

Supplementary Information for

Mechanical activation of mitochondrial energy metabolism during cell differentiation

Zong-Heng Wang, Christian Combs, Jay Knutson, Mary Lilly, and Hong Xu

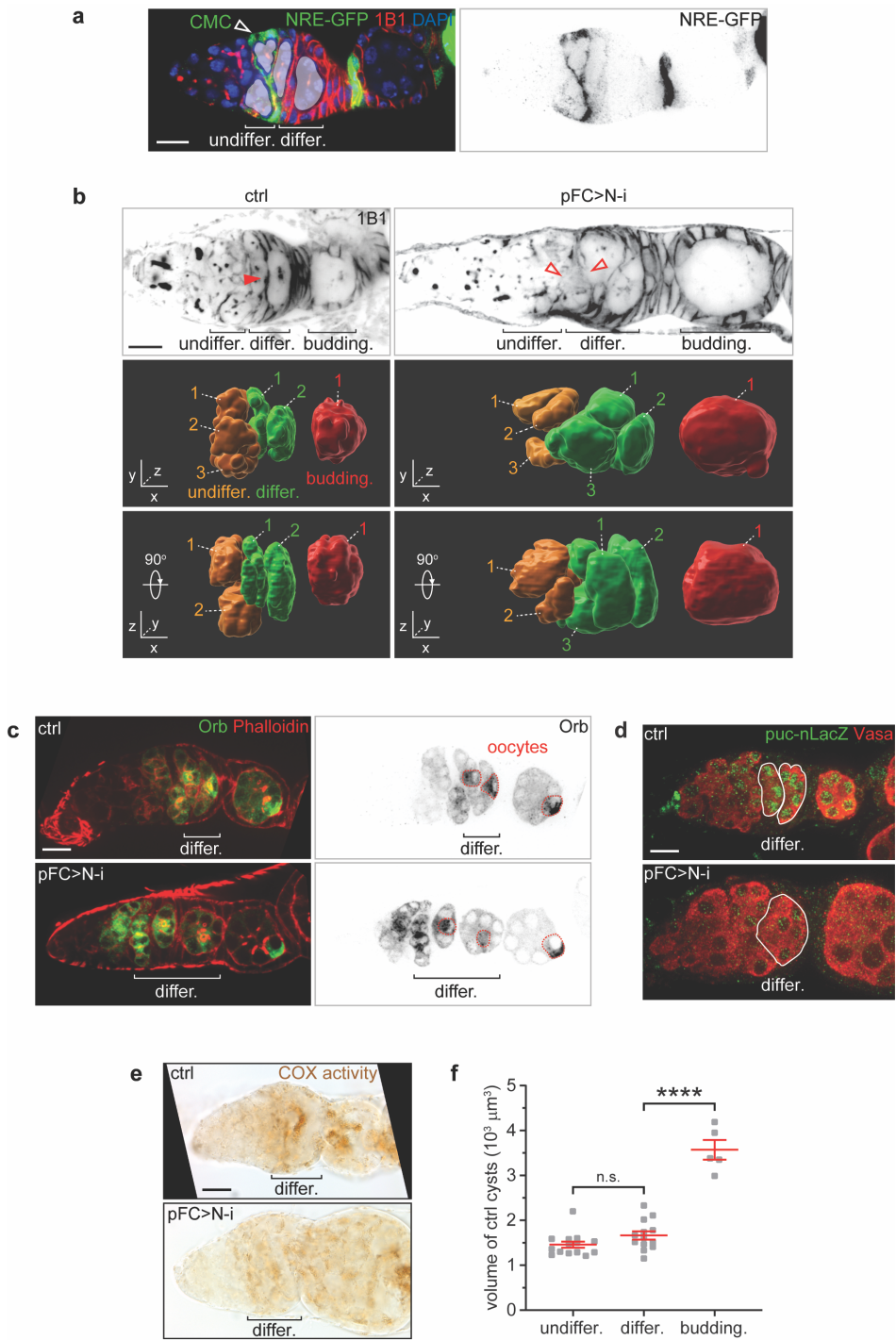
This PDF file includes:

