John Peter et al., https://doi.org/10.1083/jcb.201610055

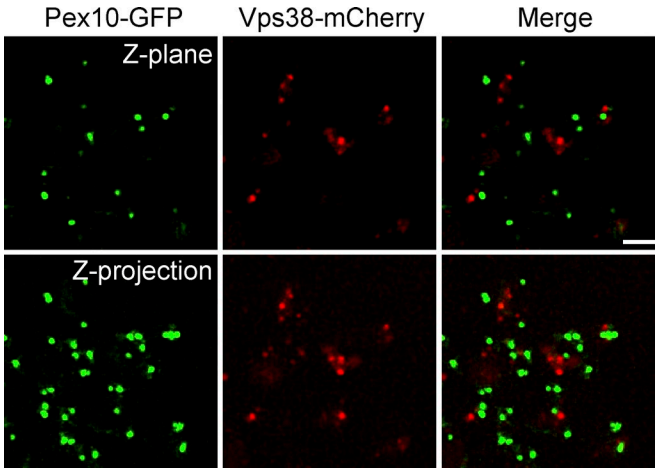

Figure S1. **Pex10 and Vps38 do not colocalize.** Pex10 and Vps38 are C-terminally tagged at their endogenous loci with GFP and mCherry, respectively. Bar, 2 µm.

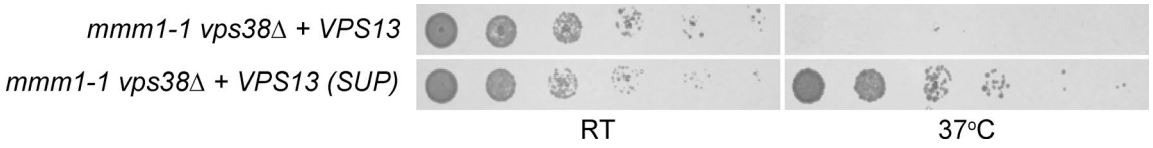

Figure S2. **Vps13 suppressor rescues loss of ERMES function in the absence of Vps38.** The ts strain *mmm1-1* with the indicated genotypes was spotted as serial dilutions on minimal media plates and grown at RT and 37°C. Both WT Vps13 and the suppressor version are expressed from a low-copy plasmid.

