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(54) **SINGLE-CHAIN MULTIVALENT BINDING
PROTEINS WITH EFFECTOR FUNCTION**

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590, filed on Mar. 3, 2008, now abandoned.

(71) Applicant: **EMERGENT PRODUCT
DEVELOPMENT SEATTLE, LLC,**
SEATTLE, WA (US)

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(72) Inventors: **PETER ARMSTRONG THOMPSON,**
BELLEVUE, WA (US); **JEFFREY A.**
LEDBETTER, SHORELINE, WA (US);
MARTHA SUSAN
HAYDEN-LEDBETTER,
SHORELINE, WA (US); **WILLIAM**
BRADY, BOTHELL, WA (US)

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(73) Assignee: **EMERGENT PRODUCT
DEVELOPMENT SEATTLE, LLC,**
SEATTLE, WA (US)

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(57) **ABSTRACT**

Related U.S. Application Data

The present disclosure relates to single-chain multivalent
binding proteins, compositions comprising the single-chain
multivalent binding proteins, and methods of making and
using the single chain-multivalent binding proteins.

(63) Continuation of application No. 12/041,590, filed on
Mar. 3, 2008, now abandoned, which is a continuation-



Figure 1.
Schematic Structure of a
Multispecific Binding Protein
AKA “Scorpion”



Figure 2.
Western Blot of Non-Reduced SDS PAGE
COS supernatants, 30 ul/well

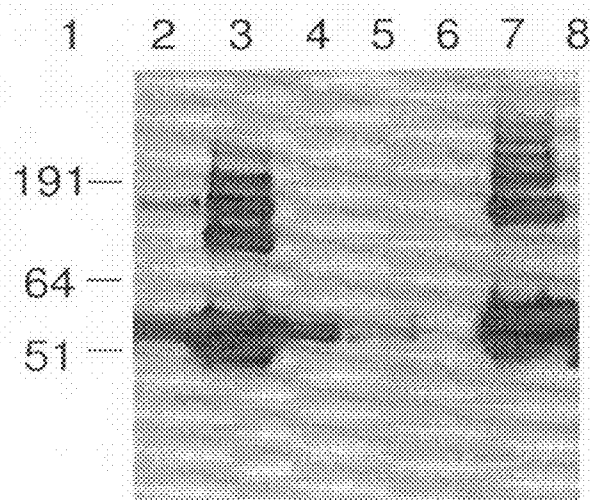


Figure 3.
Binding of Protein Expressed in COS Supernatants to
Cells Expressing Target Antigens

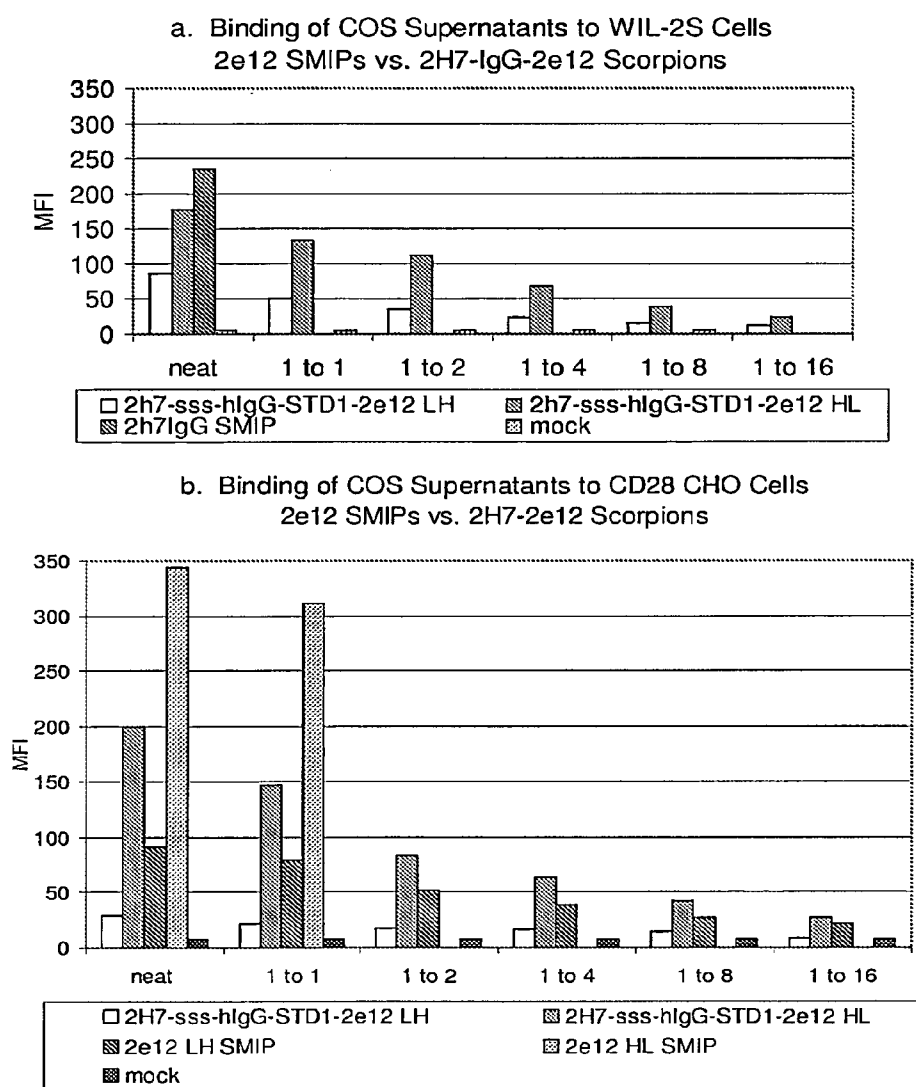


FIGURE 4

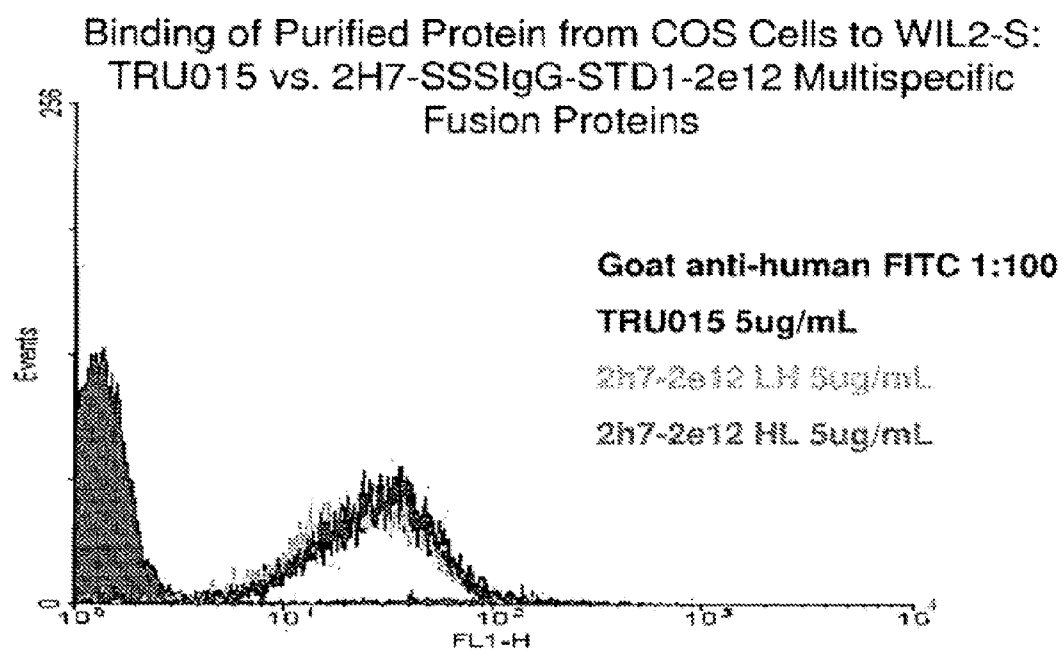


FIGURE 5

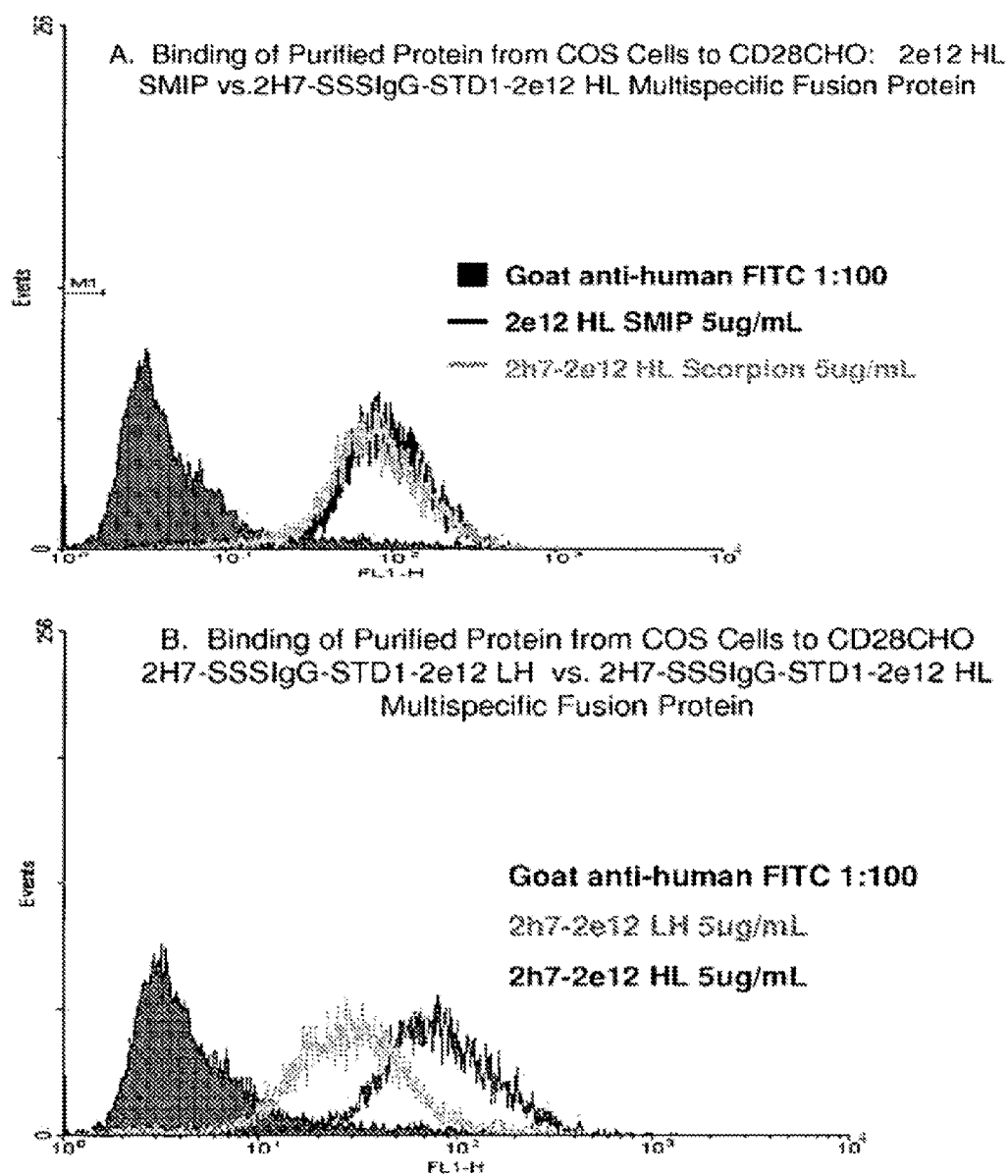


Figure 6. A

Table Identifying Functional Elements For Multispecific Fusion Proteins



BD1 Binding Domain 1	Linker 1	EFD Effector function domain	Linker 2 ID	Linker Length (aa) linker plus r. site	CH3 COOH	Linker 2 sequence (- restriction site)	Fusion Junction with BD2 (L2 RS---SVQ 2e12)	BD2 Binding Domain 2
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	STD	20	FGK	NYGGGSSGGGSSGGGSSG	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	STD2	38	FGK	NYGGGSSGGGSSGGGSSG NYGGGSSGGGSSGGGSSG	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	H1 L1=2e12 V _L V _L	2 (RS)	FGK	---	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	H2 L2=2e12 V _L V _L	8	FGK	GGGSSG	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	H3 L3=2e12 V _L V _L	10	FGK	NYGGGSSG	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	H4 L4=2e12 V _L V _L	13	FGK	GGGSSGGGGG	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	H5 L5=2e12 V _L V _L	15	FGK	NYGGGSSGGGSSG	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	H6 L6=2e12 V _L V _L	18	FGK	GGGSSGGGSSGGGSSG	NS QVQ	2e12 V _L V _L
2H7 V _L -V _L	Modified hIgG1 hinge CCC→SSS	hIgG1-CH2-CH3	H7	8	FGK	GCFFCF	NS QVQ	2e12 V _L V _L

Figure 6B (1/5)
Constructs

<i>Name</i>	<i>BD1</i>	<i>Hinge</i>	<i>EFD</i>	<i>Linker</i>	<i>BD2</i>
2H7-sss-hlgG-STD1-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-sss-hlgG-STD1-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD1	2e12 (VH-VL)
2H7-sss-hlgG-STD2-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD2	2e12 (VL-VH)
2H7-sss-hlgG-STD2-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD2	2e12 (VH-VL)
2H7-csc-hlgG-STD1-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-csc-hlgG-STD2-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD2	2e12 (VL-VH)
2H7-csc-hlgG-STD1-2e12 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-csc-hlgG-STD2-2e12 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD2	2e12 (VH-VL)
2H7-sss-hlgG-H1-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H1	2e12 (VL-VH)
2H7-csc-hlgG-H1-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H1	2e12 (VL-VH)
2H7-sss-hlgG-H1-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H1	2e12 (VH-VL)
2H7-csc-hlgG-H1-2e12 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H1	2e12 (VH-VL)
2H7-sss-hlgG-H2-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H2	2e12 (VL-VH)
2H7-csc-hlgG-H2-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H2	2e12 (VL-VH)
2H7-sss-hlgG-H2-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-csc-hlgG-H2-2e12 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-sss-hlgG-H3-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H3	2e12 (VL-VH)
2H7-csc-hlgG-H3-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H3	2e12 (VL-VH)
2H7-sss-hlgG-H3-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H3	2e12 (VH-VL)
2H7-csc-hlgG-H3-2e12 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H3	2e12 (VH-VL)
2H7-sss-hlgG-H4-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H4	2e12 (VL-VH)
2H7-csc-hlgG-H4-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H4	2e12 (VL-VH)
2H7-sss-hlgG-H4-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H4	2e12 (VH-VL)
2H7-csc-hlgG-H4-2e12 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H4	2e12 (VH-VL)

Figure 6B (2/5)

<i>Name</i>	<i>BD1</i>	<i>Hinge</i>	<i>EFD</i>	<i>Linker</i>	<i>BD2</i>
2H7-sss-hlgG-H5-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H5	2e12 (VL-VH)
2H7-csc-hlgG-H5-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H5	2e12 (VL-VH)
2H7-sss-hlgG-H5-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H5	2e12 (VH-VL)
2H7-csc-hlgG-H5-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H5	2e12 (VH-VL)
2H7-sss-hlgG-H6-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H6	2e12 (VL-VH)
2H7-csc-hlgG-H6-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H6	2e12 (VL-VH)
2H7-sss-hlgG-H6-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H6	2e12 (VH-VL)
2H7-csc-hlgG-H6-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H6	2e12 (VH-VL)
2H7-sss-hlgG-H7-2e12 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H7	2e12 (VL-VH)
2H7-csc-hlgG-H7-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H7	2e12 (VL-VH)
2H7-sss-hlgG-H7-2e12 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H7	2e12 (VH-VL)
2H7-csc-hlgG-H7-2e12 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H7	2e12 (VH-VL)
2H7-sss-hlgG-STD1-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-sss-hlgG-STD1-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD1	G28-1 (VH-VL)
2H7-sss-hlgG-STD2-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD2	G28-1 (VL-VH)
2H7-sss-hlgG-STD2-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-csc-hlgG-STD1-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-csc-hlgG-STD2-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-csc-hlgG-STD1-G28-1 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-csc-hlgG-STD2-G28-1 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-sss-hlgG-H1-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H1	G28-1 (VL-VH)
2H7-csc-hlgG-H1-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H1	G28-1 (VH-VL)
2H7-sss-hlgG-H1-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H1	G28-1 (VL-VH)
2H7-csc-hlgG-H1-G28-1 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H1	G28-1 (VH-VL)
2H7-sss-hlgG-H2-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H2	G28-1 (VL-VH)

Figure 6B (3/5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-csc-hlgG-H2-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H2	G28-1 (VH-VL)
2H7-sss-hlgG-H2-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H2	G28-1 (VL-VH)
2H7-csc-hlgG-H2-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H2	G28-1 (VH-VL)
2H7-sss-hlgG-H3-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H3	G28-1 (VL-VH)
2H7-csc-hlgG-H3-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H3	G28-1 (VH-VL)
2H7-sss-hlgG-H3-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H3	G28-1 (VL-VH)
2H7-csc-hlgG-H3-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H3	G28-1 (VH-VL)
2H7-sss-hlgG-H4-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H4	G28-1 (VL-VH)
2H7-csc-hlgG-H4-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H4	G28-1 (VH-VL)
2H7-sss-hlgG-H4-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H4	G28-1 (VL-VH)
2H7-csc-hlgG-H4-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H4	G28-1 (VH-VL)
2H7-sss-hlgG-H5-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H5	G28-1 (VL-VH)
2H7-csc-hlgG-H5-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hlgG-H5-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H5	G28-1 (VL-VH)
2H7-csc-hlgG-H5-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hlgG-H6-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H6	G28-1 (VL-VH)
2H7-csc-hlgG-H6-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H6	G28-1 (VH-VL)
2H7-sss-hlgG-H6-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H6	G28-1 (VL-VH)
2H7-csc-hlgG-H6-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H6	G28-1 (VH-VL)
2H7-sss-hlgG-H7-G28-1 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H7	G28-1 (VL-VH)
2H7-csc-hlgG-H7-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H7	G28-1 (VH-VL)
2H7-sss-hlgG-H7-G28-1 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H7	G28-1 (VL-VH)
2H7-csc-hlgG-H7-G28-1 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H7	G28-1 (VH-VL)
2H7-sss-hlgG-STD1-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-sss-hlgG-STD1-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD1	G19-4 (VH-VL)

Figure 6B (4/5)

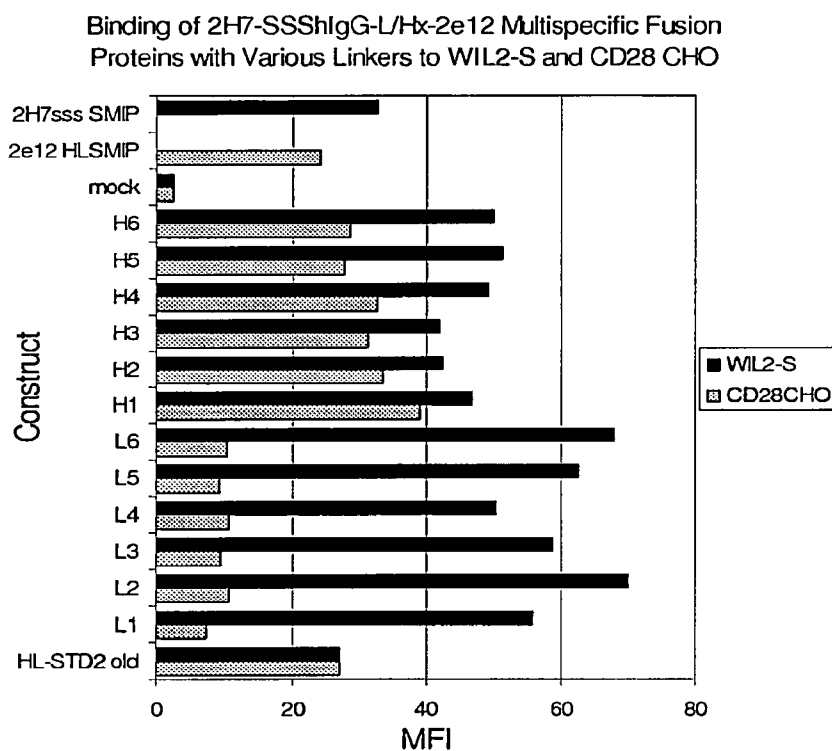
Name	BD1	Hinge	EFD	Linker	BD2
2H7-sss-hlgG-STD2-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD2	G19-4 (VL-VH)
2H7-sss-hlgG-STD2-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-csc-hlgG-STD1-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-csc-hlgG-STD2-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-csc-hlgG-STD1-G19-4 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-csc-hlgG-STD2-G19-4 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-sss-hlgG-H1-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H1	G19-4 (VL-VH)
2H7-csc-hlgG-H1-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H1	G19-4 (VH-VL)
2H7-sss-hlgG-H1-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H1	G19-4 (VL-VH)
2H7-csc-hlgG-H1-G19-4 HL	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H1	G19-4 (VH-VL)
2H7-sss-hlgG-H2-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-csc-hlgG-H2-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H2	G19-4 (VH-VL)
2H7-sss-hlgG-H2-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-csc-hlgG-H2-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H2	G19-4 (VH-VL)
2H7-sss-hlgG-H3-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H3	G19-4 (VL-VH)
2H7-csc-hlgG-H3-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H3	G19-4 (VH-VL)
2H7-sss-hlgG-H3-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H3	G19-4 (VL-VH)
2H7-csc-hlgG-H3-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H3	G19-4 (VH-VL)
2H7-sss-hlgG-H4-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)		G19-4 (VL-VH)
2H7-csc-hlgG-H4-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H4	G19-4 (VH-VL)
2H7-sss-hlgG-H4-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H4	G19-4 (VL-VH)
2H7-csc-hlgG-H4-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H4	G19-4 (VH-VL)
2H7-sss-hlgG-H5-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H5	G19-4 (VL-VH)
2H7-csc-hlgG-H5-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H5	G19-4 (VH-VL)
2H7-sss-hlgG-H5-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H5	G19-4 (VL-VH)

Figure 6B (5/5)

<i>Name</i>	<i>BD1</i>	<i>Hinge</i>	<i>EFD</i>	<i>Linker</i>	<i>BD2</i>
2H7-csc-hlgG-H5-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H5	G19-4 (VH-VL)
2H7-sss-hlgG-H6-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H6	G19-4 (VL-VH)
2H7-csc-hlgG-H6-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H6	G19-4 (VL-VH)
2H7-sss-hlgG-H6-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H6	G19-4 (VH-VL)
2H7-csc-hlgG-H6-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H6	G19-4 (VH-VL)
2H7-sss-hlgG-H7-G19-4 LH	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H7	G19-4 (VL-VH)
2H7-csc-hlgG-H7-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H7	G19-4 (VL-VH)
2H7-sss-hlgG-H7-G19-4 HL	2H7 (VL-VH)	hlgG1 -SSS	hlgG or hlgG (P238S/P331S)	H7	G19-4 (VH-VL)
2H7-csc-hlgG-H7-G19-4 LH	2H7 (VL-VH)	hlgG1 -csc	hlgG or hlgG (P238S/P331S)	H7	G19-4 (VH-VL)

Figure 7.

Altering the Linker after the EFD and the orientation of V regions in BD2 Affects Binding Efficiency of Multispecific Fusion Proteins



**All fusion proteins used at 0.72 ug/ml, Protein A purified, generated from transient COS supernatants.

Figure 8.
Effect of Gly₄Ser linker length on 2H7-ssslgG-Hx-2e12 HL Protein
Increased Cleavage of Linker Region with Increasing Linker Length

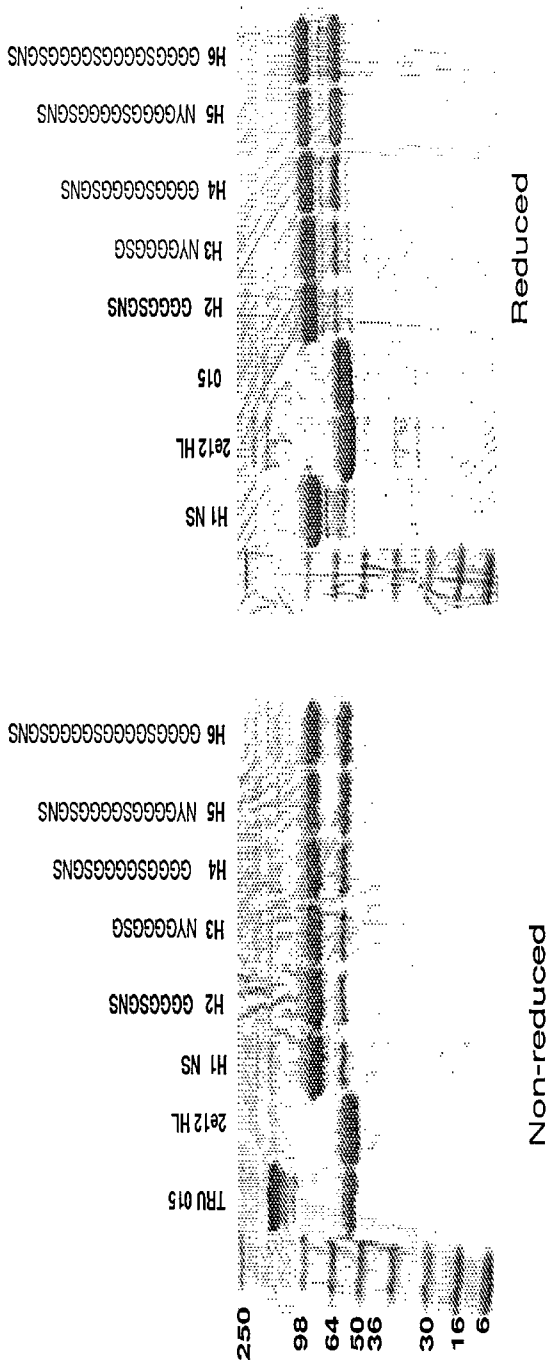


Figure 9.

Western Blots of Multispecific Fusion Proteins With H6 Linker

A. Absence of SMIP or smaller CD28 detectable forms

B. Presence of a SMIP sized form using CD20 anti-id Fab

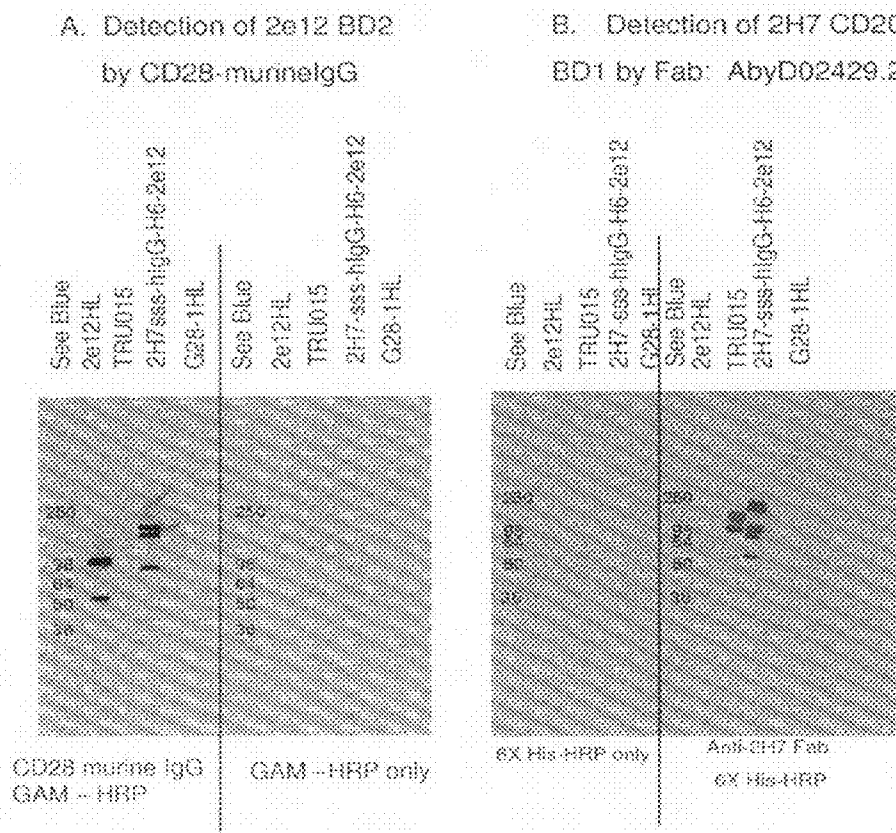
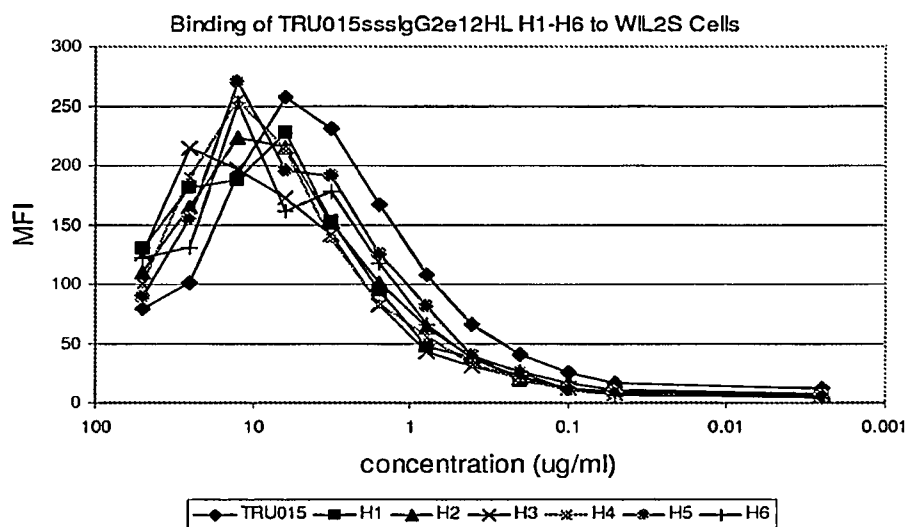


Figure 10.

A. Binding of Multispecific Fusion Proteins With Variant Linkers to WIL-2S Cells



B. Binding of Multispecific Fusion Protein With Variant Linkers to CD28 CHO Cells

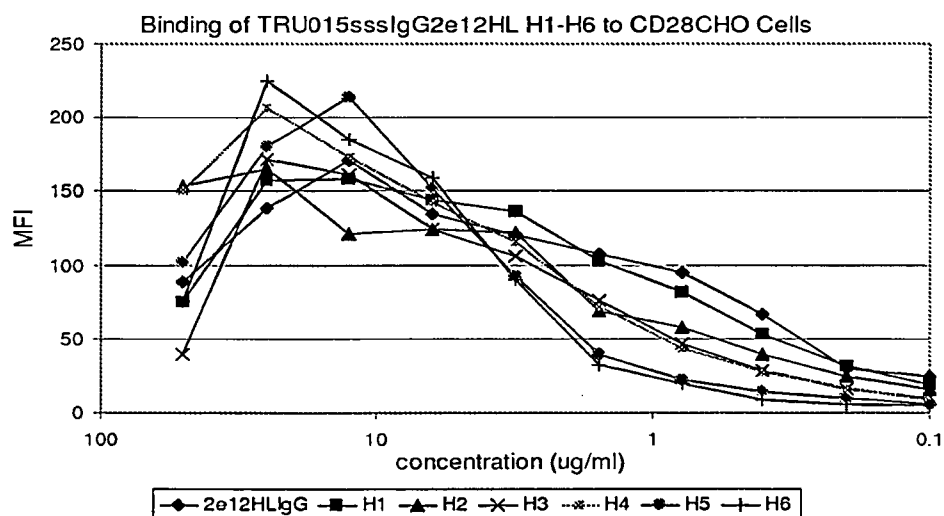


Figure 11.
Summary of SEC Fractionation of 2H7-ssslgG-2e12 HL
Multispecific Fusion Proteins with Variant Linkers

Linker ID	Retention Time for POI	% POI	% Other Forms	SDS-PAGE Analysis of Cleavage at EFD/BD2 junction
H1	7.620	70.9	29.0	(Yes)
H2	7.589	67.5	32.5	(Yes)
H3	7.605	68.7	31.4	Yes
H4	7.622 (shoulder)	68.0	32.0	Yes
H5	7.933/7.680 (doublet with shoulder)	32.42/33.94	33.63	YES
H6	7.901/7.69 (doublet with shoulder)	34.5/29.3	36.1	YES
H7	7.788	84.2	15.8 (HMW)	NO

Figure 12.
Binding of [2H7-sss-hlgG-H_x-2e12 HL] Fusion
Proteins with Different Linkers to Cells Expressing
Target Antigen for BD1 or BD2

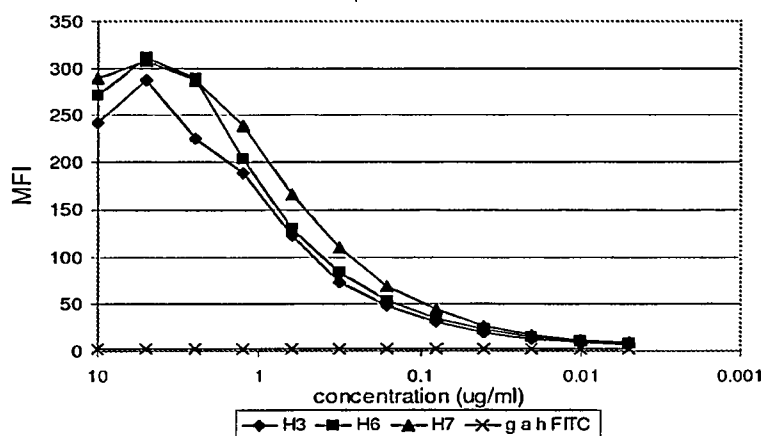
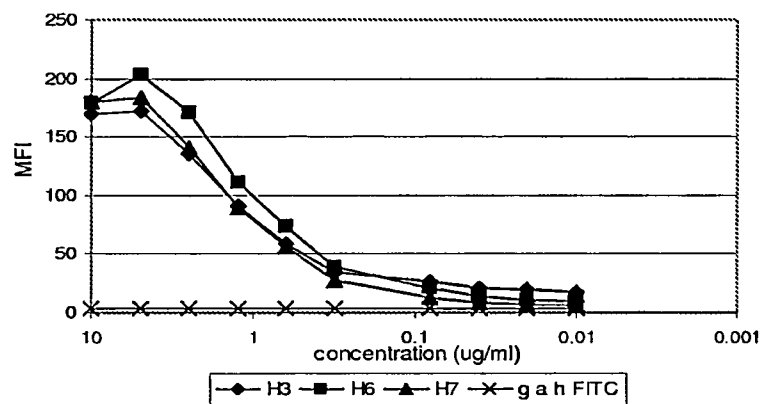


Figure 13.
Simultaneous Binding of BD1 and BD2:

Binding of [2H7-sss-hlgG-H_x-2e12 HL] Fusion Proteins with H3, H6,
and H7 Linkers to WIL-2S Cells can be detected with
CD28mIgG + FITC anti-mouse

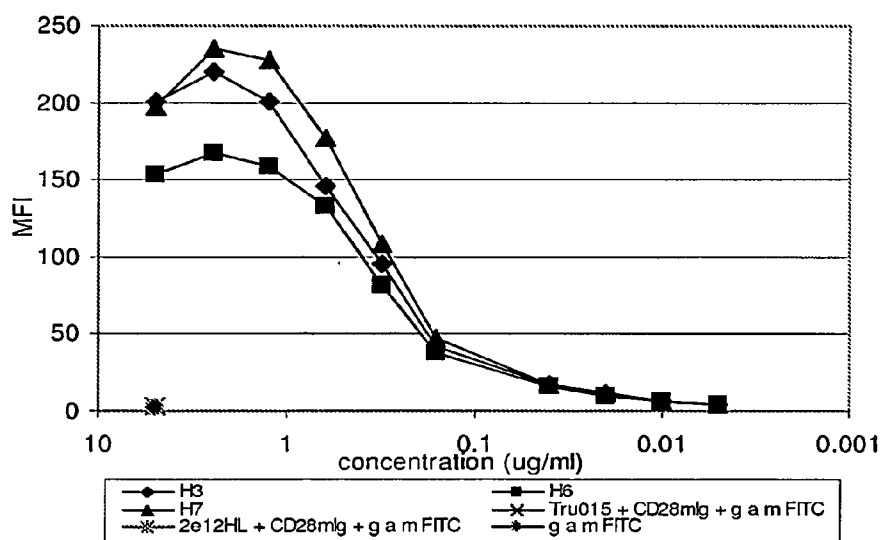
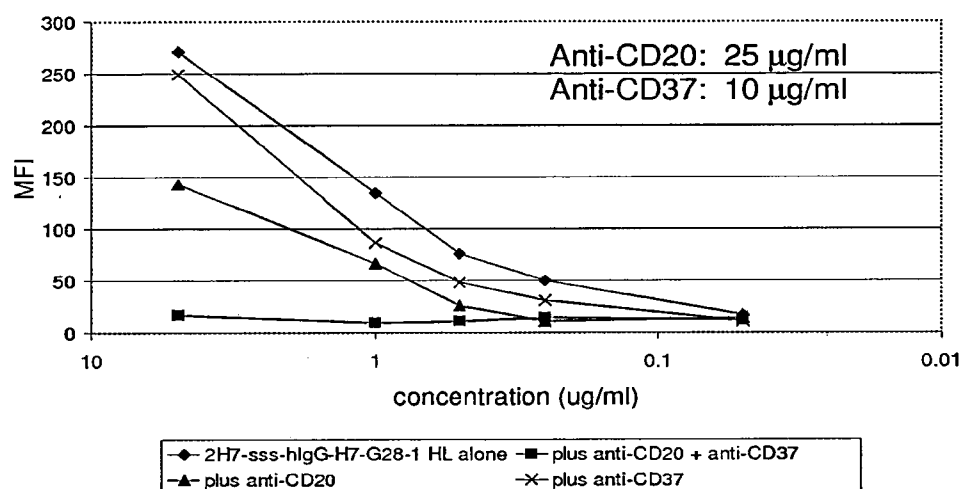


Figure 14.

A. Blocking of Binding of 2H7-sss-hlgG-H7-G28-1 HL Protein to Ramos Cells by CD20 and/or CD37 Targeted Antibodies



B. Blocking of Binding of 2H7-sss-hlgG-H7-G28-1 HL to BJAB Cells by CD20 and/or CD37 Targeted Antibodies

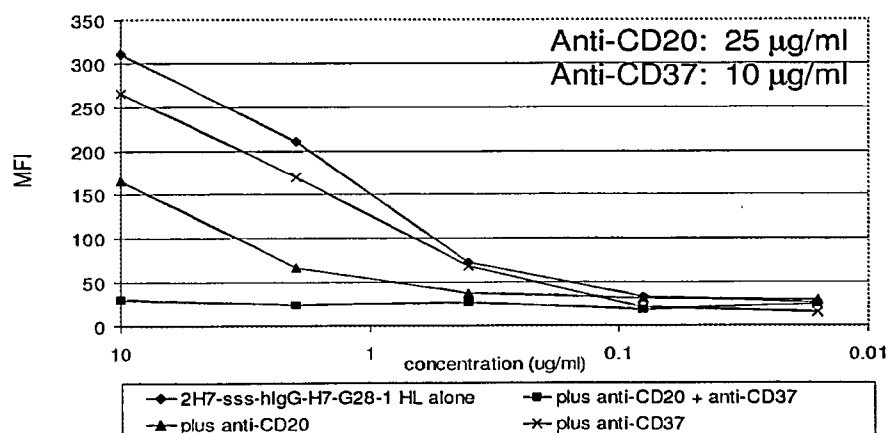


Figure 15.

ADCC Activity of 2H7-sss-hlgG1-STD1-G28-1 Variants
against BJAB Targets

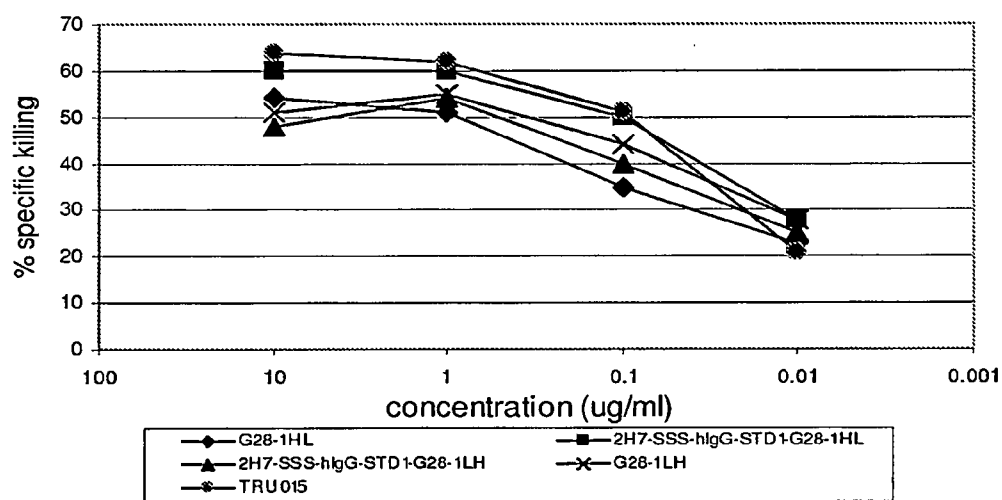


Figure 16.

The Percent CD3, CD19, and CD40 Positive Lymphocytes
Present in Culture after Incubation with TRU-015, G28-1 SMIP,
TRU-015+G28-1 SMIP, or 2H7-ssshlgG1-H7-G28-1 HL

24 Hours	CD3⁺	CD19⁺	CD40⁺
Media	77%	8%	7%
TRU015	76%	8.5%	7.9%
G28-1 SMIP	78%	8.9%	7.8%
TRU015+G28-1 SMIP	76.3%	6.1%	7.5%
2H7-ssshlgG1-G28-1 HL (H7)	78.4%	8.9%	7.9%

72 Hours	CD3⁺	CD19⁺	CD40⁺
Media	78.5%	7.9%	7.7%
TRU015	85.2%	2.3%	2.7%
G28-1 SMIP	86.7%	2.2%	2.3%
TRU015+G28-1 SMIP	87.1%	1.1%	1.1%
2H7-ssshlgG1-G28-1 HL (H7)	96.7%	.08%	.77%

Figure 17.

The Percent Annexin and/or PI Positive B Cells in Culture after 24 Hour Incubation with Single or Multispecific Fusion Proteins.

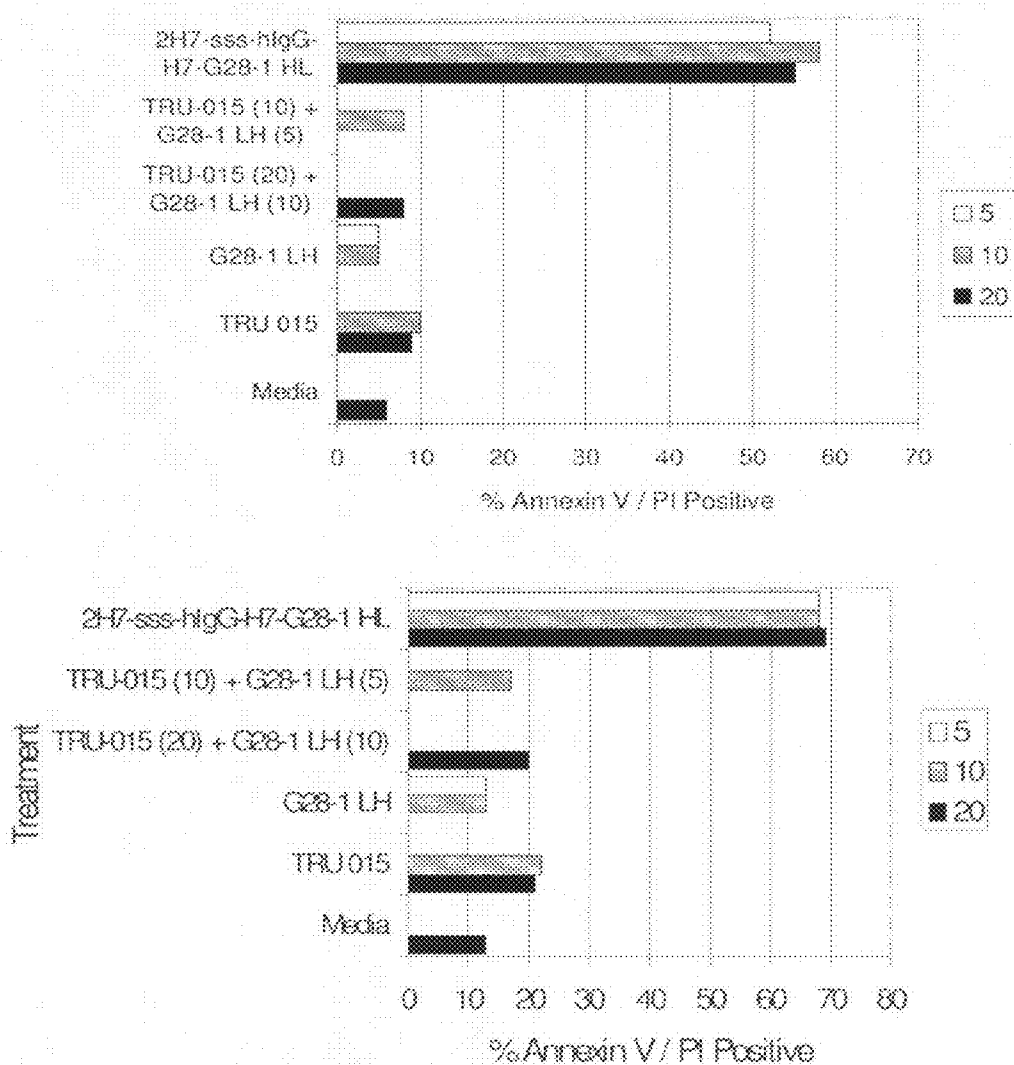


Figure 18.

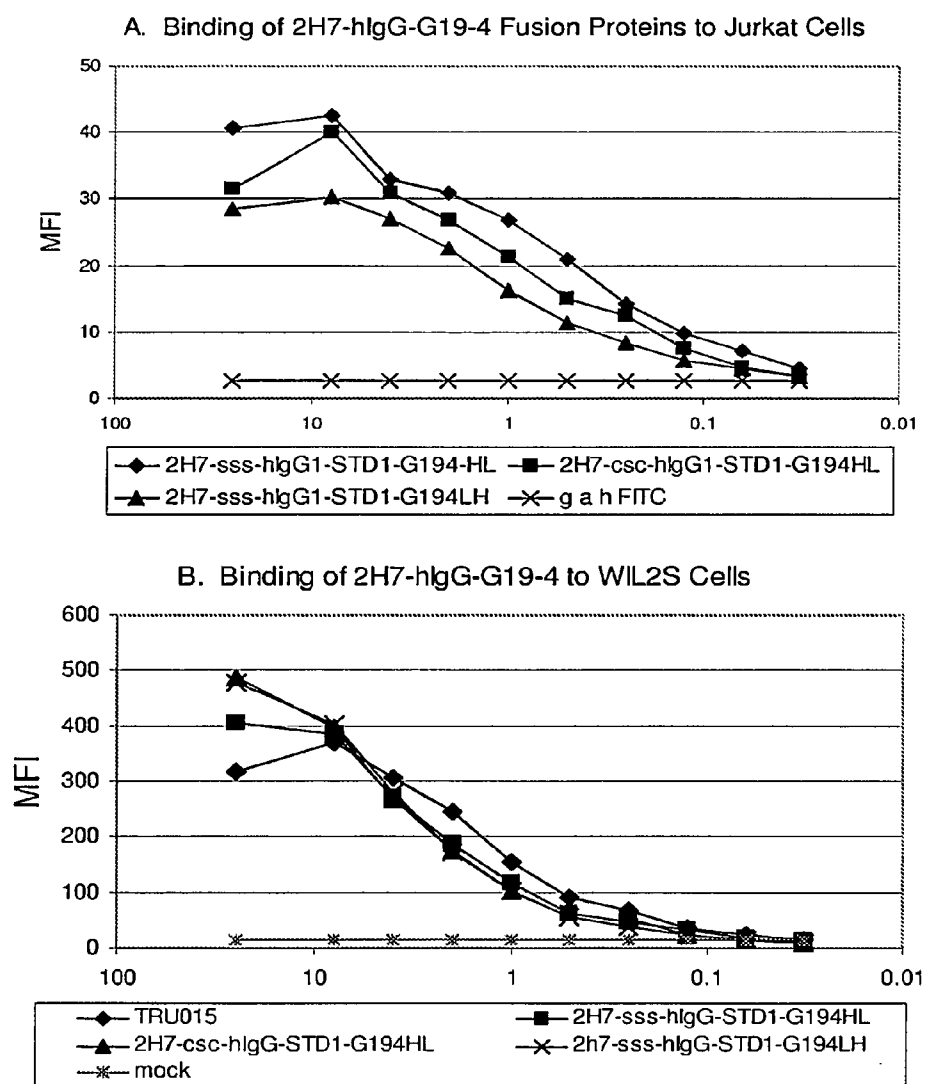
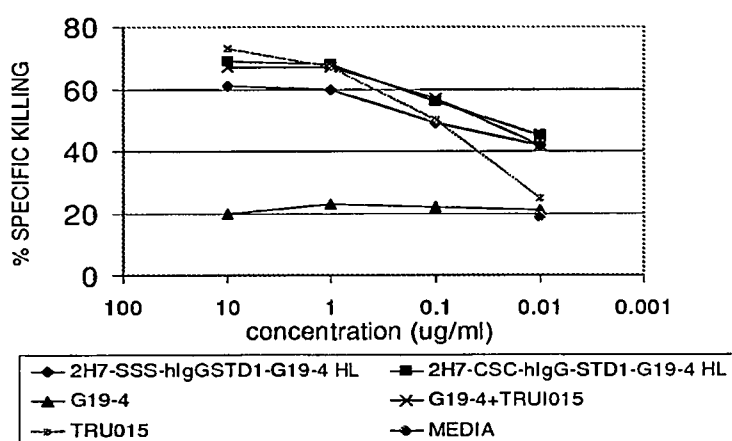


Figure 19.



B. ADCC Activity in NK Cell Depleted PBMC Effector Cultures Using BJAB Targets (30:1, E:T) and 2H7-G19-4 Fusion Proteins

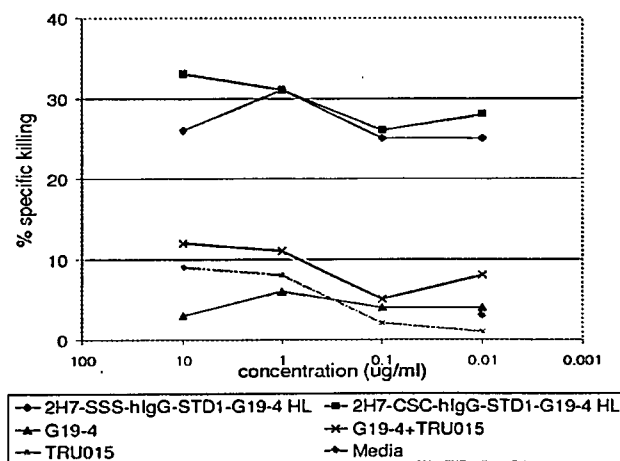


Figure 20

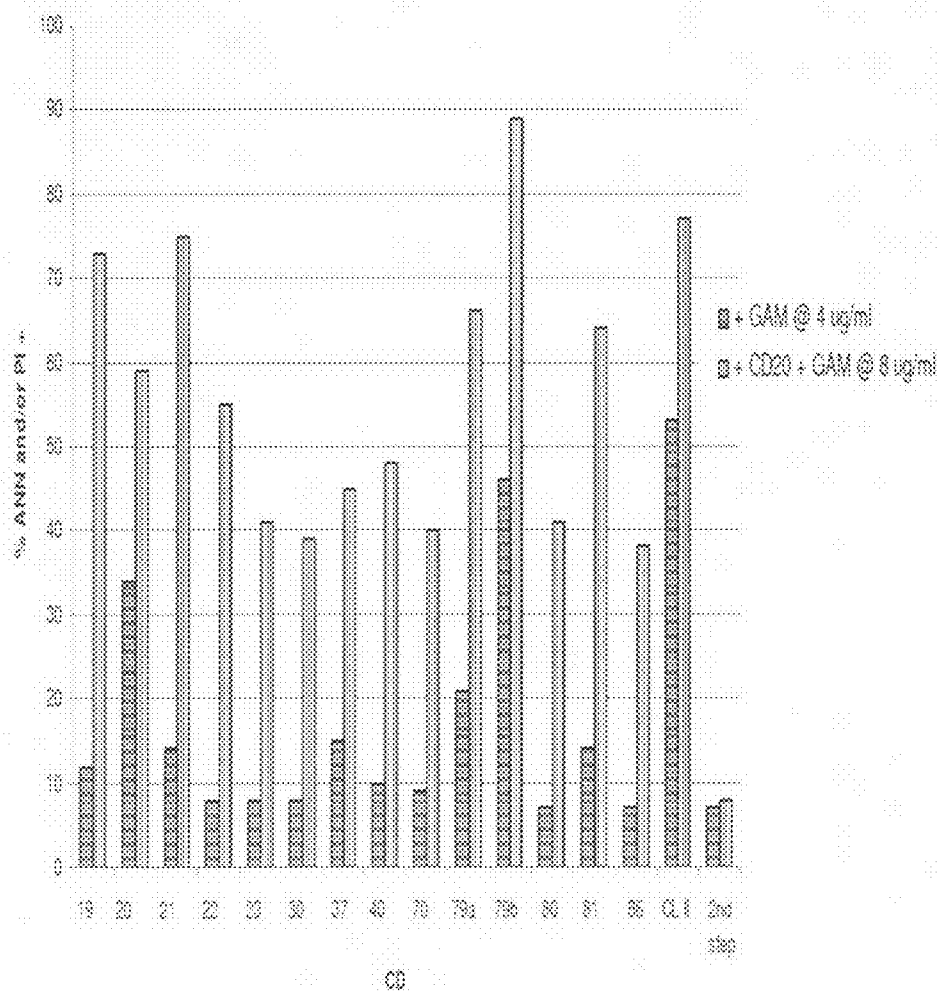


Figure 21

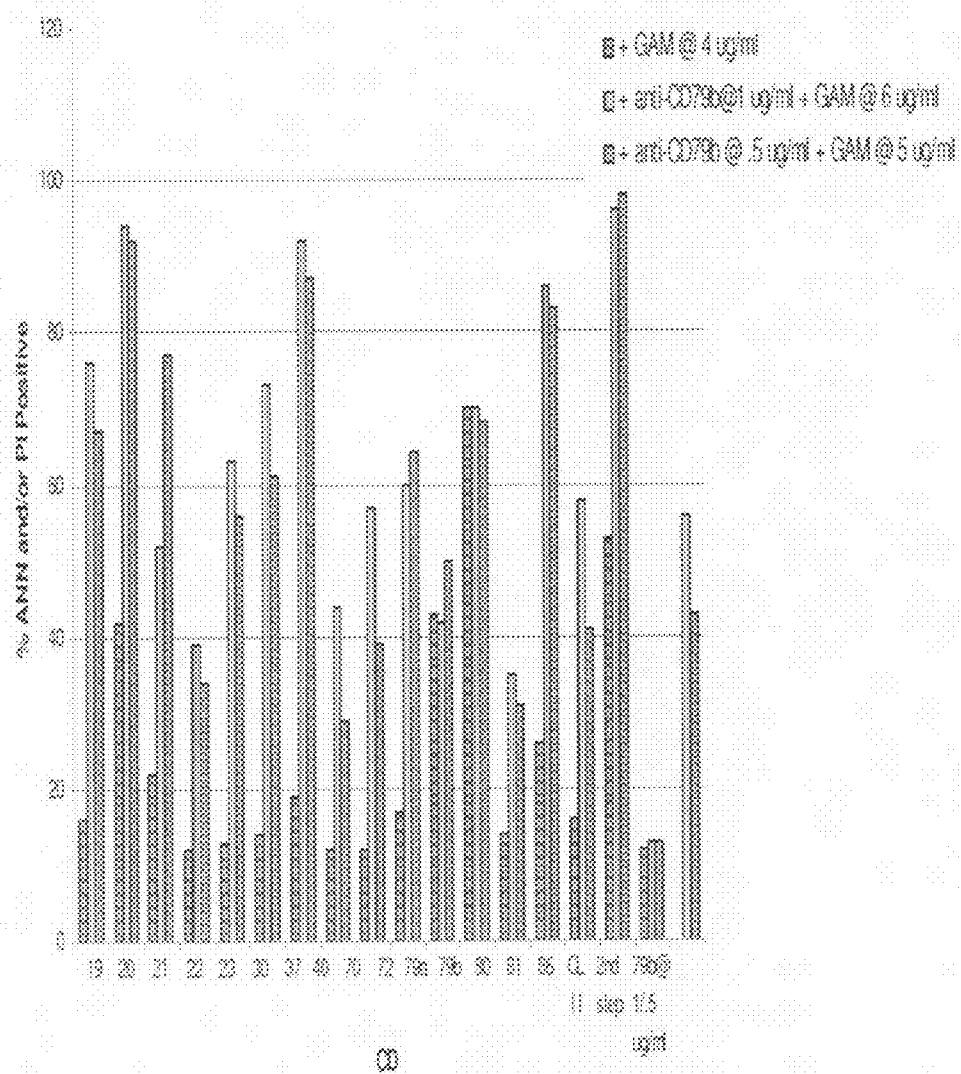


Figure 22

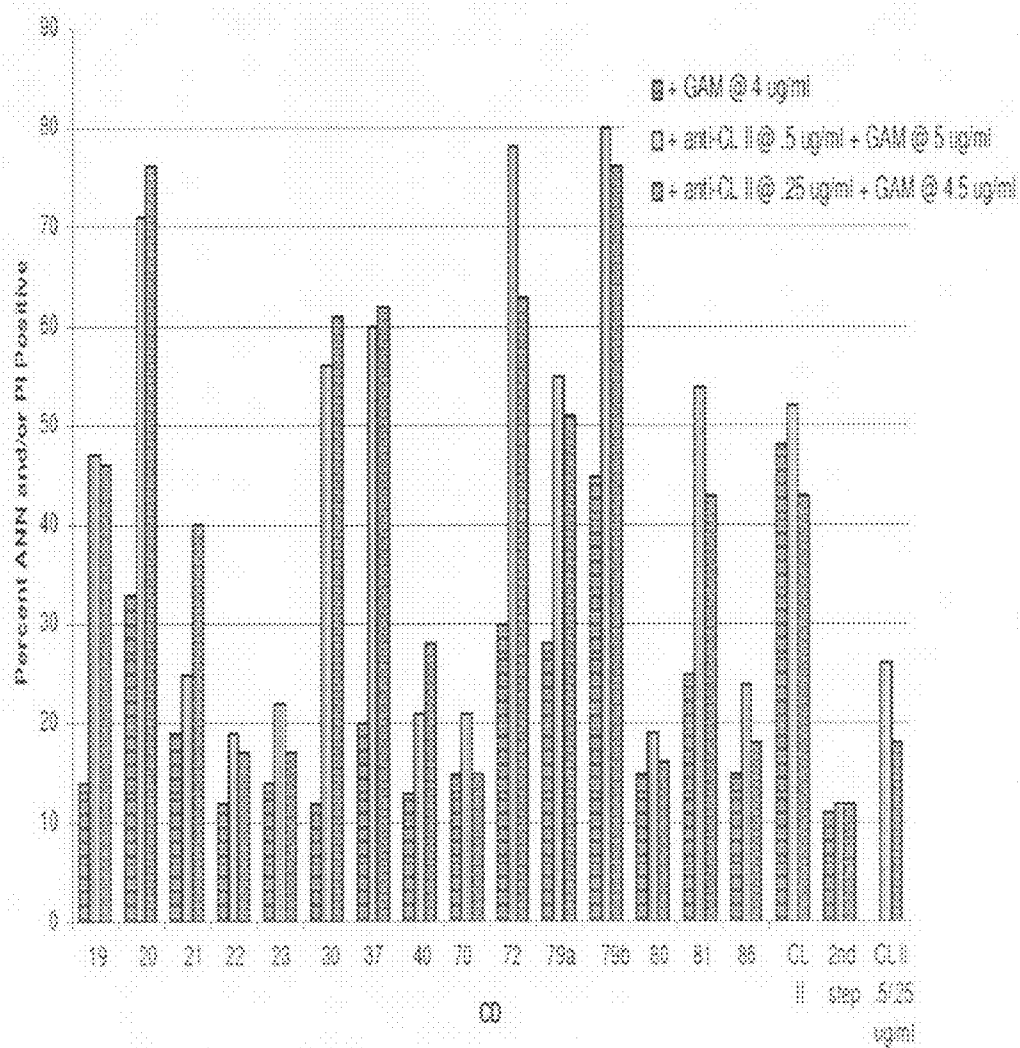


Figure 23

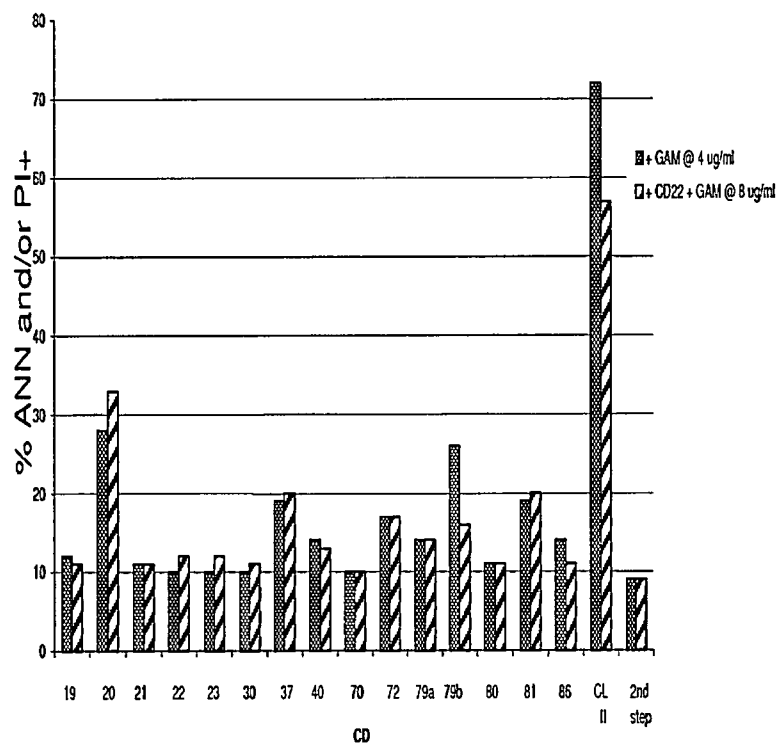


Figure 24

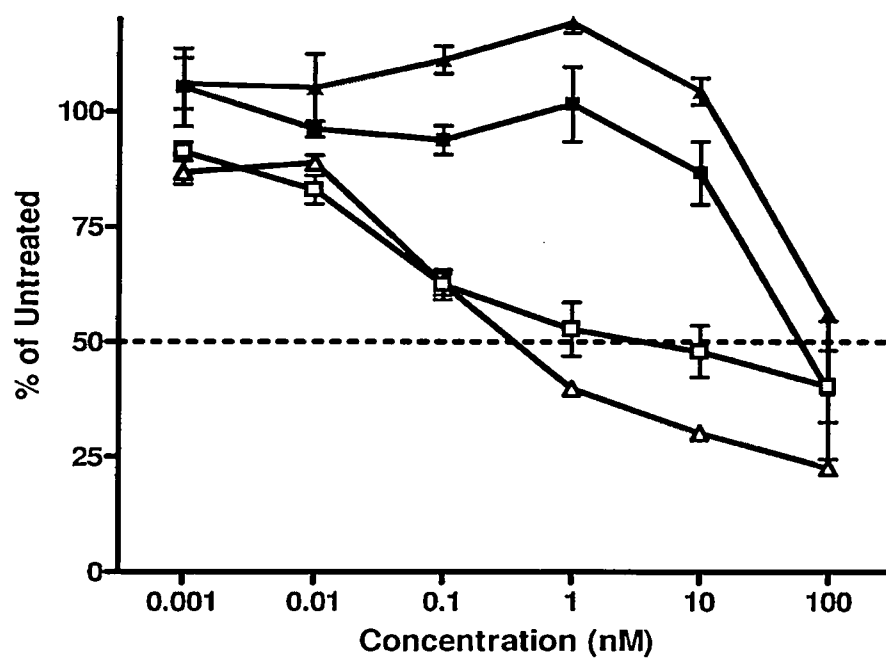


Figure 25

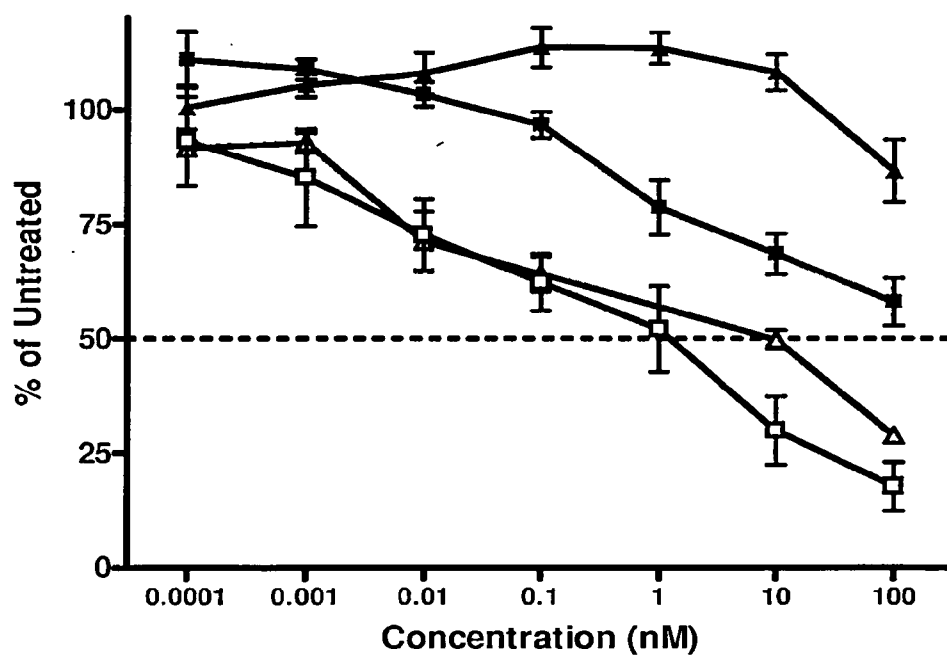


Figure 26

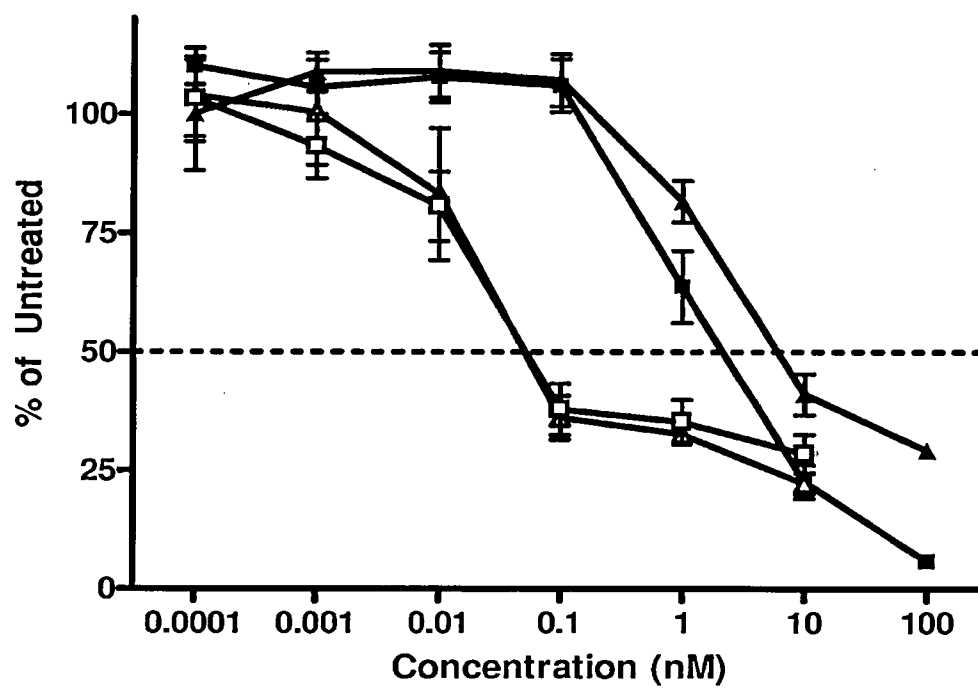


Figure 27

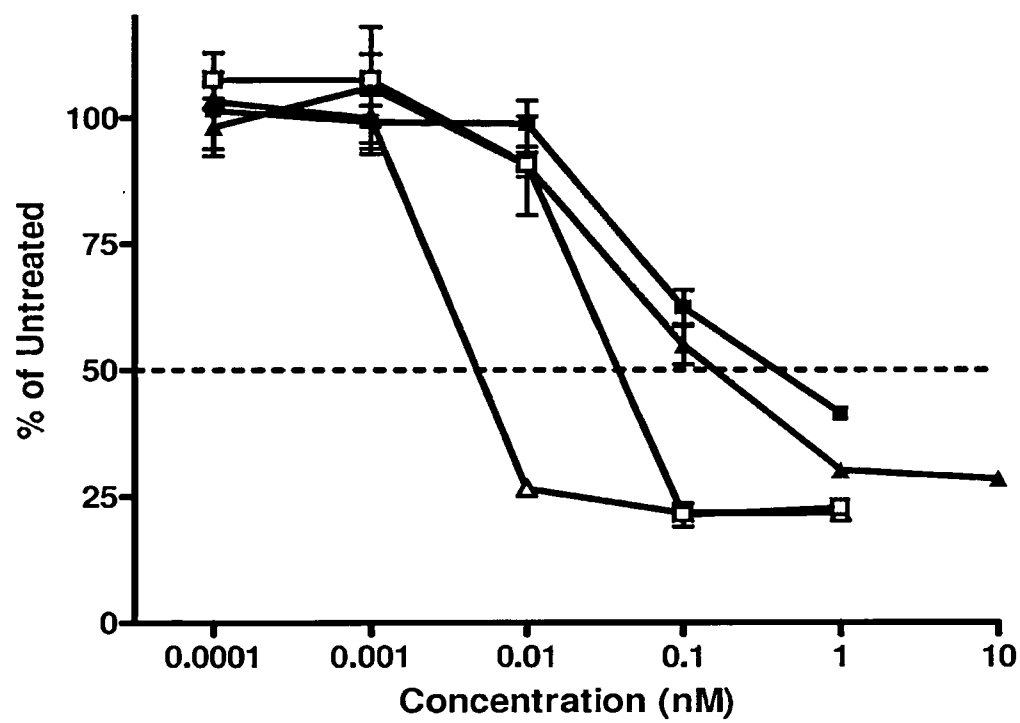


Figure 28

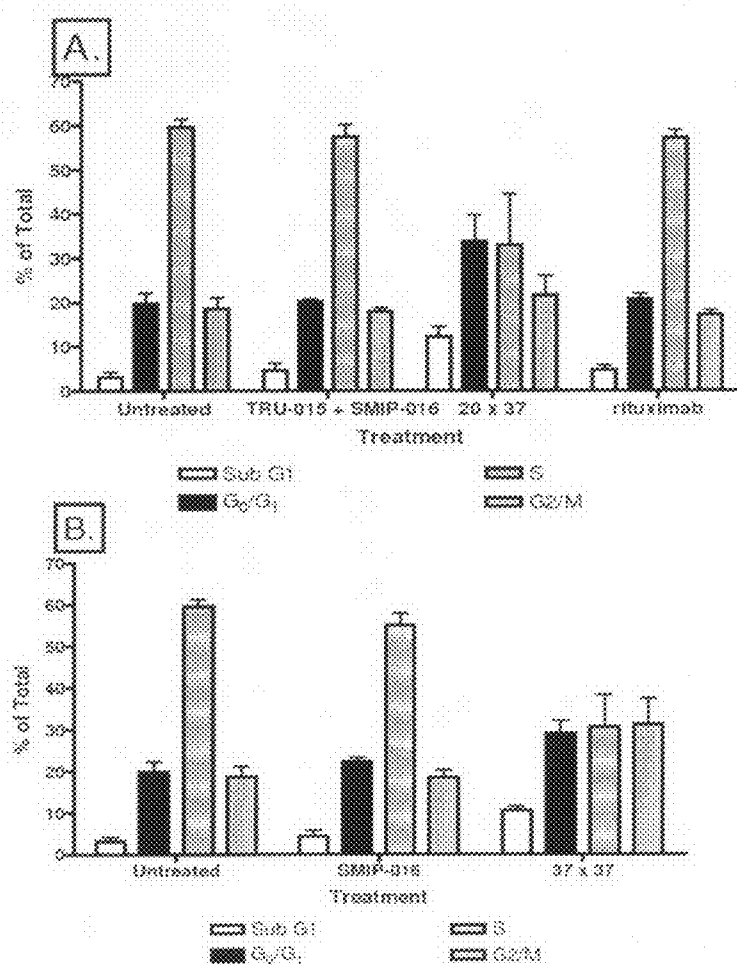


Figure 29

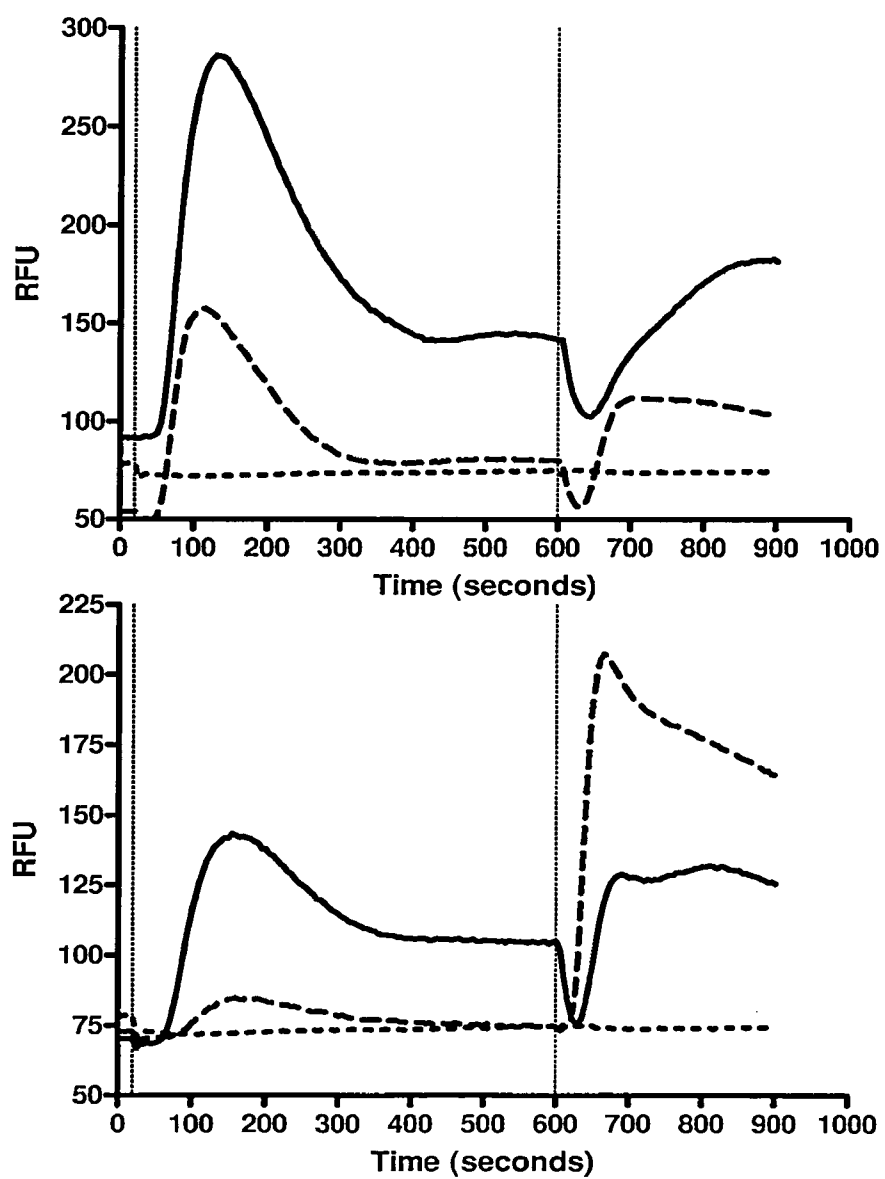


Figure 30

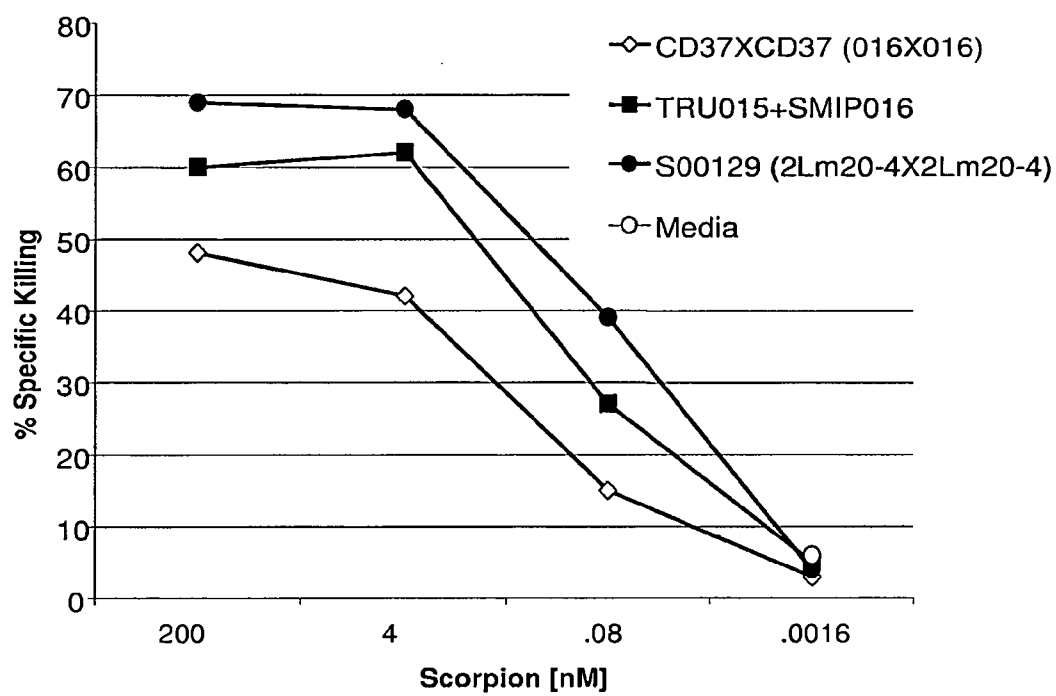


Figure 31

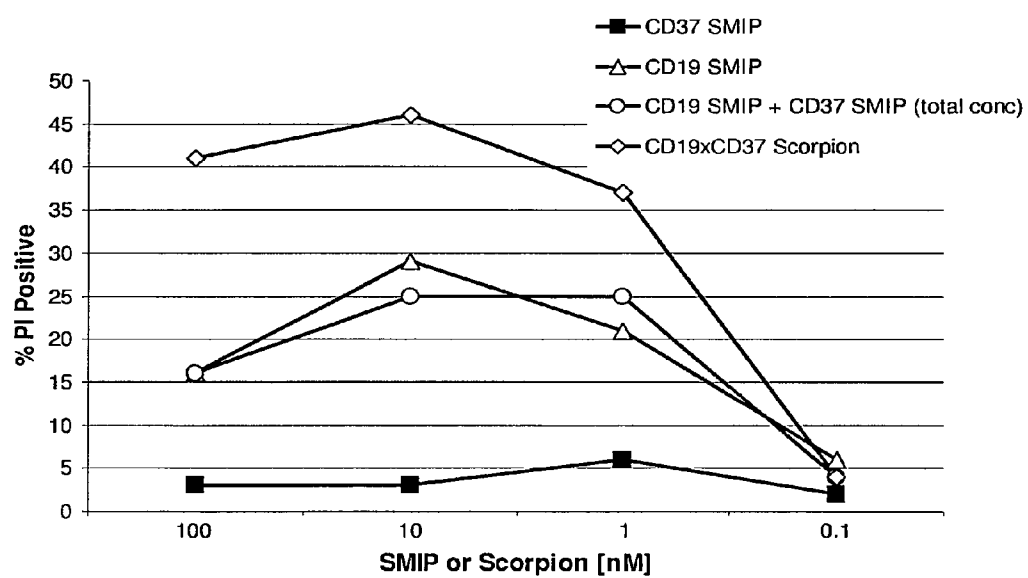


Figure 32A

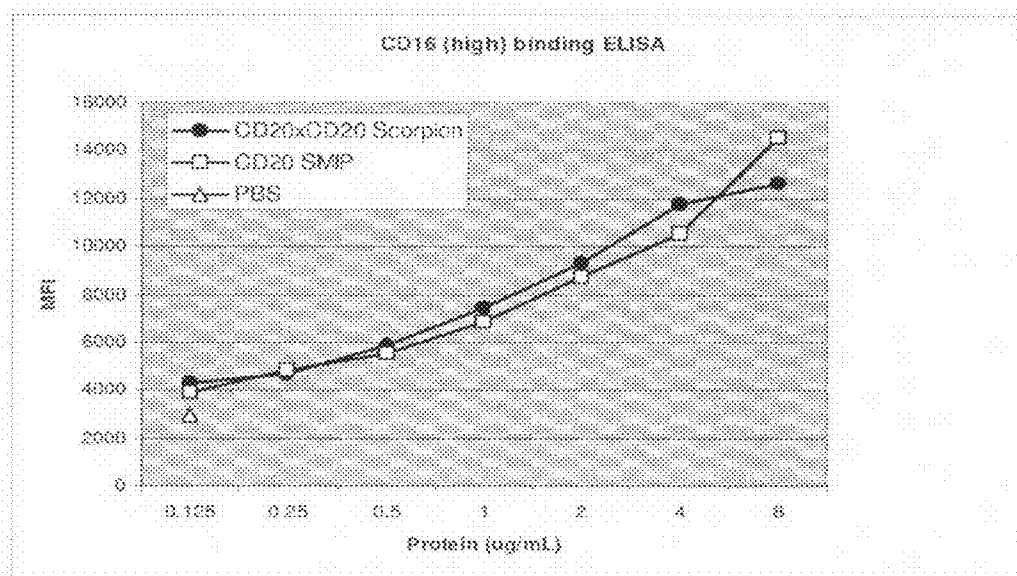


Figure 32B

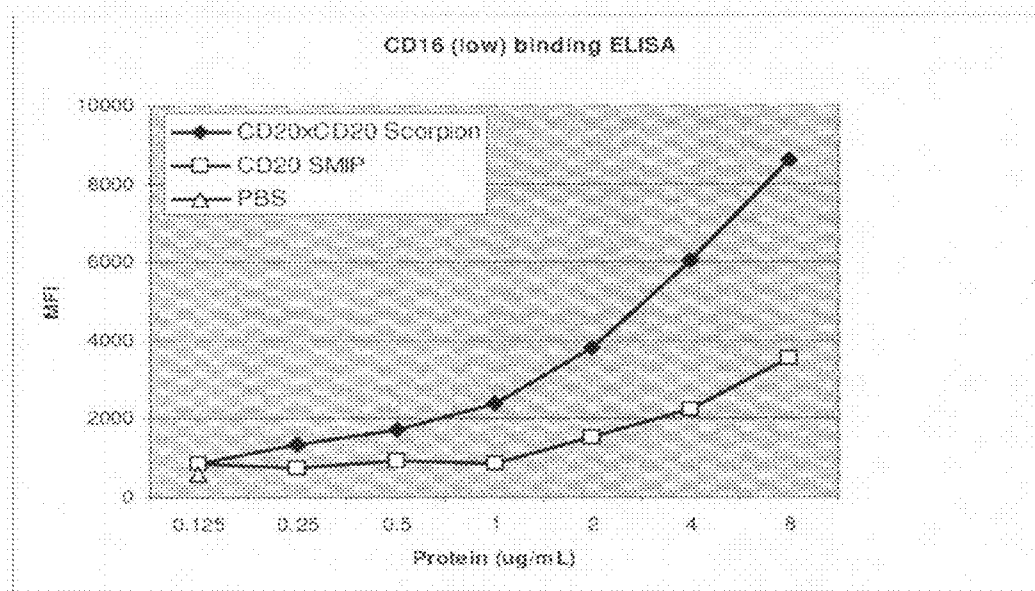


Figure 33

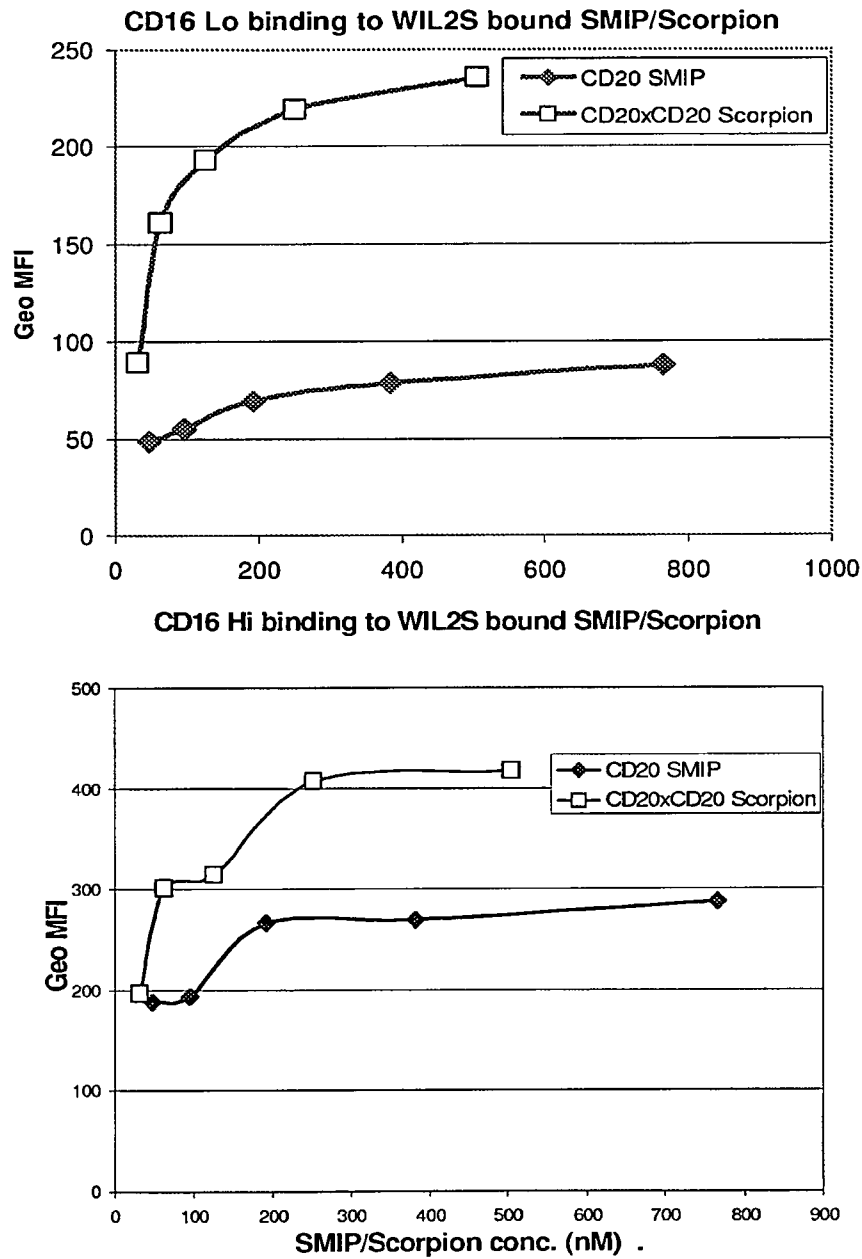


Figure 34

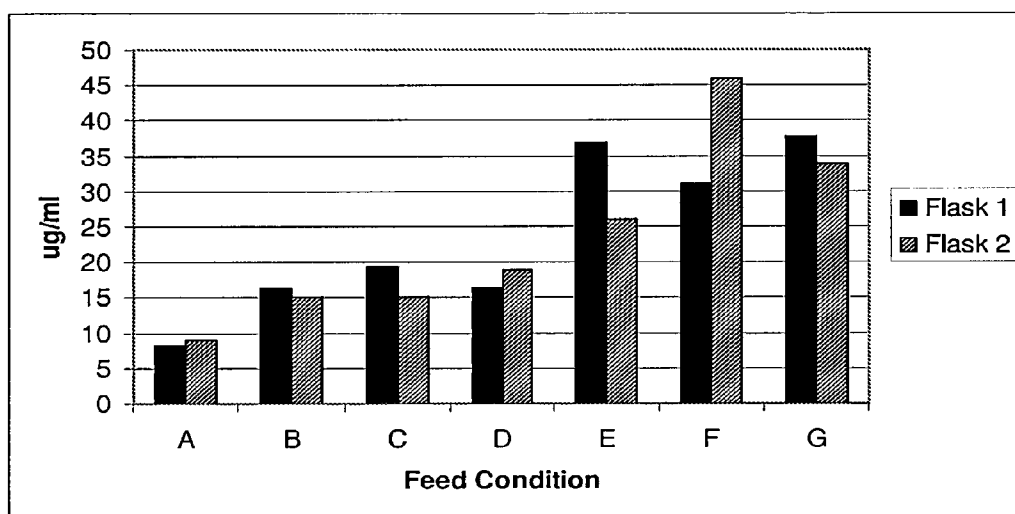
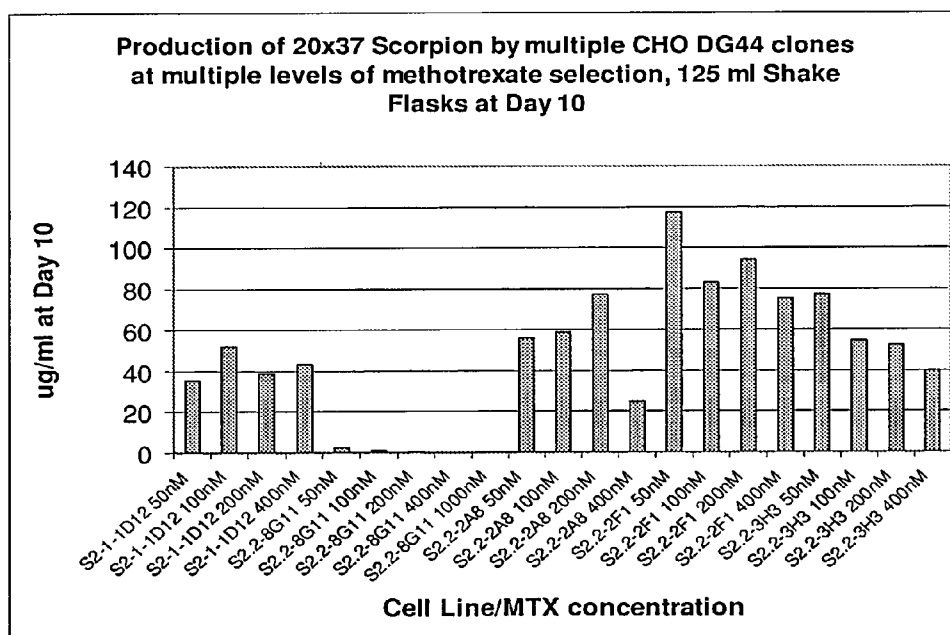


Figure 35



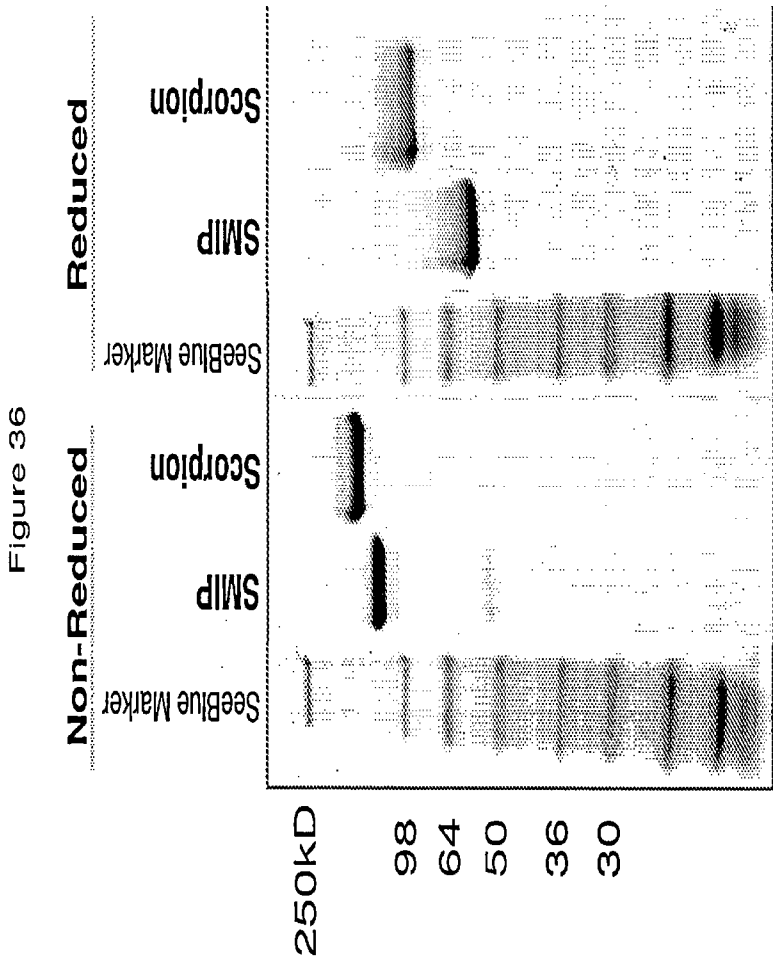


Figure 37

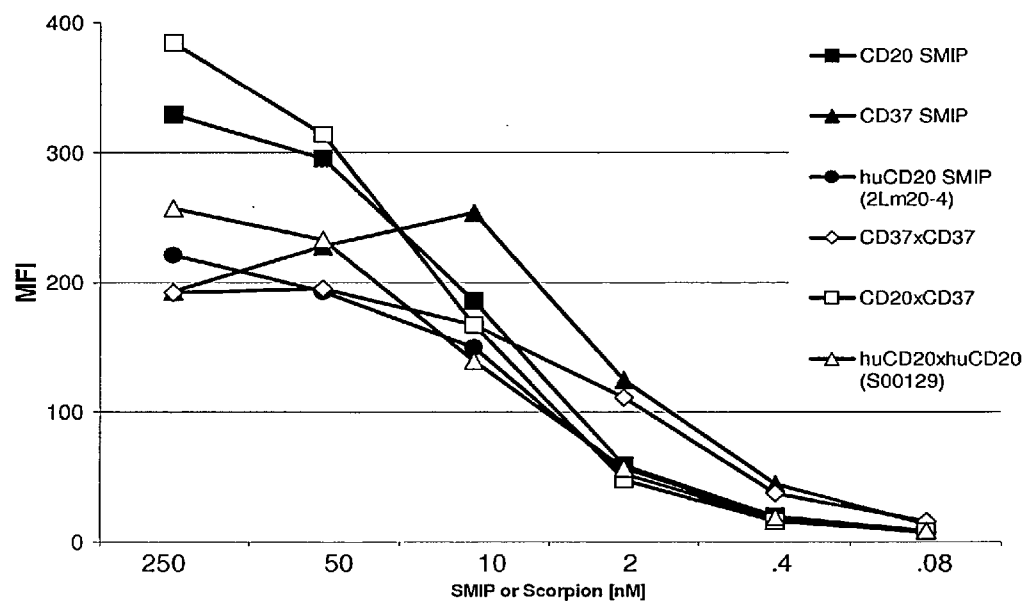


Figure 38

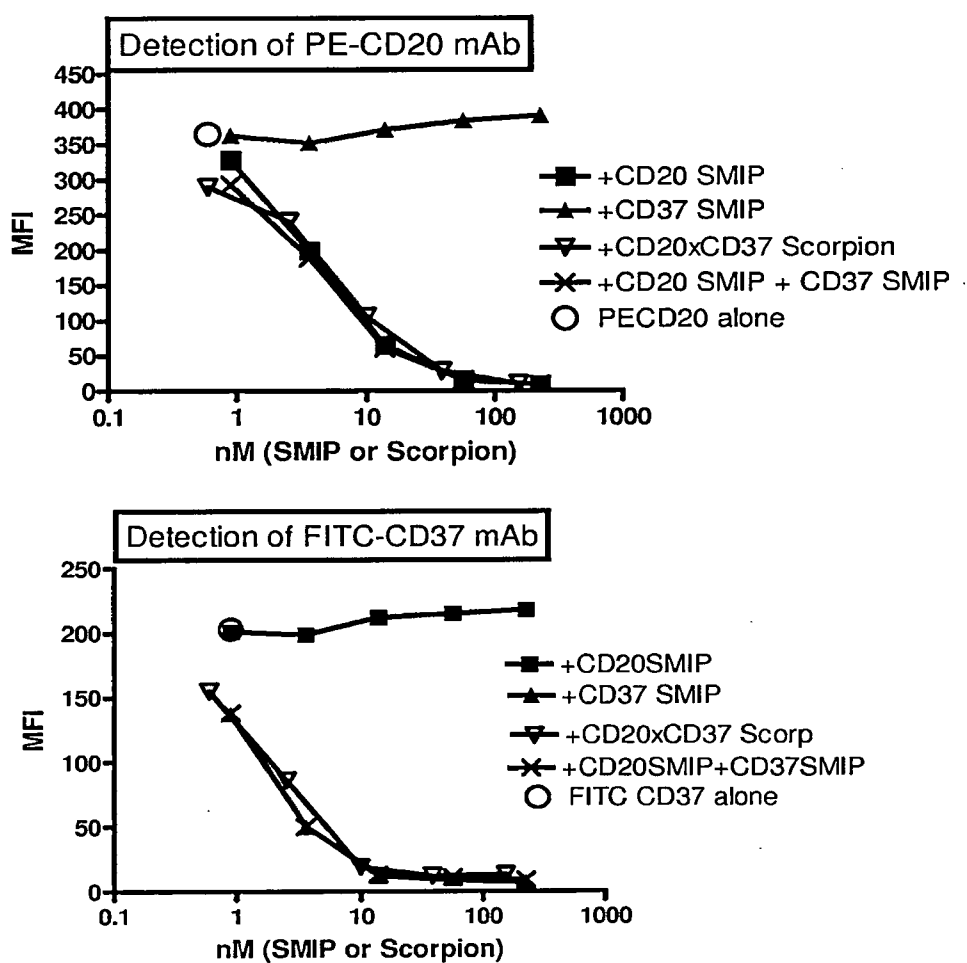
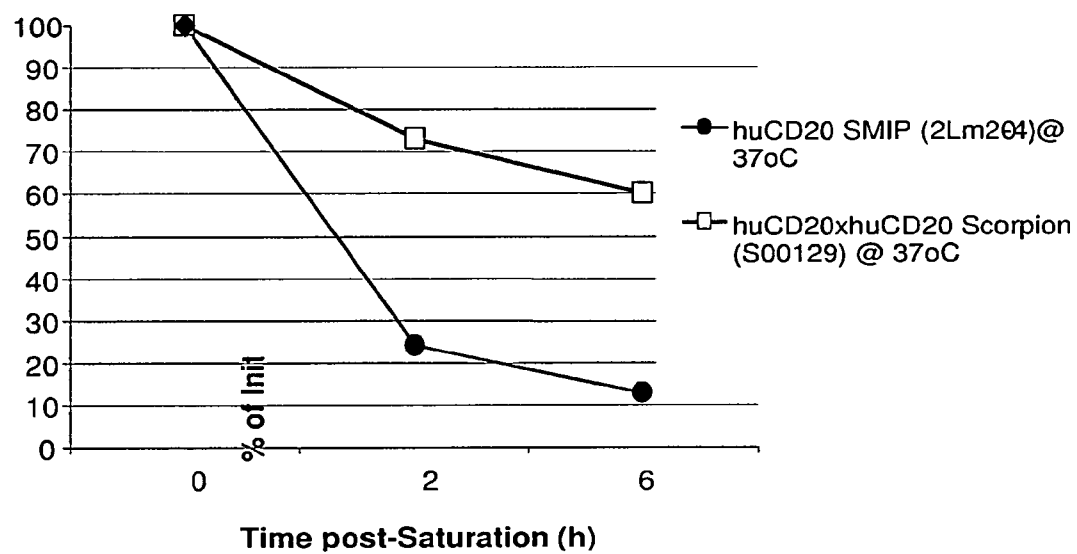


