

Enhancement of Migration and Invasion of Gastric Cancer Cells by IQGAP3

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Table S1 Up-regulation of IQGAP3 protein expression in gastric cancers based on tissue microarray analysis.

Immunohistochemical staining of IQGAP3 in 50 gastric neoplasms			
histology	positive (%)	negative (%)	total
adenocarcinoma			
intestinal type	19 (90.5)	2 (9.5)	21
diffuse type	4 (80)	1 (20)	5
signet-ring cell type	10 (47.6)	11 (52.4)	21
GIST	0 (0)	3 (100)	3
Total	33	17	50

GIST: Gastrointestinal stromal tumor

Table S2 Clinicopathological correlation of IQGAP3 protein expression with invasive phenotypes of gastric cancers based on tissue microarray analysis.

Association of IQGAP3 expression with stage of the tumors*			
Stage**	positive (%)	negative (%)	total
IA, IB	12 (100)	0 (0)	12
II, IIIA, IIIB, IV	11 (78.6)	3 (21.4)	14
Total	23	3	26

*The tumors included 21 intestinal carcinomas and 5 diffuse type carcinomas

**Stage grouping (UICC)

Fisher's exact test two-tailed p value = 0.22

Association of IQGAP3 expression with lymph node involvement of the tumors*			
Lymph node involvement	positive (%)	negative (%)	total
positive	12 (85.7)	2 (14.3)	14
negative	11 (91.7)	1 (8.3)	12
Total	23	3	26

*The tumors included 21 intestinal carcinomas and 5 diffuse type carcinomas

Fisher's exact test two-tailed p value = 1.00

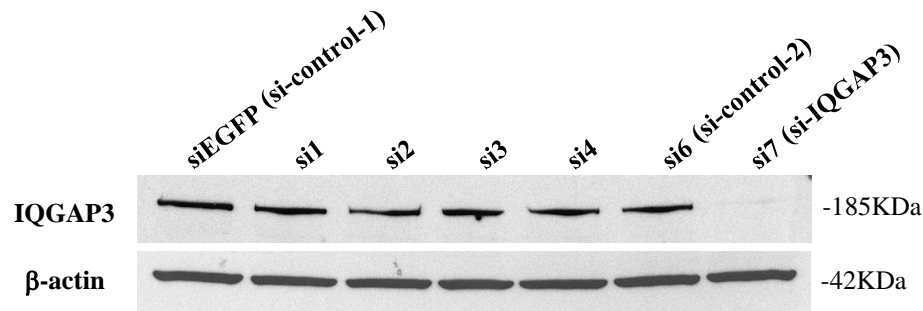


Figure S1. The knock-down effect of *IQGAP3*-siRNAs used in this study. Immunoblot analysis of *IQGAP3*, which was performed using TMK1 cells treated with different siRNAs designed to target *IQGAP3* transcripts (si1-si7) showed that only si7 successfully reduced the *IQGAP3* protein in TMK1 cells. β-actin served as a quantitative control. Si7 was then referred to as si-IQGAP3, while siEGFP and si6 were used as control siRNAs and referred to as si-control-1 and si-control-2, respectively, in Figure 5.

