CLAIMS

What is claimed is:

1. A multivalent single-chain binding protein with effector function comprising

a. a first binding domain derived from an immunoglobulin or immunoglobulin-like molecule;

b. a constant sub-region providing an effector function, the immunoglobulin constant sub-region located C-terminal to the first binding domain;

c. a scorpion linker located C-terminal to the constant sub-region; and

d. a second binding domain derived from an immunoglobulin 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.

2. The protein according to claim 1 wherein the first binding domain comprises a variable light region derived from a first immunoglobulin and a variable heavy region derived from a second immunoglobulin.

3. The protein according to claim 2 wherein the first immunoglobulin and the second immunoglobulin are the same immunoglobulin.

4. The protein according to claim 1 wherein the second binding domain comprises a variable light region derived from a first immunoglobulin and a variable heavy region derived from a second immunoglobulin.

5. The protein according to claim 4 wherein the first immunoglobulin and the second immunoglobulin are the same immunoglobulin.

6. The protein according to claim 1 wherein the first binding domain and the second binding domain recognize the same molecular target.

7. The protein according to claim 1 wherein the first binding domain and the second binding domain recognize the same epitope.

8. The protein according to claim 1 wherein the first binding domain and the second binding domain recognize different molecular targets on the same eukaryotic cell, prokaryotic cell, virus, carrier, or object.

9. The protein according to claim 1 wherein the first binding domain and the second binding domain recognize molecular targets associated with physically distinct eukaryotic cells, prokaryotic cells, viruses, carriers or objects.

10. The protein according to claim 1 wherein at least one of the first binding domain and the second binding domain recognize at least one molecular target not associated with a eukaryotic cell, prokaryotic cell, virus, carrier or object.

11. The protein according to claim 1 wherein the first binding domain, the second binding domain and the constant sub-region are each derived from a human immunoglobulin.

12. The protein according to claim 1 wherein at least one of the first binding domain and the second binding domain recognizes a target on a cancer cell.

13. The protein according to claim 11 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.

14. The protein according to claim 13 wherein the tumor antigen is selected from the group consisting of SQUAMOUS CELL CARCINOMA ANTIGEN 1, SQUAMOUS CELL CARCINOMA ANTIGEN 2, Ovarian carcinoma antigen CA125, MUCIN 1, 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, MAGE-B1 ANTIGEN, MAGE-B2 ANTIGEN, 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, Neuro-oncological ventral antigen 2, 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.

15. The protein according to claim 13 wherein the B cell target is 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.

16. The protein according to claim 13 wherein 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.

17. The protein according to claim 13 wherein the is Hedgehog family member is selected from the group consisting of Patched and Smoothened.

18. The protein according to claim 13 wherein the receptor tyrosine kinase is selected from the group consisting of Axl, FGF R4, C1q R1/CD93, FGF R5, DDR1, Flt-3, DDR2, HGF R, Dtk, IGF-I R, EGF R, IGF-II R, Eph, INSRR, 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.

19. The protein according to claim 13 wherein the proteoglycan-related molecule is selected from the group consisting of proteoglycans and regulators thereof.

20. The protein according to claim 13 wherein 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 RIIb, Endoglin/CD105 and TGF-beta RIII.

21. The protein according to claim 13 wherein the Wnt-related molecule is 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.

22. The protein according to claim 13 wherein 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, PlGF, Neuropilin-2, PlGF-2, PDGF, PDGF-A, VEGF, PDGF-B, VEGF-B, PDGF-C, VEGF-C, PDGF-D, VEGF-D and PDGF-AB.

23. The protein according to claim 13 wherein the T-cell receptor 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.

24. The protein according to claim 13 wherein 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.

25. The protein according to claim 13 wherein the monocyte/macrophage cell receptor 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, LIMPII/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 RI/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 RII, 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, LIMPII/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 RI/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.

26. The protein according to claim 13 wherein the Dendritic cell receptor 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, LIMPII/SR-B2, RP105, TLR4, TLR1, TLR5, TLR2, TLR6, TLR3, TLR9, 4-1BB Ligand/TNFSF9, 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 RI/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.

27. The protein according to claim 13 wherein 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-1, 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 RI/TNFRSF1A, EPCR, TNF RII/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.

