SUPPLEMENTARY MATERIAL

Figure S1. KIF9-CT402-GFP contacts podosomes. (A) Images from confocal time lapse movie of a primary human macrophage expressing KIF9-CT402-EGFP (green) and mRFP-Lifeact (red), labelling podosomes (see marked region of interest in suppl. videos 3+4). A KIF9-CT402-GFP decorated vesicle (arrowhead) contacting several podosomes (arrow) was monitored over time. Scale bar: 2 µm. (B) Intensity profile along a line (highlighted in yellow) across a KIF9-CT402-GFP decorated vesicle, which is in close contact to a podosome; observed at time point T9. Intensity values of GFP and mRFP fluorescence were plotted over of the length of the line drawn. (C) Intensity profile along a line (highlighted in yellow) across a KIF9-CT402-EGFP decorated vesicle, which is in very close contact to a podosome; observed at time point T84. Intensity values of GFP and mRFP fluorescence were plotted over of the length of the line drawn.

Figure S2. Effects of KIF9 expression constructs on podosomal matrix degradation. Confocal laser scanning micrographs of primary human macrophages expressing EGFP (A), KIF9-CT402-GFP(B), or KIF9 NT709-GFP (C) seeded on rhodamine-labeled gelatin matrix (red). Matrix degradation is visible as dark areas, upper insets show respective F-actin stainings by Cy5-labeled phalloidin (white), lower insets show respective GFP signals (green). White bar, 10 µm. (D) Evaluation of matrix degradation in cells treated with eGFP expression constructs. The degree of matrix degradation was analyzed by fluorescence measurements of each time 5 x 30 cells. Complete absence of labeled matrix beneath cells was set as 100% degradation. Cells were scored into groups according to matrix degradation (0-40%; 41-100%). For differences betweeen control values and values gained with eGFP constructs, a p-value < 0.05 was considered significant. Values are given as mean percentage ± SD of total counts in Table 1.

Figure S3. Subcellular localization of KIF9-NT709-GFP. (A, B, C) Images from confocal time lapse movie of a primary human macrophage expressing KIF9-NT709-GFP (green) and KIF9-mCherry (red). KIF9-NT709-GFP (A) and KIF9-mCherry (B) colocalize at vesicles in living cells (C). Scale bar: 5 µm. (D, D') Images from confocal time lapse movie of a primary human macrophage expressing KIF9-NT709-GFP (green) and mRFP-Lifeact (red), labelling podosomes. In the subcellular region indicated by white box, KIF9-NT709-GFP decorated vesicles (arrowhead) contacting several podosomes (arrow) were monitored over time (see suppl. video 5). Note repeated, non-random contact of KIF9 NT709-GFP-decorated vesicles with podosomes. Scale bar: 1 µm.

Figure S4. Reggie-1 knockdown does not affect Golgi integrity. Evaluation of Golgi architecture in primary human macrophages transfected with luciferase-specific siRNA, or siRNA specific for reggie-1, siRNA. Influence of each siRNA was evaluated 72 h after transfection. For each value, 3 x 30 cells were evaluated. Values are given as mean percentage ± SD of total counts (luciferase siRNA: 85.6 % ± 1.9 % for compact Golgi,14.4 % ± 1.9 % for dispersed Golgi; reggie-1 siRNA: 84.4 % ± 3.9 % for compact Golgi, 15.6 % ± 3.9 % for dispersed Golgi).

Figure S5. Knock down of reggie proteins does not influence podosome numbers. Evaluation of podosome formation in primary human macrophages transfected with luciferase-specific siRNA, siRNA specific for reggie-1, siRNA specific for reggie-2 or a combination of both. Influence of each siRNA was evaluated 72 h after transfection. For each value, 3 x 30 cells were evaluated. Values are given as mean percentage ± SD of total counts in Table 1.

Figure S6. Reggie1-GFP does not colocalize with matrix metalloproteinases. Confocal micrographs of macrophages overexpressing reggie1-GFP and stained for endogenous MMP-2 (A-C), MMP-7 (D-F), MMP-8 (G-I), MMP-9 (J-L), MMP-12 (M-O), MT1-MMP (P-R), or MT4-MMP (S-U) using specific primary antibodies and Alexa 568-labeled secondary antibody. Merged images are shown in (A,D,G,J,M,P,S), with single channel images of reggie1-GFP shown in (B,E,H,K,N,Q,T; green) and of respective MMPs in (C,F,I,L,O,R,U; red). White boxes in (A,D,G,J,M,P,S) indicate detail images in (Ai-Ci,Di-Fi,Gi-Ii,Ji-Li,Mi-Oi,Pi-Ri,Si-Ui). White bars indicate 10 μm.

