The novel chimeric natriuretic peptide CU-NP reduces cardiomyocyte hypertrophy through the NHE-1-calcineurin pathway

Ana Kilić, Venkatesh Rajapurohitam, Sharon S Sandberg, Asad Zeidan, J Craig Hunter, Nazo Said Faruq, Candace Y Lee, John C Burnett Jr, Morris Karmazyn

Supplemental Figures

Suppl Figure 1. ANP and CNP inhibit cardiomyocyte hypertrophy. Panels A to C (cell surface area) and D to F (ANP expression) show that both ANP (100 nM) and CNP (100 nM) inhibit cardiomyocyte hypertrophy in response to 24 h treatment with PE (10 µM), ang II (100 nM) or ET-1 (10 nM). Insets indicate a lack of direct effect of CNP or ANP on cell surface area or ANP expression in the absence of hypertrophic stimuli. Values for panels D to F represent fold increase and indicate means ± SEM. N=6 per group; *P<0.05 vs control, #P<0.05 vs hypertrophic stimuli. Ctl, control; PE, phenylephrine; Ang II, angiotensin II; ET-1, endothelin 1.

Suppl Figure 2. ANP and CNP inhibit increased NHE-1 expression in response to hypertrophic stimuli. Panel A (gene expression) and B (protein expression) show that both ANP (100 nM) and CNP (100 nM) inhibit NHE-1 upregulation in response to 24 h treatment with PE (10 µM), ang II (100 nM) or ET-1 (10 nM). Results obtained with ANP and CNP were identical and therefore pooled for both natriuretic peptides (NP) for conciseness. Values represent fold increase and indicate means ± SEM. Representative Western blots are given above bars in panel B. N=6 per group; *P<0.05 vs control, #P<0.05 vs hypertrophic stimuli. Ctl, control; PE, phenylephrine; Ang II, angiotensin II; ET-1, endothelin 1.

Suppl Figure 3. ANP and CNP inhibit increased NHE-1 activity in response to hypertrophic stimuli. Panels show intracellular pH (pH_i) recoveries after NH_4Cl pulsing in the presence of ANP (100 nM, A-C) or CNP (100 nM, D-F). Final PE, ang II and ET-1 concentrations were 10 µM, 100 nM and10 nM, respectively. Values indicate means ± SEM. N=6 per group; *P<0.05 vs other treatment groups. PE, phenylephrine; Ang II, angiotensin II; ET-1, endothelin 1.

Suppl Figure 4. ANP and CNP inhibit calcineurin activation and NFAT translocation in response to hypertrophic stimuli. Panel A shows that both ANP (100 nM) and CNP (100 nM)

