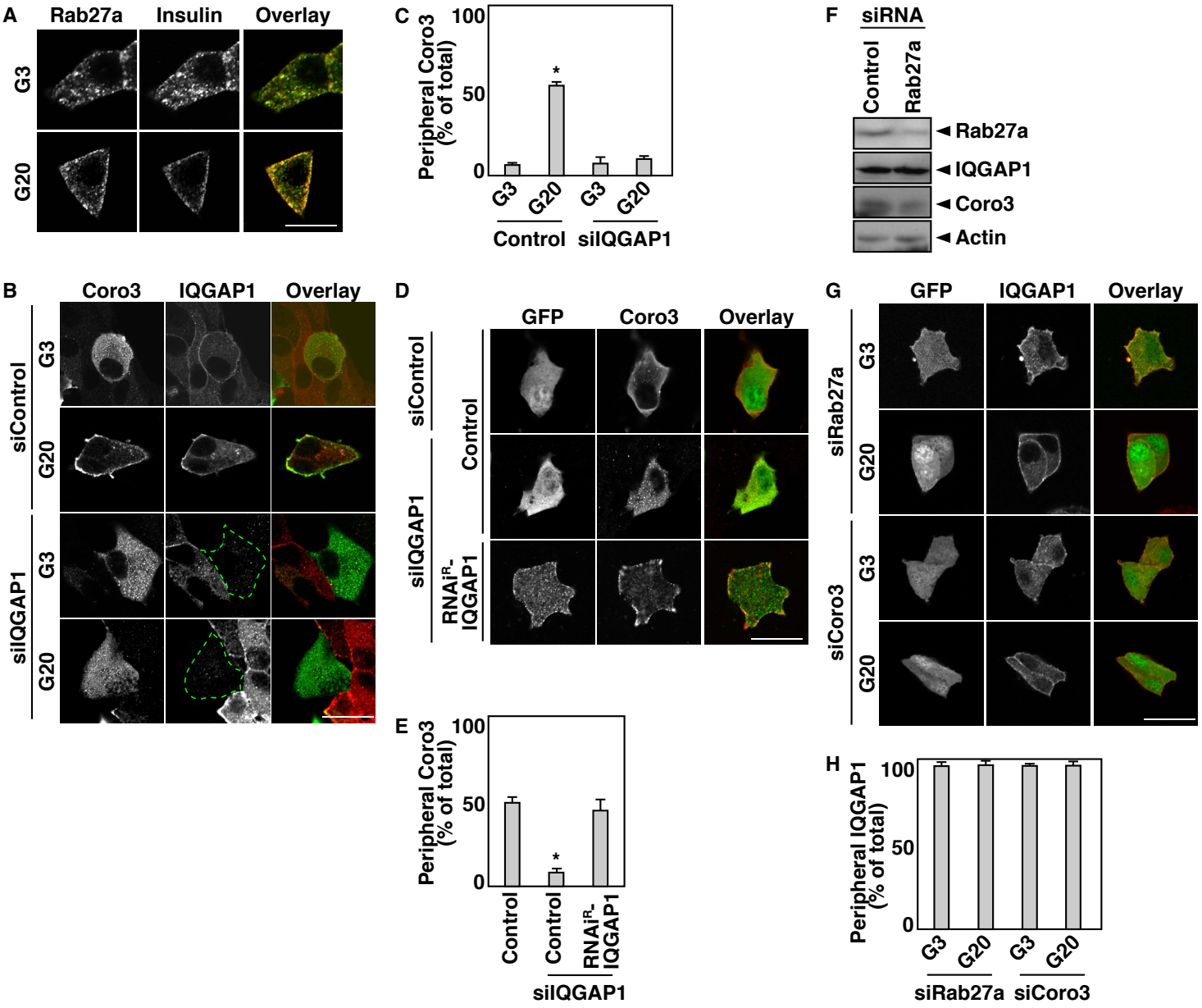
Fig. S1

IQGAP1

MSAAEEVDGL GVVRPHYGSV LDNERLTAEE MDERRRQNVA YEYLCHLEEA
KRWMEACLGE DLPPTTELEE GLRNGVYLAK LGNFFSPKVV SLKKIYDREQ
TRYKATGLHF RHTDNVIQWL NAMDEIGLPK IFYPETTDIY DRKNMPRCIY
CIHALSLYLF KLGLAPQIQD LYGKVDFTEE EINNMKIELE KYGIQMPAFS
KIGGILANEL SVDEAALHAA VIAINEAIDR RVAADTFTAL KNPNAMLVNL
EEGLAPTYQD VLYQAKQDKM TNAKNRTENS DRERDVYEEL LTQAEIQGNV
NKVNTSSALA NISLALEQGC AVTLLKALQS LALGLRGLQT QNSDWYMKQL
QSDLQQKRQS GQTDPLQKEE VQAGVDAANS AAQQYQRRLA AVAAINAAIQ
KGIAEKTVLE LMNPEAQLPQ VYPFAADLYQ KELATLQQQS PEHSLTHPEL
TVAVEMLSSV ALINRALESG DMTTVWKQLS SSVTGLTNIE EENCQRYLDE
LMKLKAQAHA ENNAFITWND IQACVDHVNL VVHEEHERIL AIGLINEALD
EGDAQKTLQA LQIPAAKLEG VLAEVAQHYQ DTLIRAKREK AQETQDESAV
LWLDEIQGGI WQSNKDTQEA QRFALGISAI NEAVDSGDVG RTLSALRSPD
VGLYGVIPEC GETYQSDLAE AKKKRLAAGD NNSKWVKHWV KGGYHYYHNL
ETQAGGWAEP PDFVQNSVQL SREEIQSSIS GVTAAYNREQ LWLANEGLIT
KLQACCRGYL VRQEFRSRMN FLKKQIPAIT CIQSQWRGYK QKKAYQDRLA
