

Supplementary Information

Role of phosphatase activity of soluble epoxide hydrolase in regulating simvastatin-activated endothelial nitric oxide synthase

Hsin-Han Hou, Yi-Jen Liao, Sheng-Huang Hsiao, Song-Kun Shyue, Tzong-Shyuan Lee

Fig S3. Role of sEH phosphatase in regulating simvastatin-induced eNOS phosphorylation.

Ⓟ eNOS1179
Veh

Simvastatin 0 5 10 15 30 60 120

Ⓟ eNOS1179

Ⓟ Akt

Ⓟ Akt

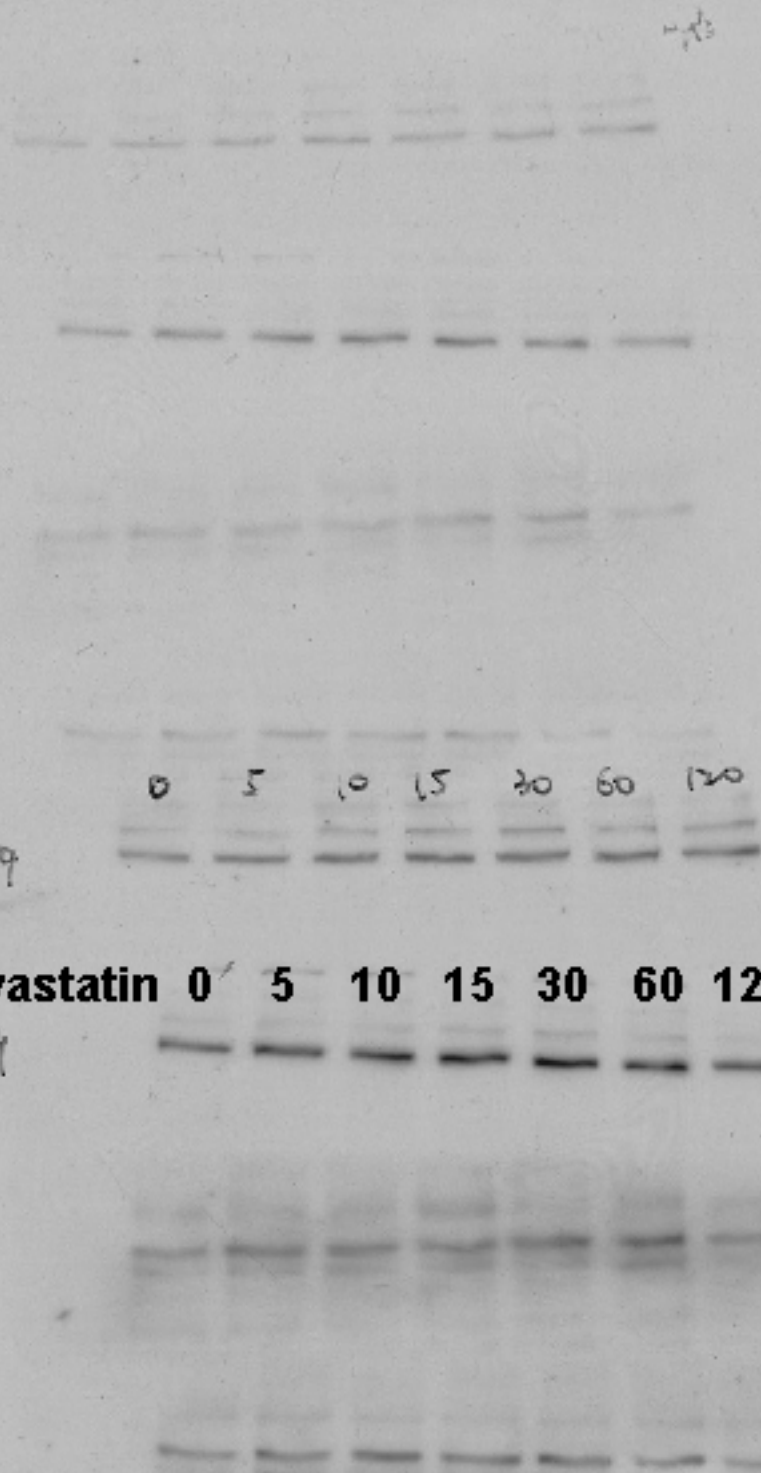
0 5 10 15 30 60 120

E₂

p-eNOS

E₂

Simva



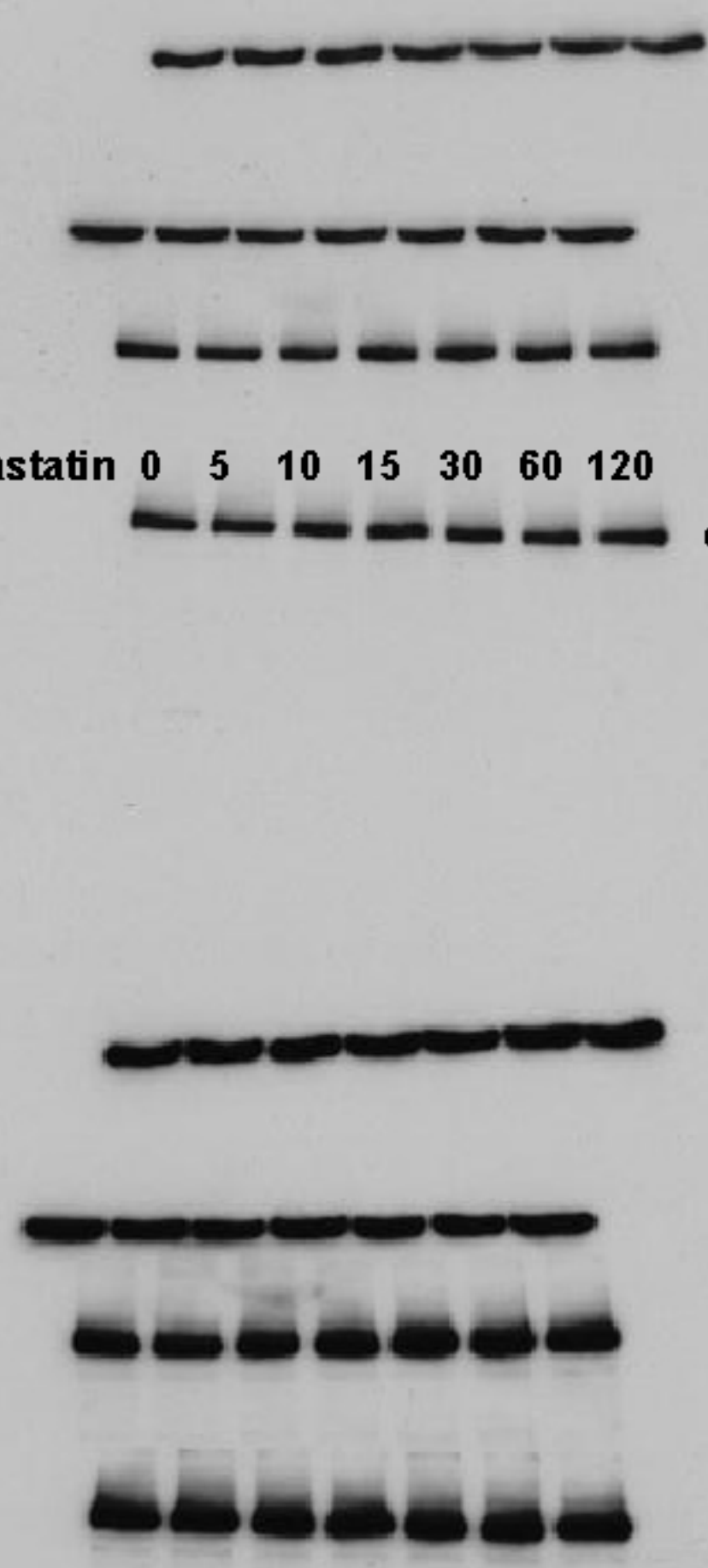
Veh

Simvastatin 0 5 10 15 30 60 120

tub

eNOS

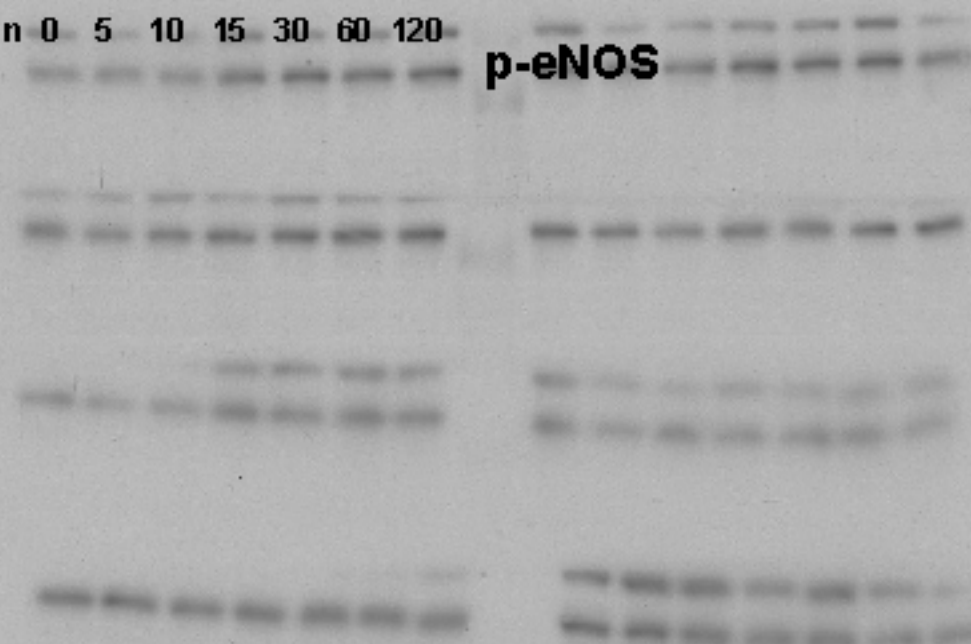
eNOS

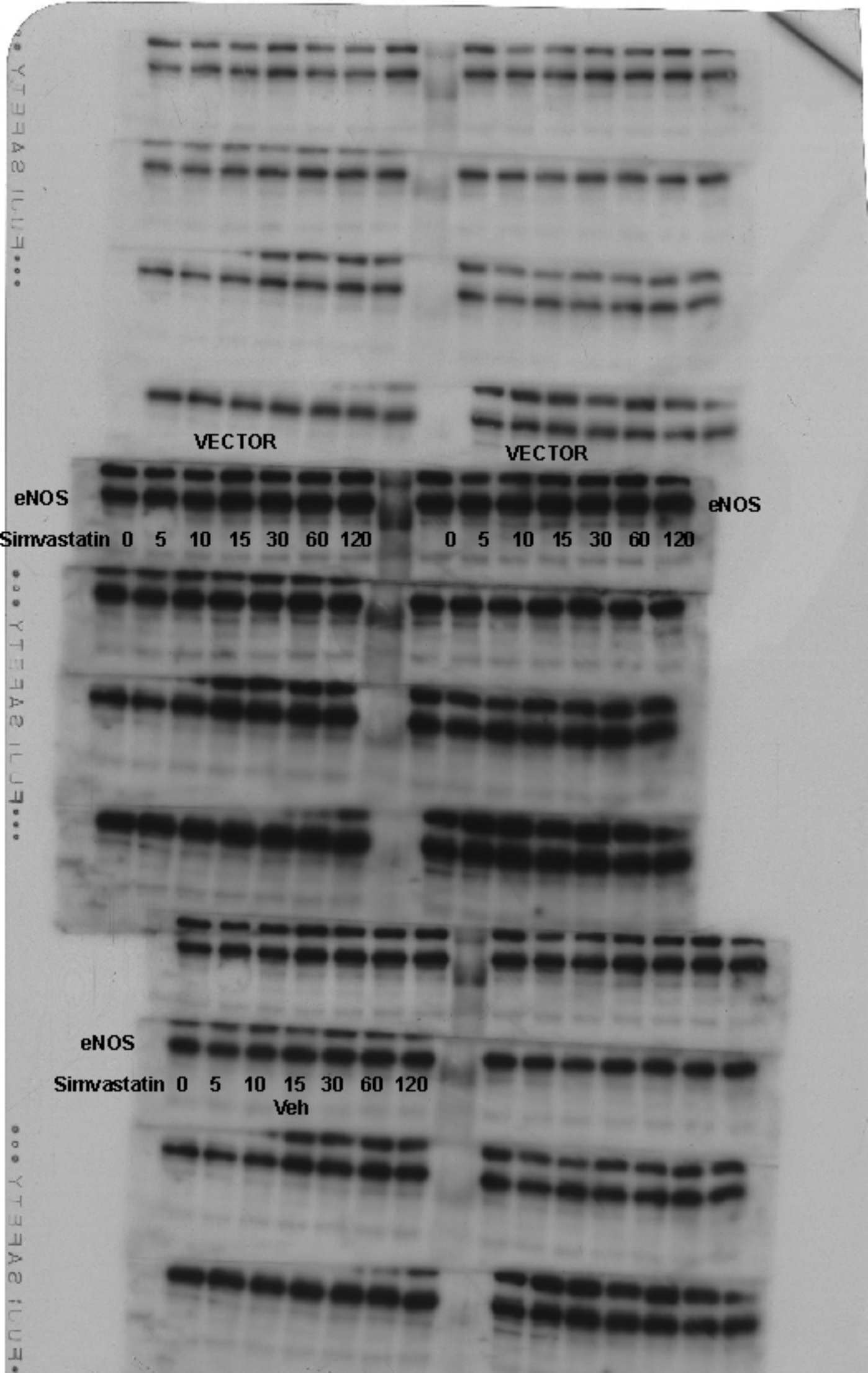


AFC

Simvastatin 0 5 10 15 30 60 120

p-eNOS





VECTOR

VECTOR

eNOS

eNOS

Simvastatin 0 5 10 15 30 60 120

0 5 10 15 30 60 120

eNOS

Simvastatin 0 5 10 15 30 60 120
Veh

AUDA

Simvastatin 0 5 10 15 30 60 120



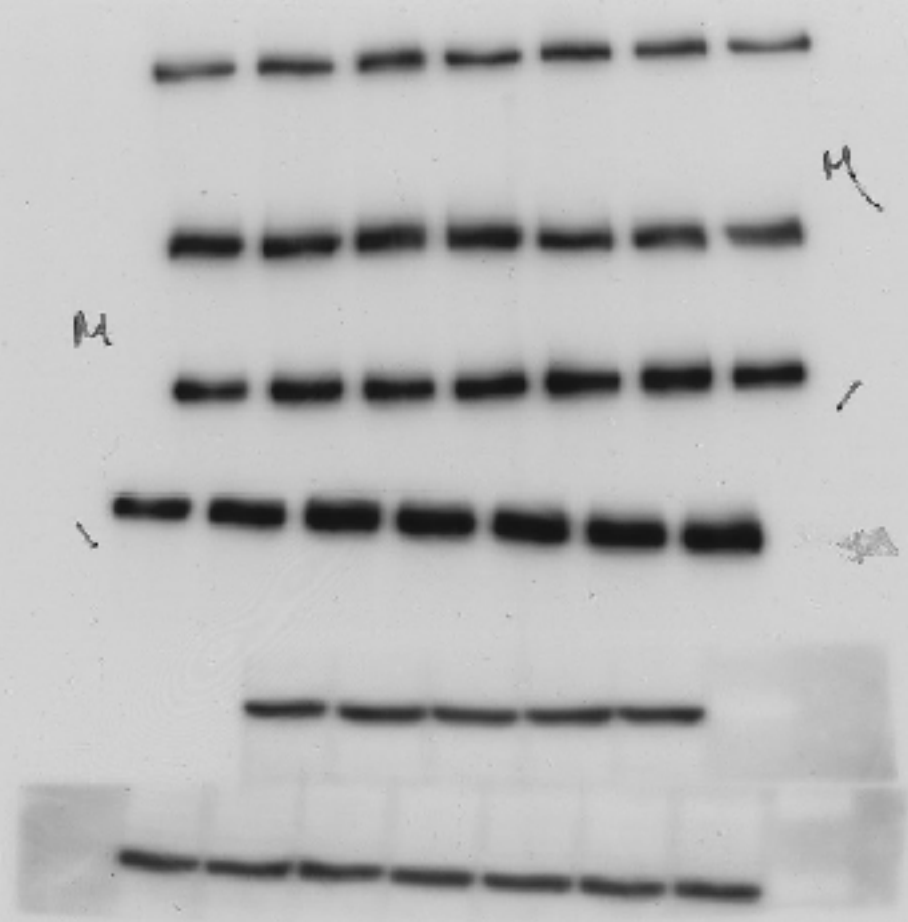
Western blot analysis showing protein levels of p-eNOS, AFC, and AUDA in response to Simvastatin treatment. The blot is divided into three main sections. The top section shows p-eNOS and AFC levels, with p-eNOS levels increasing and AFC levels decreasing over time. The middle section shows AFC levels, which remain relatively constant. The bottom section shows AUDA levels, which increase over time. Molecular weight markers are indicated on the right side of the blot.

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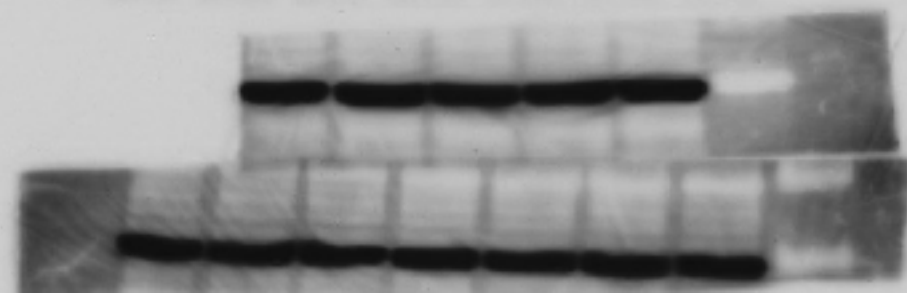
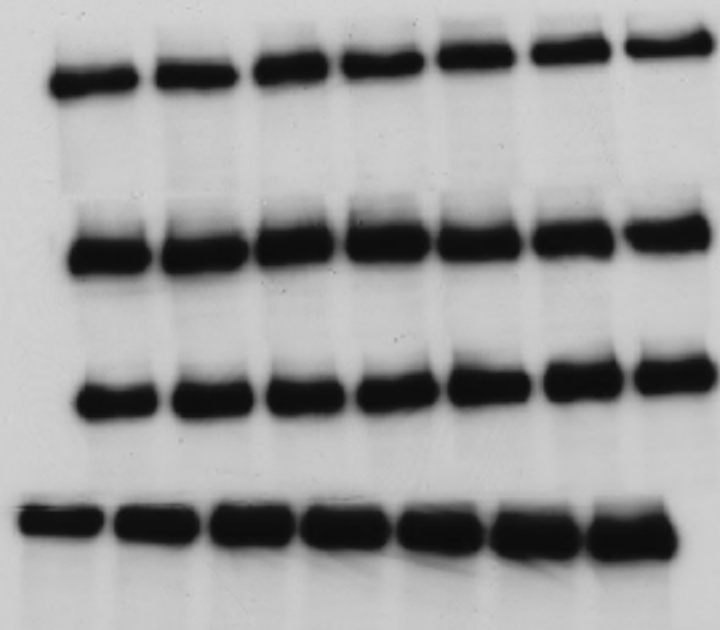
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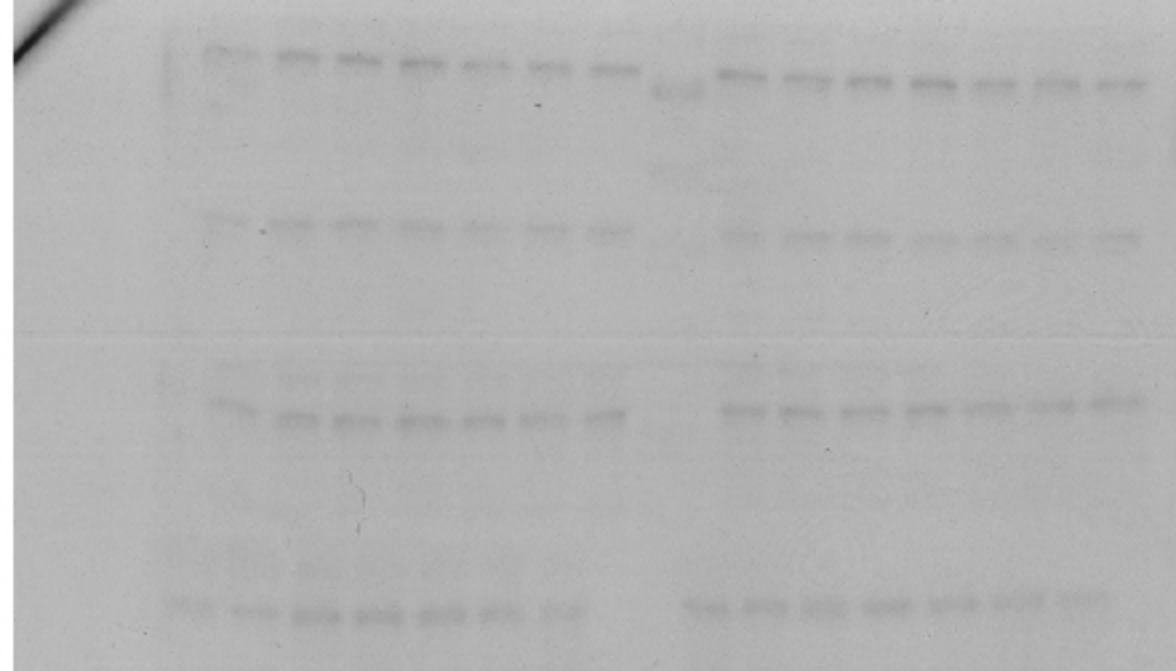
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Simvastatin 0 5 10 15 30 60 120 eNOS

