

## **SUPPLEMENTARY FILE**

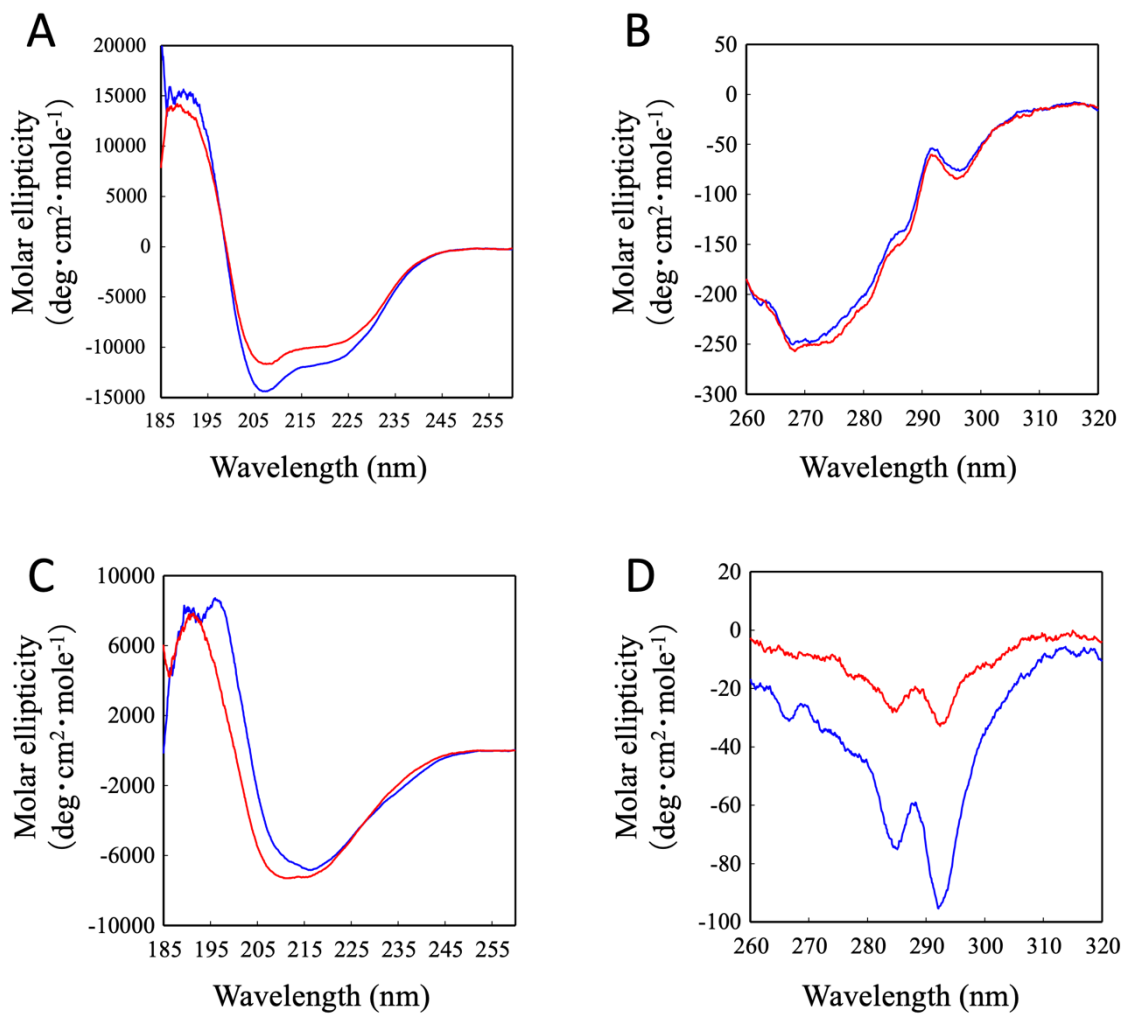
### **Two Major Bovine Milk Whey Proteins Induce Distinct Responses in IEC-6**

#### **Intestinal Cells**

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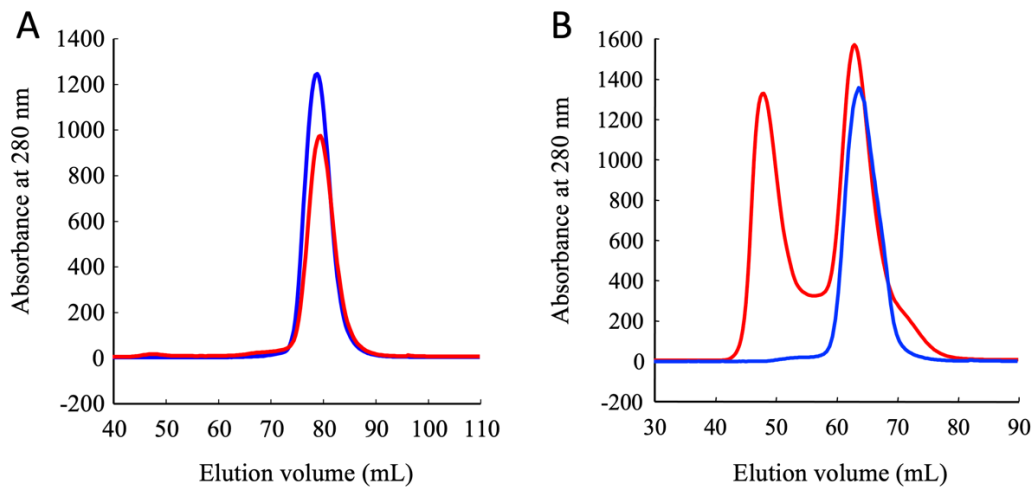
#### **Supplementary Materials and Methods**

Purification and 2,2,2-trifluoroethanol (TFE) treatment of native whey proteins was performed as described in the main manuscript. Conformational changes were evaluated by circular dichroism (CD) spectra using a JASCO J-820 CD spectrometer (JASCO, Tokyo, Japan) as previously described by Inagaki *et al.* (2017). The molecular sizes of native and TFE-treated whey proteins were analysed with high-performance liquid chromatography using a HiLoad 16/60 Superdex 75 prep grade column (GE Healthcare, Chalfont St. Giles, UK) as previously described by Xu *et al.* (2005).



### Supplementary Fig. S1

CD spectra of whey proteins measured in the far ultraviolet (UV) (A and C) and near UV (B and D) regions. CD spectra were recorded in 20 mM sodium phosphate buffer, pH 6.5, at 0.3 mg/mL (A and B,  $\alpha$ -LA; C and D,  $\beta$ -LG): native protein (blue lines) and the TFE-treated protein (red lines).



### Supplementary Fig. S2

Comparison of molecular sizes between native and TFE-treated whey proteins (A,  $\alpha$ -LA; B,  $\beta$ -LG). Size exclusion chromatography of native and TFE-treated whey proteins using a Superdex 75 prep grade column: native protein (blue lines) and the TFE-treated protein (red lines).

### References

Inagaki M, Kawai S, Xijier, Fukuoka M, Yabe T, Iwamoto S and Kanamaru Y (2017)

Effects of heat treatment on conformation and cell growth activity of alpha-lactalbumin and beta-lactoglobulin from market milk. *Biomedical Research (Tokyo, Japan)* **38**, 53-59.

Xu M, Sugiura Y, Nagaoka S and Kanamaru Y (2005) IEC-6 intestinal cell death induced by bovine milk  $\alpha$ -lactalbumin. *Bioscience, Biotechnology, and Biochemistry* **69**, 1082-1089.