Supplementary Materials for

Targeting mitochondrial RNAs enhances the efficacy of the DNA-demethylating agents

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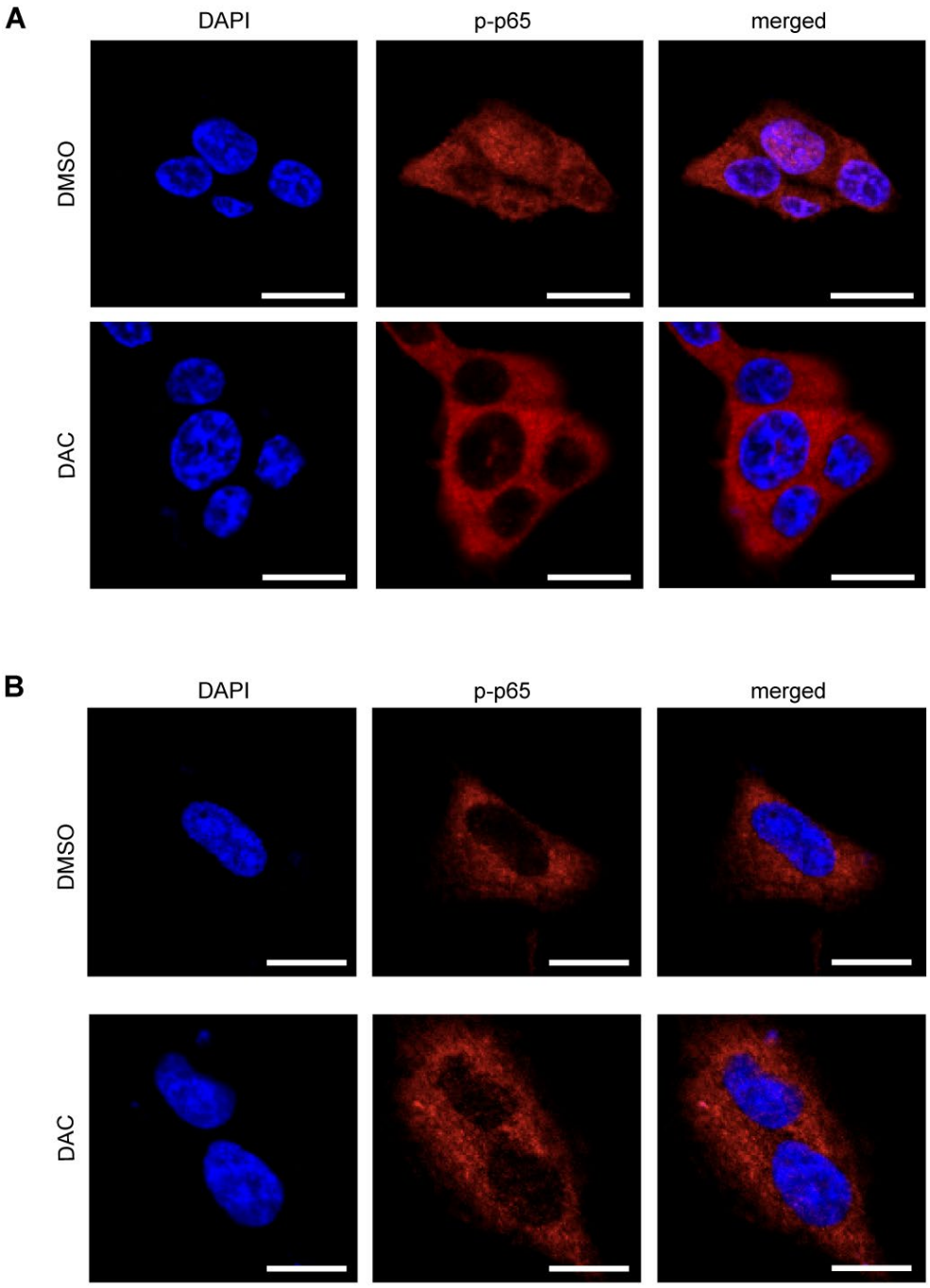


Supplementary Figure S1. Subcellular localization of p-p65 upon DAC treatment. (A, B) Nuclear localization of p-p65 in DMSO and DAC-treated HCT116 (A) and A549 cells (B) analyzed via immunocytochemistry with p-p65 antibody. DAPI was used to counterstain the nuclei. Scale bar indicates 20 μm.

