

## Dioscin-mediated autophagy alleviates MPP<sup>+</sup>-induced neuronal degeneration: an *in vitro* Parkinson's disease model

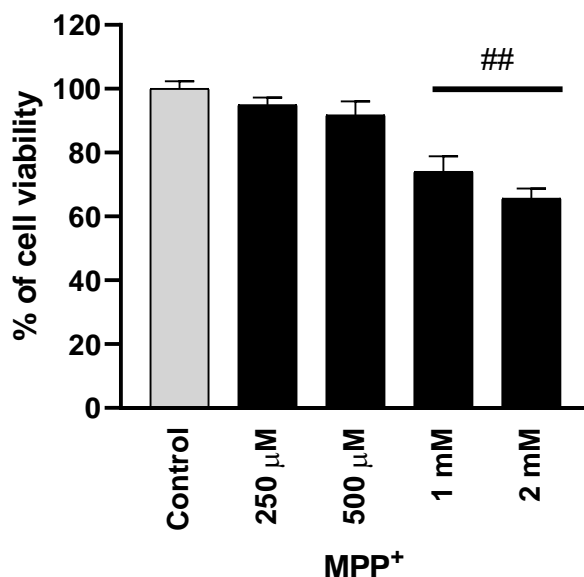
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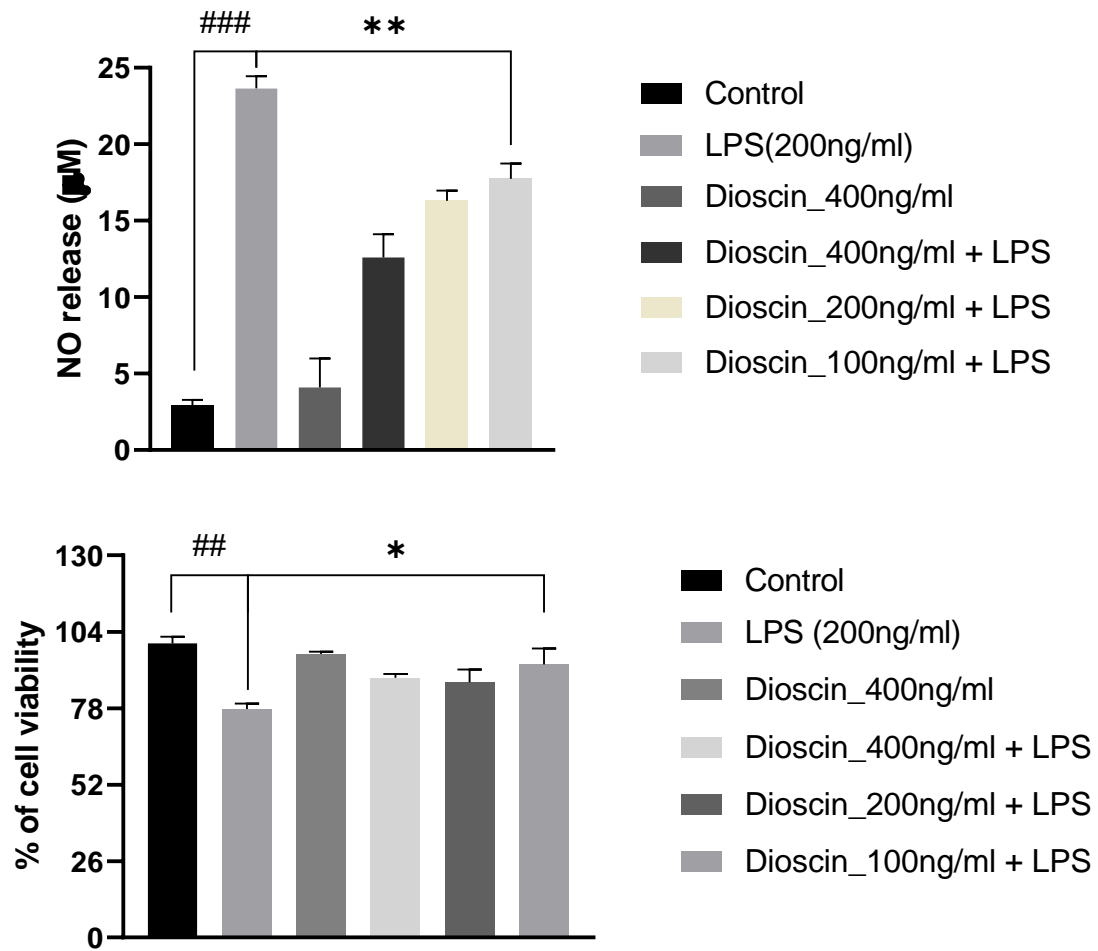