AUDA





VECTOR

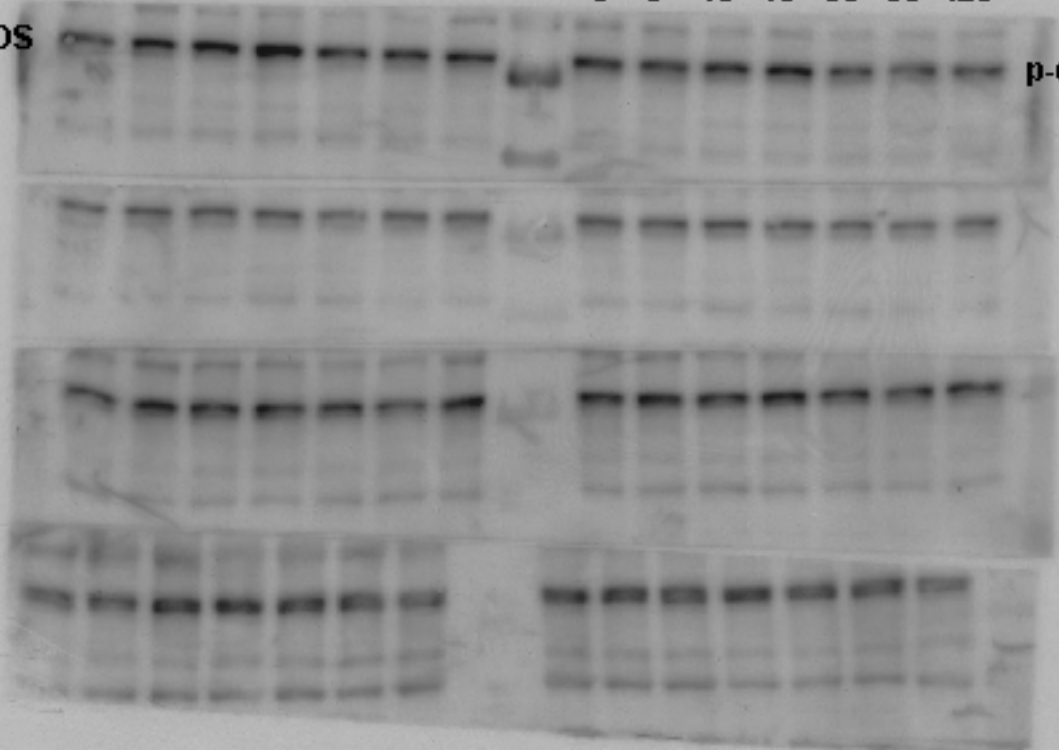
VECTOR

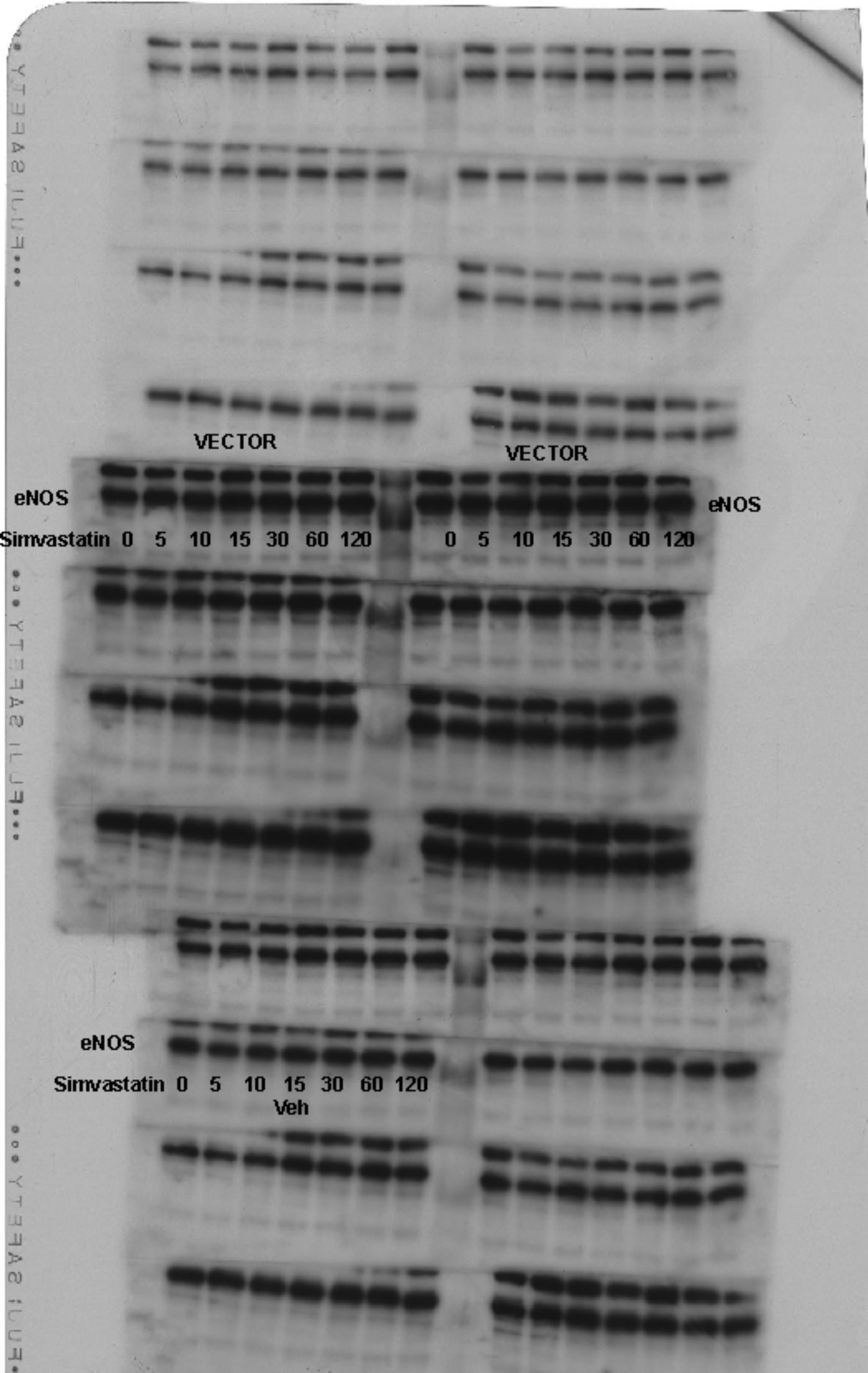
Simvastatin 0 5 10 15 30 60 120

0 5 10 15 30 60 120

p-eNOS

p-eNOS





VECTOR

VECTOR

eNOS

eNOS

Simvastatin 0 5 10 15 30 60 120

0 5 10 15 30 60 120

eNOS

Simvastatin 0 5 10 15 30 60 120
Veh



Simvastatin

0

5

10

15

30

60

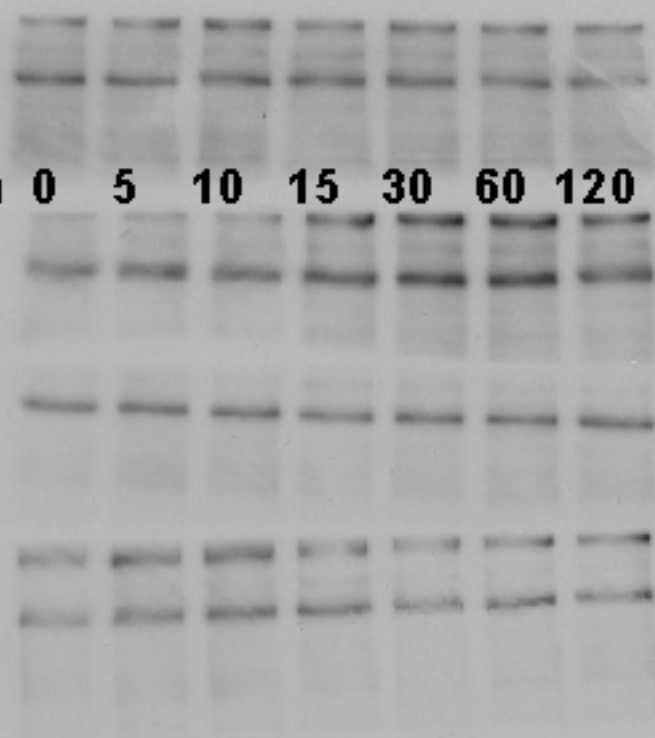
120

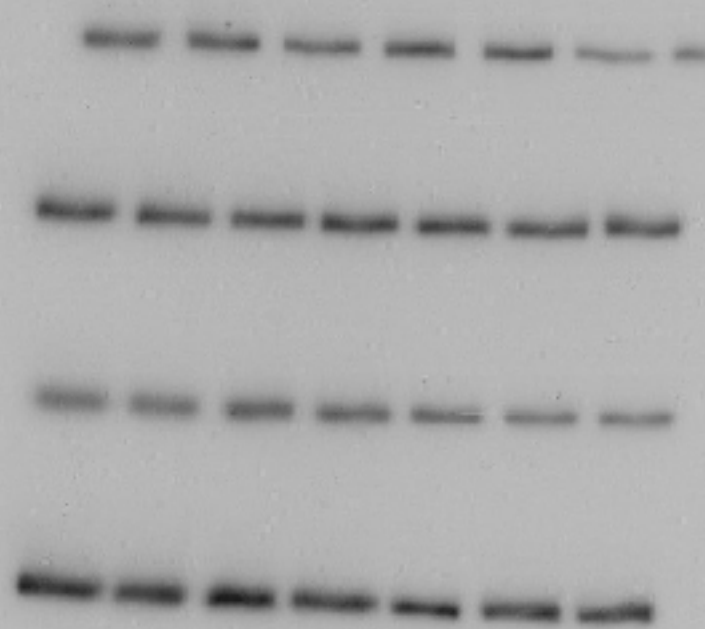
PTmut

N-ter

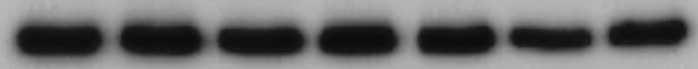
p-eNOS

p-eNOS





Simvastatin 0 5 10 15 30 60 120



PTmut eNOS



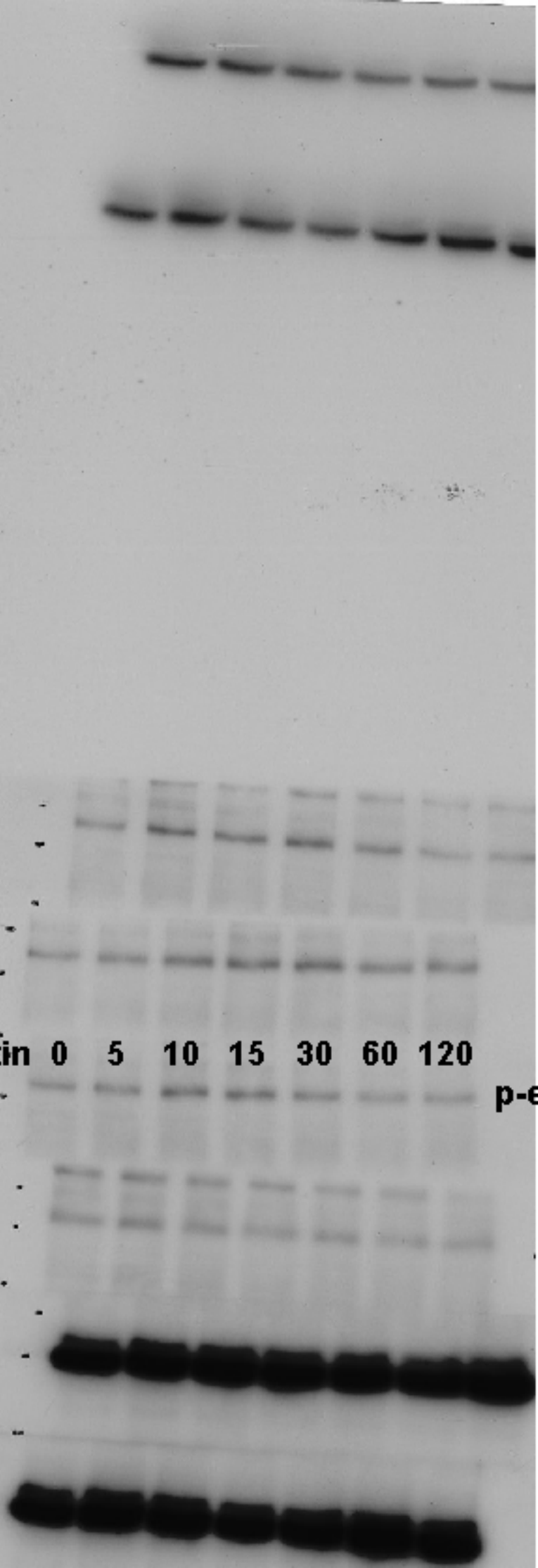
N-ter eNOS



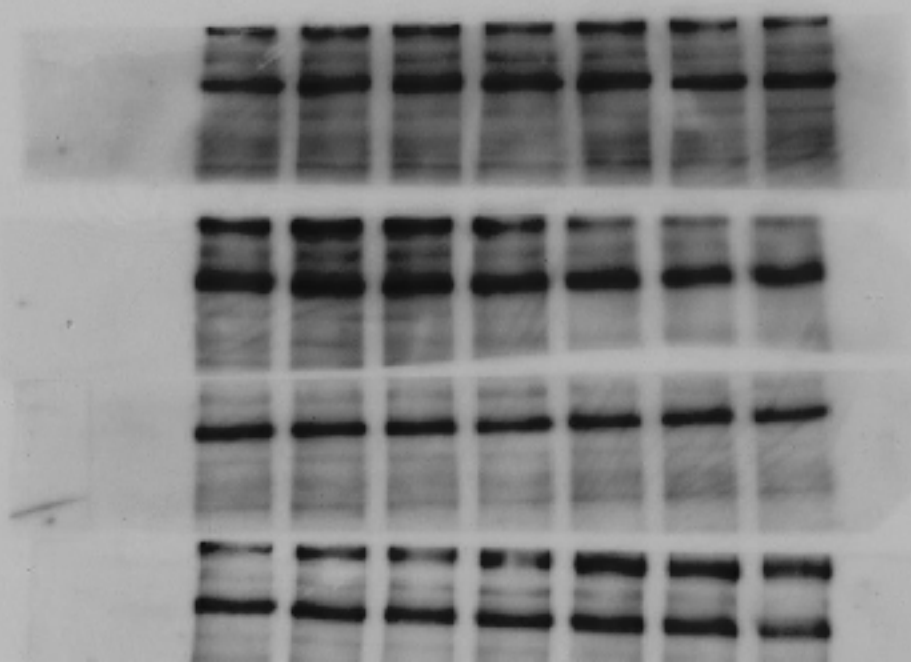
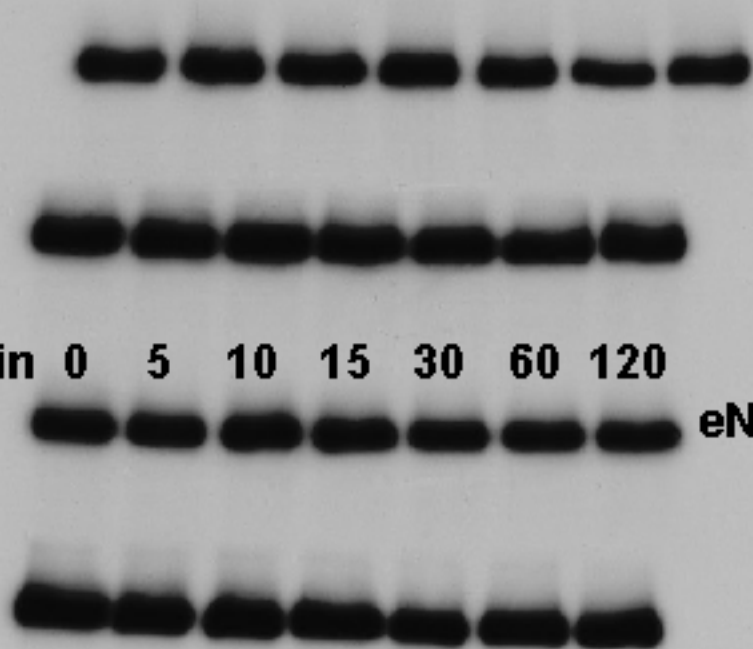
Simvastatin 0 5 10 15 30 60 120

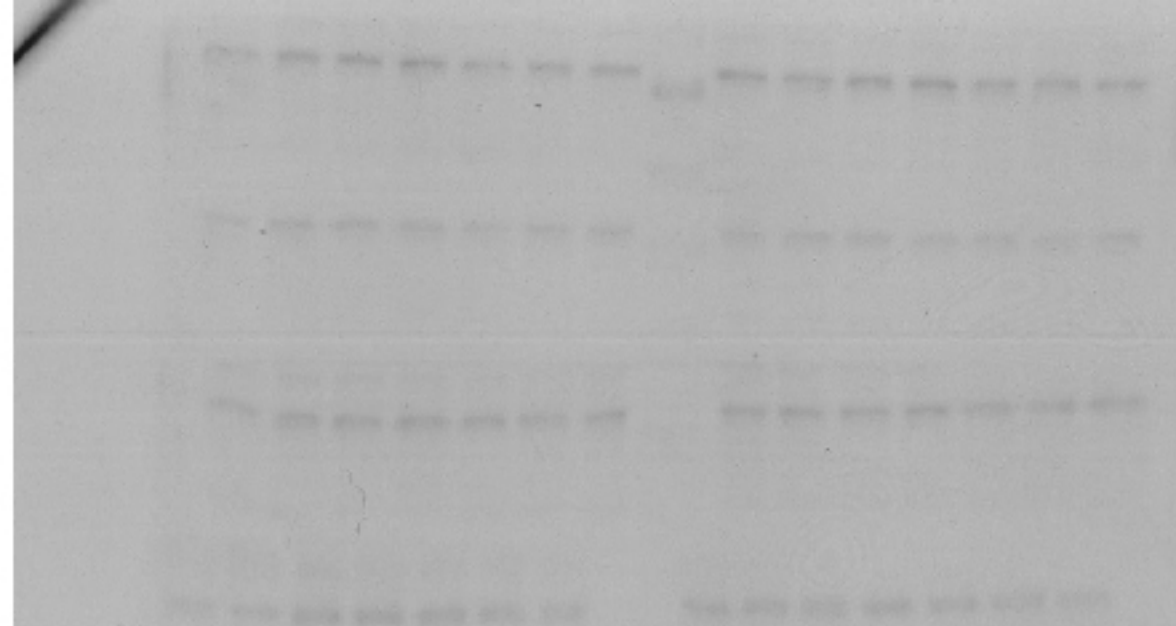
EHmut

p-eNOS



Simvastatin 0 5 10 15 30 60 120
EHmut eNOS





VECTOR

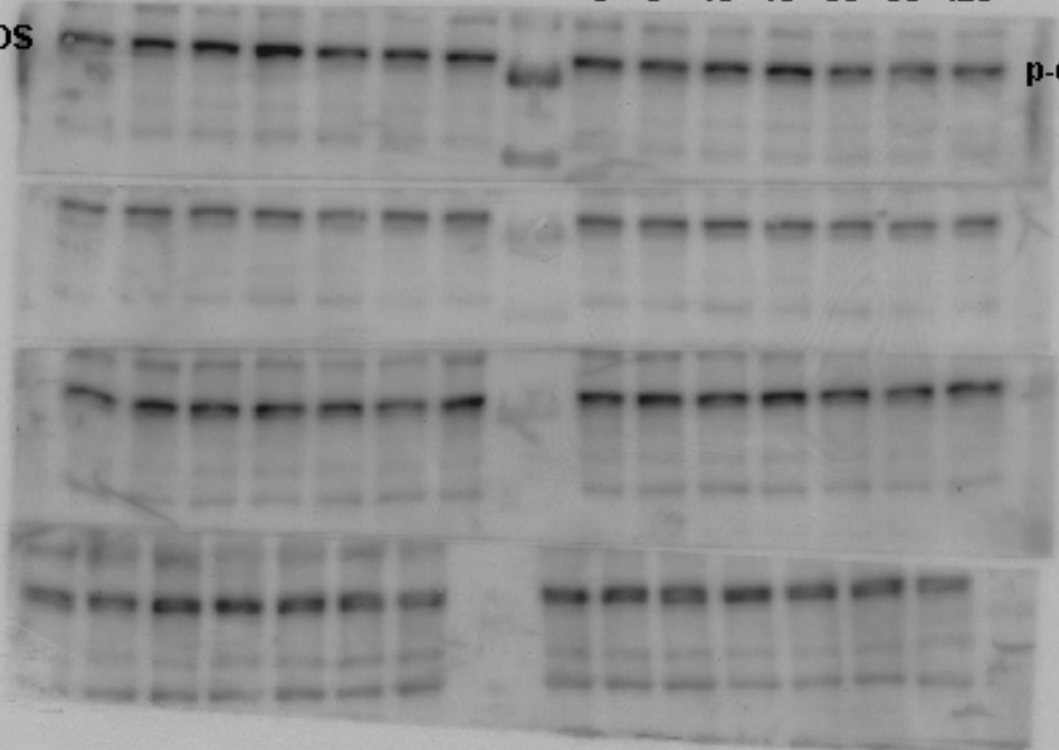
VECTOR

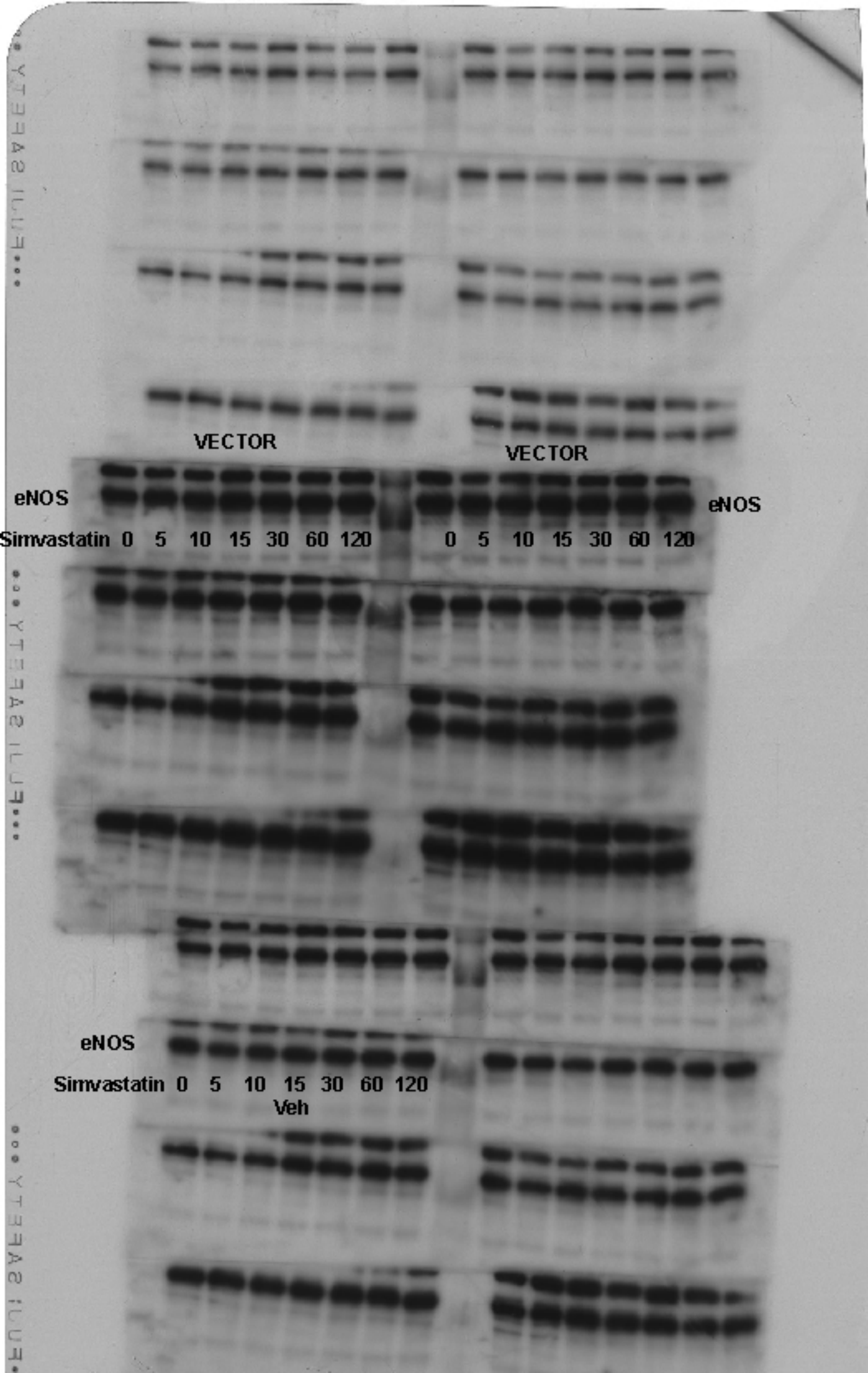
Simvastatin 0 5 10 15 30 60 120

0 5 10 15 30 60 120

p-eNOS

p-eNOS





VECTOR

VECTOR

eNOS

eNOS

Simvastatin 0 5 10 15 30 60 120

0 5 10 15 30 60 120

eNOS

Simvastatin 0 5 10 15 30 60 120
Veh



Simvastatin

0

5

10

15

30

60

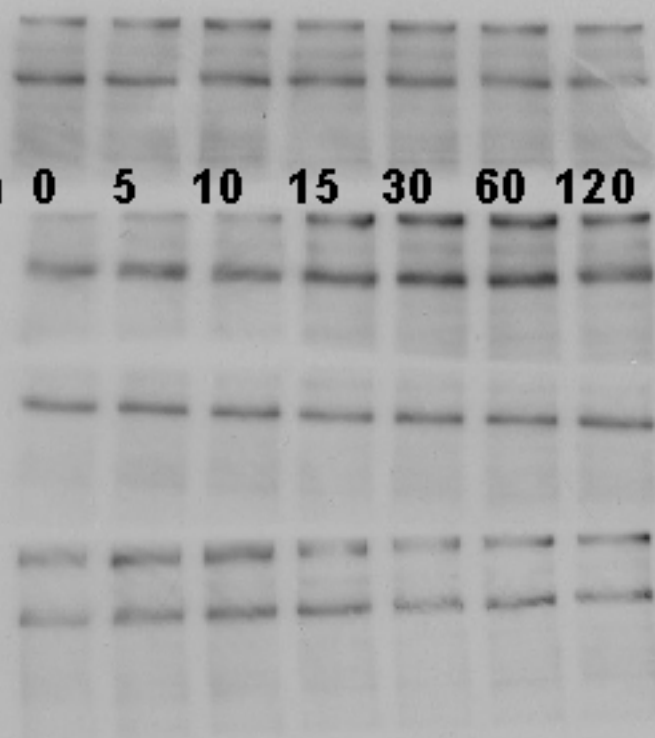
120

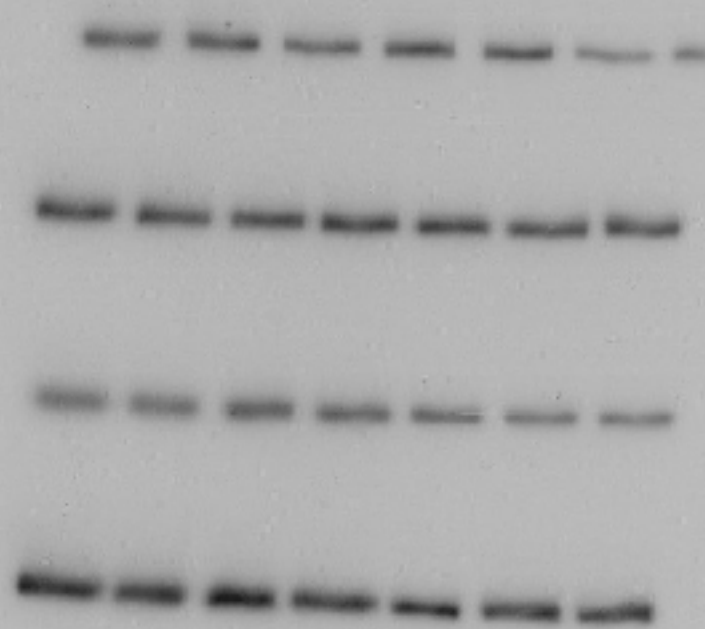
PTmut

N-ter

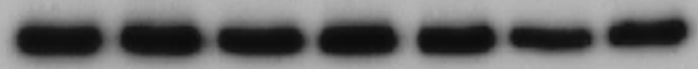
p-eNOS

p-eNOS





Simvastatin 0 5 10 15 30 60 120

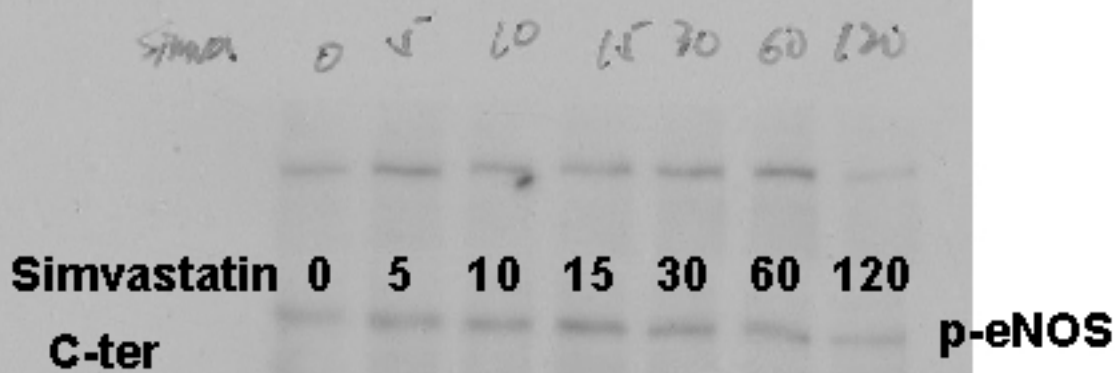


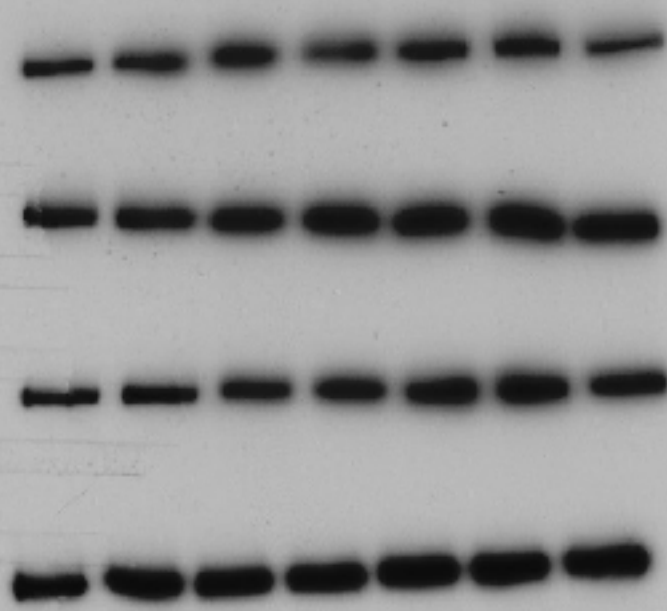
PTmut eNOS



N-ter eNOS







Simvastatin 0 5 10 15 30 60 120
C-ter **eNOS**

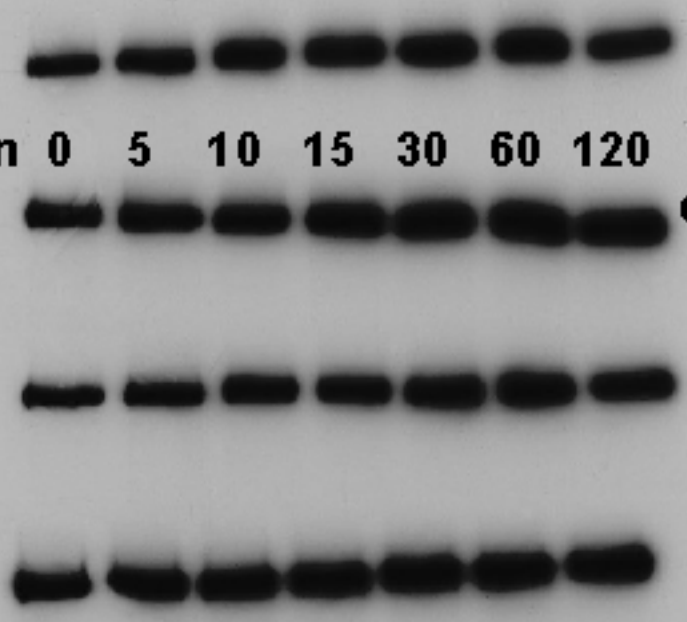
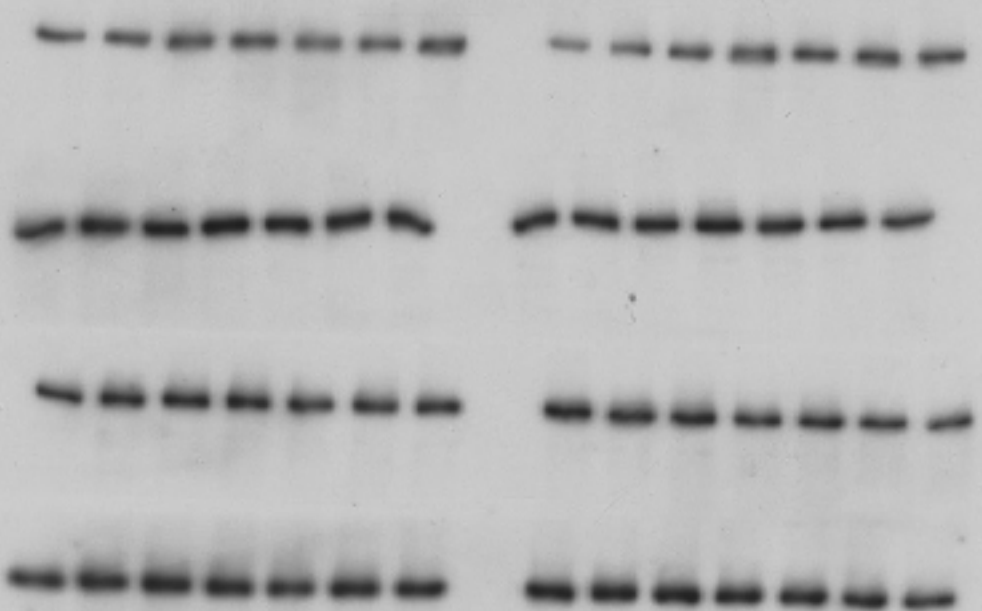
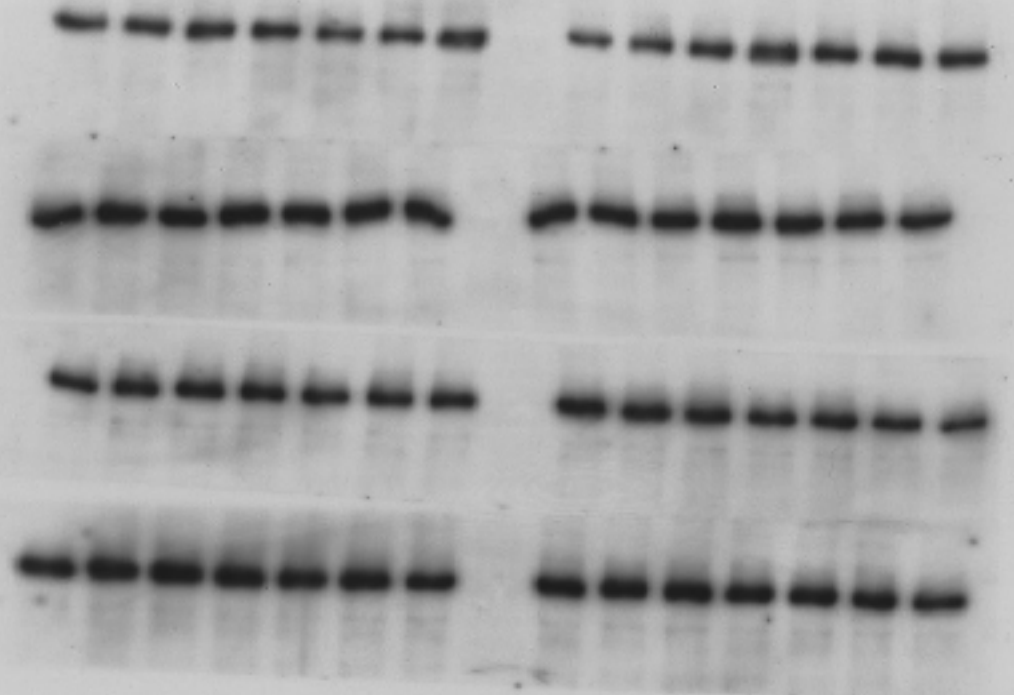


Fig S4. sEH phosphatase plays a crucial role in regulating simvastatin-induced Akt and AMPK phosphorylation.