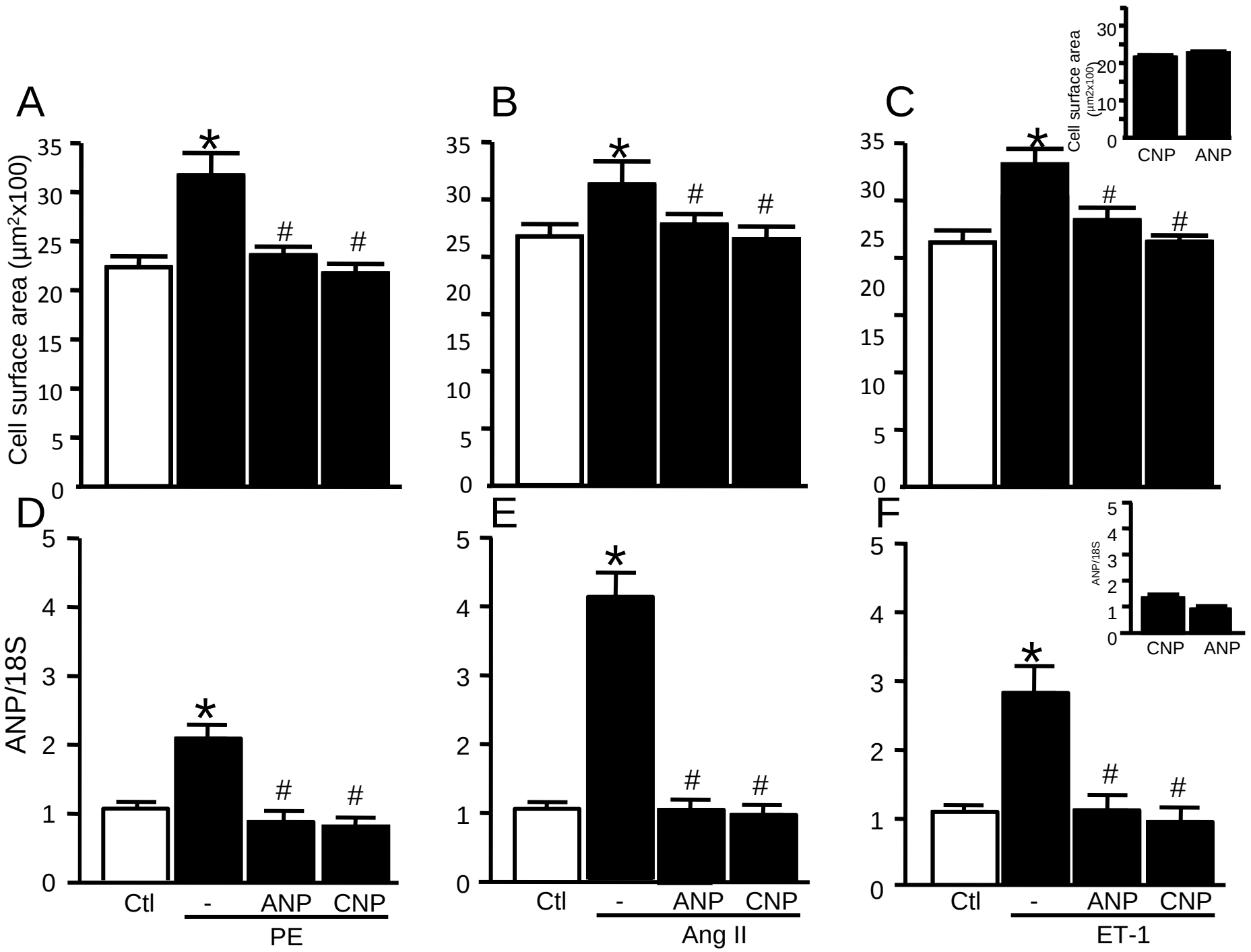
inhibit calcineurin activation in response to 24 h treatment with PE (10 μM), ang II (100 nM) or ET-1 (10 nM). Panel B depicts NFAT immunofluorescence staining for NFAT in the presence of hypertrophic stimuli and ANP whereas panel C shows ratio of the fluorescence intensity of the nuclear region to that of the entire region of the cell on a confocal plane. Results obtained with ANP and CNP were identical and therefore pooled for both natriuretic peptides (NP) for conciseness. Values indicate fold increase and are given as means ± SEM. N=6. *P<0.05 vs control, #P<0.05 vs hypertrophic stimuli. Ctl, control; PE, phenylephrine; Ang II, angiotensin II; ET-1, endothelin 1.

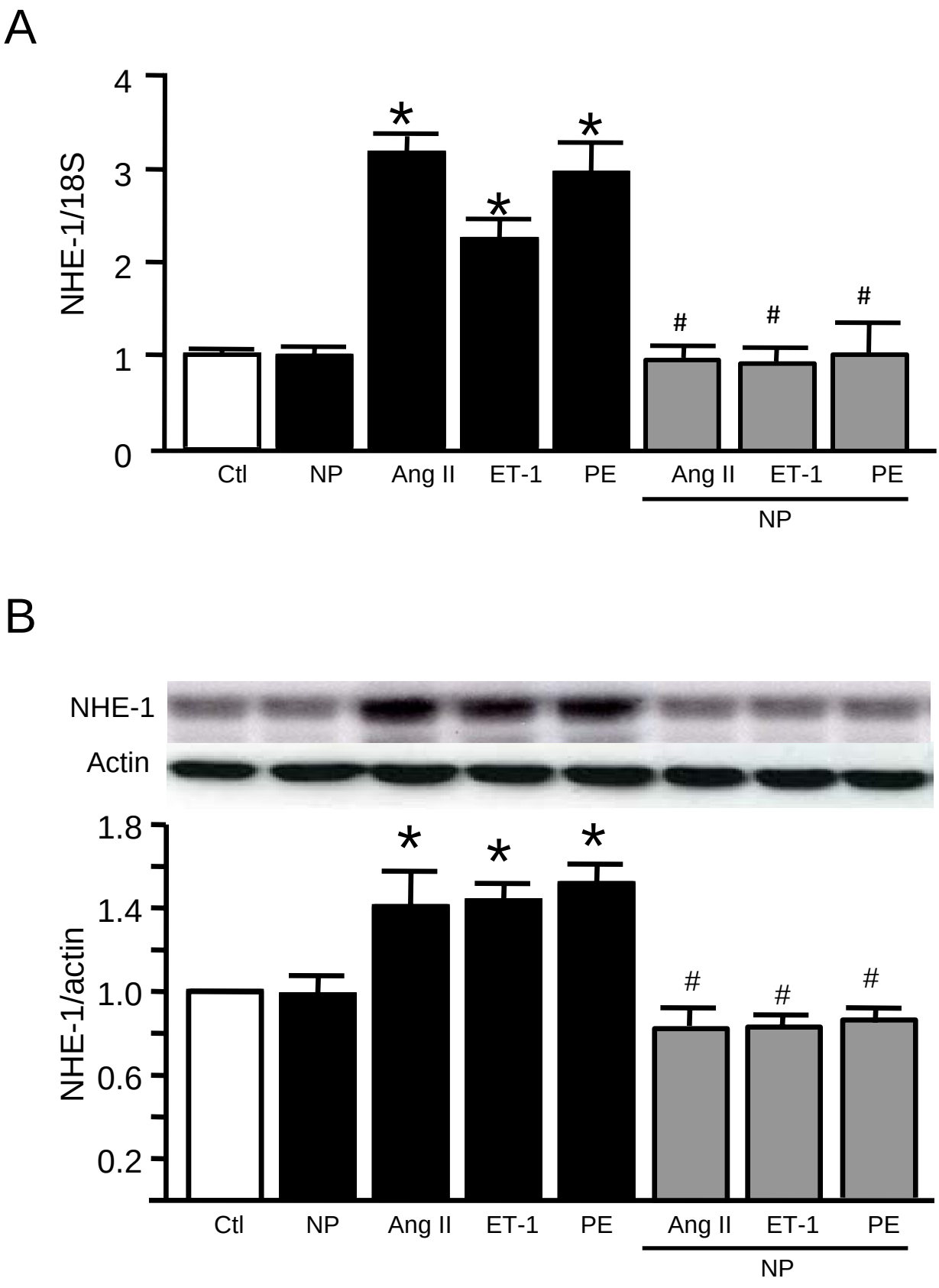
Suppl Figure 5. Lack of effect of CU-NP on hypertrophic responses in the presence of the natriuretic receptor blocker lysophosphatidic acid (LPA, 10 μM) in terms of cell surface area (panel A) and ANP expression (panel B). Data represent 24 h treatment with PE (10 μM), ang II (100 nM) or ET-1 (10 nM) and indicate means ± SEM. Values for ANP indicate fold increase. N=6 per group; *P<0.05 vs control. Ctl, control; PE, phenylephrine; Ang II, angiotensin II; ET-1, endothelin 1.

