

Supplementary Information

Title:

**Autophagy activation by novel inducers prevents BECN2-mediated
drug tolerance to cannabinoids**

Kenta Kuramoto, Nan Wang, Yuying Fan, Weiran Zhang, Frank J. Schoenen, Kevin J. Frankowski, Juan Marugan, Yifa Zhou, Sui Huang and Congcong He*

*Correspondence should be addressed to C.H. (congcong.he@northwestern.edu)

Supplementary Figure Legends

Figure S1. Body weight of WT, *Becn1*^{+/-} and *Becn2*^{+/-} mice during chronic WIN treatment. N=9-13 mice/group. Results represent mean±s.e.m.

Figure S2. Knockdown efficiency of *Becn2* and cannabinoid-induced CNR1 signaling in the absence of BECN2. **(A)** Western blot detection of BECN2 in HEK293 cells transfected with nontargeting control (NC) or *Becn2* siRNA. **(B)** *Becn2*^{+/-} and WT mice were injected with daily WIN for 14 d and then received an acute dosage of WIN 1 h prior to western blot analyses on brain lysates. Representative images (upper) and quantification (lower) of 3 mice in each group are shown.

Figure S3. Characterization of novel autophagy-inducing agents ML246 and Rg2. **(A)** EC₅₀ of ML246 in autophagy induction. Quantification of GFP-LC3 puncta in GFP-LC3 HeLa cells after 3-h treatment of ML246 at the indicated concentrations. **(B)** Representative images (left) and quantification (right) of GFP-LC3 puncta in brain sections of GFP-LC3 mice injected with vehicle or 20 mg/kg Rg2 once daily for 3 d. Scale bar: 20 μm. Results represent mean±s.d. N≥4 mice. **, *P*<0.01 (t-test). **(C)** Western blot detection of LC3 and SQSTM1 in brain samples of mice injected with vehicle, ML246 or Rg2 once daily for 3 d, or starved for 48 h. **(D)** Western blot detection of LC3 and SQSTM1 in HeLa cells cultured in normal or starvation medium, or in normal medium supplied with 0.5 μM ML246 or 100 μM Rg2 in the presence or absence of the lysosomal inhibitor bafilomycin A₁ for 3 h. S, starvation; ML, ML246.

Figure S4. Body weight and autophagy activity of WT mice treated with autophagy inducers. **(A)** Body weight of WT mice injected with vehicle, 10 mg/kg ML246, or 20 mg/kg Rg2 during chronic WIN treatment. **(B)** Body weight of WT mice housed under normal fed-and-resting conditions, or subjected to intermittent starvation or voluntary wheel exercise during chronic WIN treatment. N=8-14 mice. **(C)** Representative images (upper) and quantification (lower) of GFP-LC3 puncta in brain and skeletal muscle of GFP-LC3 transgenic mice housed under normal conditions, or subjected to either a single bout of 48 h starvation or 4 cycles of “2 days on–1 day off” periodic

starvation. Scale bar: 20 μm . N=4-5. Results represent mean \pm s.e.m. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; NS, not significant (t-test).

Supplementary Video Legends

Video S1. Analgesic tolerance test of WT mice on day 0 by the Hargreaves apparatus. A movable infrared source under the glass floor was positioned beneath the paw to generate pain. When the mouse felt pain and withdrew its paw, the infrared source switched off and the timer stopped. The latency time to the nearest 0.1 s was automatically recorded.

Video S2. Analgesic tolerance test of *Becn2*^{+/-} mice on day 0.

Video S3. Analgesic tolerance test of *Becn1*^{+/-} mice on day 0.

Video S4. Analgesic tolerance test of WT mice on day 1.

Video S5. Analgesic tolerance test of *Becn2*^{+/-} mice on day 1.

Video S6. Analgesic tolerance test of *Becn1*^{+/-} mice on day 1.

Video S7. Analgesic tolerance test of WT mice on day 14.

Video S8. Analgesic tolerance test of *Becn2*^{+/-} mice on day 14.

Video S9. Analgesic tolerance test of *Becn1*^{+/-} mice on day 14.

Video S10. Analgesic tolerance test of vehicle-treated WT mice on day 14.

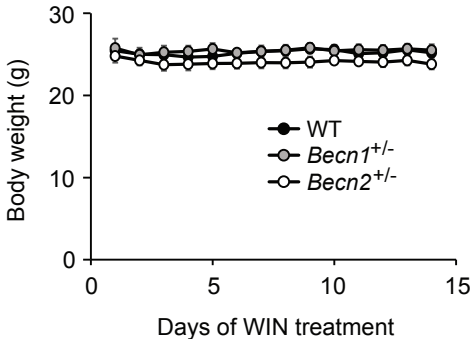
Video S11. Analgesic tolerance test of ML246-treated WT mice on day 14.

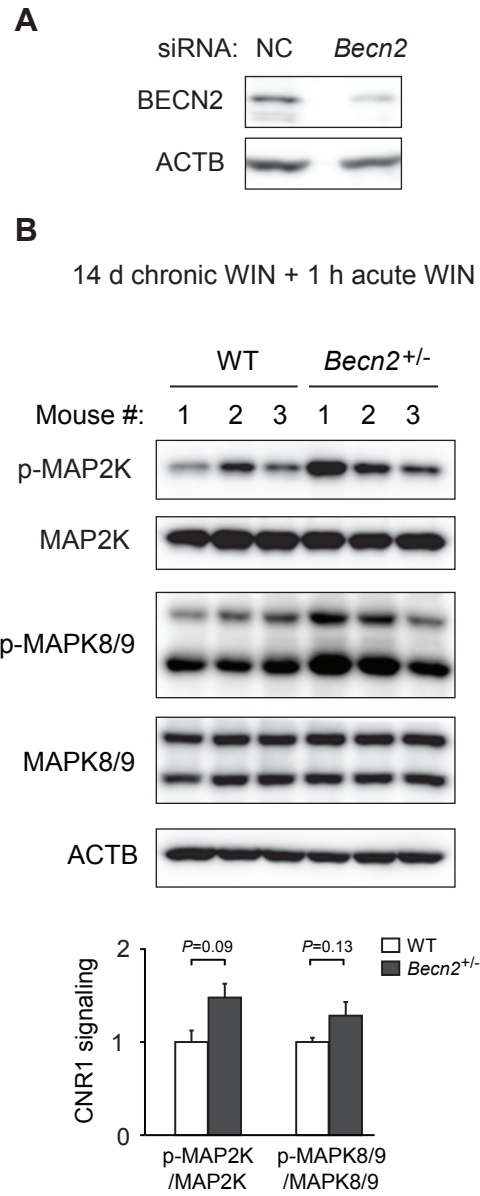
Video S12. Analgesic tolerance test of Rg2-treated WT mice on day 14.

Video S13. Analgesic tolerance test of WT mice housed under fed and resting conditions on day 14.

Video S14. Analgesic tolerance test of WT mice subject to intermittent starvation on day 14.

Video S15. Analgesic tolerance test of WT mice subject to running wheel exercise on day 14.





Supplementary Figure 3

