Supporting Information

Lee et al. 10.1073/pnas.0911262107

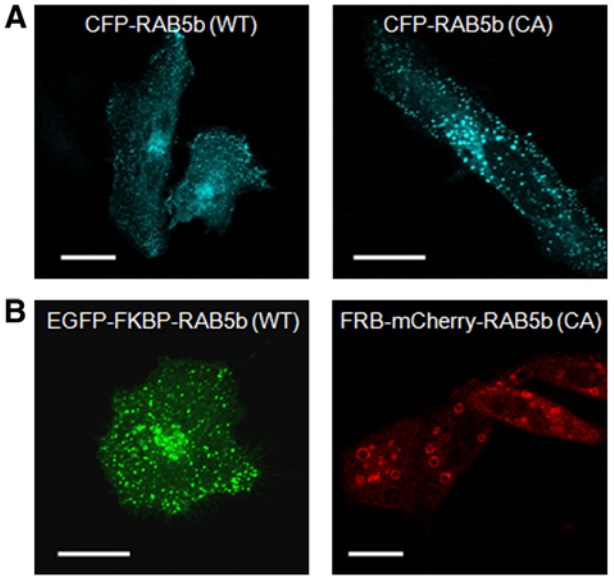


Fig. S1. Selection of endosome anchor. (*A*) Wild-type and constitutively active forms of Rab5b were expressed in HeLa cells. Constitutively active Rab5b enhanced the size and number of endosomes. (*B*) FRB-conjugated Rab5b (wild-type and constitutively active forms) proteins were expressed in HeLa cells. FRB-conjugated RAB5b was observed at large endosomes, generating a "circle". Scale bars = 10 μm.

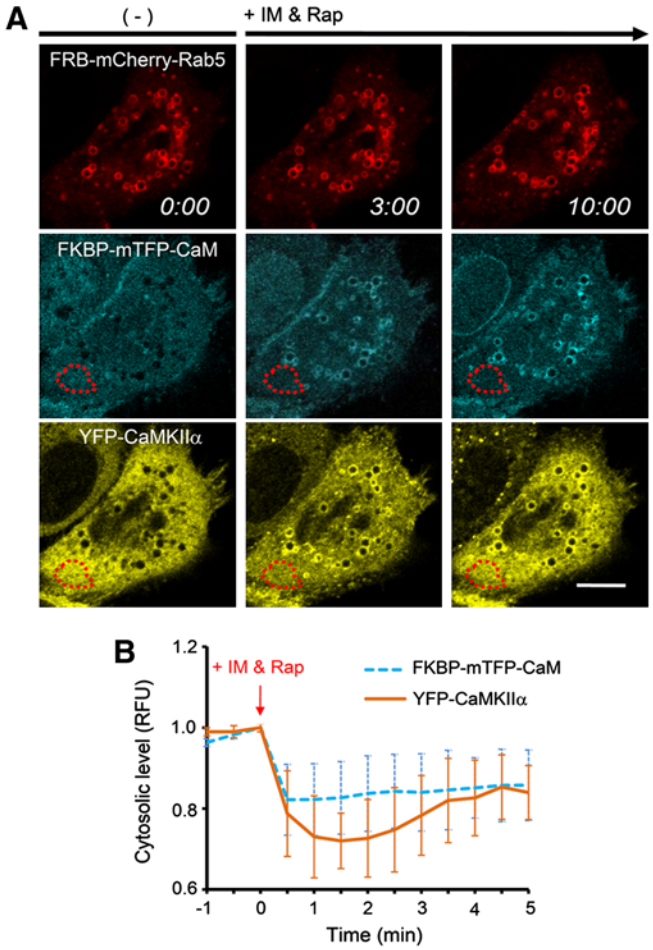


Fig. S2. Visualization of changes in cytosolic CaMKIIα levels using a three-hybrid assay. Interaction between CaM kinase IIα (YFP-CaMKIIα) and calmodulin (FKBP-mTFP-CaM). Following induction with 500 nM rapamycin and 1 μM ionomycin, cytosolic levels of CaMKIIα decreased dramatically and then showed a slight recovery. (*A*) Images were captured prior to induction and at three and 10 min after induction. Scale bars = 10 μm. (*B*) Quantitative analysis using the area surrounded by *red dots* to indicate the Region of Intensity (ROI). RFU were normalized against fluorescence before induction ($n = 4$; error bars indicate S.E.).