Figure S7. Immunoprecipitations of different KIF9 constructs. Lysates of primary human macrophages immunoprecipitated with anti-GFP antibody coupled to magnetic beads. Silver-stained PAA gel, left lane: cells transfected with full length KIF9-GFP; right lane: cells transfected with GFP-KIF9-CT81 construct. Arrow indicates band subsequently identified by mass spectrometry as reggie-1/-2. Arrowheads indicate bands corresponding to KIF9-GFP GFP (left lane) and GFP-KIF9-CT81 (right lane), as judged by their mobility on PAA gels. Molecular mass in kilodaltons is indicated on the left.

Video 1. video 1.mov

KIF9-GFP vesicles move along microtubules. Primary human macrophage expressing KIF9-GFP (green) and α-tubulin-mCherry (red), labeling microtubules. Confocal time lapse series of detail region indicated in Fig. 3B. (exposure time: 350 ms for green (491 nm), 350 ms for red (561 nm), frame rate: 4 f/s; sequence: 231 s).

Video 2. video 2.mov

KIF9-GFP contacts podosomes. Primary human macrophage expressing KIF9-GFP (green) and β-actin-mRFP (red), labelling podosomes. Confocal time lapse series of substrate

attached part of the cell (exposure time 1000 ms for green (491 nm), 4000 ms for red (561 nm), frame rate: 10 f/s; sequence: 1225 s).

Video 3. video 3.mov

KIF9-CT402-GFP positive vesicles contact podosomes. Primary human macrophage co-expressing KIF9-CT402-GFP (green) and mRFP-Lifeact (red). Note repeated, non-random contact of KIF9 construct-decorated vesicles with podosomes (labeled by mRFP-Lifeact). A dual channel confocal time lapse movie was acquired using an asynchronous mode as follows: exposure time 250ms for green (491nm) and 350ms for red (561nm), acquisition frame rate of 20 time points per minute for the green and 5 time points per minute for the red channel. Movie replay frame rate: 6 f/s; sequence length: 300 s.

Video 4. video 4.mov

Video of detail region shown in video-3.avi.

Video 5. video 5.mov

KIF9-NT709-GFP positive vesicles contact podosomes. Primary human macrophage co-expressing KIF9-NT709-GFP (green) and mRFP-Lifeact (red). Note repeated, non-random contact of KIF9 construct-decorated vesicles with podosomes (labeled by mRFP-Lifeact). A dual channel confocal time lapse movie was acquired using an asynchronous mode as follows: exposure time 300ms for green (491nm) and 250ms for red (561nm), acquisition frame rate of 20 time points per minute for the green and 5 time points per minute for the red channel. Movie replay frame rate: 4 f/s; sequence length: 111 s.

Video 6. video 6.mov

Reggie1-GFP and KIF9-mCherry vesicles contact each other. Primary human macrophage expressing reggie1-GFP and KIF9-mCherry. Confocal time lapse series of detail region (G´) indicated in Fig. 9G (exposure time: 500 ms for green (491nm), 1 s for red (561nm), frame rate: 6 f/s; sequence length: 10 min).

