Table S1. Sample characteristics of discovery and validation cohorts.

	DISCOVERY COHORTS		VALIDATION COHORTS					
	ARIC AA (N=1567)	ARIC EA (N=940)	CHS	CHS AA (N=239)	CHS EA (N=294)	FHS	FHS (N=1995)	
	mean (range)	*mean (range)*		*mean (range)*	*mean (range)*		*mean (range)*	
Age	57.2 (47 - 71)	60.2 (47 - 72)		72.4 (65 - 92)	72.3 (65 - 95)		61.4 (20-91)	
mtDNA-CN (SD units)	0.04 (-6.47 - 2.88)	0.04 (-4.41 - 2.87)		0.05 (-3.19 - 2.69)	0.05 (-2.28 - 2.76)		0.01 (-2.28 -8.11)	
Sex	*N (percentage)*	*N (percentage)*		*N (percentage)*	*N (percentage)*		*N (percentage)*	
Male	604 (38.5%)	381 (40.5%)		97 (41.8%)	112 (38.9%)		901 (45.16%)	
Female	963 (61.5%)	559 (59.5%)		135 (58.2%)	176 (61.1%)		1094 (54.64%)	
Collection Site			**Collection Site**			**Collection Site**		
Forsyth County, NC	188 (12%)	832 (88.5%)	Bowman Gray	74 (31.9%)	67 (23.3%)	Framingham Town	1995 (100%)	
Suburbs of Minneapolis, MN	0 (0%)	86 (9.2%)	Davis	71 (30.6%)	71 (24.7%)			
Jackson, MS	1379 (88%)	0 (0%)	Hopkins	0 (0%)	83 (28.8%)			
Washington County, MD	0 (0%)	22 (2.3%)	Pittsburgh	87 (37.5%)	67 (23.3%)			
Smoking Status								
Current Smoker	655 (41.8%)	181 (19.3%)		32 (13.8%)	26 (9.0%)		205 (10.4%)	
Former Smoker	484 (30.9%)	366 (38.9%)		89 (38.4%)	116 (40.3%)		947 (47.47%)	
Never Smoker	428 (27.3%)	392 (41.7%)		85 (36.6%)	133 (46.2%)		838 (42.0%)	
Unknown	0 (0.0%)	1 (0.1%)		26 (11.2%)	13 (4.5%)		5 (0.2%)	
Phenotypes (# of cases)								
Mortality	605 (38.6%)	224 (23.8%)		194 (83.6%)	263 (91.3%)		217 (10.87%)	
CVD Prevalent	154 (9.8%)	49 (5.2%)		N/A	N/A		94 (4.71%)	
Incident	296 (18.9%)	108 (11.5%)		83 (35.8%)	99 (34.4%)		94 (4.71%)	
CHD Prevalent	112 (7.1%)	40 (4.3%)		N/A	N/A		94 (4.71%)	
Incident	193 (12.3%)	83 (8.8%)		48 (20.7%)	57 (19.8%)		68 (3.41%)	
Cell Type Proportions	*mean (range)*	*mean (range)*		*mean (range)*	*mean (range)*		*mean (range)*	
CD8T Lymphocytes	0.15 (0.00 - 0.48)	0.10 (0.00 - 0.27)		0.09 (0.00 - 0.38)	0.06 (0.00 - 0.22)		0.10 (0.00-0.36)	
CD4T Lymphocytes	0.19 (0.00 - 0.52)	0.16 (0.00 - 0.44)		0.20 (0.00 - 0.48)	0.15 (0.00 - 0.52)		0.19 (0.02-0.44)	
B-cells	0.07 (0.00 - 0.58)	0.06 (0.00 - 0.56)		0.08 (0.00 - 0.26)	0.06 (0.00 - 0.76)		0.04 (0.00-0.52)	
Monocytes	0.13 (0.02 - 0.26)	0.09 (0.02 - 0.19)		0.10 (0.00 - 0.27)	0.09 (0.01 - 0.35)		0.12 (0.05-0.30)	
Granulocytes	0.45 (0.15 - 0.98)	0.55 (0.16 - 0.93)		0.44 (0.11 - 0.75)	0.57 (0.03 - 0.92)		0.49 (0.02-0.85)	
Natural Killer cells	N/A	0.07 (0.00 - 0.36)		0.12 (0.01 - 0.38)	0.09 (0.00 - 0.36)		0.02 (0.00-0.13)	

CVD: Cardiovascular disease. CHD: Coronary Heart Disease.

