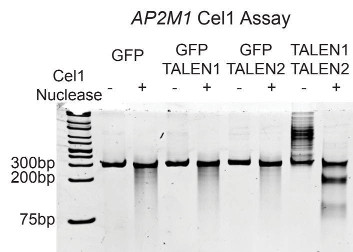
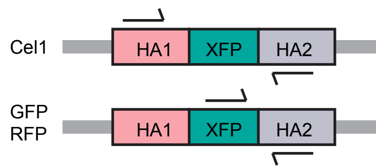
# Supplemental Figure 1

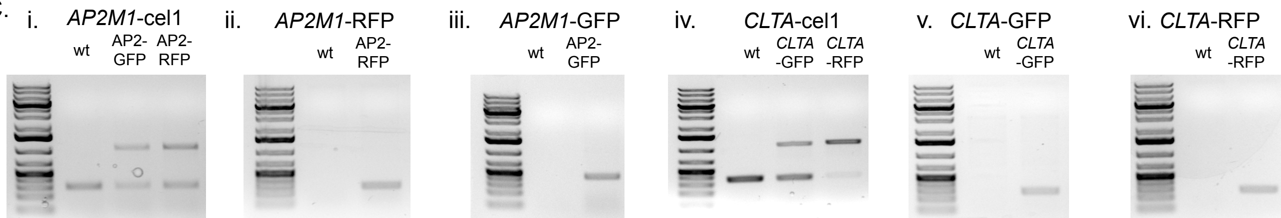
A.




B.




C.




D. Example of reciprocal track association.

i. y (um) 6.2 6.1 6.0 5.9; x (um) 3.9 4.0 4.1 4.2 4.3

ii.

iii. x (um) 4.4 4.2 4.0 3.8; 60 64 68 72 76 80 84 88 82 96

iv. y (um) 6.2 6.1 6.0 5.9; 60 64 68 72 76 80 84 88 82 96; time (s)

v. RFP movie strip

vi. GFP movie strip

vii. RFP montage

viii. GFP montage

Supplemental Figure 2

A. SVM classifier for AP2-RFP from AP2-RFP/clathrin-GFP cell line

i.
Feature 2
Max intensity (AU)
2.5
2.0
1.5
1.0
0.5
50 100 150
Feature 1
Track lifetime (sec)

ii.
Feature 3
Overall displacement (um)
1.0
0.8
0.6
0.4
0.2
50 100 150
Feature 1
Track lifetime (sec)

iii.
Feature 4
Intensity change (AU)
0.6
0.4
0.2
50 100 150
Feature 1
Track lifetime (sec)

iv.
Feature 3
Overall displacement (um)
1.0
0.8
0.6
0.4
0.2
0.5 1.0 1.5 2.0 2.5
Feature 2
Max intensity (AU)

v.
Feature 4
Intensity change (AU)
0.6
0.4
0.2
0.5 1.0 1.5 2.0 2.5
Feature 2
Max intensity (AU)

vi.
Feature 4
Intensity change (AU)
0.6
0.4
0.2
0.2 0.4 0.6 0.8 1.0
Feature 3
Overall displacement (um)

B. SVM classifier for clathrin-GFP from AP2-RFP/clathrin-GFP cell line

i.
Feature 2
Max intensity (AU)
2.0
1.5
1.0
0.5
50 100 150
Feature 1
Track lifetime (sec)

ii.
Feature 3
Overall displacement (um)
1.0
0.8
0.6
0.4
0.2
50 100 150
Feature 1
Track lifetime (sec)

iii.
Feature 4
Intensity change (AU)
0.8
0.6
0.4
0.2
50 100 150
Feature 1
Track lifetime (sec)

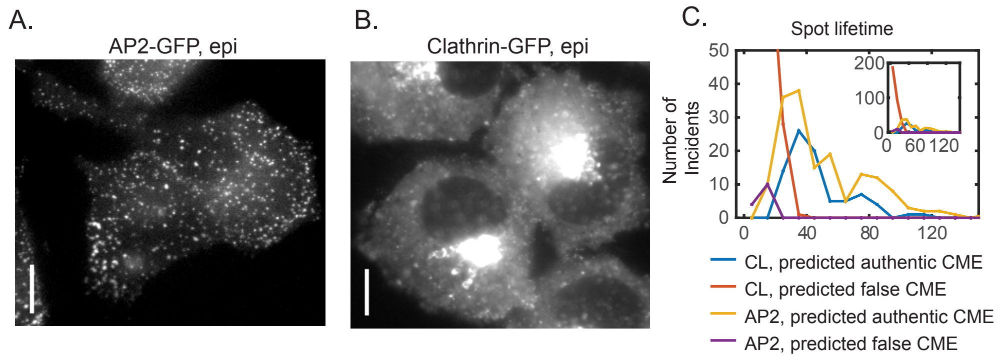
iv.
Feature 3
Overall displacement (um)
1.0
0.8
0.6
0.4
0.2
0.5 1.0 1.5 2.0
Feature 2
Max intensity (AU)

v.
Feature 4
Intensity change (AU)
0.8
0.6
0.4
0.2
0.5 1.0 1.5 2.0
Feature 2
Max intensity (AU)

