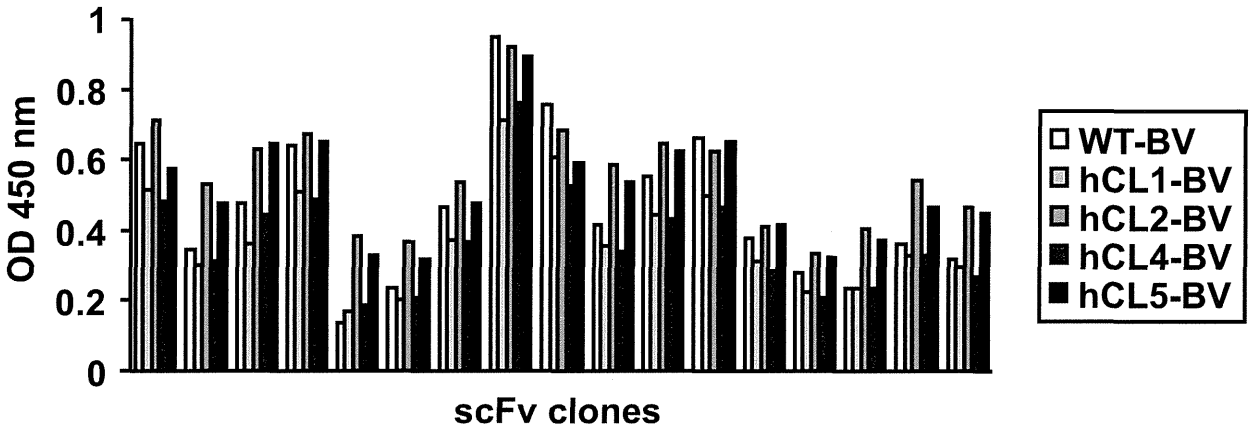

Figure 12 CL-binding characterization of the isolated hCL2 binders. Monoclonal analysis of scFv phage. Phage clones after 3rd round panning with hCL2 -BV were adopted to the WT-, hCL1-, hCL2-, hCL4-, hCL5-BV-coated immunoplates. Phage clones bound to the CL-BV-coated immunoplates were detected by ELISA with an anti-M13 mAb.

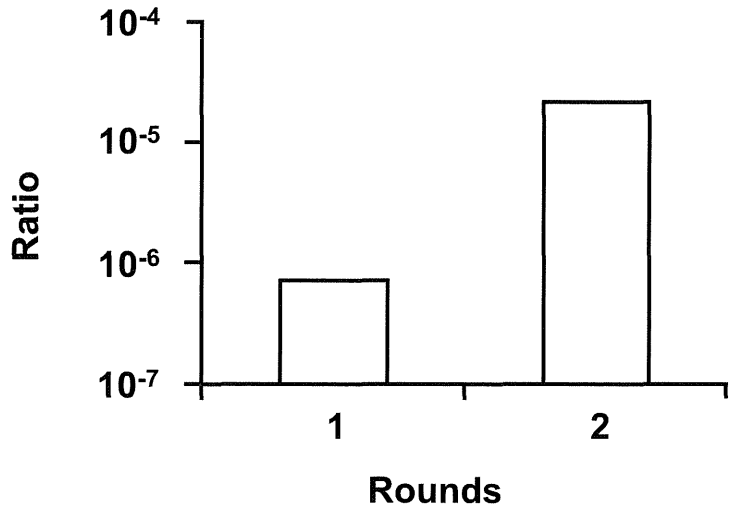

Figure 13 Panning of a hCL2 binder.
Enrichment of phages with affinity to hCL2-BV. Immunoplate coated with hCL2-BV were incubated with the scFv phage library at 8.1×10E11 CFU titer (1st input phage). The phages bound to hCL2-BV were recovered (1st output phage). The hCL2-BV-binding phages were subjected to another additional cycle of the incubation and wash step, resulting in 2nd output phage. The ratio of output phage to input phage titers was calculated.

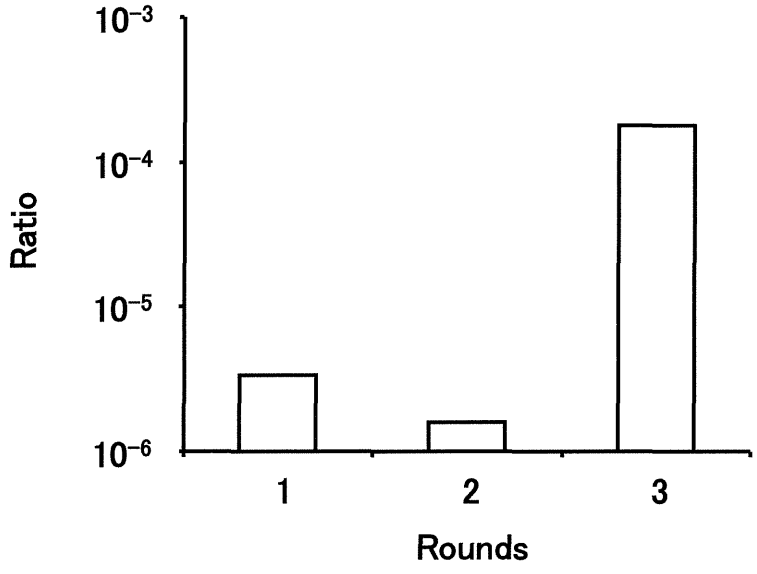

Figure 14 Panning of a hCL1 binder.
Enrichment of phages with affinity to hCL1-BV. Immunoplate coated with hCL1-BV were incubated with the scFv phage library at 8.1×10E11 CFU titer (1st input phage). The phages bound to hCL1 -BV were recovered (1st output phage). The hCL1-BV-binding phages were subjected to another additional cycle of the incubation and wash step, resulting in 2nd, 3rd output phage. The ratio of output phage to input phage titers was calculated.

