

Supplementary Materials

Piperlongumine inhibits lung tumor growth via inhibition of nuclear factor kappa B signaling pathway

Jie Zheng¹, Dong Ju Son¹, Sun Mi Gu¹, Ju Rang Woo²,
Young Wan Ham³, Hee Pom Lee¹, Wun Jae Kim⁴, Jae
Kyung Jung¹, Jin Tae Hong^{1*}

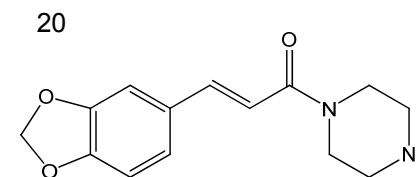
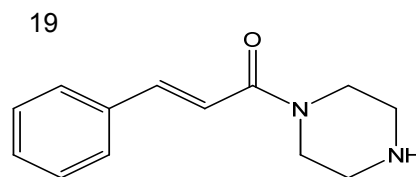
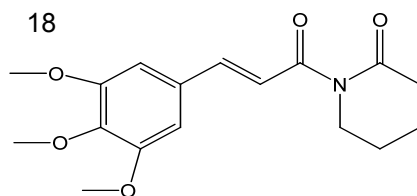
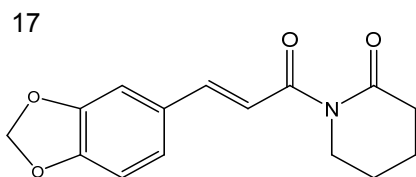
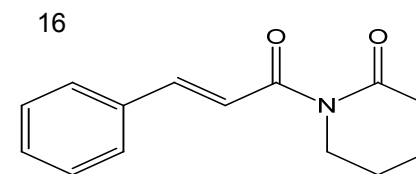
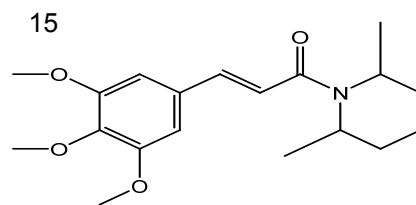
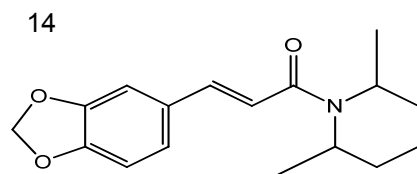
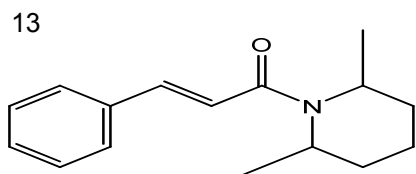
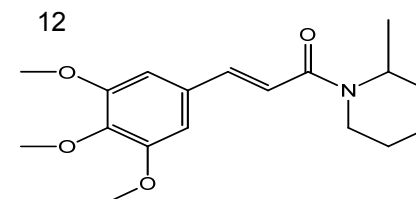
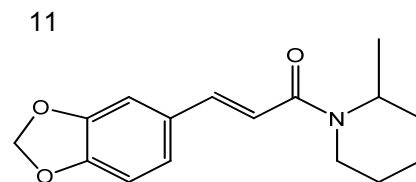
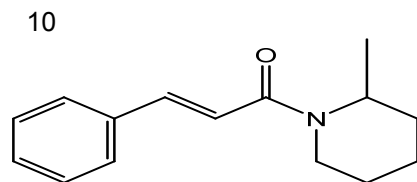
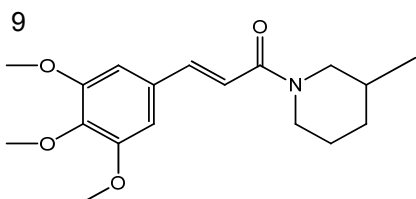
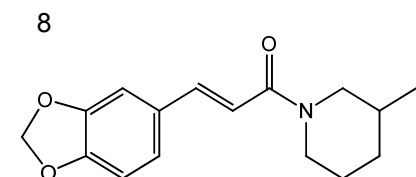
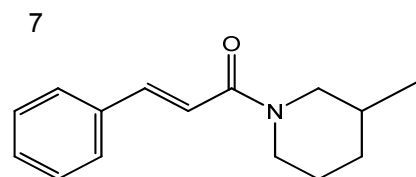
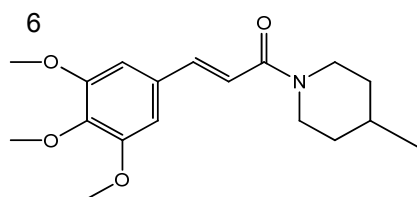
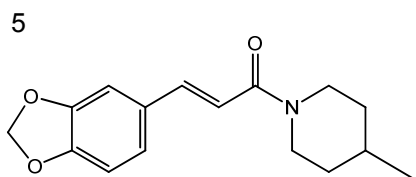
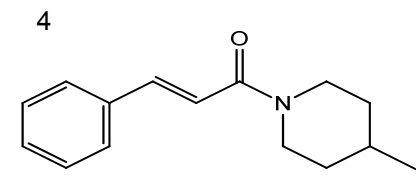
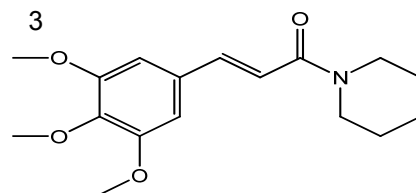
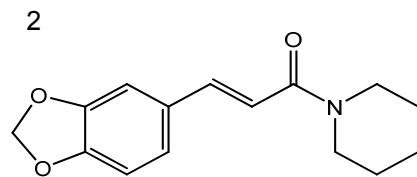
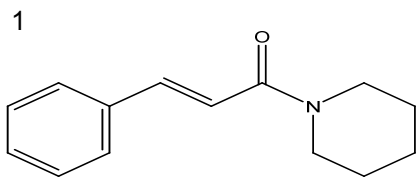
Supplementary Figure 1A: List of PL analogues. (1) 3-phenyl-1-(piperidin-1-yl)prop-2-en-1-one. (2) 3-(3,4-methylenedioxyphenyl)-1-(piperidin-1-yl)prop-2-en-1-one. (3) 3-(3,4,5-trimethoxyphenyl)-1-(piperidin-1-yl)prop-2-en-1-one. (4) 3-phenyl-1-(4-methylpiperidin-1-yl)prop-2-en-1-one. (5) 3-(3,4-methylenedioxyphenyl)-1-(4-methylpiperidin-1-yl)prop-2-en-1-one. (6) 3-(3,4,5-trimethoxyphenyl)-1-(4-methylpiperidin-1-yl)prop-2-en-1-one. (7) 3-phenyl-1-(3-methylpiperidin-1-yl)prop-2-en-1-one. (8) 3-(3,4-methylenedioxyphenyl)-1-(3-methylpiperidin-1-yl)prop-2-en-1-one. (9) 3-(3,4,5-trimethoxyphenyl)-1-(3-methylpiperidin-1-yl)prop-2-en-1-one. (10) 3-phenyl-1-(2-methylpiperidin-1-yl)prop-2-en-1-one. (11) 3-(3,4-methylenedioxyphenyl)-1-(2-methylpiperidin-1-yl)prop-2-en-1-one. (12) 3-(3,4,5-trimethoxyphenyl)-1-(2-methylpiperidin-1-yl)prop-2-en-1-one. (13) 3-phenyl-1-(2,6-dimethylpiperidin-1-yl)prop-2-en-1-one. (14) 3-(3,4-methylenedioxyphenyl)-1-(2,6-dimethylpiperidin-1-yl)prop-2-en-1-one. (15) 3-(3,4,5-trimethoxyphenyl)-1-(2,6-dimethylpiperidin-1-yl)prop-2-en-1-one. (16) 3-phenyl-1-(2-piperidon-1-yl)prop-2-en-1-one. (17) 3-(3,4-methylenedioxyphenyl)-1-(2-piperidon-1-yl)prop-2-en-1-one. (18) 3-(3,4,5-trimethoxyphenyl)-1-(2-piperidon-1-yl)prop-2-en-1-one. (19) 3-phenyl-1-(piperazin-1-yl)prop-2-en-1-one. (20) 3-(3,4-methylenedioxyphenyl)-1-(piperazin-1-yl)prop-2-en-1-one.

