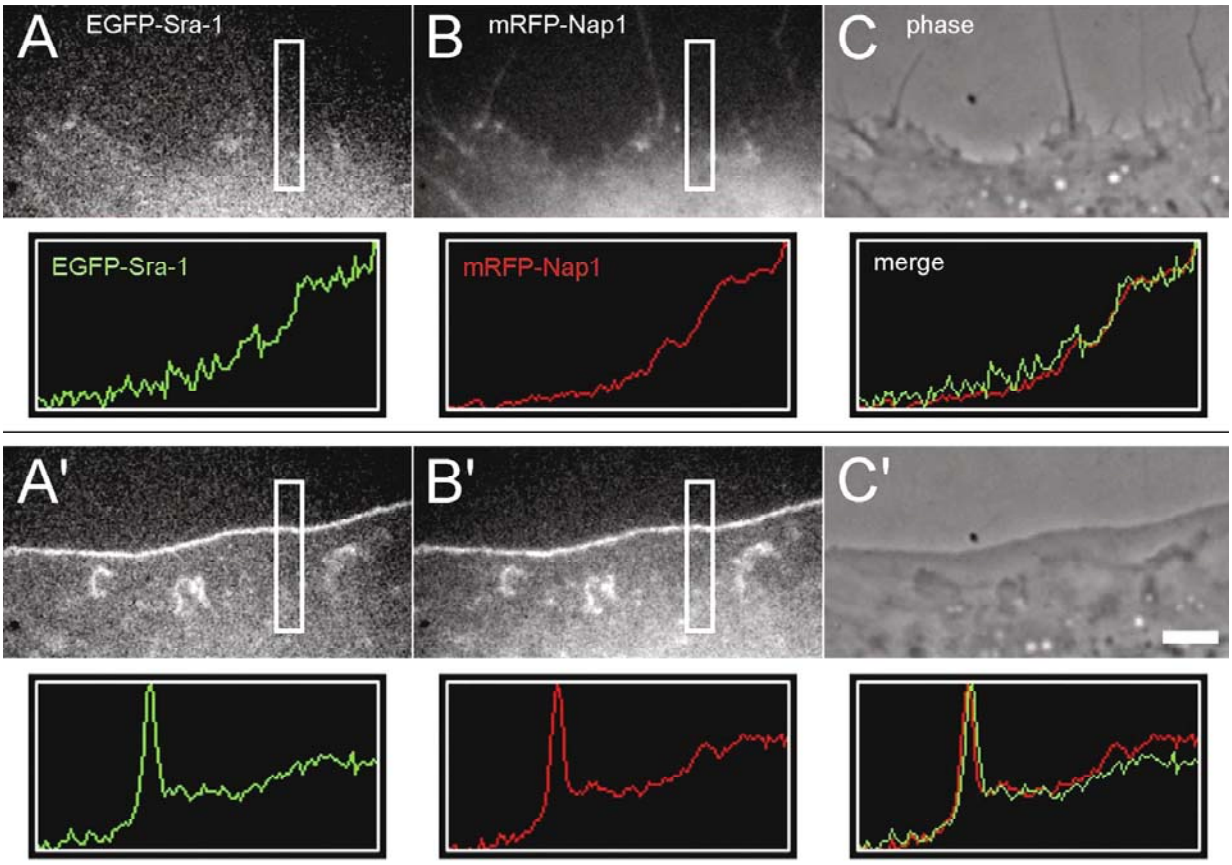

Figure S3 Co-translocation of Sra-1 and Nap1 upon injection of L61-Rac1.

Representative example of a NIH 3T3 fibroblast expressing both EGFP-tagged Sra-1 and mRFP-Nap1, which was analysed by two-colour fluorescence and phase contrast video microscopy during microinjection of constitutively active L61Rac1 as indicated. Panels shown represent video frames taken 1.5 minutes before (**A**-**C**) or 12 minutes after injection (**A'-C'**). Both Sra-1 (**A**) and Nap1 (**B**) were not enriched at the cell periphery before injection due to the absence of significant lamellipodia (**C**), but were dramatically co-translocated (**A'**, **B'**) upon induction of lamellipodia formation (**C'**) by injection of L61-Rac1. Line intensity scans obtained from regions as indicated by white rectangles (**A'**-**B'**) were generated as described in Supplementary Methods. The intensity scans for both EGFP-Sra-1 and mRFP-Nap1 upon Rac injection showed a sharp peak which corresponds to the lamellipodium tip.

Note that the slight shift of the mRFP-peak, which becomes apparent in the merged scan after Rac injection (bottom right panel) is due to consecutive imaging of EGFP-Sra-1 and mRFP-

Nap1, since – for technical reasons - image acquisition in the mRFP-channel was delayed by 6 sec as compared to the EGFP-channel leading to continuous leftward movement of the peaks due to continuous forward protrusion of the lamellipodium (see also Supplemental Movie 3).

The data demonstrate virtually identical degrees of Rac-triggered co-translocation of both proteins to the distal edge of the lamellipodium as compared to more proximal regions. The scale bar equals 5 µm.