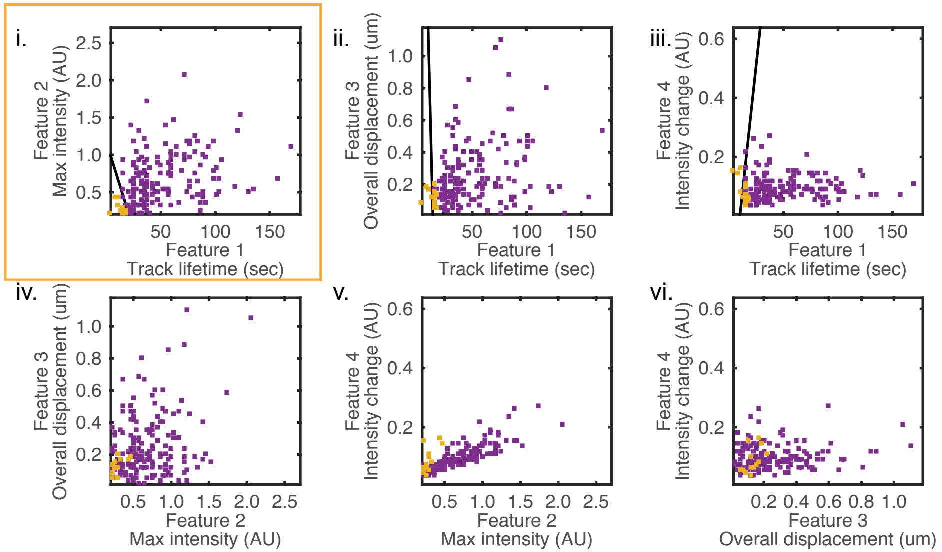
vi.
Feature 4
Intensity change (AU)
0.8
0.6
0.4
0.2
0.5 1.0
Feature 3
Overall displacement (um)

