

<Figure S1>

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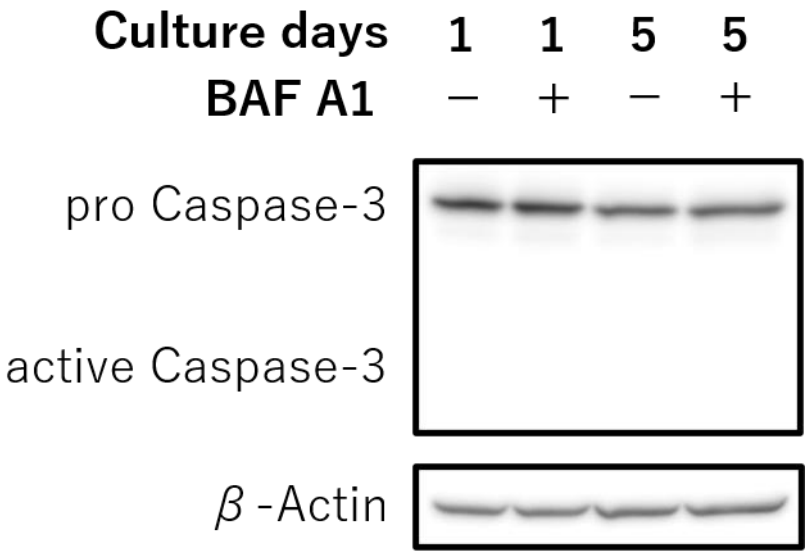


Figure S1A: Evaluation of Caspase-3 in PHT with BAF A1 during syncytialization

Primary human trophoblasts (PHT) were cultured for 1- and 5-day to induce syncytialization. The cells were treated with or without Bafilomycin A1 (BAF A1, 20nM) for 24 hours to confirm apoptosis. The protein levels were detected by Western blotting.

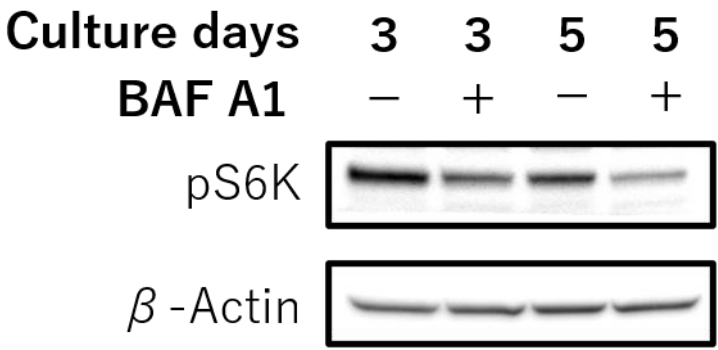


Figure S1B: Reduction of phosphorylated ribosomal protein (pS6K) in primary human trophoblast (PHT) during syncytialization. PHT was cultured for 3 and 5 days to induce syncytialization. The cells were treated with Bafilomycin A1 (BAF A1, 20nM) for 24 hours.

<Figure S2>

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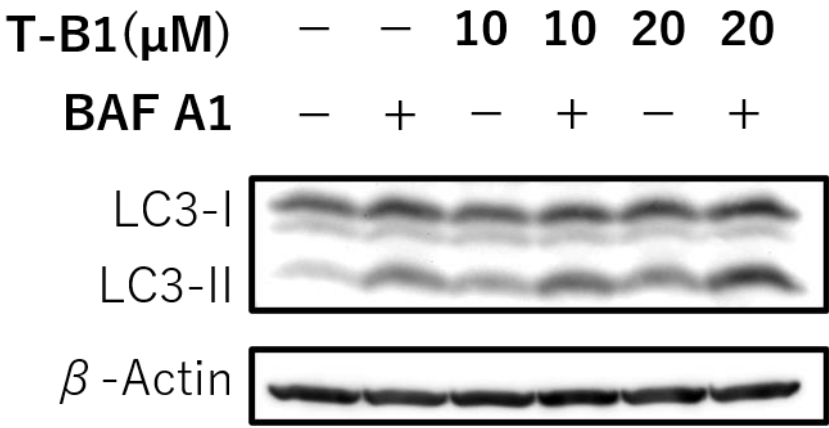


Figure S2A: Autophagy flux in BeWo cells.

The autophagy activator, Tat-Becn1 (T-B1, 10 μ M, 20 μ M), was administered to evaluate the activation of autophagy in BeWo cells. LC3 was evaluated to assess autophagy flux in the presence of Bafilomycin A1 (BAF A1, 20nM).