Supplementary Table S1. Sequences of sgRNAs

Gene	Target sequence #1 (5′-3′)	Target sequence #2 (5′-3′)
	5′-CACCG-*Target sequence*-3′ (Complement sequence: 5′-AAAC-*Reverse complement target sequence*-C-3′)	
Negative control (NC)	AAATGTGAGATCAGAGTAAT	-
POLRMT	CTAGAGCGCGAGGTGTACGA	ACAAGCCGAGAGGAACGATG
TEFM	ATTATGTAAGGCCCAGTACA	GACCGTTGGAGTTTAATGAG
TFAM	TAAACGTTATAAGCTGAACG	ATATCAAGATGCTTATAGGG
TFB2M	TTAAGCGTTACGTAACCGAT	GTTGGAATGTTCCCAAGTAG
ELAC2	TCTGAGACACTACCTTACAG	ACAGATGAAAGCTACGACCA
PTCD1	TTTGCGGAATAGTCTCCGGG	CTGCAAGAAGTACCAGTACG
PTCD2	GTGTTACGAGTTGGATCTCG	AAGCCGCGGATACCTCCGAG
MRPP1	TAAGCCATGTCAAAAACCAA	CAAGCTGCTAGAAACCACTG
MRPP3	TATAAGACTTTAGAACCTAG	AGCAACGAATCTGATTGCCA
PNPT1	GATGTCCTAGCAATTAATGG	CAAAATAAGATTAGATACGG
SUPV3L1	GGAGCTTATGTACACAACGG	AGTCGGCAGATTGAAATTCG
REXO2	ATAATCAGGTTAGGACCCTG	TGGGAGTCACGGACGGTTCG
MTPAP	TGGTGTGAGAACCTCACGAG	CTTCTGAACGGTCACGCGTA

Supplementary Table S2. Primer sequences for RT-qPCR and mtDNA copy number

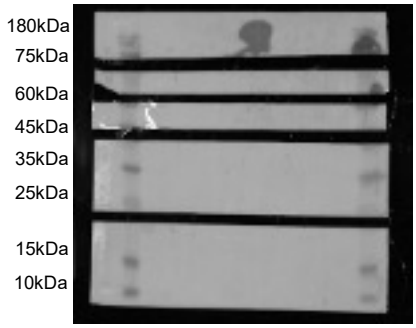
Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
ND1	TCAAACTCAAACTACGCCCTG	GTTGTGATAAGGGTGGAGAGG
ND4	CTCACACTCATTCTCAACCCC	TGTTTGTCGTAGGCAGATGG
ND5	CTAGGCCTTCTTACGAGCC	TTTGGGTTGAGGTGATGATG
CYTB	CAATTATACCCTAGCCAACCCC	GGATAGTAATAGGGCAAGGACG
CO1	GCCATAACCCAATACCAAACG	TTGAGGTTGCGGTCTGTTAG
CO2	CTAGTCCTGTATGCCCTTTTCC	GTAAAGGATGCGTAGGGATGG
CO3	CCTTTTACCACTCCAGCCTAG	CTCCTGATGCGAGTAATACGG
ISG15	CTCTGAGCATCCTGGTGAGGAA	AAGGTCAGCCAGAACAGGTCGT
IRF7	CTGTGGACACCTGTGACACC	TGCCCTCTCAGGAGCCAA
IFI6	TGCTACCTGCTGCTCTTCAC	CGAGCTCTCCGAGCACTTTT
IFI27	ATCAGCAGTGACCAGTGTGG	TGGCCACAACTCCTCCAATC
OASL	GCGGAGCCCATCACGGTCAC	AGGACCACCGCAGGCCTTGA
tRNA$^{(Gly)}$-ND3	CCGTTAACTTCCAATTAACTAGTTTTG	TGTAGCCGTTGAGTTGTGGTAG
tRNA$^{(Asp)}$-CO2	GAAAAACCATTTCATAACTTTGTCA	GGAAAATGATTATGAGGGCG
tRNA$^{(Phe)}$-mt-RNR1	TGTAGCTTACCTCCTCAAAGCA	AGGGTGAACTCACTGGAACG
CO2-tRNA$^{(Lys)}$	AGGGCCCGTATTTACCCTAT	TCACTGTAAAGAGGTGTTGGTTCT
POLRMT	AGCTGCTCAGGGACGTGTAT	CAGTGCTTTCTCCCATTGGT
TCF21	GAGGCAGATCCTGGCTAACG	TTTCCCGGCCACCATAAAGG
RASSF1A	ACCTCTGTGGCGACTTCATC	CGGTAGTGGCAGGTGAACTT
ACTB	CCTGTACGCCAACACAGTGC	ATACTCCTGCTTGCTGATCC

