## Supplementary materials

0.5

0

Vehicle

GA-DM

## С Α LC3-I (17 kDa) Pro-caspase 3 (32-35 kDa) LC3-II (14 kDa) Active caspase 3 (17 kDa) β-actin (42 kDa) β-actin (42 kDa) В D 2.5 1.6 p < 0.0001p < 0.0001 Active caspase 3 1.4 2 Relative density (LC3-I and LC3-II) 1.2 LC3-I 1 1.5 LC3-II 0.8 1 0.6

0.4

0.2

0

Vehicle

GA-DM

Toledo cells

Figure S1. Ganoderic acid DM (GA-DM) treatment activates caspase processing in human Toledo B-cell lymphoma. (A) Western blot analysis showing caspase-3 protein expression and cleavage of active caspase 3 in Toledo lymphoma cells treated with GA-DM (30 μM) or vehicle alone (control) as described in the methods. β-actin was used as a loading control. (B) Quantitative analysis of active caspase 3 protein band by ImageJ software (C) Western blot analysis also shows that GA-DM treatment upregulates autophagic protein LC3 in Toledo cells. (D) Quantitative analysis of LC3 protein bands by ImageJ software. The data are representative of three separate experiments. Significant differences were calculated by Student's *t*-test, and p < 0.05 is considered statistically significant.

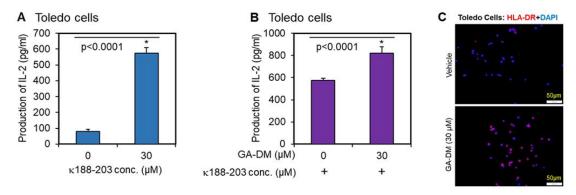


Figure S2. Ganoderic acid DM (GA-DM) treatment enhances HLA class II-restricted antigen presentation in Toledo B-cell lymphoma. (A)  $IgG\kappa_{188-203}$  peptide presentation and immune recognition of HLA-DR4 expressing Toledo cells. Cells were incubated with the  $IgG\kappa_{188-203}$  peptide for overnight, washed, and cocultured with the  $IgG\kappa_{188-203}$  peptide-specific T cell hybridoma for 24 h. (B) Cells were treated with GA-DM (30  $\mu$ M) or vehicle alone for overnight, followed by incubation with  $IgG\kappa_{188-203}$  peptide for another 4 h, washed, and co-cultured with the peptide-specific T cell hybridoma (2.18a) for 24 h. T cell production of IL-2 was measured by ELISA and expressed as  $pg/mL \pm SD$  of triplicate wells of at least three independent experiments. Effects of GA-DM on Ag presentation were calculated as compared to vehicle controls. (C) Toledo cells treated with vehicle alone or GA-DM (30  $\mu$ M) for overnight were also stained with HLA-DR (L243) and anti-mouse IgG DyLight 594, as described in the methods. Nuclear staining was performed using DAPI. Representative images were taken under a fluorescence microscope at  $20 \times magnification$ . Bar =  $50 \mu m$ .