Supplementary Materials: In vitro Evaluation of The Effects of Cadmium on Endocytic Uptakes of Proteins into Cultured Proximal Tubule Epithelial Cells

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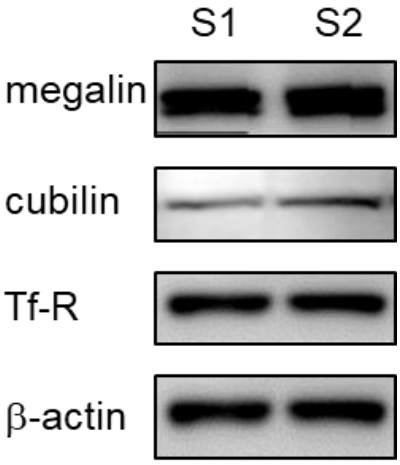

Figure S1. Expression levels of the proteins involved in endocytosis in S1 and S2 cells. Whole cell lysates of S1 and S2 cells were subjected to Western blot analysis. β-actin was used as a loading control. The expression levels of megalin, cubilin, and transferrin receptor (TfR) were almost the same between S1 and S2 cells.

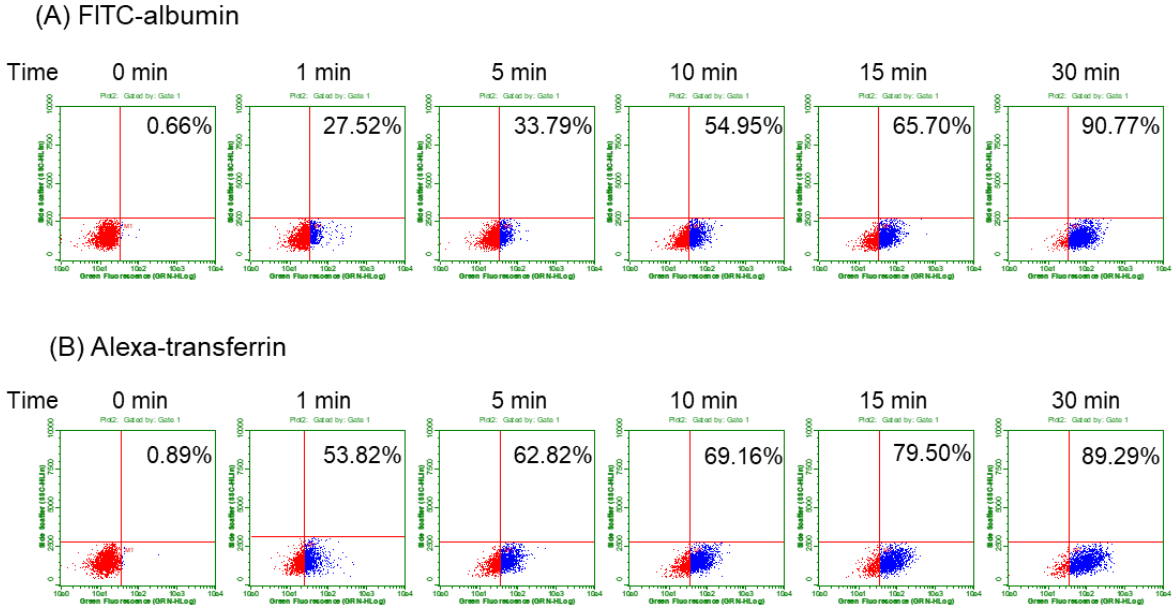

Figure S2. Time-dependent changes in endocytic uptakes of FITC-albumin (**A**) and Alexa-transferrin (**B**). S1 cells were incubated with fluorescent-labeled proteins for 1, 5, 10, 15, and 30 min. The percentages of fluorescent-positive cells were calculated in the same way as in Figure 2. Typical quadrant data were shown here. The cell populations of both proteins in the lower-right, indicative of endocytic uptakes, increased in a time-dependent manner.