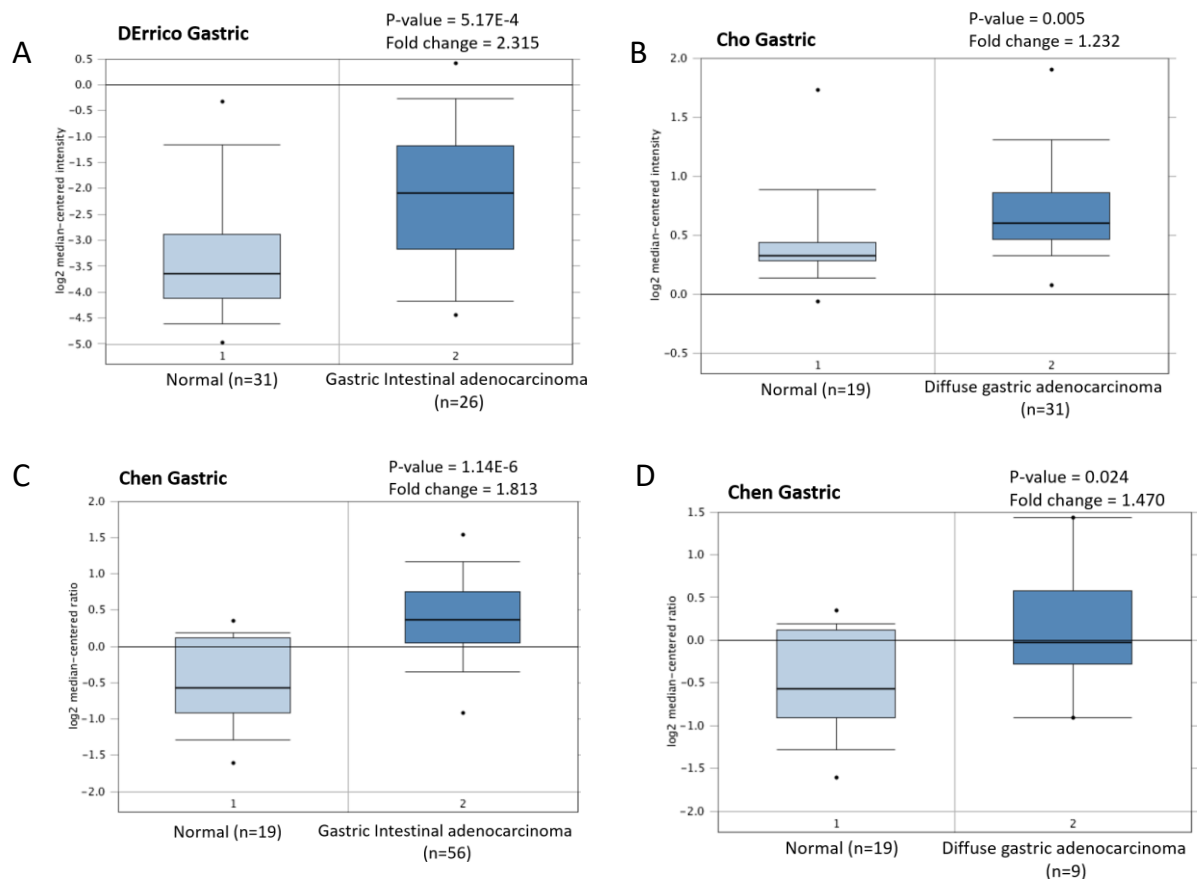


Figure. S2. Expression of *IQGAP3* in diffuse and intestinal subtypes of gastric cancer. The RNA-Seq data analyses were performed in Oncomine (<https://www.oncomine.org/resource/login.html>). Data from different studies, i.e. Derrico (A), Cho (B), and Chen et al.(C-D), all show statistically significantly higher expression in both subtypes of gastric cancer comparing to normal controls.

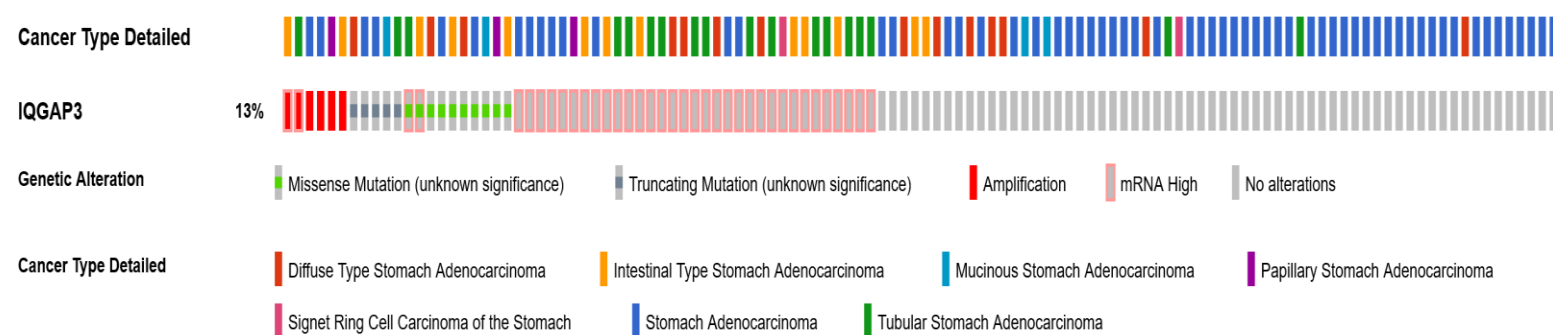


Figure S3. Frequencies and types of genetic alteration identified in *IQGAP3*. cBioportal (<https://www.cbioportal.org/>) was used to summarize all genetic alterations identified in gastric cancer patients in TCGA database.