YLHSHKDEVV KIQSLARMHQ ARKRYRDRLQ YFRDHINDII KIQAFIRANK
ARDDYKTLIN AEDPPMIVVR KFVHLLDQSD QDFQEELDLM KMREEVITLI
RSNQQLENDL NLMDIKIGLL VKNKITLQDV VSHSKKLTKK NKEQLSDMMM
INKQKGGLKA LSKEKREKLE AYQHLFYLLQ TNPTYLAKLI FQMPQNKSTK
FMDSVIFTLY NYASNQREEY LLLRLFQTAL QEEIKSKVDQ IQEIVTGNPT
VIKMVVSFNR GARGQNALRQ ILAPVVKEIM DDKSLNIKTD PVDIYKSWVN
QMESQTGEAS KLPYDVTPEQ ALSHEEVKTR LDNSIRNMRA VTDKFLSAIV
SSVDKIPYGM RFIAKVLKDS LHEKFPDAGE DELLKIIGNL LYYRYMNPAI
VAPDAFDIID LSAGGQLTTD QRRNLGSIAK MLQHAASNKM FLGDNAHLSI
INEYLSQSYQ KFRRFFQVAC DVPELQDKFN VDEYSDLVTL TKPVIYISIG
EIINTHTLLL DHQDAIAPEH NDPIHELLDD LGEVPTIESL IGESCGNSND
PNKEALAKTE VSLTLTNKFD VPGDENAEMD ARTILLNTKR LIVDVIRFQP
GETLTEILET PATNEQEAEH QRAMQRRAIR DAKTPDKMKK SKPMKEDNNL
SLQEKKEKIQ TGLKKLTELG TVDPKNRYQE LINDIAKDIR NQRRYRQRRK
AELVKLQQTY SALNSKATFY GEQVDYYKSY IKTCLDNLAS KGKVSKKPRE
MKGKKSKKIS LKYTAARLHE KGVLLEIEDL QANQFKNVIF EIGPTEEVGD
FEVKAKFMGV QMETFMLHYQ DLLQLQYEGV AVMKLFDRAK VNVNLLIFLL
NKKFYGK