Extended Data Figures and Figure Legends

Supplementary Tables and Table Legends

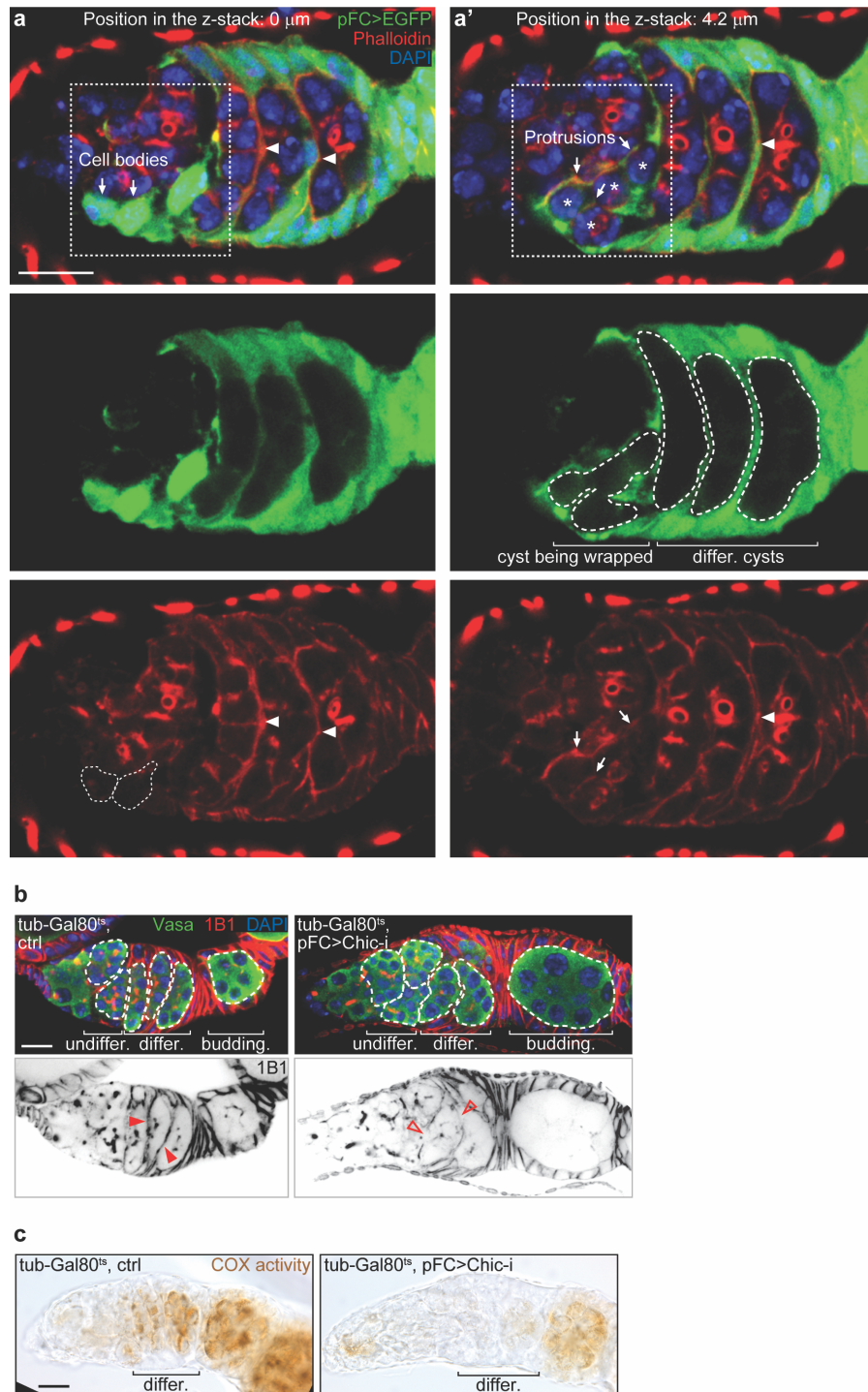
Supplementary Videos Captions

Extended Data Figures and Legends



Extended Data Fig. 1. pFCs' wrapping is required for compression and OXPHOS induction, but not differentiation, of the female germline cysts.

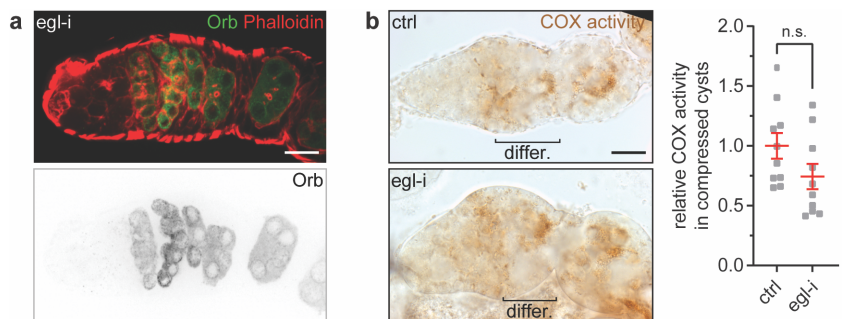
21 **a**, Representative image of Notch signaling activity (NRE-GFP) in the germarium. CMC, cross-migrating
22 pFC. **b**, 1B1 staining and three-dimensional reconstructions of the 16-cell cysts from Fig. 1c,d. Red Arrow
23 head points to a pFC inserted between germline cysts. Hollow arrow heads indicate that pFCs are absent
24 between cysts. **c**, Cyst differentiation visualized by Orb (oocyte marker) immunostaining. **d**, Visualization
25 of JNK activity by *puc*-nLacZ in germaria. **e**, Representative images of COX activity histochemistry in
26 differentiating cysts. **f**, Cyst sizes in ctrl germaria. $n = 14$ (undifferentiated cysts), $n = 13$ (differentiating
27 cysts), and $n = 5$ (budding egg chamber cysts) from 5 ctrl germaria. **** $P < 0.0001$ and n.s., not significant
28 (Mann-Witney test). Scale bars, 10 μm .



Extended Data Fig. 2. F-actin in CMCs and the pFCs between differentiating cysts.

