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Supplemental information

**Involvement of actin cytoskeletal modifications
in the inhibition of triple-negative breast
cancer growth and metastasis by nimbolide**

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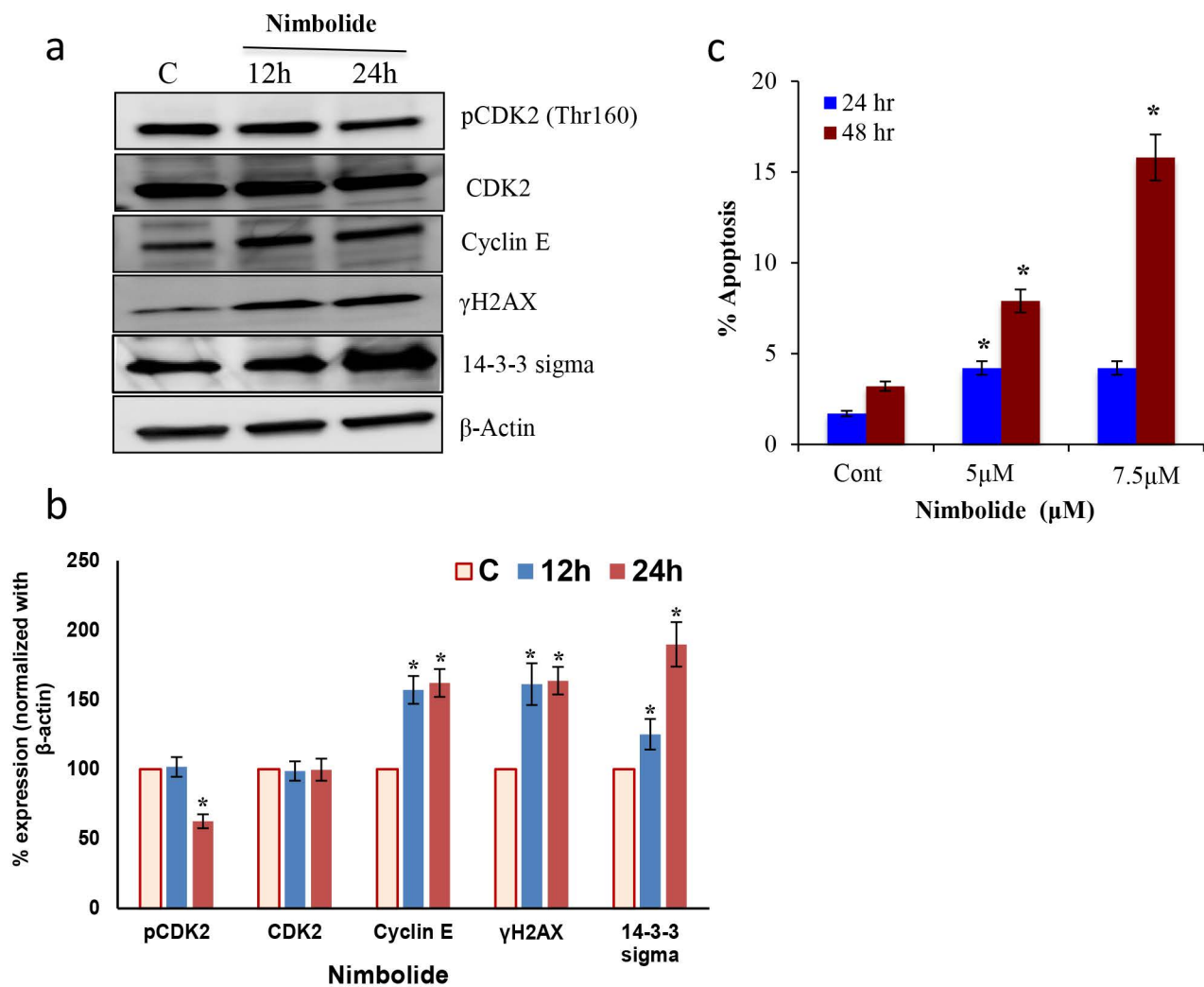


Figure. S1:

a. Analysis of cell cycle regulatory proteins in response to nimbolide treatment.

b. Densitometric analysis of Western blot data represented in Figure S1a

c. Nimbolide treatment increased apoptosis.

