APPENDIX

Chemical targeting of NEET proteins reveals their function in mitochondrial morphodynamics

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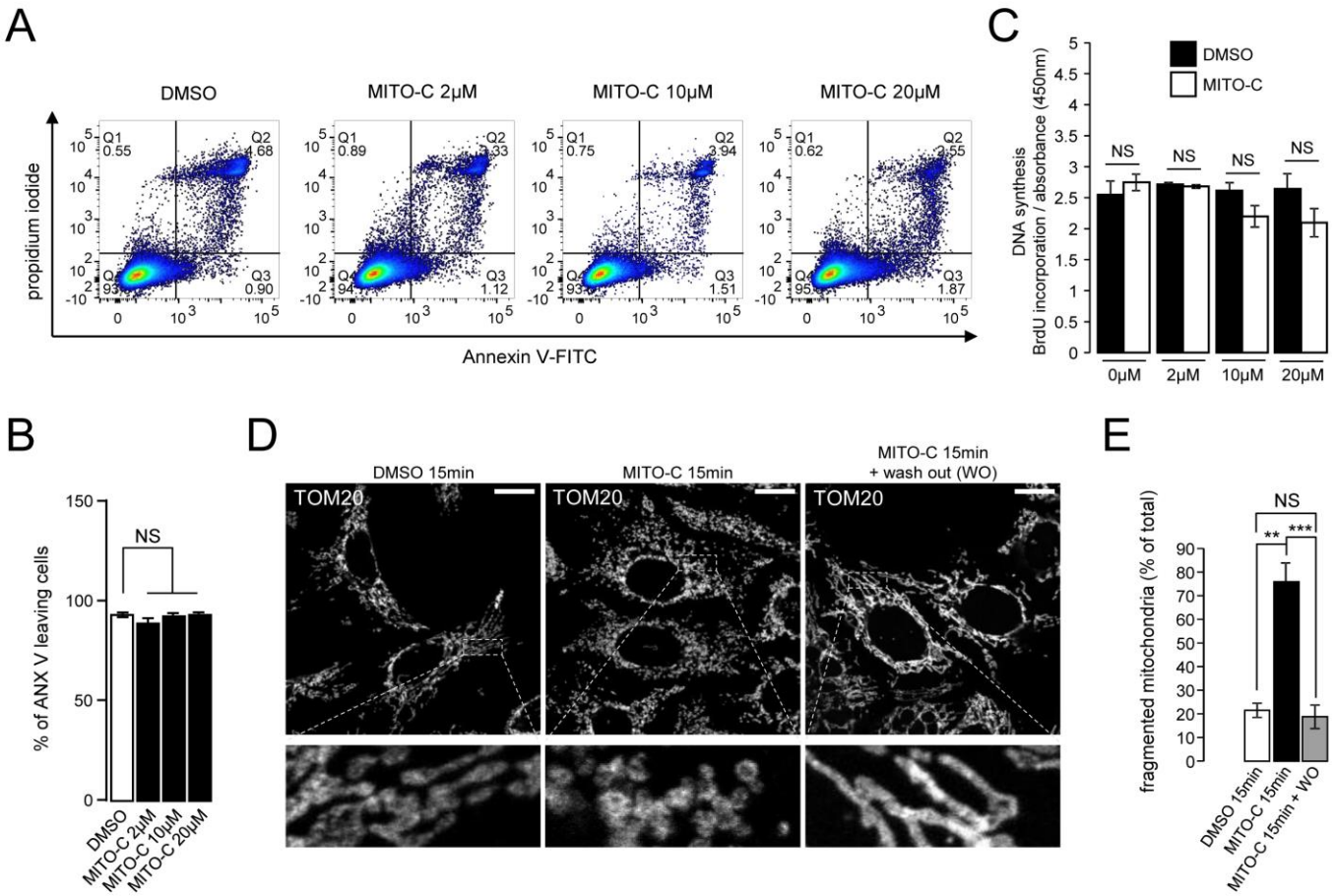


Appendix Figure S1: Mito-C treatment is reversible and does not induce cell death

(A), (B) and (C): Cells treated for 24h with DMSO or indicated concentrations of Mito-C were stained with propidium iodide (PI as shown in A), annexin V (as shown in B) or 5-Bromo-2'-deoxyuridine (BrdU, as shown in C) and analyzed by cytometry (A and B) or absorbance was measured at 450nm in a spectrofluorometer (C).

