Artificially inducing close apposition of endoplasmic reticulum and mitochondria induces mitochondrial fragmentation.

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Abbreviations: CCCP - carbonyl cyanide *m*-chlorophenylhydrazone; ER – endoplasmic reticulum; ERES – ER exit site; FRAP - fluorescence recovery after photo-bleaching.

Summary

Cycles of mitochondrial fission and fission are essential for normal cell physiology. Defects in the machinery controlling these processes lead to neurodegenerative disease. While we are beginning to understand the machinery that drives fission, our knowledge of the spatial and temporal control of this event is lacking. Here we use a rapamycin-inducible heterodimerization system comprising both ER and mitochondrial transmembrane components to bring the ER membrane into close physical proximity with mitochondria. We show that this artificial apposition of membranes is sufficient to cause rapid mitochondrial fragmentation. Resulting mitochondrial fragments are shown to be distinct entities using fluorescence recovery after photobleaching. We also show that these fragments retain a mitochondrial membrane potential. In contrast, inducible tethering of the peripheral ER exit site protein TFG does not cause mitochondrial fragmentation suggesting that very close apposition of the two membranes is required.

29   **INTRODUCTION**

30   Mitochondria routinely under-go cycles of fission and fusion (Bereiter-Hahn and Voth, 1994;

31   Hermann and Shaw, 1998). Although a traditional depiction of a cell shows individual isolated

32   mitochondria, in live cells they form a dynamic and connected tubular network that extends

33   through-out the cytoplasm. Mitochondria provide energy for cells through the generation of ATP

34   and have important roles in other cellular functions including apoptosis and autophagy (Nunnari and

35   Suomalainen, 2012). Regulation of mitochondrial fission and fusion is important both for the normal

36   physiology of the cell and for quality control of mitochondrial, with damaged mitochondria being

37   removed by mitophagy (Yang et al., 2008; Youle and van der Bliek, 2012). Altering the balance of

38   mitochondrial fission and fusion is sufficient to trigger cell cycle defects (Qian et al., 2012) and

39   mutations in several of the genes encoding the machinery involved are linked to neurodegenerative

40   diseases (Alexander et al., 2000; Delettre et al., 2000; Deng et al., 2008; Waterham et al., 2007;

41   Zuchner et al., 2004).

42   In yeast, contacts between the ER and mitochondria are facilitated by the ER-Mitochondria

43   Encounter Structure (ERMES) (Kornmann et al., 2009). These sites are required for mitophagy in

44   yeast (Bockler and Westermann, 2014). However, the role of this complex in some aspects of

45   mitochondrial metabolism is unclear (Nguyen et al., 2012) and no such stable structure has been

46   defined in mammalian cells. ER tubules mark sites of mitochondrial divisions in both yeast and

47   higher eukaryotes (Friedman et al., 2011). From this and other work, it has been proposed that

48   wrapping of the ER membrane around the mitochondrion may generate initial constriction prior to

49   the recruitment and action of the fission machinery including dynamin-related protein Drp1 (Lackner

50   and Nunnari, 2009). More recent work has identified roles for actin through the action of the ER-

51   localized formin INF2 (Korobova et al., 2013) and for myosin-2 (Korobova et al., 2014) in

52   mitochondrial fission. A role has also been proposed for ER-mitochondrial contacts in the initiation

53   of autophagy (Korobova et al., 2013) and more specifically mitophagy (Zuchner et al., 2004). COPII-

54   coated secretory cargo exit sites on the ER membrane (ERES (Brandizzi and Barlowe, 2013)) have

55   been linked to the initiation of autophagy (Tan et al., 2013) as have the membranes of the ERGIC

56   which lie in close apposition to ERES (Ge et al., 2013). Consequently, one can imagine a scenario in

57   which ERES spatially organize the apposition of ER and mitochondrial membranes and coordinate

58   this with the initiation of mitophagy. TFG is a key component of the ERES and importantly appears to

59   act as a mesh around newly formed COPII vesicles to maintain a structural integrity to these sites

60   (Witte et al., 2011).

