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Report

UBAP1 Is a Component of an Endosome-Specific ESCRT-I Complex that Is Essential for MVB Sorting

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Summary

Endosomal sorting complexes required for transport (ESCRTs) regulate several events involving membrane invagination, including multivesicular body (MVB) biogenesis, viral budding, and cytokinesis [1]. In each case, upstream ESCRTs combine with additional factors, such as Bro1 proteins [2–4], to recruit ESCRT-III and the ATPase VPS4 in order to drive membrane scission [5]. A clue to understanding how such diverse cellular processes might be controlled independently of each other has been the identification of ESCRT isoforms. Mammalian ESCRT-I comprises TSG101, VPS28, VPS37A–D [6–8], and MVB12A/B [9]. These could generate several ESCRT-I complexes, each targeted to a different compartment and able to recruit distinct ESCRT-III proteins. Here we identify a novel ESCRT-I component, ubiquitin-associated protein 1 (UBAP1), which contains a region conserved in MVB12 [10]. UBAP1 binds the endosomal Bro1 protein His domain protein tyrosine phosphatase (HDPTP) [4], but not Alix, a Bro1 protein involved in cytokinesis [11, 12]. UBAP1 is required for sorting EGFR to the MVB and for endosomal ubiquitin homeostasis, but not for cytokinesis. UBAP1 is part of a complex that contains a fraction of total cellular TSG101 and that also contains VPS37A but not VPS37C. Hence, the presence of UBAP1, in combination with VPS37A, defines an endosome-specific ESCRT-I complex.

Results and Discussion

Several Bro1 proteins exist in higher eukaryotes. One of these, Alix, is required for cytokinesis [11, 12], whereas another, His domain protein tyrosine phosphatase (HDPTP), supports multivesicular body (MVB) formation [4]. Hence, these proteins could recruit endosomal sorting complexes required for transport (ESCRTs) selectively to cellular sites. The Bro-V domain of Alix is important for effector binding [3, 13]. To identify components involved in MVB formation, we screened this region of HDPTP by yeast two-hybrid analysis against a cDNA library. Five interacting clones contained in-frame coding regions of ubiquitin-associated protein 1 (UBAP1), a ubiquitously expressed protein containing two to three C-terminal UBA domains [14] (Figure 1A). Although the function of UBAP1 is unknown, it is a risk factor for frontotemporal lobar degeneration (FTLD) [15]. All clones contained a central region (aa 159–308) of UBAP1. Directed yeast two-hybrid analysis showed that full-length UBAP1 interacted with the Bro-V region of HDPTP, but not Alix, and with full-length HDPTP (Figure 1B; see also Figure S1A available online).

The UBAP1 binding region is confined to the putative V domain of HDPTP (Figure 1B; Figure S1A). Alix V domain contains a conserved pocket that binds a YPXnL motif within the late domain of HIV [3, 13]. One amino acid that is essential for viral binding is F676, and a corresponding residue, F678, is found in HDPTP. A F678D mutation abolished HDPTP binding to UBAP1 (Figure 1B; Figure S1A), but not to other interactors (data not shown). To assess the importance of F678 for HDPTP function, we treated cells with small interfering RNA (siRNA) to deplete HDPTP and then rescued them using siRNA-resistant HDPTP. Loss of HDPTP caused accumulation of ubiquitinated proteins and internalized EGF on enlarged and/or clustered endosomes ([4]; Figure S1B). These phenotypes were rescued by wild-type (WT) HDPTP, but not by HDPTP lacking a V domain, and only marginally by HDPTP(F678D) (Figure 1C).