AFC

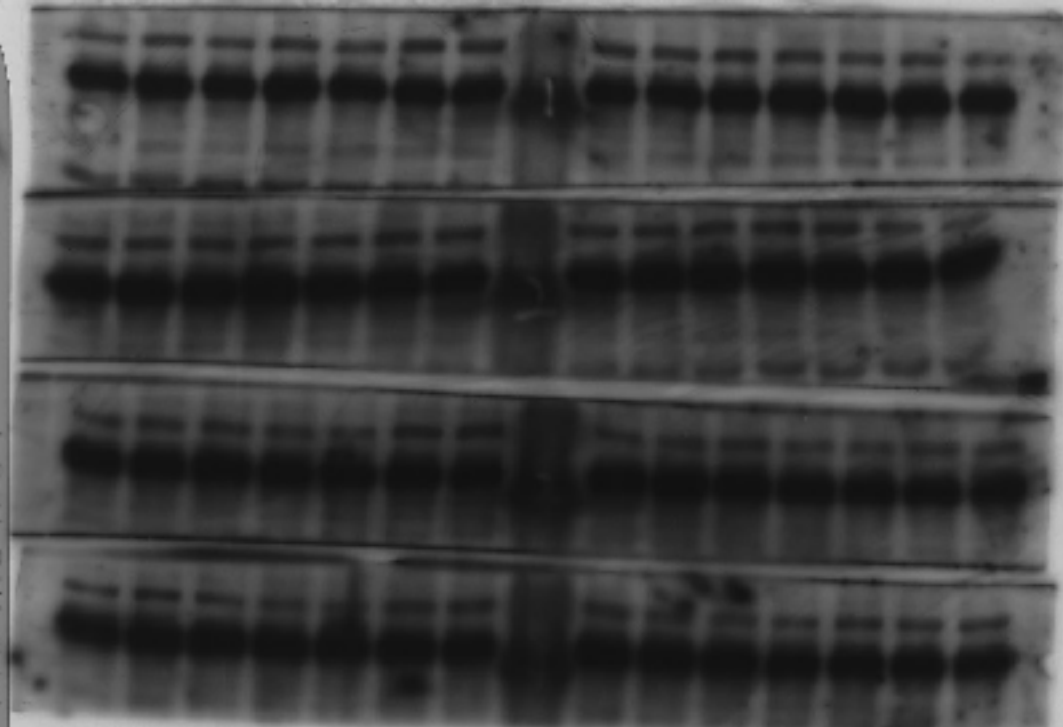
Simvastatin 0 5 10 15 30 60 120

p-Akt

Veh

Simvastatin 0 5 10 15 30 60 120

p-Akt



AFC

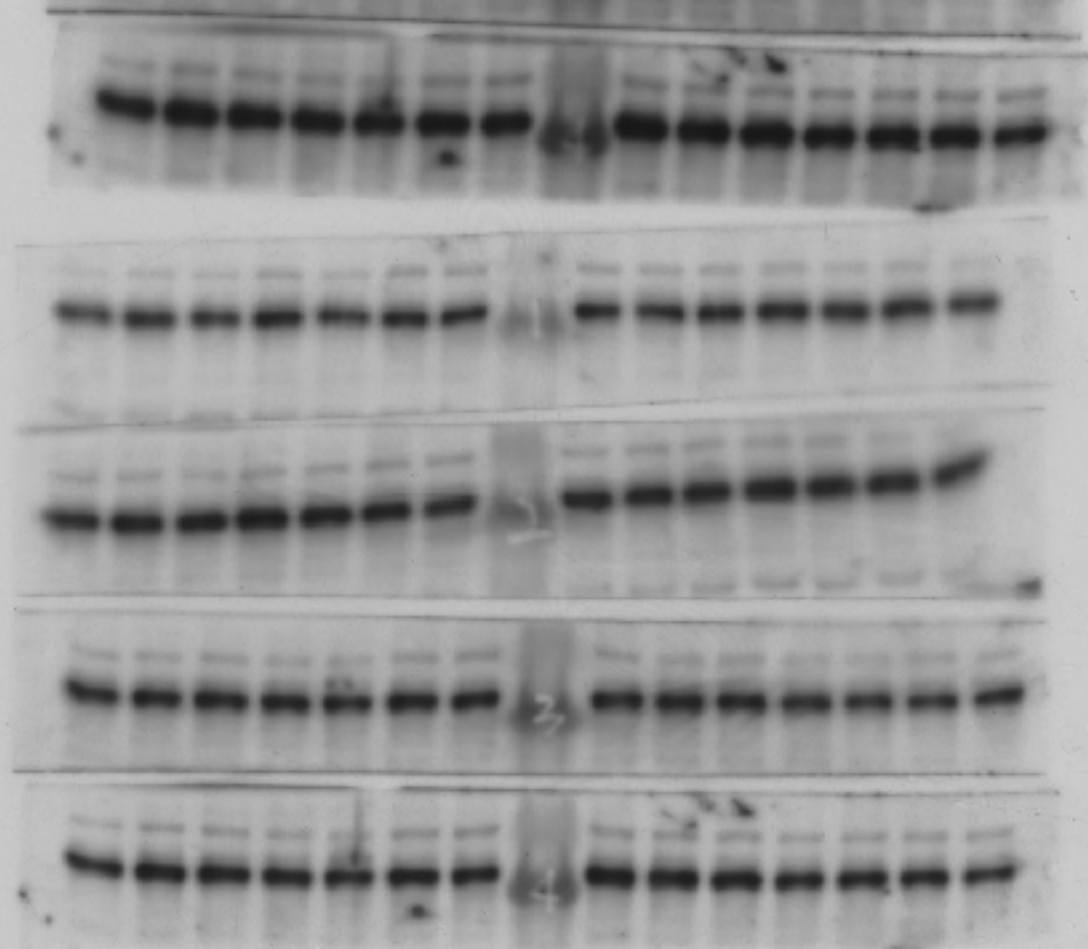
Simvastatin 0 5 10 15 30 60 120

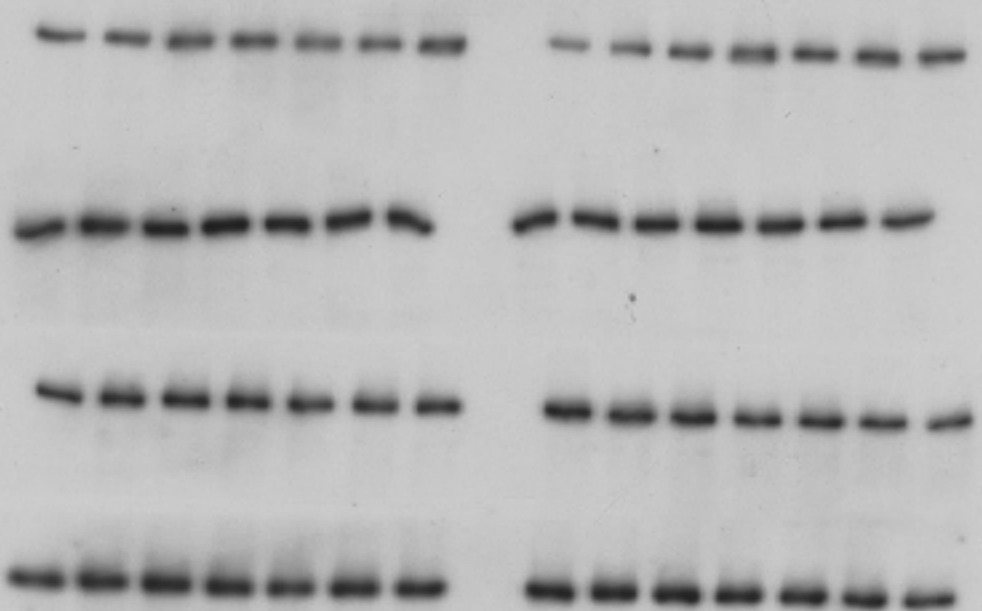
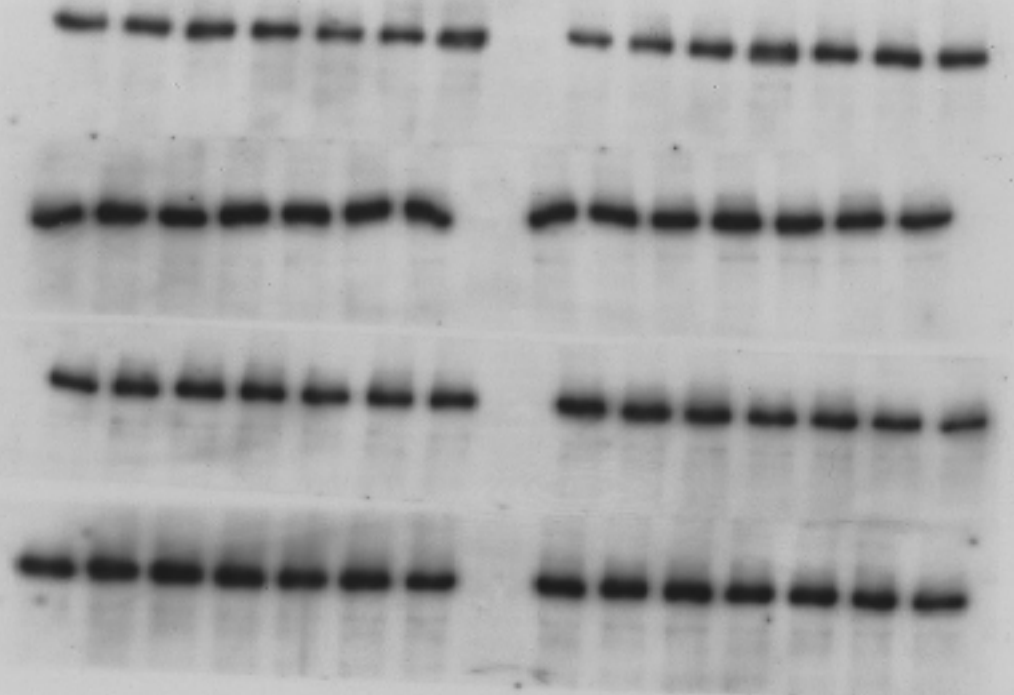
Akt

Veh

Simvastatin 0 5 10 15 30 60 120

Akt





AFC

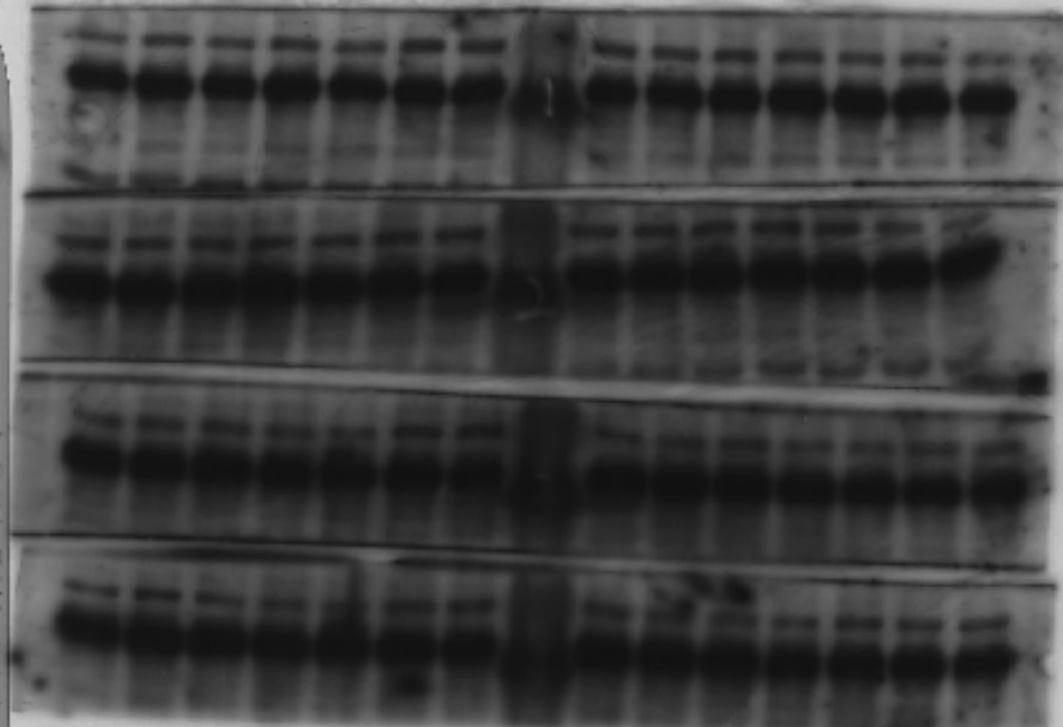
Simvastatin 0 5 10 15 30 60 120

p-Akt

Veh

Simvastatin 0 5 10 15 30 60 120

p-Akt



AFC

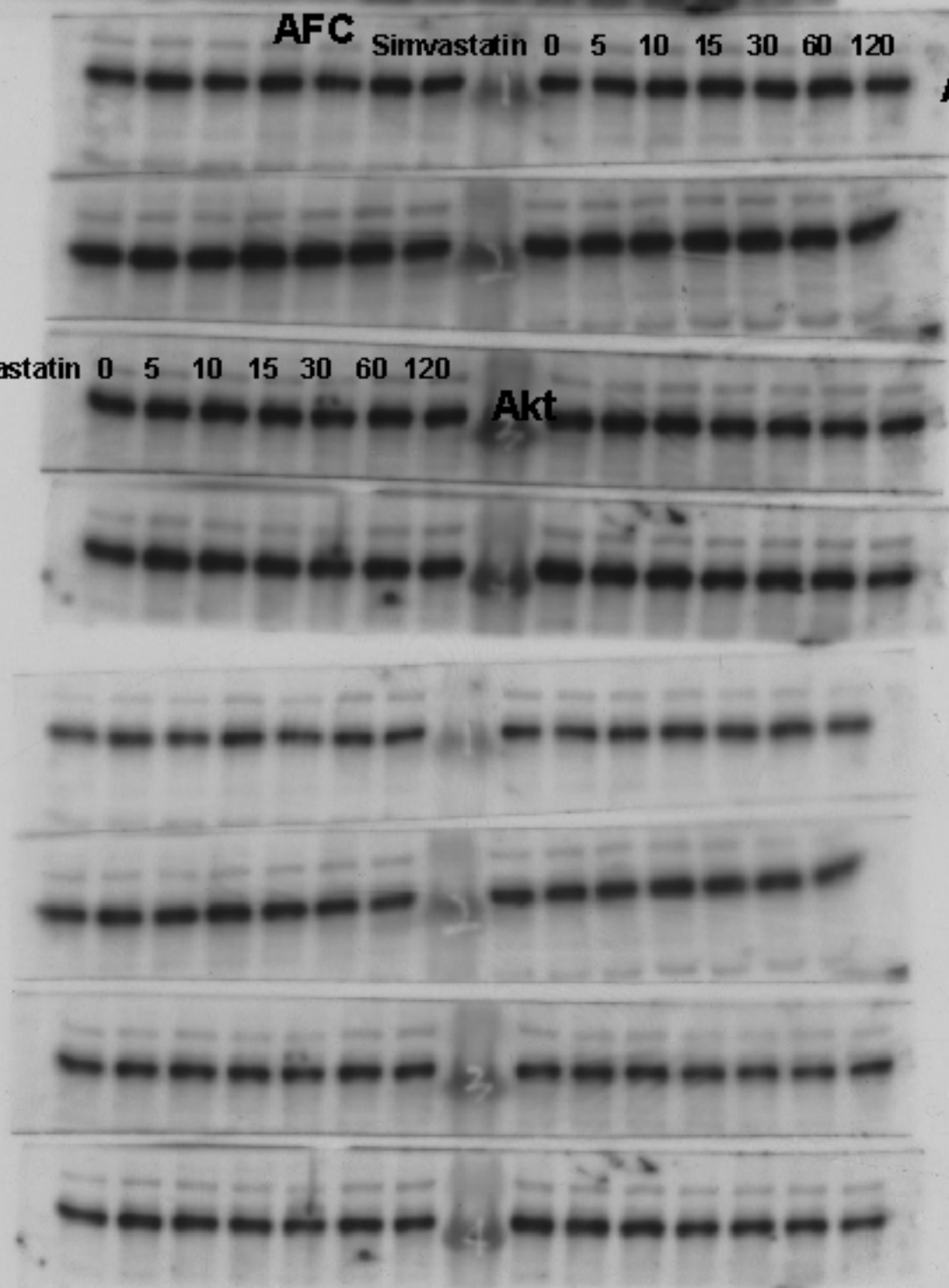
Simvastatin 0 5 10 15 30 60 120

Akt

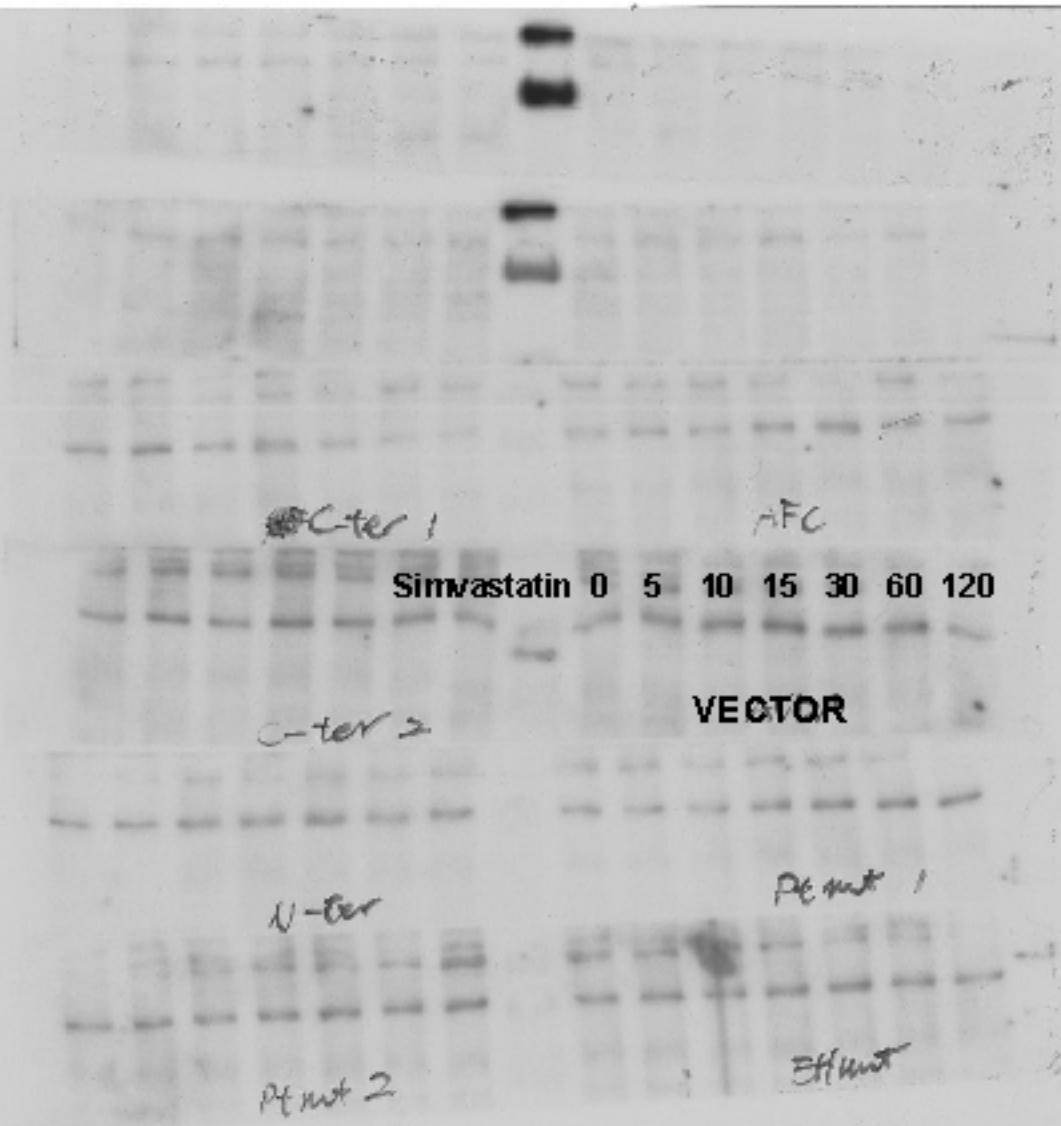
Veh

Simvastatin 0 5 10 15 30 60 120

Akt



STIMULATED



C-ter 1

AFC

Simvastatin 0 5 10 15 30 60 120

p-Akt

C-ter 2

VECTOR

N-ter

PE mut 1

PE mut 2

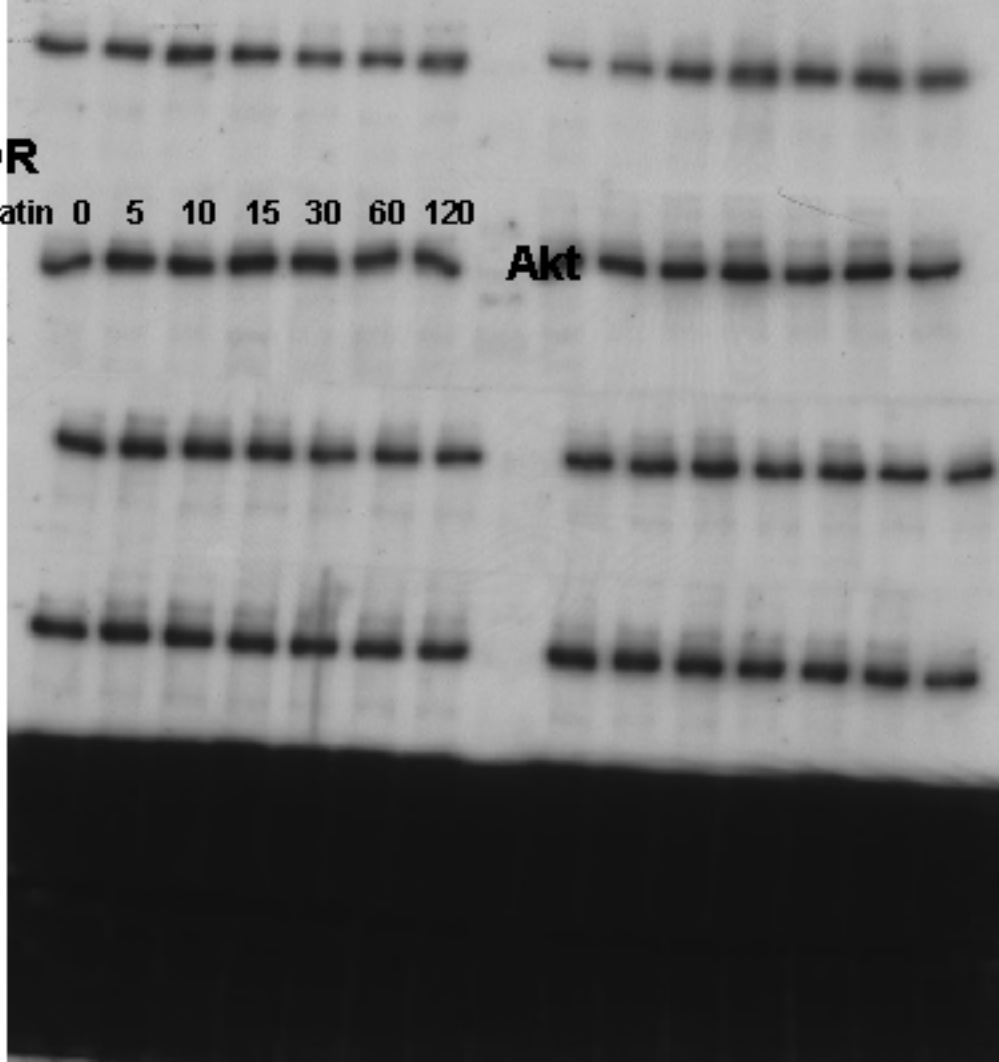
Eff mut

0 5 10 15 30 60 120

VECTOR

Simvastatin 0 5 10 15 30 60 120

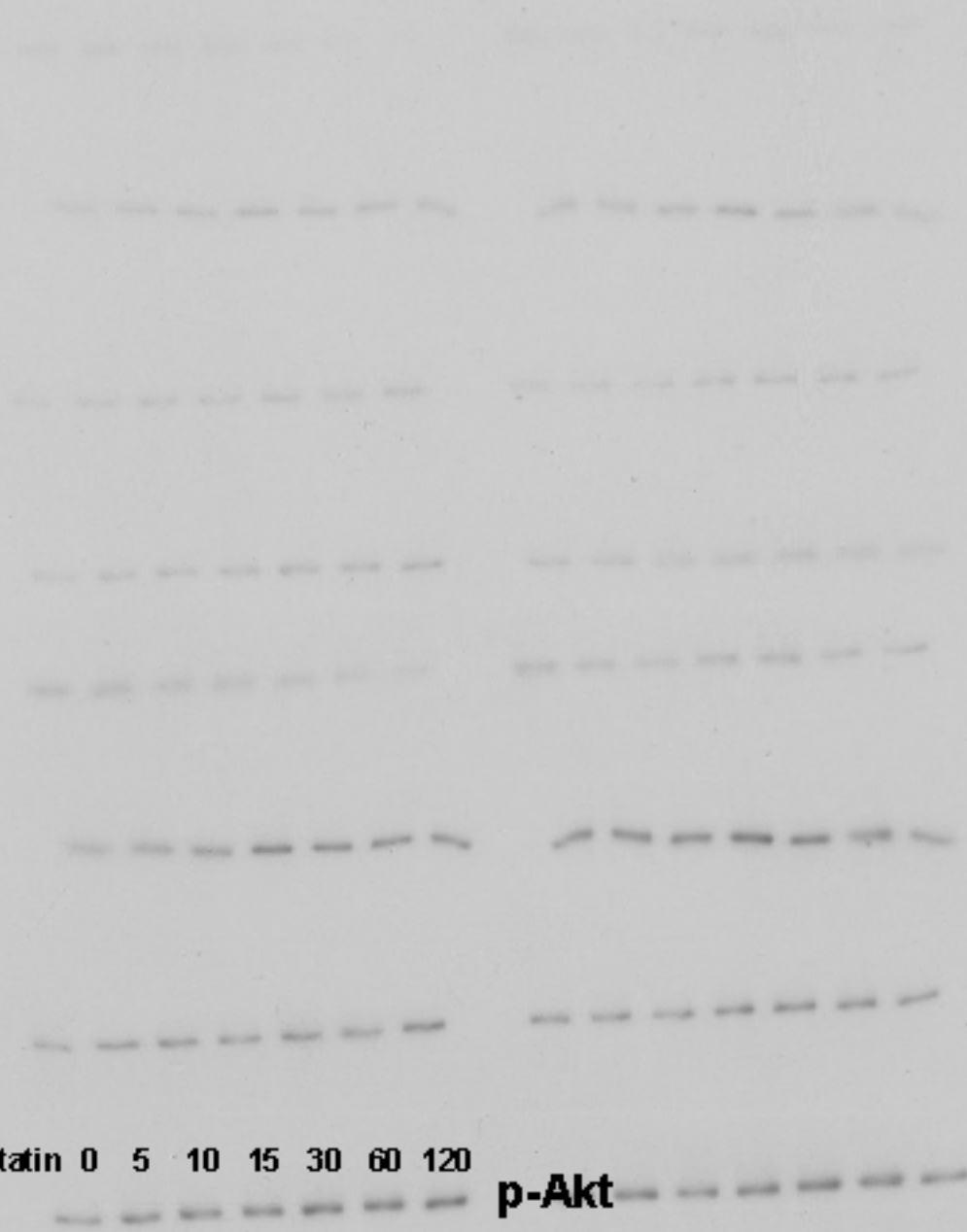
Akt

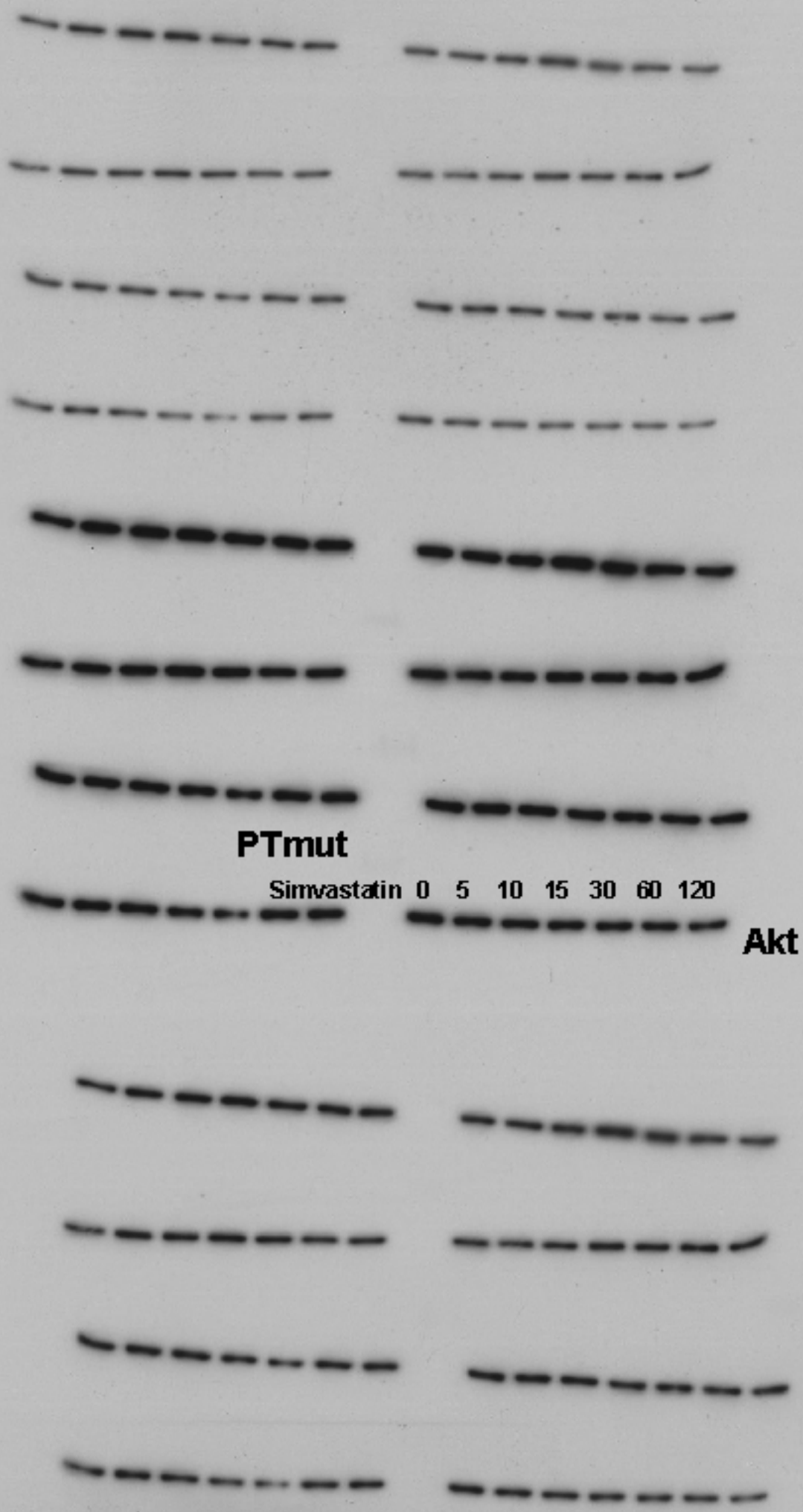


PTmut

Simvastatin 0 5 10 15 30 60 120

p-Akt





PTmut

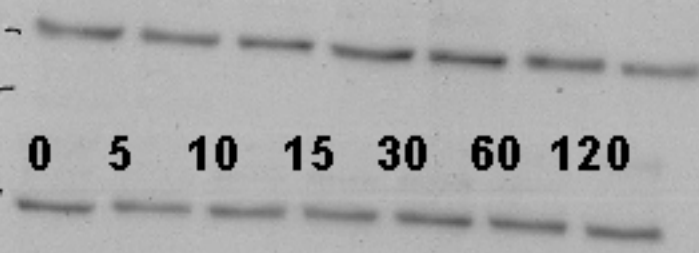
Simvastatin 0 5 10 15 30 60 120

Akt

Simvastatin 0 5 10 15 30 60 120

N-ter

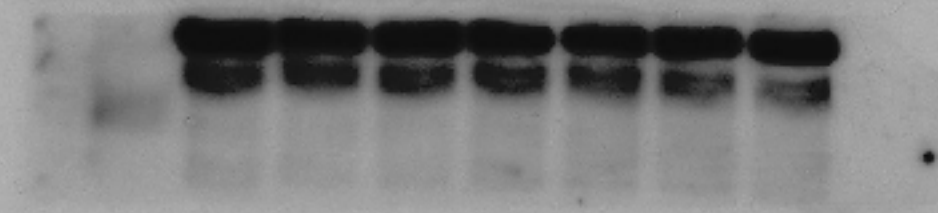
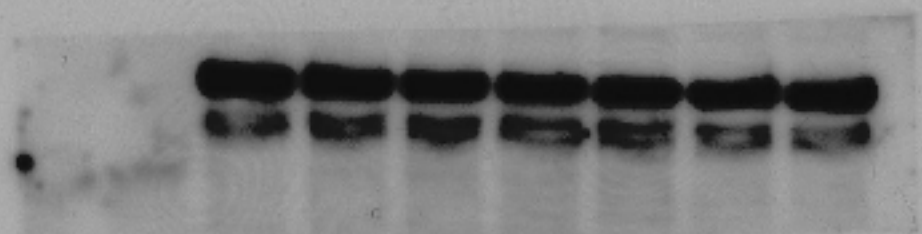
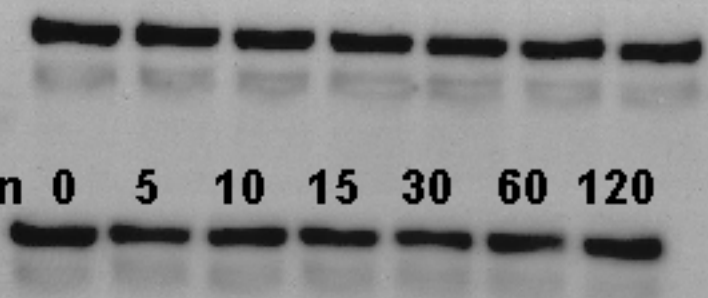
p-Akt



