Supplementary Figure Legends

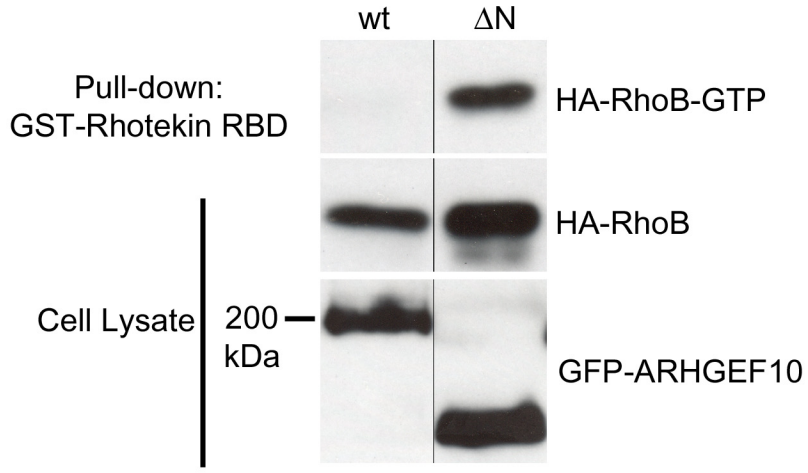
Figure S1. **Activation of RhoB and RhoC by ARHGEF10 ΔN in HEK293T cells.** *A*. HEK293T cells were transiently co-transfected with the plasmids encoding GFP-tagged ARHGEF10 wt or ΔN, and HA-tagged RhoB. After 48 h, cells were lysed with lysis buffer. The supernatants were mixed with GST-fused Rhotekin-RBD, and active form of RhoB (RhoB-GTP) was pulled-down. The amounts of active form of RhoB were determined by immunoblotting with anti-HA antibody. Expressions of HA-RhoB and GFP-ARHGEF10 in cell lysates were also detected by immunoblotting with anti-HA and anti-GFP antibodies, respectively. The representative image of three independent experiments is shown. *B*. HEK293T cells were transiently co-transfected with the plasmids encoding GFP-ARHGEF10 wt or ΔN, and HA-RhoC. After 48 h, cells were lysed with lysis buffer. The supernatants were mixed with GST-fused Rhotekin-RBD, and active form of RhoC (RhoC-GTP) was pulled-down. The amounts of active form of RhoC were determined by immunoblotting with anti-HA antibody. Expressions of HA-RhoC and GFP-ARHGEF10 in cell lysates were also detected by immunoblotting with anti-HA and anti-GFP antibodies, respectively. The representative image of three independent experiments is shown.

Figure S2. **Activation of RhoA and cell contraction by ARHGEF10 in HeLa cells**. *A*. Quantitative analyses of cell contraction of HeLa cells. HeLa cells were transiently transfected with the plasmid encoding GFP-ARHGEF10 wt, T332I, T332I ΔDH, T332I/S407A, or T332I/L547A. The proportion of cell contraction was scored as a percentage of the rounded cells of GFP positive cells. Cells floating in the culture medium were excluded from counting. The data represent the mean ± S.E. from three independent experiments. *$p < 0.05$. For each experiment, >100 cells were counted. *B*, HeLa cells were transiently transfected with the plasmid encoding GFP-ARHGEF10 wt, T332I, T332I ΔDH, T332I/S407A, or T332I/L547A. After 24 h, fluorescence images of living cells were observed. Rounded cells were indicated by arrowheads. *Scale bar*, 10 μm. *C*, HeLa cells were transiently transfected with the plasmid encoding GFP-ARHGEF10 wt or T332I. After 24 h, cells were fixed and co-stained with TRITC-phalloidin (red) and Hoechst33258 (blue). Transfected cells were shown by the fluorescence of GFP (green). *Scale bar*, 10 μm. *D*, HeLa cells were transiently co-transfected with the plasmid encoding GFP-ARHGEF10 wt, T332I, T332I ΔDH, T332I/S407A, or T332I/L547A, pSRE.L-luciferase reporter plasmid encoding firefly luciferase, and pRL-TK control vector encoding *Renilla* luciferase. The firefly luciferase activities were normalized to the *Renilla* luciferase activities, and values are expressed as fold induction compared with wt. The data are the mean ± S.E. from three independent experiments. *$p < 0.05$.

