

Supplementary data

Effects of autophagy-inhibiting chemicals on sialylation of Fc-fusion glycoprotein in recombinant CHO cells

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Running title: Effect of autophagy inhibition on sialylation in rCHO cells

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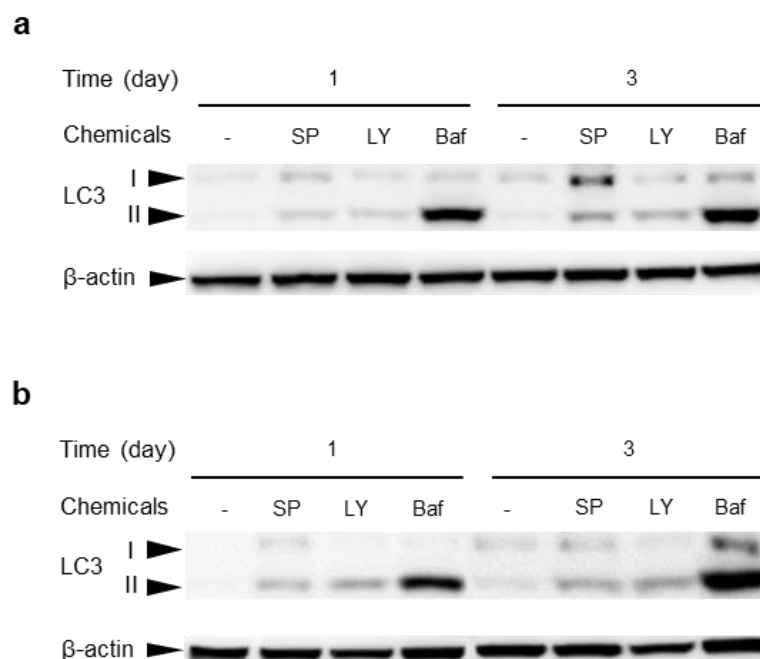


Fig. S1 Western blot analysis of the total cellular proteins from cultures in the absence and presence of three autophagy inhibitors in **a** DUKX-Fc cells and **b** DG44-Fc cells. Without autophagy inhibitor (-), with SP600125 (SP), with LY294002 (LY), and with BafA1 (Baf). An equal amount of cellular proteins from cell lysates at days 1 and 3 was separated on a 12% Bis-Tris NuPAGE gel.

