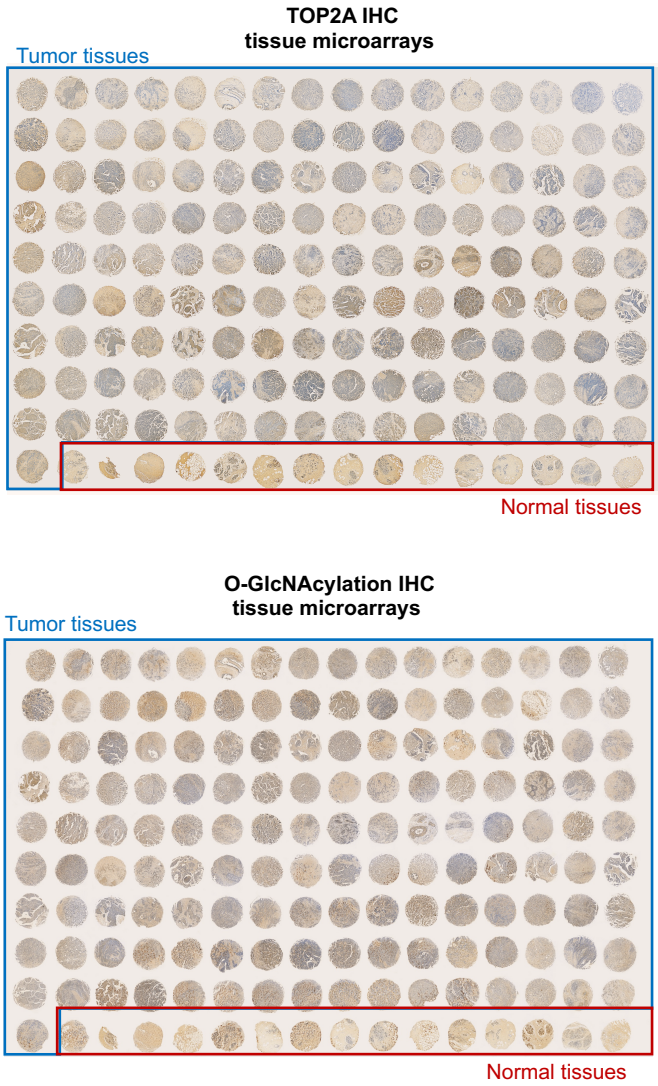
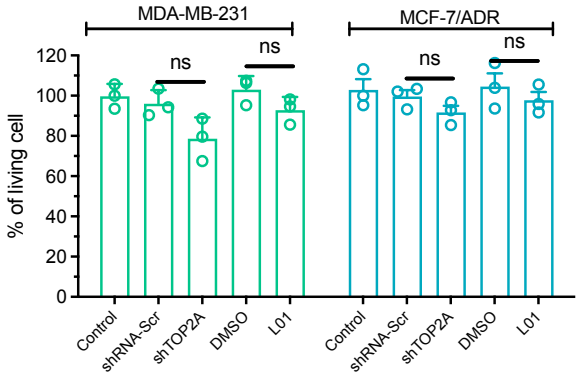
Appendix

O-GlcNAcylation promotes topoisomerase IIα catalytic activity in breast cancer chemoresistance

