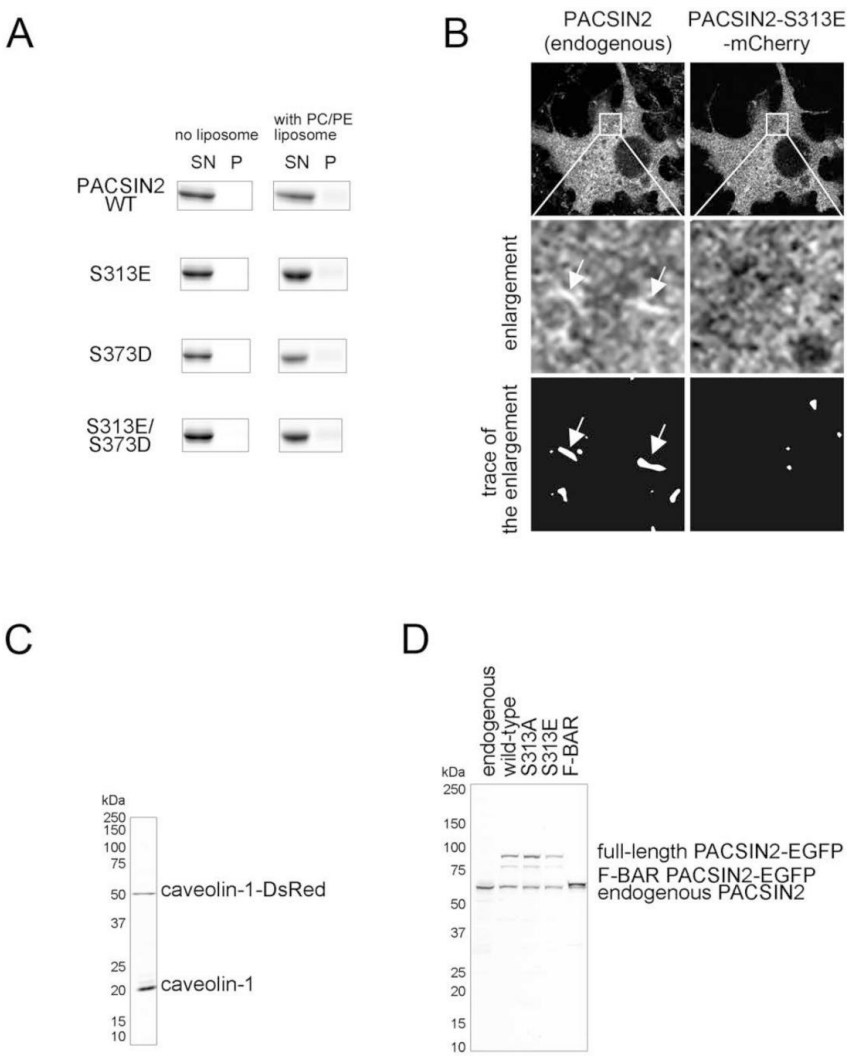


Figure S1. Characterization of the PACSIN2 phosphorylation.

(**A**) PACSIN2 binding to liposomes made of PC and PE. PACSIN2 binding to liposomes made of PC and PE was analyzed as in Figure 3A. (**B**) Endogenous PACSIN2 tubules. Non-transfected Hela cells (left) or HeLa cells expressing PACSIN2 S313E-mCherry were stained with an anti-PACSIN2 antibody to detect endogenous PACSIN2. The area marked with rectangles in the upper images was enlarged in the middle images. The tubular structures of the endogenous PACSIN2 are shown with arrows. For better visualization, higher signals of the middle images are shown on the bottom. (**C**) Exogenous caveolin-1 expression in Hela cells. Representative western blot with the anti-caveolin-1 antibody in Hela cells stably expressing caveolin-1-DsRedm. Based on this blot, we estimate that caveolin-1-DsRedm expressed at a level of approximately 25% compared to endogenous caveolin-1. (**D**) Exogenous PACSIN2 expression in Hela cells. Representative western blot with the anti-PACSIN2 antibody detecting PACSIN2-GFP and endogenous PACSIN2. Since the transfection efficiency was approximately 50-70%, the amount of exogenously expressed PACSIN2 was approximately 2 times higher than that of the endogenous protein.

