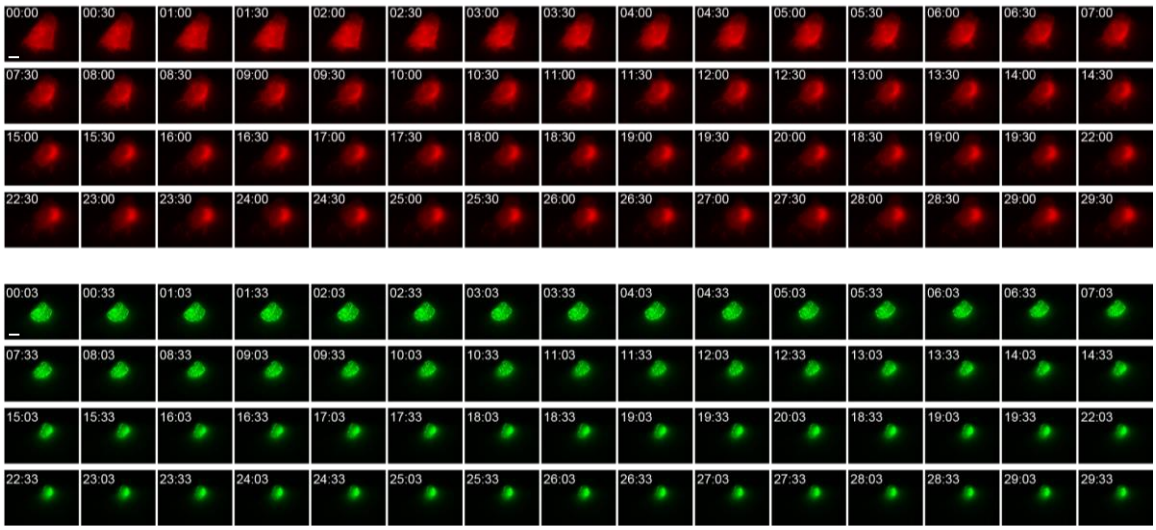

Fig. S1. Imaging profiles associated with morphological changes in cells during the execution phase of apoptosis.

Serial fluorescence images of HeLa cells expressing fluorescent proteins, RFP in the plasma membrane (upper) and EGFP in the nucleus (lower) were are displayed from top left to bottom right in chronological order. The resolution of these images was 348×260. Ttransfected cells was were treated with anti-Fas antibody and cycloheximide (CHX) and fluorescence images were captured every 30 sec for 30 min under a fluorescencet microscope using a CCD camera. Numbers indicate times after taking the first image. Scale bars indicate 10 μm.

