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Supplementary Materials for

Endoplasmic reticulum mediates mitochondrial transfer within the osteocyte dendritic network

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/11/eaaw7215/DC1)

Supplementary Figure 1.

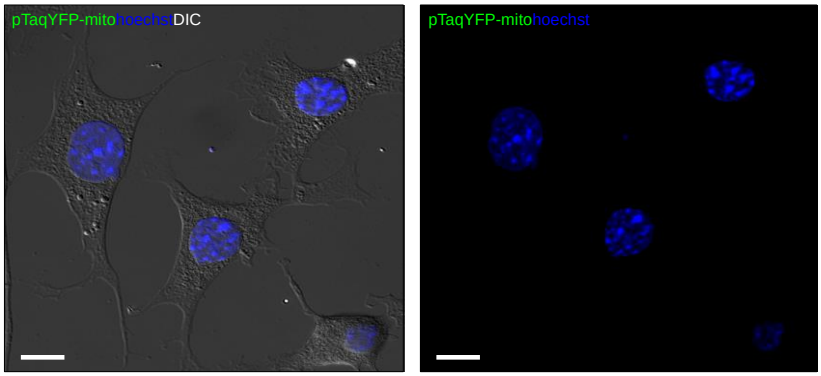

Fig. S1. No mitochondrial transfer from conditioned medium to MLO-Y4 cells. Recipient cells were co-cultured with conditioned medium isolated from MLO-Y4 cells expressing pTaqYFP-mito. No pTaqYFP-mito-labeled mitochondria were present in recipient cells. Scale bar = 10 μm.

Supplementary Figure 2.

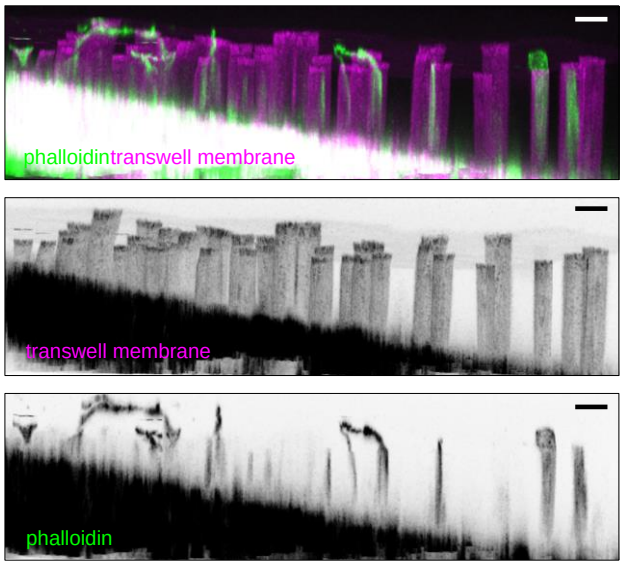

Fig. S2. Osteocytes do not pass through the pores of the transwell membrane. 3D confocal images demonstrate no osteocytes in the pores of the transwell membrane co-culture system. Scale bar = 10 μm.

Supplementary Figure 3.

