

**CANNABIDIOL INDUCES AUTOPHAGY VIA ERK1/2 ACTIVATION  
IN NEURAL CELLS**

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**Running title:** Cannabidiol induces autophagy via ERK1/2 activation.

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## Supplementary material

**Supplementary Table 1**

Three-way ANOVA effect		<i>F</i> statistic	<i>df</i>	<i>p</i> -value
CBD <i>versus</i> AM251 <i>versus</i> NH <sub>4</sub> Cl interaction		8.541	(1,40)	<i>p</i> = 0.006
CBD <i>versus</i> AM630 <i>versus</i> NH <sub>4</sub> Cl interaction		3.923	(1,32)	<i>p</i> = 0.047
CBD <i>versus</i> CPZ <i>versus</i> NH <sub>4</sub> Cl interaction		4.249	(1,40)	<i>p</i> = 0.045

Pairwise comparisons (Sidak's <i>post hoc</i> test)			
<b><i>CBD versus AM251 versus NH<sub>4</sub>Cl experiment</i></b>			<b><i>p</i>-value</b>
CBD 10 μM <sup>(-)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(-)</sup> / AM251 <sup>(-)</sup> / CTR	<i>p</i> = 0.024
CBD 10 μM <sup>(+)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(-)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>p</i> = 0.003
CBD 10 μM <sup>(+)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(+)</sup> / AM251 <sup>(+)</sup> / NH <sub>4</sub> Cl	<i>p</i> = 0.001
<b><i>CBD versus AM630 versus NH<sub>4</sub>Cl experiment</i></b>			<b><i>p</i>-value</b>
CBD 10 μM <sup>(-)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(-)</sup> / AM630 <sup>(-)</sup> / CTR	<i>p</i> = 0.018
CBD 10 μM <sup>(+)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(-)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>p</i> < 0.001
CBD 10 μM <sup>(+)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(+)</sup> / AM630 <sup>(+)</sup> / NH <sub>4</sub> Cl	<i>p</i> < 0.001
<b><i>CBD versus CPZ versus NH<sub>4</sub>Cl experiment</i></b>			<b><i>p</i>-value</b>
CBD 10 μM <sup>(-)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(-)</sup> / CPZ <sup>(-)</sup> / CTR	<i>p</i> < 0.001
CBD 10 μM <sup>(+)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(-)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>p</i> < 0.001
CBD 10 μM <sup>(+)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl	<i>vs</i>	CBD 10 μM <sup>(+)</sup> / CPZ <sup>(+)</sup> / NH <sub>4</sub> Cl	<i>p</i> < 0.001

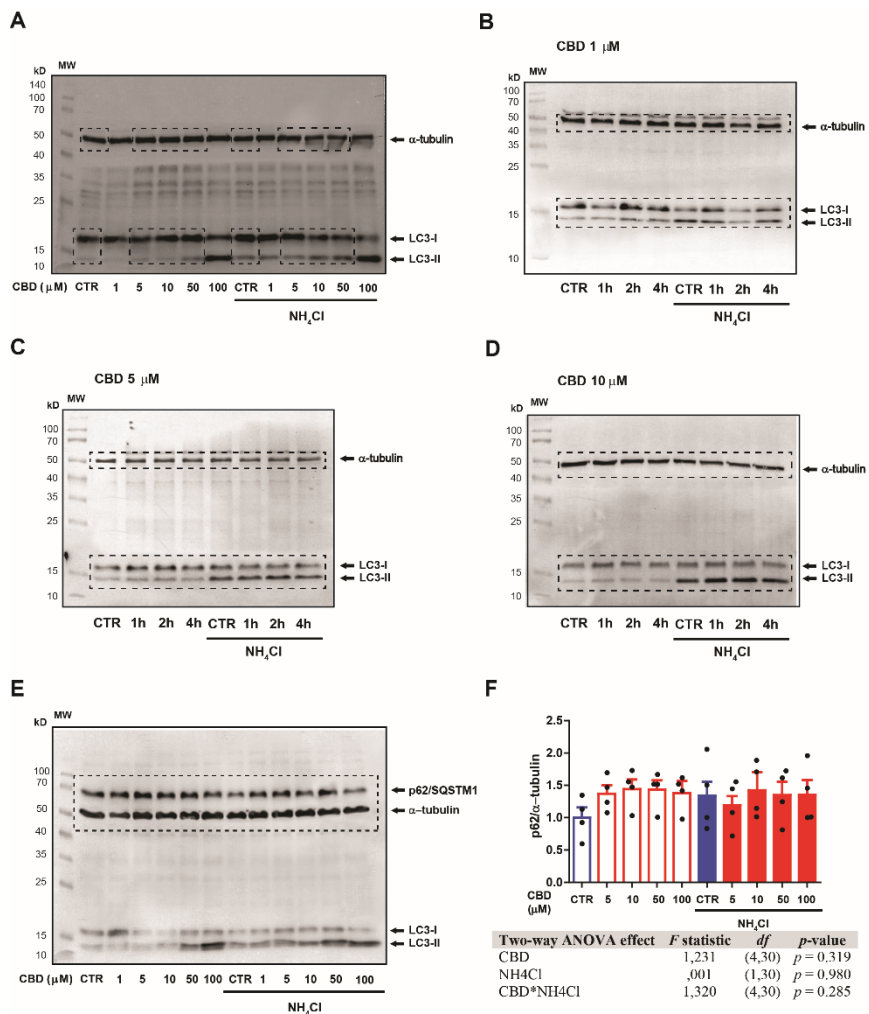
**Table S1.** Statistical summary for CBD *versus* antagonists (AM251, AM630, CPZ) under the presence or not of NH<sub>4</sub>Cl autophagic flux blockade in SH-SY5Y cells. Three-way ANOVA followed by Sidak's *post hoc* test for pairwise comparisons; respective *F* statistic, degree of freedom (*df*), and *p*-values are indicated in the table for every hypothesis test.

