SUPPLEMENTAL MATERIAL

Table S1. mtDNA variants observed in participants with muscle biopsy (n = 44).

[Please see excel spreadsheet.]

Table S2. Coefficients from mixed effect models estimating the difference in heteroplasmy rate (heteroplasmy count per base) across mtDNA regions among study participants (n = 42) adjusted for age, sex, PAD and Diabetes status. The same model is estimated in each column with a different mtDNA region used as the reference region and indicated in the top row.

Coefficient	D-loop β (se)	p	rRNA β (se)	p	ND 1-6 β (se)	p	COX 1-3 β (se)	p
(Intercept)	**1.01×10^{-2} (6.19×10^{-4})**	**<.001**	2.38×10^{-4} (6.19×10^{-4})	0.702	5.92×10^{-4} (6.19×10^{-4})	0.342	-8.69×10^{-5} (6.19×10^{-4})	0.889
D-loop			**9.83×10^{-3} (5.01×10^{-4})**	**<.001**	**9.48×10^{-3} (5.01×10^{-4})**	**<.001**	**1.02×10^{-2} (5.01×10^{-4})**	**<.001**
rRNA	**-9.83×10^{-3} (5.01×10^{-4})**	**<.001**			-3.55×10^{-4} (5.01×10^{-4})	0.480	3.25×10^{-4} (5.01×10^{-4})	0.518
ND 1-6	**-9.48×10^{-3} (5.01×10^{-4})**	**<.001**	3.55×10^{-4} (5.01×10^{-4})	0.480			6.79×10^{-4} (5.01×10^{-4})	0.176
COX 1-3	**-1.02×10^{-2} (5.01×10^{-4})**	**<.001**	-3.25×10^{-4} (5.01×10^{-4})	0.518	-6.79×10^{-4} (5.01×10^{-4})	0.176		
ATP 8/6	**-1.00×10^{-2} (5.01×10^{-4})**	**<.001**	-2.05×10^{-4} (5.01×10^{-4})	0.683	-5.60×10^{-4} (5.01×10^{-4})	0.265	1.19×10^{-4} (5.01×10^{-4})	0.812
Cyt b	**-7.39×10^{-3} (5.01×10^{-4})**	**<.001**	**2.45×10^{-3} (5.01×10^{-4})**	**<.001**	**2.09×10^{-3} (5.01×10^{-4})**	**<.001**	**2.77×10^{-3} (5.01×10^{-4})**	**<.001**
tRNA	**-9.83×10^{-3} (5.01×10^{-4})**	**<.001**	3.53×10^{-7} (5.01×10^{-4})	0.999	-3.54×10^{-4} (5.01×10^{-4})	0.480	3.25×10^{-4} (5.01×10^{-4})	0.517
Age*	-9.41×10^{-5} (3.77×10^{-4})	0.804	-9.41×10^{-5} (3.77×10^{-4})	0.804	-9.41×10^{-5} (3.77×10^{-4})	0.804	-9.41×10^{-5} (3.77×10^{-4})	0.804
Male	-2.14×10^{-4} (5.21×10^{-4})	0.683	-2.14×10^{-4} (5.21×10^{-4})	0.683	-2.14×10^{-4} (5.21×10^{-4})	0.683	-2.14×10^{-4} (5.21×10^{-4})	0.683
PAD	**2.13×10^{-3} (6.37×10^{-4})**	**0.002**	**2.13×10^{-3} (6.37×10^{-4})**	**0.002**	**2.13×10^{-3} (6.37×10^{-4})**	**0.002**	**2.13×10^{-3} (6.37×10^{-4})**	**0.002**
Diabetes	-9.31×10^{-4} (4.91×10^{-4})	0.065	-9.31×10^{-4} (4.91×10^{-4})	0.065	-9.31×10^{-4} (4.91×10^{-4})	0.065	-9.31×10^{-4} (4.91×10^{-4})	0.065

