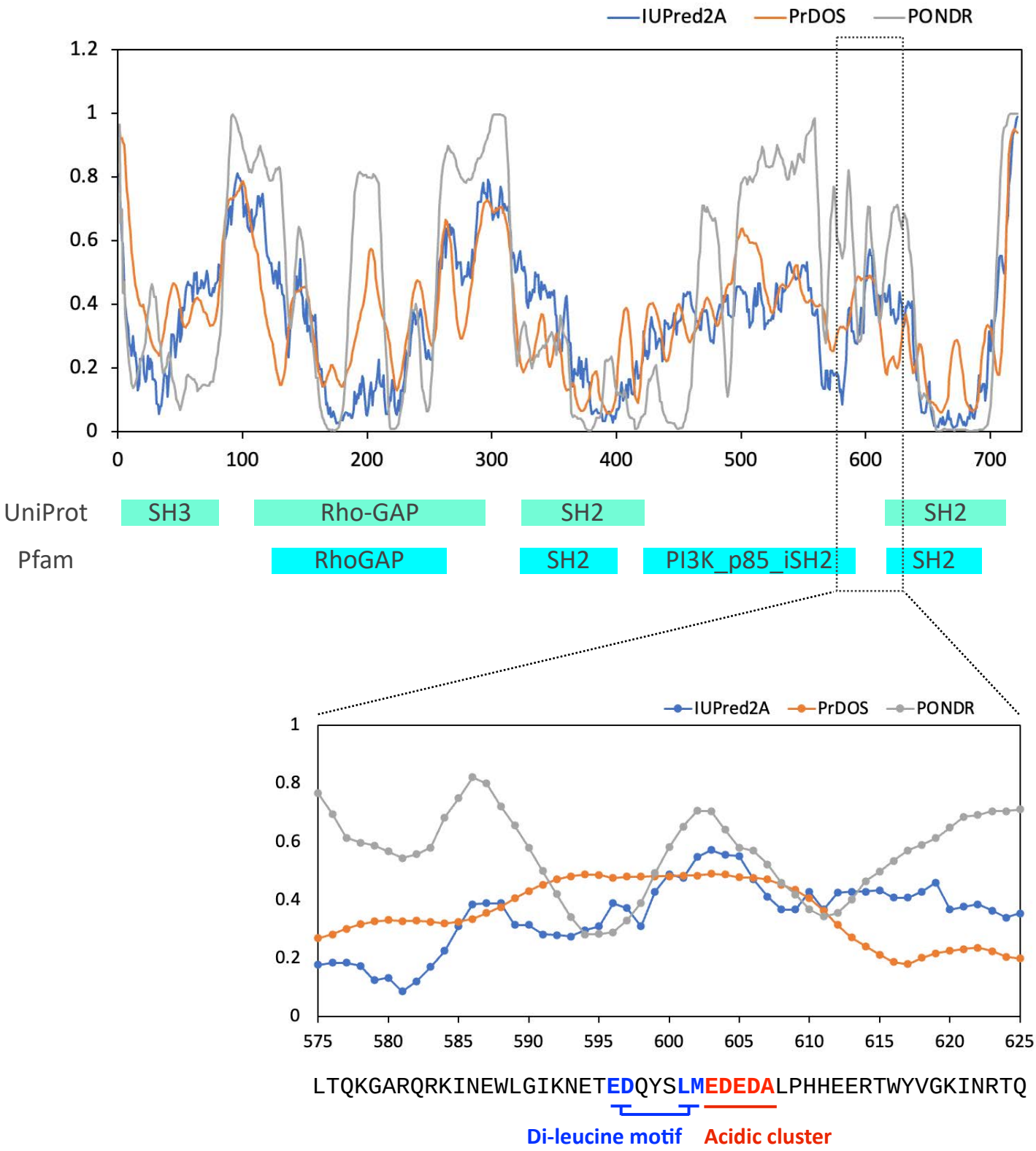
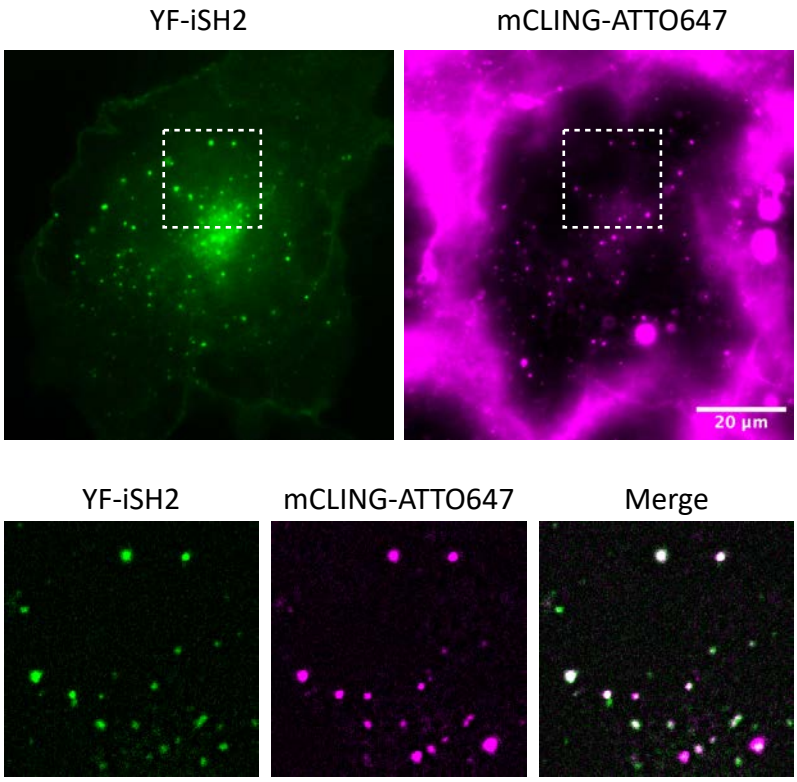


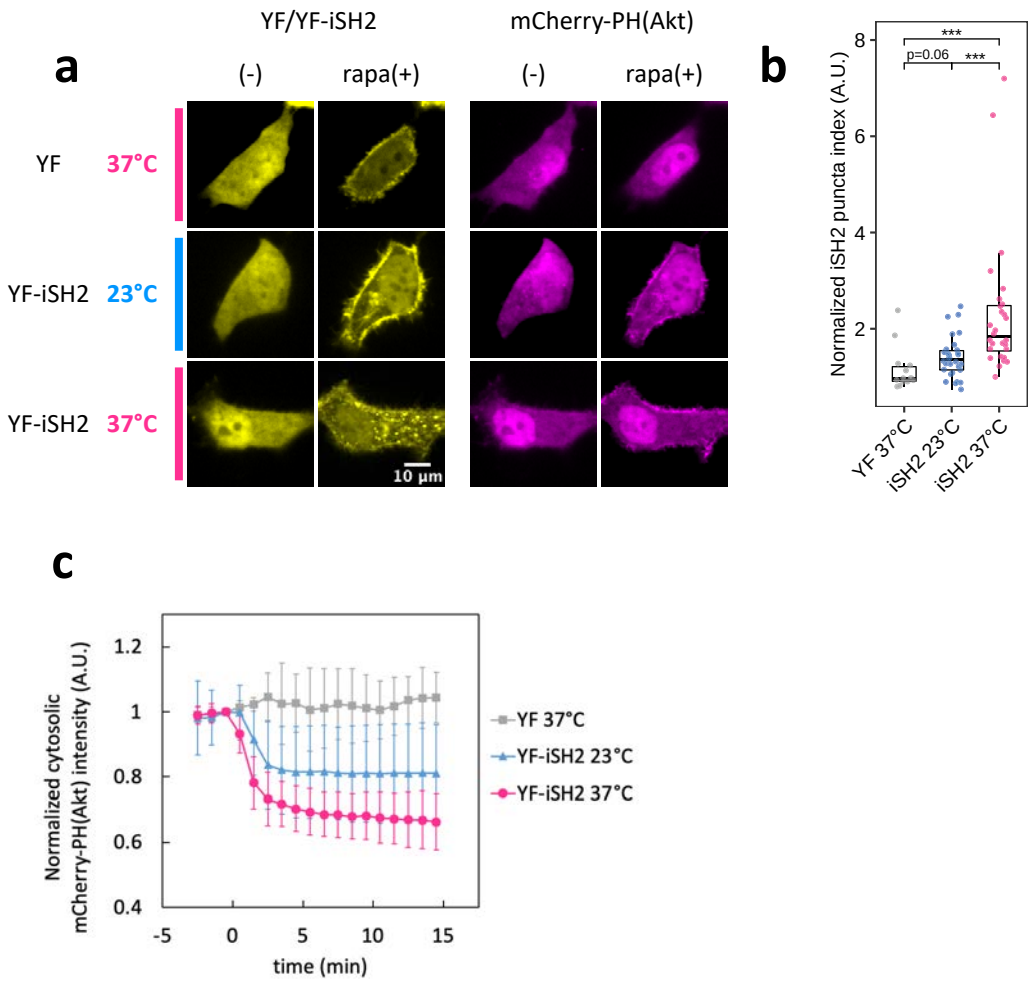
Extended Data Figure 1: Prediction of intrinsically disordered regions. Intrinsically disordered region of mouse p85β (PIK3R2) was analyzed by three algorithms, IUPred2A, PrDOS, and PONDR.