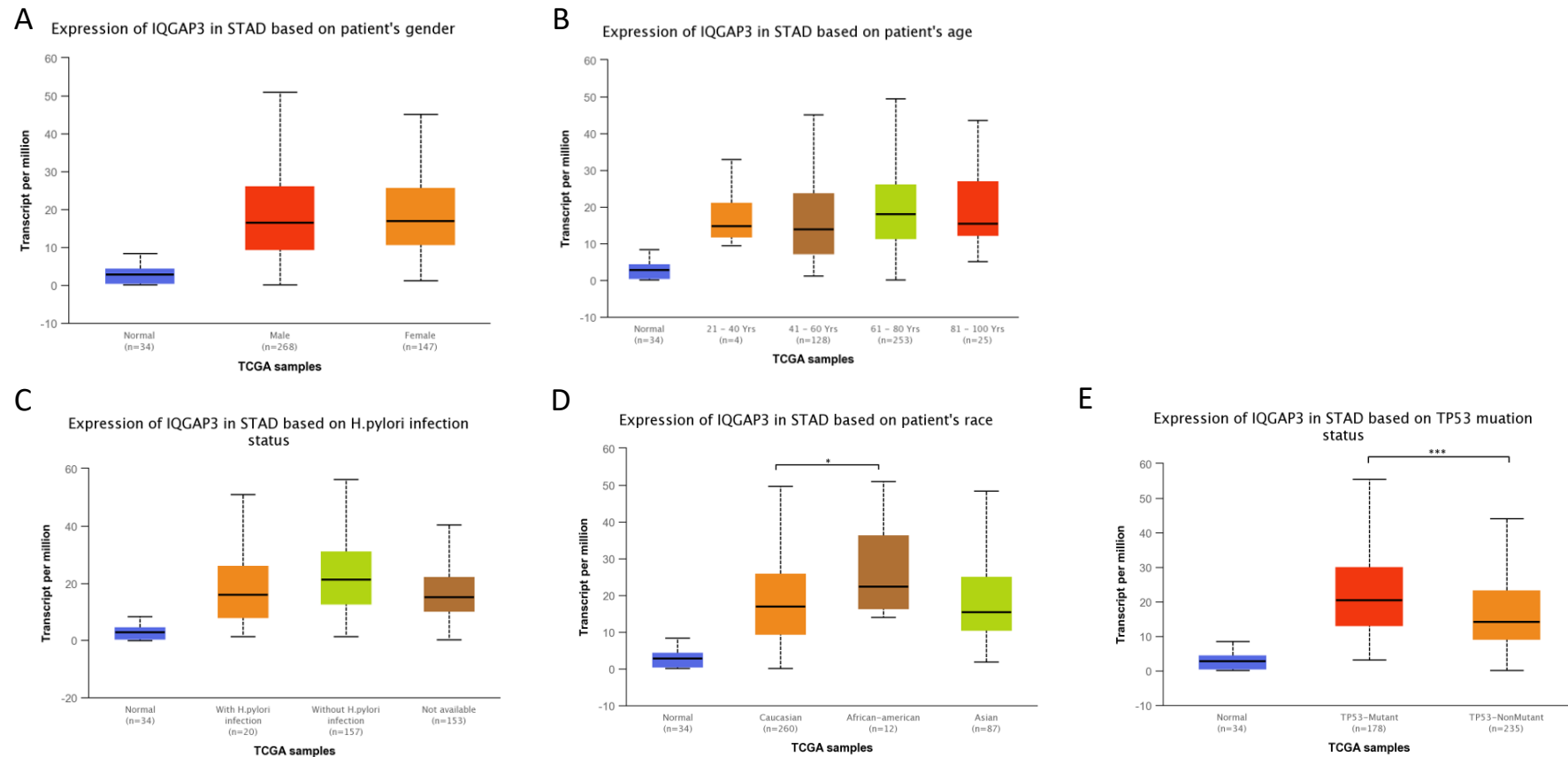


Figure S4. Expression of *IQGAP3* in patients with different clinical characteristics analyzed by UALCAN (<http://ualcan.path.uab.edu/>). RNA-Seq expression of *IQGAP3* in patients with different clinical characteristics, including (A) gender, (B) age, (C) H.pylori infection status, (D) race, and (E) *TP53* mutation status, as compared to normal controls. Of note, patients with gastric cancer all have statistically significantly higher expression of *IQGAP3* comparing to normal controls ($p < 0.05$); however, this information is not indicated in the plots above. Only the statistically significant results between groups (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) were demonstrated.

