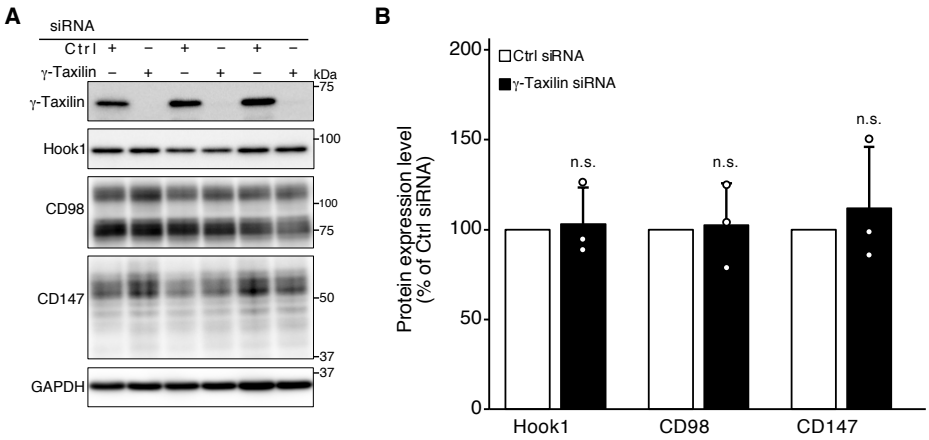


Fig. S1. γ-Taxilin depletion does not affect the expression levels of Hook1, CD98, or CD147 in HeLa cells.

(A) HeLa cells were transfected with control (Ctrl) or γ-taxilin (γ-Taxilin #1) siRNA. Cell lysates were subjected to SDS-PAGE followed by western blotting with the indicated antibodies.

(B) Quantitative analyses of protein expression levels in A. Results shown are the mean ± SD of the ratio of each protein level in γ-taxilin-depleted cells to that in control cells ($n = 3$). n.s., not significant by two-tailed Student's *t*-test.

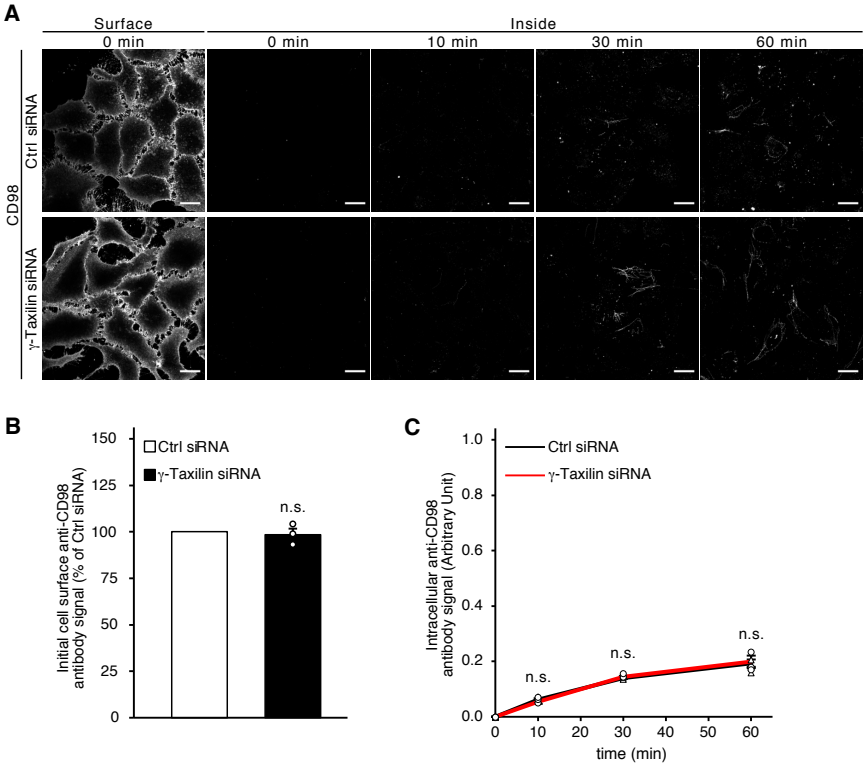


Fig. S2. γ-Taxilin depletion does not affect the internalization of CD98.

