Supplementary Information

Supplementary Figures and Legends

Figure S1

Macara IG

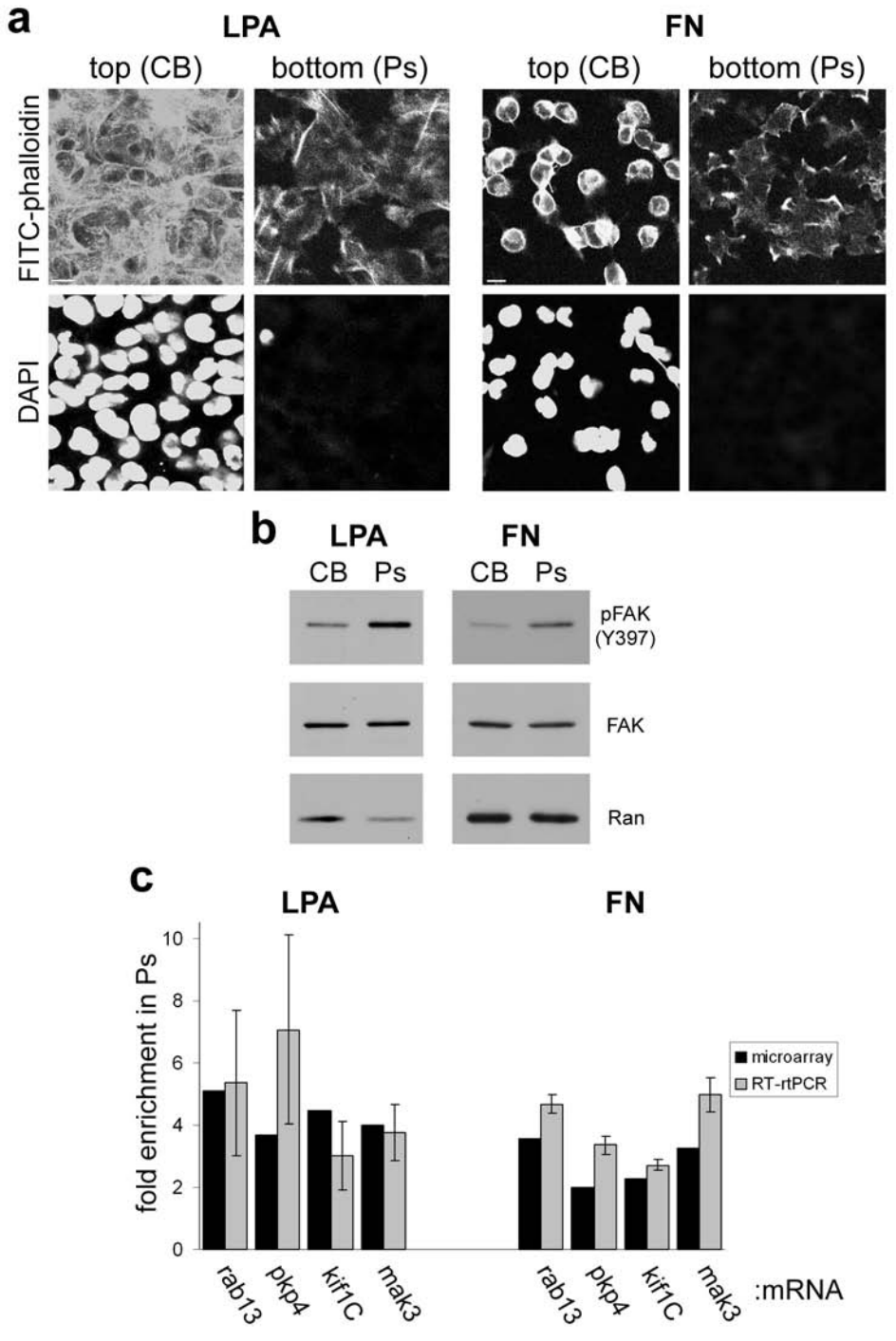

Figure S1 a, NIH/3T3 cells extending protrusions in response to LPA or fibronectin (FN), as described in Fig. 1a, were fixed and stained with FITC-phalloidin and DAPI. Confocal images of the top and bottom side of the filter are shown. Scale bar: 10μm. **b,** Cells were induced to extend pseudopodial protrusions in response to either LPA or FN. Ps and CB fractions were isolated and equal amounts of protein from each fraction were analyzed by Western blot to detect the indicated proteins. **c,** NIH/3T3 cells were induced to extend pseudopodial protrusions in response to either LPA or FN. Total RNA was isolated from Ps and CB fractions and the indicated mRNAs were detected by reverse transcription and real-time PCR (RT-rtPCR). Enrichment in the Ps fraction was calculated after normalization to the control GAPDH mRNA. Values represent averages from two independent experiments. Black bars represent fold enrichment values based on the microarray analyses, for comparison. Error bars: SEM.

Figure S2

Macara IG

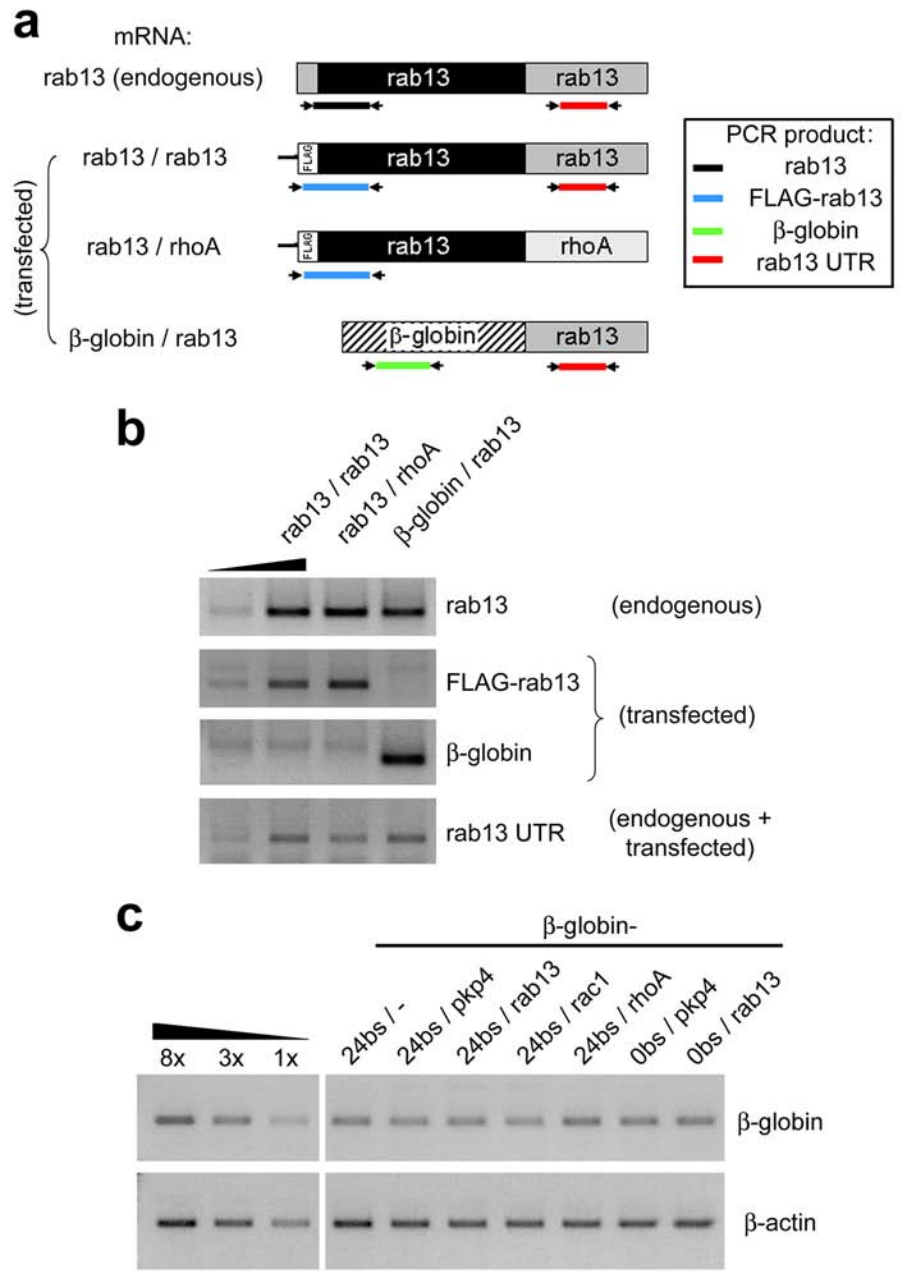

Figure S2 a, Schematic of the endogenous rab13 mRNA and of the mRNAs transcribed from constructs rab13/rab13, rab13/rhoA and β-globin/rab13. Depicted also are the positions of primer sets used in RT-PCR analysis as well as the PCR products which result from amplification of either the endogenous mRNA (rab13), the transfected RNAs (FLAG-rab13 and β-globin) or both the endogenous and the transfected RNAs carrying the rab13 3'UTR (rab13 UTR). **b,** Cells were transfected with constructs rab13/rab13, rab13/rhoA or β-globin/rab13 and analyzed by RT-PCR (as described in **a**) to detect the products indicated on the left. Increasing amounts of the first sample were amplified (lanes 1 and 2) to ensure linearity of the amplification. **c,** Cells were co-transfected with constructs encoding mRFP, MS2-GFP and β-globin mRNA carrying 24 MS2-binding sites and various 3'UTRs (see Fig. 2b, c). Total RNA was analyzed by RT-PCR to detect the expression levels of the transfected β-globin mRNA as well as of the endogenous β-actin mRNA, as a control. Various amounts of the first sample were amplified in parallel (lanes 1, 2 and 3) to ensure linearity of the amplification.