## Supplementary Table 2

Three-way ANOVA effect		<i>F</i> statistic	<i>df</i>	<i>p</i> -value
CBD <i>versus</i> AM251 <i>versus</i> NH <sub>4</sub> Cl interaction		4,352	(1,32)	<i>p</i> = 0.045
CBD <i>versus</i> AM630 <i>versus</i> NH <sub>4</sub> Cl interaction		6,674	(1,32)	<i>p</i> = 0.015
CBD <i>versus</i> CPZ <i>versus</i> NH <sub>4</sub> Cl interaction		5,498	(1,32)	<i>p</i> = 0.025
Pairwise comparisons (Sidak's <i>post hoc</i> test)				
CBD <i>versus</i> AM251 <i>versus</i> NH <sub>4</sub> Cl experiment				<i>p</i> -value
CBD 10 μM <sup>(-)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(-)</sup> / AM251 <sup>(-)</sup> / CTR		<i>p</i> < 0.001
CBD 10 μM <sup>(+)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(-)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl		<i>p</i> = 0.001
CBD 10 μM <sup>(+)</sup> / AM251 <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(+)</sup> / AM251 <sup>(+)</sup> / NH <sub>4</sub> Cl		<i>p</i> = 0.001
CBD <i>versus</i> AM630 <i>versus</i> NH <sub>4</sub> Cl experiment				<i>p</i> -value
CBD 10 μM <sup>(-)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(-)</sup> / AM630 <sup>(-)</sup> / CTR		<i>p</i> < 0.001
CBD 10 μM <sup>(+)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(-)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl		<i>p</i> < 0.001
CBD 10 μM <sup>(+)</sup> / AM630 <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(+)</sup> / AM630 <sup>(+)</sup> / NH <sub>4</sub> Cl		<i>p</i> < 0.001
CBD <i>versus</i> CPZ <i>versus</i> NH <sub>4</sub> Cl experiment				<i>p</i> -value
CBD 10 μM <sup>(-)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(-)</sup> / CPZ <sup>(-)</sup> / CTR		<i>p</i> = 0.002
CBD 10 μM <sup>(+)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(-)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl		<i>p</i> = 0.001
CBD 10 μM <sup>(+)</sup> / CPZ <sup>(-)</sup> / NH <sub>4</sub> Cl	vs	CBD 10 μM <sup>(+)</sup> / CPZ <sup>(+)</sup> / NH <sub>4</sub> Cl		<i>p</i> = 0.001