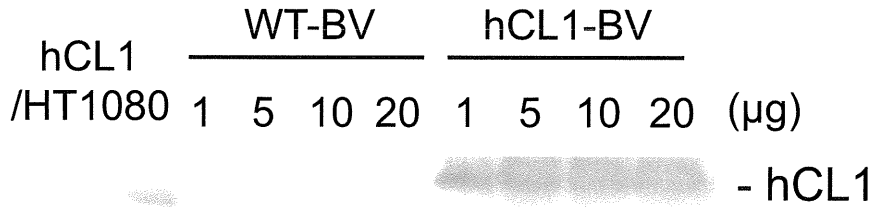

Figure 15 Preparation of CL1-expressing BVs.
A) WT-BV and hCL1-BV were subjected to SDS-PAGE, followed by immunoblot. The lysate of hCL1/HT1080 cells was used as a positive control.

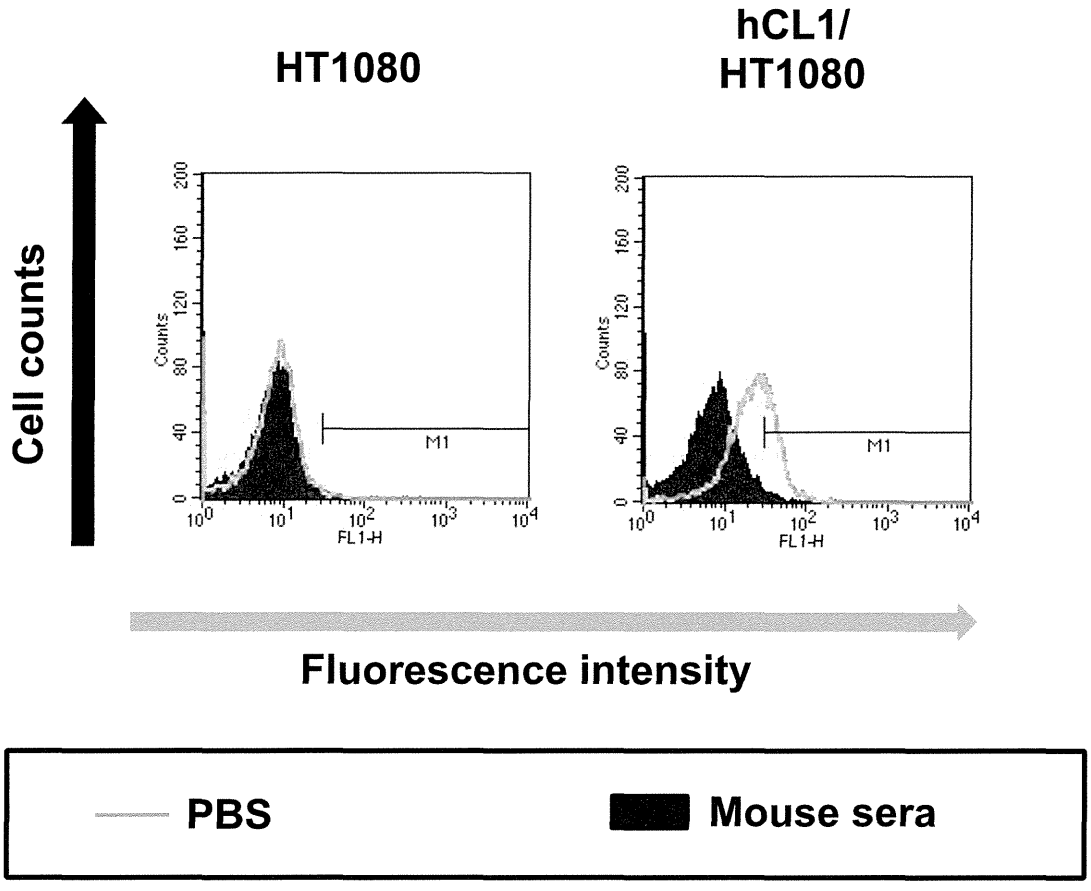

Figure 16 Detection of anti-hCL1 antibodies in the sera of gp64Tg mouse immunized with hCL1-dysplayed budded baculovirus.

hCL1/HT1080 cells were incubated with 1000-fold dilution of the sera of the sera of gp64Tg mouse immunized with hCL1-dysplayed budded baculovirus, and FITC-conjugated goat anti-mouse IgG (H+L). The antibodies-bound cells were detected using a flow cytometer. As a control, cells were incubated with phosphate buffered saline (PBS).

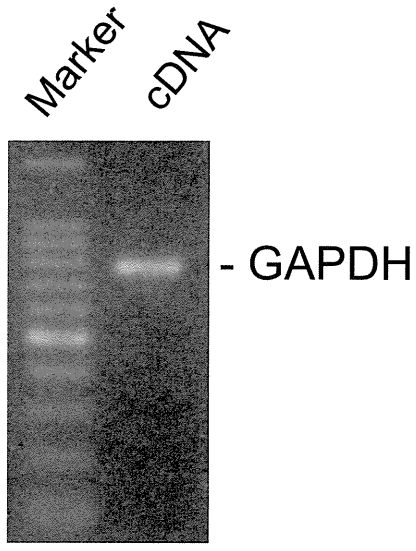

Figure 17 Preparation of cDNA for construction of scFv phage display library.
cDNA was made from mRNA purified from spleen in gp64Tg mice immunized with hCL1-BV. By using the cDNA, GAPDH expression was analysed by RT-PCR. The PCR products were subjected to agarose gel electrophoresis, followed by staining with ethidium bromide.

