SUPPLEMENTAL MATERIALS

Figure and Movie Legends

FIGURE S1. Shape changes of wild-type (KAx-3) and Roco2 mutant strains. **A.** Chemotaxis. Shape difference analysis between frames taken at 6 sec intervals from recordings of representative indicated strains of cells migrating in an exponential cAMP gradient towards a micropipette. Protrusions and retractions are illustrated in green and red, respectively, as determined by DIAS analysis (Wessels *et al*., 1998). Arrows from the cell indicate the direction of movement. The black dot indicates the direction of the gradient. **B.** Random cell motility. *Top*. DIAS computer analysis performed on digital time-lapse video DIC microscopy movies with a 40X objective of cells moving randomly. See the legend to Figure 1 for details. *Bottom*. Shape difference analysis between frames taken at 6 sec intervals of recordings of the indicated strain moving randomly. Protrusions and retractions are illustrated in green and red, respectively, as described above. GFP fusions of the wild-type and kinase dead Roco2 were used. The scale bar is 10 μm for every frame.

FIGURE S2. Development of wild-type and *roco2*$^-$ cells plated on non-nutrient agar. A. Washed vegetative cells were plated at the densities indicated and photographed at 6 hrs after plating. B. Cells were pulsed for 6 hrs, washed, and plated at the densities shown. Images were taken at the times indicated

FIGURE S3. Cytokinesis defects of *roco2*$^-$ cells. **A.** Numeric data from FACS analysis of DNA content per cell for substrate and suspension growth conditions. FACS profiles were obtained for KAx-3 and *roco2*$^-$ cells stained with propidium iodide (PI) under both growth conditions. **B.** *Top*, images of *roco2*$^-$ cells in the late cytokinesis stage presenting a long cytoplasmic bridge (>70 μm). The right image shows an abnormal cytoplasmic area (that never exists in the wild type) in the middle of the connecting bridge. *Bottom*, diagram showing cytoplasmic bridge length distribution in wild-type and *roco2*$^-$ strains. **C.** *Top*, time lapse images of a *roco2*$^-$ cell undergoing retro-cytokinesis event of visualized by DIC microscopy. *Bottom*, histogram presenting the retrocytokinesis percentage in wild-type and *roco2*$^-$ cells. Arrows indicate a cytoplasmic domain in the

cytoplasmic bridge like structure often observed in abnormal *roco2*$^{-}$ cytokinesis. The scale bar is 10 μm for each image.

FIGURE S4. Roco2$^{\Delta LRR}$ motility. **A.** Time-lapse recording of randomly moving vegetative Roco2$^{\Delta LRR}$-GFP/*roco2*$^{-}$ cells. The images were taken at 1 min intervals using DIAS software (Wessels *et al*., 1998). **B**. DIAS computer analysis performed on digital time-lapse video DIC microscopy movies with a 40X objective of cells moving randomly. See the legend to Figure 1 for details. **C and D.** Shape difference analysis between frames taken at 6 sec intervals of recordings of Roco2$^{\Delta LRR}$/*roco2*$^{-}$ cells moving randomly (**C**) or migrating in an exponential cAMP gradient (**D**). Protrusions and retractions are illustrated in green and red, respectively, as determined by DIAS analysis (Wessels et al, 1998). Arrows indicate the direction of movement. The black dot indicates the direction of the gradient. The red asterisk shows the frame in which the leading edge is lifted off of the substratum. The scale bar is 10 μm for all images.

FIGURE S5. Mass spectrometry analysis of Roco2 associated proteins and abp120 (filamin) chemotaxis defects. **A.** Partial list of Roco2 associated proteins identified by mass spectrometry analysis. **B.** Shape difference analysis between frames taken at 6 sec intervals of recordings of filamin (*abpC*) null cells and *abpC*$^{-}$ cells expressing Roco2$^{\Delta LRR}$ migrating in an exponential cAMP gradient. Protrusions and retractions are illustrated in green and red, respectively, as determined by DIAS analysis (Wessels *et al*., 1998). Arrows indicate the direction of movement. The green dot indicates the direction of the gradient.

FIGURE S6. Analysis of Rab1A. **A.** ClustalW alignment of human and *Dictyostelium* Rab1A sequences. Grey boxes indicate point mutations corresponding to S22N (GDP-bound form) and Q67L (GTP-bound form) of *Dictyostelium* Rab1A. **B**. Western blot of wild-type cells and wild-type cells expressing FLAG-Rab1A. Lane 1, wild-type cells; lane 2, wild-type cells expressing FLAG-Rab1A; lane 3, wild-type cells expressing FLAG-Rab1A. FLAG-Rab1A was immunoprecipitated with anti-FLAG antibody before analyzing by SDS-

PAGE/Western blot. **C**. Western blot of recombinant GST and GST-DdRab1A.

