Peroxisomes move by hitchhiking on early endosomes using the novel linker protein Pxda

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Accessibility
Eukaryotic cells use microtubule-based intracellular transport for the delivery of many subcellular cargos, including organelles. The canonical view of organelle transport is that organelles directly recruit molecular motors via cargo-specific adaptors. In contrast with this view, we show here that peroxisomes move by hitchhiking on early endosomes, an organelle that directly recruits the transport machinery. Using the filamentous fungus *Aspergillus nidulans* we found that hitchhiking is mediated by a novel endosome-associated linker protein, PxdA. PxdA is required for normal distribution and long-range movement of peroxisomes, but not early endosomes or nuclei. Using simultaneous time-lapse imaging, we find that early endosome-associated PxdA localizes to the leading edge of moving peroxisomes. We identify a coiled-coil region within PxdA that is necessary and sufficient for early endosome localization and peroxisome distribution and motility. These results present a new mechanism of microtubule-based organelle transport in which peroxisomes hitchhike on early endosomes and identify PxdA as the novel linker protein required for this coupling.

**Introduction**

Eukaryotic cells rely on the microtubule cytoskeleton to move intracellular components over long distances. Microtubules are dynamic polar structures, with “plus” ends usually located near the cell periphery and “minus” ends typically embedded in perinuclear microtubule organizing centers. Dynein motors move cargos toward the minus ends of microtubules, whereas most kinesin motors move in the opposite direction. Cytoplasmic dynein-1 (referred to here as dynein) and a relatively small number of kinesins are responsible for the transport of vesicles, organelles, proteins, and mRNAs (Vale, 2003; Cianfrocco et al., 2015).

In mammalian cells, a variety of organelles have been shown to depend on dynein and kinesin for transport, including endosomes, mitochondria, peroxisomes, Golgi, endoplasmic reticulum, autophagosomes, lysosomes, and nuclei (Harada et al., 1998; Tanaka et al., 1998; Roghi and Allan, 1999; Schrader et al., 2000; Kural et al., 2005; Maday and Holzbaur, 2012; Neuhaus et al., 2015). In some cases, the adaptors that link the molecular motors to their cargos have been identified (Kardon and Vale, 2009; Fu and Holzbaur, 2014; Cianfrocco et al., 2015). For example, in the case of mitochondria, TRAK/Milton proteins recruit kinesin-1 and dynein (Glater et al., 2006; Wang and Schwarz, 2009; van Sprosen et al., 2013), and in the case of early endosomes (EEs), Hook proteins have been shown to recruit dynein and kinesin-3 (Bielska et al., 2014; Zhang et al., 2014). This has led to the idea that each type of cargo uses distinct machinery to recruit molecular motors. Our goal is to use the model fungus *Aspergillus nidulans* to identify how different cargos engage the transport machinery.

*A. nidulans* and other filamentous fungi have proven to be excellent model systems for studying microtubule-based transport (Egan et al., 2012a). In these fungi, microtubules are used to transport cellular cargos through highly polarized, multinucleate cells, called hyphae. Furthermore, rapid forward genetics are possible, and genome engineering is fast and simple (Horio and Oakley, 2005; Nayak et al., 2006). In *A. nidulans*, microtubules are uniformly polarized from the hyphal tip to the tip-proximal nucleus, such that plus ends are located at hyphal tips and minus ends are embedded in the nuclear membrane (Egan et al., 2012b). As a result of this organization, defects in dynein-mediated transport generally lead to accumulation of cargo at the hyphal tip, whereas defects in kinesin-3/UncA transport can cause accumulation of cargo at the tip-proximal nucleus (Fig. 1; Egan et al., 2012a).

We took advantage of these clear organelle distribution phenotypes to conduct a forward genetic screen in *A. nidulans* to identify novel genes required for the microtubule-based movement of dynein and kinesin cargos (Tan et al., 2014). For our screen, we focused on three well-characterized cargos of dynein and kinesin: EEs, peroxisomes, and nuclei (Xiang et al., 2014).
In this study, we focused on hits from our screen that caused peroxisome-specific phenotypes. Peroxisomes are responsible for β-oxidation of long-chain fatty acids and guard against toxic reactive oxygen species (Liu et al., 2008). Their distribution in the cell (Neuhaus et al., 2015). We identified a microtubule-based motility is likely important to achieve even long-range movement (Baumann et al., 2012; Higuchi et al., 2015), but the molecules mediating this behavior were not known. Our data demonstrate that PxdA is a novel linker protein mediating peroxisomal hitchhiking on EEs.