Figure S3
Macara IG

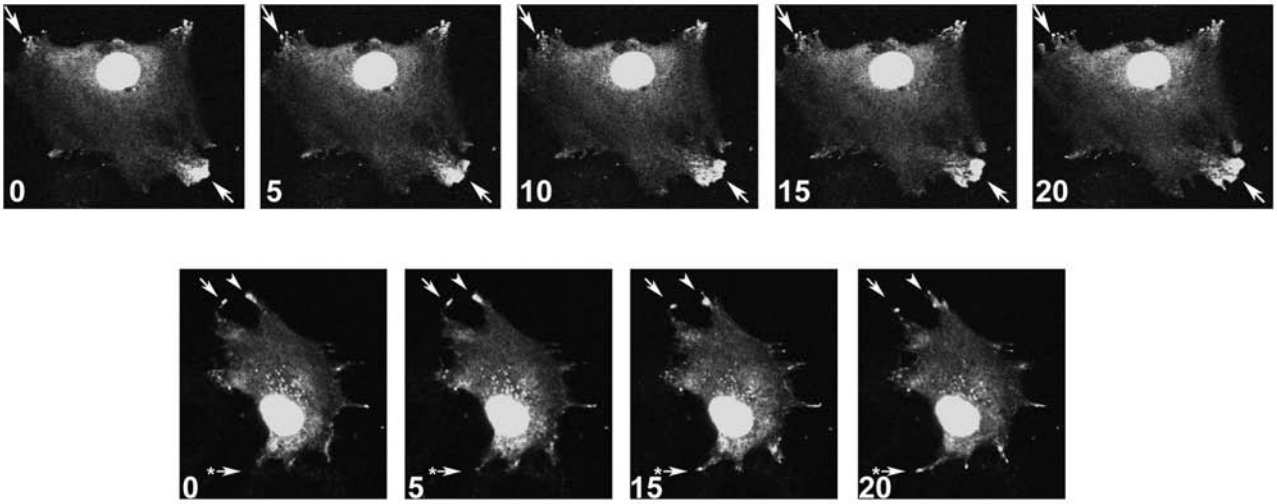

Figure S3 Cells transfected with constructs encoding MS2-GFP and the β-globin-24bs/pkp4 RNA were plated on a fibronectin-coated surface and GFP fluorescence was monitored over time. RNA granules at protrusions persisted (arrows), appeared (*) or disappeared (arrowheads) during the course of observation. Numbers indicate time in minutes. Scale bar: 10μm.

Figure S4
Macara IG

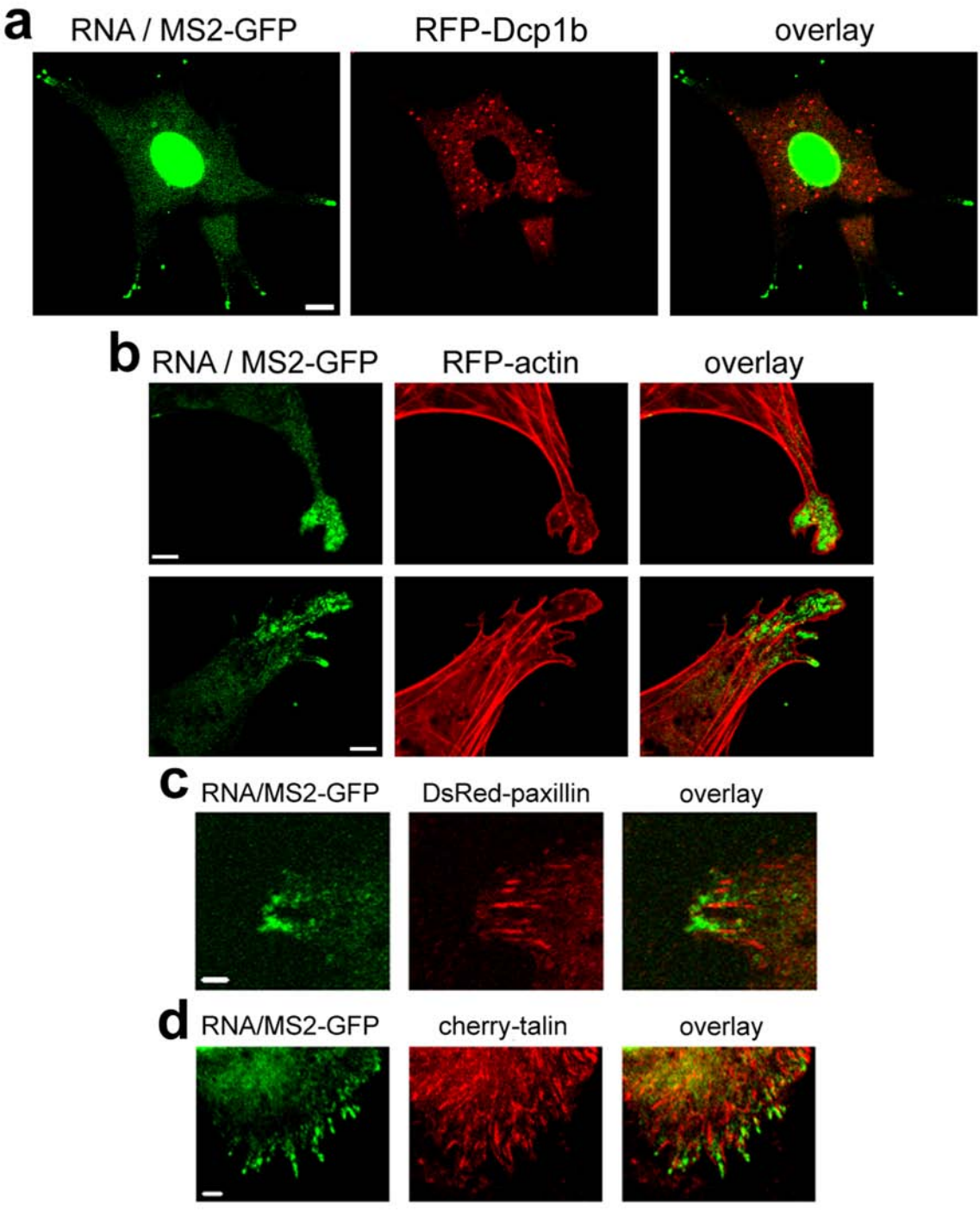

Figure S4 Confocal fluorescence images of cells expressing MS2-GFP, the β-globin-24bs/pkp4 RNA and either **a,** RFP-Dcp1b, **b,** RFP-actin, **c,** DsRed-paxillin or **d,** cherry-talin. Panels in **b-d** show edges of protrusive areas. Scale bar: 8μm (**a**), 4μm (**b**), 3μm (**c, d**).

Figure S5

Macara IG

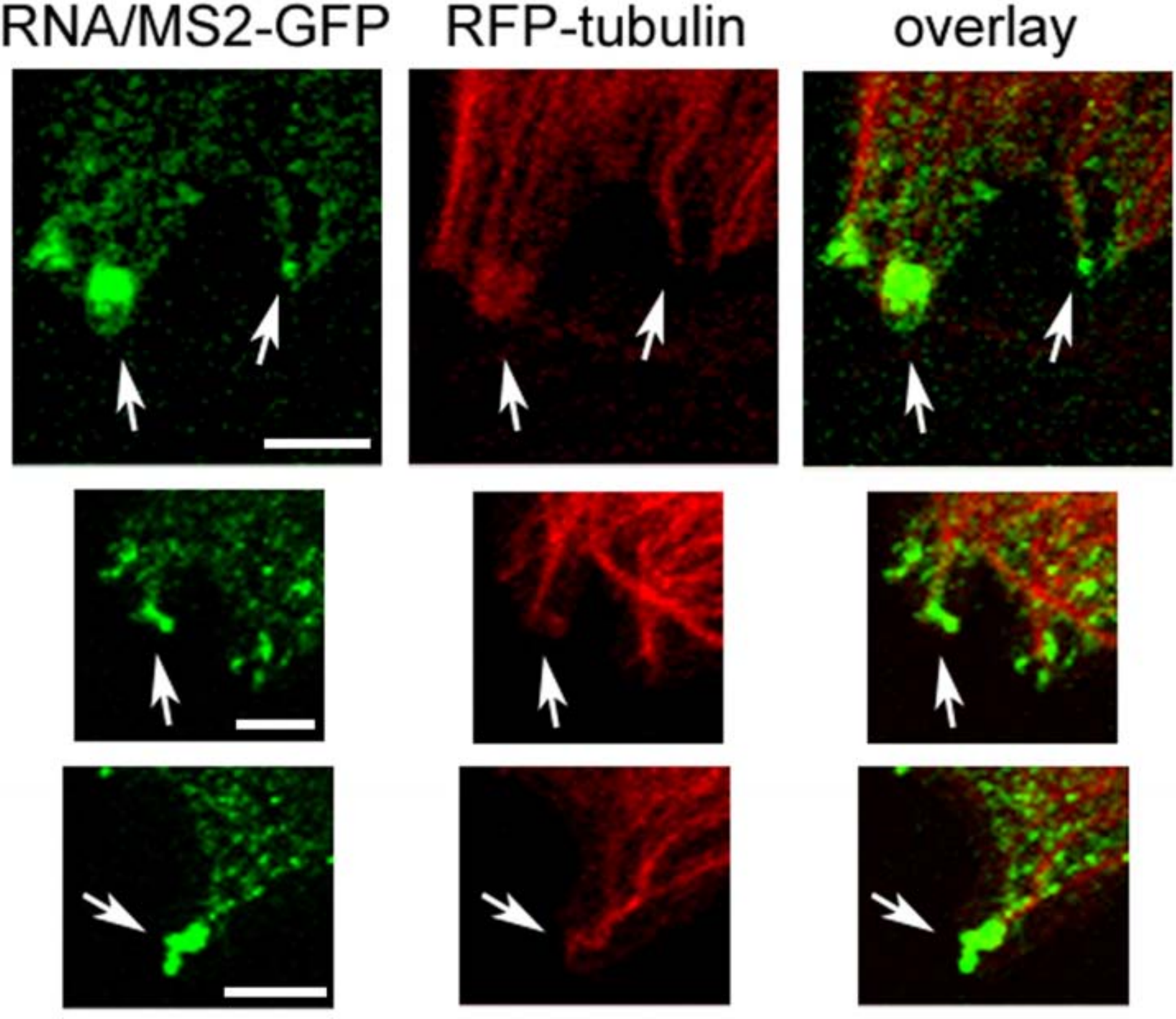

Figure S5 Confocal fluorescence images of cells expressing MS2-GFP, the β-globin-24bs/pkp4 RNA and RFP-tubulin. Panels show edges of protrusive areas. Scale bar: 4μm.

