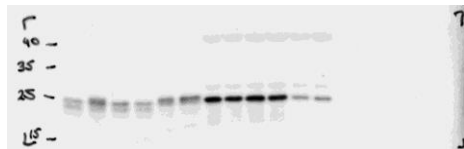


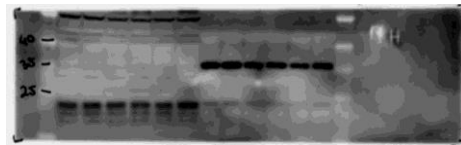
Fig 3b

H2122_2h treatment-raw data

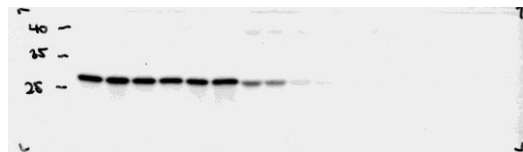
KRAS



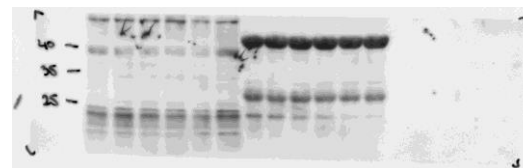
HRAS



MRAS



NRAS



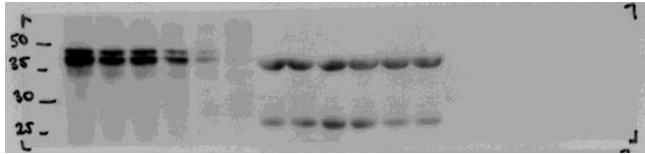
1st Antibody	MW [kD]	used dilution	Company	Cat #	2nd Antibody	Company	Cat #
GAPDH	37 kDa	1:1000	Cell Signalling Technologies	#2118	rabbit	Dako	P0448
KRAS	21 kDa	1:1000	LSBio	LS-C175665	mouse	Dako	P0447
HRAS	21 kDa	1:500	proteintech	18295-1-AP	rabbit	Dako	P0448
NRAS	21 kDa	1:1000	abcam	ab167136	rabbit	Dako	P0448
MRAS	21 kDa	1:200	abcam	ab176570	rabbit	Dako	P0448

GAPDH



H2122_2h treatment-raw data

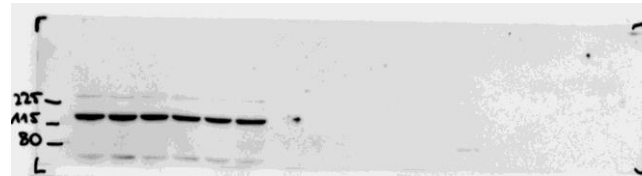
pERK



ERK

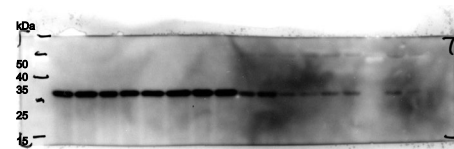
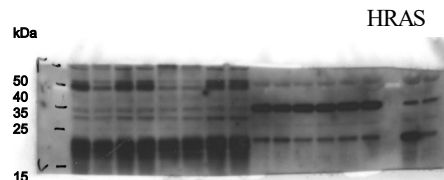
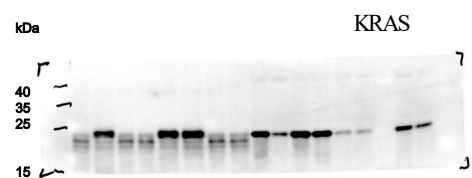


Vinculin

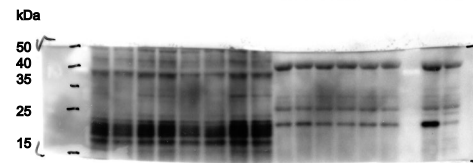


1st Antibody	MW [kD]	used dilution	Incubation	Species	Company	Cat #
Vinculin	124 kDa (it appears at 115 kDa)	1:200	ON at 4°C	rabbit	CST	#4650
pERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#4370
ERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#9102

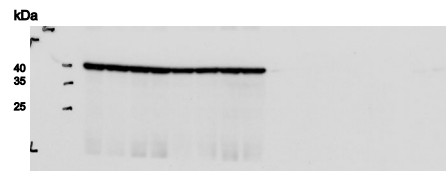
H2122_48h treatment-raw data



MRAS



NRAS

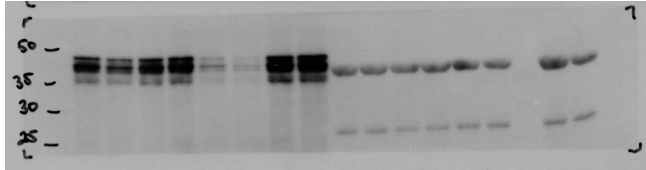


GAPDH

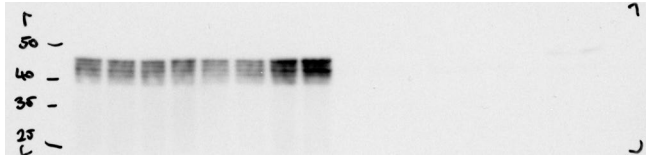
1st Antibody	MW [kD]	used dilution	Company	Cat #	2nd Antibody	Company	Cat #
GAPDH	37 kDa	1:1000	Cell Signalling Technologies	#2118	rabbit	Dako	P0448
KRAS	21 kDa	1:1000	LSBio	LS-C175665	mouse	Dako	P0447
HRAS	21 kDa	1:500	proteintech	18295-1-AP	rabbit	Dako	P0448
NRAS	21 kDa	1:1000	abcam	ab167136	rabbit	Dako	P0448
MRAS	21 kDa	1:200	abcam	ab176570	rabbit	Dako	P0448

H2122_48h treatment-raw data

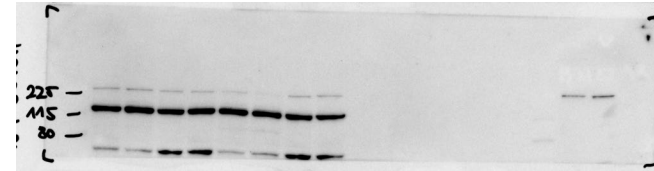
pERK



ERK



Vinculin



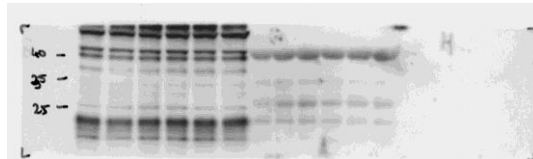
1st Antibody	MW [kD]	used dilution	Incubation	Species	Company	Cat #
Vinculin	124 kDa (it appears at 115 kDa)	1:200	ON at 4°C	rabbit	CST	#4650
pERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#4370
ERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#9102

H358_2h treatment - raw data

KRAS



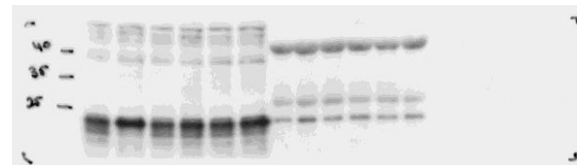
HRAS



MRAS

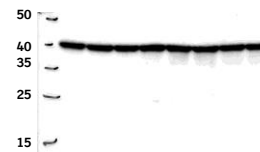


NRAS



1st Antibody	MW [kD]	used dilution	Company	Cat #	2nd Antibody	Company	Cat #
GAPDH	37 kDa	1:1000	Cell Signalling Technologies	#2118	rabbit	Dako	P0448
KRAS	21 kDa	1:1000	LSBio	LS-C175665	mouse	Dako	P0447
HRAS	21 kDa	1:500	proteintech	18295-1-AP	rabbit	Dako	P0448
NRAS	21 kDa	1:1000	abcam	ab167136	rabbit	Dako	P0448
MRAS	21 kDa	1:200	abcam	ab176570	rabbit	Dako	P0448

kDa



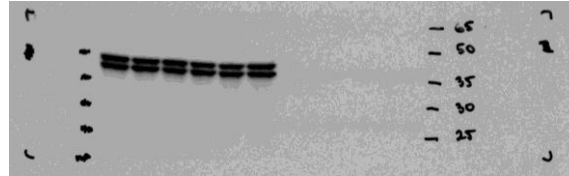
GAPDH

H358 2h treatment-raw data

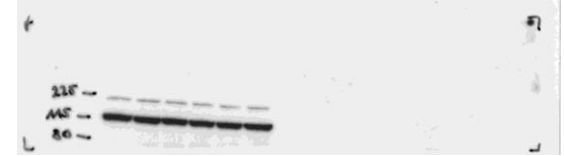
pERK



ERK

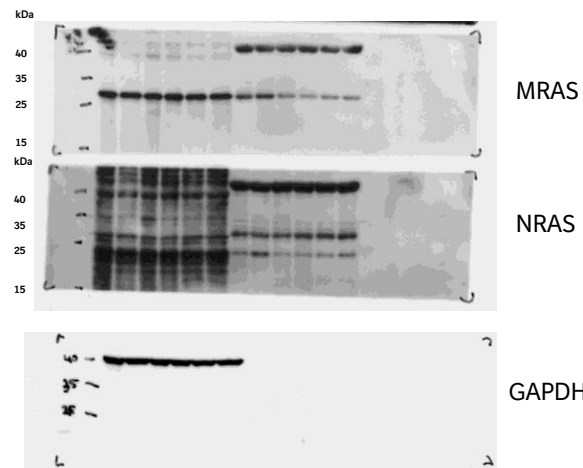
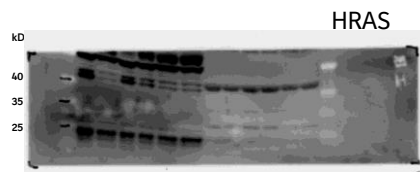
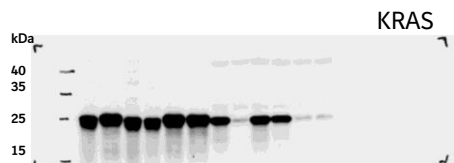


Vinculin



1st Antibody	MW [kD]	used dilution	Incubation	Species	Company	Cat #
Vinculin	124 kDa (it appears at 115 kDa)	1:200	ON at 4°C	rabbit	CST	#4650
pERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#4370
ERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#9102

H358_48h treatment - raw data



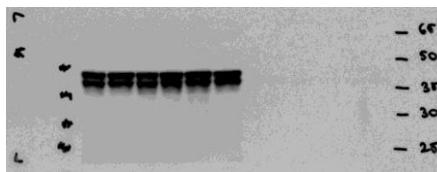
1st Antibody	MW [kDa]	used dilution	Company	Cat #	2nd Antibody	Company	Cat #
GAPDH	37 kDa	1:1000	Cell Signalling Technologies	#2118	rabbit	Dako	P0448
KRAS	21 kDa	1:1000	LSBio	LS-C175665	mouse	Dako	P0447
HRAS	21 kDa	1:500	proteintech	18295-1-AP	rabbit	Dako	P0448
NRAS	21 kDa	1:1000	abcam	ab167136	rabbit	Dako	P0448
MRAS	21 kDa	1:200	abcam	ab176570	rabbit	Dako	P0448

