LETTER

REPLY TO FILADI ET AL.:

Does Mitofusin 2 tether or separate endoplasmic reticulum and mitochondria?

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We thank Filadi et al. for their comments (1) on our paper (2), where we address whether the discrepancies between their paper (3) and our original discovery of Mitofusin (Mfn) 2 as an endoplasmic reticulum (ER)–mitochondria tether (4) resulted from: (*i*) clonal effects of chronic *Mfn2* ablation, (*ii*) proximity measurement inappropriateness, or (*iii*) changes in mitochondrial Ca^{2+} uniporter (MCU) levels in WT and $Mfn2^{-/-}$cells. Filadi et al. (1) conclude that we fell short in solving the issue and that our data reinforce Mfn2 function as an ER–mitochondria spacer (3).

First, Filadi et al. (1) reason that we did not measure contacts number upon *Mfn2* ablation. However, contact surface (which depends on contact number and extent) can be extracted from the ER–mitochondria contact coefficient and data in our paper (2). The average mitochondrial surface contacting ER is: WT cells, 7.9%; $Mfn1^{-/-}$, 8.4%; $Mfn2^{-/-}$, 5.9%; $Mfn1,2^{-/-}$, 5.0% (data from table S1 in ref. 2). Also using data from tables S2 and S3 in our paper (2), we conclude that *Mfn2* ablation decreases the ER-contacting mitochondrial surface by ∼20–35%, potentially explaining the confocal microscopy juxtaposition reduction.

Second, Filadi et al. (1) question conclusions based on fluorescent organelle proximity probes. ddGFP and FRET-based indicator of ER–mitochondria proximity (FEMP) do not artificially juxtapose organelles: ddGFP K_{OFF} is >> K_{ON}, implying that dimerization depends on proximity and not vice versa (5); FEMP does not spontaneously and stably dimerize, as confirmed by its response to rapamycin (see ref. 6 and figure S2 in ref. 2). Mathematically, the lower FRET ratio upon *Mfn2* ablation (figures 1 and 2 in ref. 2) results from lower $FRET_{basal}$ and $FRET_{maximal}$ values (Tables 1 and 2), not from increased $FRET_{maximal}$. Thus, ddGFP and FEMP are reliable organelle proximity sensors.

Finally, Filadi et al. (1) raise technical concerns on presented data. First, in the same experimental conditions, mitochondrial Ca^{2+} peak does not span two orders-of-magnitude as stated in their letter (1): it is 160 nM in figure 3B of ref. 2 and 390 ± 150 nM in figure 3C of ref. 2 (average of five independent experiments ± SEM). Panel F of figure 3 in ref. 2 cannot be compared with panels A and B because conditions were different (as described in the legend to the figure): Cre-infected $Mfn2^{flx/flx}$ cells were preincubated in Ca^{2+}-free media to equalize cytosolic Ca^{2+} peaks (figure 3 D and E of ref. 2). Second, we excluded respiration defects in purified *Mfn2* liver knockout mitochondria ($Mfn2^{LKO}$; figure S4 of ref. 2) that, as suggested by Filadi et al. (1), could limit mitochondrial Ca^{2+} uptake in Mg^{2+}-free media. Third, mitochondrial Ca^{2+} uptake rates are not "clearly slower" in $Mfn2^{LKO}$ mitochondria (1), but superimposable to the WT ones (figure 3 I–K in ref. 2; WT: 11.3 ± 0.6, $Mfn2^{LKO}$: 11.3 ± 0.9 s^{-1}). Fourth, in WT cells, MCU levels are indeed affected by density (1), but at confluency are lower than in $Mfn2^{-/-}$ cells (figure S5 of ref. 2) and not vice versa (3). Mitochondrial Ca^{2+} transients are lesser in $Mfn2^{-/-}$ cells even upon MCU overexpression (figure 5 D and E of ref. 2): reduced MCU levels cannot therefore explain the decreased mitochondrial Ca^{2+} uptake in $Mfn2^{-/-}$ cells.

The careful Filadi et al. analysis (1, 3) highlights the ER–mitochondria interface complexity. We maintain that our acute *Mfn2* genetic deletion experiments, reliable organelle proximity probes, and Ca^{2+} measurements (2) address the raised issues in their letter (3) and add to multiple independent papers reporting ER–mitochondria tethering by Mfn2 (4, 5, 7–10). A deeper knowledge of the ER–mitochondria interface architecture could help resolve this controversy.

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Table 1. Basal and maximal FEMP FRET values upon *Mfn2* ablation: Figure 1H in ref. 2

WT		*Mfn2*$^{-/-}$	
$FRET_{basal}$	$FRET_{maximal}$	$FRET_{basal}$	$FRET_{maximal}$
1.1 ± 0.05	1.7 ± 0.01	0.76 ± 0.01*	1.03 ± 0.02*

*$P < 0.005$ in a two-tailed *Student's t* test vs. WT $FRET_{basal}$ or $FRET_{maximal}$.

Table 2. Basal and maximal FEMP FRET values upon *Mfn2* ablation: Figure 2A in ref. 2

Scr		Mfn2shRNA1		Mfn2shRNA2	
$FRET_{basal}$	$FRET_{maximal}$	$FRET_{basal}$	$FRET_{maximal}$	$FRET_{basal}$	$FRET_{maximal}$
0.78 ± 0.05	1.43 ± 0.04	0.66 ± 0.02*	0.83 ± 0.01*	0.67 ± 0.01	0.85 ± 0.01*

*$P < 0.005$ in a two-tailed Student's T test vs. Scr $FRET_{basal}$ or $FRET_{maximal}$.

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