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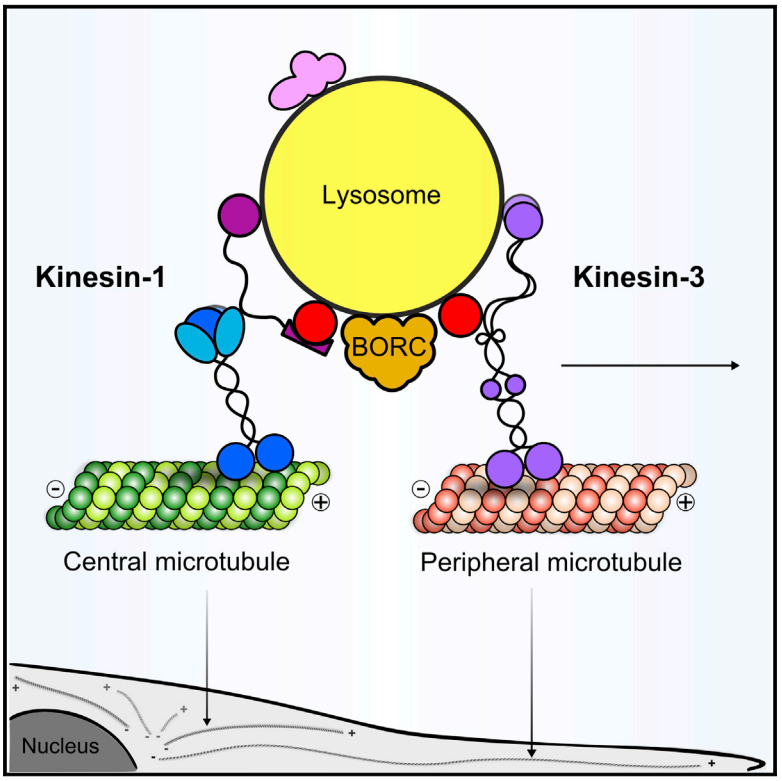
Cell Reports

BORC Functions Upstream of Kinesins 1 and 3 to Coordinate Regional Movement of Lysosomes along Different Microtubule Tracks

Graphical Abstract



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In Brief

Guardia et al. report that the multi-subunit complex BORC promotes centrifugal movement of lysosomes through coupling to kinesins 1 and 3. Remarkably, these kinesins drive lysosome movement along different microtubule tracks and in different regions of the cell. BORC thus exerts regional control of lysosome movement throughout the cytoplasm.

Highlights

- Multiple functions of lysosomes depend on their ability to move within the cytoplasm
- BORC and Arl8 promote lysosome movement through coupling to kinesins 1 and 3
- Kinesins 1 and 3 move lysosomes on spatially distinct microtubule arrays
- BORC is a master regulator of lysosome movement in different regions of the cell

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BORC Functions Upstream of Kinesins 1 and 3 to Coordinate Regional Movement of Lysosomes along Different Microtubule Tracks

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SUMMARY

The multiple functions of lysosomes are critically dependent on their ability to undergo bidirectional movement along microtubules between the center and the periphery of the cell. Centrifugal and centripetal movement of lysosomes is mediated by kinesin and dynein motors, respectively. We recently described a multi-subunit complex named BORC that recruits the small GTPase Arl8 to lysosomes to promote their kinesin-dependent movement toward the cell periphery. Here, we show that BORC and Arl8 function upstream of two structurally distinct kinesin types: kinesin-1 (KIF5B) and kinesin-3 (KIF1Bβ and KIF1A). Remarkably, KIF5B preferentially moves lysosomes on perinuclear tracks enriched in acetylated α-tubulin, whereas KIF1Bβ and KIF1A drive lysosome movement on more rectilinear, peripheral tracks enriched in tyrosinated α-tubulin. These findings establish BORC as a master regulator of lysosome positioning through coupling to different kinesins and microtubule tracks. Common regulation by BORC enables coordinate control of lysosome movement in different regions of the cell.

INTRODUCTION

Classical electron and light microscopy techniques, recently enhanced by the development of super-resolution microscopy, have produced a detailed view of the spatial organization of cytoplasmic organelles within eukaryotic cells. Live-cell imaging methodologies have further revealed that this organization is highly dynamic (van Bergeijk et al., 2016). Indeed, organelles move around the cytoplasm, change their size and shape, and establish transient contacts with one another, all under precise regulatory controls. Prime examples of such dynamic organelles are late endosomes and lysosomes (herein referred to simply as "lysosomes"), membrane-enclosed vacuoles that function in the degradation of biological macromolecules in the endomembrane system (Ballabio, 2016). A fraction of lysosomes move back and forth between the center and the periphery of the cell along microtubule tracks (Matteoni and Kreis, 1987). This bidirectional movement is important for the distribution of the degradative activity of lysosomes to all regions of the cytoplasm, as well as for the involvement of lysosomes in other cellular processes such as autophagy, antigen presentation, microbial killing, metabolic signaling, plasma membrane repair, cell adhesion, cell migration, and tumor invasion and metastasis (Reddy et al., 2001; Steffan et al., 2010; Garg et al., 2011; Korolchuk et al., 2011; Mrakovic et al., 2012; Pu et al., 2015). In most non-polarized cells, centripetal (retrograde or inward) transport and centrifugal (anterograde or outward) transport of lysosomes along microtubules are mediated by coupling to the minus-end-directed dynein (Harada et al., 1998; Jordens et al., 2001) and plus-end-directed kinesin motors (Hollenbeck and Swanson, 1990), respectively. Mammalian genomes encode a single dynein heavy chain, but more than 40 different kinesin heavy chains or KIFs (Hirokawa et al., 2009). Notably, centrifugal transport of lysosomes has been shown to depend on several kinesins, including kinesin-1 (e.g., KIF5B) (Nakata and Hirokawa, 1995; Tanaka et al., 1998; Rosa-Ferreira and Munro, 2011), kinesin-2 (e.g., KIF3A) (Brown et al., 2005), kinesin-3 (e.g., KIF1A, KIF1Bβ) (Korolchuk et al., 2011; Matsushita et al., 2004; Bentley et al., 2015), and kinesin-13 (e.g., KIF2A, KIF2Aβ) (Korolchuk et al., 2011; Santama et al., 1998) (Figure 1A).

The coupling of cytoplasmic organelles to kinesins is most often mediated by adaptor proteins. In the case of lysosomes, we recently discovered a multi-subunit complex named BORC (for BLOC-one-related complex) that promotes kinesin-mediated lysosome movement toward the cell periphery (Pu et al., 2015). BORC is composed of eight small (12–37 kDa) coiled-coil subunits named BLOS1, BLOS2, KXD1, SNAPIN, myrlysin (LOH12CR1), lyspersin (C17orf59), diaskedin (C10orf32), and MEF2BNB (these subunits are also named BORCS1 to BORCS8, respectively), and associates with the cytosolic face of the lysosomal membrane in part through a myristoyl group at the N terminus of myrlysin (Pu et al., 2015). BORC promotes the recruitment of the Arf-like small GTPase Arl8b from the cytosol to the lysosomal membrane (Pu et al., 2015). Silencing of BORC subunits or Arl8b impairs centrifugal transport of lysosomes, resulting in their accumulation in the pericentriolar area (Pu et al., 2015; Rosa-Ferreira and Munro, 2011). These effects have been attributed to a role of BORC and

