Coronin2A links actin-based endosomal processes to the EHD1 fission machinery

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ABSTRACT Fission of transport vesicles from endosomes is a crucial step in the recycling of lipids and receptors to the plasma membrane, but this process remains poorly understood. Although key components of the fission machinery, including the actin cytoskeleton and the ATPase Eps15 homology domain protein 1 (EHD1), have been implicated in endosomal fission, how this process is coordinately regulated is not known. We have identified the actin regulatory protein Coronin2A (CORO2A) as a novel EHD1 interaction partner. CORO2A localizes to stress fibers and actin microfilaments but also can be observed in partial overlap with EHD1 on endosomal structures. siRNA knockdown of CORO2A led to enlarged lamellae-like actin-rich protrusions, consistent with a role of other Coronin family proteins in attenuating actin-branching. Moreover, CORO2A depletion also caused a marked decrease in the internalization of clathrin-dependent cargo but had little impact on the uptake of clathrin-independent cargo, highlighting key differences in the role of branched actin for different modes of endocytosis. However, CORO2A was required for recycling of clathrin-independent cargo, and its depletion led to enlarged endosomes, supporting a role for CORO2A in the fission of endosomal vesicles. Our data support a novel role for CORO2A in coordinating endosomal fission and recycling with EHD1.

INTRODUCTION

The recycling of internalized cargo to the plasma membrane is an essential function for all eukaryotic cells, ensuring their ability to respond to extracellular cues as well as maintaining homeostasis of the plasma membrane and proper cell size through the retrieval of lipids from intracellular compartments (Grant and Donaldson, 2009; Naslavsky and Caplan, 2018). In addition to maintenance of cell shape and the return of receptors to the cell surface, recycling has been implicated in a host of crucial cellular processes such as polarity (Wang et al., 2000) and cell migration (Caswell and Norman, 2008) as well as events leading to cell division (Skop et al., 2001; Montagnac et al., 2008). Accordingly, the regulation of endosomal recycling at the endosome is a tightly controlled process involving many steps and culminating in a complex series of events that include endosomal fission.

Major advances have been made in recent years to enhance our understanding of endosomal recycling (Weeratunga et al., 2020). Studies published over the last several years support a new model in which recycling is a highly active process, and a variety of proteins have been identified and characterized for their roles in cargo selection and the shunting of cargo to the recycling pathways. In particular, proteins such as ACAP1 (Dai et al., 2004; Li et al., 2007; Bai et al., 2012) and various sorting nexin (SNX) proteins have been identified as key regulators of cargo sorting into the recycling pathways (McNally et al., 2017; Wang et al., 2018; Simonetti et al., 2019) that coordinate receptor retrieval to the plasma membrane through the Retromer and newly identified complexes known as Retriever and CCC complexes (McNally et al., 2017; Singla et al., 2019). Despite these advances in understanding the mechanisms involved in the partitioning of membranes and the sorting of receptors, one realm of endosomal studies that remains more incompletely understood...
is the mode by which endosomes undergo fission to release vesicles that are recycled to the plasma membrane.

The fission of clathrin-coated vesicles (CCVs) at the plasma membrane during internalization is a well-characterized process, and a key role has been attributed to dynamin in the release of CCVs from the membrane (Mettlen et al., 2018). However, considerably less is understood about the mode by which endosomal membrane buds are cleaved to release transport vesicles. Studies have suggested a role for the GTPase dynamin (Derivery et al., 2009; Derivery and Gautreau, 2010a), although the extent of dynamin’s involvement in endosomal fission remains unclear. An increasing number of studies has provided evidence for involvement of the actin cytoskeleton in the endosomal constriction process leading to fission (Derivery et al., 2009; Derivery and Gautreau, 2010a,b; Derivery et al., 2012; Jia et al., 2012; Hao et al., 2013; Wang et al., 2018), and recent studies also implicate the endoplasmic reticulum in endosomal fission (Rowland et al., 2014; Hoyer et al., 2018; Gong et al., 2021; Kawasaki et al., 2022). Moreover, a role for the Eps15 homology domain protein, EHD1 (along with several of its paralogs), has also been documented in the fission of tubular and vesicular endosomes in cells (Jakobsson et al., 2011; Cai et al., 2012, 2013, 2014a; Dhawan et al., 2020; Jones et al., 2020; Kawasaki et al., 2022) and using in vitro systems (Cai et al., 2013; Deo et al., 2018; Kamerkar et al., 2019). However, few studies have addressed the coordination of endosomal fission by the different proteins involved, in particular attempting to integrate actin-based membrane constriction with subsequent steps including nucleotide-hydrolyzed fission by GTPases or ATPases.

Herein, based on the presence of an asparagine-proline-phenylalanine-aspartic acid-aspartic acid (NPFFD) sequence motif known to preferentially bind to EHD1 (Kieken et al., 2007, 2009, 2010; Henry et al., 2010), we identify Coronin2A (CORO2A) as a novel EHD1 interaction partner that partially overlaps with EHD1 on endocytic structures. CORO2A is a type-II Coronin protein (Figure 1A) that contains an N-terminal β-propeller region with seven WD40 repeats, a unique region (U region), and a C-terminal coiled-coil region (CC). While not as extensively studied as some of the other mammalian Coronin family members, CORO2A has been localized to stress fibers and partially to focal adhesions and has also been implicated in actin regulation and related events such as focal adhesion turnover and cell migration in coordination with cofillin (Marshall et al., 2009; Huang et al., 2011; Deng et al., 2020). We demonstrate that CORO2A displays significant localization to actin microfilaments, and that siRNA knockdown of CORO2A leads to exacerbated actin-rich cell protrusions that resemble lamellipodia, consistent with the roles of other Coronin family members and particularly CORO1B, which has been implicated in disassembly of Arp2/3-containing branched actin filaments (Cai et al., 2008). Depletion of CORO2A caused a marked decrease in the internalization of clathrin-dependent cargo such as transferrin and epidermal growth factor (EGF) receptor but had little impact on the uptake of clathrin-independent cargo such as major histocompatibility class 1 (MHC-1) receptors and CD98 receptors, highlighting key differences in the role of actin for different modes of endocytosis. However, CORO2A was required for the recycling of clathrin-independent cargo, and its depletion led to enlarged endosomes, supporting a role for CORO2A in the fission of endosomal vesicles. Our data support a novel role for CORO2A in coordination of endosomal fission and recycling with EHD1.

RESULTS

To test whether CORO2A and EHD1 interact in cells, we performed co-immunoprecipitations (IPs). Antibodies specific for endogenous EHD1 detected a ~60-kDa band both in HeLa cell lysates (Figure 1B; top panel, left lane) and upon IP with anti-EHD1 antibodies (Figure 1B; IP EHD1, top panel, second lane from the left). In addition, IP with anti-CORO2A antibodies (validated in Supplemental Figure S1A) also pulled down EHD1 (Figure 1B; IP CORO2A, top panel, third lane from the left) as well as pulling down CORO2A (Figure 1B; IP CORO2A, top right panel). As a control, the anti-TOM20 antibodies pulled down TOM20 (Figure 1B; bottom panel, right lane) but failed to precipitate EHD1 (Figure 1B; top panel, lane four from the left). These data demonstrate that EHD1 and CORO2A reside in a complex in cells.

