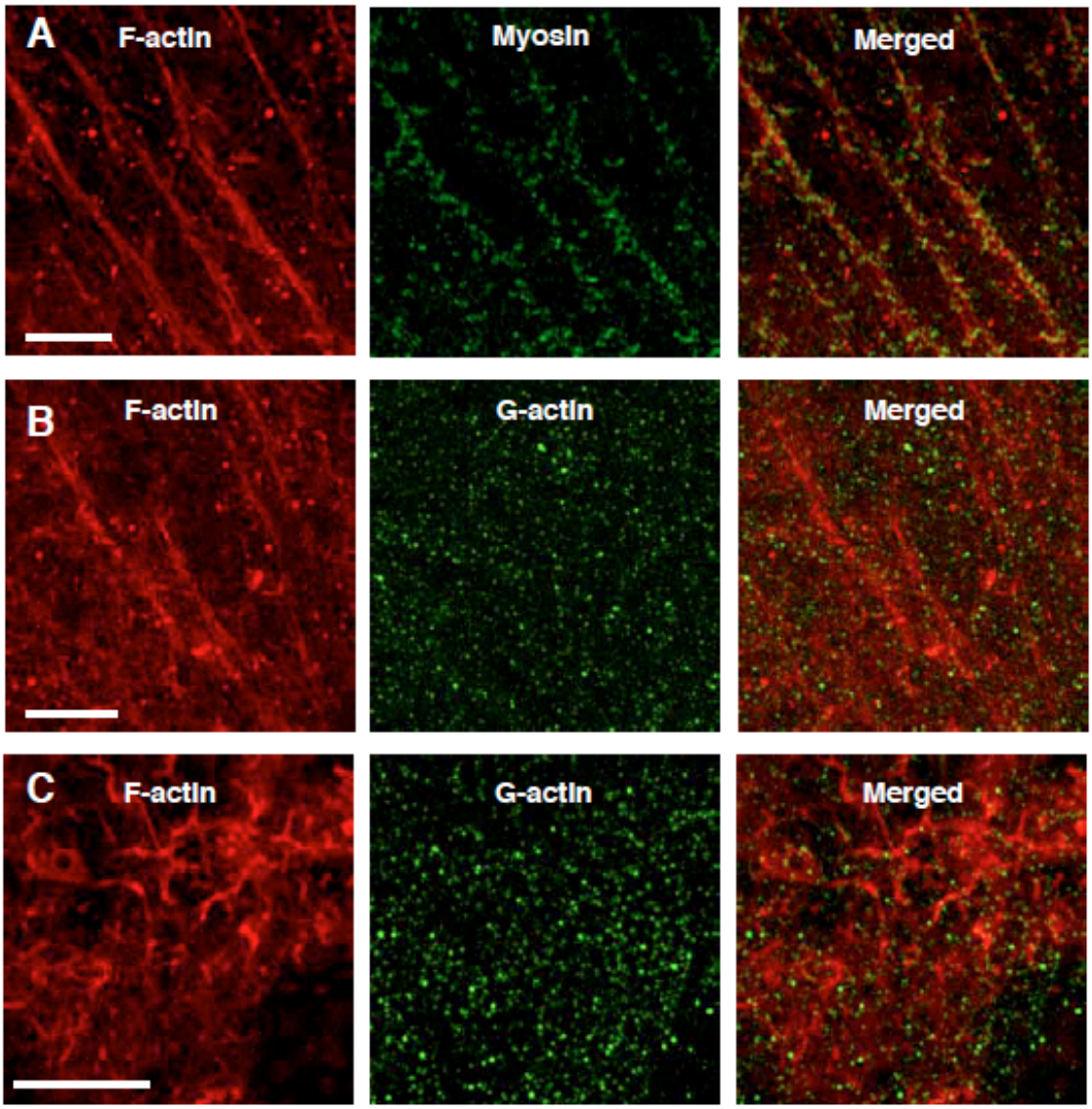

Fig. S1: Verification of anti-G-actin antibody.

A: Fixed osteoclast-like cells were stained with rhodamine-phalloidin (red) and non-muscle myosin IIA antibody (green) for N-SIM. Stress fibers were decorated with many possible dimmers of non-muscle myosin IIA. **B:** Fixed osteoclast-like cells were stained with rhodamine-phalloidin (red) and anti beta-actin antibody (green) for N-SIM. The anti-beta-actin antibody did not stain the stress fibers. **C:** Fixed osteoclast-like cells were stained with rhodamine-phalloidin (red) and anti beta-actin antibody (green) for N-SIM. The anti beta-actin antibody did not stain the microplicae. Bars, 5 μm.

