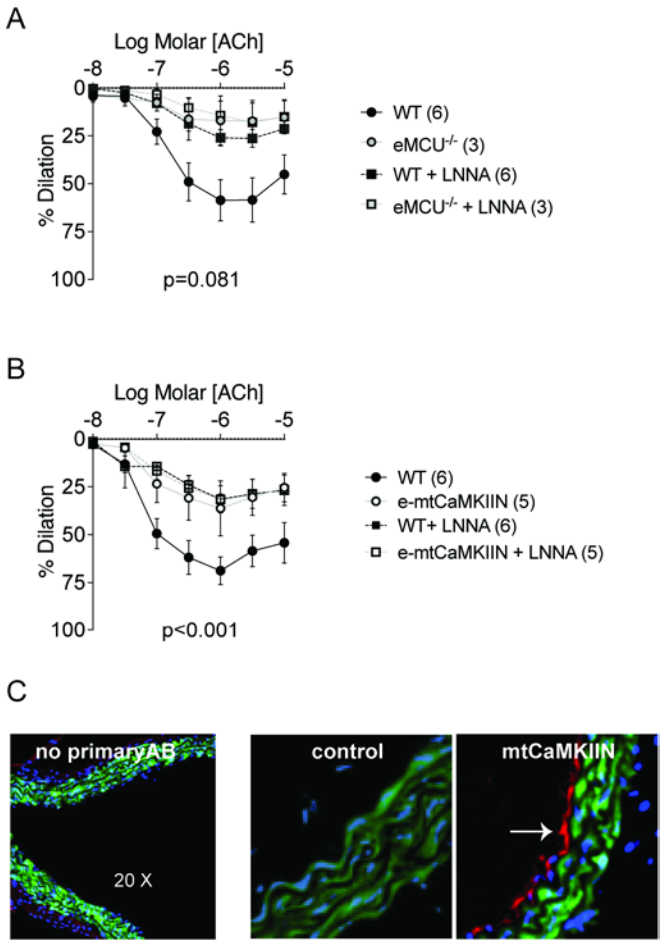
SUPPLEMENTAL MATERIAL

Table S1. 3-Way Grouped ANOVA for concentration of agonist (Ach, SNP, KCl), sex and genotype. Results for the main effects and the interaction between sex and genotype.

WT vs e-MCU$^{-/-}$	**Ach**		**SNP**		**KCl**	
	p-value	% of variance	p-value	% of variance	p-value	% of variance
concentration	<0.0001	18.88	<0.0001	72.44	<0.0001	83.35
sex	0.0682	3.79	0.7509	0.09	0.9273	0.004
genotype	0.0227	6.20	0.6643	0.17	0.0548	1.90
sex x genotype	0.4335	0.65	0.2049	1.47	0.4925	0.22

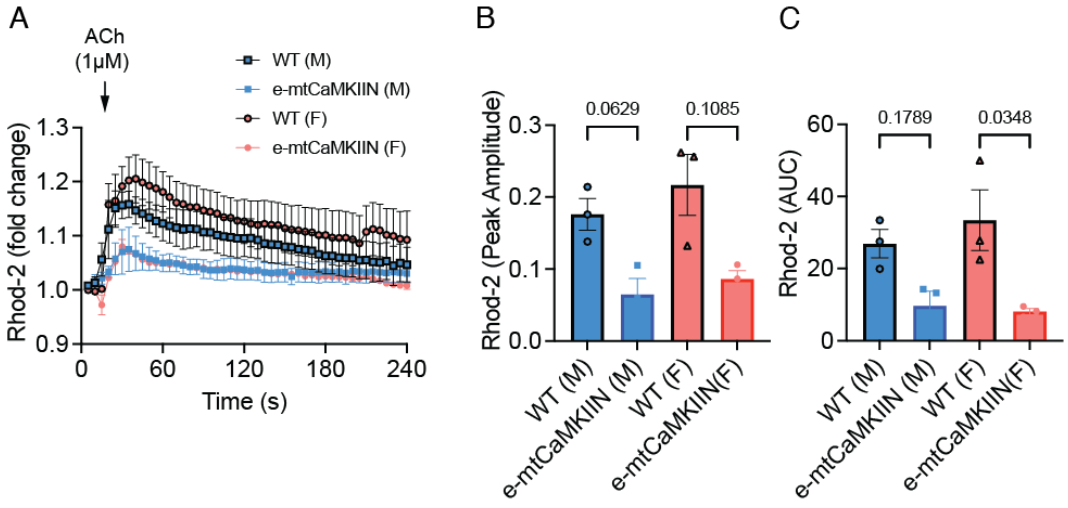
WT vs e-mtCaMKIIN	**Ach**		**SNP**		**KCl**	
	p-value	% of variance	p-value	% of variance	p-value	% of variance
concentration	<0.0001	7.01	<0.0001	50.63	<0.0001	78.48
sex	0.1182	7.87	0.4040	1.45	0.7390	0.08
genotype	0.0112	22.93	0.1587	4.31	0.1157	2.02
sex x genotype	0.5600	0.99	0.3925	1.52	0.6710	0.14