Supplementary Figure 1B: List of PL analogues. (21) 3-(3,4,5-trimethoxyphenyl)-1-(piperazin-1-yl)prop-2-en-1-one (22) 3-phenyl-1-(4-methylpiperazin-1-yl)prop-2-en-1-one (23) 3-(3,4-methylenedioxyphenyl)-1-(4-methylpiperazin-1-yl)prop-2-en-1-one (24) 3-(3,4,5-trimethoxyphenyl)-1-(4-methylpiperazin-1-yl)prop-2-en-1-one (25) 3-phenyl-1-(2,6-dimethylpiperazin-1-yl)prop-2-en-1-one (26) 3-(3,4-methylenedioxyphenyl)-1-(2,6-dimethylpiperazin-1-yl)prop-2-en-1-one (27) 3-(3,4,5-trimethoxyphenyl)-1-(2,6-dimethylpiperazin-1-yl)prop-2-en-1-one (28) 3-phenyl-1-(morpholin-4-yl)prop-2-en-1-one (29) 3-(3,4-methylenedioxyphenyl)-1-(morpholin-4-yl)prop-2-en-1-one (30) 3-(3,4,5-trimethoxyphenyl)-1-(morpholin-4-yl)prop-2-en-1-one (31) 3-phenyl-1-(thiomorpholin-4-yl)prop-2-en-1-one (32) 3-(3,4-methylenedioxyphenyl)-1-(thiomorpholin-4-yl)prop-2-en-1-one (33) 3-(3,4,5-trimethoxyphenyl)-1-(thiomorpholin-4-yl)prop-2-en-1-one (34) 3-phenyl-1-(3-methylpiperazin-1-yl)prop-2-en-1-one (35) 3-(3,4-methylenedioxyphenyl)-1-(3-methylpiperazin-1-yl)prop-2-en-1-one (36) 3-(3,4,5-trimethoxyphenyl)-1-(3-methylpiperazin-1-yl)prop-2-en-1-one

Supplementary Figure 2: Effect of PL analogues on the growth of A549 NSCLC cells and on NF- κ B luciferase activities. (A) A549 Cells were plated in 96-well and treated with 36 PL analogues and PL for 24 h, cell viability was determined by MTT assay. Data was expressed as the mean \pm S.D. of three experiments. (B) A549 cells were transfected with p-NF- κ B-Luc plasmid (5x NF- κ B), and the treated with PL (10 μ M) for 24 h. Luciferase activity was then determined as described in the materials and methods.

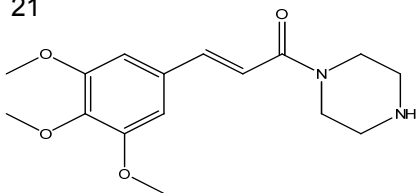
Supplementary Figure 3: Effect of Fas siRNA or DR4 siRNA on the expression of each DR. Expression of DR was determined by Western blotting with antibodies against Fas, DR4 and β -actin (internal control). For the cropped images, samples were run in the same gels under same experimental conditions and processed in parallel. Each band is representative for three experiments.