Extended Data Figure 1



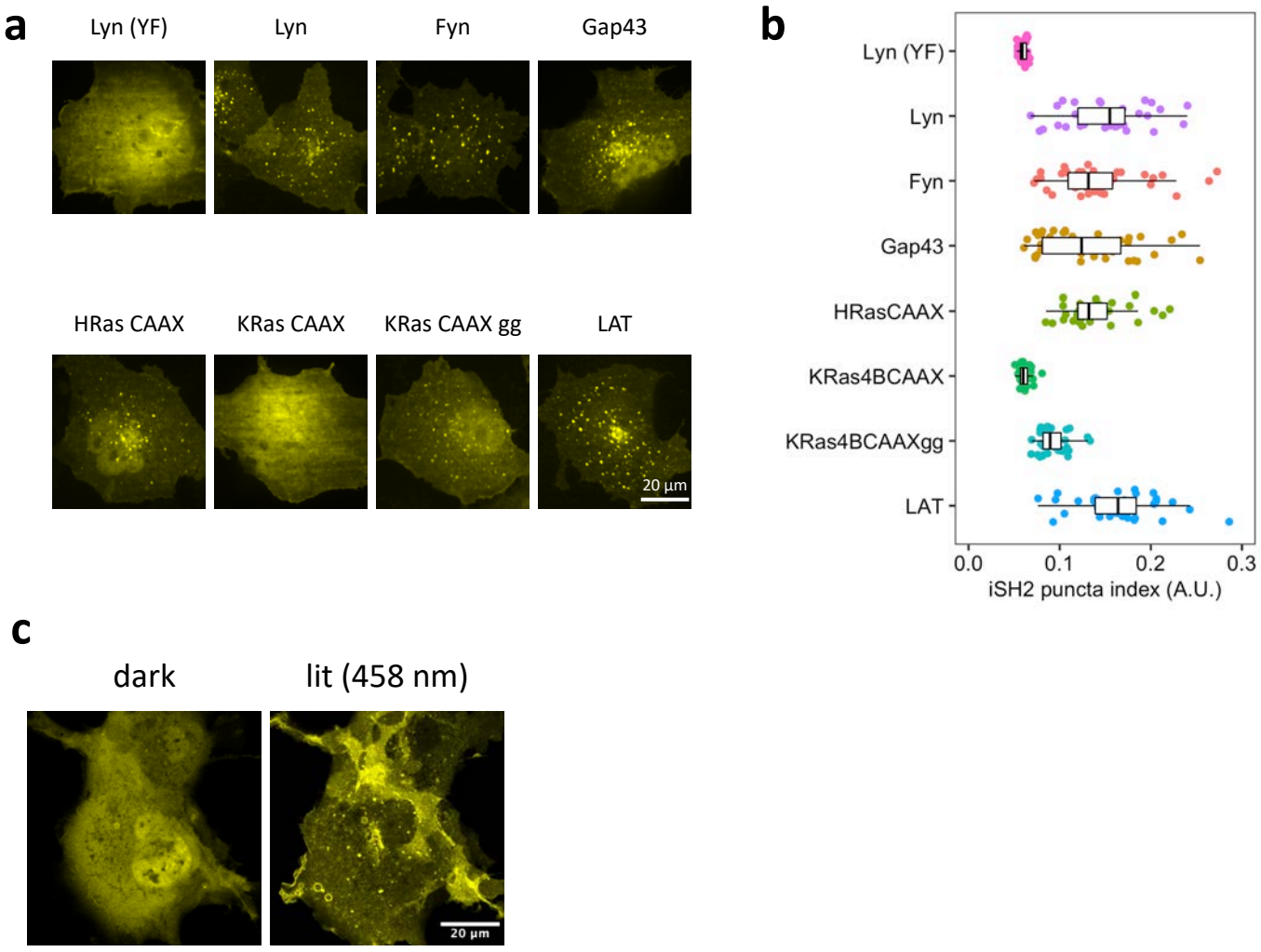
Extended Data Figure 2: iSH2-vesicles co-localize with mCLING dye. Epi-fluorescence microscopy images of iSH2-vesicles co-localized with extracellularly added mCLING-ATTO647. Cos7 cells were transiently transfected with Lyn-ECFP-FRB, EYFP-FKBP-iSH2, mCherry-PH(Akt). After mCLING addition, iSH2 translocation and vesicle formation was induced by 100 nM rapamycin. 30 min after rapamycin addition, the samples were chilled, washed, and fixed with 4% paraformaldehyde . Top: raw image of a transfected cell. Bottom: enlarged images of dashed line area of top images. To reduce background noise, median filtered values were subtracted from the raw images.

Extended Data Figure 2



Extended Data Figure 3: Temperature dependency of iSH2-mediated endocytosis. (a) Confocal images of endocytic vesicle production and PH(Akt) translocation. HeLa cells were transiently transfected with Lyn-ECFP-FRB, mCherry-PH(Akt), and EYFP-FKBP or EYFP-FKBP-iSH2. (-) before rapamycin addition, rapa(+) 20 min after adding 100 nM rapamycin. (b) Quantified iSH2-mediated endocytosis indices. The values were normalized by time=0. Box whisker plots represent median, 1st, 3rd quartiles and 1.5x inter-quartile range. P-value ***: < 0.001. Steel-Dwass test. (c) Time course of PH(Akt) translocation. Cytosolic intensity of mCherry-PH(Aki) was quantified and normalized by time=0. Error bars represent standard deviation. YF 37°C, n=15 cells. YF-iSH2 23°C, n=30 cells. YF-iSH2 37°C, n=28 cells.