Fig. 39



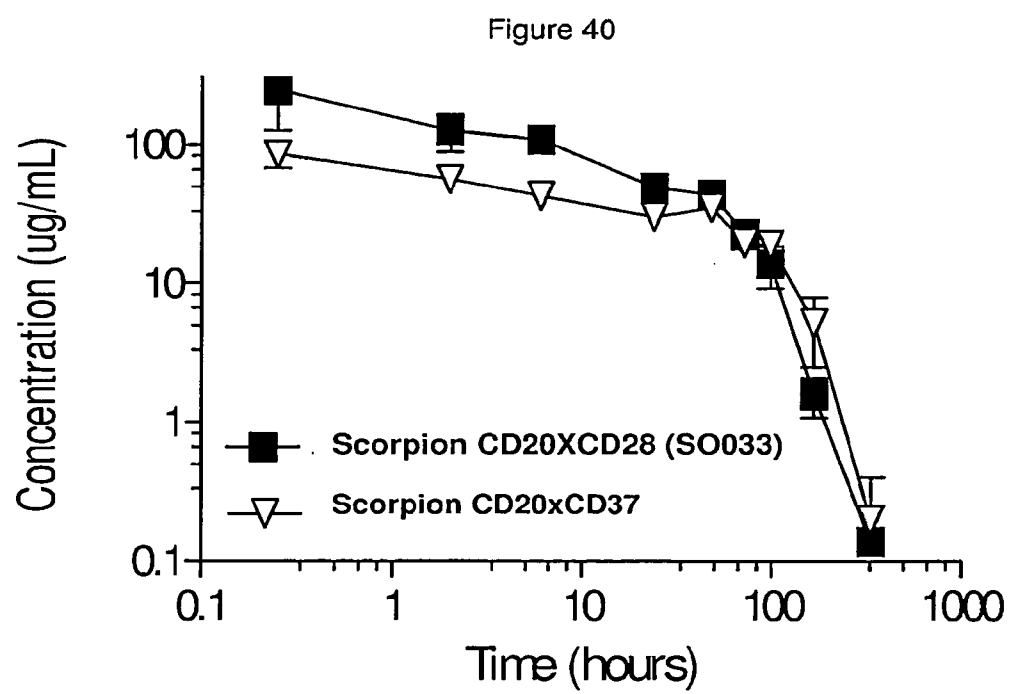


Figure 41

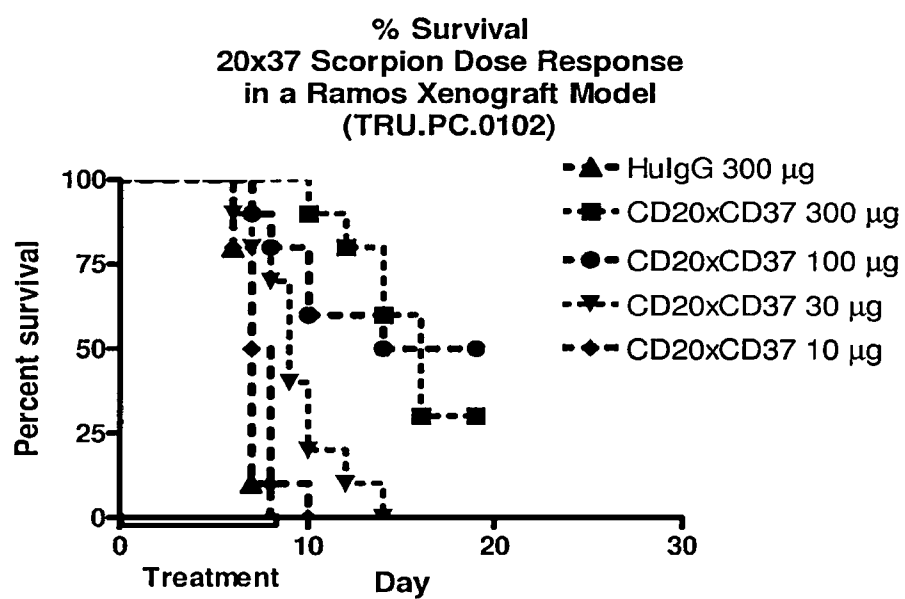
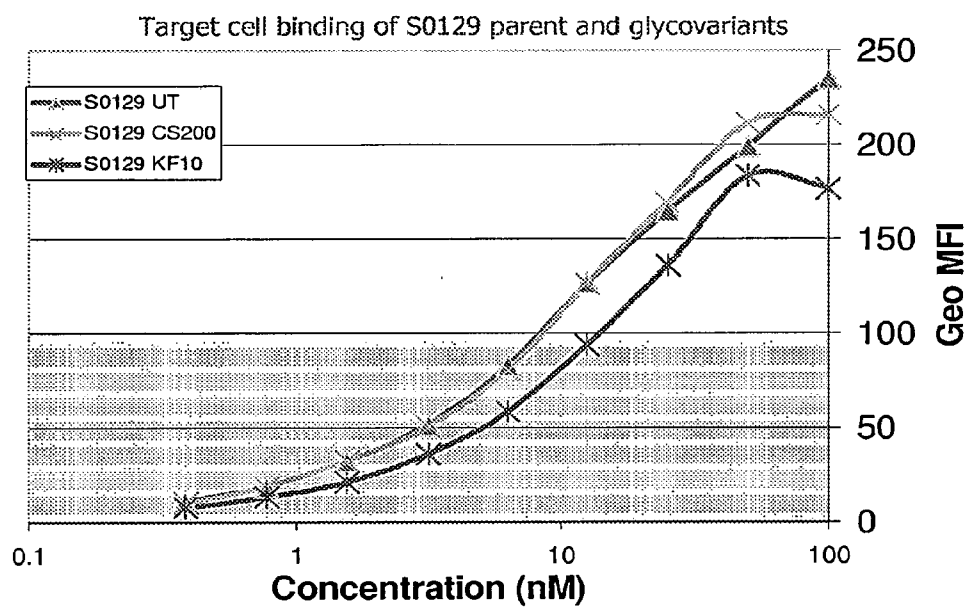


Figure 42



WIL2S staining with S0129 +/- carbohydrate modification Kifunensine@10 uM (excess), Castanospermine@200 uM, UT (untreated)

Figure 43

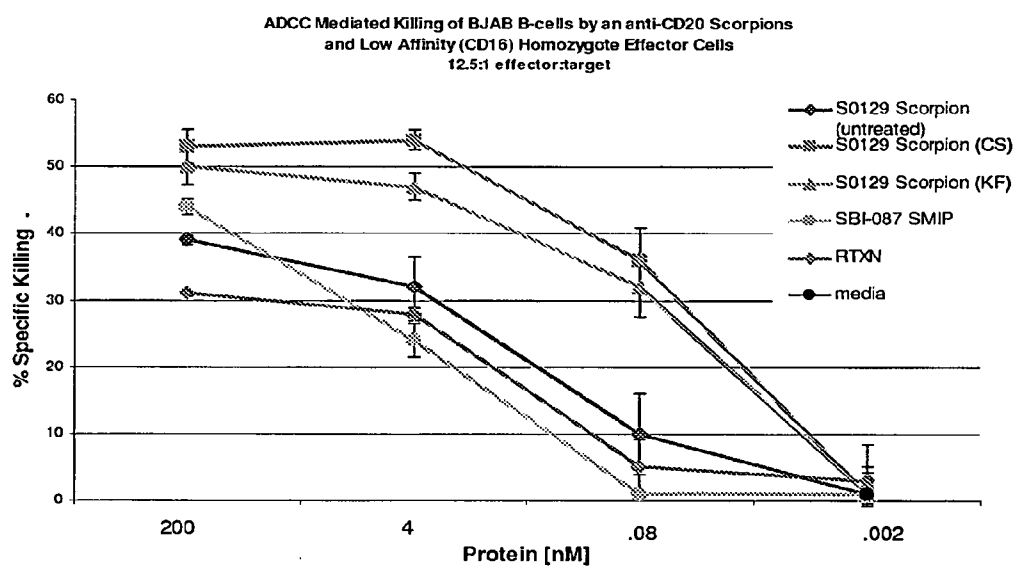


Figure 44

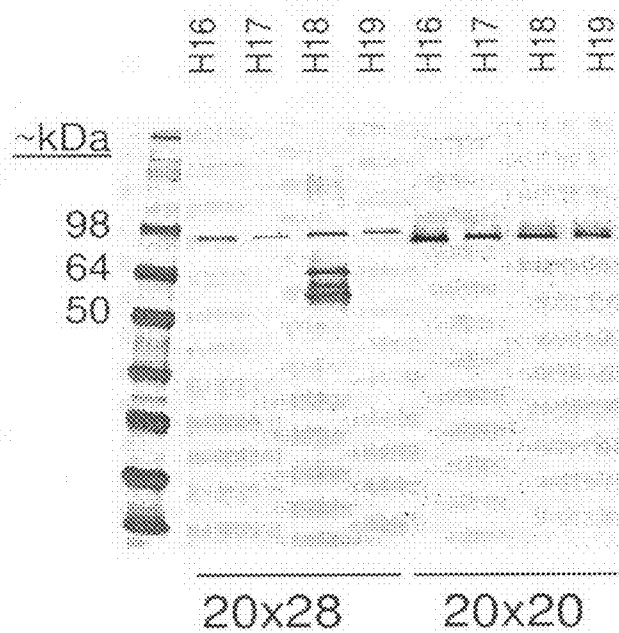


Figure 45

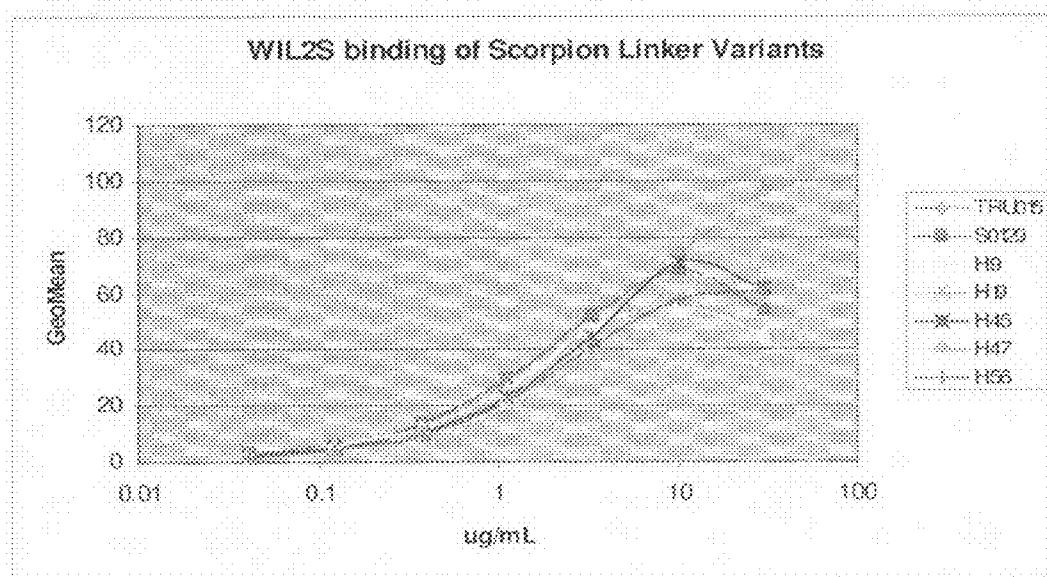


Figure 46

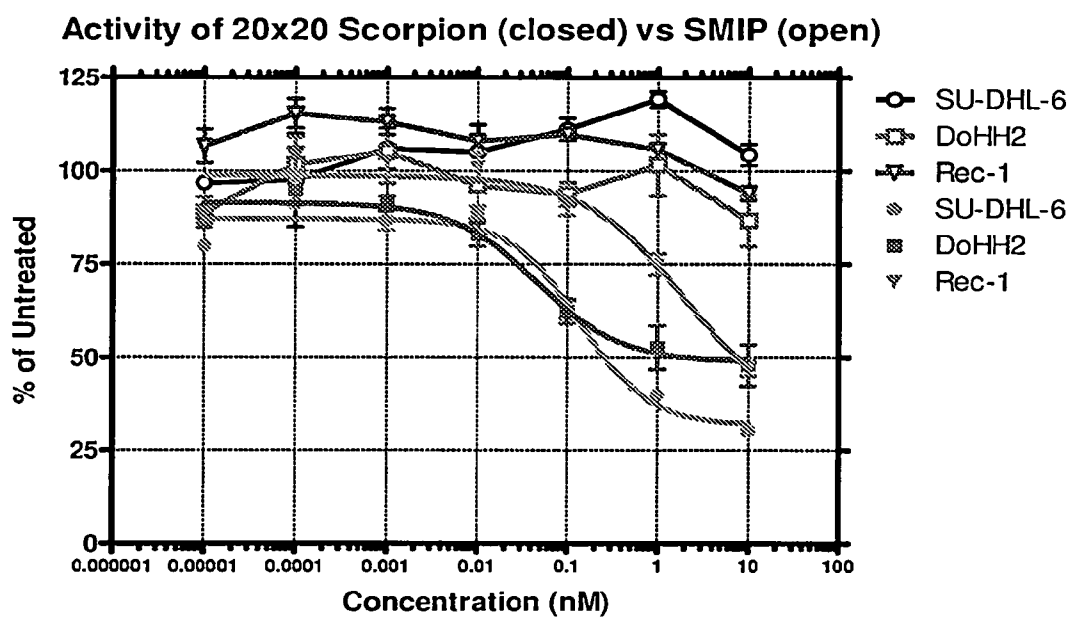


Figure 47

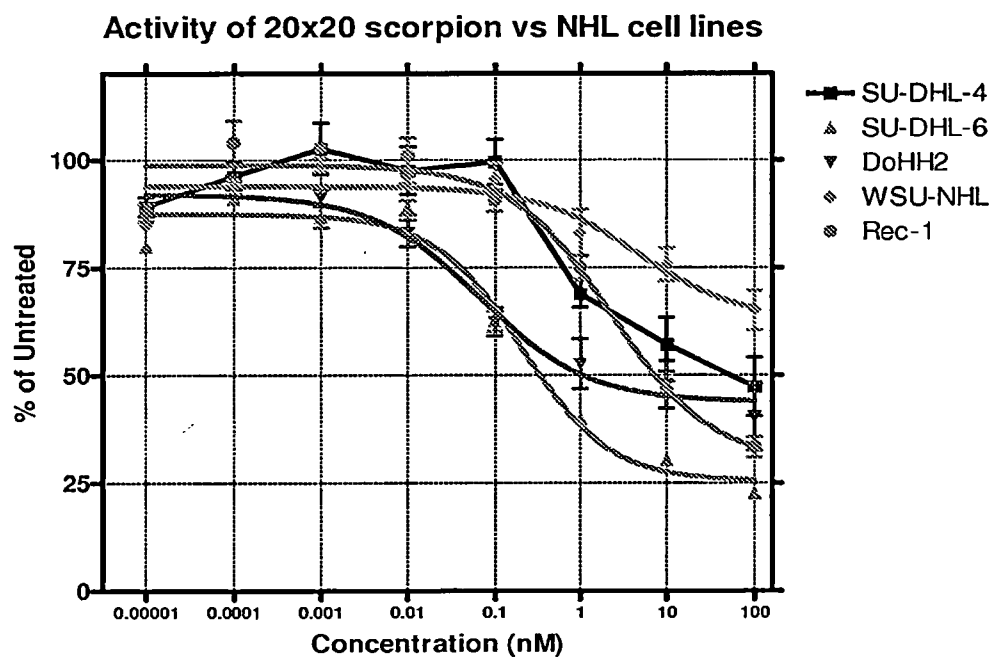


Figure 48

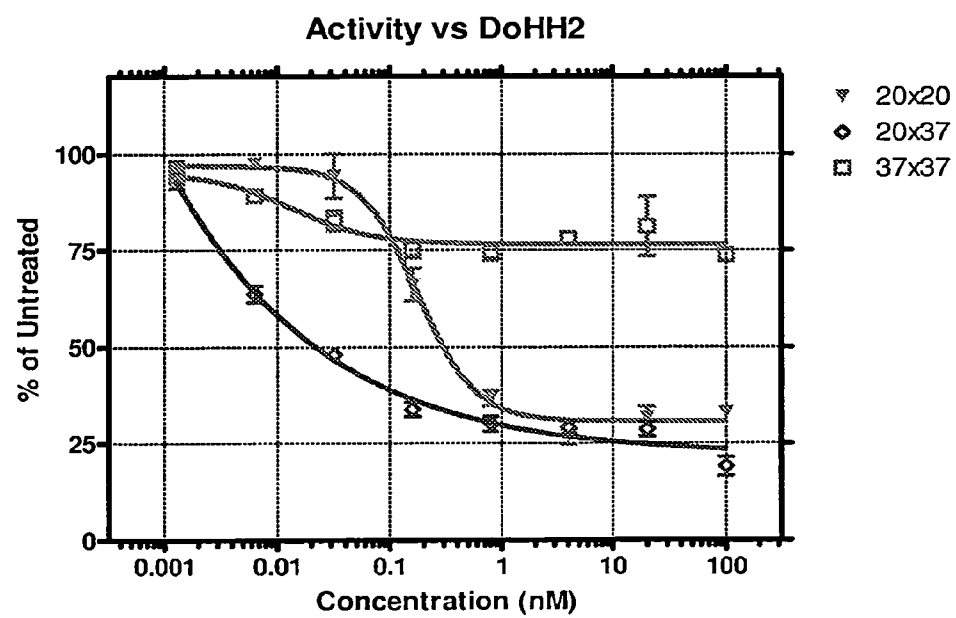


Figure 49

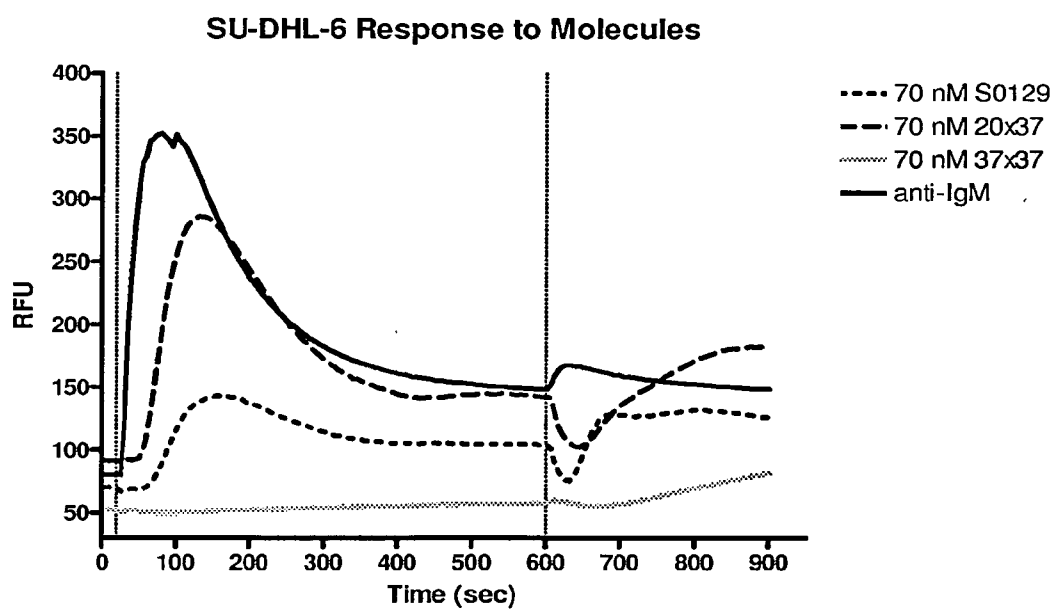


Figure 50

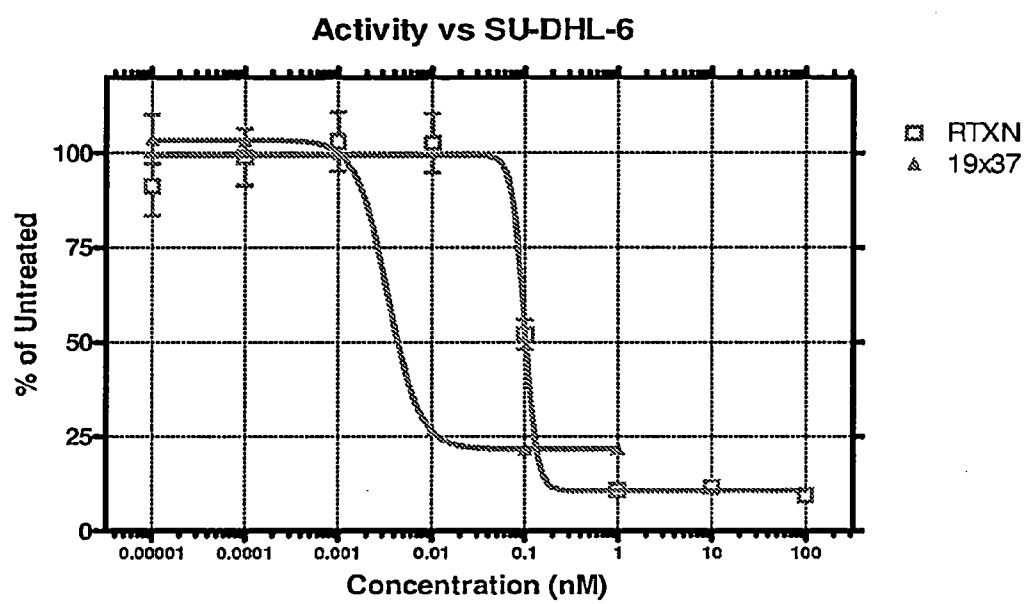


Figure 51

DHL6 Cells Treated with 20x37
Scorpion

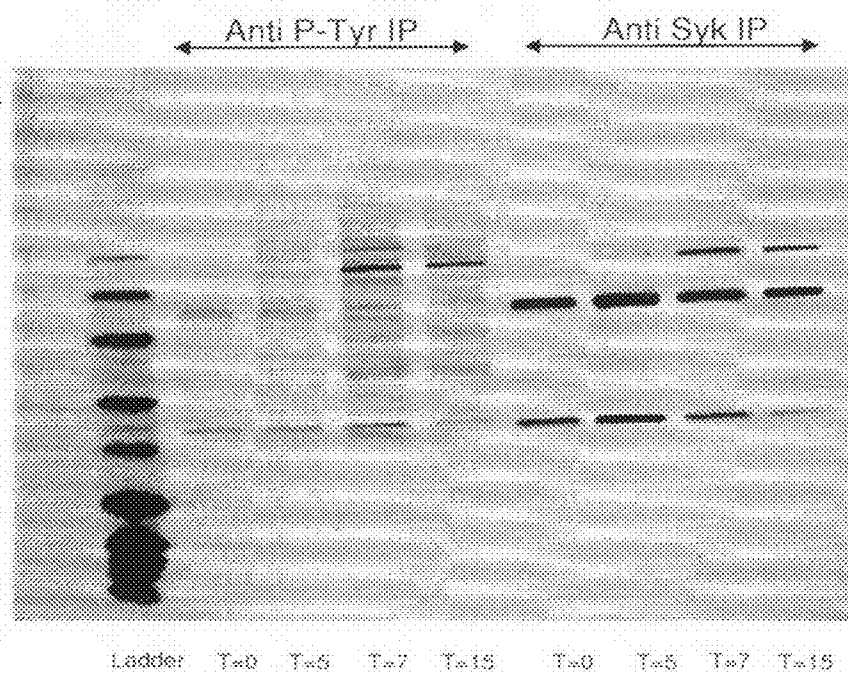
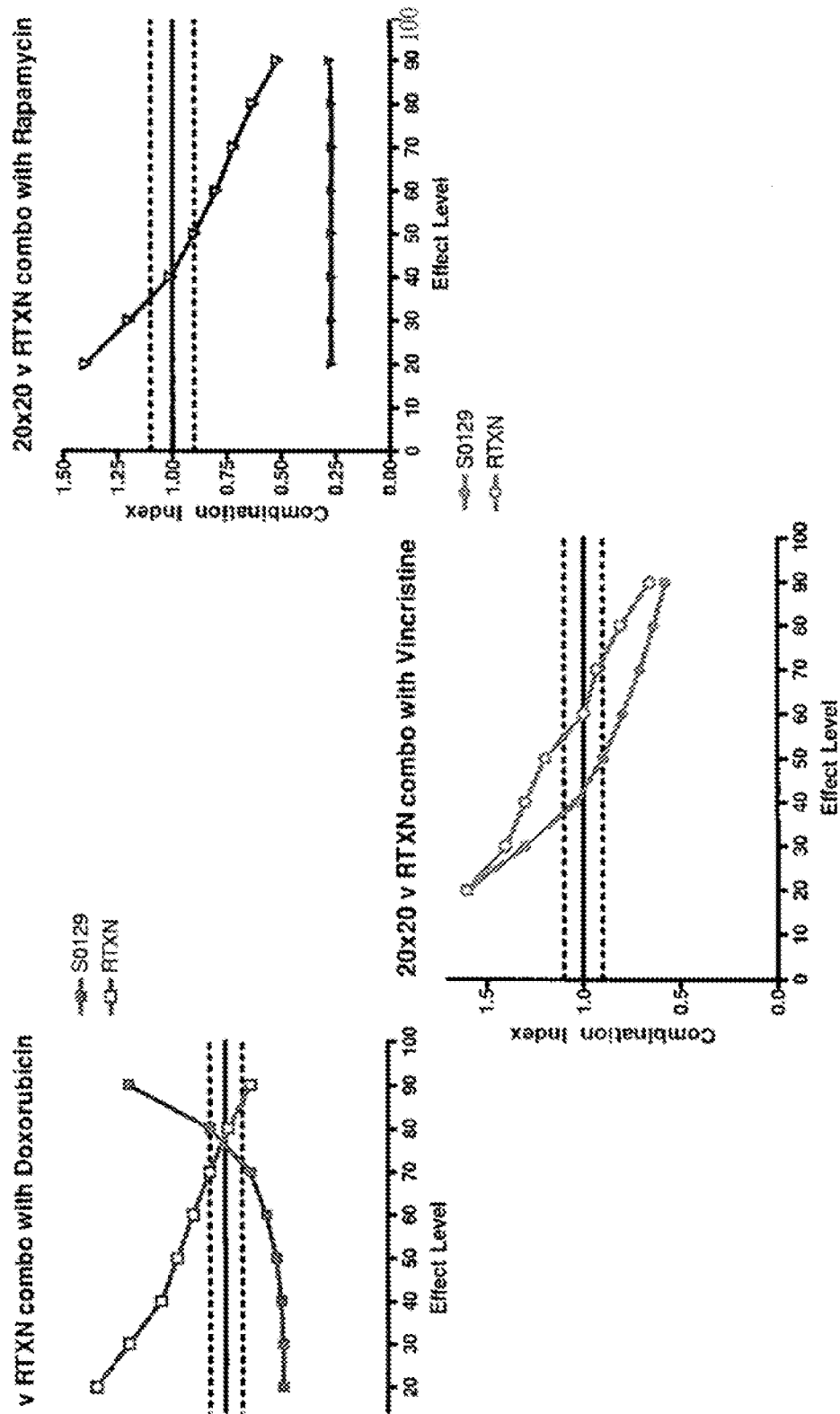


FIGURE 52



SINGLE-CHAIN MULTIVALENT BINDING PROTEINS WITH EFFECTOR FUNCTION

[0001] This application is a continuation-in-part of International (PCT) Patent Application No. PCT/US07/71052, filed Jun. 12, 2007, which claims the priority benefit of both provisional U.S. Patent Application No. 60/853,287, filed Oct. 10, 2006, and provisional U.S. Patent Application No. 60/813,261, filed Jun. 12, 2006. Each of the above-identified patent applications is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates generally to the field of multivalent binding molecules and therapeutic applications thereof.

[0003] The sequence listing is being submitted as a text file and as a PDF file in compliance with applicable requirements for electronic filing. The sequence listing is incorporated herein by reference in its entirety.

BACKGROUND

[0004] In a healthy mammal, the immune system protects the body from damage from foreign substances and pathogens. In some instances though, the immune system goes awry, producing traumatic insult and/or disease. For example, B-cells can produce antibodies that recognize self-proteins rather than foreign proteins, leading to the production of the autoantibodies characteristic of autoimmune diseases such as lupus erythematosus, rheumatoid arthritis, and the like. In other instances, the typically beneficial effect of the immune system in combating foreign materials is counterproductive, such as following organ transplantation. The power of the mammalian immune system, and in particular the human immune system, has been recognized and efforts have been made to control the system to avoid or ameliorate the deleterious consequences to health that result either from normal functioning of the immune system in an abnormal environment (e.g., organ transplantation) or from abnormal functioning of the immune system in an otherwise apparently normal environment (e.g., autoimmune disease progression). Additionally, efforts have been made to exploit the immune system to provide a number of target-specific diagnostic and therapeutic methodologies, relying on the capacity of antibodies to specifically recognize and bind antigenic targets with specificity.

[0005] One way in which the immune system protects the body is by production of specialized cells called B lymphocytes or B-cells. B-cells produce antibodies that bind to, and in some cases mediate destruction of, a foreign substance or pathogen. In some instances though, the human immune system, and specifically the B lymphocytes of the human immune system, go awry and disease results. There are numerous cancers that involve uncontrolled proliferation of B-cells. There are also numerous autoimmune diseases that involve B-cell production of antibodies that, instead of binding to foreign substances and pathogens, bind to parts of the body. In addition, there are numerous autoimmune and inflammatory diseases that involve B-cells in their pathology, for example, through inappropriate B-cell antigen presentation to T-cells or through other pathways involving B-cells. For example, autoimmune-prone mice deficient in B-cells do not develop autoimmune kidney disease, vasculitis or autoantibodies. (Shlomchik et al., *J. Exp. Med.* 1994, 180:1295-

306). Interestingly, these same autoimmune-prone mice which possess B-cells but are deficient in immunoglobulin production, do develop autoimmune diseases when induced experimentally (Chan et al., *J. Exp. Med.* 1999, 189:1639-48), indicating that B-cells play an integral role in development of autoimmune disease.

[0006] B-cells can be identified by molecules on their cell surface. CD20 was the first human B-cell lineage-specific surface molecule identified by a monoclonal antibody. It is a non-glycosylated, hydrophobic 35 kDa B-cell transmembrane phosphoprotein that has both its amino and carboxy ends situated inside the cell. Einfeld et al., *EMBO J.* 1988, 7:711-17. CD20 is expressed by all normal mature B-cells, but is not expressed by precursor B-cells or plasma cells. Natural ligands for CD20 have not been identified, and the function of CD20 in B-cell biology is still incompletely understood.

[0007] Another B-cell lineage-specific cell surface molecule is CD37. CD37 is a heavily glycosylated 40-52 kDa protein that belongs to the tetraspanin transmembrane family of cell surface antigens. It traverses the cell membrane four times forming two extracellular loops and exposing its amino and carboxy ends to the cytoplasm. CD37 is highly expressed on normal antibody-producing (slg+)B-cells, but is not expressed on pre-B-cells or plasma cells. The expression of CD37 on resting and activated T cells, monocytes and granulocytes is low and there is no detectable CD37 expression on NK cells, platelets or erythrocytes. See, Belov et al., *Cancer Res.*, 61(11):4483-4489 (2001); Schwartz-Albiez et al., *J. Immunol.*, 140(3): 905-914 (1988); and Link et al., *J. Immunol.*, 137(9): 3013-3018 (1988). Besides normal B-cells, almost all malignancies of B-cell origin are positive for CD37 expression, including CLL, NHL, and hairy cell leukemia (Moore, et al. 1987; Merson and Brochier 1988; Faure, et al. 1990). CD37 participates in regulation of B-cell function, since mice lacking CD37 were found to have low levels of serum IgG1 and to be impaired in their humoral response to viral antigens and model antigens. It appears to act as a nonclassical costimulatory molecule or by directly influencing antigen presentation via complex formation with MHC class II molecules. See Knobeloch et al., *Mol. Cell. Biol.*, 20(15):5363-5369 (2000).

[0008] Research and drug development has occurred based on the concept that B-cell lineage-specific cell surface molecules such as CD37 and CD20 can themselves be targets for antibodies that would bind to, and mediate destruction of, cancerous and autoimmune disease-causing B-cells that have CD37 and CD20 on their surfaces. Termed "immunotherapy," antibodies made (or based on antibodies made) in a non-human animal that bind to CD37 or CD20 were given to a patient to deplete cancerous or autoimmune disease-causing B-cells.

[0009] Monoclonal antibody technology and genetic engineering methods have facilitated development of immunoglobulin molecules for diagnosis and treatment of human diseases. The domain structure of immunoglobulins is amenable to engineering, in that the antigen binding domains and the domains conferring effector functions may be exchanged between immunoglobulin classes and subclasses. Immunoglobulin structure and function are reviewed, for example, in Harlow et al., Eds., *Antibodies: A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988). An extensive introduction as well as detailed information about all aspects of recombinant antibody technology

can be found in the textbook "Recombinant Antibodies" (John Wiley & Sons, NY, 1999). A comprehensive collection of detailed antibody engineering lab Protocols can be found in R. Kontermann and S. Dübel (eds.), "The Antibody Engineering Lab Manual" (Springer Verlag, Heidelberg/New York, 2000).

[0010] An immunoglobulin molecule (abbreviated Ig), is a multimeric protein, typically composed of two identical light chain polypeptides and two identical heavy chain polypeptides (H_2L_2) that are joined into a macromolecular complex by interchain disulfide bonds, i.e., covalent bonds between the sulfhydryl groups of neighboring cysteine residues. Five human immunoglobulin classes are defined on the basis of their heavy chain composition, and are named IgG, IgM, IgA, IgE, and IgD. The IgG-class and IgA-class antibodies are further divided into subclasses, namely, IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2, respectively. Intrachain disulfide bonds join different areas of the same polypeptide chain, which results in the formation of loops that, along with adjacent amino acids, constitute the immunoglobulin domains. At the amino-terminal portion, each light chain and each heavy chain has a single variable region that shows considerable variation in amino acid composition from one antibody to another. The light chain variable region, has a single antigen-binding domain and associates with the variable region of a heavy chain, V_H (also containing a single antigen-binding domain), to form the antigen binding site of the immunoglobulin, the F_v .

[0011] In addition to variable regions, each of the full-length antibody chains has a constant region containing one or more domains. Light chains have a constant region containing a single domain. Thus, light chains have one variable domain and one constant domain. Heavy chains have a constant region containing several domains. The heavy chains in IgG, IgA, and IgD antibodies have three domains, which are designated C_{H1} , C_{H2} , and C_{H3} ; the heavy chains in IgM and IgE antibodies have four domains, C_{H1} , C_{H2} , C_{H3} and C_{H4} . Thus, heavy chains have one variable domain and three or four constant domains. Noteworthy is the invariant organization of these domains in all known species, with the constant regions, containing one or more domains, being located at or near the C-terminus of both the light and heavy chains of immunoglobulin molecules, with the variable domains located towards the N-termini of the light and heavy chains. Immunoglobulin structure and function are reviewed, for example, in Harlow et al., Eds., *Antibodies: A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988).

[0012] The heavy chains of immunoglobulins can also be divided into three functional regions: the F_d region (a fragment comprising V_H and C_{H1} , i.e., the two N-terminal domains of the heavy chain), the hinge region, and the F_c region (the "fragment crystallizable" region). The F_c region contains the domains that interact with immunoglobulin receptors on cells and with the initial elements of the complement cascade. Thus, the F_c region or fragment is generally considered responsible for the effector functions of an immunoglobulin, such as ADCC (antibody-dependent cell-mediated cytotoxicity), CDC (complement-dependent cytotoxicity) and complement fixation, binding to F_c receptors, greater half-life in vivo relative to a polypeptide lacking an F_c region, protein A binding, and perhaps even placental transfer. Capon et al., *Nature*, 337: 525-531, (1989). Further, a polypeptide containing an F_c region allows for dimerization/multimeriza-

tion of the polypeptide. These terms are also used for analogous regions of the other immunoglobulins.

[0013] Although all of the human immunoglobulin isotypes contain a recognizable structure in common, each isotype exhibits a distinct pattern of effector function. IgG, by way of nonexhaustive example, neutralizes toxins and viruses, opsonizes, fixes complement (CDC) and participates in ADCC. IgM, in contrast, neutralizes blood-borne pathogens and participates in opsonization. IgA, when associated with its secretory piece, is secreted and provides a primary defense to microbial infection via the mucosa; it also neutralizes toxins and supports opsonization. IgE mediates inflammatory responses, being centrally involved in the recruitment of other cells needed to mount a full response. IgD is known to provide an immunoregulatory function, controlling the activation of B cells. These characterizations of isotype effector functions provide a non-comprehensive illustration of the differences that can be found among human isotypes.

[0014] The hinge region, found in IgG, IgA, IgD, and IgE class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses. For example, the length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 encompasses amino acids 216-231 and, because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, is relatively short, and contains a rigid poly-proline double helix, stabilized by extra inter-heavy chain disulfide bridges. These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG 1 hinge), containing 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3, the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge regions reportedly decreases in the order IgG3>IgG1>IgG4>IgG2. The four IgG subclasses also differ from each other with respect to their effector functions. This difference is related to differences in structure, including differences with respect to the interaction between the variable region, Fab fragments, and the constant Fc fragment.

[0015] According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. Shin et al., 1992 *Immunological Reviews* 130:87. The upper hinge region includes amino acids from the carboxyl end of C_{H1} to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end of the C_{H2} domain and includes residues in C_{H2} . Id. The core hinge

region of wild-type human IgG 1 contains the sequence Cys-Pro-Pro-Cys which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains five glycosylation sites within a 17-amino-acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin.

[0016] Conformational changes permitted by the structure and flexibility of the immunoglobulin hinge region polypeptide sequence may also affect the effector functions of the Fc portion of the antibody. Three general categories of effector functions associated with the Fc region include (1) activation of the classical complement cascade, (2) interaction with effector cells, and (3) compartmentalization of immunoglobulins. The different human IgG subclasses vary in the relative efficacies with which they fix complement, or activate and amplify the steps of the complement cascade. See, e.g., Kirschfink, 2001 *Immunol. Rev.* 180:177; Chakraborti et al., 2000 *Cell Signal* 12:607; Kohl et al., 1999 *Mol. Immunol.* 36:893; Marsh et al., 1999 *Curr. Opin. Nephrol. Hypertens.* 8:557; Speth et al., 1999 *Wien Klin. Wochenschr.* 111:378.

[0017] Exceptions to the H₂L₂ structure of conventional antibodies occur in some isotypes of the immunoglobulins found in camelids (camels, dromedaries and llamas; Hamers-Casterman et al., 1993 *Nature* 363:446; Nguyen et al., 1998 *J. Mol. Biol.* 275:413), nurse sharks (Roux et al., 1998 *Proc. Nat. Acad. Sci. USA* 95:11804), and in the spotted raffish (Nguyen, et al., 2002 *Immunogenetics* 54(1):39-47). These antibodies can apparently form antigen-binding regions using only heavy chain variable region, i.e., these functional antibodies are homodimers of heavy chains only (referred to as "heavy-chain antibodies" or "HCAbs"). Despite the advantages of antibody technology in disease diagnosis and treatment, there are some disadvantageous aspects of developing whole-antibody technologies as diagnostic and/or therapeutic reagents. Whole antibodies are large protein structures exemplified by the heterotetrameric structure of the IgG isotype, containing two light and two heavy chains. Such large molecules are sterically hindered in certain applications. For example, in treatments of solid tumors, whole antibodies do not readily penetrate the interior of the tumor. Moreover, the relatively large size of whole antibodies presents a challenge to ensure that the in vivo administration of such molecules does not induce an immune response. Further, generation of active antibody molecules typically involves the culturing of recombinant eukaryotic cells capable of providing appropriate post-translational processing of the nascent antibody molecules, and such cells can be difficult to culture and difficult to induce in a manner that provides commercially useful yields of active antibody.

[0018] Recently, smaller immunoglobulin molecules have been constructed to overcome problems associated with whole immunoglobulin methodologies. A single-chain variable antibody fragment (scFv) comprises an antibody heavy chain variable domain joined via a short peptide to an antibody light chain variable domain (Huston et al., *Proc. Natl. Acad. Sci. USA*, 1988, 85: 5879-83). Because of the small size of scFv molecules, they exhibit more effective penetration into tissues than whole immunoglobulin. An anti-tumor scFv showed more rapid tumor penetration and more even

distribution through the tumor mass than the corresponding chimeric antibody (Yokota et al., *Cancer Res.* 1992, 52:3402-08).

[0019] Despite the advantages that scFv molecules bring to serotherapy, several drawbacks to this therapeutic approach exist. An scFv is rapidly cleared from the circulation, which may reduce toxic effects in normal cells, but such rapid clearance impedes delivery of a minimum effective dose to the target tissue. Manufacturing adequate amounts of scFv for administration to patients has been challenging due to difficulties in expression and isolation of scFv that adversely affect the yield. During expression, scFv molecules lack stability and often aggregate due to pairing of variable regions from different molecules. Furthermore, production levels of scFv molecules in mammalian expression systems are low, limiting the potential for efficient manufacturing of scFv molecules for therapy (Davis et al, *J. Biol. Chem.* 1990, 265: 10410-18); Traunecker et al., *EMBO J.* 1991, 10: 3655-59). Strategies for improving production have been explored, including addition of glycosylation sites to the variable regions (Jost, C. R. U.S. Pat. No. 5,888,773, Jost et al, *J. Biol. Chem.* 1994, 69: 26267-73).

[0020] Another disadvantage to using scFv for therapy is the lack of effector function. An scFv without a cytolytic function, such as the antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent-cytotoxicity (CDC) associated with the constant region of an immunoglobulin, may be ineffective for treating disease. Even though development of scFv technology began over 12 years ago, currently no scFv products are approved for therapy.

[0021] Alternatively, it has been proposed that fusion of an scFv to another molecule, such as a toxin, could take advantage of the specific antigen-binding activity and the small size of an scFv to deliver the toxin to a target tissue. Chaudary et al., *Nature* 1989, 339:394; Batra et al., *Mol. Cell. Biol.* 1991, 11:2200. Conjugation or fusion of toxins to scFvs has thus been offered as an alternative strategy to provide potent, antigen-specific molecules, but dosing with such conjugates or chimeras can be limited by excessive and/or non-specific toxicity due to the toxin moiety of such preparations. Toxic effects may include supraphysiological elevation of liver enzymes and vascular leak syndrome, and other undesired effects. In addition, immunotoxins are themselves highly immunogenic upon administration to a host, and host antibodies generated against the immunotoxin limit potential usefulness for repeated therapeutic treatments of an individual.

[0022] Nonsurgical cancer therapy, such as external irradiation and chemotherapy, can suffer from limited efficacy because of toxic effects on normal tissues and cells, due to the lack of specificity these treatments exhibit towards cancer cells. To overcome this limitation, targeted treatment methodologies have been developed to increase the specificity of the treatment for the cells and tissues in need thereof. An example of such a targeted methodology for in vivo use is the administration of antibody conjugates, with the antibody designed to specifically recognize a marker associated with a cell or tissue in need of treatment, and the antibody being conjugated to a therapeutic agent, such as a toxin in the case of cancer treatment. Antibodies, as systemic agents, circulate to sensitive and undesirable body compartments, such as the bone marrow. In acute radiation injury, destruction of lymphoid and hematopoietic compartments is a major factor in the development of septicemia and subsequent death. More-

over, antibodies are large, globular proteins that can exhibit poor penetration of tissues in need of treatment.

[0023] Human patients and non-human subjects suffering from a variety of end-stage disease processes frequently require organ transplantation. Organ transplantation, however, must contend with the untoward immune response of the recipient and guard against immunological rejection of the transplanted organ by depressing the recipient's cellular immune response to the foreign organ with cytotoxic agents which affect the lymphoid and other parts of the hematopoietic system. Graft acceptance is limited by the tolerance of the recipient to these cytotoxic chemicals, many of which are similar to the anticancer (antiproliferative) agents. Likewise, when using cytotoxic antimicrobial agents, particularly antiviral drugs, or when using cytotoxic drugs for autoimmune disease therapy, e.g., in treatment of systemic lupus erythematosus, a serious limitation is the toxic effects of the therapeutic agents on the bone marrow and the hematopoietic cells of the body.

[0024] Use of targeted therapies, such as targeted antibody conjugate therapy, is designed to localize a maximum quantity of the therapeutic agent at the site of desired action as possible, and the success of such therapies is revealed by the relatively high signal-to-background ratio of therapeutic agent. Examples of targeted antibodies include diagnostic or therapeutic agent conjugates of antibody or antibody fragments, cell- or tissue-specific peptides, and hormones and other receptor-binding molecules. For example, antibodies against different determinants associated with pathological and normal cells, as well as associated with pathogenic microorganisms, have been used for the detection and treatment of a wide variety of pathological conditions or lesions. In these methods, the targeting antibody is directly conjugated to an appropriate detecting or therapeutic agent as described, for example, in Hansen et al., U.S. Pat. No. 3,927, 193 and Goldenberg, U.S. Pat. Nos. 4,331,647, 4,348,376, 4,361,544, 4,468,457, 4,444,744, 4,460,459, 4,460,561, 4,624,846 and 4,818,709.

[0025] One problem encountered in direct targeting methods, i.e., in methods wherein the diagnostic or therapeutic agent (the "active agent") is conjugated directly to the targeting moiety, is that a relatively small fraction of the conjugate actually binds to the target site, while the majority of conjugate remains in circulation and compromises in one way or another the function of the targeted conjugate. To ensure maximal localization of the active agent, an excess of the targeted conjugate is typically administered, ensuring that some conjugate will remain unbound and contribute to background levels of the active agent. A diagnostic conjugate, e.g., a radioimmunoscintigraphic or magnetic resonance imaging conjugate that does not bind its target can remain in circulation, thereby increasing background and decreasing resolution of the diagnostic technique. In the case of a therapeutic conjugate having a toxin as an active agent (e.g., a radioisotope, drug or toxic compound) attached to a long-circulating targeting moiety such as an antibody, circulating conjugate can result in unacceptable toxicity to the host, such as marrow toxicity or systemic side effects.

[0026] U.S. Pat. No. 4,782,840 discloses a method for reducing the effect of elevated background radiation levels during surgery. The method involves injection of a patient with antibodies specific for neoplastic tissue, with the antibodies labeled with radioisotopes having a suitably long half-life, such as Iodine-125. After injection of the radiolabeled

antibody, the surgery is delayed at least 7-10 days, preferably 14-21 days, to allow any unbound radiolabeled antibody to be cleared to a low background level.

[0027] U.S. Pat. No. 4,932,412 discloses methods for reducing or correcting for non-specific background radiation during intraoperative detection. The methods include the administration to a patient who has received a radiolabeled primary antibody, of a contrast agent, subtraction agent or second antibody which binds the primary antibody.

[0028] Apart from producing the antibodies described above, the immune system includes a variety of cell types that have powerful biological effects. During hematopoiesis, bone marrow-derived stem cells differentiate into either mature cells of the immune system ("B" cells) or into precursors of cells that migrate out of the bone marrow to mature in the thymus ("T" cells).

[0029] B cells are central to the humoral component of an immune response. B cells are activated by an appropriate presentation of an antigen to become antibody-secreting plasma cells; antigen presentation also results in clonal expansion of the activated B cell. B cells are primarily responsible for the humoral component of an immune response. A plasma cell typically exhibits about 10^5 antibody molecules (IgD and IgM) on its surface.

[0030] T lymphocytes can be divided into two categories. The cytotoxic T cells, Tc lymphocytes or CTLs (CD8+ T cells), kill cells bearing foreign surface antigen in association with Class I MHC and can kill cells that are harboring intracellular parasites (either bacteria or viruses) as long as the infected cell is displaying a microbial antigen on its surface. Tc cells kill tumor cells and account for the rejection of transplanted cells. Tc cells recognize antigen-Class I MHC complexes on target cells, contact them, and release the contents of granules directly into the target cell membrane, which lyses the cell.

[0031] A second category of T cells is the helper T cell or Th lymphocyte (CD4+ T cells), which produces lymphokines that are "helper" factors in the maturation of B cells into antibody-secreting plasma cells. Th cells also produce certain lymphokines that stimulate the differentiation of effector T lymphocytes and the activity of macrophages. Th1 cells recognize antigen on macrophages in association with Class II MHC and become activated (by IL-1) to produce lymphokines, including the IFN- γ that activates macrophages and NK cells. These cells mediate various aspects of the cell-mediated immunity response including delayed-type hypersensitivity reactions. Th2 cells recognize antigen in association with Class II MHC on an antigen presenting cell or APC (e.g., migratory macrophages and dendritic cells) and then produce interleukins and other substances that stimulate specific B-cell and T-cell proliferation and activity.

[0032] Beyond serving as APCs that initiate T cell interactions, development, and proliferation, macrophages are involved in expression of cell-mediated immunity because they become activated by IFN- γ produced in a cell-mediated immune response. Activated macrophages have increased phagocytic potential and release soluble substances that cause inflammation and destroy many bacteria and other cells. Natural Killer cells are cytotoxic cells that lyse cells bearing new antigen, regardless of their MHC type, and even lyse some cells that bear no MHC proteins. Natural Killer T cells, or NK cells, are defined by their ability to kill cells displaying a foreign antigen (e.g., tumor cells), regardless of MHC type, and regardless of previous sensitization (expo-

sure) to the antigen. NK cells can be activated by IL-2 and IFN- γ , and lyse cells in the same manner as cytotoxic T lymphocytes. Some NK cells have receptors for the Fc domain of the IgG antibody (e.g., CD16 or Fc γ RIII) and are thus able to bind to the Fc portion of IgG on the surface of a target cell and release cytolytic components that kill the target cell via antibody-dependent cell-mediated cytotoxicity.

[0033] Another group of cells is the granulocytes or polymorphonuclear leukocytes (PMNs). Neutrophils, one type of PMN, kill bacterial invaders and phagocytose the remains. Eosinophils are another type of PMN and contain granules that prove cytotoxic when released upon another cell, such as a foreign cell. Basophils, a third type of PMN, are significant mediators of powerful physiological responses (e.g., inflammation) that exert their effects by releasing a variety of biologically active compounds, such as histamine, serotonin, prostaglandins, and leukotrienes. Common to all of these cell types is the capacity to exert a physiological effect within an organism, frequently by killing, and optionally scavenging, deleterious compositions such as foreign cells.

[0034] Although a variety of mammalian cells, including cells of the immune system, are capable of directly exerting a physiological effect (e.g., cell killing, typified by Tc, NK, some PMN, macrophage, and the like), other cells indirectly contribute to a physiological effect. For example, initial presentation of an antigen to a naïve T cell of the immune system requires MHC presentation that mandates cell-cell contact. Further, there often needs to be contact between an activated T cell and an antigen-specific B cell to obtain a particular immunogenic response. A third form of cell-cell contact often seen in immune responses is the contact between an activated B cell and follicular dendritic cells. Each of these cell-cell contact requirements complicates the targeting of a biologically active agent to a given target.

[0035] Complement-dependent cytotoxicity (CDC) is believed to be a significant mechanism for clearance of specific target cells such as tumor cells. CDC is a series of events that consists of a collection of enzymes that become activated by each other in a cascade fashion. Complement has an important role in clearing antigen, accomplished by its four major functions: (1) local vasodilation; (2) attraction of immune cells, especially phagocytes (chemotaxis); (3) tagging of foreign organisms for phagocytosis (opsonization); and (4) destruction of invading organisms by the membrane attack complex (MAC attack). The central molecule is the C3 protein. It is an enzyme that is split into two fragments by components of either the classical pathway or the alternative pathway. The classical pathway is induced by antibodies, especially IgG and IgM, while the alternative pathway is nonspecifically stimulated by bacterial products like lipopolysaccharide (LPS). Briefly, the products of the C3 split include a small peptide C3a which is chemotactic for phagocytic immune cells and results in local vasodilation by causing the release of C5a fragment from C5. The other part of C3, C3b, coats antigens on the surface of foreign organisms and acts to opsonize the organism for destruction. C3b also reacts with other components of the complement system to form an MAC consisting of C5b, C6, C7, C8 and C9.

[0036] There are problems associated with the use of antibodies in human therapy because the response of the immune system to any antigen, even the simplest, is “polyclonal,” i.e., the system manufactures antibodies of a great range of structures both in their binding regions as well as in their effector regions.

[0037] Two approaches have been used in an attempt to reduce the problem of immunogenic antibodies. The first is the production of chimeric antibodies in which the antigen-binding part (variable regions) of a mouse monoclonal antibody is fused to the effector part (constant region) of a human antibody. In a second approach, antibodies have been altered through a technique known as complementarity determining region (CDR) grafting or “humanization.” This process has been further improved to include changes referred to as “reshaping” (Verhoeyen, et al., 1988 *Science* 239:1534-1536; Riechmann, et al., 1988 *Nature* 332:323-337; Tempest, et al., *Bio/Technol* 1991 9:266-271), “hyperchimerization” (Queen, et al., 1989 *Proc Natl Acad Sci USA* 86:10029-10033; Co, et al., 1991 *Proc Natl Acad Sci USA* 88:2869-2873; Co, et al., 1992 *J Immunol* 148:1149-1154), and “veneering” (Mark, et al., In: Metcalf B W, Dalton B J, eds. *Cellular adhesion: molecular definition to therapeutic potential*. New York: Plenum Press, 1994:291-312).

[0038] An average of less than one therapeutic antibody per year has been introduced to the market beginning in 1986, eleven years after the publication of monoclonal antibodies. Five murine monoclonal antibodies were introduced into human medicine over a ten year period from 1986-1995, including “muromonab-CD3” (OrthoClone OKT3®) for acute rejection of organ transplants; “edrecolomab” (Panorex®) for colorectal cancer; “odulimomab” (Antilfa®) for transplant rejection; and, “ibritumomab” (Zevalin® yiu-xetan) for non-Hodgkin’s lymphoma. Additionally, a monoclonal Fab, “abciximab” (ReoPro®) has been marketed for preventing coronary artery reocclusion. Three chimeric monoclonal antibodies were also launched: “rituximab” (Rituxan®) for treating B cell lymphomas; “basiliximab” (Simulect®) for transplant rejection; and “infliximab” (Remicade®) for treatment of rheumatoid arthritis and Crohn’s disease. Additionally, “abciximab” (ReoPro®), a 47.6 kD Fab fragment of a chimeric human-murine monoclonal antibody is marketed as an adjunct to percutaneous coronary intervention for the prevention of cardiac ischemic complications in patients undergoing percutaneous coronary intervention. Finally, seven “humanized” monoclonal antibodies have been launched. “Daclizumab” (Zenapax®) is used to prevent acute rejection of transplanted kidneys; “palivizumab” (Synagis®) for RSV; “trastuzumab” (Herceptin®) binds HER-2, a growth factor receptor found on breast cancers cells; “gemtuzumab” (Mylotarg®) for acute myelogenous leukemia (AML); and “alemtuzumab” (MabCampath®) for chronic lymphocytic leukemia; “adalimumab” (Humira® (D2E7)) for the treatment of rheumatoid arthritis; and, “omalizumab” (Xolair®), for the treatment of persistent asthma.

[0039] Thus, a variety of antibody technologies have received attention in the effort to develop and market more effective therapeutics and palliatives. Unfortunately, problems continue to compromise the promise of each of these therapies. For example, the majority of cancer patients treated with rituximab relapse, generally within about 6-12 months, and fatal infusion reactions within 24 hours of rituximab infusion have been reported. Acute renal failure requiring dialysis with instances of fatal outcome has also been reported in treatments with rituximab, as have severe, occasionally fatal, mucocutaneous reactions. Additionally, high doses of rituximab are required for intravenous injection

because the molecule is large, approximately 150 kDa, and diffusion into the lymphoid tissues, where many tumor cells may reside is limited.

[0040] Trastuzumab administration can result in the development of ventricular dysfunction, congestive heart failure, and severe hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events. Daclizumab immunosuppressive therapy poses an increased risk for developing lymphoproliferative disorders and opportunistic infections. Death from liver failure, arising from severe hepatotoxicity, and from veno-occlusive disease (VOD), has been reported in patients who received gemtuzumab.

[0041] Hepatotoxicity was also reported in patients receiving alemtuzumab. Serious and, in some rare instances fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab therapy. Alemtuzumab can also result in serious infusion reactions as well as opportunistic infections. In patients treated with adalimumab, serious infections and sepsis, including fatalities, have been reported, as has the exacerbation of clinical symptoms and/or radiographic evidence of demyelinating disease, and patients treated with adalimumab in clinical trials had a higher incidence of lymphoma than the expected rate in the general population. Omalizumab reportedly induces malignancies and anaphylaxis.

[0042] Cancer includes a broad range of diseases, affecting approximately one in four individuals worldwide. Rapid and unregulated proliferation of malignant cells is a hallmark of many types of cancer, including hematological malignancies. Although patients with a hematologic malignant condition have benefited from advances in cancer therapy in the past two decades, Multani et al., 1998 *J. Clin. Oncology* 16:3691-3710, and remission times have increased, most patients still relapse and succumb to their disease. Barriers to cure with cytotoxic drugs include, for example, tumor cell resistance and the high toxicity of chemotherapy, which prevents optimal dosing in many patients.

[0043] Treatment of patients with low grade or follicular B cell lymphoma using a chimeric CD20 monoclonal antibody has been reported to induce partial or complete responses in patients. McLaughlin et al., 1996 *Blood* 88:90a (abstract, suppl. 1); Maloney et al., 1997 *Blood* 90:2188-95. However, as noted above, tumor relapse commonly occurs within six months to one year. Further improvements in serotherapy are needed to induce more durable responses, for example, in low grade B cell lymphoma, and to allow effective treatment of high grade lymphoma and other B cell diseases.

[0044] Another approach has been to target radioisotopes to B cell lymphomas using monoclonal antibodies specific for CD20. While the effectiveness of therapy is reportedly increased, associated toxicity from the long in vivo half-life of the radioactive antibody increases, sometimes requiring that the patient undergo stem cell rescue. Press et al., 1993 *N. Eng. J. Med.* 329:1219-1224; Kaminski et al., 1993 *N. Eng. J. Med.* 329:459-65. Monoclonal antibodies to CD20 have also been cleaved with proteases to yield F(ab')₂ or Fab fragments prior to attachment of radioisotope. This has been reported to improve penetration of the radioisotope conjugate into the tumor and to shorten the in vivo half-life, thus reducing the toxicity to normal tissues. However, these molecules lack effector functions, including complement fixation and/or ADCC.

[0045] Autoimmune diseases include autoimmune thyroid diseases, which include Graves' disease and Hashimoto's thyroiditis. In the United States alone, there are about 20 million people who have some form of autoimmune thyroid disease. Autoimmune thyroid disease results from the production of autoantibodies that either stimulate the thyroid to cause hyperthyroidism (Graves' disease) or destroy the thyroid to cause hypothyroidism (Hashimoto's thyroiditis). Stimulation of the thyroid is caused by autoantibodies that bind and activate the thyroid stimulating hormone (TSH) receptor. Destruction of the thyroid is caused by autoantibodies that react with other thyroid antigens. Current therapy for Graves' disease includes surgery, radioactive iodine, or antithyroid drug therapy. Radioactive iodine is widely used, since antithyroid medications have significant side effects and disease recurrence is high. Surgery is reserved for patients with large goiters or where there is a need for very rapid normalization of thyroid function. There are no therapies that target the production of autoantibodies responsible for stimulating the TSH receptor. Current therapy for Hashimoto's thyroiditis is levothyroxine sodium, and lifetime therapy is expected because of the low likelihood of remission. Suppressive therapy has been shown to shrink goiters in Hashimoto's thyroiditis, but no therapies that reduce autoantibody production to target the disease mechanism are known.

[0046] Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the joints, leading to swelling, pain, and loss of function. RA affects an estimated 2.5 million people in the United States. RA is caused by a combination of events including an initial infection or injury, an abnormal immune response, and genetic factors. While autoreactive T cells and B cells are present in RA, the detection of high levels of antibodies that collect in the joints, called rheumatoid factor, is used in the diagnosis of RA. Current therapy for RA includes many medications for managing pain and slowing the progression of the disease. No therapy has been found that can cure the disease. Medications include nonsteroidal anti-inflammatory drugs (NSAIDs), and disease modifying antirheumatic drugs (DMARDs). NSAIDs are useful in benign disease, but fail to prevent the progression to joint destruction and debility in severe RA. Both NSAIDs and DMARDs are associated with significant side effects. Only one new DMARD, Leflunomide, has been approved in over 10 years. Leflunomide blocks production of autoantibodies, reduces inflammation, and slows progression of RA. However, this drug also causes severe side effects including nausea, diarrhea, hair loss, rash, and liver injury.

[0047] Systemic Lupus Erythematosus (SLE) is an autoimmune disease caused by recurrent injuries to blood vessels in multiple organs, including the kidney, skin, and joints. SLE is estimated to affect over 500,000 people in the United States. In patients with SLE, a faulty interaction between T cells and B cells results in the production of autoantibodies that attack the cell nucleus. These include anti-double stranded DNA and anti-Sm antibodies. Autoantibodies that bind phospholipids are also found in about half of SLE patients, and are responsible for blood vessel damage and low blood counts. Immune complexes accumulate in the kidneys, blood vessels, and joints of SLE patients, where they cause inflammation and tissue damage. No treatment for SLE has been found to cure the disease. NSA/DS and DMARDs are used for therapy depending upon the severity of the disease. Plasmapheresis with plasma exchange to remove autoantibodies can cause temporary improvement in SLE patients. There is general

agreement that autoantibodies are responsible for SLE, so new therapies that deplete the B cell lineage, allowing the immune system to reset as new B cells are generated from precursors, would offer hope for long lasting benefit in SLE patients.

[0048] Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands. Sjogren's syndrome is one of the most prevalent autoimmune disorders, striking up to an estimated 4 million people in the United States. About half of the people stricken with Sjogren's syndrome also have a connective tissue disease, such as RA, while the other half have primary Sjogren's syndrome with no other concurrent autoimmune disease. Autoantibodies, including anti-nuclear antibodies, rheumatoid factor, anti-fodrin, and anti-muscarinic receptor are often present in patients with Sjogren's syndrome. Conventional therapy includes corticosteroids, and additional more effective therapies would be of benefit.

[0049] Immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction. Some cases of ITP are caused by drugs, and others are associated with infection, pregnancy, or autoimmune disease such as SLE. About half of all cases are classified as being of idiopathic origin. The treatment of ITP is determined by the severity of the symptoms. In some cases, no therapy is needed although in most cases immunosuppressive drugs, including corticosteroids or intravenous infusions of immune globulin to deplete T cells, are provided. Another treatment that usually results in an increased number of platelets is removal of the spleen, the organ that destroys antibody-coated platelets. More potent immunosuppressive drugs, including cyclosporine, cyclophosphamide, or azathioprine are used for patients with severe cases. Removal of autoantibodies by passage of patients' plasma over a Protein A column is used as a second line treatment in patients with severe disease. Additional more effective therapies are needed.

[0050] Multiple sclerosis (MS) is also an autoimmune disease. It is characterized by inflammation of the central nervous system and destruction of myelin, which insulates nerve cell fibers in the brain, spinal cord, and body. Although the cause of MS is unknown, it is widely believed that autoimmune T cells are primary contributors to the pathogenesis of the disease. However, high levels of antibodies are present in the cerebrospinal fluid of patients with MS, and some predict that the B cell response leading to antibody production is important for mediating the disease. No B cell depletion therapies have been studied in patients with MS, and there is no cure for MS. Current therapy is corticosteroids, which can reduce the duration and severity of attacks, but do not affect the course of MS over time. New biotechnology interferon (IFN) therapies for MS have recently been approved but additional more effective therapies are required.

[0051] Myasthenia Gravis (MG) is a chronic autoimmune neuromuscular disorder that is characterized by weakness of the voluntary muscle groups. MG affects about 40,000 people in the United States. MG is caused by autoantibodies that bind to acetylcholine receptors expressed at neuromuscular junctions. The autoantibodies reduce or block acetylcholine receptors, preventing the transmission of signals from nerves to muscles. There is no known cure for mg. Common treatments include immunosuppression with corticosteroids, cyclosporine, cyclophosphamide, or azathioprine. Surgical removal of the thymus is often used to blunt the autoimmune response. Plasmapheresis, used to reduce autoantibody levels

in the blood, is effective in mg, but is short-lived because the production of autoantibodies continues. Plasmapheresis is usually reserved for severe muscle weakness prior to surgery. New and effective therapies would be of benefit.

[0052] Psoriasis affects approximately five million people, and is characterized by autoimmune inflammation in the skin. Psoriasis is also associated with arthritis in 30% (psoriatic arthritis). Many treatments, including steroids, uv light retinoids, vitamin D derivatives, cyclosporine, and methotrexate have been used but it is also clear that psoriasis would benefit from new and effective therapies. Scleroderma is a chronic autoimmune disease of the connective tissue that is also known as systemic sclerosis. Scleroderma is characterized by an overproduction of collagen, resulting in a thickening of the skin, and approximately 300,000 people in the United States have scleroderma, which would also benefit from new and effective therapies.

[0053] Apparent from the foregoing discussion are needs for improved compositions and methods to treat, ameliorate or prevent a variety of diseases, disorders and conditions, including cancer and autoimmune diseases.

SUMMARY

[0054] The invention satisfies at least one of the aforementioned needs in the art by providing proteins containing at least two specific binding domains, wherein those two domains are linked by a constant sub-region derived from an antibody molecule attached at its C-terminus to a linker herein referred to as a scorpion linker, and nucleic acids encoding such proteins, as well as production, diagnostic and therapeutic uses of such proteins and nucleic acids. The constant sub-region comprises a domain derived from an immunoglobulin C_{H2} domain, and preferably a domain derived from an immunoglobulin C_{H3} domain, but does not contain a domain or region derived from, or corresponding to, an immunoglobulin C_{H1} domain. Previously, it had been thought that the placement of a constant region derived from an antibody in the interior of a protein would interfere with antibody function, such as effector function, by analogy to the conventional placement of constant regions of antibodies at the carboxy termini of antibody chains. In addition, placement of a scorpion linker, which may be an immunoglobulin hinge-like peptide, C-terminal to a constant sub-region is an organization that differs from the organization of naturally occurring immunoglobulins. Placement of a constant sub-region (with a scorpion linker attached C-terminal to the constant region) in the interior of a polypeptide or protein chain in accordance with the invention, however, resulted in proteins exhibiting effector function and multivalent (mono- or multi-specific) binding capacities relatively unencumbered by steric hindrances. Moreover, the proteins exhibiting mono-specific binding capabilities have more than one binding domain recognizing the specific target and thereby, through avidity, bind more tightly and target more specifically, or selectively, that target than do binding proteins having a single binding domain. The proteins exhibiting multi-specific binding domains can exhibit greater target binding specificity, or selectivity, for the simultaneous binding of different specificities through avidity or can be designed to have a wider target specificity of cells that bear one or multiple targets than proteins having a single binding domain and than proteins having a single type of binding domain, regardless of whether there is one or more copies of that binding domain in the molecule. As will be apparent to one of skill in the art upon

consideration of this disclosure, such proteins are modular in design and may be constructed by selecting any of a variety of binding domains for binding domain 1 or binding domain 2 (or for any additional binding domains found in a particular protein according to the invention), by selecting a constant sub-region having effector function, and by selecting a scorpion linker, hinge-like (derived from a hinge region of an immunoglobulin superfamily member such as an immunoglobulin, i.e., antibody) or non-hinge like (e.g., C-type lectin stalk region peptides derived from Type II membrane proteins), with the protein exhibiting a general organization of N-binding domain 1-constant sub-region-scorpion linker-binding domain 2-C. Those of skill will further appreciate that proteins of such structure, and the nucleic acids encoding those proteins, will find a wide variety of applications, including medical and veterinary applications.

[0055] One aspect of the invention is drawn to a multivalent single-chain binding protein with effector function, or scorpion (the terms are used interchangeably), comprising a first binding domain derived from an immunoglobulin (e.g., an antibody) or an immunoglobulin-like molecule (e.g., an immunoglobulin superfamily member), a constant sub-region providing an effector function, the constant sub-region located C-terminal to the first binding domain; a scorpion linker located C-terminal to the constant sub-region; and a second binding domain derived from an immunoglobulin (such as an antibody) or immunoglobulin-like molecule, located C-terminal to the constant sub-region; thereby localizing the constant sub-region between the first binding domain and the second binding domain. The single-chain binding protein may be multispecific, e.g., bispecific in that it could bind two or more distinct targets, or it may be monospecific, with two binding sites for the same target. Moreover, all of the domains of the protein are found in a single chain, but the protein may form homo-multimers, e.g., by interchain disulfide bond formation. In some embodiments, the first binding domain and/or the second binding domain is/are derived from variable regions of light and heavy immunoglobulin chains from the same, or different, immunoglobulins (e.g., antibodies). The immunoglobulin(s) may be from any vertebrate, such as a mammal, including a human, and may be chimeric, humanized, fragments, variants or derivatives of naturally occurring immunoglobulins.

[0056] The invention contemplates proteins in which the first and second binding domains are derived from the same, or different immunoglobulins (e.g., antibodies), and wherein the first and second binding domains recognize the same, or different, molecular targets (e.g., cell surface markers, such as membrane-bound proteins). For binding domains composed of at least two regions, such as binding domains derived from immunoglobulin superfamily member binding domains, the regions may be derived from the same, or different, Ig superfamily members. Further, the first and second binding domains may recognize the same, or different, epitopes. The first and second molecular targets may be associated with first and second target cells, viruses, carriers and/or objects. In preferred embodiments according to this aspect of the invention, each of the first binding domain, second binding domain, and constant sub-region is derived from a human immunoglobulin, such as an IgG antibody. In yet other embodiments, the multivalent binding protein with effector function has at least one of the first binding domain and the second binding domain that recognizes at least one cell-free molecular target, e.g., a protein unassociated with a cell, such as a deposited

protein or a soluble protein. Cell-free molecular targets include, e.g., proteins that were never associated with a cell, e.g., administered compounds such as proteins, as well as proteins that are secreted, cleaved, present in exosomes, or otherwise discharged or separated from a cell.

[0057] The target molecules recognized by the first and second binding domains may be found on, or in association with, the same, or different, prokaryotic cells, eukaryotic cells, viruses (including bacteriophage), organic or inorganic target molecule carriers, and foreign objects. Moreover, those target molecules may be on physically distinct cells, viruses, carriers or objects of the same type (e.g., two distinct eukaryotic cells, prokaryotic cells, viruses or carriers) or those target molecules may be on cells, viruses, carriers, or objects that differ in type (e.g., a eukaryotic cell and a virus). Target cells are those cells associated with a target molecule recognized by a binding domain and includes endogenous or autologous cells as well as exogenous or foreign cells (e.g., infectious microbial cells, transplanted mammalian cells including transfused blood cells). The invention comprehends targets for the first and/or second binding domains that are found on the surface of a target cell(s) associated with a disease, disorder or condition of a mammal such as a human. Exemplary target cells include a cancer cell, a cell associated with an autoimmune disease or disorder, and an infectious cell (e.g., an infectious bacterium). A cell of an infectious organism, such as a mammalian parasite, is also contemplated as a target cell. In some embodiments, a protein of the invention is a multivalent (e.g., multispecific) binding protein with effector function wherein at least one of the first binding domain and the second binding domain recognizes a target selected from the group consisting of a tumor antigen, a B-cell target, a TNF receptor superfamily member, a Hedgehog family member, a receptor tyrosine kinase, a proteoglycan-related molecule, a TGF-beta superfamily member, a Wnt-related molecule, a receptor ligand, a T-cell target, a Dendritic cell target, an NK cell target, a monocyte/macrophage cell target and an angiogenesis target.

[0058] In some embodiments of the above-described protein, the tumor antigen is selected from the group consisting of SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1), (PROTEIN T4-A), SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA-2), Ovarian carcinoma antigen CA125 (1A1-3B) (KIAA0049), MUCIN 1 (TUMOR-ASSOCIATED MUCIN), (CARCINOMA-ASSOCIATED MUCIN), (POLYMORPHIC EPITHELIAL MUCIN), (PEM), (PEMT), (EPISIALIN), (TUMOR-ASSOCIATED EPITHELIAL MEMBRANE ANTIGEN), (EMA), (H23AG), (PEANUT-REACTIVE URINARY MUCIN), (PUM), (BREAST CARCINOMA-ASSOCIATED ANTIGEN DF3), CTCL tumor antigen se1-1, CTCL tumor antigen se14-3, CTCL tumor antigen se20-4, CTCL tumor antigen se20-9, CTCL tumor antigen se33-1, CTCL tumor antigen se37-2, CTCL tumor antigen se57-1, CTCL tumor antigen se89-1, Prostate-specific membrane antigen, 5T4 oncofetal trophoblast glycoprotein, Orf73 Kaposi's sarcoma-associated herpesvirus, MAGE-C1 (cancer/testis antigen CT7), MAGE-B1 ANTIGEN (MAGE-XP ANTIGEN) (DAM 10), MAGE-B2 ANTIGEN (DAM6), MAGE-2 ANTIGEN, MAGE-4a antigen, MAGE-4b antigen, Colon cancer antigen NY-CO-45, Lung cancer antigen NY-LU-12 variant A, Cancer associated surface antigen, Adenocarcinoma antigen ART1, Paraneoplastic associated brain-testis-cancer antigen (onconeural antigen MA2; paraneoplastic neuronal anti-

gen), Neuro-oncological ventral antigen 2 (NOVA2), Hepatocellular carcinoma antigen gene 520, TUMOR-ASSOCIATED ANTIGEN CO-029, Tumor-associated antigen MAGE-X2, Synovial sarcoma, X breakpoint 2, Squamous cell carcinoma antigen recognized by T cell, Serologically defined colon cancer antigen 1, Serologically defined breast cancer antigen NY-BR-15, Serologically defined breast cancer antigen NY-BR-16, Chromogranin A; parathyroid secretory protein 1, DUPAN-2, CA 19-9, CA 72-4, CA 195 and L6.

[0059] Embodiments of the above-described method comprise a B cell target selected from the group consisting of CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD38, CD39, CD40, CD72, CD73, CD74, CDw75, CDw76, CD77, CD78, CD79a/b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD89, CD98, CD126, CD127, CDw130, CD138 and CDw150.

[0060] In other embodiments of the above-described method, the TNF receptor superfamily member is selected from the group consisting of 4-1BB/TNFRSF9, NGF R/TNFRSF16, BAFF R/TNFRSF13C, Osteoprotegerin/TNFRSF11B, BCMA/TNFRSF17, OX40/TNFRSF4, CD27/TNFRSF7, RANK/TNFRSF11A, CD30/TNFRSF8, RELT/TNFRSF19L, CD40/TNFRSF5, TACI/TNFRSF13B, DcR3/TNFRSF6B, TNF RI/TNFRSF1A, DcTRAIL R1/TNFRSF23, TNF RII/TNFRSF1B, DcTRAIL R2/TNFRSF22, TRAIL R1/TNFRSF10A, DR3/TNFRSF25, TRAIL R2/TNFRSF10B, DR6/TNFRSF21, TRAIL R3/TNFRSF10C, EDAR, TRAIL R4/TNFRSF10D, Fas/TNFRSF6, TROY/TNFRSF19, GITR/TNFRSF18, TWEAK R/TNFRSF12, HVEM/TNFRSF14, XEDAR, Lymphotoxin beta R/TNFRSF3, 4-1BB Ligand/TNFSF9, Lymphotoxin, APRIL/TNFSF13, Lymphotoxin beta/TNFSF3, BAFF/TNFSF13C, OX40 Ligand/TNFSF4, CD27 Ligand/TNFSF7, TL1A/TNFSF15, CD30 Ligand/TNFSF8, TNF-alpha/TNFSF1A, CD40 Ligand/TNFSF5, TNF-beta/TNFSF1B, EDA-A2, TRAIL/TNFSF10, Fas Ligand/TNFSF6, TRANCE/TNFSF11, GITR Ligand/TNFSF18, TWEAK/TNFSF12 and LIGHT/TNFSF14.

[0061] The above-described method also includes embodiments in which the Hedgehog family member is selected from the group consisting of Patched and Smoothened. In yet other embodiments, the proteoglycan-related molecule is selected from the group consisting of proteoglycans and regulators thereof.

[0062] Additional embodiments of the method are drawn to processes in which the receptor tyrosine kinase is selected from the group consisting of Ax1, FGF R4, C1q R1/CD93, FGF R5, DDR1, Flt-3, DDR2, HGF R, Dtk, IGF-I R, EGF R, IGF-II R, Eph, INSR, EphA1, Insulin R/CD220, EphA2, M-CSF R, EphA3, Mer, EphA4, MSP R/Ron, EphA5, MuSK, EphA6, PDGF R alpha, EphA7, PDGF R beta, EphA8, Ret, EphB1, ROR1, EphB2, ROR2, EphB3, SCF R/c-kit, EphB4, Tie-1, EphB6, Tie-2, ErbB2, TrkA, ErbB3, TrkB, ErbB4, TrkC, FGF R1, VEGF R1/Flt-1, FGF R2, VEGF R2/Flk-1, FGF R3 and VEGF R3/Flt-4.

[0063] In other embodiments of the method, the Transforming Growth Factor (TGF)-beta superfamily member is selected from the group consisting of Activin RIA/ALK-2, GFR alpha-1, Activin RIB/ALK-4, GFR alpha-2, Activin RIIA, GFR alpha-3, Activin RIIB, GFR alpha-4, ALK-1, MIS RII, ALK-7, Ret, BMPR-IA/ALK-3, TGF-beta RI/ALK-5, BMPR-IB/ALK-6, TGF-beta RII, BMPR-II, TGF-beta Endoglin/CD105 and TGF-beta RIII.

[0064] Yet other embodiments of the method comprise a Wnt-related molecule selected from the group consisting of Frizzled-1, Frizzled-8, Frizzled-2, Frizzled-9, Frizzled-3, sFRP-1, Frizzled-4, sFRP-2, Frizzled-5, sFRP-3, Frizzled-6, sFRP-4, Frizzled-7, MFRP, LRP 5, LRP 6, Wnt-1, Wnt-8a, Wnt-3a, Wnt-10b, Wnt-4, Wnt-11, Wnt-5a, Wnt-9a and Wnt-7a.

[0065] In other embodiments of the method, the receptor ligand is selected from the group consisting of 4-1BB Ligand/TNFSF9, Lymphotoxin, APRIL/TNFSF13, Lymphotoxin beta/TNFSF3, BAFF/TNFSF13C, OX40 Ligand/TNFSF4, CD27 Ligand/TNFSF7, TL1A/TNFSF15, CD30 Ligand/TNFSF8, TNF-alpha/TNFSF1A, CD40 Ligand/TNFSF5, TNF-beta/TNFSF1B, EDA-A2, TRAIL/TNFSF10, Fas Ligand/TNFSF6, TRANCE/TNFSF11, GITR Ligand/TNFSF18, TWEAK/TNFSF12, LIGHT/TNFSF14, Amphiregulin, NRG1 isoform GGF2, Betacellulin, NRG1 Isoform SMDF, EGF, NRG1-alpha/HRG1-alpha, Epigen, NRG1-beta 1/HRG1-beta 1, Epiregulin, TGF-alpha, HB-EGF, TMEFF1/Tomoregulin-1, Neuregulin-3, TMEFF2, IGF-I, IGF-II, Insulin, Activin A, Activin B, Activin AB, Activin C, BMP-2, BMP-7, BMP-3, BMP-8, BMP-3b/GDF-10, BMP-9, BMP-4, BMP-15, BMP-5, Decapentaplegic, BMP-6, GDF-1, GDF-8, GDF-3, GDF-9, GDF-5, GDF-11, GDF-6, GDF-15, GDF-7, Artemin, Neurturin, GDNF, Persephin, TGF-beta, TGF-beta 2, TGF-beta 1, TGF-beta 3, LAP (TGF-beta 1), TGF-beta 5, Latent TGF-beta 1, Latent TGF-beta bp1, TGF-beta 1.2, Lefty, Nodal, MIS/AMH, FGF acidic, FGF-12, FGF basic, FGF-13, FGF-3, FGF-16, FGF-4, FGF-17, FGF-5, FGF-19, FGF-6, FGF-20, FGF-8, FGF-21, FGF-9, FGF-23, FGF-10, KGF/FGF-7, FGF-11, Neuropilin-1, P1GF, Neuropilin-2, P1GF-2, PDGF, PDGF-A, VEGF, PDGF-B, VEGF-B, PDGF-C, VEGF-C, PDGF-D, VEGF-D and PDGF-AB.

[0066] In still other embodiments, the T-cell target is selected from the group consisting of 2B4/SLAMF4, IL-2 R alpha, 4-1BB/TNFRSF9, IL-2 R beta, ALCAM, B7-1/CD80, IL-4 R, B7-H3, BLAME/SLAMF8, BTLA, IL-6 R, CCR3, IL-7 R alpha, CCR4, CXCR1/IL-8 RA, CCR5, CCR6, IL-10 R alpha, CCR7, IL-10 R beta, CCR8, IL-12 R beta 1, CCR9, IL-12 R beta 2, CD2, IL-13 R alpha 1, IL-13, CD3, CD4, ILT2/CD85j, ILT3/CD85k, ILT4/CD85d, ILT5/CD85a, Integrin alpha 4/CD49d, CD5, Integrin alpha E/CD103, CD6, Integrin alpha M/CD11b, CD8, Integrin alpha X/CD11c, Integrin beta 2/CD18, KIR/CD158, CD27/TNFRSF7, KIR2DL1, CD28, KIR2DL3, CD30/TNFRSF8, KIR2DL4/CD158d, CD31/PECAM-1, KIR2DS4, CD40 Ligand/TNFSF5, LAG-3, CD43, LAIR1, CD45, LAIR2, CD83, Leukotriene B4 R1, CD84/SLAMF5, NCAM-L1, CD94, NKG2A, CD97, NKG2C, CD229/SLAMF3, NKG2D, CD2F-10/SLAMF9, NT-4, CD69, NTB-A/SLAMF6, Common gamma Chain/IL-2 R gamma, Osteopontin, CRACC/SLAMF7, PD-1, CRTAM, PSGL-1, CTLA-4, RANK/TNFRSF11A, CX3CR1, CX3CL1, L-Selectin, CXCR3, SIRP beta 1, CXCR4, SLAM, CXCR6, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, Fas/TNFRSF6, TIM-3, Fas Ligand/TNFSF6, TIM-4, Fc gamma RIII/CD16, TIM-6, GITR/TNFRSF18, TNF RI/TNFRSF1A, Granulysin, TNF RII/TNFRSF1B, HVEM/TNFRSF14, TRAIL R1/TNFRSF10A, ICAM-1/CD54, TRAIL R2/TNFRSF10B, ICAM-2/CD102, TRAIL R3/TNFRSF10C, IFN-gamma R1, TRAIL R4/TNFRSF10D, IFN-gamma R2, TSLP, IL-1 RI and TSLP R.

[0067] In other embodiments, the NK cell receptor is selected from the group consisting of 2B4/SLAMF4,

KIR2DS4, CD155/PVR, KIR3DL1, CD94, LMIR1/CD300A, CD69, LMIR2/CD300c, CRACC/SLAMF7, LMIR3/CD300LF, DNAM-1, LMIR5/CD300LB, Fc epsilon RII, LMIR6/CD300LE, Fc gamma RI/CD64, MICA, Fc gamma RIIb/CD32b, MICB, Fc gamma RIIc/CD32c, MULT-1, Fc gamma RIIa/CD32a, Nectin-2/CD112, Fc gamma RIII/CD16, NKG2A, FcRH1/IRTA5, NKG2C, FcRH2/IRTA4, NKG2D, FcRH4/IRTA1, NKp30, FcRH5/IRTA2, NKp44, Fc Receptor-like 3/CD16-2, NKp46/NCR1, NKp80/KLRF1, NTB-A/SLAMF6, Rae-1, Rae-1 alpha, Rae-1 beta, Rae-1 delta, H60, Rae-1 epsilon, ILT2/CD85j, Rae-1 gamma, ILT3/CD85k, TREM-1, ILT4/CD85d, TREM-2, ILT5/CD85a, TREM-3, KIR/CD158, TREML1/TLT-1, KIR2DL1, ULBP-1, KIR2DL3, ULBP-2, KIR2DL4/CD158d and ULBP-3.

[0068] In other embodiments, the monocyte/macrophage cell target is selected from the group consisting of B7-1/CD80, ILT4/CD85d, B7-H1, ILT5/CD85a, Common beta Chain, Integrin alpha 4/CD49d, BLAME/SLAMF8, Integrin alpha X/CD11c, CCL6/C10, Integrin beta 2/CD18, CD155/PVR, Integrin beta 3/CD61, CD31/PECAM-1, Latexin, CD36/SR-B3, Leukotriene B4 R1, CD40/TNFRSF5, LIMP2/SR-B2, CD43, LMIR1/CD300A, CD45, LMIR2/CD300c, CD68, LMIR3/CD300LF, CD84/SLAMF5, LMIR5/CD300LB, CD97, LMIR6/CD300LE, CD163, LRP-1, CD2F-10/SLAMF9, MARCO, CRACC/SLAMF7, MD-1, ECF-L, MD-2, EMMPRIN/CD147, MGL2, Endoglin/CD105, Osteoactivin/GPNMB, Fc gamma R1/CD64, Osteopontin, Fc gamma RIIb/CD32b, PD-L2, Fc gamma RIIc/CD32c, Siglec-3/CD33, Fc gamma RIIa/CD32a, SIGNR1/CD209, Fc gamma RIII/CD16, SLAM, GM-CSF R alpha, TCCR/WSX-1, ICAM-2/CD102, TLR3, IFN-gamma R1, TLR4, IFN-gamma R2, TREM-1, IL-1 MI, TREM-2, ILT2/CD85j, TREM-3, ILT3/CD85k, TREML1/TLT-1, 2B4/SLAMF4, IL-10 R alpha, ALCAM, IL-10 R beta, Aminopeptidase N/ANPEP, ILT2/CD85j, Common beta Chain, ILT3/CD85k, C1q R1/CD93, ILT4/CD85d, CCR1, ILT5/CD85a, CCR2, Integrin alpha 4/CD49d, CCR5, Integrin alpha M/CD11b, CCR8, Integrin alpha X/CD11c, CD155/PVR, Integrin beta 2/CD18, CD14, Integrin beta 3/CD61, CD36/SR-B3, LAIR1, CD43, LAIR2, CD45, Leukotriene B4 R1, CD68, LIMP2/SR-B2, CD84/SLAMF5, LMIR1/CD300A, CD97, LMIR2/CD300c, CD163, LMIR3/CD300LF, Coagulation Factor III/Tissue Factor, LMIR5/CD300LB, CX3CR1, CX3CL1, LMIR6/CD300LE, CXCR4, LRP-1, CXCR6, M-CSF R, DEP-1/CD148, MD-1, DNAM-1, MD-2, EMMPRIN/CD147, MMR, Endoglin/CD105, NCAM-L1, Fc gamma R1/CD64, PSGL-1, Fc gamma RIII/CD16, RP105, G-CSF R, L-Selectin, GM-CSF R alpha, Siglec-3/CD33, HVEM/TNFRSF14, SLAM, ICAM-1/CD54, TCCR/WSX-1, ICAM-2/CD102, TREM-1, IL-6 R, TREM-2, CXCR1/IL-8 RA, TREM-3 and TREML1/TLT-1.

[0069] In yet other embodiments of the method, a Dendritic cell target is selected from the group consisting of CD36/SR-B3, LOX-1/SR-E1, CD68, MARCO, CD163, SR-AI/MSR, CD5L, SREC-I, CL-P1/COLEC12, SREC-II, LIMP2/SR-B2, RP105, TLR4, TLR1, TLR5, TLR2, TLR6, TLR3, TLR9, 4-1BB Ligand/TNFRSF9, IL-12/IL-23 p40, 4-Amino-1,8-naphthalimide, ILT2/CD85j, CCL21/6CKine, ILT3/CD85k, 8-oxo-dG, ILT4/CD85d, 8D6A, ILT5/CD85a, A2B5, Integrin alpha 4/CD49d, Aag, Integrin beta 2/CD18, AMICA, Langerin, B7-2/CD86, Leukotriene B4 R1, B7-H3, LMIR1/CD300A, BLAME/SLAMF8, LMIR2/CD300c, C1q R1/CD93, LMIR3/CD300LF, CCR6, LMIR5/CD300LB,

CCR7, LMIR6/CD300LE, CD40/TNFRSF5, MAG/Siglec-4a, CD43, MCAM, CD45, MD-1, CD68, MD-2, CD83, MDL-1/CLEC5A, CD84/SLAMF5, MMR, CD97, NCAM-L1, CD2F-10/SLAMF9, Osteoactivin/GPNMB, Chem 23, PD-L2, CLEC-1, RP105, CLEC-2, Siglec-2/CD22, CRACC/SLAMF7, Siglec-3/CD33, DC-SIGN, Siglec-5, DC-SIGNR/CD299, Siglec-6, DCAR, Siglec-7, DCIR/CLEC4A, Siglec-9, DEC-205, Siglec-10, Dectin-1/CLEC7A, Siglec-F, Dectin-2/CLEC6A, SIGNR1/CD209, DEP-1/CD148, SIGNR4, DLEC, SLAM, EMMPRIN/CD147, TCCR/WSX-1, Fc gamma R1/CD64, TLR3, Fc gamma RIIb/CD32b, TREM-1, Fc gamma RIIc/CD32c, TREM-2, Fc gamma RIIa/CD32a, TREM-3, Fc gamma RIII/CD16, TREML1/TLT-1, ICAM-2/CD102 and Vanilloid R1.

[0070] In still other embodiments of the method, the angiogenesis target is selected from the group consisting of Angiopoietin-1, Angiopoietin-like 2, Angiopoietin-2, Angiopoietin-like 3, Angiopoietin-3, Angiopoietin-like 7/CDT6, Angiopoietin-4, Tie-1, Angiopoietin-like 1, Tie-2, Angiogenin, iNOS, Coagulation Factor III/Tissue Factor, nNOS, CTGF/CCN2, NOV/CCN3, DANCE, OSM, EDG-1, Plfr, EG-VEGF/PK1, Proliferin, Endostatin, ROBO4, Erythropoietin, Thrombospondin-1, Kininostatin, Thrombospondin-2, MFG-E8, Thrombospondin-4, Nitric Oxide, VG5Q, eNOS, EphA1, EphA5, EphA2, EphA6, EphA3, EphA7, EphA4, EphA8, EphB1, EphB4, EphB2, EphB6, EphB3, Ephrin-A1, Ephrin-A4, Ephrin-A2, Ephrin-A5, Ephrin-A3, Ephrin-B1, Ephrin-B3, Ephrin-B2, FGF acidic, FGF-12, FGF basic, FGF-13, FGF-3, FGF-16, FGF-4, FGF-17, FGF-5, FGF-19, FGF-6, FGF-20, FGF-8, FGF-21, FGF-9, FGF-23, FGF-10, KGF/FGF-7, FGF-11, FGF R1, FGF R4, FGF R2, FGF R5, FGF R3, Neuropilin-1, Neuropilin-2, Semaphorin 3A, Semaphorin 6B, Semaphorin 3C, Semaphorin 6C, Semaphorin 3E, Semaphorin 6D, Semaphorin 6A, Semaphorin 7A, MMP, MMP-11, MMP-12, MMP-12, MMP-2, MMP-13, MMP-3, MMP-14, MMP-7, MMP-15, MMP-8, MMP-16/MT3-MMP, MMP-9, MMP-24/MT5-MMP, MMP-10, MMP-25/MT6-MMP, TIMP-1, TIMP-3, TIMP-2, TIMP-4, ACE, IL-13 R alpha 1, IL-13, C1q R1/CD93, Integrin alpha 4/CD49d, VE-Cadherin, Integrin beta 2/CD18, CD31/PECAM-1, KLF4, CD36/SR-B3, LYVE-1, CD151, MCAM, CL-P1/COLEC12, Nectin-2/CD112, Coagulation Factor III/Tissue Factor, E-Selectin, D6, P-Selectin, DC-SIGNR/CD299, SLAM, EMMPRIN/CD147, Tie-2, Endoglin/CD105, TNF R1/TNFRSF1A, EPCR, TNF R11/TNFRSF1B, Erythropoietin R, TRAIL R1/TNFRSF10A, ESAM, TRAIL R2/TNFRSF10B, FABP5, VCAM-1, ICAM-1/CD54, VEGF R2/Flk-1, ICAM-2/CD102, VEGF R3/Flt-4, IL-1 RI and VG5Q.

[0071] Other embodiments of the method provide multivalent binding proteins wherein at least one of binding domain 1 and binding domain 2 specifically binds a target selected from the group consisting of Prostate-specific Membrane Antigen (Folate Hydrolase 1), Epidermal Growth Factor Receptor (EGFR), Receptor for Advanced Glycation End products (RAGE, also known as Advanced Glycosylation End product Receptor or AGER), IL-17 A, IL-17 F, P19 (IL23A and IL12B), Dickkopf-1 (Dkk1), NOTCH1, NG2 (Chondroitin Sulfate Proteoglycan 4 or CSPG4), IgE (IgHE or IgH2), IL-22R (IL22RA1), IL-21, Amyloid β oligomers (Ab oligomers), Amyloid β Precursor Protein (APP), NOGO Receptor (RTN4R), Low Density Lipoprotein Receptor-Related Protein 5 (LRP5), IL-4, Myostatin (GDF8), Very Late Antigen 4, an alpha 4, beta 1 integrin (VLA4 or ITGA4), an

alpha 4, beta 7 integrin found on leukocytes, and IGF-1R. For example, a VLA4 target may be recognized by a multivalent binding protein in which at least one of binding domain 1 and binding domain 2 is a binding domain derived from Natalizumab (Antegren).

[0072] In some embodiments, the cancer cell is a transformed, or cancerous, hematopoietic cell. In certain of these embodiments, at least one of the first binding domain and the second binding domain recognizes a target selected from the group consisting of a B-cell target, a monocyte/macrophage target, a dendritic cell target, an NK-cell target and a T-cell target, each as herein defined. Further, at least one of the first binding domain and the second binding domain can recognize a myeloid cell target, including but not limited to, CD5, CD10, CD11b, CD11c, CD13, CD14, CD15, CD18, CD21, CD23, CD25, CD27, CD29, CD30, CD31, CD33, CD34, CD35, CD38, CD43, CD45, CD64, CD66, CD68, CD70, CD80, CD86, CD87, CD88, CD89, CD98, CD100, CD103, CD111, CD112, CD114, CD115, CD116, CD117, CD118, CD119, CD120a, CD120b, CDw123, CDw131, CD141, CD162, CD163, CD177, CD312, IRTA1, IRTA2, IRTA3, IRTA4, IRTA5, B-B2, B-B8, and a MHC Class II.