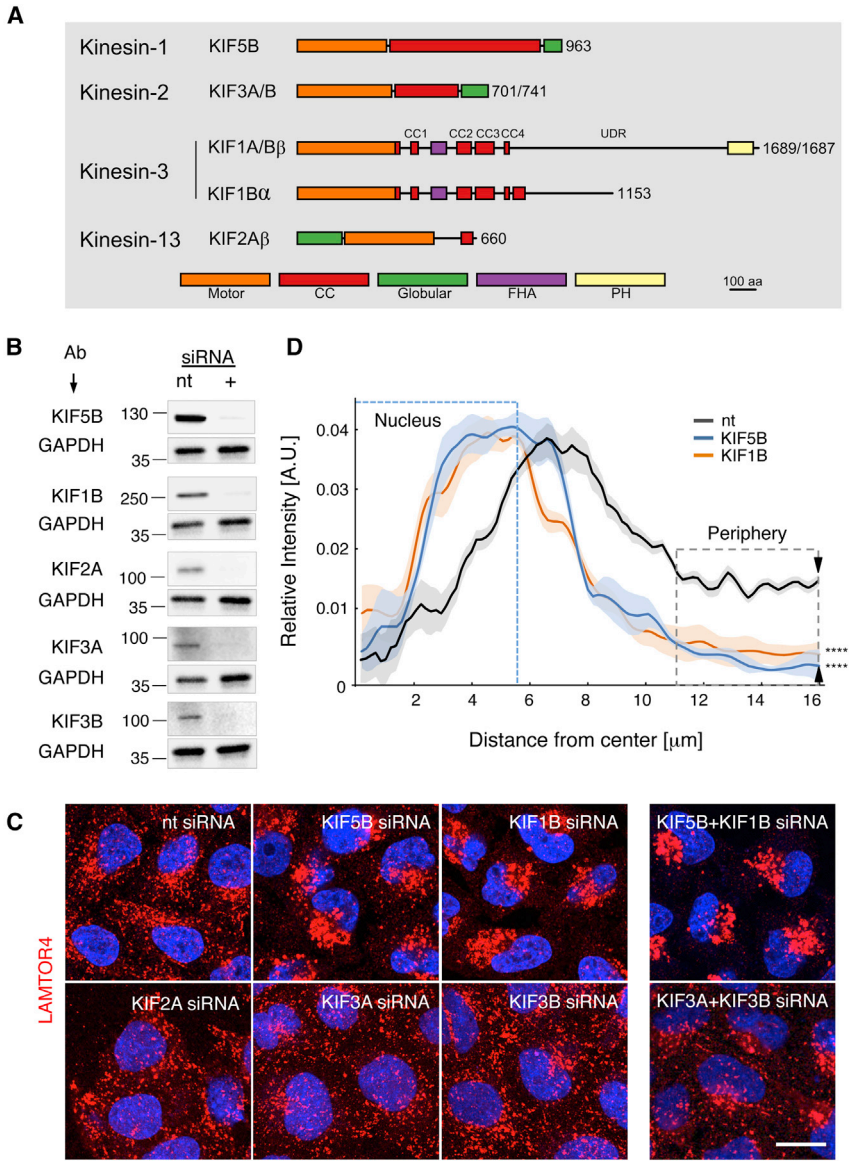

Figure 1. KIF5B and KIF1B Are Required for Peripheral Distribution of Lysosomes

(A) Schematic representation of kinesins previously implicated in lysosome transport, indicating their structural domains and amino acid numbers. CC, coiled-coil; FHA, forkhead-associated; PH, pleckstrin homology; UDR, undefined region.

(B) Immunoblot analysis of siRNA KD. nt, non-targeting siRNA. The KIF1B and KIF2A siRNAs target all isoforms of these kinesins. GAPDH was used a loading control. The positions of molecular mass markers (in kilodaltons) are indicated at left.

(C) Confocal microscopy of HeLa cells transfected with siRNA pools for the indicated kinesins and immunostained for endogenous LAMTOR4 (lysosomal marker, red) and DAPI (nuclear marker, blue). Scale bar, 10 μm.

(D) Lysosome distribution as a function of distance from the center of the nucleus, quantified from confocal images using the ImageJ plugin Radial Profile. Graphs show the average distribution ± SEM of lysosomes in 30 different cells from three independent experiments. Student's t tests (siRNA treatments versus nt control) were performed at the last point of each distribution to determine the statistical significance of the differences in the cell periphery (arrowheads).

Arl8b in the sequential recruitment of the linker protein SKIP (PLEKHM2) and kinesin-1 (a $KIF5_2$-KLC_2 heterotetramer; DeBoer et al., 2008) to lysosomes (Pu et al., 2015; Rosa-Ferreira and Munro, 2011; Dumont et al., 2010; Tuli et al., 2013). However, because multiple kinesins participate in lysosome movement, it remains to be determined which ones actually function downstream of BORC and Arl8 in this process. Moreover, it is also unclear why so many kinesins would be involved in moving lysosomes toward the cell periphery.

In this study, we performed a systematic analysis of the kinesins that mediate lysosome dispersal and their dependence on BORC and Arl8b in HeLa cells. The results of our study show that the kinesin-1 KIF5B and kinesin-3 KIF1A and KIF1Bβ proteins are the most important for centrifugal movement of lysosomes in these cells, and that both kinesin types are subject to regulation by BORC and Arl8 (both Arl8a and Arl8b isoforms). Strikingly, each of these kinesins preferentially links lysosomes to a different set of microtubule tracks: more convoluted and centrally located for KIF5B; more rectilinear and peripheral for KIF1A. These tracks are characterized by their enrichment in acetylated and tyrosinated α-tubulin, respectively. We conclude that BORC and Arl8 act together to control regional transport of lysosomes through coupling to different kinesins and, in turn, different microtubule tracks. Regulated association of the same intracellular organelle with different kinesins may represent a general principle for the control of regional movement within cells.

RESULTS

Kinesin-1 KIF5B and Kinesin-3 KIF1B Are the Most Important Motors for Peripheral Distribution of Lysosomes in HeLa Cells

As a first step to identify kinesins that function downstream of BORC and Arl8 in lysosome movement toward the cell periphery, we examined the effect of RNAi of candidate kinesins on the distribution of lysosomes in HeLa cells. The kinesins tested included members of the kinesin-1, -2, -3, and -13 families (Figure 1A) that were previously implicated in lysosome movement using different methodologies (Korolchuk et al., 2011; Nakata and Hirokawa, 1995; Tanaka et al., 1998; Rosa-Ferreira and

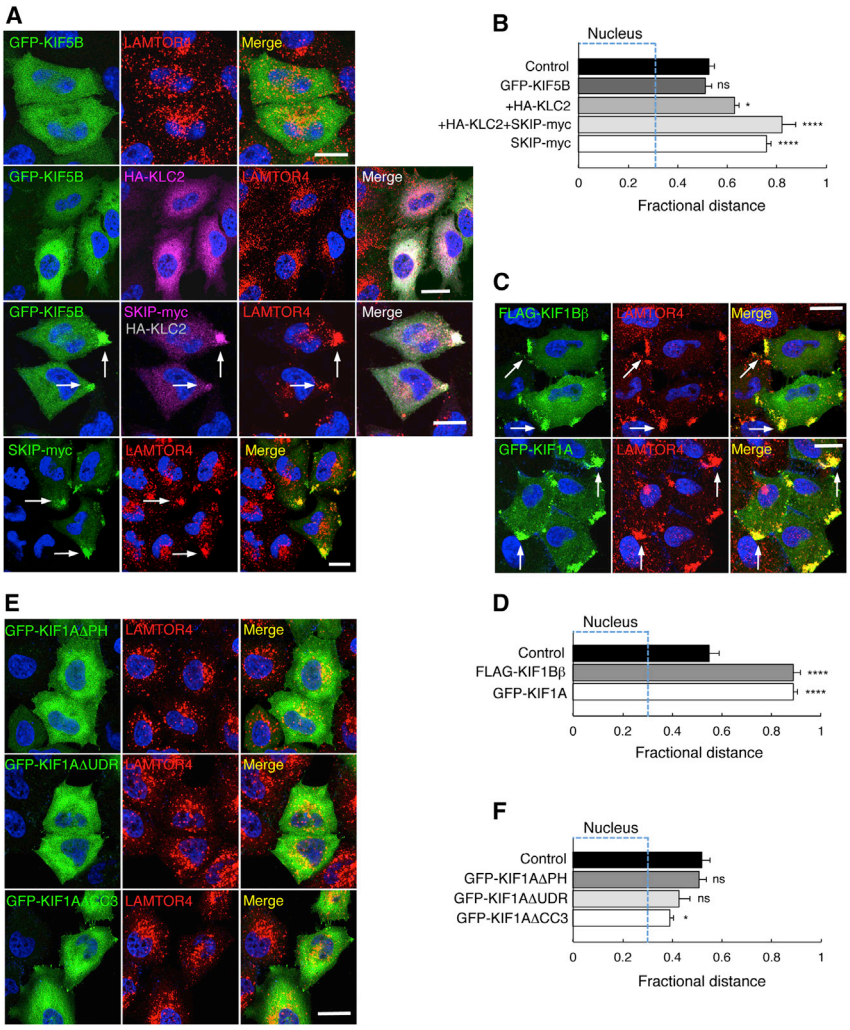

Figure 2. Overexpression of KIF5B-KLC2-SKIP, KIF1Bβ, or KIF1A Causes Accumulation of Lysosomes at the Cell Periphery

(A and B) HeLa cells expressing GFP-KIF5B, alone or in combinations with HA-KLC2 and SKIP-myc, were analyzed by immunofluorescence microscopy (A), and mean fractional distance ± SEM of lysosomes from the cell center was quantified from 30 cells in three independent experiments (B).