Table S1. **Yeast strains used in the study**

Strain	Genotype	Reference
ByK45	*BY4741 MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf
ByK46	*BY4742 MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Euroscarf
ByK498	*ByK45 VPS13^GFP*	Lang et al., 2015
ByK486	*ByK45 VPS13^GFP vps38Δ::KANMX*	This study
ByK495	*ByK45 VPS13^GFP VPS38::mCHERRY-HIS*	This study
ByK497	*ByK45 VPS13^GFP(L1627S) VPS38::mCHERRY-HIS*	This study
ByK566	*ByK45 VPS13^GFP PEX10::mCHERRY-NATNT2*	This study
ByK567	*ByK45 VPS13^GFP(L1627S) PEX10::mCHERRY-NATNT2*	This study
ByK499	*ByK45 VPS13^GFP VPS39::KANMX-GPDpr-VPS39*	This study
ByK393	*ByK45 VPS13^GFP MCP1::NATNT2-GPDpr-MCP1*	This study
ByK226	*YH8 ura3-167 trp1-A1 leu2-A1 his3-A200 mmm1-1*	This study
ByK394	*ByK226 mmm1-1 MCP1::NATNT2-GPDpr-MCP1*	This study
ByK258	*ByK226 mmm1-1 vps13Δ::HIS*	This study
ByK395	*ByK226 mmm1-1 vps13Δ::HIS MCP1::NATNT2-GPDpr-MCP1*	This study
ByK405	*ByK226 mmm1-1 MCP1::NATNT2-GPDpr-MCP1 vps39Δ::KANMX*	This study
ByK539	*ByK226 mmm1-1 MCP1::NATNT2-GPDpr-MCP1 vps38Δ::KANMX*	This study
ByK627	*ByK226 mmm1-1 MCP1::NATNT2-GPDpr-MCP1 pex1Δ::KANMX*	This study
ByK638	*ByK226 mmm1-1 VPS39::NATNT2-GPDpr-VPS39*	This study
ByK636	*ByK226 mmm1-1 vps13Δ::HIS VPS39::NATNT2-GPDpr-VPS39*	This study
ByK676	*ByK226 mmm1-1 vps38Δ::KANMX*	This study
ByK447	*ByK45 VPS39::GFP-KANMX*	This study
ByK392	*ByK45 MCP1::NATNT2-GPDpr-MCP1*	This study
ByK448	*ByK45 MCP1::NATNT2-GPDpr-MCP1 VPS39::GFP-KANMX*	This study
ByK536	*ByK45 MCP1::6xHA-HPHNT1*	This study
ByK537	*ByK45 VPS13^GFP MCP1::6xHA-HPHNT1*	This study
ByK538	*ByK45 VPS13^GFP(L1627S) MCP1::6xHA-HPHNT1*	This study
ByK433	*ByK45 MCP1::NATNT2-GPDpr-MCP1 VPS13::GFP-KANMX*	This study
ByK424	*ByK45 MCP1::NATNT2-GPDpr-MCP1 VPS13(1-2766aa)::GFP-KANMX*	This study
ByK462	*ByK45 MCP1::NATNT2-GPDpr-MCP1 VPS13(1-2599aa)::GFP-KANMX*	This study
ByK460	*ByK45 MCP1::NATNT2-GPDpr-MCP1 VPS13(1-2447aa)::GFP-KANMX*	This study
ByK458	*ByK45 MCP1::NATNT2-GPDpr-MCP1 VPS13(1-2247aa)::GFP-KANMX*	This study
ByK413	*ByK45 VPS13::NATNT2-CYC1pr-yeGFP-VPS13*	This study
ByK469	*ByK45 VPS13::NATNT2-CYC1pr-yeGFP-VPS13 (2247-3144aa)*	This study
ByK655	*ByK45 MCP1::KANMX-GPDpr-MCP1 VPS13::NATNT2-CYC1pr-yeGFP- VPS13*	This study
ByK470	*ByK45 MCP1::KANMX-GPDpr-MCP1 VPS13::NATNT2-CYC1pr-yeGFP-VPS13 (2247-3144aa)*	This study
ByK719	*ByK499 MCP1::NATNT2-GPDpr-MCP1*	This study
ByK720	*ByK719 VPS39::mCHERRY-HIS3MX*	This study
ByK324	*ByK45 VPS39::NATNT2-GPDpr-GFP*	This study
ByK325	*ByK324 vps13Δ::KANMX*	This study
ByK660	*ByK46 x ByK324 (diploid)*	This study
ByK661	*ByK660 mdm10Δ::KANMX*	This study
ByK630	*ByK226 mmm1-1 mcp1Δ::KANMX*	This study

Table S2. **Plasmids used in the study**

Plasmid	Genotype	Reference
pBK417	pRS413-TEFpr-mCHERRY-UBC6(TA)	This study
pBK250	pRS413-TEFpr-MCP1(1-61)-mCHERRY-UBC6(TA)	This study
pBK251	pRS413-TEFpr-MCP1(122-173)-mCHERRY-UBC6(TA)	This study
pBK416	pRS413-TEFpr-mCHERRY-FIS1(TA)	This study
pBK415	pRS413-TEFpr-MCP1(1-61)-mCHERRY-FIS1(TA)	This study
pBK336	pVPS13^GFP	This study
pBK239	pVPS13^GFP (L1627S)	This study
pBK509	pRS415-GPDpr-MCP1(WT)	This study
pBK510	pRS415-GPDpr-MCP1(H187A)	This study
pBK511	pRS415-GPDpr-MCP1(P195A)	This study
pBK512	pRS415-GPDpr-MCP1(H237A)	This study
pBK513	pRS415-GPDpr-MCP1(G241A)	This study