Authentic CME False CME

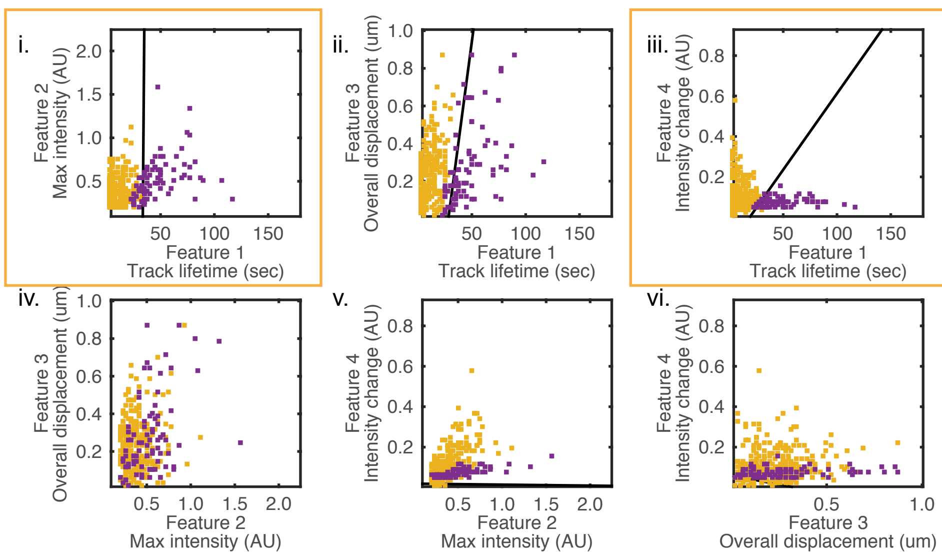
Supplemental Figure 3




D. SVM classifier for AP2-GFP from AP2-GFP cell line




E. SVM classifier for clathrin-GFP from clathrin-GFP cell line




■ Predicted true CME ■ Predicted false CME

Supplemental Figure 4

A. AP2 intensity profiles

AP2 only, 0-10sec

AP2 only, 10-20sec

AP2 only, 20-30sec

AP2 only, 30-40sec

Productive, 0-10 sec

Productive, 10-20 sec

Productive, 20-30 sec

Productive, 30-40 sec

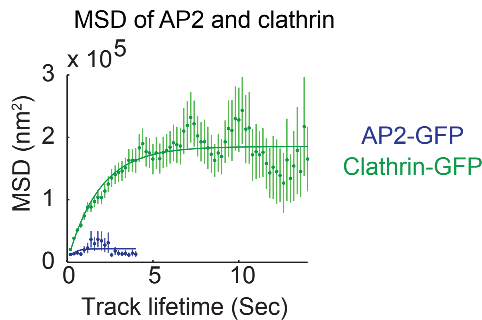
Fluorescence Intensity (AU)

4000
3000
2000
1000
0

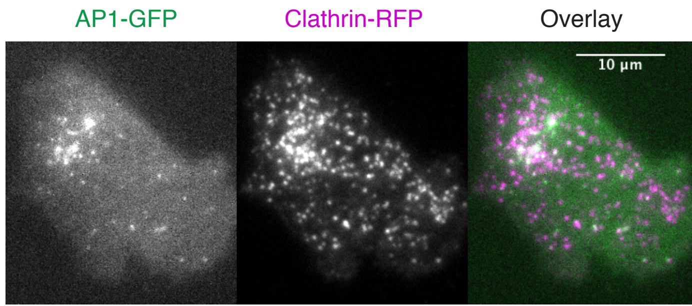
-20 0 20 40

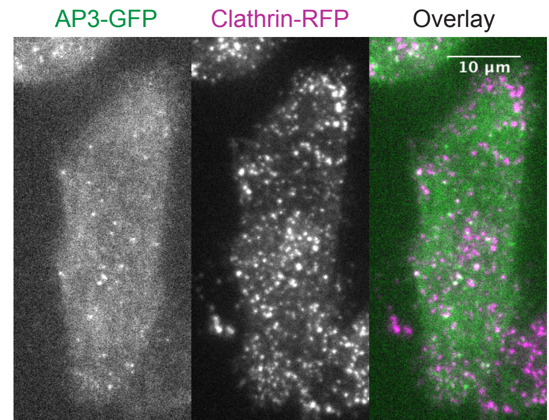
time (sec)

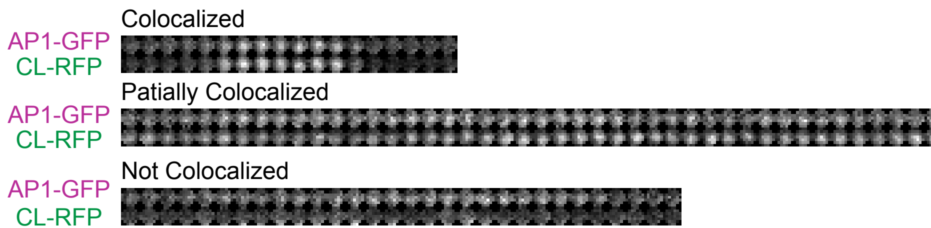
B.

