

108

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Human-DNM1L-O00429 QRIIQHCSNYSTOELLRFPKLHDAIVEVVT 470
Rat-DNM1L-O35303 QRIIQHCSNYSTOELLRFPKLHDAIVEVVT 483
Mouse-DNM1L-Q8K1M6 QRIIQHCSNYSTOELLRFPKLHDAIVEVVT 476
Chick-DNM1L-E1BV15 INTVRQCT---KKLSQYPHLREEMERIVTT 462
Darne-DNM1L-E9QF63 VNTVRQCT---KKLQYPHLREEMERIVTQ 462
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109

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Human-DNM1L-O00429 LLDVPVPVARKLSAREORDCEVIERLI 651
Rat-DNM1L-O35303 LLDVPVPVARKLSAREORDCEVIERLI 670
Mouse-DNM1L-Q8K1M6 LLDVPVPVARKLSAREORDCEVIERLI 657
Chick-DNM1L-E1BV15 -ENGSDSFMHSMDPQLERQVETIRNLV 666
Darne-DNM1L-E9QF63 DESSSDGMHSMDPQLERQVETIRNLV 667
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110

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Human-DNM1L-O00429 SVLESVGRDLPRGTGIVTRRPLILQLVH 69
Rat-DNM1L-O35303 SVLESVGRDLPRGTGVVTRRPLILQLVH 69
Mouse-DNM1L-Q8K1M6 SVLESVGRDLPRGTGVVTRRPLILQLVH 69
Chick-DNM1L-E1BV15 SVLENFVGRDLPRGSGIVTRRPLVLQLVN 76
Darne-DNM1L-E9QF63 SVLENFVGRDLPRGSGIVTRRPLVLQLIN 76
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111

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Human-FIS1-Q9Y3D6 MEAVLNELVSVEDLLKFEKKFQSEKAAGSV 30
Rat-FIS1-P84817 MEAVLNELVSVEDLKNFERKFQSEQAAGSV 30
Mouse-FIS1-Q9CQ92 MEAVLNELVSVEDLKNFERKFQSEQAAGSV 30
Darne-FIS1-E9QGI1 MEAVVSDIVAPEDLKFEKKYNAELVKGPV 30
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112

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Human-FIS1-Q9Y3D6 IRKGIVLLELLPKGSKEEQRDYVFYLA 80
Rat-FIS1-P84817 IRRGIVLLELLPKGSKEEQRDYVFYLA 80
Mouse-FIS1-Q9CQ92 IRRGIVLLELLPKGSKEEQRDYVFYLA 80
Darne-FIS1-E9QGI1 IVKGIQLLEELVHTTSKDDQRDFLFYLA 80
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113

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Human-FIS1-Q9Y3D6 IRKGIVLLEELLPKGSKEEQRDYVFYLA 80
Rat-FIS1-P84817 IRRGIVLLEELLPKGSKEEQRDYVFYLA 80
Mouse-FIS1-Q9CQ92 IRRGIVLLEELLPKGSKEEQRDYVFYLA 80
Darne-FIS1-E9QGI1 IVKGIQLLEELVHTSKKDDQRDFLYLA 80
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Fig S1. Sequence conservation of Drp1/Fis1 peptides. Sequence alignment of all the peptides between Homo sapiens Drp1 (O00429), Rat (O35303), Mouse (Q8K1M6), Chicken (E1BV15) Zebrafish (E9QF63) and Yeast (P54861); and Fis1 Homo sapiens (Q9Y3D6), Rat (P84817), Mouse (Q9CQ92), Zebrafish (E9QGI1) and Yeast (P40515). Amino acids are represented by the one-letter code; star (*) indicate identical amino acids; two points (:) indicate high similarity between amino acids.

