Supplemental material to:

ELMO recruits Actin Crosslinking Family 7 (ACF7) at the cell membrane for microtubule capture and stabilization of cellular protrusions

Yoran Margaron, Nadine Fradet and Jean-François Côté

-Supplemental figure legends

-Video legends

-Supplemental materials and methods

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Endogenous ACF7 binds Myc-ELMO1. HEK293T cells were transfected with empty Myc-vector, Myc-ELMO1 WT and Myc-ELMO1$^{\alpha N}$. Cell lysates were subjected to anti-Myc purification and bound proteins were eluted with ammonium hydroxide and identified by mass spectrometry. We restricted our analyses to ELMO, ACF7 and DOCK1. The tables report the number of peptides identified for each protein and the corresponding Mascot score.

Figure S2. ELMO1^{I204D} and ACF7 synergize to promote membrane protrusions dynamic. (**A**) Serum-starved CHO LR73 cells transfected with pDsRed-ELMO1^{I204D}±pEGFPC1A-ACF7 were plated on fibronectin and visualized by live imaging using a laser-scanning microscope. A selection of time-lapse acquisitions from a representative experiment is shown (see movies FigS2.video1 and FigS2.video2 for the entire video sequences). Inset: overlay of DsRed±GFP. Scale bar, 20 μm. (**B**) Membrane velocity maps. (**c**) Autocorrelation maps. Maps were cropped from ΔCell periphery (-180 to 180 degree) and from ΔTime (0 to 40 min).

Figure S3. ELMO2 is the only ELMO family member expressed in MDA-MB-231 cells. RT-PCR analyses demonstrate that ELMO2 is the only ELMO-family member expressed in MDA-MB-231 cells.

Figure S4. The open conformation ELMO1 mutant has no additional effect on microtubule stability. CHO LR73 cells transfected with the indicated plasmids were plated on fibronectin for 2 h. Cells were stained for β-tubulin, ELMO and ACF7. Picture: β-tubulin staining. Left inset: overlay of ELMO1, ACF7, and DAPI staining. See Figure 7B for quantification. Right insert: magnification of the dashed area. Dark-dashed lines are overlays of the cell sides. Scale bar, 10 μm.

VIDEO LEGENDS (Main article)

Fig3.video1. Overexpression of ACF7 has no effect on cell morphology. CHO-LR73 cells transfected with pEGFPC1A-ACF7 (green) were plated on fibronectin-coated plates and immediately visualized by live imaging with a laser-scanning microscope (LSM 710, Zeiss). Frames were taken every minute for 45 min. Inset: GFP signal. Scale bar, 20 μm.

Fig3.video2. ELMO1 expression induces membrane ruffling. CHO-LR73 cells transfected with pDsRed-ELMO1 (Red) were plated on fibronectin-coated plates. Images were taken by live imaging with a laser-scanning microscope (LSM 710, Zeiss). Frames were taken every minute for 45 min. Inset: DsRed signal. Scale bar, 20 μm.

Fig3.video3. Overexpression of ELMO1/ACF7 induces the formation of long membrane protrusions. CHO-LR73 cells transfected with pDsRed-ELMO1 (Red) and pEGFPC1A-ACF7 (green) were plated on fibronectin-coated plates and immediately visualized by live imaging with a laser-scanning microscope (LSM 710, Zeiss). Frames were taken every minute for 45 min. Inset: overlay of DsRed and GFP signals. Scale bar, 20 μm.

Fig3.video4. The ACF7-binding defective mutant of ELMO1 fails to synergize with ACF7. CHO-LR73 cells transfected with pDsRed-ELMO1^{PxxP} (Red) and pEGFPC1A-ACF7 (green) were plated on fibronectin-coated plates and immediately visualized by live imaging with a laser-scanning microscope (LSM 710, Zeiss). Frames were taken every minute for 45 min. Inset: overlay of DsRed and GFP signals. Scale bar, 20 μm.

