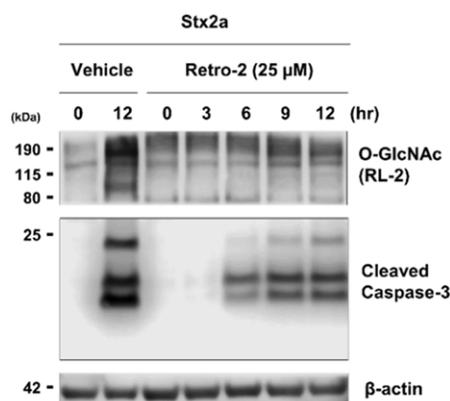


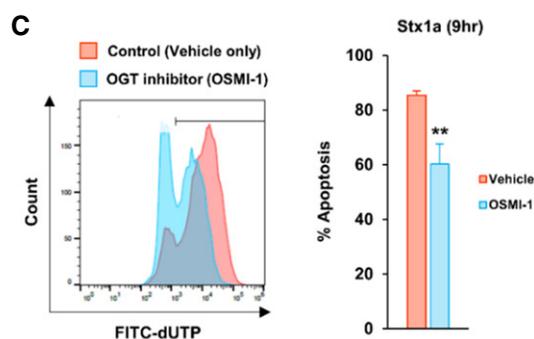
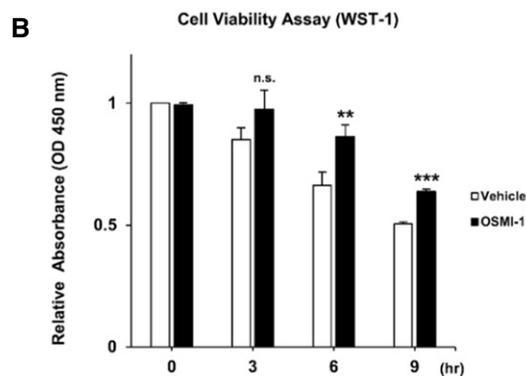
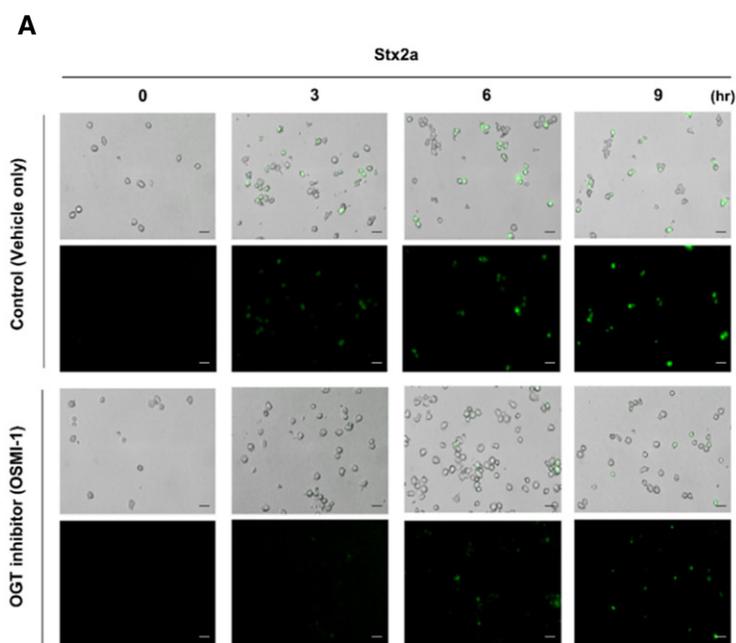
## Expanded View Figures



