

## Supplementary Information

### **Chk1 KA1 domain auto-phosphorylation stimulates biological activity and is linked to rapid proteasomal degradation**

Eun-Yeung Gong<sup>1#</sup>, Beatriz Hernández<sup>1</sup>, Jessica Hernández Nielsen<sup>1</sup>, Veronique AJ Smits<sup>1</sup>,  
Raimundo Freire<sup>1</sup>, and David A Gillespie<sup>2\*</sup>

<sup>1</sup>Unidad de Investigación, Hospital Universitario de Canarias, Ofra s/n, La Cuesta, La Laguna  
38320, Tenerife, Spain

<sup>2</sup>Instituto de Tecnologías Biomédicas, Centro de Investigaciones Biomédicas de Canarias,  
Facultad de Medicina, Campus Ciencias de la Salud, Universidad de La Laguna, La Laguna  
38071, Tenerife, Spain

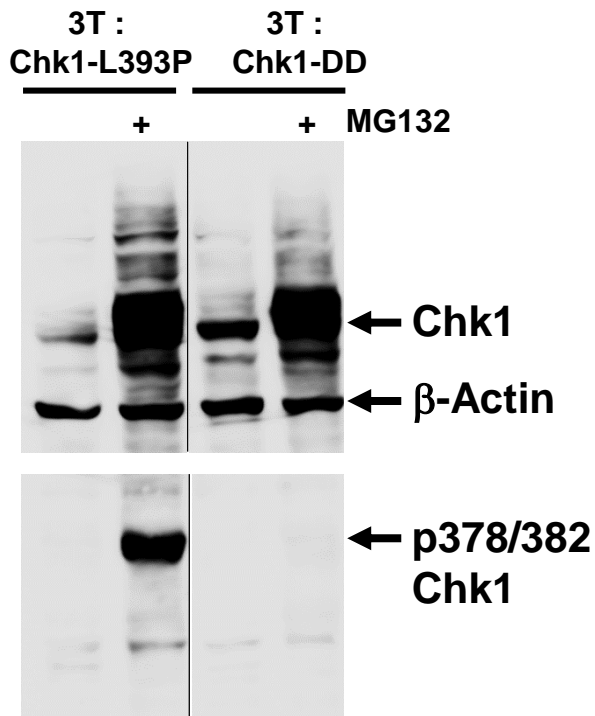
\*Corresponding author. Email: [dgillesp@ull.edu.es](mailto:dgillesp@ull.edu.es) Tel: +34 922 678 111 Fax: +34 922 647  
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# Nano-Bio Resources Center, Sookmyung Women's University, Chungpa-Dong 2-Ka,  
Yongsan-ku, Seoul 04310, Republic of Korea

Running title: Chk1 regulation by auto-phosphorylation

Key words: Chk1, auto-phosphorylation, DNA damage, cell cycle arrest, proteasomal protein degradation.

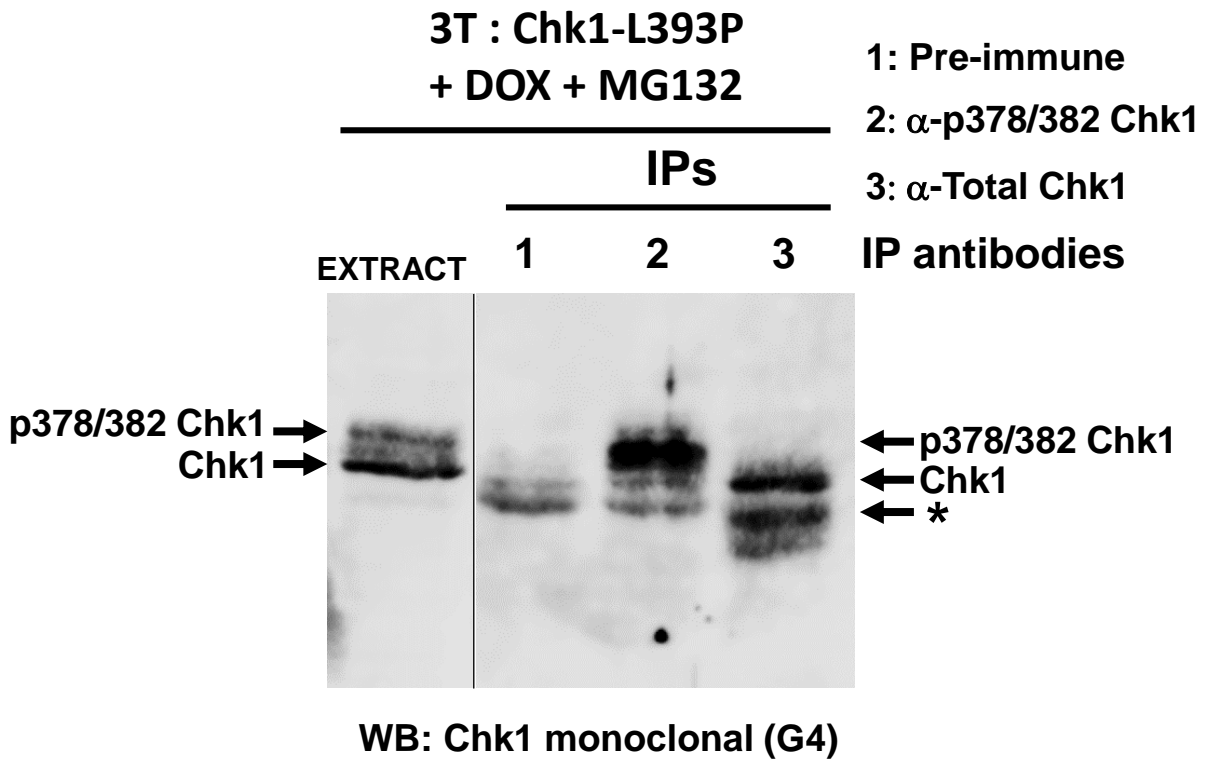
# Supplementary Figure 1



## Recognition of auto-phosphorylated Chk1 by anti-pT378/382 antiserum is dependent on threonines 378 and 382

3T: Chk1-L393P and 3T: Chk1-DD DT40 cell lines were treated for 16 hours with DOX plus or minus MG132 to inhibit protein degradation via the proteasome. Cell extracts were prepared, 30mg resolved by SDS-PAGE, and analysed by western blotting using antibodies against total Chk1 and  $\beta$ -Actin (upper panel) and anti-pT378/382 phospho-specific Chk1 antiserum (lower panel). Auto-phosphorylated Chk1-L393 protein is stabilized by MG132 and detected by anti-pT378/382. Chk1-DD is also stabilized by MG132 but is not detected by anti-p378/382. Samples were resolved on two separate western blots (see Supplementary Information for original images).