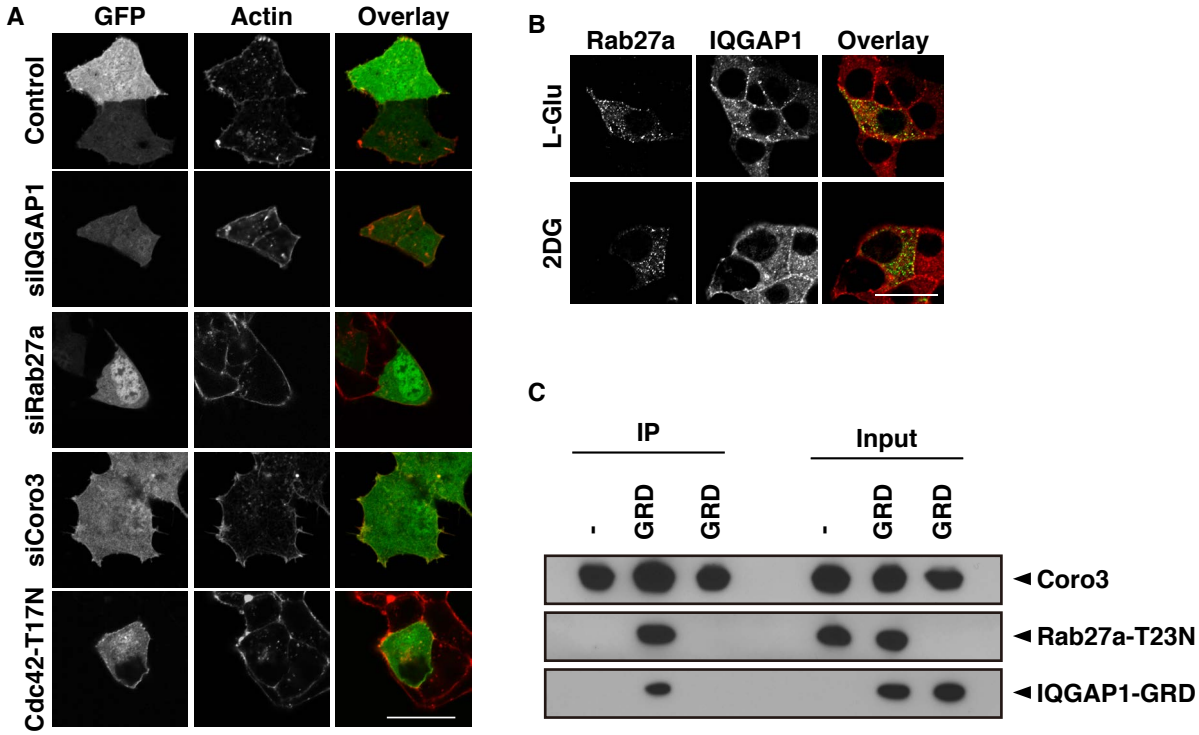
Supplemental Figure S1. Identification of the p180 protein as IQGAP1 by peptide mass fingerprinting. The p180 protein that bound to the GDP-bound Rab27a column was analyzed by peptide mass fingerprinting and identified as IQGAP1. The pink letters indicate the peptide sequences that matched the peptide masses detected by TOF-MS analysis.