[0073] Other embodiments of the invention are drawn to the multivalent binding protein, as described herein, comprising a sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 103, 105, 107, 109, 333, and 334.

[0074] In other embodiments, the multivalent and multi-specific binding protein with effector function has a first binding domain and a second binding domain that recognize a target pair selected from the group consisting of EPHB4-KDR and TIE-TEK. In such embodiments, the protein has a first binding domain recognizing EPHB4 and a second binding domain recognizing KDR or a first binding domain recognizing KDR and a second binding domain recognizing EPHB4. Analogously, the protein may have a first binding domain recognizing TIE and a second binding domain recognizing TEK, or a first binding domain recognizing TEK and a second binding domain recognizing TIE.

[0075] In a related aspect, the invention provides a multivalent binding protein with effector function, wherein the constant sub-region recognizes an effector cell Fc receptor (e.g., $F_{C\gamma}RI$, $F_{C\gamma}RII$, $F_{C\gamma}RIII$, $F_{C\alpha}R$, and $F_{C\epsilon}RI$). In particular embodiments, the constant sub-region recognizes an effector cell surface protein selected from the group consisting of CD16, CD32, CD64, CD89, $F_{C\epsilon}RI$, and $F_{C\gamma}Rn$. The constant sub-region may comprise a C_{H2} domain and a C_{H3} domain derived from the same, or different, immunoglobulins, antibody isotypes, or allelic variants. In some embodiments, the C_{H3} domain is truncated and comprises a C-terminal sequence selected from the group consisting of SEQ ID NOS: 366, 367, 368, 369, 370 and 371. Preferably, the C_{H2} domain and the scorpion linker are derived from the same class, or from the same sub-class, of immunoglobulin, when the linker is a hinge-like peptide derived from an immunoglobulin.

[0076] Some proteins according to the invention are also contemplated as further comprising a scorpion linker of at least about 5 amino acids attached to the constant sub-region and attached to the second binding domain, thereby localizing the scorpion linker between the constant sub-region and the second binding domain. Typically, the scorpion linker peptide length is between 5-45 amino acids. Scorpion linkers include peptides derived from interdomain regions of an immunoglobulin superfamily member, e.g., hinge-like peptides derived from immunoglobulin hinge regions, such as IgG1, IgG2,

IgG3, IgG4, IgA, and IgE hinge regions. Preferably, a hinge-like scorpion linker will retain at least one cysteine capable of forming an interchain disulfide bond under physiological conditions. Scorpion linkers derived from IgG1 may have 1 cysteine or two cysteines, and will preferably retain the cysteine corresponding to an N-terminal hinge cysteine of wild-type IgG1. Non-hinge-like peptides are also contemplated as scorpion linkers, provided that such peptides provide sufficient spacing and flexibility to provide a single-chain protein capable of forming two binding domains, one located towards each protein terminus (N and C) relative to a more centrally located constant sub-region domain. Exemplary non-hinge-like scorpion linkers include peptides from the stalk region of C-type lectin stalk regions of Type II membrane proteins, such as the stalk regions of CD69, CD72, CD94, NKG2A and NKG2D. In some embodiments, the scorpion linker comprises a sequence selected from the group consisting of SEQ ID NOS:373, 374, 375, 376, 377, 380 and 381.

[0077] The proteins may also comprise an N-terminal proximal linker (relative to the scorpion linker) of at least about 5 amino acids attached to the constant sub-region and attached to the first binding domain, thereby localizing the linker between the constant sub-region and the first binding domain. In some embodiments, linkers are found between the constant sub-region and each of the two binding domains, and those linkers may be of the same or different sequence, and of the same or different lengths.

[0078] The constant sub-region of the proteins according to the invention provides at least one effector function. Any effector function known in the art to be associated with an immunoglobulin (e.g., an antibody) is contemplated, such as an effector function selected from the group consisting of antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), relatively extended in vivo half-life (relative to the same molecule lacking a constant sub-region), FcR binding, protein A binding, and the like. In some embodiments, the extended half-lives of proteins of the invention are at least 28 hours in a human. Of course, proteins intended for administration to non-human subjects will exhibit relatively extended half-lives in those non-human subjects, and not necessarily in humans.

[0079] In general, the proteins (including polypeptides and peptides) of the invention exhibit a binding affinity of less than 10^{-9} M, or at least 10^{-6} M, for at least one of the first binding domain and the second binding domain.

[0080] Another aspect of the invention is drawn to a pharmaceutical composition comprising a protein as described herein and a pharmaceutically acceptable adjuvant, carrier or excipient. Any adjuvant, carrier, or excipient known in the art is useful in the pharmaceutical compositions of the invention.

[0081] Yet another aspect of the invention provides a method of producing a protein as described above comprising introducing a nucleic acid encoding the protein into a host cell and incubating the host cell under conditions suitable for expression of the protein, thereby expressing the protein, preferably at a level of at least 1 mg/liter. In some embodiments, the method further comprises isolating the protein by separating it from at least one protein with which it is associated upon intracellular expression. Suitable host cells for expressing the nucleic acids to produce the proteins of the invention include, but are not limited to, a host cell selected from the group consisting of a VERO cell, a HeLa cell, a CHO cell, a COS cell, a W138 cell, a BHK cell, a HepG2 cell, a 3T3

cell, a RIN cell, an MDCK cell, an A549 cell, a PC12 cell, a K562 cell, a HEK293 cell, an N cell, a *Spodoptera frugiperda* cell, a *Saccharomyces cerevisiae* cell, a *Pichia pastoris* cell, any of a variety of fungal cells and any of a variety of bacterial cells (including, but not limited to, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and a *Streptomyces*).

[0082] The invention also provides a method of producing a nucleic acid encoding the protein, as described above, comprising covalently linking the 3' end of a polynucleotide encoding a first binding domain derived from an immunoglobulin variable region to the 5' end of a polynucleotide encoding a constant sub-region, covalently linking the 5' end of a polynucleotide encoding a scorpion linker to the 3' end of the polynucleotide encoding the constant sub-region, and covalently linking the 5' end of a polynucleotide encoding a second binding domain derived from an immunoglobulin variable region to the 3' end of the polynucleotide encoding the scorpion linker, thereby generating a nucleic acid encoding a multivalent binding protein with effector function. Each of these coding regions may be separated by a coding region for a linker or hinge-like peptide as part of a single-chain structure according to the invention. In some embodiments, the method produces a polynucleotide encoding a first binding domain that comprises a sequence selected from the group consisting of SEQ ID NO: 2 (anti-CD20 variable region, oriented V_L - V_H), SEQ ID NO: 4 (anti-CD28 variable region, oriented V_L - V_H) and SEQ ID NO: 6 (anti-CD28 variable region, oriented V_H - V_L) in single-chain form, rather than requiring assembly from separately encoded polypeptides as must occur for heteromultimeric proteins, including natural antibodies. Exemplary polynucleotide sequences encoding first binding domains are polynucleotides comprising any of SEQ ID NOS: 1, 3 or 5.

[0083] This aspect of the invention also provides methods for producing encoding nucleic acids that further comprise a linker polynucleotide inserted between the polynucleotide encoding a first binding domain and the polynucleotide encoding a constant sub-region, the linker polynucleotide encoding a peptide linker of at least 5 amino acids. Additionally, these methods produce nucleic acids that further comprise a linker polynucleotide inserted between the polynucleotide encoding a constant sub-region and the polynucleotide encoding a second binding domain, the linker polynucleotide encoding a peptide linker of at least 5 amino acids. Preferably, the encoded peptide linkers are between 5 and 45 amino acids.

[0084] The identity of the linker regions present either between BD1 and EFD or EFD and BD2 may be derived from other sequences identified from homologous -Ig superfamily members. In developing novel linkers derived from existing sequences present in homologous members of the -Ig superfamily, it may be preferable to avoid sequence stretches similar to those located between the end of a C-like domain and the transmembrane domain, since such sequences are often substrates for protease cleavage of surface receptors from the cell to create soluble forms. Sequence comparisons between different members of the -Ig superfamily and subfamilies can be compared for similarities between molecules in the linker sequences that join multiple V-like domains or between the V and C like domains. From this analysis, conserved, naturally occurring sequence patterns may emerge; these sequences when used as the linkers between subdomains of the multivalent fusion proteins should be more protease resistant, might facilitate proper folding between Ig loop regions, and

would not be immunogenic since they occur in the extracellular domains of endogenous cell surface molecules.

[0085] The nucleic acids themselves comprise another aspect of the invention. Contemplated are nucleic acids encoding any of the proteins of the invention described herein. As such, the nucleic acids of the invention comprise, in 5' to 3' order, a coding region for a first binding domain, a constant sub-region sequence, and a coding region for a second binding domain. Also contemplated are nucleic acids that encode protein variants wherein the two binding domains and the constant sub-region sequences are collectively at least 80%, and preferably at least 85%, 90%, 95%, or 99% identical in amino acid sequence to the combined sequences of a known immunoglobulin variable region sequence and a known constant sub-region sequence. Alternatively, the protein variants of the invention are encoded by nucleic acids that hybridize to a nucleic acid encoding a non-variant protein of the invention under stringent hybridization conditions of 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68° C. or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 50% formamide at 42° C. Variant nucleic acids of the invention exhibit the capacity to hybridize under the conditions defined immediately above, or exhibit 90%, 95%, 99%, or 99.9% sequence identity to a nucleic acid encoding a non-variant protein according to the invention.

[0086] In related aspects, the invention provides a vector comprising a nucleic acid as described above, as well as host cells comprising a vector or a nucleic acid as described herein. Any vector known in the art may be used (e.g., plasmids, phagemids, phasmids, cosmids, viruses, artificial chromosomes, shuttle vectors and the like) and those of skill in the art will recognize which vectors are particularly suited for a given purpose. For example, in methods of producing a protein according to the invention, an expression vector operable in the host cell of choice is selected. In like manner, any host cell capable of being genetically transformed with a nucleic acid or vector of the invention is contemplated. Preferred host cells are higher eukaryotic host cells, although lower eukaryotic (e.g., yeast) and prokaryotic (bacterial) host cells are contemplated.

[0087] Another aspect of the invention is drawn to a method of inducing damage to a target cell comprising contacting a target cell with a therapeutically effective amount of a protein as described herein. In some embodiments, the target cell is contacted in vivo by administration of the protein, or an encoding nucleic acid, to an organism in need. Contemplated within this aspect of the invention, and more generally within aspects of the invention drawn to methods of modulating a target cell behavior, are methods wherein the multivalent single-chain binding protein induces at least an additive amount of damage to the target cell, which is at least that amount of damage expected from the sum of the damage attributable to separate antibodies comprising one or the other of the binding domains. Also contemplated are methods wherein the multivalent single-chain binding protein induces a synergistic amount of damage to the target cell (or modulation of another target cell behavior) compared to the sum of the damage (or modulation in another target cell behavior) induced by a first antibody comprising the first binding domain but not the second binding domain and a second antibody comprising the second binding domain but not the first binding domain. A preferred pair of binding domains exhibits at least an additive effect, i.e., either an additive effect or a synergistic effect. Such preferred binding pairs include

CD20-CD19, CD20-CD21, CD20-CD22, CD20-CD23, CD20-CD30, CD20-CD37, CD20-CD40, CD20-CD70, CD20-CD79a, CD20-CD79b, CD20-CD80, CD20-CD81, CD20-CD86, CD79b-CD19, CD79b-CD23, CD79b-CD30, CD79b-CD37, CD79b-CD72, CD79b-CD81, CD79b-major histocompatibility complex class II (CL II), CL II-CD19, CL 11-CD30, CL 11-CD37, CL 11-CD72, CL 11-CD79a, CL 11-CD81, CD19-CD37, CD19-CD79a, CD37-CD79a, CD81-CD37, and CD81-CD72. Also preferred is a binding pair exhibiting a synergistic effect, exemplified by CD20-CD19, CD20-CD21, CD20-CD22, CD20-CD79a, CD20-CD79b, CD20-CD81, CD79b-CD37, major histocompatibility complex class II (CL II)-CD19, CL 11-CD30, CL 11-CD37, and CL 11-CD72. Yet another preferred binding pair is a binding pair exhibiting an additive effect, which includes such binding pairs as CD20-CD22, CD20-CD23, CD20-CD30, CD20-CD37, CD20-CD70, CD20-CD80, CD20-CD86, CD79b-CD19, CD79b-CD23, CD79b-CD30, CD79b-major histocompatibility complex class II (CL II), CD79b-CD81, CD79b-major histocompatibility complex class II (CL II), CL II-CD79a, and CL 11-CD81. In some embodiments, the multivalent single-chain binding protein is multispecific and comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD20-CD40, CD20-CD79a, CD20-CD79b, CD20-CD81, CD79b-CD37, CD79b-CD81, major histocompatibility complex class II (CL II)-CD37, CL 11-CD72 and CL 11-CD79b.

[0088] This aspect of the invention also comprehends methods wherein the multispecific, multivalent single-chain binding protein induces an inhibited amount of damage (or modulation of another target cell behavior) to the target cell compared to the sum of the damage (or modulation of another target cell behavior) induced by a first antibody comprising the first binding domain but not the second binding domain and a second antibody comprising the second binding domain but not the first binding domain. Exemplary embodiments include methods wherein the multi-specific, multivalent single-chain binding protein comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD22-CD19, CD22-CD21, CD22-CD23, CD22-CD30, CD22-CD37, CD22-CD40, CD22-CD70, CD22-CD72, CD22-CD79a, CD22-CD79b, and CD22-major histocompatibility complex class II (CL II).

[0089] In a related aspect, the invention provides a method of treating a cell proliferation disorder, e.g., cancer, or of treating a disorder selected from the group consisting of a cancer, an autoimmune disorder, an infectious disease and inflammation comprising administering a therapeutically effective amount of a protein (as described herein), or an encoding nucleic acid, to an organism in need. Those of skill in the art, including medical and veterinary professionals, are proficient at identifying organisms in need of treatment. Disorders contemplated by the invention as amenable to treatment include a disorder selected from the group consisting of a cancer, an autoimmune disorder, an infectious disease and inflammation. In some embodiments, the protein is administered by in vivo expression of a nucleic acid encoding the protein as described herein. The invention also comprehends administering the protein by a route selected from the group consisting of intravenous injection, intraarterial injection, intramuscular injection, subcutaneous injection, intraperitoneal injection and direct tissue injection.

[0090] Another aspect of the invention is directed to a method of ameliorating a symptom associated with a disorder noted above comprising administering a therapeutically effective amount of a protein, as described herein, to an organism in need. Those of skill in the art are also proficient at identifying those disorders, or diseases or conditions, exhibiting symptoms amenable to amelioration. In some embodiments, the symptom is selected from the group consisting of pain, heat, swelling and joint stiffness.

[0091] Yet another aspect of the invention is drawn to a method of treating an infection associated with an infectious agent comprising administering a therapeutically effective amount of a protein according to the invention to a patient in need, wherein the protein comprises a binding domain that specifically binds a target molecule of the infectious agent. Infectious agents amenable to treatment according to this aspect of the invention include prokaryotic and eukaryotic cells, viruses (including bacteriophage), foreign objects, and infectious organisms such as parasites (e.g., mammalian parasites).

[0092] A related aspect of the invention is directed to a method of ameliorating a symptom of an infection associated with an infectious agent comprising administering an effective amount of a protein according to the invention to a patient in need, wherein the protein comprises a binding domain that specifically binds a target molecule of the infectious agent. Those of skill in the medical and veterinary arts will be able to determine an effective amount of a protein on a case-by-case basis, using routine experimentation.

[0093] Yet another related aspect of the invention is a method of reducing the risk of infection attributable to an infectious agent comprising administering a prophylactically effective amount of a protein according to the invention to a patient at risk of developing the infection, wherein the protein comprises a binding domain that specifically binds a target molecule of the infectious agent. Those of skill in the relevant arts will be able to determine a prophylactically effective amount of a protein on a case-by-case basis, using routine experimentation.

[0094] Another aspect of the invention is drawn to the above-described multivalent single-chain binding protein wherein at least one of the first binding domain and the second binding domain specifically binds an antigen selected from the group consisting of CD19, CD20, CD21, CD22, CD23, CD30, CD37, CD40, CD70, CD72, CD79a, CD79b, CD80, CD81, CD86, and a major histocompatibility complex class II peptide.

[0095] In certain embodiments, one of the first binding domain and the second binding domain specifically binds CD20, and in some of these embodiments, the other binding domain specifically binds an antigen selected from the group consisting of CD19, CD20, CD21, CD22, CD23, CD30, CD37, CD40, CD70, CD72, CD79a, CD79b, CD80, CD81, CD86, and a major histocompatibility complex class II peptide. For example, in one embodiment, the first binding domain is capable of specifically binding CD20 while the second binding domain is capable of specifically binding, e.g., CD19. In another embodiment, the first binding domain binds CD19 while the second binding domain binds CD20. An embodiment in which both binding domains bind CD20 is also contemplated.

[0096] In certain other embodiments according to this aspect of the invention, one of the first binding domain and the second binding domain specifically binds CD79b, and in

some of these embodiments, the other binding domain specifically binds an antigen selected from the group consisting of CD19, CD20, CD21, CD22, CD23, CD30, CD37, CD40, CD70, CD72, CD79a, CD79b, CD80, CD81, CD86, and a major histocompatibility complex class II peptide. Exemplary embodiments include distinct multi-specific, multivalent single-chain binding proteins in which a first binding domain:second binding domain specifically binds CD79b:CD19 or CD19:CD79b. A multivalent binding protein having first and second binding domains recognizing CD79b is also comprehended.

[0097] In still other certain embodiments, one of the first binding domain and the second binding domain specifically binds a major histocompatibility complex class II peptide, and in some of these embodiments, the other binding domain specifically binds an antigen selected from the group consisting of CD19, CD20, CD21, CD22, CD23, CD30, CD37, CD40, CD70, CD72, CD79a, CD79b, CD80, CD81, CD86, and a major histocompatibility complex class II peptide. For example, in one embodiment, the first binding domain is capable of specifically binding a major histocompatibility complex class II peptide while the second binding domain is capable of specifically binding, e.g., CD19. In another embodiment, the first binding domain binds CD19 while the second binding domain binds a major histocompatibility complex class II peptide. An embodiment in which both binding domains bind a major histocompatibility complex class II peptide is also contemplated.

[0098] In yet other embodiments according to this aspect of the invention, one of the first binding domain and the second binding domain specifically binds CD22, and in some of these embodiments, the other binding domain specifically binds an antigen selected from the group consisting of CD19, CD20, CD21, CD22, CD23, CD30, CD37, CD40, CD70, CD72, CD79a, CD79b, CD80, CD81, CD86, and a major histocompatibility complex class II peptide. Exemplary embodiments include distinct multi-specific, multivalent single-chain binding proteins in which a first binding domain:second binding domain specifically binds CD22:CD19 or CD19:CD22. A multivalent binding protein having first and second binding domains recognizing CD22 is also comprehended.

[0099] A related aspect of the invention is directed to the above-described multivalent single-chain binding protein wherein the protein has at least an additive (i.e., additive or synergistic) effect on a target cell behavior relative to the sum of the effects of each of the binding domains. In some embodiments, the protein comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD20-CD19, CD20-CD21, CD20-CD22, CD20-CD23, CD20-CD30, CD20-CD37, CD20-CD40, CD20-CD70, CD20-CD79a, CD20-CD79b, CD20-CD80, CD20-CD81, CD20-CD86, CD79b-CD19, CD79b-CD23, CD79b-CD30, CD79b-CD37, CD79b-CD72, CD79b-CD81, CD79b-major histocompatibility complex class II (CL II), CL 11-CD19, CL 11-CD30, CL 11-CD37, CL 11-CD72, CL 11-CD79a, CL II-CD81, CD19-CD37, CD19-CD79a, CD37-CD79a, CD81-CD37, and CD81-CD72. Also contemplated are binding pairs that exhibit a synergistic effect, which include the binding pairs CD20-CD19, CD20-CD21, CD20-CD22, CD20-CD79a, CD20-CD79b, CD20-CD81, CD79b-CD37, major histocompatibility complex class II (CL II)-CD19, CL 11-CD30, CL 11-CD37, and CL 11-CD72.

[0100] The invention further comprehends a multivalent single-chain binding protein as described above wherein the protein has an additive effect on a target cell behavior relative to the sum of the effects of each of the binding domains. Embodiments according to this aspect of the invention include multi-specific proteins comprising a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD20-CD22, CD20-CD23, CD20-CD30, CD20-CD37, CD20-CD70, CD20-CD80, CD20-CD86, CD79b-CD19, CD79b-CD23, CD79b-CD30, CD79b-major histocompatibility complex class II (CL II), CD79b-CD81, CD79b-major histocompatibility complex class II (CL II), CL 11-CD79a, and CL 11-CD81.

[0101] Yet another related aspect of the invention is a multivalent single-chain binding protein as described above wherein the protein has an inhibitory effect on a target cell behavior relative to the sum of the effects of each of the binding domains. In some embodiments, the protein is multispecific and comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD22-CD19, CD22-CD21, CD22-CD23, CD22-CD30, CD22-CD37, CD22-CD40, CD22-CD70, CD22-CD72, CD22-CD79a, CD22-CD79b, and CD22-major histocompatibility complex class II (CL II).

[0102] Another aspect of the invention is a method of identifying at least one of the binding domains of the multivalent binding molecule, such as a multispecific binding molecule, described above comprising: (a) linking an antibody specifically recognizing a first antigen and an antibody specifically recognizing a second antigen, such as by contacting both antibodies with an anti-isotypic antibody; (b) contacting a target comprising at least one of said antigens with the composition of step (a); and (c) measuring an activity of the target, wherein the activity is used to identify at least one of the binding domains of the multivalent binding molecule. In some embodiments, the target is a diseased cell, such as a cancer cell (e.g., a cancerous B-cell) or an auto-antibody-producing B-cell.

[0103] In each of the foregoing methods of the invention, it is contemplated that the method may further comprise a plurality of multivalent single-chain binding proteins. In some embodiments, a binding domain of a first multivalent single-chain binding protein and a binding domain of a second multivalent single-chain binding protein induce a synergistic, additive, or inhibitory effect on a target cell, such as a synergistic, additive, or inhibitory amount of damage to the target cell. The synergistic, additive or inhibitory effects of a plurality of multivalent single-chain binding proteins is determined by comparing the effect of such a plurality of proteins to the combined effect of an antibody comprising one of the binding domains and an antibody comprising the other binding domain.

[0104] A related aspect of the invention is directed to a composition comprising a plurality of multivalent single-chain binding proteins as described above. In some embodiments, the composition comprises a plurality of multivalent single-chain binding proteins wherein a binding domain of a first multivalent single-chain binding protein and a binding domain of a second multivalent single-chain binding protein are capable of inducing a synergistic, additive, or inhibitory effect on a target cell, such as a synergistic, additive or inhibitory amount of damage to the target cell.

[0105] The invention further extends to a pharmaceutical composition comprising the composition described above

and a pharmaceutically acceptable carrier, diluent or excipient. In addition, the invention comprehends a kit comprising the composition as described herein and a set of instructions for administering said composition to exert an effect on a target cell, such as to damage the target cell.

[0106] Finally, the invention also comprehends a kit comprising the protein as described herein and a set of instructions for administering the protein to treat a cell proliferation disorder or to ameliorate a symptom of the cell proliferation disorder.

[0107] Other features and advantages of the present invention will be better understood by reference to the following detailed description, including the examples.

BRIEF DESCRIPTION OF THE DRAWING

[0108] FIG. 1 shows a schematic representation of the multivalent single-chain molecules envisioned by the invention. Individual subdomains of the fusion protein expression cassette are indicated by separate shapes/blocks on the figure. BD1 refers to binding domain 1, linker 1 refers to any potential linker or hinge like peptide between the N-terminal proximal BD1 and the “effector function domain”, indicated as EFD. This subdomain is usually an engineered form of the Fc domain of human IgG1, but may include other subdomains with one or more effector functions as defined herein. Linker 2 refers to the scorpion linker sequence, if any, present between the carboxy terminus of the EFD and the C-terminal proximal binding domain 2, BD2.

[0109] FIG. 2 shows a Western blot of non-reduced proteins expressed in COS cells. Protein was secreted into the culture medium, and culture supernatants isolated after 48-72 hours from transiently transfected cells by centrifugation. Thirty microliters of crude supernatant were loaded into each well of the gel. Lane identifications: 1-molecular weight markers, with numerals indicating kilodaltons; 2-2H7-IgG-STD1-2E12 LH; 3-2H7-IgG-STD1-2E12 HL; 4-2H7-IgG-STD2-2E12 LH; 5-2H7-IgG-STD2-2E12 HL; 6-2E12 LH SMIP; 7-2E12 HL SMIP; 8-2H7 SMIP.

[0110] “2H7” refers to a single-chain construct, where BD 1 encodes the CD20 specific binding domain (2H7) in the VLVH orientation; “2E12” refers to a binding domain specific for CD28;—in these specific studies, IgG-refers to a single-chain construct, with a hinge encoding a sequence where all C are mutated to S (sss), and the CH2 and CH3 domains of IgG 1 contain mutations which eliminate both ADCC and CDC effector functions (P238S and P331 S), “STD1” refers to a 20-amino-acid (18+restriction site-encoded) scorpion linker located in linker position 2, as shown in FIG. 6A, row 2; STD2 indicates the 38-amino-acid (36+ restriction site-encoded) scorpion linker located in linker position 2, as shown in FIG. 6A, row 3 inserted adjacent to the BD2 in the VL-VH orientation, or 2E12 (V_L - V_H). “STD1-HL” refers to a similar construct as just described, but with the BD2 V regions in the VH-VL orientation as follows: 2H7-sssIgG (P238/331S)-20-amino-acid linker-2E12 (V_H - V_L). “STD2-LH” refers to 2H7-sssIgG (P238/331S)-38-amino-acid linker-2E12 (V_L - V_H); “STD2-LH” refers to 2H7-sssIgG (P238/331SS)-38-amino-acid linker-2E12 (V_H - V_L); “SMIP” refers to small modular immunopharmaceutical comprising BD 1, a linker and the C_{H2} and C_{H3} domains of an immunoglobulin such as IgG; and “H” generally refers to V_H , while “L” generally refers to V_L . Unless otherwise indicated, all protein orientations are N-terminal to C-terminal orientations.

[0111] FIG. 3 shows two columnar graphs illustrating the CD20 and CD28 binding properties of the 2H7-sssIgG (P238S/P331S)-STD1-2e12 LH and HL derivatives expressed from COS cells. These experiments were performed with crude culture supernatants rather than purified proteins. Serial dilutions from undiluted to 16-fold dilutions of the culture supernatants were incubated with either CD20 expressing cells (A; WIL-2S) or CD28 expressing cells (B; CD28 CHO). Binding activity in the supernatants was compared to control samples testing binding of the relevant single specificity SMIP, such as TRU-015 (binding CD20), or 2e12 VLVH (binding CD28), or 2e12VHVH SMIPs. Binding in each sample was detected using fluorescein isothiocyanate (FITC) conjugated goat anti-human IgG at a dilution of 1:100.

[0112] FIG. 4 is a histogram showing the binding pattern of protein A purified versions of the proteins tested in FIG. 3 to WIL-2S cells. “TRU015” is a SMIP specific for CD20. Two multispecific binding proteins with effector function were also analyzed: “2H7-2E12 LH” has binding domain 2, specific for CD28, in V_L - V_H orientation; “2H7-2E12 HL” has binding domain 2, specific for CD28, in V_H - V_L orientation. Each of the proteins was tested for binding at 5 μ g/ml, and binding detected with FITC goat anti-human IgG at 1:100. Control is FITC goat anti-human IgG alone. See the description for FIG. 2 above for more complete descriptions of the modules of the molecules tested.

[0113] FIG. 5 shows two histograms illustrating the binding by protein A purified multispecific binding proteins with effector function to CHO cells expressing CD28. “2H7-2E12 LH” has binding domain 2, specific for CD28, in V_L - V_H orientation; “2H7-2E12 HL” has binding domain 2, specific for CD28, in V_H - V_L orientation. Each of the proteins was tested for binding at 5 μ g/ml, and binding was detected with FITC goat anti-human IgG at 1:100. Control is FITC goat anti-human IgG alone. See the descriptions in FIG. 2 for a more complete description of the modules of the molecules tested.

[0114] FIG. 6A) The diagram above shows binding domain 1 (BD1) separated from the effector function domain (EFD) by linker 1, and the EFD separated from BD2 by linker 2 or the Scorpion linker. The table identifies the linkers joining the constant sub-region and binding domain 2. The linkers are identified by name, sequence, sequence identifier, sequence length, and the sequence of the fusion with binding domain 2. The scorpion linkers STD1 and STD2 are identified in rows 2 and 3, respectively. B) Shows a table identifying a variety of constructs identifying elements of exemplified molecules according to the invention. In addition to identifying the multivalent binding molecules by name, the elements of those molecules are disclosed in terms of binding domain 1 (BD1), the constant sub-region (hinge and effector domain or EFD), a linker (see FIG. 6A for additional information regarding the linkers), and binding domain 2 (BD2). The sequences of a number of exemplified multivalent binding proteins are provided, and are identified in the figure by a sequence identifier. Other multivalent binding proteins have altered elements, or element orders, with predictable alterations in sequence from the disclosed sequences.

[0115] FIG. 7 shows a composite columnar graph illustrating the binding of purified proteins at a single, fixed concentration to CD20 expressing WIL-2S cells and to CHO cells expressing CD28. “H1-H6” refers to the 2H7-sss-hIgG-Hx-2e12 molecules where Hx represents the H1-H6 linkers and

the 2e12 V regions in the orientation of V_H - V_L . "L1-L6" refers to the 2H7-sss-hIgG-Lx-2e12 molecules where Hx represents the L1-L6 linkers and the 2e12 V regions in the orientation of V_L - V_H . All the molecules were tested at a concentration of 0.72 $\mu\text{g/ml}$, and the binding detected using FITC conjugated goat anti-human IgG at 1:100. The mean fluorescence intensity for each sample was then plotted as paired bar graphs for the two target cell types tested versus each of the multivalent constructs being tested, L1-L6, or H1-H6.

[0116] FIG. 8 shows photographs of Coomassie stained non-reducing and reducing SDS-PAGE gels. These gels show the effect of the variant scorpion linker sequences/lengths on 2H7-sss-hIgG-Hx-2e12 HL protein stability. The amounts of the two predominant protein bands are visualized on the gel. The controls TRU-015 and 015 refer to a CD20-directed SMIP, and 2e12HL refers to a CD28-directed SMIP.

[0117] FIG. 9 shows Western Blots of the [2H7-sss-hIgG-H6-2e12] scorpion proteins and the relevant single specificity SMIPs probed with either (a) 2e12 cognate antigen CD28mIgG, or with (b) an anti-Id Fab reactive with the 2H7 specificity. The results show that the presence of the H6 linker results in the generation of cleaved forms of the multivalent constructs which are missing the CD28 binding specificity.

[0118] FIG. 10 shows binding curves of the different scorpion linker variants for [TRU015-sss-IgG-Hx-2e12 HL] H1-H6 linker forms. Scorpion binding specificities are to CD20 (BD1; TRU-015) and CD37 (BD2; 2e12). Panel A shows the binding curves for binding to CD20 expressing WIL-2S cells. Panel B shows the binding curves for binding of the different forms to CD28-expressing CHO cells. These binding curves were generated with serial dilutions of protein A-purified fusion protein, and binding was detected using FITC conjugated goat anti-human IgG at 1:100. The apparent decrease in binding at concentrations >10 mg/ml reflects supersaturation of target cells.

[0119] FIG. 11 shows a table summarizing the results of SEC fractionation of 2H7-sss-IgG-2e12 HL multispecific fusion proteins with variant linkers H1-H7. Each row in the table lists a different linker variant of the [2H7-sss-IgG-Hx-2e12-HL] fusion proteins. The retention time of the peak of interest (POI), and the percentage of the fusion protein present in POI, and the percentage of protein found in other forms is also tabulated. The cleavage of the molecules is also listed, with the degree of cleavage indicated in a qualitative way, with (Yes), Yes, and YES, or No being the four possible choices, corresponding to low, moderate, severe and no detectable proteolysis, respectively.

[0120] FIG. 12 shows two graphs with binding curves for [2H7-sss-hIgG-Hx-2e12] multispecific fusion proteins with variant linkers H3, H6, and H7 linkers to cells expressing CD20 or CD28. Serial dilutions of the protein A purified fusion proteins from 10 $\mu\text{g/ml}$ down to 0.005 $\mu\text{g/ml}$ were incubated with either CD20 expressing WIL-2S cells or CD28 CHO cells. Binding was detected using FITC conjugated goat anti-human IgG at 1:100. gah FITC indicates control FITC-conjugated goat anti-human IgG alone. Panel A shows the binding to WIL-2S cells, and panel B shows the binding to CD28 CHO cells.

[0121] FIG. 13 shows the results of an alternative binding assay generated by the molecules used for FIG. 12. In this case, the fusion proteins were first bound to WIL-2S CD20-expressing cells, the second binding domain was then detected by addition of its cognate antigen, CD28, fused to

mouse antibody. Binding was then detected with FITC anti-mouse reagent (gam FITC) at 1:100. These results demonstrate the simultaneous binding to both BD1 (CD20) and BD2 (CD28) in the same scorpion molecule.

[0122] FIG. 14 shows results obtained using another multispecific fusion construct variant. In this case, modifications were made in the specificity for BD2, so that the V regions for the G28-1 antibody were used to create a CD37 specific binding domain. Shown are two graphs which illustrate the relative ability of CD20 and/or CD37 antibodies to block the binding of the [2H7-sss-IgG-Hx-G28-1] multispecific fusion (scorpion) protein to Ramos or BJAB cells expressing the CD20 and CD37 targets. Each cell type was preincubated with either the CD20 specific antibody (25 $\mu\text{g/ml}$) or the CD37 specific antibody (10 $\mu\text{g/ml}$) or both reagents (these are mouse anti-human reagents) prior to incubation with the multispecific fusion protein. Binding of the multispecific fusion protein was then detected with a FITC goat anti-human IgG reagent at 1:100, (preadsorbed to mouse to eliminate cross-reactivity).

[0123] FIG. 15 shows the results of an ADCC assay performed with BJAB target cells, human PBMC effector cells, and with the CD20-hIgG-CD37 specific scorpion molecule as the test reagent. The 2H7 binding domain is specific for CD20 and the G28-1 binding domain is specific for CD37. For a full description of the procedure see the appropriate example. The graph plots the concentration of fusion protein versus the % specific killing at each dosage tested for the single specificity SMIP reagents, and for the [2H7-sss-hIgG-STD1-G28-1] LH and HL variants. Each data series plots the dose-response effects for one of these single specificity or multispecific single-chain fusion proteins.

[0124] FIG. 16 shows a table tabulating the results of a co-culture experiment where human PBMC were cultured in the presence of TRU 015, G28-1 SMIP, both molecules together, or the dual-specificity [2H7-sss-IgG-H7-G28-1HL] variant. The scorpions were used at 20 $\mu\text{g/ml}$, and incubated for 24 hours or 72 hours. Samples were then stained with CD3 antibodies conjugated to FITC to detect remaining CD20/CD37-negative T cells, and either CD19 or CD40 specific antibodies conjugated to PE to detect residual CD37/CD20-positive B cells, then subjected to flow cytometry. The percentage of cells in each gate was then tabulated.

[0125] FIG. 17 shows two columnar graphs of the effects on B cell line apoptosis after 24 hour incubation with the [2H7-sss-hIgG-H7-G28-1 HL] molecule or control single CD20 and/or CD37 specificity SMIPs alone or in combination. The percentage of annexin V-propidium iodide positive cells is plotted as a function of the type of test reagent used for the coincubation experiments. Panel A shows the results obtained using Ramos cells, and panel B shows those for Daudi cells. Each single CD20 or CD37 directed SMIP is shown at the concentrations indicated; in addition, where combinations of the two reagents were used, the relative amount of each reagent is shown in parentheses. For the multispecific CD20-CD37 fusion protein, concentrations of 5, 10, and 20 $\mu\text{g/ml}$ were tested.

[0126] FIG. 18 shows two graphs of the [2H7-hIgG-G19-4] scorpion molecule variants and their binding to either CD3 expressing cells (Jurkats; recognized by BD G19-4) or CD20 expressing cells (WIL-2S; recognized by BD 2H7). The molecules include [2H7-sss-hIgG-STD1-G19-4 HL], LH, and [2H7-csc-hIgG-STD1-G19-4 HL]. Protein A purified fusion proteins were titrated from 20 $\mu\text{g/ml}$ down to 0.05 $\mu\text{g/ml}$, and

the binding detected using FITC goat anti-human IgG at 1:100. MFI (mean fluorescence intensity) is plotted as a function of protein concentration.

[0127] FIG. 19 shows the results of ADCC assays performed with the [2H7-hIgG-STD1-G19-4 HL] molecule variants with either an SSS hinge or a CSC hinge, BJAB target cells, and either total human PBMC as effector cells (A) or NK cell depleted PBMC as effector cells (B). Killing was scored as a function of concentration of the multispecific fusion proteins. The killing observed with these molecules was compared to that seen using G19-4, TRU 015, or a combination of these two reagents. Each data series plots a different test reagent, with the percent specific killing plotted as a function of protein concentration.

[0128] FIG. 20 shows the percentage of Ramos B-cells that stained positive with Annexin V (Ann) and/or propidium iodide (PI) after overnight incubation with each member of a matrix panel of B-cell antibodies (2 μ g/ml) in the presence, or absence, of an anti-CD20 antibody (present at 2 μ g/ml where added). Goat-anti-mouse secondary antibody was always present at a two-fold concentration ratio relative to other antibodies (either matrix antibody alone, or matrix antibody and anti-CD20 antibody). Vertically striped bars—matrix antibody (2 μ g/ml) denoted on X-axis and goat anti-mouse antibody (4 μ g/ml). Horizontally striped bars—matrix antibody (2 μ g/ml) denoted on X-axis, anti-CD20 antibody (2 μ g/ml), and goat anti-mouse antibody (4 μ g/ml). The “2nd step” condition served as a control and involved the addition of goat anti-mouse antibody at 4 μ g/ml (vertically striped bar) or 8 μ g/ml (horizontally striped bar), without a matrix antibody or anti-CD20 antibody. “CL II” (MHC class II) in the figures refers to a monoclonal antibody cross-reactive to HLA DR, DQ and DP, i.e., to MHC Class II antigens.

[0129] FIG. 21 shows the percentage of Ramos B-cells that stained positive with Annexin V (Ann) and/or propidium iodide (PI) after overnight incubation with each member of a matrix panel of B-cell antibodies (2 μ g/ml) in the presence, or absence, of an anti-CD79b antibody (present at either 0.5 or 1.0 μ g/ml where added). See the description of FIG. 20 for identification of “CL II” and “2nd step” samples. Vertically striped bars—matrix antibody (2 μ g/ml) and goat anti-mouse antibody (4 μ g/ml); horizontally striped bars—matrix antibody (2 μ g/ml), anti-CD79b antibody (1.0 μ g/ml) and goat anti-mouse antibody (6 μ g/ml); stippled bars—matrix antibody (2 μ g/ml), anti-CD79b antibody (0.5 μ g/ml) and goat anti-mouse antibody (5 μ g/ml).

[0130] FIG. 22 shows the percentage of Ramos B-cells that stained positive with Annexin V (Ann) and/or propidium iodide (PI) after overnight incubation with each member of a matrix panel of B-cell antibodies (2 μ g/ml) in the presence, or absence, of an anti-CL II antibody (present at either 0.25 or 0.5 μ g/ml where added). See the description of FIG. 20 for identification of “CL II” and “2nd step” samples. Vertically striped bars—matrix antibody (2 μ g/ml) and goat anti-mouse antibody (4 μ g/ml); horizontally striped bars—matrix antibody (2 μ g/ml), anti-CL II antibody (0.5 μ g/ml) and goat anti-mouse antibody (5 μ g/ml); stippled bars—matrix antibody (2 μ g/ml), anti-CL II antibody (0.25 μ g/ml) and goat anti-mouse antibody (4.5 μ g/ml).

[0131] FIG. 23 shows the percentage of DHL-4 B-cells that stained positive with Annexin V (Ann) and/or propidium iodide (PI) after overnight incubation with each member of a matrix panel of B-cell antibodies (2 μ g/ml) in the presence, or absence, of an anti-CD22 antibody (present at 2 μ g/ml where

added). See the description of FIG. 20 for identification of “CL II” and “2nd step” samples. Solid bars—matrix antibody (2 μ g/ml) and goat anti-mouse antibody (4 μ g/ml); slant-striped bars—matrix antibody (2 μ g/ml), anti-CD22 antibody (2 μ g/ml) and goat anti-mouse antibody (8 μ g/ml).

[0132] FIG. 24 provides a graph demonstrating direct growth inhibition of lymphoma cell lines Su-DHL6 (triangles) and DoHH2 (squares) by free CD20 SMIP (closed symbols) or monospecific CD20 $\times$ CD20 scorpion (open symbols).

[0133] FIG. 25 is a graph showing direct growth inhibition of CD37-positive lymphoma cell lines Su-DHL-6 (triangles) and DoHH2 (squares) by free anti-CD37 SMIP (closed symbols) or monospecific anti-CD37 scorpion (open symbols). Cells were exposed under standard cell culture conditions to increasing concentrations of either SMIPs or Scorpions and percentage of viable cells remaining at 96 h compared to those in untreated control wells was determined.

[0134] FIG. 26 presents a graph showing direct growth inhibition of lymphoma cell lines Su-DHL-6 (triangles) and DoHH2 (squares) by a combination of two different monospecific SMIPs, i.e., CD20-SMIP and CD37 SMIP (closed symbols), or by a bispecific CD20-CD37 scorpion (open symbols).

[0135] FIG. 27 is a graph revealing direct growth inhibition of lymphoma cell lines Su-DHL-6 (triangles) and WSU-NHL (squares) by free CD19 SMIP and CD37 SMIP combination (closed symbols) or bispecific CD19 $\times$ CD37 scorpion (open symbols).

[0136] FIG. 28 provides histograms showing the cell-cycle effects of scorpions. Samples of DoHH2 lymphoma cells were separately left untreated, treated with SMIP 016 (CD37-specific) or treated with the monospecific CD37 $\times$ CD37 scorpion. Open bars: sub-G₁ phase of the cell cycle; black bars: G₀/G₁ phase; light-shaded: S phase; and dark-shaded: G₂M phase.

[0137] FIG. 29 presents graphs of data establishing that treatment of lymphoma cells with scorpions resulted in increased signaling capacity compared to free SMIPs, as measured by calcium ion flux. Upper panel: dotted line: unstimulated; dashed line: CD20 and CD37 SMIPs; solid line: CD20 $\times$ CD37 bi-specific scorpion. Lower panel: dotted line: unstimulated; dashed line: CD20 SMIP; solid line: CD20 $\times$ CD20 mono-specific scorpion.

[0138] FIG. 30 provides graphs demonstrating scorpion-dependent cellular cytotoxicity

[0139] FIG. 31 shows graphs of data indicating that scorpions mediate Complement Dependent Cytotoxicity.

[0140] FIG. 32 provides data in graphical form showing comparative ELISA binding of a SMIP and a scorpion to soluble, recombinant low-(B) and high-affinity (A) isoforms of Fc γ RIII (CD16).

[0141] FIG. 33 presents graphs establishing the binding of a SMIP and a scorpion to soluble, recombinant low (A)- and high (B)-affinity isoforms of Fc γ RIII (CD16) in the presence of target cells.

[0142] FIG. 34 is a histogram showing the expression level of a CD20 $\times$ CD20 scorpion in two experiments (flask 1 and flask 2) under six different culturing conditions. Solid black bars: flask 1; shaded bars: flask 2. The A samples contained basal CHO media and samples B-G had glucose and/or trace mineral feeds added to the basal media.

[0143] FIG. 35 provides a histogram showing the production yield of a CD20 $\times$ CD37 scorpion.

[0144] FIG. 36 presents SDS-PAGE gels (under reducing and non-reducing conditions) of a purified SMIP and a purified scorpion.

[0145] FIG. 37 provides a graph showing that scorpions retain the capacity to bind to target cells. Filled squares: CD20 SMIP; filled triangles: CD37 SMIP; filled circles: humanized CD20(2Lm20-4) SMIP; open diamond: CD37×CD37 mono-specific scorpion; open squares: CD20×CD37 bi-specific scorpion; and open triangles: humanized CD20(2Lm20-4)×humanized CD20(2Lm20-4) scorpion.

[0146] FIG. 38 contains graphs showing the results of competitive binding assays establishing that both N- and C-terminal scorpion binding domains participate in target cell binding.

[0147] FIG. 39 presents data in the form of graphs showing that scorpions have lower off-rates than SMIPs.

[0148] FIG. 40 shows a graph establishing that scorpions are stable in serum in vivo, characterized by a reproducible, sustained circulating half-life for the scorpion.

[0149] FIG. 41 provides a dose-response graph for a CD20×CD37 bispecific scorpion, demonstrating the in vivo efficacy of scorpion administration against human tumor B cells in immunocompromised mice.

[0150] FIG. 42 shows target B-cell binding by a monospecific CD20×CD20 scorpion (S0129) and glycovariants.

[0151] FIG. 43 provides graphs illustrating CD20×CD20 scorpions (untreated (UT) and glycovariants (KF10; CS200)) inducing ADCC-mediated killing of BJAB B-cells. Also shown are ADCC mediated killing by untreated anti-CD20 SMIP (SBI-0870 and anti-CD20 mAb rituximab (RTXN, or Rituxan®)).

[0152] FIG. 44 shows a gel revealing the effects on scorpion stability arising from changes in the scorpion linker, including changing the sequence of that linker and extending the linker by adding an H7 sequence to the linker, indicated by a "+" in the H7 line under the gel.

[0153] FIG. 45 shows the binding to CD20-positive WIL2S cells of a CD20×CD20 scorpion (S0129) and scorpion linker variants thereof.

[0154] FIG. 46 shows the direct cell killing of a variety of CD20-positive B-cells by a CD20×CD20 scorpion and by a CD20 SMIP.

[0155] FIG. 47 reveals the direct cell killing of additional B-cell lines by a monospecific CD20×CD20 scorpion.

[0156] FIG. 48 shows the direct cell killing capacities of each of two monospecific scorpions, i.e., CD20×CD20 and CD37×CD37, and a bispecific CD20×CD37 scorpion, the latter exhibiting increased killing at lower concentrations.

[0157] FIG. 49 graphically depicts the time-course and level of free intracellular Ca⁺⁺ released in of Su-DHL-6 B-cells to each of a CD20×CD20 (S0129), a CD37×CD37, and a CD20×CD37 scorpion.

[0158] FIG. 50 shows the capacity of a bispecific CD19×CD37 scorpion and Rituxan® (RTXN) to directly kill Su-DHL-6 B-cells.

[0159] FIG. 51 is a gel electrophoretogram of immunoprecipitates obtained with each of an anti-phosphotyrosine antibody and an anti-SYK antibody. The immunoprecipitates were obtained from lysates of B-cells contacted with CD20-binding scorpions, as indicated in the figure.

[0160] FIG. 52 provides combination index plots of CD20-binding scorpions in combination therapies with each of doxorubicin, vincristine and rapamycin.

DETAILED DESCRIPTION

[0161] The present invention provides compositions of relatively small peptides having at least two binding regions or domains, which may provide one or more binding specificities, derived from variable binding domains of immunoglobulins, such as antibodies, disposed terminally relative to an effector domain comprising at least part of an immunoglobulin constant region (i.e., a source from which a constant sub-region, as defined herein, may be derived), as well as nucleic acids, vectors and host cells involved in the recombinant production of such peptides and methods of using the peptide compositions in a variety of diagnostic and therapeutic applications, including the treatment of a disorder as well as the amelioration of at least one symptom of such a disorder. The peptide compositions advantageously arrange a second binding domain C-terminal to the effector domain, an arrangement that unexpectedly provides sterically unhindered or less hindered binding by at least two binding domains of the peptide, while retaining an effector function or functions of the centrally disposed effector domain.

[0162] The first and second binding domains of the multivalent peptides according to the invention may be the same (i.e., have identical or substantially identical amino acid sequences and be monospecific) or different (and be multispecific). Although different in terms of primary structure, the first and second binding domains may recognize and bind to the same epitope of a target molecule and would therefore be monospecific. In many instances, however, the binding domains will differ structurally and will bind to different binding sites, resulting in a multivalent, multispecific protein. Those different binding sites may exist on a single target molecule or on different target molecules. In the case of the two binding molecules recognizing different target molecules, those target molecules may exist, e.g., on or in the same structure (e.g., the surface of the same cell), or those target molecules may exist on or in separate structures or locales. For example, a multispecific binding protein according to the invention may have binding domains that specifically bind to target molecule on the surfaces of distinct cell types. Alternatively, one binding domain may specifically bind to a target on a cell surface and the other binding domain may specifically bind to a target not found associated with a cell, such as an extracellular structural (matrix) protein or a free (e.g., soluble or stromal) protein.

[0163] The compositions described herein are capable of binding two targets simultaneously utilizing the pairs of binding domains at the N- and C-terminus of the molecule. In so doing, for cell-surface targets, the composition can 'cross-link' or cause the physical co-approximation of the targets. It can be appreciated by those skilled in the art, that many receptor systems are activated upon such cross-linking and induced to signal, causing changes in cellular phenotype. The design of the compositions disclosed herein was intentionally chosen in part to maximize such signaling and control the resultant phenotype.

[0164] Approximate dimensions of domains of these compositions, as well as estimates of interdomain flexibility in terms of ranges of interdomain angles have been published and were considered in designing the compositions disclosed herein. For a scorpion using scFv binding domains for BD1 and BD2, an IgG1 N-terminal hinge (joining BD 1 and the constant sub-region), and the H7 scorpion linker, the binding domain at the N-terminus and a binding domain at the C-terminus by the composition may be maximally about 150-

180Å apart and minimally about 20-30Å apart. Binding domains at the N-terminus may be maximally about 90-100Å apart and minimally about 10-20 Å apart (see Deisenhofer, J., Colman, P. M., Huber, R., Haupt, H., Schwick, G. (1976) Crystallographic Structural Studies of a Human Fc-Fragment I. An Electron-Density Map at 4 Å Resolution and a Partial Model. Hoppe-Seyler's Z. Physiol. Chem. Bd. 357, S. 435-445.; Gregory L, Davis KG, Sheth B, Boyd J, Jefferis R, Nave C, Burton D. R. (1987) The solution conformations of the subclasses of human IgG deduced from sedimentation and small angle X-ray scattering studies, Mol. Immunol. August; 24(8):821-9.; R. J. Poljak, L. M. Amzel, H. P. Avey, B. L. Chen, R. P. Phizackerly, F. Saul (1973) Three-Dimensional Structure of the Fab' Fragment of a Human Immunoglobulin at 2.8 Å Resolution, Proc. Natl. Acad. Sci. (USA) (1973) 70: 3305-3310; Bongini, L, Fanelli, F, Piazza, F, De Los Rios, P, Sandin, S, Skoglund, U. (2004) Freezing Immunoglobulins to see them move. Proc. Natl. Acad. Sci. (USA) 101:6466-6471; Kienberger, F, Mueller, H, Pastushenko, V, Hinterdorfer, P. (2004) Following single antibody binding to purple membranes in real time, EMBO Reports 5:579-583). The choice of these dimensions was done in part to allow for receptor-receptor distances of less than about 50 Å in receptor complexes bound by the scorpion as distances less than this may be optimal for maximal signaling of certain receptor oligomers (see Paar, J M, Harris, N T, Holowka, D, Baird, B. (2002) Bivalent ligands with rigid double-stranded spacers reveal structural constraints on signaling by FcεR1, J. Immunol. 169:856-864) while allowing for the incorporation of Fc structures required for effector function.

[0165] The binding domains at the N- and C-terminus of the compositions of the invention are designed to be flexible structures to facilitate target binding and to allow for a range of geometries of the bound targets. It will also be appreciated by those skilled in the art that flexibility between the N- or C-terminal binding domains (BD1 and BD2, respectively) and between the binding domains and the Fc domain of the molecule as well as the maximal and minimal distances between receptors bound by BD1 and/or BD2 can be modified for example by choice of N-terminal hinge domain (linker joining BD 1 and the constant sub-region) and, by structural analogy, the C-terminal linker domain (scorpion linker). For example, hinge domains from IgG1, IgG2, IgG3, IgG4, IgE, IgA2, synthetic hinges and the hinge-like C_{H2} domain of IgM show different degrees of flexibility, as well as different lengths (Roux, K H, Stretlets, L, Michaelsen, T E. (1997) Molecular flexibility of human IgG subclasses, J. Immunol. 159:3372; Roux, K H, Stretlets, L, Brekke, O H, Sandlic, I, Michaelsen, T E. (1998) Comparisons of the Ability of Human IgG3 Hinge Mutants, IgM, IgE, and IgA2, to Form Small Immune Complexes: A Role for Flexibility and Geometry. J. Immunol. 161:4083-4090). Those skilled in the art will understand that the optimal choice of an N-terminal hinge domain and H2 will depend upon the receptor system (s) with which the scorpion is designed to interact as well as the desired signaling phenotype induced by scorpion binding. As a non-limiting example, it may be desirable to prevent bound receptors from approaching within about 50 Å of each other to intentionally create submaximal signals (see Paar, J M, Harris, N T, Holowka, D, Baird, B. (2002) Bivalent ligands with rigid double-stranded spacers reveal structural constraints on signaling by FcεR1, J. Immunol. 169:856-864)

and, in such a case choices of N-terminal hinge domain and scorpion linker that are shorter and less flexible may be appropriate.

[0166] Beyond the range of dimensions and flexibility enabled by compositions of the present invention, the compositions allow independent optimization of binding kinetics of BD1 and BD2 that are important in invoking desired properties upon binding to targets. Binding kinetics can be understood in terms of rate constants for binding and dissociation from the target (k_{ON} and k_{OFF} , respectively). These properties can be varied independently for BD1 and BD2 and can have independent effects on the phenotype induced upon binding to cell surface receptors on a target cell.

[0167] k_{ON} determines the likelihood of binding of an unbound BD to its target receptor given the average time an unbound receptor occupies a position within the area within which the free binding site is capable of binding its target and the concentration of unbound receptors on this area of the cell surface. The area within which the free binding site is capable of binding its target will be determined in part by the flexibility and length of the associated N-terminal linker (e.g., hinge) and scorpion linker, as described above. Given receptor concentrations in the range of 10-10,000 receptors/ μm^2 , and receptor diffusivity in the range of 10^{-9} - 10^{-13} cm^2/s (see Kusumi, A, Sako, Y, Yamamoto, M. (1993) Confined lateral diffusion of membrane receptors as studied by single particle tracking (nanovid microscopy) Effects of calcium-induced differentiation in cultured epithelial cells, Biophysical J. 65:2021-2040) it can be seen that desirable values of k_{ON} may be greater than about $10^3/\text{M}\cdot\text{s}$. k_{ON} for a binding domain, which can be varied by methods known to those skilled in the art, for example by modifying the electrostatic complementarity between the binding domain and its binding site on the receptor (Kiel, C., Selzer, T., Shaul, Y., Schreiber, G., Hermann, C. Electrostatically Optimized Ras-binding Ral guanine dissociation mutants increase the rate of association by stabilizing the encounter enthalpy, Proc. Natl. Acad. Sci. (USA) 101:9223-9228 (2004); Selzer, T, Albeck, S, Schreiber, G. (2000) Rational design of faster associating and tighter binding protein complexes, Nature Structural Biology. 7: 537-514), and can be screened for in libraries using surface plasmon resonance (Leonard P, Safsten P, Hearty S, McDonnell B, Finlay W, O'Kennedy R. High throughput ranking of recombinant avian scFv antibody fragments from crude lysates using the Biacore A100, J Immunol Methods (2007) May 15; [Epub ahead of print]). In the case where the binding domain is an scFv, k_{ON} can be chosen from a range on the order of $<10^4$ - $10^7/\text{M}\cdot\text{s}$ (Ulrik B. Nielsen, Gregory P. Adams, Louis M. Weiner and James D. Marks, Targeting of Bivalent Anti-ErbB2 Diabody Antibody Fragments to Tumor Cells Is Independent of the Intrinsic Antibody Affinity, Cancer Research 60:6434-6440 (2000); K. Asish Xavier and Richard C. Willson (1998) Association and Dissociation Kinetics of Anti-Hen Egg Lysozyme Monoclonal Antibodies HyHEL-5 and HyHEL-10, Biophys J, 74:2036-2045).

[0168] The k_{OFF} for a binding domain will determine the rate of receptor complex dissociation and, thus, the 'dwell time' of the receptor complex bound by the scorpion. It will be appreciated by those skilled in the art that changes in dwell time can affect the signaling phenotype induced (see Kersh, E N, Shaw, A S, Allen, P M. (1998) Fidelity of T-cell receptor activation thru multistep T-cell receptor zeta phosphorylation, Science 281: 572-575). The k_{OFF} should be low enough to allow for dimeric and/or higher receptor complexes bound

by scorpion molecules disclosed herein to remain in the complex longer than it takes to accumulate reaction products in the target signaling cascade(s) above the threshold required for induction of the desired signaling phenotype and should be high enough to allow the receptor complex to dissociate prior to the time required to accumulate reaction products in signaling cascade(s) that suppress or inhibit the induction of the desired signaling phenotype. Typically, k_{OFF} will be chosen to be between about 10^{-1} to 10^{-7} /sec (see Graff C P, Chester K, Begent R, Wittrup K D (2004) Directed evolution of an anti-carcinoembryonic antigen scFv with a 4-day monovalent dissociation half-time at 37 degrees C., *Protein Eng Des Sel.* 17(4):293-304). A low value of k_{OFF} will maximize the concentration of scorpion-induced cross-linked receptor oligomers (dimers or higher) for any concentration of the scorpion and low k_{OFF} rates will broaden the dose range over which ligand-mediated cross-linked species predominate over forms with engagement of BD1 or BD2 alone (see Perelson, A S. (1980) Receptor Clustering on a Cell Surface II. Theory of Receptor Cross-Linking by Ligands Bearing Two Chemically Distinct Functional Groups. *Mathematical Biosciences* 49:87-110). It may also be of interest to select binding domains with high values of k_{OFF} . For example, a high k_{OFF} may be desired for a receptor where a short dwell time in the complex is optimal for invoking the desired signaling phenotype (ex. See Matsui, K, Boniface, J J, Steffner, P, Reay, P A, Davis M M. (1994) Kinetics of T-cell receptor binding to peptide/I-E^k complexes: correlation of the dissociation rate with T-cell responsiveness, *Proc. Natl. Acad. Sci. (USA)* 91:12862-12866; Lyons, D S et al., (1996) A TCR binds to antagonist ligands with lower affinities and faster dissociation rates than to agonists, *Immunity* 5:53-61). It will be appreciated by those skilled in the art that k_{OFF} can be independently controlled for BD1 and BD2 for a composition comprising a scorpion. For a further non-limiting example, it may be desirable for one of BD 1 or BD2 to have a very low k_{OFF} and the other to have a relatively high k_{OFF} . This can allow the scorpion to have a long residence time on the cell surface bound to receptors corresponding to the low k_{OFF} BD while serially engaging receptors corresponding to the high k_{OFF} BD. This can have the effect of augmenting signaling when scorpion concentrations are low (see Kalergis, A M et al., (2001) Efficient T-cell activation requires an optimal dwell-time of interaction between the TCR and the pMHC complex, *Nature Immunol.* 2:229-234). It will be appreciated by those skilled in the art that k_{OFF} can be modified by engineering binding domains and/or by screening for binding domains with the desired kinetic properties (see Su B, Hrin R, Harvey B R, Wang Y J, Ernst R E, Hampton R A, Miller M D, Strohl W R, An Z, Montgomery D L. (2007) Automated high-throughput purification of antibody fragments to facilitate evaluation in functional and kinetic based assays, *J Immunol Methods* 322(1-2):94-103; Steukers M, Schaus J M, van Gool R, Hoyoux A, Richalet P, Sexton D J, Nixon A E, Vanhove M. (2006) Rapid kinetic-based screening of human Fab fragments, *J. Immunol. Methods*, 310(1-2):126-35; Jeremutis L, Honegger A, Schwesinger F, Hanes J, Pliickthun A. (2001) Tailoring in vitro evolution for protein affinity or stability, *Proc. Natl. Acad. Sci. (USA)* 98(1):75-80).

[0169] The first and second binding domains are derived from one or more regions of the same, or different, immunoglobulin protein structures such as antibody molecules. The first and/or second binding domain may exhibit a sequence identical to the sequence of a region of an immunoglobulin, or

may be a modification of such a sequence to provide, e.g., altered binding properties or altered stability. Such modifications are known in the art and include alterations in amino acid sequence that contribute directly to the altered property such as altered binding, for example by leading to an altered secondary or higher order structure for the peptide. Also contemplated are modified amino acid sequences resulting from the incorporation of non-native amino acids, such as non-native conventional amino acids, unconventional amino acids and imino acids. In some embodiments, the altered sequence results in altered post-translational processing, for example leading to an altered glycosylation pattern.

[0170] Any of a wide variety of binding domains derived from an immunoglobulin or immunoglobulin-like polypeptide (e.g., receptor) are contemplated for use in scorpions. Binding domains derived from antibodies comprise the CDR regions of a V_L and a V_H domain, seen, e.g., in the context of using a binding domain from a humanized antibody. Binding domains comprising complete V_L and V_H domains derived from an antibody may be organized in either orientation. A scorpion according to the invention may have any of the binding domains herein described. For scorpions having at least one binding domain recognizing a B-cell, exemplary scorpions have at least one binding domain derived from CD3, CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD38, CD39, CD40, CD72, CD73, CD74, CDw75, CDw76, CD77, CD78, CD79a/b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD89, CD98, CD126, CD127, CDw130, CD138 or CDw150. In some embodiments, the scorpion is a multivalent binding protein comprising at least one binding domain having a sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 103, 105, 107 and 109. In some embodiments, a scorpion comprises a binding domain comprising a sequence selected from the group consisting of any of SEQ ID NOS: 333-334, 336, and 343.

[0171] For embodiments in which either, or both, of the binding domains are derived from more than one region of an immunoglobulin (e.g., an Ig V_L region and an Ig V_H region), the plurality of regions may be joined by a linker peptide. Moreover, a linker may be used to join the first binding domain to a constant sub-region. Joinder of the constant sub-region to a second binding domain (i.e., binding domain 2 disposed towards the C-terminus of a scorpion) is accomplished by a scorpion linker. The scorpion linker, as a domain of a scorpion molecule that joins the C-terminus of the constant sub-region to the N-terminus of binding domain 2, provides more than the simple spacing function characteristic of peptide linkers. In addition to providing a spacing function, the scorpion linker provides the flexibility or rigidity suitable for development of the proper orientation of domains of the scorpion protein both within the scorpion molecule and between or among the scorpion and its target(s). Further, the scorpion linker supports expression of the full-length molecule and stability of the purified protein both in vitro and in vivo following administration to an organism in need, such as human, and is preferably non-immunogenic or poorly immunogenic in those same organisms. As noted herein, these functions have been found in peptides derived from either the interdomain region of an immunoglobulin superfamily member (e.g., an antibody hinge region) or the stalk region of C-type lectins, a family of type H membrane proteins. These scorpion linkers are preferably between about 2-45 amino acids, or 2-38 amino acids, or 5-45 amino acids. For example, the H1 linker is 2 amino acids in length and the STD2 linker

is 38 amino acids in length. Beyond general length considerations, a scorpion linker region suitable for use in the scorpions according to the invention includes an antibody hinge region selected from the group consisting of IgG, IgA, IgD and IgE hinges and variants thereof. For example, the scorpion linker may be an antibody hinge region selected from the group consisting of human IgG 1, human IgG2, human IgG3, and human IgG4, and variants thereof. In some embodiments, the scorpion linker region has a single cysteine residue for formation of an interchain disulfide bond. In other embodiments, the scorpion linker has two cysteine residues for formation of interchain disulfide bonds. In some embodiments, a scorpion linker region is derived from an immunoglobulin interdomain region (e.g., antibody hinge region) or a Type II C-type lectin stalk region (derived from a Type II membrane protein) and comprises a sequence selected from the group consisting of SEQ ID NOS:111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 287, 289, 297, 305, 307, 309, 310, 311, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 346, 373, 374, 375, 376, 377, 380 and 381. More generally, any sequence of amino acids identified in the sequence listing as providing a sequence derived from an immunoglobulin interdomain region (e.g., an antibody hinge region) is contemplated for use as a scorpion linker in the scorpion molecules according to the invention. In addition, a scorpion linker derived from an Ig hinge is a hinge-like peptide domain having at least one free cysteine capable of participating in an interchain disulfide bond. Preferably, a scorpion linker derived from an Ig hinge peptide retains a cysteine that corresponds to the hinge cysteine disposed towards the N-terminus of that hinge. Preferably, a scorpion linker derived from an IgG1 hinge has one cysteine or has two cysteines corresponding to hinge cysteines. Additionally, a scorpion linker is a stalk region of a C-type lectin domain (derived from a Type II membrane protein). In some embodiments, a scorpion comprises a scorpion linker having a sequence selected from the group consisting of SEQ ID NOS:373-377, 380 and 381. In general terms, a scorpion linker may be derived from an interdomain region of an immunoglobulin superfamily member, such as the interdomain region constituting a hinge in an immunoglobulin, or a scorpion linker may be derived from the extracellular stalk region of type 2 membrane proteins that are C-type lectin family members. Exemplary scorpion linkers are provided in Table A.

Scorpion linker source	Exemplary scorpion linkers*	Characteristics
IgG1 hinge	H7-H11, H17, H19, H21, H23, H25, H27, H33, H36-H46, H53, and H54	Immunoglobulin Mid-hinge region
CD2	H20, HG21, and H28	Type 1 membrane protein Immunoglobulin superfamily member Scorpion linkers derived from region linking IgV and IgC2
CD80	H16, H17, H24, H25, H47, and H48	Type 1 membrane protein Immunoglobulin superfamily member Scorpion linkers derived from region linking IgV and IgC

-continued

Scorpion linker source	Exemplary scorpion linkers*	Characteristics
CD86	H18, H19, H26, H27, H50, H51, H52, H53, H54, H55, and H56	Type 1 membrane protein Immunoglobulin superfamily member Scorpion linkers derived from region linking IgV and IgC
CD22	H22, H23, H30, H32, H33, and H57	Type 1 membrane protein Siglec (Sialic acid binding Ig-like lectin) Scorpion linkers derived from region linking non-Ig domain and IgC2 domain
CD69	H60	Type 2 membrane protein C-type lectin Scorpion linkers derived from region linking transmembrane domain and lectin C domain
CD72	H58, H59 and H70	Type 2 membrane protein C-type lectin Scorpion linkers derived from region linking transmembrane domain and lectin C domain
CD94	H61	Type 2 membrane protein C-type lectin Scorpion linkers derived from region linking transmembrane domain and lectin C domain
NKG2A	H62 and H69 (variant of H62)	Type 2 membrane protein C-type lectin Scorpion linkers derived from region linking transmembrane domain and lectin C domain
NKG2D	H63	Type 2 membrane protein C-type lectin Scorpion linkers derived from region linking transmembrane domain and lectin C domain

*Designations further described below, including the sequence listing.

[0172] The centrally disposed constant sub-region is derived from a constant region of an immunoglobulin protein. The constant sub-region generally is derived from a C_{H2} portion of a C_H region of an immunoglobulin in the abstract, although it may be derived from a C_{H2} - C_{H3} portion. Optionally, the constant sub-region may be derived from a hinge- C_{H2} or hinge- C_{H2} - C_{H3} portion of an immunoglobulin, placing a peptide corresponding to an Ig interdomain region (e.g., an antibody hinge region) N-terminal to the constant sub-region and disposed between the constant sub-region and binding domain 1. Also, portions of the constant sub-region may be derived from the C_H regions of different immunoglobulins. Further, the peptide corresponding to an Ig C_{H3} may be truncated, leaving a C-terminal amino acid sequence selected from the group consisting of SEQ ID NOS:366-371. It is preferred, however, that in embodiments in which a scorpion hinge is a hinge-like peptide derived from an immunoglobulin hinge, that the scorpion linker and the constant sub-region be derived from the same type of immunoglobulin. The constant sub-region provides at least one activity associated with a C_H region of an immunoglobulin, such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), protein A binding, binding to at least one F_C receptor, reproducibly detectable stability relative to a protein according to the invention except for the absence of a constant sub-region, and perhaps placental transfer where generational transfer of a molecule according to the invention would be advantageous, as recognized by one of skill in the art. As with the above-described

binding domains, the constant sub-region is derived from at least one immunoglobulin molecule and exhibits an identical or substantially identical amino acid sequence to a region or regions of at least one immunoglobulin. In some embodiments, the constant sub-region is modified from the sequence or sequences of at least one immunoglobulin (by substitution of one or more non-native conventional or unconventional, e.g., synthetic, amino acids or imino acids), resulting in a primary structure that may yield an altered secondary or higher order structure with altered properties associated therewith, or may lead to alterations in post-translational processing, such as glycosylation.

[0173] For those binding domains and constant sub-regions exhibiting an identical or substantially identical amino acid sequence to one or more immunoglobulin polypeptides, the post-translational modifications of the molecule according to the invention may result in a molecule modified relative to the immunoglobulin(s) serving as a basis for modification. For example, using techniques known in the art, a host cell may be modified, e.g. a CHO cell, in a manner that leads to an altered polypeptide glycosylation pattern relative to that polypeptide in an unmodified (e.g., CHO) host cell.

[0174] Provided with such molecules, and the methods of recombinantly producing them in vivo, new avenues of targeted diagnostics and therapeutics have been opened to allow, e.g., for the targeted recruitment of effector cells of the immune system (e.g., cytotoxic T lymphocytes, natural killer cells, and the like) to cells, tissues, agents and foreign objects to be destroyed or sequestered, such as cancer cells and infectious agents. In addition to localizing therapeutic cells to a site of treatment, the peptides are useful in localizing therapeutic compounds, such as radiolabeled proteins. Further, the peptides are also useful in scavenging deleterious compositions, for example by associating a deleterious composition, such as a toxin, with a cell capable of destroying or eliminating that toxin (e.g., a macrophage). The molecules of the invention are useful in modulating the activity of binding partner molecules, such as cell surface receptors. This is shown in FIG. 17 where apoptotic signaling through CD20 and/or CD37 is markedly enhanced by a molecule of the present invention. The effect of this signaling is the death of the targeted cell. Diseases and conditions where the elimination of defined cell populations is beneficial would include infectious and parasitic diseases, inflammatory and autoimmune conditions, malignancies, and the like. One skilled in the art would recognize that there is no limitation of the approach to the enhancement of apoptotic signaling. Mitotic signaling and signaling leading to differentiation, activation, or inactivation of defined cell populations can be induced by molecules of the present invention through the appropriate selection of binding partner molecules. Further consideration of the disclosure of the invention will be facilitated by a consideration of the following express definitions of terms used herein.

[0175] A “single-chain binding protein” is a single contiguous arrangement of covalently linked amino acids, with the chain capable of specifically binding to one or more binding partners sharing sufficient determinants of a binding site to be detectably bound by the single-chain binding protein. Exemplary binding partners include proteins, carbohydrates, lipids and small molecules.

[0176] For ease of exposition, “derivatives” and “variants” of proteins, polypeptides, and peptides according to the invention are described in terms of differences from proteins and/or polypeptides and/or peptides according to the inven-

tion, meaning that the derivatives and variants, which are proteins/polypeptides/peptides according to the invention, differ from underivatized or non-variant proteins, polypeptides or peptides of the invention in the manner defined. One of skill in the art would understand that the derivatives and variants themselves are proteins, polypeptides and peptides according to the invention.

[0177] An “antibody” is given the broadest definition consistent with its meaning in the art, and includes proteins, polypeptides and peptides capable of binding to at least one binding partner, such as a proteinaceous or non-proteinaceous antigen. An “antibody” as used herein includes members of the immunoglobulin superfamily of proteins, of any species, of single- or multiple-chain composition, and variants, analogs, derivatives and fragments of such molecules. Specifically, an “antibody” includes any form of antibody known in the art, including but not limited to, monoclonal and polyclonal antibodies, chimeric antibodies, CDR-grafted antibodies, humanized antibodies, single-chain variable fragments, bi-specific antibodies, diabodies, antibody fusions, and the like.

[0178] A “binding domain” is a peptide region, such as a fragment of a polypeptide derived from an immunoglobulin (e.g., an antibody), that specifically binds one or more specific binding partners. If a plurality of binding partners exists, those partners share binding determinants sufficient to detectably bind to the binding domain. Preferably, the binding domain is a contiguous sequence of amino acids.

[0179] An “epitope” is given its ordinary meaning herein of a single antigenic site, i.e., an antigenic determinant, on a substance (e.g., a protein) with which an antibody specifically interacts, for example by binding. Other terms that have acquired well-settled meanings in the immunoglobulin (e.g., antibody) art, such as a “variable light region,” variable heavy region,” “constant light region,” “constant heavy region,” “interdomain region of an immunoglobulin superfamily member,” “antibody hinge region,” “complementarity determining region,” “framework region,” “antibody isotype,” “Fc region,” “single-chain variable fragment” or “scFv,” “diabody,” “chimera,” “CDR-grafted antibody,” “humanized antibody,” “shaped antibody,” “antibody fusion,” and the like, are each given those well-settled meanings known in the art, unless otherwise expressly noted herein.

[0180] Terms understood by those in the art as referring to antibody technology are each given the meaning acquired in the art, unless expressly defined herein. Examples of such terms are “V_L” and “V_H”, referring to the variable binding region derived from an antibody light and heavy chain, respectively; and C_L and C_H, referring to an “immunoglobulin constant region,” i.e., a constant region derived from an antibody light or heavy chain, respectively, with the latter region understood to be further divisible into C_{H1}, C_{H2}, C_{H3} and C_{H4} constant region domains, depending on the antibody isotype (IgA, IgD, IgE, IgG, IgM) from which the region was derived.

[0181] CDR means “complementarity determining region.” A “hinge region” is derived from the amino acid sequence interposed between, and connecting, the C_{H1} and C_{H2} regions of a single chain of an antibody, which is known in the art as providing flexibility, in the form of a “hinge,” to whole antibodies.

[0182] A “constant sub-region” is a term defined herein to refer to a peptide, polypeptide, or protein sequence that corresponds to, or is derived from, one or more constant region

domains of an antibody. Thus, a constant sub-region may include any or all of the following domains: a C_{H1} domain, a hinge region, a C_{H2} domain, a C_{H3} domain (IgA, IgD, IgG, IgE, and IgM), and a C_{H4} domain (IgE, IgM). A constant sub-region as defined herein, therefore, can refer to a polypeptide region corresponding to an entire constant region of an antibody, or a portion thereof. Typically, a constant sub-region of a polypeptide, or encoding nucleic acid, of the invention has a hinge, C_{H2} domain, and C_{H3} domain.

[0183] An “effector function” is a function associated with or provided by a constant region of an antibody. Exemplary effector functions include antibody-dependent cell-mediated cytotoxicity (ADCC), complement activation and complement-dependent cytotoxicity (CDC), F_C receptor binding, and increased plasma half-life, as well as placental transfer. An effector function of a composition according to the invention is detectable; preferably, the specific activity of the composition according to the invention for that function is about the same as the specific activity of a wild-type antibody with respect to that effector function, i.e., the constant sub-region of the multivalent binding molecule preferably has not lost any effector function relative to a wild-type antibody]

[0184] A “linker” is a peptide, or polynucleotide, that joins or links other peptides or polynucleotides. Typically, a peptide linker is an oligopeptide of from about 2-50 amino acids, with typical polynucleotide linkers encoding such a peptide linker and, thus, being about 6-150 nucleotides in length. Linkers join the first binding domain to a constant sub-region domain. An exemplary peptide linker is $(Gly_4Ser)_3$. A scorpion linker is used to join the C-terminal end of a constant sub-region to a second binding domain. The scorpion linker may be derived from the interdomain region of an immunoglobulin superfamily member such as an immunoglobulin hinge region or from the stalk region of a C-type lectin, as described in greater detail below.

[0185] A “target” is given more than one meaning, with the context of usage defining an unambiguous meaning in each instance. In its narrowest sense, a “target” is a binding site, i.e., the binding domain of a binding partner for a peptide composition according to the invention. In a broader sense, “target” or “molecular target” refers to the entire binding partner (e.g., a protein), which necessarily exhibits the binding site.

[0186] Specific targets, such as “CD20,” “CD37,” and the like, are each given the ordinary meaning the term has acquired in the art. A “target cell” is any prokaryotic or eukaryotic cell, whether healthy or diseased, that is associated with a target molecule according to the invention. Of course, target molecules are also found unassociated with any cell (i.e., a cell-free target) or in association with other compositions such as viruses (including bacteriophage), organic or inorganic target molecule carriers, and foreign objects.

[0187] Examples of materials with which a target molecule may be associated include autologous cells (e.g., cancer cells or other diseased cells), infectious agents (e.g., infectious cells and infectious viruses), and the like. A target molecule may be associated with an enucleated cell, a cell membrane, a liposome, a sponge, a gel, a capsule, a tablet, and the like, which may be used to deliver, transport or localize a target molecule, regardless of intended use (e.g., for medical treatment, as a result of benign or unintentional provision, or to further a bioterrorist threat). “Cell-free,” “virus-free,” “car-

rier-free,” “object-free,” and the like refer to target molecules that are not associated with the specified composition or material.

[0188] “Binding affinity” refers to the strength of non-covalent binding of the peptide compositions of the invention and their binding partners. Preferably, binding affinity refers to a quantitative measure of the attraction between members of a binding pair.

[0189] An “adjuvant” is a substance that increases or aids the functional effect of a compound with which it is in association, such as in the form of a pharmaceutical composition comprising an active agent and an adjuvant. An “excipient” is an inert substance used as a diluent in formulating a pharmaceutical composition. A “carrier” is a typically inert substance used to provide a vehicle for delivering a pharmaceutical composition.

[0190] “Host cell” refers to any cell, prokaryotic or eukaryotic, in which is found a polynucleotide, protein or peptide according to the invention.

[0191] “Introducing” a nucleic acid or polynucleotide into a host cell means providing for entry of the nucleic acid or polynucleotide into that cell by any means known in the art, including but not limited to, in vitro salt-mediated precipitations and other forms of transformation of naked nucleic acid/polynucleotide or vector-borne nucleic acid/polynucleotide, virus-mediated infection and optionally transduction, with or without a “helper” molecule, ballistic projectile delivery, conjugation, and the like.

[0192] “Incubating” a host cell means maintaining that cell under environmental conditions known in the art to be suitable for a given purpose, such as gene expression. Such conditions, including temperature, ionic strength, oxygen tension, carbon dioxide concentration, nutrient composition, and the like, are well known in the art.

[0193] “Isolating” a compound, such as a protein or peptide according to the invention, means separating that compound from at least one distinct compound with which it is found associated in nature, such as in a host cell expressing the compound to be isolated, e.g. by isolating spent culture medium containing the compound from the host cells grown in that medium.

[0194] An “organism in need” is any organism at risk of, or suffering from, any disease, disorder or condition that is amenable to treatment or amelioration with a composition according to the invention, including but not limited to any of various forms of cancer, any of a number of autoimmune diseases, radiation poisoning due to radiolabeled proteins, peptides and like compounds, ingested or internally produced toxins, and the like, as will become apparent upon review of the entire disclosure. Preferably, an organism in need is a human patient.

[0195] “Ameliorating” a symptom of a disease means detectably reducing the severity of that symptom of disease, as would be known in the art. Exemplary symptoms include pain, heat, swelling and joint stiffness.

[0196] Unless clear from context, the terms “protein,” “peptide,” and “polypeptide” are used interchangeably herein, with each referring to at least one contiguous chain of amino acids. Analogously, the terms “polynucleotide,” “nucleic acid,” and “nucleic acid molecule” are used interchangeably unless it is clear from context that a particular, and non-interchangeable, meaning is intended.

[0197] “Pharmaceutically acceptable salt” refers to salts of the compounds of the present invention derived from the

combination of such compounds and an organic or inorganic acid (acid addition salts) or an organic or inorganic base (base addition salts).

[0198] Using the terms as defined above, a general description of the various aspects of the invention is provided below. Following the general description, working examples are presented to provide supplementary evidence of the operability and usefulness of the invention disclosed herein.

[0199] Proteins and Polypeptides

[0200] In certain embodiments of the invention, there are provided any of the herein-described multivalent binding proteins with effector function, including binding domain-immunoglobulin fusion proteins, wherein the multivalent binding protein or peptide with effector function comprises two or more binding domain polypeptide sequences. Each of the binding domain polypeptide sequences is capable of binding or specifically binding to a target(s), such as an antigen(s), which target(s) or antigen(s) may be the same or may be different. The binding domain polypeptide sequence may be derived from an antigen variable region or it may be derived from immunoglobulin-like molecules, e.g., receptors that fold in ways that mimic immunoglobulin molecules. The antibodies from which the binding domains are derived may be antibodies that are polyclonal, including monospecific polyclonal, monoclonal (mAbs), recombinant, chimeric, humanized (such as CDR-grafted), human, single-chain, catalytic, and any other form of antibody known in the art, as well as fragments, variants or derivatives thereof. In some embodiments, each of the binding domains of the protein according to the invention is derived from a complete variable region of an immunoglobulin. In preferred embodiments, the binding domains are each based on a human Ig variable region. In other embodiments, the protein is derived from a fragment of an Ig variable region. In such embodiments, it is preferred that each binding domain polypeptide sequence correspond to the sequences of each of the complementarity determining regions of a given Ig variable region. Also contemplated within the invention are binding domains that correspond to fewer than all CDRs of a given Ig variable region, provided that such binding domains retain the capacity to specifically bind to at least one target.

[0201] The multivalent binding protein with effector function also has a constant sub-region sequence derived from an immunoglobulin constant region, preferably an antibody heavy chain constant region, covalently juxtaposed between the two binding domains in the multivalent binding protein with effector function.

[0202] The multivalent binding protein with effector function also has a scorpion linker that joins the C-terminal end of the constant sub-region to the N-terminal end of binding domain 2. The scorpion linker may be derived from an antibody hinge region, from a region connecting binding domains of an immunoglobulin, or from the stalk region of C-type lectin. The scorpion linker may be derived from a wild-type hinge region of an immunoglobulin, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgD or an IgE hinge region. In other embodiments, the invention provides multivalent binding proteins with altered hinges. One category of altered hinge regions suitable for inclusion in the multivalent binding proteins is the category of hinges with an altered number of Cysteine residues, particularly those Cys residues known in the art to be involved in interchain disulfide bond formation in immunoglobulin counterpart molecules having wild-type hinges. Thus, proteins may have an IgG 1 hinge in which one

of the three Cys residues capable of participating in interchain disulfide bond formations is missing. To indicate the Cysteine sub-structure of altered hinges, the Cys subsequence is presented from N- to C-terminus. Using this identification system, the multivalent binding proteins with altered IgG hinges include hinge structures characterized as exc, xxc, ccx, xxc, xcx, cxx, and xxx. The Cys residue may be either deleted or substituted by an amino acid that results in a conservative substitution or a non-conservative substitution. In some embodiments, the Cysteine is replaced by a Serine. For proteins with scorpion linkers comprising IgG1 hinges, the number of cysteines corresponding to hinge cysteines is reduced to 1 or 2, preferably with one of those cysteines corresponding to the hinge cysteine disposed closest to the N-terminus of the hinge.

[0203] For proteins with scorpion linkers comprising IgG2 hinges, there may be 0, 1, 2, 3, or 4 Cys residues. For scorpion linkers comprising altered IgG2 hinges containing 1, 2 or 3 Cys residues, all possible subsets of Cys residues are contemplated. Thus, for such linkers having one Cys, the multivalent binding proteins may have the following Cys motif in the hinge region: cxxx, xcxx, xxcx, or xxxc. For scorpion linkers comprising IgG2 hinge variants having 2 or 3 Cys residues, all possible combinations of retained and substituted (or deleted) Cys residues are contemplated. For multivalent binding proteins with scorpion linkers comprising altered IgG3 or altered IgG4 hinge regions, a reduction in Cys residues from 1 to one less than the complete number of Cys residues in the hinge region is contemplated, regardless of whether the loss is through deletion or substitution by conservative or non-conservative amino acids (e.g., Serine). In like manner, multivalent binding proteins having a scorpion linker comprising a wild-type IgA, IgD or IgE hinge are contemplated, as are corresponding altered hinge regions having a reduced number of Cys residues extending from 0 to one less than the total number of Cys residues found in the corresponding wild-type hinge. In some embodiments having an IgG1 hinge, the first, or N-terminal, Cys residue of the hinge is retained. For proteins with either wild-type or altered hinge regions, it is contemplated that the multivalent binding proteins will be single-chain molecules capable of forming homo-multimers, such as dimers, e.g., by disulfide bond formation. Further, proteins with altered hinges may have alterations at the termini of the hinge region, e.g., loss or substitution of one or more amino acid residues at the N-terminus, C-terminus or both termini of a given region or domain, such as a hinge domain, as disclosed herein.

[0204] In another exemplary embodiment, the constant sub-region is derived from a constant region that comprises a native, or an engineered, IgD hinge region. The wild-type human IgD hinge has one cysteine that forms a disulfide bond with the light chain in the native IgD structure. In some embodiments, this IgD hinge cysteine is mutated (e.g., deleted) to generate an altered hinge for use as a connecting region between binding domains of, for example, a bispecific molecule. Other amino acid changes or deletions or alterations in an IgD hinge that do not result in undesired hinge inflexibility are within the scope of the invention. Native or engineered IgD hinge regions from other species are also within the scope of the invention, as are humanized native or engineered IgD hinges from non-human species, and (other non IgD) hinge regions from other human, or non-human, antibody isotypes, (such as the llama IgG2 hinge).

[0205] The invention further comprehends constant sub-regions attached to scorpion linkers that may be derived from hinges that correspond to a known hinge region, such as an IgG1 hinge or an IgD hinge, as noted above. The constant sub-region may contain a modified or altered (relative to wild-type) hinge region in which at least one cysteine residue known to participate in inter-chain disulfide bond linkage is replaced by another amino acid in a conservative substitution (e.g., Ser for Cys) or a non-conservative substitution. The constant sub-region does not include a peptide region or domain that corresponds to an immunoglobulin C_{H1} domain.

[0206] Alternative hinge and linker sequences that can be used as connecting regions are from portions of cell surface receptors that connect immunoglobulin V-like or immunoglobulin C-like domains. Regions between Ig V-like domains where the cell surface receptor contains multiple Ig V-like domains in tandem, and between Ig C-like domains where the cell surface receptor contains multiple tandem Ig C-like regions are also contemplated as connecting regions. Hinge and linker sequences are typically from 5 to 60 amino acids long, and may be primarily flexible, but may also provide more rigid characteristics. In addition, linkers frequently provide spacing that facilitates minimization of steric hindrance between the binding domains. Preferably, these hinge and linker peptides are primarily a helical in structure, with minimal sheet structure. The preferred sequences are stable in plasma and serum and are resistant to proteolytic cleavage. The preferred sequences may contain a naturally occurring or added motif such as the CPPC motif that confers a disulfide bond to stabilize dimer formation. The preferred sequences may contain one or more glycosylation sites. Examples of preferred hinge and linker sequences include, but are not limited to, the interdomain regions between the Ig V-like and Ig C-like regions of CD2, CD4, CD22, CD33, CD48, CD58, CD66, CD80, CD86, CD150, CD166, and CD244.

[0207] The constant sub-region may be derived from a camelid constant region, such as either a llama or camel IgG2 or IgG3.

[0208] Specifically contemplated is a constant sub-region having the C_{H2} - C_{H3} region from any Ig class, or from any IgG subclass, such as IgG1 (e.g., human IgG1). In preferred embodiments, the constant sub-region and the scorpion linker derived from an immunoglobulin hinge are both derived from the same Ig class. In other preferred embodiments, the constant sub-region and the scorpion linker derived from an immunoglobulin hinge are both derived from the same Ig sub-class. The constant sub-region also may be a CH3 domain from any Ig class or subclass, such as IgG1 (e.g., human IgG1), provided that it is associated with at least one immunoglobulin effector function.

[0209] The constant sub-region does not correspond to a complete immunoglobulin constant region (i.e., C_{H1} -hinge- C_{H2} - C_{H3}) of the IgG class. The constant sub-region may correspond to a complete immunoglobulin constant region of other classes, IgA constant domains, such as an IgA1 hinge, an IgA2 hinge, an IgA C_{H2} and an IgA C_{H3} domains with a mutated or missing tailpiece are also contemplated as constant sub-regions. Further, any light chain constant domain may function as a constant sub-region, e.g., C_K or any C_L . The constant sub-region may also include JH or JK, with or without a hinge. The constant sub-region may also correspond to engineered antibodies in which, e.g., a loop graft has been constructed by making selected amino acid substitutions using an IgG framework to generate a binding site for a

receptor other than a natural F_C R (CD16, CD32, CD64, $F_C\epsilon R1$), as would be understood in the art. An exemplary constant sub-region of this type is an IgG C_{H2} - C_{H3} region modified to have a CD89 binding site.

[0210] This aspect of the invention provides a multivalent binding protein or peptide having effector function, comprising, consisting essentially of, or consisting of (a) an N-terminally disposed binding domain polypeptide sequence derived from an immunoglobulin that is fused or otherwise connected to (b) a constant sub-region polypeptide sequence derived from an immunoglobulin constant region, which preferably includes a hinge region sequence, wherein the hinge region polypeptide may be as described herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region polypeptide sequence, in turn fused or otherwise connected to (c) a C-terminally disposed second native or engineered binding domain polypeptide sequence derived from an immunoglobulin.

[0211] The centrally disposed constant sub-region polypeptide sequence derived from an immunoglobulin constant region is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, complement fixation, and F_C receptor binding, and the binding domain polypeptides are each capable of binding or specifically binding to a target, such as an antigen, wherein the targets may be the same or different, and may be found in effectively the same physiological environment (e.g., the surface of the same cell) or in different environments (e.g., different cell surfaces, a cell surface and a cell-free location, such as in solution).

[0212] This aspect of the invention also comprehends variant proteins or polypeptides exhibiting an effector function that are at least 80%, and preferably 85%, 90%, 95% or 99% identical to a multivalent protein with effector function of specific sequence as disclosed herein.

[0213] Polynucleotides

[0214] The invention also provides polynucleotides (isolated or purified or pure polynucleotides) encoding the proteins or peptides according to the invention, vectors (including cloning vectors and expression vectors) comprising such polynucleotides, and cells (e.g., host cells) transformed or transfected with a polynucleotide or vector according to the invention. In encoding the proteins or polypeptides of the invention, the polynucleotides encode a first binding domain, a second binding domain and an F_C domain, all derived from immunoglobulins, preferably human immunoglobulins. Each binding domain may contain a sequence corresponding to a full-length variable region sequence (either heavy chain and/or light chain), or to a partial sequence thereof, provided that each such binding domain retains the capacity to specifically bind. The F_C domain may have a sequence that corresponds to a full-length immunoglobulin F_C domain sequence or to a partial sequence thereof, provided that the F_C domain exhibits at least one effector function as defined herein. In addition, each of the binding domains may be joined to the F_C domain via a linker peptide that typically is at least 8, and preferably at least 13, amino acids in length. A preferred linker sequence is a sequence based on the Gly₄Ser motif, such as (Gly₄Ser)₃.

[0215] Variants of the multivalent binding protein with effector function are also comprehended by the invention. Variant polynucleotides are at least 90%, and preferably 95%, 99%, or 99.9% identical to one of the polynucleotides of defined sequence as described herein, or that hybridizes to one of those polynucleotides of defined sequence under strin-

gent hybridization conditions of 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68° C. or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 50% formamide at 42° C. The polynucleotide variants retain the capacity to encode a multivalent binding protein with effector function.

[0216] The term “stringent” is used to refer to conditions that are commonly understood in the art as stringent. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of stringent conditions for hybridization and washing are 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68° C. or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 50% formamide at 42° C. See Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989).

[0217] More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used; however, the rate of hybridization will be affected. In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary stringent hybridization conditions include washing in 6×SSC, 0.05% sodium pyrophosphate at 37° C. (for 14-base oligonucleotides), 48° C. (for 17-base oligonucleotides), 55° C. (for 20-base oligonucleotides), and 60° C. (for 23-base oligonucleotides).

[0218] In a related aspect of the invention, there is provided a method of producing a polypeptide or protein or other construct of the invention, for example, including a multivalent binding protein or peptide having effector function, comprising the steps of (a) culturing a host cell as described or provided for herein under conditions that permit expression of the construct; and (b) isolating the expression product, for example, the multivalent binding protein or peptide with effector function from the host cell or host cell culture.

[0219] Constructs

[0220] The present invention also relates to vectors, and to constructs prepared from known vectors, that each include a polynucleotide or nucleic acid of the invention, and in particular to recombinant expression constructs, including any of various known constructs, including delivery constructs, useful for gene therapy, that include any nucleic acids encoding multivalent, for example, multispecific, including bi-specific, binding proteins and polypeptides with effector function, as provided herein; to host cells which are genetically engineered with vectors and/or other constructs of the invention and to methods of administering expression or other constructs comprising nucleic acid sequences encoding multivalent, for example, multispecific, including bi-specific, binding proteins with effector function, or fragments or variants thereof, by recombinant techniques.

[0221] Various constructs of the invention including multivalent, for example, multispecific binding proteins with effector function, can be expressed in virtually any host cell, including in vivo host cells in the case of use for gene therapy, under the control of appropriate promoters, depending on the nature of the construct (e.g., type of promoter, as described above), and on the nature of the desired host cell (e.g., post-mitotic terminally differentiated or actively dividing; e.g., maintenance of an expressible construct as an episome or integrated into the host cell genome).

[0222] Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, in Sambrook, et al., *Molecular Cloning: A Labora-*

tory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989). Exemplary cloning/expression vectors include, but are not limited to, cloning vectors, shuttle vectors, and expression constructs, that may be based on plasmids, phagemids, phasmids, cosmids, viruses, artificial chromosomes, or any nucleic acid vehicle suitable for amplification, transfer, and/or expression of a polynucleotide contained therein that is known in the art. As noted herein, in preferred embodiments of the invention, recombinant expression is conducted in mammalian cells that have been transfected, transformed or transduced with a nucleic acid according to the invention. See also, for example, Machida, C A., “Viral Vectors for Gene Therapy: Methods and Protocols”; Wolff, J A., “Gene Therapeutics: Methods and Applications of Direct Gene Transfer” (Birkhauser 1994); Stein, U and Walther, W (eds., “Gene Therapy of Cancer: Methods and Protocols” (Humana Press 2000); Robbins, P D (ed.), “Gene Therapy Protocols” (Humana Press 1997); Morgan, J R (ed.), “Gene Therapy Protocols” (Humana Press 2002); Meager, A (ed.), “Gene Therapy Technologies, Applications and Regulations: From Laboratory to Clinic” (John Wiley & Sons Inc. 1999); MacHida, C A and Constant, J G, “Viral Vectors for Gene Therapy: Methods and Protocols” (Humana Press 2002); “New Methods Of Gene Therapy For Genetic Metabolic Diseases NIH Guide,” Volume 22, Number 35, Oct. 1, 1993. See also U.S. Pat. Nos. 6,384,210; 6,384,203; 6,384,202; 6,384,018; 6,383,814; 6,383,811; 6,383,795; 6,383,794; 6,383,785; 6,383,753; 6,383,746; 6,383,743; 6,383,738; 6,383,737; 6,383,733; 6,383,522; 6,383,512; 6,383,481; 6,383,478; 6,383,138; 6,380,382; 6,380,371; 6,380,369; 6,380,362; 6,380,170; 6,380,169; 6,379,967; and 6,379,966.

[0223] Typically, expression constructs are derived from plasmid vectors. One preferred construct is a modified pNASS vector (Clontech, Palo Alto, Calif.), which has nucleic acid sequences encoding an ampicillin resistance gene, a polyadenylation signal and a T7 promoter site. Other suitable mammalian expression vectors are well known (see, e.g., Ausubel et al., 1995; Sambrook et al., supra; see also, e.g., catalogues from Invitrogen, San Diego, Calif.; Novagen, Madison, Wis.; Pharmacia, Piscataway, N.J.). Presently preferred constructs may be prepared that include a dihydrofolate reductase (DHFR)-encoding sequence under suitable regulatory control, for promoting enhanced production levels of the multivalent binding protein with effector function, which levels result from gene amplification following application of an appropriate selection agent (e.g., methotrexate).

[0224] Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, as described above. A vector in operable linkage with a polynucleotide according to the invention yields a cloning or expression construct. Exemplary cloning/expression constructs contain at least one expression control element, e.g., a promoter, operably linked to a polynucleotide of the invention. Additional expression control elements, such as enhancers, factor-specific binding sites, terminators, and ribosome binding sites are also contemplated in the vectors and cloning/expression constructs according to the invention. The heterologous structural sequence of the polynucleotide according to the invention is assembled in appropriate phase with translation initiation and termination sequences. Thus, for example, the multivalent binding protein-encoding nucleic acids as provided herein

may be included in any one of a variety of expression vector constructs as a recombinant expression construct for expressing such a protein in a host cell. In certain preferred embodiments the constructs, are included in formulations that are administered in vivo. Such vectors and constructs include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies, or replication deficient retroviruses as described below. However, any other vector may be used for preparation of a recombinant expression construct, and in preferred embodiments such a vector will be replicable and viable in the host.

[0225] The appropriate DNA sequence(s) may be inserted into a vector, for example, by a variety of procedures. In general, a DNA sequence is inserted into an appropriate restriction endonuclease cleavage site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are contemplated. A number of standard techniques are described, for example, in Ausubel et al. (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, Mass.); Sambrook et al. (1989 *Molecular Cloning*, Second Ed., Cold Spring Harbor Laboratory, Plainview, N.Y.); Maniatis et al. (1982 *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, N.Y.); Glover (Ed.) (1985 *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK); Hames and Higgins (Eds.), (1985 *Nucleic Acid Hybridization*, IRL Press, Oxford, UK); and elsewhere.