Table S6. Neuroactive-ligand receptor interaction genes as identified by KEGG analysis in each approach, number of genes used in each analysis and neuroactive ligand enrichment *P*-value are included in brackets.

ARIC Meta-Analysis (300 CpGs, *P*=5.24x10^{-12})	*TFAM* Methylation (300 CpGs, *P*=4.41x10^{-4})	*TFAM* Expression (169 genes, *P*=4.30x10^{-4})	*TFAM* Integrated (Methylation/Expression) (188 genes, *P*=8.77x10^{-6})
CHRM2	GABBR1	GABRB1	GABRB1
CHRM3	GRIN3B	MC2R	MC2R
CTSG	GABRA5	GH1	GH1
AGTR1	GABRB1	GABRA2	GABRA2
GABRG3	GABRG3	GABRG1	GABRG1
GHR	GABRB3	ADRB2	
GRIA2	GALR1	MC4R	
GRIA4	NPBWR1		
P2RX1	GRIK1		
P2RY2	GRIN2D		
PTGER2	HTR1E		
HTR1B	TRHR		
NTSR1	TSHR		
	VIPR2		
	CCKBR		

Table S7. Methylation Status of Validated CpGs in *TFAM* KO cell lines (N=6). Bolded entries indicate differential expression *P*<0.05.

Marker Name	All Cohort Meta-Analysis				Average Methylation in Negative Control Lines	Average Methylation in *TFAM* Knockout Lines	*TFAM* Differential Expression	
	Mean Methylation	Estimate	Standard Error	*P*-value			Beta Estimate	*P*-Value
cg03964851 (surrogate for cg21051031)	0.83	0.0038	0.0004	7.34E-27	0.7685	0.7710	-0.0025	9.42E-01
cg26094004	0.55	-0.0079	0.0007	4.13E-28	0.6504	0.9001	-0.2497	2.91E-05
cg26563141	0.37	-0.0060	0.0008	2.20E-14	0.3071	0.4187	-0.1116	1.25E-02
cg14575356	0.55	0.0033	0.0005	1.22E-09	0.7906	0.7918	-0.0013	9.40E-01
cg23513930	0.35	0.0020	0.0003	3.71E-09	Not on EPIC array and no surrogate available			
cg08899667	0.58	-0.0041	0.0006	1.55E-12	0.7931	0.7014	0.0917	3.33E-03

*Note: Mean methylation for cg21051031 = 0.85

Table S8. Differentially expressed genes (*P*<0.05) within 1 Mb of differentially methylated CpGs in *TFAM* knockout cell lines. Shading indicates most differentially expressed gene for each CpG.

