Additional File 1: Supplementary Information

Mitochondrial DNA and Inflammatory Proteins are Higher in Extracellular Vesicles from Frail Individuals

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Supplementary Table 1. Primer sequences for circulating cell-free mitochondrial DNA qPCR

Gene	Primer Name	Forward Sequence / Reverse Sequence	Probe	Size (bp)	Ref.
MT-RNR2/MT-TL1	Mito_3164	5'CCTTCCCCCGTAAATGATATCA3' / 5'GCCATCTTAACAAACCCTGTTCTT3'	5'FAM-AACTTAGTATTATACCCACACCC-MGB3'	76	(1)
MT-ND2	Mito_4625	5'CACAGAAGCTGCCATCAAGTA3' / 5'CCGGAGAGTATATTGTTGAAGAG3'	5'FAM-CCTCACGCAAGCAACCGCATCC-BLACKHOLE-3'	89	(2)
MT-COX2	Mito_7878	5'AATCAATTGGCGACCAATGG3' / 5'CGCCTGGTTCTAGGAATAATGG3'	5'FAM-ACTGAACCTACGAGTACAC-MGB-3'	100	(3)
MT-ATP8	Mito_8446	5'AATATTAAACACAAACTACCACCTACCT3' / 5'TGGTTCTCAGGGTTTGTTATAA3'	5'-FAM-CCTCACCAAAGCCCATA-MGB-3'	79	(4)

Supplementary Table 2. Inflammatory proteins detected in plasma EVs

Protein Symbol	Protein Name	Protein function	Ref
CCL28	C-C motif chemokine ligand 28	chemokine; antimicrobial activity; immune responses	(5)
CD5	T-cell surface glycoprotein CD5	scavenger receptor; immunomodulatory function, pattern recognition receptors	(6)
CD8A	T-cell surface glycoprotein CD8 alpha chain	cell surface receptor on cytotoxic T cells; mediates immune cell-cell interactions	(7)
CD40	Cluster of differentiation 40	co-stimulatory receptor on antigen-presenting cells	(8)
CD244	Natural killer cell receptor 2B4	receptor involved in immune regulation; cytotoxicity; cytokine production	(9)
CXCL1	C-X-C motif chemokine ligand 1	chemokine; angiogenesis, inflammatory responses	(10)
CXCL5	C-X-C motif chemokine ligand 5	chemokine; promotes angiogenesis and tumorigenesis, remodels connective tissue	(11)
CXCL6	C-X-C motif chemokine ligand 6	chemokine; recruits neutrophils for anti-microbial actions	(12)
CXCL11	C-X-C motif chemokine ligand 11	proinflammatory chemokines; inflammation and response to infection	(13)
LAP-TGF-β-1	Latency-assoc. peptide transforming growth factor β-1	binds and maintains TGFβ latency	(14)
MCP-4	Monocyte chemotactic protein 4	chemokine; role in chronic inflammatory diseases	(15)
MMP-1	Matrix metalloproteinase-1	breaks down extracellular matrix proteins in physiological and pathological processes	(16)
uPA	Urokinase-type plasminogen activator	serine protease that converts inactive plasminogen to plasmin	(17)
VEGFA	Vascular endothelial growth factor A	mitogen that acts on endothelial cells and promotes vasculogenesis, angiogenesis	(18)

Inflammatory proteins detected in EVs are listed. These 14 proteins met our threshold for being present in more than 65% of all the EV samples. General functions of proteins are listed, not necessarily functions attributed to these proteins in EVs. Note: MCP-4 is also known as CCL23 and CD244 is also known as SLAMF4. assoc. = associated