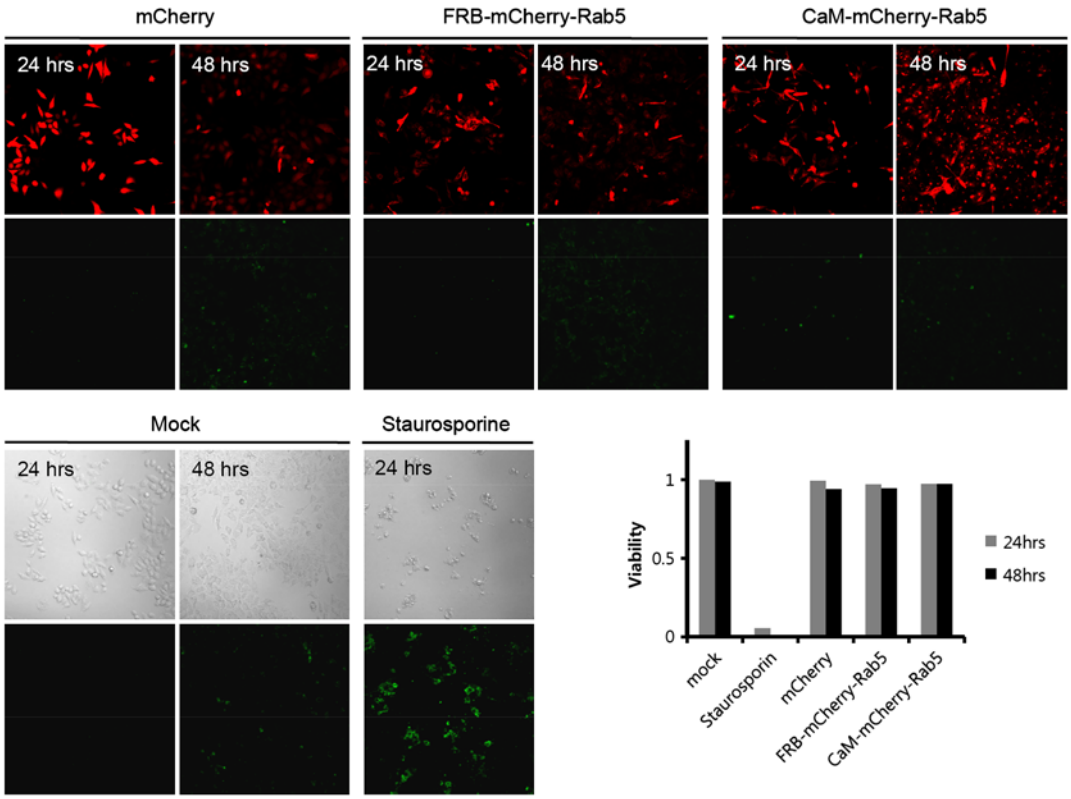


Fig. S3. Cytotoxicity test during ECLISPE assay. The transfected cells with mCherry, FRB-mCherry-Rab5 and CaM-mCherry-Rab5 was stained by Annexin V-FITC. The cell as positive control was treated by 1 μM of staurosporine for 24 hrs. The viability was calculated by the ratio of nonlabeled cell to total cells. At least 300 cells were counted for each set.