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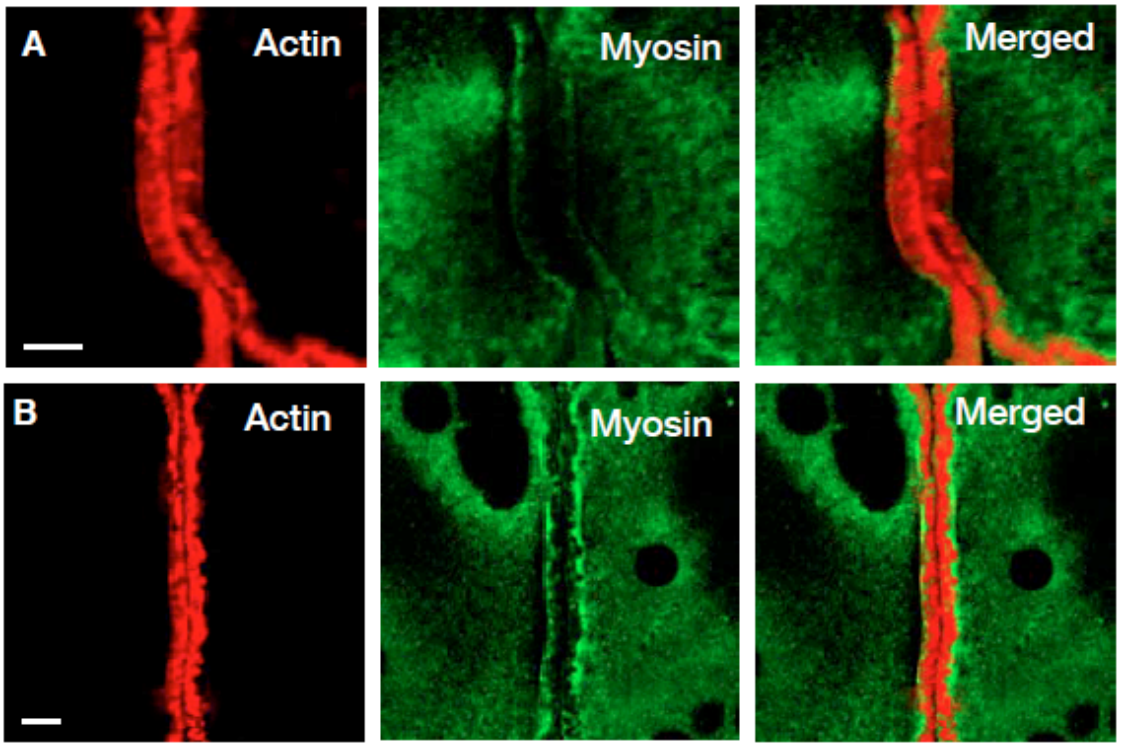

Fig. S2: Effect of ML-7 on the ZLS.

Osteoclastogenesis was performed in the presence and absence of 3 μM ML-7. The fixed cells were stained with anti-nonmuscle myosin IIA (green) and rhodamine-phalloidin (red). **A:** Confocal image of the ZLS. Control. **B:** Confocal image of the ZLS treated with 3 μM ML-7. Bars, 10 μm.

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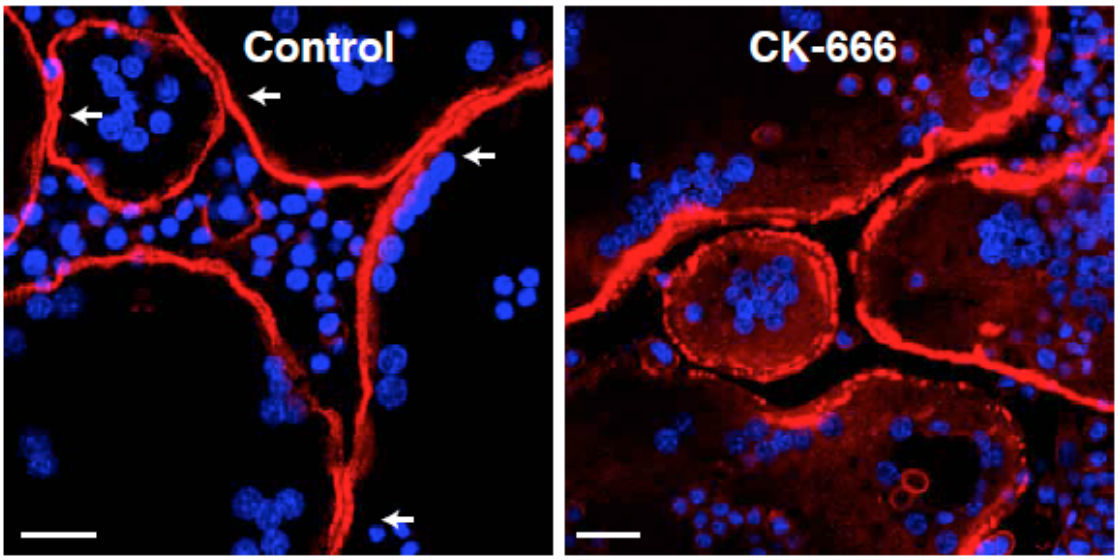

Fig. S3: Effect of CK-666 on the cell-cell interaction of multinucleated cells. OCLs were incubated with 100 μM CK-666 for 40 min. The cells were fixed and stained with rhodamine-phalloidin and DAPI for confocal microscopy. Arrows indicate the ZLS. Note the disruption of the ZLS and the detachment of two osteoclasts in the presence of 100 μM CK-666. F-actin shown in red and the nucleus in blue. Bars, 20 μm.