Fig. S2



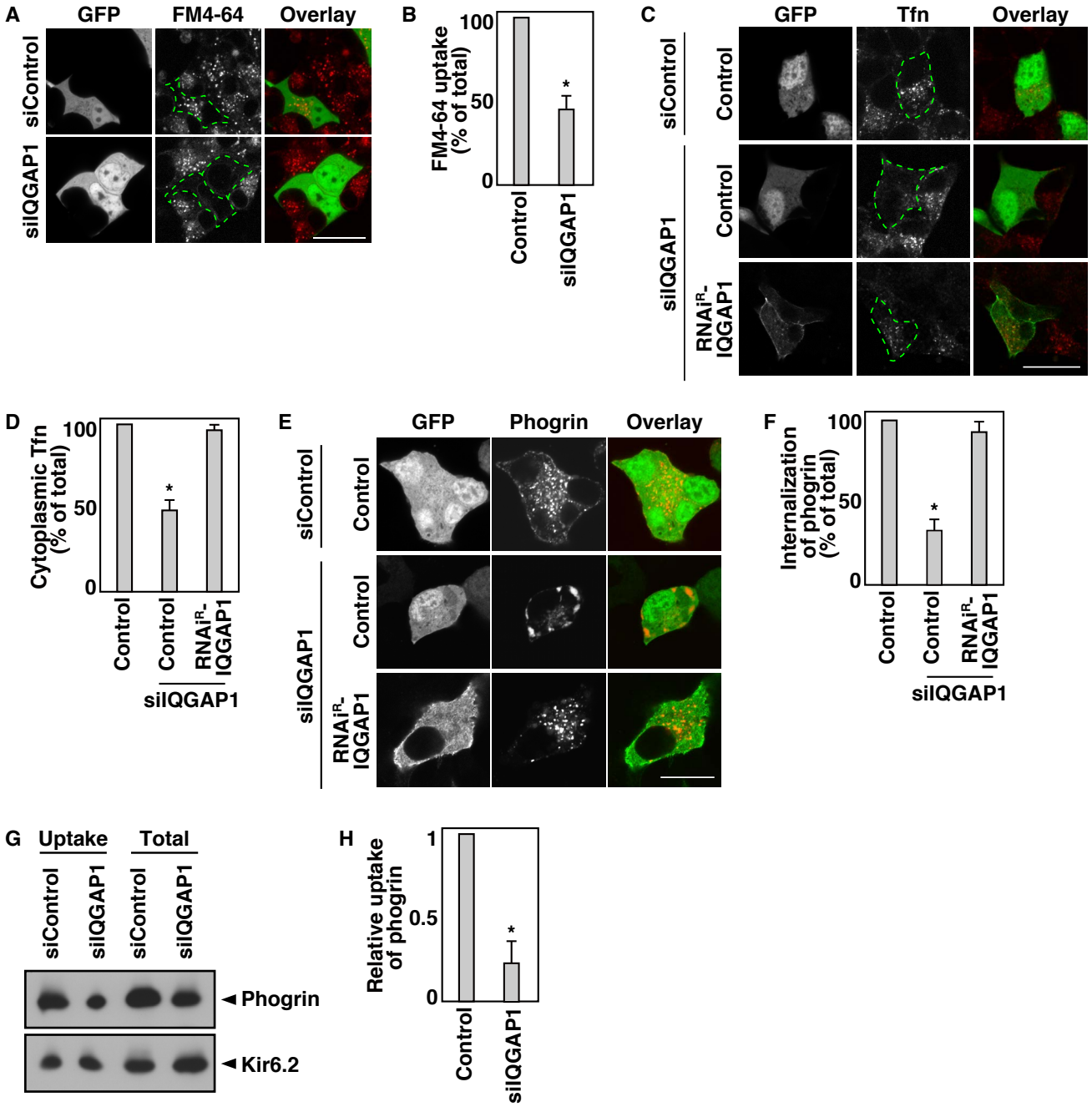
Supplemental Figure S2. IQGAP1 is required for glucose-induced redistribution of coronin 3. (A) MIN6 cells were incubated with 3 or 20 mM glucose for 5 min. The cells were immunostained with anti-Rab27a (green) and anti-insulin (red) antibodies. (B) IQGAP1-silenced or control MIN6 cells expressing flag-coronin 3 were incubated with 3 or 20 mM glucose for 5 min. The cells were immunostained with anti-flag (green) and anti-IQGAP1 (red) antibodies. (C) The percentage of cells displaying a peripheral distribution of flag-coronin 3 was analyzed. (D) IQGAP1-silenced MIN6 cells expressing flag-coronin 3 and GFP or GFP-RNAi^{R}-IQGAP1 were incubated with 20 mM glucose for 5 min. The cells were immunostained with anti-flag (red) antibody. (E) The percentage of cells displaying a peripheral distribution of flag-coronin 3 was analyzed. (F) Rab27a-silenced or control MIN6 cells were analyzed by immunoblotting with anti-Rab27a, anti-IQGAP1, anti-coronin 3 and anti-actin antibodies. (G) Rab27a- or coronin-3-silenced MIN6 cells that expressed GFP as a transfection marker were incubated with 3 or 20 mM glucose for 5 min. The cells were immunostained with anti-IQGAP1 (red) antibody. (H) The percentage of cells displaying a peripheral distribution pattern of IQGAP1 was analyzed. Statistical analysis were performed as described in the legend to Fig. 3C. Scale bars, 10 μm.

