

Boosting Proteasome Activity: A Novel Mechanism of NMDAR Antagonists Against Neurodegeneration

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Main text Western Blots

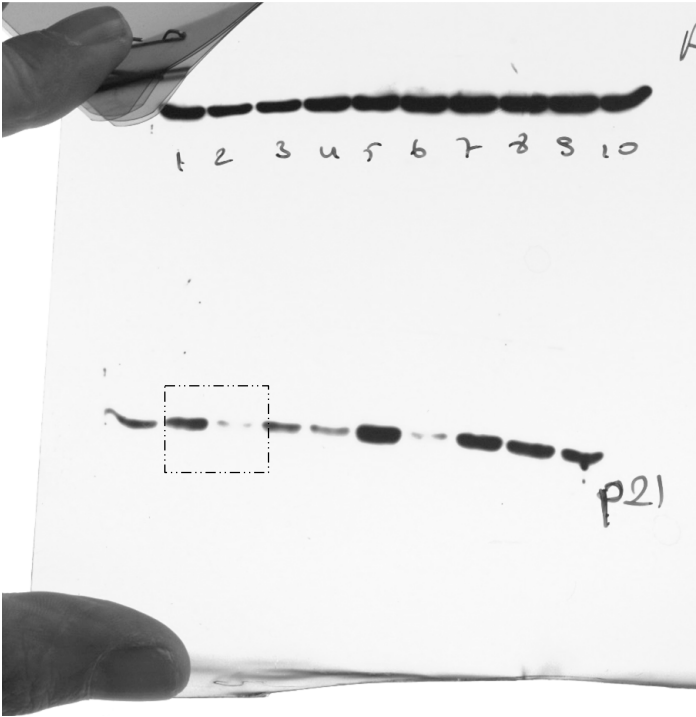
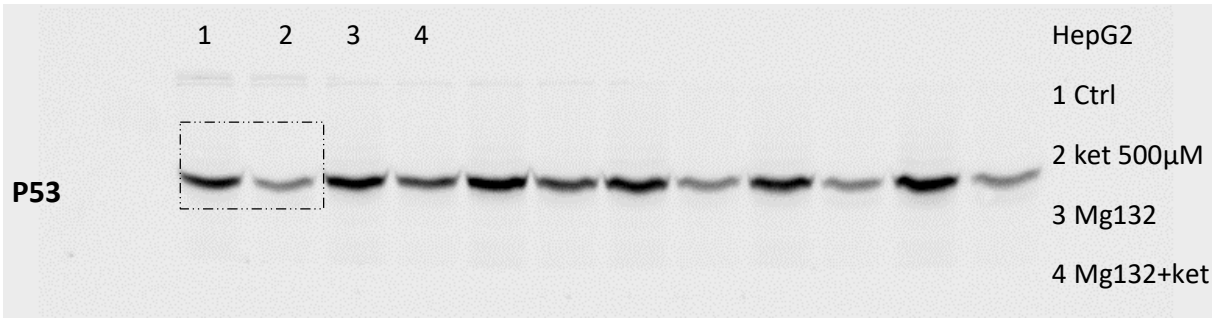
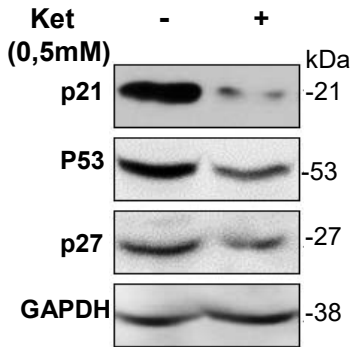


Fig. 1A

- 1-loading cell prs
- 2-ctrl
- 2-ket 500μM
- 3-ctrl
- 4-Memantine
- 5-h2o2
- 6-h2o2+ket
- 7-10-Ca2+ related



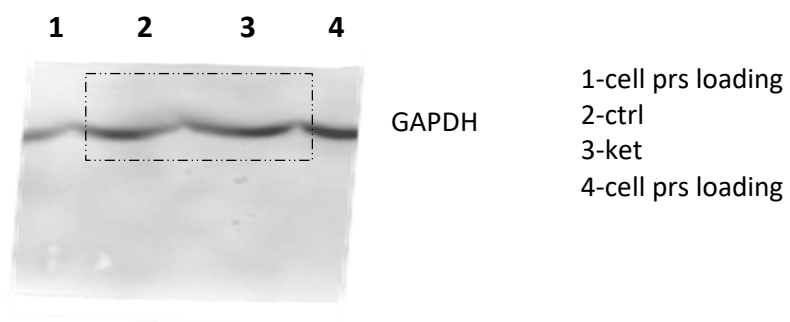
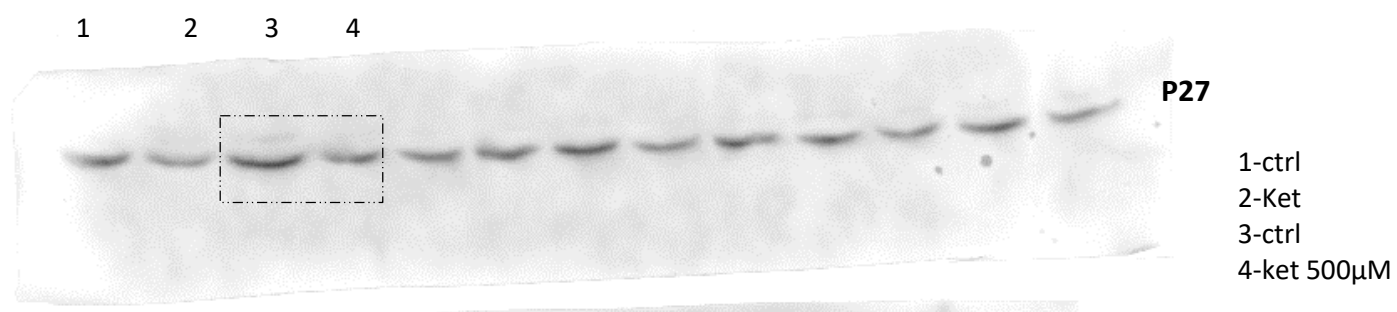
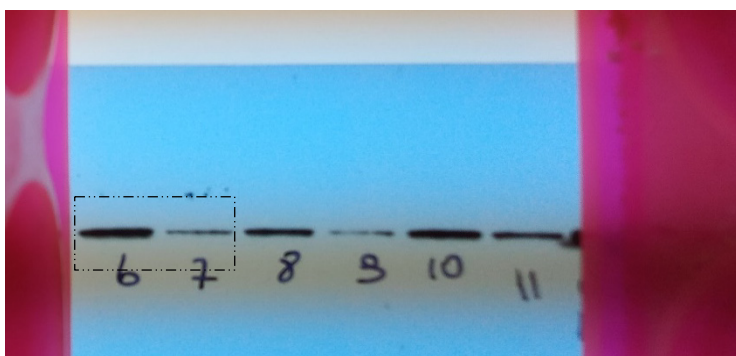
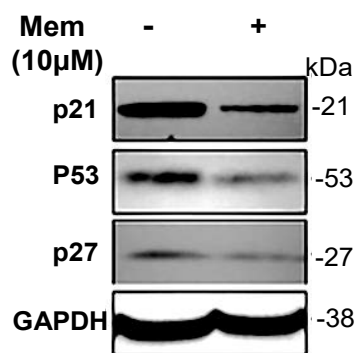
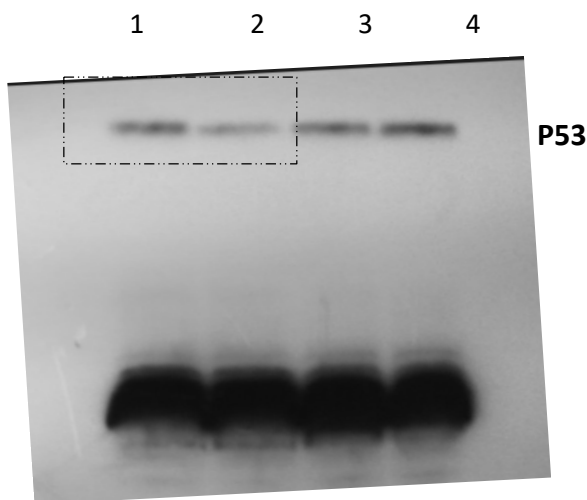


Fig. 1B

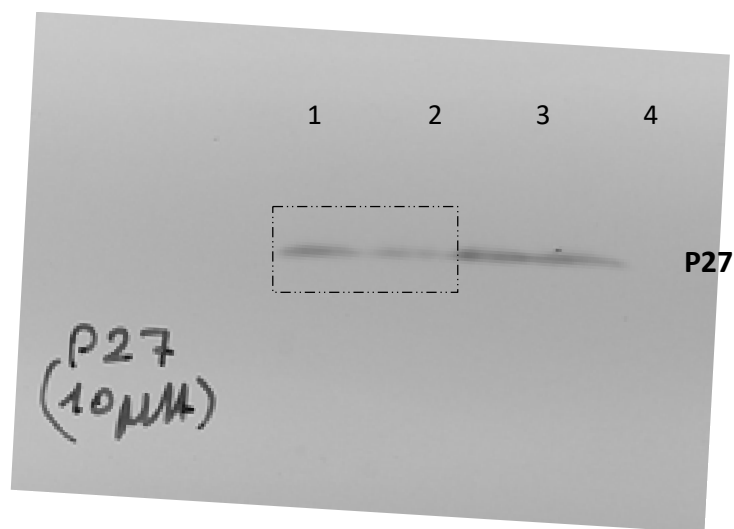


P21

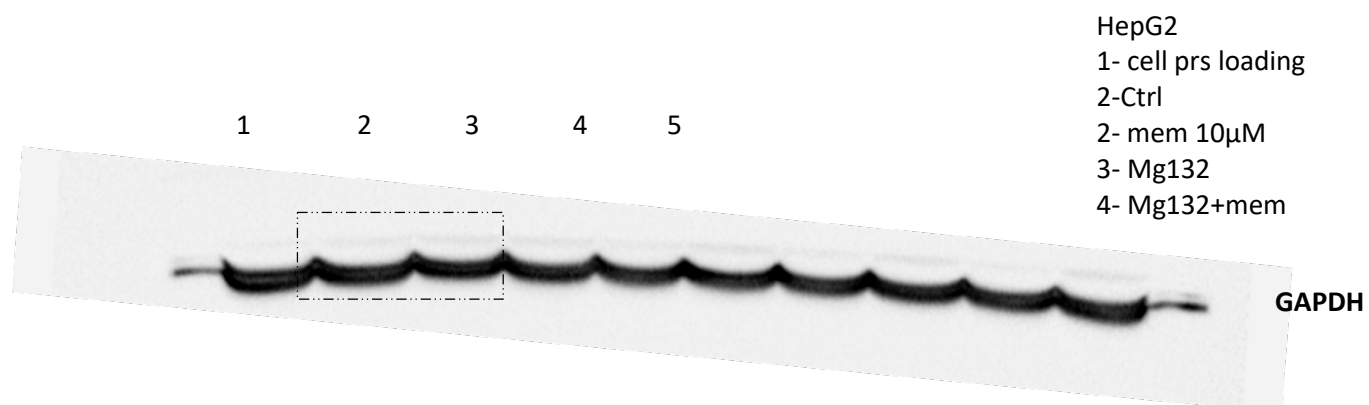
6-ctrl
7-memantin10μM
8-ctrl
9-ket 500μM
10-ctrl
11-MK801



HepG2
1- Ctrl
2- mem 10μM
3- Mg132+mem
4- Mg132

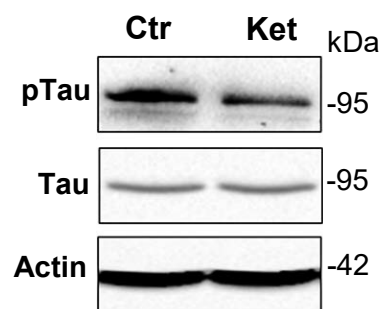
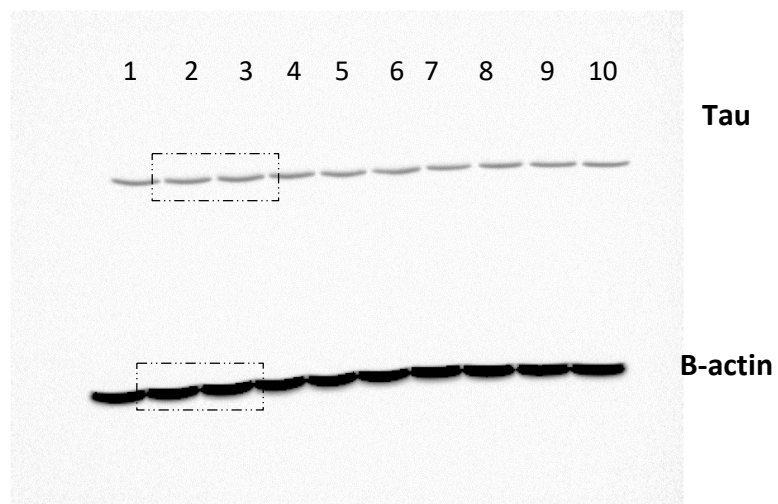


HepG2
 1- Ctrl
 2- memantin 10μM
 3- Mg132
 4- Mg132 +mem



HepG2
 1- cell prs loading
 2- Ctrl
 2- mem 10μM
 3- Mg132
 4- Mg132+mem

Fig. 1C



1-ctrl

2 ctrl

3-ket

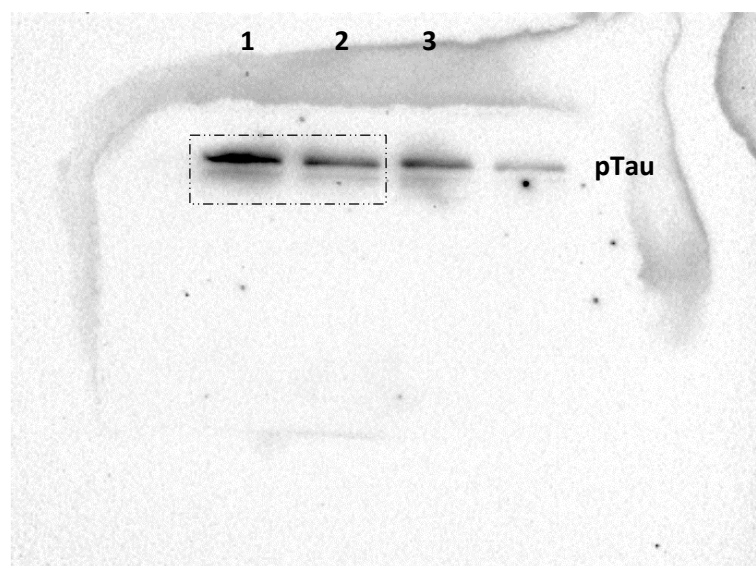
4 ket

5-ctrl

6-ctrl

7-mem

8-mem



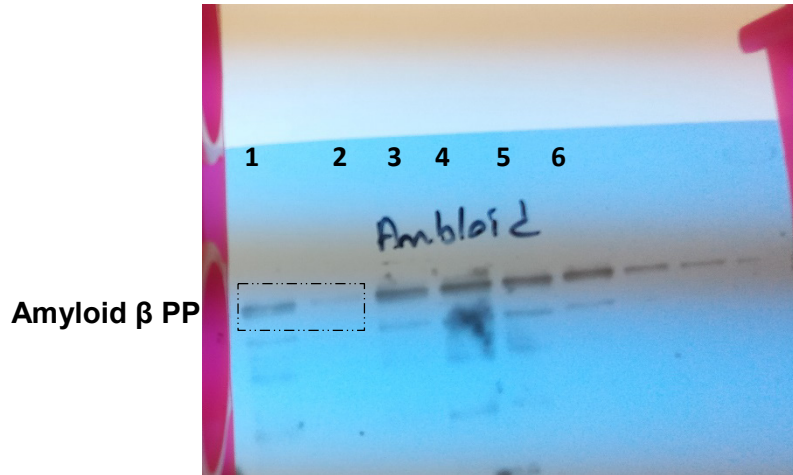
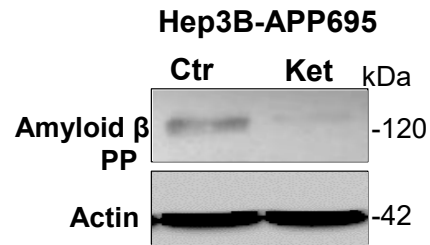
HepG2-pTau

1-ctrl

2-ket

3-mem

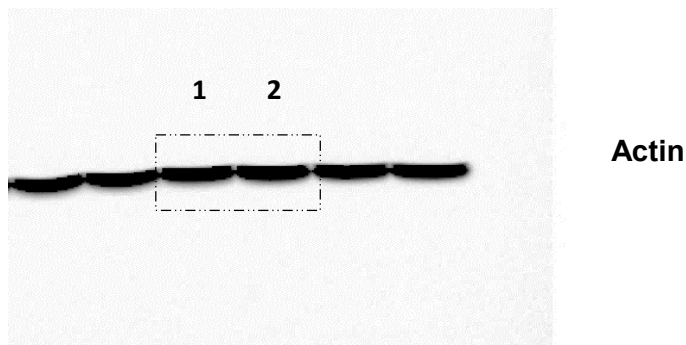
Fig. 1D

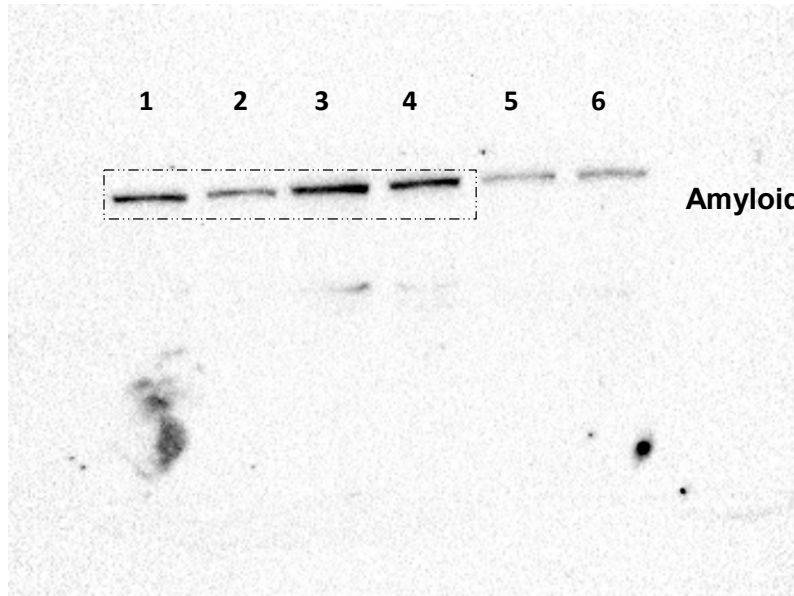


1-ctrl (Cell-Hep3B-APP695)

2-ket (Cell-Hep3B-APP695)

**3-... APP695 expressing
different Hep3B cell lines**





Other cells (not shown in the manuscript) expressing APP695

1-ctrl (Cell-HepG2-APP695)