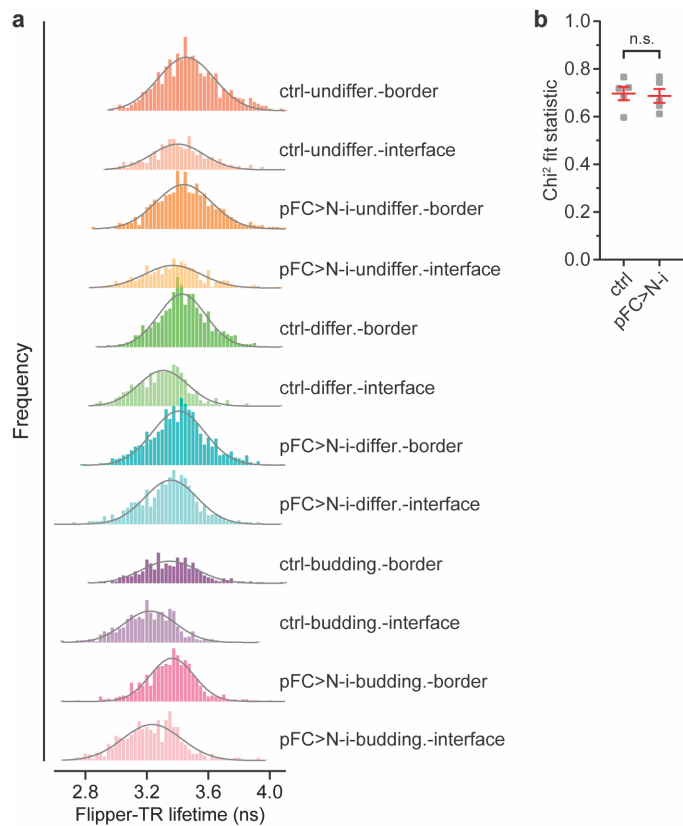
a,a', Representative images of a germarium expressing EGFP in pFCs. Arrows indicate CMC cell bodies or protrusions. Asterisks show the nuclei of cells in a cyst wrapped by CMCs. Arrowheads point to the pFCs between differentiating cysts. Germline cysts are outlined in the EGFP image, CMC cell bodies are outlined in the phalloidin image. **b**, Germlaria stained for Vasa, 1B1, and DAPI. Red arrow head points to

- 37 pFCs inserted between germline cysts. Hollow arrow heads indicate that pFCs are absent between cysts.
38 **e**, Representative images of COX activity histochemistry in differentiating cysts. Scale bars, 10 μ m.



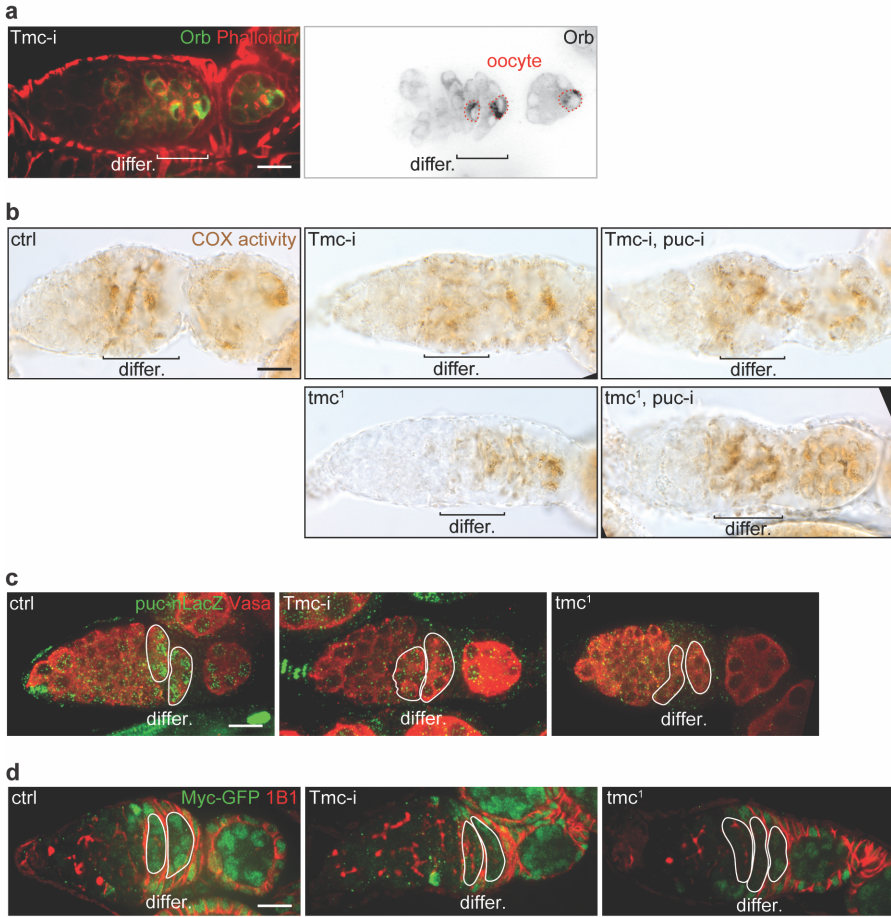
Extended Data Fig. 3. OXPHOS induction is independent of cyst differentiation.

a, Cyst differentiation is disrupted by *egl* RNAi. **b**, COX activity in compressed cysts. *n* = 10 germaria for each genotype. n.s., not significant (Mann-Witney test). Scale bars, 10 μ m.



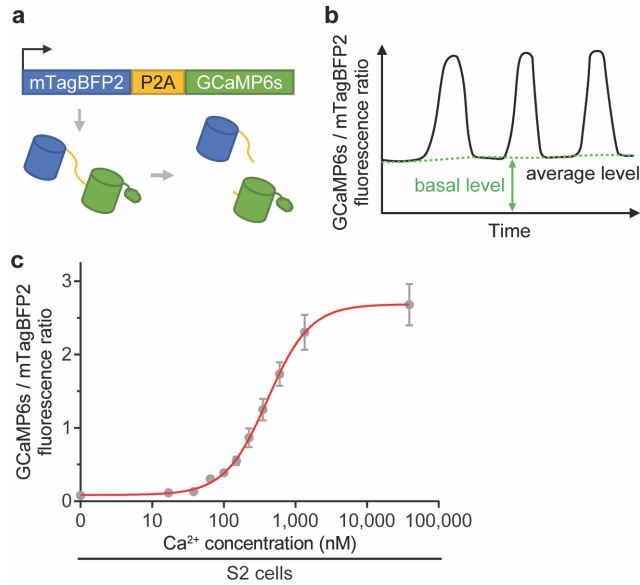
Extended Data Fig. 4. Cell membranes are expanded in compressed differentiating cysts.

a, Frequency distribution of Flipper-TR fluorescence lifetimes with Gaussian fits in ROIs. $n = 8, 8, 7, 7, 9, 9, 8, 8, 5, 5, 5, 5$ cysts, respectively. **b**, Chi² measurements for goodness of fits for the single images of germlaria stained with Flipper-TR membrane tension probe. n.s., not significant (Mann-Witney test).



