coil/K-coil-Fc Region-containing diabodies characterized in the following examples. Each multivalent DR5-Binding Molecule is composed of two pairs of polypeptide chains.

[00367] The first polypeptide chain has the general sequence: [VL1 Domain]—[GGGSGGGG]—[VH2 Domain]—[ASTKG]—[EVAACEK(EVAALEK)₃]—[LEPKSS]—[DKTHTCPPCP]—Fc Region (Wild-Type or L234A/L235A double mutant) starting from 231 EU numbering), where VL1 is from an anti-DR5 antibody, [GGGSGGGG] is SEQ ID NO:33, VH2 is from an anti-DR5 antibody, [ASTKG] is SEQ ID NO:47, [EVAACEK(EVAALEK)₃] is SEQ ID NO:41, [LEPKSS] is SEQ ID NO:49, [DKTHTCPPCP] is SEQ ID NO:48, and the Fc Region is SEQ ID NO:1 (wild-type) or SEQ ID NO:102 (L234A/L235L mutant) and optionally lacks the C-terminal amino acid residue.

[00368] The second polypeptide chain has the general sequence: [VL2 Domain]—[GGGSGGGG]—[VH1 Domain]—[ASTKG]—[KVAACEK(KVAALKE)₃], where VL2 is from an anti-DR5 antibody, [GGGSGGGG] is SEQ ID NO:33, VH1 is from an anti-DR5 antibody, [ASTKG] is SEQ ID NO:47, [KVAACEK(KVAALKE)₃] is SEQ ID NO:42.

[00369] The chains assemble as shown in **Figure 4B**. The VL1 Domain of the first polypeptide chain interacts with the VH1 Domain of the second polypeptide chain to form a first functional antigen-binding site that is specific for DR5. Likewise, the VL2 Domain of the second polypeptide chain interacts with the VH2 Domain of the first

polypeptide chain in order to form a second functional antigen-binding site that is also specific for DR5. Thus, the selection of the VL and VH Domains of the first and second polypeptide chains is coordinated, such that the two polypeptide chains of the diabody collectively comprise VL and VH Domains capable of binding to at least one epitope of DR5. Molecules having all the same VL and VH Domains (*e.g.*, all VL and VH Domains are from DR5 mAb 1 or from DR5 mAb 2) are monospecific (*i.e.*, bind a single epitope of DR5) and tetravalent for DR5. Molecules having VL and VH Domains of DR5 mAb 1 and DR5 mAb 2 are bispecific (*i.e.*, binds two different epitopes of DR5) but are still tetravalent for DR5. Exemplary E-coil/K-coil-Fc Region-containing diabodies comprising the VL and VH Domains of DR5 mAb 1 and DR5 mAb 2 are provided. However, it will be understood that E-coil/K-coil-Fc Region-containing diabodies which are tetravalent for DR5 may be prepared using the VL and VH Domains of any anti-DR5 antibody. Similarly, alternative constructs such as those disclosed above comprising the VL and VH Domains of one or more anti-DR5 antibody such as those disclosed herein may be prepared.

[00370] The VL, VH and Fc Region, as well as the SEQ ID NOs: (polypeptide), of the first and second chains for each E-coil/K-coil-Fc Region-containing diabody are summarized in **Table 9**. Also provided is the unique designator for the assembled molecule. The complete sequence for the polypeptide chains and the polynucleotides encoding the same is provided above.” While several of the molecules provided in **Table 9** are bispecific, that is they bind two different DR5 epitopes, all are tetravalent with respect to DR5.

Table 9			
Molecules Binding Two Different DR5 Epitopes			
Designation	Portion	First Chain	Second Chain
DR5 mAb 1 x DR5 mAb 2 Fc diabody	VL	DR5 mAb 1 [SEQ ID NO:3]	DR5 mAb 2 [SEQ ID NO:13]
	VH	DR5 mAb 2 [SEQ ID NO:18]	DR5 mAb 1 [SEQ ID NO:8]
	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:116]	[SEQ ID NO:118]

DR5 mAb 1 x DR5 mAb 2 Fc diabody (AA)	VL	DR5 mAb 1 [SEQ ID NO:3]	DR5 mAb 2 [SEQ ID NO:13]
	VH	DR5 mAb 2 [SEQ ID NO:18]	DR5 mAb 1 [SEQ ID NO:8]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	Polypeptide Sequence	[SEQ ID NO:120]	[SEQ ID NO:118]
DR5 mAb 2 x DR5 mAb 1 Fc diabody	VL	DR5 mAb 2 [SEQ ID NO:13]	DR5 mAb 1 [SEQ ID NO:3]
	VH	DR5 mAb 1 [SEQ ID NO:8]	DR5 mAb 2 [SEQ ID NO:18]
	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:122]	[SEQ ID NO:124]
DR5 mAb 2 x DR5 mAb 1 Fc diabody (AA)	VL	DR5 mAb 2 [SEQ ID NO:13]	DR5 mAb 1 [SEQ ID NO:3]
	VH	DR5 mAb 1 [SEQ ID NO:8]	DR5 mAb 2 [SEQ ID NO:18]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	SEQ ID NOs:	[SEQ ID NO:126]	[SEQ ID NO:124]
Molecules Binding One DR5 Epitope			
Designation	Portion	First Chain	Second Chain
DR5 mAb 1 x DR5 mAb 1 Fc diabody	VL	DR5 mAb 1 [SEQ ID NO:3]	DR5 mAb 1 [SEQ ID NO:3]
	VH	DR5 mAb 1 [SEQ ID NO:8]	DR5 mAb 1 [SEQ ID NO:8]
	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:128]	[SEQ ID NO:130]
DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA)	VL	DR5 mAb 1 [SEQ ID NO:3]	DR5 mAb 1 [SEQ ID NO:3]
	VH	DR5 mAb 1 [SEQ ID NO:8]	DR5 mAb 1 [SEQ ID NO:8]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	Polypeptide Sequence	[SEQ ID NO:132]	[SEQ ID NO:130]
DR5 mAb 2 x DR5 mAb 2 Fc diabody	VL	DR5 mAb 2 [SEQ ID NO:13]	DR5 mAb 2 [SEQ ID NO:13]
	VH	DR5 mAb 2 [SEQ ID NO:18]	DR5 mAb 2 [SEQ ID NO:18]

	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:134]	[SEQ ID NO:136]
DR5 mAb 2 x DR5 mAb 2 Fc diabody (AA)	VL	DR5 mAb 2 [SEQ ID NO:13]	DR5 mAb 2 [SEQ ID NO:13]
	VH	DR5 mAb 2 [SEQ ID NO:18]	DR5 mAb 2 [SEQ ID NO:18]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	Polypeptide Sequence	[SEQ ID NO:138]	[SEQ ID NO:136]
hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc diabody	VL	hDR5 mAb 2 VL-2 [SEQ ID NO:23]	hDR5 mAb 2 VL-2 [SEQ ID NO:23]
	VH	hDR5 mAb 2 VH-2 [SEQ ID NO:31]	hDR5 mAb 2 VH-2 [SEQ ID NO:31]
	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:140]	[SEQ ID NO:142]
hDR5 mAb 2.2 x hDR5 mAb 2.2 Fc diabody (AA)	VL	hDR5 mAb 2 VL-2 [SEQ ID NO:23]	hDR5 mAb 2 VL-2 [SEQ ID NO:23]
	VH	hDR5 mAb 2 VH-2 [SEQ ID NO:31]	hDR5 mAb 2 VH-2 [SEQ ID NO:31]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	Polypeptide Sequence	[SEQ ID NO:144]	[SEQ ID NO:142]
hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc diabody	VL	hDR5 mAb 2 VL-3 [SEQ ID NO:25]	hDR5 mAb 2 VL-3 [SEQ ID NO:25]
	VH	hDR5 mAb 2 VH-2 [SEQ ID NO:31]	hDR5 mAb 2 VH-2 [SEQ ID NO:31]
	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:146]	[SEQ ID NO:148]
hDR5 mAb 2.3 x hDR5 mAb 2.3 Fc diabody (AA)	VL	hDR5 mAb 2 VL-3 [SEQ ID NO:25]	hDR5 mAb 2 VL-3 [SEQ ID NO:25]
	VH	hDR5 mAb 2 VH-2 [SEQ ID NO:31]	hDR5 mAb 2 VH-2 [SEQ ID NO:31]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	Polypeptide Sequence	[SEQ ID NO:150]	[SEQ ID NO:148]
hDR5 mAb 2.4 x hDR5 mAb 2.4 Fc diabody	VL	hDR5 mAb 2 VL-4 [SEQ ID NO:27]	hDR5 mAb 2 VL-4 [SEQ ID NO:27]
	VH	hDR5 mAb 2 VH-2	hDR5 mAb 2 VH-2

		[SEQ ID NO:31]	[SEQ ID NO:31]
	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:152]	[SEQ ID NO:154]
hDR5 mAb 2.4 x hDR5 mAb 2.4 Fc diabody (AA)	VL	hDR5 mAb 2 VL-4 [SEQ ID NO:27]	hDR5 mAb 2 VL-4 [SEQ ID NO:27]
	VH	hDR5 mAb 2 VH-2 [SEQ ID NO:31]	hDR5 mAb 2 VH-2 [SEQ ID NO:31]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	Polypeptide Sequence	[SEQ ID NO:156]	[SEQ ID NO:154]
hDR5 mAb 2.5 x hDR5 mAb 2.5 Fc diabody	VL	hDR5 mAb 2 VL-5 [SEQ ID NO:29]	hDR5 mAb 2 VL-5 [SEQ ID NO:29]
	VH	hDR5 mAb 2 VH-2 [SEQ ID NO:31]	hDR5 mAb 2 VH-2 [SEQ ID NO:31]
	Fc	Wild-Type [SEQ ID NO:1]	n/a
	Polypeptide Sequence	[SEQ ID NO:158]	[SEQ ID NO:160]
hDR5 mAb 2.5 x hDR5 mAb 2.5 Fc diabody (AA)	VL	hDR5 mAb 2 VL-5 [SEQ ID NO:29]	hDR5 mAb 2 VL-5 [SEQ ID NO:29]
	VH	hDR5 mAb 2 VH-2 [SEQ ID NO:31]	hDR5 mAb 2 VH-2 [SEQ ID NO:31]
	Fc	L234A/L235A [SEQ ID NO:102]	n/a
	Polypeptide Sequence	[SEQ ID NO:162]	[SEQ ID NO:160]

K. Example 11: Tumor Cell Specificity of a Bispecific Tetravalent DR5-Binding Molecules Containing DR5 mAb 1 and DR5 mAb 2

[00371] The tumor cell specificity of a representative bispecific tetravalent DR5-Binding Molecule (DR5 mAb 2 x DR5 mAb 1 Fc diabody) was investigated. Normal tissue was contacted with labeled DR5 mAb 2 x DR5 mAb 1 Fc diabody or with a labeled control diabody (4-4-20 x CD3 mAb 2, described in Example 4) at 0.625 µg/mL and the extent of staining was visualized. As shown in **Figure 15A-B**, DR5 mAb 2 x DR5 mAb 1 Fc diabody and the control diabody both failed to label cells of the normal tissue. Similar results were seen in additional samples of normal tissues, including liver as shown in **Figure 15B**. In contrast, DR5 mAb 2 x DR5 mAb 1 Fc diabody was found to strongly label cells of breast cancer tissue (**Figure 16, Panel A**), colon cancer tissue (**Figure 16, Panel B**), lung cancer tissue (**Figure 16, Panel C**) and prostate cancer

tissue (**Figure 16, Panel D**). In contrast, the control diabody failed to label either tissue (**Figure 16, Panels E-H**). A summary of the histology observations is provided in **Table 10**. The results presented in **Figures 15A-15B, 16**, and **Table 10**, thus indicate that a tetravalent DR5-Binding Molecule (bispecific for two epitopes of DR5) was capable of specifically binding to cancer cells.

Table 10		
Tissue / Cells	DR5 mAb 2 x DR5 mAb 1 Fc diabody 0.625 ug/mL	Control DART 0.625 ug/mL
Colon (6N)	-	±/-
Lung (6N)	Macrophage 2+ (c) rare; endo 2+ (c) very rare; others (-)	-
Liver (6N)	-	-
Kidney (6N)	-	-
Heart (6N)	-	-
Pancreas (6N)	Secreted acinar epi ± (c) very rare	±/-
COLO205	4+ (m)	-
CHO	-	-
MDA-MB-175VII	1+	-
MDA-MD-231	3+ (c,m)	-
Liver (3N)	-	-
Lung	Endo 1+ (c) very rare; others (-)	-
Lung	Macrophage 3+ (c,m) occasional; possible type II pneumocytes 2-3+ (c,m) rare	-
Lung	Macrophage 3+ (c) occasional mixed with type II pneumocytes	-

L. Example 12: Cytotoxicity of Tetravalent DR5-Binding Molecules

[00372] The cytotoxicity of tetravalent DR5-Binding Molecules of the present invention was investigated. The activity of two exemplary bispecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 2 Fc diabody; and DR5 mAb 2 x DR5 mAb 1 Fc diabody), and four exemplary monospecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 1 Fc diabody; DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA); DR5 mAb 2 x DR5 mAb 2 Fc diabody; and DR5 mAb 2 x DR5 mAb 2 Fc diabody (AA), where “AA” refers to the L234A/L235A mutation), on a number of cell lines was examined using the non-radioactive cell proliferation assay essentially as described above except that no cross-

linking antibody was added to any of the test samples. His-tagged TRAIL (R&D systems) was used as a positive control.

[00373] Cell viability of cells treated with the test articles is normalized to the negative control (medium only) which is set to 100% to give the “% Medium Ctrl.” The % inhibition = 100% - % Medium Ctrl, and is provided in **Table 11**, where larger values indicate a greater inhibition of growth reflecting the cytotoxicity of the test article. Similar studies were performed over a range of anti-DR5 mAb concentrations from $\sim 10^{-3}$ nM to $\sim 10^2$ nM. **Figure 17** shows the data for several responsive cell lines, COLO205 (**Figure 17A**), A498 (**Figure 17B**), and SKMES (**Figure 17C**).

Table 11			
% Inhibition (Average)		DR5 mAb 1 x DR5 mAb 2 Fc diabody	DR5 mAb 2 x DR5 mAb 1 Fc diabody
Breast	BT474	3.96	2.62
	MCF7	6.03	6.27
	MDA-MB-175VII	3.36	2.96
	MDA-MB-231	15.24	14.03
	MDA-MB-361	5.35	3.61
	SKBR3	13.07	8.47
Stomach	NCI-N87	10.62	5.01
	Hs746T	5.94	5.09
Pancreas	AsPC1	15.01	12.7
	HPAFII	10.05	10.1
	Hs700T	17.89	13.38
Colon	COLO205	88.89	88.53
	HT29	16.2	11.02
	SW48	39.81	33.53
	SW948	78.33	77.91
Kidney	786O	5.62	5.13
	A498	81.47	74.27
	CaKi2	36.9	31.66
Lung	A549	2.63	2.8
	Calu3	15.18	18.01
	SKMES	75.6	68.97
Ovary	ES2	8.44	12.25
	SKOV3	13.02	9.95
Prostate	22RV1	1.83	2.95
	DU145	12.14	12.34
	LNCap	11.82	12.71
	PC3	10.54	10.79

[00374] The results indicate that all the tetravalent DR5-Binding Molecules have potent cytotoxicity in a number of cell lines. Indeed, all the tetravalent DR5-Binding Molecules were more potent than TRAIL itself. In particular, the growth of COLO205, SW48, SW948, A498, CaKi2 and SKMES were dramatically reduced when treated with tetravalent DR5-Binding Molecules of the invention. Tetravalent DR5-Binding Molecules possessing the L234A/L235A Fc Region exhibited similar, or slightly higher cytotoxicity as the counter part molecules possessing a wild-type Fc Region, indicating that Fc Regions having reduced binding to FcγRs and/or reduced effector function can be incorporated into tetravalent DR5-Binding Molecules where binding to FcγRs and/or effector function is not required and/or desirable.

M. Example 13: Tetravalent DR5-Binding Molecules Induce Apoptosis

[00375] The ability of tetravalent DR5-Binding Molecules of the present invention to induce apoptosis was investigated. The activity of two exemplary bispecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 2 Fc diabody; and DR5 mAb 2 x DR5 mAb 1 Fc diabody), and two exemplary monospecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 1 Fc diabody; and DR5 mAb 2 x DR5 mAb 2 Fc diabody), was examined in the COLO205, A496, SKMES, LNCap, MDA-MB-231 and Hs700T cell lines. This investigation was performed using the nucleosome enrichment assay essentially as described above except that no cross-linking antibody was added to the test samples. His-tagged TRAIL (R&D systems) was used as a positive control. The enrichment factor (calculated as described above) is plotted in **Figure 18**.

[00376] The results indicate that all the tetravalent DR5-Binding Molecules are potent inducers of Apoptosis. Indeed, all the tetravalent DR5-Binding Molecules had an enrichment factor similar to that seen for the positive control in the same cell line.

N. Example 14: Cytotoxicity of Tetravalent DR5-Binding Molecules

[00377] The cytotoxicity of multivalent DR5-Binding Molecules of the present invention was compared to that of the previously reported antibodies DR5 mAb 8 (KMTR2) and DR5 mAb 4 (conatumumab) in a cell proliferation assay. The activity

of one exemplary bispecific tetravalent E-coil/K-coil-Fc Region-containing diabody (DR5 mAb 1 x DR5 mAb 2 Fc diabody (AA)); two exemplary monospecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA); and DR5 mAb 2 x DR5 mAb 2 Fc diabody (AA)); the anti-DR5 antibody DR5 mAb 8 (AA) (KMTR2); and the anti-DR5 antibody DR5 mAb 4 (AA) (conatumumab) with and without cross-linking (where “AA” refers to the L234A/L235A mutation) on COLO205 was examined over a range of concentrations from approximately 10^{-3} nM to approximately 10^2 nM, using the non-radioactive cell proliferation assay essentially as described above except that cross-linking antibody was added only to one test sample of DR5 mAb 4. His-tagged TRAIL (R&D systems) was used as a positive control.

[00378] Cell viability of cells treated with the test articles is normalized to the negative control (medium only) which is set to 100% to give the “% Medium Ctrl.” The % inhibition = $100\% - \% \text{ Medium Ctrl}$ is plotted in **Figure 19**.

[00379] The results indicate that all the tetravalent DR5-Binding Molecules tested have potent cytotoxicity that is independent of cross-linking and are more potent than the previously described anti-DR5 antibodies DR5 mAb 8 (KMTR2); and DR5 mAb 4 (conatumumab). In particular, the tetravalent DR5-Binding Molecules were significantly more potent than even cross-linked DR5 mAb 4.

O. Example 15: Cytotoxicity of Tetravalent DR5-Binding Molecules On Cancer Stem Cell-Like (CSCL) Cells

[00380] The cytotoxicity of multivalent DR5-Binding Molecules of the present invention on cancer stem cell-like (CSLC) cells was investigated. RECA0201 are CSCL cells isolated from a moderately differentiated rectal adenocarcinoma (mutated APC and KRAS; CD44^{hi}, CD133⁺ and A33⁺). RECA0201 cells are tumorigenic and capable to recapitulate tumor morphology and multi-lineage differentiation in vivo or organoid formation in vitro.

[00381] The cytotoxic activity of one exemplary bispecific tetravalent E-coil/K-coil-Fc Region-containing diabody (DR5 mAb 1 x DR5 mAb 2 Fc diabody (AA)); two exemplary monospecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA); and DR5 mAb 2 x DR5 mAb 2 Fc

diabody (AA)); two anti-DR5 antibodies (DR5 mAb 8 (AA) (KMTR2); and DR5 mAb 4 (AA) (conatumumab), (where “AA” refers to the L234A/L235A mutation) on RECA0201 cells was examined over a range of concentrations, using a non-radioactive cytotoxicity assay. Briefly, 20,000 cells RECA0201 colon cancer CACL cells stably transfected with constitutively expressed luciferase are plated per well and exposed to indicated concentrations of test article. After 48 hours the level of cell viability is determined through measurement of luciferase using Promega STEADY GLO® substrate reagent on a Victor Plate reader, essentially as described by the manufacturer. The results are plotted in **Figure 20**.

[00382] As shown in **Figure 20**, the tetravalent DR5-Binding Molecules displayed potent cytotoxicity on CACL RECA0201 cells. Indeed, the results indicate that all the tetravalent DR5-Binding Molecules are more potent than the previously described anti-DR5 antibodies DR5 mAb 8 (KMTR2); and DR5 mAb 4 (conatumumab), and are more potent than TRAIL itself.

P. Example 16: Inhibition of Tumor Growth by a Tetravalent DR5-Binding Molecule in Mice Implanted with COLO205 Tumor Cells

[00383] The anti-tumor activity of an exemplary monospecific tetravalent E-coil/K-coil-Fc Region-containing diabody (DR5 mAb 1 x DR5 mAb 1 Fc diabody (AA)); and two anti-DR5 antibodies (DR5 mAb 8 (AA) (KMTR2), and DR5 mAb 4 (AA) (conatumumab)) (where “AA” refers to the L234A/L235A mutation) were evaluated in a xenograft tumor model. Briefly, female hCD16A FOX N1 mice (n = 7/group) were implanted subcutaneously (SC) with 5 million COLO205 cells suspended in 200 μ L of Ham’s F12 medium mixed 1:1 with Matrigel on Day 0. The tumors were measured every 3 – 4 days with calipers. On Study Day 3, the mice were randomized based on tumor size and treated twice a week (intravenous (IV) injection) with the indicated dose levels of test article or vehicle (sterile saline containing 0.5% bovine serum albumin). Tumor volume was monitored over the course of the study and is plotted in **Figure 21** as a group mean $\pm$ SEM. The tetravalent DR5-Binding Molecule was seen to dramatically inhibit tumor growth over the course of the study and the tumors were seen to regress in the 0.5 and 0.05 mg/kg treatment groups.

Q. Histone deacetylase Inhibitors Synergizes with Tetravalent DR5-Binding Molecules

[00384] Histone deacetylase (HDAC) inhibitors, such as vorinostat, have been reported to sensitize tumor cells to apoptosis induced via the DR5. The cytotoxic activity of DR5 mAb 1, DR5 mAb 2, and several tetravalent DR5-Binding Molecules in combination with the HDAC inhibitor vorinostat was investigated using a non-radioactive cell proliferation assay.

[00385] The activity of DR5 mAb 1, DR5 mAb 2, two exemplary bispecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 2 Fc diabody; and DR5 mAb 2 x DR5 mAb 1 Fc diabody), and two exemplary monospecific tetravalent E-coil/K-coil-Fc Region-containing diabodies (DR5 mAb 1 x DR5 mAb 1 Fc diabody; and DR5 mAb 2 x DR5 mAb 2 Fc diabody) was examined in these studies. His-tagged TRAIL (R&D systems) was used as a positive control.

[00386] For the first study COLO205 cells were plated at $\sim 2 \times 10^4$ cells/well (in 96-well plates), and incubated overnight in F12/DMEM media supplemented with 10% FBS. Separate wells (in triplicate) were treated with 0, or 1 $\mu\text{g/ml}$ of DR5 mAb 1 or DR5 mAb 2, 10 ng/ml tetravalent DR5-Binding Molecule (100 fold less than used in the previous cytotoxicity study), or His-tagged TRAIL $\pm$ 0.1 or 1 μM vorinostat and incubated for one day. Cell viability was determined using Promega CELLTITER-GLO® Luminescent Cell Viability Assay (Cat # G5430) essentially as described in the manufacturer's instructions to assay the amount of ATP present, which is a measure of the number of viable cells in the culture. Briefly, an CELLTITER-GLO® Reagent was added to the wells and mixed for two minutes to induce lysis and the luminescence was read in a PerkinElmer EnVision multilabel plate reader.

[00387] Cell viability of cells treated with the test articles is normalized to the corresponding negative control (medium $\pm$ vorinostat) which is set to 100% and reported in **Table 12** as % of control for each test agent alone or % control for each test agent in combination with vorinostat. % of control values less than 100% indicate a reduction in viability and reflect the cytotoxicity of the test article alone or in combination with vorinostat.

Table 12			
COLO205	Vorinostat		
Average % Ctrl	0μM[‡]	0.1μM[§]	1μM[§]
10% FBS	100%	100%	100%
DR5 mAb 1 x DR5 mAb 2 Fc diabody	64.4%	52.6%	44.8%
DR5 mAb 2 x DR5 mAb 1 Fc diabody	84.1%	70.7%	48.9%
DR5 mAb 1 x DR5 mAb 1 Fc diabody	64.2%	49.7%	42.9%
DR5 mAb 2 x DR5 mAb 2 Fc diabody	75.0%	50.8%	45.6%
DR5 mAb 1	96.8%	96.4%	93.3%
DR5 mAb 2	96.4%	96.5%	95.5%
R&D TRAIL/His	68.7%	54.1%	44.8%

[‡] % Medium Ctrl (10% FBS)

[§] % Medium Ctrl (1/10 μ M vorinostat)

[00388] The Net Gain (Average % Growth Inhibition) = % Medium Ctrl (10% FBS) - % Medium Ctrl (1/10 μ M vorinostat), and represents increased cytotoxicity of the test article in combination with vorinostat over cells treated with vorinostat alone. The net gain for the first study is provided in **Table 13**.

Table 13		
COLO205	Vorinostat	
Net Gain (Average % Growth Inhibition)	0.1μM	1μM
10% FBS	0	0
DR5 mAb 1 x DR5 mAb 2 Fc diabody	12	20
DR5 mAb 2 x DR5 mAb 1 Fc diabody	13	35
DR5 mAb 1 x DR5 mAb 1 Fc diabody	15	21
DR5 mAb 2 x DR5 mAb 2 Fc diabody	24	29
DR5 mAb 1	0	3
DR5 mAb 2	0	1
R&D TRAIL/His	15	24

[00389] The results indicate that the HDAC inhibitor vorinostat synergizes with low dose tetravalent DR5-Binding Molecules to enhance their cytotoxicity in cells sensitive to tetravalent DR5-Binding Molecules. However, vorinostat did not synergize with non-cross-linked antibodies DR5 mAb 1 and DR5 mAb 2.

[00390] For the second study DR5 mAb 1, DR5 mAb 2, and several tetravalent DR5-Binding Molecules in combination with vorinostat were tested on a number of cell lines including several previously shown to be insensitive to multivalent DR5-Binding

Molecules. The assay was performed essentially as described above except that the cells were treated with 0, or 1 $\mu\text{g/ml}$ of DR5 mAb 1 or DR5 mAb 2 or tetravalent DR5-Binding Molecule, or His-tagged TRAIL $\pm$ 1 or 10 μM vorinostat.

[00391] For this study the Net Gain (calculated as described above) in growth inhibition is reported in **Table 14** (treatment in combination with 10 μM vorinostat) and **Table 15** (treatment in combination with 1 μM vorinostat).

