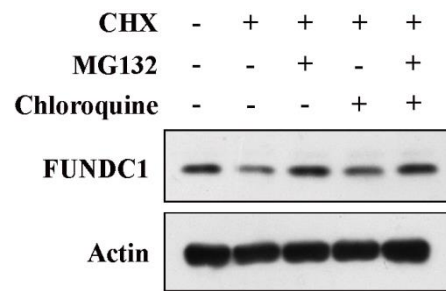


## **Appendix Contents**

Appendix Figure S1 and the figure legend.

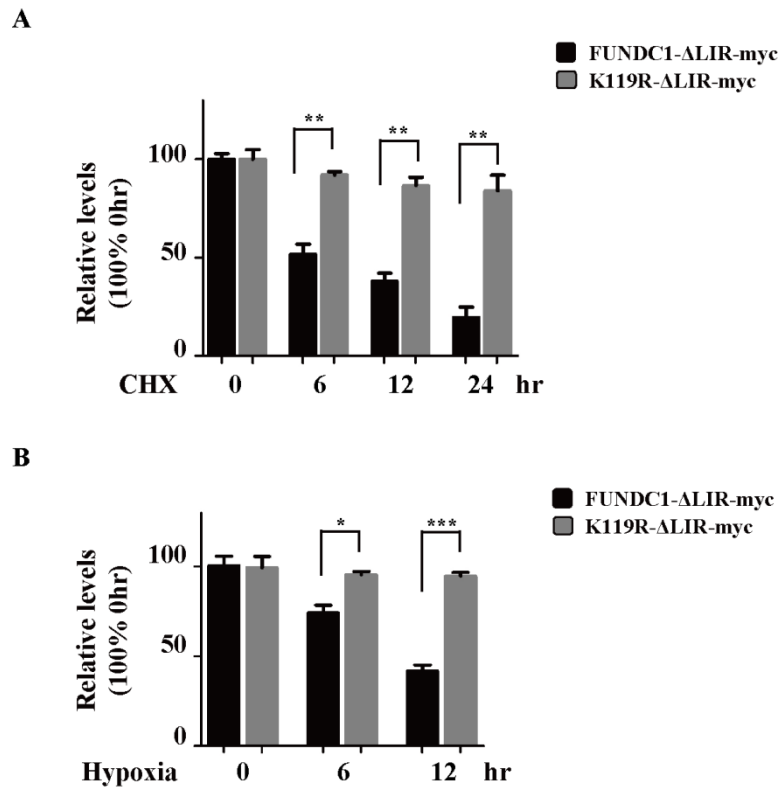
Appendix Figure S2 and the figure legend.

Appendix Figure S3 and the figure legend.



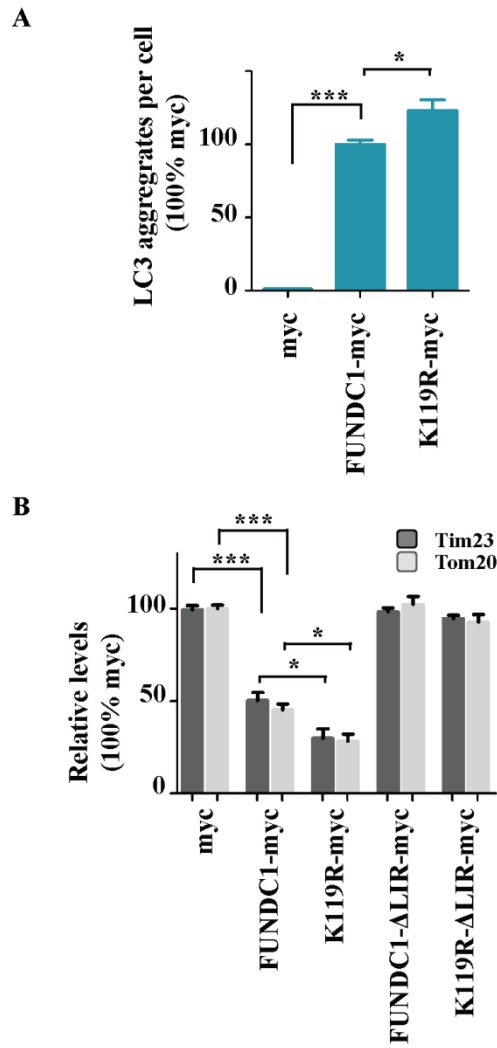
**Appendix Figure S1 MG132 and chloroquine both block FUNDC1 degradation.**

HeLa cells were treated with 10  $\mu$ M CHX for 12 hr, with MG132 (10  $\mu$ M) or chloroquine (10  $\mu$ M), and then the FUNDC1 protein levels were detected by western blotting.



**Appendix Figure S2 Mutation of K119 to R strongly inhibited FUNDC1 degradation.**

(A) Quantification of FUNDC1-ΔLIR-myc and K119R-ΔLIR-myc as indicated in Fig 4D (mean ± SEM, from 3 independent experiments, Student's t-test, \*\* $P < 0.01$ ). (B) Quantification of FUNDC1-ΔLIR-myc and K119R-ΔLIR-myc as indicated in Fig 4E (mean ± SEM, from 3 independent experiments, Student's t-test, \* $P < 0.05$ , \*\*\* $P < 0.001$ )



**Appendix Figure S3 K119R mutant was more effective at inducing mitophagy than wild-type FUNDC1.**

(A) Quantification of GFP-LC3 aggregates surrounding mitochondria in myc-vector, FUNDC1-myc or K119R-myc positive cells treated as in (Fig 4G) with ImageJ (mean  $\pm$  SEM; n = 200 cells from three independent experiments, Student's t-test, \*  $P < 0.05$ , \*\*\*  $P < 0.001$ ). (B) Quantification of Tim23 and Tom20 as indicated in Fig 4H (mean  $\pm$  SEM, from 3 independent experiments, Student's t-test, \*  $P < 0.05$ , \*\*\*  $P < 0.001$ ).