Extended Data Figure 3



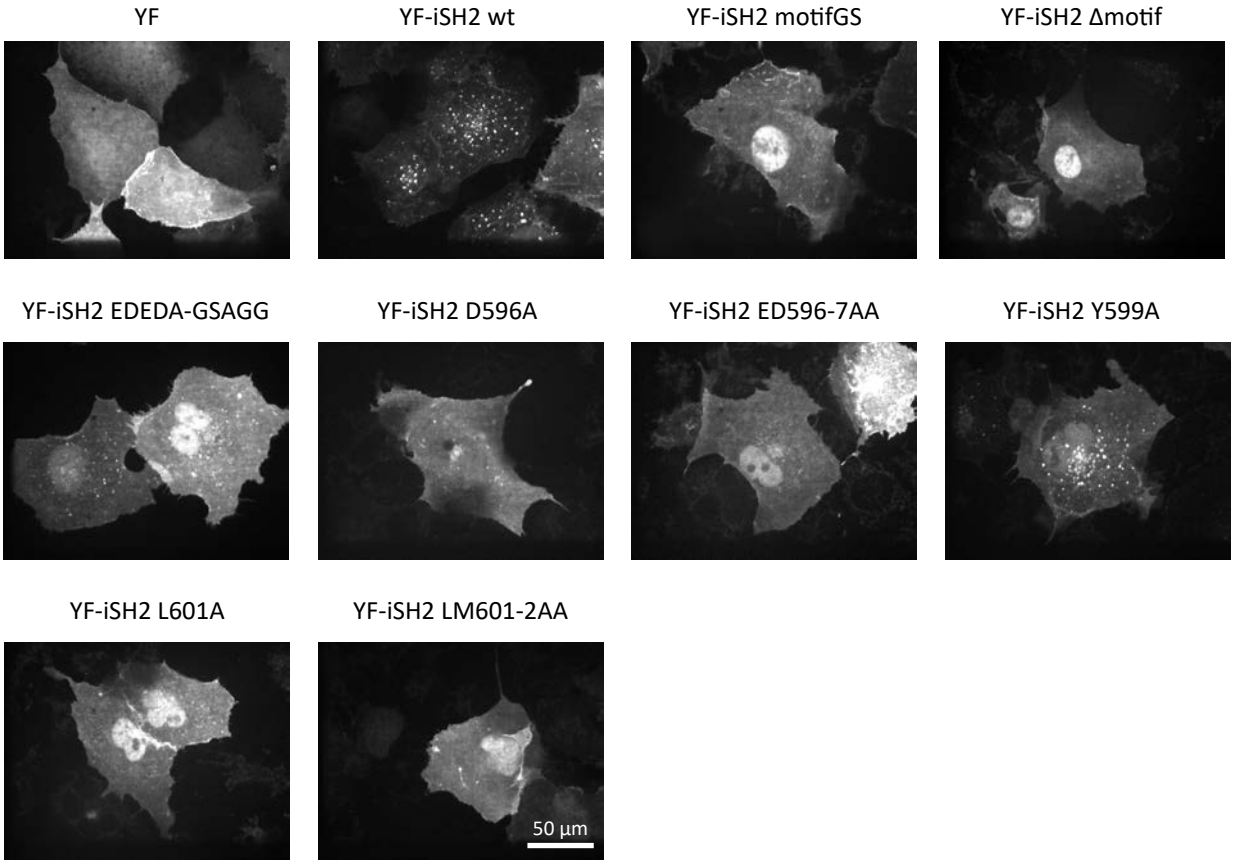
Extended Data Figure 4: Generality of iSH2-mediated endocytosis. (a, b) Confocal images of iSH2-vesicles produced with different plasma membrane anchors and the quantified iSH2 puncta index. Cos7 cells were transiently transfected with EYFP-FKBP-iSH2, mCherry-PH(Akt), and ECFP-FRB fused with different types of plasma membrane anchors. 15 min after adding 100 nM rapamycin, cells were chilled, washed, and fixed with 4% paraformaldehyde and 0.15% glutaraldehyde. Box whisker plots represent median, 1st, 3rd quartiles and 1.5×inter-quartile range. (c) Confocal images of iSH2-vesicles induced by iLID/SspB system. Cos7 cells were transiently transfected with Lyn-iLID and EYFP-SspB-iSH2. dark: before light stimulation. lit (458 nm): 15 min after 458 nm light illumination. EYFP-SspB-iSH2 shows punctate structure in the cytosol.

Extended Data Figure 4

a

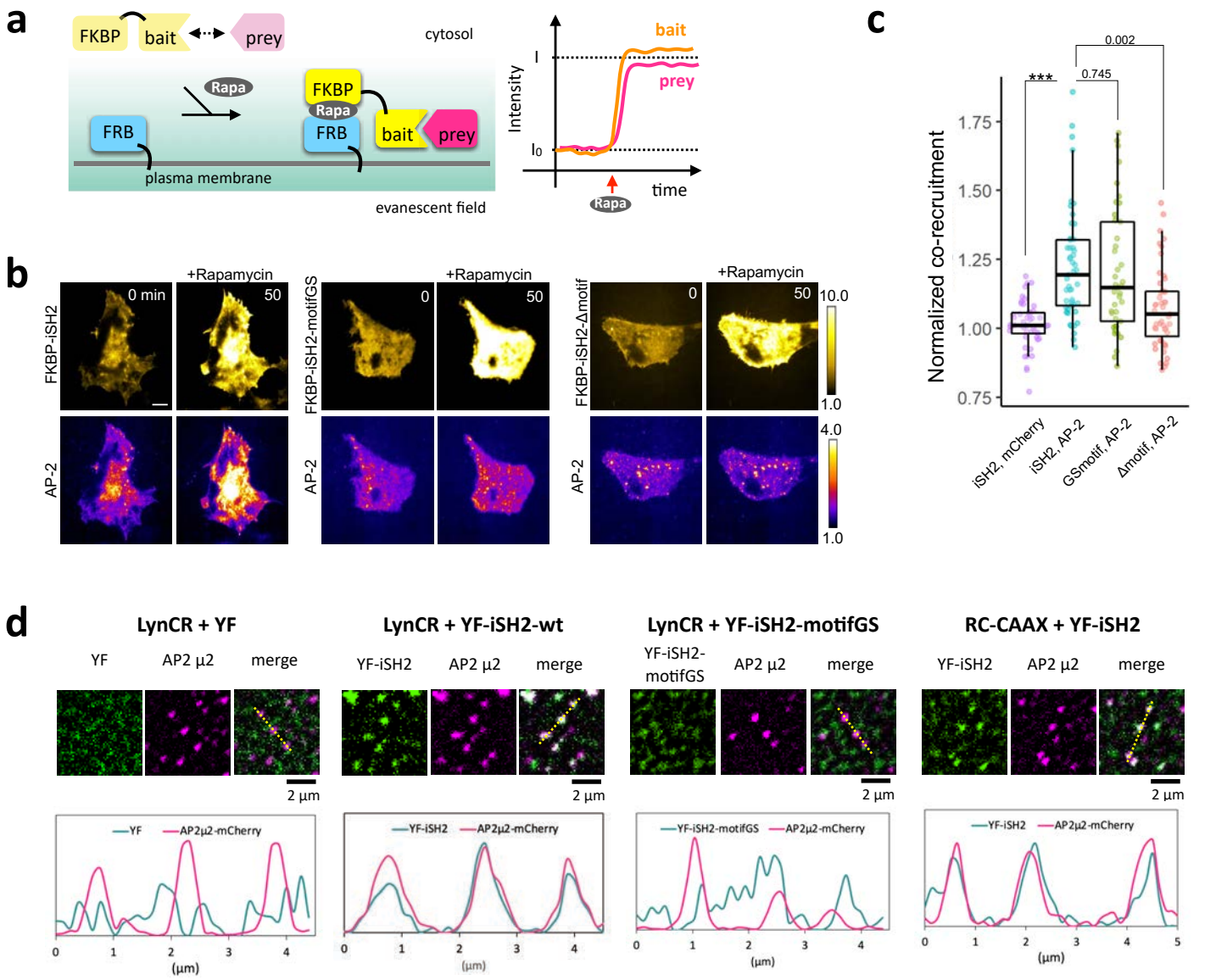
			Endocytosis
	Di-leucine motif	Acidic cluster	
	Yxxϕ		
wt	594 ETEDQYSLMEDEDALP 609		+++
motifGS	ETSAGGSAGGSAGGLP		-
Δmotif	ET----------LP		-
EDEDA-GSAGG	ETEDQYSLMGSAGGLP		+
D596A	ETEAQYSLMEDEDALP		-
ED596-7AA	ETAAQYSLMEDEDALP		-
Y599A	ETEDQASLMEDEDALP		+++
L601A	ETEDQYSAMEDEDALP		-
LM601-2AA	ETEDQYSAAEDEDALP		-