We next asked whether EHD1 and CORO2A directly interact with one another. Accordingly, we incubated purified GST-CORO2A or purified GST as a control, together with purified His-tagged EHD1 (His-EHD1) to perform in vitro binding assays (Figure 1C). In addition to WT GST-CORO2A, we also purified two mutant proteins with amino acid substitutions within the NPF motif, a region predicted to interact with EH domains. Because GST-CORO2A NPF-to-APA substitutions led to unstable proteins, we made substitutions within the acidic residues that immediately follow the NPF (DD), which have been identified as crucial for the interaction of other proteins with C-terminal EH proteins (Kieken et al., 2007; Kieken et al., 2009, 2010; Henry et al., 2010). Accordingly, in addition to WT GST-CORO2A (NPFFD), both GST-CORO2A NPFA and NPFDD were purified (Figure 1C; see 25% input lanes). Using equal concentrations of His-EHD1 bound to nickel beads (Figure 1C, bottom panel, 4 lanes on the right), we showed that His-EHD1 pulled down GST-CORO2A (Figure 1C, top panel, 3rd lane from the right), whereas His-EHD1 pulled down no detectable GST (Figure 1C, top panel, 4th lane from the right, negative control). Significantly, both GST-CORO2A NPFA and GST-CORO2A NPFDD showed markedly reduced binding to EHD1. These data provide evidence that EHD1 and CORO2A are capable of a direct interaction likely via the EHD1 EH domain and NPFFD motif of CORO2A, and that these proteins can reside within a complex in cells.

The actin-binding protein, CORO2A, belongs to the exotic Type II coronin family and its precise function in cells is complex and remains only partially understood (Marshall et al., 2009; Chan et al., 2011; Huang et al., 2011; Rastetter et al., 2015). Accordingly, we sought to characterize its intracellular localization. In the absence of effective antibodies for localization of the endogenous protein, we overexpressed FLAG-CORO2A in HeLa cells (green channel) and immunostained the cells with the filamentous actin-binding reagent, Phalloidin (red channel). Typically, we observed CORO2A localized to many of the Phalloidin-labeled actin stress fibers (Figure 2, A–C; see inset for more details and see Supplemental Figure S1, B–D for a field of cells). To quantify the overlap between CORO2A and Phalloidin, we applied Imaris software to obtain their 3D reconstructed surfaces and measured the contact events at 0 μm surface to surface distance (overlapping surfaces). CORO2A surfaces were selected as target surfaces (green), and Phalloidin (red) was tracked for any surface contacts that overlapped (yellow) with those of CORO2A (as an example, see Figure 2, D and E and Supplemental Video S1). Overall, we observed ~80% of CORO2A surfaces overlap (make contacts) with those of Phalloidin (quantified in Figure 4F). To validate the localization of CORO2A to Phalloidin-labeled actin structures, we treated FLAG-CORO2A transfected HeLa cells with cytochalasin D, an inhibitor of actin polymerization (Figure 2, F–K). Untreated cells displayed a filamentous actin distribution pattern throughout the cell, including cortical actin and stress fibers and the CORO2A primarily localized with the latter actin-based structures (Figure 2, F–H). However, upon treatment with cytochalasin D, a lack of stress fibers was observed with remaining filamentous actin mostly localized to
cortical actin beneath the plasma membrane, with little remaining overlap with CORO2A (Figure 2, I–K).

Recent studies suggest that Type I Coronins attenuate Arp2/3-dependent actin branching by inhibiting Arp2/3 docking and/or facilitating debranching (Liu et al., 2011; Sokolova et al., 2017). Since CORO2A localizes to actin filaments, we asked if it affects actin organization similar to Type I Coronins. To investigate this, we took advantage of the very elaborate actin cytoskeleton observed in U251 Glioblastoma cells (Fraley et al., 2005) and either mock-treated (untreated) the U251 cells or subjected them to CORO2A siRNA knockdown (as validated in Figure 3C) and examined their filamentous actin distribution with Phalloidin-568 (Figure 3, A and B). As observed previously in HeLa cells, the mock U251 glioblastoma cells also displayed a typical filamentous actin distribution pattern, with many stress fibers throughout the cells and some cortical actin and actin-rich protrusions at the plasma membrane (Figure 3A). Upon CORO2A knockdown, however, we observed dramatically expanded actin-rich protrusions at the plasma membrane, suggesting a role for CORO2A in attenuation of actin branching (Figure 3B). To quantify the size and frequency of these expanded actin-rich protrusions in an unbiased manner, we applied 3D filament reconstruction tool for actin using Imaris (Supplemental Figure S2, A and B). The filament tracer tool reconstructs actin filaments as dendrites (violet) and picks up expanded actin protrusions as dendrite beginning points (light green cones) above a set threshold. The frequency and mean diameter of these actin-rich protrusions (dendrite beginnings) were calculated in an automated manner and plotted (Figure 3, D and E). We also plotted the frequency over the mean inter-val of actin-rich protrusions diameter (Figure 3F). The distribution plot is shifted to the right (see inset) upon CORO2A depletion with siRNA (red bars) with actin-rich protrusions increasing both in size and frequency relative to Mock cells (black bars). Overall, these data imply that CORO2A negatively regulates actin branching.

Given the interaction between CORO2A and EHD1 and their potential to coordinate fission events at endosomes, we next assessed whether the two proteins could be visualized on common subcellular structures. Accordingly, we used our CRISPR/Cas9 gene-edited RFP-EHD1 HeLa cells (expression validated in Figure 4G, right lane) and transfected them with FLAG-CORO2A to address this question. Note that the gene-edited cells are contained within a pool of cells, and thus both RFP-EHD1 and unmodified endogenous EHD1 can be detected by immunoblot. As demonstrated (Figure 4, A–C and insets), CORO2A (green channel) localizes to the endosomal tubules containing EHD1 (red channel), as marked by yellow arrows. However, localization patterns for both EHD1 and CORO2A may be heterogeneous, and larger fields of cells may show cells with some differences in distribution (see Supplemental Figure S3). Using 3D surface rendering of z-stacks with Imaris, we examined surface contacts of CORO2A with EHD1 (Figure 4, D and E; yellow and
FIGURE 2: Cytoskeletal localization of CORO2A. (A–C) HeLa cells transfected with FLAG-CORO2A were fixed and immunostained with anti-FLAG together with the corresponding secondary antibody and Phalloidin-568 and imaged by confocal microscopy. The insets show an enlarged portion of the same cell. (D, E) Z-stacks were acquired and processed by Imaris; 3D surface reconstruction was performed simultaneously for both channels to capture Phalloidin and CORO2A voxels. Contact events at 0 μm surface to surface distance were measured using the Distance Transform program in Imaris XT bundle “Kiss and Run” integrated with MATLAB Compiler Runtime and launched on Imaris. CORO2A surfaces were selected as target surfaces, and Phalloidin was tracked for any surface contacts that overlap with those of CORO2A (highlighted in yellow). (F–K) FLAG-CORO2A transfected HeLa cells were either left untreated (F–H) or treated with 1 μm cytochalasin D for 20 min at 37°C prior to fixation (I–K) followed by immunostaining with anti-FLAG and Phalloidin-568. Data shown are representative of three independent experiments.
Supplemental Video S2) and plotted the percent surface contacts (overlap) with those of EHD1, suggesting a subpopulation of endosomes harbors both CORO2A and EHD1 (compared with ~80% surface contact overlap with actin). However, despite the interactions between CORO2A and EHD1, EHD1 knockdown had little effect on CORO2A localization (Supplemental Figure S4), and CORO2A depletion had no discernable impact on EHD1 localization (Supplemental Figure S5). Overall, these data support the idea that CORO2A interacts with EHD1 on endosomes and may be involved in the regulation of endosomal fission.