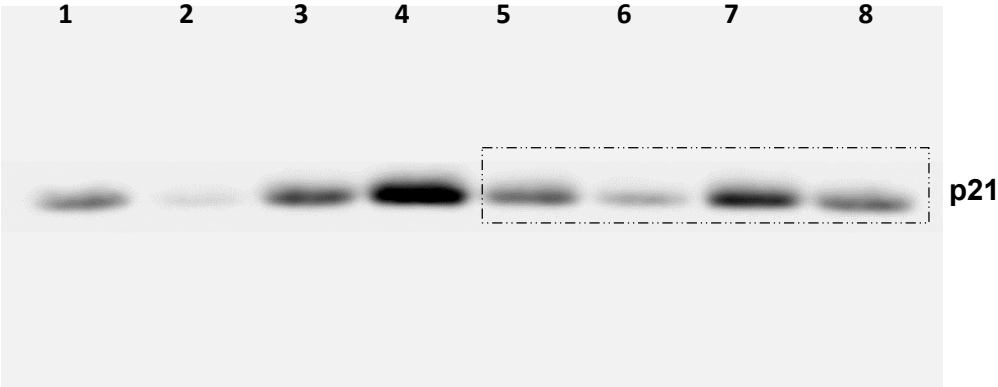
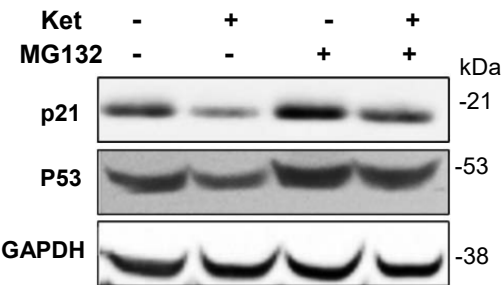
2-ketamin (Cell-HepG2-APP695)

3-Ctrl (Cell-SH-SY5Y-APP695)

4-Ketamin (Cell-SH-SY5Y-APP695)

5-6-APP695 expressing T98G cell lines

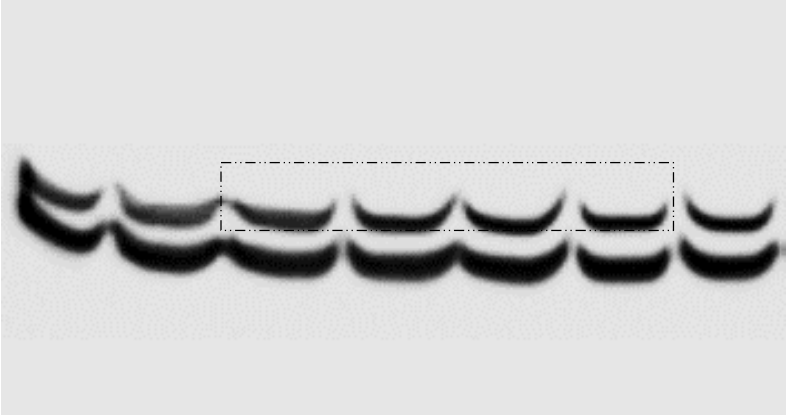
Fig. 1H



- 1-ctrl
- 2-ket
- 3-MG132+ket
- 4-MG132
- 5-ctrl
- 6-ket
- 7-MG132
- 8-MG132+ket



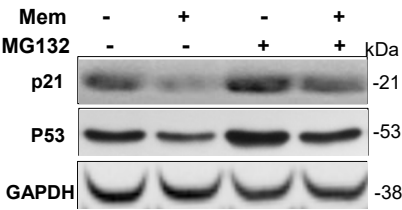
- 1-ctrl
- 2-ket
- 3-MG132
- 4-MG132+ket



GAPDH

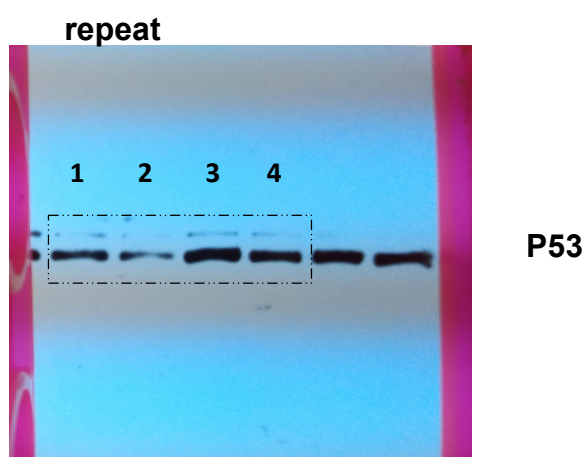
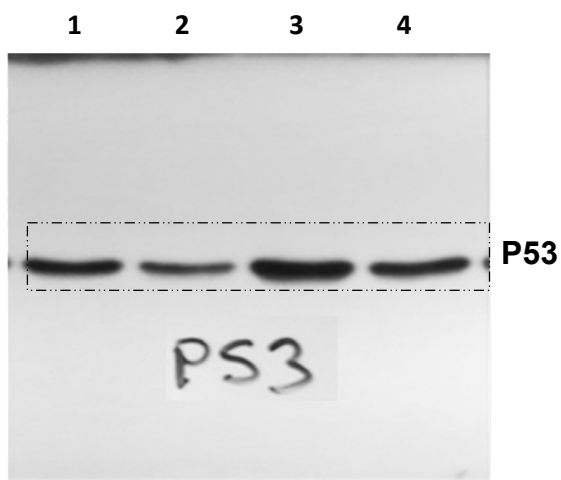
1-ctrl
2-ket
3-MG132
4-MG132+ket

Fig. 1I



p21

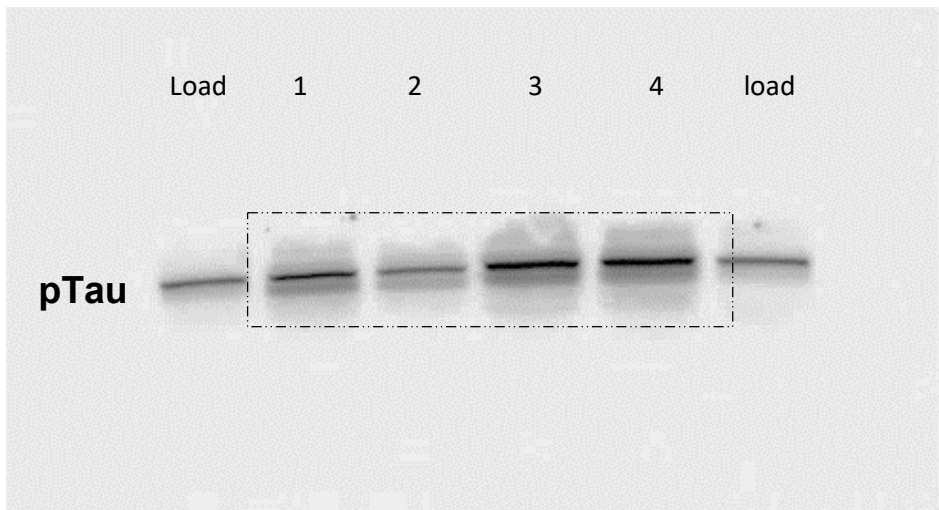
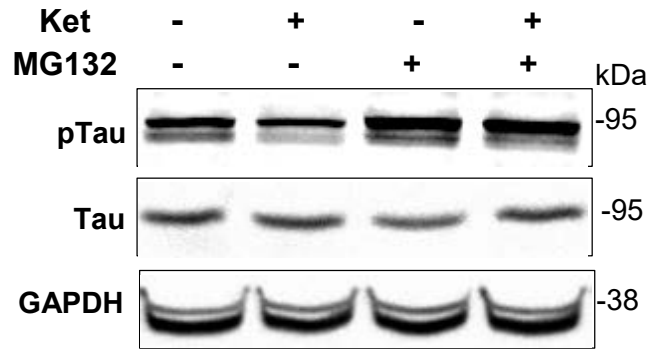
1-ctrl
2-mem
3-MG132
4-MG132+mem



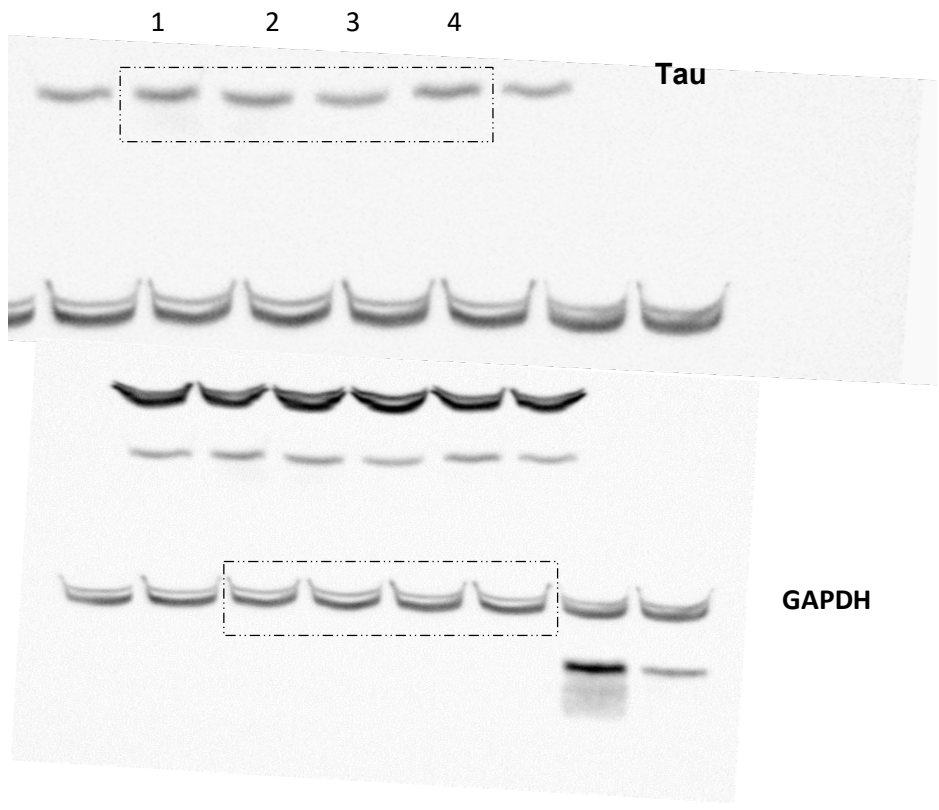
1-ctrl
2-mem
3-MG132
4-MG132+mem



Fig. 1M



pTau
1-ctrl
2-ket
3-MG132
4-MG132+Ket



HepG2
1-ctrl
2-ket
3-MG132
4-MG132+Ket

Fig. 2A

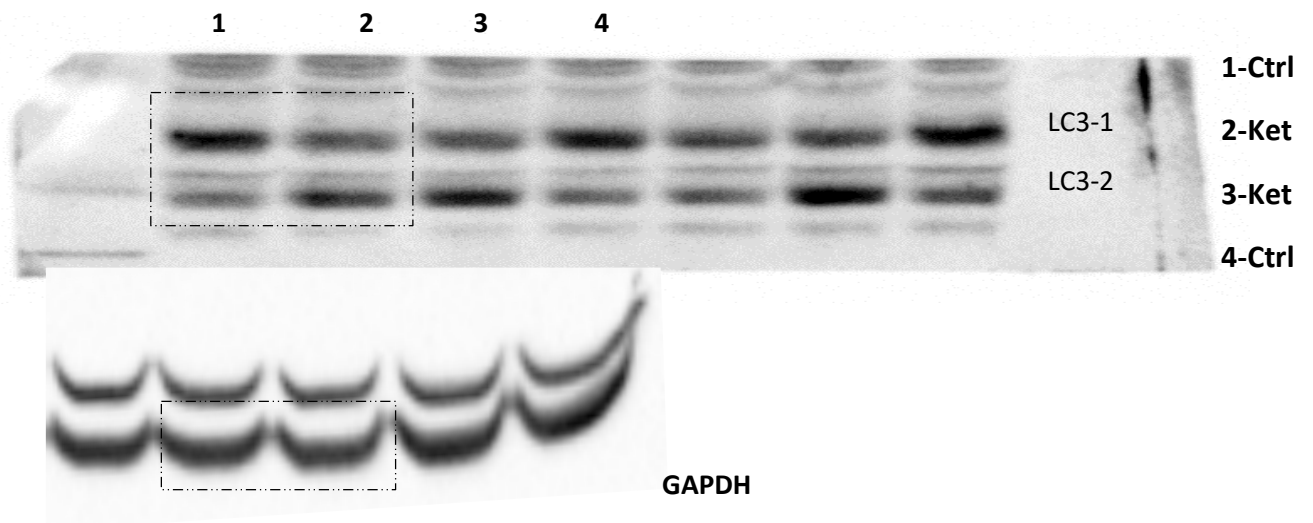
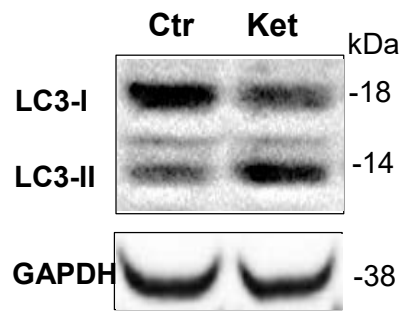
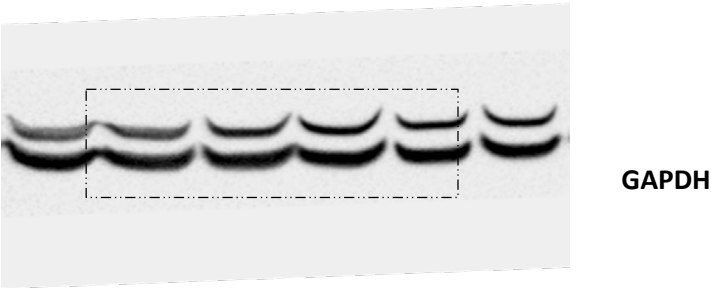
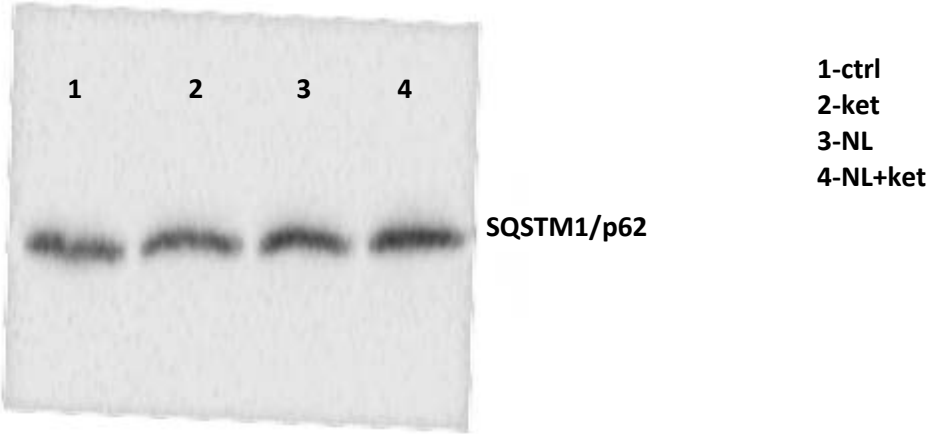
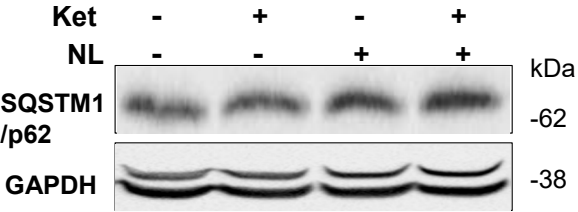


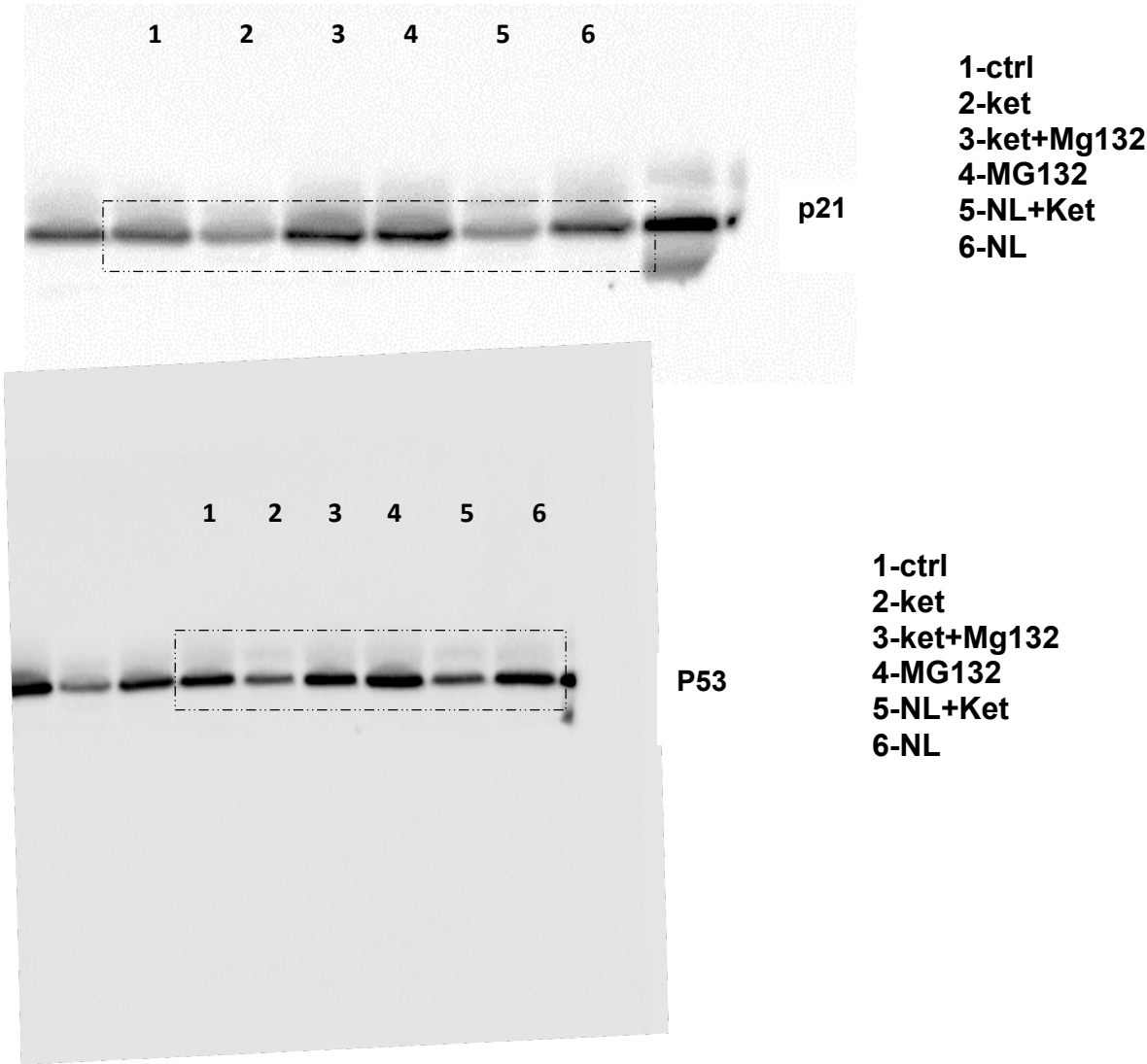
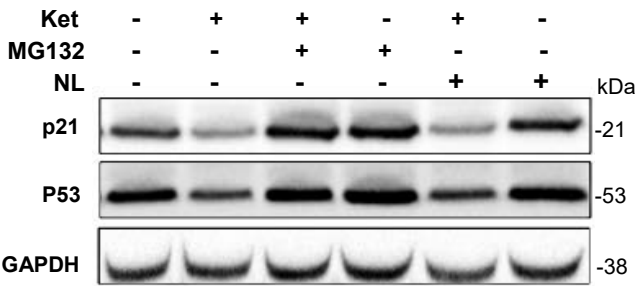
Fig. 2B



