Supplemental Materials

Molecular Biology of the Cell

Chiang et al.

SUPPLEMENTARY FIGURE LEGENDS

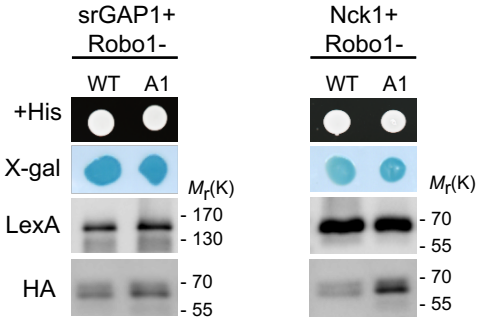
SUPPLEMENTARY FIGURE 1. The Arl4A-binding deficient Robo1 mutant did not affect the Robo1-srGAP1 or Robo1-Nck1 interactions. The interaction abilities of the Robo1-WT, Robo1-A1 mutant, and Robo1-A2 mutant constructs with srGAP1 were tested using yeast two-hybrid assays. The levels of proteins expressed by the transforming plasmids were confirmed by immunoblotting. Lamin was used as the negative control. After co-transformation with the indicated plasmids, interactions were verified by growth of the yeast on a synthetic His+ plate and a His^- plate followed by filter assays for β-galactosidase activity. Twenty micrograms of total protein were loaded onto a 10-well gel to detect proteins.

SUPPLEMENTARY FIGURE 2. Arl4A and Robo1 are important for cell migration.
(A and C) Representative images of wound healing assays. HeLa cells were transfected with the indicated plasmids or siRNA for 18 hours and then subjected to wound healing migration assays. Scale bar = 45 μm. Histogram: Wound healing migration assay data were quantified based on three biological replicates. Scatter plots represent the mean±SD. *: $p<0.05$, ***: $p<0.001$ (two-tailed Student's t-test). (B) Q-PCR analysis of Arl4A mRNA expression in HeLa cells transfected with the indicated siRNAs. GAPDH was used as the internal control. Data are presented as the mean±SD. ***: $p<0.001$ (two-tailed Student's t-test). (D) Forty micrograms of total protein were loaded onto a 10-well gel to detect proteins. Western blot analysis of lysates from HeLa cells transfected with the indicated plasmids was performed to confirm equal expression. The percentage of Robo1 after siRNA treatment was 7.14±0.3%.

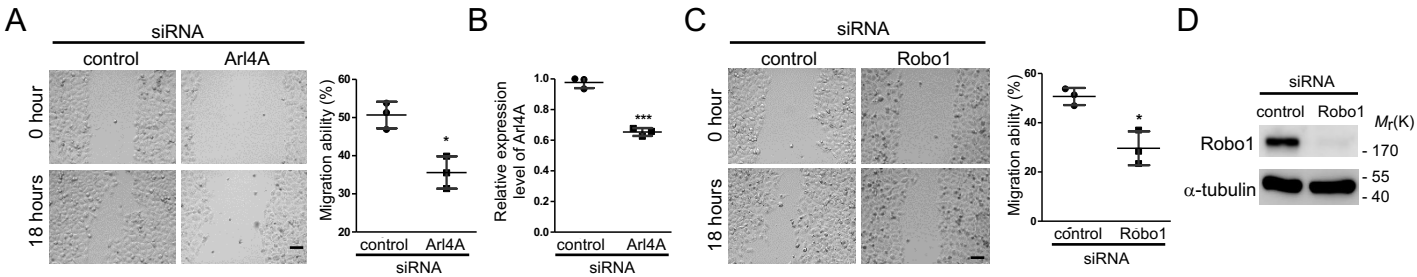
SUPPLEMENTARY FIGURE 3. The Arl4A-Robo1 interaction is required for Cdc42 activation. HeLa cells were transiently transfected with Cdc42-HA, Arl4A, Flag-Robo1-WT, and Flag-Robo1-A1. The cells were lysed, and Cdc42 activity pull-down assays were performed. Equal amounts of GST beads and cell lysates were used in each experiment as shown by Coomassie Blue staining. Histogram: Active Cdc42 was quantified based on each independent experiment with three replicates. Scatter plots represent the mean±SD. *: $p<0.05$, **: $p<0.005$, ***: $p<0.001$ (one-way ANOVA with Dunnett's post hoc multiple comparison test).

SUPPLEMENTARY FIGURE 4. Representative images of HEK293T cells transfected with the indicated plasmids and siRNA and then subjected to the transwell assay. Purified Slit2 (0.6 μg/mL) was added to the lower chamber of the transwell apparatus. The number of migrated cells in a field was calculated using ImageJ software after 22 hours of migration. Histogram: Migration assay data were quantified based on three biological replicates. Scatter plots represent the mean±SD. *: $p<0.01$ (one-way ANOVA with Dunnett's post hoc multiple comparison test).

