Supplementary Figure 1. HUVECs were transfected with MYADM-GFP for 48 h, fixed and stained for PECAM-1 (A), the adherens junction markers β-catenin and p120-catenin (B) the tight junction marker occludin (C) and nectin-2 (D).

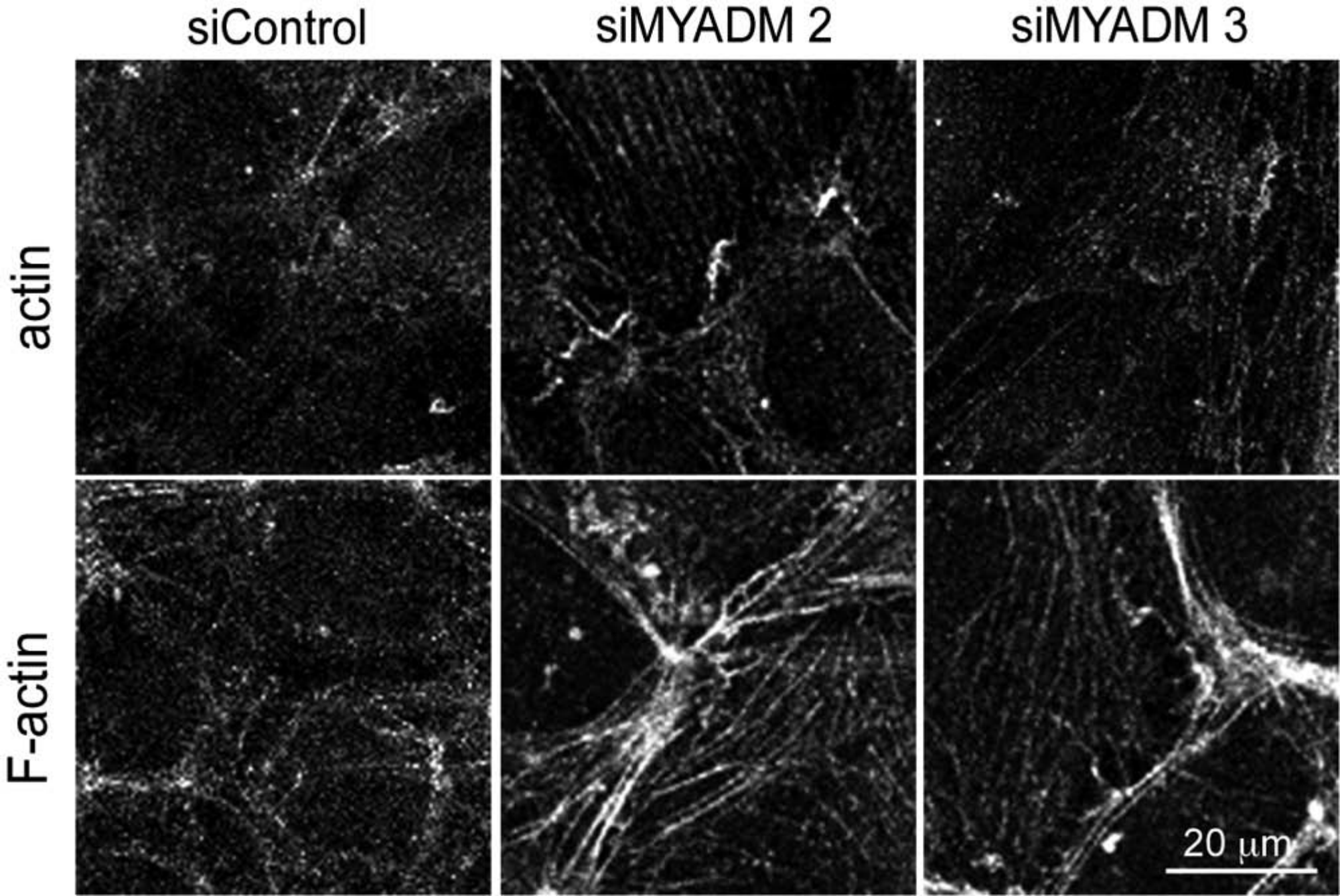
Supplementary Figure 2. HUVECs were transfected with the indicated siRNAs for 72 h, fixed (see materials and methods) and stained for total actin with anti-actin antibody, or for F-actin with TRITC-phalloidin. The F-actin/total actin ratio of three different experiments are shown in Figure 2F.