Table 14: (10 μM Vorinostat)								
Net Gain (Average % Growth Inhibition)		DR5 mAb 1 x DR5 mAb 2 Fc DART	DR5 mAb 2 x DR5 mAb 1 Fc DART	DR5 mAb 1 x DR5 mAb 1 Fc DART	DR5 mAb 2 x DR5 mAb 2 Fc DART	DR5 mAb 1	DR5 mAb 2	R&D TRAIL /His
Breast	BT474	75.26	64.18	71.80	54.94	2.15	2.23	50.24
	MCF7	4.96	4.35	4.00	8.76	4.55	5.00	0.79
	MDA-MB-361	83.21	80.65	87.06	62.67	5.11	5.11	64.85
Stomach	Hs746T	38.78	25.69	49.15	22.05	0.20	0.20	31.81
Pancreas	HPAFII	49.19	45.01	55.42	37.51	1.75	2.86	46.71
Lung	A549	36.34	34.95	30.24	20.29	6.48	6.48	26.71
Ovary	ES2	87.60	89.61	88.11	74.12	4.17	4.17	79.12
Prostate	22RV1	5.36	6.32	6.81	7.45	7.49	7.49	8.91
	DU145	1.32	3.43	2.92	3.88	4.18	4.18	4.57
	LNCap	20.41	16.63	20.96	14.37	3.81	4.49	28.73

Table 15: (1 μM Vorinostat)								
Net Gain (Average % Growth Inhibition)		DR5 mAb 1 x DR5 mAb 2 Fc DART	DR5 mAb 2 x DR5 mAb 1 Fc DART	DR5 mAb 1 x DR5 mAb 1 Fc DART	DR5 mAb 2 x DR5 mAb 2 Fc DART	DR5 mAb 1	DR5 mAb 2	R&D TRAIL /His
Breast	MDA-MB-175VII	10.84	10.63	15.84	15.11	0.95	0.95	24.17
	SKBR3	38.50	37.04	42.42	34.85	0.69	0.69	43.10
Stomach	NCI-N87	31.47	25.28	38.47	36.43	0.12	0.36	35.97
Pancreas	AsPC1	14.27	12.91	10.71	12.30	5.11	5.11	11.86
	Hs700T	46.91	43.04	49.75	48.54	0.22	0.22	15.83
Kidney	786O	21.12	13.49	27.69	18.24	0.64	2.33	31.16
Lung	Calu3	10.13	11.03	10.67	13.22	0.11	0.11	9.96
Ovary	SKOV3	55.63	55.97	60.65	55.49	1.75	1.75	33.93
Prostate	PC3	11.32	10.22	11.22	10.85	1.59	1.59	12.49

[00392] The results indicate that the HDAC inhibitor vorinostat synergizes with the tetravalent DR5-Binding Molecules to enhance their cytotoxic activity on cells, including cells insensitive to tetravalent DR5-Binding Molecules alone. In particular, nine cell lines (MDA-MB-175VII, SKBR3, NCI-N87, AsPC1, Hs700T, 786O, Calu3, SKOV3 and PC3) were seen to respond to the tetravalent DR5-Binding Molecules in combination with just 1 μ M vorinostat. Another three cell line (BT474, MDA-MB-361, Hs746T, HPAFII, A549, ES2 and LNCap) were seen to respond to the tetravalent DR5-Binding Molecules in combination with 10 μ M vorinostat.

[00393] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

What Is Claimed Is:

- Claim 1. A multivalent DR5-Binding Molecule that is a bispecific binding molecule, capable of simultaneously binding to two different epitopes of human Death Receptor 5 (DR5), wherein said multivalent DR5-Binding Molecule comprises four antigen-binding domains each capable of binding human DR5.
- Claim 2. An multivalent DR5-Binding Molecule that is a monospecific binding molecule, capable of binding to an epitope of human DR5, wherein said multivalent DR5-Binding Molecule comprises four antigen-binding domains each capable of binding human DR5.
- Claim 3. The multivalent DR5-Binding Molecule of claim 1 or 2, wherein said multivalent DR5-Binding Molecule is capable of simultaneously binding to two, three, or four human DR5 polypeptides.
- Claim 4. The multivalent DR5-Binding Molecule of any one of claims 1-3, wherein said multivalent DR5-Binding Molecule is an Fc Region-containing diabody, said diabody being a covalently bonded complex that comprises two pairs of polypeptides, wherein each pair comprises a first polypeptide chain and a second polypeptide chain.
- Claim 5. The multivalent DR5-Binding Molecule of claim 4, wherein:
- (A) the first polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - (i) a variable light chain (VL) Domain of a monoclonal antibody capable of binding to a first DR5 epitope (VL1);
 - (ii) a first peptide linker (Linker 1);
 - (iii) a variable heavy chain (VH) Domain of a monoclonal capable of binding to a second DR5 epitope (VH2);
 - (iv) a second peptide linker (Linker 2);
 - (v) a Heterodimer-Promoting Domain comprising a E-coil Domain or a K-coil Domain;
 - (vi) a third peptide linker (Linker 3); and

- (vii) a polypeptide portion of an IgG Fc Region having CH2 and CH3 domains of an IgG immunoglobulin Fc Region; and
- (B) the second polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - (i) a VL Domain of a monoclonal antibody capable of binding to said second DR5 epitope (VL2);
 - (ii) a first peptide linker (Linker 1);
 - (iii) a VH Domain of a monoclonal capable of binding to said first DR5 epitope (VH1);
 - (iv) a second peptide linker (Linker 2); and
 - (v) a Heterodimer-Promoting Domain comprising a E-coil Domain or a K-coil Domain, wherein said Heterodimer-Promoting Domain of said first polypeptide chain and said Heterodimer-Promoting Domain of said second polypeptide chain are not both E-coil Domains or both K-coil Domains;

and wherein:

- (a) the VL1 Domain of said first polypeptide chain and the VH1 Domain of said second polypeptide chain form an Antigen-Binding Domain capable of specific binding to a first epitope of DR5;
- (b) said VH2 Domain of said first polypeptide chain and said VL1 Domain of said second polypeptide chain form an Antigen-Binding Domain capable of specific binding to a second epitope of DR5; and
- (c) the CH2-CH3 portions of the pair of first polypeptide chains form an IgG Fc Region.

- Claim 6. The multivalent DR5-Binding Molecule of claim 5, wherein said VL1 comprises a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} Domain, and said VH1 comprises a CDR_{H1} Domain, a CDR_{H2} Domain and a CDR_{H3} Domain, wherein:
- (i) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**; or
 - (ii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
 - (iii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of hDR5 mAb 2 VL-3, and, respectively have the amino acid sequences: **SEQ ID NO:162**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of hDR5 mAb 2 VH-3, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
 - (iv) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:56**, and **SEQ ID NO:57**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and

respectively have the amino acid sequences: **SEQ ID NO:59**, **SEQ ID NO:60**, and **SEQ ID NO:61**; or

- (v) said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:63**, **SEQ ID NO:64**, and **SEQ ID NO:65**, and said CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:67**, **SEQ ID NO:68**, and **SEQ ID NO:69**; or
- (vi) said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:71**, **SEQ ID NO:72**, and **SEQ ID NO:73**, and said CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:75**, **SEQ ID NO:76**, and **SEQ ID NO:77**; or
- (vii) said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:79**, **SEQ ID NO:80**, and **SEQ ID NO:81**, and said CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:83**, **SEQ ID NO:84**, and **SEQ ID NO:85**; or
- (viii) said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:87**, **SEQ ID NO:88**, and **SEQ ID NO:89**, and said CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:91**, **SEQ ID NO:92**, and **SEQ ID NO:93**; or
- (ix) said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the

amino acid sequences: **SEQ ID NO:95**, **SEQ ID NO:96**, and **SEQ ID NO:97**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:99**, **SEQ ID NO:100**, and **SEQ ID NO:101**.

Claim 7. The multivalent DR5-Binding Molecule of claim 5 or 6, wherein said VL2 comprises a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} Domain, and said VH2 comprises a CDR_{H1} Domain, a CDR_{H2} Domain and a CDR_{H3} Domain, wherein:

- (i) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**; or
- (ii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
- (iii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:162**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; or
- (iv) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the

amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:56**, and **SEQ ID NO:57**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:59**, **SEQ ID NO:60**, and **SEQ ID NO:61**; or

- (v) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:63**, **SEQ ID NO:64**, and **SEQ ID NO:65**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:67**, **SEQ ID NO:68**, and **SEQ ID NO:69**; or
- (vi) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:71**, **SEQ ID NO:72**, and **SEQ ID NO:73**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:75**, **SEQ ID NO:76**, and **SEQ ID NO:77**; or
- (vii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:79**, **SEQ ID NO:80**, and **SEQ ID NO:81**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:83**, **SEQ ID NO:84**, and **SEQ ID NO:85**; or
- (viii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:87**, **SEQ ID NO:88**, and **SEQ ID NO:89**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and

respectively have the amino acid sequences: **SEQ ID NO:91**, **SEQ ID NO:92**, and **SEQ ID NO:93**; or

- (ix) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:95**, **SEQ ID NO:96**, and **SEQ ID NO:97**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:99**, **SEQ ID NO:100**, and **SEQ ID NO:101**.

Claim 8. The multivalent DR5-Binding Molecule of claim 7, wherein said VL1 and said VL2 comprise the same CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain, and wherein said VH1 and said VH2 comprise the same CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain.

Claim 9. The multivalent DR5-Binding Molecule of claim 8, wherein said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**.

Claim 10. The multivalent DR5-Binding Molecule of claim 8, wherein said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**.

Claim 11. The multivalent DR5-Binding Molecule of claim 8, wherein said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid

sequences: **SEQ ID NO:162**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**.

Claim 12. The multivalent DR5-Binding Molecule of claim 7, wherein said VL1 and said VL2 do not comprise the same CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain, and wherein said VH1 and said VH2 do not comprise the same CDR_{H1} Domain, CDR_{H2} Domain, CDR_{H3} Domain.

Claim 13. The multivalent DR5-Binding Molecule of claim 12, wherein:

- (i) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL1 are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of VH1 are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**; and
- (ii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL2 are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of VH2 are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**.

Claim 14. The multivalent DR5-Binding Molecule of claim 12, wherein:

- (i) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL1 are the Light Chain CDRs of DR5 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**, and said CDR_{H1} Domain, CDR_{H2}

Domain, and CDR_{H3} Domain of VH1 are the Heavy Chain CDRs of DR5 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**; and

- (ii) said CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of VL2 are the Light Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**, and said CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of VH2 are the Heavy Chain CDRs of DR5 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**.

Claim 15. The multivalent DR5-Binding Molecule of claim 5, wherein:

- (i) said VL1 has the amino acid sequence of **SEQ ID NO:3**, and said VH1 has the amino acid sequence of **SEQ ID NO:8**; or
- (ii) said VL1 has the amino acid sequence of **SEQ ID NO:13**, and said VH1 has the amino acid sequence of **SEQ ID NO:18**; or
- (iii) said VL1 has the amino acid sequence of **SEQ ID NO:23**, and said VH1 has the amino acid sequence of **SEQ ID NO:31**; or
- (iv) said VL1 has the amino acid sequence of **SEQ ID NO:25**, and said VH1 has the amino acid sequence of **SEQ ID NO:31**; or
- (vi) said VL1 has the amino acid sequence of **SEQ ID NO:27**, and said VH1 has the amino acid sequence of **SEQ ID NO:31**; or
- (vii) said VL1 has the amino acid sequence of **SEQ ID NO:29**, and said VH1 has the amino acid sequence of **SEQ ID NO:31**; or
- (viii) said VL1 has the amino acid sequence of **SEQ ID NO:54**, and said VH1 has the amino acid sequence of **SEQ ID NO:58**; or
- (ix) said VL1 has the amino acid sequence of **SEQ ID NO:62**, and said VH1 has the amino acid sequence of **SEQ ID NO:66**; or
- (x) said VL1 has the amino acid sequence of **SEQ ID NO:70**, and said VH1 has the amino acid sequence of **SEQ ID NO:74**; or

- (xi) said VL1 has the amino acid sequence of **SEQ ID NO:78**, and said VH1 has the amino acid sequence of **SEQ ID NO:82**; or
- (xii) said VL1 has the amino acid sequence of **SEQ ID NO:86**, and said VH1 has the amino acid sequence of **SEQ ID NO:90**; or
- (xiii) said VL1 has the amino acid sequence of **SEQ ID NO:94**, and said VH1 has the amino acid sequence of **SEQ ID NO:98**.

Claim 16. The multivalent DR5-Binding Molecule of claim 5 or 6, wherein:

- (i) said VL2 has the amino acid sequence of **SEQ ID NO:3**, and said VH2 has the amino acid sequence of **SEQ ID NO:8**; or
- (ii) said VL2 has the amino acid sequence of **SEQ ID NO:13**, and said VH2 has the amino acid sequence of **SEQ ID NO:18**; or
- (iii) said VL2 has the amino acid sequence of **SEQ ID NO:23**, and said VH2 has the amino acid sequence of **SEQ ID NO:31**; or
- (iv) said VL2 has the amino acid sequence of **SEQ ID NO:25**, and said VH2 has the amino acid sequence of **SEQ ID NO:31**; or
- (vi) said VL2 has the amino acid sequence of **SEQ ID NO:27**, and said VH2 has the amino acid sequence of **SEQ ID NO:31**; or
- (vii) said VL2 has the amino acid sequence of **SEQ ID NO:29**, and said VH2 has the amino acid sequence of **SEQ ID NO:31**; or
- (viii) said VL2 has the amino acid sequence of **SEQ ID NO:54**, and said VH2 has the amino acid sequence of **SEQ ID NO:58**; or
- (ix) said VL2 has the amino acid sequence of **SEQ ID NO:62**, and said VH2 has the amino acid sequence of **SEQ ID NO:66**; or
- (x) said VL2 has the amino acid sequence of **SEQ ID NO:70**, and said VH1 has the amino acid sequence of **SEQ ID NO:74**; or
- (xi) said VL2 has the amino acid sequence of **SEQ ID NO:78**, and said VH1 has the amino acid sequence of **SEQ ID NO:82**; or
- (xii) said VL2 has the amino acid sequence of **SEQ ID NO:86**, and said VH1 has the amino acid sequence of **SEQ ID NO:90**; or
- (xiii) said VL2 has the amino acid sequence of **SEQ ID NO:94**, and said VH2 has the amino acid sequence of **SEQ ID NO:98**.

- Claim 17. The multivalent DR5-Binding Molecule of claim 16, wherein said VL1 and said VL2 have the same amino acid sequence, and wherein said VH1 and said VH2 have the same amino acid sequence.
- Claim 18. The multivalent DR5-Binding Molecule of claim 16, wherein said VL1 and said VL2 do not have the same amino acid sequence, and wherein said VH1 and said VH2 do not have the same amino acid sequence.
- Claim 19. The multivalent DR5-Binding Molecule of any one of claims 5-18, wherein:
- (i) said Linker 1 has the amino acid sequence of **SEQ ID NO:33**,
 - (ii) said Linker 1 has the amino acid sequence of **SEQ ID NO:47**,
 - (iii) said E-coil Domain has the amino acid sequence of **SEQ ID NO:41**,
 - (iv) said K-coil Domain has the amino acid sequence of **SEQ ID NO:42**,
 - (v) said Linker 3 has the amino acid sequence of **SEQ ID NO:51**, and
 - (vi) said CH2-CH3 domain has the amino acid sequence of **SEQ ID NO:1** or **SEQ ID NO:102**, wherein the C-terminal residue is optionally included.
- Claim 20. The multivalent DR5-Binding Molecule of any one of claims 4-19, wherein said Fc Region comprises one or more amino acid modifications that reduce the affinity of the variant Fc Region for an FcγR or stabilizes said Fc Region.
- Claim 21. The multivalent DR5-Binding Molecule of any one of claims 20, wherein said modifications comprise the substitution of L234A; L235A; or L234A and L235A.
- Claim 22. The multivalent DR5-Binding Molecule of claim 5, wherein:
- (i) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:116** or **SEQ ID NO:120**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:118**; or

- (ii) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:122** or **SEQ ID NO:126**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:124**; or
- (iii) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:128** or **SEQ ID NO:132**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:130**; or
- (iv) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:134** or **SEQ ID NO:138**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:136**; or
- (v) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:140** or **SEQ ID NO:144**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:142**; or
- (vi) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:146** or **SEQ ID NO:150**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:148**; or
- (vii) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:152** or **SEQ ID NO:156**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:154**; or
- (vi) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:158** or **SEQ ID NO:162**, and said second polypeptide chain has the amino acid sequence of **SEQ ID NO:160**.

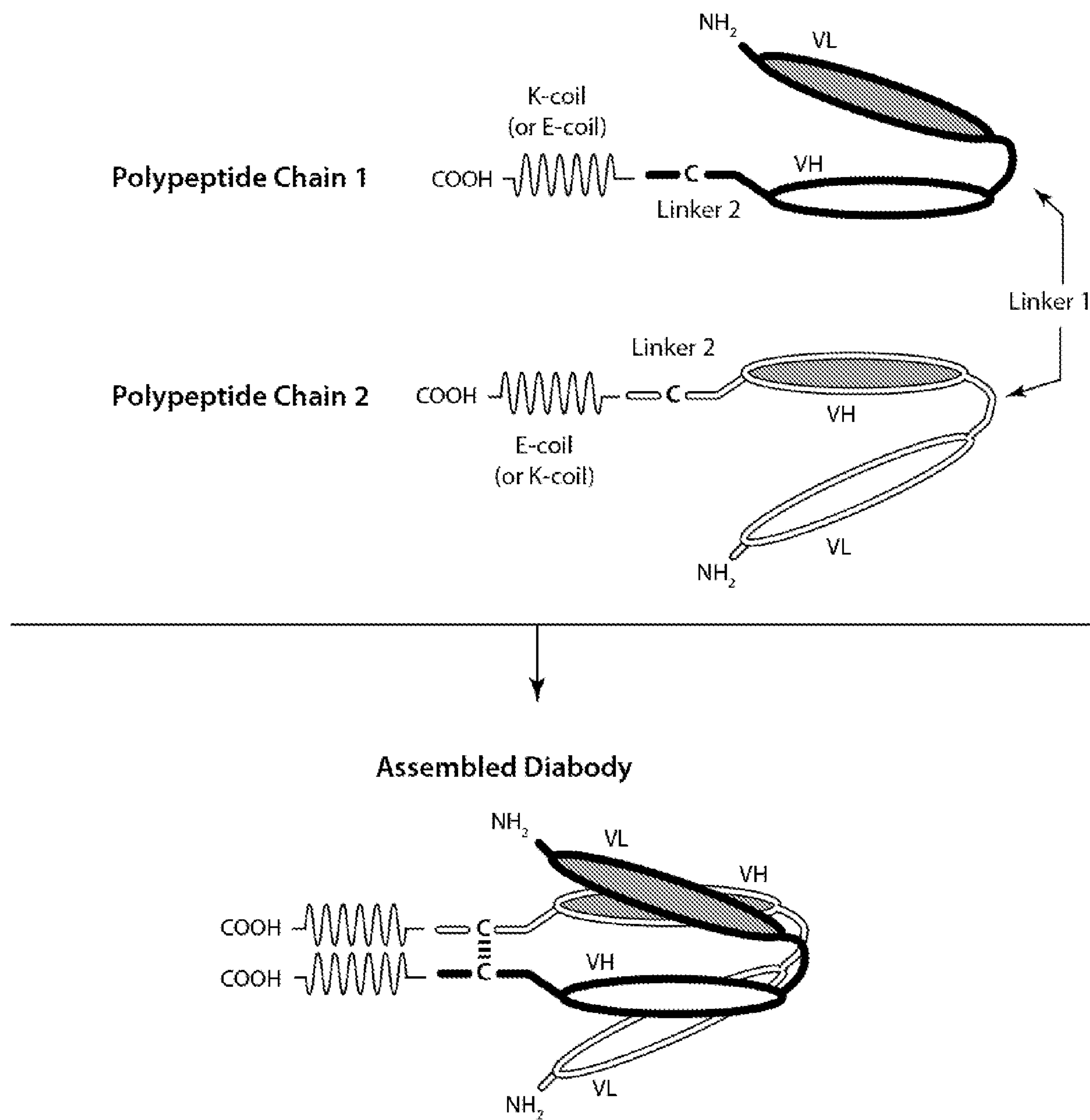
- Claim 23. A composition comprising the multivalent DR5-Binding Molecule of any one of claims 1-22 and an excipient.
- Claim 24. The composition of claim 23, further comprising a histone deacetylase inhibitor.
- Claim 25. A method of promoting cell death comprising exposing said cell to the multivalent DR5-Binding Molecule of any one of claims 1-21.
- Claim 26. The method of claim 25, wherein said cell is a tumor cell.
- Claim 27. The method of claim 25, wherein said tumor cell is a cancer stem cell-like cell.

- Claim 28. The method of claim 25, 26, or 27, wherein the method further comprises exposing said cell to a histone deacetylase inhibitor.
- Claim 29. The multivalent DR5-Binding Molecule of any one of claims 1-21, wherein said molecule is used in the treatment of cancer.
- Claim 30. The multivalent DR5-Binding Molecule of claim 29, wherein said molecule is used in combination with a histone deacetylase inhibitor.
- Claim 31. The multivalent DR5-Binding Molecule of any one of claims 1-21, wherein said molecule is detectably labeled and is used in the diagnosis or prognosis of cancer.
- Claim 32. The multivalent DR5-Binding Molecule of any of claims 29-31, wherein said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocyoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor,

a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

Claim 33. The multivalent DR5-Binding Molecule of any of claims 29-31, wherein said cancer is acolorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer or a rectal cancer.

Claim 34. The multivalent DR5-Binding Molecule of any of claims 29-31, wherein said cancer is acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantel cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

**Figure 1**

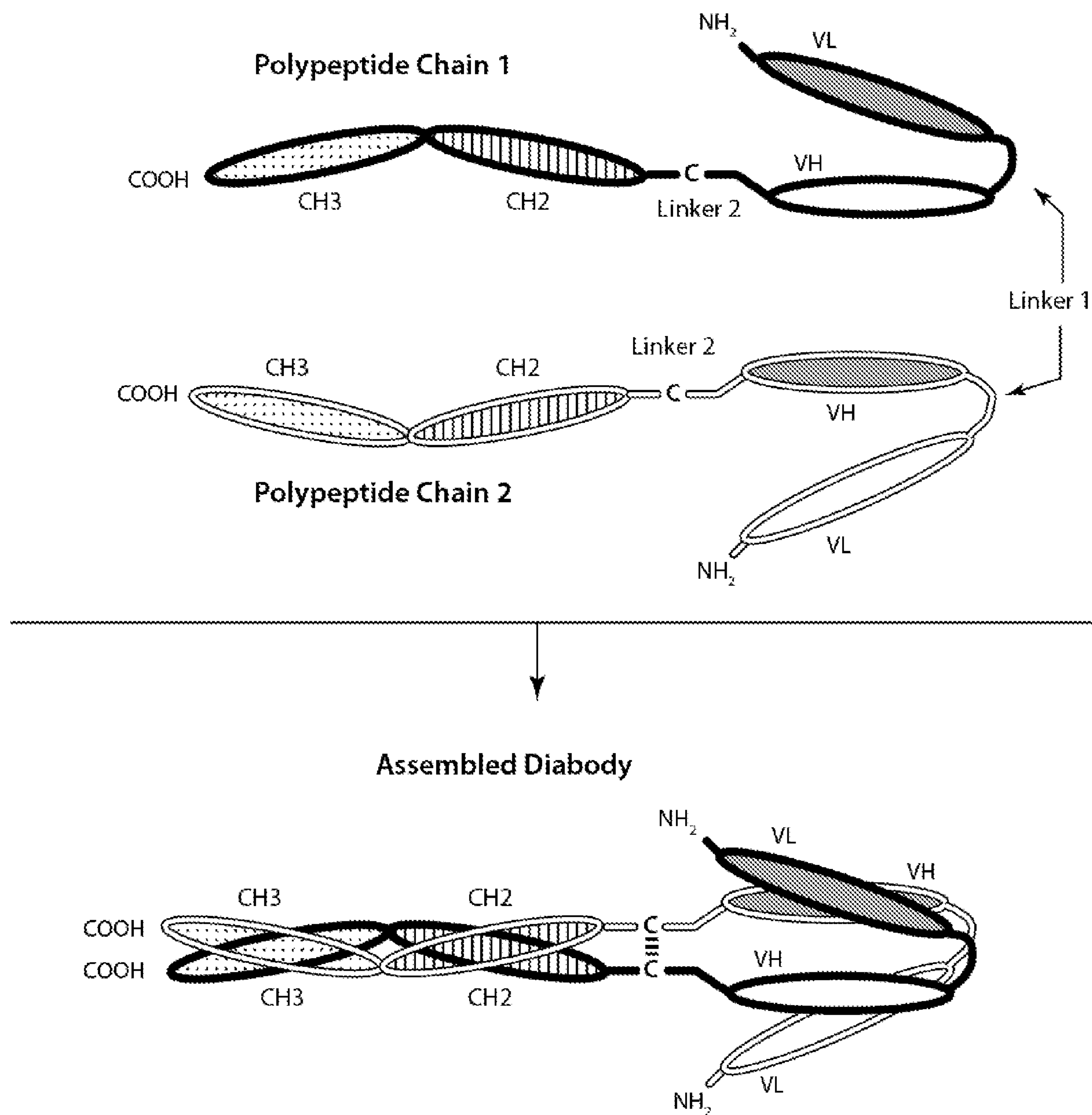


Figure 2

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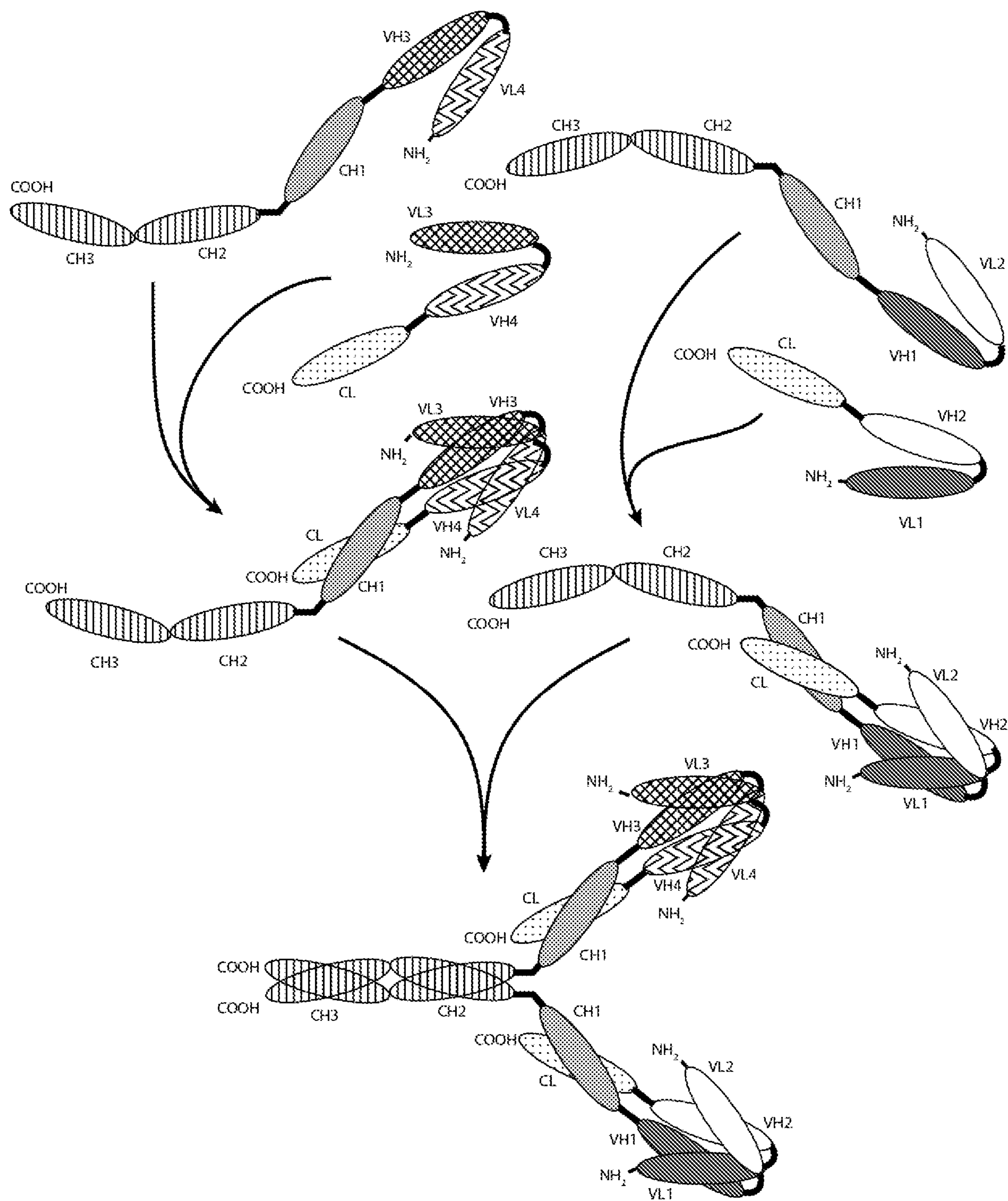


