PLOS ONE

CORRECTION

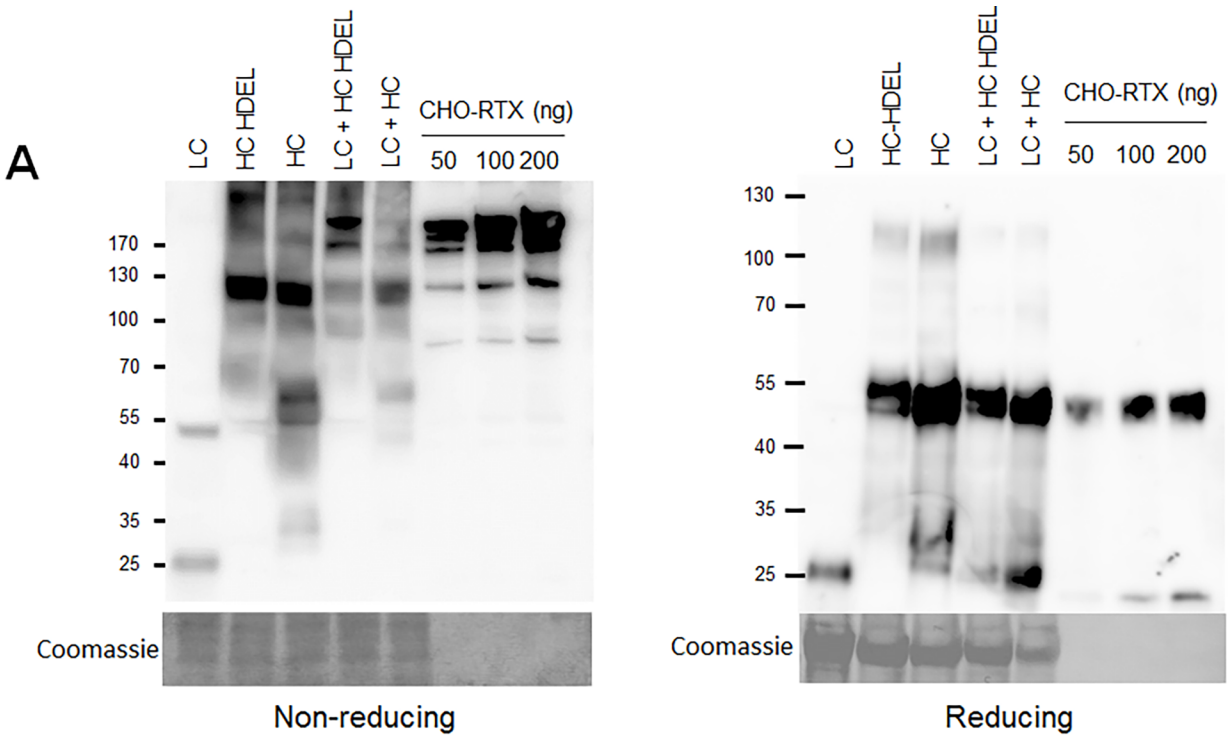
Correction: The B cell death function of obinutuzumab-HDEL produced in plant (*Nicotiana benthamiana* L.) is equivalent to obinutuzumab produced in CHO cells

Jin Won Lee, Woon Heo, Jinu Lee, Narae Jin, Sei Mee Yoon, Ki Youl Park, Eun Yu Kim, Woo Taek Kim, Joo Young Kim

Fig 2, Fig 4, and Fig 5 are incorrect. The authors have provided the corrected versions here.

Citation: Lee JW, Heo W, Lee J, Jin N, Yoon SM, Park KY, et al. (2018) Correction: The B cell death function of obinutuzumab-HDEL produced in plant (*Nicotiana benthamiana* L.) is equivalent to obinutuzumab produced in CHO cells. PLoS ONE 13(4): e0195917. https://doi.org/10.1371/journal.pone.0195917

