



**Supplementary Information for**

**SLC-30A9 is required for Zn<sup>2+</sup> homeostasis, Zn<sup>2+</sup> mobilization and mitochondria health**

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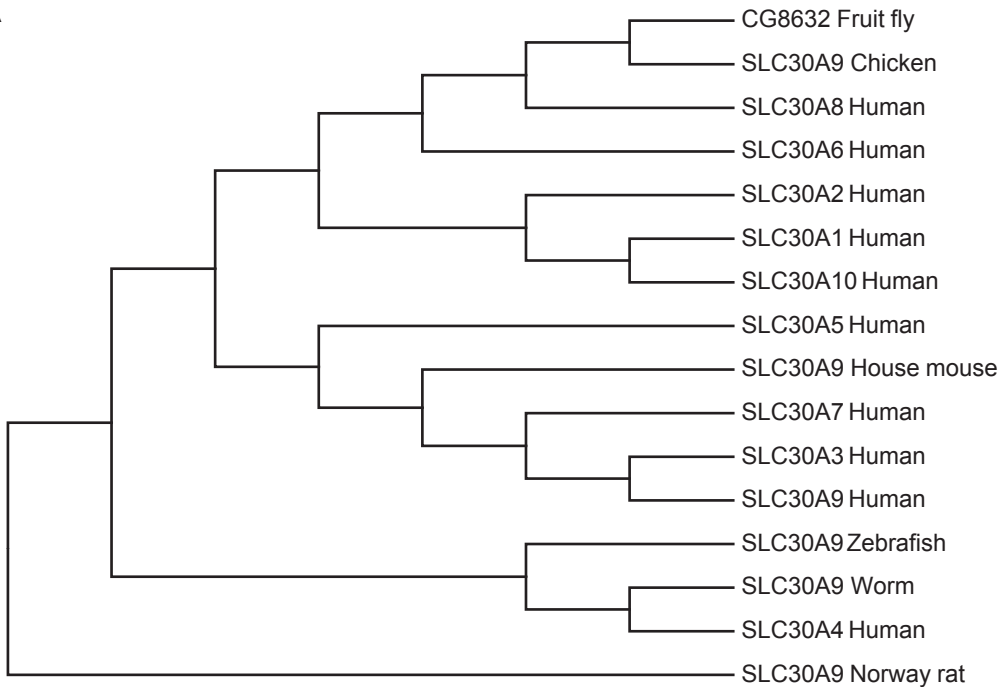
**This PDF file includes:**

Figures S1 to S6

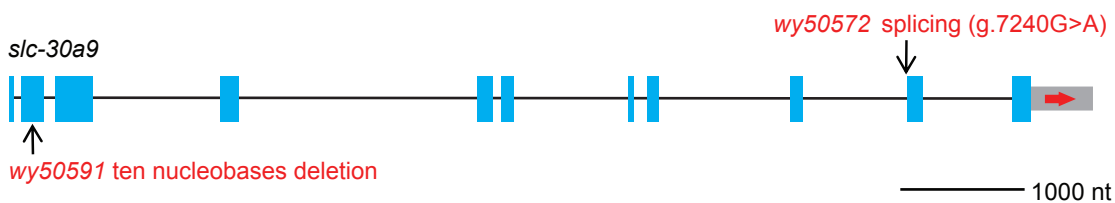
**Other supplementary materials for this manuscript include the following:**

