Figure S1. Correlation between fluorescent signals from F-actin cortex and PM in several positions along the edge of the oscillating and nonoscillating cell for two time points. (left) Merged images of fluorescent signal from PM (red) and F-cortex (green) for single oscillating (in total, \( n = 45 \) oscillating cells from 12 experiments were recorded) and nonoscillating cells (\( n = 8 \) from four experiments). Yellow rectangles show the positions where the fluorescent signal was analyzed. (right) Plots present the averaged line scans of Lifeact-GFP (green) and PMT-mRFP (red) fluorescent intensity inside the corresponding rectangles going from the inside to the outside of the cell at two time points. Values are normalized to the maximum value within the frame. The rectangles are 6 x 1.2 \( \mu m \).
Figure S2. **F-cortex and PM dynamics during blebbing.** (A) Merged image of fluorescent signals from PM (red) and F-cortex (green) for rounded CHO cell undergoing blebbing. Yellow rectangle shows the position where the fluorescent signal was analyzed. Bar, 5 µm. (B) Plots present the averaged line scans of Lifeact-GFP (green) and PMT-mRFP (red) fluorescent intensity inside the rectangle (going from the inside to the outside of the cell) at four time points for one bleb. Inserts present the corresponding fluorescent images of the analyzed bleb. The rectangle is 3 x 0.15 µm. (C) Correlation plot shows no correlation between maximum intensity of F-actin and PM fluorescence at the bleb margin for four blebs on the same cell (A) at several time points during bleb development and retraction (correlation coefficient R = 0.115, n = 28). In total, five cells with blebbing in three independent experiments were analyzed. Mean correlation coefficient R = −0.040 ± 0.260, n = 82.
Figure S3. Images of Lifeact-GFP and PMT-mRFP fluorescent signals taken on a Revolution XD spinning disk confocal microscope at 100-ms intervals. Kymographs 1 and 2 were assembled for the same cell using different directions (top middle). Total time of recording was 72.6 s. Kymograph 3 is a merged image of two kymographs assembled for F-actin (green) and PM (red), along the line shown in the merged image. Total time of recording was 99 s.
Figure S4.  TEM images of Cho cells. (A–C) Rounded cells were fixed during oscillations. Arrows point to the small organelles trapped inside the folds. (D) Cells spread for 24 h on a plastic culture dish; note the absence of surface folds. (E) Magnified view of the area inside the box in D.
Figure S5. **Degree of membrane-cortex compaction in folded regions.** (A and B) Estimation of degree of membrane-cortex folding–unfolding using dual membrane (red) and F-actin (green) signals measured in a 2-µm-wide band along the perimeter. (A) Two fiduciary markers observed in the DIC images at positions P1 and P2 were separated by 6.1 µm. (B) The fluorescent intensity between P1 and P2 had mean value 650 ± 339 a.u. for the F-actin signal and 567 ± 212 a.u. for the PM signal (top). After 19 s, this part of the perimeter expanded by a factor of 2.2 to 13.5 µm, whereas the mean fluorescence signal decreased by a factor of 2.5 and 2.6 for the F-actin and PM signals, respectively (bottom). The measurements of PM and F-actin fluorescent intensities around the cell perimeter (for \( n = 15 \) cells from four independent experiments) were used for distribution analysis (see text). (C) Approximate amount of membrane compaction from TEM images. The green line indicates typical regions of the cell periphery without folding, the light brown line indicates typical regions of the cell periphery where folds store up to three times more membrane-cortex than unfolded regions, the medium brown line indicates typical regions of the cell periphery that store from three to five times more membrane-cortex than unfolded regions, and the dark brown line indicates typical regions of the cell periphery that store more than five times more membrane-cortex than unfolded regions. (D) Measuring a contour length that includes folds (red, total length 13,319 px) compared with one that does not (green, 2,601 px), which results in a membrane-cortex compaction ratio of 5.12. In total, the contour of 12 TEM images were measured.
Video 1. **Coordinated F-actin and myosin dynamics during cell periodic protrusions.** Time-lapse imaging data demonstrates cytoskeletal dynamics during oscillation. Merged fluorescent image of F-actin (Lifeact-GFP, green) and myosin (MyosinRLC-RFP, red). Note the striking repeatability of the F-actin and myosin distributions in each period. Video was recorded on a Nipkow-type spinning disk confocal scan head with a 60x, 1.40 NA objective and a 1,024 × 1,024 camera. Images were acquired at 1-s intervals and play back at 10 fps.

Video 2. **Video demonstrates the coordination between the PM and F-actin dynamics during periodic cell protrusions.** Time-lapse laser scanning confocal microscope imaging data of dual fluorescent signals from F-actin (Lifeact-GFP, green) and membrane (PMT-mRFP) of an oscillating cell. Video was recorded on a FluoView FV1000MPE with a LUMPLEF 60x W\IR NA:0.90 at 14-s intervals using the sequential line scanning mode to ensure the separation of GFP and RFP signals. The initial imaging data contained 1,600 × 1,600 pixels and was resized for the demonstration. Video plays back at 3 fps.

Video 3. **Visualization of cell cortex dynamics using fast acquisition.** Time-lapse record of the F-actin fluorescent signal from a periodically protruding cell recorded on an Andor spinning disk confocal microscope, a 60x objective, with images acquired every 100 ms. Video plays back at 50 fps.