Published: April 11, 2018



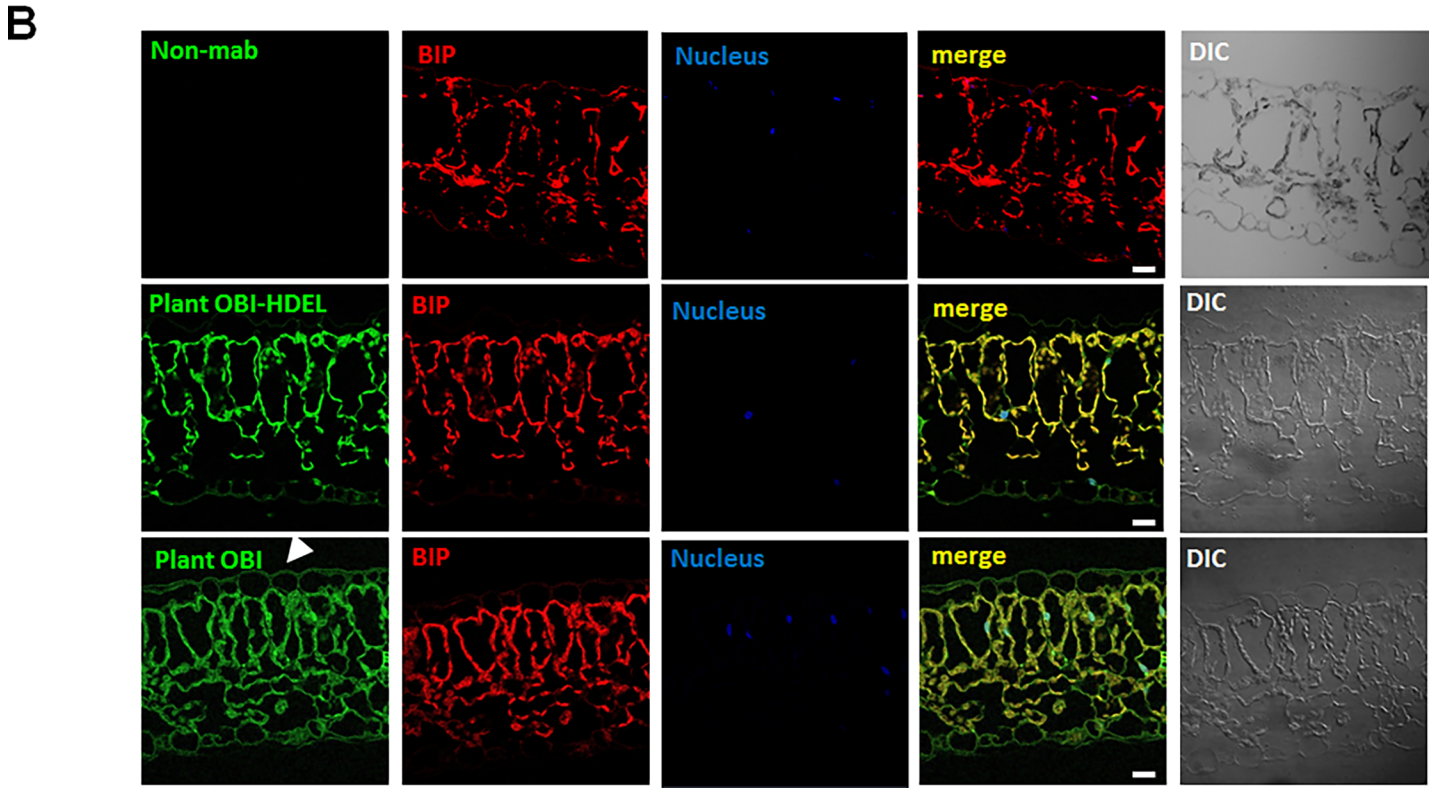


Fig 2. Comparison of the integrity and expression of light and heavy chains with or without HDEL. (A) Total protein extracts were subjected to PAGE under non-reducing and reducing conditions. Immunoblot was performed using HRP-conjugated human Ig Fc-specific antibody and HRP-conjugated mouse Ig Fc-specific antibody to detect both chains. CHO-rituximab (50 ng, 100 ng, and 200 ng) was used as the standard. Coomassie-stained gel images were used to show equivalent loading of proteins. (B) Comparison of localization and expression between plant-obinutuzumab-HDEL and plant-obinutuzumab from N. benthamiana leaves. Immunohistochemistry was performed to detect the localisation of plant-obinutuzumab-HDEL and plant-obinutuzumab in N. benthamiana leaves. Formalin-fixed and

paraffinised N. benthamiana leaves expressing plant-obinutuzumab-HDEL and plant-obinutuzumab were sectioned at 10 μm thickness and immunostained. Fluorescein isothiocyanate (FITC; green)-conjugated anti-human Ig Fc-specific 2nd antibody was used for detection. BiP protein fused with Ds-RED (red) was used to indicate the localisation of ER in N. benthamiana leaves. DraQ was used to indicate the nucleus (blue). Bar: 20 μm.