Table S3. **Primers used in the study**

Number	Name	Sequence
Primers for genomic tagging		
1	Vps38_F1_pringle	5′-TGATGGTTTTACCTATTAGGGATAGTAATCATAATTTAAAAATATGCGGATCCCCGGGTTAATTAA-3′
2	Vps38_R1_pringle	5′-GAAAAGATTAAATGGCAGTCCAAAAGAGATTTTTGATTTTCAGTCTAGAATTCGAGCTCGTTTAAAC-3′
3	Vps38_F2_pringle	5′-CTGATATATTGCAATTTAAACAGATTCTGGAGAATTACCGTCCAACACGGATCCCCGGGTTAATTAA-3′
4	Pex10_F2_pringle	5′-GTGCAGACAACACTGTCAACCACAGGAAATTCTGGTCCTGCGGCAACGGATCCCCGGGTTAATTAA-3′
5	Pex10_R1_pringle	5′-GTGGACAATGCTAAAAGAGTAGTCAAATTATTGATTAGTTCCTCTAGAATTCGAGCTCGTTTAAAC-3′
6	Vps39_F1_pringle	5′-AGCAAAAACCCTTCAAAATATCAATTTATACCAAAAATTACGGATCCCCGGGTTAATTAA-3′
7	Vps39_R1_pringle	5′-TAAGAAATACTAACAACAATAACAGCAGCTGTTAAGGGATGAATTCGAGCTCGTTTAAAC-3′
8	Vps39_F2_pringle	5′-AGAATTAAACAGAAGACTTTGGGAGAAGTTATAAATGAGCTAAATAATAAGCGGATCCCCGGGTTAATTAA-3′
9	Mcp1_S1_knop	5′-GAATTACTAGGGCATATACCAGTTAGCCCAGAGTTTTGTTTATTACGATGCGTACGCTGCAGGTCGAC-3′
10	Mcp1_S4_knop	5′-GAGACTAGCAGGGTCTACTGGTTCTGGAGGCACTTCATGCAACTTTATCATCGATGAATTCTCTGTCG-3′
11	Mcp1_S3_knop	5′-CAATTCGAAAGCATTTTCAAAAAGATCCGGTTGCTGTTGCACGTGAATCGTACGCTGCAGGTCGAC-3′
12	Mcp1_S2_knop	5′-GAATTGTCGCAAATTAATAGCTTCACACTACTCCTACAAGGATTCCTAATCGATGAATTCGAGCTCG-3′
13	Vps13_pringle_F1	5′-AAAGGGAAAAGGCAGAAAAAAGGAAAATTAAGAACAGTTACGGATCCCCGGGTTAATTAA-3′
14	Vps13_pringle_F2	5′-CTACATAGTGTACAAAAGCGGGTATATACTTTCATATGTGGAATTCGAGCTCGTTTAAAC-3′
15	Pex1_F1_pringle	5′-GAAGGGACGGCAGTAACAAGAAACACCTGAGGAACTGCTCTTTCAAATGCGGATCCCCGGGTTAATTAA-3′
16	Pex1_R1_pringle	5′-TGCCCTTTAAAGGGAAACGCGCTTTGTTCTTTTCTTCTTCCTTTTCAGAATTCGAGCTCGTTTAAAC-3′
17	Vps13_S3_cknop_2247aa	5′-GCACAATCCAAGATTTTGCTTGAAAACGAAAAAAGATATACTATTCCACGTACGCTGCAGGTCGAC-3′
18	Vps13_S3_cknop_2447aa	5′-CCATGACTTTATATTTTACCAAAGAGATCCTCGTAAAGTTAGTGATCCACGTACGCTGCAGGTCGAC-3′
19	Vps13_S3_cknop_2599aa	5′-CACAGATAGTTGTTTCGTTCAAAGGAGTTGGTATCTCATTAATCAATGGTCGTACGCTGCAGGTCGAC-3′
20	Vps13_S3_cknop_2766aa	5′-CATAGTACAATGATGACGAAAATGACTCCTCGGTCTCTTCAGCCAATCCCATCGATGAATTCTCTGTCG-3′
21	Vps13_S3_cknop	5′-CGCCATTGCTGTTAGAGAATACAATAAGTACTGTGAAGCTATCCTACGTACGCTGCAGGTCGAC-3′
22	Vps13_S2_cknop	5′-ATAGCTACATAGTGTACAAAAGCGGGTATATACTTTCATATGTGATCAATCGATGAATTCGAGCTCG-3′
23	Vps13_S1_knop	5′-GTGACTACCAAAAGGGAAAAGGCAGAAAAAAGGAAAATTAAGAACAGTTAAATGCGTACGCTGCAGGTCGAC-3′