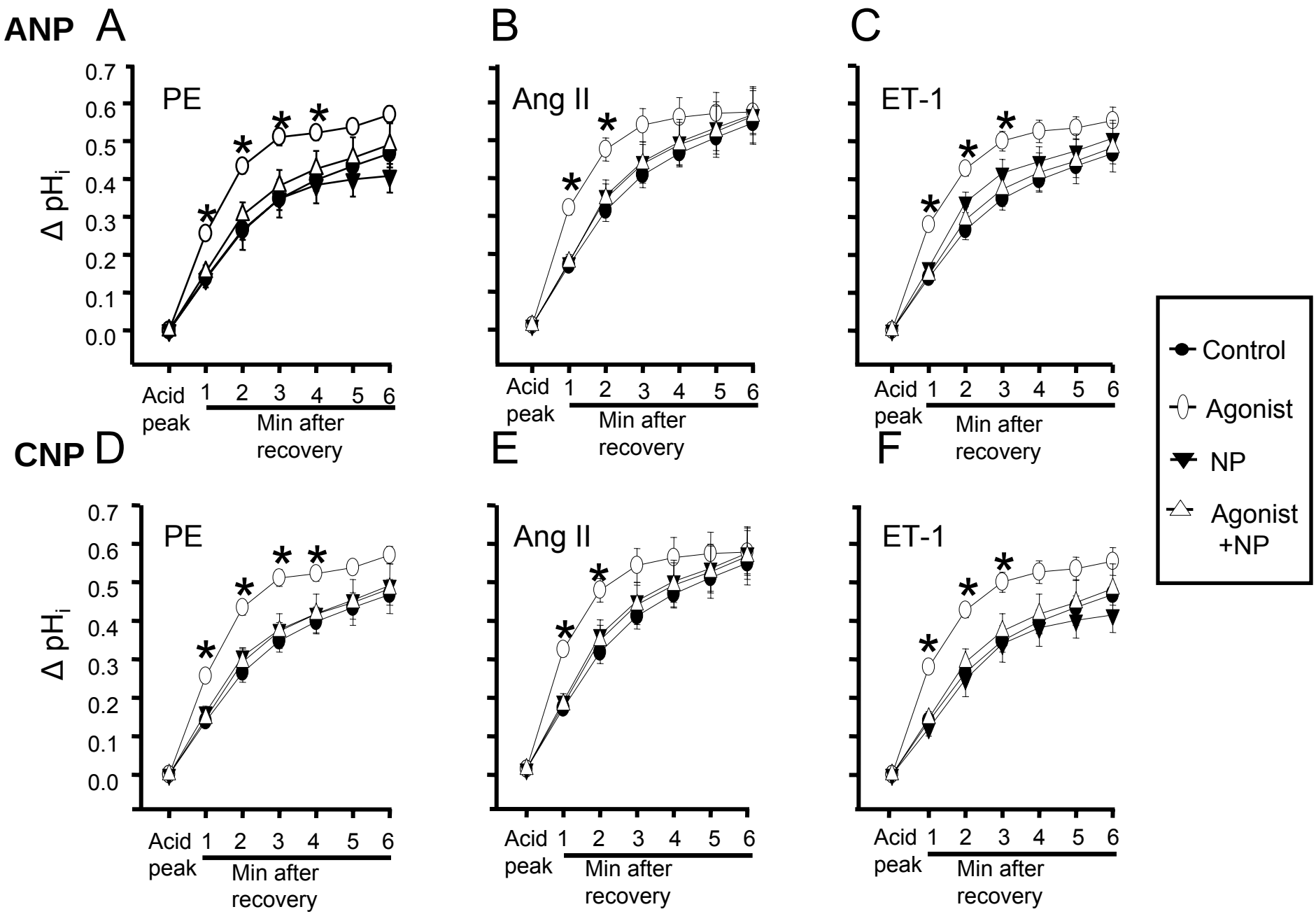
Suppl Figure 6. Lack of effect of CU-NP on NHE-1 upregulation in the presence of the natriuretic receptor blocker lysophosphatidic acid (LPA, 10 μM) in terms of NHE-1 gene expression (panel A), protein expression (panel B) and NHE-1 activity following NH_4Cl pulsing (panel C). Data represent 24 h treatment with PE (10 μM), ang II (100 nM) or ET-1 (10 nM) and indicate means ± SEM. Values in panels A and B indicate fold increase. Data with hypertrophic agonists in panel C were pooled for clarity as all agonists produced identical effects on pH_i recovery and all treatments were carried out in the presence of LPA. N=6 (panels A and B) or 9

