Supplementary Materials

Figure S1. HY-CHAL induced caspase cascade in MEL-HO cells.

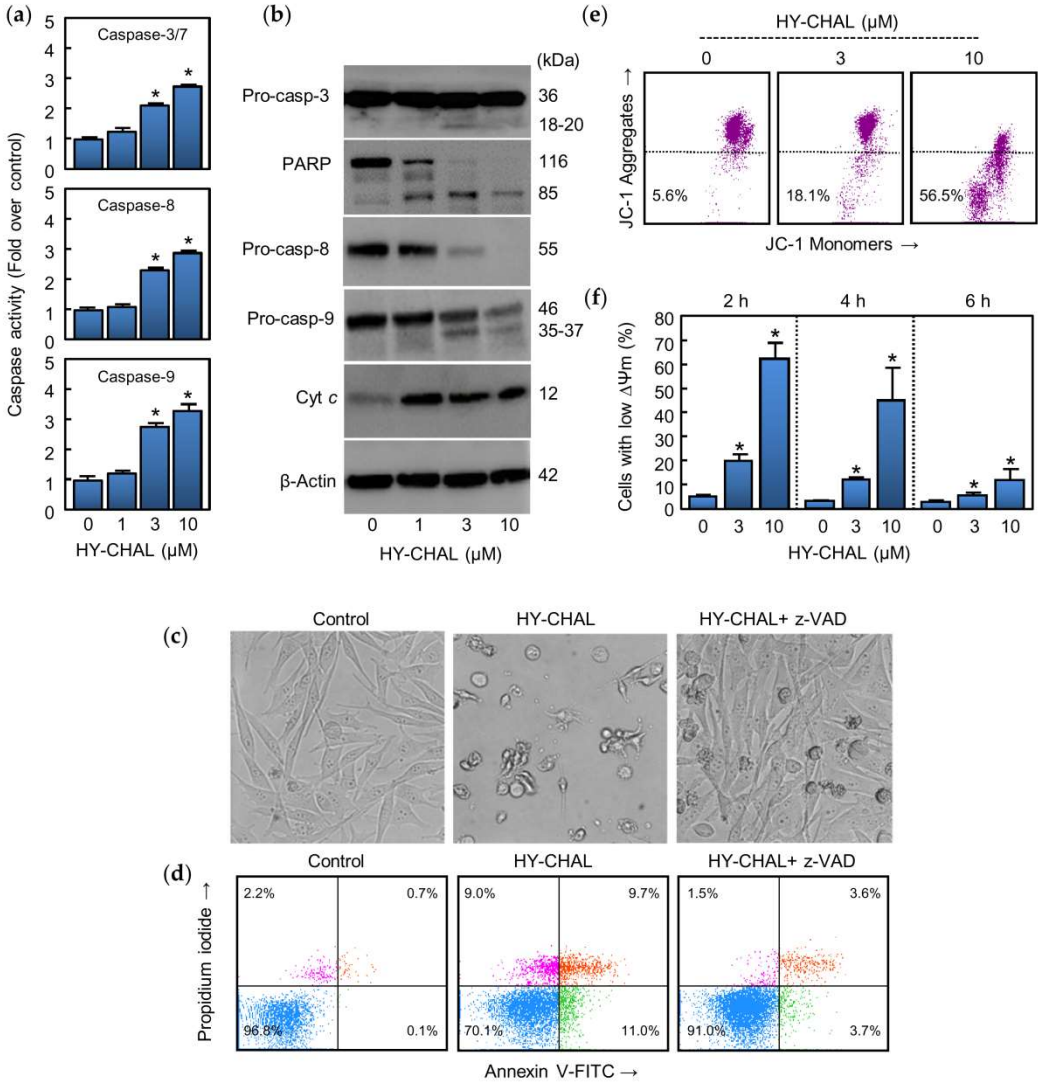
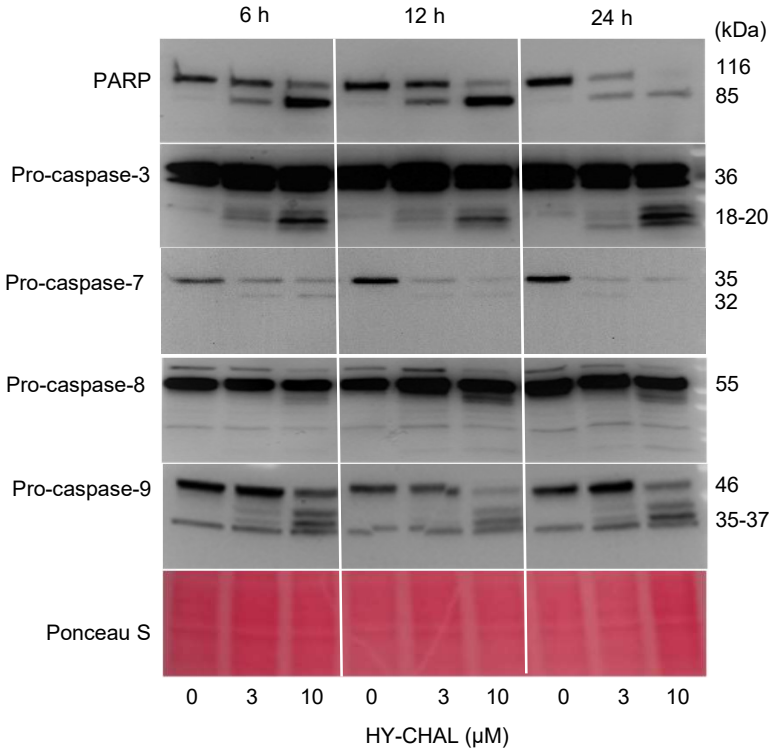
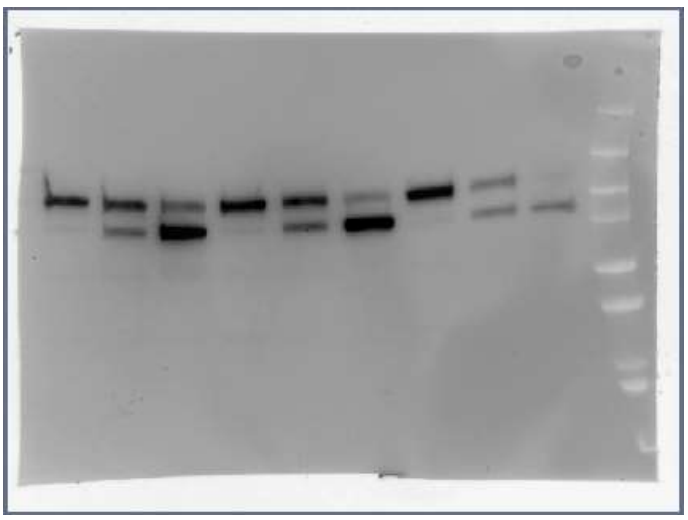


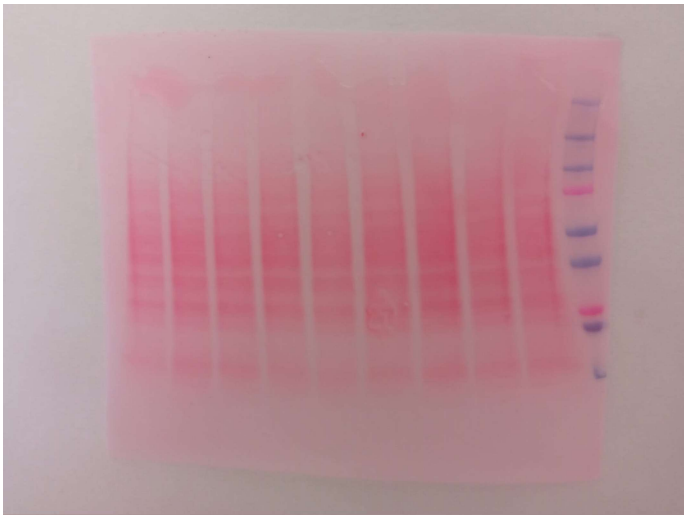
Figure S1. HY-CHAL induced caspase cascade in MEL-HO cells. (**a**) Cells were treated with the specified concentrations of HY-CHAL, harvested at 24 h and total cell lysates were assayed for caspase-3/7, -8, and -9 activities. Results are expressed as factorial increases in caspase activity compared with the control. Values represent means ± SE of two independent experiments each performed in triplicate; (**b**) Cells were treated as in (**a**) and whole cell lysates or cytosolic fractions (in the case of cytochrome *c*) were analyzed by immunoblotting. β-Actin was used as a loading control; (**c**) Cells were pretreated with 100 μM z-VAD-fmk for 1 h and then incubated in the absence or in the presence of 3 μM HY-CHAL for 24 h and images were obtained with an inverted phase-contrast microscope; (**d**) Cells were treated as in (**c**) and analyzed by flow cytometry after staining with annexin V-FITC and propidium iodide; (**e**) Cells were incubated with the indicated concentrations of HY-CHAL for 2 h and $\Delta\Psi_m$ analyzed by flow cytometry after staining with the JC-1 probe; (**f**) Cells were incubated in control conditions or in the presence of the indicated concentrations of HY-CHAL for the specified times, and the percentage of cells with reduced $\Delta\Psi_m$ was quantified by flow cytometry using JC-1. * Indicates $p < 0.05$ for comparison with untreated control.