Figure 3

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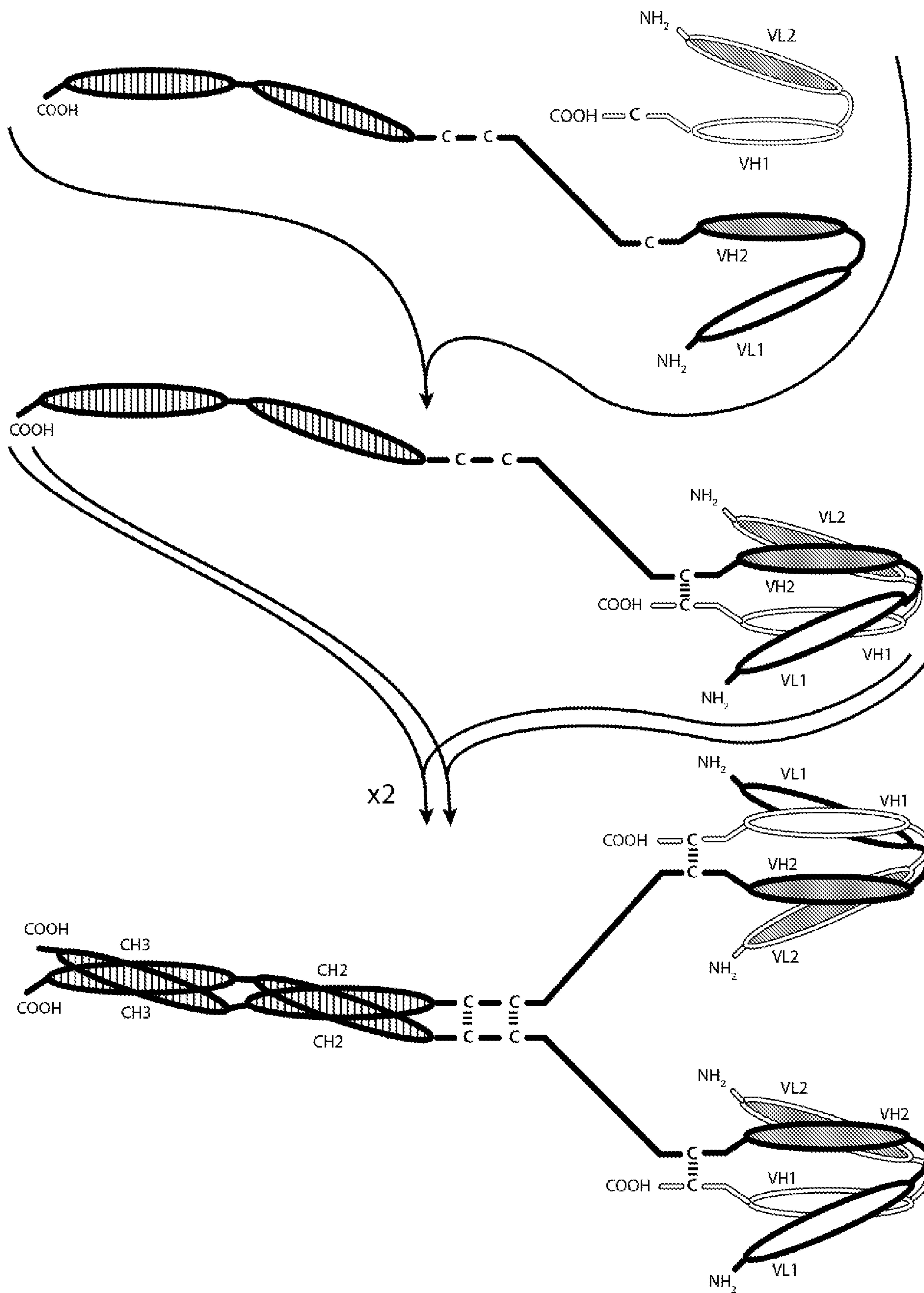


Figure 4A

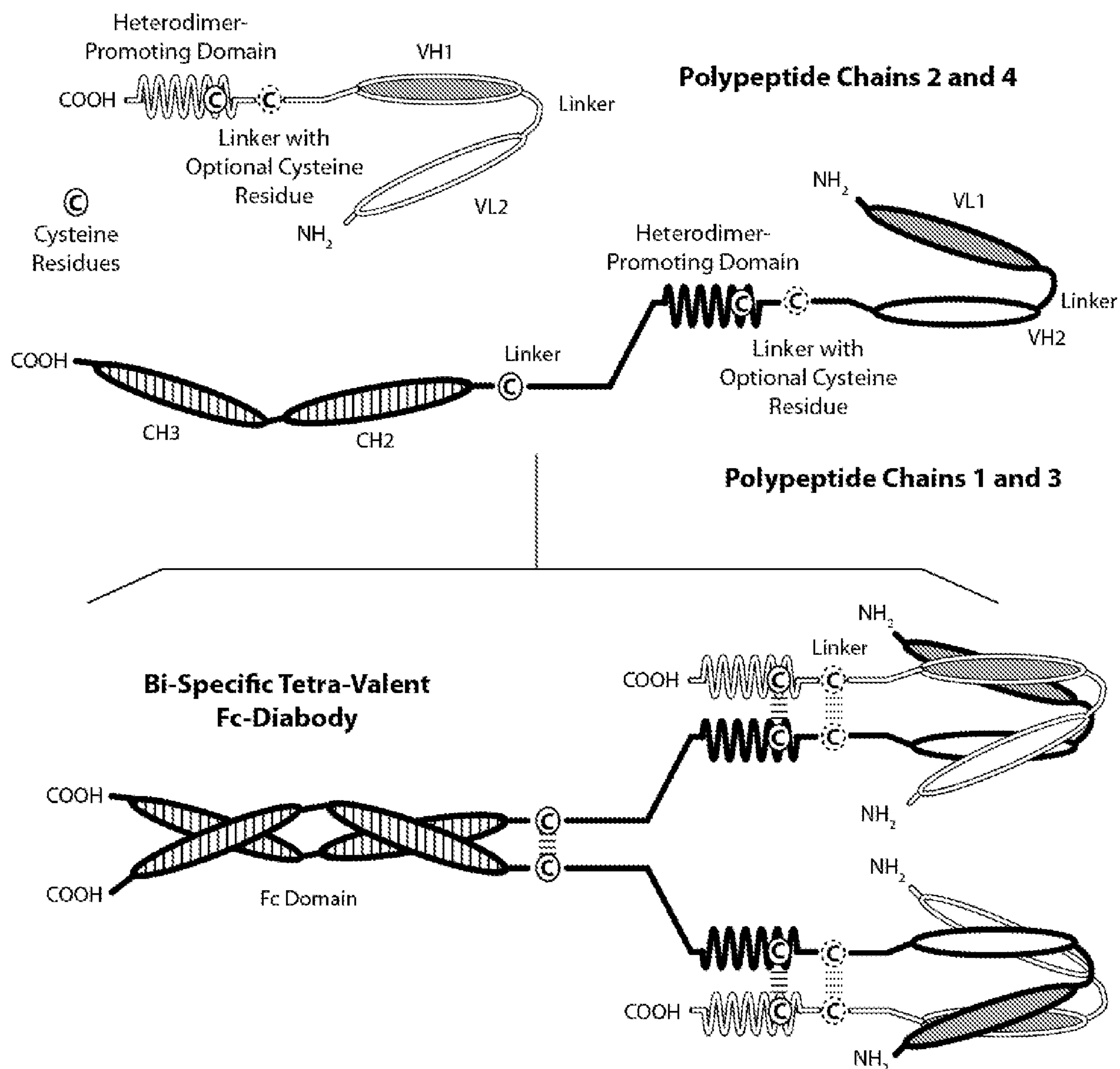
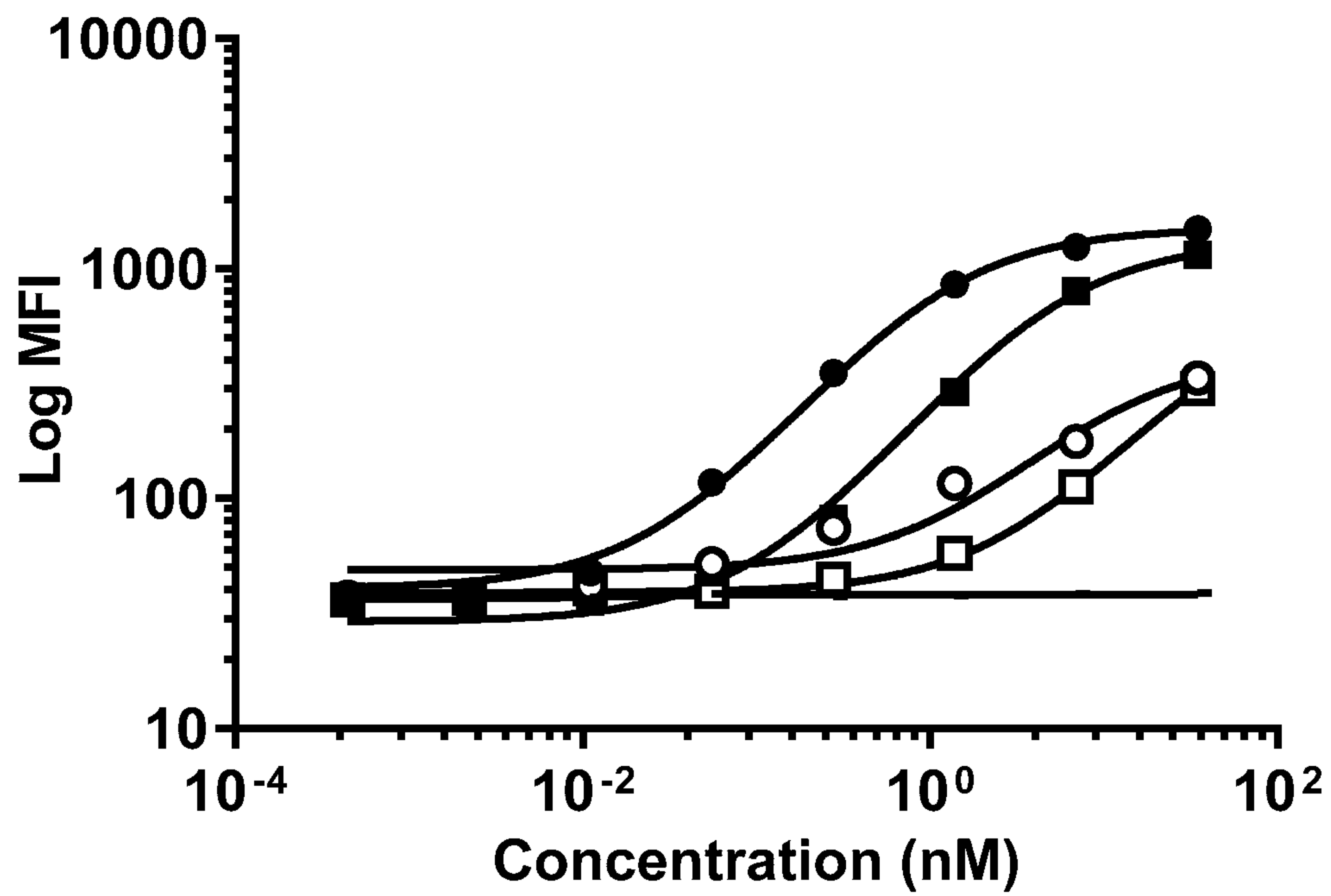


Figure 4B

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- mIgG
- DR5 mAb 1 (Human)
- DR5 mAb 1 (Cyno)
- DR5 mAb 2 (Human)
- DR5 mAb 2 (Cyno)

Figure 5

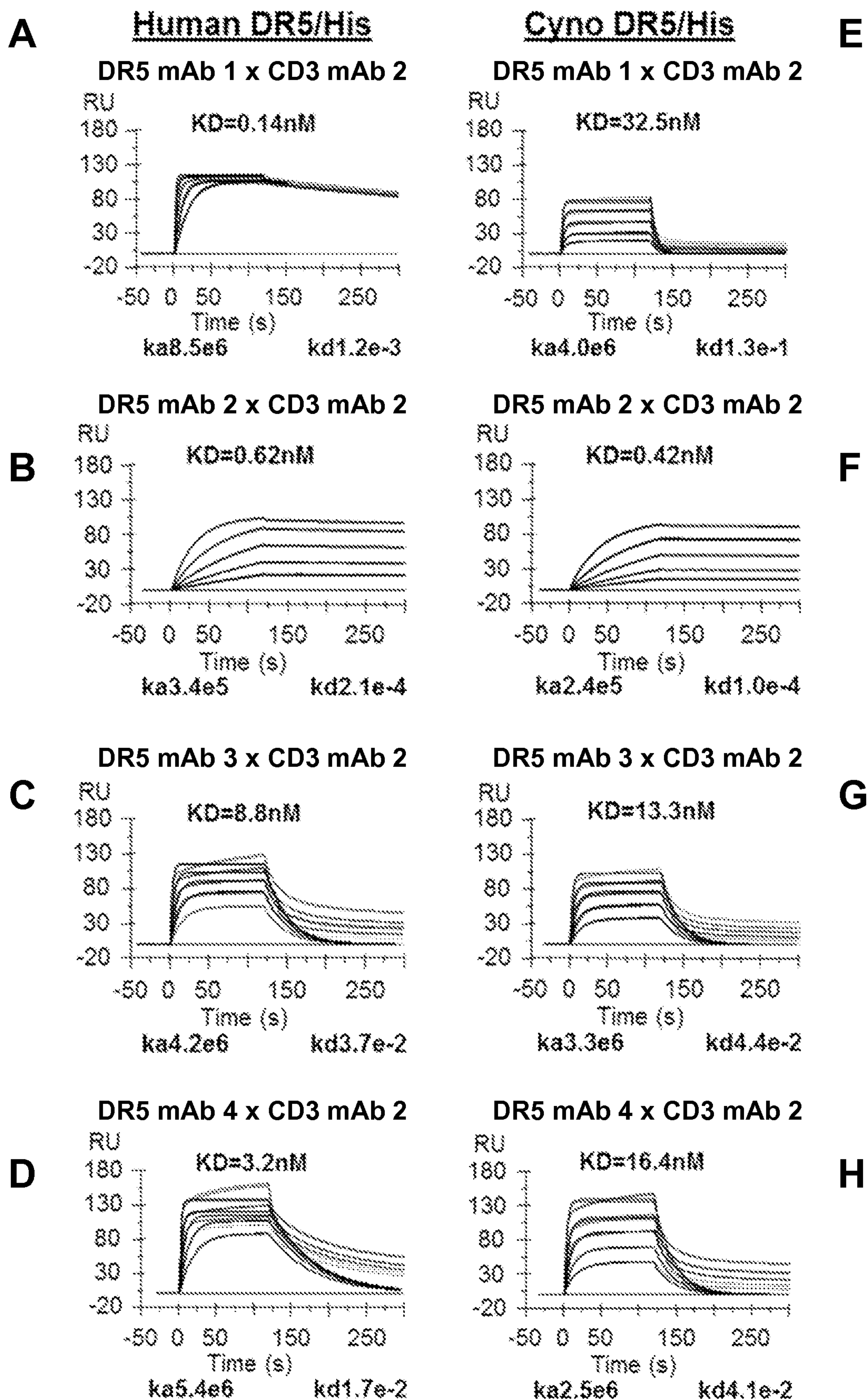


Figure 6

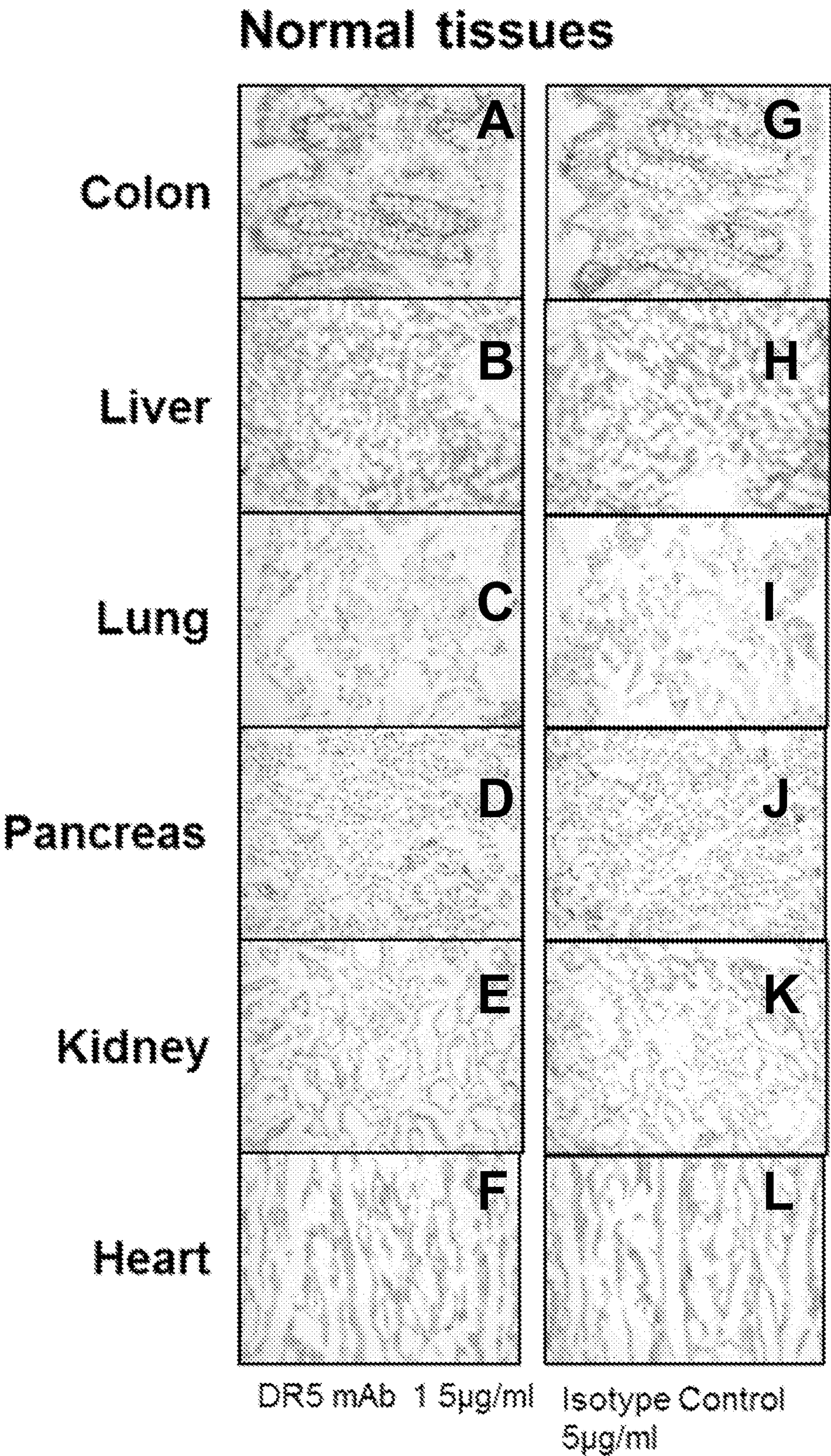
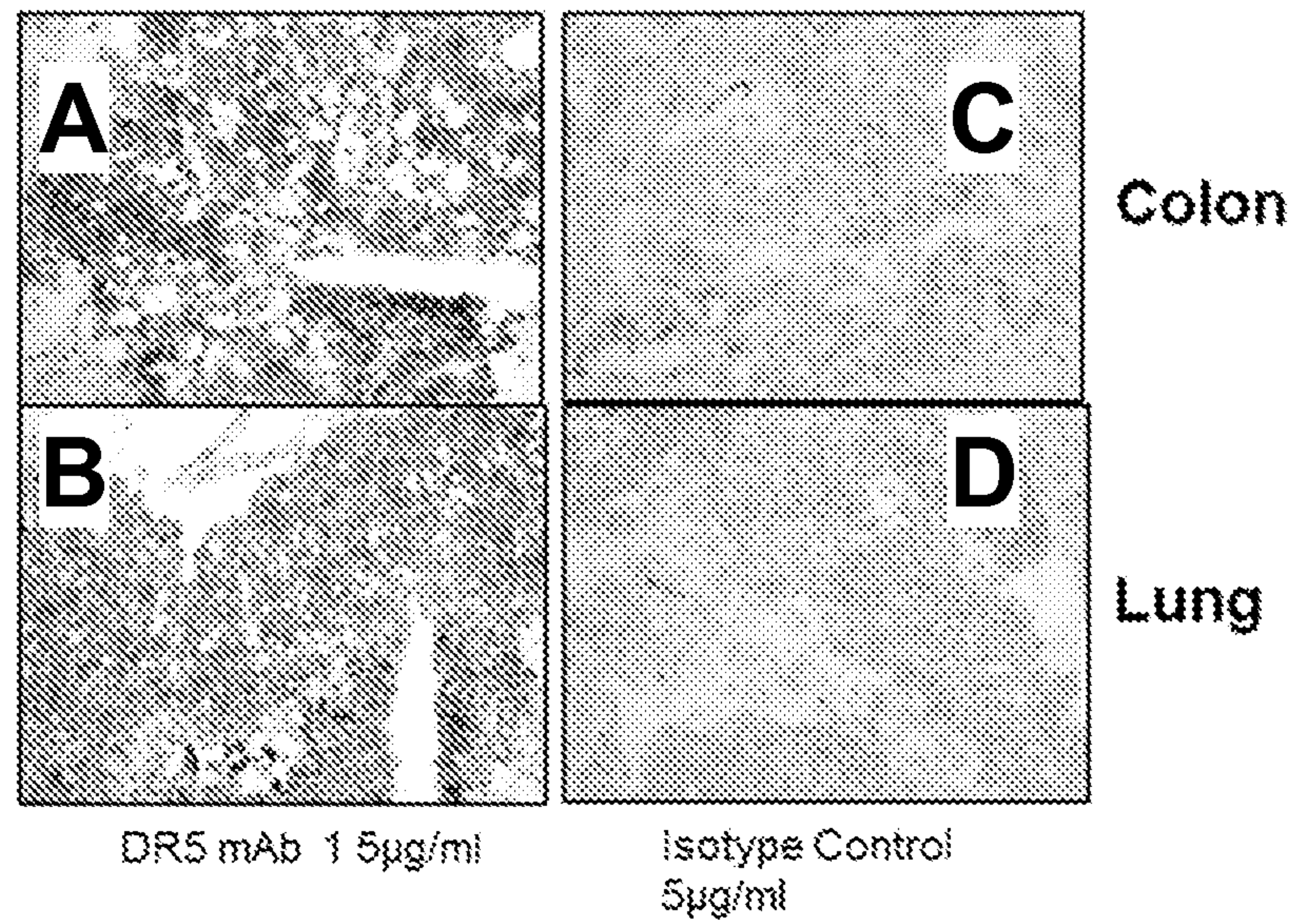


Figure 7A

Tumor tissues**Figure 7B**

Normal tissues (DR5 Mab 2 5µg/ml)

