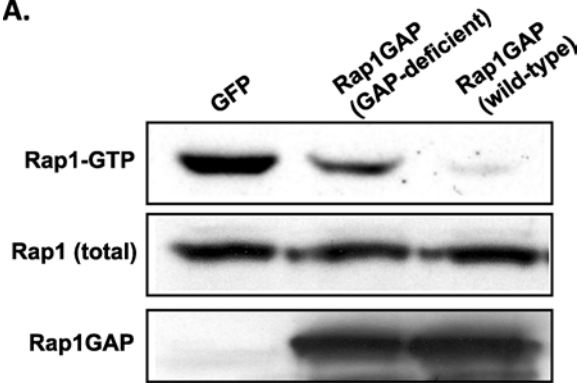


SUPPLEMENTARY FIGURES

A.



B.

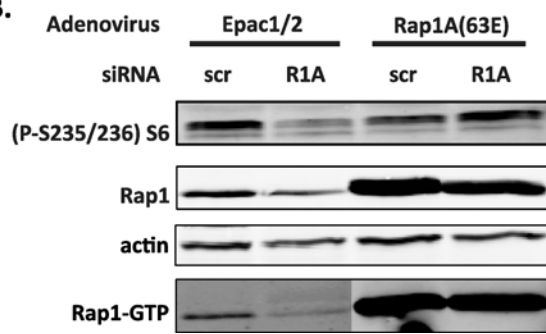
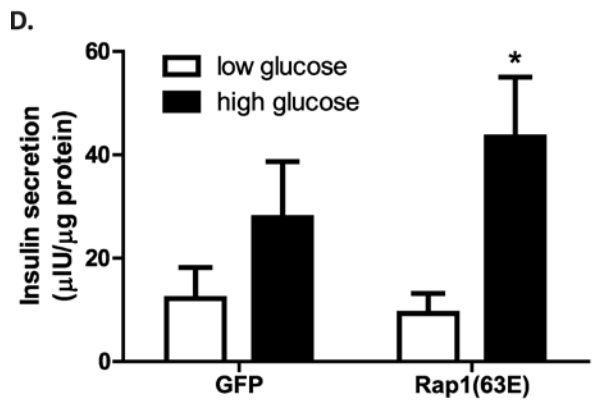
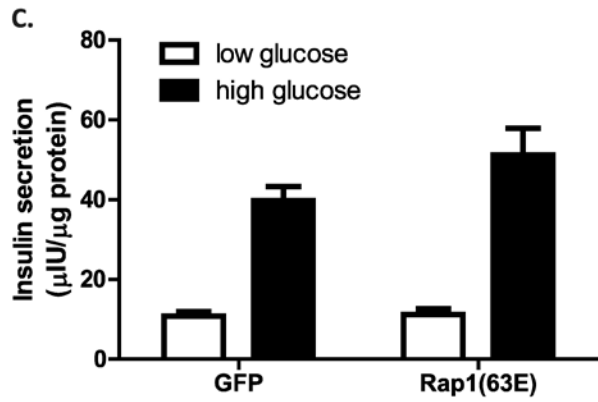
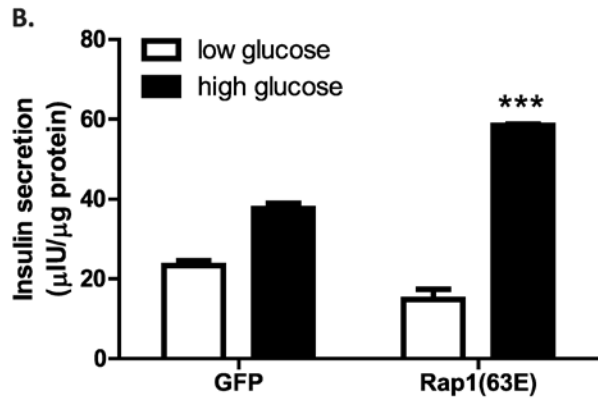
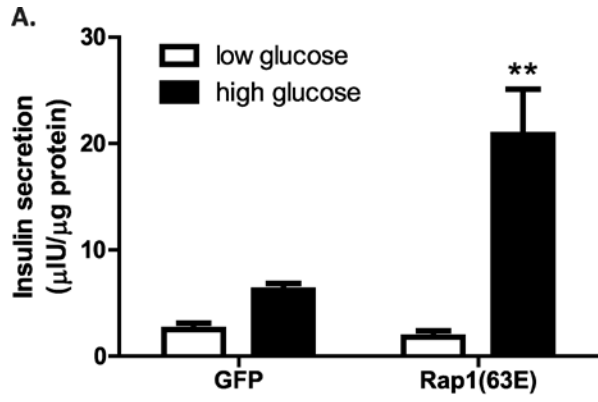


Figure S1

Figure S2



SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Validation of adenoviral constructs in Ins-1 cells. (A) Overexpression of the Rap1GAP adenovirus in the Ins-1 cell line blocks the ability of Rap1 to be activated. Ins-1 cells were seeded at a density of 4×10^6 in 10 cm dishes and allowed to grow for ~48 h. The cells were infected with the indicated adenoviruses at an M.O.I of 10 for 2 h, and then incubated for 18 h in starvation medium with the addition of 100 ng/ml pertussis toxin to dramatically raise cAMP levels. The cells were washed once with ice-cold PBS, and lysed in a buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 1 mM EDTA, 10 mM MgCl₂, 5% glycerol, 10 µg/ml leupeptin, and 10 µg/ml aprotinin. Rap1 activity assays were performed essentially as in {Wittchen, 2005 #89}. (B) Rap1-63E restores Epac1/2-stimulated RPS6 phosphorylation in Rap1A-siRNA treated cells. Ins-1 cells were transfected with siRNA specific for Rap1A (R1A) or a scrambled control (scr). After 48 h, the cells were transduced with adenoviruses expressing Epac1 & 2 or Rap1A-63E. The cells were then serum- and glucose-starved into quiescence, lysed, and subjected to immunoblotting for the indicated proteins. Lysate aliquots were also used in pulldown assays for activated Rap1-GTP. In the Rap1-GTP immunoblot, note the different exposures necessary to image the levels of endogenously-activated Rap1-GTP and Rap1A-63E in the linear range of the Odyssey® instrument. The immunoblots shown are representative of three independent experiments.

Fig. S2. Activation of Rap1 promotes GSIS in cultured human islets. Human islets were obtained through the National Institutes of Health- and Juvenile Diabetes Research Foundation-supported Islet Cell Research Consortium (<http://icr.coh.org>). The use of these human islets is

exempt from IRB approval. On arrival, islets were washed with RPMI medium and incubated in RPMI Medium containing 7 mM glucose, 1% penicillin/streptomycin and 10% FBS. Islets were hand-picked and incubated overnight in RPMI medium with GFP or Rap1A-63E-expressing adenoviruses as described in the *Materials and Methods* section for isolated rat islets. Insulin secretion was measured from islets from three separate donors (*A*, *B*, and *C*) expressing the indicated proteins after a 2 h incubation in low glucose (2.5 mM) or high glucose (12 mM) buffer. (D) The mean potentiation of GSIS by Rap1A-63E of all three donors. While calculating the mean \pm S.E. of the average of all experiments introduces significant variation due to the widely-varying secretory capacities of individual human islet preps, the potentiation of GSIS by Rap1A-63E is still significant. *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$.