FIGURE S7. Effect of expression of Rab1A mutants in roco2-. **A, B.** Fraction of time the leading edge appears detached from the substratum. Part **A** shows the number of cells in which the leading edge is off of the substratum: 0, 1, 2, or 3 times within a 10 min window (50 cells total). In **B**, the amount of time (in sec within a 10 min window) that the leading edges of individual cells are off of the substratum ("floating" leading edge) (Total of 50 cells). **C**. Chemotaxis of *roco2*$^{-}$ cells expressing Rab1A^{Q67L}. **D**. Chemotaxis of wild-type cells co-expressing Rab1A^{S22N} and Roco2$^{\Delta LRR}$. The scale bar is 10 μm for all images. The dot indicates the direction of the micropipette. The arrows indicate the direction of movement.

Movie legends:

MOVIE M1. DIC microscopy recording of KAx-3 chemotaxing cells (one frame/6 sec).

MOVIE M2. DIC microscopy recording of *roco2*$^{-}$ chemotaxing cells (one frame/6 sec).

MOVIE M3. Recording of wild-type chemotaxing cells in a Dunn chamber with a gradient of 0-100 nM cAMP (one frame/6 sec). Trough with 100 nM cAMP on the right.

MOVIE M4. Recording of *roco2*$^{-}$ chemotaxing cells in a Dunn chamber with a gradient of 0-100 nM cAMP (one frame/6 sec). Trough with 100 nM cAMP on the right.

MOVIE M5. DIC microscopy recording of KAx-3 cell during cytokinesis (one frame/6 sec).

MOVIE M6. DIC microscopy recording of *roco2*$^{-}$ dividing cells that finally abort (retro-cytokinesis; one frame/6 sec).

Movie M7. Fluorescent confocal microscopy recording of *roco2*$^{-}$ cells expressing Roco2-GFP and RFP-coronin (one frame/12 sec).

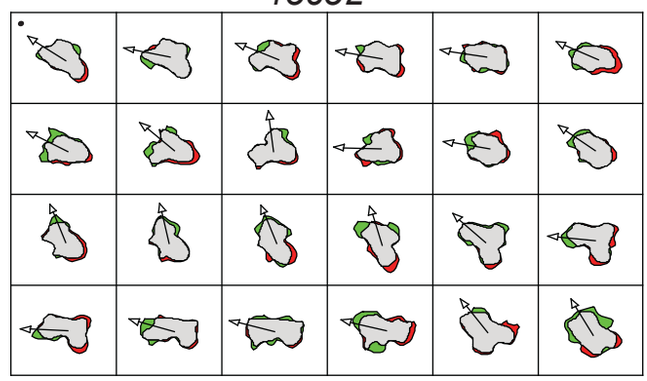
MOVIE M8. Fluorescent confocal microscopy recording of Roco2-GFP/*roco2*$^{-}$ cells during late stage of cytokinesis (one frame/12 sec).

MOVIE M9. DIC microscopy recording of Roco2$^{\Delta LRR}$/*roco2*$^{-}$ chemotaxing cells (one frame/6 sec).