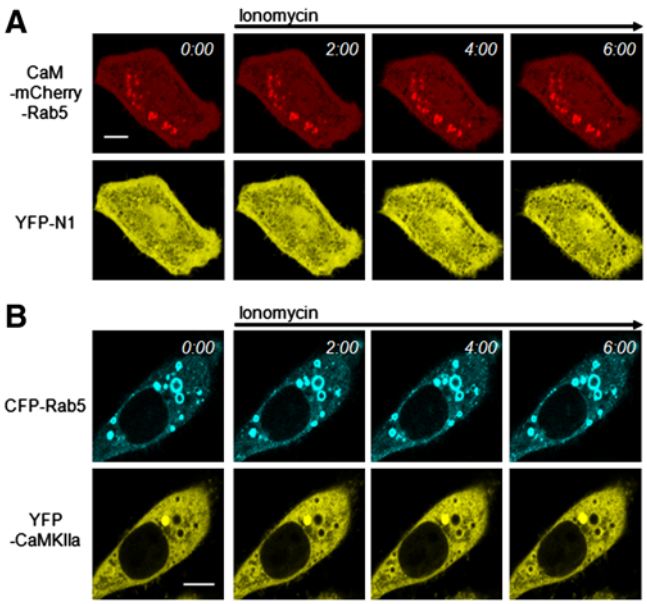


Fig. S4. Negative control experiments to verify that endosomal localization of CaM-binding proteins requires Ca^{2+}-dependent interactions. Induction with 1 μM ionomycin. No prey localization was observed in the absence of calmodulin-binding proteins (*A*) or calmodulin (*B*). Only HeLa cells containing both calmodulin and calmodulin-binding proteins showed localization of prey. Scale bars = 10 μm.

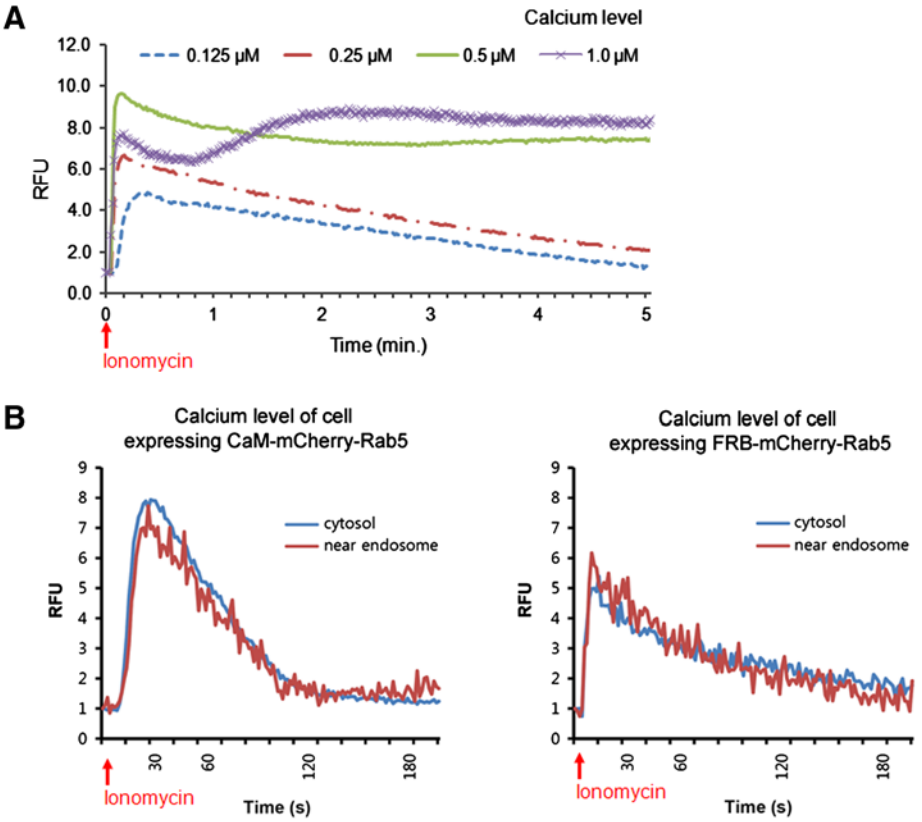


Fig. S5. In HeLa cells, the interaction rate between calmodulin and CaM kinase IIα is influenced by cytosolic Ca^{2+} levels. (*A*) Cytosolic Ca^{2+} levels were measured using Fluo-4 Ca^{2+} chelating agent. ($n = 3$). (*B*) The Ca^{2+} levels at the cytosol and near endosome compared using Fluo-3 Ca^{2+} chelating agent. The transfected cells with FRB-mCherry-Rab5 or CaM-mCherry-Rab5 was employed ($n = 2$). The addition of ionomycin was considered to be time = 0 min. RFU were normalized against the fluorescence before induction.