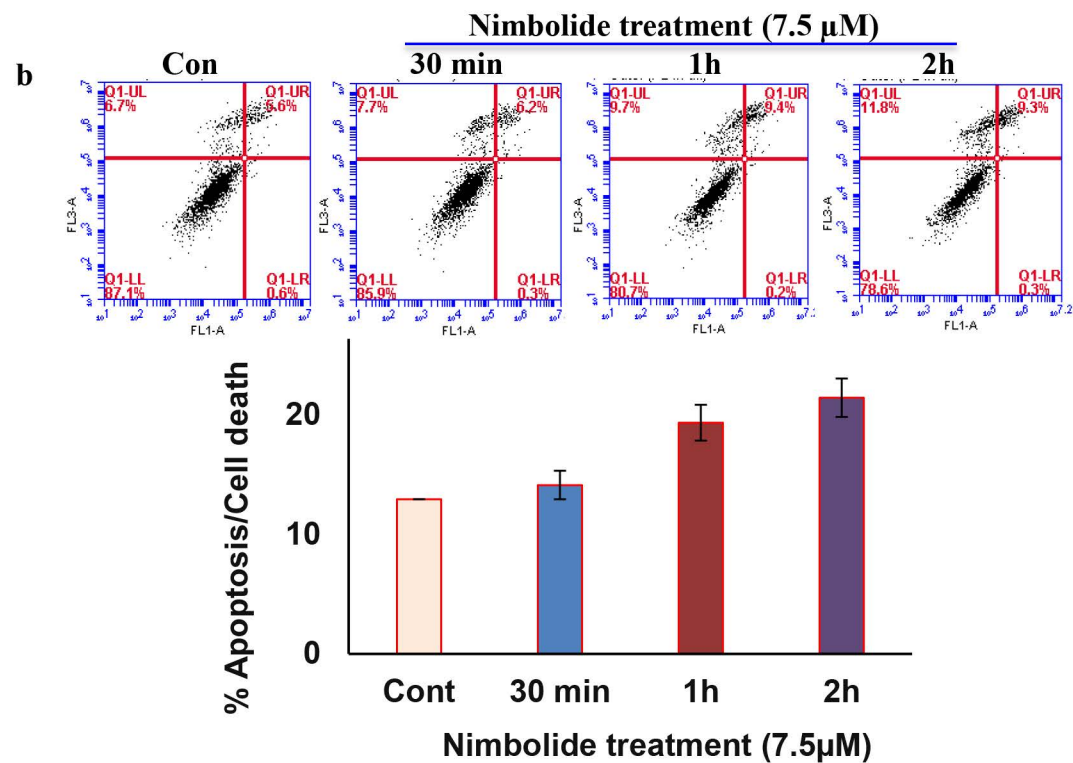
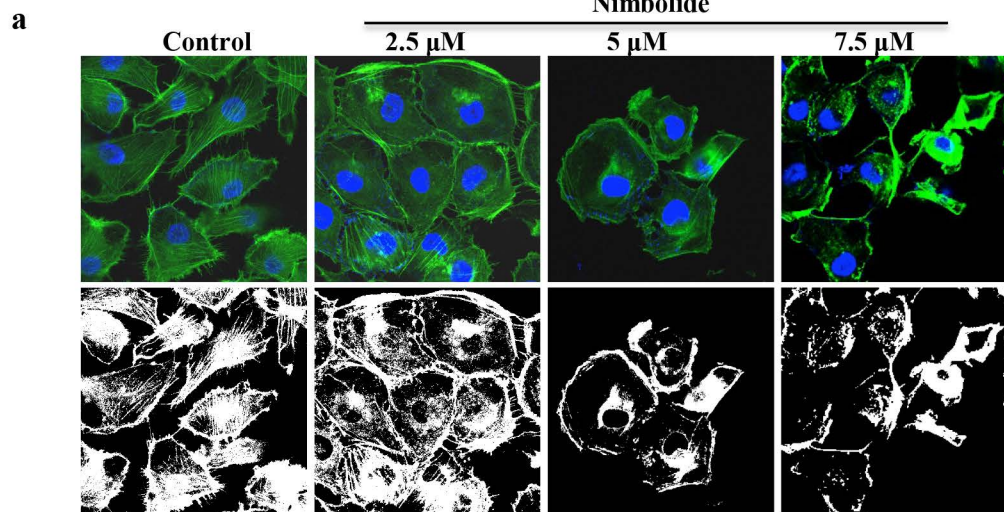


Figure S2

a: Immunofluorescent imaging of actin indicated that nimbolide treatment disrupted the actin network in a dose-dependent manner.
 b: Flowcytometric apoptosis analysis using AnnexinV/PI staining indicated that nimbolide initiates apoptosis at early timepoints.