Simvastatin 0 5 10 15 30 60 120

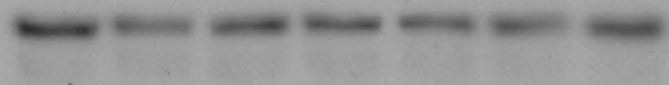
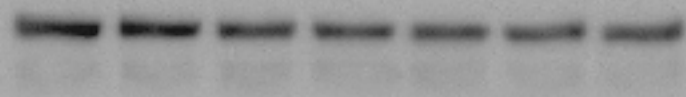
N-ter

Akt



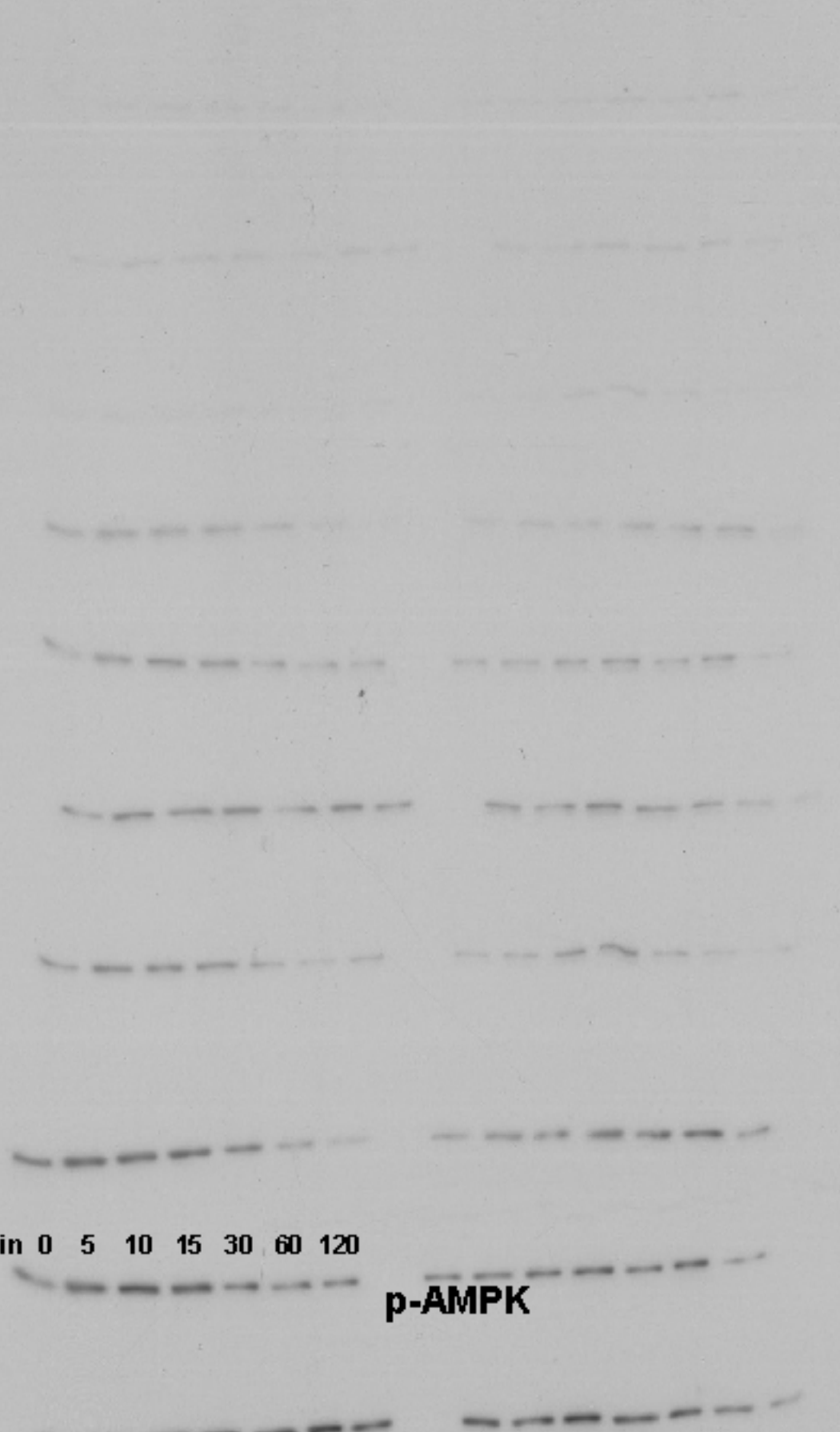
Western blot showing protein levels in the third row of the experiment. The bands are consistent in intensity across all time points, indicating no significant change in protein levels.

Western blot showing protein levels in the fourth row of the experiment. The bands are consistent in intensity across all time points, indicating no significant change in protein levels.



Simvastatin 0 5 10 15 30 60 120

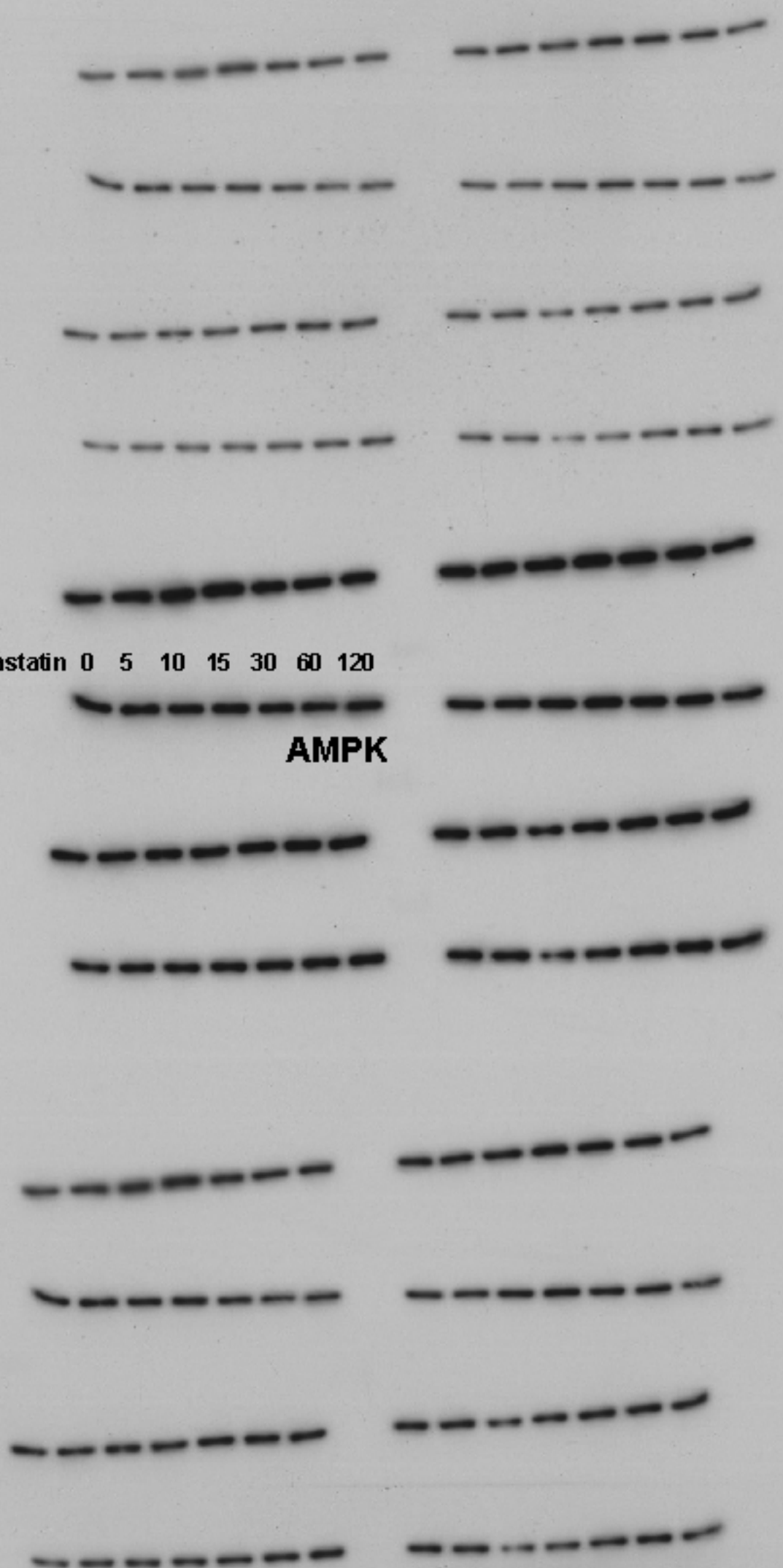
p-AMPK



The image shows a Western blot with 14 lanes. The first lane is the 0 time point. The next seven lanes (5, 10, 15, 30, 60, 120) represent the time course of Simvastatin treatment. The final lane is a control. The p-AMPK bands are visible in all lanes, with a noticeable increase in intensity in the 5, 10, 15, and 30-minute treatment groups compared to the 0 time point and the control lane. The intensity appears to decrease slightly at 60 and 120 minutes but remains higher than the 0 time point.

Simvastatin 0 5 10 15 30 60 120

AMPK



PTmut

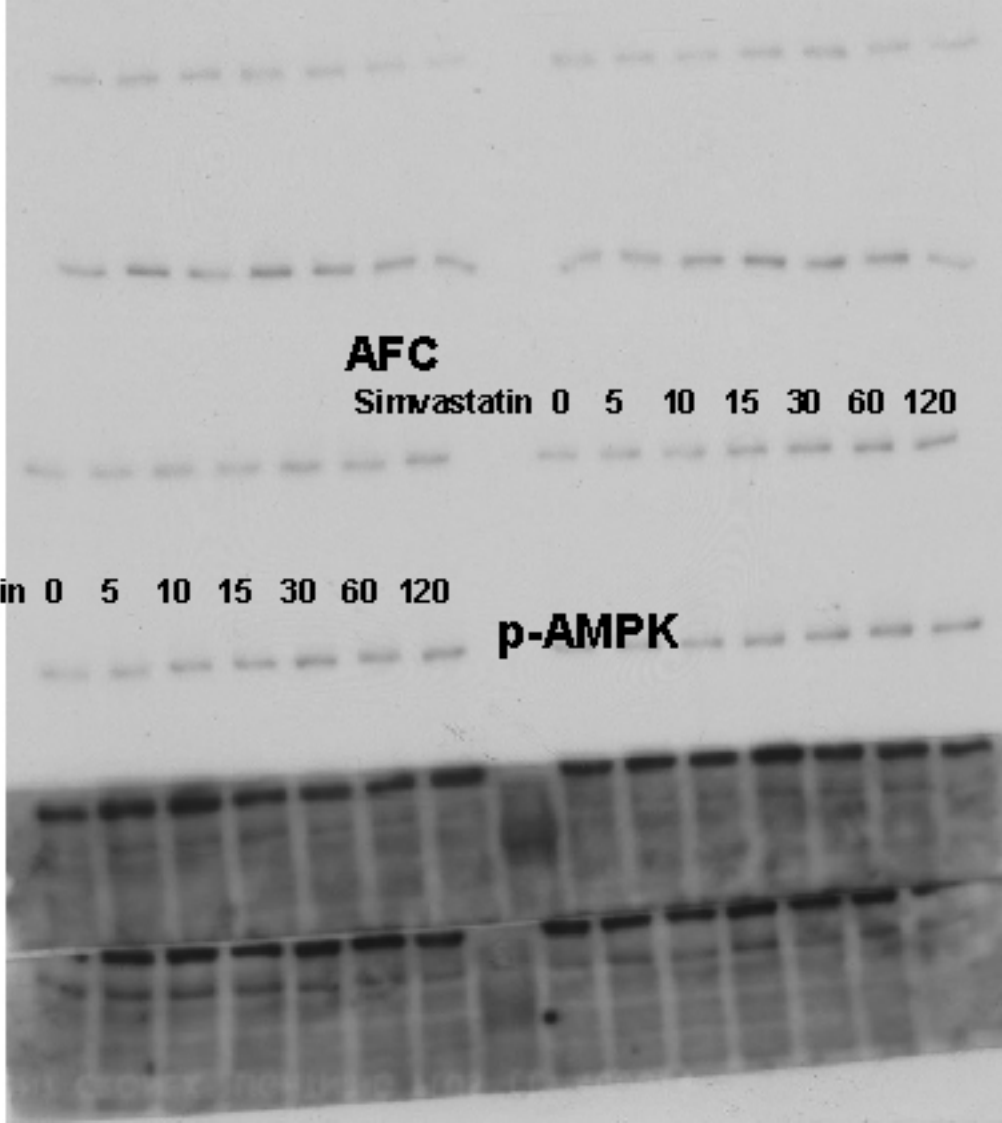
Simvastatin 0 5 10 15 30 60 120

p-AMPK

AFC

Simvastatin 0 5 10 15 30 60 120

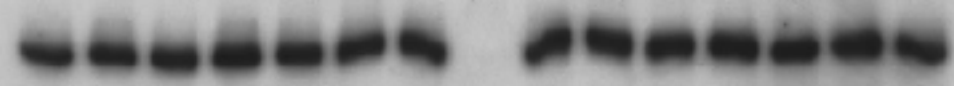
p-AMPK





AFC

Simvastatin 0 5 10 15 30 60 120

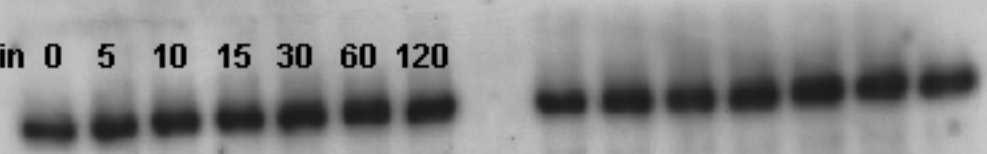


AMPK

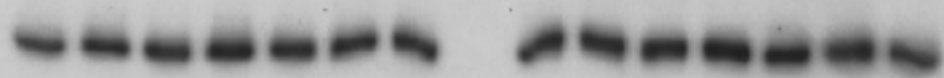
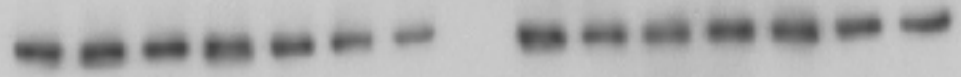


PTmut

Simvastatin 0 5 10 15 30 60 120



AMPK



AUDA

Etnot

AFC

pe mt

AUAZ

Etnot 2

p-AMPK

Simvastatin 0 5 10 15 30 60 120

Spina

p-AMPK

Vector

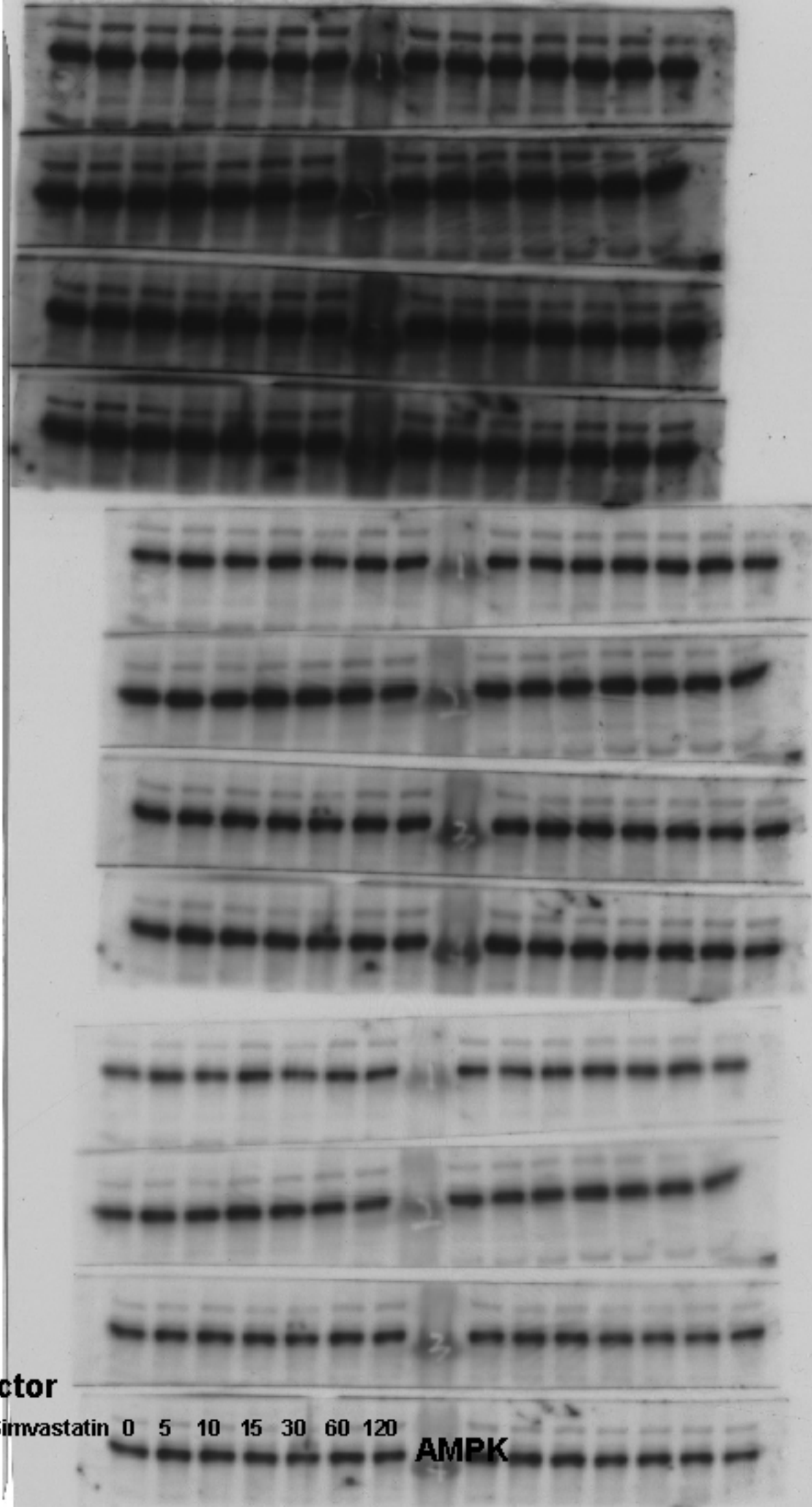
C-ter

0 5 10 15 30 60 120

Vector

Simvastatin 0 5 10 15 30 60 120

AMPK



PTmut

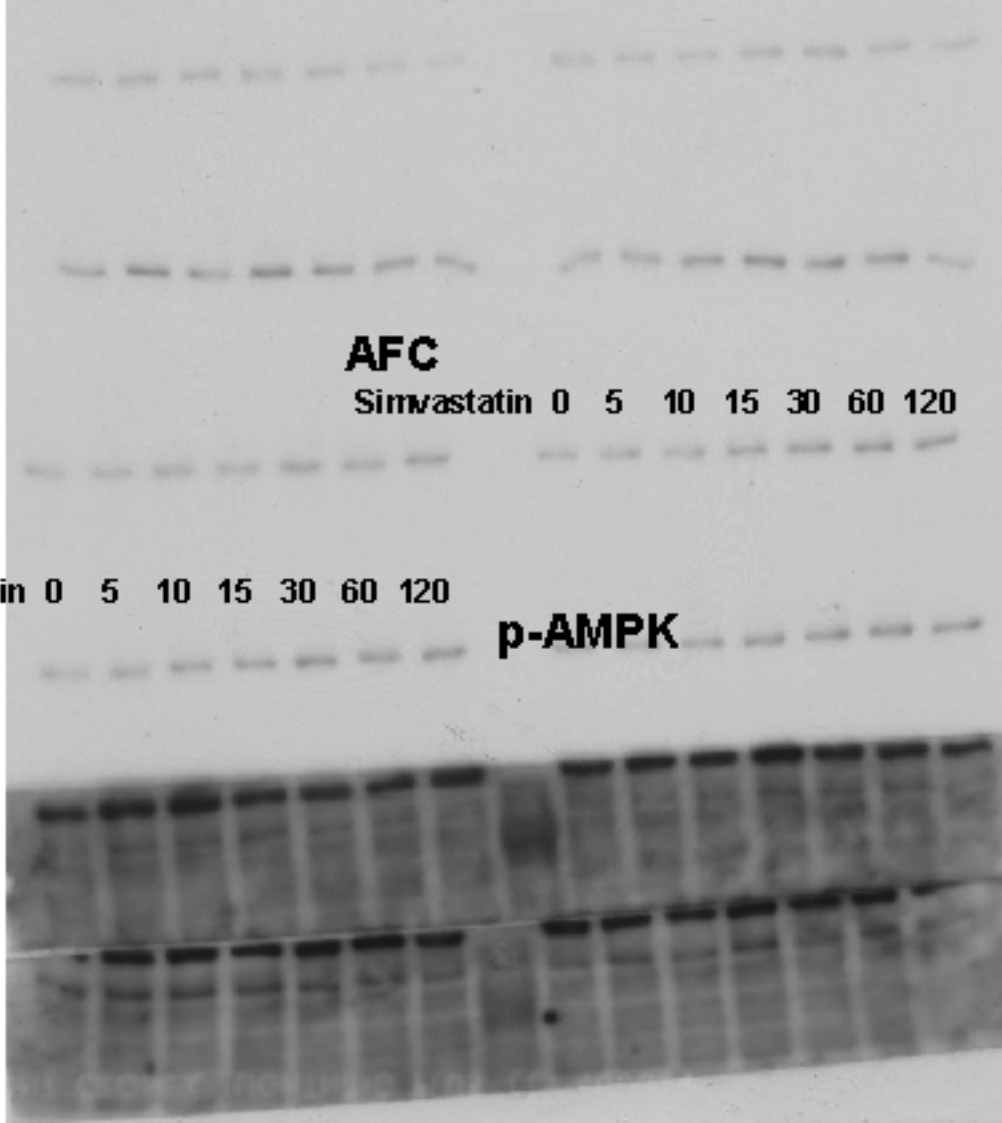
Simvastatin 0 5 10 15 30 60 120

p-AMPK

AFC

Simvastatin 0 5 10 15 30 60 120

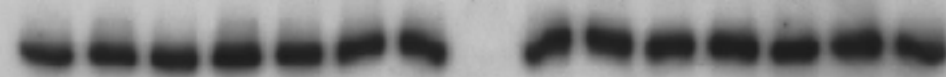
p-AMPK





AFC

Simvastatin 0 5 10 15 30 60 120

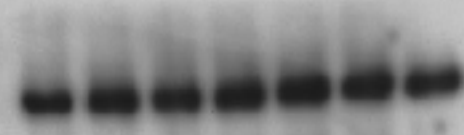


AMPK

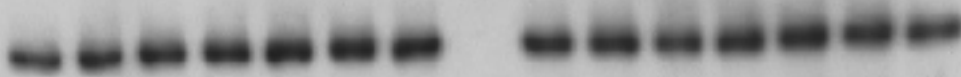
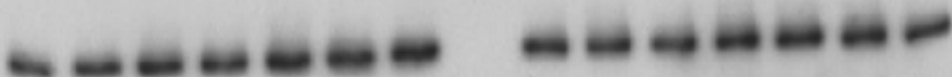
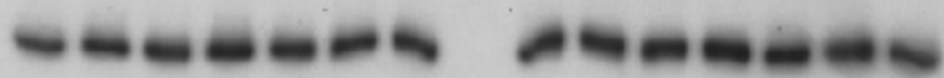


PTmut

Simvastatin 0 5 10 15 30 60 120



AMPK



Simvastatin 0 5 10 15 30 60 120

N-ter

p-AMPK

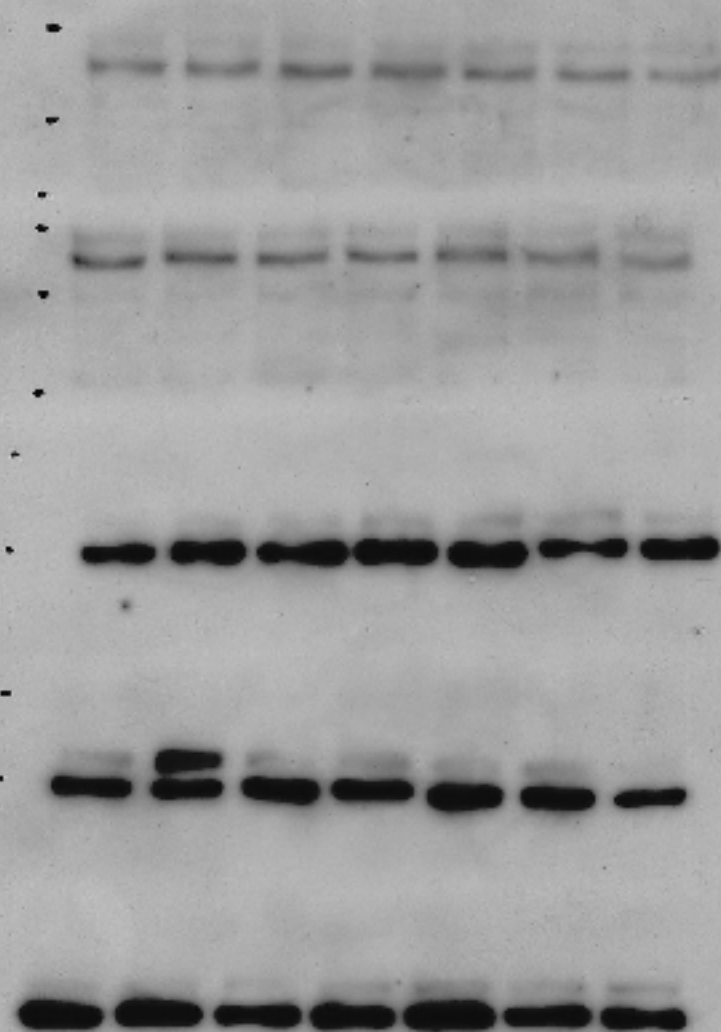
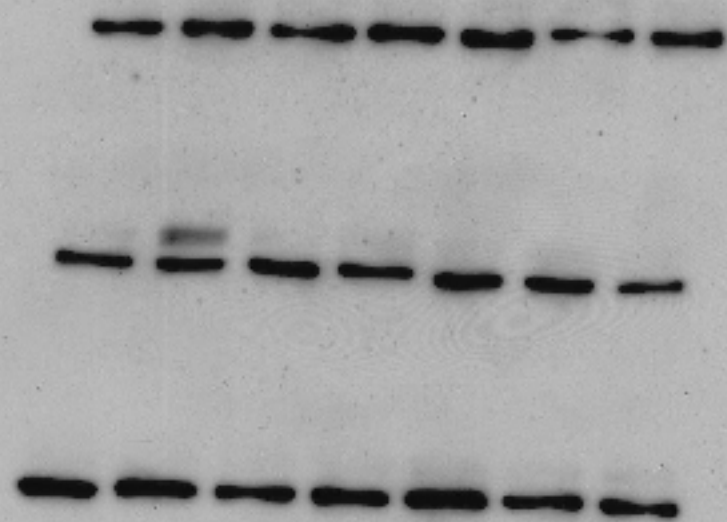
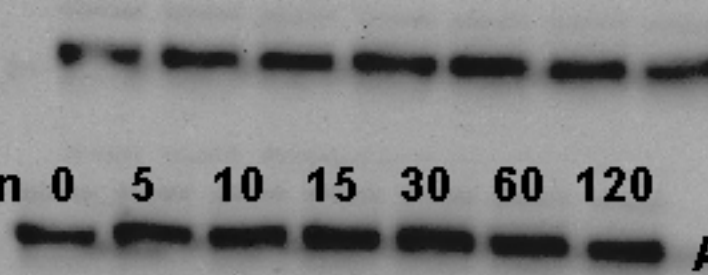


Fig S5. Simvastatin promotes the formation of an sEH-eNOS-Akt-AMPK complex.