Coefficient	ATP8/6 β (se)	p	Cytb β (se)	p	tRNA β (se)	p
(Intercept)	3.26×10^{-5} (6.19×10^{-4})	0.958	**2.68×10^{-3} (6.19×10^{-4})**	**<.001**	2.38×10^{-4} (6.19×10^{-4})	0.702
D-loop	**1.00×10^{-2} (5.01×10^{-4})**	**<.001**	**7.39×10^{-3} (5.01×10^{-4})**	**<.001**	**9.83×10^{-3} (5.01×10^{-4})**	**<.001**
rRNA	2.05×10^{-4} (5.01×10^{-4})	0.683	**-2.45×10^{-3} (5.01×10^{-4})**	**<.001**	-3.53×10^{-7} (5.01×10^{-4})	0.999
ND 1-6	5.60×10^{-4} (5.01×10^{-4})	0.265	**-2.09×10^{-3} (5.01×10^{-4})**	**<.001**	3.54×10^{-4} (5.01×10^{-4})	0.480
COX 1-3	-1.19×10^{-4} (5.01×10^{-4})	0.812	**-2.77×10^{-3} (5.01×10^{-4})**	**<.001**	-3.25×10^{-4} (5.01×10^{-4})	0.517
ATP 8/6			**-2.65×10^{-3} (5.01×10^{-4})**	**<.001**	-2.05×10^{-4} (5.01×10^{-4})	0.682
Cyt b	**2.65×10^{-3} (5.01×10^{-4})**	**<.001**			**2.45×10^{-3} (5.01×10^{-4})**	**<.001**
tRNA	2.05×10^{-4} (5.01×10^{-4})	0.682	**-2.45×10^{-3} (5.01×10^{-4})**	**<.001**		
Age*	-9.41×10^{-5} (3.77×10^{-4})	0.804	-9.41×10^{-5} (3.77×10^{-4})	0.804	-9.41×10^{-5} (3.77×10^{-4})	0.804
Male	-2.14×10^{-4} (5.21×10^{-4})	0.683	-2.14×10^{-4} (5.21×10^{-4})	0.683	-2.14×10^{-4} (5.21×10^{-4})	0.683
PAD	**2.13×10^{-3} (6.37×10^{-4})**	**0.002**	**2.13×10^{-3} (6.37×10^{-4})**	**0.002**	**2.13×10^{-3} (6.37×10^{-4})**	**0.002**
Diabetes	-9.31×10^{-4} (4.91×10^{-4})	0.065	-9.31×10^{-4} (4.91×10^{-4})	0.065	-9.31×10^{-4} (4.91×10^{-4})	0.065

Table S3. Coefficients from multivariable linear regression models estimating the association between the natural log of mtDNA heteroplasmy count and PAD status by mtDNA region and frequency after exclusion of participants with non-diabetes comorbidities* (n = 30).

Coefficient	All		Microheteroplasmy		All High frequency heteroplasmy		D-loop		Gene region	
	β (se)	p	β (se)	p	β (se)	p	β (se)	p	β (se)	p
(Intercept)	2.89 (0.26)	<.001	2.77 (0.26)	<.001	0.33 (0.56)	0.570	2.04 (0.30)	<.001	2.29 (0.30)	<.001
PAD	**0.70 (0.25)**	**0.010**	**0.68 (0.26)**	**0.013**	-0.35 (0.55)	0.533	0.55 (0.29)	0.067	**0.79 (0.29)**	**0.012**
Age†	0.01 (0.16)	0.974	0.04 (0.16)	0.802	-0.44 (0.35)	0.230	0.02 (0.19)	0.931	0.04 (0.19)	0.831
Male	0.08 (0.21)	0.699	0.07 (0.21)	0.750	0.11 (0.45)	0.807	-0.17 (0.24)	0.476	0.22 (0.24)	0.362
Black	0.14 (0.21)	0.510	0.04 (0.21)	0.869	0.88 (0.46)	0.066	0.10 (0.24)	0.670	0.18 (0.24)	0.460
Diabetes	-0.29 (0.21)	0.183	-0.25 (0.22)	0.263	0.02 (0.47)	0.969	-0.31 (0.24)	0.218	-0.36 (0.25)	0.159

* Angina, myocardial infarction, heart failure or pulmonary disease.

†Age centered at 70 years and scaled to 10 years.

Table S4. Coefficients from multivariable linear regression models estimating the association between heteroplasmy count or copy number and ABI as well as measures of walking performance among participants with PAD (n = 33).

