Supplemental Materials

Molecular Biology of the Cell

Karunanithi et al.

Supplemental Figure Legends:

Figure S1: Development of GST-Rim1 RBD pull-down assay. (A) Schematic of effector pull-down assay. Immobilized Rab10 effector selectively isolates the GTP-bound active Rab10 from whole cell lysate. (B) COS-1 cells were transiently transfected with Myc-Rim1-RBD and/or HA-Rab10 WT, QL, TN as indicated. Whole cell lysates (lower panels) or anti-HA immunoprecipitates were subjected to immunoblotting with anti-Myc or anti-HA antibodies. (C) COS-1 cells were transiently transfected with HA-Rab10 construct and cell lysates bound to GDP or GTPγS were incubated with immobilized GST or GST-Rim1-RBD as indicated. Associated proteins were resolved on SDS-PAGE and determined by Western Blot with anti-HA antibody. (D) Cos-1 cell lysates overexpressing Flag-AS160 WT and/or Myr-Akt in the presence of HA-Rab10. GST-Rim-RBD beads were used to pull down active Rab10. Levels of proteins in the total cell lysates and pull down are shown. (E) Quantification of blots in 'D' from at least three independent experiments ($n=3$, $*p<0.05$).

Figure S2: RalA does not affect Rab10 activity. GST-Rim1-RBD pull down assay was performed on Cos-1 lysates expressing HA-Rab10 together with wild type, active (GV) or inactive (SN) RalA. Rab10 levels in total cell lysates and active Rab10 are determined by western blotting.

Figure S3: Rlf interacts with active Rab10. Myc-Rab10 (WT or QL) was expressed alone or together with HA-Rlf. Immunoprecipitation experiment with anti-Myc antibody was performed and co-precipitated HA-Rlf was analyzed in an immunoblot.

Figure S4: Rab10 has a localized effect on RalA. (A) Cos-1 cells overexpressing vector, pRK5-Myc-Rlf or Rlf-CAAX were subjected to sub-cellular fractionation. Crude membrane (M) and

cytosolic (C) fractions were subjected to SDS-PAGE and immunoblotted. Expression in whole cell lysates is shown (lower panels). (B) Flag-RalA was co-expressed with vector, wild type Rlf or Rlf-CAAX. Cell lysates were pulled down using Sec5-RBD beads to assess RalA activity using immunoblots. (C) Cos-1 cells transfected with RFP vector, RFP-AS160GAP or RFP-AS160GAP-Rab10tail together with Flag-RalA. Pull down experiment was performed with GST-RalBP1 beads and subjected to immunoblotting.

Supplemental Methods:

Oligo Sequences:

