

FIGURE 3 PANEL B-LEFT (Hela)

Hela : 63-78 x 30 min

gel 1

DMSO 500nM 2 5uM 10uM 25uM 50uM

— — — — —

— — — — —

Exposure for
P-ACC used in
the paper
(2.5 min)
11.5.10

P-ACC



OK

-180

-60 PAMPK

-50

FIGURE 3 PANEL B - LEFT (Hela)

2

Hela HT 63-78 30 min
gel 2

4 5000 120 5 2500 5000

Exposure
used for
ACC and
RAPTOR
in the paper
(30 sec)
11.5.10

180 -

120 -

85 -

60 -

50 -

— — — — —

— — — — —

— — — — —

OK
ACC (2)

Raptor
OK

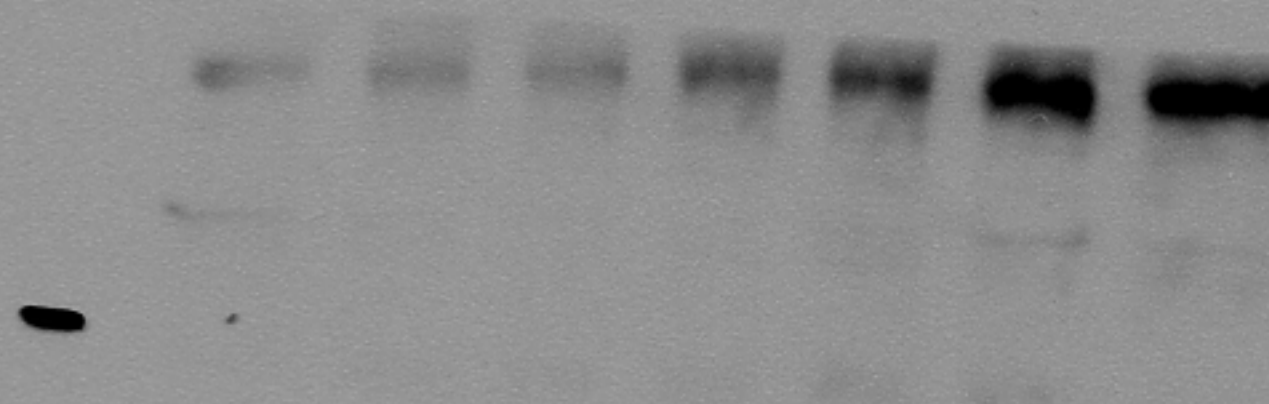
AMPK

FIGURE 3 PANEL B LEFT (Hela cells)

Exposure for
Ⓟ Raptor
used in the
paper (3 min)
11.6.2010

Ⓟ Raptor → 180
120

1 2 3 4 5 6 7



• Hela
OK

EC

3 min

11/6/2010

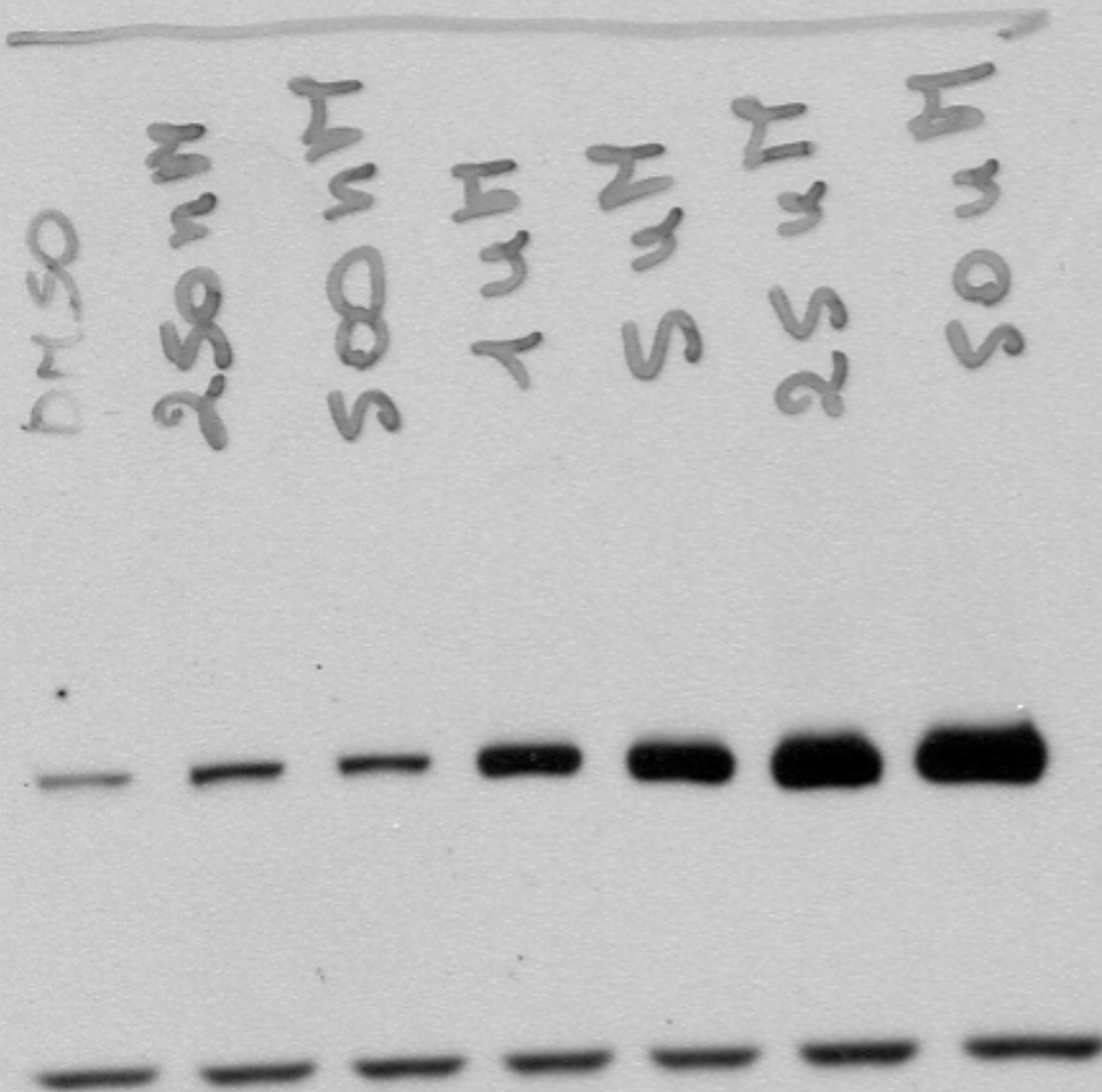
1 = 0
2 = 250 uN
3 = 500 uN
4 = 1 uN
5 = 15 uN
6 = 25 uN
7 = 50 uN