(A) Internalization of CD98. HeLa cells treated with the indicated siRNA were treated with anti-CD98 antibody for 1 h at 4°C. After washing out unbound antibody, the cells were incubated for the indicated time periods at 37°C. In the case of detection of surface anti-CD98 antibody at time zero, cells were fixed and stained without permeabilization. In the case of detection of internalized anti-CD98 antibody, surface anti-CD98 antibody was stripped by an acid wash. Then, the cells were fixed, permeabilized, and stained. Scale bars: 20 μm.

(B,C) Quantitative analyses of anti-CD98 antibody signal in A. The intensity of anti-CD98 antibody signals was measured as signal intensity per μm^2. In B, the results shown are the mean ± s.e.m. of the ratio of the cell surface anti-CD98 antibody signal in γ-taxilin-depleted cells to that in control cells (n = 3; >40 cells were analyzed in each experiment). In C, the intensity of the cell surface anti-CD98 antibody signal at time zero was set to 1.0 for control and γ-taxilin-depleted cells, respectively. The results shown are the mean ± s.e.m. of the ratio of the internalized anti-CD98 antibody signal at each time point to the cell surface anti-CD98 antibody signal at time zero (n = 3; >40 cells were analyzed in each experiment). n.s., not significant by two-tailed Student's t-test.

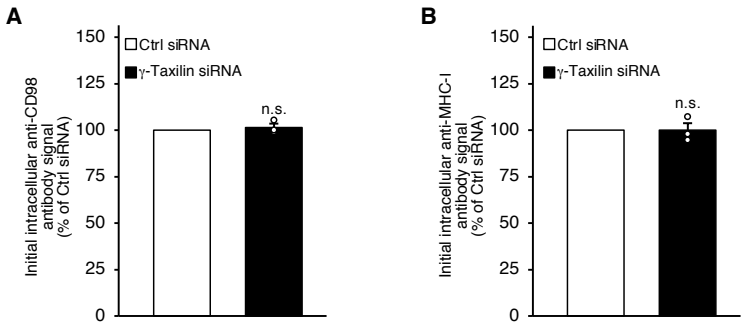


Fig. S3. γ-Taxilin depletion does not affect the amount of internalized CD98 and MHC-I at the starting point of the recycling assay in Fig 5.

(A,B) Quantitative analysis of internalized anti-CD98 and anti-MHC-I antibody signals. In A, the results shown are the mean ± s.e.m. of the ratio of the anti-CD98 antibody signal in γ-taxilin-depleted cells at time zero to that in control cells at time zero (n = 3; >40 cells were analyzed in each experiment). In B, the results shown are the mean ± s.e.m. of the ratio of the anti-MHC-I antibody signal in γ-taxilin-depleted cells at time zero to that in control cells at time zero (n = 3; >40 cells were analyzed in each experiment). n.s., not significant by two-tailed Student's t-test.

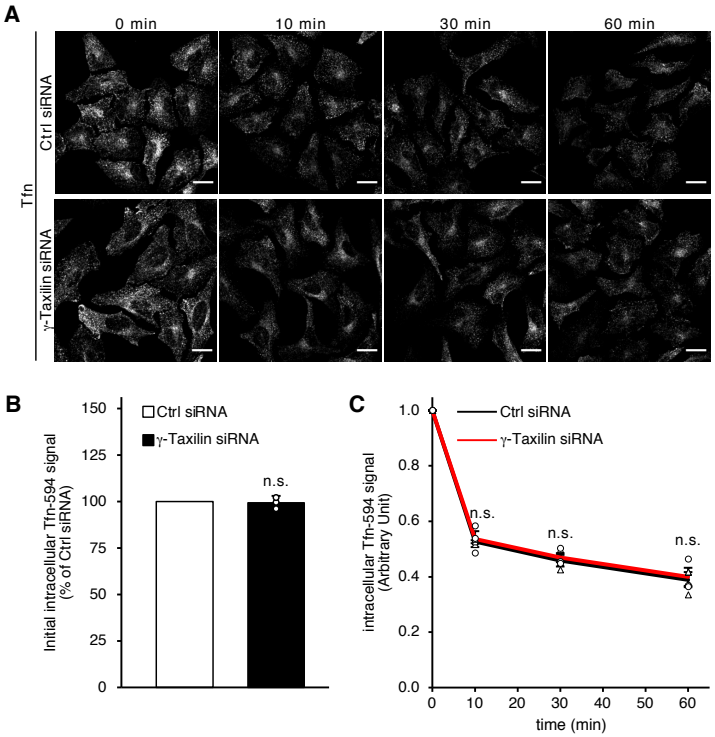


Fig. S4. γ-Taxilin depletion does not affect the recycling of TfnR.