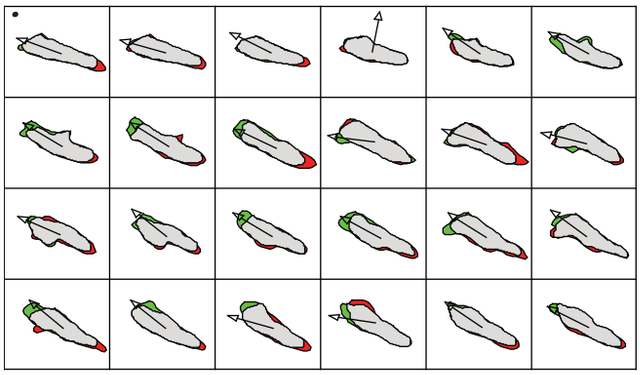
A Chemotaxis

KAx-3

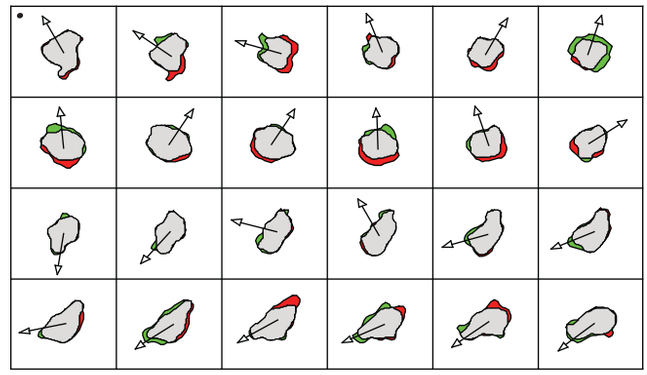
roco2^{-}



Roco2/*roco2*$^{-}$



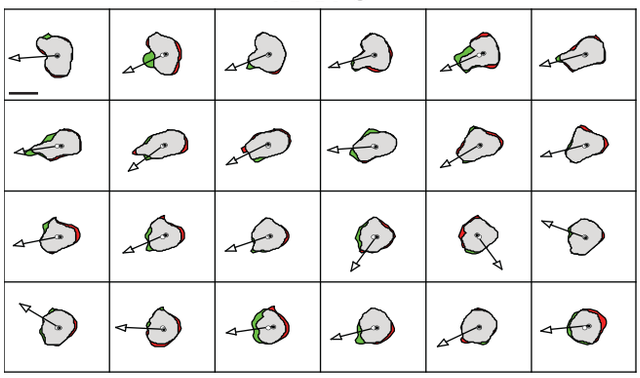
Roco2^{KD}/*roco2*$^{-}$



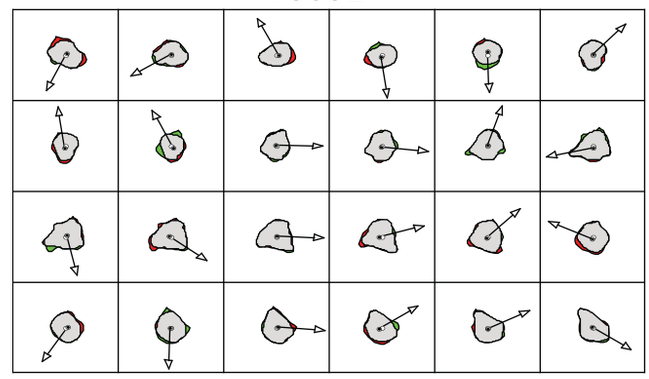
B Random motility

	Speed (µm/min)	Direction Change (deg.)	Persistence (µm/min-deg)	Roundness (%)	Area (sq.µm)
KAx-3	6.6 +/- 1.3	31.8 +/- 5.8	0.83 +/- 0.19	77.8 +/- 2.3	123 +/- 14
roco2^{-}	3.6 +/- 1.3	50.8 +/- 2.3	0.28 +/- 0.34	87.0 +/- 4.3	106 +/- 18
Roco2/*roco2*$^{-}$	7.4 +/- 1.2	34.0 +/- 3.7	0.79 +/- 0.2	77.3 +/- 4.7	110 +/- 10
Roco2^{KD}/*roco2*$^{-}$	4.1 +/- 1	49.0 +/- 3.3	0.34 +/- 0.28	82.0 +/- 2.4	109 +/- 10

