Supplementary Material

Increased O-GlcNAcylation of c-Myc Promotes Pre-B Cell Proliferation

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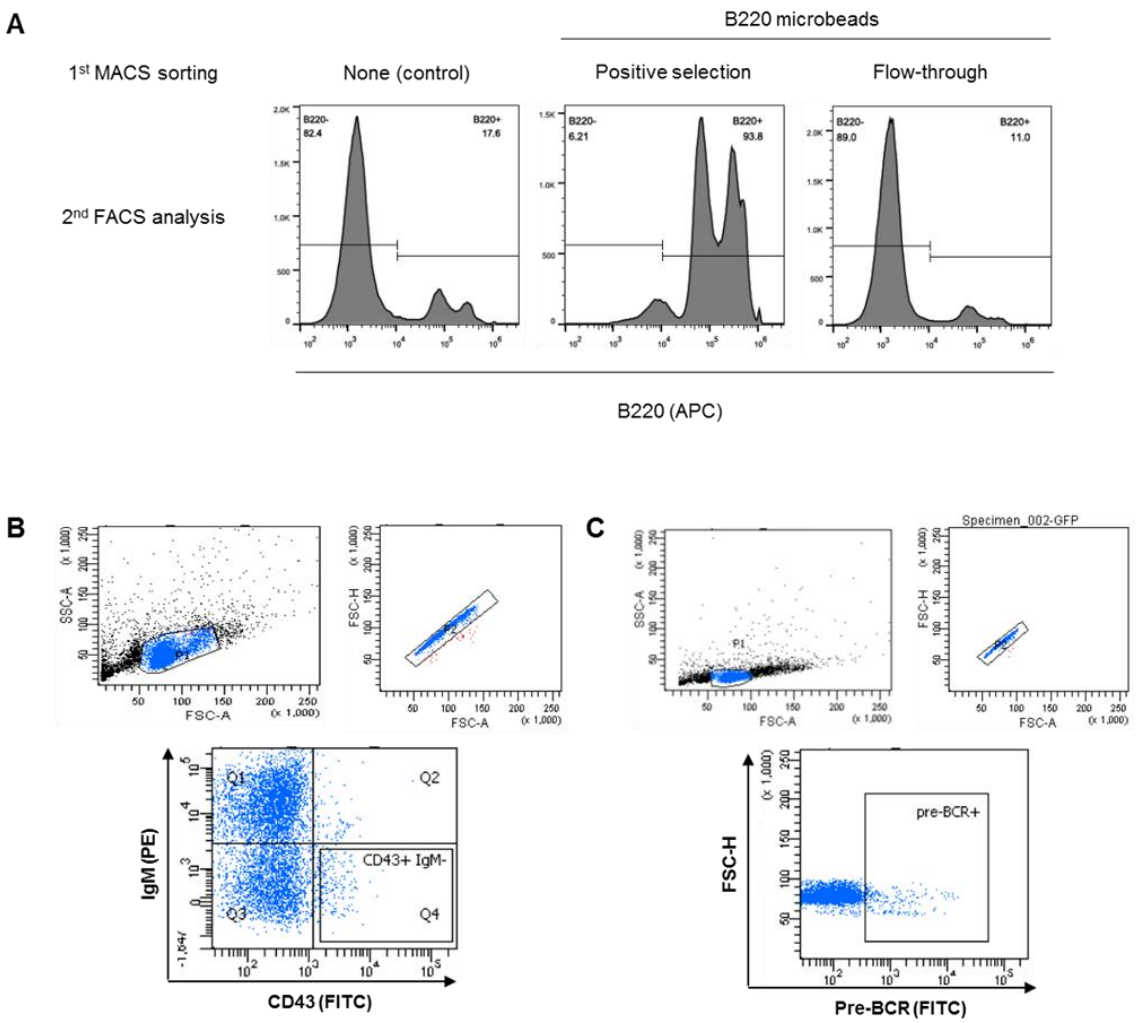

Figure S1. Pro-B cells and large pre-B cells were sequentially sorted by MACS using B220 microbeads and then by FACS using CD43 and IgM or pre-BCR as markers

(A) Representative plot of the FACS analysis using B220-APC shows the purity of B220-microbeads positive cells from mouse bone marrow sorted by MACS. (B) Pro-B cells ($CD43^+IgM^-$) were isolated by FACS using CD43-FITC and IgM-PE after B220 enrichment by MACS. (C) Large pre-B cells were pre-BCR-$FITC^+$ sorted by FACS after B220 enrichment by MACS.