(C–F) HeLa cells expressing FLAG-KIF1Bβ, GFP-KIF1A, or GFP-KIF1A deletion mutants were analyzed by immunofluorescence microscopy (C and E), and mean fractional distance ± SEM of lysosomes from the cell center was quantified as above (D and F).

In (A), (C), and (E), lysosomes were stained with antibody to endogenous LAMTOR4, and nuclei were stained with DAPI (blue). Scale bars, 10 μm. Arrows point to cell protrusions where proteins co-localize.

Munro, 2011; Brown et al., 2005; Matsushita et al., 2004; Bentley et al., 2015; Santama et al., 1998). RNAi was performed by treating cells with pools of four small interfering RNAs (siRNAs) targeting one or more members of each family. All the siRNA pools depleted their targets to 10%–20% of their normal levels (Figure 1B). Lysosomes were stained with an antibody to the LAMTOR4 (C7orf59) subunit of the ragulator complex, a well-established lysosome marker (Bar-Peled et al., 2012). In control cells, lysosomes were scattered throughout the cytoplasm, although with a higher concentration in the juxtanuclear area (Figures 1C and 1D). Treatment with siRNA pools targeting the kinesin-1 KIF5B or kinesin-3 KIF1B (both α and β isoforms) caused depletion of lysosomes from the peripheral cytoplasm and their increased clustering in the juxtanuclear area in ~40% and ~85% of the cells, respectively (Figures 1C and 1D). Similar results were obtained by depletion of KIF5B and KIF1B with any of the four individual siRNAs that make up the corresponding pool (Figure S1). Live-cell imaging showed that juxtanuclear clustering of lysosomes in KIF5B-, KIF1B-, and double KIF5B-KIF1B-knockdown (KD) cells was due to impaired long-range movement toward the cell periphery (Movie S1). Treatment with siRNAs to KIF2A, KIF3A, or KIF3B had little or no effect, although a combination of siRNAs to KIF3A and KIF3B did cause some lysosome clustering (Figure 1C). From these experiments, we concluded that KIF5B and KIF1B are the most important kinesins for centrifugal movement of lysosomes in HeLa cells.

Overexpression of KIF5B in Conjunction with KLC and SKIP Is Sufficient to Drive Lysosomes toward the Cell Periphery

KIF5B (Figure 1A) is the only kinesin-1 member that is expressed in most cells, whereas the related KIF5A and KIF5C are specifically expressed in neurons (Kanai et al., 2000). The ability of KIF5B to interact with cargo is most often dependent on its assembly with one of four kinesin light chains (DeBoer et al., 2008), of which KLC2 is the predominant non-neuronal form (Rahman et al., 1999). Coupling of kinesin-1 to lysosomes involves an additional adaptor protein named SKIP (Boucrot et al., 2005; Rosa-Ferreira and Munro, 2011). We observed that, despite the requirement of KIF5B for lysosome dispersal (Figures 1C and 1D), overexpression of GFP-tagged KIF5B, alone or together with HA-tagged KLC2 (Figure S1C), had no effect on the distribution of lysosomes (Figures 2A and 2B). Additional expression of myc-tagged SKIP (Figure S1C), however, redistributed KIF5B-KLC2 and lysosomes to cell protrusions (Figures 2A, arrows, and 2B). Expression of myc-SKIP alone also caused lysosome dispersal to the cell periphery (Figure 2A), albeit to a lesser extent than when co-expressed with KIF5B and KLC2 (Figure 2B). In line with the critical role of SKIP in this process, siRNA-mediated depletion of this protein caused juxtanuclear clustering of lysosomes (Figure S1F). To ascertain the identity of these organelles, we performed similar experiments staining for other lysosomal markers such as LAMP-1 or CD63, and the more specific

late-endosomal/multivesicular-body marker TSG101 (a component of the ESCRT-I complex). Overexpression of the KIF5B-KLC2-SKIP ensemble also drove a fraction of all of these markers to cell protrusions (Figure S2A). KIF5B and lysosomes that accumulated in these protrusions were flanked by focal adhesion proteins such as paxillin (Figure S2B), indicating that they occurred in the vicinity of cell adhesion and/or motility structures. Other organelles such as the Golgi complex, mitochondria, and endoplasmic reticulum (ER) were not affected by overexpression of KIF5B-KLC2-SKIP (Figure S2A). Thus, in line with previous studies (Rosa-Ferreira and Munro, 2011; Boucrot et al., 2005), these experiments showed that KIF5B, in conjunction with KLC2 and SKIP, is specifically capable of driving a population of late endosomes and lysosomes toward the peripheral cytoplasm. The neuronal KIF5A and KIF5C proteins also promoted lysosome dispersal when co-expressed with various KLC isoforms and SKIP (C.M.G., unpublished data), indicating that this is a conserved property of the kinesin-1 family.

Overexpressed KIF1Bβ and KIF1A Are Also Capable of Driving Lysosomes to the Cell Periphery

KIF1B is a ubiquitous kinesin-3 that occurs as two main splice variants, KIF1Bα and KIF1Bβ. The two proteins have an identical N-terminal motor domain but differ in their C-terminal portions (Zhao et al., 2001) (Figure 1A). The neuronal KIF1A is encoded by a different gene, but has a structure similar to that of KIF1Bβ (Okada et al., 1995) (Figures 1A and S3). We observed that overexpression of KIF1Bβ or KIF1A alone (Figures S1D and S1E) was sufficient to drive lysosomes to the cell periphery, particularly to cell protrusions (Figures 2C, arrows, 2D). The concentration in cell protrusions was even more extensive than that elicited by overexpression of KIF5B-KLC2-SKIP (Figure 2A, arrows). This effect of KIF1A was independent of SKIP (Figure S1G). In contrast, overexpression of the related KIF1Bα had no effect on lysosome distribution (Figure S4A). This indicated that the C-terminal portion of KIF1Bβ and KIF1A specifies lysosome recognition. Of note, despite sharing the same motor domain, KIF1Bβ accumulated in cell protrusions together with lysosomes (Figure 2C), whereas KIF1Bα remained diffusely distributed (Figure S4A). These observations are consistent with the notion that association with cargo activates the motor activity of kinesins (Friedman and Vale, 1999). As described above for KIF5B-KLC2-SKIP, overexpression of KIF1A also caused accumulation at cell protrusions of the late-endosomal/lysosomal markers LAMP-1, CD63, and TSG101, but not Golgi, mitochondria, or ER markers, demonstrating the specificity of these effects (Figure S2A). Accumulation also occurred in the vicinity of focal adhesions labeled for paxillin (PXN) (Figure S2B). Overexpression of two other ubiquitous members of the kinesin-3 family, KIF16B and KIF13A, which are mostly associated with early and recycling endosomes (Hoepfner et al., 2005; Delevoye et al., 2014), had no effect on the distribution of lysosomes (C.M.G., unpublished data), indicating that the effects of KIF1Bβ and KIF1A are limited to these family members.