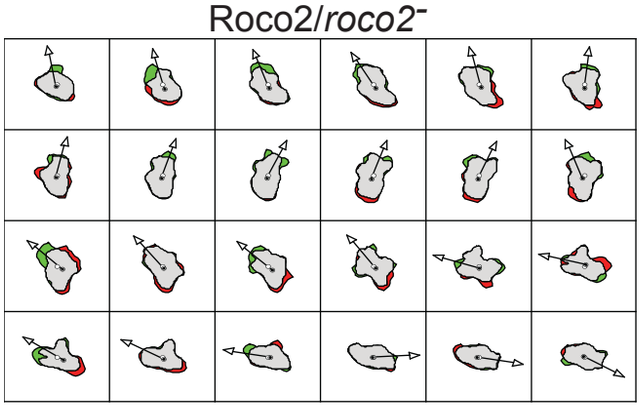
KAx-3



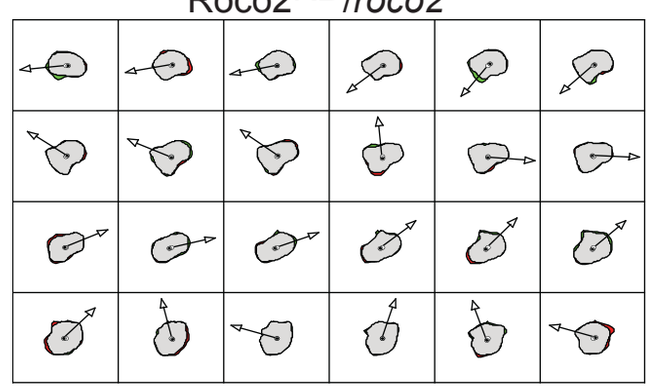
roco2^{-}



Roco2/*roco2*$^{-}$

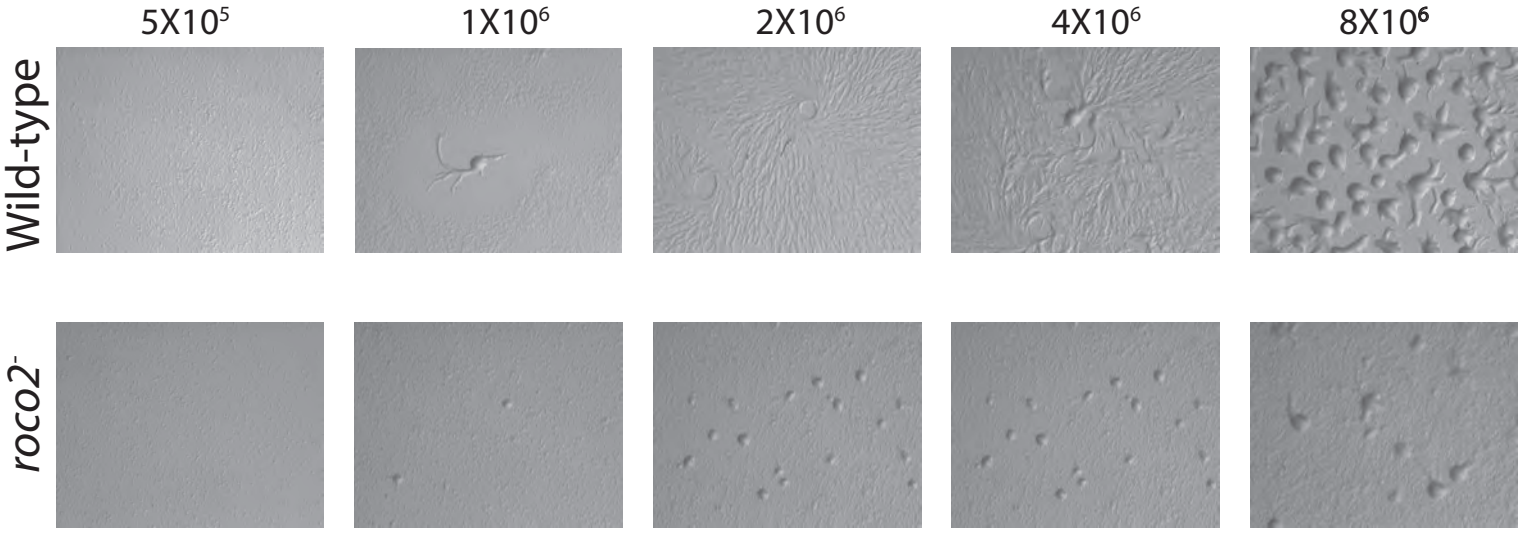


Roco2^{KD}/*roco2*$^{-}$

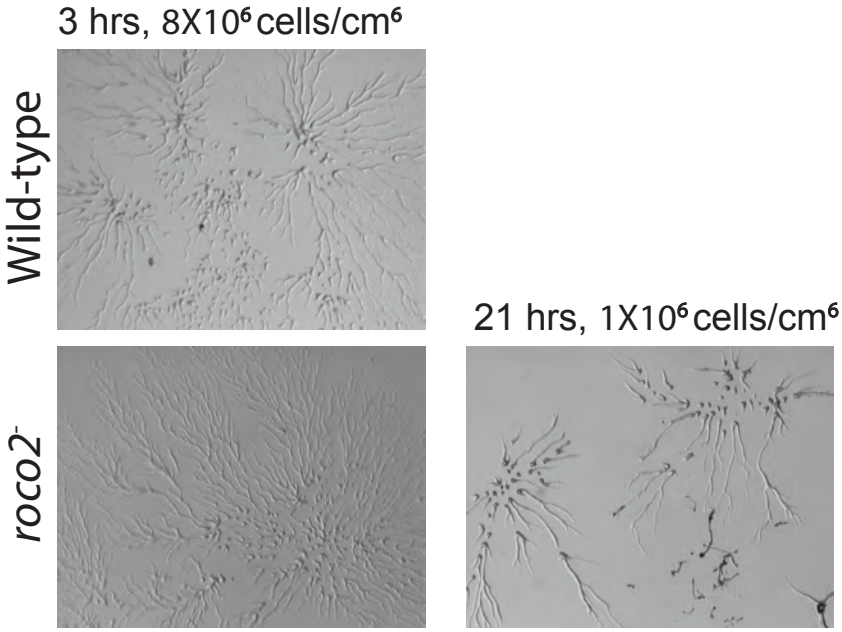


Kicka *et al*, Fig. S1

A. 6 hour development (cells/cm^2)



B. Cells pulsed for 6 hrs and plated for development (cells/cm^2)



Kicka *et al*, Fig. S2

A

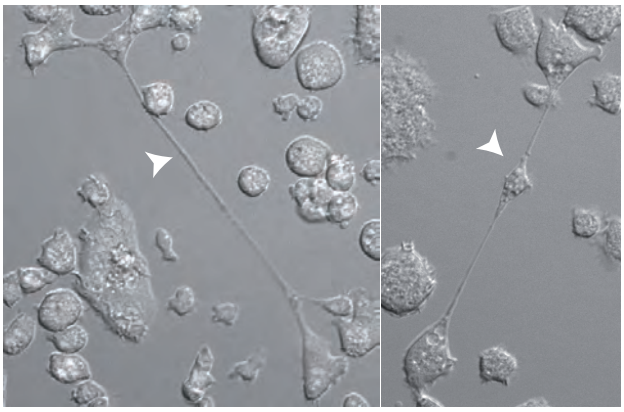
Substrate

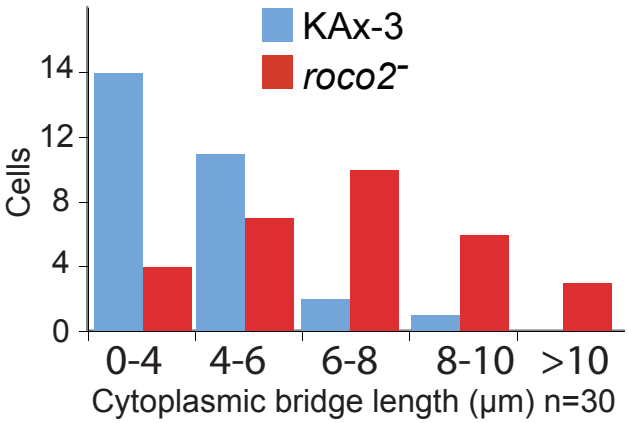
	DNA content						
	1-2n	2-4n	4-6n	6-8n	8-10n	10-12n	12-14n
KAx-3	34.19	57.60	6.59	1.07	0.55	0	0
Roco2/*roco2*$^-$	33.75	52.41	10.14	2.25	0.76	0	0
roco2$^-$	16.97	49.24	22.96	7.80	2.50	0.53	0
Roco2^{KD}/*roco2*$^-$	27.20	45.52	15.49	6.48	2.52	1.19	0.68
myosinII$^-$	26.75	50.44	18.16	4.20	0.45	0	0

Suspension

	DNA content									
	1-2n	2-4n	4-6n	6-8n	8-10n	10-12n	12-14n	14-16n	16-18n	18-20n
KAx-3	21.44	45.04	22.42	8.42	2.20	0.48	0	0	0	0
Roco2/*roco2*$^-$	14.90	44.11	27.80	10.93	2.02	0.24	0	0	0	0
roco2$^-$	4.87	32.15	29.22	17.93	10.69	4.05	0.94	0.15	0	0
Roco2^{KD}/*roco2*$^-$	8.49	38.66	29.14	13.62	6.75	1.71	1.16	0.47	0	0
myosinII$^-$	2.96	20	24.91	21.82	15.52	6.74	3.85	2.81	1.12	0.27