28. The protein according to claim 1 wherein at least one of the first binding domain and the second binding domain specifically binds a target selected from the group consisting of Prostate-specific Membrane Antigen (Folate Hydrolase 1), Epidermal Growth Factor Receptor, Receptor for Advanced Glycation End products, IL-17 A, IL-17 F, P19, Dickkopf-1, NOTCH1, NG2, IgHE, IL-22R, IL-21, an Amyloid β oligomer, an Amyloid β Precursor Protein, NOGO Receptor (RTN4R), Low Density LipoproteinReceptor-Related Protein 5, IL-4, Myostatin, Very Late Antigen 4 and IGF-1R.

29. The protein according to claim 12 wherein the cancer cell is a transformed hematopoietic cell.

30. The protein according to claim 29 wherein 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 T-cell target, a dendritic cell target, or an NK-cell target.

31. The protein according to claim 29 wherein at least one of the first binding domain and the second binding domain recognizes a target selected from the group consisting of CD5, CD10, CD11c, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD27, CD30, CD38, CD45, CD70, CD80, CD86, CD103, IRTA1, IRTA2, IRTA3, IRTA4, IRTA5, B-B2, B-B8, and B-cell antigen receptor.

32, The protein according to claim 1 comprising a sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 103, 105, 107, 109, 332, 333, 334, and 345.

33. The proteinaccording to claim 1 comprising a sequence selected from the group consisting of SEQ ID NOS:355, 356, 357, 358, 359, 360, 361, 362, 363, 364 and 365.

34. The protein according to claim 1 wherein the constant sub-region recognizes an effector cell F_C receptor.

35. The protein according to claim 1 wherein the constant sub-region recognizes an effector cell surface protein selected from the group consisting of CD16, CD32a, CD32b, CD64, CD89, FcεR1, FcRn, and pIgR.

36. The protein according to claim 1 wherein the constant sub-region comprises a C_{H2} domain and a C_{H3} domain.

37. The protein according to claim 36, wherein 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.

38. The protein according to claim 1, wherein the scorpion linker is derived from an immunoglobulin hinge.

39. The protein according to claim 38, wherein the scorpion linker has a reduced, but non-zero, number of cysteines relative to the hinge from which the scorpion linker is derived, and wherein one cysteine corresponds to the N-terminal hinge cysteine of the immunoglobulin hinge.

40. The protein according to claim 39 wherein the scorpion linker is derived from an IgG1 hinge region and wherein the linker comprises 1 or 2 cysteines, further wherein the linker retains the cysteine corresponding to an N-terminal hinge cysteine of the IgG1 hinge region.

41. The protein according to claim 1, wherein the scorpion linker is derived from the stalk region of a C-lectin.

42. The protein according to claim 1, wherein the scorpion linker comprises a sequence selected from the group consisting of SEQ ID NOS:373, 374, 375, 376 and 377.

43. The protein according to claim 1, wherein the scorpion linker comprises a sequence selected from the group consisting of SEQ ID NOS:351, 352, 353 and 354.

44. The protein according to claim 1 further comprising a 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.

45. The protein according to claim 1 in which the binding affinity of at least one of the first binding domain and the second binding domain is less than 10^{-9} M.

46. The protein according to claim 1 in which the binding affinity of at least one of the first binding domain and the second binding domain is at least 10^{-6} M.

47. The protein according to claim 1, wherein the effector function is selected from the group consisting of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

48. The protein according to claim 1, wherein the *in vivo* half-life of the protein is at least 28 hours in a human.

49. A pharmaceutical composition comprising the protein according to claim 1 and a pharmaceutically acceptable adjuvant, carrier or excipient.

50. A nucleic acid encoding a protein according to claim 1.

51. A vector comprising a nucleic acid according to claim 50.

52. A host cell comprising a vector according to claim 51.

53. A method of producing the protein according to claim 1 comprising:

a. introducing a nucleic acid encoding the protein according to claim 1 into a host cell; and

b. incubating the host cell under conditions suitable for expression of the protein, thereby expressing the protein at a level of at least 1 mg/ml.

54. The method according to claim 53 further comprising isolating the protein.

55. The method according to claim 53 wherein the host cell is 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, a fungal cell and a bacterial cell.