b



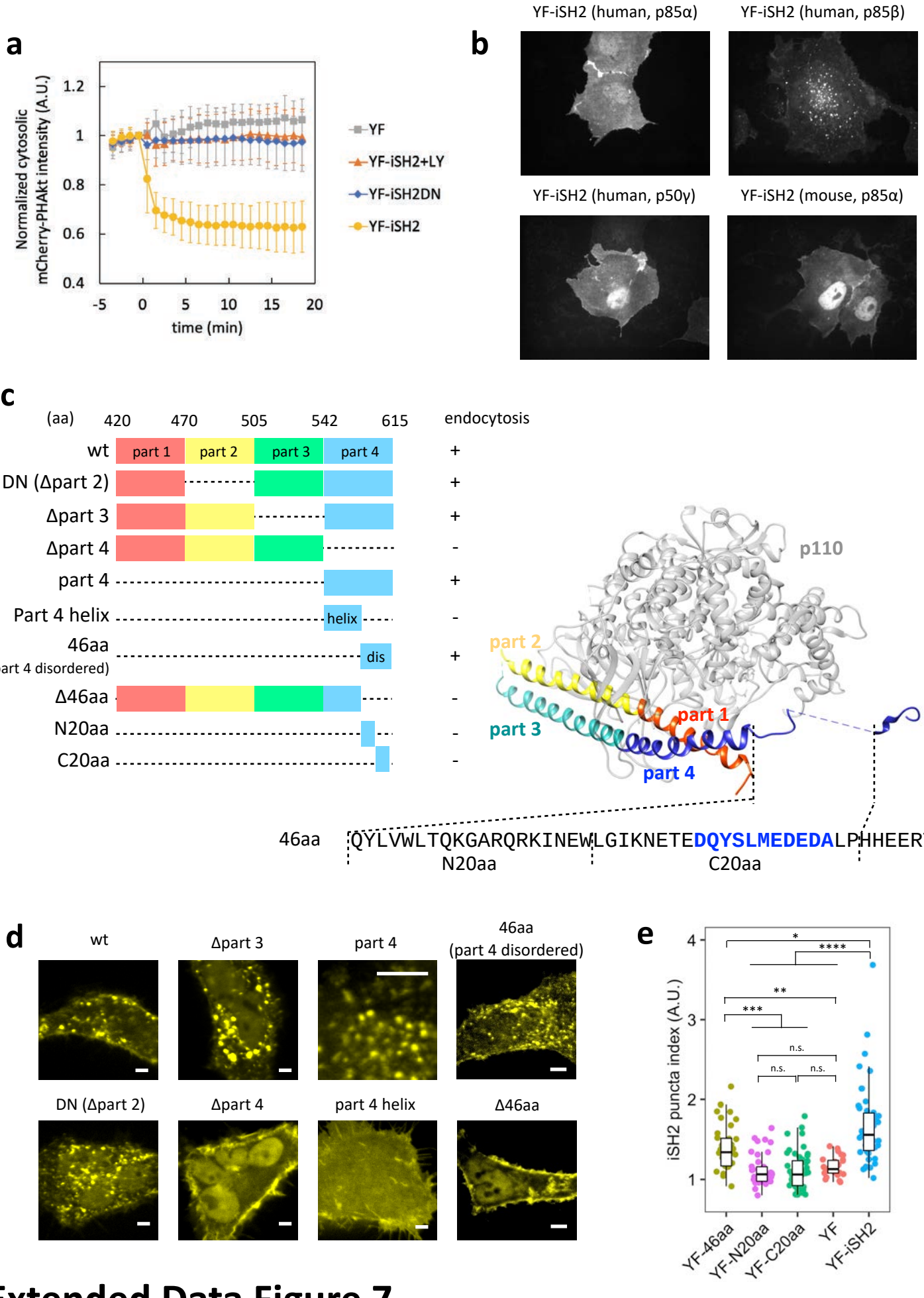
Extended Data Figure 5: Vesicle formation with iSH2 variants. (a) List of the tested iSH2 mutants. Underlines indicate mutation sites. Here, wild type is derived from iSH2 domain of mouse p85β. (b) Confocal images of iSH2-vesicles produced with wild type and mutant iSH2. Cos7 cells were transiently transfected with Lyn-ECFP-FRB, EYFP-FKBP-iSH2, and mCherry-PH(Akt). 15 min after adding 100 nM rapamycin, cells were chilled, washed, and fixed with 4% paraformaldehyde and 0.15% glutaraldehyde.