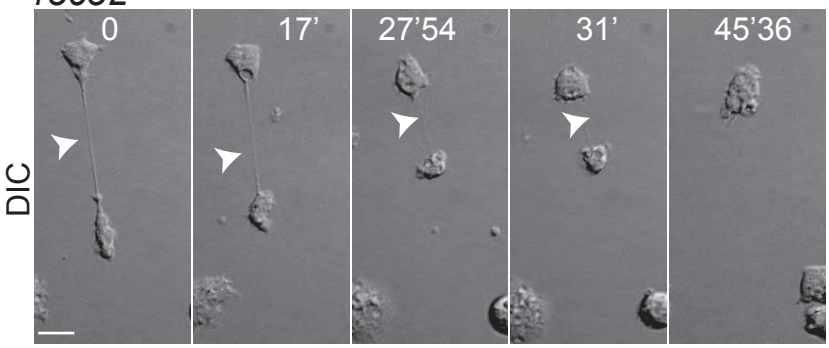
B *roco2*$^-$

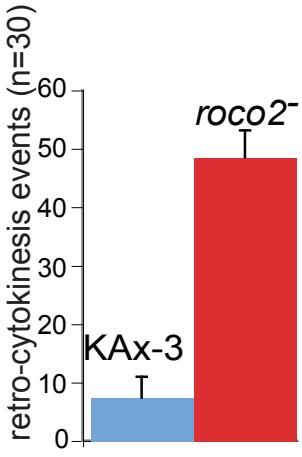




C

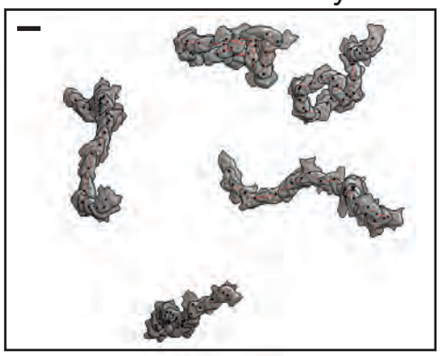
roco2$^-$





A

Random motility

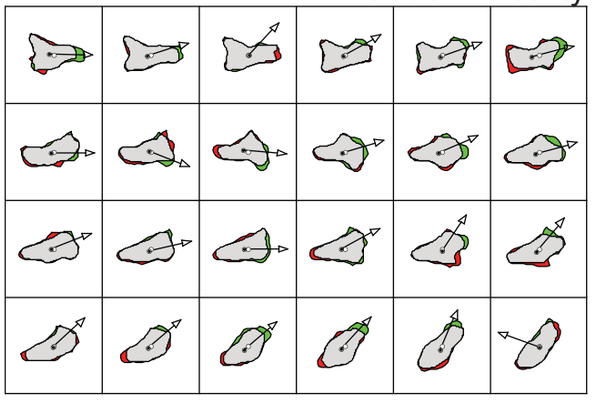


B

	Speed (µm/min)	Direction Change (deg.)	Persistence (µm/min-deg)	Roundness (%)	Area (sq.µm)
KAx-3	6.6 +/- 1.3	31.8 +/- 5.8	0.83 +/- 0.19	77.8 +/- 2.3	123 +/- 14
roco2^{-}	3.6 +/- 1.3	50.8 +/- 2.3	0.28 +/- 0.34	87 +/- 4.3	106 +/- 18
Roco2$^{\Delta LRR}$/*roco2*$^{-}$	7.3 +/- 1.4	31.3 +/- 3.5	0.81 +/- 0.2	73 +/- 2.3	122 +/- 12