MT 62-38

FIGURE 3 PANEL B - RIGHT (Hela cell-)

Hela - 30 min

gel 1



Exposure for
p-AKPK used
in the paper
(5 min)
11.5.2010

p-ACC

- 186

- 66

- 50

p-AKPK ← • OK

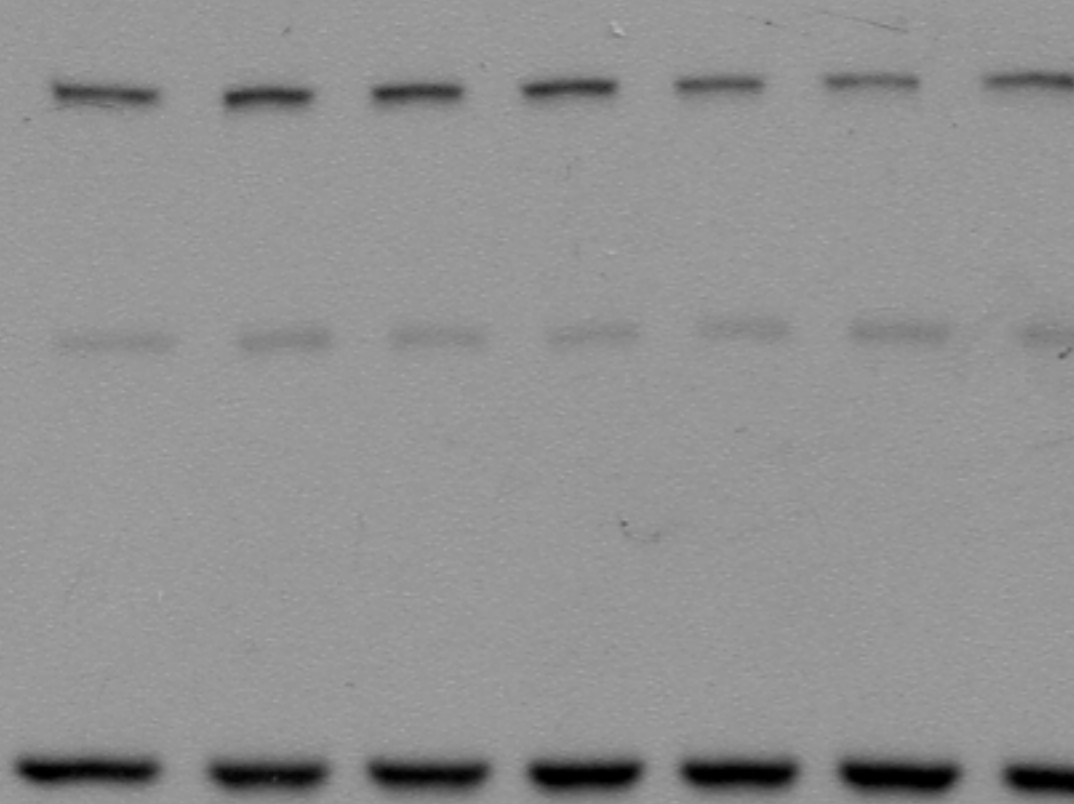
FIGURE 3 PANEL B - LEFT (Hela)