H358_48h treatment - raw data

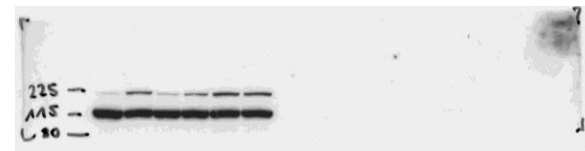
pERK



ERK



Vinculin



1st Antibody	MW [kD]	used dilution	Incubation	Species	Company	Cat #
Vinculin	124 kDa (it appears at 115 kDa)	1:200	ON at 4°C	rabbit	CST	#4650
pERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#4370
ERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#9102

SW837 2h treatment - raw data

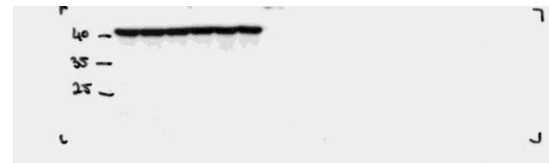
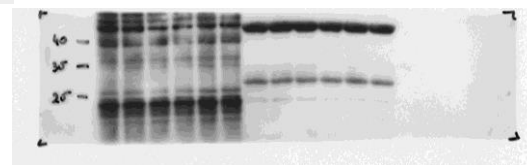
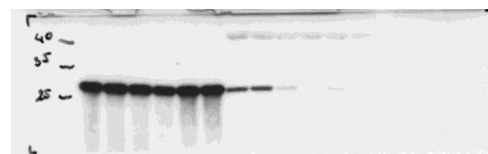
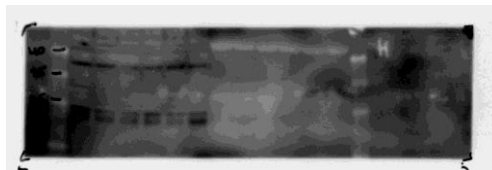
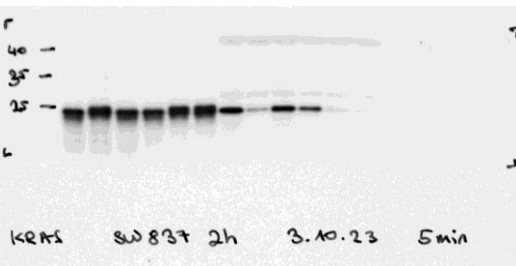
KRAS

HRAS

MRAS

NRAS

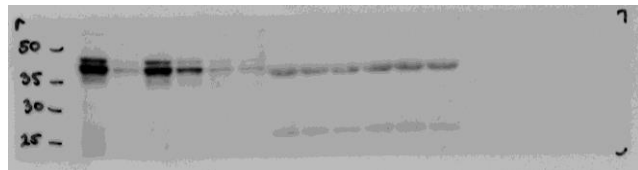
GAPDH



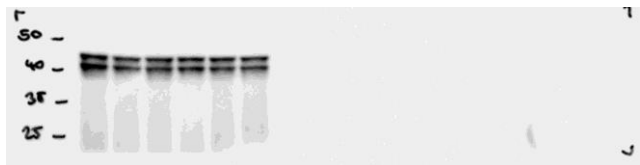
1st Antibody	MW [kDa]	used dilution	Company	Cat #	2nd Antibody	Company	Cat #
GAPDH	37 kDa	1:1000	Cell Signalling Technologies	#2118	rabbit	Dako	P0448
KRAS	21 kDa	1:1000	LSBio	LS-C175665	mouse	Dako	P0447
HRAS	21 kDa	1:500	proteintech	18295-1-AP	rabbit	Dako	P0448
NRAS	21 kDa	1:1000	abcam	ab167136	rabbit	Dako	P0448
MRAS	21 kDa	1:200	abcam	ab176570	rabbit	Dako	P0448

SW837 2h treatment - raw data

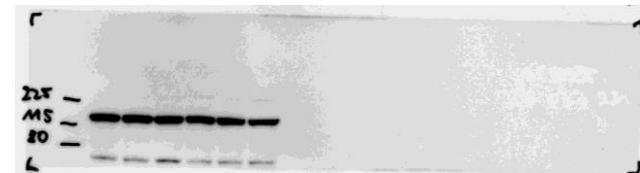
pERK



ERK

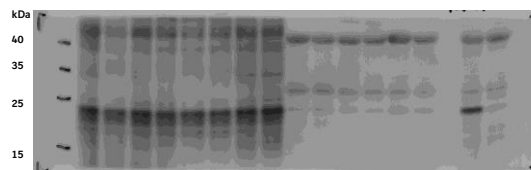
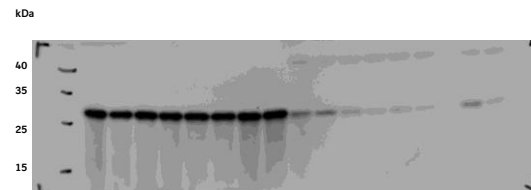
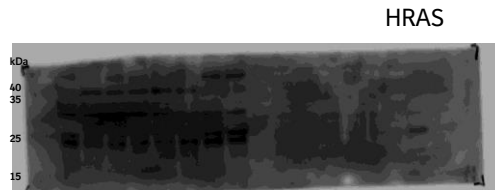
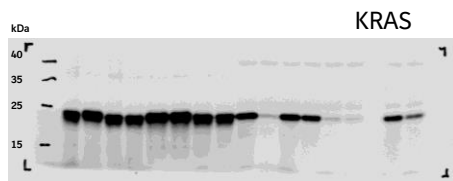


Vinculin

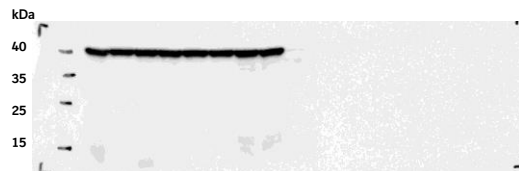


1st Antibody	MW [kD]	used dilution	Incubation	Species	Company	Cat #
Vinculin	124 kDa (it appears at 115 kDa)	1:200	ON at 4°C	rabbit	CST	#4650
pERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#4370
ERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#9102

SW837_48h treatment-raw data

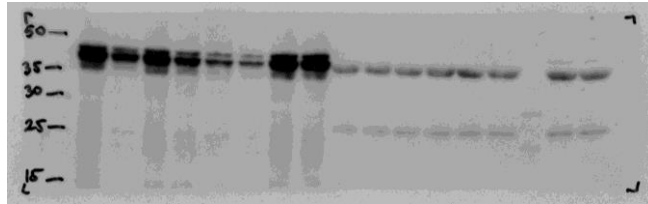


1st Antibody	MW [kD]	used dilution	Company	Cat #	2nd Antibody	Company	Cat #
GAPDH	37 kDa	1:1000	Cell Signalling Technologies	#2118	rabbit	Dako	P0448
KRAS	21 kDa	1:1000	LSBio	LS-C175665	mouse	Dako	P0447
HRAS	21 kDa	1:500	proteintech	18295-1-AP	rabbit	Dako	P0448
NRAS	21 kDa	1:1000	abcam	ab167136	rabbit	Dako	P0448
MRAS	21 kDa	1:200	abcam	ab176570	rabbit	Dako	P0448