(D): Cells were treated with DMSO or 2 µM Mito-C for 15min, with 2 µM Mito-C for 15min then washed-out (WO) overnight, treatments were blocked by fixation and mitochondria visualized by immunostaining with anti-TOM20.

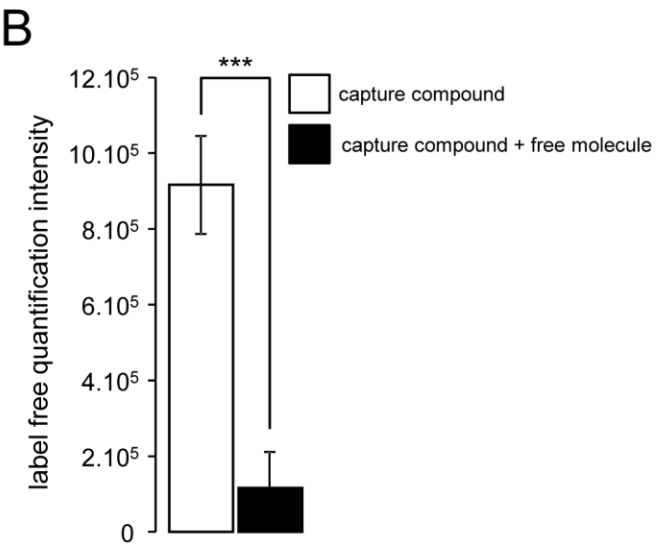
(E): Quantification of mitochondrial fragmentation following treatment or treatment followed by removal of compound as illustrated in (D) (n=120-140).

Data information: to evaluate significance of differences in B and C we used t-test, NS for non-significant. In e one-way Anova was used (NS for non-significant and ***p<0.0001). Scale bars = 10 µm.

A

Hit	Description	Main sub-cellular location
MiNT/CISD3	CDGSH iron-sulfur domain-containing protein 3	Mitochondria
CAT	Catalase	Peroxisome
CDSN	Corneodesmosin	Extracellular region
DSC1	Desmocollin-1	Plasma membrane
KPRP	Keratinocyte proline-rich protein	Extracellular region

