Supplemental Tables

TABLE S1. Probe and primer sequences for qRT-PCR analysis

Gene name	Gene symbol	Probe[a]	Primers[b]
TGF-β1	*Tgfb1*	AGTGGCTGAACCAAGGAGACGGAA	*f* GACGTCACTGGAGTTGTACGG
			r GCTGAATCGAAAGCCCTGT
CTGF	*Ctgf*	CCTGGGAAATGCTGCAAGGAGTGG	*f* TTCCCGAGAAGGGTCAAGCT
			r TCCTTGGGCTCGTCACACA
Fibronectin	*Fn1*	CCGACGAAGAGCCCTTACAGTTCCA	*f* ATCATTTCATGCCAACCAGTT
			r TCGCACTGGTAGAAGTTCCA
Collagen type I alpha 1	*Col1a1*	TGCTGTGCTTTCTGCCCGGA	*f* GTCCCAACCCCCAAAGAC
			r CATCTTCTGAGTTTGGTGATACGT
Collagen type III	*Col3a1*	TCCCACTCTTATTTTGGCACAGCAGTC	*f* TGGTTTCTTCTCACCCTTCTTC
			r TGCATCCCAATTCATCTACGT
18S rRNA	*Rn18s*	ACCGGCGCAAGACGGACCAG	*f* GCCGCTAGAGGTGAAATTCTTG
			r CATTCTTGGCAAATGCTTTCG

Sequences are listed 5' to 3'. [a]Probes were 5' end-labeled with FAM (reporter) and 3' end-labeled with TAMRA (quencher). [b]Forward primers are designated by f and reverse primers by r.

TABLE S2. The plasma and organ concentration of Y-27632 in wild-type and cGK $I^{+/-}$ mice

Genotype	n	Plasma (μmol/L)	Aorta (μmol/kg)	Heart (μmol/kg)	Kidney (μmol/kg)	Lung (μmol/kg)
WT	3	0.81 ± 0.13	16.59 ± 6.97	4.93 ± 0.58	11.97 ± 0.08	11.61 ± 2.05
cGK $I^{+/-}$	4	0.63 ± 0.14	14.96 ± 1.61	3.57 ± 0.36[a]	10.00 ± 1.41	7.77 ± 1.82

Values are means ± SEM. [a]$P = 0.0165$ vs. WT mice.

TABLE S3. The hemodynamic profile of cGK $I^{+/-}$ mice

Genotype and treatment	n	BW (g)	sBP (mmHg)	mBP (mmHg)	dBP (mmHg)	HR (beats/min)
WT	4	31.4 ± 1.4	109.0 ± 2.5	77.1 ± 1.9	61.1 ± 1.7	554.0 ± 13.4
WT + AII	10	27.2 ± 0.5[a]	141.6 ± 6.7[a]	101.9 ± 6.6[a]	82.2 ± 6.8[a]	522.3 ± 37.7
WT + AII + Y	5	27.3 ± 0.4[a]	145.1 ± 2.0[a]	105.9 ± 3.8[a]	86.4 ± 5.0[a]	599.3 ± 63.9
cGK $I^{+/-}$	5	31.8 ± 1.5	108.0 ± 7.8	75.1 ± 4.5	58.7 ± 3.2	539.8 ± 36.5
cGK $I^{+/-}$ + AII	10	27.4 ± 0.7[b]	162.4 ± 3.1[bc]	122.1 ± 4.1[bc]	101.6 ± 4.9[bc]	555.7 ± 42.4
cGK $I^{+/-}$ + AII + Y	6	27.7 ± 1.1[b]	163.8 ± 4.4[bc]	118.9 ± 2.0[bc]	97.2 ± 1.6[bc]	540.5 ± 20.1

Values are means ± SEM. AII, angiotensin II; Y, Y-27632; BW, body weight; sBP, systolic blood pressure; mBP, mean blood pressure; dBP, diastolic blood pressure; HR, heart rate. [a]$P < 0.01$ vs. WT mice. [b]$P < 0.01$ vs. cGK $I^{+/-}$ mice. [c]$P < 0.01$ vs. WT mice with AII infusion.