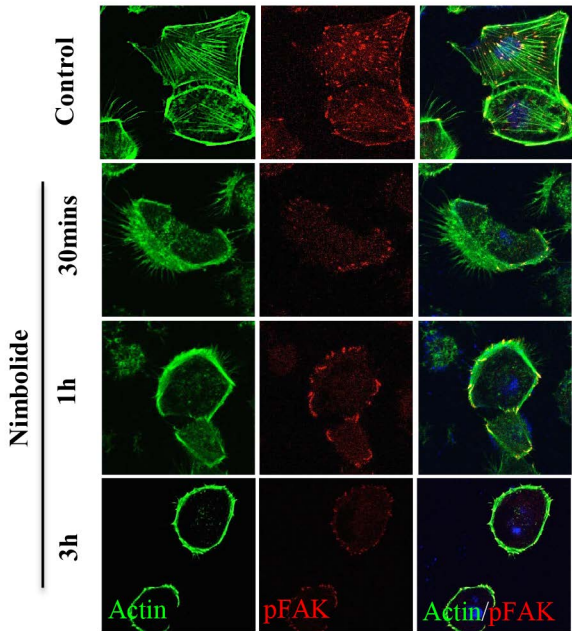


Figure S3: Immunofluorescence of pFAK and actin

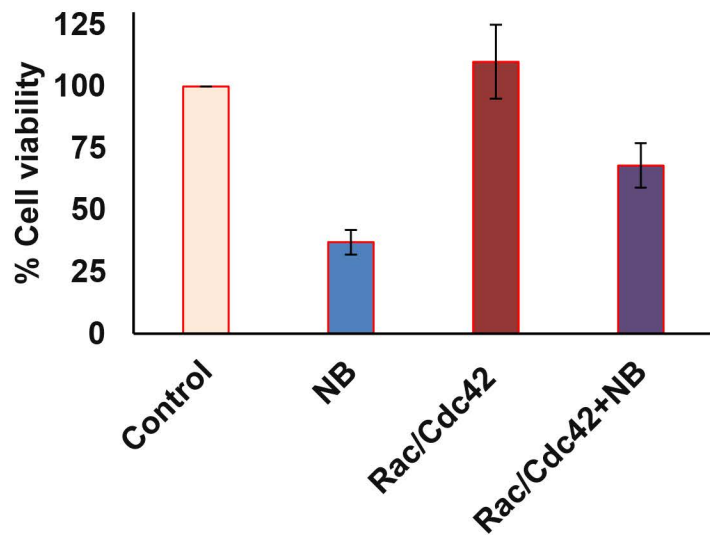
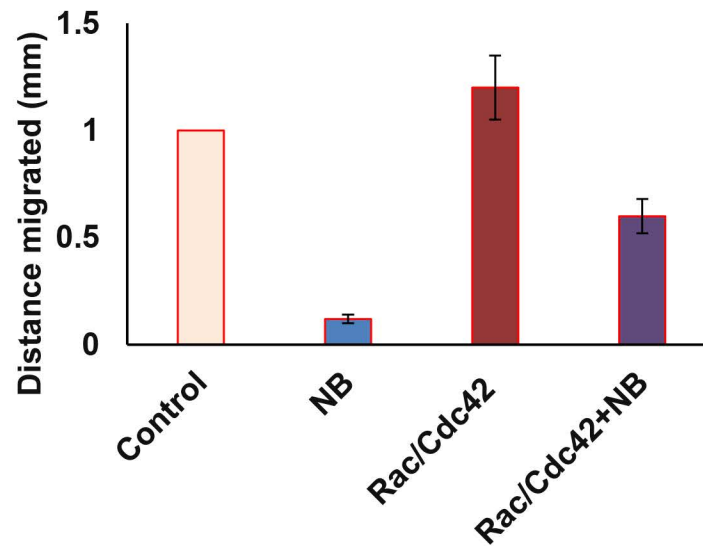
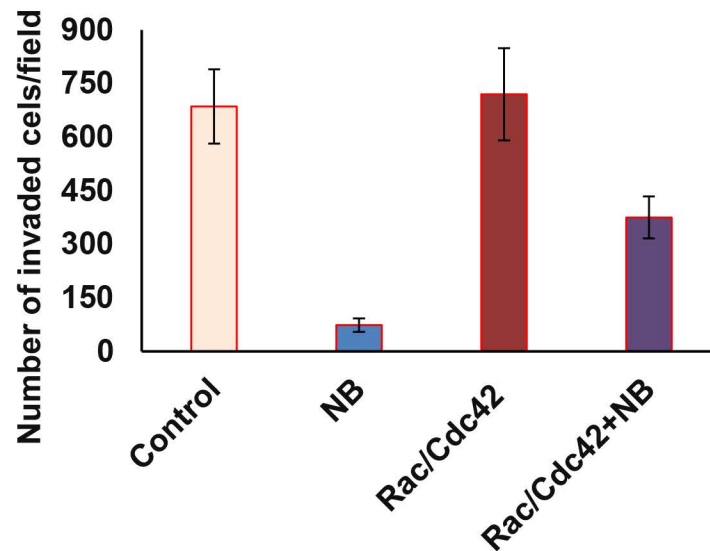
a**b****c**

Figure S4: Rac1/Cdc42 activation rescued the anticancer effect of nimbolide a: cell viability, b: migration and c: invasion.

Supplemental Information:

Supplemental Movie Legends:

Movie S1: Live cell imaging (Actin): Nimbolide treatment induced actin cytoskeletal modification started as early as 10 minutes and affected almost all the cells by 90 minutes.

Movie S2: Control Actin-Actinin (Z-stalk): Confocal microscopy data indicated that in the control cells the expression and co-localization of α -actinin was primarily found inside the cells and mainly at the base of lamellipodia structures.

Movie S3: Nimbolide treatment: Actin-Actinin (Z-stalk): Nimbolide treatment resulted in localization of α -actinin at the cell surface and most of the α -actinin was co-localized with actin indicating the strong stress fiber formation at the cell boundary.

Movie S4: Actin-Actinin (Volume-stalk): Immunofluorescence showing elongated focal adhesion points and prominent interaction with actin cytoskeleton in control cells.

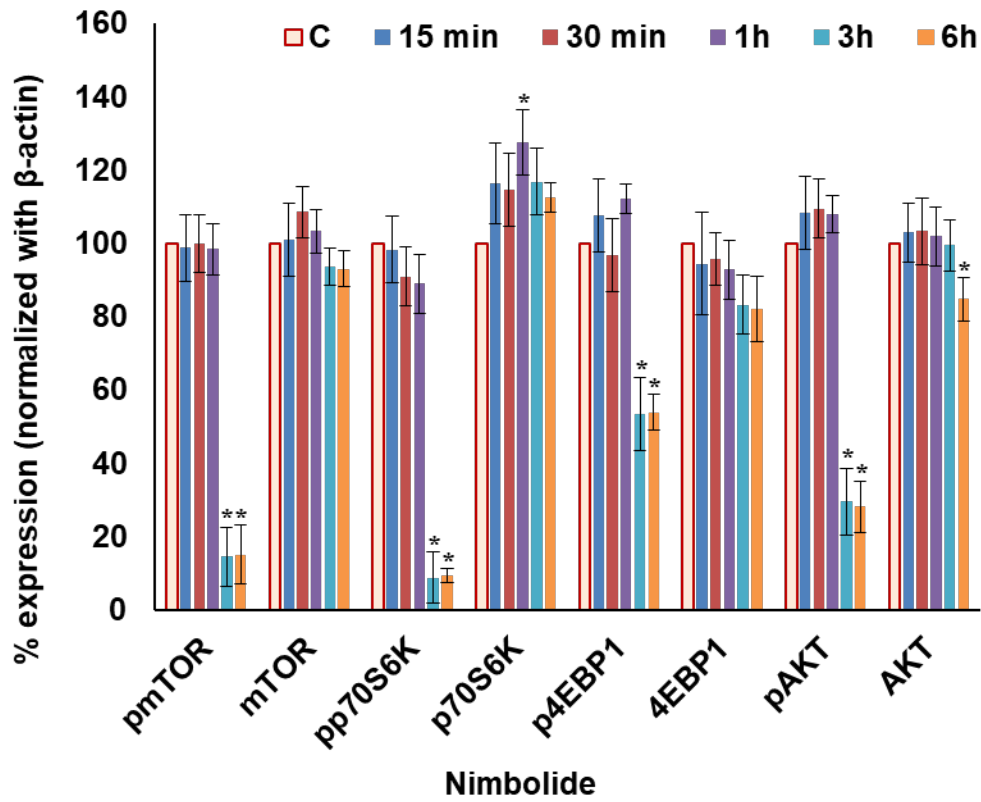
Movie S5: Actin-Actinin (Volume-stalk): Nimbolide treatment strikingly down regulates the expression of pFAK and the actin networks are not complete.

