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TMEM16A is Implicated in the Regulation of Coronary Flow and is Altered in Hypertension – Supplemental Figures

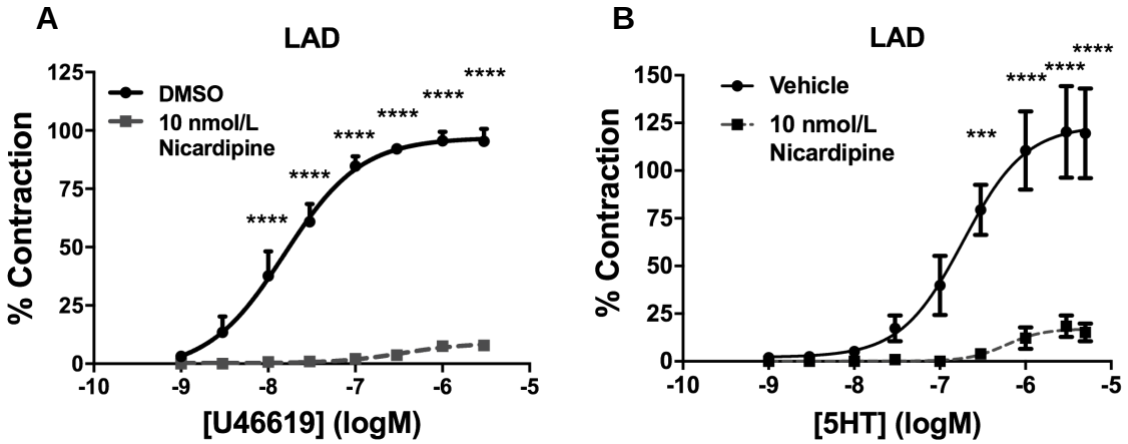

Figure S1. U46619- and 5HT-induced contractions are Nicardipine-sensitive.

(A) Concentration-effect curves of U46619 in LAD coronary artery segments in the presence and absence of 10 nM Nicardipine. (B) Concentration-effect curves of 5HT in LAD coronary artery segments in the presence and absence of 10 nM Nicardipine. Data are expressed as a percentage of the peak of a previous contraction in the same vessel segment in response to the a maximal concentration of the same vasoconstrictor, ± SEM; n=3 for both U46619 and 5HT. * indicates significant statistical difference ($p<0.05$) between percentage contractions of the two groups at the corresponding concentrations. Data were analysed using a 2-way ANOVA followed by a post hoc Bonferroni test for multiple comparisons.

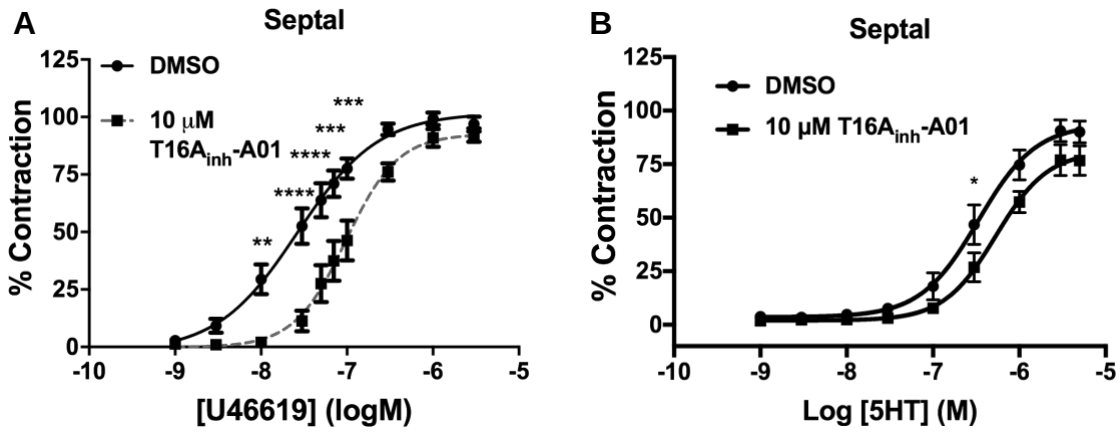

Figure S2. Effects of $T16A_{inh}$-A01 on U46619- and 5HT-induced contractions in Septal coronary artery segments. (A and B) 10 μM $T16A_{inh}$-A01 significantly inhibited the ability of Septal coronary artery segments to contract to both U46619 (n=8 for both DMSO and $T16A_{inh}$-A01) and 5HT (n=7 for both DMSO and $T16A_{inh}$-A01). Data are expressed as a percentage of the peak of a previous contraction in the same vessel segment in response to the a maximal concentration of the same vasoconstrictor, ± SEM. * indicates significant statistical difference ($p<0.05$) between percentage contractions of the two groups at the corresponding concentrations. Data were analysed using a 2-way ANOVA followed by a post hoc Bonferroni test for multiple comparisons.