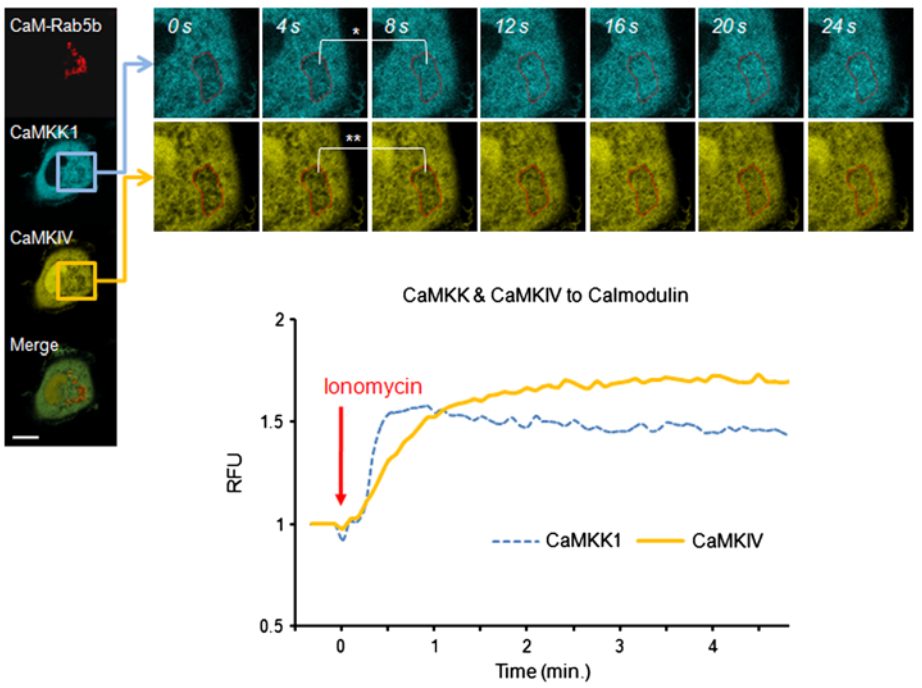


Fig. S6. Interactions between calmodulin and CaM kinase kinase1 (ECFP-CaMKK1) or CaM kinase IV (EYFP-CaMKIV) were monitored in HeLa cells using the ECLIPSE assay. Endosomal localization of the two CaM-binding proteins was induced simultaneously by 1 μM ionomycin. Asterisks indicate the beginning of endosomal localization of CaMKK1(*) and CaMKIV(**). The area surrounded by *red dots* indicates the ROI. RFU were normalized against the fluorescence before induction ($n = 1$). Scale bars = 10 μm.