AS160_mouse: CGACCUCACCUACUUUGCCUAUUUG

Rab10_mouse_1: GACGAUGCCUUCAAUACCACCUUUA

Rab10_mouse_2: AACAGAUUGCAAGGGAGCAUGGUAU

Rlf_mouse_1: GAACCUUCCUGAAGGACCUCGUGAU

Rlf_mouse_2: CCACACUCAGCUAACGUCUUCUAUG

RalA_mouse_1: GGAAGAAGUGCAGAUCGACAUCUUA

RalA_mouse_2: UAACUAUGUGGAGACGUCUGCUAAA

Rlf_human_1: GCUCCGAAACUUCUCUUCAGUUUAU

Rlf_human_2: GCCAUGGAUGGAGCUUCACACGAUU

Figure S1

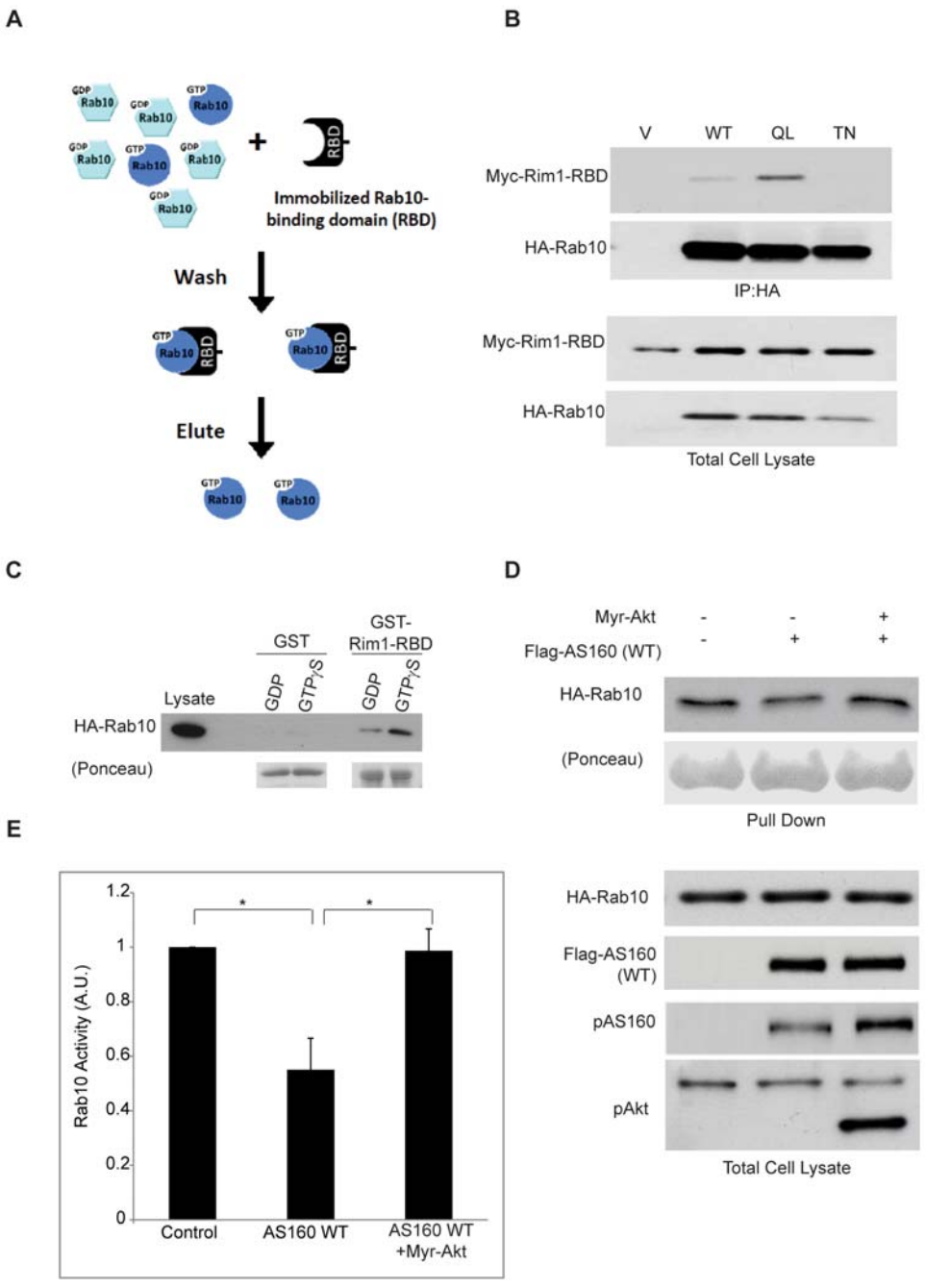

A
GDP Rab10
GTP Rab10
RBD
Immobilized Rab10-binding domain (RBD)
Wash
Elute
B
V
WT
QL
TN
Myc-Rim1-RBD
HA-Rab10
IP:HA
Total Cell Lysate
C
GST
GST-Rim1-RBD
GDP
GTPγS
Lysate
HA-Rab10
(Ponceau)
D
Myr-Akt
Flag-AS160 (WT)
HA-Rab10
(Ponceau)
Pull Down
Flag-AS160 (WT)
pAS160
pAkt
Total Cell Lysate
E
Rab10 Activity (A.U.)
Control
AS160 WT
AS160 WT +Myr-Akt

Figure S2

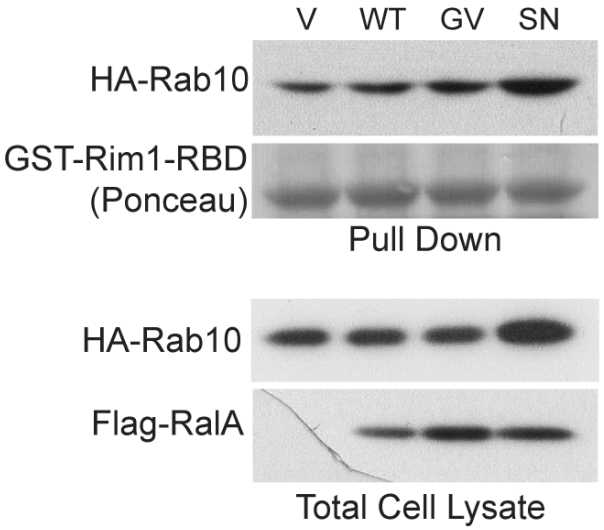

V
WT
GV
SN
HA-Rab10
GST-Rim1-RBD
(Ponceau)
Pull Down
HA-Rab10
Flag-RalA
Total Cell Lysate

