

**SI Guide** (*Nature Manuscript # 2017-03-03707D*)

**Mitochondria-lysosome contacts regulate mitochondrial fission  
via Rab7 GTP hydrolysis**

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**Supplementary Information:**  
**Supplementary Videos 1-6**

## **Supplementary Video Legends:**

### **Video 1: Live imaging of mitochondria-lysosome contact.**

Confocal microscopy imaging of a stable mitochondria-lysosome contact which remains in contact for over 1 min (60 sec) in a living HeLa cell expressing Lamp1-mGFP (lysosome; green) and mApple-TOM20 (mitochondria; red). Video was acquired at 1 frame/2 seconds for 60 sec and played back at 5 frames/second (10x speed). Video corresponds to Fig. 1d. Scale bar, 1  $\mu\text{m}$ .

### **Video 2: Live imaging of mitochondria-lysosome contact formation.**

Time-lapse confocal image of a lysosome approaching mitochondria, forming a stable contact for 24 sec, and subsequently leaving mitochondria, in a living HeLa cell expressing Lamp1-mGFP (lysosome; green) and mApple-TOM20 (mitochondria; red). Video was acquired at 1 frame/2 seconds for 34 sec and played back at 5 frames/second (10x speed). Video corresponds to Fig. 2a. Scale bar, 0.5  $\mu\text{m}$ .

### **Video 3: Live imaging of increased duration of mitochondria-lysosome contact with Rab7 Q67L GTP hydrolysis mutant.**

Time-lapse confocal image of a lysosome (left) in cytosol approaching mitochondria to form a stable contact of increased duration for > 2 min (152 sec) before leaving mitochondria in a living HeLa cell expressing constitutively active mutant Rab7Q67L-GFP unable to undergo GTP hydrolysis (lysosome; green) and mApple-TOM20 (mitochondria; red). Video was acquired at 1 frame/2 seconds for 178 sec and played back at 5 frames/second (10x speed). Video corresponds to Fig. 2c. Scale bar, 0.5  $\mu\text{m}$ .

### **Video 4: Live imaging of increased duration of mitochondria-lysosome contact with TBC1D15 D397A (TBC domain mutant) lacking GAP activity.**

Time-lapse confocal image of a stable mitochondria-lysosome contact which remains in contact for increased duration of > 5 min (326 sec) in a living HeLa cell expressing Lamp1-mGFP (lysosome; green), mApple-TOM20 (mitochondria; red) and TBC1D15/Rab7-GAP D397A (TBC domain mutant) lacking GAP activity. Video was acquired at 1 frame/2 seconds for 326 sec and played back at 10 frames/second (20x speed). Video corresponds to Fig. 3b. Scale bar, 0.5  $\mu\text{m}$ .

### **Video 5: Live imaging of mitochondria-lysosome contact at site of mitochondrial fission.**

Confocal microscopy imaging of a mitochondria-lysosome contact at the site of mitochondrial fission in a living HeLa cells expressing Lamp1-mGFP (lysosome; green) and mApple-TOM20 (mitochondria; red). Video was acquired at 1 frame/2 seconds for 12 sec and played back at 4 frames/second (8x speed). Video corresponds to Fig. 4a. Scale bar, 0.5  $\mu\text{m}$ .

### **Video 6: Live imaging of mitochondria-lysosome contact at site of mitochondrial fission.**

Time-lapse confocal image of a mitochondria-lysosome contact at the site of mitochondrial fission in a living HeLa cells expressing Lamp1-mGFP (lysosome; green) and mApple-TOM20 (mitochondria; red). Video was acquired at 1 frame/2 seconds for 20 sec and played back at 4 frames/second (8x speed). Video corresponds to Fig. 4b. Scale bar, 0.5  $\mu\text{m}$ .