Results and discussion

PxdA is a novel protein required for peroxisome distribution and motility

In our original screen, 19 mutants were categorized as only affecting peroxisome distribution, making them excellent candidates for cargo-specific regulators (Tan et al., 2014). Whole-genome sequencing of two mutants (RPA604 and RPA628) with a strong peroxisome distribution phenotype identified independent mutations in the uncharacterized gene AN1156; both mutations were predicted to result in premature stop codons. AN1156 mutants displayed hyphal tip accumulation of peroxisomes, but normal distribution of RabA5a-positive EEs.
and nuclei (Fig. S1 A). Based on this phenotype, we named AN1156 pxdA (peroxisome distribution mutant A). To confirm that PxdA is expressed, we performed immunoblots of lysates from an HA-tagged pxdA strain and detected an ~250-kD band (Fig. S1 B), which corresponds well to the larger of two predicted open reading frames for AN1156.

To verify that pxdA plays a role in regulating peroxisome distribution, we deleted the endogenous gene in haploid strains containing fluorescently labeled EEs, peroxisomes, or nuclei. Consistent with the phenotype of RPA604 and RPA628, peroxisomes accumulated in ΔpxdA strains (Fig. S1, C and D). We conclude that PxdA specifically regulates the distribution of peroxisomes, but not EEs or nuclei.

Peroxisome distribution and motility in A. nidulans requires the microtubule-based motors cytoplasmic dynein/NudA and kinesin-3a/UncA (Egan et al., 2012a,b). To determine whether the loss of pxdA affects peroxisome motility and distribution, we imaged peroxisomes in wild-type (WT) and ΔpxdA strains. In WT hyphae, peroxisomes exhibit both long-range runs and oscillatory movements (Video 1). In WT hyphae, 12% (n = 486) of peroxisomes exhibit long-range movements (distances >3 µm), consistent with previous studies in mammalian cells in which only 10%–15% of peroxisomes are motile (Rapp et al., 1996; Bharti et al., 2011). These long-range movements are reduced by >90% in ΔpxdA hyphae (Fig. 1, C and D; and Video 2). Furthermore, the velocity and run length of the small pool of peroxisomes that move in ΔpxdA hyphae are also reduced compared with WT (Figs. 1 E and S1 E). In contrast, the flux and velocity of EEs are similar in WT and ΔpxdA strains (Fig. 1, F–H; and Videos 3 and 4).

Taken together, our findings demonstrate that PxdA is required for the proper distribution and long-range movement of peroxisomes, but it is not required for the distribution or motility of EEs or nuclei. Our data also suggest that PxdA is not a core component of the transport machinery because deletions of this type would be predicted to result in defects in the transport or distribution of EEs and nuclei as well. Consistent with this, we find that the localization of dynein, characteristically seen as “comets” near the microtubule-plus end (Xiang et al., 2000), is intact in ΔpxdA hyphae (Fig. S1 F).
Indeed, we find that PxdA accumulates at the hyphal tip in strains. hookAΔ EE strains, PxdA would accumulate at hyphal tips in 2014). We reasoned that if most PxdA protein is associated with of HookA, EE accumulates near the hyphal tip (Zhang et al., 2014), and its vertebrate homolog (Hook3) is an activator kinesin-3 in filamentous fungi (Bielska et al., 2014; Zhang et al., 2014), and the peroxisome (Fig. 2 D, middle and right).

Because we observed many more moving PxdA puncta than moving peroxisomes, and because PxdA motility was reminiscent of EE motility (Abenza et al., 2009; Zhang et al., 2010; Egan et al., 2012b, 2015), we next asked whether PxdA also colocalizes with EE. To test this, we analyzed kymographs from simultaneous time-lapse movies of labeled PxdA and EE (Video 7). Ninety percent of PxdA puncta colocalized with EE at the leading edge of moving peroxisomes (Fig. 2 D, middle and right).