Given that EHD1 is an endosomal fission protein that regulates recycling of receptors internalized via both clathrin-dependent and -independent pathways (Cai et al., 2012, 2013, 2014a; Kamerkar et al., 2019), we hypothesized that CORO2A may similarly control endosomal fission and receptor recycling. We therefore first assessed whether CORO2A is required for the internalization of clathrin-dependent cargo and measured rates of transferrin and EGF receptor internalization in mock- and CORO2A-depleted cells (validated in Figure 5A). Mock (untreated) U251 glioblastoma cells and CORO2A-depleted cells were incubated with fluorophore-labeled transferrin for either 5 min (Figure 5, C and D) or 20 min (Figure 5, E and F) to induce transferrin receptor internalization. In these assays, we observed significantly lower fluorescence intensity of endocytosed transferrin in the siRNA-treated cells compared with the mock cells (quantified in Figure 5B), indicating a slower rate of transferrin internalization in the absence of CORO2A. Recent studies suggest that the Type I coronin protein which lacks an NPF motif, Coronin 1C (CORO1C), plays a role in regulating endoplasmic reticulum-dependent endosomal fission (Puthenveedu et al., 2010; Hoyer et al., 2018). Accordingly, we compared the effects of CORO2A and CORO1C on the internalization of EGF receptors. EGF receptor internalization was compared in cells subjected to scrambled siRNA, CORO2A siRNA, and CORO1C siRNA (reduced expression validated in Figure 6E) after 15 min of uptake. Similar to the internalization assays for transferrin, we observed significantly lower intensities of internalized EGF in both CORO2A siRNA- and CORO1C siRNA-treated cells (Figure 6, A–C, quantified in Figure 6D), indicating that both Type I and Type II Coronins alter the rate of internalization of EGF receptors. Since the rates of transferrin and EGF internalization were slower in the siRNA-treated cells, we could not assess the impact of the knockdowns on these cargo in recycling. Overall, these data suggest that CORO2A regulates the rate of internalization of clathrin-dependent cargo.
We next addressed whether CORO2A is required for the internalization and/or recycling of clathrin-independent cargos such as major histocompatibility class-1 (MHC-1) receptors. To test this, we allowed mock (untreated) and CORO2A siRNA-treated glioblastoma cells (reduced expression validated in Figure 7G) to internalize antibodies that recognize peptide-loaded MHC-1 for 15 min, followed by a brief acid rinse to exclusively reveal internalized MHC-1.

Alternatively, after the MHC-1 antibody binding and acid rinse, the cells were chased for 90 min, followed by an additional acid rinse to quantify the remaining intracellular MHC-1 as a measure of recycling (Figure 7, A–D). In this assay, we observed that the signal intensity of intracellular MHC-1 was similar in the mock and siRNA-treated cells at chase 0, indicating that CORO2A depletion does not significantly impact MHC-1 internalization (Figure 7E; chase 0). However, the CORO2A siRNA-treated cells showed significantly greater fluorescence intensity than the Mock cells when chased for 90 min (Figure 7E; chase 90), signifying delayed MHC-1 recycling in the absence of CORO2A. Moreover, we demonstrated that the average size of endosomes containing MHC-1 was significantly increased in the absence of CORO2A when chased for 90 min (Figure 7F). This is likely due to more MHC-1 remaining associated with the endosomes which continue to receive new endocytic cargo (Figure 7F). Accordingly, our data support the notion that CORO2A depletion impairs MHC-1 endosomal fission. Additionally, we compared the effects of CORO1C and CORO2A on the internalization and recycling of another clathrin-independent cargo, CD98 (Eyster et al., 2009). The internalization of CD98 was compared in cells subjected to scrambled siRNA, CORO2A siRNA, and CORO1C siRNA (reduced expression validated in Figure 8H). Similar to the internalized MHC-1, we observed no significant difference in the intensities of internalized CD98 in scrambled siRNA-, CORO2A siRNA-, and CORO1C siRNA-treated cells after 25 min of uptake (Figure 8, A–C; quantified in Figure 8G), indicating that depletion of either Type I and Type II Coronins used in the assay did not affect the rate of internalization of CD98 receptors. However, significantly greater intensities of remaining intracellular CD98 were observed in CORO2A- and CORO1C-depleted cells than in the mock cells when chased for 40 min (Figure 8, D–F; quantified in Figure 8G), suggesting delayed CD98 receptor recycling in the absence of CORO2A and CORO1C. We validated the effects of CORO2A and CORO1C on the internalization of an additional clathrin-independent cargo that plays an important role in regulation of the immune complement system (Cai et al., 2014b), CD59, and observed no significant effect on its internalization in the absence of CORO2A and CORO1C (Supplemental Figure S6). Together, these data suggest that CORO2A regulates recycling of receptors endocytosed via clathrin-independent pathways but has no significant impact on their internalization.

We postulate that CORO2A interacts with EHD1 to regulate the recycling of receptors by restricting actin branching and facilitating
Observing and quantifying fission in cells is a challenging task and there are few assays that have been described in the literature. Live imaging has been used successfully (Rowland et al., 2014), but this method measures limited individual events and also requires overexpression. While the experiments described in Figure 9 suggest a role for CORO2A in endosomal fission, they quantify endosome size over the course of the siRNA knockdown (48 h), and we rationalized that designing an assay that can measure large numbers of fission events acutely (in a short time frame) would help us to address the role of CORO2A. Accordingly, we designed a novel fission assay for cell populations where transferrin was internalized into enlarged endosomes induced by the reversible PI3K inhibitor, LY294002. After washout of the LY294002 inhibitor, the enlarged endosomes acutely undergo fission and return to a normal size distribution. We initially calibrated this assay with mock and EHD1 knockout cells, since EHD1 has been well characterized in fission both in vitro (Cai et al., 2013; Deo et al., 2018; Kamkar et al., 2019) and in cells (Jakobsen et al., 2011; Cai et al., 2012, 2013, 2014a; Dhawan et al., 2020; Jones et al., 2020; Kawasaki et al., 2022). To this aim, we used Imaris software to measure the mean interval of transferrin-containing structures from 3D images upon transferrin uptake in both mock and EHD1 knockout cells and following washout of the inhibitor (Supplemental Figure S7, B and C, and representative images provided in Supplemental Figure S7, D–G). As demonstrated from the graphs, endosome size distribution was similar for the transferrin uptake, but upon initiation of fission (washout of the inhibitor), the frequency of large endosomal structures (a measure of impaired fission) was greatly enhanced for the EHD1 knockout cells, consistent with a role for EHD1 as a fission protein.