**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.**

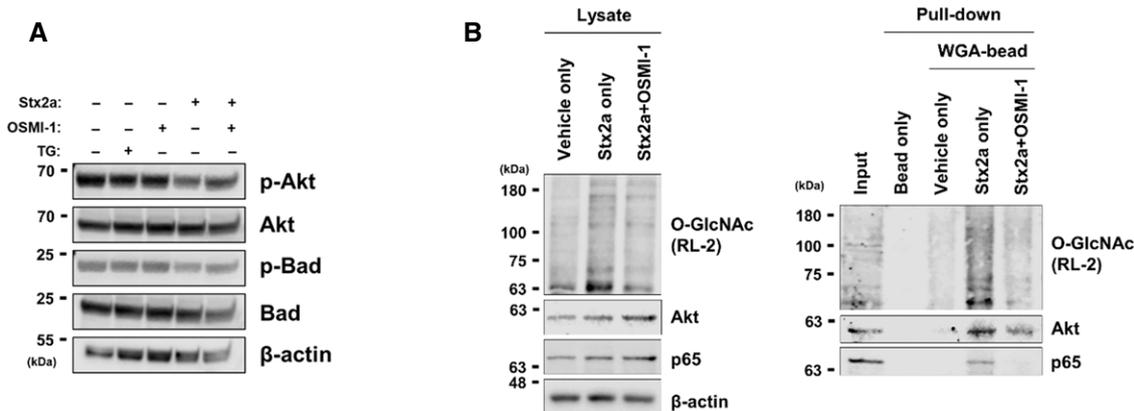
A Representative images showing TUNEL staining of Stx2a-treated THP-1 cells cultured in the presence of OSMI-1 or the vehicle control at 0, 3, 6, and 9 h time point each. FITC-dUTP staining (green fluorescence) in the TUNEL assay indicates active progression of apoptosis. Scale bars: 40 μm.

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  $\pm$  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  $\mu$ M, final) or OGA inhibitor Thiamet G (2  $\mu$ M, 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  $\mu$ M, final).

Source data are available online for this figure.

**A** Immune response

Gene	Fold change over (-)Stx2a			Gene	Fold change over (-)Stx2a		
	Vehicle	OSMI-1	OSMI-1/Vehicle		Vehicle	OSMI-1	OSMI-1/Vehicle
UBD	35.208	14.477	0.411	TNF	1.894	1.232	0.65
LCP1	25.662	13.729	0.535	IST1	1.888	1.244	0.659
NPPB	16.079	5.943	0.37	ITGB2	1.731	1.120	0.647
CXCL8	14.602	3.847	0.263	ICOSLG	1.685	2.704	1.605
LTB	13.403	5.696	0.425	TNFAIP6	1.643	0.998	0.608
IL34	11.623	6.627	0.57	OAS3	1.641	1.013	0.617
APOBEC3B	10.774	7.075	0.657	IFI6	1.533	0.618	0.403
TNFSF18	10.355	3.073	0.297	TRIM29	1.401	0.752	0.537
EB3	8.726	2.976	0.341	ITM2A	1.189	0.653	0.549
IL23A	7.892	4.689	0.594	EDN1	1.114	0.642	0.576
IL1RL1	7.573	4.151	0.548	RAB9B	1.054	0.683	0.648
BCL3	7.229	4.498	0.622	CXCL6	0.962	0.593	0.617
CX3CL1	7.005	4.178	0.596	SEMA7A	0.880	0.575	0.653
GLIPR1	5.550	3.318	0.598	TEC	0.868	1.342	1.546
CCL20	5.519	3.367	0.61	CSF2	0.857	0.200	0.234
PTX3	4.763	2.210	0.464	HSPA1A	0.854	1.302	1.524
LIME1	4.513	2.741	0.607	HSPA1B	0.832	1.369	1.645
PIK3CG	4.458	2.830	0.635	ULBP3	0.830	1.491	1.795
IL32	4.362	2.853	0.654	TRIM68	0.815	1.232	1.513
KYNU	4.156	2.691	0.647	RHOA	0.704	0.439	0.624
CCL2	3.446	1.530	0.444	IFI30	0.573	0.924	1.612
IL6	3.318	1.488	0.448	VCAM1	0.559	0.360	0.643
ILTR	2.819	1.219	0.432	S100A7	0.442	1.100	2.49
GBP1	2.806	1.677	0.598	SIGLEC15	0.442	0.285	0.644
CXCL11	2.629	1.597	0.607	PTGDS	0.334	0.662	1.983
IFI27	2.602	1.550	0.596	RAB20	0.252	0.443	1.751
BCL2	2.467	1.617	0.655	FTL	0.252	0.390	1.551
CXCL1	2.278	0.753	0.33	FTH1	0.150	0.233	1.553
SEMA3C	2.049	1.330	0.649				



**Figure EV4. Stx2a-mediated immune responses in primary human renal cells are regulated through O-GlcNAcylation.**

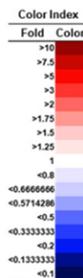
A Heatmaps representing the comparative expression levels for DEGs in the presence or absence of OSMI-1 treatment upon Stx2a intoxication related to the immune responses.