Whole Western blots. Uncropped western blots

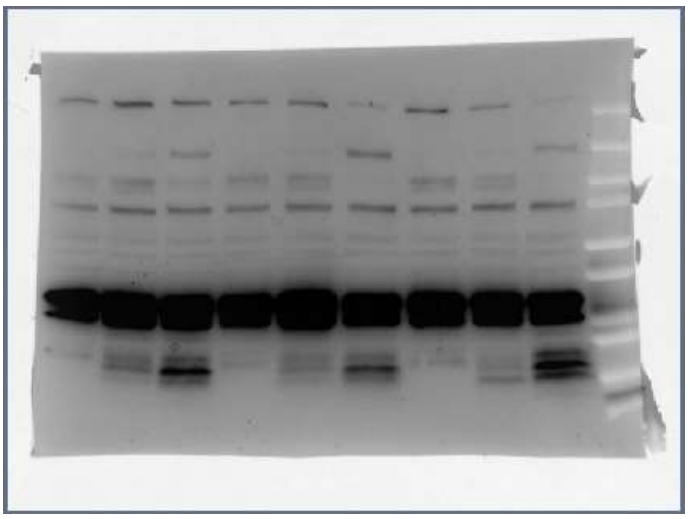


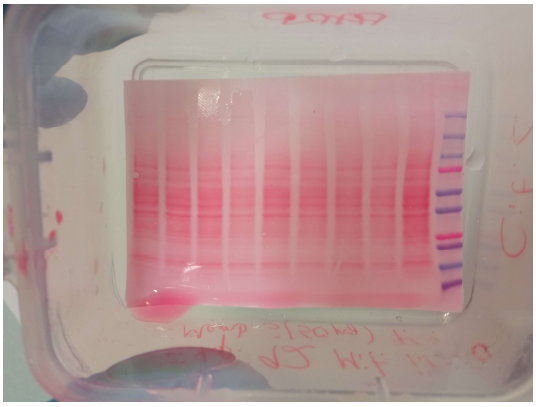
PARP





Pro-caspase-3





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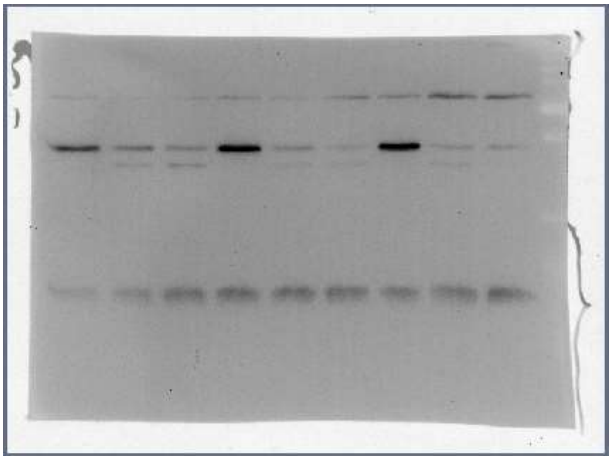

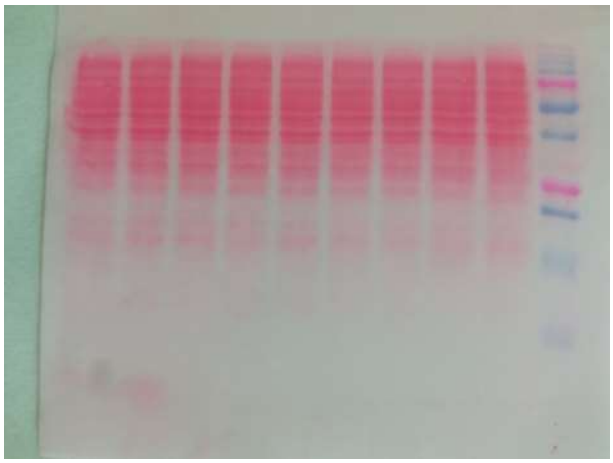

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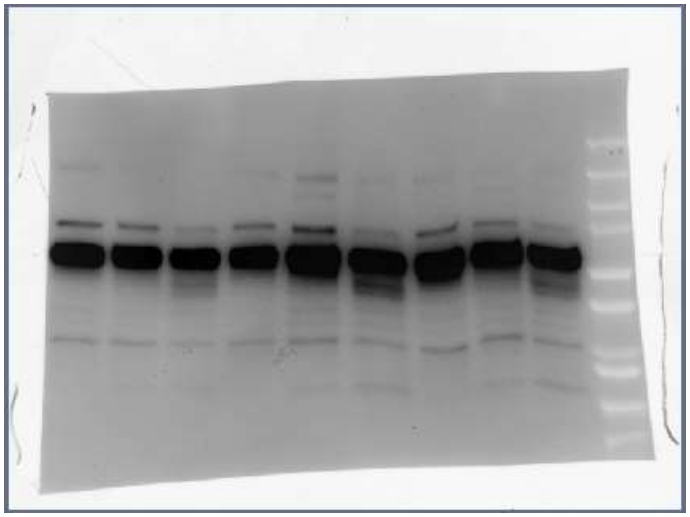
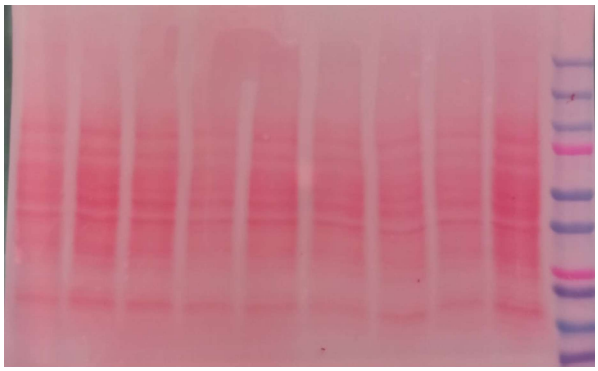
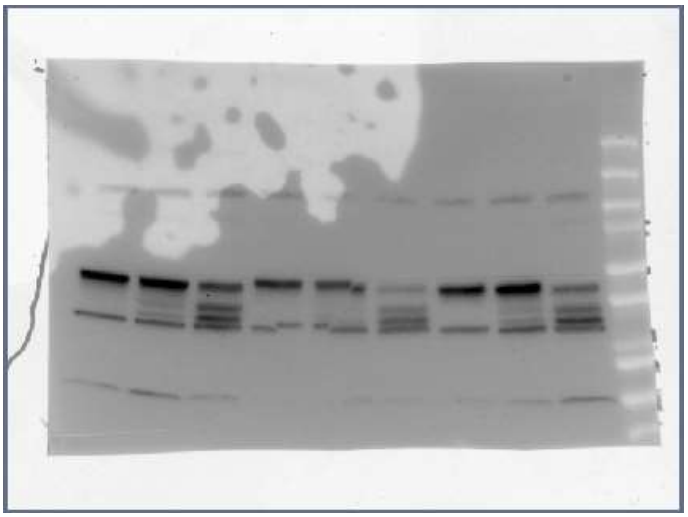
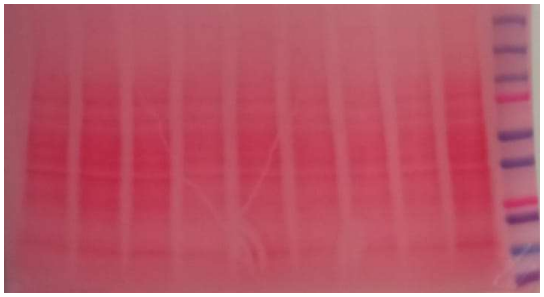
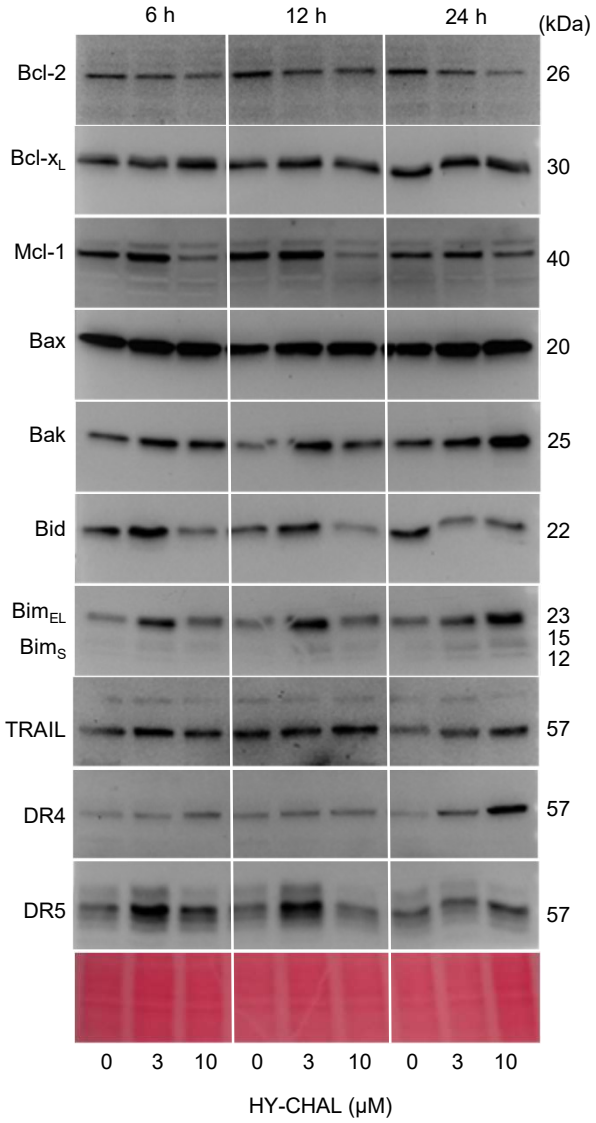