Extended Data Fig. 5. OXPHOS induction in differentiating cysts requires *Tmc*.

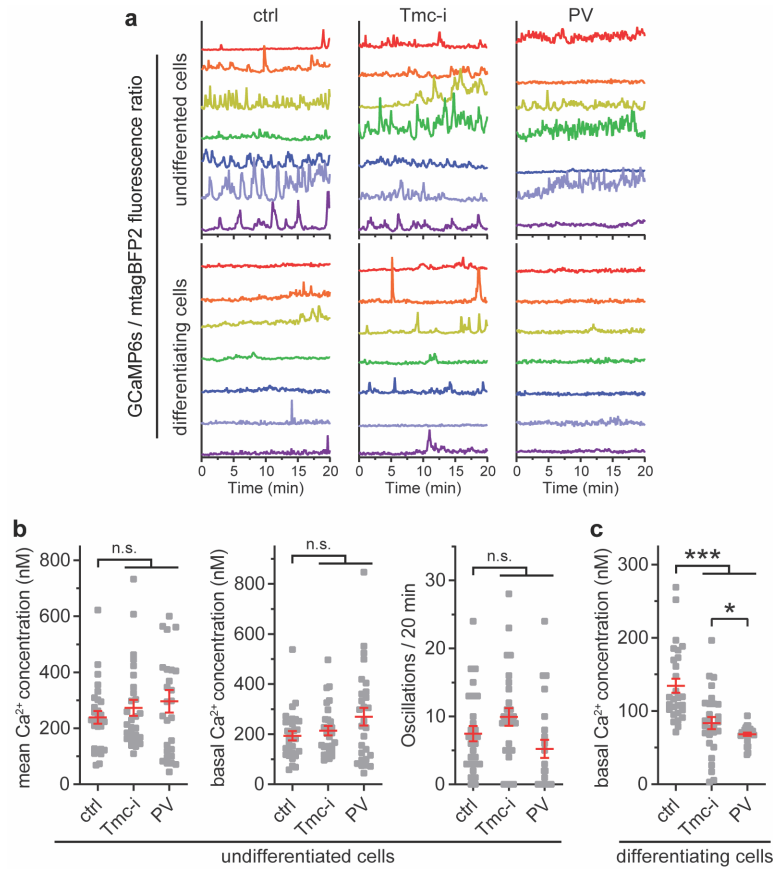
a, Cyst differentiation is normal in *Tmc* RNAi germarium. **b**, COX activity histochemistry in germaria. **c**, Representative images of JNK activity in germaria visualized by *puc-nLacZ*. **d**, Representative images of endogenous Myc-GFP expression in germaria. Scale bars, 10 μ m.



59
60
61
62
63
64
65

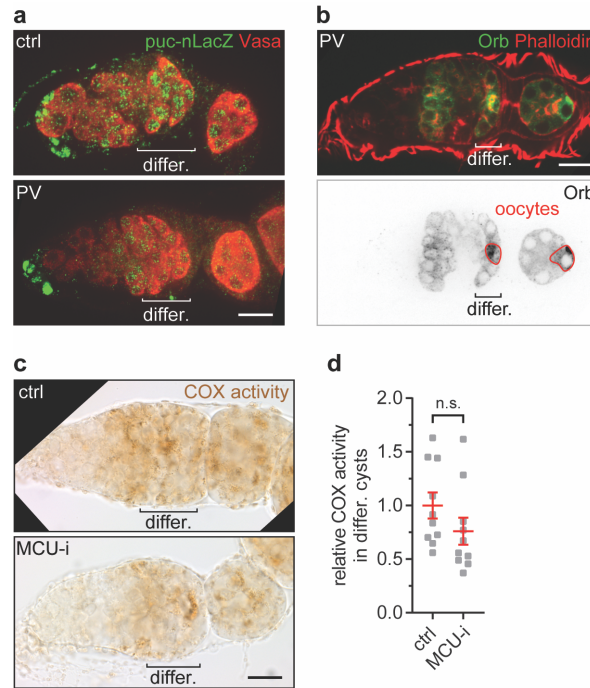
Extended Data Fig. 6. The UASz-mTagBFP2-P2A-GCaMP6s fusion protein and its calibration in S2 cells.

a, Schematic of UASz-mTagBFP2-P2A-GCaMP6s. **b**, Ca^{2+} oscillation parameters. **c**, Ca^{2+} calibrations of the mTagBFP2-P2A-GCaMP6s fusion protein in S2 cells. The standard curve is shown in red.