Datasets S1

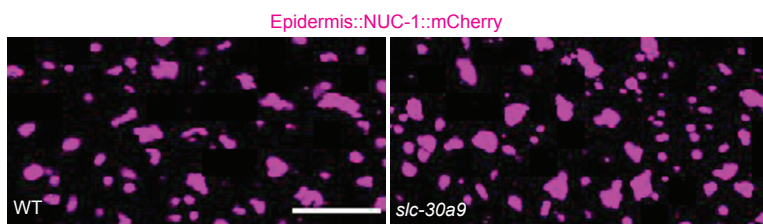
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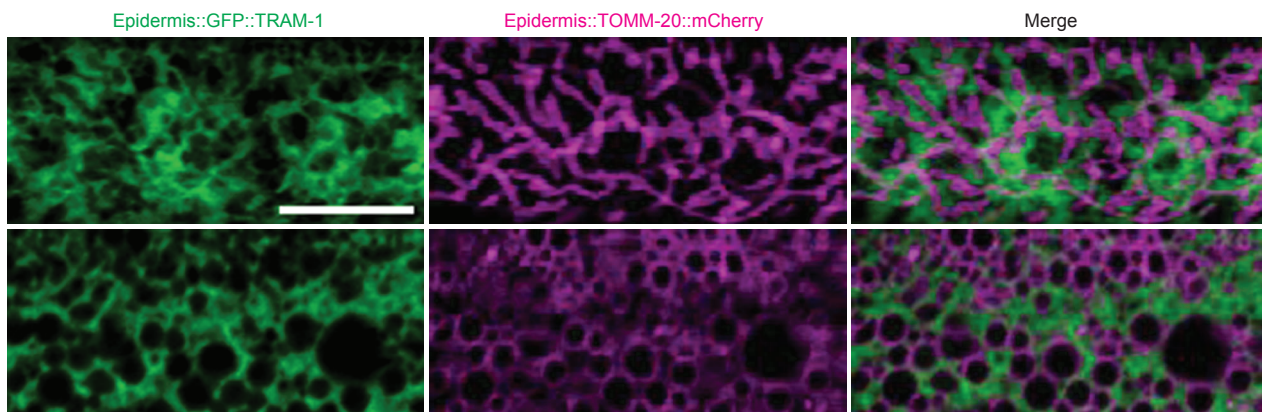
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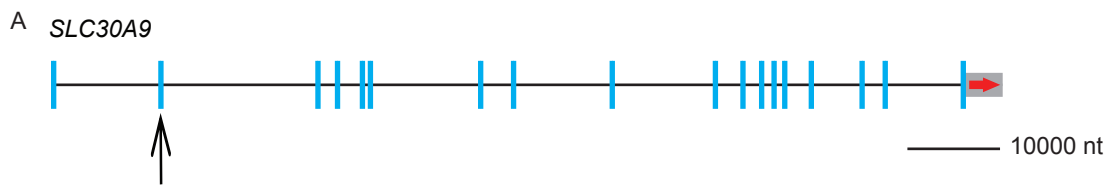
C



D



**Fig. S1.** (A) A phylogenetic tree of human SLC30 family and SLC-30A9 orthologues in various species. (B) Schematic representation of the *C. elegans slc-30a9* gene. Filled boxes represent exons and lines indicate introns. The deletion and point mutation sites of *wy50591* and *wy50572* are indicated with arrows. (C) Representative confocal images showing lysosome morphology in *C. elegans* epidermis (Epidermis::NUC-1::mCherry) in wild type and *slc-30a9(wy50572)*. Scale bar: 10  $\mu\text{m}$ . (D) Representative confocal images showing ER (GFP::TRAM-1) and mitochondria (TOMM-20(1-54AA)::mCherry) morphology in *C. elegans* epidermis in wild type and *slc-30a9(wy50572)*. Scale bar: 10  $\mu\text{m}$ .



