mTOR interacts with AIF to positively regulate autophagy

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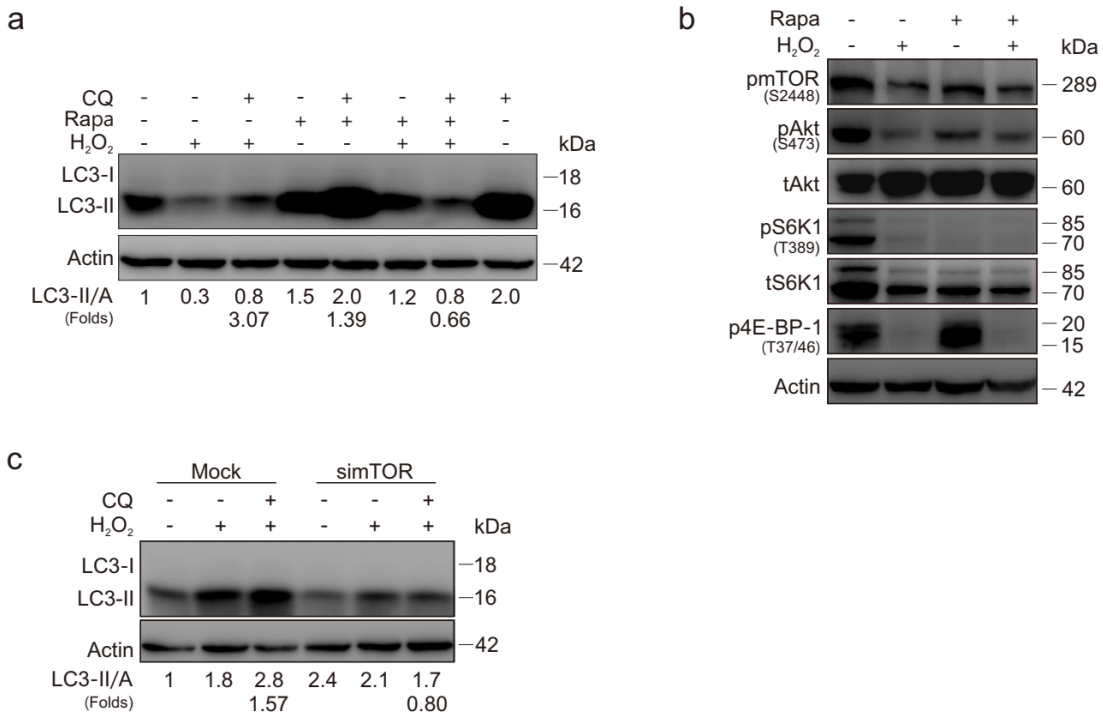
This Supplementary information includes:

Supplementary Figure: Figure 1-4

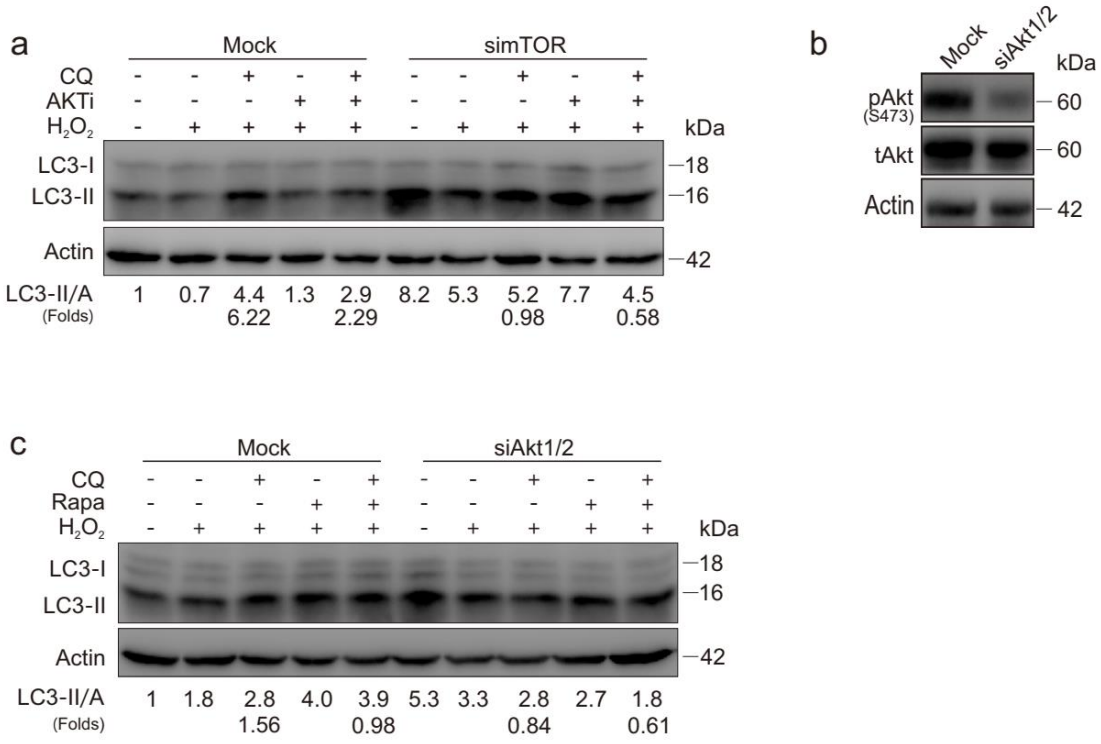
Scanned raw images for the western blot

Supplemental 1



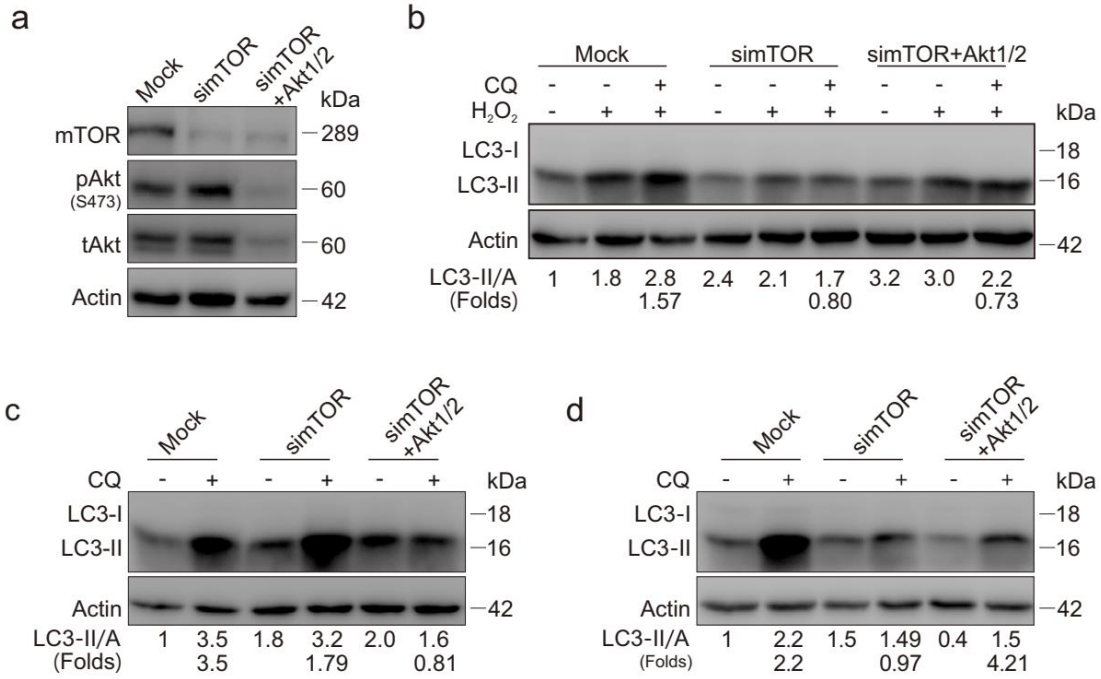
Supplemental Figure 1. Deprivation of mTOR abolishes H_2O_2- induced autophagy. (**a and b**) Following treatments with H_2O_2 (0.5 mM) or together with Rapa (200 nM) for 4 h in the presence or absence of CQ (20 μM, 2 h), HeLa cells were lysed and analyzed by immunoblotting using the indicated antibodies; actin was used as a loading control. (**c**) HeLa cells were transfected with mTOR siRNAs for 48 h, Cell lysates were analyzed by immunoblotting following treatments with H_2O_2 (0.5 mM) for 4 h in the presence or absence of CQ (20 μM, 2 h), actin was used as a loading control.

Supplemental 2



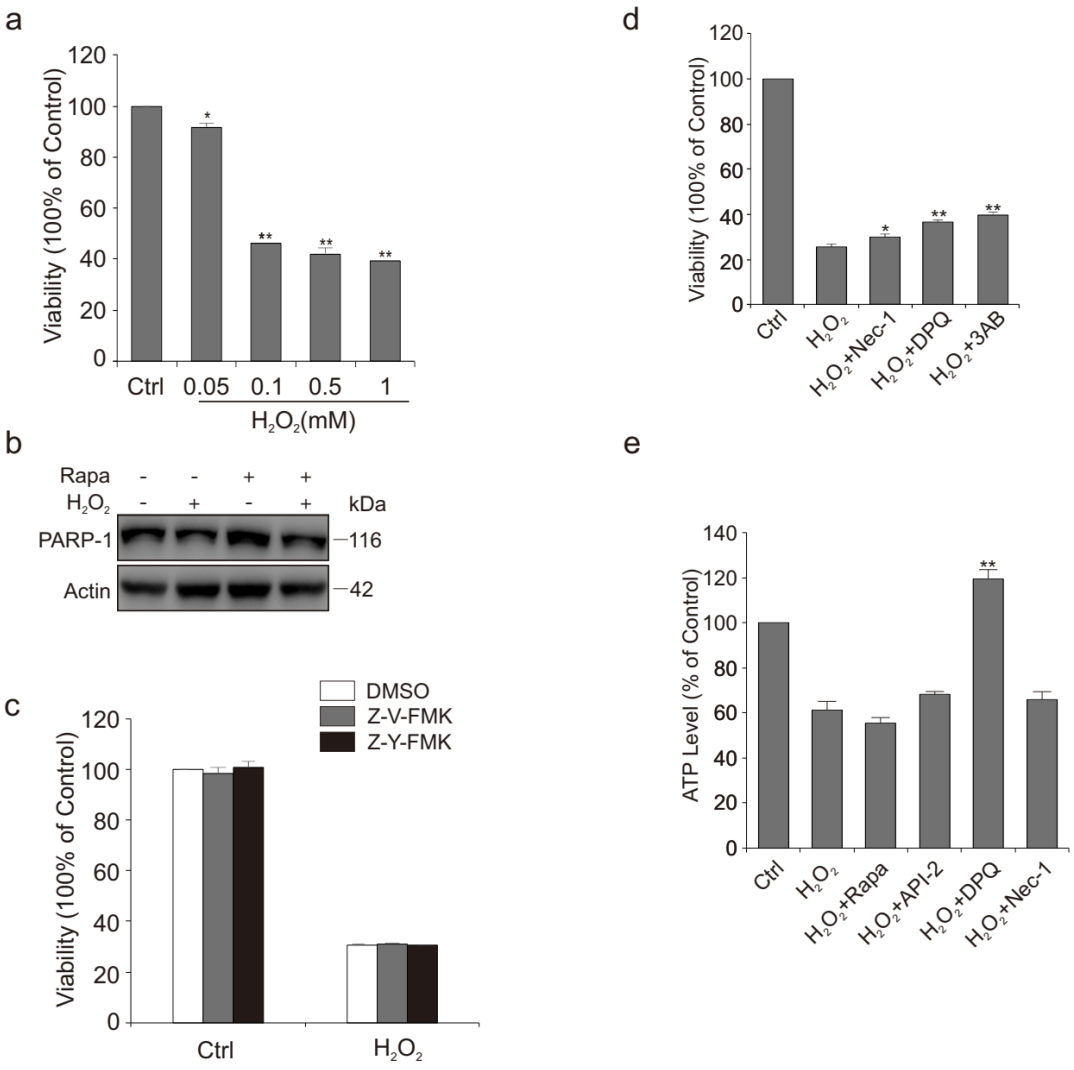
Supplemental Figure 2. Inhibition of Akt fails to rescue the induced autophagy under the condition of mTOR suppression. (**a-c**) 143B cells were transfected with the siRNA of mTOR (**a**, simTOR) or Akt1/2 (**b** and **c**, siAkt1/2) for 48 h followed by indicated treatments then cells were lysed and analyzed by immunoblotting with antibodies indicated, actin was used as a loading control.

Supplemental 3



Supplemental Figure 3. mTOR positively regulates autophagy in an Akt-dependent and independent manner. (**a-d**) HeLa (**a-c**) and 143B (**d**) cells were transfected with the siRNA of mTOR or mTOR plus Akt1/2 for 48 h. Following treatments of H_2O_2 (0.5 mM) for 4 h in the presence or absence of CQ (20 μM, 2 h), and then cells were lysed and analyzed by immunoblotting with antibodies indicated, actin was used as a loading control. Lines 1-4 of (**b**) also appeared in Supplemental Figure **1c**. Lines 1-4 of (**c**) also appeared in Figure **1e**. Lines 1-4 of (**d**) also appeared in Figure **1c**.

Supplemental 4



Supplemental Figure 4. DPQ blocks H_2O_2-induced loss of cell viability and ATP.

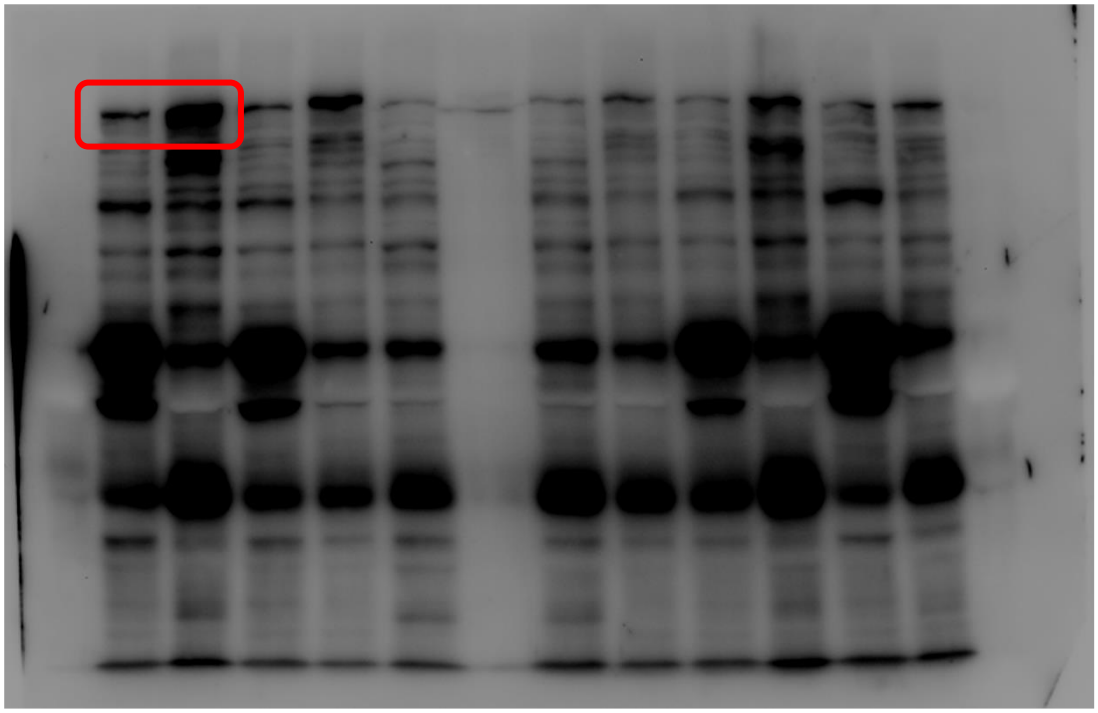
(**a**) 143B cells were treated with H_2O_2 (0.01-0.5 mM) for 4 h, cell viability was analyzed by MTS assay as described in Materials and Methods. (**b**) 143B cells were treated with H_2O_2 (0.5 mM) with or without Rapa (200 nM) for 4 h, then cell lysates were prepared and analyzed by immunoblotting using PARP1 antibody. (**c**) 143B cells were treated with 0.5 mM H_2O_2 in the presence or absence of Z-VAD-FMK (20 μM), or Z-YVAD-FMK (20 μM) for 4 h. Cell viability was analyzed by MTS assay as described in Materials and Methods. (**d and e**) HeLa cells were treated with 0.5 mM

H_2O_2 with or without Nec-1 (30 μM), DPQ (30 μM) and 3-AB (1 mM), Rapa (200 μM), or API-2 (10 μM) for 4 h, and then cell viability was analyzed by MTS assay (**d**), and ATP levels were measured as described in materials and methods (**e**). Data representing the mean ± S.D. were shown in graph. **P < 0.01 versus Ctrl group; *P < 0.05 versus Ctrl group.

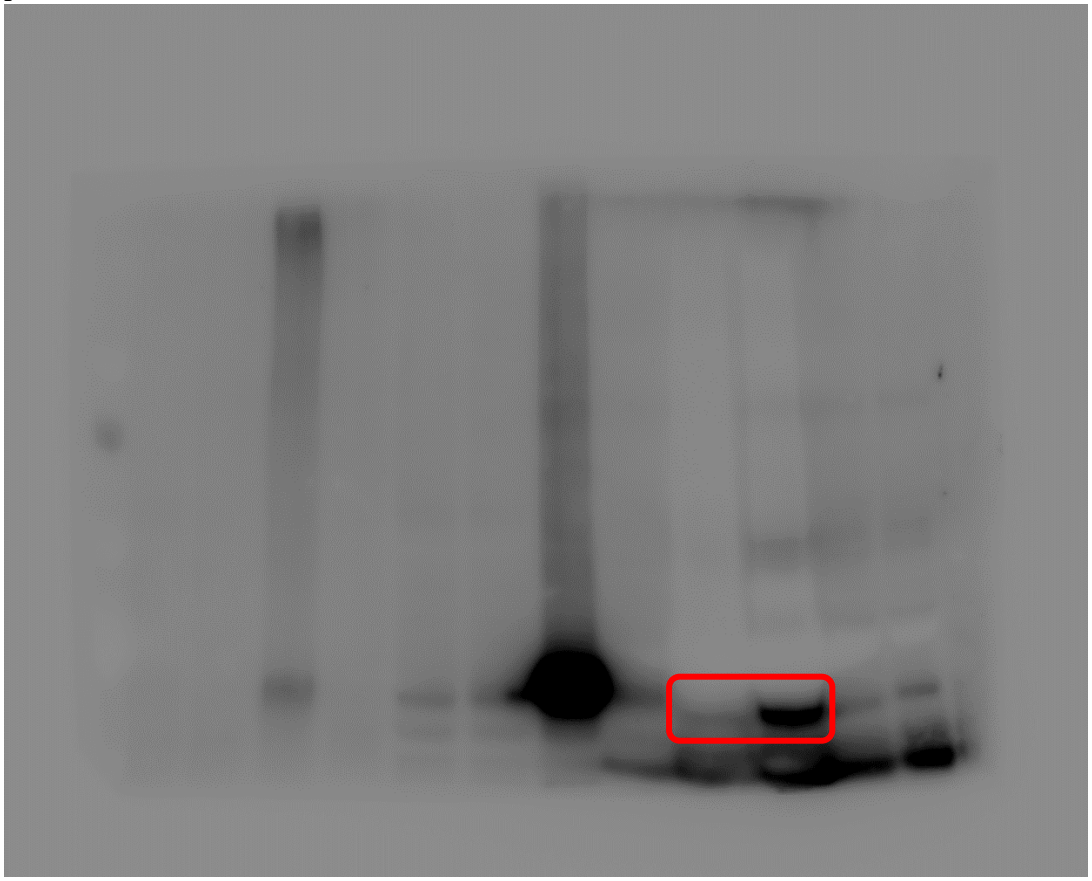
Scanned raw images for the western blot

Figure 1

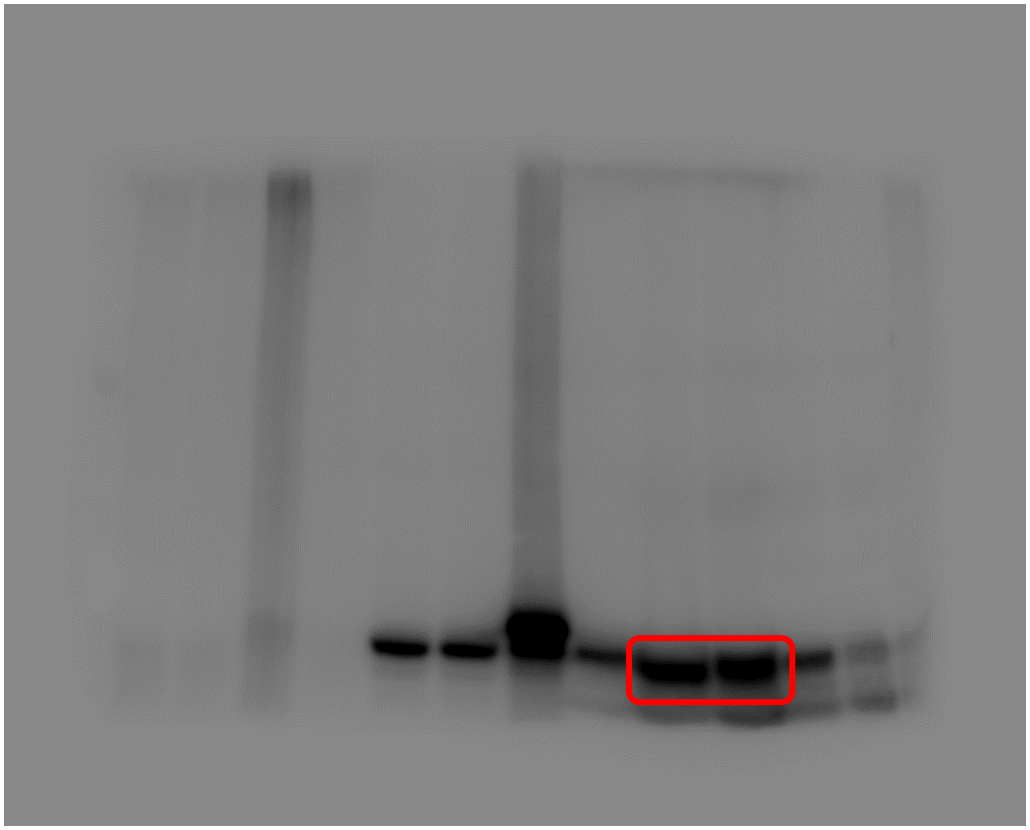
A p-mTOR



p-AKT



tAkt



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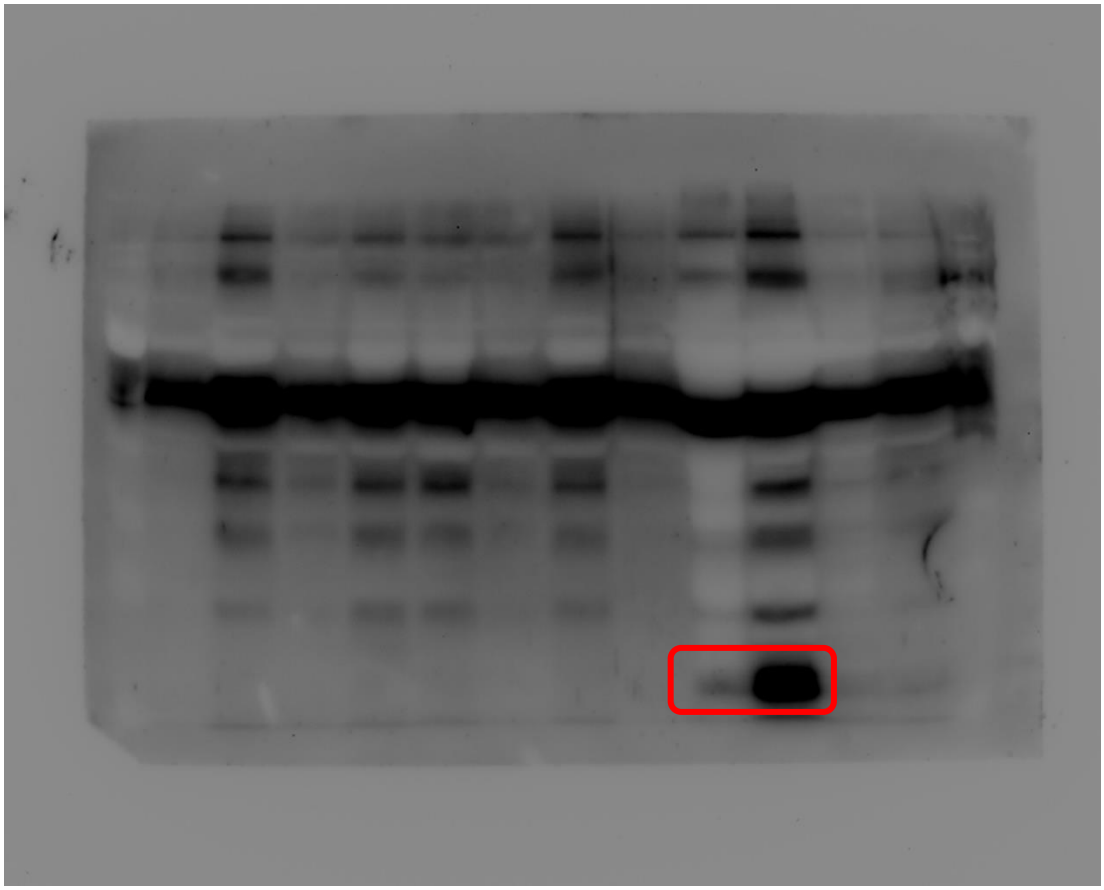

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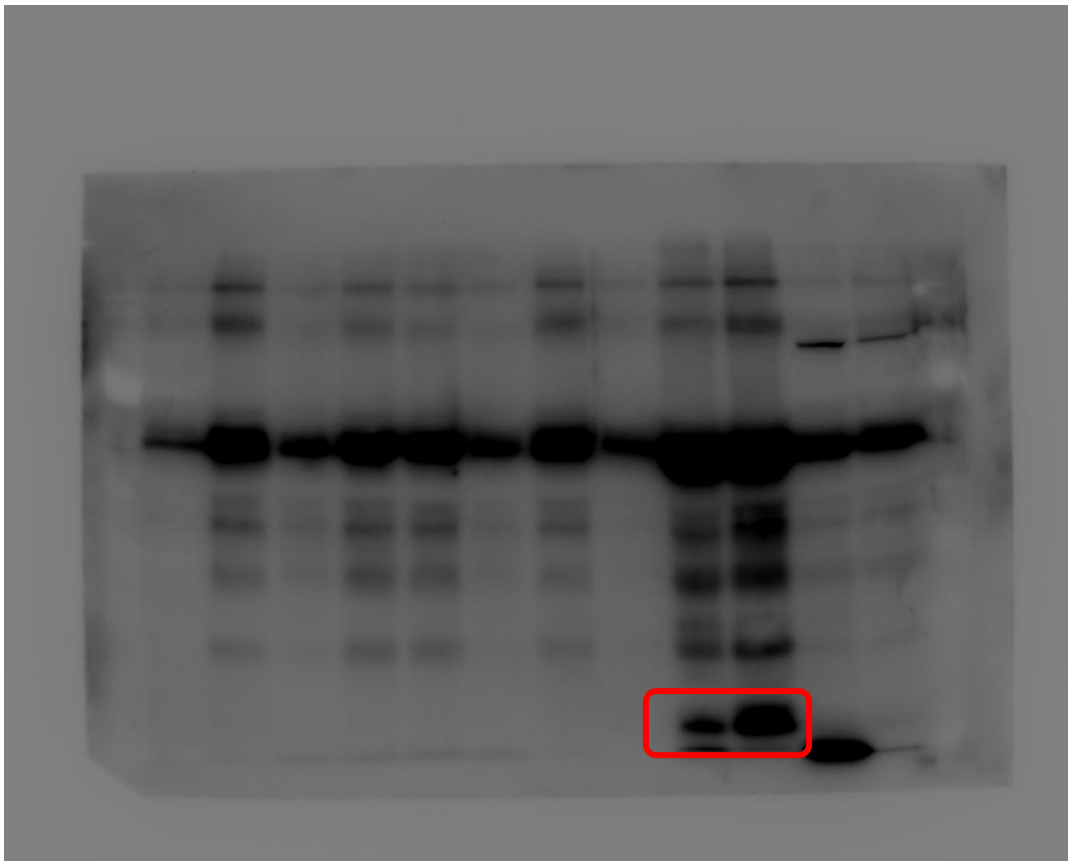
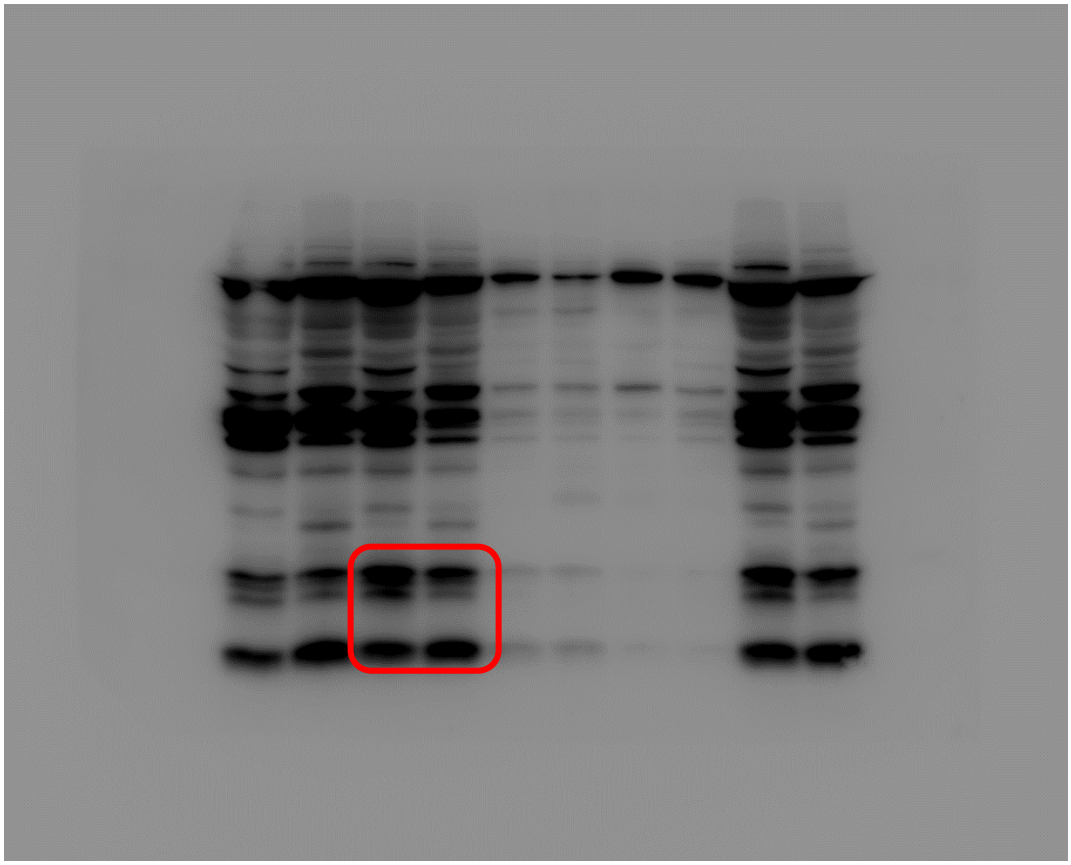


LC3



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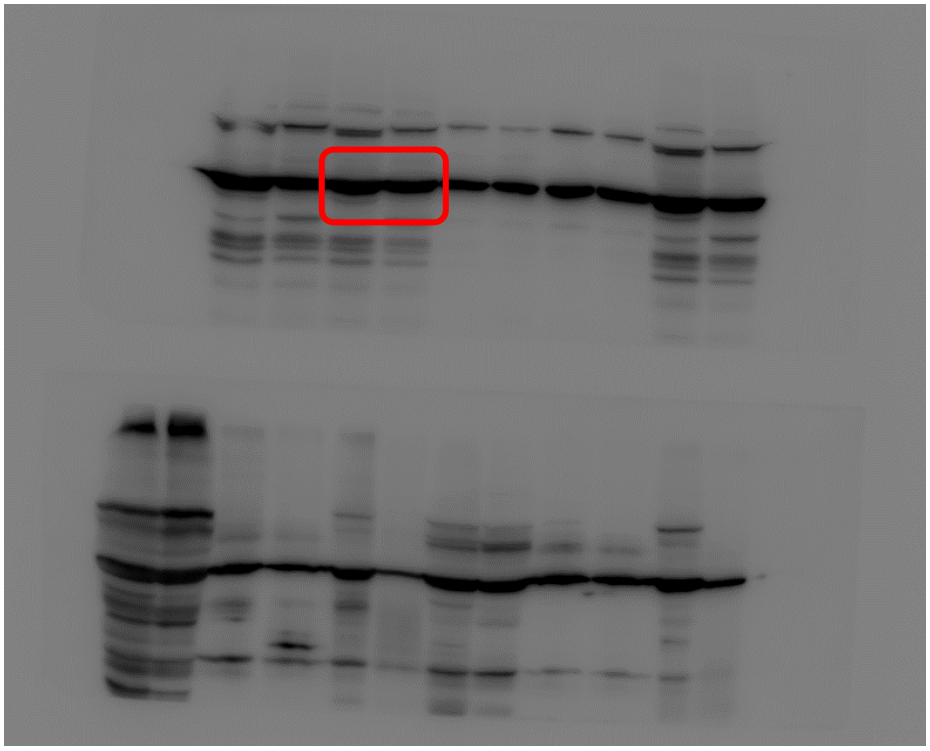
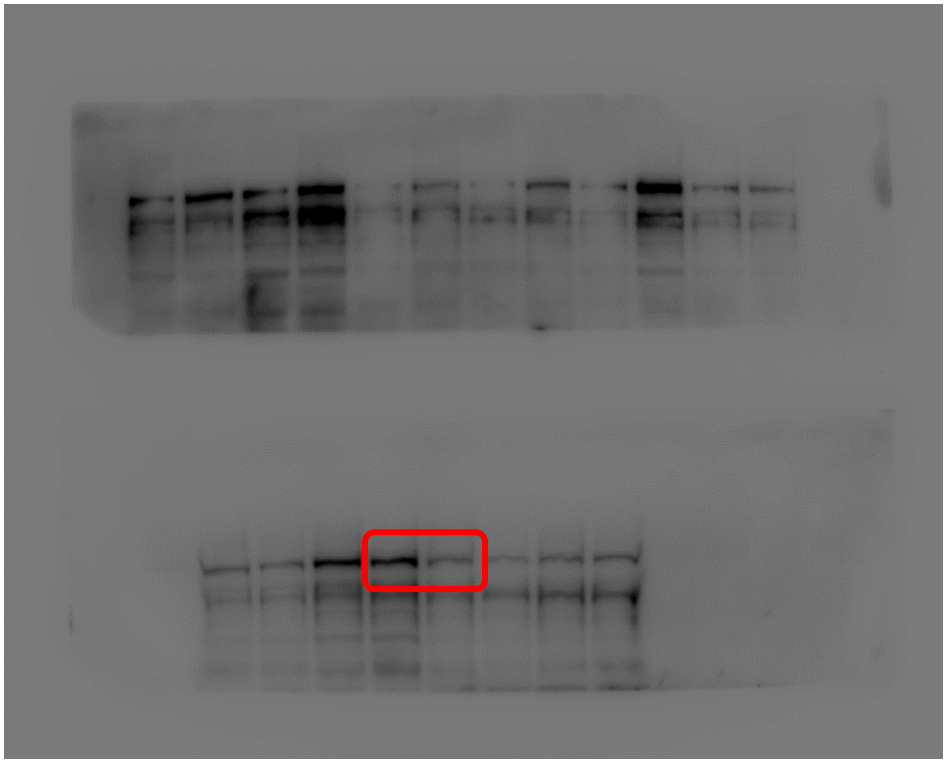


B

786-O

mTOR



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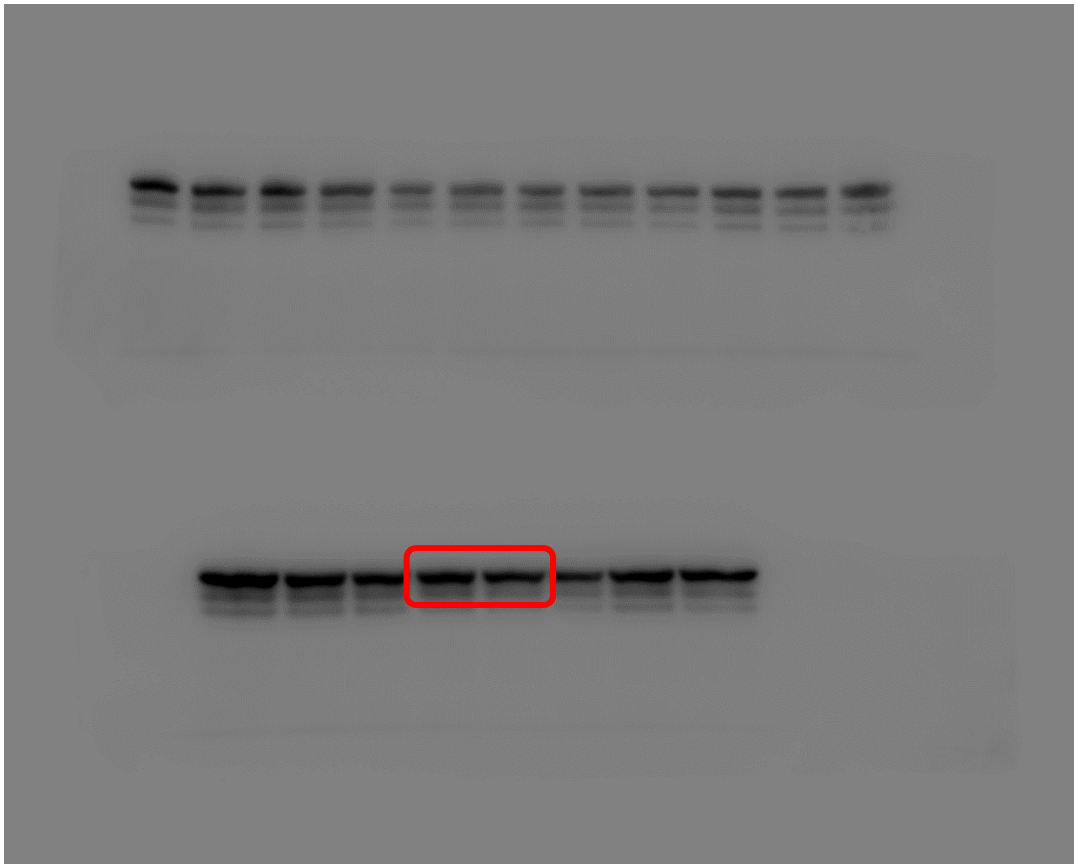
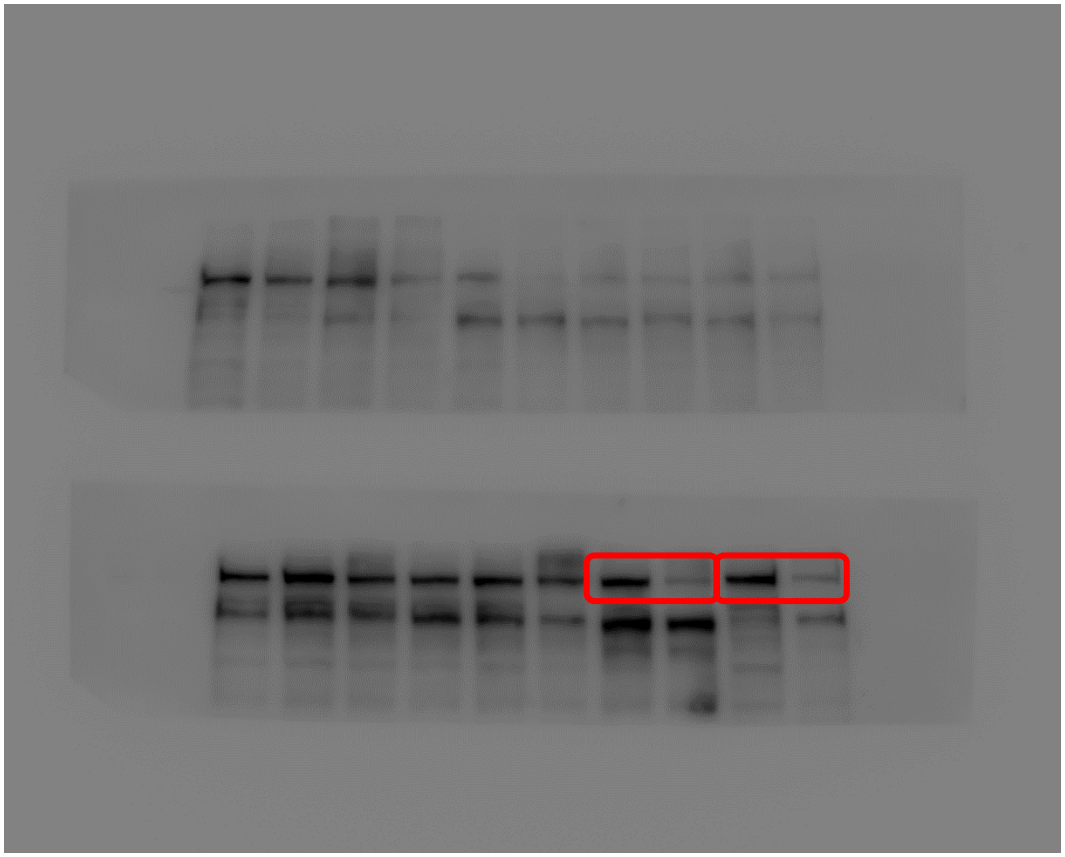


