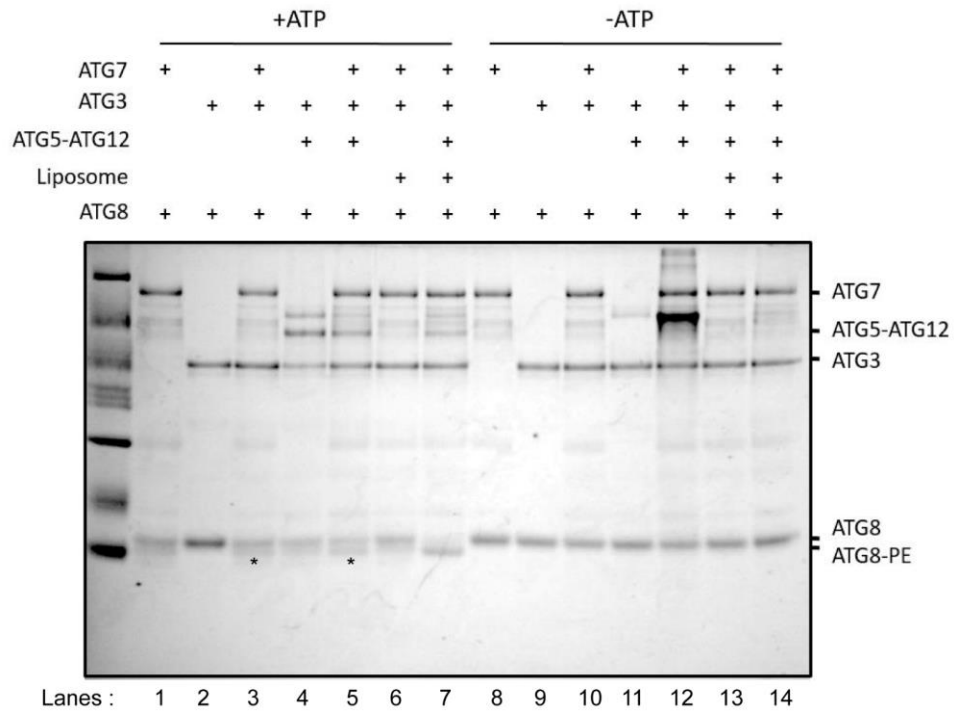


## **Supplementary materials**

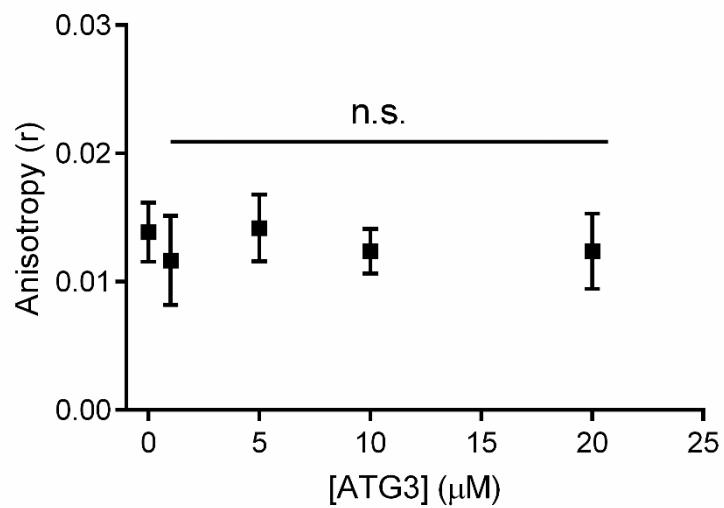
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**Supplementary Figure S1. Atg8 intermediates were observed in the absence of liposome.**

**Supplementary Figure S2. Atg3 displays no interaction with Alexa488-SN2.**



**Supplementary Figure S1. Atg8 intermediates were observed in the absence of liposome.** Shown is the example of *in vitro* Atg8 lipidation assay with various condition as indicated on the top of the chart. Asterisks indicate potential Atg8 intermediates in lanes 3 and 5.



**Supplementary Figure S2. Atg3 displays no interaction with Alexa488-SN2.** The interaction between Atg3 and Alexa488-SN2 was measured by fluorescent anisotropy. 0.2 μM Alexa488-SN2 was incubated with different concentrate of Atg3 as indicated. The excitation and emission wavelengths were set to 485 nm and 520 nm, respectively. Data are presented as means  $\pm$  s.e.m..One-way ANOVA was used for statistical analysis. n.s., not significant (n = 3).