Supplemental Materials

Molecular Biology of the Cell

Valdivia et al.

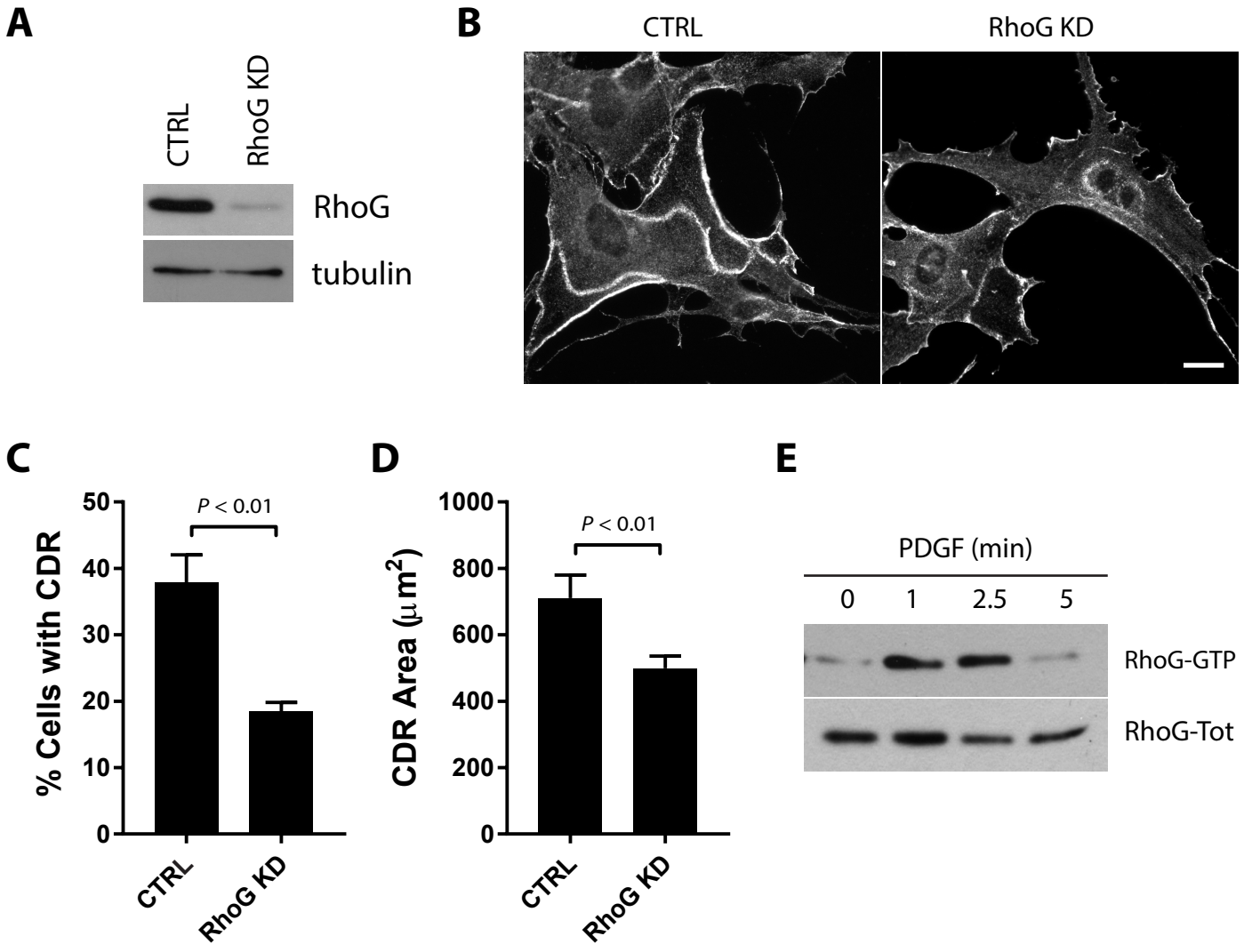
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. PDGF induces RhoG activation in human fibroblasts. (A) Cell lysates from human MRC5 cells stably expressing non-targeting (CTRL) or a RhoG-specific shRNA were analyzed by Western blotting with anti-RhoG antibodies. Anti-tubulin was used as a loading control. **(B)** CTRL and RhoG KD MRC5 cellswere starved for 2 h and then treated with PDGF-BB (20ng/ml) for 2.5 min. Cells were fixed and processed for immunofluorescence using anti-cortactin as marker of dorsal ruffles. Scale bar = 20 μm. **(C)** Number of cells with circular dorsal ruffles (CDR) expressed as percent (%) of cells that stained positive for at least one CDR, **(D)** Average CDR area. Results are shown as mean ± SEM from three independent experiments (n≥200 cells per condition/experiment). Error bars represent SEM.**(E)** Human MRC5 cells were starved for 2 h and then stimulated with PDGF-BB (20 ng/ml) for the indicated times and active RhoG (RhoG-GTP) was precipitated from total lysates using GST-ELMO and immunoblotted with anti-RhoG. RhoG-GTP is calculated as the ratio of active/total RhoG signal and expressed in arbitrary units (A.U.).

