Supplementary information

Experimental & Molecular Medicine

Rac-mediated actin remodeling and myosin II are involved in K_{ATP} channel trafficking in pancreatic β-cells

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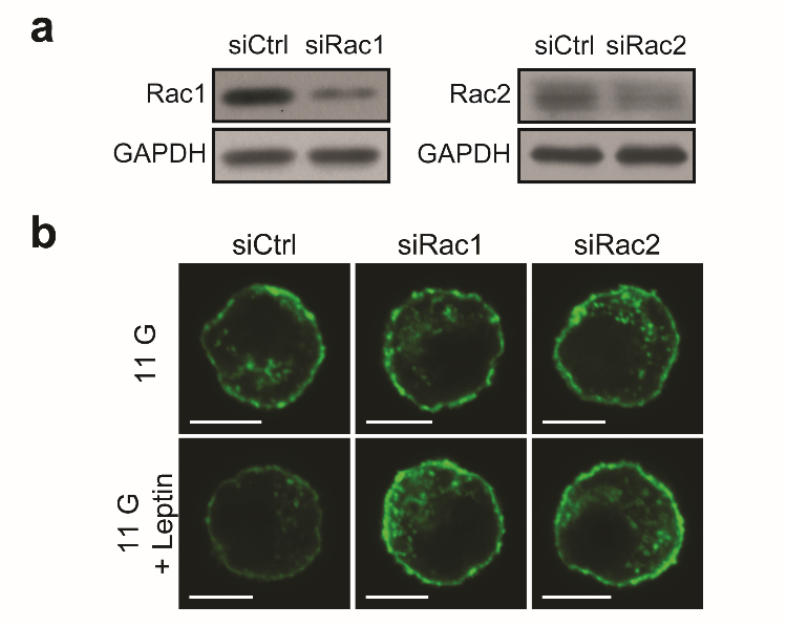
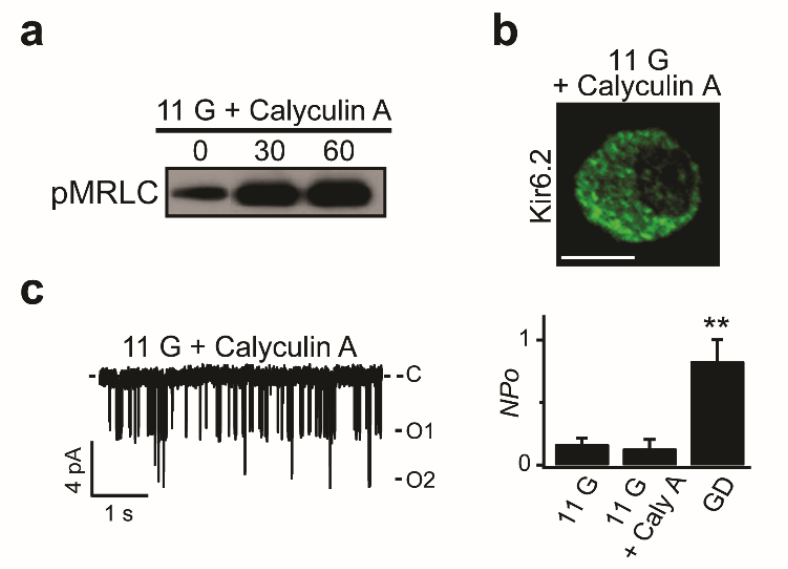
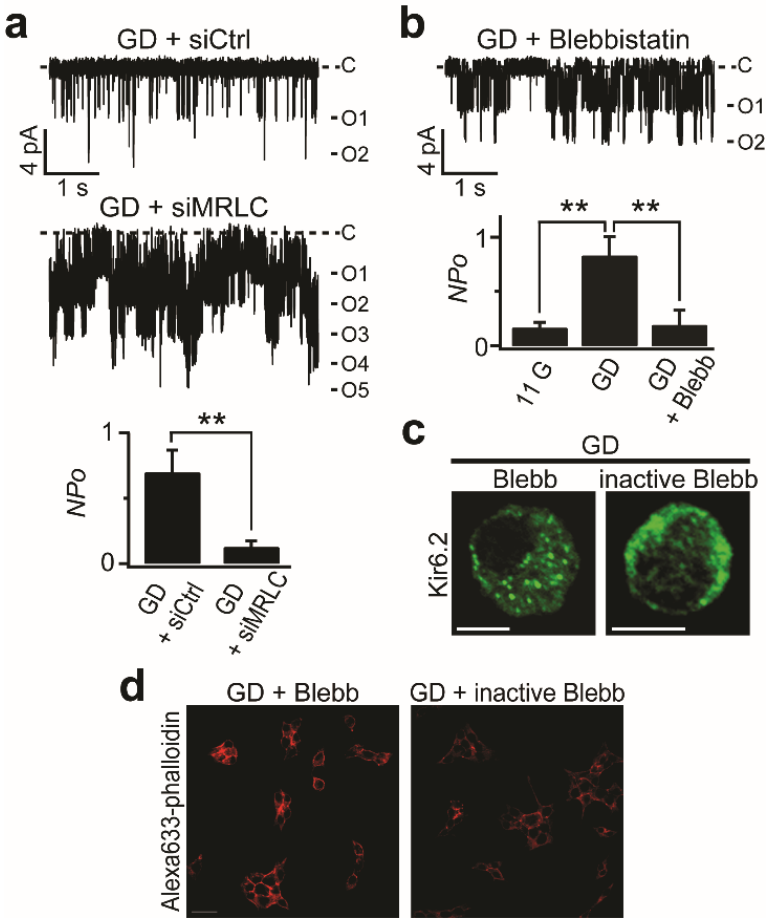


