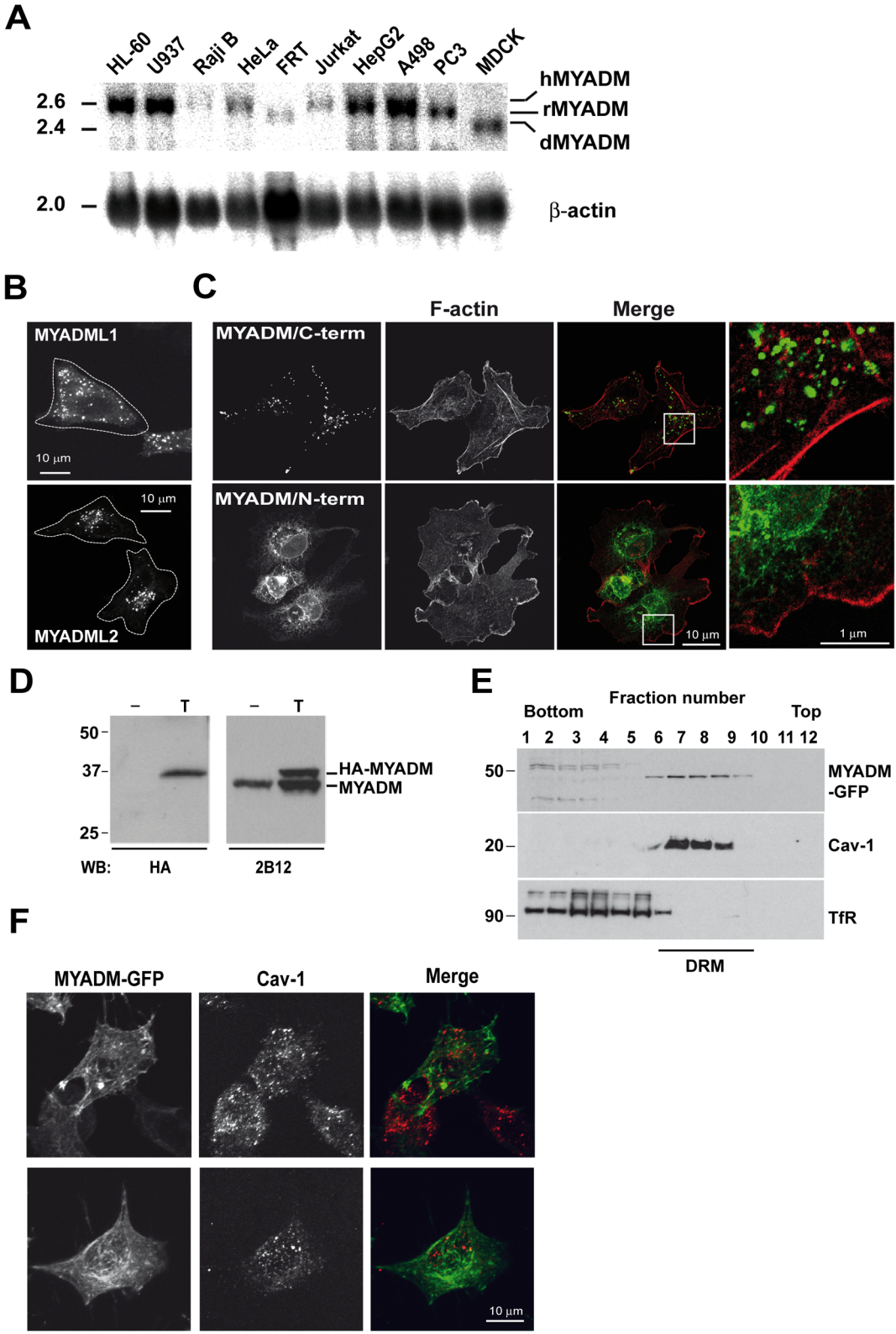
A
HL-60
U937
Raji B
HeLa
FRT
Jurkat
HepG2
A498
PC3
MDCK
2.6
2.4
hMYADM
rMYADM
dMYADM
2.0
β-actin
B
MYADML1
10 μm
10 μm
MYADML2
C
F-actin
Merge
MYADM/C-term
MYADM/N-term
10 μm
1 μm
D
–
T
–
T
50
37
25
HA-MYADM
MYADM
WB:
HA
2B12
E
Fraction number
Bottom
Top
1 2 3 4 5 6 7 8 9 10 11 12
50
20
90
MYADM
-GFP
Cav-1
TfR
DRM
F
MYADM-GFP
Cav-1
Merge
10 μm