repeat



Fig. 2D



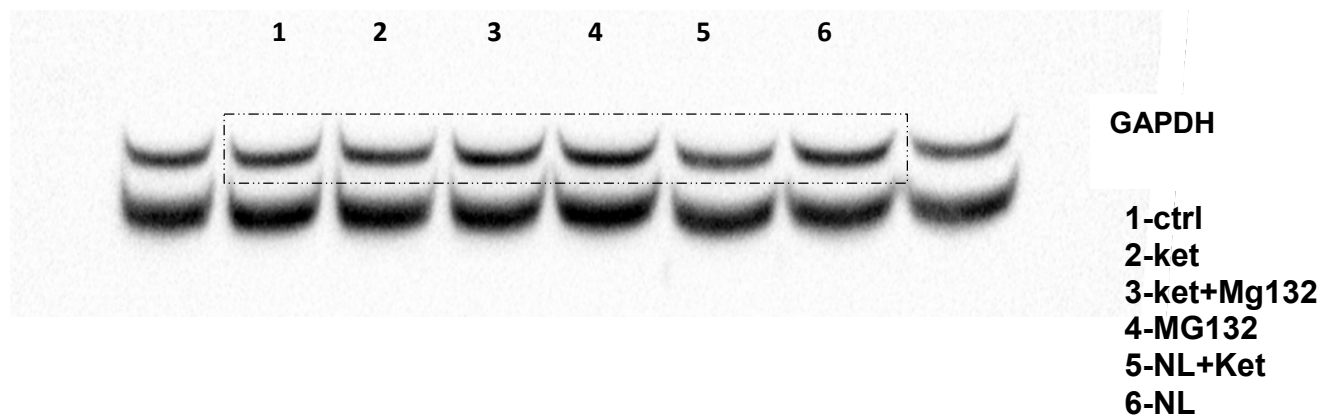


Fig. 3B

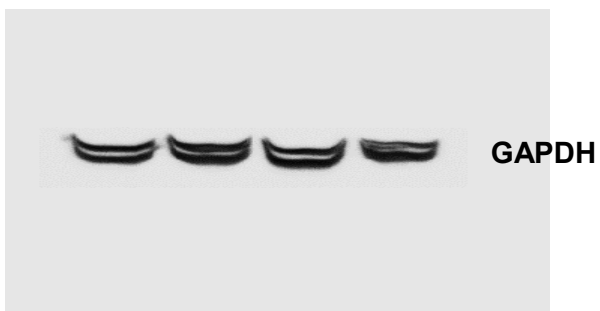
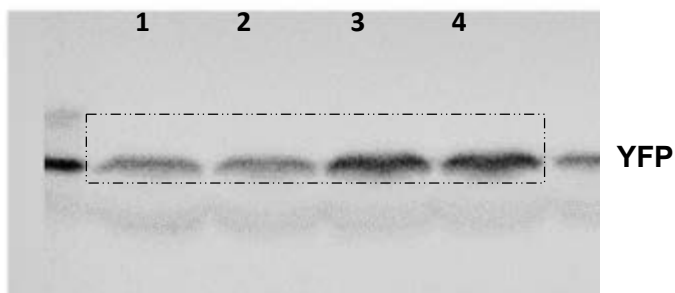
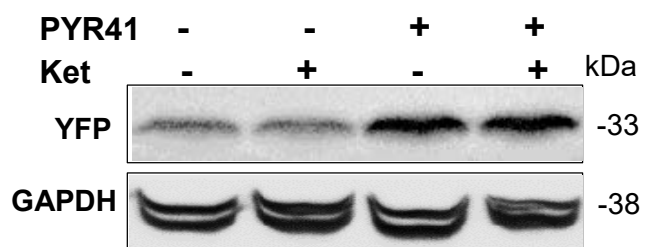
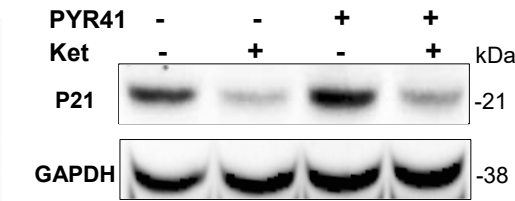
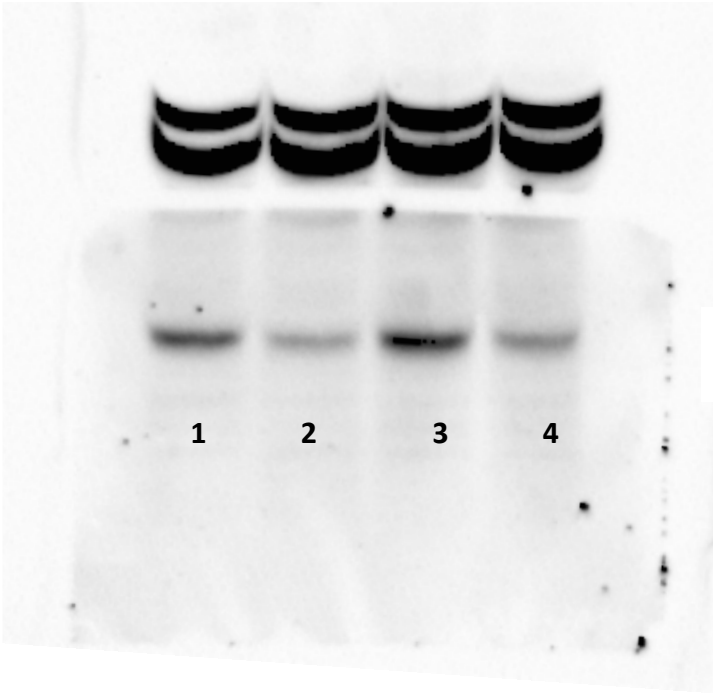
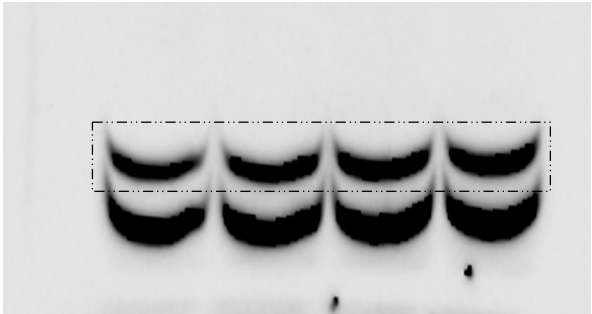


Fig. 3C



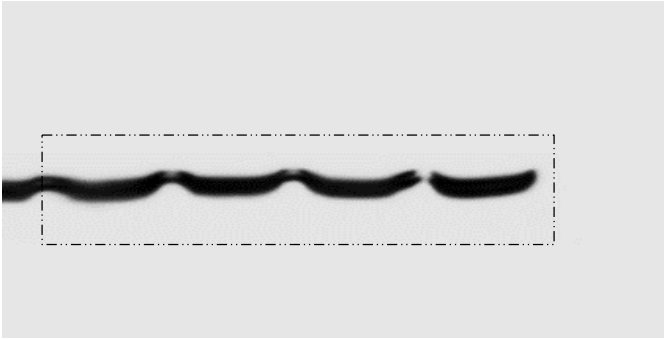
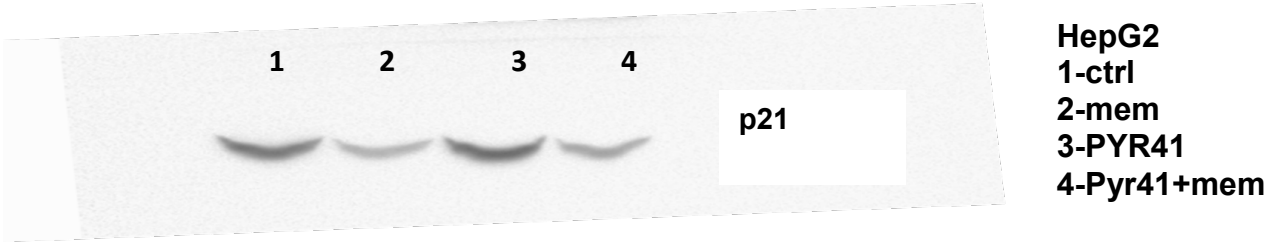
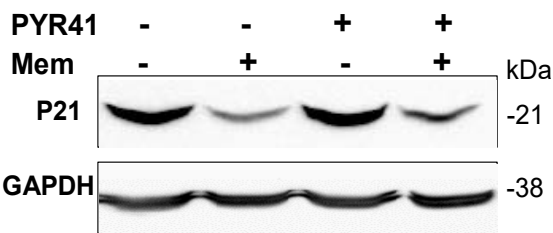
p21

HepG2
1-ctrl
2-ket
3-PYR41
4-Pyr41+ket



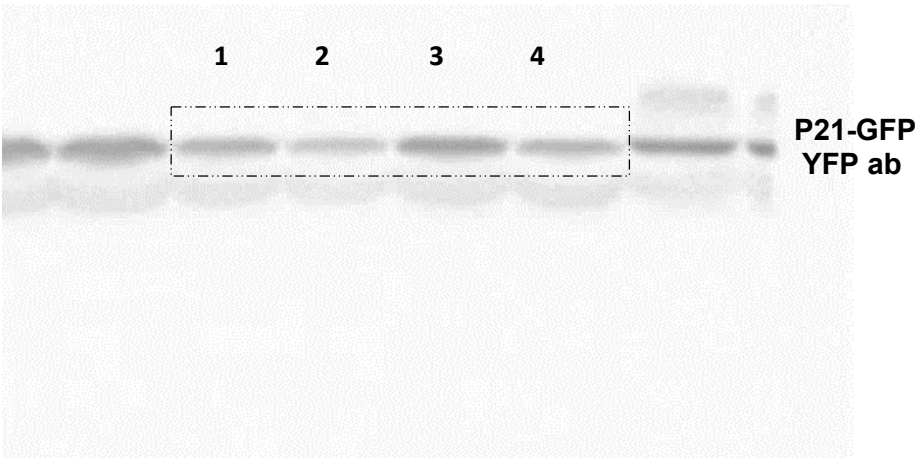
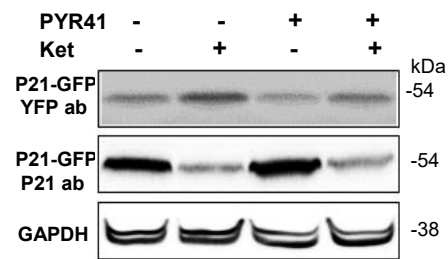
GAPDH

Fig. 3D



GAPDH

Fig. 3F



Hep3B-p21-GFP
1-ctrl
2-ket
3-PYR41
4-PYR41+ket

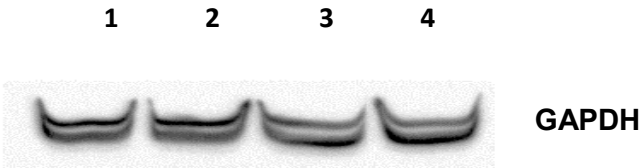
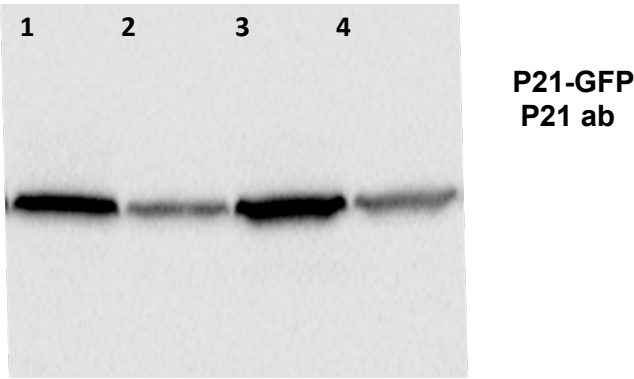
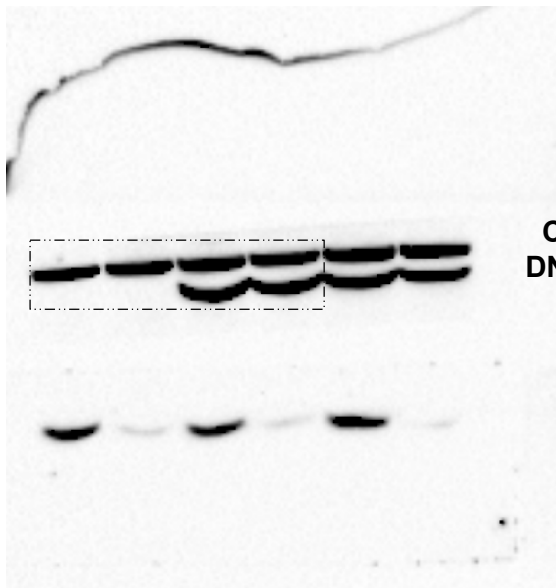
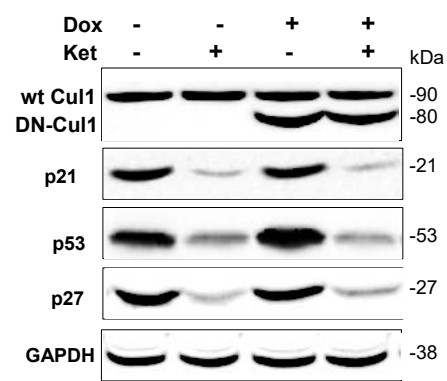
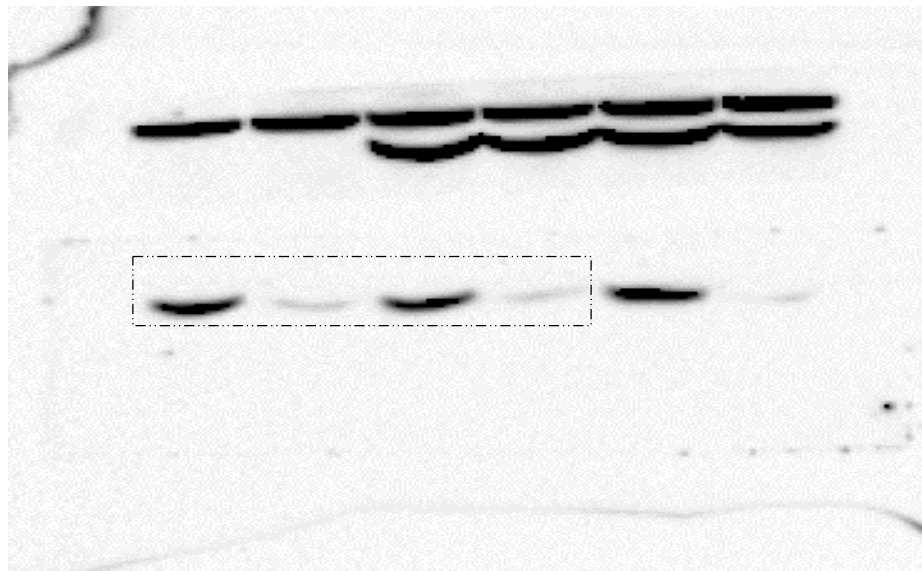