https://doi.org/10.1371/journal.pone.0195917.g001

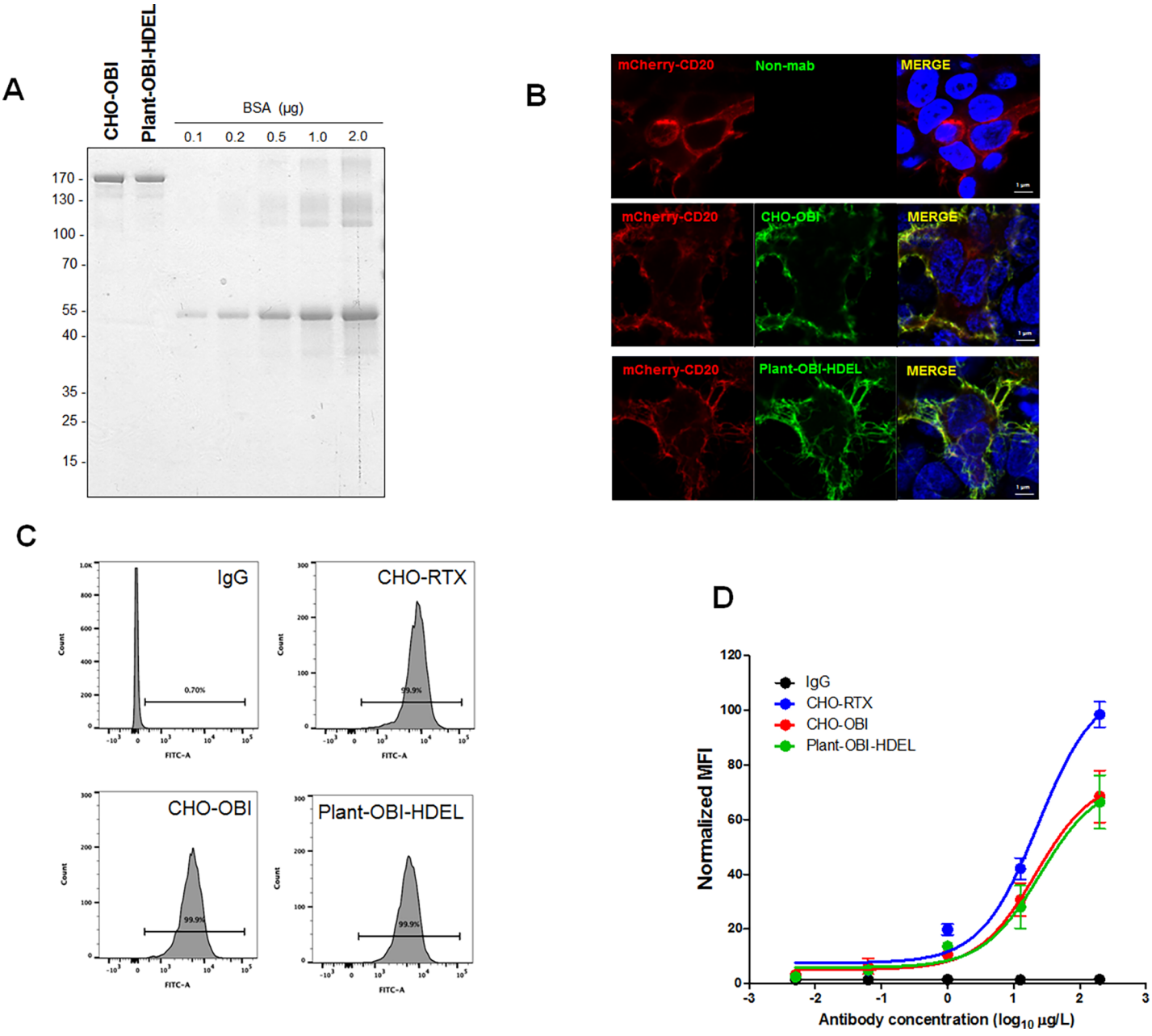
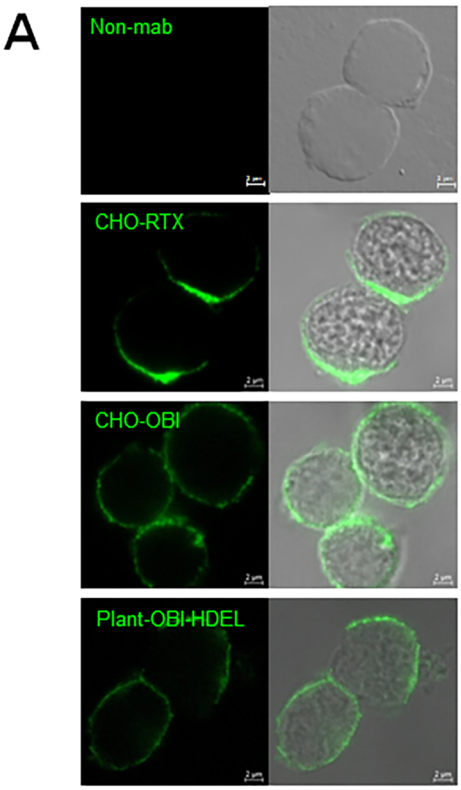
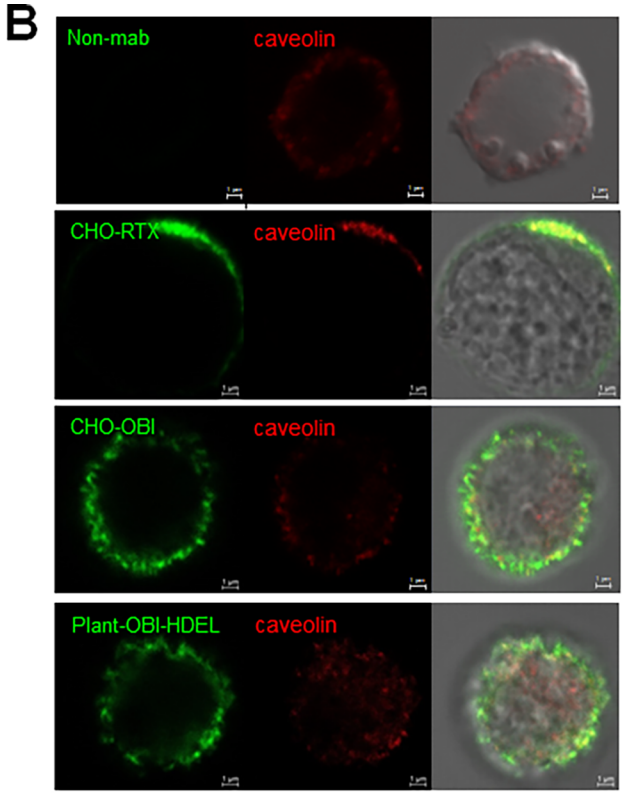
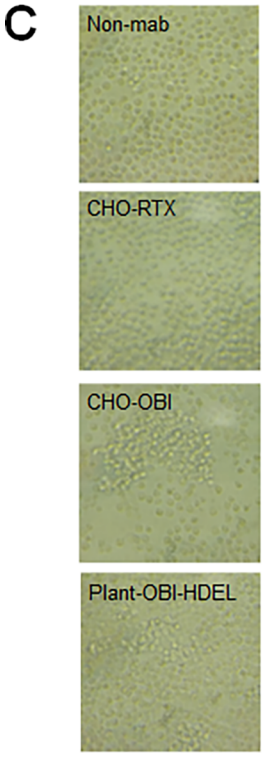


