



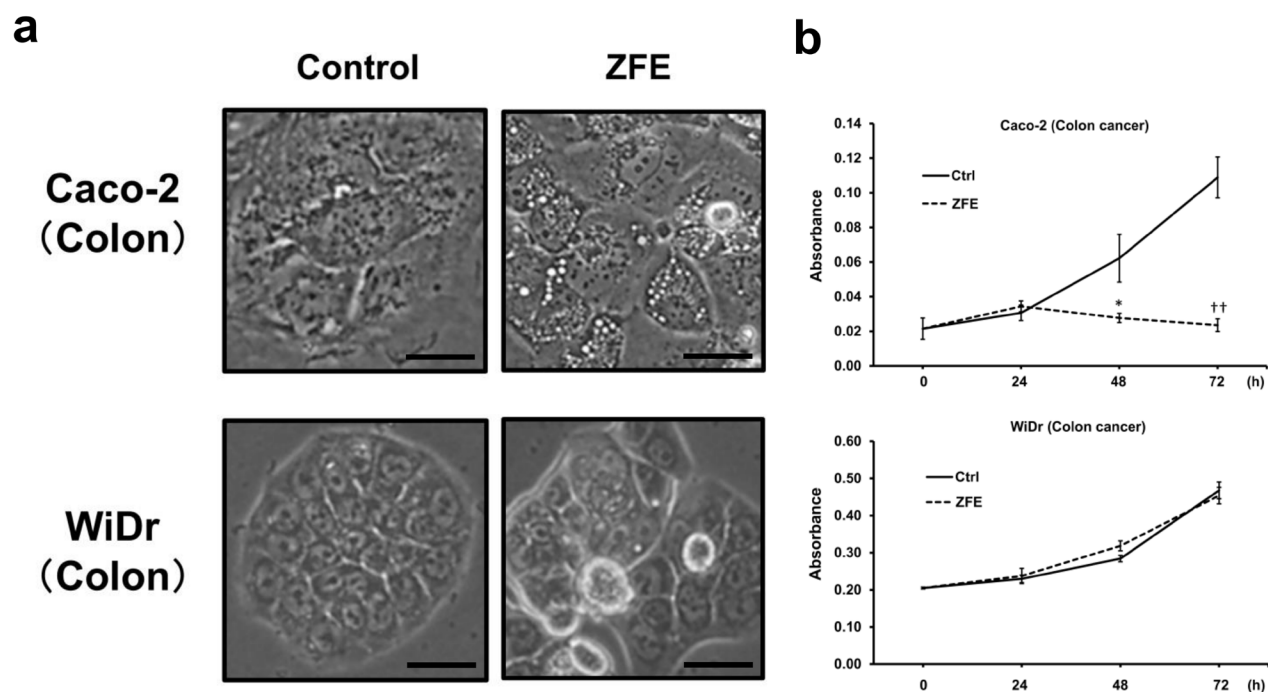
# HOKKAIDO UNIVERSITY

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| Title            | Zanthoxylum fruit extract from Japanese pepper promotes autophagic cell death in cancer cells                                       |
| Author(s)        | Nozaki, Reo; Kono, Toru; Bochimoto, Hiroki et al.                                                                                   |
| Citation         | Oncotarget, 7(43), 70437-70446<br><a href="https://doi.org/10.18632/oncotarget.11926">https://doi.org/10.18632/oncotarget.11926</a> |
| Issue Date       | 2016-10-26                                                                                                                          |
| Doc URL          | <a href="https://hdl.handle.net/2115/64034">https://hdl.handle.net/2115/64034</a>                                                   |
| Rights(URL)      | <a href="https://creativecommons.org/licenses/by/3.0/">https://creativecommons.org/licenses/by/3.0/</a>                             |
| Type             | journal article                                                                                                                     |
| File Information | supplemental materials.pdf, supplemental materials                                                                                  |