24	Vps13_S4_knop	5′-CCACATATGAGCCTAGCAATCGGTTTAGCAAATTAGCAGCTAAAGACTCTAACATCGATGAATTCTCTGTCG-3′
25	Vps13_S4_knop_2247	5′-ACGAGCTCTGTTACTTTTATCATCTTCTTTATCAAATGAAAACATTTTCATCGATGAATTCTCTGTCG-3′
26	Vps39_F2_pringle	5′-AGAATTAAACAGAAGACTTTGGGAGAAGTTATAAATGAGCTAAATAATAAGCGGATCCCCGGGTTAATTAA-3′
27	Vps39_R1_pringle	5′-TAAGAAATACTAACAACAATAACAGCAGCTGTTAAGGGATGAATTCGAGCTCGTTTAAAC-3′
Primers used for cloning		
17	mcp1_1-61_ov1TEF_f	5′-CAATCTAATCTAAGTTTTCTAGAACTAGTGGATCCATGATAAAGTTGCATGAAGTGC-3′
18	mcp1_1-61_ov1mCh_r	5′-CACCATGTTAATTAAACCAGCACCGTCACCGATGTCGACCGAAATCTTTTGACAGTTCCA-3′
19	mcp1_122-173_ov1TEF_f	5′-CATAGCAATCTAATCTAAGTTTTCTAGAACTAGTGGATCCCGAATCGTGAACAA CTGGAAC-3′
20	mcp1_122-173_ov1mCh_r	5′-CCATGTTAATTAAACCAGCACCGTCACCGATGTCGACTTGTGGAGGAATGCGGAAG-3′
21	TEFovl_mChATG_f	5′-TAATCTAAGTTTTCTAGAACTAGTGGATCCATGATCGGTGACGGTGCTGGT TT-3′
22	XhoI_Ubc6TA_mCh_rv	5′-GACCCTCGAGTCATTTCATAAAAAGGCCAACCAAAAACAAAAAAATAGCGATACCAATATAAACCATTGAAGAACTCT TGTACAGCTCGTCCATG-3′
23	mCh_Fis1TM_XhoI_r	5′-GACCCTCGAGTCATTTCATAAAAAGGCCAACCAAAAACAAAAAAATAGCGATACCAATATAAACCATTGAAGAACTCT TGTACAGCTCGTCCATG-3′
24	GPD_MCP1_gaprep_f	5′-TTTTTAGTTTTAAAACACCAAGAACTTAGTTTCGAATGATAAAGTTGCATGAAGTGCCTCC-3′
25	pRS4 × 5_MCP1_gaprep_r	5′-GCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGCTAATTCACGTGCAACAGCAACC-3′

Provided online are four alignments in one PDF and three scripts in one Word file. Alignments S1 and S2 show homology search results for Vps13 (1–3,000 aa) and Vps13 (1,501–3,144 aa), searched against the Pfam database and yeast proteome, respectively. Alignments S3 and S4 show the results for the C terminus of Vps13 (3,030–3,144 aa) and full-length Mcp1, respectively, searched against the Protein Data Bank. All homology alignments were generated using the HHPred server. Scripts S1, S2, and S3 are ImageJ macros used to calculate the percentage of colocalization between Vps13^GFP/Pex10-mCherry foci, Vps13^GFP/Vps38-mCherry foci, and Pex10-GFP/Vps38-mCherry foci, respectively.

Reference

Lang, A.B., A.T. John Peter, P. Walter, and B. Kornmann. 2015. ER-mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. *J. Cell Biol.* 210:883–890. http://dx.doi.org/10.1083/jcb.201502105