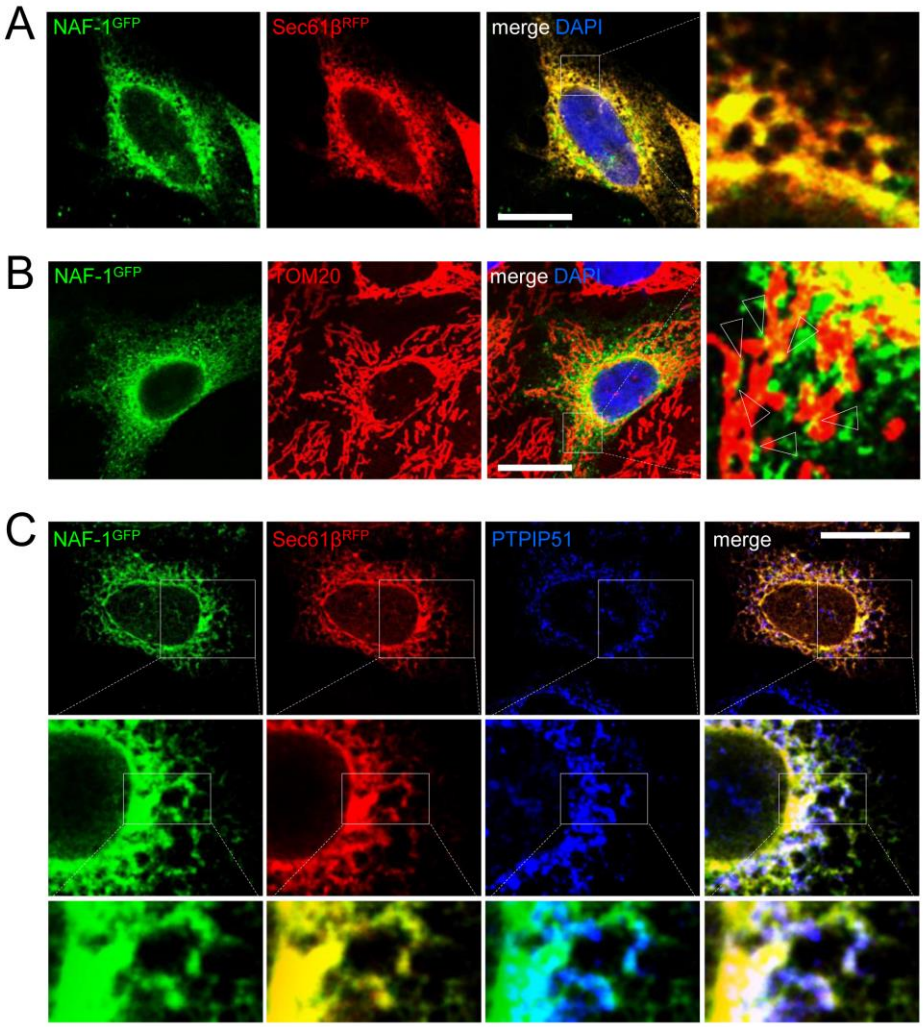
B



Appendix Figure S2: **Targets of Mito-C and competition assay**

(A): Capture experiments were conducted with 10 µM of capture compound incubated 1h on living A549 cells. The 5 captured proteins identified in area of significance are listed in the table. Label free quantification (LFQ) intensities are used to calculate fold change (*FC*) and p-values for comparison between assay and competition and no capture compound controls. Specific criteria for consideration as specific binders are FC>2 and p<0.05.

(B): Bar charts of protein amount with an excess of free molecule shows competition, indicating that MiNT/CISD3 is a likely binding partner of the ENYO compound (concentration of capture compound 10 µM, concentration of free molecule 20 µM).

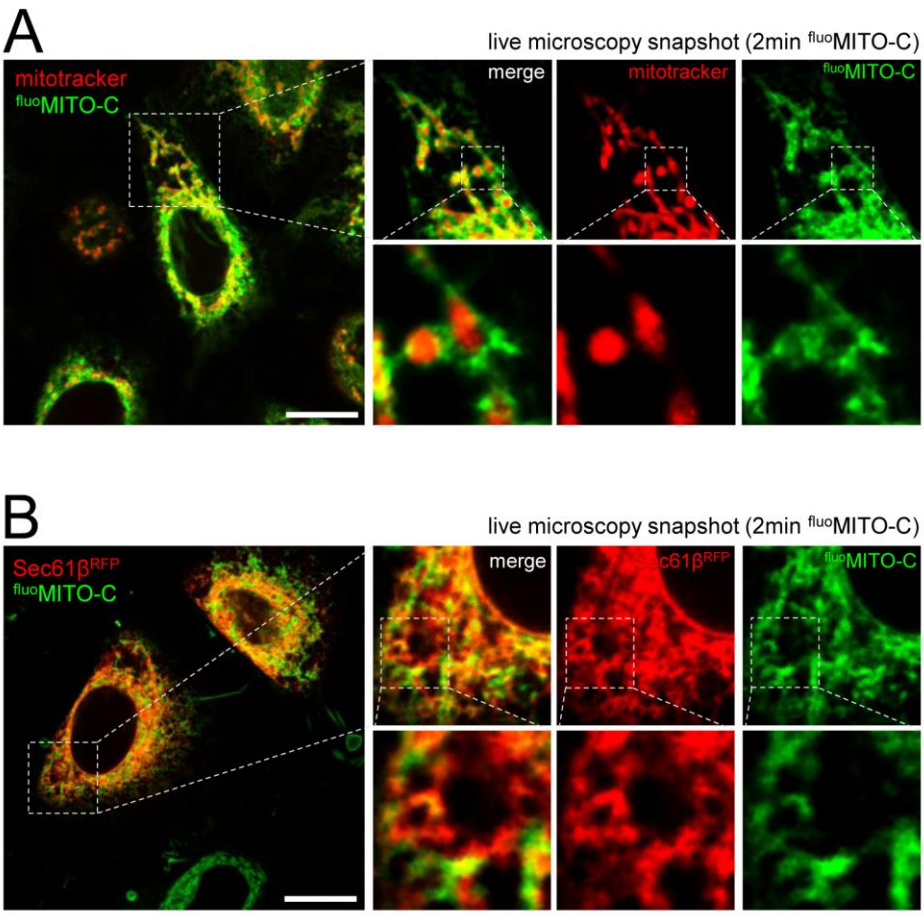


Appendix Figure S3: NAF-1 localizes to the ER-MT contact sites

(A): HeLa cells were transfected with Sec61βRFP (ER marker, red) and NAF-1-GFP (green), fixed and analyzed by confocal microscopy.

(B): HeLa cells were transfected with NAF-1-GFP (green), fixed, immunostained with TOM20 antibody (red) and analyzed by confocal microscopy.