56. A method of producing a nucleic acid encoding the protein according to claim 1 comprising:

a. 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;

b. 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

c. 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.

57. The method according to claim 56 wherein the polynucleotide encoding a first binding domain comprises a sequence selected from the group consisting of SEQ ID NO:1 (anti-CD20 variable region), SEQ ID NO:3 (anti-CD28 variable region, oriented V_L-V_H) and SEQ ID NO:5 (anti-CD28 variable region, oriented V_H-V_L).

58. The method according to claim 56, further comprising 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.

59. The method according to claim 56, further comprising 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.

60. A method of inducing damage to a target cell comprising contacting a target cell with a therapeutically effective amount of a protein according to claim 1.

61. The method according to claim 60 wherein the target cell is contacted *in vivo* by administration of the protein to an organism in need.

62. A method of treating a cell proliferation disorder comprising administering a therapeutically effective amount of a protein according to claim 1 to an organism in need.

63. The method according to claim 62 wherein the disorder is selected from the group consisting of a cancer, an autoimmune disorder, Rous Sarcoma Virus infection and inflammation.

64. The method according to claim 62 wherein the protein is administered by *in vivo* expression of a nucleic acid encoding the protein according to claim 1.

65. The method according to claim 62 wherein the protein is administered by a route selected from the group consisting of intravenous injection, intraarterial injection, intramuscular injection, subcutaneous injection, intraperitoneal injection and direct tissue injection.

66. A method of ameliorating a symptom associated with a cell proliferation disorder comprising administering a therapeutically effective amount of a protein according to claim 1 to an organism in need.

67. The method according to claim 66, wherein the symptom is selected from the group consisting of pain, heat, swelling and joint stiffness.

68. A method of treating an infection associated with an infectious agent comprising administering a therapeutically effective amount of the protein according to claim 1 to a patient in need, wherein the protein comprises a binding domain that specifically binds a target molecule of the infectious agent.

69. A method of ameliorating a symptom of an infection associated with an infectious agent comprising administering an effective amount of the protein according to claim 1 to a patient in need, wherein the protein comprises a binding domain that specifically binds a target molecule of the infectious agent.

70. A method of reducing the risk of infection attributable to an infectious agent comprising administering a prophylactically effective amount of the protein according to claim 1 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.

71. A kit comprising the protein according to claim 1 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.

72. The multivalent single-chain binding protein according to claim 1 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.

73. The protein according to claim 72 wherein one of the first binding domain and the second binding domain specifically binds CD20.

74. The protein according to claim 73 wherein 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.

75. The protein according to claim 72 wherein one of the first binding domain and the second binding domain specifically binds CD79b.

76. The protein according to claim 75 wherein 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.

77. The protein according to claim 72 wherein one of the first binding domain and the second binding domain specifically binds a major histocompatibility complex class II peptide.

78. The protein according to claim 77 wherein 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.

79. The protein according to claim 72 wherein one of the first binding domain and the second binding domain specifically binds CD22.

80. The protein according to claim 79 wherein 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.

81. The protein according to claim 72 wherein the protein has a synergistic effect on a target cell behavior relative to the sum of the effects of each of the binding domains, and wherein 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-CD40, CD20-CD79a, CD20-CD79b and CD20-CD81.

82. The protein according to claim 72 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, and wherein the protein comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD20-CD23, CD20-CD30, CD20-CD37, CD20-CD70, CD20-CD80, CD20-CD86, CD79b-CD37, CD79b-CD81, major histocompatibility complex class II peptide-CD30, and major histocompatibility complex class II peptide-CD72.