Fig. S3



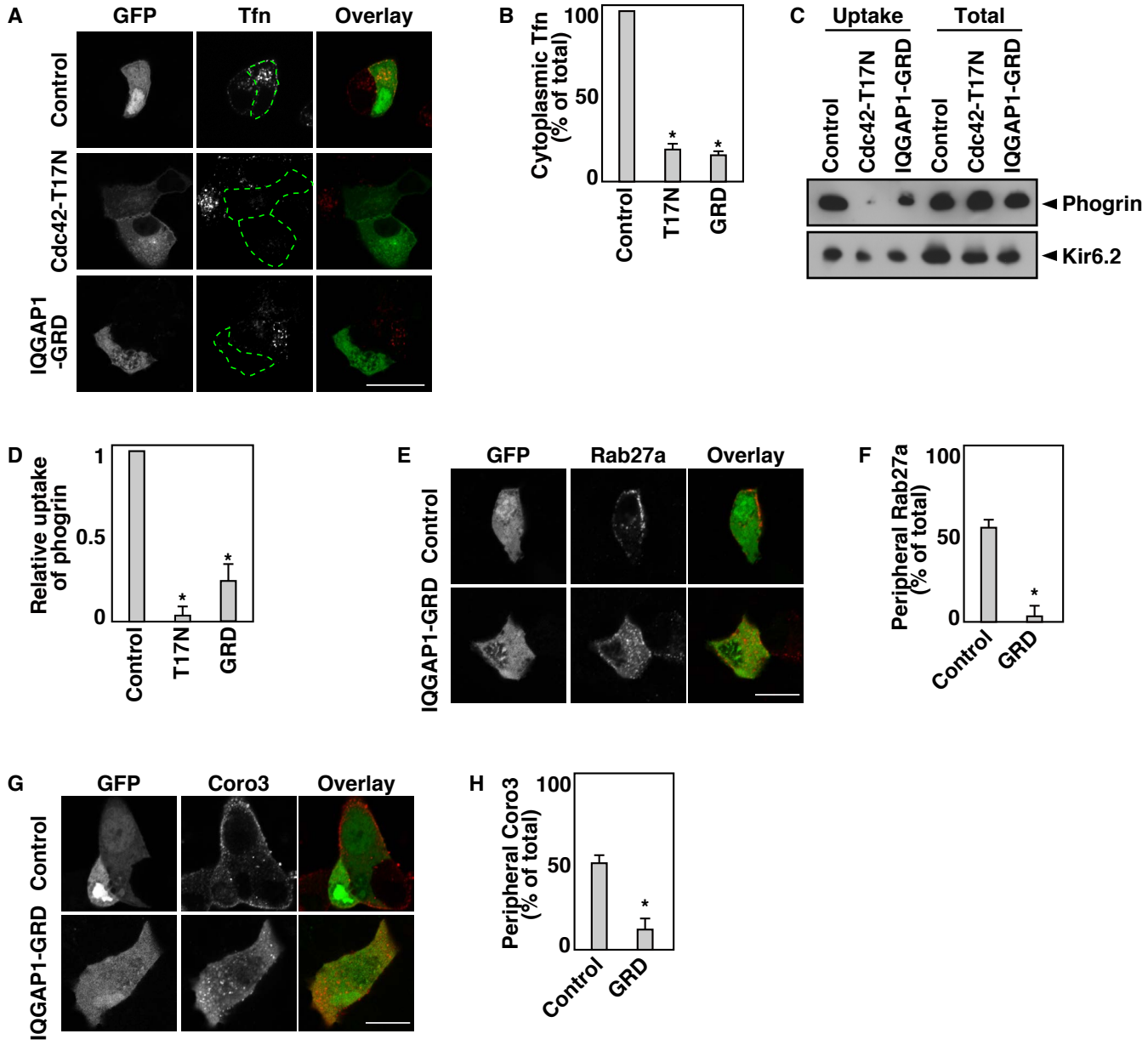
Supplemental Figure S3. GDP-Rab27a, IQGAP1 and coronin 3 form a trimeric complex.
(A) IQGAP1-, Rab27a- or coronin-3-silenced MIN6 cells that expressed GFP as a transfection marker or GFP-Cdc42-T17N-expressing MIN6 cells were incubated with 20 mM glucose for 5 min. The cortical actin network was evaluated by phalloidin-TRITC staining (red). (B) MIN6 cells expressing flag-Rab27a were incubated with 20 mM L-glucose (L-Glu) or 2-Deoxyglucose (2DG) for 5 min. The cells were immunostained with anti-flag (green) and anti-IQGAP1 (red) antibodies. (C) COS-7 extracts expressing flag-coronin 3 and GFP-Rab27a-T23N with or without GFP-IQGAP1-GRD were immunoprecipitated with an anti-flag antibody. The immunocomplexes were analyzed by immunoblotting using anti-flag and anti-GFP antibodies. 2.0% (T23N) and 0.8% (GRD) of the input protein were co-immunoprecipitated. Scale bar, 10 μm.