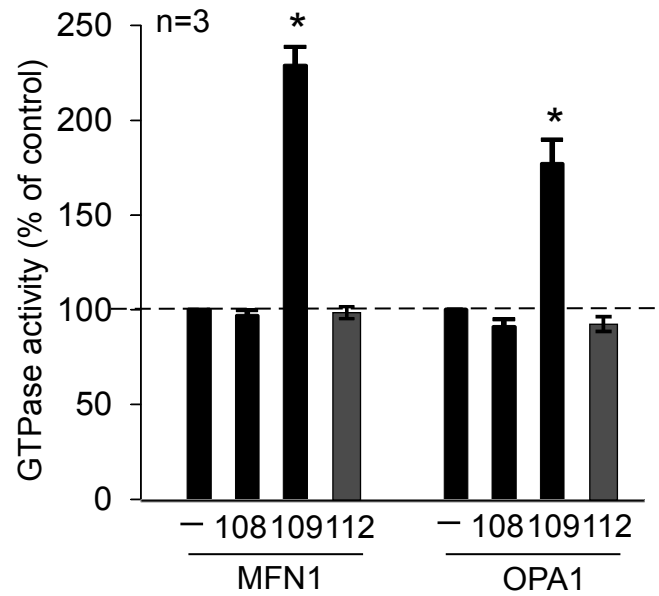


Fig S2. Drp1 P109 increases MFN1 and OPA1 GTPase activity. MFN1 (25ng) and OPA1 (25 ng) recombinant proteins were incubated with peptide 108, 109 or 112. GTPase activity of the proteins was determined. The data are expressed as mean \pm SE of three independent experiments. *, $p < 0.05$ vs. MFN1 or OPA1 protein alone.

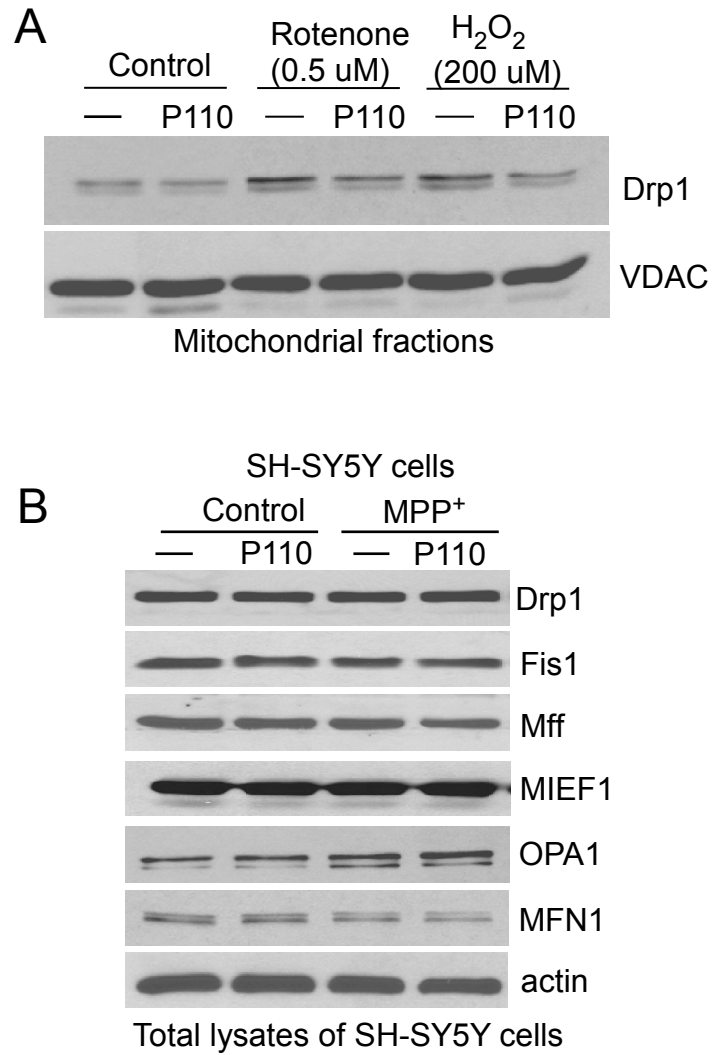


Fig S3. P110 blocked Drp1 translocation to the mitochondria in response to H₂O₂ or Rotenone. (A) SH-SY5Y cells were treated with P110 (1 μ M) followed by exposure to Rotenone (0.5 μ M, 1 hour) and H₂O₂ (200 μ M, 2 hours). Mitochondria were isolated and the mitochondrial level of Drp1 was determined by western blot. VDAC was used as a loading control. (B) Total lysates harvested from above cells and mitochondrial fusion/fission related proteins were determined by western blot.

