

**Supplementary Materials: Movies**

All movies show single confocal z-planes. Scale bars = 10  $\mu$ m.

**Movie 1:** Wide-field imaging of HeLa cells co-transfected with FKBP-FP17 and mito-YFP-FRB (green), and stained with MitoTracker (red) prior to and following addition of 200 nM rapamycin. Images were taken at 30 second intervals, changing to 5 minute intervals, then returning to 30 seconds.

**Movie 2:** Wide-field imaging of HeLa cell co-transfected with FKBP-FP17 and mito-YFP-FRB (green), additionally stained with MitoTracker (red), prior to and following addition of 200 nM rapamycin. Images taken at 3 second intervals.

**Movie 3:** Wide-field imaging of HeLa cells immediately following on from the end of Movie 1, with addition of 100 nM CCCP. Images taken at 30 second intervals.

**Movie 4:** Confocal imaging of HeLa cell co-transfected with co-transfected with mito-YFP-FRB, FKBP-ER17 and DsRed-Mito plasmids and incubated with rapamycin for 1 hr prior to imaging. Red channel (DsRed-Mito) is shown. Cell was exposed to 5 pre-bleach frames and 6 bleach frames at a frame rate of 1 frame per 2 seconds.

**Movie 5:** Same experimental conditions as Movie 5. Cell exposed to 3 pre-bleach and 8 bleach frames at a frame rate of 1 frame per 2 seconds.

**Movie 6:** Wide-field imaging of HeLa cell cells co-transfected with FKBP-TFG and mito-YFP-FRB (green), additionally stained with MitoTracker (red), prior to and following addition of 200 nM rapamycin and with addition of 100 nM CCCP; CCCP was added 55 mins after the addition of rapamycin. Images taken at 30 second intervals.