143B HeLa mTOR



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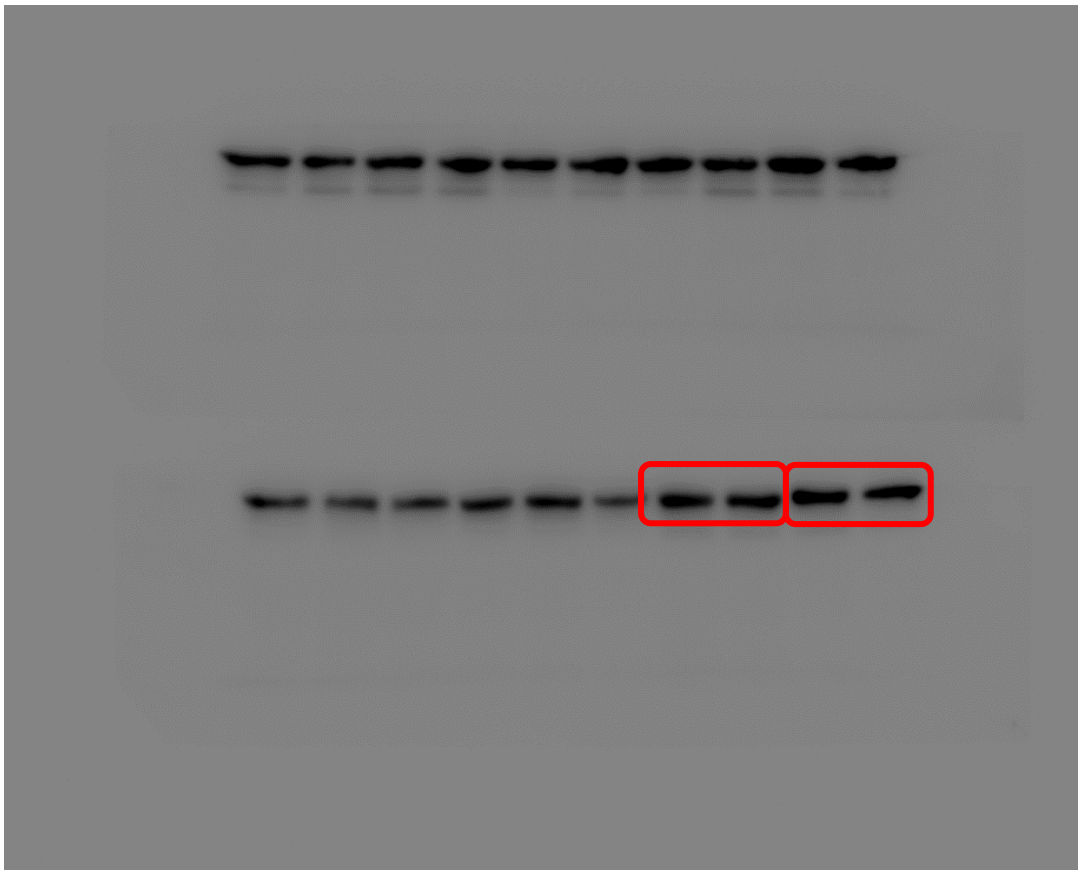
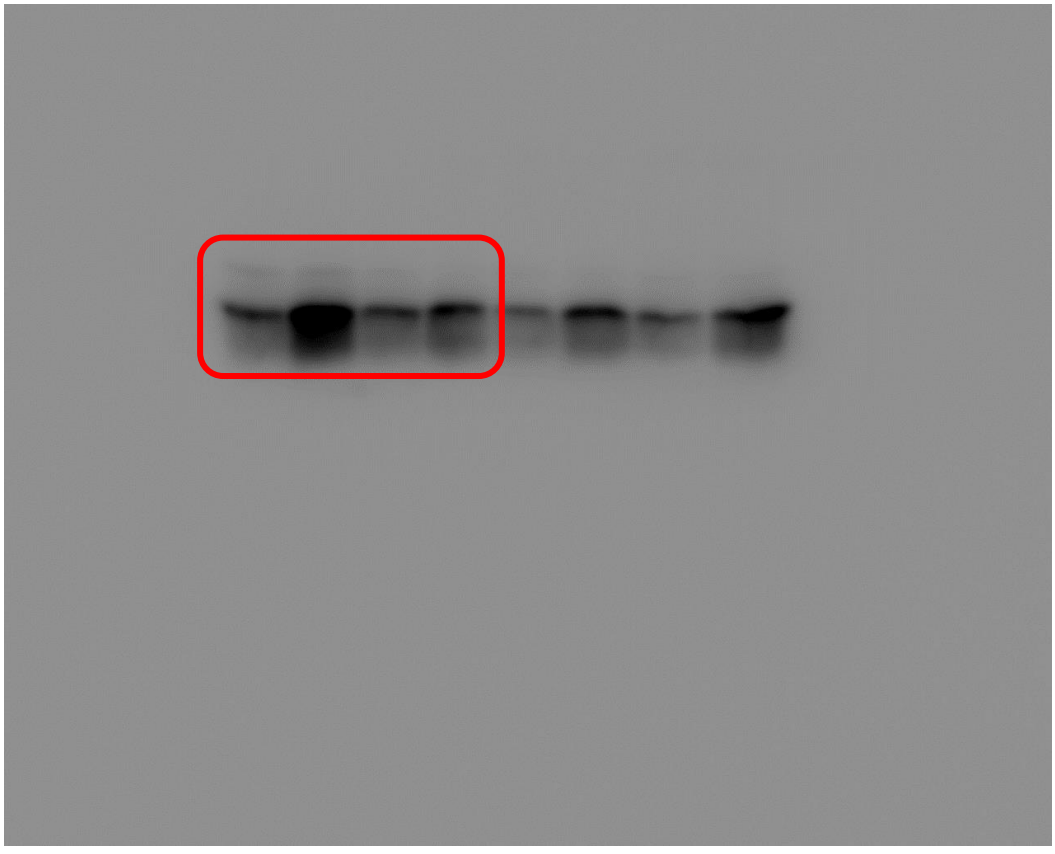


C

LC3



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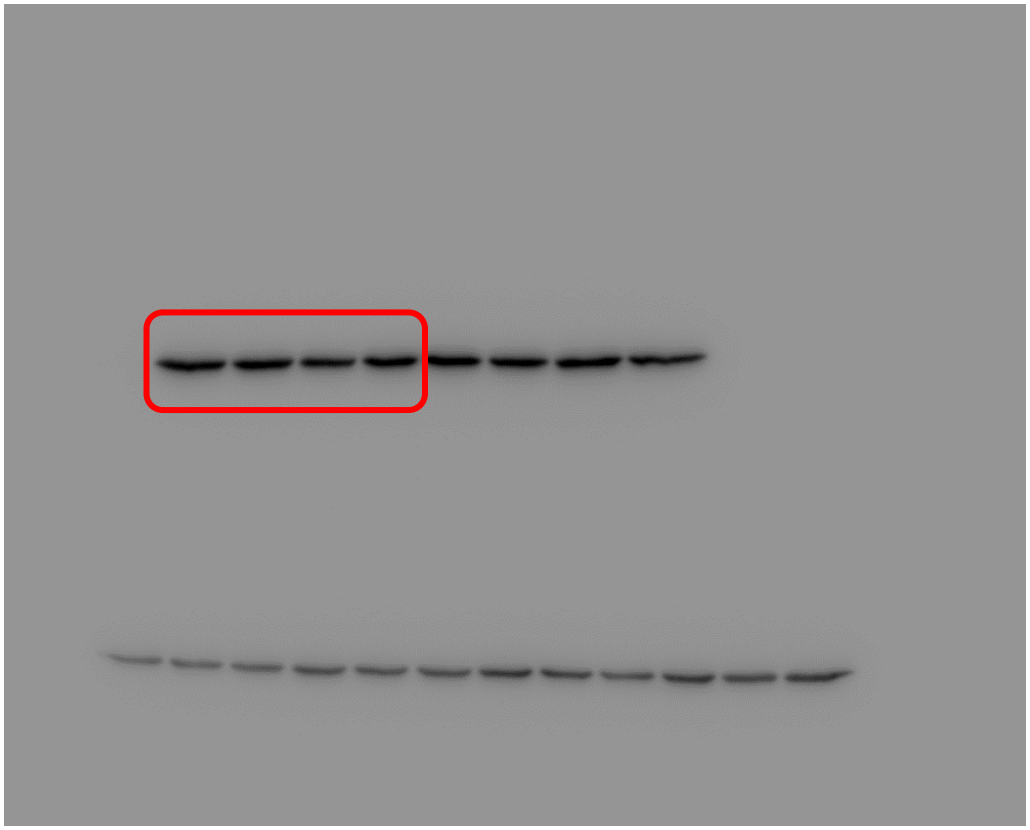
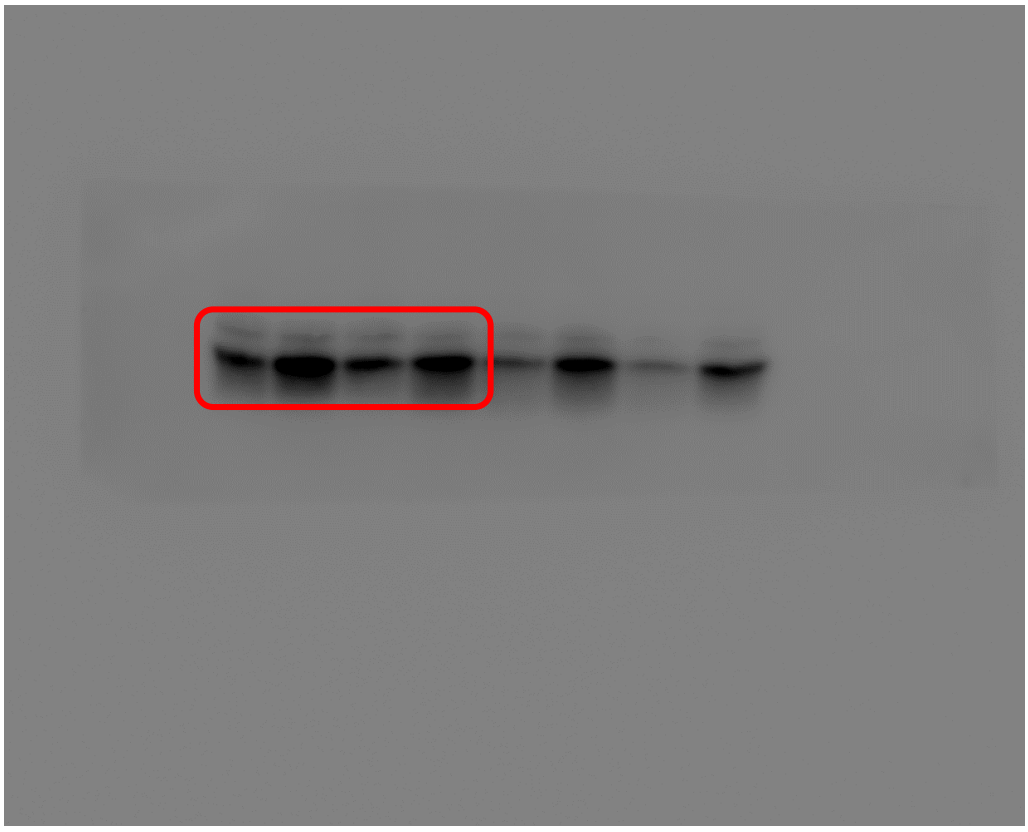


D

LC3



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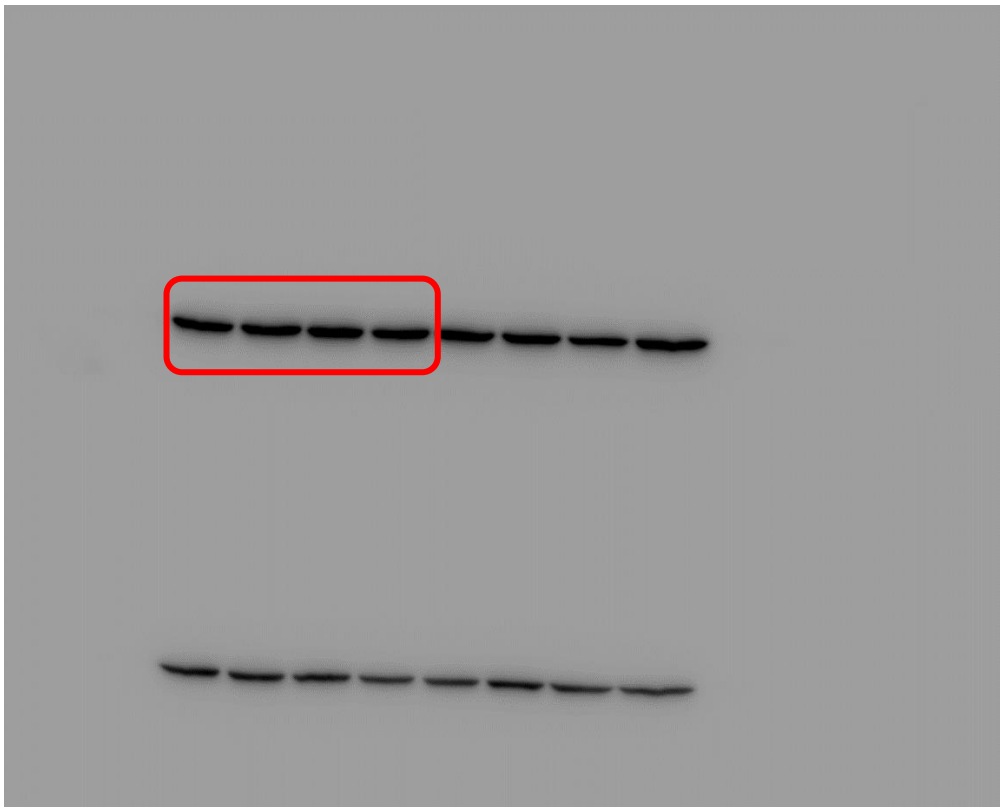
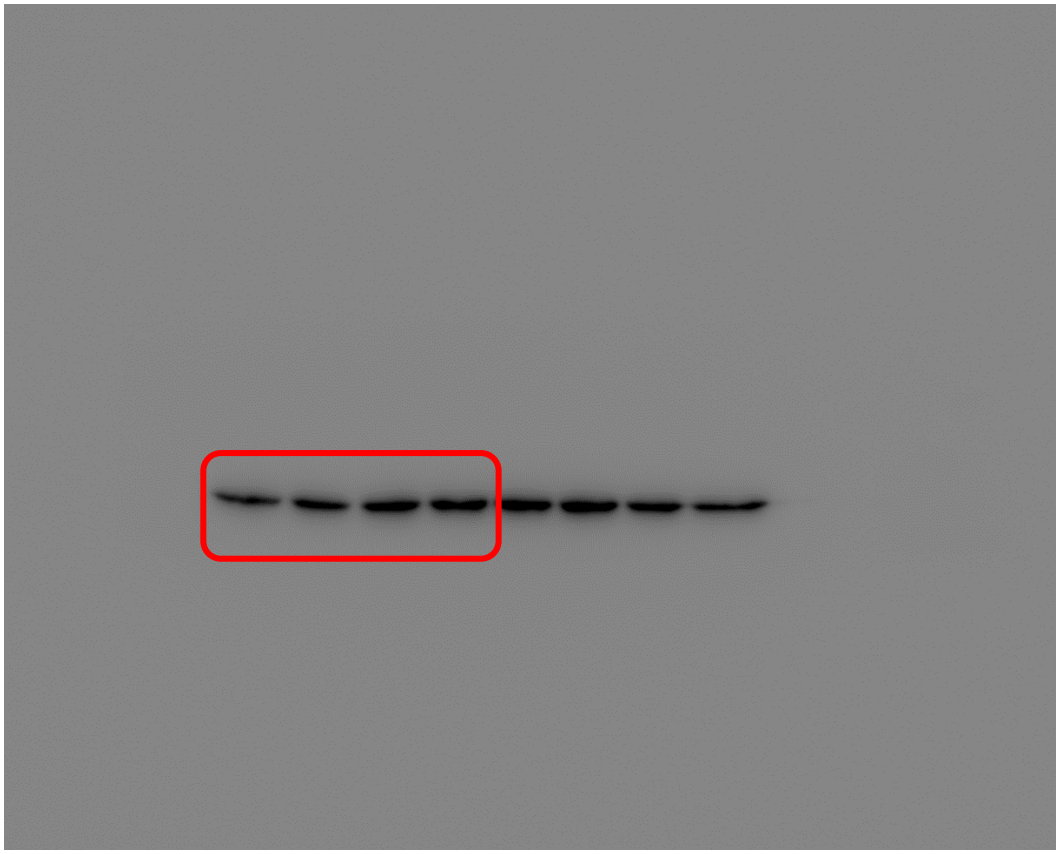


E

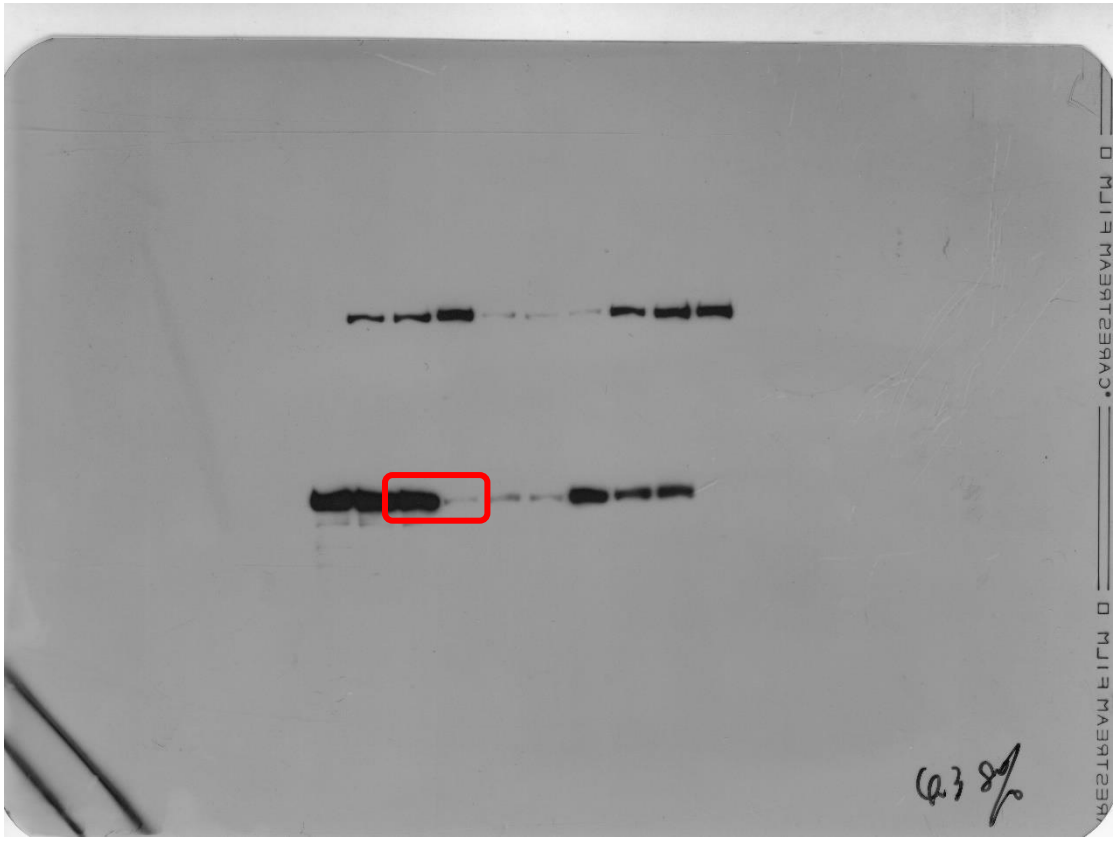
LC3

Actin



F

PAE mTOR



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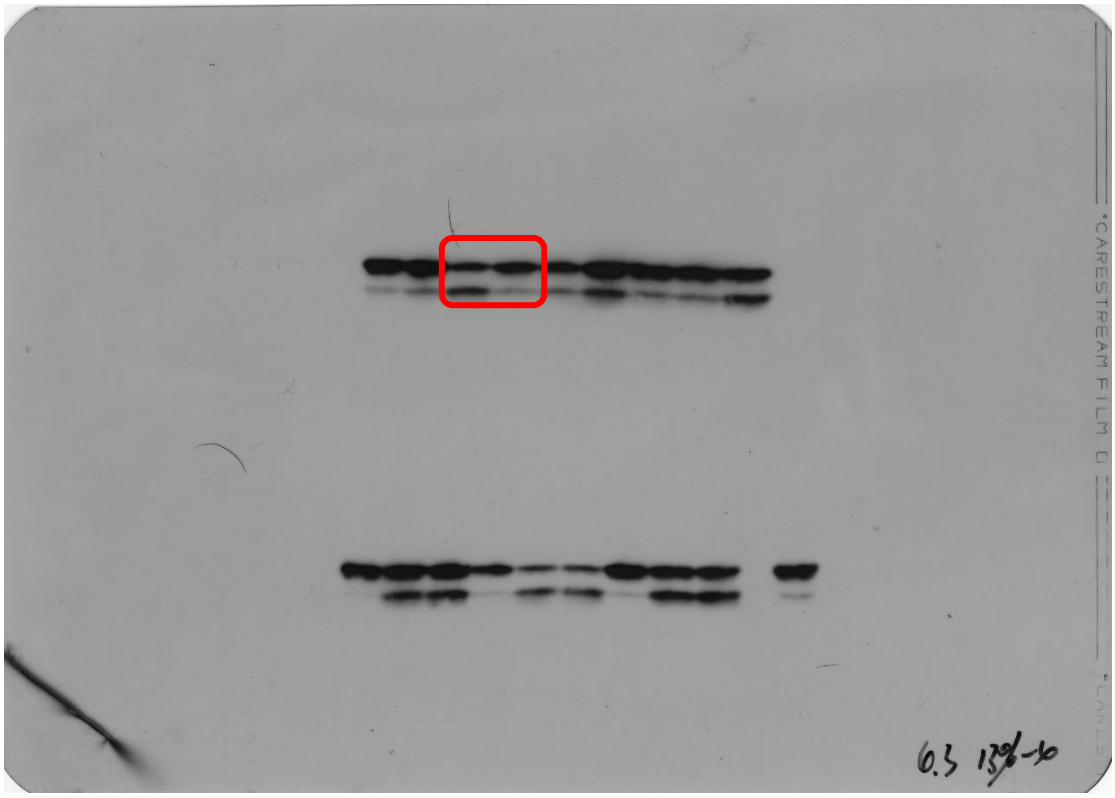

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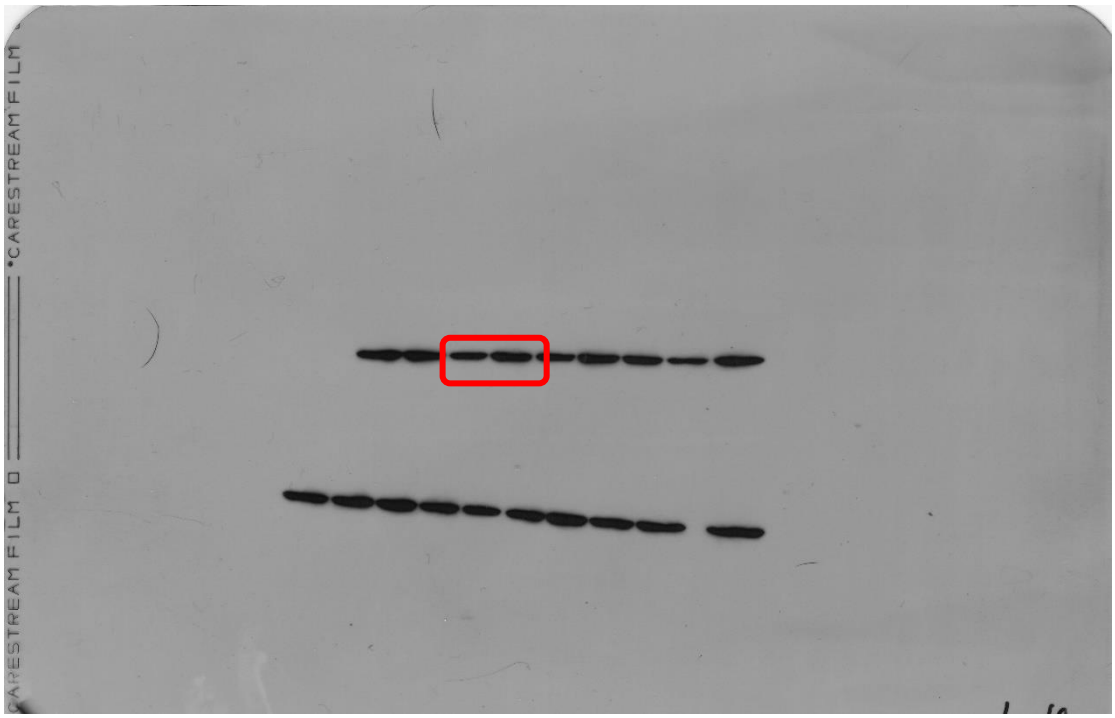
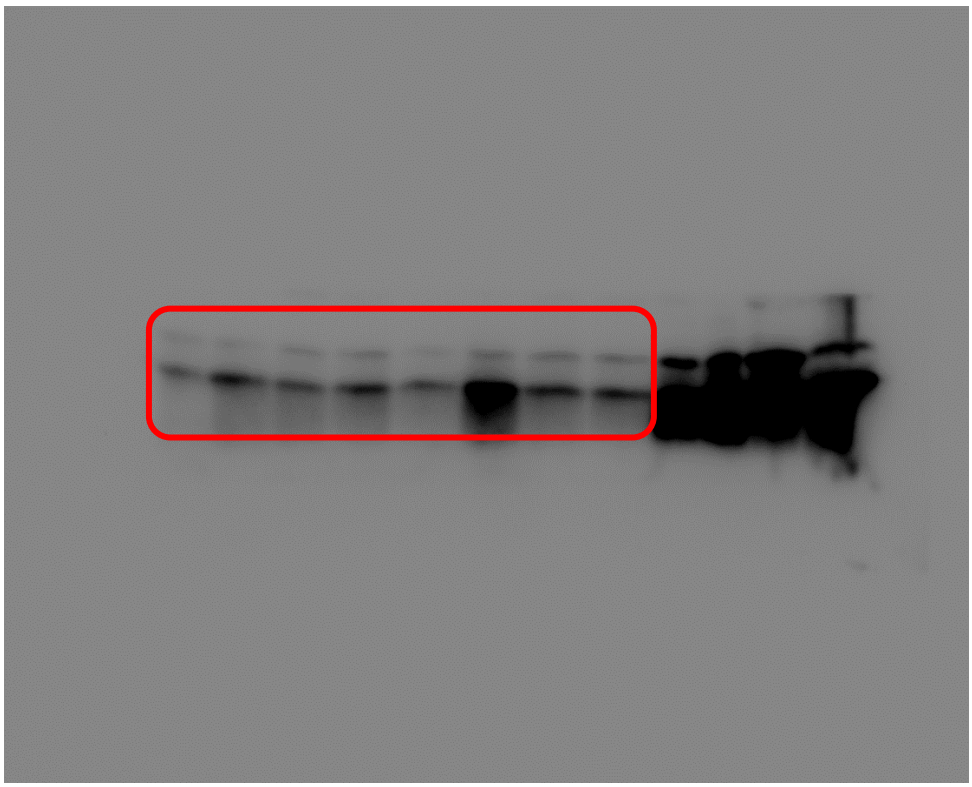


PVDF membranes were cut into stripes for different antibodies staining. f was exposed by X-ray, a-e were exposed by Thermo fisher Luminometer. Figure 1a is the result of the same batch of samples running with SDS-PAGE on different days.

Figure2

A

LC3



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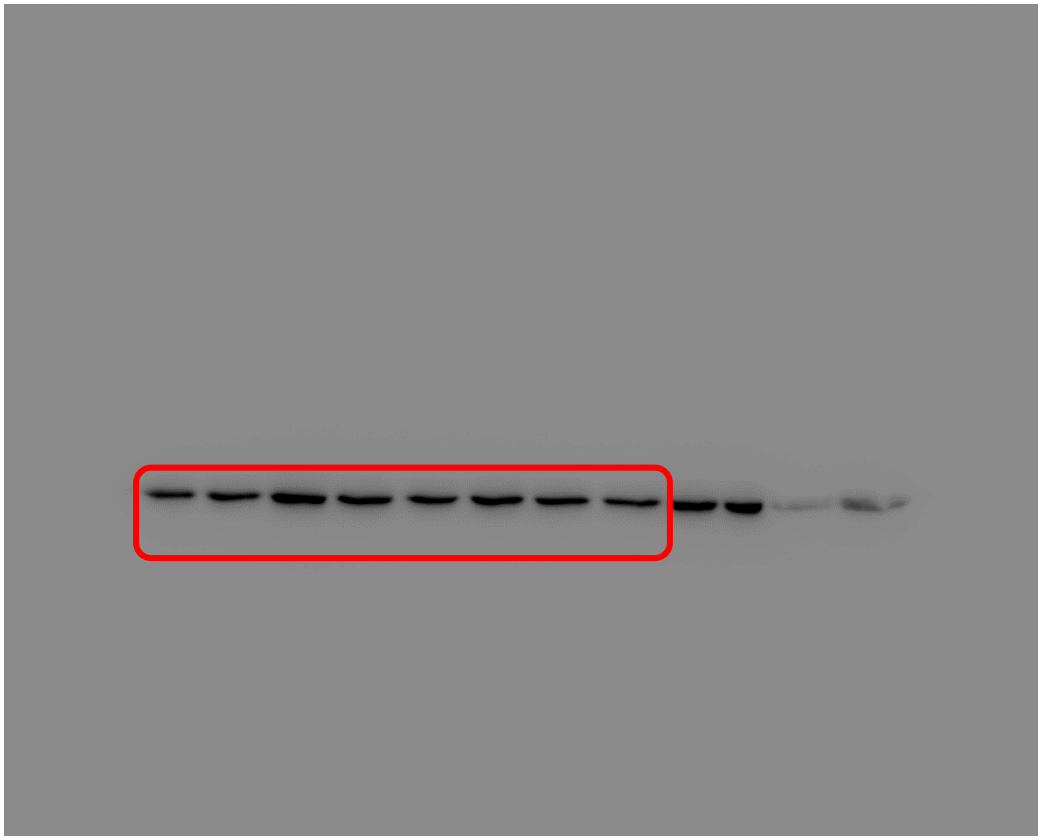
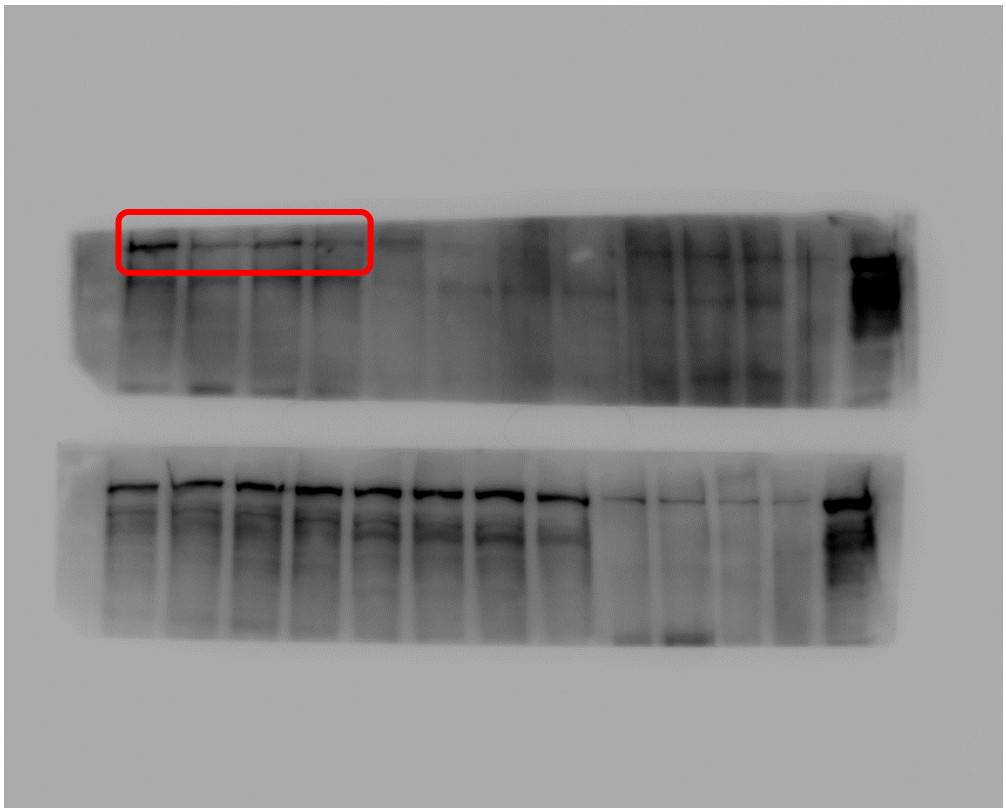
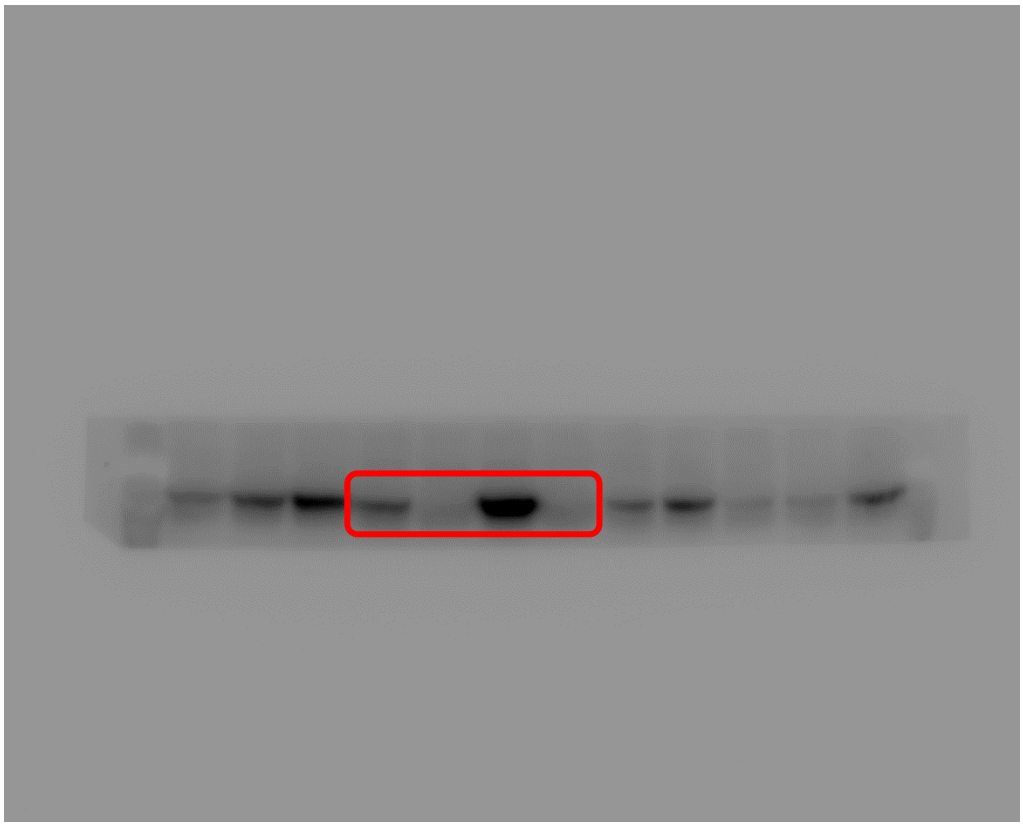


B

pmTOR



PAkt



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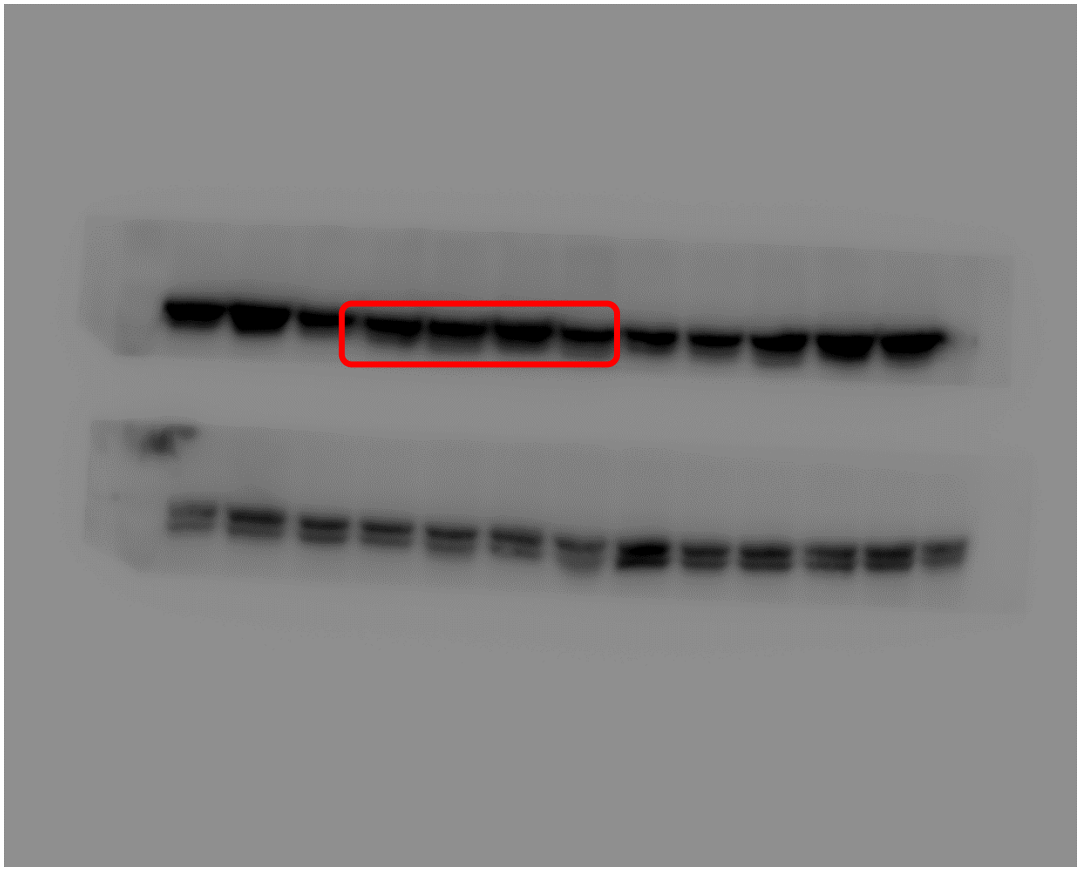

