SUPPLEMENTARY INFORMATION

C2238/αANP modulates Apolipoprotein E through Egr-1/miR199a in vascular smooth muscle cells in vitro

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Running title: C2238/αANP and Apolipoprotein E

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Supplementary Figure legends

Supplementary Figure 1

Role of oxidative stress on Egr-1 stimulation by CC2238/αANP

Cells exposed to CC2238/αANP showed significant increase of Egr-1 expression levels, which was abolished in the presence of apocynin. Number of independent experiments=3.

CC2238/αANP = variant αANP; Egr-1= early growth response protein-1; CTR= control

Supplementary Figure 2

Modulation of proteins involved in apoptosis, necrosis and inflammation by CC2238/αANP in HUVSMCs

Panels A-F. Representative western blots of cleaved-caspase-3, CREG, JNK, p38MAPK, Nf-kBp65, Smad4 under CC2238/αANP both in the presence and in the absence of NPR-C. Corresponding densitometric analysis is shown below each blot.

Data are expressed as mean ± SD. Number of independent experiments=6.

** $p<0.0001$ for CC2238/αANP vs all other points.

CC2238/αANP = variant αANP; siRNA1=NPRC gene silencer 1; siRNA2=NPRC gene silencer 2; CREG=cellular repressor of E1A-stimulated gene; JNK=c-Jun N-terminal kinase; CTR=control.

Supplementary Figure 3

Modulation of proteins involved in apoptosis, necrosis and inflammation by CC2238/αANP in CAMSCs

Panels A-F. Representative western blots of cleaved-caspase-3, CREG, JNK, MAPKp38, Nf-kB, Smad4 under CC2238/αANP both in the presence and in the absence of NPR-C. Corresponding densitometric analysis is shown below each blot.

Data are expressed as mean ± SD. Number of independent experiments=6.

** p<0.0001 for CC2238/αANP vs all other points.

CC2238/αANP = variant αANP; siRNA1=NPRC gene silencer 1; siRNA2=NPRC gene silencer 2; CREG=cellular repressor of E1A-stimulated gene; JNK=c-Jun N-terminal kinase; CTR= control.

Supplementary Figure 4

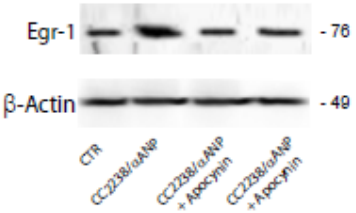
Results of ApoE gene sequencing in both HUVSMCs (panel A) and CASMCs (panel B).

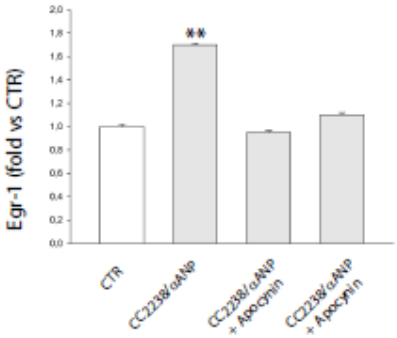
HUVSMCs express both ApoE2 (Cys112/Cys158) and ApoE3 (Cys112/Arg158) isoforms.

CASMCs express both ApoE3 (Cys112/Arg158) and ApoE4 (Arg112/Arg158) isoforms.