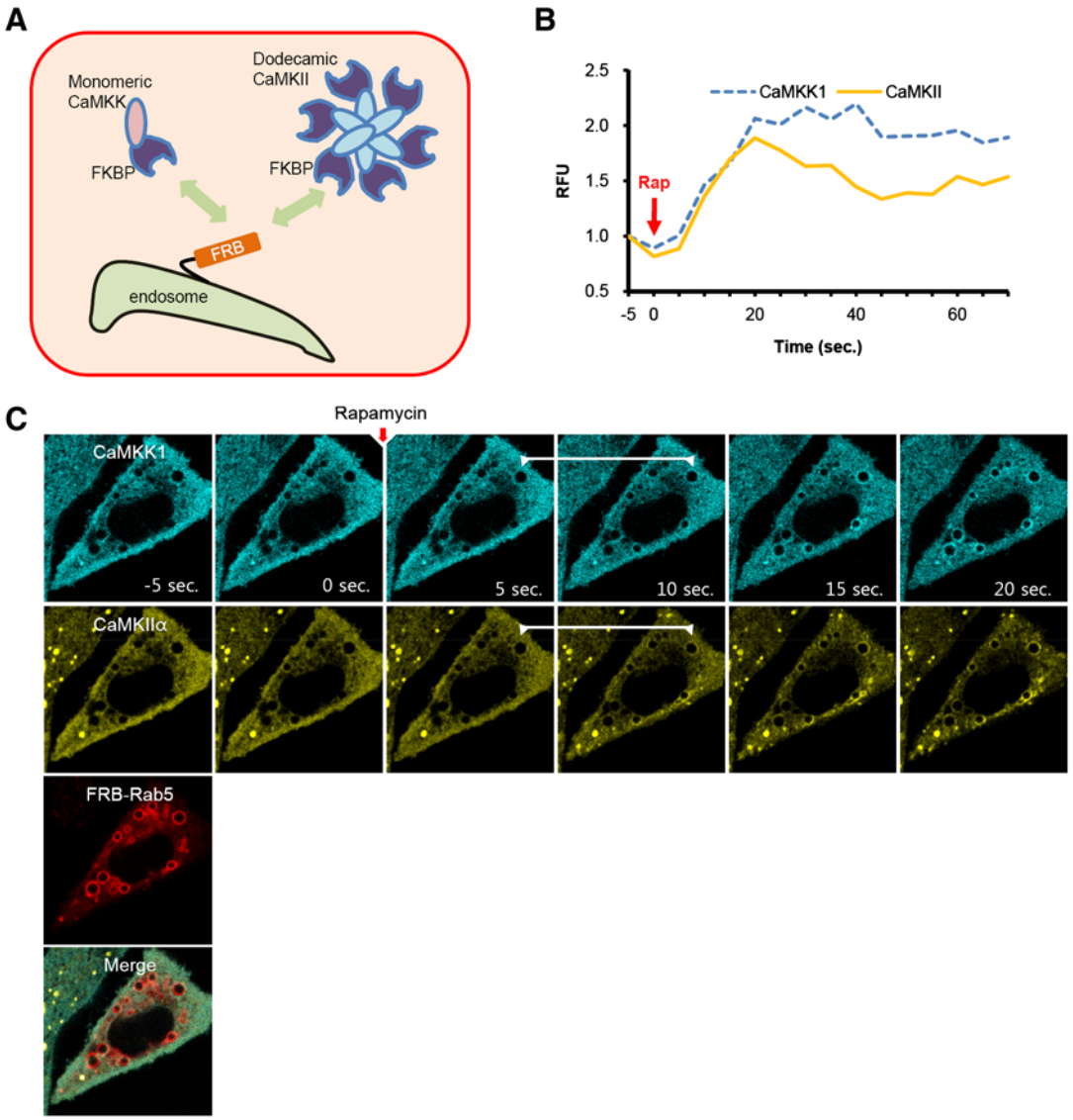
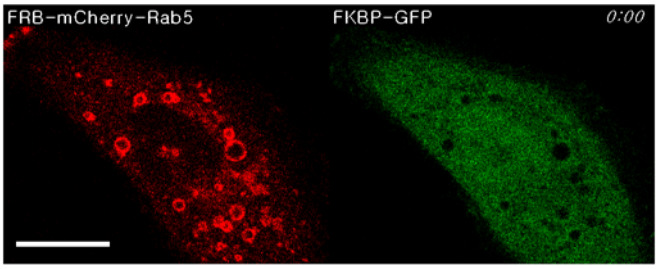


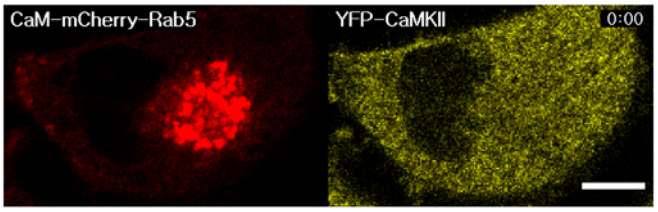
Fig. S7. The alternative test for comparing mobility caused from bulkiness. (*A*) Schematic concept of assay. When the same leading force (FKBP-FRB interaction by rapamycin) cause the endosomal location of two proteins which have different bulkiness from each other, their movements can be compared. From the assay with monomeric CaMKK1 and dodecamic CaMKIIα, two kinds of proteins recruited to the endosome at same time: quantitative analysis (*B*) and images (*C*). The addition of rapamycin was considered to be time = 0 min. RFU were normalized against the fluorescence before induction.