Figure S6

Macara IG

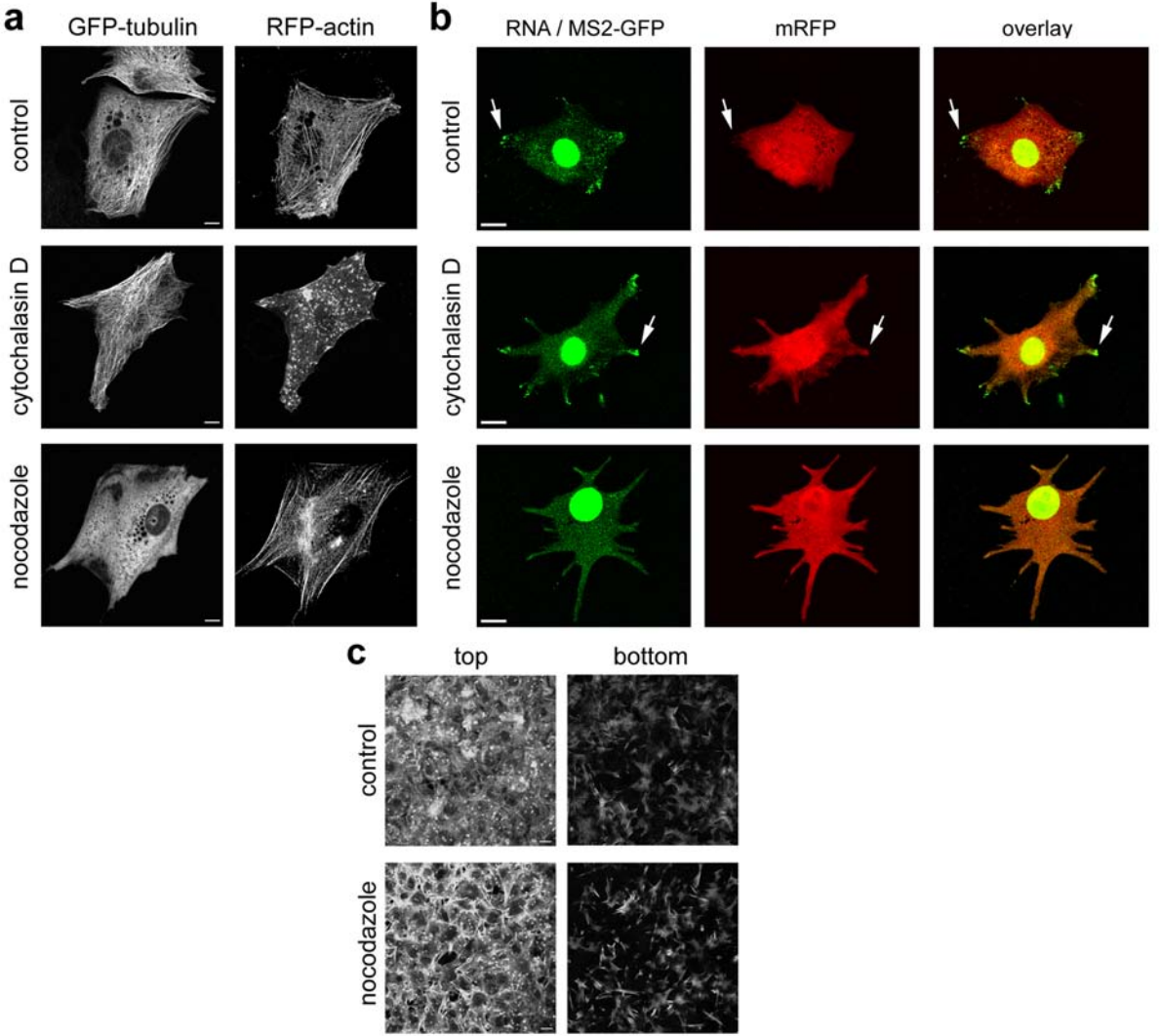

Figure S6 a, NIH/3T3 cells transfected with constructs encoding GFP-tubulin and RFP-actin were treated with DMSO (control), 1μM cytochalasin D or 10μM nocodazole. Live cells were imaged after 30 minutes of treatment. **b,** Cells were co-transfected with plasmids encoding mRFP, MS2-GFP and the β-globin-24bs/pkp4 RNA. Live cells were imaged during spreading on fibronectin after 30 min of exposure to 1μM cytochalasin D or 10μM nocodazole. Arrows indicate protrusions with accumulation of reporter RNA. Scale bar: 13 μm. **c,** Cells were plated on microporous filters and LPA was added in the bottom chamber for 70 min to induce pseudopodial extensions. During the last 25 minutes, the cells were exposed either to DMSO (control) or to 10μM nocodazole. Cells were fixed and stained with FITC-phalloidin. Confocal images of the top and bottom side of the filter are shown. Scale bar: 15μm.

Figure S7
Macara IG

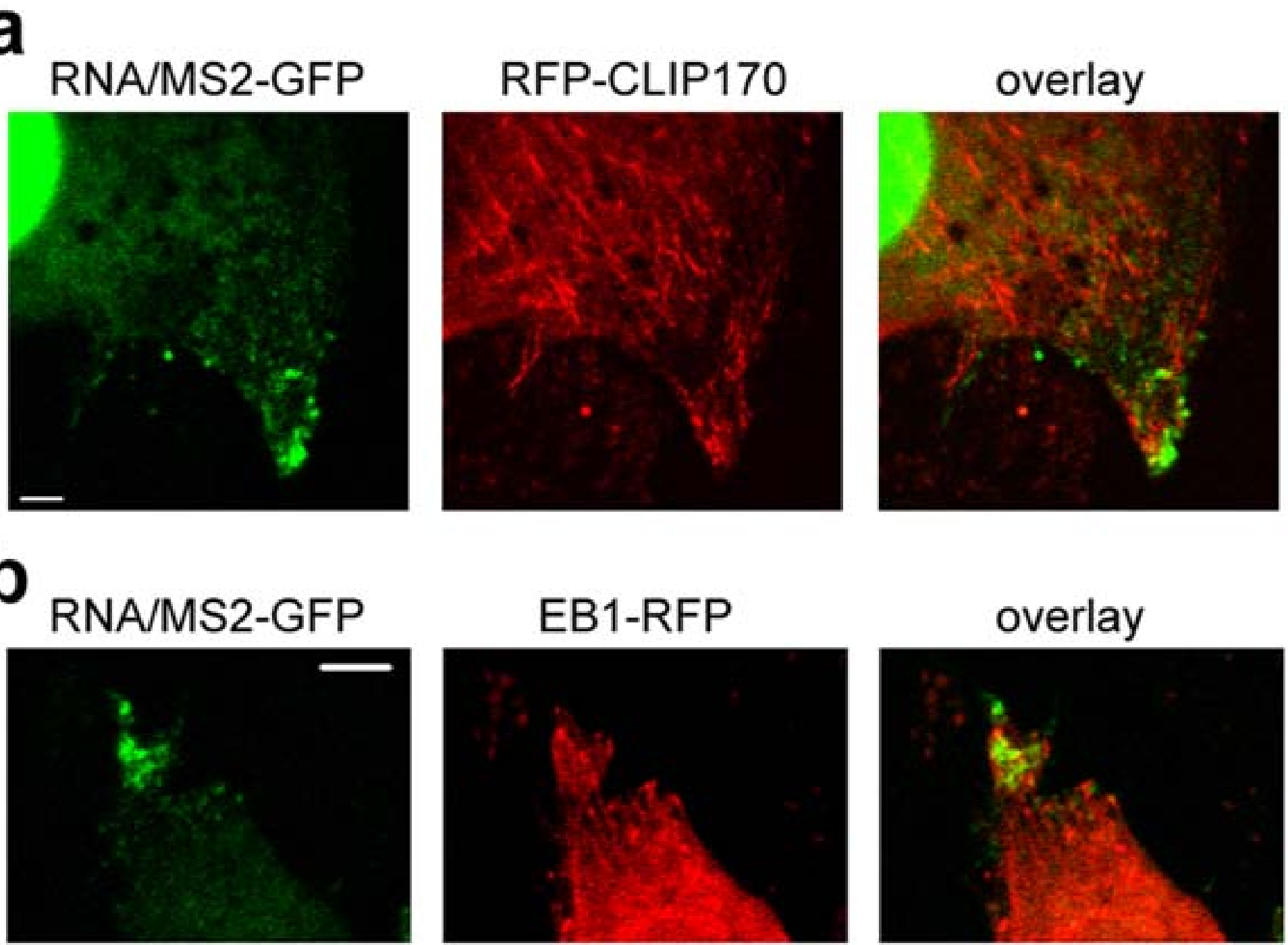

Figure S7 Confocal fluorescence images of cells expressing MS2-GFP, the β-globin-24bs/pkp4 RNA and either RFP-CLIP170 **(a)** or EB1-RFP **(b)**. Panels show edges of protrusive areas. Scale bar: 4μm.