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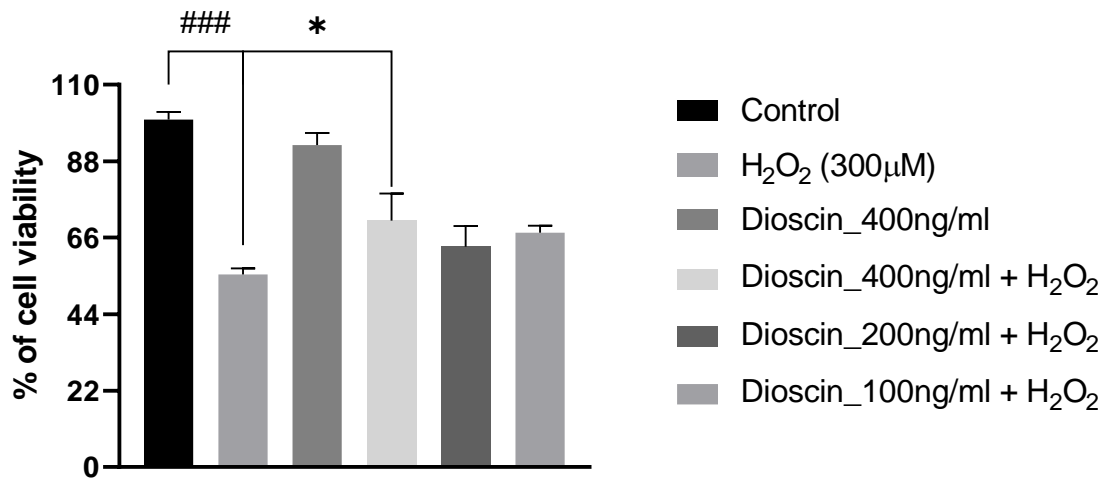
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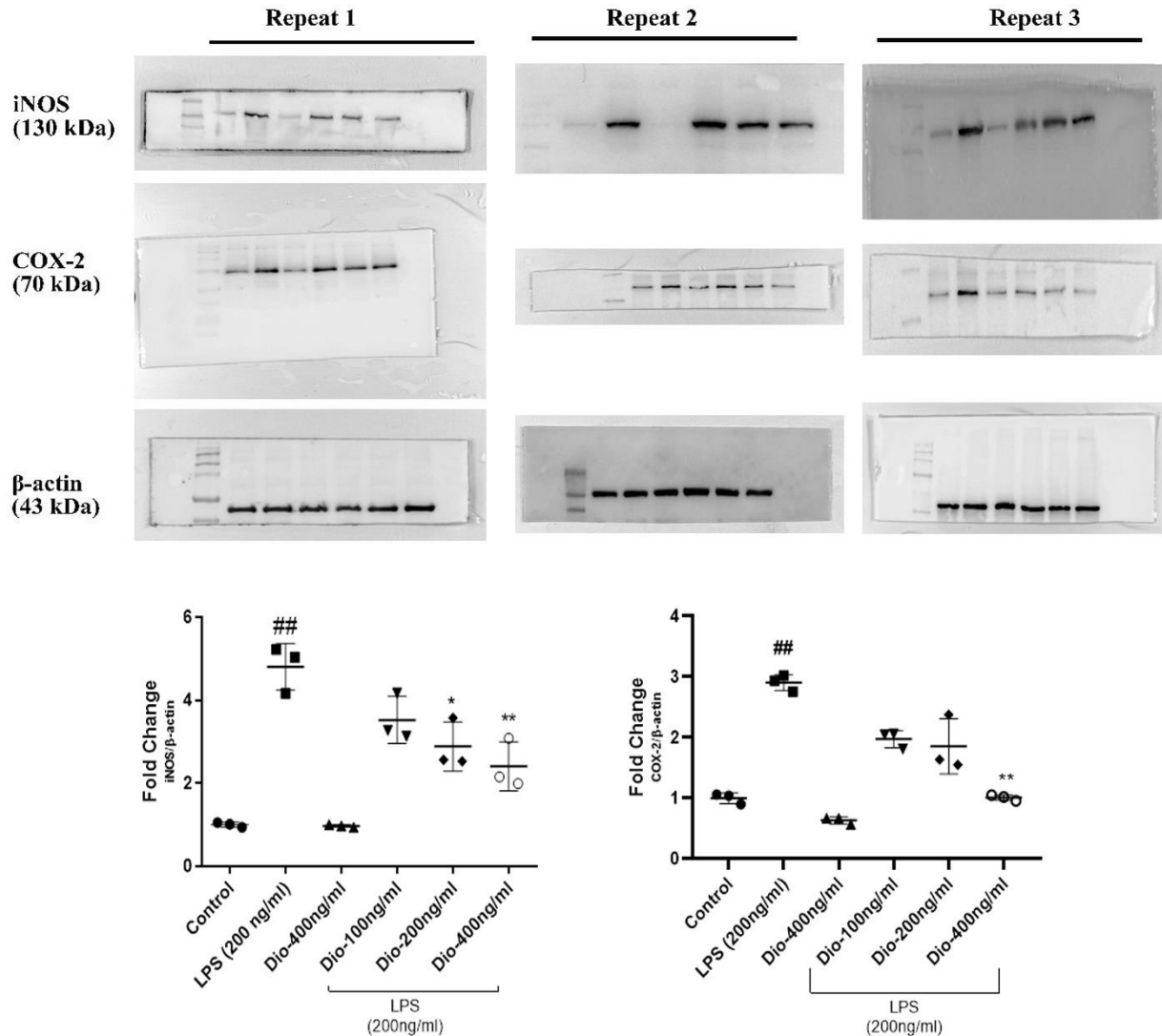
**Figure S1: Dose-dependent toxicity of MPP<sup>+</sup> in SH-SY5Y cells at 24 h.** For dose determination of MPP<sup>+</sup>, different doses of MPP<sup>+</sup> was treated to SH-SY5Y cells for 24 h; n=3; ##p<0.001 compared between non-treated (control) vs treatment (MPP<sup>+</sup>), statistical analysis was done using one-way ANOVA.