Extended Data Figure 5

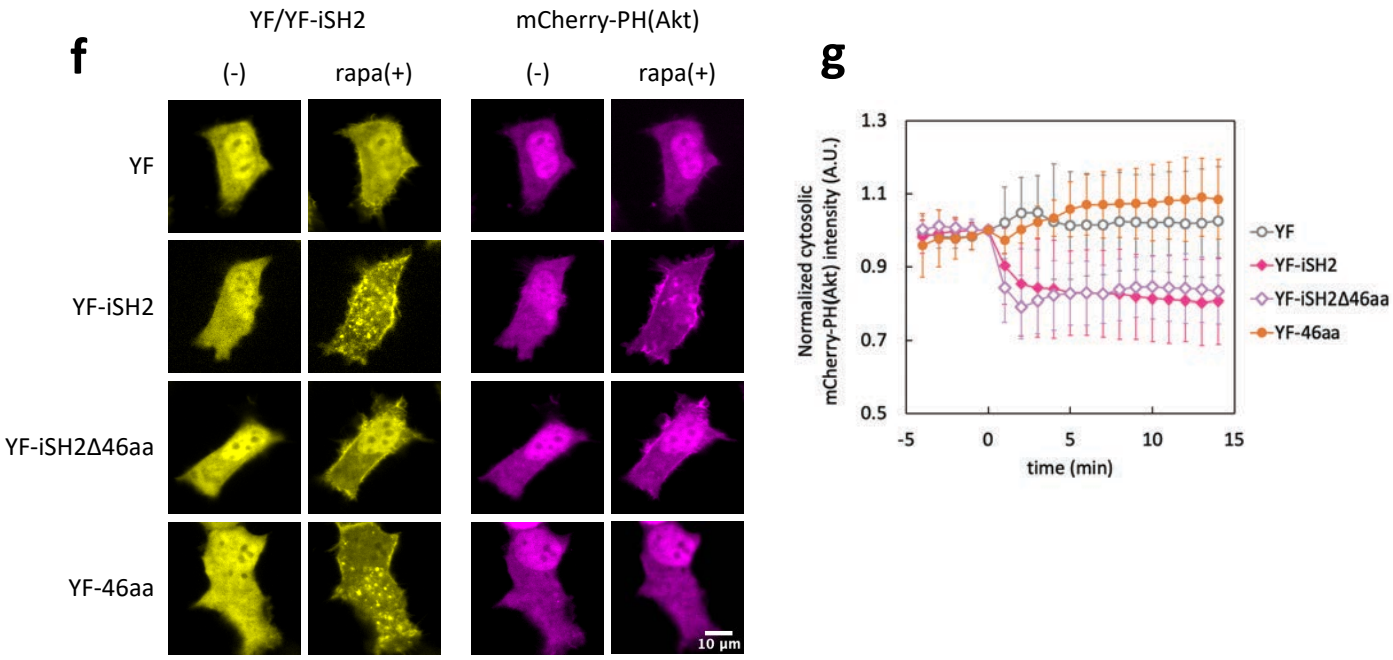


Extended Data Figure 6: iSH2 recruits AP-2 to plasma membrane. (a) Schematic of co-recruitment assay. Interaction between bait and prey was evaluated by rapamycin-dependent increase in plasma membrane intensify of prey, here AP-2-mCherry. (b) Representative images showing changes in TIRF fluorescence intensities on plasma-membrane recruitment of EYFP-FKBP-iSH2, EYFP-FKBP-iSH2-motifGS and EYFP-FKBP-iSH2-Δmotif and corresponding changes in AP2-mCherry intensities. Scale bar: 10 µm. (c) Co-recruitment levels (I/I_0) of mCherry with EYFP-FKBP-iSH2 and of AP2-mCherry with EYFP-FKBP-iSH2, EYFP-FKBP-iSH2-GSmotif and EYFP-FKBP-iSH2-Δmotif using the live cell co-recruitment assay. ***, P < 0.001 or as shown, Student's t test. (d) TIRF images showing co-localization between YF-iSH2 and mCherry-AP-2(µ2). During live cell imaging, images were taken 1 min after 100 nM rapamycin addition. To reduce noise, median filtered images were subtracted from raw images. Graphs show line scan of dashed lines in merge images. (e) Pearson's correlation between YFP signal and mCherry-AP-2(µ2) signal of (d). Calculation was performed on raw images. For each cells, 10 µm (80 pixels) x 10 µm (80 pixels) areas were selected for the quantification. Steel-Dwass test. P-values: *: < 0.05, **: < 0.01, ***: < 0.001, ****: < 0.0001.

Extended Data Figure 6



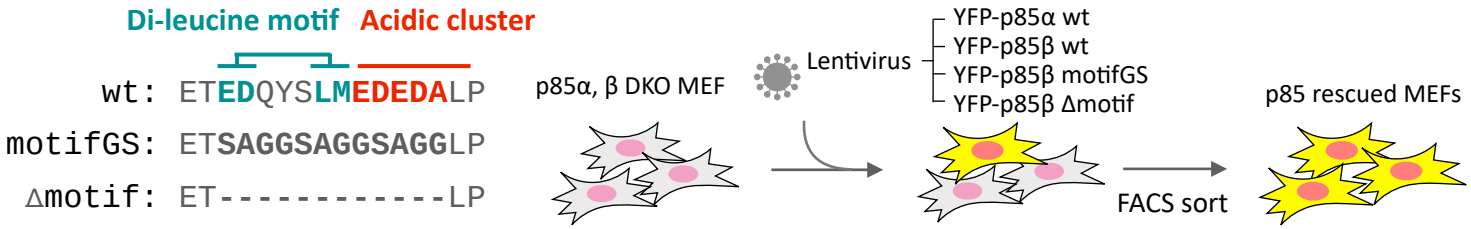
Extended Data Figure 7



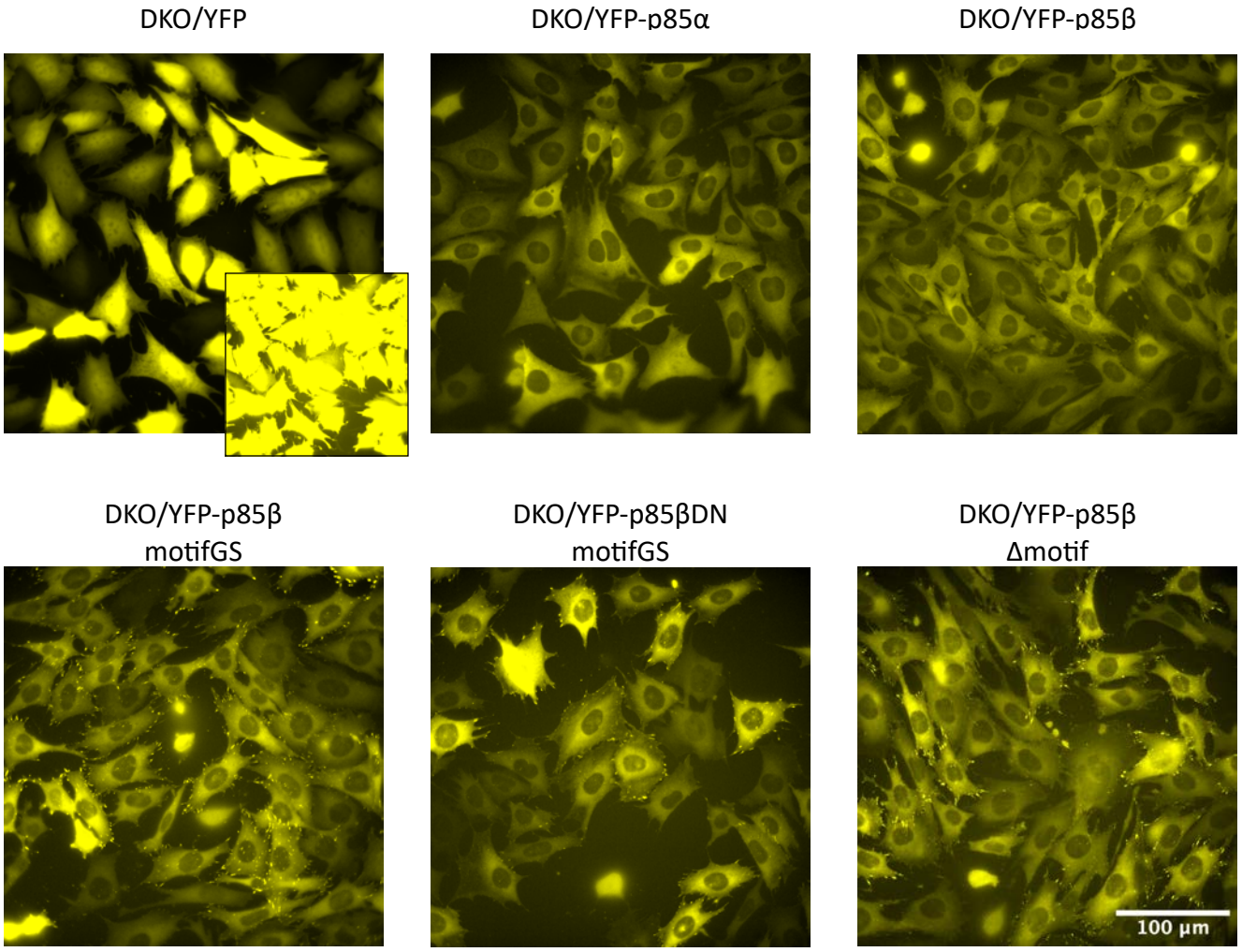