Figure S8

Macara IG

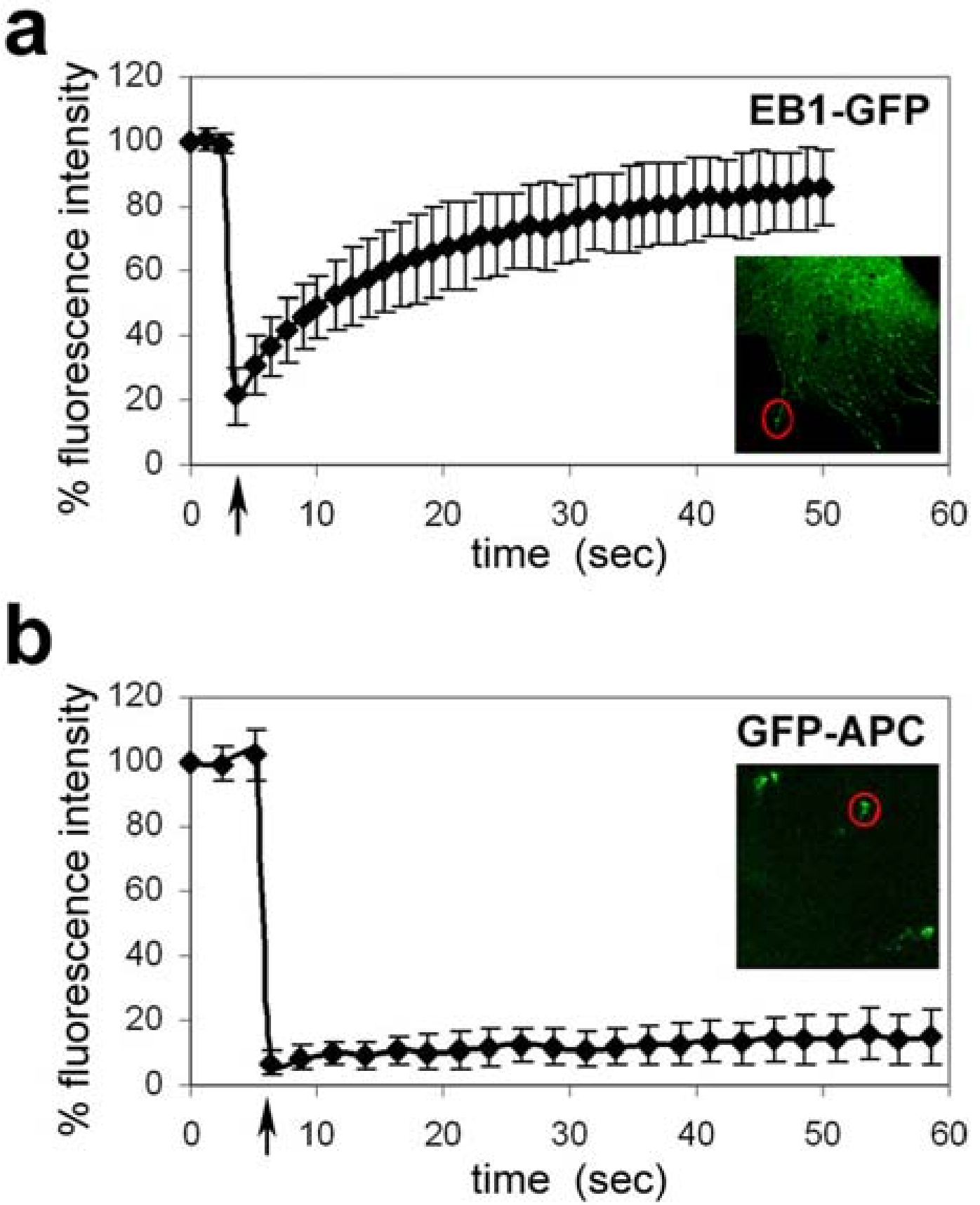

Figure S8 Fluorescence intensity was monitored before and after photobleaching of protrusive areas (red circles) of cells expressing EB1-GFP (**a**) or GFP-APC (**b**). Arrow indicates time of bleach. Curves represent average values of 10 independent experiments. Error bars: SD.

Figure S9

Macara IG

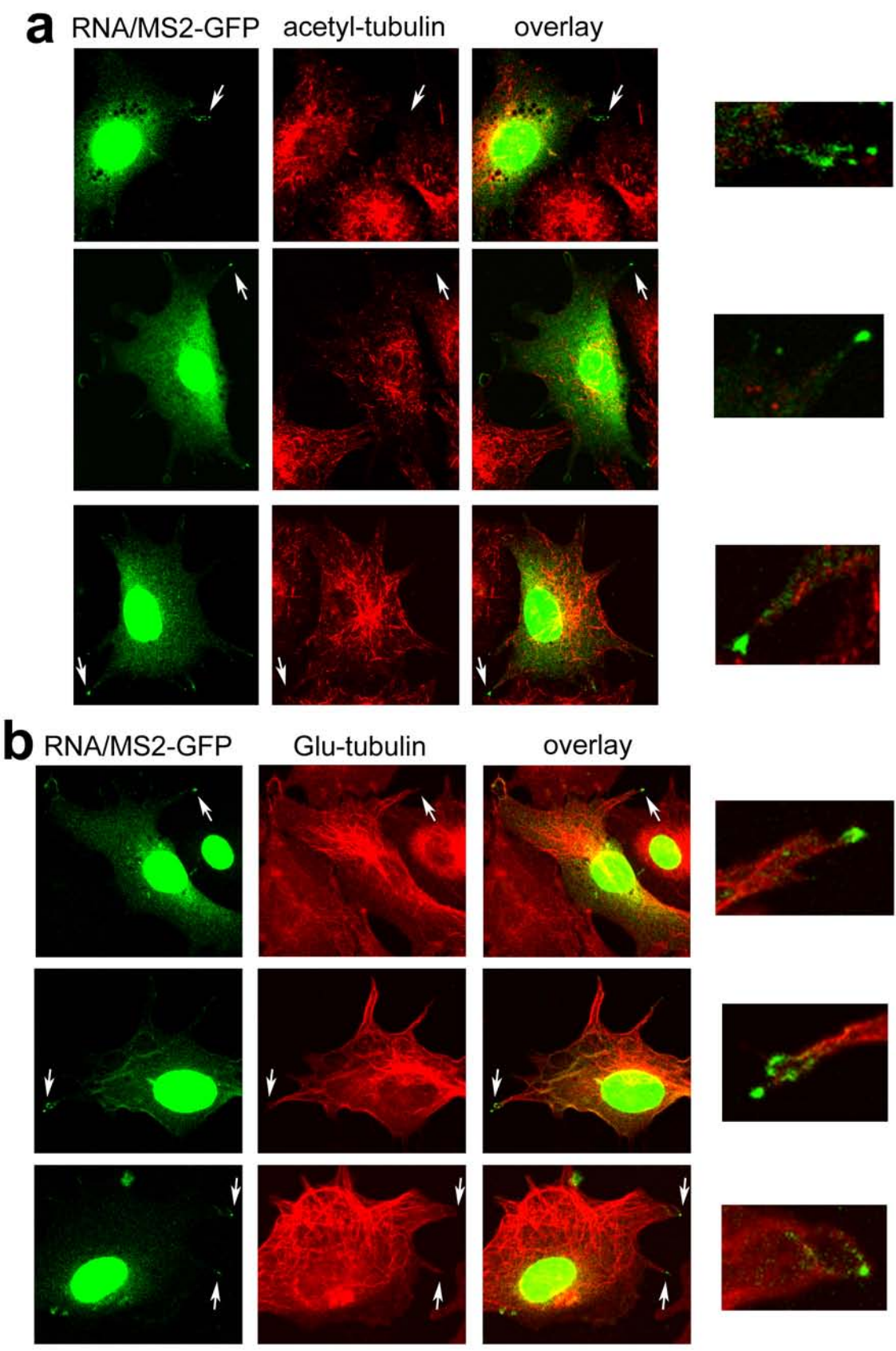

Figure S9 Cells expressing MS2-GFP and the β-globin-24bs/pkp4 RNA were fixed and stained with antibodies against acetylated-tubulin (**a**) or detyrosinated-tubulin (Glu-tubulin) (**b**). Areas indicated by arrows are shown in higher magnification to the right. (Note that after fixation RNA granules at tips of protrusions are not as pronounced as they are in live cells.)