Table S1. Primers used

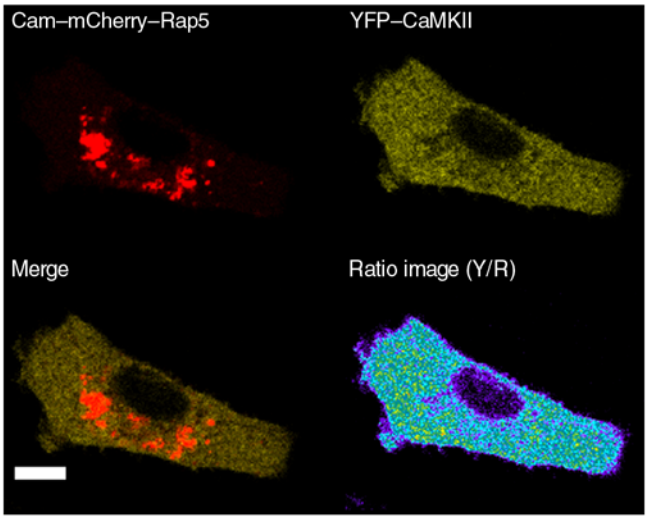
FKBP-Forward	5′-ATGCTCGCTAGCCCACCATGGGAGTGCAGGTGG-3′
FKBP-Reverse	5′-ATGCTCACCGGTGCTTCCAGTTTTAGAAGCTCCAC AT-3′
FRB-Forward	5′-GACTGCTAGCCCACCATGGCCTCCTACCC-3′
FRB-Reverse	5′-GACTACCGGTAGGGCGCCGGCGCCGGCGCCCTTTGAGATTCGTCGGAAC-3′
WASP(CRIB)-Forward	5′-GACTCTCGAGCCACCATGAAGAAGAAGATCAGCAAAGC-3′
WASP(CRIB)-Reverse	5′-GACTGGATCCTTGGCGCCGGCGCCGGCGCCAGAGGTCTCGGCGTCGG-3′
CaM-Forward	5′-GACTCTCGAG CTTCCGCCAT GGCTGAC-3′
CaM-Reverse	5′-GACTGGATCCTTGGCGCCGGCGCCGGCGCCCTTTGCAGTCATCATCTGTAC-3′



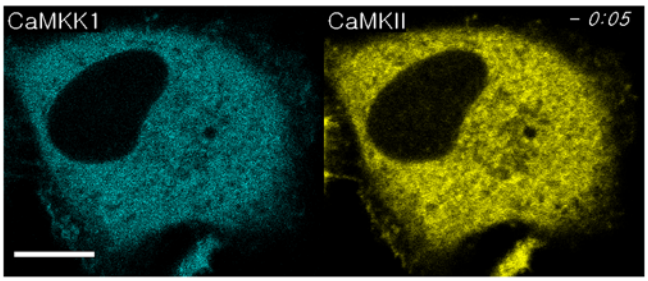
Movie S1. Proof-of-concept: endosomal localization with FKBP-Rapamycin-FRB interaction. The fluorescent fusion proteins, FRB-mCherry-Rab5 (*red*) and FKBP-EGFP (*green*), were coexpressed in HeLa cells. In the absence of induction, FRB localized to the enlarged endosomes, while FKBP-EGFP remained dispersed throughout the cytoplasm. The treatment with rapamycin induced immediate FKBP-EGFP recruitment to endosomes.
Movie S1 (MOV)



Movie S2. Two-hybrid format with calmodulin-CaMKIIα interaction. The simplified format was designed employing the calmodulin-Rab5 fusion protein (CaM-mCherry-Rab5) and YFP-conjugated CaMKIIα. The reversible interaction between calmodulin and CaMKIIα could be observed clearly by enhancing cytosolic Ca^{2+} levels.
Movie S2 (MOV)



Movie S3. The oscillatory interaction of calmodulin and CaMKIIα. The interactions between calmodulin and CaMKIIα were monitored over 30 min. After addition of ionomycin, the magnitude of CaMKIIα recruitment increased in four separate oscillations.
Movie S3 (MOV)



Movie S4. The sequential interaction of CaMKIIα and CaMKK1 to calmodulin. It was visualized the interactions of CaMKK1 and CaMKIIα to calmodulin in a single cell. Two kinases showed different initial rates of colocalization and CaMKK1 bound calmodulin faster than CaMKIIα in response of 1 μM of ionomycin.
Movie S4 (GIF)