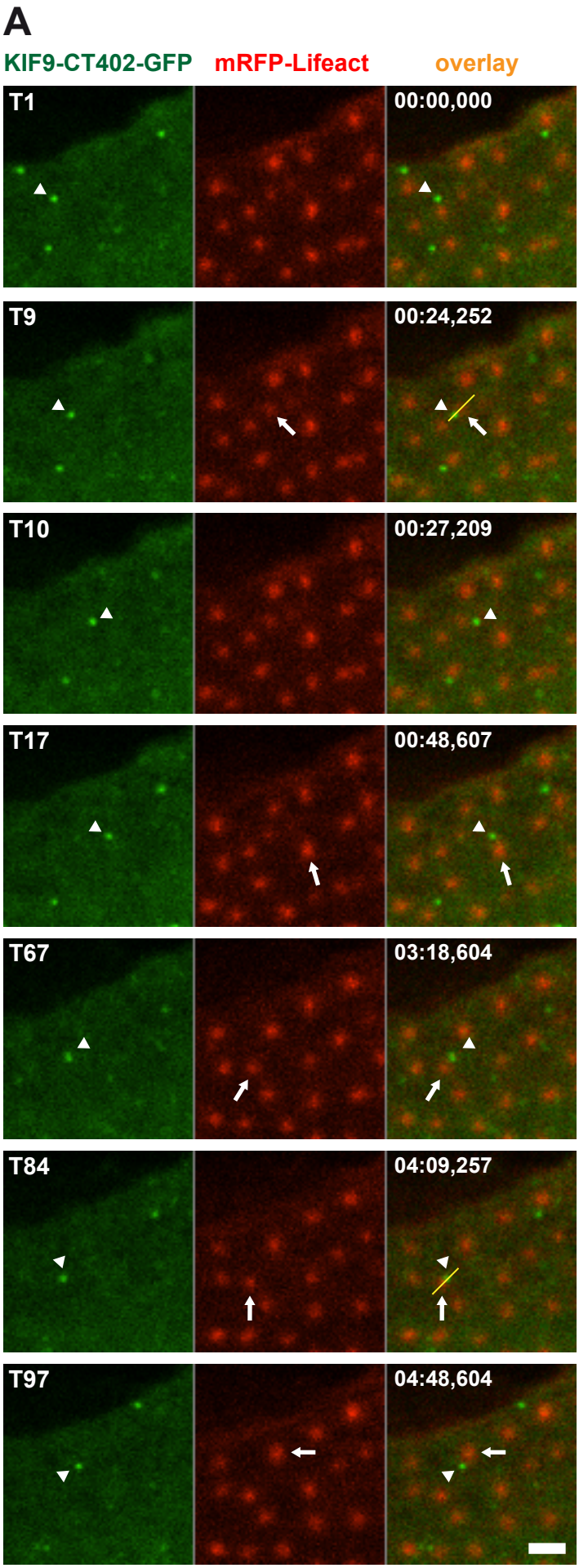
Video 7. video 7.mov

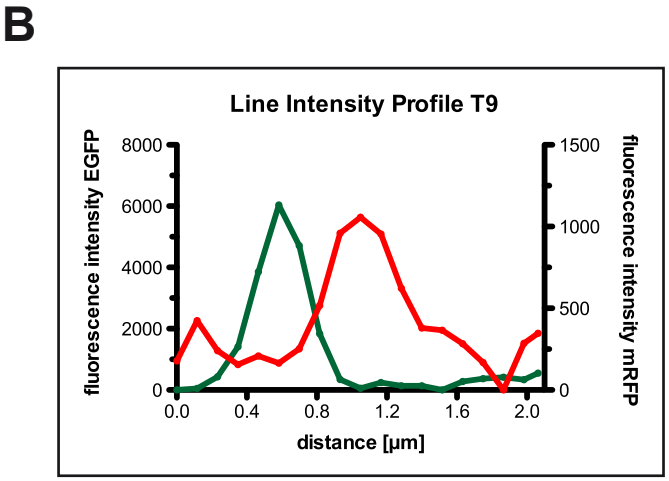
Reggie1-GFP and KIF9-mCherry vesicles contact each other. Primary human macrophage expressing reggie1-GFP and KIF9-mCherry. Confocal time lapse series of detail region (H´) indicated in Fig. 9H (exposure time: 250ms for green (491nm), 750ms for red (561nm), frame rate: 6 f/s; sequence length: 5 min).

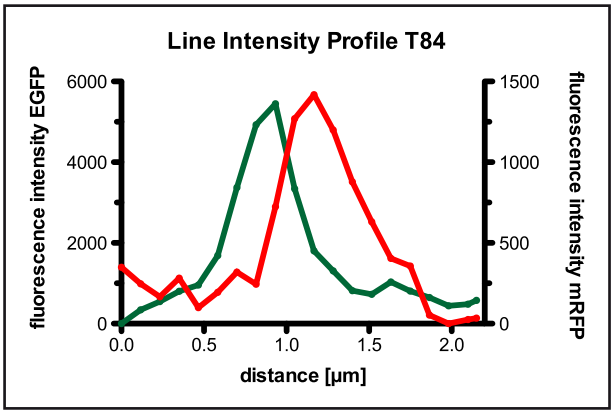
Video 8. video 1.mov

Reggie1-GFP and KIF9-mCherry vesicles contact each other. Primary human macrophage expressing reggie1-GFP and KIF9-mCherry. Confocal time lapse series of detail region (H´´) indicated in Fig. 9H (exposure time: 250 ms for green (491nm), 750 ms for red (561nm), frame rate: 6 f/s; sequence length: 5 min).