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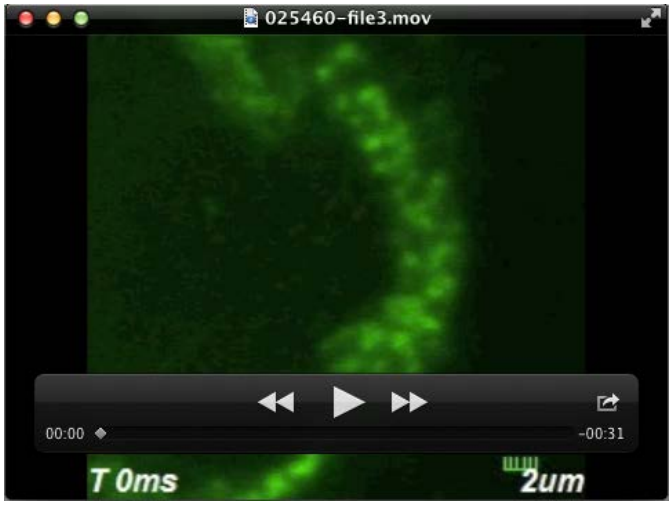
Movies

Movie 1

Actin dynamics in the ZLS. RAW 264.7 cells transfected with EGFP-actin. EGFP fluorescence was acquired every 4 sec by an Olympus FV10i. Corresponds to Fig. 1A.



Movie 2

Actin dynamics in the podosome belt. RAW 264.7 cells transfected with EGFP-actin. EGFP fluorescence was acquired every 4 sec by an Olympus FV10i. Corresponds to Fig. 1B.

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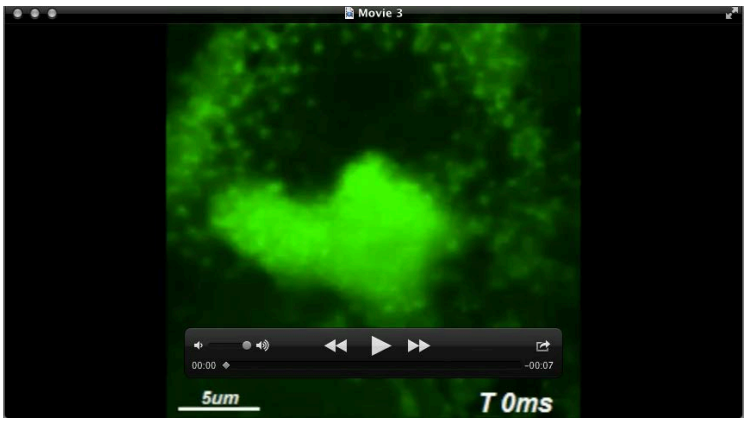
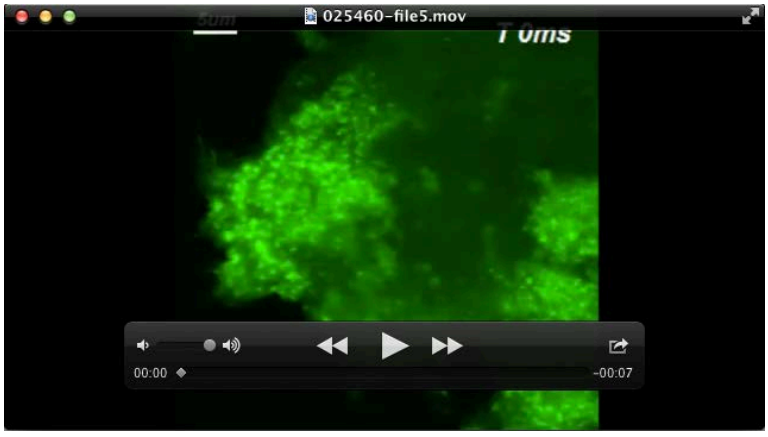


Movie 3

Actin dynamics in the podosome ring. RAW 264.7 cells transfected with EGFP-actin. EGFP fluorescence was acquired every 4 sec by an Olympus FV10i. Corresponds to Fig. 1C.



Movie 4

Actin movement in the podosome cluster. The actin clouds spontaneously moved in the podosome cluster. RAW 264.7 cells transfected with EGFP-actin. EGFP fluorescence was acquired every 4 sec by an Olympus FV10i. Corresponds to Figure 1D.

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Table S1

Dimensions of the podosomal proteins in the zipper-like structure

Component protein	Width (μm)	N
F-actin	3.52 ± 1.22	65
Non-muscle myosin IIA	1.18 ± 0.41	45
Cortactin	3.31 ± 0.96	17
Vinculin	1.55 ± 0.87	25
Paxillin	1.62 ± 0.73	17
Zxyin	0.66 ± 0.43	11

The widths of the podosomal component proteins in the half of the zipper-like structure were determined from the projected confocal images. Details are described in Materials and Methods. Width is expressed in the average ± SD. N indicates the number of osteoclasts measured. Corresponds to Figure 4E.

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