Supplementary Information


Figure S1 Model of RalBP1-dependent mitotic mitochondrial fission.

Supplementary Information

A

B

C
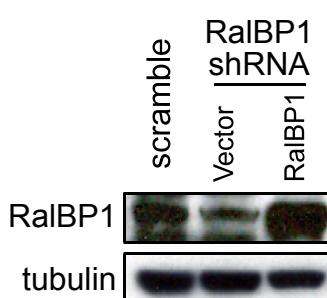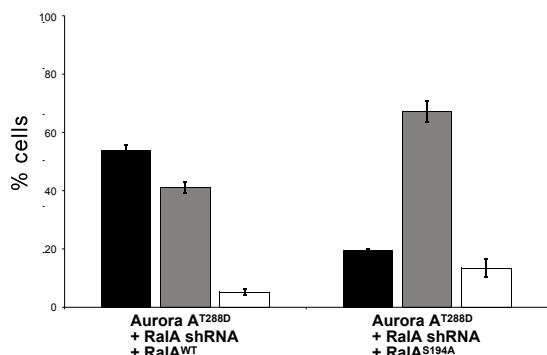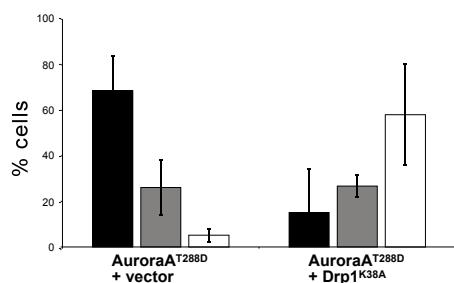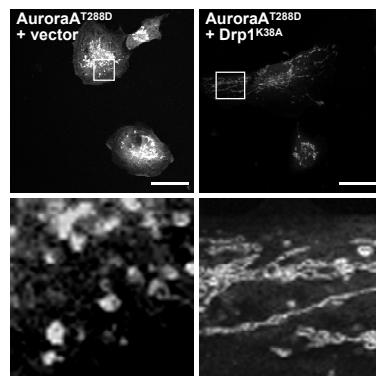
D
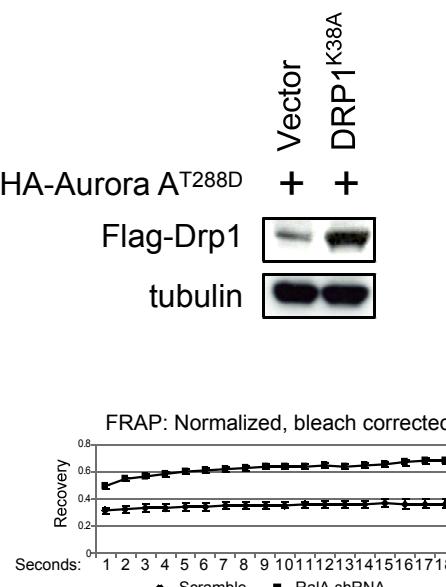
Figure S2 (a) Top- Immunoblot of RalA from HEK-TtH cells stably expressing scramble, RalA shRNA rescued with vector, shRNA-resistant RalA^{WT} or RalA^{S194A}, RalA^{WT} or RalA^{S194D}. Bottom- Immunoblot of RalBP1 and tubulin from HEK-TtH cells stably expressing scramble control or RalBP1 shRNA rescued with vector or shRNA-resistant RalBP1. (b) Top- MitoTracker Red staining of HEK-TtH cells expressing the indicated transgenes (scale bar = 2μm). Middle- Graph: % of cells (mean ± SD) exhibiting highly fragmented (■) intermediate (■) or highly interconnected (□) mitochondrial morphologies from 3 independent experiments (>100 cells). Bottom- Immunoblots of HEK-TtH cells stably expressing Aurora^{T288D} and either vector or DRP1^{K38A}. (c) Quantitation of mitochondrial morphologies of HEK-TtH cells expressing Aurora^{T288D}, RalA shRNA and shRNA-resistant RalA^{WT} or RalA^{S194A}. Graph: % of cells (mean ± SD) exhibiting highly fragmented (■) intermediate (■) or highly interconnected (□) mitochondrial morphologies from 3 independent experiments (>100 cells). (d) Mitochondrial network connectivity in either scramble or RalA shRNA HEK-TtH cells transiently transfected with mito-YFP.