TABLE S4. The hemodynamic profile of RhoA-Tg and BNP-Tg mice

Genotype	n	BW (g)	sBP (mmHg)	mBP (mmHg)	dBP (mmHg)	HR (beats/min)
NTg	5	25.3 ± 0.4	119.8 ± 1.9	91.6 ± 2.1	77.8 ± 2.6	637.4 ± 23.3
$RhoA^{WT}$-Tg	8	25.4 ± 0.7	117.2 ± 5.7	88.5 ± 4.0	74.3 ± 3.4	601.4 ± 26.4
$RhoA^{A188}$-Tg	7	23.9 ± 1.6	120.3 ± 3.7	91.5 ± 2.3	77.1 ± 1.8	645.7 ± 22.3
BNP-Tg	11	25.8 ± 0.8	94.7 ± 2.3[a]	71.9 ± 1.8[a]	60.3 ± 1.7[a]	544.0 ± 28.1[a]
$RhoA^{WT}$/BNP-Tg	5	25.1 ± 0.8	99.5 ± 2.0[b]	69.4 ± 1.7[b]	54.2 ± 1.7[b]	527.8 ± 13.9
$RhoA^{A188}$/BNP-Tg	5	25.0 ± 0.9	88.2 ± 3.1[c]	66.5 ± 1.5[c]	55.6 ± 1.5[c]	523.6 ± 51.8[c]

Values are means ± SEM. Abbreviations are as depicted in the footnote for Table S3. [a]$P < 0.05$ vs. NTg. [b]$P < 0.05$ vs. $RhoA^{WT}$-Tg. [c]$P < 0.01$ vs. $RhoA^{A188}$-Tg.

Supplemental figure legends

Supplemental FIG. S1. Effect of cGK I hemizygosity on the myocardial interstitial fibrosis. Representative images (A) and the quantification (B) of Sirius Red-stained heart sections from cGK I+/– and WT mice untreated or treated with Ang II and Y-27632. Scale bar in (A), 0.1 mm. #, $P < 0.001$. Values are means ± SEM of 5 to 46 replicates.

Supplemental FIG. S2. The transgene expression is confined to arterial media in RhoA-Tg mouse hearts. Immunostaining with anti-myc tag antibody of the heart section from $RhoA^{A188}$-Tg mice. a, artery. v, vein. Scale bar, 50 μm.

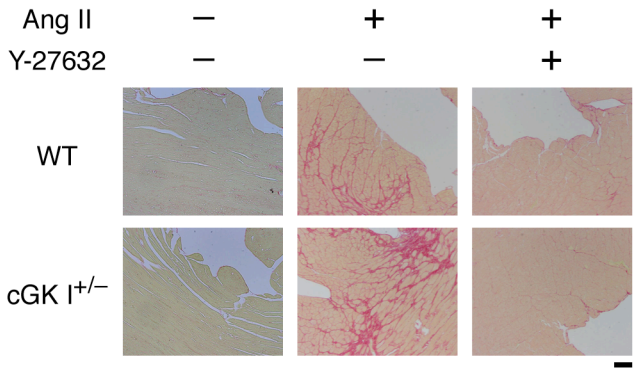
Supplemental FIG. S3. The transgene expression and activity in the MASMCs derived from RhoA-Tg mice. (A) Immunoblotting analysis of the MASMCs whole cell lysates with anti-RhoA antibody (upper left). Densitometric quantification of the endogenous RhoA protein abundance (lower left), the myc-RhoA protein abundance (upper right), and the myc-RhoA/endogenous RhoA ratio (lower right). Values are means ± SEM of triplicates. (B) MASMCs were untreated (Pre) or treated (Post) for 5 minutes with thrombin (1 U/ml), the well established RhoA agonist, and subjected to cellular fractionation. Left panel; the protein level of endogenous RhoA and myc-RhoA in the membrane and cytosolic fractions was determined by immunoblotting analysis. Right panel; densitometric quantification of RhoA protein abundance in the membrane fraction of thrombin-stimulated cells. Values represent means of duplicates.