WT (NM\_006345.4) 11117

TGGCAGAATTTAGTGACATTTGGAAGCTTTTCAAACATGGTTCCTGTAGTCATCCATATATTGGTACCCTGAGTCAAG

Protein sequence (NP\_006336.3) 38: WQNLVTFGFSFSNMVPCSHPIYIGTLSQ

*SLC30A9* haploid KO #1

TGGCAGAATTTAGTGACATTTGGAAGCTTTTCAAACATG-----GTCATCCATATATTGGTACCCTGAGTCAAG

Protein sequence: WQNLVTFGFSFSNMVIHILVP\*VK

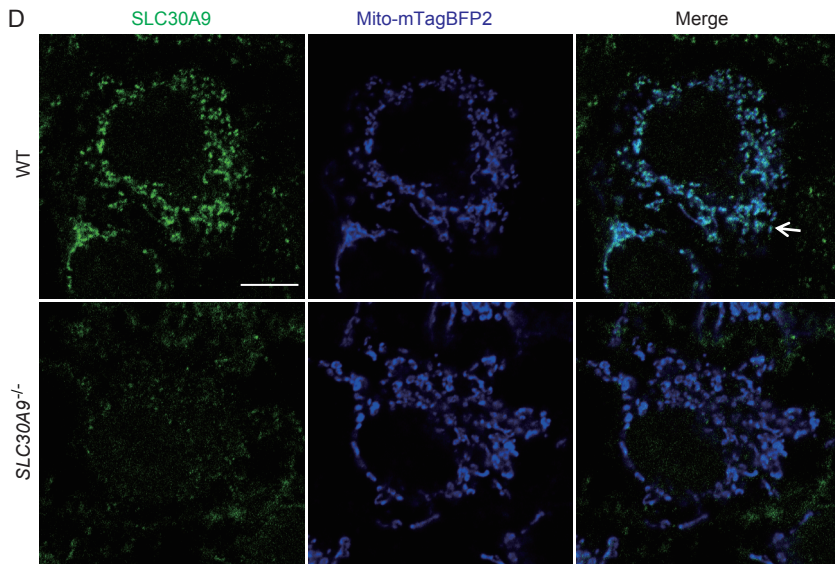
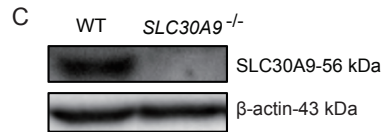
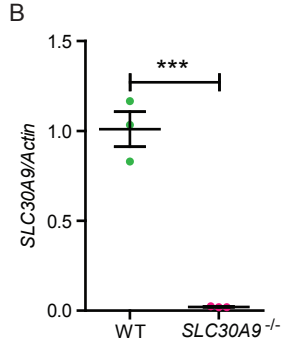
*SLC30A9* haploid KO #2

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GTAAAGTTGTACTCCACAAATGTTCCAGAAAGAAGGACAGGGATCACAAACACTCAGAGTGGAAAAAGTACCATCATT

GAAACAG

Protein sequence: WQNLVTFGFSFNVPVPSIYWPESSKVV LHKCSERRTGITNTQSGKSTII\***N**



**Fig. S2.** (A) Generation of *SLC30A9* knockout cell clones *SLC30A9*<sup>-/-</sup> using a CRISPR/Cas9 system in the human HeLa cell. A single guide RNA sequence was designed against exon 2 of the *SLC30A9* locus. Sequences of the parental and *SLC30A9*<sup>-/-</sup> cell clones were analyzed, and the results were shown below. Represented deletion and nucleotides were labeled red, and the green \* indicates stop codon. (B) *SLC30A9*-KO cells were validated by Q-PCR. Data are shown as mean±SEM (n=3). \*\*\*p<0.001 by unpaired t test. (C) Western blot of *SLC30A9* expression in HeLa wild type and *SLC30A9*<sup>-/-</sup> cells. (D) Immunofluorescence of *SLC30A9* in HeLa wild type and *SLC30A9*<sup>-/-</sup> cells. The arrow indicates mitochondria. Scale bar: 10 μm.