Supplementary Information

A

B

myc-RalA: WT S194A

	U	M	U	M	
myc					input
P-S194 RalA					IP: myc
myc					

C

shRNA: Scram RalA RalBP1

	U	M	U	M	U	M
P-Histone H3 (S10)						
Histone H3						
RalA						
RalBP1						

D

shRNA: Scram Aurora A

	U	M	U	M
P-Histone H3 (S10)				
Histone H3				
Aurora A				

E

shRNA: scram Fis1

| actin | | |
| Fis1 | | |

shRNA: scram Plk1

| actin | | |
| Plk1 | | |

F
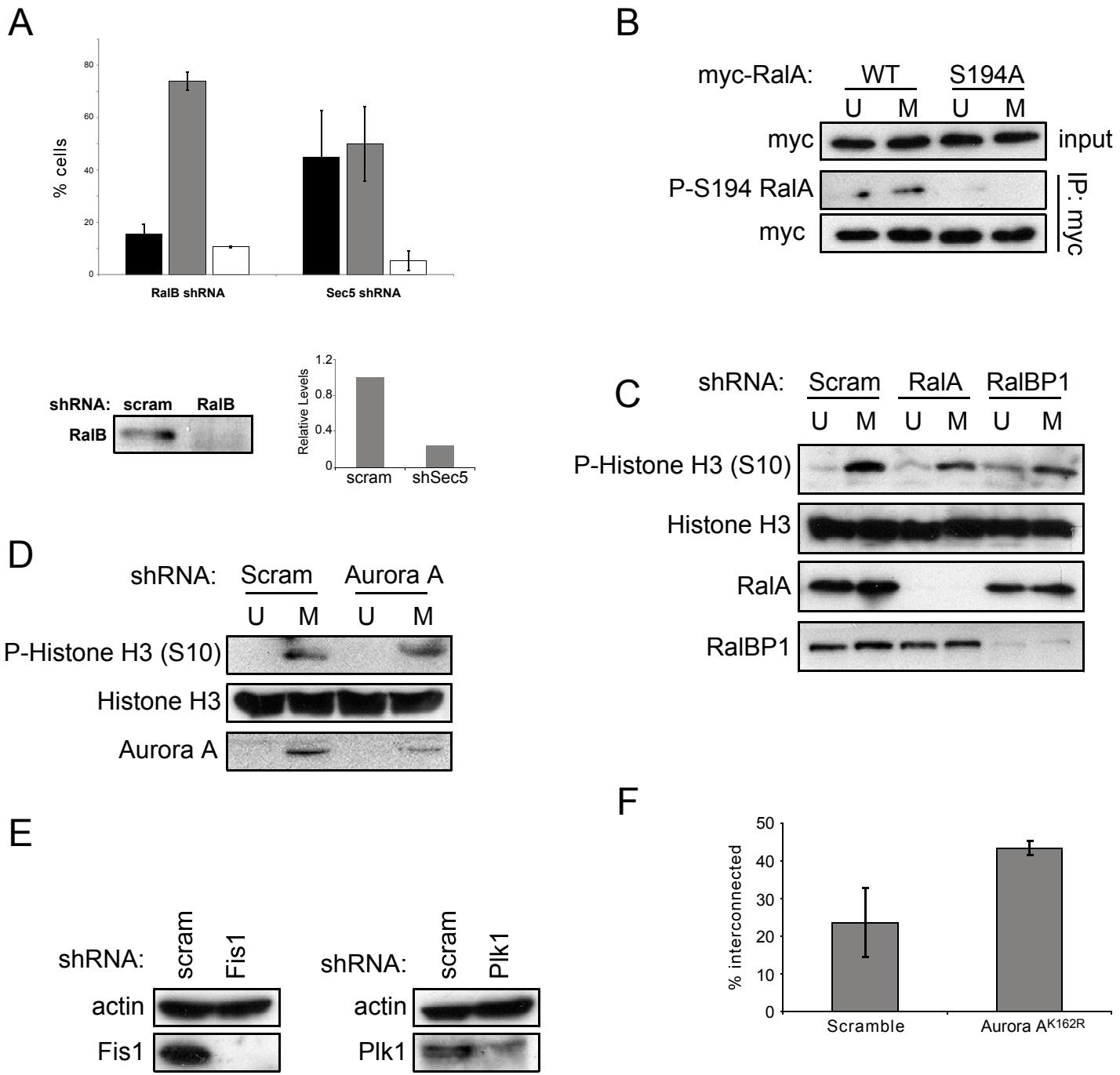
Figure S3 (a) Top- Quantitation of mitochondrial morphology of HEK-TtH cells expressing RalB or Sec5 shRNA. Graph: % of cells (mean ± SD) exhibiting highly fragmented (■) intermediate (■) or highly interconnected (□) mitochondrial morphologies from 3 independent experiments (>100 cells). Bottom- Immunoblot of RalB from HEK-TtH cells stably expressing scramble control or RalB shRNA and mRNA levels of Sec5 assessed by qRT-PCR of HEK-TtH cells stably expressing scramble control or Sec5 shRNA. (b) Immunoblot of whole cell extracts or myc immunoprecipitates from unsynchronized and mitotic HeLa cells expressing myc-RalA or myc-RalA^{S194A}. (c) Immunoblot of the indicated proteins in unsynchronized and mitotic HeLa cell expressing the indicated transgenes. (d) Immunoblot of the indicated proteins in unsynchronized and mitotic HeLa cell expressing inducible scramble or Aurora A shRNA. (e) Immunoblot analysis of Fis1, Plk1 and Actin from HeLa cells stably expressing scramble control, Fis1 or Plk1 shRNA. (f) Quantitation of the percent of HeLa cells stably expressing vector or Aurora A^{K162R} (n=50, mean ± SD from 3 experiments) exhibiting mitochondrial elongation during metaphase.