Supplemental FIG. S4. Transgenic expression of A188 RhoA causes exaggerated activation of ROCK, unresponsiveness to BNP, and increased Ser188 phosphorylation of endogenous RhoA. (A) Immunoblot showing the P-moesin and moesin level in the aortas of NTg and $RhoA^{A188}$-Tg mice untreated or treated with Ang II. (B, C) Immunoblot analysis of the aortas of NTg, $RhoA^{wt}$-Tg, $RhoA^{A188}$-Tg, BNP-Tg, $RhoA^{wt}$/BNP-Tg and $RhoA^{A188}$/BNP-Tg mice. (B) The level of P-moesin and moesin. (C) The level of P-RhoA and RhoA. (D) Immunoblot showing the level of P-VASP Ser237, P-RhoA, cGK I and ROCK in the aortas of $RhoA^{wt}$-Tg and $RhoA^{A188}$-Tg mice. Values are means ± SEM of 3 to 6 replicates (A) or triplicates (B-D). *, $P < 0.05$.

Supplemental FIG. S5. Impact of BNP and ROCK on Ang II-induced effects in RhoA-Tg/BNP-Tg-background mouse tissues. (A) The relative ROCK activity of the aortas from the indicated mouse groups untreated or treated with Ang II was determined as P-moesin/moesin ratio by immunoblotting analysis. (B) Quantitative RT-PCR analysis of pro-fibrotic gene mRNA expression in the hearts of the RhoA-Tg/BNP-Tg-background NTg mice untreated or treated with Ang II and Y-27632. Values are means ± SEM of 3 to 4 replicates. *, $P < 0.05$.

Supplemental FIG. S6. Unresponsiveness of $RhoA^{A188}$-Tg MASMCs to the cGMP-mediated inhibition of LPA-induced actin polymerization. (A, B) Verification of the integrity of MASMC preparation by immunofluorescence of smooth muscle alpha-actin (A) and myc-tag (B). Nuclear staining was conducted with Hoechst 33258. (C) Phalloidin staining to visualize F-actin of serum-starved MASMCs without or with 2-hr pretreatment with 8-Br cGMP (0.5 mM) and 15-min stimulation with LPA (10 μM). Scale bar, 25 μm.

A


Ang II
Y-27632
WT
cGK I+/–

B

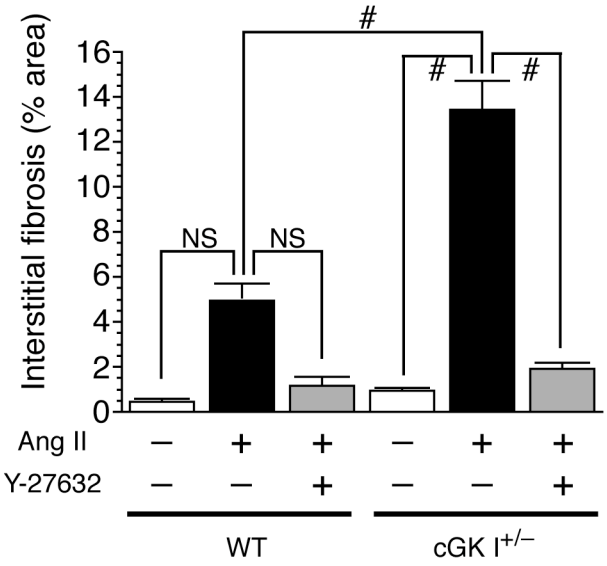

Interstitial fibrosis (% area)
16
14
12
10
8
6
4
2
0
#
#
#
NS
NS
Ang II
Y-27632
WT
cGK I+/–