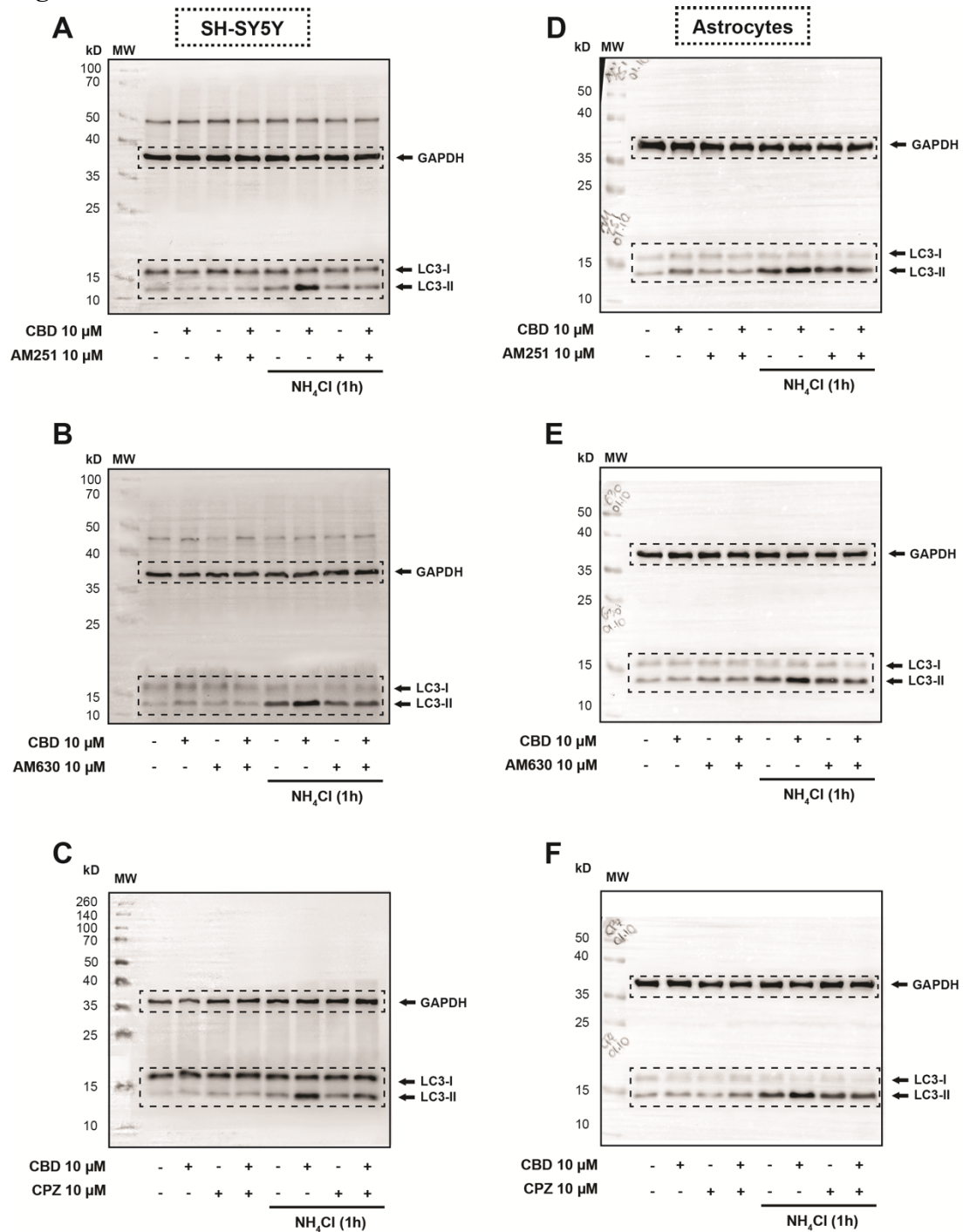
**Table S2.** Statistical summary for CBD *versus* antagonists (AM251, AM630, CPZ) under the presence or not of NH<sub>4</sub>Cl autophagic flux blockade in immortalized astrocytes cells. Three-way ANOVA followed by Sidak's *post hoc* test for pairwise comparisons; respective *F* statistic, degree of freedom (*df*), and *p*-values are indicated in the table for every hypothesis test.

