ONLINE SUPPLEMENT

RACK1 REGULATES CELL PROLIFERATION BY MODULATING CALCIUM SIGNALING

Dongmei Cheng, Xiao Zhu, Federica Barchiesi, Delbert G. Gillespie, Raghvendra K. Dubey, and Edwin K. Jackson

Departments of Pharmacology and Chemical Biology (DC, XZ, EKJ)
University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15219
and
Department of Obstetrics and Gynecology (FB, RKD)
University Hospital Zurich, Zurich, Switzerland
Running title: **RACK1 Regulates Cell Proliferation**

Address for Correspondence:
Dr. Edwin K. Jackson
Department of Pharmacology and Chemical Biology
100 Technology Drive, Room 514
University of Pittsburgh School of Medicine
Pittsburgh, PA 15219
Tel: 412-648-1505
Fax: 412-624-7050
E-mail: edj@pitt.edu

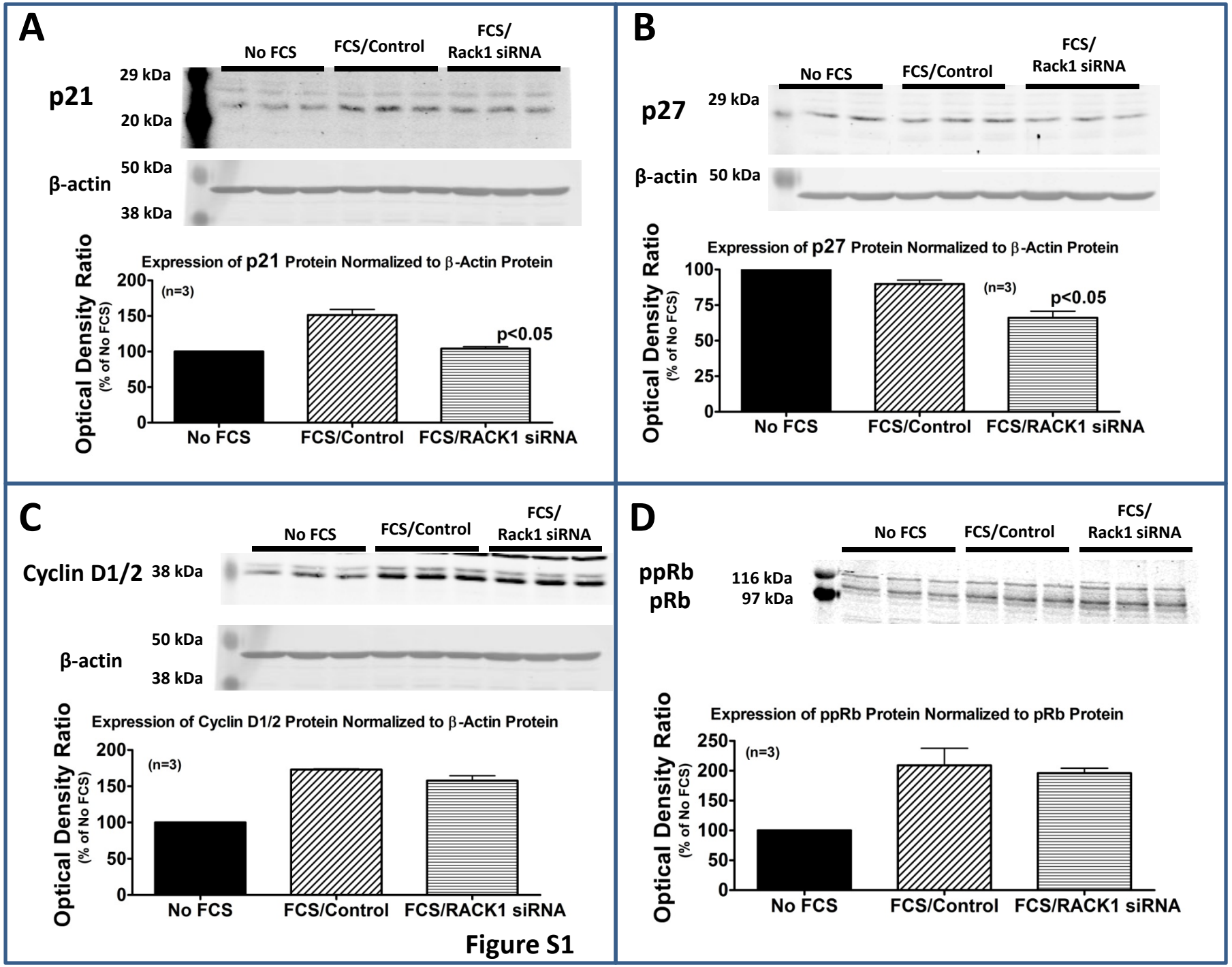
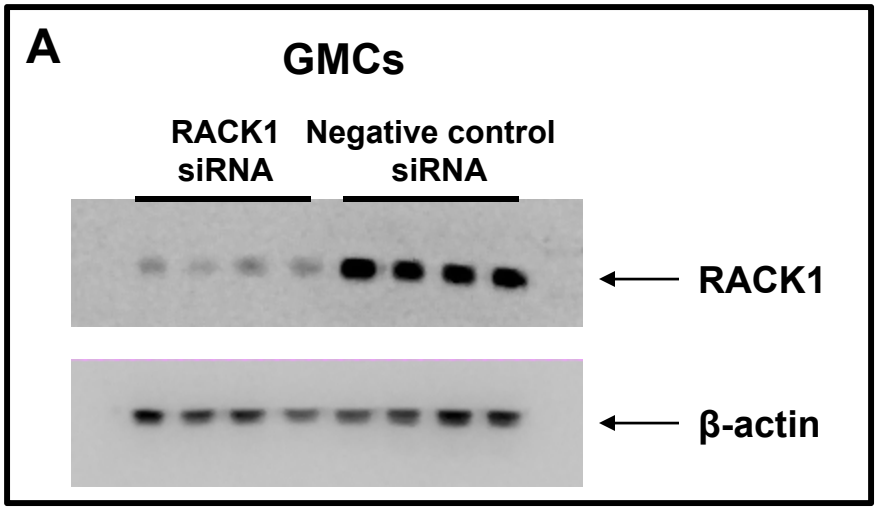
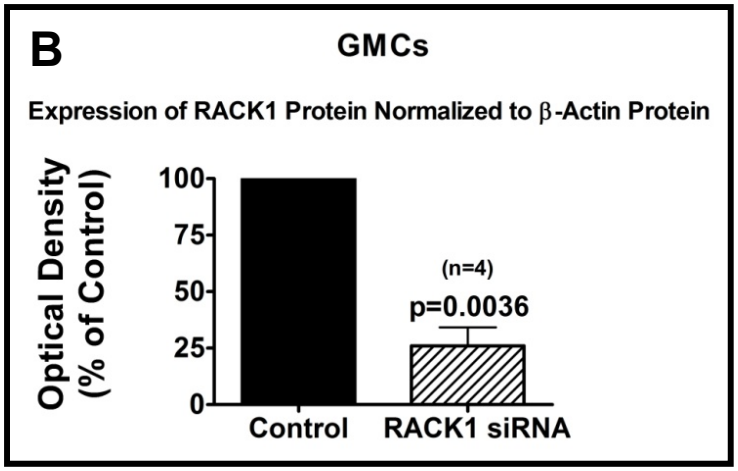