Figure S1

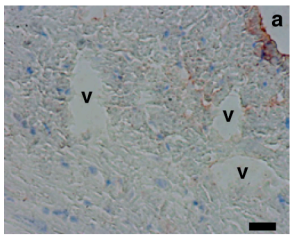


Figure S2

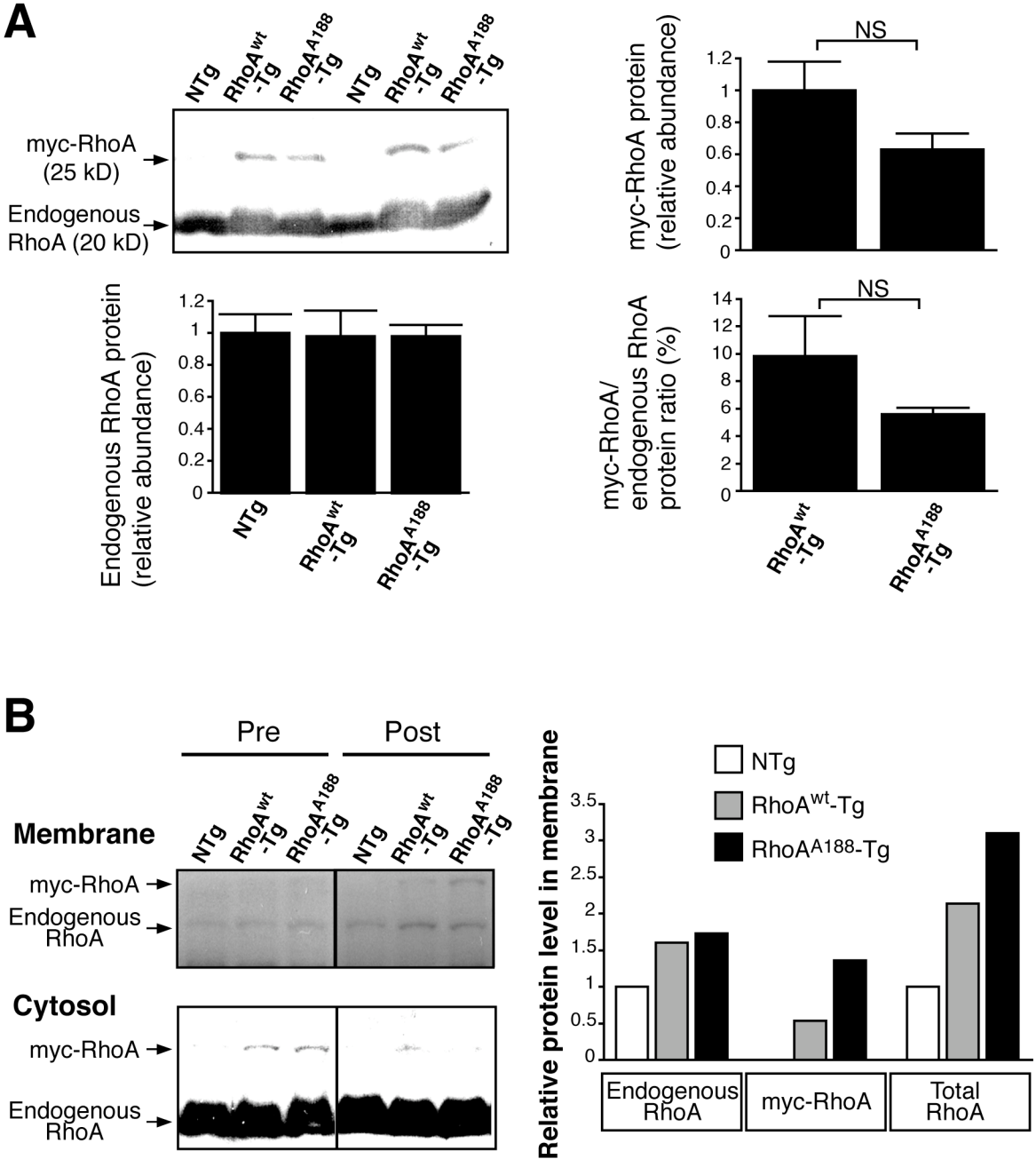

A
NTg
RhoA^wt -Tg
RhoA^A188 -Tg
myc-RhoA (25 kD)
Endogenous RhoA (20 kD)
myc-RhoA protein (relative abundance)
NS
Endogenous RhoA protein (relative abundance)
myc-RhoA/ endogenous RhoA protein ratio (%)
B
Pre
Post
Membrane
Cytosol
myc-RhoA
Endogenous RhoA
Relative protein level in membrane
NTg
RhoA^wt-Tg
RhoA^A188-Tg
Endogenous RhoA
myc-RhoA
Total RhoA