Supplementary Information
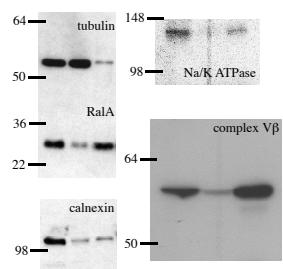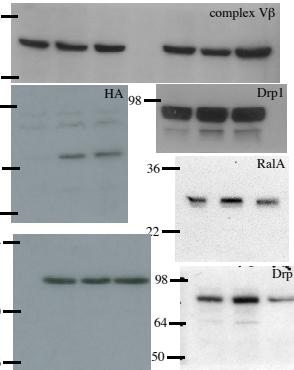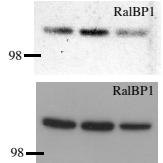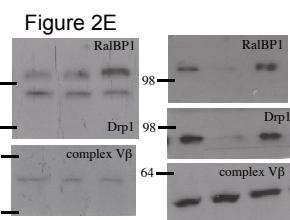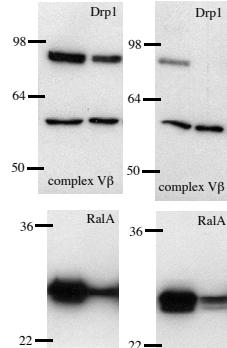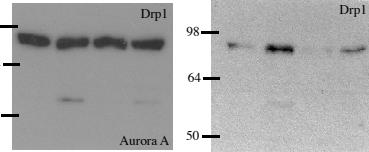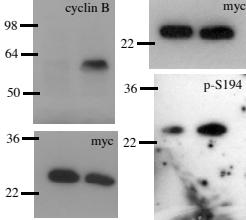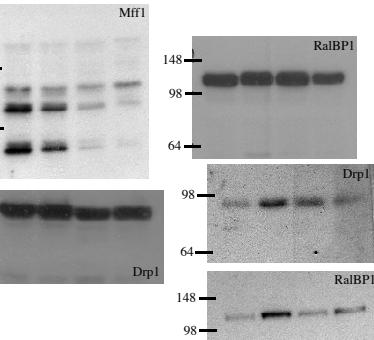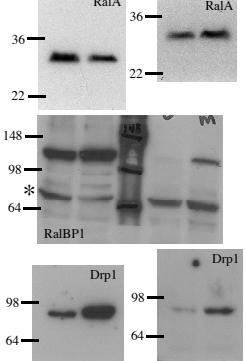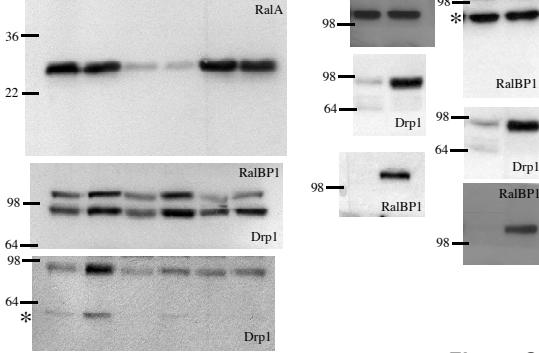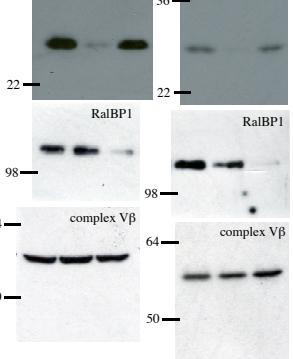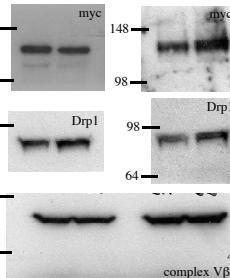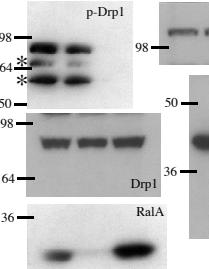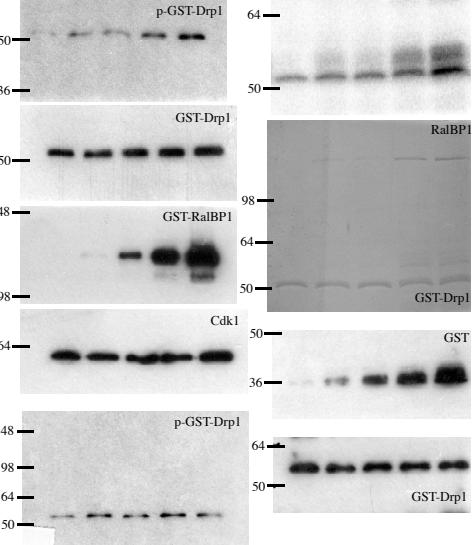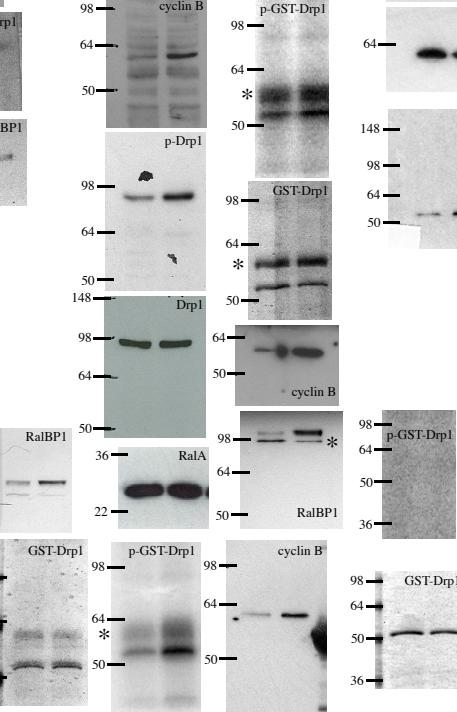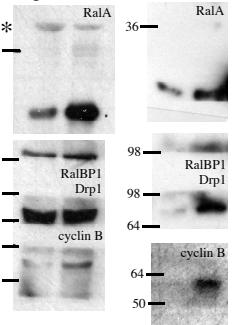
Figure S4 Full scans of immunoblot data. In most experiments, membranes were cut into several strips, each probed with a separate antibody. When present, nonspecific bands are indicated by an (*).

Supplementary Movie Legends

Movies S1-S2 Mitochondrial morphology visualized by live cell video microscopy of HeLa cells stably expressing RFP-Mito and scramble control shRNA.

Movies S3-S4 Mitochondrial morphology visualized by live cell video microscopy of HeLa cells stably expressing RFP-Mito and RalA shRNA.

Movies S5-S6 Mitochondrial morphology visualized by live cell video microscopy of HeLa cells stably expressing RFP-Mito and RalBP1 shRNA.