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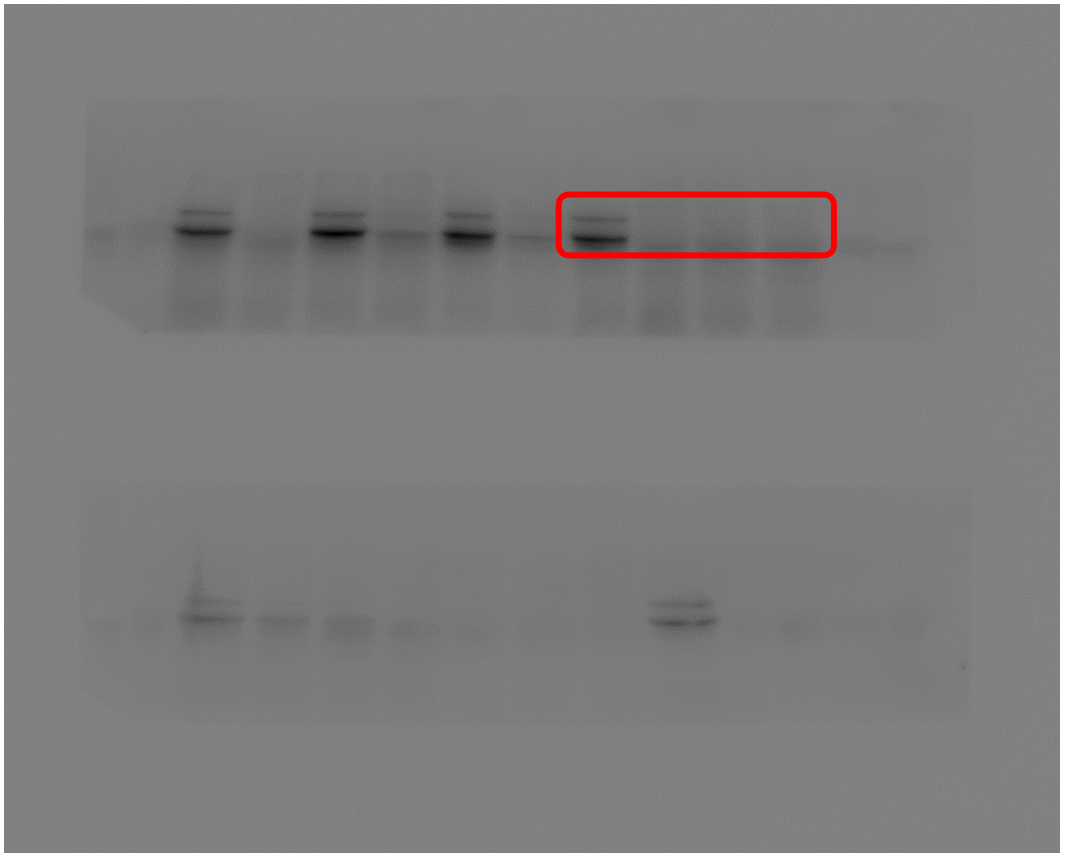

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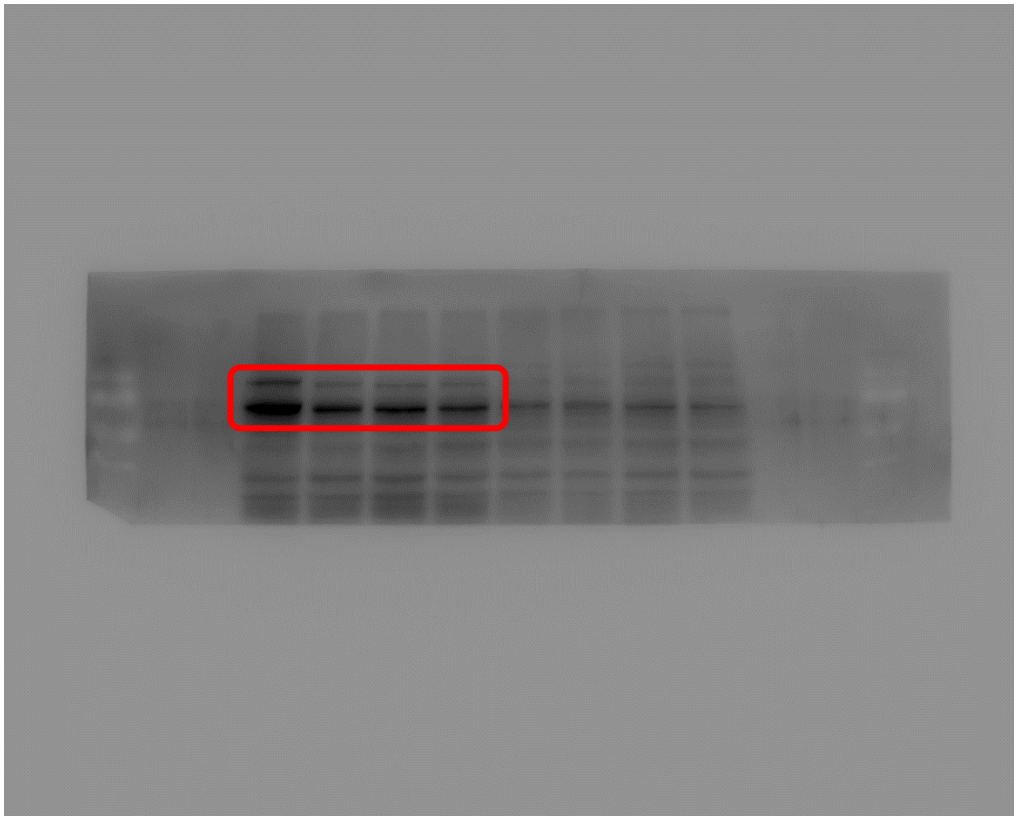

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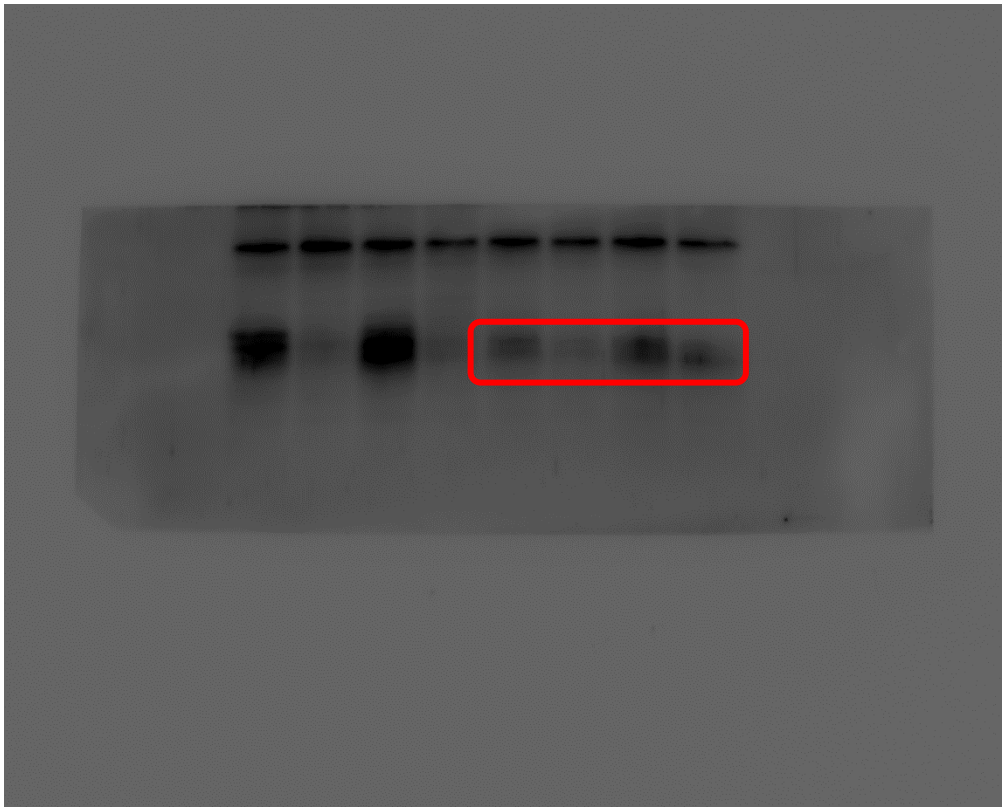
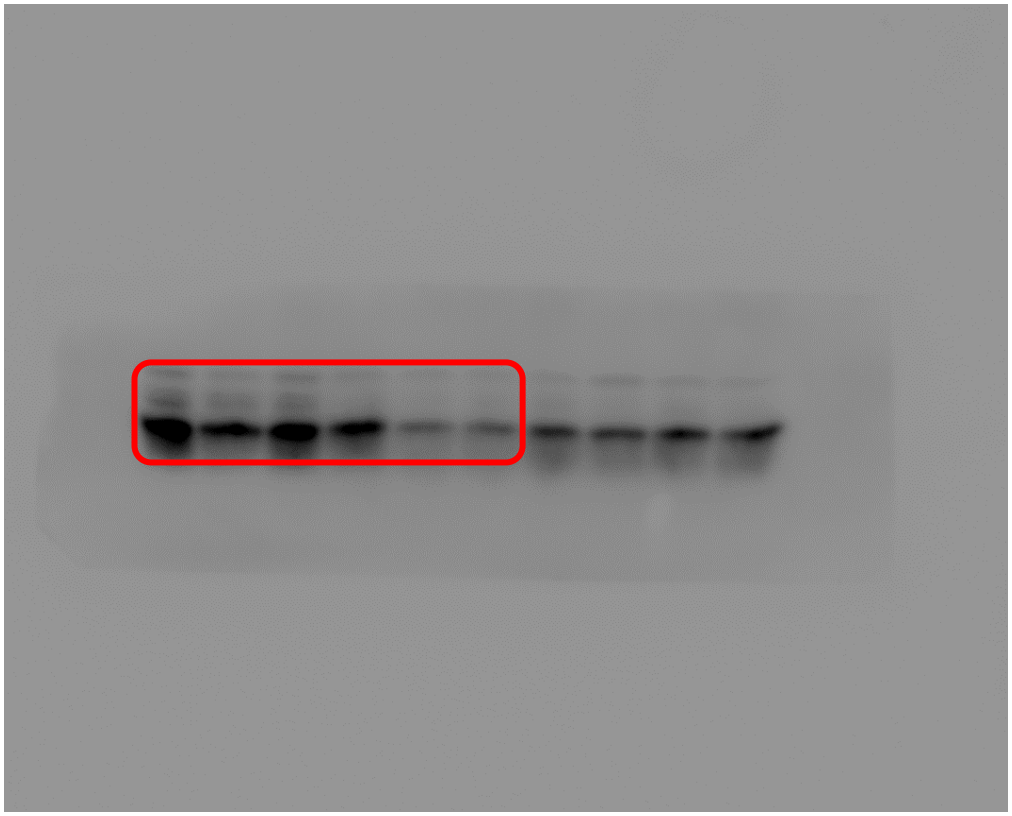


Actin

E
LC3



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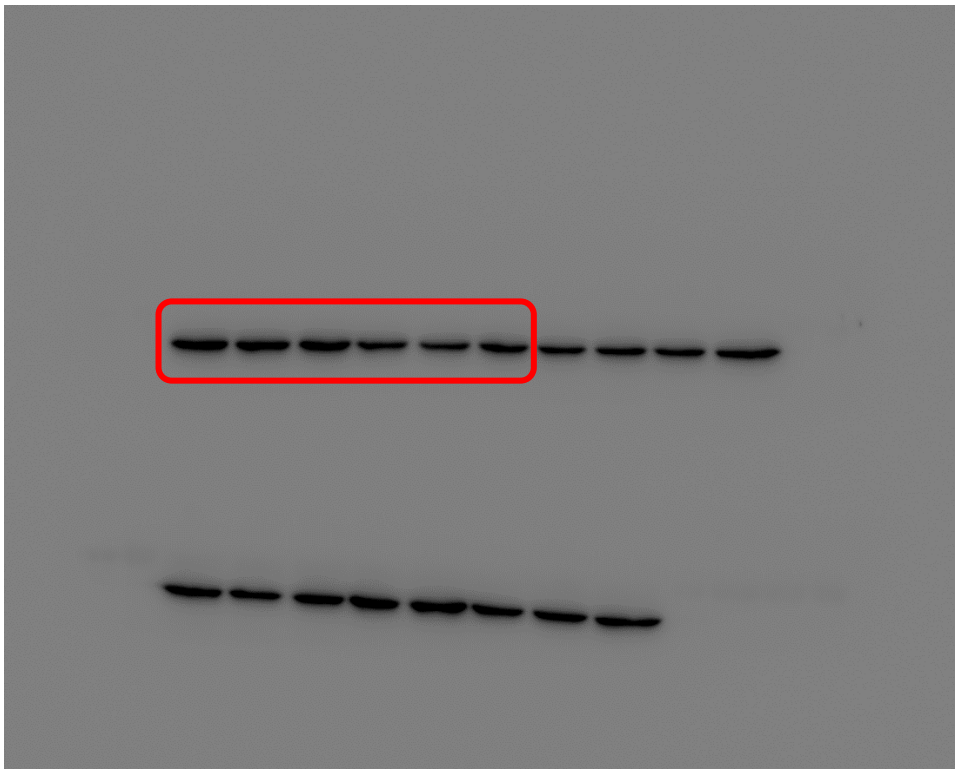
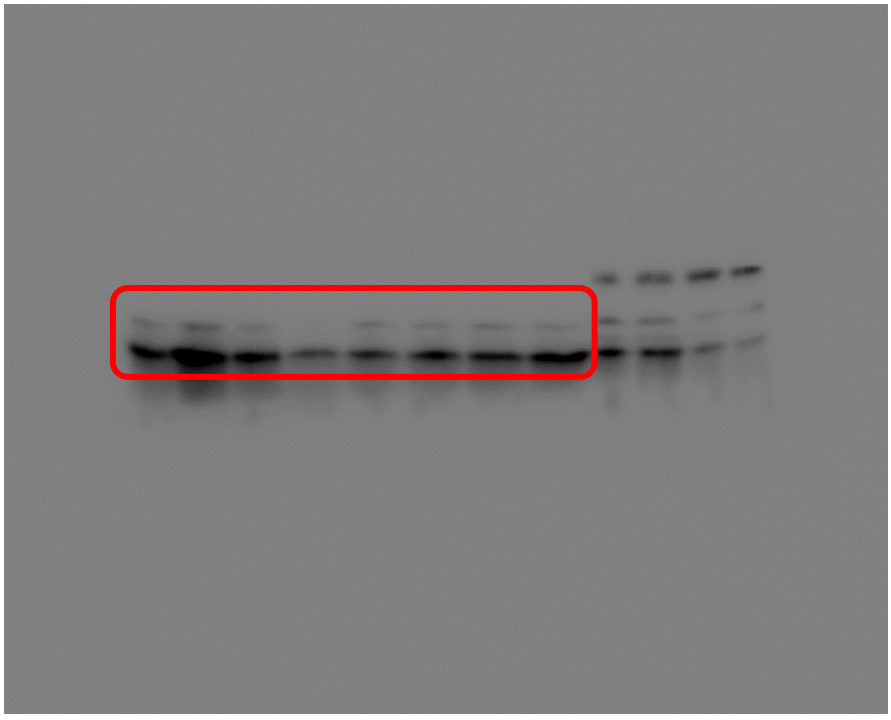


PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer. Figure 2b is the result of the same batch of samples running with SDS-PAGE on different days.

Figure3
A
LC3



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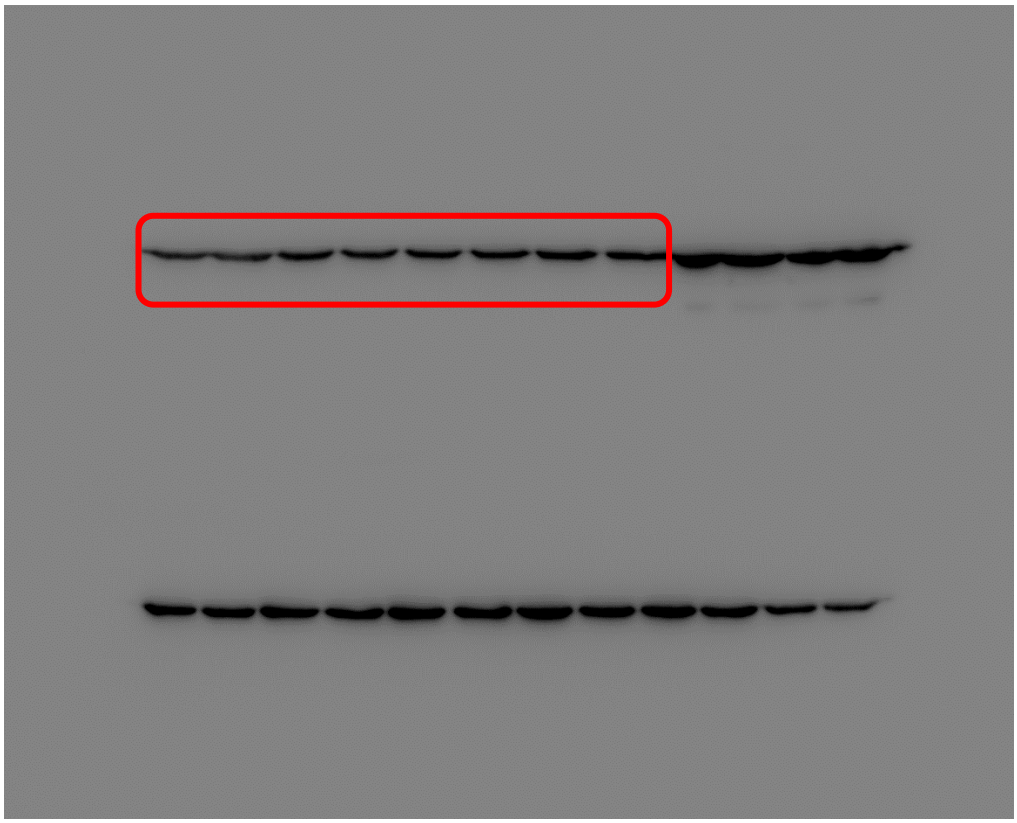
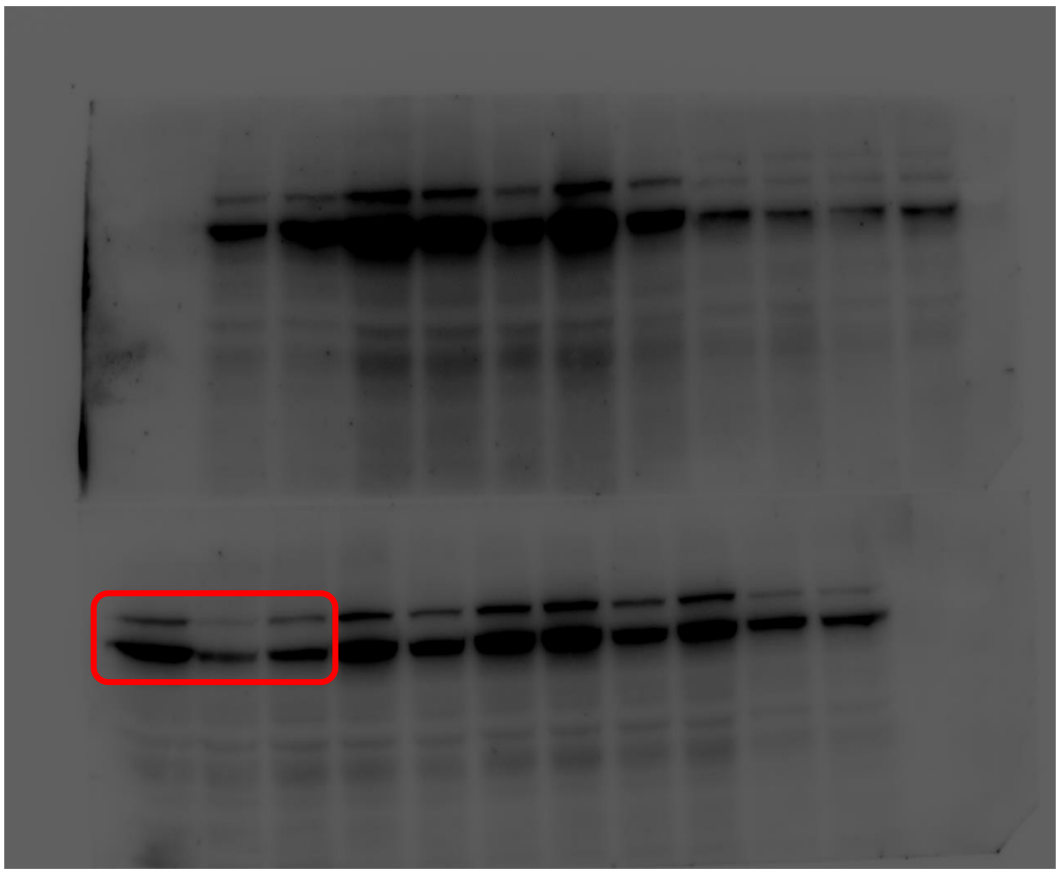
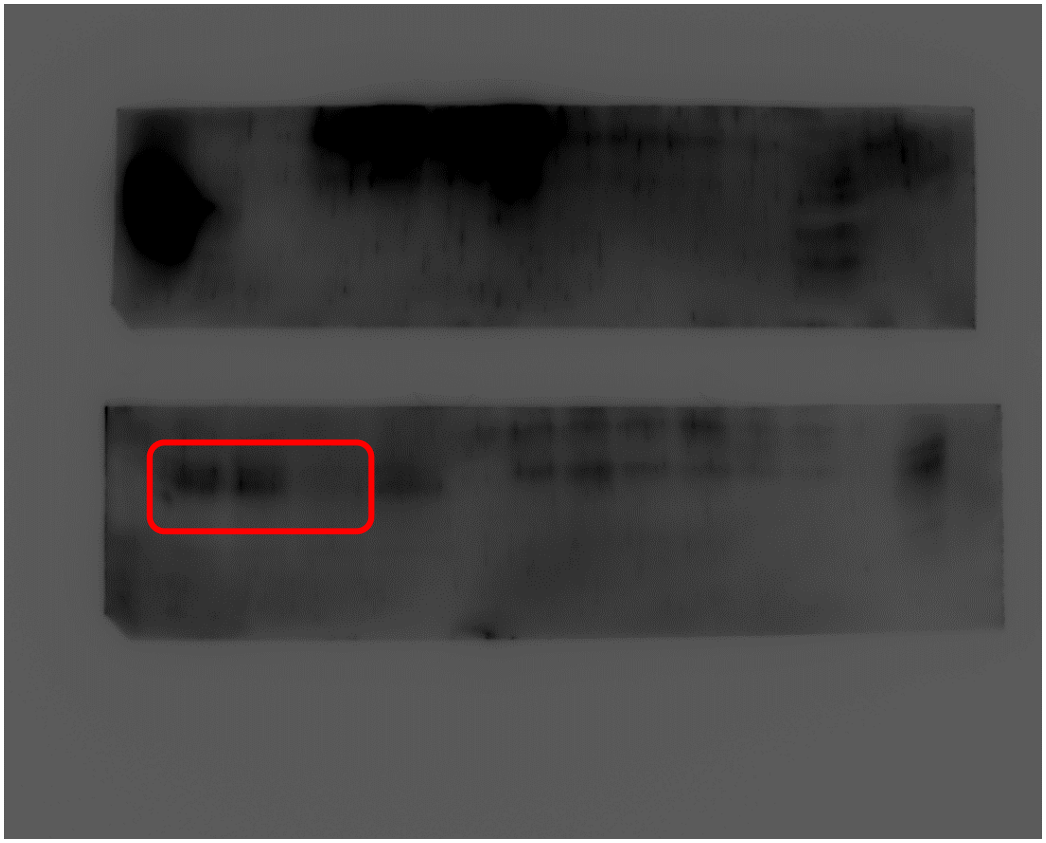


B

S6K1



4EBP1



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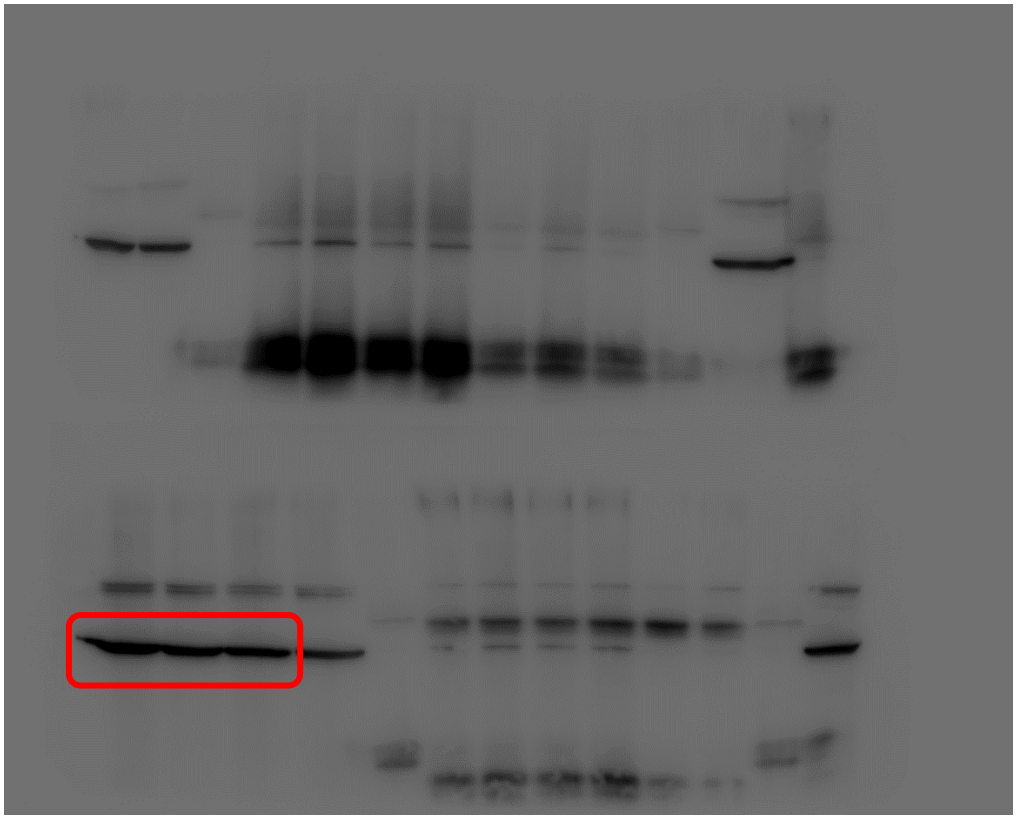
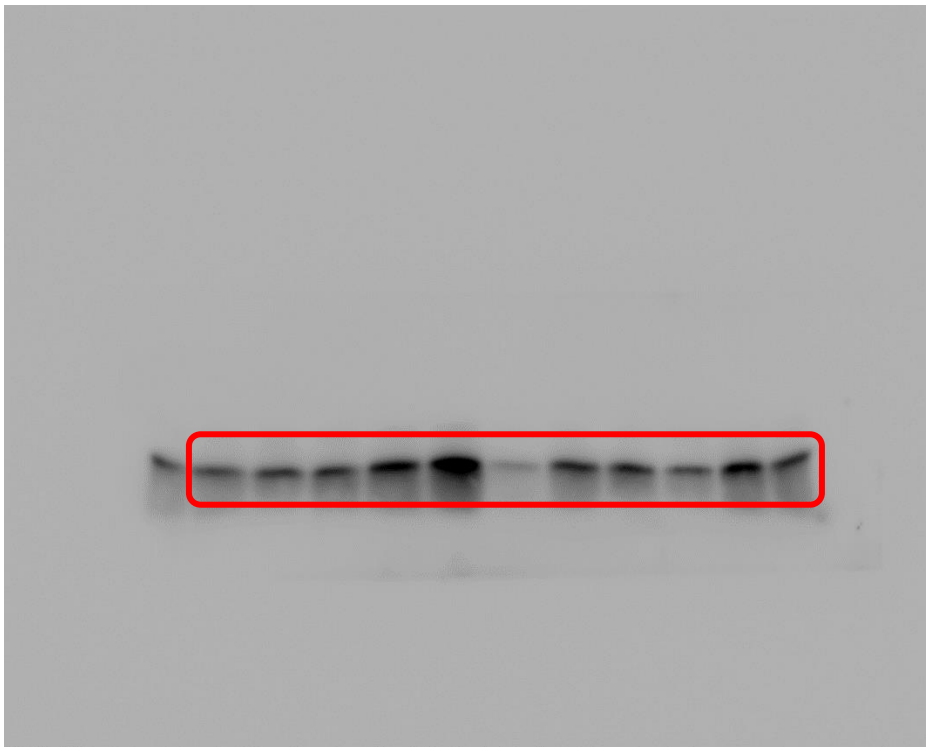


C

LC3



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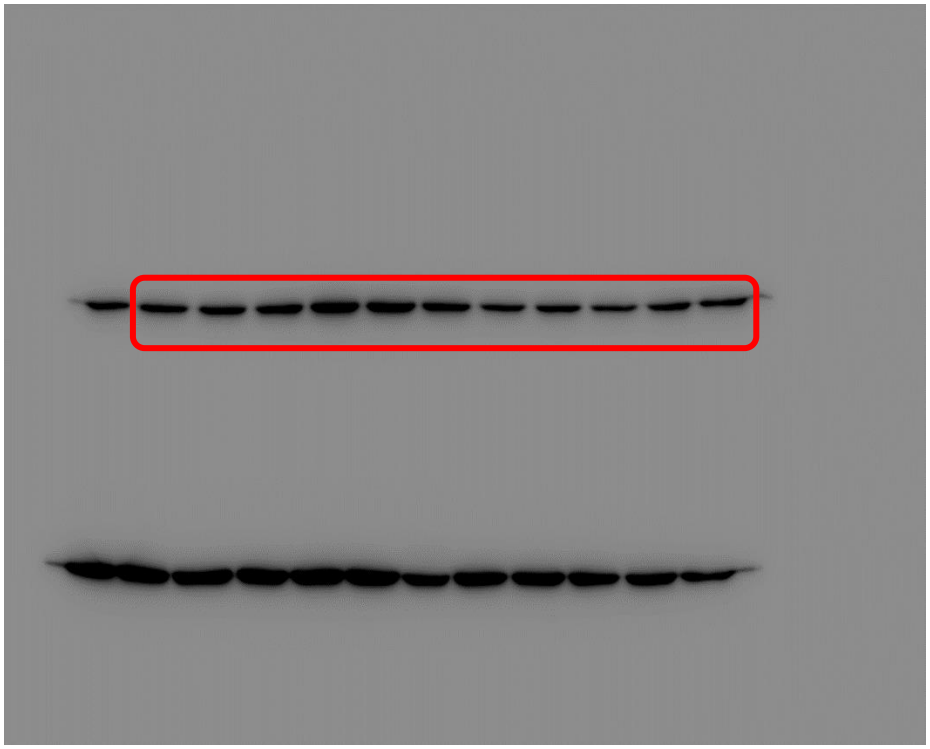
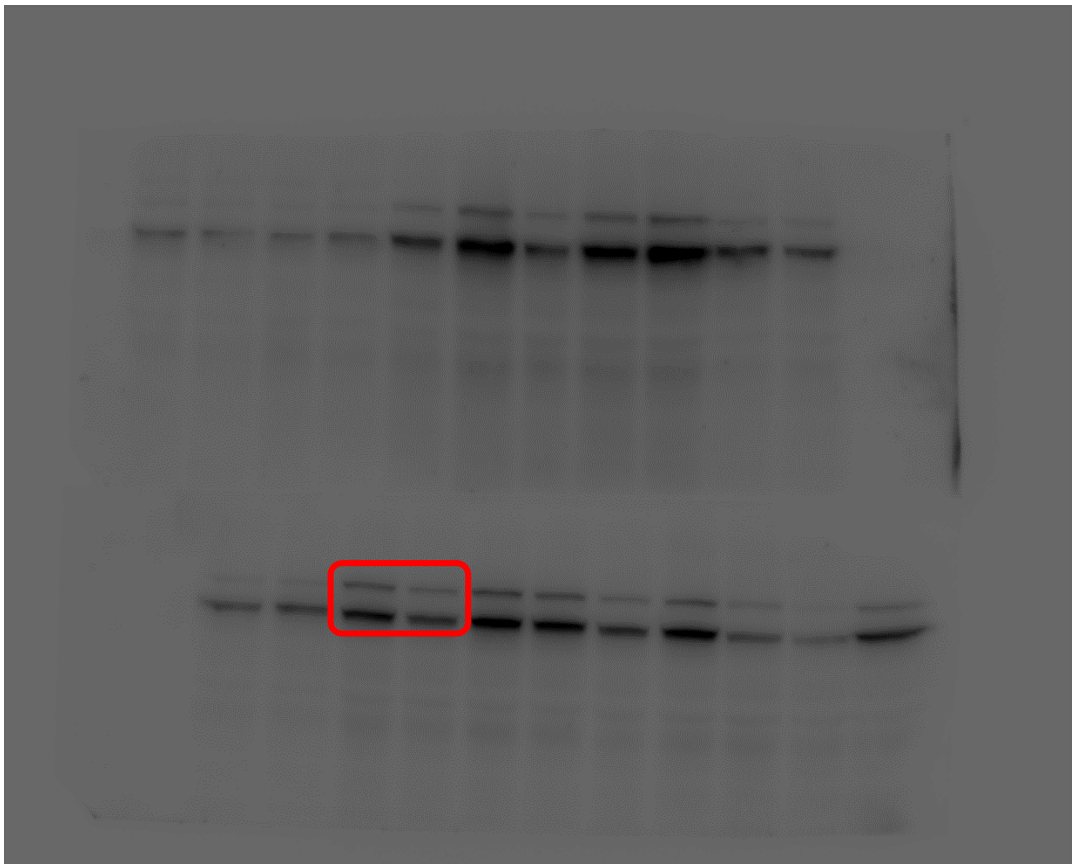
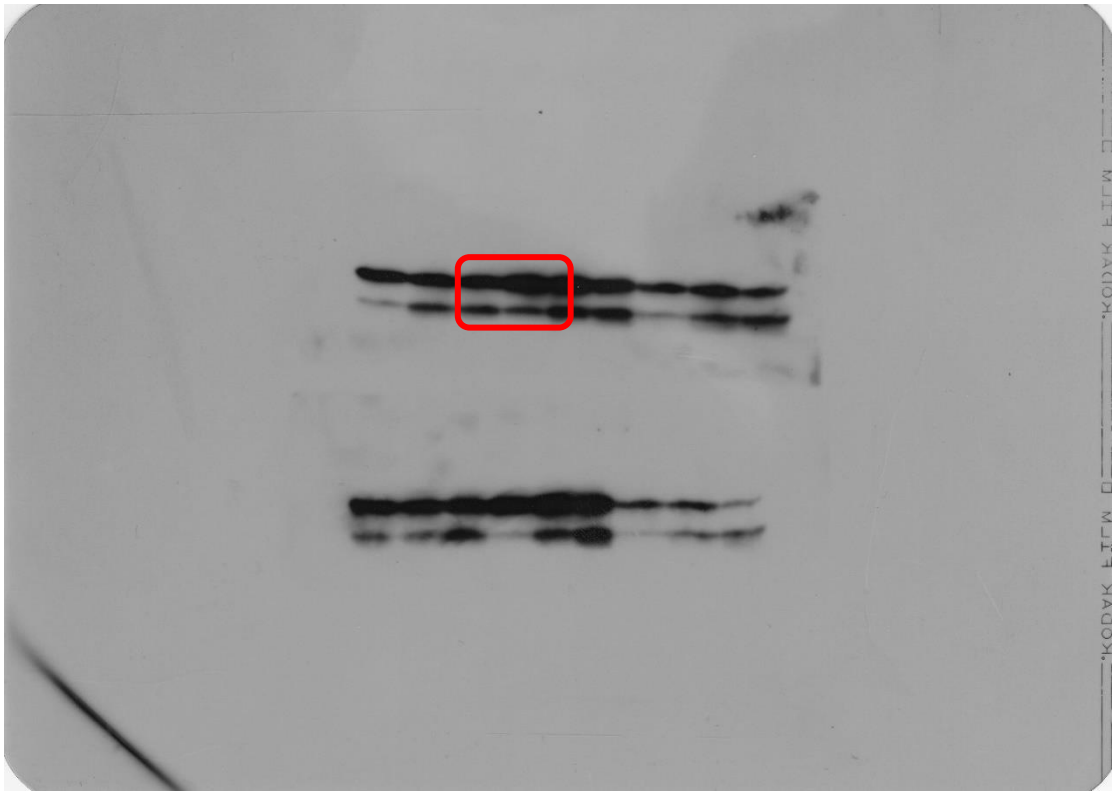


D

S6K1



LC3



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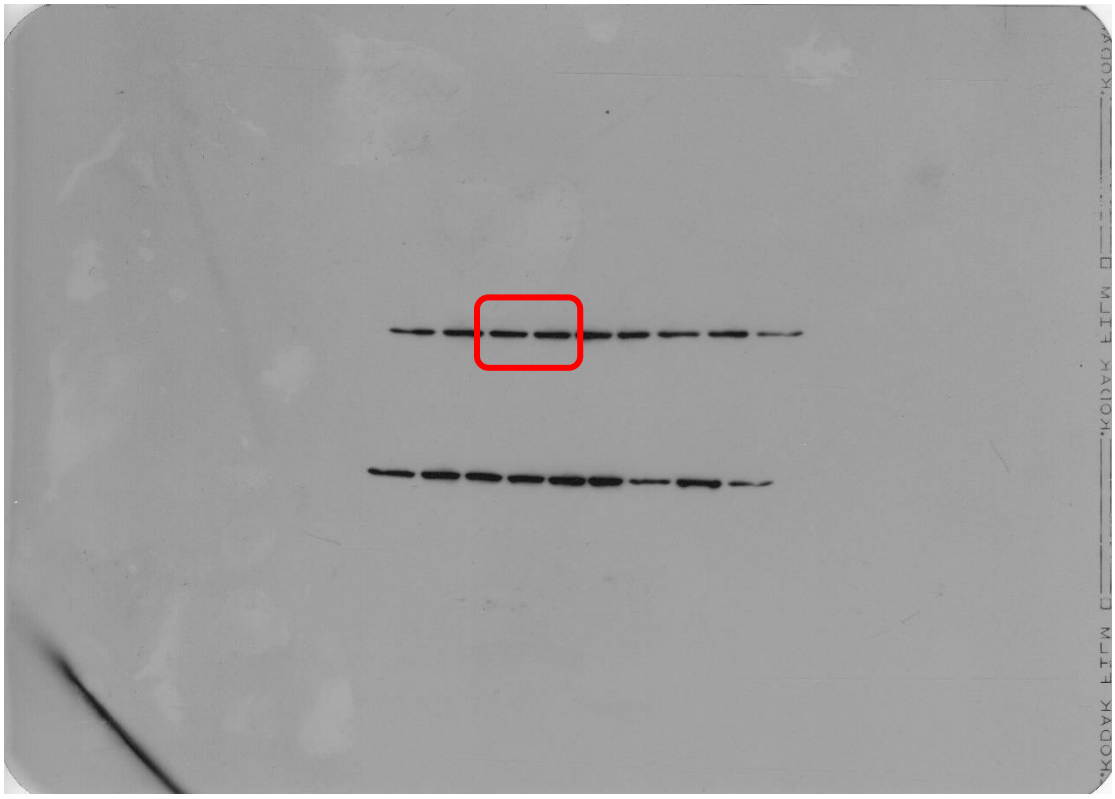
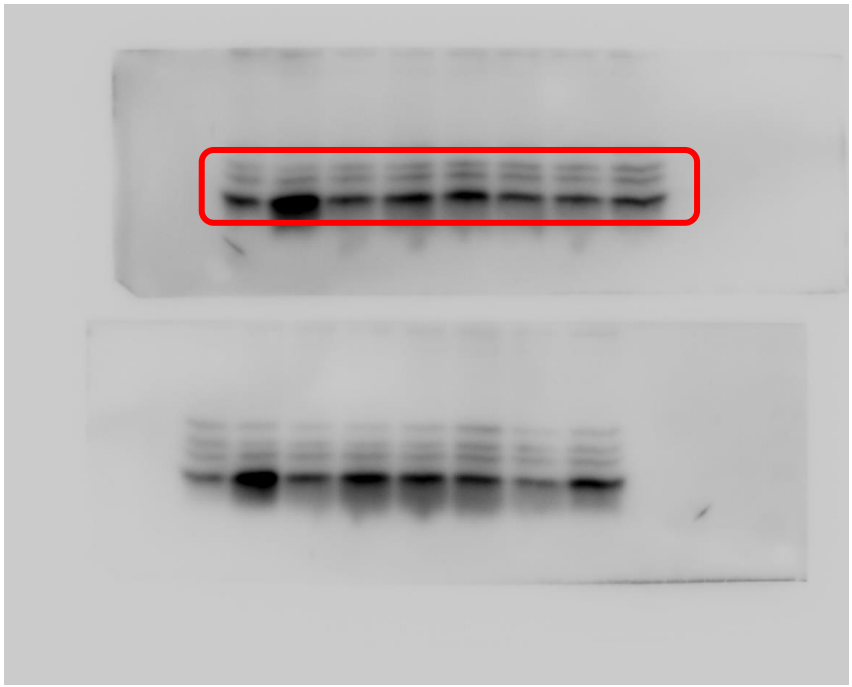


PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer.