	ABI		Normal 4-meter walk speed		Rapid 4-meter walk speed		6-minute walk distance	
Coefficient	β (se)	p	β (se)	p	β (se)	p	β (se)	p
(Intercept)	0.76 (0.13)	<.001	0.90 (0.11)	<.001	1.23 (0.15)	<.001	1298 (198)	<.001
Heteroplasmy*	-0.01 (0.02)	0.500	-0.01 (0.01)	0.521	-0.02 (0.02)	0.184	1 (23)	0.974
Diabetes	-0.29 (0.14)	0.042	-0.09 (0.11)	0.451	-0.22 (0.16)	0.184	-241 (209)	0.262
Heteroplasmy* × Diabetes	0.08 (0.03)	0.014	0.02 (0.03)	0.456	0.04 (0.04)	0.315	72 (48)	0.151
Age†	0.06 (0.05)	0.247	-0.08 (0.04)	0.055	-0.01 (0.06)	0.936	-19 (74)	0.804
Male	-0.002 (0.07)	0.978	-0.04 (0.05)	0.461	-0.06 (0.08)	0.441	-64 (102)	0.536
Black	0.01 (0.06)	0.861	-0.07 (0.05)	0.160	-0.21 (0.07)	0.009	-124 (96)	0.210
BMI†	0.003 (0.01)	0.655	-0.01 (0.01)	0.189	-0.003 (0.01)	0.734	-12 (11)	0.279
Former smoker	-0.09 (0.09)	0.319	0.08 (0.07)	0.257	0.25 (0.10)	0.022	27 (134)	0.844
Current smoker	-0.08 (0.10)	0.454	0.06 (0.08)	0.511	0.25 (0.12)	0.046	16 (155)	0.918
	β (se)	p	β (se)	p	β (se)	p	β (se)	p
(Intercept)	0.60 (0.12)	<.001	0.85 (0.09)	<.001	1.08 (0.13)	<.001	1173 (171)	<.001
Copy number*	0.01 (0.01)	0.191	-0.004 (0.01)	0.348	-0.01 (0.01)	0.188	-6 (9)	0.513
Diabetes	0.04 (0.06)	0.553	-0.01 (0.05)	0.772	-0.07 (0.06)	0.266	-1 (88)	0.989
Copy number* × Diabetes	-0.003 (0.01)	0.836	0.01 (0.01)	0.647	0.02 (0.02)	0.302	19 (20)	0.358
Age†	0.07 (0.05)	0.229	-0.08 (0.04)	0.063	0.003 (0.06)	0.962	-1 (78)	0.987
Male	0.03 (0.08)	0.723	-0.03 (0.06)	0.596	-0.03 (0.09)	0.723	3 (117)	0.981
Black	0.05 (0.07)	0.504	-0.07 (0.05)	0.149	-0.21 (0.07)	0.006	-76 (97)	0.441
BMI†	0.003 (0.01)	0.714	-0.01 (0.01)	0.240	-0.002 (0.01)	0.825	-10 (12)	0.407
Former smoker	-0.01 (0.09)	0.889	0.10 (0.07)	0.143	0.30 (0.10)	0.006	112 (134)	0.411
Current smoker	-0.07 (0.11)	0.536	0.06 (0.08)	0.474	0.27 (0.12)	0.035	32 (163)	0.848

* Heteroplasmy scaled to 10 and copy number centered at 3000 and scaled to 1000. MtDNA copy number is expressed as the ratio between mitochondrial DNA copy number and nuclear DNA copy number.

† Age centered at 70 years and scaled to 10 years, BMI centered at 30.

Table S5. Coefficients from multivariable linear regression models estimating the association between an indicator for high heteroplasmy count and/or high copy number and ABI as well as measures of walking performance among participants with PAD (n = 33).

Coefficient	ABI		Normal 4-meter walk speed		Rapid 4-meter walk speed		6-minute walk distance	
	β (se)	p	β (se)	p	β (se)	p	β (se)	p
(Intercept)	0.64 (0.15)	<.001	1.00 (0.09)	<.001	1.36 (0.13)	<.001	1470 (190)	<.001
mtDNA category*	-0.01 (0.10)	0.911	**-0.16 (0.06)**	**0.020**	**-0.28 (0.09)**	**0.004**	**-277 (129)**	**0.043**
Diabetes	0.01 (0.13)	0.939	-0.12 (0.08)	0.134	-0.28 (0.11)	0.016	-192 (160)	0.242
mtDNA category* × Diabetes	0.02 (0.15)	0.894	0.14 (0.09)	0.160	0.28 (0.13)	0.042	248 (190)	0.206
Age†	0.07 (0.06)	0.253	-0.07 (0.04)	0.055	0.01 (0.05)	0.905	1 (72)	0.984
Male	0.03 (0.08)	0.759	-0.04 (0.05)	0.385	-0.06 (0.07)	0.378	-49 (102)	0.637
Black	0.04 (0.07)	0.583	-0.09 (0.05)	0.059	-0.25 (0.06)	0.001	-115 (92)	0.225
BMI†	0.01 (0.01)	0.588	-0.01 (0.01)	0.153	-0.004 (0.01)	0.604	-10 (11)	0.345
Former smoker	-0.02 (0.10)	0.876	0.08 (0.06)	0.223	0.24 (0.09)	0.011	55 (126)	0.667
Current smoker	-0.07 (0.12)	0.557	0.07 (0.07)	0.335	0.29 (0.10)	0.011	45 (151)	0.766