EWAS CpG	Chr: Position	Number of Genes Within 1 Mb	*P*-Value for *TFAM* Methylation Difference	Gene	Test Statistic for *TFAM* Expression	*P*-value for *TFAM* Expression	Direction of Effect (following KO)*	Distance from CpG (Kb)	Description
cg26094004	17: 42,075,116	42	2.91E-05	ACLY	4.9871	2.55E-02	Negative	144.6	ATP citrate lyase [Source:HGNC Symbol;Acc:HGNC:115]
				KAT2A	5.0487	2.46E-02	Negative	38.0	lysine acetyltransferase 2A [Source:HGNC Symbol;Acc:HGNC:4201]
				HSPB9	8.3116	3.94E-03	Negative	46.3	heat shock protein family B (small) member 9 [Source:HGNC Symbol;Acc:HGNC:30589]
				KCNH4	9.5817	1.97E-03	Negative	81.8	potassium voltage-gated channel subfamily H member 4 [Source:HGNC Symbol;Acc:HGNC:6253]
				COASY	4.3154	3.78E-02	Negative	486.4	Coenzyme A synthase [Source:HGNC Symbol;Acc:HGNC:29932]
				CCR10	4.5647	3.26E-02	Positive	603.8	C-C motif chemokine receptor 10 [Source:HGNC Symbol;Acc:HGNC:4474]
				RAMP2	11.9339	5.51E-04	Positive	683.3	receptor activity modifying protein 2 [Source:HGNC Symbol;Acc:HGNC:9844]
				AOC3	4.2112	4.02E-02	Positive	776.1	copper containing 3 [Source:HGNC Symbol;Acc:HGNC:550]
				IFI35	16.9886	3.76E-05	Positive	931.6	interferon induced protein 35 [Source:HGNC Symbol;Acc:HGNC:5399]
				RND2	8.9365	2.80E-03	Positive	950.1	Rho family GTPase 2 [Source:HGNC Symbol;Acc:HGNC:18315]
				BRCA1	4.0986	4.29E-02	Positive	969.2	DNA repair associated [Source:HGNC Symbol;Acc:HGNC:1100]
cg26563141	2: 88,124,876	4	1.25E-02	RPIA	20.8215	5.04E-06	Negative	566.8	ribose 5-phosphate isomerase A [Source:HGNC Symbol;Acc:HGNC:10297]
cg08899667	6: 31,761,055	32	3.33E-03	CCHCR1	8.0190	4.63E-03	Positive	602.8	coiled-coil alpha-helical rod protein 1 [Source:HGNC Symbol;Acc:HGNC:13930]
				HLA-C	4.6442	3.12E-02	Positive	488.9	major histocompatibility complex: class I: C [Source:HGNC Symbol;Acc:HGNC:4933]
				HLA-B	9.4318	2.13E-03	Positive	403.9	major histocompatibility complex: class I: B [Source:HGNC Symbol;Acc:HGNC:4932]
				ATP6V1G2	7.1309	7.58E-03	Positive	212.6	ATPase H+ transporting V1 subunit G2 [Source:HGNC Symbol;Acc:HGNC:862]
				NFKBIL1	5.4633	1.94E-02	Positive	202.2	NFKB inhibitor like 1 [Source:HGNC Symbol;Acc:HGNC:7800]
				CLIC1	4.2279	3.98E-02	Positive	21.3	chloride intracellular channel 1 [Source:HGNC Symbol;Acc:HGNC:2062]
				MSH5	13.4158	2.50E-04	Positive	1.8	mutS homolog 5 [Source:HGNC Symbol;Acc:HGNC:7328]
				HSPA1L	4.7247	2.97E-02	Positive	48.6	heat shock protein family A (Hsp70) member 1 like [Source:HGNC Symbol;Acc:HGNC:5234]
				C2	5.9338	1.49E-02	Positive	136.7	complement C2 [Source:HGNC Symbol;Acc:HGNC:1248]
				FKBPL	8.1388	4.33E-03	Positive	367.7	FK506 binding protein like [Source:HGNC Symbol;Acc:HGNC:13949]
				EGFL8	4.5363	3.32E-02	Negative	403.5	EGF like domain multiple 8 [Source:HGNC Symbol;Acc:HGNC:13944]
				HLA-DRA	10.6612	1.09E-03	Positive	678.8	major histocompatibility complex: class II: DR alpha [Source:HGNC Symbol;Acc:HGNC:4947]
				HLA-DRB5	24.7584	6.50E-07	Negative	756.3	major histocompatibility complex: class II: DR beta 5 [Source:HGNC Symbol;Acc:HGNC:4953]
				HLA-DRB1	4.1996	4.04E-02	Positive	817.7	major histocompatibility complex: class II: DR beta 1 [Source:HGNC Symbol;Acc:HGNC:4948]

*Positive beta indicates that after the decrease in mtDNA-CN (*TFAM* Knockout), expression has increased as compared to controls.