(A) Recycling of TfnR. After serum starvation for 30 min at 37°C, HeLa cells treated with the indicated siRNA were incubated with Alexa Fluor 594-labeled Tfn (Tfn-594) for 1 h at 37°C. After washing out surface-bound Tfn-594, the cells were further incubated for the indicated time periods at 37°C and then fixed. Scale bars: 20 µm.

(B,C) Quantitative analyses of Tfn-594 signal in A. The intensity of the Tfn-594 signal was measured as signal intensity per μm^2. In B, the results shown are the mean ± s.e.m. of the ratio of the Tfn-594 signal in γ-taxilin-depleted cells at time zero to that in control cells at time zero (n = 3; >40 cells were analyzed in each experiment). In C, the intensity of the Tfn-594 signal at time zero was set to 1.0 for control and γ-taxilin-depleted cells, respectively. The results shown are the mean ± s.e.m. of the ratio of the Tfn-594 signal at each time point to that at time zero (n = 3; >40 cells were analyzed in each experiment). n.s., not significant by two-tailed Student's t-test.

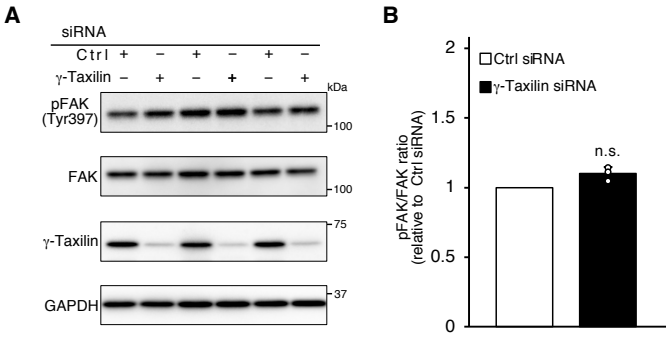


Fig. S5. γ-Taxilin depletion does not affect basal FAK phosphorylation levels in HeLa cells.

(A) HeLa cells were transfected with control (Ctrl) or γ-taxilin (γ-Taxilin #1) siRNA. Cell lysates were subjected to SDS-PAGE followed by western blotting with the indicated antibodies.

(B) Quantitative analysis of the phosphorylation of FAK on Tyr397 in A. The results shown are the mean ± SD of the ratio of pFAK to FAK relative to that when using Ctrl siRNA (n = 3). n.s., not significant by two-tailed Student's t-test.

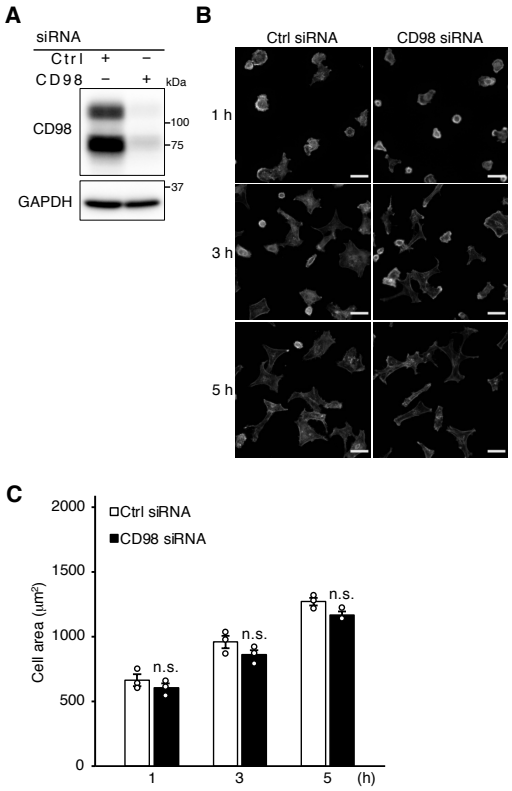


Fig. S6. CD98 depletion hardly affects cell spreading of HeLa cells.

(A) Depletion of CD98 by siRNA. HeLa cells were transfected with control (Ctrl) or CD98 siRNA. Cell lysates were subjected to SDS-PAGE followed by western blotting with the indicated antibodies.

(B) Cell spreading assay. HeLa cells treated with each siRNA were trypsinized and resuspended. The suspended cells were replated and incubated for the indicated time periods. Cells were visualized using Alexa Fluor 594-labeled phalloidin. Scale bars: 40 μm.