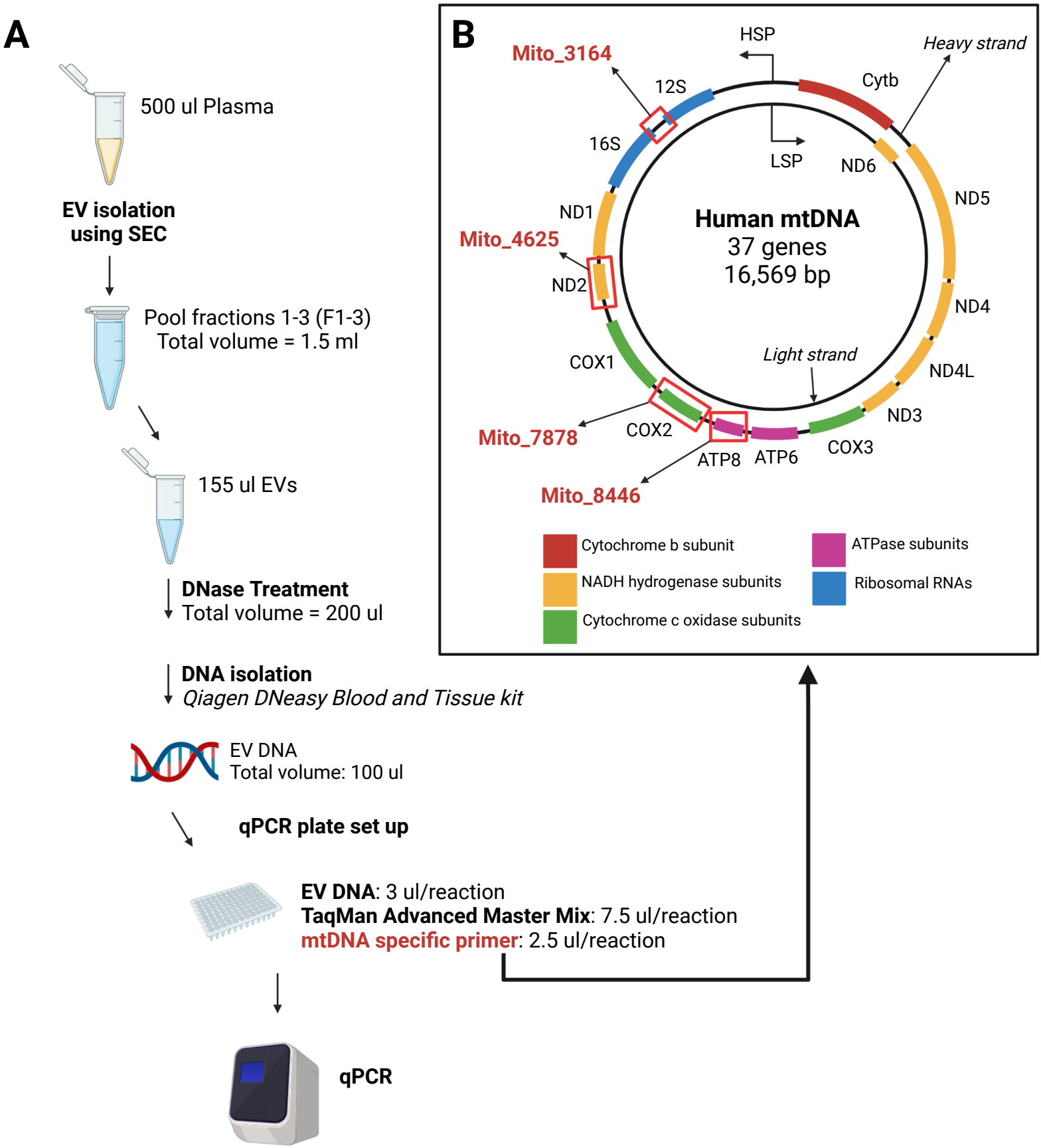
Supplementary Table 3. Significant interactions of EV inflammatory proteins

Protein	Sex × Poverty	Frailty × Sex	Frailty × Race	Frailty × Race × Poverty
CCL28		*		
CD5	*		**	
CD8A	*		*	
CD40				
CD244			**	
CXCL1	**	*	**	
CXCL5				
CXCL6[+]			*	
CXCL11			*	
LAP-TGF-beta-1	*		**	
uPA	**			*
MCP-4			*	
MMP-1				
VEGFA				