Figure S3

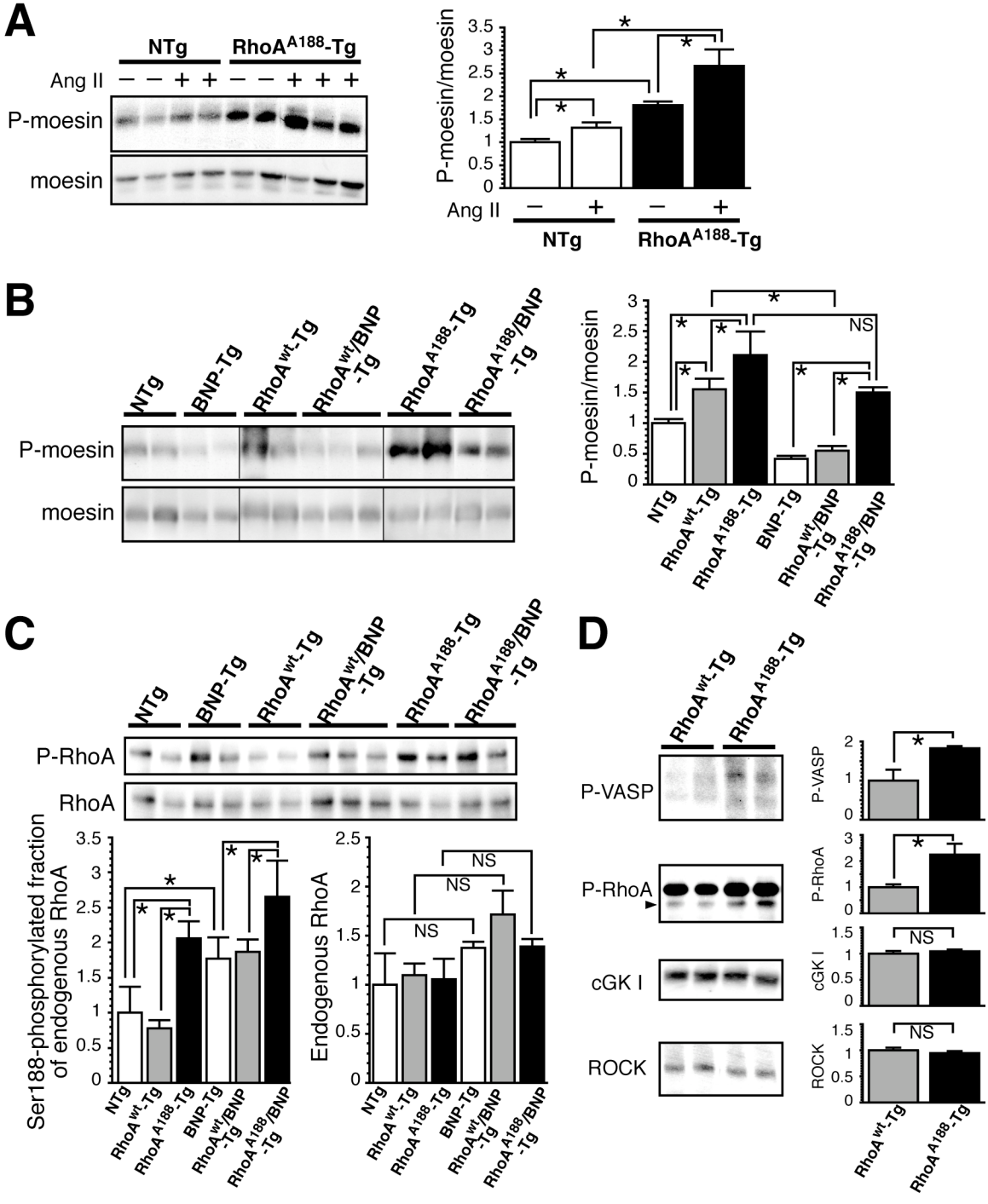

A
NTg
RhoA^A188-Tg
Ang II
P-moesin
moesin
P-moesin/moesin
B
NTg
BNP-Tg
RhoA wt-Tg
RhoA wt/BNP -Tg
RhoA A188-Tg
RhoA A188/BNP -Tg
NS
C
P-RhoA
RhoA
Ser188-phosphorylated fraction of endogenous RhoA
Endogenous RhoA
D
RhoA wt-Tg
RhoA A188-Tg
P-VASP
P-RhoA
cGK I
ROCK

