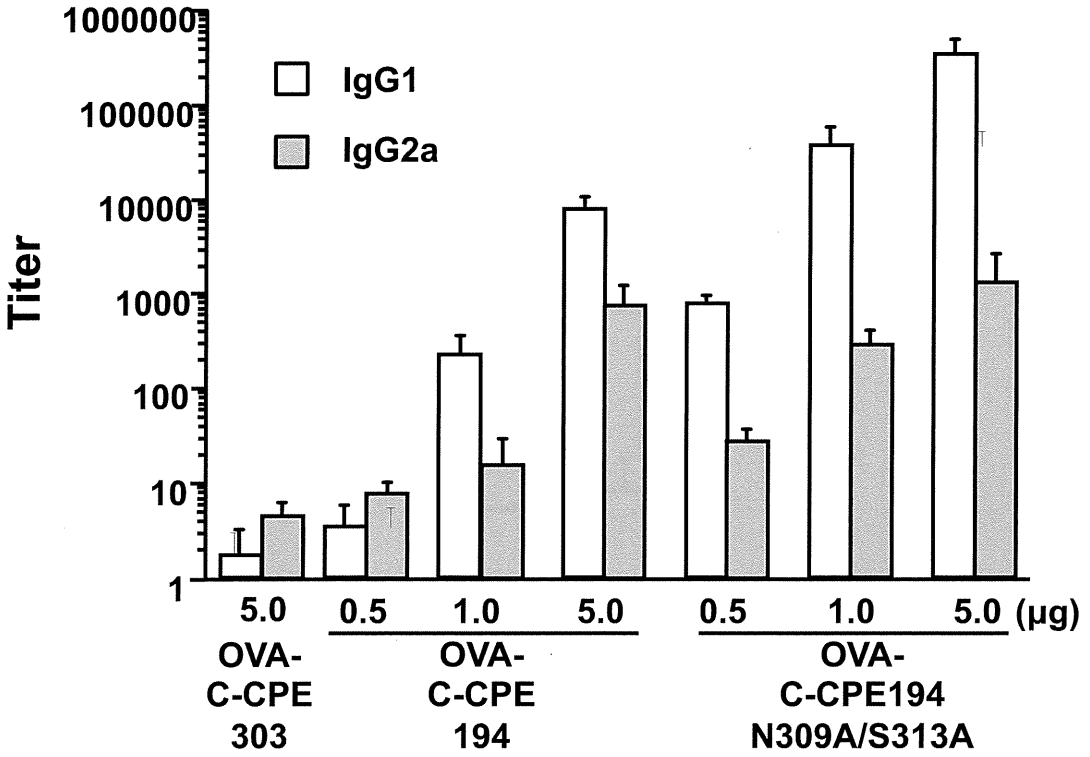


Fig.5 Serum IgG subclass induced by OVA-C-CPE 194 N309A/ S313A.
Mice were nasally immunized with OVA-C-CPE 303, OVA-C-CPE 194 or OVA-C-CPE 194 N309A/S313A (5, 1 or 0.5 mg of OVA) once a week for three weeks. Seven days after the last immunization, serum were collected. Serum IgG subclass (IgG1 and 2a) was determined by ELISA. Data are means ± SEM (n=3~5). The results are representative of three independent experiments.

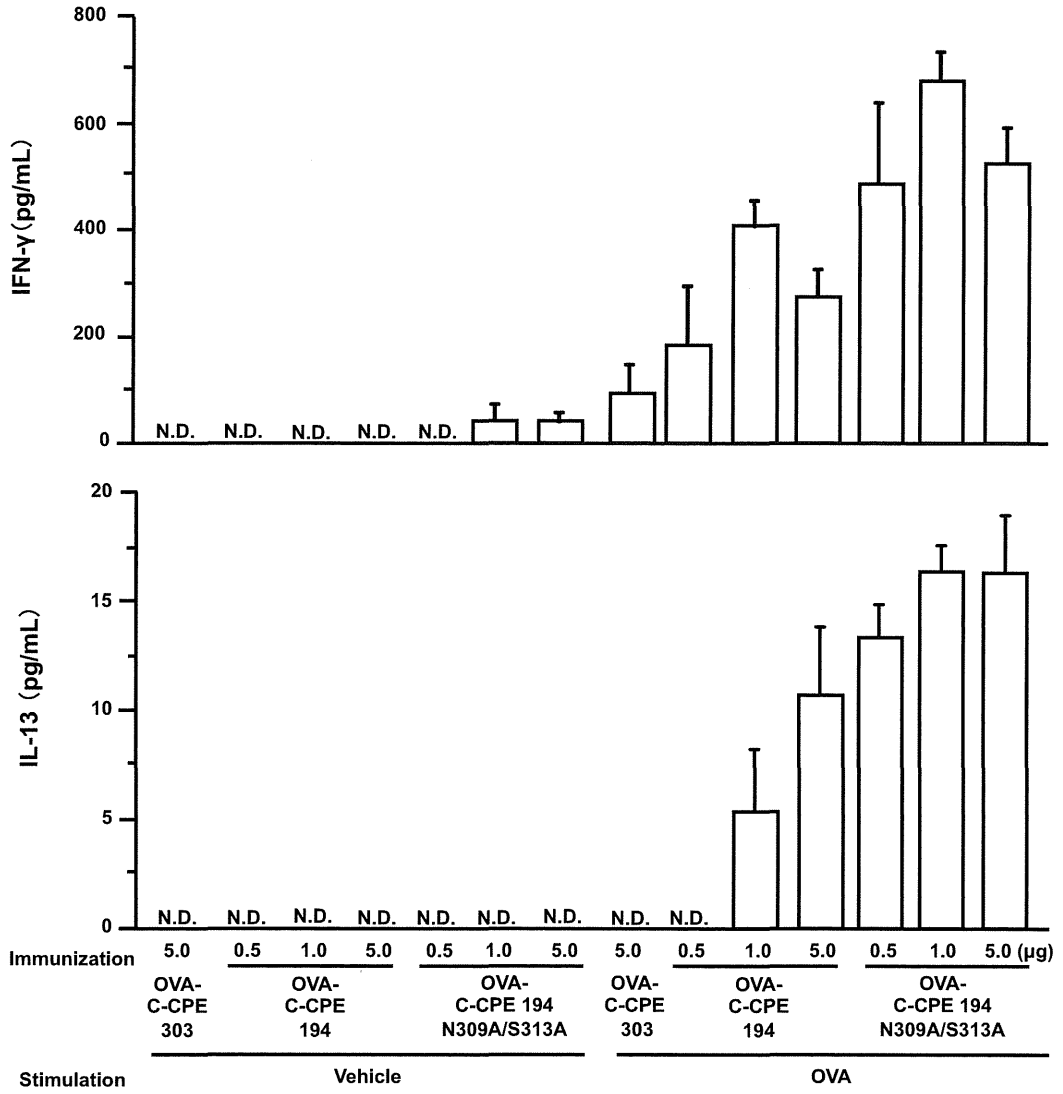


Fig.6 Th1 and Th2 responses induced by OVA-C-CPE 194 N309A/S313A.

Mice were nasally immunized with OVA-C-CPE 303, OVA-C-CPE 194 or OVA-C-CPE 194 N309A/S313A (5, 1 or 0.5 mg of OVA) once a week for three weeks. Seven days after the last immunization splenocytes were collected. The splenocytes isolated from the immunized BALB/c mice were stimulated with vehicle or OVA (1 mg/ml) for 24 hr, and the cytokine (IFN-γ and IL-13) in the conditioned medium were measured by ELISA. Data are means ± SEM (n=3~5). The results are representative of three independent experiments. N.D., not detected.