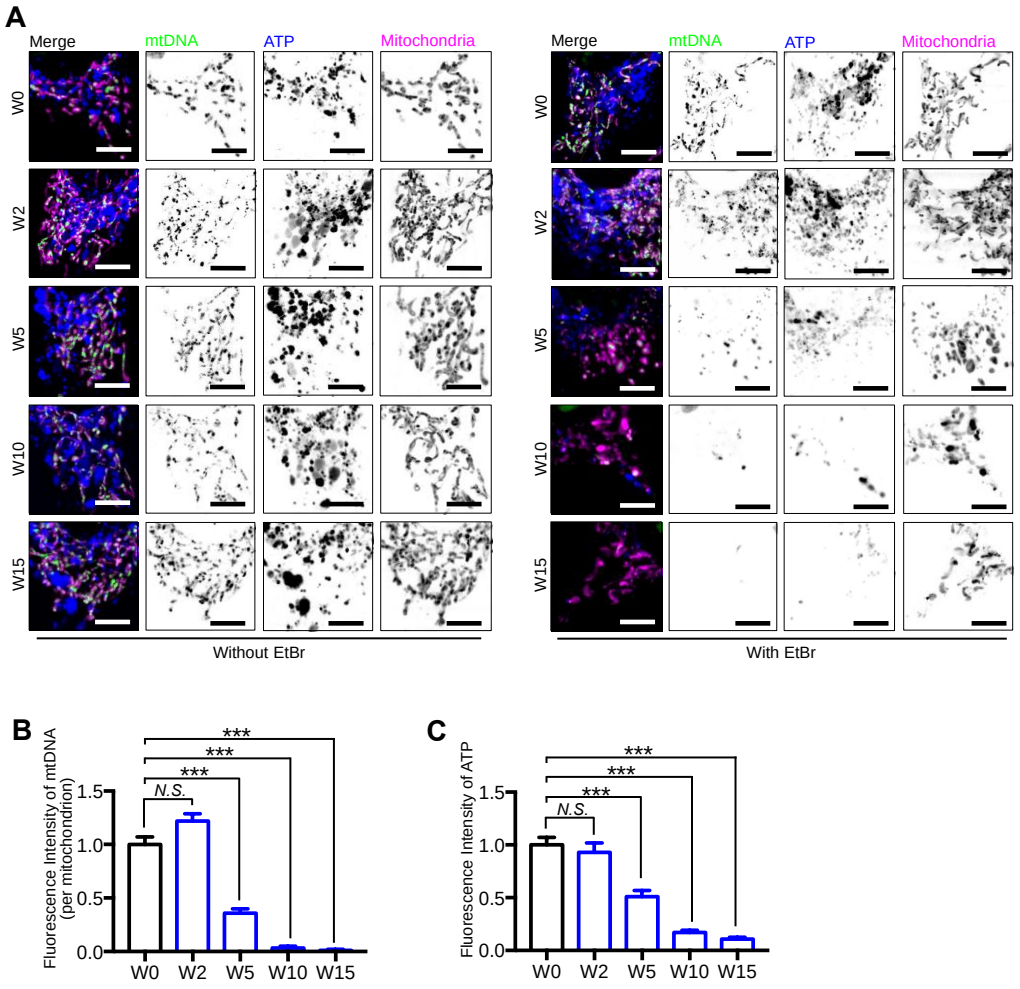

Fig. S3. Depleted mtDNA and impeded ATP production in MLO-Y4 ρ° cells. (**A**) Confocal images of MLO-Y4 cells cultured with/without EtBr for up to 15 weeks (W15). (**B**) Quantitation of mtDNA. Kruskal-Wallis test with Dunn's post hoc. Data presented as mean ± SEM. (**C**) Quantitative analysis of ATP production. Kruskal-Wallis test with Dunn's post hoc. Data presented as mean ± SEM. Scale bar = 5 μm.

Supplementary Figure 4.