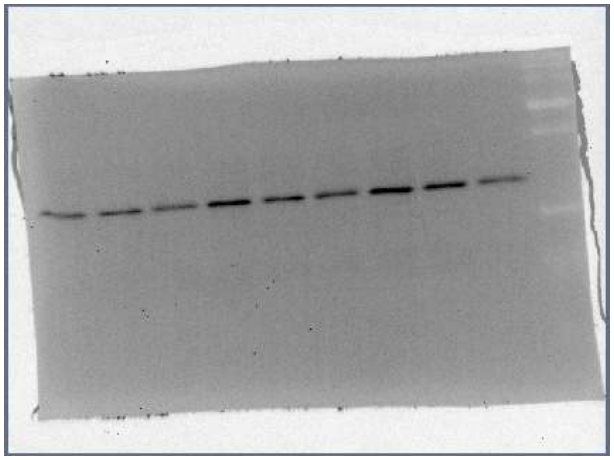


Pro-caspase-9






6 h
12 h
24 h
(kDa)
Bcl-2
Bcl-xL
Mcl-1
Bax
Bak
Bid
BimEL
BimS
TRAIL
DR4
DR5
26
30
40
20
25
22
23
15
12
57
57
57
0 3 10 0 3 10 0 3 10
HY-CHAL (μM)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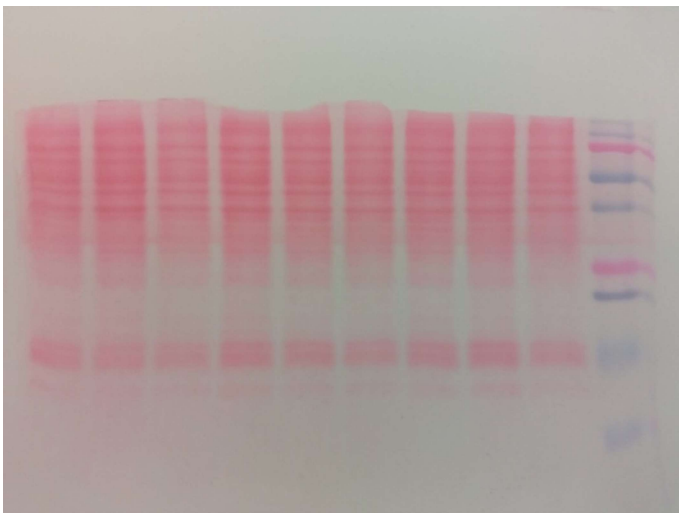
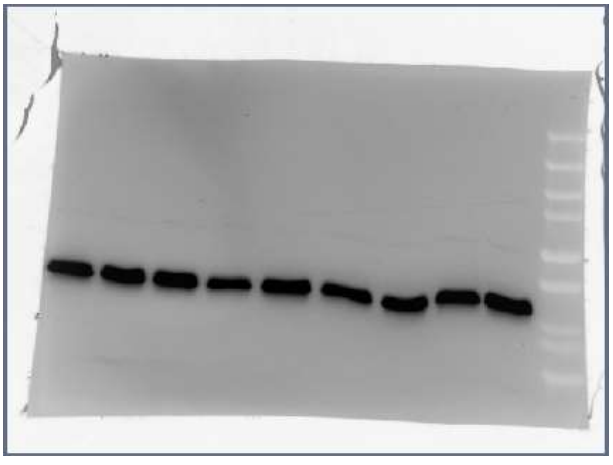
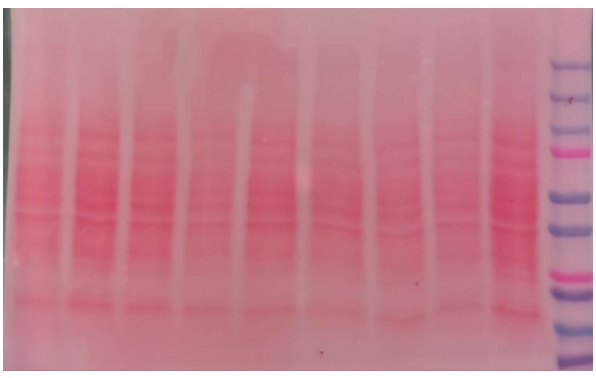
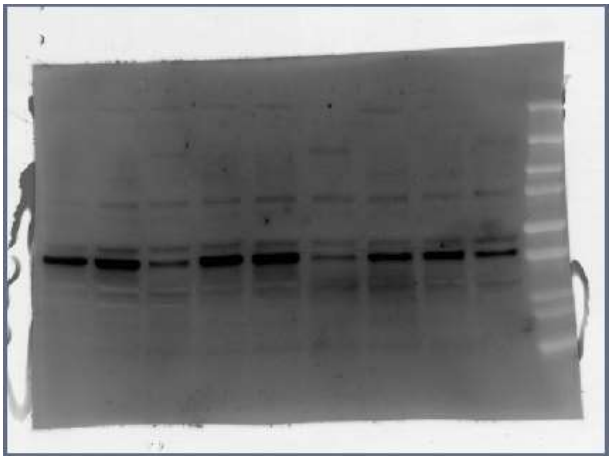
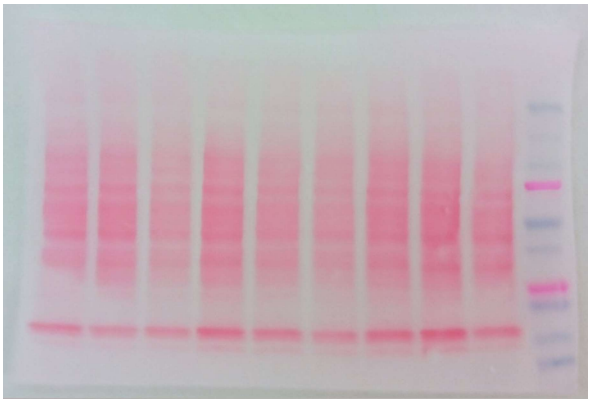