We next conducted a deletion analysis to identify the regions of KIF1A that are required for promoting lysosome movement to the cell periphery. In addition to the N-terminal motor domain, KIF1A and KIF1Bβ comprise several coiled-coil segments (CC1 to CC4), a forkhead-associated (FHA) domain, an "undefined region" (UDR), and a C-terminal PH domain (Huo et al., 2012) (Figures 1A, S1E, and S3). We found that deletion of the CC3, UDR, or PH domain (Figure S1E), but not the CC4 domain (Figure S3C), abrogated the ability of KIF1A to concentrate at cell protrusions and to drive lysosomes into the protrusions (Figures 2E, 2F, and S3C).

From these experiments, we concluded that the kinesin-3 proteins KIF1Bβ and KIF1A are also capable of driving lysosome movement to the cell periphery, and that, unlike KIF5B, they can do so without co-expression of any accessory proteins. For both KIF5B and KIF1Bβ/KIF1A, the ability to drive lysosome movement is dependent on their motor domains (Figure S4B). Moreover, the motorless forms of these kinesins have a dominant-negative effect, causing juxtanuclear clustering of lysosomes (Figure S4B).

Lysosome Movement by Both KIF5B-KLC2-SKIP and KIF1A Is Dependent on Arl8a/b and BORC

Additional experiments showed that overexpression of KIF3A, KIF3B, or KIF2Aβ (Figure 1A) had no effect on the distribution of lysosomes (Figure S4A). These results, together with the smaller effects of depleting these kinesins on lysosome positioning (Figure 1C), prompted us to focus our analyses on the relationship of KIF5B-KLC2-SKIP and KIF1A to Arl8 and BORC. KIF5B-KLC2-SKIP and KIF1A share the property of interacting with Arl8-GTP via SKIP (Rosa-Ferreira and Munro, 2011) and the CC3 domain (Wu et al., 2013), respectively. Human cells, including HeLa, express two Arl8 isoforms named Arl8a and Arl8b (Hofmann and Munro, 2006). We observed that overexpression of either Arl8a or Arl8b caused lysosome movement to the cell periphery (Figure S5A), as previously reported (Hofmann and Munro, 2006; Bagshaw et al., 2006). Inactivation of the Arl8b gene using CRISPR/Cas9 (Figure 3A) caused lysosome clustering in the juxtanuclear area (Figure S5B), consistent with this isoform being the most abundant in HeLa cells (Rosa-Ferreira and Munro, 2011). However, overexpression of KIF5B-KLC2-SKIP (Figure 3A) or KIF1A (Figure 3B) still drove some lysosomes to the cell periphery in the Arl8b-knockout (KO) cells. Treatment of the Arl8b-KO cells with siRNA to Arl8a (Figure S1H) completely abolished the effects of both kinesins on lysosome positioning (Figures 3A, 3B, and 3E), indicating that both Arl8 isoforms contribute to the ability of KIF5B-KLC2-SKIP and KIF1A to move lysosomes in HeLa cells.

In previous work, we found that BORC functions to recruit Arl8b to the lysosomal membrane (Pu et al., 2015). CRISPR/Cas9-KO of the gene expressing the myrlysin subunit of BORC (Figure S1H) impaired the association of not only Arl8b but also Arl8a with the lysosomal membrane, and the ability of both isoforms to promote lysosome movement to the cell periphery (Figure S5C). Consistent with these results, KO of the myrlysin or diaskedin (C10orf32) subunits of BORC (Figure S1H) caused juxtanuclear collapse of the lysosome population and abrogated lysosome dispersal induced by KIF5B-KLC2-SKIP (Figures 3C and 3F) or KIF1A (Figures 3D and 3F).

These experiments thus demonstrated that BORC and Arl8a/b function upstream of both KIF5B-KLC2-SKIP and KIF1A to promote lysosome movement toward the cell periphery.

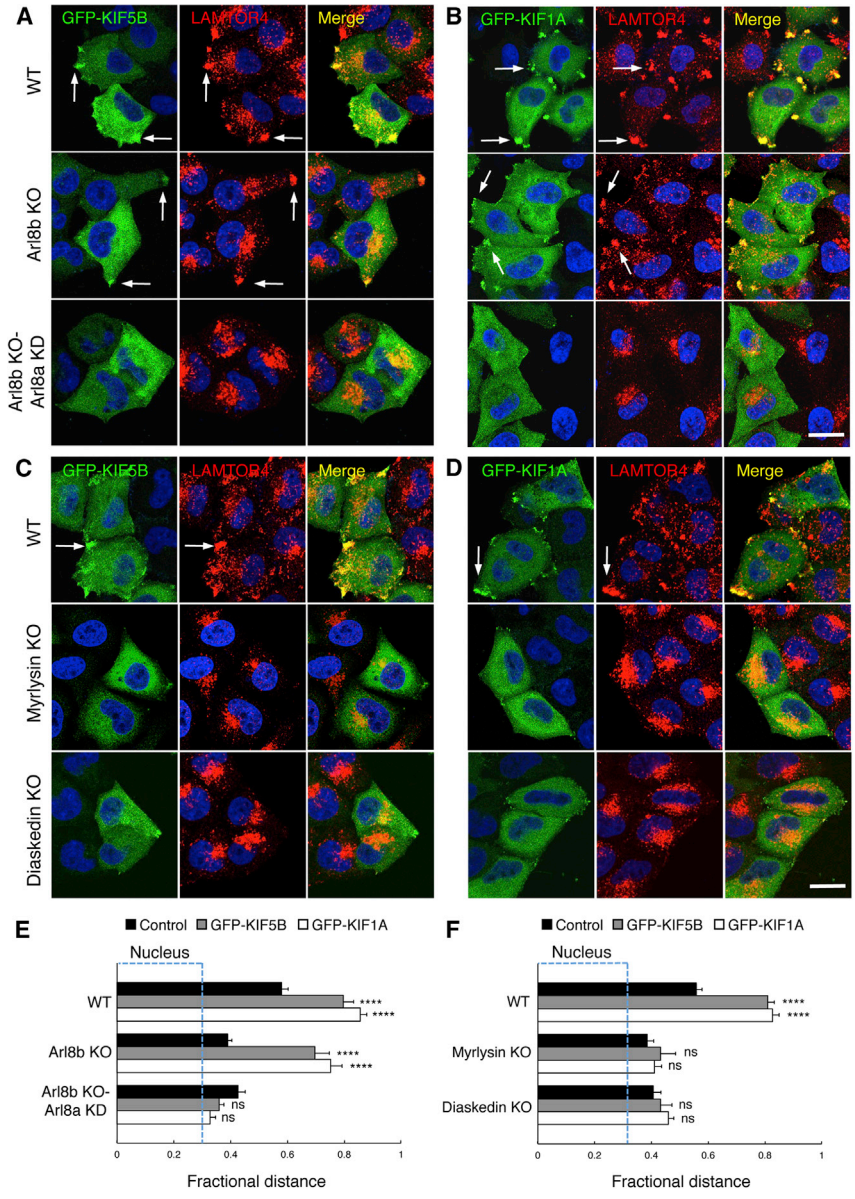

Figure 3. Peripheral Accumulation of Lysosomes Driven by Both KIF5B-KLC2-SKIP and KIF1A Is Dependent on Arl8a/b and BORC

(A–D) Immunofluorescence microscopy of lysosomes stained with antibody to endogenous LAMTOR4 in WT, Arl8b-KO, Arl8b-KO-Arl8a-KD, myrlysin-KO, and diaskedin-KO HeLa cells expressing GFP-KIF5B+HA-KLC2+SKIP-myc or GFP-KIF1A. Nuclei were stained with DAPI (blue). Scale bars, 10 μm. Arrows point to cell protrusions where proteins co-localize.

(E and F) Quantification of mean fractional distance ± SEM of lysosomes from the cell center from 30 cells in three independent experiments such as those in (A–D).

by a BORC-independent process over the long course of the experiment. This effect of p150-CC1 indicated that dynein remained active and contributed to the juxtanuclear clustering of lysosomes in BORC-deficient cells.

