

Supplemental Information:

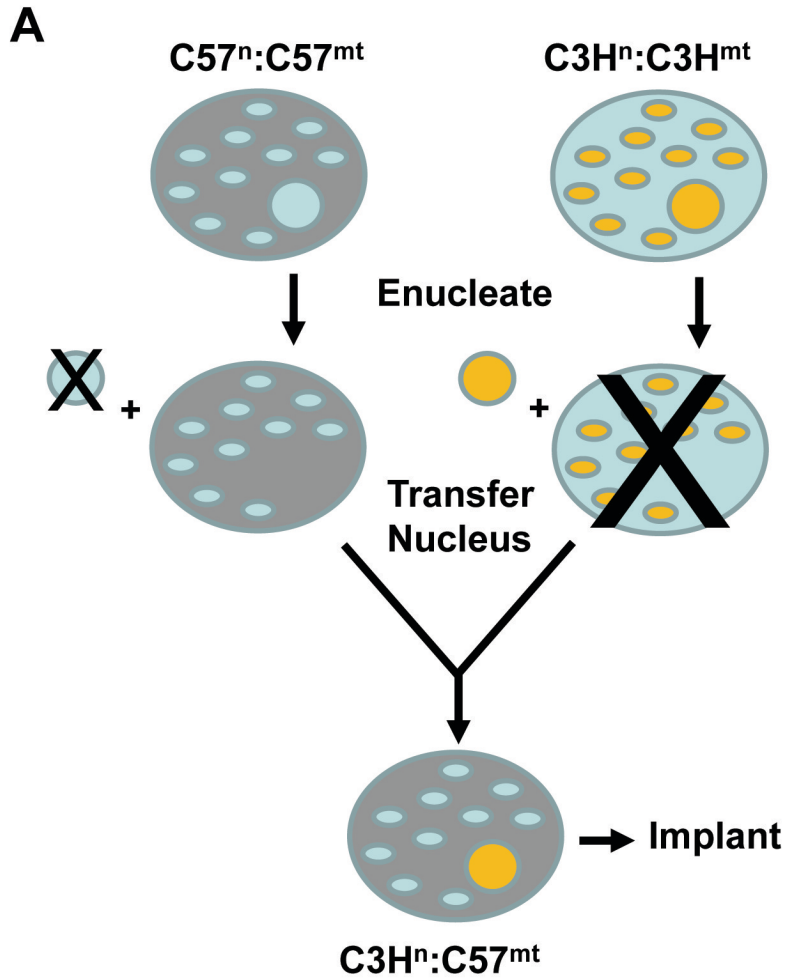
Mitochondrial Genetic Background Modulates Bioenergetics and Susceptibility to Acute Cardiac Volume – Overload (Fetterman et al)

Figure Legends:

Figure S1: Generation of Mitochondrial Nuclear eXchange (MNX) Mice. (A) MNX mice were produced by enucleating fertilized oocytes from C57BL/6 (C57ⁿ:C57^{mt} or C57) and C3H/HeN (C3Hⁿ:C3H^{mt} or C3H) mice and the C3H nucleus transferred to enucleated C57 oocytes yielding C3Hⁿ:C57^{mt} oocytes, which were implanted into surrogate females to generate MNX progeny. The reciprocal process was followed to generate the C57ⁿ:C3H^{mt} MNX. MNX nomenclature is indicated by strain nuclear (strainⁿ):strain mtDNA (strain^{mt}), e.g. mice with C3H nuclear DNA and C57 mtDNA are indicated by C3Hⁿ:C57^{mt}. Nuclear genotyping and mtDNA haplotyping of all originating founding females and F1 progeny was determined by nuclear SNP analysis of a panel of 38 distinguishing nuclear SNPs and complete sequencing of the mtDNA. (B) MNX mice; coat color segregates with the nuclear genome (C3H-brown, C57-black), whereas mtDNA haplotype verified by restriction enzyme length polymorphism analysis using *AspI* and *BclI* to confirm mitochondrial haplotype (C-D). (C) PCR products from C57 mtDNAs are cleaved by *AspI* to yield 274 bp and 111 bp fragments whereas C3H mtDNAs remain uncut (385 bp). (D) PCR products from C57 mtDNAs remain uncut (204 bp) whereas C3H mtDNAs are cleaved by *BclI* to yield 166 bp and 38 bp fragments. (E) *AspI* (upper panel) and *BclI* (lower panel) digest products of PCR products generated from genomic DNA isolated from heart tissues. Numbered lanes correspond to C57ⁿ:C57^{mt} (1,2), C3Hⁿ:C57^{mt} (3-5), C3Hⁿ:C3H^{mt} (6,7) and C57ⁿ:C3H^{mt} (8-10) mice, respectively.