Bcl-xL

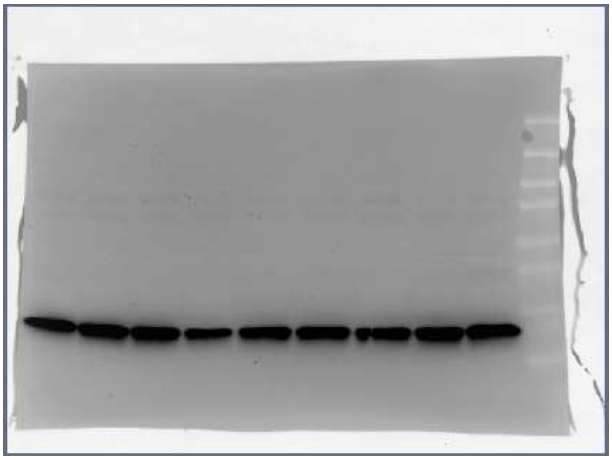


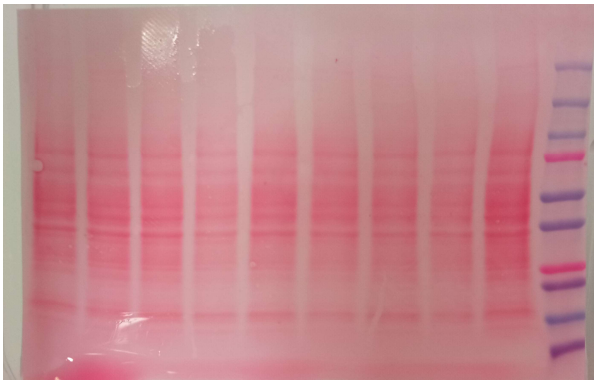
Mcl-1





Bax





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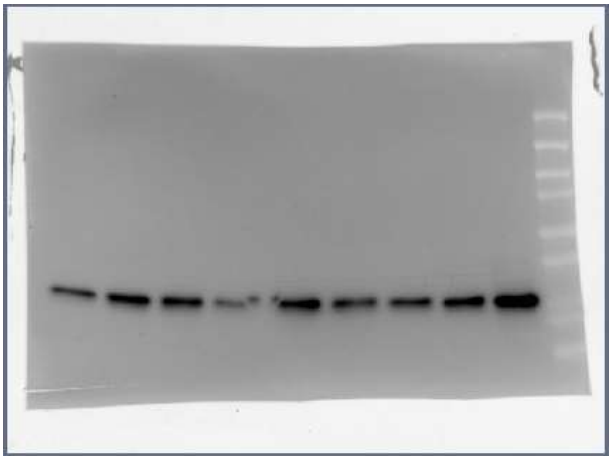

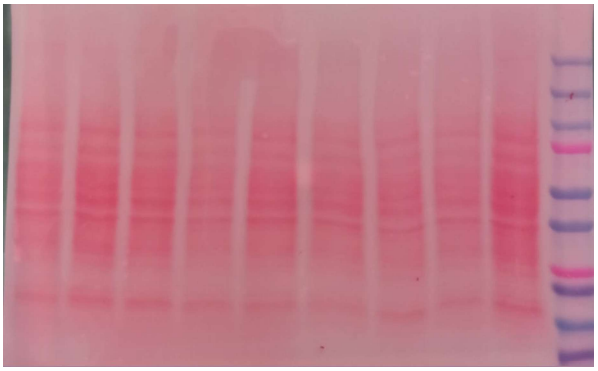

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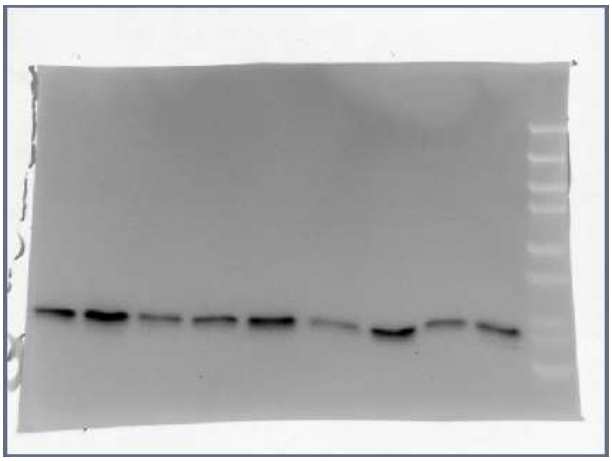
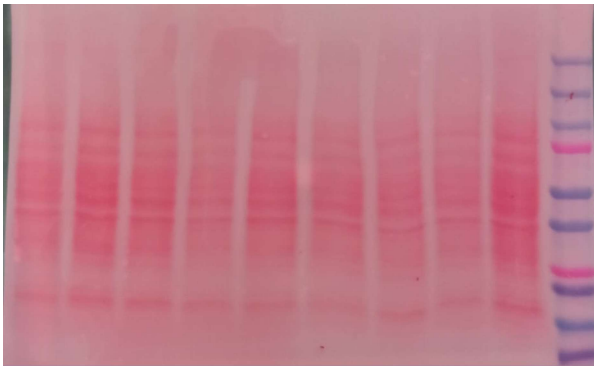
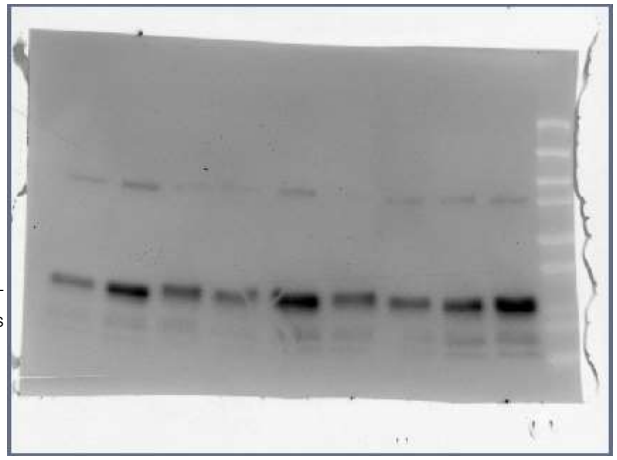
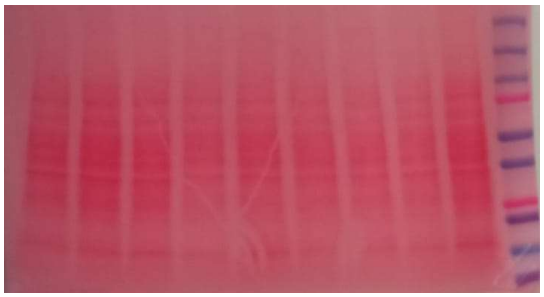
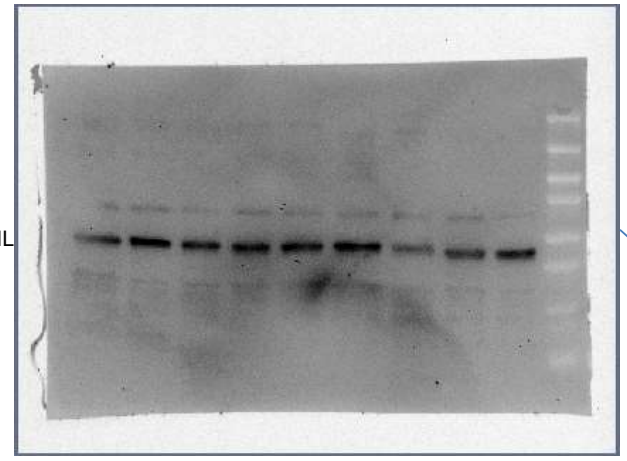





Bim_{EL}
Bim_S

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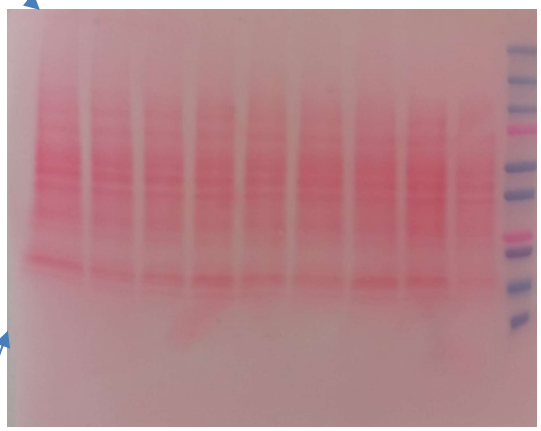