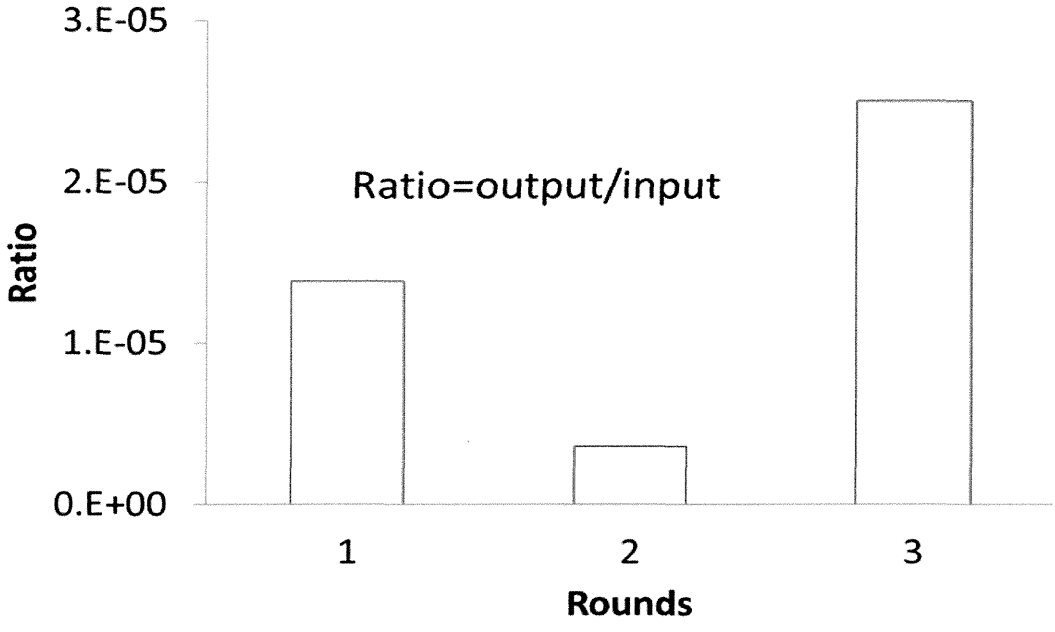

Figure 18 Panning of a hCL1 binder.
Enrichment of phages with affinity to hCL1-BV. Immunoplate coated with hCL1-BV were incubated with the scFv phage library at 1.1×10E12 CFU titer (1st input phage). The phages bound to hCL1 - BV were recovered (1st output phage). The hCL1-BV-binding phages were subjected to another additional cycle of the incubation and wash step, resulting in 2nd, 3rd output phage. The ratio of output phage to input phage titers was calculated.

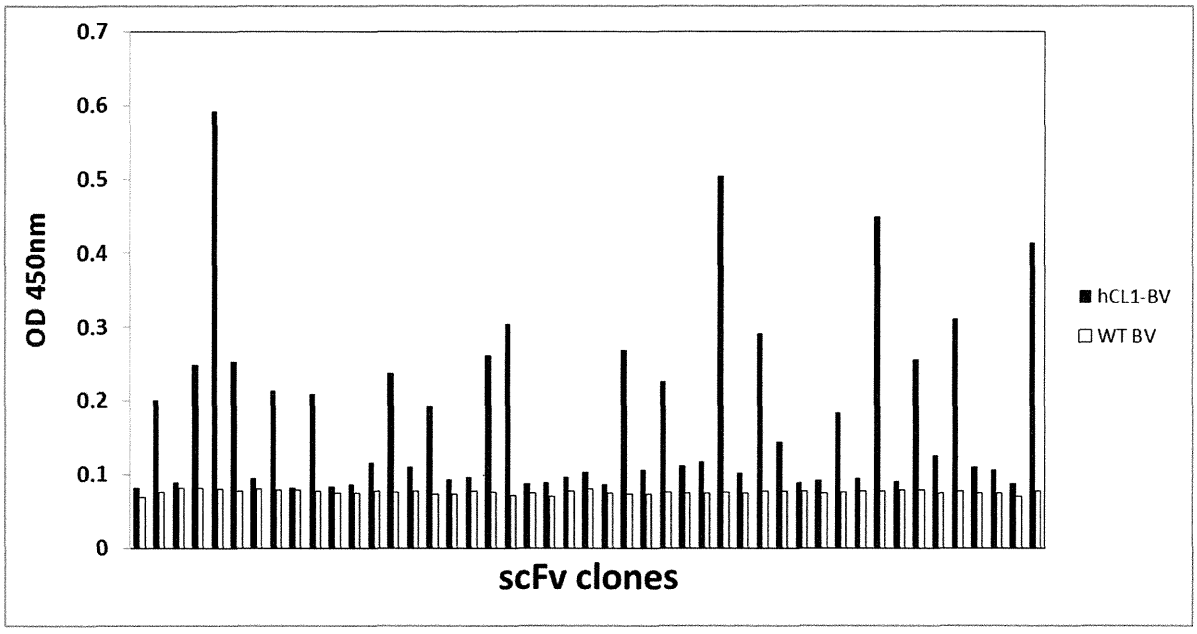

Figure 19 Screening of a hCL1 binder.
Monoclonal analysis of scFv phage. Phage clones after 3rd round panning with hCL1 -BV were adopted to the WT- or hCL1-BV-coated immunoplates. Phage clones bound to the CL-BV-coated immunoplates were detected by ELISA with an anti-M13 mAb.

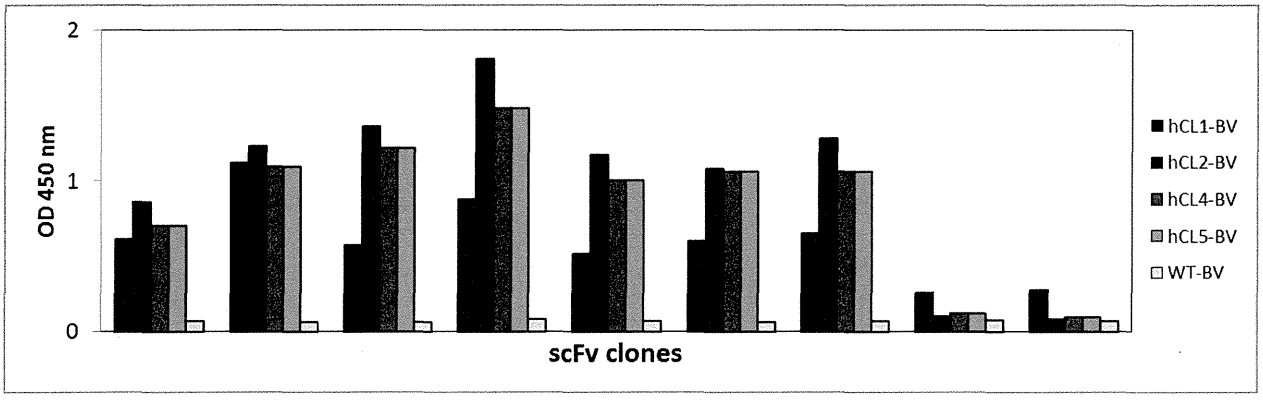

Figure 20 CL-binding characterization of the isolated hCL1 binders.