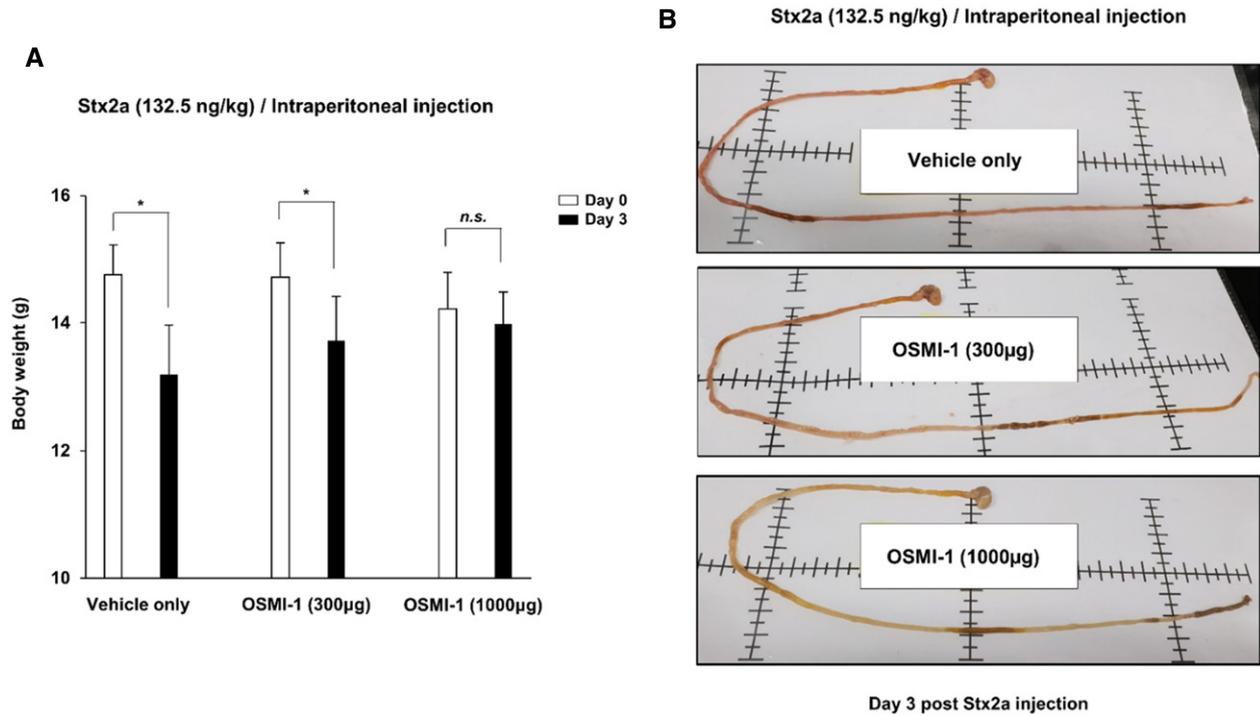
B Heatmaps representing the comparative expression levels for DEGs in the presence or absence of OSMI-1 treatment without Stx2a exposure related to the inflammatory response (left) and apoptotic process (right).

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.

**B** Inflammatory response      Apoptotic process

(-)Stx2a		(-)Stx2a	
Gene	OSMI-1/Vehicle	Gene	OSMI-1/Vehicle
PROK2	1	TRAF1	1.317
CXCL8	0.63	TNFRSF9	1.289
IL34	1	TOP2A	0.995
IL23A	0.932	BCL3	1.057
CX3CL1	1.205	PIK3CG	2.081
CCL20	0.995	IL6	0.551
PTX3	0.793	IFI27	1.536
PIK3CG	2.081	BCL2	0.942
CCL2	0.82	TNFRSF19	0.849
IL6	0.551	TGFB2	0.645
CXCL11	1	CAV1	0.910
CXCL1	0.557	INHBA	0.646
TSPAN2	0.653	TNF	1.161
TNF	1.161	PPP1R13L	0.859
PTGS1	1.045	ITGB2	0.966
ALOX5AP	0.658	GDF6	0.459
ITGB2	0.966	IFI6	1.028
TNFAIP6	0.771	PARP1	1.006
NOX4	0.826	HK2	0.869
UGT1A1	0.981	TP53NP1	1.154
CXCL6	0.777	SGK1	0.552
SEMA7A	0.895	DNAH2	1.126
ITGB6	0.995	SST	0.404
VCAM1	0.977	HMOX1	1.338
HMOX1	1.338	MAL	1.124
		TNFRSF11B	0.736





**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  $\pm$  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.