Table S1: List of Antibodies used in the study

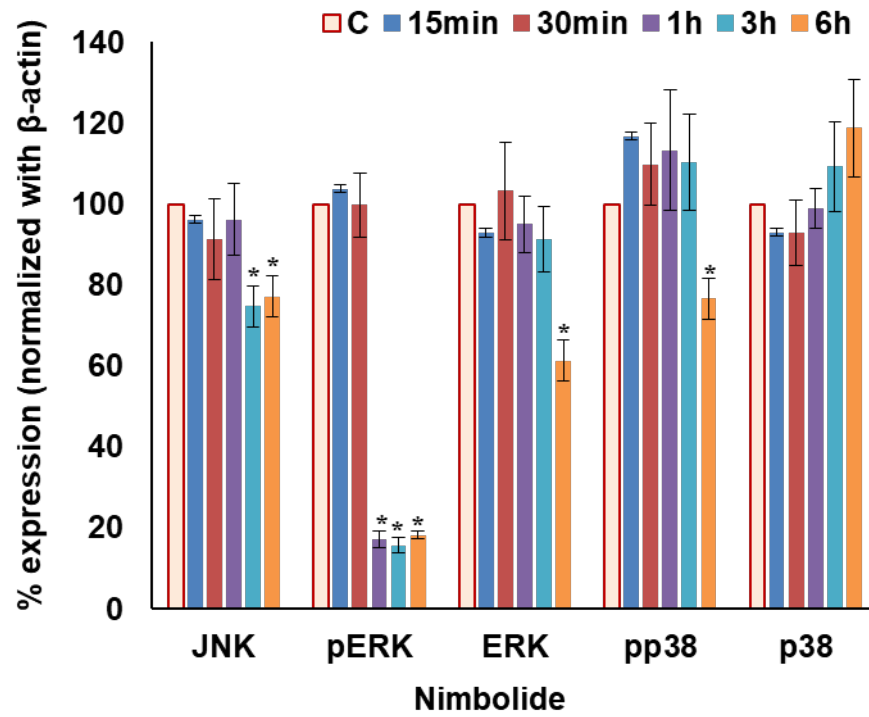
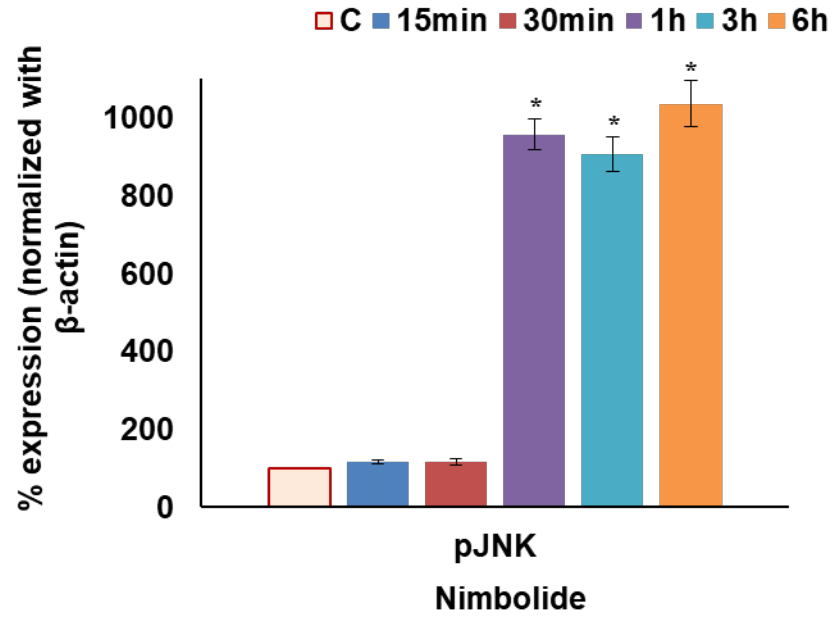
Antibody	Company	Dilution
AKT	Cell Signaling 4685	1:1000
pAKT S473	Cell Signaling 4060	1:1000
mTOR	Cell Signaling 4517	1:1000
pmTOR S2448	Cell Signaling 5536	1:1000
p70 S6 kinase	Cell Signaling 9202	1:1000
pp70 S6 kinase (T389)	Cell Signaling 9206	1:1000
4EBP1	Cell Signaling 9644	1:1000
p4EBP1 (T37/46)	Cell Signaling 2855	1:1000
JNK1	Cell Signaling 3708	1:1000
p-SAPK/JNK (T183/Y185)	Cell Signaling 9255	1:2000
P44/42 MAPK (Erk ½)	Cell Signaling 9102	1:1000
pERK T202/Y204	Cell Signaling 4370	1:1000
p38 MAPK	Cell Signaling 9212	1:1000
pp38 MAPK (T180/Y182)	Cell Signaling 9211	1:1000
Cleaved PARP	Cell Signaling 5625	1:1000
Cleaved Caspase 3	Cell Signaling 9661	1:500
Procaspase 3	Abcam ab32150	1:1000
Bim	Cell Signaling 2933	1:1000
Bad	Santa Cruz SC-8044	1:200
Bak	Cell Signaling 12105	1:1000
Bax	Santacruz SC-7480	1:500
Bcl2	Santacruz SC-578	1:500
BclXL	Cell Signaling 4477	1:500
E-cadherin	Cell Signaling 3195	1:1000
Slug	Cell Signaling 9585	1:1000
Zeb1	Santa Cruz SC-515797	1:500
β-catenin	Cell Signaling 8480	1:1000
Integrin αV	Cell Signaling 4711	1:1000
Integrin β3	Cell Signaling 4702	1:1000
Integrin β4	Santa Cruz SC-6628	1:500
ILK	Cell Signaling 3856	1:1000
FAK	Cell Signaling 3285	1:1000
pFAK (Y397)	Cell Signaling 3283	1:1000
Rac1	Abcam ab33186	1:1000
Rac1+Cdc42	Abcam ab76535	1:1000
PAK1 phospho (S144)	Abcam ab40795	1:5000
PAK1	Abcam ab40852	1:500
Cofilin	Cell Signaling 5175	1:1000
pCofilin (S3)	Cell Signaling 3313	1:1000
pVASP S157	Cell Signaling 3111	1:1000