Figure S4

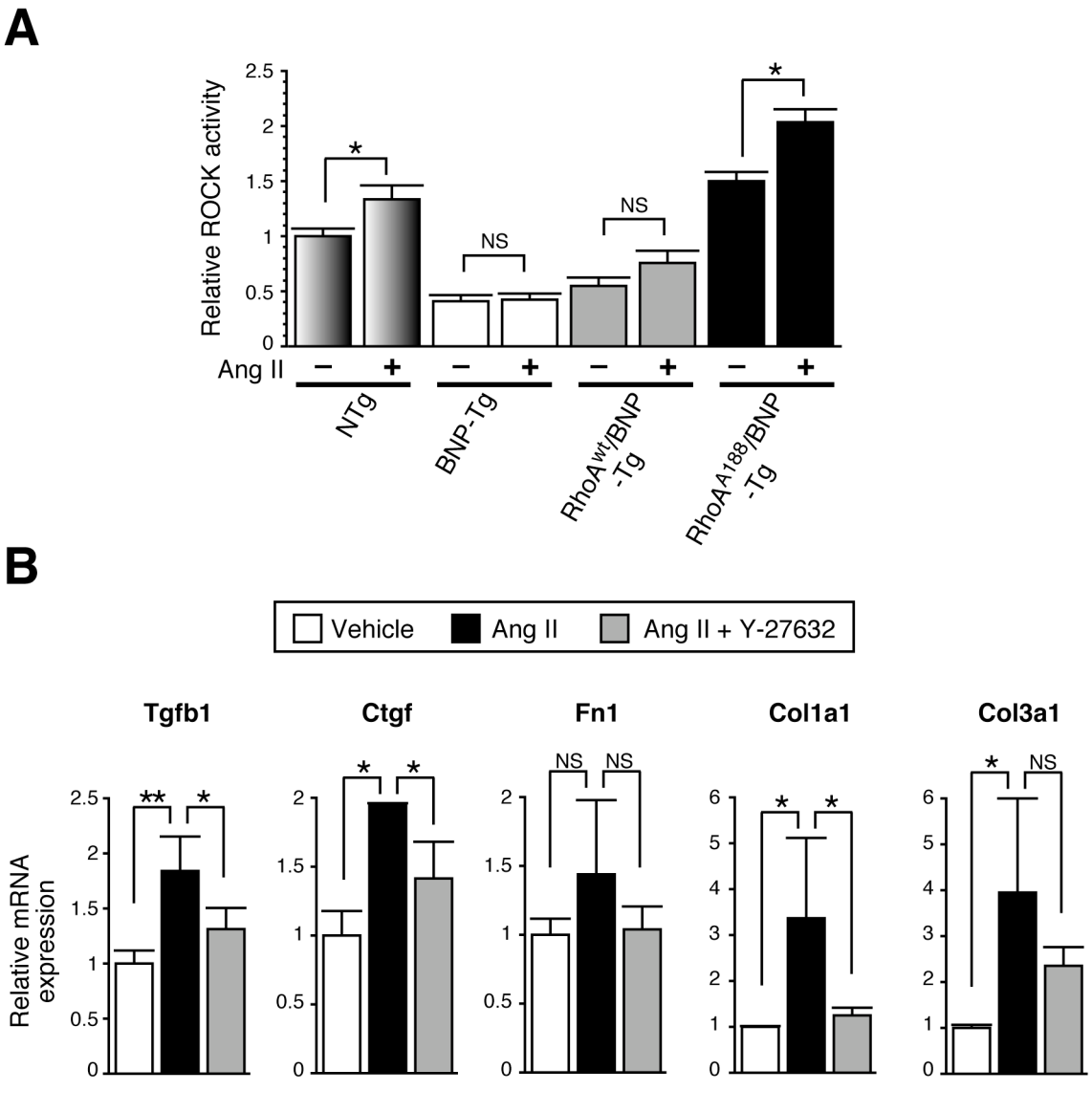

A
Relative ROCK activity
2.5
2
1.5
1
0.5
0
*
NS
NS
*
Ang II
–
+
–
+
–
+
–
+
NTg
BNP-Tg
RhoA^wt/BNP -Tg
RhoA^A188/BNP -Tg
B
Vehicle
Ang II
Ang II + Y-27632
Tgfb1
Ctgf
Fn1
Col1a1
Col3a1
Relative mRNA expression
**
*
NS
NS
6
5
4
3
2
1
0