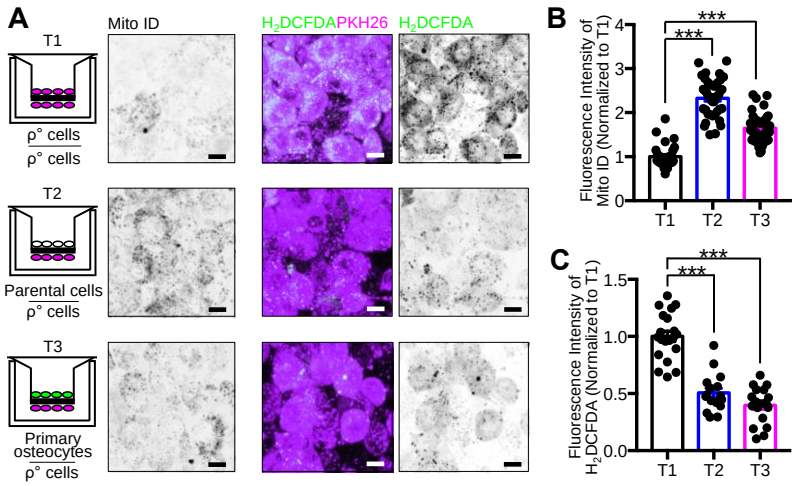

Fig. S4. Parental MLO-Y4 cells and primary osteocytes can transfer mitochondria to MLO-Y4 ρ° cells. (**A**) T1: MLO-Y4 ρ° cells (ρ° cells) co-cultured with ρ° cells in the transwell culture system; T2: ρ° cells co-cultured with healthy MLO-Y4 cells (parental cells); T3: ρ° cells co-cultured with primary osteocytes. Quantitative analysis of 3D confocal images show that healthy osteocytes (**B**) restore the fluorescence signal of an oxygen sensor (labeled by Mito ID) and (**C**) attenuate the accumulation of ROS (labeled by H_2DCFDA) in ρ° cells. Kruskal-Wallis test with Dunn's post hoc. Data presented as mean ± SEM. Scale bar = 10 μm.

Supplementary Figure 5.

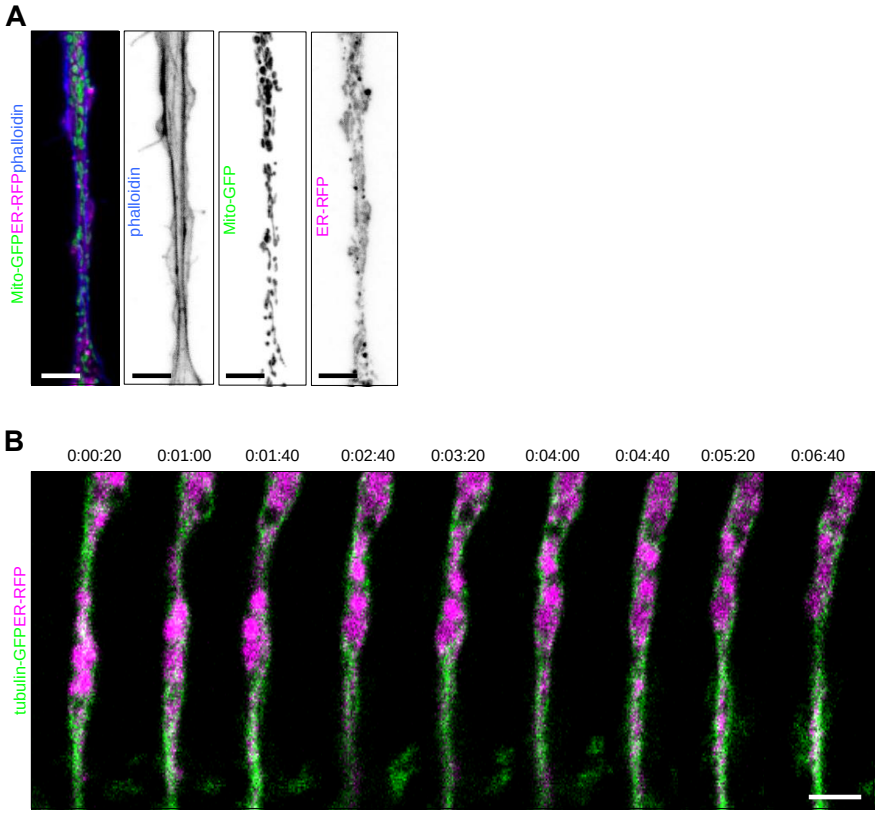

Fig. S5. Mitochondrial distribution and ER movement in osteocyte dendrites. (**A**) GFP-labeled mitochondria are co-localized with RFP-labeled ER within the phalloidin-labeled dendrite. (**B**) RFP-labeled ER dynamically moves along with GFP-labeled tubulin in the MLO-Y4 cell dendrite. Scale bar = 2 μm.

Supplementary Figure 6.