[0226] The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequence (e.g., a constitutive promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include promoters of eukaryotic cells or their viruses, as described above. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding a protein or polypeptide according to the invention is described herein.

[0227] Transcription of the DNA encoding proteins and polypeptides of the invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Examples include the SV40 enhancer on the late side of the replication origin by 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

[0228] Gene therapies using the nucleic acids of the invention are also contemplated, comprising strategies to replace defective genes or add new genes to cells and/or tissues, and is being developed for application in the treatment of cancer, the correction of metabolic disorders and in the field of immunotherapy. Gene therapies of the invention include the use of various constructs of the invention, with or without a separate carrier or delivery vehicle or constructs, for treatment of the

diseases, disorders, and/or conditions noted herein. Such constructs may also be used as vaccines for treatment or prevention of the diseases, disorders, and/or conditions noted herein. DNA vaccines, for example, make use of polynucleotides encoding immunogenic protein and nucleic acid determinants to stimulate the immune system against pathogens or tumor cells. Such strategies can stimulate either acquired or innate immunity or can involve the modification of immune function through cytokine expression. In vivo gene therapy involves the direct injection of genetic material into a patient or animal, typically to treat, prevent or ameliorate a disease or symptoms associated with a disease. Vaccines and immune modulation are systemic therapies. With tissue-specific in vivo therapies, such as those that aim to treat cancer, localized gene delivery and/or expression/targeting systems are preferred. Diverse gene therapy vectors that target specific tissues are known in the art, and procedures have been developed to physically target specific tissues, for example, using catheter-based technologies, all of which are contemplated herein.

[0229] Ex vivo approaches to gene therapy are also contemplated herein and involve the removal, genetic modification, expansion and re-administration of a subject's, e.g., human patient's, own cells. Examples include bone marrow transplantation for cancer treatment or the genetic modification of lymphoid progenitor cells. Ex vivo gene therapy is preferably applied to the treatment of cells that are easily accessible and can survive in culture during the gene transfer process (such as blood or skin cells).

[0230] Useful gene therapy vectors include adenoviral vectors, lentiviral vectors, Adeno-associated virus (AAV) vectors, Herpes Simplex Virus (HSV) vectors, and retroviral vectors. Gene therapies may also be carried out using "naked DNA," liposome-based delivery, lipid-based delivery (including DNA attached to positively charged lipids), electroporation, and ballistic projection.

[0231] In certain embodiments, including but not limited to gene therapy embodiments, the vector may be a viral vector such as, for example, a retroviral vector. Miller et al., 1989 *BioTechniques* 7:980; Coffin and Varmus, 1996 *Retroviruses*, Cold Spring Harbor Laboratory Press, NY. For example, retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

[0232] Retroviruses are RNA viruses which can replicate and integrate into the genome of a host cell via a DNA intermediate. This DNA intermediate, or provirus, may be stably integrated into the host cell DNA. According to certain embodiments of the present invention, an expression construct may comprise a retrovirus into which a foreign gene that encodes a foreign protein is incorporated in place of normal retroviral RNA. When retroviral RNA enters a host cell coincident with infection, the foreign gene is also introduced into the cell, and may then be integrated into host cell DNA as if it were part of the retroviral genome. Expression of this foreign gene within the host results in expression of the foreign protein.

[0233] Most retroviral vector systems that have been developed for gene therapy are based on murine retroviruses. Such retroviruses exist in two forms, as free viral particles referred

to as virions, or as proviruses integrated into host cell DNA. The virion form of the virus contains the structural and enzymatic proteins of the retrovirus (including the enzyme reverse transcriptase), two RNA copies of the viral genome, and portions of the source cell plasma membrane containing viral envelope glycoprotein. The retroviral genome is organized into four main regions: the Long Terminal Repeat (LTR), which contains cis-acting elements necessary for the initiation and termination of transcription and is situated both 5' and 3' to the coding genes, and the three genes encoding gag, pol, and env. These three genes, gag, pol, and env, encode, respectively, internal viral structures, enzymatic proteins (such as integrase), and the envelope glycoprotein (designated gp70 and p15e) which confers infectivity and host range specificity of the virus, as well as the "R" peptide of undetermined function.

[0234] Separate packaging cell lines and vector-producing cell lines have been developed because of safety concerns regarding the uses of retroviruses, including uses in expression constructs. Briefly, this methodology employs the use of two components, a retroviral vector and a packaging cell line (PCL). The retroviral vector contains long terminal repeats (LTRs), the foreign DNA to be transferred and a packaging sequence (y). This retroviral vector will not reproduce by itself because the genes which encode structural and envelope proteins are not included within the vector genome. The PCL contains genes encoding the gag, pol, and env proteins, but does not contain the packaging signal "y." Thus, a PCL can only form empty virion particles by itself. Within this general method, the retroviral vector is introduced into the PCL, thereby creating a vector-producing cell line (VCL). This VCL manufactures virion particles containing only the foreign genome of the retroviral vector, and therefore has previously been considered to be a safe retrovirus vector for therapeutic use.

[0235] A "retroviral vector construct" refers to an assembly which is, within preferred embodiments of the invention, capable of directing the expression of a sequence(s) or gene (s) of interest, such as multivalent binding protein-encoding nucleic acid sequences. Briefly, the retroviral vector construct must include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis and a 3' LTR. A wide variety of heterologous sequences may be included within the vector construct including, for example, sequences which encode a protein (e.g., cytotoxic protein, disease-associated antigen, immune accessory molecule, or replacement gene), or which are useful as a molecule itself (e.g., as a ribozyme or antisense sequence).

[0236] Retroviral vector constructs of the present invention may be readily constructed from a wide variety of retroviruses, including for example, B, C, and D type retroviruses as well as spumaviruses and lentiviruses (see, e.g., RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Such retroviruses may be readily obtained from depositories or collections such as the American Type Culture Collection ("ATCC"; Rockville, Md.), or isolated from known sources using commonly available techniques. Any of the above retroviruses may be readily utilized in order to assemble or construct retroviral vector constructs, packaging cells, or producer cells of the invention, given the disclosure provided herein and standard recombinant techniques (e.g., Sambrook et al, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Kunkle, 1985 *Proc. Natl. Acad. Sci. (USA)* 82:488).

[0237] Suitable promoters for use in viral vectors generally may include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., 1989 *Biotechniques* 7:980-990, or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters that may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein, and may be from among either regulated promoters or promoters as described above.

[0238] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ -AM, PA12, T19-14x, VT-19-17-H2, ψ CRE, ψ CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, *Human Gene Therapy*, 1:5-14 (1990). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[0239] The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence (s) encoding the multivalent binding proteins with effector function. Such retroviral vector particles then may be employed to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the protein or polypeptide. Eukaryotic cells that may be transduced include, but are not limited to, embryonic stem cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, circulating peripheral blood mononuclear and polymorphonuclear cells including myelomonocytic cells, lymphocytes, myoblasts, tissue macrophages, dendritic cells, Kupffer cells, lymphoid and reticuloendothelial cells of the lymph nodes and spleen, keratinocytes, endothelial cells, and bronchial epithelial cells.

[0240] Host Cells

[0241] A further aspect of the invention provides a host cell transformed or transfected with, or otherwise containing, any of the polynucleotides or cloning/expression constructs of the invention. The polynucleotides and cloning/expression constructs are introduced into suitable cells using any method known in the art, including transformation, transfection and transduction. Host cells include the cells of a subject undergoing ex vivo cell therapy including, for example, ex vivo gene therapy. Eukaryotic host cells contemplated as an aspect of the invention when harboring a polynucleotide, vector, or protein according to the invention include, in addition to a subject's own cells (e.g., a human patient's own cells), VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines (including modified CHO cells capable of modifying the glycosylation pattern of expressed multivalent binding molecules, see Published US Patent Application No. 2003/0115614 A1), incorporated herein by reference, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562, HEK293 cells, HepG2 cells, N cells, 3T3 cells, *Spodoptera frugiperda* cells (e.g., SD cells), *Saccharomyces cerevisiae* cells, and any other eukaryotic cell known in the art to be useful in expressing, and optionally isolating, a

protein or peptide according to the invention. Also contemplated are prokaryotic cells, including but not limited to, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, a *Streptomyces*, or any prokaryotic cell known in the art to be suitable for expressing, and optionally isolating, a protein or peptide according to the invention. In isolating protein or peptide from prokaryotic cells, in particular, it is contemplated that techniques known in the art for extracting protein from inclusion bodies may be used. The selection of an appropriate host is within the scope of those skilled in the art from the teachings herein.

[0242] The engineered host cells can be cultured in a conventional nutrient medium modified as appropriate for activating promoters, selecting transformants, or amplifying particular genes. The culture conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan. Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, 1981 *Cell* 23:175, and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and, optionally, enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences, for example as described herein regarding the preparation of multivalent binding protein expression constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, calcium phosphate transfection, DEAE-Dextran-mediated transfection, or electroporation (Davis et al., 1986 *Basic Methods in Molecular Biology*).

[0243] In one embodiment, a host cell is transduced by a recombinant viral construct directing the expression of a protein or polypeptide according to the invention. The transduced host cell produces viral particles containing expressed protein or polypeptide derived from portions of a host cell membrane incorporated by the viral particles during viral budding.

[0244] Pharmaceutical Compositions

[0245] In some embodiments, the compositions of the invention, such as a multivalent binding protein or a composition comprising a polynucleotide encoding such a protein as described herein, are suitable to be administered under conditions and for a time sufficient to permit expression of the encoded protein in a host cell in vivo or in vitro, for gene therapy, and the like. Such compositions may be formulated into pharmaceutical compositions for administration according to well known methodologies. Pharmaceutical compositions generally comprise one or more recombinant expression constructs, and/or expression products of such constructs, in combination with a pharmaceutically acceptable carrier, excipient or diluent. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. For nucleic acid-based formulations, or for formulations comprising expression products according to the invention, about 0.01 µg/kg to about 100 mg/kg body weight will be administered, for example, by the intradermal, subcutaneous, intramuscular

or intravenous route, or by any route known in the art to be suitable under a given set of circumstances. A preferred dosage, for example, is about 1 µg/kg to about 1 mg/kg, with about 5 µg/kg to about 200 µg/kg particularly preferred.

[0246] It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the host. Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and the like may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid may be added as preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be used. Id. The compounds of the present invention may be used in either the free base or salt forms, with both forms being considered as being within the scope of the present invention.

[0247] The pharmaceutical compositions that contain one or more nucleic acid constructs of the invention, or the proteins corresponding to the products encoded by such nucleic acid constructs, may be in any form which allows for the composition to be administered to a patient. For example, the composition may be in the form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral (e.g., sublingually or buccally), sublingual, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrathecal, intrameatal, intraurethral injection or infusion techniques. The pharmaceutical composition is formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of the invention in aerosol form may hold a plurality of dosage units.

[0248] For oral administration, an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose and ethyl cellulose. Coloring and/or flavoring agents may be present. A coating shell may be employed.

[0249] The composition may be in the form of a liquid, e.g., an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred compositions contain, in addition to one or more binding domain-immunoglobulin fusion construct or expressed product, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

[0250] A liquid pharmaceutical composition as used herein, whether in the form of a solution, suspension or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or

other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

[0251] It may also be desirable to include other components in the preparation, such as delivery vehicles including, but not limited to, aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and liposomes. Examples of immunostimulatory substances (adjuvants) for use in such vehicles include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), lipopolysaccharides (LPS), glucan, IL-12, GM-CSF, gamma interferon and IL-15.

[0252] While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration and whether a sustained release is desired. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Pat. Nos. 4,897,268 and 5,075,109. In this regard, it is preferable that the microsphere be larger than approximately 25 microns.

[0253] Pharmaceutical compositions may also contain diluents such as buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates (e.g., glucose, sucrose or dextrins), chelating agents (e.g., EDTA), glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents.

[0254] The pharmaceutical compositions according to the invention also include stabilized proteins and stable liquid pharmaceutical formulations in accordance with technology known in the art, including the technology disclosed in Published US Patent Application No. 2006/0008415 A1, incorporated herein by reference. Such technologies include derivatization of a protein, wherein the protein comprises a thiol group coupled to N-acetyl-L-cysteine, N-ethyl-maleimide, or cysteine.

[0255] As described above, the subject invention includes compositions capable of delivering nucleic acid molecules encoding multivalent binding proteins with effector function. Such compositions include recombinant viral vectors, e.g., retroviruses (see WO 90/07936, WO 91/02805, WO 93/25234, WO 93/25698, and WO 94/03622), adenovirus (see Berkner, 1988 *Biotechniques* 6:616-627; Li et al., 1993 *Hum. Gene Ther.* 4:403-409; Vincent et al., *Nat. Genet.* 5:130-134; and Kolls et al., 1994 *Proc. Natl. Acad. Sci. USA* 91:215-

219), pox virus (see U.S. Pat. No. 4,769,330; U.S. Pat. No. 5,017,487; and WO 89/01973), recombinant expression construct nucleic acid molecules complexed to a polycationic molecule (see WO 93/03709), and nucleic acids associated with liposomes (see Wang et al., 1987 *Proc. Natl. Acad. Sci. USA* 84:7851). In certain embodiments, the DNA may be linked to killed or inactivated adenovirus (see Curiel et al., 1992 *Hum. Gene Ther.* 3:147-154; Cotton et al., 1992 *Proc. Natl. Acad. Sci. USA* 89:6094). Other suitable compositions include DNA-ligand (see Wu et al., 1989 *J. Biol. Chem.* 264:16985-16987) and lipid-DNA combinations (see Feigner et al., 1989 *Proc. Natl. Acad. Sci. USA* 84:7413-7417).

[0256] In addition to direct in vivo procedures, ex vivo procedures may be used in which cells are removed from a host (e.g., a subject, such as a human patient), modified, and placed into the same or another host animal. It will be evident that one can utilize any of the compositions noted above for introduction of constructs of the invention, either the proteins/polypeptides or the nucleic acids encoding them into tissue cells in an ex vivo context. Protocols for viral, physical and chemical methods of uptake are well known in the art.

[0257] Generation of Antibodies

[0258] Polyclonal antibodies directed toward an antigen polypeptide generally are produced in animals (e.g., rabbits, hamsters, goats, sheep, horses, pigs, rats, gerbils, guinea pigs, mice, or any other suitable mammal, as well as other non-mammal species) by means of multiple subcutaneous or intraperitoneal injections of antigen polypeptide or a fragment thereof and an adjuvant. Adjuvants include, but are not limited to, complete or incomplete Freund's adjuvant, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, and dinitrophenol. BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum* are also potentially useful adjuvants. It may be useful to conjugate an antigen polypeptide to a carrier protein that is immunogenic in the species to be immunized; typical carriers include keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-antigen polypeptide antibody titer using conventional techniques. Polyclonal antibodies may be utilized in the sera from which they were detected, or may be purified from the sera using, e.g., antigen affinity chromatography.

[0259] Monoclonal antibodies directed toward antigen polypeptides are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. For example, monoclonal antibodies may be made by the hybridoma method as described in Kohler et al., *Nature* 256:495 [1975]; the human B-cell hybridoma technique (Kosbor et al., *Immunol Today* 4:72, 1983; Cote et al., *Proc Natl Acad Sci* 80: 2026-2030, 1983) and the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R Liss Inc, New York N.Y., pp 77-96, (1985).

[0260] When the hybridoma technique is employed, myeloma cell lines may be used. Cell lines suited for use in hybridoma-producing fusion procedures preferably do not produce endogenous antibody, have high fusion efficiency, and exhibit enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). For example, where the immunized animal is a mouse, one may use

P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 41, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; for rats, one may use R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6 are all useful in connection with cell fusions.

[0261] In an alternative embodiment, human antibodies can be produced from phage-display libraries (Hoogenboom et al., J. Mol. Biol. 227: 381 [1991]; Marks et al., J. Mol. Biol. 222: 581, see also U.S. Pat. No. 5,885,793). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT Application No. PCT/US98/17364, filed in the name of Adams et al., which describes the isolation of high affinity and functional agonistic antibodies for MPL- and msk-receptors using such an approach. In this approach, a complete repertoire of human antibody genes can be created by cloning naturally rearranged human V genes from peripheral blood lymphocytes as previously described (Mullinax, et al., Proc. Natl. Acad. Sci. (USA) 87: 8095-8099 [1990]).

[0262] Alternatively, an entirely synthetic human heavy chain repertoire can be created from unrearranged V gene segments by assembling each human VH segment with D segments of random nucleotides together with a human J segment (Hoogenboom, et al., J. Mol. Biol. 227:381-388 [1992]). Likewise, a light chain repertoire can be constructed by combining each human V segment with a J segment (Griffiths, et al, EMBO J. 13:3245-3260 [1994]). Nucleotides encoding the complete antibody (i.e., both heavy and light chains) are linked as a single-chain Fv fragment and this polynucleotide is ligated to a nucleotide encoding a filamentous phage minor coat protein. When this fusion protein is expressed on the surface of the phage, a polynucleotide encoding a specific antibody can be identified by selection using an immobilized antigen.

[0263] Beyond the classic methods of generating polyclonal and monoclonal antibodies, any method for generating any known antibody form is contemplated. In addition to polyclonals and monoclonals, antibody forms include chimerized antibodies, humanized antibodies, CDR-grafted antibodies, and antibody fragments and variants.

[0264] Variants and Derivatives of Specific Binding Agents

[0265] In one example, insertion variants are provided wherein one or more amino acid residues supplement a specific binding agent amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the specific binding agent amino acid sequence. Variant products of the invention also include mature specific binding agent products, i.e., specific binding agent products wherein leader or signal sequences are removed, and the resulting protein having additional amino terminal residues. The additional amino terminal residues may be derived from another protein, or may include one or more residues that are not identifiable as being derived from a specific protein. Polypeptides with an additional methionine residue at position-1 (e.g., Met-1-multivalent binding peptides with effector function) are contemplated, as are polypeptides of the invention with additional methionine and lysine residues at positions-2 and -1 (Met-2-Lys-1-multivalent binding proteins with effector function). Variants of the polypeptides of the invention having additional Met, Met-Lys, or Lys residues (or one or more basic

residues in general) are particularly useful for enhanced recombinant protein production in bacterial host cells.

[0266] The invention also embraces specific polypeptides of the invention having additional amino acid residues which arise from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of a glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at position-1 after cleavage of the GST component from the desired polypeptide. Variants which result from expression in other vector systems are also contemplated, including those wherein histidine tags are incorporated into the amino acid sequence, generally at the carboxy and/or amino terminus of the sequence.

[0267] In another aspect, the invention provides deletion variants wherein one or more amino acid residues in a polypeptide of the invention are removed. Deletions can be effected at one or both termini of the polypeptide, or from removal of one or more residues within the amino acid sequence. Deletion variants necessarily include all fragments of a polypeptide according to the invention.

[0268] Antibody fragments refer to polypeptides having a sequence corresponding to at least part of an immunoglobulin variable region sequence. Fragments may be generated, for example, by enzymatic or chemical cleavage of polypeptides corresponding to full-length antibodies. Other binding fragments include those generated by synthetic techniques or by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding partial antibody variable regions. Preferred polypeptide fragments display immunological properties unique to, or specific for, a target as described herein. Fragments of the invention having the desired immunological properties can be prepared by any of the methods well known and routinely practiced in the art.

[0269] In still another aspect, the invention provides substitution variants of multivalent binding polypeptides having effector function. Substitution variants include those polypeptides wherein one or more amino acid residues in an amino acid sequence are removed and replaced with alternative residues. In some embodiments, the substitutions are conservative in nature; however, the invention embraces substitutions that are also non-conservative. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table B (see WO 97/09433, page 10, published Mar. 13, 1997 (PCT/GB96/02197, filed Sep. 6, 1996), immediately below.

TABLE B

Conservative Substitutions I		
SIDE CHAIN	CHARACTERISTIC	AMINO ACID
Aliphatic	Non-polar	G A P I L V
	Polar-uncharged	S T M N Q
	Polar-charged	D E K R

TABLE B-continued

Conservative Substitutions I		
SIDE CHAIN	CHARACTERISTIC	AMINO ACID
Aromatic		H F W Y
Other		N Q D E

[0270] Alternatively, conservative amino acids can be grouped as described in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY: NY (1975), pp. 71-77] as set out in Table C, immediately below.

TABLE C

Conservative Substitutions II		
SIDE CHAIN	CHARACTERISTIC	AMINO ACID
Non-polar (hydrophobic)	A. Aliphatic:	A L I V P
	B. Aromatic	F W
	C. Sulfur-	M
	containing	
Uncharged-polar	D. Borderline	G
	A. Hydroxyl	S T Y
	B. Amides	N Q
	C. Sulfhydryl	C
Positively Charged (Basic)	D. Borderline	G
		K R H
Negatively Charged (Acidic)		D E
Conservative Substitutions II		
SIDE CHAIN	CHARACTERISTIC	AMINO ACID
Non-polar (hydrophobic)	A. Aliphatic:	A L I V P
	B. Aromatic:	F W
	C. Sulfur-	M
	containing:	
Uncharged-polar	D. Borderline:	G
	A. Hydroxyl:	S T Y
	B. Amides:	N Q
	C. Sulfhydryl:	C
Positively Charged (Basic)	D. Borderline:	G
		K R H
Negatively Charged (Acidic)		D E

[0271] The invention also provides derivatives of specific binding agent polypeptides. Derivatives include specific

binding agent polypeptides bearing modifications other than insertion, deletion, or substitution of amino acid residues. Preferably, the modifications are covalent in nature, and include for example, chemical bonding with polymers, lipids, other organic, and inorganic moieties. Derivatives of the invention may be prepared to increase circulating half-life of a specific binding agent polypeptide, or may be designed to improve targeting capacity for the polypeptide to desired cells, tissues, or organs.

[0272] The invention further embraces multivalent binding proteins with effector function that are covalently modified or derivatized to include one or more water-soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol, as described U.S. Pat. Nos. 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192 and 4,179,337. Still other useful polymers known in the art include monomethoxy-polyethylene glycol, dextran, cellulose, and other carbohydrate-based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide copolymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, as well as mixtures of these polymers. Particularly preferred are polyethylene glycol (PEG)—derivatized proteins. Water-soluble polymers may be bonded at specific positions, for example at the amino terminus of the proteins and polypeptides according to the invention, or randomly attached to one or more side chains of the polypeptide. The use of PEG for improving therapeutic capacities is described in U.S. Pat. No. 6,133,426 to Gonzales, et al.

[0273] Target Sites for Immunoglobulin Mutagenesis

[0274] Certain strategies are available to manipulate inherent properties of an antigen-specific immunoglobulin (e.g., an antibody) that are not available to non-immunoglobulin-based binding molecules. A good example of the strategies favoring, e.g., antibody-based molecules, over these alternatives is the in vivo modulation of the affinity of an antibody for its target through affinity maturation, which takes advantage of the somatic hypermutation of immunoglobulin genes to yield antibodies of increasing affinity as an immune response progresses. Additionally, recombinant technologies have been developed to alter the structure of immunoglobulins and immunoglobulin regions and domains. Thus, polypeptides derived from antibodies may be produced that exhibit altered affinity for a given antigen, and a number of purification protocols and monitoring screens are known in the art for identifying and purifying or isolating these polypeptides. Using these known techniques, polypeptides comprising antibody-derived binding domains can be obtained that exhibit decreased or increased affinity for an antigen. Strategies for generating the polypeptide variants exhibiting altered affinity include the use of site-specific or random mutagenesis of the DNA encoding the antibody to change the amino acids present in the protein, followed by a screening step designed to recover antibody variants that exhibit the desired change, e.g., increased or decreased affinity relative to the unmodified parent or referent antibody.

[0275] The amino acid residues most commonly targeted in mutagenic strategies to alter affinity are those in the complementarity-determining region (CDR) or hyper-variable region of the light and the heavy chain variable regions of an antibody. These regions contain the residues that physico-chemically interact with an antigen, as well as other amino acids that affect the spatial arrangement of these residues. However, amino acids in the framework regions of the vari-

able domains outside the CDR regions have also been shown to make substantial contributions to the antigen-binding properties of an antibody, and can be targeted to manipulate such properties. See Hudson, P. J. *Curr. Opin. Biotech.*, 9: 395-402 (1999) and references therein.

[0276] Smaller and more effectively screened libraries of antibody variants can be produced by restricting random or site-directed mutagenesis to sites in the CDRs that correspond to areas prone to “hyper-mutation” during the somatic affinity maturation process. See Chowdhury, et al., *Nature Biotech.*, 17: 568-572 (1999) and references therein. The types of DNA elements known to define hyper-mutation sites in this manner include direct and inverted repeats, certain consensus sequences, secondary structures, and palindromes. The consensus DNA sequences include the tetrabase sequence Purine-G-Pyrimidine-A/T (i.e., A or G-G-C or T-A or T) and the serine codon AGY (wherein Y can be C or T).

[0277] Thus, another aspect of the invention is a set of mutagenic strategies for modifying the affinity of an antibody for its target. These strategies include mutagenesis of the entire variable region of a heavy and/or light chain, mutagenesis of the CDR regions only, mutagenesis of the consensus hypermutation sites within the CDRs, mutagenesis of framework regions, or any combination of these approaches (“mutagenesis” in this context could be random or site-directed). Definitive delineation of the CDR regions and identification of residues comprising the binding site of an antibody can be accomplished though solving the structure of the antibody in question, and the antibody:ligand complex, through techniques known to those skilled in the art, such as X-ray crystallography. Various methods based on analysis and characterization of such antibody crystal structures are known to those of skill in the art and can be employed to approximate the CDR regions. Examples of such commonly used methods include the Kabat, Chothia, AbM and contact definitions.

[0278] The Kabat definition is based on sequence variability and is the most commonly used definition to predict CDR regions. Johnson, et al., *Nucleic Acids Research*, 28: 214-8 (2000). The Chothia definition is based on the location of the structural loop regions. (Chothia et al., *J. Mol. Biol.*, 196: 901-17 [1986]; Chothia et al., *Nature*, 342: 877-83 [1989].) The AbM definition is a compromise between the Kabat and Chothia definitions. AbM is an integral suite of programs for antibody structure modeling produced by the Oxford Molecular Group (Martin, et al., *Proc. Natl. Acad. Sci. (USA)* 86:9268-9272 [1989]; Rees, et al., *ABMTM*, a computer program for modeling variable regions of antibodies, Oxford, UK; Oxford Molecular, Ltd.). The AbM suite models the tertiary structure of an antibody from primary sequence using a combination of knowledge databases and ab initio methods. An additional definition, known as the contact definition, has been recently introduced. See MacCallum et al., *J. Mol. Biol.*, 5:732-45 (1996). This definition is based on an analysis of the available complex crystal structures.

[0279] By convention, the CDR domains in the heavy chain are typically referred to as H1, H2 and H3, and are numbered sequentially in order moving from the amino terminus to the carboxy terminus. The CDR regions in the light chain are typically referred to as L1, L2 and L3, and are numbered sequentially in order moving from the amino terminus to the carboxy terminus.

[0280] The CDR-H1 is approximately 10 to 12 residues in length and typically starts 4 residues after a Cys according to the Chothia and AbM definitions, or typically 5 residues later

according to the Kabat definition. The H1 is typically followed by a Trp, typically Trp-Val, but also Trp-Ile, or Trp-Ala. The length of H1 is approximately 10 to 12 residues according to the AbM definition, while the Chothia definition excludes the last 4 residues.

[0281] The CDR-H2 typically starts 15 residues after the end of H1 according to the Kabat and AbM definitions. The residues preceding H2 are typically Leu-Glu-Trp-Ile-Gly but there are a number of variations. H2 is typically followed by the amino acid sequence Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala. According to the Kabat definition, the length of H2 is approximately 16 to 19 residues, where the AbM definition predicts the length to be typically 9 to 12 residues.

[0282] The CDR-H3 typically starts 33 residues after the end of H2 and is typically preceded by the amino acid sequence Cys-Ala-Arg. H3 is typically followed by the amino acid Gly. The length of H3 ranges from 3 to 25 residues.

[0283] The CDR-L1 typically starts at approximately residue 24 and will typically follow a Cys. The residue after the CDR-L1 is always Trp and will typically begin one of the following sequences: Trp-Tyr-Gln, Trp-Leu-Gln, Trp-Phe-Gln, or Trp-Tyr-Leu. The length of CDR-L1 is approximately 10 to 17 residues.

[0284] The CDR-L2 starts approximately 16 residues after the end of L1. It will generally follow residues Ile-Tyr, Val-Tyr, Ile-Lys or Ile-Phe. The length of CDR-L2 is approximately 7 residues.

[0285] The CDR-L3 typically starts 33 residues after the end of L2 and typically follows a Cys. L3 is typically followed by the amino acid sequence Phe-Gly-XXX-Gly. The length of L3 is approximately 7 to 11 residues.

[0286] Various methods for modifying antibodies have been described in the art, including, e.g., methods of producing humanized antibodies wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is 65% to 95% identical to the sequence of the donor immunoglobulin heavy chain variable region framework. Each humanized immunoglobulin chain will usually comprise, in addition to the CDRs, amino acids from the donor immunoglobulin framework that are, e.g., capable of interacting with the CDRs to effect binding affinity, such as one or more amino acids that are immediately adjacent to a CDR in the donor immunoglobulin or those within about 3 angstroms, as predicted by molecular modeling. The heavy and light chains may each be designed by using any one or all of various position criteria. When combined into an intact antibody, humanized immunoglobulins are substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen, such as a protein or other compound containing an epitope.

[0287] In one example, methods for the production of antibodies, and antibody fragments, are described that have binding specificity similar to a parent antibody, but which have increased human characteristics. Humanized antibodies are obtained by chain shuffling using, for example, phage display technology and a polypeptide comprising the heavy or light chain variable region of a non-human antibody specific for an antigen of interest, which is then combined with a repertoire of human complementary (light or heavy) chain variable regions. Hybrid pairings which are specific for the antigen of interest are identified and human chains from the selected pairings are combined with a repertoire of human complementary variable domains (heavy or light). In another embodiment, a component of a CDR from a non-human

antibody is combined with a repertoire of component parts of CDRs from human antibodies. From the resulting library of antibody polypeptide dimers, hybrids are selected and may be used in a second humanizing shuffling step; alternatively, this second step is eliminated if the hybrid is already of sufficient human character to be of therapeutic value. Methods of modification to increase human character are known in the art.

[0288] Another example is a method for making humanized antibodies by substituting a CDR amino acid sequence for the corresponding human CDR amino acid sequence and/or substituting a FR amino acid sequence for the corresponding human FR amino acid sequences.

[0289] Yet another example provides methods for identifying the amino acid residues of an antibody variable domain that may be modified without diminishing the native affinity of the antigen binding domain while reducing its immunogenicity with respect to a heterologous species and methods for preparing these modified antibody variable regions as useful for administration to heterologous species.

[0290] Modification of an immunoglobulin such as an antibody by any of the methods known in the art is designed to achieve increased or decreased binding affinity for an antigen and/or to reduce immunogenicity of the antibody in the recipient and/or to modulate effector activity levels. In one approach, humanized antibodies can be modified to eliminate glycosylation sites in order to increase affinity of the antibody for its cognate antigen (Co, et al., *Mol. Immunol.* 30:1361-1367 [1993]). Techniques such as "reshaping," hyperchimerization," and "veneering/resurfacing" have produced humanized antibodies with greater therapeutic potential. Vaswami, et al., *Annals of Allergy, Asthma, & Immunol* 81:105 (1998); Roguska, et al., *Prot. Engineer.* 9:895-904 (1996)]. See also U.S. Pat. No. 6,072,035, which describes methods for reshaping antibodies. While these techniques diminish antibody immunogenicity by reducing the number of foreign residues, they do not prevent anti-idiotypic and anti-allotypic responses following repeated administration of the antibodies. Alternatives to these methods for reducing immunogenicity are described in Gilliland et al., *J. Immunol.* 62(6): 3663-71 (1999).

[0291] In many instances, humanizing antibodies results in a loss of antigen binding capacity. It is therefore preferable to "back mutate" the humanized antibody to include one or more of the amino acid residues found in the original (most often rodent) antibody in an attempt to restore binding affinity of the antibody. See, for example, Saldanha et al., *Mol. Immunol.* 36:709-19 (1999).

[0292] Glycosylation of immunoglobulins has been shown to affect effector functions, structural stability, and the rate of secretion from antibody-producing cells (see Leatherbarrow et al., *Mol. Immunol.* 22:407 (1985), incorporated herein by reference). The carbohydrate groups responsible for these properties are generally attached to the constant regions of antibodies. For example, glycosylation of IgG at Asn 297 in the C_{H2} domain facilitates full capacity of the IgG to activate complement-dependent cytotoxicity (Tao et al., *J. Immunol.* 143:2595 (1989)). Glycosylation of IgM at Asn 402 in the C_{H3} domain, for example, facilitates proper assembly and cytolytic activity of the antibody (Muraoka et al., *J. Immunol.* 142:695 (1989)). Removal of glycosylation sites at positions 162 and 419 in the C_{H1} and C_{H3} domains of an IgA antibody led to intracellular degradation and at least 90% inhibition of secretion (Taylor et al., *Wall, Mol. Cell. Biol.* 8:4197 (1988)). Accordingly, the molecules of the invention include muta-

tionally altered immunoglobulins exhibiting altered glycosylation patterns by mutation of specific residues in, e.g., a constant sub-region to alter effector function. See Co et al., *Mol. Immunol.* 30:1361-1367 (1993), Jacquemon et al., *J. Thromb. Haemost.* 4:1047-1055 (2006), Schuster et al., *Cancer Res.* 65:7934-7941 (2005), and Warnock et al., *Biotechnol Bioeng.* 92:831-842 (2005), each incorporated herein by reference.

[0293] The invention also includes multivalent binding molecules having at least one binding domain that is at least 80%, preferably 90% or 95% or 99% identical in sequence to a known immunoglobulin variable region sequence and which has at least one residue that differs from such immunoglobulin variable region, wherein the changed residue adds a glycosylation site, changes the location of one or more glycosylation site(s), or preferably removes a glycosylation site relative to the immunoglobulin variable region. In some embodiments, the change removes an N-linked glycosylation site in a an immunoglobulin variable region framework, or removes an N-linked glycosylation site that occurs in the immunoglobulin heavy chain variable region framework in the region spanning about amino acid residue 65 to about amino acid residue 85, using the numbering convention of Co et al., *J. Immunol.* 148: 1149, (1992).

[0294] Any method known in the art is contemplated for producing the multivalent binding molecules exhibiting altered glycosylation patterns relative to an immunoglobulin referent sequence. For example, any of a variety of genetic techniques may be employed to alter one or more particular residues. Alternatively, the host cells used for production may be engineered to produce the altered glycosylation pattern. One method known in the art, for example, provides altered glycosylation in the form of bisected, non-fucosylated variants that increase ADCC. The variants result from expression in a host cell containing an oligosaccharide-modifying enzyme. Alternatively, the Potelligent technology of BioWa/Kyowa Hakko is contemplated to reduce the fucose content of glycosylated molecules according to the invention. In one known method, a CHO host cell for recombinant immunoglobulin production is provided that modifies the glycosylation pattern of the immunoglobulin F_C region, through production of GDP-fucose. This technology is available to modify the glycosylation pattern of a constant sub-region of a multivalent binding molecule according to the invention.

[0295] In addition to modifying the binding properties of binding domains, such as the binding domains of immunoglobulins, and in addition to such modifications as humanization, the invention comprehends the modulation of effector function by changing or mutating residues contributing to effector function, such as the effector function of a constant sub-region. These modifications can be effected using any technique known in the art, such as the approach disclosed in Presta et al., *Biochem. Soc. Trans.* 30:487-490 (2001), incorporated herein by reference. Exemplary approaches would include the use of the protocol disclosed in Presta et al. to modify specific residues known to affect binding in one or more constant sub-regions corresponding to $FC\gamma RI$, $FC\gamma RII$, $FC\gamma RIII$, $FC\epsilon R$, and FUR .

[0296] In another approach, the Xencor XmAb technology is available to engineer constant sub-regions corresponding to F_C domains to enhance cell killing effector function. See Lazar et al., *Proc. Natl. Acad. Sci. (USA)* 103(11):4005-4010 (2006), incorporated herein by reference. Using this approach, for example, one can generate constant sub-regions

optimized for F_CγR specificity and binding, thereby enhancing cell killing effector function.

[0297] Production of Multivalent Binding Proteins with Effector Function

[0298] A variety of expression vector/host systems may be utilized to contain and express the multivalent binding protein (with effector function) of the invention. These systems include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, cosmid, or other expression vectors; yeast transformed with yeast expression or shuttle vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems. Mammalian cells that are useful in recombinant multivalent binding protein productions include, but are not limited to, VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and HEK293 cells. Exemplary protocols for the recombinant expression of the multivalent binding protein are described herein below.

[0299] An expression vector can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, a promoter, enhancer, or factor-specific binding site, (2) a structural or sequence that encodes the binding agent which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant multivalent binding protein is expressed without a leader or transport sequence, it may include an amino terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final multivalent binding protein.

[0300] For example, the multivalent binding proteins may be recombinantly expressed in yeast using a commercially available expression system, e.g., the Pichia Expression System (Invitrogen, San Diego, Calif.), following the manufacturer's instructions. This system also relies on the pre-pro-α sequence to direct secretion, but transcription of the insert is driven by the alcohol oxidase (AOX1) promoter upon induction by methanol. The secreted multivalent binding peptide may be purified from the yeast growth medium by, e.g., the methods used to purify the peptide from bacterial and mammalian cell supernatants.

[0301] Alternatively, the cDNA encoding the multivalent binding peptide may be cloned into the baculovirus expression vector pVL1393 (PharMingen, San Diego, Calif.). This vector can be used according to the manufacturer's directions (PharMingen) to infect *Spodoptera frugiperda* cells in SF9 protein-free medium and to produce recombinant protein. The multivalent binding protein can be purified and concentrated from the medium using a heparin-Sepharose column (Pharmacia, Piscataway, N.J.). Insect systems for protein expression, such as the SF9 system, are well known to those of skill in the art. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) can be used as a vector to express foreign genes in the *Spodoptera frugiperda* cells or in *Trichoplusia larvae*. The multivalent binding peptide cod-

ing sequence can be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the multivalent binding peptide will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can be used to infect *S. frugiperda* cells or *Trichoplusia larvae* in which peptide is expressed (Smith et al., J Virol 46: 584, 1983; Engelhard et al., Proc Nat Acad Sci (USA) 91: 3224-7, 1994).

[0302] In another example, the DNA sequence encoding the multivalent binding peptide can be amplified by PCR and cloned into an appropriate vector, for example, pGEX-3X (Pharmacia, Piscataway, N.J.). The pGEX vector is designed to produce a fusion protein comprising glutathione-S-transferase (GST), encoded by the vector, and a multivalent binding protein encoded by a DNA fragment inserted into the cloning site of the vector. The primers for the PCR can be generated to include for example, an appropriate cleavage site. Where the multivalent binding protein fusion moiety is used solely to facilitate expression or is otherwise not desirable as an attachment to the peptide of interest, the recombinant multivalent binding protein fusion may then be cleaved from the GST portion of the fusion protein. The pGEX-3X/multivalent binding peptide construct is transformed into *E. coli* XL-1 Blue cells (Stratagene, La Jolla Calif.), and individual transformants isolated and grown. Plasmid DNA from individual transformants is purified and may be partially sequenced using an automated sequencer to confirm the presence of the desired multivalent binding protein-encoding nucleic acid insert in the proper orientation.

[0303] The fused multivalent binding protein, which may be produced as an insoluble inclusion body in the bacteria, can be purified as follows. Host cells can be harvested by centrifugation; washed in 0.15 M NaCl, 10 mM Tris, pH 8, 1 mM EDTA; and treated with 0.1 mg/ml lysozyme (Sigma Chemical Co.) for 15 minutes at room temperature. The lysate can be cleared by sonication, and cell debris can be pelleted by centrifugation for 10 minutes at 12,000×g. The multivalent binding protein fusion-containing pellet can be resuspended in 50 mM Tris, pH 8, and 10 mM EDTA, layered over 50% glycerol, and centrifuged for 30 minutes at 6000 g. The pellet can be resuspended in standard phosphate buffered saline solution (PBS) free of Mg⁺⁺ and Ca⁺⁺. The multivalent binding protein fusion can be further purified by fractionating the resuspended pellet in a denaturing SDS polyacrylamide gel (Sambrook et al.). The gel is soaked in 0.4 M KCl to visualize the protein, which is excised and electroeluted in gel-running buffer lacking SDS. If the GST/multivalent binding peptide fusion protein is produced in bacteria as a soluble protein, it can be purified using the GST Purification Module (Pharmacia Biotech).

[0304] The multivalent binding protein fusion is preferably subjected to digestion to cleave the GST from the multivalent binding peptide of the invention. The digestion reaction (20-40 μg fusion protein, 20-30 units human thrombin (4000 U/mg (Sigma) in 0.5 ml PBS) can be incubated 16-48 hours at room temperature and loaded on a denaturing SDS-PAGE gel to fractionate the reaction products. The gel can be soaked in 0.4 M KCl to visualize the protein bands. The identity of the protein band corresponding to the expected molecular weight of the multivalent binding peptide can be confirmed by amino acid sequence analysis using an automated sequencer (Applied Biosystems Model 473A, Foster City, Calif.). Alterna-

tively, the identity can be confirmed by performing HPLC and/or mass spectrometry of the peptides.

[0305] Alternatively, a DNA sequence encoding the multivalent binding peptide can be cloned into a plasmid containing a desired promoter and, optionally, a leader sequence (see, e.g., Better et al., *Science*, 240:1041-43, 1988). The sequence of this construct can be confirmed by automated sequencing. The plasmid can then be transformed into a suitable *E. coli* strain, such as strain MC1061, using standard procedures employing CaCl_2 incubation and heat shock treatment of the bacteria (Sambrook et al.). The transformed bacteria can be grown in LB medium supplemented with carbenicillin or another suitable form of selection as would be known in the art, and production of the expressed protein can be induced by growth in a suitable medium. If present, the leader sequence can effect secretion of the multivalent binding peptide and be cleaved during secretion. The secreted recombinant protein can be purified from the bacterial culture medium by the methods described herein below.

[0306] Mammalian host systems for the expression of the recombinant protein are well known to those of skill in the art and are preferred systems. Host cell strains can be chosen for a particular ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Different host cells such as CHO, HeLa, MDCK, 293, WI38, and the like, have specific cellular machinery and characteristic mechanisms for such post-translational activities and can be chosen to ensure the correct modification and processing of the foreign protein.

[0307] It is preferable that the transformed cells be used for long-term, high-yield protein production and, as such, stable expression is desirable. Once such cells are transformed with vectors that preferably contain at least one selectable marker along with the desired expression cassette, the cells are grown for 1-2 days in an enriched medium before being switched to selective medium. The selectable marker is designed to confer resistance to selection and its presence allows growth and recovery of cells that successfully express the foreign protein. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell.

[0308] A number of selection systems can be used to recover the cells that have been transformed for recombinant protein production. Such selection systems include, but are not limited to, HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk-, hgprt- or aprt-cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G418 and confers resistance to chlorosulfuron; and hygromycin, which confers resistance to hygromycin. Additional selectable genes that may be useful include trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histidinol in place of histidine. Markers that give a visual indication for identification of transformants include anthocyanins, β -glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin.

[0309] Purification of Proteins

[0310] Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level,

the crude fractionation of the polypeptide and non-polypeptide fractions. Having separated the multivalent binding polypeptide from at least one other protein, the polypeptide of interest is purified, but further purification using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity) is frequently desired. Analytical methods particularly suited to the preparation of a pure multivalent binding peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; and isoelectric focusing. Particularly efficient methods of purifying peptides are fast protein liquid chromatography and HPLC.

[0311] Certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of an encoded multivalent binding protein or peptide. The term "purified multivalent binding protein or peptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the multivalent binding protein or peptide is purified to any degree relative to its naturally obtainable state. A purified multivalent binding protein or peptide therefore also refers to a multivalent binding protein or peptide, free from the environment in which it may naturally occur.

[0312] Generally, "purified" will refer to a multivalent binding protein composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation refers to a multivalent binding protein composition in which the multivalent binding protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or more of the protein, by weight, in the composition.

[0313] Various methods for quantifying the degree of purification of the multivalent binding protein will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific binding activity of an active fraction, or assessing the amount of multivalent binding polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a multivalent binding protein fraction is to calculate the binding activity of the fraction, to compare it to the binding activity of the initial extract, and to thus calculate the degree of purification, herein assessed by a "-fold purification number." The actual units used to represent the amount of binding activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed multivalent binding protein or peptide exhibits a detectable binding activity.

[0314] Various techniques suitable for use in multivalent binding protein purification are well known to those of skill in the art. These include, for example, precipitation with ammonium sulfate, PEG, antibodies and the like, or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of these and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified multivalent binding protein.

[0315] There is no general requirement that the multivalent binding protein always be provided in its most purified state. Indeed, it is contemplated that less substantially multivalent binding proteins will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in greater purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of multivalent binding protein product, or in maintaining binding activity of an expressed multivalent binding protein.

[0316] It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi et al., *Biochem. Biophys. Res. Comm.*, 76:425, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified multivalent binding protein expression products may vary.

[0317] Effector Cells

[0318] Effector cells for inducing, e.g., ADCC, ADCP (antibody-dependent cellular phagocytosis), and the like, against a target cell include human leukocytes, macrophages, monocytes, activated neutrophils, activated natural killer (NK) cells, and eosinophils. Effector cells express Fc γ R(CD89), Fc γ RI, Fc γ RII, Fc γ RIII, and/or Fc ϵ RI and include, for example, monocytes and activated neutrophils. Expression of Fc γ RI, e.g., has been found to be up-regulated by interferon gamma (IFN- γ). This enhanced expression increases the cytotoxic activity of monocytes and neutrophils against target cells. Accordingly, effector cells may be activated with (IFN- γ) or other cytokines (e.g., TNF- α or β , colony stimulating factor, IL-2) to increase the presence of Fc γ RI on the surface of the cells prior to being contacted with a multivalent protein of the invention.

[0319] The multivalent proteins of the invention provide an antibody effector function, such as antibody-dependent effector cell-mediated cytotoxicity (ADCC), for use against a target cell. Multivalent proteins with effector function are administered alone, as taught herein, or after being coupled to an effector cell, thereby forming an "activated effector cell." An "activated effector cell" is an effector cell, as defined herein, linked to a multivalent protein with effector function, also as defined herein, such that the effector cell is effectively provided with a targeting function prior to administration.

[0320] Activated effector cells are administered in vivo as a suspension of cells in a physiologically acceptable solution. The number of cells administered is on the order of 10^8 - 10^9 , but will vary depending on the therapeutic purpose. In general, the amount will be sufficient to obtain localization of the effector cell at the target cell, and to provide a desired level of effector cell function in that locale, such as cell killing by ADCC and/or phagocytosis. The term physiologically acceptable solution, as used herein, is intended to include any carrier solution which stabilizes the targeted effector cells for administration in vivo including, for example, saline and aqueous buffer solutions, solvents, antibacterial and antifungal agents, isotonic agents, and the like.

[0321] Accordingly, another aspect of the invention provides a method of inducing a specific antibody effector function, such as ADCC, against a cell in a subject, comprising

administering to the subject a multivalent protein (or encoding nucleic acid) or activated effector cell in a physiologically acceptable medium. Routes of administration can vary and suitable administration routes will be determined by those of skill in the art based on a consideration of case-specific variables and routine procedures, as is known in the art.

[0322] Cell-Free Effects

[0323] Cell-free effects are also provided by the multivalent molecules of the invention, e.g., by providing a CDC functionality. The complement system is a biochemical cascade of the immune system that helps clear foreign matter such as pathogens from an organism. It is derived from many small plasma proteins that work together in inducing cytolysis of a target cell by disrupting the target cell's plasma membrane. The complement system consists of more than 35 soluble and cell-bound proteins, 12 of which are directly involved in the complement pathways. The proteins are active in three biochemical pathways leading to the activation of the complement system: the classical complement pathway, the alternate complement pathway, and the mannose-binding lectin pathway. Antibodies, in particular the IgG1 class, can also "fix" complement. A detailed understanding of these pathways has been achieved in the art and will not be repeated here, but it is worth noting that complement-dependent cytotoxicity is not dependent on the interaction of a binding molecule with a cell, e.g., a B cell, of the immune system. Also worth noting is that the complement system is regulated by complement regulating proteins. These proteins are present at higher concentrations in the blood plasma than the complement proteins. The complement regulating proteins are found on the surfaces of self-cells, providing a mechanism to prevent self-cells from being targeted by complement proteins. It is expected that the complement system plays a role in several diseases with an immune component, such as Barraquer-Simons Syndrome, Alzheimer's disease, asthma, lupus erythematosus, various forms of arthritis, autoimmune heart disease, and multiple sclerosis. Deficiencies in the terminal pathway predispose an individual to both autoimmune disease and infections (particularly meningitis).

[0324] Diseases, Disorders and Conditions

[0325] The invention provides a multivalent binding proteins with effector function, and variant and derivative thereof, that bind to one or more binding partners and those binding events are useful in the treatment, prevention, or amelioration of a symptom associated with a disease, disorder or pathological condition, preferably one afflicting humans. In preferred embodiments of these methods, the multivalent (and multispecific) binding protein with effector function associates a cell bearing a target, such as a tumor-specific cell-surface marker, with an effector cell, such as a cell of the immune system exhibiting cytotoxic activity. In other embodiments, the multispecific, multivalent binding protein with effector function specifically binds two different disease-, disorder- or condition-specific cell-surface markers to ensure that the correct target is associated with an effector cell, such as a cytotoxic cell of the immune system. Additionally, the multivalent binding protein with effector function can be used to induce or increase antigen activity, or to inhibit antigen activity. The multivalent binding proteins with effector function are also suitable for combination therapies and palliative regimes.

[0326] In one aspect, the present invention provides compositions and methods useful for treating or preventing diseases and conditions characterized by aberrant levels of anti-

gen activity associated with a cell. These diseases include cancers and other hyperproliferative conditions, such as hyperplasia, psoriasis, contact dermatitis, immunological disorders, and infertility. A wide variety of cancers, including solid tumors and leukemias are amenable to the compositions and methods disclosed herein. Types of cancer that may be treated include, but are not limited to: adenocarcinoma of the breast, prostate, and colon; all forms of bronchogenic carcinoma of the lung; myeloid; melanoma; hepatoma; neuroblastoma; papilloma; apudoma; choristoma; branchioma; malignant carcinoid syndrome; carcinoid heart disease; and carcinoma (e.g., Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell).

[0327] Additional types of cancers that may be treated include: histiocytic disorders; leukemia; histiocytosis malignant; Hodgkin's disease; immunoproliferative small; non-Hodgkin's lymphoma; plasmacytoma; reticuloendotheliosis; melanoma; chondroblastoma; chondroma; chondrosarcoma; fibroma; fibrosarcoma; giant cell tumors; histiocytoma; lipoma; liposarcoma; mesothelioma; myxoma; myxosarcoma; osteoma; osteosarcoma; chordoma; craniopharyngioma; dysgerminoma; hamartoma; mesenchymoma; mesonephroma; myosarcoma; ameloblastoma; cementoma; odontoma; teratoma; thymoma; trophoblastic tumor. Further, the following types of cancers are also contemplated as amenable to treatment: adenoma; cholangioma; cholesteatoma; cyclindroma; cystadenocarcinoma; cystadenoma; granulosa cell tumor; gynandroblastoma; hepatoma; hidradenoma; islet cell tumor; Leydig cell tumor; papilloma; sertoli cell tumor; theca cell tumor; leiomyoma; leiomyosarcoma; myoblastoma; myomma; myosarcoma; rhabdomyoma; rhabdomyosarcoma; ependymoma; ganglioneuroma; glioma; medulloblastoma; meningioma; neurilemmoma; neuroblastoma; neuroepithelioma; neurofibroma; neuroma; paraganglioma; paraganglioma nonchromaffin. The types of cancers that may be treated also include, but are not limited to, angiokeratoma; angiolymphoid hyperplasia with eosinophilia; angioma sclerosing; angiomatosis; glomangioma; hemangioendothelioma; hemangioma; hemangiopericytoma; hemangiosarcoma; lymphangioma; lymphangiomyoma; lymphangiosarcoma; pinealoma; carcinosarcoma; chondrosarcoma; cystosarcoma phyllodes; fibrosarcoma; heman-giosarcoma; leiomyosarcoma; leukosarcoma; liposarcoma; lymphangiosarcoma; myosarcoma; myxosarcoma; ovarian carcinoma; rhabdomyosarcoma; sarcoma; neoplasms; neurofibromatosis; and cervical dysplasia.

[0328] The invention further provides compositions and methods useful in the treatment of other conditions in which cells have become immortalized or hyperproliferative due to abnormally high expression of antigen.

[0329] Exemplifying the variety of hyperproliferative disorders amenable to the compositions and methods of the invention are B-cell cancers, including B-cell lymphomas (such as various forms of Hodgkin's disease, non-Hodgkins lymphoma (NHL) or central nervous system lymphomas), leukemias (such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia and chronic myeloblastic leukemia) and myelomas (such as multiple myeloma). Additional B cell cancers include small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, solitary plasmacytoma of

bone, extraosseous plasmacytoma, extra-nodal marginal zone B-cell lymphoma of mucosa-associated (MALT) lymphoid tissue, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, Burkitt's lymphoma/leukemia, B-cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, and post-transplant lymphoproliferative disorder.

[0330] Disorders characterized by autoantibody production are often considered autoimmune diseases. Autoimmune diseases include, but are not limited to: arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, poly-chondritis, psoriatic arthritis, psoriasis, dermatitis, polymyositis/dermatomyositis, inclusion body myositis, inflammatory myositis, toxic epidermal necrolysis, systemic scleroderma and sclerosis, CREST syndrome, responses associated with inflammatory bowel disease, Crohn's disease, ulcerative colitis, respiratory distress syndrome, adult respiratory distress syndrome (ARDS), meningitis, encephalitis, uveitis, colitis, glomerulonephritis, allergic conditions, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE), subacute cutaneous lupus erythematosus, discoid lupus, lupus myelitis, lupus cerebritis, juvenile onset diabetes, multiple sclerosis, allergic encephalomyelitis, neuromyelitis optica, rheumatic fever, Sydenham's chorea, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including Wegener's granulomatosis and Churg-Strauss disease, agranulocytosis, vasculitis (including hypersensitivity vasculitis/angiitis, ANCA and rheumatoid vasculitis), aplastic anemia, Diamond Blackfan anemia, immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, central nervous system (CNS) inflammatory disorders, multiple organ injury syndrome, myasthenia gravis, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Behcet disease, Castleman's syndrome, Goodpasture's syndrome, Lambert-Eaton Myasthenic Syndrome, Reynaud's syndrome, Sjorgen's syndrome, Stevens-Johnson syndrome, solid organ transplant rejection, graft versus host disease (GVHD), bullous pemphigoid, pemphigus, autoimmune polyendocrinopathies, seronegative spondyloarthropathies, Reiter's disease, stiff-man syndrome, giant cell arteritis, immune complex nephritis, IgA nephropathy, IgM polyneuropathies or IgM mediated neuropathy, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), Henoch-Schonlein purpura, autoimmune thrombocytopenia, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism; autoimmune endocrine diseases including autoimmune thyroiditis, chronic thyroiditis (Hashimoto's Thyroiditis), subacute thyroiditis, idiopathic hypothyroidism, Addison's disease, Grave's disease, autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), Type I diabetes also referred to as insulin-dependent diabetes mellitus (IDDM) and Sheehan's syndrome; autoimmune hepatitis, lymphoid interstitial pneu-

monitis (HIV), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré Syndrome, large vessel vasculitis (including polymyalgia rheumatica and giant cell (Takayasu's) arteritis), medium vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa), polyarteritis nodosa (PAN) ankylosing spondylitis, Berger's disease (IgA nephropathy), rapidly progressive glomerulonephritis, primary biliary cirrhosis, Celiac sprue (gluten enteropathy), cryoglobulinemia, cryoglobulinemia associated with hepatitis, amyotrophic lateral sclerosis (ALS), coronary artery disease, familial Mediterranean fever, microscopic polyangiitis, Cogan's syndrome, Whiskott-Aldrich syndrome and thromboangiitis obliterans.

[0331] Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the joints, leading to swelling, pain, and loss of function. Patients having RA for an extended period usually exhibit progressive joint destruction, deformity, disability and even premature death. Beyond RA, inflammatory diseases, disorders and conditions in general are amenable to treatment, prevention or amelioration of symptoms (e.g., heat, pain, swelling, redness) associated with the process of inflammation, and the compositions and methods of the invention are beneficial in treating, preventing or ameliorating aberrant or abnormal inflammatory processes, including RA.

[0332] Crohn's disease and a related disease, ulcerative colitis, are the two main disease categories that belong to a group of illnesses called inflammatory bowel disease (IBD). Crohn's disease is a chronic disorder that causes inflammation of the digestive or gastrointestinal (GI) tract. Although it can involve any area of the GI tract from the mouth to the anus, it most commonly affects the small intestine and/or colon. In ulcerative colitis, the GI involvement is limited to the colon. Crohn's disease may be characterized by antibodies against neutrophil antigens, i.e., the "perinuclear antineutrophil antibody" (pANCA), and *Saccharomyces cerevisiae*, i.e. the "anti-*Saccharomyces cerevisiae* antibody" (ASCA). Many patients with ulcerative colitis have the pANCA antibody in their blood, but not the ASCA antibody, while many Crohn's patients exhibit ASCA antibodies, and not pANCA antibodies. One method of evaluating Crohn's disease is using the Crohn's disease Activity Index (CDAI), based on 18 predictor variables scores collected by physicians. CDAI values of 150 and below are associated with quiescent disease; values above that indicate active disease, and values above 450 are seen with extremely severe disease [Best et al., "Development of a Crohn's disease activity index." *Gastroenterology* 70:439-444 (1976)]. However, since the original study, some researchers use a 'subjective value' of 200 to 250 as an healthy score.

[0333] Systemic Lupus Erythematosus (SLE) is an autoimmune disease caused by recurrent injuries to blood vessels in multiple organs, including the kidney, skin, and joints. In patients with SLE, a faulty interaction between T cells and B-cells results in the production of autoantibodies that attack the cell nucleus. There is general agreement that autoantibodies are responsible for SLE, so new therapies that deplete the B-cell lineage, allowing the immune system to reset as new B-cells are generated from precursors, would offer hope for long lasting benefit in SLE patients.

[0334] Multiple sclerosis (MS) is also an autoimmune disease. It is characterized by inflammation of the central nervous system and destruction of myelin, which insulates nerve cell fibers in the brain, spinal cord, and body. Although the

cause of MS is unknown, it is widely believed that autoimmune T cells are primary contributors to the pathogenesis of the disease. However, high levels of antibodies are present in the cerebral spinal fluid of patients with MS, and some theories predict that the B-cell response leading to antibody production is important for mediating the disease.

[0335] Autoimmune thyroid disease results from the production of autoantibodies that either stimulate the thyroid to cause hyperthyroidism (Graves' disease) or destroy the thyroid to cause hypothyroidism (Hashimoto's thyroiditis). Stimulation of the thyroid is caused by autoantibodies that bind and activate the thyroid stimulating hormone (TSH) receptor. Destruction of the thyroid is caused by autoantibodies that react with other thyroid antigens.

[0336] Additional diseases, disorders, and conditions amenable to the benefits provided by the compositions and methods of the invention include Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands. Further, immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction, and this condition is suitable for application of the materials and methods of the invention. Myasthenia Gravis (MG), a chronic autoimmune neuromuscular disorder characterized by autoantibodies that bind to acetylcholine receptors expressed at neuromuscular junctions leading to weakness of the voluntary muscle groups, is a disease having symptoms that are treatable using the composition and methods of the invention, and it is expected that the invention will be beneficial in treating and/or preventing MG. Still further, Rous Sarcoma Virus infections are expected to be amenable to treatment, or amelioration of at least one symptom, with the compositions and methods of the invention.

[0337] Another aspect of the present invention is using the materials and methods of the invention to prevent and/or treat any hyperproliferative condition of the skin including psoriasis and contact dermatitis or other hyperproliferative disease. Psoriasis, is characterized by autoimmune inflammation in the skin and is also associated with arthritis in 30% of cases, as well as scleroderma, inflammatory bowel disease, including Crohn's disease and ulcerative colitis. It has been demonstrated that patients with psoriasis and contact dermatitis have elevated antigen activity within these lesions (Ogoshi et al., *J. Inv. Dermatol.*, 110:818-23 [1998]). The multispecific, multivalent binding proteins can deliver a cytotoxic cell of the immune system, for example, directly to cells within the lesions expressing high levels of antigen. The multivalent, e.g., multispecific, binding proteins can be administered subcutaneously in the vicinity of the lesions, or by using any of the various routes of administration described herein and others which are well known to those of skill in the art.

[0338] Also contemplated is the treatment of idiopathic inflammatory myopathy (IIM), including dermatomyositis (DM) and polymyositis (PM). Inflammatory myopathies have been categorized using a number of classification schemes. Miller's classification schema (Miller, *Rheum Dis Clin North Am.* 20:811-826, 1994) identifies 2 idiopathic inflammatory myopathies (IIM), polymyositis (PM) and dermatomyositis (DM).

[0339] Polymyositis and dermatomyositis are chronic, debilitating inflammatory diseases that involve muscle and, in the case of DM, skin. These disorders are rare, with a reported annual incidence of approximately 5 to 10 cases per million adults and 0.6 to 3.2 cases per million children per year in the

United States (Targoff, *Curr Probl Dermatol.* 1991, 3:131-180). Idiopathic inflammatory myopathy is associated with significant morbidity and mortality, with up to half of affected adults noted to have suffered significant impairment (Gottdiener et al., *Am J. Cardiol.* 1978, 41:1141-49). Miller (Rheum Dis Clin North Am. 1994, 20:811-826 and Arthritis and Allied Conditions, Ch. 75, Eds. Koopman and Moreland, Lippincott Williams and Wilkins, 2005) sets out five groups of criteria used to diagnose IIM, i.e., Idiopathic Inflammatory Myopathy Criteria (IIMC) assessment, including muscle weakness, muscle biopsy evidence of degeneration, elevation of serum levels of muscle-associated enzymes, electromagnetic triad of myopathy, evidence of rashes in dermatomyositis, and also includes evidence of autoantibodies as a secondary criteria.

[0340] IIM associated factors, including muscle-associated enzymes and autoantibodies include, but are not limited to, creatine kinase (CK), lactate dehydrogenase, aldolase, C-reactive protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and antinuclear autoantibody (ANA), myositis-specific antibodies (MSA), and antibody to extractable nuclear antigens.

[0341] Preferred autoimmune diseases amenable to the methods of the invention include Crohn's disease, Guillain-Barré syndrome (GBS; also known as acute inflammatory demyelinating polyneuropathy, acute idiopathic polyradiculoneuritis, acute idiopathic polyneuritis and Landry's ascending paralysis), lupus erythematosus, multiple sclerosis, myasthenia gravis, optic neuritis, psoriasis, rheumatoid arthritis, hyperthyroidism (e.g., Graves' disease), hypothyroidism (e.g., Hashimoto's disease), Ord's thyroiditis (a thyroiditis similar to Hashimoto's disease), diabetes mellitus (type 1), aplastic anemia, Reiter's syndrome, autoimmune hepatitis, primary biliary cirrhosis, antiphospholipid antibody syndrome (APS), opsoclonus myoclonus syndrome (OMS), temporal arteritis (also known as "giant cell arteritis"), acute disseminated encephalomyelitis (ADEM), Goodpasture's syndrome, Wegener's granulomatosis, coeliac disease, pemphigus, canine polyarthritis, warm autoimmune hemolytic anemia. In addition, the invention contemplates methods for the treatment, or amelioration of a symptom associated with, the following diseases, endometriosis, interstitial cystitis, neuromyotonia, scleroderma, vitiligo, vulvodynia, Chagas' disease leading to Chagasic cardiopathy (cardiomegaly), sarcoidosis, chronic fatigue syndrome, and dysautonomia.

[0342] The complement system is believed to play a role in many diseases with an immune component, such as Alzheimer's disease, asthma, lupus erythematosus, various forms of arthritis, autoimmune heart disease and multiple sclerosis, all of which are contemplated as diseases, disorders or conditions amenable to treatment or symptom amelioration using the methods according to the invention.

[0343] Certain constant sub-regions are preferred, depending on the particular effector function or functions to be exhibited by a multivalent single-chain binding molecule. For example, IgG (IgG1, 2, or 3) and IgM are preferred for complement activation, IgG of any subtype is preferred for opsonization and toxin neutralization; IgA is preferred for pathogen binding; and IgE for binding of such parasites as worms.

[0344] By way of example, F_CRs recognizing the constant region of IgG antibodies have been found on human leukocytes as three distinct types of Fcγ receptors, which are distinguishable by structural and functional properties, as well as

by antigenic structures detected by CD monoclonal antibodies. They are known as FcγRI, FcγRII, and FcγRIII, and are differentially expressed on (overlapping) subsets of leukocytes.

[0345] FcγRI (CD64), a high-affinity receptor expressed on monocytes, macrophages, neutrophils, myeloid precursors and dendritic cells, comprised isoforms Ia and Ib. FcγRI has a high affinity for monomeric human IgG1 and IgG3. Its affinity for IgG4 is about 10 times lower, while it does not bind IgG2. FcγRT does not show genetic polymorphism.

[0346] FcγRII (CD32), comprised of isoforms IIa, IIb1, IIb2, IIb3 and IIc, is the most widely distributed human FcγR type, being expressed on most types of blood leukocytes, as well as on Langerhans cells, dendritic cells and platelets. FcγRII is a low-affinity receptor that only binds aggregated IgG. It is the only FcγR class able to bind IgG2. FcγRIIa shows genetics polymorphism, resulting in two distinct allotypes, FcγRIIa-H131 and FcγRIIa-R131, respectively. This functional polymorphism is attributable to a single amino acid difference: a histidine (H) or an arginine (R) residue at position 131, which is critical for IgG binding. FcγRIIa readily binds human IgG and IgG3 and appears not to bind IgG4. The FcγRIIa-H131 has a much higher affinity for complexed IgG2 than the FcγRIIa-R131 allotype.

[0347] FcγRIII (CD16) has two isoforms or allelotypes, both of which are able to bind IgG1 and IgG3. The FcγRIIa, with an intermediate affinity for IgG, is expressed on macrophages, monocytes, natural killer (NK) cells and subsets of T cells. FcγRIIIb is a low-affinity receptor for IgG, selectively expressed on neutrophils. It is a highly mobile receptor with efficient collaboration with other membrane receptors. Studies with myeloma IgG dimers have shown that only IgG1 and IgG3 bind to FcγRIIIb (with low affinity), while no binding of IgG2 and IgG4 has been found. The FcγRIIIb bears a co-dominant, bi-allelic polymorphism, the allotypes being designated NA1 (Neutrophil Antigen) and NA2.

[0348] Yet another aspect of the invention is use of the materials and methods of the invention to combat, by treating, preventing or mitigating the effects of, infection, resulting from any of a wide variety of infectious agents. The multivalent, multispecific binding molecules of the invention are designed to efficiently and effectively recruit the host organism's immune system to resist infection arising from a foreign organism, a foreign cell, a foreign virus or a foreign inanimate object. For example, a multispecific binding molecule may have one binding domain that specifically binds to a target on an infectious agent and another binding domain that specifically binds to a target on an Antigen Presenting Cell, such as CD40, CD80, CD86, DC-SIGN, DEC-205, CD83, and the like). Alternatively, each binding domain of a multivalent binding molecule may specifically bind to an infectious agent, thereby more effectively neutralizing the agent. In addition, the invention contemplates multispecific, multivalent binding molecules that specifically bind to a target on an infectious agent and to a non-cell-associated binding partner, which may be effective in conjunction with an effector function of the multispecific binding molecule in treating or preventing infection arising from an infectious agent.

[0349] Infectious cells contemplated by the invention include any known infectious cell, including but not limited to any of a variety of bacteria (e.g., pathogenic *E. coli*, *S. typhimurium*, *P. aeruginosa*, *B. anthracis*, *C. botulinum*, *C. difficile*, *C. perfringens*, *H. pylori*, *V. cholerae*, and the like),

mycobacteria, mycoplasma, fungi (including yeast and molds), and parasites (including any known parasitic member of the Protozoa, Trematoda, Cestoda and Nematoda). Infectious viruses include, but are not limited to, eukaryotic viruses (e.g., adenovirus, bunyavirus, herpesvirus, papovavirus, paramyxovirus, picornavirus, poxvirus, reovirus, retroviruses, and the like) as well as bacteriophage. Foreign objects include objects entering an organism, preferably a human, regardless of mode of entry and regardless of whether harm is intended. In view of the increasing prevalence of multi-drug-resistant infectious agents (e.g., bacteria), particularly as the causative agents of nosocomial infection, the materials and methods of the invention, providing an approach to treatment that avoids the difficulties imposed by increasing antibiotic resistance.

[0350] Diseases, conditions or disorders associated with infectious agents and amenable to treatment (prophylactic or therapeutic) with the materials and methods disclosed herein include, but are not limited to, anthrax, aspergillosis, bacterial meningitis, bacterial *pneumoniae* (e.g., *chlamydia pneumoniae*), blastomycosis, botulism, brucellosis, candidiasis, cholera, coccidioidomycosis, cryptococcosis, diaphtherogenic, enterohemorrhagic or enterotoxigenic *E. coli*, diphtheria, glanders, histoplasmosis, legionellosis, leprosy, listeriosis, nocardiosis, pertussis, salmonellosis, scarlet fever, sporotrichosis, strep throat, toxic shock syndrome, traveler's diarrhea, and typhoid fever.

[0351] Additional aspects and details of the invention will be apparent from the following examples, which are intended to be illustrative rather than limiting. Example 1 describes recombinant cloning of immunoglobulin heavy and light chain variable regions. Example 2 describes the construction of Small Modular ImmunoPharmaceuticals. Example 3 describes the construction of a prototype cassette for a multivalent binding protein with effector function. Example 4 describes binding and expression studies with this initial prototype molecule. Example 5 describes construction of alternative constructs derived from this initial prototype molecule where the sequence of the linker region between the EFD and BD2 was changed in both length and sequence. In addition, it describes alternative forms where the orientation of V regions in binding domain 2 were also altered. Example 6 describes subsequent binding and functional studies on these alternative constructs with variant linker forms, identifying a cleavage in the linker region in several of these derivative forms, and the new sequence variants developed to address this problem. Example 7 describes the construction of an alternative preferred embodiment of the multispecific, multivalent fusion proteins, where both BD1 and BD2 bind to antigens on the same cell type (CD20 and CD37), or another multispecific fusion protein where the antigen binding specificity for BD2 has been changed to human CD3 instead of CD28. Example 8 describes the binding and functional studies performed with the CD20-hIgG-CD37 multispecific constructs. Example 9 describes the binding and functional studies with the CD20-hIgG-CD3 multivalent fusion protein constructs. Example 10 discloses multivalent binding molecules having linkers based on specific regions of the extracellular domains of members of the immunoglobulin superfamily. Example 11 discloses assays for identifying binding domains expected to be effective in multivalent binding molecules in achieving at least one beneficial effect identified as being associated with such molecules (e.g., disease treatment).

Example 1

Cloning of Immunoglobulin Heavy and Light Chain Variable Regions

[0352] Any methods known in the art can be used to elicit antibodies to a given antigenic target. Further, any methods known in the art can be used to clone the immunoglobulin light and/or heavy chain variable regions, as well as the constant sub-region of an antibody or antibodies. The following method provides an exemplary cloning method.

[0353] A. Isolation of Total RNA

[0354] To clone the immunoglobulin heavy and light chain variable regions, or the constant sub-region, total RNA is isolated from hybridoma cells secreting the appropriate antibody. Cells (2×10^7) from the hybridoma cell line are washed with 1xPBS and pelleted via centrifugation in a 12x75 mm round bottom polypropylene tube (Falcon no. 2059). TRIzol™ Total RNA Isolation Reagent (Gibco BRL, Life Technologies, Cat no. 15596-018) is added (8 ml) to each tube and the cells are lysed via repeated pipetting. The lysate is incubated for 5 minutes at room temperature prior to the addition of 1.6 ml (0.2x volume) of chloroform and vigorous shaking for 15 seconds. After standing 3 minutes at room temperature, the lysates are centrifuged at 9,000 rpm for 15 minutes in a 4° C. pre-chilled Beckman JA-17 rotor in order to separate the aqueous and organic phases. The top aqueous phase (about 4.8 ml) is transferred into a new tube and mixed gently with 4 ml of isopropanol. After a 10 minute incubation at room temperature, the RNA is precipitated by centrifugation at 9,000 rpm in a 4° C. JA-17 rotor for 11 minutes. The RNA pellet is washed with 8 ml of ice-cold 75% ethanol and re-pelleting by centrifugation at 7,000x rpm for 7 minutes in a JA-17 rotor at 4° C. The ethanol wash is decanted and the RNA pellets are air-dried for 10 minutes. The RNA pellets are resuspended in 150 µl of diethylpyrocabonate (DEPC)-treated ddH₂O containing 1 µl of RNase Inhibitor (Catalog No. 799017; Boehringer Mannheim/Roche) per 1 ml of DEPC-treated ddH₂O. The pellets are resuspended by gentle pipetting and are incubated for 20 minutes at 55° C. RNA samples are quantitated by measuring the OD_{260 nm} of diluted aliquots (1.0 OD_{260 nm} unit=40 µg/ml RNA).

[0355] B. Rapid Amplification of cDNA Ends

[0356] 5' RACE is carried out to amplify the ends of the heavy and light chain variable regions, or the constant sub-region. The 5' RACE System for Rapid Amplification of cDNA Ends Kit version 2.0 (Life Technologies, cat. no. 18374-058) is used according to the manufacturer's instructions. Degenerate 5' RACE oligonucleotide primers are designed to match, e.g., the constant regions of two common classes of mouse immunoglobulin heavy chains (IgG1 and IgG2b) using the oligonucleotide design program Oligo version 5.1 (Molecular Biology Insights, Cascade CO). Primers are also designed to match the constant region of the mouse IgG kappa light chain. This is the only class of immunoglobulin light chain, so no degeneracy is needed in the primer design. The sequences of the primers are as follows:

Name Sequence	SEQ ID NO
Heavy Chain GSP1 5' AGGTGCTGGAGGGACAGTCACTGAGCTGC3'	7

-continued

Name Sequence	SEQ ID NO
Nested Heavy Chain 5'GTCACWGTCACTGRCCTCAGGGAARTAGC3' (W = A or T; R = A or G)	8
Light Chain GSP 1 5'GGGTGCTGCTCATGCTGTAGGTGCT- GTCTTTGC3'	9
Nested Light Chain 5'CAAGAAGCACACGACTGAGGCACCTCCAGATG3'	10
5' Race Abridged Anchor Primer 5'GGCCACGCGTCGACTAGTACGGGNNGGGNNGGN NG3'	11

[0357] To amplify the mouse immunoglobulin heavy chain component, the reverse transcriptase reaction is carried in a 0.2 ml thin-walled PCR tube containing 2.5 pmoles of heavy chain GSP1 primer (SEQ ID NO: 7), 4 pig of total RNA isolated from a suitable hybridoma clone (e.g., either clone 4A5 or clone 4B5), and 12 μ l of DEPC treated ddH₂O. Likewise, for the mouse light chain component, the reverse transcriptase reaction is carried out in a 0.2 ml thin-walled PCR tube containing 2.5 pmoles of a light chain GSP1 primer (SEQ ID NO: 9), 4 pig of total RNA from a suitable hybridoma clone (e.g., either clone 4A5 or clone 4B5), and 12 μ l of DEPC treated ddH₂O.

[0358] The reactions are carried out in a PTC-100 programmable thermal cycler (MJ research Inc., Waltham, Mass.). The mixture is incubated at 70° C. for 10 minutes to denature the RNA and then chilled on wet ice for 1 minute. The tubes are centrifuged briefly in order to collect moisture from the lids of the tubes. Subsequently, the following components are added to the reaction: 2.5 μ l of 10 $\times$ PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 2.5 μ l of 25 mM MgCl₂, 1 μ l of 10 mM dNTP mix, and 2.5 μ l of 0.1 M DTT. After mixing each tube by gentle pipetting, the tubes are placed in a PTC-100 thermocycler at 42° C. for 1 minute to pre-warm the mix. Subsequently, 1 μ l (200 units) of SuperScript™ II Reverse Transcriptase (Gibco-BRL; cat no. 18089-011) is added to each tube, gently mixed by pipetting, and incubated for 45 minutes at 42° C. The reactions are cycled to 70° C. for 15 minutes to terminate the reaction, and then cycled to 37° C. RNase mix (1 μ l) is then added to each reaction tube, gently mixed, and incubated at 37° C. for 30 minutes.

[0359] The first-strand cDNA generated by the reverse transcriptase reaction is purified with the GlassMAX DNA Isolation Spin Cartridge (Gibco-BRL) according to the manufacturer's instructions. To each first-strand reaction, 120 μ l of 6 M NaI binding solution is added. The cDNA/NaI solution is then transferred into a GlassMAX spin cartridge and centrifuged for 20 seconds at 13,000 $\times$ g. The cartridge inserts are carefully removed and the flow-through is discarded from the tubes. The spin cartridges are then placed back into the empty tubes and 0.4 ml of cold (4° C.) 1 $\times$ wash buffer is added to each spin cartridge. The tubes are centrifuged at 13,000 $\times$ g for 20 seconds and the flow-through is discarded. This wash step is repeated three additional times. The GlassMAX cartridges are then washed 4 times with 0.4 ml of cold (4° C.) 70% ethanol. After the flow-through from the final 70% ethanol wash is discarded, the cartridges are placed back in the tubes and centrifuged at 13,000 $\times$ g for an

additional 1 minute in order to completely dry the cartridges. The spin cartridge inserts are then transferred to a fresh sample recovery tube where 50 μ l of 65° C. (pre-heated) DEPC-treated ddH₂O is quickly added to each spin cartridge. The cartridges are centrifuged at 13,000 $\times$ g for 30 seconds to elute the cDNA.

[0360] C. Terminal Deoxynucleotidyl Transferase (TdT) Tailing

[0361] For each first-strand cDNA sample, the following components are added to a 0.2 ml thin-walled PCR tube: 6.5 μ l of DEPC-treated ddH₂O, 5.0 μ l of 5 $\times$ tailing buffer, 2.5 μ l of 2 mM dCTP, and 10 μ l of the appropriate GlassMAX-purified cDNA sample. Each 24 μ l reaction is incubated 2-3 minutes in a thermal cycler at 94° C. to denature the DNA, and chilled on wet ice for 1 minute. The contents of the tube are collected by brief centrifugation. Subsequently, 1 μ l of terminal deoxynucleotidyl transferase (TdT) is added to each tube. The tubes are mixed via gentle pipetting and incubated for 10 minutes at 37° C. in a PTC-100 thermal cycler. Following this 10 minute incubation, the TdT is heat inactivated by cycling to 65° C. for 10 minutes. The reactions are cooled on ice and the TdT-tailed first-strand cDNA is stored at -20° C.

[0362] D. PCR of dC-Tailed First-Strand cDNA

[0363] Duplicate PCR amplifications (two independent PCR reactions for each dC-tailed first-strand cDNA sample) are performed in a 50 μ l volume containing 200 μ M dNTPs, 0.4 μ M of 5' RACE Abridged Anchor Primer (SEQ ID NO: 11), and 0.4 μ M of either Nested Heavy Chain GSP2 (SEQ ID NO: 8) or Nested Light Chain GSP2 (SEQ ID NO: 10), 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl, 5 μ l of dC-tailed cDNA, and 5 units of Expand™ Hi-Fi DNA polymerase (Roche/Boehringer Mannheim GmbH, Germany). The PCR reactions are amplified using a "Touch-down/Touch-up" annealing temperature protocol in a PTC-100 programmable thermal cycler (MJ Research Inc.) with the following conditions: initial denaturation of 95° C. for 40 seconds, 5 cycles at 94° C. for 20 seconds, 61° C.-2° C./cycle for 20 seconds, 72° C. for 40 seconds+1 second/cycle, followed by 5 cycles at 94° C. for 25 seconds, 53° C.+1° C./cycle for 20 seconds, 72° C. for 46 seconds+1 second/cycle, followed by 20 cycles at 94° C. for 25 seconds, 55° C. for 20 seconds, 72° C. for 51 seconds+1 second/cycle, and a final incubation of 72° C. for 5 minutes.

[0364] E. TOPO TA-Cloning

[0365] The resulting PCR products are gel-purified from a 1.0% agarose gel using the QIAQuick Gel purification system (QIAGEN Inc., Chatsworth, Calif.), TA-cloned into pCR2.1 using the TOPO TA Cloning® kit (Invitrogen, San Diego, Calif., cat. no. K4550-40), and transformed into *E. coli* TOP10 F' cells (Invitrogen), according to manufacturers' instructions. Clones with inserts are identified by blue/white screening according to the manufacturer's instructions, where white clones are considered positive clones. Cultures of 3.5 ml liquid Luria Broth (LB) containing 50 μ g/ml ampicillin are inoculated with white colonies and grown at 37° C. overnight (about 16 hours) with shaking at 225 rpm.

[0366] The QIAGEN Plasmid Miniprep Kit (QIAGEN Inc., cat. no. 12125) is used to purify plasmid DNA from the cultures according to the manufacturer's instructions. The plasmid DNA is suspended in 34 μ l of 1 $\times$ TE buffer (pH 8.0) and then positive clones sequenced as previously described by fluorescent dideoxy nucleotide sequencing and automated detection using ABI Big Dye Terminator 3.1 reagents at 1:4-1:8 dilutions and analyzed using an ABI 3100 DNA

sequencer. Sequencing primers used include T7 (5'GTAATACGACTCACTATAGG3'; SEQ ID NO: 12) and M13 Reverse (5'CAGGAAACAGCTATGACC3'; SEQ ID NO: 13) primers. Sequencing results will confirm that the clones correspond to mouse IgG sequences.

[0367] F. De Novo Gene Synthesis Using Overlapping Oligonucleotide Extension PCR

[0368] This method involves the use of overlapping oligonucleotide primers and PCR using either a high fidelity DNA polymerase or a mix of polymerases to synthesize an immunoglobulin V-region or other gene. Starting at the middle of the V-region sequence, 40-50 base primers are designed such that the growing chain is extended by 20-30 bases, in either direction, and contiguous primers overlap by a minimum of 20 bases. Each PCR step requires two primers, one priming on the anti-sense strand (forward or sense primer) and one priming on the sense strand (reverse or anti-sense primer) to create a growing double-stranded PCR product. During primer design, changes can be made in the nucleotide sequence of the final product to create restriction enzyme sites, destroy existing restriction enzyme sites, add flexible linkers, change, delete or insert bases that alter the amino acid sequence, optimize the overall DNA sequence to enhance primer synthesis and conform to codon usage rules for the organism contemplated for use in expressing the synthetic gene.

[0369] Primer pairs are combined and diluted such that the first pair are at 5 μ M and each subsequent pair has a 2-fold greater concentration up to 80 μ M. One from each of these primer mixes is amplified in a 50 μ L PCR reaction using Platinum PCR SuperMix-High Fidelity (Invitrogen, San Diego, Calif., cat. no. 12532-016). After a 2-minute initial denaturation at 94° C., 30 cycles of PCR are performed using a cycling profile of 94° C. for 20 seconds, 60° C. for 10 seconds; and 68° C. for 15 seconds. PCR products are purified using Qiaquick PCR Purification columns (Qiagen Inc., cat. no. 28704) to remove excess primers and enzyme. This PCR product is then reamplified with the next set of similarly diluted primer pairs using PCR conditions exactly as described above, but increasing the extension time of each cycle to 68° C. for 30 seconds. The resultant PCR product is again purified from primers and enzymes as described above and TOPO-TA cloned and sequenced exactly as described in section E above.

Example 2

Construction of Small Modular Immunopharmaceuticals (SMIPs)

[0370] A multispecific, multivalent binding protein with effector function was constructed that contained a binding domain 1 in the form of a single-chain recombinant (murine/human) scFv designated 2H7 (VL-linker-VH). The scFv 2H7 is a small modular immunopharmaceutical (SMIP) that specifically recognizes CD20. The binding domain was based on a publicly available human CD20 antibody sequence GenBank Accession Numbers, M17953 for VH, and M17954 for VL. CD20-specific SMIPs are described in co-owned US Patent Publications 2003/133939, 2003/0118592 and 2005/0136049, incorporated herein in their entireties by reference. The peptide linker separating VL and VH was a 15-amino acid linker encoding the sequence: Asp-Gly₃Ser-(Gly₄Ser)₂. Binding domain 1 was located at the N-terminus of the multispecific binding protein, with the C-terminus of that domain

linked directly to the N-terminus of a constant sub-region containing a hinge, C_{H2} and C₁₋₁₃ domains (in amino-to-carboxy orientation). The constant sub-region was derived from an IgG1 antibody, which was isolated by PCR amplification of human IgG1 from human PBMCs. The hinge region was modified by substituting three Ser residues in place of the three Cys residues present in the wild type version of the human IgG1 hinge domain, encoded by the 15 amino acid sequence: EPKSCDKTHTCPPCP (SEQ ID NO: 14; the three Cys residues replaced by Ser residues are indicated in bold). In alternative embodiments, the hinge region was modified at one or more of the cysteines, so that SSS and CSC type hinges were generated. In addition, the final proline was sometimes substituted with a serine as well as the cysteine substitutions.

[0371] The C-terminal end of the C_{H3} domain was covalently attached to a series of alternative linker domains juxtaposed between the constant sub-region C-terminus and the amino terminus of binding domain 2. Preferred multivalent binding proteins with effector function will have one of these linkers to space the constant sub-region from binding domain 2, although the linker is not an essential component of the compositions according to the invention, depending on the folding properties of BD2. For some specific multivalent molecules, the linker might be important for separation of domains, while for others it may be less important. The linker was attached to the N-terminal end of scFv 2E12 (V_H-linker-V_L), which specifically recognizes CD28. The linker separating the VH and VL domains of the scFv 2E12 part of the multivalent binding molecule was a, 20-amino acid linker (Gly₄Ser)₄, rather than the standard (Gly₄Ser)₃ linker usually inserted between V domains of an scFv. The longer linker was observed to improve the binding properties of the 2E12 scFv in the VH-VL orientation.

[0372] The multispecific, multivalent binding molecule as constructed contained a binding domain 1, which comprises the 2E12 leader peptide sequence from amino acids 1-23 of SEQ ID NO: 171; the 2H7 murine anti-human CD20 light chain variable region, which is reflected at position 24 in SEQ ID NO: 171; an Asp-Gly₃Ser-(Gly₄Ser)₂ linker, beginning at residue 130 in SEQ ID NO: 171, the 2H7 murine anti-human CD20 heavy chain variable region with a leucine to serine (VHL11S) amino acid substitution at residue 11 in the variable domain for VH, and which has a single serine residue at the end of the heavy chain region (i.e., VTVS where a canonical sequence would be VTVSS) (Genbank Acc. No. M17953), and interposed between the two binding domains BD 1 (2H7) and BD 2 (2E 12) is a human IgG 1 constant sub-region, including a modified hinge region comprising a "CSC" or an "SSS" sequence, and wild-type C_{H2} and C_{H3} domains. The nucleotide and amino acid sequences of the multivalent binding protein with effector function are set out in SEQ ID NOS: 228 and 229 for the CSC forms, respectively and SEQ ID NOS: 170 and 171, for the SSS forms.

[0373] Stably expressing cell lines were created by transfection via electroporation of either uncut or linearized, recombinant expression plasmid into Chinese hamster ovary cells (CHO DG44 cells) followed by selection in methotrexate containing medium. Bulk cultures and master wells producing the highest level of multivalent binding protein were amplified in increasing levels of methotrexate, and adapted cultures were subsequently cloned by limiting dilution. Transfected CHO cells producing the multivalent binding protein were cultured in bioreactors or wave bags using serum-free medium obtained from JRH Biosciences (Excell

302, cat. no. 14324-1000M, supplemented with 4 mM glutamine (Invitrogen, 25030-081), sodium pyruvate (Invitrogen 11360-070, diluted to 1×), non-essential amino acids (Invitrogen, 11140-050, final dilution to 1×), penicillin-streptomycin 100 IU/ml (Invitrogen, 15140-122), and recombinant insulin at 1 µg/ml (Invitrogen, 97-503311). Other serum free CHO basal medias may also be used for production, such as CD-CHO, and the like.

[0374] Fusion protein was purified from spent CHO culture supernatants by Protein A affinity chromatography. The multivalent binding protein was purified using a series of chromatography and filtration steps, including a virus reduction filter. Cell culture supernatants were filtered, then subjected to protein A Sepharose affinity chromatography over a GE Healthcare XK 16/40 column. After binding of protein to the column, the column was washed in dPBS, then 1.0 M NaCl, 20 mM sodium phosphate pH 6.0, and then 25 mM NaCl, 25 mM NaOAc, pH 5.0 to remove nonspecific binding proteins. Bound protein was eluted from the column in 100 mM Glycine (Sigma), pH 3.5, and brought to pH 5.0 with 0.5 M 2-(N-Morpholino) ethanesulfonic acid (MES), pH 6.0. Protein samples were concentrated to 25 mg/ml in preparation for GPC purification. Size exclusion chromatography was performed on a GE Healthcare AKTA Explorer 100 Air apparatus, using a GE healthcare XK column and Superdex 200 preparative grade (GE healthcare).

[0375] The material was then concentrated and formulated with 20 mM sodium phosphate and 240 mM sucrose, with a resulting pH of 6.0. The composition was filtered before filling into sterile vials at various concentrations, depending on the amount of material recovered.

Example 3

Construction of Scorpion Expression Cassette

[0376] A nucleic acid containing the synthetic 2H7 scFv (anti-CD20; SEQ ID NO: 1) linked to a constant sub-region as described in Example 2 has been designated TRU-015. TRU-015 nucleic acid, as well as synthetic scFv 2E12 (anti-CD28 VL-VH; SEQ ID NO: 3) and synthetic scFv 2E12 (anti-CD28 VH-VL; SEQ ID NO: 5) nucleic acids encoding small modular immunopharmaceuticals, were used as templates for PCR amplification of the various components of the scorpion cassettes. The template, or scaffold, for binding domain 1 and the constant sub-region was provided by TRU-015 (the nucleic acid encoding scFv 2H7 (anti-CD20) linked to the constant sub-region) and this template was constructed in the expression vector pD18. The above-noted nucleic acids containing scFv 2E12 in either of two orientations (V_L - V_H and V_H - V_L) provided the coding region for binding domain 2.

TRU 015 SSS hinge $C_{H2}C_{H3}$ for BD2/Linker Insertion

[0377] A version of the synthetic 2H7 scFv IgG1 containing the SSS hinge was used to create a scorpion cassette by serving as the template for addition of an EcoRI site to replace the existing stop codon and XbaI site. This molecule was amplified by PCR using primer 9 (SEQ ID NO: 23; see Table 1) and primer 87 (SEQ ID NO: 40; see Table 1) as well as a Platinum PCR High Fidelity mix (Invitrogen). The resultant 1.5 Kbp fragment was purified and cloned into the vector pCR2.1-TOPO (Invitrogen), transformed into *E. coli* strain TOP10 (Invitrogen), and the DNA sequence verified.

TABLE 1

Oligonucleotide primers used to construct CD20-CD28 scorpion cassette. Primers are separated into 2 groups, PCR and Sequencing. PCR primers were used to construct the cassette and sequencing primers were used to confirm the DNA sequence of all intermediates and final constructs.			
No.	Name	Sequence 5'-3'	SEQ ID NO.
<u>PCR Primers</u>			
1	hVK3L-F3H3	GCGATAAAGCTTGCCGCCATGGAA GCACCAGCGCAGCTTCTCTTCC	15
2	hVK3L-F2	ACCAGCGCAGCTTCTCTTCTCTCTG CTACTCTGGCTCCAGATACCACCG	16
3	hVK3L-F1-2H7VL	GGCTCCCAGATACCACCGGTCAAAT TGTTCTCTCCAGTCTCCAG	17
4	2H7VH-NheF	GCGATAGCTAGCCAGGCTTATCTAC AGCAGTCTGG	18
5	G4S-NheR	GCGATAGCTAGCCCCACCTCTCCA GATCCACCACCGCCCGAG	19
6	015VH-XhoR	GCGTACTCGAGGAGACGGTGACCGT GGTCCCTGTG	20
7	G1H-C-XHO	GCAGTCTCGAGCGAGCCCAATCTTG TGACAAAACCTC	21
8	G1H-S-XHO	GCAGTCTCGAGCGAGCCCAATCTTC TGACAAAACCTC	22
9	CH3R-EcoR1	GCGTGAGAATTCTTACCCGGAGACAGG GAGAGGCTC	23
10	G1-XBA-R	GCGACGTCTAGAGTCATTTACCCGGAG ACAGG	24
11	G4SLinkR1-S	AATTATGGTGGCGGTGGCTCGGGCGGT GGTGGATCTGGAGGAGGTGGGAGTGGG	25
12	G4SLinkR1-AS	AATCCCCACTCCACCTCTCCAGAT CCACCACCGCCGAGCCACCGCCACCAT	26
13	2E12VLXbaR	GCGTGTCTAGATTACGTTTGATTTTC CAGCTTGGTG	27
14	2E12VLR1F	GCGATGAATTCTGACATTGTGCTCAC CCAATCTCC	28
15	2E12VHR1F	GCGATGAATTCTCAGTGCAGCTGAA GGAGTCAG	29
16	2E12VHXbaR	GCGAGTCTAGATTAAAGAGGAGACGGT GACTGAGGTTTC	30
17	2e12VHdXbaR1	GGGTCTGGAGTGGCTGGGAATGATATG	31
18	2e12VHdXbaR1AT	TCCCAGCCACTCCAGACCCCTTCTCTG	32
19	IgBsrG1F	GAGAACCCAGGTGTACACCCCTG	33
20	IgBsrG1R	GCAGGGTGTACACCTGTGGTTCTCG	34
<u>Sequencing Primers</u>			
82	M13R	CAGGAAACAGCTATGAC	35
83	M13F	GTAAAACGACGGCCAGTG	36

TABLE 1-continued

Oligonucleotide primers used to construct CD20-CD28 scorpion cassette. Primers are separated into 2 groups, PCR and Sequencing. PCR primers were used to construct the cassette and sequencing primers were used to confirm the DNA sequence of all intermediates and final constructs.		
No.	Name	Sequence 5'-3'
84	T7	GTAATACGACTCACTATAGG
85	pD18F-17	AACTAGAGAACCCACTG
86	pD18F-20	GCTAACTAGAGAACCCACTG
87	pD18F-1	ATACGACTCACTATAGGG
88	pD18R-s	GCTCTAGCATTTAGGTGAC
89	CH3seqF1	CATGAGGCTCTGCACAAC
90	CH3seqF2	CCTCTACAGCAAGCTCAC
91	CH3seqR1	GGTTCTTGGTCAGCTCATC
92	CH3seqR2	GTGAGCTTGCTGTAGAGG

n2H7 V_K and Human V_{K3} Leader Sequence Fusion

[0378] Oligonucleotide-directed PCR mutagenesis was used to introduce an AgeI (ACCGGT) restriction site at the 5' end of the coding region for TRU 015 VK and an Nhe I (GCTAGC) restriction site at the 3' end of the coding region for the (G4S)₃ linker using primers 3 and 5 from Table 1. Since primer 3 also encodes the last 6 amino acids of the human VK3 leader (gb:X01668), overlapping PCR was used to sequentially add the N-terminal sequences of the leader including a consensus Kozak box and HindIII (AAGCTT) restriction site using primers 1, 2 and 5 from Table 1.

n2H7 e-C_{H2}C_{H3} Construction

[0379] Primers 4 and 6 (SEQ ID NOS: 18 and 20, respectively; Table 1) were used to re-amplify the TRU-015 V_H with an NheI site 5' to fuse with the V_K for TRU-015 and an Xho I (5'-CTCGAG-3') site at the 3' end junction with the IgG1 hinge-C_{H2}C_{H3} domains. Likewise, the IgG1 hinge-C_{H1}-C_{H3} region was amplified using primers 8 and 9 from Table 1, introducing a 5' Xho I site and replacing the existing 3' end with an EcoRI (5'-GAATTC-3') site for cloning, and destroying the stop codon to allow translation of Binding Domain 2 attached downstream of the CH3 domain. This version of the scorpion cassette is distinguished from the previously described cassette by the prefix "n."

[0380] In addition to the multivalent binding protein described above, a protein according to the invention may have a binding domain, either binding domain 1 or 2 or both, that corresponds to a single variable region of an immunoglobulin. Exemplary embodiments of this aspect of the invention would include binding domains corresponding to the V_H domain of a camelid antibody, or a single modified or unmodified V region of another species antibody capable of binding to the target antigen, although any single variable domain is contemplated as useful in the proteins of the invention.

2E12 VL-VH and VH-VL Constructions

[0381] In order to make the 2E12 scFvs compatible with the cassette, an internal Xba I (5'-TCTAGA-3') site had to be

destroyed using overlapping oligonucleotide primers 17 and 18 from Table 1. These two primers in combination with primer pairs 14/16 (VL-VH) or 13/15 (VH-VL) were used to amplify the two oppositely oriented binding domains such that they both carried EcoRI and XbaI sites at their 5' and 3' ends, respectively. Primers 13 and 16 also encode a stop codon (TAA) immediately in front of the Xba I site.