Figure S5

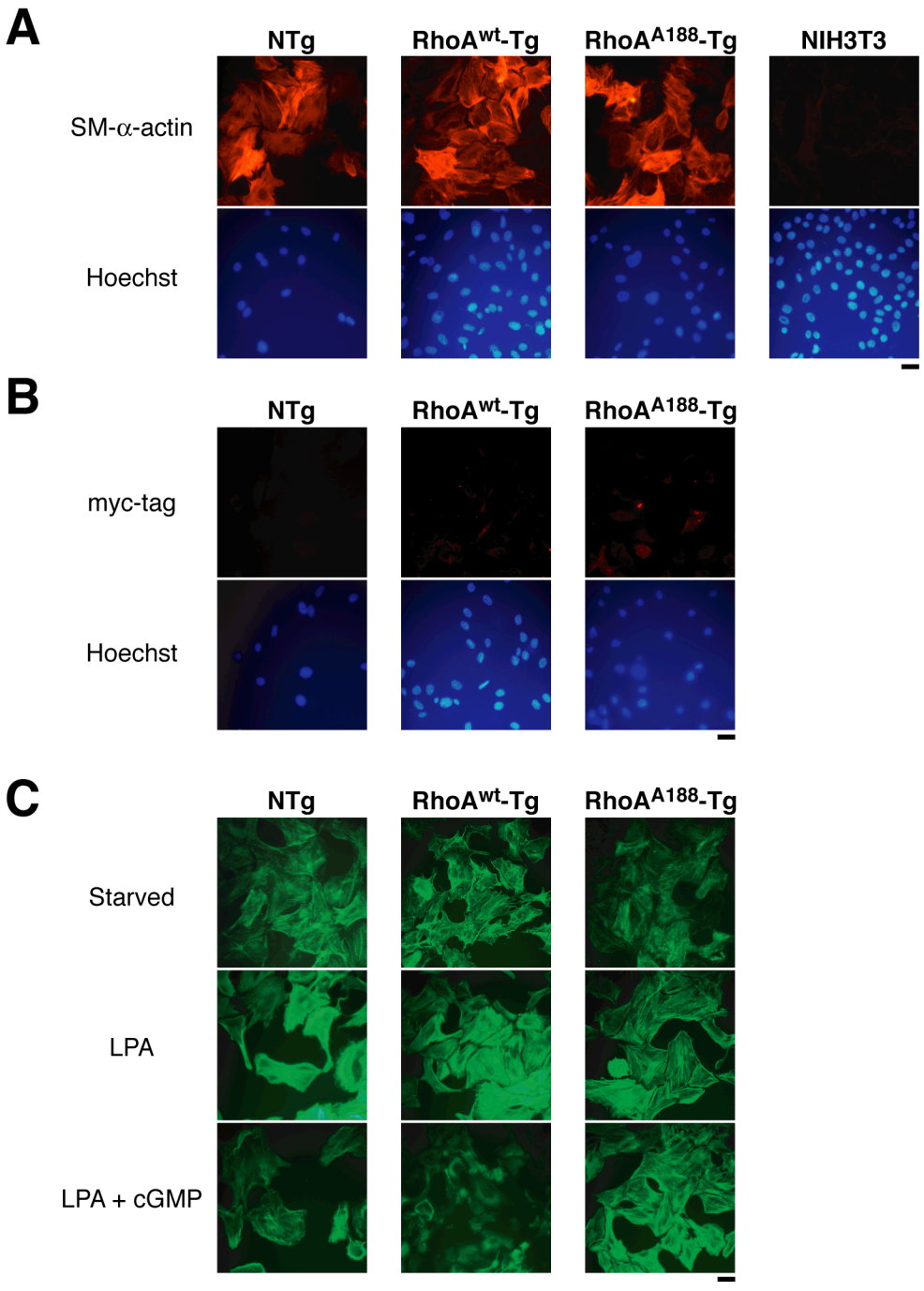

A
NTg
RhoA^wt-Tg
RhoA^A188-Tg
NIH3T3
SM-α-actin
Hoechst
B
NTg
RhoA^wt-Tg
RhoA^A188-Tg
myc-tag
Hoechst
C
NTg
RhoA^wt-Tg
RhoA^A188-Tg
Starved
LPA
LPA + cGMP

Figure S6