Supplementary materials to Rai et al.

Dictyostelium Dynamin B Modulates Cytoskeletal Structures and Membranous Organelles

SUPPLEMENTARY FIGURE AND MOVIE LEGENDS

Fig. 1S Effect of dynamin B on cell growth. **(A)** Cell growth in suspension culture. AX2 (open symbols) and AX2 dymB$^-$ (filled symbols) cells were grown in suspension culture on HL-5C media. Error bars represent the standard deviations for three independent cultures grown in parallel. **(B)** Dynamin B deletion increases bacterial uptake compared to wild-type cells. 100 cells of AX2 (open symbols) and *dym*B$^-$ (filled symbols) were mixed with a *E. coli* B suspension, plated on 1/3 MS-agar. Plaque size was measured at indicated times. Error bars represent the standard deviations for three independent experiments. **(C)** Dynamin B null cells show better survival in hypotonic media compared to wild-type cells. Cell survival experiments were done on isotonic, hypertonic and hypotonic media. AX2 and *dym*B$^-$ cells were incubated in suspension culture with different osmolarity for one hour. The number of viable cells was determined by plaque formation on bacterial lawns and is shown relative to the initial number of cells. Error bars represent the standard deviations for three independent experiments.

Fig. 2S Effect of dynamin B-depletion on the distribution of myosin-2 in AX2 and *dymB*$^-$ cells. Dynamin B null cells display stronger but diffuse myosin-2 staining. A single confocal plane close the ventral surface is shown. Scale bars, 10 μm.

Supplementary Movie 1. 3D confocal reconstitution of AX2 cells showing peroxisomes and tubulin cytoskeleton morphology and their distribution. AX2 cells were fixed and probed with antibodies as described in material and methods. Peroxisomes are shown in green and microtubuli in red. The movie shows a 3D reconstitution based on 30 confocal sections covering the entire cell volume. Movie speed is 2 frames per second. Scale bar 5 μm.

Supplementary Movie 2. 3D confocal reconstitution of *dym*B$^-$ cells showing peroxisomes and tubulin cytoskeleton morphology and their distribution. AX2 dymB$^-$ cells were fixed and probed as described in Material and Methods. Peroxisomes are shown in green and α-tubulin in red. Movie shows 3D reconstitution from 30 confocal sections covering the entire cell volume. Movie speed is 2 frames per second. Scale bar 5 μm.

Supplementary Movie 3. Confocal time–lapse series of AX2 cells overproducing GFP-PTS1. Cells are showing normal peroxisomal morphology and distribution. Movie speed is 3 frames per second. Scale bar 5 μm.

Supplementary Movie 4. Confocal time–lapse series of *dym*B$^-$ cells overproducing GFP-PTS1. The dynamin B-depleted cells show a marked increase in the size of individual peroxisomes and changes in organelle dynamics. Movie speed is 3 frames per second. Scale bar 5 μm.

Supplementary Movie 5. Time–lapse RIC microscopy of AX2 cells showing the dynamic changes that occur in the cell-substratum contact area during migration. Additionally, the dynamic behavior of the darker appearing vesiculotubular osmoregulatory apparatus on the

ventral side of the cell is visible in the movie. Movie speed is 3 frames per second. Scale bar 5 μm.

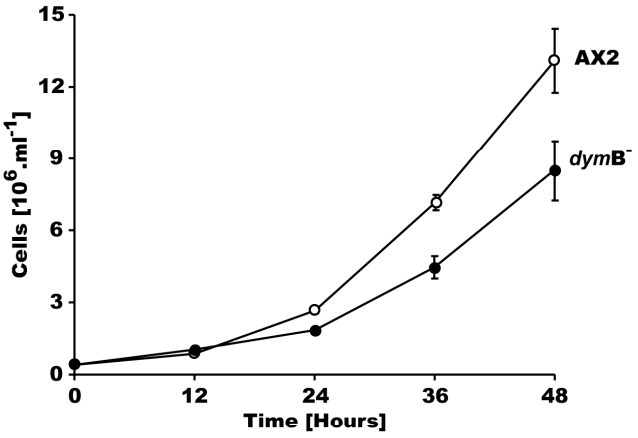
Supplementary Movie 6. Time–lapse RIC microscopy of *dym*B^{-} cells showing the dynamic changes in the cell-substratum contact area during migration. Additionally, the dynamic behavior of the darker appearing vesiculotubular osmoregulatory apparatus on the ventral side of the cell is visible in the movie. Movie speed is 3 frames per second. Scale bar 5 μm.

Supplementary Movie 7. Time–lapse TIRF microscopy of AX2 cells overproducing GFP-actin. Dynamic changes in actin distribution close to the cell surface are visualized during cell migration. Cells were starved for 1 hour in MES buffer and images were recorded every 2 seconds over 240 seconds in TIRF microscopy mode. Movie speed is 10 frames per second. Scale bar 20 μm.

Supplementary Movie 8. Time–lapse TIRF microscopy of *dym*B^{-} cells overproducing GFP-actin. Dynamin B–depleted cells display a decrease in actin dynamics and an increase in contact area with the glass surface on which the cells move. Cells were starved for one hour in MES buffer and images were recorded every 2 seconds over 240 seconds. Movie speed is 10 frames per second. Scale bar 20 μm.

Supplementary Figure 1

A



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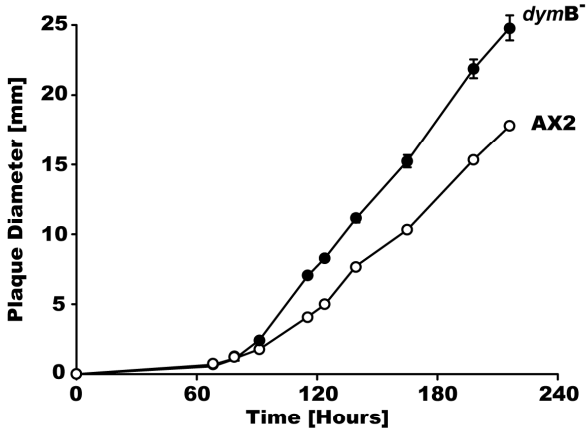

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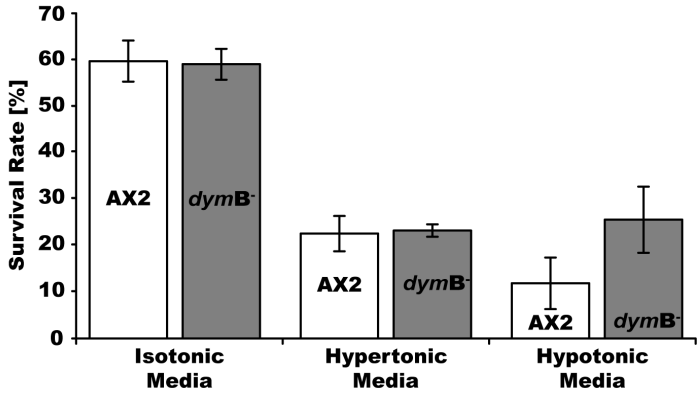

Supplementary Figure 2