

Figure S1 Localization of HA-Atg5 during the early stage of sexual reproduction of *Tetrahymena thermophila*. Cells collected after 2 and 4 h of mixing were fixed and processed for immunofluorescence staining with anti-HA primary and FITC-conjugated secondary antibodies. Cellular nuclei were stained with DAPI to visualize DNA. Fluorescent images were taken with a DeltaVision deconvolution microscope. Scale bar, 10  $\mu$ m.

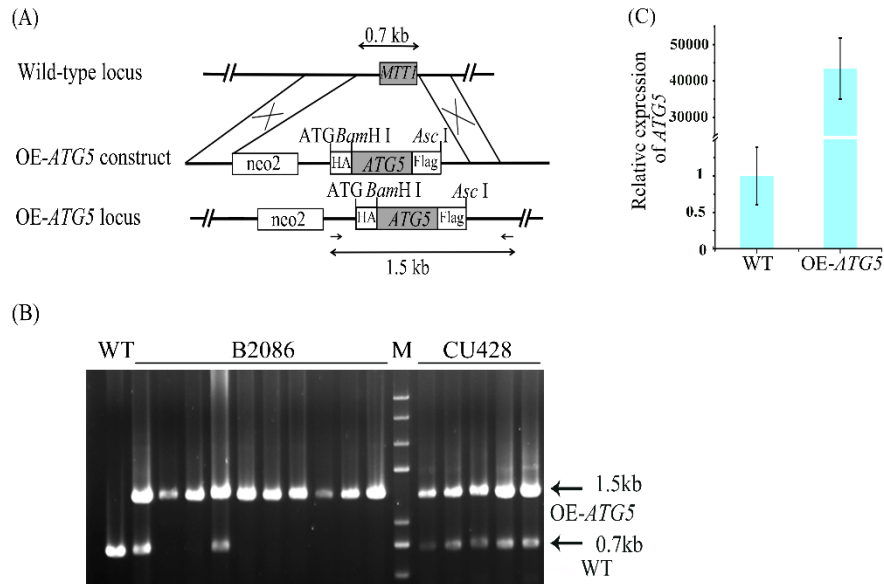


Figure S2 Construction and identification of *Tetrahymena* strains overexpressing *ATG5*. A: diagram of the *ATG5* construct at the *MTT1* locus. B: PCR identification of *ATG5*-overexpressing mutants. Arrowheads indicate positive recombinant fragments (1.5 Kb) and WT fragments (0.7 Kb), respectively. M: Trans 2K plus DNA marker;

WT: wild type; OE-*ATG5*-B/C: *ATG5*-overexpressing mutants of B2086 or CU428; C: expression level of *ATG5* was analyzed by qRT-PCR. Total RNA was isolated from conjugating cells after mixing for 8 h.

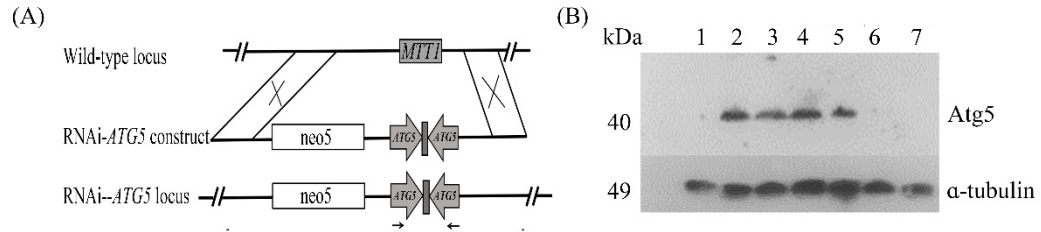


Figure S3 Construction of *ATG5* RNAi knockdown cell lines and Western blot analysis. A: diagram of the *ATG5* RNAi knockdown construct and the wild-type *ATG5* locus. B: The expression levels of HA-Atg5 protein in conjugated cells were detected using Western blot.  $\alpha$ -Tubulin served as the internal reference. Lanes 1: Wild type; Lanes 2–4: Samples were collected at 6, 8, and 10 h after wild-type cells were paired with the OE-*ATG5* mutants; Lanes 5–7: Samples were collected at 6, 8, and 10 h after *atg5i* mutants were paired with the OE-*ATG5* mutants.

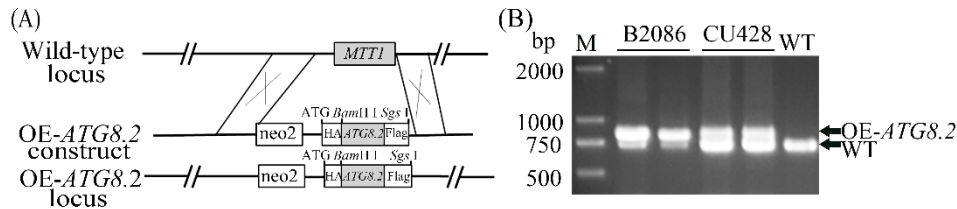


Figure S4 Construction and identification of *Tetrahymena* strains overexpressing *ATG8.2*. A: diagram of the *ATG8.2* construct at the *MTT1* locus. B: PCR identification of *ATG8.2* overexpressing mutants. Arrowheads indicate positive recombinant fragments (0.85 Kb) and WT fragments (0.7 Kb), respectively. M: Trans 2K plus DNA marker; WT: wild type.

**Table S1 Primers used in the study**

Primer name	Sequence ( 5'→3')
OE- <i>ATG5</i> -F:	GGATCC GCAGAGGAACAAAAATAAACAGATTTTG
OE- <i>ATG5</i> -R:	GGCGCGCCTTTTTATATACATAGATAAGTGAAGCCAT
RNAi- <i>ATG5</i> -3'F:	CTGCAGTAGGACCCATCTATGAAAGGAGAAT

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RNAi-*ATG5*-3'R: CCCGGGGATGAGAGAGTTATATACGACCTATAT  
RNAi-*ATG5*-5'F: GGATCCTAGGACCCATCTATGAAAGGAGAAT  
RNAi-*ATG5*-5'R: GTTTAAACGATGAGAGAGTTATATACGACCTATAT  
*MTT1*-F: GCTACGTGATTCACGATTTATGCAATG  
*MTT1*-R: CGAAACTGATTTTATGCAATTATGAATTAC  
OE- *ATG8.2*-F: GGATCC ATGGACGCTCAAATTATAAACCCCTT  
OE- *ATG8.2*-R: GGCGCGCC TCAAATTGATCCAAATACTTCTTGATC

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Underlines indicate restriction endonuclease sites: *Bam*H I: GGATCC, *Sgs* I: GGCGCGCC,

*Pst* I: CTGCAG, *Sma* I: CCCGGG, *Pme* I: GTTTAAAC. RNAi for RNA interference