

**Notoginsenoside R1 inhibits vascular smooth muscle cell proliferation, migration  
and neointimal hyperplasia through PI3K/Akt signaling**

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**Running title:** NGR1 reduces neointimal hyperplasia

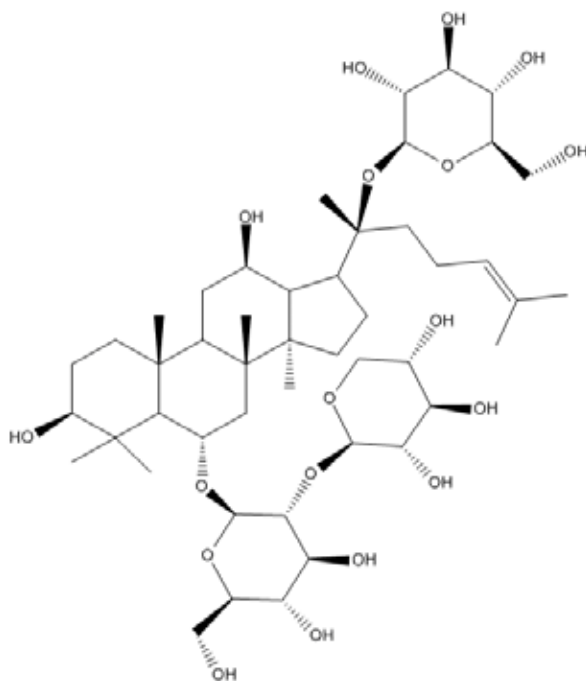
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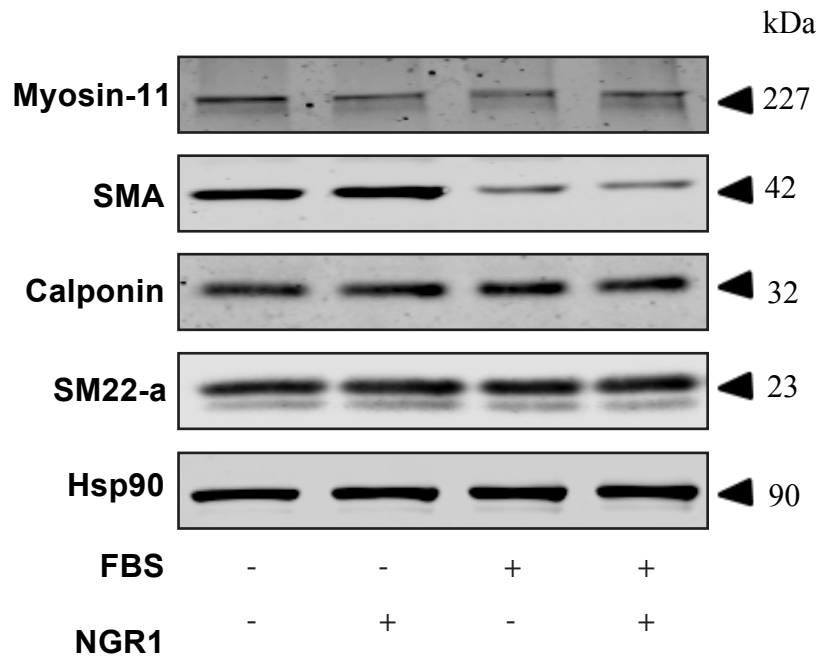
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## Supplement Figure 1



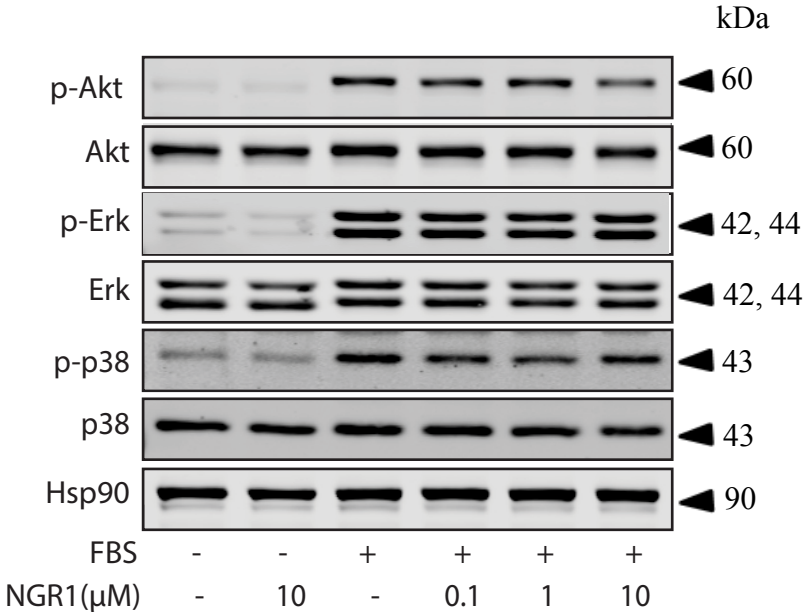
Supplement Figure 1. The chemical structure of NGR1.