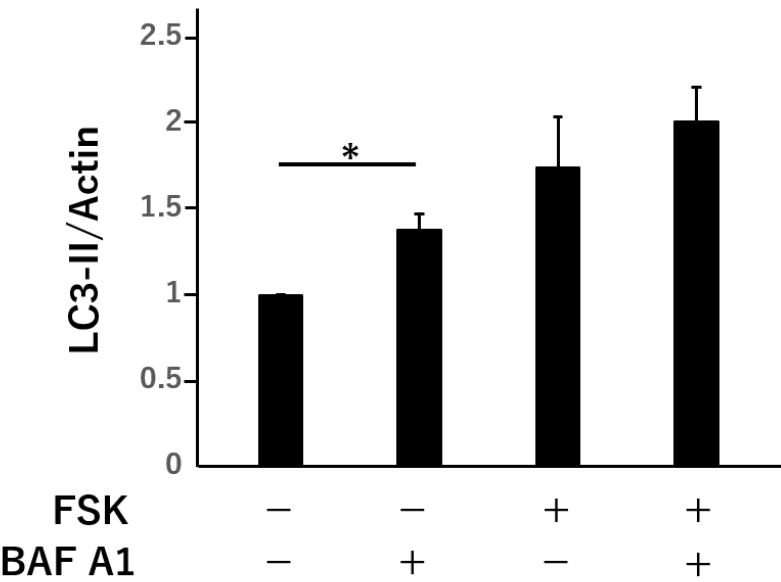


Figure S2B: Autophagy flux in BeWo cells during syncytialization

BeWo cells were treated with Forskolin (FSK, 25 μ M) to induce syncytialization. LC3 was evaluated to assess autophagy flux in the presence of Bafilomycin A1 (BAF A1 20nM). The bar graphs show the quantified protein levels of LC3.

<Figure S3>

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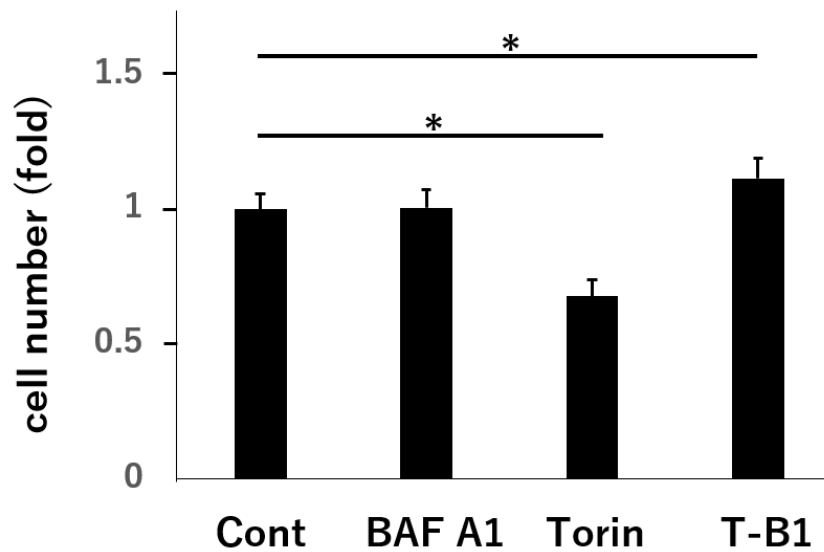


Figure S3A: Cell number in BeWo cells with the autophagy-modulators

Cell proliferation assay was performed on BeWo cells cultured with BAF A1 20nM, Torin 10 μ M, and T-B1 20 μ M in the presence of Forskolin 25 μ M. The graphs showed the cell viability with each treatment. The graphs were obtained from six independent results. Data expressed as mean \pm S.D. A significance test was performed: *P<0.05.

