

**Supplemental Fig. S1:** HEK293 Flp-In lines induced to express GFP-DLC1 WT, K626A/R627G and  $\Delta$ PBR were harvested by hypotonic lysis and supernatant (cytosol) and pellet fractions (containing cellular membranes) were obtained by 100,000xg centrifugation of post-nuclear supernatants. The amount of GFP-tagged protein was determined before and after centrifugation. Data shown are the mean of two independent experiments. Error bars represent SE.

**Supplemental movie 1:** Non-induced HEK293 Flp-In cells were plated onto collagen-coated glass bottom dishes. After 5 min, bright field images were taken every 30 seconds for 90 min. The movie runs at 10 frames per second. The dotted rectangle corresponds to the area shown in Figure 8B.

**Supplemental movie 2:** HEK293 Flp-In DLC1 WT cells induced with doxycycline were plated onto collagen-coated glass bottom dishes. After 5 min, bright field images were taken every 30 seconds for 90 min. The movie runs at 10 frames per second. The dotted rectangle corresponds to the area shown in Figure 8B.

**Supplemental movie 3:** HEK293 Flp-In DLC1  $\Delta$ PBR cells induced with doxycycline were plated onto collagen-coated glass bottom dishes. After 5 min, bright field images were taken every 30 seconds for 90 min. The movie runs at 10 frames per second. The dotted rectangle corresponds to the area shown in Figure 8B.

# Supplemental Figure S1