Having calibrated this new cell-based quantitative fission assay, we next applied it to address the role of CORO2A in acute endosomal fission. As demonstrated in Figure 10, upon successful depletion of CORO2A (Figure 10A), endosome size distribution was not markedly changed from that of mock-treated cells (Figure 10, B and C, compare black to red bars and lines; representative images in Figure 10, D–G). However, when the LY294002 inhibitor was washed out, the frequency of remaining, large, 100–200 μm² endosomes was dramatically higher in the CORO2A knockout cells (representative images in Figure 10, F and G; compare the pink bars and line in B and C with the gray bars and line). Overall, these data provide additional evidence that CORO2A is required in the cell to promote efficient endosomal fission.
FIGURE 6: CORO2A regulates EGF internalization. (A–C) U251 Glioblastoma cells were either treated with scrambled-siRNA (A) subjected to CORO2A-siRNA (B) or CORO1C-siRNA (C) for 48 h followed by incubation with EGF-Rhodamine for 15 min at 37°C prior to fixation. Representative micrographs consisting of fields of cells were obtained by confocal microscopy. Insets (dashed squares) are zoomed in (bottom panel) to highlight endosomal intensity differences. (D) The graph compares the mean arithmetic fluorescence intensity of endocytosed EGF in scrambled, CORO2A-, and CORO1C-siRNA-treated cells. The p values were determined by two-tailed t tests. (E) Immunoblot demonstrates reduced expression of CORO2A and CORO1C in siRNA-treated cells. Data shown are representative of three independent experiments, each using five images.
DISCUSSION

The Coronin family of proteins has seven mammalian paralogs generally classified into Type I, Type II, and Type III Coronins (Morgan and Fernandez, 2008; Xavier et al., 2008; Chan et al., 2011). While the single Saccharomyces cerevisiae Coronin protein and the Type I Coronins (including CORO1C) have been studied extensively and have well-documented roles in the regulation of F-actin dynamics (Heil-Chapdelaine et al., 1998; Liu et al., 2011; Mikati et al., 2015; Behrens et al., 2016; Sokolova et al., 2017), less information is available on the function of Type II Coronins, including CORO2A and CORO2B. Nevertheless, CORO2B has been implicated in the regulation of actin polymerization to facilitate dendrite outgrowth (Chen et al., 2020), and CORO2A has been linked to actin regulation and subsequent control of a variety of cellular pathways, from migration to control of inflammatory response genes (Marshall et al., 2009; Huang et al., 2011; Rastetter et al., 2015; Deng et al., 2020).

The Type II Coronins display 68% identity at the amino acid level, but only CORO2A contains an NPFDD motif of the form that is recognized by C-terminal EHD proteins, such as EHD1 (Kieken et al., 2007, 2009, 2010). Indeed, CORO2A may have evolved via gene duplication to pick up additional functions at the endosome through its interaction with EHD1. Interestingly, CORO2A displays nearly 50% identity with CORO1C, which has been implicated in sorting endosome fission (Puthenveedu et al., 2010) as well as fission of late endosomes marked by Rab7 in coordination with actin and the endoplasmic reticulum (Hoyer et al., 2018; Striepen and Voeltz, 2022).

It remains unclear if CORO1C and CORO2A are redundant or have overlapping roles in endosomal fission. Mass spectrometry analysis of lysates pulled down with antibodies to CORO2A showed that in addition to CORO2A, both EHD1 and CORO1C could be precipitated, although we anticipate that CORO1C might do so through hetero-oligomerization with CORO2A rather than directly interacting with EHD1 (unpublished observations). We have demonstrated that CORO2A directly binds to EHD1 in an in vitro system with

FIGURE 7: Recycling of MHC-1 is impaired upon CORO2A depletion. (A–D) U251 glioblastoma cells were either mock-treated (A, C) or subjected to CORO2A-siRNA (B, D) and incubated with anti-MHC-1 antibody for 15 min at 37°C. Cells were then acid-stripped and either fixed (A and B) or “chased” in the absence of anti-MHC-1 antibody for 90 min at 37°C (C, D) followed by fixation. Representative micrographs consisting of fields of cells were obtained by confocal microscopy. (E) The graph compares the mean arithmetic fluorescence intensity of internalized (chase 0) and remaining MHC-1 (chase 90) in mock- and CORO2A-siRNA-treated cells. (F) The graph compares the mean MHC-1 endosome size at chase 0 and chase 90 in mock- and CORO2A-siRNA-treated cells, as measured by ImageJ (see Materials and Methods for details). The p values were determined by two-tailed t tests. (G) Immunoblot demonstrates reduced expression of CORO2A in siRNA-treated cells. On the left, FLAG-CORO2A-transfected cell lysate depicts the molecular weight reference for CORO2A. Data shown are representative of four independent experiments, each using five images.
FIGURE 8: CD98 receptor recycling is impaired upon CORO2A depletion. (A–F) U251 glioblastoma cells were either treated with scrambled-siRNA (A, D) subjected to CORO2A- (B, E), or CORO1C-siRNA (C, F) and incubated with anti-CD98 antibody for 25 min at 37°C. Cells were then acid-stripped and either fixed (A–C) or chased in the absence of anti-CD98 antibody for 40 min at 37°C (D–F) followed by fixation. Representative micrographs consisting of fields of cells were obtained by confocal microscopy. Insets are included to highlight endosomal intensity differences. (G) The graph compares the arithmetic fluorescence intensity of internalized (chase 0) and remaining CD98 (chase 40) in scrambled, CORO2A-, and CORO1C-siRNA-treated cells, as measured by Zen software. Two-tailed t tests were performed for the sets, Mock/CORO2A-siRNA and Mock/CORO1C-siRNA at chase 0 and 40 min to derive p values that correspond to 0.337360 (chase 0), <0.000001 (chase 40) and 0.562981 (chase 0), <0.000001 (chase 40), respectively. (H) Immunoblot demonstrates reduced expression of CORO2A (left panel) and CORO1C (right panel) in siRNA-treated cells. Data shown are representative of three independent experiments, each using 10 images.
purified proteins and requires the NPFDD motif in CORO2A. Moreover, the partial localization of CORO2A to endosomes strongly hinted at a potential role in the fission process, suggesting that the interaction between these proteins may be required for optimal endosomal fission and recycling.

In our study, CORO2A depletion had a dramatic effect on actin organization beneath the plasma membrane. The large numbers of wide, lamellipodia-like actin-rich structures observed and measured in the absence of CORO2A support its role in attenuating branched actin. These data appear consistent with a very recent study demonstrating that CORO1B- and CORO1C-null cells have increased branched actin density and altered lamellipodial protrusion dynamics (King et al., 2022). Indeed, perhaps not surprisingly, CORO2A (and CORO1C) knockdown had a marked effect on the internalization of clathrin-dependent cargo receptors, consistent with previous studies indicating a major role for actin in endocytosis. Perhaps more surprisingly, however, CORO2A (and CORO1C) depletion had little impact upon the internalization rate of cargo typically considered to be taken up through clathrin-independent pathways. A number of studies have documented a role for actin regulatory proteins in clathrin-independent internalization including the GTP-binding proteins Rac1, RhoA, and Cdc42, as well as Myosin II (Saharanjanak et al., 2002; Soriano-Castell et al., 2017; Chan Wah Hak et al., 2018; Wayt et al., 2021). Nonetheless, our data highlight a major difference in the requirements for actin regulation in the internalization of clathrin-dependent and -independent cargo, at least with regard to the need for attenuation of branched actin to facilitate the internalization process. While internalization of clathrin-independent cargo remained unaffected upon CORO2A knockdown, strikingly these cargo displayed retarded rates of recycling from endosomes, consistent with a role for CORO2A in the endosomal fission process. Indeed, our initial experiments supported a role for CORO2A in the regulation of endosomal fission derived from measurements of endosomal size, with enlarged endosomes serving as an in vivo proxy for impaired fission. However, since these data rely on chronic (48 h) knockdown of CORO2A before endosome size is measured, we applied our newly developed quantitative assay to acutely measure fission of endosomes, and directly demonstrated impaired fission upon reduced CORO2A expression (Figure 10 and Supplemental Figure S7).

