TAB3 O-GlcNAcylation promotes metastasis of triple negative breast cancer

Supplementary Materials

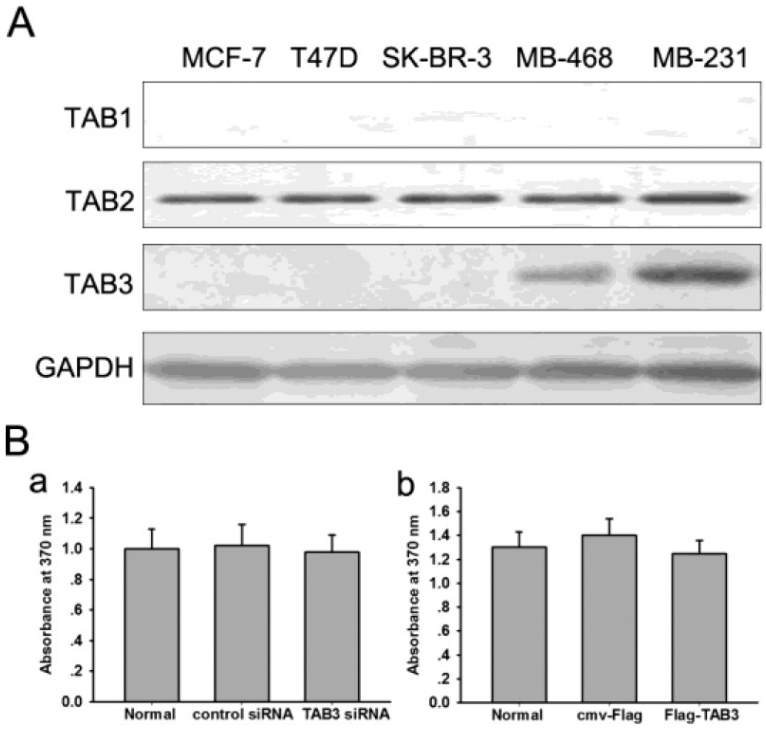
SUPPLEMENTARY MATERIALS AND METHODS

Reagents and antibodies

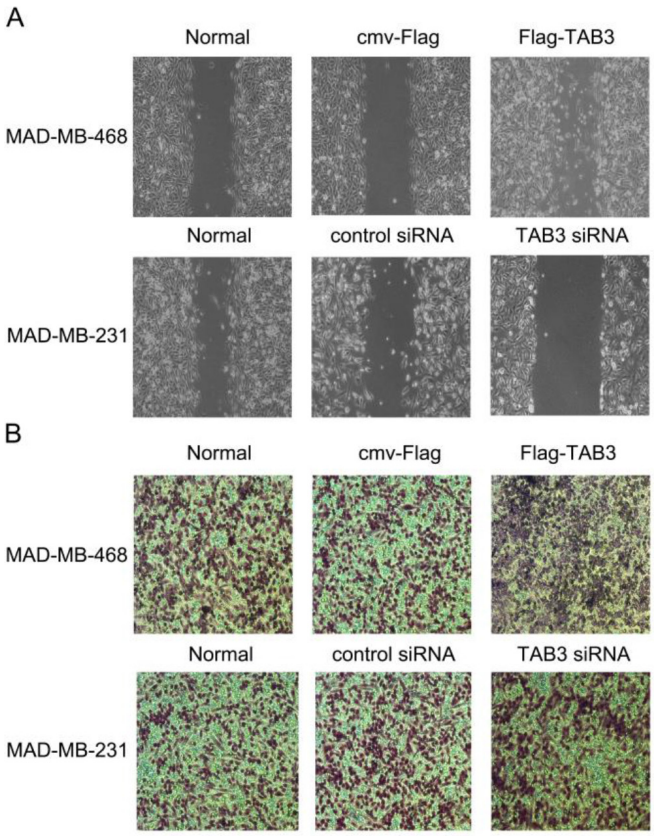
GlcNAcstatin was obtained from GlycoBioChem. Human IL-1β was from Protech. Click-iT™ O-GlcNAc Enzymatic Labeling System and Click-iT™ Biotin Protein Analysis Detection Kit were obtained from Invitrogen. GlycoProfile β-elimination kit was obtained from Sigma. The antibodies used in this study include: rabbit monoclonal anti-Flag antibody, rabbit polyclonal anti-HA antibody (Sigma), rabbit polyclonal anti-GAPDH antibodies (Santa Cruz), rabbit monoclonal anti-TAB3 antibody, rabbit monoclonal anti-TAK1/phosphorylated-TAK1 antibodies, rabbit monoclonal anti-NF-κBp65/phosphorylated-NF-κBp65 antibodies, rabbit monoclonal anti-p38/phosphorylated-p38 antibodies mouse monoclonal anti-O-GlcNAcylation CTD110.6 antibody (Cell Signaling Technology). The rabbit polyclonal anti-Thr404 phosphorylated-TAB3 antibodies and rabbit polyclonal anti-Ser408 O-GlcNAcylated-TAB3 antibody were made according to the classic approach.

Cell proliferation assay

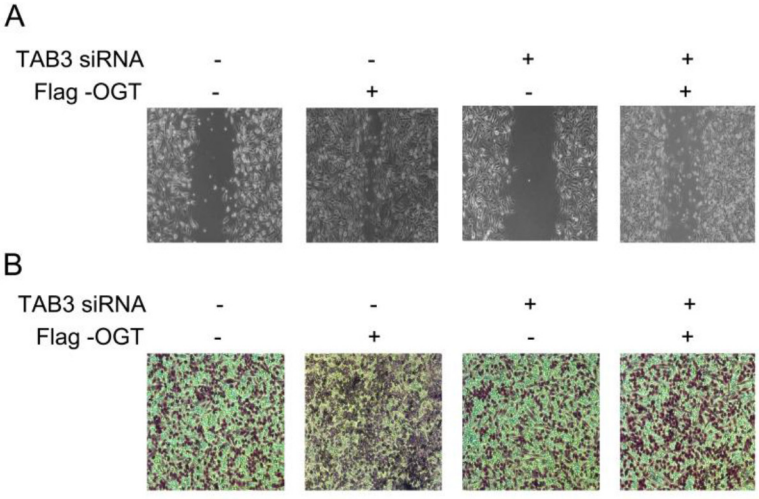
Cell proliferation was measured using the MTT assay. Briefly, cells were seeded onto 96-well cell culture cluster plates at a concentration of 10^4 cells/well in volumes of 100 μL, and grown overnight. MTT Reagents were added to the subset of wells, and then incubated for 2 hours at 37°C, the DMSO was added to detergent. Absorbances were quantified using an automated plate reader at 370 nm.



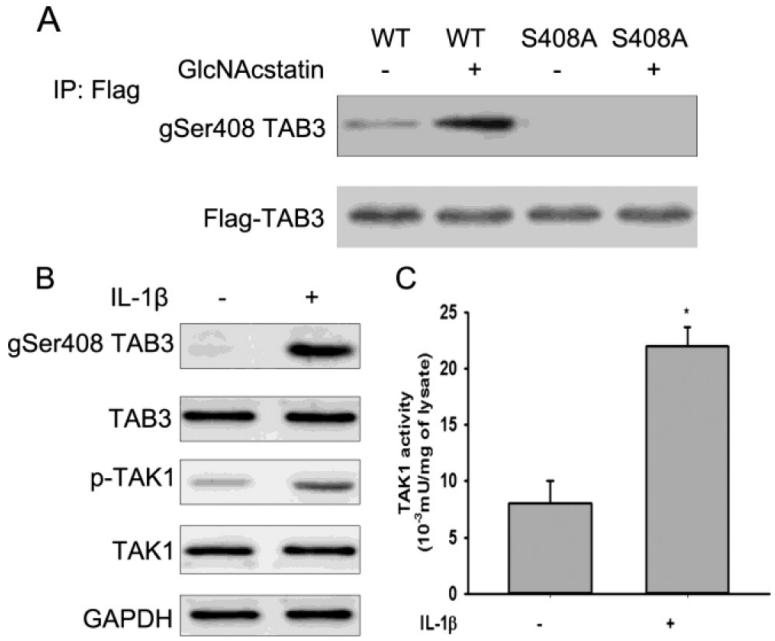
Supplementary Figure S1: Disturbed the TAB3 expression has no effect in TNBC cell proliferation. (**A**) A representative Western blot image of TAB1, TAB2 and TAB3 expression in 5 human breast cancer cell lines. (**B**) Equal numbers of indicated stably transfected MAD-MB-468 cells (a) and MAD-MB-231 cells (b) were seed on 96 well plates. MTT assay shown the cell viabilities when TAB3 was knockdown or overexpression. Data were shown as means ± SEM.



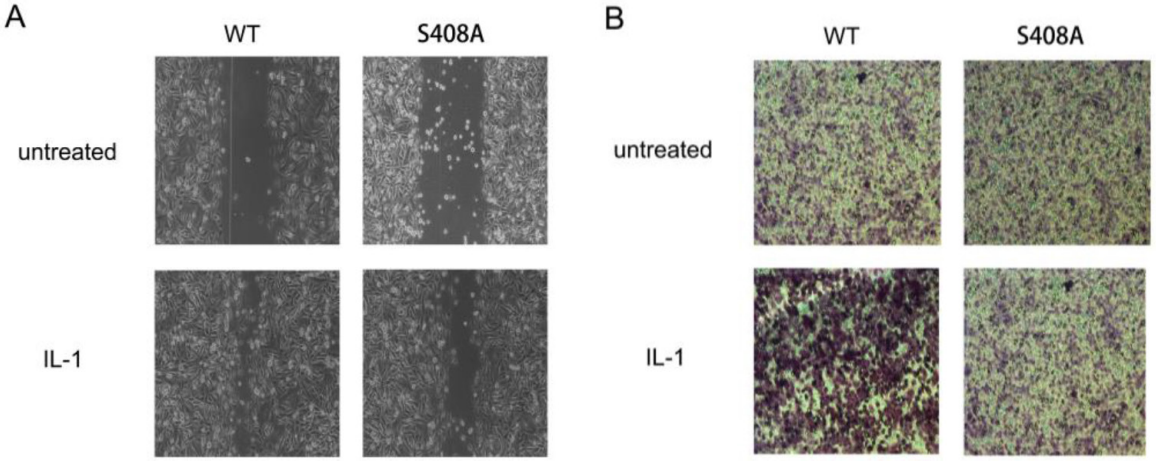
Supplementary Figure S2: TAB3 promoted TNBC cell migration and invasion *in vitro*. (**A**) Equal numbers of indicated stably transfected MAD-MB-468 cells and MAD-MB-231 cells seeded into six-well tissue culture plates. Wound healing assay was performed to detect cell migration ability. (**B**) 10^6 stably transfected MAD-MB-468 cells and MAD-MB-231 cells cultured in matrigel chambers. Transwell assay was performed to detect cell invasion ability.



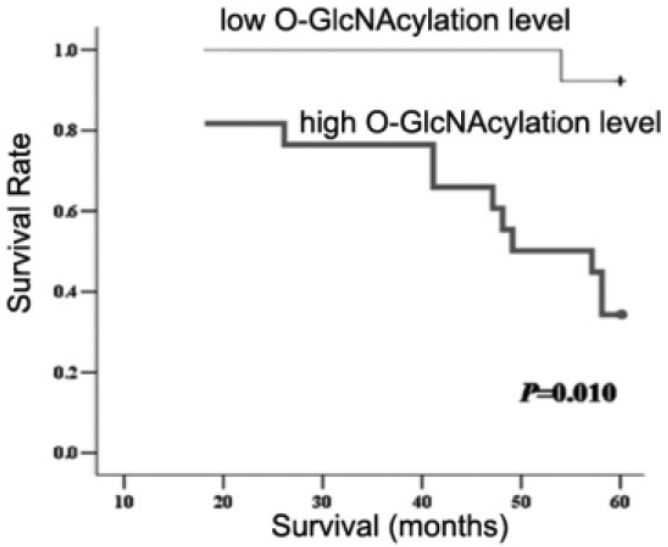
Supplementary Figure S3: TAB3 involved in OGT promoted TNBC cells migration and invasion. MDA-MB-231 cells were transfected with Flag-OGT and/or TAB3 siRNA vector. Cell migration (**A**) and invasion (**B**) were assessed as described.



Supplementary Figure S4: IL-1β promotes TAB3 O-GlcNAcylation and TAK1 activation in TNBC cells. (**A**) The specificity of the antibody which against TAB3 O-GlcNAc S408 was determined using cell lysates prepared from cells transfected with WT TAB3 and S408A TAB3 treated with or without 1 mM GlcNAcstatin. (**B**) MAD-MB-231 cells were serum starved for 6 h, and then stimulated by IL-1β for 15 min. Cell lysate were immunoblotted for O-GlcNAcylated-TAB3 (gSer408 TAB3), phosphorylated TAK1and total TAB3 and TAK1. Densitometries for TAB3 OGlcNAcylation and TAK1 phosphorylation were normalized against total TAB3 and TAK1 levels. (**C**) The TAK1 complexes were pulled down from the cell extracts (1 mg of protein extract) using glutathione-sepharose beads, and TAK1 activity assays were performed as described in the materials and methods section.



Supplementary Figure S5: TAB3 O-GlcNAcylation promoted TNBC cell migration and invasion *in vitro.* (**A**) Equal numbers of WT and S408A TAB3 stably transfected MAD-MB-231 cells seeded into six-well culture plates in the presence or absence of IL-1β. Wound healing assay was performed to detecte cell migration viability. (**B**) 10^6 stably transfected and MAD-MB-231 cells cultured in matrigel chambers with or without IL-1β. Transwell assay was performed to detect cell invasion ability.



Supplementary Figure S6: The total O-GlcNAcylation level is correlated with the poorer prognosis in TNBC patients. Kaplan-Meier survival curve of breast cancer patients with low and high total O-GlcNAcylation level.

Supplementary Table S1: Primers used in the manuscript

For cloning the human GST-TAB3	
Forward primer	CCGGGATTCATGGCTTATTCAGAAGAGCATA
Reversed primer	CCGCTCGAGCTACAGTTCAATCTCGAATGTC
For cloning the human Flag-TAB3	
Forward primer	CCGGAATTCGGATGTCCCGCAGCGCGGCGGCC
Reversed primer	CCGCTCGAGACAGTTCAATCTCGAATGTC
For cloning the human HA/Flag-OGT	
Forward primer	CCGGAATTCATGTCCCGCAGCGCGGCGGCC
Reversed primer	CCGCTCGAGACTACAGTTCAATCTCGAATGTC
For cloning the human Flag-S408A TAB3	
Forward primer	TCCCGCAGCGCGGCGGCCGACATG
Reversed primer	TACAGTTCAATCTCGAAGGTCGAG
For cloning the human Flag-T404A TAB3	
Forward primer	GCTTATTCAGAAGAGCATACGAGCG
Reversed primer	CCGAGATCCTCCAGGTCAGTTAAGT