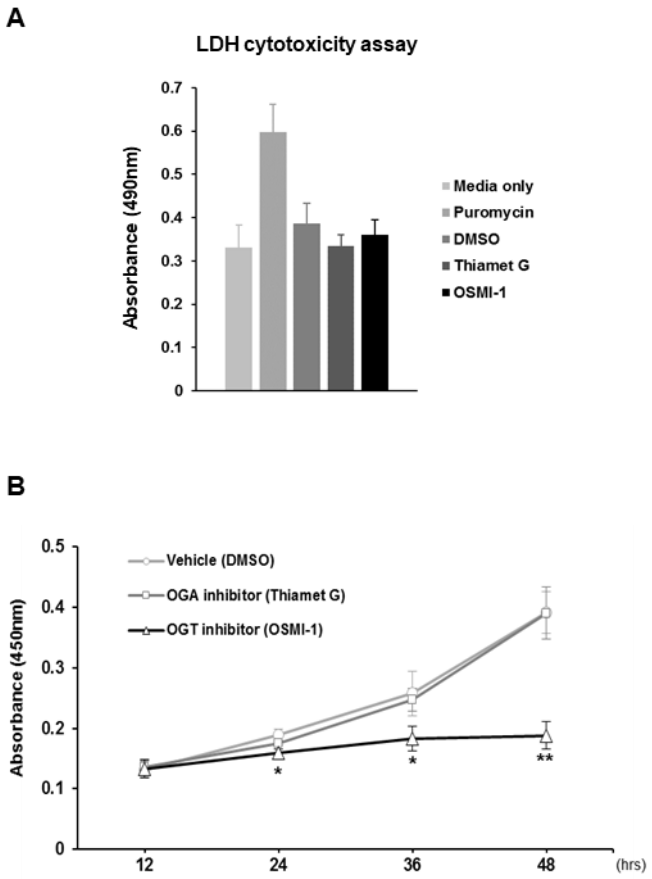

Figure S2. Pre-B cell proliferation is significantly decreased by inhibition of O-GlcNAcylation without any significant cell death

(A) LDH cytotoxicity assay performed after a 48-h culture following inhibitor treatment [Thiamet G (2 μM, final) and OSMI-1 (10 μM, final)]. Cells were incubated with puromycin (10 μg/mL) for 5 h to artificially induce cell damage as a positive control, resulting in a ~35% death rate. Background levels of LDH activity were established using culture medium alone. Data are represented as mean ± SD (n = 3). Comparison of each treatment with medium alone control. n.s. = not significant. (B) WST-1 dye-based cell-proliferation assay. The growth of PD36 pre-B cells was monitored for 48 h following inhibitor treatment [Thiamet G (OGA inhibitor; 2 μM, final) and OSMI-1 (OGT inhibitor; 10 μM, final)]. Data represent the mean ± SD (*n* = 3). Comparison of OSMI-1 treatment with DMSO control at each time point.

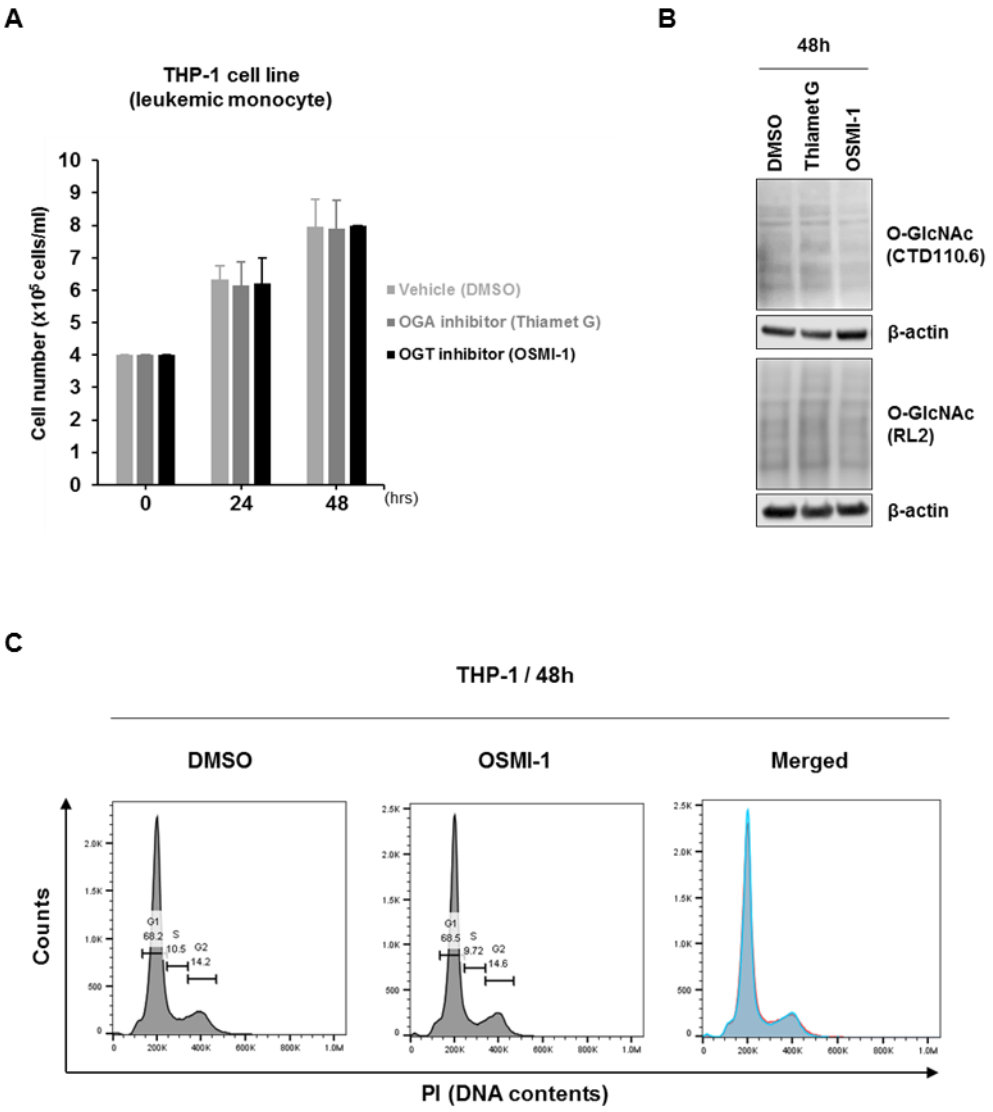

Figure S3. THP-1 cell growth is unaffected by treatments of O-GlcNAc related inhibitors

(A) The growth of THP-1 cells was monitored for 48 h following inhibitor treatment [Thiamet G (2 μM, final) and OSMI-1 (10 μM, final)]. Data represent the mean ± SD (n = 3). (B) Representative western blot monitoring changes in O-GlcNAcylation levels. (C) Representative plot of the flow cytometric analysis of THP-1 cell cycle progression following staining with propidium iodide (PI) at 48 h after treatment with OSMI-1 (10 μM, final). The percentages of cells at the G1, S, and G2/M phases are shown. Merged plots revealed no change in the cell cycle stage according to treatment.

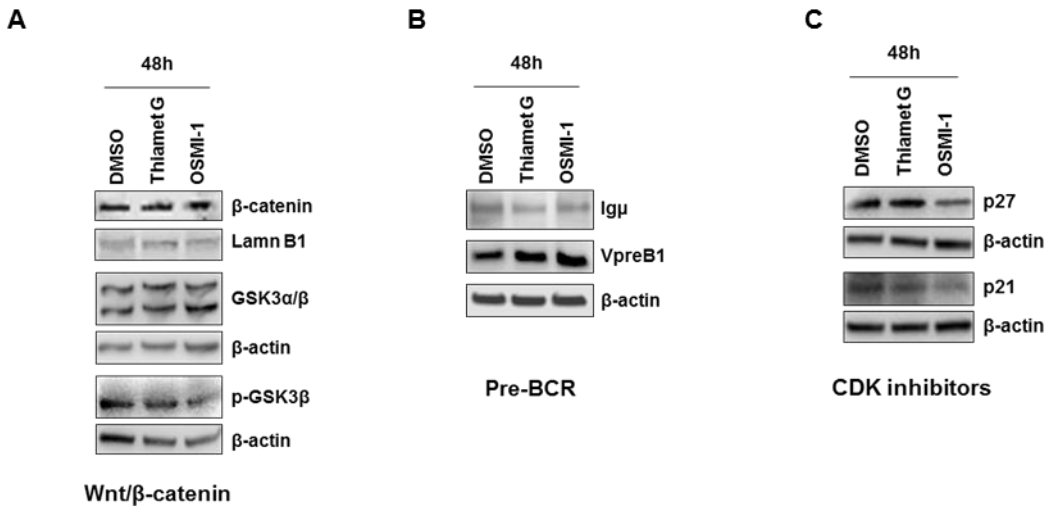

Figure S4. Unchanged expression of factors regulating pre-B cell proliferation following inhibitor treatment

(A-C) Representative western blot monitoring protein levels associated with Wnt/β-catenin signaling (A), pre-BCR signaling (B), or CDK inhibitors like p21 and p27 (C) at 48-h post-treatment with inhibitors [Thiamet G (2 μM, final) and OSMI-1 (10 μM, final)].

Name	Description	Use	Sequence(5'→3')
SP042	FOR for Cyclin D2 qPCR (mouse)	qPCR	GGA CAT CCA ACC GTA CAT G
SP043	REV for Cyclin D2 qPCR (mouse)	qPCR	GAA AGA CCT CTT CTT CAC AC
SP083	FOR for Cyclin D3 qPCR (mouse)	qPCR	GCT TTG CAT CTA TAC GGA CC
SP084	REV for Cyclin D3 qPCR (mouse)	qPCR	GAA TCA AGG CCA GGA AGT C
SP099	FOR for Cyclin E1 qPCR (mouse)	qPCR	CCGTGTTTTTGCAAGACCCAG
SP100	REV for Cyclin E1 qPCR (mouse)	qPCR	GGTCTGATTTTCCGAGGCTG
SP069	FOR for Cyclin E2 qPCR (mouse)	qPCR	CAC CCC ATA AAG AAA TAG GAA CAA G
SP070	REV for Cyclin E2 qPCR (mouse)	qPCR	CCC AGC TTA AAT CTG GCA GAG
SP087	FOR for Cyclin A2 qPCR (mouse)	qPCR	CTC AAG ACT CGA CGG/ GTT
SP088	REV for Cyclin A2 qPCR (mouse)	qPCR	CTC CAT TTC CCT AAG GTA CG
SP101	FOR for VpreB qPCR (mouse)	qPCR	GCCCACCTCACAGGTTGT
SP102	REV for VpreB qPCR (mouse)	qPCR	CCTGGCCGTATCTTTGGACC
SP005	FOR for λ5 qPCR (mouse)	qPCR	CTT GAG GGT CAA TGA AGC TCA GAA GA
SP006	REV for λ5 qPCR (mouse)	qPCR	CTT GGG CTG ACC TAG GAT TG
SP052	FOR for IL-7Rα qPCR (mouse)	qPCR	GCC CAG CAA GGG GTG AAA GCA AC
SP053	REV for IL-7Rα qPCR (mouse)	qPCR	GGC AAG ACA GGA TCC CAT CCT CC
SP007	FOR for EBF qPCR (mouse)	qPCR	GCT GTG GCA ACC GAA ATG AG
SP008	REV for EBF qPCR (mouse)	qPCR	CCG TGC TTG GAG TTA TTG TGG AC
SP119	FOR for c-Myc_T58A	site-directed mutagenesis	CCCGCCCCGCCCCTGTCCCCG
SP120	REV for c-Myc	site-directed mutagenesis	AAGCAGCTCGAATTTCTTCCA

Table S1. Primers used in this study