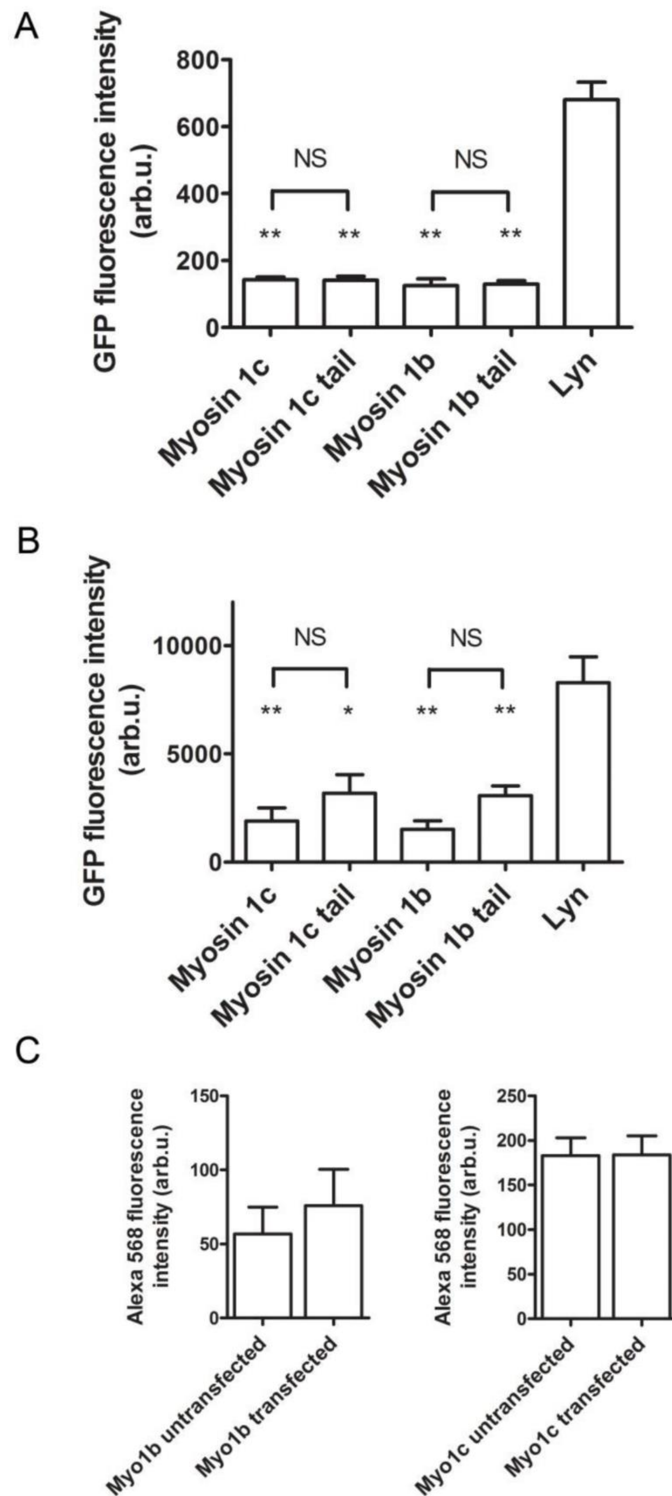

Supplementary figure 1: Expression of myosin 1b and 1c constructs

A) The expression of different constructs was compared by measuring the fluorescence intensity of cells transfected with either GFP-coupled myosin 1 constructs or lyn-GFP using