Fig. 3G



HepG2-DN-Cul1

- 1-ctrl
- 2-ket
- 3-Dox
- 4-Dox+ket



HepG2-DN-Cul1

- 1-ctrl
- 2-ket
- 3-Dox
- 4-Dox+ket

p21

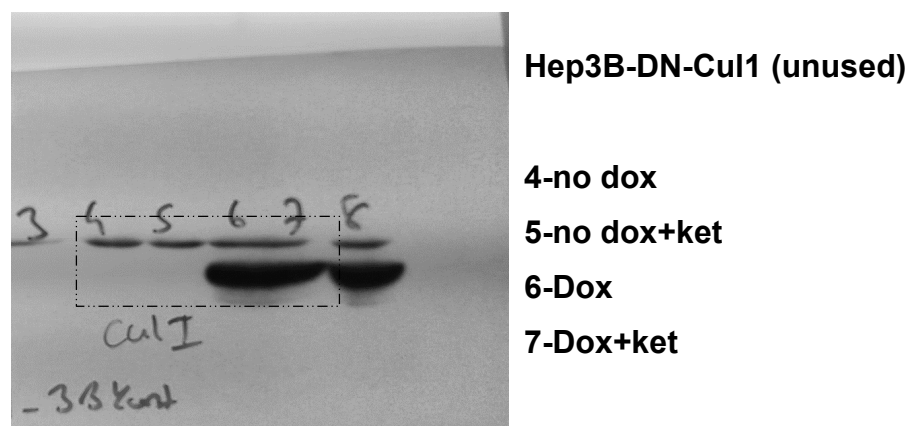
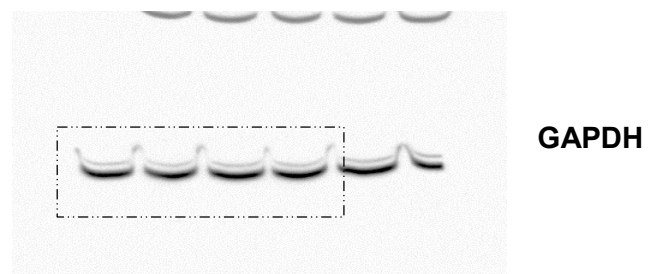
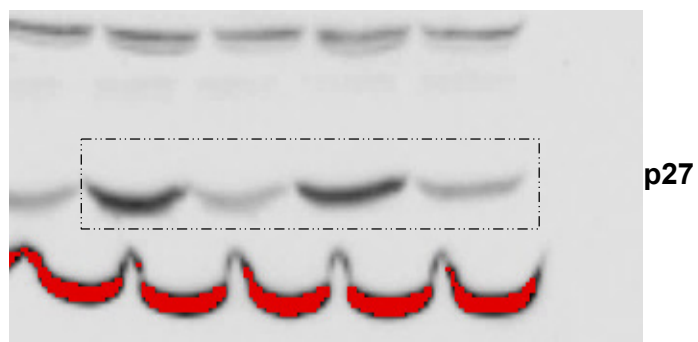
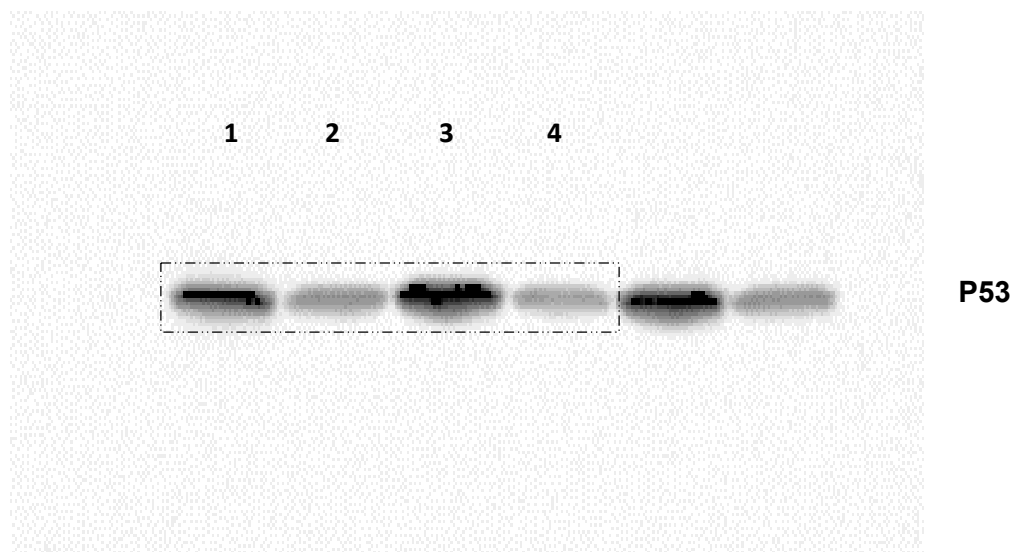
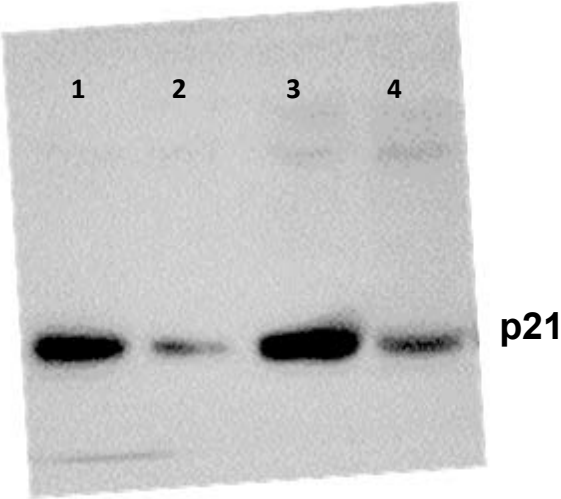
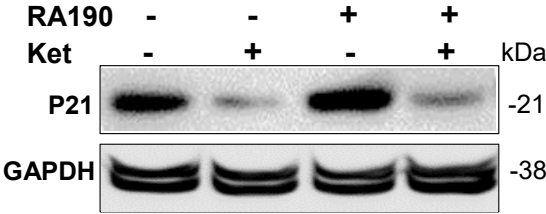


Fig. 4A



1-ctrl
2-ket
3-RA190
4-RA190+ket



Fig. 4D

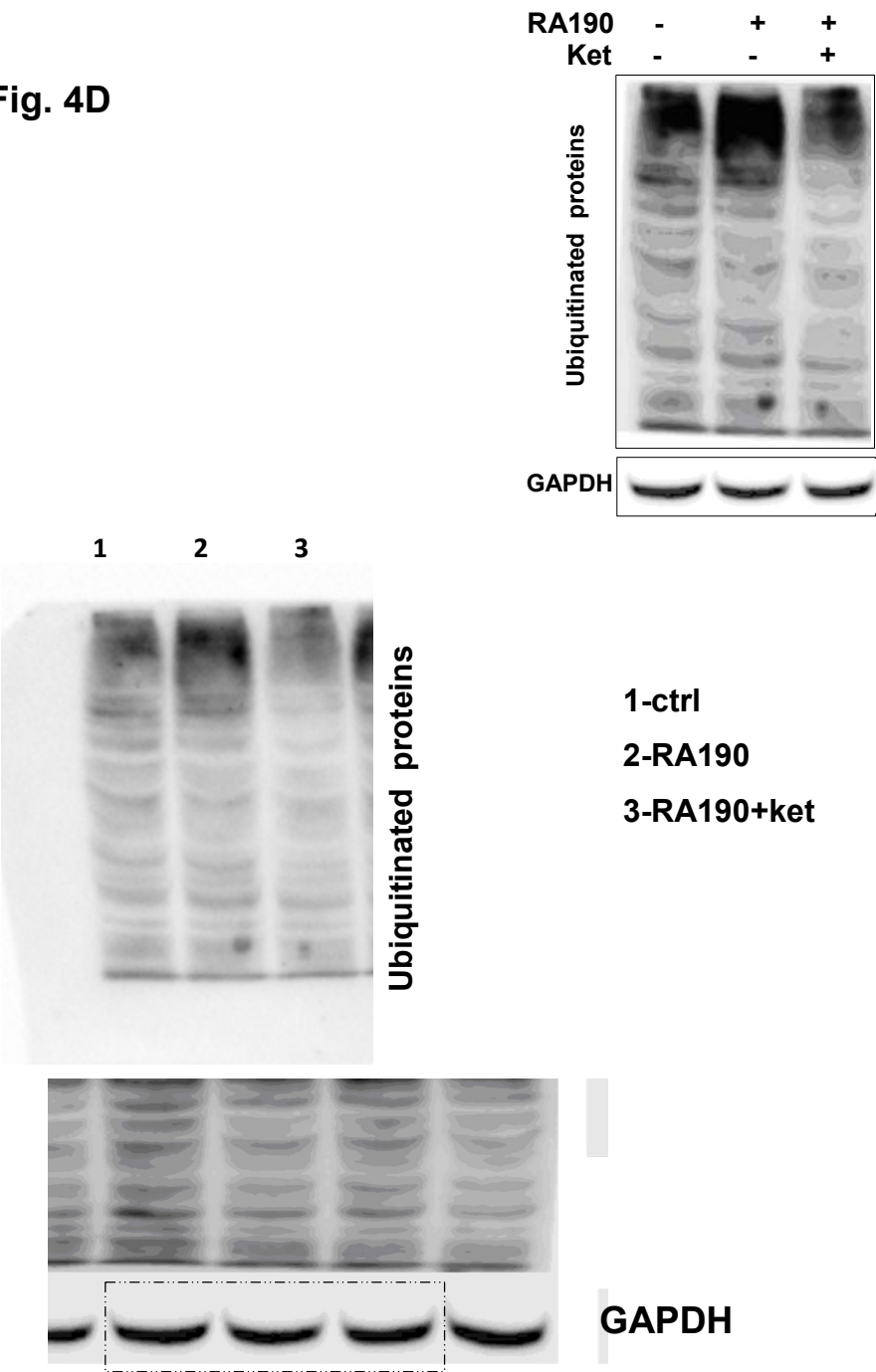


Fig. 4E

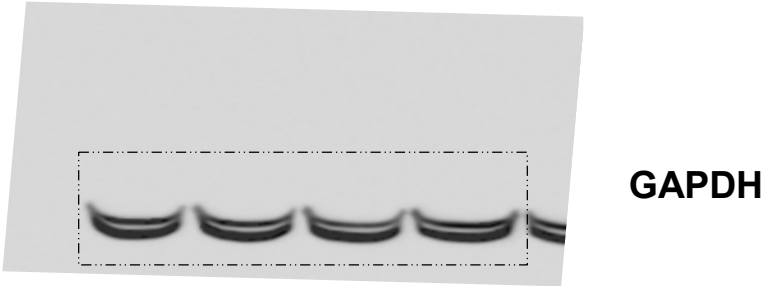
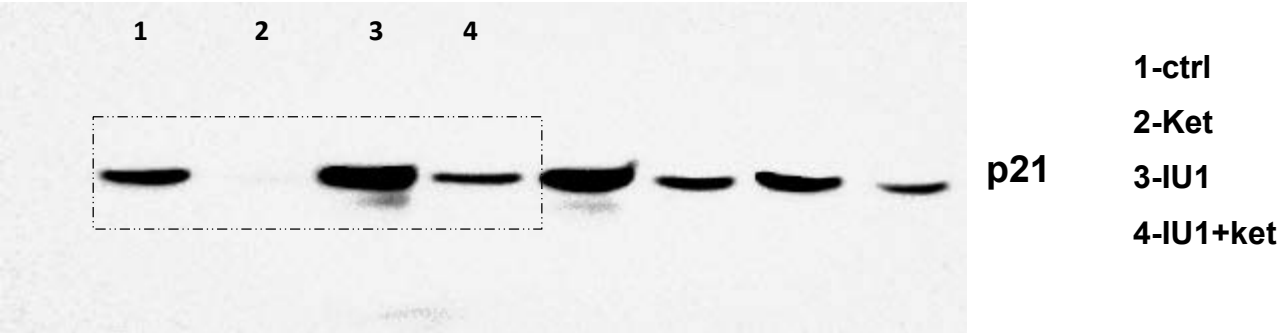
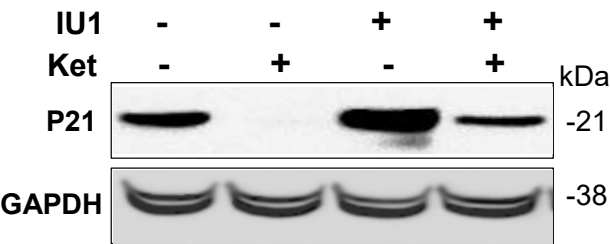
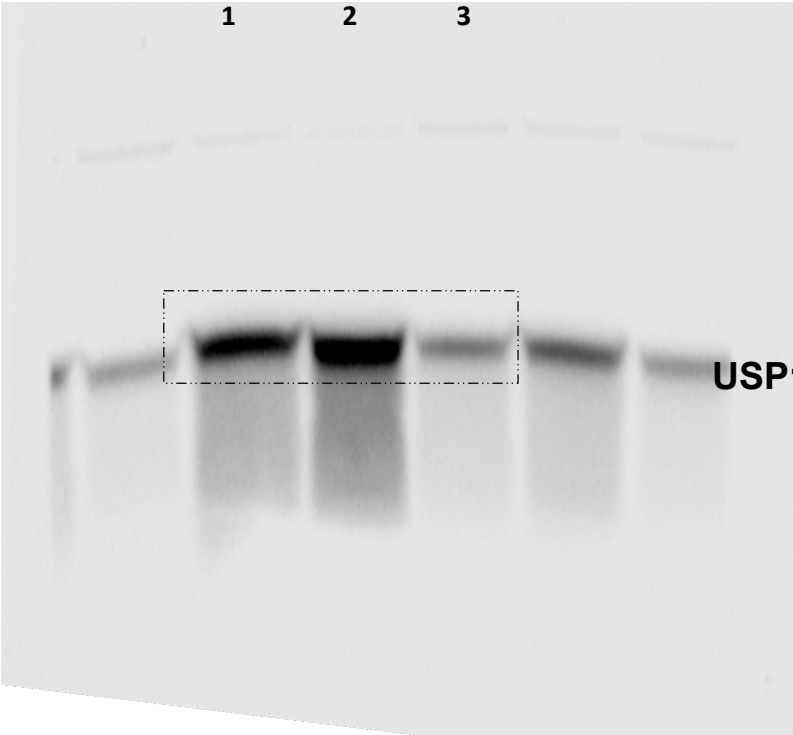
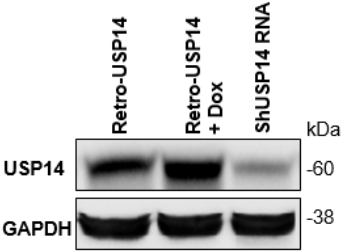
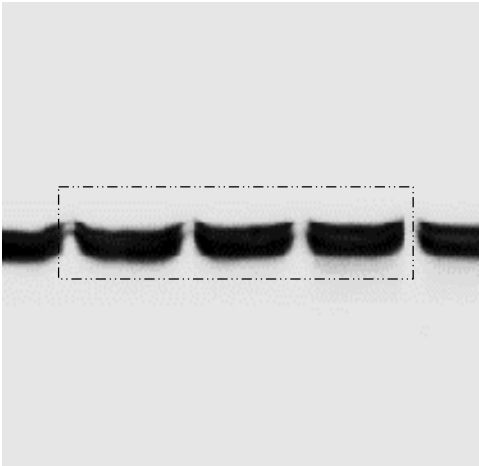


Fig. 4F



1-HepG2-Retro-USP14
2-HepG2-Retro-USP14+ Dox
3-ShUSP14 RNA



GAPDH

Fig. 4G

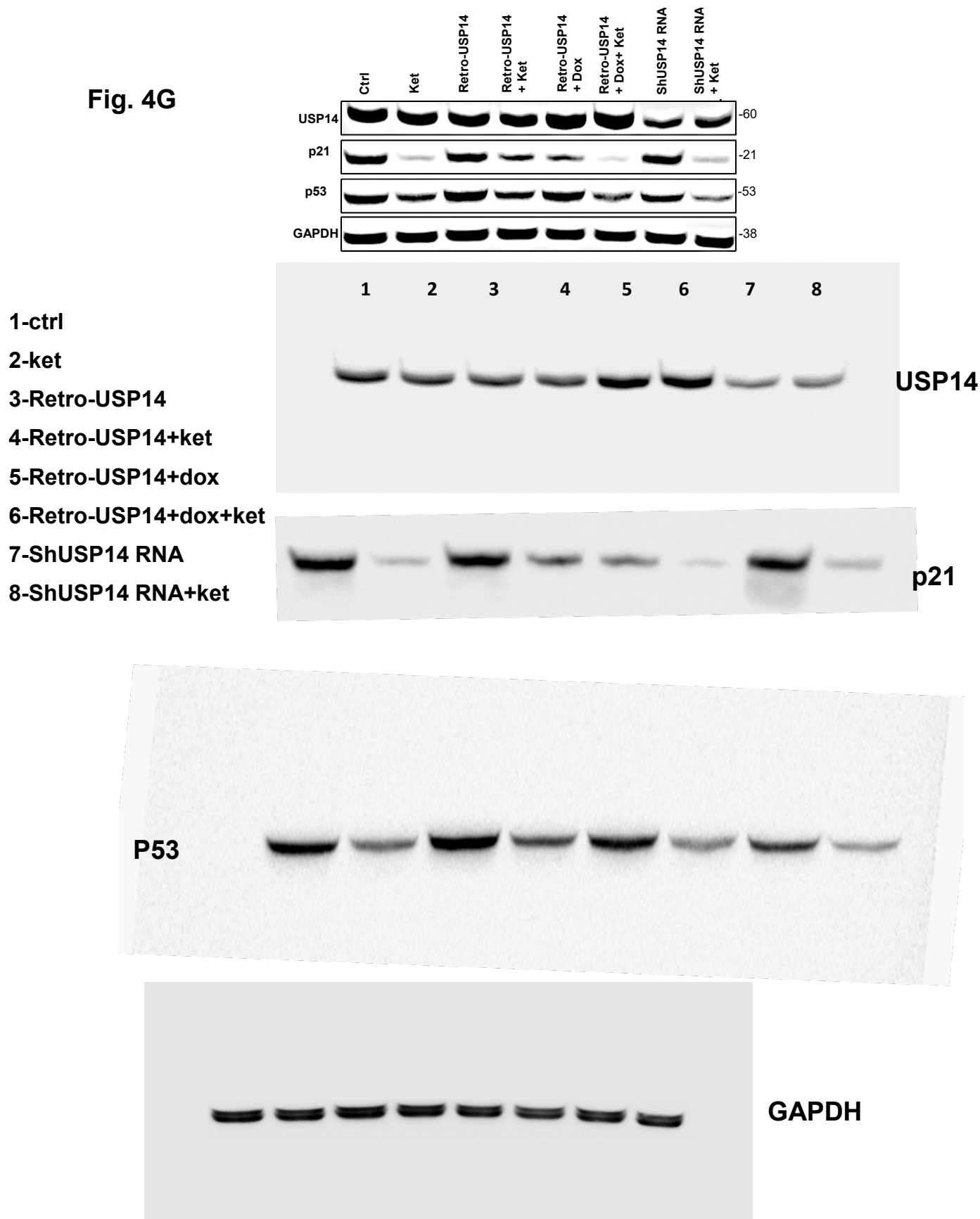
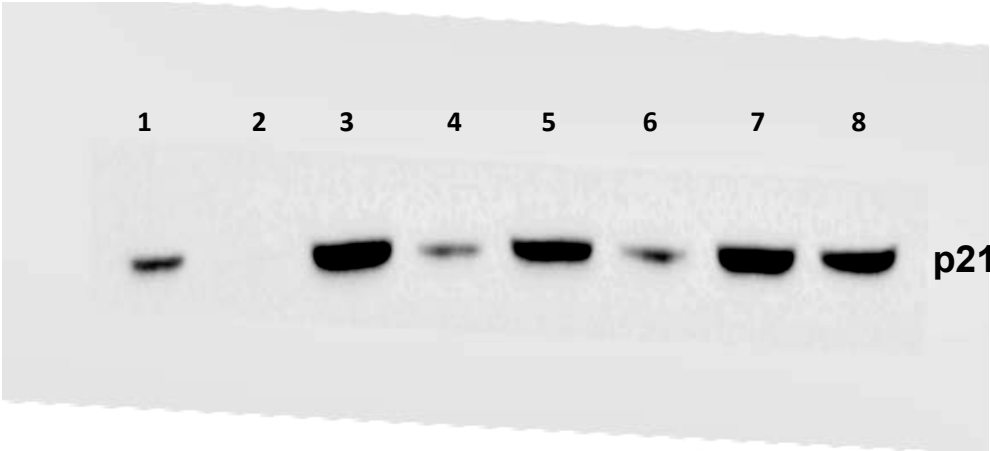
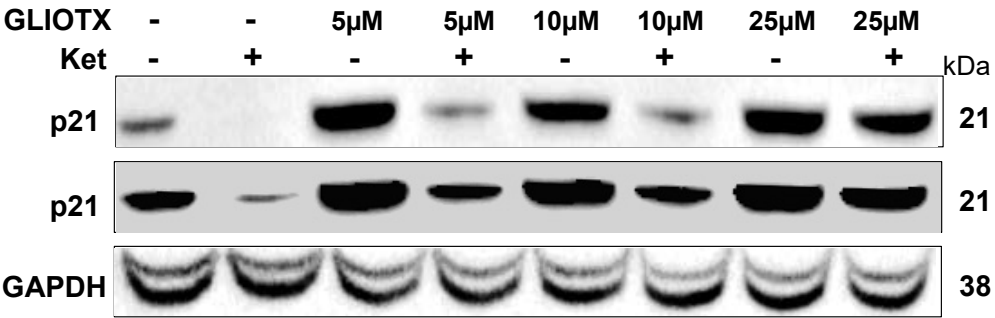