Our study unexpectedly highlights a significant difference in the requirement of branched actin attenuation for clathrin-dependent and -independent internalization. In addition, we identified...
CORO2A as a member of the endosomal fission machinery that links the ATPase EHD1 with actin-based endosomal constriction. Recruitment of the WASH complex and activation of ARP2/3 leading to actin branching is clearly crucial for initiating the early steps of endosomal fission (Duleh and Welch, 2010), with nucleation of branched actin key to membrane constriction. However, evidence suggests that the final steps of fission may be mediated by enzymes that carry out nucleotide hydrolysis, such as EHD1.

**MATERIALS AND METHODS**

Request a protocol through Bio-protocol.

**Antibodies and reagents**

The following antibodies were used: anti-EHD1 (109311, Abcam), affinity-purified rabbit polyclonal peptide antibody directed against the C-terminus of CORO2A (RELTORQAKQLEIKK; LifeTein for IP and immunoblotting), anti-TOM20 (sc-11415, Santa Cruz Biotechnology), anti-HA (T501, SAB), anti-FLAG (F1804, Sigma for immunofluorescence; ab205606, Abcam for immunoblotting), anti-GST-HRP (A01380, GenScript), anti-GAPDH-HRP (HRP-60004, Proteintech), anti-MHC-1 (purified W6/32, Leinco Technologies), anti-CD98 (315602, BioLegend), anti-CD59 (A4-233-C100, Exbio), anti-EEA1 (H00085377-B01P, Novus), donkey anti-mouse-HRP (715-035-151, Jackson), mouse anti-rabbit IgG light chain-HRP (211-032-171, Jackson), Alexa Fluor 568–conjugated goat anti-rabbit (A11036, Molecular Probes), Alexa Fluor 568–conjugated donkey anti-mouse (A21202, Molecular Probes). The following reagents were used: CF-568–conjugated Phalloidin (44-T VWR, Biotium), cytochalasin D (Calbiochem), Alexa fluor 568–conjugated transferrin (Molecular Probes), Rhodamine-tagged EGF(Invitrogen), and Blasticidin S HCl (ThermoFisher Scientific).

**Cell lines**

The HeLa cervical cancer cell line was obtained from the American Type Culture Collection (ATCC) and grown in complete DMEM (high glucose) containing 10% fetal bovine serum (FBS), 1x penicillin-streptomycin (Invitrogen), and 2 mM glutamine. CRISPR/Cas9 was applied using the TrueTag DNA Donor kit (RFP, ThermoFisher Scientific) to generate the HeLa cell line expressing endogenous levels of EHD1 with RFP attached to its N-terminus, following the
FLAG-CORO2A (bp 1–1575) was obtained from GenScript (clone# OHu03313D). pET28a-EHD1 was generated from the original EHD1 constructs designed (Caplan et al., 2002). Primers were designed using the Takara primer design tool for PCR amplification of the ORF encoding human CORO2A (bp 1-1575) and EH-domain of EHD1 (bp 1330–1603). The amplified product, CORO2A, was cloned in pGEX-4T-1 while EH-domain of EHD1 was cloned in pET-28a. Amino acid substitutions were made in pGEX-4T-1-CORO2A from aspartagine-proline-phenylalanine-aspartic acid-aspartic acid (residues 91–95) to asparagine-proline-phenylalanine-alanine-aspartic acid (NPFAD) and asparagine-proline-phenylalanine-alanine-alanine (NPFDD) using the Q5 site-directed mutagenesis kit (NEB, catalogue no. E0554S) following the manufacturer’s protocol.

**Transfection and siRNA treatment**

Wild-type HeLa cells and pooled CRISPR/Cas9 gene-edited HeLa cells expressing RFP-EHD1 were grown for 24 h in complete DMEM and then transfected with FLAG-CORO2A for 24 h at 37°C using Lipofectamine 2000 (Invitrogen). U251MG glioblastoma cells were grown for 24 h in complete IMEM. The cells were then either treated with predesigned scrambled siRNA (SICO01, Sigma), subjected to a combination of custom human CORO2A siRNA oligonucleotides (5′-CUGAGUACGCCGUCCAUAAA-3′ and 5′-GAGUAGCCGGU-GGAGU-3′, Sigma), or a combination of predesigned CORO1C siRNA oligonucleotides (SASI_Hs01_00150715 and SASI_Hs01_00150716, Sigma) for 72 h at 37°C using Dharmafect (ThermoFisher Scientific), following the manufacturer’s protocol. The efficiency of knockdown was determined by immunoblotting.

**Recombinant gene expression and protein purification**

The recombinant DNA constructs were expressed in *Escherichia coli* Rosetta (R2) strain and purified by affinity chromatography, in separate experiments. Briefly, a freshly transformed colony of *E. coli* was inoculated in 50 ml of Luria-Bertani (LB) broth (with 50 μg/ml kanamycin for recombinant pET-28a expression plasmids and 100 μg/ml ampicillin for recombinant pGEX-4T-1 expression plasmids) and cultured overnight at 37°C with continuous shaking (primary culture). Next, primary culture was inoculated in 1000 ml fresh LB-broth in a 1:100 dilution and incubated at 37°C with continuous shaking until readings of 0.4–0.6 at 600 nm OD. The culture was then induced with 1 mM IPTG overnight either at 18°C for recombinant pGEX-4T-1 expression plasmids or at 25°C for recombinant pET-28a expression plasmids. The cells were then centrifuged at 2100 x g for 15 min at 4°C. The bacterial pellet obtained was resuspended in ice-cold lysis buffer containing 1 tablet/10 ml protease inhibitor cocktail (Roche). The composition of lysis buffer for recombinant pET-28a expression plasmids was 50 mM Tris, 200 mM NaCl, and 50 mM Imidazole, pH 8.0, whereas for recombinant pGEX-4T-1 expression plasmids the composition of lysis buffer was 1x PBS, pH 7.4. Sample lysis was performed by six cycles of sonication on ice (2 min bursts/2 min cooling/200-200 W in a Branson Sonicator, USA). The lysate was centrifuged at 18,000 x g for 30 min at 4°C, which allowed separation of clear supernatant and cellular debris (inclusion bodies are pelleted by centrifugation). The supernatant was then mixed and allowed to bind with either Ni²⁺-NTA (pET-28a plasmids) or Glutathione Sepharose resin (pGEX-4T-1 plasmids) for 4 h at 4°C. To ensure the removal of any nonspecifically bound proteins, the beads were then washed extensively with 10 bed volumes of wash buffer. For pGEX-4T-1 plasmids, trichloroacetic acid precipitation followed by resuspension in 1x PBS, while for pET-28a plasmids, trichloroacetic acid precipitation followed by washing with 100 mM Imidazole, 50 mM Tris, and 200 mM

**Isolation of genomic DNA from CRISPR/Cas9 gene-edited HeLa cells**

Cells were collected by trypsinization and centrifuged at 8000 x g for 10 min followed by a wash with 1x phosphate-buffered saline (PBS). The pellet was then resuspended in 1x TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA, pH 8.0) containing 0.1% Triton X-100. The sample was boiled at 100°C for 5 min and then centrifuged at 13,000 x g for 10 min. The supernatant (containing genomic DNA) was collected and subjected to PCR.