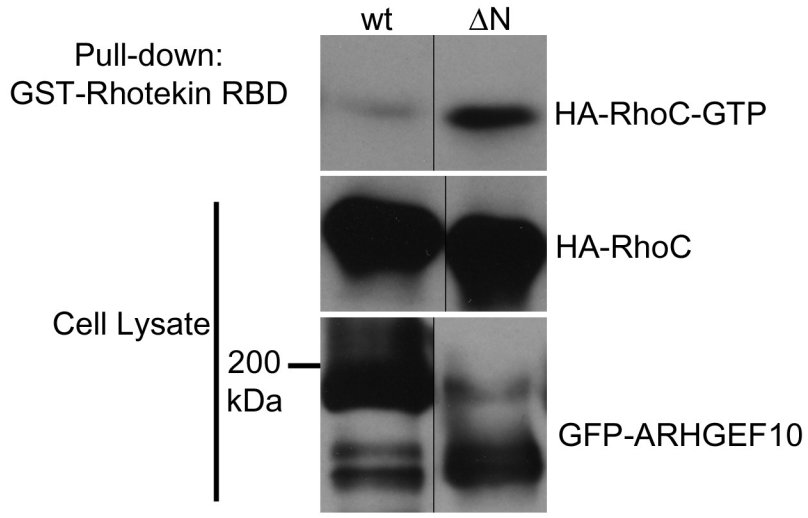
Figure S3. **Inhibition of ARHGEF10 T332I-induced cell contraction by a ROCK inhibitor in HeLa cells.** *A*, HeLa cells were transiently transfected with the plasmid encoding GFP-ARHGEF10 T332I, and cells were treated with or without 10 μM Y27632 for 8 h. After then, fluorescence images of living cells were observed. Rounded cells were indicated by arrowheads. Cells floating in the culture medium were excluded from counting (arrows). *Scale bar*, 10 μm. *B*, a quantitative analysis of cell contraction of HeLa cells. HeLa cells were transiently transfected with the plasmid encoding GFP-ARHGEF10 T332I, and cells were treated with or without 10 μM Y27632 for 8 h. The proportion of cell contraction was scored as a percentage of the rounded cells of GFP positive cells. Cells floating in the culture medium were excluded from counting. The data represent the mean ± S.E. from three independent experiments. *$p < 0.05$. For each experiment, >100 cells were counted.

Figure S1

A


wt
ΔN
Pull-down:
GST-Rhotekin RBD
HA-RhoB-GTP
HA-RhoB
Cell Lysate
200
kDa
GFP-ARHGEF10

B


wt
ΔN
Pull-down:
GST-Rhotekin RBD
HA-RhoC-GTP
HA-RhoC
Cell Lysate
200
kDa
GFP-ARHGEF10