Figure S1. In mesenteric resistance arteries, pre-incubation with the eNOS inhibitor L-NNA abolishes sex differences in vasodilation in response to ACh.



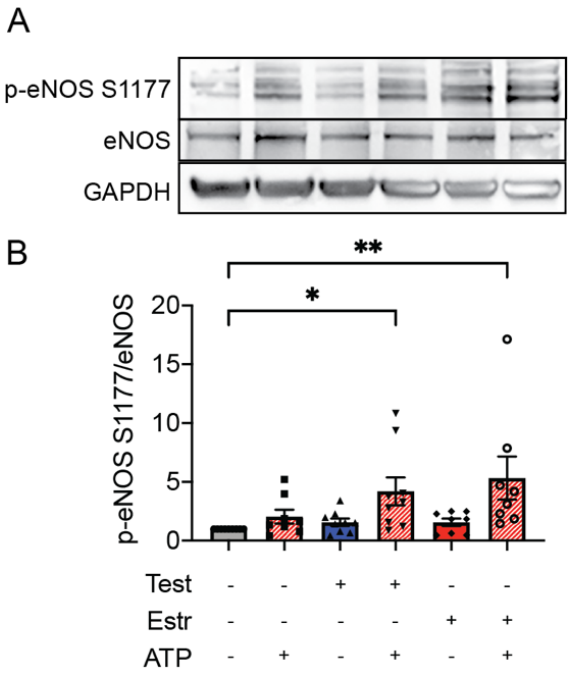
Vasodilation in response to cumulative doses of ACh after preincubation with the eNOS inhibitor L-NNA (100 μM) for 30 min, in mesenteric resistance arteries from (**A**) e-MCU$^{-/-}$ and littermate WT mice, and (**B**) e-mtCaMKIIN and WT mice. Data of untreated vascular segments correspond to those in Fig. 1, 2. P-value for source of variation with LNNA by stacked 3-Way ANOVA. A significant interaction between LNNA and genotype was seen in (B). **C)** Immunofluorescence for CaMKIIN (red) (GFP green, DAPI blue) in e-mtCaMKIIN mouse without (control) and after Tamoxifen treatment. Before cre recombination, eGFP is expressed in all tissues (control). Upon recombination by Tamoxifen, eGFP cDNA is excised and only the transgene expressed. 63 X. Arrow denotes arterial lumen.

Figure S2. In second-order mesenteric arteries from e-mt-CaMKIIN mice, mitochondrial Ca^{2+} transients are reduced.