Figure S1

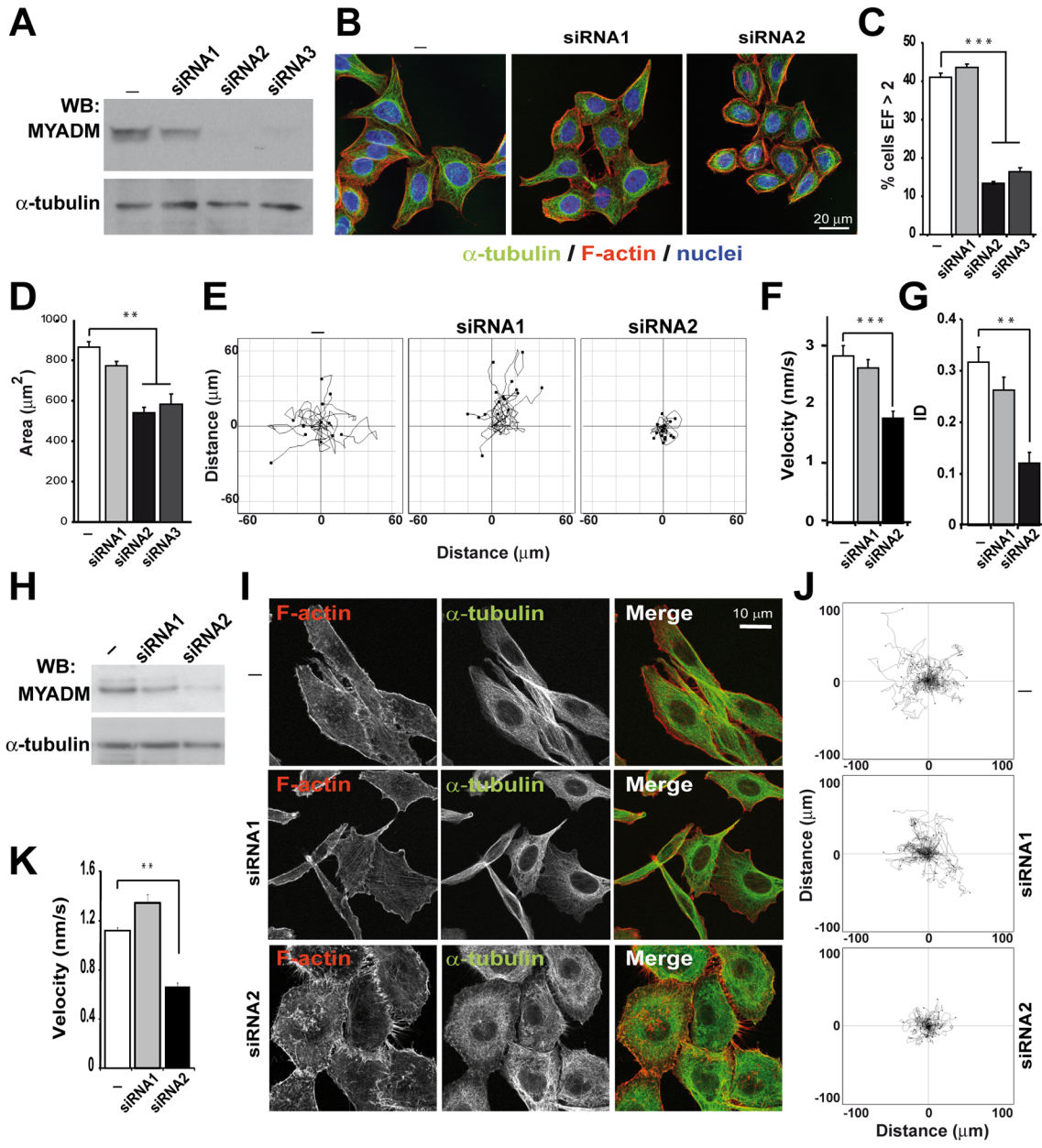
A
–
siRNA1
siRNA2
siRNA3
WB:
MYADM
α-tubulin
B
–
siRNA1
siRNA2
20 μm
α-tubulin / F-actin / nuclei
C
% cells EF > 2
***
D
Area (μm2)
**
E
–
siRNA1
siRNA2
Distance (μm)
F
Velocity (nm/s)
***
G
ID
**
H
WB:
MYADM
α-tubulin
I
F-actin
α-tubulin
Merge
10 μm
J
Distance (μm)
K
Velocity (nm/s)
**