Figure S3

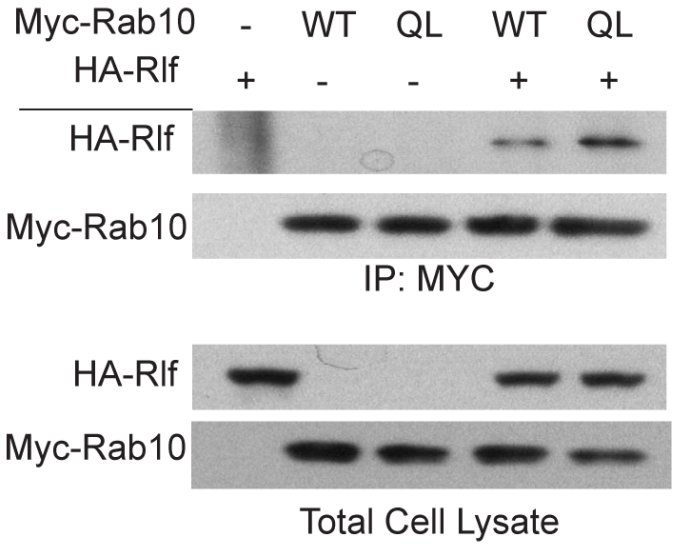
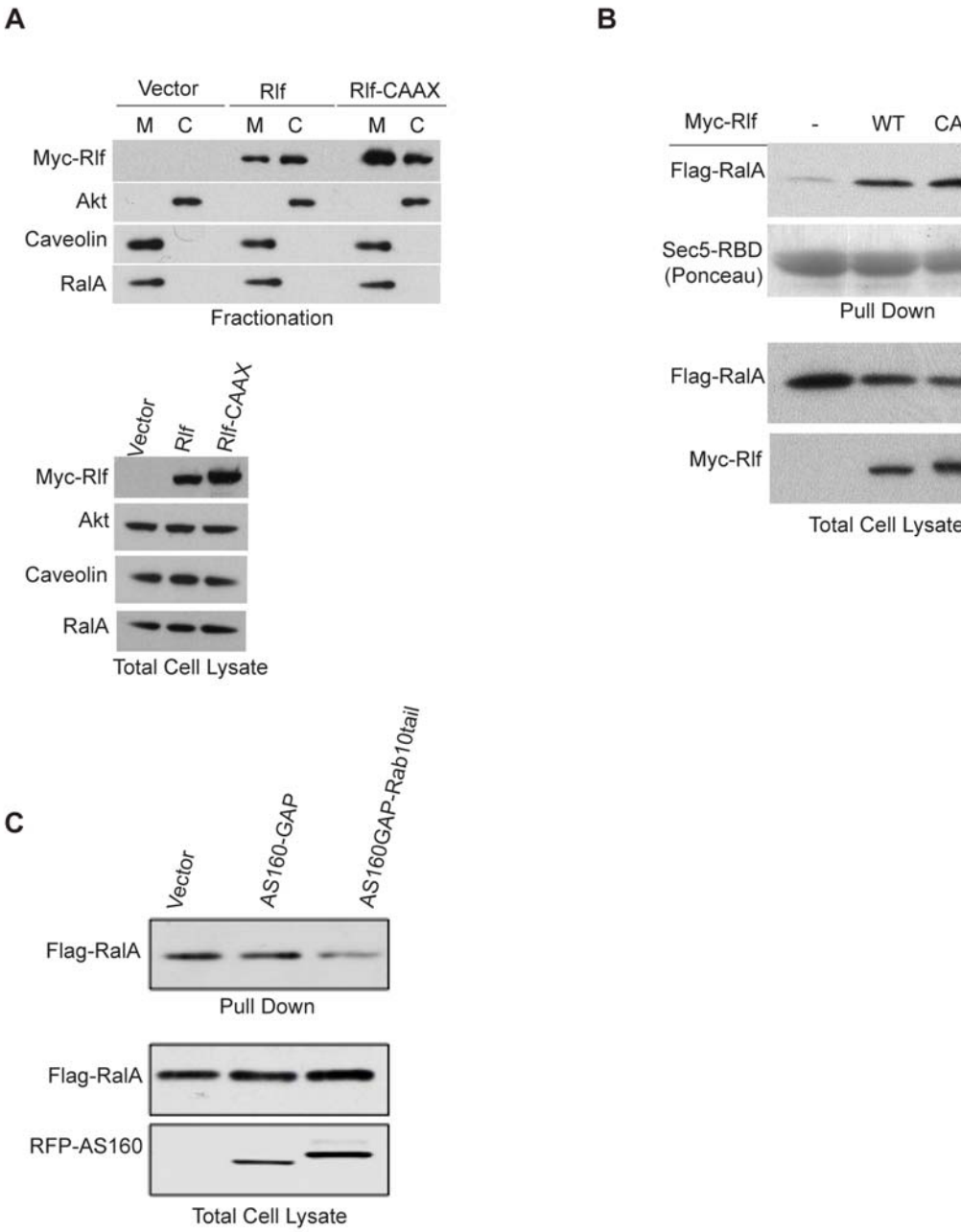

Myc-Rab10 - WT QL WT QL
HA-Rlf + - - + +
HA-Rlf
Myc-Rab10
IP: MYC
HA-Rlf
Myc-Rab10
Total Cell Lysate

Figure S4


A
Vector
Rlf
Rlf-CAAX
M C M C M C
Myc-Rlf
Akt
Caveolin
RalA
Fractionation
Vector
Rlf
Rlf-CAAX
Myc-Rlf
Akt
Caveolin
RalA
Total Cell Lysate
B
Myc-Rlf - WT CAAX
Flag-RalA
Sec5-RBD (Ponceau)
Pull Down
Flag-RalA
Myc-Rlf
Total Cell Lysate
C
Vector
AS160-GAP
AS160GAP-Rab10tail
Flag-RalA
Pull Down
Flag-RalA
RFP-AS160
Total Cell Lysate