61   Given the extensive spread of both ER and mitochondria throughout most cells, the tight control of

62   the role of ER-mitochondrial contact in driving fission is essential. How contact between these

63   organelles drives fission versus other known functions such as calcium homeostasis or lipid transfer

64   is also unclear. It is also unclear how some contact sites might exist to facilitate specific functions

65   while others trigger mitochondrial fission. Indeed, the mechanisms that control how the number and

66   location of sites of mitochondrial fission are dictated are not known.

67   Rapamycin-inducible heterodimerization uses rapamycin-binding domains to ectopically join two

68   proteins together. It is most commonly used to sequester proteins away from their normal site of

69   action (Inoue et al., 2005; Robinson et al., 2010). Here we have applied the 'knock-sideways' system

70   to test whether driving the ER membrane into close proximity to the mitochondrial membrane is

71   sufficient to direct mitochondrial fisison. We observe a rapid fission of mitochondria (initial

72   constriction of mitochondria is observed within 60 seconds progressing to fragmentation from 20

73   minutes after the addition of rapamycin) consistent with a direct role for the close apposition of

74   these membranes in triggering this event. This inducible system provides a means to interrogate the

75   mechanisms that control the location and activity mitochondrial fission machinery in mammalian

76   cells and to examine the wider function of ER-mitochondrial contacts in the cell.

77   **RESULTS**

78   We anchored the rapamycin-binding domain of FKBP12 to the cytoplasmic face of the ER membrane

79   using a single transmembrane domain of 17 uncharged amino acids (Fig. 1A) (Bulbarelli et al., 2002;

80   Ronchi et al., 2008). When expressed in cells this construct, which we have called FKBP-ER17,

81   localizes to the ER membrane with a distribution indistinguishable from that of the related GFP-FP17

82   (FP for fluorescent protein (Ronchi et al., 2008)) (Fig. 1B). Rapamycin-dependent dimerization of the

83   FKBP domain with the FKBP and rapamycin-binding (FRB) domain from mTOR is used in the

84   knocksideways system to retarget FKBP-fusion proteins to the mitochondria. This is achieved

85   through expression of a mito-YFP-FRB fusion that is constitutively associated with mitochondria by

86   virtue of the import signal of outer mitochondrial membrane protein Tom70 (Robinson et al., 2010)).

87   Incorporation of YFP into this fusion allows visualization of mitochondria and the fate of the FRB

88   fusion during these experiments. Here (Fig. 1C), we have engineered the system to drive close

89   apposition of ER membranes (through FKBP-FP17) with mitochondrial membranes (with mito-YFP-

90   FRB).

91   Cells were imaged following the addition of rapamycin to determine the effect of directing close

92   apposition of ER and mitochondrial membranes on mitochondrial morphology. Fig 2A shows

93   maximum intensity projections of cells stained with MitoTracker® Red CMXRos (referred to

94   hereafter as MitoTracker) immediately before and 49 minutes after incubation with 200 nM

95   rapamycin. In some cases fragmented mitochondria formed a characteristic 'doughnut' shape. This is

96   particularly evident in the enlargements (Fig 2A, insets). This change in mitochondrial morphology

97   required that cells were transfected with plasmids expressing both mito-YFP-FRB and FKBP-ER17;

98   Fig. 2B shows a non-expressing cell from the same field (with no visible mito-YFP-FRB protein) is

99   shown as a control for the effects of both the rapamycin and the imaging procedure.

100   In the absence of rapamycin, mitochondria in both cells form a linked network (Fig. 2A and B

101   showing a maximum intensity projection and Fig 2C showing a single z-plane). After the addition of

102   rapamycin the mitochondria in the transfected cell fragment (initial constriction of mitochondria is

103   observed within 60 seconds, with fragmentation apparent from 20 minutes after the addition of

104   rapamycin), while the mitochondrial network in the control cell remains intact. Time-lapse images

105   were taken of this process (Fig. 2D showing enlargements of the boxed region in Fig. 2C; see also

106   Movie 1). These data show clear constriction of the mitochondria followed by apparent fission (Fig.

107   2D, arrow marks a mitochondrion post-fission).

108   Immediately after addition of rapamycin, we observed that the mito-YFP-FRB, which is freely

109   diffusible within the mitochondrial outer membrane, 'clustered' into discrete patches on the

4

110   mitochondria surface within seconds of the addition of rapamycin (Fig. 3A and B and Movie 2). We

111   conclude that bringing the ER and mitochondria into close proximity results in constriction of the

112   mitochondrial membrane and subsequently results in mitochondrial fission.