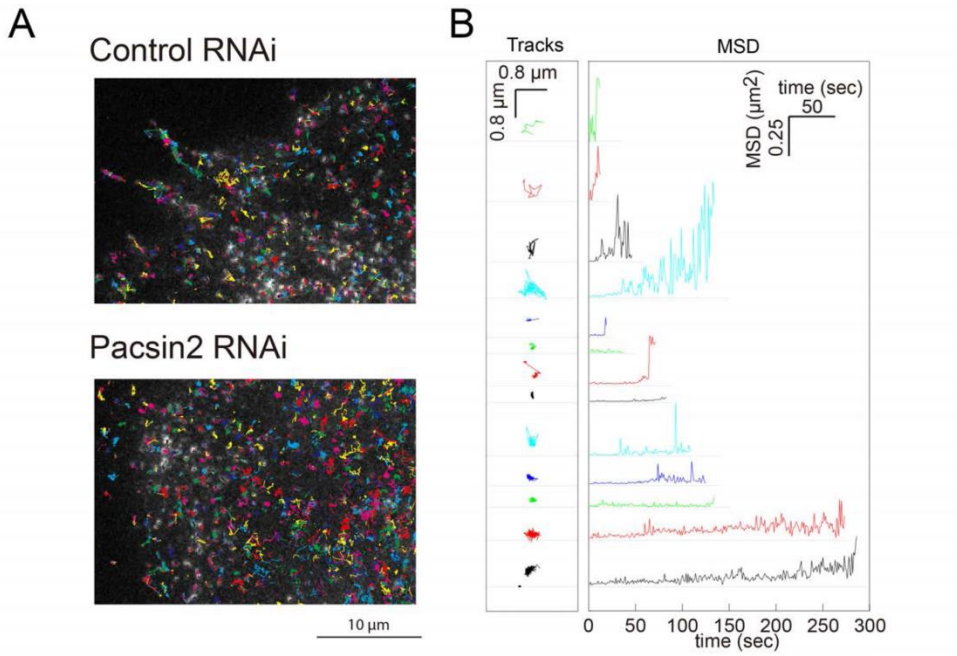


Figure S2. Representative results of caveolin-1 spot tracking.

(**A**) Caveolin-1 spots on control or PACSIN2 RNAi-treated cells were tracked, and the tracks formed during 25 seconds are shown. Each track is differentially colored, and colors are not related to the tracked durations.

(**B**) Plots of MSDs and tracks from randomly chosen caveolae over time from a cell treated with control RNAi.

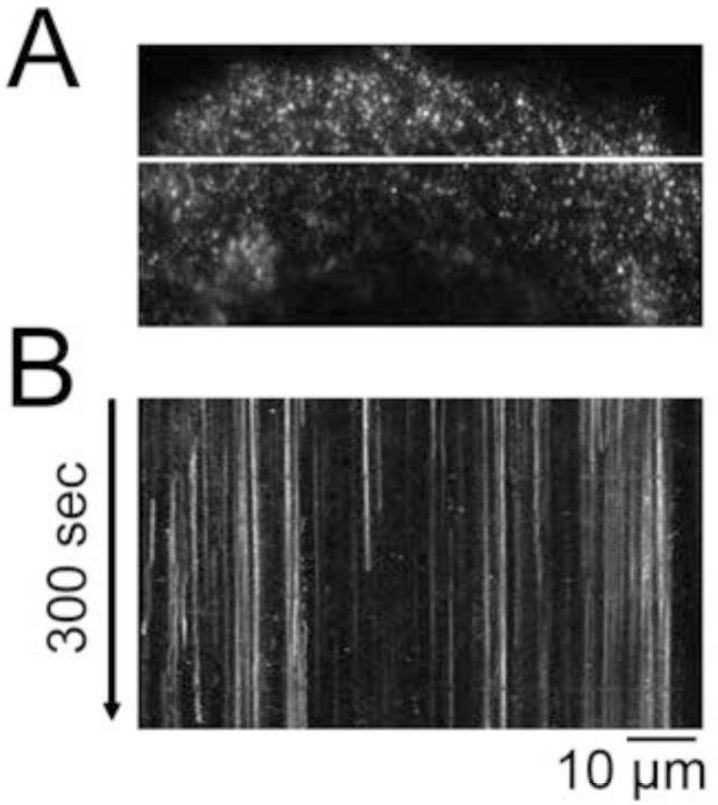


Figure S3. PMA treatment of caveolin-1-GFP-expressing Flp-in T-REx Hela cells. (**A**) Caveolin-1-GFP fluorescence was monitored for 300 sec after cells were treated with PMA for 1 min, as in Figure 5. (**B**) The kymograph over 300 seconds was generated from the indicated line. Dotted vertical lines in kymographs indicate shorter tracking durations of caveolae.