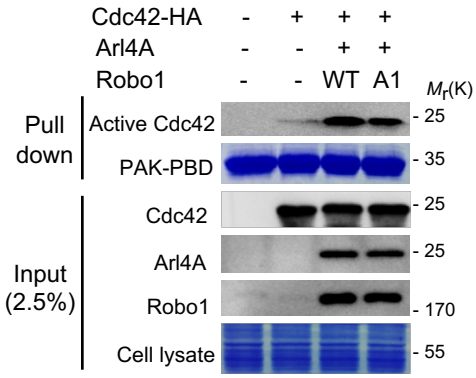
S Figure 1

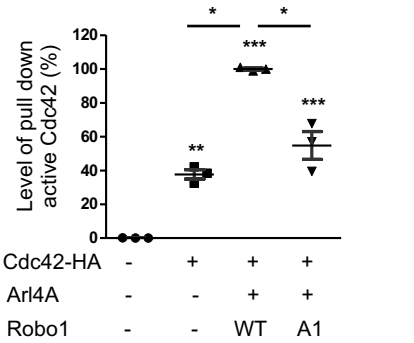

srGAP1+
Robo1-
Nck1+
Robo1-
WT
A1
WT
A1
+His
X-gal
LexA
HA
Mr(K)
- 170
- 130
- 70
- 55
Mr(K)
- 70
- 55
- 70
- 55

S Figure 2

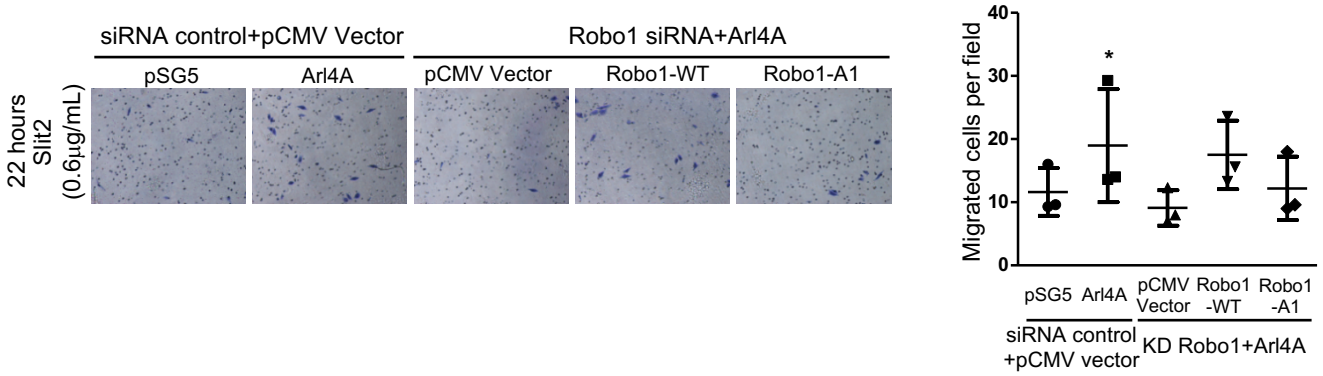

A
siRNA
control
Arl4A
0 hour
18 hours
Migration ability (%)
60
50
40
30
20
*
control
Arl4A
siRNA
B
Relative expression level of Arl4A
1.0
0.8
0.6
0.4
0.2
0.0
***
control
Arl4A
siRNA
C
siRNA
control
Robo1
0 hour
18 hours
Migration ability (%)
60
40
20
0
*
control
Robo1
siRNA
D
siRNA
control
Robo1
Mr(K)
Robo1
- 170
α-tubulin
- 55
- 40

S Figure 3


Cdc42-HA - + + +
Arl4A - - + +
Robo1 - - WT A1
Mr(K)
Pull down
Active Cdc42
PAK-PBD
Input (2.5%)
Cdc42
Arl4A
Robo1
Cell lysate
25
35
25
25
170
55


Level of pull down active Cdc42 (%)
120
100
80
60
40
20
0
*
*
***
**
***
Cdc42-HA - + + +
Arl4A - - + +
Robo1 - - WT A1

S Figure 4


siRNA control+pCMV Vector
Robo1 siRNA+Arl4A
pSG5
Arl4A
pCMV Vector
Robo1-WT
Robo1-A1
22 hours
Slit2
(0.6μg/mL)

Migrated cells per field
40
30
20
10
0
*
pSG5
Arl4A
pCMV Vector
Robo1 -WT
Robo1 -A1
siRNA control +pCMV vector
KD Robo1+Arl4A