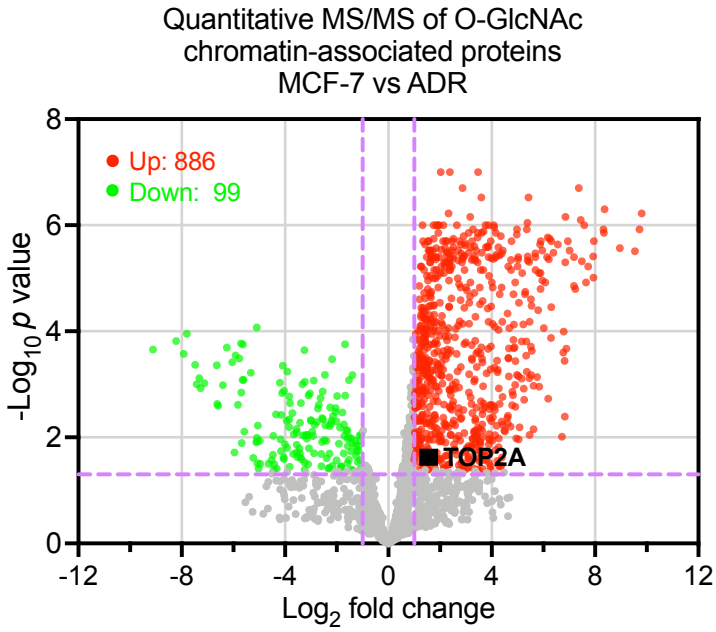
Table of content



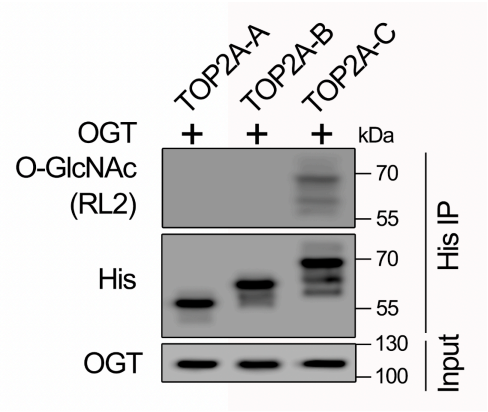
Appendix Figure S1 Layout and IHC staining of TOP2A and cellular O-GlcNAcylation on a tissue microarray containing 145 breast tumor and 15 adjacent samples.



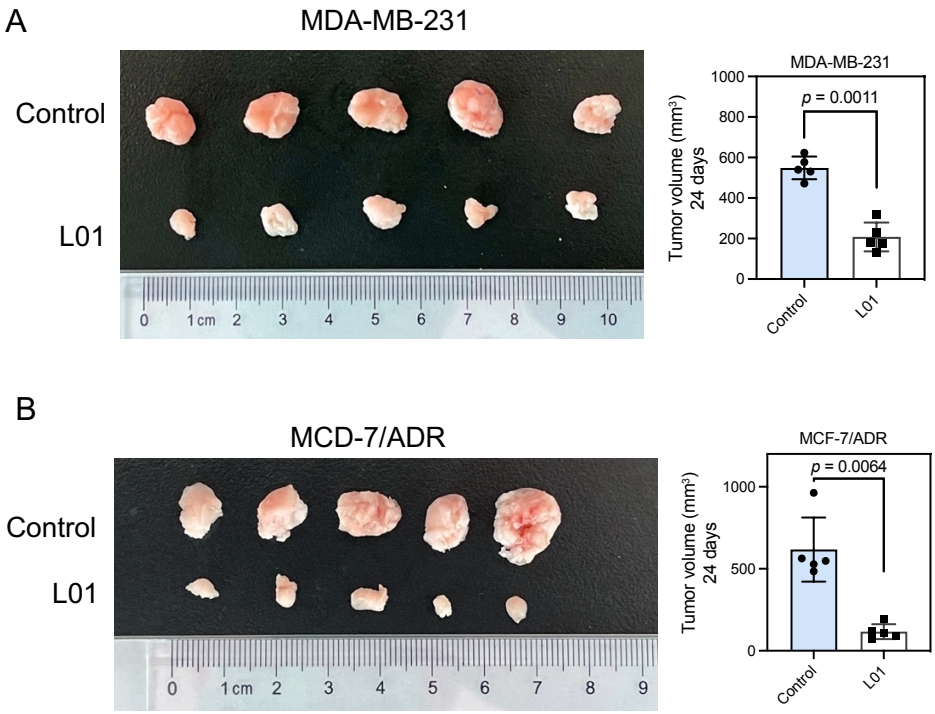
Appendix Figure S2 MDA-MB-231 and MCF-7/ADR cells were transfected with scrambled shRNA (shRNA-Scr) or TOP2A shRNA (shTOP2A) for 48 h. Or the cells were treated with 50 μM L01 for 48 h. The cell viability was assessed through a CCK-8 assay. Protein expression were shown in Figure 2D and E. OGT inhibition itself or TOP2A shRNA transfection itself did not have significant impact on the cell viability in MDA-MB-231 and MCF-7/ADR cells. n = 3 biologically replicates. Unpaired t-test was used for statistical comparison. *p* value was indicted.



Appendix Figure S3 TOP2A was previously reported to be a O-GlcNAcylated chromatin-associated protein (*Liu Y, Chen Q, Zhang N, et al. Proteomic profiling and genome-wide mapping of O-GlcNAc chromatin-associated proteins reveal an O-GlcNAc-regulated genotoxic stress response[J]. Nature communications, 2020, 11, 5898*). Volcano plot of label-free relative quantitative proteomics data of O-GlcNAc chromatin-associated proteins in MCF-7 and MCF-7/ADR cells (n = 9 biologically replicates, unpaired t-test). TOP2A was labelled.



Appendix Figure S4 His-tagged TOP2A-A, -B and -C expression plasmids were transfected with the OGT expression plasmid in HEK-293T cells. After 48 h, His-tag IP was performed, and the immunoprecipitated fractions were analyzed by Western blot for the indicated proteins.



Appendix Figure S5 The effects of L01 on tumor xenografts in nude mice. (A) MDA-MB-231 cells were injected subcutaneously into the axillae of nude mice (n = 5 for each group). 1 mg/kg L01 was administrated by tail vein injection every other day for 22 days. Control group was treated with DMSO. Volumes of tumors were monitored with caliper twice a week until 24 days. Unpaired t-test was used for statistical comparison. *p* value was indicted. (B) MCF-7/ADR cells were injected subcutaneously into the axillae of nude mice (n = 5 for each group). 4 mg/kg Adm and 1 mg/kg L01was administrated by tail vein injection. Control group was treated with DMSO. Volumes of tumors were monitored with caliper twice a week until 24 days. Unpaired t-test was used for statistical comparison. *p* value was indicted.

Appendix Table S1

The relationship between *TOP2A* mRNA expression and clinicopathological factors in 1072 TCGA breast cancer patients (female).

Variables	No. of patients	TOP2A		Chi-Square	*p* value
		low	high		
Age					
< 50	292	139	153	0.9225	0.3368
≥ 50	780	397	383		
Stage					
I	188	115	73	11.38	0.0007***
II + III + IV + X	884	421	463		
T classification					
T1	278	168	110	16.34	< 0.0001****
T2 + T3 + T4	794	368	426		
N classification					
NX + N0	523	265	258	0.1829	0.6689
N1 + N2 + N3	549	271	278		

The cutoff for high and low TOP2A expression were ≥ median or < median, ***p < 0.001, ****p < 0.0001

Appendix Table S2

The relationship between TOP2A protein expression and clinicopathological factors in 145 breast tumor samples on a tissue microarray.

Variables	No. of patients (female)	TOP2A		Chi-Square	*p* value
		low	high		
Age					
< 50	70	30	40	0.6109	0.4344
≥ 50	75	37	38		
Grade					
1	31	20	11	5.318	0.0211*
2 + 3	114	47	67		
Stage					
0 + I	10	6	4	0.3341	0.5633
II + III	135	61	74		
T classification					
Tis + T1 + T2	93	50	43	5.957	0.0147*
T3 + T4	52	17	35		
N classification					
N0 + N1	119	56	63	0.1938	0.6598
N2 + N3	26	11	15		
Lymph node metastasis					
No	96	50	46	3.947	0.047*
Yes	49	17	32		
PR					
Negative	107	46	61	1.699	0.1924
Positive	38	21	17		
ER					
Negative	110	52	58	0.2083	0.6481

Positive	35	15	20		
HER2					
Negative	65	33	32	0.9866	0.3206
Positive	80	34	46		

High group: IHC score ≥ 4 (median score), low group: IHC score < 3, *$p < 0.05$.

Appendix Table S3

The relationship between cellular O-GlcNAcylation expression and clinicopathological factors in 145 breast tumor samples on a tissue microarray.

Variables	No. of patients (female)	O-GlcNAc		Chi-Square	*p* value
		low	high		
Age					
< 50	70	32	38	0.002119	0.9633
≥ 50	75	34	41		
Grade					
1	31	19	12	3.956	0.0467*
2 + 3	114	47	67		
Stage					
0 + I	10	6	4	0.3895	0.5326
II + III	135	60	75		
T classification					
Tis + T1 + T2	93	48	45	3.886	0.0487*
T3 + T4	52	18	34		
N classification					
N0 + N1	119	62	57	11.6	0.0007***
N2 + N3	26	4	22		
Lymph node metastasis					
No	96	51	45	6.63	0.01*
Yes	49	15	34		
PR					
Negative	107	43	64	4.678	0.0306*
Positive	38	23	15		
ER					
Negative	110	47	63	1.43	0.2317

Positive	35	19	16		
HER2					
Negative	65	29	36	0.03864	0.8442
Positive	80	37	43		

High group: IHC score ≥ 3 (median score), low group: IHC score < 3, *p < 0.05, ***p < 0.001.

Appendix Table S4

Clinical characteristics of breast cancer patient samples.

Patient no.	Gender	Age	Grade	Stage	ER	PR	HER2	Chemotherapy-resistant /relapsed
1#	Female	67	2	II	Positive	Positive	Positive	No
2#	Female	52	2	II	Negative	Negative	Positive	No
3#	Female	44	3	III	Negative	Negative	Positive	Yes
4#	Female	48	3	III	Negative	Negative	Negative	Yes
5#	Female	58	3	III	Positive	Positive	Positive	Yes

Appendix Table S5

Full-length human wild type and O-GlcNAcylation site mutants TOP2A	Forward	Reverse
Subclone into pLVX-IRES-Neo vector	TTCCTCGAGGCCACCATGTACCCATACGATGTTCCAGATTACG	ATCCGCGGCCGCTTAAAACAGATCATCTTCATCTGAC
O-GlcNAcylation site mutant (Ser1469 → Ala) TOP2A	GAATCGCCGCAAAAGGAAGCCAGCAACTTCTGATGATTCTGACTC	GAGTCAGAATCATCAGAAGTTGCTGGCTTCCTTTTGCGGCGATTC
Truncated TOP2A-A	Forward	Reverse
Subclone into pET28a(+) vector	TTCGAGCTCATGGAAGTGTCACCATTGCAG	CTTGTCGACAATACCACAGCCAATGGCAG
Subclone into pCMV vector	CGGGTCGACATGGAAGTGTCACCATTGCAG	CTTGGGCCCTCAATGGTGATGGTGATGATGAATACCACAGCCAATGGCAG
Truncated TOP2A-B	Forward	Reverse
Subclone into pET28a(+) vector	TTCGAGCTCGTAGAAAGCATACTAAACTGGG	CTTGTCGACGGTTGTAGAATTAAGAATAGCTAC
Subclone into pCMV vector	CGGGTCGACATGGTAGAAAGCATACTAAACTGGG	CTTGGGCCCTCAATGGTGATGGTGATGATGGGTTGTAGAATTAAGAATAGCTAC
Truncated TOP2A-C	Forward	Reverse
Subclone into pET28a(+) vector	TTCGAGCTCATTGAAATCTCAGAGCTTCCC	CTTGTCGACAAACAGATCATCTTCATCTGAC
Subclone into pCMV vector	CGGGTCGACGCCACCATGATTGAAATCTCAGAGCTTCCC	CTTGGGCCCTCAATGGTGATGGTGATGATGAAACAGATCATCTTCATCTGAC
Flag-tagged human full-length OGT	Forward	Reverse
Subclone into pcDNA3.1 vector	CTTGGTACCATGGCGTCTTCCGTGGGC	AGTGGATCCTCACTTATCGTCGTCATCCTTGTAATCTGCTGACTCAGTGACTTCAAC