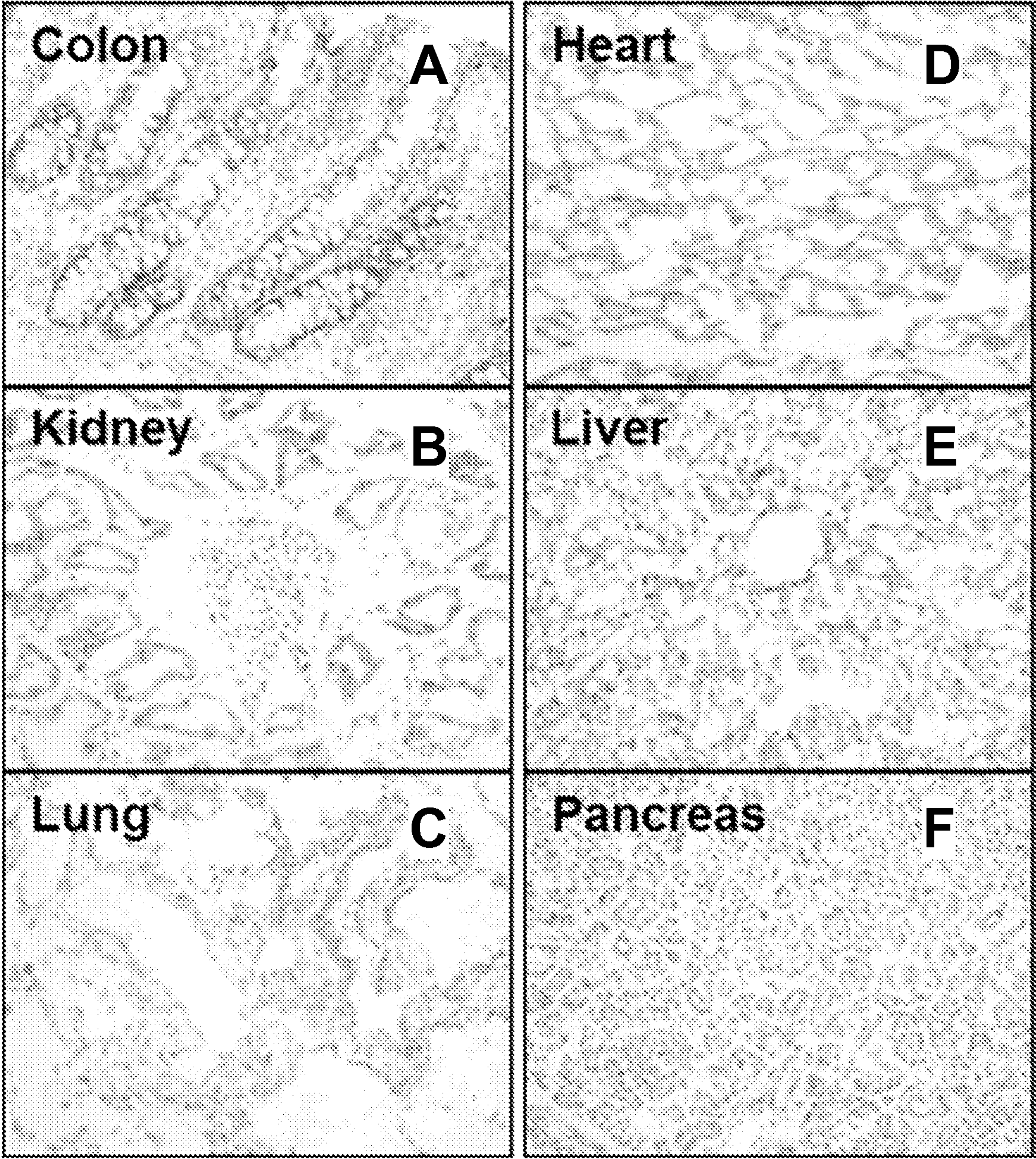


Figure 8A

Tumor Tissues

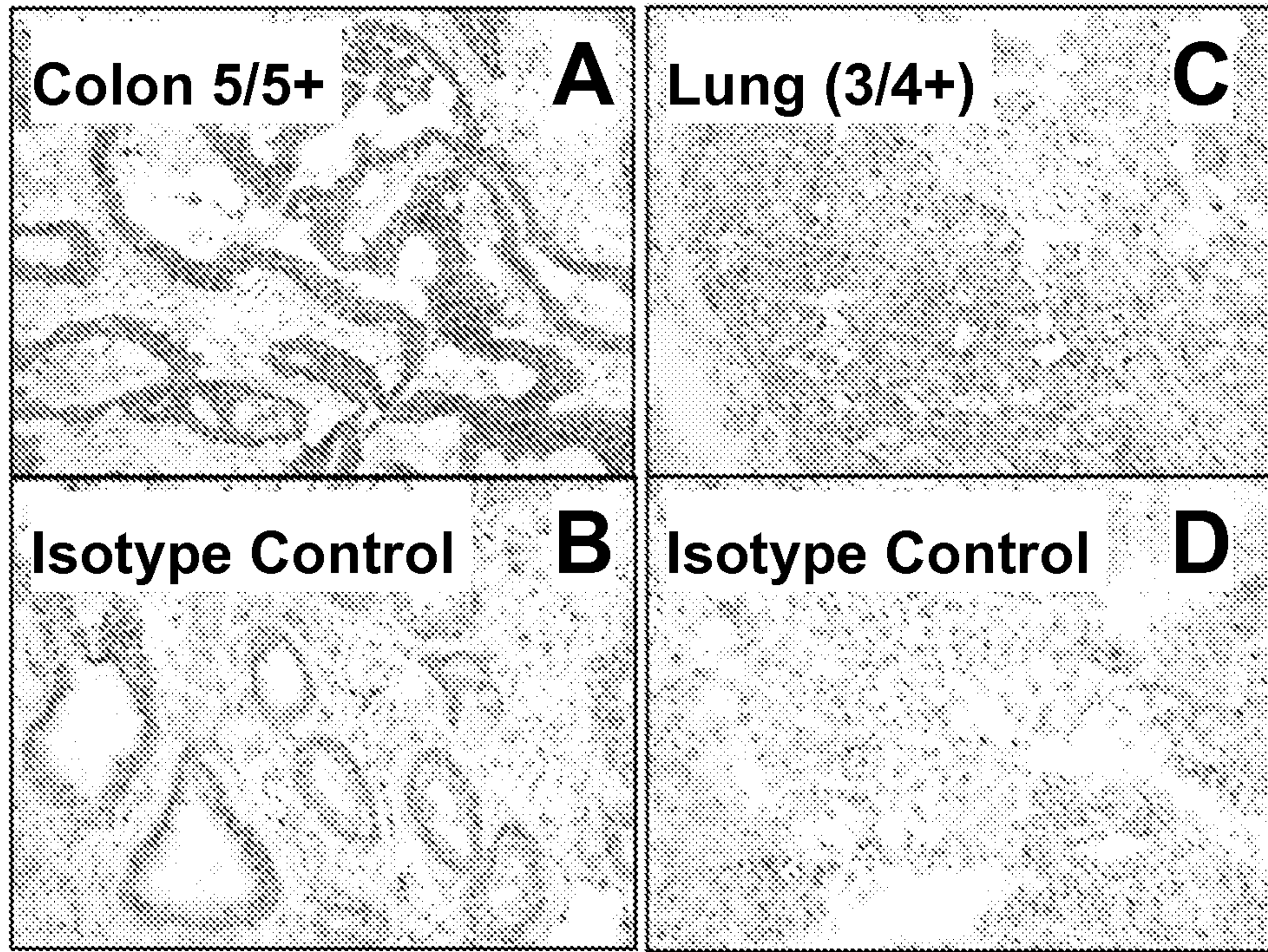
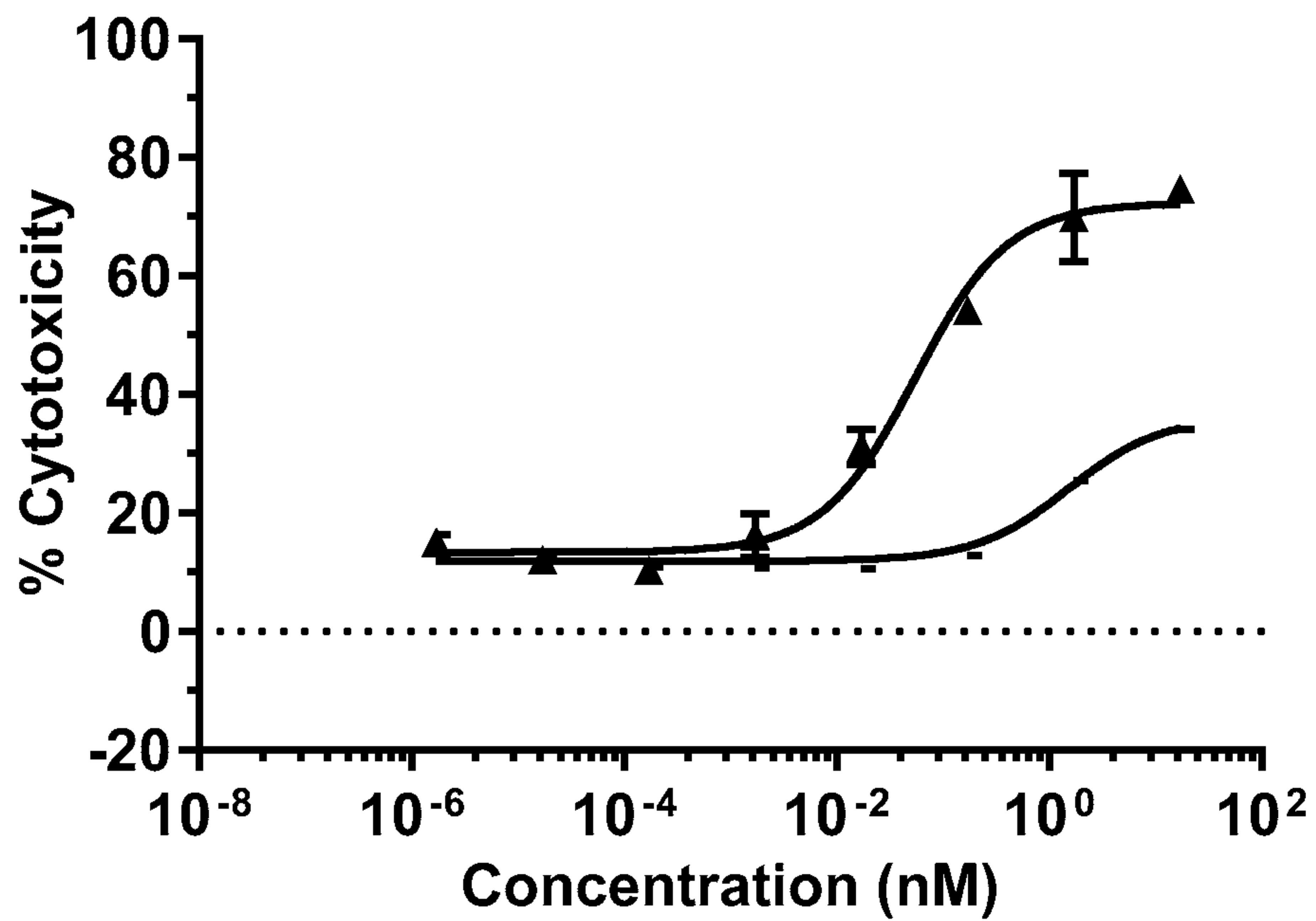


Figure 8B

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786O + PBMC (LDH) 24h
D#38263 E:T=30:1

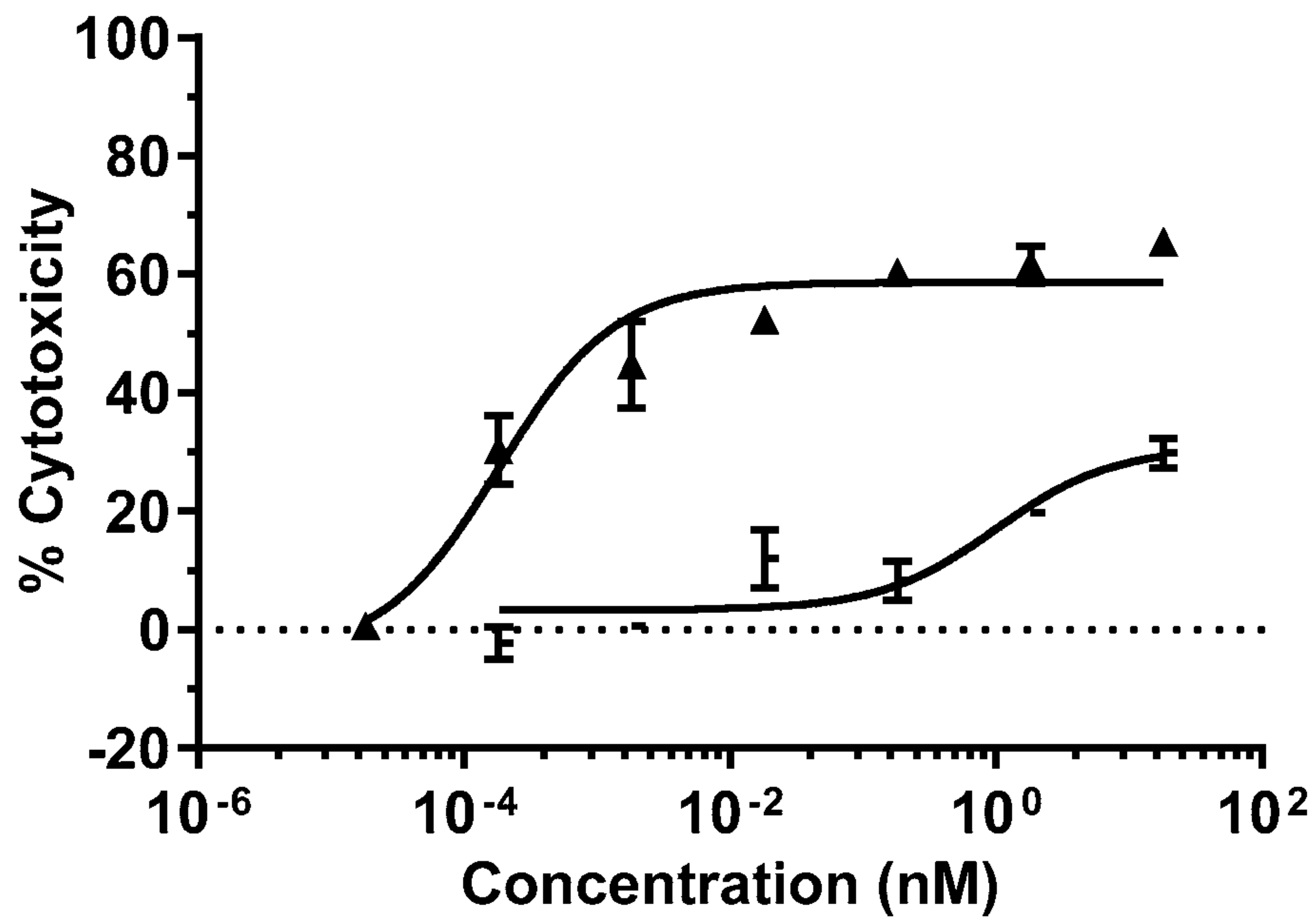


▲ DR5 mAb 2 x CD3 mAb 2 Diabody
— Control Diabody (4-4-20 x CD3 mAb 2)

Figure 9A

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A498 + PBMCs (LDH) 24h
D#46593 E:T=20:1

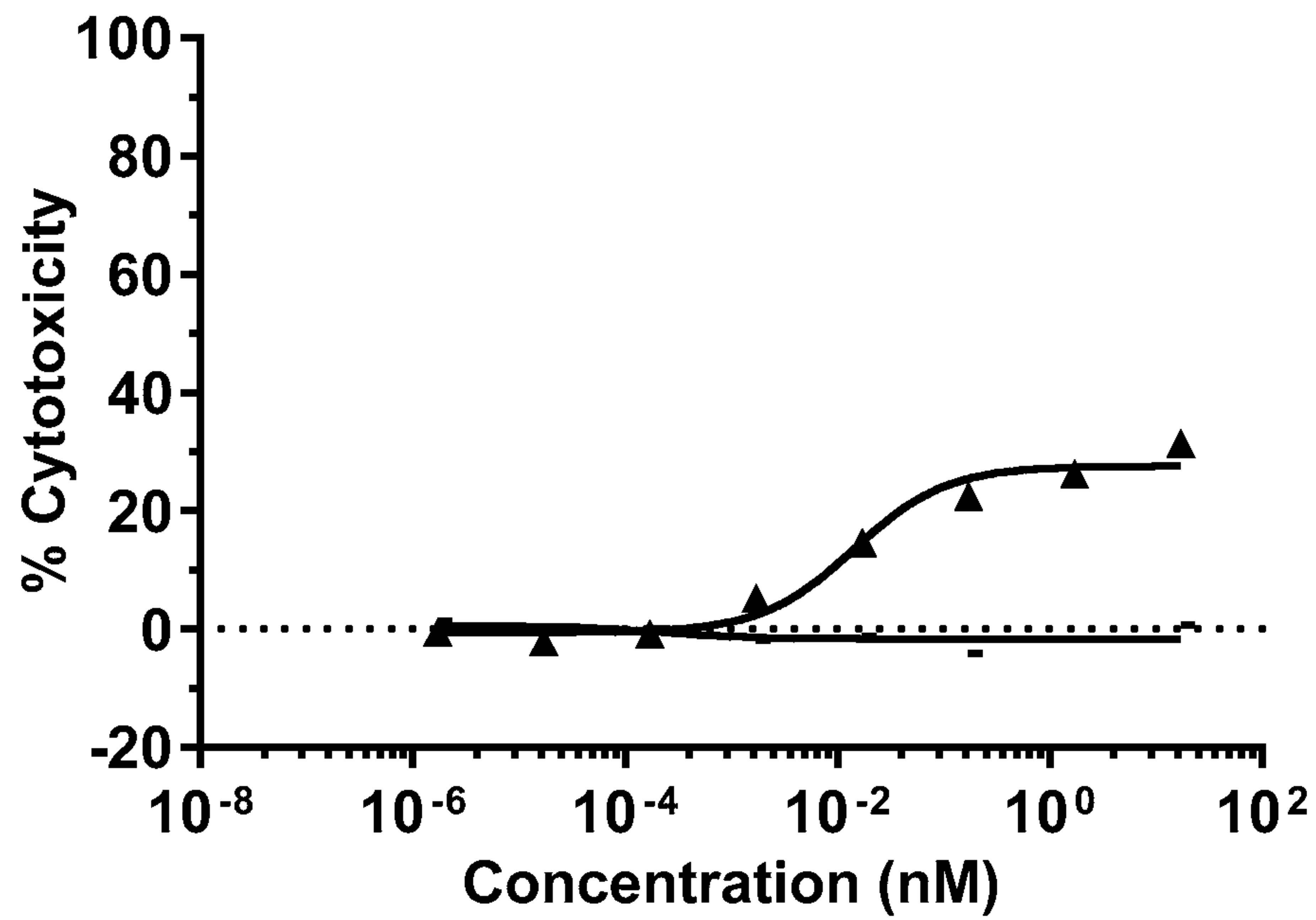


—▲ DR5 mAb 2 x CD3 mAb 2 Diabody
—■ Control Diabody (4-4-20 x CD3 mAb 2)

Figure 9B

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AsPC1 + PBMCs (LDH) 24h
D#57301 E:T=30:1

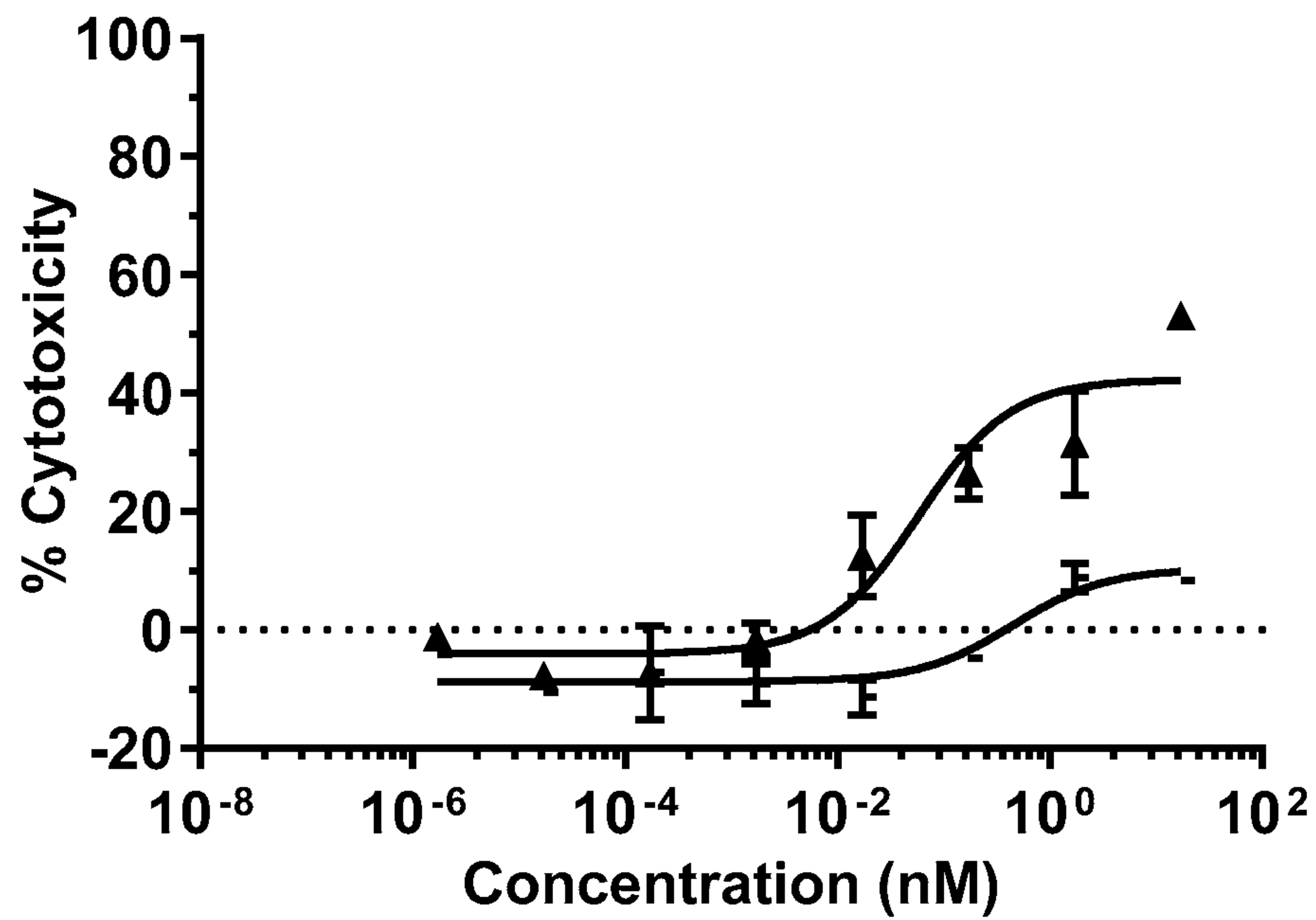


▲ DR5 mAb 2 x CD3 mAb 2 Diabody
— Control Diabody (4-4-20 x CD3 mAb 2)

Figure 9C

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LNCap + PBMC (LDH) 24h
D#38263 E:T=30:1

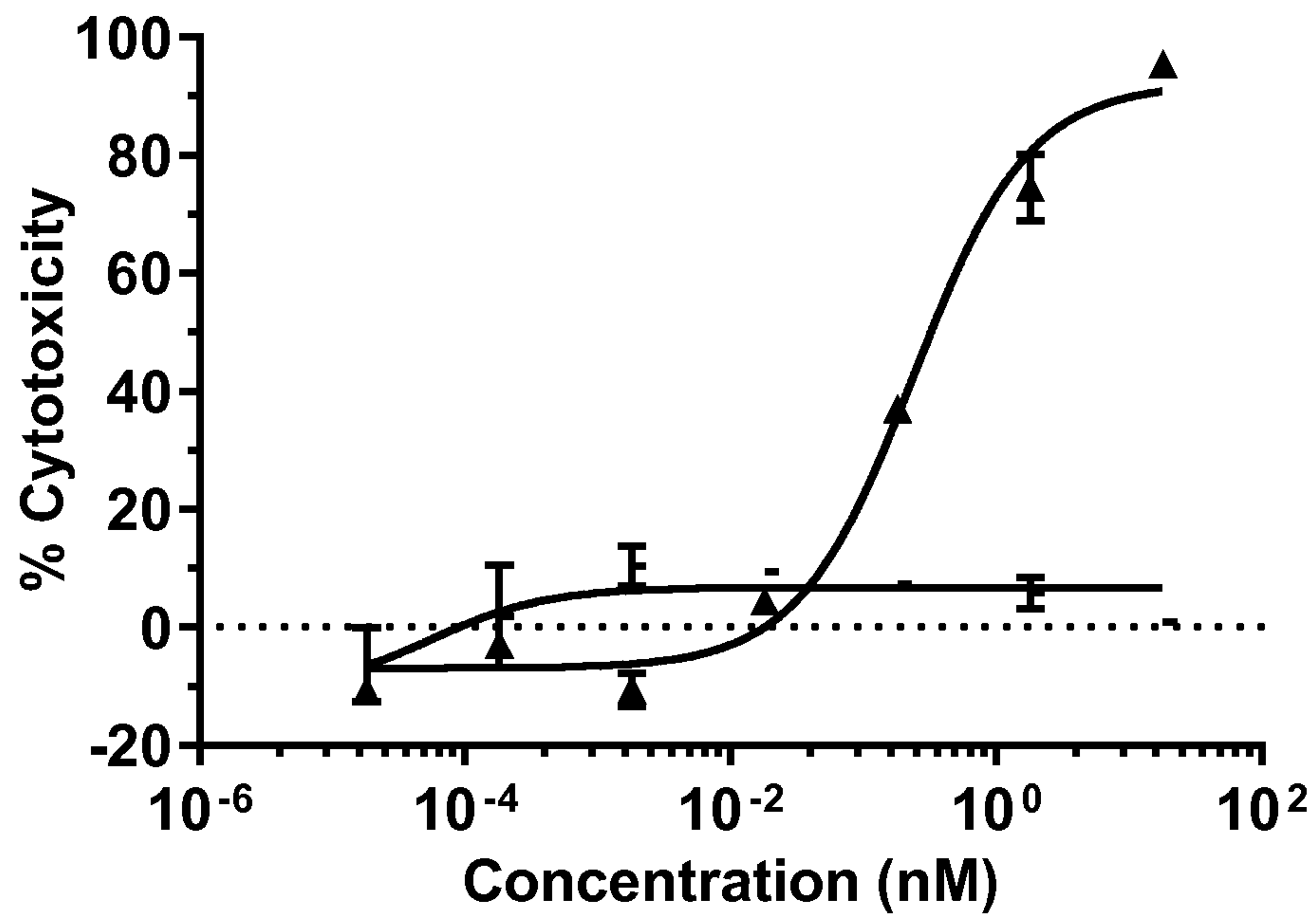


▲ DR5 mAb 2 x CD3 mAb 2 Diabody
— Control Diabody (4-4-20 x CD3 mAb 2)

Figure 9D

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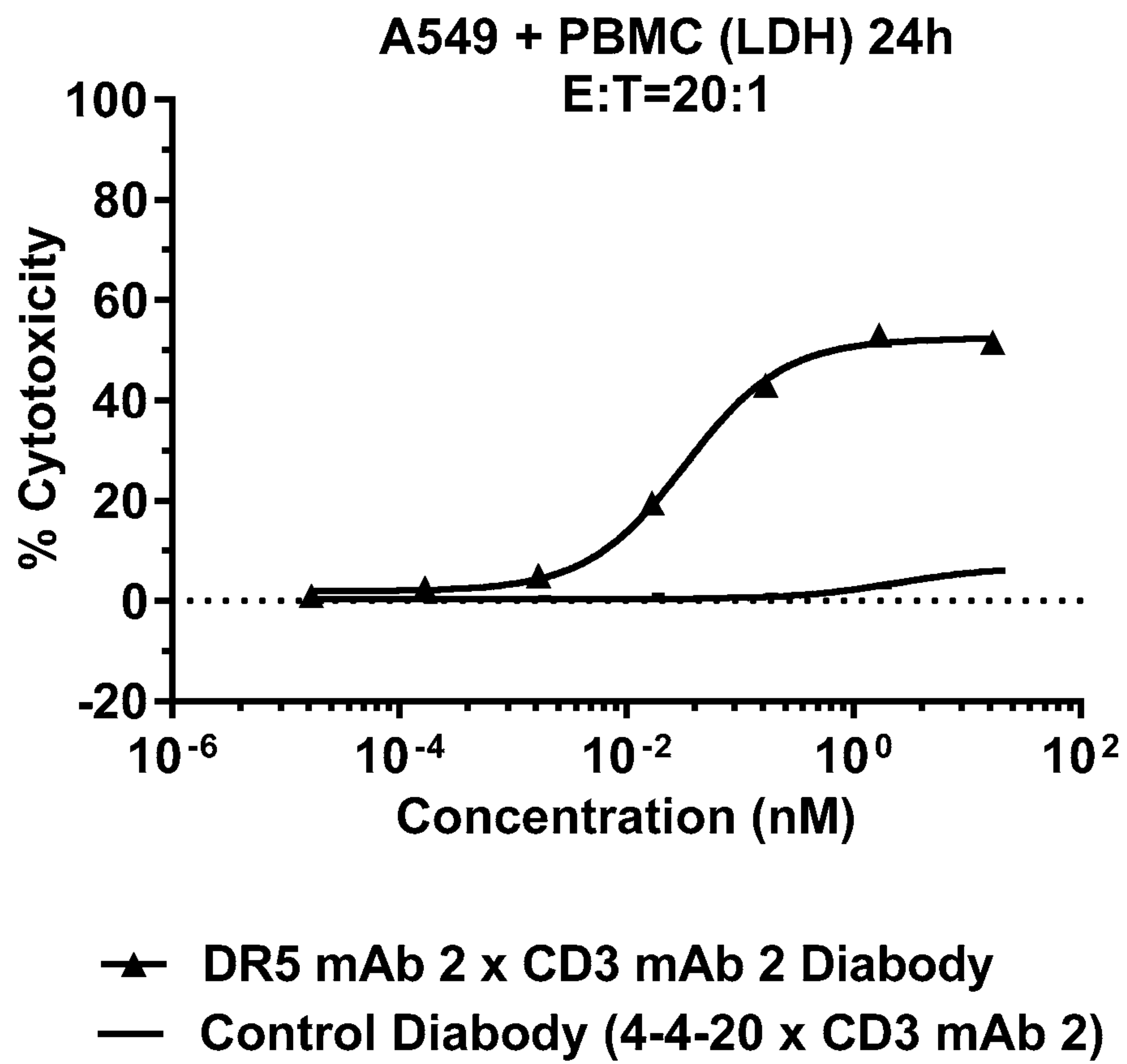
SW48 + PBMC (LDH) 24h
D#46593 E:T=20:1



- ▲ DR5 mAb 2 x CD3 mAb 2 Diabody
— Control Diabody (4-4-20 x CD3 mAb 2)