**Figure S1**



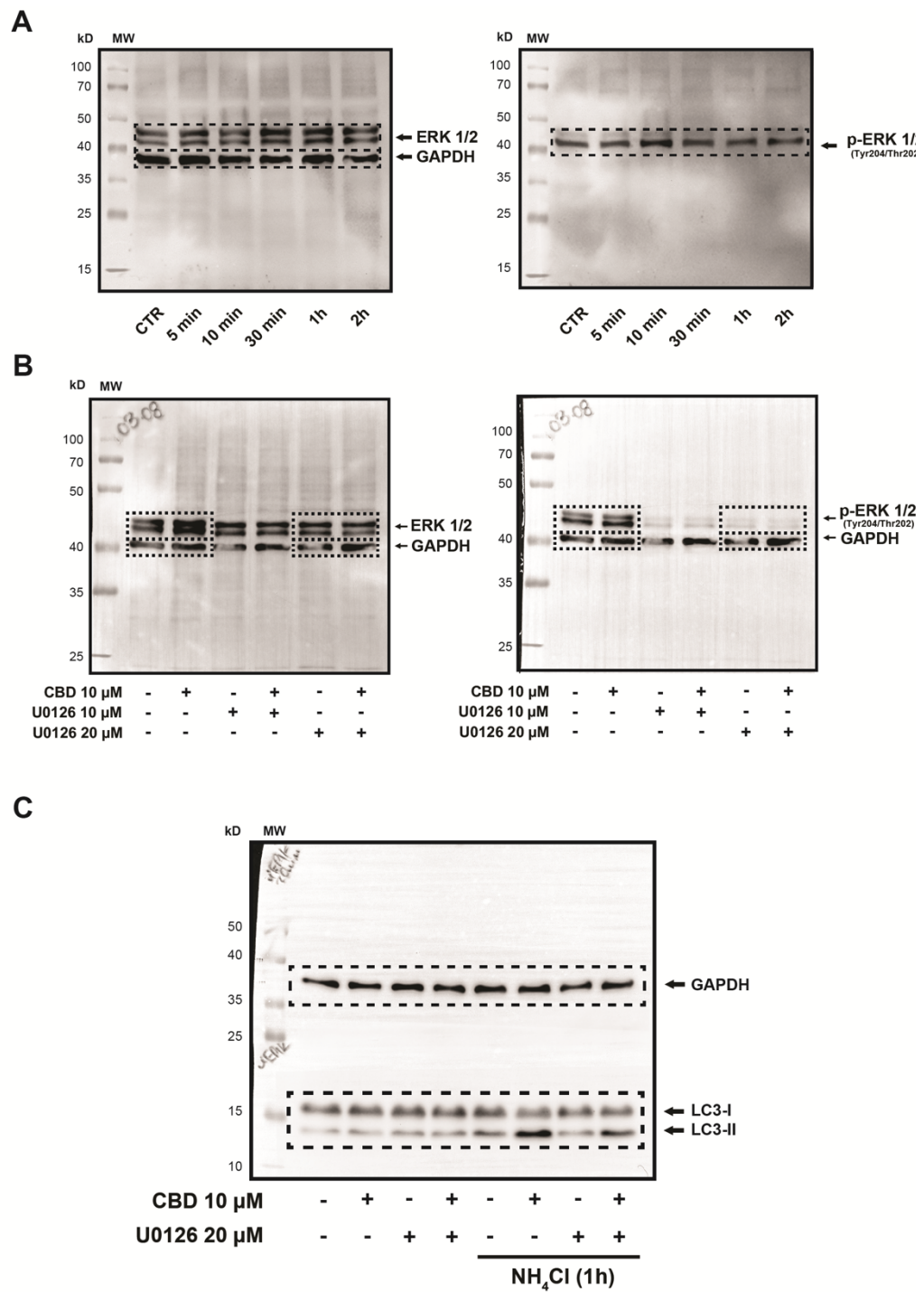
**Figure S1. (A-D)** Full scans of originals Western blots for data Figure 2. The dashed lines on the panels correspond to the figures in the paper. **(E)** Representative blot and **(F)** p62/SQSTM1 expression under different concentrations of CBD. p62/SQSTM1 expression was not modulated either by CBD or  $\text{NH}_4\text{Cl}$  treatment.