113   Damage leading to depolarization can also cause mitochondrial fragmentation (Benard et al., 2007;

114   Hackenbrock, 1966; Hackenbrock, 1968). Fluorescence of the MitoTracker dye that we used here is

115   dependent on the mitochondrial membrane potential. Live cell imaging confirmed that artificial

116   mitochondrial fragmentation using this system was not associated with a loss of mitochondrial

117   membrane potential. We added the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP;

118   Fig. 4, Movie 3) 49 minutes following the addition of rapamycin. The cell with fragmented

119   mitochondria shows a rapid decrease in MitoTracker signal in the red channel (starting 4 minutes

120   after the addition of 100 nM CCCP, with no further decrease in signal after 7.5 minutes) while the

121   mito-YFP-FRP signal is unchanged. The control cell depolarized at a similar rate. This demonstrates

122   that the fragmentation we observe is not due to mitochondrial depolarization, nor does this

123   artificially induced mitochondrial fragmentation cause subsequent loss of membrane potential.

124   To confirm that the mitochondrial fragments generated by inducible tethering via FKBP-ER17 were

125   indeed individual elements and not contiguous with the rest of the mitochondrial network, we used

126   fluorescence recovery after photobleaching (FRAP). Regions were bleached and afterwards imaged

127   to detect signs of recovery in cells co-transfected with mito-YFP-FRB, FKBP-ER17 and DsRed-Mito

128   plasmids and incubated with rapamycin for 1 hr prior to imaging (Fig. 5). To label the mitochondria

129   independently of the mito-YFP-FRB fusion, we used DsRed-Mito, which is formed of the

130   mitochondrial targeting sequence from subunit VIII of human cytochrome c oxidase fused to the red

131   fluorescent protein DsRed. We bleached DsRed-mito selecting regions that appeared to be isolated

132   (which we expected not to show signal recovery) in addition to connected regions (to confirm that

133   recovery could occur). This approach enabled us to photobleach the DsRed-mito fusion without

134   affecting the mito-YFP-FRB thereby allowing us to track the photobleached mitochondrial fragments

135   using the YFP channel.

136   We tested 96 regions over 20 cells; 60 regions that appeared to be isolated and 33 regions that

137   appeared to be connected. 93% of connected regions showed recovery of fluorescent signal within

138   35s post bleach, while 98% of isolated regions did not.

139   Fig. 5A shows a cell with representative isolated regions, while Fig. 5B shows a cell with a fully-intact

140   mitochondrial network. Despite the connected regions being bleached for a slightly longer time

141   period in this instance (8 seconds compared to 6 for the isolated regions) recovery was not seen

142   after 35 seconds, at which time point recovery was seen in the connected regions, and no recovery

143   was seen even almost 2 minutes after bleaching (Movies 4 and 5). Full recovery of fluorescence was

144   not seen within the time-scale of these experiments. This is consistent with previous work using

145   FRAP on mitochondria (Collins and Bootman, 2003). These results demonstrate that the

146   mitochondrial fragments generated by inducible tethering of the ER to the mitochondria are distinct

147   entities isolated from the remainder of the mitochondrial network.

148   We then sought to determine whether this fission event was driven by close apposition of the two

149   membranes. ER exit sites are continuous with the ER membrane but several of the components of

150   the COPII budding machinery are localized directly adjacent to the ER membrane at these sites,

151   rather than being contiguous with it. An example of this is Trk-fused gene (TFG) which associates

152   tightly with the COPII budding machinery that drives secretory cargo exit from the ER and has been

153   proposed to facilitate COPII assembly at ER exit sites (ERES) (Witte et al., 2011). ERES are spaced

154   throughout the ER network and therefore could explain the relatively regular spacing of sites of

155   fission seen in Figures 2 and 3. We sought to define whether we could trigger mitochondrial fission

156   using an FKBP-TFG fusion.

157   When expressed in cells, FKBP-TFG forms discrete puncta similar to those seen with GFP-TFG ((Witte

158   et al., 2011) and our unpublished data). These puncta co-localize with the ERES protein Sec31A (Fig.