Figure S2

Legends to Supplemental Figures

Figure S1. Characterization and expression of MYADM. (**A**) Expression of the MYADM gene in various cell lines. Total RNA was hybridized to cDNA probes specific to MYADM or β-actin. FRT and MDCK cells are of rat and dog origin, respectively. The rest of the cell lines in the panel are of human origin. The positions of the h (human), r (rat) and d (dog) MYADM mRNA species are indicated. (**B**) Constructs expressing GFP fusions of MYADML1 and MYADML2-GFP were transiently expressed in Cos-7 cells and analyzed by confocal microscopy. Dotted lines indicate the cell contour. (**C**) Cos-7 cells were transfected with constructs encoding HA-tagged forms of either the C-terminal (MYADM/C-term) or the N-terminal (MYADM/N-term) halves of the MYADM molecule and stained with anti-HA antibodies to detect the MYADM fragments and with fluorescent phalloidin to visualize F-actin. An enlargement of the boxed regions is shown in the right panels. (**D**) Characterization of a novel mAb to MYADM. Extracts from untransfected (-) or transfected (T) Cos-7 cells transiently expressing human MYADM tagged with the HA epitope were subjected to immunoblot analysis with either mAb 2B12 or anti-HA mAb. A protein band corresponding to endogenous MYADM was observed in the two extracts immunoblotted with mAb 2B12. (**E**) HeLa cells stably expressing MYADM-GFP were extracted with 1% Triton X-100 at 4ºC and centrifuged to equilibrium in sucrose density gradients. Aliquots from each fraction were analyzed by immunoblotting for MYADM (2B12 mAb) and for caveolin-1 (Cav-1) and transferrin receptor (TfR), used as markers of the DRM and soluble fractions, respectively. (**F**) HeLa cells stably expressing MYADM-GFP were stained for caveolin-1. Two images from different fields are shown.

Figure S2. Effect of MYADM siRNA expression on cell morphology and migration in HeLa and PC3 cells. (**A**) HeLa cells were transfected with the indicated siRNA and, 48 h later, MYADM levels were analyzed by immunoblot with mAb 2B12. The levels of α-tubulin were analyzed as a loading control. (**B**) Untransfected (-), siRNA1- and siRNA2-transfected cells were fixed and stained for F-actin, α-tubulin and nuclei. (**C**, **D**) Percentage of cells with an elliptical factor (EF) > 2 (C) and spreading area (D). (**E-G**) Untransfected (-), siRNA1- and siRNA2-transfected HeLa cells were analyzed by

time-lapse videomicroscopy. The trajectories of 12-16 cells were tracked (E). The velocity of the cells (F) and their index of directionality (G) were determined. (**H**-**K**) PC3 cells were transfected with the indicated siRNA and 48 h later analyzed by immunoblotting with anti-MYADM mAb 2B12 or anti-α-tubulin antibodies (H), or stained for F-actin and α-tubulin (I), or examined in random migration assays (J, K). The velocity of the cells is shown (K). The trajectories of 23-34 cells per condition are presented in (J). The mean ± SEM from three independent experiments are presented in (C, D, F, G, K); **, $p < 0.01$; ***, $p < 0.001$.