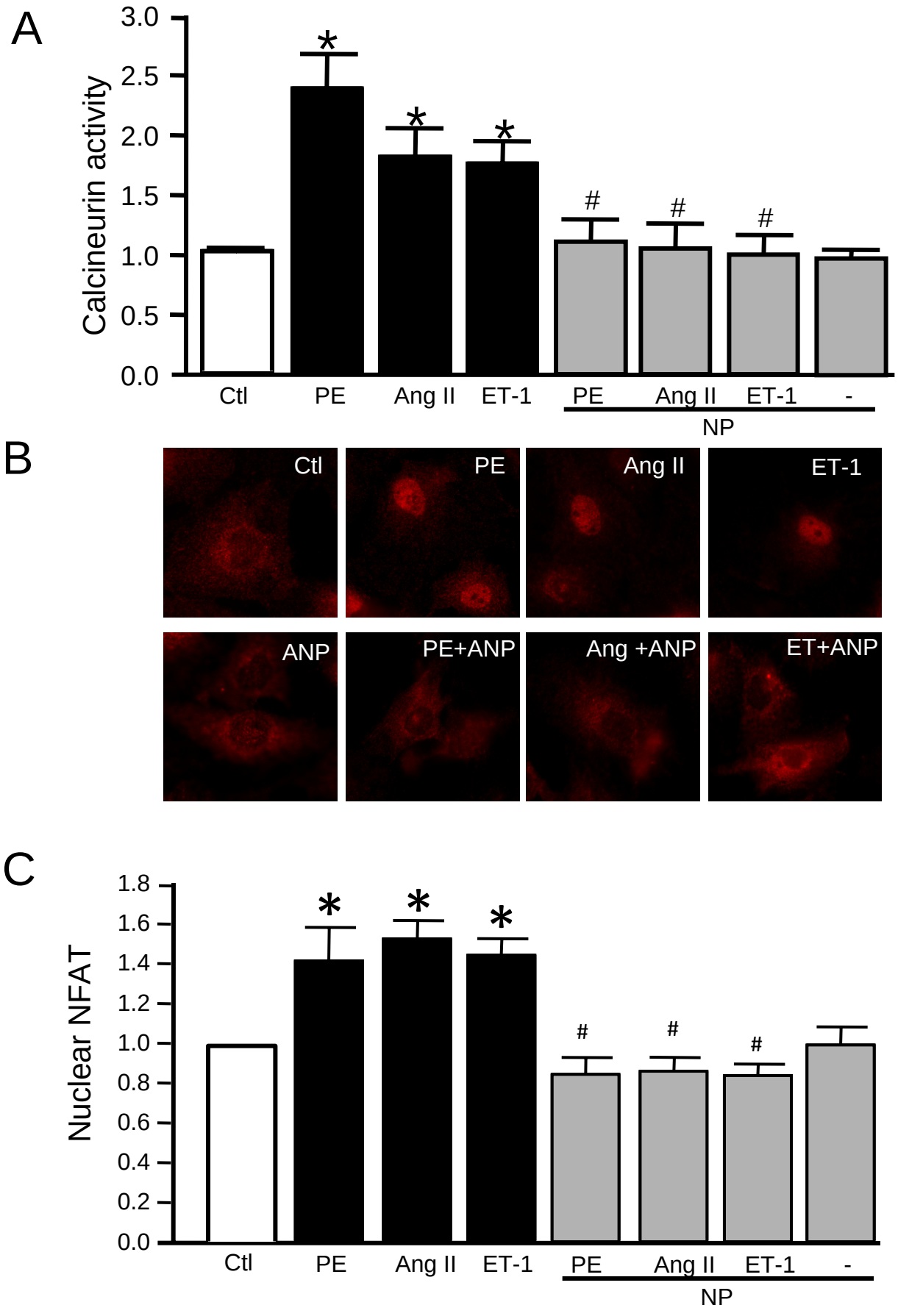
(panel C) per group; *P<0.05 vs control. Ctl, control; PE, phenylephrine; Ang II, angiotensin II; ET-1, endothelin 1.