Supplementary Figure 2.Effect of Src inhibition on PDGF-mediated RhoG activation. (A) A7r5 cells were serum starved for 2 h and then pretreated with 2.5 μM SU6656 for 30 min and then stimulated with PDGF for 1 min. Active RhoG (RhoG-GTP) was determined as described above.Antibodies against total and active Src (Src and pY416-Src) were used to determine the efficiency of the inhibitor. Antibodies against Akt and phospho-Akt (pS473) were used to monitor PDGF stimulation. **(B)**The graph shows the quantification of 3 independent assays. Active RhoG is calculated as the ratio of active/total RhoG signal and expressed in arbitrary units (A.U.).Error bars represent SEM.

Supplementary Figure 3. Efficiency of knockdown and overexpression of GTPases. The expression levels of the indicated GTPases were evaluated in parallel to each immunofluorescence assay described in Figure 5. In brief, **(A-B)** A7r5 cells were transfected with siRNA against Cdc42, Rac1, RhoG, or Cdc42 and RhoG combined. Control, cells were transfected with a non-targeting siRNA (siCTRL). The expression levels of the GTPases were evaluated by immunoblot using the indicated antibodies. Tubulin was used as a loading control.**(C)** Alternatively, cells were infected with a myc-tagged adenovirus control (CTRL) or with the same adenoviral vector encoding mycRhoG, mycRhoG Q61L, mycCdc42, mycCdc42 Q61L, mycRac1, mycRac1 Q61L, or mycRhoG and mycCdc42 combined. The expression levels of the GTPases were evaluated by immunoblot using anti-myc antibodies.Tubulin was used as a loading control. Figures are representative result from three independent experiments. Panel (C) image was acquired digitally as opposed to film, which is why there is no grey background (see Methods).