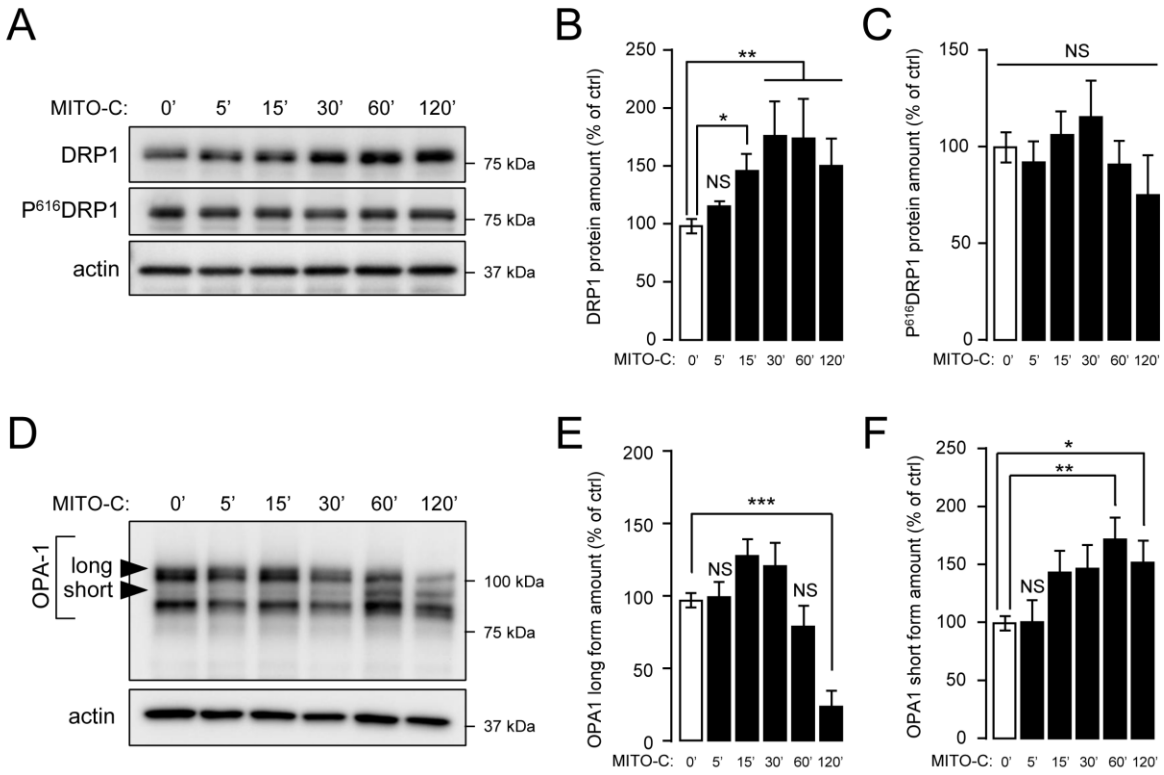
(C): HeLa cells were transfected with Sec61βRFP (ER marker, red), and NAF-1GFP (green), fixed, immunostained for PTPIP51 (ER-MT contact sites marker, blue) and analyzed by confocal microscopy. Scale bars = 10 µm.



Appendix Figure S4: Mito-C localizes to both mitochondria and Endoplasmic Reticulum

(A): HeLa cells were treated with ^{fluo}Mito-C (green), incubated with Mitotracker Redox (red) and analyzed by live microscopy.

(B): HeLa cells were transfected with Sec61βRFP (as ER marker), treated with ^{fluo}Mito-C (green), and analyzed by live microscopy Scale bar are 10 µm.



Appendix Figure S5: Expression of DRP1 and OPA1 during Mito-C treatment

(A): Western blot analysis of DRP1 and ^{phospho616}DRP1 expression in total extracts from cells treated with 2µM Mito-C for the times indicated.

(B), (C): Quantifications by Western blot with anti-DRP1 and anti-^{phospho616}DRP1 (n=5).

(D): Western blot analysis of OPA1 expression in total extracts from cells treated with 2µM Mito-C during time course indicated; long and short isoforms are indicated.

(E) (F): Quantification of Western blot for long and short isoforms of OPA1 shown in D.

Data information: to evaluate significance of differences observed in B, C, E and F we used a one-way Anova followed by Dunn's post-test in A and Bonferroni's post-test in C, E and F (ns for non-significant *p<0.01, (**p<0.001***p<0.0001).