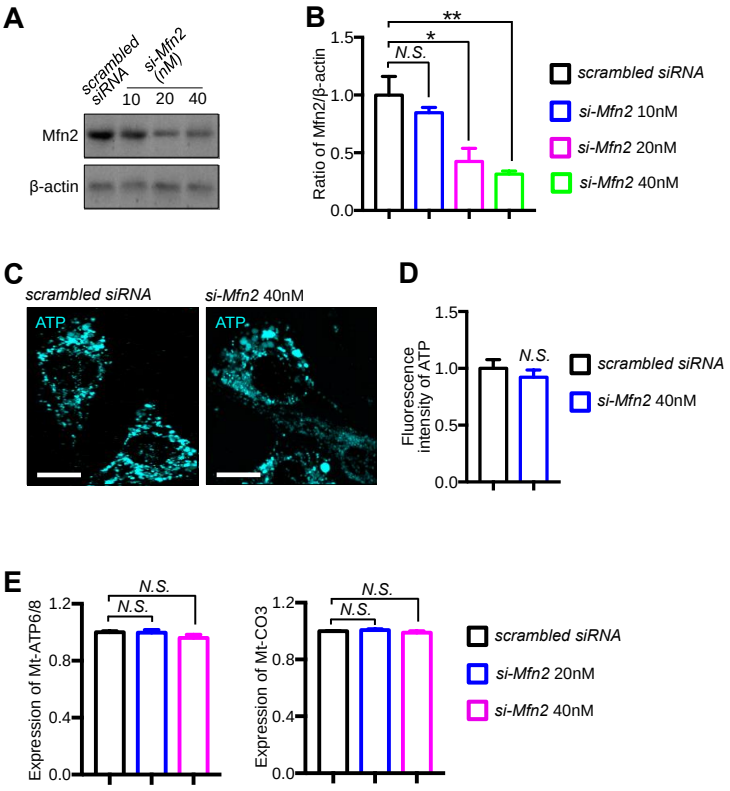

Fig. S6. Knockdown of Mfn2 has no effect on ATP production and the level of mtDNA. (**A** and **B**) Immunoblotting and quantitative analysis of Mfn2 expression in MLO-Y4 cells. One-way ANOVA with Dunnett's multiple comparisons test. Data presented as mean ± SEM. (**C** and **D**) Confocal images and quantitative analysis of images show that Mfn2 knockdown does not alter the ATP production in MLO-Y4 cells. Two-tailed Student's t test. Data presented as mean ± SEM. (**E**) qPCR results show that knockdown of Mfn2 has no significant effects on the expression of mitochondrial genes Mt-ATP6/8 and Mt-CO3. One-way ANOVA with Dunnett's multiple comparisons test. Data presented as mean ± SEM. Scale bar = 10 μm.

Supplementary Figure 7.