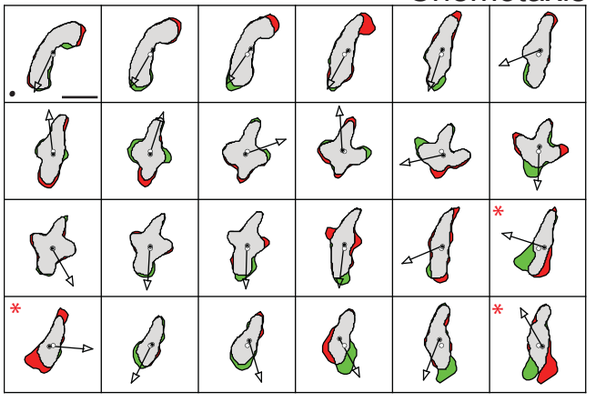
C

Roco2$^{\Delta LRR}$/*roco2*$^{-}$ Random motility



D

Roco2$^{\Delta LRR}$/*roco2*$^{-}$ Chemotaxis

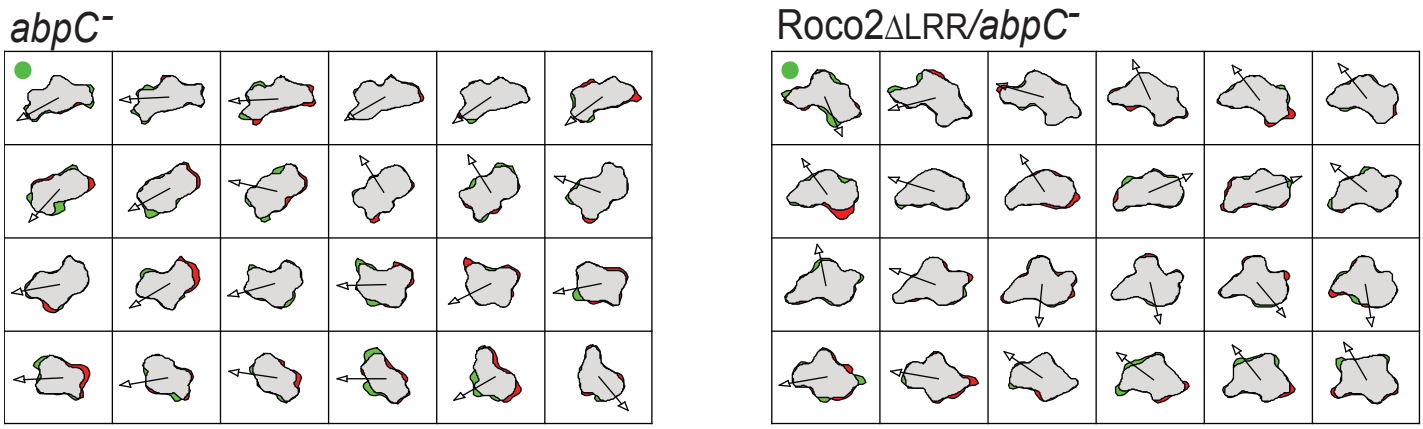


Kicka *et al*, Fig. S4

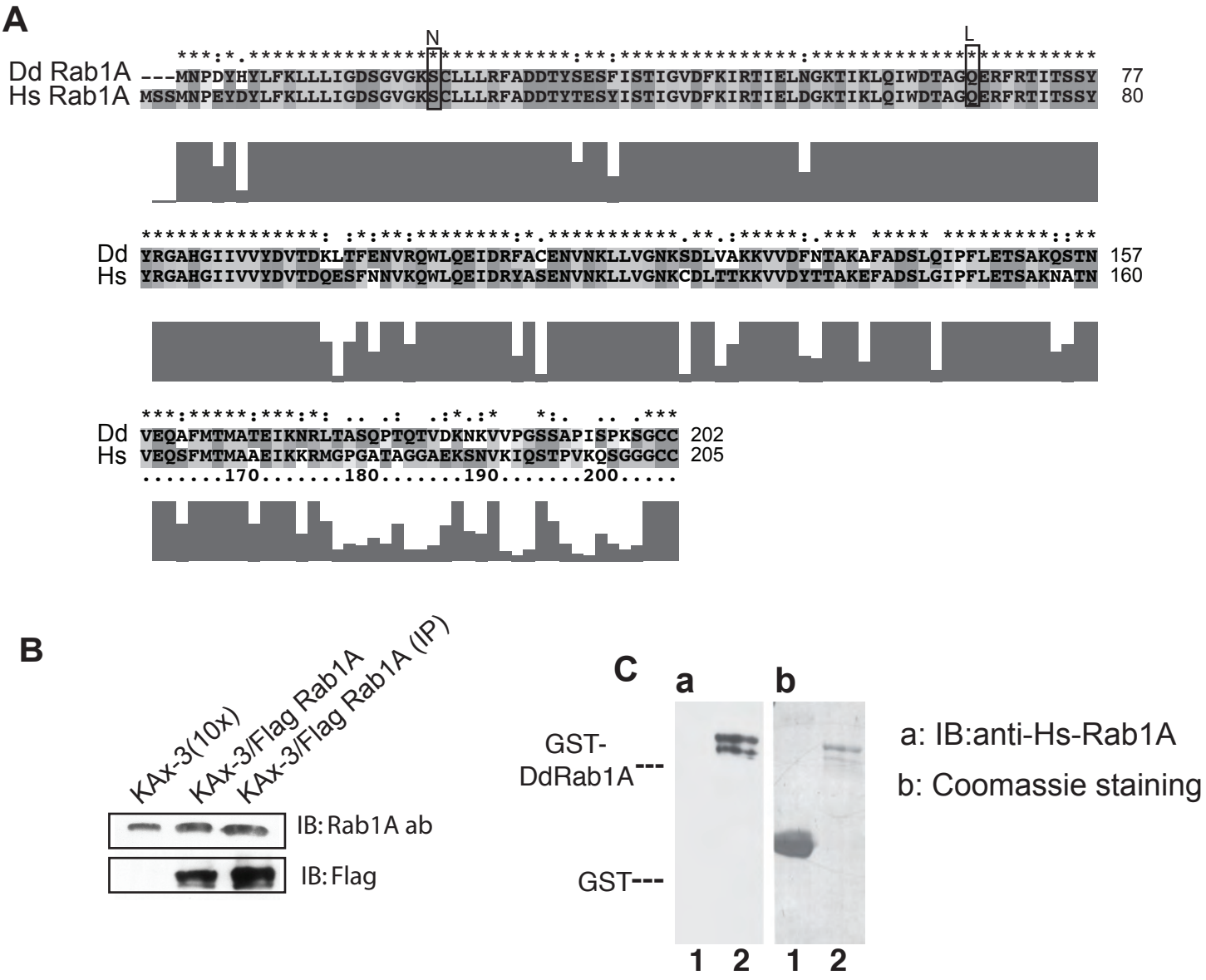
A

spectra KAx-3	spectra T7-Roco2	Protein MW (Da)	Database	Distinct Peptides	protein
0	588	174818.4	DDB0185215	69	Roco2
0	13	22562.9	DDB0191476	5	rab1A
0	7	92207.2	DDB0201554	5	Filamin (abpC)
0	11	92659.2	DDB0191363	9	ef-2A (elong. factor)
0	25	45669	DDB0215016	16	DJ-1 (chaperone)