Hela: 63-78 x 30 min gel 2 (4)

PMSO
500M
10M
50M
100M
250M
500M

Exposure for
AMPK used
in the
paper
(5 sec) 11.5.10

190 -
120 -
85 -
60 -
50 -



Acc

Raptor

AMPK ● OK

FIGURE 3 PANEL B
RIGHT (Hela)

Exposure for
vinculin used
in the paper
(Spec)
4.10.10

120-



VINCULIN

(gel 1)

Hela - 30 min

HT 63-78

dmsO 250nM 500nM 1uM 5uM 25uM 50uM

Hela x 30 min (63-78)

- 30ug

2 Film / Spec
ECL
11.10.10

OK
re-blot

FIGURE 3 PANEL B RIGHT (DU145)

Exposure
for

Ⓟ ACC

used in
the paper

(10 sec)

du145
250uH
500uH
1uH
5uH
25uH
50uH

gel 1

180
+

— — — — —

Ⓟ ACC.

OK

64.

— — — — —

Ⓟ ANPK

DUI45 - 30min

MT 63-78

Raptor total
ACC to E

3

cut at HARKER

180
180
120

dsup
250nM
500nM
1uM
5uM
25uM
50uM

gel 2

• ACC total OK

• Raptor total OK

FIGURE 3 PANEL B - RIGHT
(DUI45) Exposure for ACC and RAPTOR
7.9.13

FIGURE 3 PANEL B - RIGHT (DU145)

exposure for $\textcircled{P}$ Raptor
used in the paper
(4 min) 7.9.10

DU145

HT 63-78 30 min

cut it smaller

120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400
410
420
430
440
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460
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580
590
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610
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630
640
650
660
670
680
690
700
710
720
730
740
750
760
770
780
790
800
810
820
830
840
850
860
870
880
890
900
910
920
930
940
950
960
970
980
990
1000

duiso
250nm
500nm
1um
5um
25um
50um

gel 1

P-ACC

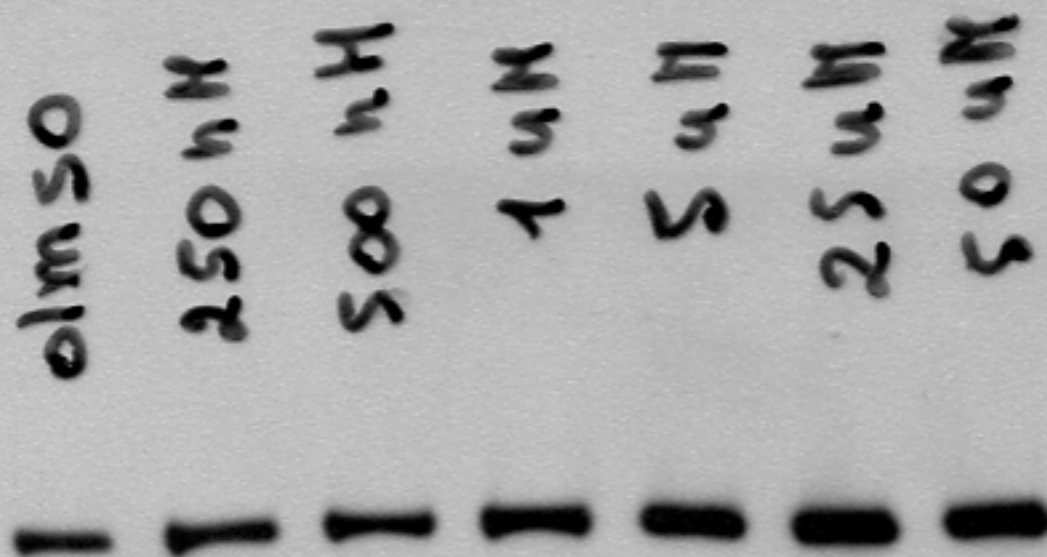
$\textcircled{P}$ -Raptor 2.0
OK

FIGURE 3 - PANEL B

RIGHT (DUI45)

exposure for
 (P) AMPK used
 in the
 paper (30 sec)
 7.9.2010

DUI45 gel 1
 HT6 3-78 (30 min)



(P) ACC(2)

64 -



● (P) AMPK OK

FIGURE 3
PANEL B RIGHT
(DU145)

AMPK tot AMPKtot
① 46BP1 ①
② ALL

DU145 - 30 min

HT 63-78

gel 2

Exposure for
AMPK total used
in the paper (10sec)