Figure 9E

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**Figure 9F**

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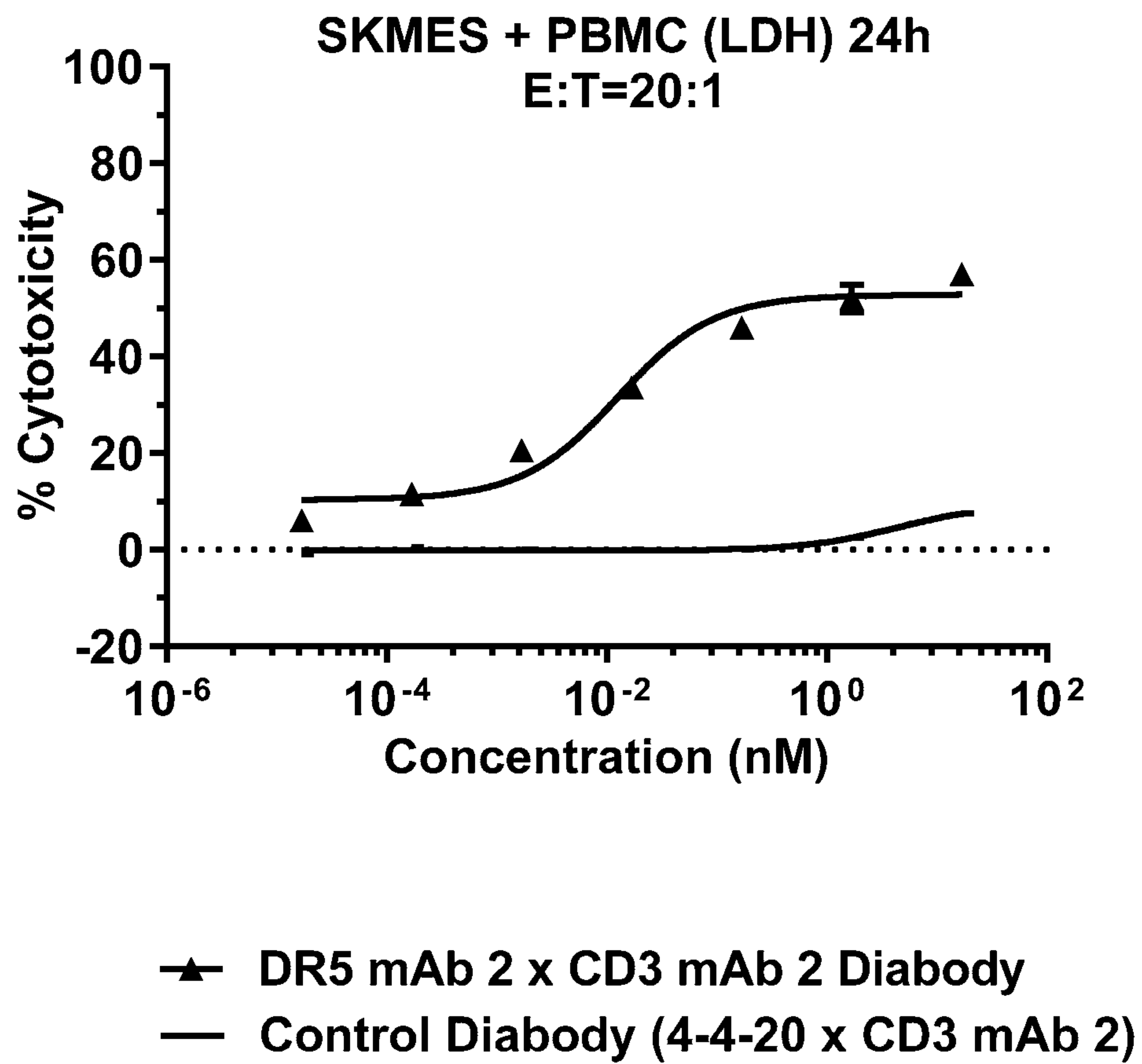


Figure 9G

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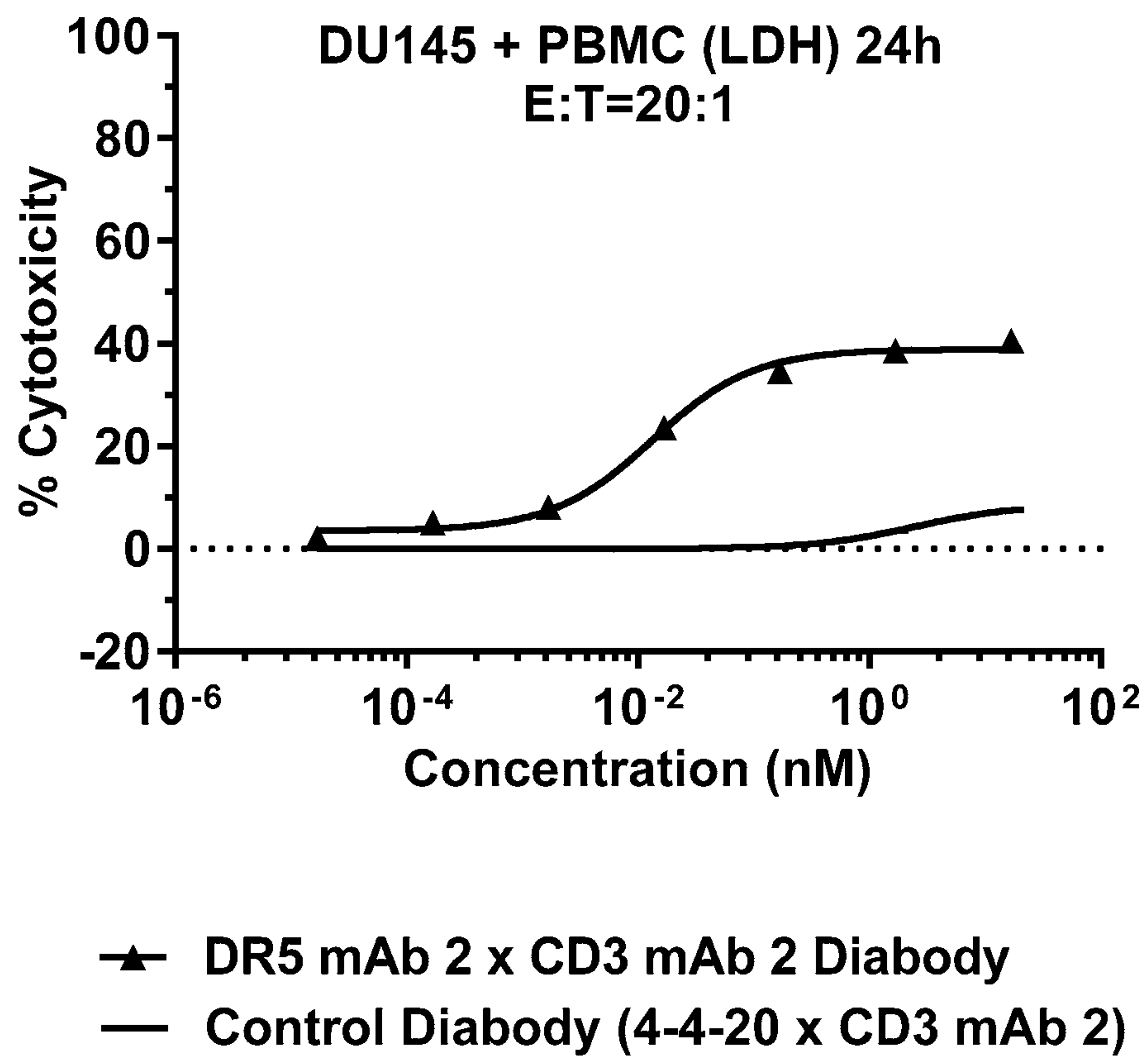


Figure 9H

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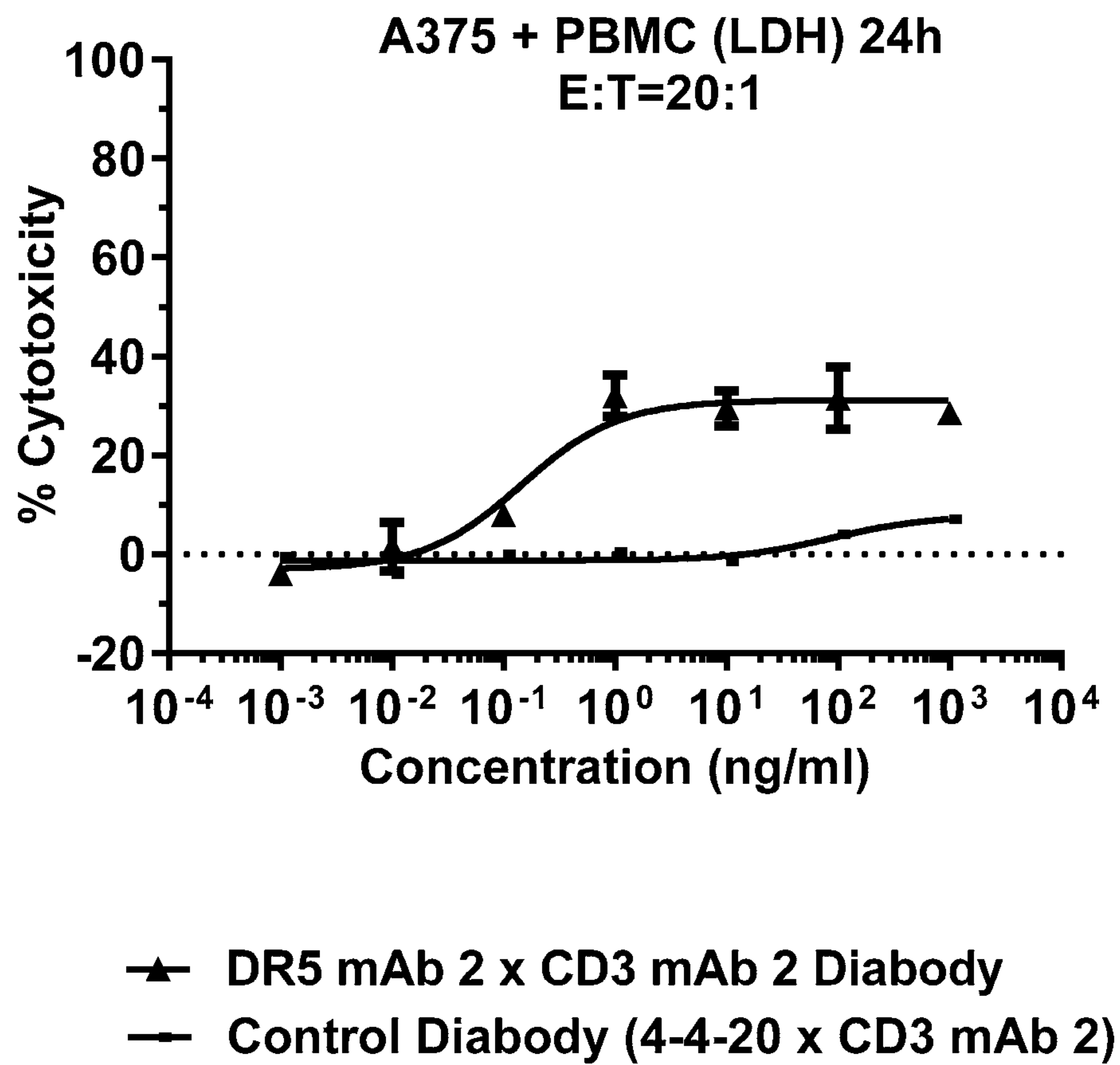


Figure 9I

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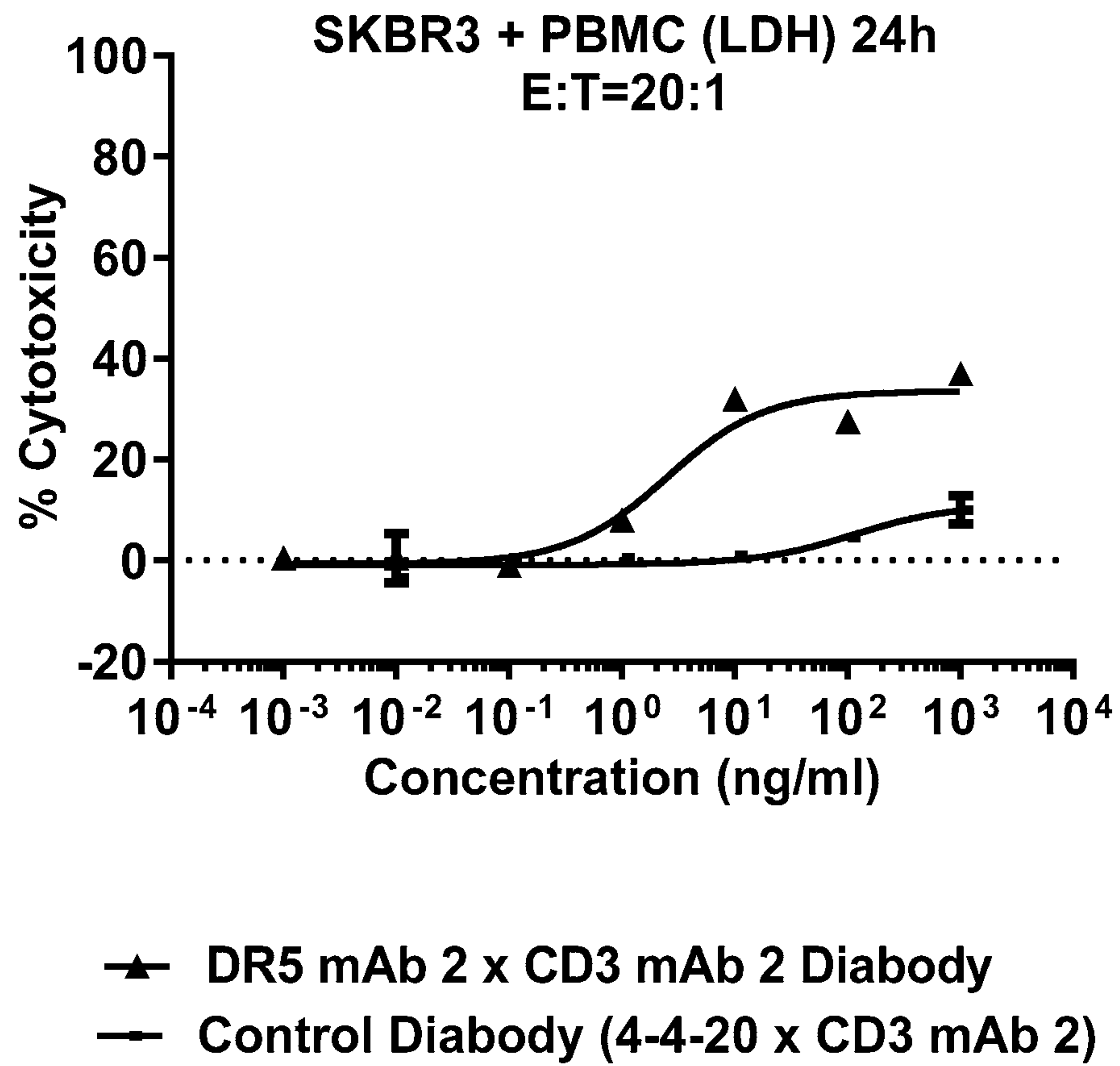


Figure 9J

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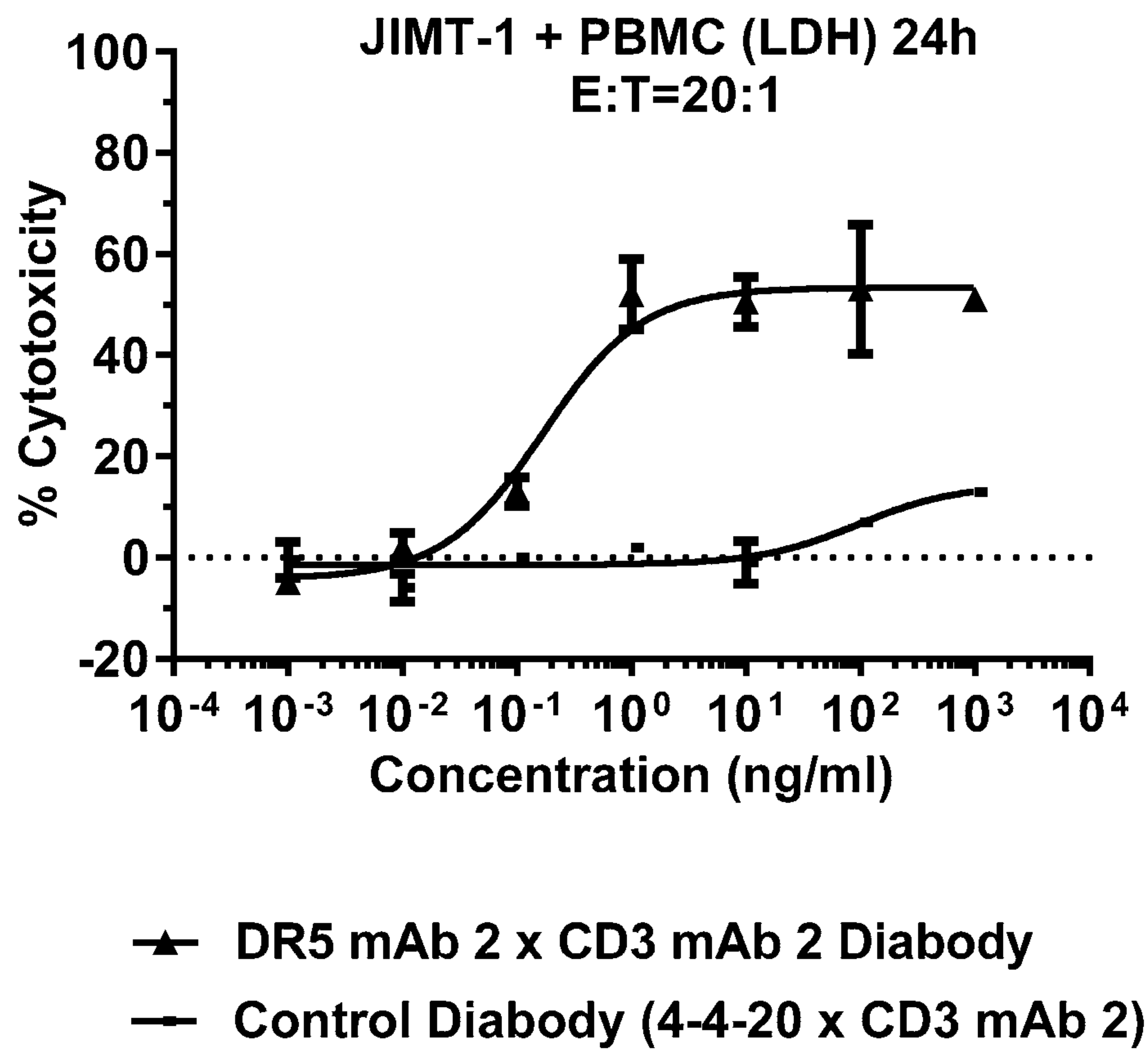


Figure 9K

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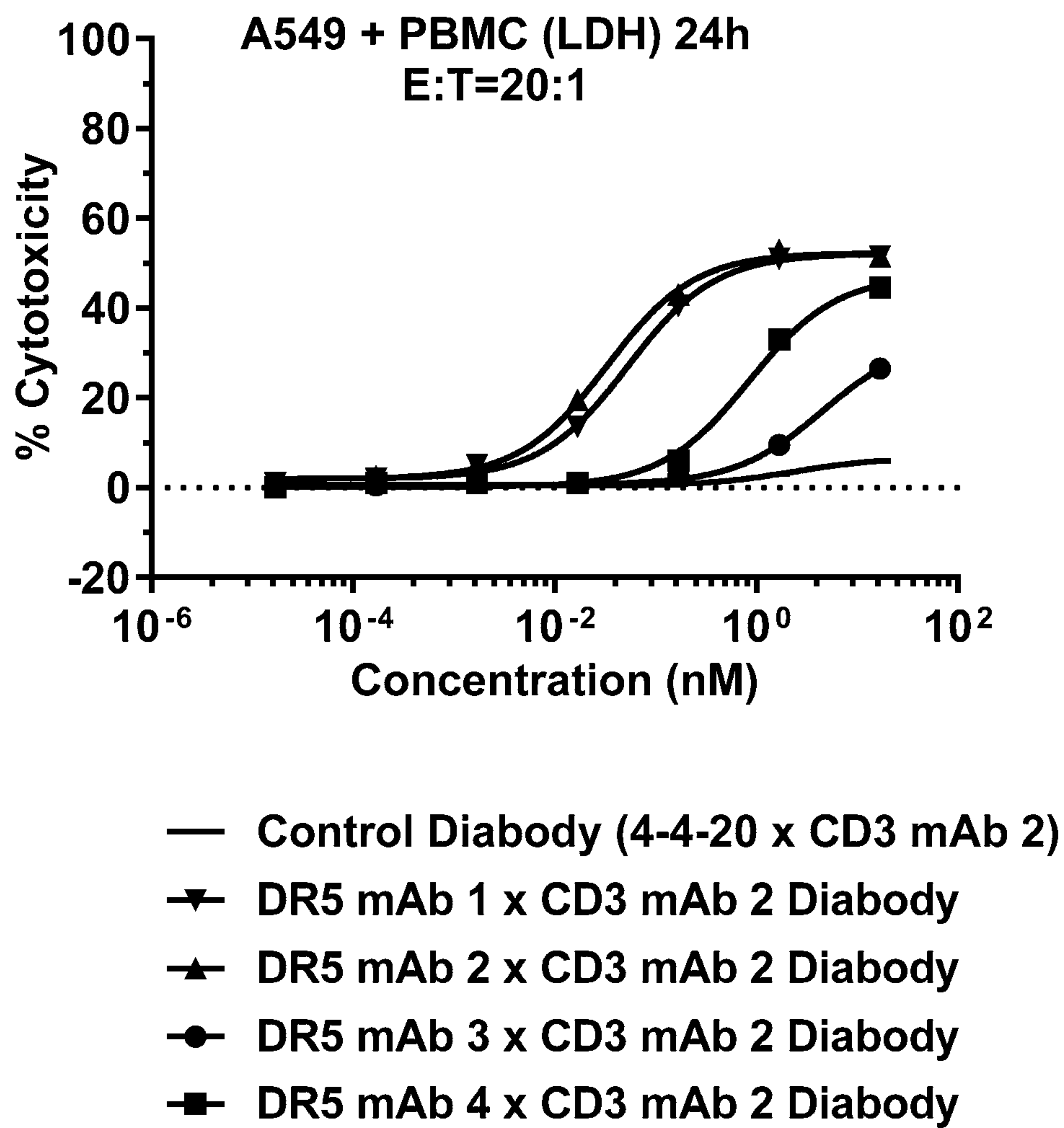


Figure 10A

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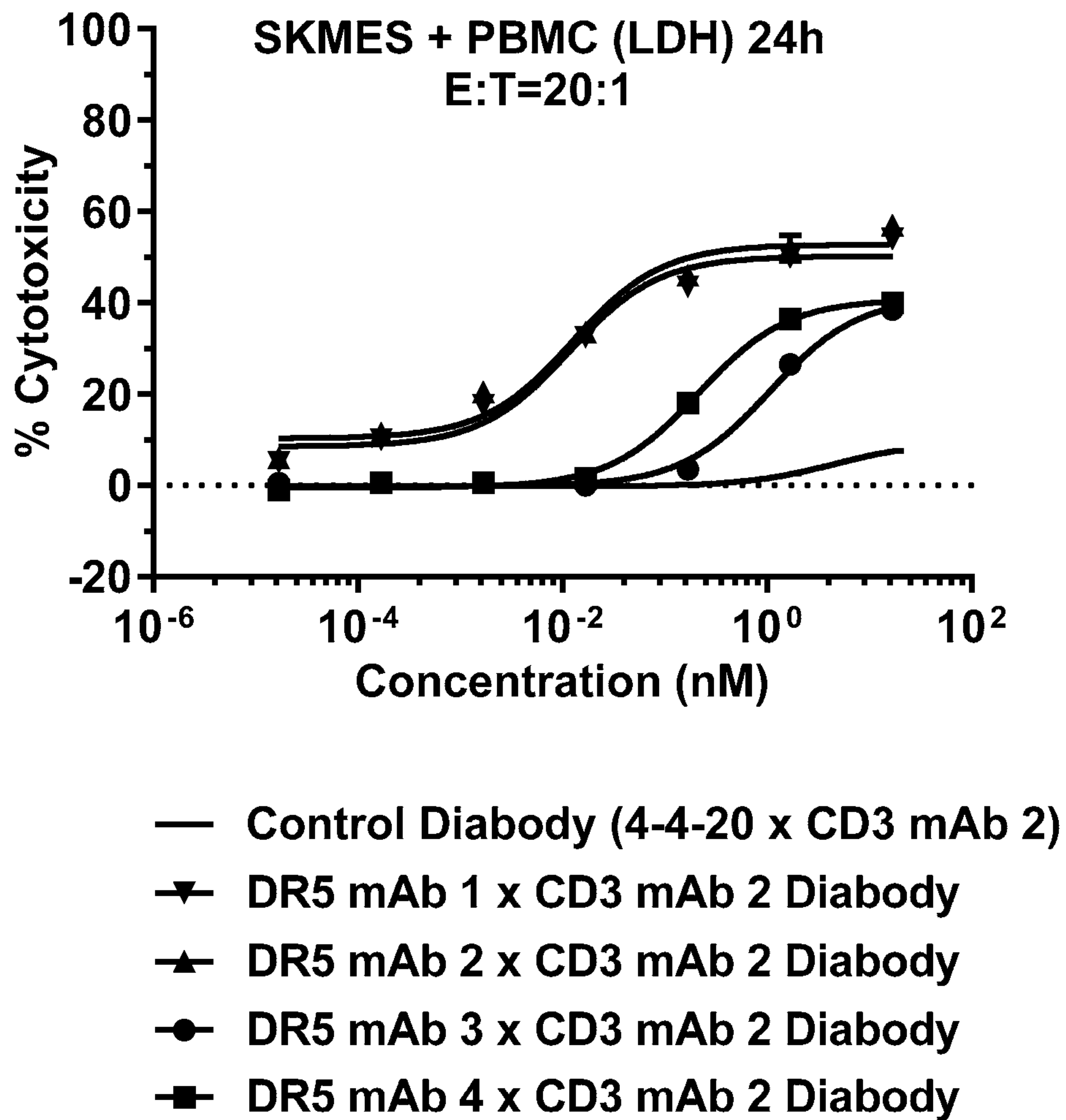
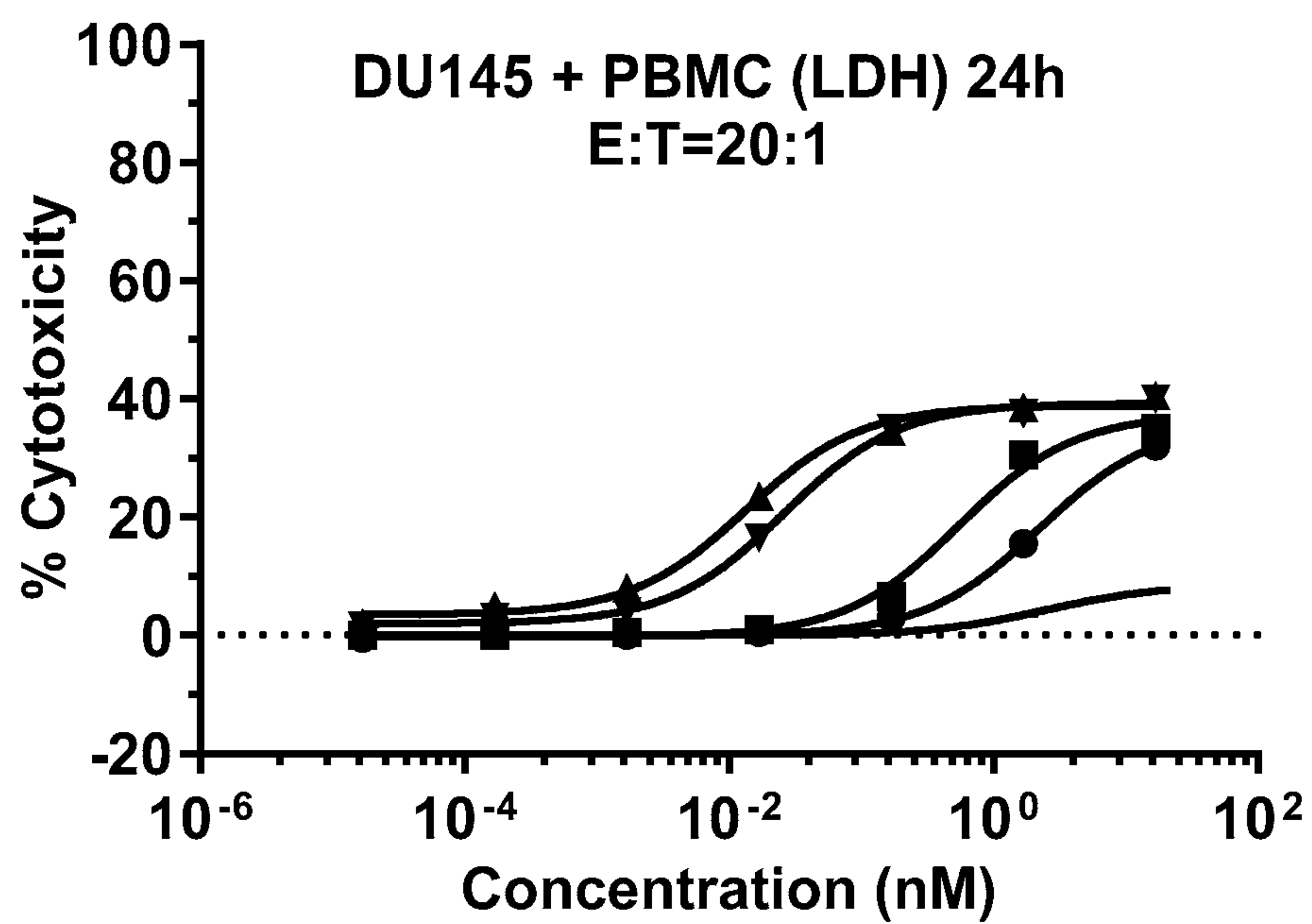