2H7 SSS IgG12e12 LH/HL Construction

Effector Domain-Binding Domain 2 Linker Addition. (STD Linkers—STD1 and STD2)

[0382] Complementary primers 11 and 12 from Table 1 were combined, heated to 70° C. and slow-cooled to room temperature to allow annealing of the two strands. 5' phosphate groups were added using T4 polynucleotide kinase (Roche) in 1× Ligation buffer with 1 mM ATP (Roche) using the manufacturer's protocol. The resulting double-stranded linker was then ligated into the EcoRI site between the coding regions for the IgG1 C_{H3} terminus and the beginning of Binding Domain 2 using T4 DNA ligase (Roche). The resultant DNA constructs were screened for the presence of an EcoRI site at the linker-BD2 junction and the nucleotide sequence GAATTA at the C_{H3}-linker junction. The correct STD 1 linker construct was then re-digested with EcoRI and the linker ligation repeated to produce a molecule that had a linker composed of two (STD 2) identical iterations of the Lx1 sequence. DNA constructs were again screened as above.

Example 4

Expression Studies

[0383] Expression studies were performed on the nucleic acids described above that encode multivalent binding proteins with effector function. Nucleic acids encoding multivalent binding proteins were transiently transfected into COS cells and the transfected cells were maintained under well known conditions permissive for heterologous gene expression in these cells. DNA was transiently transfected into COS cells using PEI or DEAE-Dextran as previously described (PEI=Boussif O. et al., PNAS 92: 7297-7301, (1995), incorporated herein by reference; Pollard H. et al., JBC 273: 7507-7511, (1998), incorporated herein by reference). Multiple independent transfections of each new molecule were performed in order to determine the average expression level for each new form. For transfection by PEI, COS cells were plated onto 60 mm tissue culture plates in DMEM/10% FBS medium and incubated overnight so that they would be approximately 90% confluent on the day of transfection. Medium was changed to serum free DMEM containing no antibiotics and incubated for 4 hours. Transfection medium (4 ml/plate) contained serum free DMEM with 50 µg PEI and 10-20 µg DNA plasmid of interest. Transfection medium was mixed by vortexing, incubated at room temperature for 15 minutes, and added to plates after aspirating the existing medium. Cultures were incubated for 3-7 days prior to collection of supernatants. Culture supernatants were assayed for protein expression by SDS-PAGE, Western blotting, binding verified by flow cytometry, and function assayed using a variety of assays including ADCC, CDC, and coculture experiments.

SDS-PAGE Analysis and Western Blotting Analysis

[0384] Samples were prepared either from crude culture supernatants (usually 30 μ l/well) or purified protein aliquots, containing 8 μ g protein per well, and 2 $\times$ Tris-Glycine SDS Buffer (Invitrogen) was added to a 1 $\times$ final concentration. Ten (10) μ l SeeBlue Marker (Invitrogen, Carlsbad, Calif.) were run to provide MW size standards. The multivalent binding (fusion) protein variants were subjected to SDS-PAGE analysis on 4-20% Novex Tris-glycine gels (Invitrogen, San Diego, Calif.). Samples were loaded using Novex Tris-glycine SDS sample buffer (2 $\times$) under reducing or non-reducing conditions after heating at 95 $^{\circ}$ C. for 3 minutes, followed by electrophoresis at 175V for 60 minutes. Electrophoresis was performed using 1 $\times$ Novex Tris-Glycine SDS Running Buffer (Invitrogen).

[0385] After electrophoresis, proteins were transferred to PVDF membranes using a semi-dry electroblotter apparatus (Ellard, Seattle, Wash.) for 1 hour at 100 mAmp. Western transfer buffers included the following three buffers present on saturated Whatman filter paper, and stacked in succession: no. 1 contains 36.34 g/liter Tris, pH 10.4, and 20% methanol; no. 2 contains 3.02 g/liter Tris, pH 10.4, and 20% methanol; and no. 3 contains 3.03 g/liter Tris, pH 9.4, 5.25 g/liter s-amino caproic acid, and 20% methanol. Membranes were blocked in BLOTTO=5% nonfat milk in PBS overnight with agitation. Membranes were incubated with HRP conjugated goat anti-human IgG (Fc specific, Caltag) at 5 μ g/ml in BLOTTO for one hour, then washed 3 times for 15 minutes each in PBS-0.5% Tween 20. Wet membranes were incubated with ECL solution for 1 minute, followed by exposure to X-omat film for 20 seconds. FIG. 2 shows a Western Blot of proteins expressed in COS cell culture supernatant (30 μ l/well) electrophoresed under non-reducing conditions. Lanes are indicated with markers 1-9 and contain the following samples: Lane 1 (cut off=See Blue Markers, kDa are indicated to the side of the blot. Lane 2=2H7-ssslgG P238S/P331S-STD1-2e12 VLVH; lane 3=2H7-ssslgG P238S/P331S-STD1-2e12 VHVL, Lane 4=2H7-ssslgG P238S/P331S-STD2-2e12 VLVH; Lane 5=2H7-ssslgG P238S/P331S-STD2-2e12 VHVL; Lane 6=2e12 VLVH SMIP; Lane 7=2e12 VHVL SMIP; Lane 8=2H7 SMIP. 2H7 in these constructs is always in the $V_L V_H$ orientation, ssslG indicates the identity of the hinge/linker located at linker position 1 as shown in FIG. 5, P238S/P331S indicates the version of human IgG 1 with mutations from wild type (first aa listed) to mutant (second aa listed) and the amino acid position at which they occur in wild type human IgG1 C_{H2} and CH3 domains, STD1 indicates the 20-amino-acid (18+restriction site) linker located in linker position 2 as shown in FIG. 5, and STD2 indicates the 38 amino acid (36+restriction site) linker located in linker position 2 as shown in FIG. 6.

Binding Studies

[0386] Binding studies were performed to assess the bispecific binding properties of the CD20/CD28 multispecific, multivalent binding peptides. Initially, WIL2-S cells were added to 96 well plates and centrifuged to pellet cells. To the seeded plates, CD20/CD28 purified protein was added, using two-fold titrations across the plate from 20 μ g/ml down to 0.16 μ g/ml. A two-fold dilution series of TRU-015 (source of binding domain 1) purified protein was also added to seeded plate wells, the concentration of TRU-015 extending from 20

μ g/ml down to 0.16 μ g/ml. One well containing no protein served as a background control.

[0387] Seeded plates containing the proteins were incubated on ice for one hour. Subsequently, the wells were washed once with 200 μ l 1% FBS in PBS. Goat anti-human antibody labeled with FITC (Fc Sp) at 1:100 was then added to each well, and the plates were again incubated on ice for one hour. The plates were then washed once with 200 μ l 1% FBS in PBS and the cells were re-suspended in 200 μ l 1% FBS and analyzed by FACS.

[0388] To assess the binding properties of the anti-CD28 peptide 2E12 $V_H V_L$, CD28-expressing CHO cells were plated by seeding in individual wells of a culture plate. The CD20/CD28 purified protein was then added to individual wells using a two-fold dilution scheme, extending from 20 μ g/ml down to 0.16 μ g/ml. The 2E12IgG-VHVL SMIP purified protein was added to individual seeded wells, again using a two-fold dilution scheme, i.e., from 20 μ g/ml down to 0.16 μ g/ml. One well received no protein to provide a background control. The plates were then incubated on ice for one hour, washed once with 200 μ l 1% FBS in PBS, and goat anti-human antibody labeled with FITC (Fc Sp, CalTag, Burlingame, Calif.) at 1:100 was added to each well. The plates were again incubated on ice for one hour and subsequently washed once with 200 μ l 1% FBS in PBS. Following re-suspension of the cells in 200 μ l 1% FBS, FACS analysis was performed. The results showed that multivalent binding proteins with the N-terminal CD20 binding domain 1 bound CD20; those proteins having the C-terminal CD28 binding domain 2 in the $N-V_H V_L-C$ orientation also bound CD28.

[0389] The expressed proteins were shown to bind to CD20 presented on WIL-2S cells (see FIG. 3) and to CD28 presented on CHO cells (refer to FIG. 3) by flow cytometry (FACS), thereby demonstrating that either BD 1 or BD2 could function to bind the specific target antigen. Each data set on the graphs in FIG. 3 shows the binding of serial dilutions of the different multivalent binding (fusion) proteins over the titration ranges indicated. The data obtained using these initial constructs indicate that multivalent binding (fusion) proteins with the binding domain 2 version using 2e12 in the VHVL orientation express better and bind better to CD28 than the form in the VLVH orientation at equivalent concentrations.

[0390] FIG. 4 shows a graphical presentation of the results of binding studies performed with purified proteins from each of these transfections/constructs. The figure shows binding profiles of the proteins to CD20 expressing WIL-2S cells, demonstrating that the multivalent molecule binds to CD20 as well as the single specificity SMIP at the same concentration. The top and bottom panels for FIG. 5 show the binding profiles of the BD2 specificity (2e12=CD28) to CD28 CHO cells. For binding of binding domain 2 to CD28, the orientation of the V regions affected binding of the 2e12. 2H7-ssslgG-STD1-2e12 multivalent binding proteins with the 2e12 in the VH-VL (HL) orientation showed binding at a level equivalent to the single specificity SMIP, while the 2e12 LH molecule showed less efficient binding at the same concentration.

Example 5

Construction of Various Linker Forms of the Multivalent Fusion Proteins

[0391] This example describes the construction of the different linker forms listed in the table shown in FIG. 6.

Construction of C_{H3}-BD2 Linkers H1 Through H7

[0392] To explore the effect of C_{H3}-BD2 linker length and composition on expression and binding of the scorpion molecules, an experiment was designed to compare the existing molecule 2H7sssIgG1-Lx1-2e12HL to a larger set of similar constructs with different linkers. Using 2H7sssIgG1-Lx1-2e12HL as template, a series of PCR reactions were performed using the primers listed in Oligonucleotide Table 2, which created linkers that varied in length from 0 to 16 amino acids. These linkers were constructed as nucleic acid fragments that spanned the coding region for C_{H3} at the BsrGI site to the end of the nucleic acid encoding the linker-BD2 junction at the EcoRI site.

TABLE 2

Sequences of primers used to generate CH3-BD2 linker variants.			
No.	Name PCR Primers	Sequence 5'-3'	SEQ ID NO.
1	L1-11R	GCGATAGAATTCCTCCAGATCCACCACCGC CCGAGCCACCGCCACCATAATTC	46
2	L1-6R	GCGATAGAATTCCTCCAGAGCCACCGCCAC CATAATTC	47
3	L3R	GCGTATGAATTCCTCCGAGCCACCGCCAC CCTTACCCGGAGACAGG	48
4	L4R	GCGTATGAATTCCTCCAGATCCACCACCGC CCGAGCCACCGCCACCTTAC	49
5	L5R	GCGTATGAATTCCTCCGCTGCTCCTCCCC CAGATCCACCACCGCC	50
6	IgBsrGI	GAGAACCACAGGTGTACACCTG	51
7	L-CPPCPR	GCGATAGAATTCGGACAAGGTGGACACC CCTTACCCGGAGACAGGGAGAG	52

[0393] FIG. 6 diagrams the schematic structure of a multivalent binding (fusion) protein and shows the orientation of the V regions for each binding domain, the sequence present at linker position 1 (only the Cys residues are listed), and the sequence and identifier for the linker(s) located at linker position 2 of the molecules.

Example 6

Binding and Functional Studies with Variant Linker Forms of the 2H7-IgG-2e12 Prototype Multivalent Fusion Proteins

[0394] This example shows the results of a series of expression and binding studies on the “prototype” 2H7-sssIgG-Hx-2e12 VHVL construct with various linkers (H1-H7) present in the linker position 2. Each of these proteins was expressed by large-scale COS transient transfection and purification of the molecules using protein A affinity chromatography, as described in the previous examples. Purified proteins were then subjected to analyses including SDS-PAGE, Western blotting, binding studies analyzed by flow cytometry, and functional assays for biological activity.

Binding Studies Comparing the Different BD2 Orientations

[0395] Binding studies were performed as described in the previous examples, except that protein A-purified material

was used, and a constant amount of binding (fusion) protein was used for each variant studied, i.e., 0.72 ug/ml. FIG. 7 shows a columnar graph comparing the binding properties of each linker variant and 2e12 orientation variant to both CD20 and CD28 target cells. H1-H6 refer to constructs with the H1-H6 linkers and 2e12 in the VH-VL orientation. L 1-L6 refer to constructs with the H1-H6 linkers and 2e12 in the VL-VH orientation. The data demonstrate that the binding domain 2 specificity for 2e12 binds much more efficiently when present in the HL orientation (samples H1-H6) than when in the LH orientation (samples L1-L6). The effect of linker length is complicated by the discovery, as shown in the next set of figures, that molecules with the longer linkers contain some single-specificity cleaved molecules which are missing the CD28 binding specificity at the carboxy terminus. Other experiments were performed which address the binding of selected linkers, with the results shown in FIGS. 10, 12, and 13.

SDS-PAGE Analysis of Purified H1-H7 Linker Variants

[0396] Samples were prepared from purified protein aliquots, containing 8 µg protein per well, and 2× Tris-Glycine SDS Buffer (Invitrogen) was added to a 1× final concentration. For reduced samples/gels, 10× reducing buffer was added to 1× to samples plus Tris-Glycine SDS buffer. Ten (10) µl SeeBlue Marker (Invitrogen, Carlsbad, Calif.) was run to provide MW size standards. The multivalent binding (fusion) protein variants were subjected to SDS-PAGE analyses on 4-20% Novex Tris-glycine gels (Invitrogen, San Diego, Calif.). Samples were loaded using Novex Tris-glycine SDS sample buffer (2×) under reducing or non-reducing conditions after heating at 95° C. for 3 minutes, followed by electrophoresis at 175V for 60 minutes. Electrophoresis was performed using 1× Novex Tris-Glycine SDS Running Buffer (Invitrogen). Gels were stained after electrophoresis in Coomassie SDS PAGE R-250 stain for 30 minutes with agitation, and destained for at least one hour. FIG. 8 shows the non-reduced and reduced Coomassie stained gels of the [2H7-sss-hIgG P238S/P331S-Hx-2e12 VHVL] multivalent binding (fusion) protein variants, along with TRU-015 and 2e12 HLSPMP as control samples. As the linker length is increased, the amount of protein running at approximately SMTP size (or 52 kDa) increases. The increase in the amount of protein in this band corresponds with a decrease in the amount of protein in the upper band running at about 90 kDa. The gel data indicate that the full-length molecule is being cleaved at or near the linker, to generate a molecule which is missing the BD2 region. A smaller BD2 fragment is not present, indicating (1) that the nucleotide sequence within the linker sequence may be creating a cryptic splice site that removes the smaller fragment from the spliced RNA transcript, or (2) that the protein is proteolytically cleaved after translation of the full-size polypeptide, and that the smaller BD2 fragment is unstable, i.e., susceptible to proteolytic processing. Western blotting of some of these molecules indicates that the proteins all contain the CD20 BD 1 sequence, but the smaller band is missing the CD28 BD2 reactivity. No smaller band migrating at “bare” scFv size (25-27 kDa) was observed on any gels or blots, indicating that this smaller peptide fragment is not present in the samples.

Western Blot Binding of BD1 and BD2 by 2H7 Specific Fab or CD28mIg

[0397] FIG. 9 shows the results of Western blotting of the 2H7-sss-hIgG-H6 multivalent binding (fusion) proteins com-

pared to each single-specificity SMIP. Electrophoresis was performed under non-reducing conditions, and without boiling samples prior to loading. After electrophoresis, proteins were transferred to PVDF membranes using a semi-dry electroblotter apparatus (Ellard, Seattle, Wash.) for 1 hour at 100 mA. Membranes were blocked in BLOTTO (5% nonfat milk in PBS) overnight with agitation. FIG. 9A: Membranes were incubated with the AbyD02429.2, a Fab directed to the 2H7 antibody, at 5 μ g/ml in BLOTTO for one hour, then washed 3 times for 5 minutes each in PBS-0.5% Tween 20. Membranes were then incubated in 6xHis-HRP for one hour at a concentration of 0.5 μ g/ml. Blots were washed three times for 15 minutes each in PBST. Wet membranes were incubated with ECL solution for 1 minute, followed by exposure to X-omat film for 20 seconds.

[0398] FIG. 9B: Membranes were incubated with CD28Ig (Ancell, Bayport, Minn.) at 10 μ g/ml in BLOTTO, then washed three times for 15 minutes each in PBS-0.5% Tween 20. Membranes were then incubated in goat anti-mouse HRP conjugate (CalTag, Burlingame, Calif.) at 1:3000 in BLOTTO. Membranes were washed three times, for 15 minutes each, then incubated in ECL solution for 1 minute, followed by exposure to X-omat film for 20 seconds. The results from the Western blots indicated that the CD28 binding domain was present in the multivalent "monomer" fraction migrating at approximately 90 kDa, and in higher order forms. No band was detectable migrating at the position expected for a single SMIP or bare scFv size fragment. When the CD20 anti-idiotypic Fab was used, a SMIP-sized fragment was detected, indicating the presence of a peptide fragment containing (2H7-sss-hlgG), and missing the CD28 scFv BD2 portion of the fusion protein.

Binding Studies on Selected Linkers

[0399] FIG. 10 shows the results of binding studies performed on the purified 2H7-sss-hlgG-Hx-2e12 fusion proteins. Binding studies were performed to assess the bispecific binding properties of the CD20/CD28 multispecific binding peptides. Initially, WIL-2S cells were plated using conventional techniques. To the seeded plates, CD20/CD28 purified protein was added, using two-fold titrations across the plate from 20 μ g/ml down to 0.16 μ g/ml. A two-fold dilution series of TRU-015 (source of binding domain 1) purified protein was also added to seeded plate wells, the concentration of TRU-015 extending from 20 μ g/ml down to 0.16 μ g/ml. One well containing no protein served as a background control.

[0400] Seeded plates containing the proteins were incubated on ice for one hour. Subsequently, the wells were washed once with 200 μ l 1% FBS in PBS. Goat anti-human antibody labeled with FITC (Fc Sp) at 1:100 was then added to each well, and the plates were again incubated on ice for one hour. The plates were then washed once with 200 μ l 1% FBS in PBS and the cells were re-suspended in 200 μ l 1% FBS and analyzed by FACS.

[0401] To assess the binding properties of the anti-CD28 peptide 2E12 V_HV_L , CD28-expressing CHO cells were plated by seeding in individual wells of a culture plate. The CD20/CD28 purified protein was then added to individual wells using a two-fold dilution scheme, extending from 20 μ g/ml down to 0.16 μ g/ml. The 2E12IgGvHvL SMIP purified protein was added to individual seeded wells, again using a two-fold dilution scheme, i.e., from 20 μ g/ml down to 0.16 μ g/ml. One well received no protein to provide a background control. The plates were then incubated on ice for one hour,

washed once with 200 μ l 1% FBS in PBS, and goat anti-human antibody labeled with FITC (Fc Sp) at 1:100 was added to each well. The plates were again incubated on ice for one hour and subsequently washed once with 200 μ l 1% FBS in PBS. Following re-suspension of the cells in 200 μ l 1% FBS, FACS analysis was performed. The expressed proteins were shown to bind to CD20 presented on WIL-2S cells (see FIG. 10A) and to CD28 presented on CHO cells (refer to FIG. 10B) by flow cytometry (FACS), thereby demonstrating that either BD1 or BD2 could function to bind the specific target antigen. In addition, the linker used (H1-H6) was not found to significantly affect binding avidity to target antigen.

[0402] SEC Fractionation of Multivalent Binding (Fusion) Proteins. The binding (fusion) protein was purified from cell culture supernatants by protein A Sepharose affinity chromatography over a GE Healthcare XK 16/40 column. After binding of protein to the column, the column was washed in dPBS, then 1.0 M NaCl, 20 mM sodium phosphate pH 6.0, and then 25 mM NaCl, 25 mM NaOAc, pH 5.0, to remove nonspecific binding proteins. Bound protein was eluted from the column in 100 mM Glycine (Sigma), pH 3.5, and brought to pH 5.0 with 0.5 M 2-(N-Morpholino) ethanesulfonic acid (MES), pH 6.0. Protein samples were concentrated to 25 mg/ml using conventional techniques in preparation for GPC purification. Size exclusion chromatography (SEC) was performed on a GE Healthcare AKTA Explorer 100 Air apparatus, using a GE healthcare XK column and Superdex 200, preparative grade (GE healthcare).

[0403] FIG. 12 shows a table summarizing the results of SEC fractionation of the different binding (fusion) proteins. With increasing linker length, the complexity of the molecules in solution also increases, making it difficult to isolate peak of interest, or POI from higher order forms by HPLC. The H7 linker seems to resolve much of this complexity into a more homogeneous form in solution, so that the soluble forms migrate mostly as a single POI at approximately 172 kDa.

Additional Binding Studies

[0404] A second series of experiments was performed (see FIGS. 12 and 13) with a smaller subset of multivalent binding (fusion) proteins, this time comparing linkers H3, H6, and H7. The data demonstrate that the binding level drops more significantly for CD28 than for CD20 binding, but both drop slightly as linker length increases. Further, the data showed that the H7 linker exhibited the highest level of binding to both antigens. These data were obtained using protein A-purified multivalent binding (fusion) proteins, but were not further purified by SEC, so multiple forms of the molecules may have been present in solution. The results also indicated that the truncated form may have been less stable than the true multivalent polypeptide, since the binding curves do not appear to fully reflect the significant amount of single specificity form present in solution for linker H6.

Demonstration of Multispecific Binding from a Single Molecule

[0405] An alternative binding assay was performed (see FIG. 13), where binding to CD20 on the surface of WIL-2S cells was detected with a reagent specific for the CD28 BD2, thereby demonstrating that simultaneous binding may occur to both target antigens, engaging both BD1 and BD2 on the same multispecific binding (fusion) protein (refer to FIG. 12). This assay demonstrates the multispecific binding property of the proteins.

Example 7

Construction of Multispecific Binding (Fusion)
Proteins with Alternative Specificities in BD2

[0406] In addition to the prototype CD20-CD28 multispecific binding molecule, two other forms were made with alternative binding domain 2 regions, including CD37 and CD3 binding domains. The molecules were also made with several of the linker domains described for the [2H7-sss-IgG-Hx/STDx-2e12 HL] multispecific binding (fusion) proteins. The construction of these additional multispecific binding (fusion) molecules are described below.

Anti-CD37 Binding Domain Construction

[0407]

TABLE 3

Oligonucleotide primers used to generate G28-1 anti-CD37 binding domains for both SMIP molecules and scorpions.			
No.	Name	Sequence	SEQ ID NO.
23	G281LH-NheR	ACTGCTGCAGCTGGACCGCTAGC TCCGCCGCCACCGAC	53
24	G281LH-NheF	GGCGGAGCTAGCGGCTCCAGCTGC AGCAGTCTGGACCTG	54
25	G281-LH- LPinF	GCGATACCGGTGACATCCAGAT GACTCAGTCTCCAG	55
26	G281-LH- HXhoR	GCGATACTCGAGGAGACGGTGAC TGAGGTTCTTGAC	56
27	G281-LH- LEcoF	GCGATCGAATTCAGACATCCAGATG ACTCAGTCTCCAG	57
28	G281-LH- HkbaR	GCGATTCTAGATTAGGAAGAGACG GTGACTGAGGTTCTTGAC	58
29	G281-HL-HF	GCGATAACCGGTGCGGTCCAGCTG CAGCAGTCTGGAC	59
30	G281-HL-HR3	GACCCACCAACCGCCGAGCCACCG CCACCAGAAGAGACGGTGACTGAGG TTC	60
31	G281-HL-HR2	ACTCCCGCCTCCTCCTGATCCGCCG CCACCCGACCCACCCGCCCCGAG	61
32	G281-HL- HNheR	GAGTCATCTGGATGTGCTAGCACTC CCGCCTCCTCCTGATC	62
33	G281-HL- LNheF	ATCAGGAGGAGGCGGGAGTGCTAGC GACATCCAGATGACTCAGTC	63
34	G281-HL- LXhoR	GCGATACTCGAGCCTTTGATCTCCAG TTCGGTGCCTC	64
35	G281-HL- LXbaR	GCGATATCTAGACTCAACCTTTGATCT CCAGTTCCGGTGCCTC	65
36	G281-HL- EcoF	GCGATAGAATTCGCGGTCCAGCTGCA GCAGTCTGGAC	66

[0408] The G28-1 scFv (SEQ ID NO:102) was converted to the G28-1 LH SMIP by PCR using the primers in Table 3 above. Combining primers 23 and 25 with 10 ng G28-1 scFv, the VK was amplified for 30 cycles of 94 C, 20 seconds, 58 C,

15 seconds, 68 C, 15 seconds using Platinum PCR Supermix Hi-Fidelity PCR mix (Invitrogen, Carlsbad, Calif.) in an ABI 9700 Thermalcycler. The product of this PCR had the restriction sites PinAI (AgeI) at the 5' end of the VK and NheI at the end of the scFv (G4S)₃ linker. The VH was similarly altered by combining primers 24 and 26 with 10 ng G28-1 scFv in a PCR run under the identical conditions as with the VK above. This PCR product had the restriction sites NheI at the 5' end of the VH and XhoI at the 3' end. Because significant sequence identity overlap was engineered into primers 23 and 24, the VK and VH were diluted 5-fold, then added at a 1:1 ratio to a PCR using the flanking primers 25 and 26 and a full-length scFv was amplified as above by lengthening the 68 C extension time from 15 seconds to 45 seconds. This PCR product represented the entire G28-1 scFv as a PinAI-XhoI fragment and was purified by MinElute column (Qiagen) purification to remove excess primers, enzymes and salts. The eluate was digested to completion with PinAI (Invitrogen) and XhoI (Roche) in 1× H buffer (Roche) at 37 C for 4 hours in a volume of 50 µL. The digested PCR product was then electrophoresed in a 1% agarose gel, the fragment was removed from the gel and re-purified on a MinElute column using buffer QG and incubating the gel-buffer mix at 50C for 10 minutes with intermittent mixing to dissolve the agarose after which the purification on the column was identical for primer removal post-PCR. 3 µL PinAI-XhoI digested G28-1 LH was combined with 1 µL PinAI-XhoI digested pD18-n2H7sssIgG1 SMIP in a 10 µL reaction with 5 µL 2× LigaFast Ligation Buffer (Promega, Madison, Wis.) and 1 µL T4 DNA ligase (Roche), mixed well and incubated at room temperature for 10 minutes. 3 µL of this ligation was then transformed into competent TOP 10 (Invitrogen) using the manufacturer's protocol. These transformants were plated on LB agar plates with 100 µg/ml carbenicillin (Teknova) and incubated overnight at 37 C. After 18 hours of growth, colonies were picked and inoculated into 1 ml T-Broth (Teknova) containing 100 µg/ml carbenicillin in a deep well 96-well plate and grown overnight in a 37 C shaking incubator. After 18-24 hours of growth, DNA was isolated from each overnight culture using the QIAprep 96 Turbo Kit (Qiagen) on the BioRobot8000 (Qiagen). 10 µL, from each clone was then digested with both HindIII and XhoI restriction enzymes in 1× B buffer in a 15 µL reaction volume. The digested DNA was electrophoresed on 1% agarose E-gels (Invitrogen, CA) for restriction site analysis. Clones that contained a HindIII-XhoI fragment of the correct size were sequence verified. The G28-1 HL SMIP was constructed in a similar manner by placing a PinAI site on the 5' end and a (G4S)₄ linker ending in an Nhe I site of the G28-1 VH using primers 29, 30 31 and 32 from Table 3 above. The VK was altered by PCR using primers 33 and 34 from Table 3 such that an NheI site was introduced at the 5' end of the VK and XhoI at the 3' end. These PCRs were then combined as above and amplified with the flanking primers 29 and 34 to yield an intact G28-1 scFv DNA in the VH-VL orientation which was cloned into PinAI-XhoI digested pD18-(n2H7)sssIgG1 SMIP exactly as with the G28-1 LH SMIP.

2H7sssIgG1-STD1-G28-1 LH/HL Construction

[0409] Using the G28-1 LH and G28-1 HL SMIPs as templates, the LH and HL anti-CD37 binding domains were altered by PCR such that their flanking restriction sites were compatible with the scorpion cassette. An EcoRI site was introduced at the 5' end of each scFv using either primer 27 (LH) or 36 (HL) and a stop codon/XbaI site at the 3' end using

either primer 28 (LH) or 35 (HL). The resulting DNAs were cloned into EcoRI-XbaI digested pD18-2H7ssIgG-STD1. 2H7ssIgG1-Hx-G28-1 HL Construction 2H7ssIgG1-Hx-2e12 HL DNAs were digested with BsrGI and EcoRI and the 325 bp fragment consisting of the C-terminal end of the IgG1 and linker. These were substituted for the equivalent region in 2H7ssIgG1-STD1-G19-4 HL by removal of the STD 1 linker using BsrGI-EcoRI and replacing it with the corresponding linkers from the 2H7ssIgG1-Hx-2e12 HL clones.

Anti-CD3 Binding Domain Construction

[0410]

TABLE 4

Oligonucleotides used to generate anti-CD3 binding domain from the G19-4 scFv sequence.			
No.	Name	Sequence	SEQ ID NO.
37	194-LH-LF1	GCGTATGAACCGGTGACATCCAGATGACACAGACTACATC	67
38	194-LF2	ATCCAGATGACACAGACTACATCCTCCCTGCTGCTGCCTCTCTGGGAGACAG	68
39	194-LF3	GTCTGCCTCTCTGGGAGACAGAGTCAACATCAGTTGCAGGGCAAGTCAGGAC	69
40	194-LF4	GTTGCAGGGCAAGTCAGGACATTCGCAATTATTTAAACTGGTATCAGCAG	70
41	194-LF5	ATTTAAACTGGTATCAGCAGAAACCAGATGGAACCTGTTAAACTCCTGATC	71
42	194-LF6	GAACTGTTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGTC	72
43	194-LF7	CAAGATTACACTCAGGAGTCCCATCAAAGTTTCAGTGGCAGTGGGTCTGGAAC	73
44	194-LR7	CAGGTTGGCAATGGTGAGAGAATAATCTGTTCCAGACCCACTGCCACTGAAC	74
45	194-LR6	GCAAAAGTAAGTGGCAATATCTTCTGGTTGCAGGTTGGCAATGGTGAGAG	75
46	194-LR5	GAACGTCCACGGAAGCGTATTACCCGTGTTGGCAAAAGTAAGTGCAATATC	76
47	194-LR4	CGTTTGGTTACCAAGTTTGGTGCCTCCACCGAACGTCCACGGAAGCGTATTAC	77
48	194-LR3	ACCACCGCCCGAGCCACCGCCACCCTGTTGGTTACCAAGTTTGGTG	78
49	194-LR2	GCTAGCGCTCCACCTCCTCCAGATCCAACACCGCCGAGCCACCGCCAC	79
50	194-LH-LR1	GTTGTCAGCTGGACCTCGCTAGCGCTCCCACCTCCTCCAGATC	80
51	194-LH-HF1	GATCTGGAGGAGGTGGGAGCGTAGCGAGGTCCAGCTGCAACAGTCTGGACCTG	81
52	194-HF2	AGCTGCAACAGTCTGGACCTGAACTGGTGAAGCTGGAGCTTCAATGAAG	82
53	194-HF3	AGCCTGGAGCTTCAATGAAGATTTCCTGCAAGGCTCTGGTTACTCATTC	83
54	194-HF4	GCAAGGCTCTGGTTACTCATTCAGTGGCTACATCGTGAAGTGAAGCAG	84

TABLE 4-continued

Oligonucleotides used to generate anti-CD3 binding domain from the G19-4 scFv sequence.			
No.	Name	Sequence	SEQ ID NO.
55	194-HF5	ATCGTGAACCTGGCTGAAGCAGAGCCATGGAAAGAACCTTGAGTGGATTGGAC	85
56	194-HF6	GAACCTTGAGTGGATTGGACTTATTAATCCATACAAAGGCTTACTACCTAC	86
57	194-HR6	AATGTGGCCTTGCCCTTGAATTTCTGTGTAGGTAGTAAGACCTTGTATG	87
58	194-HR5	CATGTAGGCTGTGCTGGATGACTTGTCTACAGTTAATGTGGCCTTGCCCTTG	88
59	194-HR4	ACTGCAGAGTCTTCAGATGTCAGACTGAGGAGCTCCATGTAGGCTGTGCTGGATG	89
60	194-HR3	ACCATAGTACCCAGATCTTGACACAGTAATAGACTGCAGAGTCTTCAGATGTC	90
61	194-HR2	GCGCCCCAGACATCGAAGTACCAGTCCGAGTACCCATAGTACCCAGATCTTG	91
62	194-LH-HR1	GCGAATACTCGAGGAGACGGTGACCGTGGTCCCTGCGCCCAGACATCGAAG	92
63	194-HL-HF1	GCGTATGAACCGGTGAGGTCCAGCTGCAACACAGTCTGGACCTG	93
64	194-HL-HR1	ACCGCCACCAGAGGAGACGGTGACCGTGGTCCCTGCGCCCAGACATCGAAGTAC	94
65	194-HL-HR0	ACCTCCTCCAGATCCACCACCGCCCGAGCCACCGCCACCAGAGAGACGGTG	95
66	194-HL-LF1	GCGGGGGAGGTGGCAGTGCTAGCGCATCCAGATGACACAGACTACATC	96
67	194-HL-LR3	Xho GCGAATACTCGAGCGTTTGGTTACCA GTTTGGTG	97
68	194-HL-LR3	Xba GCGATATCTAGATTACCGTTTGGTTAC CAGTTTGGTG	98
69	194-HL-HF1	R1 GCGTATGAGAATTCTGACATCCAGTCAACAGTCTGGACCTG	99
70	194-LH-LF1	R1 GCGTATGAGAATTCTGACATCCAGATGACACAGACTACATC	100
71	194-LH-HR1	Xba GCGTATCTAGATTAGGAGACGGTGACCGTGGTCCCTGCGCCCAGACATCGAAG	101

[0411] The G19-4 binding domain was synthesized by extension of overlapping oligonucleotide primers as described previously. The light chain PCR was done in two steps, beginning by combining primers 43/44, 42/45, 41/46 and 40/47 at concentrations of 5 μ M, 10 μ M, 20 μ M and 40 μ M, respectively, in Platinum PCR Supermix Hi-Fidelity for 30 cycles of 94° C., 20 seconds, 60° C., 10 seconds, 68° C., 15 seconds. 1 μ L of the resultant PCR product was reamplified using a primer mix of 39/48 (10 μ M), 38/49 (20 μ M) and 37/50 (40 μ M) for the LH or 66/67 (40 μ M) for the HL orientation, using the same PCR conditions with the exception of the 68 C extension which was increased to 25 seconds. The VK in the LH orientation was bounded by PinAI at the 5' end and NheI at the 3' end, while the HL orientation had NheI at the 5' end and XhoI at the 3' end.

[0412] To synthesize the heavy chain, primer mixes with the same concentrations as above were prepared by combining primers 56/57, 55/58, 54/59 and 53/60 for the first PCR step. In the second PCR, primers 52/61 (20 μ M) and 51/62 (50 μ M) were amplified with 1 μ l from the first PCR using the same PCR conditions as with the second PCR of the light chain to make the LH orientation with NheI at the 5' end and XhoI at the 3' end. Primers 52/61 (10 μ M), 63/64 (20 μ M), 63 (20 μ M)/65 (40 μ M), and 63(20 μ M)/5 (80 μ M) were combined in a second PCR with 1 μ l from the previous PCR to create the heavy chain in the HL orientation with PinAI at the 5' end and NheI at the 3' end. As with previous constructs, sufficient overlap was designed into the primers centered around the NheI site such that the G19-4 LH was synthesized by combining the heavy and light chain PCRs in the LH orientation and reamplifying with the flanking primers, 37 and 62 and the G19-4 HL was synthesized by combining the HL PCRs and re-amplifying with primers 63 and 67.

[0413] Full-length G19-4 LH/HL PCR products were separated by agarose gel electrophoresis, excised from the gel and purified with Qiagen MinElute columns as described earlier. These DNAs were then TOPO-cloned into pCR2.1 (Invitrogen), transformed into TOP10 and colonies screened first by EcoRI fragment size, then by DNA sequencing. G19-4 LH/HL were then cloned into pD18-IgG1 via PinAI-XhoI for expression in mammalian cells.

2H7ssslgG1-STD1-G19-4 LH/HL Construction

[0414] Using the G-19-4 LH and -G19-4 HL SMIPs as templates, the LH and HL anti-CD3 binding domains were altered by PCR such that their flanking restriction sites were compatible with the scorpion cassette. An EcoRI site was introduced at the 5' end of each scFv using either primer 27 (LH) or 36 (HL) and a stop codon/XbaI site at the 3' end using either primer 28 (LH) or 35 (HL). The resulting DNAs were cloned into EcoRI-XbaI digested pD18-2H7ssslgG1-STD1.

2H7ssslgG1-Hx-G19-4 HL Construction

[0415] 2H7ssslgG1-Hx-2e12 HL DNAs were digested with BsrGI and EcoRI and the 325 bp fragment consisting of the C-terminal end of the IgG1 and linker. These were substituted for the equivalent region in 2H7ssslgG1-STD1-G19-4 HL by removal of the STD1 linker using BsrGI-EcoRI and replacing it with the corresponding linkers from the 2H7ssslgG1-Hx-2e12 HL clones.

[0416] Apparent from a consideration of the variety of multivalent binding proteins disclosed herein are features of the molecules that are amenable to combination in forming the molecules of the invention. Those features include binding domain 1, a constant sub-region, including a hinge or hinge-like domain, a linker domain, and a binding domain 2. The intrinsic modularity in the design of these novel binding proteins makes it straightforward for one skilled in the art to manipulate the DNA sequence at the N-terminal and/or C-terminal ends of any desirable module such that it can be inserted at almost any position to create a new molecule

exhibiting altered or enhanced functionality compared to the parental molecule(s) from which it was derived. For example, any binding domain derived from a member of the immunoglobulin superfamily is contemplated as either binding domain 1 or binding domain 2 of the molecules according to the invention. The derived binding domains include domains having amino acid sequences, and even encoding polynucleotide sequences, that have a one-to-one correspondence with the sequence of a member of the immunoglobulin superfamily, as well as variants and derivatives that preferably share 80%, 90%, 95%, 99%, or 99.5% sequence identity with a member of the immunoglobulin superfamily. These binding domains (1 and 2) are preferably linked to other modules of the molecules according to the invention through linkers that may vary in sequence and length as described elsewhere herein, provided that the linkers are sufficient to provide any spacing and flexibility necessary for the molecule to achieve a functional tertiary structure. Another module of the multivalent binding proteins is the hinge region, which may correspond to the hinge region of a member of the immunoglobulin superfamily, but may be a variant thereof, such as the "CSC" or "SSS" hinge regions described herein. Also, the constant sub-region comprises a module of the proteins according to the invention that may correspond to a sub-region of a constant region of an immunoglobulin superfamily member, as is typified by the structure of a hinge- C_{H2} - C_{H3} constant sub-region. Variants and derivatives of constant sub-regions are also contemplated, preferably having amino acid sequences that share 80%, 90%, 95%, 99%, or 99.5% sequence identity with a member of the immunoglobulin superfamily.

[0417] Exemplary primary structures of the features of such molecules are presented in Table 5, which discloses the polynucleotide and cognate amino acid sequence of illustrative binding domains 1 and 2, as well as the primary structure of a constant sub-region, including a hinge or hinge-like domain, and a linker that may be interposed, e.g., between the C-terminal end of a constant sub-region and the N-terminal end of a binding domain 2 region of a multivalent binding protein. Additional exemplars of the molecules according to the invention include the above-described features wherein, e.g., either or both of binding domains 1 and 2 comprise a domain derived from a V_L or V_L -like domain of a member of the immunoglobulin superfamily and a V_H or V_H -like domain derived from the same or a different member of the immunoglobulin superfamily, with these domains separated by a linker typified by any of the linkers disclosed herein. Contemplated are molecules in which the orientation of these domains is V_L - V_H or V_H - V_L for BD1 and/or BD2. A more complete presentation of the primary structures of the various features of the multivalent binding molecules according to the invention is found in the table appended at the end of this disclosure. The invention further comprehends polynucleotides encoding such molecules.

TABLE 5

Primary structures (polynucleotide and cognate amino acid sequences) of exemplary features of multivalent binding molecules.			
Binding Domain	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NOS. (amino acid sequence)
2H7 LH	atggattttcaagtgcagattttcag cttcctgctaatactagtgcttcagtca	mdfgvqifsflllisavimsrgqivls qspailsaspgekvmtcrasssysm	1 (2)

TABLE 5-continued

Primary structures (polynucleotide and cognate amino acid sequences) of exemplary features of multivalent binding molecules.			
	taatgtccagaggacaaattgttctc hwyqqkpgsspkpwiapsnlasgvpa tcccagttctccagcaatcctgtctgc rfsgsgsgtsysltierveadaatyy atctccaggggagaaggtcacaaatga cqqwsfnpptfgagtklelkdggsggg cttgcaggggccagctcaagtgttaagt ggsggggssqaylqqsgaesvrpgasv tacatgcactggtaccagcagaagcc kmsckasgytftsynmhwwkqtrpggl aggatcctcccccaaacctggattt ewigaiypngdtsynqkfkkgkatltv atgccccatccaaacctggcttctgga dksstaymqlssltseedsavyfcary gtccctgctcgcttcagtggcagtgg vyysnsywyfdvwtgtttvts gtctgggacctcttactctctcaca tcagcagagtggaggctgaagatgct gccacttattactgccagcagtggag ttttaaccccccacgttcgggtctg ggaccaagctggagctgaaagatggc ggtggctcggcggtggtggtatctg aggaggtgggagctctcaggttatc tacagcagtctgggctgagtcggtg aggcctggggcctcagtgaagatgct ctgcaaggcttctgggtacacattta ccagttacaatatgactgggtaag cagacacctagacaggcctggaatg gattggagctatttatccaggaatg gtgatacttctacaatcagaagttc aagggaaggccacactgactgtaga caaatcctccagcacagcctacatgc agctcagcagcctgacatctgaagac tctgcggtctatttctgtgcaagagt ggtgtaactatagtaactcttactggt acttcgatgtctggggcacagggacc acggtcacctctct		
2e12 LH	atggattttcaagtcagattttcag MDFQVQIFSFLLISASVIMSRGVDIVL ctctcgtctaatcagtgcttcagtc TQSPASLAVSLGQRATISCRASESVEY taatgtccagaggagtcagacattgtg YVTSMLQWYQQKPGPPKLLISAASN ctcaaccaaattctccagcttctttggc ESGVVPARFSGSGSGTDFSLNIHPVEED tgtgtctctaggtcagagagccacca DIAMYPFCQSRKVPWTFGGGTKLEIKR tctcctgcagagccagtgaaagtgtt GGGGSGGGGSGGGGSGVQLKESGPGLV gaatatattatgtcacaaagtttaagca APSQSLSI TCTVSGFSLTGYGVNWVRQ gtggtaccaacagaaaccaggacagc PPGKLEWLGMWGDGSTDYNSALKSR caccacaaactcctcatctctgctgct LSI TKDNSKSVFLKMNSLQDDTARY agcaacgtagaatctggggctccctgc YCARDGYSNFHYVMDYWGQGTSTVTS caggtttagtggcagtggtctggga S cagactttagcctcaacatccatcct gtggaggaggatgatattgcaatgta tttctgtcagcaaaagtaggaaggttc catggacgttcggtggaggcaccag ctggaaatcaaacgggtggcggtgg atccggcggaggtgggtcgggtggcg gcggatctcaggtgcagctgaaggag tcaggacctggcctggtggcgccctc acagagcctgtccatcacatgcaccg tctcagggttctcattaacgggtat ggtgtaaacctgggttcgccagcctcc aggaaagggtctggagtggtgggaa tgatatggggtgatggaagcacagac tataattcagctctcaaatccagact atcgatcaccaggacaactccaaga gccaagttttcttaaaaatgaacagt ctgcaaacctgatgacacagccagata ctactgtgcccagatggttatagta actttcattactatgttatggactac tggggtaagggaacctcagtcaccgt ctctct	3 (4)	
2e12 HL	atggattttcaagtcagattttcag MDFQVQIFSFLLISASVIMSRGVQVQL ctctcgtctaatcagtgcttcagtc KESGPGGLVAPSQSLSI TCTVSGFSLTG taatgtccagaggagtcagggtgcag YGVNWVRQPPGKLEWLGMWGDGSTD ctgaaggagtcaggacctggcctggt YNSALKSRLSITKDNSKSVFLKMNSL ggcgcctcagagagcctgtccatca QDDTARYYCARDGYSNFHYVMDYWG catgcacgtctcagggttctcatta QGTSVTVSSGGGSGGGGSGGGGSGGG accggtatgggttaaacctgggttcg GSDIVLTQSPASLAVSLGQRATISCR ccagctccaggaaagggtctggagt SESVEYYVTSMLQWYQQKPGPPKLLI ggctgggaatgatatgggtgatgga SAASNVESGVPARFSGSGSGTDFSLNI agcacagactataattcagctctcaa HPVEEDDIAMYPFCQSRKVPWTFGGGT atccagactatcgatcaccaggaca KLEIKR	5 (6)	

TABLE 5-continued

Primary structures (polynucleotide and cognate amino acid sequences) of exemplary features of multivalent binding molecules.		
actccaagagccaagttttcttaaaa atgaacagctctgcaaactgatgacac agccagatactactgtgcccgagatg gttatagtaactttcattactatgtt atggactactggggtcaaggaacctc agtcacogtctcctctgggggtggag gctctggggtggcggtgatccggcgga ggtaggtcggtggcggtggatctga cattgtgctcaccatctccagctt ctttggctgtgtctctaggtcagaga gccaccatctcctgcagagccagtga aagtgttgaatatatgtcacaagt taatgcagtggtaccaacagaaacca ggacagccacccaaactcctcatctc tgctgctagcaactgagaatctgggg tcctgccaggtttagtggcagtggg tctgggacagacttagcctcaacat ccatctgtggaggaggtgatattg caatgtatttctgtcagcaagtagg aaggttccatggacgttcggtggagg caccaagctggaatcaaacgt		
G28-1 LH accgggtgacatccagatgactcagtc	DIQMTQSPASLSASVGETVTITCRTSE	102 (103)
tcagacctcctatctgcatctgtgg	NVYSYLAWYQQKQKSPQLLVSPAKTL	
gagagactgtcaccatcacatgtcga	AEGVPSRFSGSGSGTQFSLKISLQPE	
acaagtgaataatgtttacagttattt	DSGSYFCQHHSDNPWTFGGGTELEIKG	
ggcttggtatcagcagaaacagggaa	GGSGGGSGGGGSASAVQLQQSGPEL	
aatctcctcagctcctgggtctctttt	EKPGASVKISCKASGYSTGYNMNVK	
gcaaaaaccttagcagaaggtgtgcc	QNNGKSLEWIGNIDPYGGTTYNRKFK	
atcaaggttcagtggtgagtgatcag	GKATLTVDKSSSTAYMQLKSLTSEDSA	
gcacacagttttctcgaagatcagc	VYCARSVGPMDYWGQGTSTVTS	
agcctgcagcctgaagattctggaag		
ttatttctgtcaacatcattccgata		
atccgtggacgttcggtggaggacc		
gaactggagatcaaaggtggcggtgg		
ctcggtgggtgggtgggtcggtggcg		
gcggatctgctagcgagtcagctg		
cagcagctctggacctgagctggaaa		
gcctggcgcttcagtgaaatttctc		
gcaaggcttctggttactcattcact		
ggctacaatatgaactgggtgaagca		
gaataatggaagagccttgagtgga		
ttggaaatatgacatcttattatggt		
ggtactacctacaaccggaagtcca		
gggcaaggccacattgactgtagaca		
aatcctccagcacagcctacatgcag		
ctcaagagctctgacatctgaggactc		
tgcagctctattactgtgcaagatcgg		
tcggccctatggactactgggtcaa		
ggaacctcagtcaccgtctcag		
G28-1 HL accgggtgaggtccagctgcaacagtc	EVQLQQSGPELVKPGASMKISCKASGY	104 (105)
tggaacctgaactgggtgaagcctggag	SFTGYIVNWLKQSHGKNLEWIGLINPY	
cttcaatgaagatttctgcaaggcc	KGLTTYNQKFKGKATLTVDKSSSTAYM	
tctggttactcattcactggctacat	ELLSLTSEDSAVYYCARSGYIGSDWY	
cgtgaactggctgaagcagagccatg	FDVWGAGTTVTVSSGGGSGGGSGGG	
gaaagaaccttgagtggttggaactt	SGSGGGSASDIQMTQTTSSLSASLGDR	
attaatccatataaaaggtcttactac	VTISCRASQDIRNYLNWYQQKPDGTVK	
ctacaaccagaaattcaagggtcaagg	LLIYYTSRLHSGVPSRFSGSGSGTDYS	
ccacattaaactgtagacaagtcaccc	LTIANLQPEDIATYFCQQGNTLPWTFG	
agcacagcctacatggagctcctcag	GGTKLVTKRS	
tctgacatctgaagactctgcagctt		
attactgtgcaagatctgggtactat		
ggtgactcggactggtacttcgatgt		
ctggggcgagggaccacggtcacccg		
tctcctctgggtggcggtggctcgggc		
ggtgggtggatctggaggaggtgggag		
cgggggaggtggcagtgctagcgaca		
tcagatgacacagactacatcctcc		
ctgtctgcctctctgggagacagagt		
caccatcagttgcagggcaagtcagg		
acattcgcaattatttaaactggtat		
cagcagaaaccagatggaactgttaa		
actcctgatctactacacatcaagat		

TABLE 5-continued

Primary structures (polynucleotide and cognate amino acid sequences) of exemplary features of multivalent binding molecules.			
	tacactcaggagtcccatcaagggtc agtggcagtggtctggaacagatta ttctctcaccattgccaaactgcaac cagaagataattgccacttacttttgc caacagggtataacgttccgtggac gttcggtggaggcaccaaaactggtaa ccaaacgctcgag		
G19-4 LH	accggtgacatccagatgacacagac tacatcctccctgtctgcctctctgg gagacagagtcaccatcagttgcagg gcaagtcaggacattcgcaattattt aaactgggtatcagcagaaaccagatg gaactgttaaaactcctgatctactac acatcaagattacactcaggagtcctc atcaagggttcagtgaggcagtggtctg gaacagattattctctcaccattgcc aaactgcaaccagaagatattgccac ttacttttgccaacagggtataacgc ttccgtggacggttcggtggaggcacc aaactggtaacaaaacgggtggcgg tggctcgggcggtggtggatctggag gaggtgggagcgctagcgaggtccag ctgcaacagtcctggacctgaactggg gaagcctggagcttcaatgaagattt cctgcaaggcctctggttactcattc actggctacatcgtgaactggctgaa gcagagccatggaagaaccttgagt ggattggacttattaatccatacaaa ggtcttactacctacaaccagaaatt caaggccaaggccacattaactgtag acaagtcacccagcagcctacatg gagctcctcagtcgacatctgaaga ctctgcagtccttactgtgcaagat ctgggtactatgggtgactcggactgg tacttcgagtcctggggcgcaggggac cagggtcaccgtctcctcgag	DIQMTQTTSSLSASLGDRVTISCRASQ DIRNYLNWYQQKPDGTVKLLIYYTSRL HSGVPSRFSGSGSGTDYSLTIANLQPE DIATYFCQQGNTLPWTFGGGKLVTKR GGGSGGGSGGGSSASEVQLQQSGPE LVKPGASMKISCKASGYSTGYIVNWL KQSHGKNLEWIGLINPYKGLTTYNQKF KGKATLTVDKSSSTAYMELLSLTSEDS AVYYCARSGYGDSWDYFDVWGAGTTV TVSS	106 (107)
G19-4 HL	accggtgagggtccagctgcaacagtc tggaactgaactgggtgaagcctcgag cttcaatgaagatttctctgaaggcc tctggttactcattcactggctacat cgtgaactgggtgaagcagagccatg gaaagaaccttgagtggtggaactt attaatccatacaaaaggtcttactac ctacaaccagaaattcaagggaagg ccacattaaactgtagacaagtcattc agcacagcctacatggagctcctcag tctgacatctgaagactctgcagtc attactgtgcaagatctgggtactat ggtgactcggactgggtacttcgatgt ctggggcgcagggaaccaggtcacgc tctcctctggtggcggtggctcgggc ggtggtggatctggaggaggtgggag cgctagcgacatccagatgacacaga ctacatcctccctgtctgcctctctg ggagacagagtcaccatcagttgcag ggcaagtcaaggacattcgcaattatt taaaactggtatcagcagaaaccagat ggaactgttaaaactcctgatctacta cacatcaagattacactcaggagtc catcaagggttcagtggcagtggtct ggaacagattattCtctcaccattgc caacctgcaaccagaagatattgcca cttacttttgccaacagggttaatacg cttcctggagcgttcggtggaggcac caaaactggttaacaaaacgctcgag	EVQLQQSGPELVKPGASMKISCKASGY STGYIVNWLKQSHGKNLEWIGLINPY KGLTTYNQKFKGKATLTVDKSSSTAYM ELLSLTSEDSAVYYCARSGYGDSWDY FDVWGAGTTVTSSGGGSGGGSGGG GSASDIQMTQTTSSLSASLGDRVTISC RASQDIRNYLNWYQQKPDGTVKLLIYY TSRLHSGVPSRFSGSGSGTDYSLTIAN LQPEDIATYFCQQGNTLPWTFGGGKLV VTKRS	108 (109)
Hinge Region	Nucleotide Sequence	Amino Acid Sequence	SEQ ID NO. (amino acid sequence)
sss(s)-hIgG1	gagcccaaatcttctgacaaaactca cacatctccaccgagctca	EPKSSDKTHTSPPPS	230 (231)

TABLE 5-continued

Primary structures (polynucleotide and cognate amino acid sequences) of exemplary features of multivalent binding molecules.				
csc(s) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatctccaccgtgctca	EPKSCDKTHTSPPCS	232 (233)	
ssc(s) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatctccaccgtgctca	EPKSSDKTHTSPPCS	110 (111)	
scc(s) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatgtccaccgtgctca	EPKSSDKTHTCPPCS	112 (113)	
css(s) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatctccaccgagctca	EPKSCDKTHTSPSS	114 (115)	
scs(s) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatgtccaccgagctca	EPKSSDKTHTCPPSS	116 (117)	
ccc(s) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatgtccaccgtgctca	EPKSCDKTHTSPPCS	118 (119)	
ccc(p) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatgtccaccgtgccc	EPKSCDKTHTSPPCP	120 (121)	
sss(p) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatctccaccgagccc	EPKSSDKTHTSPSP	122 (123)	
csc(p) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatctccaccgtgccc	EPKSCDKTHTSPPCP	124 (125)	
ssc(p) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatctccaccgtgccc	EPKSSDKTHTSPPCP	126 (127)	
scc(p) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatgtccaccgtgccc	EPKSSDKTHTCPPCP	128 (129)	
css(p) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatctccaccgagccc	EPKSCDKTHTSPSP	130 (131)	
scs(p) - hIgG1	gagcccaaatcttctgtgacaaaactca cacatgtccaccgagccc	EPKSSDKTHTCPPSP	132 (133)	
scppcp	agttgtccaccgtgccc	SCPPCP	134 (135)	
EFD	Nucleotide Sequence	Amino Acid Sequence	Sequence Identifier (amino acid sequence)	
hIgG1 (P238S) C _{H2} C _{H3}	gcacctgaactcctgggtggatcgtc agtctctctcttcccccaaaaccca aggacaccctcatgatctcccgacc cctgaggtcacatgcgtgggtgga cgtgagccacgaagacctgaggtca agttcaactggtacgtggacggcgtg gaggtgcataaatgcaagacaaagcc gcgggaggagcagtagaacagcacgt accgtgtggtcagcgtcctcaccgtc ctgcaccaggactggctgaatggcaa ggagtacaagtgaagggtctccaaca aagccctcccagccccatcgagaaa acaatctccaaagccaaagggcagcc ccgagaaccacaggtgtacaccctgc ccccatcccgggatgagctgaccaag aaccaggtcagctgacctgacctggt caaaggcttctatcccagcgacatcg ccgtggagtgggagagcaatgggcag ccggagaacaactacaagaccagcc tcccgtgctggactccgacggctcct tcttctctacagcaagctcaccgtg gacaagagcaggtggcagcaggggaa cgtcttctcatgctccgtgatgcatg aggctctgcacaaccactacacgcag aagagcctctcctgtctccgggtaa atga	APELLGGSSVFLPPPKPDTLMIS RTP EVTGVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTI SK AKGQPREPQVYTLPPSRDLETKNQVSL TCLVKGFPYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSQVMHEALHNHYTQKSLSLSPG K	142 (143)	

TABLE 5-continued

Primary structures (polynucleotide and cognate amino acid sequences) of exemplary features of multivalent binding molecules.				
hIgG1 (P331S) C _{H2} C _{H3}	gcacctgaactcctgggtggaccgtc agtcttctcttccccccaaaaccca aggacaccctcatgatctcccgacc cctgagggtcacatgctggtggtgga cgtgagccacgaagaccctgaggtca agttcaactggtagctggacggcggtg gaggtgcataatgccaaagacaaaagcc gcgggaggagcagtagaacagcacgt accgtgtggtcagcgtcctcacgctc ctgcaccaggactggctgaatggcaa ggagtacaagtgaaggtctccaaca aagccctcccagcctccatcgagaaa acaatctccaaagccaaaggcgacc ccgagaaccacaggtgtacaccctgc ccccatccgggatgagctgaccaag aaccagggtcagcctgacctgctggt caaaaggcttctatcccagcgacatcg ccgtggagtgaggagcaatgggcag ccggagaacaaactacaagaccagcc tcccgtgctggactccgacggctcct tcttctctacagcaagctcacggtg gacaagagcaggtggcagcaggggaa cgtcttctcatgctccgtgatgcatg aggctctgcacaaccactacacgcag aagagcctctcctgtctccgggtaa atga	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDNLNGKEYCKVSNKALPASIEKTIKAKGQPREPQVYTLPPSRDLETKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHELAHNHYTQKSLSLSPG	144	(145)
hIgG1 (P238S/ P331S) C _{H2} C _{H3}	gcacctgaactcctgggtggatcgtc agtcttctcttccccccaaaaccca aggacaccctcatgatctcccgacc cctgagggtcacatgctggtggtgga cgtgagccacgaagaccctgaggtca agttcaactggtagctggacggcggtg gaggtgcataatgccaaagacaaaagcc gcgggaggagcagtagaacagcacgt accgtgtggtcagcgtcctcacgctc ctgcaccaggactggctgaatggcaa ggagtacaagtgaaggtctccaaca aagccctcccagcctccatcgagaaa accatctccaaagccaaaggcgacc ccgagaaccacaggtgtacaccctgc ccccatccgggatgagctgaccaag aaccagggtcagcctgacctgctggt caaaaggcttctatcccagcgacatcg ccgtggagtgaggagcaatgggcag ccggagaacaaactacaagaccagcc tcccgtgctggactccgacggctcct tcttctctacagcaagctcacggtg gacaagagcaggtggcagcaggggaa cgtcttctcatgctccgtgatgcatg aggctctgcacaaccactacacgcag aagagcctctcctgtctccgggtaa atga	APELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYADGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDNLNGKEYCKVSNKALPASIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG	146	(147)
Linker	Nucleotide Sequence	Amino Acid Sequence	Sequence Identifier	
STD1	aattatggtggcggtggctcgggcgg tggtggatctggaggaggtgggagtg ggaattct	NYGGGSGGGGSGGGGSGNS	148	(149)
STD2	aattatggtggcggtggctcgggcgg tggtggatctggaggaggtgggagtg ggaattatggtggcggtggctcgggc ggtggtggatctggaggaggtgggag tggaattct	NYGGGSGGGGSGGGGSGNYGGGSGG GGSGGGGSGNS	150	(151)
H1	aattct	NS	152	(153)
H2	ggtggcggtggctcggggaattct	GGGGSGNS	154	(155)
H3	aattatggtggcggtggctcgggaa ttct	NYGGGSGNS	156	(157)

TABLE 5-continued

Primary structures (polynucleotide and cognate amino acid sequences) of exemplary features of multivalent binding molecules.		
H4	ggtggcggtggtcgggcgggtggtgg GGGGSGGGSGNS atctgggaattct	158 (159)
H5	aattatggtggcggtggtcgggcgg NYGGGSGGGSGNS tggtgatctgggaattct	160 (161)
H6	ggtggcggtggtcgggcgggtggtgg GGGGSGGGSGGGSGNS atctggggaggaggcagcgggaatt ct	162 (163)
H7	gggtgtccaccttgtccgaattct GCPPCPNS	164 (165)
(G4S) 3	ggtggcggtgatccggcgagggtgg GGGSGGGSGGGS gtcgggtggcgcggtatct	166 (167)
(G4S) 4	ggtggcggtggtcgggcgggtggtgg GGGSGGGSGGGSGGGS atctggaggaggtgggagcgggggag gtggcagt	168 (169)

Example 8

Binding and Functional Studies with Alternative Multispecific Fusion Proteins

[0418] Experiments that parallel the experiments described above for the prototypical CD20-IgG-CD28 multispecific binding (fusion) molecule were conducted for each of the additional multivalent binding molecules described above. In general, the data obtained for these additional molecules parallel the results observed for the prototype molecule. Some of the salient results of these experiments are disclosed below. FIG. 14 shows results of blocking studies performed on one of the new molecules where both BD1 and BD2 bind to target antigens on the same cell or cell type, in this case, CD20 and CD37. This multispecific, multivalent binding (fusion) protein was designed with binding domain 1 binding CD20(2H7; VLVH orientation), and binding domain 2 binding CD37, G28-1 VL-VH (LH) or VH-VL (HL). The experiment was performed in order to demonstrate the multispecific properties of the protein.

[0419] Blocking Studies: Ramos or BJAB B lymphoblastoid cells (2.5×10^5) were pre-incubated in 96-well V-bottom plates in staining medium (PBS with 2% mouse sera) with murine anti-CD20 (25 $\mu\text{g/ml}$) antibody, or murine anti-CD37 (10 $\mu\text{g/ml}$) antibody, both together or staining medium alone for 45 minutes on ice in the dark. Blocking antibodies were pre-incubated with cells for 10 minutes at room temperature prior to addition of the multispecific binding (fusion) protein at the concentration ranges indicated, usually from 0.02 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$, and incubated for a further 45 minutes on ice in the dark. Cells were washed 2 times in staining medium, and incubated for one hour on ice with Caltag (Burlingame, Calif.) FITC goat anti-human IgG (1:100) in staining medium, to detect binding of the multispecific binding (fusion) proteins to the cells. The cells were then washed 2 times with PBS and fixed with 1% paraformaldehyde (cat. no. 19943, USB, Cleveland, Ohio). The cells were analyzed by flow cytometry using a FACsCalibur instrument and CellQuest software (BD Biosciences, San Jose, Calif.). Each data series plots the binding of the 2H7-ss-hIgG-STD1-G28-1 HL fusion protein in the presence of CD20, CD37, or both CD20 and CD37 blocking antibodies. Even though this experiment used one of the cleaved linkers, only the presence

of both blocking antibodies completely eliminates binding by the multispecific binding (fusion) protein, demonstrating that the bulk of the molecules possess binding function for both CD20 and CD37. The data were similar for two cell lines tested in panels A and B, Ramos and BJAB, where the CD20 blocking antibody was more effective than the CD37 blocking antibody at reducing the level of binding observed by the multispecific binding (fusion) protein.

ADCC Assays

[0420] FIG. 15 shows the results of ADCC assays performed on the CD20-CD37 multispecific binding (fusion) proteins. ADCC assays were performed using BJAB lymphoblastoid B cells as targets and human PBMC as effector cells. BJAB cells were labeled with 500 $\mu\text{Ci/ml}$ ^{51}Cr sodium chromate (250 $\mu\text{Ci}/\mu\text{g}$) for 2 hours at 37°C . in IMDM/10% FBS. The labeled cells were washed three times in RPMI. 10% FBS and resuspended at 4×10^5 cells/ml in RPMI. Heparinized, human whole blood was obtained from anonymous, in-house donors and PBMC isolated by fractionation over Lymphocyte Separation Media (LSM, ICN Biomedical) gradients. Buffy coats were harvested and washed twice in RPMI/10% FBS prior to resuspension in RPMI/10% FBS at a final concentration of 5×10^6 cells/ml. Cells were counted by trypan blue exclusion using a hemacytometer prior to use in subsequent assays. Reagent samples were added to RPMI medium with 10% FBS at 4 times the final concentration and three 10 fold serial dilutions for each reagent were prepared. These reagents were then added to 96-well U-bottom plates at 50 $\mu\text{l/well}$ for the indicated final concentrations. The ^{51}Cr -labeled BJAB cells were added to the plates at 50 $\mu\text{l/well}$ (2×10^4 cells/well). The PBMCs were then added to the plates at 100 $\mu\text{l/well}$ (5×10^5 cells/well) for a final ratio of 25:1 effector (PBMC):target (BJAB). Effectors and targets were added to medium alone to measure background killing. The ^{51}Cr -labeled cells were added to medium alone to measure spontaneous release of ^{51}Cr and to medium with 5% NP40 (cat. no. 28324, Pierce, Rockford, Ill.) to measure maximal release of ^{51}Cr . Reactions were set up in triplicate wells of a 96-well plate. Multispecific binding (fusion) proteins were added to wells at a final concentration ranging from 0.01 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$, as indicated on the graphs. Each data series plots a different multispecific binding (fusion) protein or the corre-

sponding single specificity SMIPs at the titration ranges described. Reactions were allowed to proceed for 6 hours at 37° C. in 5% CO₂ prior to harvesting and counting. Twenty-five μ l of the supernatant from each well were then transferred to a Luma Plate 96 (cat. no. 6006633, Perkin Elmer, Boston, Mass.) and dried overnight at room temperature. CPM released was measured on a Packard TopCounNXT. Percent specific killing was calculated by subtracting (cpm {mean of triplicate samples} of sample-cpm spontaneous release)/(cpm maximal release-cpm spontaneous release) $\times$ 100. Data are plotted as % specific killing versus protein concentration. The data demonstrate that the multispecific binding (fusion) protein is able to mediate ADCC activity against cells expressing the target antigen(s) as well as the single specificity SMIPs for CD20 and/or CD37, but does not show augmentation in the level of this effector function.

Co-Culture Experiments

[0421] FIG. 16 shows the results of experiments designed to look at other properties of this type of multispecific binding (fusion) protein, where having two binding domains against targets expressed on the same cell or cell type might result in synergistic effects by signaling/binding through the two surface receptors bound. The co-culture experiments were performed using PBMC isolated as described for the ADCC assays above. These PBMC were resuspended in culture medium at 2×10^6 cells/ml in a final volume of 500 μ l/well, and cultured alone or incubated with single specificity SMIPs for CD20, CD37, CD20+CD37, or the multispecific binding (fusion) protein using the H7 linker, [2H7-sss-IgG-H7-G28-1 HL]. Each of the test reagents was added at a final concentration of 20 μ g/ml. After 24 hours of culture, no real differences were seen in the % of B cells in culture; however, when the cells were subjected to flow cytometry, cell clumping was visible in the FWD X 90 staining pattern for the cultures containing the multispecific binding (fusion) protein, indicating that the B cells expressing the two target antigens were engaged in homotypic adhesion. After 72 hours in culture, the multispecific binding (fusion) protein resulted in the death of almost all the B cells present. The combination of the two single-specificity SMIPs also drastically decreased the percentage of B cells, but not to the level seen with the multispecific binding molecule. These data suggest that engaging both binding domains for CD20 and CD37 on the same multispecific molecule, results in homotypic adhesion between B cells and may also result in binding of both CD20 and CD37 antigens on the same cell. Without wishing to be bound by theory, the synergistic effect in eliminating target cells may be due (1) to the binding through binding domains 1 and 2 on the same cell types, and/or (2) to interactions of the effector function domain (constant sub-region) of the multivalent binding molecules with monocytes or other cell types in the PBMC culture that result in delayed killing. The kinetics of this killing effect are not rapid, taking more than 24 hours to be achieved, indicating that it is may be a secondary effect, requiring production of cytokines or other molecules prior to the effects being observed.

Apoptosis Assays

[0422] FIG. 17 shows the results of experiments designed to explore the induction of apoptosis after treatment of B cell lines with either the [2H7-sss-hIgG-H7-G28-1 HL] multispecific, multivalent binding (fusion) proteins or the single speci-

ficity CD20 and/or CD37 SMIPs, alone and in combination with one another. Ramos cells (panel A; ATCC No. CRL-1596), and Daudi cells (panel B; ATCC No. CCL-213) were incubated overnight (24 hours) at 37° C. in 5% CO₂ in Iscoves (Gibco) complete medium with 10% FBS at 3×10^5 cells/ml and 5, 10, or 20 μ g/ml fusion proteins. For combination experiments with the single specificity SMIPs, the proteins were used at the following concentrations: TRU-015 (CD20 directed SMIP)=10 μ g/ml, with 5 μ g/ml G28-1 LH (CD37 directed SMIP). Alternatively, TRU-015=20 μ g/ml was combined with G28-1 LH at 10 μ g/ml. Cells were then stained with Annexin V-FITC and propidium iodide using the BD Pharmingen Apoptosis Detection Kit I cat. no. 556547), and processed according to kit instructions. The cells were gently vortexed, incubated in the dark at room temperature for 15 minutes, and diluted in 400 μ l binding buffer prior to analysis. Samples were analyzed by flow cytometry on a FACsCalibur (Becton Dickinson) instrument using Cell Quest software (Becton Dickinson). The data are presented as columnar graphs plotting the percentage of Annexin V/propidium iodide positive cells versus type of treatment. Clearly, the multispecific binding (fusion) protein is able to induce a significantly higher level of apoptotic death in both cell lines than the single specificity reagents, even when used together. This increased functional activity reflects an interaction of the coordinate binding of BD1 and BD2 (specific for CD20 and CD37) receptors on the target cells.

Example 9

Binding and Functional Properties of 2H7-hIgG-G19-4 Multispecific Binding (Fusion) Proteins

[0423] This example describes the binding and functional properties of the 2H7-hIgG-G19-4 multispecific fusion proteins. The construction of these molecules is described in Example 7. Expression and purification are as described in previous Examples.

[0424] Binding experiments were performed as described for previous molecules, except that the target cells used to measure CD3 binding were Jurkat cells expressing CD3 on their surface. Refer to FIG. 18, where the top graph shows binding curves obtained for binding of the CD20-CD3 multispecific molecules to Jurkat cells using purified proteins serially diluted from 20 to 0.05 μ g/ml. The HL orientation of the G19-4 specificity seems to bind better to the CD3 antigen than does the LH orientation. The lower panel shows the binding curves obtained for the BD 1, the binding domain recognizing CD20. All of the molecules bind well, and at a level nearly equivalent to a single specificity SMIP for CD20.

ADCC Assays

[0425] For the data presented in FIG. 19, ADCC assays were performed as described in the previous Example. In this case, the fusion proteins were all 2H7-hIgG-G19-4 variants or combinations of the single-specificity SMIPs (2H7, specific for CD20) or antibodies (G 19-4, specific for CD3). In addition, for the data presented in the lower panel of FIG. 19, NK cells were depleted from PBMC prior to use, by magnetic bead depletion using a MACS (Miltenyi Biotec, Auburn, Calif.) column separation apparatus and NK cell-specific CD16 magnetic microbeads (cat no.: 130-045-701). The data presented in the two panels demonstrate that all of the CD20-

hIgG-CD3 multispecific molecules mediate ADCC, regardless of whether NK cells are depleted or total PBMC are used in the assay. For the TRU 015 or combinations of G19-4 and TRU015, only cultures containing NK cells could mediate ADCC. G19-4 did not work well in either assay against BJAB targets, which do not express CD3, although G19-4 may have bound to CD3 expressing NK T cells and activated these cells in the first assay shown. The killing observed in the lower panel for the multispecific binding (fusion) proteins is probably mediated through activation of cytotoxicity in the T cell population by binding CD3, against the BJAB targets expressing the CD20 antigen. This killing activity appears to be relatively insensitive to the dosage of the molecules over the concentration ranges used, and is still significantly different from the other molecules tested, even at a concentration of 0.01 ug/ml.

Example 10

Multivalent Binding Molecules

[0426] Other embodiments include linker domains derived from immunoglobulins. More specifically, the source sequences for these linkers are sequences obtained by comparing regions present between the V-like domains or the V- and C-like domains of other members of the immunoglobulin superfamily. Because these sequences are usually expressed as part of the extracellular domain of cell surface receptors, they are expected to be more stable to proteolytic cleavage, and should also not be immunogenic. One type of sequence that is not expected to be as useful in the role of a linker for the multivalent binding (fusion) proteins is the type of sequence expressed on surface-expressed members of the -Ig superfamily, but that occur in the intervening region between the C-like domain and the transmembrane domain. Many of these molecules have been observed in soluble form, and are cleaved in these intervening regions close to the cell membrane, indicating that the sequences are more susceptible to cleavage than the rest of the molecule.

[0427] The linkers described above are inserted into either a single specificity SMIP, between the binding domain and the effector function domain, or are inserted into one of the two possible linker positions in a multivalent binding (fusion) protein, as described herein.

[0428] A complete listing of the sequences disclosed in this application is appended, and is incorporated herein by reference in its entirety. The color coding indicating the sequence of various regions or domains of the particular polynucleotides and polypeptides are useful in identifying a corresponding region or domain in the sequence of any of the molecules disclosed herein.

Example 11

Screening Matrix for Scorpion Candidates Targeting B-Cells

[0429] Introduction

[0430] As a means of identifying combinations of paired monoclonal antibody binding domains that would most likely yield useful and potent multivalent binding molecules, or scorpions, against a target population, a series of monoclonal antibodies against B cell antigens was tested in a combination matrix against B cell lines representing various non Hodgkin's lymphomas. To ensure that all possible pairwise comparisons of antibodies known or expected to bind to the

cell of interest are assayed, a two-dimensional matrix of antibodies may be used to guide the design of studies using a given cell type. Monoclonal antibodies against numerous B cell antigens known by their cluster designations (CDs) are recorded in the left column. Some of these antibodies (designated by the antigen(s) to which they specifically bind), i.e., CD19, CD20, CD21, CD22, CD23, CD30, CD37, CD40, CD70, CD72, CD79a, CD79b, CD80, CD81, CD86, and CL II (MHC Class II), were incubated, alone or in combination with other members of this monoclonal antibody set, with antigen-positive target cells. The variable domains of these antibodies are contemplated as binding domains in exemplary embodiments of the multivalent binding molecules. Using the knowledge in the art and routine procedures, those of skill in the art are able to identify suitable antibody sequences (nucleic acid encoding sequences as well as amino acid sequences), for example in publicly available databases, to generate a suitable antibody or fragment thereof (e.g., by hybridization-based cloning, PCR, peptide synthesis, and the like), and to construct multivalent binding molecules using such compounds. Sources of exemplary antibodies from which binding domains were obtained as described herein are provided in Table 6. Typically, a cloning or synthesis strategy that realizes the CDR regions of an antibody chain will be used, although any antibody, fragment thereof, or derivative thereof that retains the capacity to specifically bind to a target antigen is contemplated.

[0431] Stated in more detail, the cloning of heavy and/or light chain variable regions of antibodies from hybridomas is standard in the art. There is no requirement that the sequence of the variable region of interest be known in order to obtain that region using conventional cloning techniques. See, e.g., Gilliland et al., *Tissue Antigens* 47(1):1-20 (1996). To prepare single-chain polypeptides comprising a variable region recognizing a murine or human leukocyte antigen, a method was devised for rapid cloning and expression that yielded functional protein within two to three weeks of RNA isolation from hybridoma cells. Variable regions were cloned by poly-G tailing the first-strand cDNA followed by anchor PCT with a forward poly-C anchor primer and a reverse primer specific for the constant region sequence. Both primers contain flanking restriction endonuclease sites for insertion into pUC19. Sets of PCR primers for isolation of murine, hamster and rat V_L and V_H genes were generated. Following determination of consensus sequences for a specific V_L and V_H pair, the V_L and V_H genes were linked by DNA encoding an intervening peptide linker (typically encoding $(Gly_4Ser)_3$) and the V_L -linker- V_H gene cassettes were transferred into the pCDM8 mammalian expression vector. The constructs were transfected into COS cells and sFvs were recovered from conditioned culture medium supernatant. This method has been successfully used to generate functional sFv to human CD2, CD3, CD4, CD8, CD28, CD40, CD45 and to murine CD3 and gp39, from hybridomas producing murine, rat, or hamster antibodies. Initially, the sFvs were expressed as fusion proteins with the hinge- C_{H2} - C_{H3} domains of human IgG 1 to facilitate rapid characterization and purification using goat anti-human IgG reagents or protein A. Active sFv could also be expressed with a small peptide, e.g., a tag, or in a tailless form. Expression of CD3 (G19-4)₂Fv tailless forms demonstrated increased cellular signaling activity and revealed that sFvs have potential for activating receptors.

[0432] Alternatively, identification of the primary amino acid sequence of the variable domains of monoclonal anti-

bodies can be achieved directly, e.g., by limited proteolysis of the antibody followed by N-terminal peptide sequencing using, e.g., the Edman degradation method or by fragmentation mass spectroscopy. N-terminal sequencing methods are well known in the art. Following determination of the primary amino acid sequence, the variable domains, a cDNA encoding this sequence is assembled by synthetic nucleic acid synthesis methods (e.g., PCR) followed by scFv generation. The necessary or preferred nucleic acid manipulation methods are standard in the art.

[0433] Fragments, derivatives and analogs of antibodies, as described above, are also contemplated as suitable binding domains. Further, any of the constant sub-regions described above are contemplated, including constant sub-regions comprising any of the above-described hinge regions. Additionally, the multivalent single-chain binding molecules described in this example may include any or all of the linkers described herein.

[0434] Monoclonal antibodies were initially exposed to cells and then cross-linked using a goat anti-mouse second-step antibody (2nd step). Optionally, one could cross-link the antibodies prior to contacting cells with the antibodies, e.g., by cross-linking the antibodies in solution. As another alternative, monoclonal antibodies could be cross-linked in a solid phase by adsorbing onto the plastic bottom of tissue culture wells or “trapped” on this plastic by means of goat anti-mouse antibody adsorbed to the plastic, followed by plate-based assays to evaluate, e.g., growth arrest or cell viability.

[0435] Inversion of phosphatidylserine from the cytosolic side of the cell membrane to the exterior cell surface of that plasma membrane is an accepted indicator of pro-apoptotic events. Progression to apoptosis leads to loss of cell membrane integrity, which can be detected by entry of a cell-impermeant intercalating dye, e.g., propidium iodide (PI). Following cell exposure to monoclonal antibodies alone or in combination, a dual, pro-apoptotic assay was performed and treated cell populations were scored for cell surface-positive annexin V (ANN) and/or PI inclusion.

Annexin V Binding/Propidium Iodide Internalization Analysis

[0436] Cells and cell culture conditions. Experiments were performed to examine the effect of cross-linking two different monoclonal antibodies against targets expressed on four human B-cell lines. Effects on cell lines were measured by determining levels of ANN and/or PI staining following exposure. The human B cell lines BJAB, Ramos (ATCC#CRL-1596), Daudi (ATCC#CCL-213), and DHL-4 (DSMZ#ACC495) were incubated for 24 hours at 37° C. in 5% CO₂ in Iscoves (Gibco) complete medium with 10% FBS. Cells were maintained at a density between 2–8×10⁵ cells/ml and a viability typically >95% prior to study.

[0437] Experiments were conducted at a cell density of 2×10⁵ cells/ml and 2 µg/ml of each comparative monoclonal antibody from a matrix against B-cell antigens. Each comparator monoclonal antibody was added at 2 µg/ml alone or individually when combined with each matrix monoclonal antibody, also at 2 µg/ml. Table 6 lists the catalog number and sources of monoclonal antibodies used in these experiments. For cross-linking these monoclonal antibodies in solution, goat anti-mouse IgG (Jackson Labs catalog no. 115-001-008) was added to each well at a concentration ratio of 2:1 (goat anti-mouse: each monoclonal antibody), e.g., a well with only one monoclonal antibody at 2 µg/ml would have goat anti-

mouse added to a final concentration of 4 µg/ml, while wells with both comparator monoclonal antibody (2 µg/ml) and a monoclonal antibody from the matrix (2 µg/ml) would have 8 µg/ml of goat anti-mouse antibody added to the well.

[0438] After 24 hours of incubation at 37° C. in 5% CO₂, cells were stained with Annexin V-FITC and propidium iodide using the BD Pharmingen Annexin V-FITC Apoptosis Detection Kit I (#556547). Briefly, cells were washed twice with cold PBS and resuspended in “binding buffer” at 1×10⁶ cells/ml. One hundred microliters of the cells in binding buffer were then stained with 5 µl of Annexin V-FITC and 5 µl of propidium iodide. The cells were gently mixed and incubated in the dark at room temperature for 15 minutes. Four hundred microliters of binding buffer were then added to each sample. The samples were then read on a FACsCalibur (Becton Dickinson) and analyzed using Cell Quest software (Becton Dickinson).

TABLE 6

Table 6. Antibodies against B cell antigens used in this study and their sources.

Name	Catalog number	Commercial supplier
Anti-CD19	#C2269-74	US Biological (Swampscott, MA)
Anti-CD20	#169-820	Ancell Corp (Bayport, MN)
Anti-CD21	#170-820	Ancell Corp (Bayport, MN)
Anti-CD22	#171-820	Ancell Corp (Bayport, MN)
Anti-CD23	#172-820	Ancell Corp (Bayport, MN)
Anti-CD30	#179-820	Ancell Corp (Bayport, MN)
Anti-CD37	#186-820	Ancell Corp (Bayport, MN)
Anti-CD40	#300-820	Ancell Corp (Bayport, MN)
Anti-CD70	#222-820	Ancell Corp (Bayport, MN)
Anti-CD72	#C2428-41B1	US Biological (Swampscott, MA)
Anti-CD79a	#235-820	Ancell Corp (Bayport, MN)
Anti-CD79b	#301-820	Ancell Corp (Bayport, MN)
Anti-CD80	#110-820	Ancell Corp (Bayport, MN)
Anti-CD81	#302-820	Ancell Corp (Bayport, MN)
Anti-CD86	#307-820	Ancell Corp (Bayport, MN)
Anti-CL II DR, DQ, DP	#131-820	Ancell Corp (Bayport, MN)

[0439] Addition of the cross-linking antibody (e.g., goat anti-mouse antibody) to monoclonal antibody A alone resulted in increased cell sensitivity, suggesting that a multivalent binding molecule, or scorpion, constructed with two binding domains recognizing the same antigen would be effective at increasing cell sensitivity. Without wishing to be bound by theory, this increased sensitivity could be due to antigen clustering and altered signaling. TNF receptor family members, for example, require homo-multimerization for signal transduction and scorpions with equivalent binding domains on each end of the molecule could facilitate this interaction. The clustering and subsequent signaling by CD40 is an example of this phenomenon in the B cell system.

[0440] As shown in FIGS. 20, 21 and 22, the addition of monoclonal antibody A and monoclonal antibody B against different antigens will produce additive or in some combinations greater than additive (i.e., synergistic) pro-apoptotic effects on treated cells. In FIG. 20, for example, the combination of anti-CD20 with monoclonal antibodies against other B cell antigens all resulted, to varying extents, in increased cell sensitivity. Some combinations, such as anti-CD20 combined with anti-CD19 or anti-CD20 combined with anti-CD21, however, produced greater than additive pro-apoptotic effects, indicating that multivalent binding molecules or scorpions composed of these binding domains

should be particularly effective at eliminating transformed B cells. Referring to FIG. 20, the percentage of cells exhibiting pro-apoptotic activities when exposed to anti-CD20 antibody alone is about 33% (vertically striped bar corresponding to “20,” i.e., the anti-CD20 antibody); the percentage of pro-apoptotic cells upon exposure to anti-CD19 antibody is about 12% (vertically striped bar in FIG. 20 corresponding to “19,” i.e., the anti-CD19 antibody); and the percentage of pro-apoptotic cells upon exposure to both anti-CD20 and anti-CD19 antibodies is about 73% (horizontally striped bar in FIG. 20 corresponding to “19”). The 73% of pro-apoptotic cells following exposure to both antibodies is significantly greater than the 45% (33%+12%) sum of the effects attributable to each individual antibody, indicating a synergistic effect attributable to the anti-CD19 and anti-CD20 antibody pair. Useful multivalent binding molecules include molecules in which the two binding domains lead to an additive effect on B-cell behavior as well as multivalent binding molecules in which the two binding domains lead to synergistic effects on B-cell behavior. In some embodiments, one binding domain will have no detectable effect on the measured parameter of cell behavior, with each of the paired binding domains contributing to distinct aspects of the activities of the multivalent binding molecule, such as a multispecific, multivalent binding molecule (e.g., binding domain A binds to a target cell and promotes apoptosis while binding domain B binds to a soluble therapeutic such as a cytotoxin). Depending on the design of a multivalent binding molecule, the issue of the type of combined effect (additive, synergistic, or inhibitory) of the two binding domains on a target cell may not be relevant because one of the binding domains is specific for a non-cellular (e.g., soluble) binding partner or is specific for a cell-associated binding partner, but on a different cell type. [0441] Exemplary binding domain pairings producing additive, synergistic or inhibitory effects, as shown in FIGS. 20-23, are apparent from Tables 7 and 8. Table 7 provides quantitative data extracted from each of FIGS. 20-23 in terms

of the percentage of cells staining positive for ANN and/or PI. Table 8 provides calculations using the data of Table 7 that provided a basis for determining whether the interaction of a given pair of antibodies yielded an additive, synergistic, or inhibitory effect, again as assessed by the percentage of cells staining positive for ANN and/or PI.

TABLE 7

Name	Anti-CD20	Anti-CD79b	Anti-CL II	Anti-CD22
Anti-CD19	13/73*	18/76/66	14/47/46	12/11
Anti-CD20	33/NA	42/94/92	33/71/76	28/33
Anti-CD21	14/75	22/50/76	18/24/40	11/11
Anti-CD22	8/55	12/39/33	12/19/17	10/12
Anti-CD23	8/41	12/63/55	14/22/17	10/12
Anti-CD30	8/38	14/72/61	12/56/61	10/11
Anti-CD37	15/45	19/92/86	20/60/62	19/20
Anti-CD40	10/48	12/44/30	13/21/28	14/13
Anti-CD70	9/40	12/56/39	15/21/15	10/10
Anti-CD72	NA	16/60/64	30/78/63	17/17
Anti-CD79a	21/66	43/42/50	28/55/51	14/14
Anti-CD79b	46/88	70/70/68	45/80/76	26/16/
Anti-CD80	7/41	14/35/30	15/19/17	11/11
Anti-CD81	14/65	25/86/83	25/54/43	19/20
Anti-CD86	7/38	16/58/42	15/24/18	14/11
Anti-CL II	53/77	52/96/98	47/52/43	72/57

*In columns 2-4 of Table 7, the numerical values reflect the heights of histogram bars in FIGS. 20-22, respectively, with the first number in each cell denoting the height of a vertically striped bar, the second number denoting the height of a horizontally striped bar and, where present, the third number reflecting the height of a stippled bar. In column 5, the first number reflects the height of a solid bars and the second number reflects the height of a slant-striped bar in FIG. 23.

TABLE 8

Name	Anti-CD20	Anti-CD79b	Anti-CL II	Anti-CD22
Anti-CD19	S: 13 + 33 = 46*	A: 18 + 56 = 74 A: 18 + 43 = 61	S: 14 + 26 = 40 S: 14 + 18 = 32	I: 12 + 10 = 22
Anti-CD20	NA	A: 42 + 56 = 98 A: 42 + 43 = 85	S: 33 + 26 = 59 S: 33 + 18 = 51	A/I: 28 + 10 = 38
Anti-CD21	S: 14 + 33 = 47	I: 22 + 56 = 78 S: 22 + 43 = 65	I: 18 + 26 = 44 A: 18 + 18 = 36	I: 11 + 10 = 21
Anti-CD22	S: 8 + 33 = 41	I: 12 + 56 = 68 I: 12 + 43 = 55	I: 12 + 26 = 38 I: 12 + 18 = 30	NA
Anti-CD23	A: 8 + 33 = 41	A: 12 + 56 = 68 A: 12 + 43 = 55	I: 14 + 26 = 40 I: 14 + 18 = 32	I: 10 + 10 = 20
Anti-CD30	A: 8 + 33 = 41	A: 14 + 56 = 70 A: 14 + 43 = 57	S: 12 + 26 = 38 S: 12 + 18 = 30	I: 10 + 10 = 20
Anti-CD37	A: 15 + 33 = 48	S: 19 + 56 = 75 S: 19 + 43 = 62	S: 20 + 26 = 46 S: 20 + 18 = 38	I: 19 + 10 = 29
Anti-CD40	A/S: 10 + 33 = 43	I: 12 + 56 = 68 I: 12 + 43 = 55	I: 13 + 26 = 39 A: 13 + 18 = 31	I: 14 + 10 = 24
Anti-CD70	A: 9 + 33 = 42	I: 12 + 56 = 68 I: 12 + 43 = 55	I: 15 + 26 = 41 I: 15 + 18 = 33	I: 10 + 10 = 20
Anti-CD72	NA	I: 16 + 56 = 72 A: 16 + 43 = 59	S: 30 + 26 = 56 S: 30 + 18 = 48	I: 17 + 10 = 27
Anti-CD79a	S: 21 + 33 = 54	I: 43 + 56 = 99 I: 43 + 43 = 86	A: 28 + 26 = 54 A: 28 + 18 = 46	I: 14 + 10 = 24
Anti-CD79b	S: 46 + 33 = 79	NA	S: 45 + 26 = 71 S: 45 + 18 = 63	I: 26 + 10 = 36
Anti-CD80	A: 7 + 33 = 40	I: 14 + 56 = 70 I: 14 + 43 = 57	I: 15 + 26 = 41 I: 15 + 18 = 33	I: 11 + 10 = 21
Anti-CD81	S: 14 + 33 = 47	A: 25 + 56 = 81 S: 25 + 43 = 68	A: 25 + 26 = 51 A: 25 + 18 = 43	I: 19 + 10 = 29

TABLE 8-continued

Name	Anti-CD20	Anti-CD79b	Anti-CL II	Anti-CD22
Anti-CD86	A: 7 + 33 = 40	I: 16 + 56 = 72 I: 16 + 43 = 59	I: 15 + 26 = 41 I: 15 + 18 = 33	I: 14 + 11 = 25
Anti-CL II	I: 53 + 33 = 86	A: 52 + 56 = 108 A: 52 + 43 = 95	NA	I: 72 + 10 = 82

"A" means an "additive" effect was observed

"S" means a "synergistic" effect was observed

"I" means an "inhibitory" effect was observed

*Equation schematic: $A + B = C$, where "A" is the percent ANN and/or PI positive cells due to matrix antibody alone, "B" is the percent ANN and/or PI positive cells due to the common antibody (anti-CD20 for FIG. 20, anti-CD79b for FIG. 21, anti-CLII for FIG. 22, and anti-CD22 for FIG. 23), and "C" is the expected additive effect. (See Table 7, above, for the quantitative data corresponding to FIGS. 20-23.) Where two equations are present in a cell, the upper equation reflects use of the higher indicated concentration of common antibody; the lower equation reflects use of the lower indicated concentration of common antibody.

[0442] In some embodiments, the two binding domains interact in an inhibitory, additive or synergistic manner in sensitizing (or de-sensitizing) a target cell such as a B cell. FIG. 23 shows the protective, or inhibitory, effects resulting from combining anti-CD22 antibody with strongly pro-apoptotic monoclonal antibodies such as the anti-CD79b antibody or anti-MHC class II (i.e., anti-CL II) antibody. For example, FIG. 23 and Table 7 show that anti-CD22 antibody alone induces no more than about 10% of cells to exhibit pro-apoptotic behavior (solid bar corresponding to "22" in FIG. 23) and anti-CD79b induces about 26% pro-apoptotic cells (solid bar corresponding to "CD79b" in FIG. 23). In combination, however, anti-CD22 and anti-CD79b induce only about 16% pro-apoptotic cells (slant-striped bar corresponding to "79b" in FIG. 23). Thus, the combined antibodies induce 16% pro-apoptotic cells, which is less than the 38% sum of the individual effects attributable to anti-CD22 (12%) and anti-CD79b (26%). Using this approach, an inspection of FIG. 23 and/or Tables 7-8 reveals that anti-CD22 antibody, and by extension a multispecific, multivalent binding molecule comprising an anti-CD22 binding domain, when used in separate combination with each of the following antibodies (or corresponding binding domains): anti-CD19, anti-CD20, anti-CD21, anti-CD23, anti-CD30, anti-CD37, anti-CD40, anti-CD70, anti-CD72, anti-CD79a, anti-CD79b, anti-CD80, anti-CD81, anti-CD86 and anti-MHC class II antibodies/binding domains, will result in an inhibited overall effect.

[0443] Without wishing to be bound by theory, the data can be interpreted as indicating that anti-CD22 antibody, or a multispecific, multivalent binding molecule comprising an anti-CD22 binding domain, will protect against, or mitigate an effect of, any of the antibodies listed immediately above. More generally, a multispecific, multivalent binding molecule comprising an anti-CD22 binding domain will inhibit the effect arising from interaction with any of CD19, CD20, CD21, CD23, CD30, CD37, CD40, CD70, CD72, CD79a, CD79b, CD80, CD81, CD86, and MHC class II molecules. It can be seen in FIG. 23 and Table 8 that anti-CD22 antibody, and by extension a binding domain comprising an anti-CD22 binding domain, will function as an inhibitor or mitigator of the activity of any antibody/binding domain recognizing a B-cell surface marker such as a CD antigen. Multivalent binding molecules, including multispecific, multivalent binding molecules, are expected to be useful in refining treatment regimens for a variety of diseases wherein the activity of a binding domain needs to be attenuated or controlled.

[0444] In addition to the inhibitory, additive or synergistic combined effect of two binding domains interacting with a target cell, typically through the binding of cell-surface

ligands, the experimental results disclosed herein establish that a given pair of binding domains may provide a different type of combined effect depending on the relative concentrations of the two binding domains, thereby increasing the versatility of the invention. For example, Table 8 discloses that anti-CD21 and anti-CD79b interact in an inhibitory manner at the higher tested concentration of anti-CD79b, but these two antibodies interact in a synergistic manner at the lower tested concentration of anti-CD79b. Although some embodiments will use a single type of multivalent binding molecule, i.e., a monospecific, multivalent binding molecule, comprising, e.g., a single CD21 binding domain and a single CD79b binding domain, the invention comprehends mixtures of multivalent binding molecules that will allow adjustments of relative binding domain concentrations to achieve a desired effect, such as an inhibitory, additive or synergistic effect. Moreover, the methods of the invention encompass use of a single multivalent binding molecule in combination with another binding molecule, such as a conventional antibody molecule, to adjust or optimize the relative concentrations of binding domains. Those of skill in the art will be able to determine useful relative concentrations of binding domains using standard techniques (e.g., by designing experimental matrices of two dilution series, one for each binding domain).

[0445] Without wishing to be bound by theory, it is recognized that the binding of one ligand may induce or modulate the surface appearance of a second ligand on the same cell type, or it may alter the surface context of the second ligand so as to alter its sensitivity to binding by a specific binding molecule such as an antibody or a multivalent binding molecule.

[0446] Although exemplified herein using B cell lines and antigens, these methods to determine optimally effective multivalent binding molecules (i.e., scorpions) are applicable to other disease settings and target cell populations, including other normal cells, their aberrant cell counterparts including chronically stimulated hematopoietic cells, carcinoma cells and infected cells.

[0447] Other signaling phenotypes such as Ca^{2+} mobilization; tyrosine phosphoregulation; caspase activation; NF- κ B activation; cytokine, growth factor or chemokine elaboration; or gene expression (e.g., in reporter systems) are also amenable to use in methods of screening for the direct effects of monoclonal antibody combinations.

[0448] As an alternative to using a secondary antibody to cross-link the primary antibodies and mimic the multivalent binding molecule or scorpion structure, other molecules that bind the Fc portion of antibodies, including soluble Fc receptors, protein A, complement components including C1q,

mannose binding lectin, beads or matrices containing reactive or cross-linking agents, bifunctional chemical cross-linking agents, and adsorption to plastic, could be used to cross-link multiple monoclonal antibodies against the same or different antigens.

Example 12

Multivalent Binding Protein with Effector Function, or Scorpion, Structures

[0449] The general schematic structure of a scorpion polypeptide is H2N-binding domain 1-scorpion linker-constant sub-region-binding domain 2. Scorpions may also have a hinge-like region, typically a peptide region derived from an antibody hinge, disposed C-terminal to binding domain 1. In some scorpion embodiments, binding domain 1 and binding domain 2 are each derived from an immunoglobulin binding domain, e.g., derived from a V_L and a V_H . The V_L and a V_H are typically joined by a linker. Experiments have been conducted to demonstrate that scorpion polypeptides may have binding domains that differ from an immunoglobulin binding domain, including an Ig binding domain from which the scorpion binding domain was derived, by amino acid sequence differences that result in a sequence divergence of typically less than 5%, and preferably less than 1%, relative to the source Ig binding domain.

[0450] Frequently, the sequence differences result in single amino acid changes, such as substitutions. A preferred location for such amino acid changes is in one or more regions of a scorpion binding domain that correspond, or exhibit at least 80% and preferably 85% or 90%, sequence identity to an Ig complementarity determining region (CDR) of an Ig binding domain from which the scorpion binding domain was derived. Further guidance is provided by comparing models of peptides binding the same target, such as CD20. Typically, alterations in a scorpion region corresponding to an Ig CDR will be screened for those scorpions exhibiting an increase in affinity for the target.