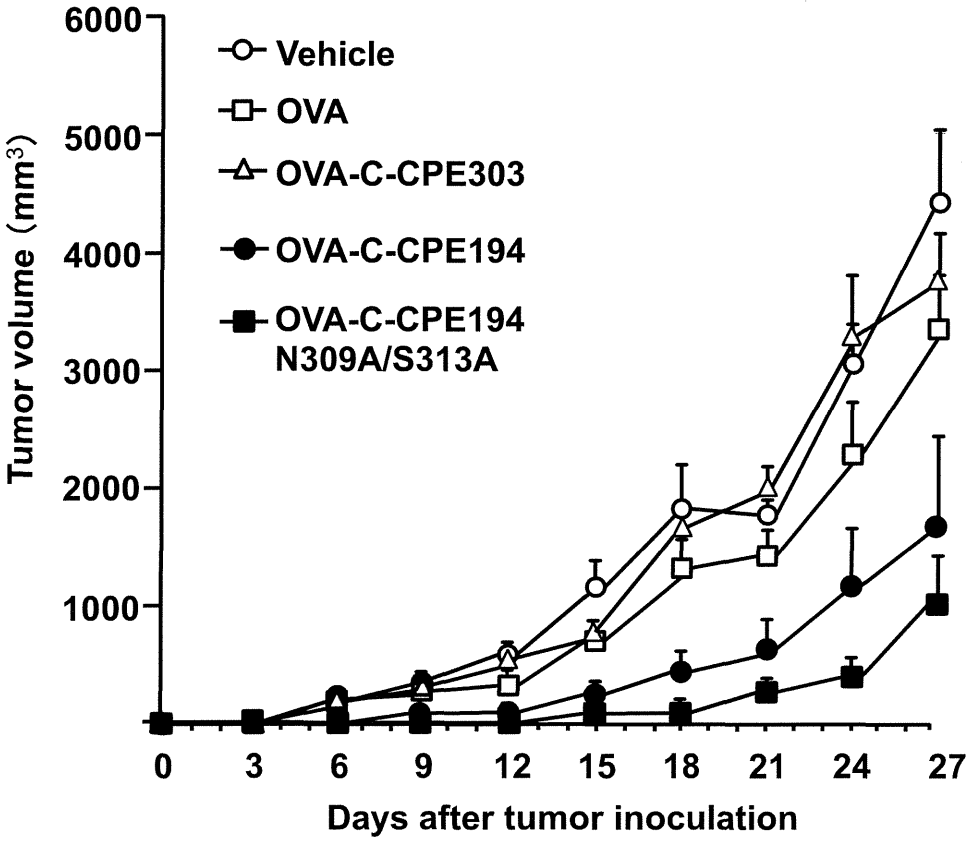
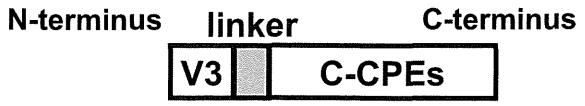


Fig.7 Anti-tumor activity induced by immunization with OVA-C-CPE 194 N309A/S313A in an E.G7-OVA cancer model.
C57BL/6 mice were nasally immunized with vehicle, OVA, OVA-C-CPE 303, OVA-C-CPE 194 or OVA-C-CPE 194 N309A/S313A (5 mg of OVA) once a week for three weeks. Seven days after the last immunization, the mice were injected s.c. on the right back with 1×10^6 E.G7-OVA cells. The tumor growth was monitored by measuring two diameters, and the tumor volumes was calculated as $a \times b \times b/2$, where a is the maximum diameter of the tumor and b is the minimum diameter of the tumor. Data are means ± SEM (n=5~6). The results are representative of three independent experiments.

A



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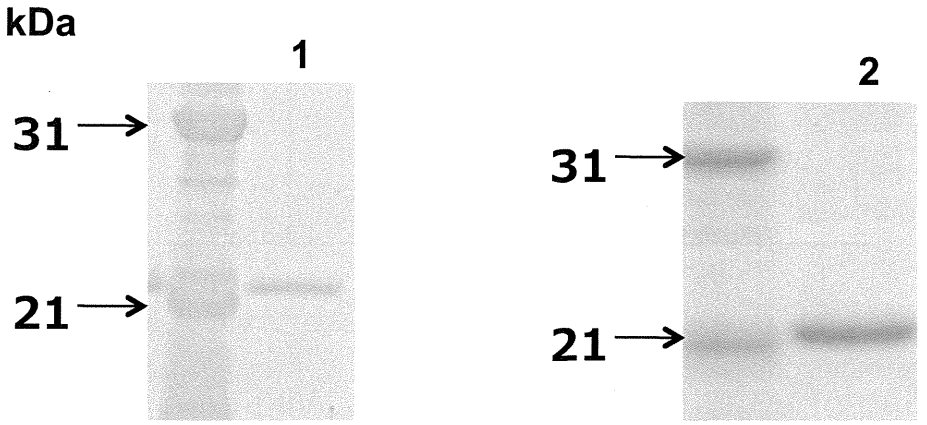


Fig.8 Preparation of V3-C-CPE mutant.
A) Schematic illustration of V3-C-CPE mutant. V3 was fused with C-CPE mutant at the N-terminal of C-CPE mutant, resulting in V3-C-CPE mutant. B) Purification of V3-C-CPE mutant. V3-C-CPE mutant was expressed in *E. coli* and isolated by anti-His tag affinity chromatography. The purification of V3-C-CPE mutant was confirmed by SDS-PAGE followed by staining with Coomassie Brilliant Blue (CBB) and by immunoblotting with an anti-his-tag antibody. Lane1, V3-C-CPE 303; lane2, V3-C-CPE 194N309A/S313A.

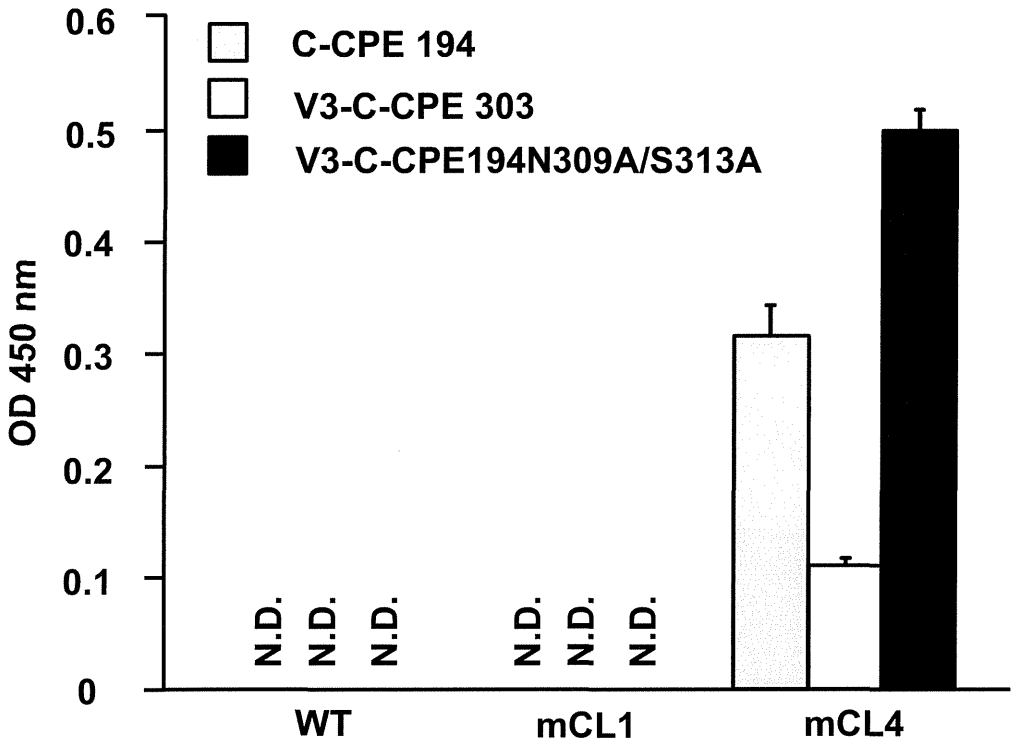


Fig.9 Binding of V3-C-CPEs to claudin-4. Wild-type BV (WT-BV), mouse claudin-1 displaying BV (mCL1-BV) or -4 (mCL4-BV) was absorbed onto immunoplate, and then V3-C-CPEs were added to the well. V3-C-CPEs bound to BV was detected by an anti-his-tag antibody followed by goat anti-mouse IgG (H+L)-HRP. Data are means ± SD (n=4)