83. The protein according to claim 72 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, and wherein the protein comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD20-major histocompatibility complex class II peptide, CD79b-CD19, CD79b-CD20, CD79b-CD21, CD79b-CD22, CD79b-CD23, CD79b-CD30, CD79b-CD40, CD79b-CD70, CD79b-CD72, CD79b-CD79a, CD79b-CD80, CD79b-CD86, CD79b-major histocompatibility complex class II peptide, major histocompatibility complex class II peptide-CD19, major histocompatibility complex class II peptide-CD20, major histocompatibility complex class II peptide-CD21, major histocompatibility complex class II peptide-CD22, major histocompatibility complex class II peptide-CD23, major histocompatibility complex class II peptide-CD37, major histocompatibility complex class II peptide-CD40, major histocompatibility complex class II peptide-CD70, major histocompatibility complex class II peptide-CD79a, major histocompatibility complex class II peptide-CD79b, major histocompatibility complex class II peptide-CD80, major histocompatibility complex class II peptide-CD81, major histocompatibility complex class II peptide-CD86, CD22-CD19, CD22-CD40, CD22-CD79b, CD22-CD86 and CD22-major histocompatibility complex class II peptide.

84. A method of identifying at least one of the binding domains of the multivalent binding molecule according to claim 1 comprising:

(a) contacting an anti-isotypic antibody with an antibody specifically recognizing a first antigen and an antibody specifically recognizing a second antigen;

(b) further contacting a target comprising at least one of the 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.

85. The method according to claim 84 wherein the target is a diseased cell.

86. The method according to claim 60 wherein the multivalent single-chain binding protein induces a synergistic amount of damage to the target cell compared to the sum of the damage 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.

87. The method according to claim 86 wherein the multivalent single-chain binding protein comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD19/CD20, CD20/CD21, CD20/CD22, CD20/CD40, CD20/CD79a, CD20/CD79b, CD20/CD81, CD21/CD79b, CD37/CD79b, CD79b/CD81, CD19/CL II, CD20/CL II, CD30/CL II, CD37/CL II, CD72/CL II, and CD79b/CL II.

88. The method according to claim 60 wherein the multivalent single-chain binding protein induces an inhibited amount of damage to the target cell compared to the sum of the damage 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.

89. The method according to claim 88 wherein the multivalent single-chain binding protein comprises a binding domain pair specifically recognizing a pair of antigens selected from the group consisting of CD20/CL II, CD21/CD79b, CD22/CD79b, CD40/CD79b, CD70/CD79b, CD72/CD79b, CD79a/CD79b,

CD79b/CD80, CD79b/CD86, CD21/CL II, CD22/CL II, CD23/CL II, CD40/CL II, CD70/CL II, CD80/CL II, CD86/CL II, CD19/CD22, CD20/CD22, CD21/CD22, CD22/CD23, CD22/CD30, CD22/CD37, CD22/CD40, CD22/CD70, CD22/CD72, CD22/79a, CD22/79b, CD22/CD80, CD22/CD86 and CD22/CL II.

90. The method according to claim 60 further comprising a plurality of multivalent single-chain binding proteins.

91. The method according to claim 90 wherein 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 amount of damage to the target cell.

92. The method according to claim 90 wherein a binding domain of a first multivalent single-chain binding protein and a binding domain of a second multivalent single-chain binding protein induce an inhibited amount of damage to the target cell.

93. A composition comprising a plurality of multivalent single-chain binding proteins according to claim 1.

94. The composition according to claim 93 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 amount of damage to a target cell.

95. The composition according to claim 93 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 an additive amount of damage to a target cell.

96. The composition according to claim 93 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 an inhibited amount of damage to a target cell.

97. A pharmaceutical composition comprising the composition according to claim 93 and a pharmaceutically acceptable carrier, diluent or excipient.

98. A kit comprising the composition according to claim 93 and a set of instructions for administering the composition to damage a target cell.

CLAIMS

What is claimed is:

1. A single-chain binding protein comprising from amino-terminus to carboxy-terminus:

a. a first binding domain comprising variable regions from an immunoglobulin or immunoglobulin-like molecule;

b. an immunoglobulin constant sub-region that comprises immunoglobulin CH2 and CH3 domains;

c. a scorpion linker peptide, wherein said scorpion linker peptide comprises an amino acid sequence derived from a hinge of an immunoglobulin or a stalk region of a type II C-lectin protein; and

d. a second binding domain comprising variable regions from an immunoglobulin or immunoglobulin-like molecule.

2. The binding protein according to claim 1, wherein the first and second binding domains specifically bind different target molecules located on the same cell.

3. The binding protein according to claim 1, wherein the first and second binding domains specifically bind different target molecules located on physically distinct cells.