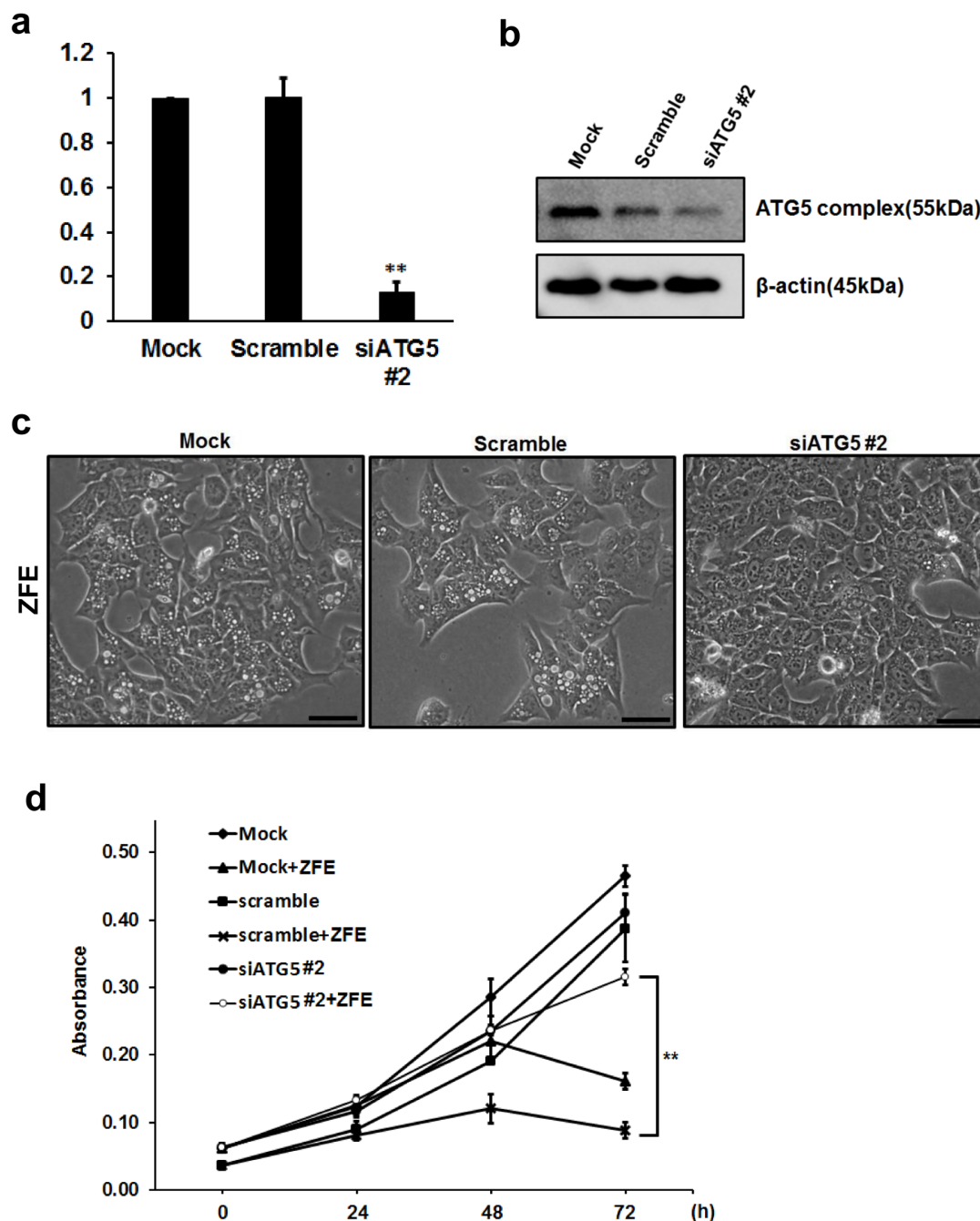


# Zanthoxylum fruit extract from Japanese pepper promotes autophagic cell death in cancer cells

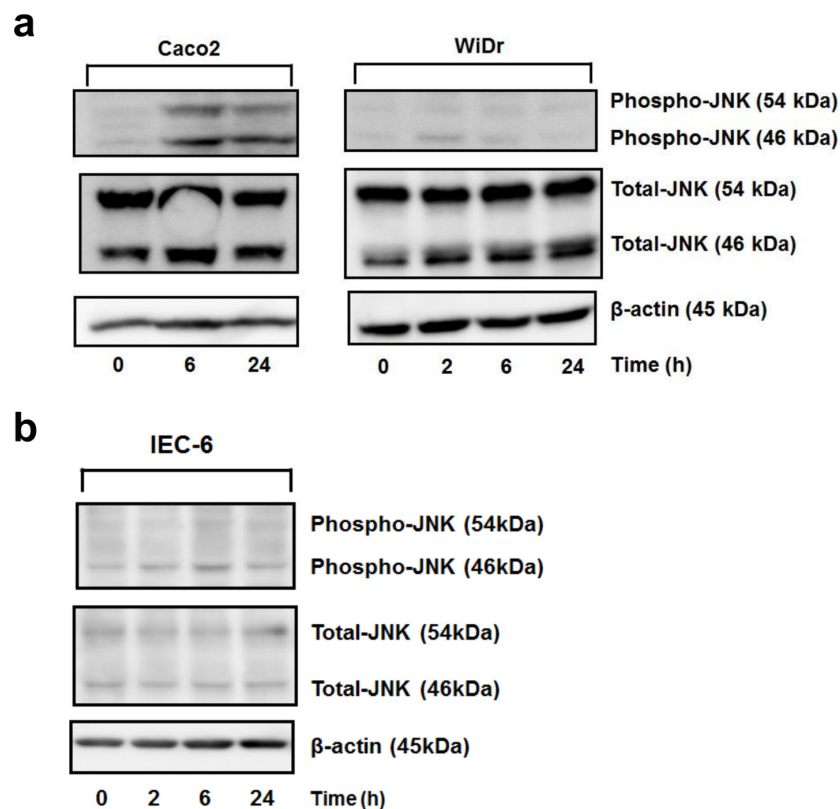
## SUPPLEMENTARY FIGURES



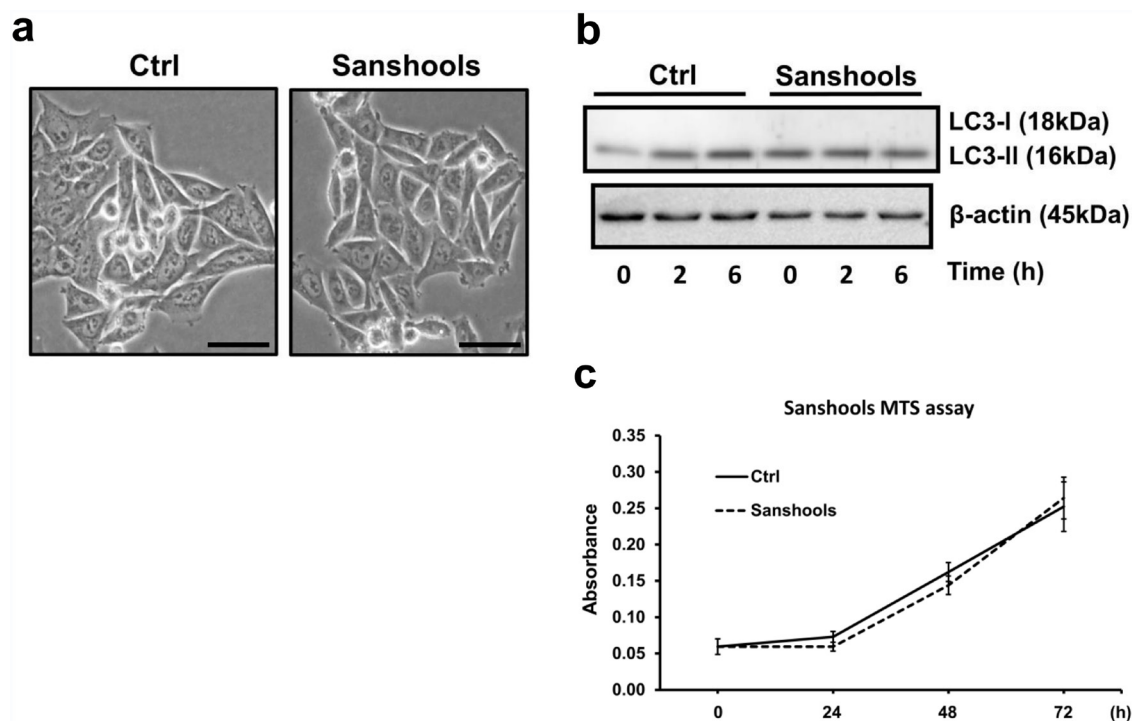
**Supplementary Figure S1: Effects of ZFE on morphology and proliferation of human colon cancer cells.** **a.** Effect of ZFE on the morphology of the indicated cells. Cells were incubated with 200  $\mu\text{g/ml}$  of ZFE or 0.2% v/v DMSO (control) for 24 h. Scale bars, 50  $\mu\text{m}$ . **b.** Cells were incubated with 200  $\mu\text{g/ml}$  of ZFE or 0.2% v/v DMSO (control) for the indicated time and cell viability was measured using a cell proliferation assay kit. Data are the mean  $\pm$  SD of three independent experiments. \* $p < 0.05$  vs. control at 48 h.  $\dagger\dagger p < 0.01$ , vs. control at 72 h (Student's t-test). Ctrl; control.



