

E08-01-0034 Cramer

Supplementary Figure 1. Cells expressing GFP-myosin II light chain co-localize with endogenous myosin II.

Live cells infected with adenovirus expressing GFP-myosin II light chain were fixed and counter-stained with an antibody to myosin II heavy chains by indirect immunofluorescence. Cells expressing GFP-myosin II were identified and co-colour images acquired and the staining patterns compared. (a, a') Counter-stain with anti-myosin II antibody. (b, b') GFP-myosin II light chain expression. (c, c') Merged images (magenta represents endogenous myosin II and green is GFP-myosin II light chain). (a', b', c') enlarged view (boxed area) of (a-c) respectively. Scale bar in (a) = 10um (a-c), and in (a') = 2um (a'-c').

Supplementary movie 1 (fig. 2a-f): Photo-bleached zone of F-actin flows retrograde through the lamellipodium then stops within the lamella in a migrating fibroblast expressing GFP-actin. 1 sec interval between frames, 177 sec duration (1-3 sec is pre-bleach and 4-177 sec is post-bleach: 4 sec total time of movie is 1 sec post-bleach in fig. 2b, f; and 143 sec total movie time is 140 sec post-bleach in fig. 2e, f).

Supplementary movie 2 (fig. 2h-l): Photo-bleached zone of F-actin within a filopodium flows retrograde through the lamellipodium then is subsequently located within a GP bundle within the lamella in a migrating fibroblast expressing GFP-actin. 1 sec interval between frames, 76 sec duration (1-3 sec is pre-bleach and 4-76 sec is post-bleach: 4 sec total movie time is 1 sec post-bleach in fig. 2i and 76 sec total movie time is 73 sec post-bleach in fig. 2l).

Supplementary movie 3 (fig. 3b): Cell migration of the GFP-actin expressing cell shown in movie 4. 10 sec interval between frames, 20 min duration

Supplementary movie 4 (fig. 3c): Timelapse sequence of a filopodium within the leading cell edge seeding the formation of a GP bundle in the lamella in a cell expressing GFP-actin (whole cell shown in movie 3). 10 sec interval between frames, 100 sec duration.

Supplementary movie 5 (fig. 3g): Higher temporal resolution of a filopodium within the leading cell edge seeding the formation of a GP bundle in the lamella in a migrating cell expressing GFP-actin; at the end of the movie (18sec in fig.3g) the GP bundle spans the lamellipodium-lamella boundary. 1 sec interval between frames.

Supplementary movie 6 (fig. 4): GP bundles expressing GFP-actin were photo-bleached at the front of the lamella. Note sub-zones within several adjacent GP bundles are bleached; one individual GP bundle is exemplified in fig. 4a-g. 1 sec interval between frames, 81 sec duration (1-3 sec is pre-bleach and 4-81 sec is post-bleach: 4 sec total movie time is 1 sec post-bleach in fig. 4a, e and 81 sec total movie time is 78 sec post-bleach in fig. 4d, e).

