Supplementary Figure 13. LPS-induced NF-κB-p65/IKKβ O-GlcNAcylation and subsequent NF-κB signaling activation in Caco-2 cells were blocked by BtGH84 and AkkGH84. Caco-2 cells were pre-treated with BtGH84, AkkGH84, BtGH84-2D or AkkGH84-2D, followed by LPS incubation. (**A**) Whole cell extracts were analyzed with immunoblots for O-GlcNAc, NF-κB-p65, IKKβ and IκBα. β-actin serves as a loading control. (**B**) O-GlcNAcylated proteins in Caco-2 were pulled down using sWGA beads. O-GlcNAc, NF-κB-p65, IKKβ and IκBα in the pull-down complexes were detected using immunoblots. (**C**) Whole cell extracts of Caco-2 were immunoprecipiated with anti-NF-κB-p65 or anti-IKKβ antibody. The O-GlcNAcylated NF-κB-p65 and IKKβ were detected using anti-O-GlcNAc antibody. (**D-F**) Cytosolic and nuclear sections were analyzed by immunoblots for IκBα and NF-κB-p65 respectively. β-actin serves as a loading control of cytosolic section; Histone-H3 serves as a loading control of nuclear. (**G**) Caco-2 were transfected with pGL3/NF-κB and pRL, followed by treated with BtGH84, AkkGH84, BtGH84-2D or AkkGH84-2D as described above. Afterward, cells were harvested for luciferase activity assay. (**H, I**) Pro-inflammatory cytokines levels in treated Caco-2.