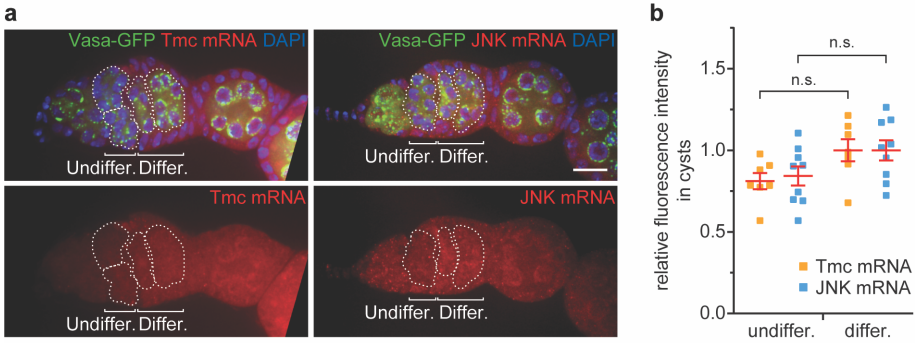
Extended Data Fig. S7. Cytosolic Ca^{2+} in differentiating but not undifferentiated cysts can be manipulated by *Tmc* knockdown or PV expression.

a, Representative traces of GCaMP6s/mTagBFP2 ratio in individual germ cells at different developmental stages. $n = 7$ germaria for each genotype. **b,c**, Mean and basal Ca^{2+} concentrations, and Ca^{2+} oscillation frequency in individual germ cells. $n = 28$ cells from 7 germaria for each genotype. $*P < 0.05$, $***P < 0.001$, and n.s., not significant [Kruskal-Wallis tests for the three groups in **b** and **c**; Mann-Witney tests between the two groups in **c**]. Scale bars, 10 μm .



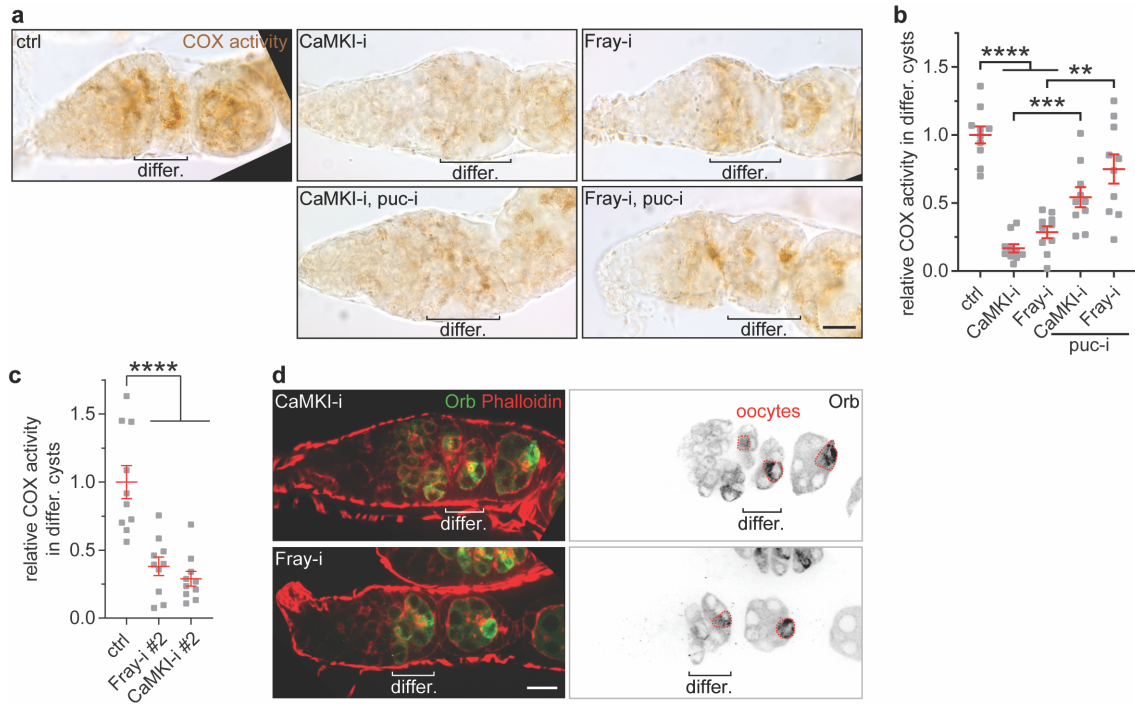
Extended Data Fig. S8. Cytosolic but not mitochondrial Ca^{2+} is essential for JNK and OXPHOS induction in differentiating cysts.

a, JNK activity is diminished in germ cells with expression of PV. **b**, Cyst differentiation is normal when PV is expressed in the germline. **c**, COX activity histochemistry in germaria. **d**, Quantification of COX activity in differentiating cysts. $n = 10$ germaria for each genotype. n.s., not significant (Mann-Witney test). Scale bars, $10\ \mu\text{m}$.



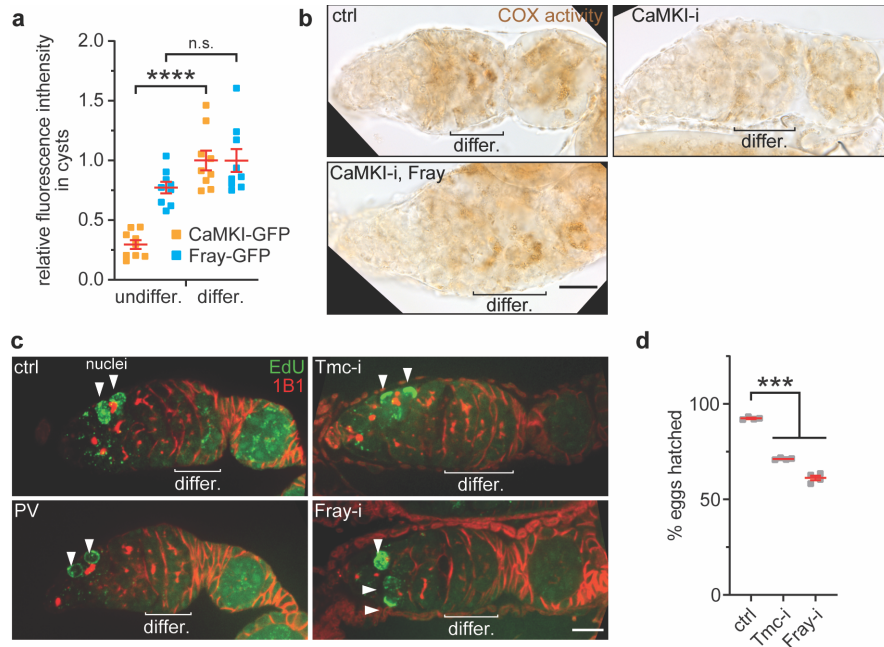
Extended Data Fig. S9. Expression of *Tmc* and *JNK* mRNA in germaria.

a, Visualization of *Tmc* and *JNK* mRNA in germaria by smFISH. **b**, Quantification of *Tmc* (n = 7 germaria) and *JNK* mRNA (n = 9 germaria) in cysts. n.s., not significant (Mann-Witney tests). Scale bars, 10 μ m.