7.09.10

dms0
250nM
500nM
1 μ M
5 μ M
25 μ M
50 μ M

— — — — —

AMPK tot

120
64
49

FIGURE 3 PANEL B

RIGHT - DU145

Exposure for
vinculin used in
the paper (1 sec)
7.12.10

120 -



re-blot on
vinculin (P-Rap Mem)
gel 1

- 120



re-blot on
vinculin (Rap Mem) • OK
gel 2

1 2 3 4 5 6 7,
DU145 - 30 min

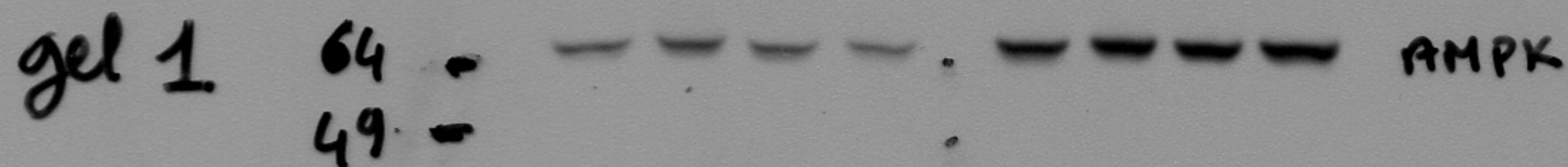
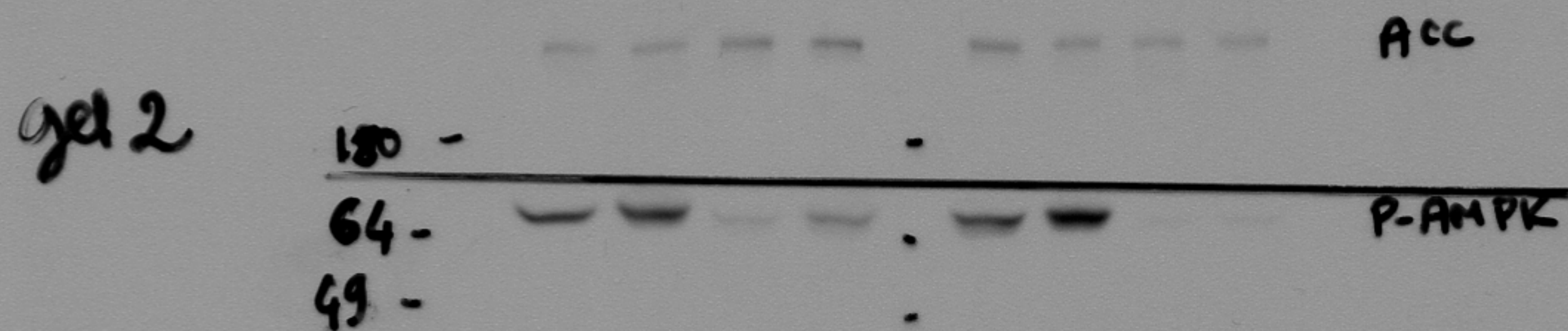
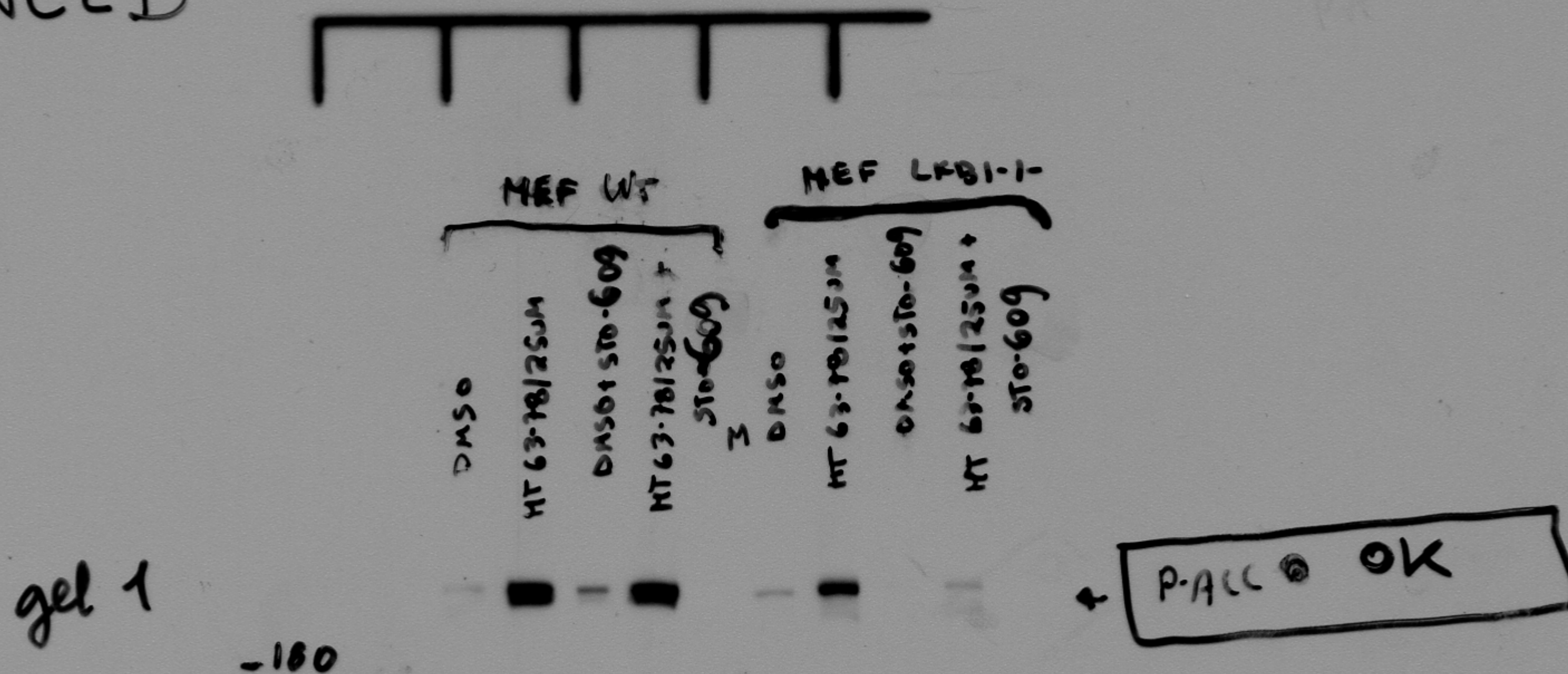
- 1 = dms0
- 2 = 250 nM
- 3 = 500 nM
- 4 = 1 uM
- 5 = 5 uM
- 6 = 25 uM
- 7 = 50 uM