Interaction between endogenous or overexpressed HDPTP and UBAP1 could not be detected by immunoprecipitation (data not shown), suggesting that it has a low affinity. However, localization experiments provided evidence that UBAP1 functions closely with HDPTP. Endogenous HDPTP colocalized partially with the early endosome marker EEA1 (Figure S1C). Although UBAP1 was cytosolic (Figure S1D), it was recruited to vacuolar, HDPTP-labeled structures when VPS4 was depleted (Figures S1E and S1F). Recruitment of UBAP1 to VPS4-induced endosomal vacuoles is consistent with the behavior of other ESCRT components and was not a trivial consequence of it binding to the pool of endosomal ubiquitin via its UBA domains, because myc-UBAP1 lacking this region was recruited as efficiently as WT myc-UBAP1 (Figure S1G).

To identify UBAP1 function, we depleted cells of UBAP1 by siRNA (Figure S2A). Like depletion of HDPTP [4], this caused clustering of early endosomes and enlargement and clustering of LAMP1-positive late endosomes and lysosomes (Figure 2A; Figure S2B). One striking feature of HDPTP depletion is the replacement of the normal complement of early endosomes and MVBs with clusters of membranes that include both tubulovesicular and vacuolar elements [4] (Figures S2C and S2D). Loss of UBAP1 caused a very similar phenotype (Figure 2B; Table S1).

Depletion of UBAP1 also interfered with ubiquitin homeostasis on early endosomes, causing dramatic accumulation of ubiquitinated proteins on compartments adjacent to or overlapping with the endosomal marker and ESCRT-0 component Hrs (hepatocyte growth factor [HGF]-regulated tyrosine kinase substrate) (Figures 3A and 3B). In addition, internalized fluorescent EGF accumulated in UBAP1-depleted cells, including in clusters that localized closely with Hrs (Figure 3C). Pulse-chase experiments using ^{125}I-labeled EGF confirmed that EGF degradation was impaired by loss of UBAP1 ($p = 0.001$) (Figure 3D). Hence, UBAP1 is important for EGF trafficking. Phenotypes were similar to those seen upon depletion of HDPTP or the ESCRT-I component TSG101 (Figures S3A

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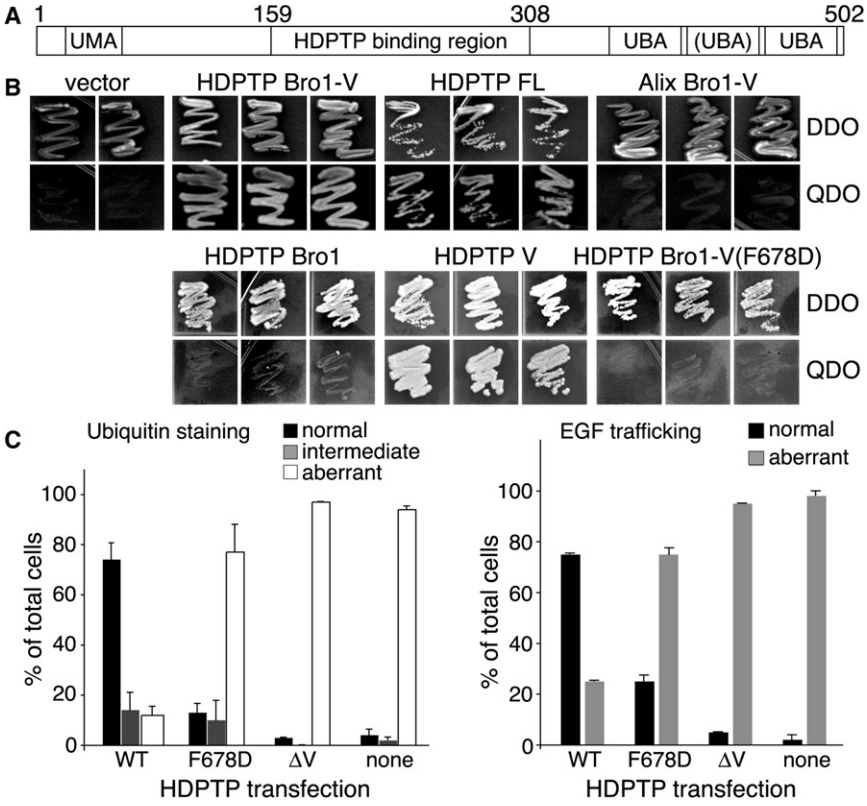

Figure 1. Interaction between HDPTP and UBAP1

(A) Diagram showing domain structure of UBAP1. The following abbreviations are used: UMA, UBAP1-MVB12-associated domain; UBA, ubiquitin-associated domain (note that the central UBA-like domain does not have the consensus MGF/Y motif).