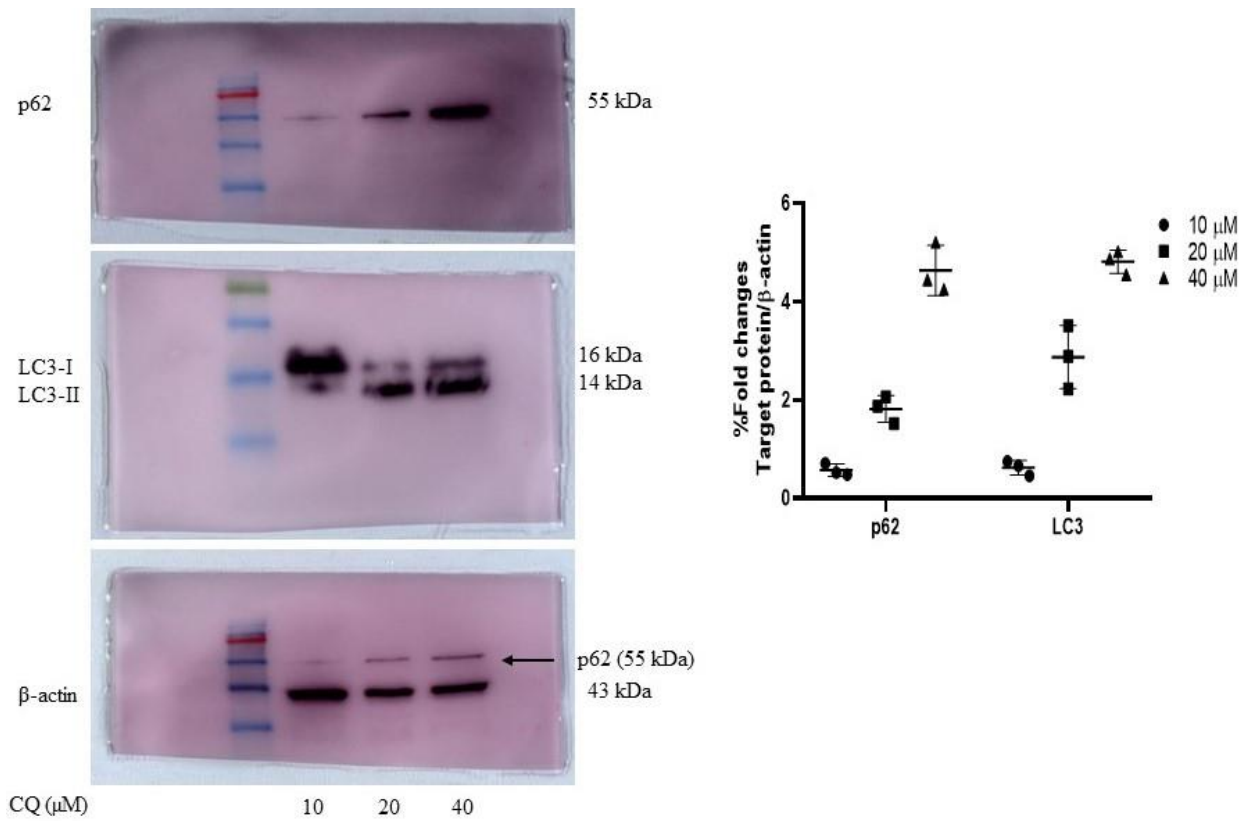
**Figure S2A: Cell viability and NO release from LPS-treated BV-2 cells after dioscin treatment at 24 h.** BV-2 cells were co-treated (LPS and dioscin) or non-treated for 24 h, LPS-activated microglial cells released NO and decreased viability.  $n=3$ ; ### $p<0.001$ , ## $p<0.01$  compared between non-treated (control) vs LPS, and \*\* $p<0.01$ , \* $p<0.05$  compared between dioscin vs LPS, statistical analysis was done using one-way ANOVA.