Extended Data Fig. S10. CaMKI and Fray are required for OXPHOS induction in differentiating cysts.

a, Representative images of COX activity histochemistry in germlaria. **b,c**, Quantification of COX activity in differentiating cysts. $n = 10$ germlaria for each genotype. **d**, Cyst differentiation is normal in *CaMKI* RNAi and *Fray* RNAi germlaria. $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$ [Kruskal-Wallis tests for **b** and **c**; Mann-Witney tests for **b**]. Scale bars, $10 \mu\text{m}$.



Extended Data Fig. S11. Fray is downstream of CaMKI and the mechanotransduction pathway is required for mtDNA replication in the germline.

a, Quantification for the expression levels of endogenous CaMKI-GFP and Fray-GFP in cysts. $n = 10$ germlaria for each protein. **b**, COX activity histochemistry in germlaria. **c**, mtDNA replication in germlaria visualized with EdU incorporation. **d**, Hatching rate of eggs laid by female flies with indicated germline RNAi. $n > 180$ eggs laid by 4 females for each genotype. *** $P < 0.001$, **** $P < 0.0001$, and n.s., not significant [Mann-Whitney tests for **a**; Kruskal-Wallis tests for **d**]. Scale bars, 10 μ m.

Supplementary Tables and Table Legends

Supplementary Table 1. List of RNAi lines for the initial candidate screen used and the resulted COX activity.

CG #	Genes	RNAi lines (BL: BDSC; v: VDRC)	% germaria with normal COX activity in differentiating cysts
CG15148	luciferase	BL31603	100
	beethoven	v19324	100
		v105152	90
CG7669	hemingway	v29201	100
		v108918	100
		BL26304	100
CG2171	Triose phosphate isomerase	BL51829	100
		BL54850	100
		v108746	100
CG15427	turtle	v25623	95
		v108835	100
		BL35200	92
CG6203	Fmr1	v110800	100
		BL53313	72
		v105280	70
CG5996	Transient receptor potential cation channel γ	v7126	100
		v100701	100
		v32433	100
CG30092	jitterbug	v102221	100
		v10278	100
		v101742	100
CG5594	kazachoc	BL27699	100
		BL50608	100
		v30922	88
CG6706	metabotropic GABA-B receptor subtype 2	v107431	100
		v12330	100
		v100619	100
CG33988	mid1	BL53312	100
		v100090	100
		BL51722	75
CG5842	nanchung	BL31689	80
		BL51835	100
		v39477	100
CG15860	painless	v6131	100
		v104775	100
		v108683	100
CG9907	paralytic	v330392	100
		v25780	42
		v105132	46
CG3478	pickpocket	BL60501	100
		v109995	100
		BL51502	100
CG44122	piezo	v110681	100
		BL52903	100
CG45783	Piezo-like		
CG10537	Resistant to dieldrin		

		v100429	100
CG1058	ripped pocket	v8549	88
		v105463	100
CG6129	Rootletin	v22694	100
		v110171	100
CG1732	GABA transporter	v13359	95
		v106638	100
CG46121	Transmembrane channel-like	v39828	20
		v102145	15
CG8663	nervana 3	BL29431	100
		BL60367	100
CG14509	julius seizure	BL28764	93
		v4434	100
CG17150	dynein, axonemal, heavy chain 3	BL65239	100
		v35624	100
CG42685	CG42685	BL35005	100
		v49858	100
CG3525	easily shocked	BL38528	91
		v34286	100
CG31509	Turandot A	BL55378	44
		BL53244	44
CG6053	dynein, axonemal, intermediate chain 2	BL67961	100
		v35050	100
CG8546	pickpocket 26	BL25825	100
		v5110	100
CG14994	Glutamic acid decarboxylase 1	BL51794	100
		v32344	100
CG5751	Transient receptor potential cation channel A1	BL66905	88
		v37249	100
CG6963	gilgamesh	BL36719	100
		v26003	89
CG9472	brivido-1	BL31496	67
		v8424	65
CG16793	brivido-2	BL31670	85
		BL31671	90
CG13762	brivido-3	BL31385	100
		v101019	100

115 **Supplementary Table 2.** List of RNAi lines for Ca²⁺ signaling and JNK regulators, and the resulted COX
 116 activity.