* A two category variable where low heteroplasmy-low copy number is the reference group, and heteroplasmy count > 36 (median level) or copy number > 3041 (median level) is the alternate category.

† Age centered at 70 years and scaled to 10 years, BMI centered at 30.

Figure S1. Distribution of mtDNA copy number by (A) PAD status and (B) heteroplasmy count among participants with (n = 33) and without PAD (n = 9).
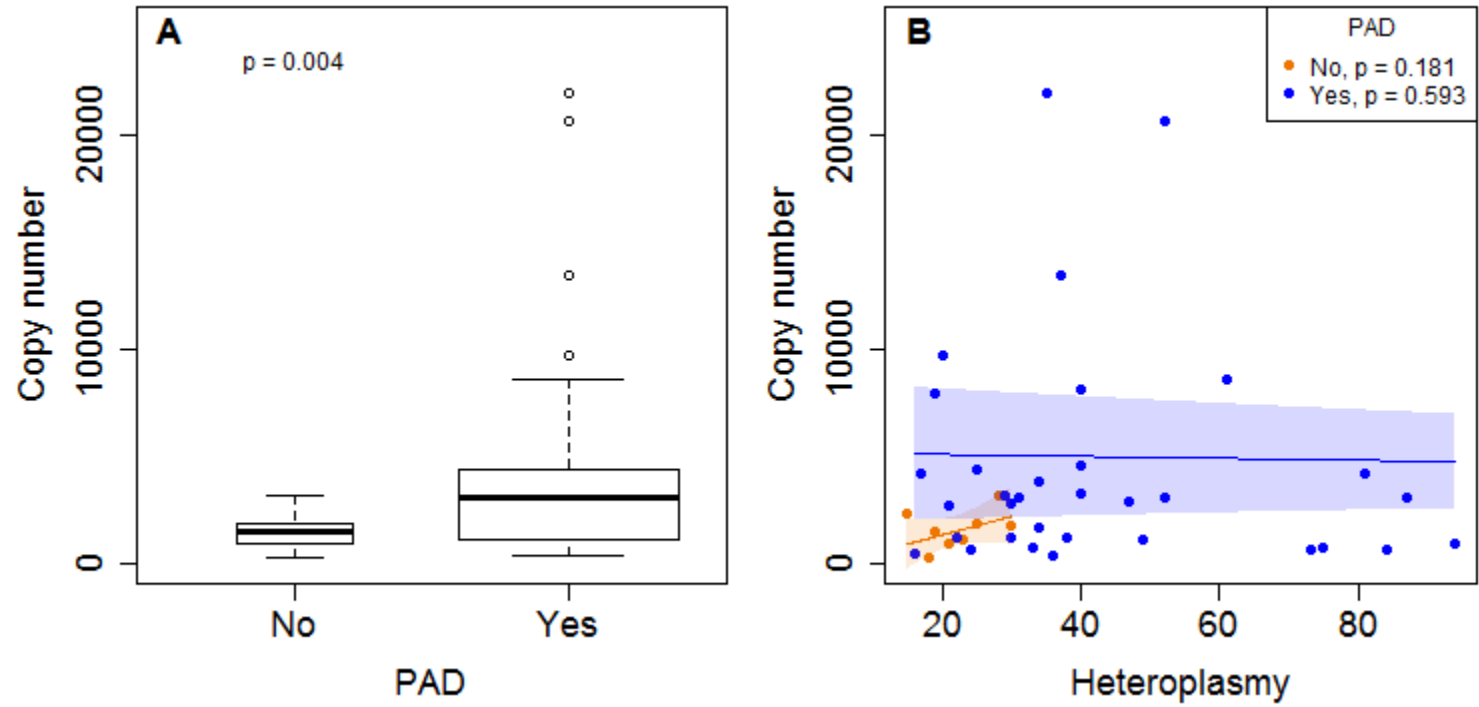
Figure S2. Scatterplots depicting relationships between mtDNA heteroplasmy and ABI, overall and according to mtDNA region in 33 participants with PAD.
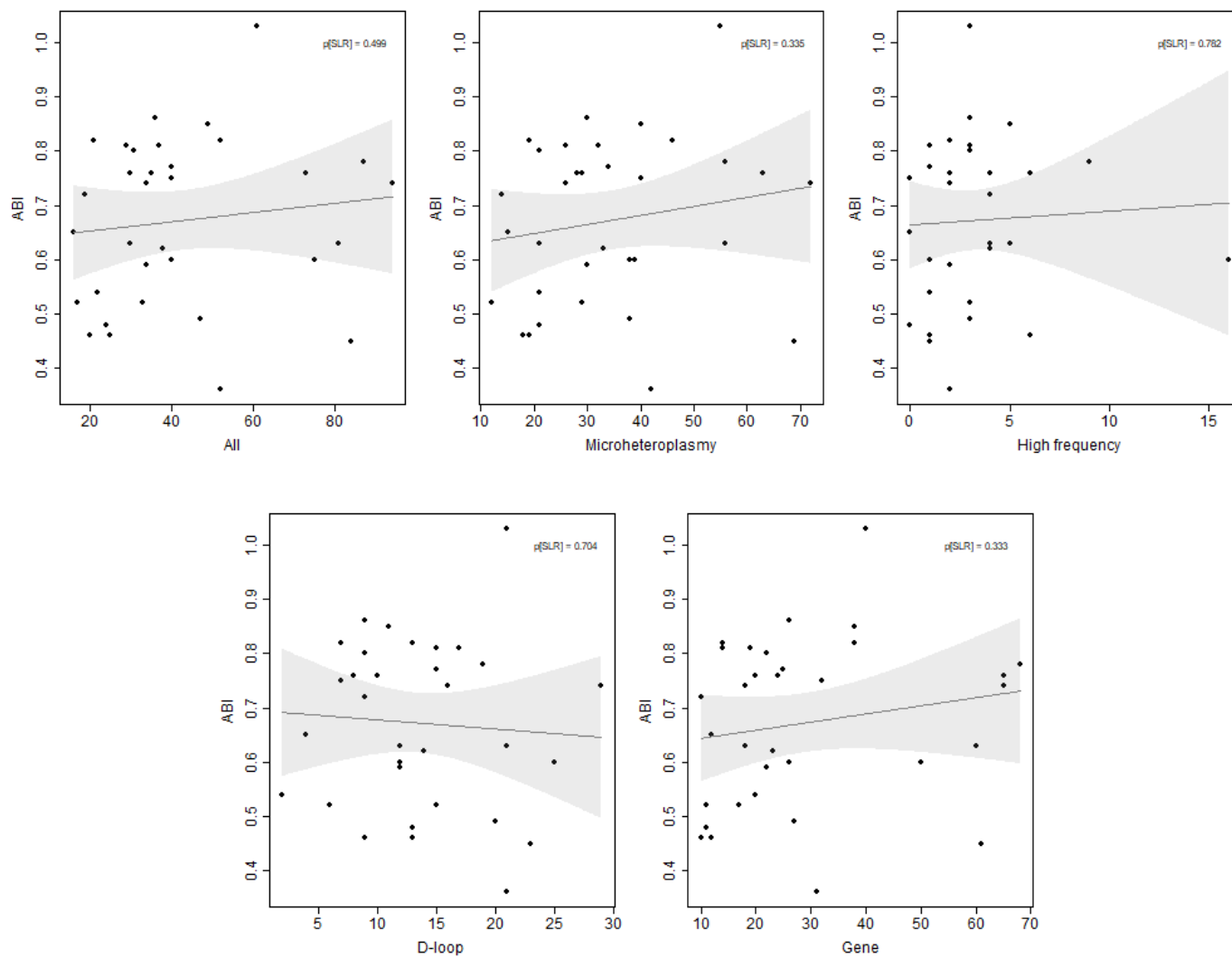
Figure S3. Normal 4-meter walking speed versus heteroplasmy count and mtDNA copy number among participants with PAD (n = 33).