(B) Yeast cultures were cotransformed in duplicate or triplicate, as indicated, and liquid cultures were streaked onto agar plates containing selection media as shown.

(C) HeLaM cells depleted of HDPTP were transfected with the indicated HDPTP constructs and scored for the accumulation of ubiquitinated proteins on endosomes (left) or retention of internalized EGF in intracellular clusters (right). Values are from three experiments in which 100 cells were scored for each condition; error bars are standard error of the mean (SEM) to indicate interexperimental error.

and S3B) and were rescued by siRNA-resistant UBAP1-GFP (Figures S3C–S3E).

To analyze EGFR transport at the ultrastructural level, we incubated cells with gold-conjugated anti-EGFR and then challenged them with EGF. In control cells, most gold conjugates localized to the lumen of MVBs after 40 min (Figure 3E; Table S2A). In contrast, in UBAP1-depleted cells, anti-EGFR gold was found mainly in tubulovesicular regions of membrane clusters or at the limiting membrane of vacuolar elements within these clusters (Figure 3F; Table S2A). Even after 3 hr, though more anti-EGFR gold was now associated with vacuolar elements within endosomal clusters, most remained at the limiting membrane (Figure 3G; Table S2A). As in unstimulated cells, endosomal clusters were much more abundant than normal MVBs (Table S2B).

Hence, UBAP1 is required for the MVB sorting of EGFR and functionally is close to TSG101 [16]. As well as TSG101, ESCRT-I contains VPS28, VPS37A–D, and MVB12A/B [6–9, 17, 18]. UBAP1 contains a region (*U*BAP1-*M*VB12-*a*ssociated [UMA] domain; aa 17–63) resembling those within MVB12A and MVB12B responsible for their incorporation into ESCRT-I [10] and thus might represent a new ESCRT-I component [10]. We confirmed the presence of UBAP1 within ESCRT-I by immunodepleting cytosolic extracts of native ESCRT-I using antibodies against TSG101 and VPS28 (Figure 4A). Under these conditions, virtually all UBAP1 was immunodepleted, along with the VPS37 isoforms VPS37A and VPS37C (Figure 4A). Relative to TSG101, UBAP1 was immunodepleted using anti-TSG101 by 95.3% ± 2.5%, VPS37A by 95.6% ± 7.5%, and VPS37C by 92% ± 6.6% (n = 3). Hence, all are stoichiometrically incorporated into ESCRT-I. To further investigate the association of UBAP1 with ESCRT-I, we overexpressed UBAP1-myc alongside two UBAP1 mutants (P37A and E59G) in which conserved UMA residues [9, 10] were altered. UBAP1-myc, but neither UMA mutant, was incorporated into native ESCRT-I (Figure 4B).

UBAP1 contains three putative UBA domains (aa 390–418, 420–452, and 455–500) [10]. The first and third possess the consensus UBA motif MGF/Y [19], whereas the second has a KGF sequence. UBAP1-myc, expressed to high levels to minimize the amount incorporated into endogenous ESCRT-I, bound selectively to ubiquitin beads (Figure 4C). Binding was unaffected by mutation of the UMA domain (E59G; 134% ± 26%; n = 3, p = 0.28), confirming that it occurred independently of ESCRT-I. In addition, binding of UBAP1-myc to ubiquitin was unaffected by prior immunodepletion of ESCRT-I (Figure S4A). In contrast, binding was reduced to 18.6% ± 5.0% (n = 3, p = 0.01) by mutation of Y404 and F472. Hence, the tandem UBA domains are largely, if not totally, responsible for the ubiquitin binding activity of UBAP1. It is possible that the central UBA domain may also bind ubiquitin, and this warrants further investigation. Cells depleted of UBAP1 by siRNA could not be rescued for endosomal phenotypes by UBAP1(E59G) or UBAP1(Y404A, F472A) (Figure 4D). Hence, UBAP1 must be incorporated into ESCRT-I and must be able to bind ubiquitin efficiently to function in endosomal sorting.