Raw blotting and stained SDS-PAGE gel images

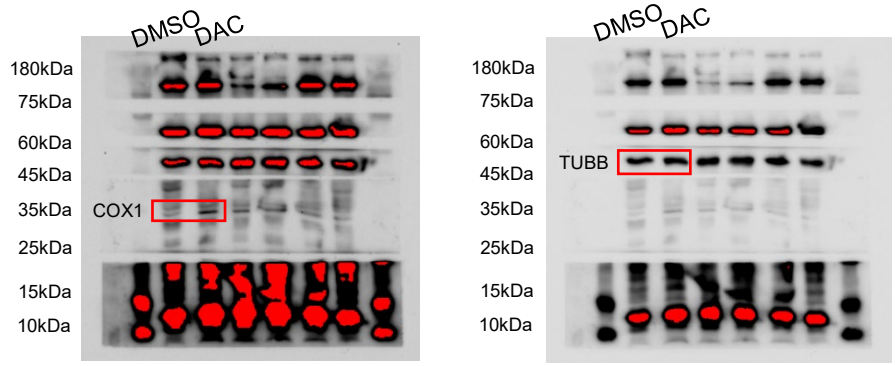
Author notes:

- SDS-PAGE membranes were cut before the hybridization of target proteins' antibodies
- Images were cropped after chemiluminescence detection by ChemiDoc imaging system
- Images for different target protein from the same gel were taken in different exposure to avoid saturation.

