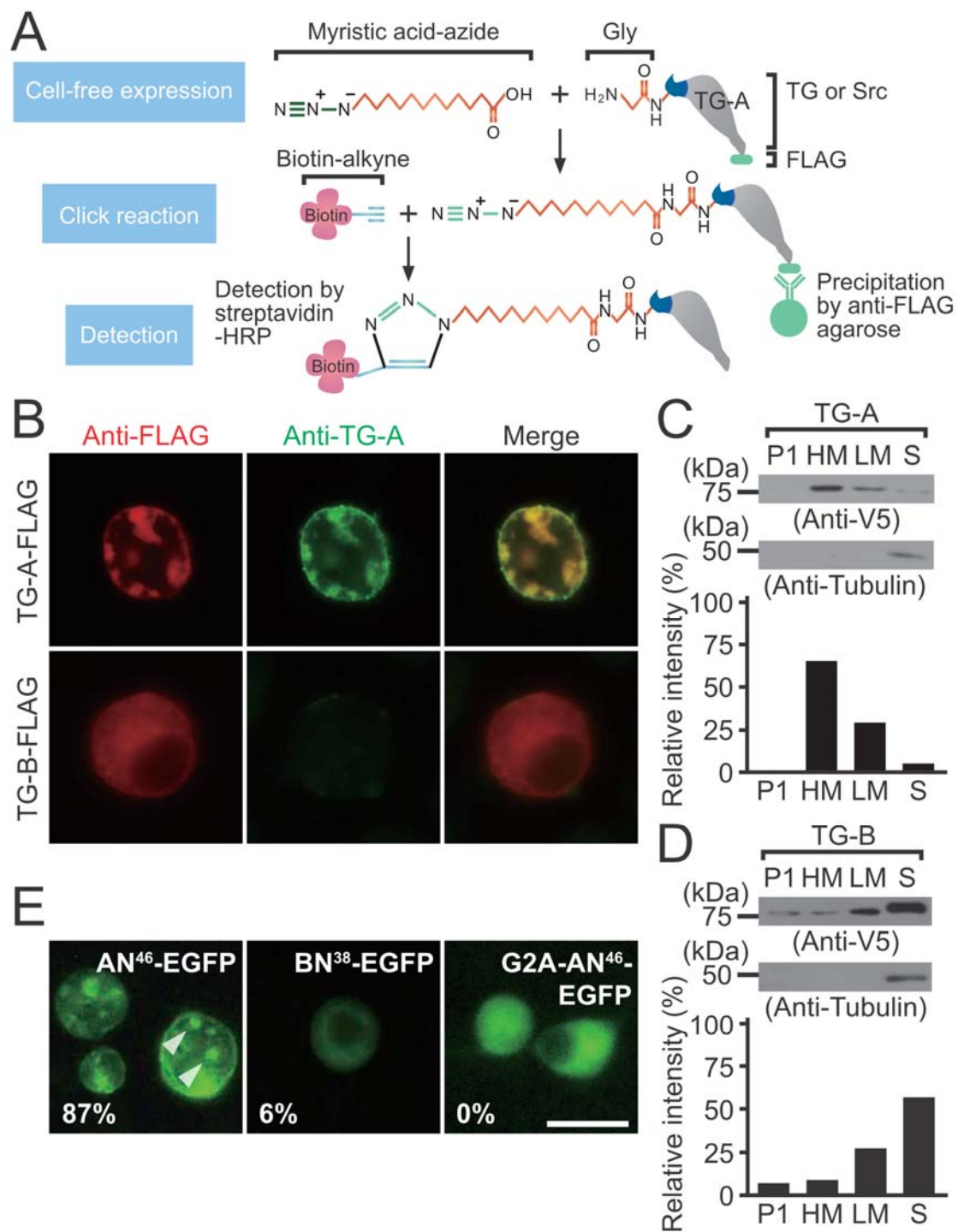
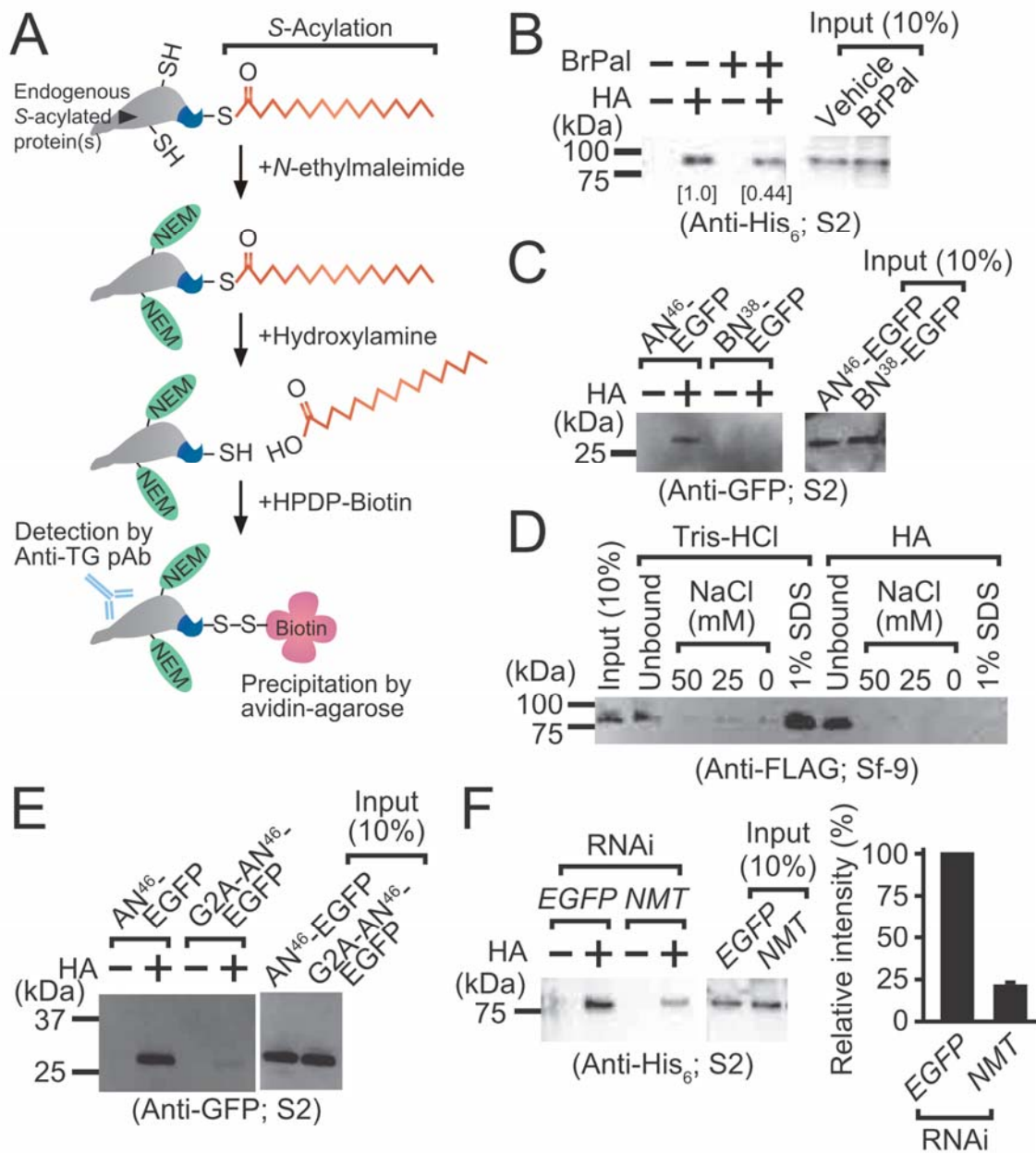