159   6). Time-lapse imaging following addition of rapamycin revealed clustering of the mito-YFP-FRB (Fig.

160   7, Movie 6) when co-expressed with FKBP-TFG. However, we did not observe mitochondrial

161   fragmentation, even 55 minutes after addition of rapamycin. As seen for FKBP-ER17, mitochondria

162   did not depolarize until addition of CCCP and this then resulted in fading of the MitoTracker signal.

163   We conclude that inducible tethering of ERES is not sufficient for mitochondrial fragmentation.

164

DISCUSSION

166   Membrane-membrane interactions cross-link many organelles in eukaryotic cells, with interactions

167   with the ER (as the largest organelle) being particularly frequent. Much recent interest has focused

168   in the functional role of ER contacts including ER-mitochondrial calcium transfer (Csordas et al.,

169   2010) and mitophagy (Bockler and Westermann, 2014) as well as their involvement in mitochondrial

170   division (Friedman et al., 2011; Murley et al., 2013). Here we have demonstrated that inducible

171   tethering of the ER membrane to mitochondria is sufficient to cause rapid mitochondrial

172   fragmentation. We observe a two-step process here. Both FKBP-TFG and FKBP-FP17 induce a

173   clustering of mito-YFP-FRB on the mitochondrial membrane but only FKBP-FP17 causes subsequent

174 fragmentation of mitochondria. This kinetic delay could be due to assembly of the fission machinery

175 or reorganization of the ER-mitochondria membrane interface to facilitate fission. Our interpretation

176 of the fact that FKBP-TFG does not induce fission (even after several hours in the presence of

177 rapamycin) is that its localization is simply not close enough to the ER membrane to direct close

178 enough proximity of the two membranes to trigger the fission event or alternately that the presence

179 of ERES is somehow incompatible with the processes required to facilitate fission.

180 We do not observe mitochondrial fission when using the FKBP-TFG fusion. Neither do we observe

181 mitochondrial fission when artificially targeting the AP2 clathrin adaptor (data not shown and see

182 (Robinson et al., 2010)). We tested the potential of an FKBP-TFG fusion to induce mitochondrial

183 fragmentation when targeted to the outer mitochondrial membrane for two reasons. Both ER-

184 mitochondrial contact sites have been implicated in the initiation of autophagy (Hamasaki et al.,

185 2013) and ER exit sites have been shown to be important sites of autophagy initiation (Tan et al.,

186 2013). We therefore postulated that ERES could act as a hub for these two processes. While we do

187 not see mitochondrial fission with the FKBP-TFG fusion, it is important to note that on addition of

188 rapamycin, FKBP-TFG does induce clustering of mito-YFP-FRB showing that this alone is not sufficient

189 to drive mitochondrial fission. We also cannot rule out that the targeting and or function of FKBP-

190 TFG is compromised compared to that of endogenous TFG. As such FKBP-TFG acts as a negative

191 control here and we do not draw any firm conclusions with regard to the role that TFG or ERES might

192 play in ER-mitochondrial contact.

193 One caveat that should be borne in mind when using the inducible systems currently available is the

194 potential effects resulting from adding rapamycin to cells, as rapamycin is a potent inhibitor of the

195 mTOR signalling pathway, to cells. mTOR co-ordinates protein turn-over and autophagy in response

196 to nutrient availability (Raught et al., 2001). In these experiments, imaging of cells expressing FKBP-

197 TFG and non-expressing cells demonstrates that the mitochondrial fragmentation we see with FKBP-

198 ER17 is not due to the effects of rapamycin.

199 Since the ER membrane has previously been shown to be intimately involved in establishing the site

200 of mitochondrial division in yeast and mammals (Friedman et al., 2011), we speculate that inducible

201 tethering of the ER membrane may trigger fission through a similar mechanism. If simply bringing

202 the ER and mitochondria into close proximity is sufficient to divide the mitochondrial network, one

203 important question that remains is how are normal ER-mitochondrial contract sites regulated to

204 prevent constitutive and uncontrolled mitochondrial division? In yeast, the cortical ER acts to tether

205 mitochondria for segregation in the nascent bud (Swayne et al., 2011). How is this achieved without

206 triggering mitochondrial fragmentation? Similarly, it is not clear why the number of sites of

207   mitochondrial fission is limited following artificial tethering of ER and mitochondrial membranes.