Fig 4. Specificity and affinity of epitope binding by plant-obinutuzumab-HDEL compared to CHO-obinutuzumab. (A) Antibody protein concentrations were measured via BCA method and quantification of PAGE gel analysis with Coomassie blue staining. To validate the concentration of each antibody, 1 μg of antibodies was subjected to PAGE under non-reducing conditions with BSA (0.1, 0.2, 0.5, 1, 2 μg) as the standard. Gels were then stained with Coomassie blue. (B) Specific epitope recognition by plant-obinutuzumab-HDEL was tested by immunocytochemistry. mCherry-tagged CD20 was expressed in HEK cells. CHO-obinutuzumab and plant-obinutuzumab-HDEL were used for immunocytochemistry with FITC-conjugated human Fc-specific secondary antibodies. Bar 1 μm. C. Representative FACS images for affinity comparison with 10 μg/ml antibodies. (C, D) Dose-dependent binding capacity of CHO-rituximab, plant-obinutuzumab-HDEL and CHO-obinutuzumab (1 ng/ml, 10 ng/ml, 100 ng/ml, 1 μg/ml, and 10 μg/ml) using flow cytometry. Representative FACS images of binding affinity with 10 μg/ml antibodies are shown in C. FITC intensities of 10 μg/ml of each antibody bound to cells are depicted by normalized mean fluorescence intensity (MFI). Results of triplicate assays are summarised in D.