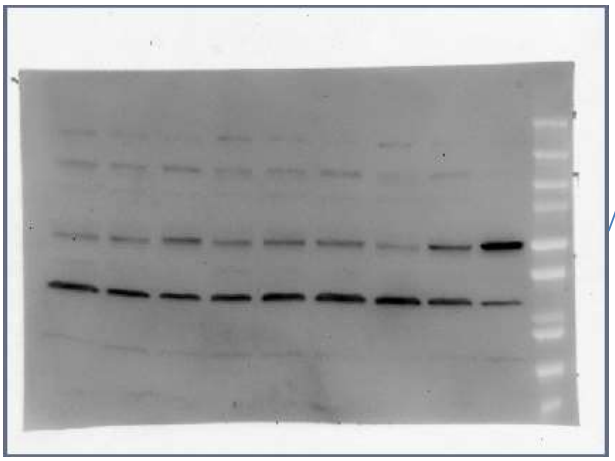
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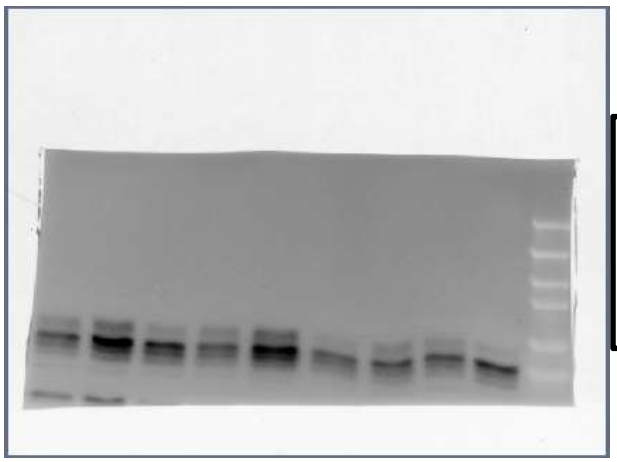
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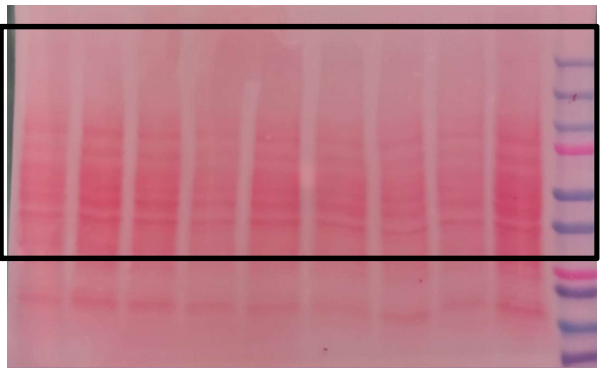
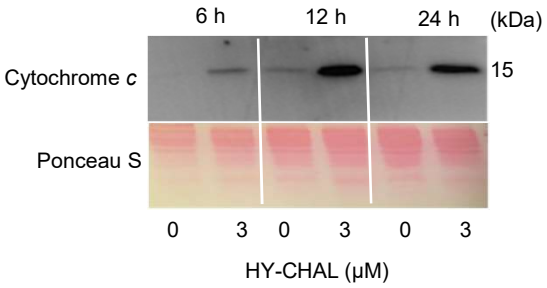
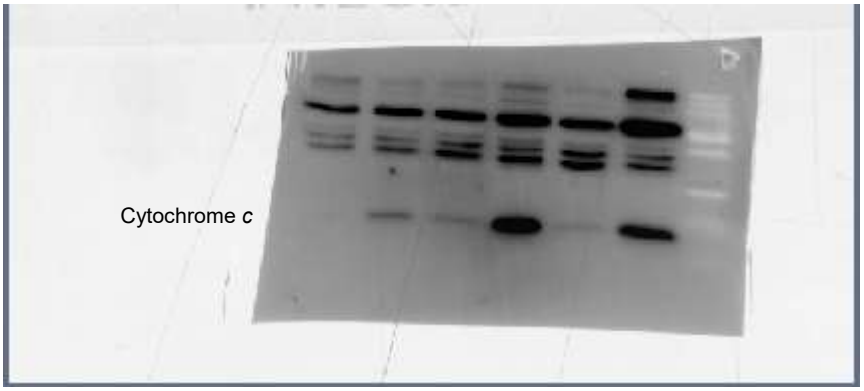
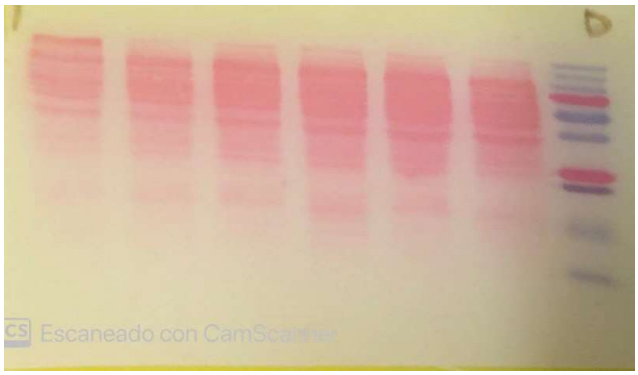
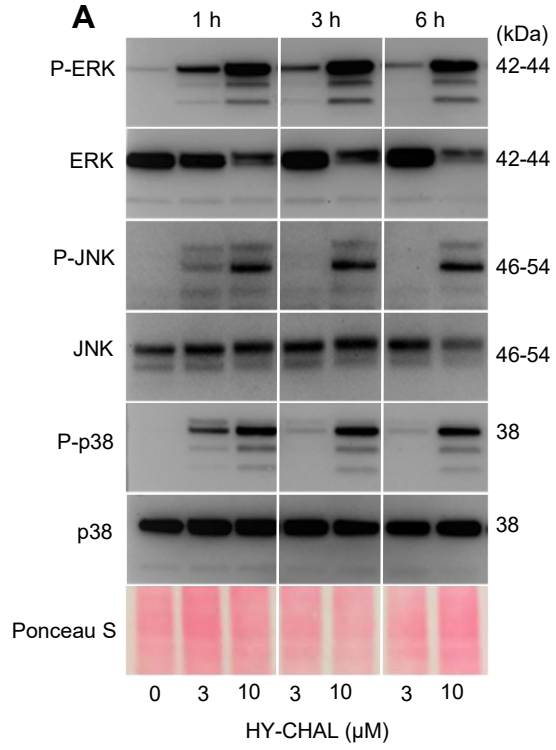
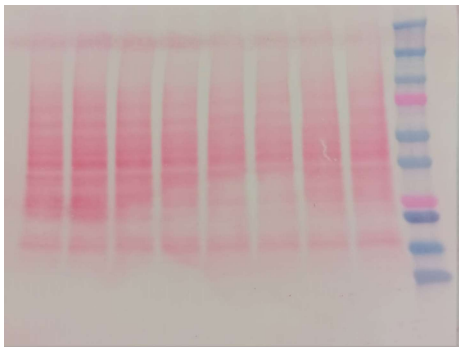


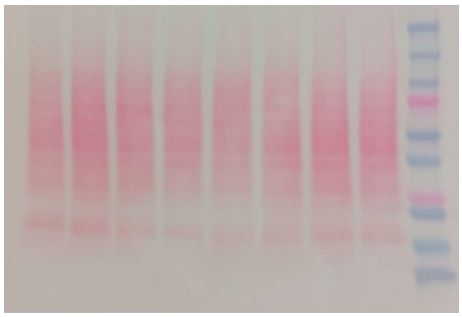

6 h
12 h
24 h
(kDa)
Cytochrome c
15
Ponceau S
0
3
0
3
0
3
HY-CHAL (μM)