Fig. 4J



- 1-ctrl
- 2-ket
- 3-Gliotoxin 5μM
- 4-Gliotoxin 5μM+Ket
- 5-Gliotoxin 10μM
- 6-Gliotoxin 10μM+Ket
- 7-Gliotoxin 25μM
- 8-Gliotoxin 25μM+ket



p21
Overexposed

GAPDH

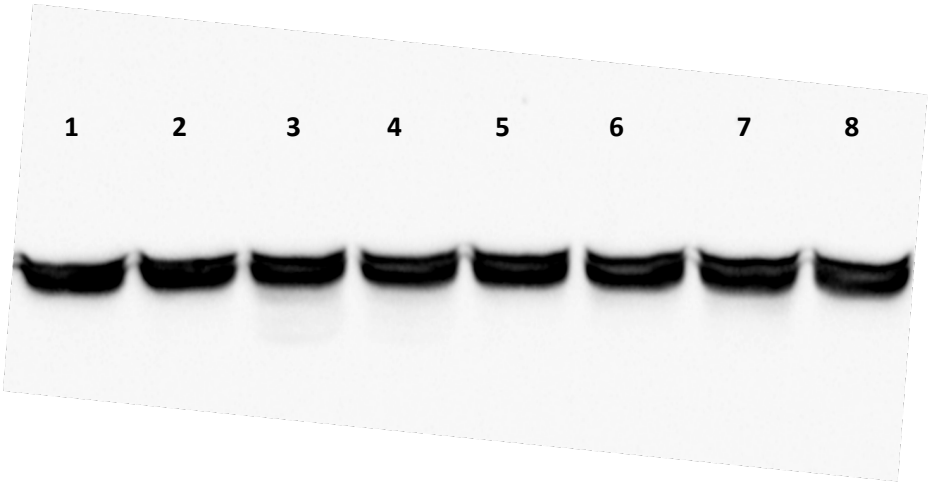
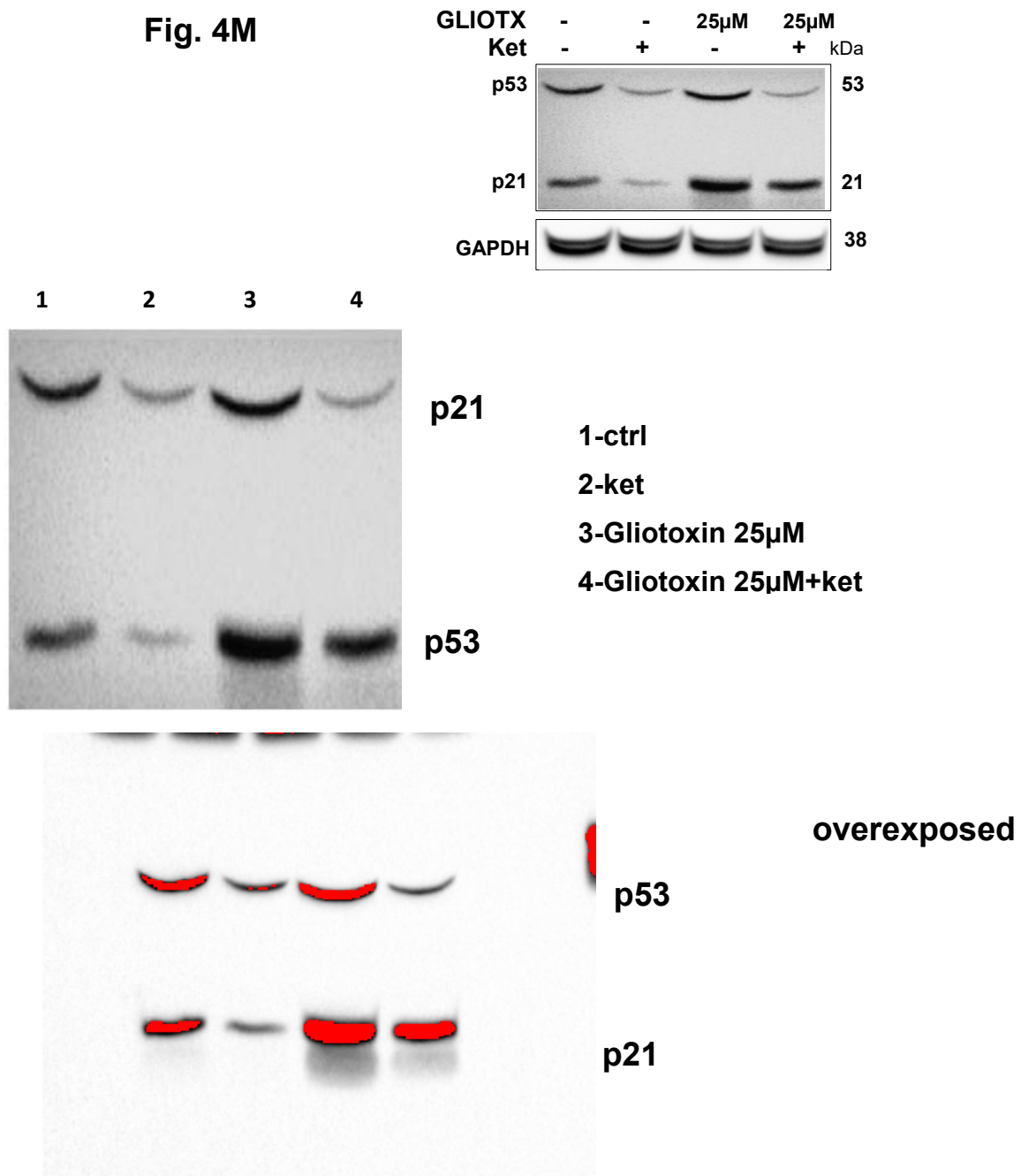
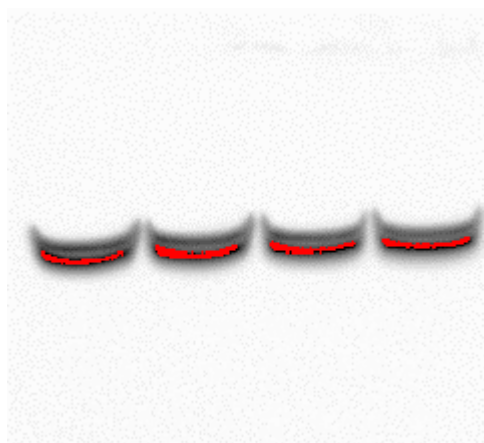


Fig. 4M



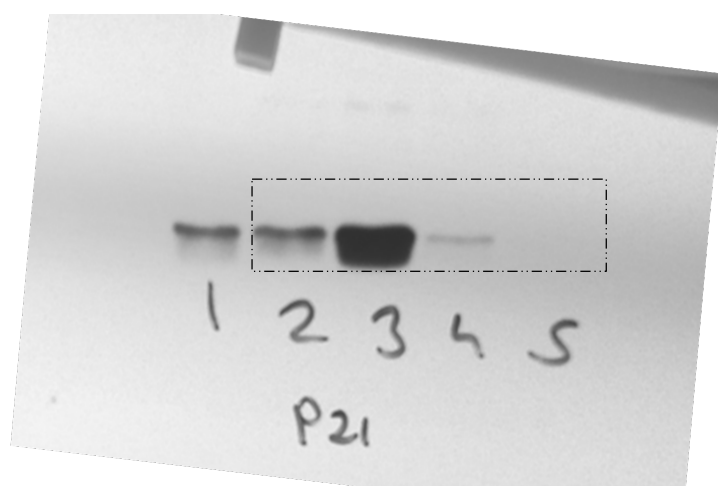
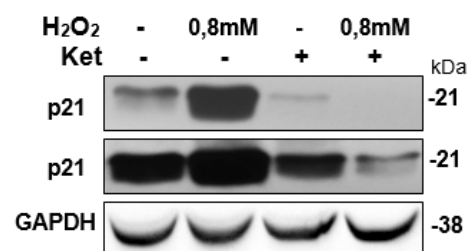
Since the p21 and p53 proteins were differently regulated by ketamine in the presence of 25 μM Gliotoxin, as shown by individual western blots using p21 or p53 antibodies separately, p21 and p53 antibodies were used on the same membrane to confirm the results.



GAPDH

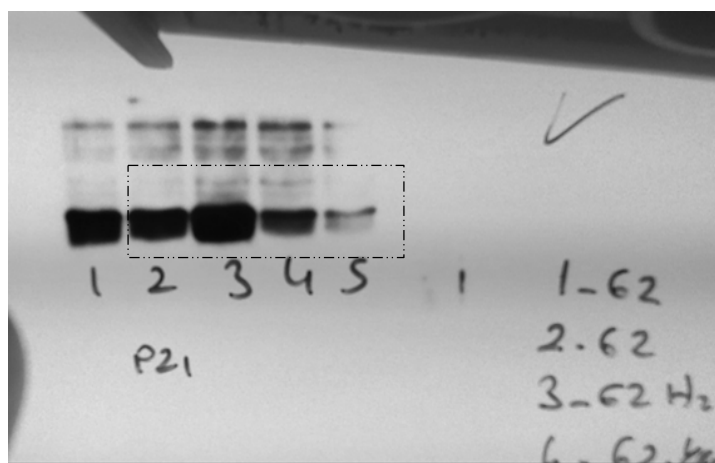


Fig. 5A



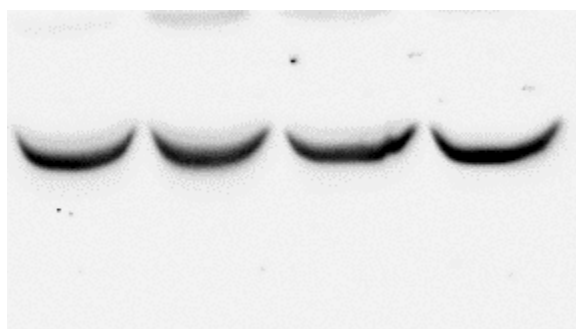
P21

- 1-HepG2 cell prs loaded
- 2-HepG2 ctrl
- 3-H₂O₂ 0,8mM
- 4-Ket
- 5-H₂O₂ 0,8mM+ket



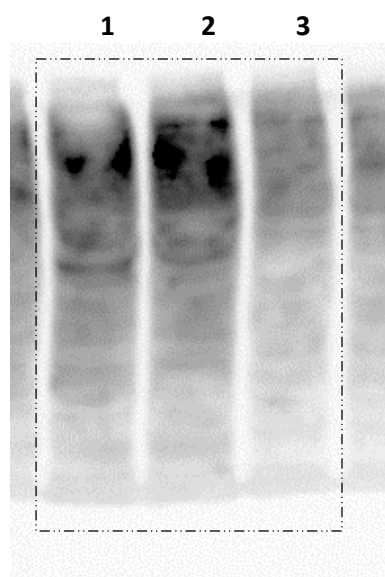
overexposed

P21

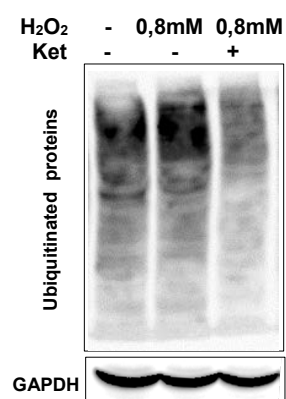


GAPDH

Fig. 5B



**Ubiquitinated
proteins**



1-ctrl

2-H₂O₂ 0,8mM

3-H₂O₂ 0,8mM+ket

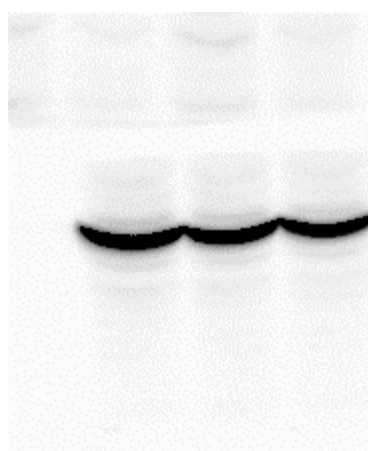


Fig. 5C

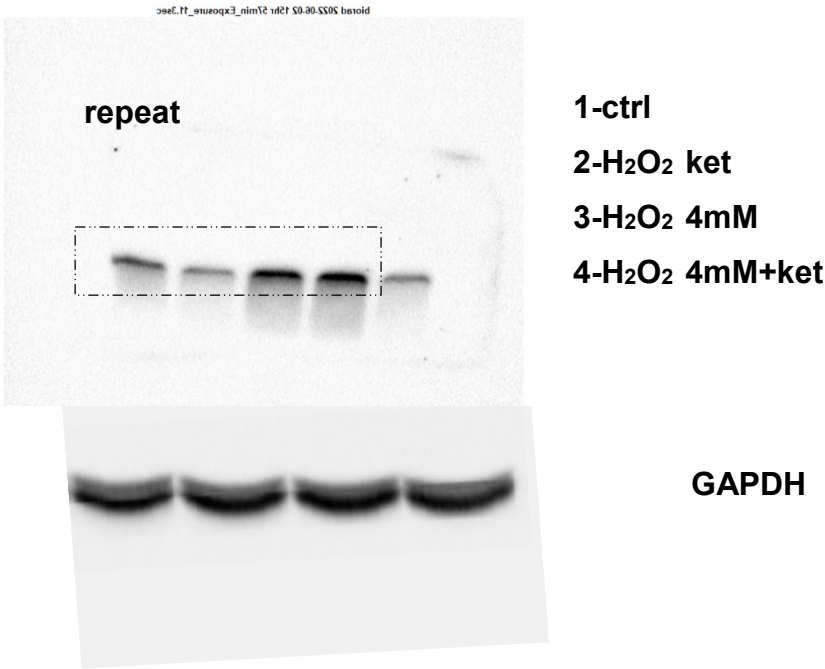
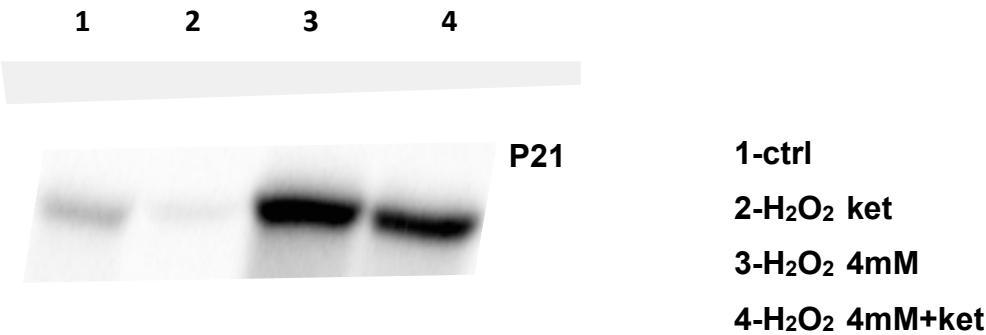
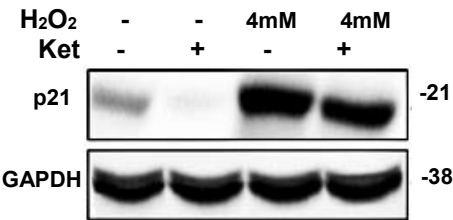