The multiple isoforms of VPS37 and MVB12 could generate a range of ESCRT-I complexes, each with unique characteristics and consequently specific cellular functions [9]. Several lines of evidence suggest that UBAP1 defines a population of ESCRT-I that also contains VPS37A. First, quantitative immunodepletion of UBAP1 from cytosol precipitated ~70% of VPS37A (Figure 4E). Likewise, although immunoprecipitation of VPS37A was rather inefficient using the reagents available, some UBAP1 was detected in VPS37A immunoprecipitates (Figure 4F). In contrast, no VPS37C coimmunoprecipitated with anti-UBAP1, and no UBAP1 coimmunoprecipitated with anti-VPS37C (Figures 4E and 4F). The UBAP1-VPS37A complex accounts for only a fraction of total ESCRT-I, because only ~10% of TSG101 coimmunoprecipitated with anti-UBAP1 (Figure 4E). Selective incorporation of UBAP1 into VPS37A-containing ESCRT-I complexes was

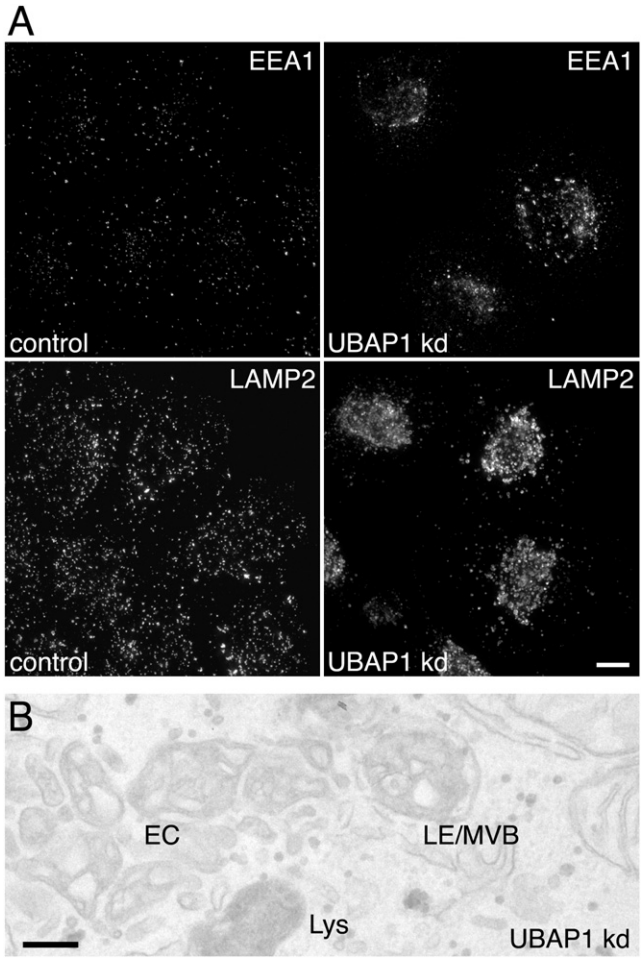

Figure 2. UBAP1 Influences Endosome Morphology

(A) Control or UBAP1-depleted HeLaM cells were stained for EEA1 or for LAMP2. Scale bar represents 10 μm.