https://doi.org/10.1371/journal.pone.0195917.g002


A
Non-mab
CHO-RTX
CHO-OBI
Plant-OBI-HDEL


B
Non-mab
caveolin
CHO-RTX
caveolin
CHO-OBI
caveolin
Plant-OBI-HDEL
caveolin


C
Non-mab
CHO-RTX
CHO-OBI
Plant-OBI-HDEL

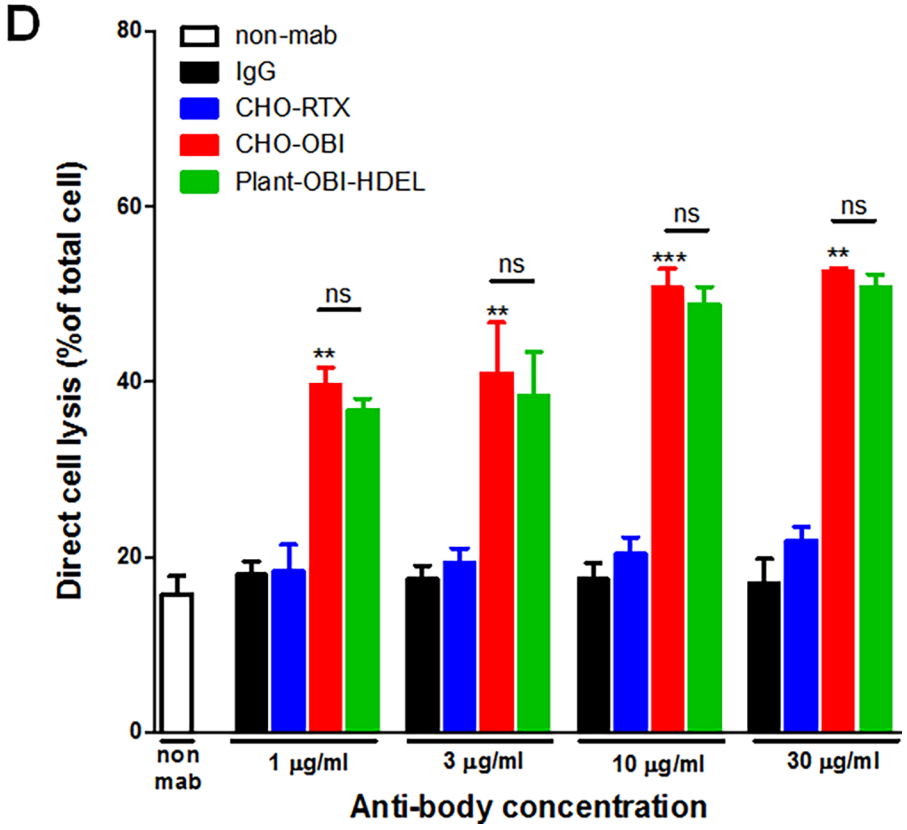

D
non-mab
IgG
CHO-RTX
CHO-OBI
Plant-OBI-HDEL
Direct cell lysis (%of total cell)
80
60
40
20
0
ns
**
ns
**
ns
***
ns
**
non mab
1 µg/ml
3 µg/ml
10 µg/ml
30 µg/ml
Anti-body concentration

Fig 5. plant-obinutuzumab-HDEL binding to Ramos cells showed typical type II CD20 antibody characteristics, similar to CHO-obinutuzumab. (A) Two-panel photomicrograph showing binding of the antibodies to CD20 localized on the surface of Ramos cells. Binding was visualised with FITC-conjugated human Fc-specific secondary antibody. Bar, 2 μm. (B) In addition to the same antibodies used in A, caveolin was stained with anti-caveolin antibody and Alexa 568-conjugated secondary antibody. Merged DIC image shows CD20 and caveolin co-localised on the surface of the Ramos cell. Bar, 1 μm. (C) Photomicrograph showing cell aggregation (homotypic adhesion: HA) 30 minutes after treatment with each antibody. (D) Direct binding-induced cell death caused by CHO-obinutuzumab and plant-obinutuzumab-HDEL were compared to IgG and CHO-rituximab. Each antibody (at 1 μg/ml, 10 μg/ml, and 30 μg/ml) was incubated with Ramos cells for 14 hours and cell death was measured by lose of calcein-AM dye via FACS analysis. Three independent experiments are shown as means ± s.e.m. $^{**}P < 0.01$; $^{***}P < 0.001$.

https://doi.org/10.1371/journal.pone.0195917.g003

Reference

1. Lee JW, Heo W, Lee J, Jin N, Yoon SM, Park KY, et al. (2018) The B cell death function of obinutuzumab-HDEL produced in plant (Nicotiana benthamiana L.) is equivalent to obinutuzumab produced in CHO cells. PLoS ONE 13(1): e0191075. https://doi.org/10.1371/journal.pone.0191075 PMID: 29324849