

## Supplementary Figure 1: Examples of neopeptides generated from frame-shift deletions in mtDNA.

**a. Deletion: 10969-14119; Neopeptide sequence: LLMLTLLIL (P14); Gene ND4**

L T T P L L M L T T L I L T L L L I T \*  
 CTA ACA ACC CCC CTC CTA ATA CTA ACT ACC CTC ATC CTA ACC CTA CTC CTA ATC ACA TAA  
 10969 14119

Deletion: 9180-14281; Neopeptide sequence: YVFTLLVPL (P9); Gene ATP6  
 M Q A Y V F T L L V P L S F M N  
 ATC CAA GCC TAG GTT TTC ACA CTT CTA GTA CCC CTC TCC TTC ATA AAT  
 9180 14281  
 Y S A S Y T I K V Y H N H H P I  
 TAT TCA GCT TCC TAC ACT ATT AAA GTT TAC CAC AAC CAC CAC CCC ATC  
 M L F H P Q H Q S Y L H R \*  
 ATA CTC TTT CAC CCA CAG CAC CAA TCC TAC CTC CAT CGC TAA

**b. Deletion : 8034-16076; Neopeptide sequence: TLLPATMNI (P5); Gene CO2**

N R V V L P I E A P I N N R Y V F R T L L P  
 AAT CGA GTA GTA CTC CCG ATT GAA GCC CCC A TC AAC AAC CGC TAT GTA TTT CGT ACA TTA CTG CCA  
 8034 16076

A T M N I V R Y H K Y L T T C S T \*  
 GCC ACC ATG AAT ATT GTA CGG TAC CAT AAA TAC TTG ACC ACC TGT AGT ACA TAA

**c. Deletion : 10952-15837; Neopeptide sequence: LLMPTISLI (P10); Gene ND4**

T F S S D P L T T P L L M P T I S L I E N K  
 ACC TTT TCC TCC GAC CCC CTA ACA ACC CCC CTC CTA ATA CCA ACT ATC TCC CTA ATT GAA AAC AAA  
 10952 15837

M L K W A C P C S M N \*  
 ATA CTC AAA TGG GCC TGT CCT TGT AGT ATA AAC TAA

**Deletion: 10961-15846; Neopeptide sequence: LLMPTISLI (P10); Gene: ND4**

S D P L T T P L L M P T I S L I E N K M L  
 TCC GAC CCC CTA ACA ACC CCC CTC CTA ATA C CA ACT ATC TCC CTA ATT GAA AAC AAA ATA CTC  
 10961 15846

K W A C P C S M N \*  
 AAA TGG GCC TGT CCT TGT AGT ATA AAC TAA

**Deletion: 10961-15846; Neopeptide sequence: PLLMPTISL (P11); Gene ND4**

S D P L T T P L L M P T I S L I E N K M L  
 TCC GAC CCC CTA ACA ACC CCC CTC CTA ATA C CA ACT ATC TCC CTA ATT GAA AAC AAA ATA CTC  
 10961 15846

K W A C P C S M N \*  
 AAA TGG GCC TGT CCT TGT AGT ATA AAC TAA

**Deletion : 10961-15837; Neopeptide sequence: PLLMPTISL (P11); Gene ND4**

T F S S D P L T T P L L M P T I S L I E N  
 ACC TTT TCC TCC GAC CCC CTA ACA ACC CCC C TC CTA ATA CCA ACT ATC TCC CTA ATT GAA AAC  
 10952 15837

K M L K W A C P C S M N \*  
 AAA ATA CTC AAA TGG GCC TGT CCT TGT AGT ATA AAC TAA

**d. Deletion : 10969-14119; Neopeptide sequence: MLTLLIL (P12); Gene ND4**

L T T P L L M L T T L I L T L L I T \*  
 CTA ACA ACC CCC CTC CTA ATA CTA ACT ACC CTC ATC CTA ACC CTA CTC CTA ATC ACA TAA  
 10969 14119

**Deletion : 10969-14119; Neopeptide sequence: LLMLTLLIL (P13); Gene ND4**

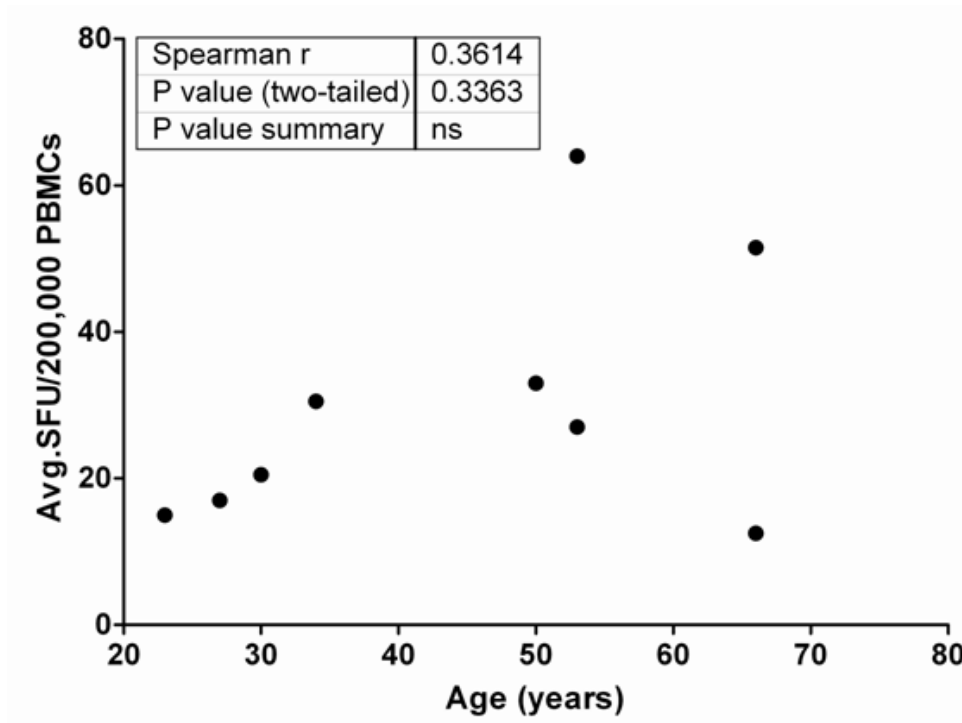
L T T P L L M L T T L I L T L L L I T \*  
 CTA ACA ACC CCC CTC CTA ATA CTA ACT ACC CTC ATC CTA ACC CTA CTC CTA ATC ACA TAA  
 10969 14119