Supplementary Figure 1A

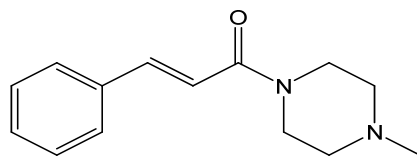


Supplementary Figure 1B

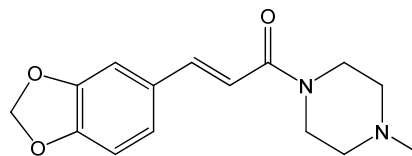
21



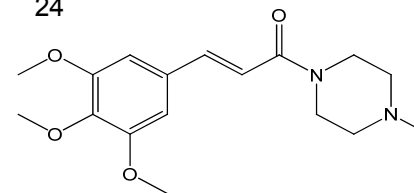
22



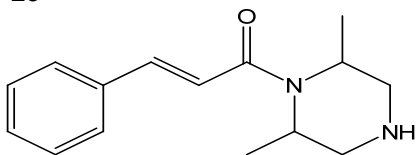
23



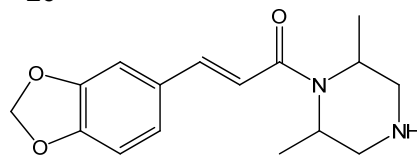
24



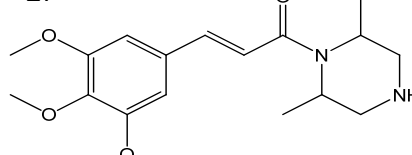
25



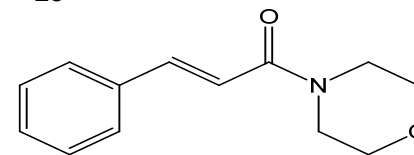
26



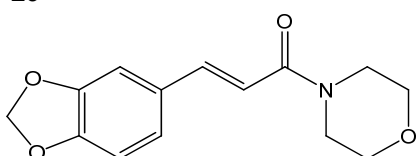
27



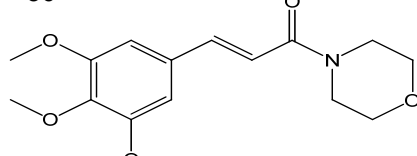
28



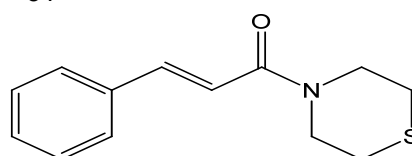
29



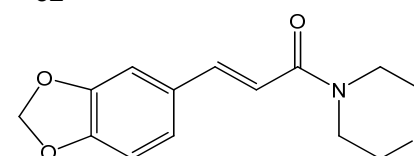
30



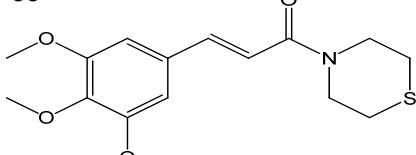
31



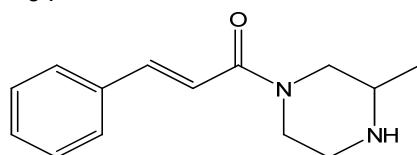
32



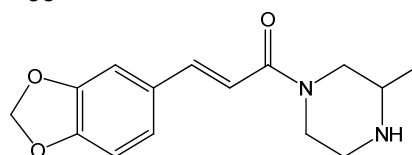
33



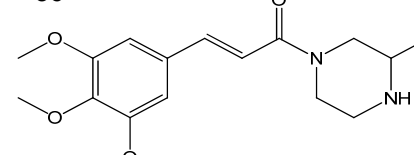
34



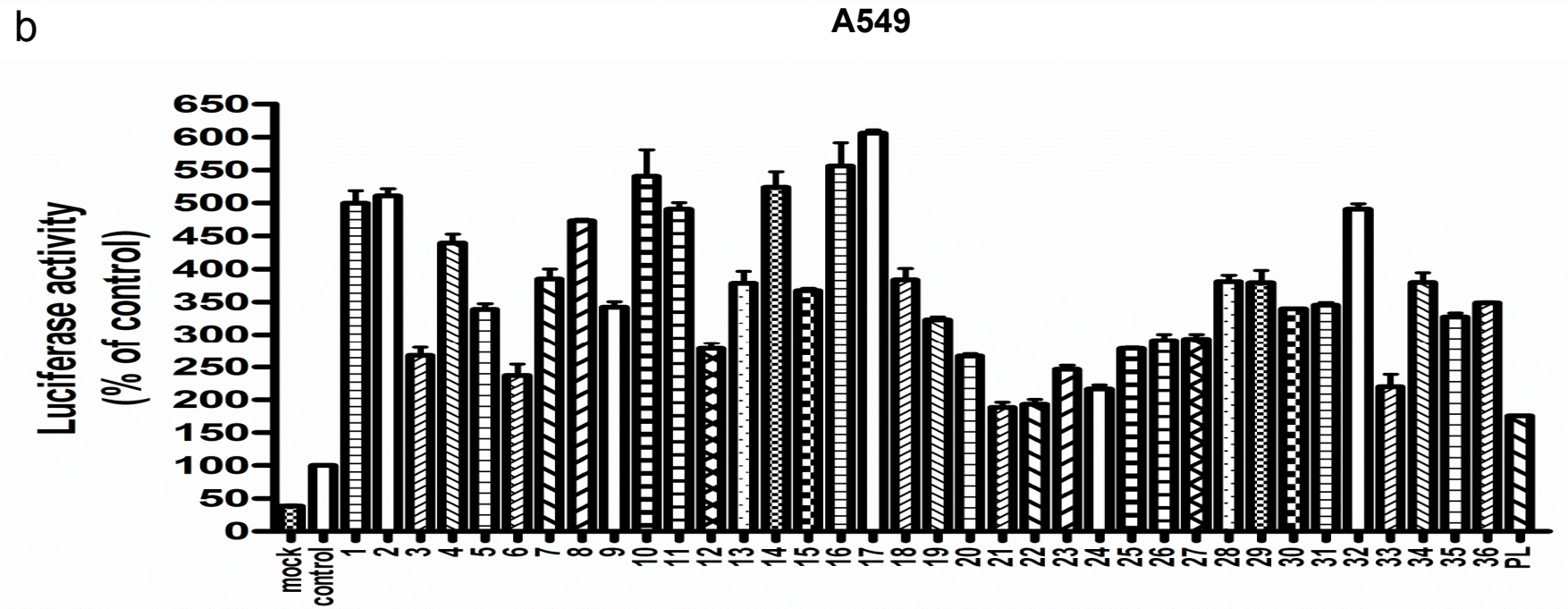
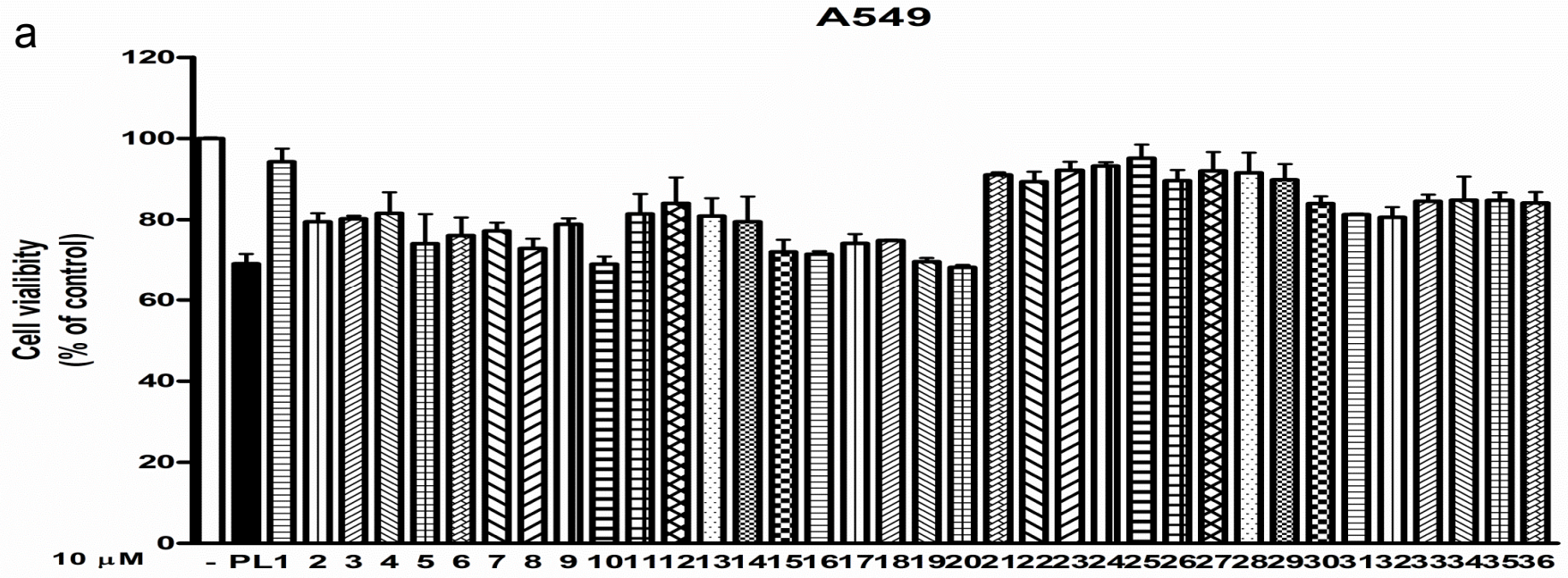
35