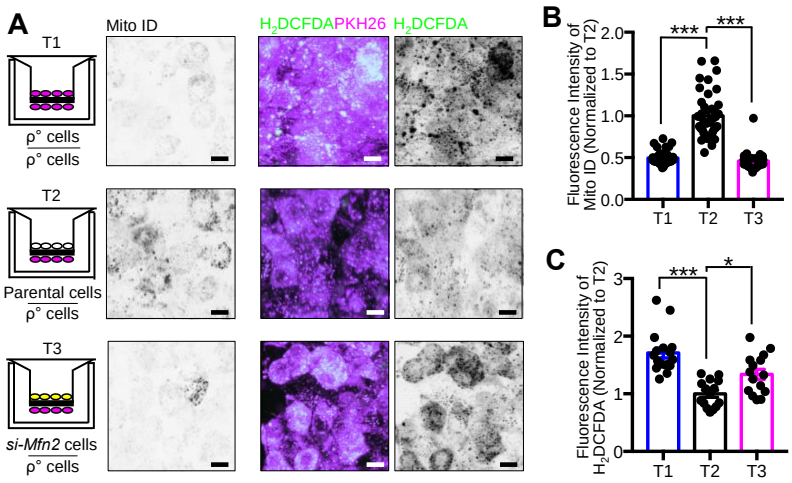

Fig. S7. Mfn2 knockdown impedes mitochondrial transfer in MLO-Y4 cells. (**A**) T1: MLO-Y4 ρ° cells (ρ° cells) co-cultured with ρ° cells; T2: ρ° cells co-cultured with healthy MLO-Y4 cells (parental cells); T3: ρ° cells co-cultured with Mfn2 knockdown MLO-Y4 cells (*si-Mfn2* cells). Quantitative analyses of 3D confocal images show that parental MLO-Y4 cells, but not cells lacking Mfn2 (**B**) restore the fluorescence signal of an oxygen sensor (labeled by Mito ID) and (**C**) attenuate the accumulation of ROS (labeled by H_2DCFDA) in ρ° cells, Kruskal-Wallis test with Dunn's post hoc. Data presented as mean ± SEM. Scale bar = 10 μm.

Supplementary Figure 8.

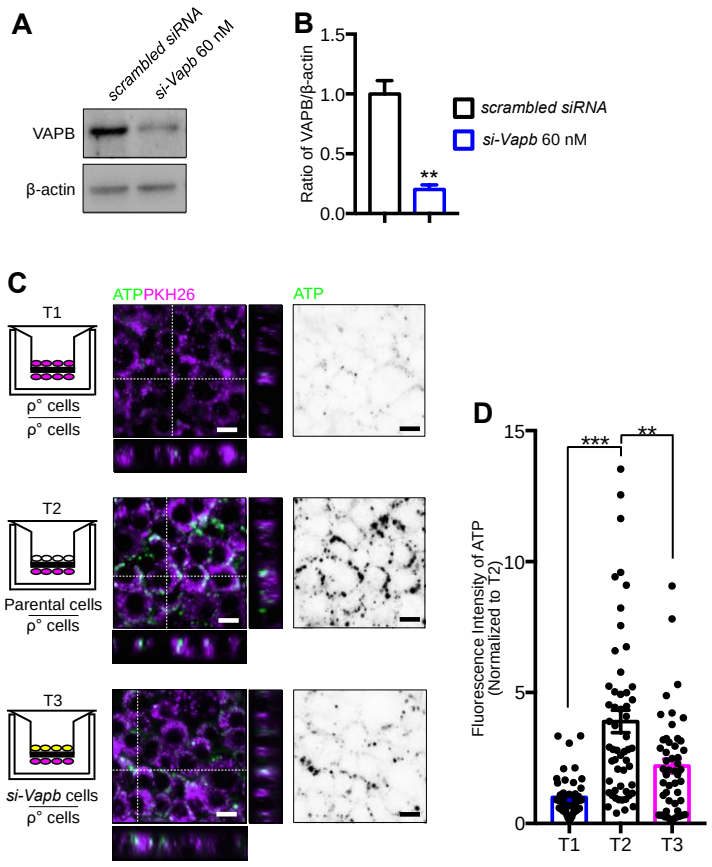

Fig. S8. VAPB knockdown impedes mitochondrial transfer in MLO-Y4 cells. (**A** and **B**) Immunoblotting and quantitative analysis demonstrate knockdown of VAPB in MLO-Y4 cells. Two-tailed Student's t test. Data presented as mean ± SEM. (**C**) T1: MLO-Y4 ρ° cells (ρ° cells) co-cultured with ρ° cells; T2: ρ° cells co-cultured with healthy MLO-Y4 cells (parental cells); T3: ρ° cells co-cultured with VAPB knockdown MLO-Y4 cells (*si-Vapb* cells). (**D**) Quantitative analysis of 3D confocal images shows healthy cells rescue the ATP production (labeled by Alexa Fluor 647) in ρ° cells, which rely on the expression of VAPB. Kruskal-Wallis test with Dunn's post hoc. Data presented as mean ± SEM. Scale bar = 10 μm.

Supplementary Figure 9.