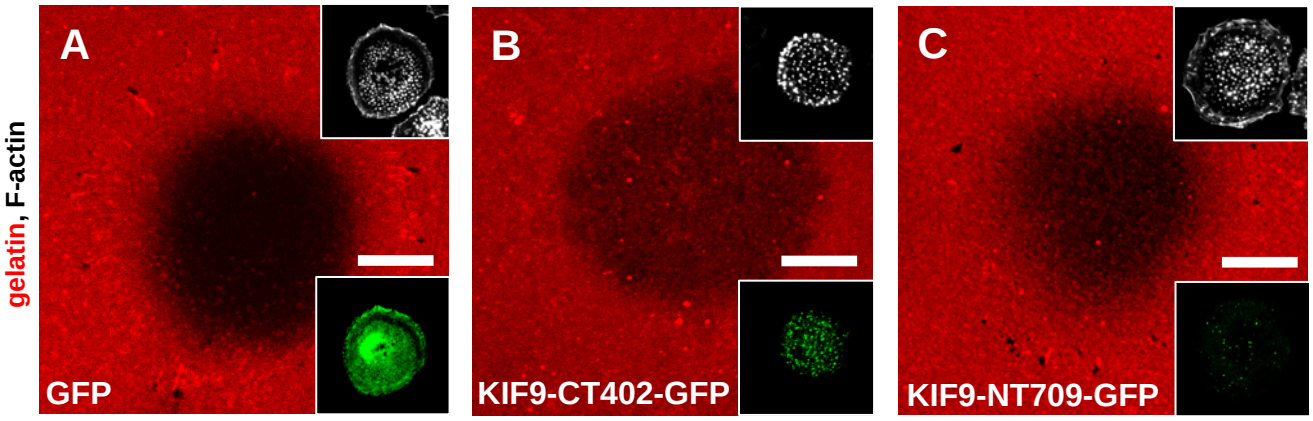
Suppl. Fig. 1

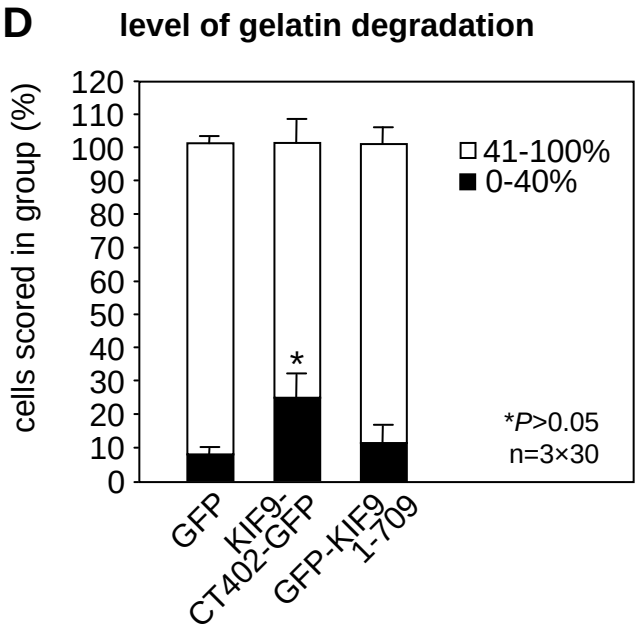

A
KIF9-CT402-GFP
mRFP-Lifeact
overlay
T1
00:00,000
T9
00:24,252
T10
00:27,209
T17
00:48,607
T67
03:18,604
T84
04:09,257
T97
04:48,604


B
Line Intensity Profile T9
fluorescence intensity EGFP
fluorescence intensity mRFP
distance [µm]
8000
6000
4000
2000
0
1500
1000
500
0
0.0
0.4
0.8
1.2
1.6
2.0