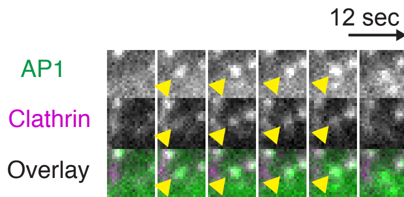


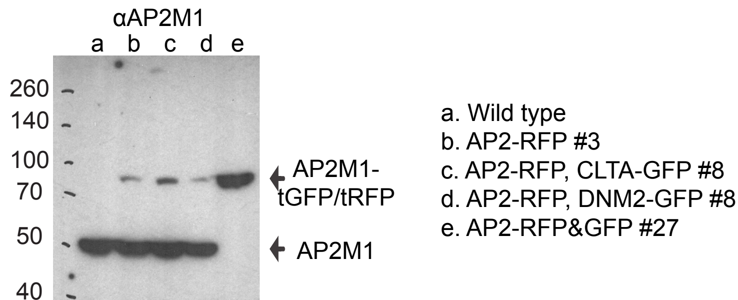
Supplemental Figure 5



## Supplemental Figure Legends

### Supplemental Figure 1, related to figure 1

**Co-labeling of AP2 and clathrin, and analysis of their dynamics.** A) Acrylamide gel showing cel1 assays of the TALENs targeting the *AP1M1* gene. The cells were transfected with GFP alone, GFP and one of the TALENs, or both of the TALENs. Lower bands representing non-homologous end-joining events were detected only when both of the TALENs were transfected. B) Schematics depicting the PCR reactions used for clone characterization. Top: cel1 primers bind homology arms (HAs) 1 and 2. They were used to determine whether the coding sequence for a fluorescent protein (GFP or RFP, shown as XFP in the figure) was inserted. If the XFP coding sequence is inserted at the target site, then the PCR band is larger than the band without it. If all of the alleles are tagged, then PCR gives one band. If a subset of alleles is tagged, then PCR gives a slower migrating band for the tagged allele and a fast migrating band for the untagged allele. Bottom: To directly test if the XFP coding sequence is inserted, a primer that binds the XFP coding sequence and another that binds HA2, were used. C) PCR reactions to test genome editing of *AP2M1* and *CLTA* genes. Ci-iii) PCR reaction for the *AP2M1* gene with cel1 primers (i), RFP primers (ii) and GFP primers (iii). Civ-vi) PCR reaction for the *CLTA* gene with cel1 primers (iv), GFP primers (v) and RFP primers (vi). D) An example track in which reciprocal searching for the associated track allowed stitching of the divided tracks. In this example, the reference track is AP2-RFP, and the associated track is clathrin-GFP. The reference track (RFP) is stitched. Di) The x-y coordinates of the associated RFP and GFP tracks. Dii) GFP/RFP tracks were overlaid

with the TIRF image from the reference channel (RFP) at the time point when the spot in the reference channel was first detected. Diii-iv) x and y positions of tracks over time. The blue arrow indicates the time point wherein the red spot was not detected by Imaris software. Dv-vi) Movie strip images showing the reference RFP (v) and the corresponding GFP (vi) tracks. The regions encompassing the tracks over their entire lifetimes were cropped from time-lapse images. Dvii-viii) Montage images centered at the spots seen in the reference RFP channel (vii) or the corresponding GFP channel (viii). Only circular areas of 7-pixel diameter are shown for montages.

**Supplemental Figure 2, related to figure 2**

**Support Vector Machine (SVM) classifiers for identification of authentic CME sites.** A-B) Projection of 4-dimensional (4D) features used for SVM classification onto a 2D feature plane. All possible combinations of two features are shown. Black lines mark the 2D slice of an SVM hyperplane (classifier) at the mean of the other two features. Purple squares represent colocalized clathrin or AP2 spots, and the yellow squares are AP2 or clathrin spots not colocalized with each other. The plots boxed with yellow lines are the ones shown in Fig. 2.

**Supplemental Figure 3, related to figure 2**

**Application of SVM classifiers to single-color cell lines.** A-B) Epifluorescence images of MDA-MB-231 cells genome-edited to express *AP2M1-GFP* (A) or *CLTA-GFP* (B). The image of AP2 is focused on the plasma membrane while the image of clathrin is focused on the middle plane of the cell. Scale bar = 5µm. C) Histogram of the lifetimes for clathrin-GFP and AP2-GFP tracks (9 cells in 3 experiments respectively). The tracks

were grouped into authentic CME sites or false CME sites based on predictions made using the SVM classifiers. The average lifetimes are shown Table S2. D-E) Projection of 4D features used for SVM classification of AP2 (D) and clathrin (E) onto a 2D feature plane. All possible combinations of two features are shown. Black lines mark the 2D slice of an SVM hyperplane (classifier) at the mean of the other two features. Purple squares represent clathrin-GFP or AP2-GFP spots that are predicted to mark authentic CME sites. The yellow squares are AP2 or clathrin spots that are predicted to be false CME sites. The plots boxed with yellow lines correspond to plots shown in Fig. 2.

**Supplemental Figure 4, related to figure 3**

**AP2 and clathrin dynamics in authentic or false CME sites.** B) All of the AP2-RFP tracks that did (lower panels) or did not (upper panels) recruit dynamin2-GFP are shown. The tracks were divided into different lifetime cohorts: 0-10s, 10-20s, 20-30s and 30-40s. For productive events (AP2 associated with dynamin2), the fluorescence intensity is brighter when the lifetimes are longer (n = 6, 10, 38 and 47 for each cohort). The numbers of analyzed AP2 tracks without dyanmin2 were 71, 22, 9 and 5 for each cohort. The tracks were aligned so that the first time point of spot detection is zero. B) MSD curves for clathrin-GFP tracks and AP2-GFP tracks individually determined in clathrin-GFP or AP2-GFP cell lines. Tracks were extracted from streaming TIRF images with 200ms exposure times for 30s. Only the tracks with lifetimes shorter than 20s that started and finished within the imaging period (30s) were included in our quantifications. The plateau values of MSD curves are shown in Table 2.