Suppl Figure 7. Lack of effect of CU-NP on calcineurin activation in the presence of the natriuretic receptor blocker lysophosphatidic acid (LPA, 10 μM). Data represent 24 h treatment with PE (10 μM), ang II (100 nM) or ET-1 (10 nM) and indicate means ± SEM. Values indicate fold increase. N=6 per group; *P<0.05 vs control. Ctl, control; PE, phenylephrine; Ang II, angiotensin II; ET-1, endothelin 1.

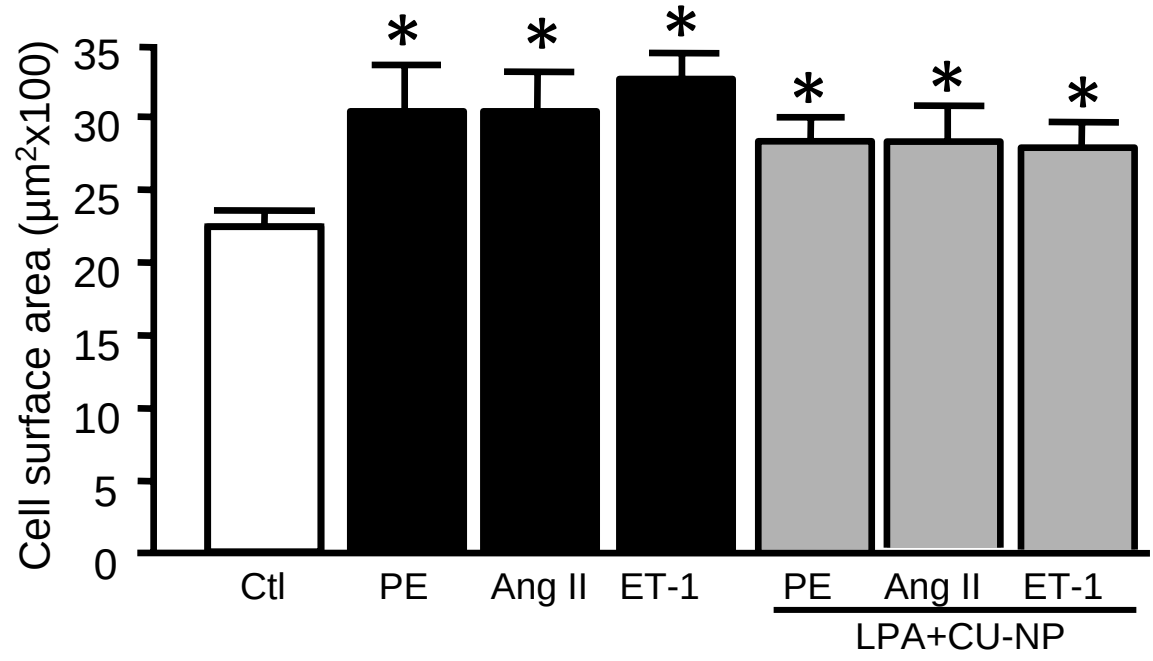

A
B
C
D
E
F
Cell surface area (μm²x100)
ANP/18S
Cell surface area (μm2x100)
ANP/18S
CNP
ANP
Ctl
-
ANP
CNP
PE
Ang II
ET-1
*
#