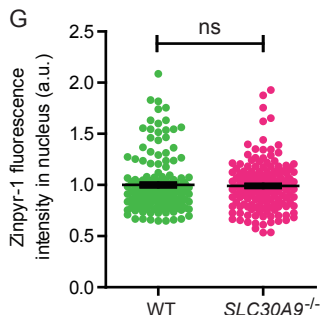
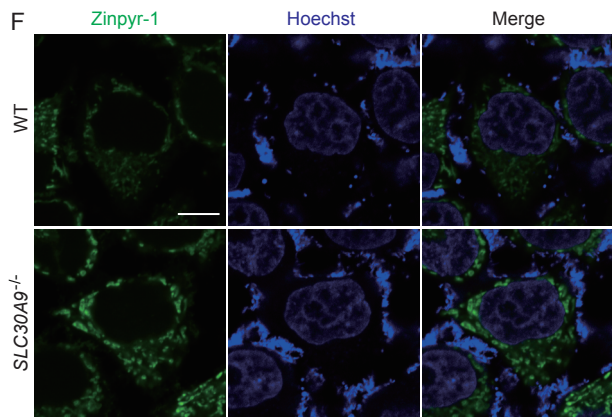
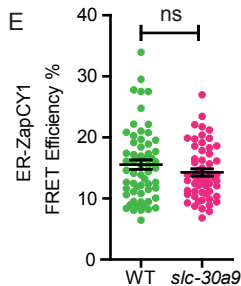
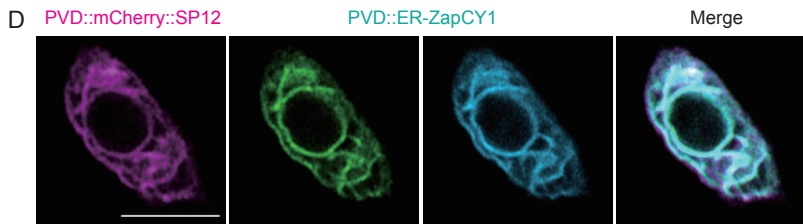
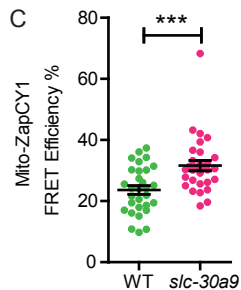
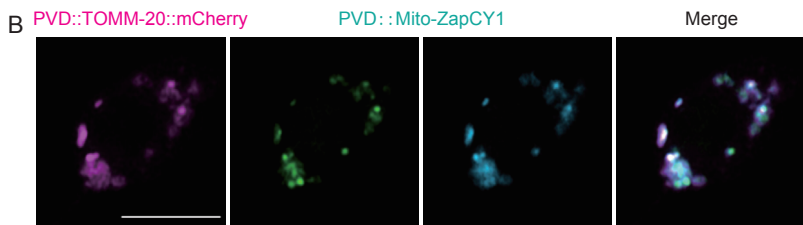
**A**

	430	440	450	460	470	480
SLC30A5_Human	YFLCLNLLFTFVELLYFYGVLTNSLGLISDGF	HMLFDCSALVMGLFAALMSRWKATRIFSYG				
SLC30A7_Human	FFLCLNLSFAFVELLYGIWNSCLGLISDSF	HMFDFDSTAILAGLAASVISKWRDNDAFSYG				
SLC30A1_Human	FLTGLCFAILLEAIER..FIEPHEMQQPLV	VLVGVGVAGLLVNVLGLCLFHHHSGFSQDSG				
SLC30A10_Human	FLTALCFITFVEAVLR..LARPERIDDP	VLIVGVVGLLVNVVGLLIFQDCAAWFACCL				
SLC30A2_Human	VASAI CLLFMIGEVEVGGYLAHSLAVMTDAA	HLLTDFASMLISLFSLWMSRPAATKTMNFG				
SLC30A8_Human	SASAI CFIFMIAEVVGGHIAHSLAVVTDAA	HLLIDLTSFLLSLFSLWSSKPPSKRLTFG				
SLC30A3_Human	AACAVCFVFMAGEVVGYYLAHSLAIMTDAH	LLADVGSMMGSLFSLWLSTRPATRTMTFG				
SLC30A4_Human	IAAVLYLLFMIGELVGGYIANS LAIMTDAH	MLTDLASAIILTLALWLSSKSPTKRFTFG				
SLC30A9_Human	VAICINGLNCFFKFLAWIYTGSA MFSEAIH	SLSDTCNQGLLALGISKSVQTPDPSPHPYG				
SLC30A9_C. elegans	IAFTLNLCDMAMKFTAAYLTGSKSLFAEAIH	SAMDTCNQQLILLGIRYSAKNPDLLFPYG				
SLC30A6_Human	VSPFWLGSQTPDLKWS THLGLPKCWDNRRE	ELPCLNSLMTCLISYWVTLRKPSPVYSFG				