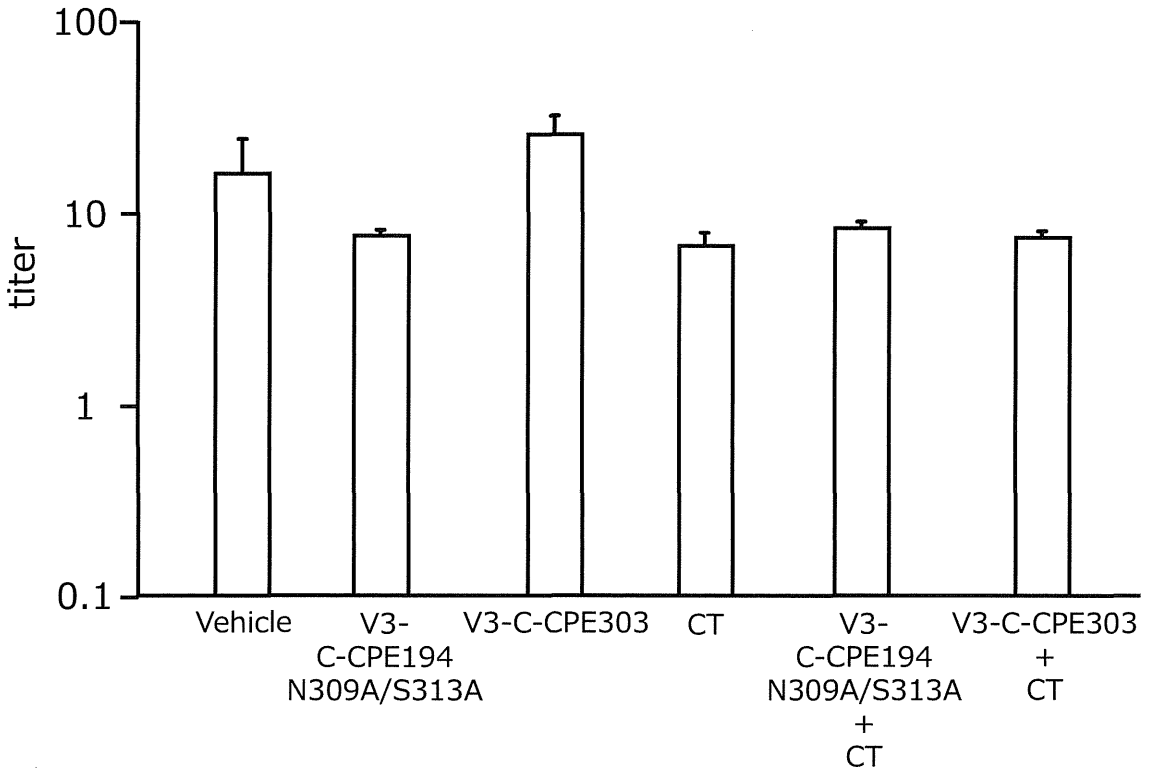


Fig.10 Production of V3 specific IgG in serum by V3-C-CPE194 N309A/S313A. Mice were nasally immunized with V3-C-CPE194 N309A/S313A, V3-C-CPE 303, cholera Toxin (CT), V3-C-CPE194 N309A/S313A and CT, V3-C-CPE 303 and CT (5 mg of V3) once a week for three weeks. Seven days after the last immunization, the levels of serum IgG were determined by ELISA. Data are means ± SEM (n=3~5).

A

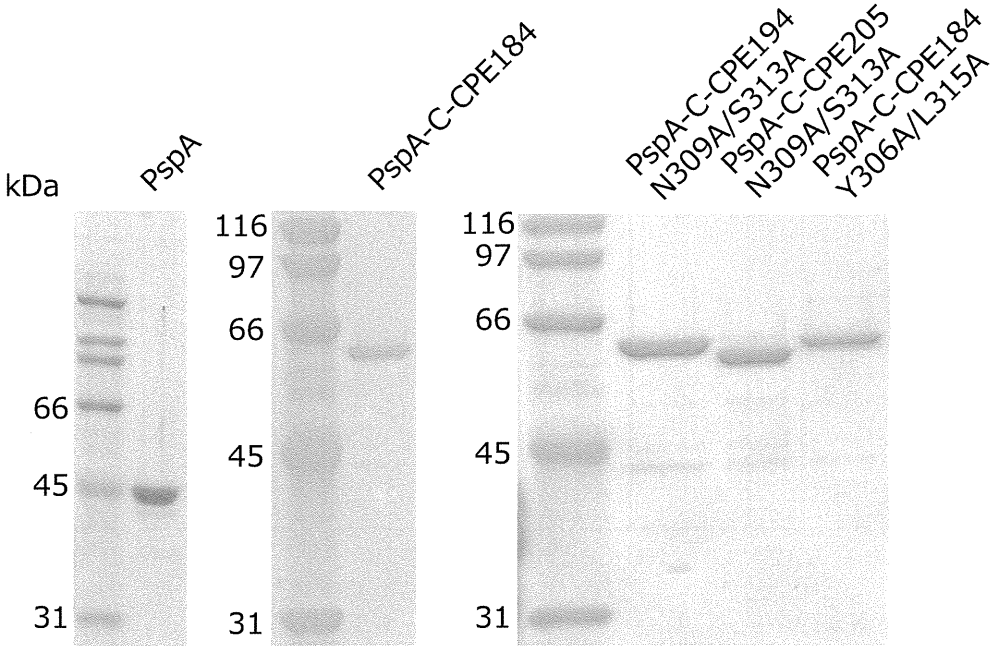


Fig.11 Preparation of PspA, PspA-C-CPE and PspA mutants. Purification of PspA, PspA-C-CPE and PspA-C-CPE mutants.
These proteins were expressed in *E. coli* and isolated by anti-His tag affinity chromatography. The purification of PspA, PspA-C-CPE and PspA mutants were confirmed by SDS-PAGE followed by staining with Coomassie Brilliant Blue (A) and by immunoblotting with an anti-his-tag antibody (B).

B

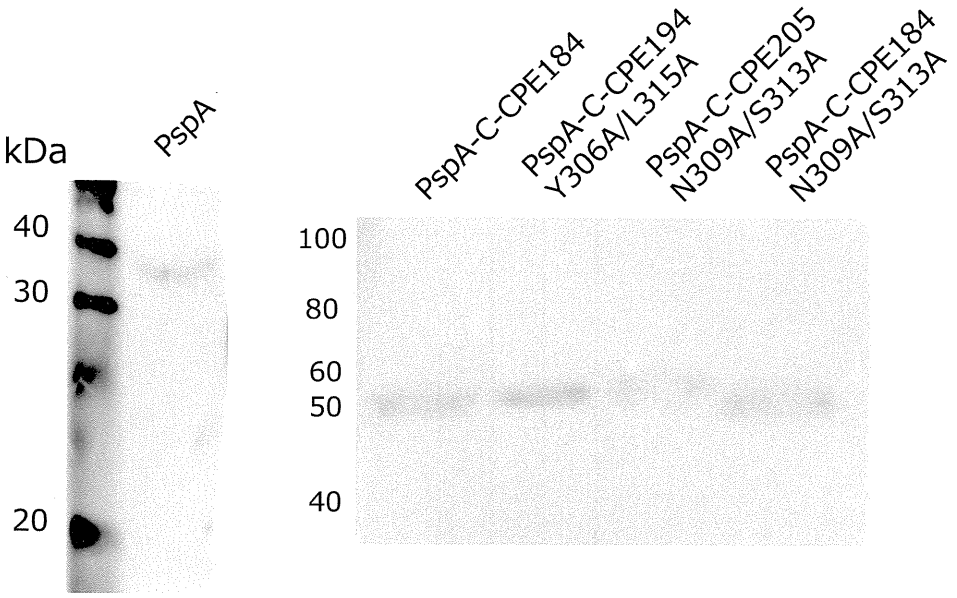


Fig.11 Preparation of PspA, PspA-C-CPE and PspA mutants. Purification of PspA, PspA-C-CPE and PspA-C-CPE mutants.
These proteins were expressed in *E. coli* and isolated by anti-His tag affinity chromatography. The purification of PspA, PspA-C-CPE and PspA mutants were confirmed by SDS-PAGE followed by staining with Coomassie Brilliant Blue (A) and by immunoblotting with an anti-his-tag antibody (B).

