Expanded View Figures

**Figure EV1.** Inhibition of retrograde trafficking blocks the aberrant Stx2a-induced increase in cellular O-GlcNAcylation and consequently delays caspase-3 activation.

Representative western blot showing changes in O-GlcNAcylation and caspase-3 activation in THP-1 cells treated with Stx2a (10 ng/ml) for 12 h in the presence or absence of the retrograde trafficking inhibitor Retro-2 (25 µM final).

Source data are available online for this figure.

**Figure EV2.** Suppression of Stx2a- and Stx1a-induced O-GlcNAcylation rescues cells from apoptosis.

A Representative images showing TUNEL staining of Stx2a-treated THP-1 cells cultured in the presence of OSMI-1 or the vehicle control at 0, 3, 6, and 9 h time point each. FITC-dUTP staining (green fluorescence) in the TUNEL assay indicates active progression of apoptosis. Scale bars: 40 µm.

B WST-1 dye-based cell viability assay of Stx2a-exposed THP-1 cells treated with or without OSMI-1 (10 µM final) at 0, 3, 6, and 9 h time point each (n = 3 biological replicates).

C Representative flow cytometric plot showing apoptosis progression in THP-1 cells detected by TUNEL upon treatment with Stx1a (100 ng/ml) in the presence or absence of OSMI-1 (10 µM final), and quantification of the percentage of apoptotic cells at 9 h (n = 3 biological replicates). The effects of OSMI-1 treatment were compared with those of the vehicle (DMSO) control.

Data information: Error bars for bar graphs are presented as mean ± SD Statistical analysis was performed using two-tailed Student’s t-test. *P < 0.05, **P < 0.01, and ***P < 0.001.

Source data are available online for this figure.
Figure EV3. Akt and p65 were directly O-GlcNAcylated in Stx2a-treated THP-1 cells.

A Representative western blot showing changes in phosphorylation status of Akt or Bad in THP-1 cells treated with Stx2a (10 ng/ml) for 3 h in the presence or absence of OSMI-1 (10 µM, final) or OGA inhibitor Thiamet G (2 µM, final).

B Representative western blot images, before (left) and after (right) pull down using WGA-lectin conjugated to agarose beads or bead-only control, to determine O-GlcNAc attachment to Akt and p65 in lysates from Stx2a (10 ng/ml)-exposed THP-1 cells for 9 h in the presence or absence of OSMI-1 (10 µM, final).

Source data are available online for this figure.
Stx2a-mediated immune responses in primary human renal cells are regulated through O-GlcNAcylation.

**A** Heatmaps representing the comparative expression levels for DEGs in the presence or absence of OSMI-1 treatment upon Stx2a intoxication related to the immune responses.

**B** Heatmaps representing the comparative expression levels for DEGs in the presence or absence of OSMI-1 treatment without Stx2a exposure related to the inflammatory response (left) and apoptotic process (right).

Data information: The numbers within the tables are normalized gene expression level compared to HRPTEpi cells maintained in the absence of either Stx2a or OSMI-1. Expression values are represented with red (upregulation) or blue (downregulation) color using FPKM values by Cufflinks, the cutoffs used a fold change of at least 1.5 followed by pairwise comparison and Student's t-test with a Benjamini and Hochberg correction. The FPKM values were normalized using EdgeR within R and visualized using ExDEGA. Data are the means from two independent replicates.
Figure EV5. Treatment with an OGT inhibitor improves severe loss of body weight due to the hemorrhagic symptoms in the intestines of mice challenged with Stx2a.

A Body weight changes in mice challenged with Stx2a in the presence or absence of OSMI-1 (300 or 1,000 µg/mouse). Data are presented as mean ± SD (n = 5 biological replicates per group, two-tailed Student’s t-test). Comparisons between days 0 and 3 were indicated for each group. *P < 0.05; **P < 0.01; and ***P < 0.001.

B Representative images of the intestines of mice at day 3 after Stx2a injection and combinatorial treatment with two doses of OSMI-1 or the vehicle alone. Source data are available online for this figure.