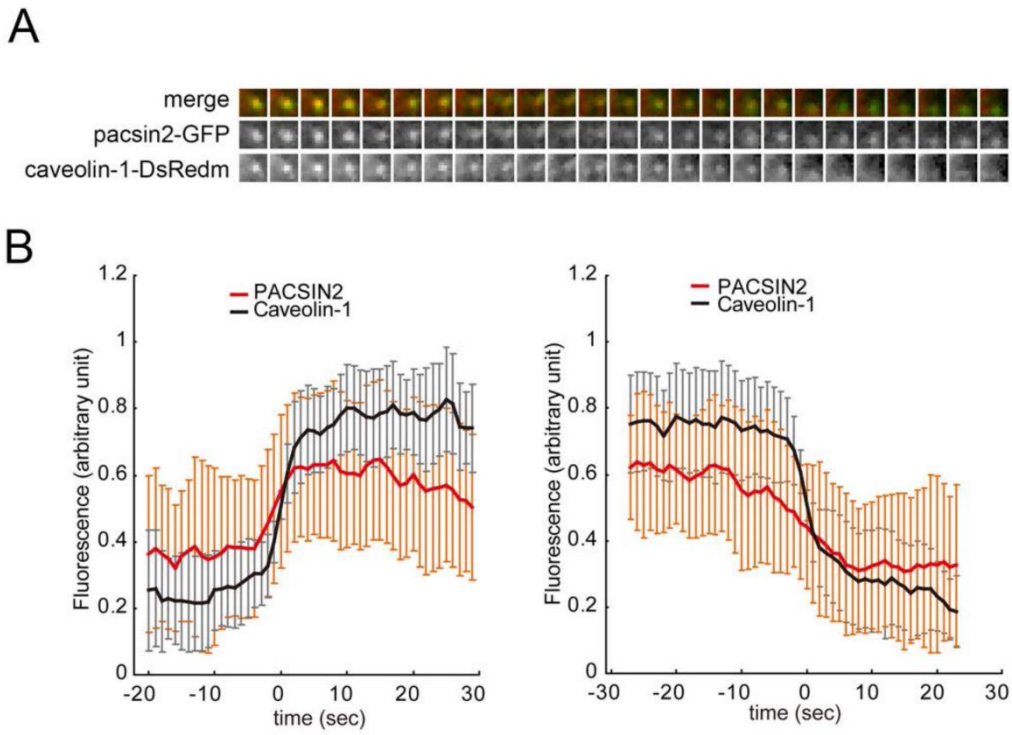
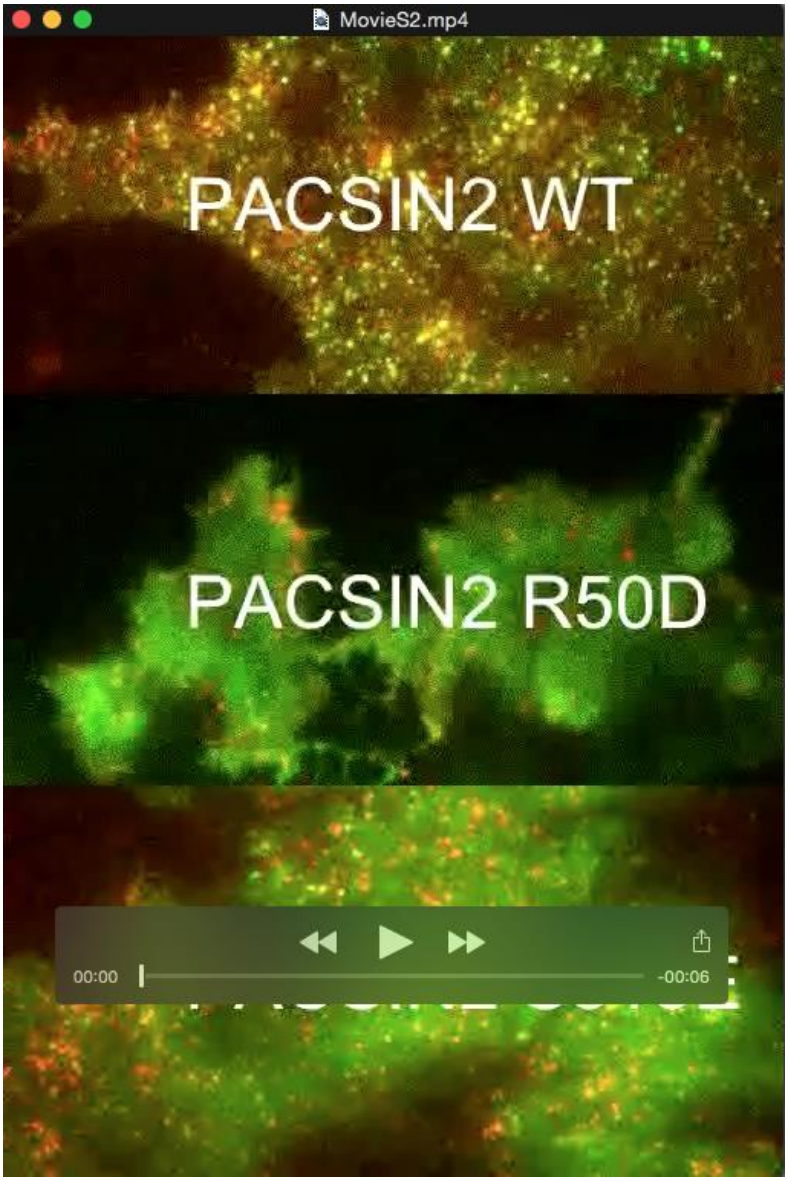