We next sought to determine whether perturbing EE localization also perturbed PxdA localization. HookA has recently been identified as a cargo adaptor between EE and dynein/dynactin (McKenney et al., 2014). In the absence of HookA, EE accumulates near the hyphal tip (Zhang et al., 2014). We reasoned that if most PxdA protein is associated with EE, PxdA would accumulate at hyphal tips in hookAΔ strains. Indeed, we find that PxdA accumulates at the hyphal tip in hookAΔ compared with WT strains (Fig. 3, C and D), suggesting that PxdA is present on EE that require HookA for motility.

To determine whether PxdA colocalizes with peroxisomes, we next visualized PxdA and peroxisomes simultaneously using dual-color time-lapse imaging. Kymograph analysis (Fig. 2 D) of time-lapse movies (Fig. 2 E and Video 6) revealed that 66% (n = 29) of moving peroxisomes co-migrate with PxdA. PxdA is rarely associated with stationary peroxisomes. Closer inspection of the time-lapse movies and kymographs revealed that PxdA localizes at the leading edge of moving peroxisomes with the peak PxdA signal leading by 560 ± 80 nm (mean ± SEM; Fig. 2 F). We also occasionally observed instances in which a moving PxdA puncta paused at a nonmotile peroxisome, an event that was followed by the movement of comigrating PxdA and the peroxisome (Fig. 2 D, middle and right).

Figure 3. EE colocalize with PxdA and moving peroxisomes. (A) Representative kymographs generated from simultaneous time-lapse movies of PxdA-mKate and GFP-RabA/5a-labeled EE. (B) Bar graph quantifying the colocalization of PxdA-mKate and EE. EE colocalized with 54.8% ± 3.8% (SEM) PxdA puncta (n = 20 kymographs from 10 cells). PxdA colocalized with 89.7% ± 2.0% EE (n = 20 kymographs from 10 cells). (C) Representative micrographs of PxdA-mKate distribution in WT versus hookAΔ hyphae. (D) Normalized line scans of WT and hookAΔ hyphae [P < 0.0001, two-way analysis of variance, Bonferroni post hoc test significant between 0.64 and 1.92 µm; n = 5 hyphae per genotype]. (E) A kymograph generated from a simultaneous time-lapse movie of peroxisomes (pex) and EE. 71.4% of moving peroxisomes (n = 28) overlapped with EE. Bars: (x axis) 5 µm; (y axis) 5 s. (F) Stills from Video 8 of peroxisomes (Pex) and EE. The peak EE signal was leading the peak peroxisome signal by 610 ± 170 nm (Fig. 3 G), comparable with what we observed for peroxisomes and PxdA (Fig. 2 F).

These results suggest that peroxisomes move by “hitchhiking” on EE and that this process is mediated by PxdA. The concept that cellular components can hitchhike on EE has been observed in U. maydis for Rrm4-containing ribonucleoprotein complexes (Baumann et al., 2012) and polysomes (Higuchi et al., 2014). In a study parallel to ours, also in U. maydis, peroxisomes, endoplasmic reticulum, and lipid droplets were observed to hitchhike on EE (Guimaraes et al., 2015).

Endosomal localization of PxdA is required for peroxisome hitchhiking

We hypothesized that PxdA tethers EE to peroxisomes to promote hitchhiking. If this were the case, we might be able to separate PxdA’s EE localization from its function in peroxisome motility. To test this, we constructed a series of PxdA mutants (Fig. 4 A).

First, we sought to determine which domains of PxdA are necessary and sufficient for peroxisome motility. PxdA encodes a 2236-aa protein containing an ∼600-aa stretch of predicted
tandem coiled-coil domains (CC1–3), flanked by a large N-terminal uncharacterized region (UR1) and a much smaller C-terminal uncharacterized region (UR2; Fig. 4 A). Initially, we truncated the entire N-terminal UR1 (ΔNT) or the entire C-terminal region, including CC1–3 and UR2 (ΔCT) or the UR2 region alone (ΔUR2). We performed peroxisome line scans and found that peroxisomes accumulated at the hyphal tip of ΔCT strains, but not full-length (FL), ΔUR2, or ΔNT strains (Fig. 4 B). The PxdA-ΔCT strain also had a significant reduction in the flux of peroxisome movements compared with the FL and ΔUR2 strains, but not full-length (FL), ΔUR2, or ΔNT strains and found that peroxisomes accumulated at the hyphal tip of ΔUR2 hyphae (Fig. 4 C).