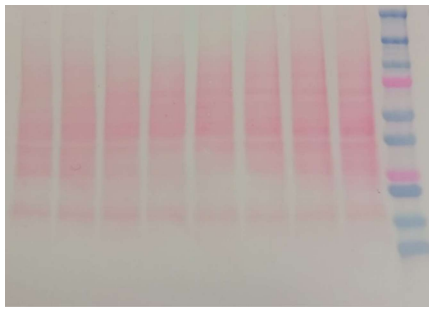

Cytochrome c

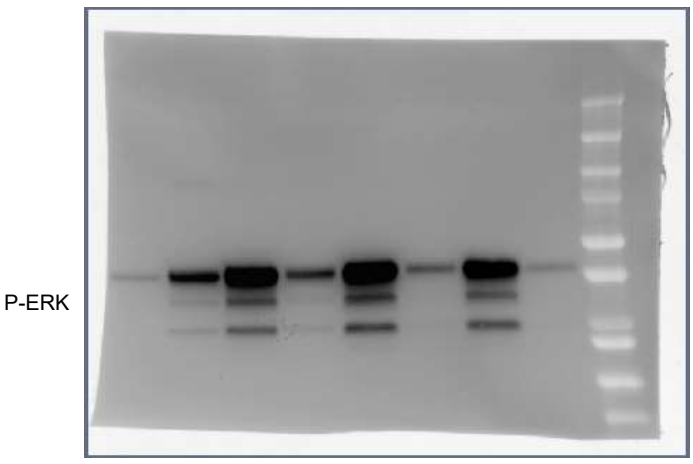

Escaneado con CamScanner

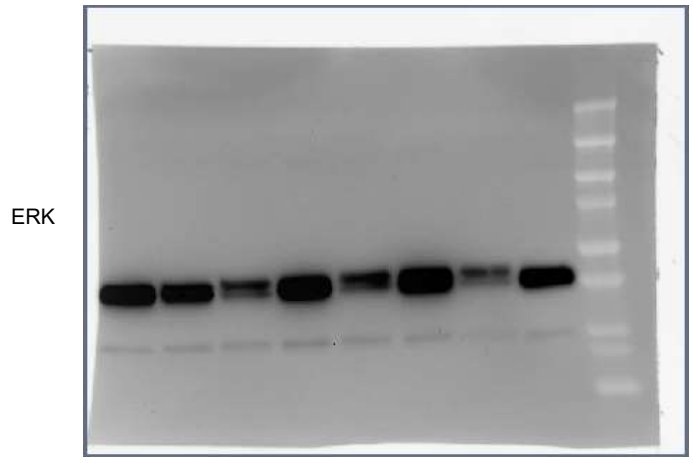












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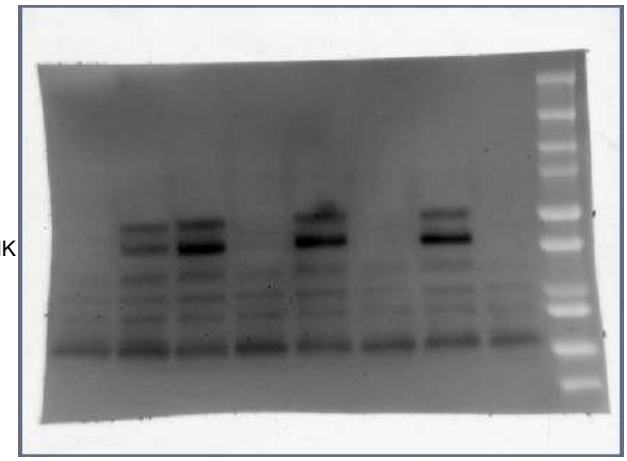
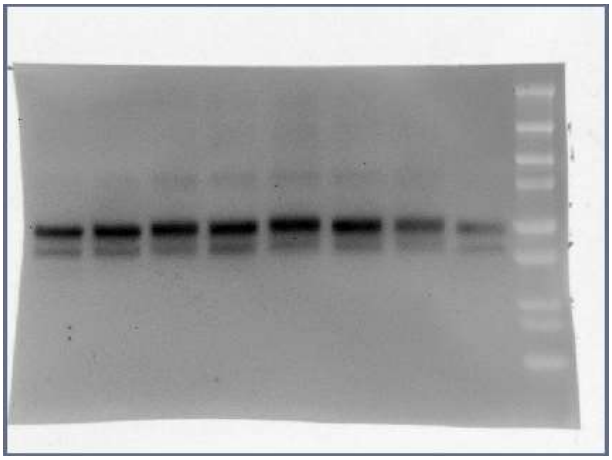
P-JNK



Last line, anisomycin, is not included in Figure 5a.

JNK



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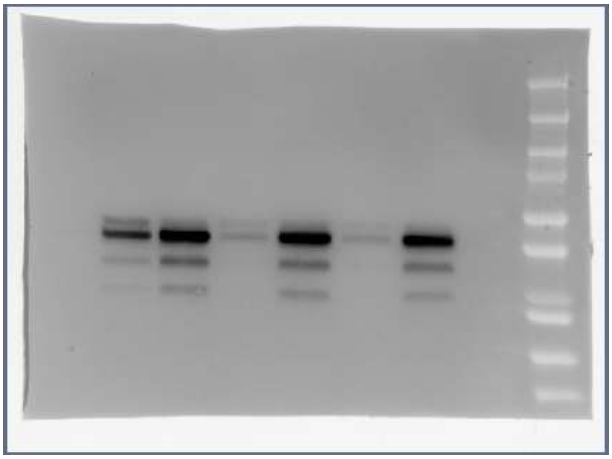
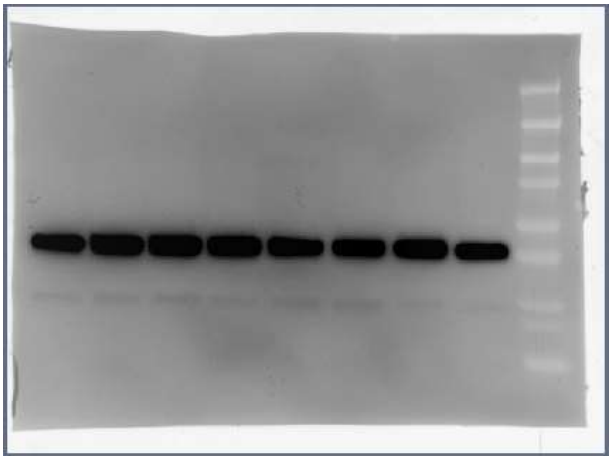
P-p38

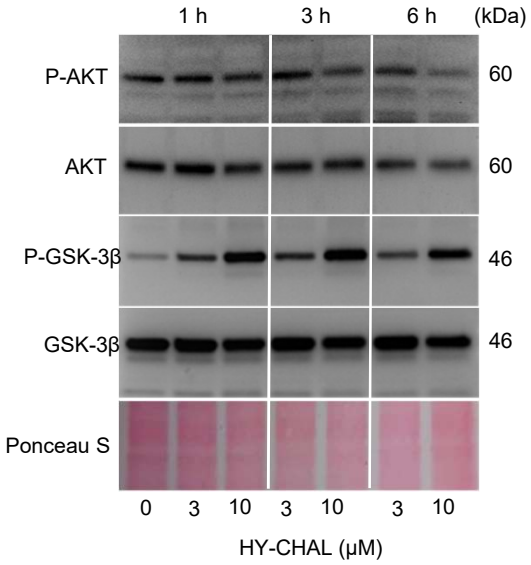


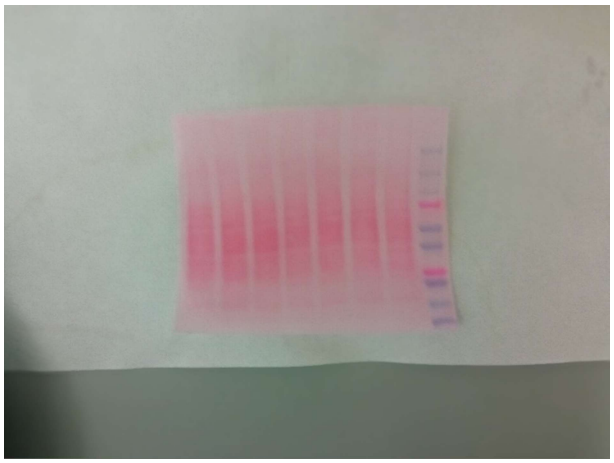
Last line, anisomycin, is not included in Figure 5a.