Figure S4. Time courses of PACSIN2 and caveolin-1 localizations.

(**A**) TIRF images of HeLa cells transfected with PACSIN2-GFP (green) and stably transfected with caveolin-1-DsRedm (red). Representative sequential images of disappearance of caveolin-1 are shown.

(**B**) Quantification of the fluorescence intensity of PACSIN2 and caveolin-1 before and after the appearance (left, 0 time point) and disappearance (right, 0 time point) of a caveolin-1 signal at the plasma membrane. Mean ± S.D. (n= 51, 71).

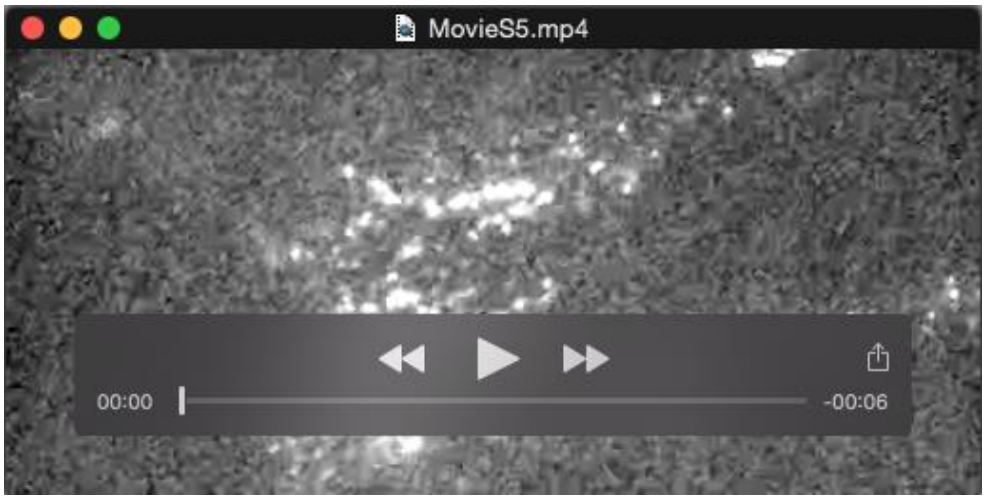
MOVIE 1. HeLa cells stably expressing caveolin-1-DsRedm after treatment with control or PACSIN2 siRNA, visualized by TIRF microscopy.



MOVIE 2. HeLa cells stably expressing caveolin-1-DsRedm (red) and WT, R50D, or S313E PACSIN2-GFP (green), visualized by TIRF microscopy.

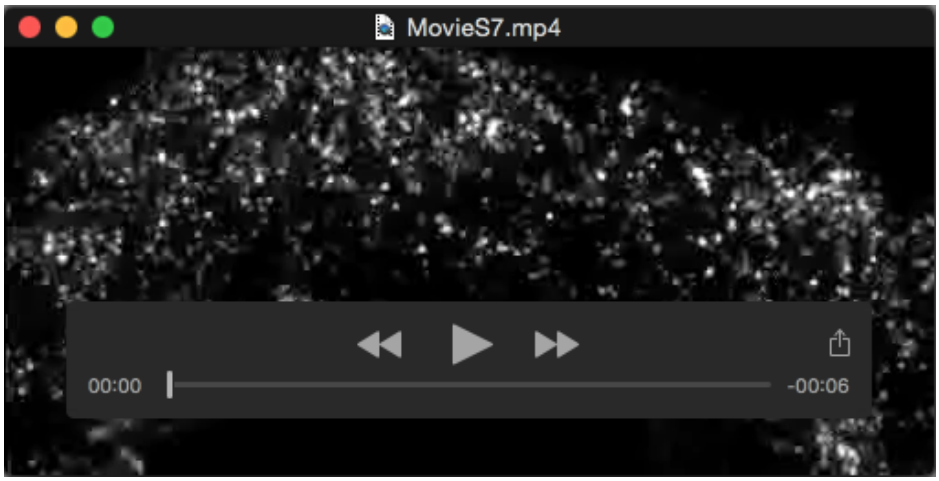
MOVIE 3. HeLa cells stably expressing caveolin-1-DsRedm after 1 min of treatment with PMA, visualized by TIRF microscopy.