(B) Electron microscopy image of UBAP1-depleted HeLa cells. The following abbreviations are used: EC, endosomal cluster; Lys, lysosome; LE/MVB, late endosome or mature MVB. Scale bar represents 200 nm.

confirmed by performing immunoprecipitations from cells expressing UBAP1-myc (Figure S4B). UBAP1-myc was immunoprecipitated by anti-TSG101 and anti-VPS37A, but not by anti-VPS37C. Because UBAP1 binds to ESCRT-I via its UMA domain, these complexes are unlikely to contain other MVB12 isoforms. Indeed, anti-MVB12A did not precipitate UBAP1 (Figure S4B). Anti-MVB12B did not work for immunoprecipitations.

Loss of a single ESCRT-I protein by siRNA might be expected to induce partial or complete degradation of the other components of that complex. Depletion of TSG101 led to almost complete degradation of VPS37A and VPS37C, and UBAP1 levels were also reduced substantially (Figure 4G). TSG101 levels were not significantly affected by depletion of VPS37A, as reported [6], or UBAP1, consistent with UBAP1-VPS37A accounting for a small proportion of total ESCRT-I. UBAP1 levels were substantially reduced upon depletion of VPS37A, but not VPS37C, and depletion of UBAP1 caused loss of ~70% of VPS37A (Figure 4G; Figure S4C). Depletion of HDPTP affected levels of UBAP1 and, to a lesser extent, VPS37A, whereas HDPTP levels were modestly affected by loss of UBAP1 (Figure 4G; Figure S4C), consistent with a functional relationship between these proteins.

Further evidence that UBAP1-VPS37A defines a biochemically distinct ESCRT-I complex was obtained using high-resolution gel filtration chromatography of cytosols. UBAP1 migrated at ~450 kDa. Although most TSG101 migrated with an apparent molecular weight of ~350 kDa (Figure 4H), in line with previous estimates for native ESCRT-I [6, 9, 17], the leading edge of the TSG101 peak coincided with the UBAP1 peak. Most VPS37A comigrated with UBAP1, though a secondary peak migrated with the bulk of TSG101 as previously reported [6], alongside VPS37C.

Cells depleted for VPS37A are deficient in EGFR degradation [6], whereas loss of VPS37C impairs HIV budding [7]. To extend these observations, we treated cells with siRNA against VPS37A or VPS37C and investigated effects on endosomal ubiquitin homeostasis and EGF trafficking. Loss of VPS37A caused a robust endosomal sorting phenotype (Figure 4I; Figure S4D), although the penetrance of this phenotype was somewhat lower than that caused by loss of UBAP1. In contrast, endosomal sorting and ubiquitin homeostasis appeared unaffected by loss of VPS37C (Figure S4E).

In addition to its role in endosomal sorting, TSG101 has been implicated in cytokinesis, where it is recruited by CEP55 and Alix [11, 12]. To test whether UBAP1 might define an endosome-specific ESCRT-I complex, we examined UBAP1-depleted cells for defects in cytokinesis. Fixed cells were analyzed for the presence and the structure of midbodies because midbodies are abundant in cells depleted of Alix, and in many midbodies the normal central gap in microtubule staining is absent [11, 12]. These phenotypes were observed upon depletion of Alix, but not UBAP1 (Figure S4F). Fluorescence-activated cell sorting (FACS) analysis provided quantitative information about the cell-cycle status of cells. Consistent with previous studies [11, 12], depletion of Alix caused a marked increase in the number of cells in G2 or M phase and in cells with higher DNA content (data not shown). Loss of TSG101 also generated an increase, albeit milder, in the proportion of cells at G2/M phase (17.6% ± 1.0% compared to 11.5% ± 0.3% for control cells; n = 3, p = 0.03), as seen by others [11, 12], but UBAP1 depletion did not (11.0% ± 0.5%). Likewise, the proportion of cells at G2/M was not increased by HDPTP depletion (9.6% ± 1.5%).