## Supplement Figure 2



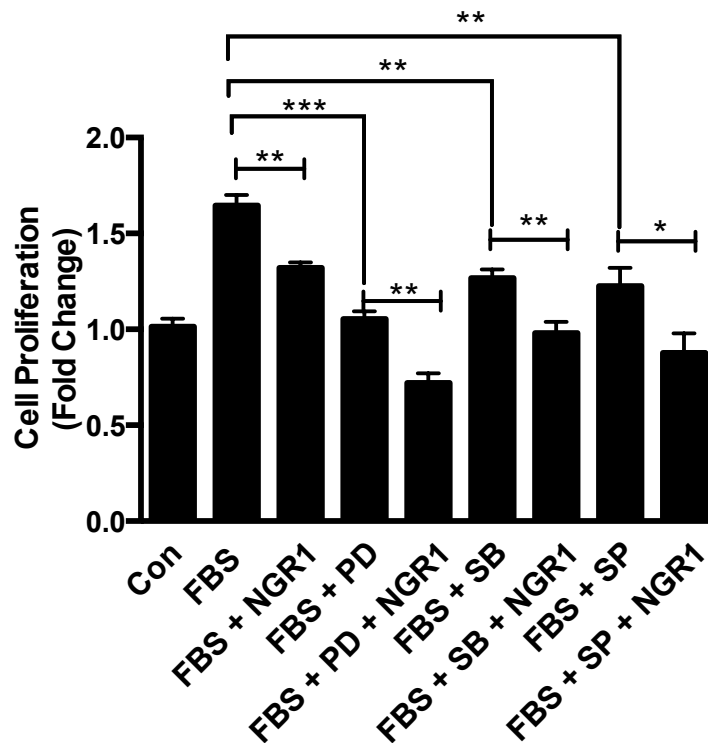
Supplement Figure 2. NGR1 treatment does not change VSMC contractile protein expression. Western blot analysis of cell lysate of hCASMCs that were treated with NGR1 for 24hrs and then stimulated with or without serum for 24hrs. The protein expression levels of Myosin-11, SMA, Calponin and SM22 alpha were blotted. Hsp90 was used as loading control. Representative western blots from 3 experiments are shown.

### Supplement Figure 3



Supplement Figure 3. NGR1 inhibits serum-induced hCASMC proliferation and migration specifically through PI3K/Akt signaling pathway in dose-dependent manner. Western blot analysis of hCASMCs that were treated with different concentration of NGR1 (0, 0.1, 1, 10 μM) for 24hrs and stimulated with 10 % FBS for 15 min. The protein expression levels of p-Akt/Akt, p-ERK/ERK, p-p38/p38 were blotted. Hsp90 was used as loading control. Representative western blots from 3 experiments are shown.

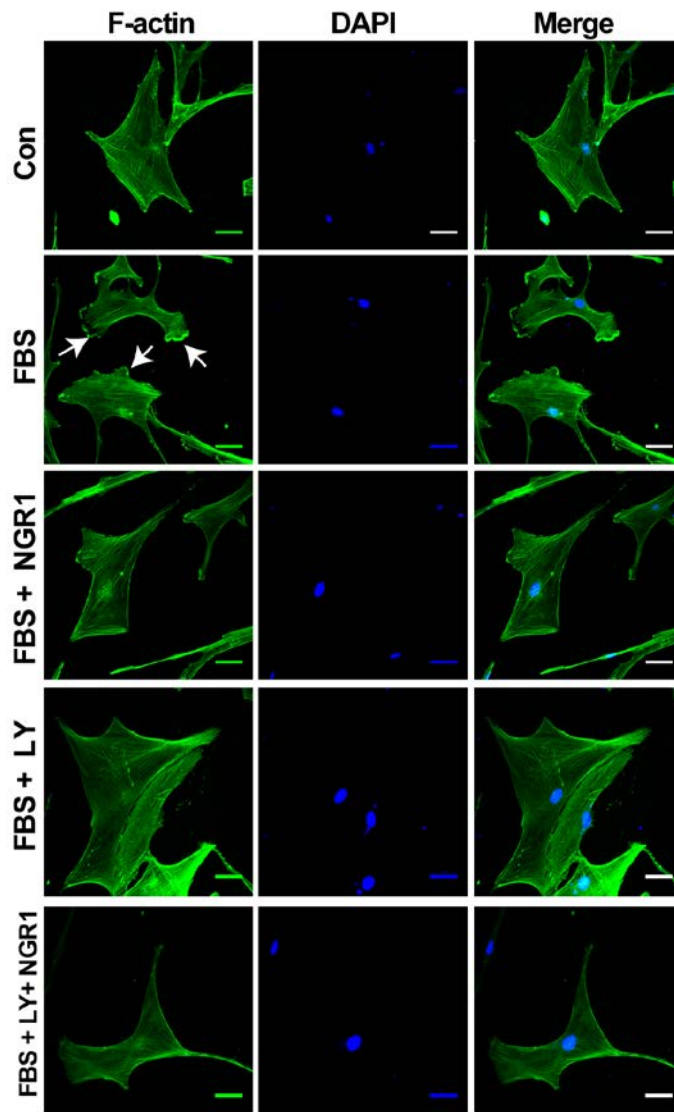
## Supplement Figure 4



Supplement Figure 4. MAPK small molecule inhibitors doses not constraint NGR1's inhibitory effect on hCASMCM proliferation.