A
NHE-1/18S
4
3
2
1
0
*
*
*
#
#
#
Ctl
NP
Ang II
ET-1
PE
Ang II
ET-1
PE
NP
B
NHE-1
Actin
NHE-1/actin
1.8
1.4
1.0
0.6
0.2
*
*
*
#
#
#
Ctl
NP
Ang II
ET-1
PE
Ang II
ET-1
PE
NP

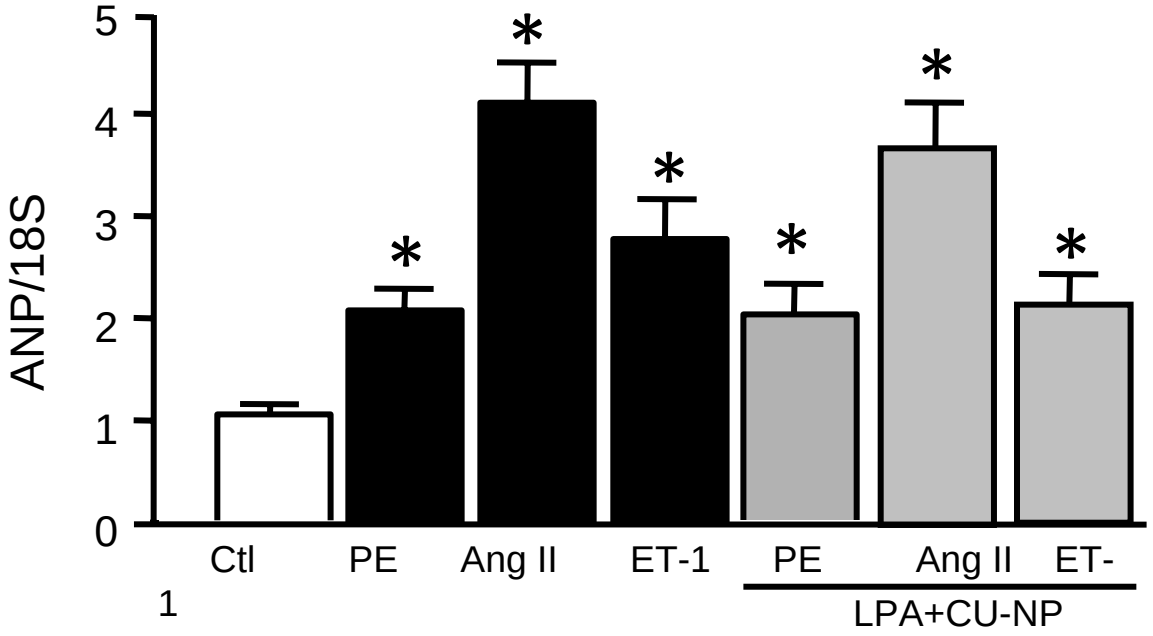

ANP
A
PE
B
Ang II
C
ET-1
CNP
D
PE
E
Ang II
F
ET-1
Δ pH$_i$
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Acid peak
1 2 3 4 5 6
Min after recovery
*
Control
Agonist
NP
Agonist +NP


A
Calcineurin activity
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
#
#
#
Ctl
PE
Ang II
ET-1
PE
Ang II
ET-1
-
NP
B
Ctl
PE
Ang II
ET-1
ANP
PE+ANP
Ang +ANP
ET+ANP
C
Nuclear NFAT
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
*
#
#
#
Ctl
PE
Ang II
ET-1
PE
Ang II
ET-1
-
NP

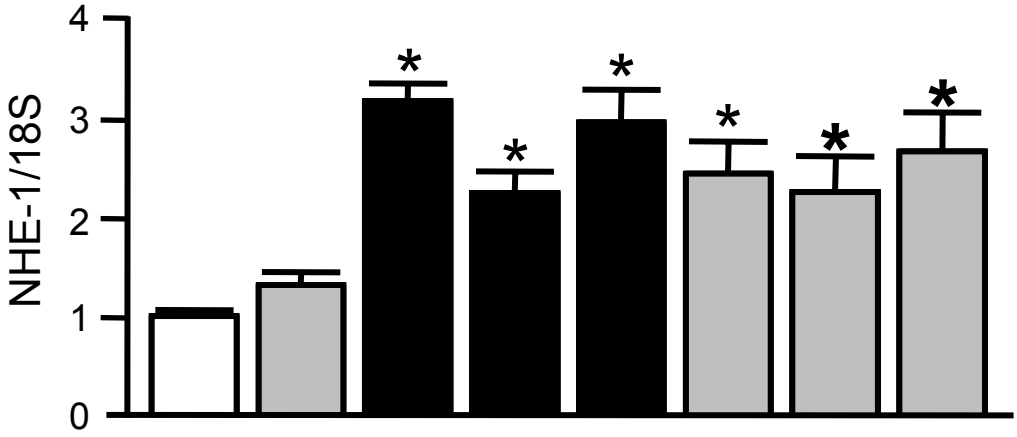
A


Cell surface area (μm²x100)
35
30
25
20
15
10
5
0
*
Ctl
PE
Ang II
ET-1
PE
Ang II
ET-1
LPA+CU-NP