Video 4. **Cell oscillations result from detachment from substrate and subsequent rounding.** Time-lapse recording of merged DIC and F-actin (Lifeact-GFP, green) fluorescent signals of cells that were plated on a glass bottom dish 1 d before experiment. During recording the media was replaced with trypsin; subsequently cells quickly detached and rounded. Immediately after this, most of the cells developed oscillatory behavior. After ~90 s trypsin was replaced with media to prevent complete cell detachment. Video was recorded on a FluoView FV1000 with a PLAPON 60× O NA:1.42 at 1.1-s intervals and plays back at 10 fps.

Video 5. **Video demonstrates the coordinated folding and unfolding of the PM- and F-actin-containing cortex.** (cell 1) Time-lapse laser scanning confocal microscope imaging data of dual fluorescent signals from F-actin (Lifeact-GFP, green) and membrane (PMT-mRFP, red) of the edge of an oscillating cell. The images at t = 7 s and 119 s show thick folded membrane-cortex couple, whereas the image at t = 77 s shows the extended membrane-cortex couple with no folds. Video was recorded on an FV1000 with an objective UPLSAPO oil 100×/NA:1.40, at 7-s intervals. (cell 2) Time-lapse of merged DIC and laser scanning confocal microscope imaging data of fluorescent signals from F-actin (Lifeact-GFP, green) on the edge of an oscillating cell. Video was recorded at 3-s intervals on a FluoView FV1000 with a PLAPON 60× O NA:1.42. (cell 3) Time-lapse laser scanning confocal microscope imaging data of fluorescent signals from F-actin (Lifeact-GFP, green) on the edge of an oscillating cell. The images show unfolding during extension. Video was recorded on an FV1000 with objective UPLSAPO oil 100×/NA:1.40, at 6-s intervals. Videos play back at 5 fps.

Video 6. **Traveling wave on the periphery of an oscillating cell.** The first part of the video presents time lapse of merged DIC and laser scanning confocal microscope imaging data of fluorescent signals from PM (red) and F-actin (Lifeact-GFP, green) showing the traveling wave on the cell periphery. The second part of the video showing only fluorescent signals demonstrates the development of large accordion-like folds on the periphery of the same cell. Video plays forward and then the same frames are played backward to allow better visual perception. Video was recorded in sequential mode on a FluoView FV1000MPE with a LUMPLEF 60x W\IR NA:0.90 at 7-s intervals and plays back at 5 fps.
Video 7. **The cortical actin traveling viewed in an XY plane and in a 3D reconstruction.** Time lapse of a 3D reconstruction of cell cortex during oscillations (left) and corresponding confocal image from F-actin (Lifeact-GFP, green) at the position Z = 12 µm from the substrate (right). On both images the propagation of a density traveling wave in a clockwise direction is clearly visible. Time lapse of Z stack imaging data from fluorescent signal arising from F-actin in an oscillating cell was recorded on a Cell Observer Spinning Disk with 63× objective and reconstructed using Axiovision software. One Z stack contains 32 planes at 1-µm intervals along the Z axis. Note the cell relocation; the red outline indicates the initial position on the last time step. Images inside the Z stack were recorded with 0.2-s intervals (7 s is the time for whole Z stack recording) and video plays back at 5 fps.

Video 8. **Video shows a 3D reconstruction of the cell cortex and F-actin traveling wave viewed in a YZ orthogonal projection.** Simultaneous time lapse of 3D reconstruction of the cell cortex during the periodic protrusive phenotype (middle), corresponding microscope confocal image (XY plane) from fluorescent F-actin (Lifeact-GFP, green) at the position Z = 8 µm from the substrate (left), and reconstructed image of YZ orthogonal projection (right). On the XY confocal images the propagation of density traveling wave is not visible, whereas on the YZ orthogonal view the wave is seen to travel in a counterclockwise direction. Time lapse of Z stack imaging data of the fluorescent signal from F-actin of an oscillating cell was recorded on a Cell Observer Spinning Disk with a 63× objective and reconstructed using Axiovision software. One Z stack contains 80 planes at 0.5-µm intervals along the Z axis. Images inside the Z stack were recorded with 0.17-s interval (14 s is the duration for whole Z stack recording) and video plays back at 5 fps.

Video 9. **The reversible loss of oscillatory behavior as a result of myosin inhibition.** Video presents time lapse of merged DIC and laser scanning confocal microscope imaging data of fluorescent signals from F-actin (Lifeact-RFP, red) showing the oscillating cells before and after 5-µM blebbistatin application. Myosin inhibition gradually stopped oscillations (~180 s). After oscillations disappeared, an intense 1-s flash of blue light led to the inhibition of blebbistatin and rescue of the oscillatory phenotype within 30 s. All four cells shown on the video were on the same microscope dish during the experiment. Video was recorded on a FluoView FV1000 with a 60× O NA:1.35 at 2-s intervals and plays back at 20 fps.

Video 10. **Periodic protrusions as a mechanism for translocation.** Video presents two separate records of DIC images of randomly migrating MDA-MB and NIH 3T3 cells plated on glass with depolymerized microtubules. Two records were combined into one video for presentation purposes. Time-lapse images were recorded every 10 s on a VivaView Incubator fluorescence microscope with a 10x objective and video plays back at 10 fps.