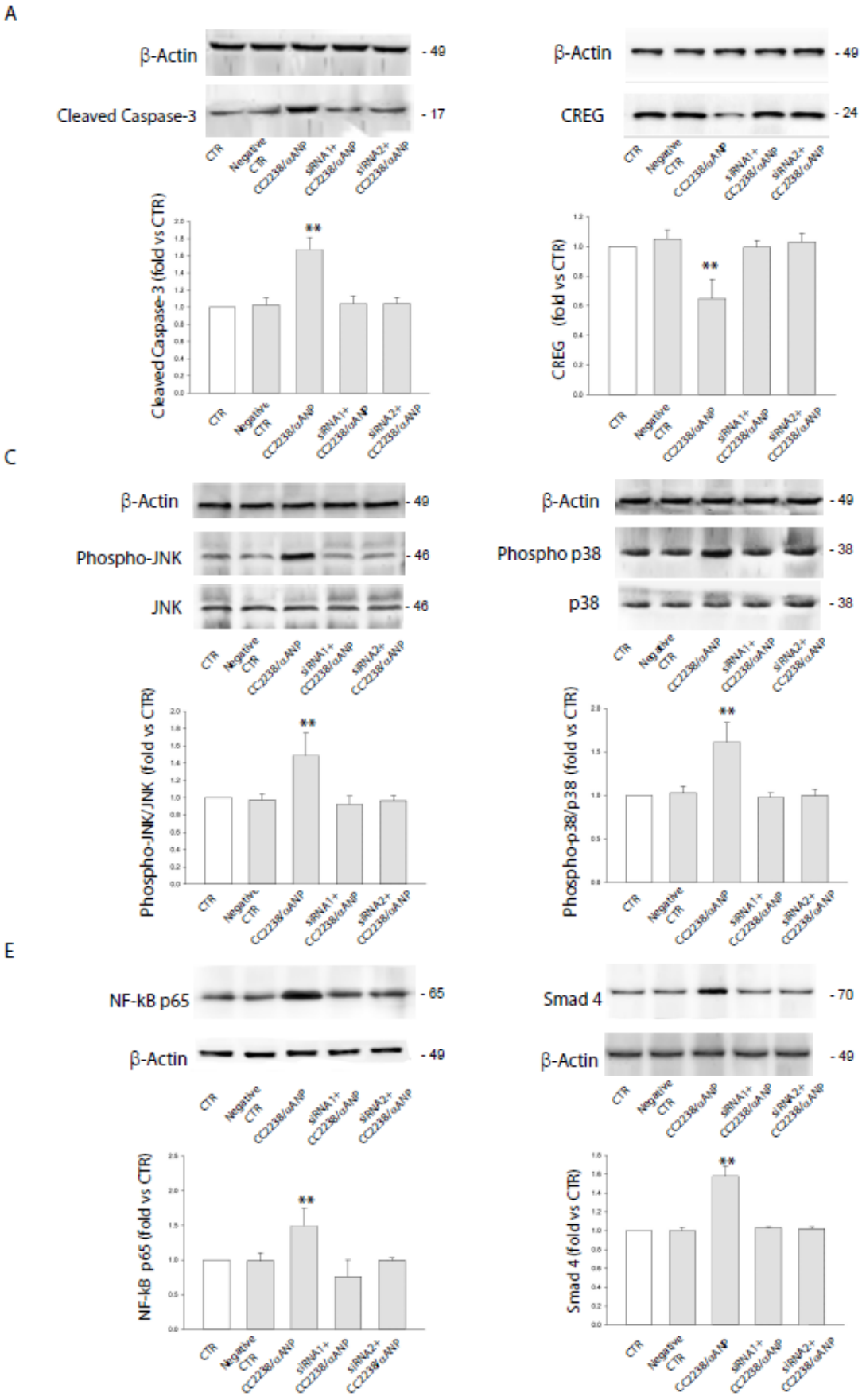
Cys=cysteine; Arg=arginine

Supplementary Figure 1

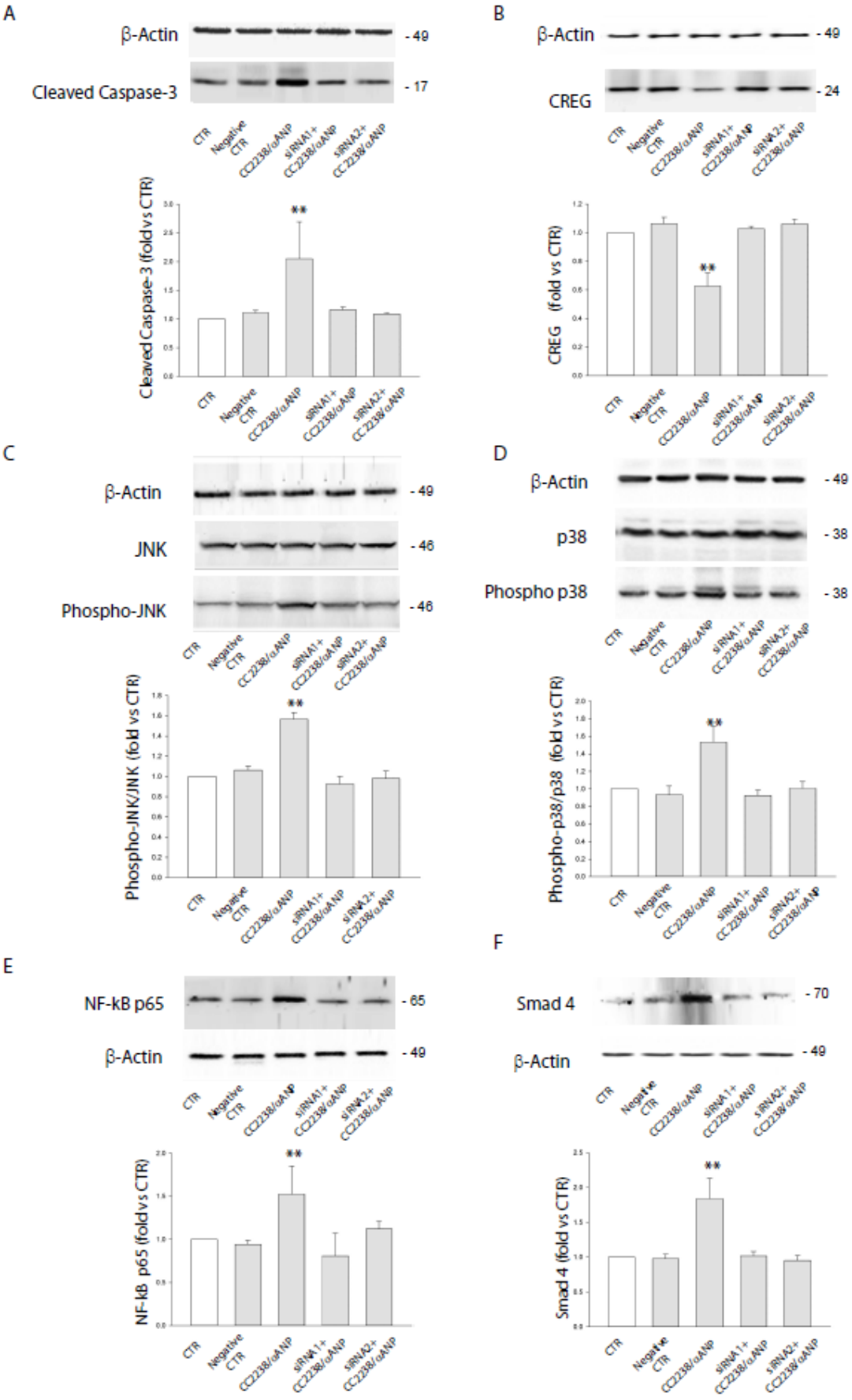




Supplementary Figure 2