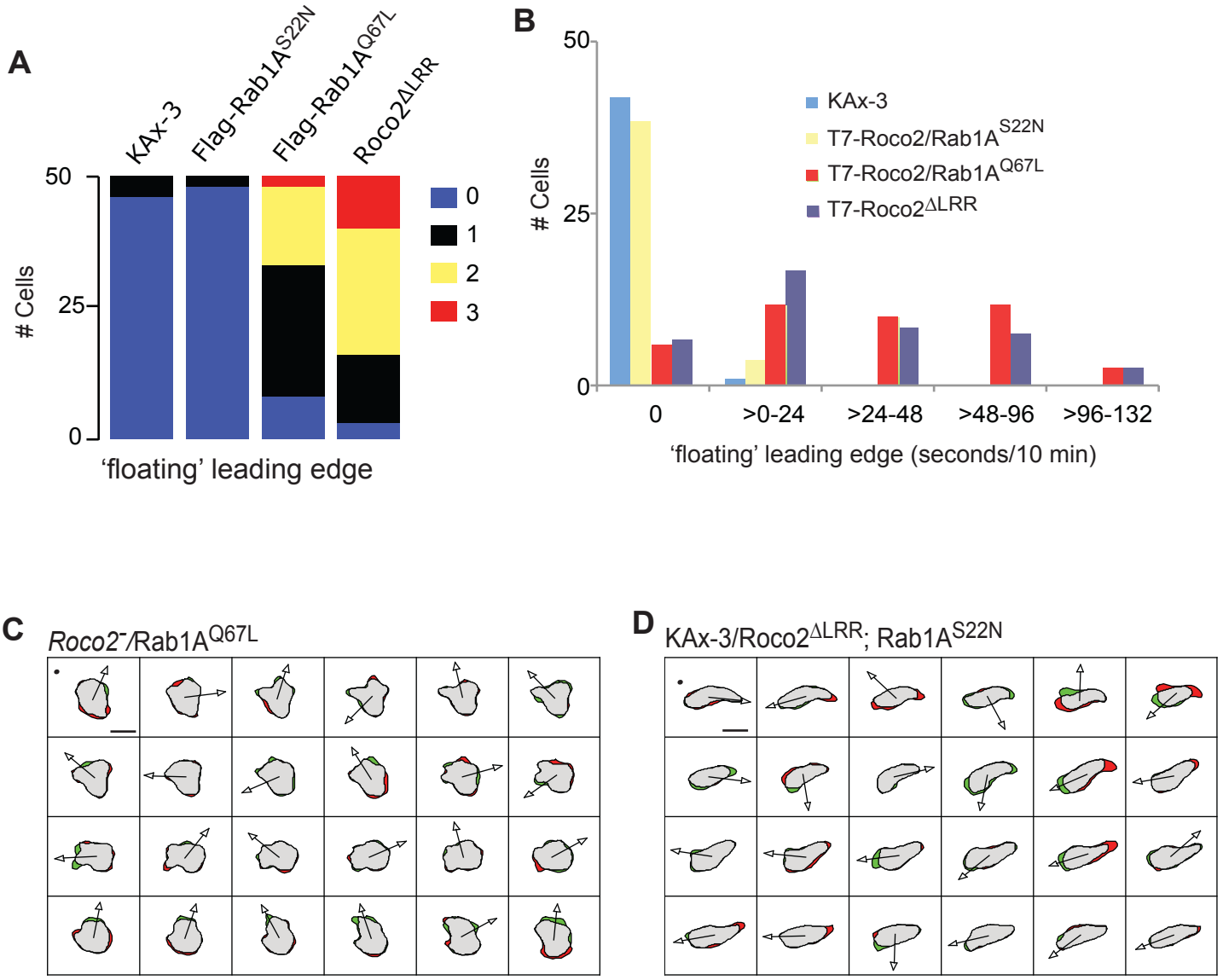
B



Kicka et al, Fig. S5



Kicka et al, Fig. S6



Kicka et al, Fig. S7