(C) Quantitative analysis of cell spreading in B. The area of spreading cells at each time point was measured. Data are expressed as the mean ± s.e.m. (n = 3; >100 cells were analyzed in each experiment). n.s., not significant by two-tailed Student's t-test.

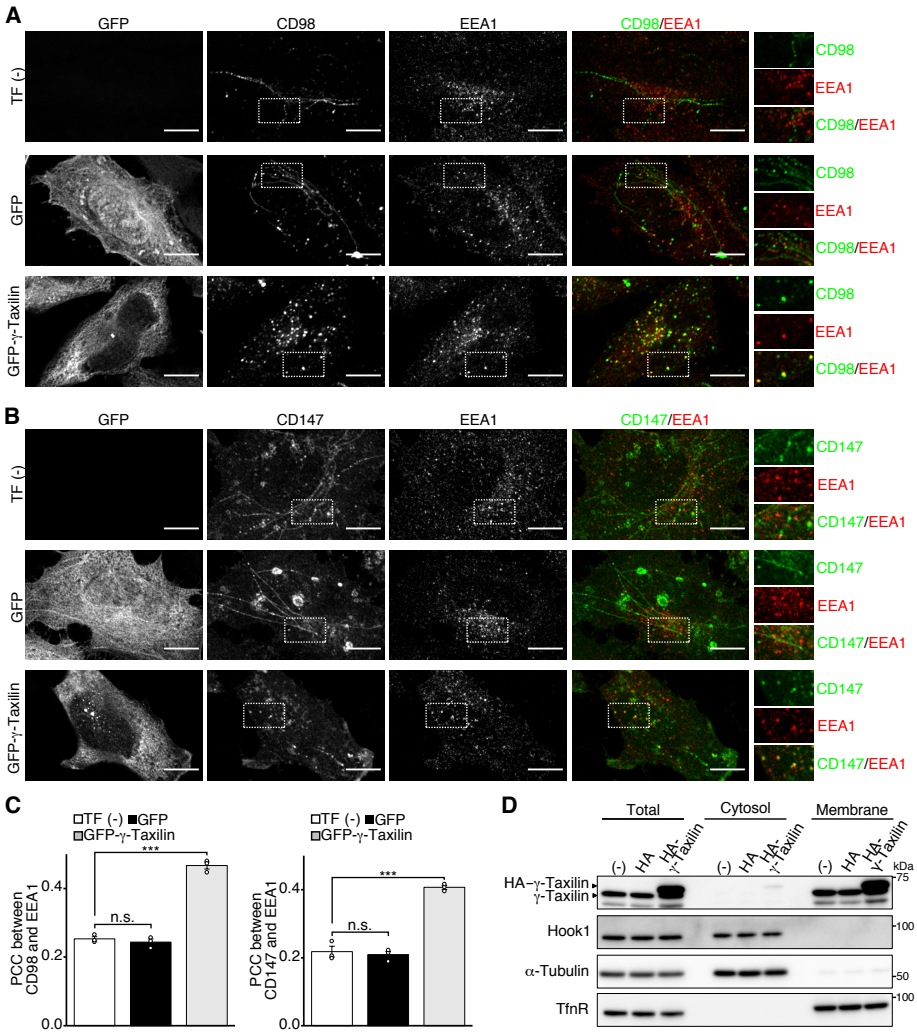


Fig. S7. Subcellular distribution of CD98, CD147, and Hook1 in cells overexpressing γ-taxilin.

(A,B) Subcellular distribution of CD98 and CD147. Hela cells expressing GFP or GFP-γ-taxilin were subjected to the antibody uptake assay followed by immunostaining with an anti-EEA1 antibody. Scale bars: 10 μm. Magnified views of the boxed areas in the panels are shown in the right panels.

(C) Pearson correlation coefficient (PCC) for the relation between CD98 or CD147 and EEA1 shown in A and B using the Fiji software. Data are expressed as the mean ± s.e.m. (n = 3; >10 cells were analyzed in each experiment) ***, $P < 0.001$; n.s., not significant by one-way ANOVA with *post-hoc* Tukey's multiple comparison test.

(D) Subcellular fractionation of Hook1. The cytosolic and membrane fractions, and the total cell lysates of HeLa cells treated with the indicated plasmids were subjected to SDS-PAGE followed by western blotting with the indicated antibodies (n=3). TfnR and α-tubulin were used as markers for membrane and cytosolic proteins, respectively.