Monoclonal analysis of scFv phage. Phage clones after 3rd round panning with hCL1 -BV were adopted to the WT- or hCL1-,hCL2-,hCL4-,hCL5-BV- coated immunoplates. Phage clones bound to the CL-BV-coated immunoplates were detected by ELISA with an anti-M13 mAb.

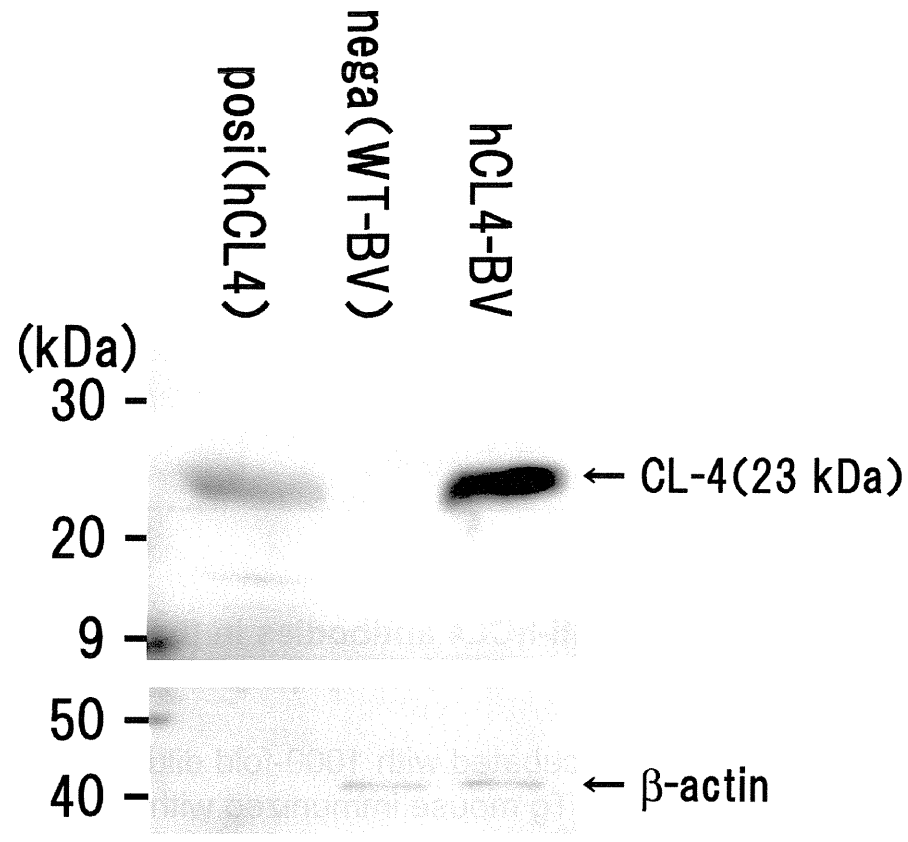

Figure 21 Preparation of CL4-expressing BVs.
A) WT-BV and hCL4-BV were subjected to SDS-PAGE, followed by immunoblot. The lysate of hCL4/HT1080 cells was used as a positive control.

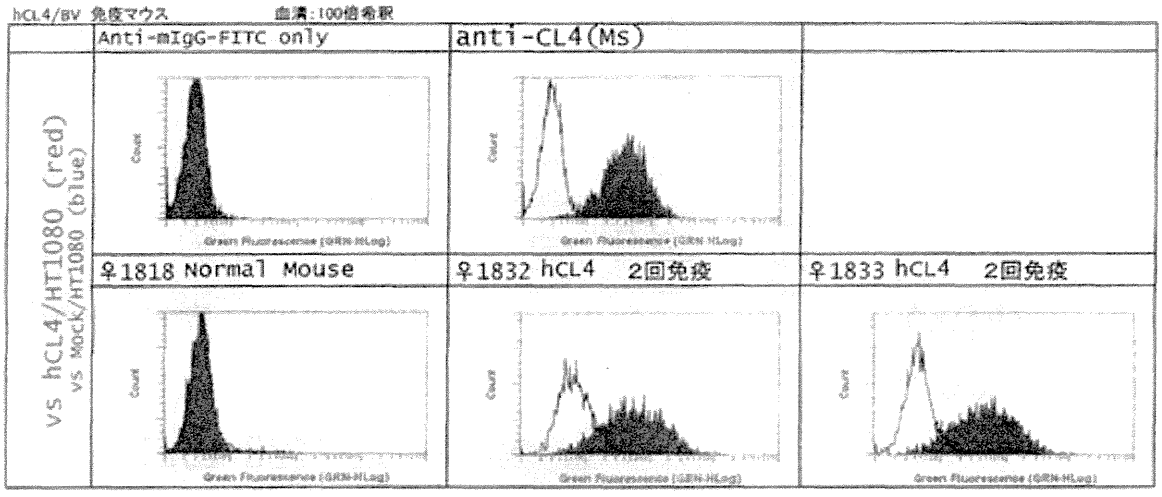

Figure 22 Detection of anti-hCL4 antibodies in the sera of gp64Tg mouse immunized with hCL4-dysplayed budded baculovirus.

hCL4/HT1080 cells were incubated with 1000-fold dilution of the sera of the sera of gp64Tg mouse immunized with hCL4-dysplayed budded baculovirus, and FITC-conjugated goat anti-mouse IgG (H+L). The antibodies-bound cells were detected using a flow cytometry. As a control, cells were incubated with phosphate buffered saline (PBS).