Simvastatin 0 5 10 15 30 60 120

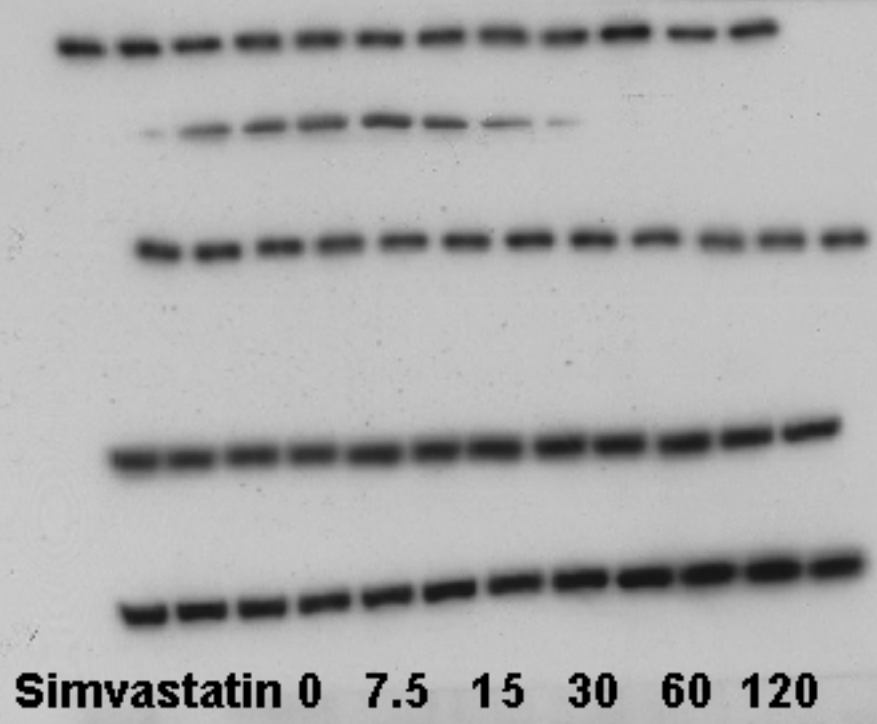


AMPK
N-ter

Simvastatin 0 7.5 15 30 60 120

IP:sEH IP:eNOS

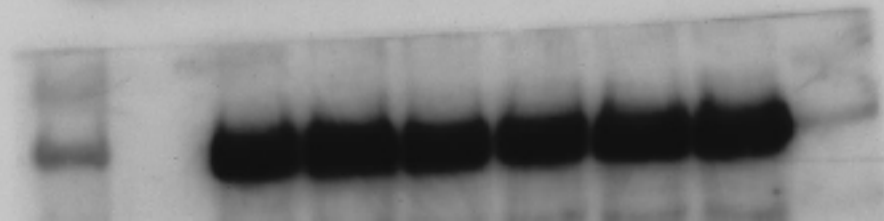
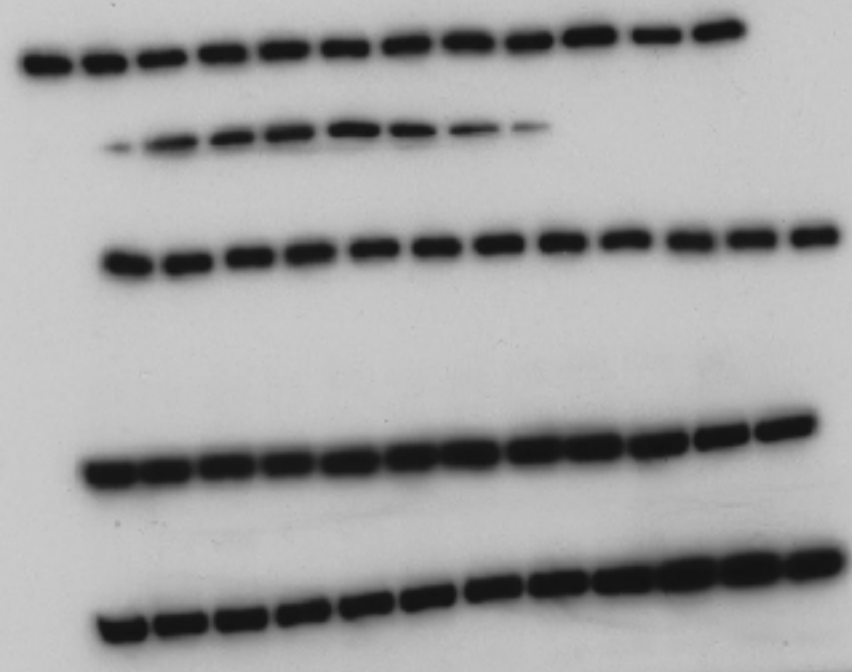




] tub
 (α-tub)
] tub

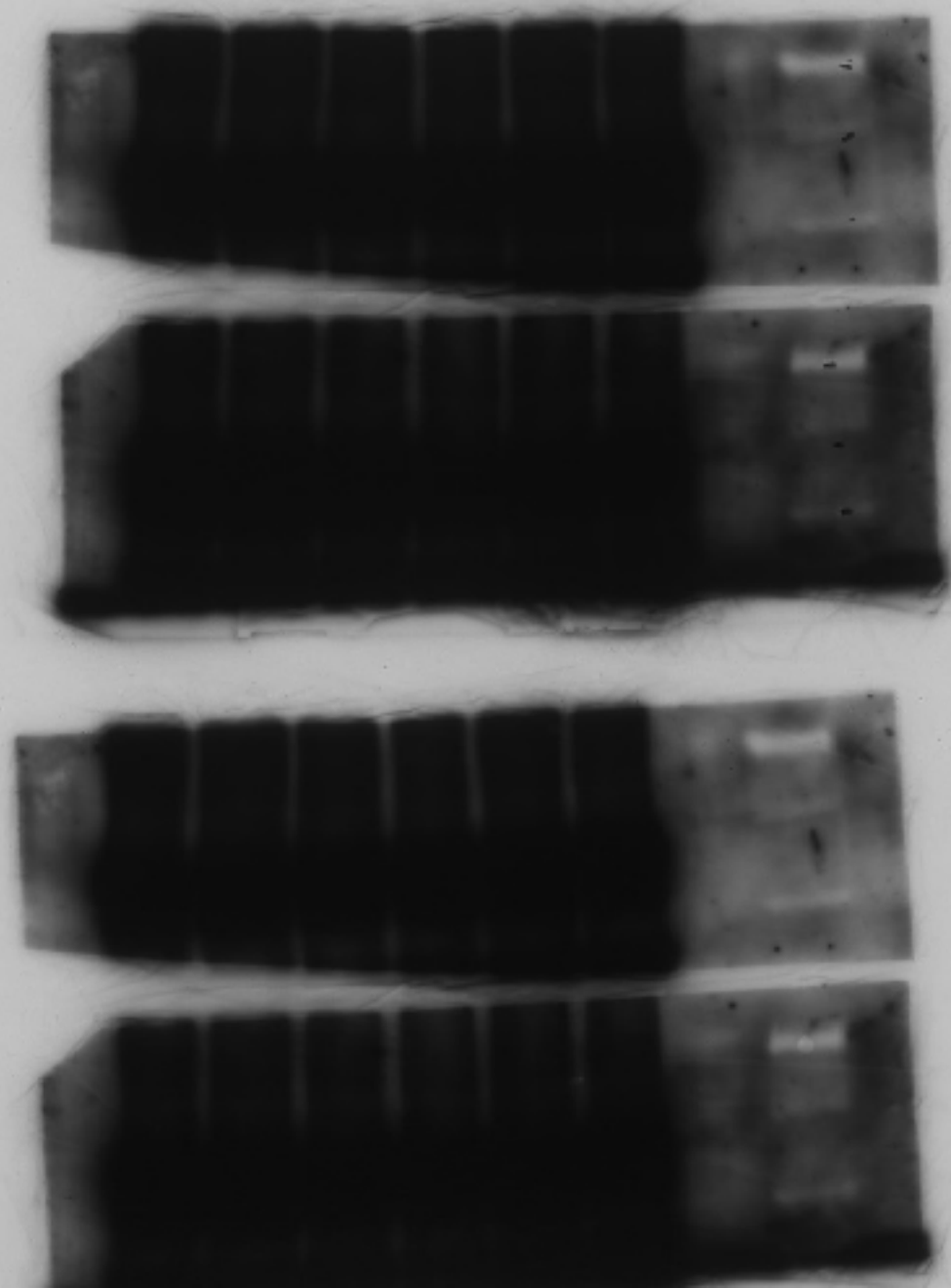
Simvastatin 0 7.5 15 30 60 120

IP: sEH^{EM} IP: sEH
 UB sEH



Simvastatin 0 7.5 15 30 60 120

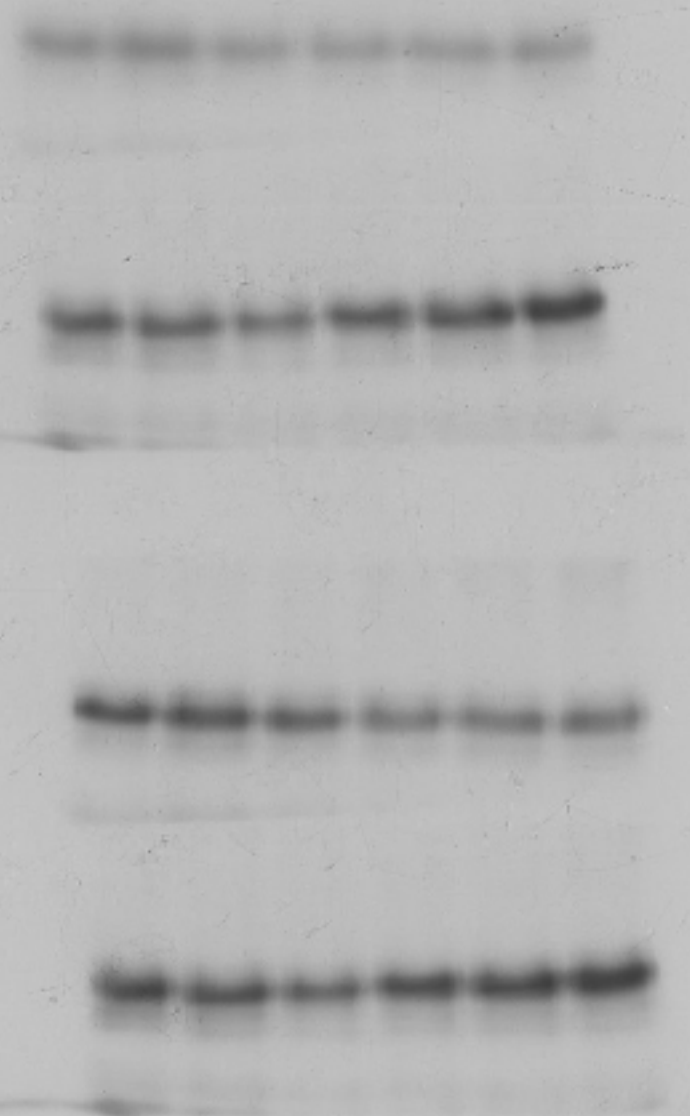
IP:sEH IP:Akt



*** Y131A2 ILU9***

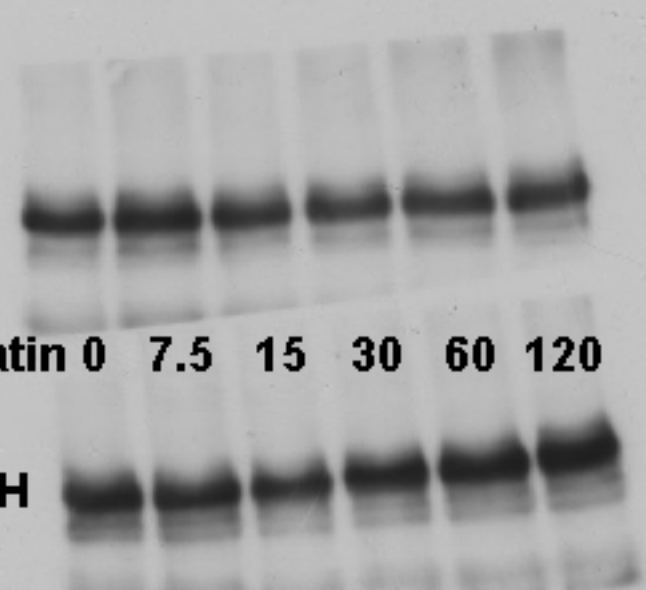
*** Y131A2 ILU9***

**



Simvastatin 0 7.5 15 30 60 120

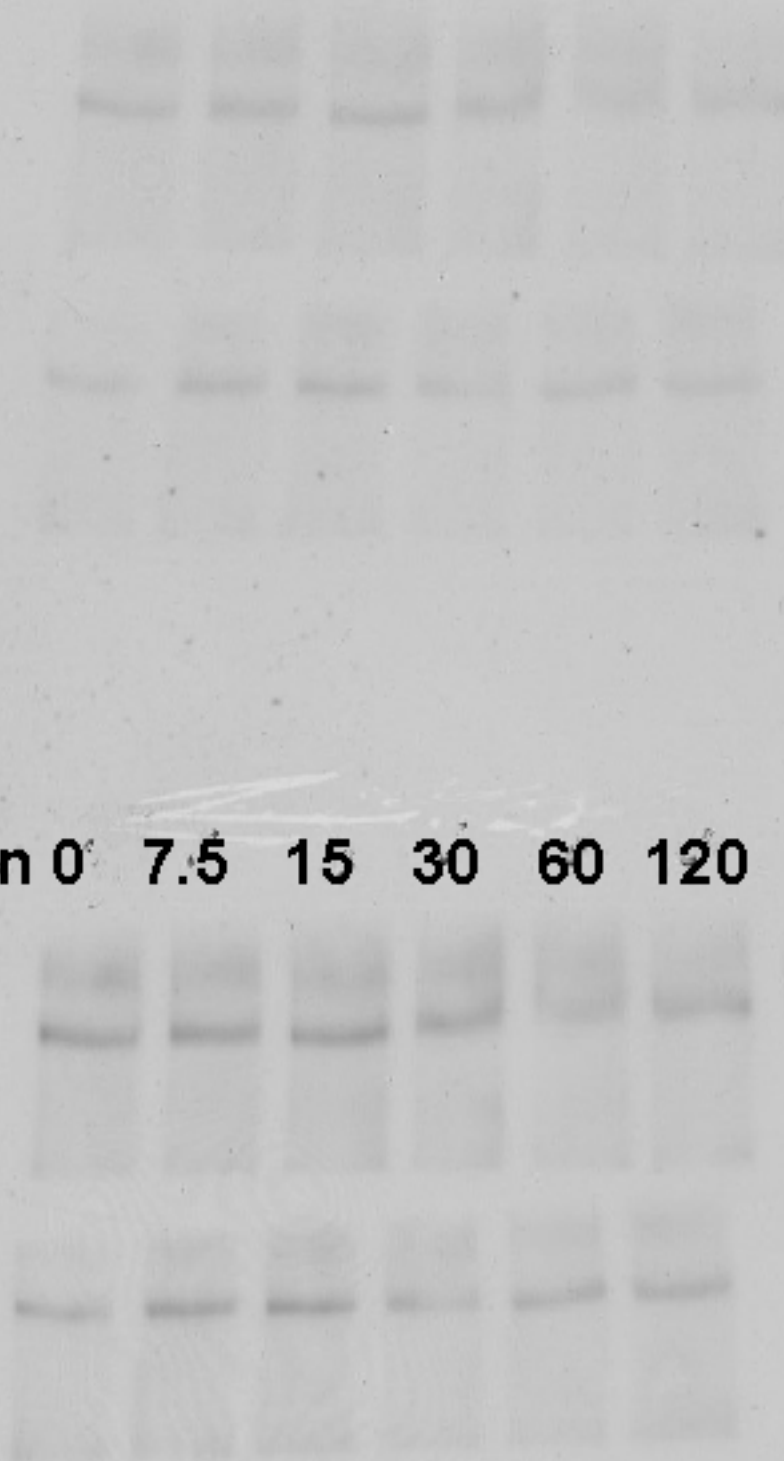
IP:sEH IP:sEH

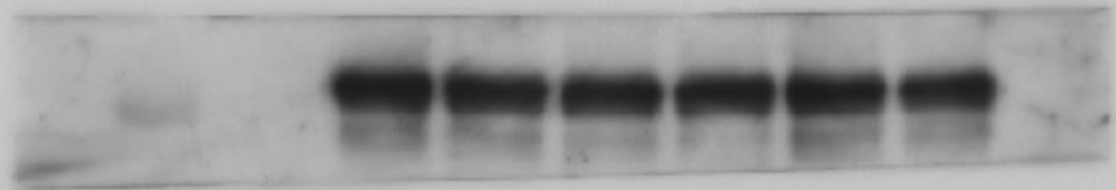


...FUJISAFETY...

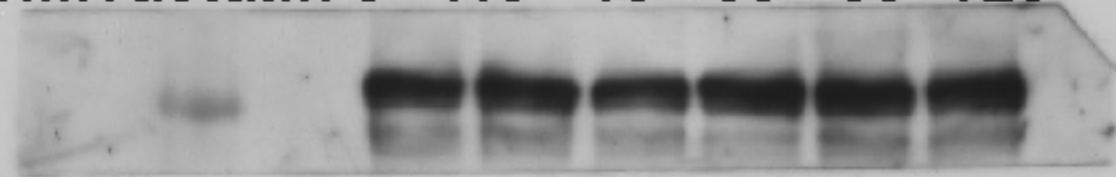
Simvastatin 0 7.5 15 30 60 120

IP:sEH IP:AMPK





Simvastatin 0 7.5 15 30 60 120

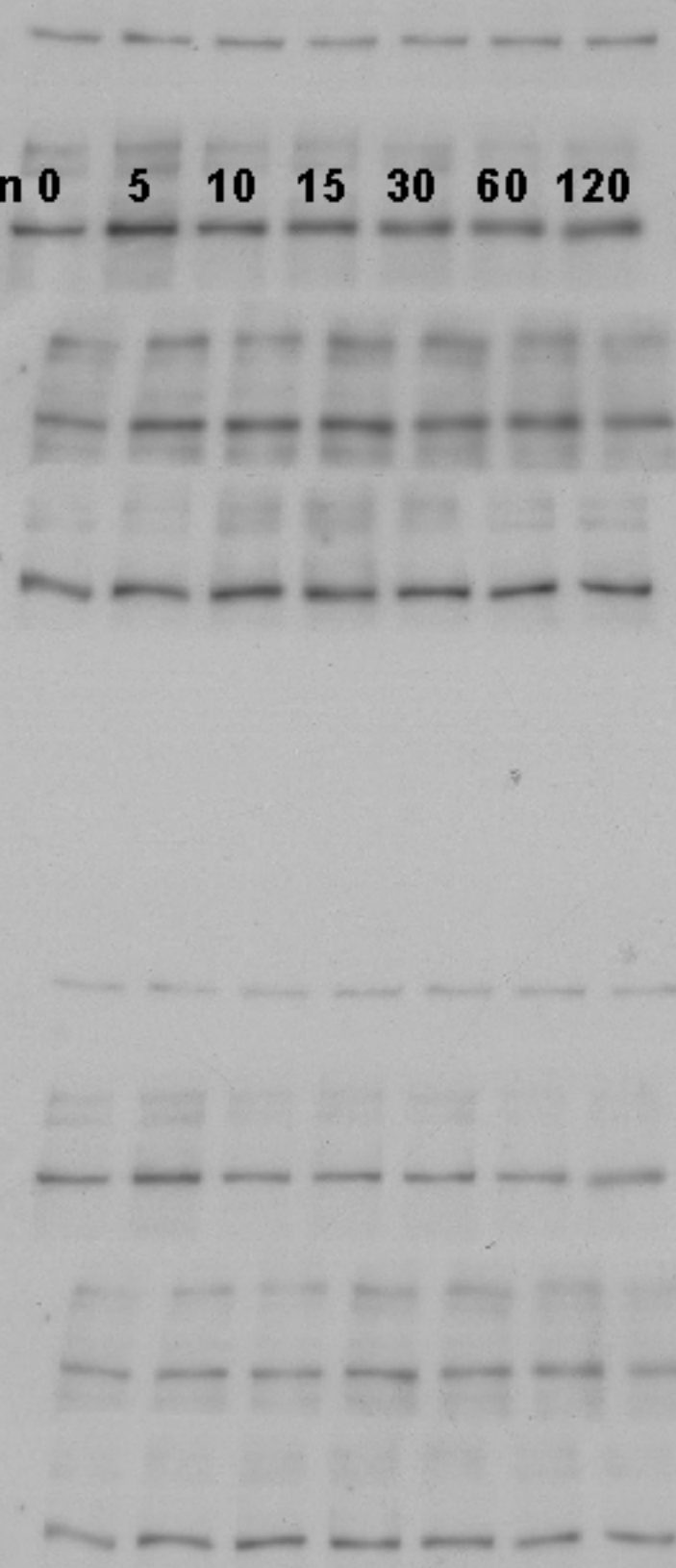


IP:sEH IP:sEH

Fig S6. Activation of epidermal growth factor receptor (EGFR)–c-Src signaling is required for simvastatin-induced increase in sEH phosphatase activity.

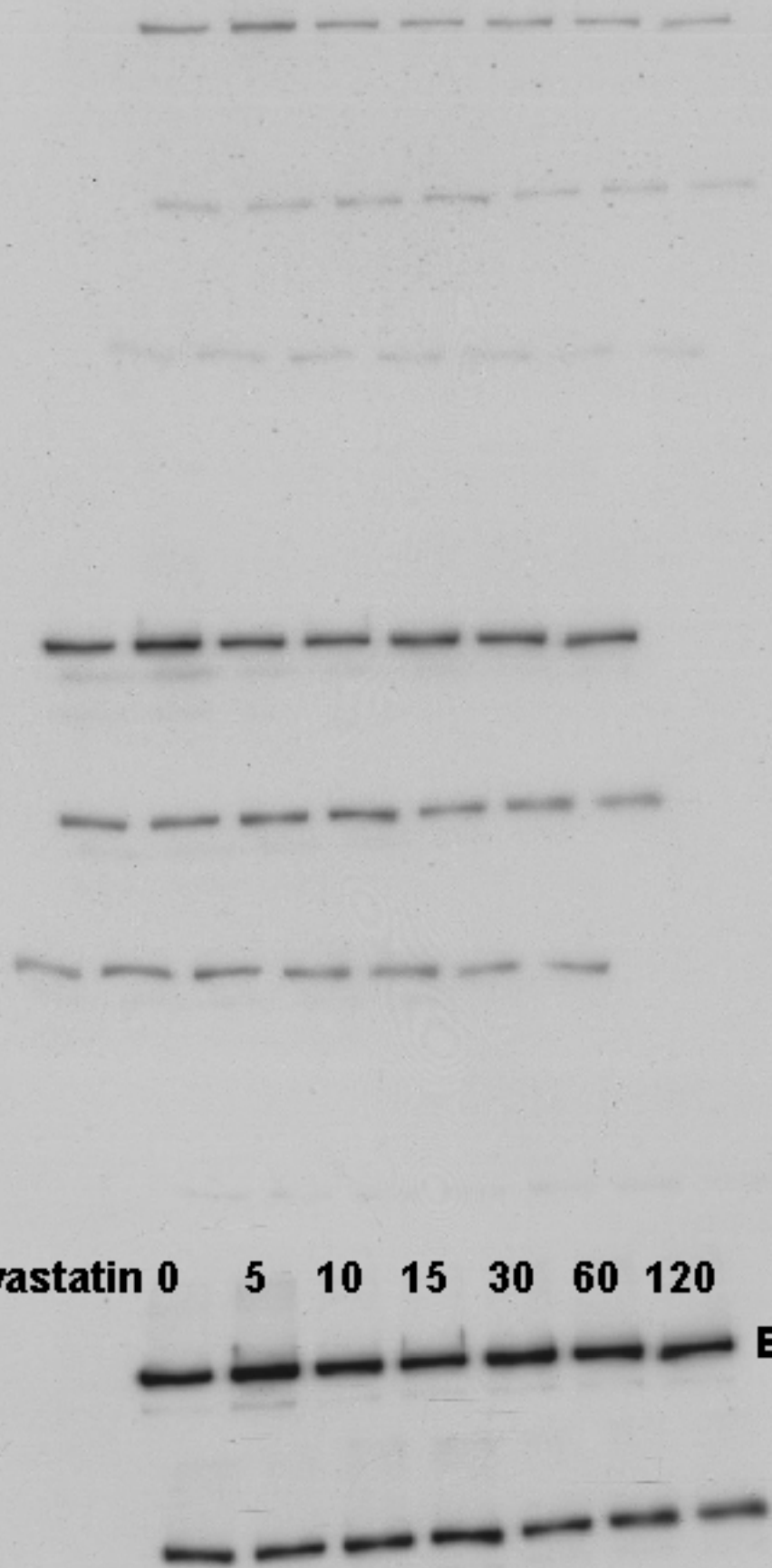
Simvastatin 0 5 10 15 30 60 120

pEGFR



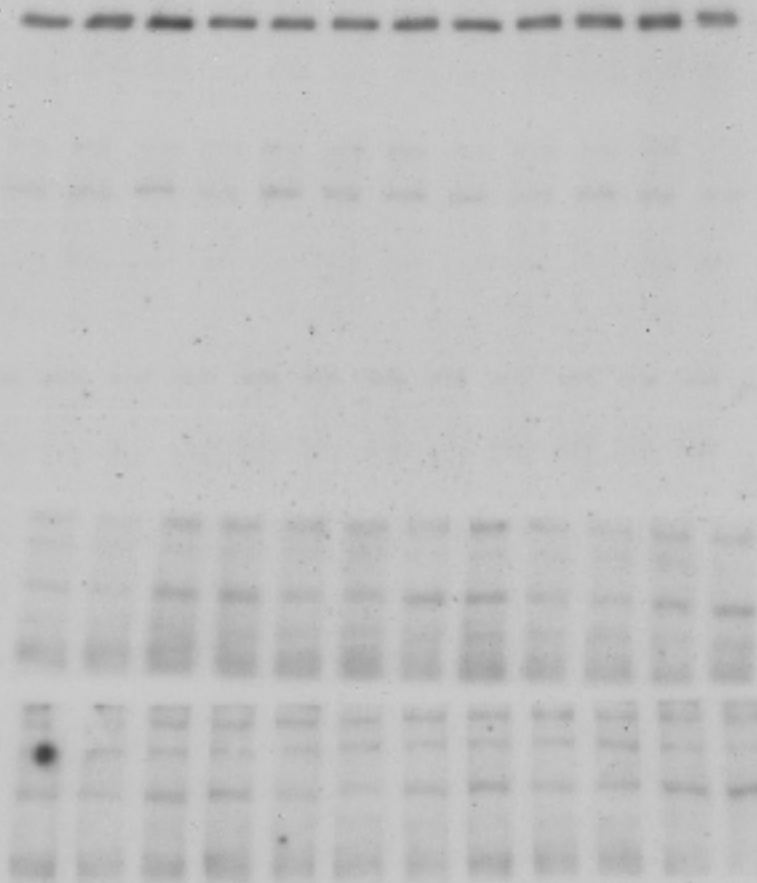
Simvastatin 0 5 10 15 30 60 120

EGFR



Simvastatin 0 5 10 15 30 60 120

p-Src



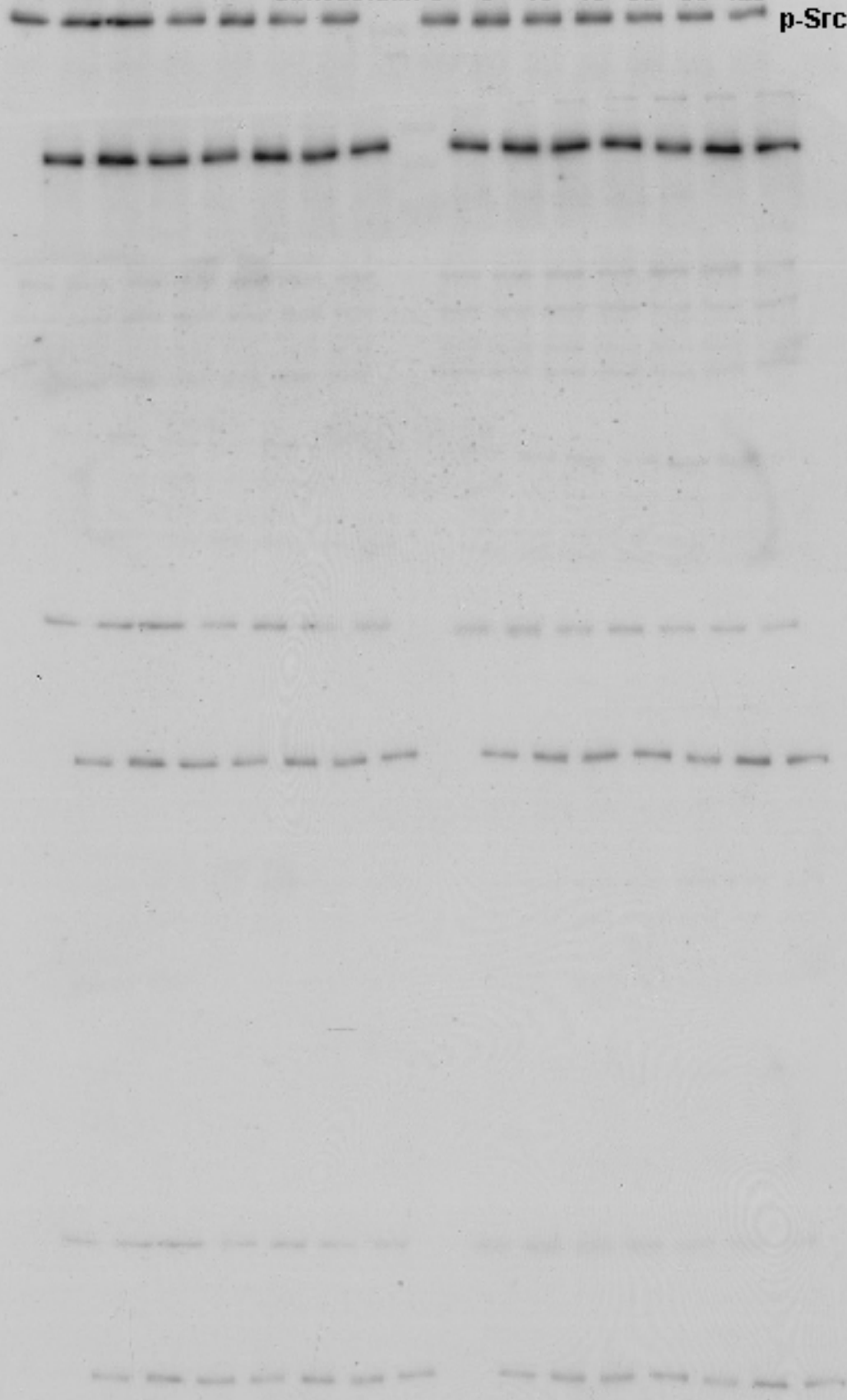
...YTRRAA2 ILUQA...

...YTRRAA2 ILUQA...

...YTRRAA2 ILUQA...

Simvastatin 0 5 10 15 30 60 120

p-Src



...FUJI SAFETY...

...FUJI SAFETY...

...FUJI SAFETY...