HT63-78

quick
ECL
7/12/10

FIGURE 3 PANEL D

Exposure for
P-ACC used in
the paper (12)



Samples were prepared in duplicate and
loaded on 2 gels (gel 1 and 2)

-25ug

-8/17/2010 samples

quick
ECL
8.24.10

FIGURE 3 PANEL D

Exposure for
ACC used in
the paper
(30 sec)

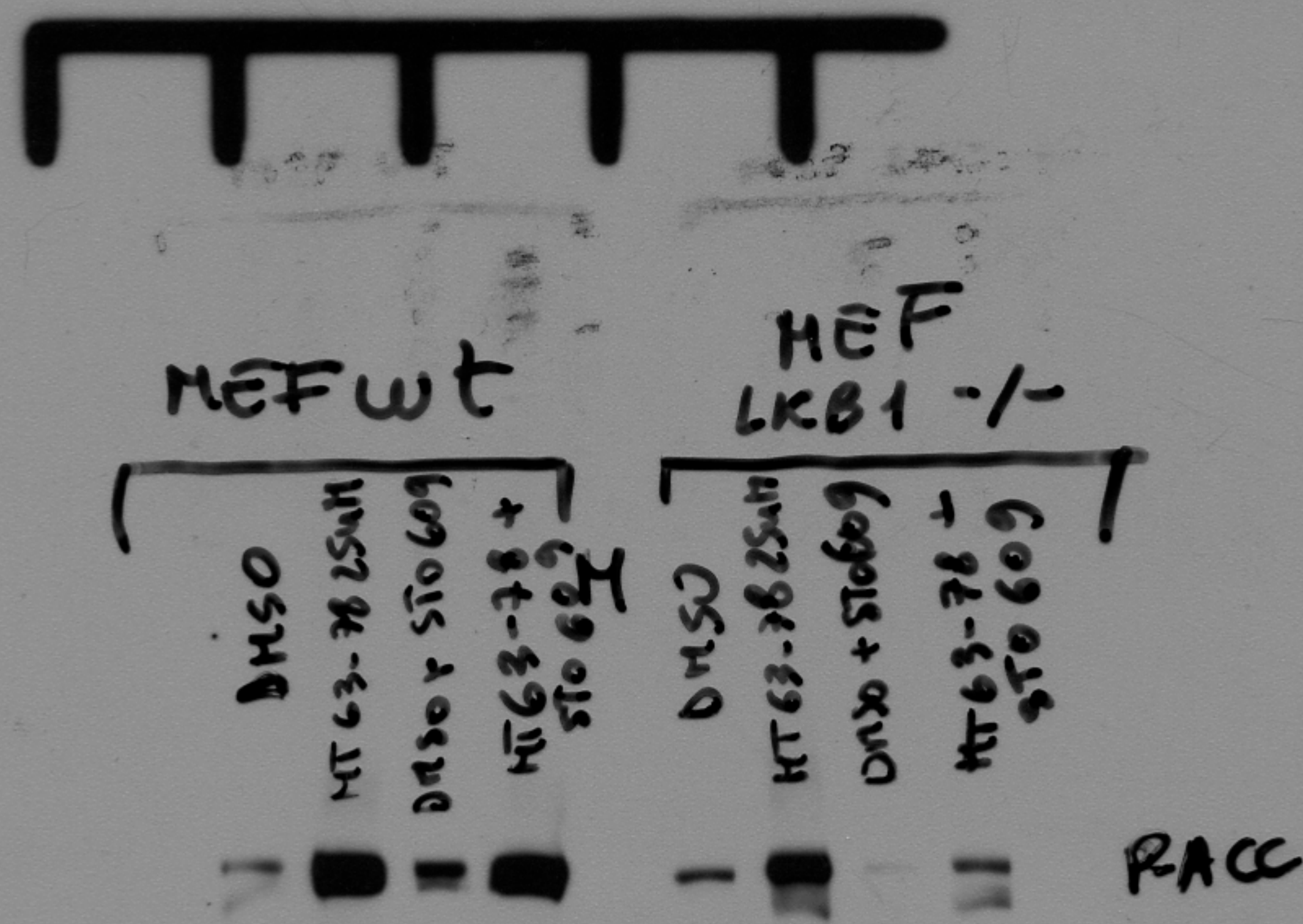
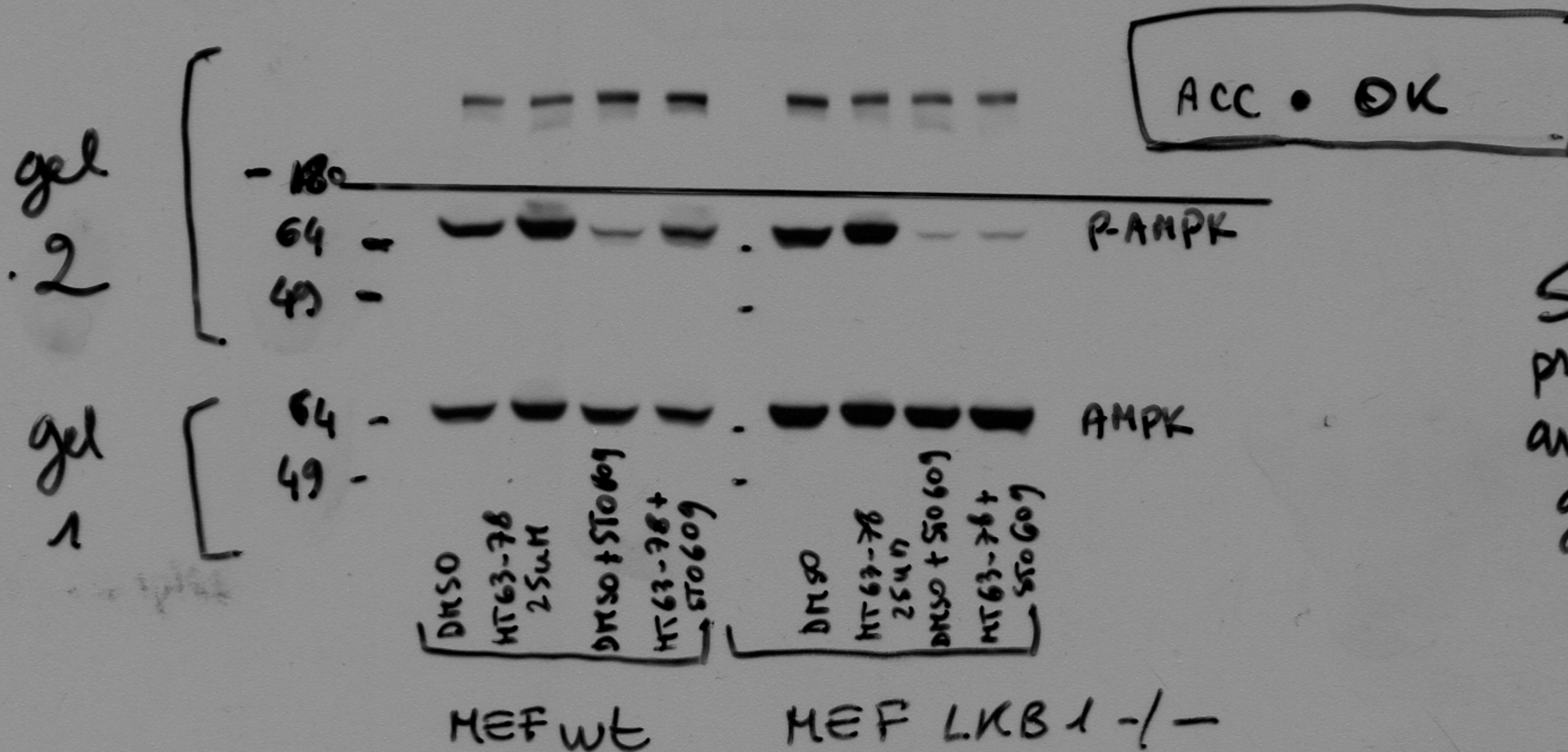


FIGURE 3
PANEL D

Exposure for
ACC used in
the paper
(30 sec)