To further assess a possible role of BORC in centripetal transport, we devised a method for reversible association of lysosomes with dynein. This method consisted of co-expressing a construct comprising LAMP-1 fused to the streptavidin-binding peptide (SBP) and GFP, with another construct comprising the motor and part of the stalk domains of KIF5B fused to streptavidin (Strep) and mCherry (Figure 4B). As expected, co-expression of these constructs forced the redistribution of lysosomes to the cell periphery in both control and myrlysin-KO cells (Figures 4C and 4D). Importantly, dissociation of the chimeras by addition of biotin caused a time-dependent reversal of the peripheral localization of lysosomes in both wild-type (WT) and myrlysin-KO cells (Figures 4C and 4D). Taken together, these results indicated that the ability of dynein to move lysosomes toward the cell center is largely independent of BORC.

Dynein-Mediated Centripetal Transport of Lysosomes Is Independent of BORC

Because some organelle adaptors regulate movement along microtubules in both directions (Bielska et al., 2014), we next examined whether BORC also regulated centripetal transport of lysosomes mediated by dynein. This was initially done by overexpressing the CC1 domain of the $p150^{Glued}$ subunit (DCTN1) of the dynein activator dynactin, a manipulation that has a dominant-negative effect on the centripetal movement of various organelles, including lysosomes (Quintyne et al., 1999; Moughamian et al., 2013). We observed that p150-CC1 overexpression caused redistribution of lysosomes to the cell periphery in control cells, and also partially in myrlysin-KO and diaskedin-KO cells (Figure 4A). The dispersal of lysosomes in BORC-deficient cells could be due to a slow drift in the lysosome population

KIF5B and KIF1A Associate with Different Microtubule Tracks in HeLa Cells

Why are two structurally different kinesin types involved in moving lysosomes to the cell periphery? We hypothesized that each kinesin could move lysosomes along different microtubule tracks or in different regions of the cells. To address this hypothesis, we compared the localization of ATPase "rigor" mutants of KIF5B (G234A) and KIF1A (G251A), which bind microtubules but cannot walk along them (Nakata and Hirokawa, 1995). The stability of the association of these kinesins allows visualization of the sites where they first bind to their preferred microtubules. Strikingly, we observed that KIF5B-rigor

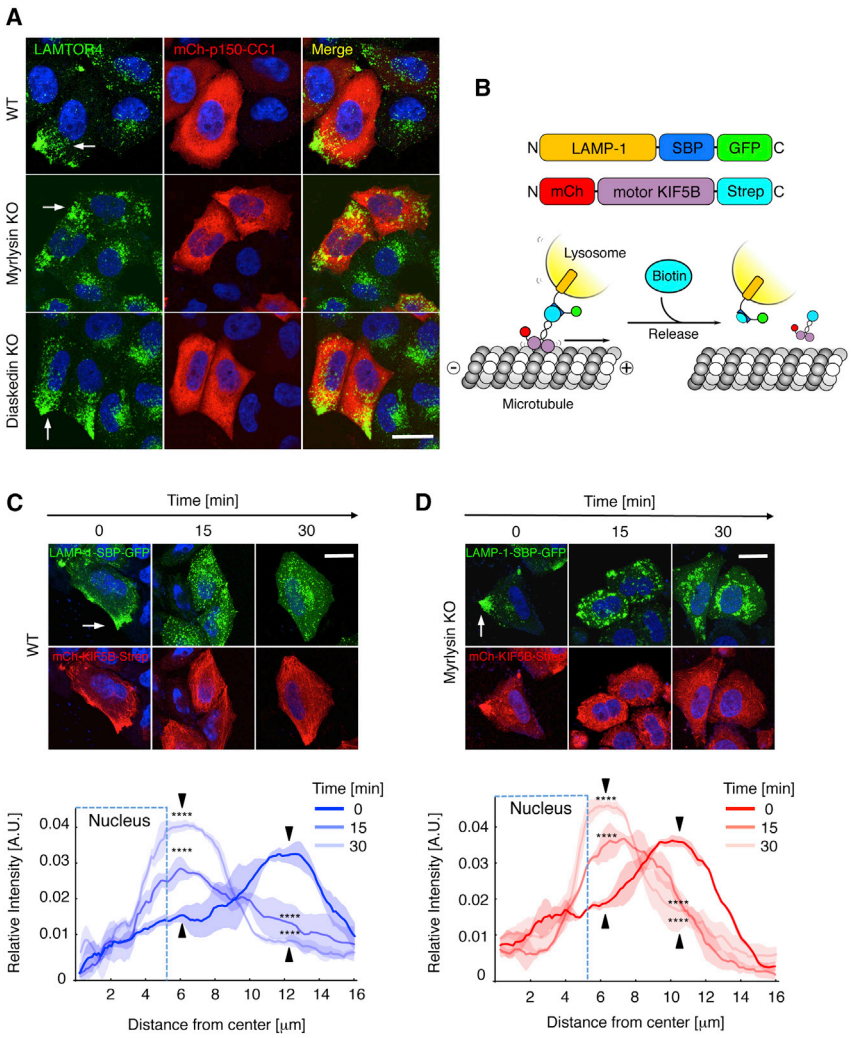

Figure 4. Dynein-Mediated Centripetal Transport of Lysosomes Is Independent of BORC

(A) A dominant-negative mutant of the dynactin p150 subunit (mCh-p150-CC1) was expressed in WT, myrlysin-KO, and diaskedin-KO HeLa cells to inactivate dynein-mediated retrograde transport of lysosomes. Cells were stained with antibody to endogenous LAMTOR4 and analyzed by immunofluorescence microscopy.

(B) Schematic representation of the constructs designed for accumulation of lysosomes at the cell periphery and their release after treatment with biotin.

(C and D) Fluorescence microscopy and quantification of lysosome distribution (performed as explained in the legend to Figure 1D) showing changes in lysosome positioning after biotin-induced release from their peripheral sites in (C) WT and (D) myrlysin-KO HeLa cells. Student's t tests (at 15 and 30 min of treatment versus initial time point) were performed near the nucleus and in the periphery to determine the statistical significance of the differences in these regions of interest (arrowheads). In (A), (C), and (D), scale bars represent 10 μm. DAPI was used as a nuclear stain (blue). Arrows point to cell protrusions where the proteins co-localize.

(KIF5B-R) and KIF1A-rigor (KIF1A-R) decorated distinct sets of microtubules (Figure 5A). KIF5B-R was predominantly associated with a population of perinuclear microtubules, whereas KIF1A-R was mainly found on more rectilinear microtubules that started at the cell center but reached into cell protrusions (Figure 5A). The spatial segregation of both kinesins was also evident when they were co-expressed in the same cells (Figure 5B). Although the rigor kinesins could alter the structure of the microtubule cytoskeleton, the populations of microtubules preferred by either kinesin thus remain distinct regardless of whether the kinesins are expressed singly or in combination. Super-resolution structured illumination microscopy (SIM) of cells co-expressing KIF5B-R and KIF1A-R confirmed the distinct spatial distribution of these kinesins, and also revealed overlapping localization on some microtubules or microtubule bundles (Figure S6).

Diverse microtubule populations can be generated by post-translational modifications (PTMs) of tubulin, a phenomenon that has been best characterized in neurons (Song and Brady, 2015). Staining of HeLa cells with antibodies to modified tubulins showed that acetylated tubulin was enriched in the more centrally located microtubules decorated by KIF5B-R but not KIF1A-R (Figure 5C). Tyrosinated tubulin also occurred in centrally located microtubules but was additionally found in more peripheral microtubules having associated KIF1A-R (Figure 5C). Calculation of the average Pearson's correlation coefficient from many cells confirmed the preference of KIF5B-R and KIF1A-R for acetylated and tyrosinated microtubules, respectively (Figure 5C). These observations indicated that PTMs may contribute to the association of KIF5B-R and KIF1A-R with distinct populations of microtubules.