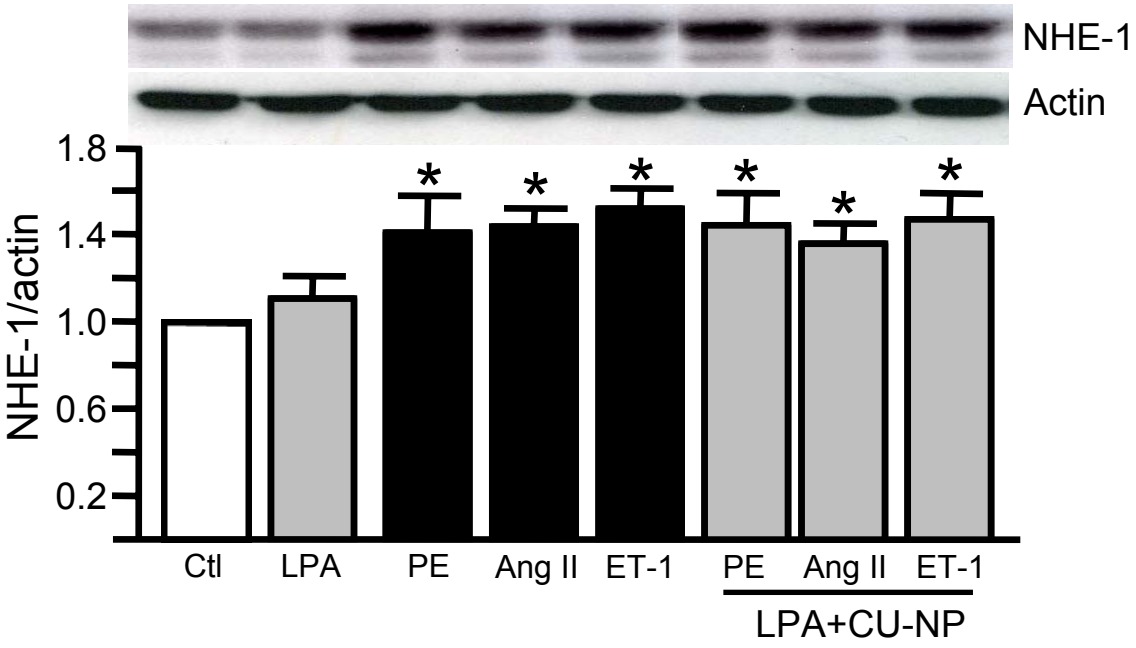
B


ANP/18S
5
4
3
2
1
0
*
Ctl
PE
Ang II
ET-1
PE
Ang II
ET-
1
LPA+CU-NP

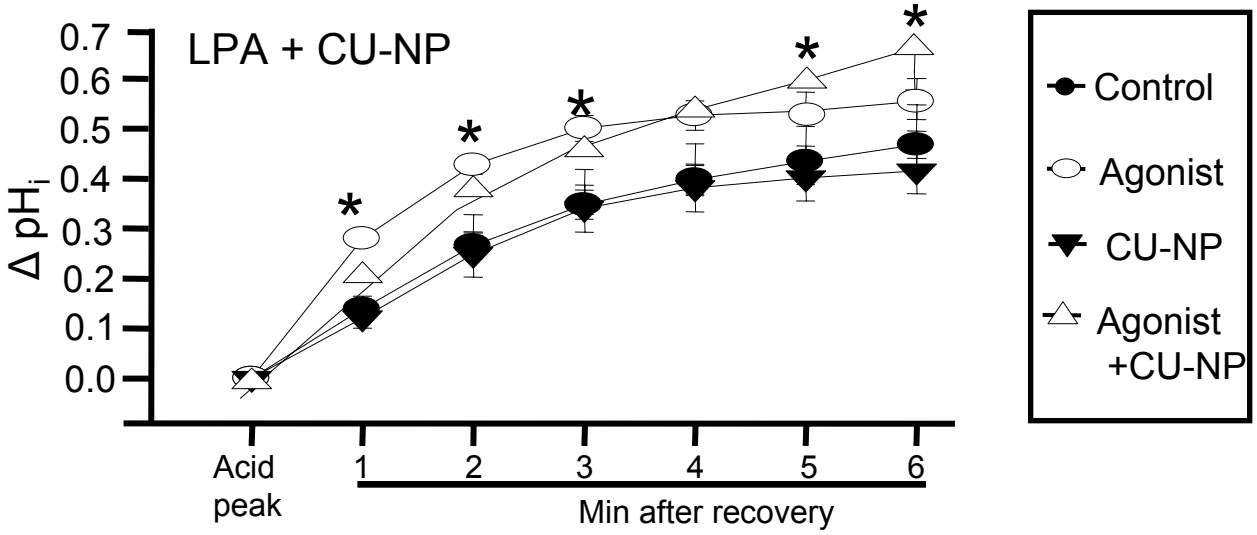
A

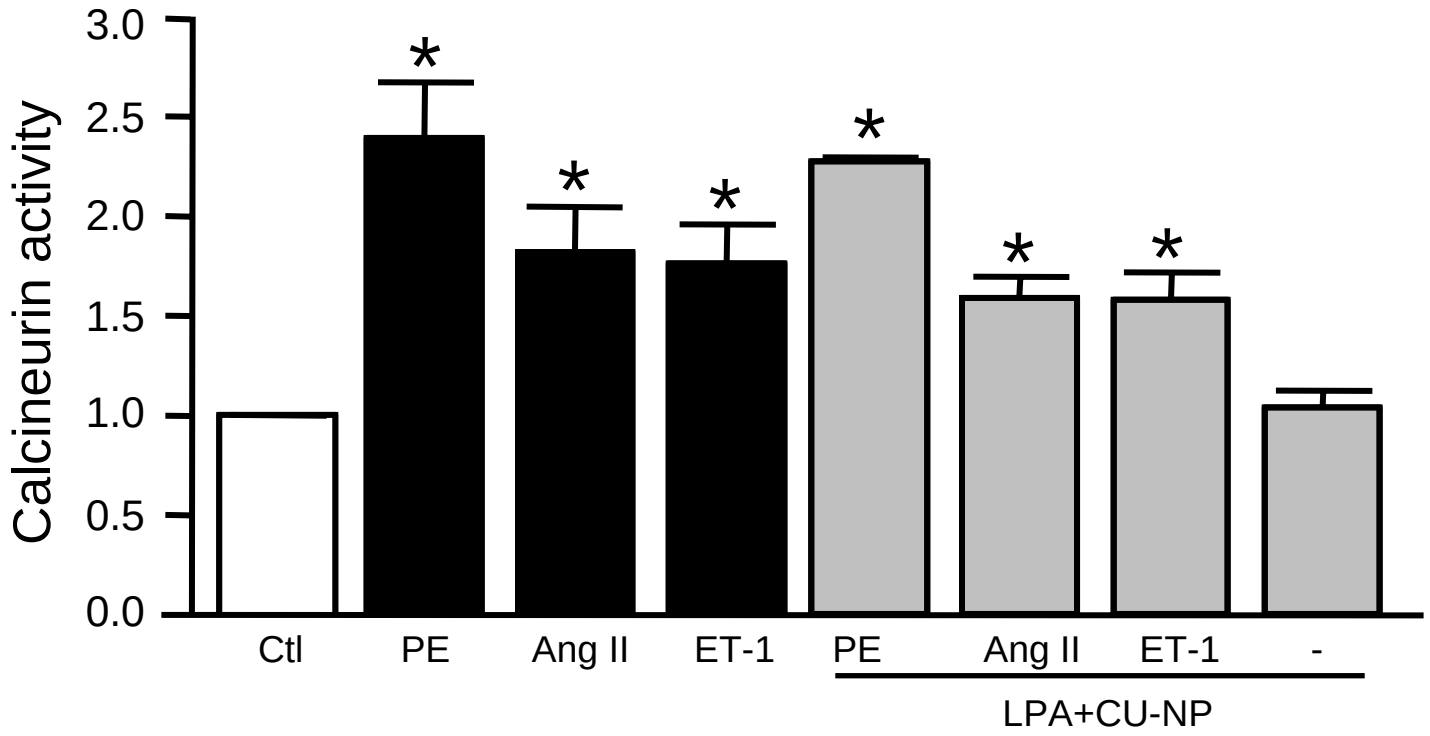

NHE-1/18S
4
3
2
1
0
*

B


NHE-1
Actin
NHE-1/actin
1.8
1.4
1.0
0.6
0.2
Ctl
LPA
PE
Ang II
ET-1
PE
Ang II
ET-1
LPA+CU-NP
*

C


LPA + CU-NP
Δ pH$_i$
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Acid peak
1
2
3
4
5
6
Min after recovery
Control
Agonist
CU-NP
Agonist +CU-NP
*


Calcineurin activity
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
*
*
*
Ctl
PE
Ang II
ET-1
PE
Ang II
ET-1
-
LPA+CU-NP