Simvastatin 0 5 10 15 30 60 120

Src

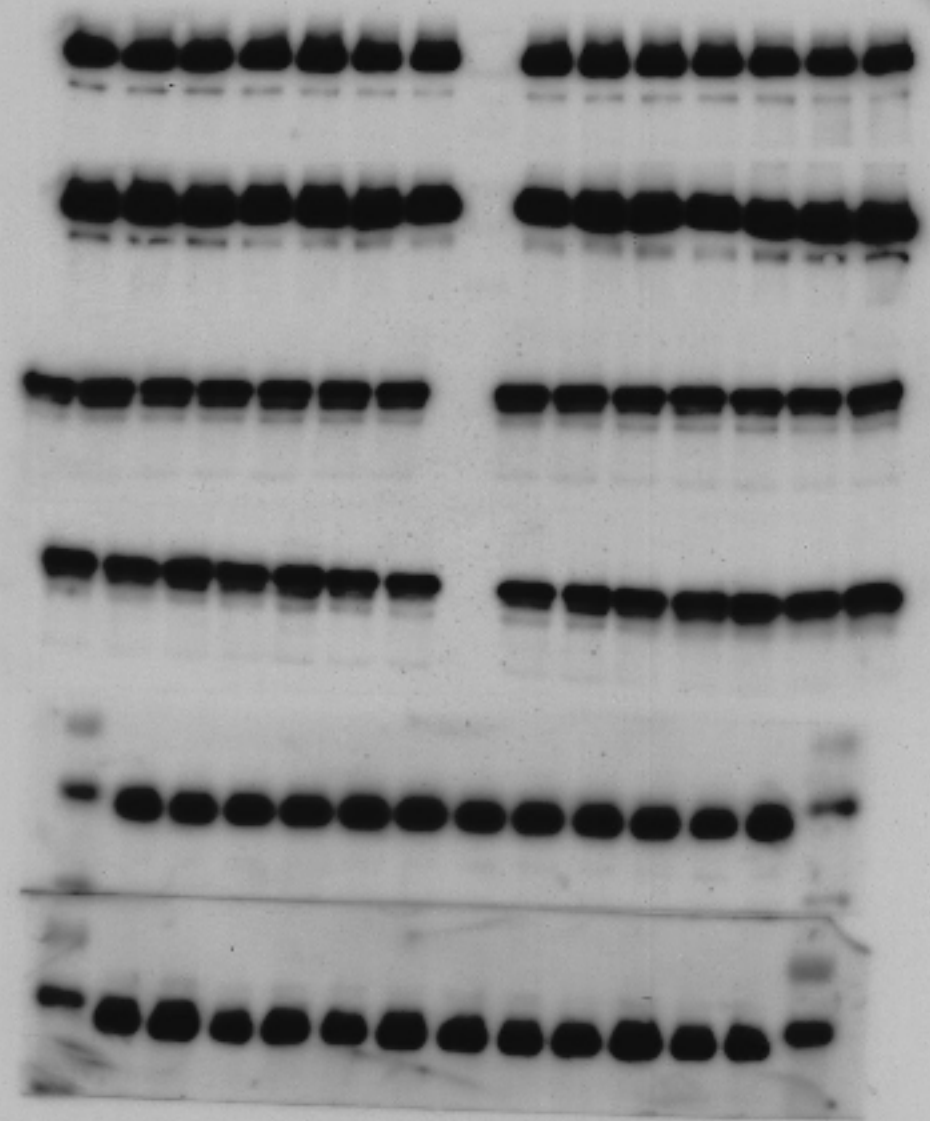
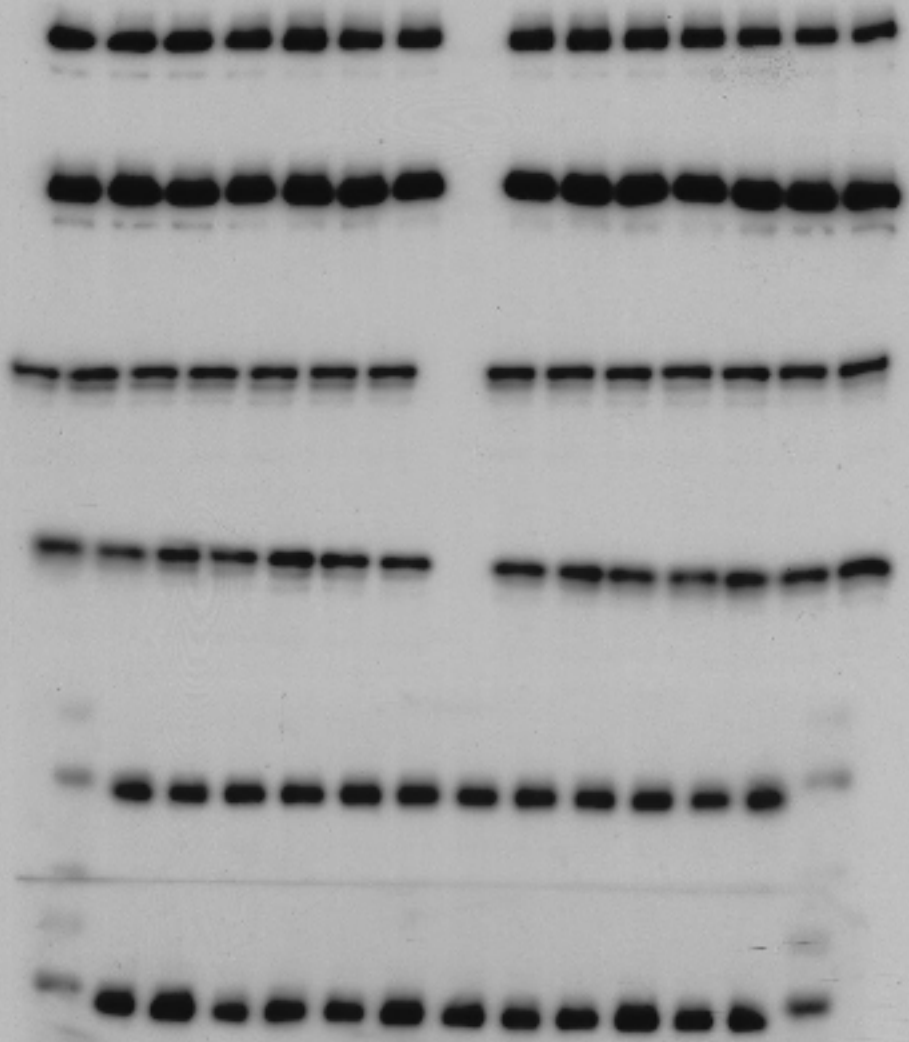
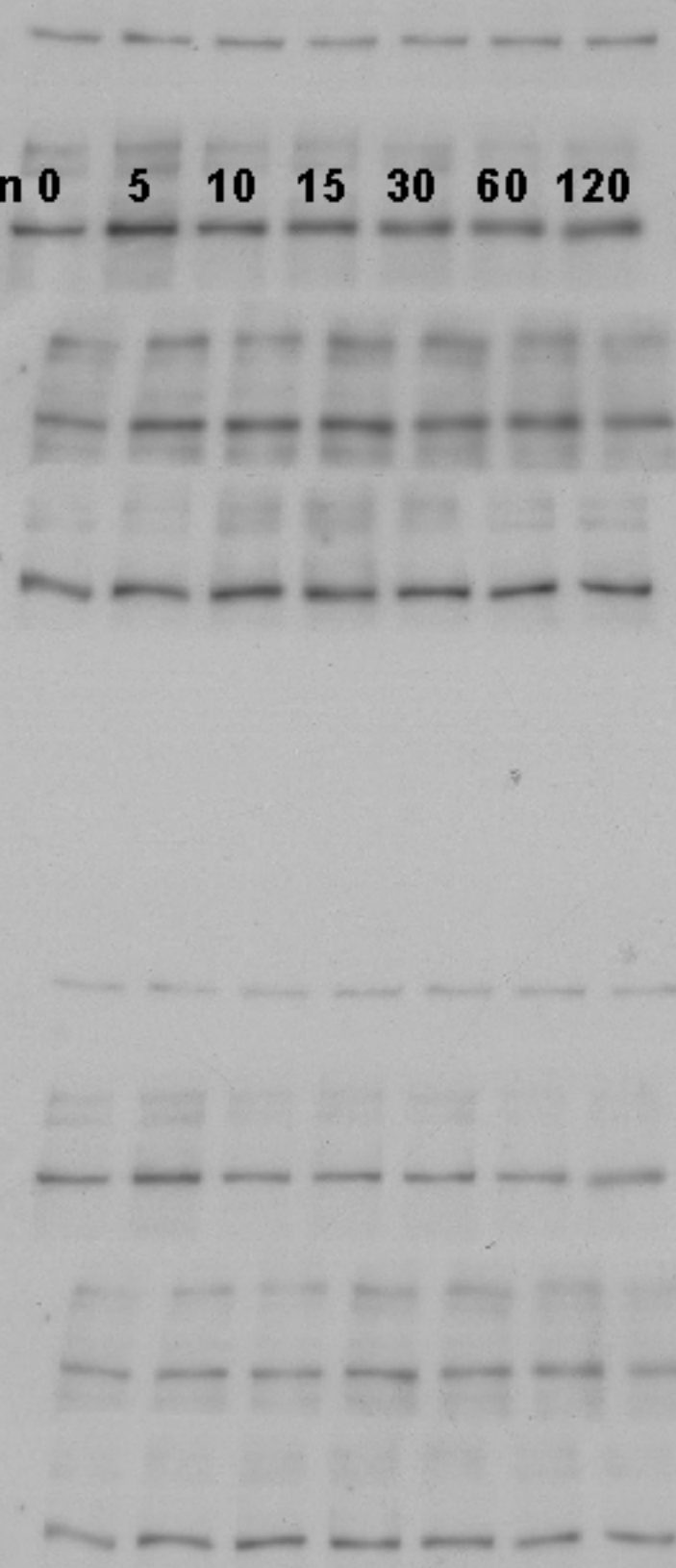


Fig S7. Simvastatin induces tyrosine phosphorylation of sEH.

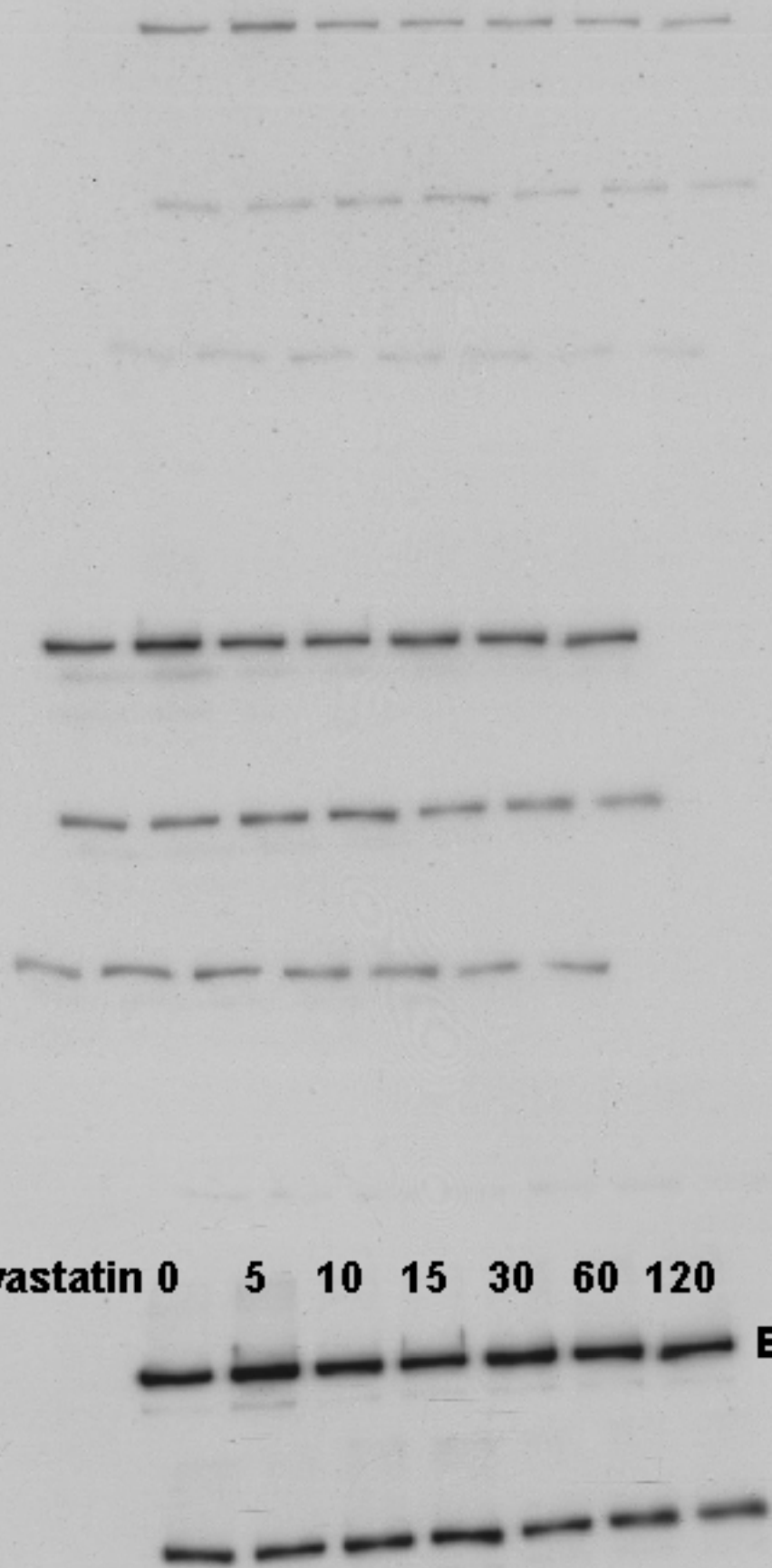
Simvastatin 0 5 10 15 30 60 120

pEGFR



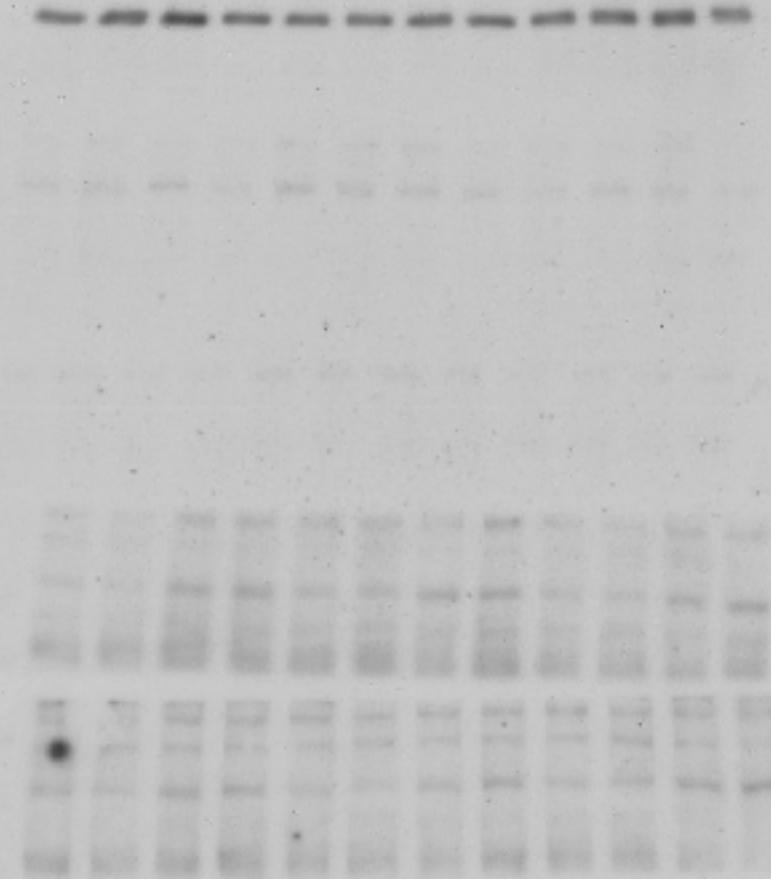
Simvastatin 0 5 10 15 30 60 120

EGFR



Simvastatin 0 5 10 15 30 60 120

p-Src

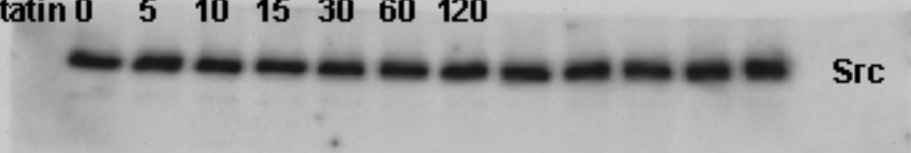


...YTRRAA2 ILUQA...

...YTRRAA2 ILUQA...

...YTRRAA2 ILUQA...

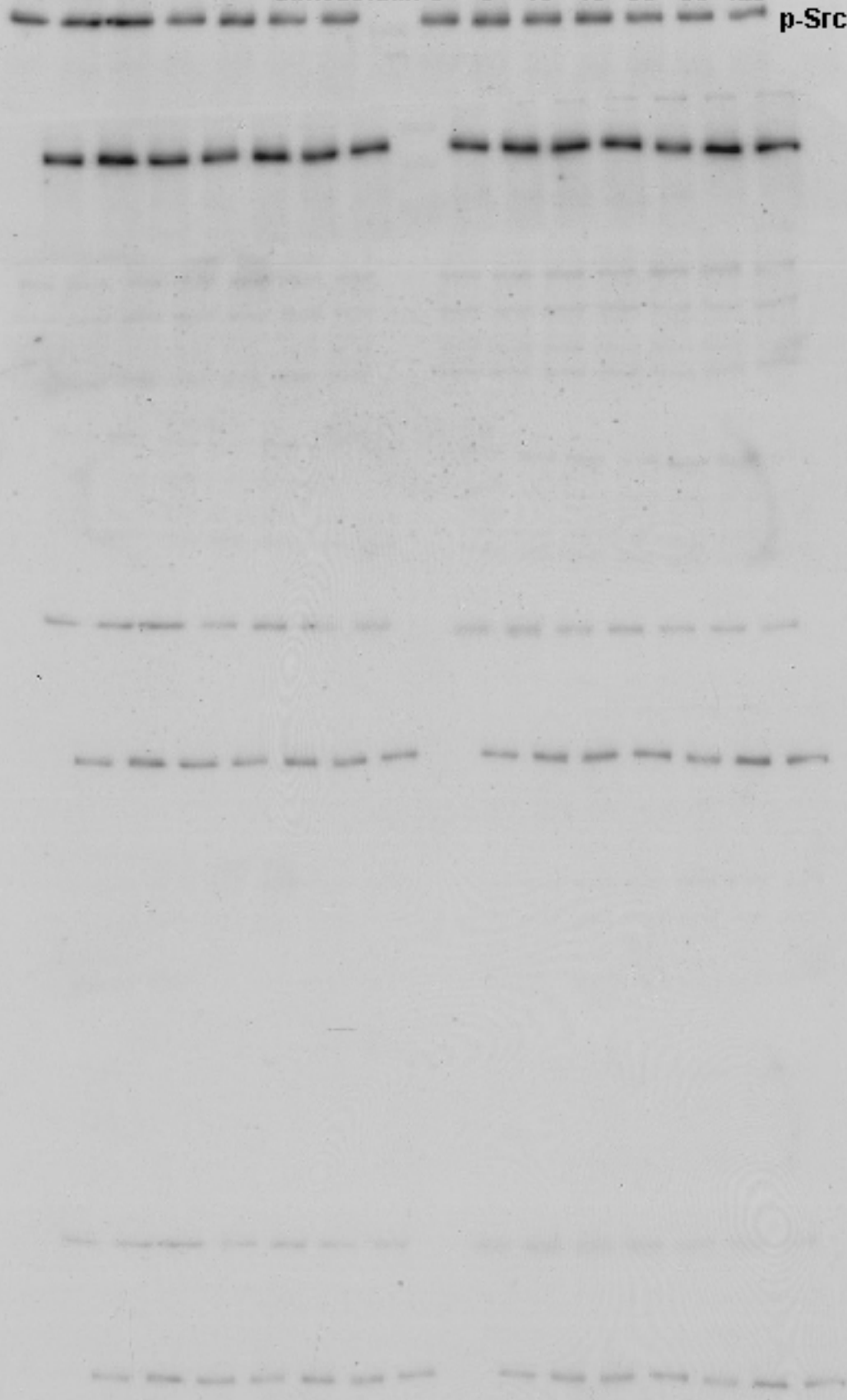
Simvastatin 0 5 10 15 30 60 120



Src

Simvastatin 0 5 10 15 30 60 120

p-Src



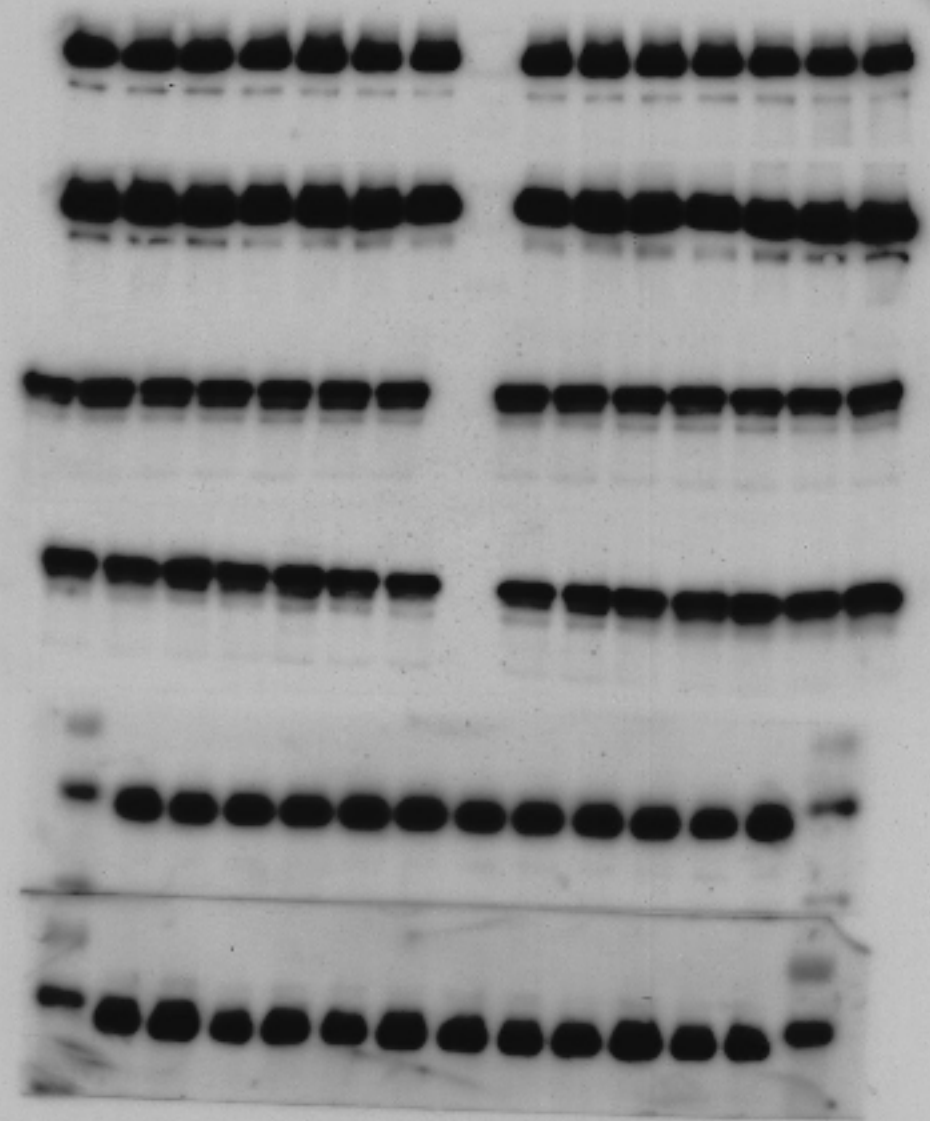
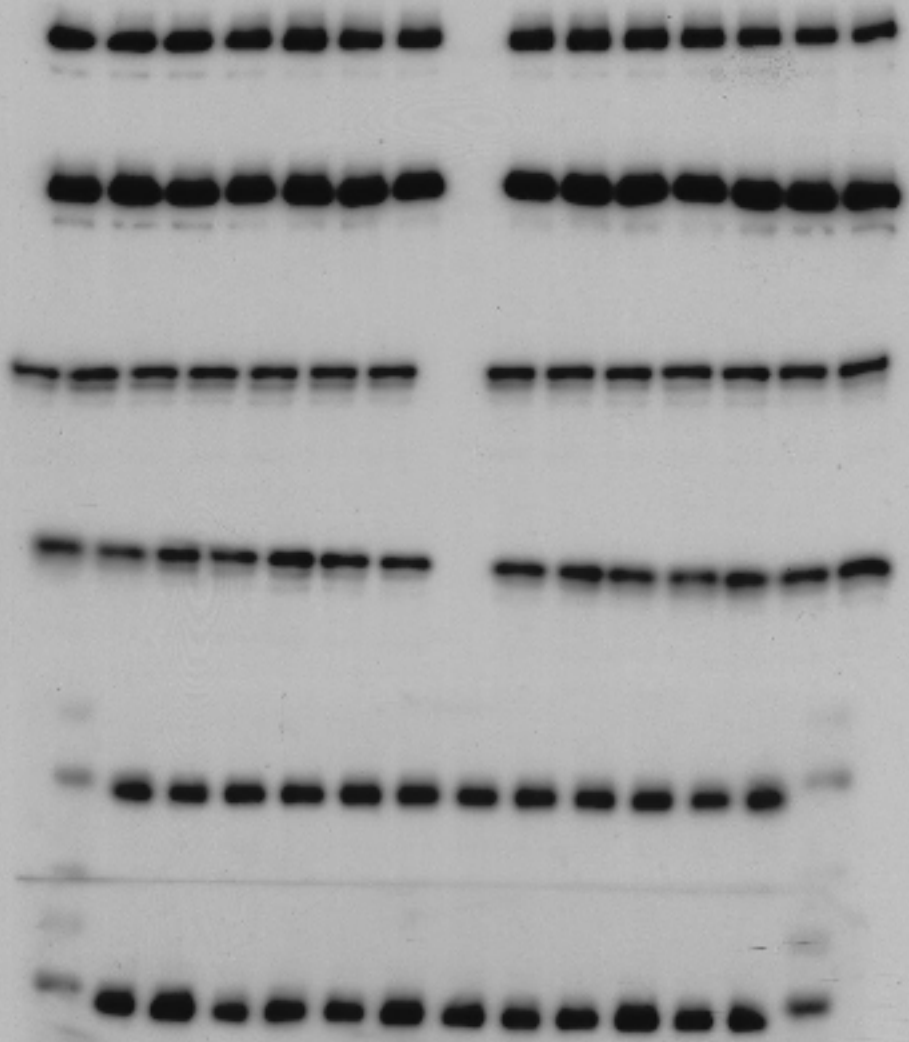
...FUJI SAFETY...

...FUJI SAFETY...

...FUJI SAFETY...

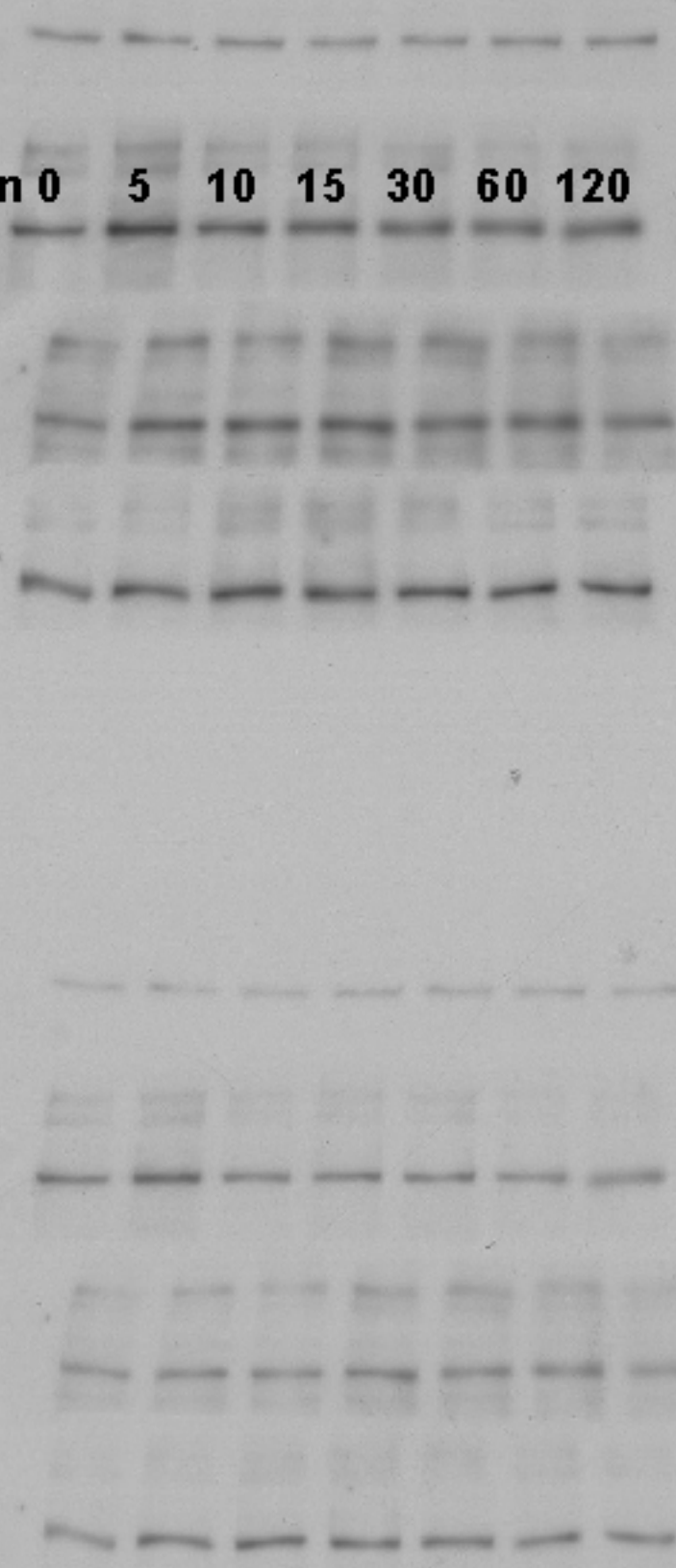
Simvastatin 0 5 10 15 30 60 120

Src



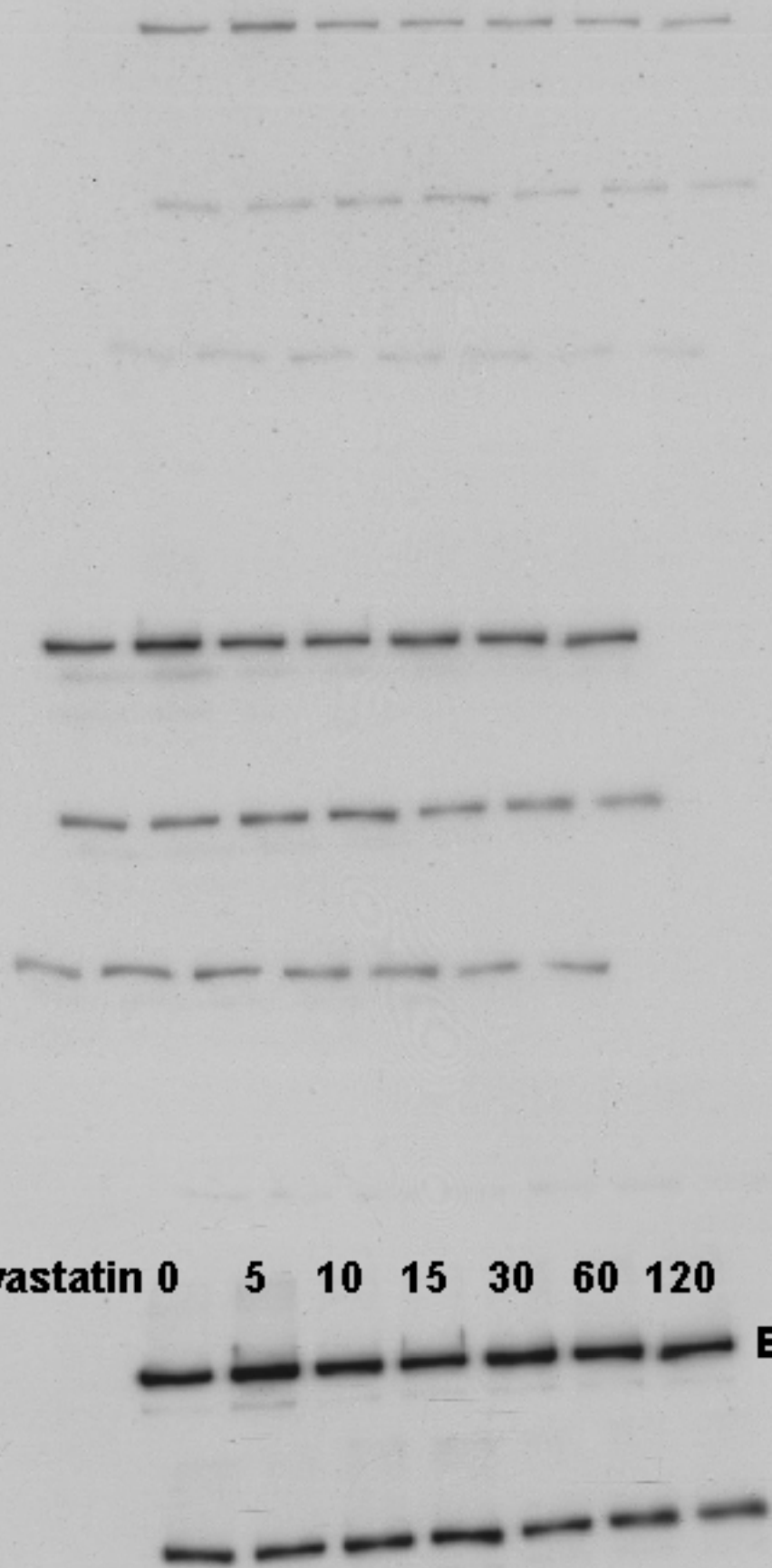
Simvastatin 0 5 10 15 30 60 120

pEGFR



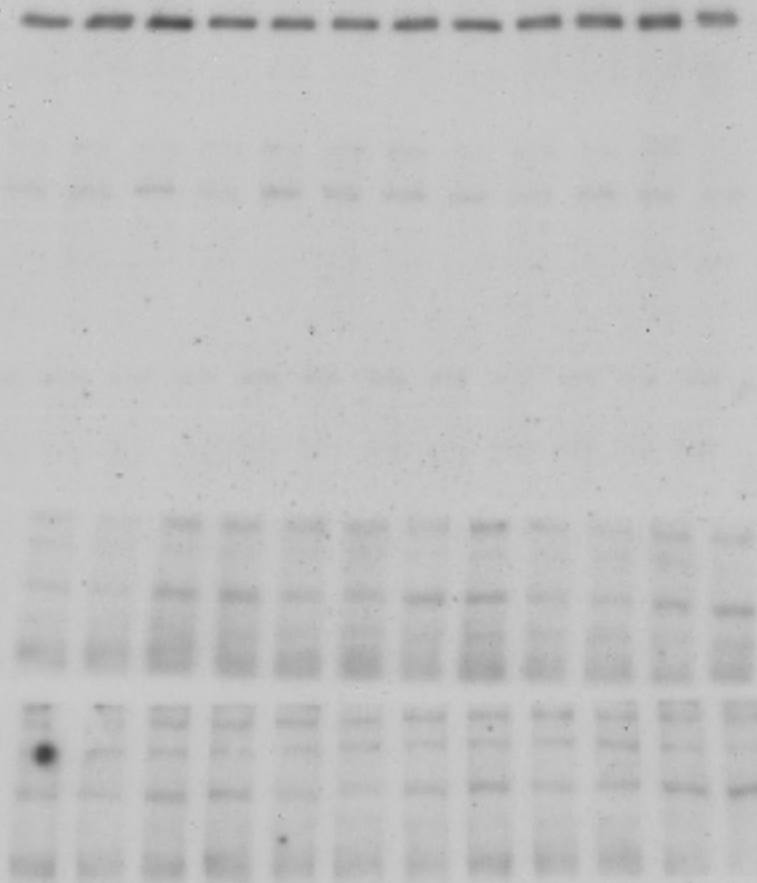
Simvastatin 0 5 10 15 30 60 120

EGFR



Simvastatin 0 5 10 15 30 60 120

p-Src

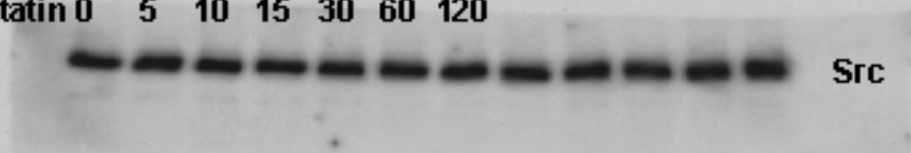


...YTRRAA2 ILUQA...