208   This could be due to the physical nature of mitochondria e.g. a finite size of the smallest fragment

209   that we can generate owing to geometric constraints or organization of nucleoids (DNA within the

210   mitochondria) that appear to localize adjacent to sites of fission (Ban-Ishihara et al., 2013; Murley et

211   al., 2013). It could also be due to a limiting quantity of the fission machinery, or due to a defined

212   number of pre-existing sites already primed for fission awaiting apposition of ER and mitochondrial

213   membranes. Clearly there are many open questions here and we hope that a synthetic system to

214   trigger time-resolved mitochondrial fission will be of value to study the machinery, regulation and

215   role of ER-associated mitochondrial fission.

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217

218 **MATERIALS AND METHODS**

219 PLASMID CONSTRUCTION

220 pLVX.FKBP was constructed by using PCR to amplify the FKBP domain from the γ-FKBP plasmid (a

221 kind gift from M. Robinson, Cambridge (Robinson et al., 2010)) and inserted into pLVX.puro

222 (Clontech, California) using XhoI/EcoRI sites. This XhoI site was destroyed in the cloning and a novel

223 XhoI site introduced downstream of the FKBP domain. Subsequently ER17 or TFG were amplified by

224 PCR from GFP-FP-17 (the kind gift of N. Borgese, Milan) or GFP-TFG (the kind gift of A. Audhya,

225 Wisconsin) and inserted into pLVX.FKBP using XhoI/XbaI or XhoI/BamHI respectively. Sequences

226 amplified by PCR were subsequently confirmed by sequencing the resultant plasmid.

227 pMito-YFP-FRB and γ-FKBP construct were a kind gift of Scottie Robinson (Robinson et al., 2010) and

228 pDsRed-Mito was obtained from Clontech.

229 CELL CULTURE

230 HeLa cells were cultured in DMEM medium (Sigma-Aldrich, Poole, UK). Plasmids were transfected

231 using Lipofectamine® 2000 (Life Technologies, Paisley, UK) as per manufacturer's instructions.

232 IMAGING

233 Glass-bottomed dishes (MatTek, Ashland, MA, USA) were used for live cell imaging. Where used,

234 MitoTracker® Red CMXRos (Life Technologies, Paisley, UK) was added to media at 1:2000 dilution for

235 3-5 min immediately prior to imaging, and then rinsed to remove excess dye. Cells were then placed

236 in imaging media (Life Technologies, Paisley, UK). Rapamycin (Sigma-Aldrich, Poole, UK) was added

237 as a 0.025 mg.ml^{-1} stock in DMSO to a final concentration of 200 nM. CCCP was added as a 1000x

238 stock to a final concentration of 100 nM.

239 Live epifluorescence imaging was performed with an Olympus IX-81 inverted microscope fitted with

240 a 37°C heated Perspex box using a 63× oil objective and an Orca-R2 CCD camera. Z-stacks are 9 x 1

241 micron slices; all time-lapse imaging is of a single focal plane. For immunofluorescence, cells were

242 methanol-fixed prior to antibody staining. Mouse anti-FKBP12 and mouse anti-Sec31A were

243 obtained from BD Transduction Laboratories, Oxford, UK; rabbit anti-TFG is from Imgenex, San

244 Diego, USA. Fixed cells were imaged with an Olympus IX-71 inverted microscope. Volocity software

245 (Perkin Elmer, version 5.4.2) was used for image acquisition and Image J (Fuji version 1.48q

246 (Schindelin et al., 2012)) for image processing.

247 FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING

248 Cells were live imaged in a 37°C heated Perspex box (Life Imaging Services, Reinach, CH) on a Leica

249 SP5 confocal imaging system (Leica Microsystems, Milton Keynes, UK) with a 63x 1.4 numerical

250 aperture blue light-corrected lens. EYFP was imaged using the 514 nm laser line and DsRed using the

251 594 nm laser line. A pinhole size of 1.8 Airy disk units was used to take images using a Leica DMI

252 6000 inverted microscope. Cells were exposed to 3-6 pre-bleach frames, 4-10 bleach frames with

253 594 nm laser at high power and at least 35 post-bleach frames at low laser power all at 1 frame per 2

254 seconds. Data was processed using Leica LAS AF Lite (Wetzlar, Germany) and Image J (Fuji version

255 1.48q (Schindelin et al., 2012)).

