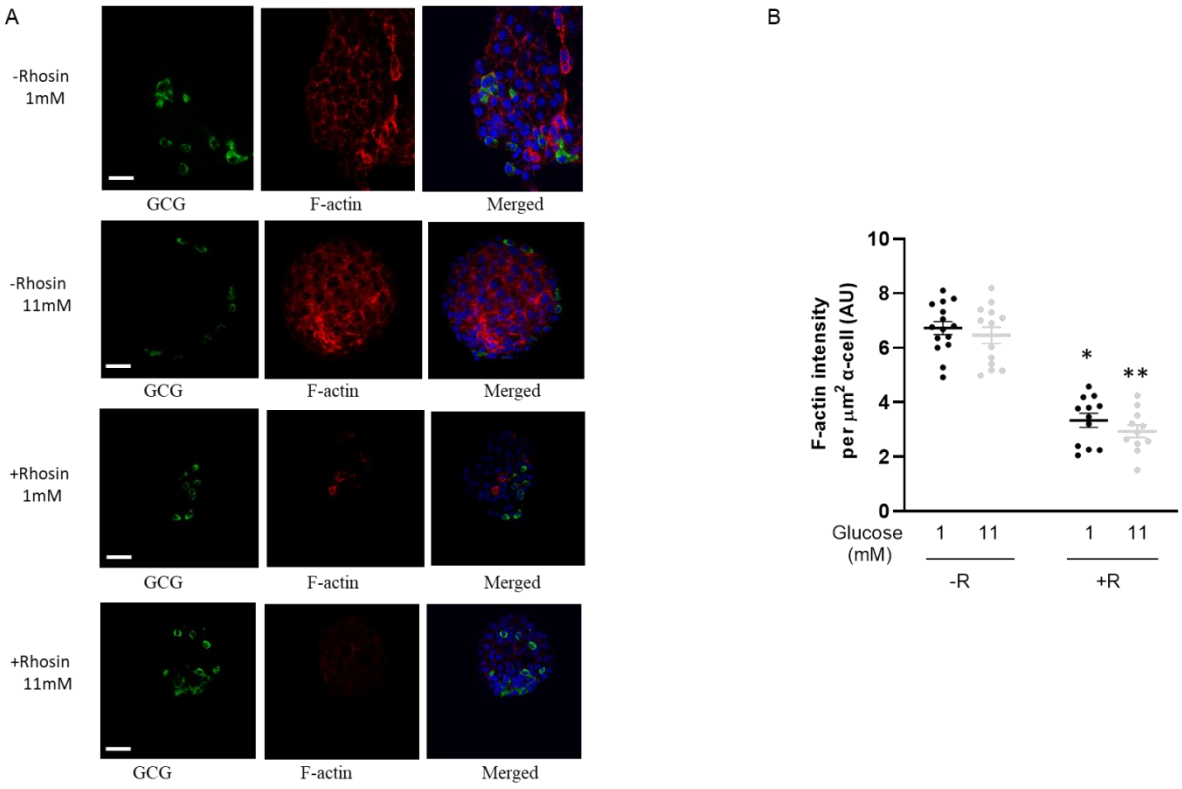
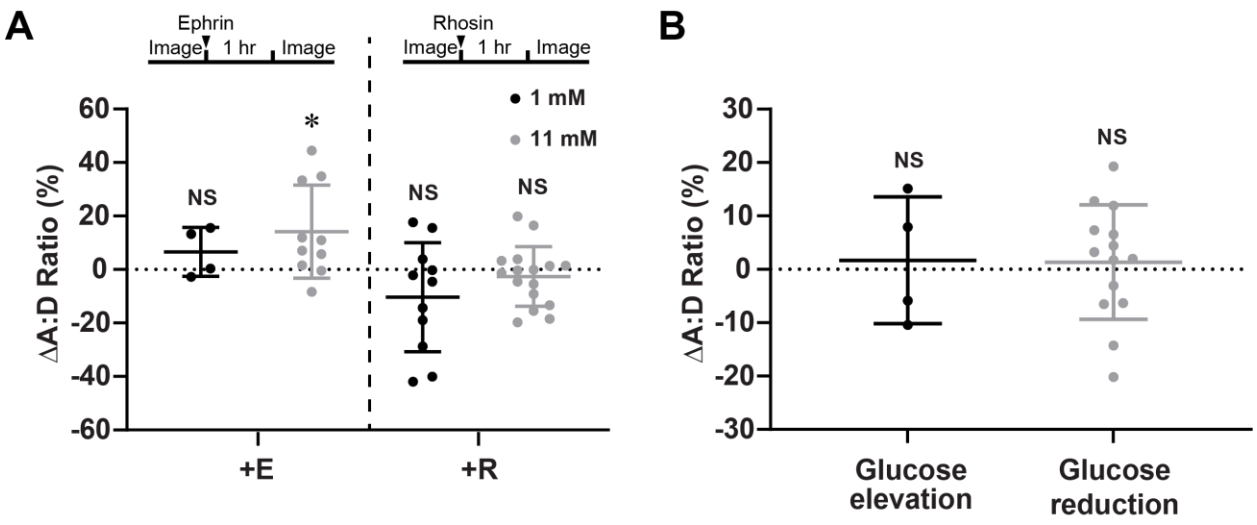
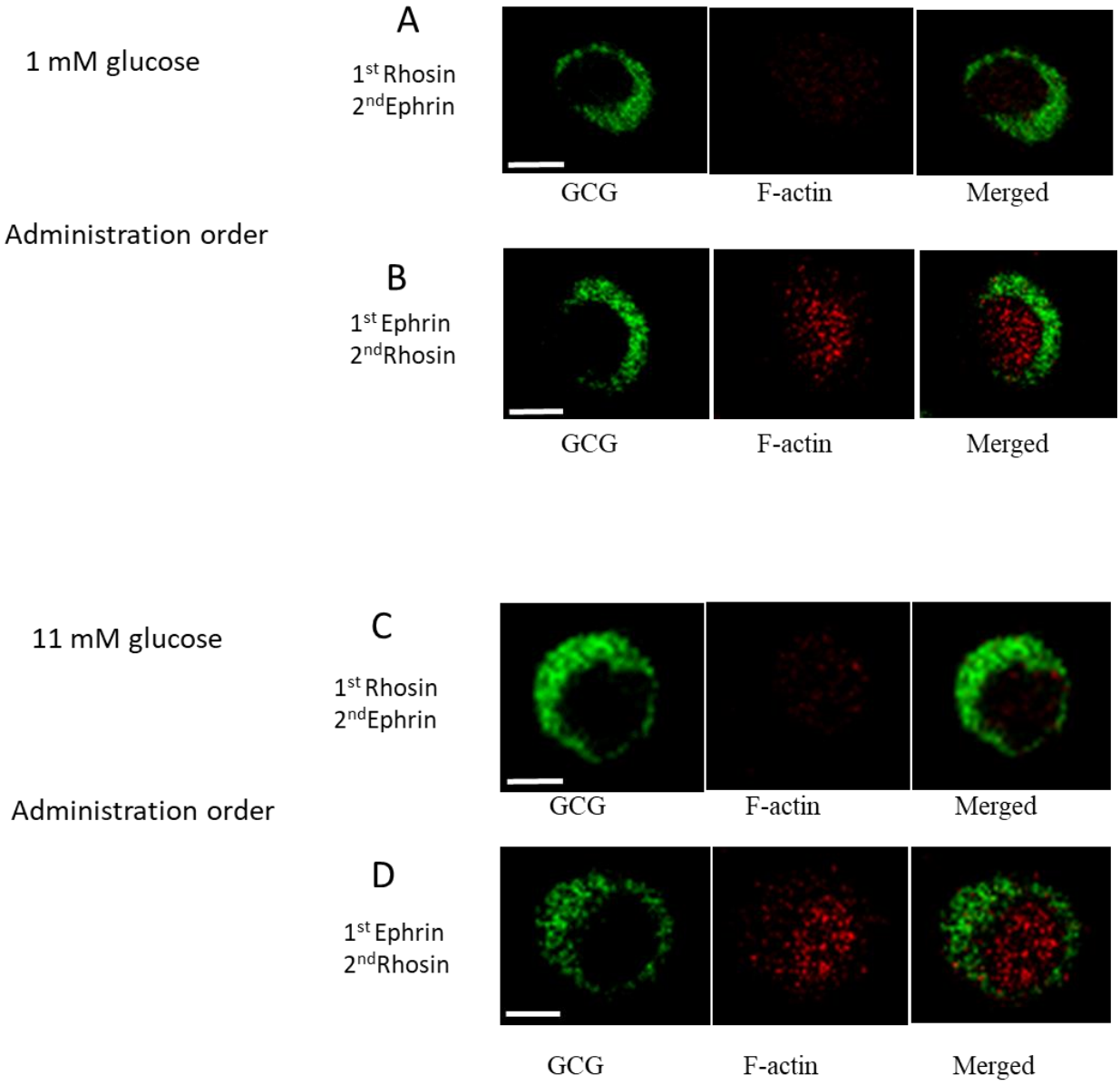