pVASP S239	Cell Signaling 3114	1:1000
Diaph1	Invitrogen PA5-21409	1:500
P44/42 MAPK (Erk ½)	Cell Signaling 9102	1:1000
CDK2	Cell Signaling 2546	1:1000
pCDK2 (Thr160)	Cell Signaling 2561	1:1000
Cyclin E2	Cell Signaling 4132	1:1000
γH2AX	Cell Signaling 2577	1:1000
14-3-3 σ	Abcam ab14123	1:1000
F-actin	Abcam ab205	1:500
β-actin	Sigma A2228	1:1000

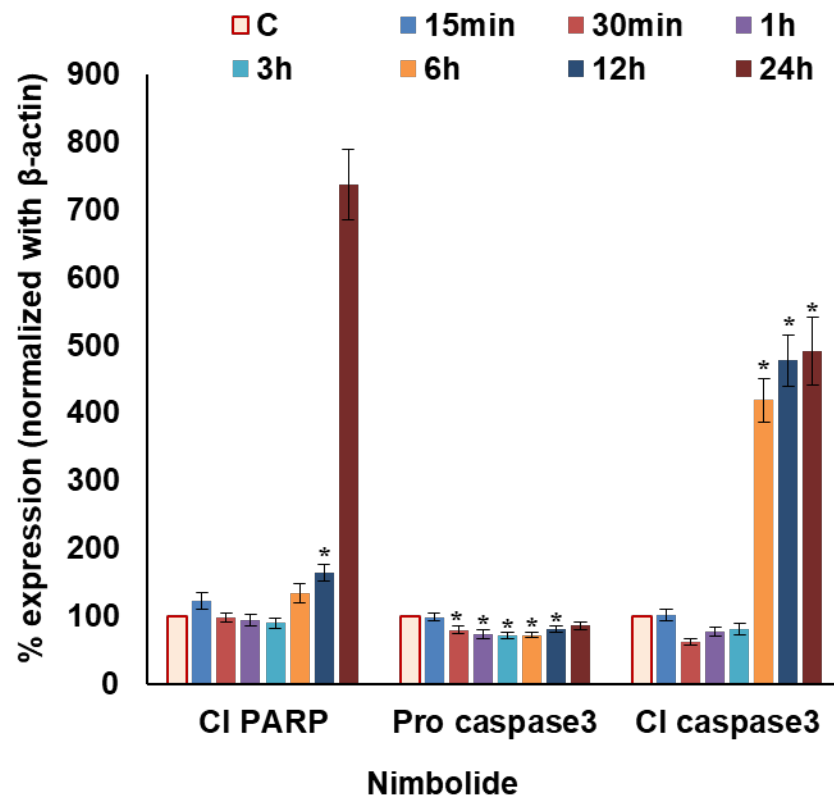
Denistometric data for all western blots