In summary, we have identified UBAP1 as a novel ESCRT-I component with a restricted cellular function, ubiquitin-dependent endosomal sorting and MVB biogenesis. The selective interaction of UBAP1 with HDPTP, and not Alix, is likely to help confer such specificity to this ESCRT. The finding that the complex also contains VPS37A is significant because this is unique among the VPS37 isoforms in containing a UEV domain, which may bind ubiquitin [8]. Combined with the UEV domain within TSG101 [20] and the tandem UBA domains of UBAP1, the endosomal ESCRT-I therefore has multiple potential ubiquitin binding modules. These may be important for binding to, and potentially clustering, cargo containing the short K63-linked polyubiquitin chains that act as a signal for cargo sorting at the MVB [21, 22]. It is possible that other ESCRT-I complexes are also involved in MVB sorting, perhaps acting in conjunction with the UBAP1-VPS37A complex. Indeed, MVB12A and MVB12B bind EGFR and are posttranslationally modified upon EGFR activation [18]. In any event, the specialization of ESCRT-I complexes that we identify here may provide one means to assemble the specific ESCRT-III subunits that have been linked to given cellular events [1, 23, 24].

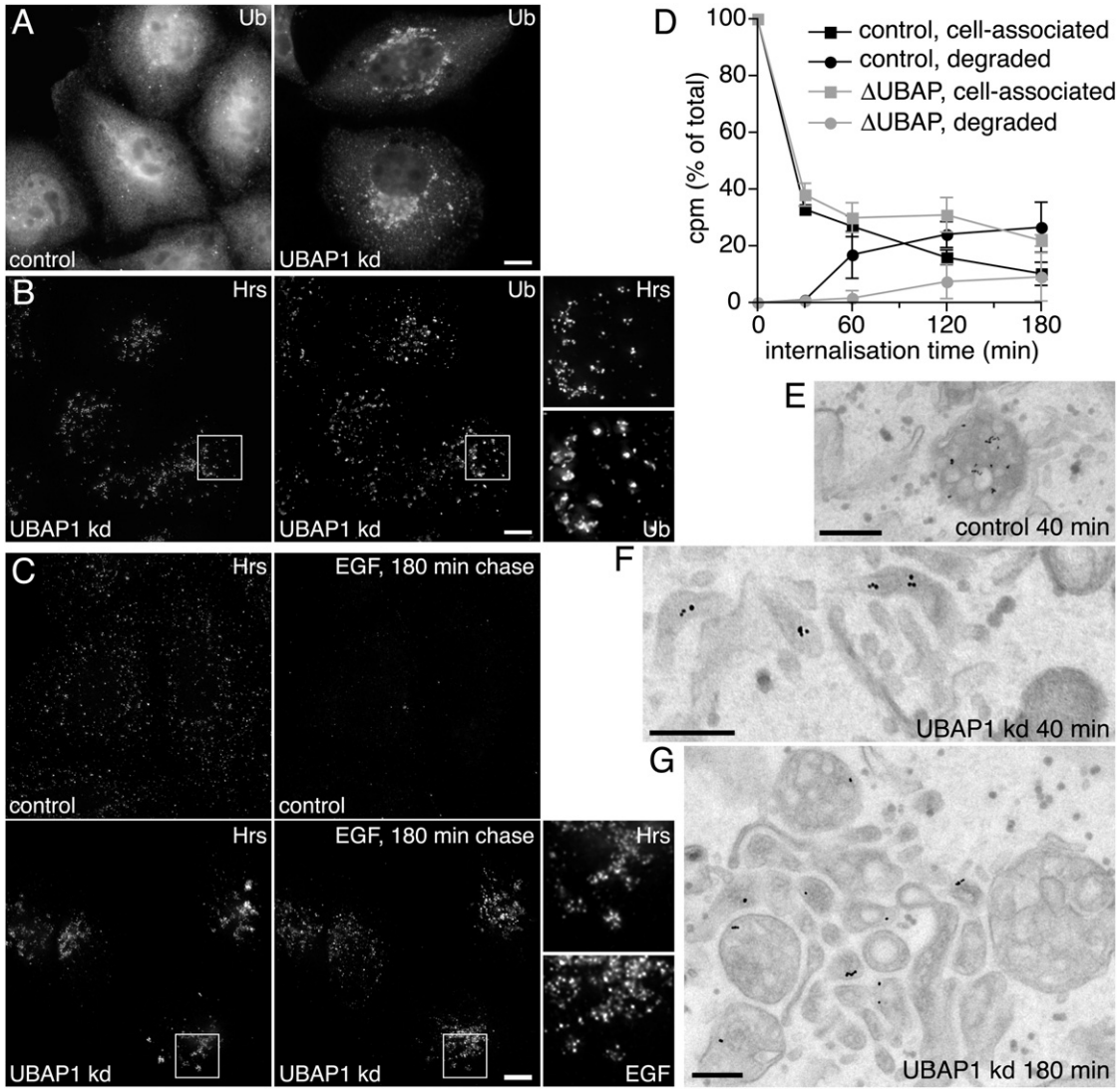