**Figure S2B: Cell viability of H<sub>2</sub>O<sub>2</sub>-treated PC12 cells after Dioscin treatment at 24 h.** For the cell viability, PC12 cells were either co-treated (H<sub>2</sub>O<sub>2</sub> and dioscin) or non-treated for 24 h; n=3, ###p<0.001 compared between control vs H<sub>2</sub>O<sub>2</sub> and \*p<0.05 compared between H<sub>2</sub>O<sub>2</sub> vs dioscin, statistical analysis was done by one-way ANOVA.



**Figure S3: Inflammatory biomarkers regulated by Dioscin against LPS-induced in BV-2 microglial cells at 24 h.** BV-2 cells were pre-treated (dioscin) for 2 h prior to incubation with LPS (200 ng/ml) or non-treated for 24 h, LPS-activated microglial cells increasing iNOS and COX-2 expression. n=3; ###p<0.001, ##p<0.01 compared between non-treated (control) vs LPS, and \*\*p<0.01, \*p<0.05 compared between dioscin vs LPS, statistical analysis was done using one-way ANOVA.

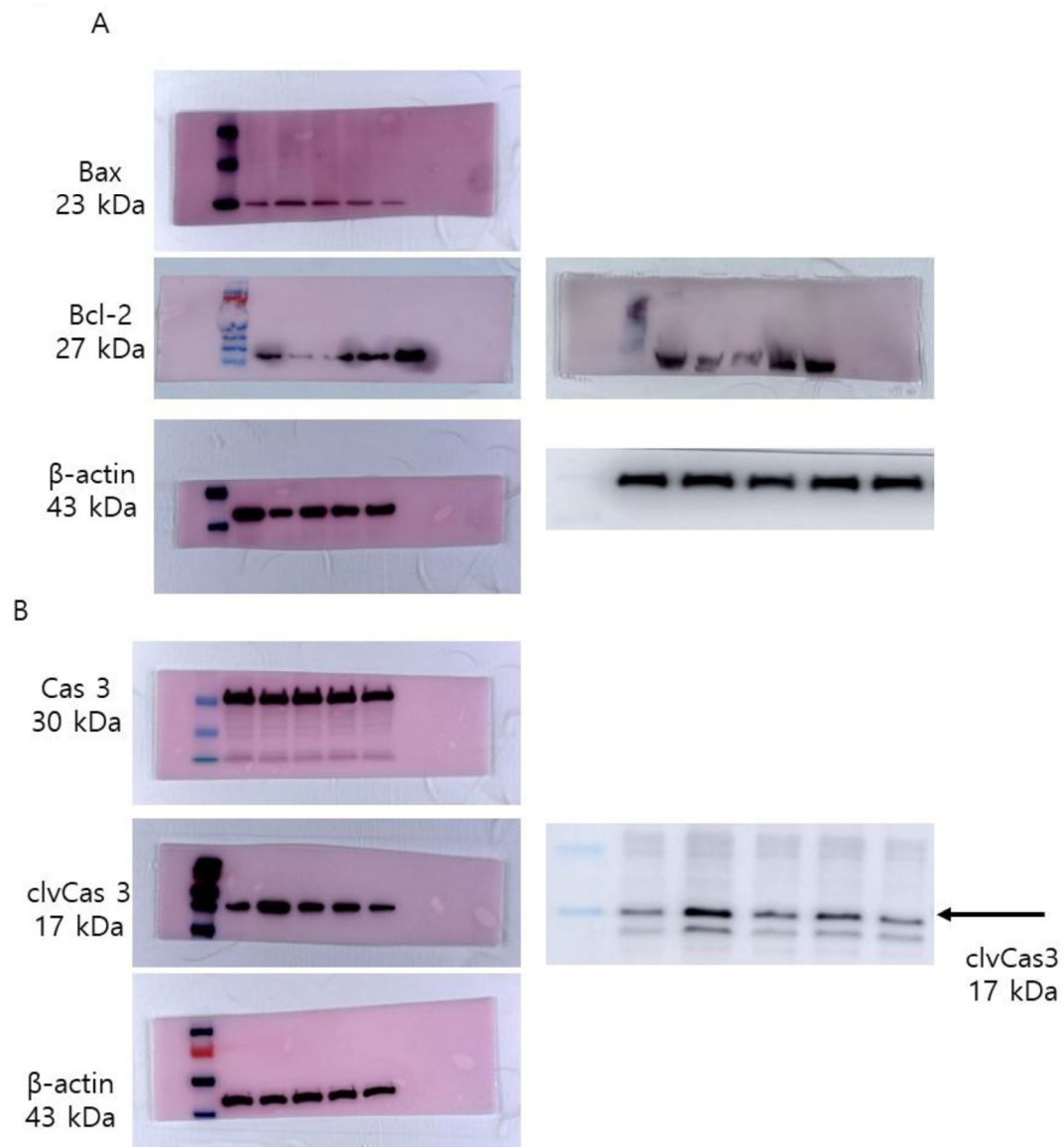


**Figure S4: Dose-dependent autophagic impairment by CQ in SH-SY5Y cells at 24 h.** For dose determination of CQ, SH-SY5Y cells were treated with different doses of CQ for 24 h and proteins were tested via WB for LC3 and p62 expression; n=3, expression of target proteins were neutralized against β-actin, no statistical analysis performed.