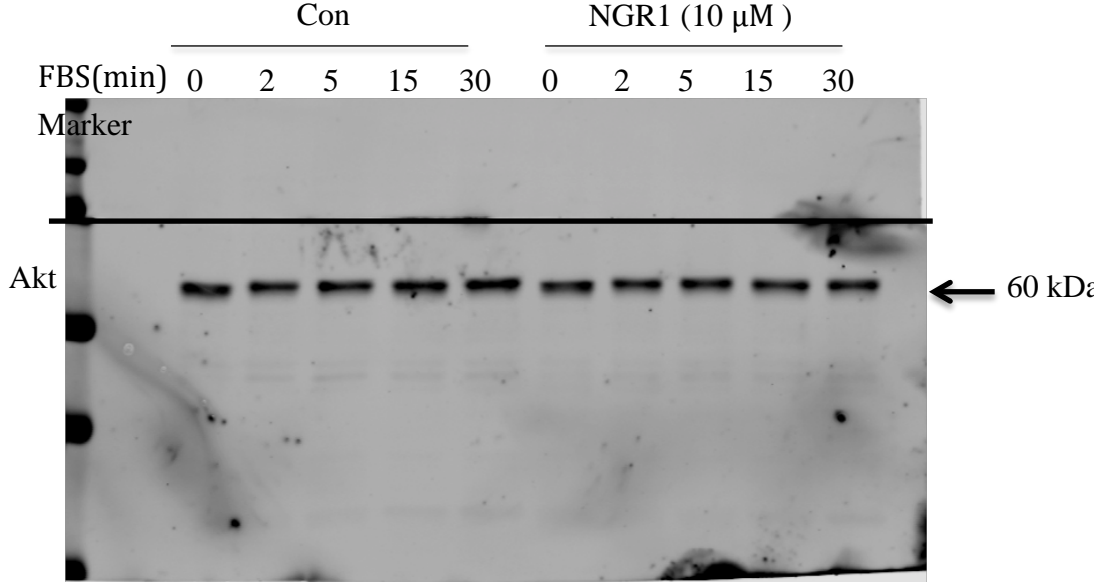
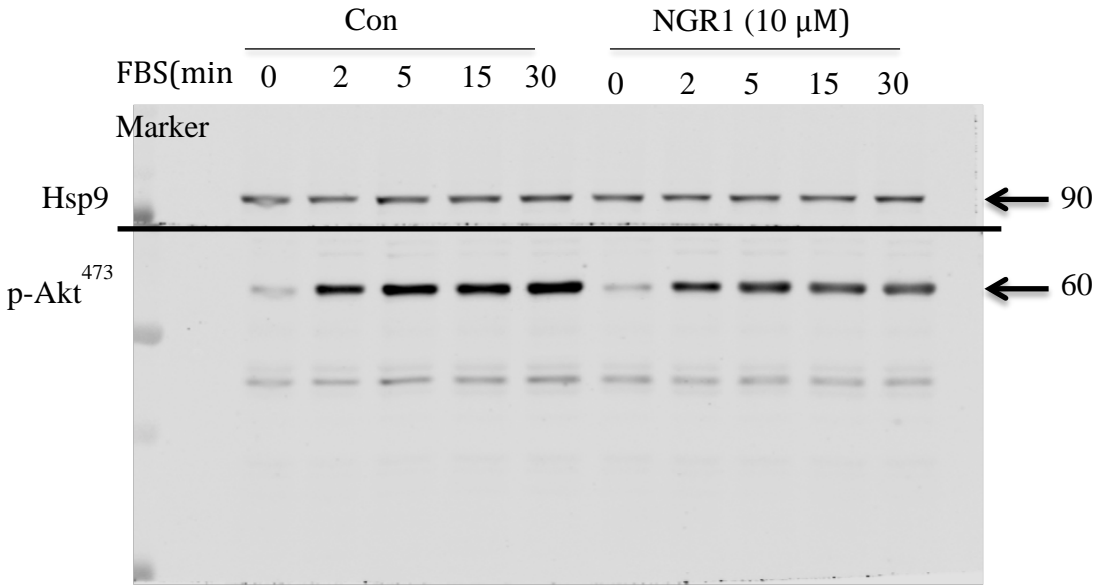
Quiescent hCASMCMs were treated with NGR1 (10  $\mu$ M) for 24hrs and incubated with PD98059 (50  $\mu$ M), SB203580 (20  $\mu$ M) or SP600125 (20  $\mu$ M) in combination of NGR1 (10  $\mu$ M) or vehicle 48hrs. Cells proliferation was determined by MTT assay. N=3 for each condition. Data shown are means  $\pm$  SEM. \*P < 0.05; \*\*P < 0.01; \*\*\* P < 0.001.

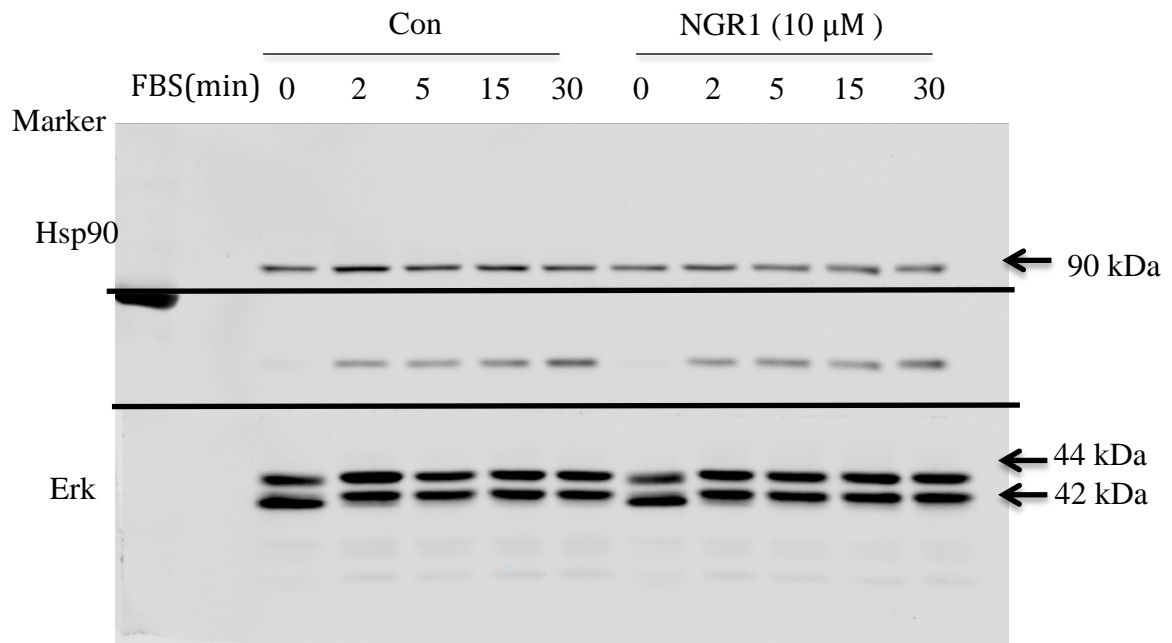
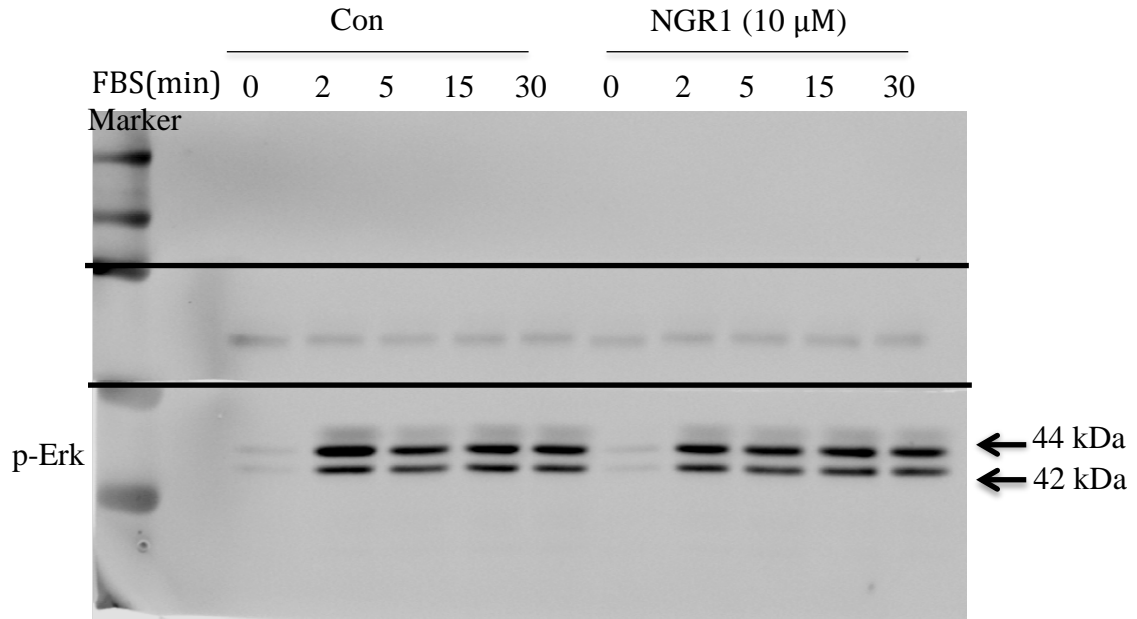
## Supplement Figure 5