Table S9. Results of Mendelian Randomization. A. Results for association between ARIC EA and AA derived independent cis meQTLs and mtDNA-CN. **B.** Results for association between ARIC meta-analysis derived independent cis meQTLs and mtDNA-CN (fixed effects model).

A.

ARIC Cohort	Chr	meQTL CpG from EWAS	CpG Position	meQTL SNP	SNP Position	MAF	Imputation Quality (R²)	mtDNA~meQTL SNP			CpG~meQTL SNP			Power for MR
								Beta Estimate	Standard Error	P-value	Beta Estimate	Standard Error	P-value	
EA (Permuted P=7.84E-04)	17	cg26094004	42,075,116	rs11654132	42,149,134	0.178	0.97	-0.0362	0.0842	0.67	0.0322	0.0043	8.80E-14	0.99
	6	cg08899667	31,761,055	rs3117574	31,725,230	0.098	1	0.0362	0.0762	0.64	0.0149	0.0031	1.76E-06	0.29
	6	cg08899667	31,761,055	rs9267653	31,840,415	0.305	0.97	-0.0127	0.0547	0.82	0.0101	0.0022	6.42E-06	0.28
	5	cg21051031	93,905,482	rs2973154	93,930,488	0.195	0.97	0.0112	0.0664	0.87	0.0073	0.0021	4.47E-04	0.75
AA (Permuted P=9.12E-04)	3	cg23513930	10,334,717	rs154236	10,356,314	0.254	0.96	-0.0692	0.0416	0.10	-0.0064	0.0009	2.23E-11	0.86
	6	cg08899667	31,761,055	rs28366163	31,704,804	0.148	0.94	0.0244	0.0474	0.61	0.0097	0.0019	4.64E-07	0.75
	6	cg14575356	130,013,903	rs1894642	130,015,331	0.41	1	0.0358	0.0353	0.31	-0.0056	0.0012	3.95E-06	0.58
	6	cg14575356	130,013,903	rs17469966	130,061,044	0.057	0.82	0.1076	0.0867	0.21	-0.0129	0.003	1.70E-05	0.49
	6	cg08899667	31,761,055	rs9267659	31,846,234	0.057	0.98	0.1007	0.0875	0.25	0.0151	0.0036	2.52E-05	0.56
	6	cg14575356	130,013,903	rs9398917	130,014,226	0.238	0.98	-0.0150	0.0393	0.70	-0.0046	0.0014	6.44E-04	0.34

B.

Chr	meQTL CpG from EWAS	CpG Position	meQTL SNP	SNP Position	Analysis	MAF	Imputation Quality (R²)	mtDNA~meQTL SNP			CpG~meQTL SNP			Power for MR
								Beta Estimate	Standard Error	P-value	Beta Estimate	Standard Error	P-value	
6	cg08899667	31,761,055	rs9267653	31,840,415	Meta			-0.0156	0.0318	0.62	0.0086	0.0013	1.99E-11	
					EA	0.305	0.97	-0.0127	0.0547	0.82	0.0101	0.0022	6.42E-06	0.28
					AA	0.246	0.97	-0.0154	0.0389	0.69	0.0078	0.0015	5.31E-07	0.75
6	cg08899667	31,761,055	rs28366163	31,704,804	Meta			0.0347	0.0428	0.42	0.0107	0.0017	1.10E-09	
					EA	0.063	0.96	0.1318	0.1033	0.20	0.0143	0.0041	4.54E-04	0.18
					AA	0.148	0.94	0.0244	0.0474	0.61	0.0096	0.0019	4.18E-07	0.75