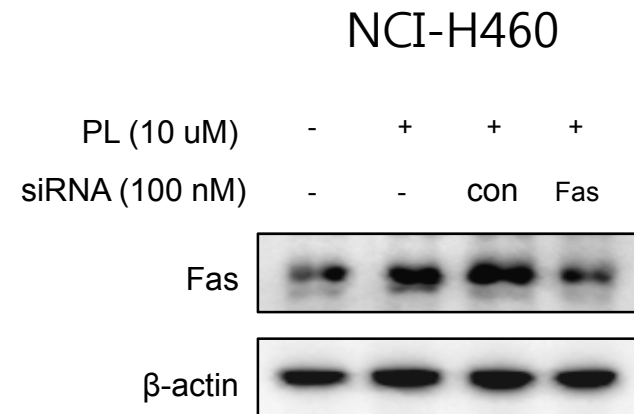
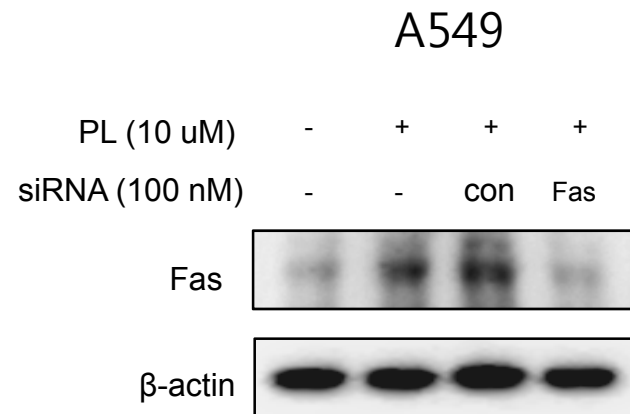
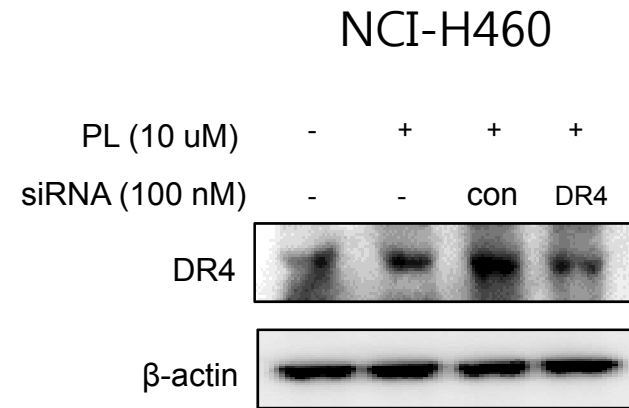
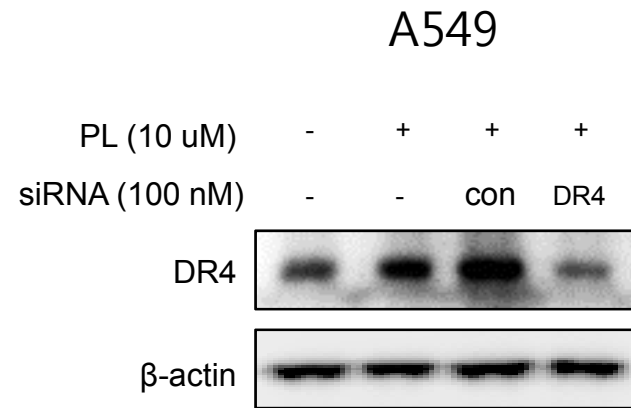
36