Figure4

A

LC3



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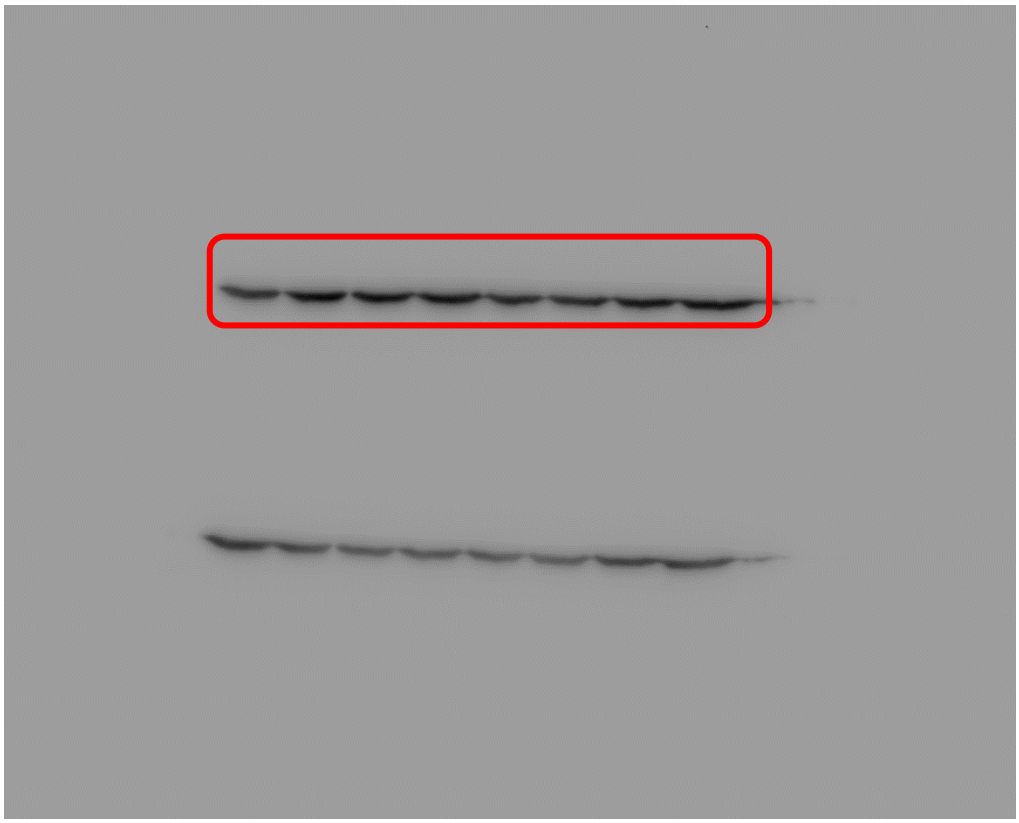
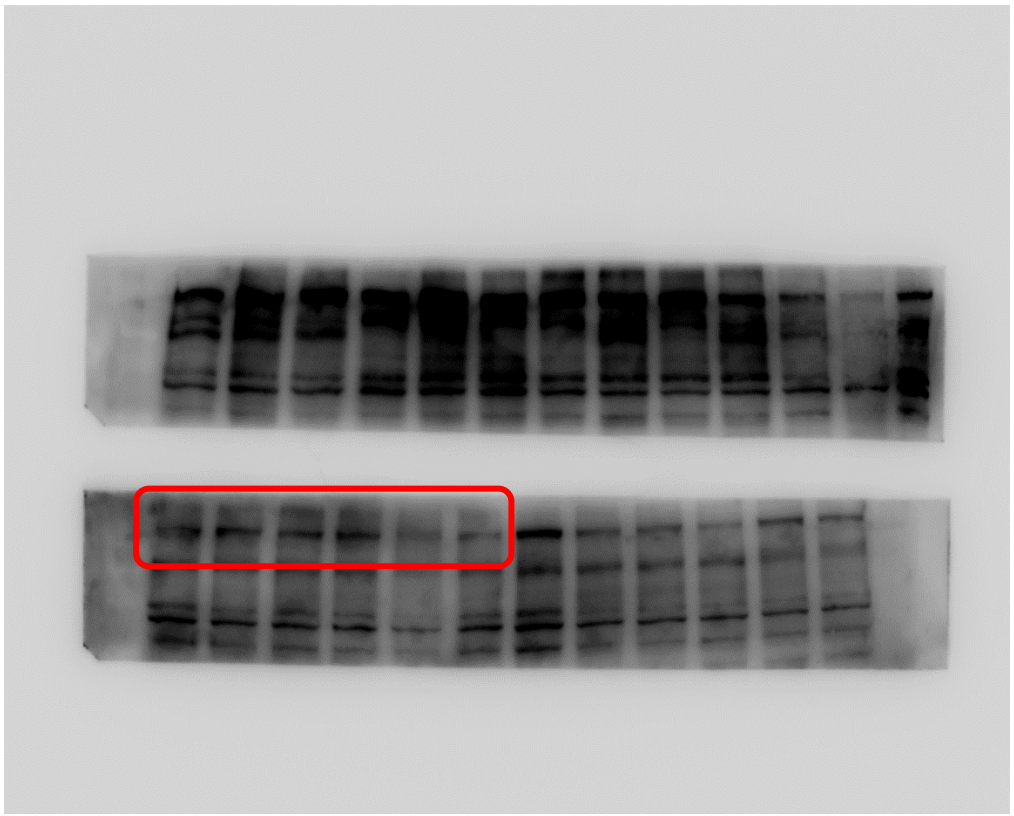


B

p-mTOR



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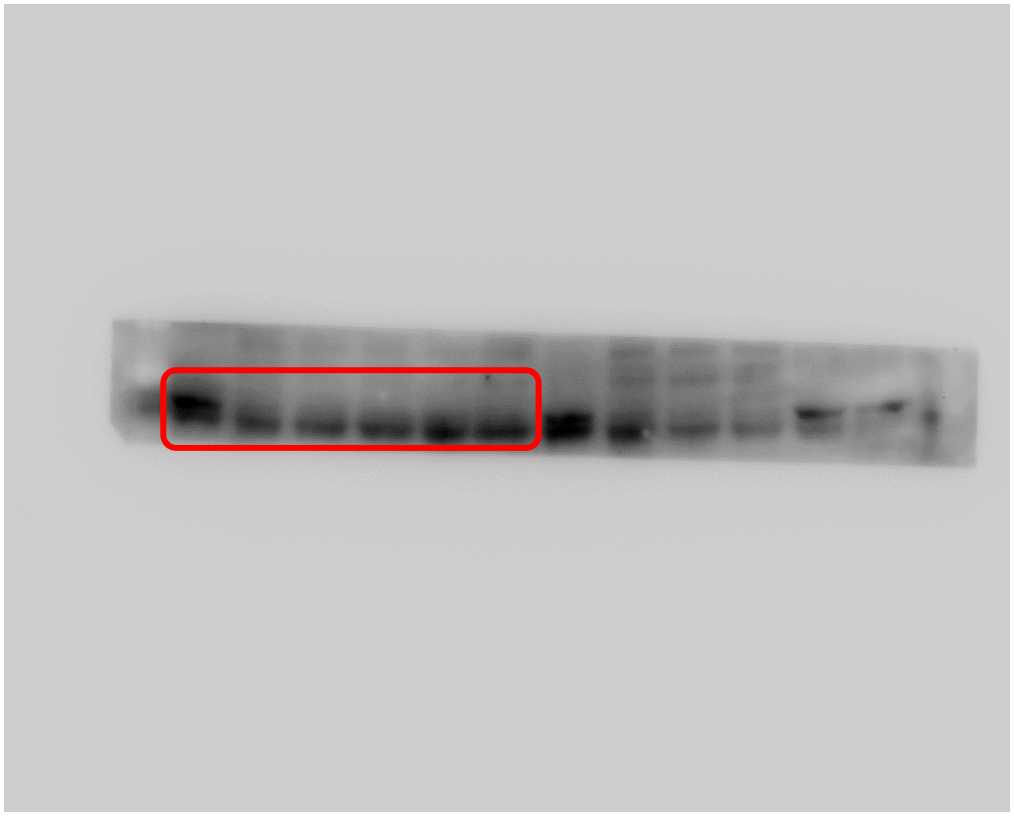

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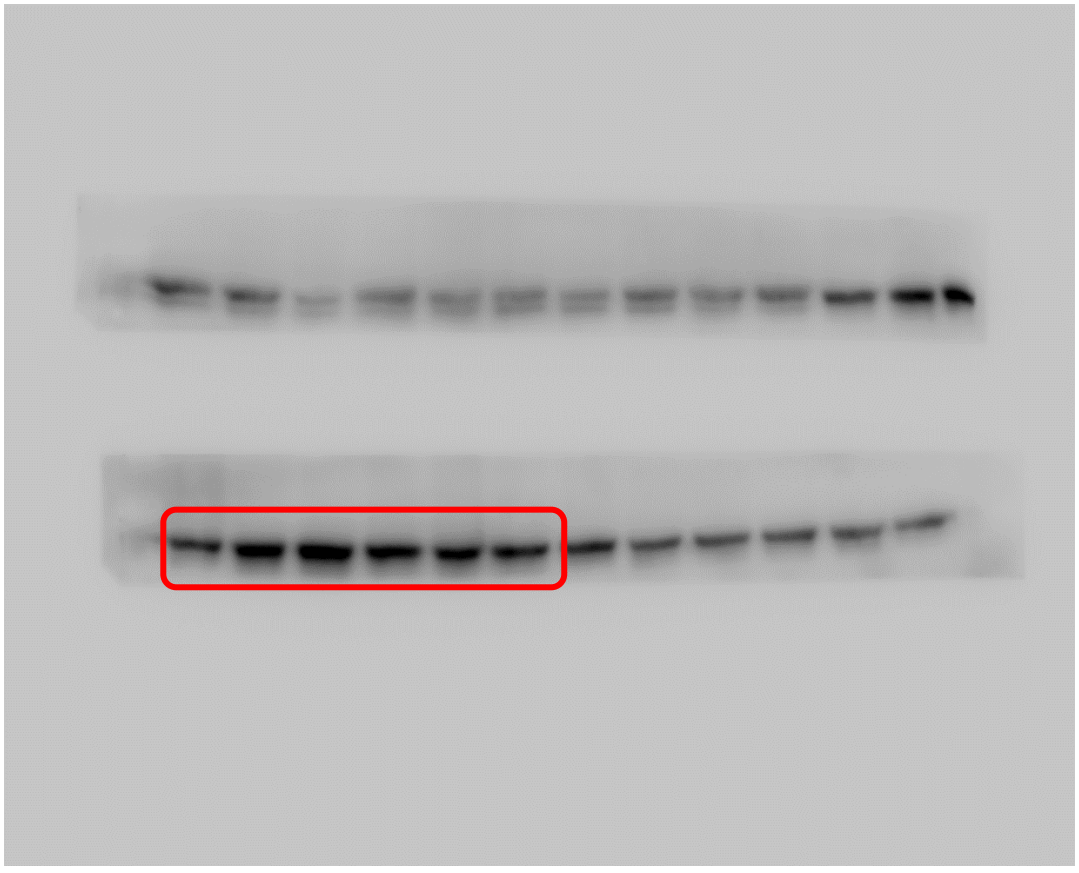

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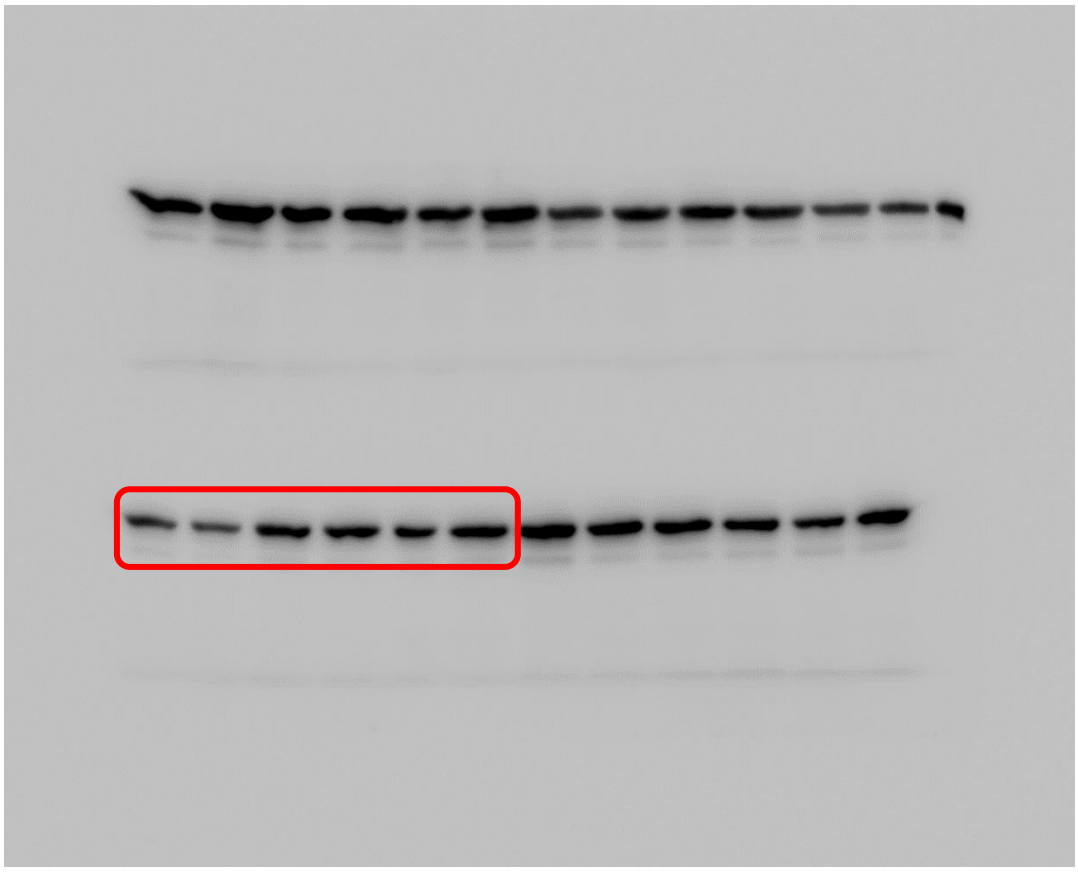
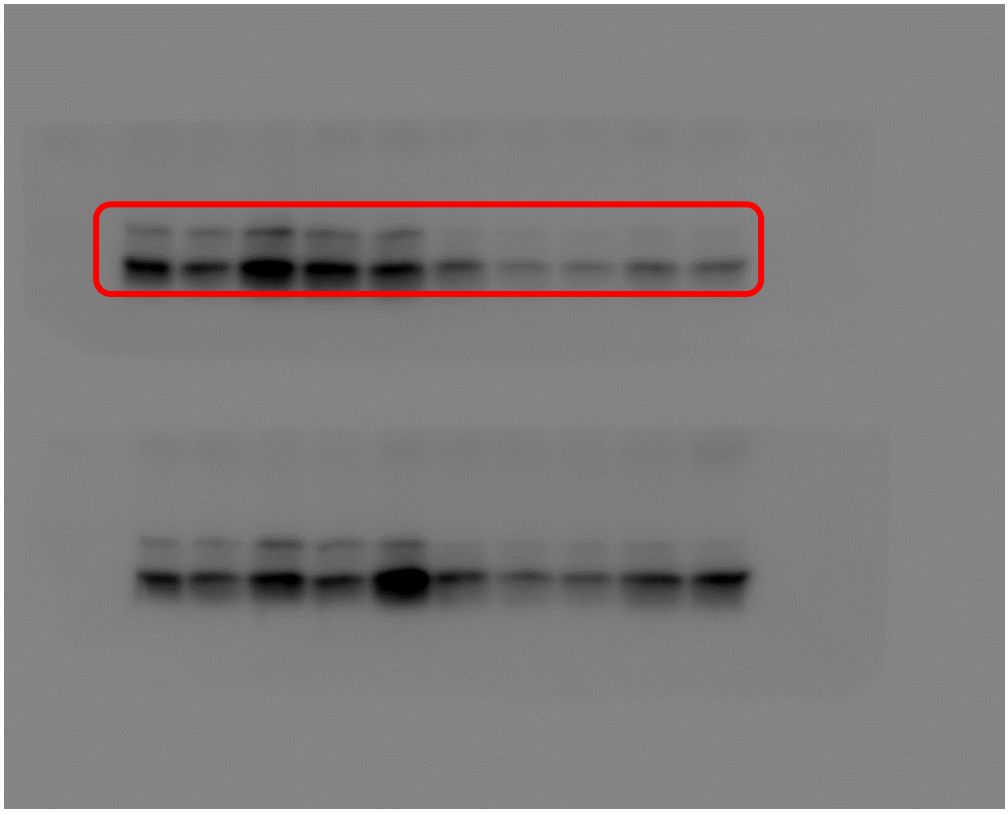


C

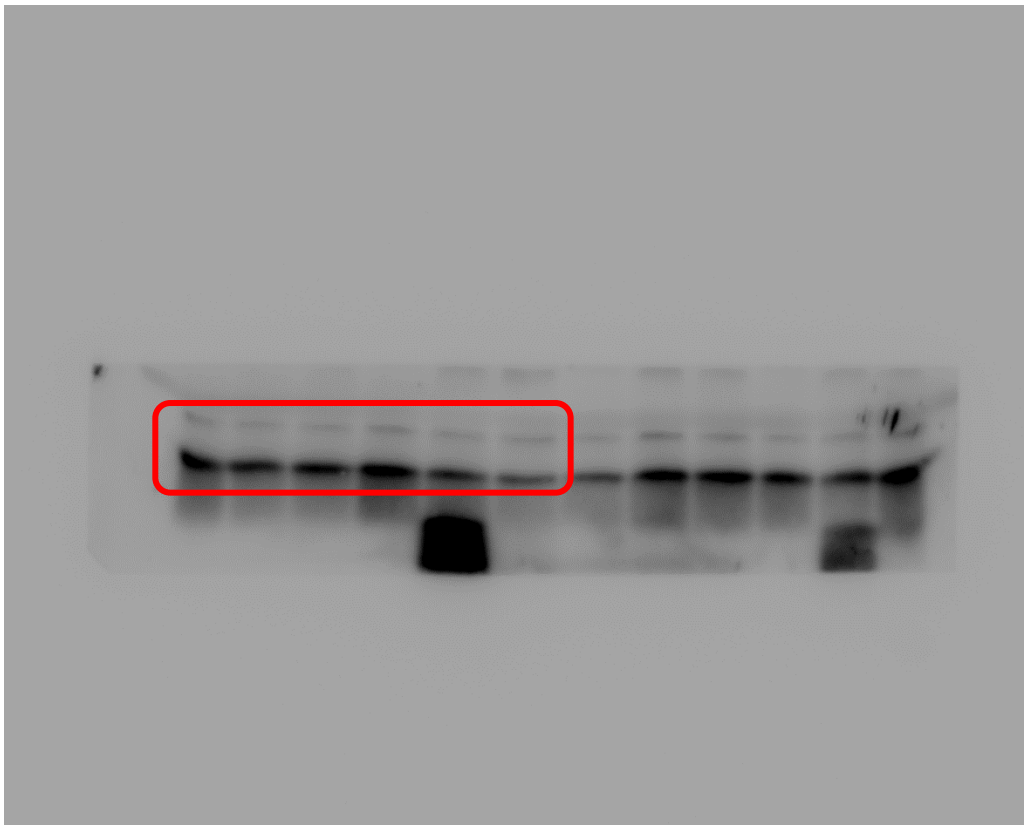
LC3



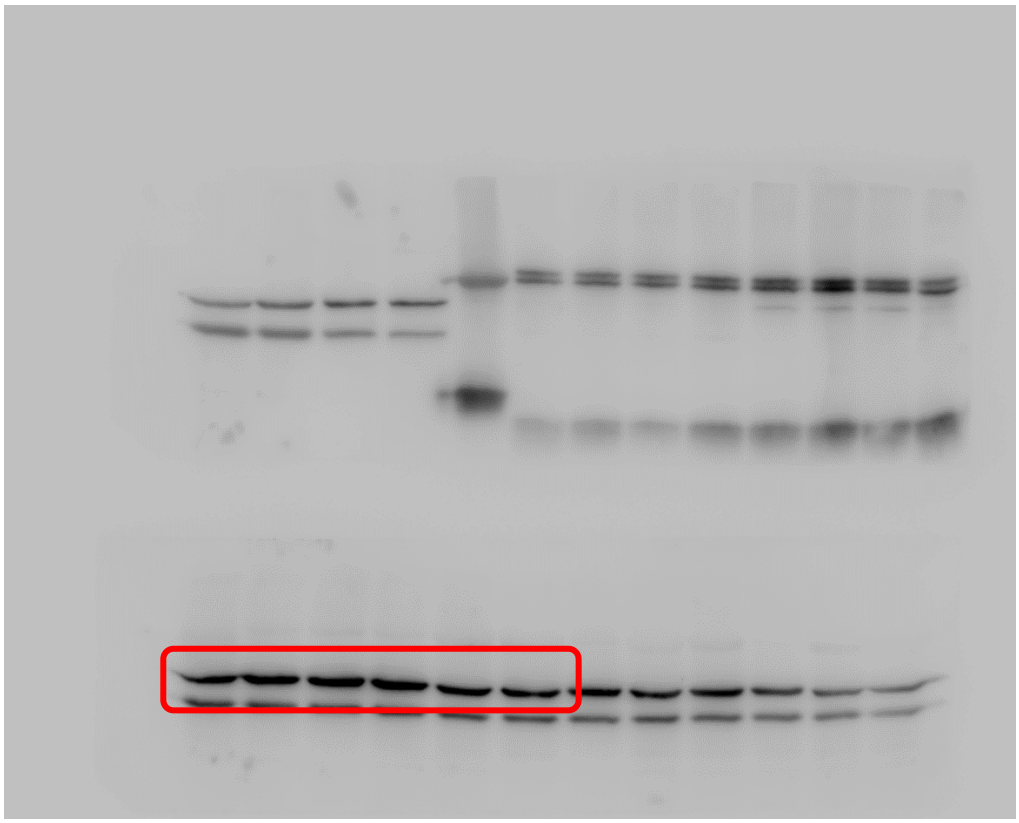
Actin

D

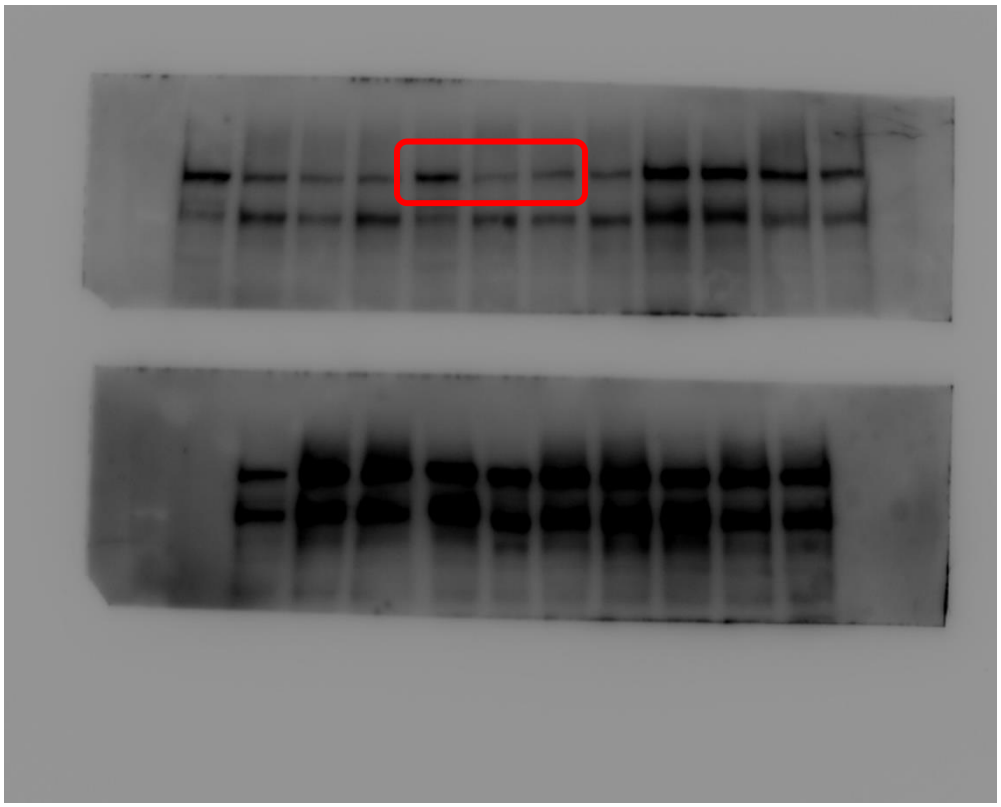
LC3



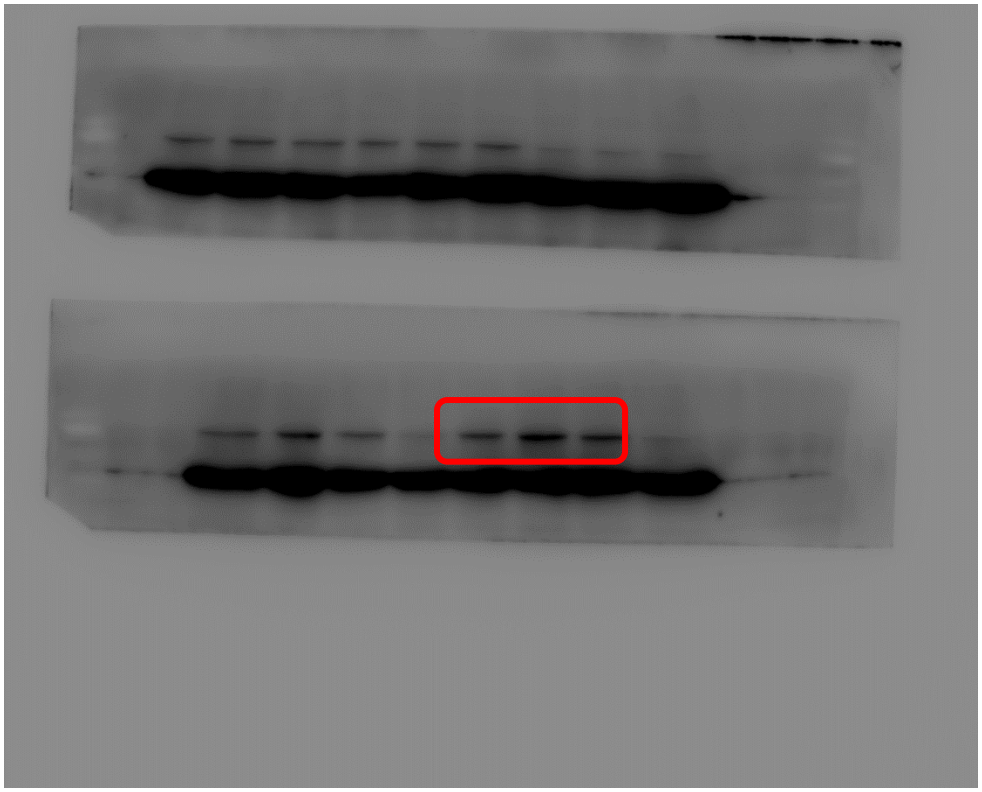
Actin



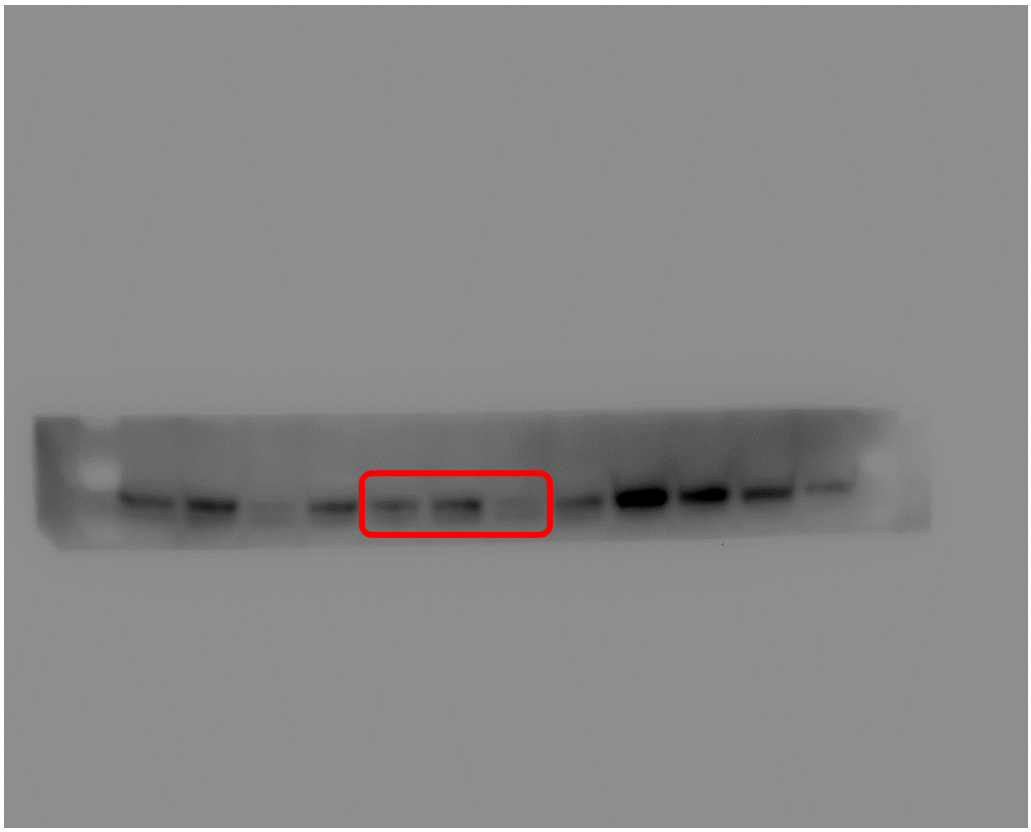
E
mTOR



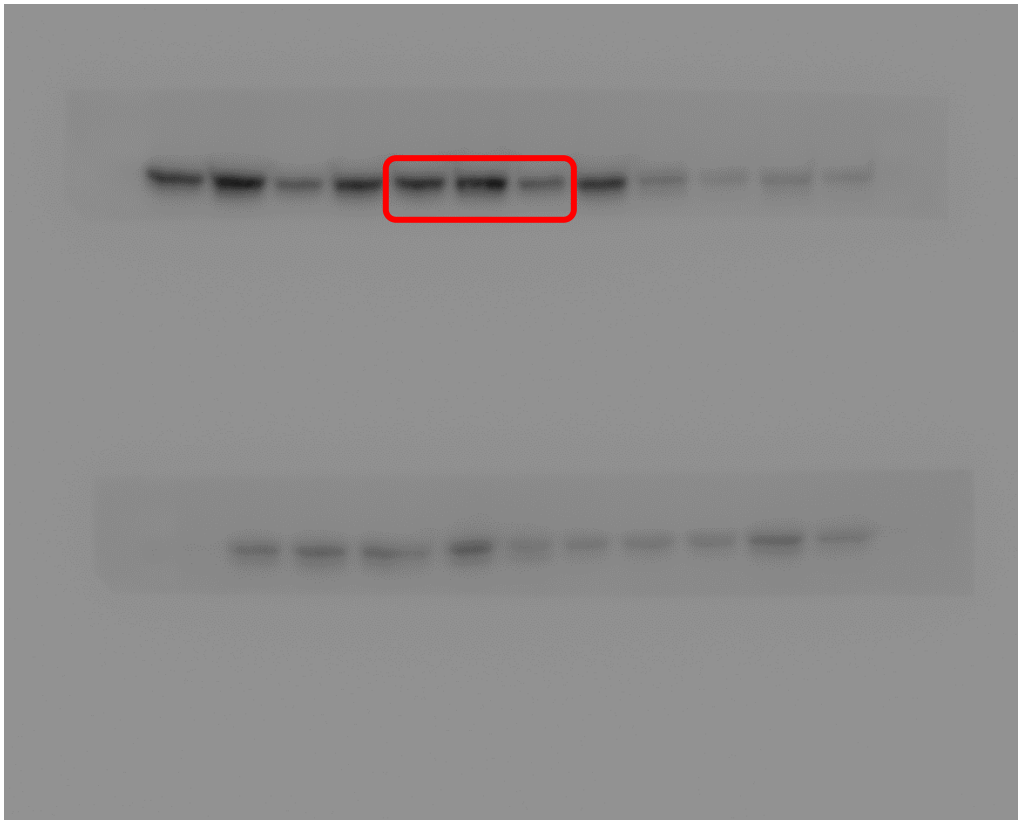
AIF



P-AKT



tAkt



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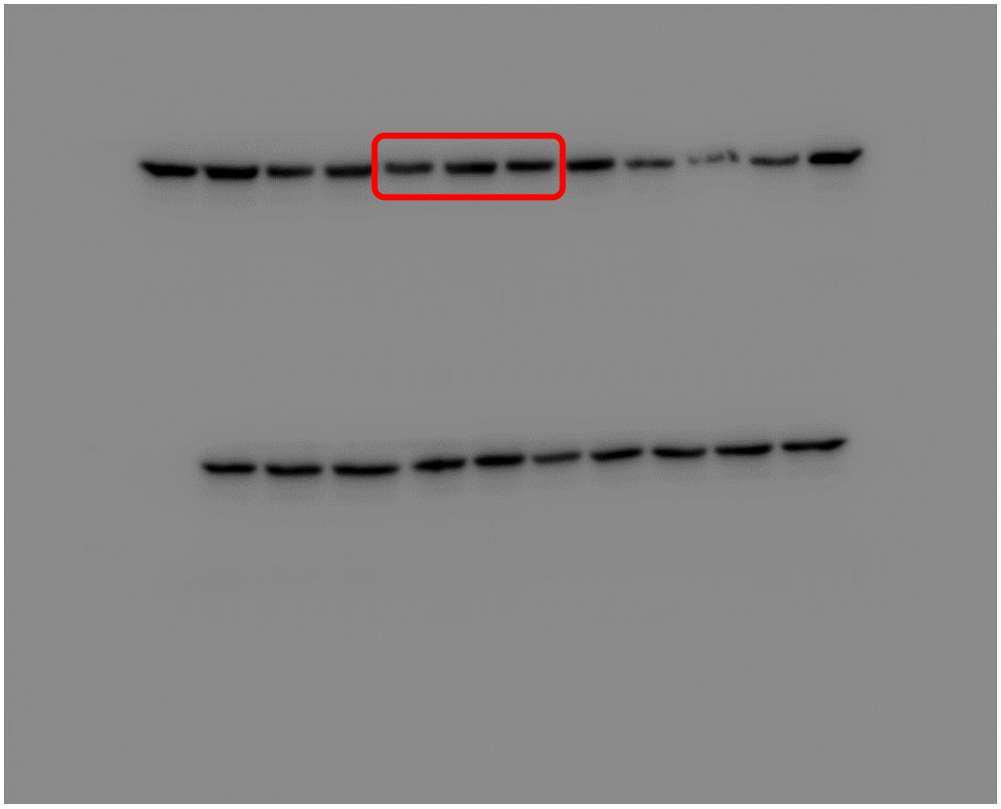
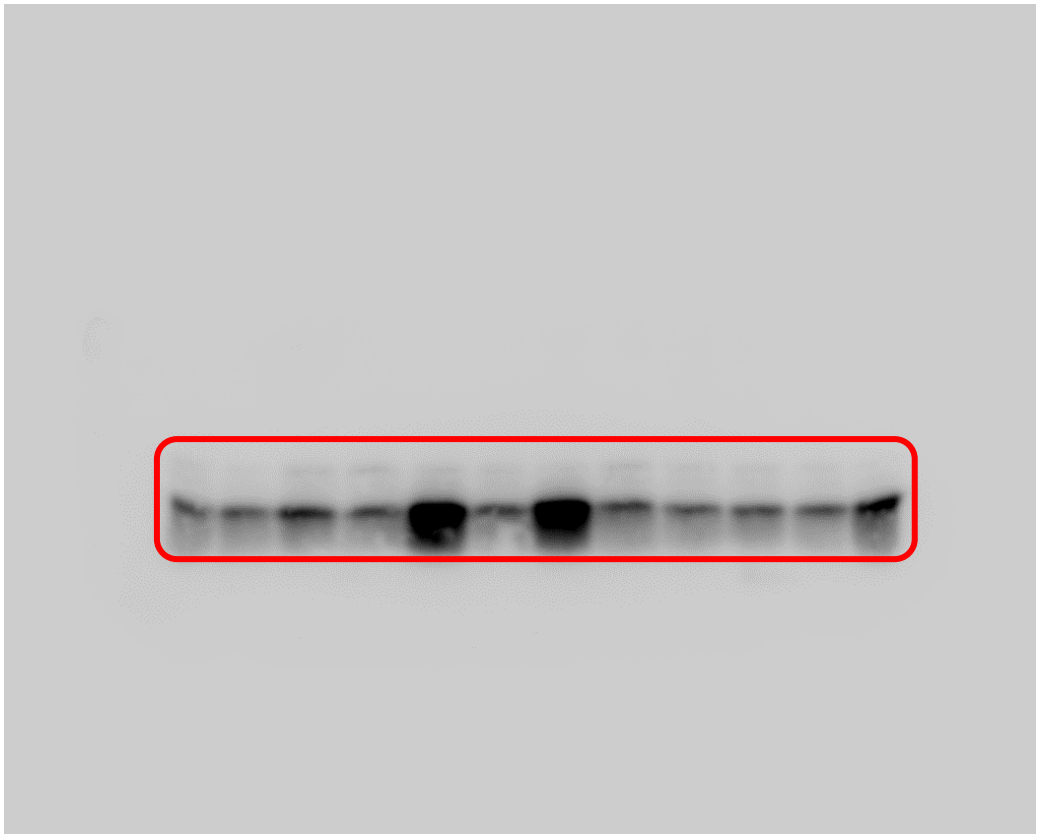


PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer.