**Supplementary Figure S2: Knockdown of ATG5 protein inhibits the anticancer effect of ZFE in DLD-1 cells.** **a.** DLD-1 cells were transfected with scrambled siRNA or ATG5 siRNA #2 (10 nM final concentration) or subjected to transfection in the absence of siRNA (Mock). Twenty-four hours after transfection, RNAs were extracted, and quantitative RT-PCR was performed to measure knockdown efficiency. Fold-changes in ATG5 mRNA levels were calculated by the  $\Delta\Delta C_t$  method using GAPDH as a reference gene. Error bars represent S.D. of mean values (n=3). \*\* $p < 0.01$  vs. control (Dunnett's test). **b.** DLD-1 cells were transfected with scrambled siRNA or ATG5 siRNA #2 (10 nM final concentration) or subjected to transfection in the absence of siRNA (Mock). Twenty-four hours after transfection, cell lysates were subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments. **c.** Effect of ZFE on the morphology of ATG5 knockdown DLD-1 cells. Transfected DLD-1 cells were incubated with 200  $\mu\text{g}/\text{ml}$  of ZFE for 24 h. Scale bars, 50  $\mu\text{m}$ . **d.** DLD-1 cells were transfected with scrambled siRNA or ATG5 siRNA #2 (10 nM final concentration) or subjected to transfection in the absence of siRNA (Mock). Twenty-four hours after transfection, the cells were harvested and treated with 200  $\mu\text{g}/\text{ml}$  of ZFE or 0.2% v/v DMSO (control) for the indicated time. Cell viability was measured using a cell proliferation assay kit. Error bars represent S.D. of mean values (n=3). \*\* $p < 0.01$  at 72 h (Student's t-test). Ctrl; control.



**Supplementary Figure S3: Effects of ZFE on phosphorylation of JNK in intestinal cells.** **a.** Cells were treated with 200  $\mu$ g/ml of ZFE for the indicated time. The cell lysates were prepared and subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments. **b.** IEC-6 cells were treated with 200  $\mu$ g/ml of ZFE for the indicated time. The cell lysates were prepared and subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments.



**Supplementary Figure S4: Sanshools have no anticancer activity in DLD-1 cells.** **a.** Effect of sanshools (mixture of HAS and HBS at the same concentration) on the morphology of DLD-1 cells. Cells were incubated with 38  $\mu$ M of sanshools or 0.2% v/v DMSO (control) for 24 h. Scale bars, 50  $\mu$ m. **b.** DLD-1 cells were treated with 38  $\mu$ M of sanshools or 0.2% v/v DMSO (control) for the indicated time. The cell lysates were prepared and subjected to Western blotting with the indicated antibodies. Similar results were obtained in three independent experiments. **c.** DLD-1 cells were incubated with 38  $\mu$ M of sanshools or 0.2% v/v DMSO (control) for the indicated time and cell viability was measured using a cell proliferation assay kit. Data are the mean  $\pm$  SD of three independent experiments. Ctrl; control.