4. The binding protein according to any one of claims 1 to 3, wherein at least one of the binding domains specifically binds a target molecule not associated with a cell.

5. The binding protein according to any one of claims 1 to 3, wherein at least one of the binding domains comprises variable regions of light and heavy chains of an immunoglobulin.

6. The binding protein according to claim 1, wherein the immunoglobulin constant sub-region is derived from a human immunoglobulin.

7. The binding protein according to claim 1, wherein at least one of the binding domains is an scFv comprising a sequence selected from SEQ ID NO: 2, 4, 6, 103, 105, 107, and 109.

8. The binding protein according to claim 1, wherein the first and second binding domains comprise chimeric, humanized, or human immunoglobulin variable domains.

9. The binding protein according to any one of claims 1 to 8, wherein the binding affinity of the first binding domain and the second binding domain is at least 10^{-6} M.

10. The binding protein according to any one of claims 1 to 9, wherein the scorpion linker peptide is derived from the stalk region of a type II C-lectin protein selected from the group consisting of: CD69, CD72, CD94, NKG2A and NKG2D.

11. The binding protein according to any one of claims 1 to 9, wherein the scorpion linker peptide comprises the sequence of SEQ ID NO: 165.

12. The binding protein according to any one of claims 1 to 9, wherein the scorpion linker peptide comprises an amino acid sequence as shown in SEQ ID NO: 373, 374, 375, 376, or 377.

13. The binding protein according to any one of claims 1 to 12, wherein the immunoglobulin constant subregion comprises an IgG1 CH2 domain and IgG1 CH3 domain.

14. The binding protein according to any one of claims 1 to 13, wherein the immunoglobulin constant subregion provides an effector function selected from antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), complement fixation, antibody-dependent cellular phagocytosis (ADCP), binding to Fc receptors, and protein A binding.

15. The binding protein according to any one of claims 1 to 14, wherein the constant sub-region further comprises an immunoglobulin hinge or hinge-like domain.

16. The binding protein according to any one of claims 1 to 15, wherein the constant sub-region does not contain an immunoglobulin CH1 domain.

17. The binding protein according to claim 1, wherein the protein comprises a binding domain pair specifically recognizing a pair of antigens selected from CD19/CD20, CD19/CD22, CD19/CL II, CD20/CD21, CD20/CD22, CD20/CD40, CD20/CD79a, CD20/CD79b, CD20/CD81, CD20/CL II, CD21/CD22, CD21/CD79b, CD21/CL II, CD22/CD23, CD22/CD30, CD22/CD37, CD22/CD40, CD22/CD70, CD22/CD72, CD22/CD79a, CD22/CD79b, CD22/CD80, CD22/CD86, CD22/CL II, CD23/CL II, CD30/CL II, CD37/CD79b, CD37/CL II, CD40/CD79b, CD40/CL II, CD70/CD79b, CD70/CL II, CD72/CD79b, CD72/CL II, CD79a/CD79b, CD79b/CD80, CD79b/CD81, CD79b/CD86, CD79b/CL II, CD80/CL II, CD19/CD37, CD20/CD28, CD20/CD37, and CD86/CL II.

18. A pharmaceutical composition comprising the protein according to any one of the preceding claims and a pharmaceutically acceptable adjuvant, carrier or excipient.

19. A nucleic acid encoding a protein according to any one of claims 1 to 17.

20. A vector comprising a nucleic acid according to claim 19.

21. A host cell comprising a vector according to claim 20.

22. Use of a single-chain multispecific binding protein according to any one of claims 1-17 or a pharmaceutical composition according to claim 18 in the manufacture of a medicament for the treatment of a cell proliferation disorder.

23. The use according to claim 22, wherein the cell proliferation disorder is selected from the group consisting of a cancer, an autoimmune disease, and an inflammatory disease.

24. The use according to claim 23, wherein the cancer is a tumor or B-cell cancer.

25. The use according to claim 23, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, osteoarthritis, psoriatic arthritis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, asthma, systemic lupus erythematosus (SLE), diabetes, multiple sclerosis, solid organ transplant rejection, and graft versus host disease (GVHD).

26. A protein dimmer comprising two single-chain binding proteins according to claim 1.