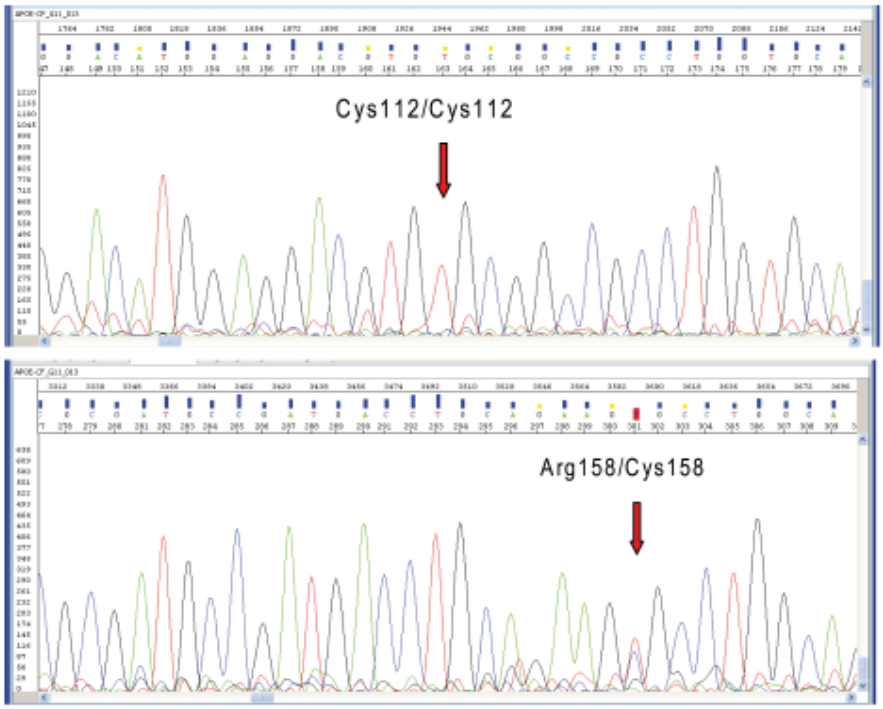


Supplementary Figure 3



Supplementary Figure 4

A



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