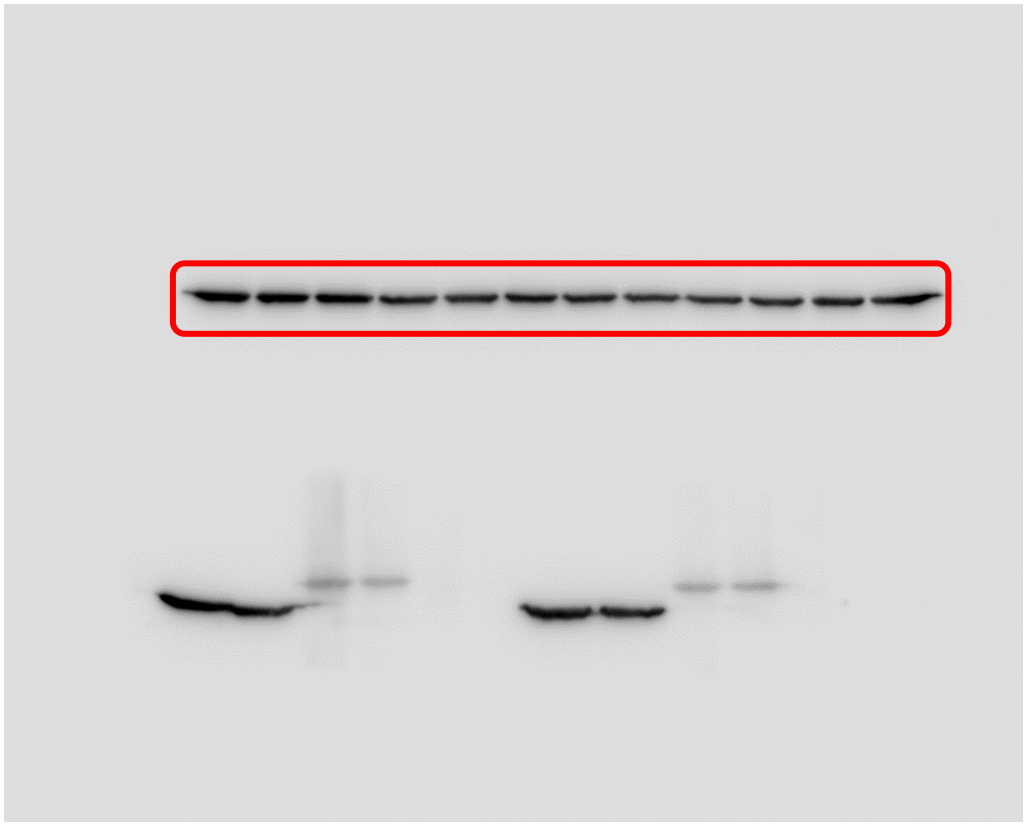
Figure5

C

LC3

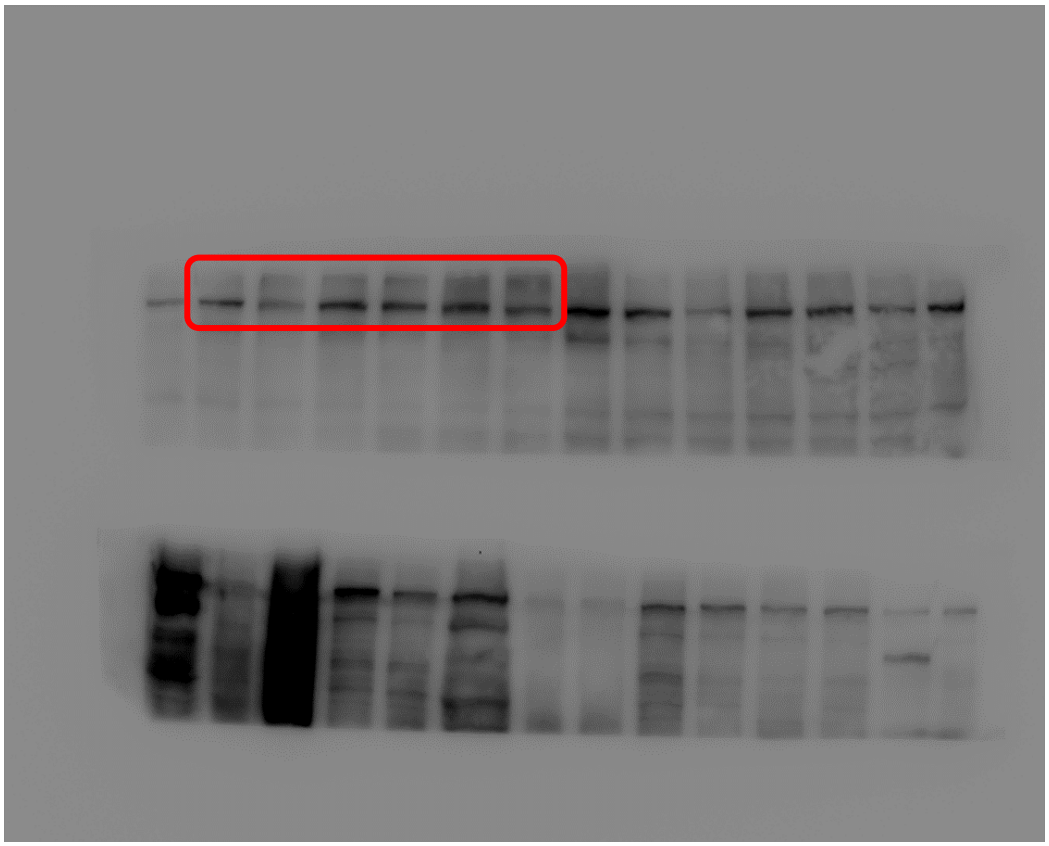


Actin



D

p-mTOR



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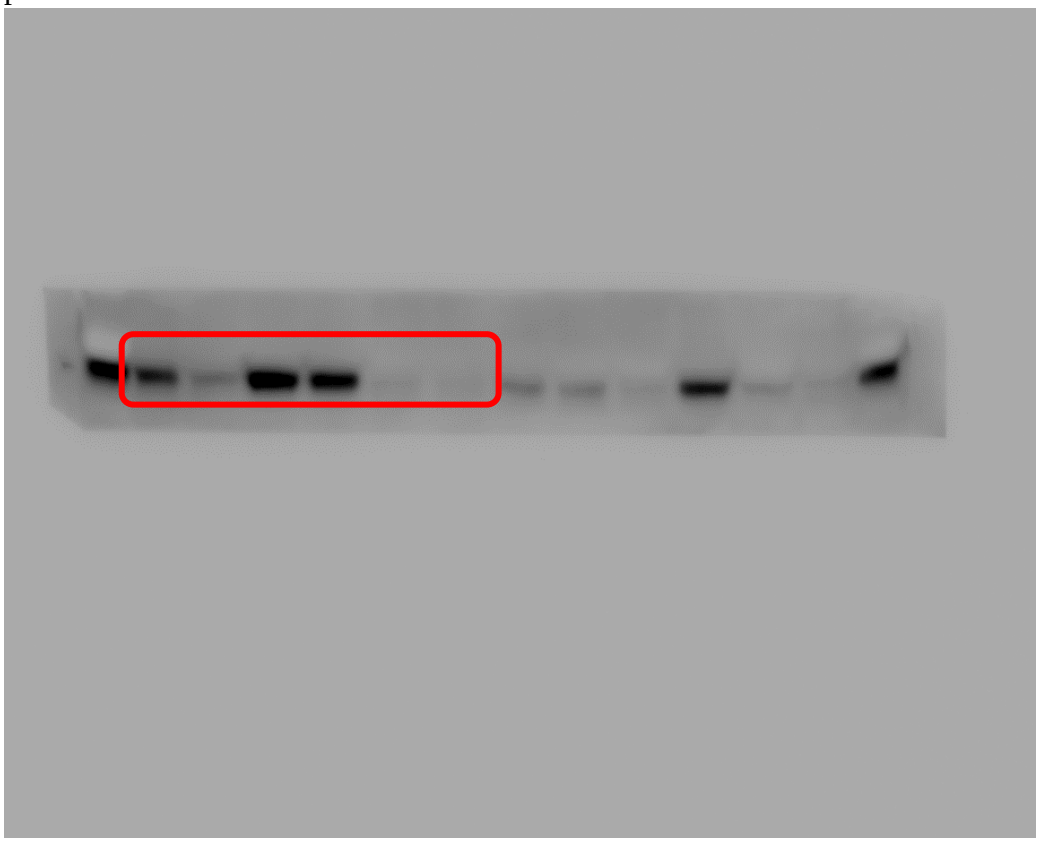

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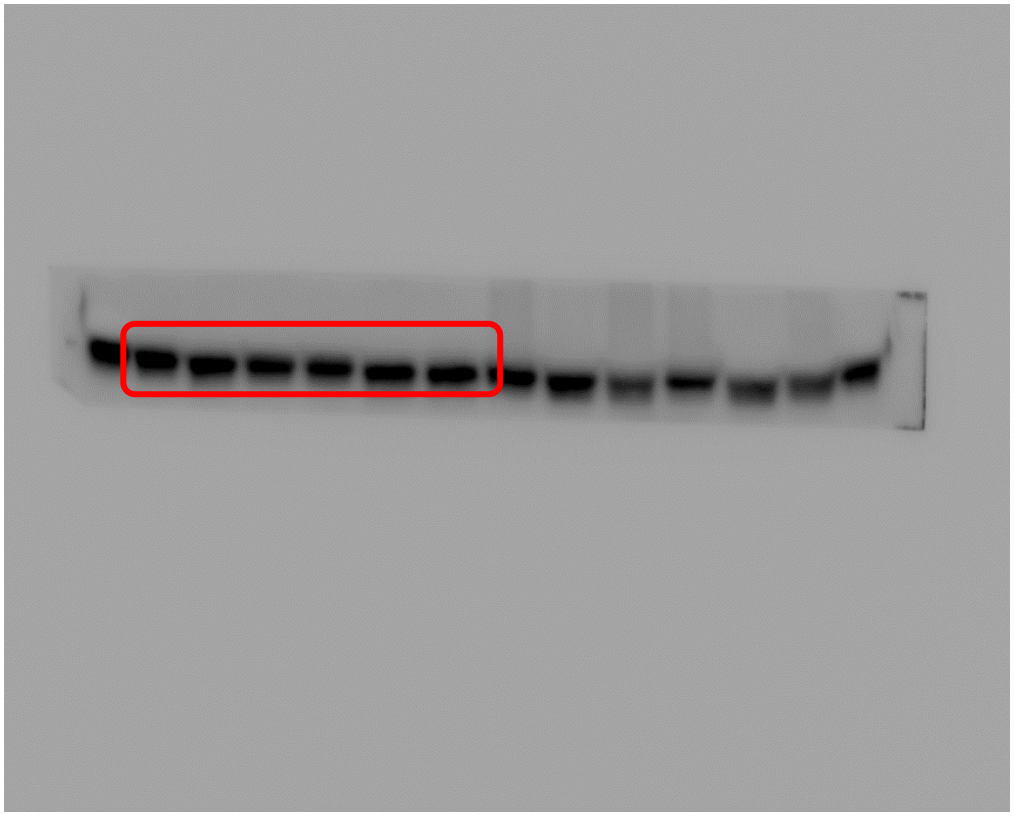

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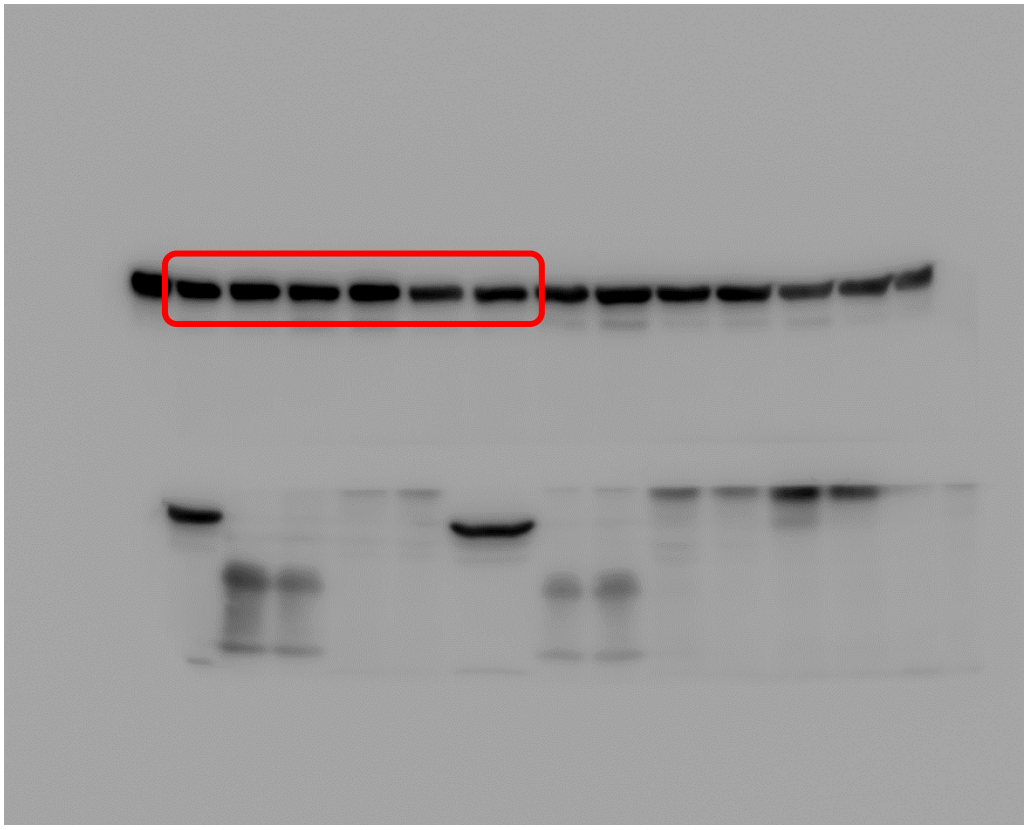
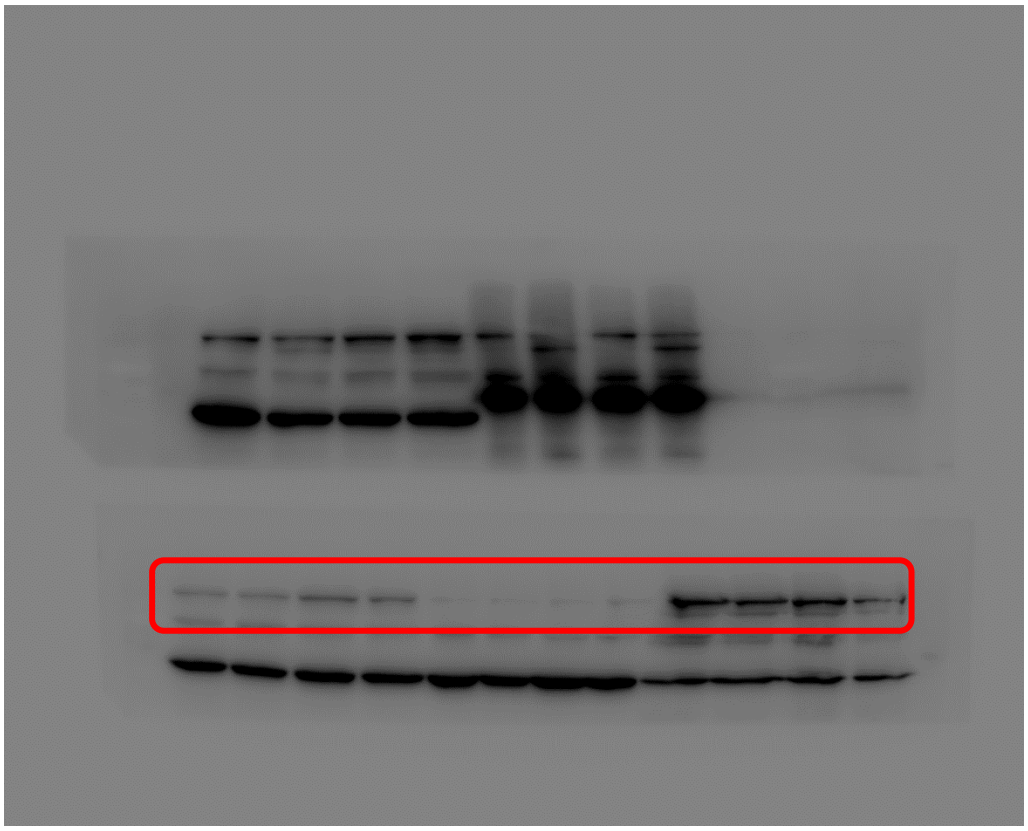
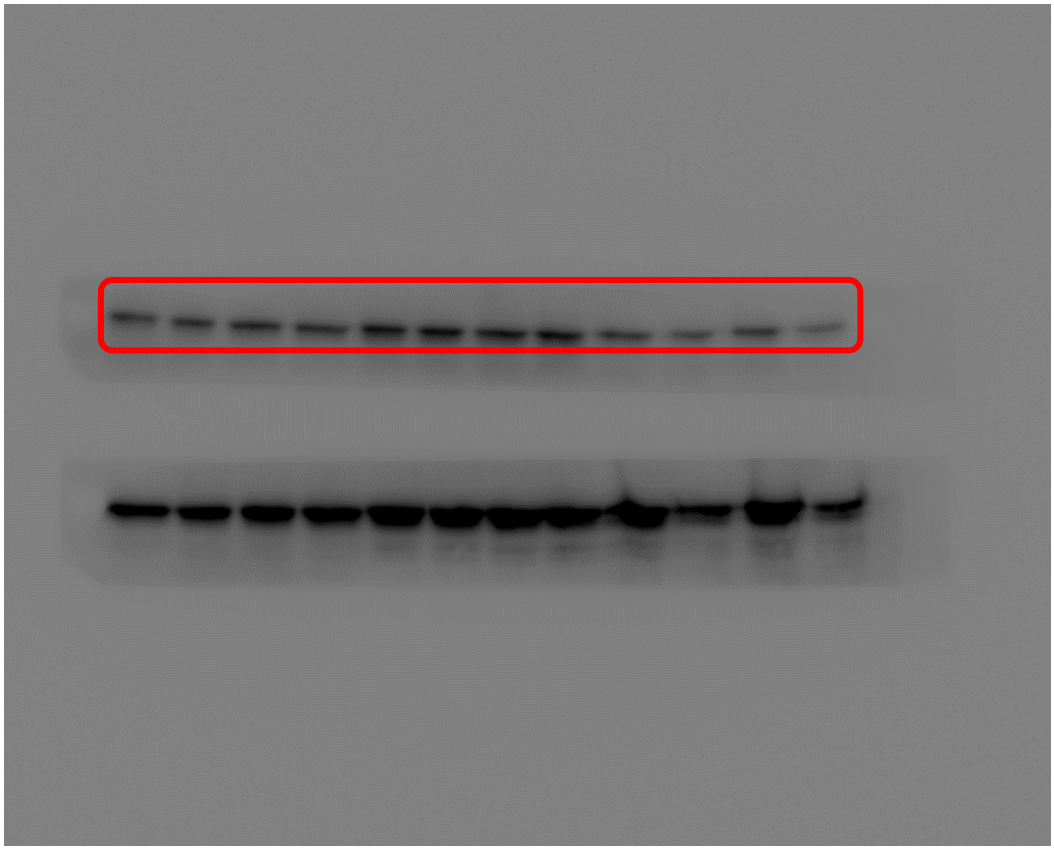


F

PARP



AIF



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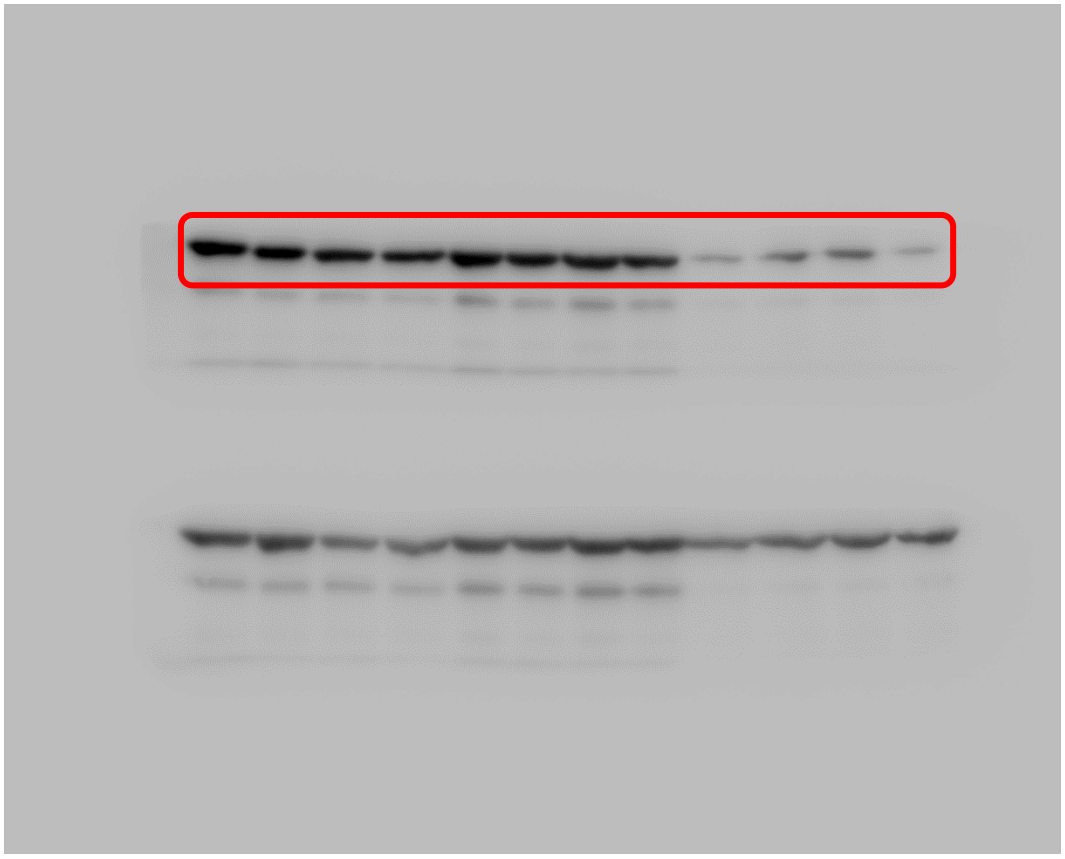
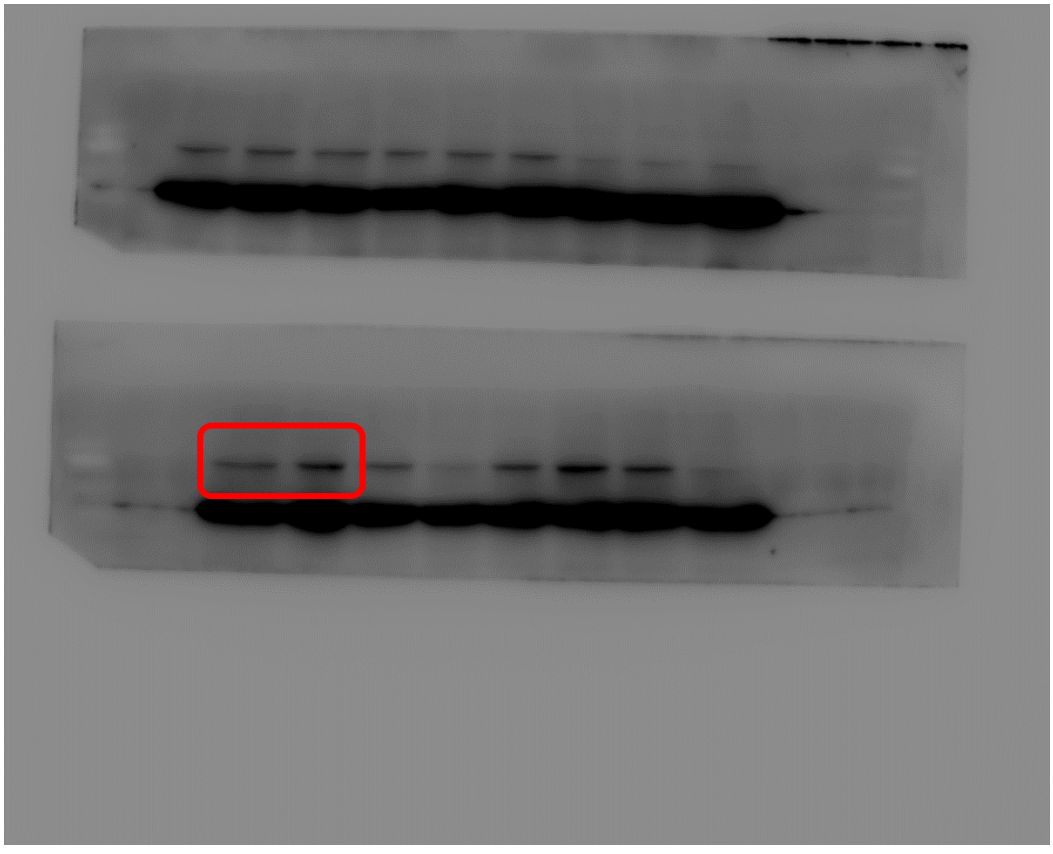
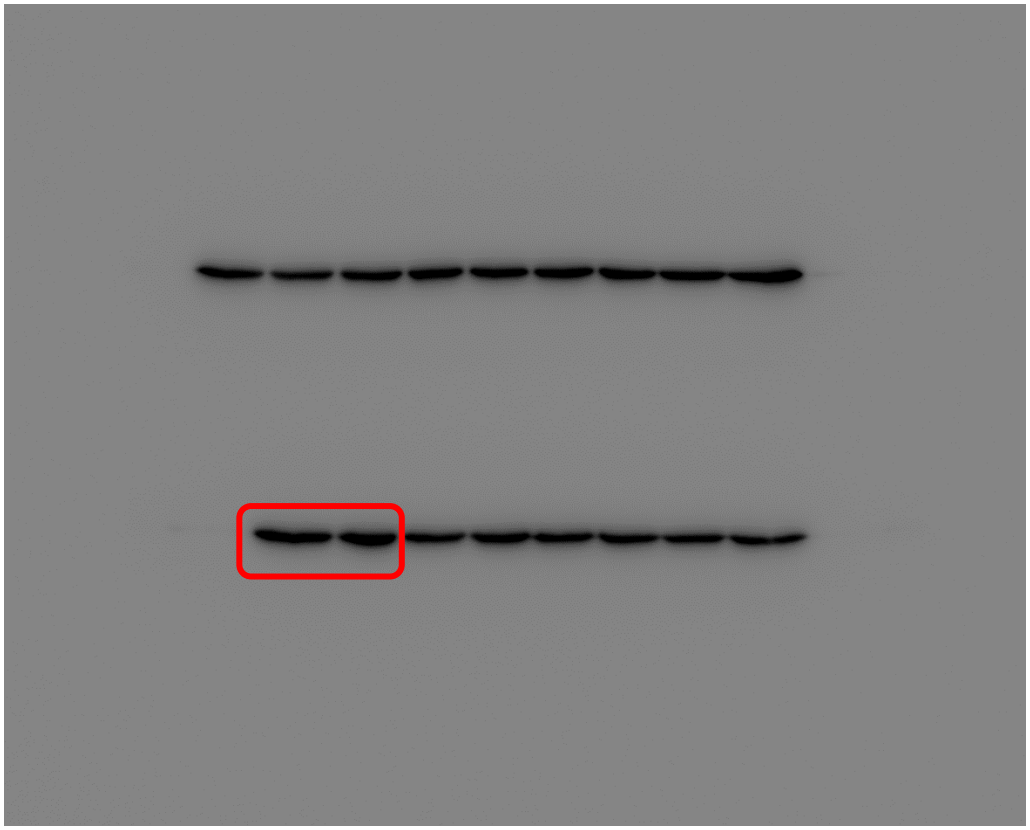


G

AIF



Actin

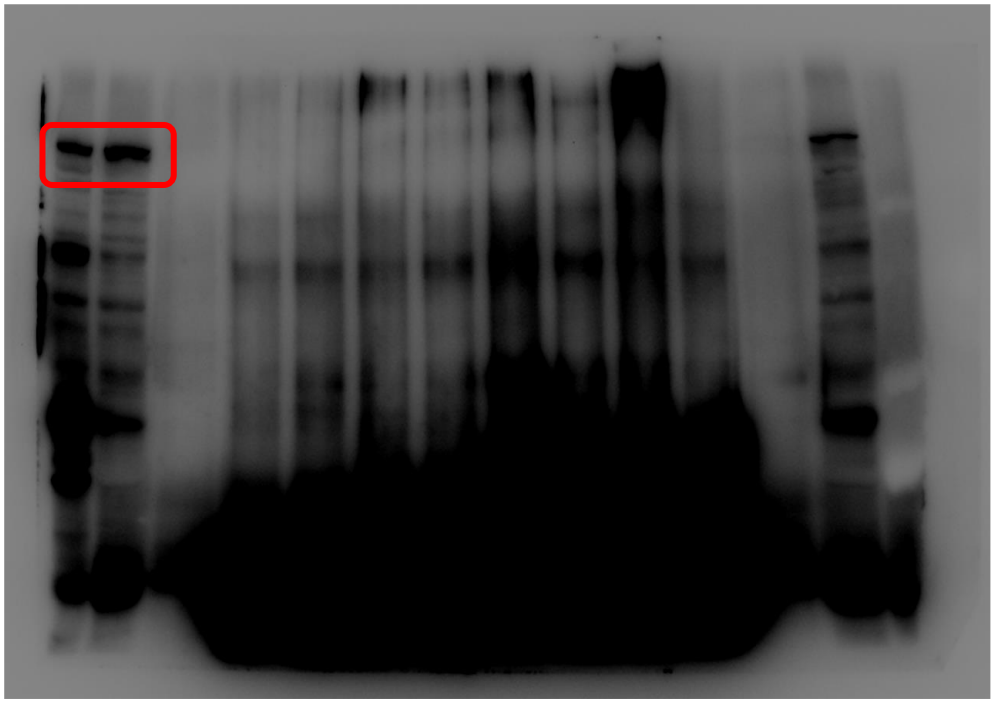


PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer.