Original membrane – Full length blot

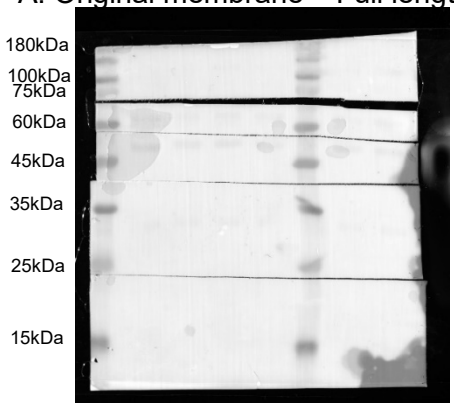


Western blot images

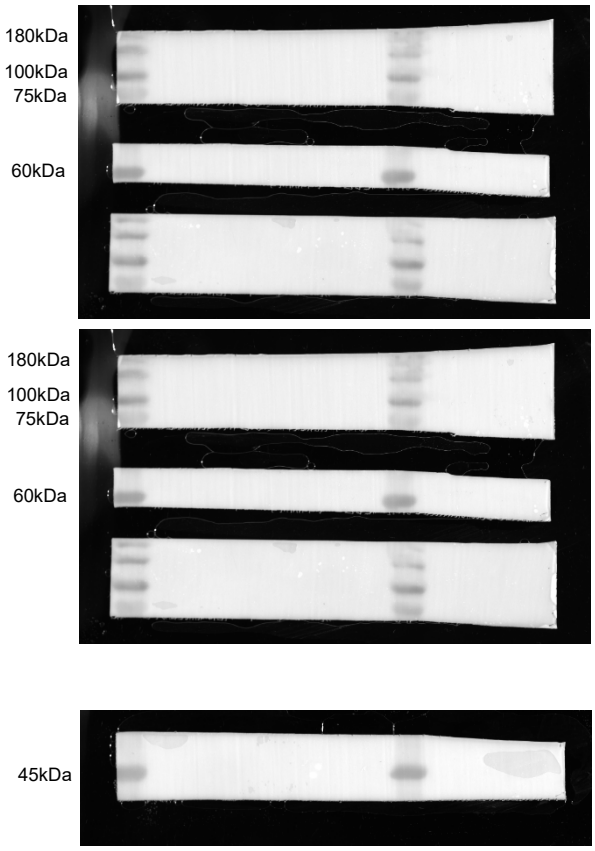


Supplementary Figure S2. Original blot images for Figure 2D. In all western blot experiments, membranes were cut prior to hybridizing with primary antibodies. Images were cropped after chemiluminescence detection by ChemiDoc imaging system. The images were taken at different exposure times to avoid saturation.

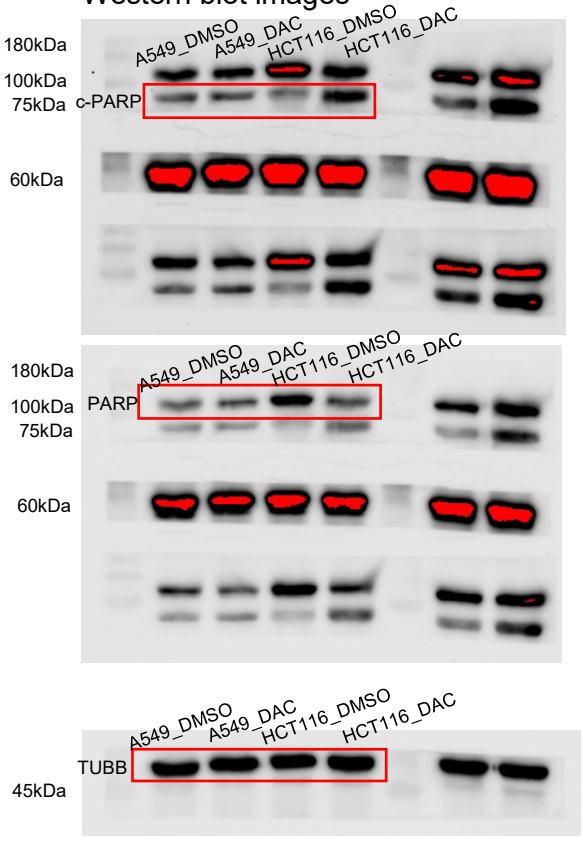
A. Original membrane – Full length blot for PARP, cleaved PARP and TUBB