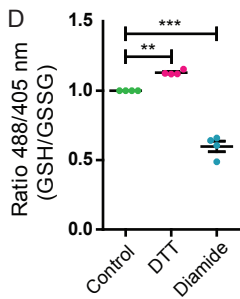
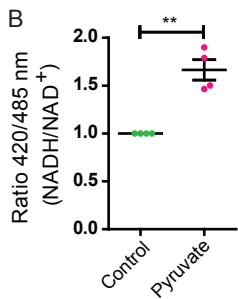
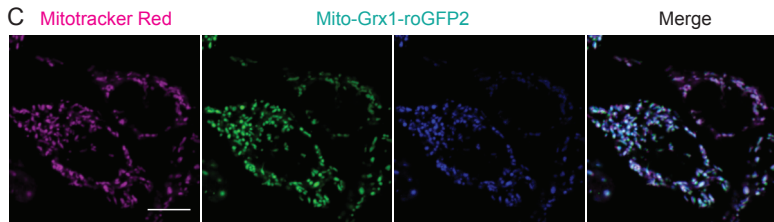
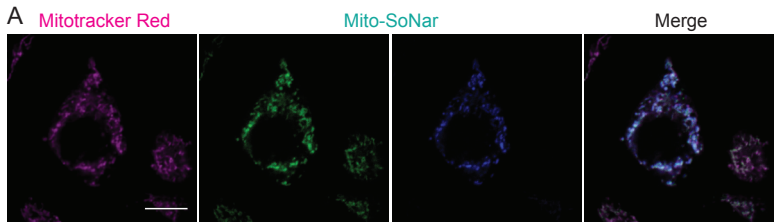
H198

	590	600	610
SLC30A5_Human	AG.....GMNANMRGVFLHVLAD	ITLGSIGVIVSTVLIEQFG.....	
SLC30A7_Human	DGP SLKET TGP SRQILQGVFLHILAD	ITLGSIGVIASAIMQNF.....	
SLC30A1_Human	.....DRAGQLNMRGVFLHVLGD	ALGSVIVVVALVFYFSWKGCEGDFCVNCPFPD	
SLC30A10_Human	.....KKSEALNIRGVLLHVMGD	ALGSVVVVITAIIFYVLPKSE.....D	
SLC30A2_Human	.....NPSVRAAFIHVIGD	FMQSMGVLVAAYILYFKP.....	
SLC30A8_Human	.....NASVRAAFVHALGD	LFQISIVLSALIIYFKP.....	
SLC30A3_Human	.....LPLGNTSVRAAFVHVLC	DLLOSFGVLAASILIIYFKP.....	
SLC30A4_Human	.....HGQDSLAVRAAFVHALGD	LVQSVGVLIYAAYIRFKP.....	
SLC30A9_Human	.....YVMESRDPSTNVILLE	DTAAVLGVI IAAATCMGLT SITG.....	
SLC30A9_C. elegans	.....YVRTSADPSLNVVLE	DTAAVTGVGIALCAISLSSFLN.....	
SLC30A6_Human	.....GLSSIFLPRMNPFLI	DLAGAFALCITYMLIEINN.....	