# Supplementary Figure 2

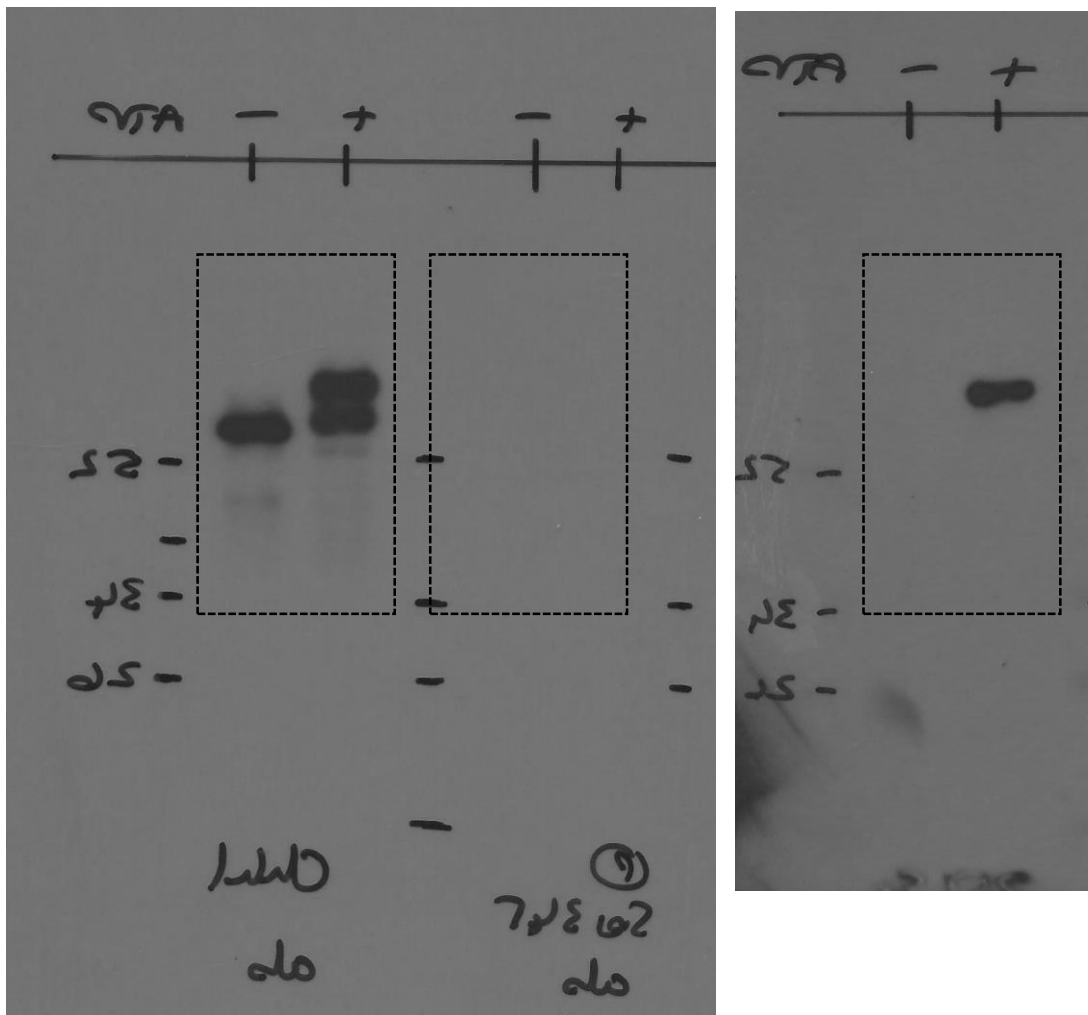


## Anti-pT378/382 antiserum selectively immuno-precipitates auto-phosphorylated Chk1

3T: Chk1-L393P DT40 cell lines were treated for 16 hours with DOX plus MG132 to inhibit protein degradation via the proteasome. Whole cell extracts were prepared, and equal portions immuno-precipitated with pre-immune serum (PI), anti-pT378/382, and a polyclonal antiserum against total Chk1. Immuno-precipitates were resolved by SDS-PAGE, transferred to nitrocellulose, and the membrane probed with a monoclonal antibody against Chk1 (G4) in combination with protein A-HRC conjugate for detection (see Supplementary Information for original images). The shifted, auto-phosphorylated form of Chk1 that is stabilized by MG132 is selectively immuno-precipitated by anti-p378/382 antiserum. Asterisk indicates precipitating antibody heavy chains that are detected weakly by the protein HRP conjugate.

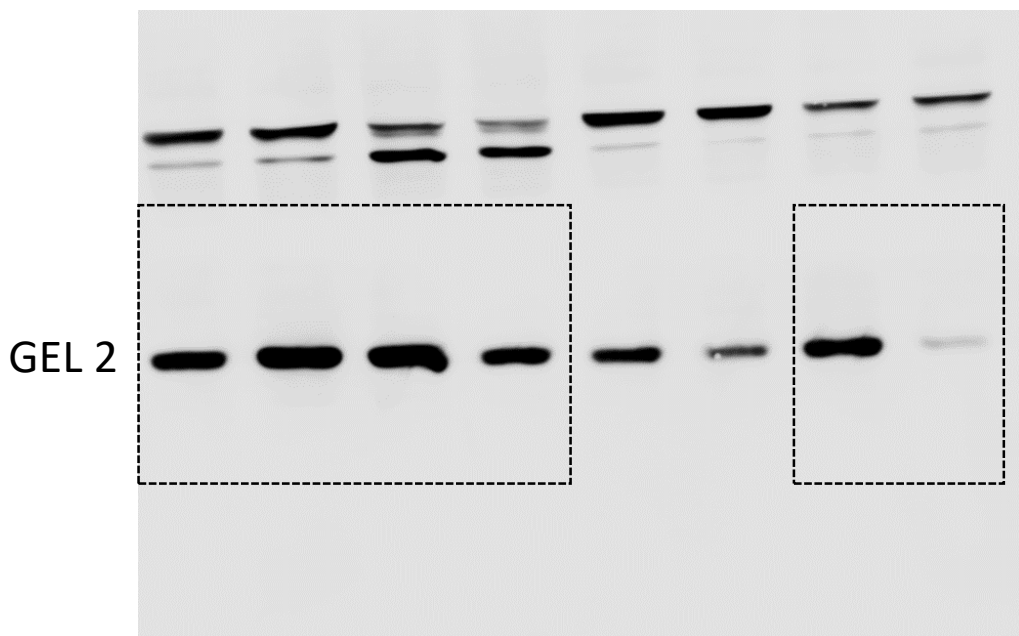
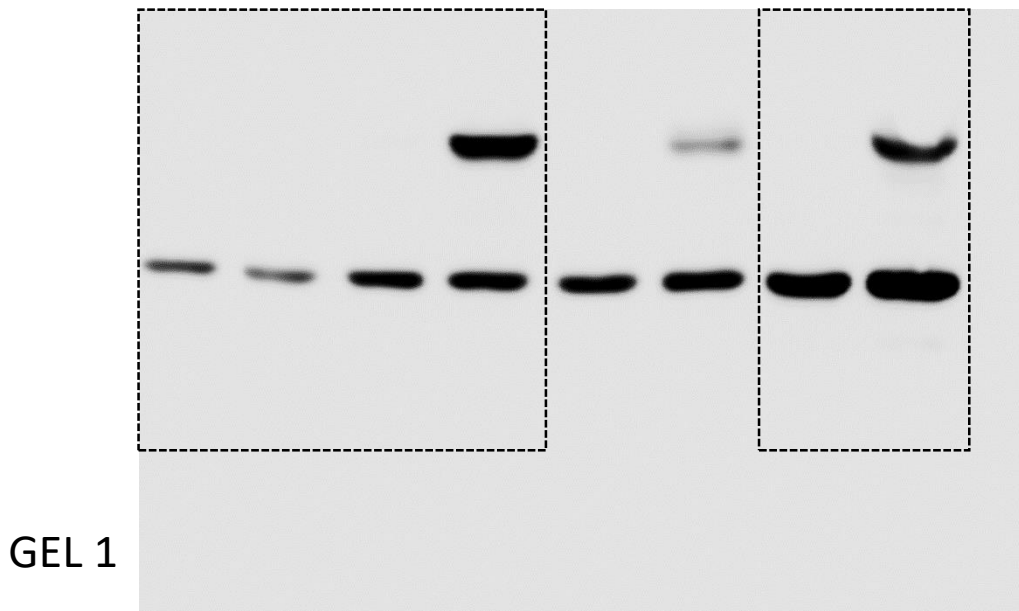
# Figure 1: Uncropped Images