**Supplemental Figure 5, related to figure 4**

**Origins of false CME sites marked by clathrin or AP2.** A-B) TIRF images of MDA-MB-231 cells wherein an RFP fusion of clathrin is expressed at endogenous levels by genome-editing, and GFP-tagged σ1 subunit of the AP1 (A) or GFP-tagged σ3 subunit of the AP3 (B) complex is transiently overexpressed. C) Representative montages of clathrin-RFP and AP1-GFP that are colocalized, partially colocalized, or not colocalized. The interval and the exposure times were both 200ms. Circular areas of 7-pixel diameter are shown. CL stands for clathrin. Fractions and mean lifetimes are shown in Table S3. D) Representative montage showing a region of TIRF image wherein colocalized AP1 and clathrin appear. E) Western blot of the cell lines expressing *AP2M1-RFP* probed with antibodies against the AP2 μ subunit.

## Extended Experimental Procedures

**TALEN design.** TALENs were designed to cut the *AP2M1* gene locus at a site corresponding to residue 236 of the AP2 μ subunit. TALENs were constructed following the protocol in (Sanjana *et al.*, 2012). In short, monomers specific for target sequences were linked together by an iterative Golden Gate cloning strategy. Nuclease activity of the TALENs was tested using the Surveyor Mutation Detection Kit (Integrated DNA Technologies, Coralville, IA, USA) to detect insertions or deletions (Fig. S1A). Phusion polymerase (New England Biolabs, Ipswich, MA, USA) was used to amplify a short region surrounding the TALEN target site in a 35-cycle PCR. PCR products were denatured, slowly re-annealed, and treated with cel1 nuclease for 1 hour at 42°C, then separated on 10% acrylamide TBE gels and visualized by ethidium bromide staining.

**Donor Design.** Donor plasmids to insert the fluorescent marker into the unstructured loop of the AP2 μ subunit were designed. In previous studies, tagging of this protein at this site was shown to minimally interfere with protein function (Motley *et al.*, 2006). Donor plasmids for TagGFP2 or TagRFP.t (Shaner *et al.*, 2008) were created by inserting the fluorescent protein coding sequences between the gene loci corresponding to residues 236 and 237 of AP2 μ subunit. A 1.63kb homology region of *AP2M1* centered at residues 236 and 237 was amplified from the genomic DNA of SK-MEL-2 cells with NdeI and SpeI restriction sites at the ends by Phusion polymerase (New England Biolabs, Ipswich, MA, USA). This homology region was inserted into the pCR8 backbone. The entire pCR8-*AP2M1* plasmid was amplified to insert a KpnI restriction

site between residues 236 and 237. TagGFP2 and TagRFP.t coding sequences were amplified with linkers GTSGGS at the N-terminus and AGSGT at the C-terminus. The fluorescent protein coding sequences were inserted at the KpnI restriction site. Sequences were verified by PCR and sequencing. For a detailed protocol see (Dambournet *et al.*, 2014).

**Generation of genome-edited cell lines.** 2μg of plasmids expressing TALENs or ZFNs, and 20μg of donor plasmids were transfected into cells using a single cuvette Amaxa Nucleofector device (Lonza, Basel, Switzerland), following the manufacturer's protocol. MDA-MB-231 cells grown to ~80% confluency were harvested by trypsinization, resuspended in Nucleofector solution V, and transfected using Amaxa Nucleofector program X-013 (Lonza, Basel, Switzerland). After transfection, cells were grown at 37°C and 5% $CO_2$ for recovery and proliferation. After 5-7 days, cells were trypsinized and sorted for RFP-positive and/or GFP-positive signals as single cells directly into 96-well plates using a DAKO-Cytomation MoFlo High Speed Sorter (Glostrup, Denmark) or BD Bioscience InFlux Sorter (Franklin Lakes, NJ, USA). Each clonal population was tested by fluorescence microscopy, genotyping and western blotting to identify true positive clones (Fig. S1C, S5E). Primer sequences used for diagnostic PCR were as follows: AP2M1-Cel1-F, 5′-GCTTTTCATAGTCTCTGGTGCC-3′; AP2M1-Cel1-R, 5′-GGAAGCAAGGTTACCAAAGAGC-3′; CLTA-Cel1-F, 5′-GCAGCAGAAGAAGCCTTTGT-3′; CLTA-Cel1-R, 5′-TTCCTCCTCTCCCTCCTCTC-3′; DNM2-Cel1-F, 5′-CCCTCCCCACCTGTCTTTAT-3′; DNM2-Cel1-R, 5′-GAGACTCCATCCCCCAAAGT-3′; TagGFP-F, 5′-AACTTCAAGACCCGCCACAACA-3′; TagRFP-F, 5′-

ATACTGCGACCTCCCTAGCAAA-3′; eGFP-F, 5′-CATGGTCCTGCTGGAGTTCGTG-3′. The antibody used for western blot analysis was Rabbit monoclonal [EP2695Y] anti-AP2M1, ab75995 at 1:250 dilution (Abcam, Cambridge, UK).