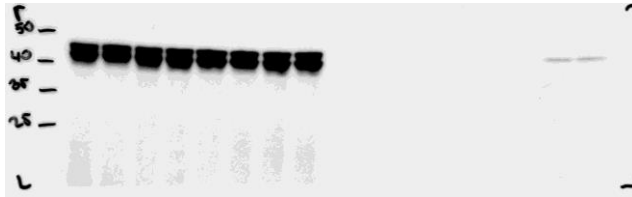


SW837_48h treatment-raw data

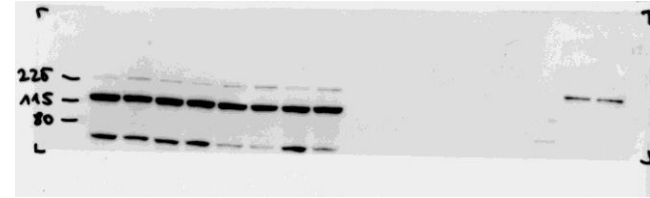
pERK



ERK



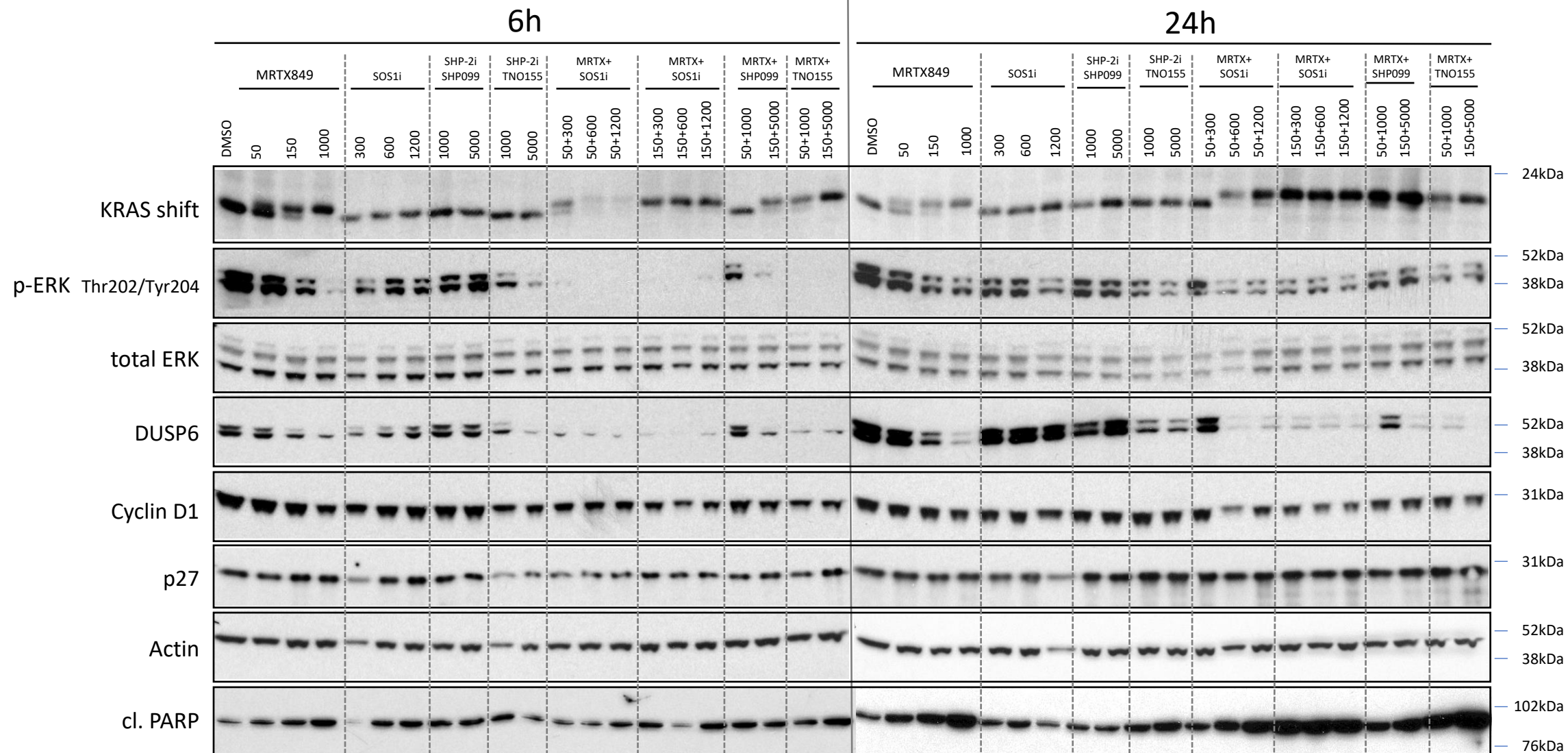
Vinculin



1st Antibody	MW [kD]	used dilution	Incubation	Species	Company	Cat #
Vinculin	124 kDa (it appears at 115 kDa)	1:200	ON at 4°C	rabbit	CST	#4650
pERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#4370
ERK	44, 42 kDa	1:1000	ON at 4°C	rabbit	CST	#9102

Figure 3c

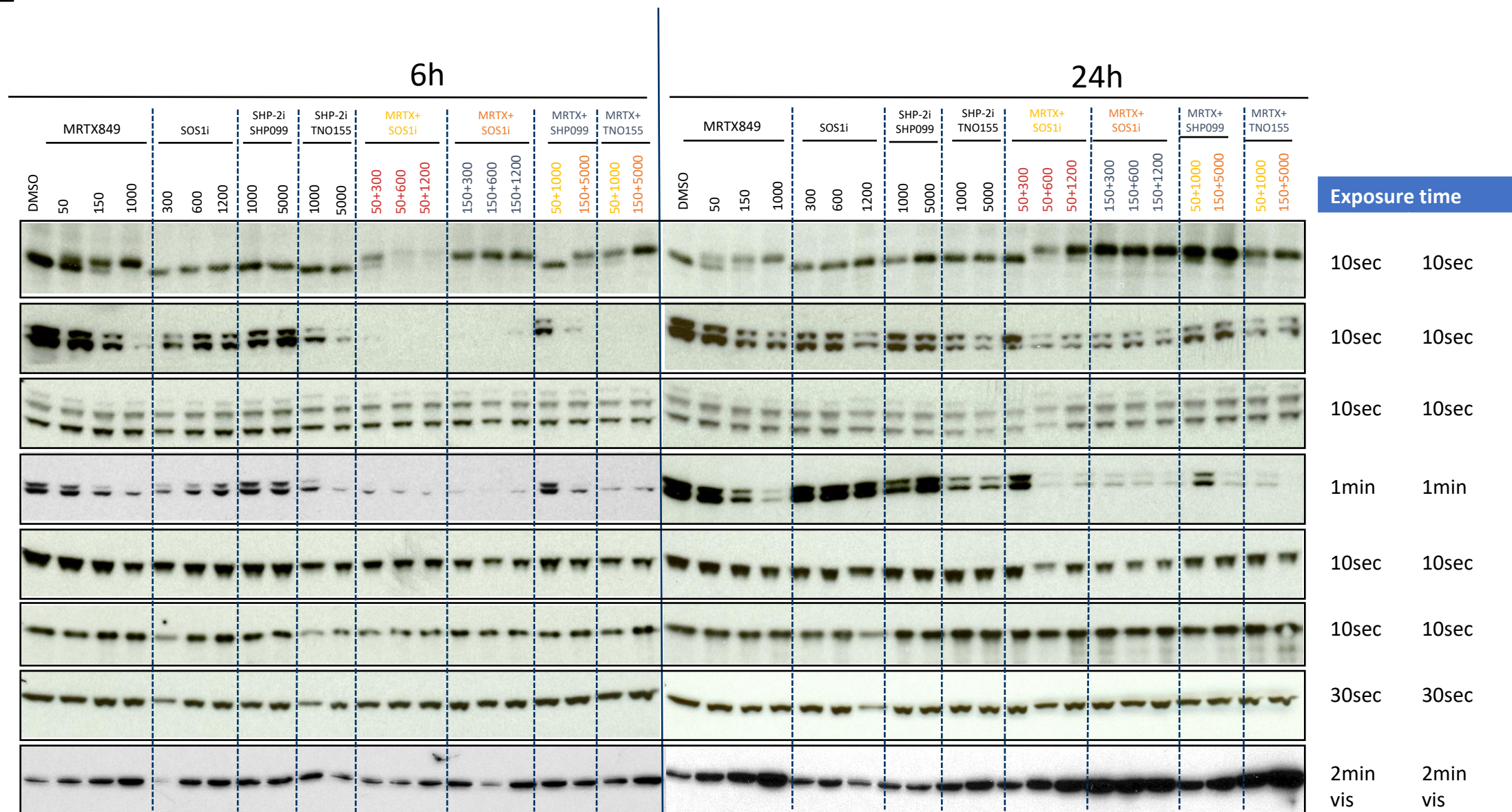
NCI-H2122



Technical Information

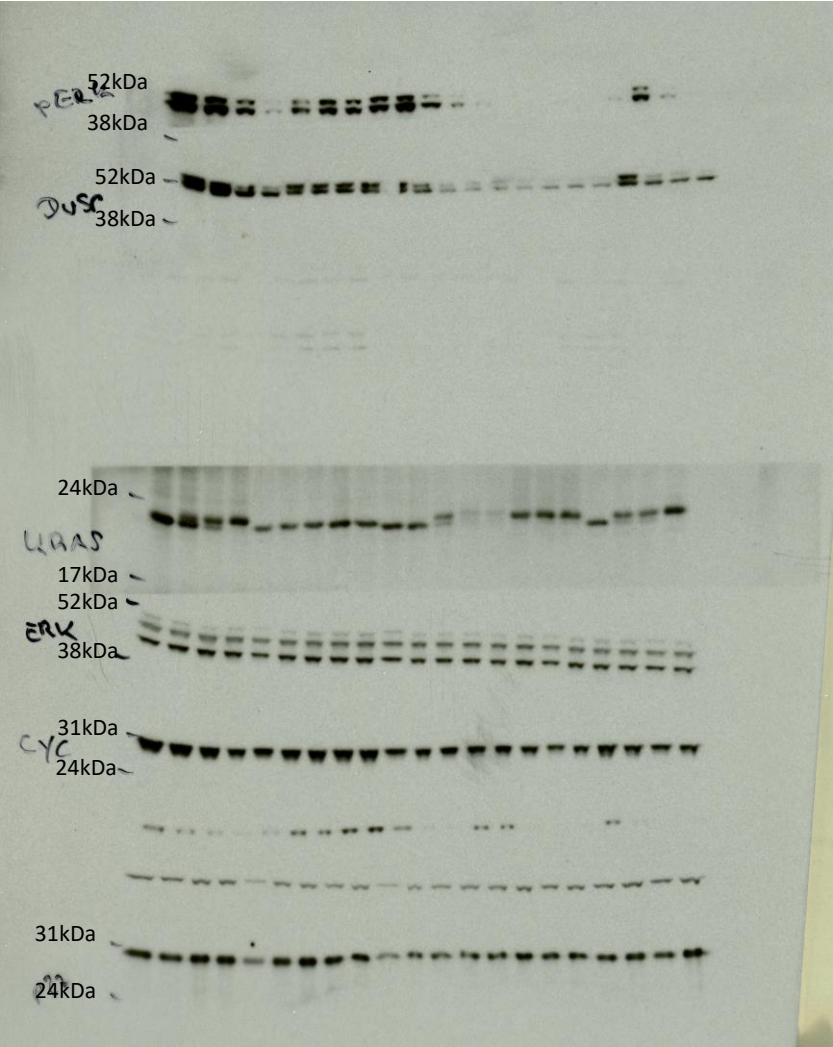
- If not otherwise mentioned 20 µg protein / lane loaded
- Same antibody dilutions used for 6 and 24h samples
- Exposure times to detect ECL signals are listed, and same exposures were used for 6 and 24h samples
- To save extract, antibodies etc. we typically cut membranes to only blot the relevant region of the membrane with the respective antibodies, that allows typically 2-4 antibodies to use per membrane
- X-ray scans were either in total or often only the relevant region scanned
- Note the colour code which indicates correlating membranes
- Sometimes several exposure or several blots are shown, the once taken for the figures are indicated in bold and have a star (*)

NCI-H2122



Scanned blots of 6h treatment:

10 seconds exposure - showing how several membrane incubated with various antibodies look on one x-ray



pERK 42/44kDa*

DUSP 6 42/44kDa not used for figure, blot was repeated (air bubble)

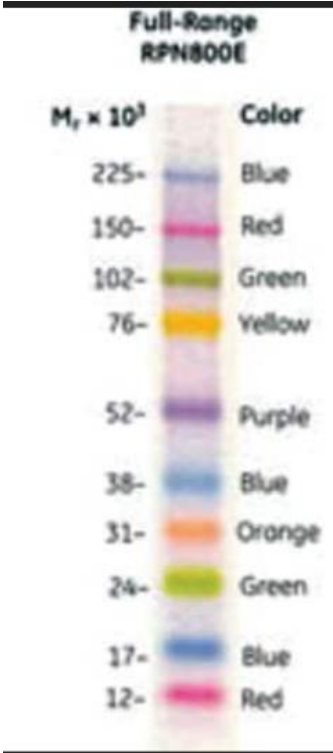
KRAS 21,7kDa*

ERK 42/44kDa*

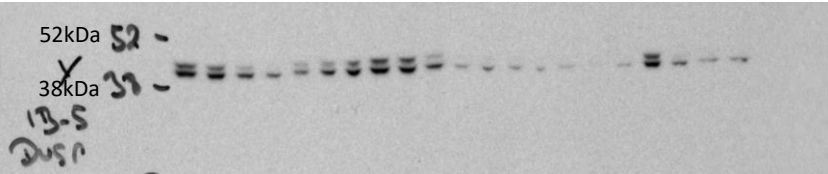
Cyclin D1 36kDa*

Actin 42kDa — not used for figure, 30sec exposure used

p27 27kDa*

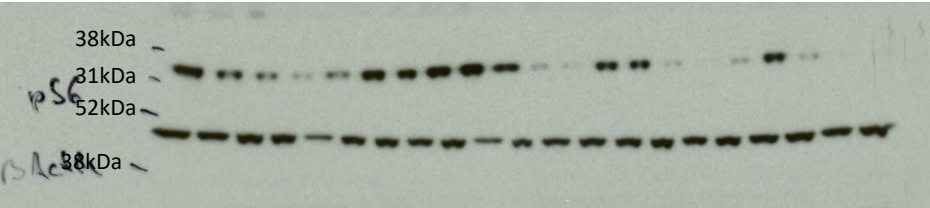


Scanned blots of 6h treatment:



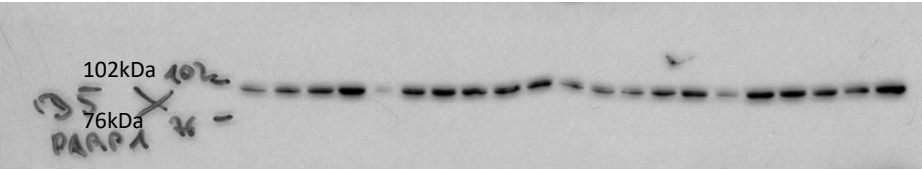
1min exposure

DUSP6* 42/44kDa



30sec exposure

Actin* 42kDa

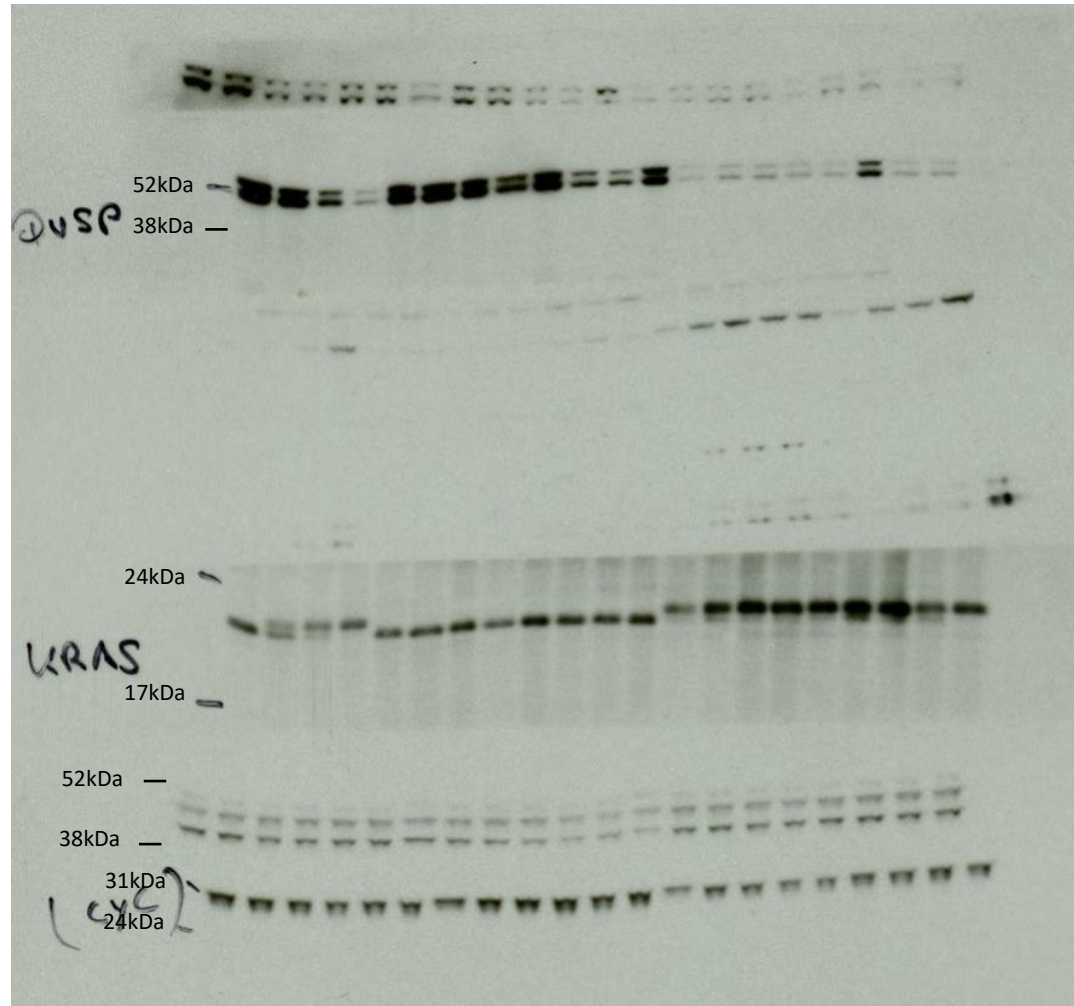


2min Visualizer exposure*

cl-.PARP* 89kDa

Scanned blots of 24h treatment:

1min exposure – showing how several membrane incubated with various antibodies look on one x-ray



DUSP 6 42/44kDa*

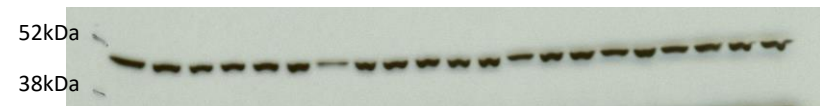
KRAS 21,7kDa (not used
10 sec exp. used)

ERK 42/44kDa (not used
10 sec exp. used)

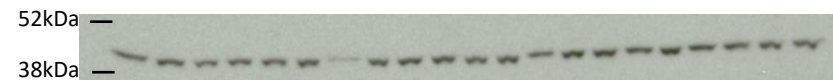
Cyclin 36kDa (not used, 10
sec exp. used)

Scanned blots of 24h treatment:

Actin 42kDa

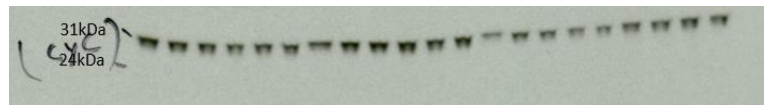


30sec exposure*



10sec exposure

Cyclin D1 36kDa



5sec exposure



10sec exposure*

ERK 42/44kDa



10sec exposure*



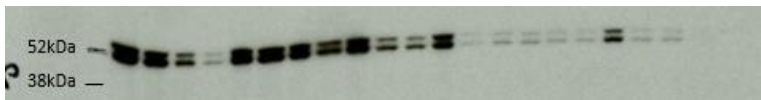
1 min exposure

p27 27kDa

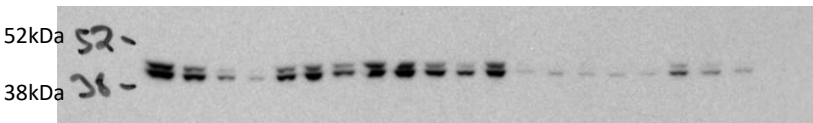


10sec exposure*

DUSP 6 42/44kDa

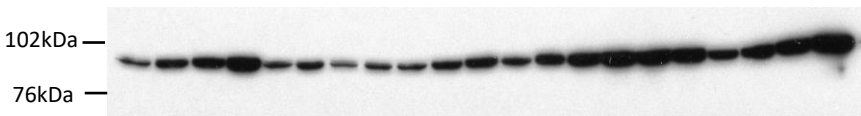


1min exposure*



1min exposure (repeated blot)

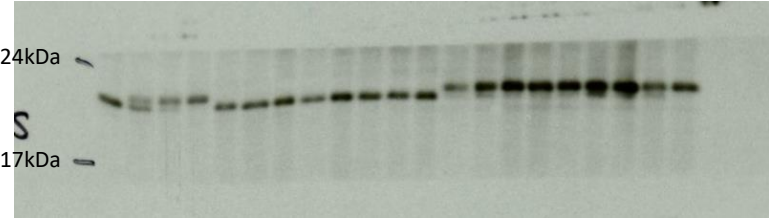
PARP 89kDa



2min Visualizer exposure*

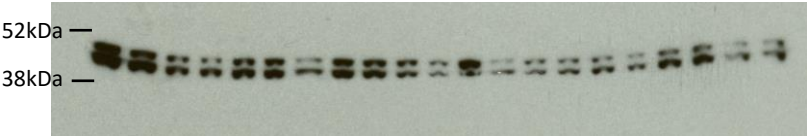
Scanned blots of 24h treatment:

KRAS 21,7kDa



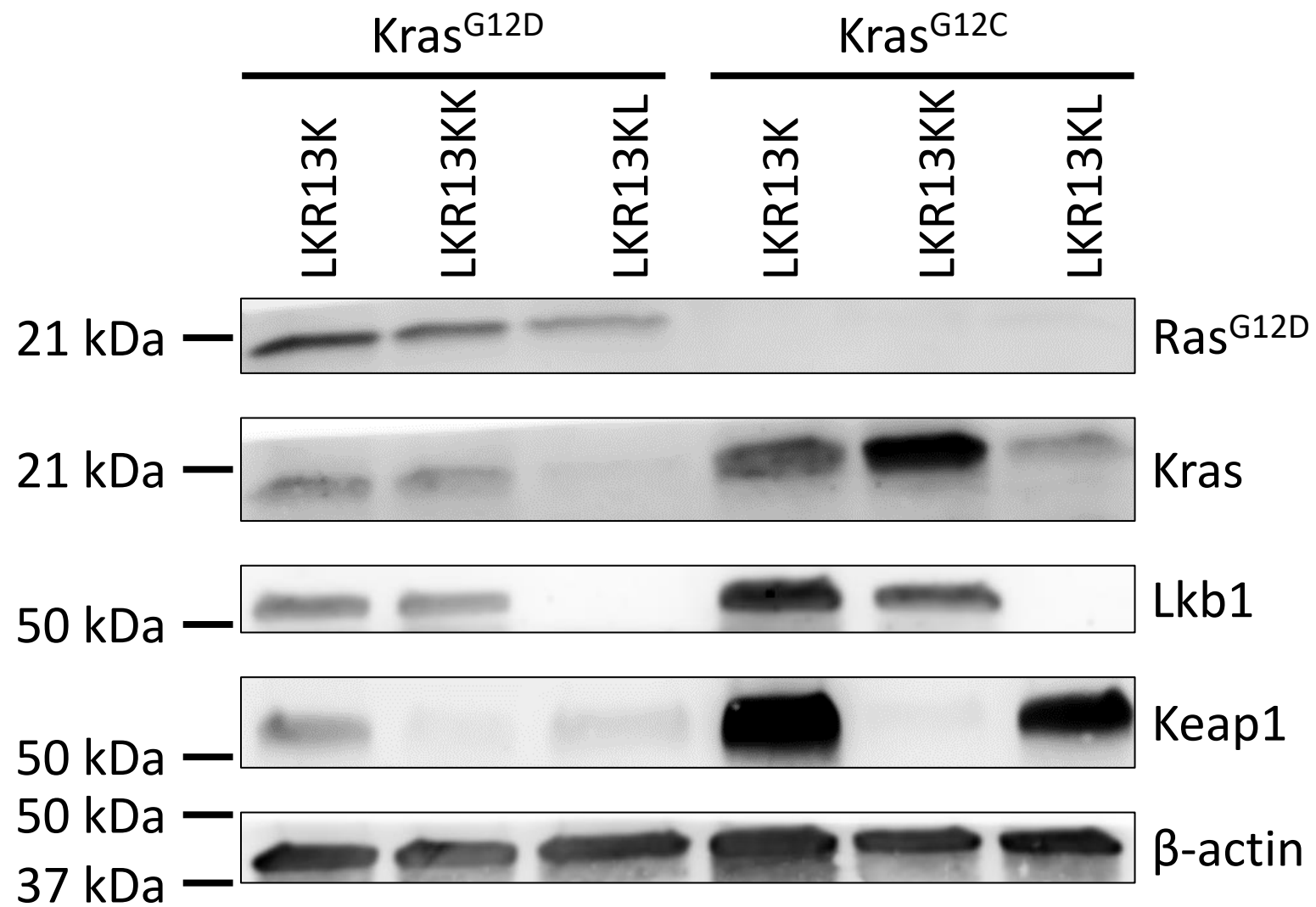
10sec exposure*

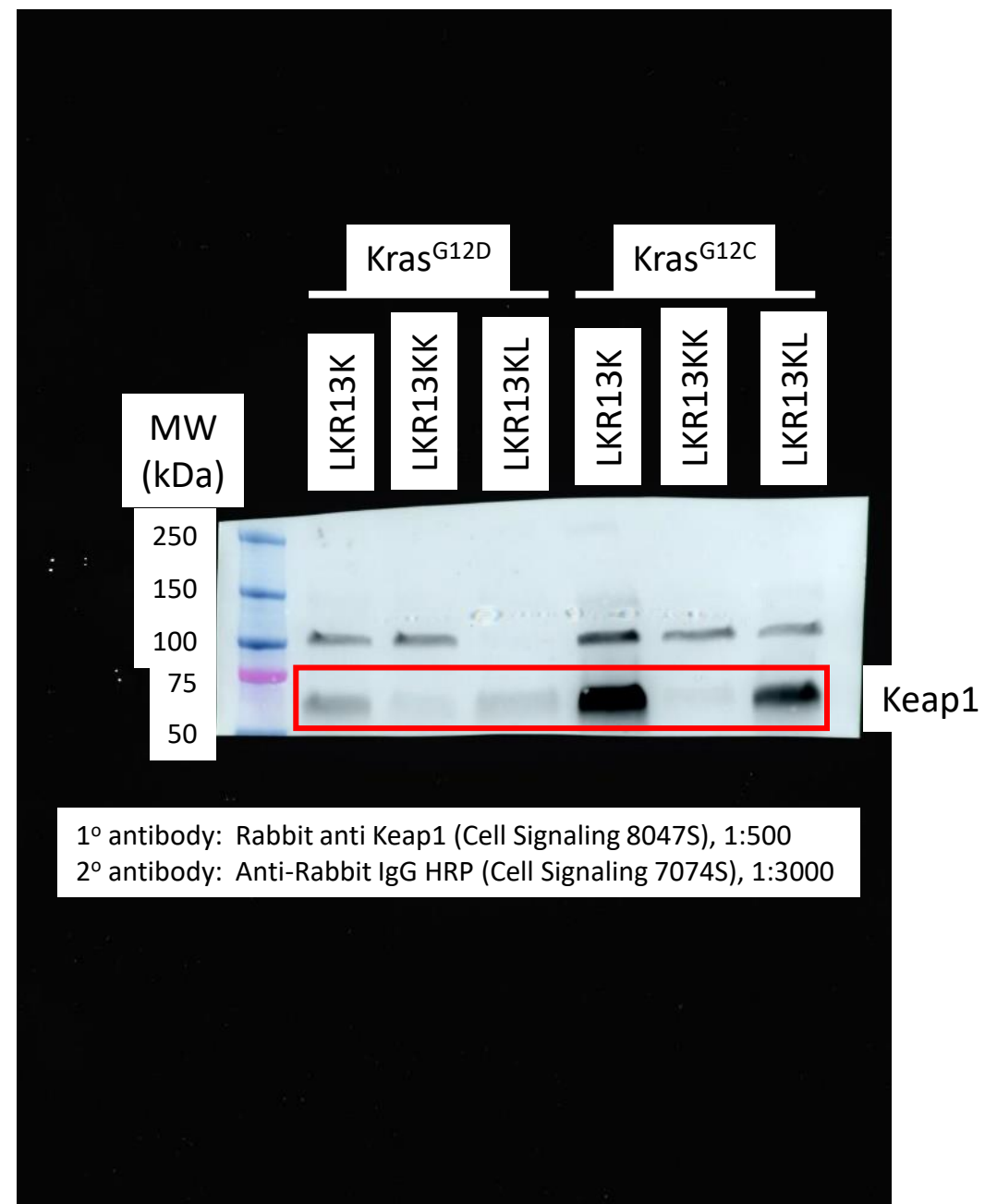
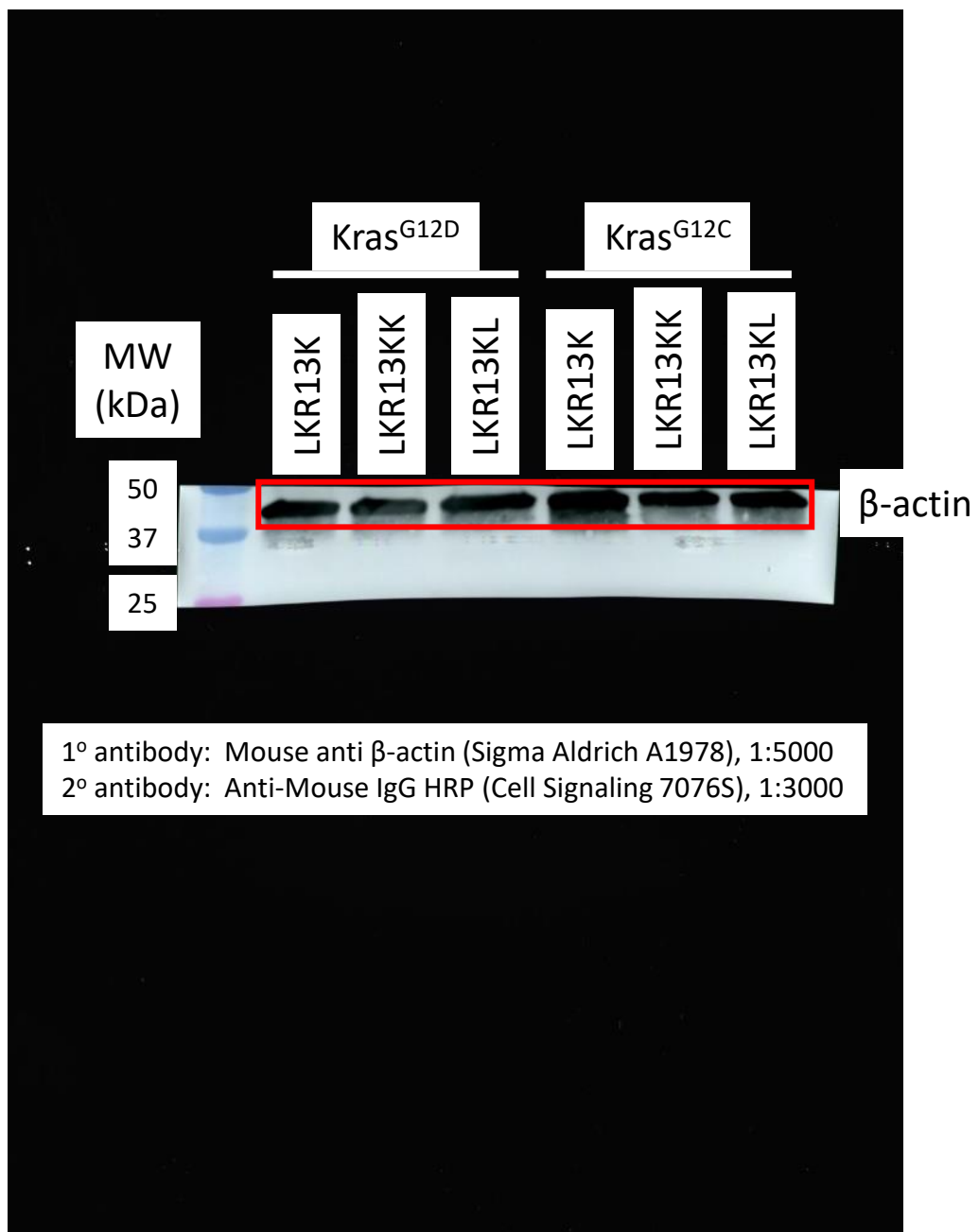
pERK 42/44kDa