Figure 10B

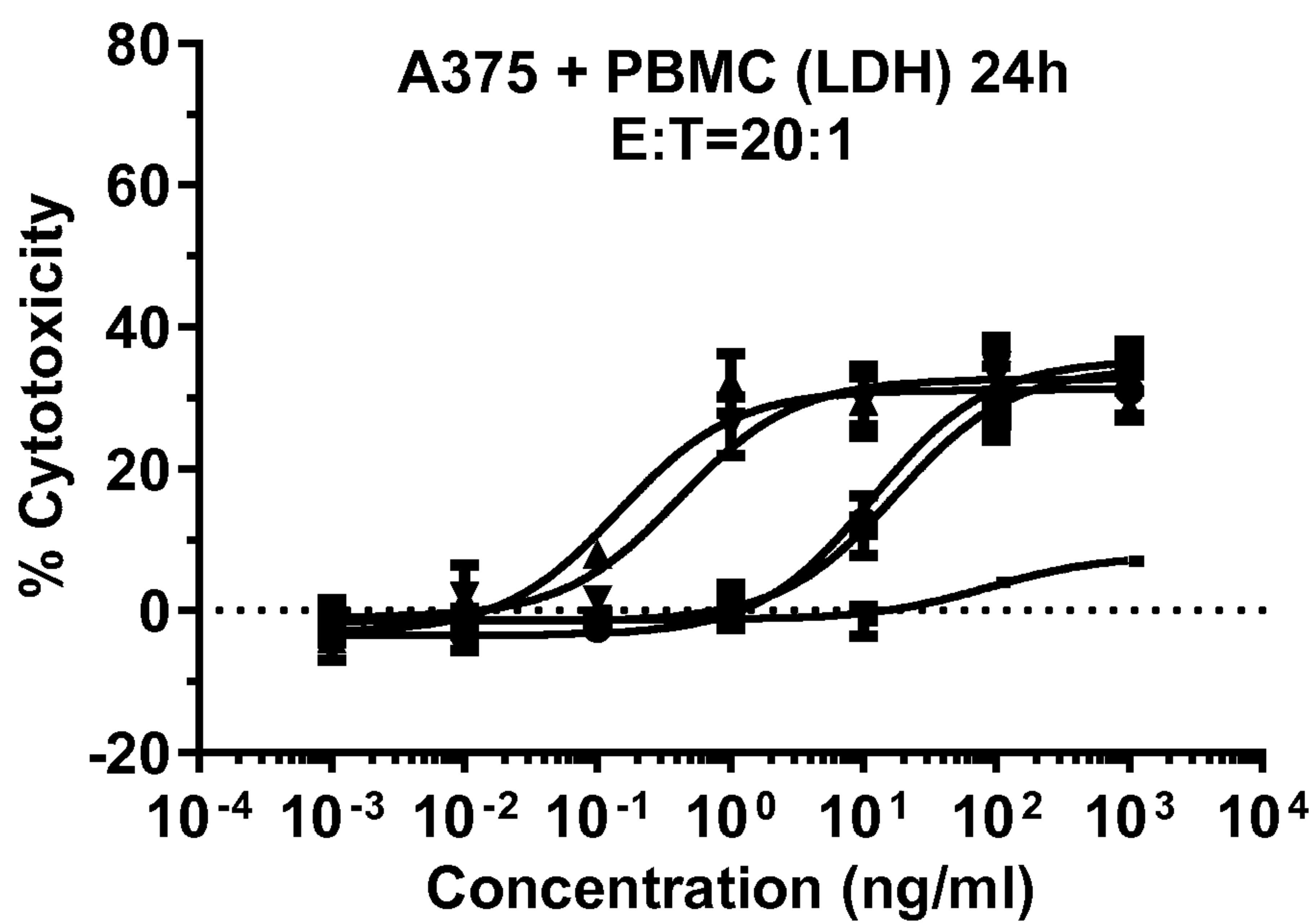
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- Control Diabody (4-4-20 x CD3 mAb 2)
- ▼— DR5 mAb 1 x CD3 mAb 2 Diabody
- ▲— DR5 mAb 2 x CD3 mAb 2 Diabody
- DR5 mAb 3 x CD3 mAb 2 Diabody
- DR5 mAb 4 x CD3 mAb 2 Diabody

Figure 10C

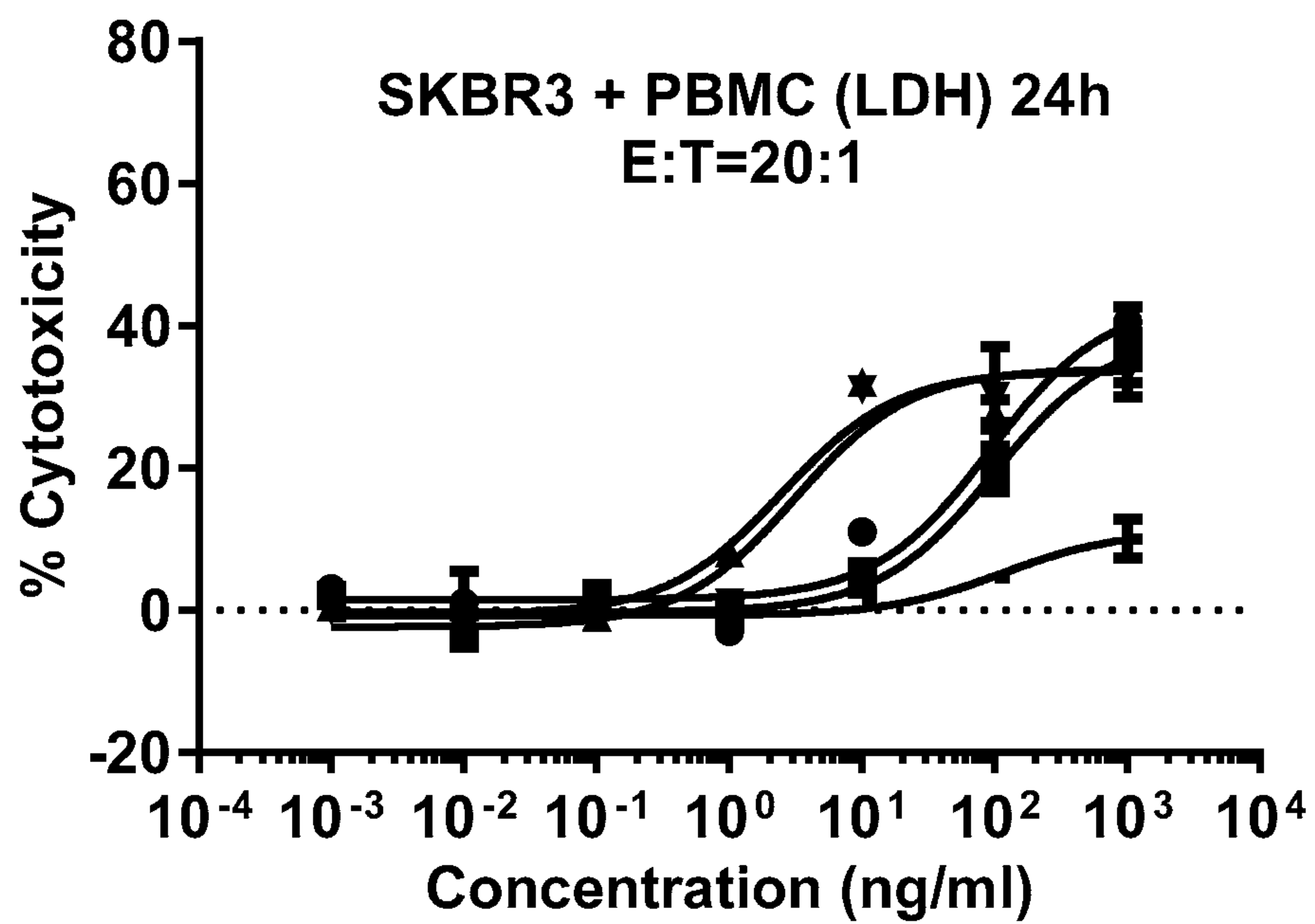
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- Control Diabody (4-4-20 x CD3 mAb 2)
- ▼— DR5 mAb 1 x CD3 mAb 2 Diabody
- ▲— DR5 mAb 2 x CD3 mAb 2 Diabody
- DR5 mAb 3 x CD3 mAb 2 Diabody
- DR5 mAb 4 x CD3 mAb 2 Diabody

Figure 10D

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- Control Diabody (4-4-20 x CD3 mAb 2)
- ▼ DR5 mAb 1 x CD3 mAb 2 Diabody
- ▲ DR5 mAb 2 x CD3 mAb 2 Diabody
- DR5 mAb 3 x CD3 mAb 2 Diabody
- DR5 mAb 4 x CD3 mAb 2 Diabody

Figure 10E

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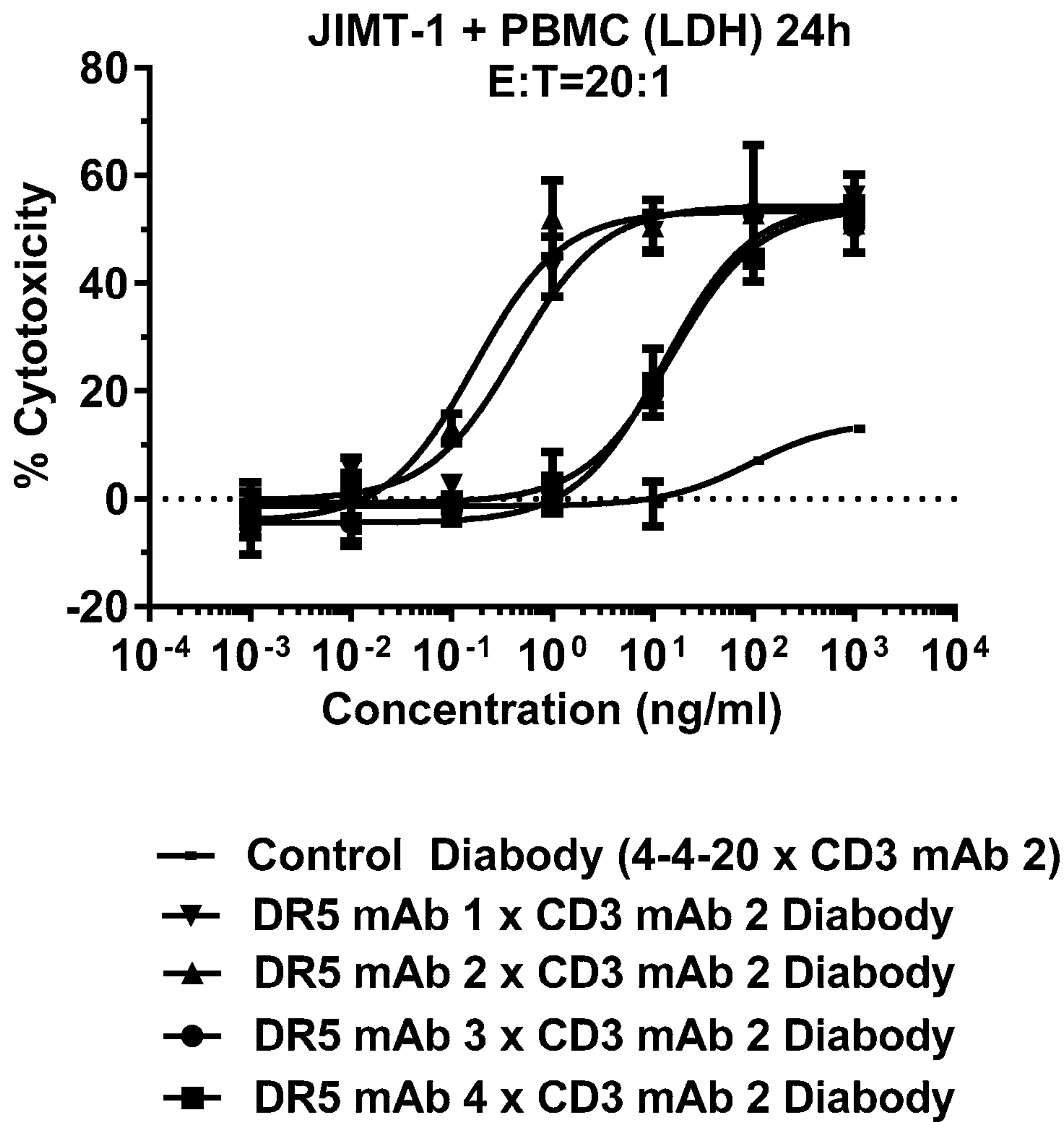
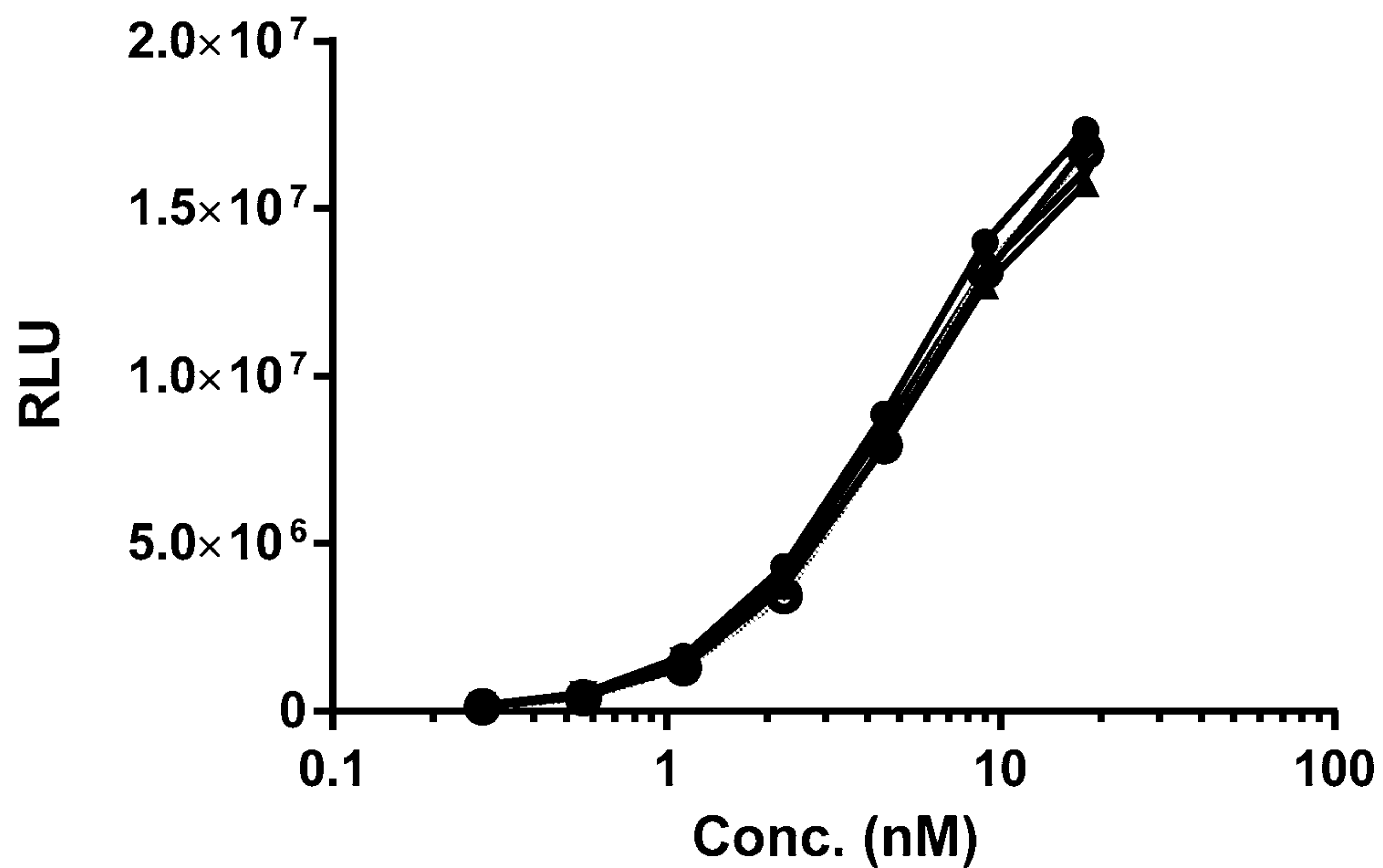


Figure 10F

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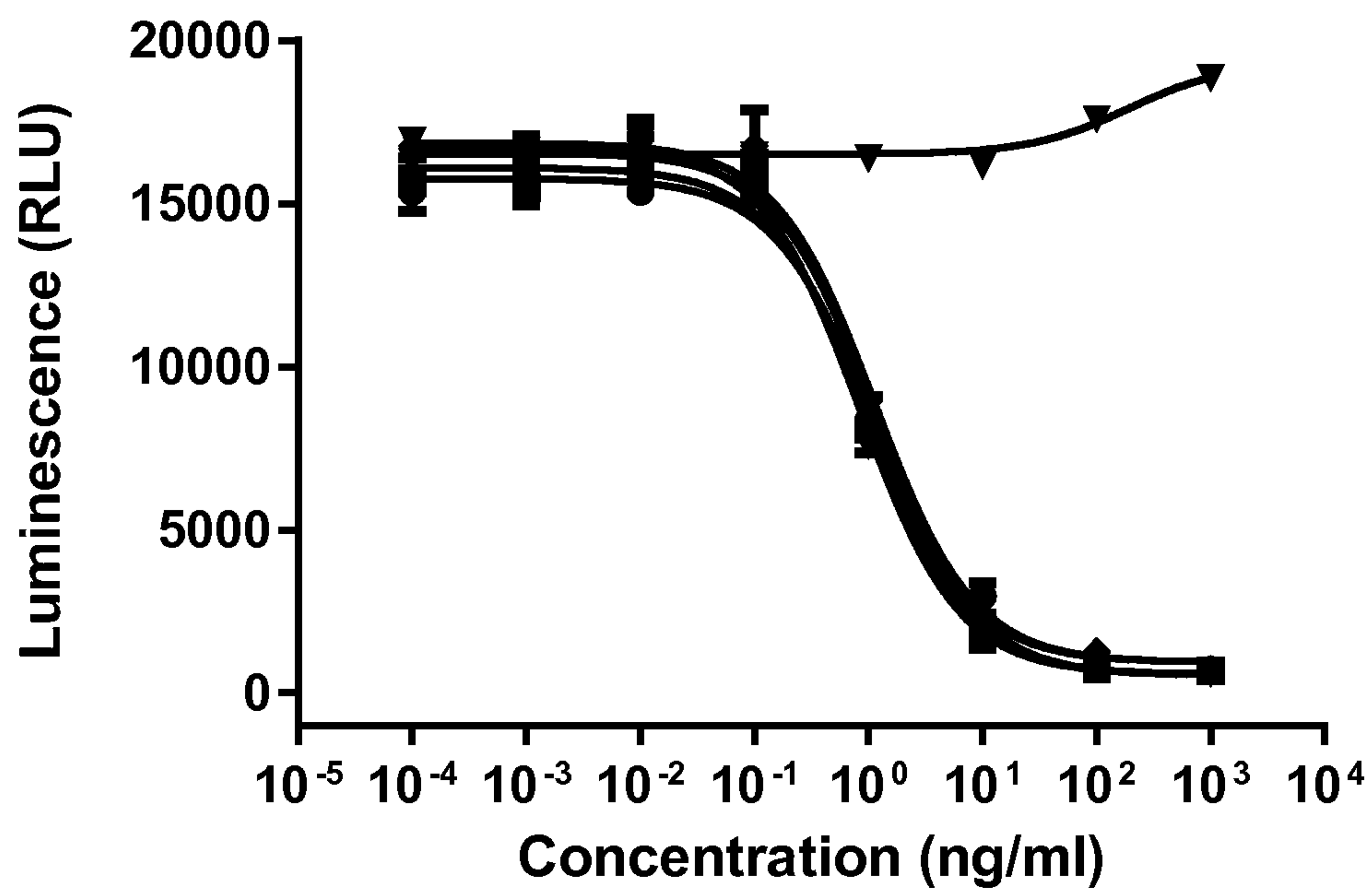
**shDR5-His (0.5 μ g/mL) Coated
and CD3-Biotin Detected**

- DR5 mAb 2 x CD3 mAb 2 Diabody
- ▲ hDR5 mAb 2 (2.2) x CD3 mAb 2 Diabody
- ◆ hDR5 mAb 2 (2.3) x CD3 mAb 2 Diabody
- hDR5 mAb 2 (2.4) x CD3 mAb 2 Diabody
- ▼ hDR5 mAb 2 (2.5) x CD3 mAb 2 Diabody

Figure 11

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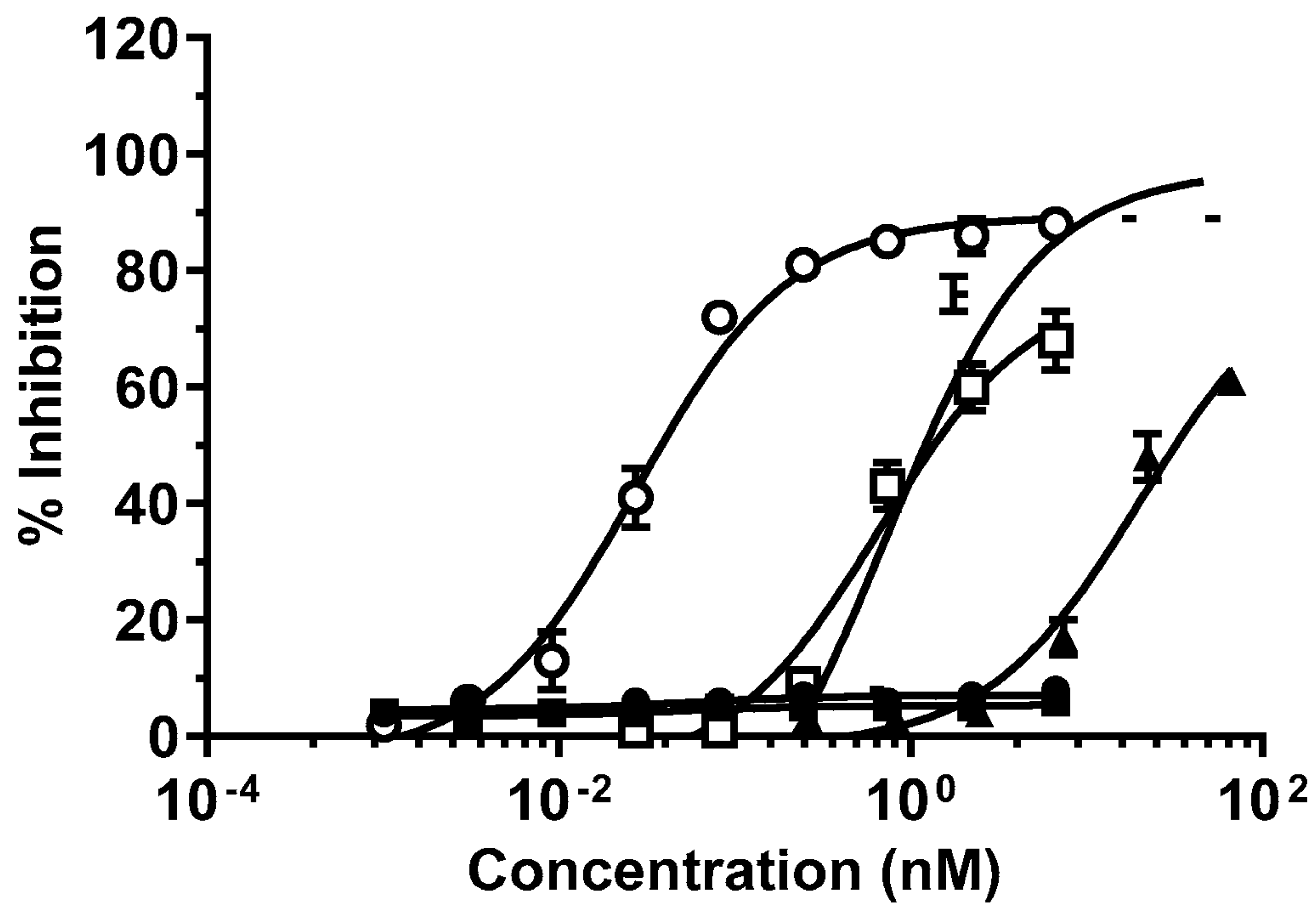
**Colo205-Luc + Pan T cells
(LUM) E:T=10:1 24h**



- DR5 mAb 2 x CD3 mAb 2 Diabody
- hDR5 mAb 2 (2.2) x CD3 mAb 2 Diabody
- ▲ hDR5 mAb 2 (2.3) x CD3 mAb 2 Diabody
- ▼ hDR5 mAb 2 (2.4) x CD3 mAb 2 Diabody
- ◆ hDR5 mAb 2 (2.5) x CD3 mAb 2 Diabody
- ▼ Control Diabody (4-4-20 x CD3 mAb 2)