**Cell culture**. Parental and genome-edited MDA-MB-231 cells were maintained under 5% $CO_2$ at 37°C in DMEM/F-12 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (HyClone, Logan, UT, USA), penicillin and streptomycin.

**Total internal reflection fluorescence (TIRF) microscopy, transient overexpression and live-cell imaging.** At 24-48 hours prior to imaging, cells were seeded onto uncoated glass coverslips (Fischer Scientific, Waltham, MA, USA) in DMEM/F-12 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (HyClone, Logan, UT, USA). For transient overexpression of σ1-GFP (Anitei *et al.*, 2010) and σ3a-GFP (Kural *et al.*, 2012), 1 day after seeding the cells onto coverslips, 1μg of the corresponding plasmids were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. The transfected cells were imaged the next day. During imaging, cells were maintained in DMEM/F-12 without phenol red (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (HyClone, Logan, UT, USA) and 10 mM HEPES (Life Technologies, Grand Island, NY, USA). TIRF microscopy images were acquired using MetaMorph software on an Olympus IX-81 microscope equipped with a 60x NA 1.49 objective (Olympus Corporation, Tokyo, Japan) and an ORCA-Flash4 camera (Hamamatsu Photonics, Hamamtsu, Japan). The system was maintained at 37°C using a WeatherStation chamber and temperature controller (Precision Control, Tacoma, WA, USA). A 488 nm

solid-state laser (Melles Griot, Albuquerque, NM, USA) and a 561 nm diode-pumped solid-state laser (Melles Griot, Albuquerque, NM, USA) were used for excitation of fluorescent proteins. Simultaneous two-color TIRF images were obtained using a DV2 image splitter (MAG Biosystems, Santa Fe, NM, USA) to separate GFP and RFP emission signals.

**CME spot tracking** For each cell, a 100x100 pixel area (117μm$^2$) was cropped and used for analysis. The fluorescent spots in each fluorescent channel were tracked using Imaris (Bitplane Scientific, Zurich, Switzerland) software. The "Spots Module" was used for spot detection. The spot diameter was set at 350nm. Then a "quality filter" was applied with a user-defined threshold to select spots for further analysis. The Brownian motion particle-tracking algorithm was applied to track movements of spots. The maximum search distance for tracking was set to 350nm. The maximum gap between time points was set to 5 frames for AP2 and dynamin2 spots, and 7 frames for clathrin, AP1 and AP3 spots. The tracking results were further screened and analyzed using custom-built Matlab software (see below).

**Association of corresponding GFP and RFP tracks and automated selection of clean tracking results**

We used custom-built Matlab programs to associate corresponding GFP and RFP tracks that were independently identified using Imaris. For each track in the reference channel, the colocalization score *s* was determined with respect to all of the tracks detected in the other channel. The score *s* was calculated as the time average of the reciprocal of the squared Euclidean distance between the RFP spot and the GFP spot.

$$s = \frac{1}{\Delta t} \sum \frac{1}{(\vec{x}_{RFP}(t) - \vec{x}_{GFP}(t))^2}$$

Here, $\Delta t$ is the duration of time when both RFP and GFP spots are detected. $\vec{x}_{XFP}(t)$ is the xy coordinate of an XFP spot at time point t. If the colocalization score *s* is higher than the threshold described below, then the tracks in the other channel were determined to be associated with the reference track. We used $1/d^2$ as the threshold where *d* was chosen as the typical size of a fluorescent spot in our imaging setup, 350nm. Occasionally GFP or RFP tracks were divided into subtracks due to weak or fluctuating signal intensity. To overcome this limitation in tracking, we reciprocally used the newly found associated tracks as references to find associated tracks in the other channel. In this manner, we were able to identify the entire track even if each track in one channel is divided into subtracks.

If a track was intersected by tracks in the vicinity or if it had a low signal-to-noise ratio (SNR), then the track was automatically excluded from further analyses. To ensure that the tracking results obscured by nearby spots were not included in our datasets, we discarded tracks for which other detected spots were within the search range (1.5 x spot size or 1.5x350nm). By expanding our search range for other spots to 5 frames before and after the span of the spot of interest, only de novo appearance of spots followed by clean disappearance but not blinking events was analyzed. The background brightness level was locally determined as the average of the fluorescence intensity of the spot location over 5 frames before spot appearance and 5 frames after the spot disappearance. Only when the spots were significantly brighter

than the background, were they subjected to further analyses. The fluorescence intensity of a CME spot was calculated as an integrated intensity within a circular region with diameter of 7 pixels (756nm), centered at the spot position determined by Imaris.

