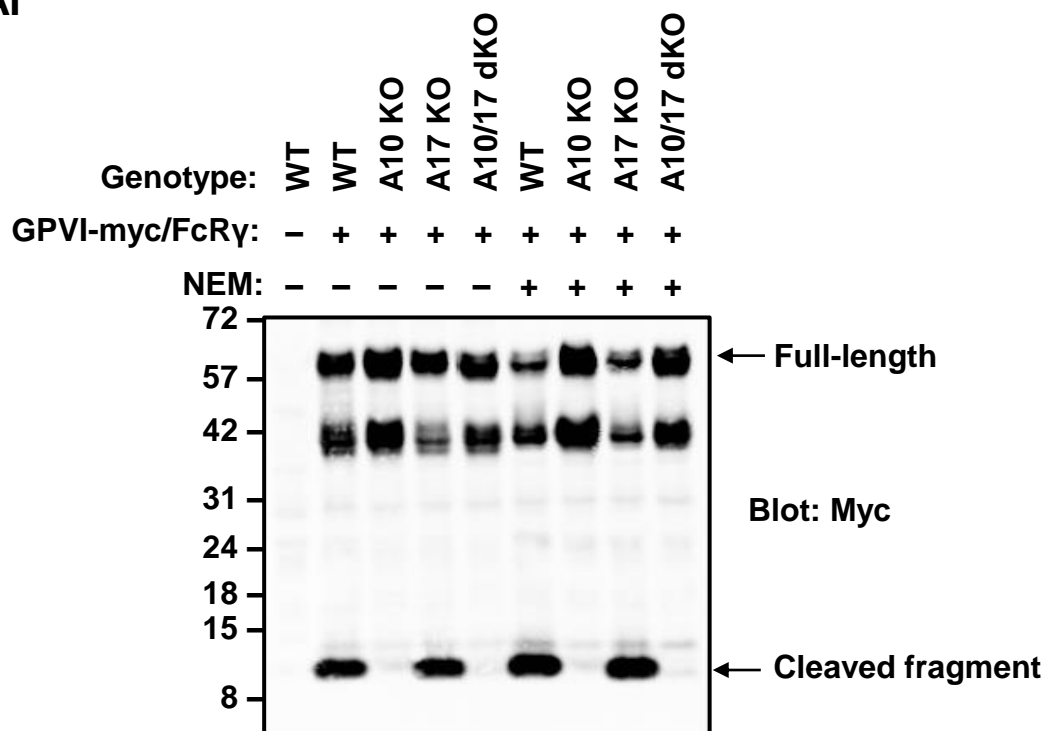
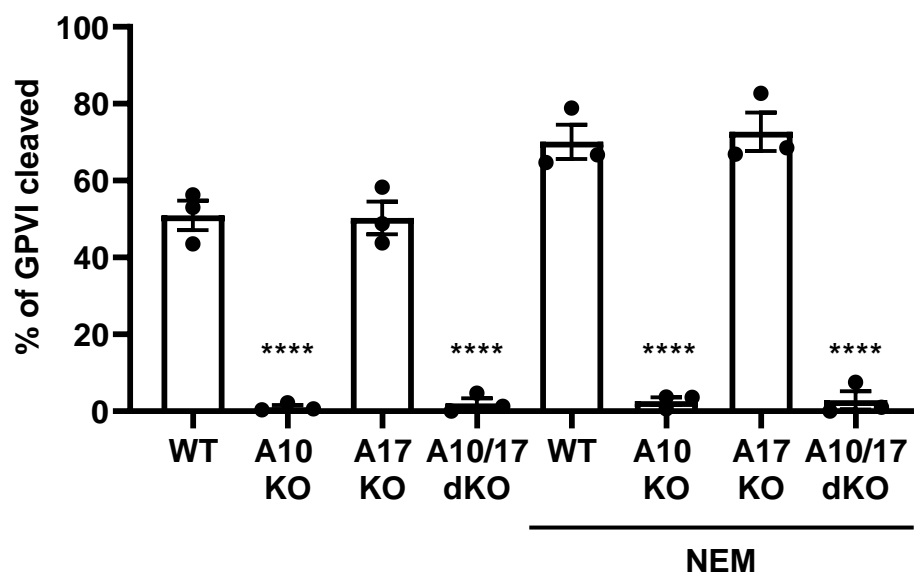