Supplementary Figure S1. **Expression pattern of TG-A and TG-B in each developmental stage and in several tissues.** *A* and *B*, The amount of mRNA expressed in each developmental stage (*A*) and tissue (*B*) for TG-A and TG-B were measured by real-time PCR analysis. 3L, third-instar larva; EP, early pupa; LP, late pupa. *w¹¹¹⁸* flies were used. Statistical analysis was performed by one-way analysis of variance followed by Bonferroni's correction for multiple comparisons to evaluate pairwise differences. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. Error bars are shown \pm SE ($n = 3$).



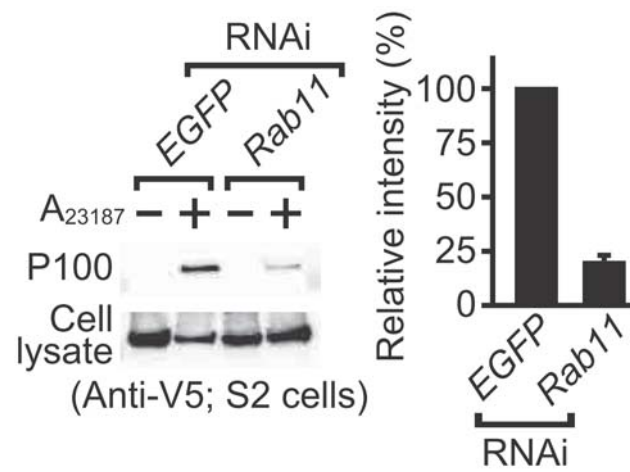
Supplementary Figure S2. **Subcellular localization of TG-A and TG-B.** A, Schematic of the method for detecting *N*-myristoylated proteins using a cell-free protein expression system. The C-terminal FLAG-tagged TG-A, TG-B, or Src (positive control) were expressed using the cell-free expression system in the presence of myristic acid-azide, an analog of myristic acid, and the