Figure 3. UBAP1 Is Important for Ubiquitin-Dependent EGFR Trafficking

(A) Control or UBAP1-depleted HeLaM cells were stained with FK2 antibody against ubiquitinated proteins.

(B) UBAP1-depleted HeLaM cells were stained with FK2 and anti-Hrs.

(C) Control or UBAP1-depleted cells were pulse chased with Alexa 555-EGF and then fixed and labeled for Hrs. Scale bars represent 10 μm. Insets are shown magnified ×3 at right.

(D) Control or UBAP1-depleted cells were incubated with ^{125}I-labeled EGF to follow EGF degradation and intracellular accumulation of EGF.

(E–G) Control or UBAP1-depleted HeLa cells were labeled with anti-EGFR gold conjugates and pulse chased with EGF as indicated. Scale bars represent 200 nm.

The importance of UBAP1 is underlined by its linkage to FTLD. Mutations in progranulin and MAPT are the most common known genetic causes of autosomal-dominant FTLD [25]. However, leading on from the identification of mutations in CHMP2b that cause FTLD and impaired endocytic trafficking in patient fibroblasts [26, 27], our data confirm that the MVB pathway is vital in maintaining neuronal health and that its dysfunction can lead to neurodegenerative disease in midlife.

Supplemental Information

Supplemental Information includes four figures, three tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.06.028.