KIF5B and KIF1A Recruit Lysosomes to Different Populations of Microtubules

Next, we performed live-cell imaging to examine the effect of expressing GFP-tagged wild-type and rigor forms of KIF5B and KIF1A on the distribution and motility of lysosomes labeled with LAMP-1-RFP (Figure 6; Movies S2 and S3). We observed that wild-type KIF5B, expressed together with KLC2 and SKIP, was associated with clusters of lysosomes at cell protrusions (Figure 6A), as shown earlier for fixed cells (Figure 2A). Despite the overall displacement toward the periphery, many lysosomes still moved bidirectionally between the periphery and the center of the cell, as seen in Movie S2 and represented by lines with positive and negative slopes in the kymographs in Figure 6A. Although weak, staining for KIF5B could be seen on some of the moving lysosomes (Movie S2; Figure 6A). KIF5B-R plus

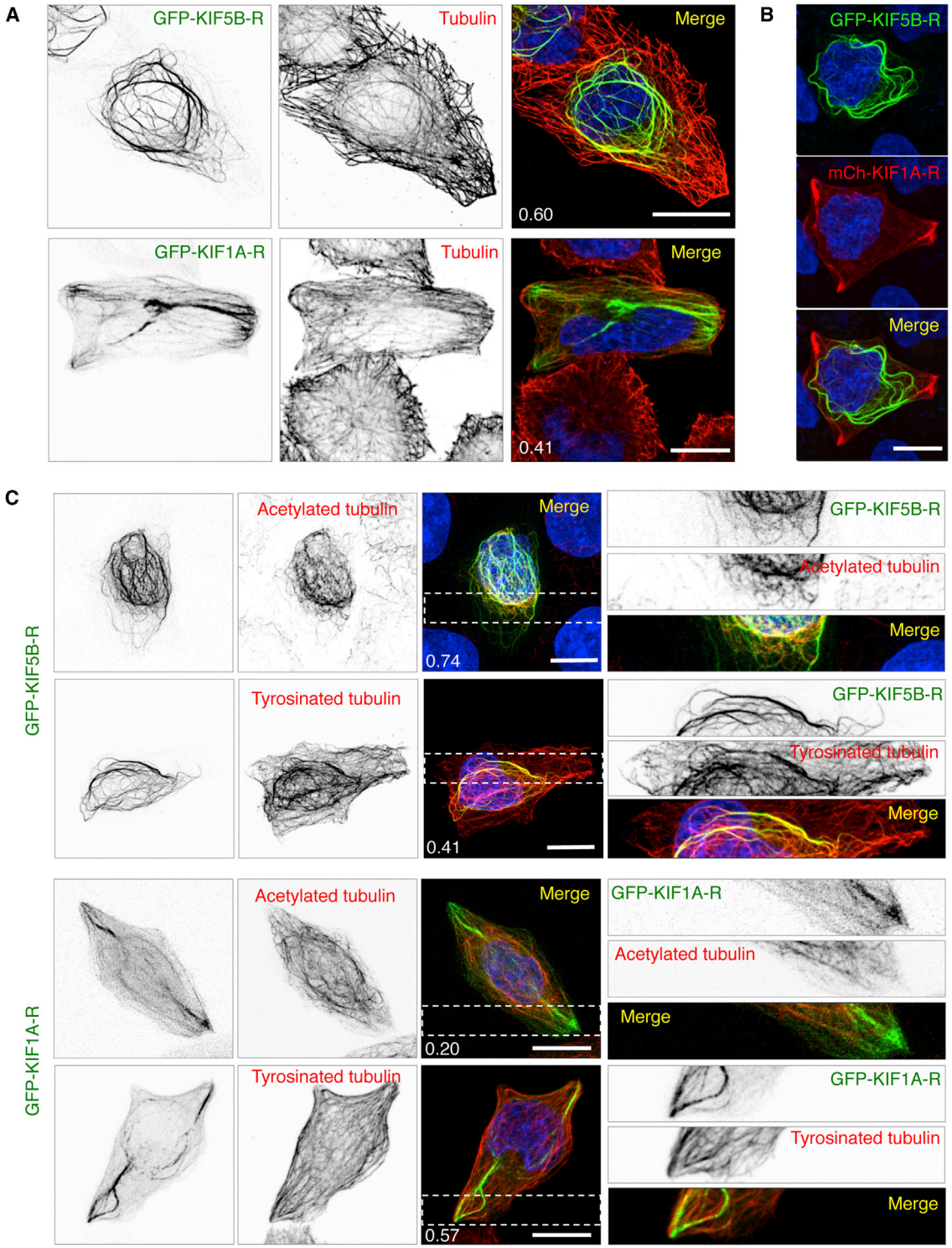

Figure 5. Rigor KIF5B and KIF1A Mutants Associate with Different Microtubule Tracks in HeLa Cells

(A) HeLa cells were transfected with plasmids encoding the kinesin rigor mutants GFP-KIF5B-R and GFP-KIF1A-R (green) and immunostained for α-tubulin (red).

(B) Co-expression of GRP-KIF5B-R (green) and mCh-KIF1A-R (red). Notice the association of each kinesin with different subsets of microtubules when expressed separately (A) or in the same cell (B).

(C) HeLa cells expressing GFP-KIF5B-R and GFP-KIF1A-R (green) were immunostained with antibodies to acetylated or tyrosinated α-tubulin (red). Magnifications of the boxed regions are shown on the right panels. Scale bars, 10 μm. Nuclei were stained with DAPI (blue). Numbers in the merge images are Pearson's correlation coefficients.