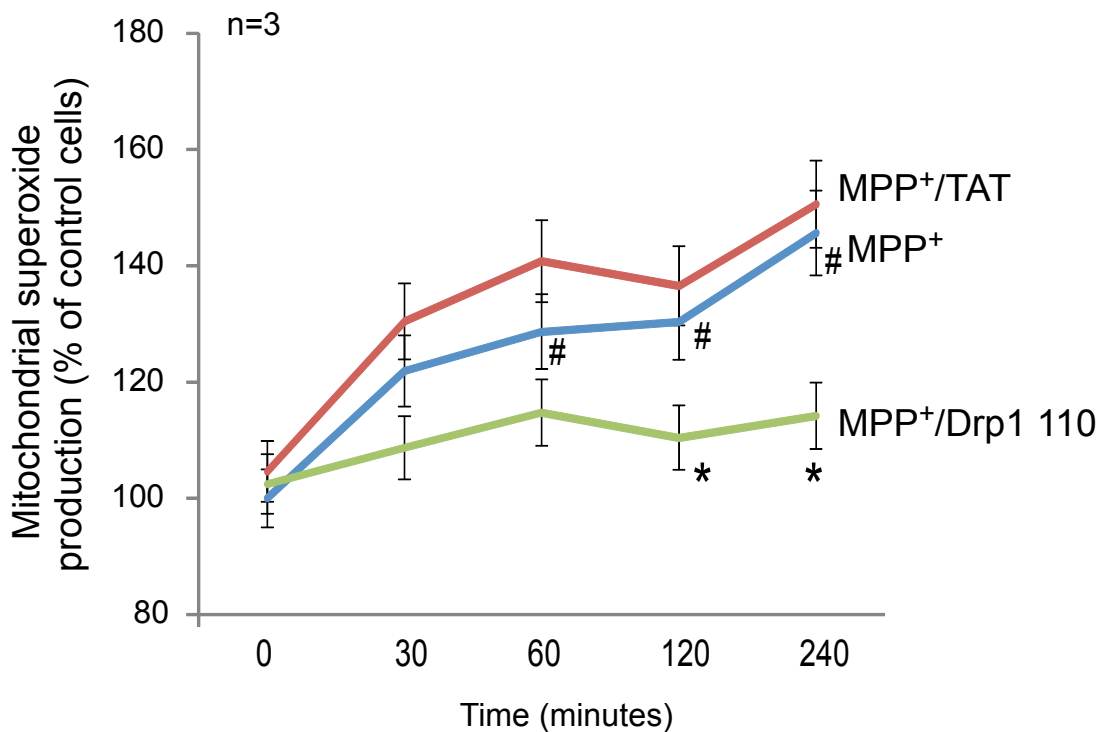


Fig S4. P110 reduced MPP⁺-induced mitochondrial ROS over time. SH-SY5Y cells were treated with P110 (1 μ M) or control peptide TAT (1 μ M) followed by incubation of MPP⁺ (2 mM) at the indicated time points. The detection of MitoSOXTM was performed using black 96-well plates in a fluorescence microplate reader at 510 nm excitation and 580 nm emission. All these measurements were normalized to the cell number counted using Hoechst staining. Data are expressed as mean \pm S.E. of three independent experiments. * p <0.05 vs. MPP⁺-treated cells; # p <0.05 vs. control cells.