Figure S2: Complex I (NADH dehydrogenase) protein levels and activity in C57 and C3H mitochondria. (A) Blue native 2D protein gels of complex I from isolated cardiac mitochondria. Molecular weight in kilodaltons is indicated on the left and spot number is indicated on the right. (B) Quantification of complex I subunits indicating no differences in the expression of isolated subunits. (C) Complex I activity measured in isolated heart mitochondria from C57 and C3H animals.

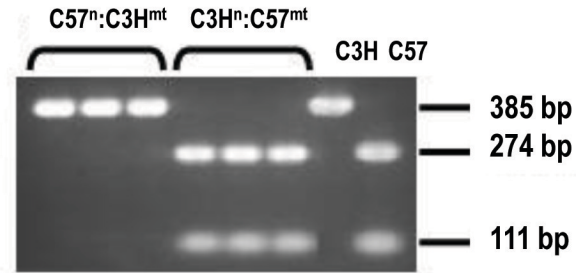
Figure S3: Complex II (Succinate dehydrogenase) activity in heart mitochondria. Complex II activity measured in isolated heart mitochondria from control (C57ⁿ:C57^{mt} and C3Hⁿ:C3H^{mt}) and MNX (C57ⁿ:C3H^{mt} and C3Hⁿ:C57^{mt}) mice. The nuclear genome for each group is indicated on the X-axis, whereas the filled or open bars indicate the presence of either the C57 mtDNA or C3H mtDNA, respectively.



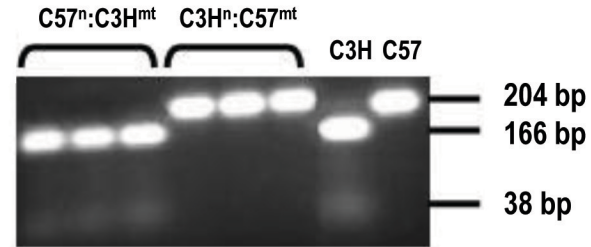
B



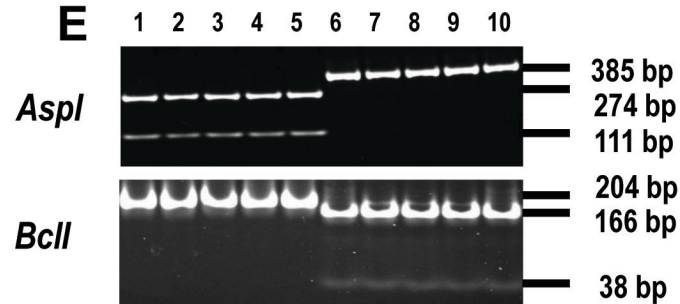
C

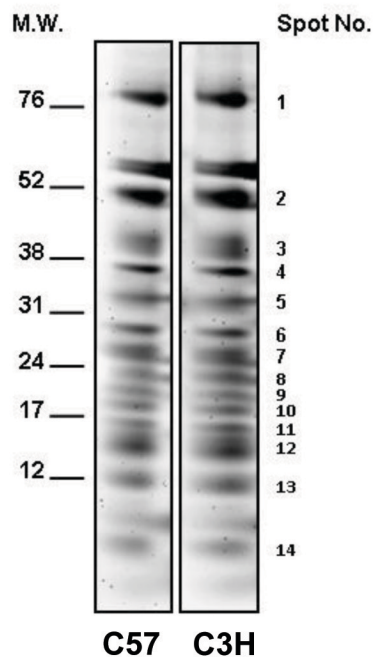
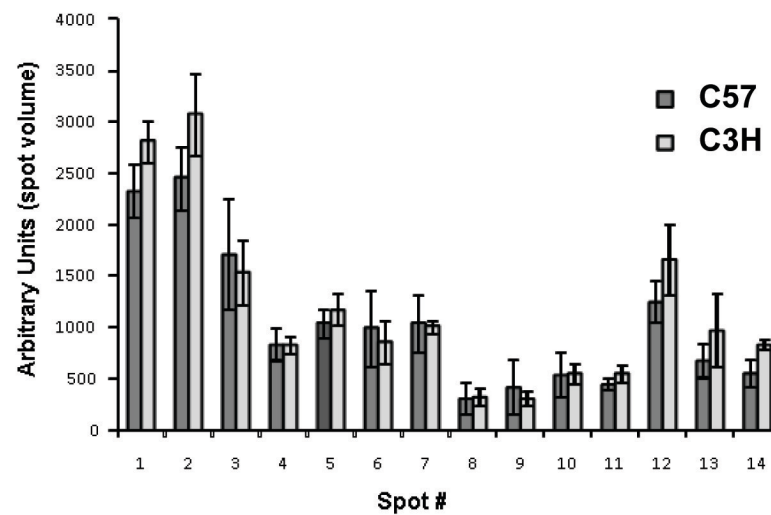
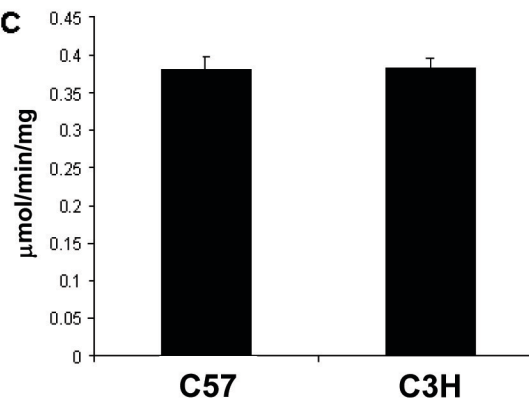