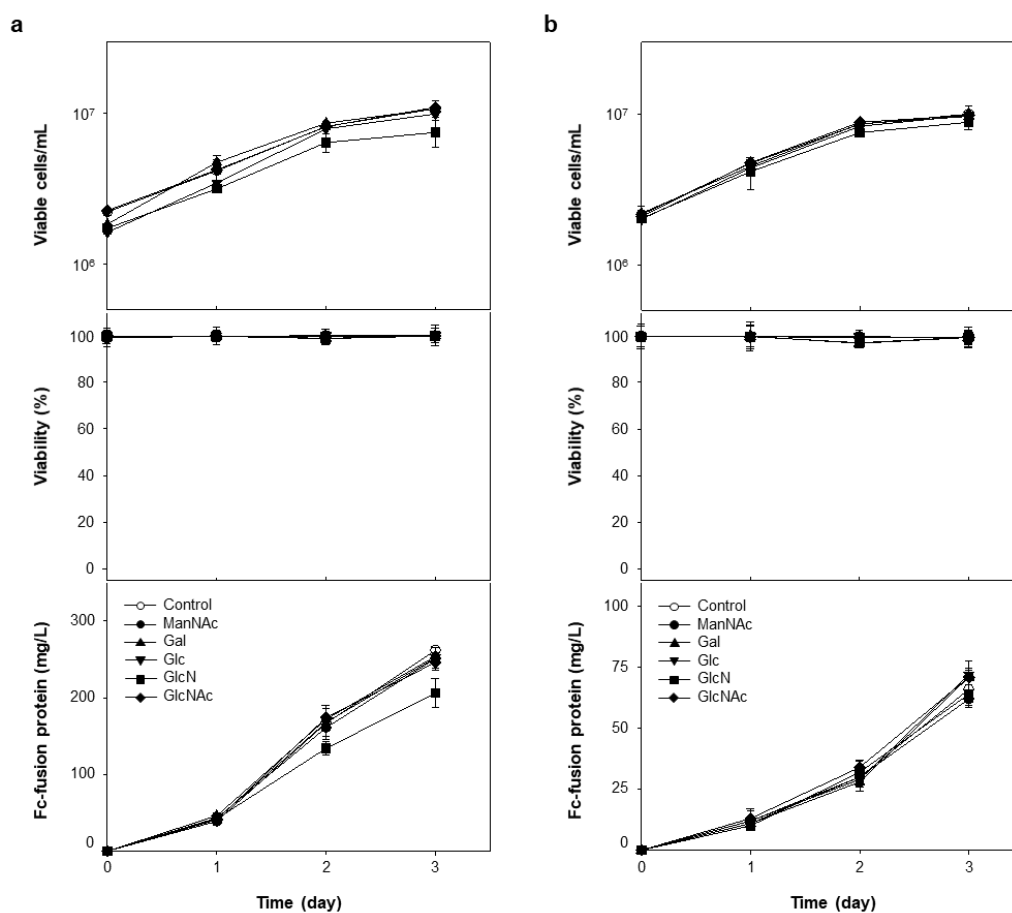


Fig. S2 Profiles of cell growth, viability, and Fc-fusion glycoprotein production of two rCHO cell lines individually supplemented with five nucleotide sugar precursors. **a** DUKX-Fc cells and **b** DG44-Fc cells. Without nucleotide sugar precursor (open circle), with ManNAc (closed circle), with Gal (closed up-triangle), with Glc (closed down-triangle), with GlcN (closed square), and with GlcNAc (closed diamond). Error bars represent the standard deviations of triplicate experiments

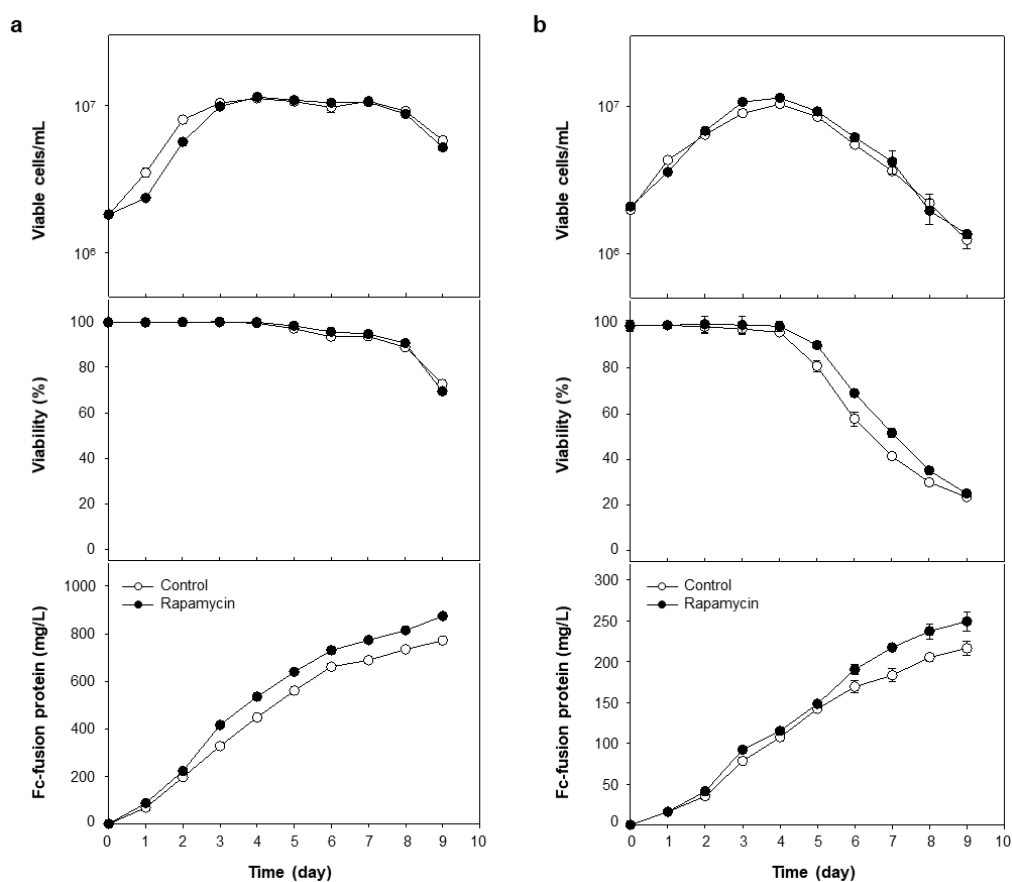


Fig. S3 Profiles of cell growth, viability, and Fc-fusion glycoprotein production of two rCHO cell lines in the absence and presence of rapamycin, a typical autophagy inducer. **a** DUKX-Fc cells and **b** DG44-Fc cells. Without rapamycin (open circle) and with 200 nM rapamycin (closed circle). Error bars represent the standard deviations of triplicate experiments