We next sought to identify the region of PxdA required for its localization to EEs. To test this directly, we expressed PxdA’s CC1–3 alone and quantified peroxisome distribution and flux along hyphae. CC1–3 is sufficient to rescue the defects in both peroxisome distribution and peroxisome flux observed in pxdAΔ hyphae (Figs. 4 D and S2 B). Furthermore, PxdA CC1–3 localizes to moving puncta that show velocity and flux similar to those of strains expressing FL PxdA (Fig. S2, C–E). These experiments show that CC1–3 is necessary and sufficient for PxdA’s function on peroxisomes.

Figure 4. Coiled-coil region of PxdA is necessary and sufficient for peroxisome motility and endosomal hitchhiking. (A) Schematic of PxdA protein constructs used in this analysis relative to FL PxdA. UR1 (white) and UR2 (yellow) are on the N and C terminus, respectively. Predicted regions of coiled-coil (CC1, 2, and 3) are indicated in orange. All strains contain a C-terminal fluorescent protein tag (either mKate or TagGFP). (B) Normalized line scans of peroxisome distribution from the hyphal tip for FL, C-terminal deletion (ΔCT), N-terminal deletion (ΔNT), and ΔUR2 PxdA strains (P < 0.0001, two-way analysis of variance, Bonferroni post hoc test significant between 0.51 and 3.71 μm for FL vs. ΔCT; n = 51–54 hyphae per genotype). (C) Bar graph of the flux of peroxisome movements in FL, ΔCT, and ΔUR2 hyphae. Peroxisome movements were 3.69 ± 0.47 (SEM)/min for FL, 0.44 ± 0.14/ min for ΔCT, and 3.10 ± 0.50/min for ΔUR2 (P < 0.0001, one-way analysis of variance; *, Bonferroni post hoc test significant for FL vs. ΔCT; FL vs. ΔUR2 was not significantly different; n = 29–35 hyphae per genotype). (D) Normalized line scans of peroxisome distribution along hyphae expressing either FL or CC1–3 PxdA (P < 0.0001, two-way analysis of variance, Bonferroni post hoc test significant between 0.87 and 4.11 μm from hyphal tip; n = 28 [FL] and n = 32 [ΔCC2/3]). (E) Normalized line scans of peroxisome distribution along hyphae expressing either FL or ΔCC2/3 PxdA (P < 0.0001, two-way analysis of variance, Bonferroni post hoc test significant between 1.19 and 3.03 μm from hyphal tip; n = 78 [FL] and n = 68 [CC1–3]). (F) Normalized line scans of peroxisome distribution along hyphae expressing either FL or ΔCC2/3 PxdA (P < 0.0001, two-way analysis of variance, Bonferroni post hoc test significant between 0.87 and 4.11 μm from hyphal tip; n = 28 [FL] and n = 32 [ΔCC2/3]). (G) Representative kymographs generated from simultaneous time-lapse movies of EEs with FL or ΔCC2/3 PxdA. Colocalization represents the number of EEs that colocalize with PxdA. FL is 54.8% ± 3.8% (Fig. 3 B) and ΔCC2/3 3.2% ± 1.3% (n = 10 kymographs). (H) Model for peroxisome (P) hitchhiking on EEs mediated by PxdA. HookA recruits the transport machinery to EEs. PxdA is required to tether EEs to peroxisomes and may have distinct binding partners (x) on each organelle. The arrow depicts the direction of movement.
Materials and methods

Fungal growth conditions

*A. nidulans* strains were grown in yeast extract and glucose medium (Szewczyk et al., 2006) or 1% glucose minimal medium (Nayak et al., 2006), supplemented with 1 mg/ml uracil, 2.4 mg/ml uridine, 2.5 µg/ml riboflavin, 1 µg/ml para-aminobenzoic acid, and 0.5 µg/ml pyridoxine when required. Glufosinate was used at a final concentration of 25 µl/ml as previously described (Nayak et al., 2006).