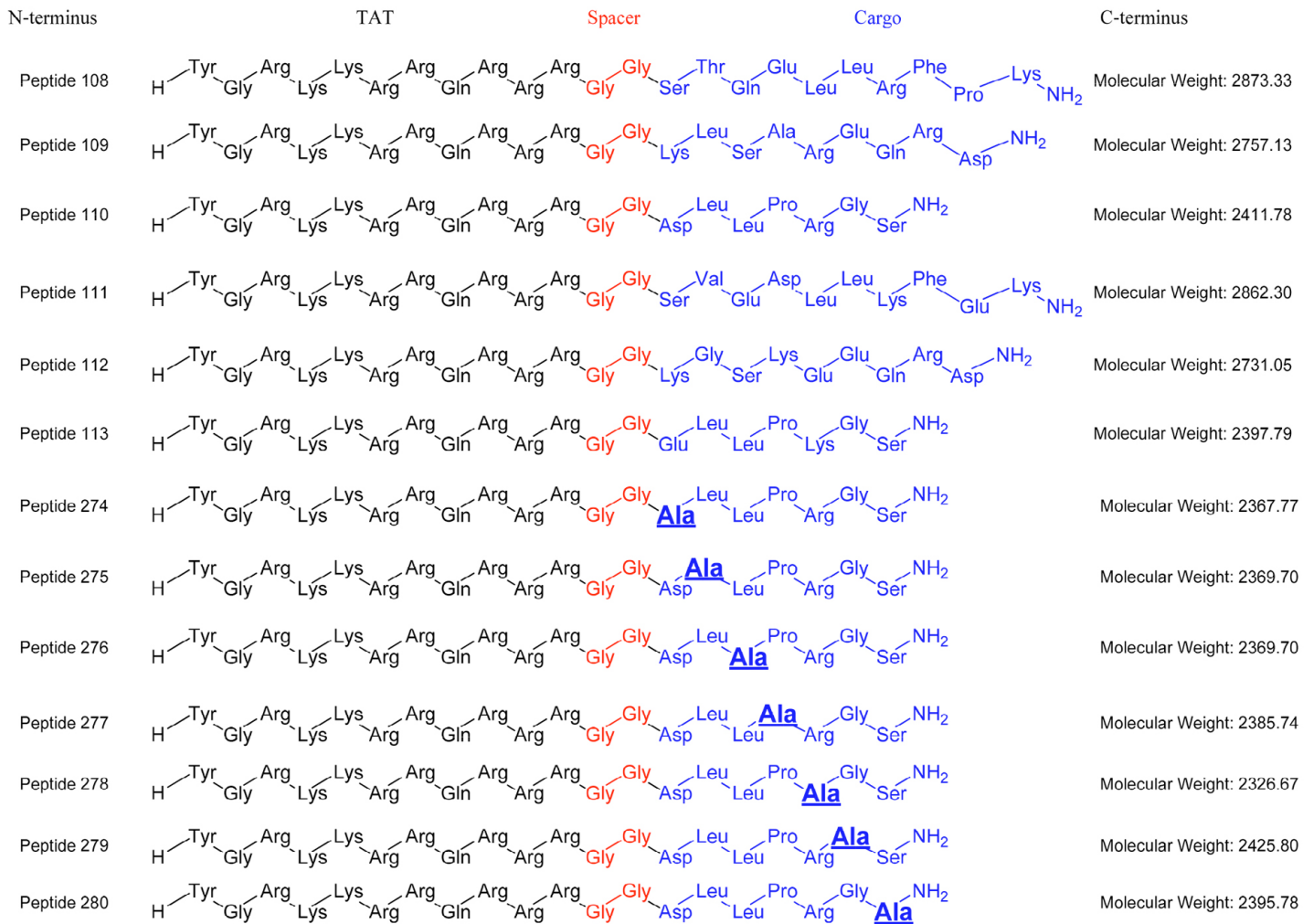


Fig S5: Chemical structure of all the peptides that were used in this study. All the peptides are present from the N-terminus to the C-terminus. TAT is on the N-terminus, spacer of two Gly amino acids after that and the cargo sequence which derived from the proteins on the C-terminus. Peptides 108-113 derived from Drp1 and Fis1 proteins. Peptides 274-280 are Ala scan of peptide 110. To peptide P110, two peptides were synthesized (one is DLLPRGT, the other is DLLPRGS). We used DLLPRGS in the current study.