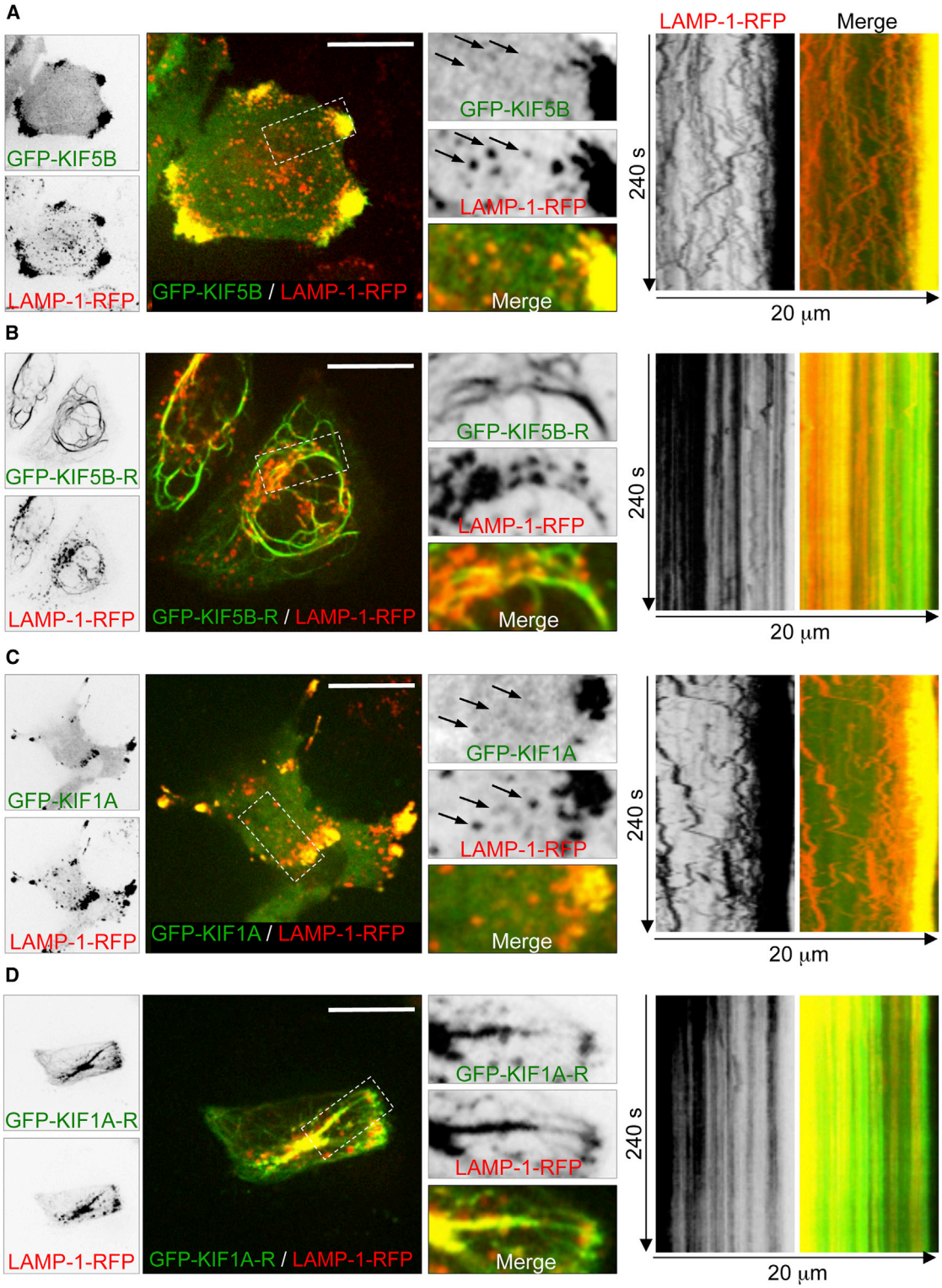

Figure 6. Rigor KIF5B and KIF1A Trap Lysosomes in Different Regions of the Cells

(A–D) HeLa cells co-expressing LAMP-1-RFP (red) with GFP-tagged (A) KIF5B+HA-KLC2+SKIP-myc, (B) KIF5B-R+HA-KLC2+SKIP-myc, (C) KIF1A, or (D) KIF1A-R (green) were analyzed by live-cell imaging on a spinning-disk confocal microscope. Panels on the left and center correspond to a single frame from Movies S2 and S3. Panels on the right are kymographs from the boxed regions in the corresponding movies. Scale bars, 10 μm. Arrows in the center panels show moving vesicles where LAMP-1-RFP and GFP-tagged kinesins co-localize.

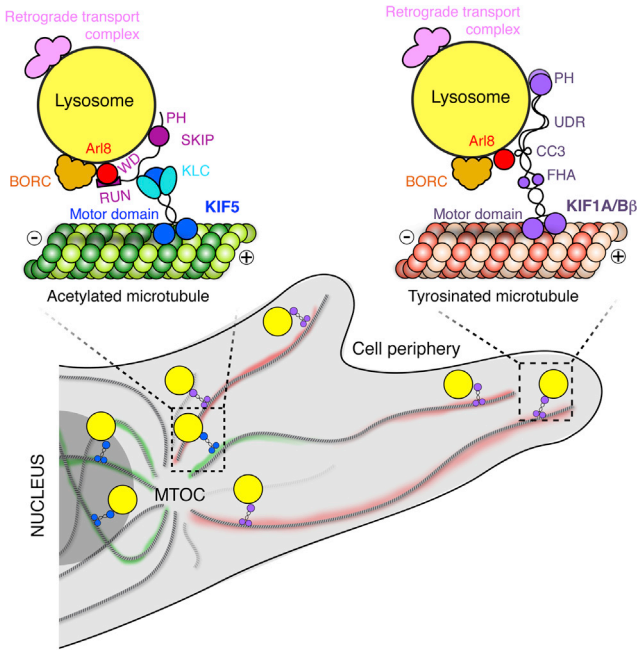

Figure 7. Model for BORC/Arl8-Dependent Coupling of Lysosomes to Different Kinesins and Microtubule Tracks

The scheme depicts the interaction chains by which BORC and Arl8 promote the recruitment of both kinesin-1 and kinesin-3 motors to lysosomes. Recruitment of kinesin-1 involves interactions of Arl8 (both a or b isoforms) with the RUN domain of SKIP, of a WD motif in an unstructured region of SKIP with KLC2, and of KLC2 with KIF5B. Recruitment of kinesin-3, on the other hand, is mediated by a direct interaction between Arl8 (a or b) and the CC3 domain of the kinesin-3 KIF1Bβ or KIF1A proteins. Kinesin-1 and kinesin-3 are shown to mediate centrifugal lysosome transport in perinuclear and peripheral regions of the cytoplasm, respectively, through association with different microtubule tracks. The function of both kinesins is countered by BORC-independent centripetal transport mediated by dynein. The schemes are based on findings in this study and previous studies (Pu et al., 2015; Rosa-Ferreira and Munro, 2011; Dumont et al., 2010; Wu et al., 2013; Pernigo et al., 2013).

KLC2 and SKIP, in contrast, exhibited an association with perinuclear microtubules, as described above (Figure 5A), and also caused lysosomes to accumulate in the perinuclear area (Figure 6B). These lysosomes were largely immobile, as shown in Movie S2 and by the vertical lines in the corresponding kymographs (Figure 6B). Wild-type KIF1A also co-localized with lysosome clusters at peripheral sites, but some lysosomes underwent bidirectional movement between these sites and the cell center (Movie S3; Figure 6C). Weak staining for KIF1A could also be seen on some of the moving lysosomes (Movie S3; Figure 6C). In contrast, KIF1A-R immobilized lysosomes on rectilinear tracks leading to the cell protrusions and in the protrusions themselves (Figure 6D; Movie S3).

Further experiments showed that the full-length form of either KIF5B (with its adaptors) or KIF1A could drive the motorless form of the other kinesin, together with lysosomes, to cell protrusions (Figure S7). In addition, Airyscan super-resolution microscopy showed co-localization of both motorless kinesins to individual lysosomes (Figure S7), indicating that both kinesins can simultaneously associate with the same lysosomes.

Taken together, the results shown in Figures 5, 6, and S7 demonstrated that KIF5B and KIF1A drive lysosome movement along distinct sets of microtubules localized to different regions of the cell and characterized by different tubulin PTMs.

DISCUSSION

BORC and Arl8 Are Master Regulators of Lysosome Positioning and Motility through Recruitment of Different Kinesins

The results of our study show that BORC and Arl8a/b function upstream of the kinesin-1 KIF5B and the kinesin-3 KIF1Bβ and KIF1A proteins to move lysosomes toward the cell periphery along different microtubule tracks and in different regions of the cell (Figure 7). Although both kinesin types share the property of having their motor domains at the N terminus, their structures are otherwise distinct (Figure 1A). To enable coupling of lysosomes to both kinesin types, Arl8 has evolved different means of interacting with these proteins: through the adaptor proteins SKIP and KLC in the case of KIF5B (Rosa-Ferreira and Munro, 2011) and through an intrinsic CC3 domain in KIF1Bβ and KIF1A (Wu et al., 2013). The Arl8-interacting modules of both kinesins, as well as the UDR and PH domains of KIF1A, are all necessary for the ability of these kinesins to drive lysosome movement (Figure 2). These findings indicate that BORC and Arl8 act as master regulators of lysosome movement through interaction with different kinesins, and explain why silencing of BORC subunits or Arl8 has a profound inhibitory effect on lysosome dispersal (Figures 3 and S5) (Pu et al., 2015; Rosa-Ferreira and Munro, 2011). Although BORC and Arl8 are common regulators of both kinesins, specific regulation of each kinesin likely occurs at the level of downstream effectors such as SKIP or the kinesins themselves.

We also examined the requirement of other kinesin types previously implicated in lysosome movement, including the kinesin-2 proteins KIF3A and KIF3B (Brown et al., 2005) and the kinesin-13 protein KIF2Aβ (Santama et al., 1998; Korolchuk et al., 2011), but found that these were less important in the HeLa cells used in our studies (Figures 1C and S4). The ability of these kinesins to drive lysosome movement may be more critical in other cell types or under different physiological conditions. In future studies, it will be of interest to determine whether BORC and Arl8 also regulate these kinesins, and, if so, by what mechanism.

Kinesin-1 and -3 Drive Lysosome Movement along Different Microtubule Tracks

We were intrigued by the involvement of different kinesin types in lysosome movement toward the cell periphery. Indeed, both kinesin-1 and -3 family members were found to be necessary (Figures 1C and 1D) and sufficient (upon overexpression) (Figures 2A–2D) for complete lysosome dispersal. However, rigor forms of KIF5B and KIF1A were preferentially associated with more centrally and peripherally located populations of microtubules, respectively (Figure 5), and selectively recruited lysosomes to the corresponding microtubule tracks (Figure 6). These findings lead us to conclude that kinesin-1 and -3 proteins preferentially drive lysosome movement in different regions of the cytoplasm through association with different microtubule tracks.

