E08-04-0430 McCulloch

SUPPLEMENTARY FIGURE LEGENDS

S1.a- Quantification of representative blots of pull down assay with GST-RalGDS to determine levels of GTP-bound Rap1 associated with collagen and vitronectin coated beads. b- Quantification of experiments with $\alpha 2\beta 1$ blocking antibody or with control antibody to show importance of $\alpha 2\beta 1$ integrin in Rap1 activation.

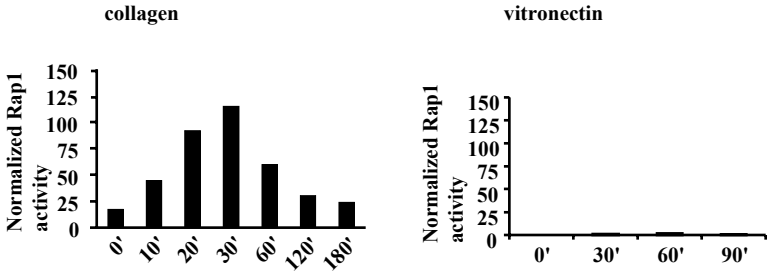
S2. Left panel: Immunoblot of Rap1 expression levels in fibroblasts treated with Rap1 siRNA (a), control siRNA (b), untreated cells (c) and cells treated with transfection reagent alone (d). Rap1-specific siRNA reduced Rap1 protein levels by 70% compared to cells treated with control siRNA in this representative experiment. Right panel: In three experiments, Rap1 was reduced by 65-75%; $p<0.05$.

S3. a-Rap1, Rap2 or β1 integrin immunoprecipitates of bead-associated proteins. Quantification (mean ± sem) is shown for 3 experiments. Collagen treated (T) and untreated (UT) samples. b- Treatment with ML-7 (25 μM) inhibited collagen-induced phosphorylation of MLC. c- Cells transfected with MLCK siRNA show 70-80% reduction in MLCK levels as evaluated by densitometry. d- Lysates prepared from fibroblasts treated with ionomycin exhibit time-dependent phosphorylation of MLC.

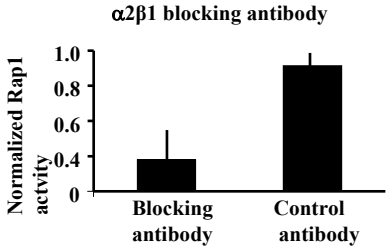
S4- GST-RalGDS fusion protein was used to determine levels of GTP-bound Rap1 in wild-type, NM II-A and NM II-B null ES cells after incubation with collagen-coated beads. Data represent the mean ± sem for the ratio of active Rap1 to total Rap1 (3 independent experiments).

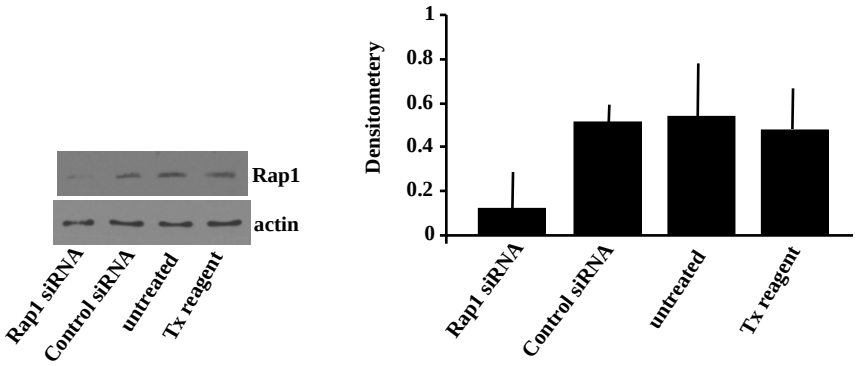
S5- a- Fibroblasts treated with blebbistatin (40μM) show recruitment of actin around beads independent of myosin activity. b- Quantitation of rhodamine phalloidin fluorescence intensity around beads.