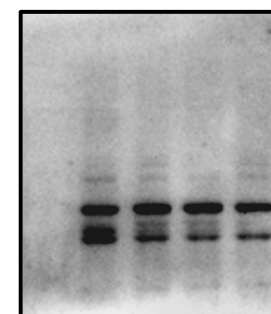
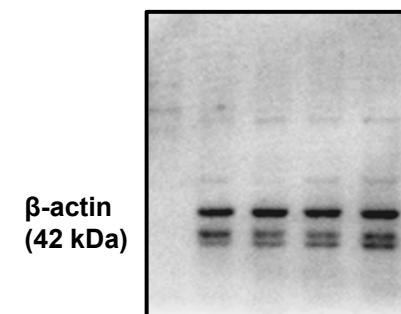
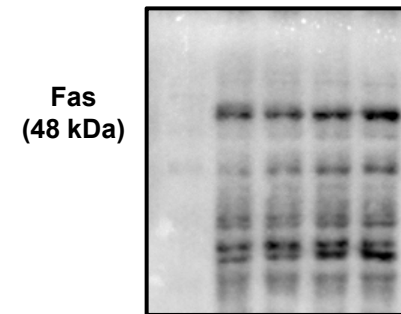
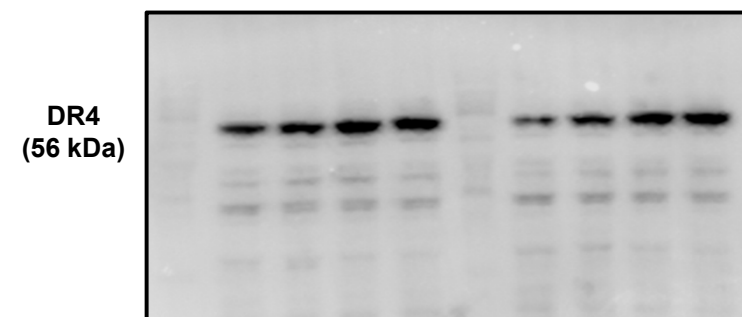
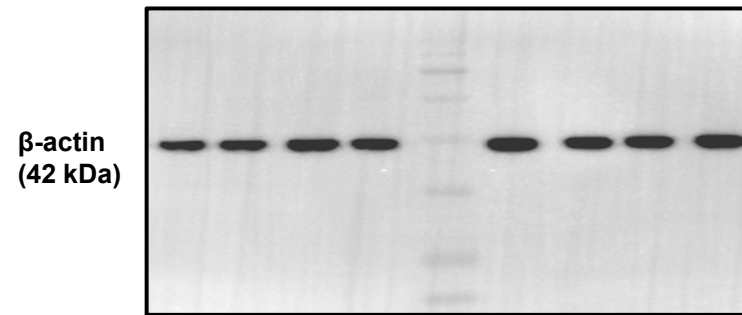
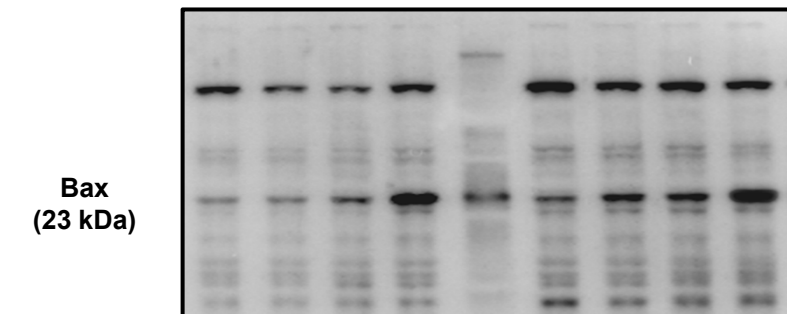
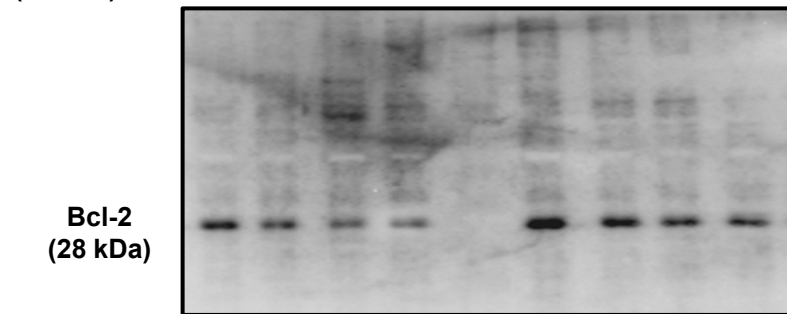
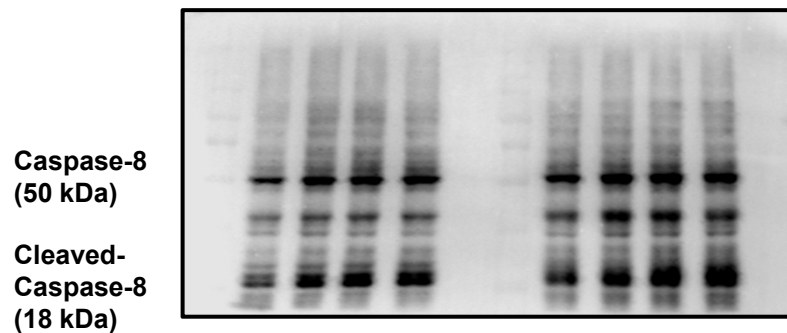
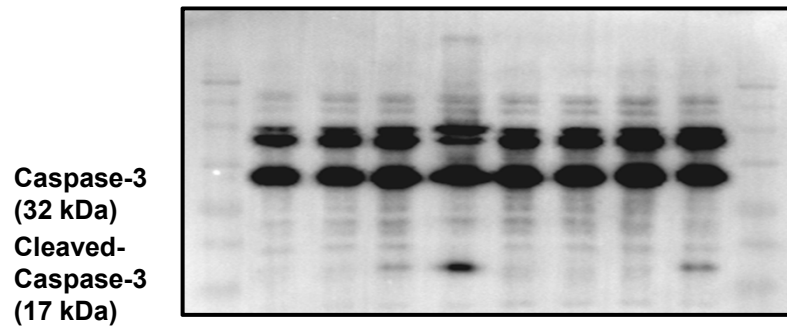
Supplementary Figure 2