...YTRRAA2 ILUQA...

...YTRRAA2 ILUQA...

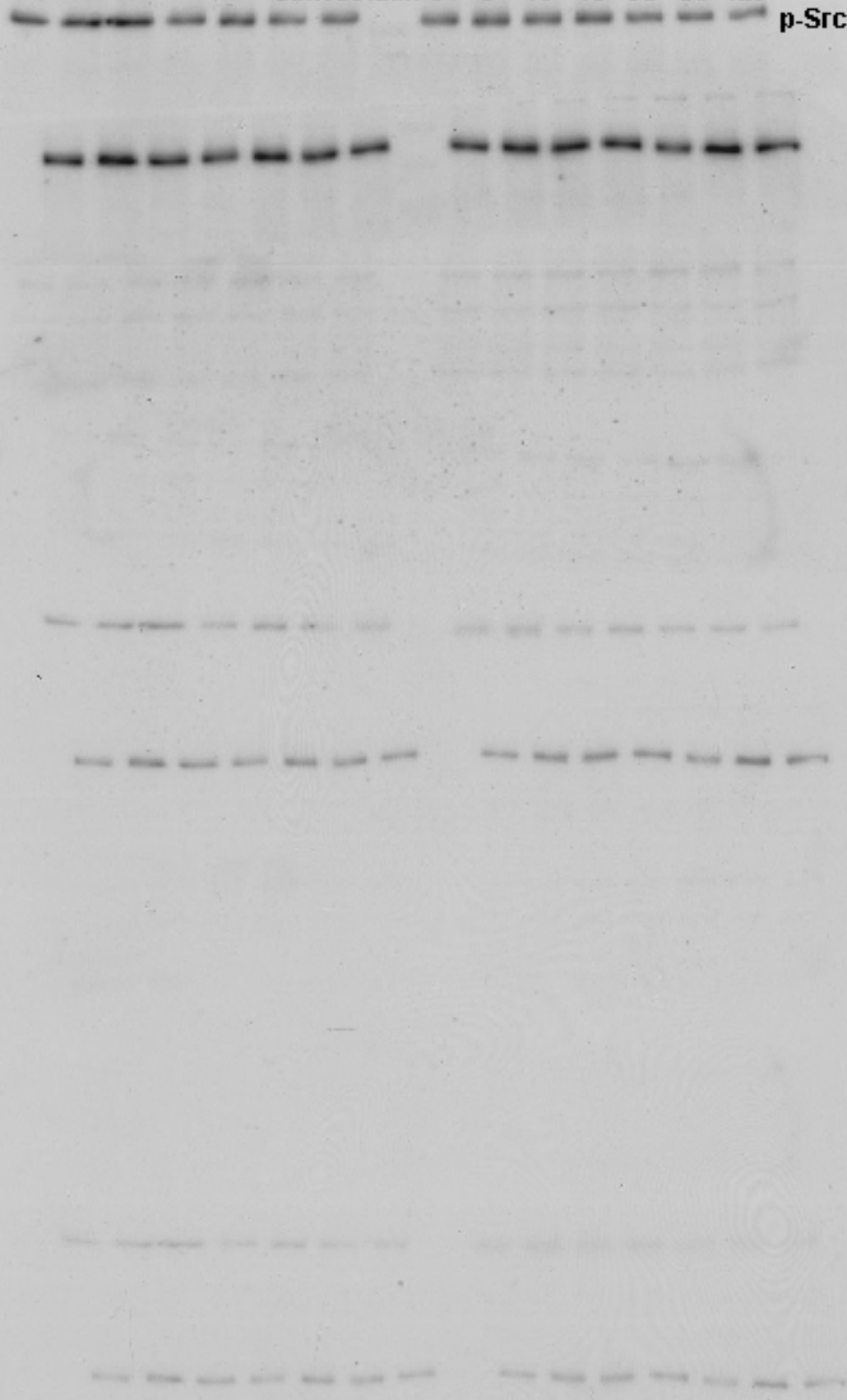
Simvastatin 0 5 10 15 30 60 120



Src

Simvastatin 0 5 10 15 30 60 120

p-Src



...FUJI SAFETY...

...FUJI SAFETY...

...FUJI SAFETY...

Simvastatin 0 5 10 15 30 60 120

Src

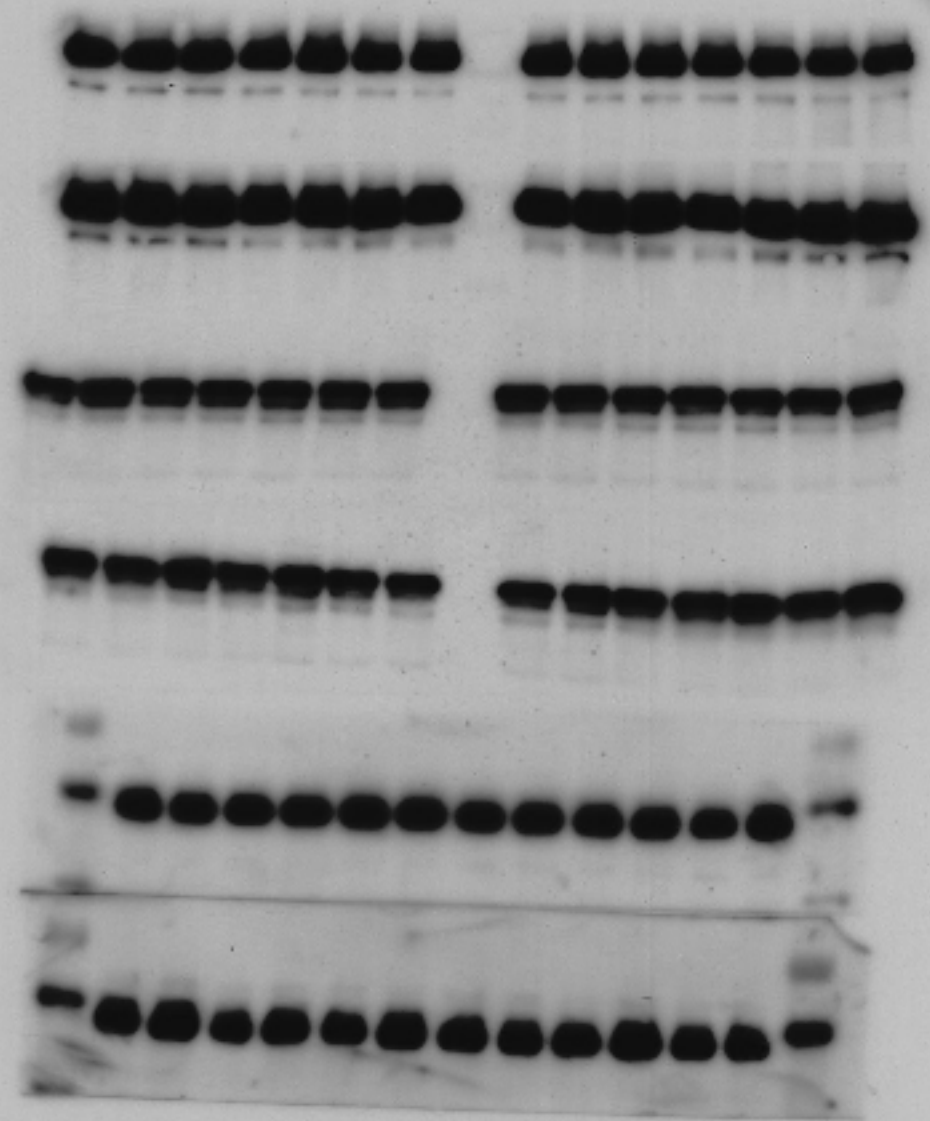
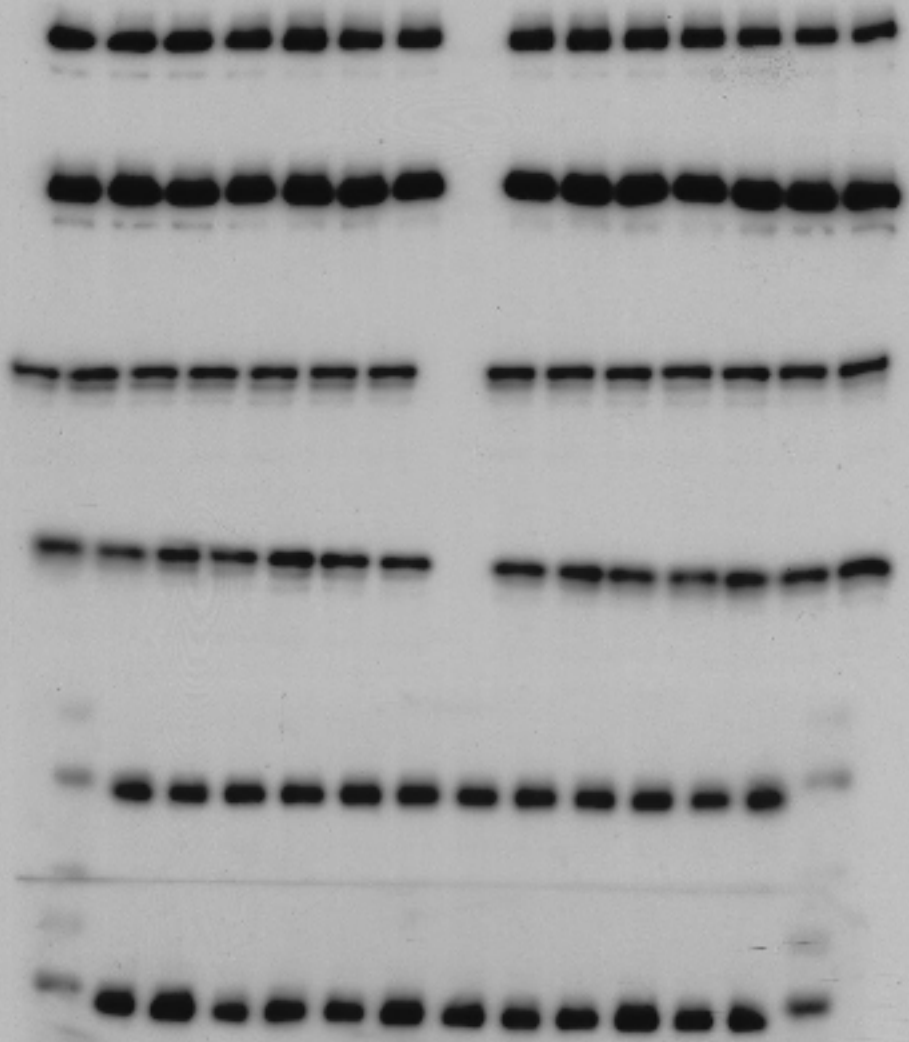
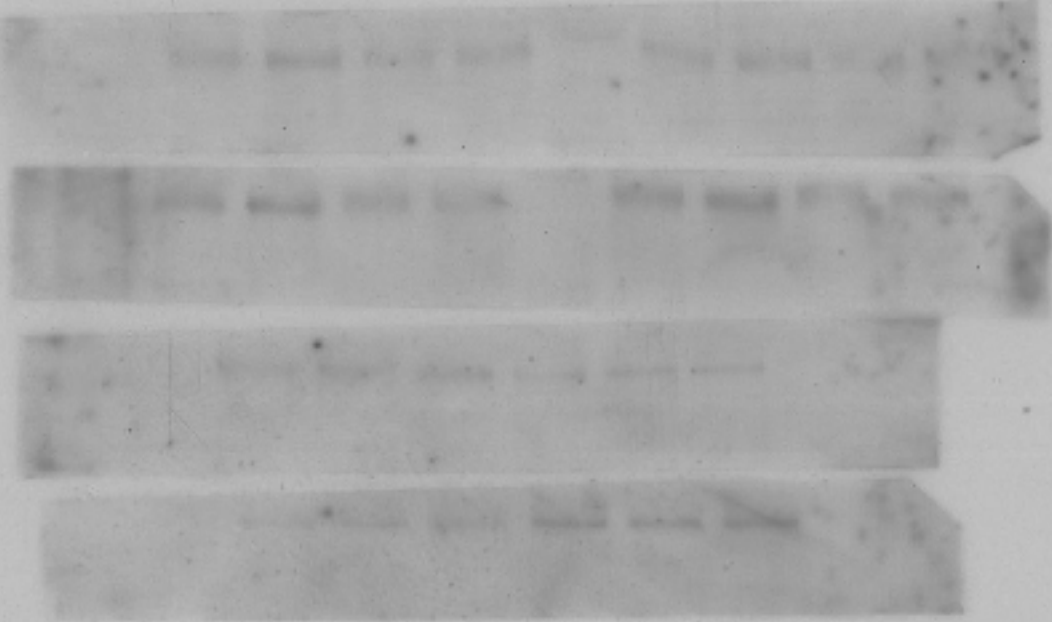
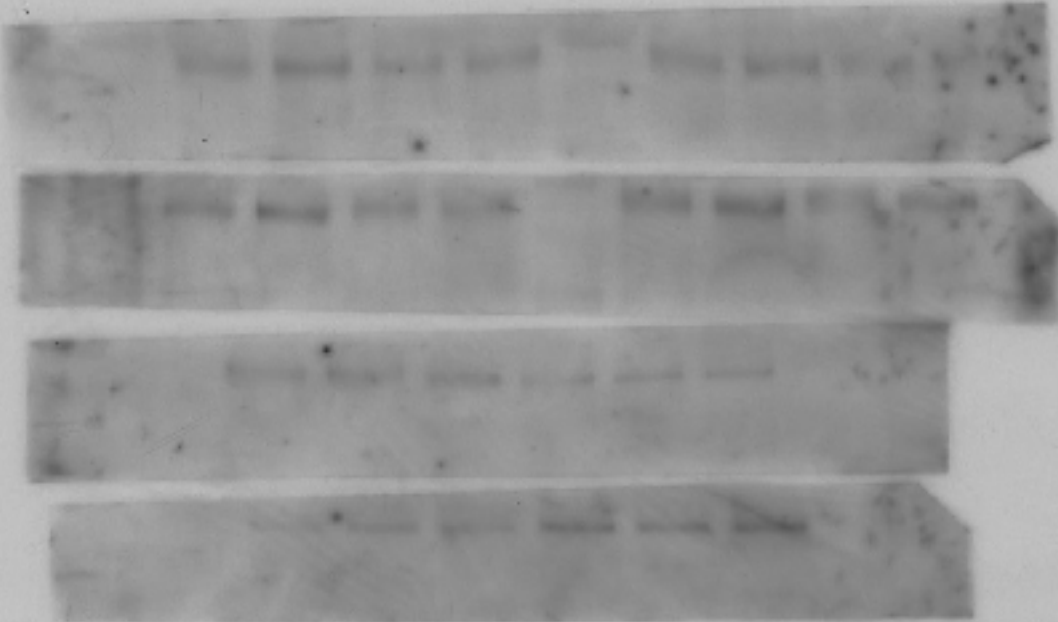


Fig S8. c-Src signaling is essential for simvastatin-induced sEH tyrosine phosphorylation and the formation of an sEH–Akt–AMPK–eNOS complex.

YTHFAQIUQF



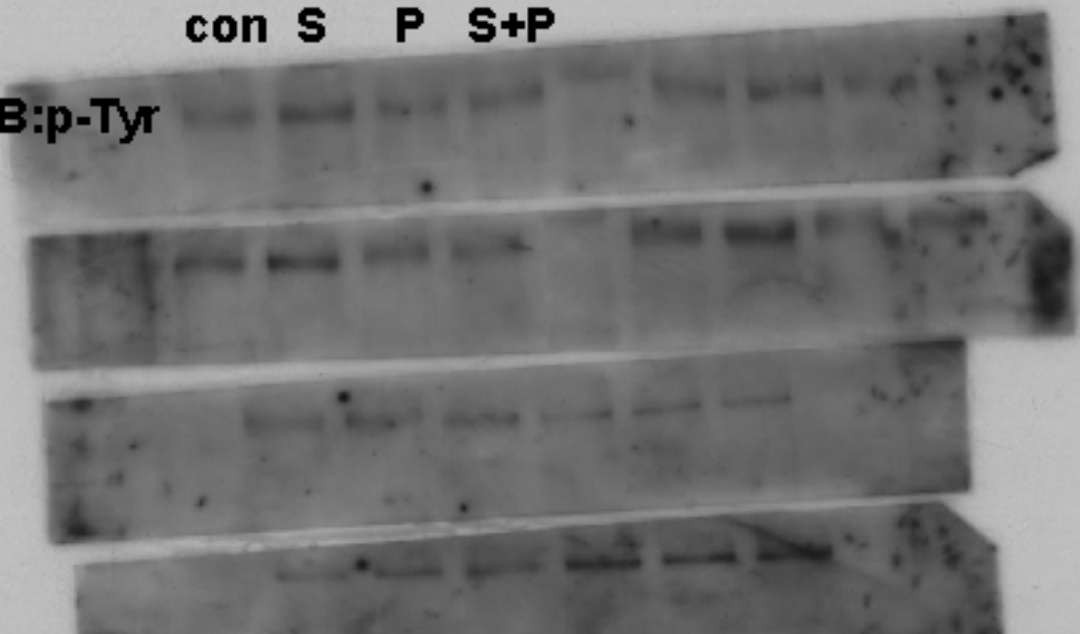
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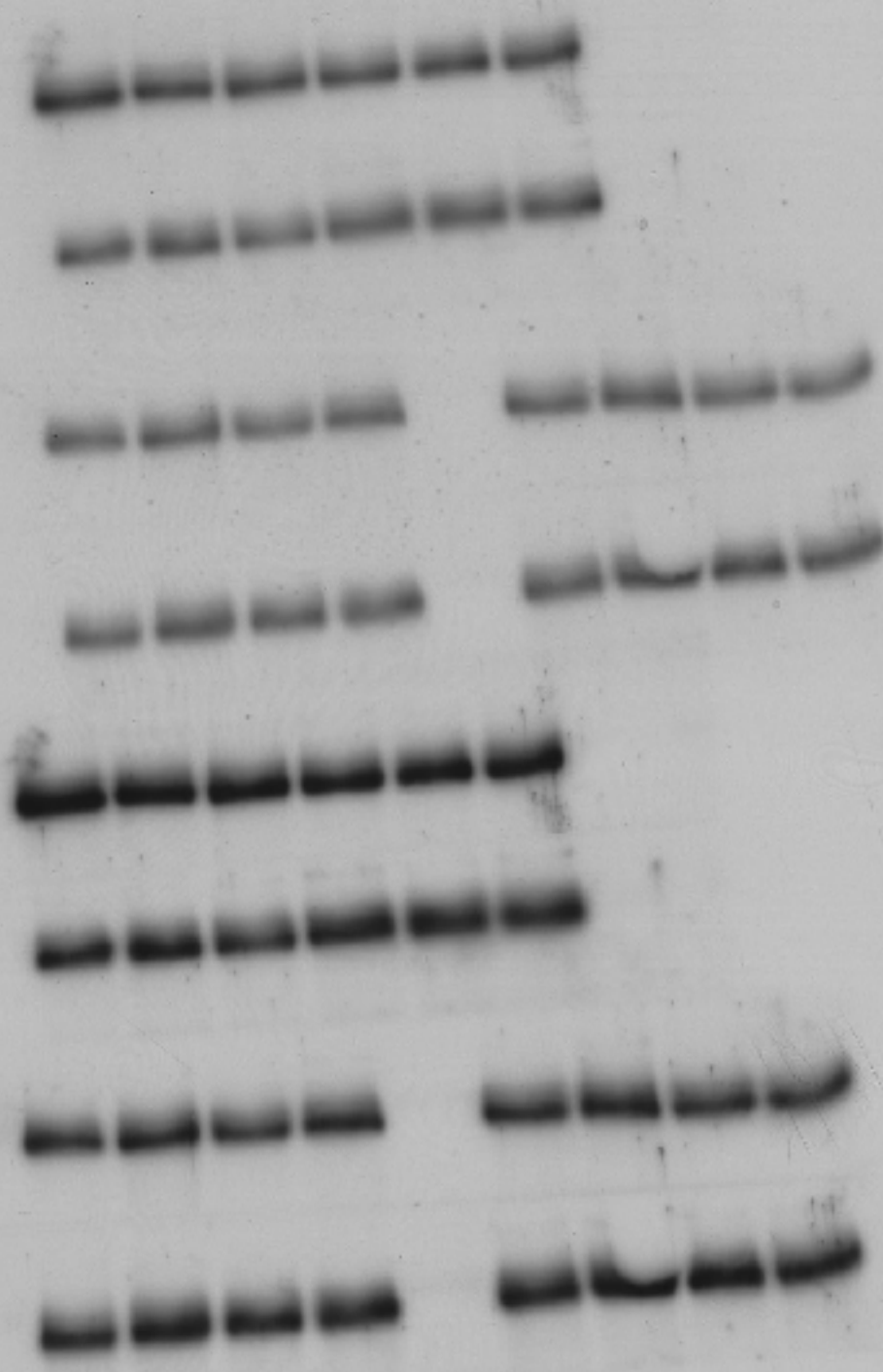


con S P S+P

IP: sEH IB:p-Tyr

YTHFAQIUQF





con S P S+P

IP: sEH IB:sEH



con S K S+K

IP: sEH IB: p-Tyr

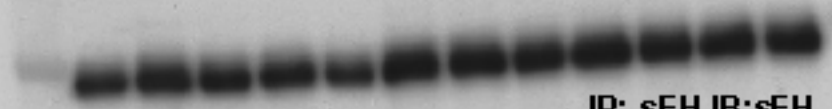
...FUJIFILM SAFETY ...

...FUJIFILM SAFETY ...

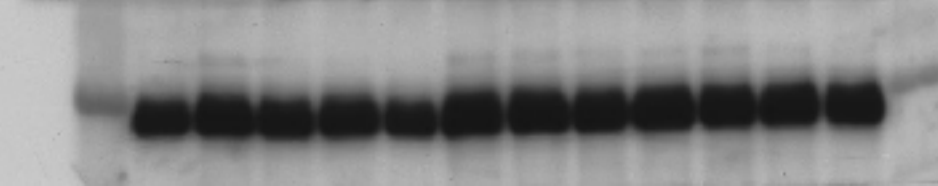
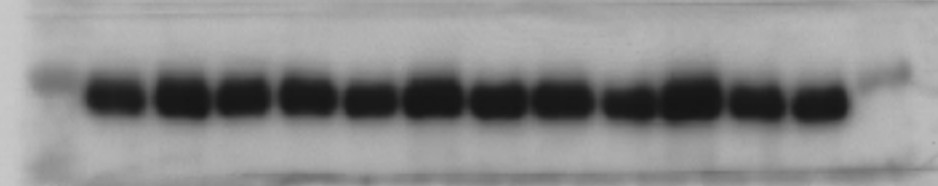
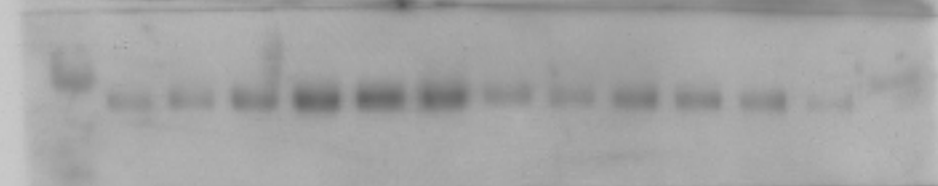
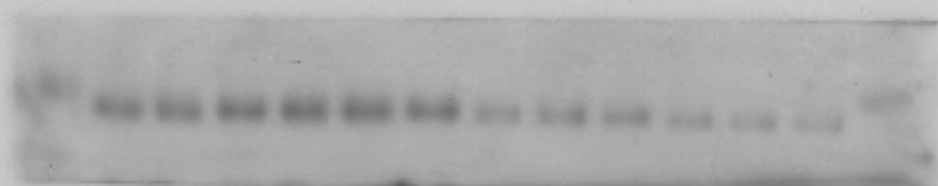
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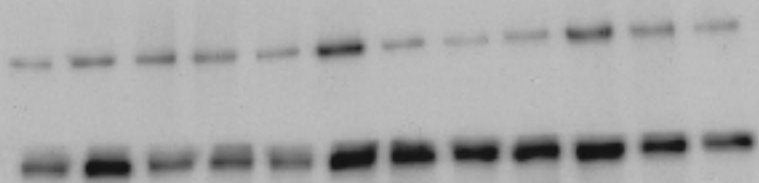
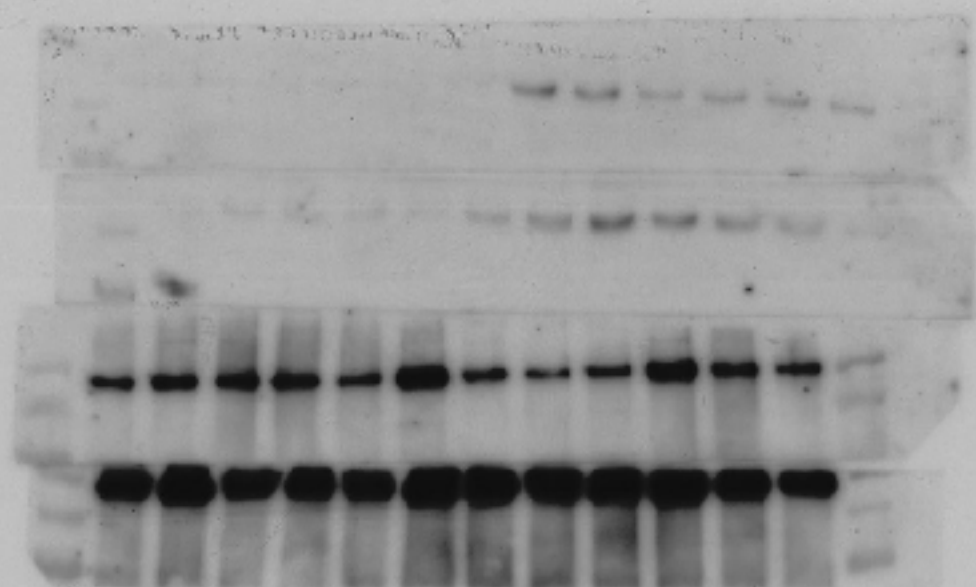


