

Figure S1. Summary of mass spectrometry data. (A) Table summarizing mass spectrometry results for each identified peptide. (B-G) Representative mass spectra for ubiquitinated IQGAP1 peptides. (B) 539-567 containing ubiquitinated Lys-556. (C) 1145-1161 containing ubiquitinated Lys-1155. (D) 1224-1239 containing ubiquitinated Lys-1230. (E) 1465-1475 containing ubiquitinated Lys-1465. (F) 1466-1477 containing ubiquitinated Lys-1475. (G) 1517-1532 containing ubiquitinated Lys-1528.

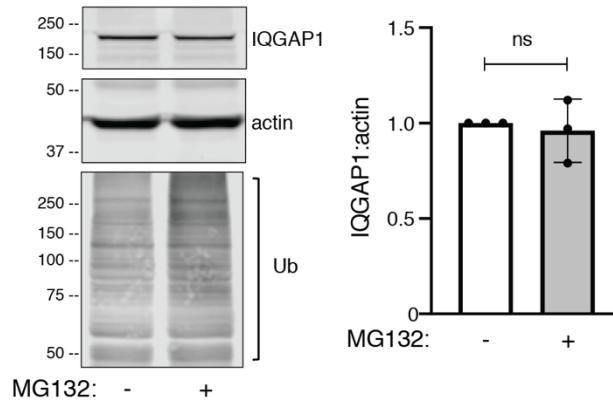


Figure S2. MG132 does not alter the amount of IQGAP1 in cell lysates.

(A) HEK293 cells were incubated with MG132 (+) or DMSO (-) for 4 h. Equal amounts of protein lysate were analyzed by Western blotting using anti-IQGAP1, anti-actin and anti-ubiquitin (Ub) antibodies. (B) The IQGAP1 bands were quantified with Image Studio 2.0 and corrected for the amount of actin in the same sample. Data are expressed as means \pm SD (n=3) and compared using Welch's t-test, with cells incubated with DMSO set as 1. ns: not significant.

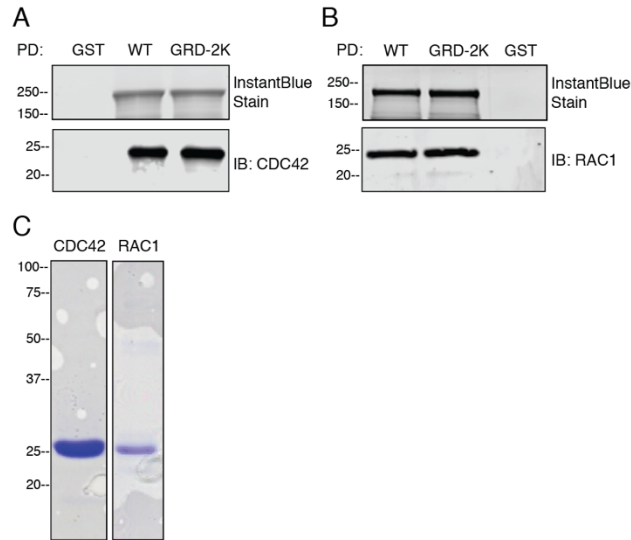


Figure S3. Lysine mutations in the GRD of IQGAP1 do not disturb interactions between IQGAP1 and CDC42 or RAC1.

(A) Purified His-CDC42(Q61L) was incubated with equal amounts of purified GST-tagged WT IQGAP1 (WT) or IQGAP1 GRD-2K (GRD-2K) or GST alone. Complexes were isolated with glutathione-Sepharose (PD) and bound proteins were analyzed by SDS-PAGE. The gel was cut at ~50kDa; the upper section of the gel was stained with InstantBlue Protein Stain, while the lower section was processed by Western blotting and probed with anti-CDC42 antibodies. (B) RAC1 pulldown was performed as described above for CDC42, except His-RAC1(Q61L) was used and the Western blot was probed with anti-RAC1 antibodies. Data are representative of 2 to 4 experiments. (C) Purified His-CDC42(Q61L) and His-RAC1(Q61L) proteins were resolved by SDS-PAGE and stained with InstantBlue Protein Stain to show purity.