[0451] Glycosylated scorpions are also contemplated and, in this context, it is contemplated that host cells expressing a scorpion may be cultured in the presence of a carbohydrate modifier, which is defined herein as a small organic compound, preferably of molecular weight less than 1000 daltons, that inhibits the activity of an enzyme involved in the addition, removal, or modification of sugars that are part of a carbohydrate attached to a polypeptide, such as occurs during N-linked carbohydrate maturation of a protein. Glycosylation is a complex process that takes place in the endoplasmic reticulum ("core glycosylation") and in the Golgi bodies ("terminal glycosylation"). A variety of glycosidase and/or mannosidase inhibitors provide one or more of desired effects of increasing ADCC activity, increasing Fc receptor binding, and altering glycosylation pattern. Exemplary inhibitors include, but are not limited to, castanospermine and kifunensine. The effects of expressing scorpions in the presence of at least one such inhibitor are disclosed in the following example.

Example 13

Scorpion Protein Expression Levels and Characterization

[0452] Scorpion protein expression levels were determined and the expressed proteins were characterized to demonstrate

that the protein design led to products having practical benefits. A monospecific CD20×CD20 scorpion and a bispecific CD20×CD37 scorpion were expressed in CHO DG44 cells in culture using conventional techniques.

[0453] Basal level, stable expression of the CD20×CD20 scorpion S0129 (2Lm20-4x2Lm20-4) in CHO DG44 cells cultured in the presence of various feed supplements was observed as shown in FIG. 34. All culture media contained 50 nM methotrexate, a concentration that maintained copy number of the scorpion-encoding polynucleotide. The polynucleotide contained a coding region for the scorpion protein that was not codon-optimized for expression in CHO DG44 cells. The polynucleotide was introduced into cells using the pD18 vector. Apparent from FIG. 34, expression levels of about 7-46 µg/ml were obtained.

[0454] Expression levels following amplification of the polynucleotide encoding a bispecific CD20×CD37 scorpion were also determined. The pD18 vector was used to clone the CD20×CD37 scorpion coding region and the plasmid was introduced into CHO DG44 cells. Amplification of the encoding polynucleotide was achieved using the dhfr-methotrexate technique known in the art, where increasing concentrations of MTX are used to select for increased copy number of the Dihydrofolate Reductase gene (dhfr), which leads to co-amplification of the tightly linked polynucleotide of interest. FIG. 35 shows that stable expression levels of the bispecific CD20×CD37 scorpion were typically observed, and the expression levels at various methotrexate levels showed that the levels were not strictly a function of copy number. Variability in yield was seen under different conditions, including methotrexate concentration used for amplification, but these variables are amenable to optimization by those of skill in the art. A variety of other scorpion molecules described herein were also subjected to expression analyses in CHO and/or COS cells, with the results provided in Table 9, below. These results demonstrate that significant yields of scorpion proteins can be obtained using conventional techniques and routine optimization of the amplification technique.

[0455] Expressed proteins were also characterized by SDS-PAGE analysis to assess the degrees of homogeneity and integrity of the expressed proteins and to confirm molecular weight of monomeric peptides. The denaturing polyacrylamide gels (4-20% Tris Glycine) were run under reducing and non-reducing conditions. The results presented in FIG. 36 reveal single protein bands for each of a 2Lm20-4 SCC SMIP and S1000 (CD20(2Lm20-4)×CD20(2Lm20-4) monospecific scorpion. S0126) of the expected monomeric molecular weights under reducing conditions. These data establish that SMIPs and scorpions are amenable to purification in an intact form. Under non-reducing conditions, a trace amount of a peptide consistent with the expected size of a monomeric SMIP was seen, with the vast majority of the protein appearing in a single well-defined band consistent with a dimeric structure. Under these non-reducing conditions, the monospecific scorpion protein showed a single well-defined band of a molecular weight consistent with a dimeric structure. The dimeric structures for both the SMIP and the scorpion are consistent with their monomeric structures, each of which contains a hinge-like scorpion linker containing at least one Cysteine capable of participating in disulfide bond formation.

[0456] The effect of scorpion linkers on the expression and integrity of scorpions was also assessed, and results are shown in Table 10. This table lists scorpion linker variants of the monospecific CD20×CD20(2Lm20-4x2Lm20-4) S0129

scorpion and the CD20×CD28 S0033 scorpion (2H7scpIgG1-H7-2e12), their integrity as single chain molecules, and their transient expression levels in COS cells relative to the parent scorpion S0129 or S0033, as appropriate, with an H7 linker (set as 100%). Table 11 provides data resulting from an evaluation of scorpion linker variants incorporated into the CD20×CD20 scorpion, along with analogous data for the CD20×CD28 scorpion. Table 13 provides data resulting from an evaluation of S0129 variants containing scorpion linkers that are not hinge-like linkers containing at least one Cysteine capable of disulfide bond formation; rather, the scorpion linkers in these molecules are derived from C-type lectin stalk regions of Type II membrane proteins. Apparent from the data presented in Table 13 is that

hinge-like scorpion linkers may be associated with scorpions expressed at higher or lower levels than an unmodified parent scorpion linker in transient expression assays. Further, some of the linker variants exhibit greater resistance to proteolytic cleavage than the unmodified parent linker, a concern for all or almost all expressed proteins. The data of Table 11 show that non-hinge-like linkers such as linkers derived from the stalk region of C-type lectins are found in scorpions that exhibit binding characteristics that vary slightly from scorpions containing hinge-like scorpion linkers. Additionally, the scorpion containing a non-hinge-like scorpion linker exhibits effector function (ADCC) that either equals or exceeds the ADCC associated with scorpions having hinge-like scorpion linkers.

TABLE 9

Linker Name	Upstream (CH3) Sequence	S0129 (2Lm20-4 × 2Lm0-4) linker variants-aa seq ¹	based on	#AAs	Expression COS ²	Cleavage ³	Expression CHO ²
H7	QKSLSLSPGK	GCPPCPNS	H7	18	100	—	100
H16	QKSLSLSPGK	LSVKADFLTPSIGN	CD80	25	174	+	
H18	QKSLSLSPGK	LSVLNFSQPEIGN	CD86	25	165	++	
H19	QKSLSLSPGK	LSVLNFSQPEISCPPSPNS	CD86 + H7	30	161	+	109
H26	QKSLSLSPGK	RIHQMNSELSVLANS	CD86	25	170	++	
H32	QKSLSLSPGK	RIHLNVSERPFPNS	CD22	25	184	++	
H47	QKSLSLSPG	LSVKADFLTPSIGN	H16	24	141	—	206
H48	QKSLSLSPG	KADFLTPSIGN	H16	21	137	—	
H50	Q	LSVLNFSQPEIGN	H18	16	21	—	
H51	QKS	LSVLNFSQPEIGN	H18	18	110	—	
H52	QKSLSLSPG	SQPEIVPISNS	H18	20	95	—	
H53	QKSLSL	SQPEIVPISCPPCPNS	H19	26	95	—	
H54	Q	SVLNFSQPEISCPPCPNS	H19	21	72	+/-	
H55	QKSLSLSPG	RIHQMNSELSVLANS	H26	24	118	+	
H56	QKSLSLSPG	QMNSELSVLANS	H26	21	130	—	163
H57	QKSLSLSPG	VSERPFPNS	H32	19	118	—	
H58	QKSLSLSPG	KPFFTCGSADTCPNS	CD72	24	103	—	
H59	QKSL	KPFFTCGSADTCPNS	CD72	20	94	—	

¹NFS is a glycosylation consensus motif

²Transient expression in COS (6 W plates), or CHO (single flask) relative to S0129-H7 (%)

³Cleavage product(s) observed by SDS-PAGE silver stain: — = none; + = faint band, ++ = major band(s), +++ >50% cleaved

TABLE 10

Linker Name	Linker S0129 (2Lm20-4 × 2Lm20-4) linker variants-aa seq	Changes in CH3?	Linker seq. based on	20 × 20 Expression ²	20 × 20 Cleavage ³
H7	GCPPCPNS	N	H7	100	—
H8	GSPPSPNS	N	H7	107	+
H9	GSPPSPNS	Y	H7	142	—
H10	EPKSTDKTHTCPNS	N	IgG1 hinge	98	—

TABLE 10-continued

Linker S0129 (2Lm20-4 × 2Lm20-4) Name	Linker variants-aa seq	Changes in CH3? ¹	Linker seq. based on	20 × 20 Expression ²	20 × 20 Cleavage? ³
H11	EPKSTDKTHTSPSPNS	N	IgG1 hinge	126	+
H16	LSVKADFLTPSIGNS	N	CD80	174	+
H17	LSVKADFLTPSISCPNPCNS	N	CD80 + H7	113	+
H18	LSVLANFSQPEIGNS	N	CD86	165	++
H19	LSVLANFSQPEISCPNPCNS	N	CD86 + H7	161	+
H20	LKIQERVSKPKISNS	N	CD2	115	+++
H21	LKIQERVSKPKISCPNPCNS	N	CD2 + H7	90	+++
H22	LVNVSERPPPHIQNS	N	CD22	149	++
H23	LDVVSERPPPHIQSCPPNPCNS	N	CD22 + H7	121	++
H24	REQLAEVTLNLSKANS	N	CD80	145	++
H25	REQLAEVTLNLSKACPPNPCNS	N	CD80 + H7	98	+
H26	RIHQMNSELSVLANS	N	CD86	170	++
H27	RIHQMNSELSVLACPPNPCNS	N	CD86 + H7	154	++
H28	DTKGKNVLEKIFSNS	N	CD2	153	+
H30	LPPETQESQEVTLNS	N	CD22	78	+
H32	RIHLNVSERPPFPNS	N	CD22	184	++
H33	RIHLNVSERPPFPPCPNPCNS	N	CD22 + H7	74	+
H36	GCPPCPGGGSNS	N	H7	110	+
H40	GCPPCPANS	Y	H7	110	+
H41	GCPPCPANS	Y	H7	102	-
H42	GCPPCPNS	Y	H7	99	-
H44	GGGASCPPCPGNS	Y	H7	108	+
H45	GGGASCPPCAGNS	Y	H7	107	-
H46	GGGASCPPCANS	Y	H7	98	-
H47	LSVKADFLTPSIGNS	Y	CD80	141	-
H48	ADFLTPSIGNS	N	CD80	137	-
H50	LSVLANFSQPEIGNS	Y	CD86	21	-
H51	LSVLANFSQPEIGNS	Y	CD86	110	-
H52	SQPEIVPISNS	Y	CD86	95	-
H53	SQPEIVPISCPNPCNS	Y	CD86 + H7	95	-
H54	SVLANFSQPEISCPNPCNS	Y	CD86 + H7	72	+/-
H55	RIHQMNSELSVLANS	Y	CD86	118	+
H56	QMNSELSVLANS	Y	CD86	130	-
H57	VSERPPFPNS	Y	CD22	118	-
H58	KPFFTCGSADTCPNS	Y	CD72	103	-
H59	KPFFTCGSADTCPNS	Y	CD72	94	-

TABLE 10-continued

Linker Name	Linker S0129 (2Lm20-4 × 2Lm20-4) variants-aa seq	Changes in CH3? ¹	Linker seq. based on	20 × 20 Expression ²	20 × 20 Cleavage? ³
H60	QYNCPGQYTFSPNS	Y	CD69	>100 ⁵	-
H61	EPAFTPGPNIELQKSDCPNS	Y	CD94	>100	-
H62	QRHNNSSLNTRTQKARHCPNS	Y	NKG2A	>100	-
H63	NSLFNQEVQIPLTESYCPNS	Y	NKG2D	>100	-

¹Additional changes to the end of CH3 such as 1-9 aa deletion and/or codon optimization²Transient expression in COS (6 W plates), relative to S0129-H7 parent (%)³Cleavage products(s) observed by SDS-PAGE/silver stain:

- = none, + = faint band, ++ = major band(s), +++ >50% cleaved

⁵H60-H63 variants compared by estimation of recovery of protein purified from COS spent media.

TABLE 11

Proteins	Description	Production Yield (ug protein purified/ml sup)	% POI (M. wt in Kd by MALS)	Improvement over S0129 wt POI	Binding to Ramos	ADCC assay	Sequence of scorpion linker
S0129 wt	H7 linker	1.6	67 (167)	—	—	—	GCPPS
S0129-CD69	CD69 stalk	2.9	66 (167)	1.8	Weaker than S0129 wt	*Slightly better than S0129 wt POI	QYNCPGQYTFSM
S0129-CD72	CD72 truncated stalk	2.0	69 (165)	1.2	Similar to S0129 wt	*Slightly better than S0129 wt POI	PFPTCGSADTC
S0129-CD94	CD94 stalk	2.9	67 (171)	1.8	Similar to S0129 wt	*Slightly better than S0129 wt POI	EPAFTPGPNIELQKSDC
S0129-NKG2A	NKG2A stalk	2.5	93 (170)	2.2	Slightly better than S0129 wt	Similar to S0129 wt POI	QRHNNSSLNTRTQKARHC
S0129-NKG2D	NKG2D stalk	1.9	70 (166)	1.2	Similar to S0129 wt	*Slightly better than S0129 wt POI	NSLFNQEVQIPLTESYC

[0457] As noted in the preceding example, production by expression of scorpions in cultures containing a carbohydrate modifier is contemplated. In exemplary embodiments, castanospermine (MW 189.21) is added to the culture medium to a final concentration of about 200 μ M (corresponding to about 37.8 μ g/mL), or concentration ranges greater than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 μ M, and up to about 300, 275, 250, 225, 200, 175, 150, 125, 100, 75, 60, or 50 μ g/mL. For example, ranges of 10-50, or 50-200, or 50-300, or 100-300, or 150-250 μ M are contemplated. In other exemplary embodiments, DMJ, for example DMJ-HCl (MW 199.6) is added to the culture medium to a final concentration of about 200 μ M (corresponding to about 32.6 μ g DMJ/mL), or concentration ranges greater than about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 μ M, and up to about 300, 275, 250, 225, 200, 175, 150, 125, 100, 75, 60, or 50 μ g/mL. For example, ranges of 10-50, or 50-200, or 50-300, or 100-300, or 150-250 μ M are contemplated. In other exemplary

embodiments, kifunensine (MW 232.2) is added to the culture medium to a final concentration of about 10 μ M (corresponding to about 2.3 μ g/mL), or concentration ranges greater than about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 μ M, and up to about 50, 45, 40, 35, 30, 25, 20, 19, 18; 17, 16, 15, 14, 13, 12, or 11 μ M. For example, ranges of 1-10, or 1-25, or 1-50, or 5-10, or 5-25, or 5-15 μ M are contemplated.

[0458] In one experiment, a monospecific CD20 $\times$ CD20 scorpion (S0129) was expressed in cells cultured in 200 μ M castanospermine (S0129 CS200) or 10 μ M (excess) kifunensine (S0129 KF 10) and the binding, or staining, of WIL2S cells by the expressed scorpion was measured, as shown in FIG. 42. In comparative binding studies, moreover, a glycosylated S0129 scorpion bound CD16 (FC γ RIII) approximately three times better than the unglycosylated S0129 scorpion.

[0459] In another study, the ADCC-mediated killing of BJAB B-cells by humanized CD20 $\times$ CD20 scorpion (S0129) was explored. The results shown in FIG. 43 establish that the

scorpion, when expressed in cells being cultured in the presence of either castanospermine or kifunensine, led to significantly more potent ADCC-mediated BJAB B-cell death for a given concentration of scorpion exposure.

Example 14

Scorpion Binding

[0460] a. Domain Spacing

[0461] Bispecific scorpions are capable of binding at least two targets simultaneously, utilizing the pairs of binding domains at the N- and C-terminus of the molecule. In so doing, for cell-surface targets, the composition can cross-link or cause the physical co-approximation of the targets. It will be appreciated by those skilled in the art that many receptor systems are activated upon such cross-linking, resulting in signal induction causing changes in cellular phenotype. The design of the compositions disclosed herein was intended, in part, to maximize such signaling and to control the resultant phenotype.

[0462] Approximate dimensions of domains of the scorpion compositions, as well as expectations of interdomain flexibility in terms of ranges of interdomain angles, are known and were considered in designing the scorpion architecture. For scorpions using scFv binding domains for binding domains 1 and 2 (BD 1 and BD2), an IgG 1 N-terminal hinge domain, and the H7 scorpion linker described herein, the binding domain at the N-terminus and the binding domain at the C-terminus may be maximally about 150-180 Å apart and minimally about 20-30 Å apart. Binding domains at the N-terminus may be maximally about 90-100 Å apart and minimally about 10-20 Å apart (Deisenhofer, et al., 1976, Hoppe-Seyler's Z. Physiol. Chem. Bd. 357, S. 435-445; Gregory, et al., 1987, Mol. Immunol. 24(8):821-9.; Poljak, et al., 1973, Proc. Natl. Acad. Sci., 1973, 70: 3305-3310; Bongini, et al., 2004, Proc. Natl. Acad. Sci. 101: 6466-6471; Kienberger, et al., 2004, EMBO Reports, 5: 579-583, each incorporated herein by reference). The choice of these dimensions was done in part to allow for receptor-receptor distances of less than about 50 Å in receptor complexes bound by the scorpion as distances less than this may be optimal for maximal signaling of certain receptor oligomers (Paar, et al., 2002, J. Immunol., 169: 856-864, incorporated herein by reference) while allowing for the incorporation of F_c structures required for effector function.

[0463] The binding domains at the N- and C-terminus of scorpions were designed to be flexible structures to facilitate target binding and to allow for a range of geometries of the bound targets. It will also be appreciated by those skilled in the art that flexibility between the N- or C-terminal binding domains (BD1 and BD2, respectively) and between the binding domains and the Fc domain of the molecule, as well as the maximal and minimal distances between receptors bound by BD 1 and/or BD2, can be modified, for example by choice of N-terminal hinge domain (connecting BD1 and the constant

sub-region) and, by structural analogy, the more C-terminally located scorpion linker domain. For example hinge domains from IgG 1, IgG2, IgG3, IgG4, IgE, IgA2, synthetic hinges and the hinge-like C_{H2} domain of IgM show different degrees of flexibility, as well as different lengths. Those skilled in the art will understand that the optimal choice of N-terminal hinge region and scorpion linker will depend upon the receptor system(s) the scorpion is designed to interact with as well as the desired signaling phenotype induced by scorpion binding.

[0464] Exemplifying the influence of the scorpion linker on scorpion stability is a study done using two scorpions, a bispecific CD20×CD28 scorpion and a monospecific CD20×CD20 scorpion. For each of these two scorpion designs, a variety of scorpion linkers were inserted. In particular, scorpion linkers H16 and H17, which primarily differ in that H17 has the sequence of H16 with the sequence of H7 appended at the C-terminus, and scorpion linkers H18 and 19, in which, analogously, the sequence of H7 is appended at the C-terminus of H18 in generating H19, were used. For each of the two scorpion backbones (20×28 and 20×20), each of the four above-described scorpion linkers were inserted at the appropriate location. Transient expression of these constructs was obtained in COS cells and the scorpion proteins found in the culture supernatants were purified on protein A/G-coated wells (Pierce SEIZE IP kit). Purified proteins were fractionated on SDS-PAGE gels and visualized by silver stain. Inspection of FIG. 44 reveals that the additional H7 sequence in the scorpion linker adds to the stability of each type of scorpion linker and each type of scorpion protein. In other words, appending H7 to the C-terminus of either H16 or H18 added to the stability of the scorpion molecule, and this observation held regardless of whether the scorpion was CD20×CD28 or CD20×CD20. In terms of target binding, the scorpion proteins having the CD20×CD20 architecture exhibited similar binding properties to the parent monospecific humanized CD20×CD20 scorpion S0129, as shown in FIG. 45.

[0465] Beyond the preceding embodiments, however, it may be desirable to prevent bound receptors from approaching within about 50 Å of each other to intentionally create submaximal signals (Paar, et al., J. Immunol., 169: 856-864). In such a case, choices of N-terminal hinge region and scorpion linker that are shorter and less flexible than those described above would be expected to be appropriate.

[0466] The same spacing considerations apply to scorpion linkers that are not hinge-like. These scorpion linkers are exemplified by the class of peptides having the amino acid sequence of a stalk region of a C-type lectin of a Type II membrane protein. Exemplary scorpion hinges comprising a C-type lectin stalk region are scorpion hinges derived from the CD72 stalk region, the CD94 stalk region, and the NKG2A stalk region. Scorpions containing such scorpion linkers were constructed and characterized in terms of expression, susceptibility to cleavage, and amenability to purification. The data are presented in Table 12.

TABLE 12

G ₄ S Codon optimization ¹	End of S0129 CH3	scorpion linkers amino acid seq	Linker seq. based on	Expression (% S0129) ²	Cleavage ³	Bench-top purification on % POI
N (17)	K	GCPPCPNS (SEQ ID NO: 165)	H7	100	—	70

TABLE 12-continued

G ₄ S Codon optimization ¹	End of S0129 linker	scorpion amino acid seq	Linker seq. based on	Expression (% S0129) ²	Cleavage ³	Bench-top purification on % POI
Y(17)	K	GCPPCPNS (SEQ ID NO: 165)	H7	114	—	ND
Y(15)	K	GCPPCPNS (SEQ ID NO: 165)	H7	90	—	66
N(17)	G	QRHNSSLNTRTQKARHCPNS (SEQ ID NO: 325)	NKG2A stalk	129	—	89
Y(17)	G	QRHNSSLNTRTQKARHCPNS (SEQ ID NO: 325)	NKG2A stalk	100	—	85
Y(15)	G	QRHNSSLNTRTQKARHCPNS (SEQ ID NO: 325)	NKG2A stalk	81	—	83
N(17)	G	EPAFTPGPNIELQKSDCPNS (SEQ ID NO: 324)	CD94 stalk	133	—	66
Y(17)	G	EPAFTPGPNIELQKSDCPNS (SEQ ID NO: 324)	CD94 stalk	200	—	64
Y(15)	G	EPAFTPGPNIELQKSDCPNS (SEQ ID NO: 324)	CD94 stalk	129	—	65
N(17)	G	RTRYLQVSQQLQQTNRVLEVT NSSLRQQLRLKITQLGQSAED LQGSRELAQSQEALQVEQRA HQAAEGQLQACQADRQKTKE TLQSEEQRRALQKLSNMEN RLKPFPTCGSADTCCPNS (SEQ ID NO: 331)	CD72 full stalk	110	—	75

¹Codon optimization of Gly₄Ser linker, with (17) or without (15) restriction site

²Estimate of expression in COS based on recovery of protein in benchtop purification

³Cleavage product(s) observed by SDS-PAGE/Coomassie Blue stain of purified protein

[0467] b. Binding of N- and C-Terminal Binding Domains

Both N- and C-Terminal Domains Participate in Target Cell Binding

[0468] The target cell binding abilities of a CD20 SMIP (TRU015), a CD37 SMIP (SMIP016), a combination of CD20 and CD37 SMIPs (TRU015+SMIP016), and the CD20×CD37 bispecific scorpion (015×016), were assessed by measuring the capacity of each of these molecules to block the binding of an antibody specifically competing for binding to the relevant target, either CD37 or CD20. The competing antibodies were FITC-labeled monoclonal anti-CD37 antibody or PE-labeled monoclonal anti-CD20 antibody, as appropriate. Ramos B-cells provided the targets.

[0469] Ramos B-cells at 1.2×10^7 /ml in PBS with 5% mouse sera (#100-113, Gemini Bio-Products, West Sacramento, Calif.) (staining media) were added to 96-well V-bottom plates (25 μ l/well). The various SMIPs and scorpions were diluted to 75 μ g/ml in staining media and 4-fold dilutions were performed to the concentrations indicated in FIG. 38. The diluted compounds were added to the plated cells in addition to media alone for control wells. The cells were incubated for 10 minutes with the compounds and then FITC anti-CD37 antibody (#186-040, Ancell, Bayport, Minn.) at 5 μ g/ml and PE anti-CD20 antibody (#555623, BD Pharmingen, San Jose, Calif.) at 3 μ g/ml (neet) were added together to the wells in 25 μ l staining media. The cells were incubated on ice in the dark for 45 minutes and then washed 2.5 times with

PBS. Cells were fixed with 1% paraformaldehyde (#19943 1 LT, USB Corp, Cleveland, Ohio) and then run on a FACs Calibur (BD Biosciences, San Jose, Calif.). The data were analyzed with Cell Quest software (BD Biosciences, San Jose, Calif.). The results shown in FIG. 38 establish that all SMIPs, SMIP combinations and scorpions containing a CD20 binding site successfully competed with PE-labeled anti-CD 20 antibody for binding to Ramos B-cells (upper panel); all SMIPs, SMIP combinations and scorpions containing a CD37 binding site successfully competed with FITC-labeled anti-CD37 antibody for binding to Ramos B-cells (lower panel). The bispecific CD20×CD37 scorpion, therefore, was shown to have operable N- and C-terminal binding sites for targets on B-cells.

[0470] c. Cell-Surface Persistence

[0471] An investigation of the cell-surface persistence of bound SMIPs and scorpions (monospecific and bispecific) on the surface of B-cells revealed that scorpions exhibited greater cell-surface persistence than SMIPs. Ramos B-cells at 6×10^6 /ml (3×10^5 /well) in staining media (2.5% goat sera, 2.5% mouse sera in PBS) were added to 96-well V-bottom plates. Test reagents were prepared at two-fold the final concentration in staining media by making a 5-fold serial dilution of a 500 nM initial stock and then were added 1:1 to the Ramos B-cells. In addition, media controls were also plated. The cells were incubated in the dark, on ice, for 45 minutes. The plates were then washed 3.5 times with cold PBS. The secondary reagent, FITC goat anti-human IgG (#H10501,

Caltag/Invitrogen, Carlsbad, Calif.) was then added at a 1:100 dilution in staining media. The cells were incubated for 30 minutes in the dark, on ice. Cells were then washed 2.5 times by centrifugation with cold PBS, fixed with a 1% paraformaldehyde solution (#19943 1 LT, USB Corp, Cleveland, Ohio) and then run on a FACs Calibur (BD Biosciences, San Jose, Calif.). The data were analyzed with CellQuest software (BD Biosciences, San Jose, Calif.). Results of the data analysis are presented in FIG. 37, which shows the binding of several SMIPs, a monospecific CD20×CD20 scorpion and a bispecific CD20×CD37 scorpion to their targets on Ramos B cells.

[0472] Two tubes of Ramos B-cells (7×10^5 /ml) were incubated for 30 minutes on ice with each of the two compounds being investigated, i.e., a humanized CD20(2Lm20-4) SMIP and a humanized CD20×CD20(2Lm20-4x2Lm20-4) scorpion, each at 25 µg/ml in Iscoves media with 10% FBS. At the end of the incubation period, both tubes were washed 3 times by centrifugation. One tube of cells was then plated into 96-well flat-bottom plates at 2×10^5 cells/well in 150 µl of Iscoves media with one plate then going into the 37° C. incubator and the other plate incubated on ice. The second tube of each set was resuspended in cold PBS with 2% mouse serum and 1% sodium azide (staining media) and plated into a 96-well V-bottom plate at 2×10^5 cells/well for immediate staining with the secondary antibody, i.e., FITC goat anti-human IgG (#H10501, Caltag/Invitrogen, Carlsbad, Calif.). The secondary antibody was added at a 1:100 final dilution in staining media and the cells were stained on ice, in the dark, for 30 minutes. Cells were then washed 2.5 times with cold PBS, and fixed with 1% paraformaldehyde (#19943 1 LT, USB Corp, Cleveland, Ohio).

[0473] At the time points designated in FIG. 39, samples were harvested from the 96-well flat-bottom plates, incubated at either 37° C. or on ice, and placed into 96-well V-bottom plates (2×10^5 cells/well). The cells were washed once with cold staining media, resuspended, and the secondary antibody was added at a final dilution of 1:100 in staining media. These cells were incubated on ice, in the dark, for 30 minutes. The cells were then washed 2.5 times by centrifugation in cold PBS, and subsequently fixed with 1% paraformaldehyde. The samples were run on a FACS Calibur (BD Biosciences, San Jose, Calif.) and the data was analyzed with CellQuest software (BD Biosciences, San Jose, Calif.). Results presented in FIG. 39 demonstrate that the binding of a SMIP and a scorpion to the surface of B-cells persists for at least six hours, with the monospecific hu CD20×CD20 (2Lm20-4x2Lm20-4) scorpion persisting to a greater extent than the hu CD20(2Lm20-4) SMIP.

Example 15

Direct Cell Killing by Monospecific and Bispecific Scorpions

[0474] Experiments were conducted to assess the capacity of monospecific and bispecific scorpion molecules to directly kill lymphoma cells, i.e., to kill these cells without involvement of ADCC or CDC. In particular, the Su-DHL-6 and DoHH2 lymphoma cell lines were separately subjected to a monospecific scorpion, i.e., a CD20×CD20 scorpion or a CD37×CD37 scorpion, or to a bispecific CD20×CD37 scorpion.

[0475] Cultures of Su-DHL-6, DoHH2, Rec-1, and WSU-NHL lymphoma cells were established using conventional techniques and some of these cultures were then individually

exposed to a monospecific CD20 SMIP, a monospecific scorpion (CD20×CD20 or CD37×CD37), or a bispecific scorpion (CD20×CD37 or CD19×CD37). The exposure of cells to SMIPs or scorpions was conducted under conditions that did not result in cross-linking. The cells remained in contact with the molecules for 96 hours, after which growth was measured by detection of ATP, as would be known in the art. The cell killing attributable to the CD20 SMIP and the CD20×CD20 monospecific scorpion are apparent in FIG. 24 and Table 13. The cell killing capacity of the CD37×CD37 monospecific scorpion is apparent from FIG. 25 and Table 13, the ability of the CD20×CD37 bispecific scorpion to kill lymphoma cells is apparent from FIG. 26 and Table 13, and the capacity of the CD19×CD37 bispecific scorpion to kill lymphoma cells is evident from FIG. 27 and Table 13. Data were pooled from three independent experiments and points represent the mean±SEM. IC₅₀ values in Table 13 were determined from the curves in FIGS. 24, 25, and 26, as noted in the legend to Table 13, and are defined as the concentration resulting in 50% inhibition compared to untreated cultures. The data in the figures and table demonstrate that scorpions are greater than 10-fold more potent in killing these cell lines than the free SMIP using the same binding domains.

TABLE 13

IC ₅₀ (nM)	Cell Line		
	SU-DHL-6	DoHH2	WSU-NHL
CD20 SMIP*	>100	60	NA
CD20×CD20 scorpion*	0.3	4.0	NA
CD37 SMIP**	>100	>100	NA
CD37×CD37 scorpion**	10	1.2	NA
CD20 SMIP and CD37 SMIP***	6	2	NA
CD20×CD37 scorpion***	0.05	0.05	NA
CD19 SMIP and CD37 SMIP****	0.16	NA	0.40
CD19×CD37 scorpion****	0.005	NA	0.04

*Data derived from FIG. 24.

**Data derived from FIG. 25.

***Data derived from FIG. 26.

****Data derived from FIG. 27.

[0476] Additional experiments with the humanized CD20×CD20 scorpion S0129 were conducted in Su-DHL-4, Su-DHL-6, DoHH2, Rec-1, and WSU-NHL cells. The results are presented in FIG. 46 and FIG. 47. The data provided in these figures extends the findings discussed above in showing that scorpions have the capacity to directly kill a variety of cell lines.

[0477] The above findings were extended to other monospecific and bispecific scorpions, with each scorpion demonstrating capacity to directly kill B cells. DoHH2 B-cells were exposed in vitro to the monospecific CD20×CD20 scorpion, a monospecific CD37×CD37 scorpion, or a bispecific CD20×CD37 scorpion. The results presented in FIG. 48 demonstrate that bispecific scorpions have kill curves that are different in form from monospecific scorpions.

[0478] Culturing Su-DHL-6 cells in the presence of 70 nM CD20×CD20 scorpion (S0129), CD20×CD37 scorpion, or CD37×CD37 scorpion also led to direct B-cell killing in an in vitro environment (FIG. 49). Consistently, Su-DHL-6 cells

exposed to either a bispecific CD19×CD37 scorpion or to Rituxan® led to direct cell killing, with the bispecific scorpion exhibiting lethality at lower doses, as revealed in FIG. 50.

Example 16

Accessory Functions Mediated by Scorpions (ADCC & CDC)

[0479] a. Scorpion-Dependent Cellular Cytotoxicity

[0480] Experiments were conducted to determine whether scorpions would mediate the killing of BJAB B lymphoma cells. BJAB B lymphoma cells were observed to be killed with CD20 and/or CD37 scorpions.

[0481] Initially, 1×10^7 /ml BJAB B-cells were labeled with 500 $\mu\text{Ci}/\text{ml}$ ^{51}Cr sodium chromate (#CJS1, Amersham Biosciences, Piscataway, N.J.) for 2 hours at 37°C . in Iscoves media with 10% FBS. The ^{51}Cr -loaded BJAB B cells were then washed 3 times in RPMI media with 10% FBS and resuspended at 4×10^5 /ml in RPMI. Peripheral blood mononuclear cells (PBMC) from in-house donors were isolated from heparinized whole blood via centrifugation over Lymphocyte Separation Medium (#50494, MP Biomedicals, Aurora, Oh), washed 2 times with RPMI media and resuspended at 5×10^6 /ml in RPMI with 10% FBS. Reagent samples were added to RPMI media with 10% FBS at 4 times the final concentration and three 10-fold serial dilutions for each reagent were prepared. These reagents were then added to 96-well U-bottom plates at 50 μl /well to the indicated final concentrations. The ^{51}Cr -labeled BJAB were then added to the plates at 50 μl /well (2×10^4 /well). The PBMC were then added to the plates at 100 μl /well (5×10^5 /well) for a final ratio of 25:1 effectors (PBMC):target (BJAB). Effectors and targets were added to media alone to measure background killing. The ^{51}Cr -labeled BJAB were added to media alone to measure spontaneous release of ^{51}Cr and to media with 5% NP40 (#28324, Pierce, Rockford, Ill.) to measure maximal release of ^{51}Cr . The plates were incubated for 6 hours at 37°C . in 5% CO_2 . Fifty μl (25 μl would also be suitable) of the supernatant from each well were then transferred to a Luma-Plate-96 (#6006633, Perkin Elmer, Boston, Mass.) and dried overnight at room temperature.

[0482] After drying, radioactive emissions were quantitated as cpm on a Packard TopCount-NXT. Sample values were the mean of triplicate samples. Percent specific killing was calculated using the following equation: % Kill = ((sample-spontaneous release)/(maximal release-spontaneous release)) $\times 100$. The plots in FIG. 30 show that BJAB B cells were killed by monospecific scorpions CD20×CD20 and CD37×CD37. The combination of CD20 SMIP and CD37 SMIP also killed BJAB B cells. These results demonstrate that scorpions exhibit scorpion-dependent cellular cytotoxicity and it is expected that this functionality is provided by the constant sub-region of the scorpion, providing ADCC activity.

[0483] b. Scorpion Role in Complement-Dependent Cytotoxicity

[0484] Experiments also demonstrated that scorpions have Complement-Dependent Cytotoxicity (CDC) activity. The experiment involved exposure of Ramos B-cells to CD19 and/or CD37 SMIPs and scorpions, as described below and as shown in FIG. 31.

[0485] The experiment was initiated by adding from 5 to 2.5×10^5 Ramos B-cells to wells of 96-well V-bottomed plates

in 50 μl of Iscoves media (no FBS). The test compounds in Iscoves, (or Iscoves alone) were added to the wells in 50 μl at twice the indicated final concentration. The cells and reagents were incubated for 45 minutes at 37°C . The cells were washed 2.5 times in Iscoves with no FBS and resuspended in Iscoves with human serum (# A113, Quidel, San Diego, Calif.) in 96-well plates at the indicated concentrations. The cells were then incubated for 90 minutes at 37°C . The cells were washed by centrifugation and resuspended in 125 μl cold PBS. Cells were then transferred to FACs cluster tubes (#4410, CoStar, Corning, N.Y.) and 125 μl PBS with propidium iodide (# P-16063, Molecular Probes, Eugene, Oreg.) at 5 $\mu\text{g}/\text{ml}$ was added. The cells were incubated with the propidium iodide for 15 minutes at room temperature in the dark and then placed on ice, quantitated, and analyzed on a FACsCalibur with CellQuest software (Becton Dickinson). The results presented in FIG. 31 establish that the CD19 SMIP, but not the CD37 SMIP, exhibits CDC activity, with a combination of the two SMIPs exhibiting approximately the same level of CDC activity as CD19 SMIP alone. The CD19×CD37 scorpion, however, exhibited significantly greater CDC activity than either SMIP alone or in combination, establishing that the scorpion architecture provides a greater level of Complement-dependent Cytotoxicity than other molecular designs.

[0486] c. Interactions of Scorpions with $\text{Fc}\gamma\text{RIII}$

[0487] ELISA studies showed that scorpions bound to $\text{Fc}\gamma\text{RIII}$ (CD16) low (a low affinity isoform or allelotype) at increased levels in the absence of target cells. ELISA plates were initially coated with either low- or high-affinity CD16mIgG using conventional techniques. The ability of this immobilized fusion protein to capture either a CD20 SMIP or a CD20×CD20 monospecific scorpion was assessed. Bound SMIPs and scorpions were detected with goat anti-human IgG (HRP) secondary antibody and mean fluorescence intensity (MFI) was determined. PBS alone (negative control) is shown as a single point. The results are presented in FIG. 32A (capture by CD16 high affinity isoform fusion) and 32B (capture by CD16 low affinity isoform fusion). Apparent from a consideration of FIGS. 32A and 32B is that both CD20 SMIP and CD20×CD20 monospecific scorpion showed increased binding to both the high- and low-affinity CD16 isoform fusions, with the CD20×CD20 scorpion showing a dramatic increase in binding to the low affinity isoform fusion with increasing protein concentration.

[0488] The binding of scorpions to the $\text{Fc}\gamma\text{RIII}$ isoforms in the presence of target cells was also assessed. The data show the increased binding of scorpions to both $\text{Fc}\gamma\text{RIII}$ (CD16) low- and high-affinity isoforms or allelotypes in the presence of target cells with increasing protein concentration.

[0489] In conducting the experiment, CD20-positive target cells were exposed to CD20 SMIPs or CD20×CD20 monospecific scorpions under conditions that allowed the binding of the SMIP or scorpion to the CD20-positive target cell. Subsequently, the SMIP- or scorpion-bearing target cell was exposed to either CD16 high- or low-affinity isoform tagged with mouse IgFc. A labeled goat anti-mouse Fc was then added as a secondary antibody to label the immobilized CD16 tagged with the mouse IgFc. Cells were then detected using flow cytometry on a FACs Calibur (BD Biosciences, San Jose, Calif.) and analyzed with Cell Quest software (BD Biosciences, San Jose, Calif.). As shown in FIG. 33, increased concentrations of each of the CD20 SMIP and the CD20×CD20 monospecific scorpion led to increased binding to the

CD16 isoforms in the presence of target cells, with the increase in binding of the CD20×CD20 scorpion being more significant than the increased binding seen with the CD20 SMIP.

Example 17

Cell-Cycle Effects of Scorpions on Target Lymphoma Cells

[0490] The cell-cycle effects of scorpions were assessed by exposing lymphoma cells to SMIPs, monospecific scorpions and bispecific scorpions. More particularly, DoHH2 lymphoma cells (0.5×10^6) were treated for 24 hours with 0.4 nM rituximab, CD20×CD37 scorpion, TRU-015 (CD20 SMIP)+ SMIP-016 combination (0.2 nM each), 100 nM SMIP-016 or 100 nM CD37×CD37 scorpion. These concentrations represent about 10-fold more than the IC50 value of the scorpion in a 96-hour growth inhibition assay (see FIGS. 24-27). Cultures were labeled for 20 minutes at 37° C. with 10 μM BrdU (bromodeoxyuridine). Following fixation, cells were stained with anti-BrdU-FITC antibody and counterstained with propidium iodide. Values in FIG. 28 are the mean+/-SD of 4 replicate cultures from 2-3 independent experiments. All sample data were analyzed at the same time and pooled for presentation using both the BrdU and PI incorporation dot plots. Plots demonstrate that a major effect of scorpion treatment is a depletion of cells in S-phase, as well as an increase in the G₀/G₁ compartment.

Example 18

Physiological Effects of Scorpions

[0491] a. Calcium Flux
[0492] Scorpion molecules were analyzed for influences on cell signaling pathways, using Ca⁺⁺ mobilization, a common feature of cell signaling, as a measure therefor. SU-DHL-6 lymphoma cells were labeled with Calcium 4 dye and treated with the test molecules identified below. Cells were read for 20 seconds to determine background fluorescence, and then SMIPs/scorpions were added (first vertical dashed line in FIG. 29) and fluorescence was measured out to 600 seconds. At 600 seconds, an 8-fold excess of cross-linked goat-anti-human F(ab)² was added and fluorescence was measured for a further 300 seconds. The upper panel of FIG. 29 shows the results obtained with a combination of CD20 SMIP and CD37 SMIP (dashed line); or obtained with a CD20×CD37 bispecific scorpion (solid line), compared with unstimulated cells (dotted line). A much more robust Ca⁺⁺ transient was induced by the heterospecific scorpion without further cross-linking as compared to the combination of the SMIPs. In the lower panel of FIG. 29, the results of treating cells with CD20 SMIP alone (dashed line) resulted in Ca⁺⁺ mobilization compared to the unstimulated control (dotted line), but, without further cross-linking, this was not as robust as the signal generated by the monospecific CD20×CD20 scorpion (solid line). The Ca⁺⁺ mobilization plots of FIG. 29 represent the fluorescence from triplicate wells treated with equimolar amounts of scorpion and SMIP/SMIP combinations.
[0493] b. Caspases 3, 7 and 9
[0494] A time series study was conducted to determine the effect of CD20 binding proteins, including a CD20×CD20 scorpion, on Caspase 3. DoHH2 or Su-DHL-6 B-cells were incubated with 10 nM CD20 binding protein (S0129 scorpion, 2Lm20-4 SMIP, or Rituxan®)+/-soluble CD16 Ig

(40nM), soluble CD16 Ig alone, or media. The cells were cultured in complete RPMI with 10% FBS at 3×10^5 /well/300 μl and harvested at 4 hours, 24 hours or 72 hours. The 72-hour time-point samples were plated in 500 μl of the test agent. Cells were washed with PBS and then stained for intracellular active caspase-3 using the BD Pharmingen Caspase 3, Active Form, mAB Apoptosis Kit:FITC (cat no. 55048, BD Pharmingen, San Jose, Calif.). Briefly, after 2 additional washes in cold PBS, the cells were suspended in cold cytofix/cytoperm solution and incubated on ice for 20 minutes. Cells were then washed by centrifugation, aspirated, and washed two times with Perm/Wash buffer at room temperature. The samples were then stained with 20 μl FITC-anti-caspase 3 in 100 μl of Perm-Wash buffer at room temperature in the dark for thirty minutes. The samples were then washed two times with Perm-Wash buffer, and resuspended in 500 μl of Perm-Wash buffer. Washed cells were then transferred to FACs tubes and run on a FACs Calibur (BD Biosciences, San Jose, Calif.) and analyzed with Cell Quest software (BD Biosciences, San Jose, Calif.). The results are shown in Table 14.

TABLE 14

Molecule (10 nM)	Percentage Caspase-3 positive cells			Percentage in live gate		
	4 hours	24 hours	48 hours	4 hours	24 hours	48 hours
RTXN and CD16 hi (4X)	7	25	7	75	53	56
CD20 SMIP (2Lm20-4)	27	47	21	79	60	43
and CD16 hi	5	5	10	89	85	81
Humanized CD20×CD20 scorpion (S00129)	28	54	21	61	60	41
and CD16 hi	7	13	14	69	68	61
Media	30	31	15	67	75	72
and CD16 hi	7	5	9	89	82	80
	6	5	9	91	83	80

The results of all of these experiments are consistent in showing that there is limited activation of caspase 3 in the absence of CD16, which does not implicate caspase 3 activation as a significant feature of the direct cell killing induced by CD20 binding scorpions.

[0495] c. SYK Phosphorylation

[0496] SYK is a phospho-regulated protein with several phosphorylation sites that functions as a transcriptional repressor. SYK is localized to the cell nucleus, but is capable of rapid relocation to the membrane upon activation. For activation, SYK must retain its nuclear localization sequence. Activated SYK has a role in suppressing breast cancer tumors and SYK is activated by pro-apoptotic signals such as ionizing radiation, BCR ligation and MHC class II cross-linking. Further, SYK has been shown to affect the PLC-γ and Ca⁺⁺ pathways. Given these observations, the capacity of CD20-binding scorpions to affect SYK was investigated.
[0497] DHL-6 B-cells were exposed to a bispecific CD20×CD37 scorpion for 0, 5, 7 or 15 hours and the cells were lysed. Lysates were immunoprecipitated with either an anti-phosphotyrosine antibody or with an anti-SYK antibody. Immunoprecipitates were fractionated by gel electrophoresis and the results are shown in FIG. 51. Apparent from an inspection of FIG. 51 is the failure of the bispecific CD20×CD37 scorpion to induce phosphorylation of SYK, thereby activating it. Consistent with the above-described studies on caspase activation and chromosomal DNA fragmentation, it does not appear that CD20-binding scorpions directly kill B-cells

using a classic apoptotic pathway, such as the caspase-dependent pathway. While not wishing to be bound by theory, it is expected that the CD20-binding scorpions directly kill B-cells through a caspase-, and SYK-, independent pathway that does not prominently feature chromosomal DNA fragmentation, at least not on the same time frame as fragmentation occurs during caspase-dependent apoptosis.

Example 19

Scorpion Applications

[0498] a. In Vivo Activity of Scorpions

[0499] The activity of scorpions was also assessed using a mouse model. Measurements of scorpion activity in vivo involved administration of 10-300 μ g scorpion and subsequent time-series determinations of serum concentrations of that scorpion. Results of these studies, presented as serum concentration curves for each of two bispecific scorpions (i.e., S0033, a CD20 $\times$ CD27 scorpion and a CD20 $\times$ CD37 scorpion) from three-week pharmacokinetic studies in mice are presented in FIG. 40. The data in FIG. 40 show that it took at least 500 hours after administration before the serum levels of each of the two bispecific scorpions fell back to baseline levels. Thus, scorpions show serum stability and reproducible, sustained circulating half-lives in vivo.

[0500] The in vivo efficacy of scorpions was also assessed. An aggressive Ramos xenograft model was used in parallel experiments with SMIPs versus historical immunoglobulin controls. The survival curves provided in FIG. 41 reveal that administration of 10 μ g bispecific scorpion had negligible influence on survival, but administration of 100-300 μ g had significant positive effect on the survival of mice bearing Ramos xenografts.

[0501] b. Combination Therapies

[0502] it is contemplated that scorpions will find application in the prevention, treatment or amelioration of a symp-

tom of, a wide variety of conditions affecting man, other mammals and other organisms. For example, CD20-binding scorpions are expected to be useful in treating or preventing a variety of diseases associated with excessive or aberrant B-cells. In fact, any disease amenable to a treatment involving the depletion of B-cells would be amenable to treatment with a CD20-binding scorpion. In addition, scorpions, e.g., CD20-binding scorpions, may be used in combination therapies with other therapeutics. To illustrate the feasibility of a wide variety of combination therapies, the monospecific CD20 $\times$ CD20 scorpion (S0129) was administered to Su-DHL-6 B-cells in combination with doxorubicin, vincristine or rapamycin. Doxorubicin is a topoisomerase H poison that interferes with DNA biochemistry and belongs to a class of drugs contemplated for anti-cancer treatment. Rapamycin (Sirolimus) is a macrolide antibiotic that inhibits the initiation of protein synthesis and suppresses the immune system, finding application in organ transplantation and as an anti-proliferative used with coronary stents to inhibit or prevent restenosis. Vincristine is a vinca alkaloid that inhibits tubule formation and has been used to treat cancer.

[0503] The experimental results shown in FIG. 52 are presented as Combination Index values for each combination over a range of effect levels. The interactions of the monospecific CD20 $\times$ CD20 scorpion S0129 are different for each drug class, while with Rituxan® (RTXN) the plots forms are similar. The effect seen with doxorubicin at high concentrations may reflect a shift towards monovalent binding. The data establish that CD20-binding scorpions may be used in combination with a variety of other therapeutics and such combinations would be apparent to one of skill in the art in view of the present disclosure.

[0504] Variations on the structural themes for multivalent binding molecules with effector function, or scorpions, will be apparent to those of skill in the art upon review of the present disclosure, and such variant structures are within the scope of the invention.

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Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
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Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
210 215 220

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
225 230 235 240

Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
245 250 255

Gly Thr Gly Thr Thr Val Thr Val Ser
260 265

<210> SEQ ID NO 3
<211> LENGTH: 813
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD28 (2e12) LH (DNA)

<400> SEQUENCE: 3

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atggattttc aagtgcagat tttcagcttc ctgctaataca gtgcttcagt cataatgtcc    60
agaggagtcg acattgtgct caccacaatct ccagcttctt tggctgtgtc tctaggtcag    120
agagccacca tctctgcag agccagtga aagtgtgaat attatgtcac aagtttaatg    180
cagtggtagc aacagaaacc aggacagcca cccaaactcc tcatctctgc tgctagcaac    240
gtagaatctg gggtcctctg caggtttagt ggcagtgggt ctgggacaga ctttagcctc    300
aacatccatc ctgtggagga ggatgatatt gcaatgtatt tctgtcagca aagtaggaag    360
gttccatgga cgttcggtag aggcaccaag ctggaaatca aacggggtag cggtggatcc    420
ggcggagggt ggtagggtag cggcggatct cagggtgcagc tgaaggagtc aggacctggc    480
ctggtggcgc cctcacagag cctgtccatc acatgcaccg tctcagggtt ctcattaacc    540
ggctatgggt taaactgggt tcgccagcct ccaggaaagg gtctggagtg gctgggaatg    600
atatgggggt atggaagcac agactataat tcagctctca aatccagact atcgatcacc    660
aaggacaact ccaagagcca agttttctta aaaatgaaca gtctgcaaac tgatgacaca    720
gccagatact actgtgcccc agatgggtat agtaactttc attactatgt tatggactac    780
tggggtcaag gaacctcagt caccgtctcc tct                                813
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<210> SEQ ID NO 4
<211> LENGTH: 271
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD28 (2e12) LH (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(135)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (136)..(150)
<223> OTHER INFORMATION: Linker

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (151)..(271)
<223> OTHER INFORMATION: VH

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<400> SEQUENCE: 4

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Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
 1             5             10             15

Val Ile Met Ser Arg Gly Val Asp Ile Val Leu Thr Gln Ser Pro Ala
 20             25             30

Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala
 35             40             45

Ser Glu Ser Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln
 50             55             60

Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn
 65             70             75             80

Val Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
 85             90             95

Asp Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met
 100            105            110

Tyr Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly
 115            120            125

Thr Lys Leu Glu Ile Lys Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly
 130            135            140

Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly
 145            150            155            160

Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly
 165            170            175

Phe Ser Leu Thr Gly Tyr Gly Val Asn Trp Val Arg Gln Pro Pro Gly
 180            185            190

Lys Gly Leu Glu Trp Leu Gly Met Ile Trp Gly Asp Gly Ser Thr Asp
 195            200            205

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser
 210            215            220

Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
 225            230            235            240

Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr Tyr
 245            250            255

Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 260            265            270

```

```

<210> SEQ ID NO 5
<211> LENGTH: 828
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD28 (2e12) HL (DNA)

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```

<400> SEQUENCE: 5

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atggattttc aagtgcagat tttcagcttc ctgctaataca gtgcttcagt cataatgtcc      60
agaggagtcc aggtgcagct gaaggagtca ggacctggcc tggtagcgcc ctcacagagc      120
ctgtccatca catgcaccgt ctcagggttc tcattaaccg gctatggtgt aaactggggt      180
cgccagcctc caggaagagg tctggagtgg ctgggaatga tatggggtga tggaagcaca      240

```

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gactataatt cagctctcaa atccagacta tcgatcacca aggacaactc caagagccaa    300
gttttcttaa aaatgaacag tctgcaaact gatgacacag ccagatacta ctgtgcccga    360
gatggttata gtaactttta ttactatggt atggactact ggggtcaagg aacctcagtc    420
accgtctcct ctgggggttg aggctctggt ggcgggtgat ccggcggagg tgggtcgggt    480
ggcggcggat ctgacattgt gctcacccaa tctccagctt ctttggctgt gtctctaggt    540
cagagagcca ccatctctg cagagccagt gaaagtgttg aatattatgt cacaagttta    600
atgcagtggg accaacagaa accaggacag ccacccaaac tcctcatctc tgctgctagc    660
aacgtagaat ctgggggtccc tgccagggtt agtggcagtg ggtctgggac agactttagc    720
ctcaacatcc atcctgtgga ggaggatgat attgcaatgt atttctgtca gcaaagtagg    780
aaggttccat ggacgttcgg tggaggcacc aagctggaaa tcaaacgt                828

```

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<210> SEQ ID NO 6
<211> LENGTH: 276
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD28 (2e12) HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(144)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(164)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (165)..(276)
<223> OTHER INFORMATION: VL

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<400> SEQUENCE: 6

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```

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1           5           10          15

Val Ile Met Ser Arg Gly Val Gln Val Gln Leu Lys Glu Ser Gly Pro
20          25          30

Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser
35          40          45

Gly Phe Ser Leu Thr Gly Tyr Gly Val Asn Trp Val Arg Gln Pro Pro
50          55          60

Gly Lys Gly Leu Glu Trp Leu Gly Met Ile Trp Gly Asp Gly Ser Thr
65          70          75          80

Asp Tyr Asn Ser Ala Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Asn
85          90          95

Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp
100         105         110

Thr Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr
115         120         125

Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
130         135         140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly

```

-continued

145	150	155	160
Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala	165	170	175
Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser	180	185	190
Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro	195	200	205
Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn Val Glu Ser	210	215	220
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser	225	230	235
Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys	245	250	255
Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu	260	265	270
Glu Ile Lys Arg	275		

<210> SEQ ID NO 7
 <211> LENGTH: 30
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic primer
 <220> FEATURE:
 <223> OTHER INFORMATION: Heavy Chain GSP1

<400> SEQUENCE: 7

aggtgctgga ggggacagtc actgagctgc

30

<210> SEQ ID NO 8
 <211> LENGTH: 28
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic primer
 <220> FEATURE:
 <223> OTHER INFORMATION: Nested Heavy Chain

<400> SEQUENCE: 8

gtcacwgtca ctgrctcagg gaartagc

28

<210> SEQ ID NO 9
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic primer
 <220> FEATURE:
 <223> OTHER INFORMATION: Light Chain GSP1

<400> SEQUENCE: 9

gggtgctgct catgctgtag gtgctgtctt tgc

33

<210> SEQ ID NO 10
 <211> LENGTH: 32
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic primer
 <220> FEATURE:
 <223> OTHER INFORMATION: Nested Light Chain

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<400> SEQUENCE: 10

caagaagcac acgactgagg cacctccaga tg

32

<210> SEQ ID NO 11

<211> LENGTH: 36

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic primer

<220> FEATURE:

<223> OTHER INFORMATION: Race Abridged Anchor Primer

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (24)..(25)

<223> OTHER INFORMATION: n is a, c, g, or t

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (29)..(30)

<223> OTHER INFORMATION: n is a, c, g, or t

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (34)..(35)

<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 11

ggccacgcgt cgactagtagt gggnnngggnn gggnnng

36

<210> SEQ ID NO 12

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic primer

<220> FEATURE:

<223> OTHER INFORMATION: T7 primer

<400> SEQUENCE: 12

gtaatacgac tcactatagg

20

<210> SEQ ID NO 13

<211> LENGTH: 18

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic primer

<220> FEATURE:

<223> OTHER INFORMATION: M13 reverse primer

<400> SEQUENCE: 13

caggaaacag ctatgacc

18

<210> SEQ ID NO 14

<211> LENGTH: 15

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: wild-type human IgG1 hinge domain

<400> SEQUENCE: 14

Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro
1				5					10				15	

<210> SEQ ID NO 15

<211> LENGTH: 46

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

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<220> FEATURE:
<223> OTHER INFORMATION: hVK3L-F3H3

<400> SEQUENCE: 15

gcgataaagc ttgccgccat ggaagcacca gcgcagcttc tcttcc 46

<210> SEQ ID NO 16
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: hVK3LF2

<400> SEQUENCE: 16

accagcgag cttctcttcc tctgtact ctggtccca gataccaccg 50

<210> SEQ ID NO 17
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: hVK3LF12HVL

<400> SEQUENCE: 17

ggctccaga taccaccggt caaatgttc tctccagtc tccag 45

<210> SEQ ID NO 18
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 2H7VHNheF

<400> SEQUENCE: 18

gcgatagcta gccaggctta tctacagcag tctgg 35

<210> SEQ ID NO 19
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G4SNheR

<400> SEQUENCE: 19

gcgatagcta gccccacctc ctccagatcc accaccgccc gag 43

<210> SEQ ID NO 20
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 015VHXhoR

<400> SEQUENCE: 20

gcgtactcga ggagacggtg accgtggtcc ctgtg 35

<210> SEQ ID NO 21
<211> LENGTH: 37
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G1HC-Xho

<400> SEQUENCE: 21

gcagtctcga gcgagcccaa atcttgtgac aaaactc 37

<210> SEQ ID NO 22
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G1H-S-Xho

<400> SEQUENCE: 22

gcagtctcga gcgagcccaa atcttctgac aaaactc 37

<210> SEQ ID NO 23
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: CH3REcoR1

<400> SEQUENCE: 23

gcgtgagaat tcttaccggg agacagggag aggctc 36

<210> SEQ ID NO 24
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G1 XBZ-R

<400> SEQUENCE: 24

gcgacgtcta gagtcattta cccggagaca gg 32

<210> SEQ ID NO 25
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G4SLinkR1-S

<400> SEQUENCE: 25

aattatggtg gcggtggctc gggcggtggt ggatctggag gaggtgggag tggg 54

<210> SEQ ID NO 26
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G4SLinkR1-AS

<400> SEQUENCE: 26

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aattcccact cccacctcct ccagatccac caccgcccga gccaccgcca ccat 54

<210> SEQ ID NO 27
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 2E12VLXbaR

<400> SEQUENCE: 27

gcgtgtctag attaacgttt gatttcagc ttggtg 36

<210> SEQ ID NO 28
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 2E12VLR1F

<400> SEQUENCE: 28

gcgatgaatt ctgacattgt gctcacccaa tctcc 35

<210> SEQ ID NO 29
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 2E12VHR1F

<400> SEQUENCE: 29

gcgatgaatt ctcaggtgca gctgaaggag tcag 34

<210> SEQ ID NO 30
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 2E12VHXbaR

<400> SEQUENCE: 30

gcgagtc tag attagagga gacggtgact gaggttc 37

<210> SEQ ID NO 31
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 2e12VHdXbaF1

<400> SEQUENCE: 31

gggtctggag tggctgggaa tgatatg 27

<210> SEQ ID NO 32
<211> LENGTH: 28
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 2e12VHdXbaR1

<400> SEQUENCE: 32

attcccagcc actccagacc ctttcctg

28

<210> SEQ ID NO 33
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: IgBsrG1F

<400> SEQUENCE: 33

gagaaccaca ggtgtacacc ctg

23

<210> SEQ ID NO 34
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: IgBsrG1R

<400> SEQUENCE: 34

gcaggggtgta cacctgtggt tctcg

25

<210> SEQ ID NO 35
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: M13R

<400> SEQUENCE: 35

caggaaacag ctatgac

17

<210> SEQ ID NO 36
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: M13F

<400> SEQUENCE: 36

gtaaaacgac ggccagtg

18

<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: T7

<400> SEQUENCE: 37

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gtaatacgac tcactatagg 20

<210> SEQ ID NO 38
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: pD18F-17

<400> SEQUENCE: 38

aactagagaa cccactg 17

<210> SEQ ID NO 39
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: pD18F-20

<400> SEQUENCE: 39

gctaactaga gaacccactg 20

<210> SEQ ID NO 40
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: pD18F-1

<400> SEQUENCE: 40

atacgactca ctataggg 18

<210> SEQ ID NO 41
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: pD18R-s

<400> SEQUENCE: 41

gctctagcat ttaggtgac 19

<210> SEQ ID NO 42
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: CH3seqF1

<400> SEQUENCE: 42

catgaggctc tgcacaac 18

<210> SEQ ID NO 43
<211> LENGTH: 18
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: CH3seqF2

<400> SEQUENCE: 43

cctctacagc aagctcac 18

<210> SEQ ID NO 44
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: CH3seqR1

<400> SEQUENCE: 44

ggttcttggt cagctcac 19

<210> SEQ ID NO 45
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: CH3seqR2

<400> SEQUENCE: 45

gtgagcttgc ttagaggg 18

<210> SEQ ID NO 46
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: L1-11R

<400> SEQUENCE: 46

gcgatagaat tccagatcc accacgccc gagccaccgc caccataatt c 51

<210> SEQ ID NO 47
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: L1-6R

<400> SEQUENCE: 47

gcgatagaat tccagagcc accgccacca taattc 36

<210> SEQ ID NO 48
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: L3R

<400> SEQUENCE: 48

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gcgtatgaat tccccgagcc accgccaccc ttaccggag acagg 45

<210> SEQ ID NO 49
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: L4R

<400> SEQUENCE: 49

gcgtatgaat tcccagatcc accaccgccc gagccaccgc cacccttac 49

<210> SEQ ID NO 50
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: L5R

<400> SEQUENCE: 50

gcgtatgaat tcccgtgccc tectccccca gatccaccac cgcc 44

<210> SEQ ID NO 51
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: IgBsrG1F

<400> SEQUENCE: 51

gagaaccaca ggtgtacacc ctg 23

<210> SEQ ID NO 52
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: L-CPPCPR

<400> SEQUENCE: 52

gcgatagaat tcggacaagg tggacacccc ttaccggag acagggagag 50

<210> SEQ ID NO 53
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: G281LH-NheR
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 53

actgctgcag ctggaccgcg ctagctccgc cgccaccga c 41

<210> SEQ ID NO 54
<211> LENGTH: 40
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281LH-NheF

<400> SEQUENCE: 54

ggcggagcta gcgcggtcca gctgcagcag tctggacctg

40

<210> SEQ ID NO 55
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-LH-LPinF

<400> SEQUENCE: 55

gcgatacccg gtgacatcca gatgactcag tctccag

37

<210> SEQ ID NO 56
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-LH-HXhoR

<400> SEQUENCE: 56

gcgatactcg aggagacggt gactgaggtt ccttgac

37

<210> SEQ ID NO 57
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-LH-LEcoF

<400> SEQUENCE: 57

gcgatcgaat tcagacatcc agatgactca gtctccag

38

<210> SEQ ID NO 58
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-LH-HXbaR

<400> SEQUENCE: 58

gcgattctag attaggaaga gacggtgact gaggttcctt gac

43

<210> SEQ ID NO 59
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-HF

<400> SEQUENCE: 59

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gcgataaccg gtgcggtcca gctgcagcag tctggac 37

<210> SEQ ID NO 60
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-HR3

<400> SEQUENCE: 60

gacccaccac cgcccgagcc accgccacca gaagagacgg tgactgaggt tc 52

<210> SEQ ID NO 61
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-HR2

<400> SEQUENCE: 61

actcccgct cctcctgac cgccgccacc cgacccacca ccgccgag 49

<210> SEQ ID NO 62
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-HNheR

<400> SEQUENCE: 62

gagtcacctg gatgtcgcta gcactccgc ctcctcctga tc 42

<210> SEQ ID NO 63
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-LNheF

<400> SEQUENCE: 63

atcaggagga ggcgaggatg ctagcgacat ccagatgact cagtc 45

<210> SEQ ID NO 64
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-LXhoR

<400> SEQUENCE: 64

gcgatactcg agcctttgat ctccagttcg gtgcctc 37

<210> SEQ ID NO 65
<211> LENGTH: 42
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-LXhoR

<400> SEQUENCE: 65

gcgatatcta gactcaacct ttgatctcca gttcgggtgcc tc 42

<210> SEQ ID NO 66
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: G281-HL-EcoF

<400> SEQUENCE: 66

gcgatagaat tcgcggtcca gctgcagcag tctggac 37

<210> SEQ ID NO 67
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LH-LF1

<400> SEQUENCE: 67

gcgtatgaac cggtgacatc cagatgacac agactacatc 40

<210> SEQ ID NO 68
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LF2

<400> SEQUENCE: 68

atccagatga cacagactac atcctccctg tctgcctctc tgggagacag 50

<210> SEQ ID NO 69
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LF3

<400> SEQUENCE: 69

gtctgcctct ctgggagaca gagtcaccat cagttgcagg gcaagtcagg ac 52

<210> SEQ ID NO 70
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<223> OTHER INFORMATION: 194-LF4

<400> SEQUENCE: 70

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gttgcagggc aagtcaggac attcgcaatt atttaaactg gtatcagcag 50

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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LF5

<400> SEQUENCE: 71

atttaaactg gtatcagcag aaaccagatg gaactgttaa actcctgac 50

<210> SEQ ID NO 72
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LF6

<400> SEQUENCE: 72

gaactgttaa actcctgac tactacacat caagattaca ctcaggagtc 50

<210> SEQ ID NO 73
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LF7

<400> SEQUENCE: 73

caagattaca ctcaggagtc ccatcaaggt tcagtggcag tgggtctgga ac 52

<210> SEQ ID NO 74
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LR7

<400> SEQUENCE: 74

caggttggca atggtgagag aataatctgt tccagacca ctgccactga ac 52

<210> SEQ ID NO 75
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LR6

<400> SEQUENCE: 75

gcaaaagtaa gtggcaatat cttctggttg caggttggca atggtgagag 50

<210> SEQ ID NO 76
<211> LENGTH: 51
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LR5

<400> SEQUENCE: 76

gaacgtccac ggaagcgtat taccctgttg gcaaaagtaa gtggcaatat c 51

<210> SEQ ID NO 77
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LR4

<400> SEQUENCE: 77

cgtttggtta ccagtttgggt gcctccaccg aacgtccacg gaagcgtatt ac 52

<210> SEQ ID NO 78
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LR3

<400> SEQUENCE: 78

accaccgccc gagccaccgc caccctgttt gggtaccagt ttggtg 46

<210> SEQ ID NO 79
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LR2

<400> SEQUENCE: 79

gctagcgctc ccacctcctc cagatccacc accgcccag ccaccgccac 50

<210> SEQ ID NO 80
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LH-LR1

<400> SEQUENCE: 80

gttgcaagctg gacctcgcta gcgctccac ctctccaga tc 42

<210> SEQ ID NO 81
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LH-HF1

<400> SEQUENCE: 81

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gatctggagg aggtgggagc gctagcgagg tccagctgca acagtctgga cctg 54

<210> SEQ ID NO 82
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HF2

<400> SEQUENCE: 82

agctgcaaca gtctggacct gaactgggtga agcctggagc ttcaatgaag 50

<210> SEQ ID NO 83
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HF3

<400> SEQUENCE: 83

agcctggagc ttcaatgaag atttcttgca aggcctctgg ttactcattc 50

<210> SEQ ID NO 84
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HF4

<400> SEQUENCE: 84

gcaaggcctc tggttactca ttcactggct acatcgtaga ctggctgaag cag 53

<210> SEQ ID NO 85
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HF5

<400> SEQUENCE: 85

atcgtagaact ggctgaagca gagccatgga aagaaccttg agtggattgg ac 52

<210> SEQ ID NO 86
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HF6

<400> SEQUENCE: 86

gaaccttgag tggattggac ttattaatcc atacaaaggt cttactacct ac 52

<210> SEQ ID NO 87
<211> LENGTH: 52
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HR6

<400> SEQUENCE: 87

aatgtggcct tgccttgaa tttctggtg taggtagtaa gacctttgta tg 52

<210> SEQ ID NO 88
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HR5

<400> SEQUENCE: 88

catgtaggct gtgctggatg acttgtctac agttaatgtg gccttgccct tg 52

<210> SEQ ID NO 89
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HR4

<400> SEQUENCE: 89

actgcagagt cttcagatgt cagactgagg agctccatgt aggctgtgct ggatg 55

<210> SEQ ID NO 90
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HR3

<400> SEQUENCE: 90

accatagtac ccagatcttg cacagtaata gactgcagag tcttcagatg tc 52

<210> SEQ ID NO 91
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HR2

<400> SEQUENCE: 91

gcgccccaga catcgaagta ccagtccgag tcaccatagt acccagatct tg 52

<210> SEQ ID NO 92
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LH-HR1

<400> SEQUENCE: 92

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gcgaatactc gaggagacgg tgacctggt cctgcgccc cagacatcga ag 52

<210> SEQ ID NO 93
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HL-HF1

<400> SEQUENCE: 93

gcgtatgaac cggtagggtc cagctgcaac agtctggacc tg 42

<210> SEQ ID NO 94
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HL-HR1

<400> SEQUENCE: 94

accgccacca gaggagacgg tgacctggt cctgcgccc cagacatcga agtac 55

<210> SEQ ID NO 95
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HL-HR0

<400> SEQUENCE: 95

acctcctcca gatccaccac cgcccagacc accgccacca gaggagacgg tg 52

<210> SEQ ID NO 96
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HL-LF1

<400> SEQUENCE: 96

gcgggggagg tggcagtgt agcgacatcc agatgacaca gactacatc 49

<210> SEQ ID NO 97
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HL-LR3Xho

<400> SEQUENCE: 97

gcgaatactc gagcgtttgg ttaccagttt ggtg 34

<210> SEQ ID NO 98
<211> LENGTH: 37
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HL-LR3Xba

<400> SEQUENCE: 98

gcgatatcta gattaccggt tggttaccag tttggtg 37

<210> SEQ ID NO 99
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-HL-HF1R1

<400> SEQUENCE: 99

gcgtatgaga attcagaggt ccagctgcaa cagtctggac ctg 43

<210> SEQ ID NO 100
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LH-LF1R1

<400> SEQUENCE: 100

gcgtatgaga attctgacat ccagatgaca cagactacat c 41

<210> SEQ ID NO 101
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: 194-LH-HR1Xba

<400> SEQUENCE: 101

gcgtatctag attaggagac ggtgaccgtg gtcctcgcg cccagacatc gaag 54

<210> SEQ ID NO 102
<211> LENGTH: 725
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: G28-1 VLVH (DNA)

<400> SEQUENCE: 102

accggtgaca tccagatgac tcagtctcca gcctccctat ctgcatctgt gggagagact 60
gtcaccatca catgtcgaac aagtgaatat gtttacagtt atttggcttg gtatcagcag 120
aaacagggaa aatctcctca gtcctgggtc tcttttgcaa aaaccttagc agaaggtgtg 180
ccatcaaggt tcagtggcag tggatcaggc acacagtttt ctctgaagat cagcagcctg 240
cagcctgaag attctggaag ttatttctgt caacatcatt ccgataatcc gtggacgttc 300
ggtggaggca ccgaactgga gatcaaaggt ggcgggtggct cgggcgggtg tgggtcgggt 360
ggcggcggat ctgctagcgc agtccagctg cagcagctctg gacctgagct ggaaaagcct 420

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ggcgcttcag tgaagatttc ctgcaaggct tctgggttact cattcactgg ctacaatatg   480
aactgggtga agcagaataa tggaaagagc cttgagtgga ttggaaatat tgatccttat   540
tatgggtgga ctacctacaa ccggaagttc aagggcaagg ccacattgac tgtagacaaa   600
tcctccagca cagcctacat gcagctcaag agtctgacat ctgaggactc tgcagtctat   660
tactgtgcaa gatcggtcgg ccctatggac tactgggggc aaggaacctc agtcaccgtc   720
tcgag                                           725

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<210> SEQ ID NO 103
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: G-28-1 VLVH (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(107)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (108)..(124)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (125)..(239)
<223> OTHER INFORMATION: VH

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<400> SEQUENCE: 103

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

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210	215	220	
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225	230	235	

<210> SEQ ID NO 104
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 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: G28-1 VHVL (DNA)

<400> SEQUENCE: 104

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cagagccatg gaaagaacct tgagtggatt ggacttatta atccatacaa aggtcttact	180
acctacaacc agaaattcaa gggcaaggcc acattaactg tagacaagtc atccagcaca	240
gcctacatgg agctcctcag tctgacatct gaagactctg cagtctatta ctgtgcaaga	300
tctgggtact atgggtgactc ggactgggtac ttcgatgtct ggggcgcagg gaccacggtc	360
accgtctcct ctgggtggcgg tggctcgggc ggtgggtggat ctggaggagg tgggagcggg	420
ggaggtggca gtgctagcga catccagatg acacagacta catcctcct gtctgcctct	480
ctgggagaca gagtcccat cagttgcagg gcaagtcagg acattcgcaa ttatttaaac	540
tggtatcagc agaaaccaga tggaactgtt aaactcctga tctactacac atcaagatta	600
cactcaggag tcccacaaag gttcagtggc agtgggtctg gaacagatta ttctctcacc	660
attgccaacc tgcaaccaga agatattgcc acttactttt gccaacaggg taatacgctt	720
ccgtggacgt tcggtggagg caccaaaactg gtaaccaaac gctcgag	767

<210> SEQ ID NO 105
 <211> LENGTH: 253
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: G28-1 VHVL (AA)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(121)
 <223> OTHER INFORMATION: VH
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (122)..(144)
 <223> OTHER INFORMATION: Linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (145)..(253)
 <223> OTHER INFORMATION: VL

<400> SEQUENCE: 105

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Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr	
20 25 30	
Ile Val Asn Trp Leu Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile	
35 40 45	
Gly Leu Ile Asn Pro Tyr Lys Gly Leu Thr Thr Tyr Asn Gln Lys Phe	

-continued

50	55	60
Lys Gly Lys Ala Thr	Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr	
65	70	75 80
Met Glu Leu Leu Ser	Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	
	85	90 95
Ala Arg Ser Gly Tyr Tyr Gly Asp Ser Asp Trp Tyr Phe Asp Val Trp		
	100	105 110
Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly		
	115	120 125
Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser		
	130	135 140
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly		
145	150	155 160
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Arg Asn Tyr		
	165	170 175
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile		
	180	185 190
Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly		
	195	200 205
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ala Asn Leu Gln Pro		
	210	215 220
Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp		
225	230	235 240
Thr Phe Gly Gly Gly Thr Lys Leu Val Thr Lys Arg Ser		
	245	250

<210> SEQ ID NO 106

<211> LENGTH: 749

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: G19-4 VLVH (DNA)

<400> SEQUENCE: 106

```

accggtgaca tccagatgac acagactaca tcctccctgt ctgcctctct gggagacaga      60
gtcaccatca gttgcagggc aagtcaggac attcgcaatt atttaactg gtatcagcag      120
aaaccagatg gaactgttaa actcctgata tactacacat caagattaca ctcaggagtc      180
ccatcaaggt tcagtggcag tgggtctgga acagattatt ctctcaccat tgccaacctg      240
caaccagaag atattgccac ttacttttgc caacagggta atacgcttcc gtggacgttc      300
gggtgaggca ccaaactggt aaccaaaccg ggtggcggtg gctcgggcgg tggtggatct      360
ggaggagggt ggagcgctag cgagggtccag ctgcaacagt ctggacctga actggtgaag      420
cctggagctt caatgaagat ttcttgcaag gcctctggtt actcattcac tggtacatc      480
gtgaactggc tgaagcagag ccatggaaag aaccttgagt ggattggact tattaatcca      540
tacaaaggtc ttactaccta caaccagaaa ttcaagggca aggccacatt aactgtagac      600
aagtcattcca gcacagccta catggagctc ctcagtctga catctgaaga ctctgcagtc      660
tattactgtg caagatctgg gtactatggt gactcggact ggtacttcca tgtctggggc      720
gcagggacca cggtcaccgt ctctcagag                                     749

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-continued

```

<210> SEQ ID NO 107
<211> LENGTH: 247
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: G-19-4 VLVH (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(108)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (109)..(125)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (126)..(247)
<223> OTHER INFORMATION: VH

<400> SEQUENCE: 107

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

```

```

<210> SEQ ID NO 108
<211> LENGTH: 752
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:

```

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<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: G19-4 VHVL (DNA)

<400> SEQUENCE: 108

```

accggtgagg tccagctgca acagtctgga cctgaactgg tgaagcctgg agcttcaatg      60
aagatttcct gcaaggcctc tggttactca ttcactggct acatcgtgaa ctggctgaag      120
cagagccatg gaaagaacct tgagtggatt ggacttatta atccatacaa aggtcttact      180
acctacaacc agaaattcaa gggcaaggcc acattaactg tagacaagtc atccagcaca      240
gctacatagg agctcctcag tctgacatct gaagactctg cagtctatta ctgtgcaaga      300
tctgggtact atggtgactc ggactggtag ttcgatgtct ggggcgcagg gaccacggtc      360
accgtctcct ctggtggcgg tggctcgggc ggtggtggat ctggaggagg tgggagcgct      420
agcgacatcc agatgacaca gactacatcc tccctgtctg cctctctggg agacagagtc      480
accatcagtt gcagggcaag tcaggacatt cgcaattatt taaactggta tcagcagaaa      540
ccagatggaa ctgttaaact cctgatctac tacacatcaa gattacactc aggagtccca      600
tcaaggttca gtggcagtggt gtctggaaca gattattctc tcaccattgc caacctgcaa      660
ccagaagata ttgccactta cttttgccaa cagggtaata cgcttccgtg gacgttcggt      720
ggaggcacca aactggtaac caaacgctcg ag                                     752

```

<210> SEQ ID NO 109

<211> LENGTH: 248

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polypeptide

<220> FEATURE:

<223> OTHER INFORMATION: G19-4 VHVL (AA)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (1)..(122)

<223> OTHER INFORMATION: VH

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (123)..(139)

<223> OTHER INFORMATION: Linker

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (140)..(248)

<223> OTHER INFORMATION: VL

<400> SEQUENCE: 109

```

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1          5          10          15
Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
20        25        30
Ile Val Asn Trp Leu Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile
35        40        45
Gly Leu Ile Asn Pro Tyr Lys Gly Leu Thr Thr Tyr Asn Gln Lys Phe
50        55        60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65        70        75        80
Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85        90        95
Ala Arg Ser Gly Tyr Tyr Gly Asp Ser Asp Trp Tyr Phe Asp Val Trp
100       105       110

```

-continued

Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
 115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Asp Ile Gln Met Thr
 130 135 140

Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile
 145 150 155 160

Ser Cys Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr Gln
 165 170 175

Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser Arg
 180 185 190

Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
 195 200 205

Asp Tyr Ser Leu Thr Ile Ala Asn Leu Gln Pro Glu Asp Ile Ala Thr
 210 215 220

Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp Thr Phe Gly Gly Gly
 225 230 235 240

Thr Lys Leu Val Thr Lys Arg Ser
 245

<210> SEQ ID NO 110
 <211> LENGTH: 45
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic primer
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <223> OTHER INFORMATION: ssc(s)-hIgG1 (DNA)

<400> SEQUENCE: 110

gagcccaaat cttctgacaa aactcacaca tctccaccgt gctca

45

<210> SEQ ID NO 111
 <211> LENGTH: 15
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <223> OTHER INFORMATION: ssc(s)-hIgG1 (AA)

<400> SEQUENCE: 111

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Cys Ser
 1 5 10 15

<210> SEQ ID NO 112
 <211> LENGTH: 45
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic primer
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <223> OTHER INFORMATION: scc(s)-hIgG1 (DNA)

<400> SEQUENCE: 112

gagcccaaat cttctgacaa aactcacaca tgtccaccgt gctca

45

<210> SEQ ID NO 113
 <211> LENGTH: 15
 <212> TYPE: PRT

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: scc(s)-hIgG1 (AA)

<400> SEQUENCE: 113

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Ser
1 5 10 15

<210> SEQ ID NO 114
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: css(s)-hIgG1 (DNA)

<400> SEQUENCE: 114

gagcccaaat cttgtgacaa aactcacaca tctccaccga gctca 45

<210> SEQ ID NO 115
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: css(s)-hIgG1 (AA)

<400> SEQUENCE: 115

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Ser Ser
1 5 10 15

<210> SEQ ID NO 116
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: scs(s)-hIgG1 (DNA)

<400> SEQUENCE: 116

gagcccaaat cttctgacaa aactcacaca tgtccaccga gctca 45

<210> SEQ ID NO 117
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: scs(s)-hIgG1 (AA)

<400> SEQUENCE: 117

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Ser Ser
1 5 10 15

<210> SEQ ID NO 118
<211> LENGTH: 45

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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 118

gagcccaaat cttgtgacaa aactcacaca tgtccaccgt gctca 45

<210> SEQ ID NO 119
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 119

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Ser
1 5 10 15

<210> SEQ ID NO 120
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 120

gagcccaaat cttgtgacaa aactcacaca tgtccaccgt gccca 45

<210> SEQ ID NO 121
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 121

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

<210> SEQ ID NO 122
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 122

gagcccaaat cttctgacaa aactcacaca tctccaccga gccca 45

<210> SEQ ID NO 123
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 123

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Pro
1 5 10 15

<210> SEQ ID NO 124
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: csc(p)-hIgG1 (DNA)

<400> SEQUENCE: 124

gagcccaaat cttgtgacaa aactcacaca tctccaccgt gccca

45

<210> SEQ ID NO 125
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: csc(p)-hIgG1 (AA)

<400> SEQUENCE: 125

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys Pro
1 5 10 15

<210> SEQ ID NO 126
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: ssc(p)-hIgG1 (DNA)

<400> SEQUENCE: 126

gagcccaaat cttctgacaa aactcacaca tctccaccgt gccca

45

<210> SEQ ID NO 127
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: ssc(p)-hIgG1 (AA)

<400> SEQUENCE: 127

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Cys Pro
1 5 10 15

<210> SEQ ID NO 128
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: ssc(p)-hIgG1 (DNA)

<400> SEQUENCE: 128

gagcccaaat cttctgacaa aactcacaca tgtccaccgt gccca

45

<210> SEQ ID NO 129
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: scc(p)-hIgG1 (AA)

<400> SEQUENCE: 129

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

<210> SEQ ID NO 130
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: css(p)-hIgG1 (DNA)

<400> SEQUENCE: 130

gagcccaaat cttgtgacaa aactcacaca tctccaccga gccca 45

<210> SEQ ID NO 131
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: css(p)-hIgG1 (AA)

<400> SEQUENCE: 131

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Ser Pro
1 5 10 15

<210> SEQ ID NO 132
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: scs(p)-hIgG1 (DNA)

<400> SEQUENCE: 132

gagcccaaat cttctgacaa aactcacaca tgtccaccga gccca 45

<210> SEQ ID NO 133
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: scs(p)-hIgG1 (DNA)

<400> SEQUENCE: 133

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Ser Pro
1 5 10 15

<210> SEQ ID NO 134
<211> LENGTH: 18
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: scppcp (DNA)
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 134

agttgtccac cgtgccca

18

<210> SEQ ID NO 135
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 135

Ser Cys Pro Pro Cys Pro
1 5

<210> SEQ ID NO 136
<211> LENGTH: 7
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 136

tgatcag

7

<210> SEQ ID NO 137
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 137

Asp Gln
1

<210> SEQ ID NO 138
<211> LENGTH: 7
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 138

ctcgagt

7

<210> SEQ ID NO 139
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 139

Ser Ser
1

<210> SEQ ID NO 140
<211> LENGTH: 654

-continued

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: hIgG1 wild type

<400> SEQUENCE: 140
gcacctgaac tcttggtgg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc      60
ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac      120
cctgagggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag      180
ccgctggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac      240
caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc      300
cccatcgaga aaacaatctc caagccaaa gggcagcccc gagaaccaca ggtgtacacc      360
ctgcccccat cccgggatga gctgaccaag aaccagggtc gcctgacctg cctgggtcaaa      420
ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac      480
tacaagacca cgctcccggt gctggactcc gacggctcct tcttctctta cagcaagctc      540
accgtggaca agagcagggt gcagcagggg aacgtcttct catgctccgt gatgcatgag      600
gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa atga          654

```

```

<210> SEQ ID NO 141
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: hIgG1 wild type

```

```

<400> SEQUENCE: 141
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1           5           10           15
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20          25          30
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35          40          45
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50          55          60
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65          70          75          80
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85          90          95
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
100         105         110
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
115         120         125
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
130         135         140
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145         150         155         160
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
165         170         175
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
180         185         190
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
195         200         205

```

-continued

Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215

<210> SEQ ID NO 142
<211> LENGTH: 654
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: hIgG1 (P238S)

<400> SEQUENCE: 142

```
gcacctgaac tcttggtgg atcgtcagtc ttcctcttcc ccccaaaacc caaggacacc 60
ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac 120
cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 180
ccgcgaggag agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac 240
caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc 300
cccatacaga aaacaatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 360
ctgcccccat cccggatga gctgaccaag aaccaggtca gcctgacctg cctgggtcaaa 420
ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac 480
tacaagacca cgctcccggt gctggactcc gacggctcct tcttccteta cagcaagctc 540
accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 600
gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa atga 654
```

<210> SEQ ID NO 143
<211> LENGTH: 217
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: hIgG1 (P238S)

<400> SEQUENCE: 143

```
Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35 40 45
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85 90 95
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
100 105 110
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
115 120 125
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
130 135 140
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145 150 155 160
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
```

-continued

	165	170	175	
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val				
	180	185	190	
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln				
	195	200	205	
Lys Ser Leu Ser Leu Ser Pro Gly Lys				
	210	215		

<210> SEQ ID NO 144
 <211> LENGTH: 654
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: hIgG1 (P331S)

<400> SEQUENCE: 144

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gcacctgaac tcttggtgg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc      60
ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac      120
cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag      180
ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac      240
caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc      300
tccatcgaga aaacaatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc      360
ctgcccccat cccgggatga gctgaccaag aaccaggtca gcctgacctg cctgggtcaaa      420
ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac      480
tacaagacca cgctcccggt gctggactcc gacggctcct tcttctctta cagcaagctc      540
accgtggaca agagcagggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag      600
gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa atga          654
  
```

<210> SEQ ID NO 145
 <211> LENGTH: 217
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: hIgG1 (P331S)

<400> SEQUENCE: 145

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys				
1	5	10	15	
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val				
	20	25	30	
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr				
	35	40	45	
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu				
	50	55	60	
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His				
	65	70	75	80
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys				
	85	90	95	
Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln				
	100	105	110	
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu				
	115	120	125	

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Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
 130 135 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
 145 150 155 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
 165 170 175

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
 180 185 190

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
 195 200 205

Lys Ser Leu Ser Leu Ser Pro Gly Lys
 210 215

<210> SEQ ID NO 146
 <211> LENGTH: 654
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: hIgG1 (P238S/P331S)

<400> SEQUENCE: 146

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gcacctaagc tctgtgggtg atcgtaagtc ttctctctcc ccccaaaacc caaggacacc    60
ctcatgatct cccggacccc tgaggtcaca tgcgtgggtg tggacgtgag ccacgaagac    120
cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag    180
ccgcggggagg agcagtacaa cagcacgtac cgtgtgggtc gcgtcctcac cgtcctgcac    240
caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc    300
tccatcgaga aaacaatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc    360
ctgcccccat cccgggatga gctgaccaag aaccagggtc gctgacctg cctgggtcaaa    420
ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac    480
tacaagacca cgctcccggt gctggactcc gacggctcct tcttctctta cagcaagctc    540
accgtggaca agagcagggt gcagcagggg aacgtcttct catgctccgt gatgcatgag    600
gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa atga        654
```

<210> SEQ ID NO 147
 <211> LENGTH: 217
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: hIgG1 (P238S/P331S)

<400> SEQUENCE: 147

Ala Pro Glu Leu Leu Gly Gly Ser Ser Val Phe Leu Phe Pro Pro Lys
 1 5 10 15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
 20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
 35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 50 55 60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
 65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
 85 90 95

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Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
100 105 110
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
115 120 125
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
130 135 140
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145 150 155 160
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
165 170 175
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
180 185 190
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
195 200 205
Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215

<210> SEQ ID NO 148
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: STD1 (DNA)

<400> SEQUENCE: 148

aattatgggtg gcggtggctc gggcggtggt ggatctggag gaggtgggag tgggaattct 60

<210> SEQ ID NO 149
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: STD1 (AA)

<400> SEQUENCE: 149

Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15
Ser Gly Asn Ser
20

<210> SEQ ID NO 150
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: STD2 (DNA)

<400> SEQUENCE: 150

aattatgggtg gcggtggctc gggcggtggt ggatctggag gaggtgggag tgggaattat 60
ggtggcggtg gctcgggcgg tgggtggatct ggaggaggtg ggagtgggaa ttct 114

<210> SEQ ID NO 151
<211> LENGTH: 38
<212> TYPE: PRT

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: STD2 (AA)

<400> SEQUENCE: 151

Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15

Ser Gly Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 20 25 30

Gly Gly Ser Gly Asn Ser
 35

<210> SEQ ID NO 152
<211> LENGTH: 6
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H1 (PN)

<400> SEQUENCE: 152

aattct

6

<210> SEQ ID NO 153
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H1 (AA)

<400> SEQUENCE: 153

Asn Ser
1

<210> SEQ ID NO 154
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H2 (PN)

<400> SEQUENCE: 154

ggtggcggtg gtcggggaa ttct

24

<210> SEQ ID NO 155
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H2 (AA)

<400> SEQUENCE: 155

Gly Gly Gly Gly Ser Gly Asn Ser
1 5

<210> SEQ ID NO 156

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<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H3 (PN)

<400> SEQUENCE: 156

aattatggtg gcggtggctc tgggaattct

30

<210> SEQ ID NO 157
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H3 (AA)

<400> SEQUENCE: 157

Asn Tyr Gly Gly Gly Gly Ser Gly Asn Ser
1 5 10

<210> SEQ ID NO 158
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H4 (PN)

<400> SEQUENCE: 158

ggtggcgggtg gctcggggcgg tgggtgatct gggaattct

39

<210> SEQ ID NO 159
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H4 (AA)

<400> SEQUENCE: 159

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Asn Ser
1 5 10

<210> SEQ ID NO 160
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H5 (PN)

<400> SEQUENCE: 160

aattatggtg gcggtggctc gggcgggtggt ggatctggga attct

45

<210> SEQ ID NO 161
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

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<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H5 (AA)

<400> SEQUENCE: 161

Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Asn Ser
1 5 10 15

<210> SEQ ID NO 162
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H6 (PN)

<400> SEQUENCE: 162

ggtagcggtg gctcggcggtg tggtaggatct gggggaggag gcagcgggaa ttct 54

<210> SEQ ID NO 163
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H6 (AA)

<400> SEQUENCE: 163

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Asn Ser

<210> SEQ ID NO 164
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H7 (PN)

<400> SEQUENCE: 164

gggtgtccac ctgtccgaa ttct 24

<210> SEQ ID NO 165
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H7 (AA)

<400> SEQUENCE: 165

Gly Cys Pro Pro Cys Pro Asn Ser
1 5

<210> SEQ ID NO 166
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer
<220> FEATURE:
<223> OTHER INFORMATION: Linker(G4S)3

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<400> SEQUENCE: 166

ggtaggcgggtg gatccggcgg aggtgggtcg ggtggcggcg gatct 45

<210> SEQ ID NO 167

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Linker(G4S)3

<400> SEQUENCE: 167

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 1 5 10

<210> SEQ ID NO 168

<211> LENGTH: 60

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic primer

<220> FEATURE:

<223> OTHER INFORMATION: Linker(G4S)4

<400> SEQUENCE: 168

ggtaggcgggtg gctcgggcgg tggtaggatct ggaggaggtg ggagcggggg aggtggcagt 60

<210> SEQ ID NO 169

<211> LENGTH: 17

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Linker(G4S)4

<400> SEQUENCE: 169

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly
 1 5 10 15

Ser

<210> SEQ ID NO 170

<211> LENGTH: 2337

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-STD1-2e12HL (DNA)

<400> SEQUENCE: 170

aagcttgccg ccatggattt tcaagtgcag attttcagct tctgctaata cagtgcattca 60

gtcataatgt ccagaggaca aattgttctc tcccagcttc cagcaatcct gtctgcatct 120

ccaggggaga aggtcacaat gacttgacagg gccagctcaa gtgtaagtta catgcactgg 180

taccagcaga agccaggatc ctcccccaaa ccctggattt atgccccatc caacctggct 240

tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc 300

agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaacca 360

cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcgggtgt 420

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ggatctggag gagtgaggag ctctcaggt tatctacagc agtctggggc tgagtcggtg	480
aggcctgggg cctcagtga gatgtcctgc aaggtctctg gctacacatt taccagttac	540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat	600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta	660
gacaaatcct ccagcacagc ctacatgcag ctacgcagcc tgacatctga agactctgcg	720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggactct cgatgtctgg	780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac	840
acatccccac cgagctcagc acctgaactc ctggggggac cgtcagtcct cctcttcccc	900
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtggtggtg	960
gacgtgagcc acgaagacc tgaggtaag ttcaactggt acgtggacgg cgtggagggtg	1020
cataatgcc agacaagcc ggggaggag cagtacaaca gcacgtaccg tgtggtcagc	1080
gtctcaccg tctgcacca ggactggctg aatggcaagg agtacaagt caaggtctcc	1140
aacaaagccc tccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccag cctcccgctg tggactccga cggctccttc	1380
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcatgagc tctgcacaac cactacacgc agaagagcct ctccctgtct	1500
ccgggtaaga attatggtgg cgggtgctcg ggcgggtggt gatctggagg aggtgggagt	1560
gggaattctc aggtgcagct gaaggagtca ggacctggcc tgggtggcgc ctccacagagc	1620
ctgtccatca catgcaccgt ctacgggttc tcattaaccg gctatggtgt aaactggggt	1680
cgccagcctc caggaaagg tctggagtg ctgggaatga tatggggtga tggaagcaca	1740
gactataatt cagctctcaa atccagacta tcgatcacca aggacaactc caagagccaa	1800
gttttcttaa aaatgaacag tctgcaaact gatgacacag ccagatacta ctgtgctcga	1860
gatgggtata gtaactttca ttactatgt atggactact ggggtcaagg aacctcagtc	1920
accgtctcct ctgggggtgg aggtctggt ggcgggtgat ccggcggagg tgggtcgggt	1980
ggcggcggat ctgacattgt gctcaccaca tctccagctt ctttggctgt gtctctaggt	2040
cagagagcca ccatctcctg cagagccagt gaaagtgtg aatattatgt cacaagtta	2100
atgcagtgtg accaacagaa accaggacag ccacccaaac tcctcatctc tgetgctagc	2160
aacgtagaat ctgggggtccc tgccagggtt agtggcagtg ggtctgggac agacttttagc	2220
ctcaacatcc atcctgtgga ggagatgat attgcaatgt atttctgtca gcaaagtagg	2280
aaggttccat ggacgttcgg tggaggcacc aagctggaaa tcaaacgtta atctaga	2337

<210> SEQ ID NO 171

<211> LENGTH: 772

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polypeptide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-STD1-2e12HL (AA)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (1)..(22)

<223> OTHER INFORMATION: Leader

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(281)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(519)
<223> OTHER INFORMATION: EPD-Scorpion
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (520)..(640)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (641)..(660)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (661)..(772)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 171

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1          5          10          15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
          20          25          30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
          35          40          45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
          50          55          60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
          65          70          75          80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
          85          90          95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
          100         105         110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
          115         120         125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
          130         135         140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
          145         150         155         160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
          165         170         175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
          180         185         190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
          195         200         205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
          210         215         220

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Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	
225					230					235					240	
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	
				245					250					255		
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser	
			260					265					270			
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Ser	Ala	Pro	Glu	Leu	Leu	Gly	
	275						280					285				
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	
	290					295					300					
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	
305					310				315						320	
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	
			325					330						335		
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	
		340						345					350			
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	
		355					360					365				
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	
	370					375					380					
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	
385					390				395						400	
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	
			405					410						415		
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	
		420						425					430			
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	
		435					440					445				
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	
	450					455					460					
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	
465					470				475						480	
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	
			485						490					495		
Pro	Gly	Lys	Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	
			500				505						510			
Gly	Gly	Gly	Ser	Gly	Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	
		515					520					525				
Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	
	530					535					540					
Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	
545					550				555						560	
Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	
			565					570						575		
Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	
			580					585					590			
Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	
		595					600					605				
Thr	Ala	Arg	Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr	
	610					615					620					
Tyr	Val	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	
625					630				635						640	

-continued

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
645 650 655

Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
660 665 670

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser
675 680 685

Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro
690 695 700

Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn Val Glu Ser
705 710 715 720

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser
725 730 735

Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys
740 745 750

Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
755 760 765

Glu Ile Lys Arg
770

<210> SEQ ID NO 172
<211> LENGTH: 772
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1(P238S/P331S)-STD1-2e12HL (w/2e12
leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
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<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
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<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(519)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (520)..(640)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (641)..(660)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (661)..(772)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 172

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Met	Asp	Phe	Gln	Val	Gln	Ile	Phe	Ser	Phe	Leu	Leu	Ile	Ser	Ala	Ser	1	5	10	15
Val	Ile	Met	Ser	Arg	Gly	Gln	Ile	Val	Leu	Ser	Gln	Ser	Pro	Ala	Ile	20	25	30	
Leu	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	Ala	Ser	35	40	45	
Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser	50	55	60	
Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Pro	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	65	70	75	80
Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	85	90	95	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	100	105	110	
Ser	Phe	Asn	Pro	Pro	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	115	120	125	
Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	130	135	140	
Gln	Ala	Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Ser	Val	Arg	Pro	Gly	Ala	145	150	155	160
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	165	170	175	
Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile	180	185	190	
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	195	200	205	
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	210	215	220	
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	225	230	235	240
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	245	250	255	
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser	260	265	270	
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Ser	Ala	Pro	Glu	Leu	Leu	Gly	275	280	285	
Gly	Ser	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	290	295	300	
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	305	310	315	320
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	325	330	335	
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	340	345	350	
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	355	360	365	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Ser	Ile	370	375	380	
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	385	390	395	400
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	405	410	415	

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Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 420 425 430
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 435 440 445
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 450 455 460
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 465 470 475 480
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 485 490 495
 Pro Gly Lys Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 500 505 510
 Gly Gly Gly Ser Gly Asn Ser Gln Val Gln Leu Lys Glu Ser Gly Pro
 515 520 525
 Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser
 530 535 540
 Gly Phe Ser Leu Thr Gly Tyr Gly Val Asn Trp Val Arg Gln Pro Pro
 545 550 555 560
 Gly Lys Gly Leu Glu Trp Leu Gly Met Ile Trp Gly Asp Gly Ser Thr
 565 570 575
 Asp Tyr Asn Ser Ala Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Asn
 580 585 590
 Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp
 595 600 605
 Thr Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr
 610 615 620
 Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 625 630 635 640
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 645 650 655
 Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
 660 665 670
 Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser
 675 680 685
 Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro
 690 695 700
 Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn Val Glu Ser
 705 710 715 720
 Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser
 725 730 735
 Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys
 740 745 750
 Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
 755 760 765
 Glu Ile Lys Arg
 770

<210> SEQ ID NO 173

<211> LENGTH: 2322

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

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<220> FEATURE:

<223> OTHER INFORMATION: 2H7ssIgG1-STD1-2e12LH (DNA)

<400> SEQUENCE: 173

aagcttgccg ccatggattt tcaagtgcag attttcagct tcctgctaata cagtgcctca	60
gtcataatgt ccagaggaca aattgttctc tcccagtctc cagcaatcct gtctgcatct	120
ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg	180
taccagcaga agccaggatc ctccccaaa cctgggattt atgccccatc caacctggct	240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc	300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaacca	360
ccccagttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcgggtgg	420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtcgggtg	480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac	540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat	600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta	660
gacaaatcct ccagcacagc ctacatgcag ctacagagcc tgacatctga agactctgcg	720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggactct cgatgtctgg	780
ggcacaggga ccacggctac cgtctctgat caggagccca aatcttctga caaaactcac	840
acatccccac cgagcccagc acctgaactc ctggggggac cgtcagctct cctcttcccc	900
ccaaaaccca aggacacct catgatctcc cggaccctcg aggtcacatg cgtgggtggg	960
gacgtgagcc acgaagaccc tgaggtcaag ttcaactggc acgtggacgg cgtggagggtg	1020
cataatgcc aagacaaagc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc	1080
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc	1140
aacaaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtacacct gcccccctcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccagc cctcccgctg tggactccga cgctccttc	1380
ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcattgagg tctgcacaac cactacacgc agaagagcct ctccctgtct	1500
ccgggtaaga attatgggtg cgggtggctg ggcgggtggg gatctggagg aggtgggagt	1560
gggaattctg acattgtgct caccatctt ccagcttctt tggctgtgct tctaggtcag	1620
agagccacca tctcctgcag agccagtga agtgttgaat attatgtcac aagtttaatg	1680
cagtggtagc aacagaaacc aggacagcca cccaaactcc tcatctctgc tgctagcaac	1740
gtagaatctg gggctccctg cagggttagt ggcagtgggt ctgggacaga ctttagcctc	1800
aacatccatc ctgtggagga ggatgatatt gcaatgtatt tctgtcagca aagtaggaag	1860
gttccatgga cgttcgggtg aggcaccaag ctggaaatca aacgggggtg cggtggatcc	1920
ggcggagggt ggtcgggtg cggcggatct cagggtgcagc tgaaggagtc aggacctggc	1980
ctgggtgggc cctcacagag cctgtccatc acatgcaccg tctcagggtt ctcatatacc	2040
ggctatgggt taaactgggt tcgccagcct ccaggaaagg gtctggagtg gctgggaaatg	2100
atatgggggt atggaagcac agactataat tcagctctca aatccagact atcgatcacc	2160

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aaggacaact ccaagagcca agttttctta aaaatgaaca gtctgcaaac tgatgacaca 2220
gccagatact actgtgctcg agatgggttat agtaactttc attactatgt tatggactac 2280
tggggtcaag gaacctcagt caccgtctcc tcttaatcta ga 2322

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<210> SEQ ID NO 174
<211> LENGTH: 767
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-STD1-2e12LH (w/2e12 leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(519)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (520)..(631)
<223> OTHER INFORMATION: VL2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (632)..(646)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (647)..(767)
<223> OTHER INFORMATION: VH2

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<400> SEQUENCE: 174

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Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1           5           10           15