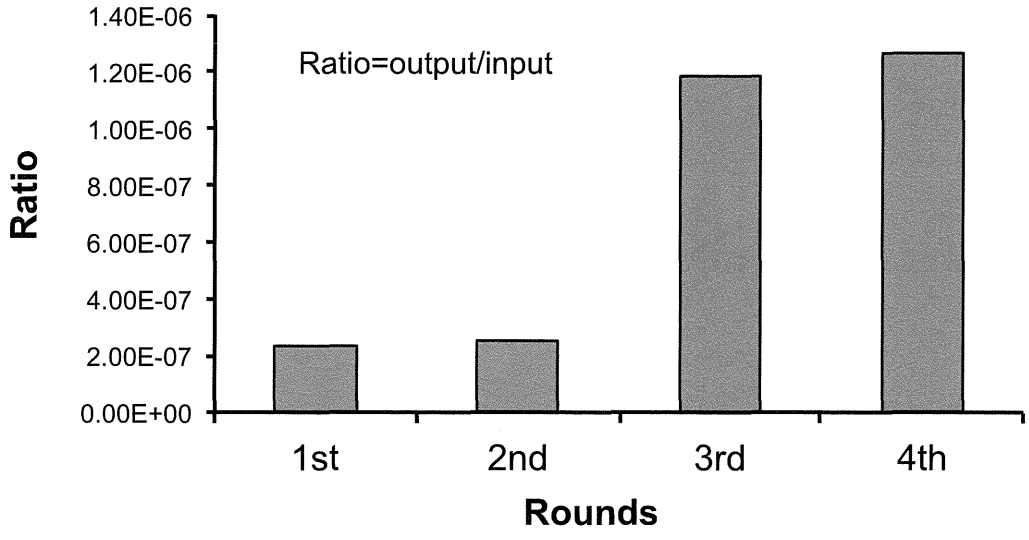

Figure 23 Panning of a hCL4 binder.
Enrichment of phages with affinity to hCL4-BV. HT1080/CL4 were incubated with the scFv phage (1st input phage). The phages bound to HT1080/CL4 were recovered (1st output phage). The HT1080/CL4 binding phages were subjected to another additional cycle of the incubation and wash step, resulting in 2nd, 3rd, and 4th output phage. The ratio of output phage to input phage titers was calculated.

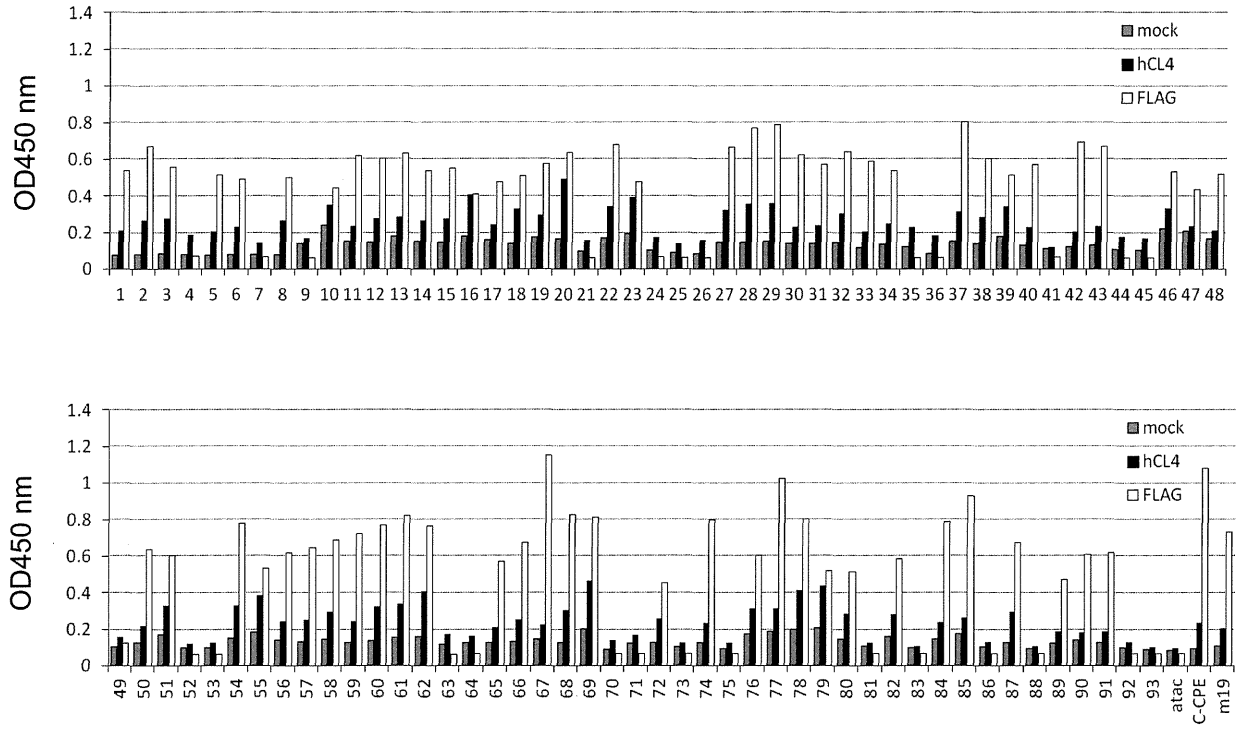

Figure 24 Screening of a hCL4 binder.
Monoclonal analysis of scFv phage. Phage clones after 3rd round panning with HT1080/CL4 were treated to the HT1080 or HT1080/CL4 cells. Phage clones bound to the HT1080 or HT1080/CL4 detected by ELISA with an anti-M13 mAb.