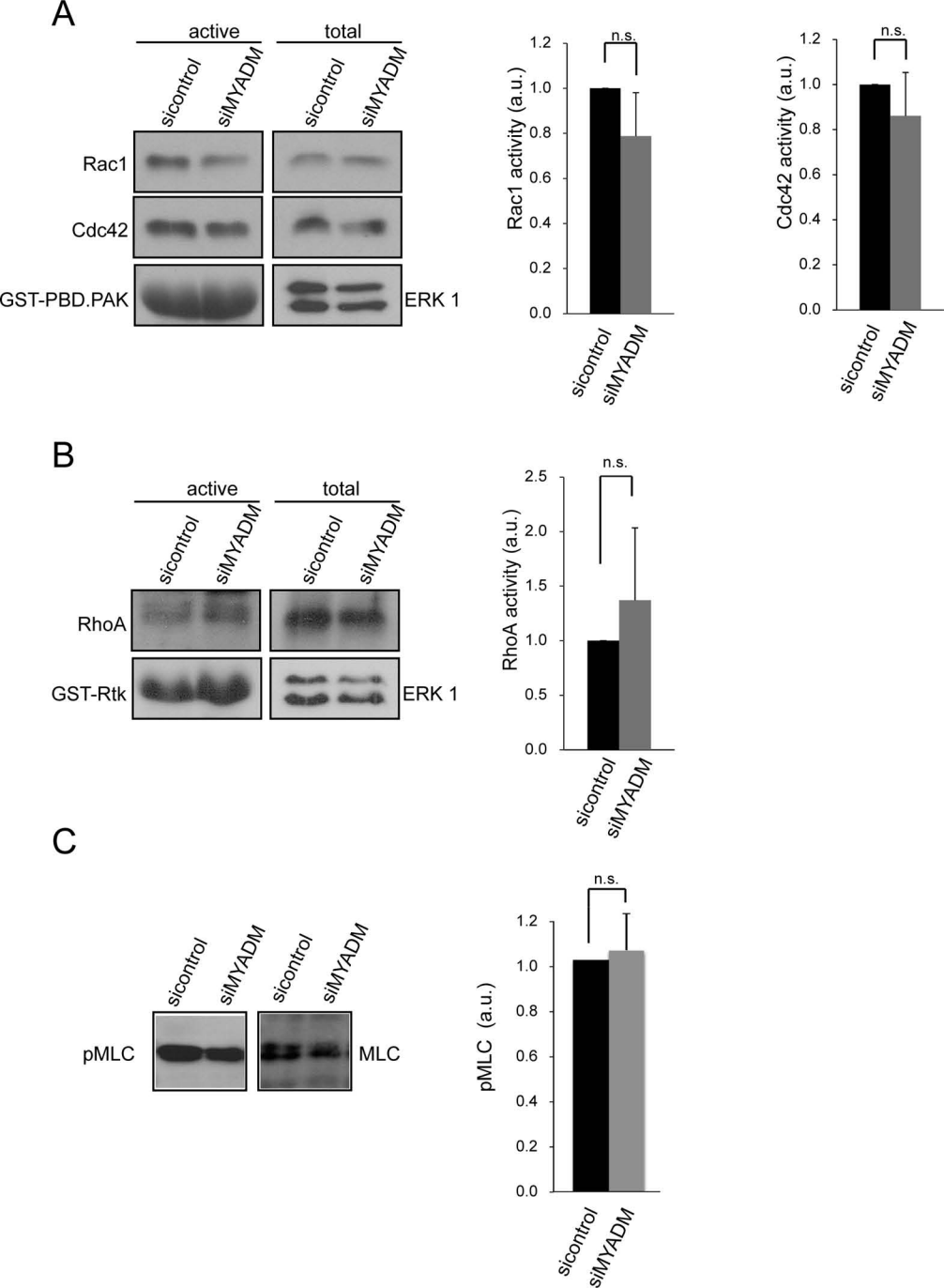
Supplementary Figure 3. HUVECs were transfected with the indicated siRNAs for 72 h, lysed and subjected to pulldown assay to analyse active, GTP-loaded Rac1 and Cdc42 (A) or active, GTP-loaded RhoA (B). (C) Western blot analysis of phosphorylated Ser-19 of MLC (pMLC) in siRNA transfected cells. The right graph show the mean + SEM from three independent experiments.