Supplementary Figure 3

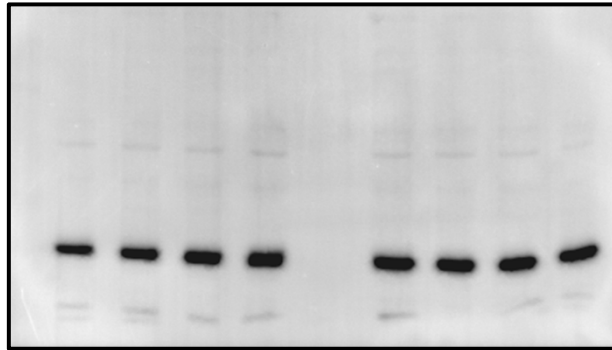


Supplementary Figure 4

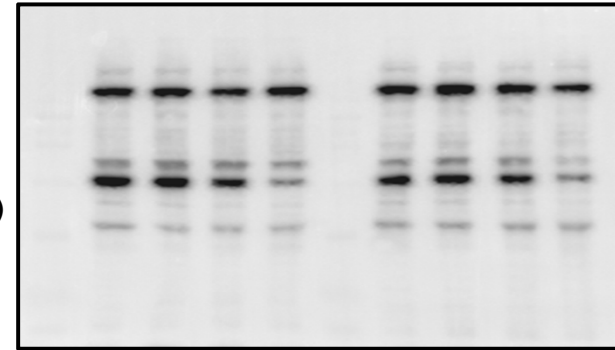


Supplementary Figure 5

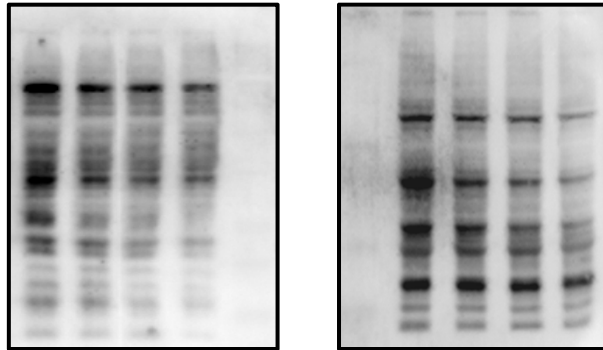
I κ B α
(31-41
kDa)



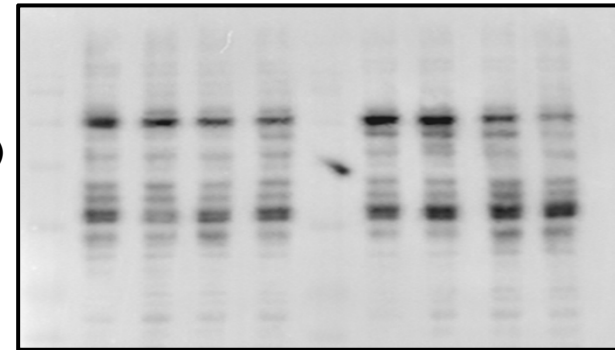
p50
(50 kDa)



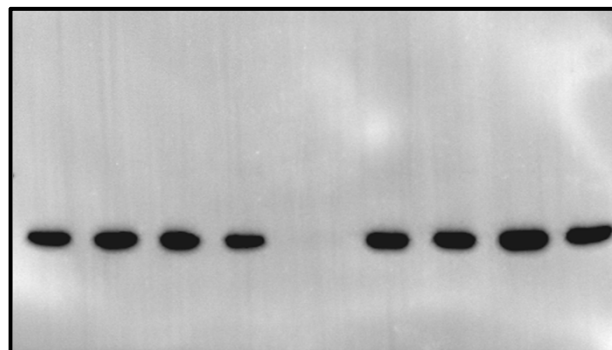
p-I κ B α
(41 kDa)



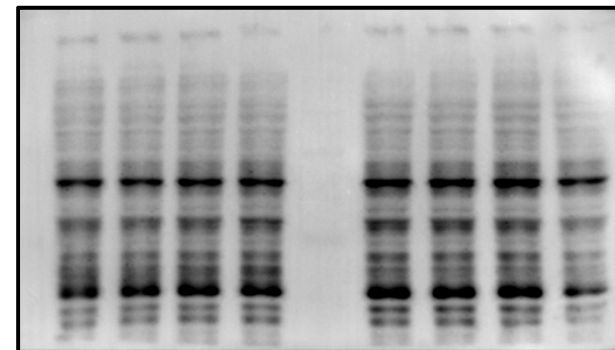
p65
(65 kDa)



β -actin
(42 kDa)

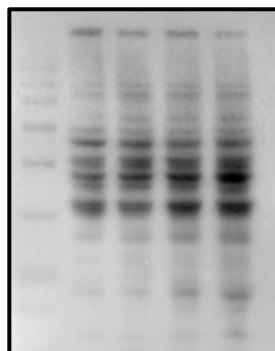
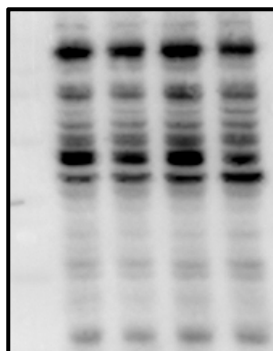


Histone-H1
(32 kDa)