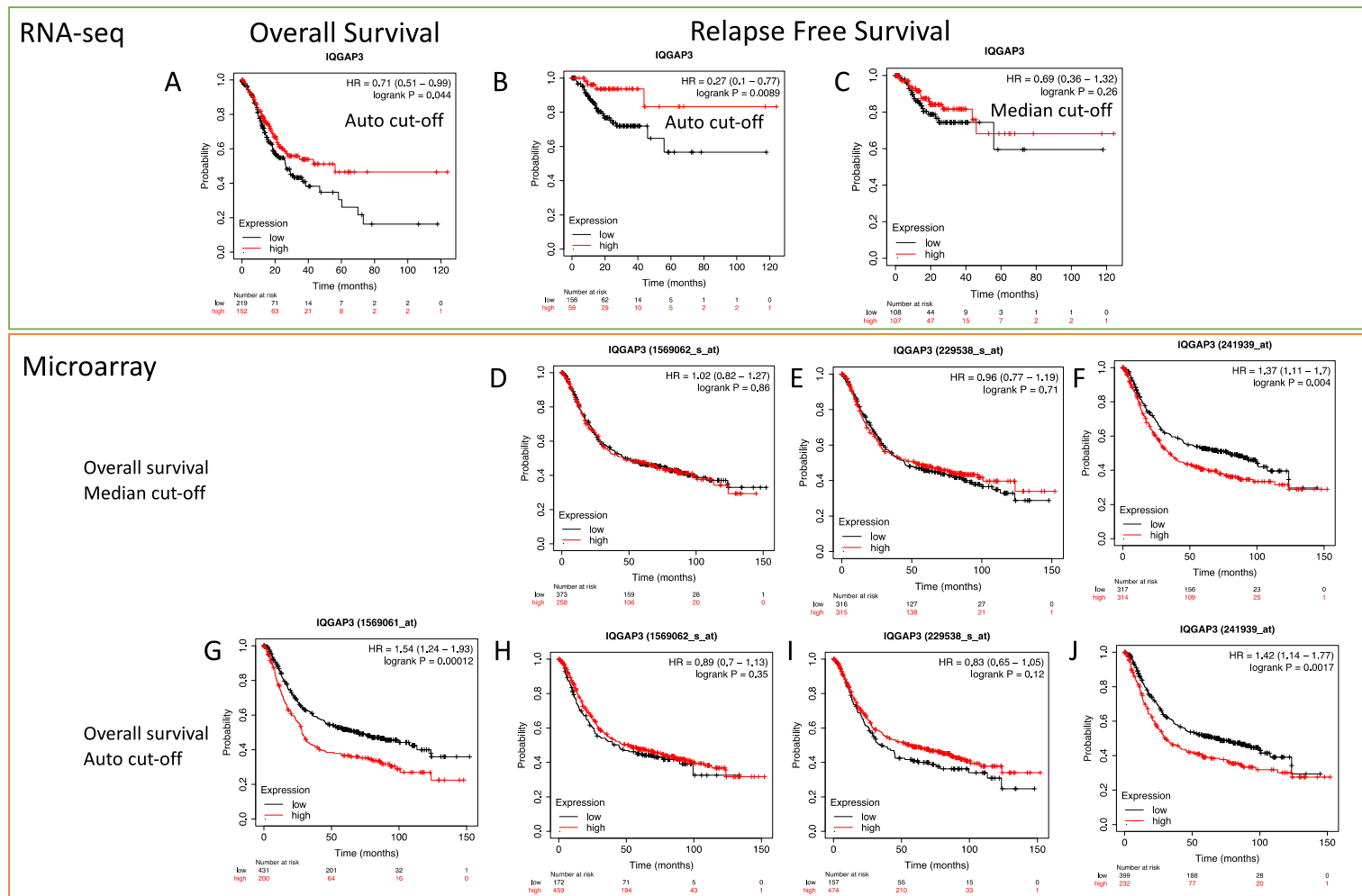


Figure S5. Kaplan-Meier plots of gastric cancer patients using microarray and RNA-Seq data from TCGA database by KMplotter (<https://kmplot.com/analysis/>). (A-C) Overall survival rates and relapse free survival rates of patients with low and high expression of *IQGAP3* from RNA-seq dataset. Different cut-off values were applied (automatic and median cut-off). (D-J) Overall survival rates of patients with low and high expression of *IQGAP3* from multiple microarray dataset. The results from all four different probes covering *IQGAP3* transcripts (Probe IDs: 1569061_at, 1569062_s_at, 229538_s_at, and 241939_at) are shown. Different cut-off values were also applied. Results of overall survival using median cut-off is shown in Figure 2E.

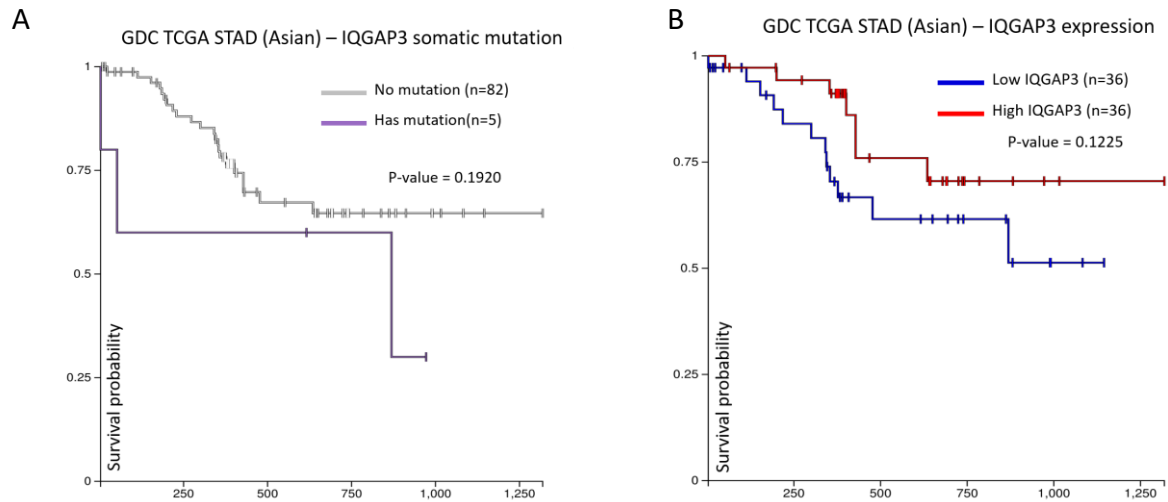


Figure S6. Overall survival of Asian patients in UCSC Xena (<https://xena.ucsc.edu/>). (A) Overall survival rate between patients with mutation in *IQGAP3* and without mutation in *IQGAP3*. (B) Overall survival rate between patients with high and low expression of *IQGAP3*.