Figure S10

Macara IG

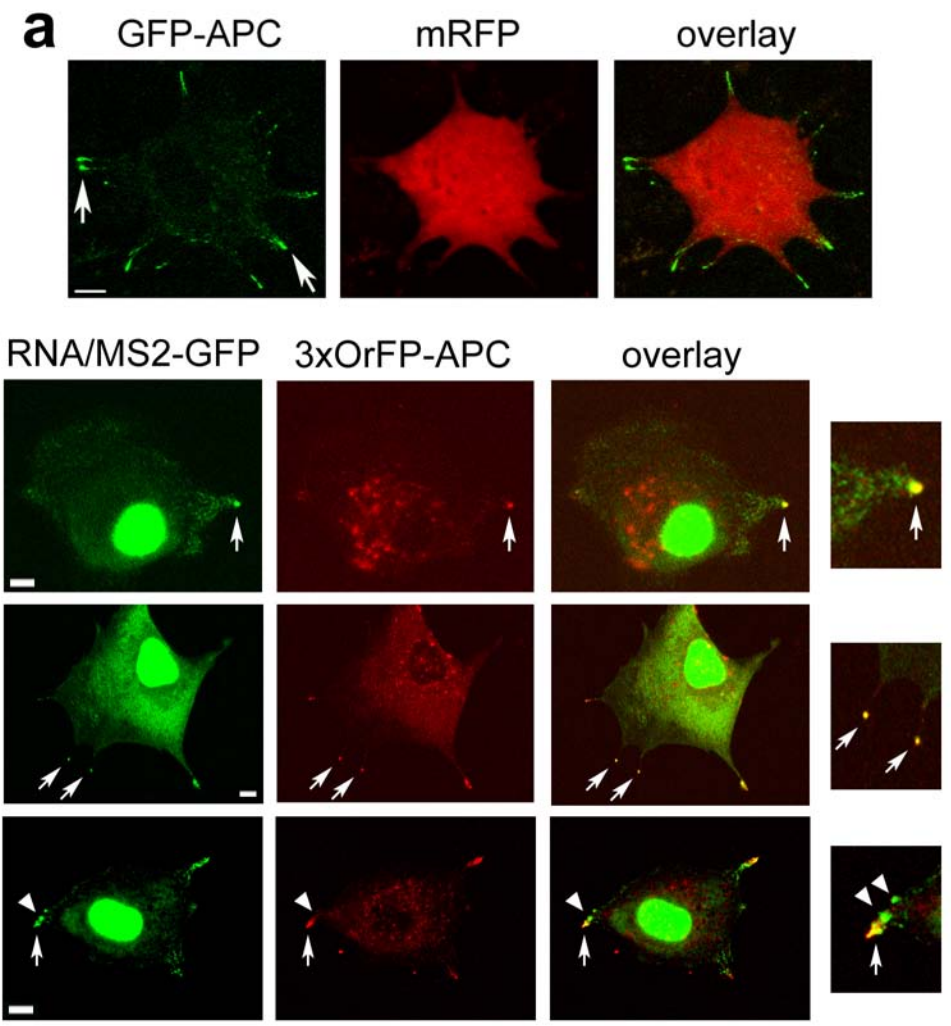

Figure S10 a, Confocal fluorescence images of cells expressing GFP-APC and mRFP. Scale bar: 9μm. Arrows point to tips of protrusions where GFP-APC accumulates. **b,** Confocal fluorescence images of cells expressing MS2-GFP, the β-globin-24bs/pkp4 RNA and 3xOrFP-APC. Scale bar: 5μm. Note that granular perinuclear fluorescence in the OrFP channel results from cellular autofluoresence. Arrows point to areas shown in higher magnification to the right. The upper two panels show whole cell images of cells presented in Fig 4a. Note that RNA granules co-localize with APC at the tips of protrusions. In some cells, however, we additionally observe RNA granules in more internal areas, which do not co-localize with APC (lower panels, arrowheads). When followed over time these granules can occasionally be seen to move and fuse with granules at the tip, but for the most part remain stationary. We cannot conclude at this point to what extent these internal granules represent transport intermediates or whether they become prominent in some cells because of saturation of binding sites at the tip due to over-expression of the reporter RNA.

Figure S11
Macara IG

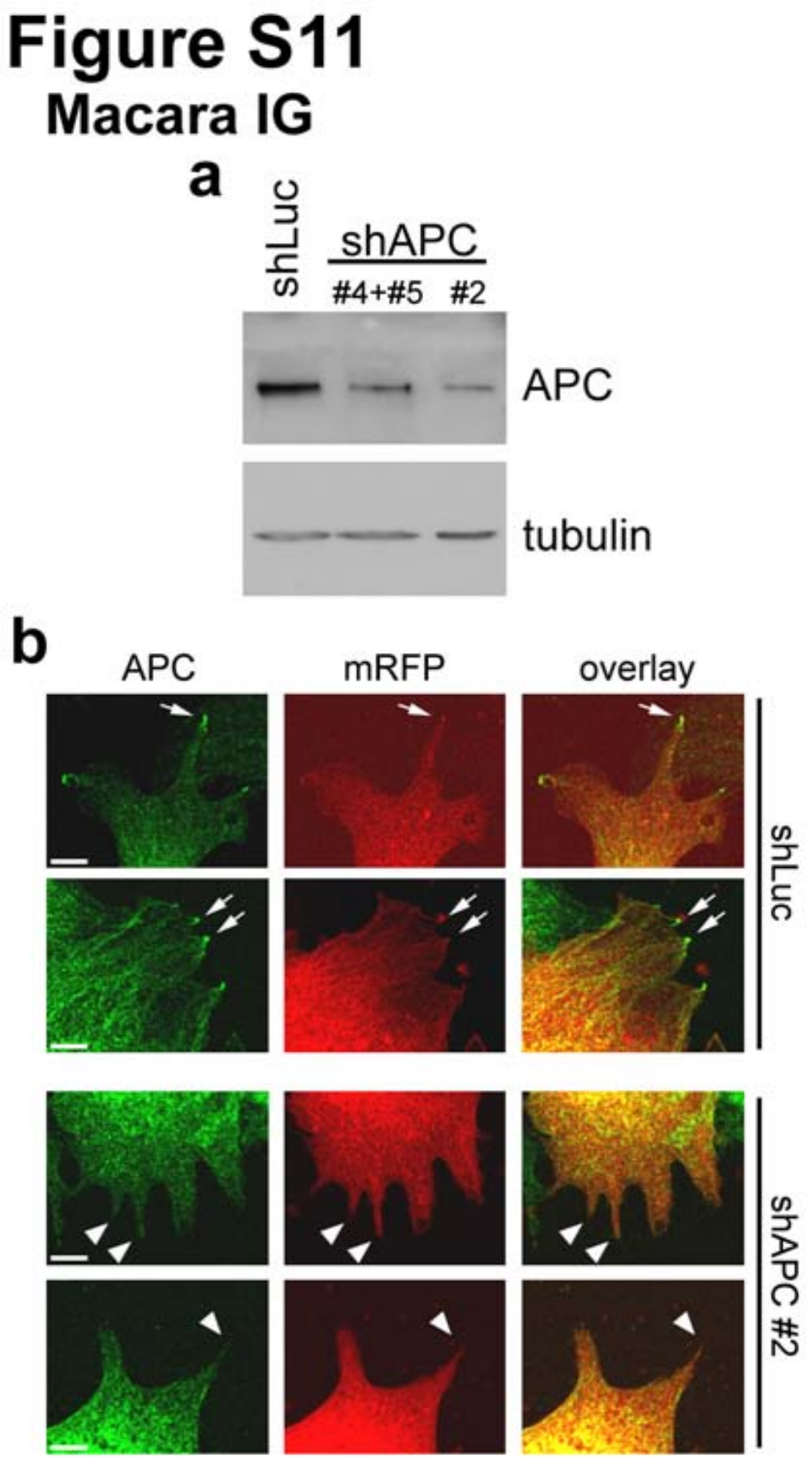

Figure S11 a, Cells expressing shRNAs against Luciferase (shLuc) or APC (shAPC), individually or in combination as indicated, were analyzed by Western blot to detect the indicated proteins. **b,** Cells were transfected with constructs expressing shRNAs against Luciferase (shLuc) or APC (shAPC#2) together with mRFP, to mark the transfected cells. 72 hours post-transfection, cells were fixed and stained with anti-APC antibody. Note that APC granules at tips of protrusions (arrows) are no longer detectable in shAPC-expressing cells (arrowheads). Non-specific cytoplasmic signal does not change between control and knockdown cells. Scale bar: 8μm.