Fig. 5D

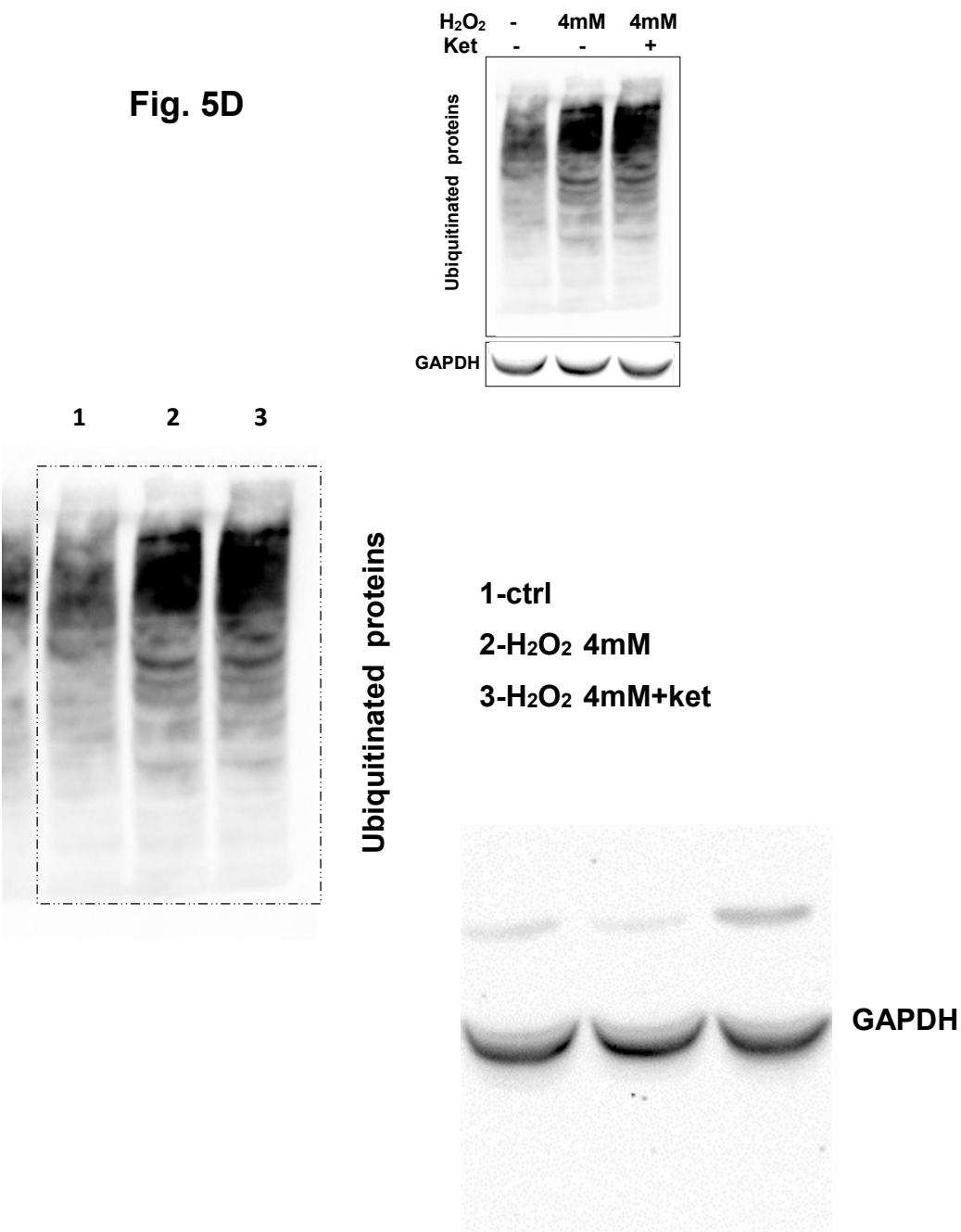


Fig. 5F

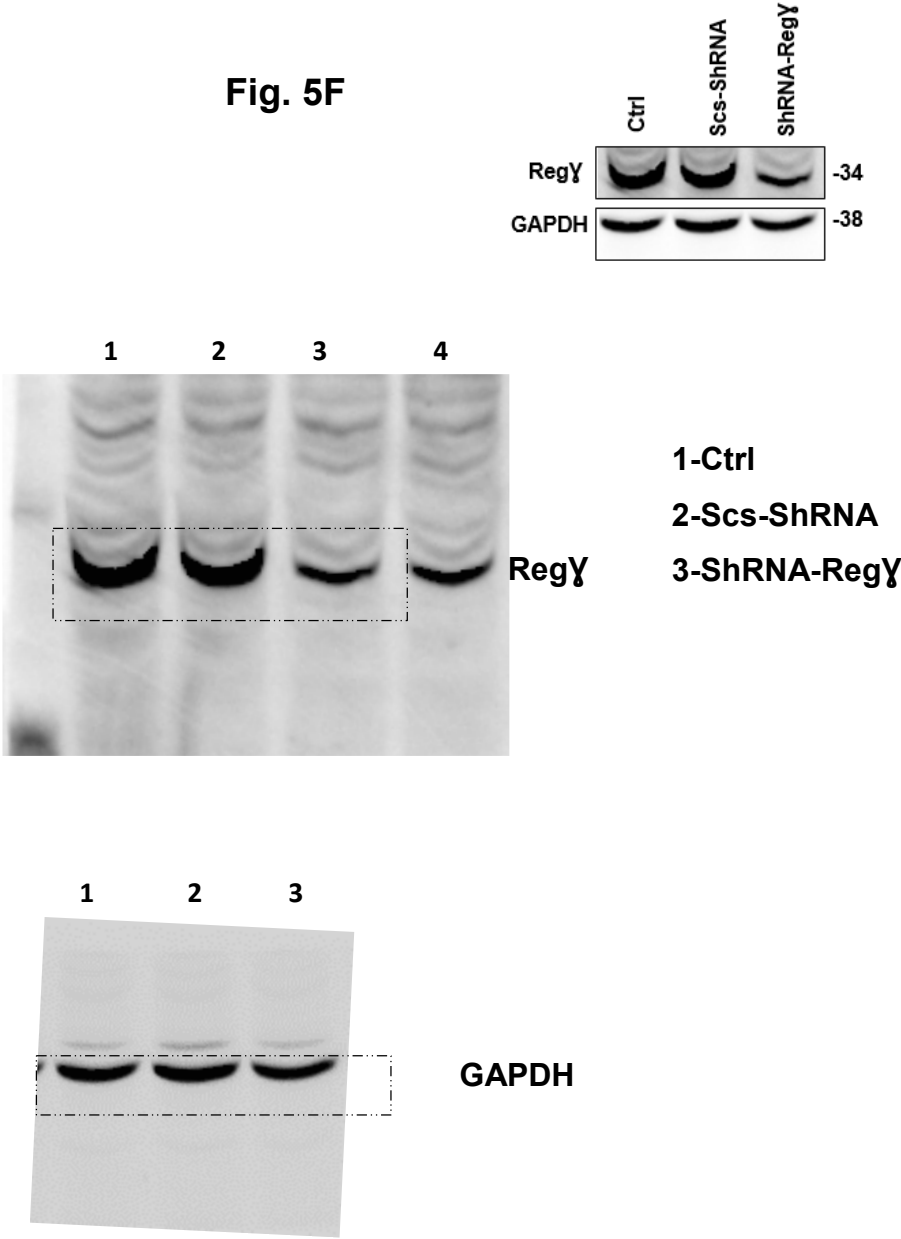
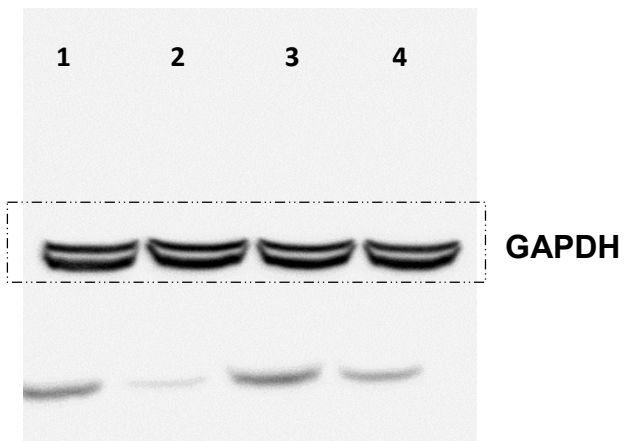
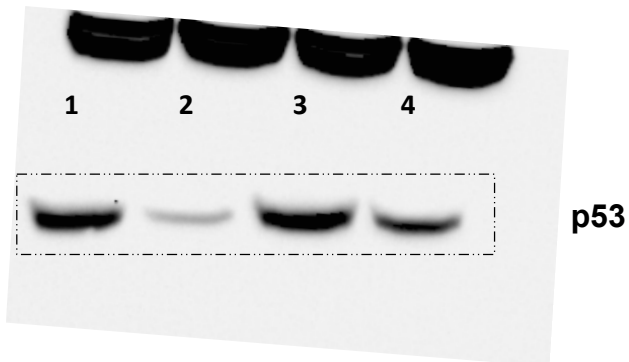
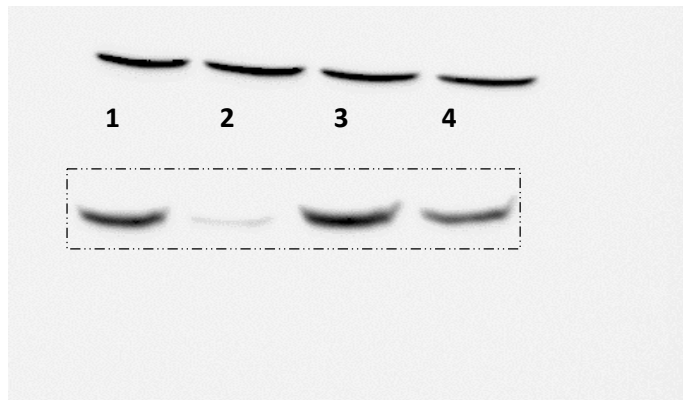
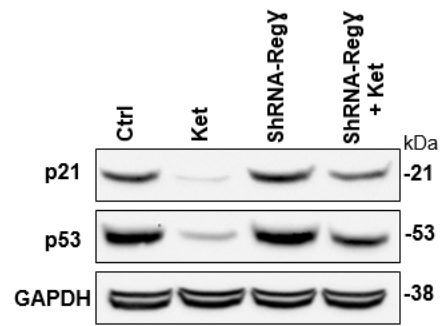
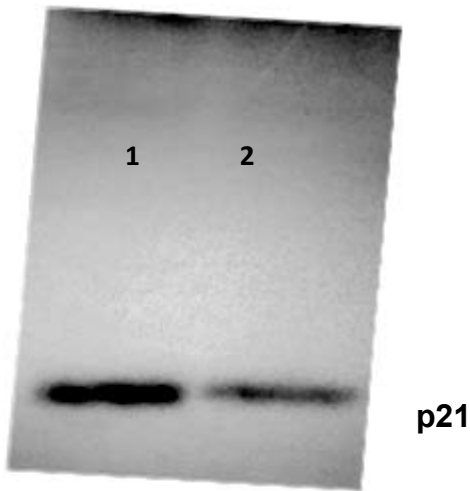


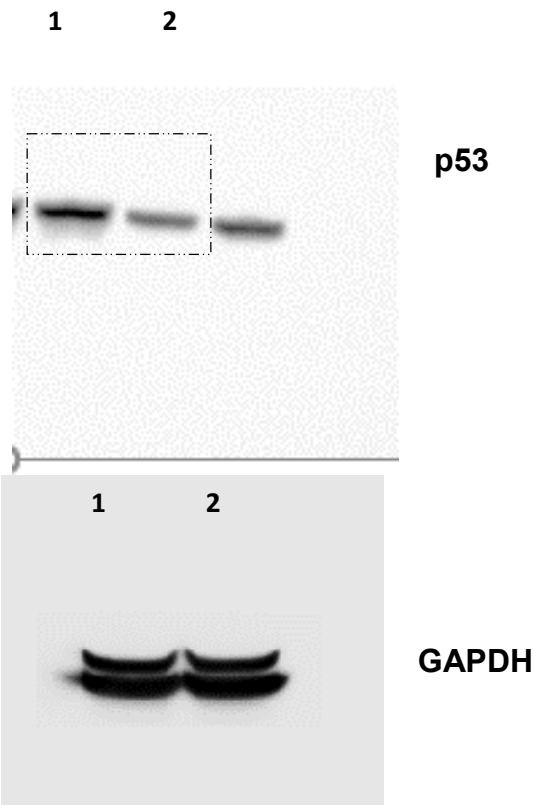
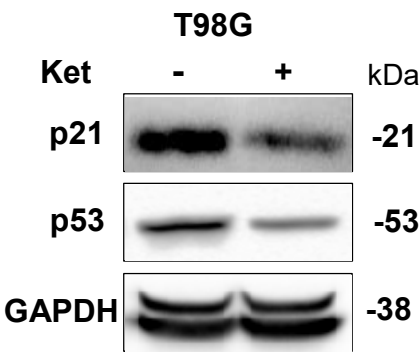
Fig. 5G



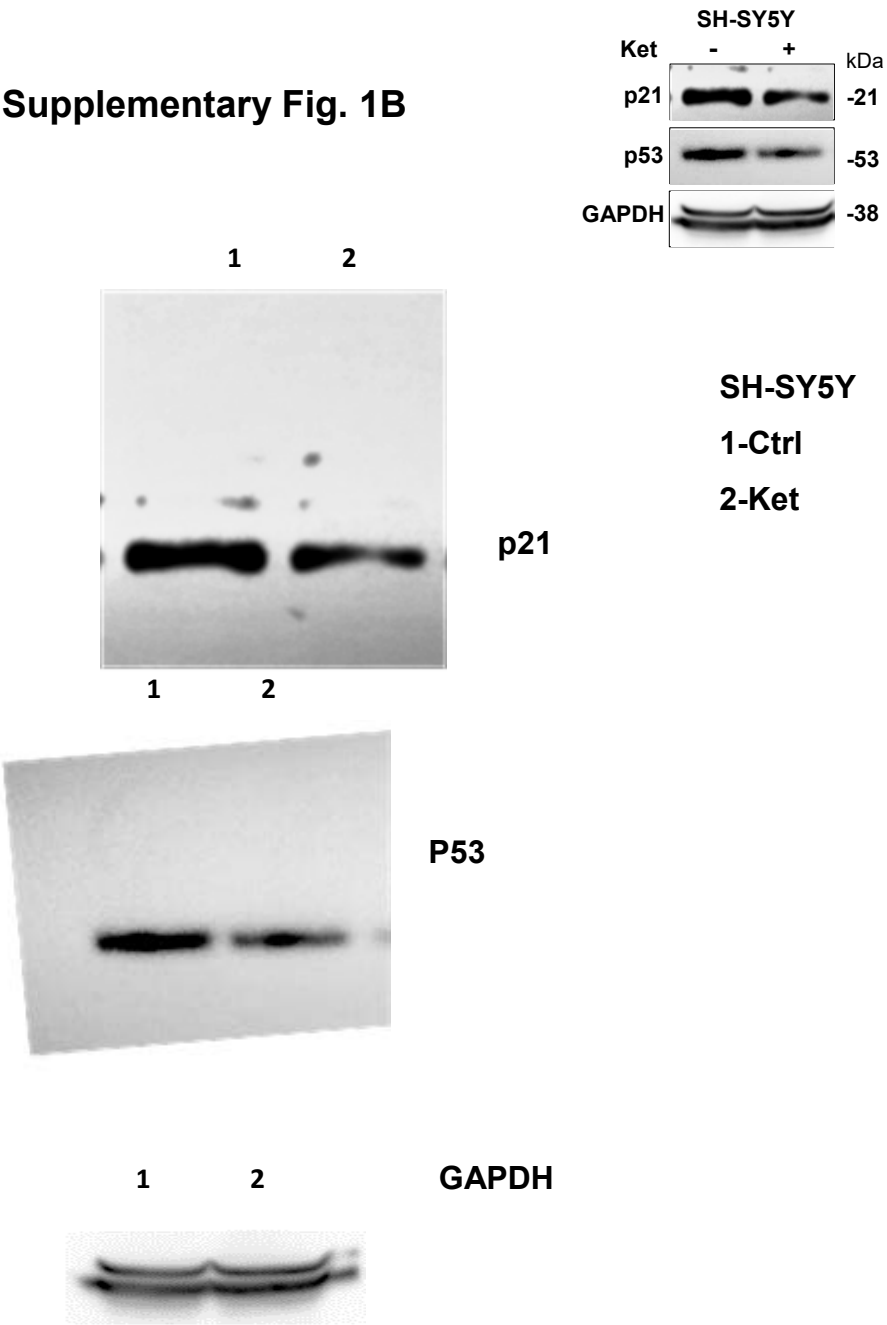
Supplementary Fig. 1A



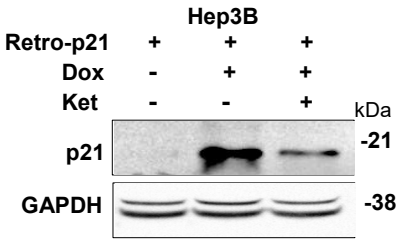
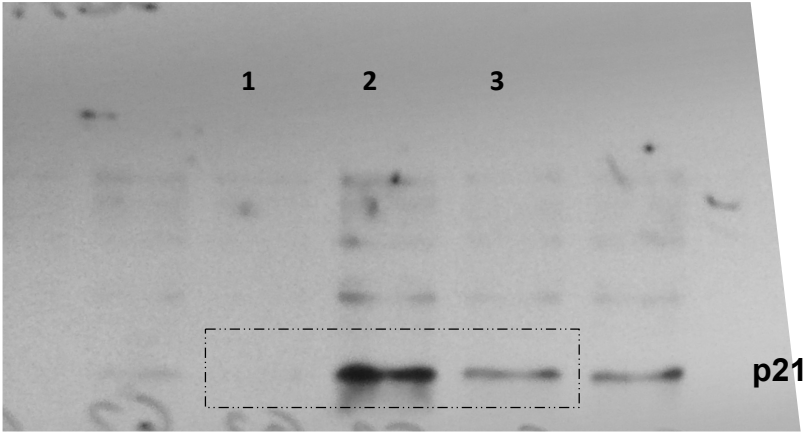
T98G
1-Ctrl
2-Ket



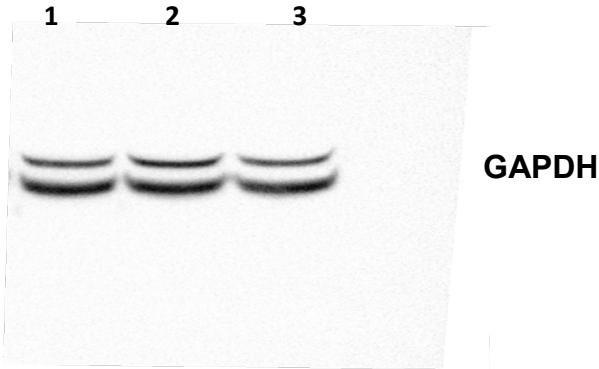
Supplementary Fig. 1B



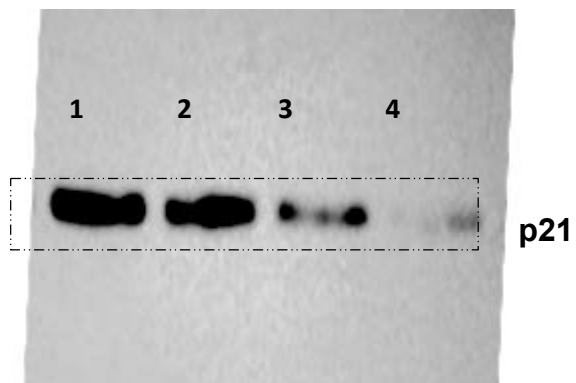
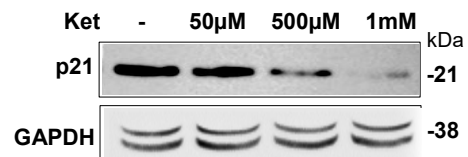
Supplementary Fig. 1C



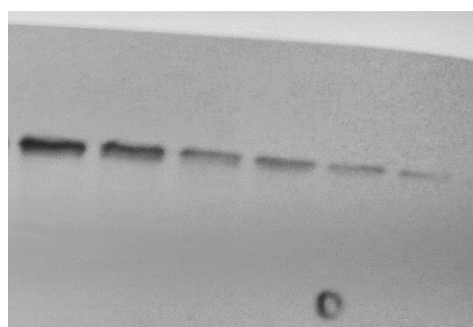
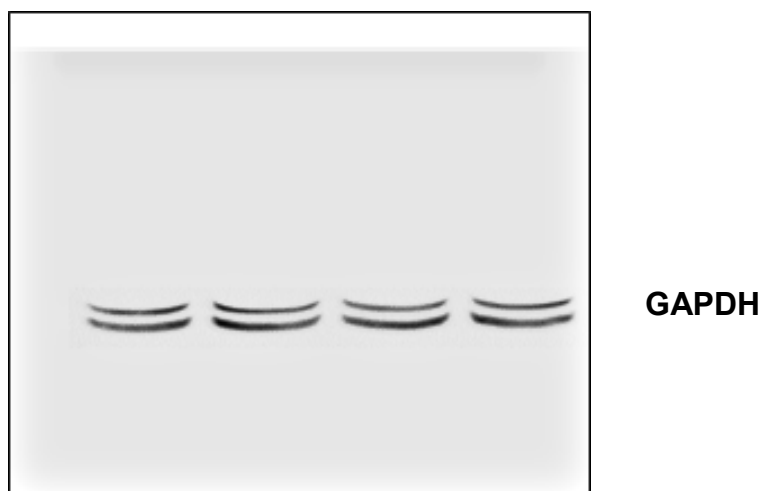
Hep3B-Retro-p21
1-Hep3B-Retro-p21
2-Hep3B-Retro-p21 +Dox
3-Hep3B-Retro-p21+ Dox+ Ket