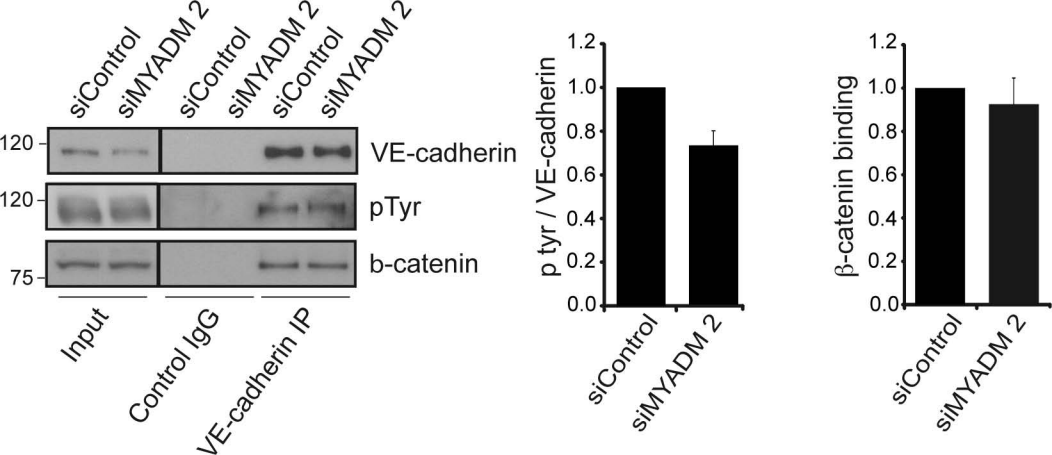
Supplementary Figure 4. HUVECs were transfected with the indicated siRNAs for 72 h, lysed and subjected to immunoprecipitation assays with a control IgG antibody or anti-VE-cadherin antibody. Lysates (input) and immunoprecipitates were immunobloted with the indicated antibodies. Right graphs show quantitations of phosphorylated (pTyr)-VE-cadherin/total VE-cadherin ratio and of β-catenin association with VE-cadherin immunoprecipitates. Graphs show the mean + SEM from two independent experiments.

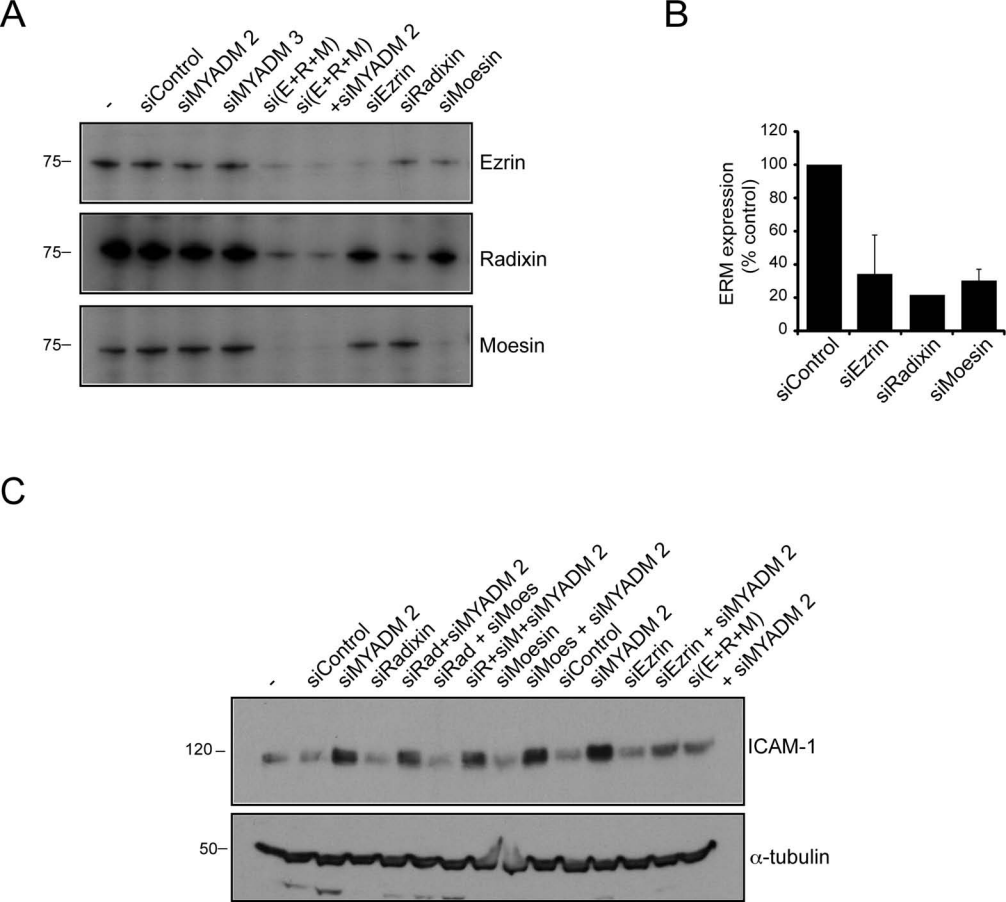
Supplementary Figure 5. Effect of single knockdown of ezrin, radixin and moesin on ERM and ICAM-1 protein expression. (A) HUVECs were transfected with the indicated siRNAs for 72 h and blotted with antibodies specific for ezrin, radixin and moesin. (B) Quantitation of the effect of siRNAs for ezrin, radixin and moesin on their respective targets. The mean + SEM from two independent experiments is shown. (C) HUVECs were transfected with the indicated siRNAs for 72 h and ICAM-1 levels analyzed by Western blot.