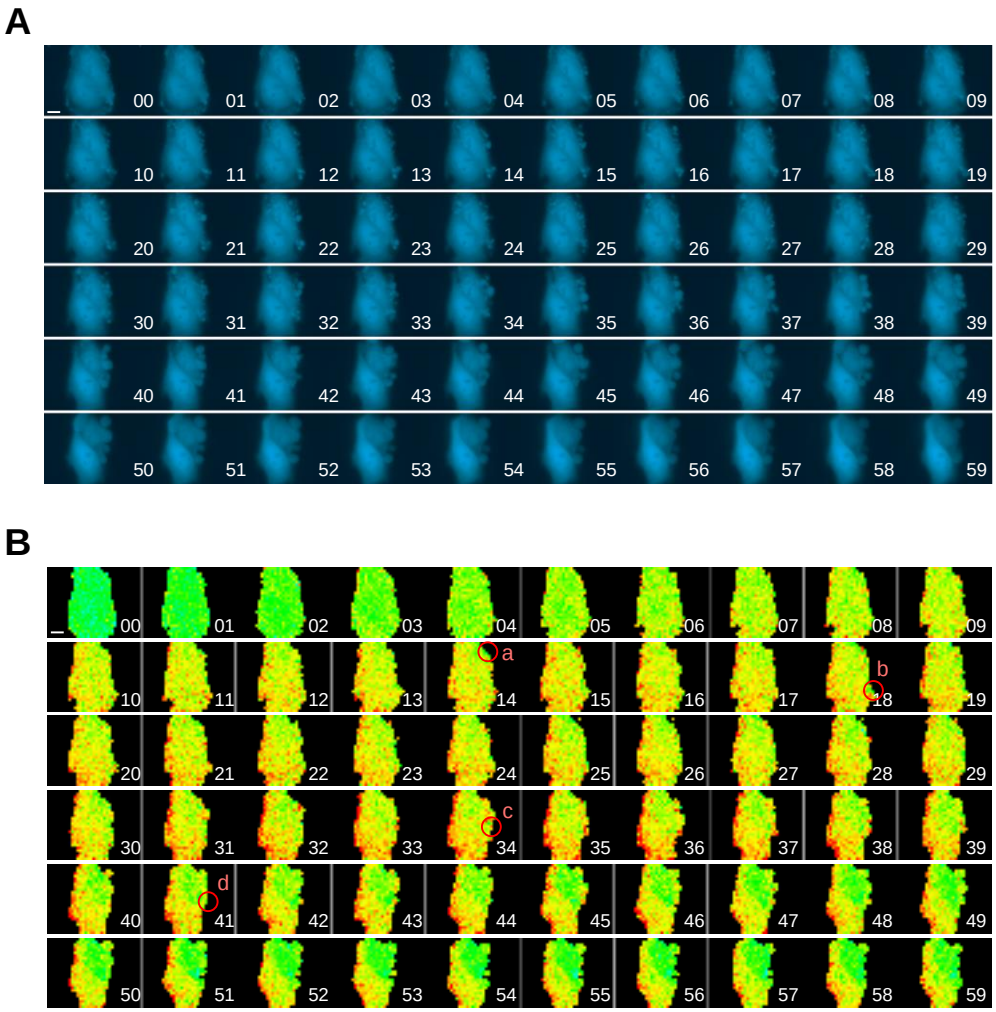

Fig. S2. Imaging profiles of the ratio change of a FRET-based indicator, CRCit in the execution phase of apoptosis.

Phosphorylation of a FRET-based CRCit biosensor in transfected cells was monitored during apoptosis. Transfected cells expressing CRCit were treated with an anti-Fas antibody and CHX, and fluorescence images were captured every 1 min for 60 min under a fluorescence microscope using a CCD camera through the specified filters (Excitation 425 nm/Emission 480 nm for ECFP and Excitation 425 nm/Emission 535 nm for ECFP → Citrine). (**A**, **B**) The serial CFP fluorescence images (A) and pseudo- colored images correlated with FRET ratios (B) are displayed. Numbers indicate the time (minutes) after taking the first image. Zero indicates the starting point of the observation of blebbing. Four membrane regions were chosen from the upper cell as (a) to (d) and marked in a red circle. Enlarged views were shown in Fig. 2C. Scale bars indicate 10 μm.