**Co-IP**

HeLa cells were grown in 100-mm dishes until confluent. Cells were lysed with lysis buffer containing 50 mM Tris, pH 7.4, 100 mM NaCl, 0.5% Triton X-100, and 1x protease cocktail inhibitor (Millipore) on ice for 30 min. Lysates were incubated with the specific antibody at 4°C overnight. Protein G beads (GE Healthcare) were added to the lysate-antibody mix at 4°C for 4 h. Samples were then washed three times with the same lysis buffer. Proteins were eluted from the protein G beads by boiling in the presence of 4x loading buffer (250 mM Tris, pH 6.8, 8% SDS, 40% glycerol, 5% β-mercaptoethanol, 0.2% bromophenol blue) for 10 min. Eluted proteins were then detected by immunoblotting.
NaCl, pH 8.0 by centrifuging at 3000 × g for 3 min at 4°C. Finally, the bound Histidine-tagged and GST-tagged proteins were subjected to elution for 4 h at 4°C in elution buffer containing 300 mM imidazole, 50 mM Tris, and 200 mM NaCl, pH 8.0, and 30 mM glutathione (reduced) in 50 mM Tris-HCl, pH 8.0, respectively, followed by centrifugation at 2100 × g for 5 min at 4°C. The purified proteins were then dialyzed against dialysis buffer (50 mM Tris, pH 8.0, 200 mM NaCl, and 0.1 mM phenylmethylsulfon fluoride [PMSF]) overnight at 4°C.

Direct interaction assay
For the protein–protein interaction assays, a 20-μl slurry of Ni²⁺-NTA beads was washed four times with 10 bed volumes of beads with TGEM buffer (20 mM Tris-HCl, pH 7.9, 20% glycerol, 1 mM EDTA, 5 mM MgCl₂, 0.1% NP-40, 1 mM dithiothreitol [DTT], 0.2 mM PMSF, 0.1 M NaCl) by centrifuging at 13,000 rpm for 30 s, followed by an addition of three bed volumes of TGEM to the packed beads to which 0.5 μg of the purified Histidine-tagged proteins was incubated for 2 h at 4°C in a tube rotator. The immobilized His-tagged bait slurry was then centrifuged at 13,000 rpm for 30 s, followed by two washes with 10 bed volumes of beads with TGEM buffer (20 mM Tris-HCl, pH 7.9, 20% glycerol, 1 mM EDTA, 5 mM MgCl₂, 0.1% NP-40, 1 mM DTT, 0.2 mM PMSF, 1 M NaCl) and then twice with TGMC buffer (20 mM Tris-HCl, pH 7.9, 20% glycerol, 5 mM CaCl₂, 0.1% NP-40, 1 mM DTT, 0.2 mM PMSF, 0.1 M NaCl). After the last wash, two bed volumes of TGMC were added to the bait along with 1 U micrococcal nuclease and incubated at 30°C for 10 min; 0.5 μg of target GST-fusion proteins was diluted in TGMC to obtain a 40-μl sample (target) volume per reaction and treated with 1 U micrococcal nuclease and incubated at 30°C for 10 min. The nuclease-treated GST-target proteins were then incubated with bait for 2 h at 4°C in a tube rotator. The samples were then washed four times with 10 bed volumes of TGEM and subjected to SDS–PAGE.

Immunofluorescence analysis
HeLa and U251MG glioblastoma cells on coverslips were treated as indicated in the text. The uptake of transferrin and EGF receptors in the cells was performed by diluting transferrin-568 and EGF-Rhodamine, respectively, diluted in complete IMEM at 37°C for the time points (chase) indicated in the text. Cells were then incubated again in stripping buffer to quantify the remaining intracellular MHC-1 and CD98 receptors followed by fixation in 4% paraformaldehyde in PBS for 10 min. After fixation, cells were rinsed three times in PBS and then incubated with appropriate fluorochrome–conjugated secondary antibodies diluted in staining buffer (PBS containing 0.5% bovine serum albumin and 0.2% saponin) for 30 min at room temperature. Cells were washed 3x in PBS and mounted in Fluoromount. Z-stack confocal imaging was performed using a Zeiss LSM 800 confocal microscope (Carl Zeiss) with a 63x/1.4 NA oil objective and the images were assessed using either Zen, ImageJ, or Imaris software. For quantification, three to five independent experiments were carried out, and the number of samples collected for quantification is described in the text.

Endosome fission assay using a phosphoinositide 3-kinase (PI3K) inhibitor
Mock and CORO2A siRNA-treated HeLa cells were incubated with 8 μM of the PI3K inhibitor LY294002 diluted in DMEM for 1 h at 37°C, followed by washing with 1× PBS. The cells were then allowed to internalize Alexa Fluor 568–conjugated transferrin for 10 min at 37°C in the presence of LY294002 and washed with PBS to remove the LY294002 inhibitor. Finally, the cells were chased for 15 min in complete DMEM at 37°C to acutely allow fission and trafficking to proceed, followed by three washes with 1× PBS and fixation with 4% paraformaldehyde in PBS for 10 min at room temperature. Frequency and distribution of endosomal size was quantified to inform on the degree of endosomal fission.

Image processing, 3D surface and filament reconstruction, and “Kiss and Run” analysis
The excitation spot that scanned across the specimen is visualized as a pixel in the final 2D images acquired from the confocal microscope. Each spot has an intensity value associated with it and the matrix of intensity values for an image is displayed as arithmetic mean intensity. These values were obtained from the “histo” bar in Zen software. Z-sections of images (7 slices) were either projected with ImageJ (National Institutes of Health, Bethesda, MD) or 3D-rendered with Imaris x64 9.1.2 software (Bitplane AG, Zurich, Switzerland) coupled with custom MATLAB (2009 and 2014) programming, as indicated in the text. Briefly, the threshold for images analyzed by ImageJ was set at Renyintropy and the average particle size was then measured in an automated manner. For Imaris analysis, the image display was adjusted for the channels and rendering quality was set to 100%; 3D surfaces for CORO2A, Phalloidin, and EHD1 were created by selecting source channel and smooth surface detail set at 0.198 μm. The background subtraction was set to 0.543 μm and the threshold at 6354 AU for surfaces to fully cover all voxels. To quantify the surface contacts (overlap) between two surfaces (EHD1 and CORO2A or Phalloidin and CORO2A), the ImarisXT bundle “Kiss and Run” was first integrated with MATLAB and launched in Imaris; 3D surface reconstructed images were then processed for kiss and run analysis using the Distance Transformation module which uses a surface mask for the target and tracks surface as well as determining contacts for each surface independently. This Xtension program analyzes contact events between surfaces that are defined by closest surface to surface distance measure, having at least one overlapping voxel (distance threshold set at 0 μm). Percentage surface contacts were then quantified and exported to Excel for further analysis. To obtain 3D-rendered surfaces for EEA1, the smooth surface detail was set at 0.200 μm and the background subtraction at 0.543 μm. For fission assays using transferrin, smooth surface detail was set at 0.200 μm and thresholding was set at an absolute intensity of 2.22 × 10⁴ AU. The surface area of the surfaces generated was quantified by Imaris and the values were exported into Excel for graphical and statistical analysis using GraphPad Prism. Finally, to measure the number and diameter of actin-rich cell protrusions, the Imaris “Filament Tracer” module was used and an autopath (no loops) algorithm was selected. The diameter of the start of a protrusion at the cell surface (dendrite beginning diameter) and the end of the filaments were manually assigned in the software. The diameter and frequency of
dendrite beginning points were quantified and exported to Excel for further analysis.