Cropped areas in Figures boxed in dashed lines.

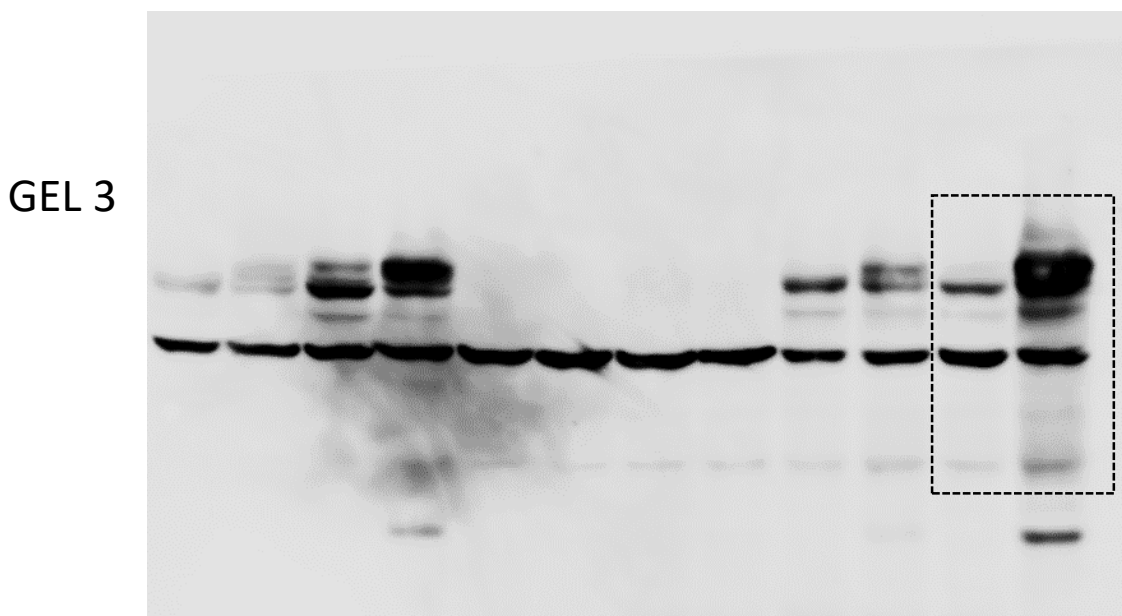
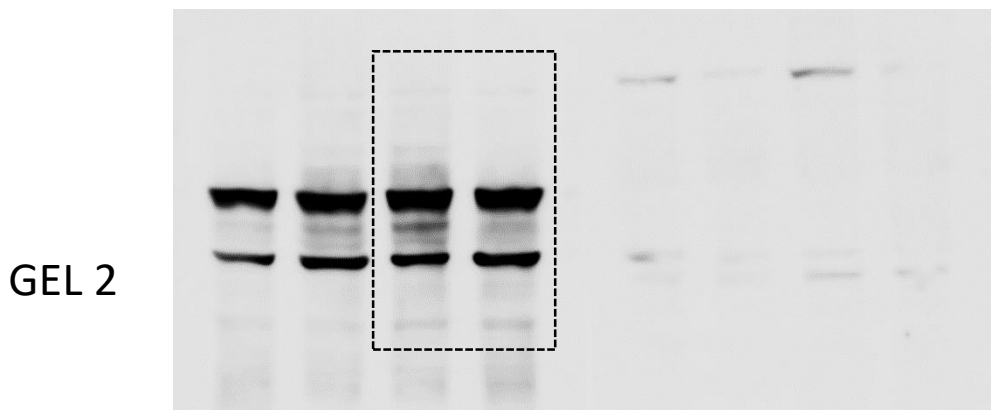
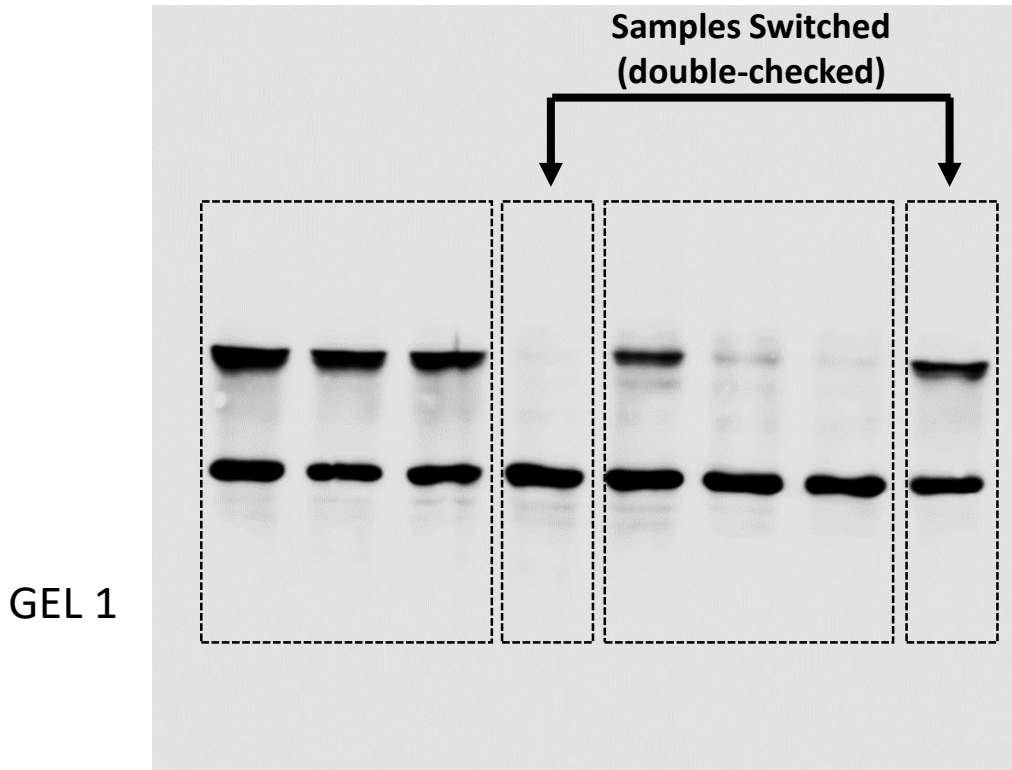