Figure 12

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- DR5 mAb 1
- DR5 mAb 1 + αmFc
- DR5 mAb 2
- DR5 mAb 2 + αmFc
- ▲ DR5 mAb 1 + DR5 mAb 2
- R&D TRAIL/His

Figure 13

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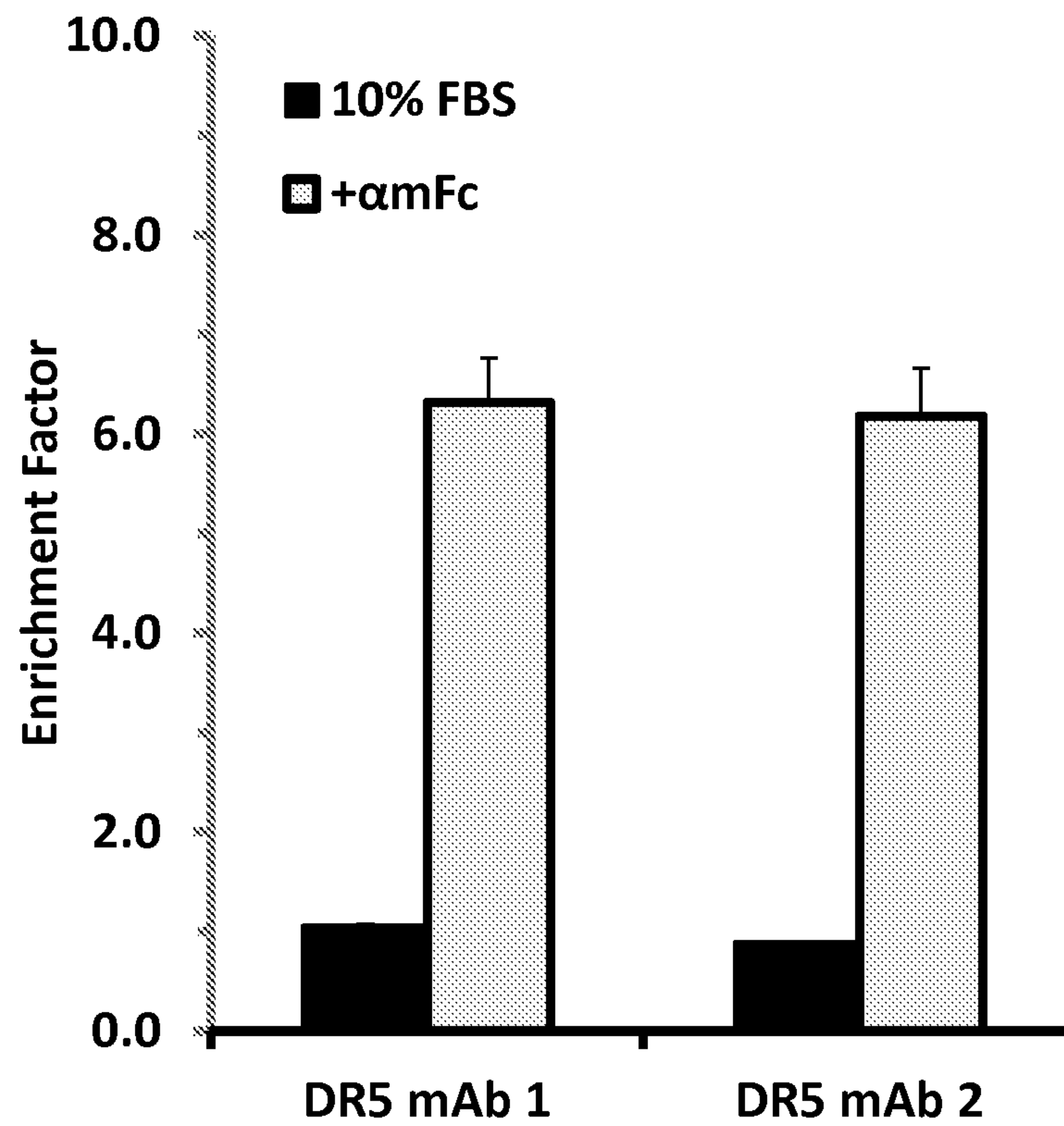


Figure 14A

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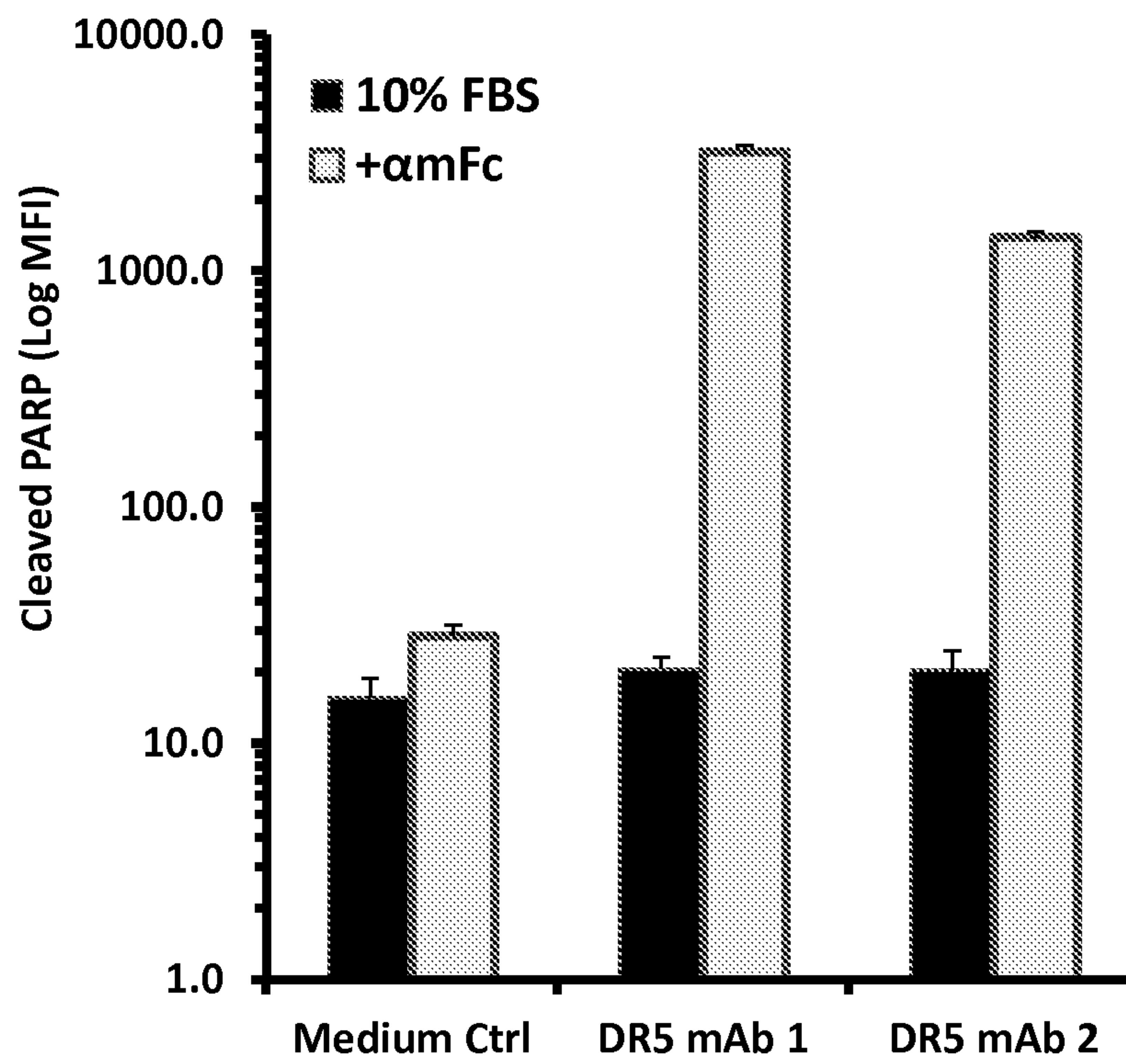


Figure 14B

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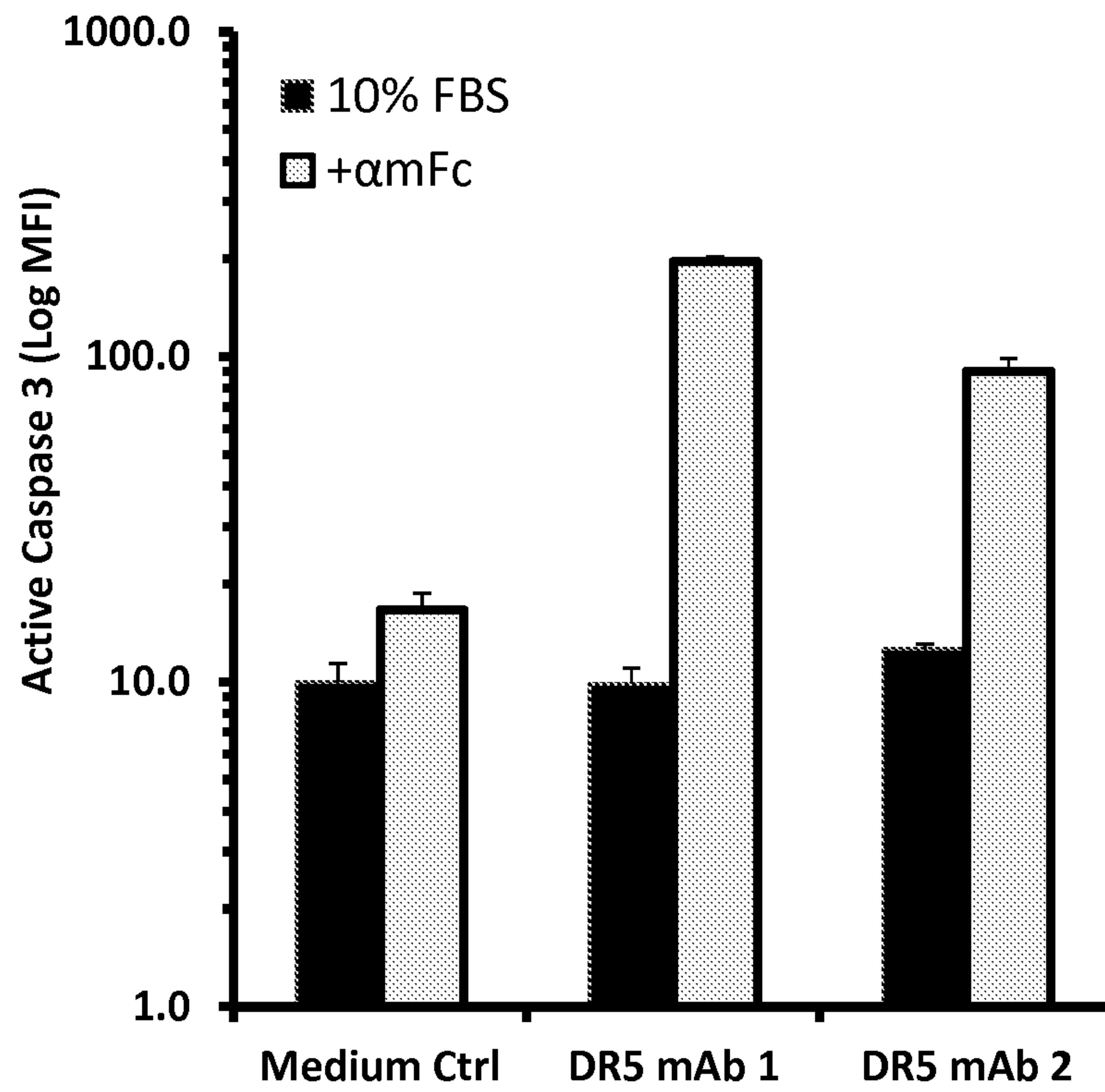


Figure 14C

Normal tissues

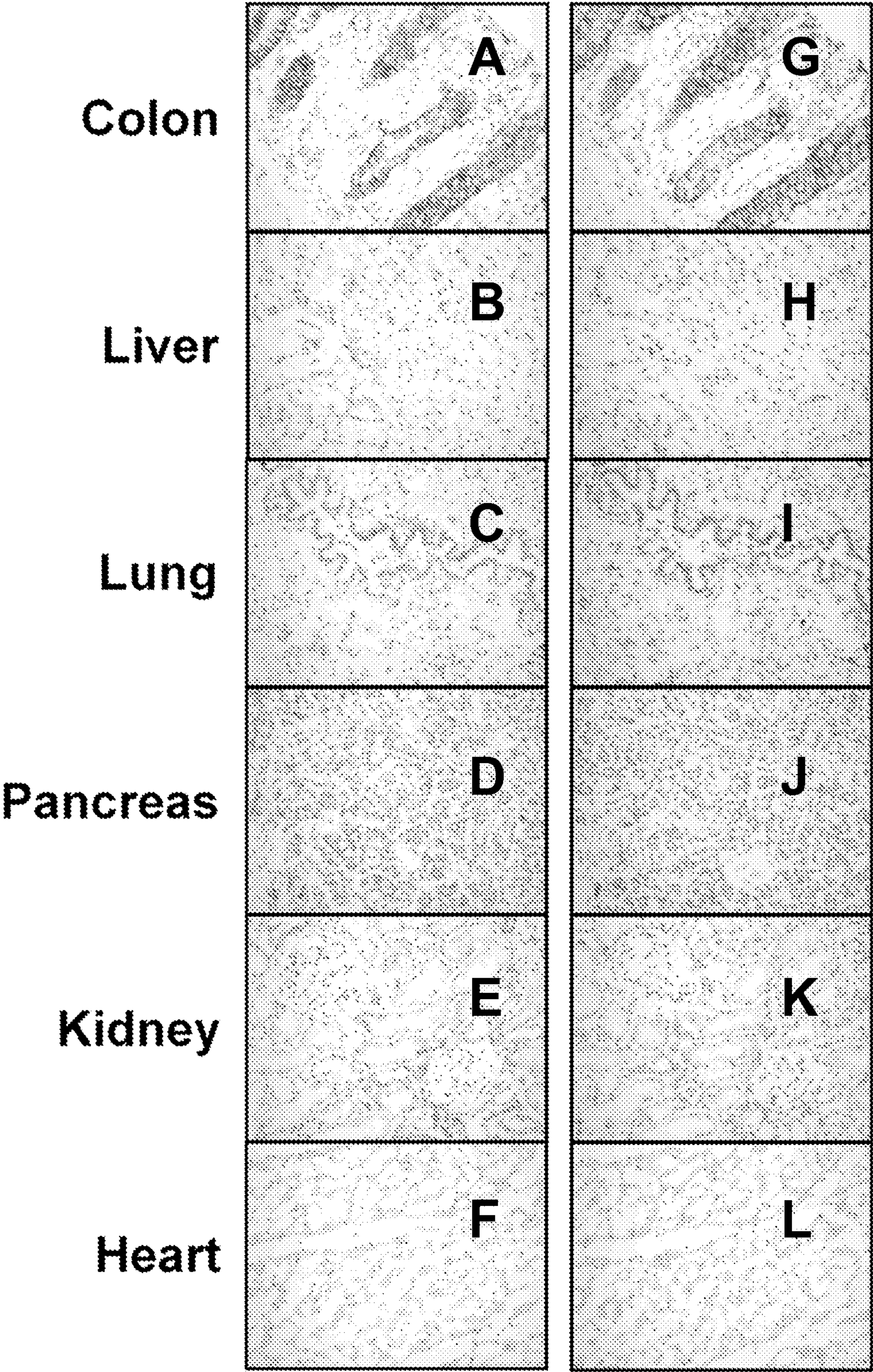


Figure 15A

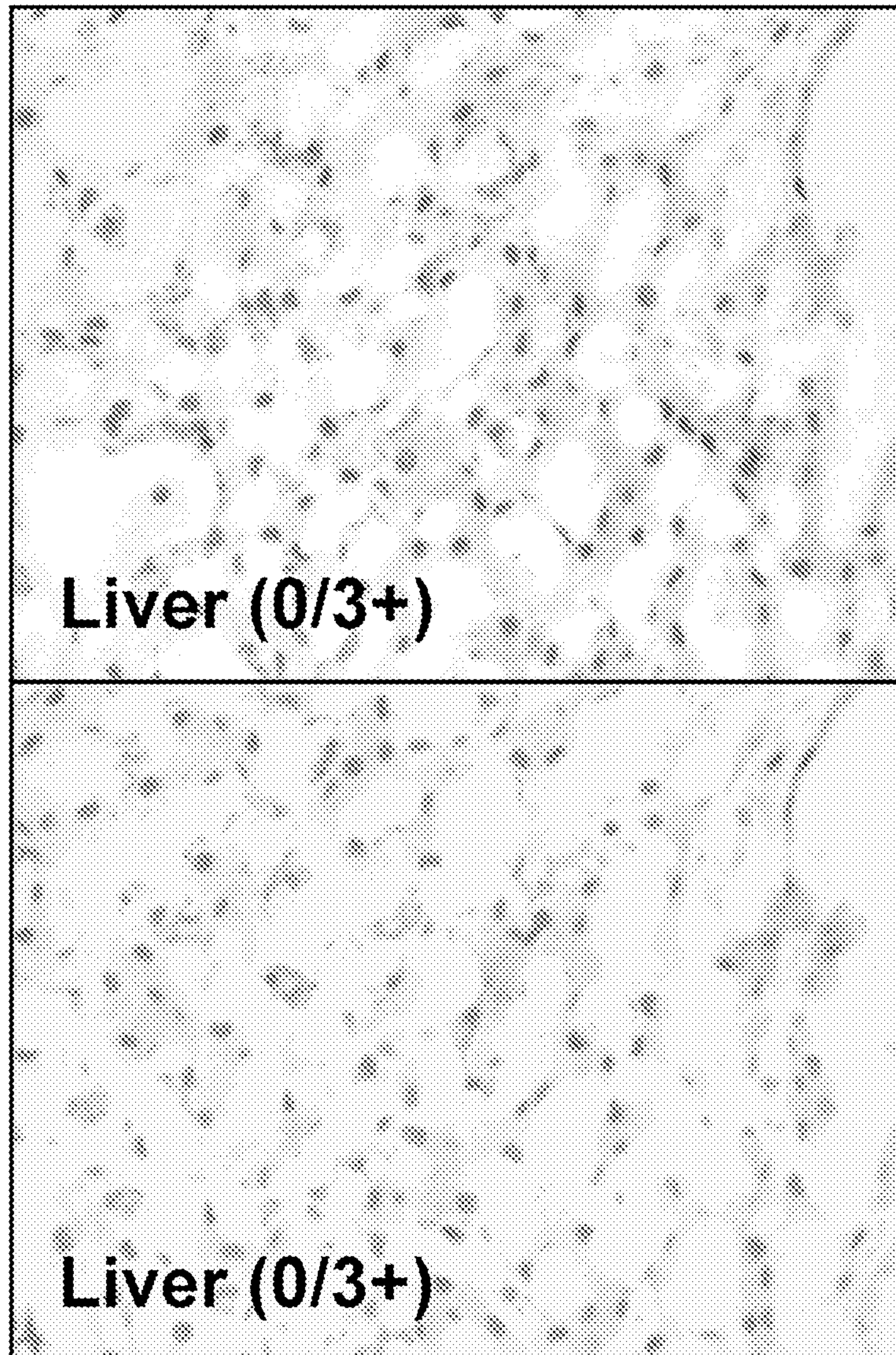


Figure 15B

Tumor tissues

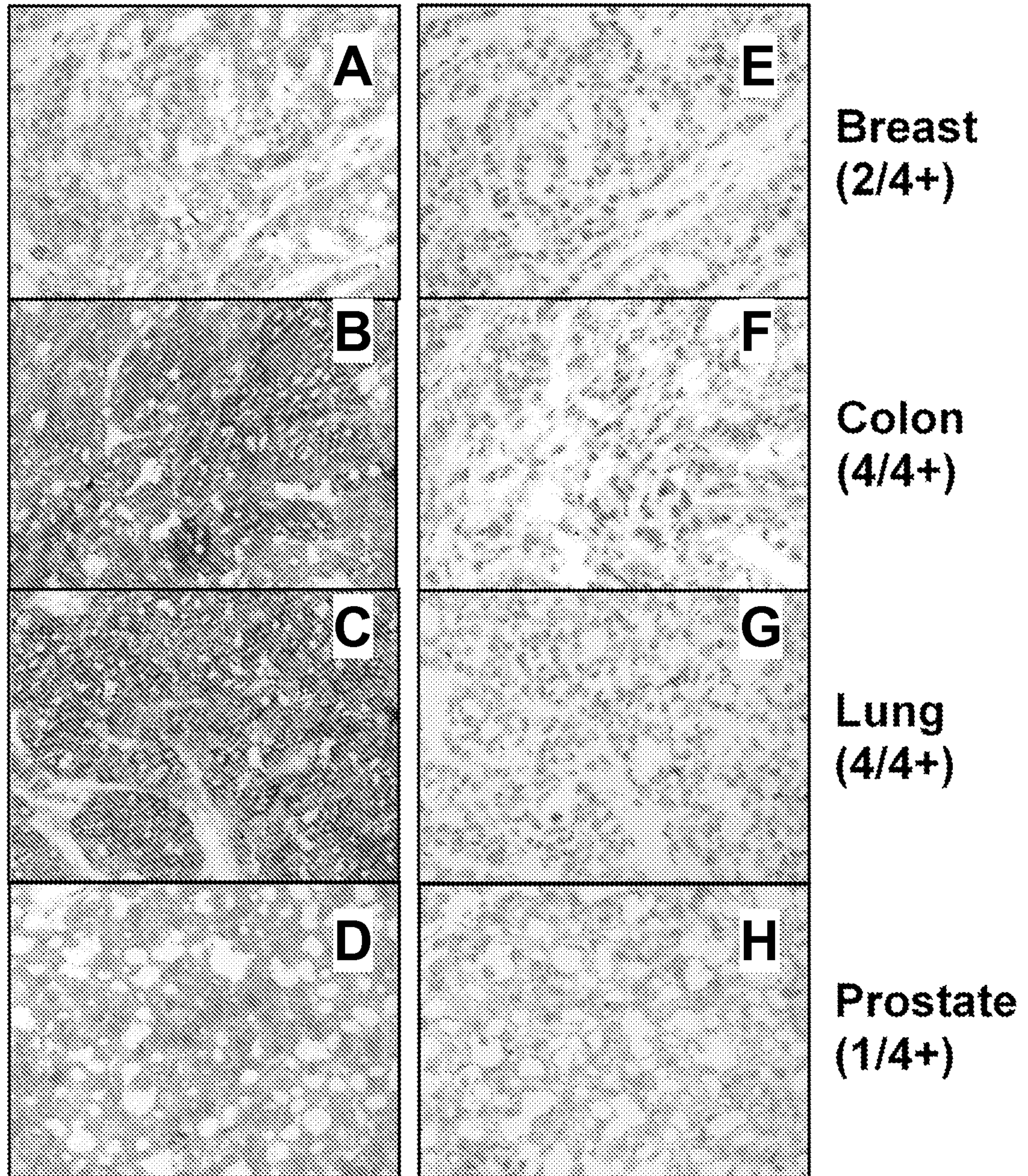


Figure 16

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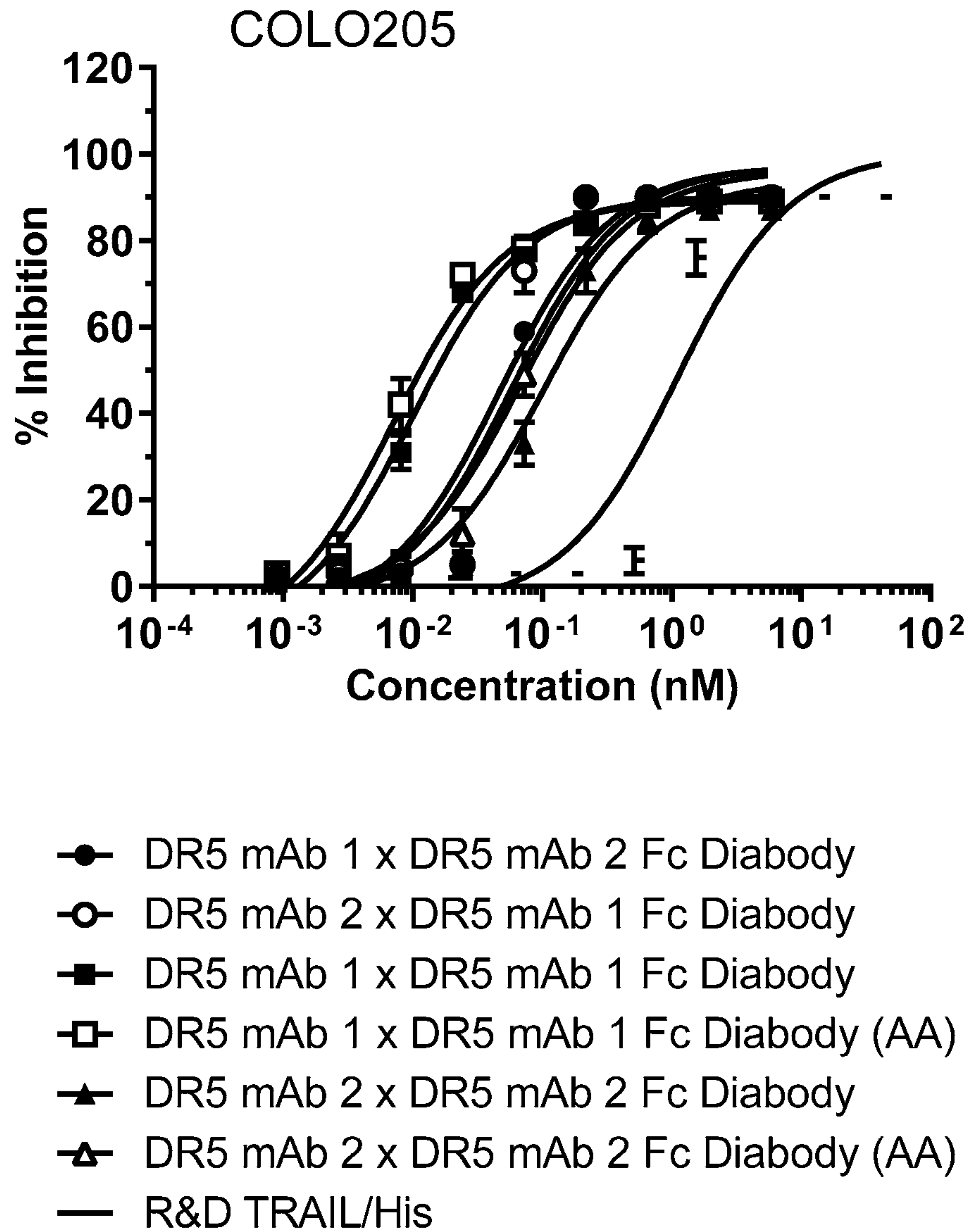