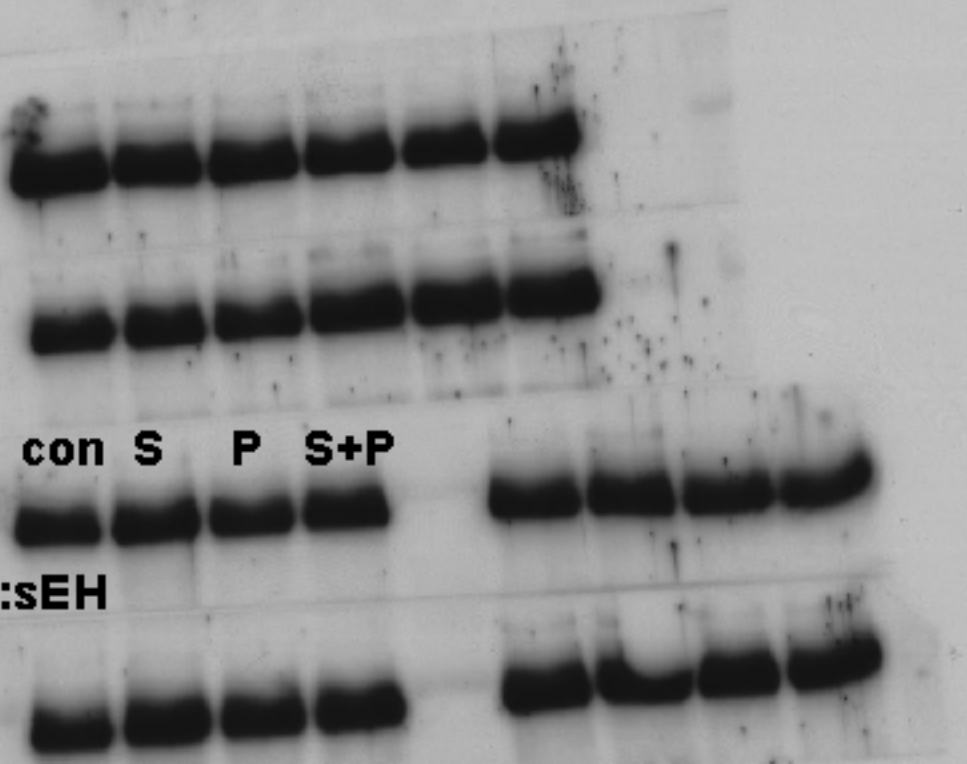
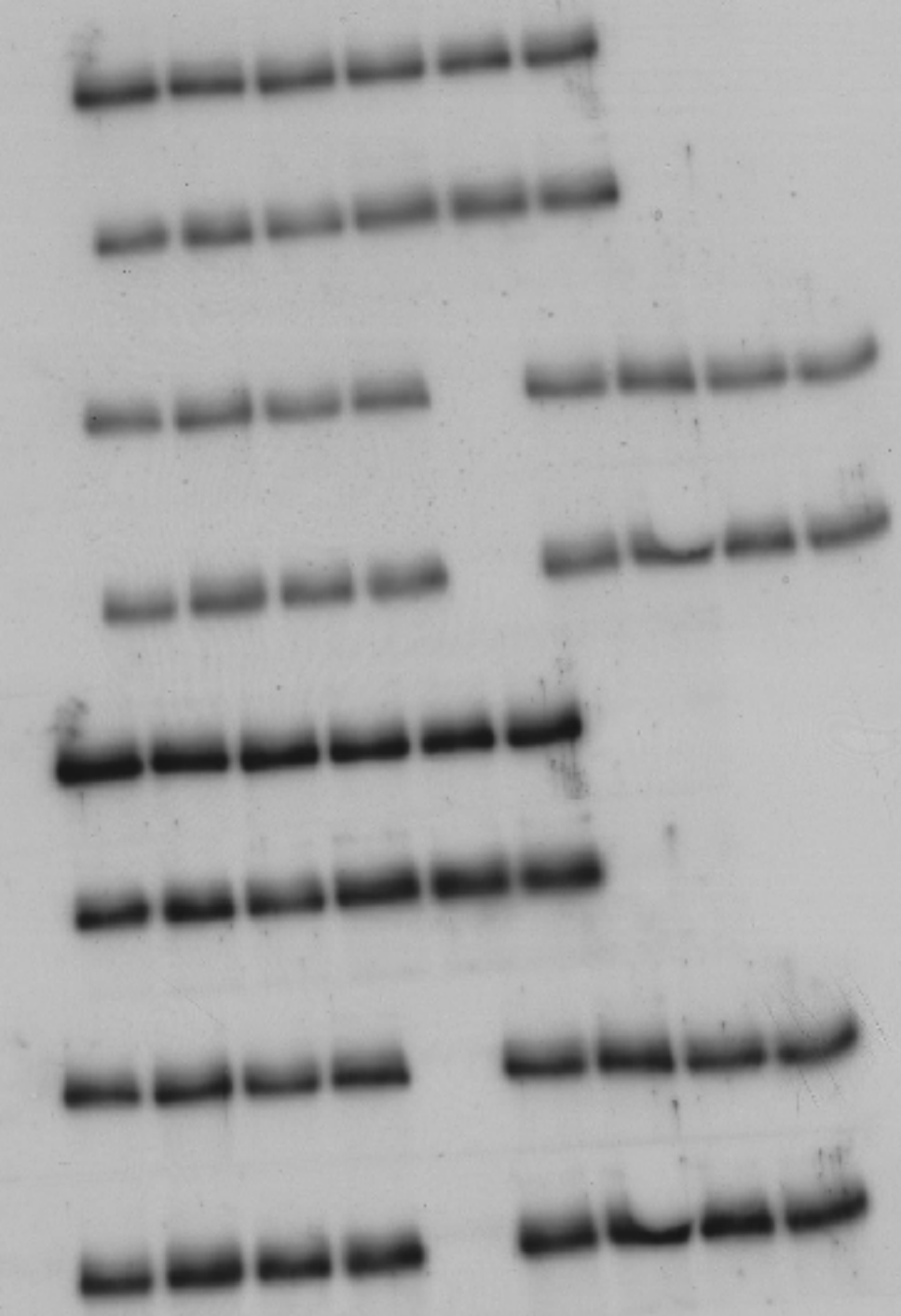
con S K S+K



IP: sEH IB:sEH

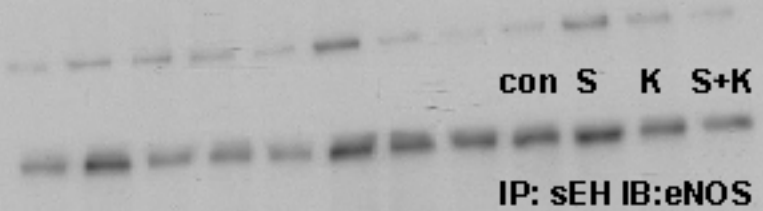




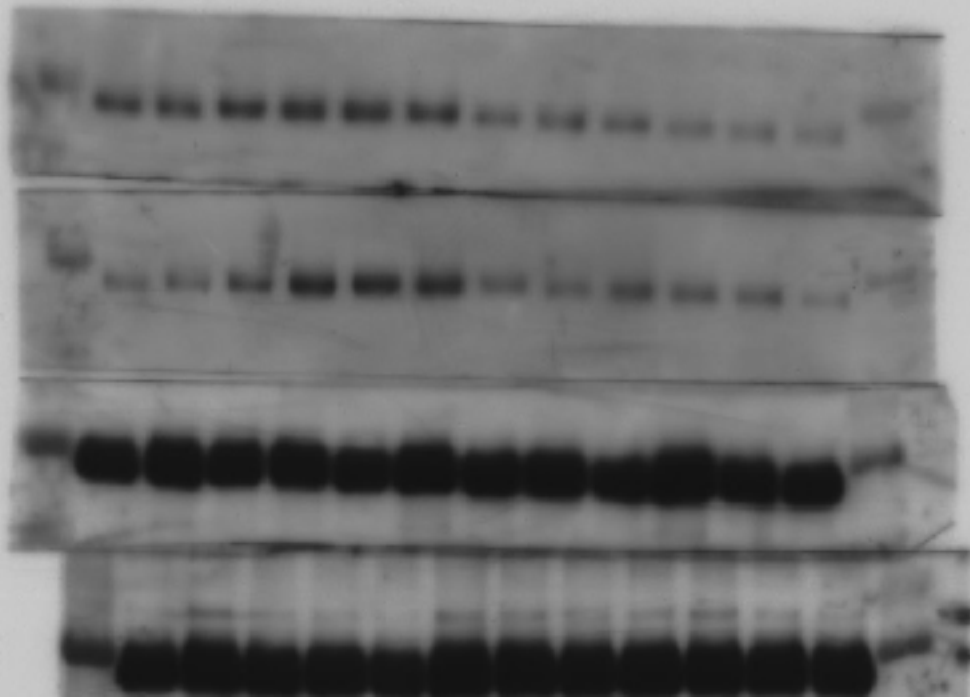
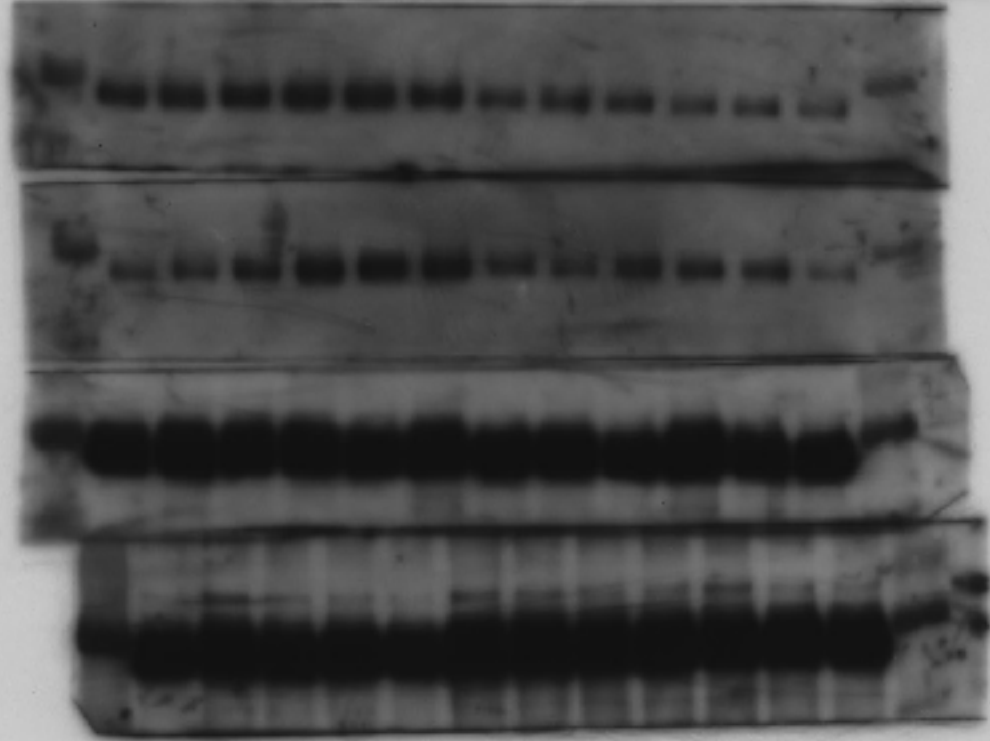
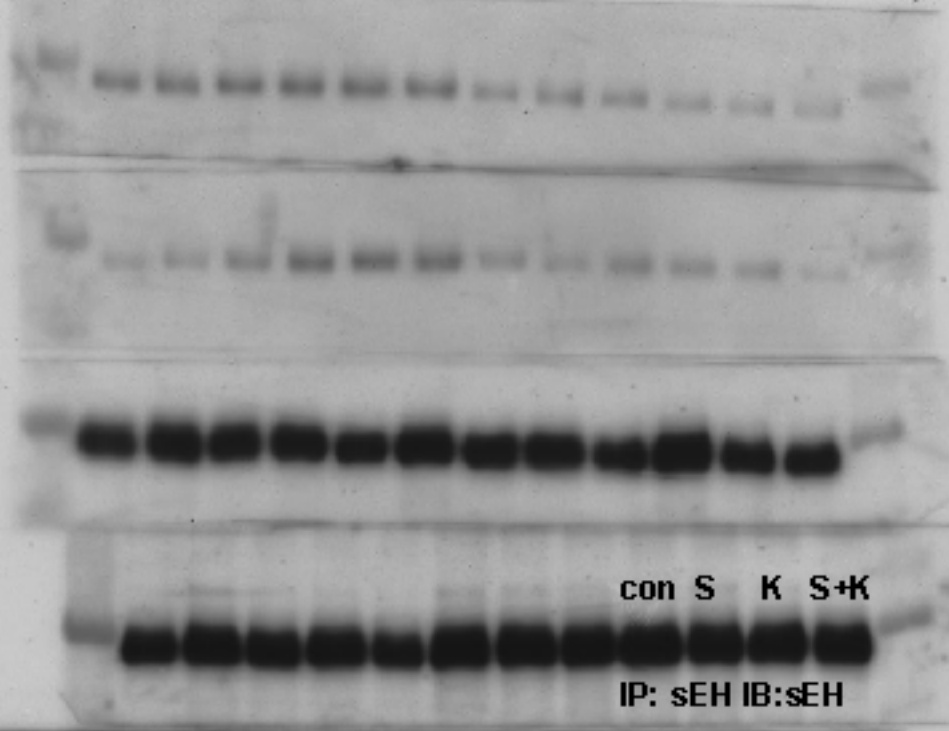


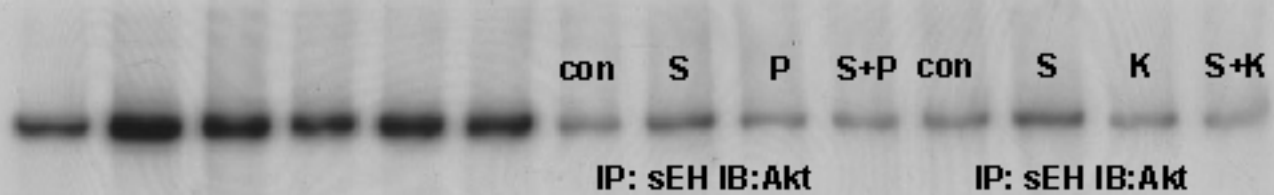
con S P S+P

IP: sEH IB:sEH



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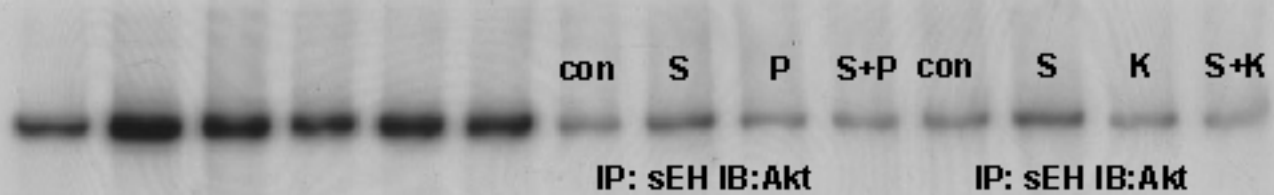
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con S P S+P con S K S+K

IP: sEH IB:sEH IP: sEH IB:sEH

23



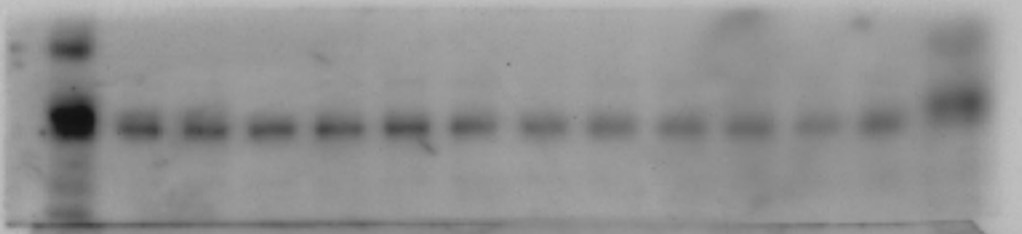
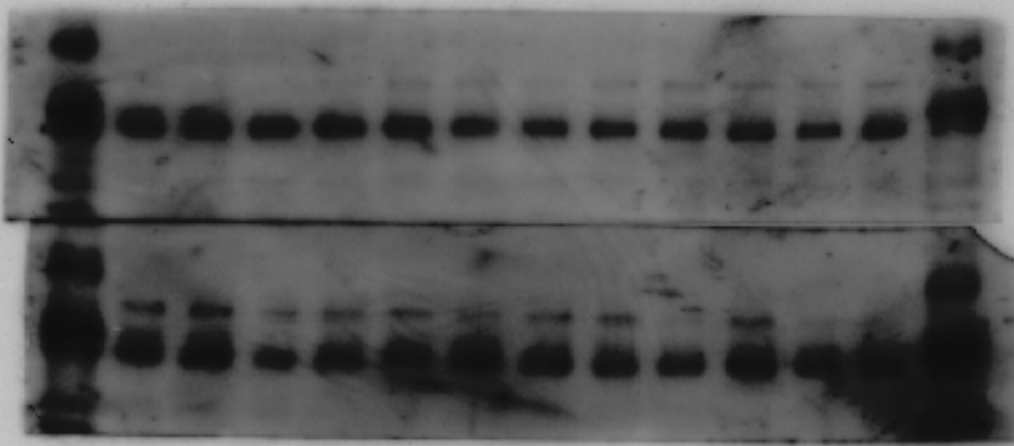
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con S P S+P con S K S+K

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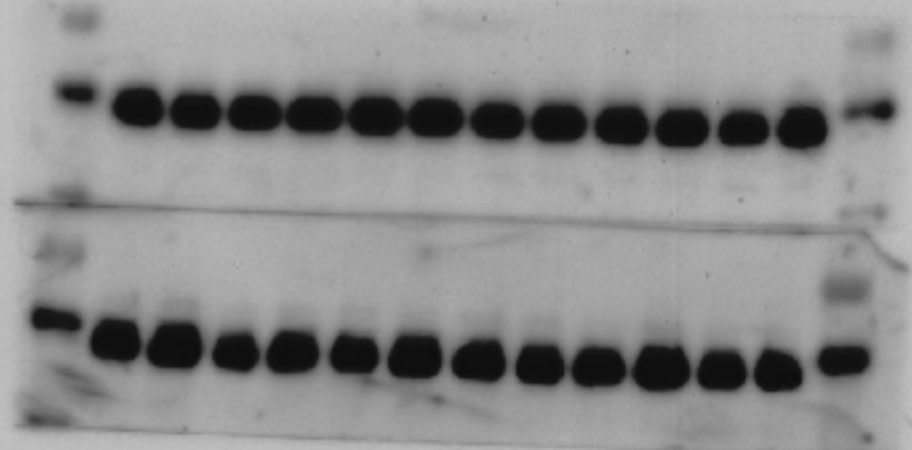
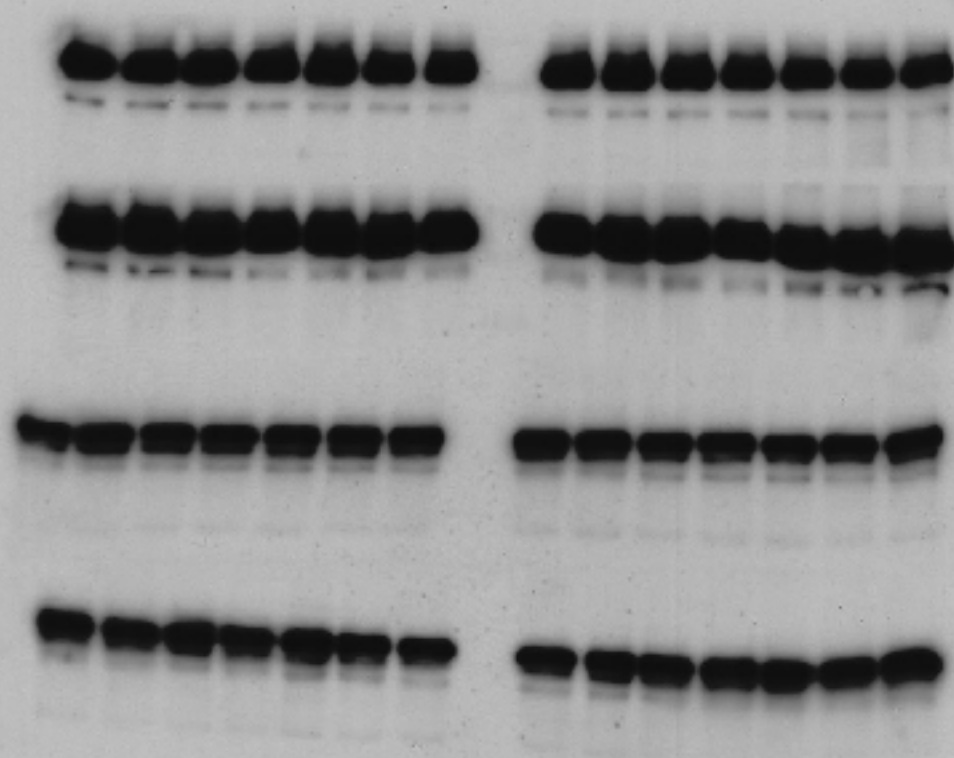
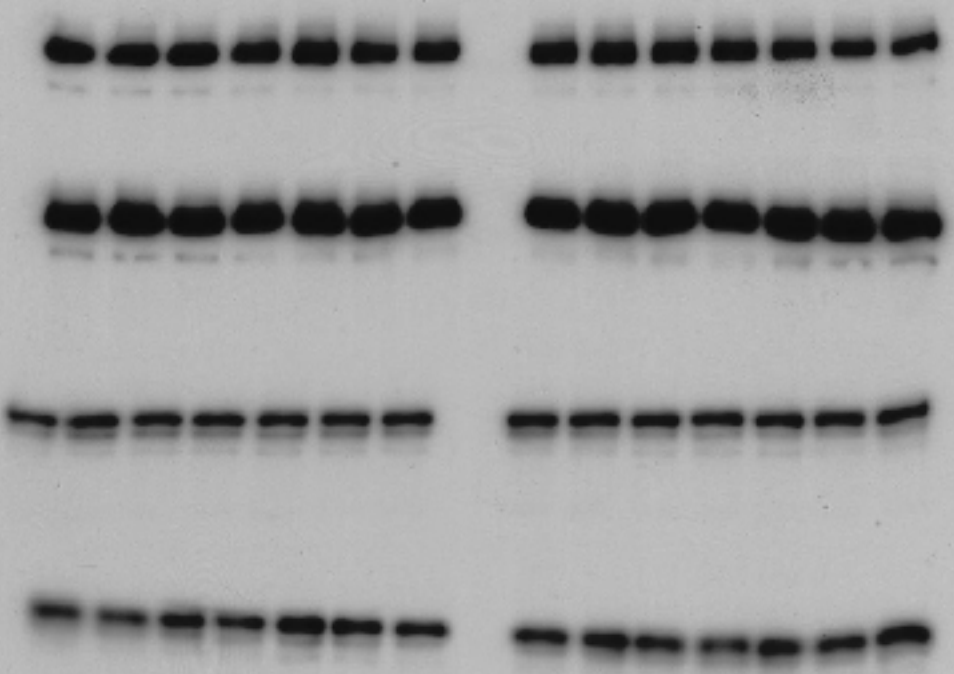
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con S P S+P con S K S+K

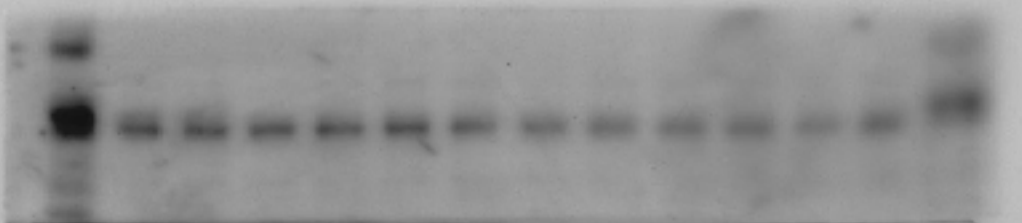
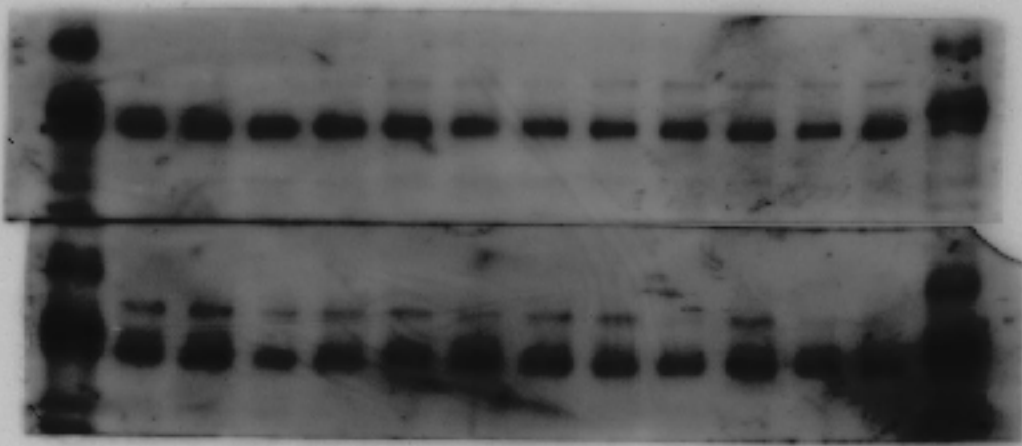
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29



con S P S+P con S K S+K

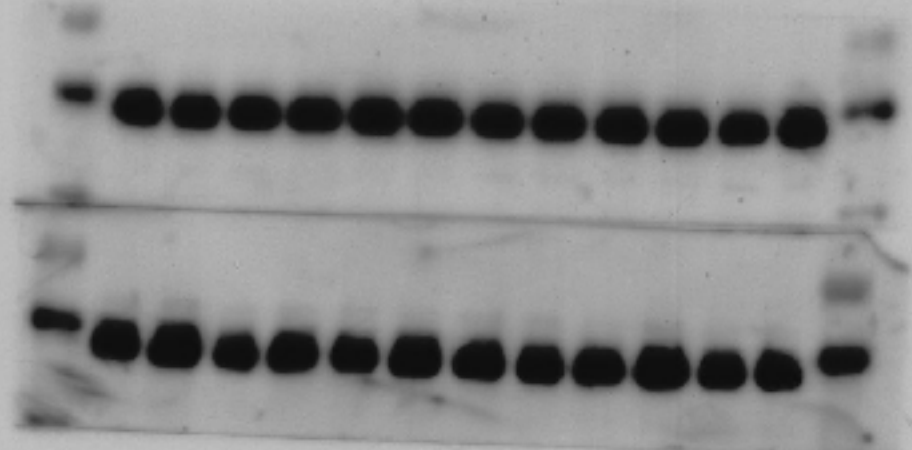
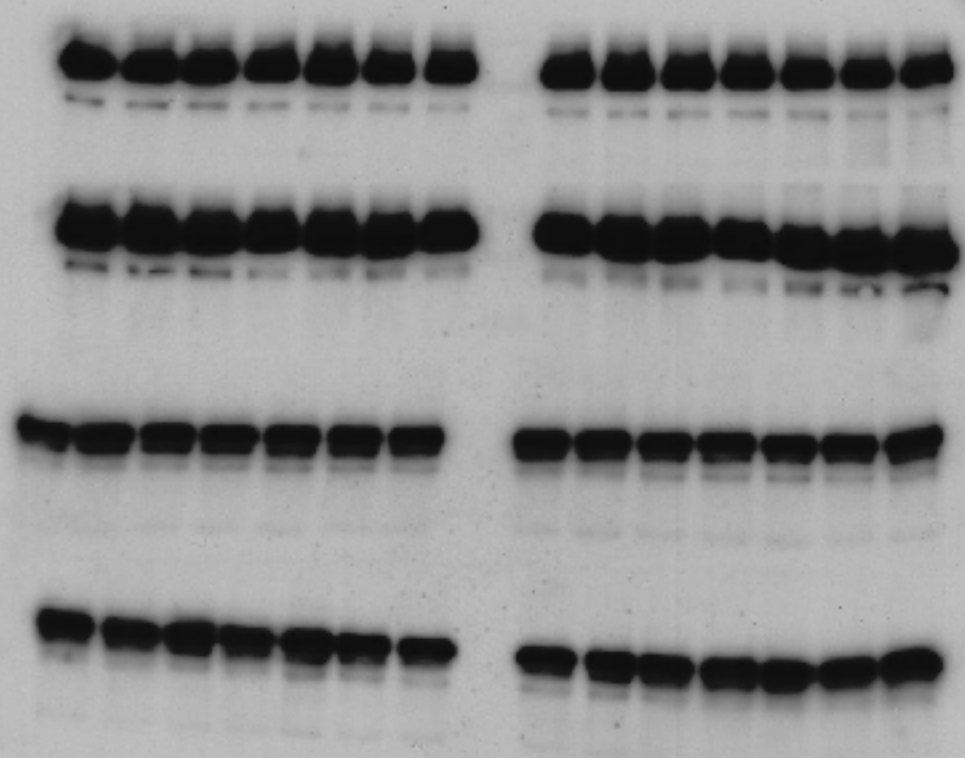
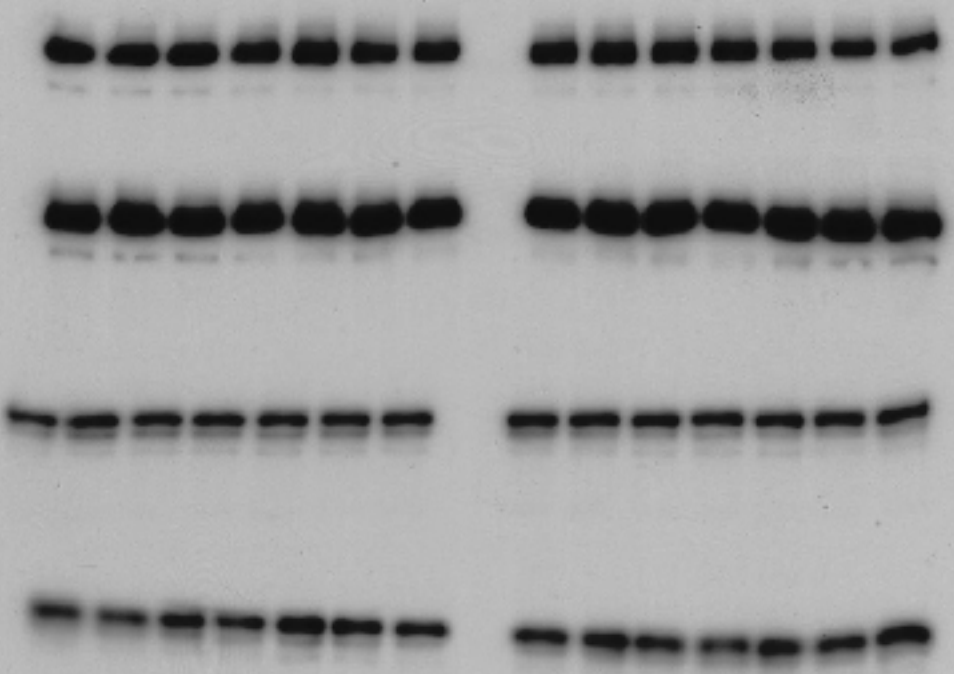
IP: sEH IB: sEH IP: sEH IB: sEH



con S P S+P con S K S+K

IP: sEH IB:AMPK IP: sEH IB:AMPK

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con S P S+P con S K S+K

IP: sEH IB: sEH IP: sEH IB: sEH