A
No FCS
FCS/Control
FCS/
Rack1 siRNA
p21
29 kDa
20 kDa
β-actin
50 kDa
38 kDa
Expression of p21 Protein Normalized to β-Actin Protein
Optical Density Ratio
(% of No FCS)
(n=3)
p<0.05
No FCS
FCS/Control
FCS/RACK1 siRNA
B
No FCS
FCS/Control
FCS/
Rack1 siRNA
p27
29 kDa
β-actin
50 kDa
Expression of p27 Protein Normalized to β-Actin Protein
Optical Density Ratio
(% of No FCS)
(n=3)
p<0.05
No FCS
FCS/Control
FCS/RACK1 siRNA
C
No FCS
FCS/Control
FCS/
Rack1 siRNA
Cyclin D1/2
38 kDa
β-actin
50 kDa
38 kDa
Expression of Cyclin D1/2 Protein Normalized to β-Actin Protein
Optical Density Ratio
(% of No FCS)
(n=3)
No FCS
FCS/Control
FCS/RACK1 siRNA
D
No FCS
FCS/Control
FCS/
Rack1 siRNA
ppRb
pRb
116 kDa
97 kDa
Expression of ppRb Protein Normalized to pRb Protein
Optical Density Ratio
(% of No FCS)
(n=3)
No FCS
FCS/Control
FCS/RACK1 siRNA