D



E



A**B****C**

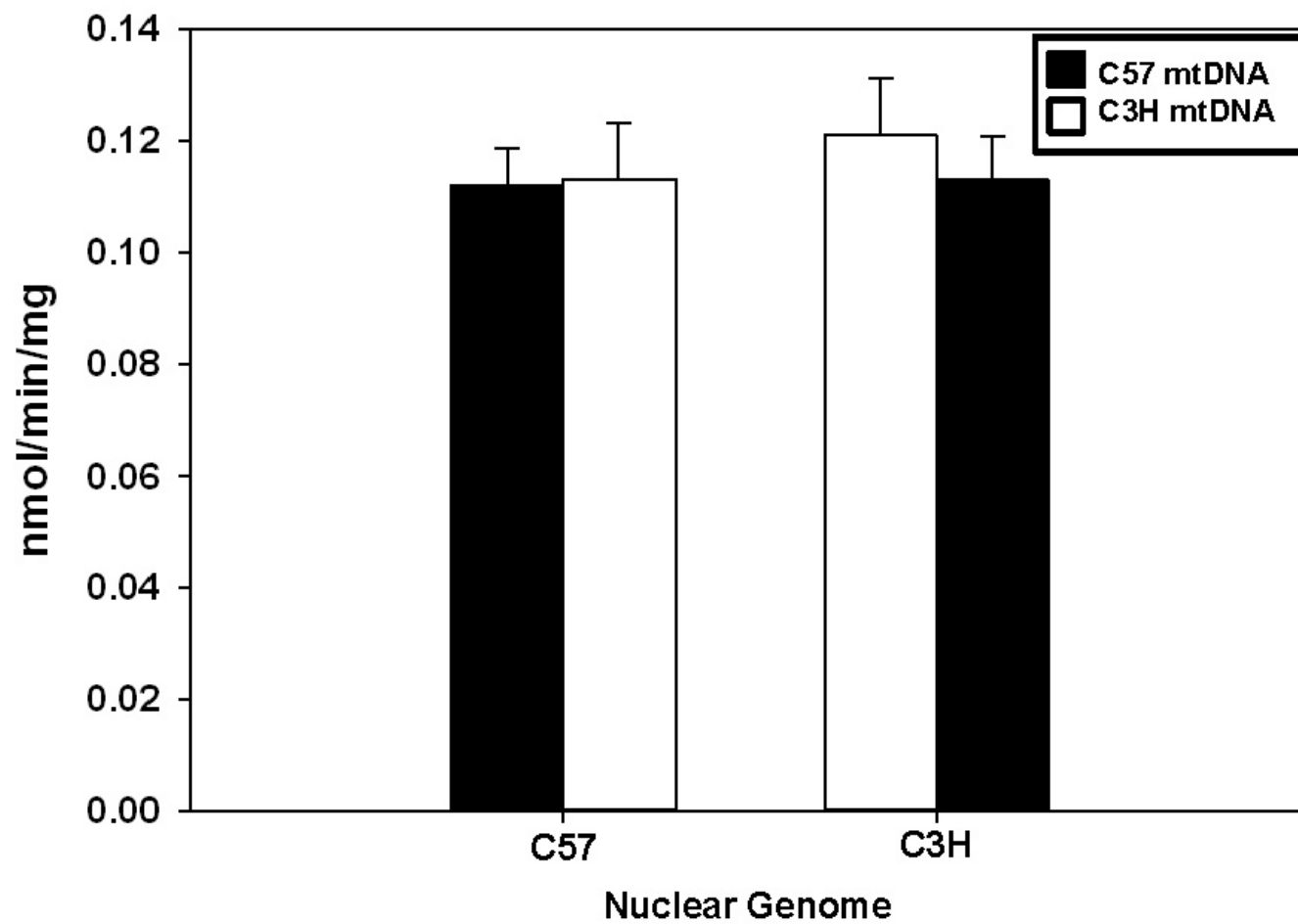


Table S1: Analysis of 38 distinguishing Nuclear SNPs in control and MNX founder females.