Figure S12
Macara IG

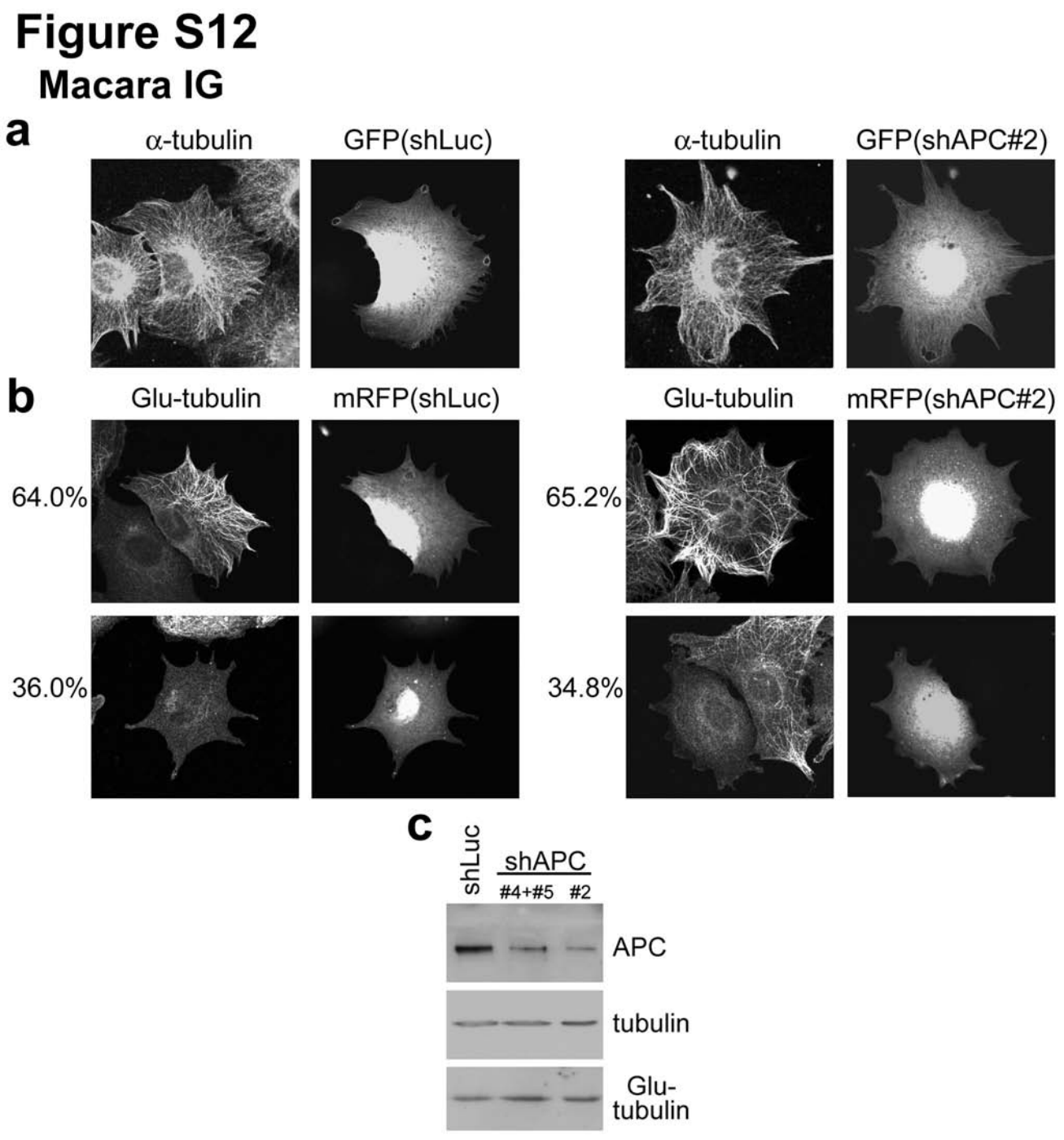

Figure S12 a, Cells were transfected with constructs expressing shRNAs against Luciferase (shLuc) or APC (shAPC#2) together with GFP, to mark the transfected cells. 72 hours post-transfection, cells were fixed and stained with anti-alpha-tubulin antibody. No significant difference in the microtubule cytoskeleton is detected between control and APC-knockdown cells. **b,** Cells were transfected with constructs expressing shRNAs against Luciferase (shLuc) or APC (shAPC#2) together with mRFP, to mark the transfected cells. 72 hours post-transfection, cells were fixed and stained with anti-detyrosinated-tubulin (Glu-tubulin) antibody. We observed either cells with a well-developed Glu-MT network (upper panels) or cells with no detectable Glu-MTs (lower panels). Percentages of cells in either category are indicated to the left (n=50 for shLuc, n=69 for shAPC#2). **c,** Cells expressing shRNAs against Luciferase (shLuc) or APC (shAPC), individually or in combination as indicated, were analyzed by Western blot to detect the indicated proteins. Note that no significant differences are detected in the organization of Glu-MTs or the amount of Glu-tubulin between control and APC-knockdown cells.

Figure S13
Macara IG

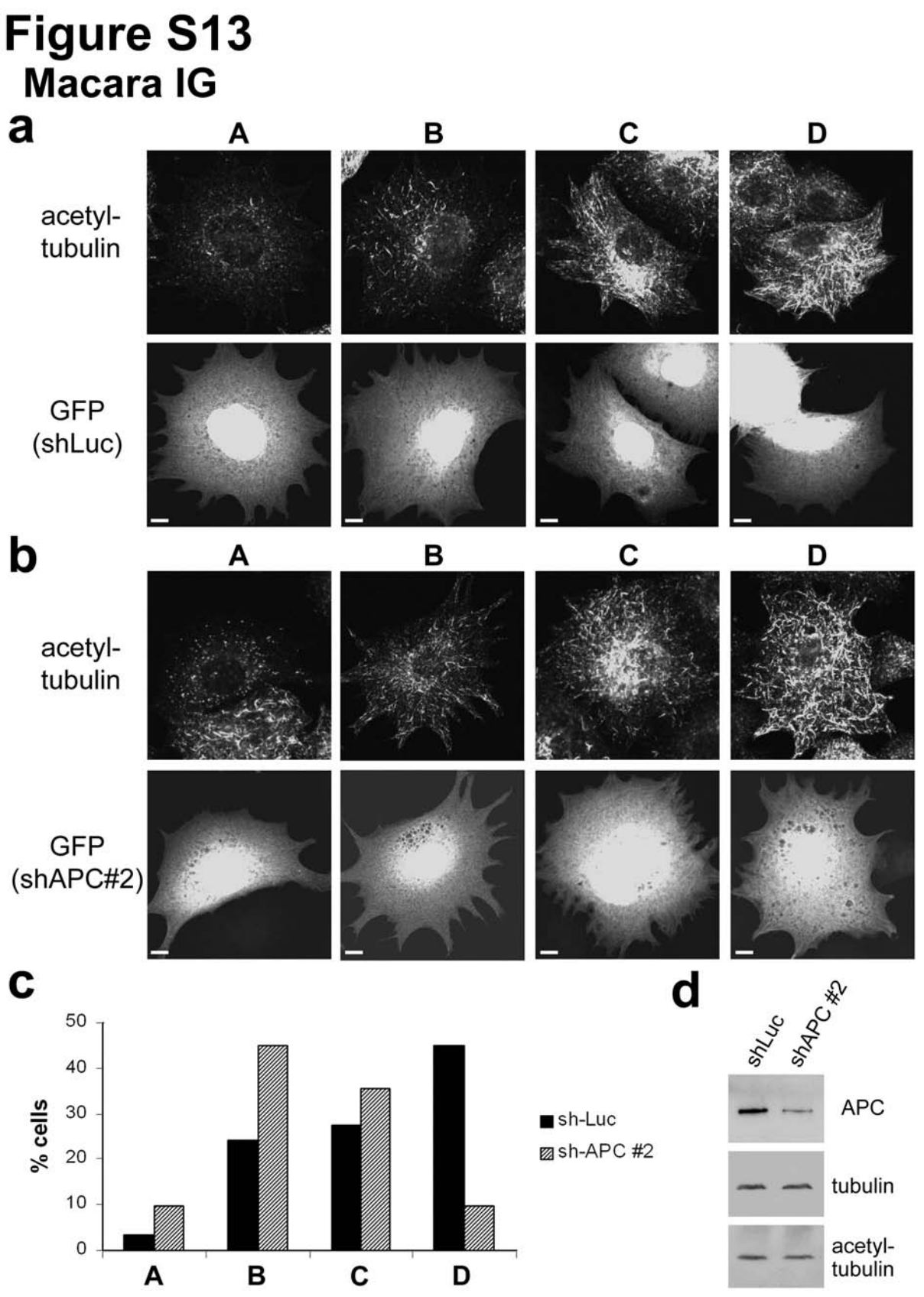

Figure S13 Cells were transfected with constructs expressing shRNAs against Luciferase (shLuc) (**a**) or APC (shAPC#2) (**b**) together with GFP, to mark the transfected cells. 72 hours post-transfection, cells were fixed and stained with anti-acetylated-tubulin antibody. Acetyl-tubulin staining varies between cells with respect to its intensity and its distribution. We thus assigned the cells into one of four different categories depending on the type of staining they displayed; (A) almost no acetylated-MTs (Ac-MTs) detectable, (B) low intensity of staining with short Ac-MTs distributed throughout the cytoplasm, (C) higher intensity of staining with most Ac-MTs clustered in the perinuclear region and few extending to the periphery and (D) high intensity of staining with Ac-MTs extending to the periphery. Scale bar: 7μm. **c,** Percentages of shLuc- and shAPC-expressing cells displaying each type of Ac-MT staining (A-D, as described above), were quantified (n=42). Note that a higher percentage of shAPC-expressing cells exhibits short Ac-MTs compared to control cells. **d,** shLuc- and shAPC-xpressing cells were analyzed by Western blot to detect the indicated proteins.

Supplementary Methods

Plasmid constructs

A plasmid encoding the human β-globin mRNA was provided by J. Lykke-Andersen (University of Colorado). The plasmid contains all the coding exons and introns of human β-globin, 54 nts of the β-globin 5'UTR, 69 nts of the β-globin 3'UTR followed by 24 repeats of the MS2-binding site cloned into the HindIII, XbaI sites of pcDNA3 (Invitrogen). To facilitate cloning, a multiple cloning site with sequence 5'-atcgatggtaccgctagcgatatcctcgag-3' was introduced between the XbaI and ApaI sites, generating plasmid β-globin-24bs/-. Various 3'UTRs were PCR amplified from mouse genomic DNA (BDbiosciences Clontech) with primers carrying appropriate restriction sites and were ligated into the NheI and XhoI sites (for the pkp4 and rab13 3'UTRs) or into the XbaI and ApaI sites (for the rac1 and rhoA 3'UTRs) of the β-globin-24bs/- plasmid, to generate plasmids β-globin-24bs/pkp4, β-globin-24bs/rab13, β-globin-24bs/rac1 and β-globin-24bs/rhoA. The exact 3'UTRs correspond to: nts 3858-4612 of NM_026361 (pkp4 UTR), nts 91295-92092 of NT_039254 (rab13 UTR), nts 776-2240 of NM_009007 (rac1 UTR) and nts 1009-2071 of NM_016802 (rhoA UTR). To generate plasmids β-globin-0bs/pkp4 and β-globin-0bs/rab13, plasmids β-globin-24bs/pkp4 and β-globin-24bs/rab13 respectively were digested with NotI and NheI (to remove the fragment containing the 24 MS2 binding sites), the ends were blunted with T4 DNA polymerase and religated. To generate constructs rab13/rab13 and rab13/rhoA, the genomic sequence containing the coding exons and intervening introns of the rab13 gene was PCR amplified from mouse genomic DNA with primers introducing an EcoRI site at

the 5' end and a KpnI site at the 3' end. A FLAG tag was ligated at the EcoRI site in frame with the rab13 ORF. The rab13 3'UTR or the rhoA 3'UTR were ligated at the KpnI site through blunt end ligation. The two resulting fragments were ligated into pEGFP-C1 vector from which the GFP sequence had been previously removed.