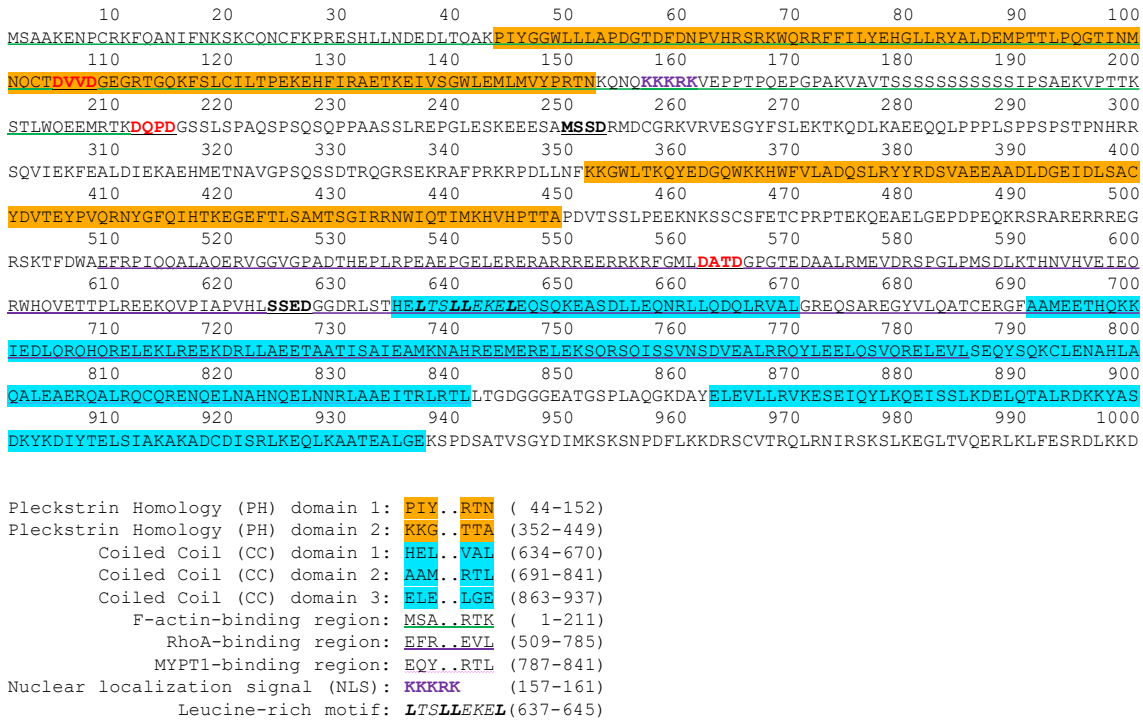

Fig. S3. Amino acid sequence of human MPRIP.

The amino acid sequence of human MPRIP used in this study is identical to the isoform CRA_d registered in GenBank (EAW55724). It comprises 1000 amino acid residues. Two pleckstrin homology (PH) domains and three coiled-coil (CC) domains are highlighted. The regions interacting with either F-actin, RhoA, or MYPT1 are underlined. The NLS sequence “KKKRK” (purple font) and a weak NES sequence “LTSLLLEKEL” (italicized) are shown.

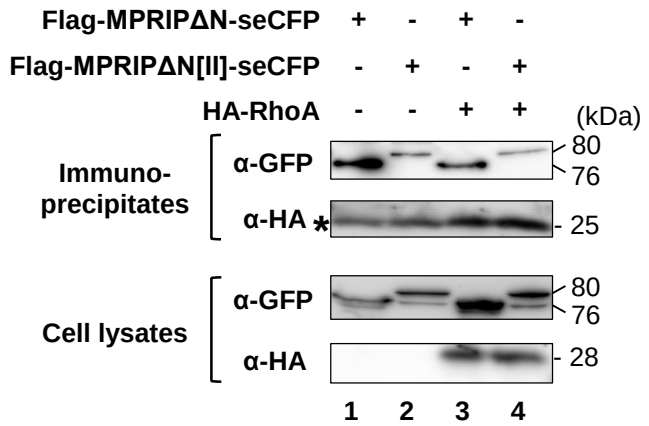

Fig. S4. Co-immunoprecipitation and immunoblot analysis. HEK293T cells were transfected with either pCI/Flag-MPRIPΔN-seCFP or pCI/Flag-MPRIPΔN[II]-seCFP in conjunction with either an empty plasmid control or pEFBOS/HA-RhoA. 48 h after transfection, cells were lysed, and Flag-tagged proteins were immunoprecipitated with an anti-Flag antibody. Whole cell lysates and immunoprecipitates were analyzed by immunoblotting with anti-GFP and anti-HA antibodies. The asterisk indicates the light chain of an antibody.

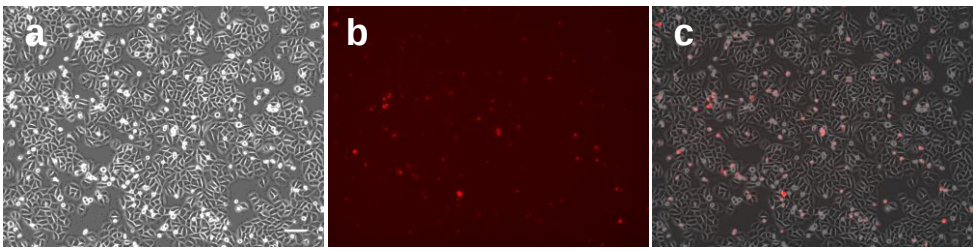

Fig. S5. Microscopic observation of HeLa cells exposed to apoptotic stimuli.

Cells were treated with a mixture of anti-Fas antibody and CHX for 3 h. For detecting CASP3 activity in dying cells, a fluorescent substrate, 4 μM NucView®530 was added to the medium 30 min before observation. The images were displayed by capturing through a bright-field channel (a) and a red fluorescent channel (b) and by merging (c). Scale bar indicates 100 μm.

Supplementary Movie

Movie S1. A series of spatiotemporal images of RFP- and EGFP-labeled cells undergoing apoptosis.
Fluorescence images of a HeLa cell expressing fluorescent proteins, RFP (plasma membrane) and EGFP (nucleus), as shown in Fig. S1, follow the progression of apoptosis.

Movie S2. Real-time movies with a FRET-based biosensor, CRCit.
Both CFP images (A) and FRET ratio images (B) of HeLa cells expressing CRCit, as shown in Fig. S2, are continuously displayed as Movie S2A and S2B, respectively.