Supplementary Figure 6. (A) HUVECs were stimulated or not with TNF-α for 8 h, lysed and fractionated into pellet, soluble (SOL) and detergent-resistant membranes (DRMs) as in Figure 1. Fractions were immunoblotted with the indicated antibodies. (B) HUVECs were stimulated with TNF-α for the indicated times, mRNA was isolated and the levels of MYADM mRNA were analysed by qPCR. Graphs show the mean + SEM from three independent experiments.

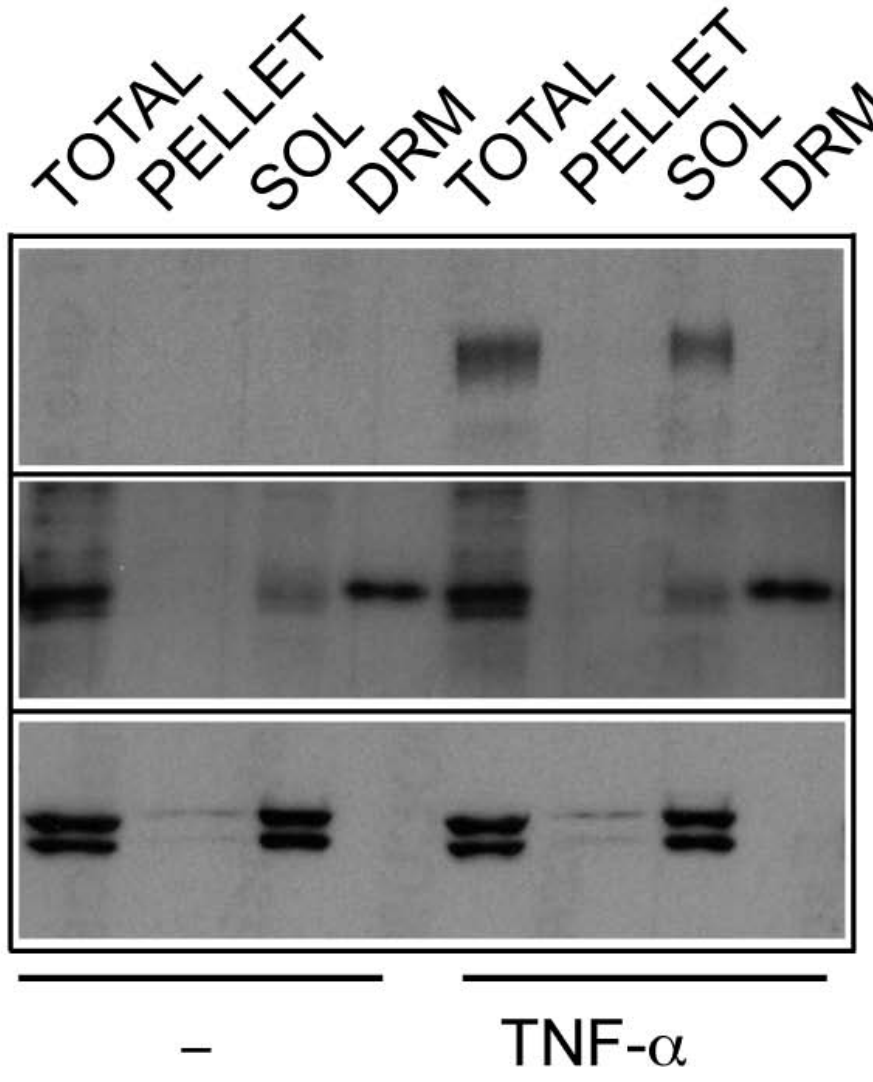

siControl
siMYADM 2
siMYADM 3
actin
F-actin
20 μm


A
active
total
sicontrol
siMYADM
sicontrol
siMYADM
Rac1
Cdc42
GST-PBD.PAK
ERK 1
Rac1 activity (a.u.)
n.s.
1.2
1.0
0.8
0.6