The plasmid pcNMS2, containing an oligomerization-defective MS2 coat protein mutant, has been described previously[32]. The GFP sequence was PCR amplified from plasmid pEGFP-N3 (Clontech) with primers introducing BamHI and XhoI sites and was ligated at the XhoI site with oligos containing the SV40 large T-Ag NLS. The GFP-NLS fragment was ligated into the BamHI and NotI sites of pcNMS2 to generate the pcNMS2-GFP-NLS plasmid expressing the MS2-GFP protein. RFP-tubulin was generated from pEGFP-tubulin (Clontech) by replacing the NheI/XhoI GFP fragment with a PCR-amplified fragment of mRFP. To generate EB1-GFP and EB1-RFP expressing plasmids, the mouse EB1 cDNA was amplified by RT-PCR from total NIH/3T3 RNA with primers introducing a KpnI site at the 5'end and a BglII site at the 3'end. The EB1 fragment was either ligated into KpnI/BamHI sites of pEGFP-N3 or into KpnI/XhoI sites of pcDNA3 (Invitrogen) together with a BglII/XhoI PCR-amplified fragment of mRFP. The plasmid expressing GFP-APC was provided by I. Nathke (University of Dundee). The SacI/BamHI APC fragment was ligated with a NheI/SacI fragment containing 3 copies of Orange fluorescent protein (OrFP) (generated through sequential PCR amplification and ligation of individual OrFP fragments) into the XbaI and BamHI sites of pKH3 to generate plasmid 3xOrFP-APC. To generate RFP-FMRP, a plasmid expressing GFP-FMRP was used (provided by R.Darnell, Rockefeller University) and the NheI/SacI fragment, encoding GFP, was replaced with a NheI/SacI PCR-amplified fragment of

mRFP. To generate FLAG-FMRP, the SacI/EcoRI fragment encoding FMRP (from the RFP-FMRP plasmid) was ligated together with a NheI/SacI fragment encoding the FLAG tag into the XbaI/EcoRI sites of pKH3. To generate RFP-Dcp1b, a plasmid expressing FLAG-Dcp1b was used (provided by J. Lykke-Andersen, University of Colorado). The BamHI(blunted with T4 DNA polymerase)/NotI fragment encoding human Dcp1b was ligated with a HindIII/NdeI(blunted with T4 DNA polymerase) fragment of mRFP into HindIII and NotI sites of pcDNA3.

For knockdown experiments, oligonucleotides were synthesized targeting different regions of the mouse APC mRNA. Sequences of the oligos are as follows: shAPC #2 sense oligo, 5'-gatcccc**gaatcaaccaggcataata**ttcaagaga**tattatgcctggttgattc**tttttggaaa-3', shAPC #2 antisense oligo, 5'-agcttttccaaaaa**gaatcaaccaggcataata**tctcttgaa**tattatgcctggttgattc**ggg-3', shAPC #4 sense oligo, 5'- gatcccc**taagtgatctgacaataga**ttcaagaga**tctattgtcagatcactta**tttttggaaa-3', shAPC #4 antisense oligo, 5'-agcttttccaaaaa**taagtgatctgacaataga**tctcttgaa**tctattgtcagatcactta**ggg-3', shAPC #5 sense oligo, 5'- gatccccc**aactacagtgaacgttat**ttcaagaga**ataacgttcactgtagttg**tttttggaaa-3', shAPC #5 antisense oligo, 5'-agcttttccaaaaa**caactacagtgaacgttat**tctcttgaa**ataacgttcactgtagttg**ggg-3'. Bold characters indicate the APC mRNA targeting sequences. Underlined characters indicate the 9bp hairpin loop. Sequences of control oligonucleotides targeting luciferase have been described previously[33]. The sense and antisense oligos were annealed and ligated into the BglII and HindIII sites of the pSuper vector.

Cell culture and transfection

NIH/3T3 cells were grown in DMEM supplemented with 10% calf serum, sodium pyruvate, penicillin and streptomycin (Invitrogen). For live cell imaging, plasmid constructs were transfected with Effectene (Qiagen) according to the manufacturer's instructions. 24 hours after transfection cells were plated for ~2 hrs on Lab-Tek coverglass chambers (Nalge nunc International) coated with 5μg/ml fibronectin and fluorescence was visualized by confocal microscopy. For pseudopodia/cell body fractionation or for shRNA-mediated knockdown experiments, cells were electroporated using the Gene Pulser II electroporation system (Bio-Rad) with plasmid constructs (5 μg / $6x10^6$ cells) or with pSuper constructs encoding shRNAs (40 μg / $6x10^6$ cells), respectively. Electroporation efficiency, based on co-transfected GFP, was generally >70%. Electroporated cells were processed after 72 hrs.

Pseudopodia and cell body isolation

To isolate pseudopodia and cell bodies of cells induced to migrate with LPA, we followed the protocol described by Cho and Klemke[15] with some modifications. Cells were serum-starved overnight and $1.5x10^6$ cells were placed in the upper compartment of a Transwell insert (24mm diameter, Costar) equipped with a 3μm porous polycarbonate membrane coated on both sides with 5μg/ml fibronectin. Cells were allowed to spread on the upper surface of the membrane for 2 hrs. LPA (150ng/ml) was then added in the bottom chamber to induce the cells to extend pseudopodial protrusions. After 1 hr the cells were briefly rinsed with PBS and fixed with 0.3 % methanol-free formaldehyde (Polysciences, Inc) in PBS for 10 min at room temperature. Glycine was added to 250

mM for 5 min at room temperature and the cells were washed twice with PBS. To isolate pseudopodia, cell bodies on the upper membrane surface were manually removed with cotton swab and laboratory paper and pseudopodia on the underside of the membrane were scraped into crosslink reversal buffer (100mM Tris pH 6.8, 5mM EDTA, 10mM DTT and 1% SDS). Cell bodies were similarly isolated except that pseudopodia on the underside of the membrane were manually removed and cell bodies were scraped into crosslink reversal buffer. Extracts were incubated at 70^{o}C for 45 min to reverse the formaldehyde-induced crosslinks. Proteins were directly analyzed by Western blot otherwise total RNA was isolated and processed as described below.

To isolate pseudopodia and cell bodies of cells migrating towards fibronectin, ~1x10^{6} cells were placed in serum-free media in the upper compartment of a Transwell insert equipped with a 3μm porous polycarbonate membrane whose underside only was coated with 5μg/ml fibronectin. Cells were allowed to extend pseudopodial protrusions towards the fibronectin-coated surface for 1 hr and were subsequently fixed and processed as described above.

RNA isolation and analysis

Total RNA or RNA from Ps and CB fractions was isolated using Trizol LS reagent (Invitrogen) and contaminating DNA was removed by treatment with RQ1 DNase (Promega) for 30 min at 37^{o}C. 1μg of RNA was reverse transcribed using SuperscriptII Reverse Transcriptase (Invitrogen) and random hexamer primers according to the manufacturer's instructions. cDNA was used for PCR reactions in a GeneAmp PCR System 9700 (Applied Biosystems), to detect different RNAs using the following primer

pairs: rab13 F: 5' gcctaccagtgttggctctt, rab13 R: 5' tccacggtaataggcggtag, rab13 UTR F: 5' aggctgctagcgagcatttcttgcctcctat, rab13 UTR R: 5' aatggctcgagccattcatttcttcttcc, pkp4 F: 5' aggctgctagccagggaagtgaggaaacc, pkp4 R: 5' aatggctcgagaaaacatgaagggcatcc, ankrd25 F: 5' ttcaaagccagaaagccaag, ankrd25 R: 5' aggtgacaaagggtggtgag, inpp1* F: 5'tttgaagtggaatggggataac, inpp1* R: 5' aatagtcagatagtcaaactcatgg, APC F: 5' cctctcaccggagtaagcag, APC R: 5' gtcgtcctgggaggtatgaa, arpc3 F: 5' cacggacattgtggatgaag, arpc3 R: 5' ccaccacttgctggctttat, FLAG rab13 F: 5' taatacgactcactataggg, FLAG rab13 R: 5' tccacggtaataggcggtag, β-globin F: 5' ttgagtcctttggggatctg, β-globin R: 5' cactggtggggtgaattctt, β-actin F: 5' tgttaccaactgggacgaca, β-actin R: 5' gctgtggtggtgaagctgta.

The identity of the amplified products was verified either by sequencing or in the case of transfected RNAs by ensuring that no product was amplified when using RNA from vector-transfected cells. PCR reactions contained 10mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM $MgCl_2$, 0.2mM each dNTPs, 0.5μM each primers and 2.5U Taq polymerase. The amount of cDNA and the number of cycles was varied for each primer pair, to ensure amplification was within the linear phase. This was verified in all experiments by including decreasing amounts of selected samples.

To calculate the enrichment of RNAs in pseudopodia, equal amounts of RNA from Ps and CB fractions were analyzed by RT-PCR, the signals were quantified using WCIF ImageJ software, normalized to the control arpc3 mRNA and enrichment in pseudopodia was defined as (Ps signal/CB signal)-1, when Ps signal>CB signal or as (-1)*[(CB signal/Ps signal)-1], when Ps signal<CB signal.

Microarray analysis

Microarray analysis was performed at the Biomolecular Research Facility at the University of Virginia. Total RNA from Ps and CB fractions was analyzed on an Agilent BioAnalyzer and was deemed to be of good quality. Biotin-labeled cRNAs were generated and hybridized to GeneChip Mouse Genome 430 2.0 arrays (Affymetrix), which contain 45,000 probe sets analyzing the expression level of over 39,000 transcripts and variants from over 34,000 well-characterized mouse genes. Details about the RNA preparation and hybridization protocols can be found at www.healthsystem.virginia.edu/internet/biomolec/microarray.cfm.

In all hybridization experiments, quality assessment variables, such as background, noise, GAPDH 3'/5' ratio, were within the acceptance limits.

For experiments analyzing Ps and CB fractions from cells induced with LPA, results were analyzed using the GeneChip Operating Software (GCOS) platform. RNAs were considered to be significantly enriched in pseudopodia if the p-value was significant, the fold change in signal intensity was greater than 2.2 and the absolute difference in signal intensity was greater than 100.

For experiments analyzing Ps and CB fractions from cells migrating towards fibronectin, results were analyzed using the dChip software (www.dchip.org). RNAs were considered to be significantly enriched in pseudopodia if the p-value was less than 0.05, the fold change in signal intensity was greater than 1.5 and the absolute difference in signal intensity was greater than 10 times the noise level.

Western Blot, Immunofluorescence and Immunoprecipitation

For Western blot and immunofluorescence, the following antibodies were used: mouse monoclonal anti-Ran (Transduction Laboratories), rabbit anti-pY397-FAK (Biosource International), monoclonal anti-FAK (BD Transduction Labs), anti-APC (C-20) (Santa Cruz Biotechnology), rabbit anti-PABP1 (Cell Signaling Technology), anti-FMRP (clone 1C3, Chemicon), anti-acetylated tubulin (Clone 6-11B-1, Sigma), anti-alpha-tubulin (clone DM1A, Sigma), anti-Glu-tubulin (provided by Gregg Gundersen, Columbia University).