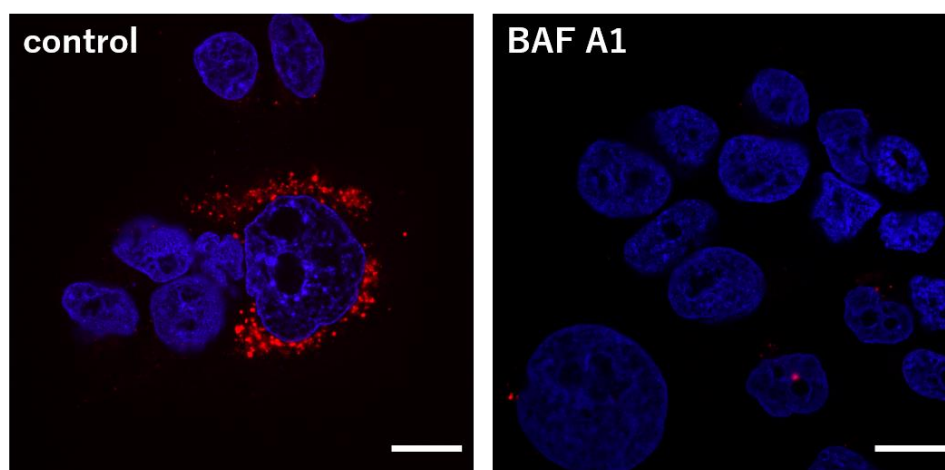


Fig S3B: BAF A1 affects acidic organelles in BeWo cells

LysoTracker was used to evaluate the acidic organelles in BeWo cells with BAF A1 20nM treatment for 24 hours. The acidic organelles, which were observed in the perinuclear area at steady, were not detected in BeWo cells with BAF A1. Scale bar: 20 μ m.

<Figure S4>

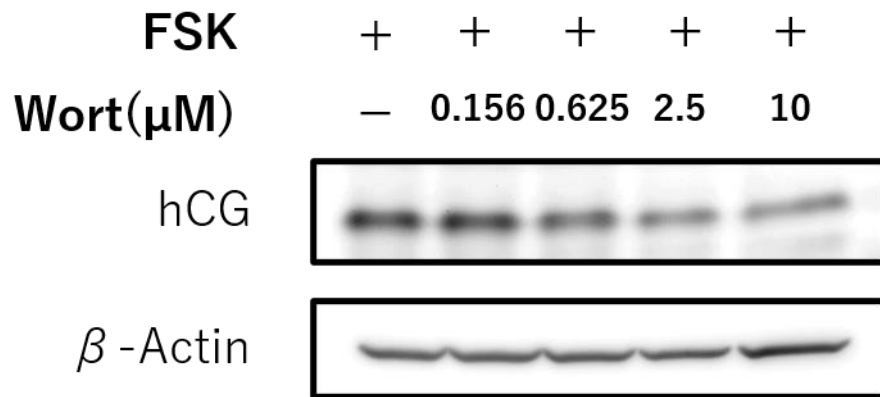


Figure S4 : Wortmannin inhibited hCG production during syncytialization in BeWo cells.

Wortmannin inhibits the generation of autophagosomes. The hCG production was evaluated in BeWo cells with the indicated concentrations of Wort in the presence of FSK 25 μ M.

<Figure S5>

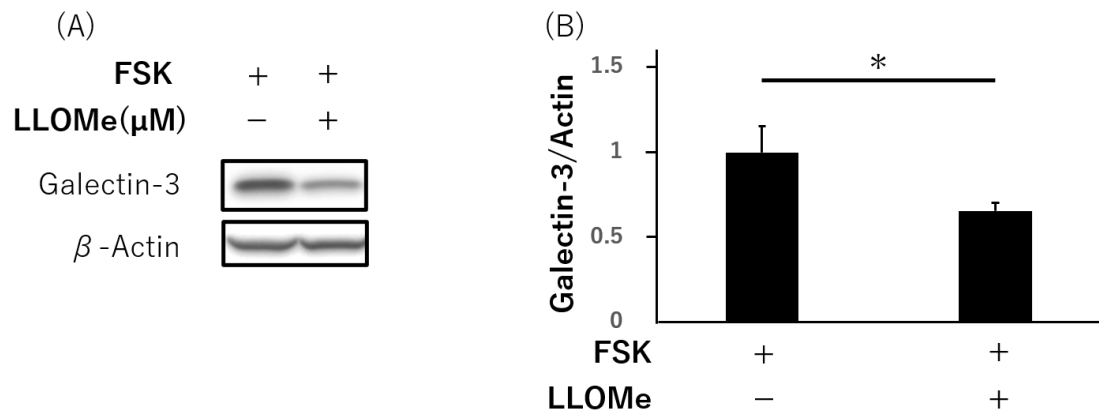


Figure S5: LLOMe downregulated Galectin-3 expression in BeWo cells.

Galectin-3 is a marker of damaged lysosomes engulfed by autophagosomes. Galectin-3 expression was evaluated in BeWo cells with LLOMe 1000 μ M in the presence of FSK 25 μ M (A). The graphs indicated the ratio of Galectin-3 to Actin in BeWo cells. This was made from the three independent results. A significance test was performed: *P<0.05.