For imaging of germlings (for all organelle distribution experiments and CC1–3 fl ux experiments), spores were resuspended in 0.5 ml of 0.01% Tween-80 solution. The spore suspension was diluted to 1:1,000 in liquid minimal medium containing appropriate auxotropic supplements. The spore and media mix (400 µl) was added to an eight-chambered Nunc Lab-Tek II coverglass (Thermo Fisher Scientific) and incubated at 30°C for 16–20 h before imaging. For imaging of mature hyphae (for velocity and flux experiments), spores were inoculated on minimal medium plates containing the appropriate auxotropic supplements and incubated at 37°C for 12–16 h. Colonies were excised from agar plates and inverted on Lab-Tek plates for imaging.

For benomyl and latrunculin A experiments, minimal media containing the drugs (2.4 µg/ml for benomyl [Sigma-Aldrich]; 12 µM for latrunculin A [Life Technologies]) or DMSO control (1.2%) were added fresh to Lab-Tek chambers containing germlings and were imaged 45 min to 1 h later. For Western blots, cells were grown in yeast extract and glucose medium for 16–20 h, strained in Miracidil (Millipore), and flash frozen in liquid nitrogen. For lysis, cells were ground in liquid nitrogen and boiled in 9 M urea denaturing buffer (125 mM Tris-HCl, pH 6.8, 9 M urea, 1 mM EDTA, pH 7.0, 4% SDS, 10 mM DTT, 10% β-mercaptoethanol, and 4% glycerol) before running on SDS-PAGE.

**Strain construction**

Strains of *A. nidulans* used in this study are listed in Table S1. All strains were confirmed by a combination of PCR and sequencing from genomic DNA isolated as previously described (Lee and Taylor, 1990). Strains were created by homologous recombination to replace the endogenous gene in strains lacking *ku70* (Nayak et al., 2006) with *AfpyrG* (*Aspergillus fumigatus* PyrG), *AfpyrA* (*Aspergillus fumigatus* PyrA), or *bar* (Straubinger et al., 1992) as selectable markers. All linearized transforming DNA for deletion strains, including *pxdA* and *hookA*, were constructed by fusion PCR (Szewczyk et al., 2006). All *PxdA* DNA constructs contained a C-terminal codon-optimized fluorescent protein tag, either TagGFP2 (Subach et al., 2008) or mKate2 (Shcherbo et al., 2009), followed by *pxdA*’s native 3’ UTR, and were inserted into the Blue Heron Biotechnology pUC vector at 5’ EcoRI and 3’ HindIII restriction sites using isothermal assembly (Gibson et al., 2009). Plasmid constructs were confirmed by sequencing. Linearized transforming DNA from plasmid constructs was created by PCR with flanking primers located at the 5’ and 3’ ends of homologous arms.

The amino acid positions for each *PxdA* construct in Fig. 4 were as follows: FL, aa 1–2,236; ∆NT, aa 1,466–2,236; ∆CT, aa 1–1,465; ∆UR2, aa 1–2,116; and ∆CC2/3, aa 1–1,946. For the CC1–3 rescue experiment (Figs. 4 D and S1, B–D), the CC1–3 construct (aa 1,466–2,116) was inserted into a *pxdAΔ* strain at the endogenous locus.

**Fluorescence microscopy**

All images were collected at 22°C. For acquisition of the distribution and motility of peroxisomes (PTS1-mCherry and PexK-GFP) and for the distribution of EE (GFP-RabA/A5a), dynemin (NudA-3xGFP), and PxdA, images were collected using a Plan Apo 60x/1.42 (Olympus) oil immersion objective on an epifluorescence DeltaVision Core microscope (GE Healthcare). GFP was excited with a 488-nm laser line (50 mW) and collected with a 525/50 FITC emission filter. mCherry fluorescence was excited with the 561-nm laser line (50 mW) and collected with a 594/45 emission filter. Images were acquired with a PCO edge sCMOS camera (Kelkem) and controlled with softWoRx software (GE Healthcare). For distribution images, 20 optical z-sections were collected with a step size of 0.5 µm. Hyphae (used for outlining cells) were imaged using brightfield.

Simultaneous multicolor time-lapse images were collected using a Plan Apo 60x/1.42 oil immersion objective on the DeltaVision OMX Blaze V4 system (GE Healthcare). GFP and mKate2/mCherry were excited simultaneously with 488- and 568-nm diode laser lines (100 mW), respectively. A series of dichroic mirrors were used to split emission light from fluorophores to different PCO Edge sCMOS cameras. An emission filter in front of both cameras was used to select appropriate wavelengths (528/48 and 609/37 for GFP and mKate/mCherry, respectively). Images were aligned and registered using the OMX alignment slide and corrected for translation, rotation, and magnification differences between channels using softWoRx software. Some images were deconvolved using the enhanced ratio method (five iterations) and/or binned (2 × 2).