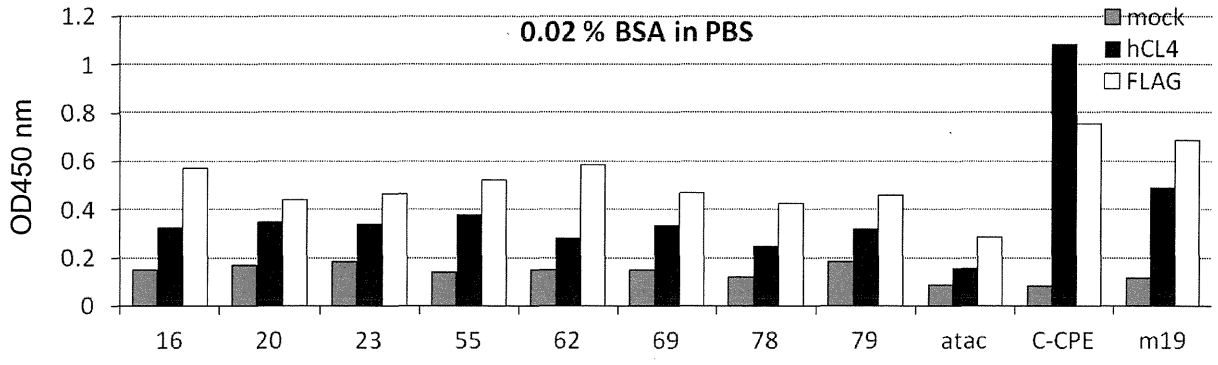

Figure 25 Retest of a hCL4 binder identified by cell panning screening.

Monoclonal analysis of scFv phage. Phage clones bind to HT1080/CL4 were treated to the HT1080 or HT1080/CL4 cells. Phage clones bound to the HT1080 or HT1080/CL4 detected by ELISA with an anti-M13 mAb.

VL	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	(G4S)3
Clone 1	RHLLTQSH KFLSTSVG NRVSITC	KASQDVST AVA	WYQQKPG QSPKLLIY	SASY RYA	GVPDRFTGSGSGTDFT FTISSVQAEDLAVYYC	QQHYS TPPT	FGGGTK LEIER	GGGGSGG GGSGGGG S
Clone 2	DILLNQSQ KFMSTSVG DRVSVSC	KASQDVDT DVA	WYQQKPG QSPKALIY	SASY RYS	GVPDRFTGSGSGTDFT LTISNVQSEDLAEYFC	QQYNS YPYT	FGGGTK LEIKR	GGGGSGG GGSGGGG S
Clone 3	DILMYQSQ KFMSTSVG DRVSVTC	KASQNVGT NVA	WYQQKPG QSPKALIY	SASY RYS	GVPDRFTGSGSGTDFT LTISNVQSEDLAEYFC	QQYNS YPYT	FGGGTK LEMKR	GGGGSGG GGSGGGG S
Clone 4	DILLTQSPS SLSVSAGEK VTMSC	KSSQSLLNS GNQKNYLA	WYQQKPG QPPKLLIY	GAST RES	GVPDRFTGSGSGTDFT LTISSVQAEDLAVYYC	QNDH SYPLT	FGAGTK LELKR	GGGGSGG GGSGGGG S
Clone 5	DILLNQSQ KFMSTSVG DRVSVSC	KASQDVDT DVA	WYQQKPG QSPALIY	SASY RYS	GVPDRFTGSGSGTDFT LTISNVQSEDLAEYFC	QQYNS YPYT	FGGGTK LEIKR	GGGGSGG GGSGGGG S
Clone 6	DIVITQSHK FMSTSVGD RVSITC	KASQDVGT AVA	WYQQKPG QSPKLLIY	SASY RYT	GVPDRFTGSGSGTDFT FTISSVQAEDLAVYYC	QQHYS TPYT	FGGGTK LEIKR	GGGGSGG GGSGGGG S

VH	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	FLAG tag
Clone 1	QVQLQQSGPE LVKPGASVKLS CKASGYTFS	NYWM Q	WVKQR PGQGL EWIG	AIYPEDGDT RYTQKFKG	KATLTADKSSTTAYMQ LSSLTSEDSAVYYCAR	DGHYGM DY	WGQGT SVTVSS	DYKDDDDK
Clone 2	QVQLQQSGA ELVRPGALVKL SCKASGFNIK	DYYMH	WVKQR PEQGLE WIG	WIDPENGN TIYDPKFQG	KASITADTSSNTAYLQL SSLTSEDTADTSSNTAY LQLSSLTSEDTAVYYCV R	DNYGYDA FGY	WGQGT LVTASS	DYKDDDDK
Clone 3	DVKLQESGAE LVRPGALVKLS CKASDFNIK	DYYMH	WVKQR PEQGLE WIG	WIDPENGN TIYDPKFQG	KASITADTSSNTAYLQL SSLTSEDTAVYYCAR	EDYGYDY VPPFDY	WGQGT TLTVSS	DYKDDDDK
Clone 4	EVMLVESGAE LVKPGASVKLS CTTSGFNIK	DYYMH	WVKQR PEQGLE WIG	WIDPENGN AIYDPKFQG	KASITAETSSNTAYLQLS SLTSEDTAVYYCAR	DNYGYYD GFAY	WGQGT LVTVSA	DYKDDDDK
Clone 5	QVQLQQSGA ELVRPGALVKL SCKASGFNIK	DYYMH	WVKQR PEQGLE WIG	WIDPENGN TIYDPKFQG	KASITADTSSNTAYLQL SSLTSEDTAVYYCAR	DNYGYDA FGY	WGQGT LVTASS	DYKDDDDK
Clone 6	EVQLQQSGAE LVRPGTSVKVS CKASGYAFT	NYLIE	WVKLR PGQGL EWIG	VINPGSGG TNYNEKFK G	KATLTADKSSSTAYMQL SSLTSDDSAVYFCAR	DGVYYRY DEGNYFA MDY	WGQGT SVTVSS	DYKDDDDK

Table 1 Amino acid sequence of hCL1 binders.
The amino acids sequences of scFv clones were analyzed.