Figure S1

Figure S1. Western blots in panels A-D illustrate effects of RACK1 siRNA (FCS/RACK1 siRNA) versus negative control siRNA (FCS/Control) on the expression in preglomerular vascular smooth muscle cells of $p21^{Cip1/Waf1}$ (p21; panel A), $p27^{Kip1}$ (p27; panel B), cyclin D1/2 (panel C) and hyperphosphorylated retinoblastoma protein (ppRb; panel D) in the presence of 2.5% fetal calf serum (FCS) in the medium. The protein expression of p21, p27 and cyclin D1/2 was normalized to β-actin protein expression by calculating the optical density ratio (optical density of p21, p27 or cyclin D1/2 band divided by optical density of β-actin band). The protein expression of ppRb was normalized to pRb protein expression by calculating the optical density ratio (optical density of ppRb band divided by optical density of pRb band). The optical density ratio for each protein was expressed as a % of the average optical density ratio for cells in the absence of FCS (No FCS). Values represent means ± SEM, and p-values (unpaired Student's t-test) compare FCS/RACK1 siRNA to FCS/Control.


A
GMCs
RACK1 siRNA
Negative control siRNA
RACK1
β-actin


B
GMCs
Expression of RACK1 Protein Normalized to β-Actin Protein
Optical Density (% of Control)
100
75
50
25
0
(n=4)
p=0.0036
Control
RACK1 siRNA

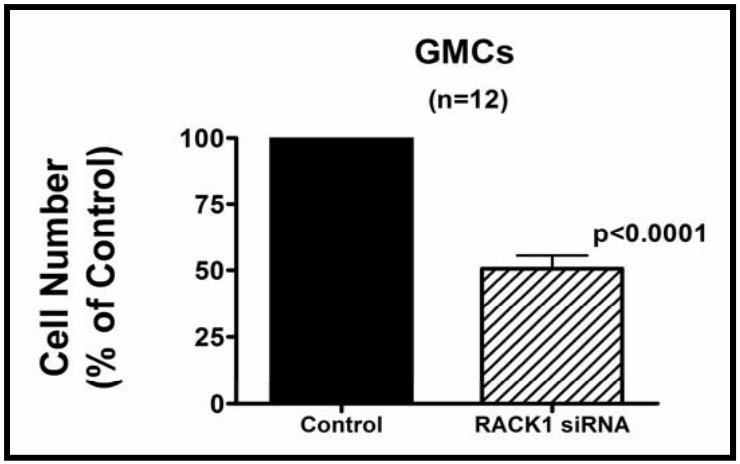

GMCs
(n=12)
Cell Number (% of Control)
100
75
50
25
0
p<0.0001
Control
RACK1 siRNA

Figure S2

Figure S2. Panel A shows Western blots demonstrating the effects of RACK1 siRNA versus negative control siRNA on RACK1 protein expression in glomerular mesangial cells (GMCs). For both negative control siRNA-treated cells (Control) and RACK1 siRNA-treated cells (RACK1 siRNA) the RACK1 protein expression was normalized to β-actin protein expression by calculating the optical density ratio (optical density of RACK1 band divided by optical density of β-actin band). In panel B, the optical density ratio for RACK1 siRNA cells was expressed as a % of the average optical density for the Control cells. Panel C depicts effects of RACK1 siRNA versus negative control siRNA (Control) on cell number in GMCs under high serum (2.5%) conditions. Cell number for RACK1 siRNA cells was expressed as a % of the average cell number for the Control cells. The cell count for control GMCs was 396,875. Values represent means ± SEM, and p-values are from unpaired Student's t-test.