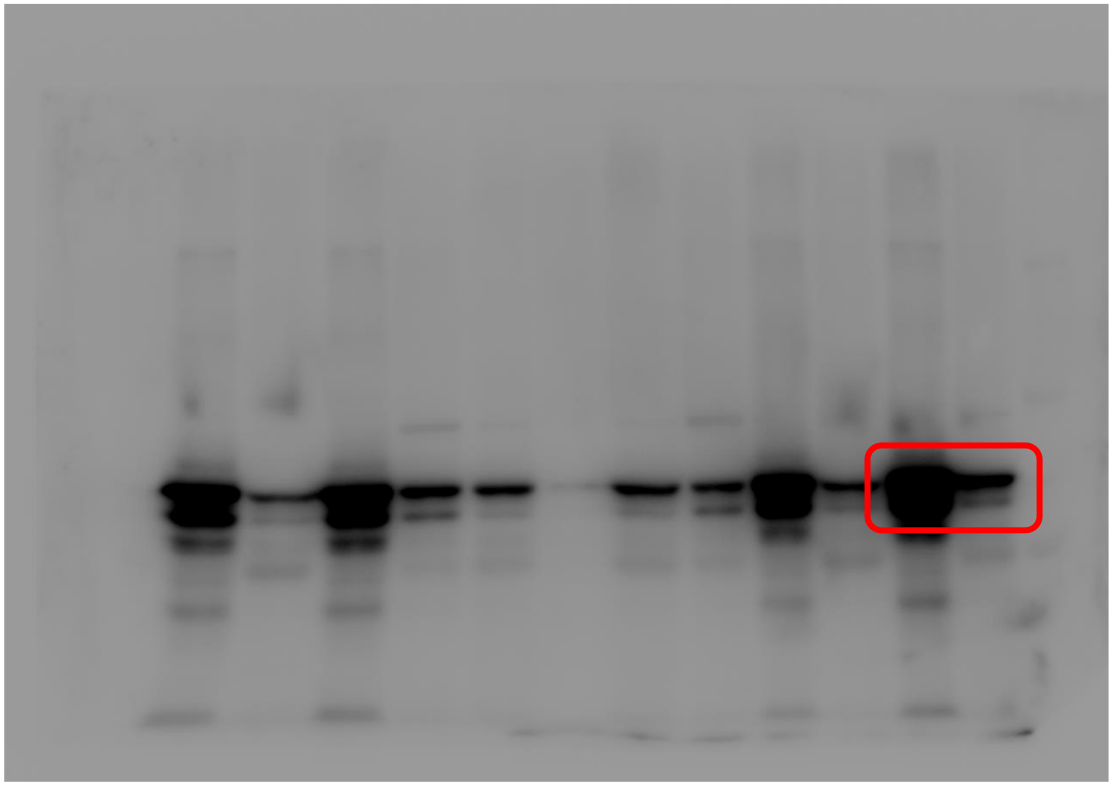
Figure6

A

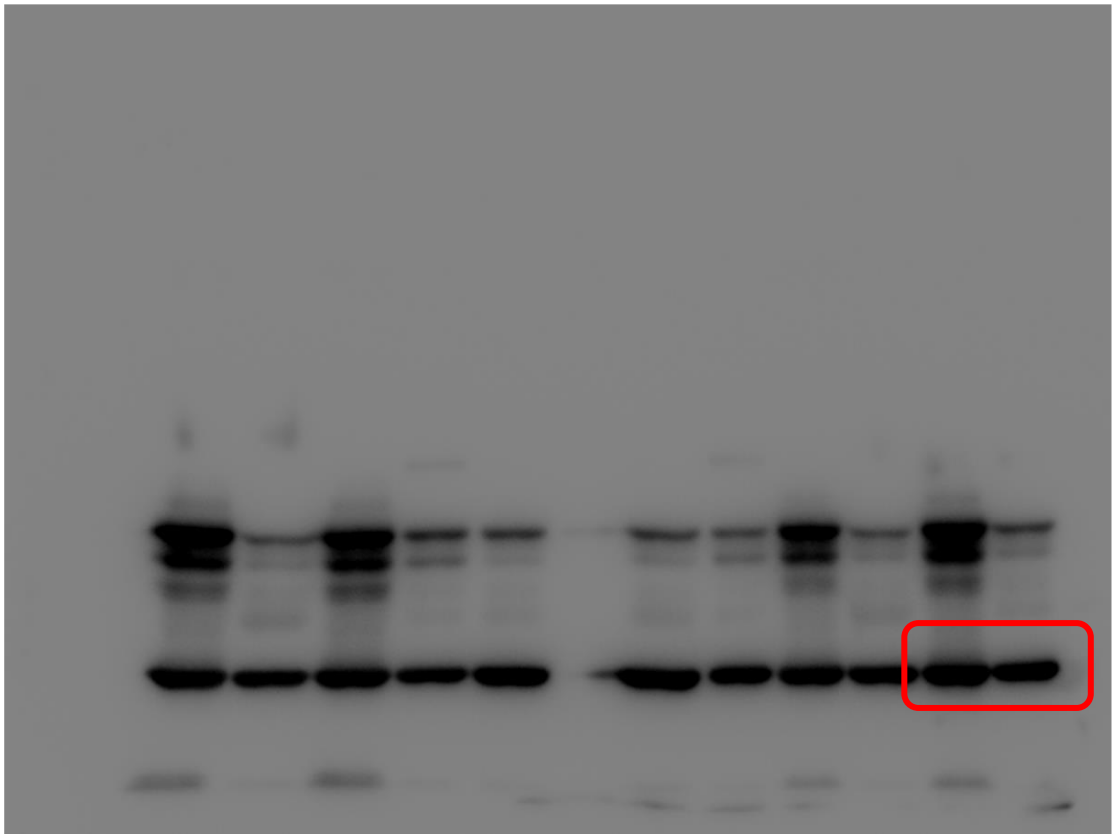
mTOR



AIF

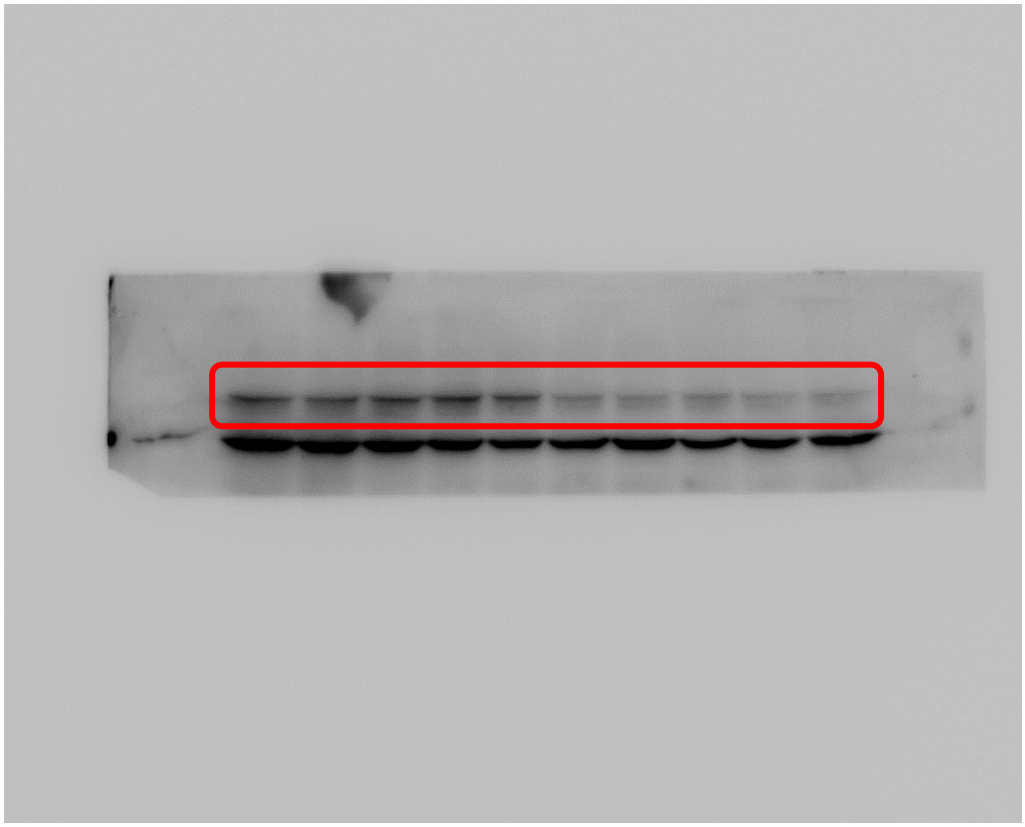


Actin

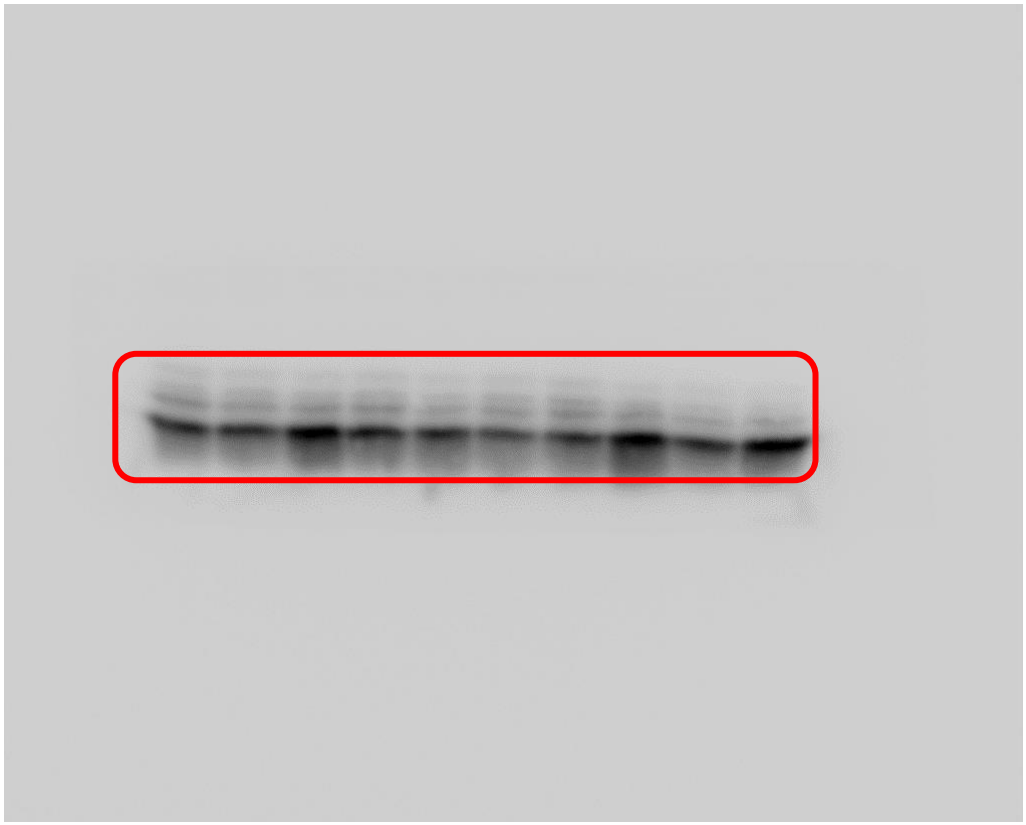


B

AIF



LC3



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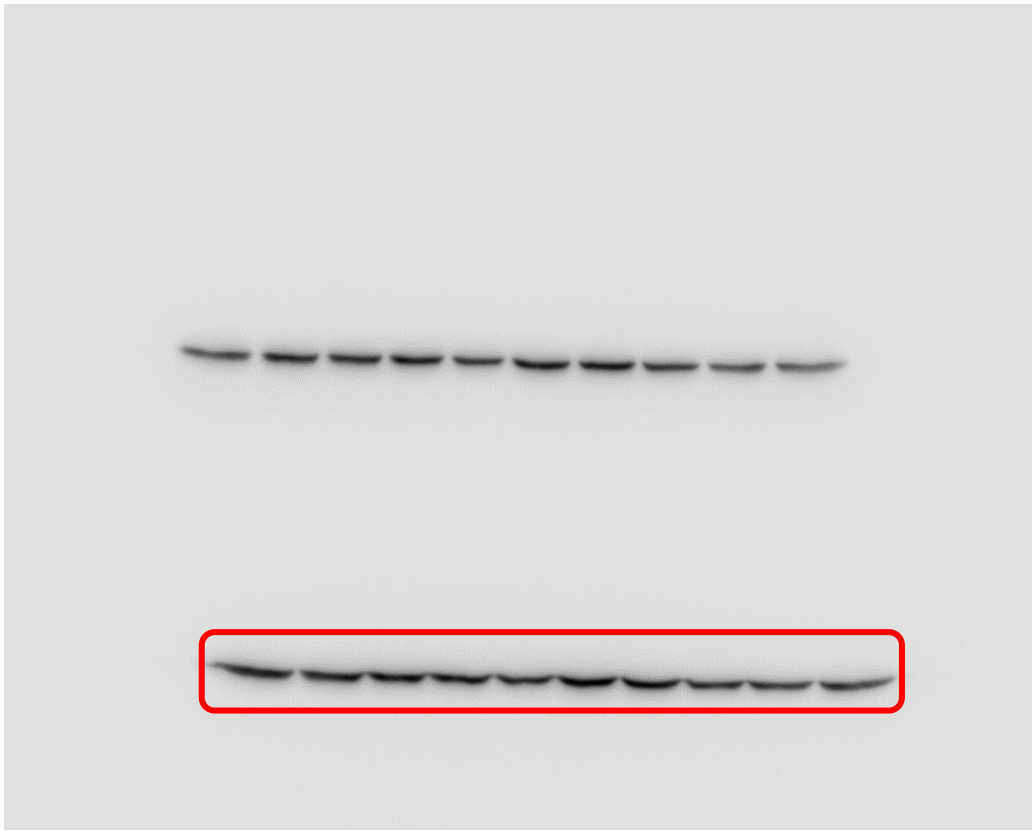
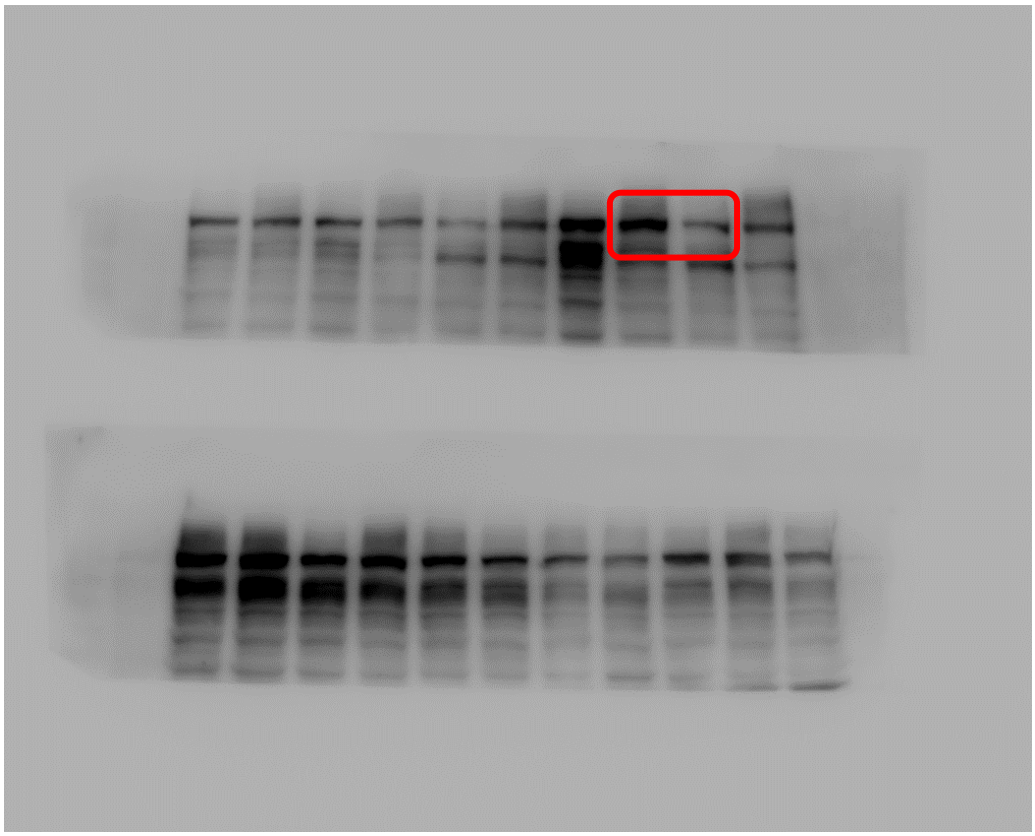


C

mTOR



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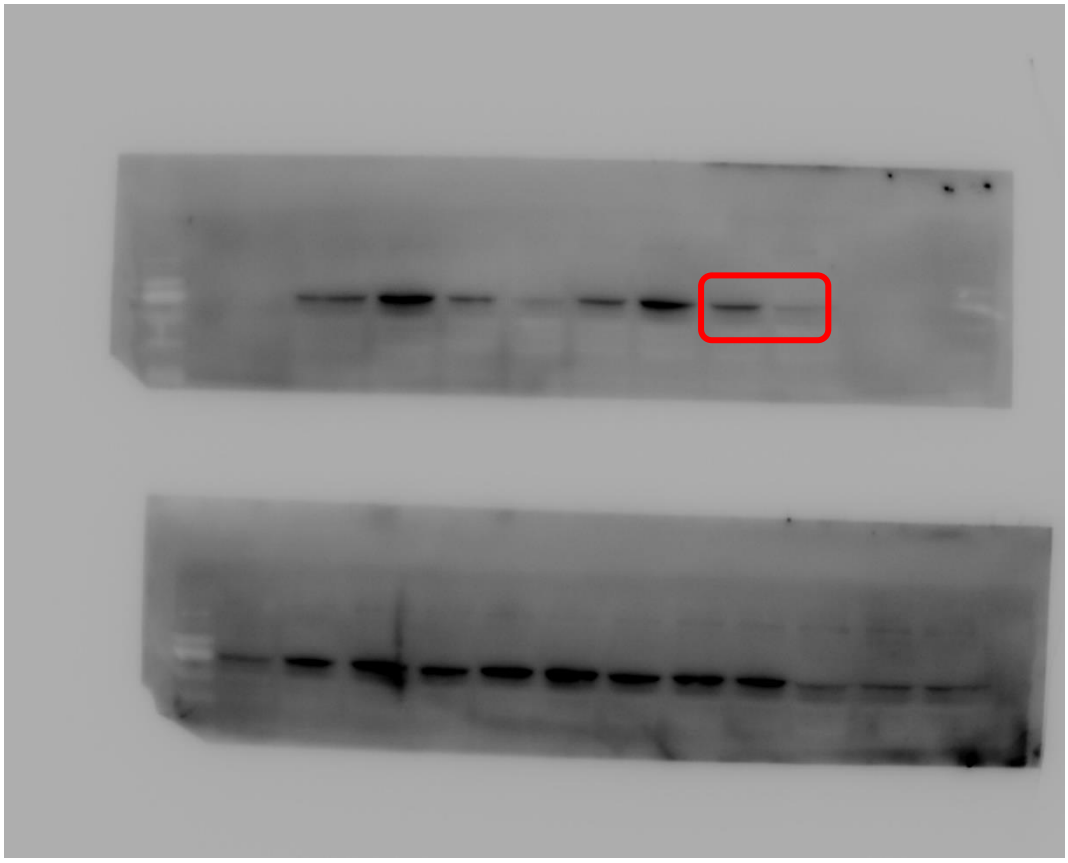

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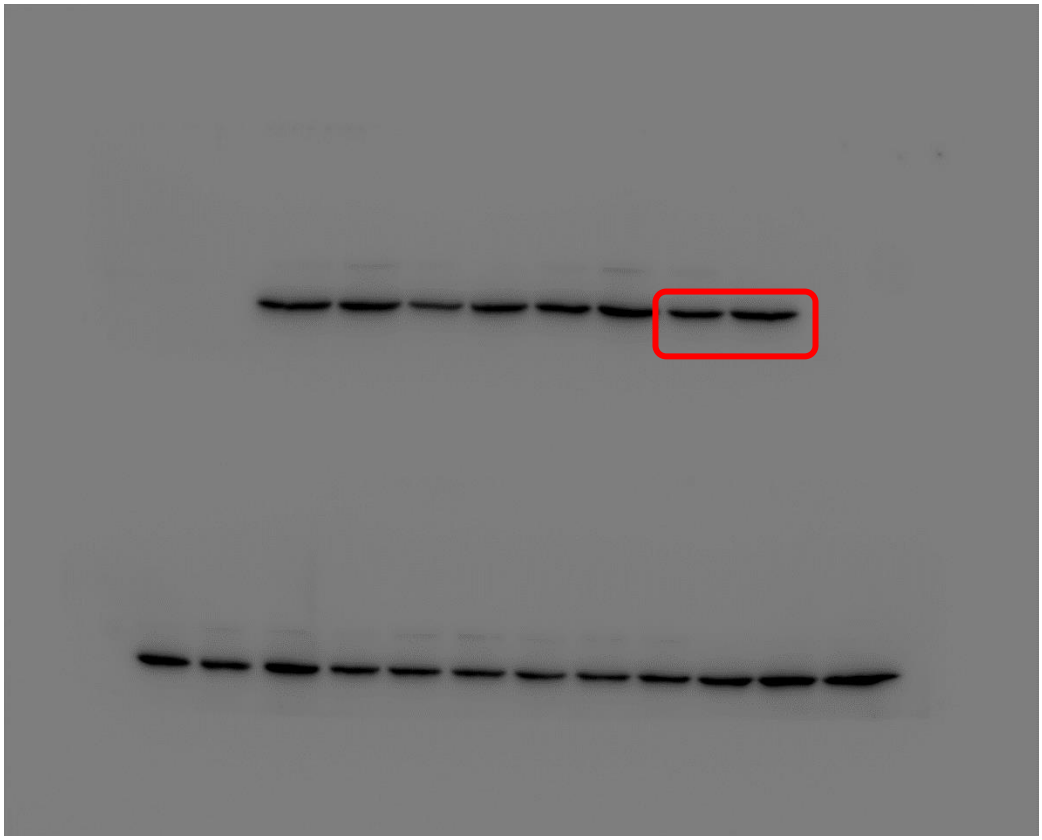
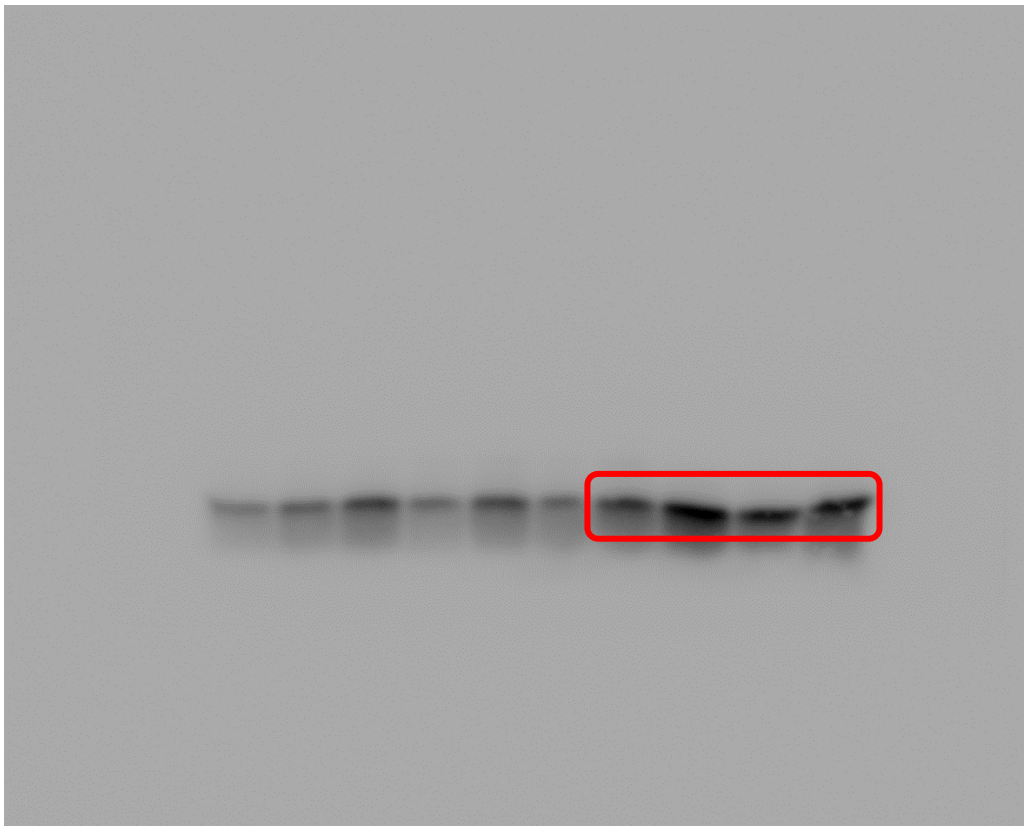


D

LC3



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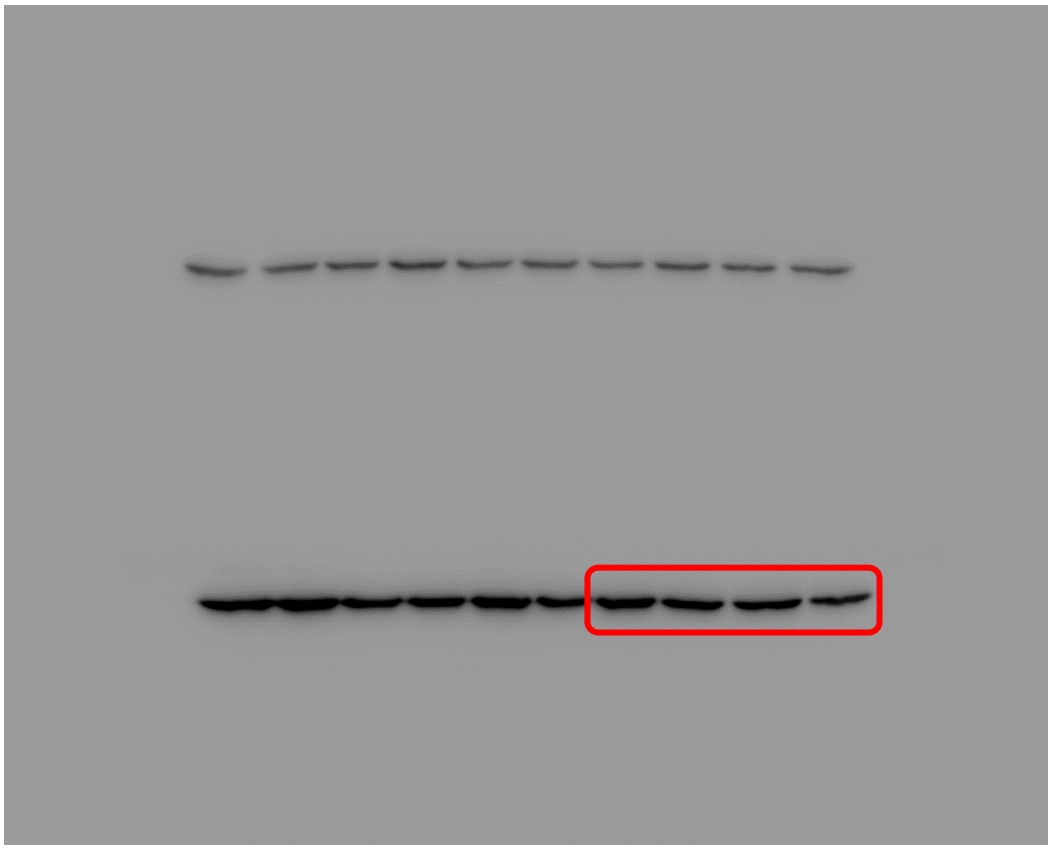
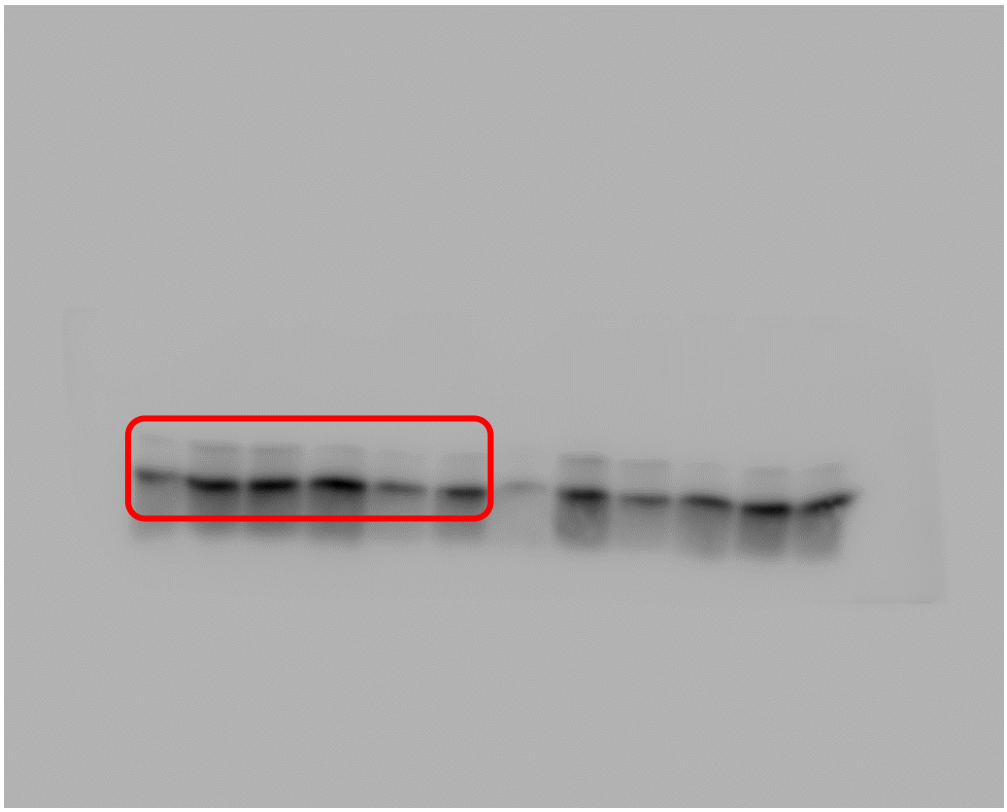
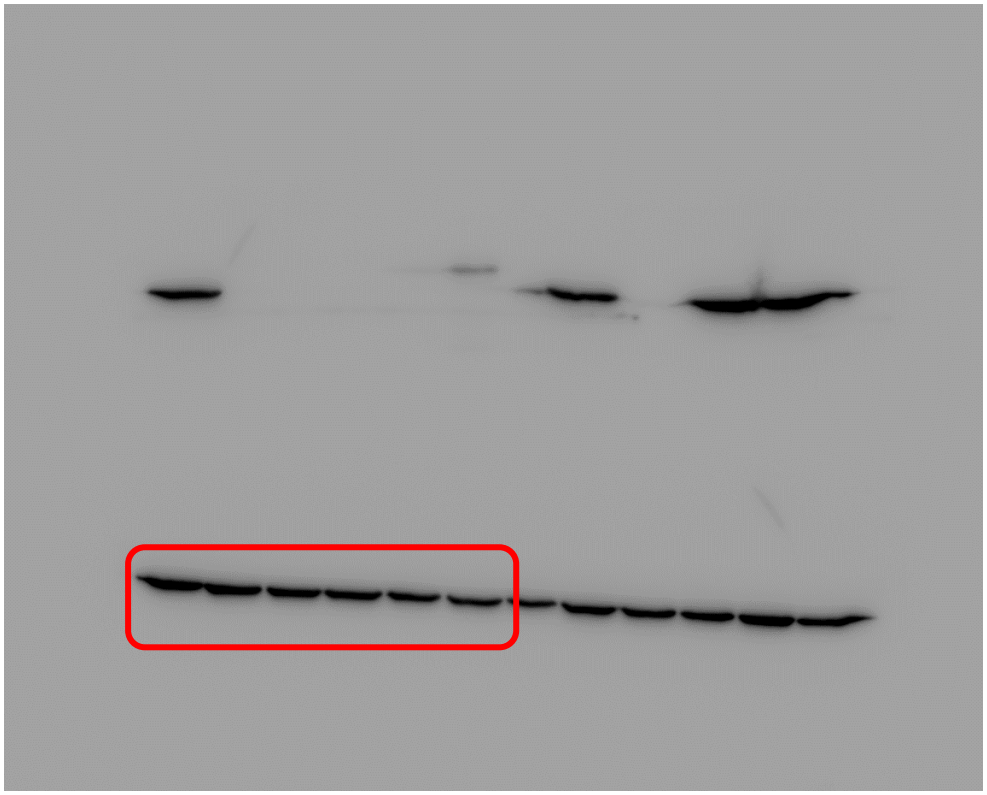


E

LC3

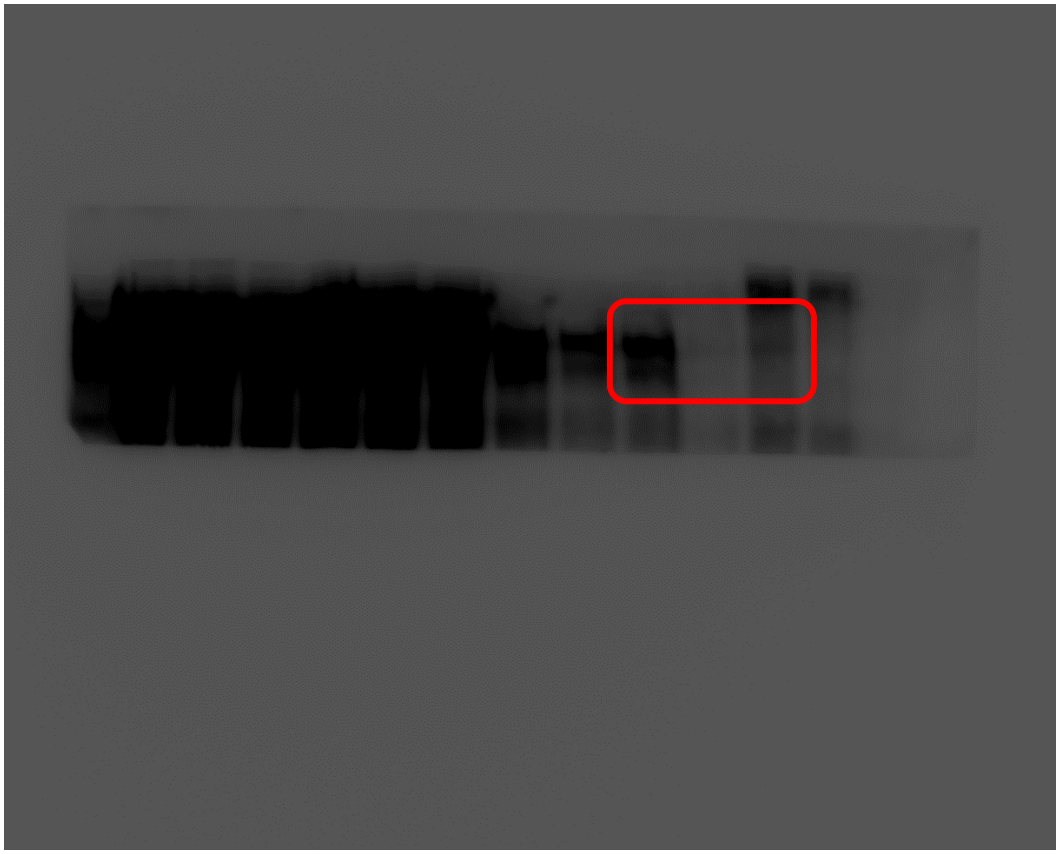


Actin

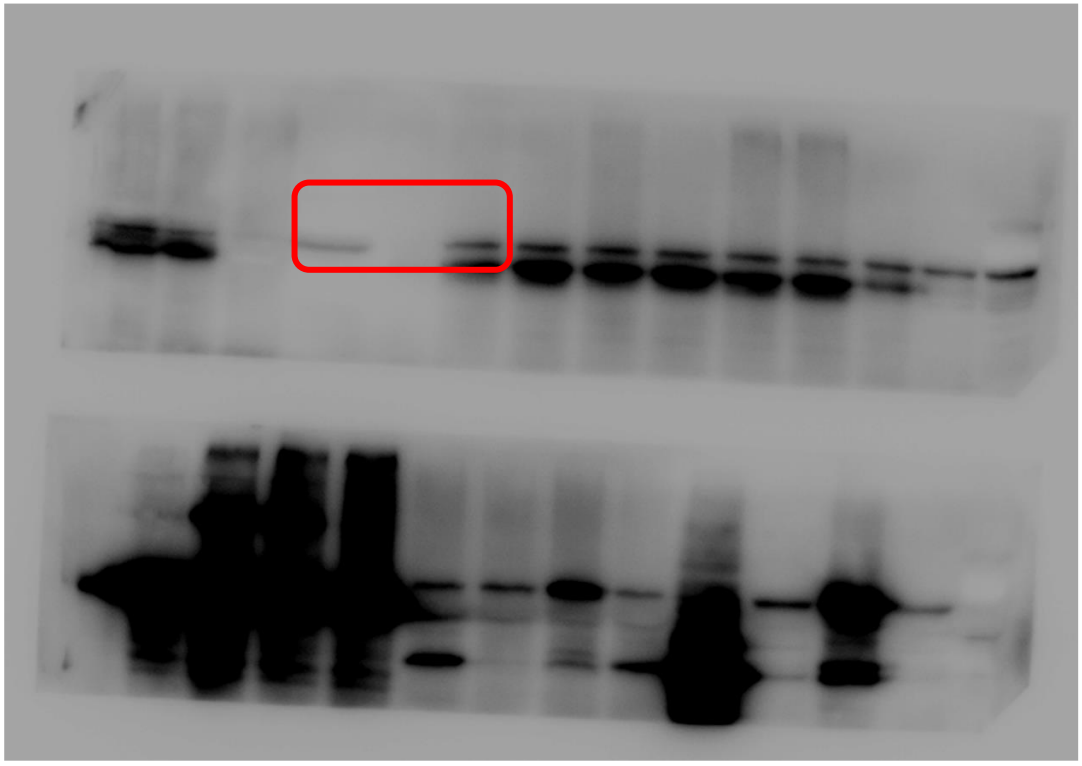


PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer.

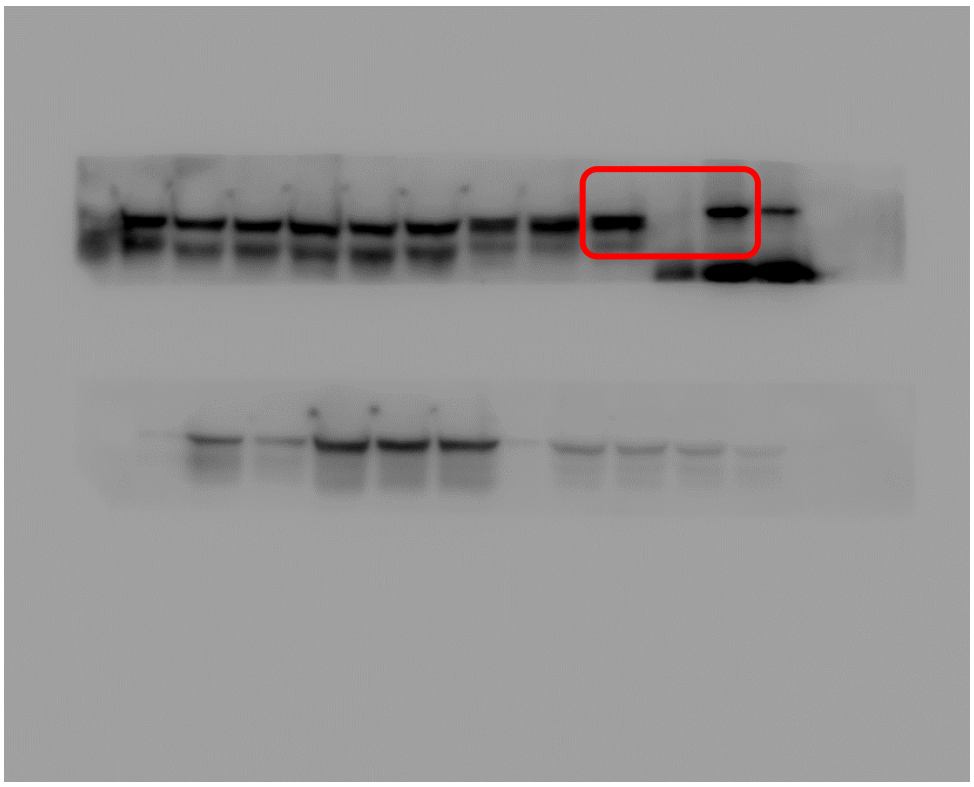
Figure7
A
pmTOR



BECN1

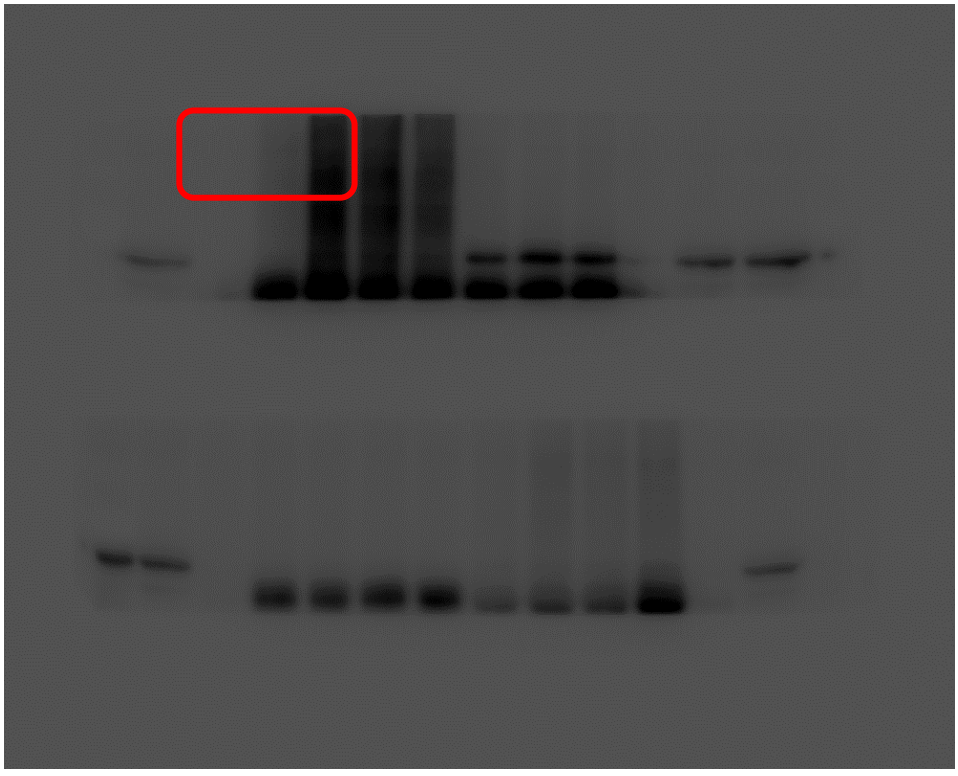


AIF

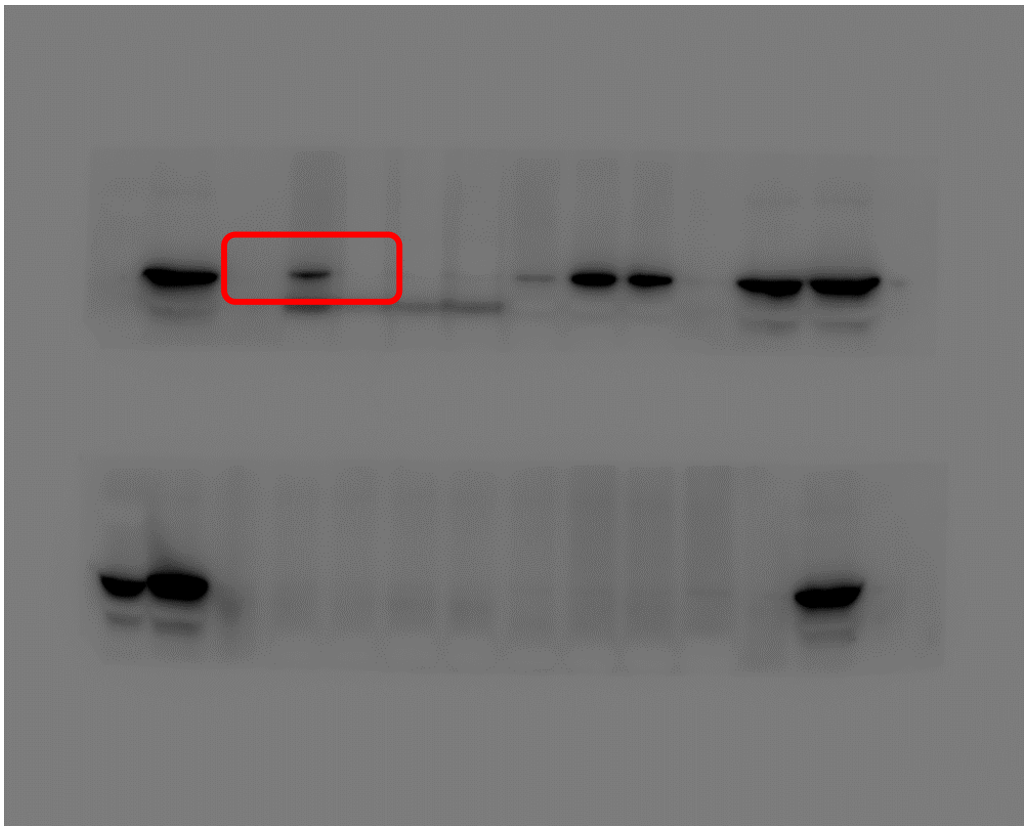


B

VPS34



AIF



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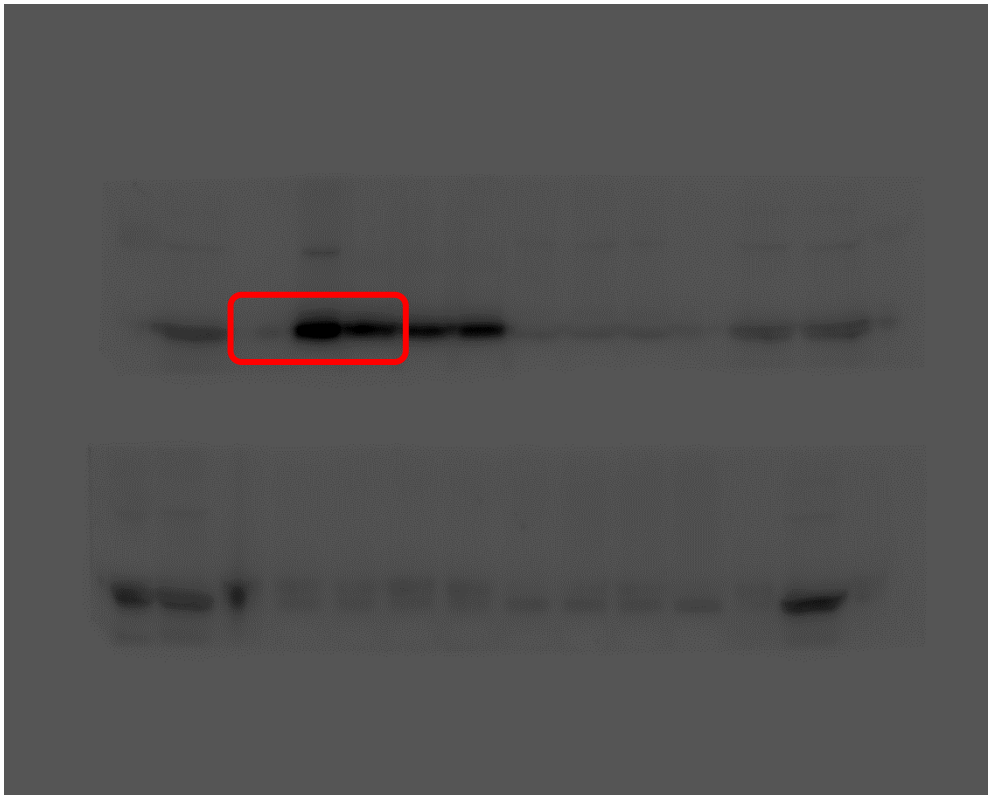
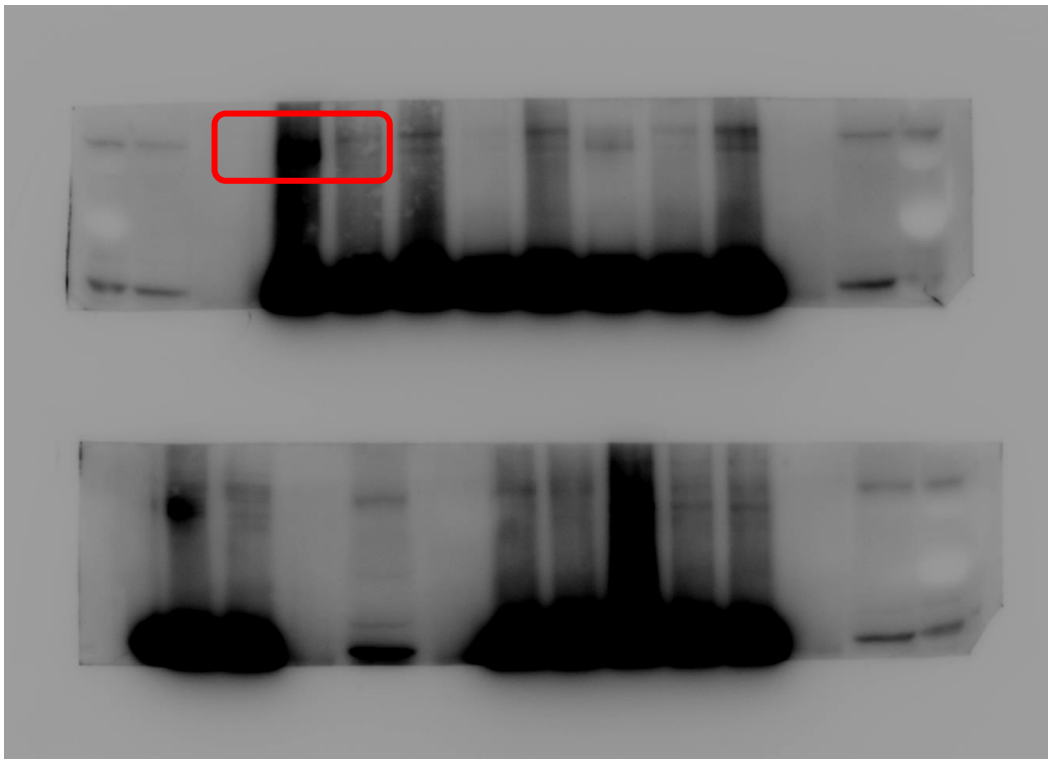