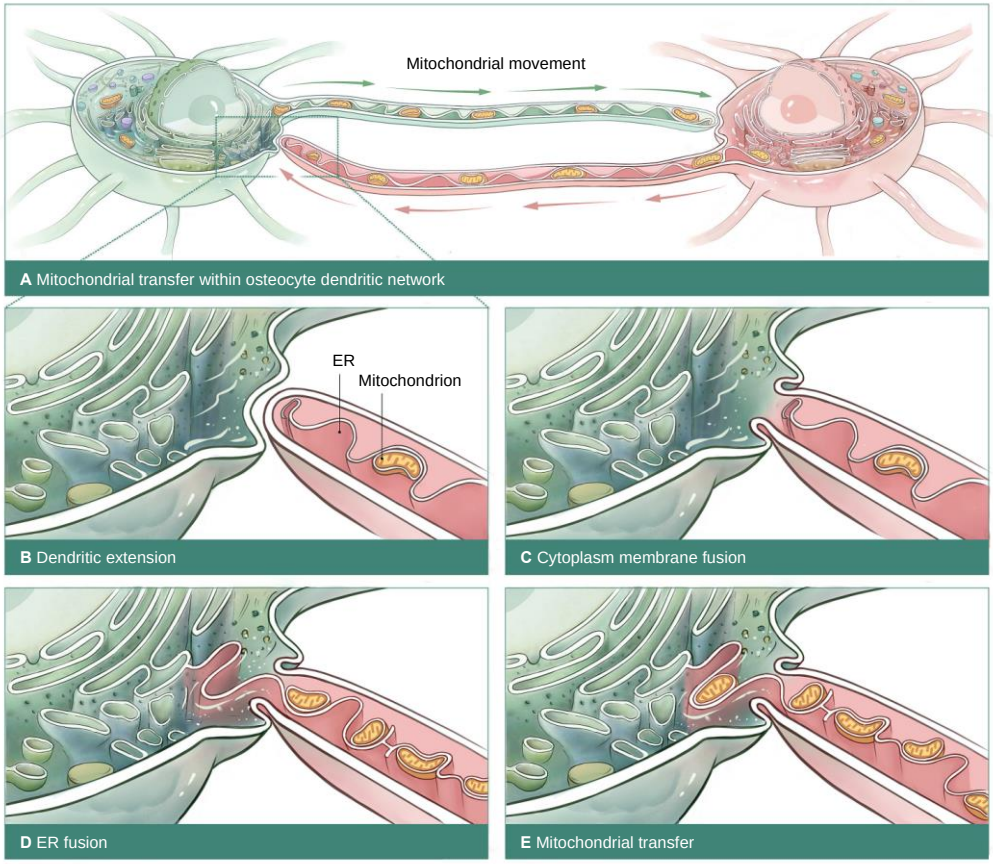

Fig. S9. Hypothetical model of ER-mediated mitochondrial transfer. (**A**) Mitochondria are shared within the osteocyte dendritic network through mitochondrial transfer. (**B** and **C**) The dendrite from donor osteocyte extends and fuses with the cytoplasm membrane of the recipient osteocyte. (**D**) ER from the donor osteocyte makes contact with the ER in the recipient osteocyte. (**E**) Mitochondria apposed by the ER in the dendrite of donor osteocyte transfer into the recipient osteocyte. The mitochondrial transfer process is associated with ER-tethering proteins such as Mfn2.

Table S1. Primer sequences for qPCR.

Gene	Species	Permer sequence	
Mt-ATP6/8	*Mus musculus*	F	5' - CAAACATTCCCACTGGCACC - 3'
		R	5' - TTGTTGGGGTAATGAATGAGGC - 3'
Mt-CO3	*Mus musculus*	F	5' -TCCAAGTCCATGACCATTAACTG - 3'
		R	5' -TATTGGTGAGTAGGCCAAGGG - 3'
β-Actin	*Mus musculus*	F	5' -GTGACGTTGACATCCGTAAAGA - 3'
		R	5' -GCCGGACTCATCGTACTCC - 3'

(F: forward primer and R: reverse primer)!