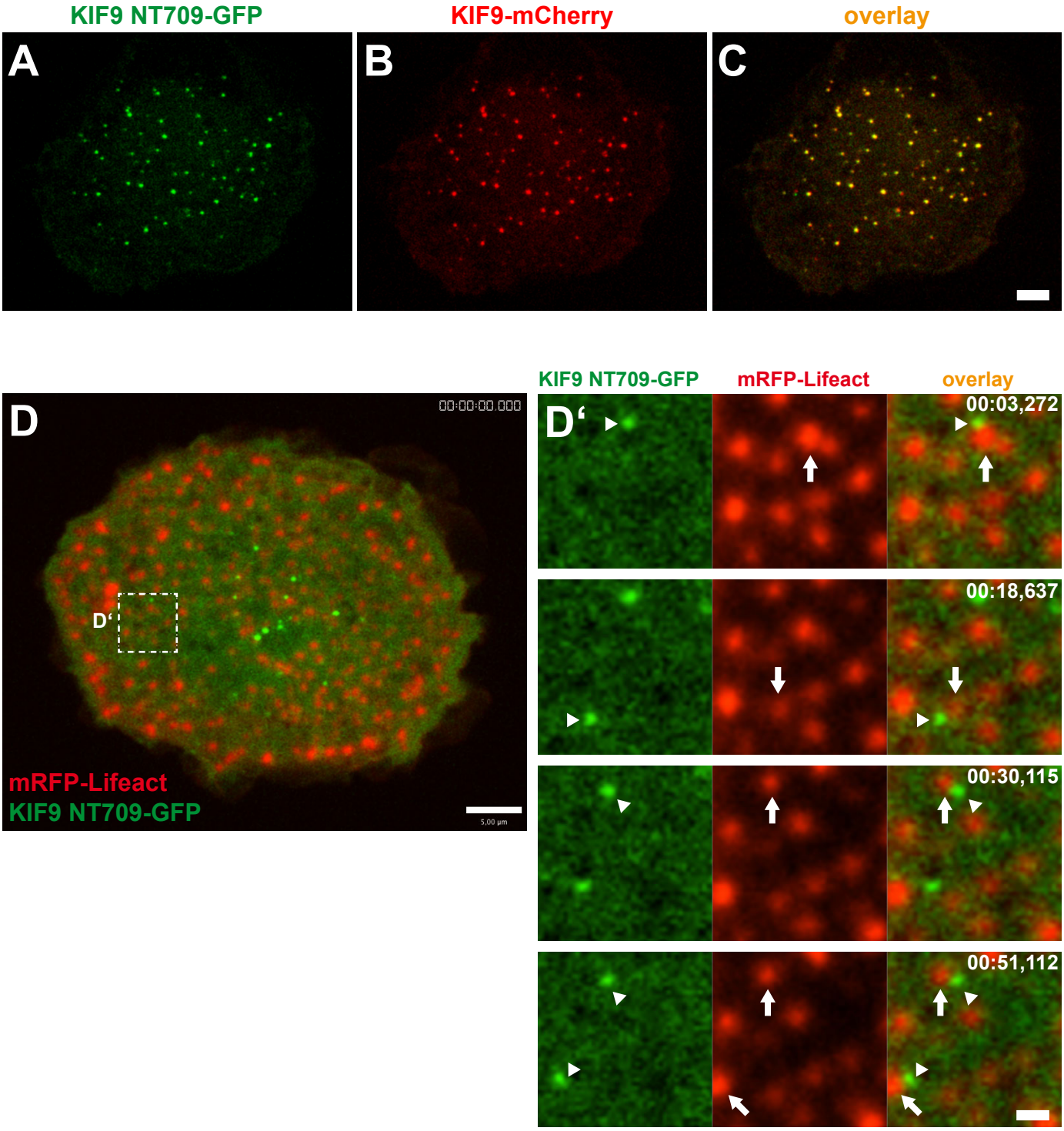

C
Line Intensity Profile T84
fluorescence intensity EGFP
fluorescence intensity mRFP
distance [µm]
6000
4000
2000
0
1500
1000
500
0
0.0
0.5
1.0
1.5
2.0