Nuclear SNP	Expected SNP		Control		C57 ⁿ :C3H ^{mt}			C3H ⁿ :C57 ^{mt}		
	C57	C3H	C57	C3H	MNX 1	MNX 2	MNX 3	MNX 4	MNX 5	MNX 6
01-004147733-M	A	T	A	T	A	A	A	T	T	T
01-187052090-M	C	A	C	A	C	C	C	A	A	A
02-047368792-G	T	C	T	C	T	T	T	C	C	C
02-162979695-M	G	A	G	A	G	G	G	A	A	A
03-007561998-N	T	C	T	C	T	T	T	C	C	C
03-157197990-M	G	A	G	A	G	G	G	A	A	A
04-004044733-M	C	T	C	T	C	C	C	T	T	T
04-137216461-M	T	C	T	C	T	T	T	C	C	C
05-013136419-M	C	T	C	T	C	C	C	T	T	T
05-147904991-M	C	A	C	A	C	C	C	A	A	A
06-012968430-M	A	G	A	G	A	A	A	G	G	G
06-146820927-N	G	A	G	A	G	G	G	A	A	A
07-004201219-N	A	G	A	G	A	A	A	G	G	G
07-135345950-N	A	G	A	G	A	A	A	G	G	G
08-076188935-M	A	T	A	T	A	A	A	T	T	T
08-126038064-N	C	G	C	G	C	C	C	G	G	G
09-063923771-M	G	A	G	A	G	G	G	A	A	A
09-123708875-M	G	A	G	A	G	G	G	A	A	A
10-012713073-N	T	C	T	C	T	T	T	C	C	C
10-100345477-M	T	C	T	C	T	T	T	C	C	C
11-033040833-M	T	C	T	C	T	T	T	C	C	C
11-095327574-N	G	T	G	T	G	G	G	T	T	T
12-005458738-M	T	C	T	C	T	T	T	C	C	C
12-071296001-M	G	A	G	A	G	G	G	A	A	A
13-032546453-N	G	A	G	A	G	G	G	A	A	A
13-110101560-N	G	T	G	T	G	G	G	T	T	T
14-022368155-G	T	C	T	C	T	T	T	C	C	C
14-112544301-N	A	G	A	G	A	A	A	G	G	G
15-005994001-M	A	C	A	C	A	A	A	C	C	C
15-103221933-M	C	G	C	G	C	C	C	G	G	G
16-012752727-C	A	G	A	G	A	A	A	G	G	G
16-093066825-C	T	C	T	C	T	T	T	C	C	C
17-011173324-G	G	C	G	C	G	G	G	C	C	C
17-093441700-M	T	C	T	C	T	T	T	C	C	C
18-006845916-M	G	A	G	A	G	G	G	A	A	A
18-086980249-M	G	A	G	A	G	G	G	A	A	A
19-007376322-N	T	C	T	C	T	T	T	C	C	C
19-059089086-M	G	A	G	A	G	G	G	A	A	A

Table S2: Left ventricle function in 3 day ACF

	C57ⁿ:C57^{mt}		C57ⁿ:C3H^{mt}		C3Hⁿ:C3H^{mt}		C3Hⁿ:C57^{mt}	
Treatment (n)	Sham (5)	ACF (5)	Sham (4)	ACF (4)	Sham (5)	ACF (5)	Sham (3)	ACF (5)
HR (bpm)	523 ± 21	549 ± 22	469 ± 44	536 ± 46	523 ± 23	491 ± 13	544 ± 19	516 ± 20
MAP (mm Hg)	77 ± 1	64 ± 5*	79 ± 1	59 ± 2*	87 ± 2	73 ± 5*	80 ± 6	65 ± 5*
LVEDP (mm Hg)	2 ± 0	7 ± 2*	4 ± 1	4 ± 1	3 ± 2	10 ± 2*	2 ± 1	9 ± 2*
LVEDD (mm)	4 ± 0.1	4.4 ± 0.1*	3.7 ± 0.2	4.1 ± 0.2	3.9 ± 0.06	4.1 ± 0.04	3.7 ± 0.08	4 ± 0.2
LV FS (%)	25 ± 3	34 ± 5*	35 ± 2	45 ± 3*	31 ± 2	33 ± 3	28 ± 1	30 ± 1
* = P < 0.05 vs. Matched Sham								

HR – heart rate

MAP – mean arterial pressure

LVEDP – left ventricular end diastolic pressure

LVEDD – left ventricular end diastolic dimension

LV FS – left ventricular fractional shortening