**Machine learning-based classification of authentic tracks.**

The following features were selected for support vector machine (SVM)-based classification.

i) Track lifetime: The length of time from the frame when the spot appeared to the frame when it disappeared. If more than one track are stitched by reciprocal search, the lifetime encompasses all of the track segments.

ii) Maximum intensity of a spot: For SVM analyses, the center intensity of a spot $I(t)$ at a time point $t$ was calculated as the sum of intensity values over the rectangular 3x3 pixel area centered at the spot location determined by Imaris software ($324 \times 324 nm^2$). For the frames before or after the duration of the track, the same area centered at the position where the spot first appeared or disappeared was used for calculation of the center intensity $I$. The average $I_{bg, mean}$ and the standard deviation $I_{bg,std}$ of the background intensity were calculated from the center intensities ($I$) from the 5 frames before the beginning and 5 frames after the end of the track. The relative center intensity $I_R$ of a spot was calculated as $I_R(t) = (I(t) - I_{bg,mean})/I_{bg,std}$. The maximum of the relative center intensity $I_R(t)$ was used as the feature 2.

iii) Overall displacement: The Euclidean distance between the point where the fluorescent spot first appeared and the point where it finally disappeared.

iv) The average rate of change in spot brightness: The rate of change in spot intensity for each time point t was calculated as $\dot{I_R}(t) = |I_R(t + \Delta t) - I_R(t)|/\Delta t$. $I_R$ is the relative center intensity defined above. Δt is the interval of the time lapse. Two vertical lines (| |) stand for absolute values. The average rate of change in intensity is the time average of $\dot{I_R}(t)$ over the lifetime of a spot.

Half of the AP2-RFP tracks were used for training of the SVM classifier. AP2-RFP tracks that colocalize with clathrin-GFP tracks were defined as authentic CME sites while AP2-RFP tracks without an associated clathrin signal are defined false CME sites. The four features explained above were calculated for AP2-RFP tracks in the training set. To keep features with large values from eclipsing the distinguishing power of other features, each feature was normalized so that the mean was 1. The Matlab function fitcsvm.m with linear kernel was used to determine the SVM classifier. To test the accuracy of the SVM classifier, the other half of the AP2-RFP/clathrin-GFP tracks were used. 2/3 of the tracks were randomly sampled 50 times as test samples to determine the range of accuracy. The SVM classifiers were applied to the test sample, and the accuracy was determined as the fraction of spots whose predicted authenticity matched the real authenticity (colocalization with clathrin-GFP). The SVM classifier for clathrin-GFP was determined and tested in the same manner.

**Fitting of MSD**

The mean squared displacement was calculated as follows:

$$MSD(t) = \frac{1}{n} \sum_{\text{particles}} (\vec{x}(t) - \vec{x}(t_0))^2$$

$t_0$ is the time when a spot is first detected. MSD was calculated up to the time point in which the sample number was at least 10. To determine the value at which the MSD curves plateau, the MSD curves were fitted using f(t)=A[1-exp(-t/τ)] using Matlab. Diffusion coefficient for non-CME clathrin spots was determined by fitting a linear curve (f(t)=6Dt+constant) to the linear part of the MSD curve (first 5 seconds).

**Colocalization analysis of clathrin with AP1 and AP3**

AP1 or AP3 spots were detected and tracked using Imaris software. Then AP1 or AP3 spots were manually classified as colocalized, partially colocalized, or not colocalized with clathrin. Matlab was used for further statistical analysis of manually classified AP1/AP3 spots.

## Supplemental Tables

**Supplemental Table 1 List of cell lines.** "All" indicates that all alleles are tagged with a fluorescent protein. "Partial" indicates that not all alleles were tagged.

| Parental cell line | Genotype | Clone # |
|---|---|---|
| MDA-MB-231 | *AP2M1*-TagGFP2$^{\text{Partial}}$ | 2 |
| MDA-MB-231 | *CLTA*-eGFP$^{\text{Partial}}$ | 2 |
| MDA-MB-231 | *CLTA*-TagRFP.t$^{\text{Partial}}$ | E |
| MDA-MB-231 | *AP2M1*-TagRFP.t$^{\text{Partial}}$, *CLTA*-eGFP$^{\text{Partial}}$ | 8 |
| MDA-MB-231 | *AP2M1*-TagRFP.t$^{\text{Partial}}$, *DNM2*-eGFP$^{\text{Partial}}$ | 8 |
| MDA-MB-231 | AP2M1-TagGFP2$^{\text{Partial}}$, AP2M1-TagRFP.t$^{\text{Partial}}$ | 27 |