Extended Data Figure 7: iSH2-mediated endocytosis is independent of PI3K catalytic activity and C-terminal 46 aa region is necessary and sufficient. (a) Time course of PH(Akt) translocation of Fig. 2a. Cytosolic intensity of mCherry-PH(Akt) was quantified and normalized by time=0. Error bars represent standard deviation. YF-iSH2, n=30 cells. YF-iSH2 + LY, n=28 cells. YF-iSH2DN, n=27 cells. YF, n=28 cells. (b) Confocal images of vesicles induced by iSH2 derived from different p85 isoforms. Cos7 cells were transiently transfected with Lyn-ECFP-FRB, EYFP-FKBP-iSH2, and mCherry-PH(Akt). 15 min after adding 100 nM rapamycin, cells were chilled, washed, and fixed with 4% paraformaldehyde and 0.15% glutaraldehyde. (c) Schematic representation of iSH2 truncates. Crystal structure of p110β-iSH2β is derived from PDB 2y3a. (d) Representative confocal image of live-cell plasma membrane recruitment of iSH2 truncates in HeLa expressing Lyn-ECFP-FRB, EYFP-FKBP-iSH2 (truncates), and mCherry-PH(Akt). Scale bar, 5 μm. (e) Quantified iSH2 puncta index of iSH2 truncates tested in Cos7 cells expressing Lyn-ECFP-FRB, EYFP-FKBP-iSH2 (truncates), and mCherry-PH(Akt). YF-46aa, n=38 cells. YF-N20aa, n=39 cells. YF-C20aa, n=48 cells. YF, n=27 cells. YF-iSH2, n=46 cells. Box whisker plots represent median, 1st, 3rd quartiles and 1.5×inter-quartile range. P-values (Steel-Dwass test): *: < 0.05, **: < 0.01, ***: < 0.001, ****: < 0.0001. n.s.: not significant. (f) Confocal live-cell images of iSH2-vesicles and PH(Akt) translocation. (g) Time course of PH(Akt) translocation of (f). Cytosolic intensity of mCherry-PH(Aki) was quantified and normalized by time=0. Error bars represent standard deviation. YF, n=17 cells. YF-iSH2, n=41 cells. YF-iSH2Δ46aa, n=39 cells. YF-46aa, n=22 cells. (f, g) Data correspond with Fig. 2c.