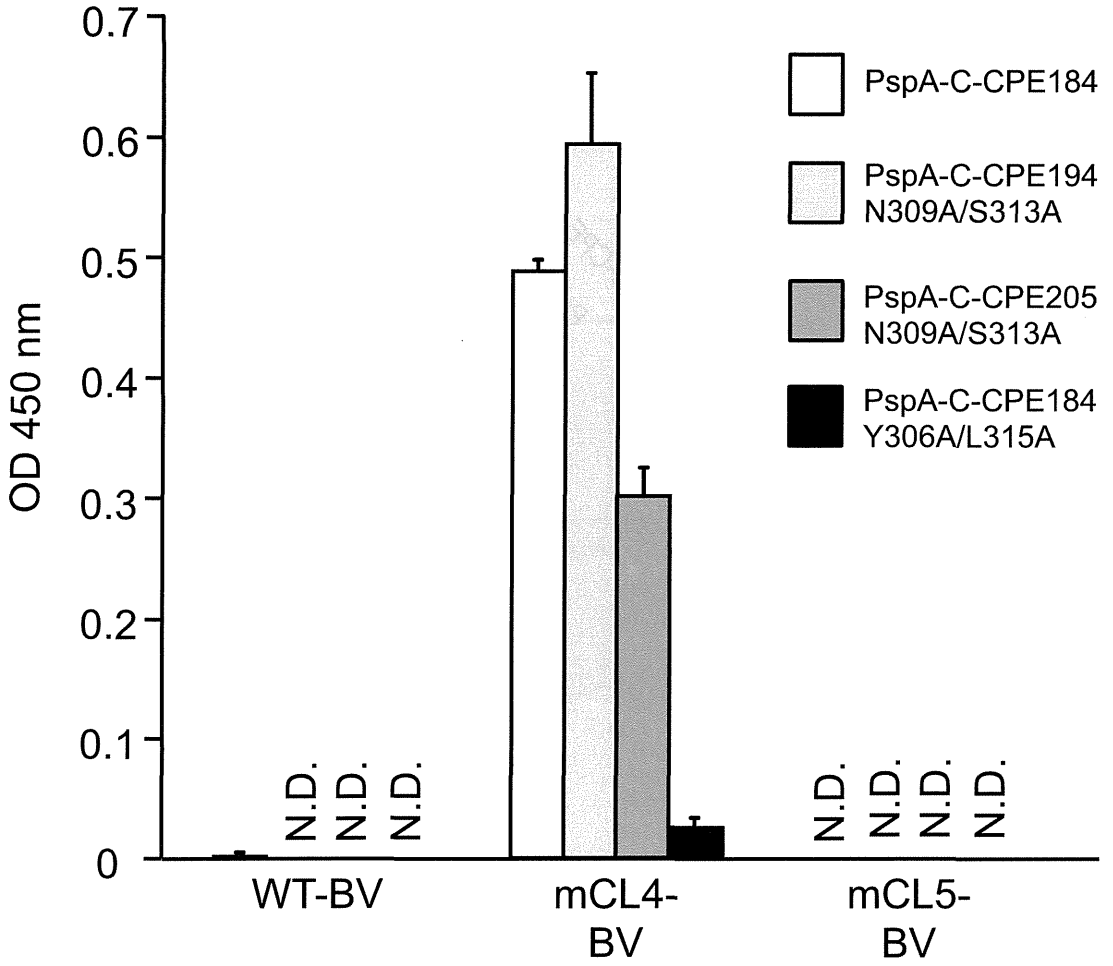


Fig.12. Binding of PspA-C-CPEs to claudin-4 displaying BV.
Wild-type BV (WT-BV), mouse claudin-4 displaying BV (mCL4-BV) or -5 (mCL5-BV) was absorbed onto immunoplate, and then PspA-C-CPEs were added to the well. PspA-C-CPEs bound to BV was detected by an anti-his-tag antibody followed by goat anti-mouse IgG (H+L)-HRP. Data are means ± SD (n=4).

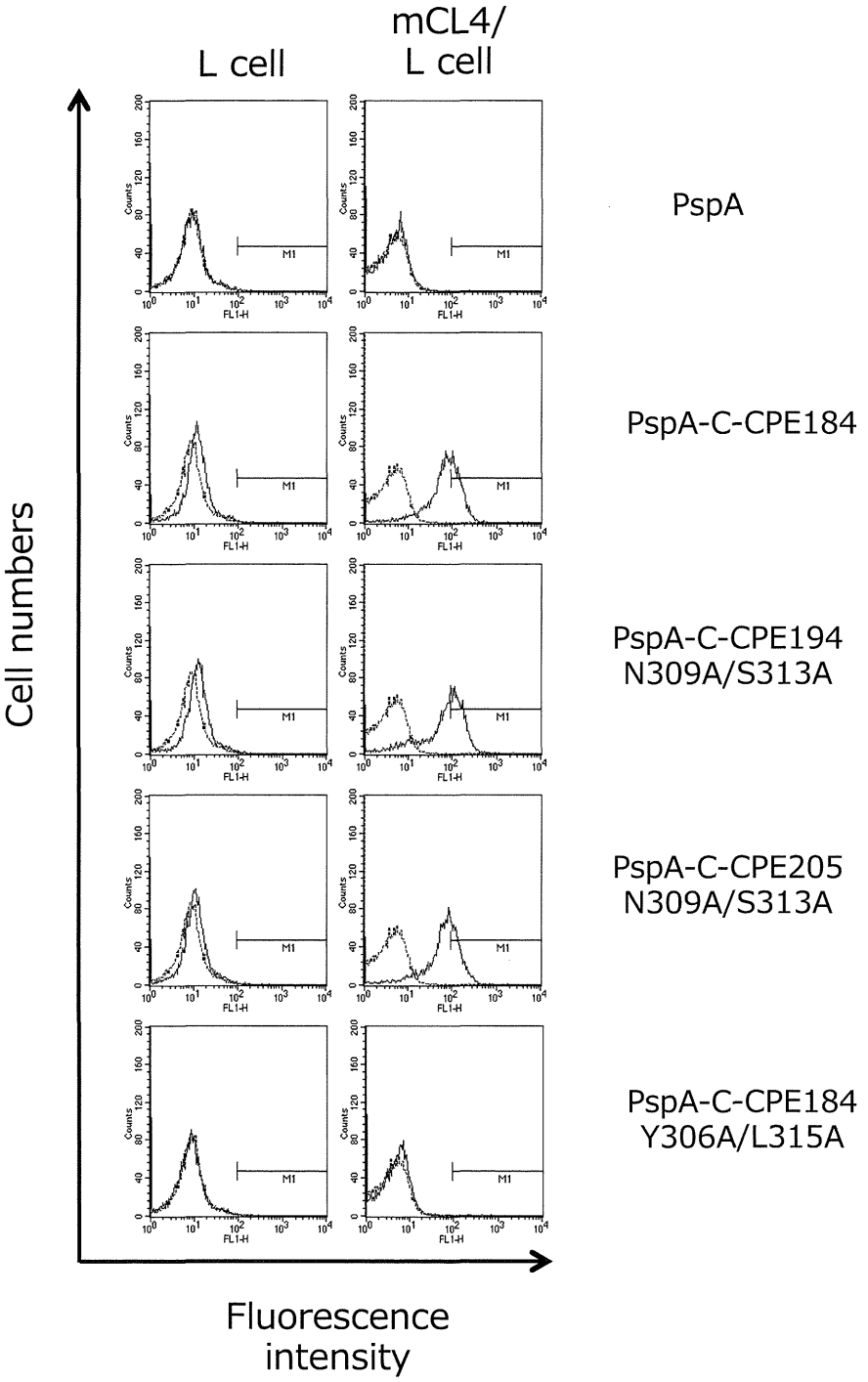


Fig. 13 Binding of PspA-C-CPEs to claudin-4-expressing cell. Claudin expressing L cells were incubated with PspA-C-CPEs followed by anti-his-tag antibody. Cells were stained with FITC-conjugated goat-anti mouse IgG (H+L) and analyzed by flow cytometry (solid line). Dotted line represents control cells incubated with the second and third step regent alone. (n=3)