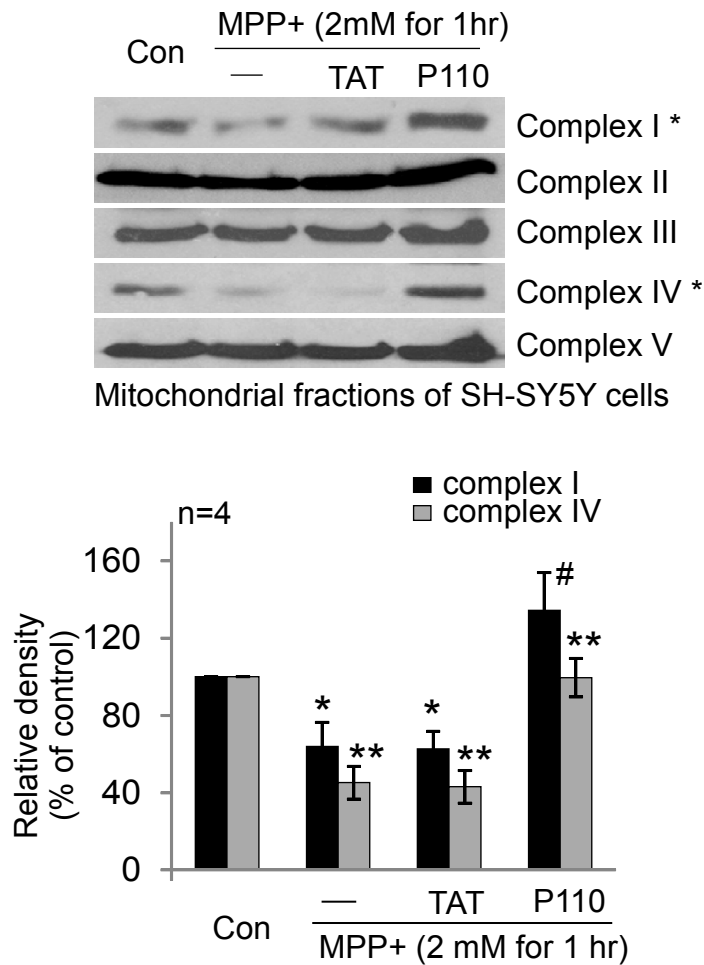


Fig S6. P110 reduced mitochondrial complex I and IV disassembly induced by MPP+. SHSY5Y cells were treated with P110 (1 μ M) or TAT (1 μ M) followed by the addition of MPP+ (2 mM, 1 hour). Mitochondria were isolated and mitochondrial complex I-V assembly was determined by a cocktail of antibodies of complex I-V (Mitosciences/Abcam, MitoProfile Total OXPHOS WB Antibody Cocktail). Upper: representative western blot; Lower histogram: the data are expressed as mean \pm S.E. of three independent experiments. The oxidative phosphorylation system in the mitochondria is responsible for generating ATP and consists of five major membrane protein complexes, the mitochondrial complexes I-V. MPP+ is a specific mitochondrial complex I inhibitor. Here, we investigated whether P110 has effects on MPP+-induced defects in mitochondrial complexes. In cultured SH-SY5Y cells, MPP+ treatment disassembled complex I and IV, as evidenced by the reduction of NDUFB8 (component of complex I) and MTCOI (component of complex IV). By contrast, treatment of P110 under the same conditions abolished the reduction of these two proteins, suggesting that P110 treatment recovered the MPP+-induced oxidative phosphorylation defect and mitochondrial integrity.