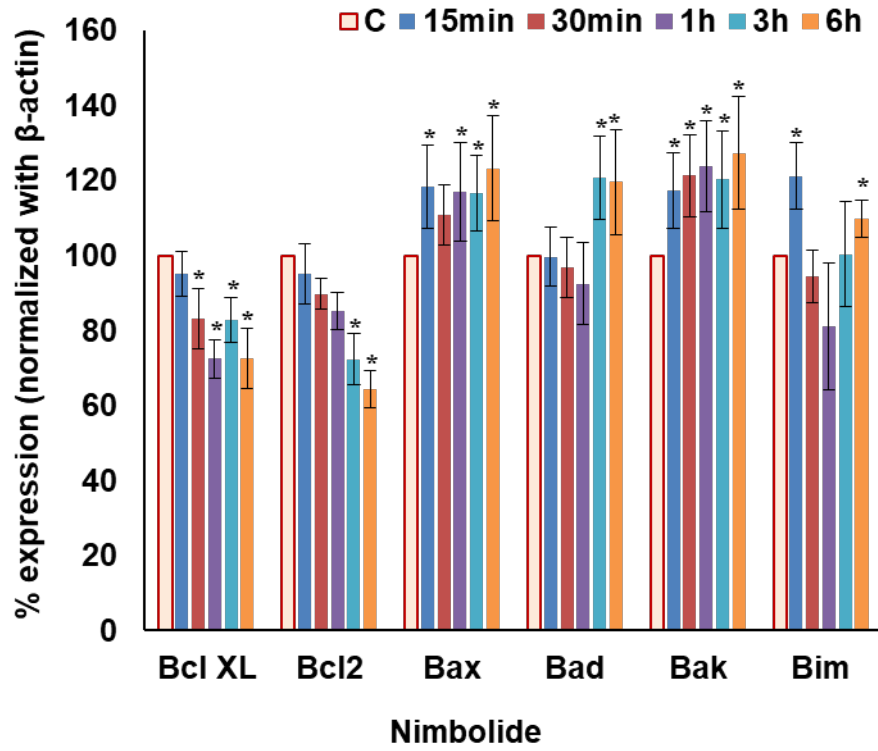
Densitometric analysis of Western blot data represented in Figure 1d.



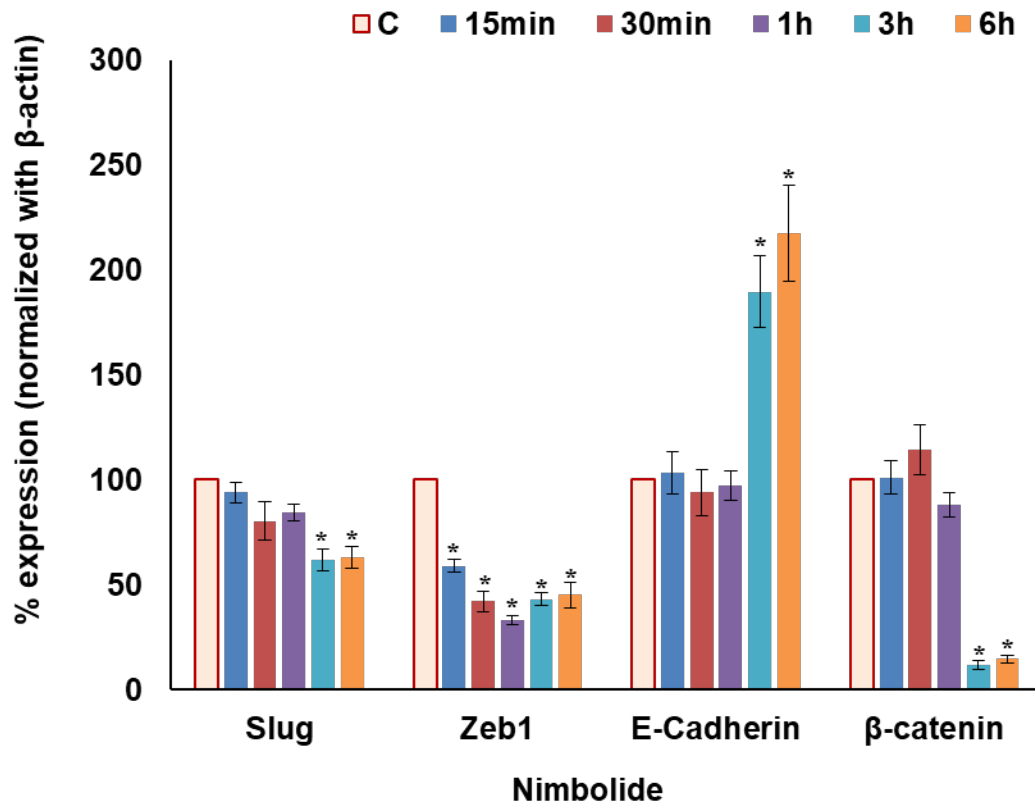
Densitometric analysis of Western blot data represented in Figure 1e.