D323

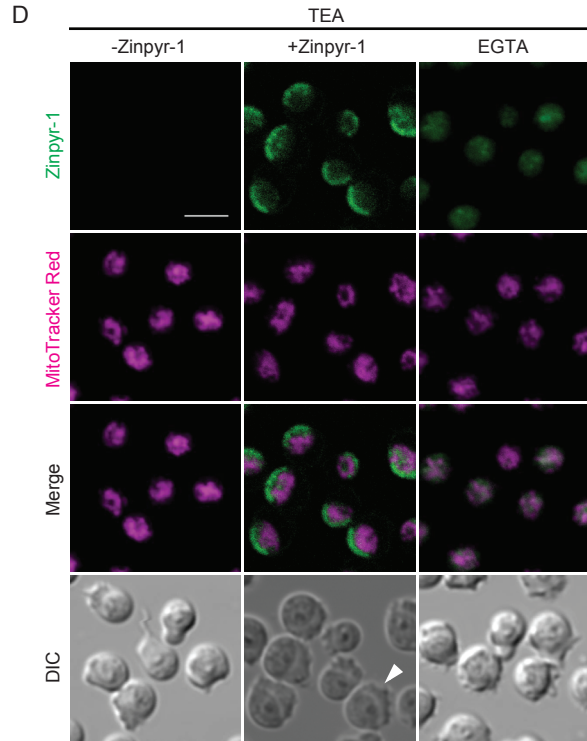
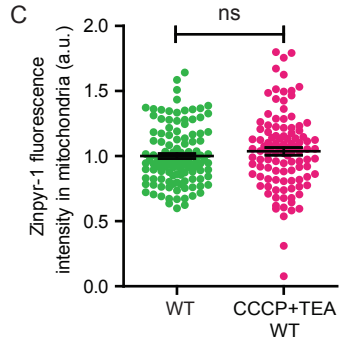
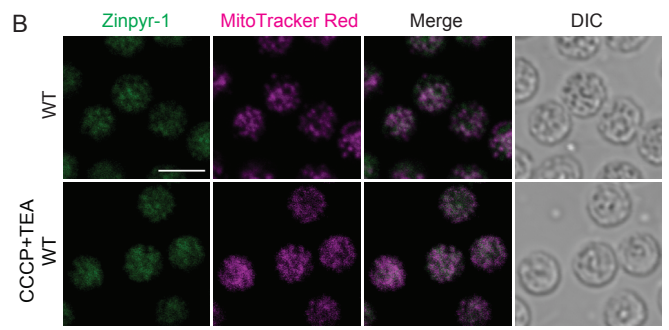
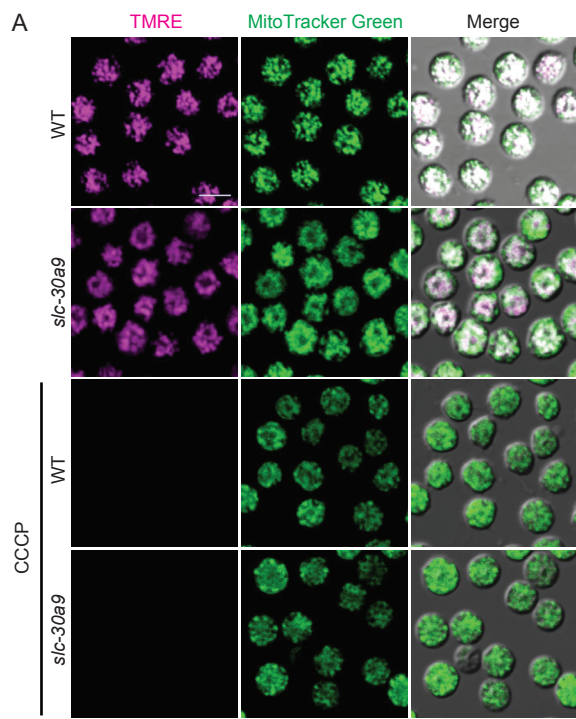