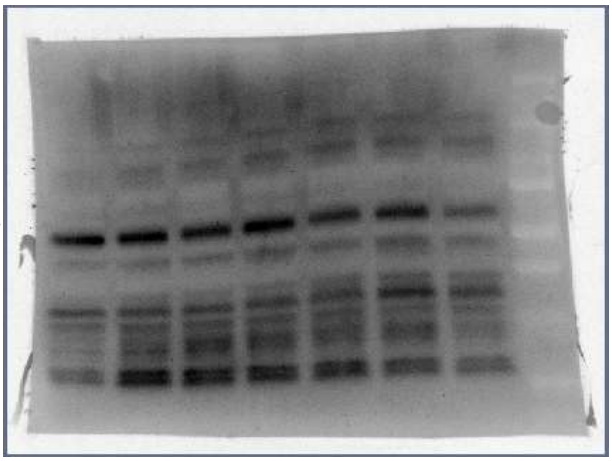
p38

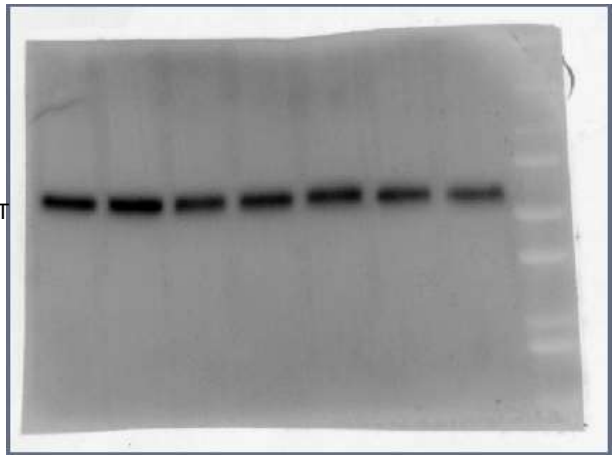


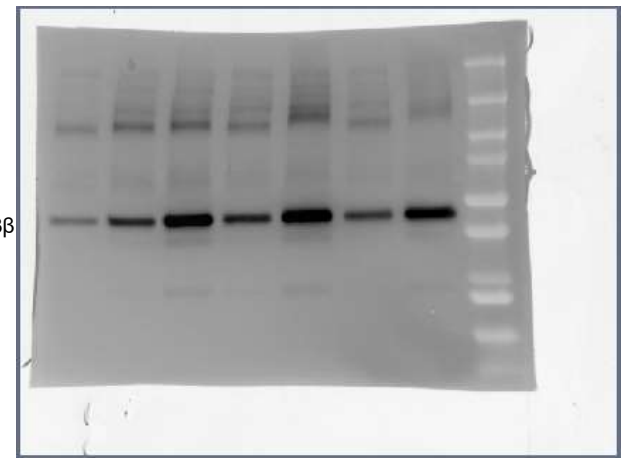
Last line, anisomycin, is not included in Figure 5a.


1 h
3 h
6 h
(kDa)
P-AKT
AKT
P-GSK-3β
GSK-3β
Ponceau S
60
60
46
46
0
3
10
3
10
3
10
HY-CHAL (μM)




P-AKT


AKT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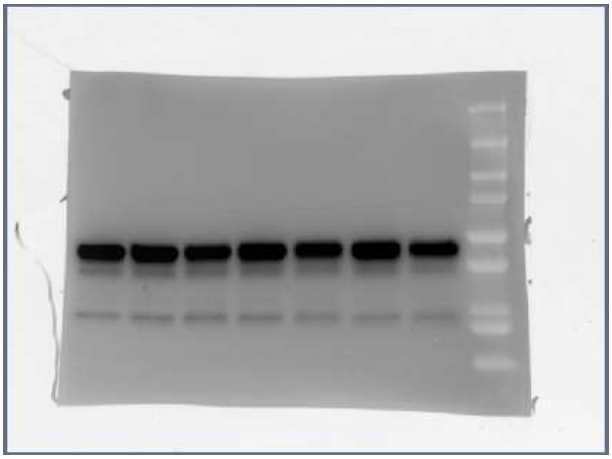
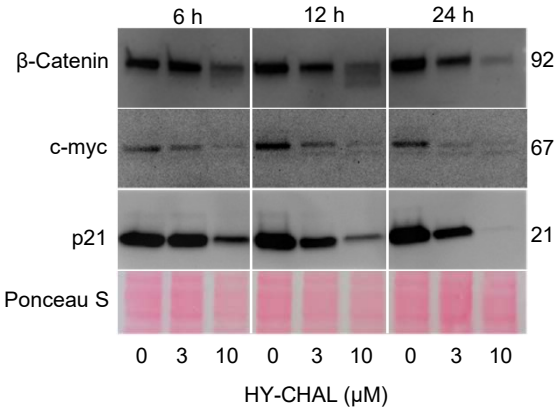
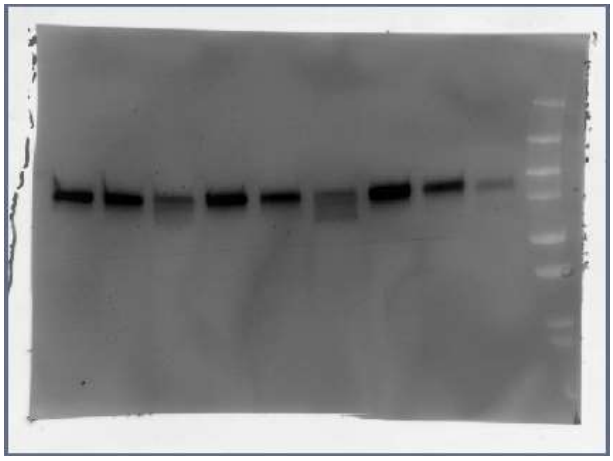
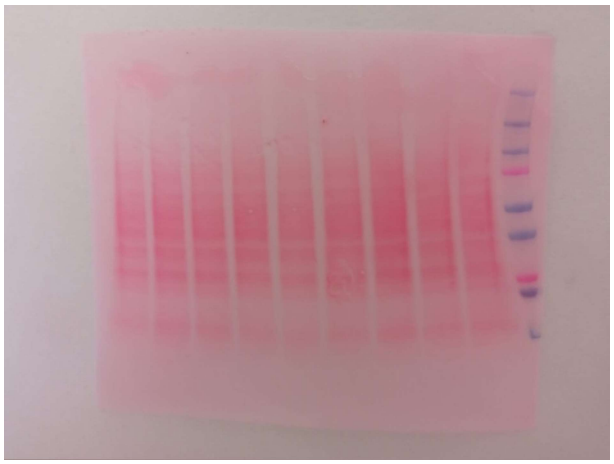