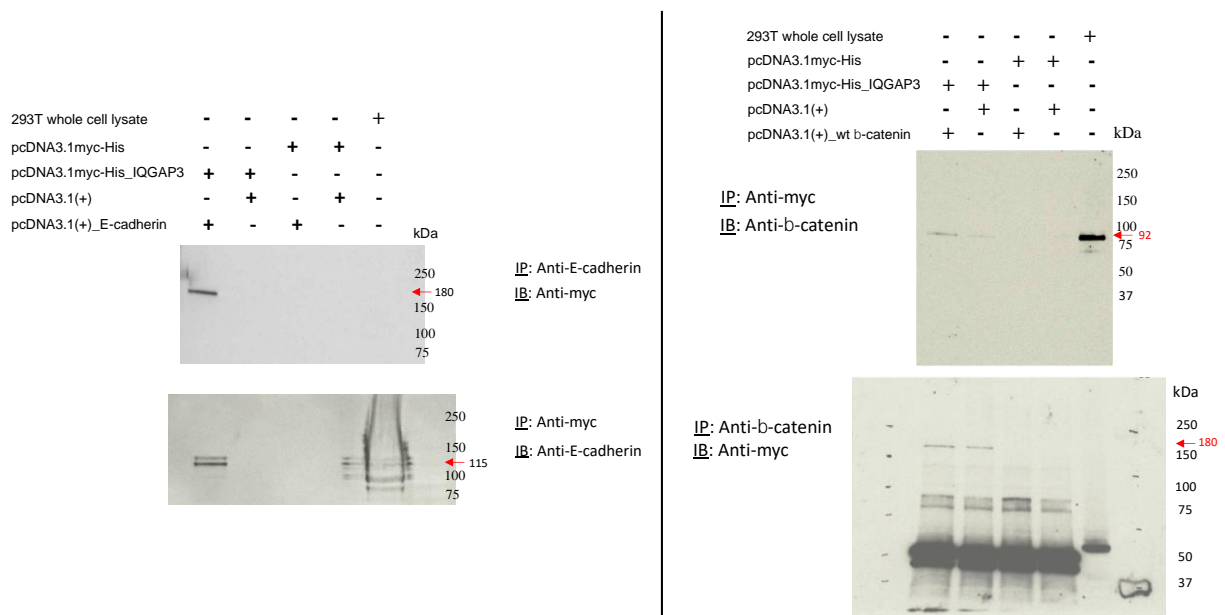
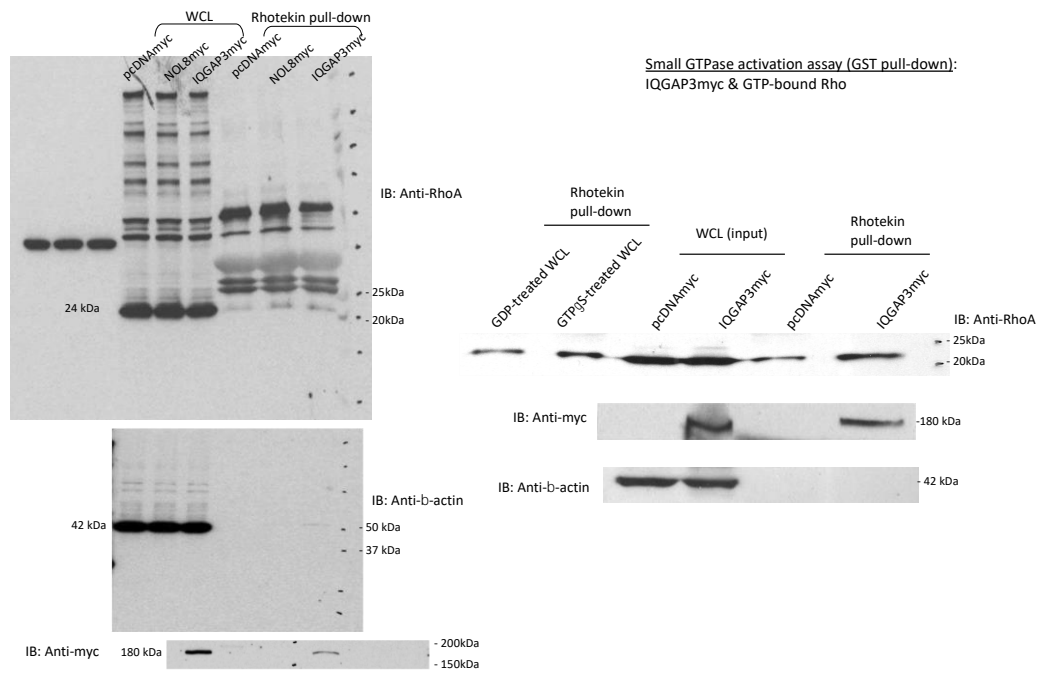