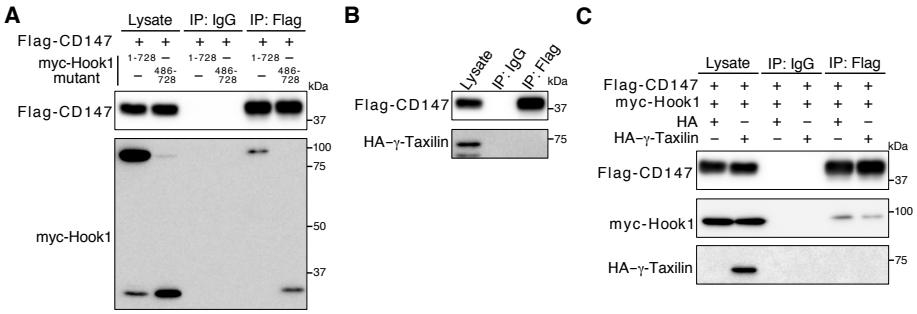
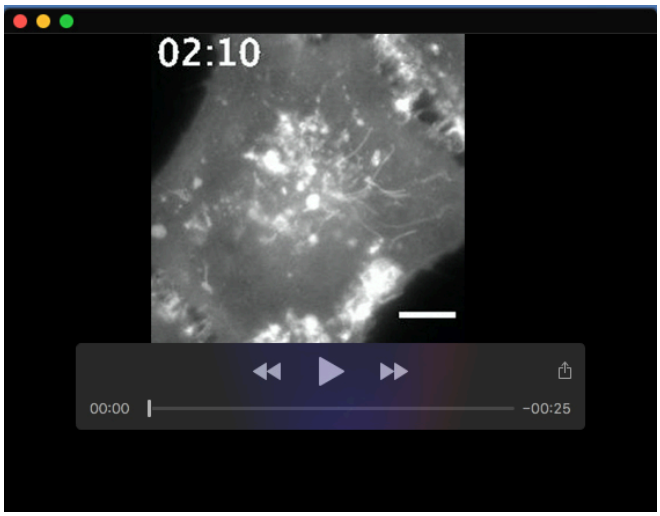


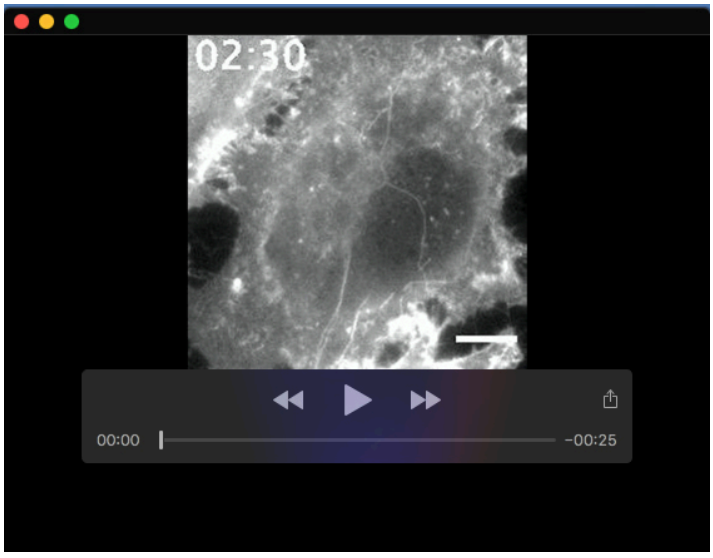
Fig. S8. γ-Taxilin binds to Hook1 competitively with CD147

(A-C) Co-immunoprecipitation assay. Cells were co-transfected with the indicated plasmids. Cell lysates were immunoprecipitated with the indicated antibodies. The immunoprecipitate was subjected to SDS-PAGE followed by western blotting with the indicated antibodies for detection of the corresponding proteins (n=3, each experiment). The amount of cell lysates used for western blotting were 2.5% of those used for IP.



Movie 1. Time-lapse imaging of Alexa Fluor 488-labelled anti-CD98 antibody in control siRNA-treated HeLa cells.

Time-lapse images were captured at 10-s intervals over 10 min. Scale bar: 10 µm.



Movie 2. Time-lapse imaging of Alexa Fluor 488-labelled anti-CD98 antibody in γ-taxilin siRNA-treated HeLa cells.

Time-lapse images were captured at 10-s intervals over 10 min. Scale bar: 10 µm.