3 replicate western blot panels

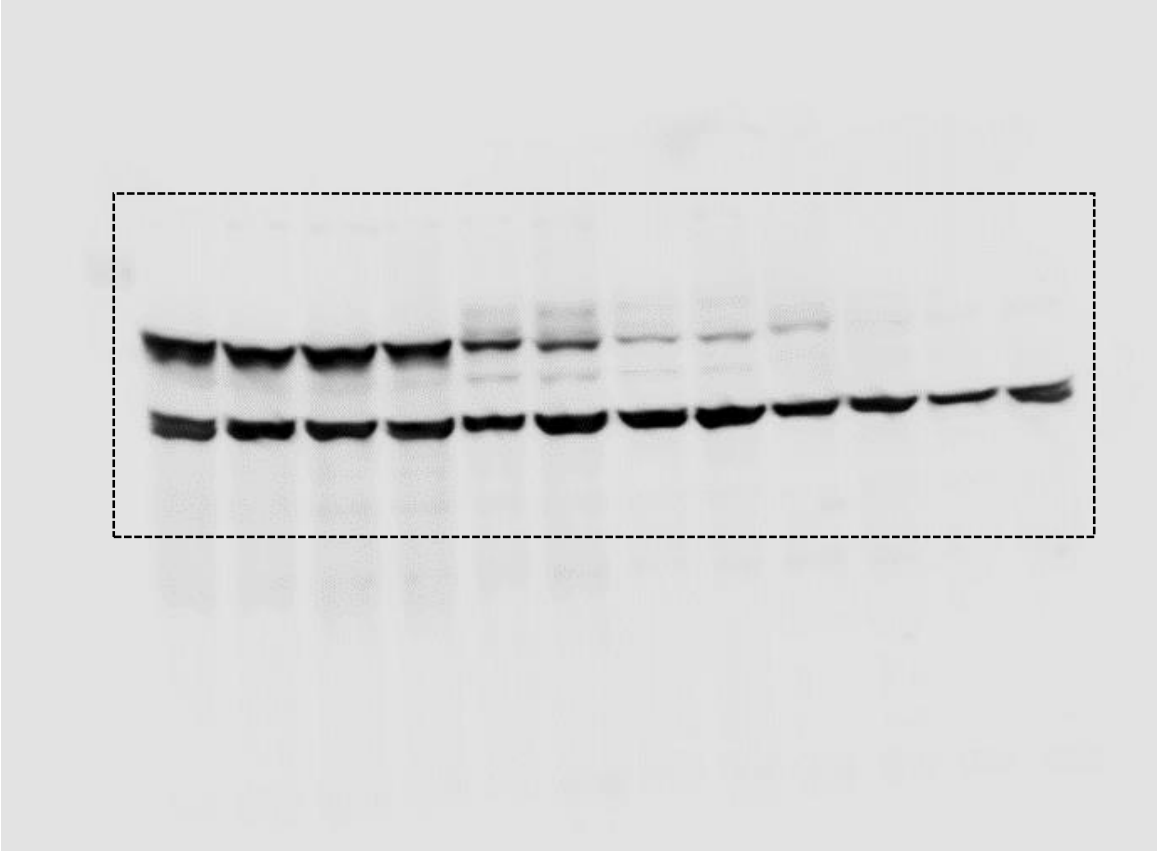
## Figure 2: Uncropped Images



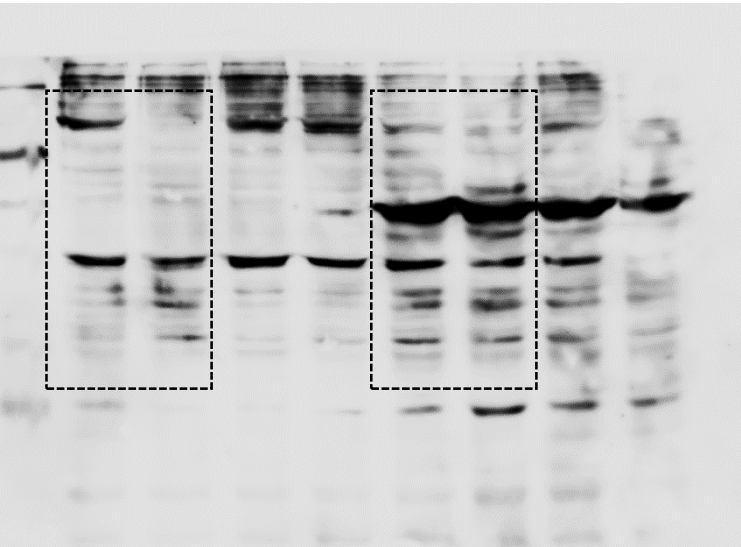
# Figure 3: Uncropped Images



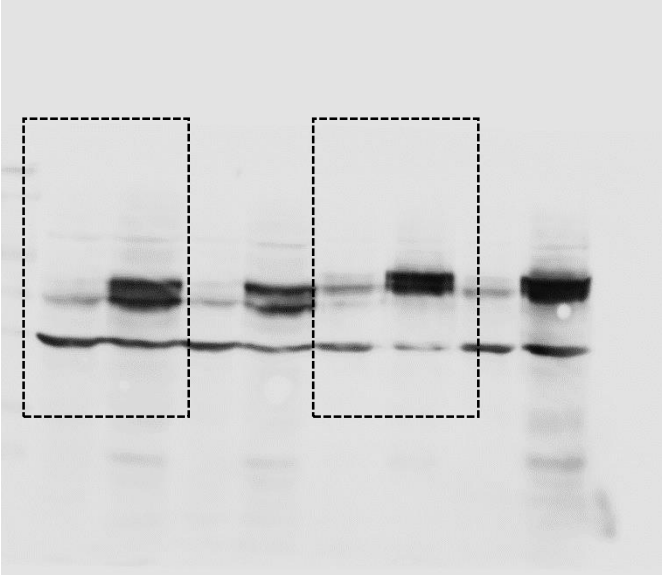