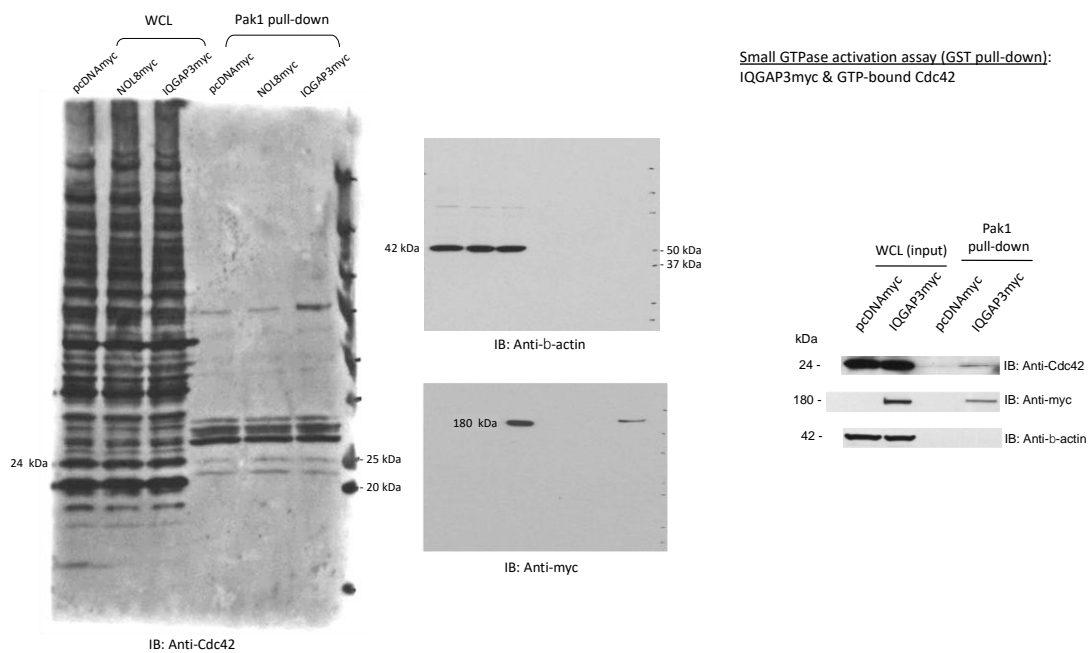