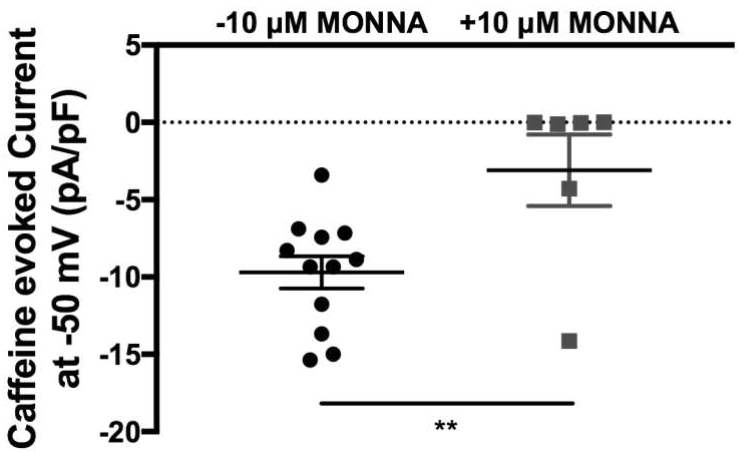

Figure S3. MONNA inhibits Caffeine-evoked $I_{Cl(Ca)}$ in pulmonary artery VSMCs. Mean maximum evoked currents in pulmonary artery VSMCs were significantly smaller when the cells were bathed in 10 µM MONNA (n=6), compared to when they were not bathed in 10 µM MONNA (n=12). Data are expressed as mean pA/pF ± SEM. * indicates statistical significant difference ($p<0.05$) between the two groups. Data were analysed using an unpaired, two-tailed student's t-test.

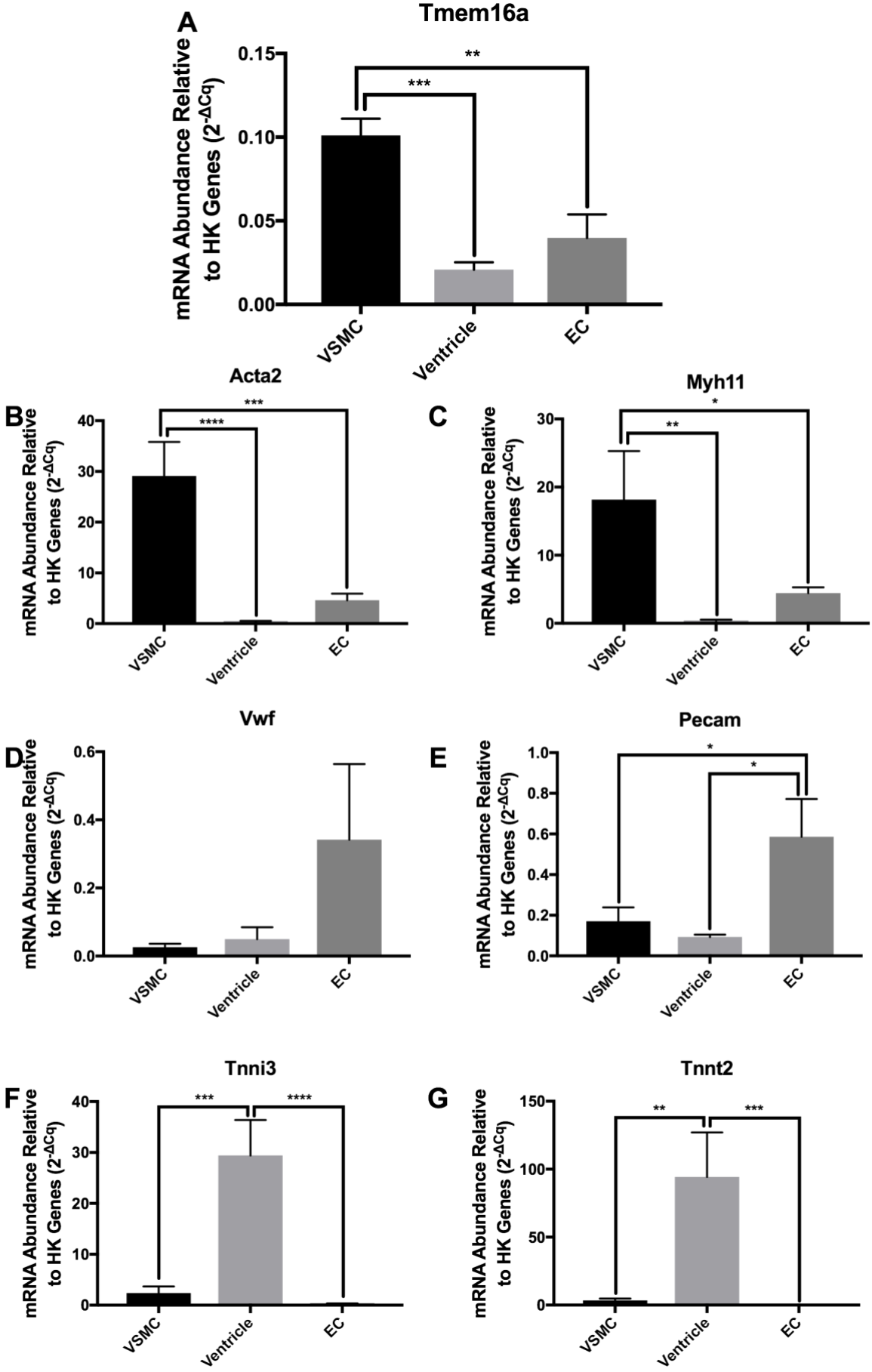
A
Tmem16a
mRNA Abundance Relative to HK Genes ($2^{-\Delta Cq}$)
0.15
0.10
0.05
0.00
**
***
VSMC
Ventricle
EC
B
Acta2
mRNA Abundance Relative to HK Genes ($2^{-\Delta Cq}$)
40
30
20
10
0
***
****
VSMC
Ventricle
EC
C
Myh11
mRNA Abundance Relative to HK Genes ($2^{-\Delta Cq}$)
30
20
10
0
*
**
VSMC
Ventricle
EC
D
Vwf
mRNA Abundance Relative to HK Genes ($2^{-\Delta Cq}$)
0.6
0.4
0.2
0.0
VSMC
Ventricle
EC
E
Pecam
mRNA Abundance Relative to HK Genes ($2^{-\Delta Cq}$)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
VSMC
Ventricle
EC
F
Tnni3
mRNA Abundance Relative to HK Genes ($2^{-\Delta Cq}$)
40
30
20
10
0
***
****
VSMC
Ventricle
EC
G
Tnnt2
mRNA Abundance Relative to HK Genes ($2^{-\Delta Cq}$)
150
100
50
0
**
***
VSMC
Ventricle
EC