For immunoprecipitation, anti-APC (C-20) or control antibody (rabbit anti-HA tag, Santa Cruz Biotechnology) were bound on protein-A beads otherwise anti-FLAG M2 agarose beads (Sigma) were used. In all cases, antibody-bound beads were pre-incubated with 200μg/ml E.coli tRNA, 200μg/ml herring sperm DNA, 200μg/ml RNase-free BSA and 50μg/ml glycogen. Cells spreading on FN-coated plates for ca 2 hrs were lysed in lysis buffer (10mM Tris pH:7.5, 100mM NaCl, 2.5mM $MgCl_2$, 0.5% Triton X-100) supplemented with protease inhibitors (leupeptin, pepstatin, aprotinin) and RNase inhibitor (0.5 U/μl). Lysates were centrifuged at 10,000 g for 10 min and incubated with antibody-bound beads at 4 C for 3 hrs. Beads were washed 5 times with lysis buffer and the immunoprecipitated material was released by incubation in lysis buffer containing 1%SDS. A fraction of the released material was analyzed by Western blot. The remainder was used for total RNA isolation and RT-PCR analysis. For RNase treatment, 1mM $CaCl_2$ was added in the lysis buffer and after the initial centrifugation, RNAse A (30μg/ml) and micrococcal nuclease (70U/ml) were added, the lysate was incubated at 30

C for 10 min and subsequently centrifuged at 10,000g for 5 min. The supernatant was used in immunoprecipitations as described above.

Confocal Microscopy and Photobleaching

Imaging and photobleaching were performed with a Zeiss LSM 510 Meta confocal microscope operated by LSM-FCS software (Carl Zeiss). Temperature was maintained at 37°C. Medium pH was controlled by addition of 25 mM HEPES buffer. EGFP was excited with the 488-nm line of an Argon laser at 30% power, 5% transmission. mRFP, OrFP and DsRed were excited with the 543-nm line of a HeNe laser at 80% transmission. For photobleaching, regions of interest (ROIs) were selected and bleached with the 488-nm laser line at 100% power, 100% transmission for two iterations. Fluorescence recovery within the ROI was monitored for ~60 seconds. For each experiment, three images were recorded pre-bleach. Mean intensities in the bleached area were measured, background signal was subtracted, intensities were corrected for bleaching during imaging and were expressed as a percentage of the pre-bleach intensity.

Table S1: RNAs significantly enriched in pseudopodia in response to both LPA and fibronectin (FN) stimulation. Fold change indicates the average fold enrichment in pseudopodia from two independent experiments for each stimulus. Listed are the identifiers in the GeneChip arrays (probe set) and the GenBank accession numbers. [a]Inpp1* indicates that the probe set likely detects an RNA isoform of inositole polyphosphate 1-phosphatase with a longer 3'UTR.

Probe set	Accession	LPA fold change	FN fold change	Gene name
				vesicle/membrane trafficking
1424894_at	BC027214	5.1	3.58	RAB13, member RAS oncogene family
1428409_at	AK013287	3.19	3.26	Mak3 homolog (S. cerevisiae)
1455478_at	BM222792	3.26	1.67	Liprin a1
1426269_at	BC003764	2.3	2.98	synaptobrevin like 1
				motors
1424746_at	BC016221	4.46	2.29	kinesin family member 1C
1418258_s_at	NM_133796	4.01	1.6	dynein light chain LC8 type 2
1418429_at	BI328541	2.84	2.05	kinesin family member 5B
1459854_s_at	AV059518	2.39	2.91	dynein light chain Tctex-type 3
				cytoskeleton organization/signalling
1442073_at	BQ266693	6.46	6.92	inositole polyphosphate 1-phosphatase* (Inpp1*)
1452209_at	AV286396	4.14	1.99	plakophilin 4
1455688_at	BB795075	4.01	2.97	discoidin domain receptor member 2
1451736_a_at	AW541674	3.81	1.93	mitogen activated protein kinase kinase 7
1433768_at	BG071905	3.16	2.24	Palladin
1419829_a_at	AW049055	2.94	1.59	GRB2-associated binding protein 2, Gab2
1437995_x_at	AV219419	2.73	1.91	septin 7
1435543_at	BM124893	2.47	2.92	adenomatosis polyposis coli
1420956_at	NM_007462	2.39	1.85	adenomatosis polyposis coli
1443694_at	BB794177	2.28	2.37	regulator of G-protein signaling 20
				RNA metabolism
1454760_at	AW543705	2.97	3.63	HIV TAT specific factor 1
1423274_at	BB381966	2.55	1.77	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26
1428262_s_at	AK011930	2.55	1.59	heterogeneous nuclear ribonucleoprotein A3
1434452_x_at	BG797460	2.47	2.82	eukaryotic translation initiation factor 2a
1416422_a_at	BG796845	2.47	3.19	La protein
1439268_x_at	BB032885	2.22	1.74	eukaryotic translation initiation factor 3, subunit 6
				various or unknown function
1452646_at	AK003956	4	3.02	tumor protein p53 inducible nuclear protein 2
1422186_s_at	NM_029787	3.52	1.96	diaphorase 1 (NADH)
1422185_a_at	NM_029787	3.04	1.83	diaphorase 1 (NADH)
1426051_a_at	BC006628	3.04	1.58	centromere autoantigen B
1434745_at	BQ175880	2.84	1.68	cyclin D2
1422618_x_at	NM_009529	2.8	4.01	Xmr, similar to Xlr-related, meiosis regulated
1420827_a_at	BG065754	2.56	2.6	cyclin G1
1456607_at	AV320529	2.46	2.53	valosin containing protein (p97)/p47 complex interacting protein 1

1448733_at	M64279	2.38	2.46	Bmi1 polycomb ring finger oncogene
1460559_at	BB038765	4.14	2.91	ankyrin repeat domain 25
1454863_at	AV335903	4	3.91	ankyrin repeat domain 11
1436382_at	BB045268	3.48	2.64	Zinc finger and BTB domain containing 12 (Zbtb12)
1424594_at	BC011507	3.48	2.5	sterile alpha motif domain containing 4 isoform 2
1436356_at	AW107196	2.64	2.18	sterile alpha motif domain containing 4 isoform 2
1423694_at	BC006935	3.16	1.56	potassium channel tetramerisation domain containing 10
1436997_x_at	BB248904	3.16	2.47	SH3-binding domain glutamic acid-rich protein like
1428107_at	AK004519	3.04	2.89	SH3-binding domain glutamic acid-rich protein like
1455354_at	BG061773	2.81	1.57	WD repeat domain 22
1449345_at	NM_026613	2.66	2.4	coiled-coil domain containing 34
1418926_at	NM_011546	2.65	2	zinc finger homeobox 1a
1454862_at	AV253284	2.55	1.63	pleckstrin homology-like domain, family B, member 2
1457260_at	BI080487	6.03	3.04	hypothetical protein LOC230757
1435265_at	BF466929	3.03	3.24	RIKEN cDNA clone 9130204C03
1452883_a_at	BQ176330	2.94	2.78	hypothetical protein
1443621_at	BG092359	2.73	2.03	Transcribed locus
1436523_s_at	AV111033	2.73	2.44	RIKEN cDNA clone 1810022K09
1456736_x_at	AV217938	2.65	2.98	hypothetical protein
1426356_at	BB667858	2.47	1.96	hypothetical protein LOC76178
1456603_at	BG070087	2.46	1.59	hypothetical protein
1438016_at	BG068512	2.22	2.39	cDNA sequence BC068171

Supplementary Notes

32. Lykke-Andersen, J., Shu, M. D. & Steitz, J. A. Human Upf proteins target an mRNA for nonsense-mediated decay when bound downstream of a termination codon. *Cell* **103**, 1121-31 (2000).

33. Chen, X. & Macara, I. G. Par-3 controls tight junction assembly through the Rac exchange factor Tiam1. *Nat Cell Biol* **7**, 262-9 (2005).

Supplementary video legends

Supplementary video 1 Confocal fluorescence time lapse imaging of a cell expressing mRFP (red), MS2-GFP (green) and the β–globin-24bs/pkp4 mRNA. Overlay images of the two channels are shown. Arrows point to protrusions with localized RNA granules. Note that the RNA granules remain stationary over the course of observation. Time is indicated on the upper left corner in seconds.

Supplementary video 2 FRAP experiment of a localized RNA granule. Shown is a cell expressing MS2-GFP and the β–globin-24bs/pkp4 mRNA and which exhibits a localized RNA granule at the end of a protrusion (indicated by an arrow). Fluorescence at this protrusion was bleached and its recovery recorded over ca 60 seconds. Note that fluorescence recovers only minimally during the course of observation. Time is indicated on the upper left corner in seconds.

Supplementary video 3 FRAP experiment of a localized RNA granule. Shown is a cell expressing MS2-GFP and the β–globin-24bs/pkp4 mRNA and which exhibits two localized RNA granules at the ends of two protrusions. Fluorescence at one of the granules (arrow) was bleached and its recovery recorded over ca 4 minutes. Note that fluorescence recovers only minimally during the course of observation while the second granule (arrowhead) remains stationary. Time is indicated on the upper left corner in seconds.

Supplementary video 4 FRAP experiment of a cell expressing EB1-GFP. Fluorescence at a protrusion (arrow) was bleached and its recovery recorded over ca 60 seconds. Note that EB1-GFP comets (representing +ends of dynamic MTs) rapidly move throughout the cell body and several of them enter into protrusions over the course of observation. Time is indicated on the upper left corner in seconds.

Supplementary video 5 FRAP experiment of a cell expressing GFP-APC which exhibits APC granules at the tips of different protrusions. Fluorescence at the tip of one protrusion (arrow) was bleached and its recovery recorded over ca 60 seconds. Note that fluorescence recovers only minimally during the course of observation while another APC granule (arrowhead) remains stationary. Time is indicated on the upper left corner in seconds.