Supplementary Figure 1. RhoA inhibition in isolated islets dramatically reduced F-actin intensities in both 1 mM and 11 mM glucose conditions. A) Confocal immunofluorescent images (20 μm scale bars) of glucagon- and F-actin-stained isolated islets in low glucose (1 mM) versus high glucose (11 mM) conditions in the absence and presence of Rhosin. B) F-actin intensities of α-cells in isolated islets in response to Rhosin in low and high glucose conditions. Statistical difference between Rhosin-treated and control islets at the same glucose concentration was determined using unpaired t-test (*$p<0.05$; **$p<0.001$). Values expressed as mean ± SEM (n = 11-15 islets).



Supplementary Figure 2. EphA forward signaling stimulates α-cell RhoA activity in intact mouse islets. A) The % changes in the acceptor:donor intensity ratio before and after treatment (either with 4 µg/mL ephrin-A5-Fc (+E) or 250 µM Rhosin (+R)) at low (black circles) and high (gray circles) glucose concentrations. B) The % changes in the acceptor:donor intensity ratio on switching glucose concentrations from 1 mM to 11 mM (Glucose elevation, black circles) and 11 mM to 1 mM (Glucose reduction, gray circles). Data presented as mean ± SD ($n \geq 4$ cells). One sample t-test was employed to determine statistical significance between no treatment value (0% change in acceptor:donor intensity ratio) and treatment conditions. *: $p < 0.05$; NS: $p > 0.05$.



Supplementary Figure 3. Ephrin-A5 prevents Rhosin-imposed depolymerization of F-actin in dispersed mouse islet cells. Dispersed islet cells were treated in a sequential order with Rhosin (+R; 100 µM) – ephrin-A5-Fc (E; 4 µg/mL) (Rhosin-Ephrin) or in a reverse order. Confocal immunofluorescent images (10 µm scale bars) represent a typical α-cell when treated in sequential order of Rhosin-Ephrin **(A)** or Ephrin-Rhosin **(B)** in low glucose (1 mM) condition and Rhosin-Ephrin **(C)** or Ephrin-Rhosin **(D)** in high glucose (11 mM) condition. Data quantified in Figure 3 M and N.