# Figure 4: Uncropped Images



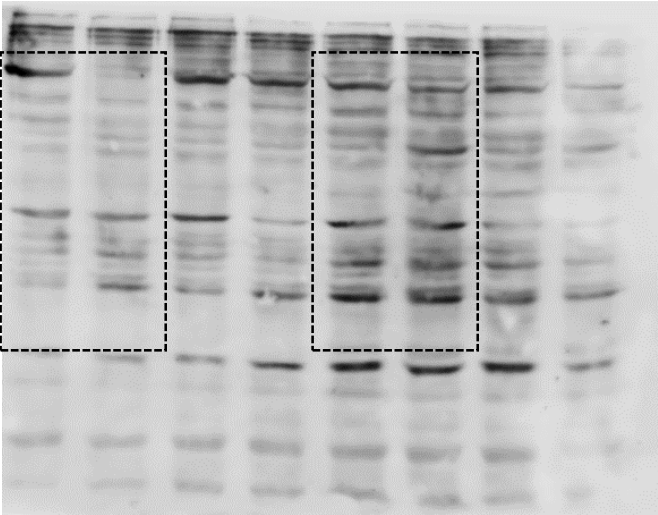
GEL 1



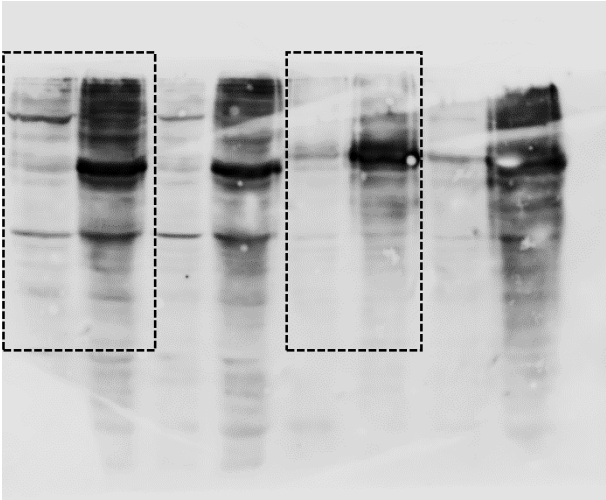
GEL 2



GEL 3

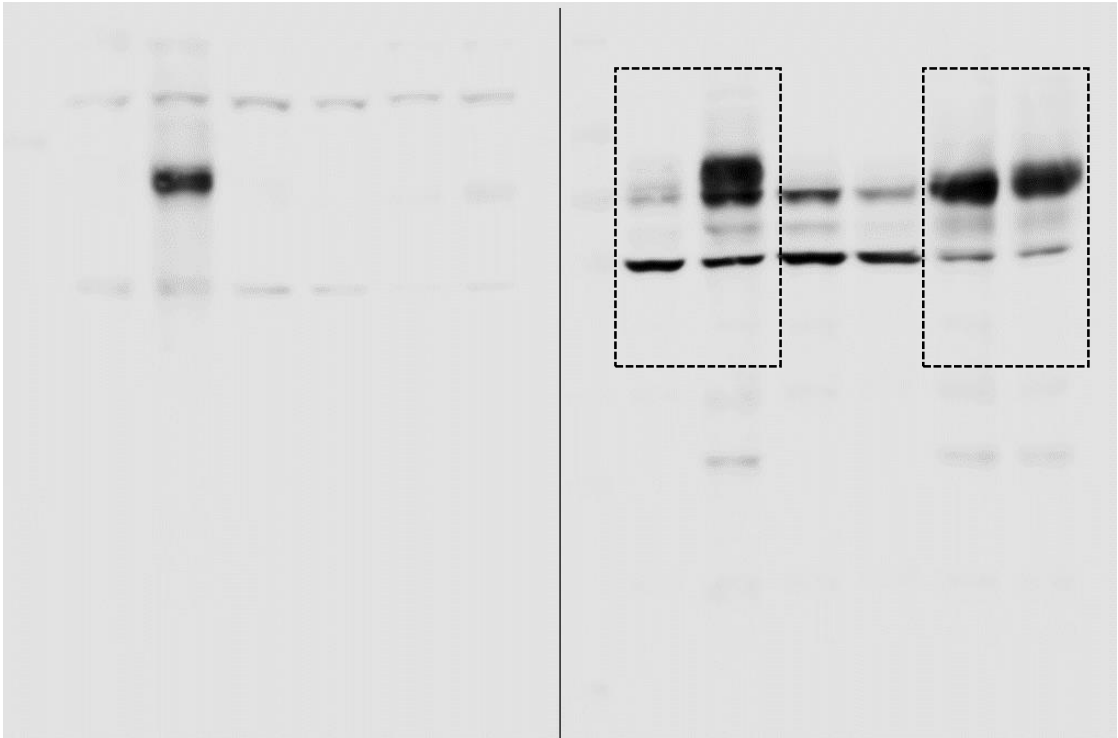


GEL 4



GEL 5

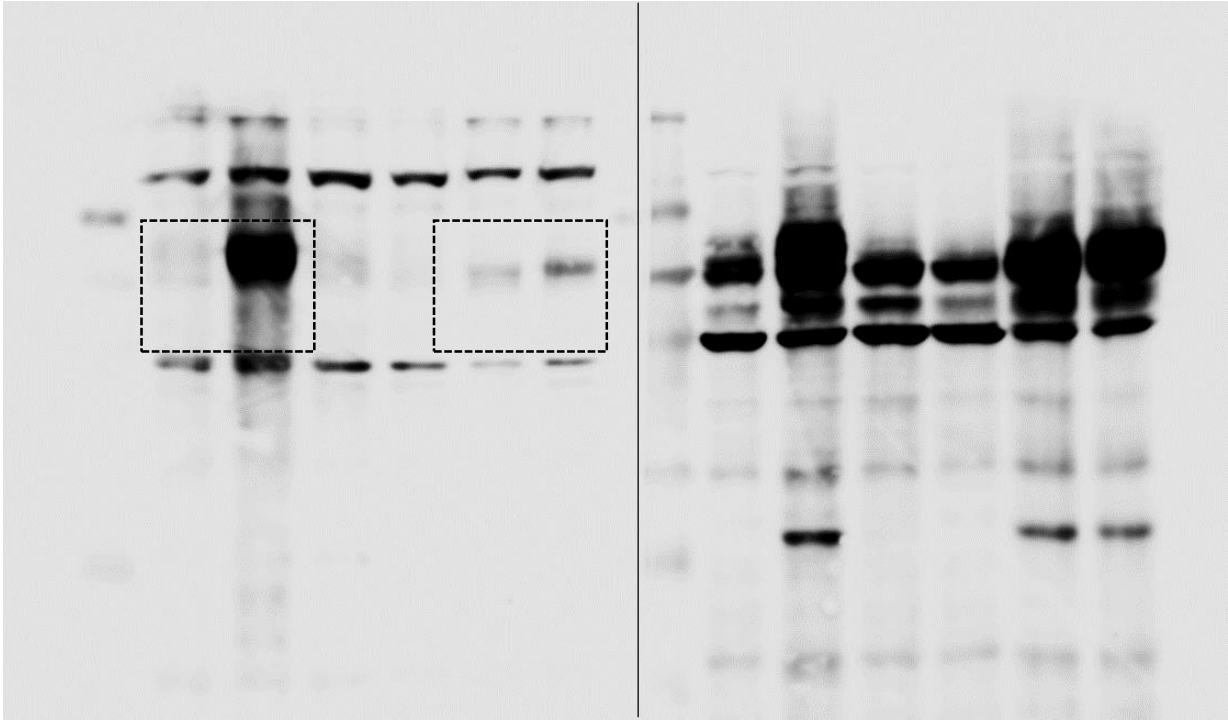