C

VPS34



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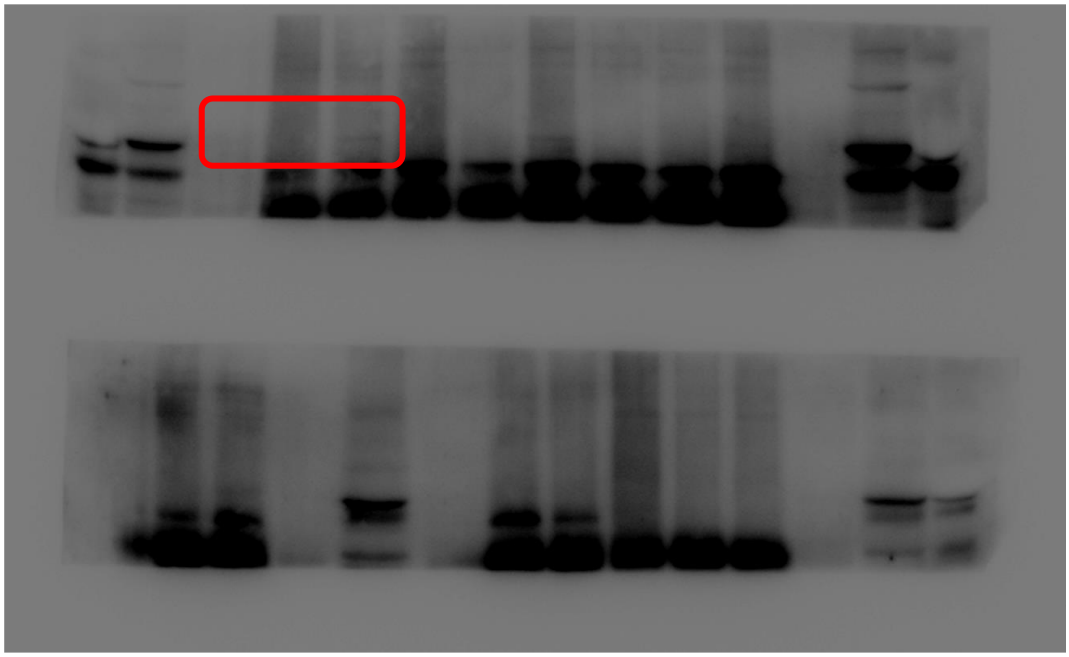
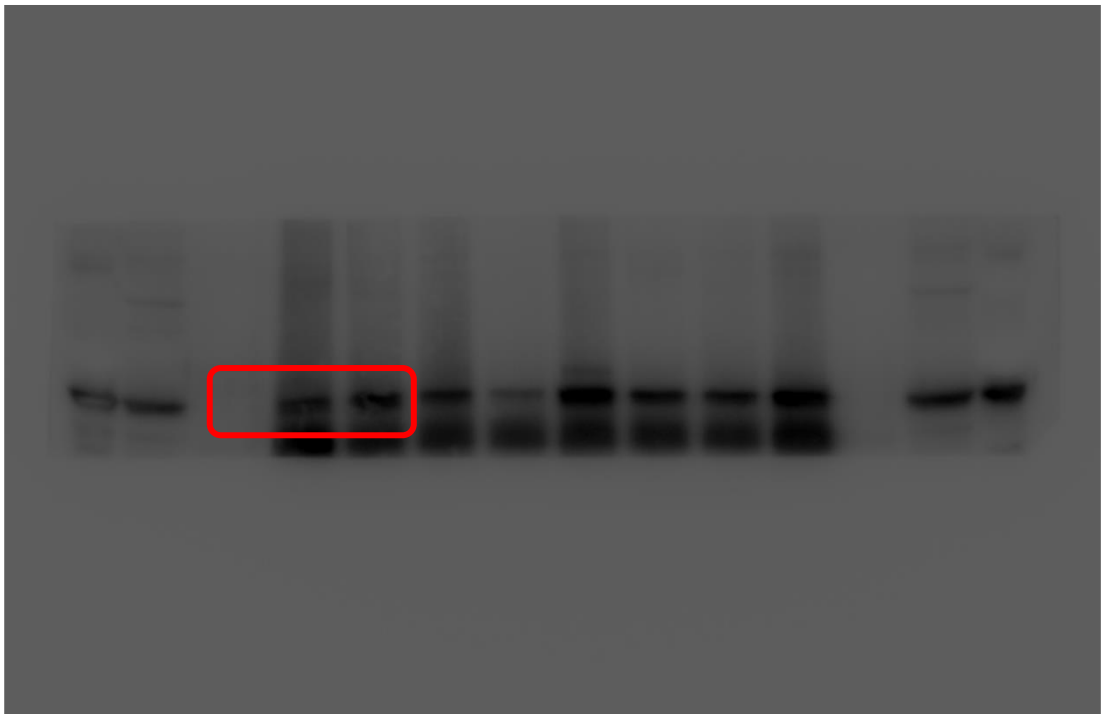
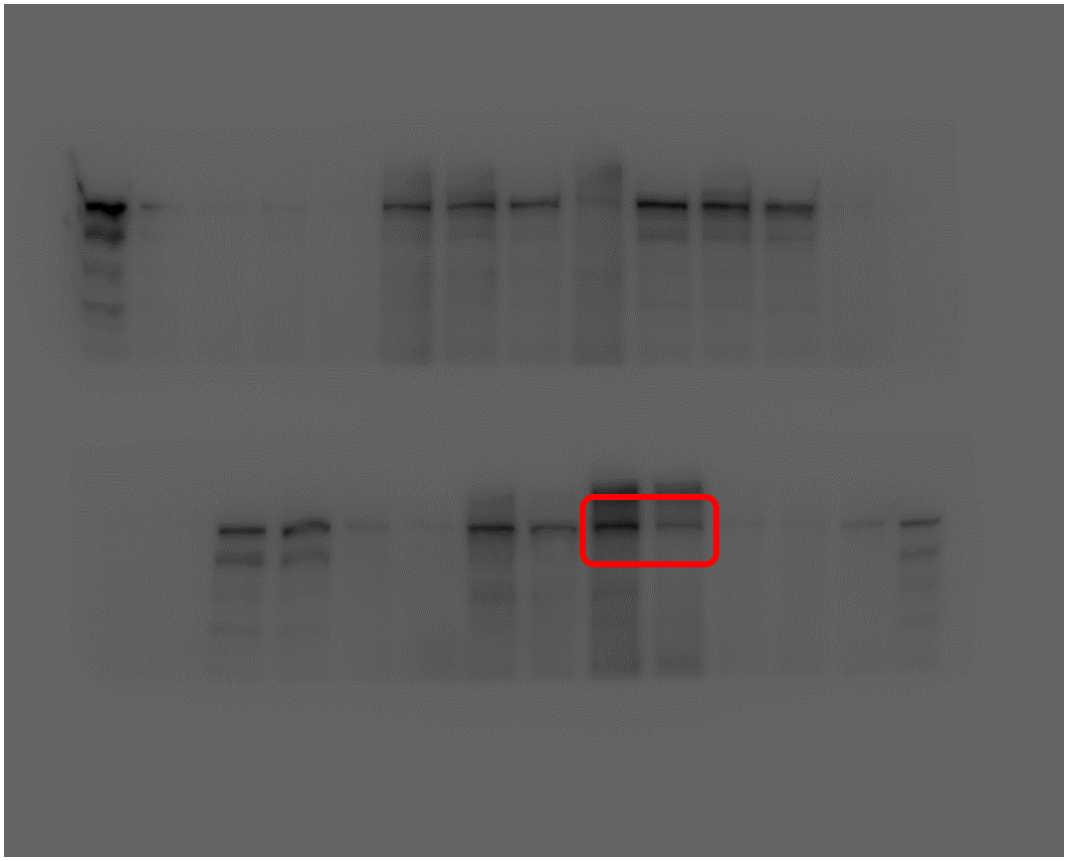


Beclin1

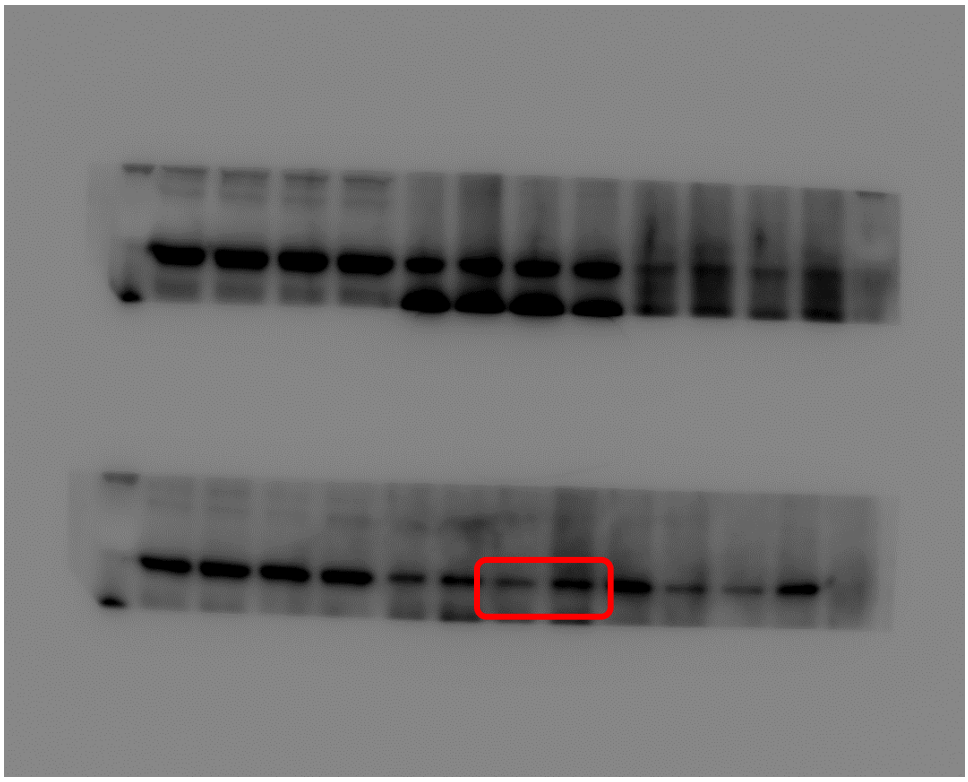


D

p-mTOR



BECN1



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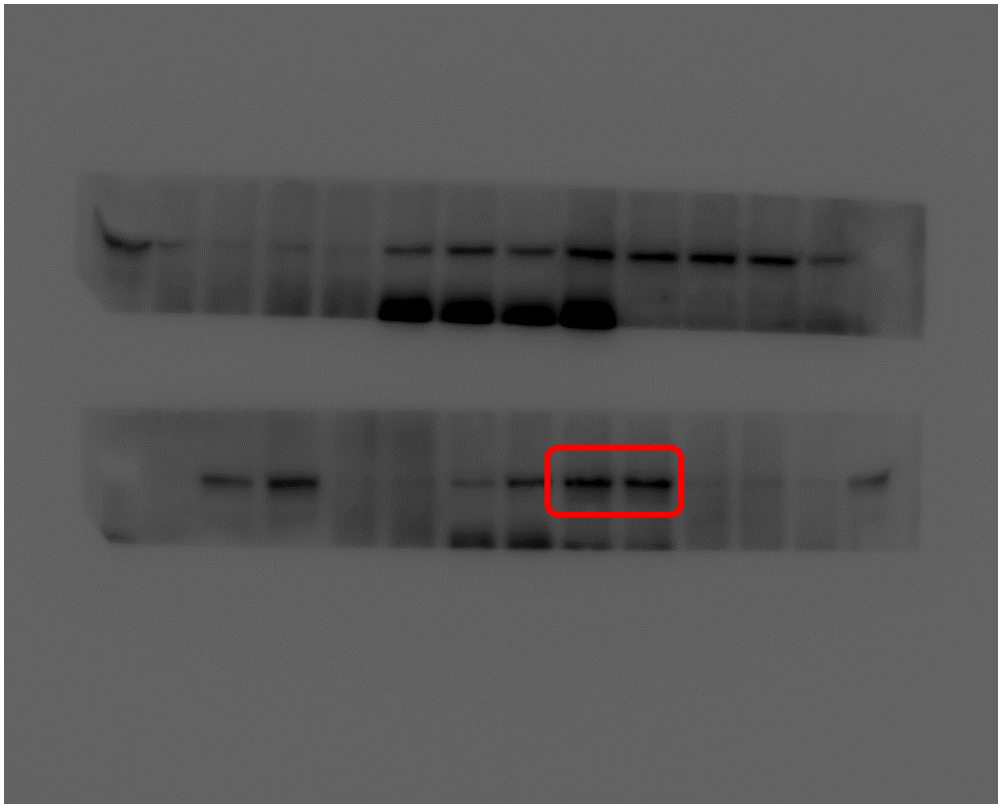
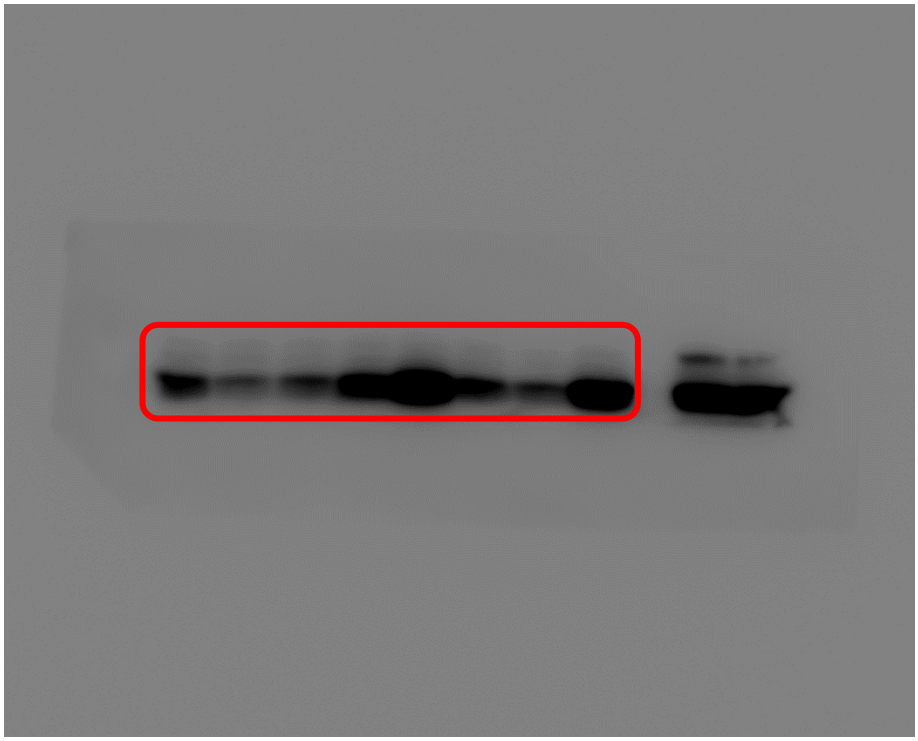


PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer. Figure 7a is the result of the same batch of samples running with SDS-PAGE on different days.

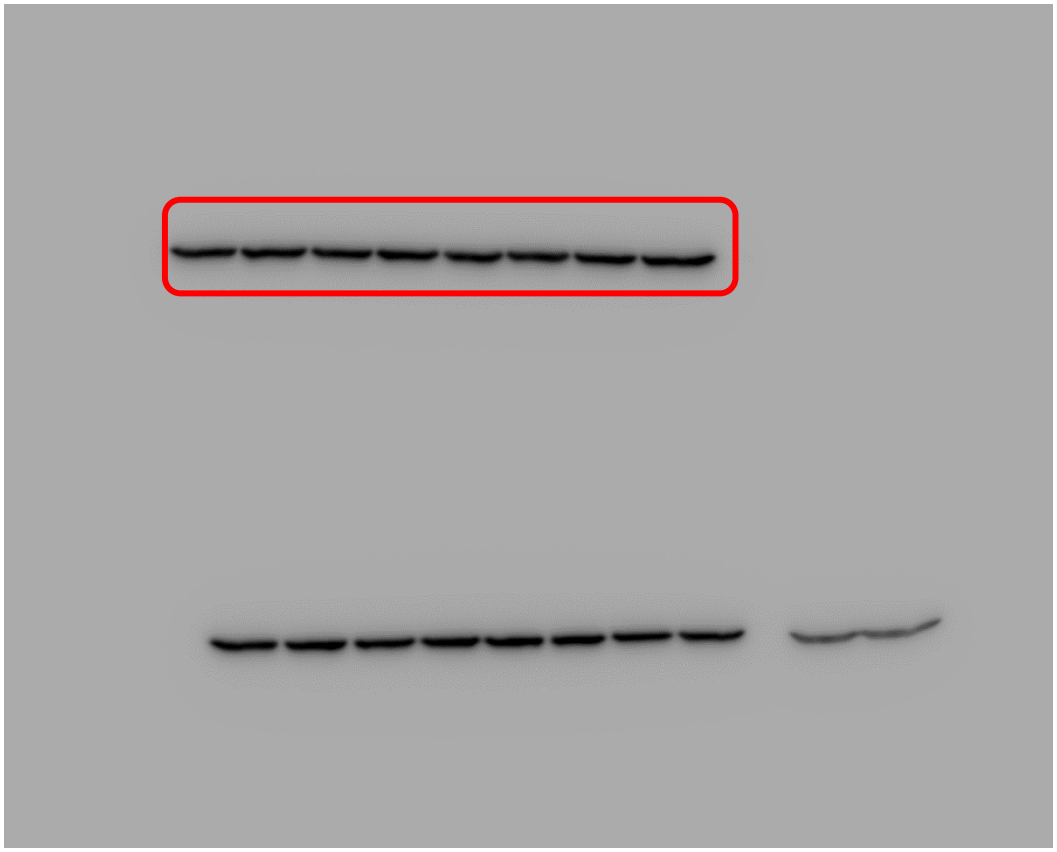
Supplemental 1

A

LC3

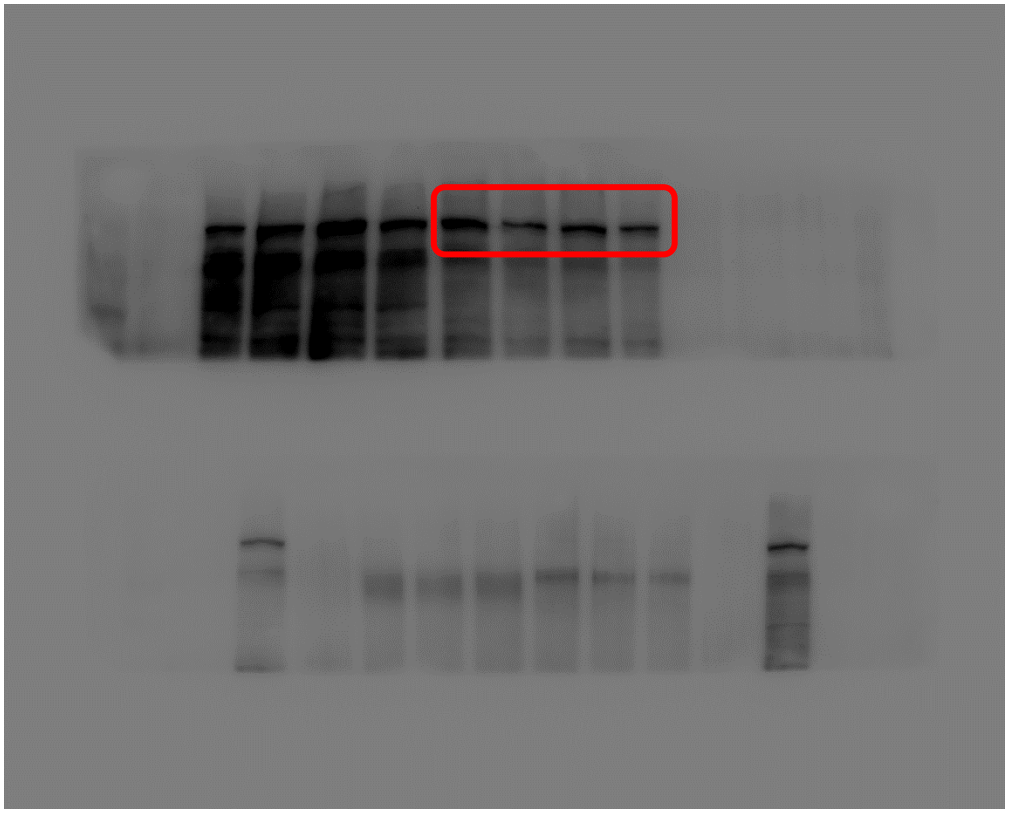


Actin

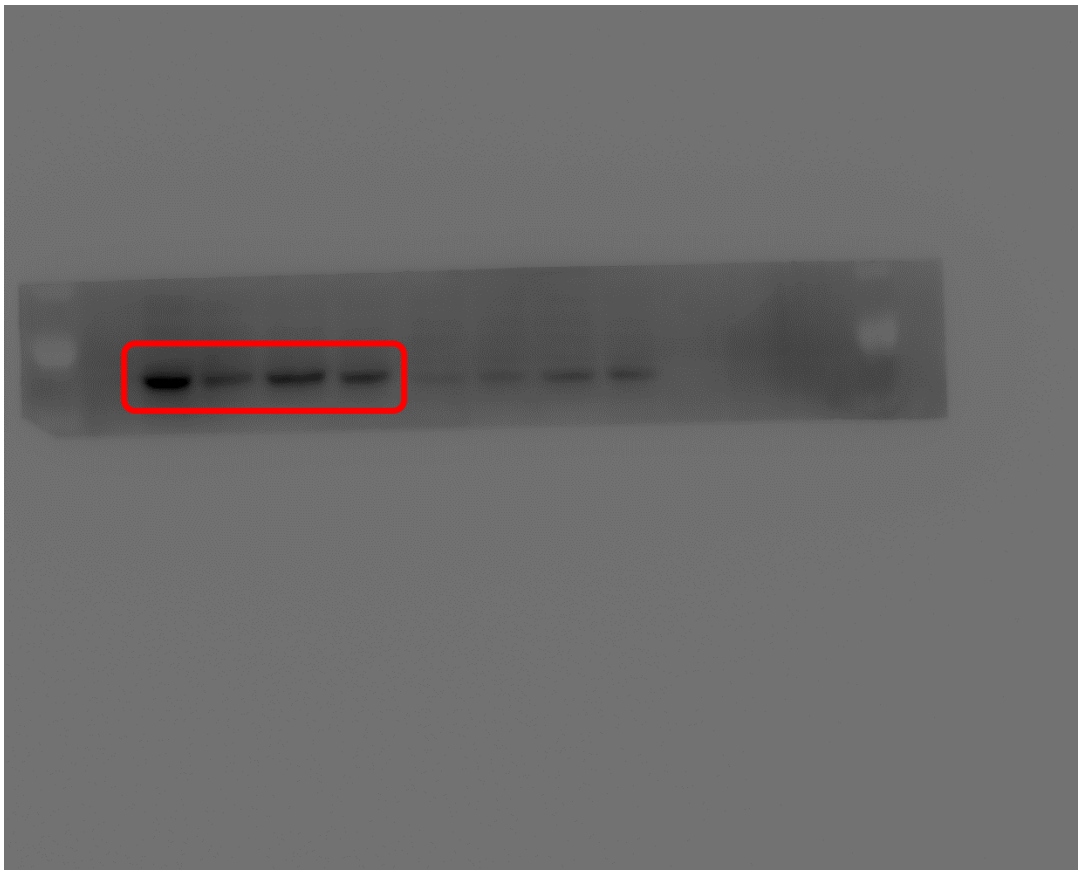


B

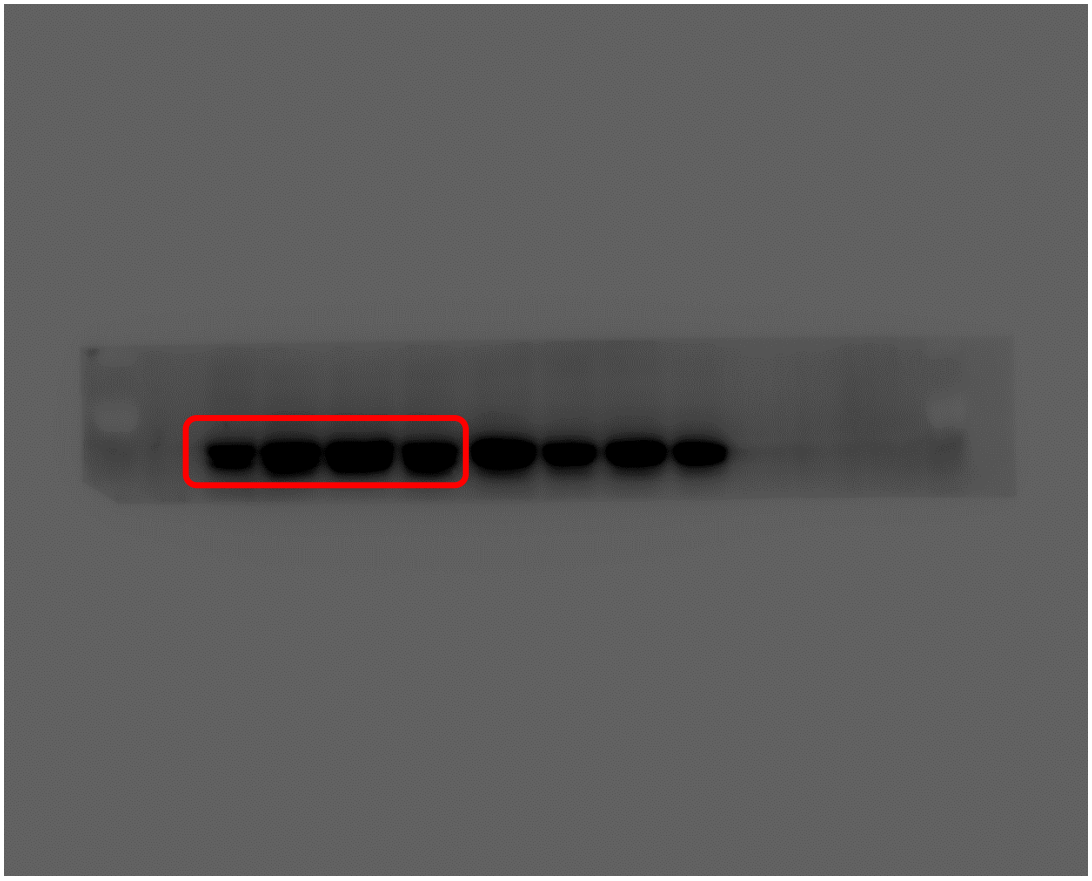
p-mTOR



p-Akt

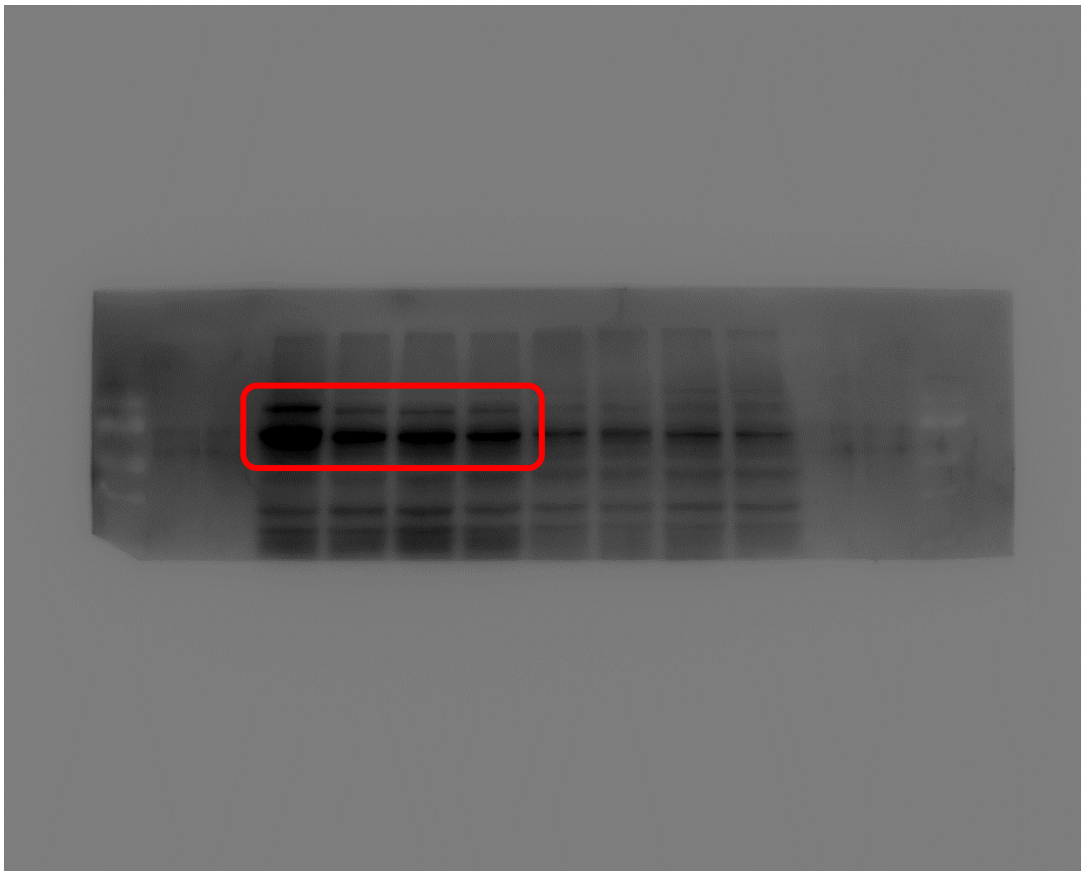


tAkt

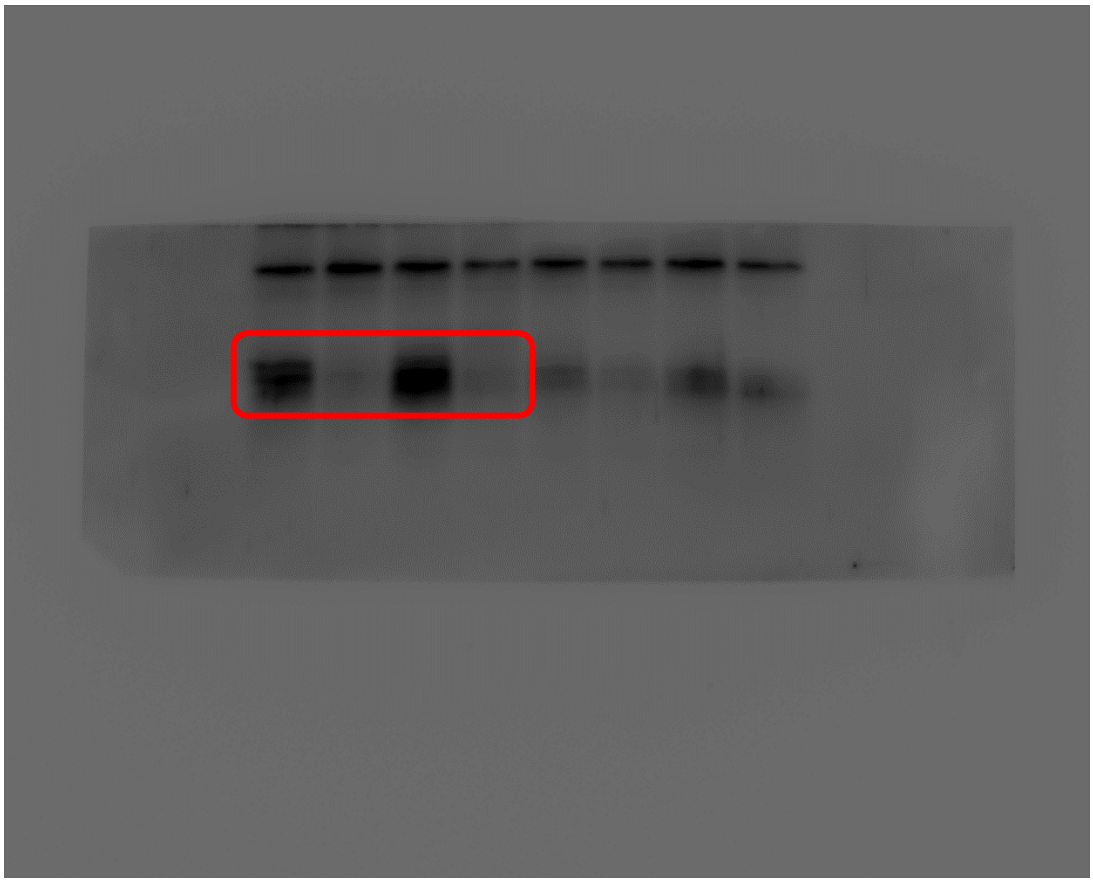


pS6K1

tS6K1



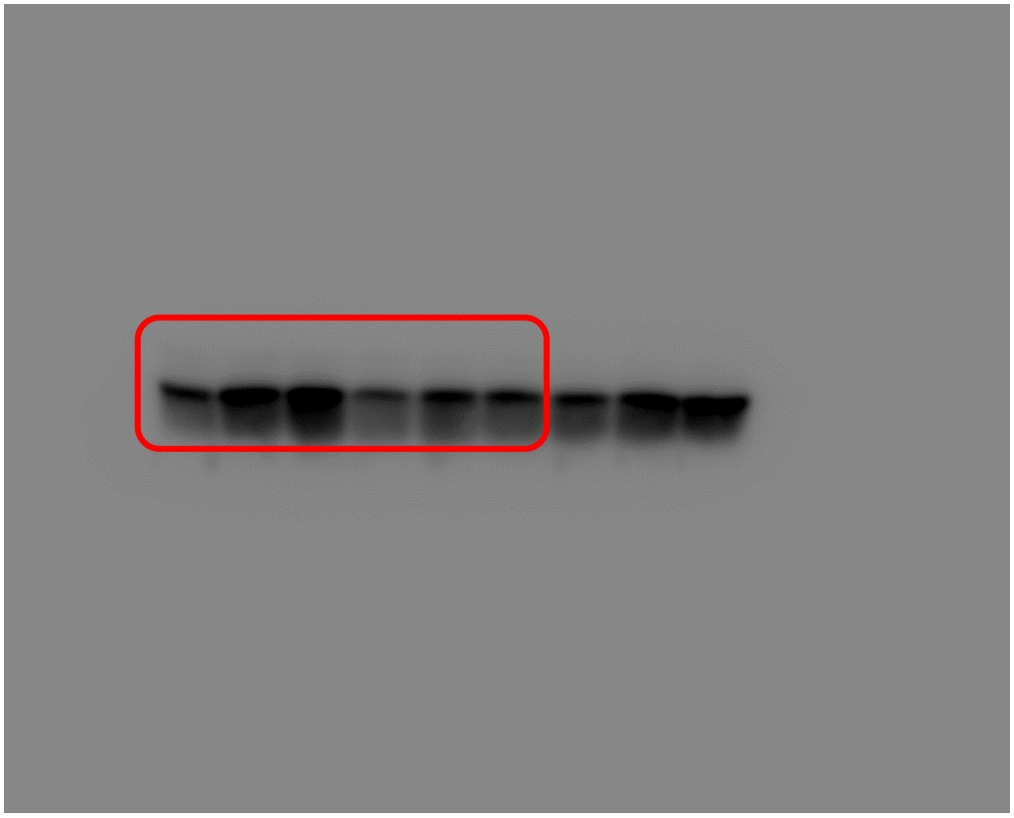
P4EBP1



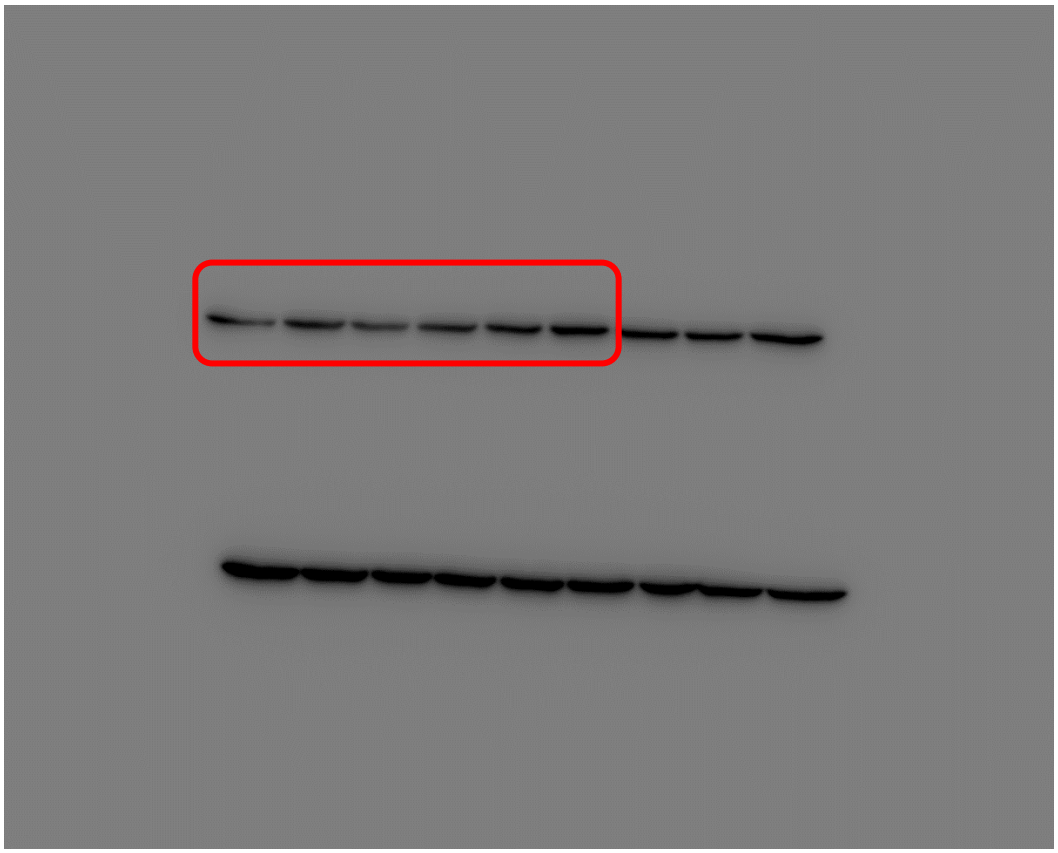
Actin

C

LC3

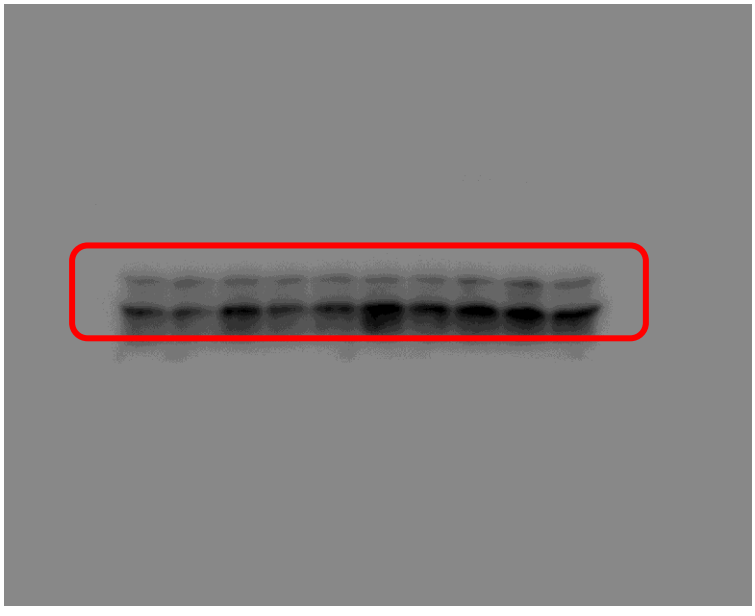


Actin



PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer. Supplemental Figure 1b is the result of the same batch of samples running with SDS-PAGE on different days.