Various kinesins exhibit preferences for microtubule tracks that are characterized by specific tubulin PTMs or associated microtubule-associated proteins (MAPs). This phenomenon has been best documented in polarized cell types such as neurons, but probably also occurs in non-polarized cells (Cai et al., 2009). In our studies, we found that rigor KIF5B was predominantly attached to more perinuclear microtubules enriched in acetylated tubulin (Figure 5C), a hallmark of stable microtubules (Piperno et al., 1987). In contrast, rigor-KIF1A was mostly associated with rectilinear and peripheral microtubules enriched in tyrosinated tubulin (Figure 5C), a feature of more dynamic microtubules (Kreis, 1987). Thus, the biochemical properties of different microtubule populations underlie the preferential recruitment of different kinesins and, in turn, the regional movement of lysosomes, even in non-polarized cells.

Physiological Implications of the Regional Control of Lysosome Movement

The regional control of lysosome movement is likely critical for cellular processes that require regulated encounters of lysosomes with other organelles in different parts of the cytoplasm. These processes include autophagy, phagocytosis, and antigen presentation, which also depend on BORC and/or Arl8 (Garg et al., 2011; Korolchuk et al., 2011; Mrakovic et al., 2012; Sasaki et al., 2013; Pu et al., 2015). Both kinesin types could also participate in a relay mechanism in which lysosomes are handed over from one kinesin to the other in order to reach cell protrusions. The role of KIF1Bβ in driving lysosomes to cell protrusions might be particularly important for the participation of lysosomes in cell adhesion and migration, processes in which BORC and Arl8 have also been implicated (Pu et al., 2015; Schiefermeier et al., 2014). In this regard, KIF1Bβ was recently shown to promote cancer cell migration and invasion by translocation of the late-endosomal, membrane type 1 matrix metalloproteinase (MT1-MMP; MMP14) to the cell surface (Dong et al., 2013; Chen et al., 2016).

The mechanism described here could have a special significance in neurons, which display biochemically and structurally different microtubule arrays in axons and dendrites. In particular, axons are enriched in acetylated microtubules (Cambray-Deakin and Burgoyne, 1987), whereas dendrites contain more tyrosinated microtubules (Burgoyne and Norman, 1986). Furthermore, the motor domain of KIF5A has a preference for axonal microtubules, whereas the motor domain of KIF1A associates with both axonal and dendritic microtubules (Nakata and Hirokawa, 2003; Jacobson et al., 2006; Huang and Banker, 2012; Lipka et al., 2016). These correlations are broadly in line with those described in our study in non-neuronal cells and suggest that a similar mechanism might be in action. The relationship of PTMs to kinesin selectivity is likely more complex and dependent on combinations of multiple PTMs, MAPs, and cargos. Nonetheless, it is tempting to propose that the association of KIF5B and KIF1A with distinct microtubule populations revealed in our study will translate into an ability to move lysosomes in different regions of the neuron, particularly in axons and dendrites.

Both the myrlysin subunit of BORC and Arl8 were also shown to promote axonal transport of synaptic vesicle precursors (SVPs) in Caenorhabditis *elegans* neurons (Wu et al., 2013; Zheng et al., 2014). In addition, KIF1A orthologs are mainly responsible for the axonal transport of SVPs in various organisms, including mammals (Okada et al., 1995; Klopfenstein and Vale, 2004; Pack-Chung et al., 2007). In light of our findings, BORC and Arl8 might also function upstream of KIF1A to drive axonal transport of SVPs.

Dynein-Mediated Centripetal Transport of Lysosomes Is Independent of BORC

The collapse of the lysosome population when either KIF5B or KIF1B are knocked down (Figure 1C) suggests that both kinesins are required to counteract the function of dynein in centripetal transport of lysosomes. In other words, when either kinesin is missing, dynein prevails. This observation, together with more direct evidence shown in Figure 4, indicates that dynein activity is largely independent of BORC in the HeLa cells used in our study. This observation is significant because the SNAPIN subunit shared by both BORC and BLOC-1 was shown to mediate dynein-mediated axonal retrograde transport of various cargos, including late endosomes (Cai et al., 2010), signaling endosomes (Zhou et al., 2012), and autophagosomes (Cheng et al., 2015). Thus, SNAPIN might have functions in neurons that are independent of BORC, perhaps as a component of BLOC-1 or some other complex.

Conclusions

Our findings are a striking example of cellular processes that depend on cooperation of multiple kinesins. Other such processes are mitosis and cytokinesis (Zhu et al., 2005; Neef et al., 2006), intraflagellar particle transport (Pan et al., 2006), and hyphal growth in filamentous fungi (Schuchardt et al., 2005). In these cases, the multiple kinesins function redundantly, alternatingly or competitively. The results reported in the present study represent yet another mechanism in which two co-regulated kinesin types drive movement of the same cytoplasmic organelle in different regions of the cell.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection

HeLa cells were cultured in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine at 37°C and 5% CO_2. Transfections were performed using 1–3 μg of plasmid and 2 μL of Lipofectamine 2000 (Life Technologies) per well of a 24-well plate; cells were analyzed 24 hr after transfection. For knockdown experiments, cells were transfected with 25 or 50 nM siRNAs (ONTARGET SMART-pools, individual siRNAs, or non-targeting control siRNA; GE Dharmacon) and 2 μL of Lipofectamine 2000, and analyzed 48 hr later.

Fluorescence Microscopy

For fixed-cell immunofluorescence experiments, HeLa cells were plated on 12-mm-diameter coverslips coated with fibronectin (Sigma) (0.1 mg/ml in PBS for 1 hr at 37°C) and grown to ~30% confluency before transfection. At 24 hr after transfection, coverslips were washed in PBS and processed under two different conditions. In most experiments, cells were treated for 12 min with 4% paraformaldehyde (PFA) in PBS. Coverslips were washed twice for 5 min in PBS and permeabilized for 15 min in 0.2% Triton X-100. For analysis of tubulin PTMs, cells were first permeabilized for 5 min at 37°C with 2 mM $MgCl_2$, 10 mM EGTA, 60 mM PIPES, pH 6.9, and 0.5% Triton X-100, and washed twice with PBS. Cells were next fixed for 5 min with methanol at −20°C. In both conditions, cells were blocked for 30 min in 1% bovine serum

albumin (BSA) in PBS and stained for 30 min with primary antibodies in 0.2% BSA in PBS (BPBS) at 37°C. Coverslips were then washed twice with PBS and incubated for 30 min at 37°C with secondary Alexa-conjugated antibodies in BPBS. Coverslips were again washed twice with PBS and mounted on slides using Fluoromount-G with DAPI (Electron Microscopy Sciences). Images were obtained on an inverted confocal laser-scanning microscope (LSM780; Carl Zeiss) fitted with a 63×, 1.4 numerical aperture (NA) objective.

In live-cell experiments, cells were imaged on a spinning-disk confocal microscope (Marianas; Intelligent Imaging) equipped with a 63×, 1.4 NA objective. Digital images were acquired with an EM-CCD camera (Evolve; Photometrics). For dual-color movies, GFP-KIFs and LAMP-1-RFP channels were sequentially exposed for 200 and 100 ms, respectively.

For analysis of the requirement of BORC for dynein function, cells co-expressing mCh-KIF5B-Strep and LAMP-1-SBP-GFP (Supplemental Experimental Procedures) were treated with 40 μM biotin (Sigma) and imaged by confocal fluorescence microscopy as described above.

Statistical Analysis

Statistical analyses were performed using Prism 6 (GraphPad). Data are represented as mean ± SEM and p values were determined using two-tailed Student's t test for unpaired data (ns: not significant; *$p \leq 0.05$; ****$p \leq 0.0001$).

Other Reagents and Procedures

Sequence and structural analyses, recombinant DNAs, antibodies, development of CRISPR/Cas9 KO cell lines, image analysis, and SIM are described in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.10.062.

AUTHOR CONTRIBUTIONS

C.M.G. and J.S.B. conceived the project. C.M.G. performed most of the experiments. G.G.F. conducted live-cell imaging experiments. R.J. and J.P. developed the CRISPR/Cas9-KO cell lines. C.M.G., G.G.F., R.J., J.P., and J.S.B. contributed to the writing of the manuscript.

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