analog-incorporated proteins were labeled with biotin alkyne using click chemistry. The resulting proteins were purified using anti-FLAG agarose, and detected using streptavidin-horseradish peroxidase. *B*, *Drosophila* S2 cells expressing TG-A or TG-B tagged with the C-terminal FLAG were analyzed by immunocytochemistry using the anti-FLAG-tag (red) or anti-TG-A-specific antibody (green). *C* and *D*, Subcellular fractionation of S2 cells expressing C-terminal V5-His₆-tagged TG-A (*C*) and TG-B (*D*). Upper panels show the results of Western blotting. Lower panels show the band intensity analyzed by Image J software. P1, low speed pellet; HM, heavy membrane; LM, light membrane; S, soluble. *E*, The C-terminal EGFP-tagged N-terminal fragment of TG-A (AN⁴⁶-EGFP), TG-B (BN³⁸-EGFP), or G2A (G2A-AN⁴⁶-EGFP) was expressed in S2 cells. EGFP signals are shown. The percentage of cells with the plasma membrane-localized signal is shown (n = 100). Arrowheads indicates MVBs. The scale bar in white is 10 μ m.

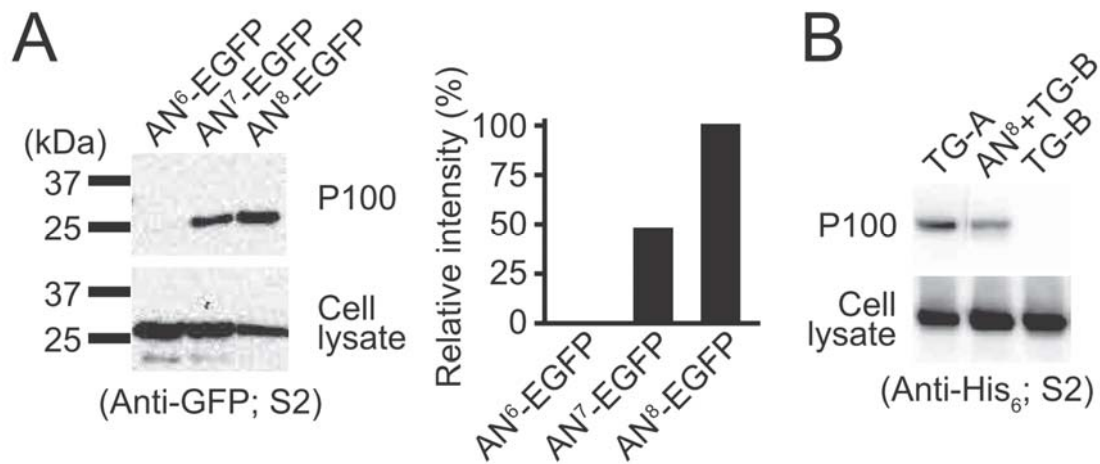


Supplementary Figure S3. **TG-A is a target for S-palmitoylation.** *A*, Schematic method of the biotin-switch assay. *B* and *F*, The biotin-switch assay for lysates of S2 cells expressing the C-terminal V5-His₆-tagged TG-A. Proteins that precipitated on avidin-immobilized agarose after the biotin-switch assay were detected by Western blotting using the anti-His₆-tag antibody. Number shows the band intensity analyzed by Image J software, and the band intensity of 2-bromopalmitate (BrPal)-untreated sample was set to 1 (*B*). Bar graph shows the band intensity analyzed by Image J software, and error bars are shown ± SE (n = 3) (*F*). *C* and *E*, The biotin-switch assay for lysates of S2 cells expressing the C-terminal EGFP-tagged AN⁴⁶-EGFP, BN³⁸-

EGFP, or G2A-AN⁴⁶-EGFP. Proteins that precipitated on avidin-immobilized agarose after the biotin-switch assay were detected by Western blotting using the anti-GFP-tag antibody. *D*, Butyl-Sepharose was used for the protein hydrophobicity assay and each fraction was analyzed by Western blotting using the anti-FLAG antibody. Lysates from Sf-9 cells expressing C-terminal FLAG-tagged TG-A were used.



Supplementary Figure S4. **TG-A is secreted in exosomes via an unconventional secretion pathway.** The P100 fraction from dsRab11 or dsEGFP (negative control)-treated C-terminal V5-His₆-tagged TG-A-expressing S2 cells were analyzed by Western blotting using the anti-V5 antibody. Bar graph shows the band intensity analyzed by Image J software, and error bars are shown \pm SE (n = 3).



Supplementary Figure S5. **The first 7 amino acids are necessary and sufficient for TG-A secretion.** *A*, The P100 fraction prepared from A_{23187} -stimulated the C-terminal EGFP-tagged AN⁶-EGFP, AN⁷-EGFP, or AN⁸-EGFP-expressing S2 cells was analyzed by Western blotting using the anti-GFP antibody. Bar graph shows the band intensity analyzed by Image J software, and the band intensity of AN⁸-EGFP was set to 100%. *B*, The P100 fraction prepared from A_{23187} -stimulated the C-terminal V5-His₆-tagged AN⁸-TG-B-expressing S2 cells was analyzed by Western blotting using the anti-His₆-tag antibody.