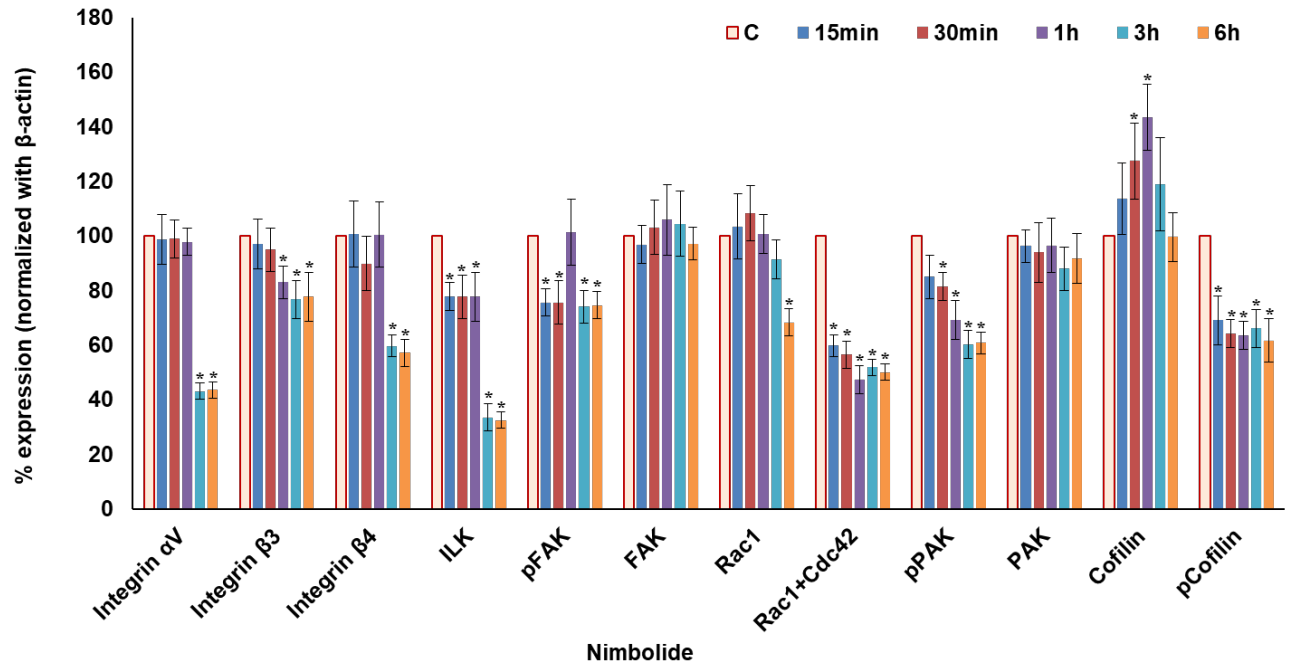
Densitometric analysis of Western blot data represented in Figure 2b.



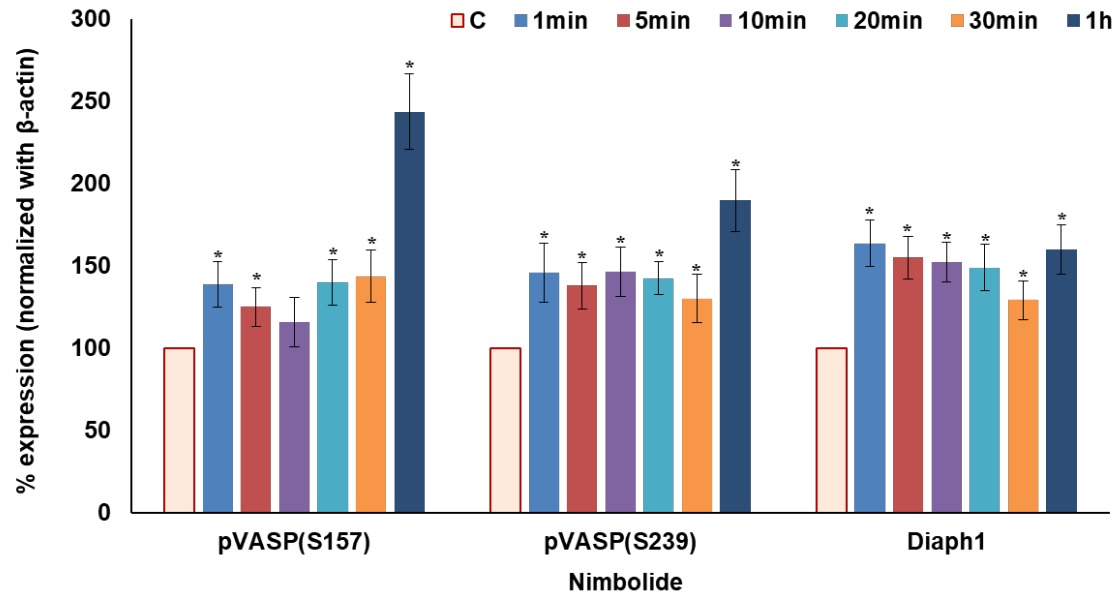
Densitometric analysis of Western blot data represented in Figure 2c.