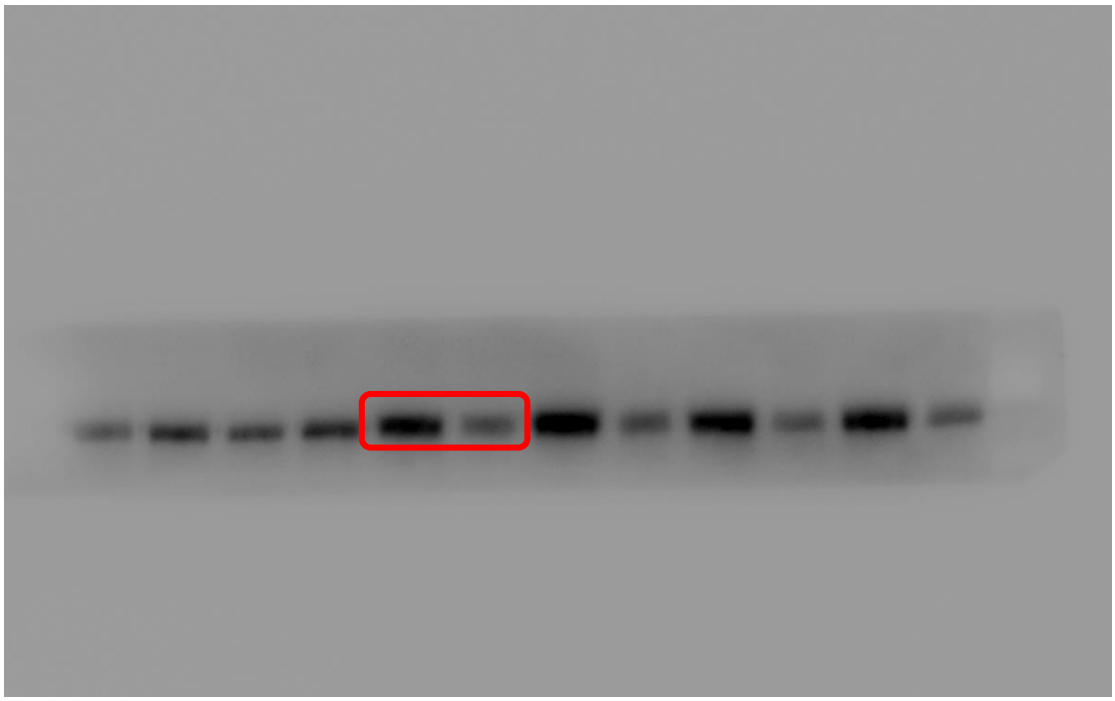
Supplemental 2
A
LC3



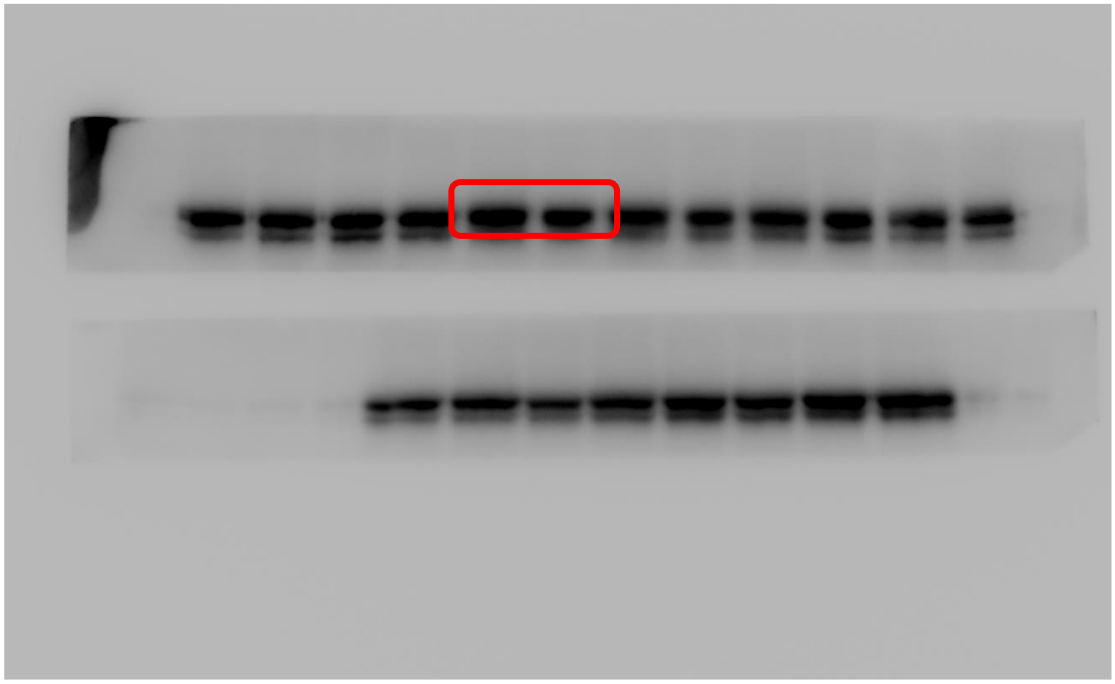
Actin

B

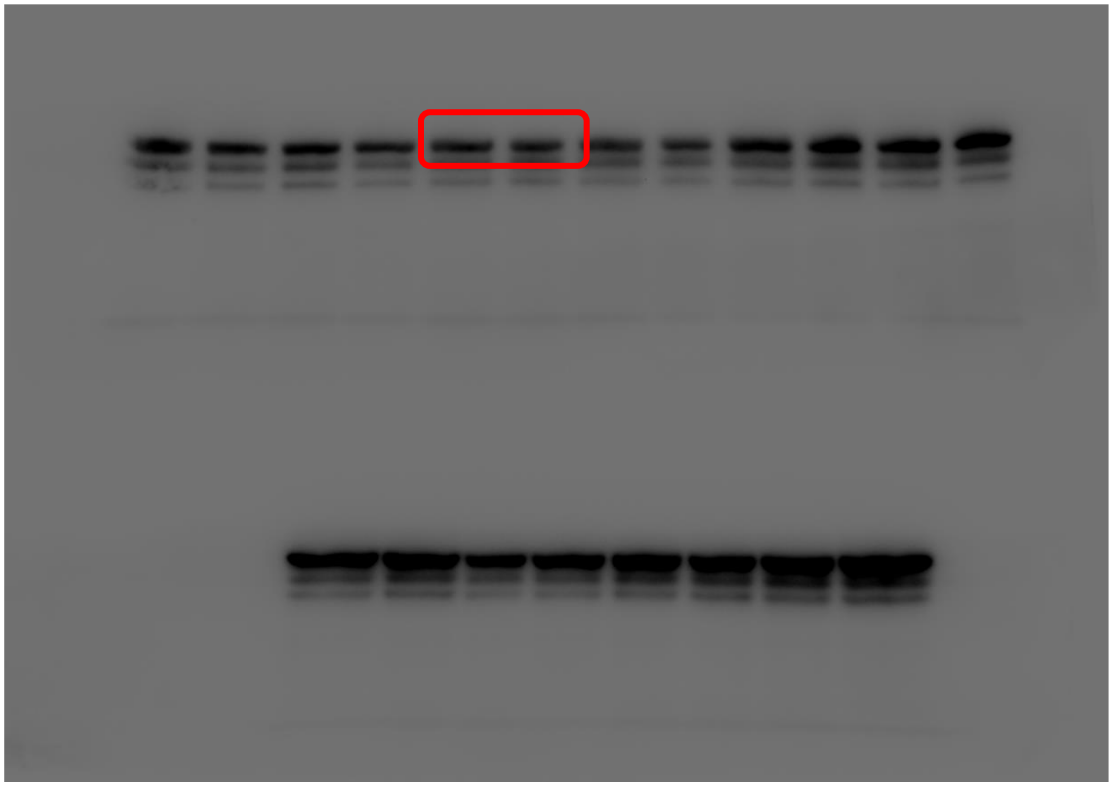
pAkt



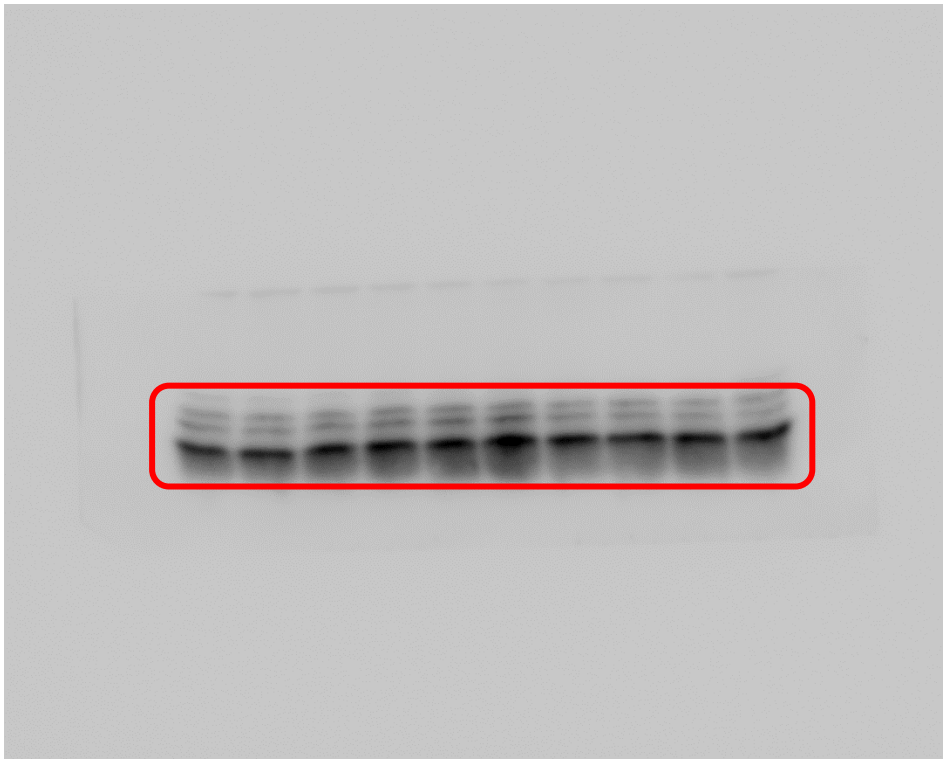
tAkt



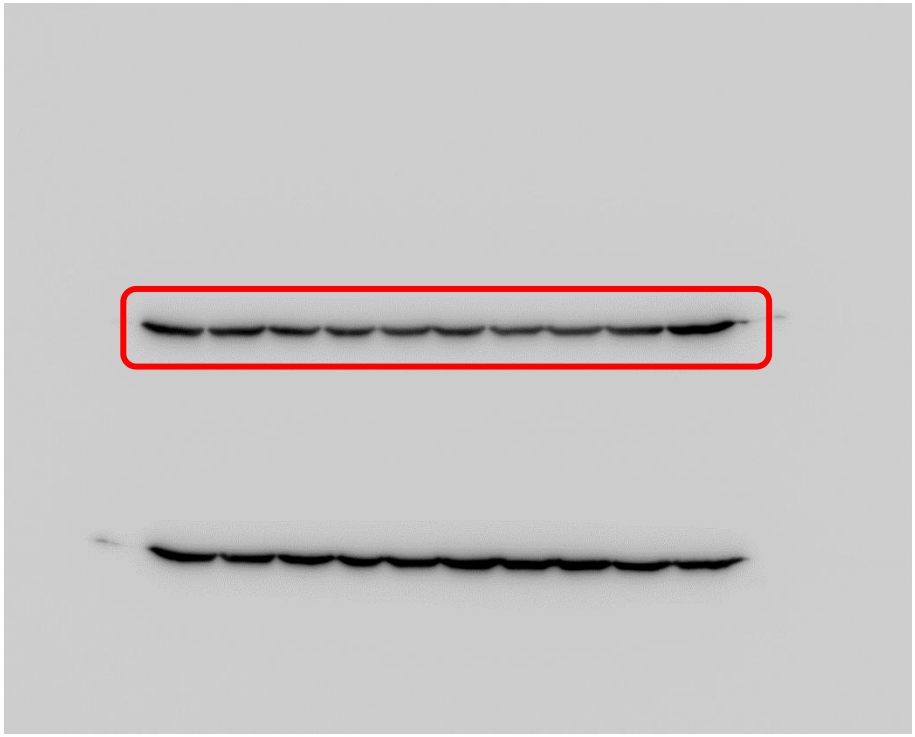
Actin



C
LC3



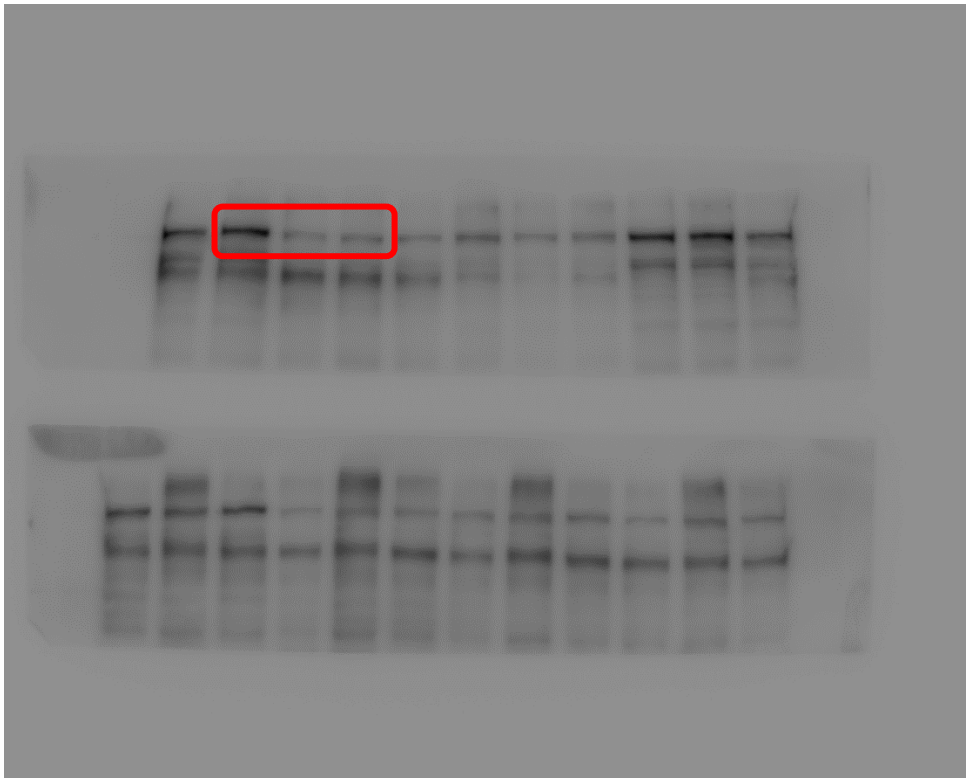
Actin



PVDF membranes were cut into stripes for different antibodies staining. All images were exposed by Thermo fisher Luminometer.

Supplemental 3

mTOR



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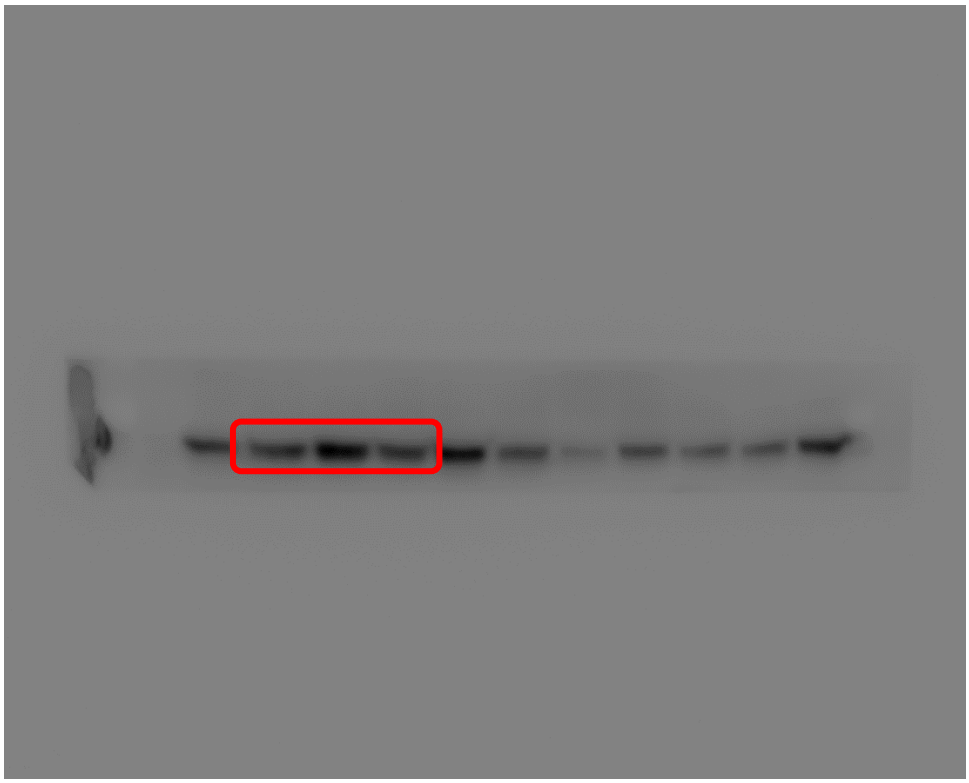

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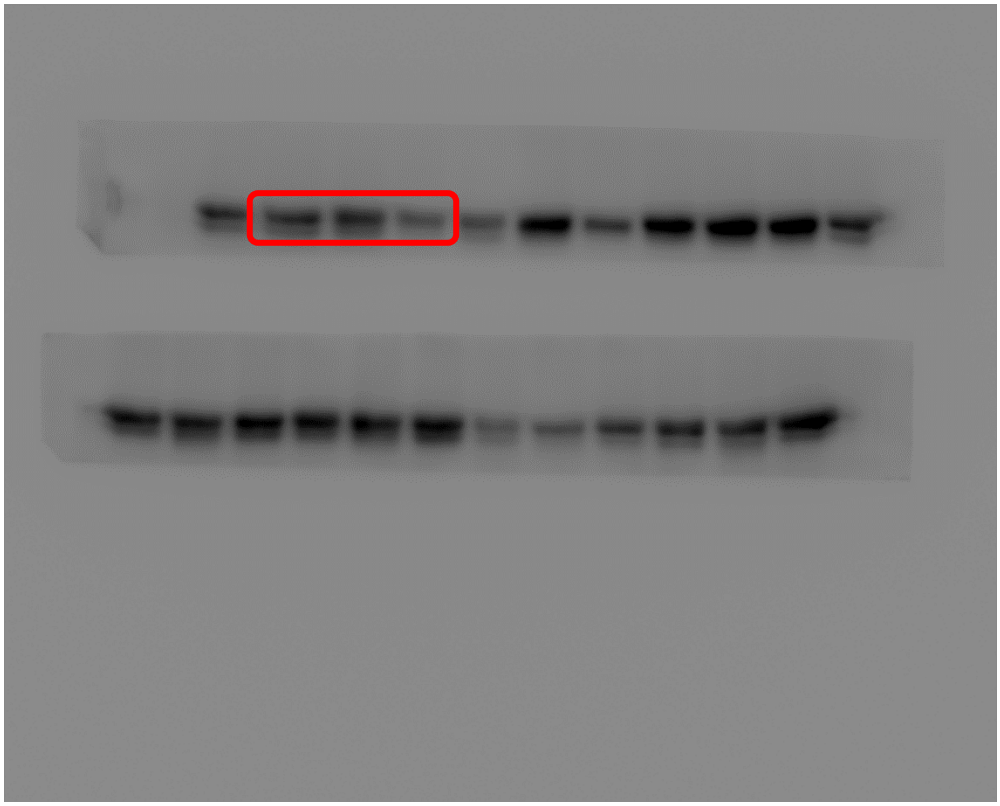

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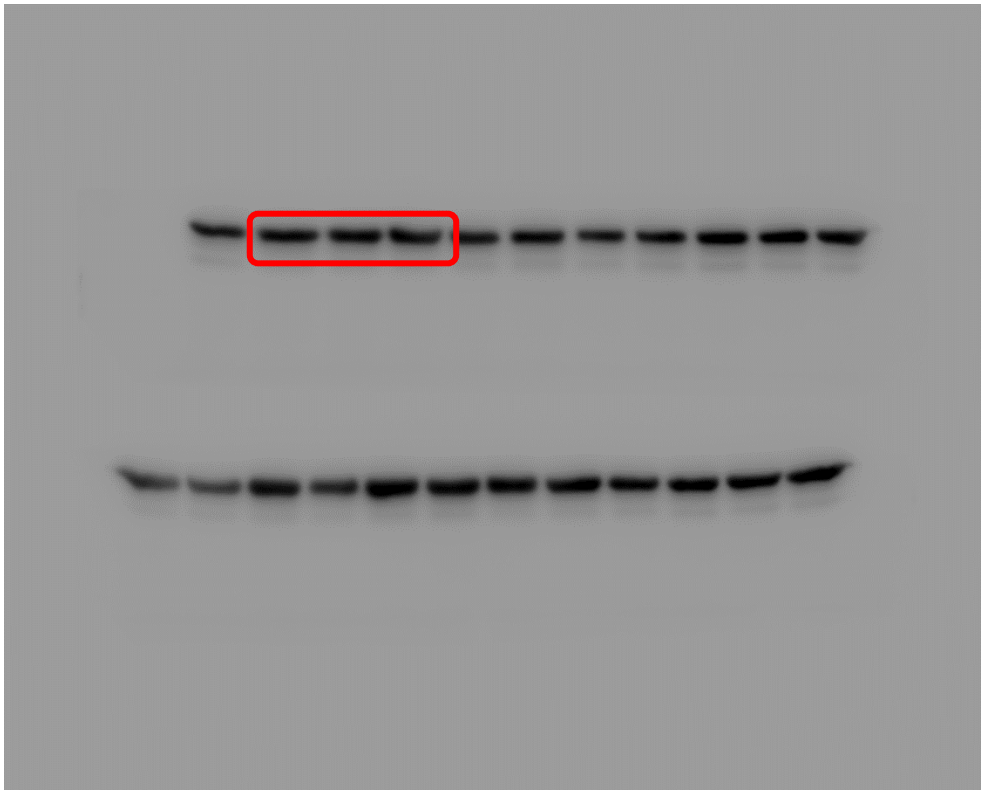

B

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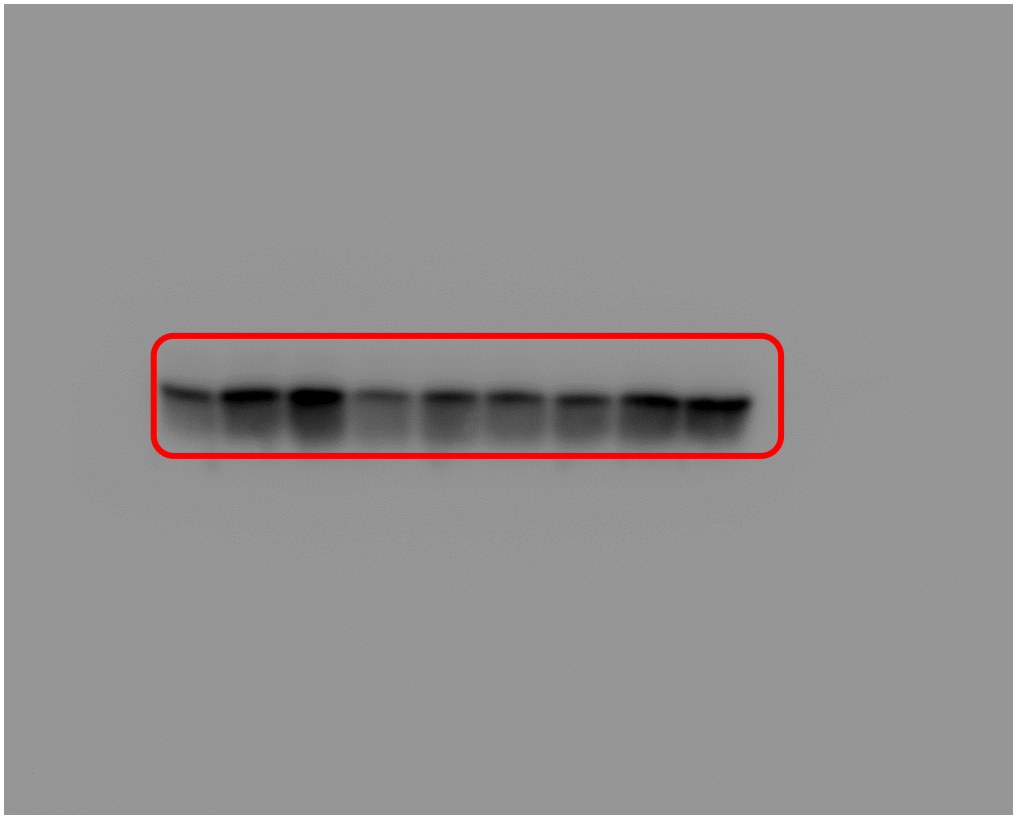

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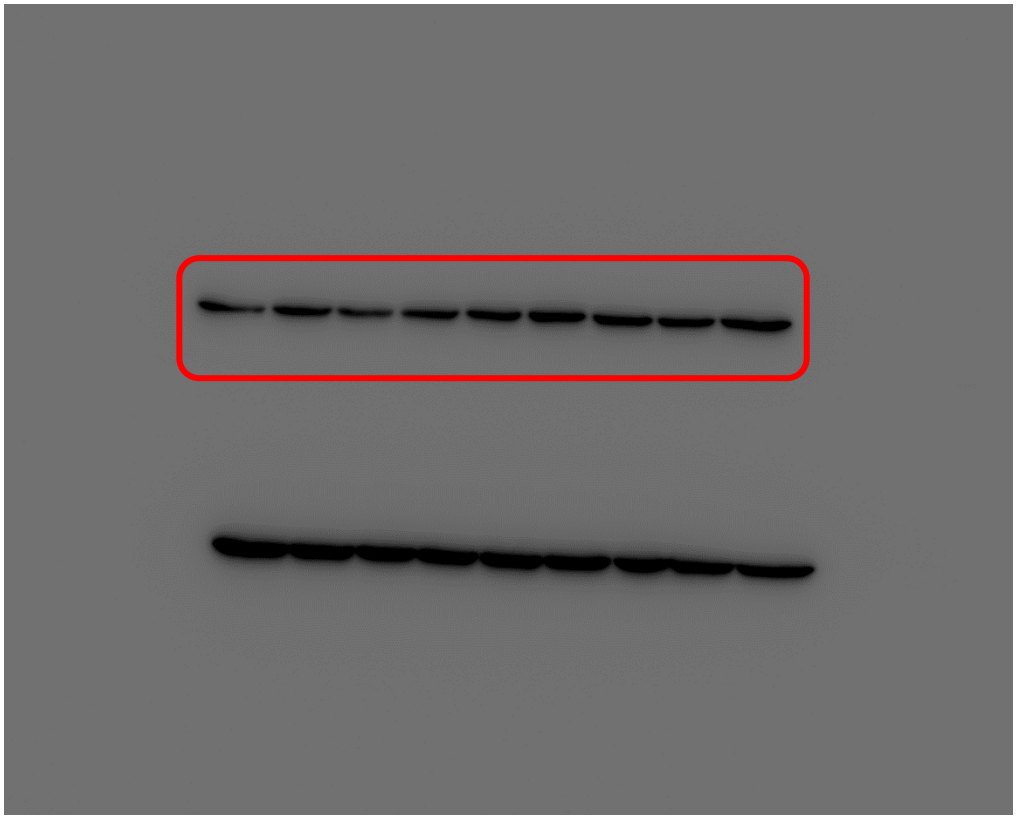

C

LC3

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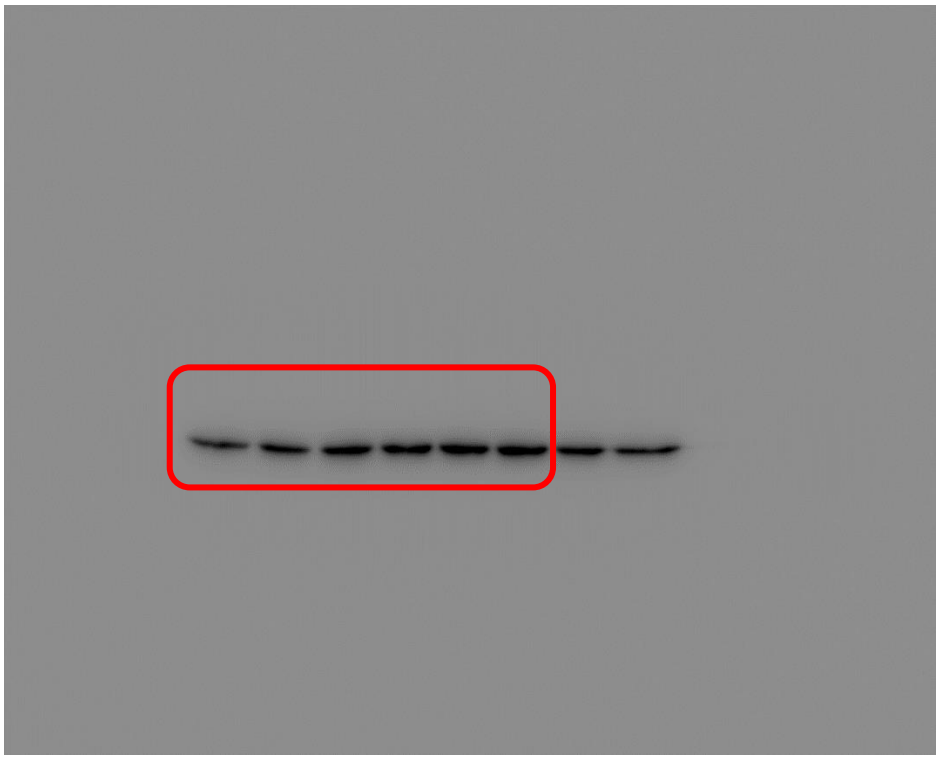

D

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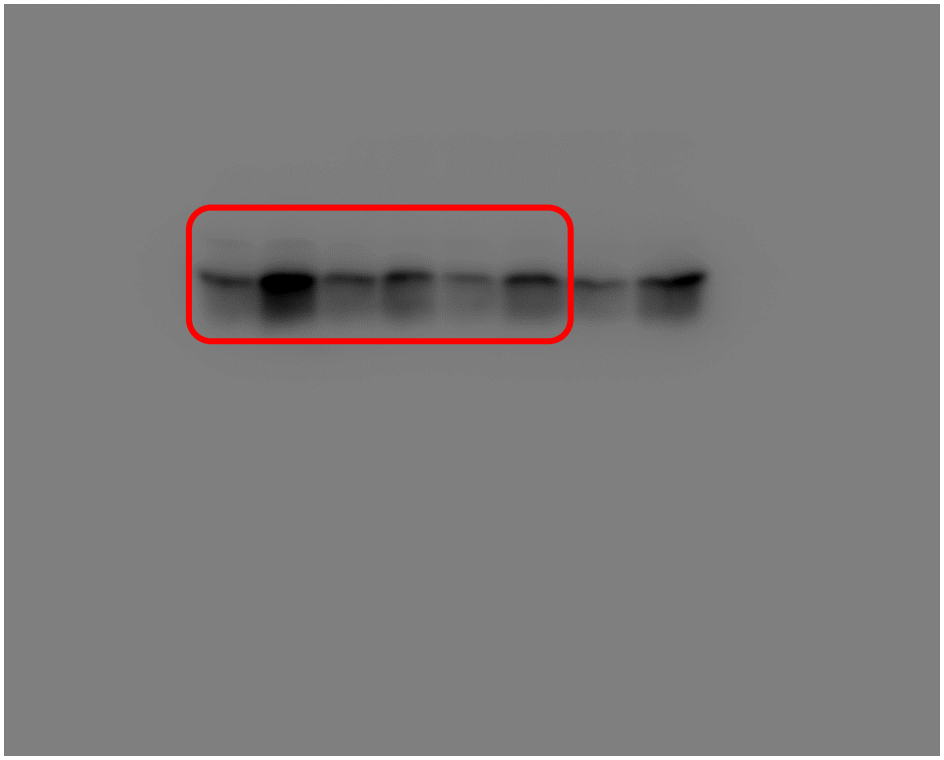

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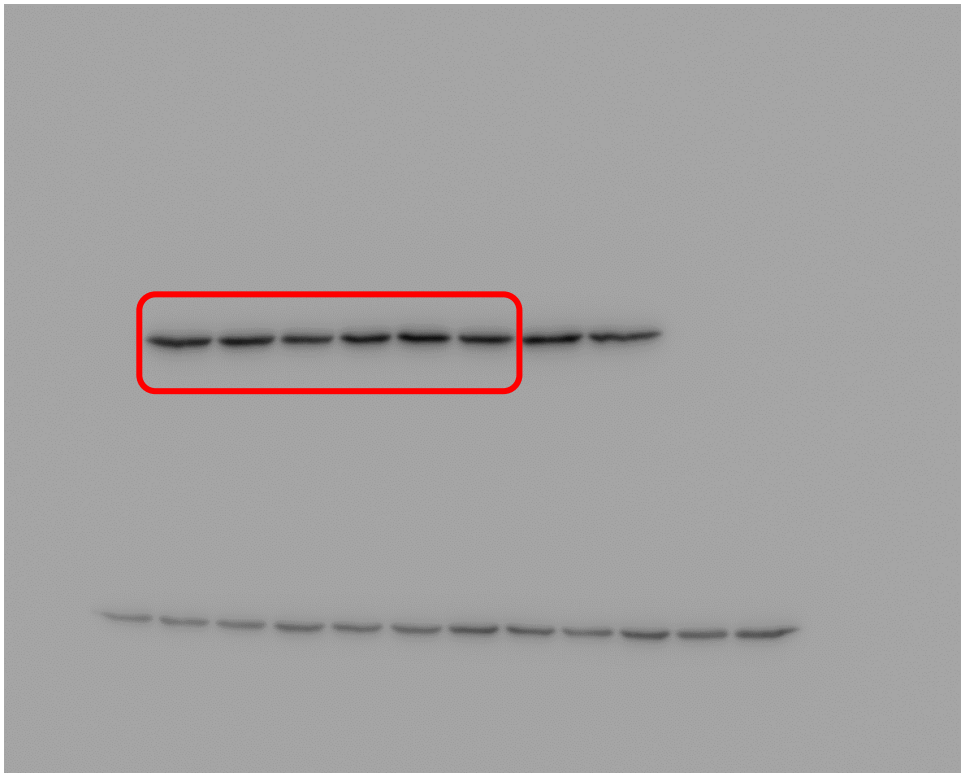

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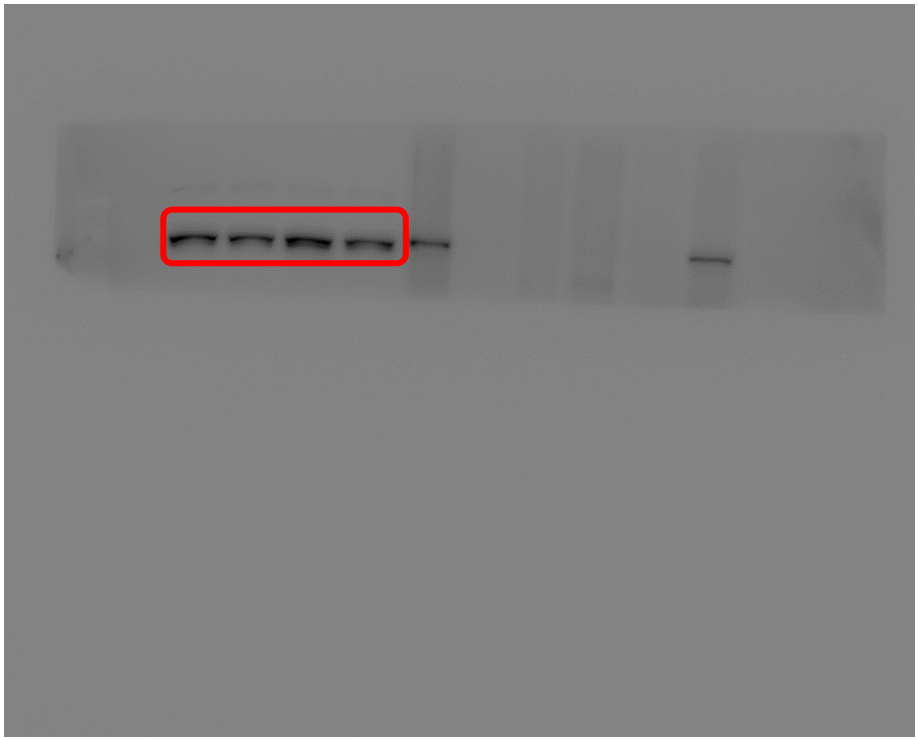
Supplemental 4

B

PARP



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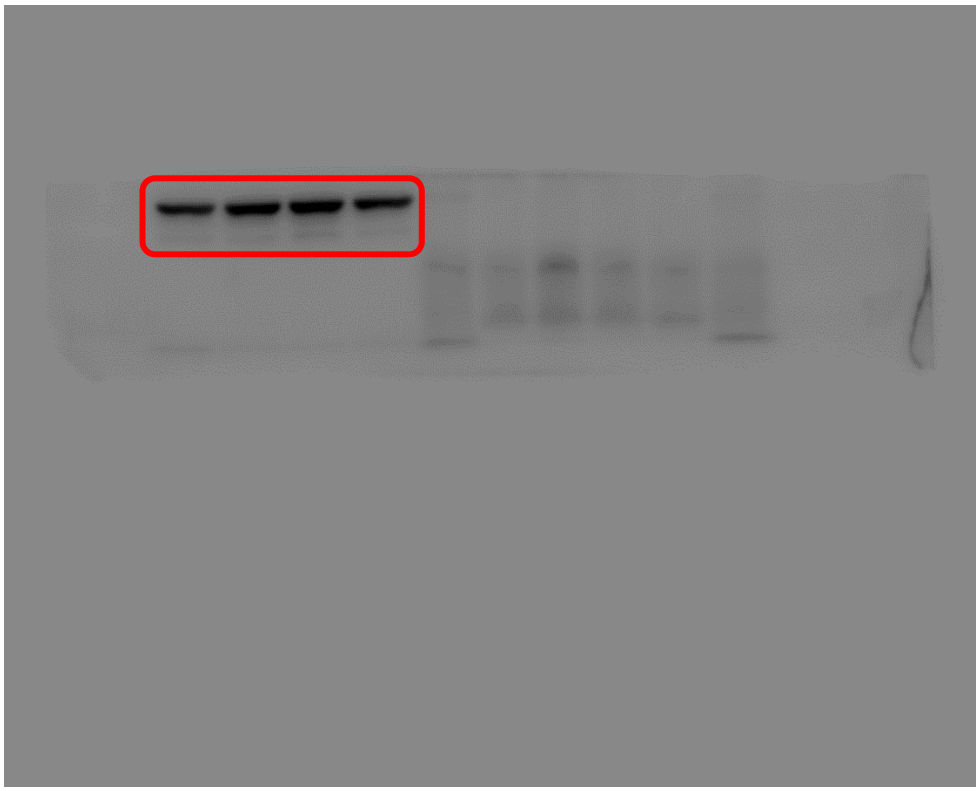

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