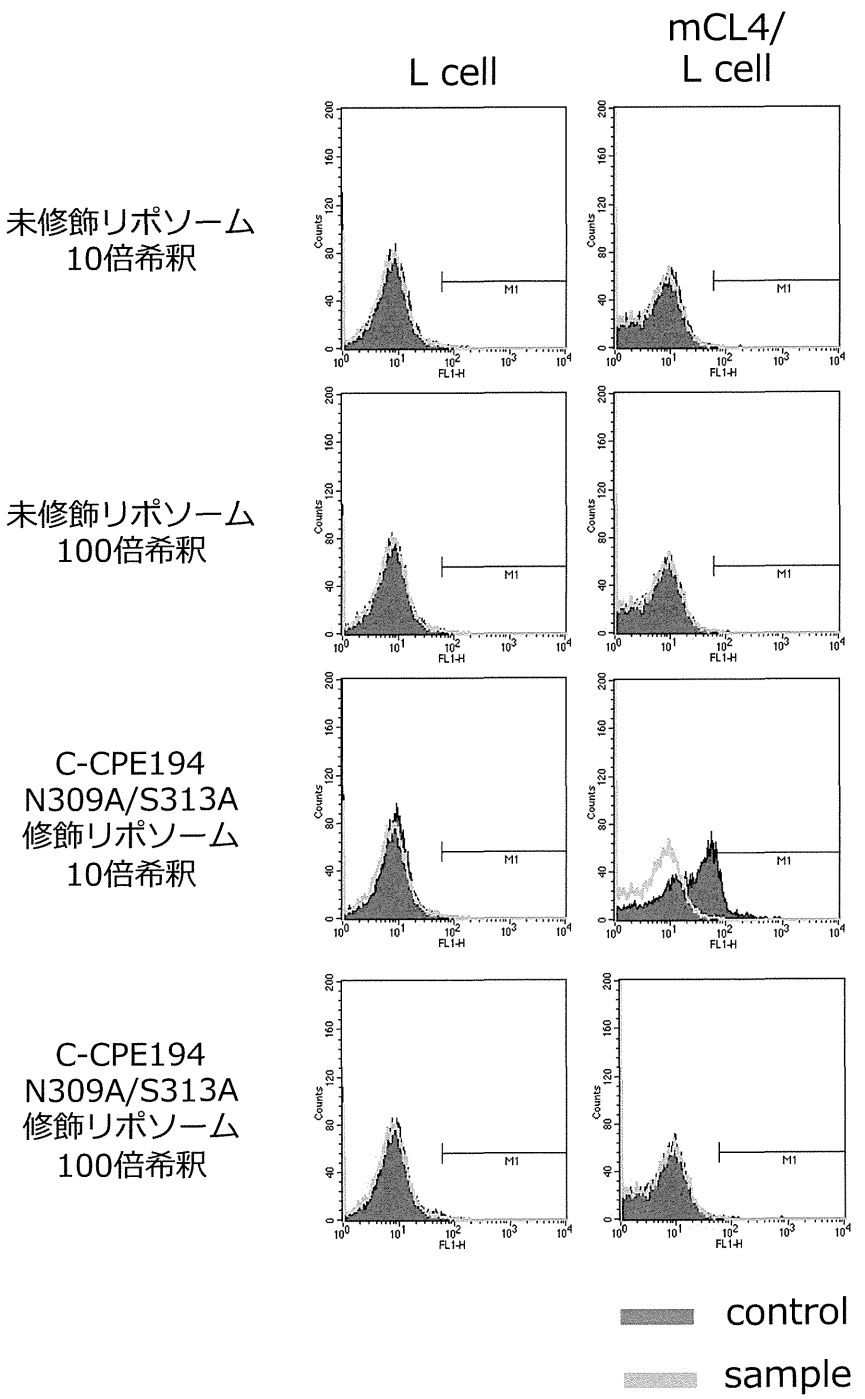


Fig. 14 C-CPE修飾リポソームのclaudin-4結合性
L細胞およびclaudin-4発現 L細胞にC-CPE194 N309A/S313Aを修飾したリポソーム（10倍希釈と100倍希釈液）を作用させた後、FACSにより結合性を確認した。

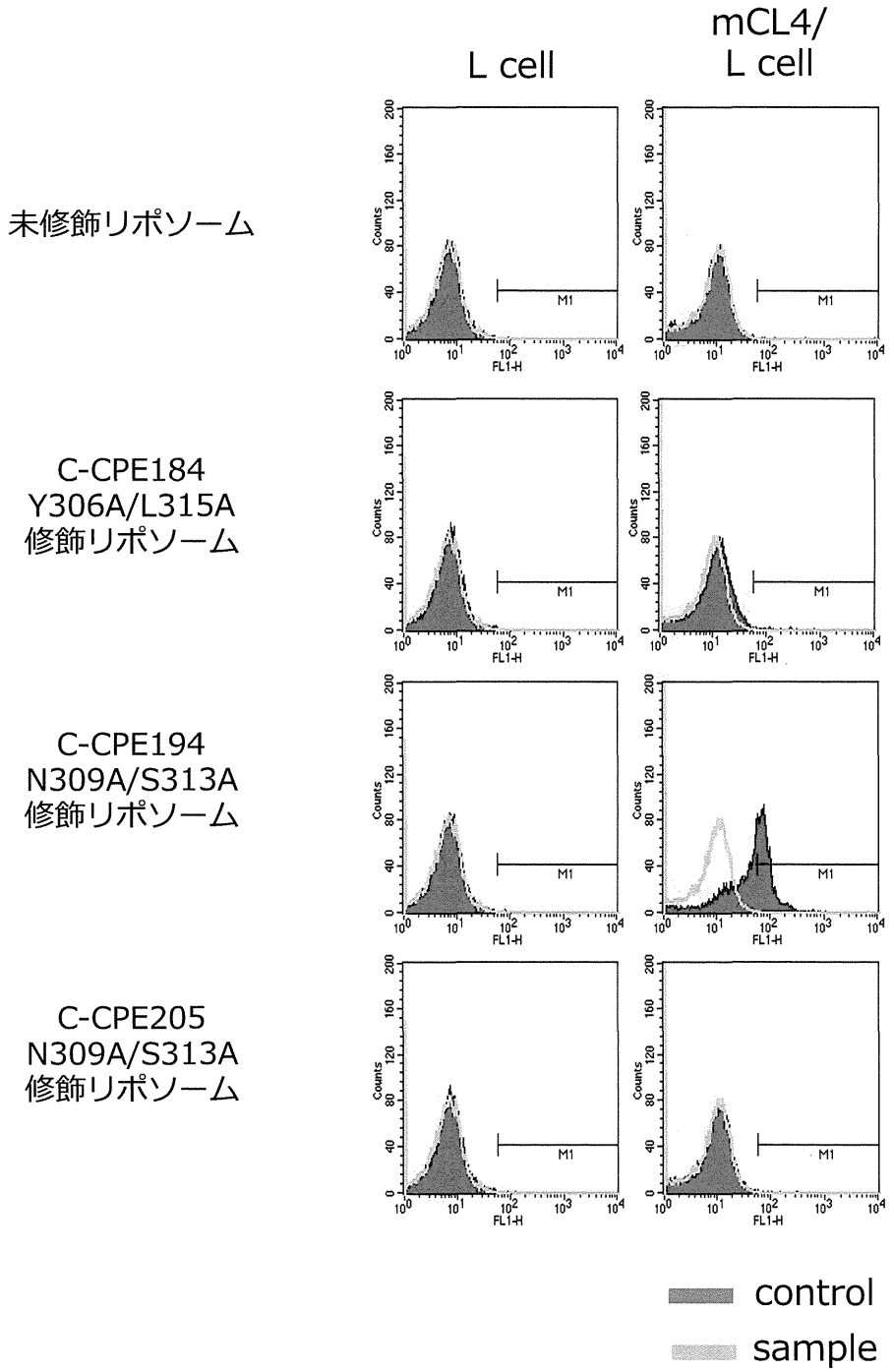


Fig. 15 OVA封入C-CPE修飾リポソームのclaudin-4結合性
L細胞およびclaudin-4発現 L細胞にC-CPE184 Y306A/L315A,
C-CPE194 N309A/S313A, C-CPE205 N309A/S313A
を修飾したOVA封入リポソームを作用させた後、FACSにより
結合性を確認した。