Supplementary Fig. 1D

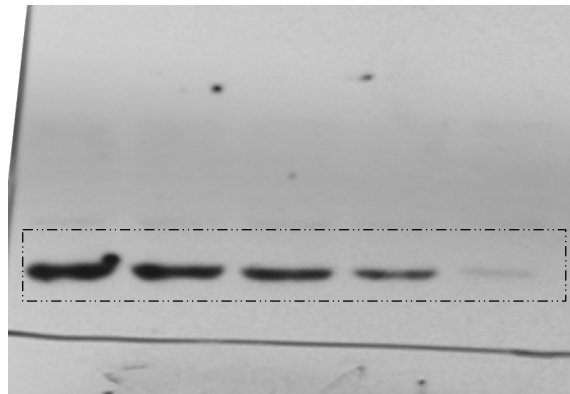


Ketamine
 1- 0
 2- 50μM
 3- 500μM
 4- 1mM



ket (500μM)
 Time (min) 0, 10, 30, 60, 120, 160

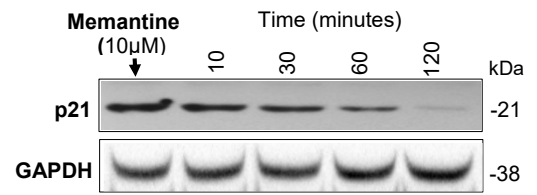
Supplementary Fig. 1E



p21



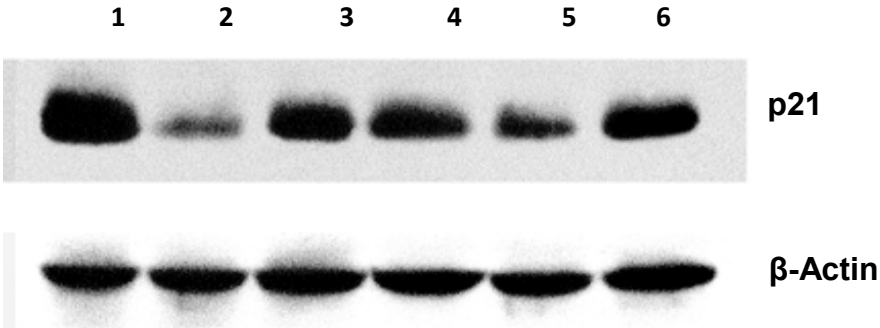
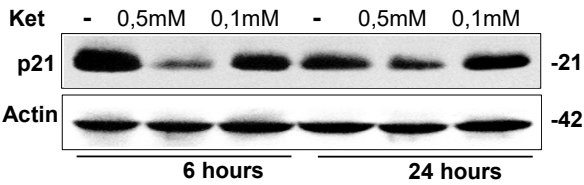
GAPDH



Memantin (10 μ M)

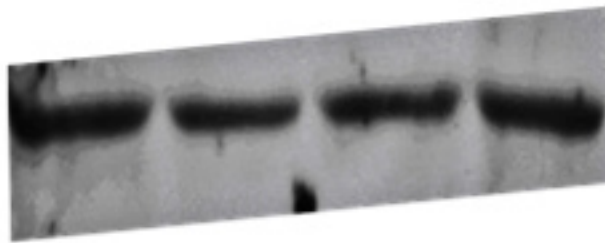
Time (min) 0, 10, 30, 60, 120

Supplementary Fig. 1F

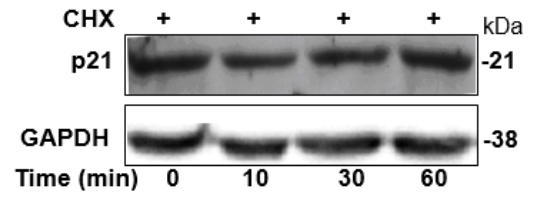


- 1- ctrl 6 hours
- 2- Ket 0,5 mM 6 hours
- 3- Ket 0,1 mM 6 hours
- 4- ctrl 24 hours
- 5- Ket 0,5 mM 24 hours
- 6- Ket 0,1 mM 24 hours

Supplementary Fig. 1G



p21

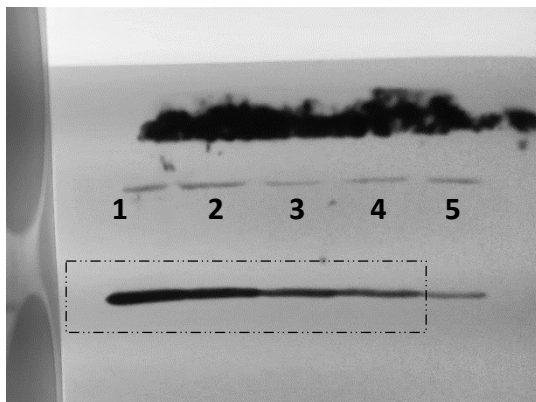


- 1- cycloheximide -0 min
- 2- cycloheximide -10 min
- 3- cycloheximide -30 min
- 4- cycloheximide -60 min



GAPDH

Supplementary Fig. 1H



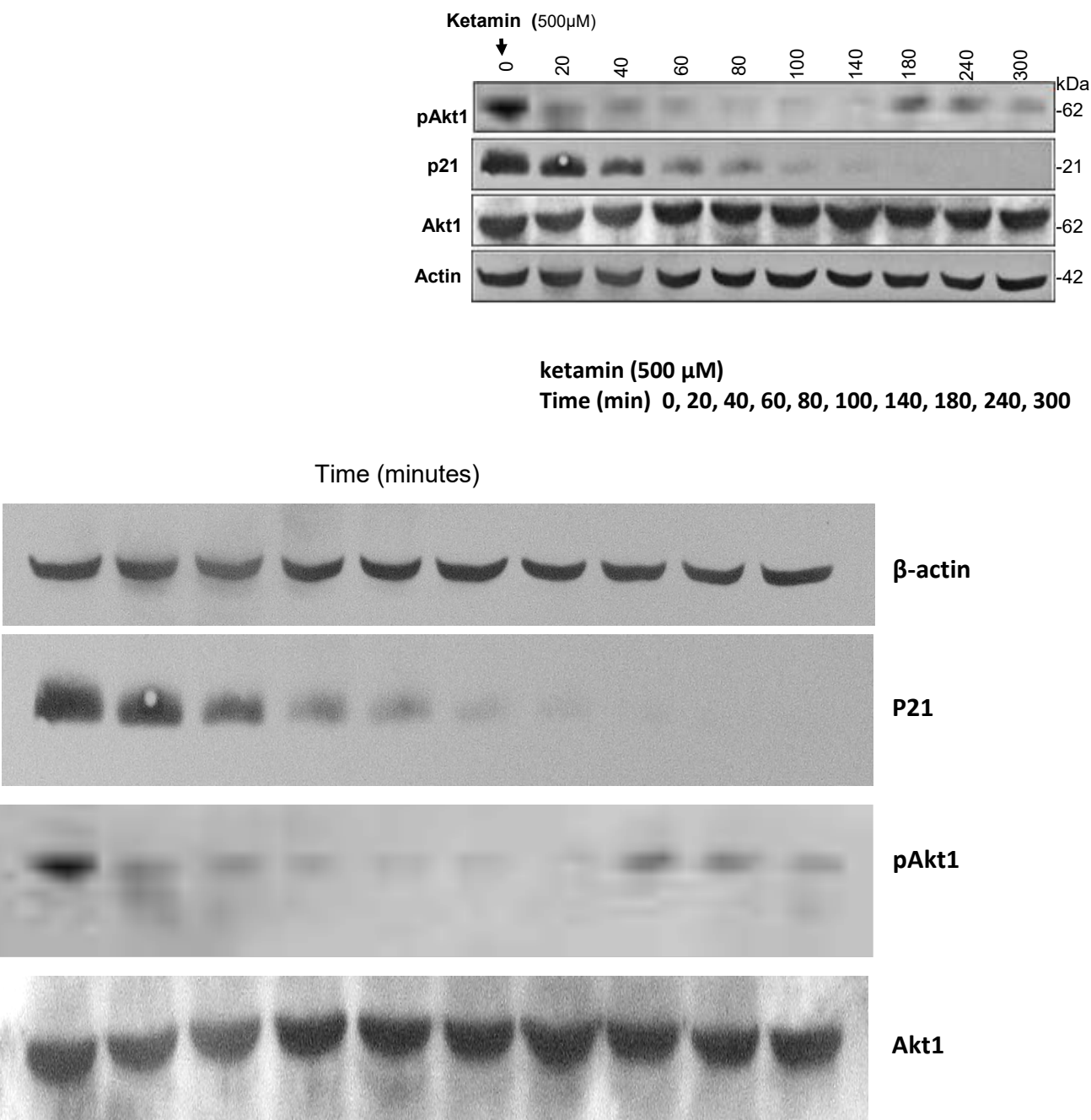
p21

- 1- cycloheximide no Ket
- 2- cycloheximide + Ket +10 min
- 3- cycloheximide + Ket +30 min
- 4- cycloheximide + Ket +60 min
- 5- cycloheximide + Ket +120 min

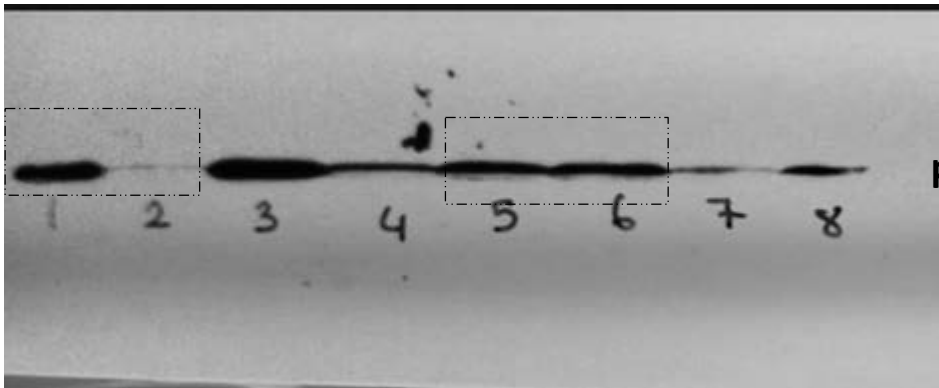
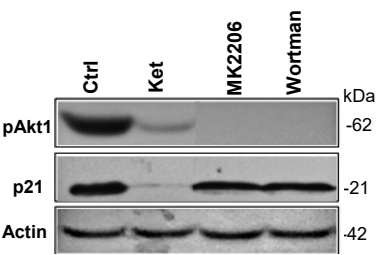


GAPDH

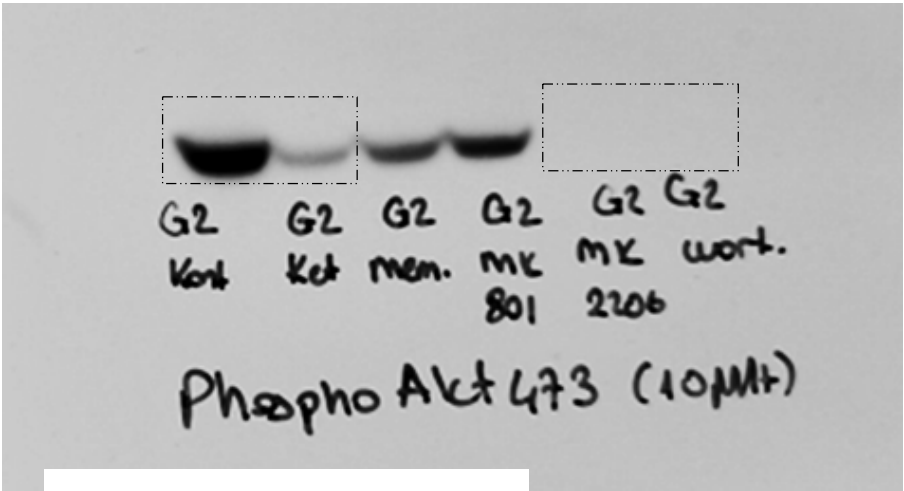
Supplementary Fig. 1l



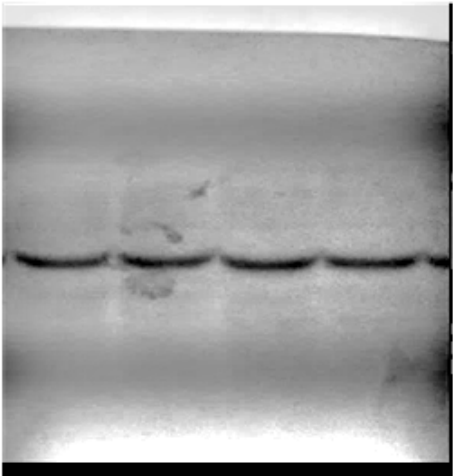
Supplementary Fig. 1J



Cell-HepG2
1-Ctrl
2-Ket
3-MG132
4-MG132+ket
5-MK2206
6-Wortmannin
7-Memantine
8-MK801

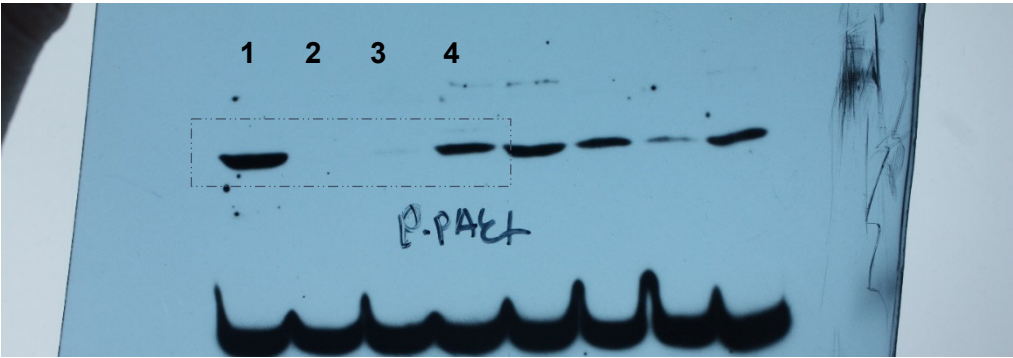
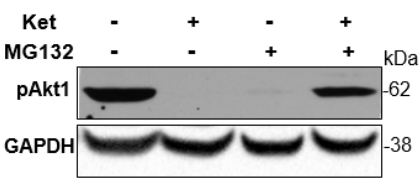


pAkt1

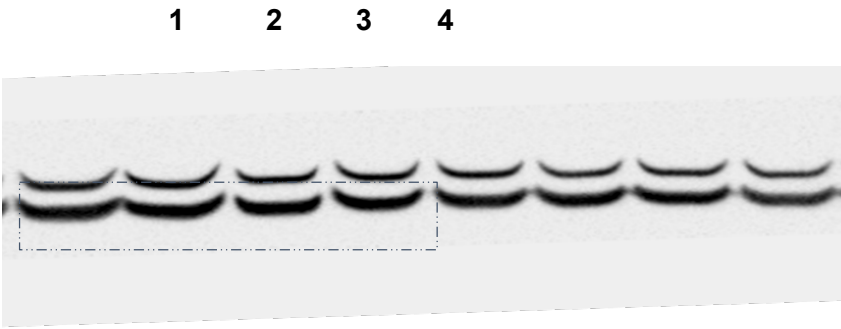


actin

Fig. 1K

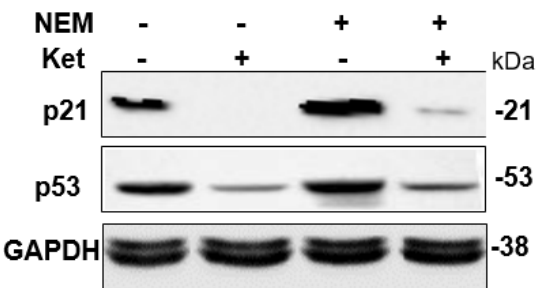
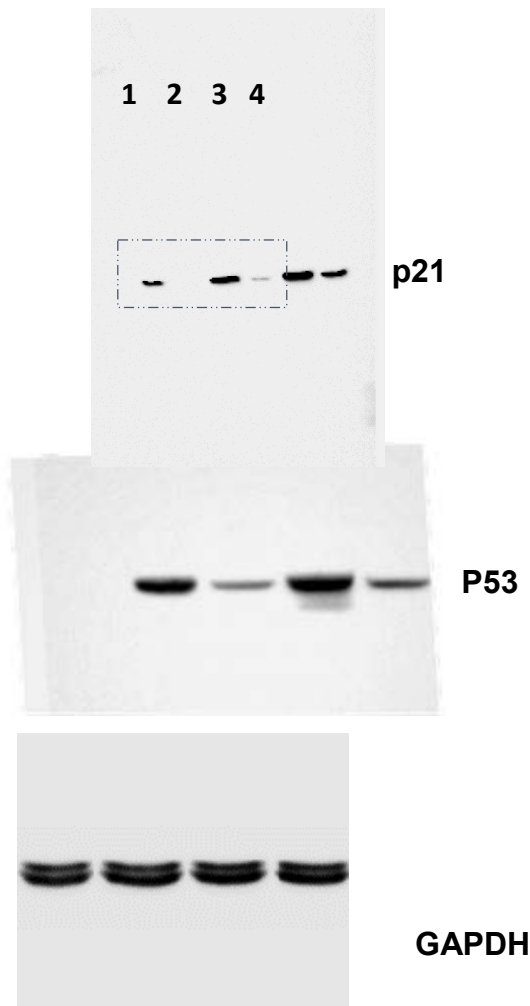


HepG2
pAkt1
1-ctrl
2-ket
3-MG132+ket
4-MG132



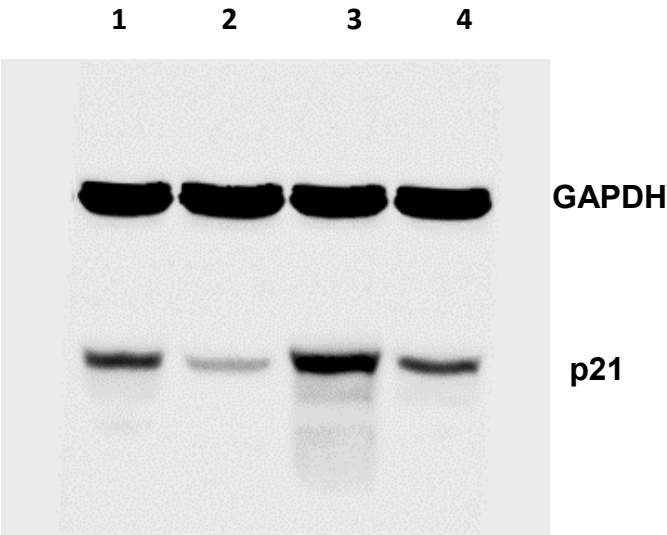
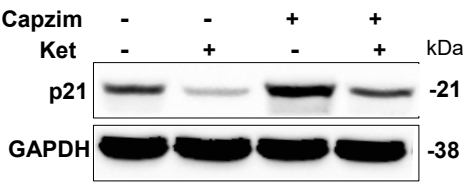
GAPDH

Supplementary Fig. 5A



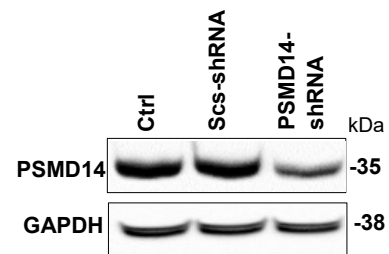
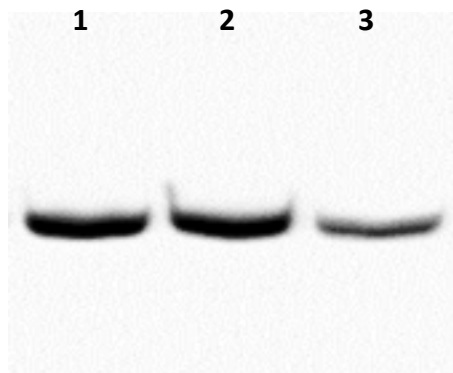
1- ctrl
2- Ket
3-N-Ethylmaleimide (NEM)
4-NEM+ Ket