a

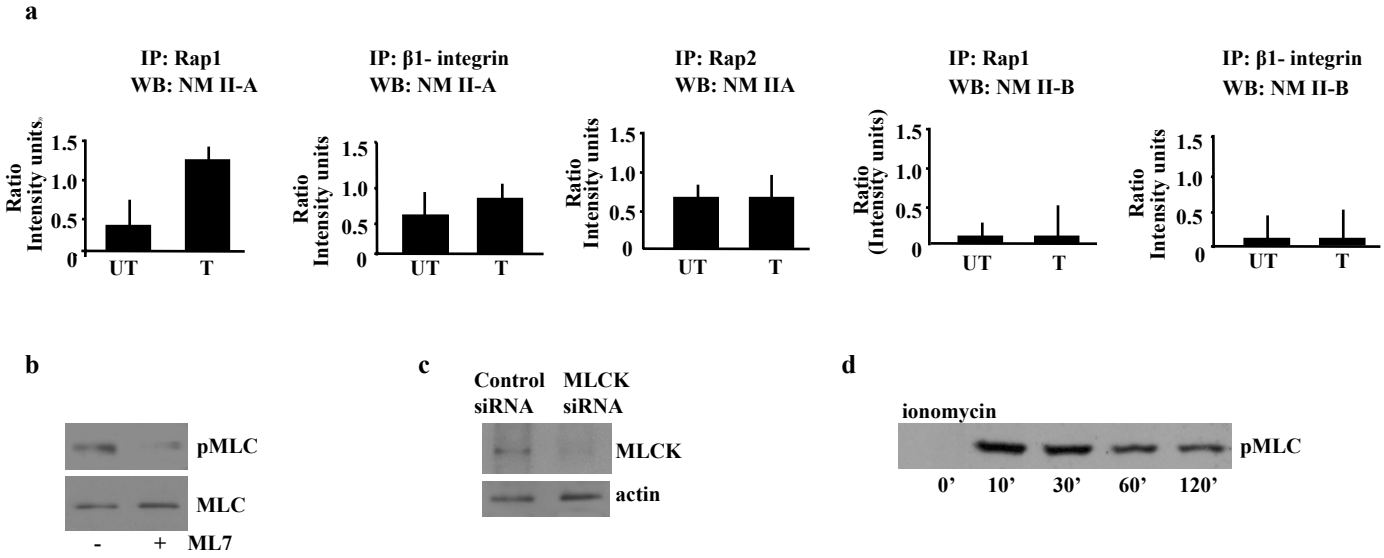

collagen
vitronectin
Normalized Rap1 activity
150
125
100
75
50
25
0
0'
10'
20'
30'
60'
120'
180'
Normalized Rap1 activity
150
125
100
75
50
25
0
0'
30'
60'
90'

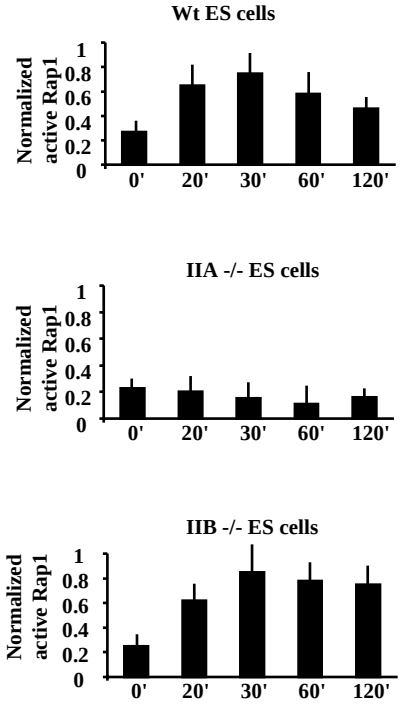
b


α2β1 blocking antibody
Normalized Rap1 actvity
1.0
0.8
0.6
0.4
0
Blocking antibody
Control antibody

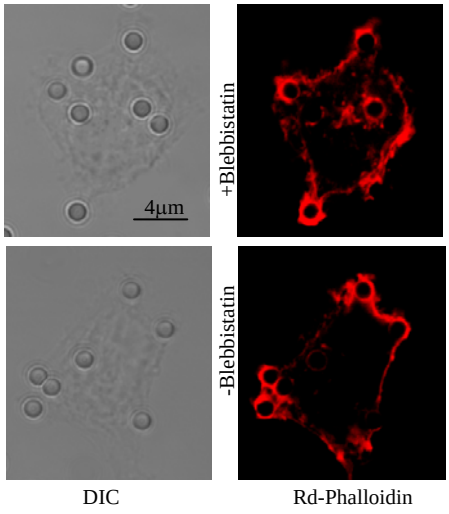

Rap1
actin
Rap1 siRNA
Control siRNA
untreated
Tx reagent
Densitometery
1
0.8
0.6
0.4
0.2
0
Rap1 siRNA
Control siRNA
untreated
Tx reagent

S 3

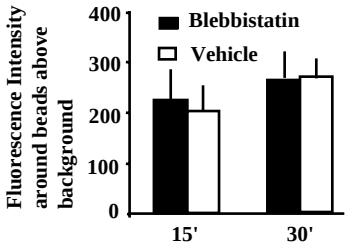

a
IP: Rap1
WB: NM II-A
IP: β1- integrin
WB: NM II-A
IP: Rap2
WB: NM IIA
IP: Rap1
WB: NM II-B
IP: β1- integrin
WB: NM II-B
Ratio Intensity units
1.5
1.0
0.5
0
UT
T
b
pMLC
MLC
-
+
ML7
c
Control siRNA
MLCK siRNA
MLCK
actin
d
ionomycin
pMLC
0'
10'
30'
60'
120'


Wt ES cells
Normalized active Rap1
1
0.8
0.6
0.4
0.2
0
0'
20'
30'
60'
120'
IIA -/- ES cells
Normalized active Rap1
1
0.8
0.6
0.4
0.2
0
0'
20'
30'
60'
120'
IIB -/- ES cells
Normalized active Rap1
1
0.8
0.6
0.4
0.2
0
0'
20'
30'
60'
120'

a


4µm
+Blebbistatin
-Blebbistatin
DIC
Rd-Phalloidin

b


Fluorescence Intensity around beads above background
400
300
200
100
0
Blebbistatin
Vehicle
15'
30'