**Figure S3.** (A) An alignment of amino acid sequences of human SLC30 members and *C. elegans* SLC-30A9. The red boxed regions indicate the amino acid critical for Zn<sup>2+</sup>/H<sup>+</sup> binding (H198 and D323). (B) Representative confocal images of PVD soma co-expressing mitochondria Zn<sup>2+</sup> sensor PVD::mito-ZapCY1 and PVD::TOMM-20(1-54AA)::mCherry. Scale bar: 5 μm. (C) Quantification of the FRET efficiency of wild type and *slc-30a9* (*wy50591*). Data are shown as mean±SEM. \*\*\*p<0.001 by unpaired t test. 30 worms were scored for each genotype. (D) Representative confocal images of PVD soma co-expressing ER Zn<sup>2+</sup> sensor PVD::ER-ZapCY1 and PVD::mCherry::SP12. Scale bar: 5 μm. (E) Quantification of the FRET efficiency of wild type and *slc-30a9* (*wy50591*). Data are shown as mean±SEM. ns means not significant by unpaired t test. 56 worms or more were scored for each genotype. (F) Representative confocal images of WT or *SLC30A9*<sup>-/-</sup> HeLa cells co-stained with Zinpyr-1 and Hoechst. Scale bar: 10 μm. (G) Quantification of the nucleus Zinpyr-1 fluorescence intensity in WT and *SLC30A9*<sup>-/-</sup>. Data are shown as mean±SEM. ns means not significant by unpaired t test. More than 159 cells were analyzed for each genotype.