VIDEO LEGENDS (Supplemental material)

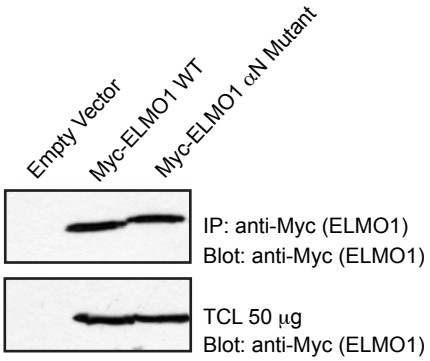
FigS2.video1. ELMO1^{I204D} induces an elongated cell shape. CHO LR73 cells transfected with pDsRed-ELMO1^{I204D} (Red) were plated on fibronectin and visualized by live imaging using a laser-scanning microscope (LSM 710, Zeiss). Frames were taken every minute for 45 min. Inset: DsRed signal. Scale bar, 20 μm.

FigS2.video2. ELMO1^{I204D} and ACF7 cooperate to promote membrane protrusions. CHO LR73 cells transfected with pDsRed-ELMO1^{I204D} (Red) and pEGFPC1A-ACF7 (green) were plated on fibronectin and visualized by live imaging using a laser-scanning microscope (LSM 710, Zeiss). Frames were taken every minute for 45 min. Inset: overlay of DsRed and GFP. Scale bar, 20 μm.

SUPPLEMENTAL MATERIALS AND METHODS

Identification of ELMO-bound ACF7 and DOCK1 by immunoprecipitation and mass spectrometry. HEK293T cells were transfected with 5 μg of pcDNA3.1 Myc, pcDNA3.1 Myc-ELMO1 WT or pcDNA3.1 Myc-ELMO1$^{\alpha N}$ mutant (two 10 cm dishes per condition) using Lipofectamine 2000. 48 hours after transfection, cells were washed with PBS and lysed in 50 mM Tris pH 7.5, 1% NP-40 and 150 mM NaCl for 10 minutes. Clarified cell lysates were filtered (.45 micron) and subjected to anti-Myc immunoprecipitation (25 μl of Proteine-A agarose and 1 μg of anti-Myc) for 90 minutes. Beads were washed three times with lysis buffer and three additional times with detergent-free lysis buffer. Bound proteins were eluted twice with NH_4OH/EDTA buffer (1% NH_4OH, 0.5 mM EDTA in water). Corresponding samples were pooled and the elution buffer was evaporated in a Speed Vac to dry and concentrate the proteins. Mass spectrometry and protein identification was performed at the IRCM Proteomic Discovery Platform according to standard protocols. The Mascot software was used to identify the proteins based on the identified peptides (n=2).

Gene expression analysis by RT-PCR. Total RNA was isolated from exponentially growing MDA-MB-231 cells using the Trizol reagent (Invitrogen). Prior to reverse transcription, an aliquot of the RNA (2 μg) was treated with DNase1 (Invitrogen). Superscript II Reverse Transcriptase was used to convert the total RNA to cDNA. Expression of ELMO family members was investigated by PCR using a dilution of this cDNA. Actin was used as a control. The following primers were used for ELMO1 (5'-TGTCACGATCACAGTGCAGA-3' and 5'-CAACTTTCAGCCCCTAGCTG-3'), ELMO2 (5'-CGTTGCCAAACCCAGAGTAT-3' and 5'-TGGAGGTGTGAGATGAGCTG-3'), ELMO3 (5'-TGACGCACTCTGAGCGTTAC-3' and 5'-CAAGGTCACACTCTCCAGCA-3') and β-actin (5'-TGATGGTGGGCATGGGTCAGAA-3' and 5'-TCCATGTCGTCCCAGTTGGTGA-3'). PCR products were amplified with TAQ polymerase (Feldan) and fractionated on agarose gels. Expected PCR fragment sizes are 525 bp (ELMO1), 550 bp (ELMO2), 467 bp (ELMO3) and 150 bp (β-actin).