VL	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	(G4S)3
Clone 1	DIVMTQTPASLV VSLGQRATISC	RASQSVSS SSYSYMH	WYQQKPGQ SPKLLIY	YASYLES	GVPARFSGSGSGTDFTL FTIPVKEEDTATYYC	QHSY STPYT	FGGGTK LELKR	GGGGSGGG GSGGGGS
Clone 2	DIVMKQSHKFM STSVGDRVSITC	KASQD MGSKVA	WYQQRPGQ SPKLLIY	WTSTRHV	GVPDRFTGSGSGTDFT LTISNVQSEDLADYFC	QQYS SYPLT	FGAGTK LELER	GGGGSGGG GSGGGGS
Clone 3	DIELTQSQKFMS TSVGDRVSITC	KASQD VSTAVA	WYQQKPGQ SPKLLIY	SASYRYT	GVPDRFTGSGSGTDFT FTISSVQAEDLAVYYC	QQHY STPYT	FGGGTK LELKR	GGGGSGGG GSGGGGS
Clone 4	DIVMTQTPASLV VSLGQRATISC	RASQSVSS SSYSYMH	WYQQKPGQ PPKLLIK	YASNLES	GVPARFSGSGSGTDFTL NIHPVKEEDTATYYC	QHSW EIPYT	FGGGTK LELKR	GGGGSGGG GSGGGGS
Clone 5	DIVMTQSHKFMS TSVGDRVSITC	KASQDV STAVA	WYQQKPGQ SPKLLIY	SASYRYT	GVPDRFTGSGSGTDFT LTISNVQSEDLAEYFC	QQYS SYMYT	FGGGTK LEIKR	GGGGSGGG GSGGGGS

VH								FLAG
Clone 1								
Clone 2	EVQGVESGGGLVKPG GSLKLSCAASGFTFS	DYGMH	WVRQAPEKGLE WVA	YISSGSSTIY YADTVKG	RFTISRDNAKNTLFLQM TSLRSEDTAMYYCAR	QLRY	WGQGTT LTVSS	○
Clone 3	EVMLVESGGGLVQPG GSLKLSCAASGFTFS	SYAMS	WVRQTPEKR LDWVA	TISDGGSYTY YPDNVKG	RFTISRDNAKNNLYLQM SHLKSEDTAMYYCAR	DPWDVG YWYFDV	WGTGTT VTVSS	○
Clone 4								
Clone 5	EVQLEESGGGLVKPG GSLKLSCAASGFTFS	DYGM	WVRQAPEKGLE WVA	YISSGSSTIY YADTVKG	RFTISRDNAKNTLFLQM TSLRSEDTAMYYCAR	IGYYGSSY NWYFDV	WGTGTP LTVFL	×

Table 2 Amino acid sequence of scFv phage library.
Phage clones were randomly picked up from the scFv phage library, and the amino acids sequences of scFv clones were analyzed.

VL	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	(G4S)3
Clone 1	GIVMTQSPT FLAVTASKK VTISC	TASESLY SSKHKVH YLA	WYQKKPE QSPKLLIY	GASNRYI	GVPDRFTGSGS GTDFTLTISSVQV EDLTHYYC	AQFYSYP LT	FGAGTK LEIKR	GGGGSGG GGSGGGG S
Clone 2	DIVMTQSQK FMSTSVGD RVSVTC	KASQNVG TNVA	WYQQKPG QSPKALIY	SASYRY S	GVPDRFTGSGS GTDFTLTISNVQS EDLADYFC	QQYSNYY T	FGGGT KLELER	GGGGSGG GSGGGGS
Clone 3	DIVMTQSHK FMSTSVGE RVNITC	KASQDVS TAVA	WYQQKPG QSPKLLIY	SASYRY T	GVPDRFTGSGS GTDFTFTISSVQA EDLAVYYC	QQYNSYP LT	FGAGTK LELKR	GGGGSGG GGSGGGG S
Clone 4	DIVMTQSHK IMSTSVGDG VSITC	KASQDVS PAVA	WYQQKPG QSPKLLIY	SASYRY S	GVPDRFTGSGS GTDFTLTISNVQS EDLAEYFC	QQYNSYP YT	FGGGT KLELKR	GGGGSGG GGSGGGG S
Clone 5	DIQMTQSH KFMSTSVG DRVSITC	KASPDVS TAVA	WYQQKPG QSPQLLIY	SASYRY T	GVPDRFTGSGS GTDFTFTISSVQA EDLAVYYC	QQHYSTP LT	FGAGTK LEIKR	GGGGSGG GGSGGGG S
Clone 6	DIVMTQSQK FMSTSVGD RVSITC	KASQDVG TAVA	WYQQKPG QSPSKLLIY	WASTRH T	GVPDRFTGSGS GTDFTLTISNVQS EDLAEYFC	QQYNTYP LT	FGAGTK LEIKR	GGGGSGG GGSGGGG S
Clone 7	DIVMTQSHK FMSTSVRD RVSITC	KASQNVG TNVA	WYQQKPG QSPKALIY	SASYRY S	GVPDRFTGSGS GTDFTLTISNVQS EDLAEYFC	QQYNYYP LT	FGAGTK LEIKR	GGGGSGG GGSGGGG S
Clone 8	DIVITQSHKF MSTSVGDR VSITC	KASQDVS TAVA	WYQQKPG QSPKLLIY	SASYRY T	GVPDRFTGSGS GTDFTFTISSVQA EDLAVYYC	QQHYSTP YT	FGGGT KLELKR	GGGGSGG GGSGGGG S