```

```

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20           25           30

```

```

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35           40           45

```

```

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
50           55           60

```

```

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65           70           75           80

```

```

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
85           90           95

```

```

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
100          105          110

```

```

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
115          120          125

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Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser
130					135					140					
Gln	Ala	Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Ser	Val	Arg	Pro	Gly	Ala
145				150					155					160	
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr
			165						170					175	
Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile
			180					185					190		
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe
		195					200					205			
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
	210					215					220				
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
225					230					235					240
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp
				245					250					255	
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser
			260					265					270		
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Ser	Ala	Pro	Glu	Leu	Leu	Gly
		275				280						285			
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
	290					295					300				
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
305					310					315					320
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
			325						330					335	
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Gln	Gln	Tyr	Asn	Ser	Thr	Tyr
			340					345					350		
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
		355					360					365			
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
	370					375					380				
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
385					390					395					400
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
			405						410					415	
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
			420					425					430		
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
		435					440					445			
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
	450					455					460				
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
465					470					475					480
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
			485					490						495	
Pro	Gly	Lys	Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly
			500					505					510		
Gly	Gly	Gly	Ser	Gly	Asn	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala
		515					520					525			
Ser	Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala

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

<210> SEQ ID NO 175
 <211> LENGTH: 767
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1(P238S/P331S)-STD1-2e12LH (w/2e12 leader) (AA)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(22)
 <223> OTHER INFORMATION: Leader
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (23)..(128)
 <223> OTHER INFORMATION: VL
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (129)..(144)
 <223> OTHER INFORMATION: Linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (145)..(265)
 <223> OTHER INFORMATION: VH
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (268)..(282)
 <223> OTHER INFORMATION: Hinge
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (500)..(519)

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<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (520) .. (631)
<223> OTHER INFORMATION: VL2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (632) .. (646)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (647) .. (767)
<223> OTHER INFORMATION: VH2

<400> SEQUENCE: 175

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1 5 10 15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20 25 30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35 40 45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
50 55 60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65 70 75 80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
85 90 95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
100 105 110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
115 120 125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
130 135 140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145 150 155 160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
165 170 175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
180 185 190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
195 200 205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
210 215 220

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
225 230 235 240

Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
245 250 255

Gly Thr Gly Thr Thr Val Thr Val Ser Asp Gln Glu Pro Lys Ser Ser
260 265 270

Asp Lys Thr His Thr Ser Pro Pro Ser Ser Ala Pro Glu Leu Leu Gly
275 280 285

Gly Ser Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
290 295 300

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
305 310 315 320

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

-continued

325								330				335					
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr		
			340				345						350				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly		
		355				360					365						
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Ser	Ile		
370					375					380							
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val		
385				390						395			400				
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser		
			405						410					415			
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu		
			420					425					430				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro		
		435					440					445					
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val		
450					455					460							
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met		
465				470						475			480				
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser		
			485						490					495			
Pro	Gly	Lys	Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly		
			500					505					510				
Gly	Gly	Gly	Ser	Gly	Asn	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala		
			515					520					525				
Ser	Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala		
530					535					540							
Ser	Glu	Ser	Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln		
545				550						555			560				
Gln	Lys	Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn		
			565						570					575			
Val	Glu	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr		
			580					585					590				
Asp	Phe	Ser	Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala	Met		
		595					600					605					
Tyr	Phe	Cys	Gln	Gln	Ser	Arg	Lys	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly		
610					615						620						
Thr	Lys	Leu	Glu	Ile	Lys	Arg	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly		
625				630						635			640				
Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly		
			645					650					655				
Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly		
			660					665					670				
Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly		
		675					680					685					
Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp		
690					695						700						
Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser		
705				710						715			720				
Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr		
			725					730					735				

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Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr Tyr
740 745 750

Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
755 760 765

<210> SEQ ID NO 176
<211> LENGTH: 2376
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-STD2-2e12LH (DNA)

<400> SEQUENCE: 176

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aagcttgccg ccatggattt tcaagtgcag attttcagct tcctgctaata cagtgccttca    60
gtcataatgt ccagaggaca aattgttctc tcccagctctc cagcaatcct gtctgcatct    120
ccaggggaga aggtcacaat gacttgacagg gccagctcaa gtgtaagtta catgcactgg    180
taccagcaga agccaggatc ctcccccaaa cctgggattt atgccccatc caacctggct    240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc    300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaaccca    360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt    420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtcgggtg    480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac    540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat    600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta    660
gacaaatcct ccagcacagc ctacatgcag ctacgcagcc tgacatctga agactctgcg    720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggactct cgatgtctgg    780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac    840
acatccccac cgagcccagc acctgaactc ctgggggggac cgtcagctct cctcttcccc    900
ccaaaaccca aggacacct catgatctcc cggaaccttg aggtcacatg cgtgggtggtg    960
gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg    1020
cataatgcc aagacaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc    1080
gtctctaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc    1140
aacaaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga    1200
gaaccacagg tgtacacct gcccccaccc cgggatgagc tgaccaagaa ccaggtcagc    1260
ctgacctgcc tggtaaaagg cttctatccc agcgacatcg cgtggagtg ggagagcaat    1320
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc    1380
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca    1440
tgctccgtga tgcatgaggc tctgcacaac cactacagc agaagagcct ctccctgtct    1500
ccgggtaaga attatggtgg cgggtggctc ggcgggtggt gatctggagg aggtgggagt    1560
gggaattatg gtggcggtgg ctcgggcggt ggtggatctg gaggaggtgg gagggggaat    1620
tctgacattg tgctaccca atctccagct tctttggctg tgtctctagg tcagagagcc    1680
accatctcct gcagagccag tgaaagtgtt gaatattatg tcacaagttt aatgcagtgg    1740
taccacaga aaccaggaca gccacccaaa ctctcatct ctgctgctag caacgtagaa    1800

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tctgggggtcc ctgccagggt tagtggcagt gggctctggga cagacttttag cctcaacatc 1860
catcctgtgg aggaggatga tattgcaatg ttttctgtc agcaaagtag gaagggtcca 1920
tggaagcttc gtggaggcac caagtggaa atcaaacggg gtggcggtgg atccggcgga 1980
ggtagggctcg gtggcgcggt atctcagggt cagctgaagg agtcaggacc tggcctggtg 2040
gcgcctctac agagcctgtc catcacatgc accgtctcag ggttctcatt aaccggctat 2100
ggtgtaaaact gggttcgcca gcctccagga aagggtctgg agtggctggg aatgatatgg 2160
ggtgatggaa gcacagacta taattcagct ctcaaatacca gactatcgat caccaaggac 2220
aactccaaga gccaaagtttt cttaaaaatg aacagtctgc aaactgatga cacagccaga 2280
tactactgtg ctcgagatgg ttatagtaac tttcattact atgttatgga ctactggggg 2340
caaggaacct cagtcaccgt ctcctcttaa tctaga 2376

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<210> SEQ ID NO 177
<211> LENGTH: 785
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-STD2-2e12LH (w/2e12 leader)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(129)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (130)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (266)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(537)
<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (538)..(649)
<223> OTHER INFORMATION: VL2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (650)..(664)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (665)..(785)
<223> OTHER INFORMATION: VH2

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<400> SEQUENCE: 177

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Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1             5             10             15

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Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20             25             30

```

```

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35             40             45

```

Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser
Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Pro	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile
Ser	Arg	Val	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp
Ser	Phe	Asn	Pro	Pro	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys
Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser
Gln	Ala	Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Ser	Val	Arg	Pro	Gly	Ala
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr
Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Ser	Ala	Pro	Glu	Leu	Leu	Gly
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val

-continued

450	455	460
Asp Lys Ser Arg Trp	Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met	
465	470	475 480
His Glu Ala Leu His	Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser	
	485	490 495
Pro Gly Lys Asn Tyr	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly	
	500	505 510
Gly Gly Gly Ser Gly	Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly	
	515	520 525
Ser Gly Gly Gly Gly	Ser Gly Asn Ser Asp Ile Val Leu Thr Gln Ser	
	530	535 540
Pro Ala Ser Leu Ala	Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys	
545	550	555 560
Arg Ala Ser Glu Ser	Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp	
	565	570 575
Tyr Gln Gln Lys Pro	Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala	
	580	585 590
Ser Asn Val Glu Ser	Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser	
	595	600 605
Gly Thr Asp Phe Ser	Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile	
	610	615 620
Ala Met Tyr Phe Cys	Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly	
625	630	635 640
Gly Gly Thr Lys Leu	Glu Ile Lys Arg Gly Gly Gly Gly Ser Gly Gly	
	645	650 655
Gly Gly Ser Gly Gly	Gly Gly Ser Gln Val Gln Leu Lys Glu Ser Gly	
	660	665 670
Pro Gly Leu Val Ala	Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val	
	675	680 685
Ser Gly Phe Ser Leu	Thr Gly Tyr Gly Val Asn Trp Val Arg Gln Pro	
	690	695 700
Pro Gly Lys Gly Leu	Glu Trp Leu Gly Met Ile Trp Gly Asp Gly Ser	
705	710	715 720
Thr Asp Tyr Asn Ser	Ala Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp	
	725	730 735
Asn Ser Lys Ser Gln	Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp	
	740	745 750
Asp Thr Ala Arg Tyr	Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His	
	755	760 765
Tyr Tyr Val Met Asp	Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser	
	770	775 780
Ser		
785		

<210> SEQ ID NO 178
 <211> LENGTH: 785
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1(P238S/P331S)-STD2-2e12LH (w/2e12 leader) (AA)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature

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<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(281)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(537)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (538)..(649)
<223> OTHER INFORMATION: VL2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (650)..(664)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (665)..(785)
<223> OTHER INFORMATION: VH2

<400> SEQUENCE: 178

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1           5           10          15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20          25          30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35          40          45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
50          55          60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65          70          75          80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
85          90          95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
100         105         110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
115         120         125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
130         135         140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145         150         155         160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
165         170         175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
180         185         190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
195         200         205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr

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210					215					220						
Met 225	Gln	Leu	Ser	Ser	Leu 230	Thr	Ser	Glu	Asp	Ser 235	Ala	Val	Tyr	Phe	Cys 240	
Ala	Arg	Val	Val	Tyr 245	Tyr	Ser	Asn	Ser	Tyr 250	Trp	Tyr	Phe	Asp	Val 255	Trp	
Gly	Thr	Gly	Thr	Thr 260	Val	Thr	Val	Ser 265	Asp	Gln	Glu	Pro	Lys 270	Ser	Ser	
Asp	Lys	Thr	His	Thr 275	Ser	Pro	Pro 280	Ser	Ser	Ala	Pro	Glu 285	Leu	Leu	Gly	
Gly	Ser 290	Ser	Val	Phe	Leu	Phe 295	Pro	Pro	Lys	Pro	Lys 300	Asp	Thr	Leu	Met	
Ile 305	Ser	Arg	Thr	Pro	Glu 310	Val	Thr	Cys	Val	Val 315	Val	Asp	Val	Ser	His 320	
Glu	Asp	Pro	Glu	Val 325	Lys	Phe	Asn	Trp	Tyr 330	Val	Asp	Gly	Val	Glu 335	Val	
His	Asn	Ala	Lys 340	Thr	Lys	Pro	Arg	Glu 345	Glu	Gln	Tyr	Asn 350	Ser	Thr	Tyr	
Arg	Val	Val 355	Ser	Val	Leu	Thr	Val 360	Leu	His	Gln	Asp	Trp 365	Leu	Asn	Gly	
Lys 370	Glu	Tyr	Lys	Cys	Lys 375	Val	Ser	Asn	Lys	Ala	Leu 380	Pro	Ala	Ser	Ile	
Glu 385	Lys	Thr	Ile	Ser	Lys 390	Ala	Lys	Gly	Gln	Pro 395	Arg	Glu	Pro	Gln	Val 400	
Tyr	Thr	Leu	Pro 405	Pro	Ser	Arg	Asp	Glu 410	Leu	Thr	Lys	Asn	Gln	Val 415	Ser	
Leu	Thr	Cys	Leu 420	Val	Lys	Gly	Phe	Tyr 425	Pro	Ser	Asp	Ile 430	Ala	Val	Glu	
Trp	Glu	Ser 435	Asn	Gly	Gln	Pro	Glu 440	Asn	Asn	Tyr	Lys	Thr 445	Thr	Pro	Pro	
Val 450	Leu	Asp	Ser	Asp	Gly 455	Ser	Phe	Phe	Leu	Tyr 460	Ser	Lys	Leu	Thr	Val	
Asp 465	Lys	Ser	Arg	Trp	Gln 470	Gln	Gly	Asn	Val	Phe 475	Ser	Cys	Ser	Val	Met 480	
His	Glu	Ala	Leu 485	His	Asn	His	Tyr	Thr 490	Gln	Lys	Ser	Leu	Ser	Leu 495	Ser	
Pro	Gly	Lys	Asn 500	Tyr	Gly	Gly	Gly	Gly 505	Ser	Gly	Gly	Gly 510	Gly	Ser	Gly	
Gly	Gly	Gly 515	Ser	Gly	Asn	Tyr	Gly 520	Gly	Gly	Gly	Ser	Gly 525	Gly	Gly	Gly	
Ser 530	Gly	Gly	Gly	Gly	Ser	Gly 535	Asn	Ser	Asp	Ile 540	Val	Leu	Thr	Gln	Ser	
Pro 545	Ala	Ser	Leu	Ala 550	Val	Ser	Leu	Gly	Gln	Arg 555	Ala	Thr	Ile	Ser	Cys 560	
Arg	Ala	Ser	Glu 565	Ser	Val	Glu	Tyr	Tyr 570	Val	Thr	Ser	Leu	Met	Gln 575	Trp	
Tyr	Gln	Gln 580	Lys	Pro	Gly	Gln	Pro	Pro 585	Lys	Leu	Leu	Ile 590	Ser	Ala	Ala	
Ser	Asn 595	Val	Glu	Ser	Gly	Val	Pro 600	Ala	Arg	Phe	Ser	Gly 605	Ser	Gly	Ser	
Gly	Thr 610	Asp	Phe	Ser	Leu 615	Asn	Ile	His	Pro	Val 620	Glu	Glu	Asp	Asp	Ile	

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Ala Met Tyr Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly
625 630 635 640

Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly Gly Ser Gly Gly
645 650 655

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Lys Glu Ser Gly
660 665 670

Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val
675 680 685

Ser Gly Phe Ser Leu Thr Gly Tyr Gly Val Asn Trp Val Arg Gln Pro
690 695 700

Pro Gly Lys Gly Leu Glu Trp Leu Gly Met Ile Trp Gly Asp Gly Ser
705 710 715 720

Thr Asp Tyr Asn Ser Ala Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp
725 730 735

Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp
740 745 750

Asp Thr Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His
755 760 765

Tyr Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
770 775 780

Ser
785

<210> SEQ ID NO 179

<211> LENGTH: 2391

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-STD2-2e12HL (DNA)

<400> SEQUENCE: 179

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aagcttgccg ccatggattt tcaagtgcag attttcagct tctgctaata cagtgcctca 60
gtcataatgt ccagaggaca aattgtttct tccagtcctc cagcaatcct gtctgcatct 120
ccaggggaga aggtcacaat gacttgacagg gccagctcaa gtgtaagtta catgcactgg 180
taccagcaga agccaggatc ctcccccaaa ccctggattt atgccccatc caacctggct 240
tctggagtc cgtctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc 300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaacca 360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt 420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtcggtg 480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac 540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat 600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta 660
gacaaatcct ccagcacagc ctacatgcag ctacgcagcc tgacatctga agactctgcg 720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggctactt cgatgtctgg 780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac 840
acatccccac cgagcccagc acctgaactc ctggggggac cgtcagctct cctcttcccc 900
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtgggtggtg 960

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gacgtgagcc acgaagaccc tgagggtcaag ttcaactggt acgtggacgg cgtggagggtg	1020
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtgggcagc	1080
gtcttcaccg tcttgccacca ggactggctg aatggcaagg agtacaagtg caaggtctcc	1140
aacaaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtacaccct gcccctatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc	1380
ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcattgagc tctgcacaac cactacacgc agaagagcct ctccctgtct	1500
ccgggtaaga attatggtgg cgggtggctcg ggcgggtggg gatctggagg aggtgggagt	1560
gggaattatg gtggcgggtg ctcggggcgg ggtggatctg gaggaggtgg gagtgggaat	1620
tctcaggtgc agctgaagga gtcaggacct ggctgggtgg cgccctcaca gagcctgtcc	1680
atcacatgca ccgtctcagg gttctcatta accggctatg gtgtaactg ggttcgccag	1740
cctccaggaa agggctctgga gtggctggga atgatatggg gtgatggaag cacagactat	1800
aattcagctc tcaaatccag actatcgatc accaaggaca actccaagag ccaagttttc	1860
ttaaaaatga acagtctgca aactgatgac acagccagat actactgtgc tcgagatggt	1920
tatagtaact ttcattacta tggtatggac tactggggtc aaggaacctc agtcaccgtc	1980
tcctctgggg gtggaggctc tgggtggcgg ggatccggcg gaggtgggtc gggtgccggc	2040
ggatctgaca ttgtgctcac ccaatctcca gcttctttgg ctgtgtctct aggtcagaga	2100
gccaccatct cctgcagagc cagtgaaggt gttgaatatt atgtcacaag tttaatgcag	2160
tggtaccaac agaaaccagg acagccaccc aaactcctca tctctgctgc tagcaacgta	2220
gaatctgggg tccctgccag gtttagtggc agtgggtctg ggacagactt tagcctcaac	2280
atccatcctg tggaggagga tgatattgca atgtatttct gtcagcaaag taggaagggt	2340
ccatggacgt tcggtggagg caccaagctg gaaatcaaac gttaatctag a	2391

<210> SEQ ID NO 180
 <211> LENGTH: 790
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1-STD2-2e12HL (w/2e12 leader) (AA)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(22)
 <223> OTHER INFORMATION: Leader
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (23)..(128)
 <223> OTHER INFORMATION: VL
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (129)..(144)
 <223> OTHER INFORMATION: Linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (145)..(265)
 <223> OTHER INFORMATION: VH
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (266)..(282)
 <223> OTHER INFORMATION: Hinge

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(537)
<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (538)..(658)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (659)..(678)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (679)..(790)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 180

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1 5 10 15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20 25 30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35 40 45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
50 55 60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65 70 75 80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
85 90 95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
100 105 110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
115 120 125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
130 135 140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145 150 155 160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
165 170 175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
180 185 190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
195 200 205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
210 215 220

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
225 230 235 240

Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
245 250 255

Gly Thr Gly Thr Thr Val Thr Val Ser Asp Gln Glu Pro Lys Ser Ser
260 265 270

Asp Lys Thr His Thr Ser Pro Pro Ser Ser Ala Pro Glu Leu Leu Gly
275 280 285

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
290 295 300

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

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305	310	315	320
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val	325	330	335
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr	340	345	350
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly	355	360	365
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile	370	375	380
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val	385	390	395
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser	405	410	415
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu	420	425	430
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro	435	440	445
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val	450	455	460
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met	465	470	475
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser	485	490	495
Pro Gly Lys Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly	500	505	510
Gly Gly Gly Ser Gly Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly	515	520	525
Ser Gly Gly Gly Gly Ser Gly Asn Ser Gln Val Gln Leu Lys Glu Ser	530	535	540
Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr	545	550	555
Val Ser Gly Phe Ser Leu Thr Gly Tyr Gly Val Asn Trp Val Arg Gln	565	570	575
Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly Met Ile Trp Gly Asp Gly	580	585	590
Ser Thr Asp Tyr Asn Ser Ala Leu Lys Ser Arg Leu Ser Ile Thr Lys	595	600	605
Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr	610	615	620
Asp Asp Thr Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe	625	630	635
His Tyr Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val	645	650	655
Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly	660	665	670
Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser	675	680	685
Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser	690	695	700
Glu Ser Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln Gln	705	710	715
			720

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Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn Val
 725 730 735

 Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
 740 745 750

 Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr
 755 760 765

 Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly Thr
 770 775 780

 Lys Leu Glu Ile Lys Arg
 785 790

<210> SEQ ID NO 181
 <211> LENGTH: 790
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1(P238S/P331S)-STD2-2e12HL (w/2e12
 leader) (AA)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(22)
 <223> OTHER INFORMATION: Leader
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (23)..(128)
 <223> OTHER INFORMATION: VL
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (129)..(144)
 <223> OTHER INFORMATION: Linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (145)..(265)
 <223> OTHER INFORMATION: VH
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (268)..(282)
 <223> OTHER INFORMATION: Hinge
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (500)..(537)
 <223> OTHER INFORMATION: EPD-Scorpion linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (538)..(658)
 <223> OTHER INFORMATION: VH2
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (659)..(678)
 <223> OTHER INFORMATION: Linker2
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (679)..(790)
 <223> OTHER INFORMATION: VL2

<400> SEQUENCE: 181

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
 1 5 10 15

 Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
 20 25 30

 Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
 35 40 45

 Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
 50 55 60

 Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro

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65	70	75	80
Ala Arg Phe Ser Gly 85	Ser Gly Ser Gly Thr 90	Ser Tyr Ser Leu Thr Ile 95	
Ser Arg Val Glu Ala Glu Asp 100	Ala Ala Thr Tyr Tyr Cys 105	Gln Gln Trp 110	
Ser Phe Asn Pro Pro Thr Phe 115	Gly Ala Gly Thr Lys Leu 120	Glu Leu Lys 125	
Asp Gly Gly Gly Ser Gly 130	Gly Gly Gly Ser Gly 135	Gly Gly Gly Ser Ser 140	
Gln Ala Tyr Leu Gln Gln Ser 145	Gly Ala Glu Ser Val Arg 150	Pro Gly Ala 155	
Ser Val Lys Met Ser Cys Lys 165	Ala Ser Gly Tyr Thr Phe 170	Thr Ser Tyr 175	
Asn Met His Trp Val Lys Gln 180	Thr Pro Arg Gln Gly Leu 185	Glu Trp Ile 190	
Gly Ala Ile Tyr Pro Gly Asn 195	Gly Asp Thr Ser Tyr Asn 200	Gln Lys Phe 205	
Lys Gly Lys Ala Thr Leu Thr 210	Val Asp Lys Ser Ser Thr 215	Ala Tyr 220	
Met Gln Leu Ser Ser Leu Thr 225	Ser Glu Asp Ser Ala Val 230	Tyr Phe Cys 235	
Ala Arg Val Val Tyr Tyr Ser 245	Asn Ser Tyr Trp Tyr Phe 250	Asp Val Trp 255	
Gly Thr Gly Thr Thr Val Thr 260	Val Ser Asp Gln Glu Pro 265	Lys Ser Ser 270	
Asp Lys Thr His Thr Ser Pro 275	Pro Ser Ser Ala Pro Glu 280	Leu Leu Gly 285	
Gly Ser Ser Val Phe Leu Phe 290	Pro Pro Lys Pro Lys Asp 295	Thr Leu Met 300	
Ile Ser Arg Thr Pro Glu Val 305	Thr Cys Val Val Val Asp 310	Val Ser His 315	
Glu Asp Pro Glu Val Lys Phe 325	Asn Trp Tyr Val Asp Gly 330	Val Glu Val 335	
His Asn Ala Lys Thr Lys Pro 340	Arg Glu Glu Gln Tyr Asn 345	Ser Thr Tyr 350	
Arg Val Val Ser Val Leu Thr 355	Val Leu His Gln Asp Trp 360	Leu Asn Gly 365	
Lys Glu Tyr Lys Cys Lys Val 370	Ser Asn Lys Ala Leu Pro 375	Ala Ser Ile 380	
Glu Lys Thr Ile Ser Lys Ala 385	Lys Gly Gln Pro Arg Glu 390	Pro Gln Val 395	
Tyr Thr Leu Pro Pro Ser Arg 405	Asp Glu Leu Thr Lys Asn 410	Gln Val Ser 415	
Leu Thr Cys Leu Val Lys Gly 420	Phe Tyr Pro Ser Asp Ile 425	Ala Val Glu 430	
Trp Glu Ser Asn Gly Gln Pro 435	Glu Asn Asn Tyr Lys Thr 440	Thr Pro Pro 445	
Val Leu Asp Ser Asp Gly Ser 450	Phe Phe Leu Tyr Ser Lys 455	Leu Thr Val 460	
Asp Lys Ser Arg Trp Gln Gln 465	Gly Asn Val Phe Ser Cys 470	Ser Val Met 475	
			480

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His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	
				485					490						495	
Pro	Gly	Lys	Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	
			500					505					510			
Gly	Gly	Gly	Ser	Gly	Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	
		515					520					525				
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	
	530					535					540					
Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	
545					550					555					560	
Val	Ser	Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	
			565					570						575		
Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	
			580					585					590			
Ser	Thr	Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	
		595				600					605					
Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	
	610					615					620					
Asp	Asp	Thr	Ala	Arg	Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	
625					630					635					640	
His	Tyr	Tyr	Val	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	
			645					650						655		
Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	
		660					665					670				
Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	
		675				680						685				
Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	
	690					695					700					
Glu	Ser	Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln	Gln	
705				710						715					720	
Lys	Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	Val	
			725					730						735		
Glu	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	
		740					745						750			
Phe	Ser	Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala	Met	Tyr	
		755				760						765				
Phe	Cys	Gln	Gln	Ser	Arg	Lys	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	
	770					775					780					
Lys	Leu	Glu	Ile	Lys	Arg											
785				790												

<210> SEQ ID NO 182

<211> LENGTH: 2283

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-H1-2e12HL (DNA)

<400> SEQUENCE: 182

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aagcttgccg ccatggattt tcaagtgcag attttcagct tcctgctaata cagtgcattca    60
gtcataatgt ccagaggaca aattgttctc tcccagcttc cagcaatcct gtctgcatct    120
ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg    180

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taccagcaga agccaggatc ctccccaaa ccttgattt atgccccatc caacctggct	240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc	300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaaccca	360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcgggtgt	420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtccgtg	480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttaac	540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat	600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta	660
gacaaatcct ccagcacagc ctacatgcag ctacgcagcc tgacatctga agactctgcg	720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggactct cgatgtctgg	780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac	840
acatccccac cgagcccagc acctgaactc ctggggggac cgtcagtctt cctcttcccc	900
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtgggtgtg	960
gacgtgagcc acgaagacct tgaggtaag ttcaactggt acgtggacgg cgtggaggtg	1020
cataatgcc aagacaaagc ggggaggag cagtacaaca gcacgtaccg tgtggtcagc	1080
gtctcaccg tctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc	1140
aacaaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccag cctcccgctg tggactccga cggctccttc	1380
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcatgagge tctgcacaac cactacagc agaagagcct ctccctgtct	1500
ccgggtaaga attctcaggt gcagctgaag gagtcaggac ctggcctggt ggcgcctca	1560
cagagcctgt ccatcacatg caccgtctca gggttctcat taaccggcta tgggtgaaac	1620
tgggttcgcc agcctccagg aaagggtctg gagtggctgg gaatgatatg ggggatgga	1680
agcacagact ataattcagc tctcaaatcc agactatcga tcaccaagga caactccaag	1740
agccaagttt tcttaaaat gaacagtctg caaactgatg acacagccag atactactgt	1800
gctcgagatg gttatagtaa ctttcattac tatgttatgg actactggg tcaaggaaac	1860
tcagtcaccg tctcctctgg ggggtgaggg tctgggtggc gtggatccgg cggaggtggg	1920
tcgggtggcg gcgcatctga cattgtgctc acccaatctc cagcttcttt ggtgtgtct	1980
ctaggtcaga gagccaccat ctctgcaga gccagtgaat gtgtgaata ttatgtcaca	2040
agtttaatgc agtggtagca acagaaacca ggacagccac ccaaaactct catctctgct	2100
gctagcaacg tagaatctgg ggtccctgcc aggttttagtg gcagtgggtc tgggacagac	2160
tttagcctca acatccatcc tgtggaggag gatgatattg caatgtatct ctgtcagcaa	2220
agtaggaagg ttccatggac gtccgggtgga ggcaccaagc tggaaatcaa acgttaatct	2280
aga	2283

<210> SEQ ID NO 183

<211> LENGTH: 754

<212> TYPE: PRT

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-H1-2e12HL (w/2e12 leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(281)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(501)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (502)..(622)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (623)..(642)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (643)..(754)
<223> OTHER INFORMATION: VL2

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<400> SEQUENCE: 183

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Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1           5           10           15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
          20           25           30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
          35           40           45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
          50           55           60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65           70           75           80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
          85           90           95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
          100          105          110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
          115          120          125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
          130          135          140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145          150          155          160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
          165          170          175

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Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile
			180					185					190		
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe
		195					200					205			
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
		210					215					220			
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
225					230					235					240
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp
				245						250				255	
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser
			260						265				270		
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly
		275					280					285			
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
	290					295					300				
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
305					310					315					320
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
				325					330					335	
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
			340					345					350		
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
		355					360					365			
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
	370					375					380				
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
385					390					395					400
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
			405						410					415	
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
		420					425						430		
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
		435					440					445			
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
	450					455						460			
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
465					470					475					480
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
			485						490					495	
Pro	Gly	Lys	Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu
			500					505					510		
Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe
		515					520					525			
Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys
	530					535						540			
Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp	Tyr
545					550					555					560
Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys
			565						570					575	
Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala
			580					585					590		

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Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr Tyr Val
 595 600 605
 Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly
 610 615 620
 Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
 625 630 635 640
 Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser
 645 650 655
 Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu
 660 665 670
 Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln
 675 680 685
 Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn Val Glu Ser Gly Val
 690 695 700
 Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn
 705 710 715 720
 Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln
 725 730 735
 Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 740 745 750

Lys Arg

<210> SEQ ID NO 184
 <211> LENGTH: 2301
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1-H2-2e12HL (DNA)

<400> SEQUENCE: 184

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aagcttgccg ccatggattt tcaagtgcag attttcagct tectgctaata cagtgccttca    60
gtcataatgt ccagaggaca aattgtttctc tcccagctctc cagcaatcct gtctgcatct    120
ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg    180
taccagcaga agccaggatc ctcccccaaa ccctggattt atgccccatc caacctggct    240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc    300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaacca    360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt    420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtcggtg    480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac    540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat    600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta    660
gacaaatcct ccagcacagc ctacatgcag ctacgcagcc tgacatctga agactctgcg    720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggtactt cgatgtctgg    780
ggcacagggg ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac    840
acatccccac cgagcccagc acctgaactc ctgggggggac cgtcagctct cctcttcccc    900
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtggtggtg    960
  
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gacgtgagcc acgaagaccc tgagggtcaag ttcaactggt acgtggacgg cgtggagggtg	1020
cataatgcc aagacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtgggtcagc	1080
gtctctaccg tctgtcacca ggactgggtg aatggcaagg agtacaagtg caaggtctcc	1140
aacaaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc	1380
ttctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcattgagc tctgcacaac cactacacgc agaagagcct ctccctgtct	1500
ccgggtaagg gtggcggtgg ctcggggaat tctcagggtc agctgaagga gtcaggacct	1560
ggcctgggtg cgcctcaca gagcctgtcc atcacatgca ccgtctcagg gttctcatta	1620
accggctatg gtgtaaactg ggttcgccag cctccaggaa aggggtctgga gtggctggga	1680
atgatattgg gtgatggaag cacagactat aattcagctc tcaaatccag actatcgatc	1740
accaaggaca actccaagag ccaagttttc ttaaaaatga acagtctgca aactgatgac	1800
acagccagat actactgtgc tcgagatggt tataagtaact ttcattacta tgttatggac	1860
tactggggtc aaggaacctc agtcaccgtc tctctggggg gtggaggctc tgggtggcgg	1920
ggatccggcg gaggtgggtc ggggtggcggc ggatctgaca ttgtgtctac ccaatctcca	1980
gcttcttttg ctgtgtctct aggtcagaga gccaccatct cctgcagagc cagtgaaggt	2040
gttgaatatt atgtcacaag tttaatgcag tggtagcaac agaaaccagg acagccaccc	2100
aaactcctca tctctgctgc tagcaacgta gaatctgggg tccttgccag gtttagtggc	2160
agtgggtctg ggacagactt tagcctcaac atccatcctg tggaggagga tgatattgca	2220
atgtatttct gtcagcaaa taggaaggtt ccatggacgt tcggtggagg caccaagctg	2280
gaaatcaaac gttaatctag a	2301

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<210> SEQ ID NO 185
<211> LENGTH: 760
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-H2-2e12HL (w/2e12 leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(127)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (128)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (266)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature

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<222> LOCATION: (500)..(507)
<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (508)..(628)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (629)..(648)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (649)..(760)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 185

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1          5          10          15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
          20          25          30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
          35          40          45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
          50          55          60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
          65          70          75          80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
          85          90          95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
          100         105         110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
          115         120         125

Asp Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
          130         135         140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
          145         150         155         160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
          165         170         175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
          180         185         190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
          195         200         205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
          210         215         220

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
          225         230         235         240

Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
          245         250         255

Gly Thr Gly Thr Thr Val Thr Val Ser Asp Gln Glu Pro Lys Ser Ser
          260         265         270

Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly
          275         280         285

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
          290         295         300

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
          305         310         315         320

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Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
			325						330			335			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
			340						345			350			
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
			355						360			365			
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
			370						375			380			
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
			385						390			395			
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
			405						410			415			
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
			420						425			430			
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
			435						440			445			
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
			450						455			460			
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
			465						470			475			
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
			485						490			495			
Pro	Gly	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Asn	Ser	Gln	Val	Gln	Leu	Lys
			500						505			510			
Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr
			515						520			525			
Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val
			530						535			540			
Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly
			545						550			555			
Asp	Gly	Ser	Thr	Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile
			565						570			575			
Thr	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu
			580						585			590			
Gln	Thr	Asp	Asp	Thr	Ala	Arg	Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser
			595						600			605			
Asn	Phe	His	Tyr	Tyr	Val	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val
			610						615			620			
Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly
			625						630			635			
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro
			645						650			655			
Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg
			660						665			670			
Ala	Ser	Glu	Ser	Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr
			675						680			685			
Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser
			690						695			700			
Asn	Val	Glu	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly
			705						710			715			
Thr	Asp	Phe	Ser	Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala
			725						730			735			

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Met Tyr Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly
 740 745 750

Gly Thr Lys Leu Glu Ile Lys Arg
 755 760

<210> SEQ ID NO 186
 <211> LENGTH: 2307
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1-H3-2e12HL (DNA)

<400> SEQUENCE: 186

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aagcttgccg ccattgattt tcaagtgcag attttcagct tcctgctaata cagtgcctca    60
gtcataatgt ccagaggaca aattgttctc tcccagcttc cagcaatcct gtctgcatct    120
ccaggggaga aggtcacaat gacttgccagg gccagctcaa gtgtaagtta catgcactgg    180
taccagcaga agccaggatc ctcccccaaa ccttgattt atgccccatc caacctggct    240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc    300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaacca    360
ccacagttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt    420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtcggtg    480
aggctctggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac    540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat    600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta    660
gacaaatcct ccagcacagc ctacatgcag ctacagagcc tgacatctga agactctgcg    720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggtaact cgatgtctgg    780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaaactcac    840
acatccccac cgagcccagc acctgaactc ctgggggggac cgtcagtcct cctcttcccc    900
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtggtggtg    960
gacgtgagcc acgaagacct tgaggtcaag ttcaactggt acgtggacgg cgtggagggtg   1020
cataatgcc aagacaaagc gggggaggag cagtacaaca gcacgtaccg tgtggtcagc   1080
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc   1140
aacaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga   1200
gaaccacagg tgtacacct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc   1260
ctgacctgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat   1320
gggcagccgg agaacaacta caagaccag cctcccgctg tggactccga cggctccttc   1380
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca   1440
tgctccgtga tgcatgagc tctgcacaac cactacagc agaagagcct ctccctgtct   1500
ccgggtaaga attatggtg cgggtggctc gggaattctc aggtgcagct gaaggagtca   1560
ggacctggcc tgggtggcgc ctcacagagc ctgtccatca catgcaccgt ctcagggttc   1620
tcattaaccg gctatggtgt aaactgggtt cgccagcctc caggaaaggg tctggagtgg   1680
ctgggaatga tatggggtga tggaagcaca gactataatt cagctctcaa atccagacta   1740

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tcgatcacca aggcacaactc caagagccaa gttttcttaa aatgaacag tctgcaaact	1800
gatgacacag ccagatacta ctgtgctcga gatggttata gtaactttca ttactatgtt	1860
atggactact ggggtcaagg aacctcagtc accgtctcct ctgggggttg aggcctctgtt	1920
ggcgggtggat cggcgaggag tgggtcgggt ggcggcggat ctgacattgt gctcacccaa	1980
tctccagctt ctttggtgt gtctctaggt cagagagcca ccattctctg cagagccagt	2040
gaaagtgttg aatattatgt cacaagtta atgcagtgg accaacagaa accaggacag	2100
ccacccaaac tcctcatctc tgctgctagc aacgtagaat ctgggggtccc tgccaggttt	2160
agtggcagtg ggtctgggac agacttttagc ctcaacatcc atcctgtgga ggaggatgat	2220
attgcaatgt atttctgtca gcaaagtagg aaggttccat ggacgttcgg tggaggcacc	2280
aagctggaaa tcaaactgta atctaga	2307

<210> SEQ ID NO 187
 <211> LENGTH: 762
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1-H3-2e12HL (w/2e12 leader) (AA)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(22)
 <223> OTHER INFORMATION: Leader
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (23)..(128)
 <223> OTHER INFORMATION: VL
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (129)..(144)
 <223> OTHER INFORMATION: Linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (145)..(265)
 <223> OTHER INFORMATION: VH
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (268)..(282)
 <223> OTHER INFORMATION: Hinge
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (500)..(509)
 <223> OTHER INFORMATION: EPD-Scorpion linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (510)..(630)
 <223> OTHER INFORMATION: VH2
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (631)..(650)
 <223> OTHER INFORMATION: Linker2
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (651)..(762)
 <223> OTHER INFORMATION: VL2

<400> SEQUENCE: 187

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
 1 5 10 15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
 20 25 30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
 35 40 45

Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser
50						55					60				
Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Pro	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
65				85	70					75					80
Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile
									90					95	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp
			100					105					110		
Ser	Phe	Asn	Pro	Pro	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys
			115				120					125			
Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser
	130					135					140				
Gln	Ala	Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Ser	Val	Arg	Pro	Gly	Ala
145					150					155					160
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr
				165					170					175	
Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile
			180					185					190		
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe
		195					200					205			
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
	210					215					220				
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
225					230					235					240
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp
				245					250					255	
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser
			260					265					270		
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly
		275					280				285				
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
	290					295					300				
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
305					310					315					320
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
				325					330					335	
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
			340					345					350		
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
		355					360					365			
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
	370					375					380				
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
385					390					395					400
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
			405					410						415	
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
			420					425					430		
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
		435					440					445			
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
	450					455					460				

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

<210> SEQ ID NO 188

<211> LENGTH: 2316

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-H4-2e12HL (DNA)

<400> SEQUENCE: 188

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aagcttgccg ccattgattt tcaagtgcag attttcagct tcctgctaata cagtgttca      60
gtcataatgt ccagaggaca aattgttctc tccagtcctc cagcaatcct gtctgcatct      120
ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg      180
taccagcaga agccaggatc ctcccccaaa ccttgattt atgccccatc caacctggct      240

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tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc	300
agcagagtgg aggctgaaga tgctgccact tattactgcc agcagtggag ttttaacca	360
ccccgcttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcgggtgg	420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtccgtg	480
aggcctgggg cctcagtga gatgtcctgc aaggtctctg gctacacatt taccagttac	540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat	600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta	660
gacaaatcct ccagcacagc ctacatgcag ctacagagcc tgacatctga agactctgcg	720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggactct cgatgtctgg	780
ggcacaggga ccacggctac cgtctctgat caggagccca aatcttctga caaaactcac	840
acatccccac cgagcccagc acctgaactc ctggggggac cgtcagctct cctcttcccc	900
ccaaaaccca aggacacct catgatctcc cggaccctcg aggtcacatg cgtgggtggg	960
gacgtgagcc acgaagacc tgaggtcaag ttcaactggc acgtggacgg cgtggagggtg	1020
cataatgcc agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc	1080
gtcctcacgg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc	1140
aacaaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtaaccct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccagc cctcccgtgc tggactccga cggtccttc	1380
ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcattgagg tctgcacaac cactacagc agaagagcct ctccctgtct	1500
ccgggtaagg gtggcggtg ctcggggcgt ggtggatctg ggaattctca ggtgcagctg	1560
aaggagtcag gacctggcct ggtggcgccc tcacagagcc tgtccatcac atgcaccgtc	1620
tcagggttct cattaaccgg ctatgggtga aactgggttc gccagcctcc aggaaagggt	1680
ctggagtggc tgggaatgat atggggatg ggaagcacag actataattc agctctcaaa	1740
tccagactat cgatcaccaa ggacaactcc aagagccaag ttttcttaa aatgaacagt	1800
ctgcaactg atgacacagc cagatactac tgtgctcgag atggttatag taactttcat	1860
tactatgta tggactactg gggtaagga acctcagtc cgtctctctc tgggggtgga	1920
ggctctgggt gcgggtggatc cggcggaggt gggtcgggtg gcggcggatc tgacattgtg	1980
ctcaccat ctccagcttc tttggctgtg tctctaggtc agagagccac catctcctgc	2040
agagccagt aaagtgtta atattatgtc acaagtttaa tgcagtggta ccaacagaaa	2100
ccaggacagc caccacaaact cctcatctct gctgctagca acgtagaatc tggggtcct	2160
gccaggttta gtggcagtg gtctgggaca gactttagcc tcaacatcca tctgtggag	2220
gaggatgata ttgcaatgta tttctgtcag caaagtagga aggttccatg gacgttcggt	2280
ggaggcacca agctggaaat caaacgttaa tctaga	2316

<210> SEQ ID NO 189

<211> LENGTH: 765

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-H4-2e12HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(512)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (513)..(633)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (634)..(653)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (654)..(765)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 189

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1 5 10 15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20 25 30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35 40 45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
50 55 60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65 70 75 80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
85 90 95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
100 105 110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
115 120 125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
130 135 140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145 150 155 160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
165 170 175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
180 185 190

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Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	195	200	205
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	210	215	220
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	225	230	235
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	245	250	255
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser	260	265	270
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	275	280	285
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	290	295	300
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	305	310	315
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	325	330	335
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	340	345	350
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	355	360	365
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	370	375	380
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	385	390	395
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	405	410	415
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	420	425	430
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	435	440	445
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	450	455	460
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	465	470	475
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	485	490	495
Pro	Gly	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Asn	Ser	500	505	510
Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	515	520	525
Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Gly	Tyr	530	535	540
Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	545	550	555
Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp	Tyr	Asn	Ser	Ala	Leu	Lys	565	570	575
Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	580	585	590
Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Arg	Tyr	Tyr	Cys	Ala			

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595	600	605
Arg Asp Gly Tyr Ser Asn Phe His Tyr Tyr Val Met Asp Tyr Trp Gly		
610	615	620
Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly		
625	630	635
Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Asp Ile Val		
645	650	655
Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala		
660	665	670
Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Tyr Tyr Val Thr Ser		
675	680	685
Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu		
690	695	700
Ile Ser Ala Ala Ser Asn Val Glu Ser Gly Val Pro Ala Arg Phe Ser		
705	710	715
Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His Pro Val Glu		
725	730	735
Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Arg Lys Val Pro		
740	745	750
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg		
755	760	765

<210> SEQ ID NO 190

<211> LENGTH: 2322

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-H5-2e12HL (DNA)

<400> SEQUENCE: 190

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aagcttgccg ccatggattt tcaagtgcag attttcagct tcctgctaata cagtgtctca    60
gtcataatgt ccagaggaca aattgttctc tcccagctctc cagcaatcct gtctgcatct    120
ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg    180
taccagcaga agccaggatc ctccccaaaa ccttgattt atgccccatc caacctggct    240
tctggagtcc ctgctcgctt cagtggcagt gggctcggga cctcttactc tctcacaatc    300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaaccca    360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt    420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtgggtg    480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac    540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat    600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta    660
gacaaatcct ccagcacagc ctacatgcag ctacagagcc tgacatctga agactctgcg    720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggtactt cgatgtctgg    780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac    840
acatccccac cgagccagc acctgaactc ctggggggac cgtcagctct cctcttcccc    900
ccaaaaccca aggacacct catgatctcc cggaccctg aggtcacatg cgtggtggtg    960
gacgtgagcc acgaagacc tgaggtcaag ttcaactggt acgtggacgg cgtggagggtg   1020

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cataatgcc	agacaaagcc	gcgggaggag	cagtacaaca	gcacgtaccg	tgtggtcagc	1080
gtcctcaccg	tcttgaccca	ggactggctg	aatggcaagg	agtacaagtg	caaggtctcc	1140
aacaaagccc	tcccagcccc	catcgagaaa	acaatctcca	aagccaaagg	gcagccccga	1200
gaaccacagg	tgtacacct	gccccatcc	cgggatgagc	tgaccaagaa	ccaggtcagc	1260
ctgacctgcc	tggtaaagg	cttctatccc	agcgacatcg	ccgtggagtg	ggagagcaat	1320
gggcagccgg	agaacaacta	caagaccacg	cctcccgtgc	tggactccga	cggctccttc	1380
ttcctctaca	gcaagctcac	cgtggacaag	agcaggtggc	agcaggggaa	cgtcttctca	1440
tgctccgtga	tgcattgagg	tctgcacaac	cactacacgc	agaagagcct	ctccctgtct	1500
ccgggtaaga	attatgggtg	cgggtggctg	ggcgggtggg	gatctgggaa	ttctcaggtg	1560
cagctgaagg	agtcaggacc	tggcctgggt	gcgcctcac	agagcctgtc	catcacatgc	1620
accgtctcag	ggttctcatt	aaccggctat	gggtgaaact	gggttcgcca	gcctccagga	1680
aagggtctgg	agtggtctgg	aatgatattg	ggatgatgaa	gcacagacta	taattcagct	1740
ctcaaatcca	gactatcgat	caccaaggac	aactccaaga	gccaagtttt	cttaaaaatg	1800
aacagtctgc	aaactgatga	cacagccaga	tactactgtg	ctcgagatgg	ttatagtaac	1860
tttcattact	atgttatgga	ctactggggg	caaggaaact	cagtcaccgt	ctcctctggg	1920
gggtggaggct	ctgggtggcg	tggatccggc	ggaggtgggt	cgggtggcgg	cggatctgac	1980
attgtgctca	cccaatctcc	agcttctttg	gctgtgtctc	taggtcagag	agccaccatc	2040
tcttgacagag	ccagtgaagg	tggtgaatat	tatgtcacia	gtttaatgca	gtggtaacca	2100
cagaaaccag	gacagccacc	caaactcttc	atctctgtctg	ctagcaacgt	agaatctggg	2160
gtccctgcc	ggtttagtgg	cagtggtgct	gggacagact	ttagcctcaa	catccatcct	2220
gtggaggagg	atgatattgc	aatgtatttc	tgtcagcaaa	gtaggaaggt	tccatggacg	2280
tcgggtggag	gcaccaagct	ggaaatcaaa	cgttaatcta	ga		2322

<210> SEQ ID NO 191
 <211> LENGTH: 767
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1-H5-2e12HL (w/2e12 leader) (AA)
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(22)
 <223> OTHER INFORMATION: Leader
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (23)..(128)
 <223> OTHER INFORMATION: VL
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (129)..(144)
 <223> OTHER INFORMATION: Linker
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (145)..(265)
 <223> OTHER INFORMATION: VH
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (268)..(282)
 <223> OTHER INFORMATION: Hinge
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (500)..(514)

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<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (515)..(635)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (636)..(655)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (656)..(767)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 191

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
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Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20          25          30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35          40          45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
50          55          60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65          70          75          80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
85          90          95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
100         105         110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
115         120         125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
130         135         140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145         150         155         160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
165         170         175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
180         185         190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
195         200         205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
210         215         220

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
225         230         235         240

Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
245         250         255

Gly Thr Gly Thr Thr Val Thr Val Ser Asp Gln Glu Pro Lys Ser Ser
260         265         270

Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly
275         280         285

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
290         295         300

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
305         310         315         320

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

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325								330				335					
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr		
			340				345						350				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly		
		355				360					365						
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile		
370					375						380						
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val		
385				390						395			400				
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser		
			405						410					415			
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu		
			420					425					430				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro		
		435					440					445					
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val		
450					455						460						
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met		
465				470						475			480				
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser		
			485						490					495			
Pro	Gly	Lys	Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly		
			500					505					510				
Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro		
			515					520					525				
Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr		
530					535						540						
Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu		
545				550						555					560		
Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp	Tyr	Asn	Ser	Ala		
			565						570					575			
Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val		
			580					585					590				
Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Arg	Tyr	Tyr		
595					600						605						
Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr	Tyr	Val	Met	Asp	Tyr		
610					615						620						
Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser		
625				630						635			640				
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp		
			645						650					655			
Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly	Gln		
			660					665					670				
Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser	Val	Glu	Tyr	Tyr	Val		
675					680						685						
Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln	Lys	Pro	Gly	Gln	Pro	Pro	Lys			
690					695						700						
Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	Val	Glu	Ser	Gly	Val	Pro	Ala	Arg		
705				710						715					720		
Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Ser	Leu	Asn	Ile	His	Pro		
			725						730					735			

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Val	Glu	Glu	Asp	Asp	Ile	Ala	Met	Tyr	Phe	Cys	Gln	Gln	Ser	Arg	Lys
			740					745					750		

Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg
		755					760					765		

<210> SEQ ID NO 192
 <211> LENGTH: 2331
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sssIgG1-H6-2e12HL (DNA)

<400> SEQUENCE: 192

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aagcttgccg ccatggattt tcaagtgcag attttcagct tcctgctaata cagtgccttca      60
gtcataatgt ccagaggaca aattgttctc tcccagctctc cagcaatcct gtctgcatct      120
ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg      180
taccagcaga agccaggatc ctcccccaaa cctgggattt atgccccatc caacctggct      240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc      300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaaccca      360
ccccagttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt      420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtcgggtg      480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac      540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat      600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta      660
gacaaatcct ccagcacagc ctacatgcag ctacgcagcc tgacatctga agactctgcg      720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggactct cgatgtctgg      780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac      840
acatccccac cgagcccagc acctgaactc ctgggggggac cgtcagctct cctcttcccc      900
ccaaaaccca aggacacct catgatctcc cggaaccttg aggtcacatg cgtgggtggtg      960
gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg      1020
cataatgcc aagacaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc      1080
gtcctcaccc tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc      1140
aacaaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga      1200
gaaccacagg tgtacacct gcccccaccc cgggatgagc tgaccaagaa ccaggtcagc      1260
ctgacctgcc tggtaaaagg cttctatccc agcgacatcg cgtggagtgg ggagagcaat      1320
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggtccttc      1380
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca      1440
tgctccgtga tgcagtaggc tctgcacaac cactacagc agaagagcct ctccctgtct      1500
ccgggtaagg gtggcggtgg ctggggcggt ggtggatctg ggggaggagg cagcggaat      1560
tctcaggtgc agctgaagga gtcaggacct ggcctggtgg cgcctcaca gagcctgtcc      1620
atcacatgca ccgtctcagg gttctcatta accggctatg gtgtaaactg ggttcgccag      1680
cctccaggaa agggctctgga gtggctggga atgatatggg gtgatggaag cacagactat      1740
aattcagctc tcaaatccag actatcgatc accaaggaca actccaagag ccaagttttc      1800
  
```

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ttaaaaaatga acagtctgca aactgatgac acagccagat actactgtgc tcgagatggt 1860
tatagtaact ttcattacta tggtatggac tactgggggc aaggaacctc agtcaccgtc 1920
tctctggggg gtggaggctc tgggtggcgtt ggatccggcg gaggtgggtc ggggtggcggc 1980
ggatctgaca ttgtgtctac ccaatctcca gcttcttttg ctgtgtctct aggtcagaga 2040
gccaccatct cctgcagagc cagtgaagt gttgaatatt atgtcacaag tttaatgcag 2100
tggtagcaac agaaaccagg acagccaccc aaactcctca tctctgctgc tagcaacgta 2160
gaatctgggg tccttgccag gtttagtggc agtgggtctg ggacagactt tagcctcaac 2220
atccatcctg tggaggagga tgatattgca atgtatttct gtcagcaaag taggaaggtt 2280
ccatggacgt tcggtggagg caccaagctg gaaatcaaac gttaatctag a 2331

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<210> SEQ ID NO 193
<211> LENGTH: 770
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-H6-2e12HL (w/2e12 leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(517)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (518)..(638)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (639)..(658)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (659)..(770)
<223> OTHER INFORMATION: VL2

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<400> SEQUENCE: 193

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Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1           5           10          15

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Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20          25          30

```

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Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35          40          45

```

```

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser

```

50					55					60					
Pro 65	Lys	Pro	Trp	Ile	Tyr 70	Ala	Pro	Ser	Asn	Leu 75	Ala	Ser	Gly	Val	Pro 80
Ala	Arg	Phe	Ser	Gly 85	Ser	Gly	Ser	Gly	Thr 90	Ser	Tyr	Ser	Leu	Thr 95	Ile
Ser	Arg	Val	Glu 100	Ala	Glu	Asp	Ala	Ala 105	Thr	Tyr	Tyr	Cys	Gln 110	Gln	Trp
Ser	Phe	Asn 115	Pro	Pro	Thr	Phe	Gly 120	Ala	Gly	Thr	Lys	Leu 125	Glu	Leu	Lys
Asp	Gly 130	Gly	Gly	Ser	Gly	Gly 135	Gly	Gly	Ser	Gly	Gly 140	Gly	Gly	Ser	Ser
Gln 145	Ala	Tyr	Leu	Gln	Gln 150	Ser	Gly	Ala	Glu	Ser 155	Val	Arg	Pro	Gly	Ala 160
Ser	Val	Lys	Met	Ser 165	Cys	Lys	Ala	Ser	Gly 170	Tyr	Thr	Phe	Thr	Ser 175	Tyr
Asn	Met	His	Trp 180	Val	Lys	Gln	Thr	Pro 185	Arg	Gln	Gly	Leu 190	Glu	Trp	Ile
Gly	Ala	Ile 195	Tyr	Pro	Gly	Asn	Gly 200	Asp	Thr	Ser	Tyr	Asn 205	Gln	Lys	Phe
Lys	Gly 210	Lys	Ala	Thr	Leu	Thr 215	Val	Asp	Lys	Ser	Ser 220	Ser	Thr	Ala	Tyr
Met 225	Gln	Leu	Ser	Ser	Leu 230	Thr	Ser	Glu	Asp	Ser 235	Ala	Val	Tyr	Phe	Cys 240
Ala	Arg	Val	Val 245	Tyr	Tyr	Ser	Asn	Ser	Tyr 250	Trp	Tyr	Phe	Asp	Val 255	Trp
Gly	Thr	Gly	Thr 260	Thr	Val	Thr	Val	Ser 265	Asp	Gln	Glu	Pro	Lys 270	Ser	Ser
Asp	Lys	Thr 275	His	Thr	Ser	Pro	Pro 280	Ser	Pro	Ala	Pro	Glu 285	Leu	Leu	Gly
Gly	Pro 290	Ser	Val	Phe	Leu	Phe 295	Pro	Pro	Lys	Pro	Lys 300	Asp	Thr	Leu	Met
Ile 305	Ser	Arg	Thr	Pro	Glu 310	Val	Thr	Cys	Val	Val 315	Val	Asp	Val	Ser	His 320
Glu	Asp	Pro	Glu 325	Val	Lys	Phe	Asn	Trp	Tyr 330	Val	Asp	Gly	Val	Glu 335	Val
His	Asn	Ala	Lys 340	Thr	Lys	Pro	Arg	Glu 345	Glu	Gln	Tyr	Asn	Ser 350	Thr	Tyr
Arg	Val	Val 355	Ser	Val	Leu	Thr	Val 360	Leu	His	Gln	Asp	Trp 365	Leu	Asn	Gly
Lys	Glu 370	Tyr	Lys	Cys	Lys	Val 375	Ser	Asn	Lys	Ala	Leu 380	Pro	Ala	Pro	Ile
Glu 385	Lys	Thr	Ile	Ser	Lys 390	Ala	Lys	Gly	Gln	Pro 395	Arg	Glu	Pro	Gln	Val 400
Tyr	Thr	Leu	Pro 405	Ser	Arg	Asp	Glu	Leu 410	Thr	Lys	Asn	Gln	Val 415	Ser	
Leu	Thr	Cys 420	Leu	Val	Lys	Gly	Phe	Tyr 425	Pro	Ser	Asp	Ile	Ala 430	Val	Glu
Trp	Glu	Ser 435	Asn	Gly	Gln	Pro	Glu 440	Asn	Asn	Tyr	Lys	Thr 445	Thr	Pro	Pro
Val	Leu 450	Asp	Ser	Asp	Gly	Ser	Phe 455	Phe	Leu	Tyr	Ser	Lys 460	Leu	Thr	Val

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Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
465					470					475					480
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
				485					490						495
Pro	Gly	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly
			500					505					510		
Gly	Ser	Gly	Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu
		515					520					525			
Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe
	530					535					540				
Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys
545					550					555					560
Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp	Tyr
				565					570					575	
Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys
			580					585					590		
Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala
		595					600					605			
Arg	Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr	Tyr	Val
	610					615					620				
Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Gly	Gly
625					630					635					640
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly
			645						650					655	
Gly	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser
			660					665					670		
Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser	Val	Glu
		675					680					685			
Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
	690					695					700				
Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	Val	Glu	Ser	Gly	Val
705					710					715					720
Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Ser	Leu	Asn
			725						730					735	
Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala	Met	Tyr	Phe	Cys	Gln	Gln
			740					745					750		
Ser	Arg	Lys	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile
		755					760					765			
Lys	Arg														
	770														

<210> SEQ ID NO 194
 <211> LENGTH: 2301
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: 2H7sscIgG1-H7-2e12HL (DNA)

<400> SEQUENCE: 194

aagcttgccg ccatggattt tcaagtgcag attttcagct tcctgctaata cagtgccttca	60
gtcataatgt ccagaggaca aattgttctc tcccagcttc cagcaatcct gtctgcatct	120
ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg	180

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taccagcaga agccaggatc ctccccaaa ccttgattt atgccccatc caacctggct	240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc	300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtggag ttttaaccca	360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt	420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtccgtg	480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac	540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat	600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta	660
gacaaatcct ccagcacagc ctacatgcag ctacgcagcc tgacatctga agactctgcg	720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggactct cgatgtctgg	780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac	840
acatccccac cgtgccacgc acctgaactc ctggggggat cgtcagtctt cctcttcccc	900
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtggtggtg	960
gacgtgagcc acgaagacct tgaggtaag ttcaactggt acgtggacgg cgtggagggtg	1020
cataatgcc aagacaaagc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc	1080
gtctcaccg tctgcacca ggactggctg aatggcaagg agtacaagtg caaggctctcc	1140
aacaaagccc tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtacacct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tggtaaaagg cttctatcca agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccag cctcccgctc tggactccga cggctccttc	1380
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcatgagge tctgcacaac cactacacgc agaagagcct ctccctgtct	1500
ccgggtaagg ggtgtccacc ttgtccgaat tctcaggtgc agctgaagga gtcagggcct	1560
ggcagcgtgg cgcctcaca gagcctgtcc atcacatgca ccgtctcagg gttctcatta	1620
accggctatg gtgtaaaact ggttcgccag cctccaggaa agggctctgga gtggctggga	1680
atgatatggg gtgatggaag cacagactat aattcagctc tcaaatccag actatcgatc	1740
accaaggaca actccaagag ccaagttttc ttaaaaatga acagtctgca aactgatgac	1800
acagccagat actactgtgc tcgagatggt tatagtaact ttcattacta tgttatggac	1860
tactggggtc aaggaaacct agtcaccgtc tctctggggg gtggaggctc tggtgccggt	1920
ggatccggcg gaggtgggtc ggggtggcgc ggatctgaca ttgtgctcac ccaatctcca	1980
gcttctttgg ctgtgtctct aggtcagaga gccaccatct cctgcagagc cagtgaaggt	2040
gttgaatatt atgtcacaag tttaatgcag tggtagcaac agaaaccagg acagccaccc	2100
aagctcctca tctctgctgc tagcaacgta gaatctgggg tccctgccag gtttagtggc	2160
agtgggtctg ggacagactt tagcctcaac atccatcctg tggaggagga tgatattgca	2220
atgtatttct gtcagcaaa taggaagggt ccatggacgt tcggtggagg caccaagctg	2280
gaaatcaaac gttaatctag a	2301

<210> SEQ ID NO 195

<211> LENGTH: 760

<212> TYPE: PRT

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sscIgG1-H7-2e12HL (w/2e12 linker ) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(507)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (508)..(628)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (629)..(648)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (649)..(760)
<223> OTHER INFORMATION: VL2

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<400> SEQUENCE: 195

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Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1           5           10           15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
          20           25           30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
          35           40           45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
          50           55           60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65           70           75           80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
          85           90           95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
          100          105          110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
          115          120          125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
          130          135          140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145          150          155          160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
          165          170          175

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Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile
			180					185					190		
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe
		195					200					205			
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
		210					215				220				
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
225					230					235					240
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp
				245					250					255	
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser
			260					265					270		
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly
		275					280					285			
Gly	Ser	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
		290				295					300				
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His
305					310					315					320
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val
				325					330					335	
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr
			340					345					350		
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly
		355					360					365			
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile
		370				375					380				
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val
385					390					395					400
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser
			405						410					415	
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
			420				425						430		
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro
		435				440						445			
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val
		450				455					460				
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met
465					470				475						480
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser
			485						490					495	
Pro	Gly	Lys	Gly	Cys	Pro	Pro	Cys	Pro	Asn	Ser	Gln	Val	Gln	Leu	Lys
			500					505					510		
Glu	Ser	Gly	Pro	Gly	Ser	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr
		515					520					525			
Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val
		530				535					540				
Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly
545					550					555					560
Asp	Gly	Ser	Thr	Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile
			565					570					575		
Thr	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu
			580					585					590		

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Gln Thr Asp Asp Thr Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser
 595 600 605
 Asn Phe His Tyr Tyr Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
 610 615 620
 Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 625 630 635 640
 Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro
 645 650 655
 Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
 660 665 670
 Ala Ser Glu Ser Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr
 675 680 685
 Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser
 690 695 700
 Asn Val Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
 705 710 715 720
 Thr Asp Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala
 725 730 735
 Met Tyr Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly
 740 745 750
 Gly Thr Lys Leu Glu Ile Lys Arg
 755 760

<210> SEQ ID NO 196

<211> LENGTH: 2283

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-H7-G194 HL (DNA)

<400> SEQUENCE: 196

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gtcataatgt ccaggagaca aattgttctc tccagtcctc cagcaatcct gtctgcatct    120
ccaggggaga aggtcacaat gacttgacag gccagctcaa gtgtaagtta catgcactgg    180
taccagcaga agccaggatc ctcccccaaa ccctggattt atgccccatc caacctggct    240
tctggagtc cgtctcgctt cagtggcagt ggggtctggga cctcttactc tctcacaatc    300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtgagg tttaaccca    360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt    420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtgggtg    480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac    540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat    600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta    660
gacaaatcct ccagcacagc ctacatgcag ctacagagcc tgacatctga agactctgcg    720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggtactt cgatgtctgg    780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac    840
acatccccac cgtgcccagc acctgaactc ctggggggat cgtcagtcct cctcttcccc    900
ccaaaaccca aggacacct catgatctcc cggaacctcg aggtcacatg cgtggtggtg    960

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gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg	1020
cataatgcca agacaaagcc gcgaggaggag cagtacaaca gcacgtaccg tgtggtcagc	1080
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc	1140
aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtacaccct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tgggtcaaagg cttctatcca agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccacg cctcccgctg tggactccga cggtccttc	1380
ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct	1500
ccgggtaagg ggtgtccacc ttgtccgaat tctgaggtcc agctgcaaca gtctggacct	1560
gaactgggtga agcctggagc ttcaatgaag atttctgca aggcctctgg ttactcattc	1620
actgggtaca tcgtgaactg gctgaagcag agccatggaa agaaccttga gtggattgga	1680
cttattaatc catacaaagg tcttactacc tacaaccaga aattcaaggg caaggccaca	1740
ttaactgtag acaagtcac cagcacagcc tacatggagc tcctcagtct gacatctgaa	1800
gactctgcag tctattactg tgcaagatct ggggtactatg gtgactcgga ctggtaactc	1860
gatgtctggg gcgcagggac cacggtcacc gtctcctctg gtggcggtgg ctggggcggt	1920
gggtgatctg gaggaggtgg gagcgctagc gacatccaga tgacacagac tacatcctcc	1980
ctgtctgcct ctctgggaga cagagtcacc atcagttgca gggcaagtca ggacattcgc	2040
aattatttaa actggtatca gcagaaacca gatggaactg ttaaactcct gatctactac	2100
acatcaagat tacactcagg agtcccacatc aggttcagtg gcagtgggtc tggaacagat	2160
tattctctca ccattgcca cctgcaacca gaagatattg ccacttactt ttgccaacag	2220
ggtaatacgc ttccgtggac gtccgggtgga ggcaccaaac tggtaaccaa acggtaatct	2280
aga	2283

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<210> SEQ ID NO 197
<211> LENGTH: 754
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-H7-G194 HL (w/2e12 leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:

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<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(507)
<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (508)..(629)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (630)..(646)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (647)..(754)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 197

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
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Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
          20          25          30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
          35          40          45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
          50          55          60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65          70          75          80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
          85          90          95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
          100         105         110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
          115         120         125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
          130         135         140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145         150         155         160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
          165         170         175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
          180         185         190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
          195         200         205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
          210         215         220

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
225         230         235         240

Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
          245         250         255

Gly Thr Gly Thr Thr Val Thr Val Ser Asp Gln Glu Pro Lys Ser Ser
          260         265         270

Asp Lys Thr His Thr Ser Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
          275         280         285

Gly Ser Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
          290         295         300

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
305         310         315         320

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Glu Asp Pro	Glu Val	Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val	
	325	330	335
His Asn Ala	Lys Thr	Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr	
	340	345	350
Arg Val Val	Ser Val	Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly	
	355	360	365
Lys Glu Tyr	Lys Cys	Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile	
	370	375	380
Glu Lys Thr	Ile Ser	Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val	
385		390	400
Tyr Thr Leu	Pro Pro	Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser	
	405	410	415
Leu Thr Cys	Leu Val	Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu	
	420	425	430
Trp Glu Ser	Asn Gly	Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro	
	435	440	445
Val Leu Asp	Ser Asp	Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val	
	450	455	460
Asp Lys Ser	Arg Trp	Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met	
465		470	480
His Glu Ala	Leu His	Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser	
	485	490	495
Pro Gly Lys	Gly Cys	Pro Pro Cys Pro Asn Ser Glu Val Gln Leu Gln	
	500	505	510
Gln Ser Gly	Pro Glu	Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser	
	515	520	525
Cys Lys Ala	Ser Gly	Tyr Ser Phe Thr Gly Tyr Ile Val Asn Trp Leu	
	530	535	540
Lys Gln Ser	His Gly	Lys Asn Leu Glu Trp Ile Gly Leu Ile Asn Pro	
545		550	560
Tyr Lys Gly	Leu Thr	Thr Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr	
	565	570	575
Leu Thr Val	Asp Lys	Ser Ser Ser Thr Ala Tyr Met Glu Leu Leu Ser	
	580	585	590
Leu Thr Ser	Glu Asp	Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr	
	595	600	605
Tyr Gly Asp	Ser Asp	Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr	
	610	615	620
Val Thr Val	Ser Ser	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly	
625		630	640
Gly Gly Gly	Ser Ala	Ser Asp Ile Gln Met Thr Gln Thr Thr Ser Ser	
	645	650	655
Leu Ser Ala	Ser Leu	Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser	
	660	665	670
Gln Asp Ile	Arg Asn	Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly	
	675	680	685
Thr Val Lys	Leu Leu	Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val	
	690	695	700
Pro Ser Arg	Phe Ser	Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr	
705		710	720
Ile Ala Asn	Leu Gln	Pro Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln	

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	725	730	735	
Gly Asn Thr Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Val Thr				
	740	745	750	
Lys Arg				
<210> SEQ ID NO 198				
<211> LENGTH: 2298				
<212> TYPE: DNA				
<213> ORGANISM: Artificial sequence				
<220> FEATURE:				
<223> OTHER INFORMATION: Synthetic polynucleotide				
<220> FEATURE:				
<223> OTHER INFORMATION: 2H7sssIgG1-H7-G281 HL (DNA)				
<400> SEQUENCE: 198				
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gtcataatgt ccagaggaca aattgttctc tcccagcttc cagcaatcct gtctgcatct				120
ccaggggaga aggtcacaat gacttgacag gccagctcaa gtgtaagtta catgcactgg				180
taccagcaga agccaggatc ctccccaaa ccttggaattt atgccccatc caacctggct				240
tctggagtcc ctgctcgctt cagtggcagt gggctctgga cctcttactc tctcacaatc				300
agcagagtgg aggtgaaga tgctgccact tattactgcc agcagtgagg ttttaacca				360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcgggtgt				420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtccgtg				480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac				540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat				600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta				660
gacaaatcct ccagcacagc ctacatgcag ctacagagcc tgacatctga agactctgcg				720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actggtaact cgatgtcttg				780
ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttctga caaaactcac				840
acatccccac cgtgcccagc acctgaactc ctggggggat cgtcagtcct cctcttcccc				900
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtggtggtg				960
gacgtgagcc acgaagacct tgaggtcaag ttcaactggt acgtggacgg cgtggagggtg				1020
cataatgcc aagacaaagc gggggaggag cagtacaaca gcacgtaccg tgtggtcagc				1080
gtcctcaccg tcttgacca ggactggctg aatggcaagg agtacaagtg caaggtctcc				1140
aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga				1200
gaaccacagg tgtacacct gcccccaccc cgggatgagc tgaccaagaa ccaggtcagc				1260
ctgacctgcc tggtaaaagg cttctatcca agcgacatcg ccgtggagtg ggagagcaat				1320
gggcagccgg agaacaacta caagaccag cctcccgtgc tggactccga cggctccttc				1380
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca				1440
tgctccgtga tgcatgagc tctgcacaac cactacagc agaagagcct ctccctgtct				1500
ccgggtaagg ggtgtccacc ttgtccgaat tctgaggtcc agctgcaaca gtctggacct				1560
gaactggtga agcctggagc ttcaatgaag atttctctga aggcctctgg ttactcattc				1620
actggctaca tcgtgaactg gctgaagcag agccatggaa agaacctga gtggattgga				1680
cttattaatc catacaaagg tcttactacc tacaaccaga aattcaaggg caaggccaca				1740

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ttaactgtag	acaagtcac	cagcacagcc	tacatggagc	tctcagtcct	gacatctgaa	1800
gactctgcag	tctattactg	tgcaagatct	gggtactatg	gtgactcgga	ctgggtacttc	1860
gatgtctggg	gcgcagggac	cacgggtcac	gtctctctctg	gtggcggttg	ctcggggcgg	1920
ggtggatctg	gaggaggttg	gagcggggga	ggtggcagtg	ctagcgacat	ccagatgaca	1980
cagactacat	cctccctgtc	tgcctctctg	ggagacagag	tcaccatcag	ttgcagggca	2040
agtcaggaca	ttcgcaatta	tttaaactgg	tatcagcaga	aaccagatgg	aactgttaaa	2100
ctctctgatc	actacacatc	aagattacac	tcaggagtcc	catcaagggt	cagtggcagt	2160
gggtctggaa	cagattatct	tctcaccatt	gccaaactgc	aaccagaaga	tattgccact	2220
tacttttgcc	aacagggtaa	tacgctctcg	tggaagctcg	gtggaggcac	caaactggta	2280
accaaaccgt	aatctaga					2292

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<210> SEQ ID NO 199
<211> LENGTH: 759
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7sssIgG1-H7-G281 HL (w/2e12 leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(507)
<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (508)..(629)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (630)..(651)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (652)..(759)
<223> OTHER INFORMATION: VL2

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<400> SEQUENCE: 199

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1 5 10 15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20 25 30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35 40 45

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Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser		
50						55					60						
Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Pro	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro		
65					70					75					80		
Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile		
				85					90					95			
Ser	Arg	Val	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp		
			100					105					110				
Ser	Phe	Asn	Pro	Pro	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys		
		115					120				125						
Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser		
	130					135					140						
Gln	Ala	Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Ser	Val	Arg	Pro	Gly	Ala		
145					150					155					160		
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr		
				165					170					175			
Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile		
			180					185					190				
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe		
		195				200						205					
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr		
	210					215					220						
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys		
225					230				235						240		
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp		
				245					250					255			
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Ser		
			260					265					270				
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly		
		275				280						285					
Gly	Ser	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met		
					295						300						
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His		
305					310				315						320		
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val		
				325					330					335			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr		
			340					345					350				
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly		
			355				360					365					
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile		
	370					375					380						
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val		
385					390				395						400		
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser		
				405					410					415			
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu		
			420					425					430				
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro		
			435				440					445					
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val		
	450					455						460					

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

<210> SEQ ID NO 200

<211> LENGTH: 1533

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2e12-sss-IgG1 HL SMIP (DNA)

<400> SEQUENCE: 200

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atggattttc aagtgcagat tttcagcttc ctgctaataca gtgcttcagt cataatgtcc      60
agaggagtcc aggtgcagct gaaggagtca ggacctggcc tggtgccgcc ctcacagagc      120
ctgtccatca catgcaccgt ctcagggttc tcattaaccg gctatggtgt aaactggggt      180
cgccagcctc caggaaaggg tctagagtgg ctgggaatga tatggggtga tggaagcaca      240

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gactataatt cagctctcaa atccagacta tcgatcacca aggacaactc caagagccaa	300
gttttcttaa aaatgaacag tctgcaaaact gatgacacag ccagatacta ctgtgctcga	360
gatggttata gtaactttca ttactatgtt atggactact ggggtcaagg aacctcagtc	420
accgtctcct ctgggggttg aggctctggt ggcggtggat ccggcggagg tgggtcgggt	480
ggcgggcggt ctgacattgt gctcacccaa tctccagctt ctttggctgt gtctctaggt	540
cagagagcca ccatctcctg cagagccagt gaaagtgttg aatattatgt cacaagttta	600
atgcagtggg accaacagaa accaggacag ccacccaaac tcctcatctc tgctgctagc	660
aacgtagaat ctgggggtccc tgccagggtt agtggcagtg ggtctgggac agactttagc	720
ctcaacatcc atcctgtgga ggaggatgat attgcaatgt atttctgtca gcaaagtagg	780
aaggttccat ggacgttcgg tggaggcacc aagctggaaa tcaaacgtga tcaggagccc	840
aaatcttctg acaaaactca cacatcccca cgtccccag cacctgaact cctgggggga	900
tcgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcctgatctc ccggaccct	960
gaggtcacat gcgtgggtgt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg	1020
tacgtggacg gcgtggagggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac	1080
agcacgtacc gtgtggtcag cgctctcacc gtctgcacc aggactggct gaatggcaag	1140
gagtacaagt gcaaggtctc caacaaagcc ctcccagcct ccctcgagaa aacaatctcc	1200
aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag	1260
ctgaccaaga accaggtcag cctgacctgc ctgggtcaaag gcttctatcc cagcgacatc	1320
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccggtg	1380
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa gagcaggtgg	1440
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg	1500
cagaagagcc tctccctgtc tccgggtaaa tga	1533

```

<210> SEQ ID NO 201
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2e12-sss-IgG1 HL SMIP (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(144)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(164)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (165)..(276)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (279)..(293)
<223> OTHER INFORMATION: Hinge
<400> SEQUENCE: 201

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Met	Asp	Phe	Gln	Val	Gln	Ile	Phe	Ser	Phe	Leu	Leu	Ile	Ser	Ala	Ser
1			5						10					15	
Val	Ile	Met	Ser	Arg	Gly	Val	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro
		20						25					30		
Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser
		35					40					45			
Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro
	50					55					60				
Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr
65					70					75					80
Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn
			85						90					95	
Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp
			100					105					110		
Thr	Ala	Arg	Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr
		115					120					125			
Tyr	Val	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser
	130					135					140				
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly
145					150					155					160
Gly	Gly	Gly	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala
			165						170					175	
Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser
		180						185					190		
Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln	Gln	Lys	Pro
		195					200					205			
Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	Val	Glu	Ser
	210					215					220				
Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Ser
225					230				235						240
Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala	Met	Tyr	Phe	Cys
			245						250					255	
Gln	Gln	Ser	Arg	Lys	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu
		260						265					270		
Glu	Ile	Lys	Arg	Asp	Gln	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr
		275					280					285			
Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Ser	Ser	Val	Phe
	290					295					300				
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro
305					310					315					320
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val
			325						330					335	
Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr
		340					345						350		
Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val
		355					360					365			
Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys
	370					375					380				
Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Ser	Ile	Glu	Lys	Thr	Ile	Ser
385					390					395					400
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro
			405						410					415	

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Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
420 425 430

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
435 440 445

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
450 455 460

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
465 470 475 480

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
485 490 495

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
500 505 510

<210> SEQ ID NO 202

<211> LENGTH: 1518

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2e12-sss-IgG1 LH SMIP (DNA)

<400> SEQUENCE: 202

```

atggattttc aagtgcagat tttcagcttc ctgctaataca gtgcttcagt cataatgtcc      60
agaggagtcg acattgtgct caccacaatct ccagcttctt tggctgtgtc tctaggtcag      120
agagccacca tctctgcag agccagtga aagtgtgaat attatgtcac aagtttaatg      180
cagtggtagc aacagaaacc aggacagcca cccaaactcc tcatctctgc tgctagcaac      240
gtagaatctg gggctccctgc cagggttagt ggcagtggtg ctgggacaga ctttagcctc      300
aacatccatc ctgtggagga ggatgatatt gcaatgtatt tctgtcagca aagtaggaag      360
gttccatgga cgttcggtag aggcaccaag ctggaaatca aacggggtag cggtggatcc      420
ggcggaggtg ggtcgggtgg cggcggatct caggtgcagc tgaaggagtc aggacctggc      480
ctggtggcgc cctcacagag cctgtccatc acatgcaccg tctcaggggt ctcattaacc      540
ggctatgggt taaactgggt tcgccagcct ccaggaaagg gtctagagtg gctgggaatg      600
atatgggggt atggaagcac agactataat tcagctctca aatccagact atcgatcacc      660
aaggacaact ccaagagcca agttttctta aaaatgaaca gtctgcaaac tgatgacaca      720
gccagatact actgtgctcg agatgggtat agtaactttc attactatgt tatggactac      780
tggtgtcaag gaacctcagt caccgtctcc tctgatcagg agcccaaata ttctgacaaa      840
actcacacat cccacccgtc ccagcacct gaactcctgg ggggaccgtc agtcttcttc      900
ttcccccaaa aacccaagga caccctcatg atctcccga cccctgaggt cacatgcgtg      960
gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg     1020
gagggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg     1080
gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcagg     1140
gtctccaaca aagccctccc agccccatc gagaaaacaa tctccaaagc caaagggcag     1200
ccccgagaac cacaggtgta caccctgccc ccatcccggg atgagctgac caagaaccag     1260
gtcagcctga cctgcctggt caaaggcttc tatcccagcg acatcgccgt ggagtgggag     1320
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc     1380

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tccttcttcc tctacagcaa gctcacccgtg gacaagagca ggtggcagca ggggaacgtc 1440
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1500
ctgtctccgg gtaaatga 1518

```

```

<210> SEQ ID NO 203
<211> LENGTH: 505
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2e12-sss-IgG1 LH SMIP (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(23)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(135)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (136)..(150)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (151)..(271)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (274)..(288)
<223> OTHER INFORMATION: Hinge

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<400> SEQUENCE: 203

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```

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1           5           10          15
Val Ile Met Ser Arg Gly Val Asp Ile Val Leu Thr Gln Ser Pro Ala
20          25          30
Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala
35          40          45
Ser Glu Ser Val Glu Tyr Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln
50          55          60
Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn
65          70          75          80
Val Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
85          90          95
Asp Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met
100         105         110
Tyr Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly
115         120         125
Thr Lys Leu Glu Ile Lys Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly
130         135         140
Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly
145         150         155         160
Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly
165         170         175
Phe Ser Leu Thr Gly Tyr Gly Val Asn Trp Val Arg Gln Pro Pro Gly
180         185         190
Lys Gly Leu Glu Trp Leu Gly Met Ile Trp Gly Asp Gly Ser Thr Asp
195         200         205

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Tyr Asn Ser Ala Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser
 210 215 220
 Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr
 225 230 235 240
 Ala Arg Tyr Tyr Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr Tyr
 245 250 255
 Val Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Asp
 260 265 270
 Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Pro
 275 280 285
 Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
 290 295 300
 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
 305 310 315 320
 Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
 325 330 335
 Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 340 345 350
 Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
 355 360 365
 Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
 370 375 380
 Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
 385 390 395 400
 Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
 405 410 415
 Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
 420 425 430
 Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
 435 440 445
 Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
 450 455 460
 Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
 465 470 475 480
 Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
 485 490 495
 Lys Ser Leu Ser Leu Ser Pro Gly Lys
 500 505

<210> SEQ ID NO 204

<211> LENGTH: 1498

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: G28-1 LH SMIP (DNA)

<400> SEQUENCE: 204

```

aagcttgccg ccatggaagc accagcgagc cttctcttcc tctgctact ctggctccca    60
gataccaccg gtgacatcca gatgactcag tctccagcct ccctatctgc atctgtggga    120
gagactgtca ccatcacatg tcgaacaagt gaaaatgttt acagttatgt ggcttggtat    180
cagcagaaac agggaaaatc tctcagctc ctggtctctt ttgcaaaaac cttagcagaa    240

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gggtgtgccaat caaggttcag tggcagtggga tcaggcacac agttttctct gaagatcagc 300
agcctgcagc ctgaagattc tggaagtat ttctgtcaac atcattccga taatccgtgg 360
acgttcgggtg gaggcaccga actggagatc aaaggtggcg gtggctcggg cgggtggggg 420
tcgggtggcg gcggatctgc tagcgcagtc cagctgcagc agtctggacc tgagctggaa 480
aagcctggcg cttcagtga gatttctgc aaggcttctg gttactcatt cactggetac 540
aatatgaact ggggtgaagca gaataatgga aagagccttg agtggattgg aaatattgat 600
ccttattatg gtggtactac ctacaaccgg aagttcaagg gcaaggccac attgactgta 660
gacaaatcct ccagcacagc ctacatgcag ctcaagagtc tgacatctga ggactctgca 720
gtctattact gtgcaagatc ggtcggccct atggactact ggggtcaagg aacctcagtc 780
accgtctcga gcgagcccaa atcttctgac aaaactcaca catgcccacc gtgcccagca 840
cctgaactcc tgggtggacc gtcagtcttc ctcttcccc caaaacccaa ggacacctc 900
atgatctccc ggacctctga ggtcacatgc gtggtgggtg acgtgagcca cgaagacct 960
gaggtcaagt tcaactggta cgtggacggc gtggagggtc ataatgcca gacaaagccg 1020
cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag 1080
gactggctga atggcaagga gtacaagtgc aaggtctcca acaagccct cccagcccc 1140
atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacacctg 1200
cccccatccc gggatgagct gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc 1260
ttctatccaa gcgacatcgc cgtggagtgg gagagcaatg ggagccgga gaacaactac 1320
aagaccacgc ctcccgctgt ggactccgac ggctccttct tcctctacag caagctcacc 1380
gtggacaaga gcagggtggc gcaggggaac gtcttctcat gctccgtgat gcatgaggct 1440
ctgcacaacc actacacgca gaagagcctc tcctgtctc cgggtaagtg actctaga 1498

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<210> SEQ ID NO 205
<211> LENGTH: 492
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: G28-1 LH SMIP (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(127)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (128)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(260)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (261)..(275)
<223> OTHER INFORMATION: Hinge

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<400> SEQUENCE: 205

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Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1           5           10           15

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Asp	Thr	Thr	Gly	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	20	25	30
Ala	Ser	Val	Gly	Glu	Thr	Val	Thr	Ile	Thr	Cys	Arg	Thr	Ser	Glu	Asn	35	40	45
Val	Tyr	Ser	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Gln	Gly	Lys	Ser	Pro	50	55	60
Gln	Leu	Leu	Val	Ser	Phe	Ala	Lys	Thr	Leu	Ala	Glu	Gly	Val	Pro	Ser	65	70	75
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Phe	Ser	Leu	Lys	Ile	Ser	85	90	95
Ser	Leu	Gln	Pro	Glu	Asp	Ser	Gly	Ser	Tyr	Phe	Cys	Gln	His	His	Ser	100	105	110
Asp	Asn	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Glu	Leu	Glu	Ile	Lys	Gly	115	120	125
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Ser	130	135	140
Ala	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Glu	Lys	Pro	Gly	Ala	145	150	155
Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Gly	Tyr	165	170	175
Asn	Met	Asn	Trp	Val	Lys	Gln	Asn	Asn	Gly	Lys	Ser	Leu	Glu	Trp	Ile	180	185	190
Gly	Asn	Ile	Asp	Pro	Tyr	Tyr	Gly	Gly	Thr	Thr	Tyr	Asn	Arg	Lys	Phe	195	200	205
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	210	215	220
Met	Gln	Leu	Lys	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	225	230	235
Ala	Arg	Ser	Val	Gly	Pro	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	245	250	255
Thr	Val	Ser	Ser	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	260	265	270
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	275	280	285
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	290	295	300
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	305	310	315
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	325	330	335
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	340	345	350
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	355	360	365
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	370	375	380
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	385	390	395
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	405	410	415
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	420	425	430

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Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> SEQ ID NO 206

<211> LENGTH: 1522

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: G28-1 HL SMIP (DNA)

<400> SEQUENCE: 206

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aagcttgccc ccattggaagc accagcgcag cttctcttcc tctgtctact ctggctccca    60
gataccaccg gtgcggtcca gctgcagcag tctggacctg agtcggaaaa gcctggcgct    120
tcagtgaaga tttcttgcaa ggcttctggt tactcattca ctggctacaa tatgaactgg    180
gtgaagcaga ataatggaaa gagccttgag tggattggaa atattgatcc ttattatggt    240
ggtactacct acaaccggaa gttcaaggcc aaggccacat tgactgtaga caaatcctcc    300
ggcacagcct acatgcagct caagagtctg acatctgagg actctgcagt ctattactgt    360
gcaagatcgg tcggccctat ggactactgg ggtcaaggaa cctcagtcac cgtctcttct    420
ggtagcgaga tccagatgac tcagtcctca gctccctat ctgcatctgt gggagagact    540
gtcaccatca catgtcgaa aagtgaataa gtttacagtt atttggcttg gtatcagcag    600
aaacagggaa aatctctca gctcctggtc tcttttgcaa aaaccttagc agaaggtgtg    660
ccatcaaggt tcagtggcag tggatcaggc acacagtttt ctctgaagat cagcagcctg    720
cagcctgaag attctggaag ttatttctgt caacatcatt ccgataatcc gtggacgttc    780
ggtaggaggc ccgaactgga gatcaaagcc tcgagcagac ccaaatcttc tgacaaaact    840
cacacatgcc caccgtgccc agcacctgaa ctctgggtg gaccgtcagt ctctctcttc    900
ccccaaaaac ccaaggacac cctcatgac tcccggaccc ctgaggtcac atgcgtggtg    960
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag   1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc   1080
agcgtctctc ccgtctctga ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc   1140
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaagccaa agggcagccc   1200
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc   1260
agcctgacct gcctgtgcaa aggcttctat ccaagcgaca tcgccgtgga gtgggagagc   1320
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc   1380
ttcttctctc acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc   1440
tcatgtctcg tgatgcataa ggctctgcac aaccactaca cgcagaagag cctctccttg   1500
tctccgggta agtgactcta ga                               1522

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<210> SEQ ID NO 207
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: G28-1 HL SMIP (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(136)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (137)..(158)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (159)..(266)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(283)
<223> OTHER INFORMATION: Hinge

<400> SEQUENCE: 207

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

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

<210> SEQ ID NO 208

<211> LENGTH: 1522

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: G19-4 LH SMIP (DNA)

<400> SEQUENCE: 208

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aagcttgccg ccatggaagc accagcgagc cttctcttcc tctgctact ctggctccca    60
gataccaccg gtgacatcca gatgacacag actacatcct cctgtctgc ctctctggga    120
gacagagtca ccatcagttg cagggcaagt caggacattc gcaattatit aaactggtat    180
cagcagaaaac cagatggaac tgttaaacct ctgatctact acacatcaag attacactca    240
ggagtcccat caaggttcag tggcagtggt tctggaacag attattctct caccattgcc    300
aacctgcaac cagaagatat tgccacttac ttttgccaac agggtaatac gcttccgtgg    360
acgttcgggtg gaggcaccaa actggttaac aaacgggggtg gcggtggctc gggcggtggt    420