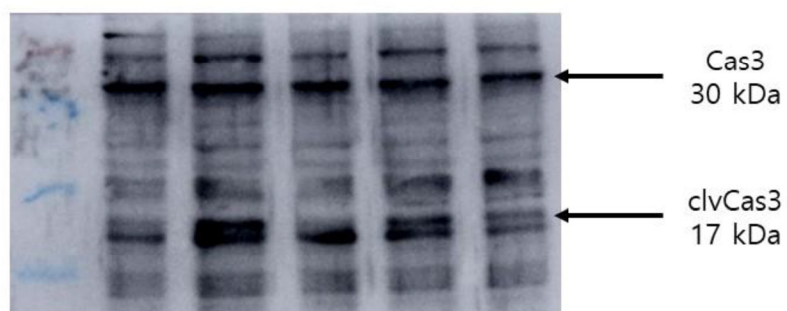
**Original blots below are containing proteins per lane as mentioned in the main manuscript.**

Sample full blots of cropped images

Figure 3.

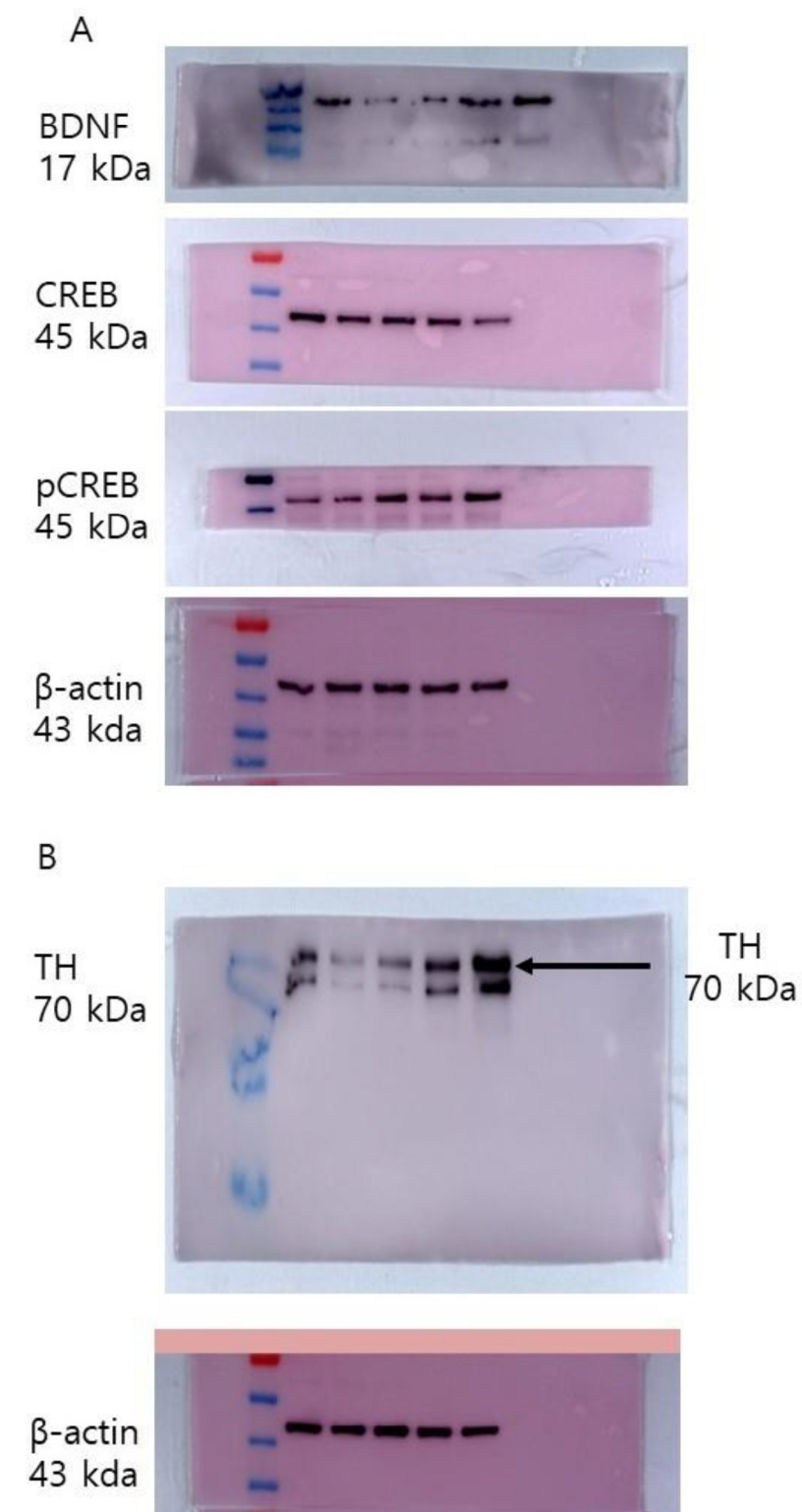


Sample full blots of Cas3/clvCas3



Sample full blots of cropped images

Figure 4.

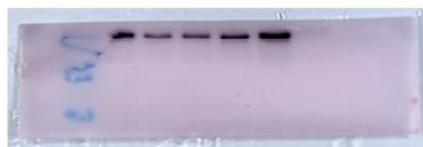


Sample full blots of cropped images

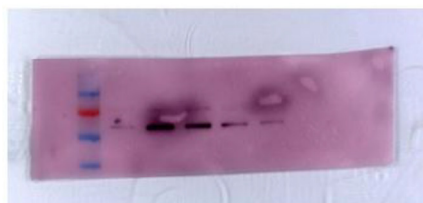