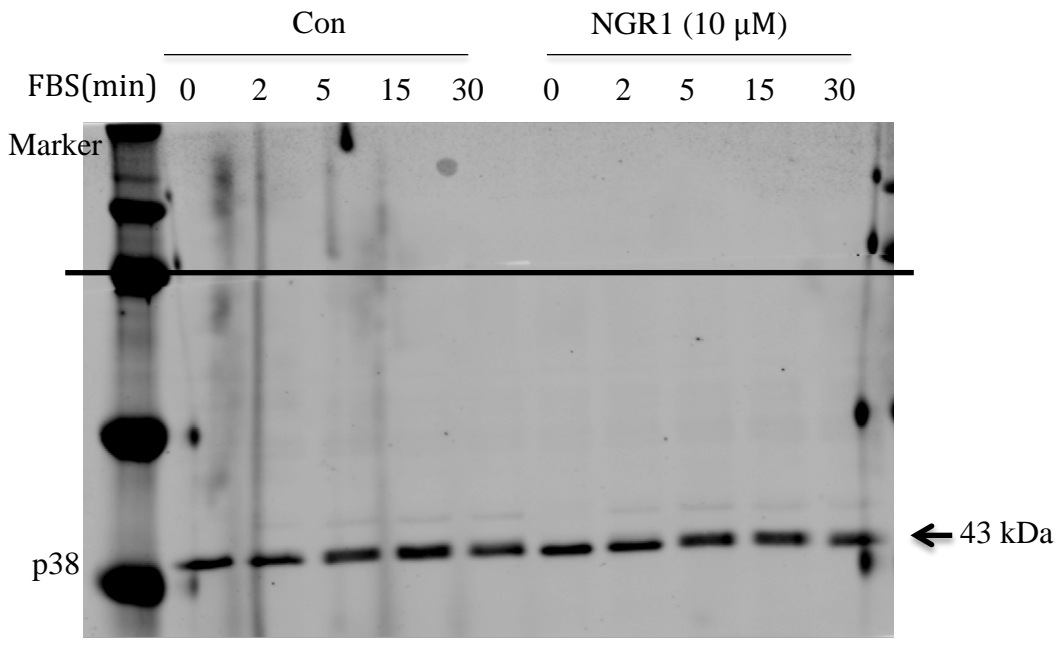
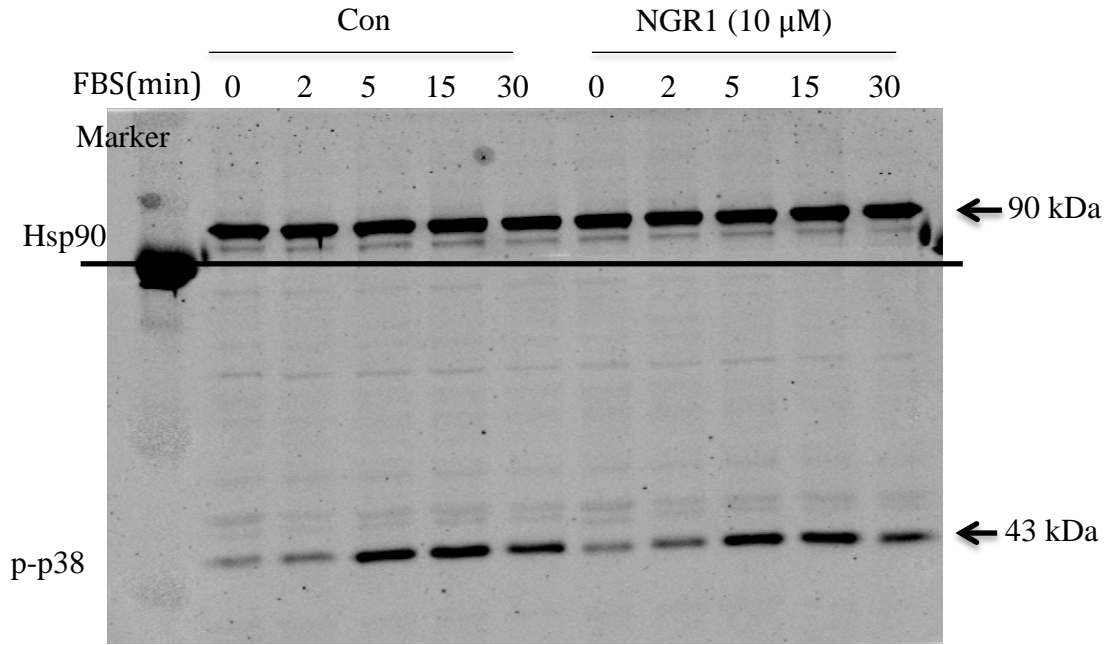
Supplement Figure 5. Representative images of F-actin (Alexa-488-tagged phalloidin) and DAPI stained hCASM cells that were non-treated (Con) or treated with FBS, FBS + NGR1 (10  $\mu$ M), FBS + LY294002 (20  $\mu$ M), or FBS + LY294002 + NGR1. Lamellipodia are indicated by the white arrows. Scale bar, 2  $\mu$ m.