Supplementary Figure 1 AMPK-induced actin disruption is mediated by Rac. (**a**) Knockdown of endogenous Rac1 or Rac2 in siRac1 or siRac2-transfected INS-1 cells. GAPDH was detected as a loading control. (**b**) Confocal fluorescent images taken by F-actin staining with Alexa 488-phalloidin in siCtrl-, siRac-, or siRac2-transfected INS-1 cells pretreated with or without 10 nM leptin. Scale bar, 5 μm.

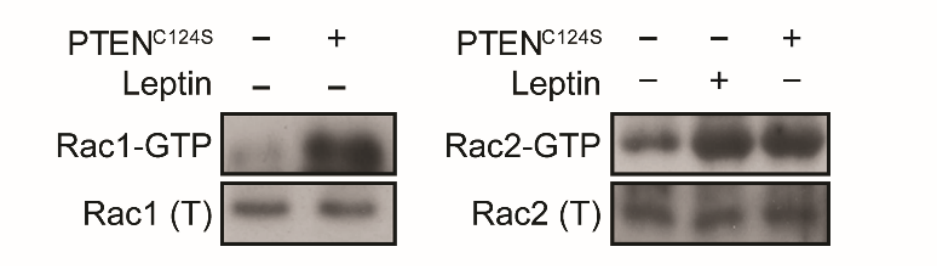


Supplementary Figure 2 Effects of calyculin A, a MLCP inhibitor, on K_{ATP} channel trafficking and activity. (**a**) Time dependence of MRLC phosphorylation after stimulation with MLCP inhibitor (calyculin A, 50 nM) (**b**) Confocal fluorescent images obtained from pancreatic β-cells immunolabeled with Kir6.2 antibody in 11G + Calyculin A. Scale bar, 5 μm. (**c**) Single K_{ATP} channel currents obtained from pancreatic β-cells pretreated with calyculin A (Caly A) in 11.1 mM glucose solution. The data are expressed as the mean ± S.E.M. (11G, n = 14; 11G + Caly A, n = 3; GD, n = 14). $^{**}P<0.01$ compared with 11G + Caly A.



Supplementary Figure 3 The inhibition of MyoII signaling blocks the AMPK-induced increases in K_{ATP} channel trafficking and actin remodeling. (**a**, **b**) The inside-out patches were recorded at –60 mV. C: closed level. O: opened level. The cells after excision were exposed to 0.25 mM diazoxide in the presence of 1μM MgATP to open K_{ATP} channels. (**a**) Representative single K_{ATP} channel currents of GD-treated INS-1 cells transfected with siCtrl or siMRLC. Mean values for *NPo*; 0.70 ± 0.17 (n = 5) in GD + siCtrl and 0.12 ± 0.05 (n = 7) in GD + siMRLC. The data are expressed as the mean ± S.E.M. (n = 3). **P<0.01 compared as indicated. (**b**) Representative

single K_{ATP} channel currents of pancreatic β-cells pretreated with 50 μM blebbistatin (Blebb) for 30 min in GD. Mean value for *NPo*; 0.19 ± 0.14 (n = 3) in GD + Blebbistatin, and it is compared to 11G or GD. The data are expressed as the mean ± S.E.M. (n = 3). $^{**}P<0.01$ compared as indicated. (**c**) Representative immunocytochemical images of pancreatic β-cells pretreated with 50 μM blebbistatin (Blebb) or 50 μM inactive blebbistatin (inactive Blebb) in GD. Scale bar, 5 μm. (**d**) Representative immunofluorescence images for F-actin staining with Alexa 633-phalloidin in INS-1 cells. The cells were pretreated with 50 μM blebbistatin (Blebb) or 50 μM inactive blebbistatin (inactive blebb) for the 30 min before fixation in GD. Scale bar, 20 μm.



Supplementary Figure 4 Activation of Rac by PTEN inhibition. Western blot analysis of Rac1 or Rac2 activity in lysates from INS-1 cells transfected with PTEN^{WT} or PTEN^{C124} construct in the presence or absence of 10 nM leptin.