For EE and PxdA motility assays, time-lapse images were collected using a Plan Apochromat TIRF 100x/1.49 oil immersion objective on an epifluorescence Ti-E motorized inverted microscope (Nikon) with the Perfect focus system (Nikon) for continuous maintenance of focus, all controlled by NIS-Elements software (Nikon). GFP fluorescence was excited using a 488-nm laser line (50 mW) and collected with a 525/50 emission filter. Images were acquired with an EM-CCD camera (DU-897; Andor).

Images were brightness and contrast adjusted using ImageJ (version 2.0; National Institutes of Health) and Photoshop CS3 (version 10.0; Adobe), and figures were compiled in Illustrator CC (2015.2; Adobe).

**Image and data analysis**

For peroxisome, EE, and PxdA line-scan distribution measurements, maximum-intensity projections of fluorescence micrographs and brightfield images (for hyphae) were obtained using ImageJ. Brightfield images of hyphae were traced using the segmented line tool (line width 25), starting from the hyphal tip and ending at a point indicated in the x axis of respective line-scan graphs. Each trace was overlaid onto the corresponding fluorescence micrograph and used to project the average fluorescence intensity. For peroxisome normalization (Fig. 4), average intensity values at each point along hyphae were normalized against that particular condition’s baseline mean (10–25 µm). For PxdA normalization (Fig. 3D), average intensity values at each point along hyphae were normalized against that particular condition’s baseline average (5–10 µm). For flux measurements, the number of puncta crossing a line 10 µm perpendicular to and from the hyphal tip was calculated (Egan et al., 2012b).
EE, peroxisomes, and PxdA velocities and run lengths were measured using ImageJ. Maximum-intensity projections were generated from time-lapse sequences to define the trajectory of particles of interest. The segmented line tool was used to trace the trajectories and map them onto the original video sequence, which was subsequently resliced to generate a kymograph. The instantaneous velocities of individual movements (for runs ≥ 2 μm in length) were calculated from the inverse of the slopes of kymograph traces, and run-length measurements were obtained from each of these traces. For measurements of normalized intensity during co-migrating runs between PtxA or EEs with peroxisomes (Figs. 2 E and 3 F), peroxisomes and associated puncta (PtxA or EEs) were traced using ImageJ at a point along the trajectory of the path (in the direction of movement) that was clear and distinct from other puncta on the micrograph. Line intensity profiles for each channel individually were generated. The “0” along the x axis corresponds to the position of peak intensity for the peroxisome signal. Intensities for each channel were normalized to that channel’s peak intensity for each comigrating run.

Data visualization and statistical analyses were performed using GraphPad Prism (6.0d; GraphPad Software, Excel (version 14.5.8; Microsoft), and ImageJ (2.0). Analysis of variance followed by Bonferroni post hoc statistical tests were used for all distribution comparisons, and nonparametric Mann-Whitney or Kolmogorov-Smirnov tests were used for all flux and velocity comparisons, respectively. All experiments were analyzed from at least three independent replicates, except for ΔCC2/3 experiments (Fig. 4, E–G) and ΔCC1–3 flux/velocity measurements (Fig. S2, B, D, and E), which were analyzed from two independent replicates.

Online supplemental material

Fig. S1 shows that peroxisomes accumulate in the hyphal tip in PtxA mutant and deletion strains, but EEs, nuclei, and cytoplasmic dynein are unaffected. Fig. S2 shows that tagging PtxA does not affect its function and that expressing its CC alone moves similarly to the FL protein. Video 1 shows peroxisome dynamics in a WT hypha. Video 2 shows peroxisome dynamics are perturbed in a ptxΔΔ hypha. Video 3 shows EEs dynamics in a WT hypha. Video 4 shows that EE dynamics are unaffected in a ptxΔΔ hypha. Video 5 shows PtxA-GFP colocalizing with EEs. Video 8 shows an EE comigrating with a moving peroxisome. Table S1 lists the A. nidulans strains used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201512020/DC1. Additional data are available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201512020.dv.

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