

SUPPLEMENTARY MOVIES

Movie 1 EGFP-actin recovery in B16-F1 cell lamellipodium as recorded by epi-illumination upon LSM-mediated photobleaching (see also Figure 1A). Time is as indicated; bar = 3 μ m.

Movie 2 Photoactivation of actin within the lamellipodium of B16-F1 cell (corresponds to Figure 1B). Cell coexpressing PA-GFP-actin (green) and mRFP-actin (red). Red rectangle (labelled “2”) marks activation region.

Movie 3 Rapid translocation of bleached actin to the cell front (corresponds to Figure 1C). B16-F1 cell expressing EGFP-actin was bleached in the lamella (as indicated by red rectangle), which rapidly translocates to the leading edge, and subsequently travels rearwards with the lamellipodium network.

Movie 4 Mathematical simulation of FRAP experiment shown in Movie 1 (corresponds to Figure 1D).

Movie 5 Movie illustrating fluorescence intensity measurements of lamellipodial regions (marked by front and back) used for treadmilling analysis. The field marked with “b” was used for measuring background intensity.

Movie 6 Representative FRAP experiment on two scan-headed confocal microscope of B16-F1 cell expressing EGFP-actin.

Movie 7 FRAP experiment of B16-F1 cell expressing EGFP-ArpC5B (corresponds to Figure 2A).

Movie 8 FRAP experiment of B16-F1 cell expressing EGFP-tagged Abi-1 (corresponds to Figure 2E).

Movie 9 FRAP experiment of B16-F1 cell expressing EGFP-tagged WAVE2, and treated with aluminium fluoride (AlF) (corresponds to Figure 2G).

Movie 10 FRAP experiment of B16-F1 cell expressing EGFP-VASP (see also Supplementary Figure 3).

Movie 11 FRAP experiment of B16-F1 cell expressing EGFP-tagged cortactin construct 1 (van Rossum et al., 2003) (corresponds to Figure 3A).

Movie 12 FRAP experiment of B16-F1 cell expressing cortactin construct 2, cortactin-EGFP-N1 (Kaksonen et al., 2000) (corresponds to Supplementary Figure 4A).

Movie 13 FRAP experiment of B16-F1 cell expressing EGFP-tagged cortactin 3 (Zhu et al., 2007) (corresponds to Supplementary Figure 4C).

Movie 14 FRAP experiment of B16-F1 cell expressing EGFP-tagged capping protein beta2 (corresponds to Figure 3C).

Movie 15 Latrunculin B treatment abolishes capping protein localization at the lamellipodium front. Latrunculin B (LatB, final concentration: 5 μ M) was added to B16-F1 cell expressing EGFP-tagged CP-beta2 (CP) at the time point indicated (top right, corresponds to Supplementary Figure 6).

Movie 16 FRAP experiment of B16-F1 cell expressing EGFP-tagged cofilin WT (wild-type) (corresponds to Figure 4A).

Movie 17 FRAP experiment of B16-F1 cell expressing EGFP-tagged, constitutively active cofilin mutant (S3A).

Movie 18 FRAP experiment of B16-F1 cell transfected with EGFP-tagged, inactive cofilin mutant (S3D).

Movie 19 FRAP experiment of MTLn3 rat carcinoma cell expressing EGFP-tagged actin and stimulated with 5nM EGF (corresponds to Figure 5A). Note the exclusive recovery of fluorescent actin from the lamellipodium front.

Movie 20 FRAP experiment of MTLn3 rat carcinoma cell expressing EGFP-tagged cofilin wild type and stimulated with 5nM EGF (corresponds to Figure 5B). Note rapid cofilin recovery without treadmilling.