MOVIE 4. HeLa cells stably expressing caveolin-1-DsRedm after 1 min of treatment with hypotonic medium, visualized by TIRF microscopy.

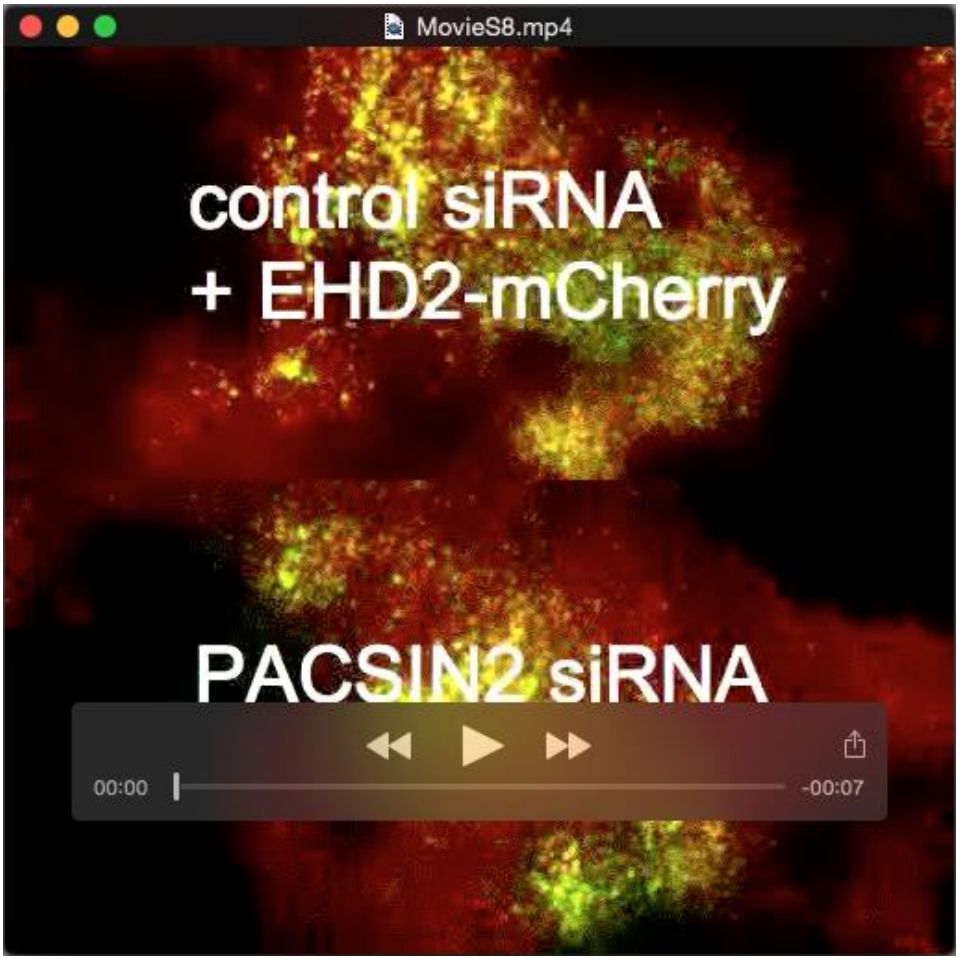


MOVIE 5. HeLa cells stably expressing caveolin-1-DsRedm after treatment with BIM and hypotonic conditions, visualized by TIRF microscopy.

MOVIE 6. HeLa cells expressing WT PACSIN2-GFP (green) and stably expressing caveolin-1-DsRedm (red) after hypotonic treatment, visualized by TIRF microscopy.



MOVIE 7. Flp-In T-REx HeLa cells expressing caveolin-1-GFP after 1 min of treatment with PMA, visualized by TIRF microscopy.



MOVIE 8. HeLa cells stably expressing caveolin-1-GFP (green) and expressing EHD2-mCherry (red) and after treatment with control or PACSIN2 siRNA, visualized by TIRF microscopy.