0.4
0.2
0.0
sicontrol
siMYADM
Cdc42 activity (a.u.)
n.s.
1.2
1.0
0.8
0.6
0.4
0.2
0.0
sicontrol
siMYADM
B
active
total
sicontrol
siMYADM
sicontrol
siMYADM
RhoA
GST-Rtk
ERK 1
RhoA activity (a.u.)
n.s.
2.5
2.0
1.5
1.0
0.5
0.0
sicontrol
siMYADM
C
sicontrol
siMYADM
sicontrol
siMYADM
pMLC
MLC
pMLC (a.u.)
n.s.
1.2
1.0
0.8
0.6
0.4
0.2
0.0
sicontrol
siMYADM

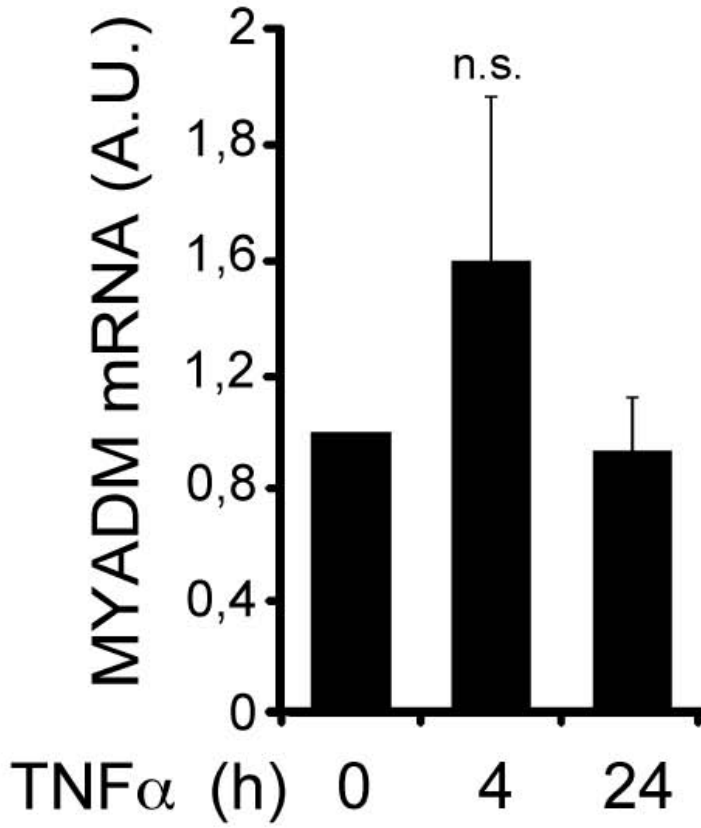

siControl
siMYADM 2
siControl
siMYADM 2
siControl
siMYADM 2
120
120
75
VE-cadherin
pTyr
b-catenin
Input
Control IgG
VE-cadherin IP
p tyr / VE-cadherin
1.2
1.0
0.8
0.6
0.4
0.2
0.0
siControl
siMYADM 2
β-catenin binding
1.2
1.0
0.8
0.6
0.4
0.2
0.0
siControl
siMYADM 2