**Deletion : 10969-14119; Neopeptide sequence: TLILTLLI (P14); Gene ND4**

L T T P L L M L T T L I L T L L I T \*  
 CTA ACA ACC CCC CTC CTA ATA CTA ACT ACC CTC ATC CTA ACC CTA CTC CTA ATC ACA TAA  
 10969 14119

**(a)** Example of candidate neopeptides including the residue(s) encoded by the deletion breakpoints. Frame-shift deletion that generated neopeptide P9 that is extensively investigated in current study is highlighted in the box. **(b)** Example of candidate neopeptide encoded by fused DNA downstream of the 3' breakpoint **(c)** Example of candidate neopeptides resulting from more than 1 deletion **(d)** Example of generating multiple neopeptides from same deletion. Neopeptides are underlined; \*denotes termination codon.

**Supplementary Figure 2.** Age versus frequency of IFN- $\gamma$  responses to neopeptide P9.



Included in analysis are HLA-A\*02 positive and negative healthy human donors whose PBMCs displayed more than 10 SFU/200,000 PBMCs in IFN- $\gamma$  ELISPOT<sup>PRO</sup> assay (details on ELISPOT<sup>PRO</sup> assay in Materials and Methods).

**Supplementary Table I.** Full details on frame-shift deletions<sup>a</sup> in mitochondrial DNA that generated neopeptides (P1-P18).

<b>Neopeptide</b>	<b>Sequence</b>	<b>Number of patients with deletion/total number of patients studied</b>
P1	LLLSFFFPL	1/1
P2	SLRILYMTL	1/28
P3	YMLPPLPKT	1/1
P4	SLLNDINTI	1/1
P5	TLLPATMNI	1/1
P6	LLIPNPPYI	1/5
P7	LLIPTMHIL	1/24
P8	PLLIPTSKL	1/2
P9	YVFTLLV PL	1/24
P10	LLMPTISLI	1/4
P11	PLLMPTISL	1/6
P12	MLTTLILTL	1/1
P13	TLILTLLLI	1/1
P14	LLMLTTLIL	1/1
P15	SLPLLLLDL	1/1;1/5
P16	YTMAFLPSL	1/24
P17	SLNPWPPCL	5/23
P18	ILLLSLNPV	1/21; 1/10

<sup>a</sup> References on deletions are available in Mitomap.org.

**Supplementary Table II:** Age, gender, and the results of MHC typing of twelve healthy human donors volunteers <sup>a</sup>.

<b>Subjects</b>	<b>Age</b>	<b>Sex</b>	<b>HLA class -I (HLA-A)</b>	<b>HLA class -II (HLA-DRB)</b>
OA1	66	Female	HLA-A*02	HLA-DRB1*15; HLA-DRB5*51
OA2	66	Male	HLA-A*02	HLA-DRB1*04; HLA-DRB3*52; HLA-DRB4*53
OA3	60	Female	HLA-A*02	HLA-DRB1*03
OA4	53	Female	HLA-A*02	HLA-DRB1*07
YA1	25	Male	HLA-A*02	HLA-DRB1*07; HLA-DRB3*52
YA2	22	Male	HLA-A*02; HLA-A*68	HLA-DRB1*13; HLA-DRB1*14; HLA-DRB3*52
YA3	23	Female	HLA-A*02; HLA-A*24	HLA-DRB1*15; HLA-DRB1*04; HLA-DRB4*53; HLA-DRB5*51
YA4	25	Male	HLA-A*02	HLA-DRB1*07; HLA-DRB3*52
NA1	34	Male	HLA-A*32	HLA-DRB1*07; HLA-DRB4*53
NA2	53	Male	HLA-A*01; HLA-A*31	HLA-DRB1*07; HLA-DRB1*13; HLA-DRB4*53
NA3	51	Female	HLA-A*30	HLA-DRB1*17; HLA-DRB1*07; HLA-DRB3*52; HLA-DRB4*53
NA4	30	Female	HLA-A*68	HLA-DRB1*08; HLA-DRB1*15; HLA-DRB5*51

<sup>a</sup>OA1: HLA-A\*02-positive old healthy human donors; YA: HLA-A\*02-positive young healthy human donors; NA: HLA-A\*02-negative healthy human donors. The HLA-type was determined using Olerup Sequence Specific Primer PCR HLA-A and HLA-DR low resolution kits (Details in Materials and Methods).