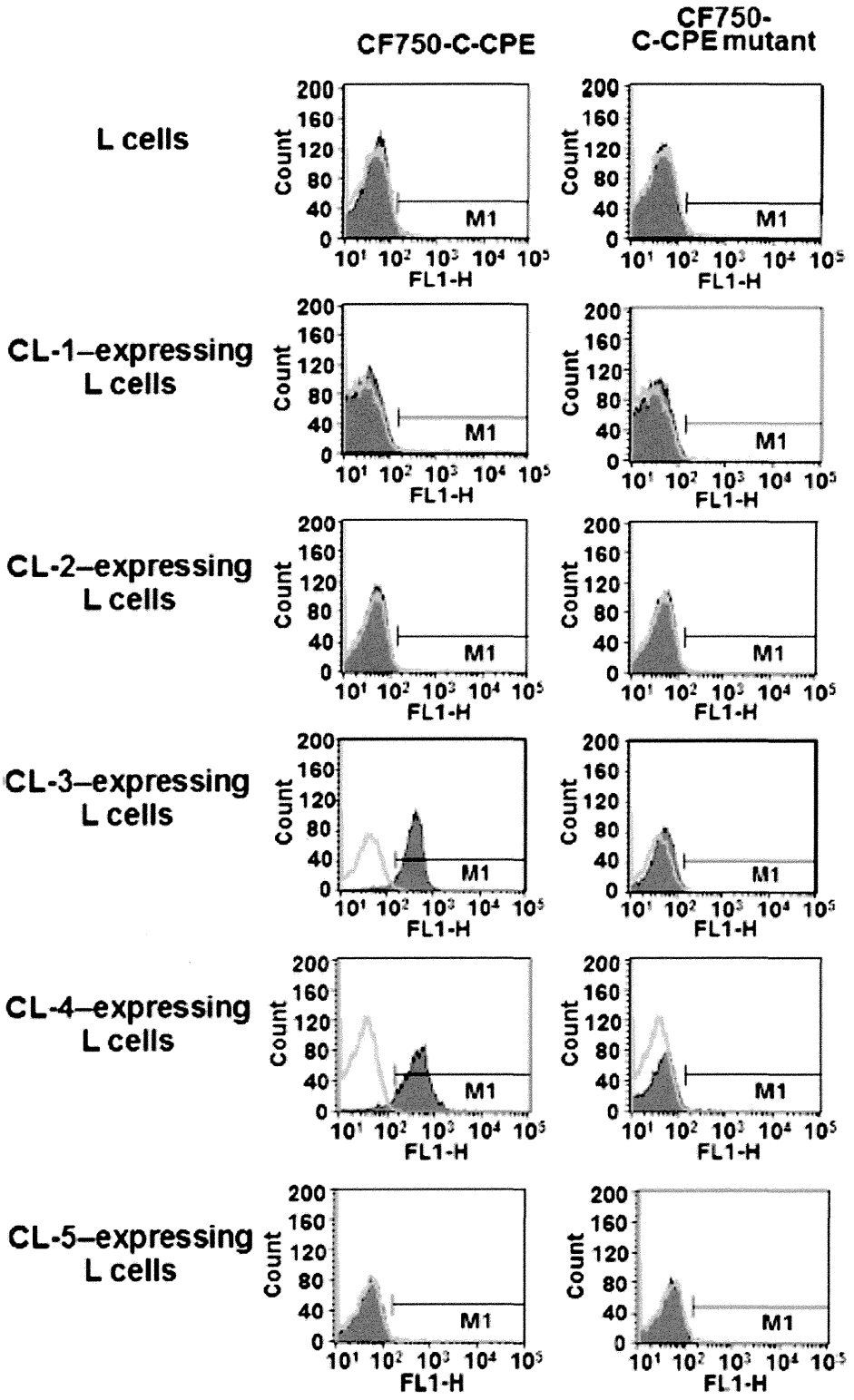


Fig. 16. Flow cytometric analysis of the interaction of claudins (CLs) with the CF750-labeled C-terminal fragment of Clostridium perfringens enterotoxin (C-CPE).
Mouse fibroblast L cells were incubated with 10 lg/ml CF750-labeled C-CPE or a mutant form of C-CPE (also labeled with CF750) for 1 h and then subjected to fluorescence-activated cell sorter analysis as described in the Materials and Methods. Unfilled curves show the results obtained when cells were not treated with C-CPE proteins. Filled curves show data from C-CPE-treated cells. FL1-H indicates fluorescent intensity and M1 indicates C-CPE-bound cells.

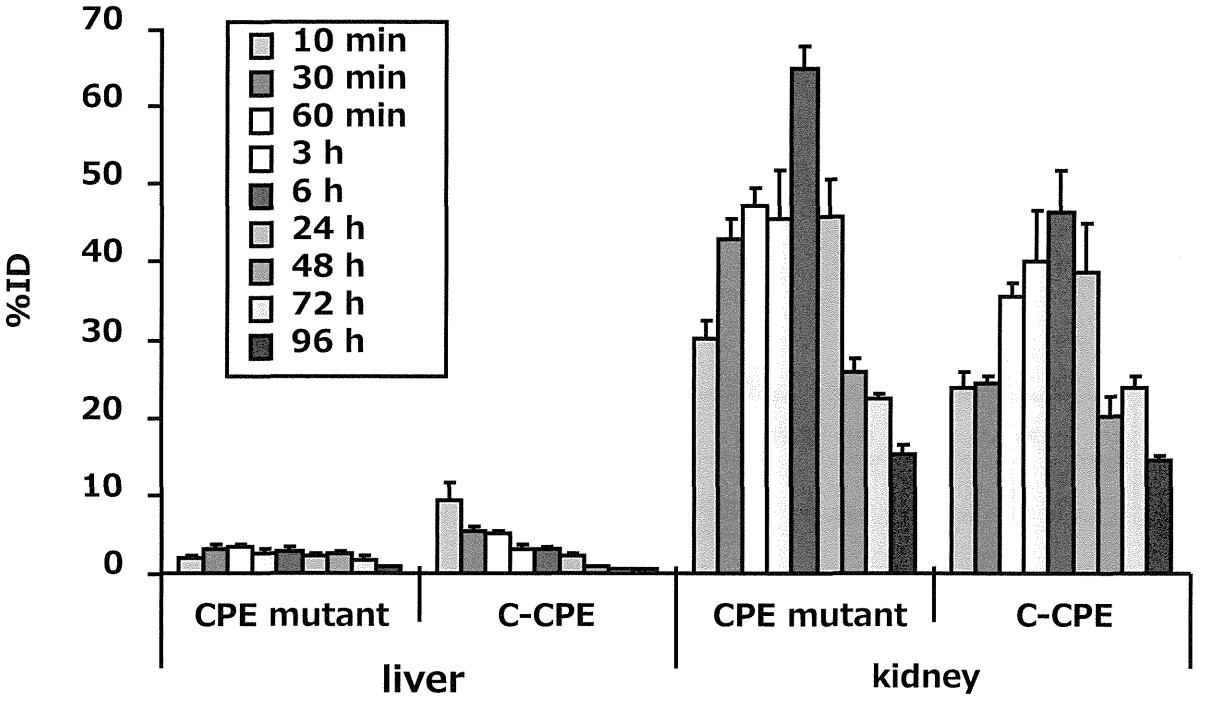


Fig. 17. *In vivo* distribution of the CF750-labeled C-terminal fragment of *Clostridium perfringens enterotoxin* (C-CPE). Mice were intravenously injected with 2 μg/mouse CF750-labeled C-CPE or a CF750-labeled C-CPE mutant. Liver and kidney were removed at the indicated times after injection and the intensity of fluorescence of each tissue was measured. Tissue C-CPE levels were calculated as percentages of injected doses. Data are means ±SEM (n = 5). ID, injected dose.

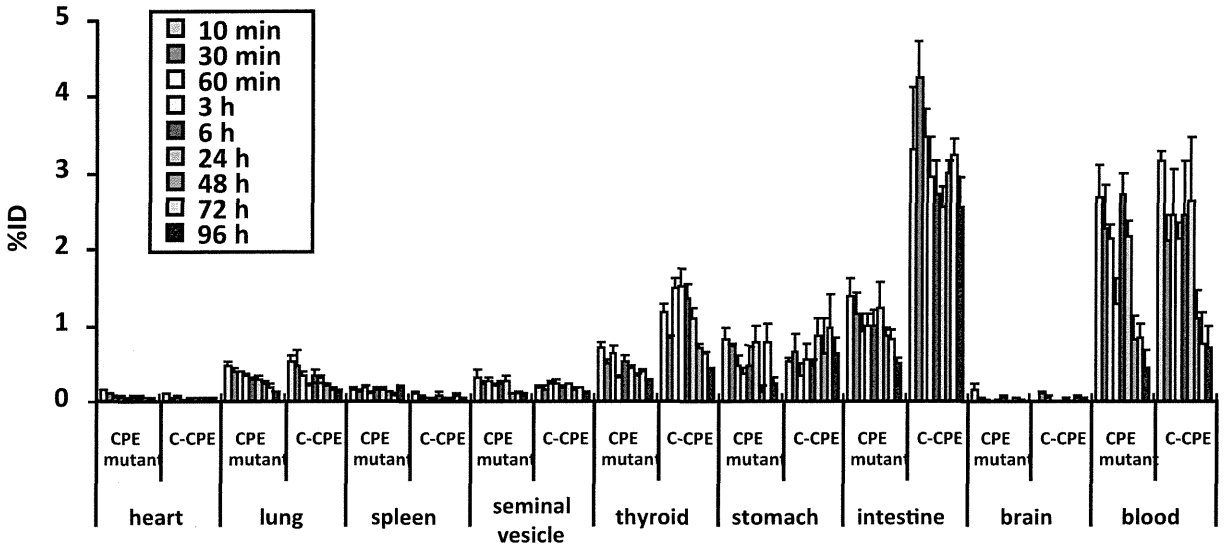
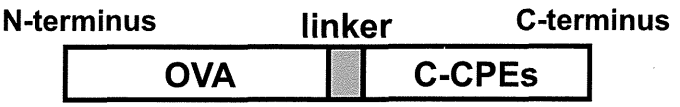


Fig. 18 The distribution of the CF750-C-CPE in mice.
Mice were intravenously injected with 2 μg/mouse CF750-labeled C-CPE or a CF750-labeled C-CPE mutant. Heart, lung, spleen, seminal vesicle, thyroid, stomach, intestine, brain, and blood were removed at the indicated times after injection and the intensity of fluorescence of each tissue was measured. Tissue C-CPE levels were calculated as percentages of injected doses. Data are means ±SEM (n = 5). ID, injected dose.