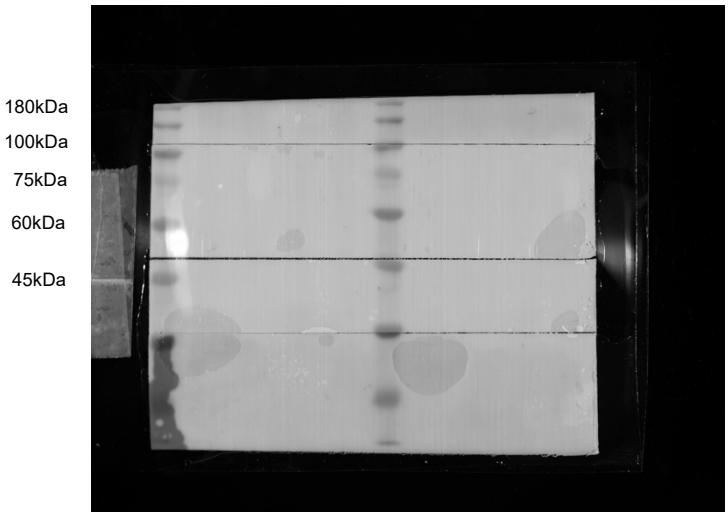
Original membrane for each image

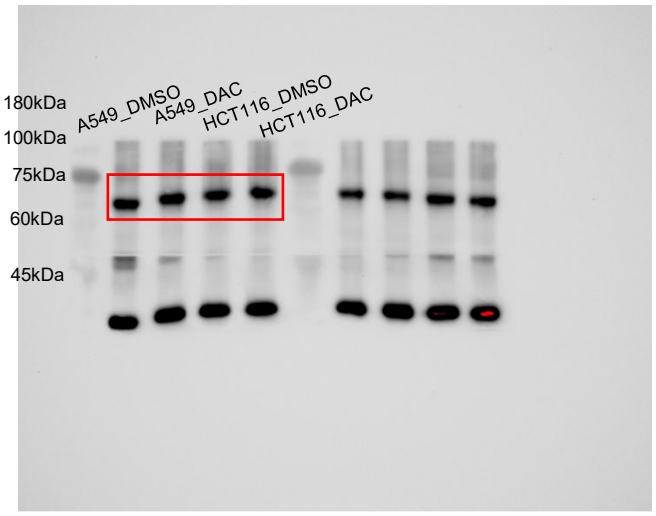


Western blot images



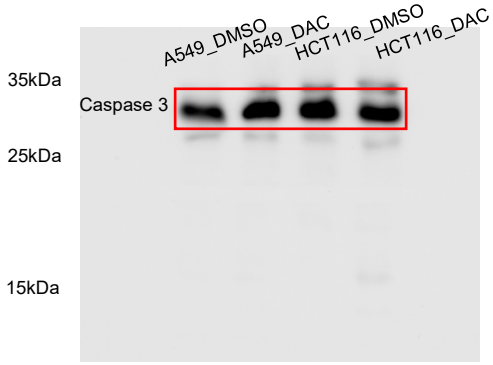
B. Original membrane for p65

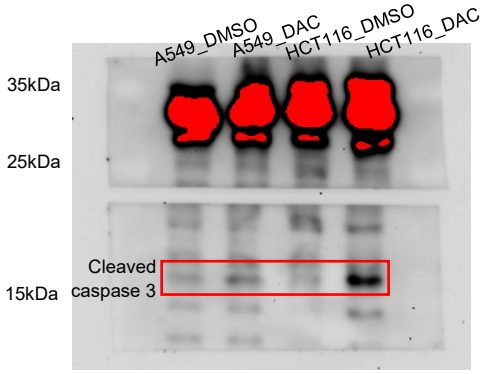




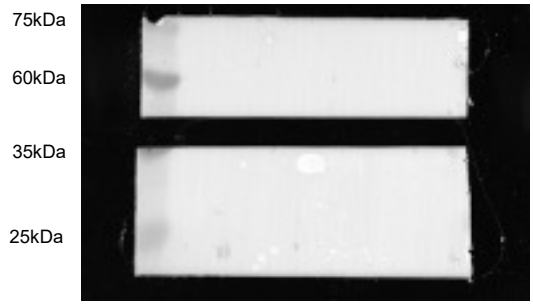
C. Original membrane for caspase 3 and cleaved caspase 3