Significant interactions are indicated for the inflammatory proteins detected in EVs. These significant interactions were determined by linear regression analysis modeled to the study design of frailty, sex, race, and poverty status. *P<0.05; **P<0.01

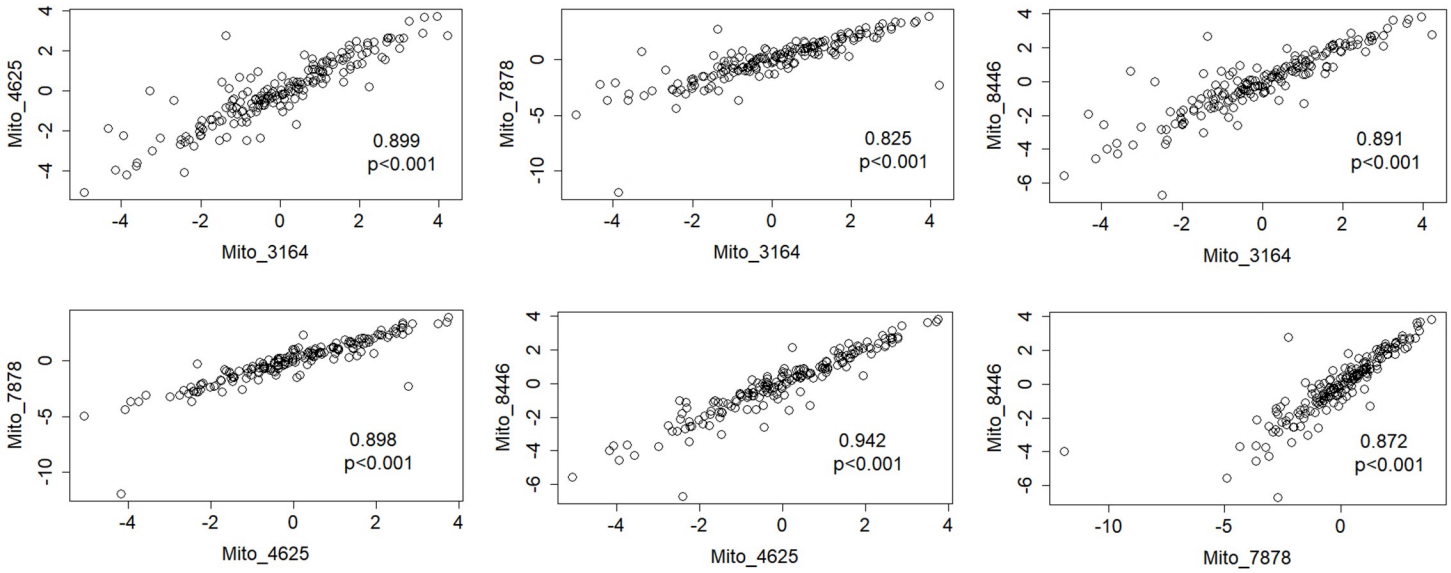
[+] CXCL6 also had a significant poverty status effect.

Supplementary Figure 1



Supplementary Figure 1. Schematic workflow and primer design for quantifying EV mtDNA levels. (A) Schematic of experimental workflow for quantifying mitochondrial DNA from size exclusion chromography (SEC) isolated extracellular vesicles (EVs). **(B)** Mitochondrial genome with mtDNA primer regions indicated by a red box and primer name denoted by starting nucleotide. qPCR=quantitative real-time PCR

Supplementary Figure 2



Supplementary Figure 2. Positive correlation between EV mtDNA levels. Plasma EVs were isolated from participants in the frailty cohort (Table 1). DNA was isolated from EVs and mtDNA levels were measured using mtDNA specific primers (Supplementary Table 1), targeting four regions of the mitochondrial genome. The relationship between EV mtDNA levels (log2 transformed) were analyzed by Pearson correlation. r values and p values are indicated.

Supplementary Information References

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