Figure S7. Original Western blots shown in Figure 4. Lt. panel: E-Cadherin; Rt. panel: β -catenin.

A



B



C

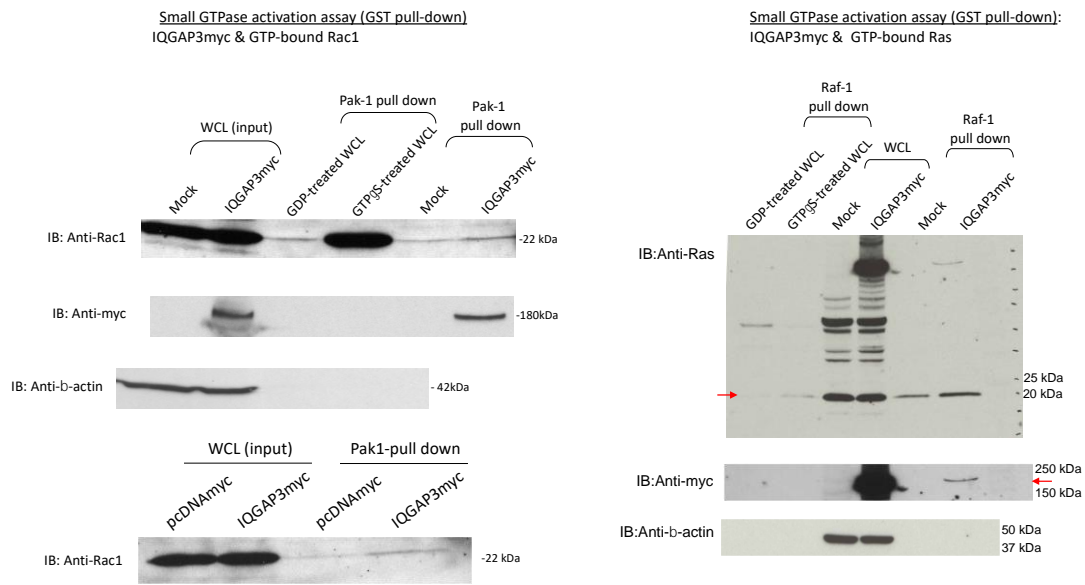


Figure S8. Representative images of original Western blots shown in Figure 6. Small GTPase activation assays. (A) RhoA activation, (B) Cdc42 activation, (C) Lt. panel: Rac1; Rt. panel: Ras.