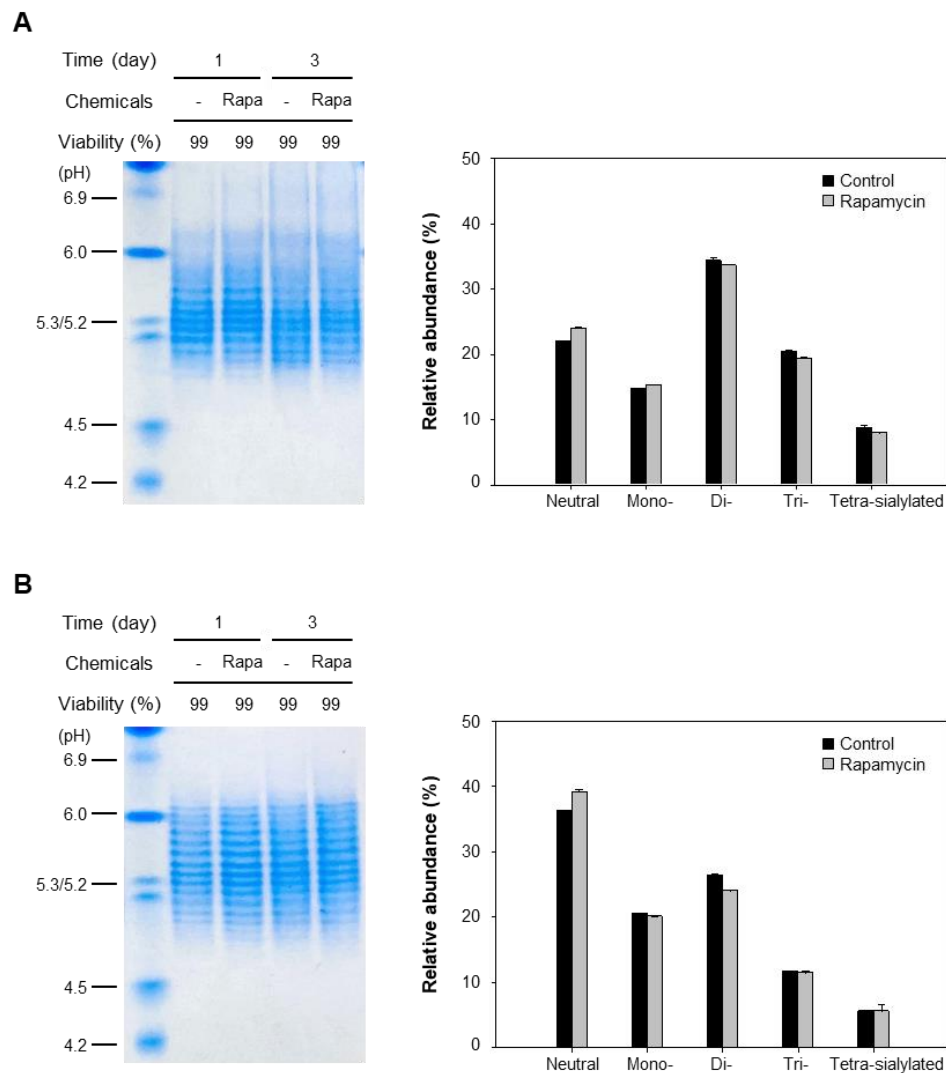


Fig. S4 Isoelectric focusing (IEF) gel analysis and sialylated *N*-glycan profiles obtained from purified Fc-fusion glycoprotein produced in the absence (- and black bar) and presence (Rapa and gray bar) of rapamycin in **a** DUKX-Fc cells and **b** DG44-Fc cells. Samples were taken on days 1 and 3 of the cultures, as shown in Fig. S2. IEF gel analysis was performed in triplicate experiments. Error bars in sialylated *N*-glycan analysis represent standard deviations calculated from triplicate experiments