Figure 5.

A

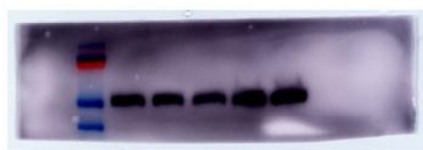
ATG5  
55 kDa



P62  
55 kDa



$\beta$ -actin  
43 kDa



B

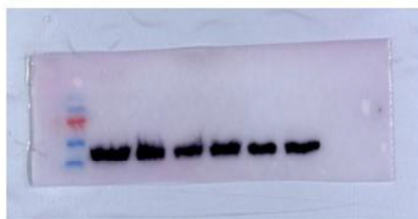
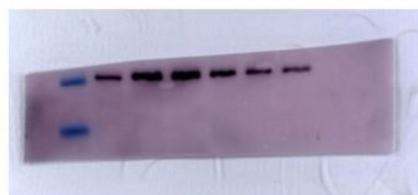
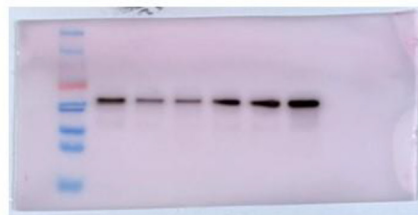
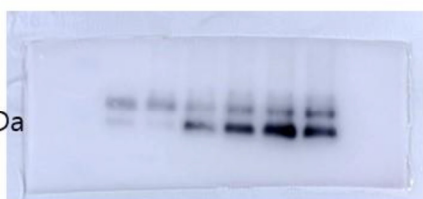
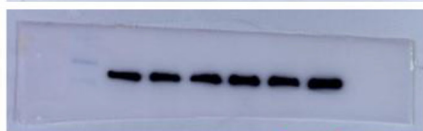


Fig. 6

LC3  
16-14 kDa



$\beta$ -actin  
43 kDa



Both lane are same  
(Dio-400 ng/ml)