VH	FR1	CDR 1	FR2	CDR2	FR3	CDR3	FR4	FLAG
Clone 1	DVKLVESGGG LVKPGGSLKLS CAASGFTFS	SYTMS	WVRQT PEKRL EWVA	TISSGGGY TYYLDTVK G	RFTISRDNAKNNLY LQMSSLRSEDTALY YCAR	RSLDGY DYWYFD V	WGAGT TLTVSS	DYKDDDDK
Clone 2	EVKLVESGGDL VKPGGSLKLS CAASGFTFS	SYGMS	WVRQT PDKRL EWVA	TISSGGSF TYYPDSVK G	RFTISRDNAKNTLH LQMSSLKSEDTAM YYCAR	HGSSYY AMDY	WGQGT SVTVSS	DYKDDDDK
Clone 3	EVQLQQSGDD LVKPGASVKLS CKASGYTFT	SYWIN	WIKQR PGQGL EWIG	RIAPGSGS TYYNEMFK G	KATLTVDTSSSTAYI QLSSLSSEDSAVYF FCAR	RGIWGS SYDYFD Y	WGQGT TLTVSS	DYKDDDDK
Clone 4	QVQLKQSGAE LVRPGALVKLS CKASGFNIK	DYFMH	WVKQ RPEQG LEWIG	WIDPENGN TIYDPKFQ G	KASITADTSSNTAYL QLSSLTSEDTAVYY CAR	RYRWYL SHFDY	WGQGT TLTVSS	DYKDDDDK
Clone 5	EVMLVESGGG LVQPGGSRKL SCAASGFTFS	NYAMS	WGRQ TPDKR LEWVA	TITSGGSY TYYPDSVK G	RFTISRANAKHTLY LRMSSLRSEDTAM YYCTR	HEDTLLR RHFDY	WGQGT TLTVSS	DYKDDDDK
Clone 6	EVKLVESGGGL VKPGGSLKLS CAASGFTFS	SYAMS	WVRQT PEKRL EWVA	TISGGGTT YYPDSVKG	RFTISRDNAKNNLY LQMSSLRSEDTALY YCAR	DDYDET GSFAY	WGQGT LVTVSS	DYKDDDDK
Clone 7	EVMLVESGGG LVKPGGSLKLS CAASGFTLS	SYAMS	WVRQ SPEKR LEWVA	EISSGGSY TYYPDTVT G	RFTISRDNAKNTLY LEMSSLRSEDTAM YYCAR	VVYYAM DY	WGQGT TLTVSA	DYKDDDDK
Clone 8	EVMLVESGGG LVKPGGSLKLS CAASGFTFS	SYAMS	WVRQT PEKRL EWVA	TISSGGSY NYYPDSVK G	RFITSRDNAKNTLY LQMSSLRSEDTAM YYCAR	QGPPFA Y	WGQGT LVSVSS	DYKDDDDK

Table 3 Amino acid sequence of scFv phage library.
Phage clones were randomly picked up from the scFv phage library, and the amino acids sequences of scFv clones were analyzed.

Clone	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	(G4S)3	Amino acids	FLAG tag
	VL									
1	MADIVMTQSH KFMSTSVGDR VTIAC	KASQDVS TAVA	WYQQKPGQ SPKLLIY	SASYRYT	GVPDRFTGSGSG TDFTFTITNVQAE DLAVYYC	QQHYTTPLT	FGAGTKLEL KR	GGGGSGGGGS GGGGS		
2	MADIVMTQSH KFMSTSVGDR VSITC	KASQDVG TAVA	WYQQKPGQ SPKLLIY	WTSTRHT	GVPDRFTGSGSG TDFTLTISNVQSE DLADYFC	QQYSSYPLT	FGAGTKLEI KR	GGGGSGGGGS GGGGS		
4	MANIVMTQSH KFMSTSVGDR VSITC	KASQDVS TAVV	WYQQKPGQ SPKLLIY	WASTRHT	GVPDRFTGSGSG TDYILTISSVQAED LALYYC	QQHYSTPLT	FGAGTKLEL KR	GGGGSGGGGS GGGGS		
5	MADILLTQSQ KFMSTSVGDR VSVTC	KASQNVG TNVA	WYQQKPGQ SPKALIY	SASYRYS	GVPDRFTGSGSG TDFTLTISNVQSE DLAEYFC	QHYITYPYT	FGGGTKLEI KR	GGGGSGGGGS GGGGS		
10	HGRYCDDPDSQIPACISRRQGYHNLQGQSECES*CSLVPTEARAVS*TADILYIQSLHWSP*SLHWQWIWDGFHFH HQHCAG*RPGTLLLSTTL*HSVDVRWRHQIGEIR（分類不可）							GGGGSGGGGS GGGGS		
	VH									
1	DVHLVESGPG LVAPSQSLSI TCTVSGFSLT	SVTVSSA AGYGVN	WVRQPPGK GLEWLGMIW G	DGSTDYNSAL	KSRLSISKDKSKS QVFLKMDSLQTD DTARYYCAR	AGYDGGYYYA MDY	WGQGT		248	○
2	EVQLVESGGG LVQPGGSRKL SCAASGFTFS	SFGMH	WVRQAPEKG LEWVA	YIGSGSSTIYYA DTV	KGRFTISRDNPKD TLFLQMTSLRSED TAMYYCAR	YALRRCLLGP RDSG	QCLCSGR		244	○
4	RFSFSSLGQSLRDLGLQ*SCPARLLATPLLATGFSG*NRGLDRVWNGLGLFILEMVI*GTLRSSRARPH*LQINPPA QPTCNSAAWHLRTLPSITVICIGSMLWTTGVKEPRSLSLQRP（分類不可）							VHが×	244	○
5	EVQGVESGGG LVKPGGSLKL SCAASGFTFS AA	SYTMS	WVRQTPEKR LEWVA	TISGGGGNTYYP DSV	KGRFTISRDNAKN TLYLQMSSLKSE DTAMYYCAS	SGSPFAY	WGQGTLVT VSA		243	○
10	EVKGVESGGG LVKPGGSLKL SCAASGFTFS	SYAMS	WVRQSPEKR LEWVA	EISSGGTYTFYP DTV	TGRFTISRDNAKN TLYLEMSSLRSED TAMYYCAR	PPYGNYEYFD V	WGAGTTLT VSSAA	VLが×	247	○

Table 4 Amino acid sequence of scFv phage library.
Phage clones were randomly picked up from the scFv phage library, and the amino acids sequences of scFv clones were analyzed.

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
	該当事項なし						

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Li X, Kondoh M, Watari A, Hasezaki T, Isoda K, Tsutsumi Y, Yagi K.	Effect of 70-nm silica particles on the toxicity of acetaminophen, tetracycline, trazodone, and 5-aminosalicylic acid in mice.	*Pharmazie*,	66 (4)	282-6	2011
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