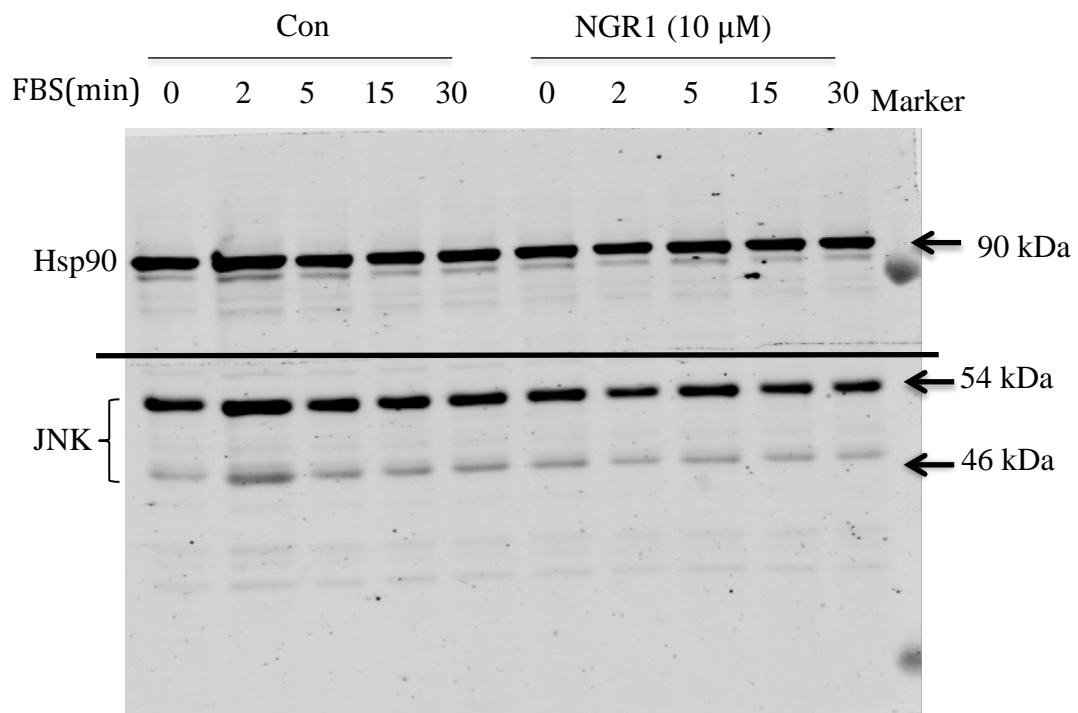
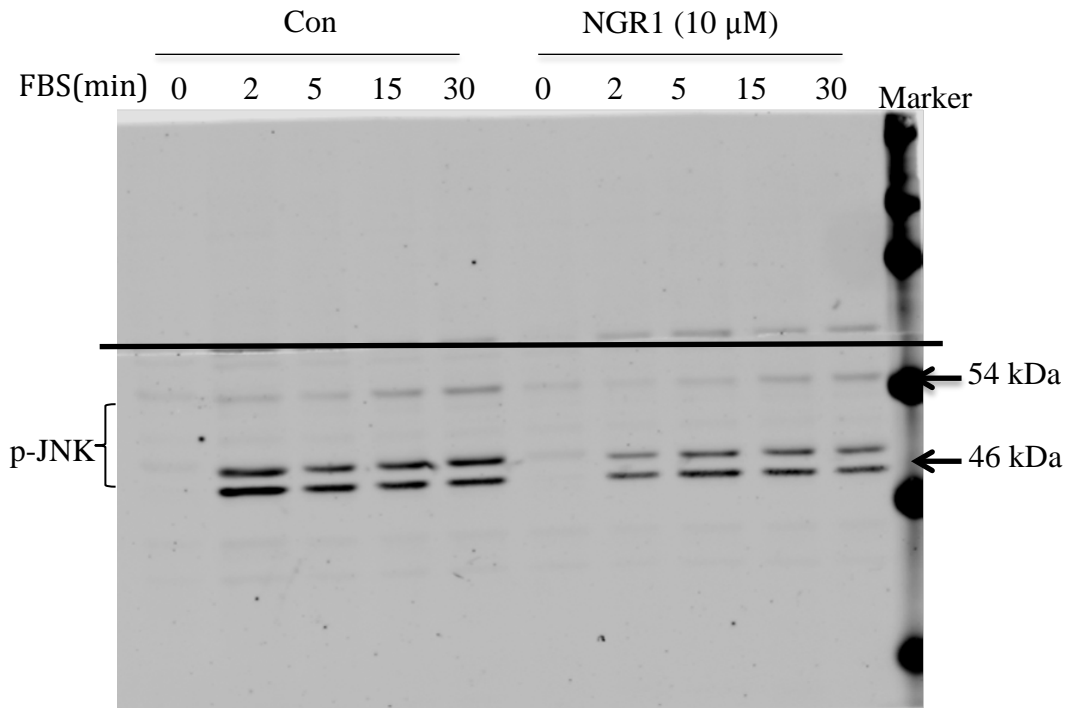
Original western blotting images for Fig. 4A.