Fig. S4



Supplemental Figure S4. IQGAP1 regulates the endocytosis of secretory membrane.
(A) IQGAP1-silenced or control MIN6 cells that expressed GFP as a transfection marker were labeled with FM4-64 (red in the overlay image) in the presence of 20 mM glucose. (B) The percentage of transfected cells that showed a cytoplasmic distribution of FM4-64 was analyzed. (C) IQGAP1-silenced or control MIN6 cells expressing GFP used as a transfection marker or GFP-RNAi^{R}-IQGAP1 were incubated with Alexa-568-labeled transferrin (red) in the presence of 20 mM glucose for 5 min. (D) The percentage of transfected cells that showed a cytoplasmic distribution of Alexa-568-labeled transferrin was analyzed. (E) IQGAP1-silenced or control MIN6 cells expressing phogrin-Cherry and GFP or GFP-RNAi^{R}-IQGAP1 were incubated with 20 mM glucose for 5 min. (F) The percentage of transfected cells that showed a cytoplasmic distribution of phogrin-Cherry was analyzed. (G) Endocytosed proteins were isolated from IQGAP1-silenced MIN6 cells, and immunoblotted for phogrin and Kir6.2. This experiment was carried out with 20 mM glucose. (H) The endocytosed phogrin was determined by densitometry. Data are expressed as means ± S.D. from 3 independent experiments. *$p<0.01$, significantly different from the siControl cells when analyzed by an unpaired Student's t-test. Statistical analysis of the data in B, D and F was performed as described in the legend to Fig. 3C. Scale bars, 10 μm.

Fig. S5



Supplemental Figure S5. The uptake of transferrin is inhibited in Cdc42-T17N- or IQGAP1-GRD-expressing cells.

(A) MIN6 cells expressing GFP, GFP-Cdc42-T17N or GFP-IQGAP1-GRD were incubated with Alexa-568-labeled transferrin (red) in the presence of 20 mM glucose for 5 min followed by immunofluorescence analysis. (B) The percentage of transfected cells that showed a cytoplasmic distribution of Alexa-568-labeled transferrin was analyzed. More than 40 randomly selected cells (more than 8 cells/experiment) were examined. Data are expressed as means ± S.D. from 4 independent experiments. *$p<0.01$, significantly different from control cells when analyzed by unpaired Student's t-test. (C) Endocytosed proteins were isolated from GFP-Cdc42-T17N or GFP-IQGAP1-GRD-expressing MIN6 cells, and immunoblotted for phogrin and Kir6.2. This experiment was carried out with 20 mM glucose. (D) The endocytosed phogrin was determined by densitometry. Statistical analysis was performed as described in the legend to Fig. S4H. (E-H) MIN6 cells expressing GFP-IQGAP1-GRD or control GFP together with flag-Rab27a (E-F) or flag-coronin 3 (G-H) were incubated with 20 mM glucose for 5 min.The cells were immunostained with anti-flag antibody and an Alexa568-labeled second antibody (red) and the percentage of cells that showed a peripheral distribution of flag-Rab27a (F) or -coronin 3 (H) was statistically analyzed as described in the legend to Fig. 3C. Scale bar, 10 μm.