256

257 **ACKNOWLEDGMENTS**

263 **CONTRIBUTIONS**

264   V.J.M. performed experimental work, analyzed the data and wrote the manuscript; D.J.S. conceived

265   and directed the project, assisted with experimental work, and helped draft the manuscript.

266 **FUNDING**

269

270   **FIGURE LEGENDS**

271   Figure 1 **Schematic of engineered ER-mitochondrial contact.** (A) Schematic of FKBP-ER17 construct,

272   adapted from (Bulbarelli et al., 2002). The FKBP domain (oval) is linked to a transmembrane domain

273   (TM) flanked by sequences up- and downstream. (B) Immunofluorescence labelling of methanol

274   fixed HeLa cells co-expressing GFP-FP17 and FKBP-ER17. (C) Schematic of rapamycin-induced

275   heterodimerization of mito-YFP-FRB and FKBP-ER17 bringing the ER and mitochondrial membranes

276   together. Scale bar = 10 μm.

277   Figure 2 **Mitochondrial fragmentation following inducible tethering of the ER membrane to**

278   **mitochondria.** (A) Maximal intensity projections of live cell imaging of HeLa cells co-transfected with

279   FKBP-FP17 and mito-YFP-FRB before and after incubation with 200 nM rapamycin. Mitochondria are

280   additionally stained with MitoTracker-Red. Images of both cells are taken from a single field of view,

281   right-hand panel shows cell not expressing mito-YFP-FRB. (B) Single-plane frames from time-lapse

282   imaging of cells shown in (A). Arrow indicates mitochondrial breakage. (C) Single-plane frames from

283   time-lapse imaging of cells. Arrows show rapid clustering of mito-YFP-FRB on addition of rapamycin.

284   Scale bars = 10 μm.

285   Figure 3 **Addition of CCCP causes clustering of mito-YFP signal.** HeLa cells co-transfected with FKBP-

286   FP17 and mito-YFP-FRB before (A) and after (B) incubation with 200 nM rapamycin. Mitochondria

287   are additionally stained with MitoTracker-Red. Single-plane frames from time-lapse imaging of cells

288   prior to (A) and (B) immediately following addition of 200 mM rapamycin. Arrows show clustering of

289   mito-YFP-FRB. Scale bar = 10 μm.

290   Figure 4 **Addition of CCCP causes depolarization of mitochondrial fragments.** Single-plane frames

291   from time-lapse images of cells shown in Fig1D, E following addition of 100 nM CCCP. Scale bar = 10

292   μm.

293   Figure 5 **FRAP analysis of mitochondrial luminal continuity**. DsRed-mito fluorescence in HeLa cells

294   additionally co-transfected with mito-YFP-FRB (not shown) and FKBP-ER17 incubated with rapamycin

295   for 1 hr. (A) Cell showing mitochondrial fragmentation phenotype. Cell was exposed to 5 pre-bleach

296   frames and 6 bleach frames. In region 1 and region 2 isolated fragments do not show recovery. In

297   region 1 a continuous area to the right is bleached and then recovers (arrow), while the curved

298   mitochondrion in the middle does not. (B) Cell with continuous mitochondrial network, where

299   regions show recovery 40 s post-bleaching. Cell exposed to 3 pre-bleach and 8 bleach frames. Frame

300   rate 1 frame per 2 seconds. Scale bars = 10 μm.

301   Figure 6 **Localisation of FKBP-TFG**. Immunofluorescence labelling of methanol fixed HeLa cells

302   expressing FKBP-TFG. A dilute concentration of anti-TFG antibody was used to compensate for the

303   over-expression of the FKBP-TFG protein. Arrows show puncta. Scale bar = 10 μm.