Suppl. Fig. 2

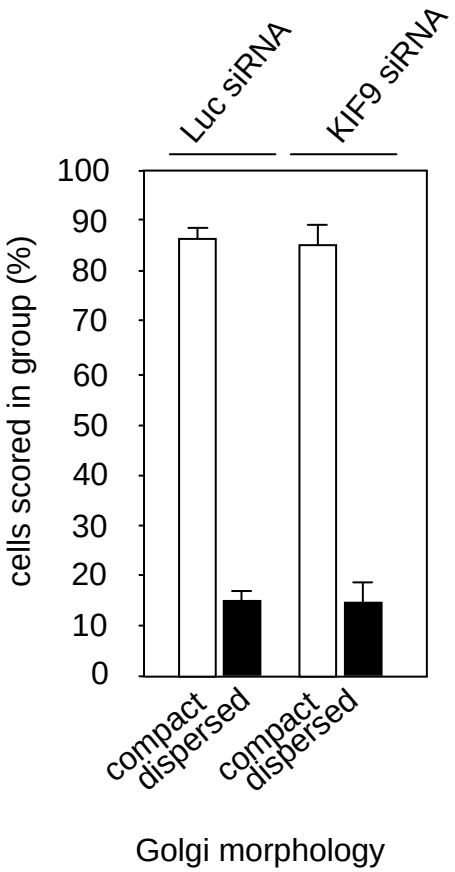

gelatin, F-actin
A
GFP
B
KIF9-CT402-GFP
C
KIF9-NT709-GFP


D
level of gelatin degradation
cells scored in group (%)
120
110
100
90
80
70
60
50
40
30
20
10
0
41-100%
0-40%
*
*P>0.05
n=3×30
GFP
KIF9-CT402-GFP
GFP-KIF9 1-709

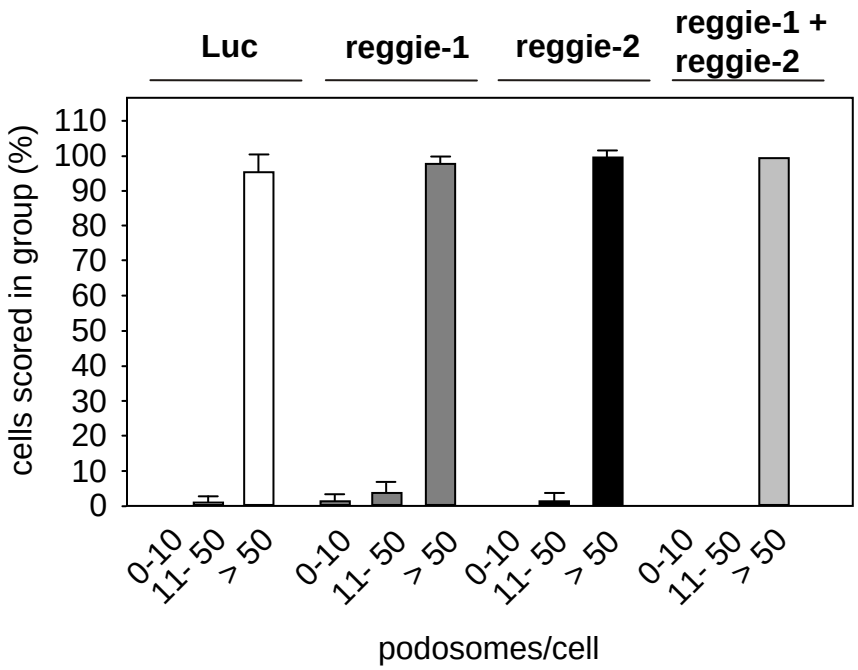
Suppl. Fig. 3


KIF9 NT709-GFP
KIF9-mCherry
overlay
A
B
C
D
00:00:00.000
D‘
mRFP-Lifeact
KIF9 NT709-GFP
5,00 µm
KIF9 NT709-GFP
mRFP-Lifeact
overlay
D‘
00:03,272
00:18,637
00:30,115
00:51,112