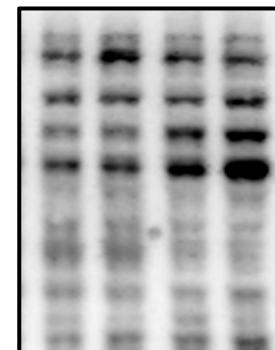
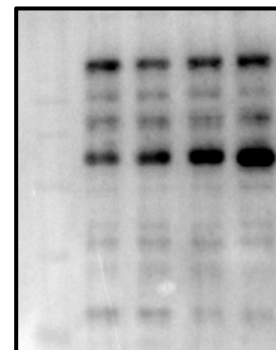


Supplementary Figure 6

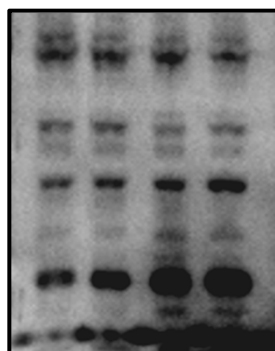
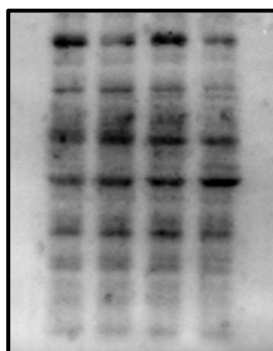
Fas
(48 kDa)



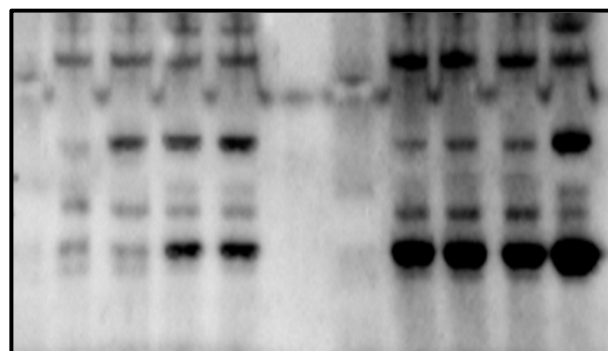
Fas
(48 kDa)



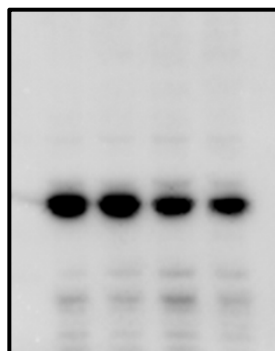
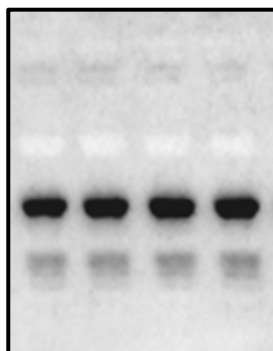
DR4
(56 kDa)



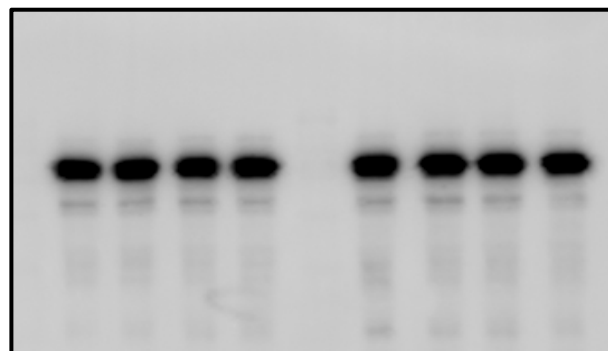
DR4
(56 kDa)



β -actin
(42 kDa)

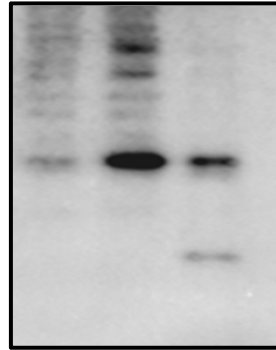
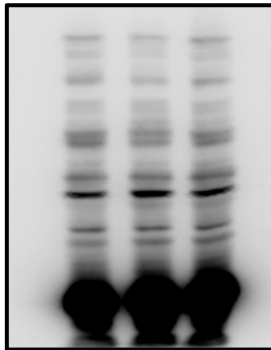


β -actin
(42 kDa)

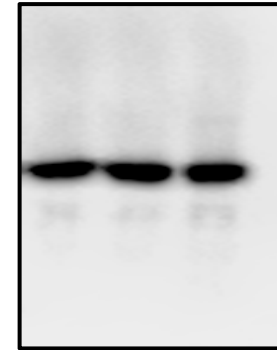
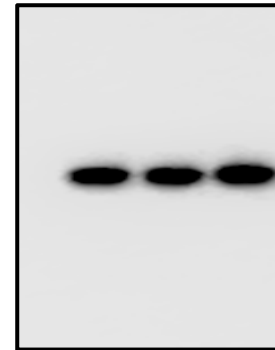


Supplementary Figure 7

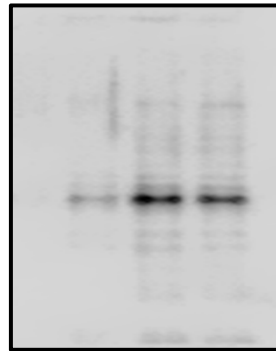
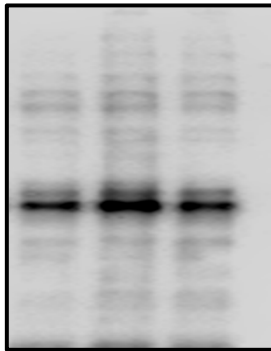
Fas
(48 kDa)



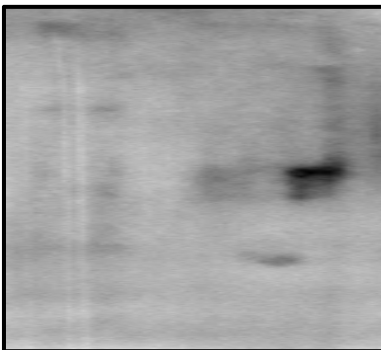
β -actin
(42 kDa)