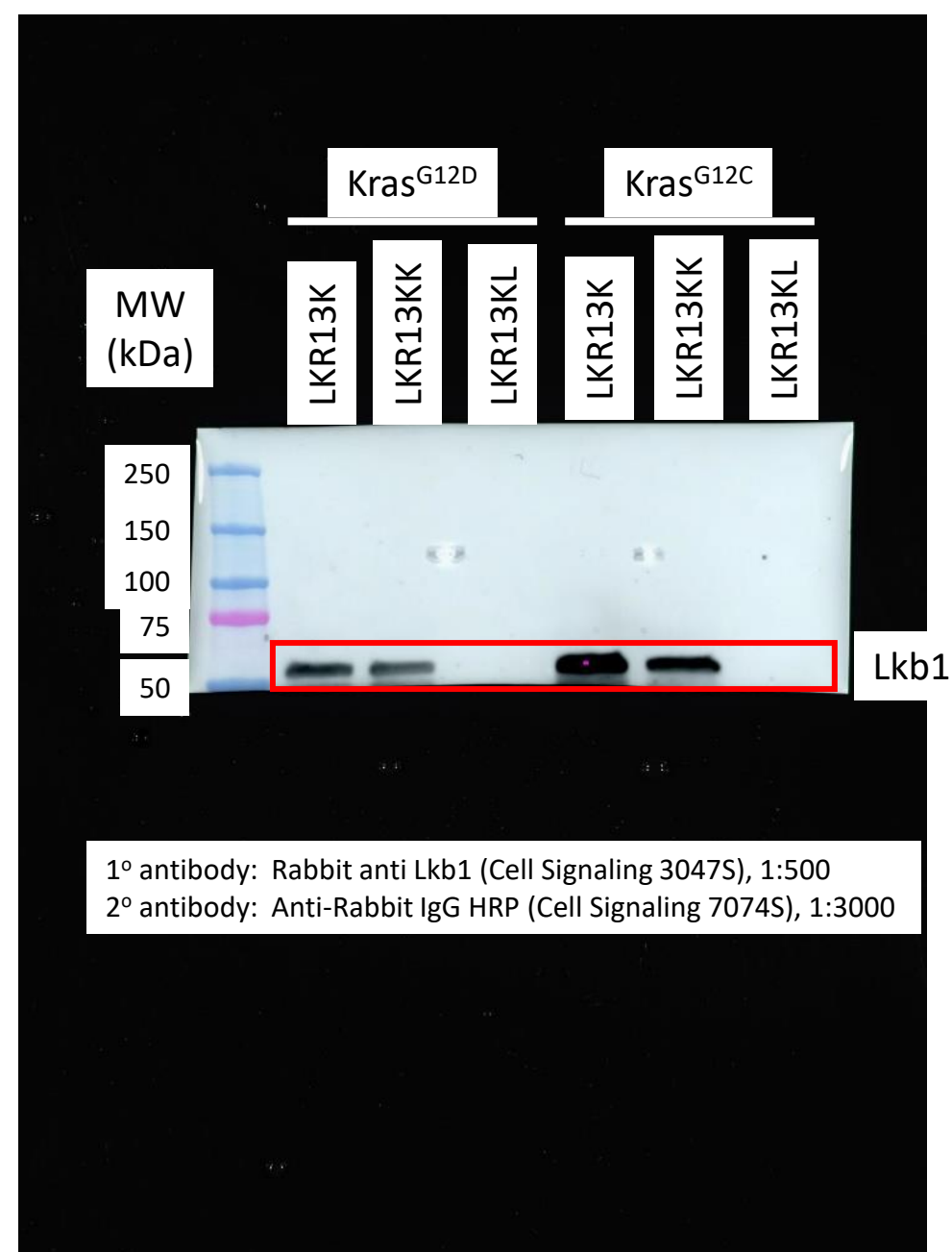
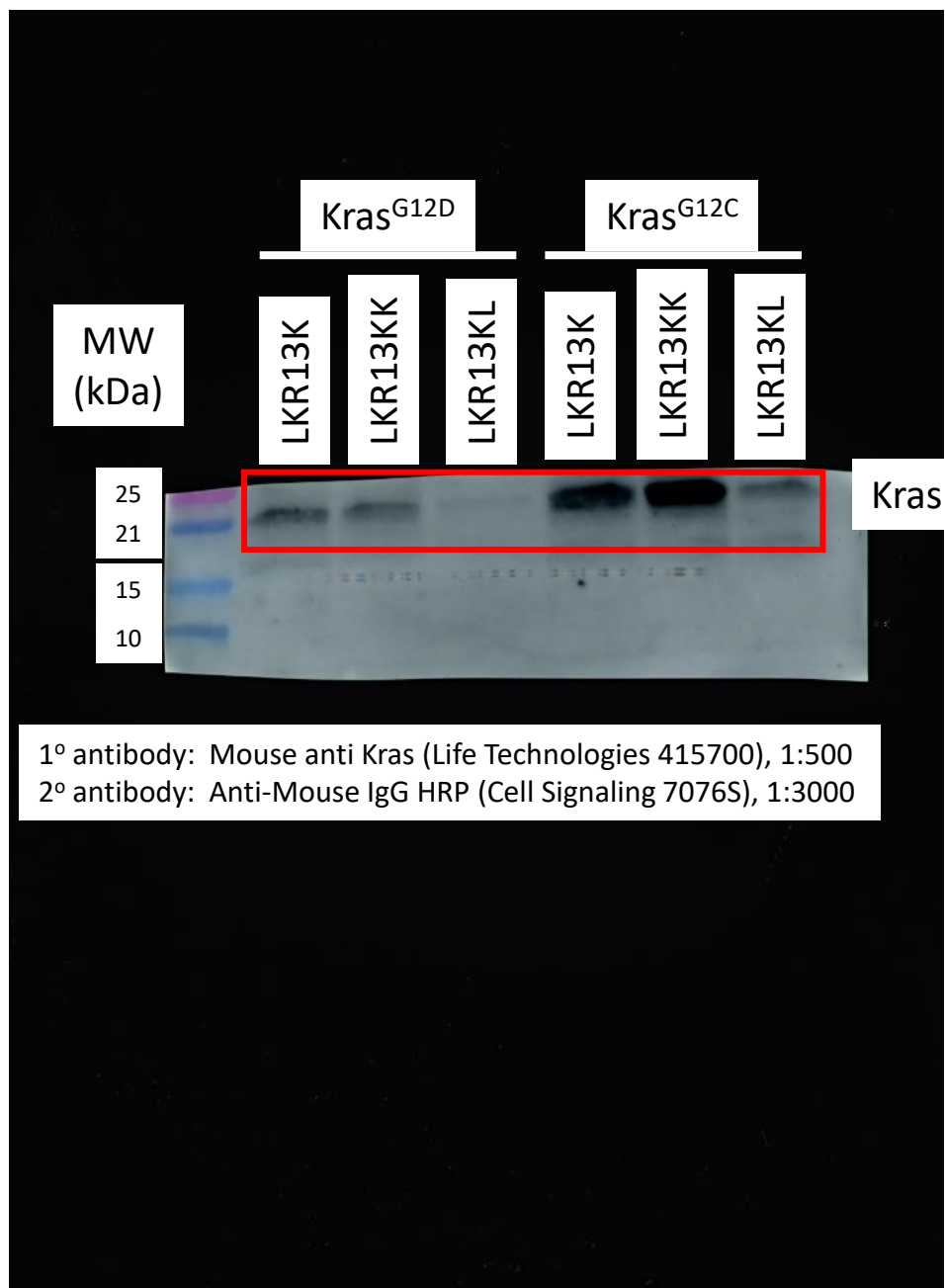


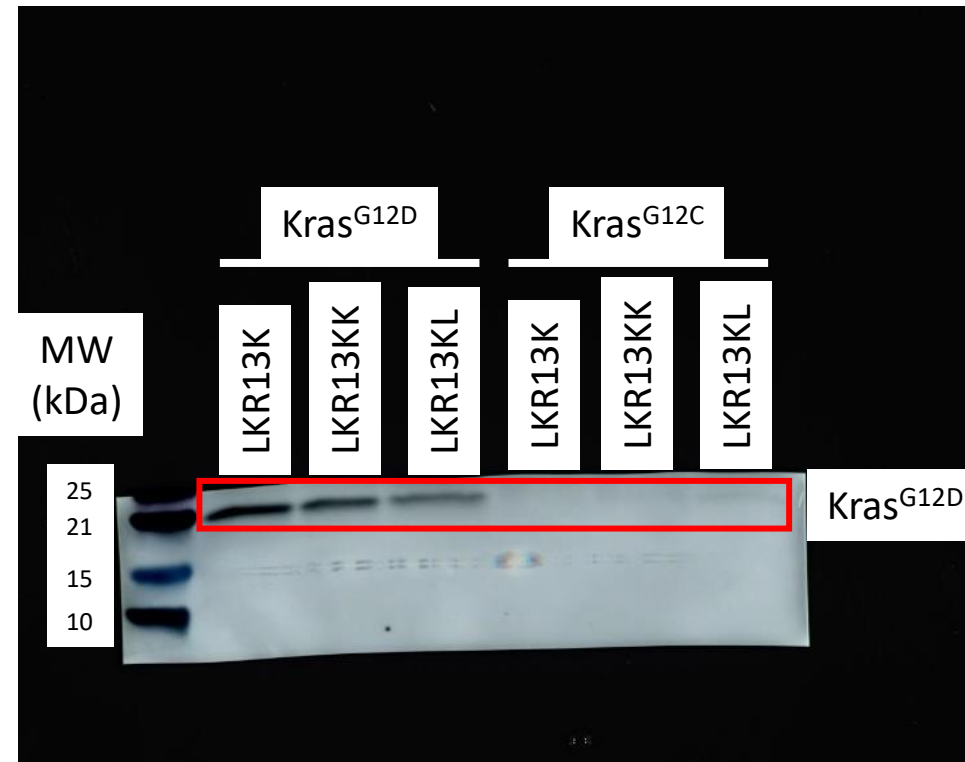
10sec exposure*

Extended Data Fig 1c









1° antibody: Rabbit anti Kras^{G12D} (Life Technologies MA536256), 1:500
2° antibody: Anti-Rabbit IgG HRP (Cell Signaling 7074S), 1:3000