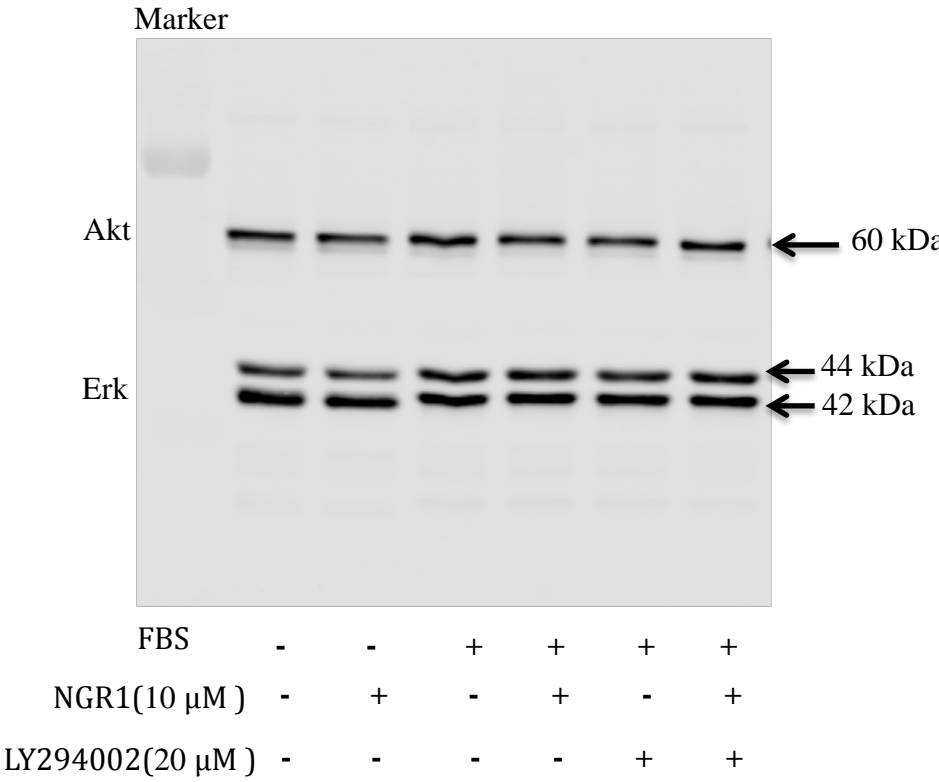
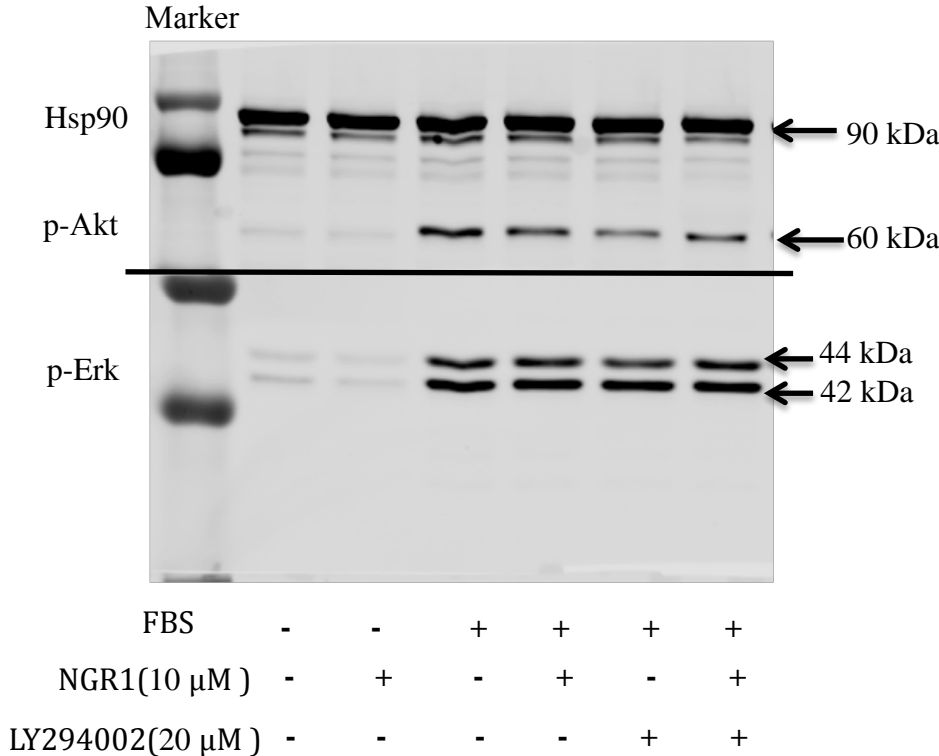