Extended Data Figure 7

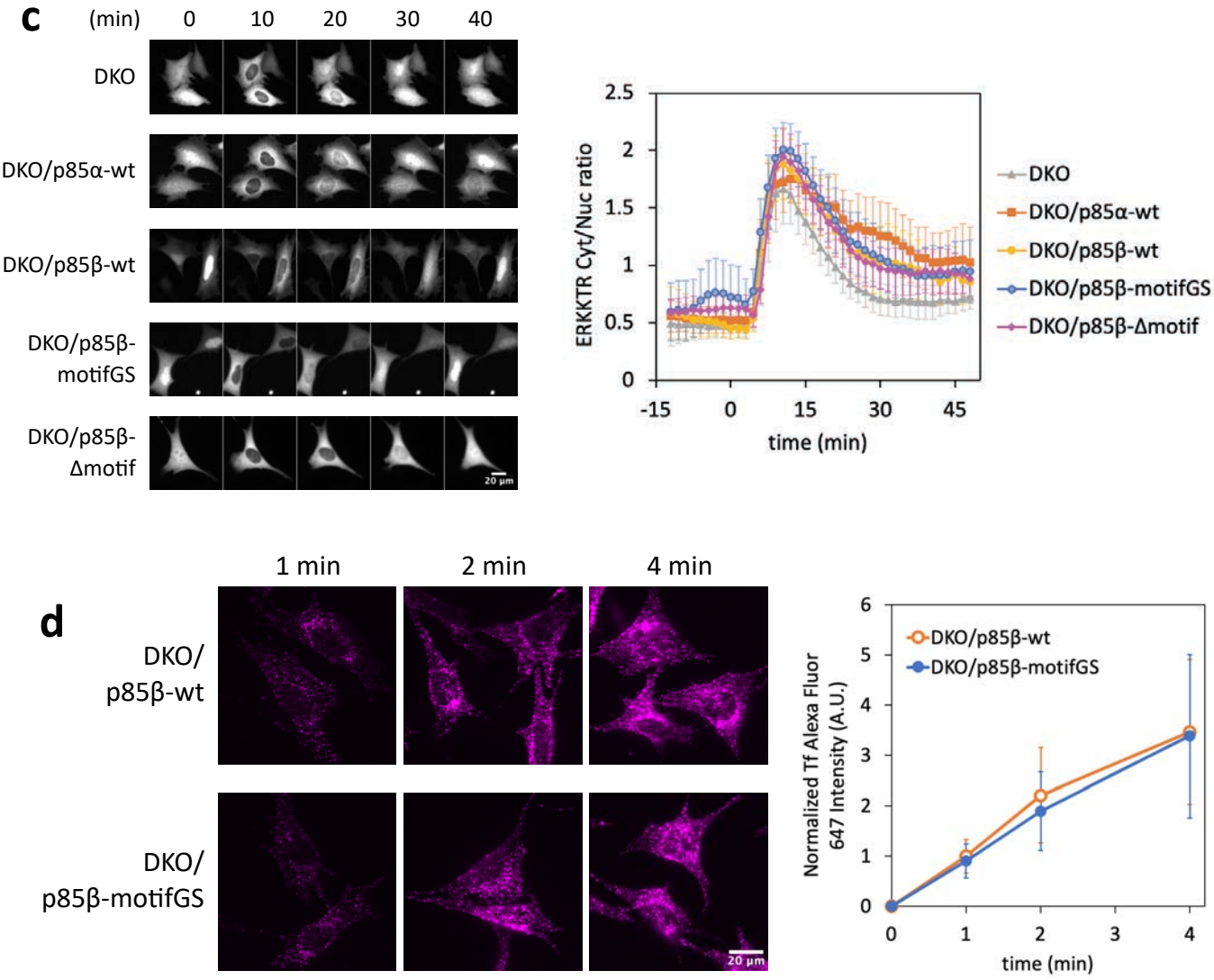
a



b

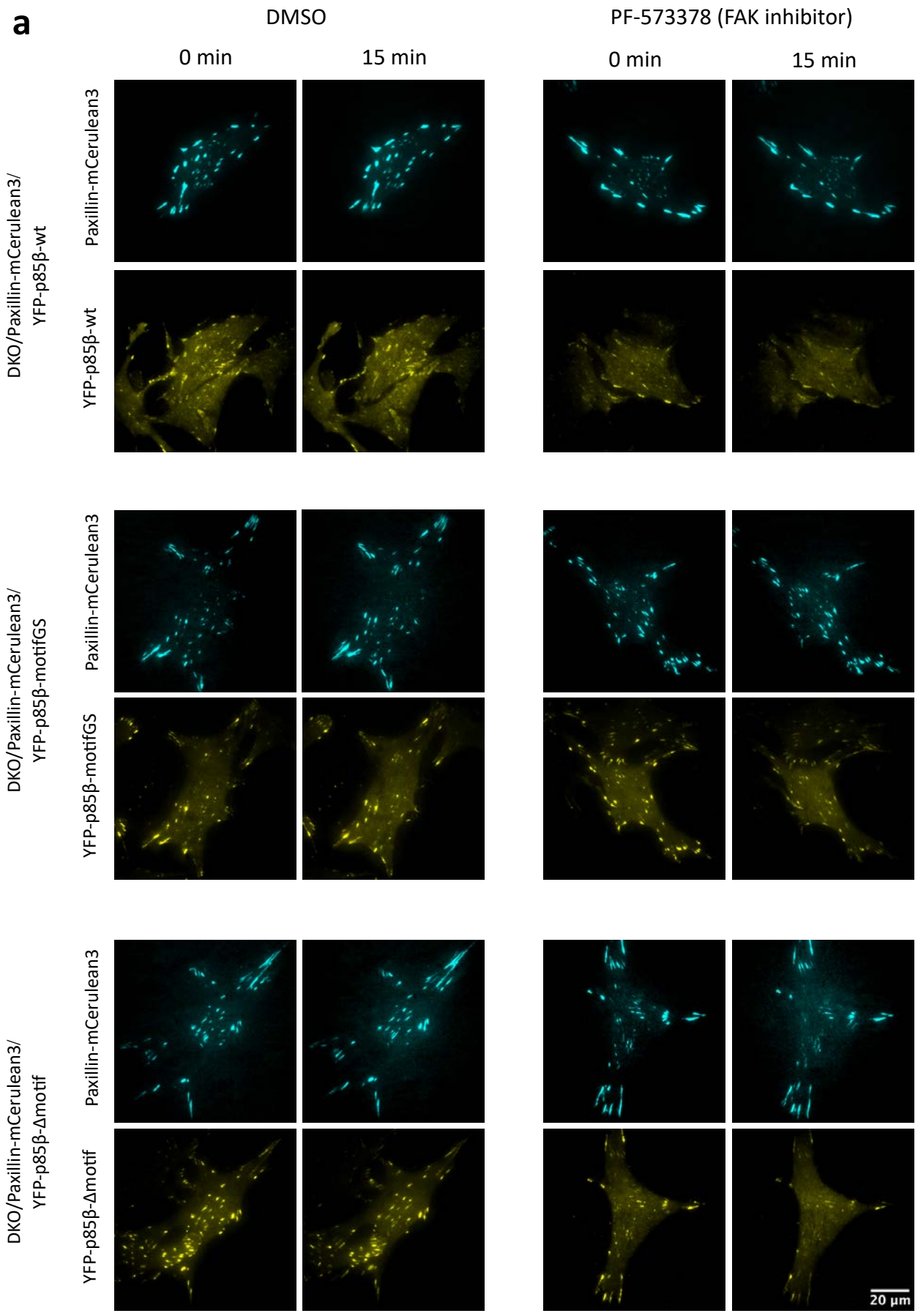


Extended Data Figure 8



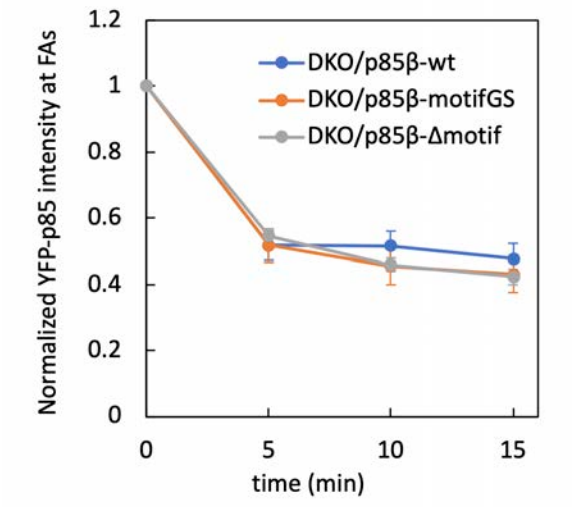
Extended Data Figure 8: Generation and Functional analysis of p85-rescued MEFs. (a) p85α, β double knockout (DKO) MEFs were infected with lentiviruses encoding YFP-p85 variants. Infected cells were FACS-sorted by YFP fluorescence. (b) Epi-fluorescence microscopy images of each cell lines. Dynamic range was adjusted between. c) ERK response to PDGF stimulation. Each cell lines were transiently transfected with mCherry-ERKKTR. The cells were serum starved and stimulated with 50 ng/mL PDGF-BB. ERKKTR response was recorded by live cell imaging at 37°C with 5% CO_2. Left: epi-fluorescence microscopy images of mCherry-ERKKTR. Right: quantified Cytosol/Nucleus ratio of mCherry-ERKKTR. Error bars represent 2x SEM (95% CI). DKO, n=18 cells. DKO/p85α-wt, n=18 cells. DKO/p85β-wt, n=18 cells. DKO/p85β-motifGS, n=19 cells. DKO/p85β-Δmotif, n=19 cells. (d) Transferrin uptake. Alexa Fluor 647-conjugated transferrin was added to serum starved cells. After the indicated time, the cells were chilled, washed with acid, and fixed with 4% paraformaldehyde. Left: epi-fluorescence microscopy images of Alexa Fluor 647-conjugated transferrin. Right: quantified Alexa Fluor 647 intensity. Error bars represent standard deviation. n>61 cells for each time point.