A



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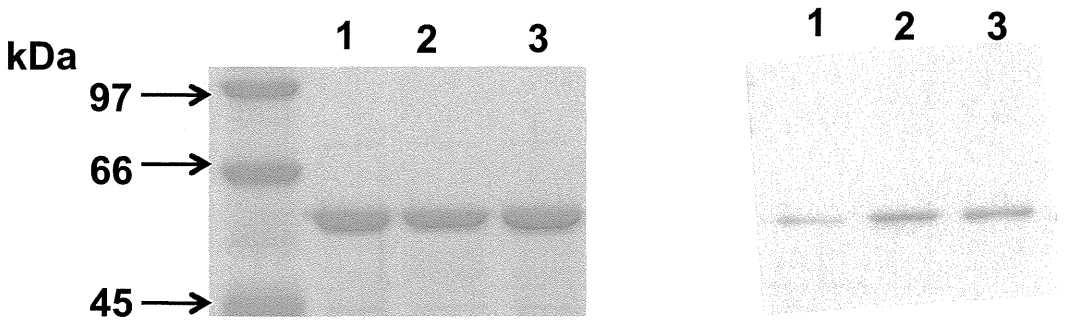

Fig.19 Preparation of OVA-C-CPE mutant.
A) Schematic illustration of OVA-C-CPE mutant. OVA was fused with C-CPE mutant at the N-terminal of C-CPE mutant, resulting in OVA-C-CPE mutant.
B) Purification of OVA-C-CPE mutant. OVA-C-CPE mutant was expressed in *E. coli* and isolated by anti-His tag affinity chromatography. The purification of OVA-C-CPE mutant was confirmed by SDS-PAGE followed by staining with Coomassie Brilliant Blue (CBB) and by immunoblotting with an anti-his-tag antibody. Lane1,OVA-C-CPE 303; lane2, OVA-C-CPE 194; lane3 OVA-C-CPE 194N309A/S313A.

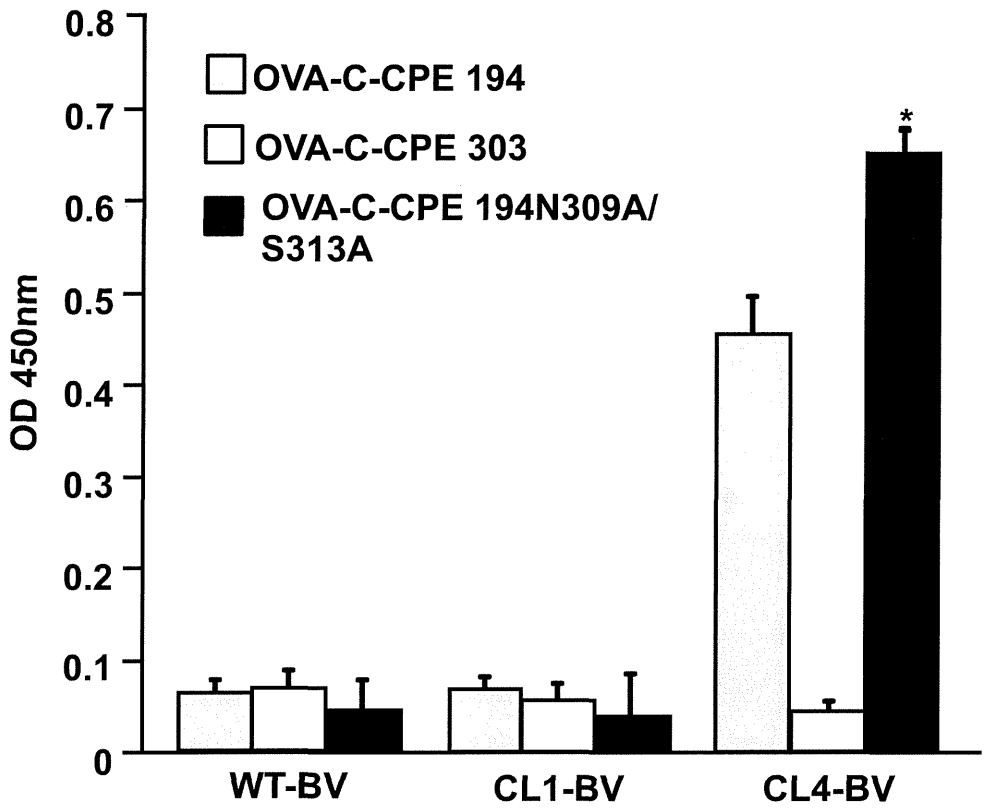

Fig.20 Binding of OVA-C-CPEs to claudin-4 displaying BV.
Wild-type BV (WT-BV), claudin-1 displaying BV (CL1-BV) or -4 (CL4-BV) was absorbed onto immunoplate, and then OVA-C-CPEs were added to the well. OVA-C-CPEs bound to BV was detected by an anti-his-tag antibody followed by goat anti-mouse IgG (H+L)-HRP.
Data are means ± SD (n=4).

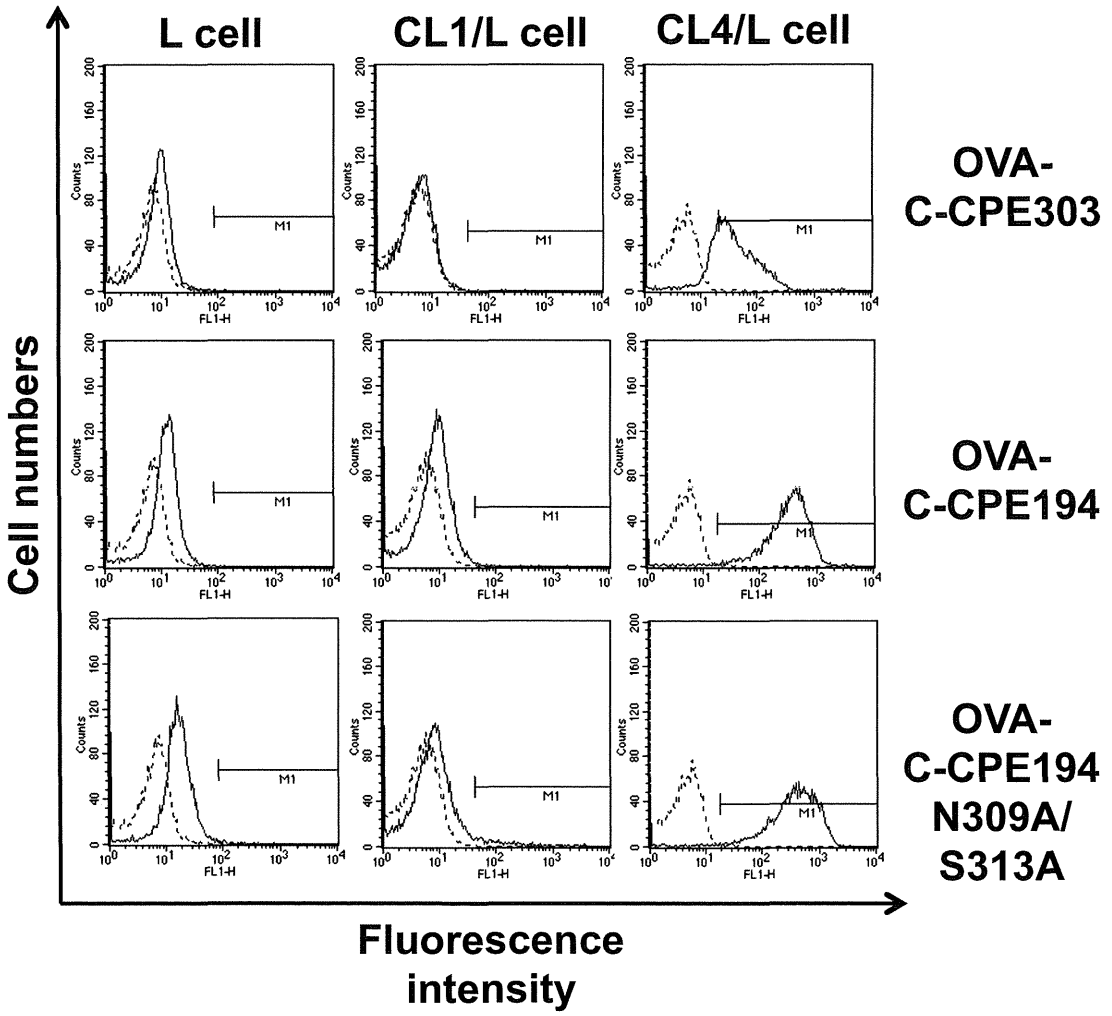

Fig. 21 Binding of OVA-C-CPEs to claudin-4-expressing cell. Claudin expressing L cells were incubated with OVA-C-CPEs followed by anti-his-tag antibody. Cells were stained with FITC-conjugated goat-anti mouse IgG (H+L) and analyzed by flow cytometry (solid line). Dotted line represents control cells incubated with the second and third step regent alone. (n=3)