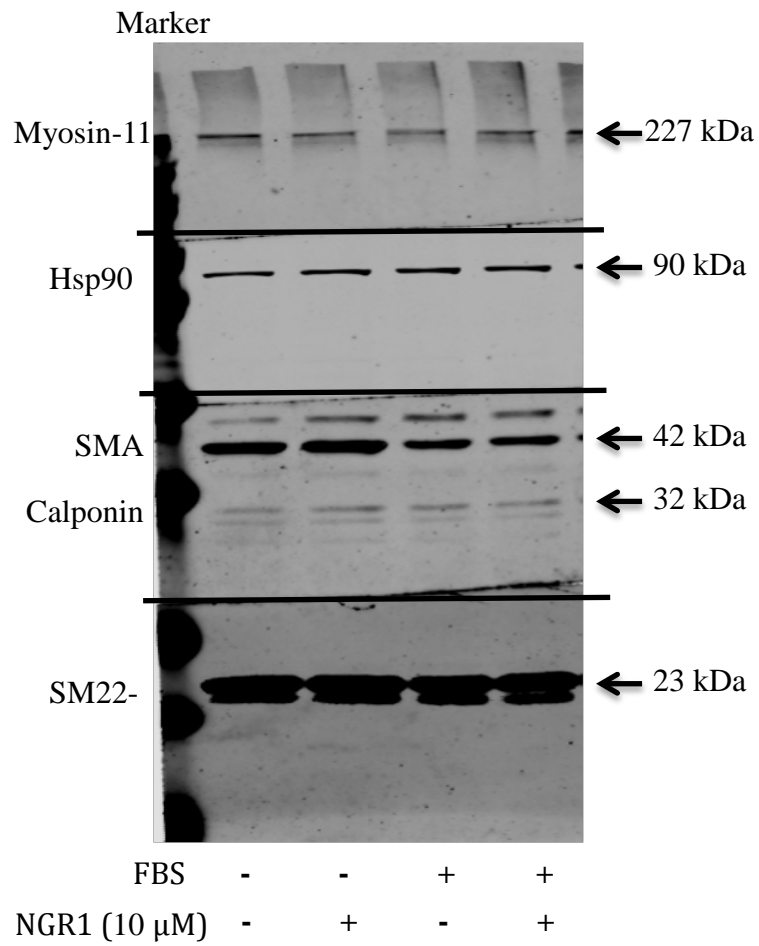




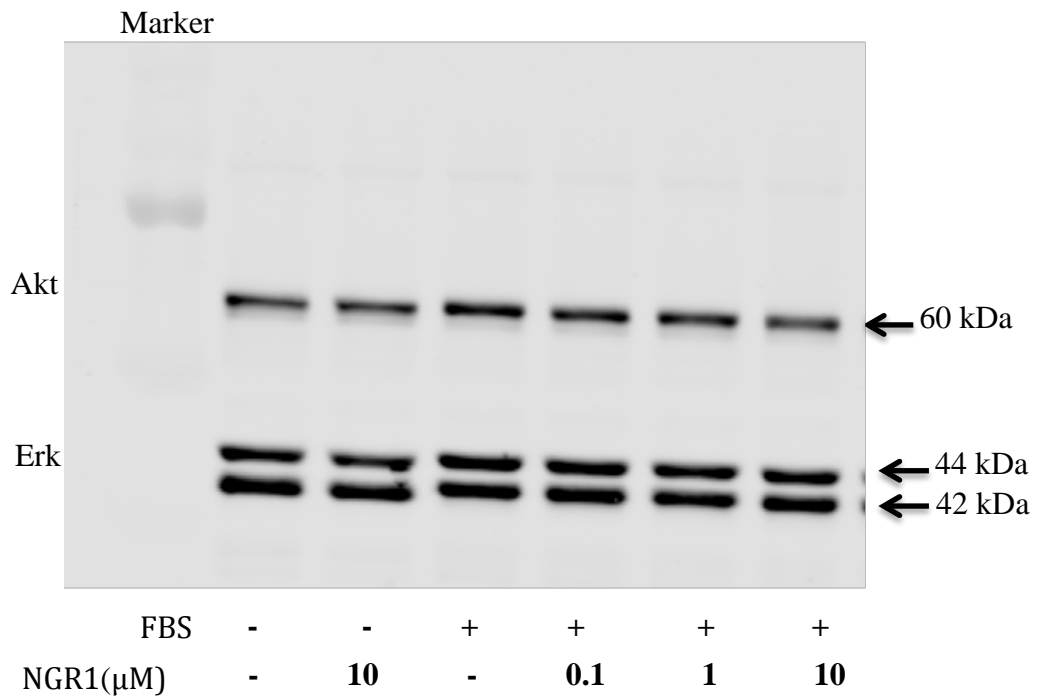
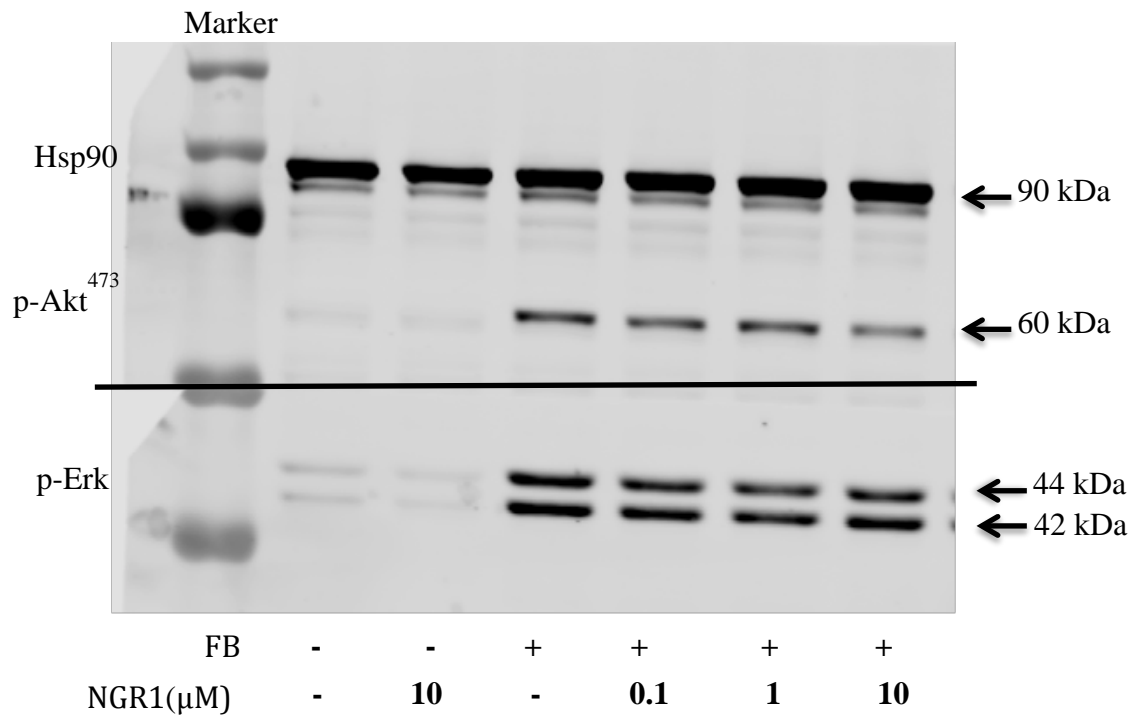
Original western blotting images for Fig. 4E.