Figure S4. Coronary artery VMSC QPCR preparations have negligible Cardiac and Endothelial contamination. (A) TMEM16A is expressed across coronary artery SMC, ventricular tissue, and endothelial cell samples, with the highest expressed in the SMC sample. (B and C) Markers of smooth muscle expression are significantly greater in the SMC preparation, with negligible expression in both the ventricular tissue and endothelial cell samples. (D and E) Endothelial cell expression markers are expressed at a greater value in the endothelial cells than in SMC or ventricular tissue samples where there was negligible expression. (F and G) The two markers of cardiac tissue, troponin T2 and troponin I3 are expressed highly in the ventricular tissue samples, with insignificant expression in both the SMC and endothelial cell samples. Data are expressed as mean relative expression ± SEM. * indicates statistical significant difference ($p<0.05$) between groups. Data were analysed using ordinary one-way ANOVA followed by a post hoc Tukey test for multiple comparisons.

Gene	GeneID	Pos	Chrom	Reference allele	Observed Allele	Type	Annotation Type	CADD score
ANO1	ENSG00000131620	16	11	T	C	SNV	CodingTranscript	29
RGL3	ENSG00000205517	35	19	G	T	SNV	CodingTranscript	26
CCDC151	ENSG00000198003	36	19	G	T	SNV	Intergenic	26
HRCT1	ENSG00000196196	75	9	C	T	SNV	CodingTranscript	25.6
LINC00961	ENSG00000235387	76	9	C	T	SNV	Intergenic	25.6
N/A	N/A	29	17	T	C	SNV	RegulatoryFeature	15.2
GOSR2	ENSG00000108433	30	17	T	C	SNV	Transcript	15.2
RP11-156P1.2	ENSG00000262633	31	17	T	C	SNV	Transcript	15.2
N/A	N/A	17	11	T	C	SNV	RegulatoryFeature	14.4
ARGHGAP42	ENSG00000165895	18	11	T	C	SNV	Transcript	14.4

Table S1. Details of 10 variations of genetic loci associated with essential hypertension with the highest CADD scores.