Supplementary Figure 4. RhoG silencing effect on Cdc42 activation.(A) A7r5 cells were serum starved for 2 h and then stimulated with PDGF-BB (20 ng/ml) for 1, 2.5 and 5 min. Active Cdc42 (Cdc42-GTP) was then precipitated from total lysates using GST-PBD and immunoblotted with Cdc42 antibodies. **(B)** A7r5 cells were transfected with siRNA targeting RhoG (RhoG KD) or with a non-targeting siRNA (CTRL). After 72 h cells were serum starved for 2 h and then treated with PDGF-BB (20 ng/ml) for 1 min. Active Cdc42 (Cdc42-GTP) was precipitated from total lysates using GST-PBD and immunoblotted with the indicated antibodies. Active Cdc42 is calculated as the ratio of active/total Cdc42 signal and expressed in arbitrary units (A.U.). Tubulin was used as loading control.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RhoG
tubulin
B
CTRL
RhoG KD
C
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40
30
20
10
0
CTRL
RhoG KD
D
CDR Area (μm²)
P < 0.01
1000
800
600
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200
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CTRL
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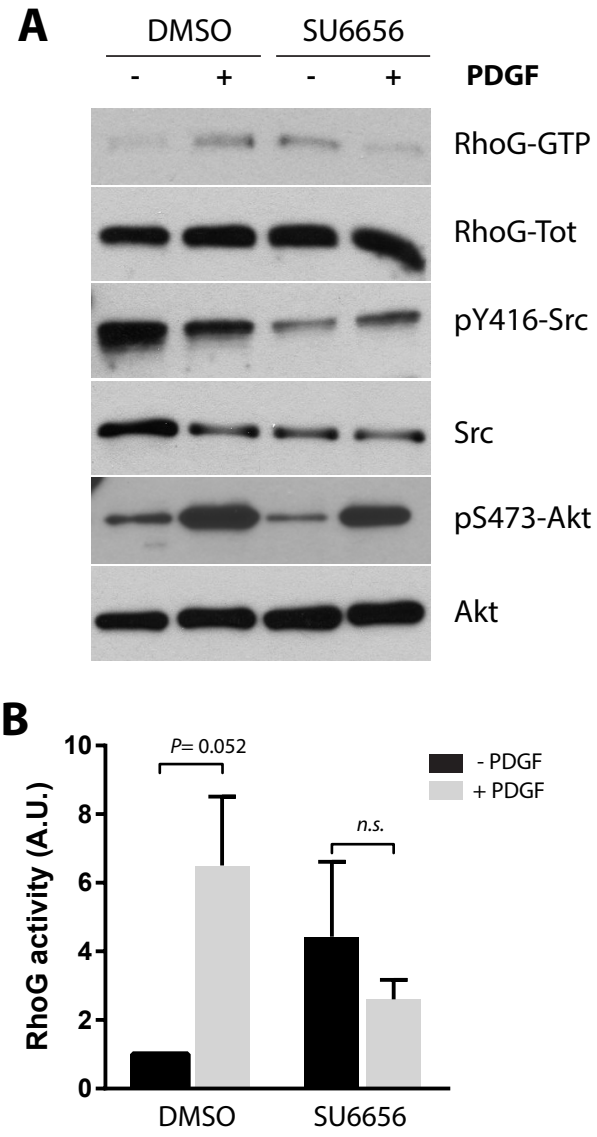
E
PDGF (min)
0
1
2.5
5
RhoG-GTP
RhoG-Tot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RhoG-Tot
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Src
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Akt
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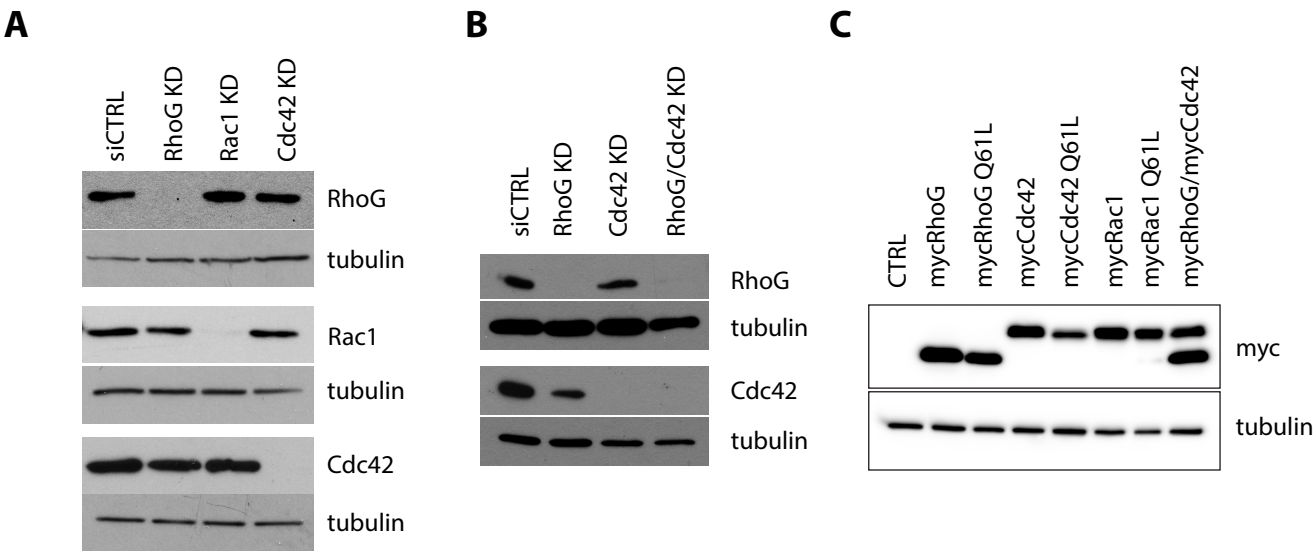
n.s.
- PDGF
+ PDGF
RhoG activity (A.U.)
10
8
6
4
2
0
DMSO
SU6656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Cdc42 KD
RhoG/Cdc42 KD
RhoG
tubulin
Cdc42
tubulin
C
CTRL
mycRhoG
mycRhoG Q61L
mycCdc42
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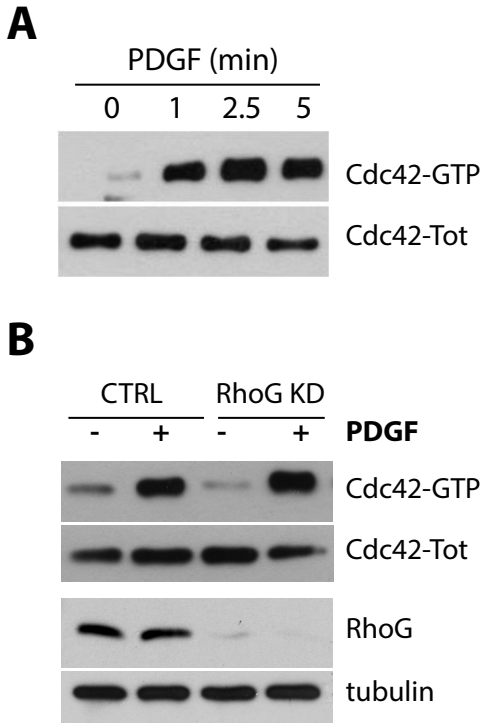
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2.5
5
Cdc42-GTP
Cdc42-Tot
B
CTRL
RhoG KD
-
+
-
+
PDGF
Cdc42-GTP
Cdc42-Tot
RhoG
tubulin