Myc Control IP (empty vector)

Protein	#Peptides	Mascot Scores
ELMO1	0	n/a
DOCK1	0	n/a
ACF7	0	n/a

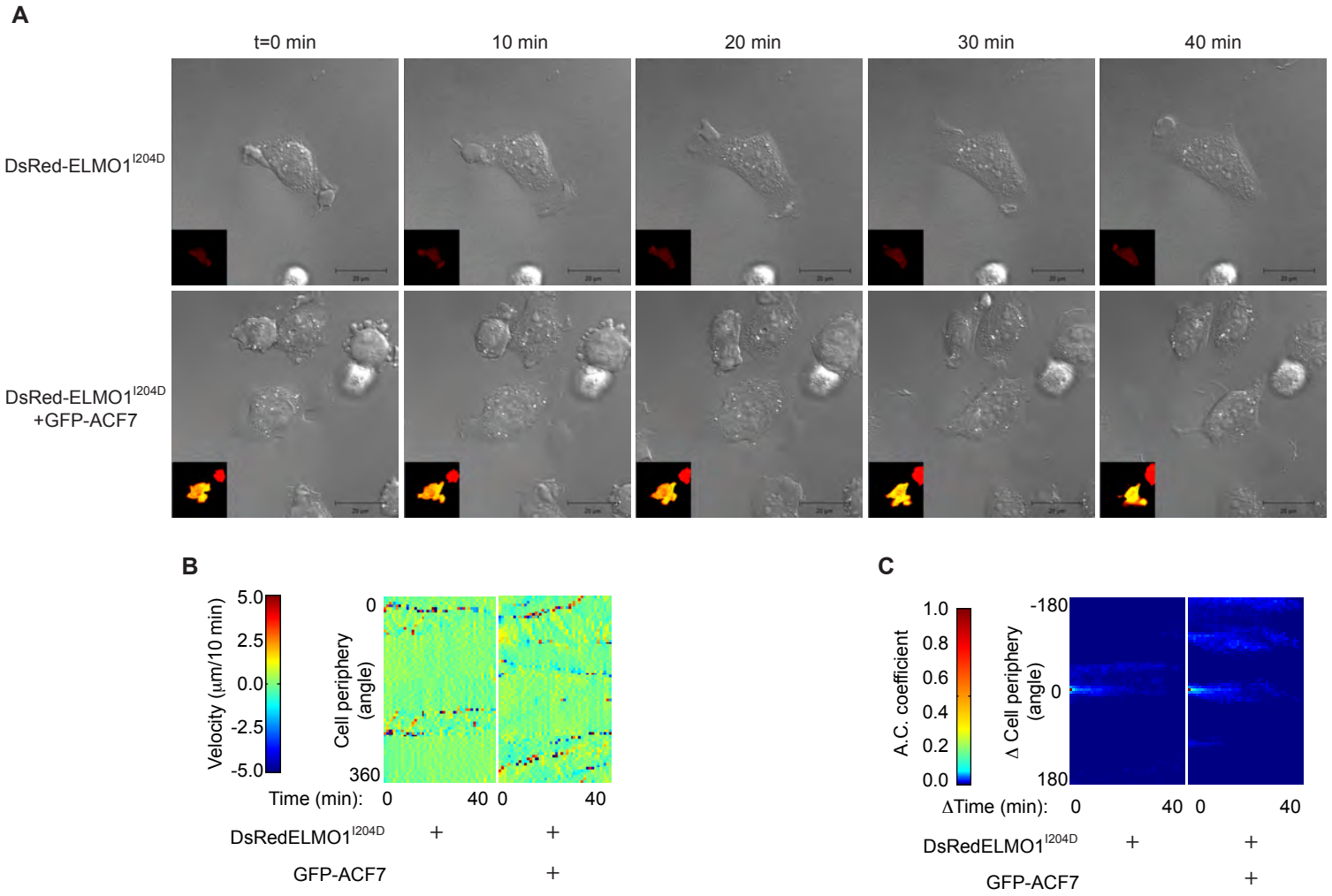
Myc ELMO1 WT IP

Protein	#Peptides	Mascot Scores
ELMO1	213	2334
DOCK1	17	96
ACF7	16	42

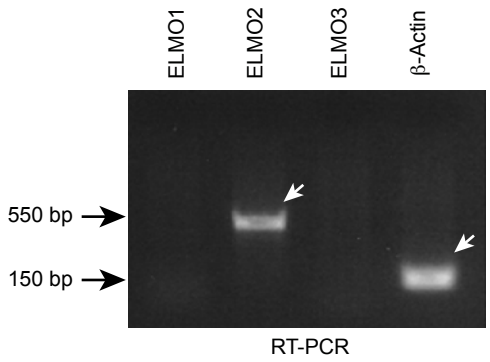
Myc ELMO1 aN Mutant IP (DOCK-binding deficient)