Western blot images

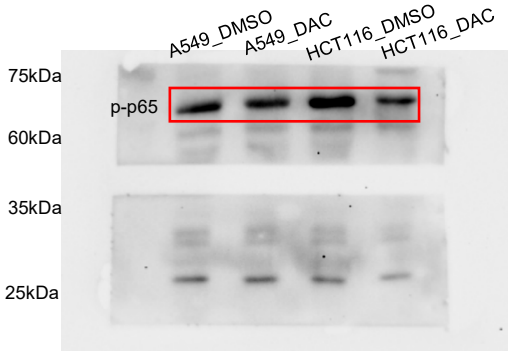


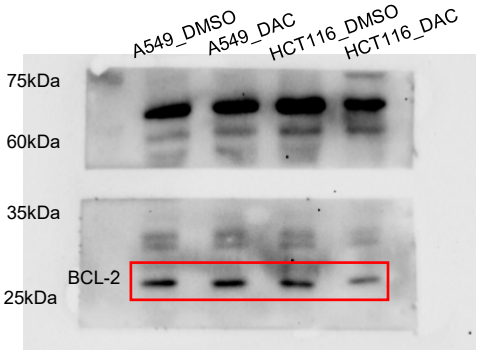


D. Original membrane for p-p65 and BCL-2



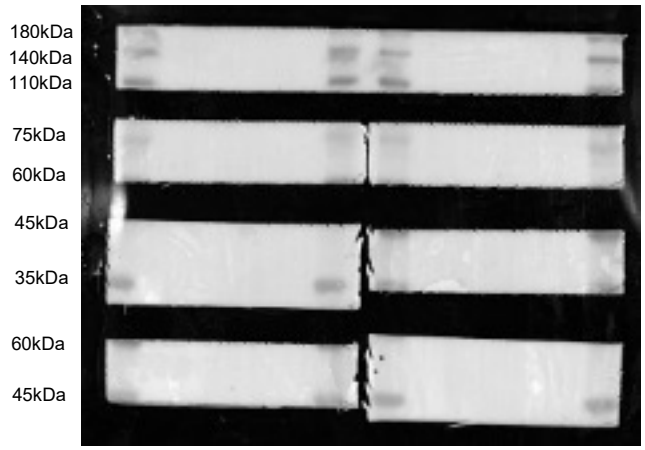
Western blot images



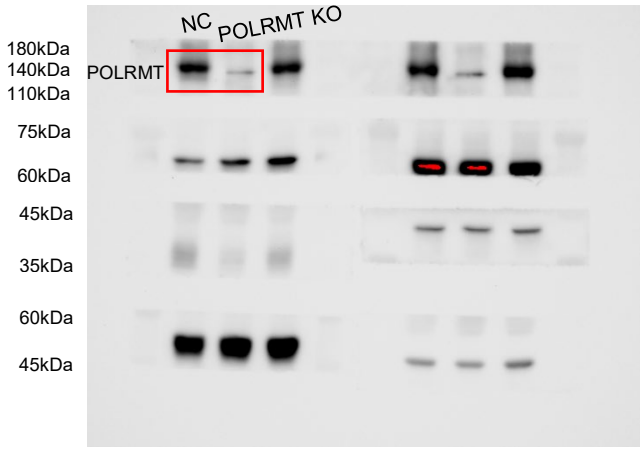


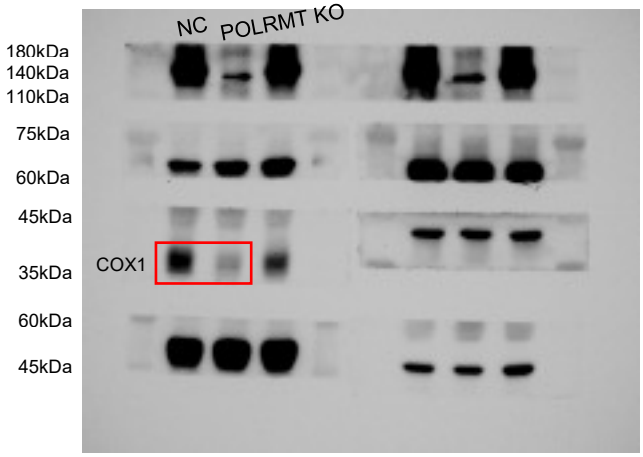
Supplementary Figure S3. Original blot images for Figure 2H. All western blots were performed using the same samples but run on different gels due to the similar sizes of the target proteins. This method is performed to provide clear and distinct data presentation. (A) The blots for PARP, cleaved PARP, and TUBB. (B) The blots for p65. (C) The blots for caspase 3 and cleaved caspase 3. (D) The blots for p-p65 and BCL-2. In each subfigure, blots were cropped from the same gel prior to the hybridization of primary antibodies and the images were taken at different exposure times to avoid saturation.