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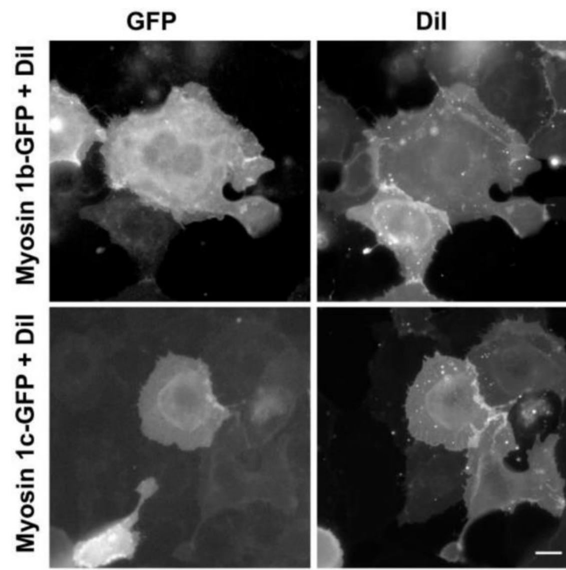
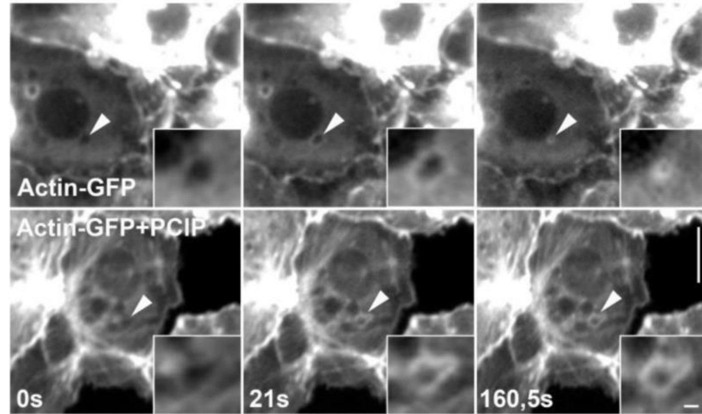
identical light intensity and exposure time settings on a fluorescence microscope and drawing a region of interest around the whole cell and measuring the mean fluorescence. The measurements were made on single transfected cells in 5 experiments from 5 cell isolations. Up to 82 cells were analyzed for every construct in each experiment. The fluorescence intensity of the control construct lyn GFP was significantly higher than the fluorescence intensity of myosin 1 constructs. The fluorescence intensities of myosin 1c or 1b wt and tail constructs were not significantly different. NS = not significant, **p<0.01, two tailed Mann-Whitney test.

B) he fluorescence intensity of cells transfected with either GFP-coupled myosin 1 constructs or lyn-GFP was measured with plate reader assay (Infinite M200 plate reader, Tecan, Crailsheim, Germany). The cells were seeded in 96 well plate (Greiner, Frickenhausen, Germany). Before experiment cells were covered with bath solution containing brilliant black BN (MP Biochemicals Inc., Illkirch, France; 5 mg/ml) and the fluorescence intensity was measured in fluorescence bottom reading mode using the settings suggested for GFP detection by the manufacturer (483 nm excitation and 535 nm emission wavelengths, 30 μs integration time) and 4 reads per well. The fluorescence intensity was measured in 5 experiments from 5 cell isolations with 3 technical replicates per experiment. The fluorescence intensity of the control construct lyn GFP was significantly higher than the fluorescence intensity of myosin 1 constructs. The fluorescence intensities of myosin 1c or 1b wt and tail constructs were not significantly different. NS = not significant, *p<0.01, **p<0.01 two tailed Mann-Whitney test.

C) TII cells were transfected with myo1c GFP or myo1b GFP constructs and subsequently immunostained with anti myo1c or anti myo1b antibody, respectively. The primary antibodies were labelled with Alexa Fluo 568 fluorescently coupled anti-rabbit secondary antibody and the fluorescence intensity of Alexa 568 was compared in cells transfected with the myosin constructs and in non-transfected cells. The data was obtained from 5 experiments from 5 cell isolations, up to 294 cells were analysed for each bar.



Supplementary figure 2: Plasma membrane localization of myosin 1b and myosin 1c. ATII cells were transfected with myo1b-GFP or myo1c-GFP and stained with plasma membrane-staining dye DiI by incubating the cells for 10 min at 1µM concentration. Both myosin 1 expression constructs localized to the plasma membrane.



Supplementary figure 3: Actin coating after PClP treatment

ATII cells were transfected with actin-GFP or transfected with actin-GFP and treated with 10 µM PClP myosin 1 inhibitor. Although actin coats formed on the vesicles in cells treated with PClP, their compression was inhibited. Inserts show enlarged view of the fused vesicle. Scale bars: 10 µm and 1 µm on the image and insert, respectively.