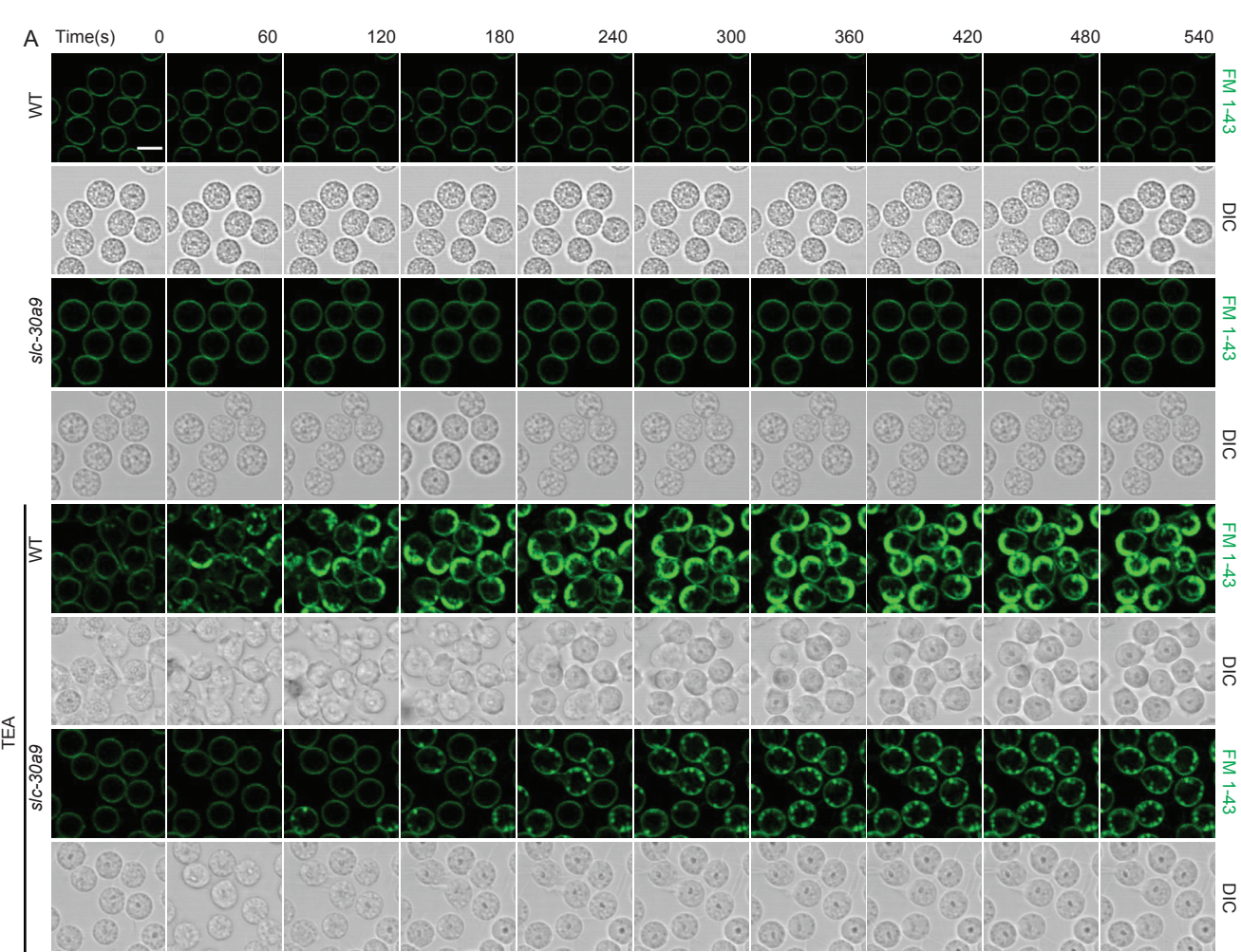




**Figure S4.** (A) Representative confocal images of HeLa cells expressing Mito-SoNar and stained with MitoTracker Red. Scale bar: 10  $\mu\text{m}$ . (B) Quantification of Mito-SoNar signal in response to pyruvate. HeLa cells expressing Mito-SoNar were treated with 200  $\mu\text{M}$  pyruvate, and the fluorescence ratio excited at 420 and 485 nm (emission at 535 nm) were detected by microplate reader. Data are shown as mean $\pm$ SEM (n=3). \*\*p<0.01 by paired t test. (C) Representative confocal images of HeLa cells expressing Mito-Grx1-roGFP2 and stained with MitoTracker Red. Scale bar: 10  $\mu\text{m}$ . (D) HeLa cells expressing Mito-Grx1-roGFP2 were treated with 5 mM DTT or 1 mM Diamide. The fluorescence excitation at 525 nm was measured with two excitation wavelength, 405 and 488 nm using a microplate reader. The ratio between 488 nm and 405 nm excitation was calculated. Data are shown as mean $\pm$ SEM (n=3). One way ANOVA with Tukey correction. \*\*p<0.01, \*\*\*p<0.001.



**Figure S5.** (A) Representative confocal images of spermatids co-stained with TMRE and MitoTracker Green in *him-5(e1490)* and *slc-30a9(wy50591); him-5(e1490)* males supplemented with SM or SM+CCCP. Scale bar: 5  $\mu$ m. (B) Representative confocal images of spermatids co-stained with MitoTracker Red and Zinpyr-1, and supplemented with the CCCP+TEA. Scale bar: 5  $\mu$ m. (C) Quantification of the Zinpyr-1 fluorescence intensity in the sperms mitochondria of *him-5(e1490)* and CCCP+TEA treatment. Data are shown as mean $\pm$ SEM (n=3). ns means not significant by unpaired t test. 100 sperms or more were scored for each genotype. (D) Representative confocal images of redistribution of labile zinc occurred during TEA sperm activation supplemented with the zinc chelator EGTA. The arrowheads indicate the pseudopods. Scale bar: 5  $\mu$ m.



**Figure S6.** (A) Representative confocal images of spermatids stained with FM 1-43 in *him-5(e1490)* and *slc-30a9(wy50591); him-5(e1490)* mutant males supplemented with SM or SM+TEA. Confocal images were collected every 60 seconds for total 540 seconds. Scale bar: 5  $\mu$ m.

**Dataset S1 (separate file).** antibodies, strains, and primers used in this paper.