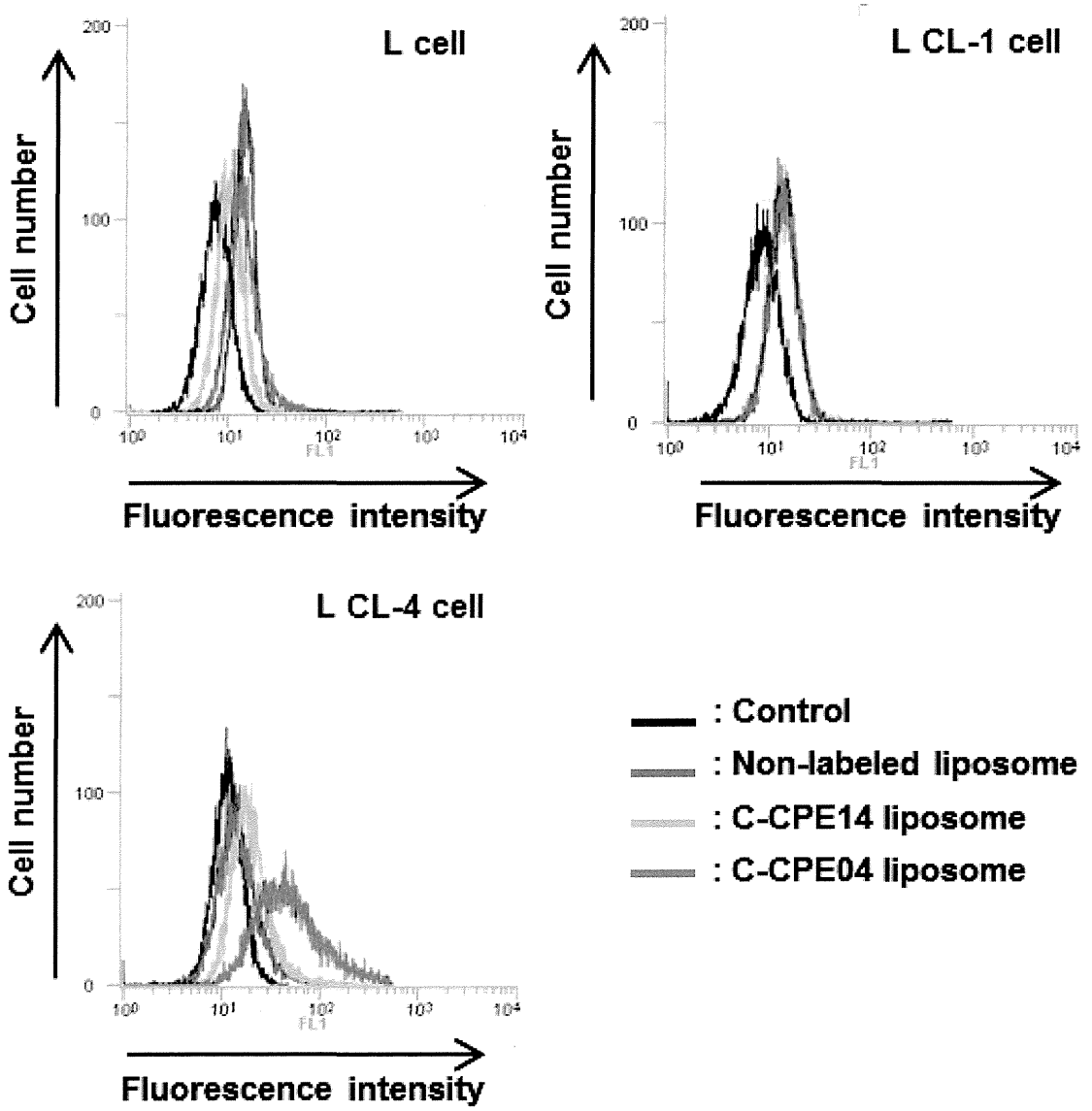

Fig. 22 OVA封入C-CPE修飾リポソームのCL-4発現細胞への結合評価
L, L CL-1, L CL-4細胞にNBD標識した各種OVA封入C-CPE修飾リポソームに添加し、4℃、1時間培養した。その後、細胞の蛍光をフローサイトメトリーにより測定した。

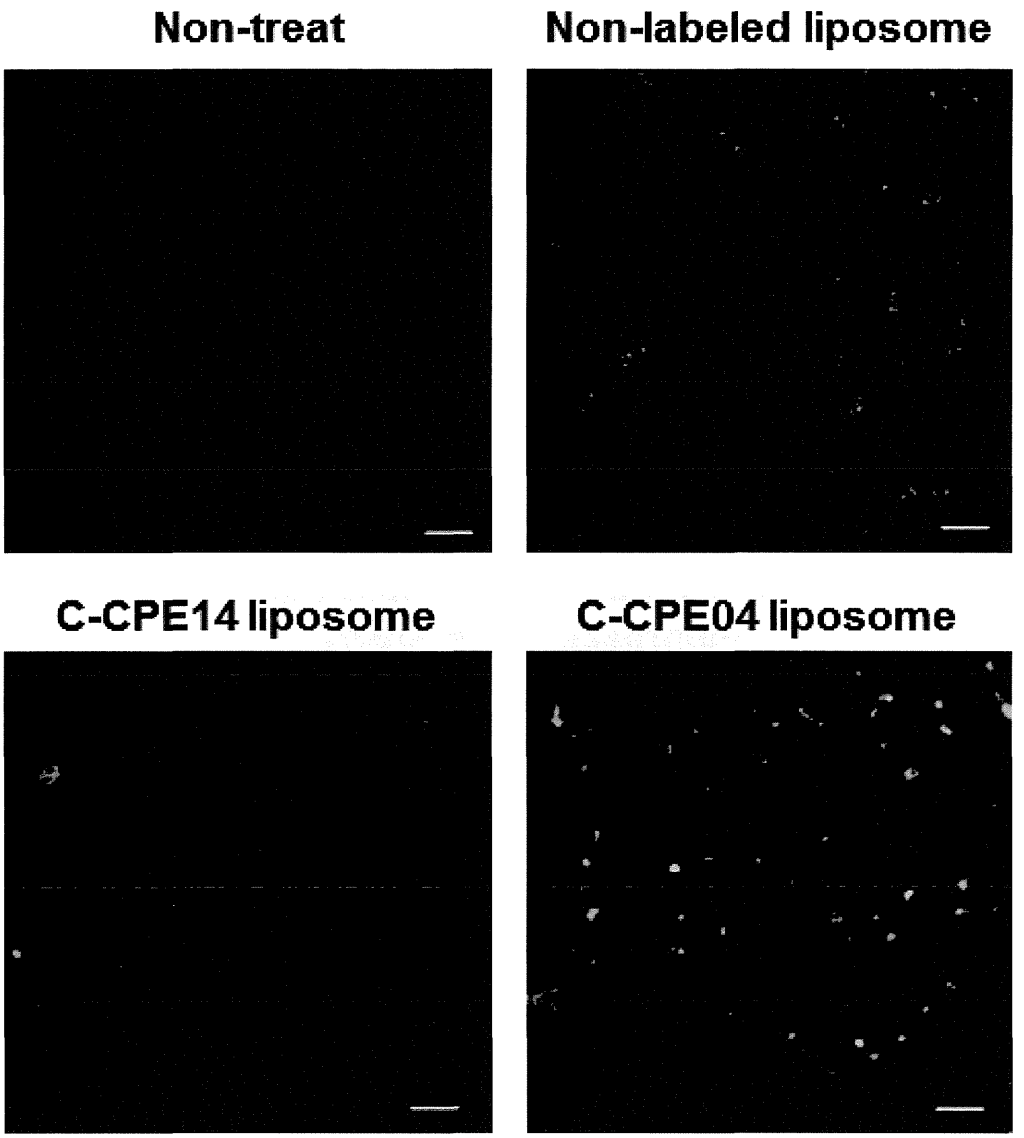

Fig. 23　OVA封入C-CPE修飾リポソームのCL-4発現細胞への結合の観察
L，L CL-1，L CL-4細胞にNBD標識した各種OVA封入C-CPE修飾リポソームに添加し、4℃、1時間培養した。その後、共焦点顕微鏡による観察を行った。
青:DAPI、緑:NBD、Scale bar:20 μm