Role of IQGAP1 ubiquitination in CDC42 regulation

| | | |
|--------|---|------|
| IQGAP1 | S KEKREKLEAY QHL FY LLQ TN PTY LAKLI FQMPQNK STK FMDSV IFTLY NY ASNQREEY L | 1021 |
| IQGAP2 | S KERRKTLE TY QQL FY LLQ TN PLY LAKLI FQMPQNK STK FMDTV IFTLY NY ASNQREEY L | 934 |
| IQGAP3 | S KEKRQKLEAY QHL FY LLQ TQ PIY LAKLI FQMPQNK TTK FMEAV IFSLY NY ASSRREAY L | 1005 |
| IQGAP1 | LLRL FK TALQE EIK SKVDQ IQEIV TGNPT VI KMVVS FNR GARGQNALRQ I LAPVVK EIMD | 1081 |
| IQGAP2 | LLKL FK TALEE EIK SKVDQ VQDIV TGNPT VI KMVVS FNR GARGQNT LRQL LAPVVK EIID | 994 |
| IQGAP3 | LLQL FK TALQE EIK SKVEQ PQDVV TGNPT VVRLVVR FYRNGRGQ SALQE I LGKV IQDVLE | 1065 |
| IQGAP1 | DKSLNI KTD PVDIY KSWN QMESQ TGEAS KL PYDVT PEQAL AHE EVKTR LDSSI RNMRAV | 1141 |
| IQGAP2 | DKSL I I NTN PVEVY KAWVN QLETQ TGEAS KL PYDVT TEQAL TYPEVKNK LEAS I ENLRRV | 1054 |
| IQGAP3 | DKVL SVHTD PVHLY KNWIN QT EAQ TGQRS HL PYDVT PEQAL SHPEVQRR LD IALRNLLAM | 1125 |
| IQGAP1 | T DKFLSAIV SSVD <u>K</u> IPYGMRF IAKVL KDS LHEKF PDAGE DE LLK I I GNLLY YRYMNP AI V | 1201 |
| IQGAP2 | T DKVLNS I I SS LDLPYGLRY IAKVL KNS IHEKF PDATE DE LLK IVGNLLY YRYMNP AI V | 1114 |
| IQGAP3 | T DKFLLAIT SSVDQ IPYGMRY VAKVL KAT LAEKFPD ATD SEVYKVV GNLLY YRFLNPAVV | 1185 |
| IQGAP1 | APDAFD IIDLS AGGQL TTDQRNLGS IA <u>K</u> MLQHAASNKMFLGDNAHLSI INEYLSQSYQK | 1261 |
| IQGAP2 | APDGF D IIDMT AGGQI NSDQRNLGS VAKVL QHAASNKL FEGENEHLSSMNNYLSETYQE | 1174 |
| IQGAP3 | APDAFD IVAMA AGGALAAPQRHALGAVALLQHAAGKAFSGSQHLRV LNDYLEE THLK | 1245 |
| IQGAP1 | FRRF FQTAC DVPELQDKFN VDEYS DLVTL TKPVI YI SIGEI INTHT LLLDHQDA IAP EHN | 1321 |
| IQGAP2 | FRKY FKEACNV PEPEEKFNMDKYTDLVTVSKPVI YI SIEEI ISTHS LLEH QDA IAP EKN | 1234 |
| IQGAP3 | FRKF IHRACQV PEPEERFAVDEYS DMVAV AKPMVYI TVGELVNT HR LLEH QDC IAPDHQ | 1305 |
| IQGAP1 | DPIHEL LDDLG EVP TIESLIGESS | 1345 |
| IQGAP2 | DLLSEL LGS LG EVP TVESFLGEGA | 1258 |
| IQGAP3 | DPLHEL LEDLGELPTI PDLIGESI | 1329 |

Figure S4. Amino acid alignments of the GRD of human IQGAPs. Sequences of the GRD of IQGAP1 (962-1345), IQGAP2 (875-1258) and IQGAP3 (946-1329) were aligned by Clustal W Multiple Alignment. Lys-1155 and Lys-1230 of IQGAP1 are underlined and colored red.

Video S1. Time-lapse wound healing assay of IQGAP1-null MEFs expressing GFP-tagged WT IQGAP1 or IQGAP1 GRD-2K. The video corresponds to the experiment shown in Fig. 6B.