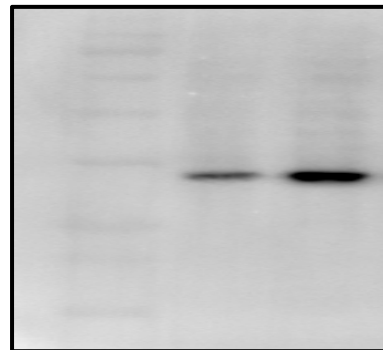
DR4
(56 kDa)



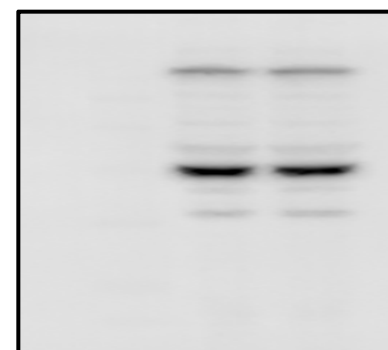
p50
(50 kDa)



p50
(50 kDa)

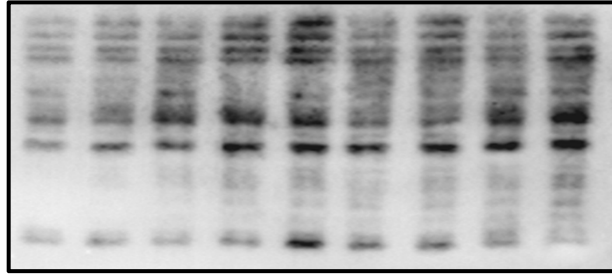


p50
(50 kDa)

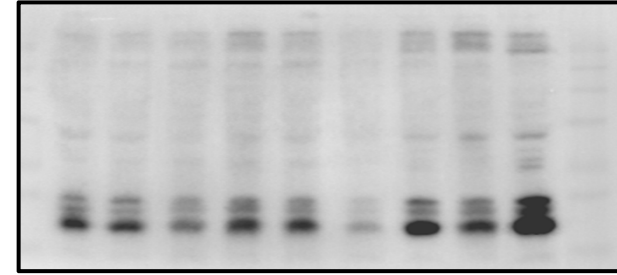


Supplementary Figure 8

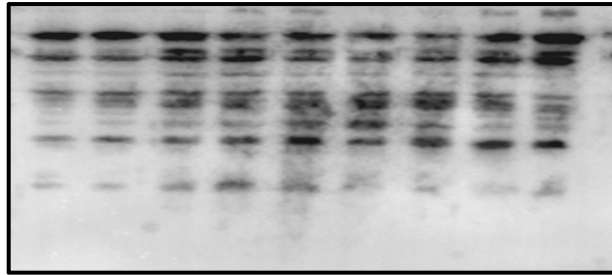
Fas
(48 kDa)



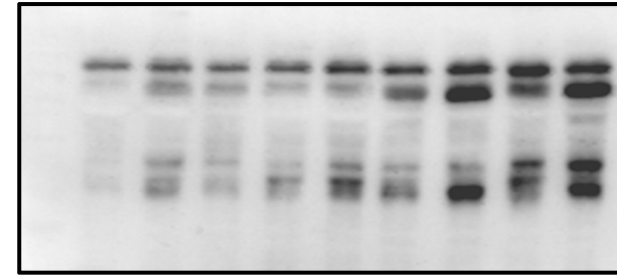
Cleaved-
Caspase-3
(11,17,20
kDa)



DR4
(56 kDa)

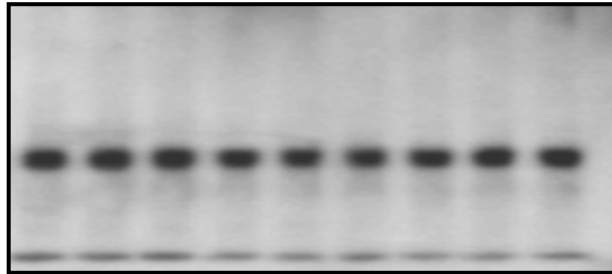


Caspase-8
(57 kDa)

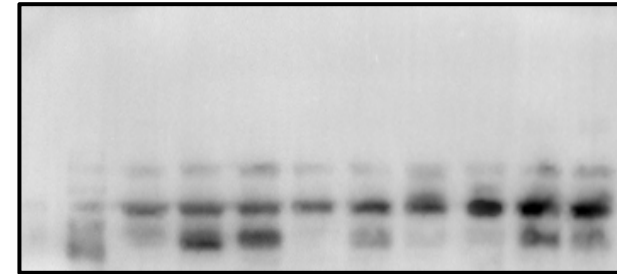


Cleaved-
Caspase-8
(18 kDa)

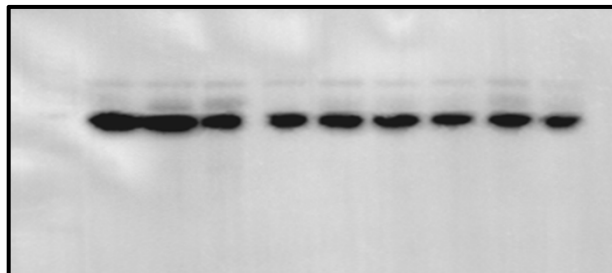
β -actin
(42 kDa)



Bax
(23 kDa)

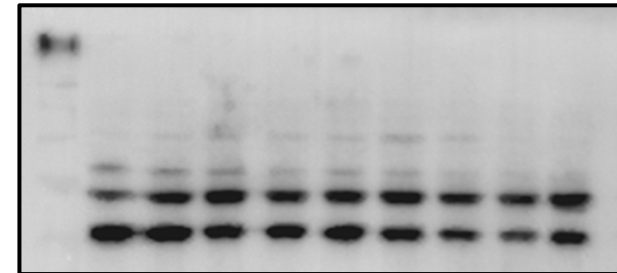


48 kDa



β -actin
(42 kDa)

Bcl-2
(28 kDa)



Supplementary Figure 9