**Supplemental Table 2, related Fig. 1, 3 and Fig. S3. Average lifetimes of AP2, clathrin and dynamin2 spots.**

| Cell line | Classification | Mean Lifetime (s) |
|---|---|---|
| AP2-RFP & Clathrin-GFP | AP2 (Authentic CME) | 46.7±2.7 |
| | AP2 (False CME) | 9.8±0.9 |
| | Clathrin (Authentic CME) | 46.7±2.7 |
| | Clathrin (False CME) | 11.6±0.4 |
| AP2-GFP | AP2 (Predicted authentic CME) | 49.7±2.3 |
| | AP2 (Predicted false CME) | 11.6±1.1 |
| Clathrin-GFP | Clathrin (Predicted authentic CME) | 45.5±2.2 |
| | Clathrin (Predicted false CME) | 8.5±0.4 |
| AP2-RFP & dynamin2-GFP | AP2 without Dynamin2 | 11.5±6.1 |
| | AP2 with Dynamin2 | 48.8±6.3 |
| | Dynamin2 without AP2 | 11.4±3.0 |
| | Dynamin2 with AP2 | 30.9±6.2 |

**Supplemental Table 3, related to Fig. S5 Dynamics of AP1 and AP3**

Fractions and mean lifetimes of AP1 or AP3 spots that are colocalized, partially colocalized or not colocalized with clathrin. The ranges for the lifetimes are the standard error of the means.

| | Fraction of tracks (%) | | Mean Lifetime (s) | |
|---|---|---|---|---|
| | AP1 | AP3 | AP1 | AP3 |
| Colocalized | 40.1 | 15.1 | 4.5±0.3 | 4.2±0.4 |
| Partially colocalized | 44.1 | 26.3 | 2.8±0.1 | 2.9±0.2 |
| Not colocalized | 15.8 | 58.6 | 1.6±0.2 | 2.0±0.1 |

## <u>Supplemental Movies</u>

**Supplemental Movie 1, related to figure 1. AP2-RFP and clathrin-GFP**

A TIRF time-lapse movie of AP2-RFP (left), clathrin-GFP (middle) and the their false-colored overlay (right). AP2-RFP is false-colored with magenta and clathrin-GFP with green. 200ms exposure. 2s interval, 4 min duration. Scale bar: 5μm

**Supplemental Movie 2, related to figure 1. AP2-RFP and clathrin-GFP, detail.**

Magnified view of a TIRF time-lapse movie of AP2-RFP (left), clathrin-GFP (middle) and the their false-colored overlay (right). AP2-RFP is false-colored with magenta and clathrin-GFP with green. 200ms exposure. 2s interval, 4 min duration. 20 frames per seconds. 5.4 x5.4 $\mu m^2$ area is shown.

**Supplemental Movie 3, related to figure 1, AP2-RFP and clathrin-GFP with tracks.**

A TIRF time-lapse movie of AP2-RFP (magenta) and clathrin-GFP (green) overlaid with the tracking results. 200ms exposure. 200ms interval, 30 sec duration. Speed: 4x of the real time.

**Supplemental Movie 4, related to figure 3, AP2-RFP and dynamin2-GFP**

A TIRF time-lapse movie of AP2-RFP (left), dynamin2-GFP (middle) and the their false-colored overlay (right). AP2-RFP is false-colored with magenta and dynamin2-GFP with green. 200ms exposure, 2s interval, 4 min duration. Scale bar: 5μm

**Supplemental Movie 5, related to supplemental figure 5, AP1-GFP and clathrin-RFP**

A TIRF time-lapse movie of a cell expressing AP1-GFP and clathrin-RFP. AP1-GFP (left), clathrin-RFP (middle) and overlay of the two false colored with green (AP1) and magenta (clathrin) are shown (right). 200ms exposure, 200ms interval, 30s duration.

**Supplemental Movie 6, related to supplemental figure 5, AP1-GFP and clathrin-RFP**

A TIRF time-lapse movie of a cell expressing AP3-GFP and clathrin-RFP. AP3-GFP (left), clathrin-RFP (middle) and overlay of the two false colored with green (AP3) and magenta (clathrin) are shown (right). 200ms exposure, 200ms interval, 30s duration.

## Supplemental References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