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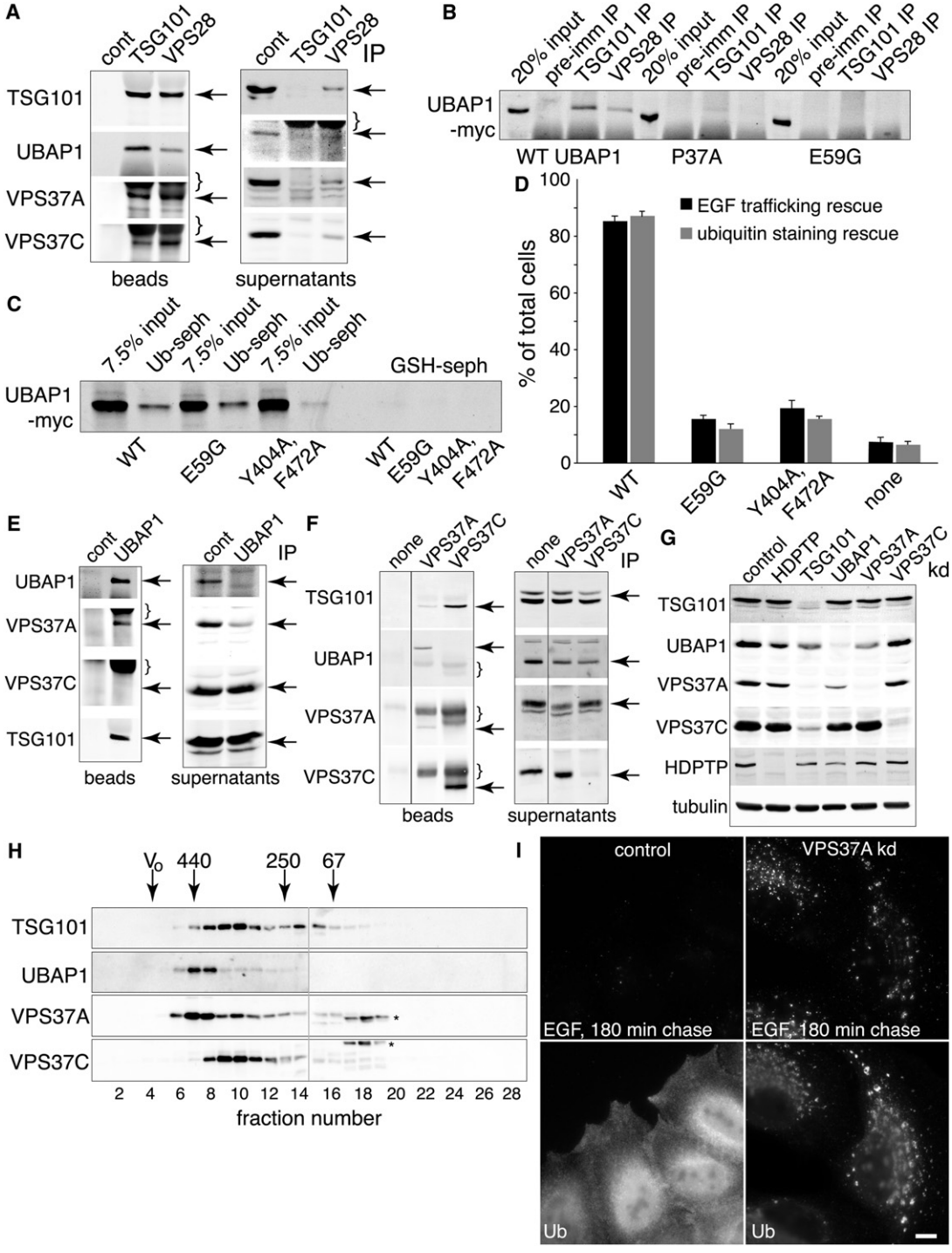

Figure 4. UBAP1 and VPS37A Are Components of an Endosomal ESCRT-I Complex

(A) K562 cytosolic extract was subjected to native immunoprecipitation, and both bead-bound fractions and supernatants were blotted for ESCRT components as indicated. Note that bead and supernatant fractions were exposed independently.

(B) HeLaM cells were transfected with the indicated UBAP1-myc constructs, and cell extracts were subjected to native immunoprecipitations as indicated.

(C) Extracts from HeLaM cells transfected with the indicated constructs were incubated with ubiquitin or control beads.

(D) HeLaM cells were treated with UBAP1 siRNA and transfected with UBAP1-myc constructs. Cells were scored for rescue of endosomal ubiquitin and EGF trafficking defects (three experiments in which 200 cells were scored for each condition; error bars are SEM to indicate interexperimental error).

(E and F) K562 cytosols were subjected to native immunoprecipitation and then blotted for ESCRT components. Note that pellet and supernatant blots were exposed independently and that control and specific immunoprecipitation samples are cropped from the same blots.

(G) HeLaM cells were depleted by siRNA, and total extracts were blotted as indicated.

(H) K562 cytosol was separated by size exclusion chromatography, and fractions were blotted. Positions of molecular weight markers are shown.

(I) Control HeLaM cells or cells depleted of VPS37A were incubated with fluorescent EGF, chased for 180 min, and then immunostained for antibody against ubiquitinated protein. Scale bar represents 10 μm.

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