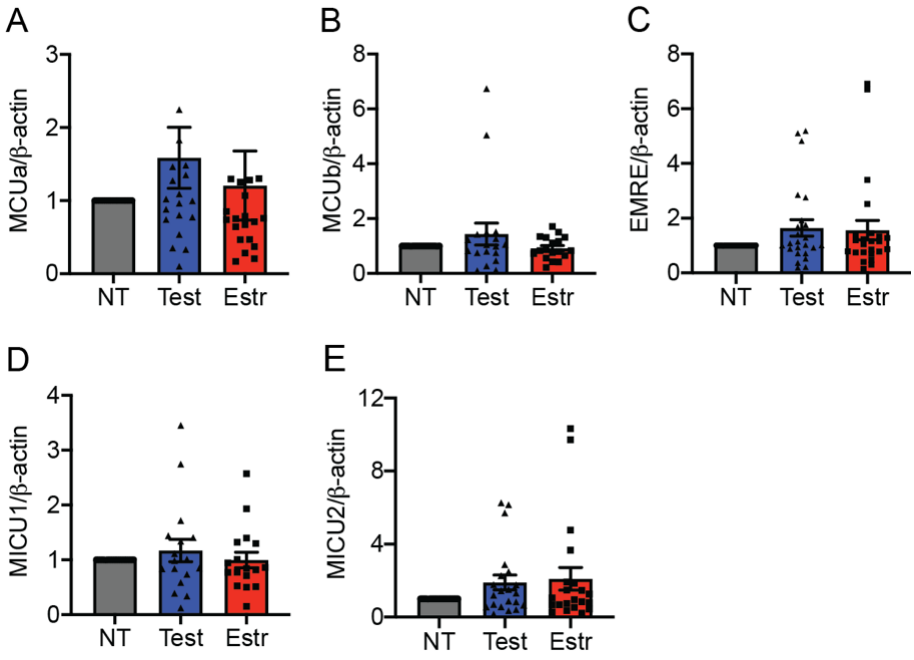
En-face endothelial Rhod-2 imaging of second-order mesenteric arteries. (**A**) Mitochondrial Ca^{2+} transients after addition of 1 μM ACh to samples from male (M) and female (F) e-mtCaMKIIN and WT female mice. (B) Peak amplitude and (C) area under the curve (AUC) for the tracings in (A). n = 3 mice per genotype. P-values by Kruskal-Wallis test with pairwise comparisons as indicated.

Figure S3. In HAECs, eNOS activation is enhanced after treatment with sex hormones.



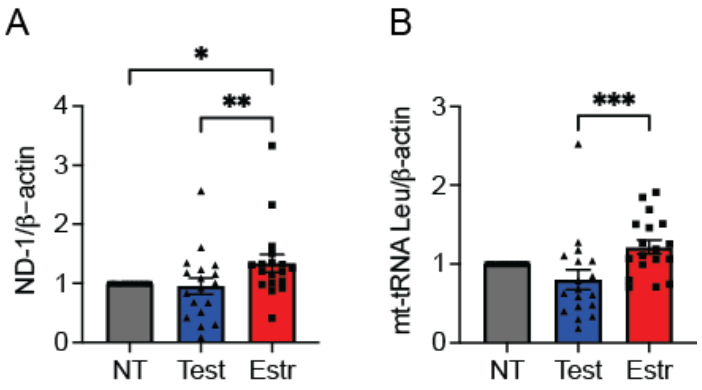
A) Representative immunoblots for activated eNOS (p-eNOS S1177), total eNOS, and GAPDH (housekeeping control) in HAECs pre-treated with testosterone, estradiol, or diluent for 72 hr and then stimulated with ATP (1 μM) for 15 min. **B**) Quantification of proteins in A. * $p<0.05$, ** $p<0.01$ by Kruskal-Wallis test.

Figure S4. In HAECs, levels of MCU subunit mRNAs are not altered by sex hormones.



qRT-PCR results for MCU subunits in HAECs treated with testosterone, estradiol, or diluent (NT) for 72 hr. Quantification of mRNA levels of (**A**) MCUa, (**B**) MCUb, (**C**) EMRE, (**D**) MICU1, and (**E**) MICU2, normalized to β-actin. N = 20 (A), 18 (B), 24 (C), 17 (D), 21 (E) biological replicates. Analysis by Friedman test.

Figure S5. In HAECs, mitochondrial DNA copy numbers are increased after estradiol treatment.



A, B) qPCR for genes encoded by mitochondrial DNA normalized to β-actin: (**A**) tRNA-Leu, and (**B**) ND1. * $p<0.05$, ** $p<0.01$ by Friedman test. n= 18 biological replicates.