Extended Data Figure 8



Extended Data Figure 9

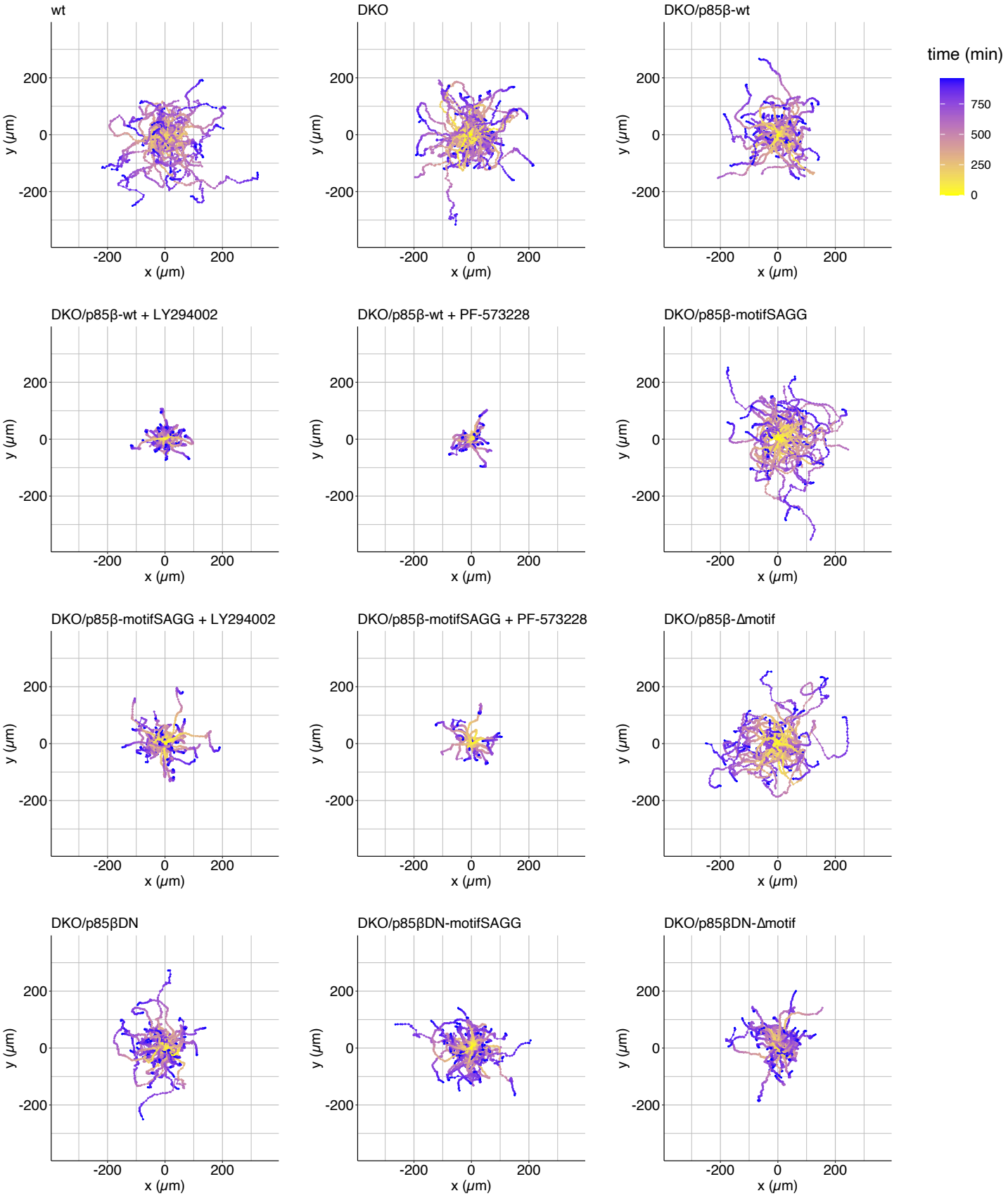
b



Extended Data Figure 9: PF-573378 (FAK inhibitor) response of p85 variants. (a) TIRF images of MEFs stably expressing Paxillin-mCerulean3 and YFP-p85 variants. The cells were serum starved and imaged at 37°C with 5% CO_2. (b) Normalized YFP-p85 intensity at focal adhesions. YFP-p85 intensity at focal adhesion was measured with image masks created by Paxillin-mCerulean3 images and normalized by time=0. Error bars represent standard deviation. DKO/p85β-wt, n=20 cells. DKO/p85β-motifGS, n=22 cells. DKO/p85β-Δmotif, n=18 cells.

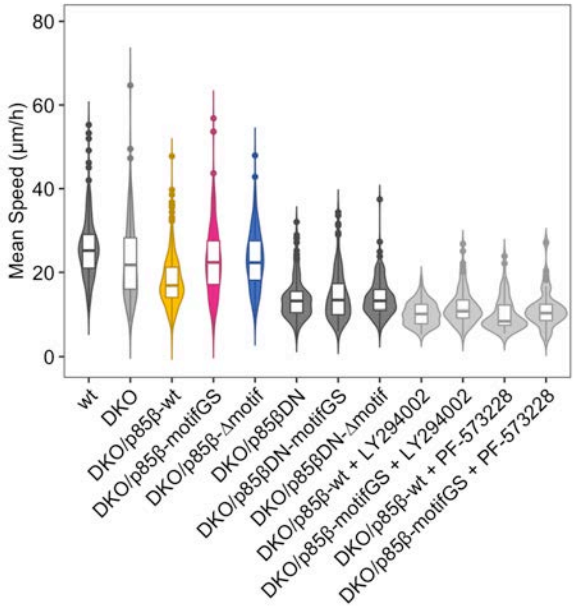
Extended Data Figure 9

a



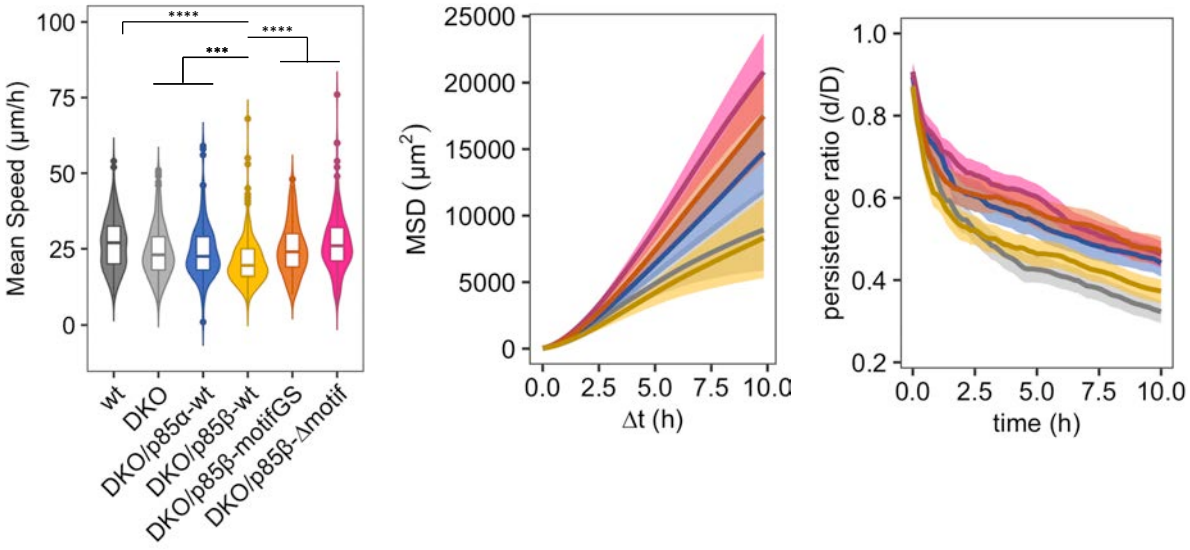
Extended Data Figure 10

b

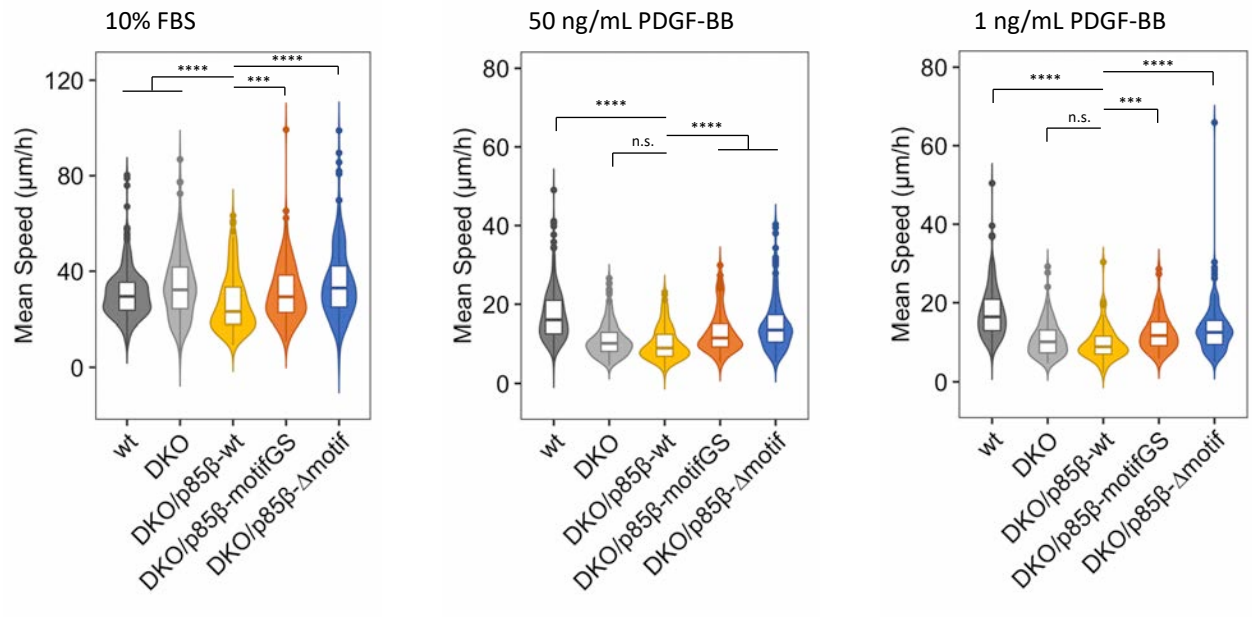


Extended Data Figure 10: Supplementary data of migration assay. (a–c) Random migration. (a) Cell track analysis of each cell lines. Data correspond with Fig. 4b-d. (b) Full data of random migration including PI3K inhibitor LY294002 data and FAK inhibitor PF-573228 data. Data correspond with Fig. 4b-d. (c) Different data set of random migration including DKO/p85α-wt. (d) Different data set with PDGF stimulation.

c



d



Extended Data Figure 10