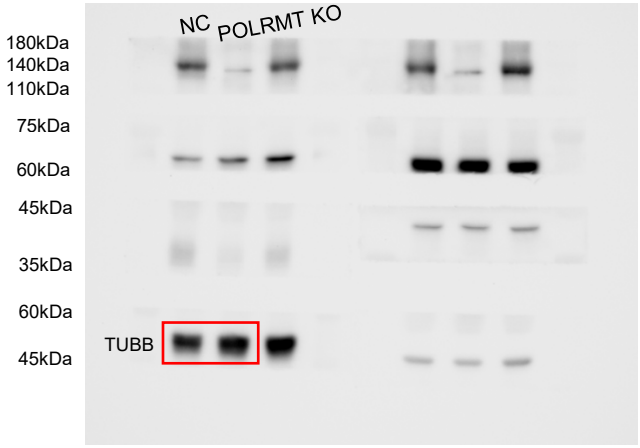
Original membrane – Full length blot



Western blot images

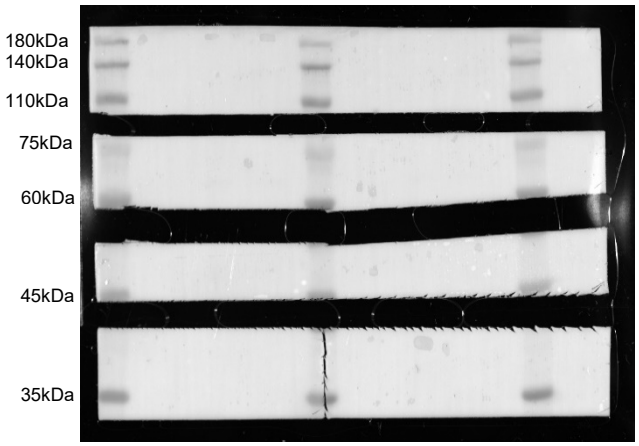




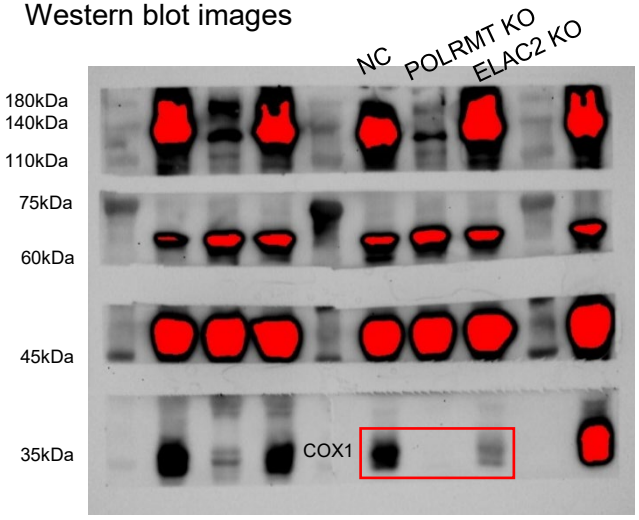


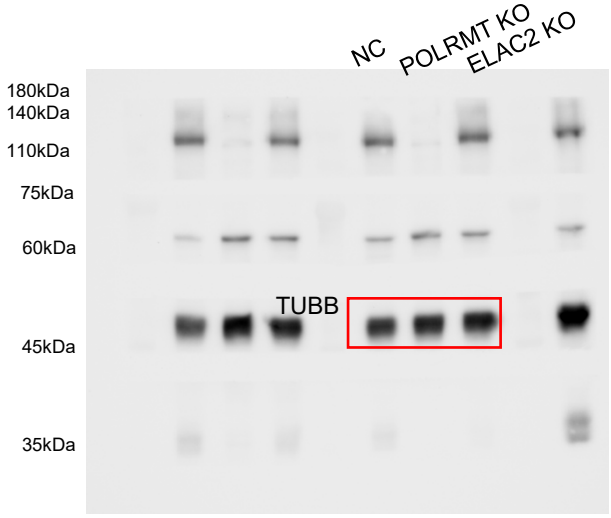
Supplementary Figure S4. Original blot images for Figure 3C. The blots were cropped from the same gel prior to the hybridization with primary antibodies and the images were taken at different exposure times to avoid saturation.

Original membrane – Full length blot



Western blot images





Supplementary Figure S5. Original blot images for Figure 4C. The blots were cropped from the same gel prior to the hybridization with primary antibodies and the images were taken at different exposure times to avoid saturation.