# Figure 5A: Uncropped Images



GEL 1

Short exposure

GEL 2



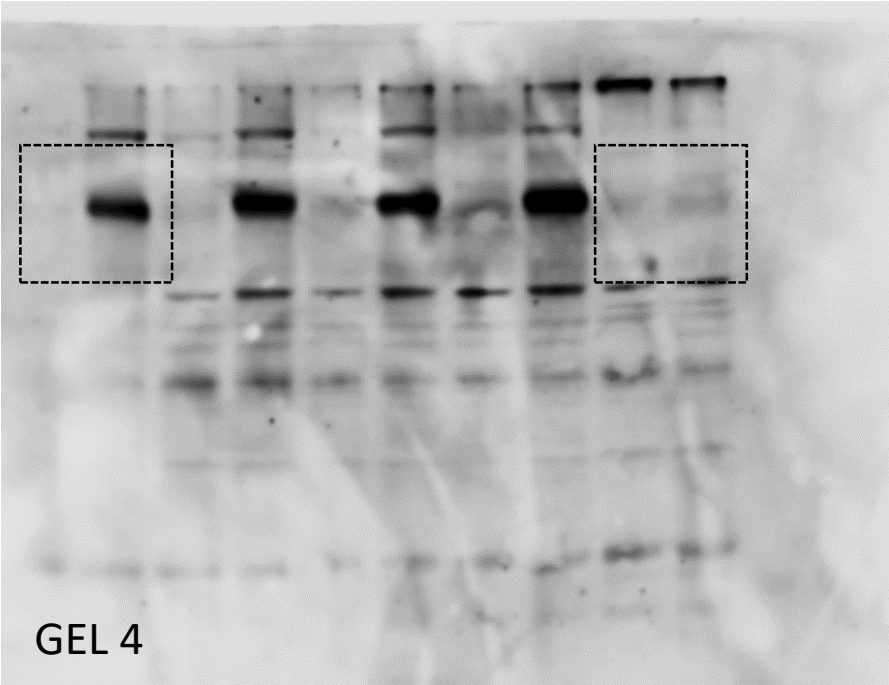
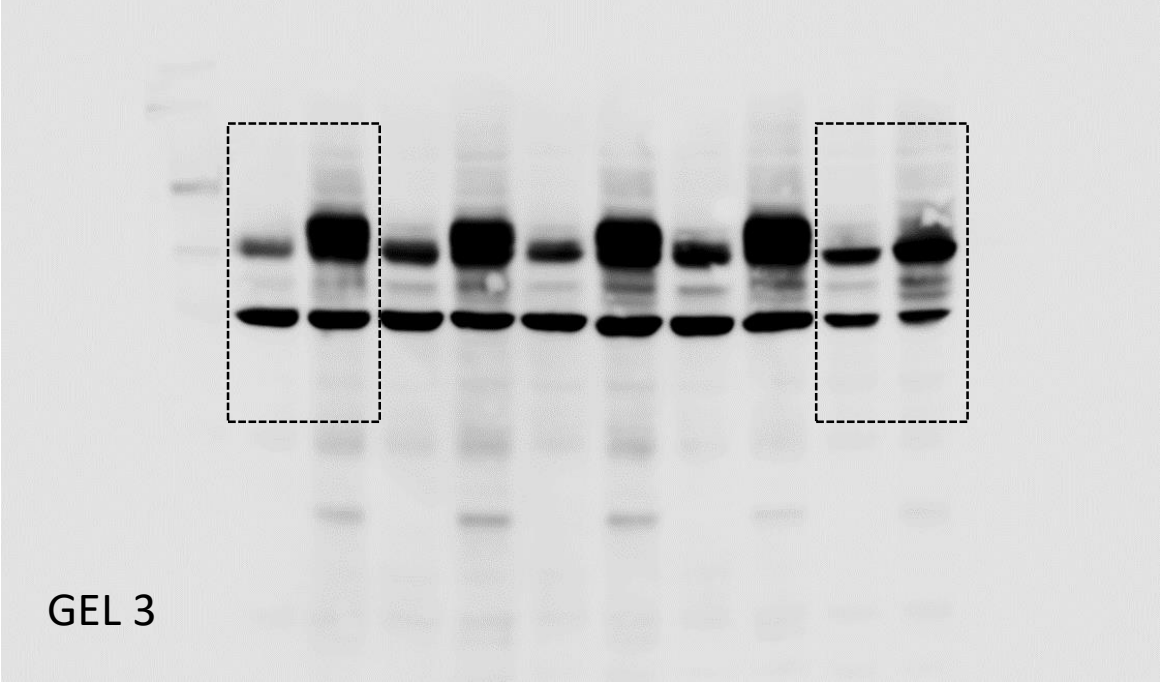
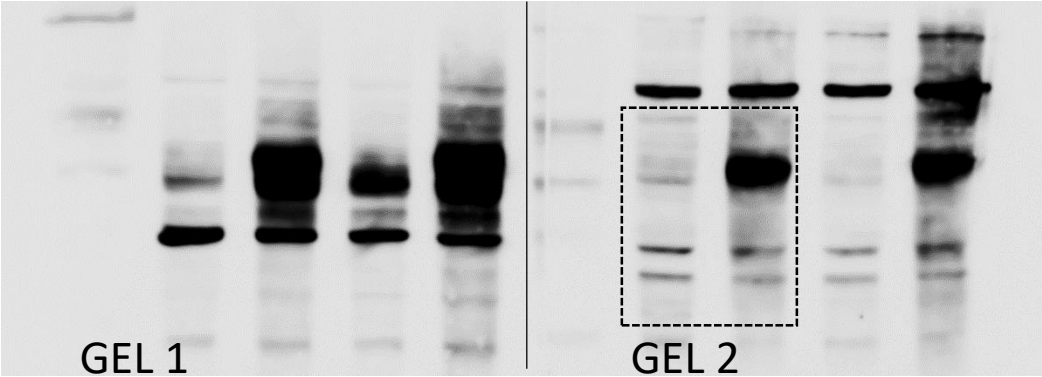
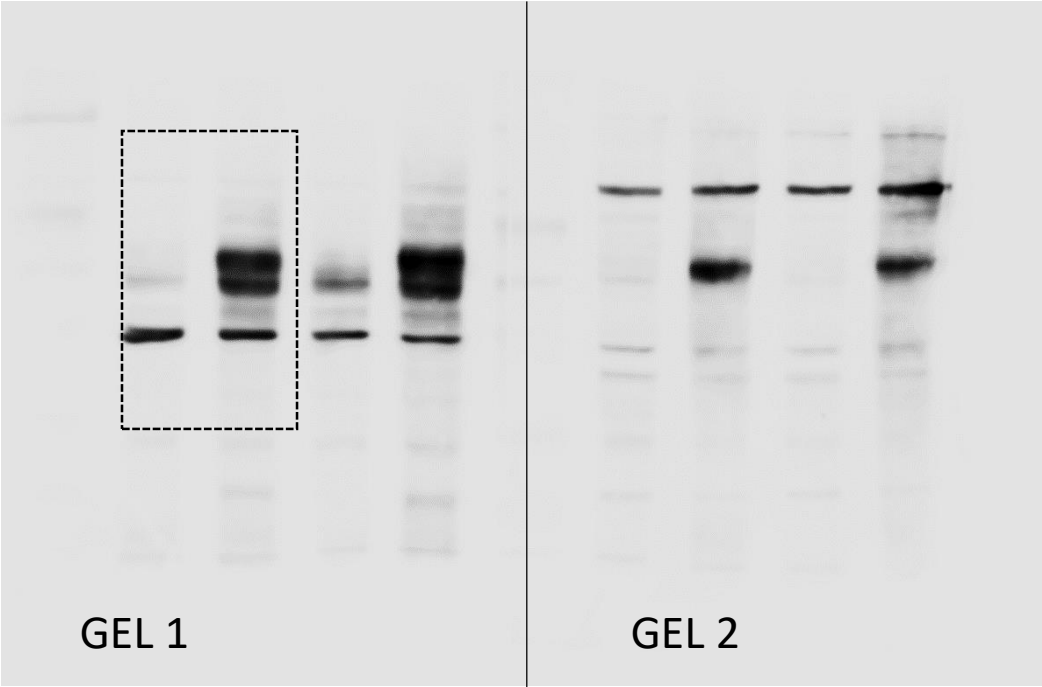
GEL 1