304   Figure 7 **Inducible tethering of TFG to mitochondria**. Single-plane frames from time-lapse imaging of

305   HeLa cells co-transfected with FKBP-TFG and mito-YFP-FRB following addition of 200 nM rapamycin

306   then 100 nM CCCP. Asterisks indicate clustering of mito-YFP-FRB. Scale bar = 10 μm.

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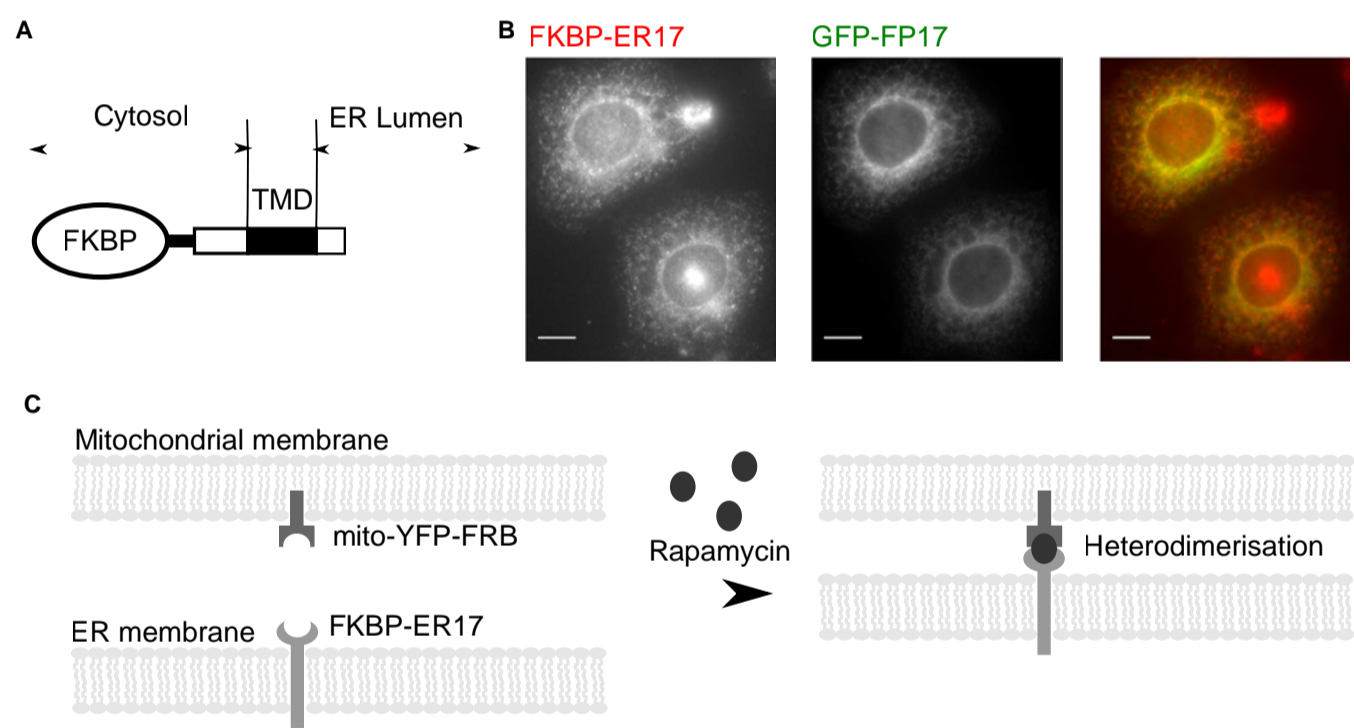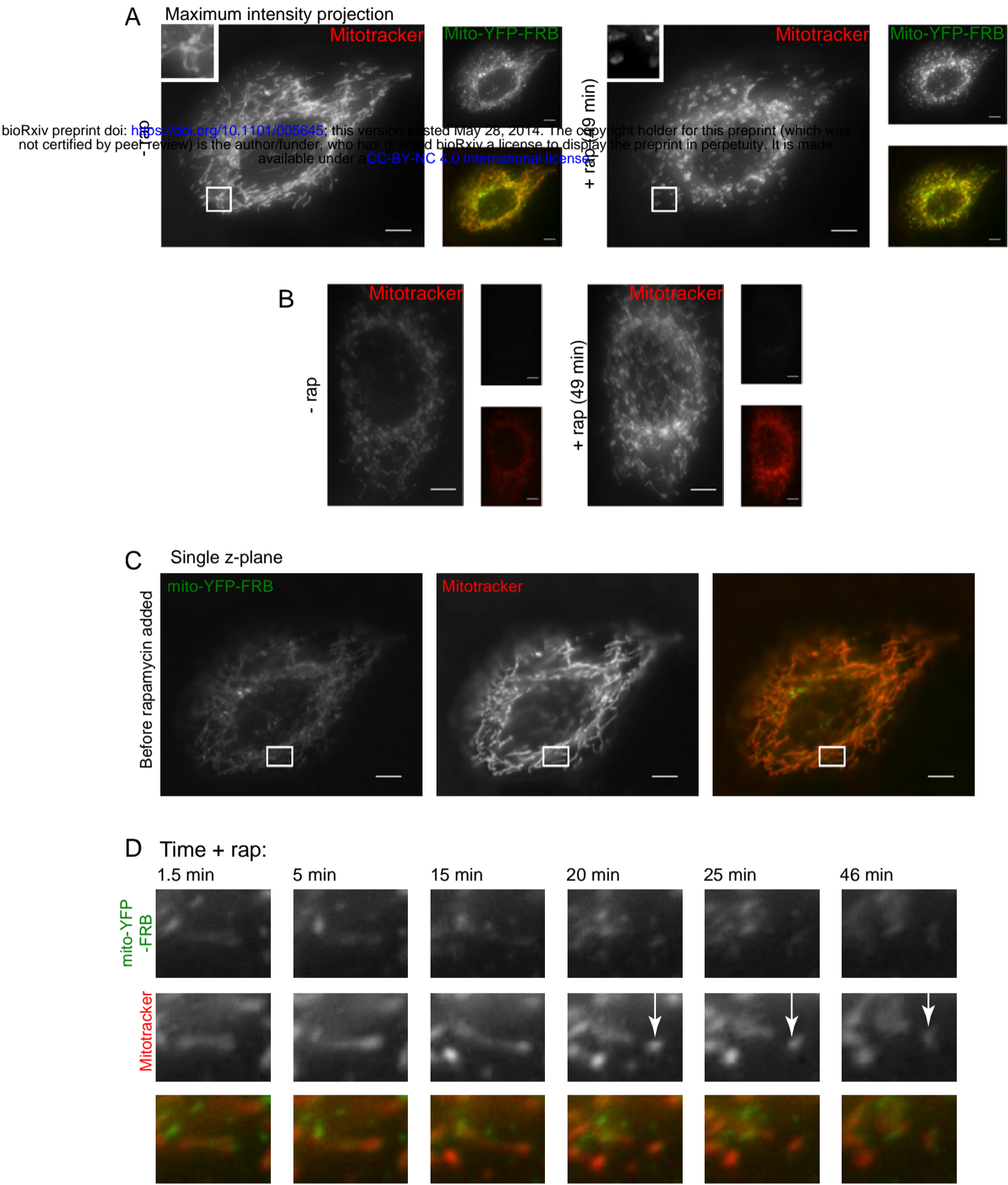414   Charcot-Marie-Tooth neuropathy type 2A. *Nat. Genet.* 36:449-451.

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416

Figure 1

A

Cytosol | ER Lumen

TMD

FKBP

B FKBP-ER17 GFP-FP17

C

Mitochondrial membrane

mito-YFP-FRB

Rapamycin

Heterodimerisation

ER membrane FKBP-ER17

Figure 2

A Maximum intensity projection

B

C Single z-plane

D Time + rap:

Figure 3

Figure 4

Cells expressing FKBP-ER17 and mito-YFP-FRB

Figure 5

A

Pre-bleach | Post-bleach 2 s | Post-bleach 40 s

DsRed-mito

Region 1

Region 2

B

Pre-bleach | Post-bleach 2 s | Post-bleach 40 s

DsRed-mito

Region 1

Region 2

Figure 6

Sec31A   FKBP-ER17

Figure 7

Time with Rapamycin | Mito-YFP-FRB | Mitotracker

0 min

2.5 min

5 min

10 min

55 min

+ CCCP 10 min