Supplementary Fig. 6A

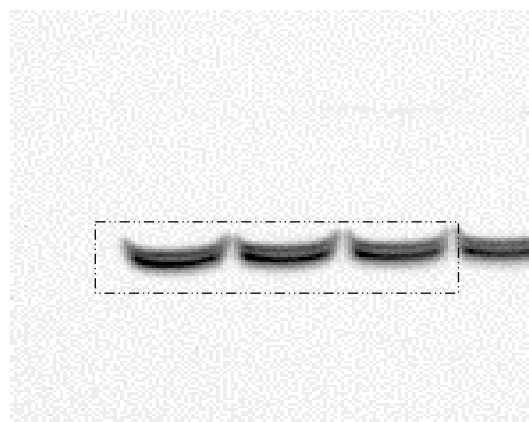


1- ctrl
2- ket
3-Capzimin
4-Capzimin+ket

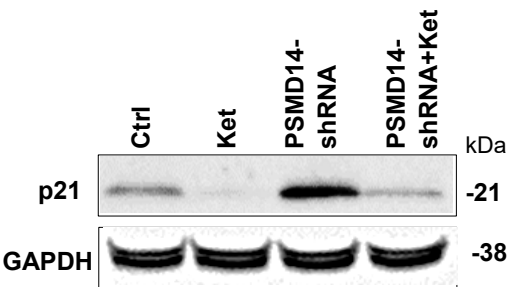
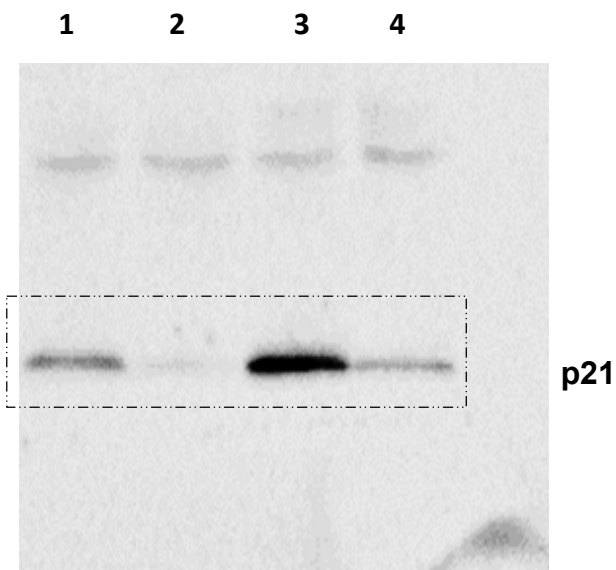
Supplementary Fig. 7A



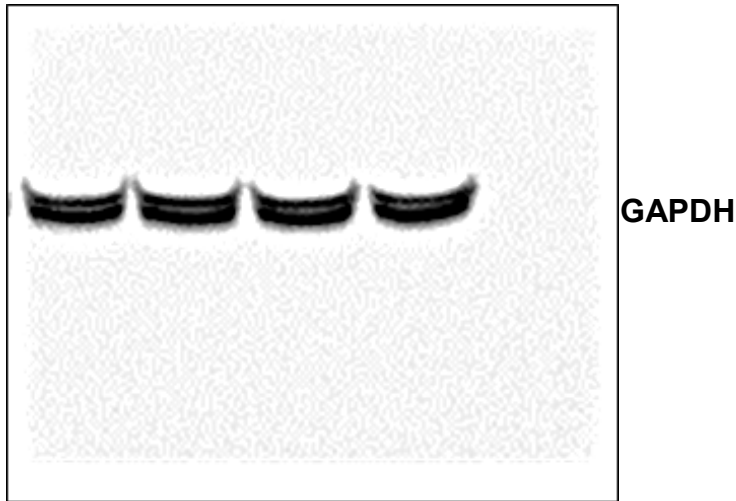
1- ctrl
2- Scs-shRNA
3-PSMD14-shRNA



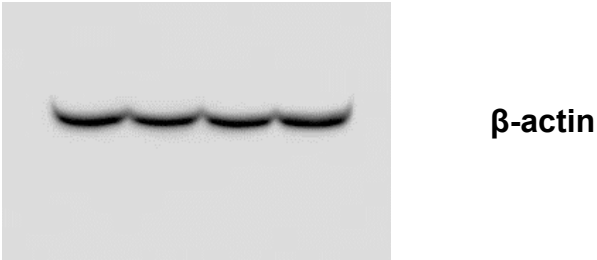
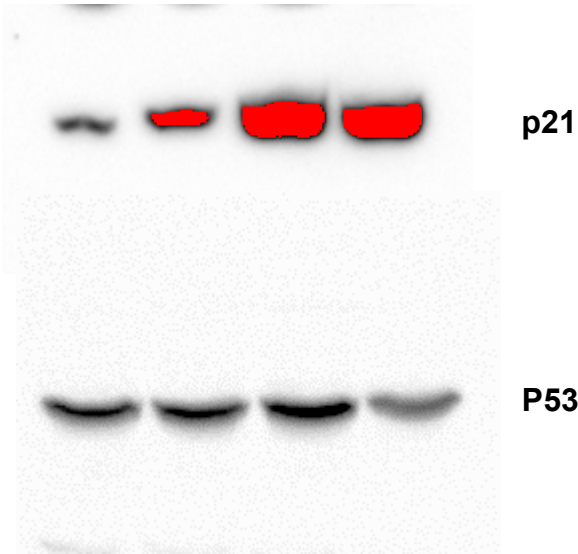
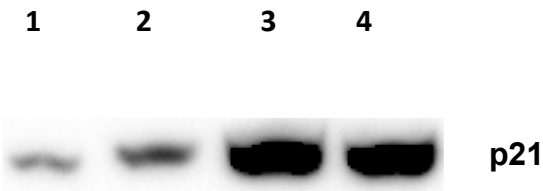
Supplementary Fig. 7B



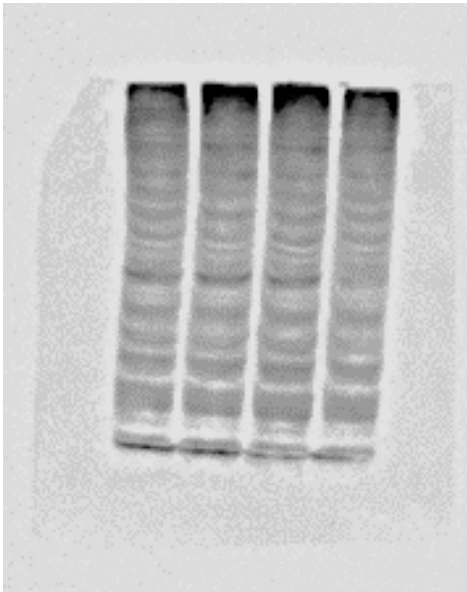
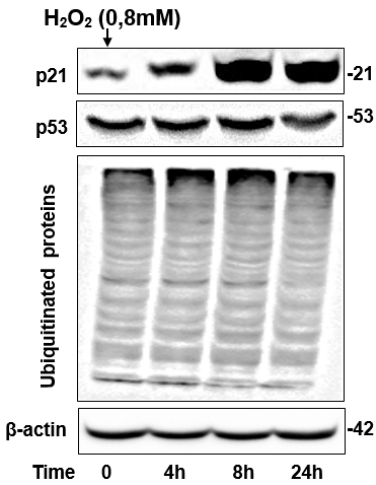
- 1- ctrl
- 2- ket
- 3-PSMD14-shRNA
- 4-PSMD14-shRNA + ket



Supplementary Fig. 8B

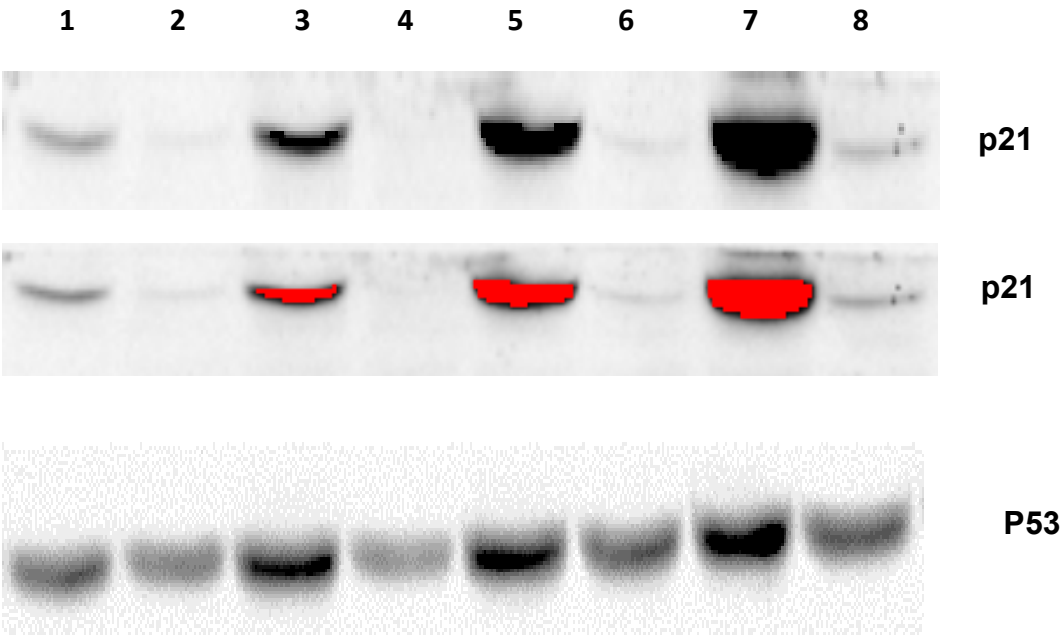
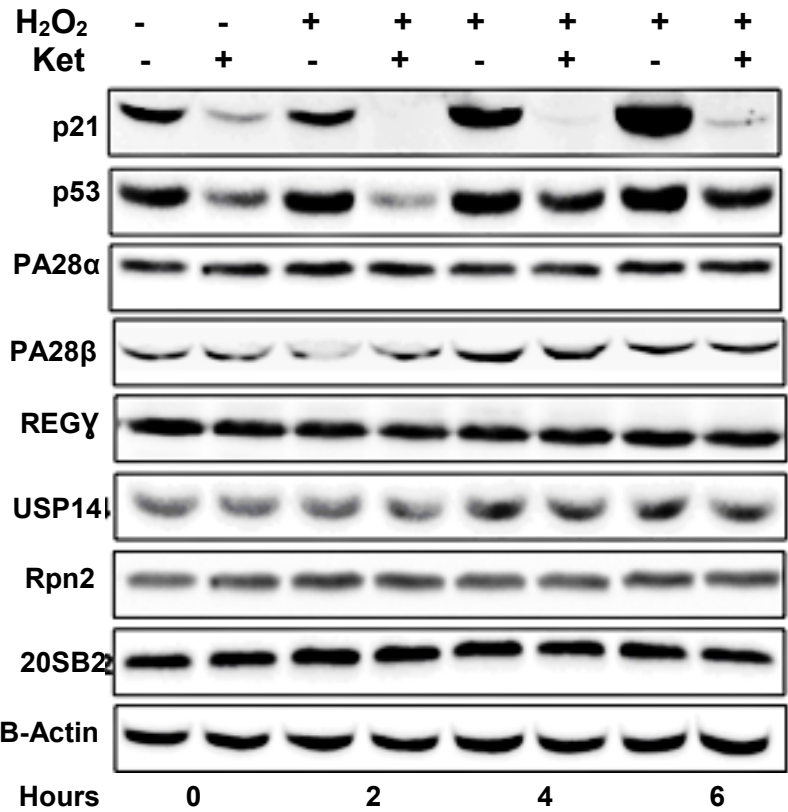


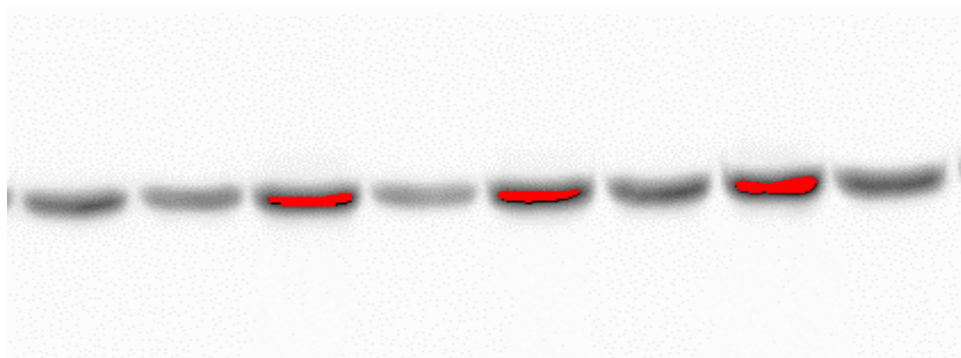
Ubiquitinated
proteins



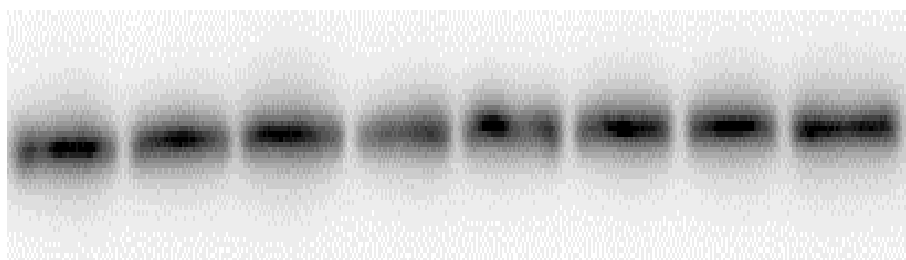
Supplementary Fig. 8C

H ₂ O ₂ (0,8mM)	added at time 0
Time	0 2h 4h 6h
1-ctrl	
2-ket	
3-H ₂ O ₂	2h
4-H ₂ O ₂ + ket	2h
5-H ₂ O ₂	4h
6-H ₂ O ₂ + ket	4h
7-H ₂ O ₂ +	6h
8-H ₂ O ₂ + ket	6h





P53



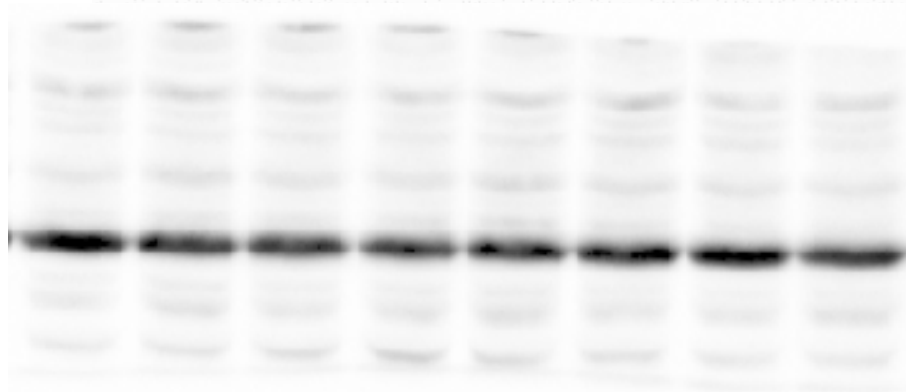
PA28α



PA28α



PA28β



Reg Gamma



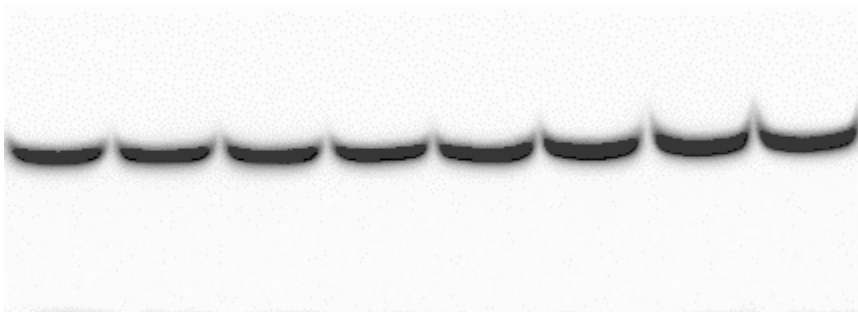
USP14



Rpn2

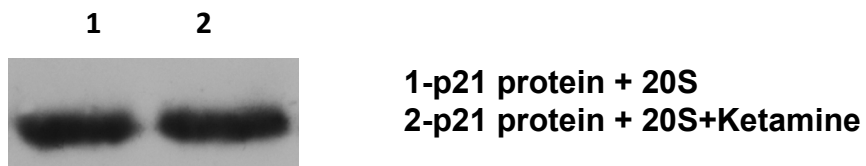


**20S proteasome
beta2**

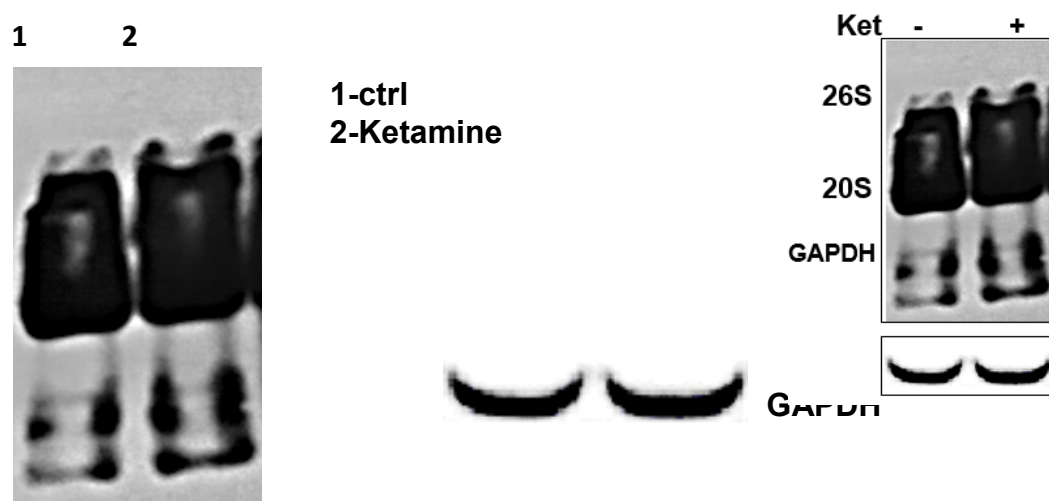


β-actin

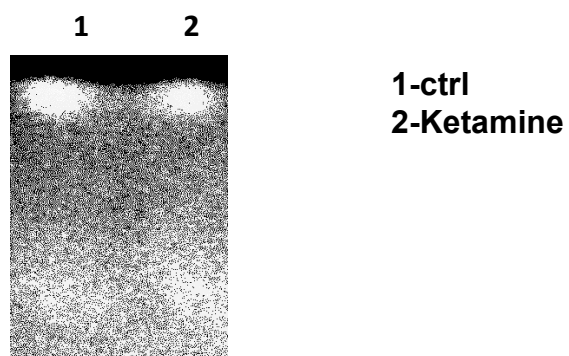
Supplementary Fig. 9B



Supplementary Fig. 9C



Supplementary Fig. 9D



Supplementary Fig. 9E