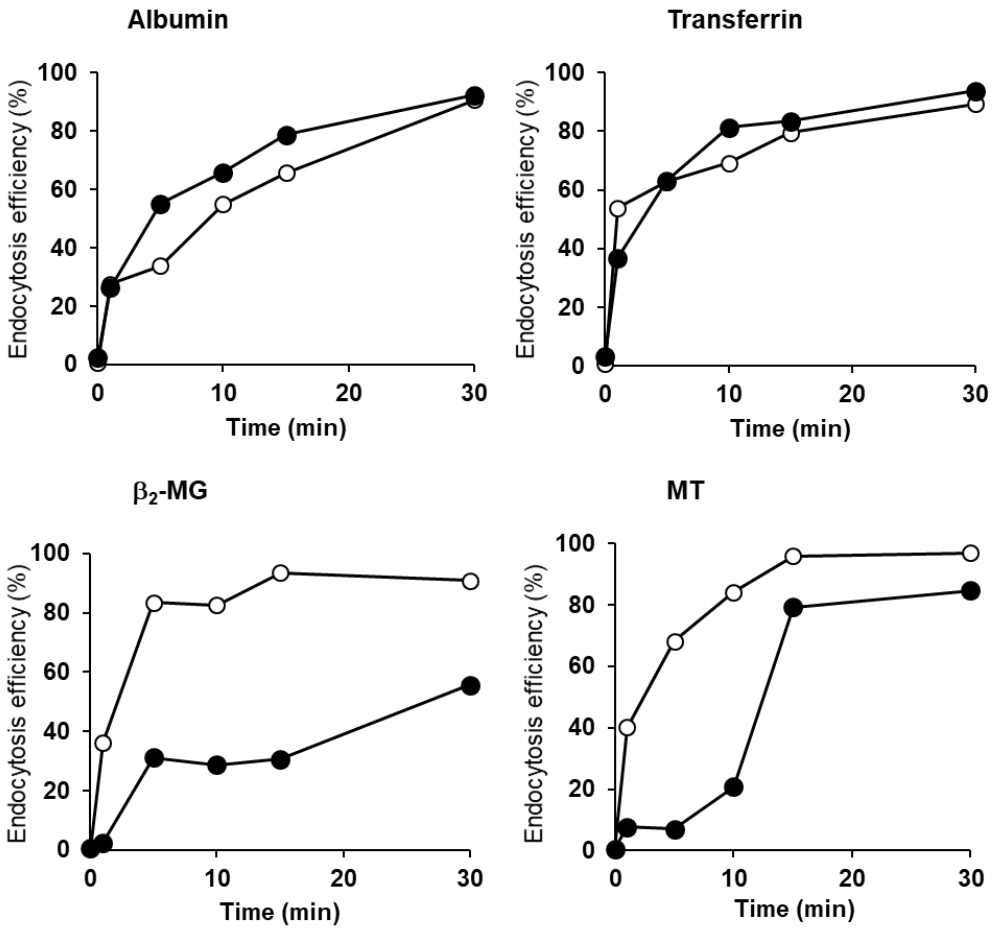

Figure S3. Time-dependent changes in endocytic uptakes of albumin, transferrin, β_2-MG, and MT. S1 and S2 cells were incubated with fluorescent-labeled proteins for 1, 5, 10, 15, and 30 min. The fluorescence-positive cells were counted by flow cytometry and expressed as percentages of the total cells. Open and closed circles represent S1 and S2 cells, respectively (n = 1–2).

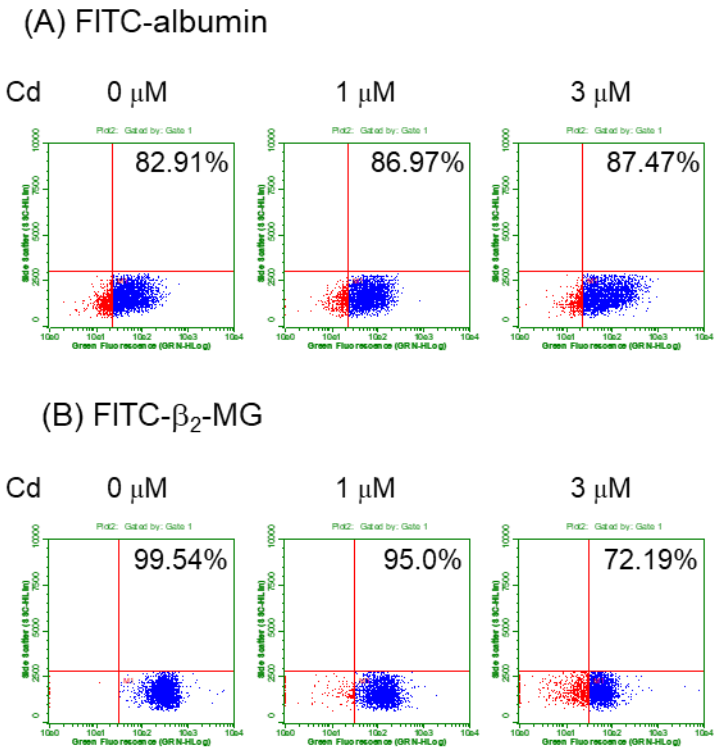

Figure S4. Effects of Cd on the endocytosis efficiencies of albumin and β2-MG into S1 cells. S1 cells were exposed to $CdCl_2$ for 3 days (1 or 3 μM), washed, harvested, and then incubated with FITC-albumin (**A**) or FITC-β2-MG (B) for 30 min. The percentages of fluorescent-positive cells were calculated in the same way as in Figure 2. Typical quadrant data were shown here. The cell populations of β2-MG (**B**), but not those of albumin (A), in the lower-right, indicative of endocytic uptakes, were decreased by exposure to Cd at 3 μM for 3 days.