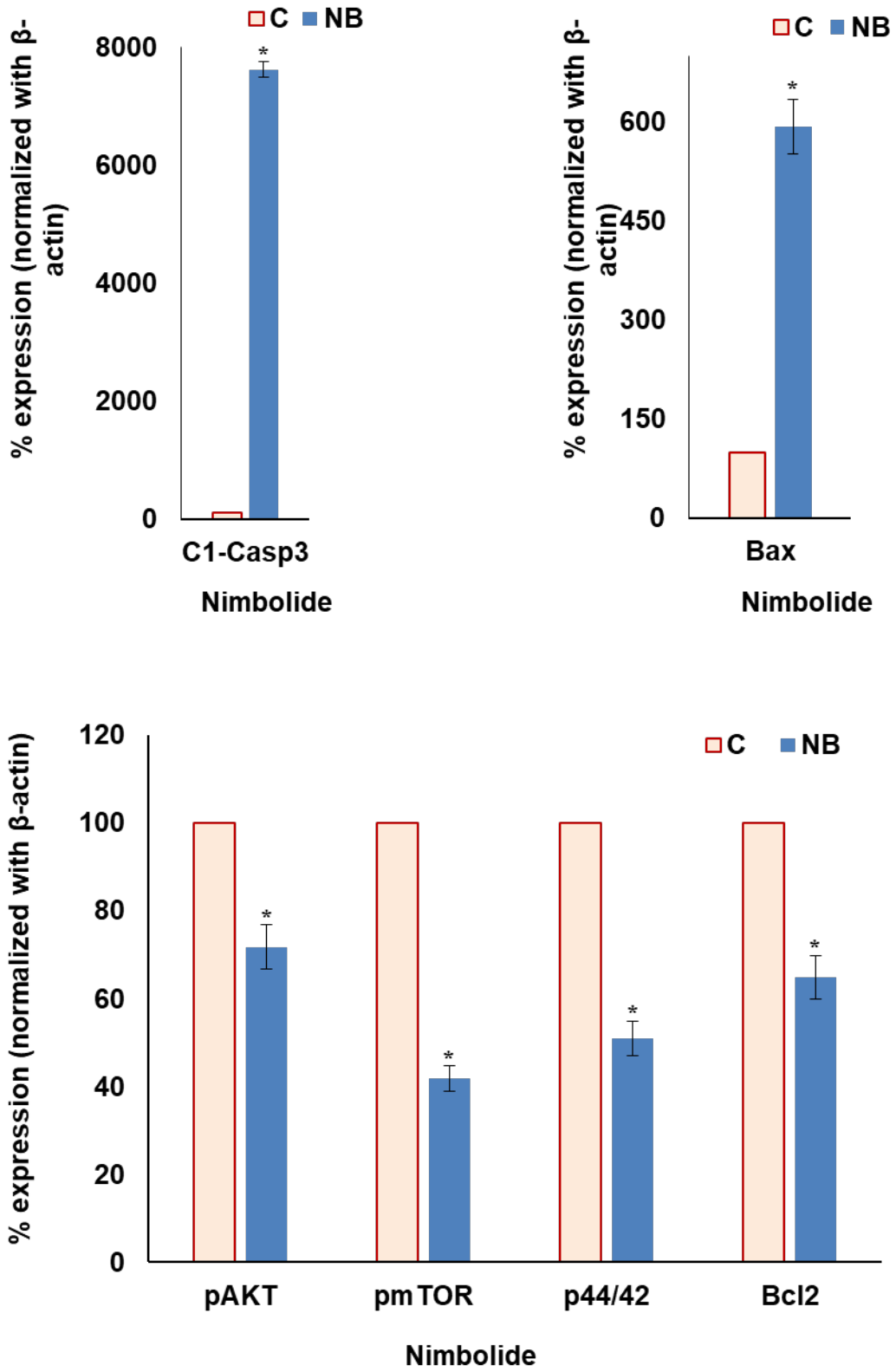
Densitometric analysis of Western blot data represented in Figure 2f.



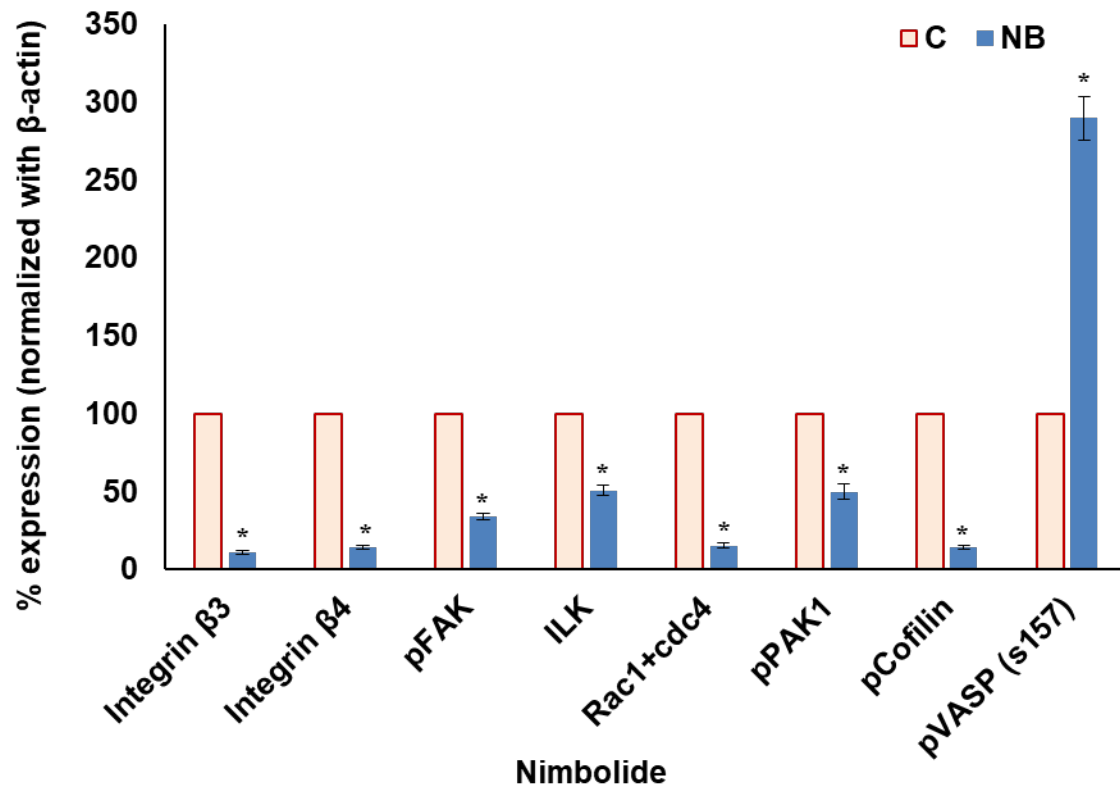
Densitometric analysis of Western blot data represented in Figure 4b.



Densitometric analysis of Western blot data represented in Figure 4c.



Densitometric analysis of Western blot data represented in Figure 6c1.



Densitometric analysis of Western blot data represented in Figure 6c2.

General pathways influenced by nimbolide. Our study demonstrates that nimbolide significantly alters FAK signaling in TNBC.