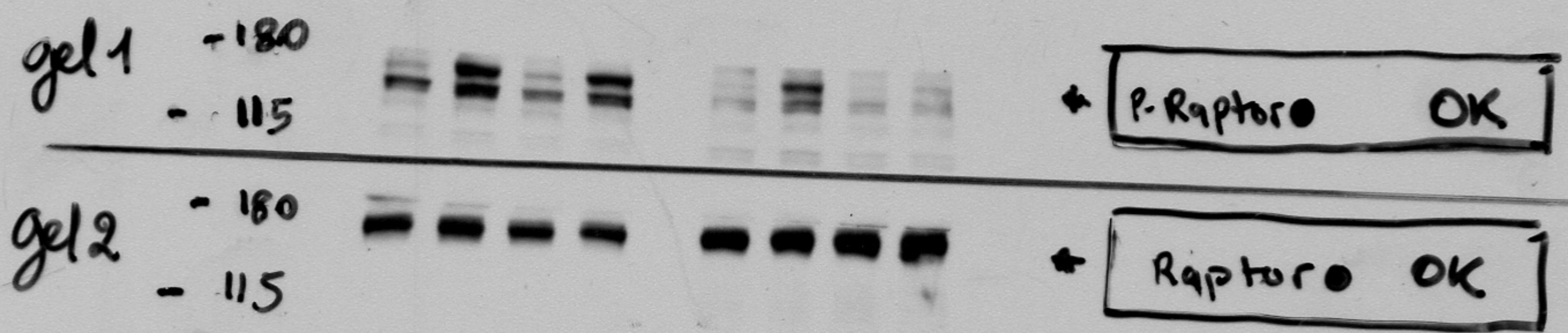
Samples were
prepared in duplicate
and loaded on 2
gels (gel 1 and 2)

FIGURE 3 PANEL D

Exposure for:
 P-Raptor and
 Raptor used in
 the paper
 (5min)



3



- 25ug
 - 8/17/2010 samples

Samples were prepared in duplicate and
 loaded on 2 gels (gel 1 and 2)

5min
 ECL
 8.24.10

- 25ug
 - 8/17/2010 samples

Samples were prepared in duplicate and
 loaded on 2 gels (gel 1 and 2)

5min
 ECL
 8.24.10

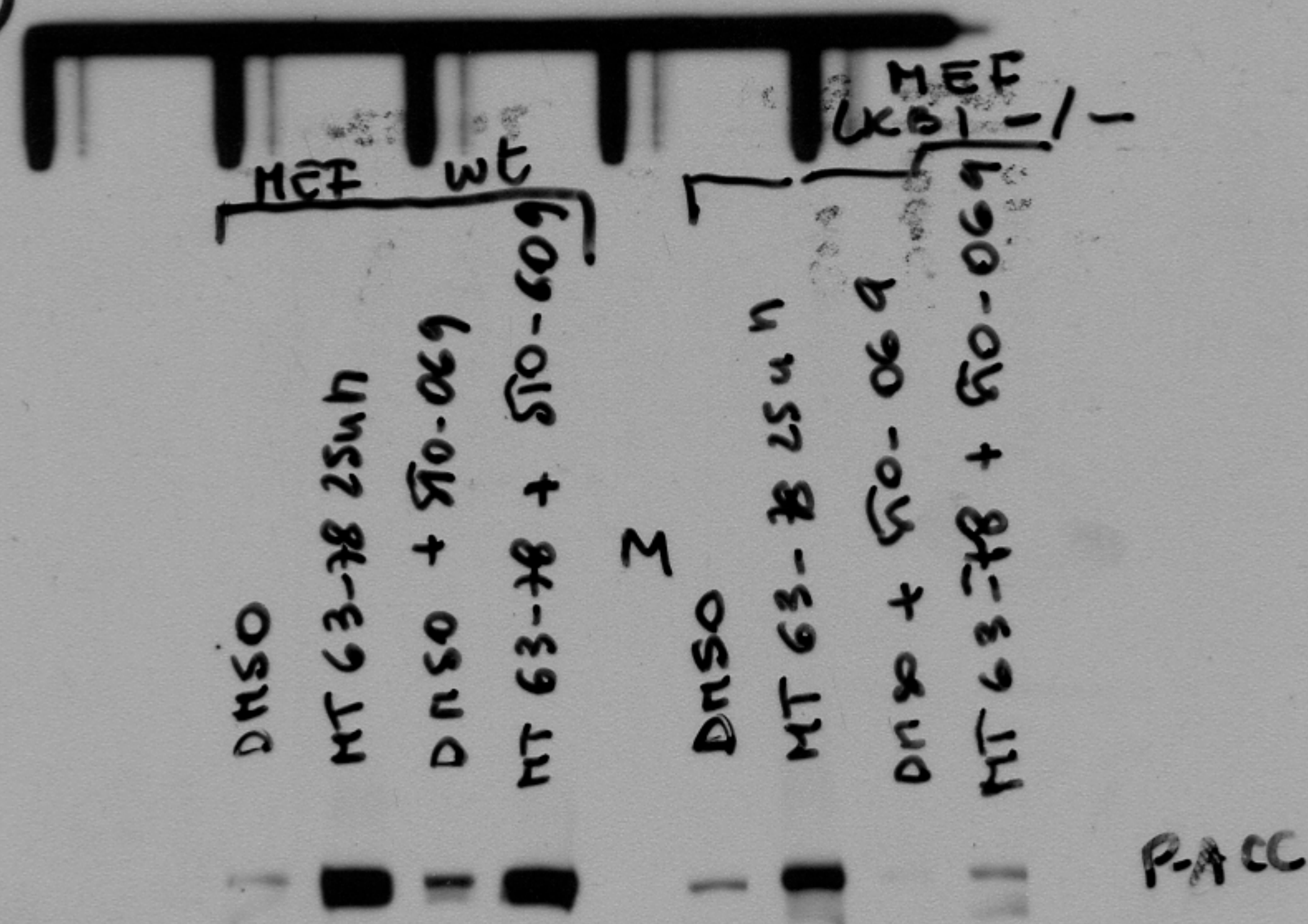
FIGURE 3, PANEL D

2

Exposure for
• P-AMPK
• AMPK used in
the paper (10 sec)