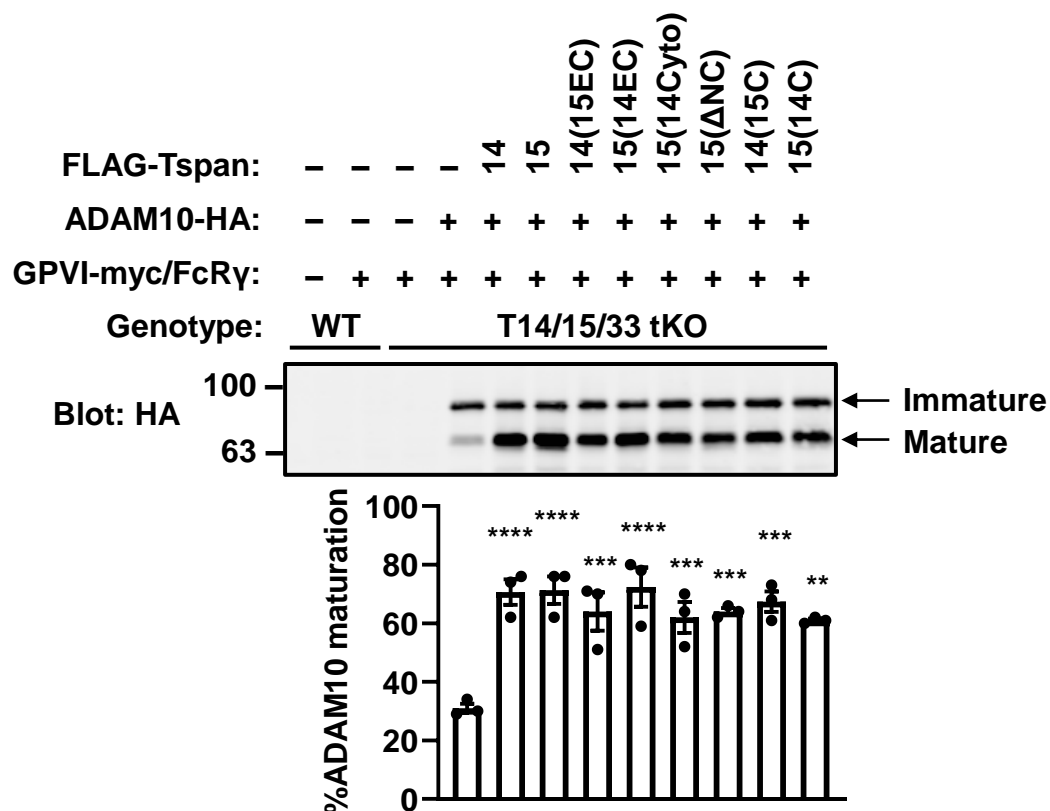
**Ai**



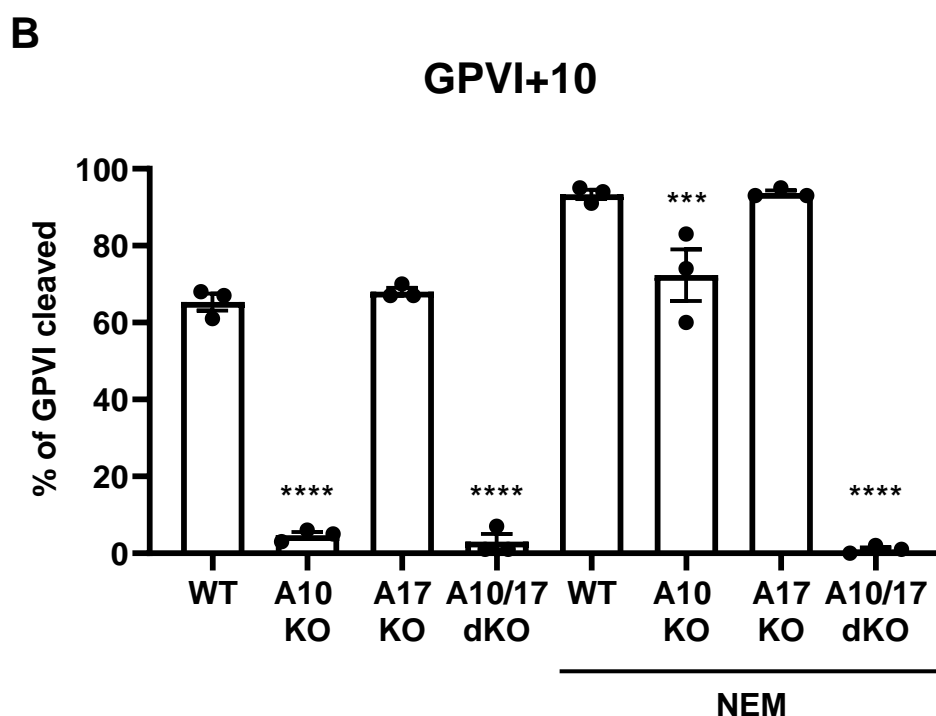
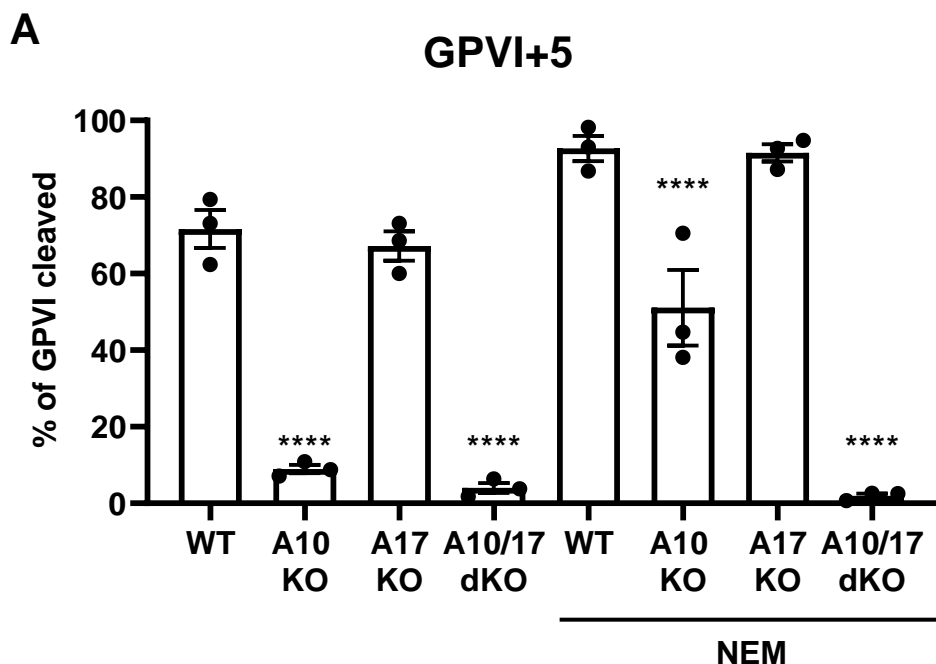
**Aii**



**Supplementary Figure S1. Cleavage of GPVI in transfected HEK-293T cells is ADAM10-dependent.** (Ai) Wild-type (WT), ADAM10-knockout (A10 KO), ADAM17-knockout (A17 KO), and ADAM10/17 double knockout (A10/17 dKO) HEK-293T cells were transfected with expression constructs for C-terminally myc-tagged human GPVI and FcRγ (+), or empty vector (-). Cells were treated with 2 mM NEM (+), or vehicle control (-) for 30 min prior to harvest. Cells were then lysed in 1% Triton X-100 and lysates subjected to Western blotting with an anti-myc antibody. (Aii) The percentage of cleaved GPVI from Ai was calculated. Error bars represent the standard error of the mean from three independent experiments. Data were arcsine-transformed and statistically analyzed using a two-way ANOVA followed by a Dunnett's multiple comparison test, compared to their respective WT controls in each stimulation condition (\*\*\*\*,  $p < 0.0001$ ).



**Supplementary Figure S2. Tspan14 and Tspan15 mutants support ADAM10 maturation.