**Figure S2**



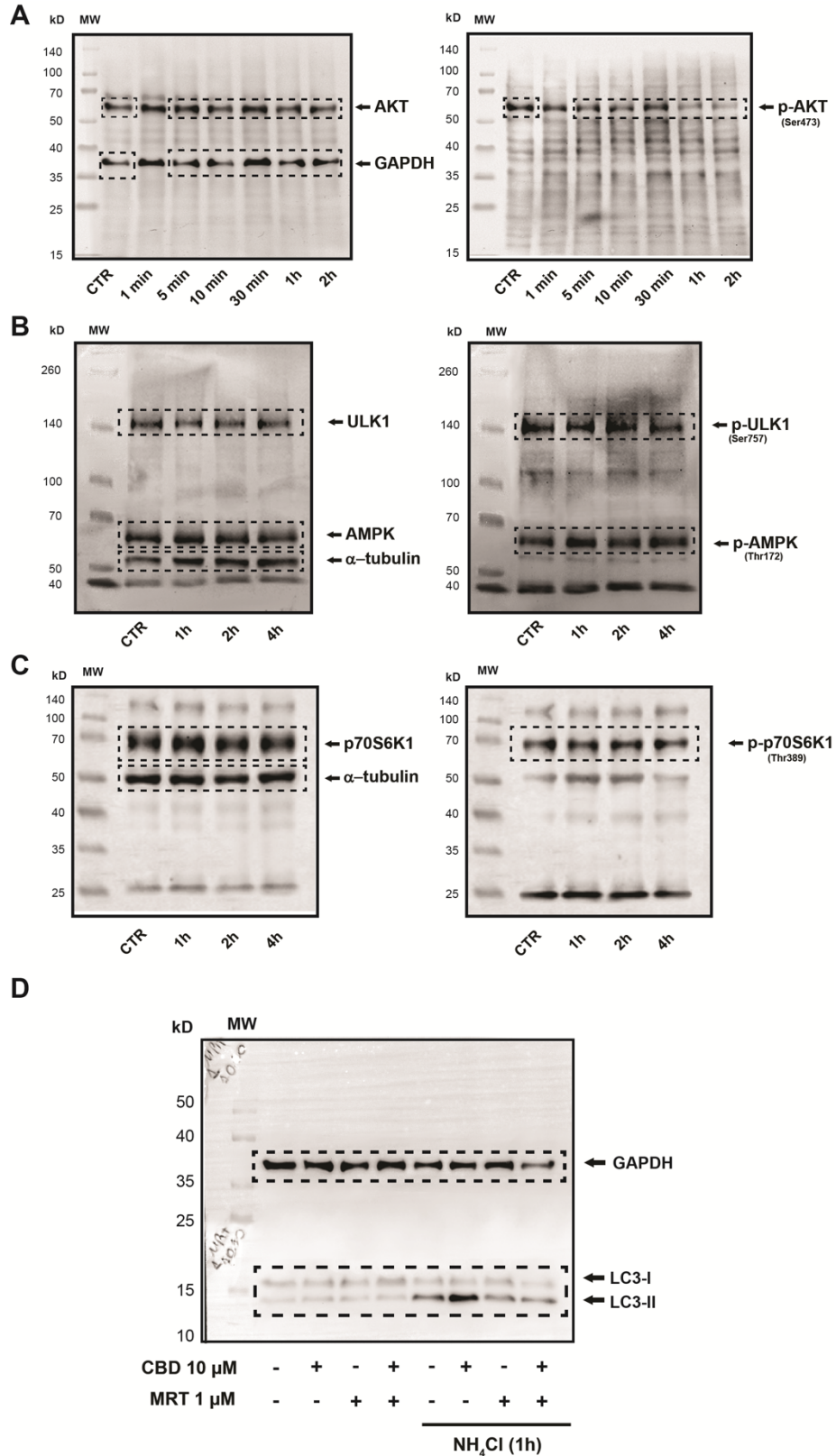
**Figure S2.** Full scans of originals Western blots for data Figure 3. The dashed lines on the panels correspond to the figures in the paper.

**Figure S3**



**Figure S3.** Full scans of originals Western blots for data Figure 4A-C. The dashed lines on the panels correspond to the figures in the paper.

**Figure S4**



**Figure S4** Full scans of original Western blots for data Figure 4D-E and 5. The dashed lines on the panels correspond to the figures in the paper.