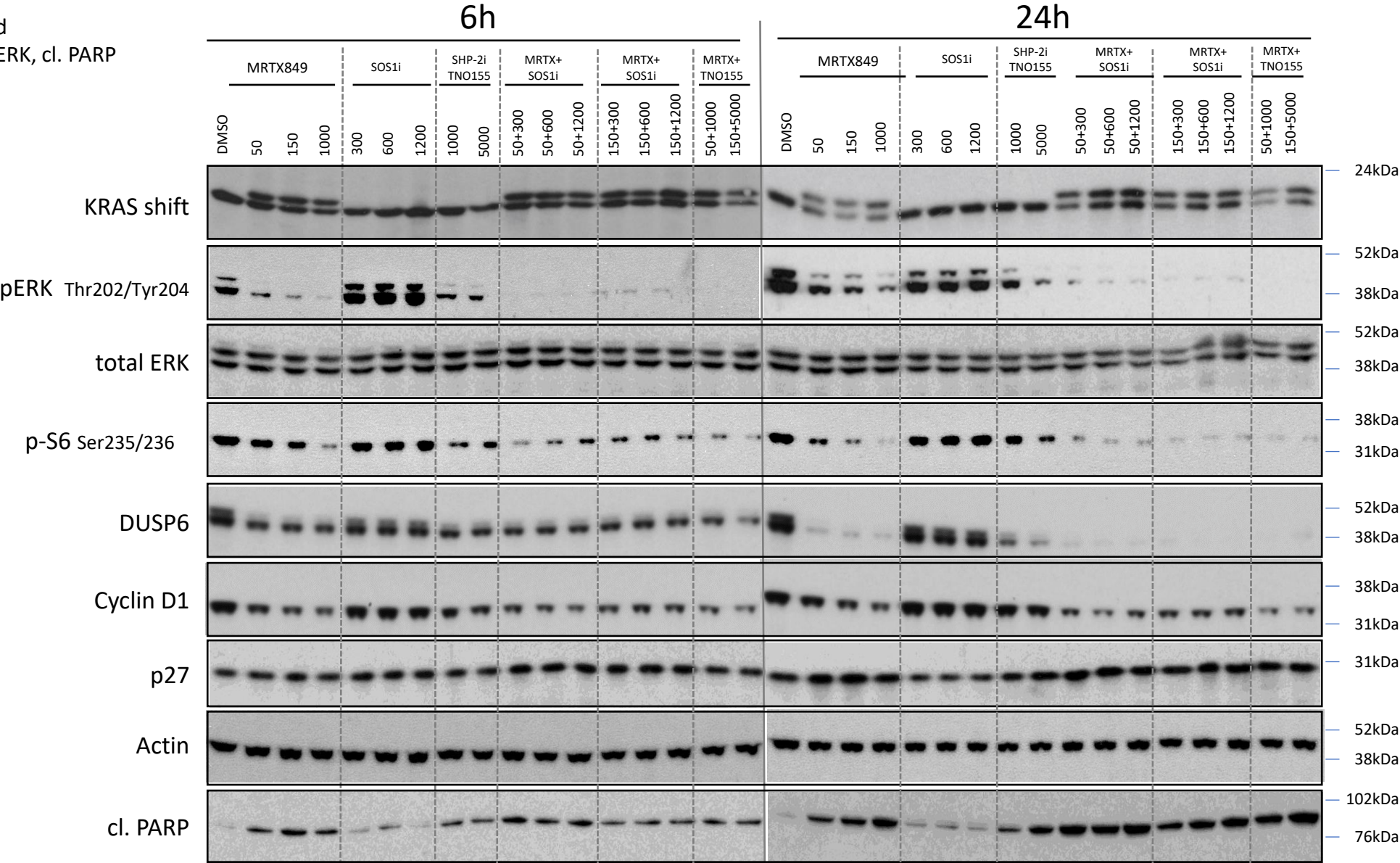
Extended Data Figure 3

Technical Information

- If not otherwise mentioned 20 µg protein / lane loaded
- Same antibody dilutions used for 6 and 24h samples
- Exposure times to detect ECL signals are listed, and same exposures were used for 6 and 24h samples
- To save extract, antibodies etc. we typically cut membranes to only blot the relevant region of the membrane with the respective antibodies, that allows typically 2-4 antibodies to use per membrane
- X-ray scans were either in total or often only the relevant region scanned
- Note the colour code which indicates correlating membranes
- Sometimes several exposure or several blots are shown, the once taken for the figures are indicated in bold and have a star (*)

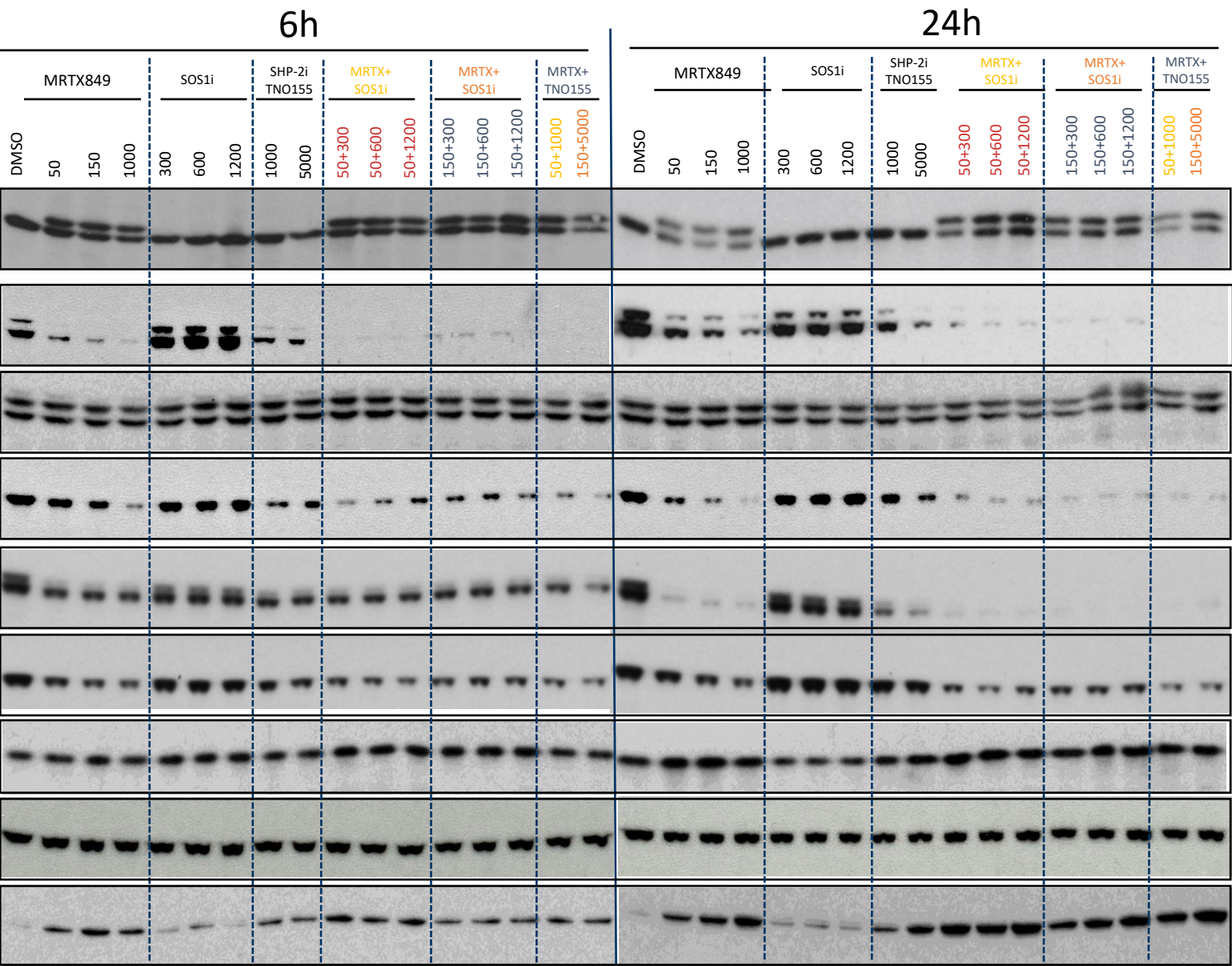
SW837

20µg/lane loaded
15 µg/lane for t-ERK, cl. PARP



SW837

20µg/lane loaded



Exposure time

1min 1min

10sec 10sec

10sec 10sec

1min 1min

30min 30min

2min 2min

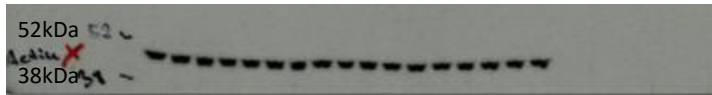
1min 1min

8min 8min

2min vis 2min vis

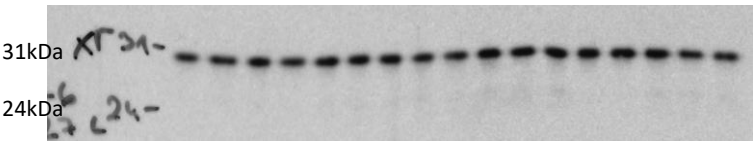
Scanned blots of 6h treatment:

Actin 42kDa



8min exposure *

p27 27kDa

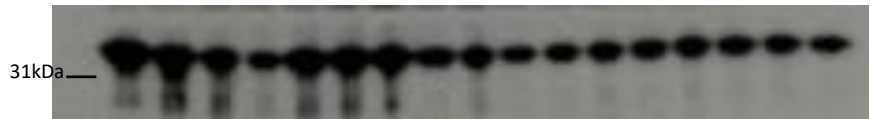
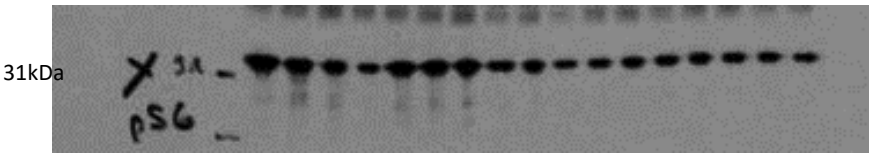


1min exposure *

pS6 32kDa



10sec exposure *



5min exposure

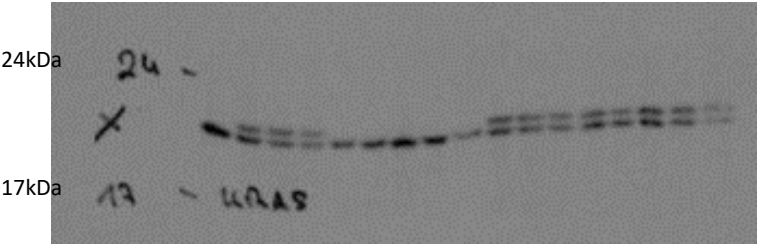
Cyclin D1 36kDa



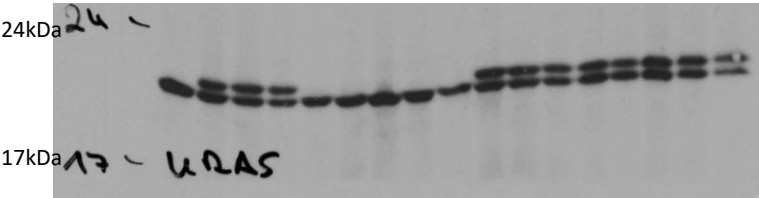
2min exposure *

Scanned blots of 6h treatment:

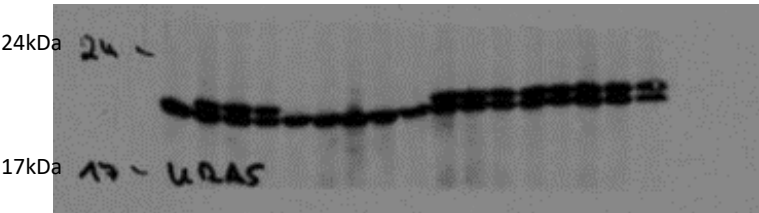
KRAS 21,7kDa



10sec exposure

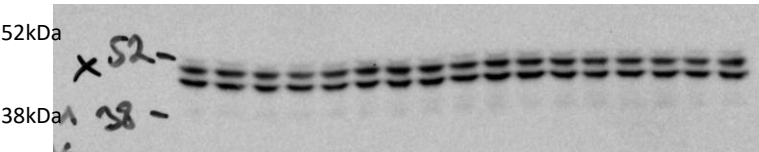


1min exposure *



5min exposure

ERK 42/44kDa

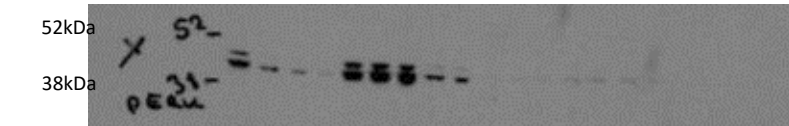


10sec exposure *

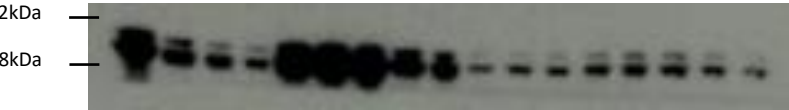
pERK 42/44kDa



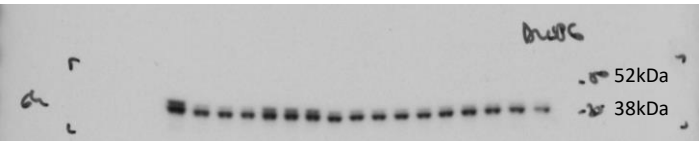
3sec exposure



10sec exposure *

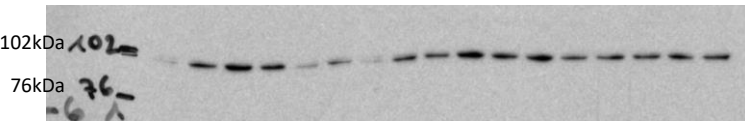


DUSP6 42/44kDa



30min exposure *

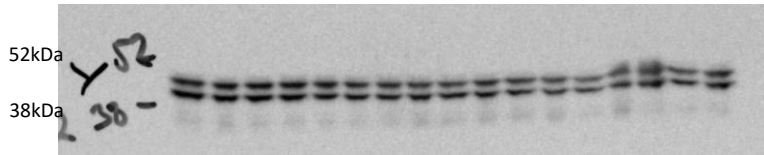
Cl-PARP 89 kDa



2min exposure *
Visualizer

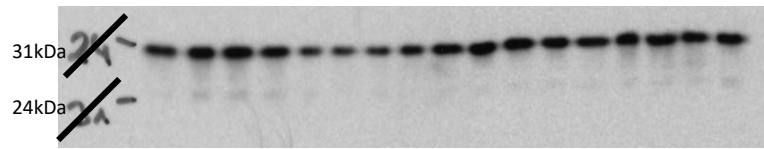
Scanned blots of 24h treatment:

ERK 42/44kDa



10sec exposure *

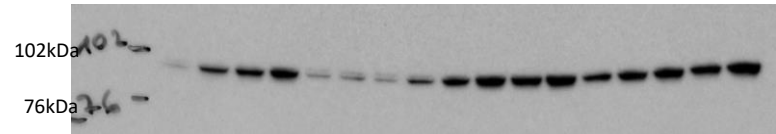
p27 27kDa



1min exposure *

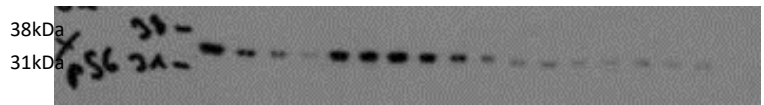
reverse and eroneous hand-written label; for reference of p27 protein migration towards position of markers please see slides 21 and 32.

CI-PARP 89 kDa



2min exposure visualizer *

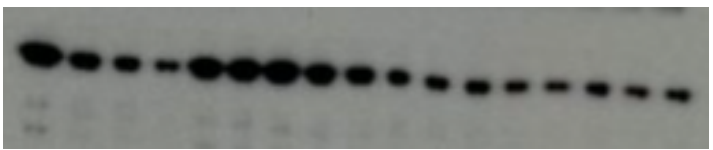
pS6 32kDa



1 min exposure *

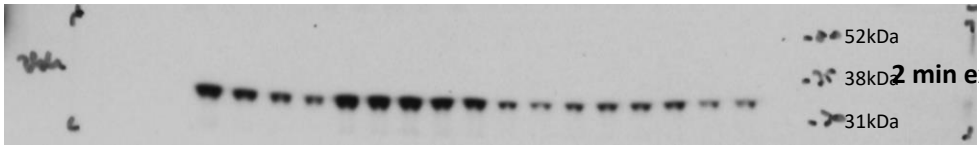


5 min exposure



8 min exposure

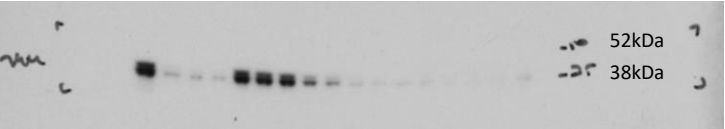
Cyclin D1 36kDa



2 min exposure *

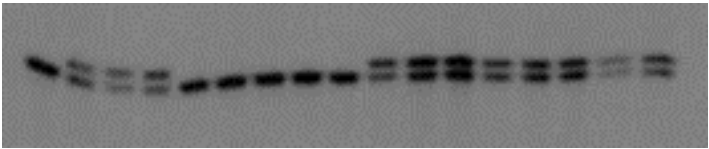
Scanned blots of 24h treatment:

DUSP6 42/44kDa

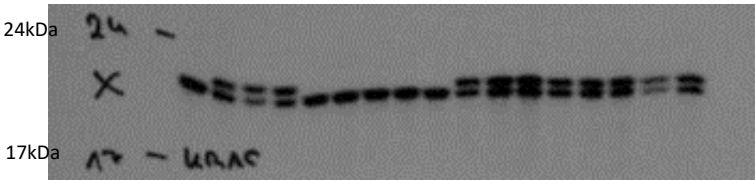


30min exposure *

KRAS 21,7kDa

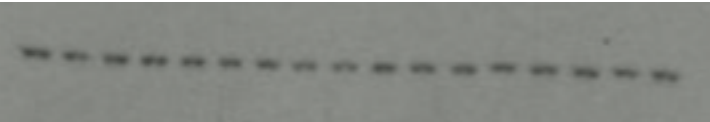


1 min exposure *

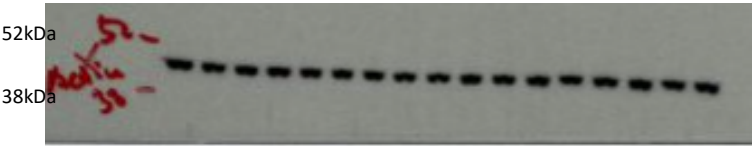


5 min exposure

Actin 42kDa



3 min exposure

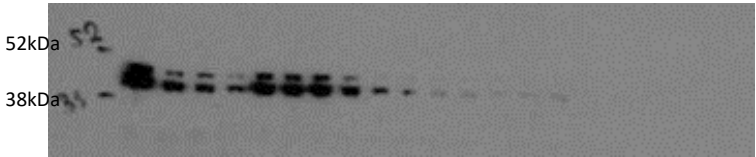


8 min exposure *

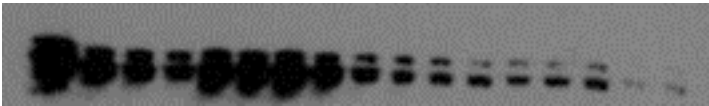


10 min exposure

pERK 42/44kDa



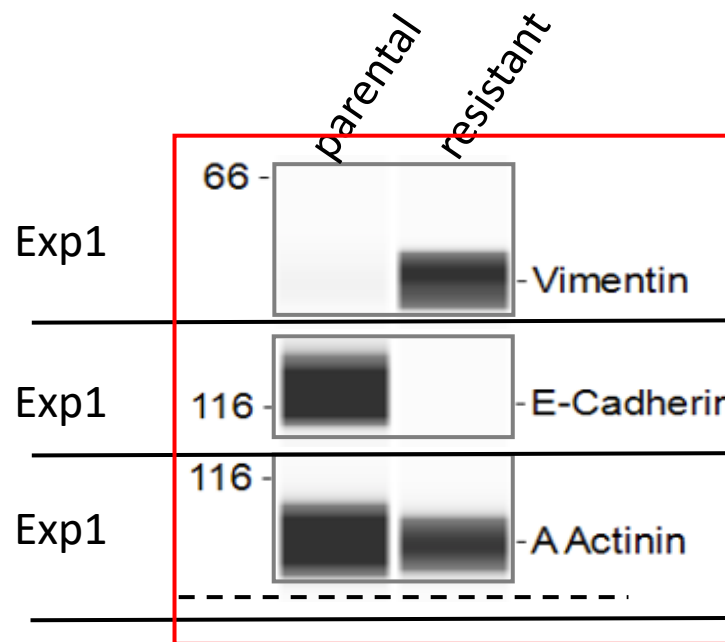
10sec exposure *



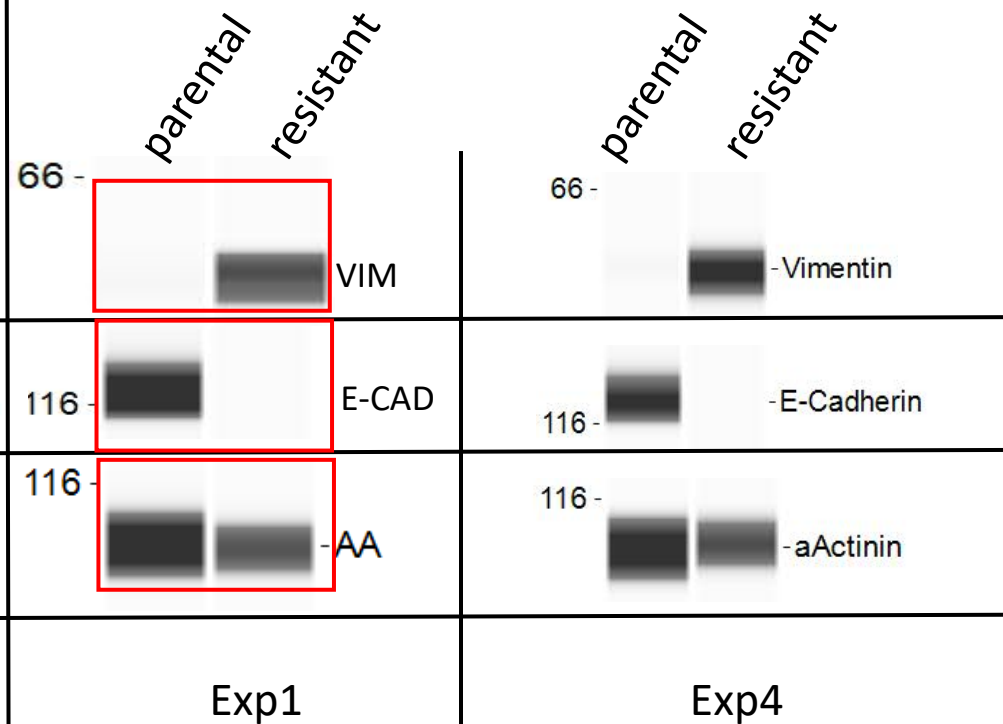
5 min exposure

Extended Data 7f

ED Fig 7f



Raw data



WES analysis: bands represents expression
seen in two experiments
EZ Standard Pack 12-230 kDa
Protein Simple #PS-ST01EZ-8

Data shown