Protein	#Peptides	Mascot Scores
ELMO1	174	1947
DOCK1	0	n/a
ACF7	22	107

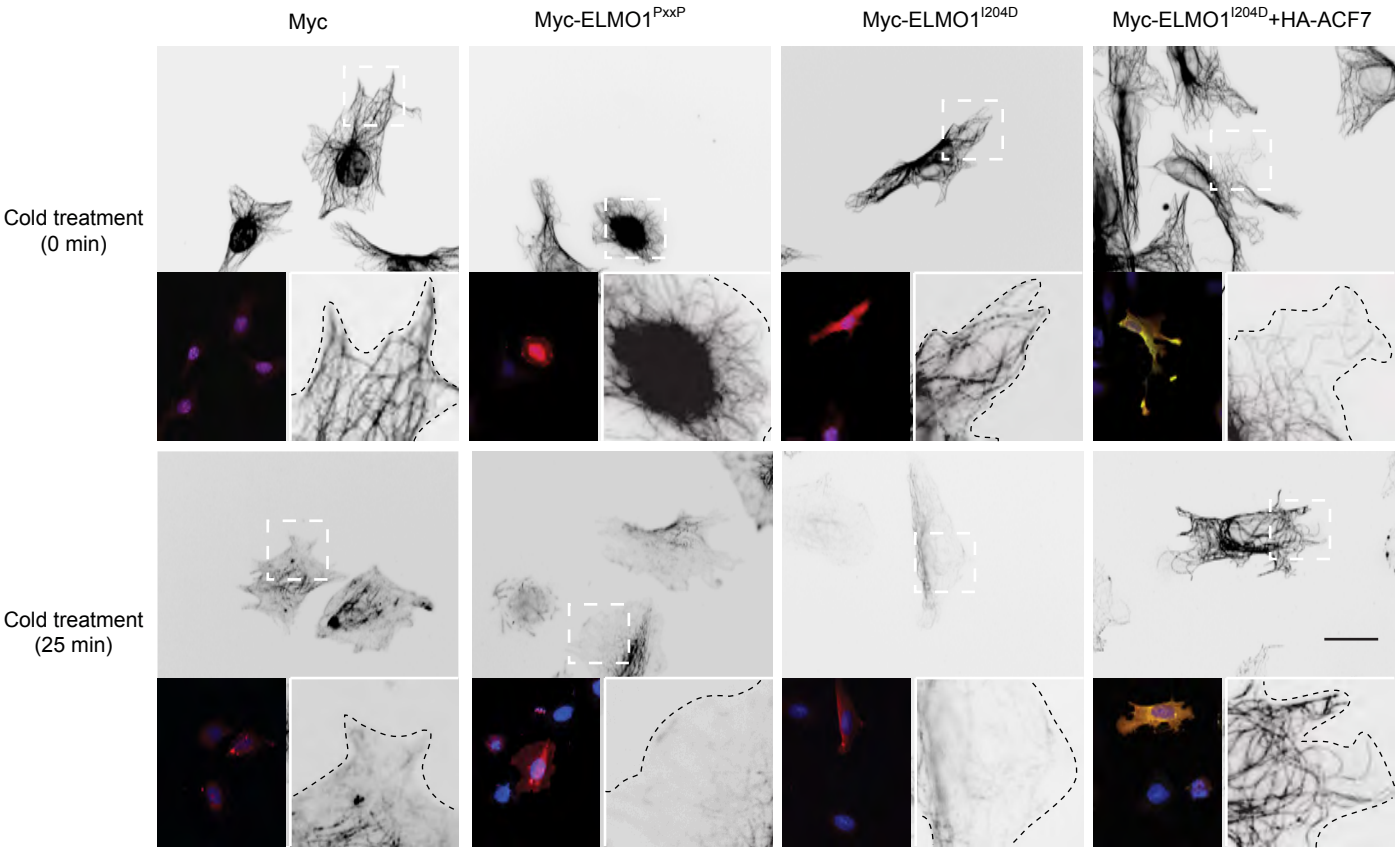
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4