GSK-3β

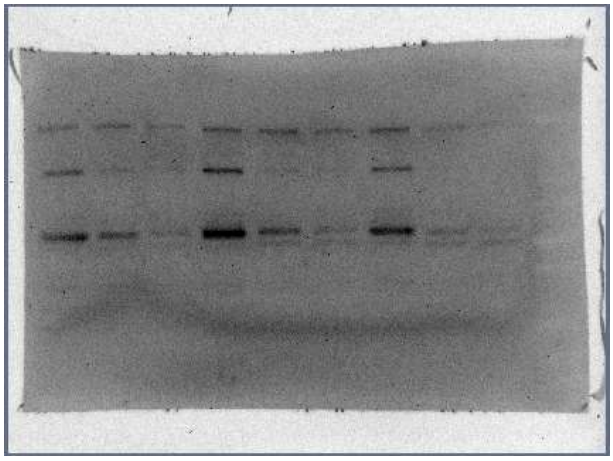


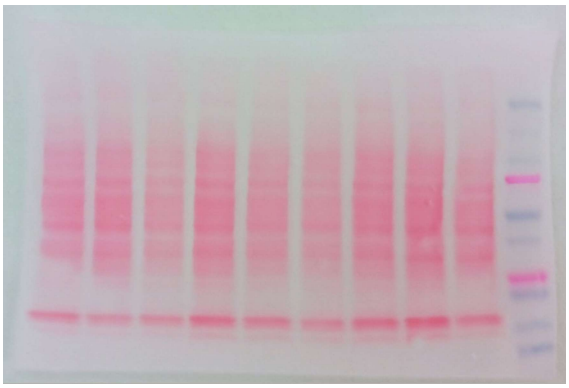
β-Catenin





c-myc





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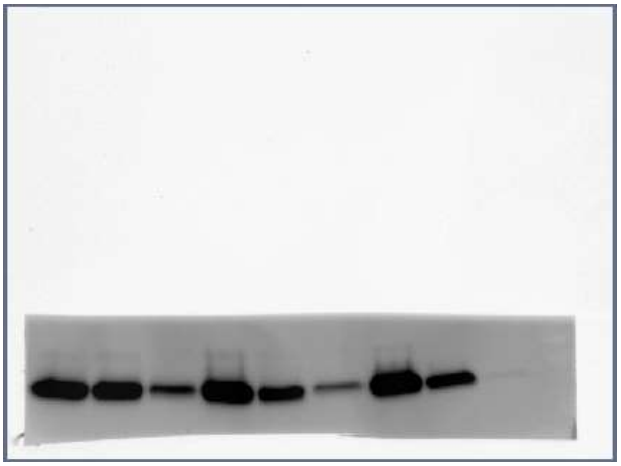
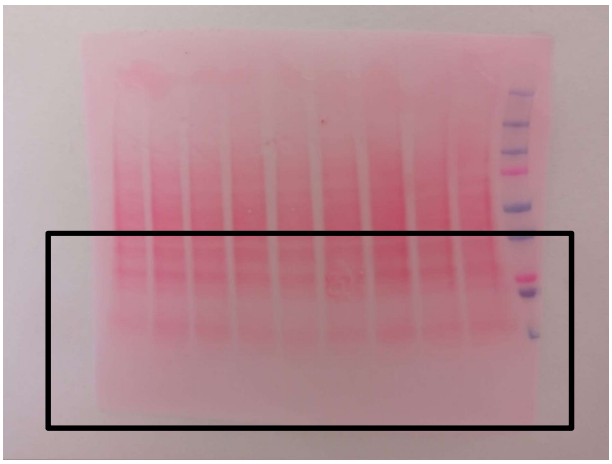
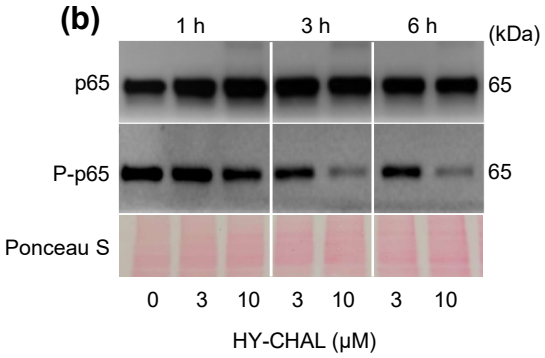
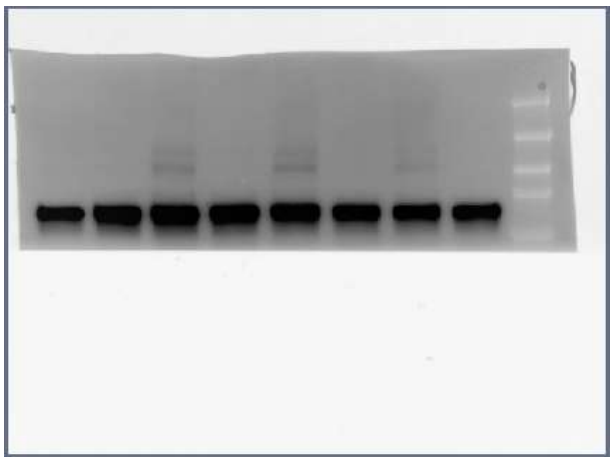
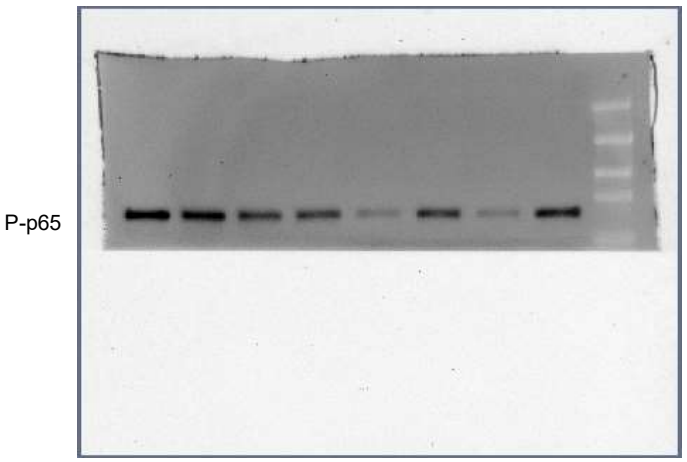
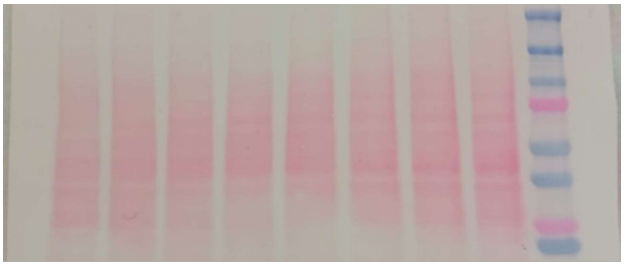




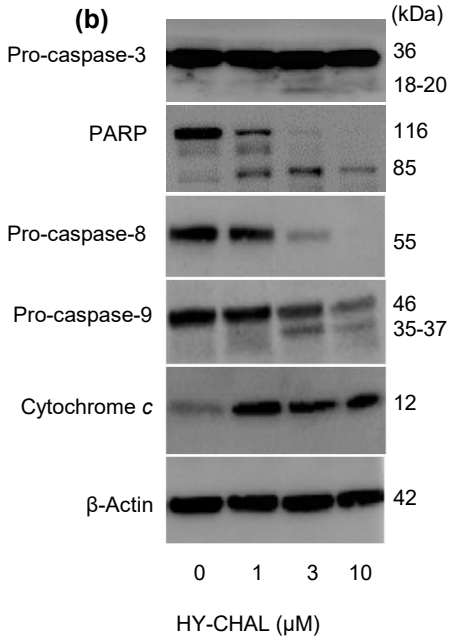


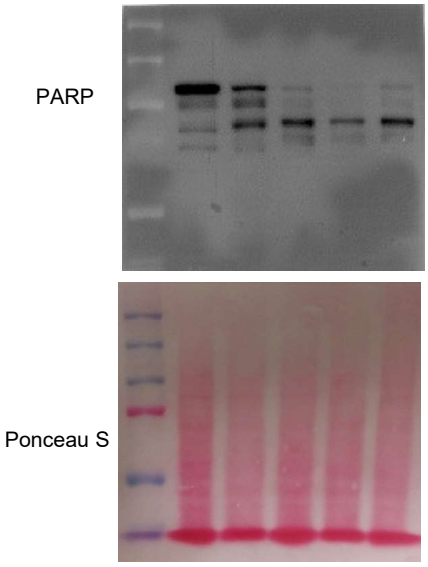




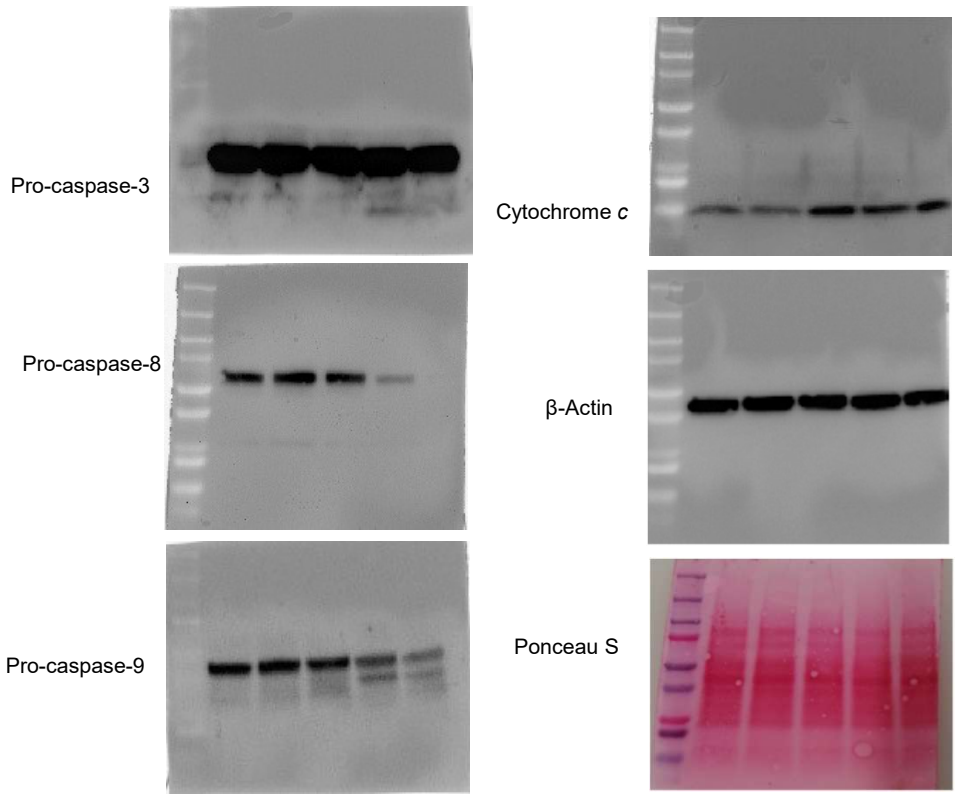


Last line (anisomycin) is not included.in Figure 6b.





Last line is not included.in Figure S1b.
Ponceau S staining is not included.



First line (control, equal to line 2) is not included in Figure S1b.
Ponceau S staining is not included.