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ggatctggag gaggtgggag cgctagcgag gtccagctgc aacagtctgg acctgaactg 480
gtgaagcctg gagcttcaat gaagatttcc tgcaaggcct ctggttactc attcactggc 540
tacatcgtga actggctgaa gcagagccat ggaaagaacc ttgagtggat tggacttatt 600
aatccataca aaggtcttac tacctacaac cagaaattca agggcaaggc cacattaact 660
gtagacaagt catccagcac agcctacatg gagctcctca gtctgacatc tgaagactct 720
gcagtctatt actgtgcaag atctgggtac tatggtgact cggactggta cttecatgtc 780
tggggcgcag ggaccacggt caccgtctcc tcgagcgagc ccaaatcttc tgacaaaact 840
cacacatgcc caccgtgccc agcacctgaa ctctgggtg gaccgtcagt ctctctcttc 900
ccccaaaaac ccaaggacac cctcatgatc tcccggaccc ctgaggtcac atcggtggtg 960
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag 1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc 1080
agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc 1140
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaagccaa agggcagccc 1200
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc 1260
agcctgacct gcctgtgcaa aggccttctat ccaagcgaca tcgccgtgga gtgggagagc 1320
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc 1380
ttcttcctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc 1440
tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg 1500
tctccgggta agtgactcta ga 1522

```

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<210> SEQ ID NO 209
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: G19-4 LH SMIP (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(145)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (146)..(267)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<400> SEQUENCE: 209

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```

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1           5           10           15
Asp Thr Thr Gly Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser
20           25           30
Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp

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35					40					45					
Ile	Arg	Asn	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Val
50						55					60				
Lys	Leu	Leu	Ile	Tyr	Tyr	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro	Ser
65					70					75				80	
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile	Ala
				85					90					95	
Asn	Leu	Gln	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly	Asn
			100					105					110		
Thr	Leu	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Val	Thr	Lys	Arg
			115					120				125			
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala
			130					135				140			
Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly
145					150					155				160	
Ala	Ser	Met	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Gly
				165					170					175	
Tyr	Ile	Val	Asn	Trp	Leu	Lys	Gln	Ser	His	Gly	Lys	Asn	Leu	Glu	Trp
			180					185					190		
Ile	Gly	Leu	Ile	Asn	Pro	Tyr	Lys	Gly	Leu	Thr	Thr	Tyr	Asn	Gln	Lys
			195				200						205		
Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala
			210				215				220				
Tyr	Met	Glu	Leu	Leu	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr
225					230					235				240	
Cys	Ala	Arg	Ser	Gly	Tyr	Tyr	Gly	Asp	Ser	Asp	Trp	Tyr	Phe	Asp	Val
			245					250						255	
Trp	Gly	Ala	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ser	Glu	Pro	Lys	Ser
			260					265						270	
Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu
			275				280					285			
Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu
			290				295					300			
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
305					310					315				320	
His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu
			325					330						335	
Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr
			340					345					350		
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn
			355				360					365			
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro
			370				375				380				
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln
385					390				395					400	
Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val
			405						410					415	
Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val
			420					425					430		
Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro
			435				440						445		

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Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr
	450					455					460				
Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val
465					470					475					480
Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu
				485					490					495	
Ser	Pro	Gly	Lys												
			500												

<210> SEQ ID NO 210
 <211> LENGTH: 1525
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: G19-4 HL SMIP (DNA)

<400> SEQUENCE: 210

aagcttgccg ccatggaagc accagcgagc cttctcttcc tcctgctact ctggctccca	60
gataccaccg gtgaggtcca gctgcaacag tctggacctg aactgggtgaa gcttgagct	120
tcaatgaaga tttctgcaa ggcctctggt tactcattca ctggctacat cgtgaactgg	180
ctgaagcaga gccatggaaa gaaccttgag tggattggac ttattaatcc atacaaaggt	240
cttactacct acaaccagaa attcaagggc aaggccacat tgactgtaga caagtcattcc	300
agcacagcct acatggagct cctcagtcgt acatctgaag actctgcagt ctattactgt	360
gcaagatctg ggtactatgg tgactcagac tgggtacttc atgtctgggg cgcagggacc	420
acagtcaccg tctcctctgg cggcggtggc tcggcggtg gtggatctgg aggaggtggg	480
agcgctagcg acatccagat gacacagact acatcctccc tgtctgcctc tctgggagac	540
agagtcacca tcagttgcag ggcaagtcag gacattcgca attattgaa ctgggtatcag	600
cagaaaccag atggaactgt taaactcctg atctactaca catcaagact acaactcagga	660
gtcccatcaa ggttcagtgg cagtgggtct ggaacagatt attctctcac cattgccaac	720
ctgcaaccag aagatattgc cacttacttt tgccaacagg gtaatacact tccttgagcg	780
ttcggtggag ggaccaaact ggtgacaaa cgctcgagcg agcccaaatc ttctgacaaa	840
actcacacat gccacacgtg ccacagacct gaactcctgg gtggaccgtc agtcttctc	900
ttcccccaa aacccaagga caccctcatg atctcccgga ccctgaggt cacatgcgtg	960
gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg	1020
gagggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg	1080
gtcagcgctc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcgaag	1140
gtctccaaca aagccctccc agcccccatc gagaaaacca tctccaaagc caaagggcag	1200
ccccgagaac cacaggtgta caccctgccc ccaccccggtg atgagctgac caagaaccag	1260
gtcagcctga cctgctgtgt caaaggcttc tatccaagcg acatcgccgt ggagtgggag	1320
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc	1380
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc	1440
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc	1500
ctgtctccgg gtaagtgact ctaga	1525

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<210> SEQ ID NO 211
<211> LENGTH: 501
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: G19-4 HL SMIP (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(142)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(159)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (160)..(267)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (270)..(284)
<223> OTHER INFORMATION: Hinge

<400> SEQUENCE: 211

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1          5          10          15

Asp Thr Thr Gly Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val
20          25          30

Lys Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser
35          40          45

Phe Thr Gly Tyr Ile Val Asn Trp Leu Lys Gln Ser His Gly Lys Asn
50          55          60

Leu Glu Trp Ile Gly Leu Ile Asn Pro Tyr Lys Gly Leu Thr Thr Tyr
65          70          75          80

Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser
85          90          95

Ser Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala
100         105         110

Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser Asp Trp Tyr
115         120         125

Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly
130         135         140

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Asp
145         150         155         160

Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp
165         170         175

Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu
180         185         190

Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr
195         200         205

Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
210         215         220

Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ala Asn Leu Gln Pro Glu
225         230         235         240

Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp Thr

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

<210> SEQ ID NO 212

<211> LENGTH: 2328

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: n2H7sssIgG1-STD1-2e12HL (DNA)

<400> SEQUENCE: 212

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aagcttgccg ccatggaagc accagcgagc ettctcttcc tctgtctact ctggctccca    60
gataccaccg gtcaaattgt tctctcccag tctccagcaa tcctgtctgc atctccaggg    120
gagaagggtca caatgacttg cagggccagc tcaagtgtaa gttacatgca ctggtaccag    180
cagaagccag gatcctcccc caaaccttgg atttatgccc catccaacct ggtctctgga    240
gtccctgctc gcttcagtgg cagtgggtct gggacctctt actctctcac aatcagcaga    300
gtggaggctg aagatgctgc cacttattac tgccagcagt ggagttttaa cccacccacg    360
ttcggtgctg ggaccaagct ggagctgaaa gatggcggtg gtcggggcgg tgggtgatct    420
ggaggagggtg gagctagcca ggcttatcta cagcagtctg gggctgagct ggtgaggcct    480

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ggggcctcag tgaagatgtc ctgcaaggct tctggctaca catttaccag ttacaatatg 540
cactgggttaa agcagacacc tagacagggc ctggaatgga ttggagctat ttatccagga 600
aatggtgata cttcctacaa tcagaagttc aagggcaagg ccacactgac tgtagacaaa 660
tcctccagca cagcctacat gcagctcagc agcctgacat ctgaagactc tgcgggtctat 720
ttctgtgcaa gagtgggtga ctatagtaac tcttactggt acttcgatgt ctggggcaca 780
gggaccacgg tcaccgtctc gagcgagccc aaatcttctg acaaaactca cacatcccca 840
ccgagcccag cactgaact cctgggggga cgcgcagtct tcctcttccc cccaaaaccc 900
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgt ggacgtgagc 960
cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1020
aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtgggtcag cgtcctcacc 1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggctct caacaaagcc 1140
ctcccagccc ccatcgagaa aacaatctcc aaagccaaag ggcagccccc agaaccacag 1200
gtgtacaccc tgccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1260
ctggtcaaa gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt ctctctctac 1380
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1440
atgcatgagg ctctgcacaa ccactacagc cagaagagcc tctccctgtc tccgggtaag 1500
aattatgggt gcggtggctc gggcggtggt ggatctggag gaggtgggag tgggaattct 1560
caggtgcagc tgaaggagtc aggacctggc ctggtggcgc cctcacagag cctgtccatc 1620
acatgcaccg tctcagggtt ctcatatacc ggctatggtg taaactgggt tcgccagcct 1680
ccaggaaaag gtctggagtg gctgggaatg atatggggtg atggaagcac agactataat 1740
tcagctctca aatccagact atcgatcacc aaggacaact ccaagagcca agttttctta 1800
aaaaatgaac gtctgcaaac tgatgacaca gccagatact actgtgctcg agatggttat 1860
agtaactttt attactatgt tatggactac tgggggtcaag gaacctcagt caccgtctcc 1920
tctgggggtg gaggtctctg tggcggtgga tccggcgag gtgggtcggg tggcggcgga 1980
tctgacattg tgctcaccca atctccagct tctttggctg tgtctctagg tcagagagcc 2040
accatctcct gcagagccag tgaaagtgtt gaatattatg tcacaagttt aatgcagtgg 2100
taccaacaga aaccaggaca gccacccaaa ctctcatct ctgctgctag caacgtagaa 2160
tctgggggtc ctgccagggt tagtggcagt ggggtctggga cagactttag cctcaacatc 2220
catcctgtgg aggaggtatg tattgcaatg tatttctgtc agcaaagtag gaaggttcca 2280
tggacgttcg gtggaggcac caagctggaa atcaaagctt aatctaga 2328

```

<210> SEQ ID NO 213

<211> LENGTH: 769

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polypeptide

<220> FEATURE:

<223> OTHER INFORMATION: n2H7sssIgG1-STD1-2e12HL (AA)

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (1)..(20)

<223> OTHER INFORMATION: Leader

<220> FEATURE:

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<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(127)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (128)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(516)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (517)..(637)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (638)..(657)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (658)..(769)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 213

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1          5          10          15

Asp Thr Thr Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser
20          25          30

Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
35          40          45

Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
50          55          60

Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
65          70          75          80

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
85          90          95

Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe
100         105         110

Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Asp Gly
115         120         125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln Ala
130         135         140

Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val
145         150         155         160

Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met
165         170         175

His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile Gly Ala
180         185         190

Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly
195         200         205

Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
210         215         220

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg

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225	230								235								240			
Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Thr					
				245					250					255						
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr					
				260					265					270						
His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser					
				275					280					285						
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg					
				290					295					300						
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro					
				305					310					315						
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala					
				325					330					335						
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val					
				340					345					350						
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr					
				355					360					365						
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr					
				370					375					380						
Ile	Ser	Lys	Ala	Lys	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu						
				385					390					395						
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys					
				405					410					415						
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser					
				420					425					430						
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp					
				435					440					445						
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser					
				450					455					460						
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala					
				465					470					475						
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys					
				485					490					495						
Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Gly					
				500					505					510						
Ser	Gly	Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu	Val					
				515					520					525						
Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser					
				530					535					540						
Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly					
				545					550					555						
Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp	Tyr	Asn					
				565					570					575						
Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys	Ser					
				580					585					590						
Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Arg					
				595					600					605						
Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr	Tyr	Val	Met					
				610					615					620						
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly					
				625					630					635						

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Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 645 650 655
 Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu
 660 665 670
 Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Tyr
 675 680 685
 Tyr Val Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro
 690 695 700
 Pro Lys Leu Leu Ile Ser Ala Ala Ser Asn Val Glu Ser Gly Val Pro
 705 710 715 720
 Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile
 725 730 735
 His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser
 740 745 750
 Arg Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 755 760 765

Arg

<210> SEQ ID NO 214
 <211> LENGTH: 2313
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: n2H7sssIgG1-STD2-2e12LH (DNA)

<400> SEQUENCE: 214

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aagcttgccg ccatggaagc accagcgagc cttctcttcc tctgctact ctggctccca    60
gataccaccg gtcaaattgt tctctccagc tctccagcaa tctgtctgc atctccaggg    120
gagaagggtca caatgacttg cagggccagc tcaagtgtaa gttacatgca ctggtaccag    180
cagaagccag gatcctcccc caaaccttgg atttatgccc catccaacct ggcttctgga    240
gtccctgctc gcttcagtgg cagtgggtct gggacctctt actctctcac aatcagcaga    300
gtggaggctg aagatgctgc cacttattac tgccagcagt ggagttttaa cccaccacg    360
ttcgggtgctg ggaccaagct ggagctgaaa gatggcggtg gctcgggcgg tgggtgatct    420
ggaggagggtg gagctagcca ggcttatcta cagcagctcg gggctgagct ggtgaggcct    480
ggggcctcag tgaagatgct ctgcaaggct tctggctaca cattaccag ttacaatatg    540
cactgggtaa agcagacacc tagacagggc ctggaatgga ttggagctat ttatccagga    600
aatggtgata cttctacaaa tcagaagttc aagggcaagg ccacactgac tgtagacaaa    660
tctccagca cagcctacat gcagctcagc agcctgacat ctgaagactc tgcggtctat    720
ttctgtgcaa gagtgggtga ctatagtaac tcttactggt acttcgatgt ctggggcaca    780
gggaccacgg tcaccgtctc gagcgagccc aaatcttctg acaaaactca cacatcccca    840
ccgagcccg cacctgaact cctgggggga ccgtcagttc tcctcttccc cccaaaaccc    900
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtggtggt ggacgtgagc    960
cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc    1020
aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc    1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggcttc caacaaagcc    1140
ctccagccc ccatcgagaa aacaatctcc aaagccaaag ggcagccccc agaaccacag    1200
  
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gtgtacaccc tgcceccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1260
ctggtcaaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1380
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaag 1500
aattatgggt gcggtggctc gggcgggtgt ggatctggag gaggtgggag tgggaattct 1560
gacattgtgc tcacccaatc tccagcttct ttggtgtgt ctctaggtca gagagccacc 1620
atctcctgca gagccagtga aagtgttgaa tattatgtca caagttaaat gcagtggtag 1680
caacagaaac caggacagcc acccaaatc ctcactctctg ctgctagcaa cgtagaatct 1740
ggggtccttg ccaggtttag tggcagtggt tctgggacag actttagcct caacatccat 1800
cctgtggagg aggatgatat tgcaatgtat ttctgtcagc aaagtaggaa ggttccatgg 1860
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gggtcgggtg gcggcggatc tcaggtgcag ctgaaggagt caggacctgg cctggtggcg 1980
ccctcacaga gcctgtccat cacatgcacc gtctcaggtt tctcattaac cggtctatgt 2040
gtaaaactggg ttgcgccacc tccaggaaaag ggtctggagt ggctgggaat gatatggggt 2100
gatggaagca cagactataa ttcagctctc aaatccagac tatcgatcac caaggacaa 2160
tccaagagcc aagttttctt aaaaatgaac agtctgcaa ctgatgacac agccagatac 2220
tactgtgctc gagatgggta tagtaacttt cattactatg ttatggacta ctgggggtcaa 2280
ggaacctcag tcaccgtctc ctcttaattc aga 2313

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<210> SEQ ID NO 215
<211> LENGTH: 764
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-STD2-2e12LH (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(126)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (127)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(516)
<223> OTHER INFORMATION: EFD-BD2 Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (517)..(628)
<223> OTHER INFORMATION: VL2
<220> FEATURE:

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<221> NAME/KEY: misc_feature
<222> LOCATION: (629) .. (643)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (644) .. (764)
<223> OTHER INFORMATION: VH2

<400> SEQUENCE: 215

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1          5          10          15

Asp Thr Thr Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser
20          25          30

Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
35          40          45

Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
50          55          60

Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
65          70          75          80

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
85          90          95

Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe
100         105         110

Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Asp Gly
115         120         125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln Ala
130         135         140

Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val
145         150         155         160

Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met
165         170         175

His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile Gly Ala
180         185         190

Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly
195         200         205

Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
210         215         220

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
225         230         235         240

Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp Gly Thr
245         250         255

Gly Thr Thr Val Thr Val Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr
260         265         270

His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
275         280         285

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
290         295         300

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
305         310         315         320

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
325         330         335

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
340         345         350

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr

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355					360					365					
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
	370					375					380				
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
385					390					395					400
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys
				405					410					415	
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
			420					425					430		
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
		435					440					445			
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
	450					455					460				
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
465					470					475					480
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys
			485						490					495	
Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
		500						505					510		
Ser	Gly	Asn	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala
		515					520					525			
Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser
	530					535					540				
Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln	Gln	Lys	Pro
545					550					555					560
Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	Val	Glu	Ser
				565					570					575	
Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Ser
		580						585					590		
Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala	Met	Tyr	Phe	Cys
	595					600						605			
Gln	Gln	Ser	Arg	Lys	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu
	610					615					620				
Glu	Ile	Lys	Arg	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly
625					630					635					640
Gly	Gly	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala
				645					650					655	
Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu
		660						665					670		
Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu
	675						680					685			
Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp	Tyr	Asn	Ser
	690					695					700				
Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys	Ser	Gln
705					710					715					720
Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Arg	Tyr
				725					730					735	
Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr	Tyr	Val	Met	Asp
		740						745					750		
Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser				
	755						760								

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<210> SEQ ID NO 216
<211> LENGTH: 2048
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-H1-2e12HL (DNA)

<400> SEQUENCE: 216

aagcttgccg ccatggaagc accagcgagc cttctcttcc tctgtctact ctggctccca	60
gataccaccg gtcaaattgt tctctcccag tctccagcaa tctgtctgc atctccagg	120
gagaagggtca caatgacttg cagggccagc tcaagtgtaa gttacatgca ctggtaccag	180
cagaagccag gatcctcccc caaaccttg atttatgccc catccaacct ggcttctgga	240
gtccctgtct gcttcagtgg cagtgggtct gggacctctt actctctcac aatcagcaga	300
gtggaggctg aagatgctgc cacttattac tggcagcagt ggagttttaa cccaccacg	360
ttcggtgctg ggaccaagct ggagctgaaa gatggcggtg gctcgggcgg tggtgatct	420
ggaggagggt gagctagcca ggcttatcta cagcagtctg gggctgagct ggtgaggcct	480
ggggcctcag tgaagatgtc ctgcaaggct tctggctaca catttaccag ttacaatatg	540
cactgggtaa agcagacacc tagacagggc ctggaatgga ttggagctat ttatccagga	600
aatgggtgata cttctacaaa tcagaagttc aagggcaagg ccacactgac tgtagacaaa	660
tcctccagca cagcctacat gcagctcagc agcctgacat ctgaagactc tgcggctctat	720
ttctgtgcaa gagtgggtga ctatagtaac tcttactggt acttcgatgt ctggggcaca	780
gggaccacgg tcaccgtctc gagcgagccc aaatcttctg acaaaactca cacatcccca	840
ccgagccca cactgaact cctgggggga cgcctcagct tcctcttccc cccaaaaccc	900
aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtgggtgt ggacgtgagc	960
cacgaagacc ctgaggtcaa gttcaactgg tacgtggagc gcgtggaggt gcataatgcc	1020
aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc	1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggctct caacaaagcc	1140
ctcccagccc ccatcgagaa aacaatctcc aaagccaaag ggcagccccc agaaccacag	1200
gtgtacaccc tgccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc	1260
ctggtcaaa gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg	1320
gagaacaact acaagaccac gcctcccggt ctggactccg acggctcctt cttcctctac	1380
agcaagctca ccgtggacaa gagcagggtg cagcagggga acgtcttctc atgctccgtg	1440
atgcatgagg ctctgcacaa ccactacagc cagaagagcc tctccctgtc tccgggtaag	1500
aattctcagg tgcagctgaa ggagtcagga cctggcctgg tggcgccctc acagagcctg	1560
tcctatcat gcaccgtctc aggggtctca ttaaccggt atggtgtaaa ctgggttcgc	1620
cagcctccag gaaagggtct ggagtggctg ggaatgatat ggggtgatgg aagcacagac	1680
tataattcag ctctcaaate cagactatcg atcaccaagg acaactccaa gagccaagtt	1740
ttcttaaaaa tgaacagtct gcaaactgat gacacagcca gatactactg tgctcgagat	1800
ggttatagta actttcatta ctatgttatg gactactggg gtcaaggaac ctcagtcacc	1860
gtctcctctg ggggtggagg ctctgggtgg ggtggatccg gcggaggtgg gtcgggtggc	1920
ggcggatctg acattgtgct caccatctc ccagcttctt tggctgtgtc tctaggtcag	1980

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agagccacca tctcctgcag agccagtgaag agtgttgaat attatgtcac aagtttaatg 2040
cagtggta 2048

<210> SEQ ID NO 217
<211> LENGTH: 751
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-H1-2e12HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(126)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (127)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(498)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (499)..(619)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (620)..(639)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (640)..(751)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 217

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1 5 10 15
Asp Thr Thr Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser
20 25 30
Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
35 40 45
Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
50 55 60
Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
65 70 75 80
Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
85 90 95
Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe
100 105 110
Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Asp Gly
115 120 125

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Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ala	Ser	Gln	Ala
130						135						140			
Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ala	Ser	Val
145					150					155					160
Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	Asn	Met
				165					170					175	
His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Ala
			180					185					190		
Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly
		195					200					205			
Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln
	210					215					220				
Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg
225					230					235					240
Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Thr
				245					250					255	
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr
			260					265					270		
His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser
		275					280					285			
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
	290					295					300				
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
305					310					315					320
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
				325					330					335	
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
			340					345					350		
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
		355					360					365			
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
	370					375					380				
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
385					390					395					400
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys
				405					410					415	
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
			420					425					430		
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
		435					440					445			
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
	450					455					460				
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
465					470					475					480
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys
				485					490					495	
Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro
			500					505					510		
Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr
			515				520					525			
Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu
	530					535					540				

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Trp Leu Gly Met Ile Trp Gly Asp Gly Ser Thr Asp Tyr Asn Ser Ala
 545 550 555 560
 Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val
 565 570 575
 Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Arg Tyr Tyr
 580 585 590
 Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr Tyr Val Met Asp Tyr
 595 600 605
 Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
 610 615 620
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
 625 630 635 640
 Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln
 645 650 655
 Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Tyr Tyr Val
 660 665 670
 Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys
 675 680 685
 Leu Leu Ile Ser Ala Ala Ser Asn Val Glu Ser Gly Val Pro Ala Arg
 690 695 700
 Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His Pro
 705 710 715 720
 Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Arg Lys
 725 730 735
 Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 740 745 750

<210> SEQ ID NO 218

<211> LENGTH: 2292

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: n2H7sssIgG1-H2-2e12HL (DNA)

<400> SEQUENCE: 218

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aagcttgccg ccattggaagc accagcgagc cttctcttcc tctgtctact ctggctccca    60
gataccaccg gtcaaatgtg tctctcccag tctccagcaa tctgtctgc atctccaggg    120
gagaagggtca caatgacttg cagggccagc tcaagtgtaa gttacatgca ctggtaccag    180
cagaagccag gatcctcccc caaaccttg atttatgccc catccaacct ggcttctgga    240
gtccctgctc gcttcagtgg cagtgggtct gggacctctt actctctcac aatcagcaga    300
gtggagggtg aagatgctgc cacttattac tgccagcagt ggagttttaa cccaccacag    360
ttcgggtgctg ggaccaagct ggagctgaaa gatggcggtg gctcgggcgg tggtgatct    420
ggaggaggtg gagctagcca ggcttatcta cagcagctcg ggcgtgagct ggtgaggcct    480
ggggcctcag tgaagatgct ctgcaaggct tctggctaca catttaccag ttacaatatg    540
cactgggtaa agcagacacc tagacagggc ctggaatgga ttggagctat ttatccagga    600
aatggtgata cttcctacaa tcagaagttc aagggcaagg ccacactgac tgtagacaaa    660
tctccagca cagcctacat gcagctcagc agcctgacat ctgaagactc tgcggtctat    720
ttctgtgcaa gagtgggtga ctatagtaac tcttactggg acttcgatgt ctggggcaca    780

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gggaccacgg tcaccgtctc gagcgagccc aaatcttctg aaaaaactca cacatcccca 840
ccgagcccgag cacctgaact cctgggggga cgcgcagtct tcctcttccc cccaaaaccc 900
aaggacaccc tcatgatctc cgggaccctc gaggtcacat gcgtgggtgg ggacgtgagc 960
cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1020
aagacaaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggctct caacaaagcc 1140
ctcccagccc ccatcgagaa aacaatctcc aaagccaaag ggcagccccc agaaccacag 1200
gtgtacaccc tgccccatc cgggatgag ctgaccaaga accaggtcag cctgacctgc 1260
ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320
gagaacaact acaagaccac gcctccctg ctggactccg acggtcctt ctctctctac 1380
agcaagctca ccgtggacaa gagcagggtg cagcagggga acgtctctc atgctccgtg 1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaag 1500
ggtggcgggt gctcgggaa ttctcagggt cagctgaagg agtcaggacc tggcctgggt 1560
gcgcctcac agagcctgtc catcacatgc accgtctcag ggttctcatt aaccggctat 1620
ggtgtaaaact gggttcgcca gcctccagga aagggtctgg agtggctggg aatgatatgg 1680
ggtgatggaa gcacagacta taattcagct ctcaaacca gactatcgat caccaaggac 1740
aactccaaga gccaaagtttt cttaaaaatg aacagtctgc aaactgatga cacagccaga 1800
tactactgtg ctcgagatgg ttatagtaac tttcattact atgttatgga ctactggggt 1860
caaggaacct cagtcaccgt ctctctctgg ggtggaggct ctggtggcgg tggatccggc 1920
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tatgtcacia gtttaatgca gtggtaccaa cagaaaccag gacagccacc caaactctc 2100
atctctgctg ctagcaacgt agaatctggg gtcctgcca ggtttagtgg cagtgggtct 2160
gggacagact ttagcctcaa catccatcct gtggaggagg atgatattgc aatgtatttc 2220
tgtcagcaaa gtaggaaggt tccatggacg ttcggtggag gcaccaagct ggaaatcaaa 2280
cgттаатсга ga 2292

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<210> SEQ ID NO 219
<211> LENGTH: 757
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-H2-2e12HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(126)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (127)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)

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<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(504)
<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (505)..(625)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (626)..(645)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (646)..(757)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 219

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
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Asp Thr Thr Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser
          20          25          30

Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
          35          40          45

Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
          50          55          60

Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
65          70          75          80

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
          85          90          95

Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe
          100          105          110

Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Asp Gly
          115          120          125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln Ala
          130          135          140

Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val
145          150          155          160

Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met
          165          170          175

His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile Gly Ala
          180          185          190

Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly
          195          200          205

Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
          210          215          220

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
225          230          235          240

Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp Gly Thr
          245          250          255

Gly Thr Thr Val Thr Val Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr
          260          265          270

His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
          275          280          285

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Val 290	Phe	Leu	Phe	Pro	Pro	Lys 295	Pro	Lys	Asp	Thr	Leu 300	Met	Ile	Ser	Arg
Thr 305	Pro	Glu	Val	Thr	Cys 310	Val	Val	Val	Asp	Val 315	Ser	His	Glu	Asp	Pro 320
Glu	Val	Lys	Phe	Asn 325	Trp	Tyr	Val	Asp	Gly 330	Val	Glu	Val	His	Asn 335	Ala
Lys	Thr	Lys	Pro 340	Arg	Glu	Glu	Gln	Tyr 345	Asn	Ser	Thr	Tyr	Arg 350	Val	Val
Ser	Val	Leu 355	Thr	Val	Leu	His	Gln 360	Asp	Trp	Leu	Asn	Gly 365	Lys	Glu	Tyr
Lys	Cys 370	Lys	Val	Ser	Asn	Lys 375	Ala	Leu	Pro	Ala	Pro 380	Ile	Glu	Lys	Thr
Ile 385	Ser	Lys	Ala	Lys	Gly 390	Gln	Pro	Arg	Glu	Pro 395	Gln	Val	Tyr	Thr	Leu 400
Pro	Pro	Ser	Arg	Asp 405	Glu	Leu	Thr	Lys	Asn 410	Gln	Val	Ser	Leu	Thr 415	Cys
Leu	Val	Lys	Gly 420	Phe	Tyr	Pro	Ser	Asp 425	Ile	Ala	Val	Glu	Trp 430	Glu	Ser
Asn	Gly 435	Gln	Pro	Glu	Asn	Asn	Tyr 440	Lys	Thr	Thr	Pro	Pro 445	Val	Leu	Asp
Ser	Asp 450	Gly	Ser	Phe	Phe	Leu 455	Tyr	Ser	Lys	Leu	Thr 460	Val	Asp	Lys	Ser
Arg 465	Trp	Gln	Gln	Gly	Asn 470	Val	Phe	Ser	Cys	Ser 475	Val	Met	His	Glu	Ala 480
Leu	His	Asn	His	Tyr 485	Thr	Gln	Lys	Ser	Leu 490	Ser	Leu	Ser	Pro	Gly 495	Lys
Gly	Gly	Gly	Gly 500	Ser	Gly	Asn	Ser	Gln 505	Val	Gln	Leu	Lys	Glu 510	Ser	Gly
Pro	Gly 515	Leu	Val	Ala	Pro	Ser	Gln 520	Ser	Leu	Ser	Ile	Thr 525	Cys	Thr	Val
Ser	Gly 530	Phe	Ser	Leu	Thr	Gly 535	Tyr	Gly	Val	Asn 540	Trp	Val	Arg	Gln	Pro
Pro 545	Gly	Lys	Gly	Leu	Glu 550	Trp	Leu	Gly	Met	Ile 555	Trp	Gly	Asp	Gly	Ser 560
Thr	Asp	Tyr	Asn 565	Ser	Ala	Leu	Lys	Ser	Arg 570	Leu	Ser	Ile	Thr	Lys 575	Asp
Asn	Ser	Lys	Ser 580	Gln	Val	Phe	Leu	Lys 585	Met	Asn	Ser	Leu	Gln 590	Thr	Asp
Asp	Thr 595	Ala	Arg	Tyr	Tyr	Cys	Ala 600	Arg	Asp	Gly	Tyr	Ser 605	Asn	Phe	His
Tyr 610	Tyr	Val	Met	Asp	Tyr	Trp 615	Gly	Gln	Gly	Thr	Ser 620	Val	Thr	Val	Ser
Ser 625	Gly	Gly	Gly	Gly	Ser 630	Gly	Gly	Gly	Gly	Ser 635	Gly	Gly	Gly	Gly	Ser 640
Gly	Gly	Gly	Gly 645	Ser	Asp	Ile	Val	Leu	Thr 650	Gln	Ser	Pro	Ala	Ser 655	Leu
Ala	Val	Ser	Leu 660	Gly	Gln	Arg	Ala	Thr 665	Ile	Ser	Cys	Arg	Ala 670	Ser	Glu
Ser	Val 675	Glu	Tyr	Tyr	Val	Thr	Ser 680	Leu	Met	Gln	Trp	Tyr 685	Gln	Gln	Lys
Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	Val	Gln

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690	695	700
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe		
705	710	715 720
Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe		
	725	730 735
Cys Gln Gln Ser Arg Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys		
	740	745 750
Leu Glu Ile Lys Arg		
755		

<210> SEQ ID NO 220

<211> LENGTH: 2298

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: n2H7sssIgG1-H3-2e12HL (DNA)

<400> SEQUENCE: 220

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aagcttgccg ccatggaagc accagcgagc cttctcttcc tctgtctact ctggctccca    60
gataccaccg gtcaaattgt tctctcccag tctccagcaa tctgtctgc atctccaggg    120
gagaagggtca caatgacttg cagggccagc tcaagtgtaa gttacatgca ctggtaccag    180
cagaagccag gatcctcccc caaaccttgg atttatgccc catccaacct ggcttctgga    240
gtccctgtctc gcttcagtgg cagtgggtct gggacctctt actctctcac aatcagcaga    300
gtggaggctg aagatgctgc cacttattac tgccagcagt ggagttttaa cccaccacg    360
ttcggtgctg ggaccaagct ggagctgaaa gatggcggtg gctcgggcgg tgggtgatct    420
ggaggagggtg gagctagcca ggcttatcta cagcagctctg gggctgagct ggtgaggcct    480
ggggcctcag tgaagatgtc ctgcaaggct tctggctaca catttaccag ttacaatatg    540
cactgggtaa agcagacacc tagacagggc ctggaatgga ttggagctat ttatccagga    600
aatggtgata cttcctacaa tcagaagtgc aagggcaagg ccacactgac tgtagacaaa    660
tctccagca cagcctacat gcagctcagc agcctgacat ctgaagactc tgcggtctat    720
ttctgtgcaa gagtgggtga ctatagtaac tcttactggt acttcgatgt ctggggcaca    780
gggaccacgg tcaccgtctc gagcgagccc aaatcttctg aaaaaactca cacatcccca    840
ccgagcccag cacctgaact cctgggggga cgtcagctct tctcttccc cccaaaaccc    900
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgg ggacgtgagc    960
cacgaagacc ctgaggtcaa gttcaactgg tacgtggagc gcgtggaggt gcataatgcc   1020
aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc   1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggctct caacaaagcc   1140
ctcccagccc ccatcgagaa aacaatctcc aaagccaaag ggcagccccg agaaccacag   1200
gtgtacaccc tgccccatc cgggatgag ctgaccaaga accaggtcag cctgacctgc   1260
ctggtcaaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg   1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acggtctctt ctctctctac   1380
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg   1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaag   1500
aattatgggt gcggtggctc tgggaattct caggtgcagc tgaaggagtc aggacctggc   1560

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ctggtggcgc cctcacagag cctgtccatc acatgcaccg tctcaggggt ctcattaacc 1620
ggctatgggtg taaactgggt tcgccagcct ccaggaaaagg gtctggagtg gctgggaatg 1680
atatgggggtg atggaagcac agactataat tcagctctca aatccagact atcgatcacc 1740
aaggacaact ccaagagcca agttttctta aaaatgaaca gtctgcaaac tgatgacaca 1800
gccagatact actgtgctcg agatgggttat agtaactttc attactatgt tatggactac 1860
tggggccaag gaacctcagt caccgtctcc tctgggggtg gaggctctgg tggcggtgga 1920
tccggcggag gtgggtcggg tggcggcgga tctgacattg tgctcaccca atctccagct 1980
tctttggctg tgtctctagg tcagagagcc accatctcct gcagagccag tgaaagtgtt 2040
gaatattatg tcacaagttt aatgcagtgg taccaacaga aaccaggaca gccacccaaa 2100
ctctcatct ctgctgctag caacgtagaa tctgggggtcc ctgccagggt tagtggcagt 2160
gggtctggga cagactttag cctcaacatc catcctgtgg aggaggatga tattgcaatg 2220
tatttctgtc agcaaagtag gaaggttcca tggacgttcg gtggaggcac caagctggaa 2280
atcaaacggt aatctaga 2298

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<210> SEQ ID NO 221
<211> LENGTH: 759
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-H3-2e12HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(126)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (127)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(506)
<223> OTHER INFORMATION: EFD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (507)..(627)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (628)..(647)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (648)..(759)
<223> OTHER INFORMATION: VL2

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<400> SEQUENCE: 221

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Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1           5           10           15

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Asp	Thr	Thr	Gly	Gln	Ile	Val	Leu	Ser	Gln	Ser	Pro	Ala	Ile	Leu	Ser	20	25	30
Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	Ala	Ser	Ser	Ser	35	40	45
Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser	Pro	Lys	50	55	60
Pro	Trp	Ile	Tyr	Ala	Pro	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ala	Arg	65	70	75
Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Arg	85	90	95
Val	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Phe	100	105	110
Asn	Pro	Pro	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Asp	Gly	115	120	125
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ala	Ser	Gln	Ala	130	135	140
Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ala	Ser	Val	145	150	155
Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	Asn	Met	165	170	175
His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Ala	180	185	190
Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	195	200	205
Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	210	215	220
Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	225	230	235
Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Thr	245	250	255
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	260	265	270
His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	275	280	285
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	290	295	300
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	305	310	315
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	325	330	335
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	340	345	350
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	355	360	365
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	370	375	380
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	385	390	395
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	405	410	415
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser			

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420					425					430						
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	
435					440					445						
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	
450					455					460						
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	
465					470					475					480	
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	
485					490					495						
Asn	Tyr	Gly	Gly	Gly	Ser	Gly	Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu		
500					505					510						
Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	
515					520					525						
Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	
530					535					540						
Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	
545					550					555					560	
Gly	Ser	Thr	Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	
565					570					575						
Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	
580					585					590						
Thr	Asp	Asp	Thr	Ala	Arg	Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	
595					600					605						
Phe	His	Tyr	Tyr	Val	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	
610					615					620						
Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	
625					630					635					640	
Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	
645					650					655						
Ser	Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	
660					665					670						
Ser	Glu	Ser	Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln	
675					680					685						
Gln	Lys	Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	
690					695					700						
Val	Glu	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	
705					710					715					720	
Asp	Phe	Ser	Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala	Met	
725					730					735						
Tyr	Phe	Cys	Gln	Gln	Ser	Arg	Lys	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	
740					745					750						
Thr	Lys	Leu	Glu	Ile	Lys	Arg										
755																

<210> SEQ ID NO 222

<211> LENGTH: 2307

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: n2H7sssIgG1-H4-2e12HL (DNA)

<400> SEQUENCE: 222

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gataccaccg	gtcaaattgt	tctctcccag	tctccagcaa	tctgtctgc	atctccagg	120
gagaaggtea	caatgacttg	cagggccagc	tcaagtgtaa	gttacatgca	ctggtaccag	180
cagaagccag	gatcctcccc	caaaccctgg	atttatgccc	catccaacct	ggcttctgga	240
gtccctgctc	gcttcagtgg	cagtgggtct	gggacctctt	actctctcac	aatcagcaga	300
gtggaggctg	aagatgctgc	cacttattac	tgccagcagt	ggagttttaa	cccaccacg	360
ttcggtgctg	ggaccaagct	ggagctgaaa	gatggcggtg	gctcgggcgg	tggtgatct	420
ggaggagggt	gagctagcca	ggcttatcta	cagcagtctg	gggtgagct	ggtgaggcct	480
ggggcctcag	tgaagatgtc	ctgcaaggct	tctggctaca	catttaccag	ttacaatatg	540
cactgggtaa	agcagacacc	tagacagggc	ctggaatgga	tggagctat	ttatccagga	600
aatgggtgata	cttctacaaa	tcagaagtgc	aagggcaagg	ccacactgac	tgtagacaaa	660
tctccagca	cagcctacat	gcagctcagc	agcctgacat	ctgaagactc	tgcggtctat	720
ttctgtgcaa	gagtgggtga	ctatagtaac	tcttactggg	acttcgatgt	ctggggcaca	780
gggaccacgg	tcaccgtctc	gagcgagccc	aatcttcttg	acaaaactca	cacatcccca	840
ccgagccca	cactgaact	cctgggggga	cgtcagttct	tctctctccc	cccaaaaccc	900
aaggacaccc	tcatgatctc	ccggaccctt	gaggtcacat	gcgtgggtgg	ggacgtgagc	960
cacgaagacc	ctgaggtcaa	gttcaactgg	tacgtggacg	gcgtggaggt	gcataatgcc	1020
aagacaaaag	cgcgggagga	gcagtacaac	agcacgtacc	gtgtggtcag	cgtcctcacc	1080
gtcctgcacc	aggactggct	gaatggcaag	gagtacaagt	gcaaggctct	caacaaagcc	1140
ctcccagccc	ccatcgagaa	aacaatctcc	aaagccaaag	ggcagccccc	agaaccacag	1200
gtgtacaccc	tgcctccatc	ccgggatgag	ctgaccaaga	accaggtcag	cctgacctgc	1260
ctggtcaaa	gcttctatcc	cagcgacatc	gccgtggagt	gggagagcaa	tgggcagccc	1320
gagaacaact	acaagaccac	gcctcccgtg	ctggactccg	acggctcctt	cttctctctac	1380
agcaagctca	cgtgggacaa	gagcaggtgg	cagcagggga	acgtctctct	atgctccgtg	1440
atgcatgagg	ctctgcacaa	ccactacacg	cagaagagcc	tctccctgtc	tccgggtaag	1500
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tcgatcacca	aggacaactc	caagagccaa	gttttcttaa	aatgaacag	tctgcaaaact	1800
gatgacacag	ccagatacta	ctgtgctcga	gatggttata	gtaactttca	ttactatgtt	1860
atggactact	gggttcaagg	aacctcagtc	accgtctcct	ctgggggttg	aggtctctgt	1920
ggcgggtgat	ccggcggagg	tgggtcgggt	ggcggcggat	ctgacattgt	gctcacccaa	1980
tctccagctt	ctttgctgtg	gtctctaggt	cagagagcca	ccatctctctg	cagagccagt	2040
gaaagtgttg	aatattatgt	cacaagttta	atgcagtggg	accaacagaa	accaggacag	2100
ccacccaaac	tcctcatctc	tgctgctagc	aacgtagaat	ctgggggtccc	tgccagggtt	2160
agtggcagtg	ggtctgggac	agacttttagc	ctcaacatcc	atcctgtgga	ggaggatgat	2220
attgcaatgt	atttctgtca	gcaaagtagg	aaggttccat	ggacgttcgg	tggaggcacc	2280
aagctggaaa	tcaaacgtta	atctaga				2307

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<210> SEQ ID NO 223
<211> LENGTH: 762
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-H4-2e12HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(126)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (127)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(509)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (510)..(630)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (631)..(650)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (651)..(762)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 223

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1           5           10           15

Asp Thr Thr Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser
20          25          30

Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
35          40          45

Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
50          55          60

Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
65          70          75          80

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
85          90          95

Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe
100         105         110

Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Asp Gly
115         120         125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln Ala
130         135         140

Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val

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145	150	155	160
Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met	165	170	175
His Trp Val Lys Lys Thr Pro Arg Gln Gly Leu Glu Trp Ile Gly Ala	180	185	190
Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly	195	200	205
Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln	210	215	220
Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg	225	230	235
Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp Gly Thr	245	250	255
Gly Thr Thr Val Thr Val Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr	260	265	270
His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser	275	280	285
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg	290	295	300
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro	305	310	315
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala	325	330	335
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val	340	345	350
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr	355	360	365
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr	370	375	380
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu	385	390	395
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys	405	410	415
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser	420	425	430
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp	435	440	445
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser	450	455	460
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala	465	470	475
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	485	490	495
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Asn Ser Gln Val Gln	500	505	510
Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser	515	520	525
Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Gly Tyr Gly Val Asn	530	535	540
Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly Met Ile	545	550	555
			560

<400> SEQUENCE: 224

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gataaccaccg	gtcaaatgtg	tctctcccg	tctccagcaa	tcctgtctgc	atctccagg	120
gagaagggtca	caatgacttg	cagggccagc	tcaagtgtaa	gttacatgca	ctgggtaccag	180
cagaagccag	gatacctccc	caaaccctgg	atttatgccc	catccaacct	ggcttctgga	240
gtccctgctc	gcttcagtg	cagtggggtct	gggacctctt	actctctcac	aatcagcaga	300
gtggaggtcg	aagatgctgc	cacttattac	tgccagcagt	ggagttttaa	cccaccacg	360
ttcggtgctg	ggaccaagct	ggagctgaaa	gatggcggtg	gctcgggcgg	tggtggatct	420
ggaggagggtg	gagctagcca	ggcttatcta	cagcagctctg	gggctgagct	ggtgaggcct	480
ggggcctcag	tgaagatgtc	ctgcaaggct	tctgggtaca	catttaccag	ttacaatatg	540
cactgggttaa	agcagacacc	tagacagggc	ctggaatgga	ttggagctat	ttatccagga	600
aatggtgata	cttcctacaa	tcagaagttc	aagggcaagg	ccacactgac	tgtagacaaa	660
tcctccagca	cagcctacat	gcagctcagc	agcctgacat	ctgaagactc	tgcggtctat	720
ttctgtgcaa	gaqtggtgta	ctatagtaac	tcttactggt	acttcgatgt	ctggggcaca	780

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gggaccacgg tcaccgtctc gagcgagccc aaatcttctg acaaaactca cacatcccca 840
ccgagcccg cactgaact cctgggggga cgtcagtc tctcttccc cccaaaaccc 900
aaggacccc tcatgatctc cgggaccct gaggtcacat gctgggtggt ggacgtgagc 960
cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gctgggaggt gcataatgcc 1020
aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1080
gtcctgcacc aggactggct gaatggcaag gactacaagt gcaaggtctc caacaaagcc 1140
ctcccagccc ccatcgagaa aacaatctcc aaagccaaag ggcagccccg agaaccacag 1200
gtgtacaccc tgccccatc cgggatgag ctgaccaaga accaggtcag cctgacctgc 1260
ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt ctctctctac 1380
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaag 1500
aattatgggt gcggtggctc gggcggtggt ggatctggga attctcaggt gcagctgaag 1560
gagtcaggac ctggcctggt ggcgcctca cagagcctgt ccacacatg caccgtctca 1620
gggttctcat taaccggcta tgggtgtaac tgggttcgcc agcctccagg aaaggggtctg 1680
gagtggtctg gaatgatatg ggggtgatga agcacagact ataattcagc tctcaaatcc 1740
agactatcga tcaccaagga caactccaag agccaagttt tcttaaaaat gaacagtctg 1800
caaaactgat acacagccag atactactgt gctcgagatg gttatagtaa ctttcattac 1860
tatgttatgg actactgggg tcaaggaacc tcagtcaccg tctcctctgg gggtgaggc 1920
tctgggtggc gtggatccgg cggaggtggg tcgggtggcg gcggatctga cattgtgctc 1980
acccaatctc cagcttcttt ggctgtgtct ctaggtcaga gagccaccat ctctgcaga 2040
gccagtgaat gtgttgaata ttatgtcaca agtttaatgc agtggtagca acagaaacca 2100
ggacagccac ccaaactcct catctctgct gctagcaacg tagaatctgg ggtccctgcc 2160
agggttagtg gcagtggtgc tgggacagac tttagcctca acatccatcc tgtggaggag 2220
gatgatattg caatgtatct ctgtcagcaa agtaggaagg ttccatggac gttcggtgga 2280
ggcaccaagc tggaaatcaa acgttaatct aga 2313

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<210> SEQ ID NO 225
<211> LENGTH: 764
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-H5-2e12HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(126)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (127)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)
<223> OTHER INFORMATION: VH

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(511)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (512)..(632)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (633)..(652)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (653)..(764)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 225

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1          5          10          15

Asp Thr Thr Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser
          20          25          30

Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
          35          40          45

Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
          50          55          60

Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
          65          70          75          80

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
          85          90          95

Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe
          100          105          110

Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Asp Gly
          115          120          125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln Ala
          130          135          140

Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val
          145          150          155          160

Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met
          165          170          175

His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile Gly Ala
          180          185          190

Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly
          195          200          205

Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
          210          215          220

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
          225          230          235          240

Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp Gly Thr
          245          250          255

Gly Thr Thr Val Thr Val Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr
          260          265          270

His Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
          275          280          285

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Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	
290						295					300					
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	
305					310					315					320	
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	
				325					330					335		
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	
			340					345					350			
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	
		355					360					365				
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	
	370					375					380					
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	
385					390					395					400	
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	
				405					410						415	
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	
			420					425					430			
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	
		435					440					445				
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	
	450					455					460					
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	
465					470					475					480	
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	
				485					490					495		
Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Asn	Ser	Gln	
			500					505					510			
Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	
		515					520					525				
Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	
	530					535					540					
Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	
545					550					555					560	
Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr	Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	
				565					570					575		
Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	
			580				585						590			
Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Arg	Tyr	Tyr	Cys	Ala	Arg	
		595					600					605				
Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr	Tyr	Val	Met	Asp	Tyr	Trp	Gly	Gln	
	610					615					620					
Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	
625					630					635					640	
Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Val	Leu	
				645					650				655			
Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	
			660					665					670			
Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser	Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	
		675					680					685				
Met	Gln	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	
	690					695					700					

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Ser Ala Ala Ser Asn Val Glu Ser Gly Val Pro Ala Arg Phe Ser Gly
 705 710 715 720

Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His Pro Val Glu Glu
 725 730 735

Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Arg Lys Val Pro Trp
 740 745 750

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 755 760

<210> SEQ ID NO 226
 <211> LENGTH: 2322
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: n2H7sssIgG1-H6-2e12HL (DNA)

<400> SEQUENCE: 226

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gagaagggtca caatgacttg cagggccagc tcaagtgtaa gttacatgca ctggtaccag    180
cagaagccag gatcctcccc caaaccttg atttatgccc catccaacct ggcttctgga    240
gtccctgctc gcttcagtgg cagtgggtct gggacctctt actctctcac aatcagcaga    300
gtggaggctg aagatgctgc cacttattac tgccagcagt ggagttttaa cccaccacg    360
ttcgggtgctg ggaccaagct ggagctgaaa gatggcggtg gctcgggcgg tggtgatct    420
ggaggagggtg gagctagcca ggcttatcta cagcagtctg gggctgagct ggtgaggcct    480
ggggcctcag tgaagatgct ctgcaaggct tctggctaca catttaccag ttacaatatg    540
cactgggttaa agcagacacc tagacagggc ctggaatgga ttggagctat ttatccagga    600
aatgggtgata cttcctacaa tcagaagttc aagggaagg ccacactgac tgtagacaaa    660
tctccagca cagcctacat gcagctcagc agcctgacat ctgaagactc tgcggtctat    720
ttctgtgcaa gagtgggtga ctatagtaac tcttactggt acttcgatgt ctggggcaca    780
gggaccacgg tcaccgtctc gagcagagcc aaatcttctg acaaaactca cacatcccca    840
ccgagcccag cacctgaact cctgggggga ccgtcagttt tctcttccc cccaaaaccc    900
aaggacaccc tcatgatctc ccggaccctt gaggtcacat gcgtgggtgg ggacgtgagc    960
cacgaagacc ctgagggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc   1020
aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtgggtcag cgtcctcacc   1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaagggtct caacaaagcc   1140
ctcccagccc ccatcgagaa aacaatctcc aaagccaaag ggcagccccg agaaccacag   1200
gtgtacaccc tgccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc   1260
ctggtcaaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg   1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acgctcctt cttcctctac   1380
agcaagctca ccgtggacaa gagcagggtg cagcagggga acgtcttctc atgctccgtg   1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaag   1500
ggtgggcggg gctcgggcgg tgggtgatct gggggaggag gcagcgggaa ttctcagggt   1560

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cagctgaagg agtcaggacc tggcctggtg ggcgcctcac agagcctgtc catcacatgc 1620
accgtctcag ggttctcatt aaccggctat ggtgtaaact gggttcgcca gctccagga 1680
aagggtctgg agtggctggg aatgatatgg ggtgatggaa gcacagacta taattcagct 1740
ctcaaatacca gactatcgat caccaaggac aactccaaga gccaaagtttt cttaaaaatg 1800
aacagtctgc aaactgatga cacagccaga tactactgtg ctcgagatgg ttatagtaac 1860
tttcattact atgttatgga ctactggggg caaggaacct cagtcaccgt ctctctggg 1920
gggtggaggct ctgggtggcg tggatccggc ggaggtgggt cgggtggcg cggtatctgac 1980
attgtgctca cccaatctcc agcttctttg gctgtgtctc taggtcagag agccaccatc 2040
tcctgcagag ccagtgaaag tgttgaatat tatgtcacia gttaaatgca gtggtaccaa 2100
cagaaaccag gacagccacc caaactctc atctctgctg ctacgaacgt agaactctggg 2160
gtccctgccg ggtttagtgg cagtgggtct gggacagact ttagcctcaa catccatcct 2220
gtggaggagg atgatattgc aatgtatttc tgtcagcaaa gtaggaaggt tccatggacg 2280
ttcggtgagg gcaccaagct ggaaatcaaa cgtaaatcta ga 2322

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<210> SEQ ID NO 227
<211> LENGTH: 767
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: n2H7sssIgG1-H6-2e12HL (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(126)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (127)..(142)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (143)..(264)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (265)..(279)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (497)..(514)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (515)..(635)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (636)..(655)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (656)..(767)
<223> OTHER INFORMATION: VL2

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<400> SEQUENCE: 227

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Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1           5           10           15

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Asp	Thr	Thr	Gly 20	Gln	Ile	Val	Leu	Ser 25	Gln	Ser	Pro	Ala	Ile 30	Leu	Ser
Ala	Ser	Pro 35	Gly	Glu	Lys	Val	Thr 40	Met	Thr	Cys	Arg	Ala 45	Ser	Ser	Ser
Val	Ser 50	Tyr	Met	His	Trp	Tyr 55	Gln	Gln	Lys	Pro	Gly 60	Ser	Ser	Pro	Lys
Pro 65	Trp	Ile	Tyr	Ala	Pro 70	Ser	Asn	Leu	Ala	Ser 75	Gly	Val	Pro	Ala	Arg 80
Phe	Ser	Gly	Ser	Gly 85	Ser	Gly	Thr	Ser	Tyr 90	Ser	Leu	Thr	Ile	Ser 95	Arg
Val	Glu	Ala	Glu 100	Asp	Ala	Ala	Thr	Tyr 105	Tyr	Cys	Gln	Gln	Trp 110	Ser	Phe
Asn	Pro	Pro 115	Thr	Phe	Gly	Ala	Gly 120	Thr	Lys	Leu	Glu	Leu 125	Lys	Asp	Gly
Gly	Gly 130	Ser	Gly	Gly	Gly	Gly 135	Ser	Gly	Gly	Gly	Gly 140	Ala	Ser	Gln	Ala
Tyr 145	Leu	Gln	Gln	Ser	Gly 150	Ala	Glu	Leu	Val	Arg 155	Pro	Gly	Ala	Ser	Val 160
Lys	Met	Ser	Cys 165	Lys	Ala	Ser	Gly	Tyr	Thr 170	Phe	Thr	Ser	Tyr	Asn 175	Met
His	Trp	Val	Lys 180	Gln	Thr	Pro	Arg	Gln 185	Gly	Leu	Glu	Trp	Ile 190	Gly	Ala
Ile	Tyr	Pro 195	Gly	Asn	Gly	Asp	Thr 200	Ser	Tyr	Asn	Gln	Lys 205	Phe	Lys	Gly
Lys	Ala 210	Thr	Leu	Thr	Val	Asp 215	Lys	Ser	Ser	Ser	Thr 220	Ala	Tyr	Met	Gln
Leu 225	Ser	Ser	Leu	Thr	Ser 230	Glu	Asp	Ser	Ala	Val 235	Tyr	Phe	Cys	Ala	Arg 240
Val	Val	Tyr	Tyr 245	Ser	Asn	Ser	Tyr	Trp	Tyr 250	Phe	Asp	Val	Trp	Gly 255	Thr
Gly	Thr	Thr	Val 260	Thr	Val	Ser	Ser	Glu 265	Pro	Lys	Ser	Ser	Asp 270	Lys	Thr
His	Thr 275	Ser	Pro	Pro	Ser	Pro	Ala 280	Pro	Glu	Leu	Leu	Gly 285	Gly	Pro	Ser
Val	Phe 290	Leu	Phe	Pro	Pro	Lys 295	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
Thr 305	Pro	Glu	Val	Thr	Cys 310	Val	Val	Val	Asp	Val 315	Ser	His	Glu	Asp	Pro 320
Glu	Val	Lys	Phe 325	Asn	Trp	Tyr	Val	Asp	Gly 330	Val	Glu	Val	His	Asn	Ala 335
Lys	Thr	Lys	Pro 340	Arg	Glu	Glu	Gln	Tyr 345	Asn	Ser	Thr	Tyr	Arg 350	Val	Val
Ser	Val 355	Leu	Thr	Val	Leu	His	Gln 360	Asp	Trp	Leu	Asn	Gly 365	Lys	Glu	Tyr
Lys	Cys 370	Lys	Val	Ser	Asn	Lys 375	Ala	Leu	Pro	Ala	Pro 380	Ile	Glu	Lys	Thr
Ile 385	Ser	Lys	Ala	Lys	Gly 390	Gln	Pro	Arg	Glu	Pro 395	Gln	Val	Tyr	Thr	Leu 400
Pro	Pro	Ser	Arg 405	Asp	Glu	Leu	Thr	Lys	Asn 410	Gln	Val	Ser	Leu	Thr	Cys
Leu	Val	Lys	Gly 420	Phe	Tyr	Pro	Ser	Asp 425	Ile	Ala	Val	Glu	Trp 430	Glu	Ser

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Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
 435 440 445
 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
 450 455 460
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
 465 470 475 480
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 485 490 495
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 500 505 510
 Asn Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro
 515 520 525
 Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr
 530 535 540
 Gly Tyr Gly Val Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu
 545 550 555 560
 Trp Leu Gly Met Ile Trp Gly Asp Gly Ser Thr Asp Tyr Asn Ser Ala
 565 570 575
 Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val
 580 585 590
 Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Arg Tyr Tyr
 595 600 605
 Cys Ala Arg Asp Gly Tyr Ser Asn Phe His Tyr Tyr Val Met Asp Tyr
 610 615 620
 Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
 625 630 635 640
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
 645 650 655
 Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln
 660 665 670
 Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Tyr Tyr Val
 675 680 685
 Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys
 690 695 700
 Leu Leu Ile Ser Ala Ala Ser Asn Val Glu Ser Gly Val Pro Ala Arg
 705 710 715 720
 Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His Pro
 725 730 735
 Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Arg Lys
 740 745 750
 Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 755 760 765

<210> SEQ ID NO 228

<211> LENGTH: 2337

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: 2H7sssIgG1-H7-G281 HL (DNA)

<400> SEQUENCE: 228

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gtcataatgt ccagaggaca aattgttctc tcccagtctc cagcaatcct gtctgcatct	120
ccaggggaga aggtcacaat gacttgacagg gccagctcaa gtgtaagtta catgactagg	180
taccagcaga agccaggatc ctccccaaa cctgggattt atgccccatc caacctggct	240
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cctcttactc tctcacaatc	300
agcagagtgg aggctgaaga tgctgccact tattactgcc agcagtggag ttttaacca	360
cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt	420
ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtgggtg	480
aggcctgggg cctcagtga gatgtcctgc aaggcttctg gctacacatt taccagttac	540
aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat	600
ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta	660
gacaaatcct ccagcacagc ctacatgcag ctacagagcc tgacatctga agactctgcg	720
gtctatttct gtgcaagagt ggtgtactat agtaactctt actgggtactt cgatgtctgg	780
ggcacaggga ccacggctac cgtctctgat caggagccca aatcttgtga caaaactcac	840
acatccccac cgtgctcagc acctgaactc ctggggggac cgtcagctct cctcttcccc	900
ccaaaaccca aggacacct catgatctcc cggaccctcg aggtcacatg cgtggtggtg	960
gacgtgagcc acgaagacc tgaggtcaag ttcaactggt acgtggacgg cgtggagggtg	1020
cataatgcc agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc	1080
gtcctcacgg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc	1140
aacaagccc tcccagcccc catcgagaaa acaatctcca aagccaaagg gcagccccga	1200
gaaccacagg tgtaaccct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc	1260
ctgacctgcc tggtaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat	1320
gggcagccgg agaacaacta caagaccag cctcccgtgc tggactccga cgctccttc	1380
ttctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca	1440
tgctccgtga tgcatgaggc tctgcacaac cactacagc agaagagcct ctccctgtct	1500
ccgggtaaga attatggtg cgggtgctcg ggcggtggtg gatctggagg aggtgggagt	1560
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ctgtccatca catgcacct ctcagggttc tcattaaccg gctatggtgt aaactgggtt	1680
cgcagcctc caggaaagg tctggagtgg ctgggaatga tatgggtga tggaagcaca	1740
gactataatt cagctctcaa atccagacta tcgatcaca aggacaactc caagagccaa	1800
gttttcttaa aaatgaacag tctgcaaaact gatgacacag ccagatacta ctgtgctcga	1860
gatgggtata gtaactttca ttactatgt atggactact ggggtcaagg aacctcagtc	1920
accgtctcct ctgggggtgg aggtctctgt ggcggtggt cggcgaggag tgggtcgggt	1980
ggcgcgggat ctgacattgt gctcacccaa tctccagctt ctttggctgt gtctctaggt	2040
cagagagcca catctcctg cagagccagt gaaagtgtg aatattatgt cacaagttta	2100
atgcagtggt accaagacaa accaggacag ccacccaaac tcctcatctc tgctgctagc	2160
aacgtagaat ctgggggtccc tgccagggtt agtggcagtg ggtctgggac agacttttagc	2220
ctcaacatcc atcctgtgga ggaggtgat attgcaatgt atttctgtca gcaaagtagg	2280
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<210> SEQ ID NO 229
<211> LENGTH: 772
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: 2H7cscIgG1-STD1-2e12HL (w/2E12 leader) (AA)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(22)
<223> OTHER INFORMATION: Leader
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(128)
<223> OTHER INFORMATION: VL
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(144)
<223> OTHER INFORMATION: Linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (145)..(265)
<223> OTHER INFORMATION: VH
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (268)..(282)
<223> OTHER INFORMATION: Hinge
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (500)..(519)
<223> OTHER INFORMATION: EPD-Scorpion linker
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (520)..(640)
<223> OTHER INFORMATION: VH2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (641)..(660)
<223> OTHER INFORMATION: Linker2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (661)..(772)
<223> OTHER INFORMATION: VL2

<400> SEQUENCE: 229

Met	Asp	Phe	Gln	Val	Gln	Ile	Phe	Ser	Phe	Leu	Leu	Ile	Ser	Ala	Ser
1				5					10					15	
Val	Ile	Met	Ser	Arg	Gly	Gln	Ile	Val	Leu	Ser	Gln	Ser	Pro	Ala	Ile
		20						25					30		
Leu	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	Ala	Ser
		35						40					45		
Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser
		50				55					60				
Pro	Lys	Pro	Trp	Ile	Tyr	Ala	Pro	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
65				70					75				80		
Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile
				85					90				95		
Ser	Arg	Val	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp
		100						105					110		
Ser	Phe	Asn	Pro	Pro	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys
		115					120					125			
Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser
	130					135						140			
Gln	Ala	Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Ser	Val	Arg	Pro	Gly	Ala
145					150					155				160	

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Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	165	170	175
Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile	180	185	190
Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	195	200	205
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	210	215	220
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	225	230	235
Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	245	250	255
Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Cys	260	265	270
Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Cys	Ser	Ala	Pro	Glu	Leu	Leu	Gly	275	280	285
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	290	295	300
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	305	310	315
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	325	330	335
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	340	345	350
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	355	360	365
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	370	375	380
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	385	390	395
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	405	410	415
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	420	425	430
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	435	440	445
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	450	455	460
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	465	470	475
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	485	490	495
Pro	Gly	Lys	Asn	Tyr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	500	505	510
Gly	Gly	Gly	Ser	Gly	Asn	Ser	Gln	Val	Gln	Leu	Lys	Glu	Ser	Gly	Pro	515	520	525
Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	530	535	540
Gly	Phe	Ser	Leu	Thr	Gly	Tyr	Gly	Val	Asn	Trp	Val	Arg	Gln	Pro	Pro	545	550	555
Gly	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Met	Ile	Trp	Gly	Asp	Gly	Ser	Thr			

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565					570					575					
Asp	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn
580					585					590					
Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp
595					600					605					
Thr	Ala	Arg	Tyr	Tyr	Cys	Ala	Arg	Asp	Gly	Tyr	Ser	Asn	Phe	His	Tyr
610					615					620					
Tyr	Val	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser
625					630					635					
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly
645					650					655					
Gly	Gly	Gly	Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala
660					665					670					
Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser
675					680					685					
Val	Glu	Tyr	Tyr	Val	Thr	Ser	Leu	Met	Gln	Trp	Tyr	Gln	Gln	Lys	Pro
690					695					700					
Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Ser	Ala	Ala	Ser	Asn	Val	Glu	Ser
705					710					715					
Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Ser
725					730					735					
Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Asp	Asp	Ile	Ala	Met	Tyr	Phe	Cys
740					745					750					
Gln	Gln	Ser	Arg	Lys	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu
755					760					765					
Glu	Ile	Lys	Arg												
770															

<210> SEQ ID NO 230
 <211> LENGTH: 45
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide
 <220> FEATURE:
 <223> OTHER INFORMATION: sss(s) -hIgG1 (DNA)

<400> SEQUENCE: 230

gagcccaaat cttctgacaa aactcacaca tctccaccga gctca

45

<210> SEQ ID NO 231
 <211> LENGTH: 15
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polypeptide
 <220> FEATURE:
 <223> OTHER INFORMATION: sss(s) -hIgG1 (AA)

<400> SEQUENCE: 231

Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Ser
1				5					10				15	

<210> SEQ ID NO 232
 <211> LENGTH: 45
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic polynucleotide

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<220> FEATURE:
<223> OTHER INFORMATION: csc(s) (DNA)

<400> SEQUENCE: 232

gagcccaaat cttgtgacaa aactcacaca tctccaccgt gctca 45

<210> SEQ ID NO 233
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: csc(s) (AA)

<400> SEQUENCE: 233

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys Ser
1 5 10 15

<210> SEQ ID NO 234
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H8 (PN)

<400> SEQUENCE: 234

gggtctccac cttctccgaa ttct 24

<210> SEQ ID NO 235
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 235

Gly Ser Pro Pro Ser Pro Asn Ser
1 5

<210> SEQ ID NO 236
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H9 (PN)

<400> SEQUENCE: 236

gggtctccac cttctccgaa ttct 24

<210> SEQ ID NO 237
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H9 (AA)

<400> SEQUENCE: 237

Gly Ser Pro Pro Ser Pro Asn Ser
1 5

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<210> SEQ ID NO 238

<400> SEQUENCE: 238

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<210> SEQ ID NO 239

<400> SEQUENCE: 239

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<210> SEQ ID NO 240

<211> LENGTH: 51

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H11 (PN)

<400> SEQUENCE: 240

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<210> SEQ ID NO 241

<211> LENGTH: 17

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H11 (AA)

<400> SEQUENCE: 241

Glu Pro Lys Ser Thr Asp Lys Thr His Thr Ser Pro Pro Ser Pro Asn
1 5 10 15

Ser

<210> SEQ ID NO 242

<211> LENGTH: 51

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H12 (PN)

<400> SEQUENCE: 242

gagccccacat ctaccgacaa aactcacaca tctccaccca gcccgaaattc t 51

<210> SEQ ID NO 243

<211> LENGTH: 17

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H12 (AA)

<400> SEQUENCE: 243

Glu Pro Thr Ser Thr Asp Lys Thr His Thr Cys Pro Pro Cys Pro Asn
1 5 10 15

Ser

-continued

<210> SEQ ID NO 244
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H13 (PN)

<400> SEQUENCE: 244

gagcccatctacccgacaa aactcacaca tctccaccca gccgaattc t 51

<210> SEQ ID NO 245
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H13 (AA)

<400> SEQUENCE: 245

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Asn
1 5 10 15

Ser

<210> SEQ ID NO 246
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H15 (PN)

<400> SEQUENCE: 246

ggcgggtggtg gctcctgtcc acctgtgccg aattct 36

<210> SEQ ID NO 247
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H15 (AA)

<400> SEQUENCE: 247

Gly Gly Gly Gly Ser Cys Pro Pro Cys Pro Asn Ser
1 5 10

<210> SEQ ID NO 248
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H16 (PN)

<400> SEQUENCE: 248

ctgtctgtga aagctgactt cctcactcca tccatcgga attct 45

<210> SEQ ID NO 249
<211> LENGTH: 15

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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H16 (AA)

<400> SEQUENCE: 249

Leu Ser Val Lys Ala Asp Phe Leu Thr Pro Ser Ile Gly Asn Ser
1 5 10 15

<210> SEQ ID NO 250
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H17 (PN)

<400> SEQUENCE: 250

ctgtctgtga aagctgactt cctcactcca tccatctcct gtccaccttg cccgaattct 60

<210> SEQ ID NO 251
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H17 (AA)

<400> SEQUENCE: 251

Leu Ser Val Lys Ala Asp Phe Leu Thr Pro Ser Ile Ser Cys Pro Pro
1 5 10 15

Cys Pro Asn Ser
20

<210> SEQ ID NO 252
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H18 (PN)

<400> SEQUENCE: 252

ctgtctgtgc tcgctaaactt cagtcagcca gagatcggga attct 45

<210> SEQ ID NO 253
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H18 (AA)

<400> SEQUENCE: 253

Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Gly Asn Ser
1 5 10 15

<210> SEQ ID NO 254
<211> LENGTH: 60
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H19 (PN)

<400> SEQUENCE: 254

ctgtctgtgc tcgctaactt cagtcagcca gagatctect gtccaccttg cccgaattct 60

<210> SEQ ID NO 255
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H19 (AA)

<400> SEQUENCE: 255

Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Ser Cys Pro Pro
1 5 10 15

Cys Pro Asn Ser
20

<210> SEQ ID NO 256
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H20 (PN)

<400> SEQUENCE: 256

ctgaaaatcc aggagagggt cagtaagcca aagatctcga attct 45

<210> SEQ ID NO 257
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H20 (AA)

<400> SEQUENCE: 257

Leu Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Asn Ser
1 5 10 15

<210> SEQ ID NO 258
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H21 (PN)

<400> SEQUENCE: 258

ctgaaaatcc aggagagggt cagtaagcca aagatctcct gtccaccttg cccgaattct 60

<210> SEQ ID NO 259
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H21 (AA)

<400> SEQUENCE: 259

Leu Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Cys Pro Pro
1 5 10 15

Cys Pro Asn Ser
20

<210> SEQ ID NO 260
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H22 (PN)

<400> SEQUENCE: 260

ctggatgtga gtgagaggcc ttttctcca cacatccaga attct 45

<210> SEQ ID NO 261
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H22 (AA)

<400> SEQUENCE: 261

Leu Asp Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Asn Ser
1 5 10 15

<210> SEQ ID NO 262
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H23 (PN)

<400> SEQUENCE: 262

ctggatgtga gtgagaggcc ttttctcca cacatccagt cctgtccacc ttgcccgat 60

tct 63

<210> SEQ ID NO 263
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H23 (AA)

<400> SEQUENCE: 263

Leu Asp Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Ser Cys Pro
1 5 10 15

Pro Cys Pro Asn Ser
20

<210> SEQ ID NO 264

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<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H24 (DNA)

<400> SEQUENCE: 264
cggggaacagc tggcagaggt cactttgagc ttgaaagcga attct 45

<210> SEQ ID NO 265
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H24 (AA)

<400> SEQUENCE: 265
Arg Glu Gln Leu Ala Glu Val Thr Leu Ser Leu Lys Ala Asn Ser
1 5 10 15

<210> SEQ ID NO 266
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H25 (PN)

<400> SEQUENCE: 266
cggggaacagc tggcagaggt cactttgagc gtgaaagctt gtccaccctg cccgaattct 60

<210> SEQ ID NO 267
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H25 (AA)

<400> SEQUENCE: 267
Arg Glu Gln Leu Ala Glu Val Thr Leu Ser Leu Lys Ala Cys Pro Pro
1 5 10 15
Cys Pro Asn Ser
20

<210> SEQ ID NO 268
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H26 (PN)

<400> SEQUENCE: 268
cggattcacc agatgaactc cgagttgagc gtgctcgcga attct 45

<210> SEQ ID NO 269
<211> LENGTH: 15
<212> TYPE: PRT

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H26 (AA)

<400> SEQUENCE: 269

Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala Asn Ser
1 5 10 15

<210> SEQ ID NO 270
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H27 (PN)

<400> SEQUENCE: 270

cggattcacc agatgaactc cgagttgagc gtgctcgctt gtccaccctg cccgaattct 60

<210> SEQ ID NO 271
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H27 (AA)

<400> SEQUENCE: 271

Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala Cys Pro Pro
1 5 10 15

Cys Pro Asn Ser
 20

<210> SEQ ID NO 272
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H28 (PN)

<400> SEQUENCE: 272

gataccaaaag ggaagaacgt cctcgagaag atcttctcga attct 45

<210> SEQ ID NO 273
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H28 (AA)

<400> SEQUENCE: 273

Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Ser Asn Ser
1 5 10 15

<210> SEQ ID NO 274
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H29 (PN)

<400> SEQUENCE: 274

gataccaaaag ggaagaacgt cctcgagaag atcttcgact cctgtccacc ttgcccgaaat 60

tct 63

<210> SEQ ID NO 275
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H29 (AA)

<400> SEQUENCE: 275

Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Ser Cys Pro
1 5 10 15

Pro Cys Pro Asn Ser
20

<210> SEQ ID NO 276
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H30 (PN)

<400> SEQUENCE: 276

ctgccacctg agacacagga gagtcaagaa gtcaccctga attct 45

<210> SEQ ID NO 277
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H30 (AA)

<400> SEQUENCE: 277

Leu Pro Pro Glu Thr Gln Glu Ser Gln Glu Val Thr Leu Asn Ser
1 5 10 15

<210> SEQ ID NO 278
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H31 (PN)

<400> SEQUENCE: 278

ctgccacctg agacacagga gagtcaagaa gtcaccctgt cctgtccacc ttgcccgaaat 60

tct 63

<210> SEQ ID NO 279
<211> LENGTH: 21

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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H31 (AA)

<400> SEQUENCE: 279

Leu Pro Pro Glu Thr Gln Glu Ser Gln Glu Val Thr Leu Ser Cys Pro
1 5 10 15

Pro Cys Pro Asn Ser
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<210> SEQ ID NO 280
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H32 (PN)

<400> SEQUENCE: 280

cggaattcacc tgaacgtgtc cgagaggccc tttcctccga attct 45

<210> SEQ ID NO 281
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H32 (AA)

<400> SEQUENCE: 281

Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe Pro Pro Asn Ser
1 5 10 15

<210> SEQ ID NO 282
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H33 (PN)

<400> SEQUENCE: 282

cggaattcacc tgaacgtgtc cgagaggccc tttcctccct gtccaccctg ccgaattct 60

<210> SEQ ID NO 283
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H33 (AA)

<400> SEQUENCE: 283

Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe Pro Pro Cys Pro Pro
1 5 10 15

Cys Pro Asn Ser
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<210> SEQ ID NO 284

<400> SEQUENCE: 284

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<210> SEQ ID NO 285

<400> SEQUENCE: 285

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<210> SEQ ID NO 286

<400> SEQUENCE: 286

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<210> SEQ ID NO 287

<400> SEQUENCE: 287

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<210> SEQ ID NO 288

<211> LENGTH: 39

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H36 (PN)

<400> SEQUENCE: 288

gggtgtccac cttgtccagg cgggtggtgga tcgaattct

39

<210> SEQ ID NO 289

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H36 (AA)

<400> SEQUENCE: 289

Gly Cys Pro Pro Cys Pro Gly Gly Gly Gly Ser Asn Ser
1 5 10

<210> SEQ ID NO 290

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<210> SEQ ID NO 291

<400> SEQUENCE: 291

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<210> SEQ ID NO 292

<400> SEQUENCE: 292

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<210> SEQ ID NO 294

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<210> SEQ ID NO 295

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<210> SEQ ID NO 296

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H40 (PN)

<400> SEQUENCE: 296

ggatgtccac cttgtcccg cgaattct

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<210> SEQ ID NO 297

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H40 (AA)

<400> SEQUENCE: 297

Gly Cys Pro Pro Cys Pro Ala Asn Ser
1 5

<210> SEQ ID NO 298

<400> SEQUENCE: 298

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<210> SEQ ID NO 299

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

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<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<221> NAME/KEY: misc_feature

<223> OTHER INFORMATION: Scorpion linker H41

<400> SEQUENCE: 299

Gly Cys Pro Pro Cys Pro Ala Asn Ser
1 5

<210> SEQ ID NO 300

<400> SEQUENCE: 300

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<210> SEQ ID NO 301
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H42 (AA)

<400> SEQUENCE: 301

Gly Cys Pro Pro Cys Pro Asn Ser
1 5

<210> SEQ ID NO 302

<400> SEQUENCE: 302

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<210> SEQ ID NO 303

<400> SEQUENCE: 303

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<210> SEQ ID NO 304
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H44 (PN)

<400> SEQUENCE: 304

ggaggaggag ctagttgtcc accttggtccc gggaattct

39

<210> SEQ ID NO 305
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H44 (AA)

<400> SEQUENCE: 305

Gly Gly Gly Ala Ser Cys Pro Pro Cys Pro Gly Asn Ser
1 5 10

<210> SEQ ID NO 306
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H45 (PN)

<400> SEQUENCE: 306

ggaggaggag ccagttgtcc accttggtgcc gggaattct

39

<210> SEQ ID NO 307

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<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H45 (AA)

<400> SEQUENCE: 307

Gly Gly Gly Ala Ser Cys Pro Pro Cys Ala Gly Asn Ser
1 5 10

<210> SEQ ID NO 308
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polynucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H46

<400> SEQUENCE: 308

ggaggaggag ccagttgtcc accttgtagc aattct 36

<210> SEQ ID NO 309
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H46

<400> SEQUENCE: 309

Gly Gly Gly Ala Ser Cys Pro Pro Cys Ala Asn Ser
1 5 10

<210> SEQ ID NO 310
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H47

<400> SEQUENCE: 310

Leu Ser Val Lys Ala Asp Phe Leu Thr Pro Ser Ile Gly Asn Ser
1 5 10 15

<210> SEQ ID NO 311
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H48

<400> SEQUENCE: 311

Lys Ala Asp Phe Leu Thr Pro Ser Ile Gly Asn Ser
1 5 10

<210> SEQ ID NO 312

<400> SEQUENCE: 312

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<210> SEQ ID NO 313
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H50

<400> SEQUENCE: 313

Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Gly Asn Ser
1 5 10 15

<210> SEQ ID NO 314
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H51

<400> SEQUENCE: 314

Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Gly Asn Ser
1 5 10 15

<210> SEQ ID NO 315
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H52

<400> SEQUENCE: 315

Ser Gln Pro Glu Ile Val Pro Ile Ser Asn Ser
1 5 10

<210> SEQ ID NO 316
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H53

<400> SEQUENCE: 316

Ser Gln Pro Glu Ile Val Pro Ile Ser Cys Pro Pro Cys Pro Asn Ser
1 5 10 15

<210> SEQ ID NO 317
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H54

<400> SEQUENCE: 317

Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Ser Cys Pro Pro Cys
1 5 10 15

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Pro Asn Ser

<210> SEQ ID NO 318
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H55

<400> SEQUENCE: 318

Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala Asn Ser
1 5 10 15

<210> SEQ ID NO 319
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H56

<400> SEQUENCE: 319

Gln Met Asn Ser Glu Leu Ser Val Leu Ala Asn Ser
1 5 10

<210> SEQ ID NO 320
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H57

<400> SEQUENCE: 320

Val Ser Glu Arg Pro Phe Pro Pro Asn Ser
1 5 10

<210> SEQ ID NO 321
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H58

<400> SEQUENCE: 321

Lys Pro Phe Phe Thr Cys Gly Ser Ala Asp Thr Cys Pro Asn Ser
1 5 10 15

<210> SEQ ID NO 322
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H59

<400> SEQUENCE: 322

Lys Pro Phe Phe Thr Cys Gly Ser Ala Asp Thr Cys Pro Asn Ser
1 5 10 15

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<210> SEQ ID NO 323
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H60

<400> SEQUENCE: 323

Gln Tyr Asn Cys Pro Gly Gln Tyr Thr Phe Ser Met Pro Asn Ser
1 5 10 15

<210> SEQ ID NO 324
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H61

<400> SEQUENCE: 324

Glu Pro Ala Phe Thr Pro Gly Pro Asn Ile Glu Leu Gln Lys Asp Ser
1 5 10 15

Asp Cys Pro Asn Ser
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<210> SEQ ID NO 325
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H62

<400> SEQUENCE: 325

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys Ala Arg
1 5 10 15

His Cys Pro Asn Ser
20

<210> SEQ ID NO 326
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H63

<400> SEQUENCE: 326

Asn Ser Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr
1 5 10 15

Cys Pro Asn Ser
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<210> SEQ ID NO 327

<400> SEQUENCE: 327

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<210> SEQ ID NO 328

<400> SEQUENCE: 328

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<210> SEQ ID NO 329

<400> SEQUENCE: 329

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<210> SEQ ID NO 330

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<210> SEQ ID NO 331

<211> LENGTH: 122

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Scorpion linker H68

<400> SEQUENCE: 331

Arg Thr Arg Tyr Leu Gln Val Ser Gln Gln Leu Gln Gln Thr Asn Arg
1 5 10 15Val Leu Glu Val Thr Asn Ser Ser Leu Arg Gln Gln Leu Arg Leu Lys
20 25 30Ile Thr Gln Leu Gly Gln Ser Ala Glu Asp Leu Gln Gly Ser Arg Arg
35 40 45Glu Leu Ala Gln Ser Gln Glu Ala Leu Gln Val Glu Gln Arg Ala His
50 55 60Gln Ala Ala Glu Gly Gln Leu Gln Ala Cys Gln Ala Asp Arg Gln Lys
65 70 75 80Thr Lys Glu Thr Leu Gln Ser Glu Glu Gln Gln Arg Arg Ala Leu Glu
85 90 95Gln Lys Leu Ser Asn Met Glu Asn Arg Leu Lys Pro Phe Phe Thr Cys
100 105 110Gly Ser Ala Asp Thr Cys Cys Pro Asn Ser
115 120

<210> SEQ ID NO 332

<400> SEQUENCE: 332

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<210> SEQ ID NO 333

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD-20 VL CDR1

<400> SEQUENCE: 333

Arg Ala Ser Ser Ser Val Ser Tyr Ile Val
1 5 10

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<210> SEQ ID NO 334
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD-20 VL CDR3

<400> SEQUENCE: 334

Gln Gln Trp Ser Phe Asn Pro Pro Thr
1 5

<210> SEQ ID NO 335

<400> SEQUENCE: 335

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<210> SEQ ID NO 336
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD-20 VH CDR2

<400> SEQUENCE: 336

Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> SEQ ID NO 337

<400> SEQUENCE: 337

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<210> SEQ ID NO 338
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD20 VH CDR3

<400> SEQUENCE: 338

Ser Val Tyr Tyr Ser Asn Tyr Trp Tyr Phe Asp Leu
1 5 10

<210> SEQ ID NO 339

<400> SEQUENCE: 339

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<210> SEQ ID NO 340

<400> SEQUENCE: 340

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<210> SEQ ID NO 341

<400> SEQUENCE: 341

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<210> SEQ ID NO 342

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<210> SEQ ID NO 343

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: anti-CD20 VH CDR3

<400> SEQUENCE: 343

Ser Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Leu
1 5 10

<210> SEQ ID NO 344

<400> SEQUENCE: 344

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<210> SEQ ID NO 345

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<210> SEQ ID NO 366

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: partial CH3 sequence

<400> SEQUENCE: 366

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
1 5 10

<210> SEQ ID NO 367

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Partial CH3 sequence

<400> SEQUENCE: 367

Gln Lys Ser Leu Ser Leu Ser Pro Gly
1 5

<210> SEQ ID NO 368

<211> LENGTH: 1

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<223> OTHER INFORMATION: Partial CH3 sequence

<400> SEQUENCE: 368

Gln

1

<210> SEQ ID NO 369

<211> LENGTH: 3

<212> TYPE: PRT

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Partial CH3 sequence

<400> SEQUENCE: 369

Gln Lys Ser
1

<210> SEQ ID NO 370
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Partial CH3 sequence

<400> SEQUENCE: 370

Gln Lys Ser Leu Ser Leu
1 5

<210> SEQ ID NO 371
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Partial CH3 sequence

<400> SEQUENCE: 371

Gln Lys Ser Leu Ser
1 5

<210> SEQ ID NO 372
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H7 scorpion linker lacking C-term PNS
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker

<400> SEQUENCE: 372

Gly Cys Pro Pro Cys
1 5

<210> SEQ ID NO 373
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H60 scorpion linker lacking C-term PNS
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker

<400> SEQUENCE: 373

Gln Tyr Asn Cys Pro Gly Gln Tyr Thr Phe Ser Met
1 5 10

<210> SEQ ID NO 374
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:

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<223> OTHER INFORMATION: CD72 variant lacking N-term K and C-term PNS
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker

<400> SEQUENCE: 374

Pro Phe Phe Thr Cys Gly Ser Ala Asp Thr Cys
1 5 10

<210> SEQ ID NO 375
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H61 scorpion linker lacking C-term PNS
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker

<400> SEQUENCE: 375

Glu Pro Ala Phe Thr Pro Gly Pro Asn Ile Glu Leu Gln Lys Asp Ser
1 5 10 15

Asp Cys

<210> SEQ ID NO 376
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H62 scorpion linker lacking C-term PNS
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker

<400> SEQUENCE: 376

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys Ala Arg
1 5 10 15

His Cys

<210> SEQ ID NO 377
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H63 scorpion linker lacking C-term PNS
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker

<400> SEQUENCE: 377

Asn Ser Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr
1 5 10 15

Cys

<210> SEQ ID NO 378
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD20 VL CDR1 (TRU-015)

<400> SEQUENCE: 378

Arg Ala Ser Ser Ser Val Ser Tyr Met His
1 5 10

<210> SEQ ID NO 379

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<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: anti-CD20 VH CDR3 (TRU-015)

<400> SEQUENCE: 379

Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
1 5 10

<210> SEQ ID NO 380
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H69, NKG2A stalk variant with
Ser substitution for Cys

<400> SEQUENCE: 380

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys Ala Arg
1 5 10 15

His Ser Pro Asn Ser
20

<210> SEQ ID NO 381
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: Scorpion linker H70, a scorpion linker CD72
variant

<400> SEQUENCE: 381

Pro Phe Phe Thr Cys Gly Ser Ala Asp Thr Cys Pro Asn Ser
1 5 10

<210> SEQ ID NO 382
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: wild-type anti-CD20 VL CDR2 (TRU-015)

<400> SEQUENCE: 382

Ala Pro Ser Asn Leu Ala Ser
1 5

<210> SEQ ID NO 383
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<223> OTHER INFORMATION: wild-type anti-CD20 VH CDR1 (TRU-015)

<400> SEQUENCE: 383

Ser Tyr Asn Met His
1 5

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<210> SEQ ID NO 384
<211> LENGTH: 1431
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic protein
<220> FEATURE:
<223> OTHER INFORMATION: 2Lm20-4 LH SMIP
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1431)

<400> SEQUENCE: 384

gaa att gtg ttg aca cag tct cca gcc acc ctg tct ttg tct cca ggt      48
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1      5      10      15

gaa aga gcc acc ctc tcc tgc agg gcc agt tcc agt gtt agc tac att      96
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Ser Tyr Ile
      20      25      30

gtt tgg tac caa cag aaa cct ggc cag gca cct agg ctc ctc atc tat     144
Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
      35      40      45

gcc cca tcc aac ctg gct tct gga att cca gcc agg ttc agt ggc agt     192
Ala Pro Ser Asn Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser
      50      55      60

gga tcc ggg aca gac ttc act ctc acc atc agc agt cta gag cct gaa     240
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
      65      70      75      80

gat ttt gca gtt tat tac tgt cag cag tgg agt ttt aac cct ccc acg     288
Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Phe Asn Pro Pro Thr
      85      90      95

ttc ggc caa ggg acc aag gtg gaa atc aaa gat ggc ggt gga tcg ggc     336
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Asp Gly Gly Gly Ser Gly
      100      105      110

ggg ggt gga tct gga gga ggt ggg agc tct cag gtg cag ctg gtg cag     384
Gly Gly Gly Ser Gly Gly Gly Ser Ser Gln Val Gln Leu Val Gln
      115      120      125

tct ggt gct gag agc aag aag cct ggg gcc tca gtg aag gtt tcc tgc     432
Ser Gly Ala Glu Ser Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys
      130      135      140

aag gct agc gga tac acc ttc acc agc tac aat atg cac tgg gtg cga     480
Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Trp Val Arg
      145      150      155      160

cag gcg cct gga caa ggg cta gag tgg atg gga gct atc tat cct gga     528
Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Ala Ile Tyr Pro Gly
      165      170      175

aat ggt gat aca tcc tac aat cag aag ttc aag ggc agg gtc acc atg     576
Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Arg Val Thr Met
      180      185      190

acc aga gac acg tcc acg agc aca gtc tac atg gag ctg agc agc ctg     624
Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu
      195      200      205

aga tct gag gac acg gcc gtg tat tac tgt gcc cga agc tac tat agt     672
Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Tyr Tyr Ser
      210      215      220

aac tct tac tgg tac ttc gat ctc tgg ggc cgc ggc acc ctg gtc act     720
Asn Ser Tyr Trp Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu Val Thr
      225      230      235      240

gtc tcc tcg agc gag ccc aaa tct tct gac aaa act cac aca tgc cca     768

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Val	Ser	Ser	Ser	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	
				245					250					255		
ccg	tgc	cca	gca	cct	gaa	ctc	ctg	ggt	gga	ccg	tca	gtc	ttc	ctc	ttc	816
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	
			260					265					270			
ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	864
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	
		275					280					285				
aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	912
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	
		290				295					300					
aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	960
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	
305					310				315					320		
cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	1008
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	
			325						330					335		
gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	1056
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	
			340					345					350			
tcc	aac	aaa	gcc	ctc	cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	1104
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	
		355					360					365				
aaa	ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	cgg	1152
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	
	370					375					380					
gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	1200
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	
385					390				395					400		
ttc	tat	cca	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	ccg	1248
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	
			405					410						415		
gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	tcc	1296
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	
			420					425					430			
ttc	ttc	ctc	tac	agc	aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	cag	1344
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	
		435					440					445				
ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	cac	1392
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	
	450					455					460					
tac	acg	cag	aag	agc	ctc	tcc	ctg	tct	ccg	ggt	aaa	tga				1431
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys					
465					470				475							

<210> SEQ ID NO 385

<211> LENGTH: 476

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic protein

<220> FEATURE:

<223> OTHER INFORMATION: 2Lm20-4 LH SMIP

<400> SEQUENCE: 385

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Leu	Ser	Pro	Gly	
1				5					10					15		

Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Ile	
		20					25						30			

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Val	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr
	35						40					45			
Ala	Pro	Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser
	50					55					60				
Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu
65					70					75				80	
Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Phe	Asn	Pro	Pro	Thr
				85					90					95	
Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Asp	Gly	Gly	Gly	Ser	Gly
			100					105					110		
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Gln	Val	Gln	Leu	Val	Gln
		115					120					125			
Ser	Gly	Ala	Glu	Ser	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val	Ser	Cys
	130					135					140				
Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	Asn	Met	His	Trp	Val	Arg
145					150					155					160
Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	Gly	Ala	Ile	Tyr	Pro	Gly
				165					170					175	
Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Arg	Val	Thr	Met
		180						185					190		
Thr	Arg	Asp	Thr	Ser	Thr	Ser	Thr	Val	Tyr	Met	Glu	Leu	Ser	Ser	Leu
		195					200					205			
Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Ser	Tyr	Tyr	Ser
	210					215					220				
Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Leu	Trp	Gly	Arg	Gly	Thr	Leu	Val	Thr
225					230					235					240
Val	Ser	Ser	Ser	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro
				245					250					255	
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe
		260						265					270		
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val
		275					280					285			
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe
	290					295					300				
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro
305					310					315					320
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr
			325						330					335	
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val
		340						345					350		
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala
		355					360					365			
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg
	370					375					380				
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly
385					390					395					400
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro
				405					410					415	
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser
			420				425						430		
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln
	435						440					445			

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Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 450 455 460

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 465 470 475

<210> SEQ ID NO 386

<211> LENGTH: 2269

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic polynucleotide

<220> FEATURE:

<223> OTHER INFORMATION: S0129 scorpion

<400> SEQUENCE: 386

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gataccaccg gtgaaattgt gttgacacag tctccagcca cctgtctttt gtctccaggt    120
gaaagagcca ccctctctcg cagggccagt tccagtgtta gctacattgt ttggtaccaa    180
cagaaacctg gccaggcacc taggctcttc atctatgccc catccaacct ggcttctgga    240
attccagcca gggtcagtg cagtggatcc gggacagact tcaactctac catcagcagt    300
ctagagcctg aagattttgc agttttattac tgtcagcagt ggagttttta cctccaccg    360
ttcggccaa ggaacaaagt ggaaatcaaa gatggcggtg gatcgggcgg tgggtgatct    420
ggaggaggtg ggagctctca ggtgcagctg gtgcagctct gtgctgagag caagaagcct    480
ggggcctcag tgaaggtttc ctgcaaggct agcggataca ccttcaccag ctacaatatg    540
cactgggtgc gacagcgccc tggacaaggg ctagagtggg tgggagctat ctatcctgga    600
aatgggtgata catcctacaa tcagaagttc aagggcaggg tcaccatgac cagagacacg    660
tccacgagca cagtctacat ggagctgagc agcctgagat ctgaggacac ggcctgtgat    720
tactgtgccc gaagctacta tagtaactct tactggtagt tcatctctct gggccgcggc    780
acctgggtca ctgtctcttc gagcgagccc aaatcttctg acaaaactca cacatgccc    840
ccgtgcccag cacctgaact cctgggtgga ccgtcagttt tctctctccc cccaaaaccc    900
aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtgggtgt ggacgtgagc    960
cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc   1020
aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc   1080
gtcctgcacc aggactgggt gaatggcaag gagtacaagt gcaaggtctc caacaaagcc   1140
ctccagcccc ccattcgaga aacctctccc aaagccaaag ggcagccccg agaaccacag   1200
gtgtacaccc tgccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc   1260
ctggtcaaa gcttctatcc aagcgacatc gccgtggagt gggagagcaa tgggcagccg   1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt ctctctctac   1380
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg   1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaag   1500
gggtgtccac cttgtccgaa ttctcagtg cagctggtgc agtctggtgc tgagagcaag   1560
aagcctgggg cctcagttaa ggtttctctc aaggccagcg gatacacctt caccagctac   1620
aatatgcact ggggtgcaca gccgcctgga caagggctag agtggatggg agtatctat   1680
cctggaaatg gtgatacatc ctacaatcag aagttcaagg gcagggtcac catgaccaga   1740

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gacacgtcca cgagcacagt ctacatggag ctgagcagcc tgagatctga ggacacggcc 1800
gtgtattact gtgccgaag ctactatagt aactcttact ggtacttcga tctctggggc 1860
cgcggaaccc tggctactgt ctctcggggg ggcgggtggat cgggcgggtgg tggatctgga 1920
ggaggtgggt ctgctagcga aattgtgttg acacagtctc cagccaccct gtctttgtct 1980
ccaggtgaaa gagccaccct ctctgcagg gccagttcca gtgttagcta cattgtttgg 2040
taccaacaga aacctggcca ggcacctagg ctctcatct atgccccatc caacctggct 2100
tctggaatac cagccagggt cagtggcagt ggatccggga cagacttcac tctcaccatc 2160
agcagtcttg agcctgaaga ttttgcagtt tattactgtc agcagtggag ttttaaccct 2220
cccacgttcg gccaaaggac caagtgga atcaaagact gagtctaga 2269

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<210> SEQ ID NO 387
<211> LENGTH: 729
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic protein
<220> FEATURE:
<223> OTHER INFORMATION: S0129 mature peptide

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<400> SEQUENCE: 387

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

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Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe
			260					265					270		
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val
		275					280					285			
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe
	290					295					300				
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro
305					310					315					320
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr
				325					330					335	
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val
			340					345					350		
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala
	355						360					365			
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg
	370					375					380				
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly
385					390					395					400
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro
				405					410					415	
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser
			420					425					430		
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln
	435						440					445			
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His
	450					455					460				
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Gly	Cys	Pro	Pro
465					470					475					480
Cys	Pro	Asn	Ser	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Ser	Lys
				485					490					495	
Lys	Pro	Gly	Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr
			500					505					510		
Phe	Thr	Ser	Tyr	Asn	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly
		515					520					525			
Leu	Glu	Trp	Met	Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr
	530				535					540					
Asn	Gln	Lys	Phe	Lys	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Thr
545					550					555					560
Ser	Thr	Val	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala
				565					570					575	
Val	Tyr	Tyr	Cys	Ala	Arg	Ser	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe
			580					585					590		
Asp	Leu	Trp	Gly	Arg	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly
	595						600					605			
Gly	Ser	Gly	Gly	Gly	Gly	Ser</									

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660							665					670					
Ser	Asn	Leu	Ala	Ser	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser		
675							680					685					
Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro	Glu	Asp	Phe		
690							695					700					
Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Phe	Asn	Pro	Pro	Thr	Phe	Gly		
705					710					715						720	
Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Asp									
725																	

1. A single-chain multivalent binding protein comprising from amino-terminus to carboxyl-terminus:

- (a) a first binding domain comprising a first light chain immunoglobulin variable region (VL1) and a first heavy chain immunoglobulin variable region (VH1), wherein said variable regions are positioned in a VH1-VL1 or a VL1-VH1 orientation and separated by a first peptide linker of 2-50 amino acids;
- (b) an immunoglobulin hinge domain;
- (c) an immunoglobulin C_{H2} domain
- (d) an immunoglobulin C_{H3} domain
- (e) a scorpion linker; and
- (f) a second binding domain comprising a second light chain immunoglobulin variable region (VL2) and a second heavy chain immunoglobulin variable region (VH2), wherein said variable regions are positioned in a VH2-VL2 or a VL2-VH2 orientation and separated by a second peptide linker of 2-50 amino acids, wherein the multivalent binding protein is capable of forming a dimer in solution.

2. The binding protein of claim 1, wherein VL1 and VH1 are from the same immunoglobulin.

3. The binding protein of claim 1, wherein VL1 and VH1 are from different immunoglobulins.

4. The binding protein of claim 1, wherein VL2 and VH2 are from the same immunoglobulin.

5. The binding protein of claim 1, wherein VL2 and VH2 are from different immunoglobulins.

6. The binding protein of claim 1, wherein the first and second binding domains are derived from different immunoglobulins.

7. The binding protein of claim 1, wherein the first and second binding domains specifically bind different target molecules located on the same cell.

8. The binding protein of claim 1, wherein the first and second binding domains specifically bind different target molecules located on physically distinct cells.

9. The binding protein of claim 1, wherein at least one binding domain specifically binds a cell-free molecular target.

10. The binding protein of claim 1, wherein at least one binding domain comprises a sequence selected from SEQ ID NO: 2, 4, 6, 103, 105, 107, and 109.

11. The binding protein of claim 1, wherein the first and/or second peptide linker is 15 amino acids in length.

12. The binding protein of claim 1, wherein the first and/or second peptide linker is 20 amino acids in length.

13. The binding protein of claim 1, wherein the first and/or second peptide linker comprises a Gly₄Ser sequence.

14. The binding protein of claim 1, wherein the first and/or second peptide linker comprises the sequence Asp-Gly₃-Ser-(Gly₄Ser)₂.

15. The binding protein of claim 1, wherein the first and/or second peptide linker comprises the sequence (Gly₄Ser)₄.

16. The binding protein of claim 1, wherein the immunoglobulin hinge domain is a hinge domain selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgE, IgA2, synthetic hinge and the hinge-like C_{H2} domain of IgM.

17. The binding protein of claim 16, wherein the hinge domain is an IgG1 hinge domain.

18. The binding protein of claim 17, wherein the hinge domain is a human IgG1 hinge domain with a mutation at one or two cysteine residues.

19. The binding protein of claim 1, wherein the immunoglobulin C_{H2} domain is a IgG1 immunoglobulin C_{H2} domain.

20. The binding protein of claim 1, wherein the immunoglobulin C_{H3} domain is a IgG1 immunoglobulin C_{H3} domain.

21. The binding protein of claim 20, wherein the C_{H3} domain is truncated and comprises a C-terminal sequence selected from the group consisting of SEQ ID NO: 366-371.

22. The binding protein of claim 1, wherein the binding protein does not comprise a C_{H1} domain.

23. The binding protein of claim 1, wherein the scorpion linker is at least 5 amino acids in length.

24. The binding protein of claim 23, wherein the scorpion linker is between 5 and 45 amino acids in length.

25. The binding protein of claim 1, wherein the scorpion linker is resistant to proteolytic cleavage.

26. The binding protein of claim 1, wherein the scorpion linker comprises an amino acid sequence derived from an immunoglobulin hinge.

27. The binding protein of claim 1, wherein the scorpion linker comprises an amino acid sequence derived from an interdomain region of an immunoglobulin superfamily member.

28. The binding protein of claim 27, wherein the immunoglobulin superfamily member is CD2, CD80, CD86, or CD22.

29. The binding protein of claim 1, wherein the scorpion linker comprises an amino acid sequence derived from a stalk region of a C-type lectin.

30. The binding protein of claim 29, wherein the C-type lectin is selected from the group consisting of CD69, CD72, CD94, NKG2A, and NKG2D.

31. The binding protein of claim 1, wherein the scorpion linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 231, 233, 235, 237, 239,

241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 287, 289, 297, 305, 307, 309, 310, 311, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 346, 373, 374, 375, 376, 377, 380, and 381.

32. The binding protein of claim **1**, wherein the first and/or second binding domains comprise chimeric, humanized, or human immunoglobulin variable domains.

33. The binding protein of claim **1**, wherein at least one binding domain specifically binds a target selected from a tumor antigen, a B-cell target, a TNF receptor superfamily member, a Hedgehog family member, a receptor tyrosine kinase, a proteoglycan-related molecule, a TGF-beta superfamily member, a Wnt-related molecule, a receptor ligand, a T-cell target, a Dendritic cell target, an NK cell target, a monocyte/macrophage cell target, a myeloid target, and an angiogenesis target.

34. The binding protein of claim **1**, wherein the binding protein is capable of cross-linking two or more targets.

35. The binding protein of claim **1**, wherein the first binding domain and the second binding domain are about 20-30 Å apart to about 150-180 Å apart.

36. The binding protein of claim **1**, wherein the first binding domain and the second binding domain are about 20-30 Å apart to less than about 50 Å apart.

37. The binding protein of claim **1**, wherein the first binding domain and the second binding domain are about 50 Å apart.

38. The binding protein of claim **1**, wherein the binding protein exhibits a binding affinity of less than 10^{-9} M or at least 10^{-6} M for at least one of the first binding domain and the second binding domain.

39. A protein dimer comprising two multivalent binding proteins of claim **1**.

40. A nucleic acid encoding the binding protein of claim **1**.

41. A vector comprising the nucleic acid of claim **40**.

42. A host cell comprising the nucleic acid of claim **40** or vector of claim **41**.

43. A composition comprising the binding protein of claim **1** and a pharmaceutically acceptable carrier.

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