Figure 17A

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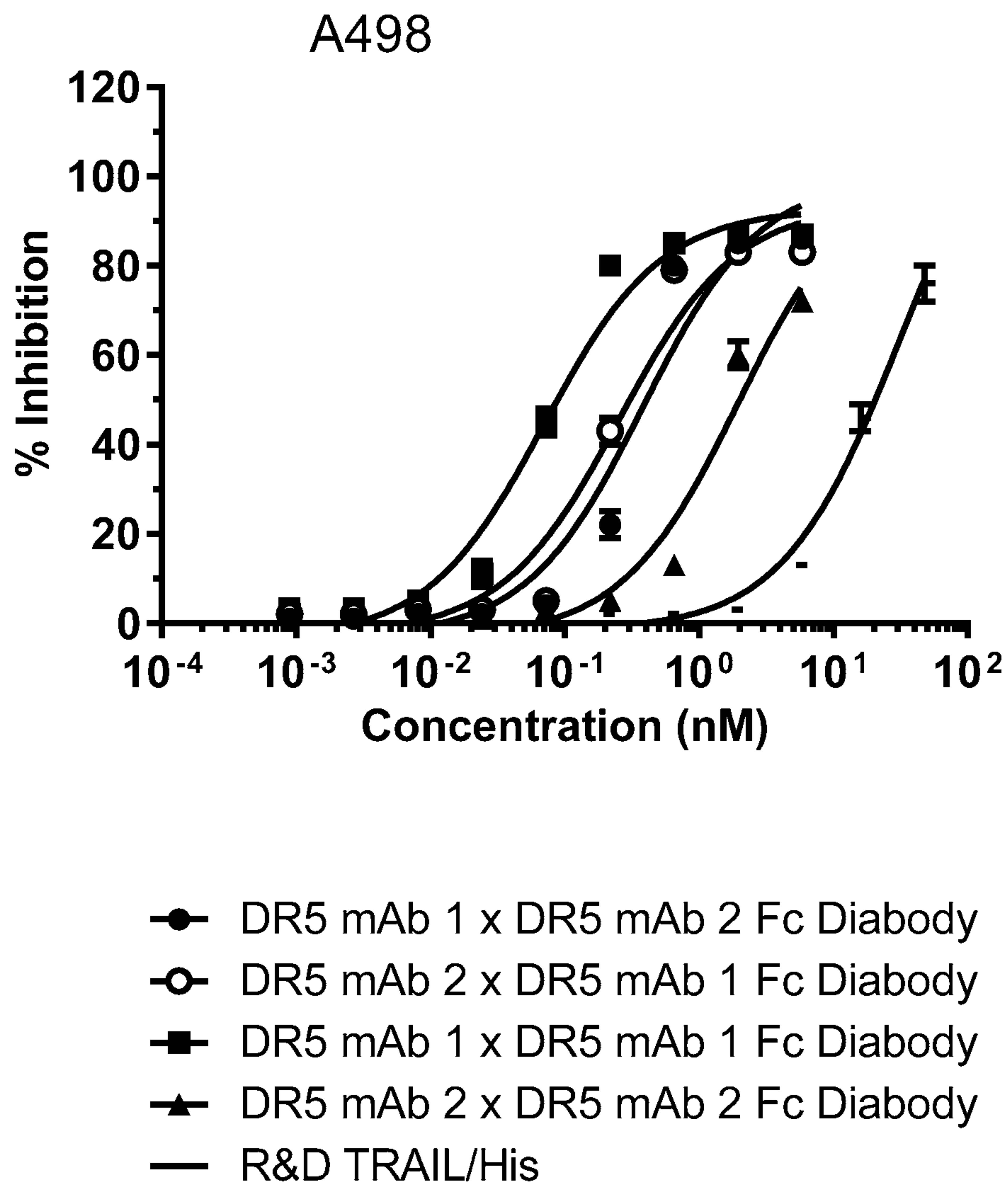


Figure 17B

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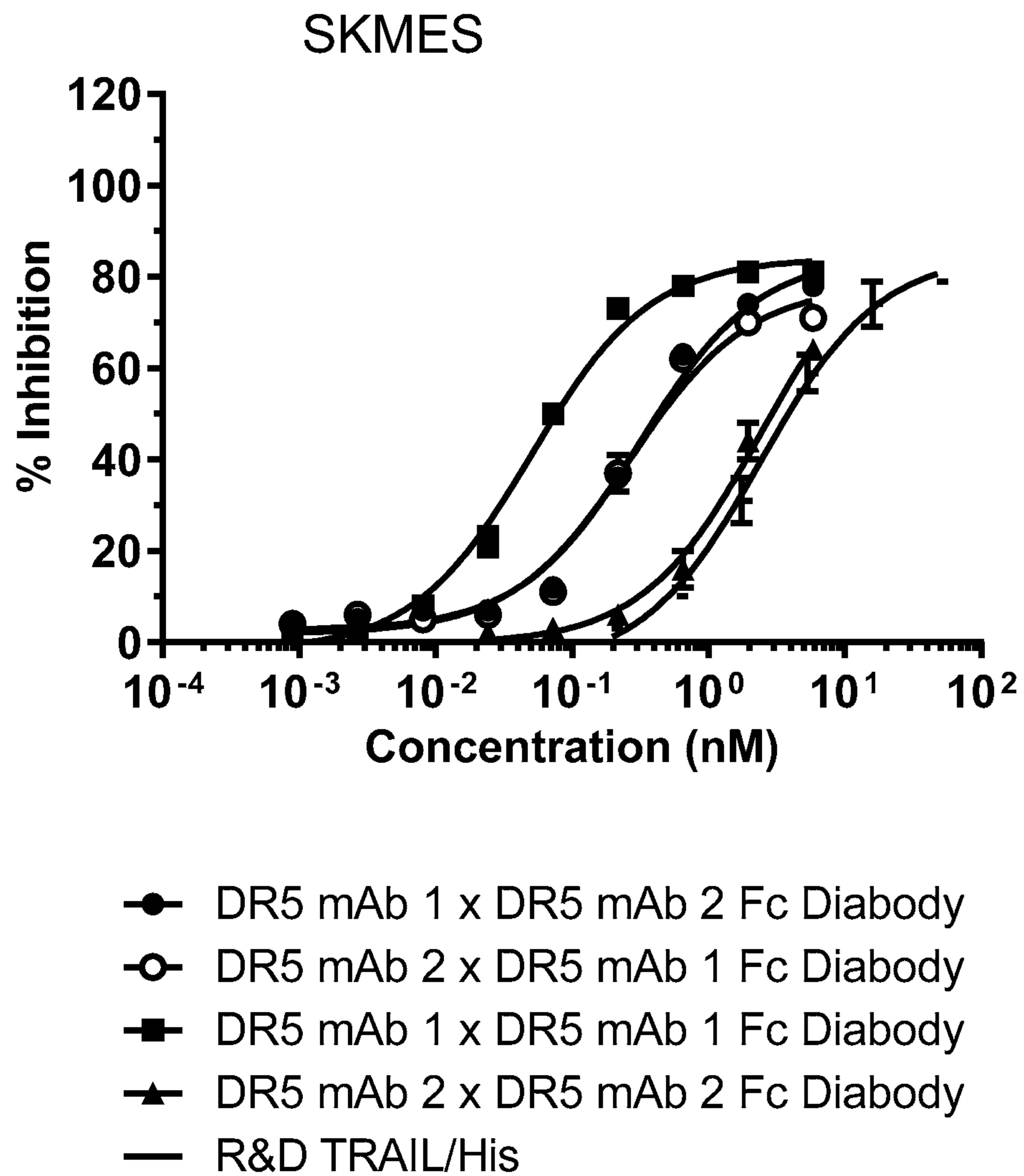


Figure 17C

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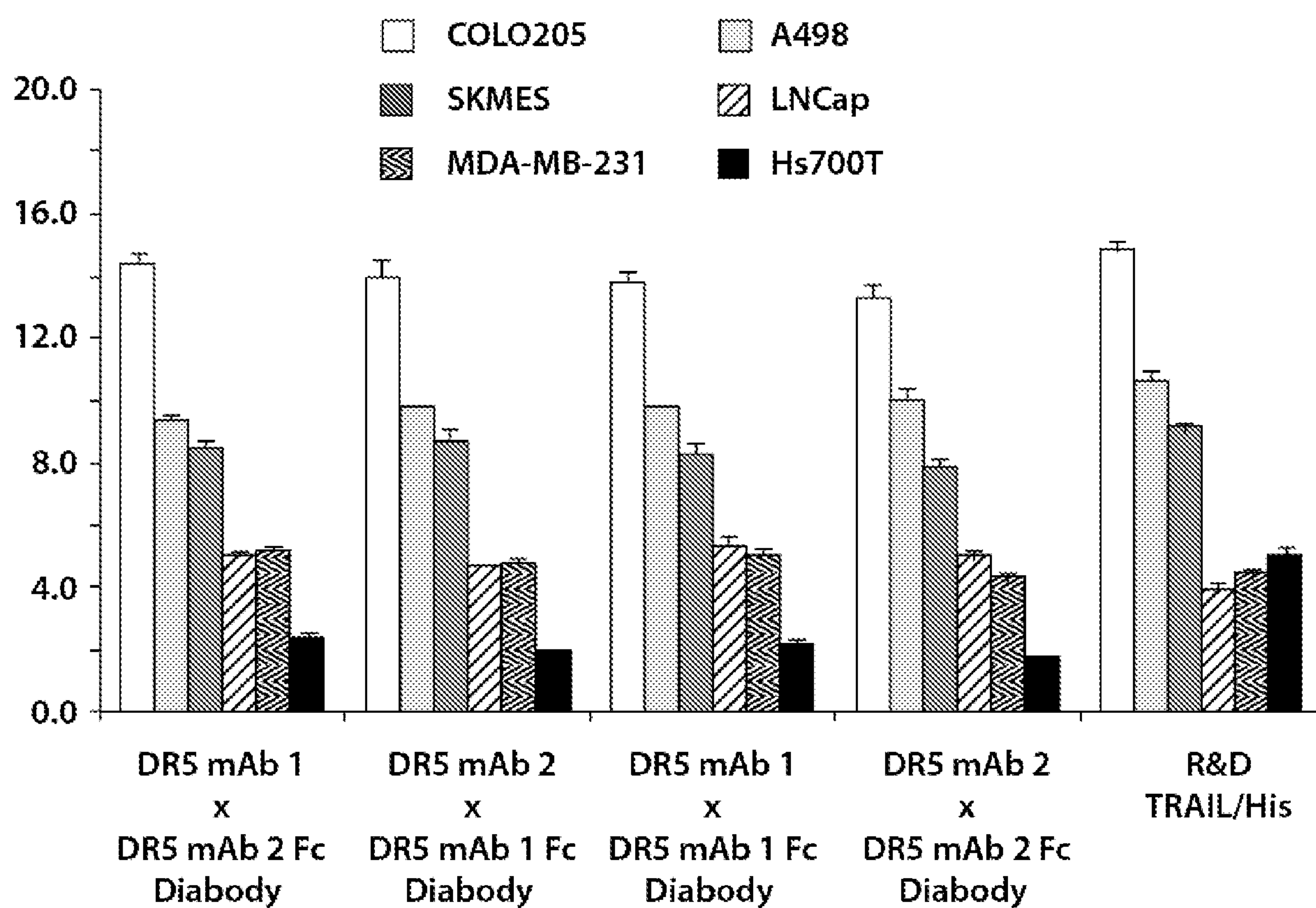
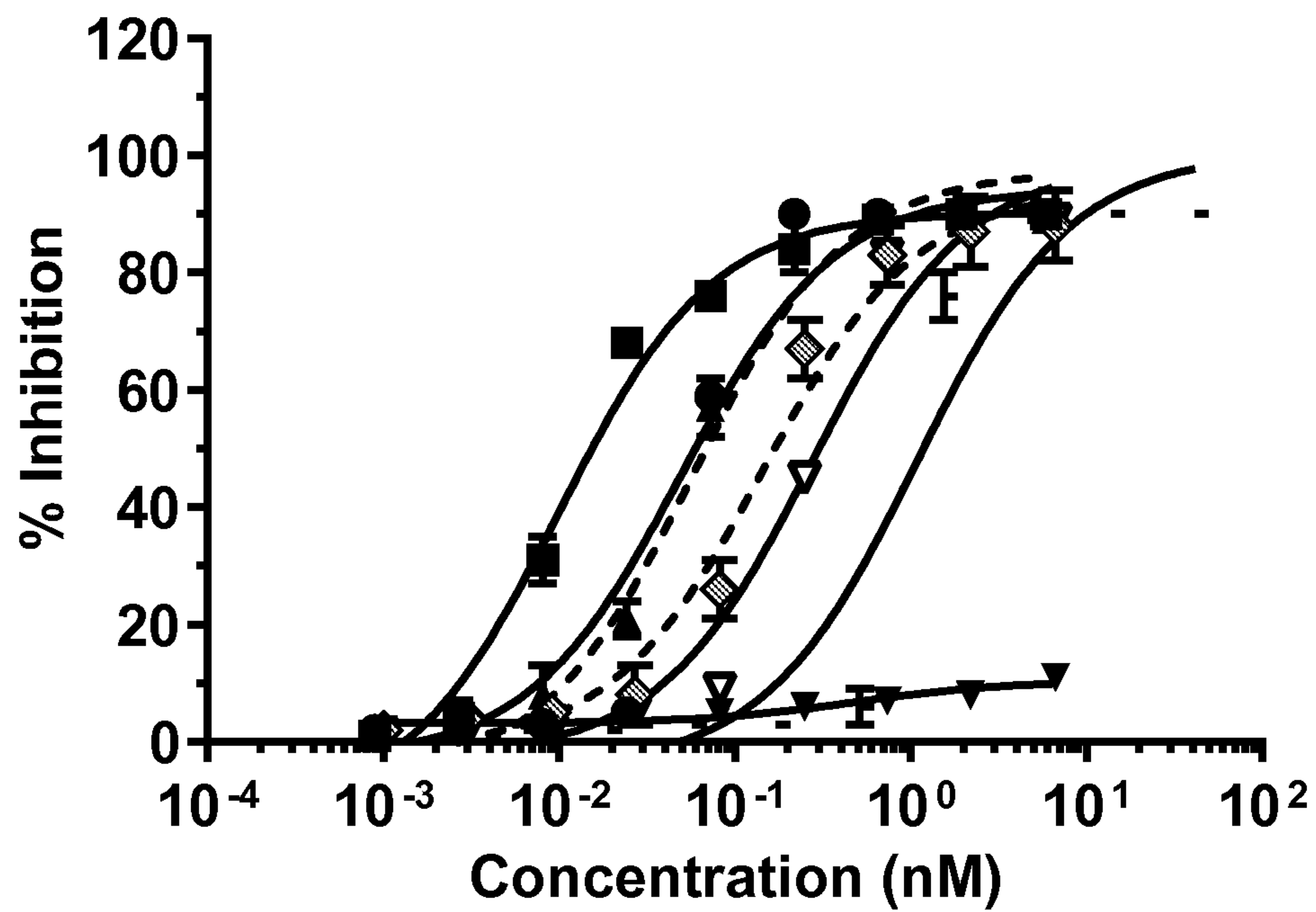


Figure 18

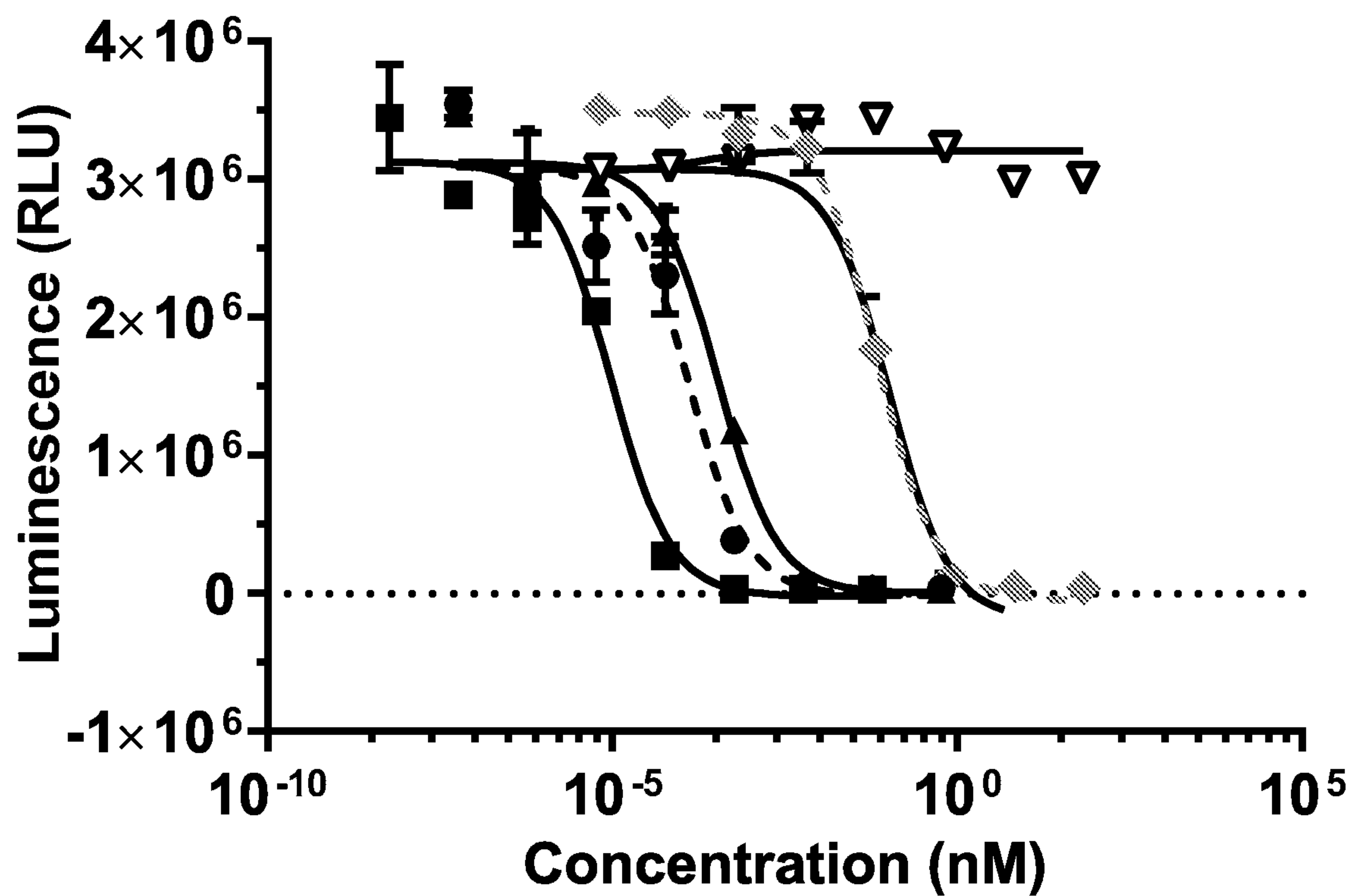
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- DR5 mAb 1 x DR5 mAb 2 Fc Diabody (AA)
- DR5 mAb 1 x DR5 mAb 1 Fc Diabody (AA)
- ▲- DR5 mAb 2 x DR5 mAb 2 Fc Diabody (AA)
- ◇- DR5 mAb 8 (AA)
- ▼- DR5 mAb 4 (AA)
- ▽- DR5 mAb 4 (AA)+ α hFc
- R&D TRAIL/His

Figure 19

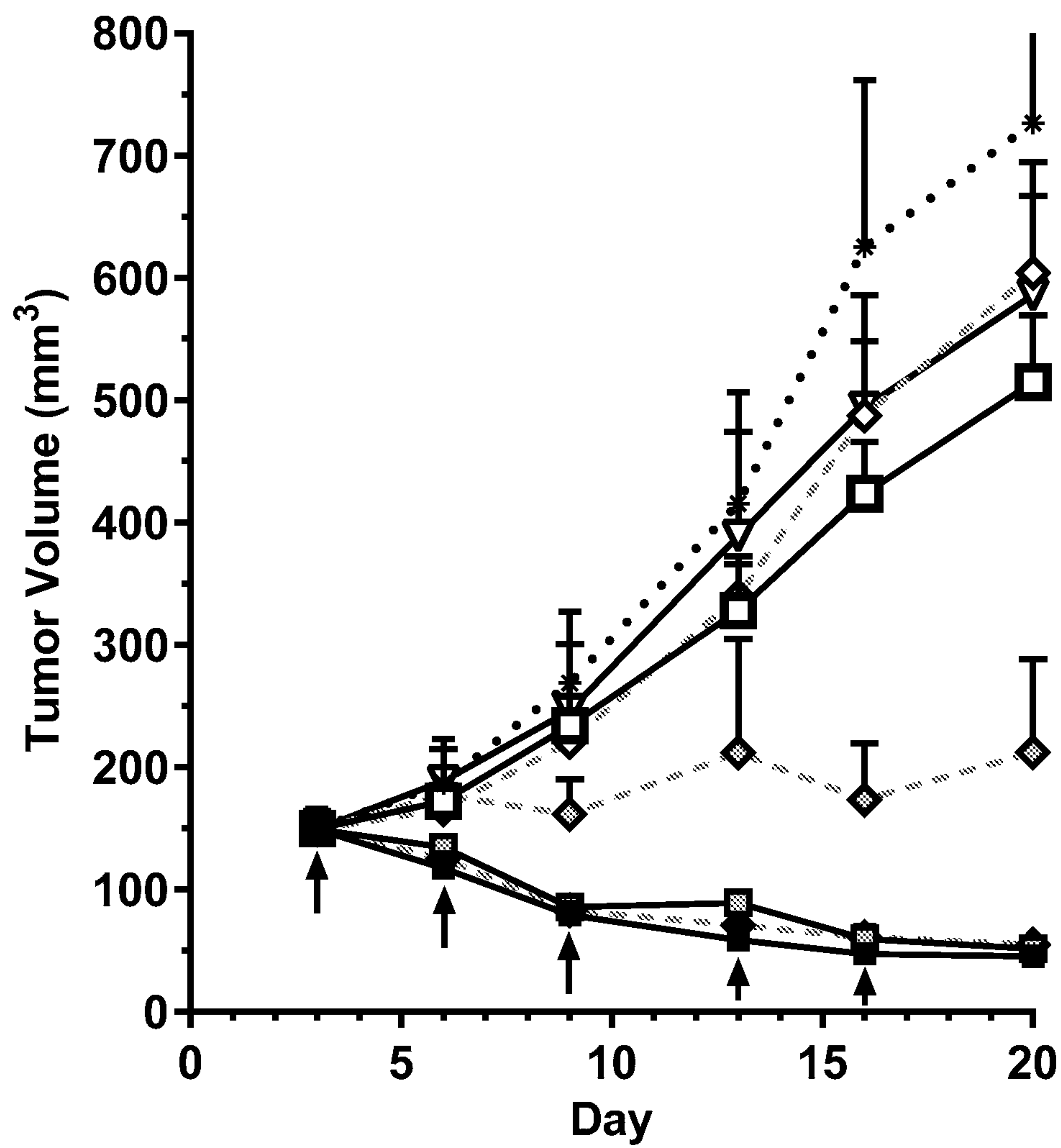
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- DR5 mAb 1 x DR5 mAb 2 Fc Diabody (AA)
- DR5 mAb 1 x DR5 mAb 1 Fc Diabody (AA)
- ▲- DR5 mAb 2 x DR5 mAb 2 Fc Diabody (AA)
- ◇- DR5 mAb 8 (AA)
- ▽- DR5 mAb 4 (AA)
- - - TRAIL/His

Figure 20

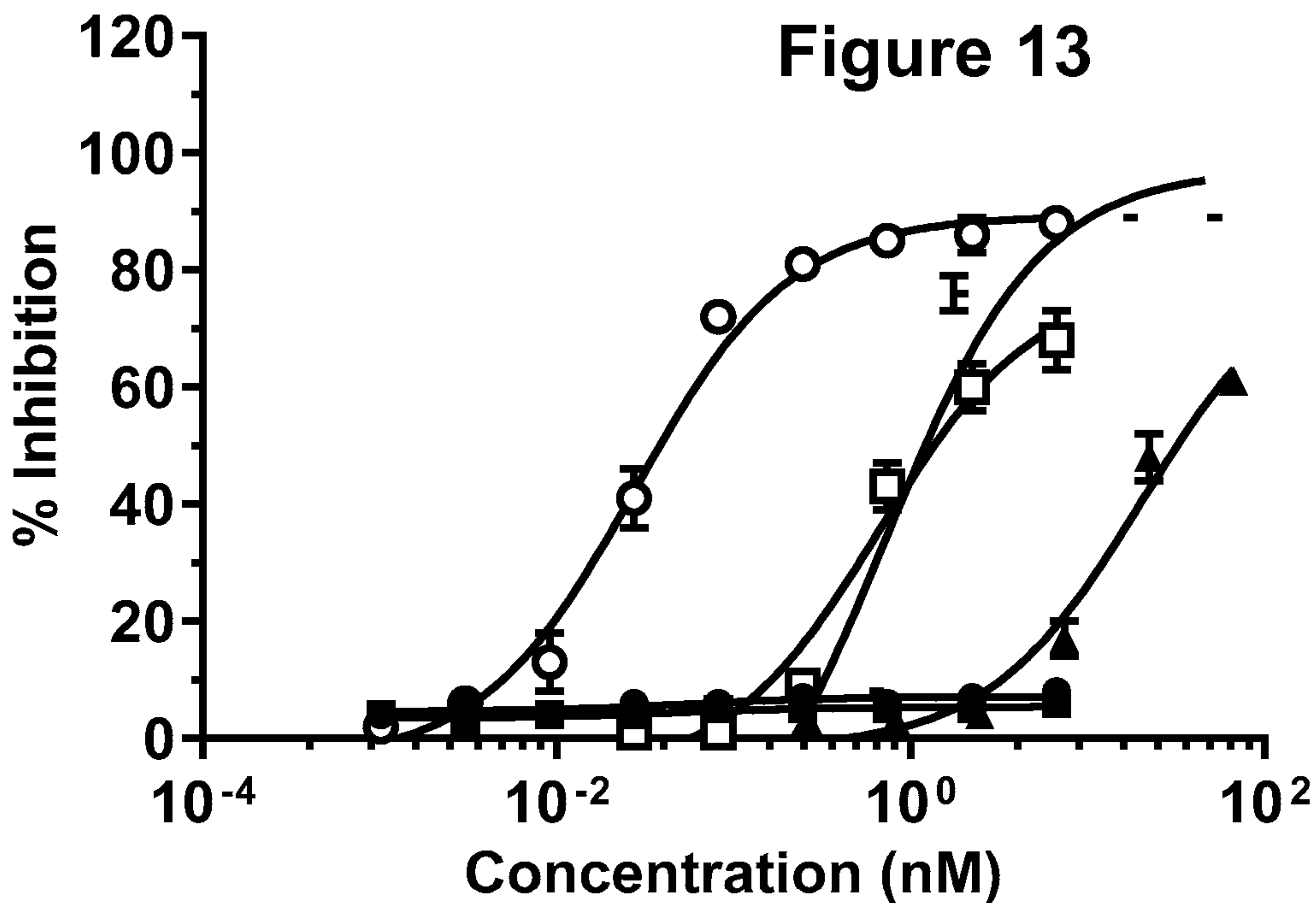
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- * • Vehicle
- DR5 mAb 1 x DR5 mAb 1 Fc Diabody (AA) (0.5mg/kg)
- ▣ DR5 mAb 1 x DR5 mAb 1 Fc Diabody (AA) (0.05mg/kg)
- DR5 mAb 1 x DR5 mAb 1 Fc Diabody (AA) (0.005mg/kg)
- ◆ DR5 mAb 8 (AA) (0.5mg/kg)
- ◈ DR5 mAb 8 (AA) (0.05mg/kg)
- ◊ DR5 mAb 8 (AA) (0.005mg/kg)
- ▽ DR5 mAb 5 (AA) (5mg/kg)

Figure 21

Figure 13



- DR5 mAb 1
- DR5 mAb 1 + α mFc
- DR5 mAb 2
- DR5 mAb 2 + α mFc
- ▲ DR5 mAb 1 + DR5 mAb 2
- R&D TRAIL/His