CG #	Genes	RNAi lines (BL: BDSC; v: VDRC)	% germaria with normal COX activity in differentiating cysts
CG12530	luciferase	BL31603	100
	Cdc42	BL35756	33
		v100794	69
CG8416	RhoA	v109420	69
		v12734	90
		v49246	100
CG2248	Rac1	BL34910	100
		BL42521	80
CG7177	Wnk kinase	BL62150	73
		BL35141	55
CG10295	p21-activated kinase	BL41714	61
		BL32948	87
		BL41605	100
CG4353	hemipterous	BL35210	100
CG9738	MAP kinase kinase 4	BL35143	85
CG7717	Mekk1	BL35402	100
CG8789	Wallenda	BL35369	100
CG9774	Rho kinase	BL35305	70
CG1495	CaMKI	BL35362	20
		v101380	20
CG18069	CaMKII	v100265	100
		BL35330	100
		BL35264	100
CG14992	Activated Cdc42 kinase	BL32929	100
CG7324	TBC1 domain family member 8/9	BL35147	100
CG14217	Tao	BL32946	100
CG1976	RhoGAP100F	BL35373	80
CG14895	Pak3	BL35381	100
CG18582	mushroom bodies tiny	BL36841	degeneration
CG8114	pebble	BL42518	100
CG16973	misshapen	BL29580	55
CG2849	Ras-like protein A	BL34375	50
		BL53946	91
CG11376	Zizimin-related	BL65086	100
CG7356	Transglutaminase	BL32947	90
CG10043	Rho-type guanine nucleotide exchange factor	BL42545	90
CG30115	Guanine nucleotide exchange factor in mesoderm	BL61831	100
CG45017	Inositol 1,4,5-triphosphate kinase 2	BL42575	100
CG17528	CG17528	BL63717	75
CG3086	MAP kinase activated protein-kinase-2	BL60003	100
CG17342	Lk6 kinase	BL28355	100
CG42349	Protein kinase C δ	BL35179	70
CG4527	Sterile20-like kinase	BL41596	100
CG5169	Germinal centre kinase III	BL42569	10
CG7693	frayed		

		BL41587	20
CG7097	happyhour	BL53699	100
CG4290	Salt-inducible kinase 2	BL55880	100
CG42856	Salt-inducible kinase 3	BL56941	100
CG4720	Apoptotic signal-regulating kinase 1	BL35331	92
CG18492	TGF- β activated kinase 1	BL53377	40
CG9842	Protein phosphatase 2B at 14D	BL40872	100
		BL25929	83
CG9819	Calcineurin A at 14F	BL58249	50
CG1455	Calcineurin A1	BL25850	90
CG4209	Calcineurin B	BL27307	100
CG11217	Calcineurin B2	BL38971	100
		BL27270	100

Supplementary Table 3. Primers used for molecular cloning in this study.

Primer	Sequence
mTagBFP2-F	ATGGTGTCTAAGGGCGAAGAGC
mTagBFP2-P2A-R	GGGGCCGGGGTTCTCCTCCACGTCGCCGGCCTGCTTCAGCAGGGAGAAGTTG GTGGCGCCATTAAGCTTGTGCCCCAGTTTGCTA
GCaMP6s-F	GAGAACCCCGGCCCCATGGTCGACTCATCACGTCGTAAG
GCaMP6s-R	TCACTTCGCTGTCATCATTTGTACAAAC
PV-F	ATGGCCCAGGTGCAGCTGC
PV-3xHA-R	TTAAGCGTAATCTGGAACGTCATATGGATAGGATCCTGCATAGTCCGGGACG TCATAGGGATAGCCCGCATAGTCAGGAACATCGTATGGATAGCCTCCACCTG CGGCCGCGCTTTTCGG
Fray-F	ATGACCTCCATACCCGCCAATC
Fray-3xHA-R	TTAAGCGTAATCTGGAACGTCATATGGATAGGATCCTGCATAGTCCGGGACG TCATAGGGATAGCCCGCATAGTCAGGAACATCGTATGGATAGCCTCCACCGT CCGTGATGGAGATCTGC

121

Supplementary Table 4. Fluorescently labeled short DNA probes for smFISH.