Graphical and statistical analysis
Arithmetic mean intensity was quantified using Zen software. NIH ImageJ was used to quantify average particle size of MHC-1 endosomes. Percentage surface contacts were calculated using Imaris XT bundle “Kiss and Run” distance transform module with CORO2A set as target surfaces and EHD1 or Phalloidin as tracked surfaces. A bar graph was plotted and a two-tailed t test was performed to evaluate significance, as indicated in Figure 4F. The surface area of EEA1 endosomes was assessed using the Imaris surface module. A frequency distribution (interleaved) graph was plotted for diameter of actin-rich cell protrusions in cells expressing CORO2A or lacking CORO2A with the first bin starting from 1 μm up to 7 μm and the bin width set at 0.5 μm. For fission assays, the frequency distribution (interleaved) graph was plotted for the area of transferrin-containing structures with the first bin starting from 100 μm$^2$ up to 200 μm$^2$ and the bin width set at 20 μm$^2$. A Gaussian curve was also plotted. All the graphical and statistical tests were done using GraphPad Prism 9.2.0.

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REFERENCES
Bai M, Pang X, Lou J, Zhou Q, Zhang K, Ma S, Ji L, Ji J, Sun F, Hsu VW (2012). Mechanistic insights into regulated cargo binding by ACAP1 protein. J Biol Chem 287, 28675–28685.
Behrens J, Solga R, Ziemann A, Rastetter RH, Berwanger C, Herrmann H, Noegel AA, Clemen CS (2016). Coronin 1C-free primary mouse fibroblasts exhibit robust rearrangements in the orientation of actin filaments, microtubules and intermediate filaments. Eur J Cell Biol 95, 239–251.
Cai B, Caplan S, Naslavsky N (2012). cPLA2alpha and EHD1 interact and regulate the vesiculation of cholesterol-rich, GPI-anchored, protein-containing endosomes. Mol Biol Cell 23, 1874–1888.
Cai B, Giridharan SS, Zhang J, Caplan S, Naslavsky N (2014a). GRAF1 forms a complex with McAC-L1 and EHD1 to cooperate in tubular recycling endosome vesiculation. Front Cell Dev Biol 2, 22.
Cai B, Xie S, Caplan S, Naslavsky N (2014a). GRAF1 forms a complex with McAC-L1 and EHD1 to cooperate in tubular recycling endosome vesiculation. Front Cell Dev Biol 2, 22.
Cai B, Xie S, Liu F, Simone LC, Caplan S, Qin X, Naslavsky N (2014b). Rapid degradation of the complement regulator, CD59, by a novel inhibitor. J Biol Chem 289, 12109–12125.
Cai L, Makrov AM, Schafer DA, Bear JE (2008). Coronin 1B antagonizes cortxin and remodels Arp2/3-containing actin branches in lamellipodia. Cell 134, 828–842.
Caplan S, Naslavsky N, Hartnell LM, Lodge R, Polischuk RS, Donaldson JG, Bonifacino JS (2002). A tubular EHD1-containing compartment involved in the recycling of major histocompatibility complex class I molecules to the plasma membrane. EMBO J 21, 2557–2567.
Caswell P, Norman J (2008). Endocytic transport of integrins during cell migration and invasion. Trends Cell Biol 18, 257–263.
Chan KT, Creed SJ, Bear JE (2011). Unraveling the enigma: progress towards understanding the coronin family of actin regulators. Trends Cell Biol 21, 481–488.
Chen Y, Xu J, Zhang Y, Ma S, Yi W, Liu S, Xu X, Wang J, Chen Y (2020). Coronin 2B regulates dendrite outgrowth by modulating actin dynamics. FEBS Lett 594, 2975–2987.
Dai J, Li J, Bos E, Porciomatto M, Premont RT, Bourgoin S, Peters PJ, Hsu VW (2004). EAP1 promotes endocytic recycling by recognizing recycling sorting signals. Dev Cell 7, 771–776.
Deng JH, Zhang HB, Zeng Y, Xu YH, Huang Y, Wang G (2020). Effects of CORO2A on cell migration and proliferation and its potential regulatory network in breast cancer. Front Oncol 10, 916.
Deo R, Kushwah MS, Kamerkar SC, Kadam NY, Dar S, Babu K, Srivastava A, Pucadyil TJ (2018). ATP-dependent membrane remodeling links EHD1 functions to endocytic recycling. Nat Commun 9, 5187.
Derivery E, Gautreau A (2010a). Evolutionary conservation of the WASH complex, an actin polymerization machinery involved in endosomal fission. Commun Integr Biol 3, 227–230.
Derivery E, Gautreau A (2010b). Generation of branched actin networks: assembly and regulation of the N-WASP and WAVE molecular machines. Bioessays 32, 119–131.
Derivery E, Helfer E, Henriot V, Gautreau A (2012). Actin polymerization controls the organization of WASH domains at the surface of endosomes. PLoS One 7, e39774.
Derivery E, Sousa C, Gautier JJ, Lombard B, Loew D, Gautreau A (2009). The Arp2/3 activator WASH controls the fission of endosomes through a large multiprotein complex. Dev Cell 17, 712–723.
Dhawan K, Naslavsky N, Caplan S (2020). Sorting nexin 17 (SNX17) links endosomal sorting to Eps15 homology domain protein 1 (EHD1)-mediated fission machinery. J Biol Chem 295, 3837–3850.
Duleh SN, Welch MD (2010). WASH and the Arp2/3 complex regulate endosome shape and trafficking. Cytoskeleton (Hoboken) 67, 193–206.
Eyster CA, Higginson JD, Huebner R, Porat-Shlom N, Weigert R, Wu WW, Shen RF, Donaldson JG (2009). Discovery of new cargo proteins that enter cells through clathrin-independent endocytosis. Traffic 10, 590–599.
Fraley TS, Pereira CB, Tran TC, Singleton C, Greenwood JA (2005). Phosphoinositide binding regulates alpha-actinin dynamics: mechanism for modulating cytoskeletal remodeling. J Biol Chem 280, 15479–15482.
Gong B, Guo Y, Ding S, Liu X, Meng A, Li D, Jia S (2021). A Golgi-derived vesicle potentiates PtdIns4P to PtdIns3P conversion for endosome fission. Nat Cell Biol 23, 782–795.
Grant BD, Donaldson JG (2009). Pathways and mechanisms of endocytic recycling. Nat Rev Mol Cell Biol 10, 597–608.
Hao YH, Doyle JM, Ramanathan S, Gomez TS, Jia D, Xu M, Chen ZJ, Billadeau DD, Rosen MK, Potts PR (2013). Regulation of WASH-dependent actin polymerization and protein trafficking by ubiquitination. Cell 152, 1051–1064.
Heil-Chapdelaine RA, Tran NK, Cooper JA (1998). The role of Saccharomyces cerevisiae coronin in the actin and microtubule cytoskeletons. Curr Biol 8, 1281–1284.
Henry GD, Corigan DJ, Dineen JV, Baleja JD (2010). Charge effects in the selection of NPF motifs by the EH domain of EHD1. Biochemistry 49, 3381–3392.
Hoyer MJ, Chitwood PJ, Ebmeier CC, Striepen JF, Qi RZ, Old WM, Voeltz GK (2018). A novel class of ER membrane proteins regulates ER-associated endosome fission. Cell 175, 254–265.e214.
Huang W, Ghisletti S, Saigo K, Gandhi M, Aouadi M, Tesz GJ, Zhang DX, Yao J, Czech MP, Goode BL, et al. (2011). Coronin 2A mediates actin-dependent de-repression of inflammatory response genes. Nature 470, 414–418.
Jakobsson J, Ackermann F, Andersson F, Larhammar D, Low P, Brodin L (2011). Regulation of synaptic vesicle budding and dynamin function by an EHD AT-Pase. J Neurosci 31, 13972–13980.
Jia D, Gomez TS, Billadeau DD, Rosen MK (2012). Multiple repeat elements within the FAM21 tail link the WASH actin regulatory complex to the retromer. Mol Biol Cell 23, 2352–2361.
Jones T, Naslavsky N, Caplan S (2020). Eps15 homology domain protein 4 (EHD4) is required for Eps15 homology domain protein 1 (EHD1)-mediated endosomal recruitment and fission. PLoS One 15, e0239657.
Kamerkar SC, Roy K, Bhattacharyya S, Pucadyil TJ (2019). A screen for membrane fusion catalysts identifies the ATPase EHD1. Biochemistry 58, 65–71.
Kawasaki A, Sakai A, Nakanishi H, Hasegawa J, Taguchi T, Sasaki J, Arai H, Sasaki T, Igarashi M, Nakatsu F (2022). PIP4/P5 countertransporter by ORP10 at ER-endosome membrane contact sites regulates endosome fission. J Cell Biol 211, e202103141.
Kieken F, Jovic M, Naslavsky N, Caplan S, Sorgen PL (2007). EH domain of coronin 2A mediates actin–endosome fission. J Cell Biol 221, e202103141.
Kieken F, Jovic M, Tonelli M, Naslavsky N, Caplan S, Sorgen PL (2009). Structural insight into the interaction of proteins containing NPF, DPF, and GPF motifs with the C-terminal EH-domain of EHD1. Protein Sci 18, 2471–2479.