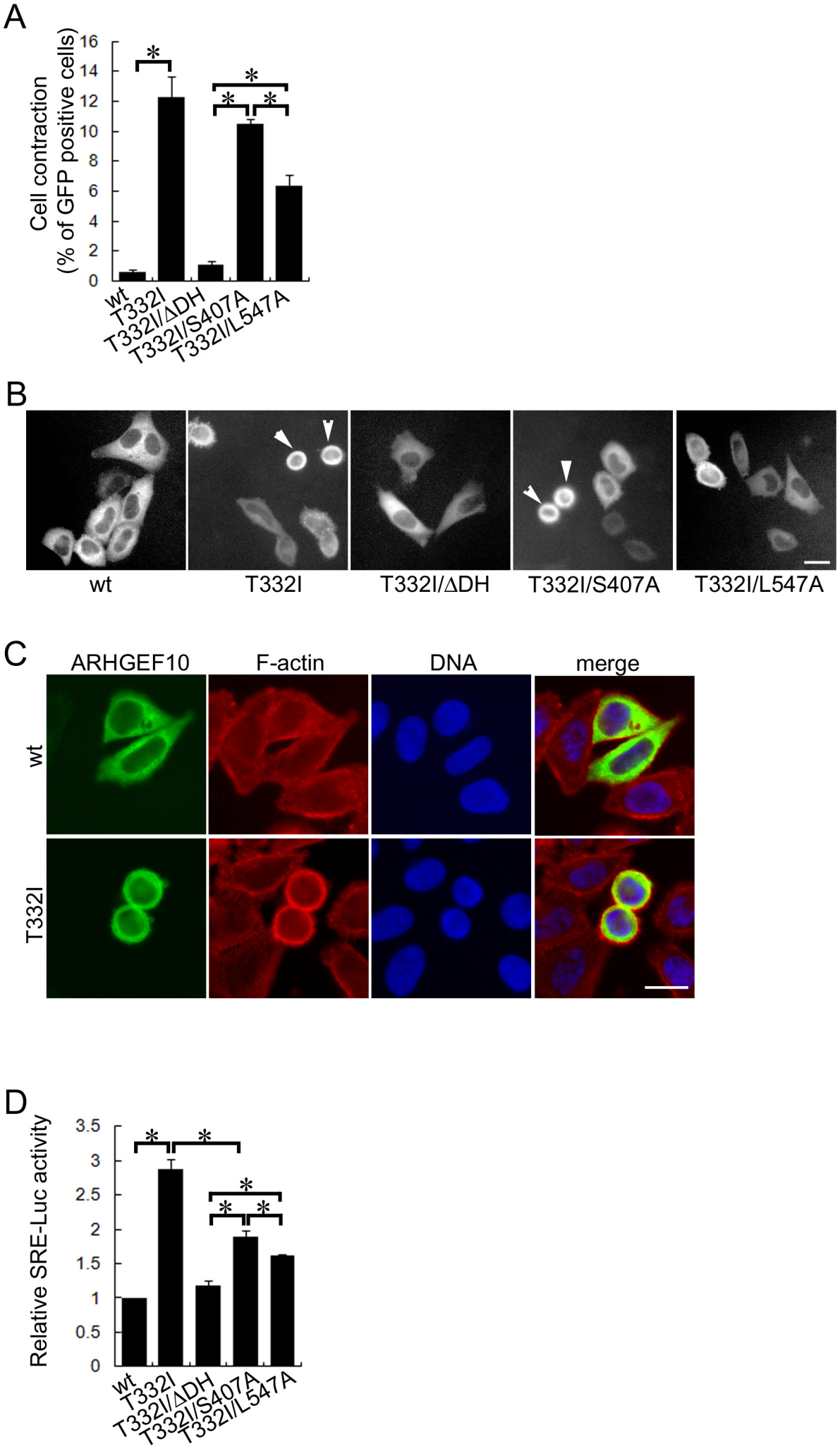
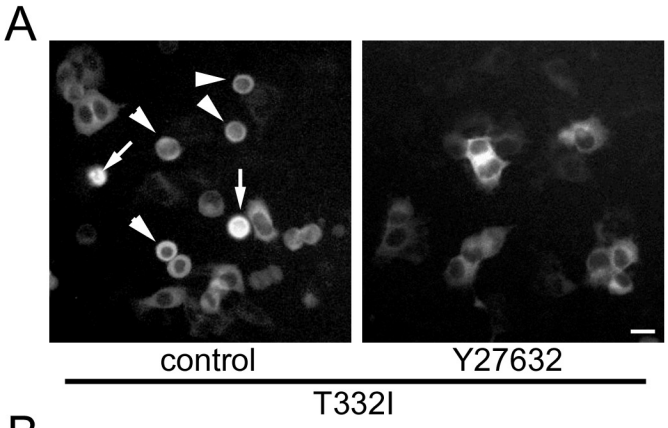
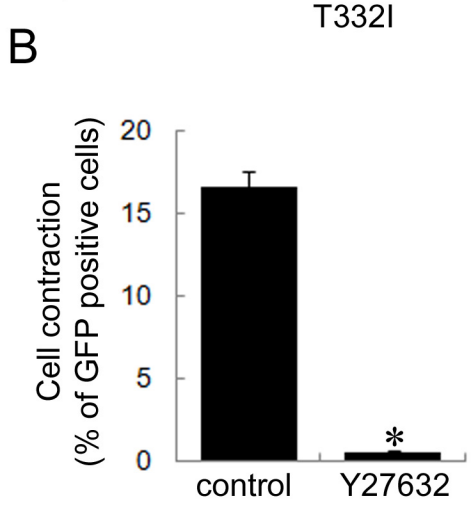

A
Cell contraction
(% of GFP positive cells)
0
2
4
6
8
10
12
14
16
*
*
*
*
wt
T332I
T332I/ΔDH
T332I/S407A
T332I/L547A
B
wt
T332I
T332I/ΔDH
T332I/S407A
T332I/L547A
C
ARHGEF10
F-actin
DNA
merge
wt
T332I
D
Relative SRE-Luc activity
0
0.5
1
1.5
2
2.5
3
3.5
*
*
*
*
*
wt
T332I
T332I/ΔDH
T332I/S407A
T332I/L547A

Figure S3


A
control
Y27632
T332I


B
Cell contraction
(% of GFP positive cells)
20
15
10
5
0
*
control
Y27632