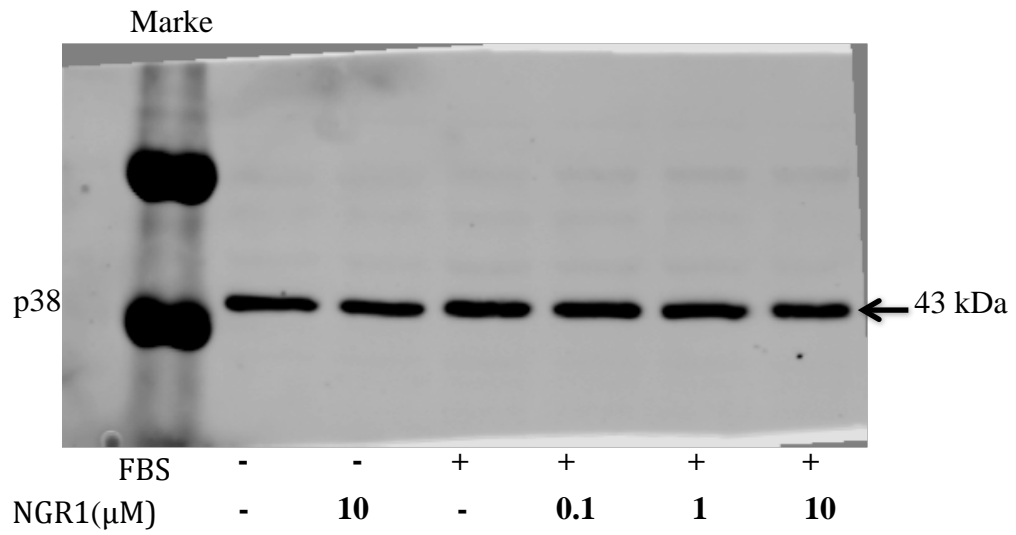
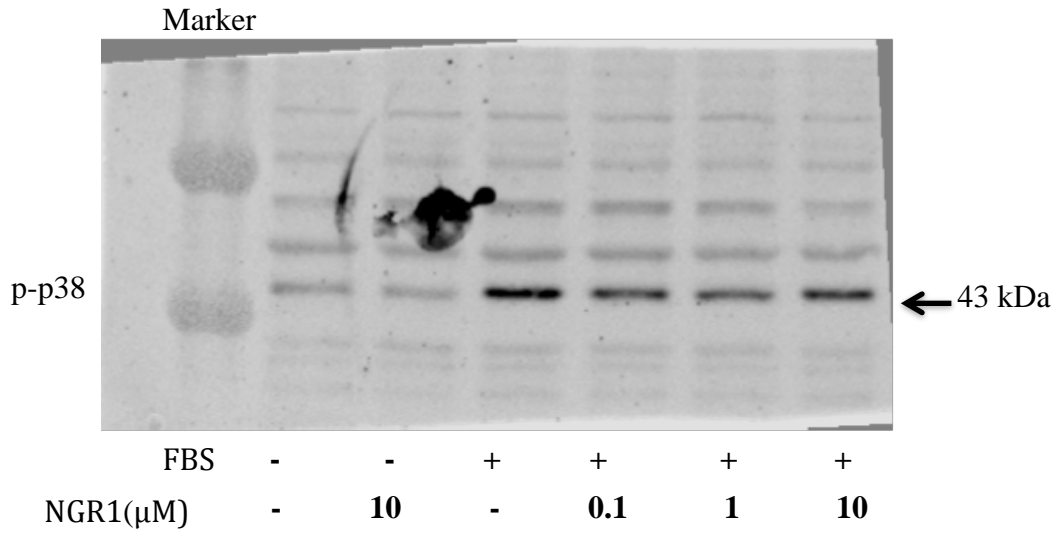


Original western blotting images for supplementary Fig. 2.



Original western blotting images for supplementary Fig. 3.





**Supplement Table 1: Antibody list**

<b>Antibody</b>	<b>Company</b>	<b>Catalog #</b>	<b>Species</b>	<b>Dilution</b>	<b>Application</b>
Hsp90	BD	610419	Mouse	1:1000	Western
p-Akt(Ser473)	Cell Signaling	4051	Mouse	1:1000	Western
Akt	Cell Signaling	4685	Rabbit	1:1000	Western
p-Erk	Cell Signaling	4377	Rabbit	1:1000	Western
Erk	Cell Signaling	4696	Mouse	1:1000	Western
p-p38	Cell Signaling	9216	Mouse	1:500	Western
p38	Cell Signaling	9212	Rabbit	1:500	Western
p-JNK	Cell Signaling	4668	Rabbit	1:1000	Western
JNK	Santa Cruz	sc-7345	Mouse	1:500	Western
Myosin-11	Abcam	ab53219	Rabbit	1:1000	Western
Calponin	Abcam	ab46794	Rabbit	1:2000	Western
SM22- $\alpha$	Abcam	ab14106	Rabbit	1:1000	Western
SM- $\alpha$ -actin	DAKO	M0851	Mouse	1:1000	Western
IRDYE 680RD second antibody	Odyssey	926-68073	Rabbit	1:5000	Western
IRDYE 800CW second antibody	Odyssey	926-32212	Mouse	1:5000	Western
Anti-BrdU	Abcam	ab6326	Rat	1:40	IF
FITC- conjugated phalloidin (F-actin)	Invitrogen	A12379		1:100	IF
Alexa Fluor® 594 conjugate Deoxyribonuclease I (G-actin)	Invitrogen	D12372		1:200	IF

**Supplement Table 2: Other reagent list**

<b>Reagents</b>	<b>Company</b>	<b>Catalog #</b>
Notoginsenoside R1	Chinese National Institute for the Control of Pharmaceutical and Biological Products	110745
rhEGF	Promega	G5021
rhFGF	Promega	G5071
BrdU	Sigma-Aldrich	19160
LY294002	Sigma-Aldrich	L9908
PD98059	Sigma-Aldrich	P215
SB203580	Sigma-Aldrich	S8307
SP600125	Sigma-Aldrich	S5567
TUNEL Andy Fluor™ 488 Apoptosis Detection Kit	GeneCopia	A050