gel 1

180 -



gel 2

-180

-64

-49

ACC

* P-AMPK OK

gel 1

-60

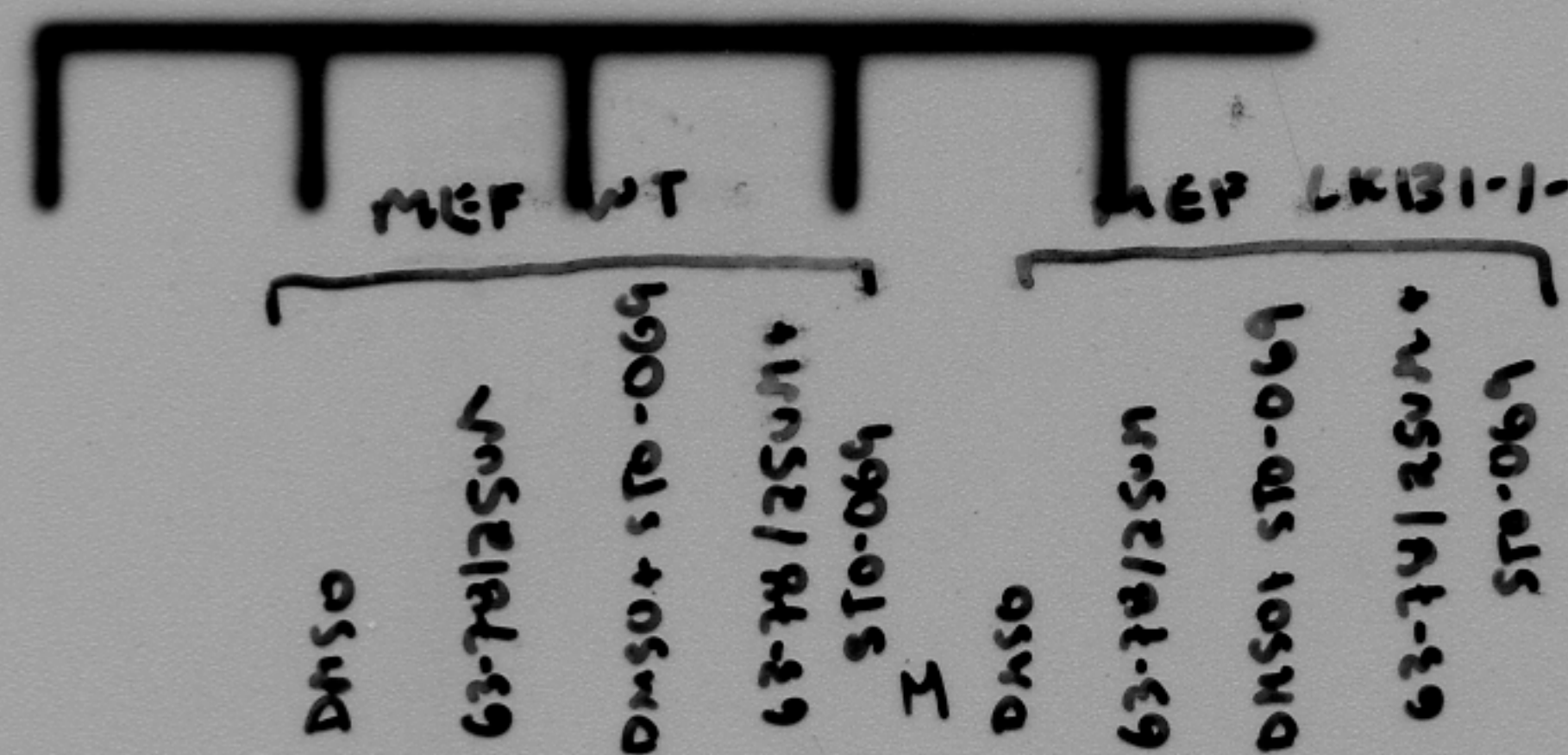
-50

* AMPK OK

Samples were prepared in duplicate and
loaded on 2 gels (gel 1 and 2)

FIGURE 3 PANEL D

Exposure for
vinculin
used in the
paper (5sec)



(S)

Re-blot

gel 1 - 115

VINCULIN re-blot on
P-Raptor Membrane

● OK

gel 2 115

VINCULIN re-blot on
Raptor membrane

- 25ug
- 8/17/2010 samples

Samples were prepared in duplicate and loaded
on 2 gels (gel 1 and 2)

- 25ug

- 8/17/2010 samples

2 films/5sec
EU
8.25.10