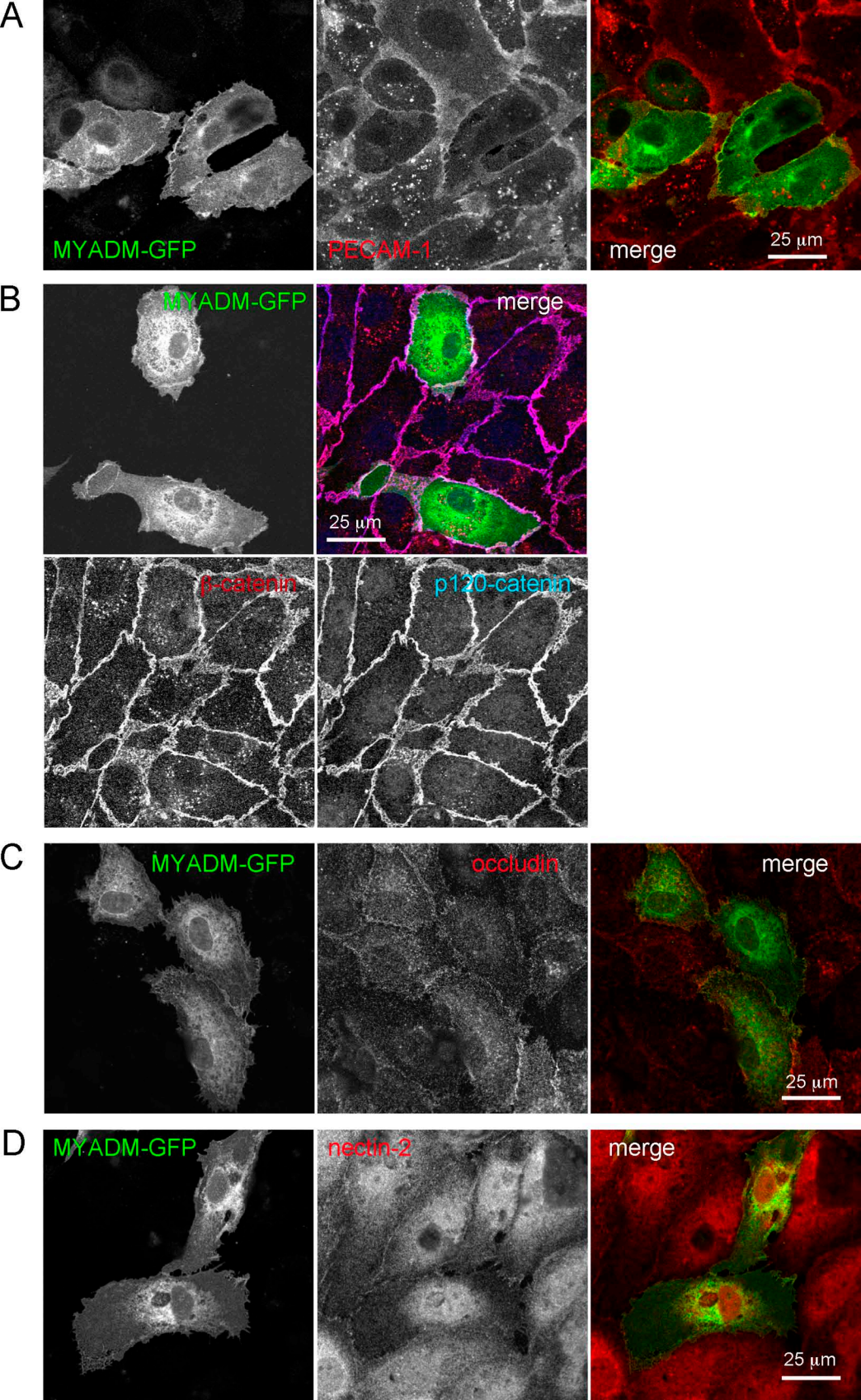

A
-
siControl
siMYADM 2
siMYADM 3
si(E+R+M)
si(E+R+M) +siMYADM 2
siEzrin
siRadixin
siMoesin
75–
Ezrin
75–
Radixin
75–
Moesin
B
ERM expression (% control)
120
100
80
60
40
20
0
siControl
siEzrin
siRadixin
siMoesin
C
-
siControl
siMYADM 2
siRadixin
siRad+siMYADM 2
siRad + siMoes
siR+siM+siMYADM 2
siMoesin
siMoes + siMYADM 2
siControl
siMYADM 2
siEzrin
siEzrin + siMYADM 2
si(E+R+M) + siMYADM 2
120–
ICAM-1
50–
α-tubulin

A


TOTAL
PELLET
SOL
DRM
TOTAL
PELLET
SOL
DRM
–
TNF-α

B


MYADM mRNA (A.U.)
2
1,8
1,6
1,2
0,8
0,4
0
n.s.
TNFα (h) 0 4 24


A
MYADM-GFP
PECAM-1
merge
25 μm
B
MYADM-GFP
merge
25 μm
β-catenin
p120-catenin
C
MYADM-GFP
occludin
merge
25 μm
D
MYADM-GFP
nectin-2
merge
25 μm