Kieken F, Sharma M, Jovic M, Giridharan SS, Naslavsky N, Caplan S, Sorgen PL (2010). Mechanism for the selective interaction of C-terminal Ephs15 homology domain proteins with specific Asn-Pro-Phe-containing partners. J Biol Chem 285, 8687–8694.

King ZT, Butler MT, Hockenberry MA, Subramanian BC, Siess PF, Graham DM, Legant WR, Bear JE (2022). Coro1B and Coro1C regulate lamellipodia dynamics and cell motility by tuning branched actin turnover. J Cell Biol 221.

Li J, Peters PJ, Bai M, Dai J, Bos E, Kirchhausen T, Kandror KV, Hsu VW (2007). An ACAP1-containing clathrin coat complex for endocytic recycling. J Cell Biol 178, 453–464.

Liu SL, Needham KM, May JR, Nolen BJ (2011). Mechanism of a concentration-dependent switch between activation and inhibition of Arp2/3 complex by coronin. J Biol Chem 286, 17039–17046.

Marshall TW, Aloor HL, Bear JE (2009). Coronin 2A regulates a subset of focal-adhesion-turnover events through the cofilin pathway. J Cell Sci 122, 3061–3069.

McNally KE, Faulkner R, Steinberg F, Gallon M, Ghai R, Pim D, Langton P, Marshall TW, Aloor HL, Bear JE (2009). Coronin 2A (CRN5) expression is associated with colorectal adenoma-adenocarcinoma sequence and oncogenic signalling. BMC Cancer 15, 638.

Rowland AA, Chitwood PJ, Phillips MJ, Voeltz GK (2014). ER contact sites define the position and timing of endosome fission. Cell 159, 1027–1041.

Sabhananjak S, Sharma P, Parton RG, Mayor S (2002). GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. Dev Cell 2, 411–423.

Simonetti B, Paul B, Chaudhari K, Weeratunga S, Steinberg F, Gorla M, Heesom KJ, Bashaw GJ, Collins BM, Cullen PJ (2019). Molecular identification of a BAR domain-containing coat complex for endosomal recycling of transmembrane proteins. Nat Cell Biol 21, 1219–1233.

Singla A, Fedoseienko A, Giridharan SS, Overlee BL, Lopez A, Jia D, Song J, Huff-Hardy K, Weisman L, Burstein E, Billadeau DD (2019). Endosomal PI(3)P regulation by the COMMD/CCDC22/CCDC93 (CCC) complex controls membrane protein recycling. Nat Commun 10, 4271.

Skop AR, Bergmann D, Mohler WA, White JG (2001). Completion of cytokinesis in C. elegans requires a brefeldin A-sensitive membrane accumulation at the cleavage furrow apex. Curr Biol 11, 735–746.

Sokolova OS, Chemeris A, Guo S, Alioto SL, Gandhi M, Padrick S, Pechiknova E, David V, Gautreau A, Goode BL (2017). Structural Basis of Arp2/3 Complex Inhibition by GMF, Coronin, and Arpin. J Mol Biol 429, 237–248.

Simonetti B, Paul B, Chaudhari K, Weeratunga S, Steinberg F, Gorla M, Heesom KJ, Bashaw GJ, Collins BM, Cullen PJ (2019). Molecular identification of a BAR domain-containing coat complex for endosomal recycling of transmembrane proteins. Nat Cell Biol 21, 1219–1233.

Singla A, Fedoseienko A, Giridharan SS, Overlee BL, Lopez A, Jia D, Song J, Huff-Hardy K, Weisman L, Burstein E, Billadeau DD (2019). Endosomal PI(3)P regulation by the COMMD/CCDC22/CCDC93 (CCC) complex controls membrane protein recycling. Nat Commun 10, 4271.

Skop AR, Bergmann D, Mohler WA, White JG (2001). Completion of cytokinesis in C. elegans requires a brefeldin A-sensitive membrane accumulation at the cleavage furrow apex. Curr Biol 11, 735–746.

Sokolova OS, Chemeris A, Guo S, Alioto SL, Gandhi M, Padrick S, Pechiknova E, David V, Gautreau A, Goode BL (2017). Structural Basis of Arp2/3 Complex Inhibition by GMF, Coronin, and Arpin. J Mol Biol 429, 237–248.

Simonetti B, Paul B, Chaudhari K, Weeratunga S, Steinberg F, Gorla M, Heesom KJ, Bashaw GJ, Collins BM, Cullen PJ (2019). Molecular identification of a BAR domain-containing coat complex for endosomal recycling of transmembrane proteins. Nat Cell Biol 21, 1219–1233.

Singla A, Fedoseienko A, Giridharan SS, Overlee BL, Lopez A, Jia D, Song J, Huff-Hardy K, Weisman L, Burstein E, Billadeau DD (2019). Endosomal PI(3)P regulation by the COMMD/CCDC22/CCDC93 (CCC) complex controls membrane protein recycling. Nat Commun 10, 4271.

Skop AR, Bergmann D, Mohler WA, White JG (2001). Completion of cytokinesis in C. elegans requires a brefeldin A-sensitive membrane accumulation at the cleavage furrow apex. Curr Biol 11, 735–746.