	JNK-RB	TMC-RD 1	TMC-RD 2
1	TCGACGGTGTAGTGTGGTG	TTTGTAGAATTCCTCTTGG	CTAAGTCGTCTGAGTCTTTC
2	TGTGGATGGTGAAGTTGGTG	TTCAGTTCGTAGGATCTGTG	TAGTCGTGGTTCGATATCTC
3	TGGGCCGTAGATTAATGTAC	CTCTGGTTTTTGCAATGGGAA	TGAATCAGTGGAACCTCTCCG
4	GCATACTATTCCTTGGGCAC	GAAATTGTGTAGCTCTCCTC	TGTCGAAGGCATTGGTAGTG
5	GGGTGATAGTATCGTAAGCG	TGTCGTATAGACACTGGAGC	GTGGTGTATTTCATCTTCTTC
6	GTTTCTTAATCGCCACATTT	CAGGCGGATGTTCTCAAAGA	CCACATAGTAGGGTCTATTC
7	ACATTTTGGAAATGGTCGAGA	CGTGCCGCCATTAAAATTTT	TCTCCATCGTAGTAGATAGT
8	TATATGCTCGCTTGGCATGG	GGGAATGAGGACGGTAAGGA	ACACTCCTCCAGAAATTCAC
9	AGCTTCATTAGCTTGAATTC	GTGTGATTTCGATTTCTTGA	GCTGCCAAAAAAGTCATCGC
10	GCCAAATTATGTTTTTATGGT	CATAAGCCATCGCAGGAATG	ACTTCCTTCAAACGCTTCTG
11	GCGGCGTAAAAGCATTAAGT	CAGCGGAATGGCAATCATGA	GTCGTCAGATTGGTTTGTAT
12	TGAAACTCTTCCAAGTTCCT	CTTTGTGGCAAAGTACTCGG	ACGATGACTTTGGAGAGCTC
13	CTCCATGACCAGGTAGACAT	AATATTTTCAGGTATCCCTCG	CGCAGAAAGTCGATGCACAG
14	TGGCAGAGATTAGCGTCCAT	CCGGATGTGCTTGAATAGTA	GCACCAGTTTTGATTGACAT
15	ATCCAAGTCCATTTGGATGA	GGCGGTAAGGAAATAGGCTA	TACTGGGGAAAGGTCTTCTC
16	GCAAATAGGACATTCTGTCTG	GCCACAAAGCTGTAGATGTA	TTCTCGGCTATCTTGAAGTC
17	ATTCCGCACAACATTTGATA	GGAAAGTTTTGAGTTGCGCG	CCCTGGTTATTAATCAACGT
18	TCCTGCTGAGTGTAATGTT	GTGAAGAGCTTCCACGAGAA	AGAAGAATATGCCCATCCAC
19	ATGGCTTTAAGTCTCTGTGA	AGTTGCGATTATCCTTCTTT	CACTATCCACGAACGGAAGT
20	AGTCGGCCTTTACAACATA	AGTATCCTCTGGAGTATGAC	GAAGTTGTTGCACTTGGAGG
21	GCCAGACCGAAATCCAAAAT	CTCAGAGTGATTAACCAGCA	ATGGTCAACAGCAGCGATAG
22	AAAGGTAGTTCCTGCAGTAC	CATGGGCAGGAAGAAGGAGA	GGGCAGGACACAAAGGAACA
23	TGACCACATAGGGAGTCATC	TCATAATGCGAGCCAATTGC	ATGAACTCAGCTATCCTGTT
24	TCTGGAGCCCTGTAATATCG	TGTTTTGTTTCGTTCTCCAAT	GCAATGGAATCACAGTGCTC
25	TGTATCCCATGCCCAAATG	CTCAGAGCCTCAATTGAACT	AGTTAGCGAAACCAGGTAGT
26	GACCATATGTCCACGTTCTC	TCCGAAGTACTTTCACCATA	CCTTAAAGATCTTCTTGCGC
27	GTATCATTTTCGCCATAATG	CAAGTTGCCGTTGATGTAGA	TACTCCTCCGTATCGAAATC
28	TTCCAGGAAACAGCACACCG	TGTAACACTTGAGACGCTGC	ATGCGTGAGATTAGCTCTTT
29	TTCCACTGATCTATATGATC	TGGTGTGTGTCAGGTTGAGAA	AAACGAATCCTCATCCGACG
30	GGTGTACCTAATTGCTCAAT	GCAATGTGGTTGGCAAAGTT	GCAATCTGAACGATGTCCAC
31	CCGTTGCATAAACGAGGGTG	GTAATGTGGTGGGCAATGTG	GGGTCTCCTTTATGGGAAAA
32	TAGTTTCGGACGGTTGGCTG	CAATGTGGTAGGCAATGCTG	ATGTTTCTCCACTTTGAAGC
33	GTATCTAGGACGATTTTCCA	GGGCAATGTAGTAGGCAATG	TGTCCTTTTTATCCTTCATG
34	GTCTATCAAATGAGTATCCA	GTAGTTGTGCGAGATGTTTC	TTTGTATCCGGATCATGTTT
35	TTTGGAACAAGCCATCGGG	TAACGTGGTCCAAGGTGATG	AACTGGCGCTGACAATTCTT
36	GGCGGCTATTCTGATTATTA	TTTATTTTCGGCAGTGGTGAT	ATGTGGAATTTGCTTTTGGC
37	AGATTTCTAGCGTCAGAAGC	CAGGGGTTTTGTAGTATCTG	CGGGGGTTTTTTTTTCATCAA
38	GCTCTGGATCAATGACTAGC	GTTGAGTTGGGTATTTTCAGT	AAGGGTTTATCTTCCACTTC
39	AAAGCCTCGTCCACAGATAT	ATTGTGCTCAAAGTGGCAGA	CTTGAACCTGCCCCAACTTC
40	CACGTTGATATATTCATGCT	TCCGGTGGTGTAGATAAAGT	AAACTTGAACACCTCTGGCT
41	CCACTTCCTCAGCATCATAC	ATCACTGCCATAATCGTAGG	CTCGTTGTTTAGGTACTCTG
42	GTCCACGCTGTGATCATATG	CGACGGTTCCGAAGAGTAAT	AAAGCACAGACAATTGCCGC
43	CATAGATTAGCTCCTTCCAC	GCTTTCCTGTTTCATCGAAAT	GTCCTTTTTGAGATTGACGG
44	TGCTTCGTAGTCCATGACTT	CTTCGTCTCATTTTCATCGA	AACTGGACACGCTGGACAAT
45	ACCGCGTTCTATTATTTGTA	CTGCTGTACTTGCTGGTAAA	AATGTAATAGGACCGGCTGT
46		CAATTAAATGGCCACCGACT	TCTGTGAATCCTGTCTCATA
47		GGTGTGGTTTGCCTAAAAGA	TGGGTATATTATCCGACCAA
48		TGGAACTCTTCTTGTGTG	CCTTATAATGTTGACCGTGG

122

123

124 **Supplementary Video Captions**

125

126 **Supplementary Video 1**

127 Representative 3D reconstruction for germline cysts in the ctrl germarium shown in Fig. 1c.

128

129 **Supplementary Video 2**

130 Representative 3D reconstruction for germline cysts in a pFC>N-i germarium shown in Fig. 1d.

131

132 **Supplementary Video 3**

133 Representative live recording of cytosolic Ca^{2+} in germ cells from the ctrl germarium shown in Fig. 3c.

134

135 **Supplementary Video 4**

136 Representative live recording of cytosolic Ca^{2+} in germ cells with *Tmc* RNAi.

137

138 **Supplementary Video 5**

139 Representative live recording of cytosolic Ca^{2+} in germ cells expressing PV protein.

140