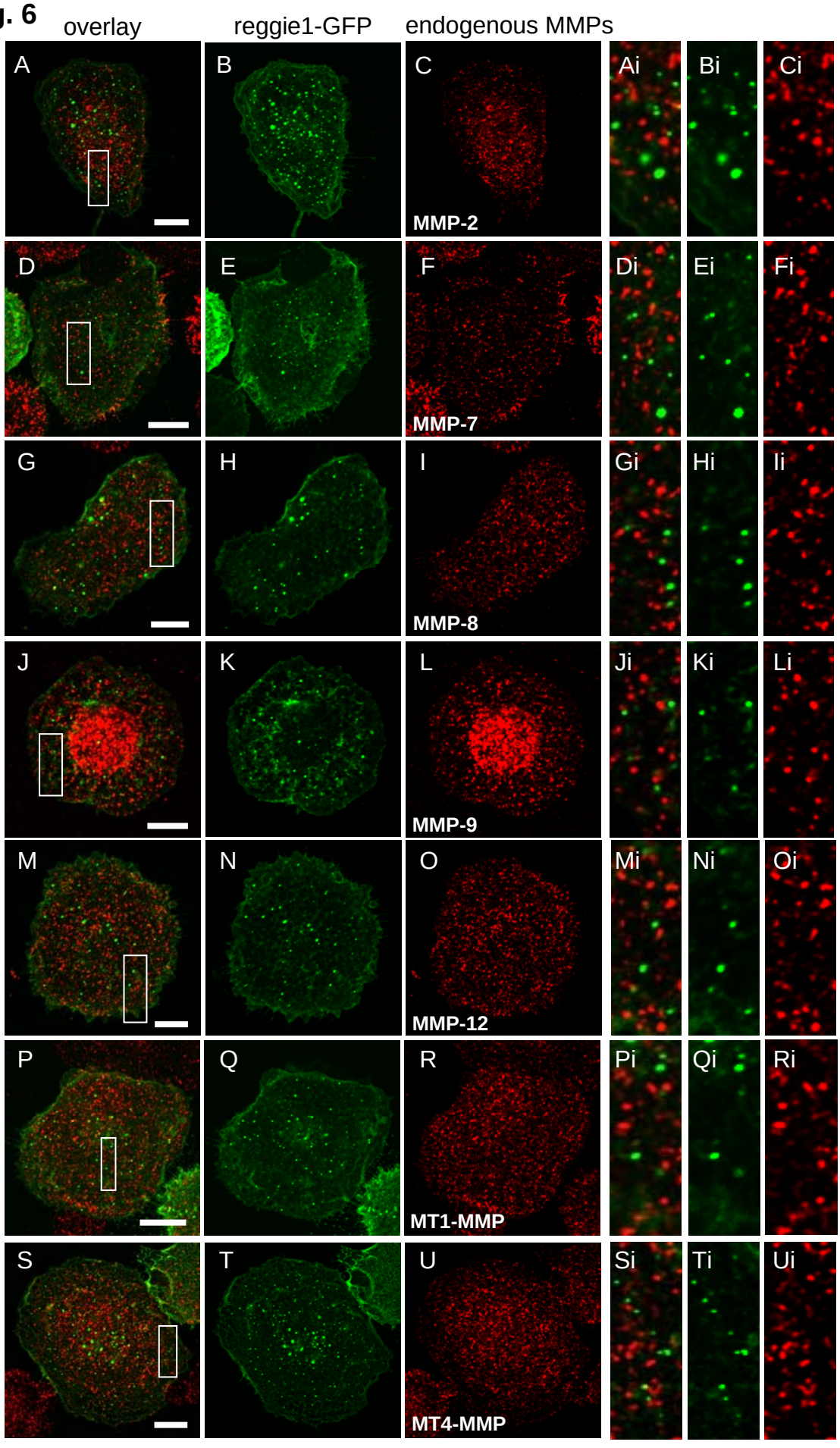
Suppl. Fig. 4


Luc siRNA
KIF9 siRNA
100
90
80
70
60
50
40
30
20
10
0
cells scored in group (%)
compact
dispersed
compact
dispersed
Golgi morphology

Suppl. Fig.5


Luc
reggie-1
reggie-2
reggie-1 +
reggie-2
cells scored in group (%)
110
100
90
80
70
60
50
40
30
20
10
0
0-10
11- 50
> 50
podosomes/cell

Suppl. Fig. 6


overlay
reggie1-GFP
endogenous MMPs
A
B
C
Ai
Bi
Ci
MMP-2
D
E
F
Di
Ei
Fi
MMP-7
G
H
I
Gi
Hi
Ii
MMP-8
J
K
L
Ji
Ki
Li
MMP-9
M
N
O
Mi
Ni
Oi
MMP-12
P
Q
R
Pi
Qi
Ri
MT1-MMP
S
T
U
Si
Ti
Ui
MT4-MMP

Suppl. Fig. 7