Long exposure

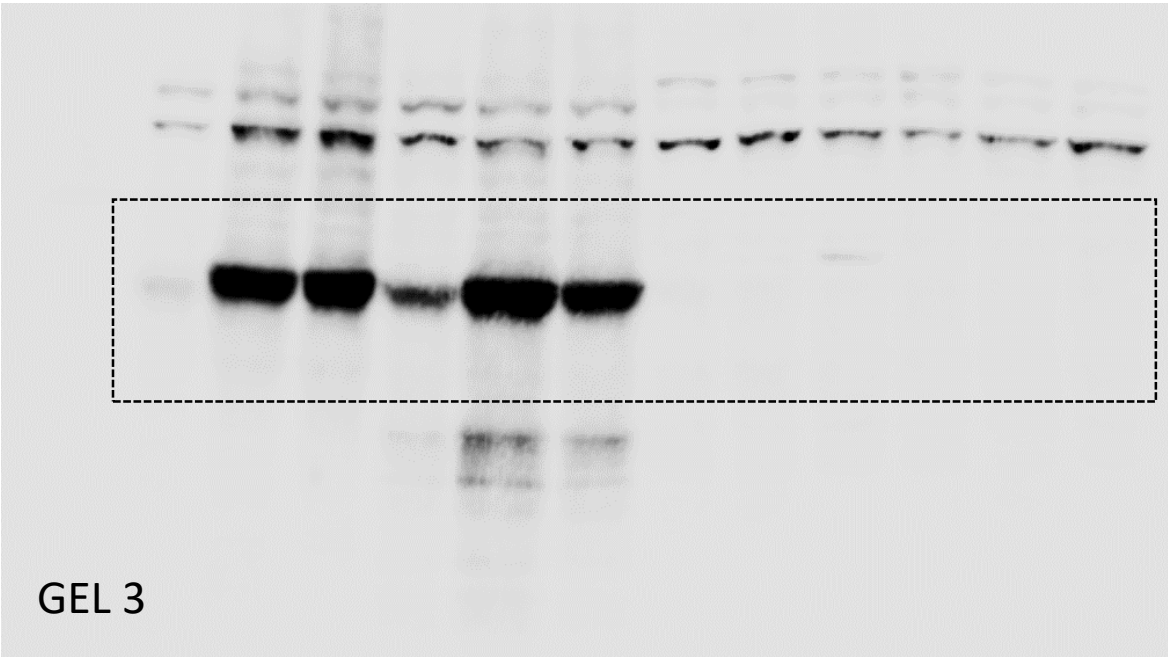
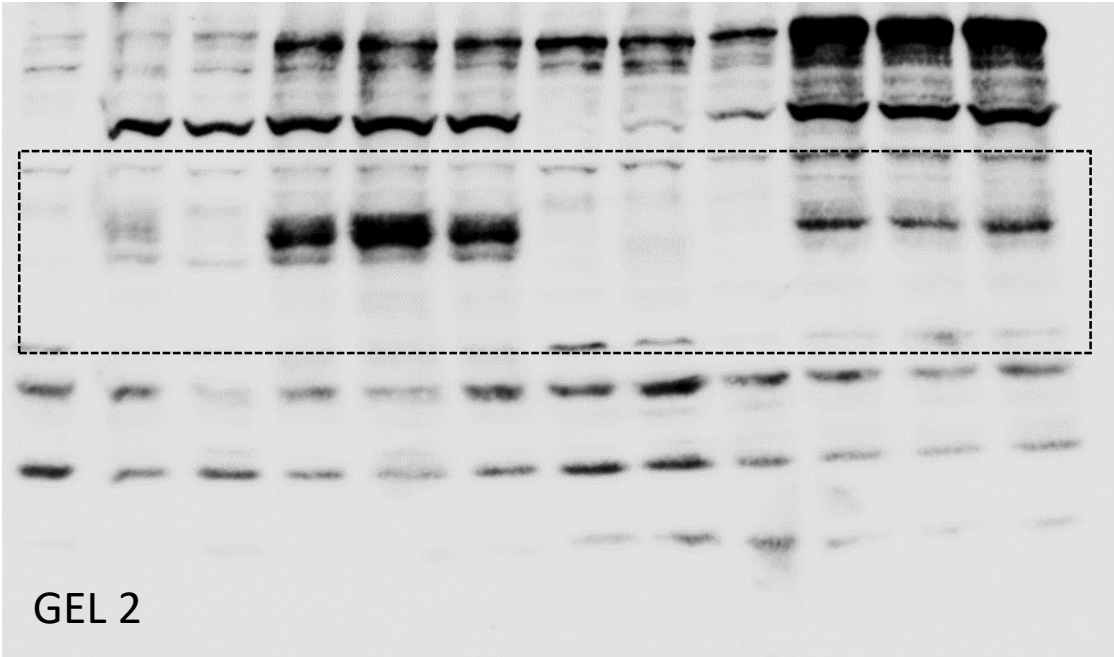
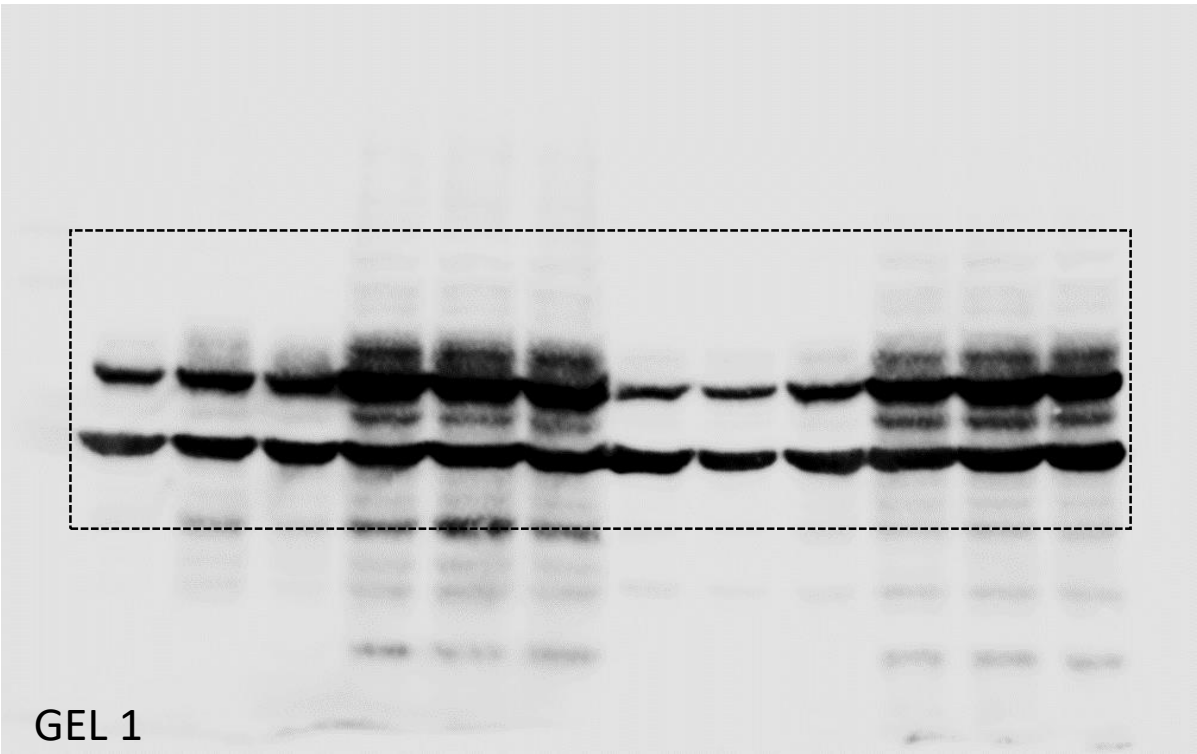
GEL 2



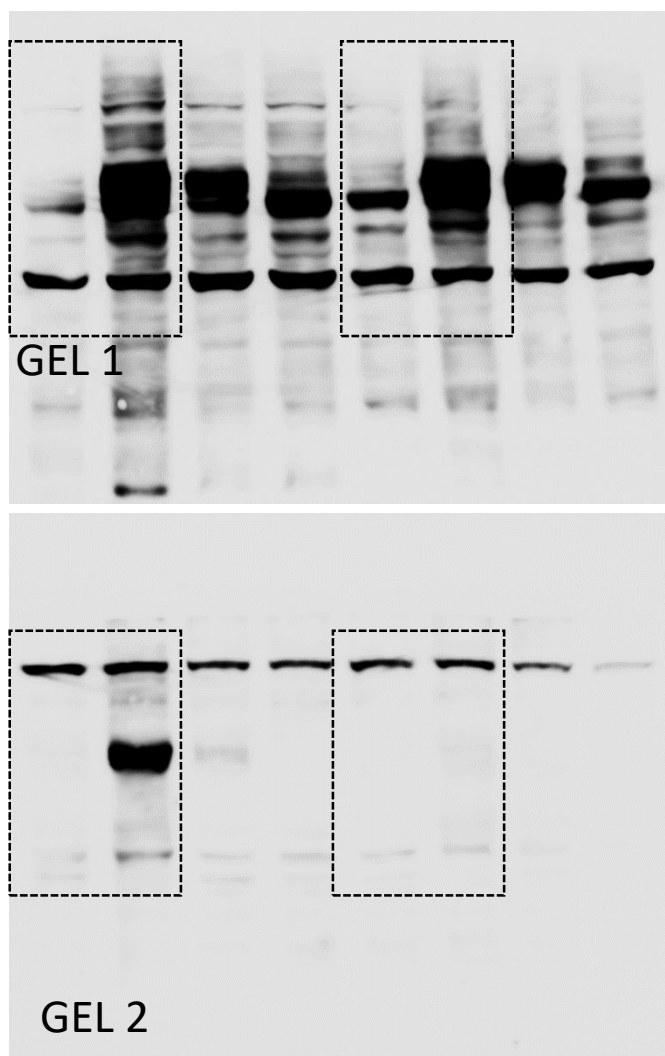
# Figure 5B: Uncropped Images



# Figure 6: Uncropped Images



## Supplementary Fig. 1: Uncropped Images



## Supplementary Fig. 2: Uncropped Images