** Cells and constructs were as described in Figure 4. The percentage of ADAM10 maturation was quantitated by expressing the smaller, mature ADAM10 as a percentage of total ADAM10 (mature and immature). Error bars represent the standard error of the the mean from three independent experiments. Data were acrsine-transformed and statistically analyzed using a one-way ANOVA followed by a Dunnett's multiple comparison test, compared to cells transfected with ADAM10 only (\*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ).



**Supplementary Figure S3. ADAM17 can cleave stalk-extended GPVI mutant in the absence of ADAM10.**

Wild-type (WT), ADAM10-knockout (A10 KO), ADAM17-knockout (A17 KO), and ADAM10/17 double knockout (A10/17 dKO) HEK-293T cells were transfected with expression constructs for C-terminally myc-tagged stalk-extension mutants, (A) GPVI+5 or (B) GPVI+10 and FcR $\gamma$  (+), or empty vector (-). Cells were treated with 2 mM NEM (+), or vehicle control (-) for 30 min prior to harvest. Cells were then lysed in 1% Triton X-100 and lysates subjected to Western blotting with an anti-myc antibody. The percentage of cleaved GPVI was calculated. Error bars represent the standard error of the mean from three independent experiments. Data were arcsine-transformed and statistically analyzed using a two-way ANOVA followed by a Dunnett's multiple comparison test, compared to their respective WT controls in each stimulation condition (\*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ).