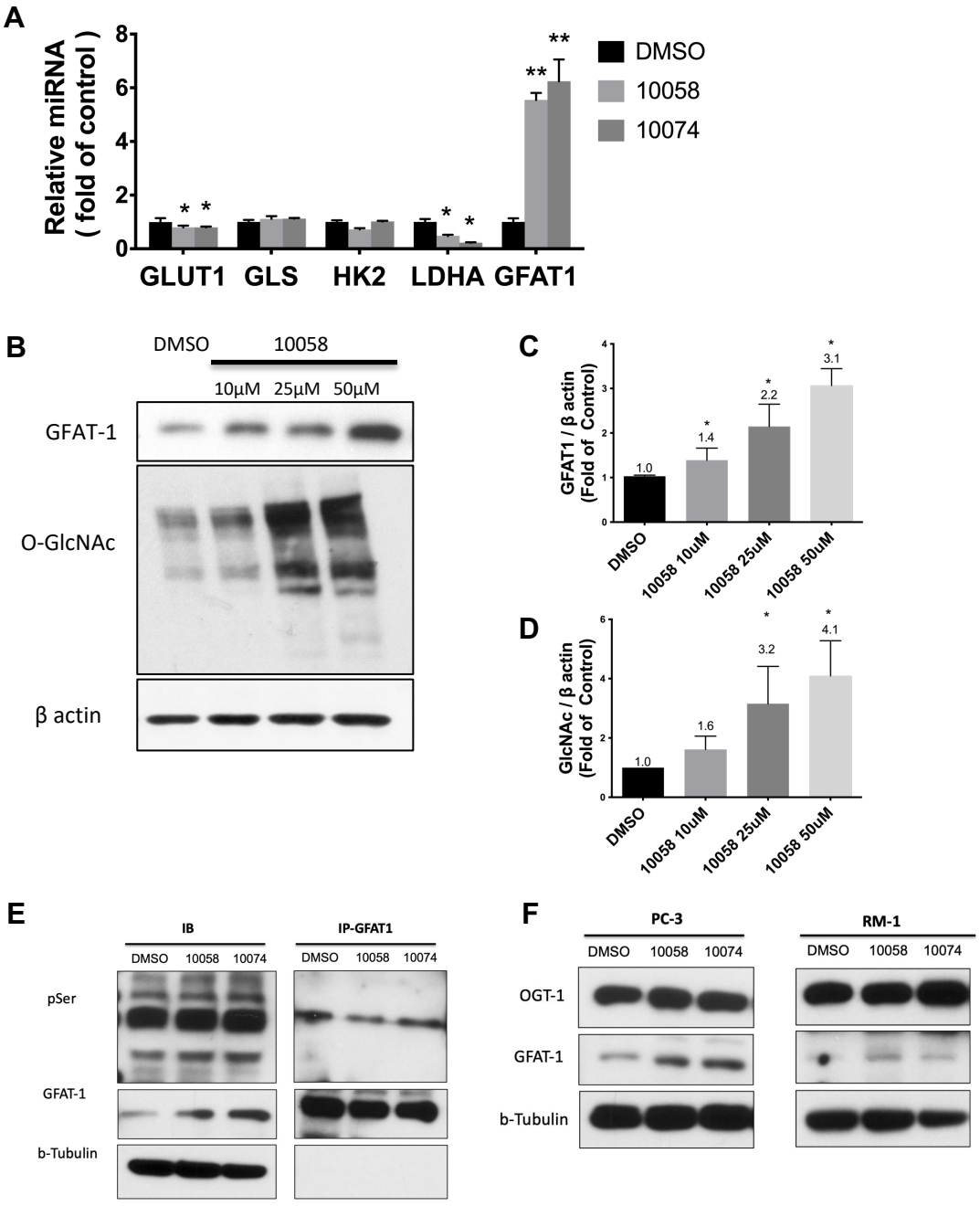



**Figure S1. Inhibition of Myc leads to GFAT1 up-regulation and increased total protein glycosylation in PC3 cells in a dose dependent manner.** (**A**) Real-time PCR analysis of pivotal genes involved in glucose metabolism in PC3 cells following treatment of Myc inhibitors. (**B-D**) Western blot assay revealed the effect of Myc inhibitor 10058 in up-regulating GFAT1 and total protein glycosylation in a dose dependent manner. (**E**) Immunoprecipitation of GFAT1 indicate that the phospho-GFAT1 was not altered by Myc inhibitors. (**F**) Western blot assay shows that 10058 and 10074 could not change OGT-1 level in prostate cell lines. The results are expressed as the mean ± SEM of triplicate measurements in each group. *p<0.05, **p<0.01, ***p<0.001.

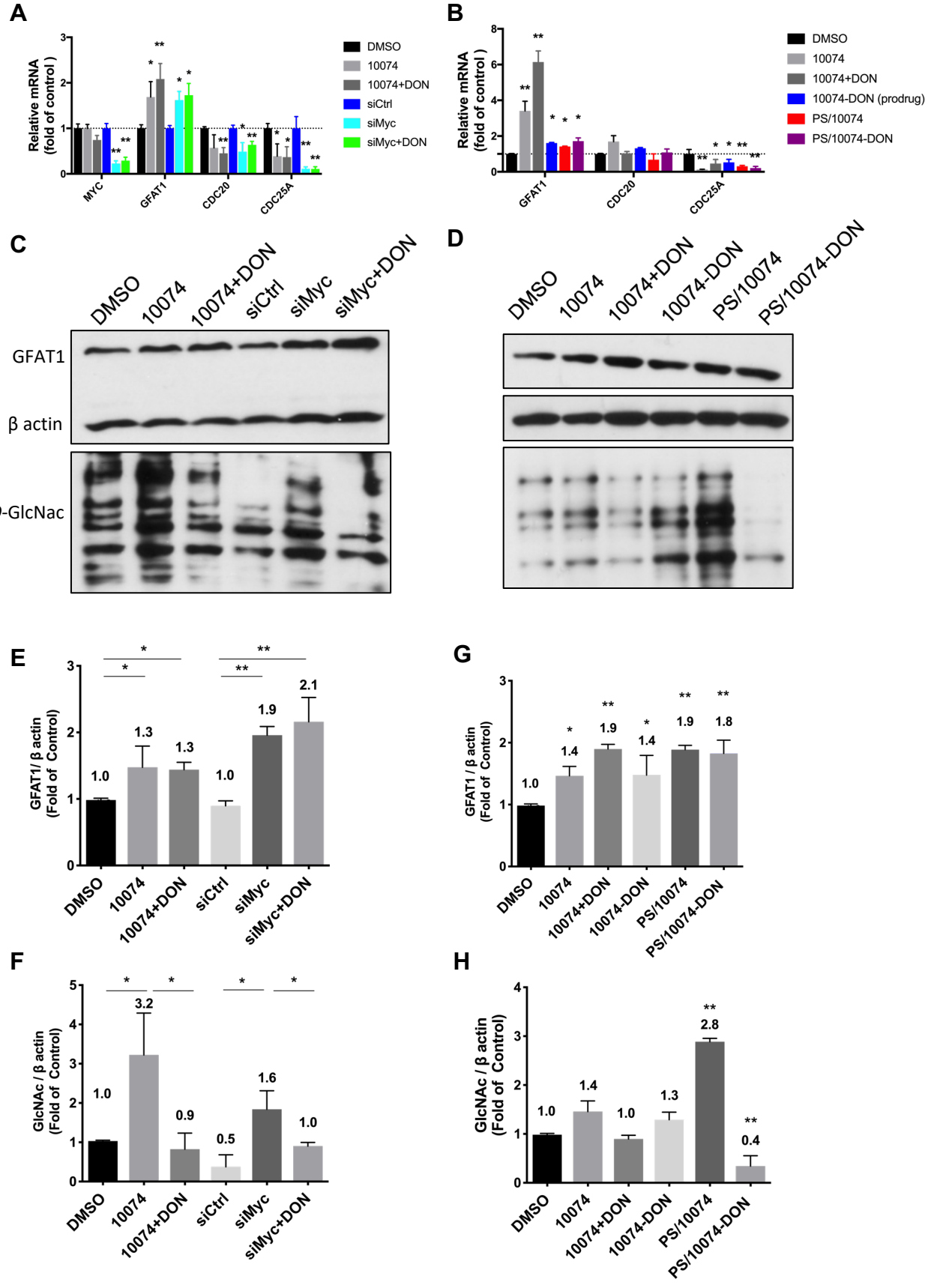

A
Relative mRNA (fold of control)
DMSO
10074
10074+DON
siCtrl
siMyc
siMyc+DON
MYC
GFAT1
CDC20
CDC25A
B
Relative mRNA (fold of control)
DMSO
10074
10074+DON
10074-DON (prodrug)
PS/10074
PS/10074-DON
GFAT1
CDC20
CDC25A
C
DMSO
10074
10074+DON
siCtrl
siMyc
siMyc+DON
GFAT1
β actin
O-GlcNac
D
DMSO
10074
10074+DON
10074-DON
PS/10074
PS/10074-DON
E
GFAT1/ β actin (Fold of Control)
1.0
1.3
1.3
1.0
1.9
2.1
DMSO
10074
10074+DON
siCtrl
siMyc
siMyc+DON
G
GFAT1 / β actin (Fold of Control)
1.0
1.4
1.9
1.4
1.9
1.8
DMSO
10074
10074+DON
10074-DON
PS/10074
PS/10074-DON
F
GlcNAc / β actin (Fold of Control)
1.0
3.2
0.9
0.5
1.6
1.0
DMSO
10074
10074+DON
siCtrl
siMyc
siMyc+DON
H
GlcNAc / β actin (Fold of Control)
1.0
1.4
1.0
1.3
2.8
0.4
DMSO
10074
10074+DON
10074-DON
PS/10074
PS/10074-DON

**Figure S2. Inhibition of Myc leads to GFAT1 up-regulation and increased total protein glycosylation in Myc-CaP cell line**: (**A-B**) Total RNAs in PC3 cells were isolated for qPCR analysis. (**A**) Myc-Cap cells were treated with 10074, 10074/DON, siMyc, or siMyc /DON combination. (**B**)The Myc-Cap cells were treated with 10074, 10074/DON combination, 10074-DON prodrug, PS/10074 or PS/10074-DON. (**C-D**) Total proteins in cell lysates were analyzed by Western blot. (**E-H**)Analysis of band intensity. The results are expressed as the mean ± SEM of triplicate measurements in each group. *p<0.05, **p<0.01, ***p<0.001.

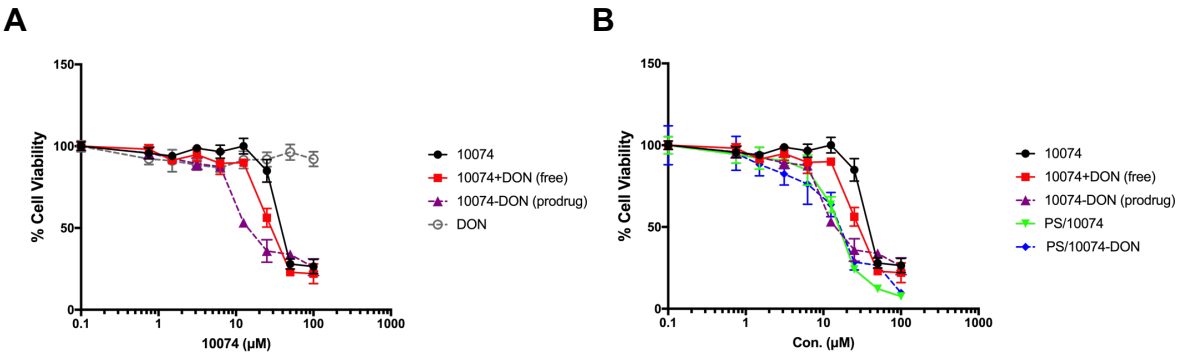



**Figure S3. Cell viability of Myc-Cap cells receiving various treatments**. (**A-B**) MTT assay of 10074 or 10074-DON in Myc-Cap cells treated with prodrug or carrier-loaded drug. The results are expressed as the mean ± SEM of triplicate measurements in each group. *p<0.05, **p<0.01, ***p<0.001.

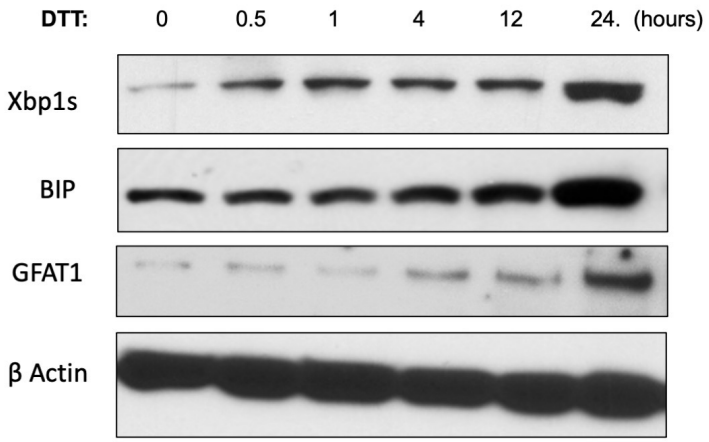



**Figure S4. ER stress inducer DTT significantly induce Xbp1s, BIP and GFAT1 protein expression in a timely-dependent manner.**

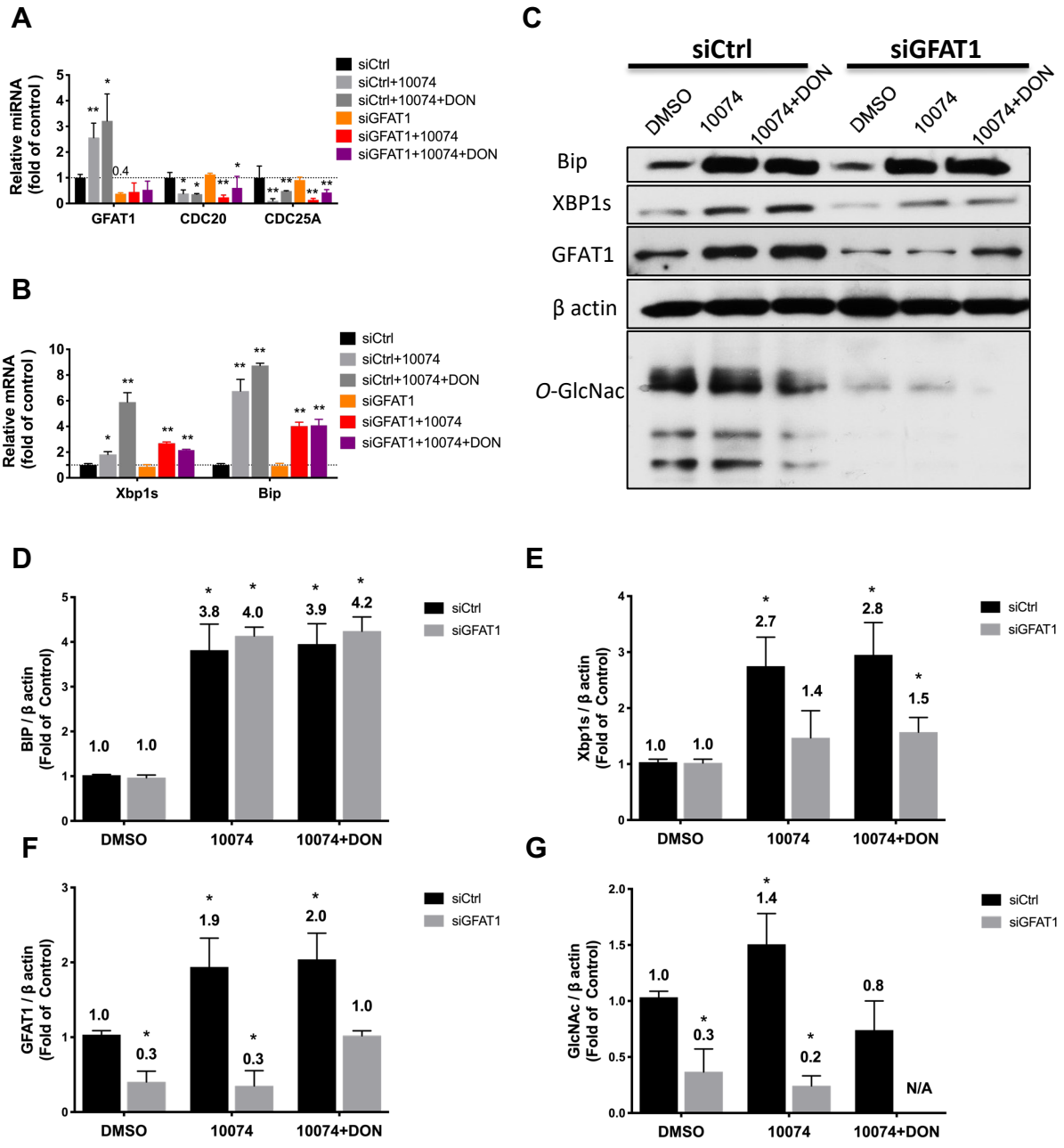
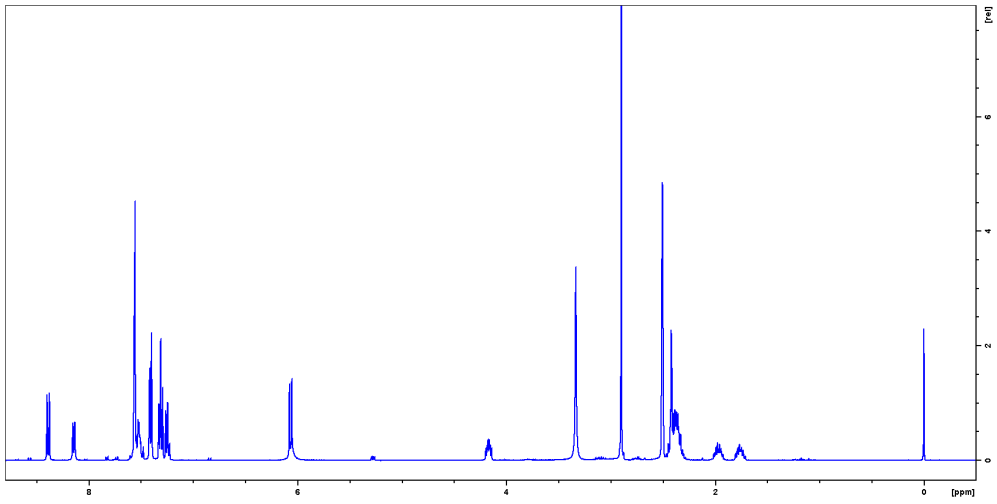



**Figure S5. Knockdown of GFAT1 by siRNA attenuated 10074-induced protein glycosylation but did not affect the expression of Myc target genes as well as the expression of XBP1s and Bip**: (**A-B**) Knockdown GFAT1 by siRNA abolished the effect of 10074 in up-regulating the expression of GFAT1 mRNA but did not affect the mRNA expression of other Myc target genes (**A**) as well as xbp1s and BIP (**B**). (**C**) Pre-transfection of GFAT-1 siRNA (siGFAT1) attenuated the effect of 10074 in up-regulating the protein level of GFAT1 and total protein glycosylation but did not affect the 10074-induced upregulation of XBP1s and BIP. (**D-G**) Quantification of western results.(The results are expressed as the mean ± SEM of triplicate measurements in each group. *p<0.05, **p<0.01.

A




B

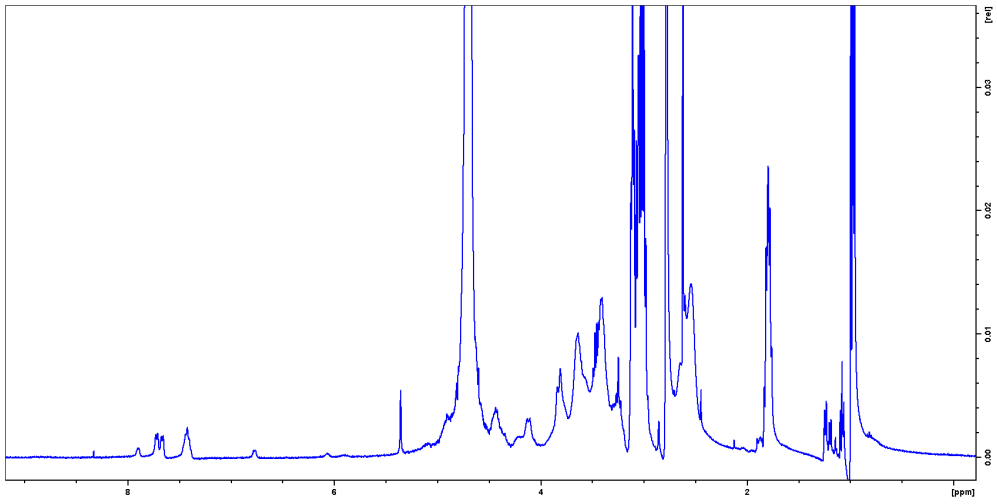



**Figure S6. NMR spectrum of 10074-DON conjugate(A) and NMR spectrum of 10074-derivatized laminarin(B)**.

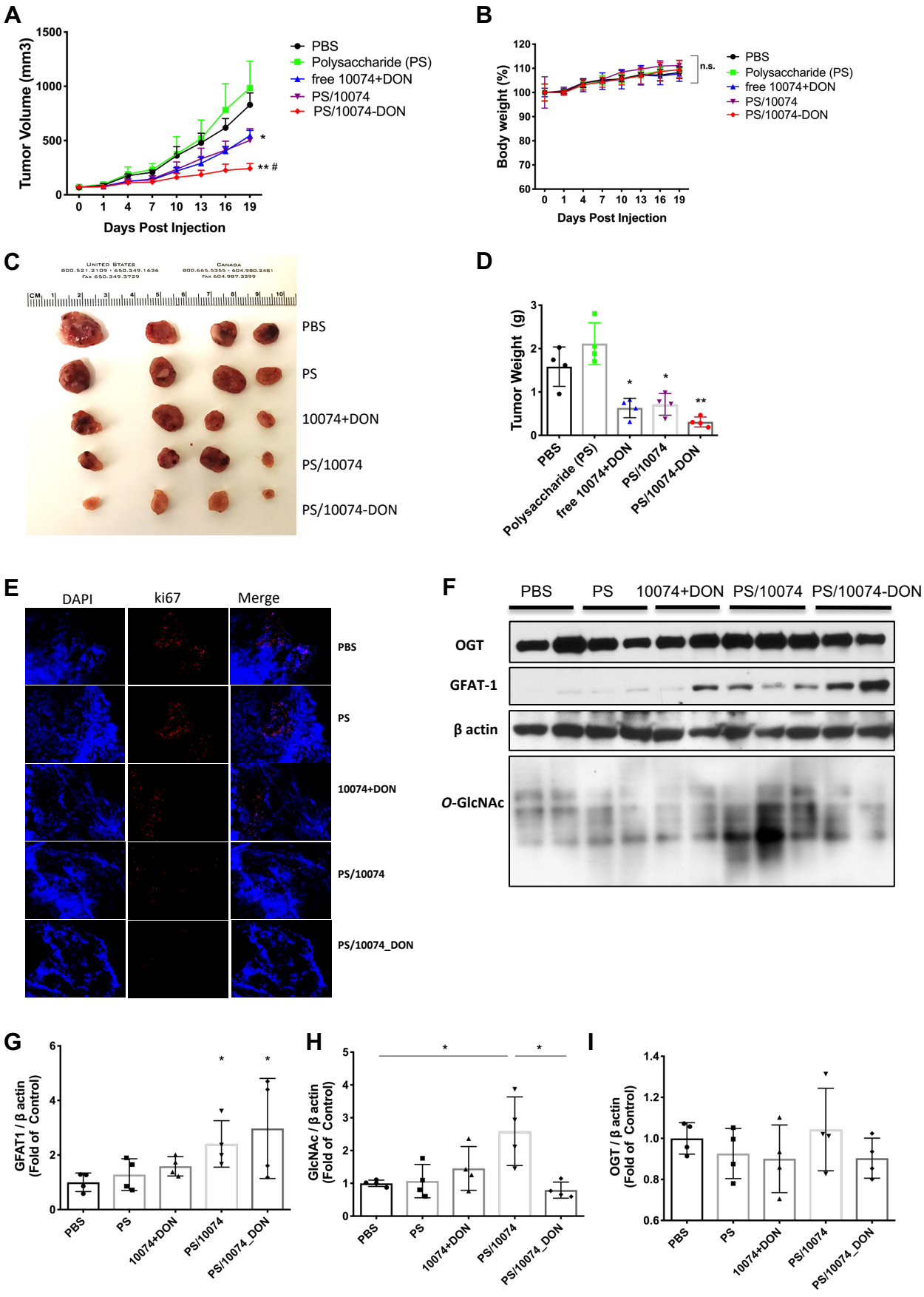

A
Tumor Volume (mm3)
1500
1000
500
0
0 1 4 7 10 13 16 19
Days Post Injection
PBS
Polysaccharide (PS)
free 10074+DON
PS/10074
PS/10074-DON
*
** #
B
Body weight (%)
120
110
100
90
80
70
60
0 1 4 7 10 13 16 19
Days Post Injection
n.s.
PBS
Polysaccharide (PS)
free 10074+DON
PS/10074
PS/10074-DON
C
PBS
PS
10074+DON
PS/10074
PS/10074-DON
D
Tumor Weight (g)
3
2
1
0
*
*
**
PBS
Polysaccharide (PS)
free 10074+DON
PS/10074
PS/10074-DON
E
DAPI
ki67
Merge
PBS
PS
10074+DON
PS/10074
PS/10074_DON
F
PBS
PS
10074+DON
PS/10074
PS/10074-DON
OGT
GFAT-1
β actin
O-GlcNAc
G
GFAT1 / β actin (Fold of Control)
6
4
2
0
*
*
PBS
PS
10074+DON
PS/10074
PS/10074_DON
H
GlcNAc / β actin (Fold of Control)
5
4
3
2
1
0
*
*
PBS
PS
10074+DON
PS/10074
PS/10074_DON
I
OGT / β actin (Fold of Control)
1.4
1.2
1.0
0.8
0.6
PBS
PS
10074+DON
PS/10074
PS/10074_DON

**Figure S7. *In vivo* anti-tumor effect of 10074-DON-loaded PS NPs in Myc-Cap tumor model**: (**A**) Tumor growth curves in Myc-Cap tumor model. (**B**) Body weight changes during the treatment. (**C**) Gross images of the endpoint tumors. (**D**) Endpoint tumor weights in RM-1 tumor model. (**E**) Ki67 immunostaining of tumor sections. (**F**) Western blot analysis of the tumor tissues collected in (**C**) and the quantification by ImageJ (**G-I**). The results are expressed as the mean ± SEM of measurements from 6 animals per group, and each dot represents a measurement from one animal. *p<0.05, **p<0.01, ***p<0.001.

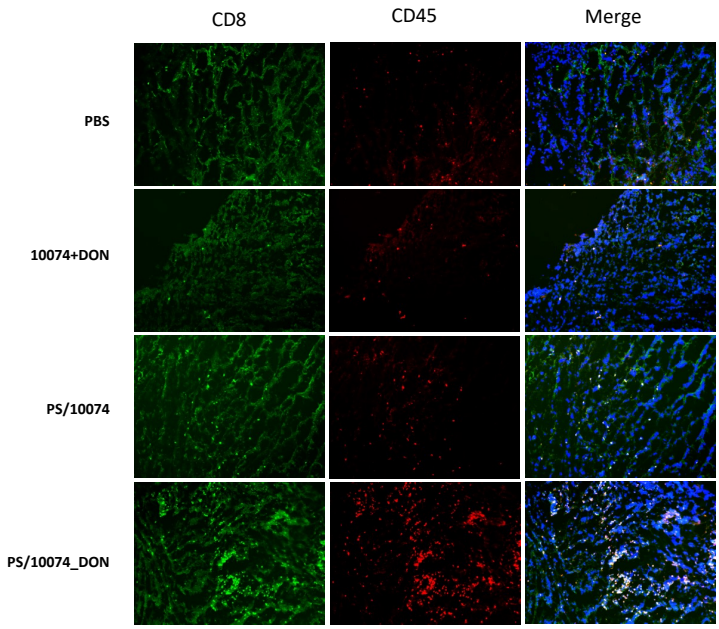



**Figure S8. Immunostaining of infiltrating immune cells in tumor tissues with various treatments**: RM-1 tumor-bearing mice received various treatments as described in **Fig. 8**. Infiltration of $CD4^+$ and $CD8^+$ T cells in tumor tissues was examined by staining of tumor sections with the respective specific antibody.

**Table S1.** Primers used in real-time PCR.

| Primer | Species | Sequence |
|---|---|---|
| GAPDH-F | human | GGTGAAGGTCGGAGTCAACG |
| GAPDH-R | human | TGGGTGGAATCATATTGGAACA |
| MYC-F | human | AATGAAAAGGCCCCCAAGGTAG |
| MYC-R | human | GTCGTTTCCGCAACAAGTCCT |
| CDC20-F | human | CGCTATATCCCCCATCGCAG |
| CDC20-R | human | CTGATAACCCTCTGGCGCAT |
| CDC25A-F | human | AGACCTGTATCTCGTGGCTG |
| CDC25A-R | human | CATTTGAGGAAAGCATCCGAGC |
| CDC45-F | human | GTGATTTGGCGGGAGTCTTG |
| CDC45-R | human | CGAAGAGAAGGACCCTCTGG |
| GFAT1-F | human | AGCAGTGCAAACCCTCCAGATGG |
| GFAT1-R | human | TGAACCCCACAATCTGTCTCCCG |
| GLUT1_F | human | TTGCAGGCTTCTCCAACTGGAC |
| GLUT1_R | human | CAGAACCAGGAGCACAGTGAAG |
| GLS_F | human | CAGAAGGCACAGACATGGTTGG |
| GLS_R | human | GGCAGAAACCACCATTAGCCAG |
| LDHA_F | human | GGATCTCCAACATGGCAGCCTT |
| LDHA_R | human | AGACGGCTTTCTCCCTCTTGCT |
| HK2_F | human | GAGTTTGACCTGGATGTGGTTGC |
| HK2_R | human | CCTCCATGTAGCAGGCATTGCT |
| XBP1u_F | human | CAGACTACGTGCACCTCTGC |
| XBP1u_R | human | CTGGGTCCAAGTTGTCCAGAAT |
| GFAT1-F | mouse | GGA ATC ATC ACC AAC TAC AAA GAC |
| GFAT1-R | mouse | AAT ACT CCA CTG CTT TTT CTT CCA C |
| XBP1u_F | mouse | CAGACTACGTGCACCTCTGC |
| XBP1u_R | mouse | CAGGGTCCAACTTGTCCAGAAT |
| XBP1s_F | mouse | GCTGAGTCCGCAGCAGGT |
| XBP1s_R | mouse | CAGGGTCCAACTTGTCCAGAAT |
| CHOP_F | mouse | AAGATGAGCGGGTGGCAGCG |
| CHOP_R | mouse | GCACGTGGACCAGGTTCTGCT |
| GRP78_F | mouse | TGCAGCAGGACATCAAGTTC |
| GRP78_R | mouse | TACGCCTCAGCAGTCTCCTT |

**Table S2.** Primary antibodies used in western blot.

| | Species | Dilution | Cat.# | Brand |
|---|---|---|---|---|
| **GFAT1** | Rabbit | 1:1000 | 3818S | Cell Signaling Technology (Danvers, MA,US) |
| **O-GlcNac** | Mouse | 1:500 | ab201995 | Abcam (Cambridge, UK) |
| **c-MYC** | Rabbit | 1:1000 | 18583S | Cell Signaling Technology (Danvers, MA,US) |
| **β Actin** | Rabbit | 1:4000 | 4970S | Cell Signaling Technology (Danvers, MA,US) |
| **Xbp1s** | Rabbit | 1:1000 | 24868-1-AP | Thermo Fisher Scientific (Waltham, MA, US ) |
| **IRE1α** | Rabbit | 1:1000 | 3294S | Cell Signaling Technology (Danvers, MA,US) |
| **pIRE1α** | Rabbit | 1:1000 | PA1-16927 | Thermo Fisher Scientific (Waltham, MA, US ) |
| **BIP** | Rabbit | 1:1000 | 3177S | Cell Signaling Technology (Danvers, MA,US) |
| **OGT** | Rabbit | 1:1000 | 24083S | Cell Signaling Technology (Danvers, MA,US) |